User Guide

ZEN 2.1 (blue edition)

We make it visible.

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1 About this help

1.1 Welcome

Welcome to the ZEN 2.1 User Guide.

ZEN 2.1 is a modular image acquisition, processing and analysis software for digital microscopy. The abbreviation ZEN stands for ZEISS Efficient Navigation and points out that the software can be used to control all microscopes and imaging systems by ZEISS. In addition to basic functionality for image acquisition, elementary image processing and annotations, image analysis and reporting functions a lot of optional modules for specific microscopy tasks are available.

This User Guide contains all the information you need to work with the installed software.

1.2 Overview of the Contents

This document is split into the following main sections:

Section	Description
About this User Guide	Introduction and overview of the documentation as well as conventions for the related information product, see *Welcome* [▶ 10].
Basic Concepts	Background information about the concepts of the software, see *Introduction* [▶ 15].
First Steps	First steps with the software. A chapter with descriptions of the user interface and how to guides ideal for beginners and users who are new to the ZEN software, see *First Steps* [▶ 17].
Image Acquisition	How to guides for the main imaging tasks with the software, see *Image Acquisition* [▶ 34].
Image Processing	How to guides for the imaging processing, see *Image Processing* [▶ 82].
Image Analysis	How to guides for Image Analysis, see *Image Analysis* [▶ 119].
Experiment Feedback	Introduction and concepts of Feedback Experiments, see *Introduction* [▶ 138]. An additional extensive tutorial with code samples and macros is available on the DVD.

Section	Description
Customizing the Application	This chapter shows the possibilities to adopt the software appearance to the users needs, see *Customizing toolbar* [▶ 140]
Open Application Development (OAD)	Introduction and Concepts of Open Application Develpoment, see *OAD* [▶ 141].
Module Tiles	An extensive documentation of the Tiles module. A lot of descriptions and how to guides can be found here, see *Module Tiles* [▶ 149].
Module Physiology	An extensive documentation of the Physiology module. A lot of descriptions and how to guides can be found here, see *Module Physiology* [▶ 243].
Module Shuttle & Find	An extensive documentation of the Shuttle & Find module. A lot of descriptions and how to guides can be found here, see *Introduction* [▶ 262].
Module CAT	Here you find an introduction and basic functional descriptions for the CAT module, see *Introduction* [▶ 296]. An extensive user guide with how-to instructions is in preparation and will be provided soon.
Software Functions and Reference	Theses chapters refer to functions and descriptions of the software, see *Software Functions* [▶ 313]. As theses topics are called up context sensitive, you just have to press F1 when you are in the software and need help for the respective function. The chapters structure is optimized therefore.
FAQ	Frequently asked questions on the software, see *FAQ* [▶ 741].

1.3 Conventions in this Document

The following text formats are used in this document:

Example	Description
Save icon	Clickable user interface elements, e.g. buttons and icons
On/off button	Hardware buttons on the microscope
Image panel	Non-clickable user interface elements, e.g. name of a dialog box

Example	Description
CTRL key	Keyboard shortcut
`Input` text	Text to be entered by the user
Link [▶ 11]	Link to further information

Additional information is indicated as follows:

i Note

Helpful additional information, e.g. about necessary additional actions.

1.4 Safety Notes and Safety Icons Conventions

The safety notes in this documentation follow a system of risk levels, that are defined as follows:

⚠ WARNING

Risk of personal injury

WARNING indicates a potentially hazardous situation which, if not avoided, could result in major personal injury or death.

⚠ CAUTION

Risk of personal injury

CAUTION indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate personal injury.

NOTICE

Risk of property damage

NOTICE indicates a property damage message. In addition, NOTICE is used for data loss or corrupt data as well.

The safety icons / labels on the device refer to potential dangers that are defined as follows:

Icon / Label	Name	Description
	**Crushing Fingers**	This icon warns you of a potential risk of crushing fingers.

1.5 Legal Notes

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Safety

Refer to the safety notes and instructions in the documentations of all necessary devices (e.g. microscope peripherals, cameras, computers, computer additionals, etc.) before installing and using the software.

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Notice of the Producer

This software product was designed, realized, verificated, validated and released in a certificated process environment. The quality management system is certified following the rule of DIN EN ISO 9001 and DIN EN ISO 13485.

The fields of application of the Software are common tasks and applications in microscopy respectively imaging (so called "Off-The-Shelf Software"). Though the user acknowledges that in any kind of use the end user of the software is responsible for the validation of the Software for the end user's dedicated intend of use considering all requirements of law and standards (e. g. FDA/21 CFR part 11, IvDD, etc.). If necessary the end user has to establish, to document, to implement and to maintain a special process to fulfill all the requirements to be conform with the validate rules of law and standards. It is pointed out that displayed measure values (eg length measurement) may not be used directly as analytical values for diagnostic results.

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2 Basic Concepts

2.1 Introduction

ZEN 2.1 is a modular image acquisition, processing and analysis software for digital microscopy. The abbreviation ZEN stands for ZEISS Efficient Navigation and points out that the software can be used to control all microscopes and imaging systems by ZEISS. In addition to basic functionality for image acquisition, elementary image processing and annotations, image analysis and reporting functions a lot of optional modules for specific microscopy tasks are available.

2.2 Image Acquisition

The software ZEN 2.1 completes all microscopes and cameras from ZEISS to efficient and tailor-made imaging systems. With little training you will interactively control the entire workflow from image acquisition, processing and analysis.

Depending on the system you capture single images, multi-channel fluorescence images or video sequences with up to 16-bit per channel image information. ZEN supports reliably: Smart Setup proposes the optimal dye and wavelength combinations for your experiment.

A range of different camera types can be used with ZEN 2.1, from simple TV cameras through to high-resolution and high-sensitivity microscope cameras. The seamless integration of cameras into the software allows you to create complex images and image sequences by one mouse click. For best results we recommend to use Axiocam microscope cameras by ZEISS.

2.3 Image Processing

After acquiring an image it is immediately displayed on your screen. It can then be optimized using a wide range of techniques:

- Contrast, brightness and color adjustment
- Noise suppression, smoothing and contour enhancement
- Sharpness enhancement/emphasizing of details
- Correction of illumination influences and white balance

ZEN 2.1 can also be used to add any annotations that you may require to the images. All elements, from scale bars and colored markings through to text and graphics, have been integrated into the program.

2.4 Image Analysis

Even with **ZEN lite** you are able to perform simple interactive measurements. The measured values (e.g. lengths, areas and perimeters) are made available in a data table, and can be processed further using spreadsheet programs.

With the optional modules **Image Analysis** and **Measurement** you can perform professional analysis tasks like generating automatic measurement procedures or measuring microscopic structures interactively.

2.5 File Format

For the ZEN software we developed a special file format called ***.czi** (Carl Zeiss Image). Besides the image data itself, the image format saves a lot of additional data, for example the date of acquisition, microscope settings, exposure values, size and scale details, contrast procedures which were used. Also all annotations and measured values are saved with the file.

To learn more about the ZEISS image file format we recommend to visit the ZEISS Microscopy Community forum in the internet (http://forums.zeiss.com/microscopy/community/forum.php). There you can join interesting discussions or download the detailed documentation of the file format.

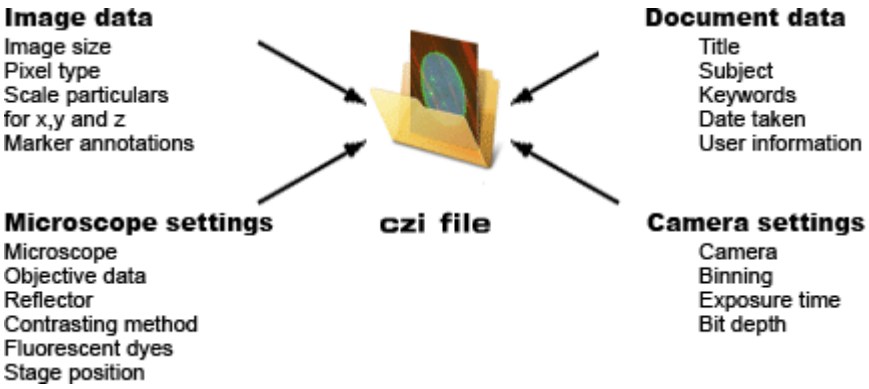


Fig. 1: CZI file format

2.6 Extensions

The extensions concept allows you to extend ZEN dynamically in its functionality. From a technical point of view the concept is comparable with plug-in`s or add-on`s. For the extensions we reserved a special area (*Extensions tab* [▶ 355]) within the software so that you can find all loaded extensions at a glance.

3 First Steps

3.1 Starting the software

Prerequisites

- You have installed ZEN 2.1 on your computer.

Procedure

1. Double click on the program icon on your desktop.

2. Alternatively click on **Start | All Programs | Carl Zeiss Microscopy | ZEN 2.1 | ZEN (blue edition)** entry (blue icon).

   The software starts. After a while you see the login screen.

3. Click on the button of the application you want to work with. The available applications depend on your licenses and system. Make sure that the hardware components you use are switched on and are ready for operation.

   The software starts. During the program start the hardware settings will be initialized.

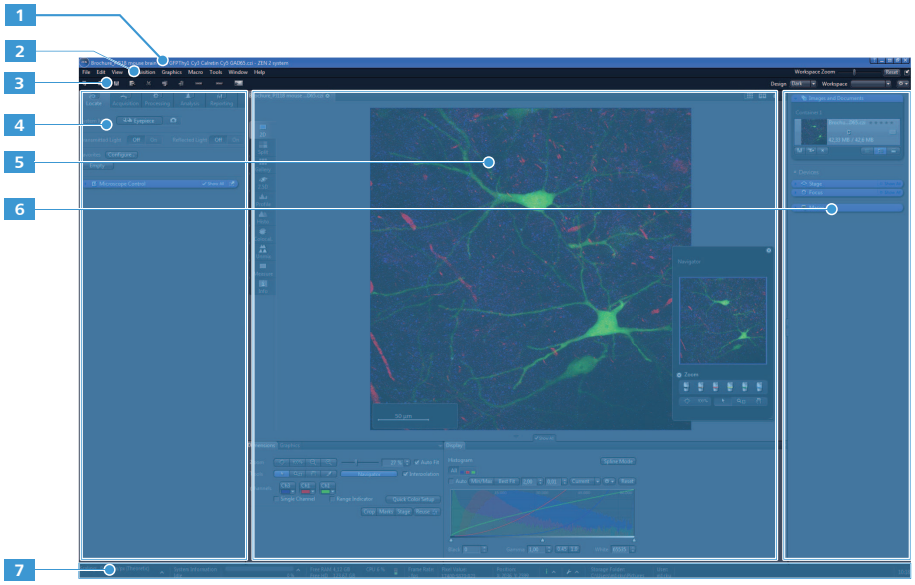
You successfully started the software.

i Note

For using pre-recorded images when starting the software, in the menu **Tools | Options | Startup**, the **Reload Last Used Documents** checkbox must be activated.

3.2 User Interface

The ZEN 2.1 user interface is divided into three main areas. Via the tabs in the **Left Tool Area (4)** you can access all the main tools for microscope control (Locate tab), acquisition (Acquisition tab), image processing (Processing tab), image analysis (Analysis tab) and report generation (Reporting tab). The **Center Screen Area (5)** is used to display your images, while the **Right Tool Area (6)** provides you with an overview of all open documents and is used for advanced file management.



1 Title bar
2 Menu bar
3 Tool bar
4 Left Tool Area
5 Center Screen Area
6 Right Tool Area
7 Status bar

3.2.1 Title bar

Parameter	Description
Help icon	Activates the "drag & drop" help function. A question mark appears beside the mouse pointer. Move the mouse pointer to a place in the software where you need help. Left-click on the desired location. The online help opens.
Minimize	Minimizes the program window.

Parameter	Description
Maximize across 2 screens	Maximizes the program window across 2 screens if available. This option is only possible if you are working with 2 screens with the same resolution.
Maximize	Maximizes the program window to the main screen.
Reduce	Reduces the program window to any selected size.
Close	Closes the program window.

3.2.2 Menu bar

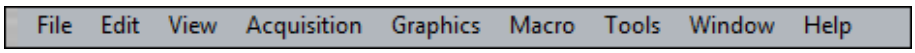


Fig. 2: Menu bar

The menu bar contains all the menus you need to manage, edit and view your projects.

3.2.3 Tool bar

Fig. 3: Tool bar

Here you gain quick access to important functions, e.g. saving or opening files. Further right you find more workspace settings, e.g. **Design** and **Workspace** selection. Read how to customize the Tool bar in chapter *Customizing toolbar* [▶ 140].

3.2.4 Left Tool Area

Here you find the main tabs for microscope and camera settings (**Locate** tab), image acquisition (**Acquisition** tab), image processing (**Processing** tab), image analysis (**Analysis** tab) and reporting (**Reporting** tab).The main tabs are organized in an order which follows the typical workflow of experiments in bioscience or material science. You can find a detailed description of each tab in the chapter *Main tabs* [▶ 337].

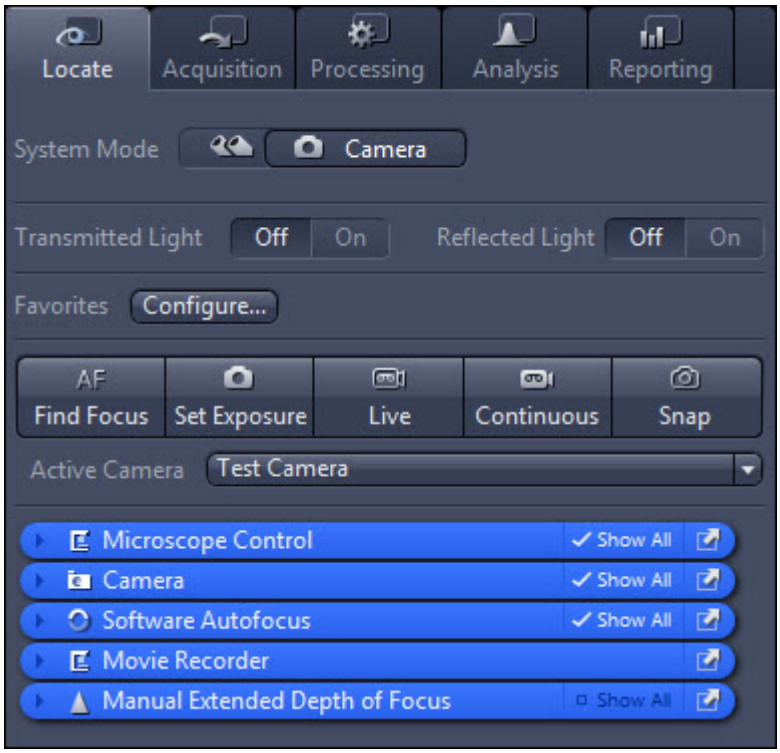


Fig. 4: Left Tool Area (Camera Mode)

3.2.5 Center Screen Area

The Center Screen Area is structured in 4 areas. The **Document bar (1)** is on top. Down the left side of the displayed image you find the tabs for the general and specific **Image Views (2)**. In the middle of Center Screen Area is the **Image Area (3)**, images, reports and tables were shown here. Under the image area you find the **General -** and **Specific View Options (4)** organized on tabs. View specific control tabs are flagged with a blue corner.

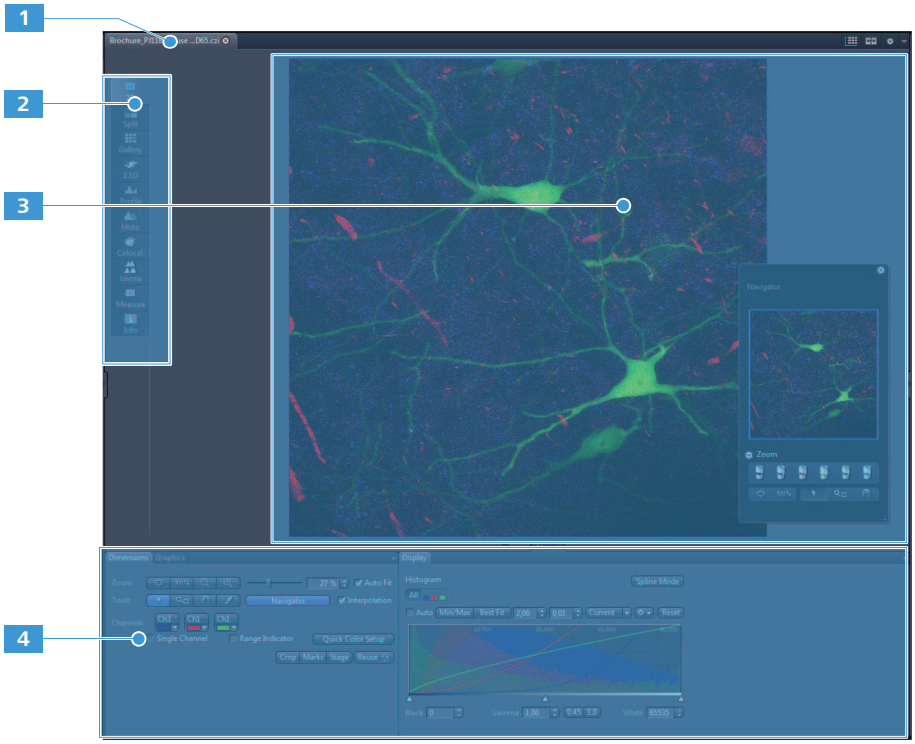


Fig. 5: Center Screen Area

1 Document bar
2 Image Views organized on tabs
3 Image Area or View Port
4 General and specific view options organized on tabs

3.2.6 Right Tool Area

Here you find the **Images and Documents** gallery, the **Macro** tool (depending on module/ license options) and the **Stage** / **Focus** control tools.

3.2.7 Document bar

Fig. 6: Document bar

Here you see tabs of all opend documents. Click on a tab to view the image/ document. On the right end of document bar you find buttons to switch view mode (**Expose** and **Splitter mode**) and further view options (**View** menu).

i Note

A **asterisk** (*) next to an image/document title indicates that unsaved changes have been made to this document.

3.2.8 Status bar

Here you will see important information on the system status:

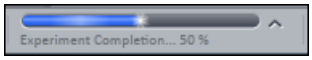
Scaling options

Displays which lateral scaling is currently being used. The **automatic** checkbox is activated by default. The scaling will be calculated automatically based on your hardware settings (i.e. objective, adapters, etc.). If the **automatic** checkbox is deactivated, you can also load/import scalings or start the scaling wizard in the **Options** menu.

System Information

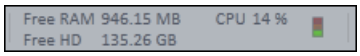
Always shows the latest, currently active process that the system is performing.

Progress bar



Displays the progress of the currently active process. Each new process added supersedes older still active processes. If you click on the up button, a window opens with a list of all processes in chronological order. You can stop a process that is running using the **Stop** button.

Performance indicators



In this group you will see an overview of the performance of individual computer components:

- **Free RAM** indicates how much physical memory is still available.
- **Free HD** indicates how much space is still available on the hard drive onto which the next image is to be acquired (see Extras/Options/Save).
- **CPU** indicates the usage of the Central Processing Unit.
- The **status bar** provides an overall assessment of the system usage.

Frame rate

Indicates the current frame rate in frames per second (fps) used by the active camera for producing new images. Please note in most cases that at speeds greater than 100 frames per second, this value cannot always be accurately determined.

Pixel Value and Position

Pixel Value displays the gray value to the image at the current position of the mouse pointer. In the case of multichannel images the gray value/channel is displayed for up to 4 channels.

Position displays the X/Y position (in pixel coordinates) of the mouse pointer in the image.

Information (i)

If you click on the icon, a window opens with a *List of System Messages* [▶ 23].

Storage folder

Displays the location where new images are automatically saved. This path can be changed in the menu **Tools** | **Options** | **Saving**.

User

Shows the Windows user name of the logged in user.

Time

Shows the current Windows system time.

3.2.8.1 List of System Messages

Important system messages are collected here.

i Note

If you **right click** on a system message the **Copy** button will appear. Left click on **Copy** button to copy the message to clipboard. Then paste it into a text file or an E-Mail. The idea behind is that you can easily send error messages to your support team for example. This copy/paste function works for all upcoming system messages or error messages within the application as well.

Information

System information that arises during normal operation. This system information does not lead to an interruption of the workflow. The information window is not displayed automatically.

Warnings

Information that requires input from the user, e.g. a prompt to change a manual microscope component. This information leads to the information window being shown briefly. However, it closes again after a few seconds.

Errors

Error messages indicate a malfunction by the system. In this case the information window opens and remains open. The system requires input from the user in order to continue.

i Note

Hundreds of messages can accumulate in the course of a session. A maximum of 300 messages are displayed. To display messages for a certain category, activate or deactivate the corresponding checkboxes.

3.2.9 Workspace configuration

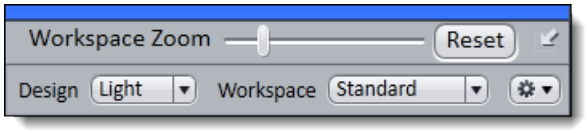


Fig. 7: Workspace Configuration

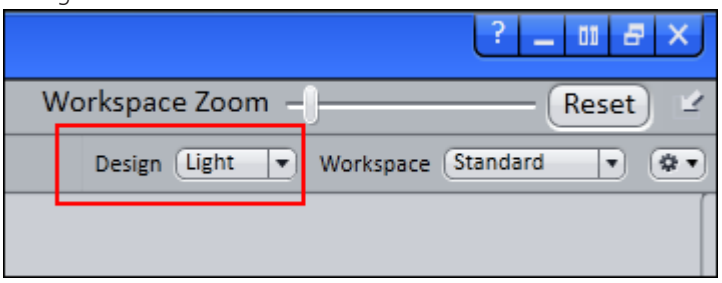
Here you find settings to adjust your workspace. Select **Light/Dark Design** of the user interface or enlarge the screen with **Workspace Zoom**. Save and reload all your personal settings as a **Workspace** configuration. With the **Dock all tool windows**  button in the top right corner you can easily dock all undocked tools back to the **Left Tool Area** by one click.

3.3 Adjusting workspace appearance

3.3.1 Selecting design

Procedure

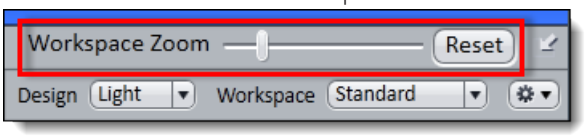
1 Select **Light/Dark** design from **Design** dropdown list in the workspace configuration area.



3.3.2 Zooming in/out workspace

Procedure

1 To zoom in or out of the workspace move the slider left or right.

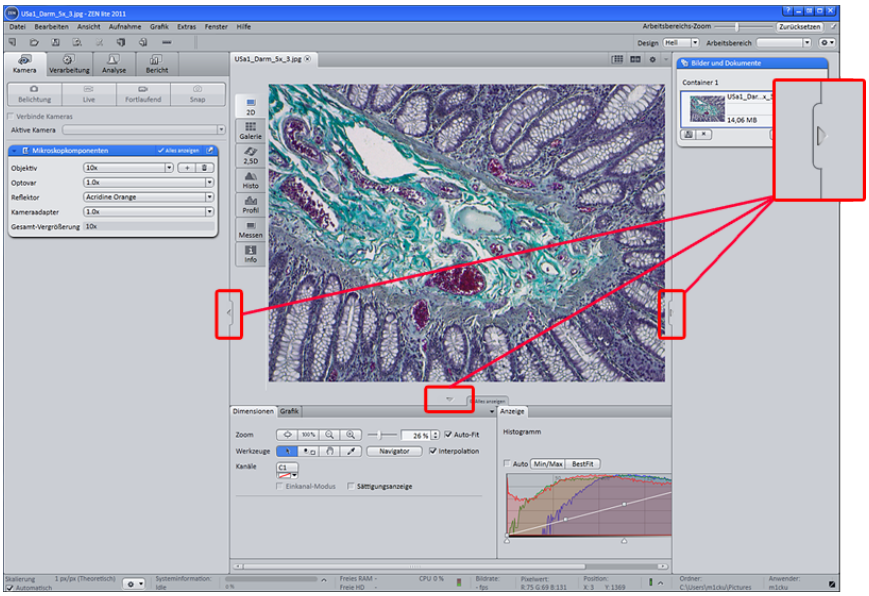


2 To reset workspace zoom to default click on **Reset** button.

3.3.3 Showing/hiding areas

Procedure

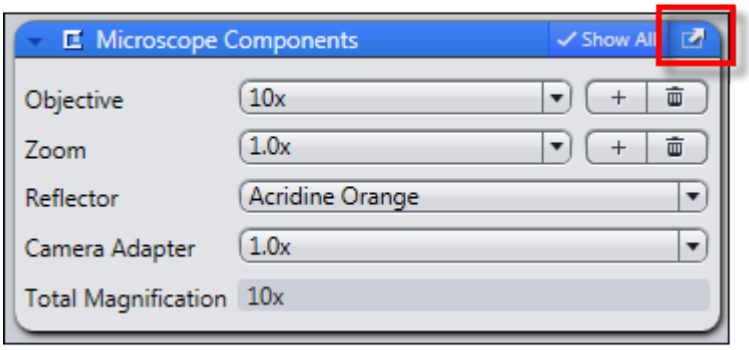
1 Click on **show/hide** buttons to show or hide areas.



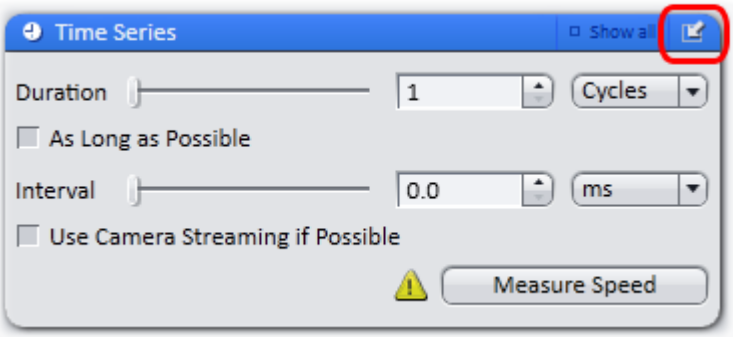
3.3.4 Undocking/docking tool window

This function allows you to undock/dock a tool window. An undocked tool window can be positioned anywhere on the screen.

Procedure **1** Click the **Undock** button to undock a tool window. Once undocked, the tool window can be moved around by clicking and dragging it on the blue bar.



2 Click the **Dock** button to dock a tool window back to its place in the left tool area.

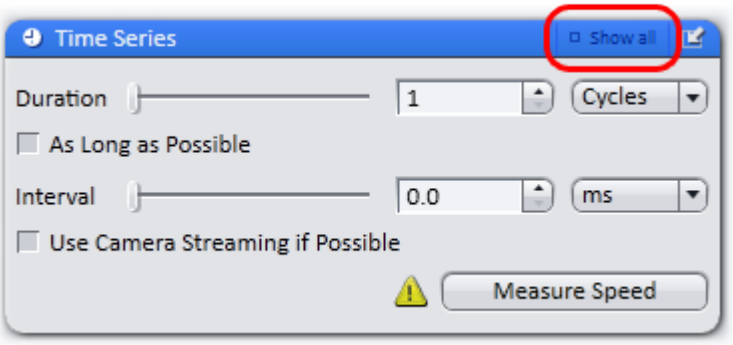


Note

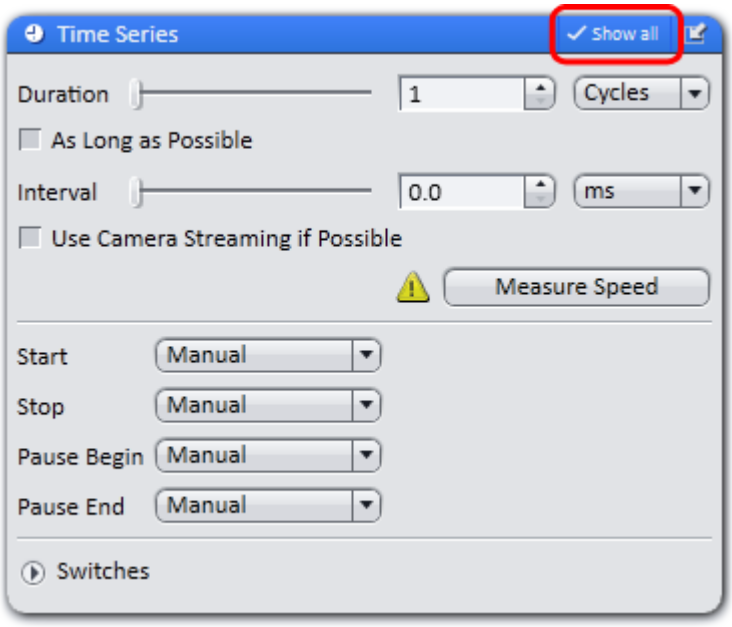
With the **dock all tools** function in the *Workspace Configuration* [▶ 24] you can globally attach all undocked tool windows back to the **Left Tool Area**.

3.3.5 Acitvating Show All mode

Procedure **1** With the **Show All** mode deactivated (default setting), only the basic functions of tool windows or view options are shown.



2 To show the advanced settings or expert functions of tool windows or view options, click on the **Show All** button.

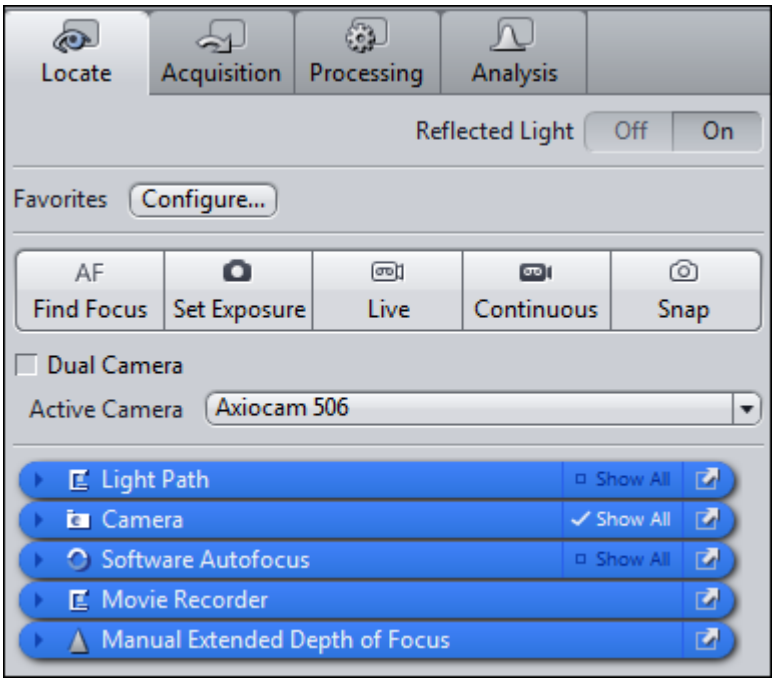


3.4 Configuring micorscope components

This chapter refers to the manual configuration of the microscope components in **ZEN lite**. All microscope components definitions will be stored in the meta data of the acquired image.

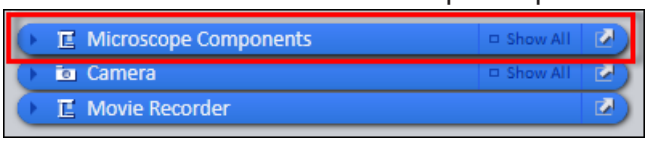
Prerequisites

- You have selected the **Camera** tab.

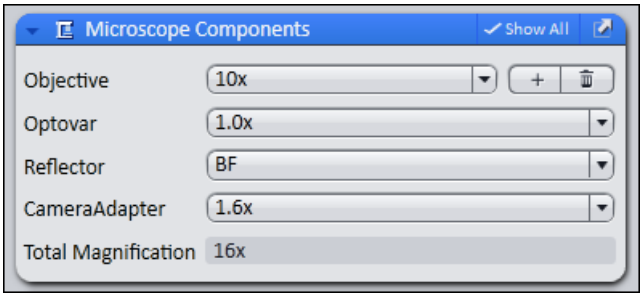


Procedure

1 Click to the blue header of the **Microscope Components** tool.



The tool will open. Consider that the button **Show all** is activated.



2 Under **Objective** select that objective you will use for your acquisitions.

3 Select all other microscope components you eventually will use (i.e. Optovar, Reflector, etc.).

Note

If you have activated the **automatic** button in the **Statusbar** under **Scaling** (standard settings), the scaling will be calculated on the basis of your definitions. If you want to perform a manual scaling, read the chapter **Create Manual Scaling**.

You have successfully configured your microscope components.

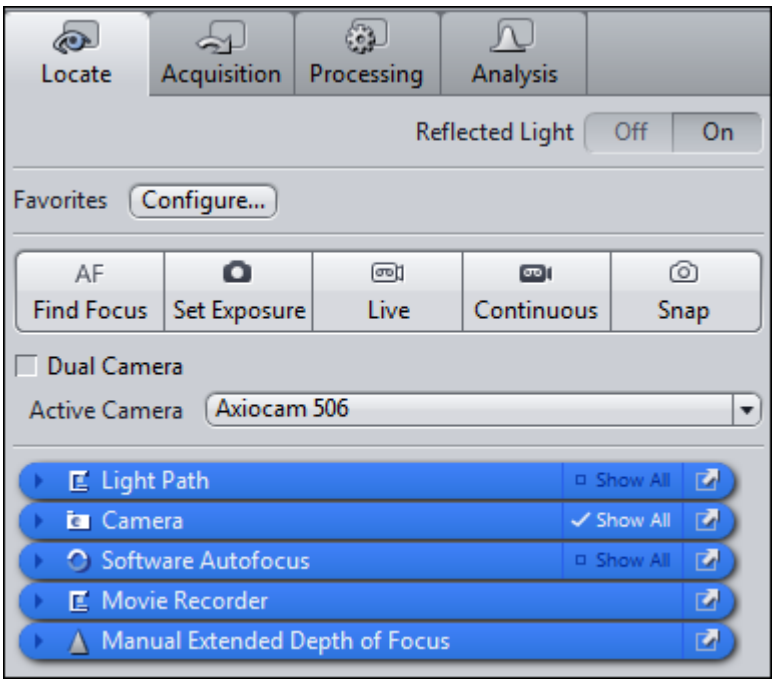
3.5 Acquire a first image

This topic guides you through acquiring your first image with ZEN 2.1 software.

Prerequisites

- You have connected and configured a microscope camera (i.e. AxioCam MR) to your system.
- You have started the software.
- You have configured the microscope components (e.g. objective, camera adapter) und you are using the automatic or manual scaling.
- You are on the **Camera** (ZEN light only) or **Locate** tab.

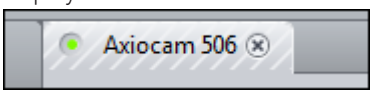
- You see your microscope camera available in the **Active Camera** section. If not, select the camera from the list.



Procedure

1. Position your sample on the microscope and adjust the microscope to see a focused image through the eyepieces.
2. Adjust the tube slider of the microscope to divert the image to the camera (e.g. **50% camera** and **50% eyepieces**).
3. Click on **Live** button.

   The **Live Mode** will be activated. You will recognize the **Live Mode** by the green signal and by the hatched tab in the *Document Bar* [▸ 21]. In the **Center Screen Area** you will see the camera live image. By default the live image shows a cross hair helping to navigate on the specimen. In the chapter *Optimize live image settings* [▸ 30] you will learn how to optimize live image display.



4. Click on **Set Exposure** button.

   The exposure time will be automatically determined and set.

i Note

If you do not see a focused image please refocus the specimen on the microscope. You may activate the focus bar as an additional aid. Open the context menu in the **Center Screen Area** via the right mouse key. There select the entry **Focus Bar**. The focus bar will be shown.

5 Click on **Snap** button.

You successfully acquired your first image with **ZEN blue**. Save the image in the file system via the menu **File | Save as**.

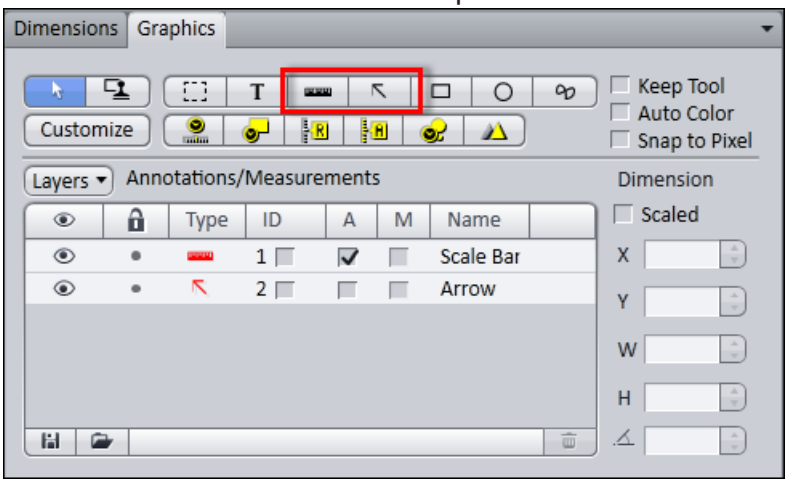
3.6 Add Annotations

Prerequisites

- You acquired an image with ZEN 2.1 .

Procedure

1 In the **Center Screen Area** select the **Graphics** tab.



2 Click on the **Scale Bar** button.

The scale bar will appear directly in the image.

i Note

Click with the right mouse key to any requested annotation in the image to edit this annotation (e.g. color, line width). This will open the context menu. Select the entry **Format Graphical Elements…** In this dialog you have numerous formatting possibilities.

3 Click on the **Draw Arrow** button.

The button will turn into blue to indicate its activation. Now you may draw an arrow into your image.

You added the annotations **Scale Bar** and **Arrow** from the toolbar to your image.

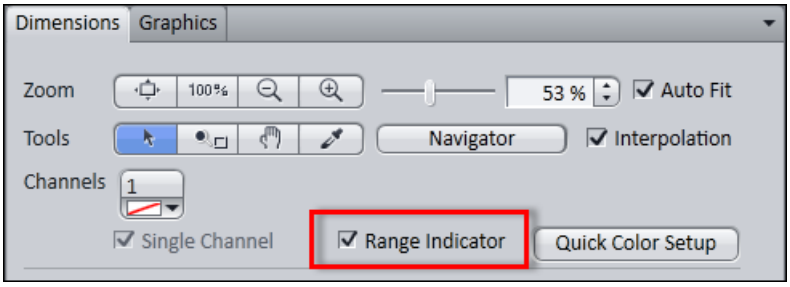
3.7 Optimize live image settings

Prerequisites

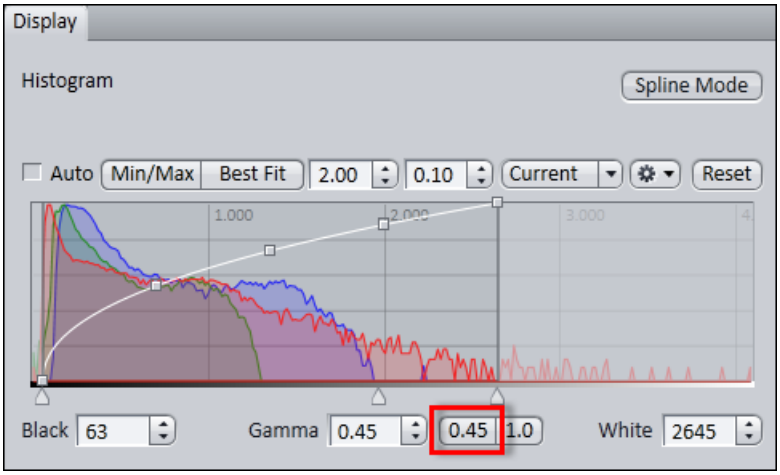
- You have started the **Live** mode via the **Live** button and see the camera's live image in the **Center Screen Area**.
- Under the image area you see the general view options on **Dimensions** tab, **Graphics** tab and **Display** tab.

Procedure

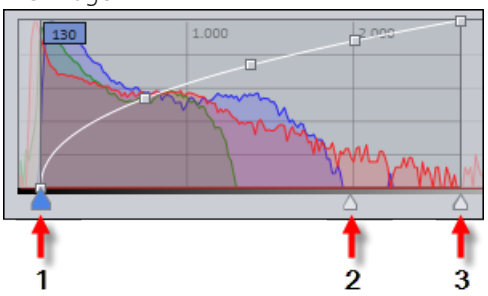
1 In the **Dimensions** tab activate the **Range Indicator** checkbox. This will mark overexposed (too bright) areas in the live image in red and underexposed (too dark) areas in blue.



2 On the **Display** tab click the **0.45** button. The display curve will be adapted to a gamma value of 0.45. This will set the optimum color presentation. If you do not see this button, activate the **Show all** mode.



3 Move the controls under the display curve left and right in order to directly adjust the values for **Brightness** (**White**), **Gamma**, and **Contrast** (**Black**) in the live image.



1 Contrast (black point) control

2 Gamma control

3 Brightness (white point) control

i Note

With the settings above the display of the live image will be adapted. These settings will also be transferred to your acquired image. This will not change the camera settings.

3.8 Create manual scaling

Prerequisites

- You oriented an object micrometer horizontally on the microscope stage.
- You selected correctly all definitions for your microscope in the **Microscope Components** tool (ZEN lite only). In our example we use an objective with a 10x magnification.

Procedure

1. Acquire an image (see *Acquire a first image* [▶ 28]) of the scale in your object micrometer using the objective to be scaled manually.
2. In the **Status** bar | **Scaling** deactivate the **Automatic** button.
3. Open the **Options** menu and click on the entry **Create New Scaling**.

   The calibration wizard will appear in the image area.
4. Click on single **Reference Line** button (selected as default) and activate the **Automatic Line Detection** button.

i Note

The function **Automatic Line Detection** calculates the theoretical maximum of the reference line's both end points to the closest scale lines in the image. Thus the distance will be calculated with sub-pixel accuracy.

5. Draw in the reference line along the scale.
6. Enter the true distance between both scale lines in the calibration wizard. In our example this is 500 micrometer.
7. Enter a name for the scaling (i.e. Obj 10x) and click the **Save Scaling** button.

You performed a manual scaling for your objective. Repeat this sequence for all objectives you will need a manual scaling for. Always ensure that you did select the correct objective in the tool **Microscope Components** and for this performed and selected the matching scaling in the status bar.

i Note

If you defined manual scalings for your available objectives, and if you activate in the **Status bar** under **Scaling** the checkbox **Automatic** again, the system will use the measured scalings instead of the theoretic ones. You will recognize this via the label "**measured**" instead of "**theoretic**" beside the pixel size.

3.9 Close software

Prerequisites

- You have acquired or processed an image, created a table or a report with ZEN blue.

Procedure

1. Click on **File | Exit** to end **ZEN blue** software. Alternatively you can press **ALT +F4** on your keyboard or click on **Close** icon in the **program bar**.

i Note

If you haven't saved your files the **Save/Keep Documents** dialog will open before the program closes. Select files you want to save or unselect files you don't want to save.

4 Image Acquisition

4.1 Acquiring Multi-Channel images

In the following chapters you will learn how to set-up and run multi-channel experiments with ZEN 2.1 quick and easy.

i Note

Make sure that you work with a fully motorized microscope system. In advance all microscope components (e.g. objectives, filters, etc.) must be configured correctly in the Microtoolbox (MTB) software.

In principle there are two variants for setting up multi-channel experiments. The first variant uses Smart Setup, while the second variant uses the **Channels** tool. Both variants have similarities and differences, which are presented in the following overview:

Commonalities

- Fluorescent dyes and transmitted light techniques can be selected from a database.
- Hardware settings for motorized microscopes, which take the properties of the selected dye and the available microscope hardware into account, can be created automatically.
- Bases for experiments can be created using both variants and experienced users can optimize settings further.

Differences

Smart Setup	Channels tool
A maximum of 4 fluorescence channels and 1 transmitted light channel are available	No restriction on the number and type of channels
Offers up to 3 proposals of variants of the experiment (depending on the selected combination of dyes and available hardware)	-
Offers more optimization of experiment settings by using the Motif buttons	-

Smart Setup	Channels tool
Graphic overview of the expected signal strength for the selected dyes	-
Graphic overview of the expected spectral crosstalk with the selected dye combinations	-
Display of the excitation and emission spectra of the selected dyes	-
-	Channels can be configured for dyes that are not supported (or not supported sufficiently well) by the available hardware

4.1.1 Set up a new experiment

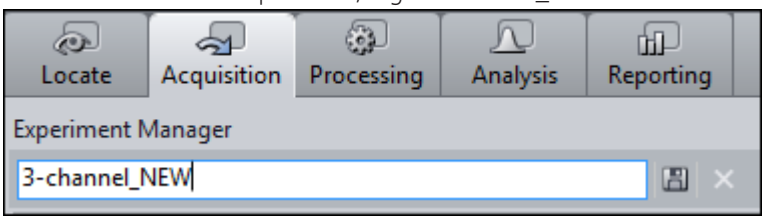
Prerequisites

- You have switched on and configured your microscope system and all components.
- You have successfully started the software.

Procedure

1. In the **Left Tool Area** click on the **Acquisition** tab.
2. In the **Experiment Manager** click on the **Options** button .

   The **Options** dropdown list opens.
3. To create a new, "empty" experiment, click on the **New** entry.
4. Enter a name for the experiment, e.g. "3-channel_NEW".



5. To create the experiment, click on the **Save** button  .

You have created a new, blank experiment. All other settings are now stored in this experiment. If you make changes to the experiment, an asterisk (*) after the file name appears. This means that the experiment was modified and not saved. Save your experiments from time to time to ensure that your settings are not lost.

4.1.2 Variant 1: Configure channels by using Smart Setup

Procedure

1. Click on the **Smart Setup** button on the **Acquisition** tab.

   The **Smart Setup** dialog opens.
2. Select the **WF** button on top of the dialog.

3 To add a channel, click on the **+** (add) button in the list under **Configure your Experiment**.

The **Add Dye or Contrasting Method** dialog opens.

4 Select the desired dye or contrast method.

5 Click on the **Add** button. Alternatively you can double-click on the entry in the dye database. The dye is then adopted directly into the experiment.

You have added a channel to your experiment. To add further channels, repeat the last 2 steps.

i Note

If you see the error message "**Smart Setup calculation failed**", it was not possible for Smart Setup to calculate any proposal. This may be because the filters and light sources available on the system do not allow an image of the dye to be acquired with a good signal strength or with little crosstalk. The channel for this dye or the contrast method cannot therefore be created. In this case, try selecting another, similar dye.

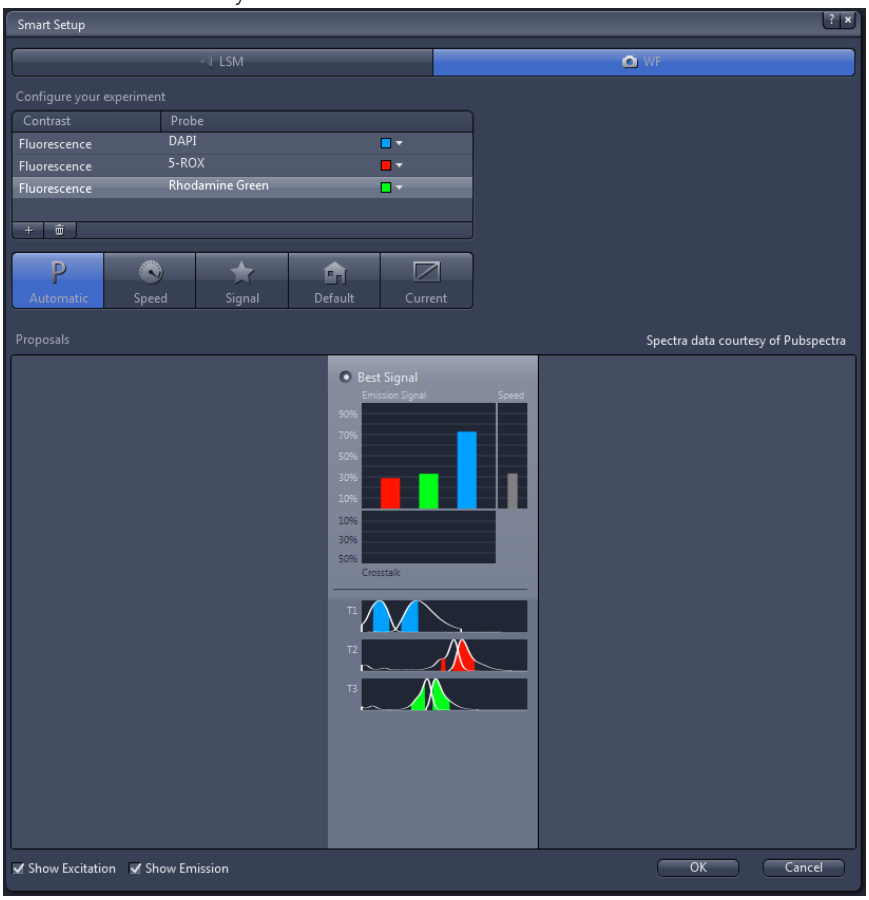
Should the error message be displayed for all dyes that you select, this may be due to one of the following causes:

- no light source has been configured or the light source is switched off

- no camera has been configured on the system, the camera is not connected or (on some models) has been switched off.

6 To return to **Smart Setup**, click on the **Close** button.

You will now see a graphic overview in the **Proposals** section. This displays the spectra of the dyes, the expected signal strengths per dye and the spectral crosstalk schematically.



Note

Depending on which dye you have selected and the microscope hardware available, up to three different proposals (Best Signal, Fastest, Best Compromise) are displayed. These differ in terms of signal strength, crosstalk and speed. Select the proposal that best meets the needs of your experiment.

7 To select a proposal (if there's more than one), activate the **radio** button on top of the proposal.

8 To optimize experiment settings additionally, click on a **Motif** button. Automatic button is set as default setting.

Note

By the **Motif** buttons you can optimize acquisition parameters and camera settings automatically either for a high quality (**Quality** button) image or a faster acquisition but reduced image quality (**Speed** button). Find a more detailed description of **Motif** buttons in Smart Setup dialog.

9 To optimize experiment settings, adopt the suggestion and leave **Smart Setup**, click on the **OK** button.

The added channels are adopted automatically into the **Channels** tool.

10 Click on the **Set Exposure** button in the **Action buttons** bar on top of the **Acquisition** tab.

The exposure time is now measured for all three channels one after the other. This is adopted into the settings for the channels. Following the measurement of the exposure time, the multi-channel image is acquired automatically and displayed in the **Center Screen Area**.

11 To save the experiment together with all the settings, click in the **Experiment Manager** on the **Options** button  .

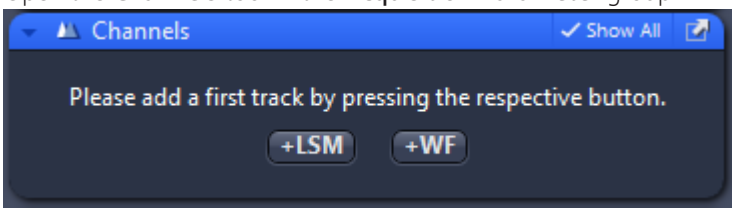
12 In the **Experiment Manager** click on the **Save** entry in the dropdown list.

You have set up the multichannel experiment using **Smart Setup**, executed it and then saved the configuration. This means that you can repeat the experiment as often as you like using the same settings.

4.1.3 Variant 2: Configure channels by using Channels tool

Procedure

1 Open the **Channels** tool in the **Acquisition Parameter** group.



2 Click on the **+WF** button.

The **Add Dye or Contrast Method** dialog opens.

3 Select the desired dye or contrast method. You can search for a dye by entering its name (or starting letter) in the **Search** input field.

4 Click on the **Add** button at the bottom of the dialog or simply double click on an entry.

The channel will be added to your experiment. To add more channels, repeat the last 2 steps.

5 Click on the **Close** button.

You will see the added channels in the **Channels** tool.

6 Click on the **Set Exposure** button in the main buttons bar on top of the **Acquisition** tab.

The exposure time is now measured for all configured channels one after the other. It will be also adopted into the settings for the channels. To set the exposure time channel specific, use the **Set Exposure** button in the channel settings of the channels tool. Following the measurement of the exposure time, the multi-channel image is acquired automatically and displayed in the **Center Screen Area**.

7 To save the experiment together with all the settings, click in the **Experiment Manager** on the **Options** button  .

8 Click on the **Save** entry in the dropdown list.

You have set up the multichannel experiment using the **Channels** tool, executed it and then saved the configuration. This means that you can repeat the experiment as often as you like using the same settings.

4.2 Acquiring Z-Stack images

4.2.1 Introduction

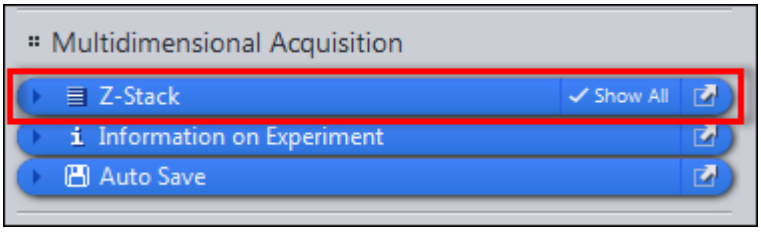
Prerequisites

- You have switched on and configured your microscopesystem and all components.
- You have *set up a new experiment* [▶ 35], at least defined one channel and adjusted focus and exposure time correctly.
- You are on the **Acquisition** tab.

Procedure

1 In the **Acquisition dimensions** section activate the **Z-Stack** checkbox.

The **Z-Stack tool** appears in the **Multidimensional Acquisition** tool group.



2 Simply click on the blue bar to open the tool.

You have successfully completed the general preparations. You can now set up Z-Stack experiments automatically or manually.

4.2.2 Configuring a Z-Stack automatically

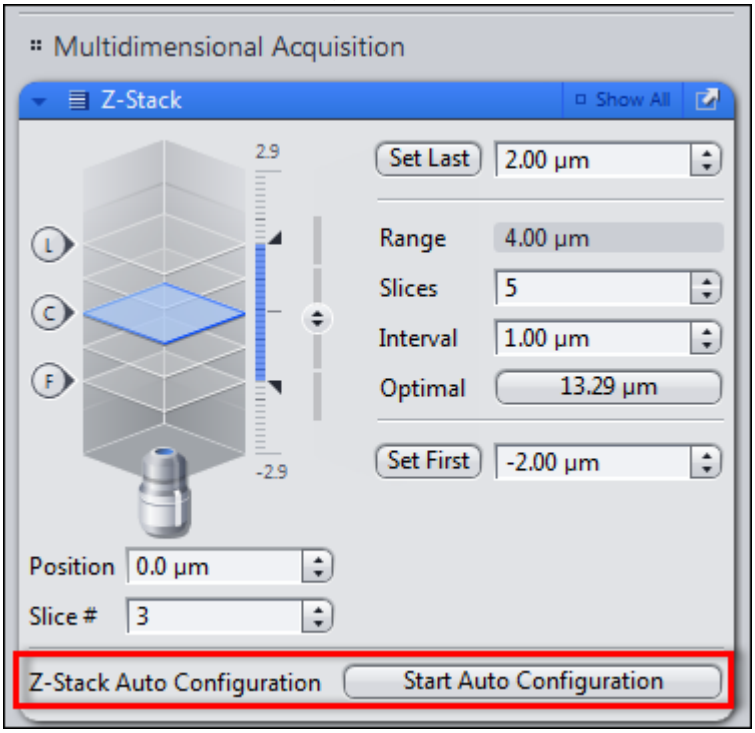
Note

Note that the automatic Z-Stack configuration will only work, if no LSM tracks are added in the **Channels** tool.

Procedure

1 Make sure that you have placed a sample in the visual field of the camera and that the sample is roughly in focus. Set the exposure time of the camera fair enough for receiving a good signal.

2 Click on the **Start Auto Configuration** button.



Confirm the system message by clicking on **OK**. The automatic configuration will be started.

Note

The auto configuration can last for a few seconds up to half a minute depending on the acquisition settings. You can check the configuration status on **Progress bar** in the **Status bar**.

The auto configuration sets the focus position for the first, last and center slice of the Z-Stack, the number of slices and the interval automatically. The Z-Stack experiment is set up successfully now.

3 Click on the **Start Experiment** button to start the experiment.

You have successfully set up and performed an Z-Stack experiment.

i Note

You can change the area of the sample (Z direction in %) covered by the Z-Stack auto configuration under **Tools | Options | Acquisition | Z-Stack** section. Smaller values will enlarge the Z-Stack. Bigger values will make the Z-Stacks smaller.

4.2.3 Configuring a Z-Stack manually

4.2.3.1 First/Last mode

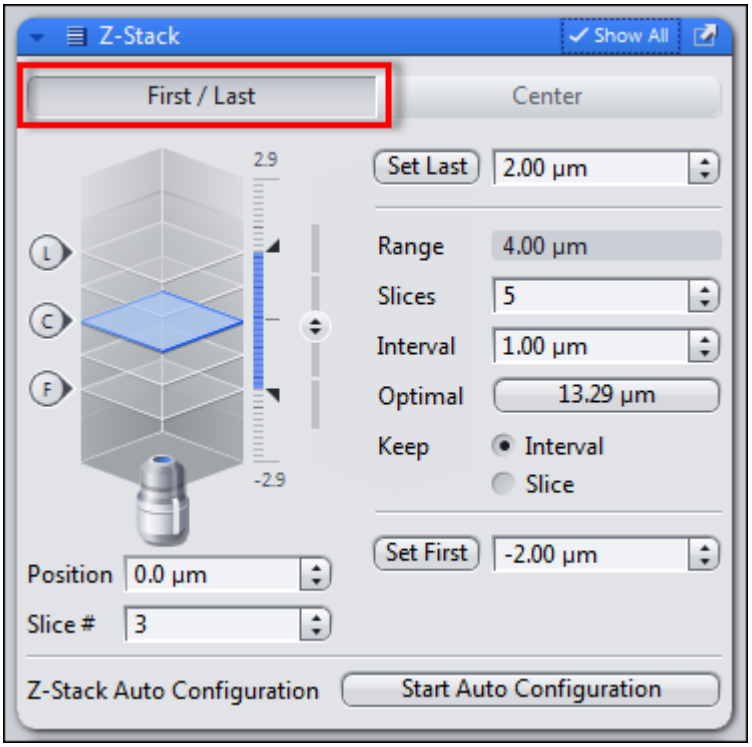
Using this mode you set the first and the last plane of the Z-Stack. This mode is suitable if you don't know the thickness of your sample exactly.

Prerequisites

- You are in the **Z-Stack tool**.

Procedure

1 Activate the **First /Last** mode by clicking on the **First /Last** button. This button is selected by default.



2 In the **Live** mode adjust the Z-drive until you have reached the upper plane of the Z-Stack. The blue plane in the illustration shows the actual focus plane.

3 Click on **Set First** button to set the adjusted Z-Position as first position of the Z-Stack.

4 In the **Live** mode adjust the Z-drive until you have reached the lower plane of the Z-Stack.

5 Click on **Set Last** button to set the adjusted Z-Position as last position of the Z-Stack.

You have set the upper and lower boundaries of the Z-Stack.

6 Click on the **Optimal** button. This will adjust the number of slices and the best interval according to the Nyquist criteria. Alternatively you can set the desired interval and number of slices in the input fields manually.

i Note

Depending on which option is selected in the **Keep** section either the **Interval** or the **Slices** will be held constantly.

7 Click on **Start Experiment** button to start the experiment.

You have successfully set up and performed an Z-Stack experiment using **First/Last** mode.

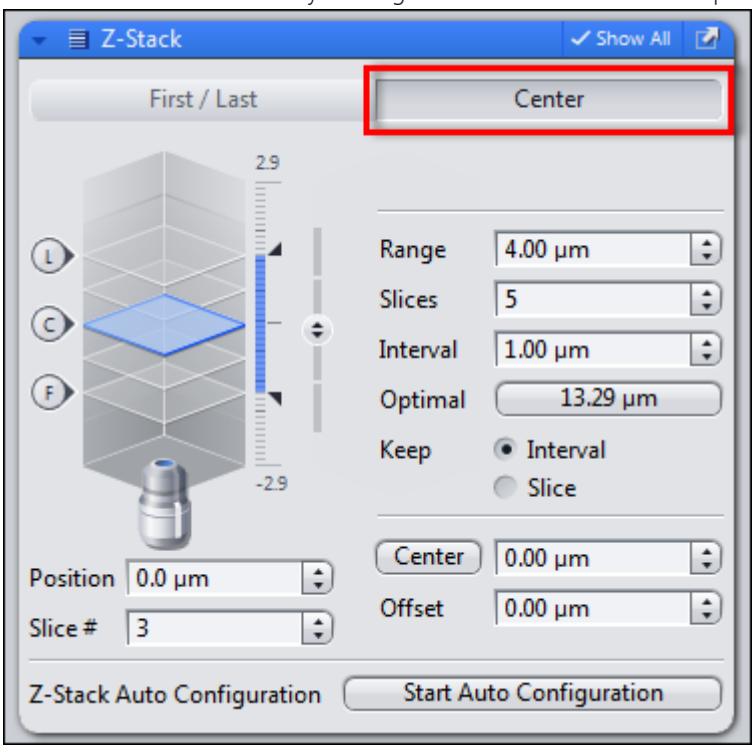
4.2.3.2 Center mode

Using this mode you set the center plane of the Z-Stack. This mode is suitable if you know the thickness of your sample. It will be the fastest method to set up a Z-Stack then.

Prerequisites
- You are in the **Z-Stack tool**.

Procedure

1 Activate the **Center** mode by clicking on the **Center** button on top of the tool.



2 In the **Live** mode adjust the Z-drive until you have focused the center of the sample exactly. The blue plane in the illustration shows the actual focus plane.

3 Click on the **Center** button under the settings section to set the actual focus position as center of the Z-Stack.

4 Click on the **Optimal** button. This will adjust the number of slices and the best interval according to the Nyquist criteria. Alternatively you can set the desired interval and number of slices in the input fields manually.

i Note

Depending on which option is selected in the **Keep** section either the **Interval** or the **Slices** will be held constantly.

5 Click on **Start Experiment** button to start the experiment.

6 You have successfully set up and performed a Z-Stack experiment using the **Center** mode.

4.3 Acquiring Time Series images

Prerequisites

- To set up **Time Series** experiments you need to license the **Time Series** module.
- You have set up a *new experiment* [▶ 35], at least *defined one channel* [▶ 34] and adjusted focus and exposure time correctly.

- You are on **Acquisition** tab.

Procedure

1. Activate the **Time Series** tool by activating the **Time Series** checkbox in the **Acquisition Dimensions** section.

   The **Time Series** tool appears in the **Left Tool Area**.

2. Open the **Time Series** tool.
3. Set length of your time series by the **Duration** slider. You are able to select an interval (days, hours, minutes, seconds) or the cycles (1-n) e.g. 10 cycles.
4. Set interval of your time series by the **Interval slider**, e.g. 5 s.
5. Click on **Start Experiment** button.

The time series experiment will be started. You`ve successfully learned the basics of how to set up time series experiments. In our example in 10 cycles after each 5 seconds an image is acquired. The time Series image also contains 10 single images.

4.4 Using Focus Strategies

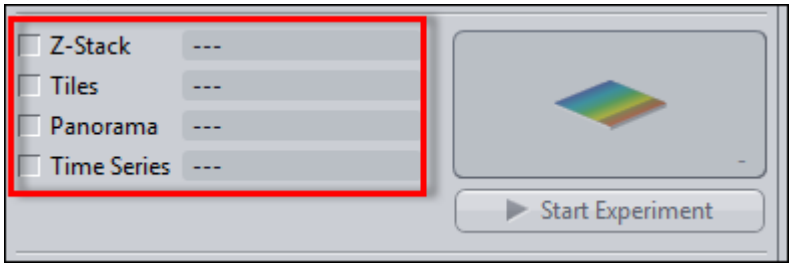
Use the **Focus Strategy** tool to define Z-positions manually or automatically and update these during the experiment.

Prerequisites

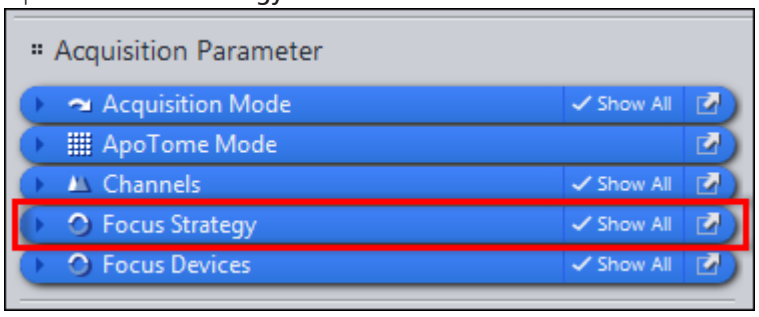
- To use focus strategies, you will need a motorized focus drive /Z-drive.
- You have *created a new experiment* [▶ 35], *defined at least one channel* [▶ 34] and adjusted the focus and exposure time.
- You are in the **Left Tool Area** on the **Acquisition** tab.

Procedure

1. Activate the acquisition dimensions (e. g. Tiles, Time Series) that you want to use for your experiment.



2. Open the **Focus Strategy** tool.



The available focus strategies are displayed in the dropdown list.

> **Note**
>
> The number of focus strategies available depends on the activated acquisition dimensions (e.g. tiles, time series), the available hardware devices (e.g. definite focus) present and software license (e.g. **Software Autofocus** and **Tiles** module allow additional focus strategies).

3 Decide which strategy is best suited to your experiment. For a detailed functional description of all strategies, read the chapter Focus Strategy tool.

You have successfully completed the general preparations. Now follow one of the following sets of instructions for specific focus strategies.

4.4.1 Using an absolute fixed Z-position

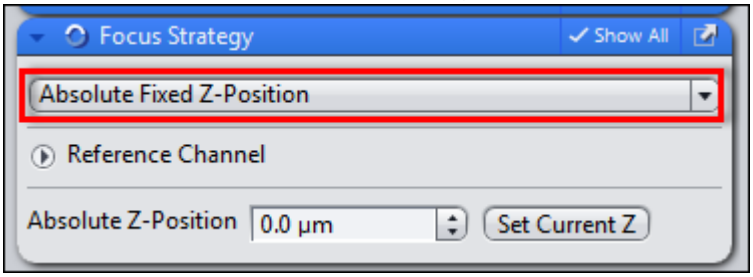
Using this strategy you can define an absolute fixed Z-Position. This position is used as reference position for all acquisitions and remains unchanged throughout the experiment. In the case of Z-Stack experiments it determines the center of the Z-Stack.

Prerequisites

- You are on the **Acquisition** tab in the **Focus Strategy** tool.

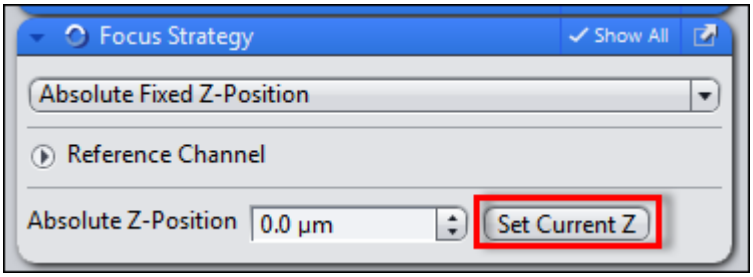
Procedure

1 Select the **Absolute Fixed Z-Position** entry from the dropdown list.



2 Use the Live mode to set the desired Z-position using the focus drive.

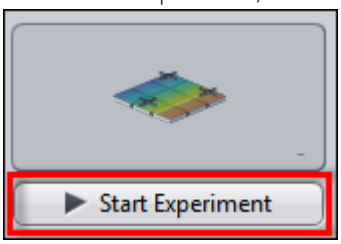
3 Click on the **Set Current Z** button.



The current Z-position is set as the **Absolute Z-Position**. Alternatively, you can enter a value in the input field.

4 You can now configure other experiment settings. For example, you can create tile regions, positions, a time series or a Z-Stack.

5 To start the experiment, click on the **Start Experiment** button.



You have successfully defined an absolute fixed Z-position as focus strategy for your experiment.

4.4.2 Using Z-positions from Tile Setup

Select this focus strategy to define individual Z-positions for positions and tile regions in the **Tiles** tool and use these for acquisition.

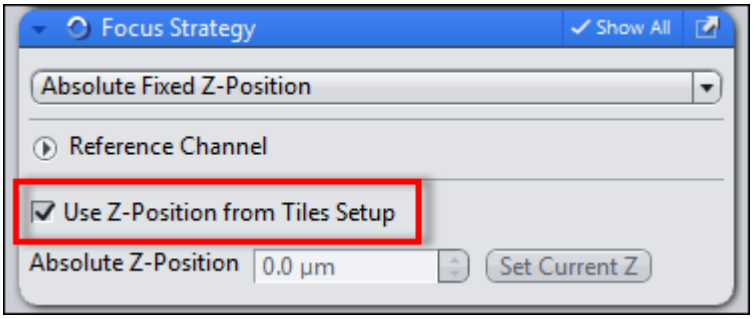
Prerequisites

- You have completed the *general preparations* [▶ 44] for using focus strategies (experiment created, at least one channel defined, acquisition dimensions activated).
- You are on the **Acquisition** tab in the **Focus Strategy** tool.

Procedure

1 Select the **Absolute Fixed Z-Position** entry from the dropdown list.

2 Activate the **Use Z-Position From Tiles Setup** checkbox.

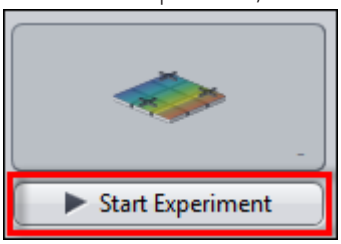


The Z-positions from the **Tiles** tool are now taken into account during acquisition as soon as you start the experiment.

3 Create your tile regions or positions using the **Tiles** tool, seeSet up tiles and positions experiments.

4 If necessary you can check and adjust the Z-positions of your positions and/or tile regions in the **Tiles** tool. Further information on this can be found under *Adjusting Z-positions of positions* [▶ 217] and *Adjusting Z-positions of tile regions* [▶ 216].

5 To start the experiment, click on the **Start Experiment** button.



The Z-positions of the tile regions and positions defined in the **Tiles** tool are taken into account during the acquisition of your tile experiment.

4.4.3 Using Software Autofocus

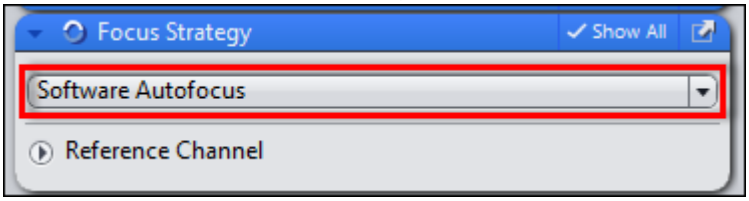
Select this focus strategy to automate the focusing of your specimen before and during acquisition with the help of the **Software Autofocus**. This is particularly useful for **Time Series** or **Tiles** experiments.

Prerequisites

- To use the **Software Autofocus** focus strategy, you will need the **Autofocus** module.
- You have completed the *general preparations* [▶ 44] for using focus strategies (experiment created, at least one channel defined, acquisition dimensions activated).
- You are on the **Acquisition** tab in the **Focus Strategy** tool.

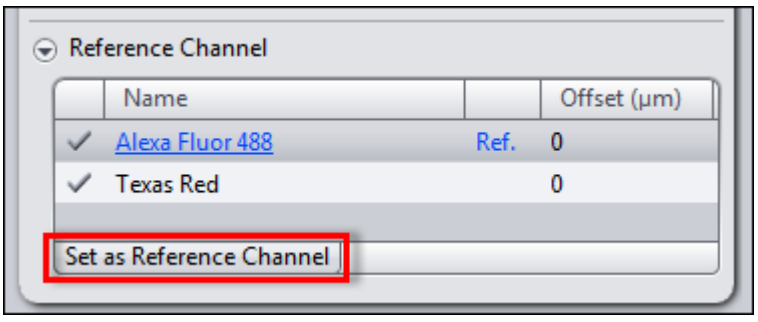
Procedure

1 Select the **Software Autofocus** entry from the dropdown list.



2 In the **Reference Channel** section select the channel that you want to use for the focus action from the list. Expand the section if you don`t see it in full.

3 Click on the **Set as Reference Channel** button.



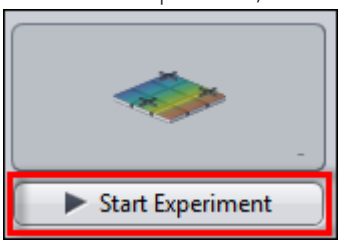
4 In the **Time Series Loop** and/or **Tiles Loop** sections of the **Focus Strategy** tool you can define when focus actions should be performed during the course of the experiment.

5 Open the **Software Autofocus** tool.

6 Adjust the autofocus settings (e.g. **Quality**, **Sampling**, **Autofocus Search Range** etc.) to your experiment conditions or use the default settings first.

7 Set up your tile and/or time series experiment.

8 To start the experiment, click on the **Start Experiment** button.



You have successfully used the Software Autofocus to bring images into focus automatically during the experiment.

4.4.4 Using Definite Focus in Time Series Experiments

Select this focus strategy to use the definite focus device to stabilize the focus in the event of temperature fluctuations during your **Time Series** experiments.

Prerequisites

- To use the **Definite Focus** focus strategy, you will need the **Definite Focus** hardware device.
- You have completed the *general preparations* [▶ 44] for using focus strategies.
- You are on the **Acquisition** tab in the **Focus Strategy** tool.

Procedure

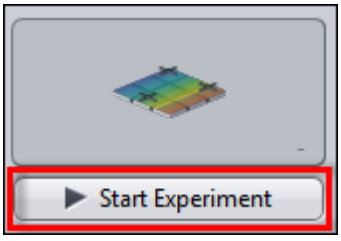
1 Select **Definite Focus** as focus strategy from the dropdown list.

2 In the **Stabilization Event Repetitions and Frequency** section select **Standard** mode. This mode will use our recommend default settings for stabilization. When selecting the **Expert** mode you can adjust all settings according to your needs.

3 Set up a **Time Series** experiment, see *Acquiring Time Series images* [▶ 43].

4 Use the **Live** mode to set the focus position using the focus drive.

5 To start the experiment, click on **Start Experiment**.



Definite Focus is initialized at the start of the experiment at the current focus position. The focus is then stabilized in accordance with your settings during the time series experiment. You will be reminded to set the focus accordingly prior to the experiment starting. You can do this by navigating to a suitable location (position or Tile region) and starting live or continuous. You can then continue with the experiment or cancel it.

You have successfully used the **Definite Focus** to stabilize the focus during a Time Series experiment.

4.4.5 Using Local or Global Focus Surfaces Defined by Tiles Setup

Using the focus strategy **Use Focus Surface Defined by Tiles Setup**, you can acquire tiles images along local or global focus surfaces (tile-region/position-specific), which you create in the **Tiles** tool.

This allows you to ensure that all tiles are in focus on tilted or irregular specimens. Local focus surfaces for tile regions are interpolated on the basis of the focus positions of reference points. Positions automatically have a horizontal focus area with the Z-value of the position.

The following guide explains how to use the strategy for local focus surfaces.

Prerequisites

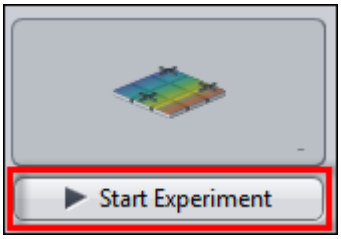
- To use the **Use Focus Surface Defined by Tiles Setup** focus strategy, you will need the **Tiles** module.
- You have completed the *general preparations* [▶ 44] for using focus strategies (experiment created, at least one channel defined, acquisition dimensions activated).
- You are on the **Acquisition** tab in the **Focus Strategy** tool.

Procedure

1. Select the **Use Focus Surface Defined by Tiles Setup** entry from the dropdown list.
2. In the **Focus Surface** section select **Local (per Region/Position)**.
3. Under **Initial Definition of Z-Values for Support Points/Positions** select**Fixed Z-Position** entry from the dropdown list.

> **Note**
>
> Alternatively, you can select the **By Software Autofocus** entry. This allows you to specify that the Z-positions of the reference points should be updated by the software autofocus immediately before acquisition.

4. Set up a **Tiles** experiment, see Set up tiles and positions experiments.
5. Create a **local focus surface** for the tile regions, see *Creating a local focus surface* [▶ 219].
6. To start the experiment, click on the **Start Experiment** button.



The tiles of tile regions and positions are acquired along the local focus surfaces.

4.5 Acquiring ApoTome images

Aim

The aim of these instructions is to show you how to calibrate the **ApoTome** for a two-channel experiment and acquire a two-channel image. This image will be used as a basis for demonstrating the processing options. After this a Z-stack will be acquired and processed with the help of ApoTome deconvolution.

Phase calibration, if it has not yet been performed, is carried out from the **Locate** tab, while the other steps are all performed from the **Acquisition** tab.

Grid Focus Calibration is an important step. It is best to perform this using the sample that you will want to acquire later, to guarantee identical optical conditions. If your sample is prone to significant bleaching, you can also use the calibration slide provided.

Background information on the ApoTome can be found here:

- *Principle of imaging using fringe projection* [▶ 50]
- *Optimum acquisition conditions* [▶ 54]
- *List of recommended objectives* [▶ 55]

4.5.1 Introduction

4.5.1.1 Principle of imaging using fringe projection

The optics of a microscope are optimized for analyzing very thin samples. For a cover-slip-corrected objective, all optical calculations are performed for very thin objects that lie directly beneath the cover slip. All cover-slip-corrected objectives from ZEISS are optimized for this particular usage, and exhibit an optimum point spread function (PSF) for the wavelengths for which the corresponding objective has been specified.

In biological applications, however, the vast majority of specimens used do not satisfy these optimum requirements. Sometimes thicker biological tissue slices are used, e.g. to analyze cells in the tissue using specific fluorescent markers.

In such cases, during microscopic analysis, and particularly during documentation, the set focal plane is hidden by parts of the image that originate from above and below the actual focal plane. As a result the image appears "faded", the contrast is reduced, and the background becomes bright. In extreme cases important structures and image details may be completely hidden.

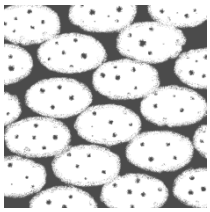


Fig. 8: Input image (schematically)

The above representation of a microscopic image of cell nuclei in tissue shows this effect. A number of methods can be used to prevent or reverse this effect, such as confocal laser scanning microscopy or 3D deconvolution.

Principle of fringe projection

With the **ApoTome 2** the principle of fringe projection has been employed. This technology involves inserting a grid structure with grid lines of a defined width into the plane of the luminous field diaphragm of the reflected light beam path. As the plane of the luminous field diaphragm is conjugated to the focal plane, when you look into the eyepiece you can therefore see the grid, overlaid with the actual sample.

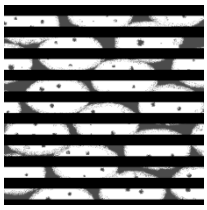
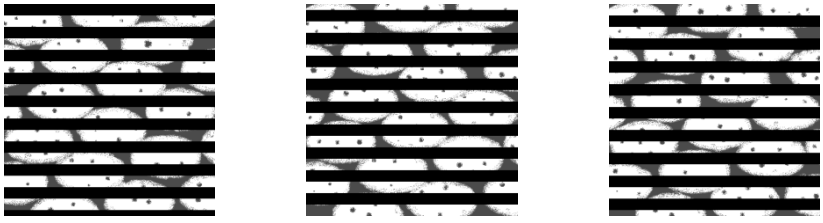


Fig. 9: Grid image

The image of the grid is shown schematically in the figure above. In reality the grid lines are much thinner.

Moving the grid structure

A scanning mechanism in the ApoTome 2 slider is used to move the grid structure in at least three defined steps within the specimen plane. The grid is moved very quickly (in less than 20 ms). A digital image is acquired at each grid position. The next figure shows the movement of the grid schematically:



Position 1 Position 2 Position 3

Optical section

The three raw images are combined online on the PC and displayed as an optical section. This combined resulting image is an optical section through the sample with the following properties:

- The grid structure has been removed from the raw images.
- The parts of the image that are out of focus are no longer visible.
- The sharpness and contrast of the image have been increased.
- The image's resolution in the axial direction has been increased.

Fig. 10: Output image (schematically)

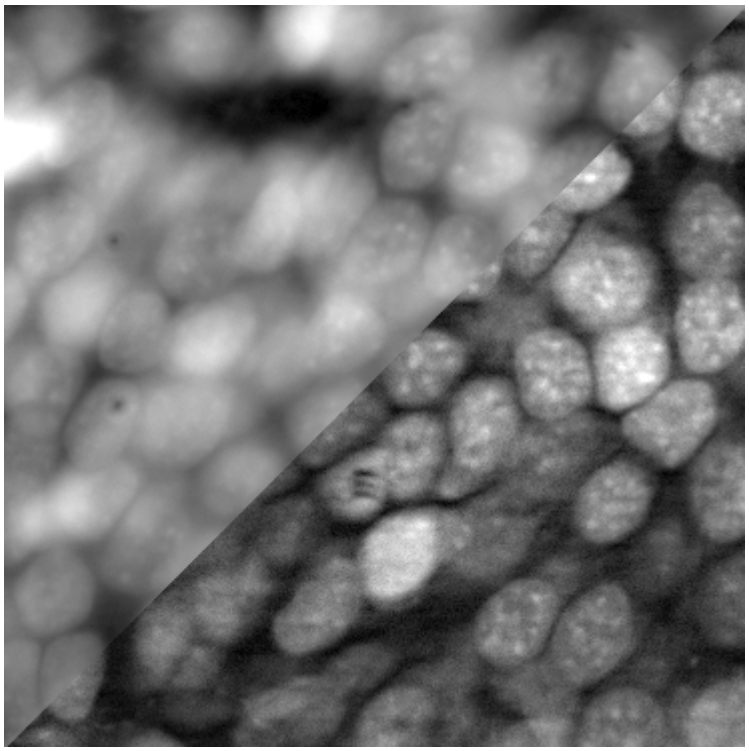


Fig. 11: Sample image: cell nuclei

The figure above shows an application image of cell nuclei (tadpole brain section) in black and white. Above left: conventional fluorescence Below right: optical section

Why is the resulting image an optical section?

Schematic image of the grid

One possible way to explain this is to use the image of the grid in the sample:

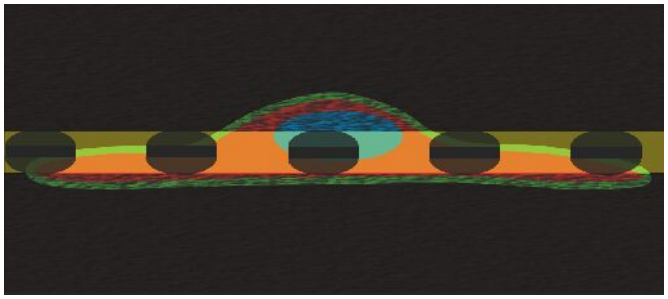


Fig. 12: Grid image (schematically)

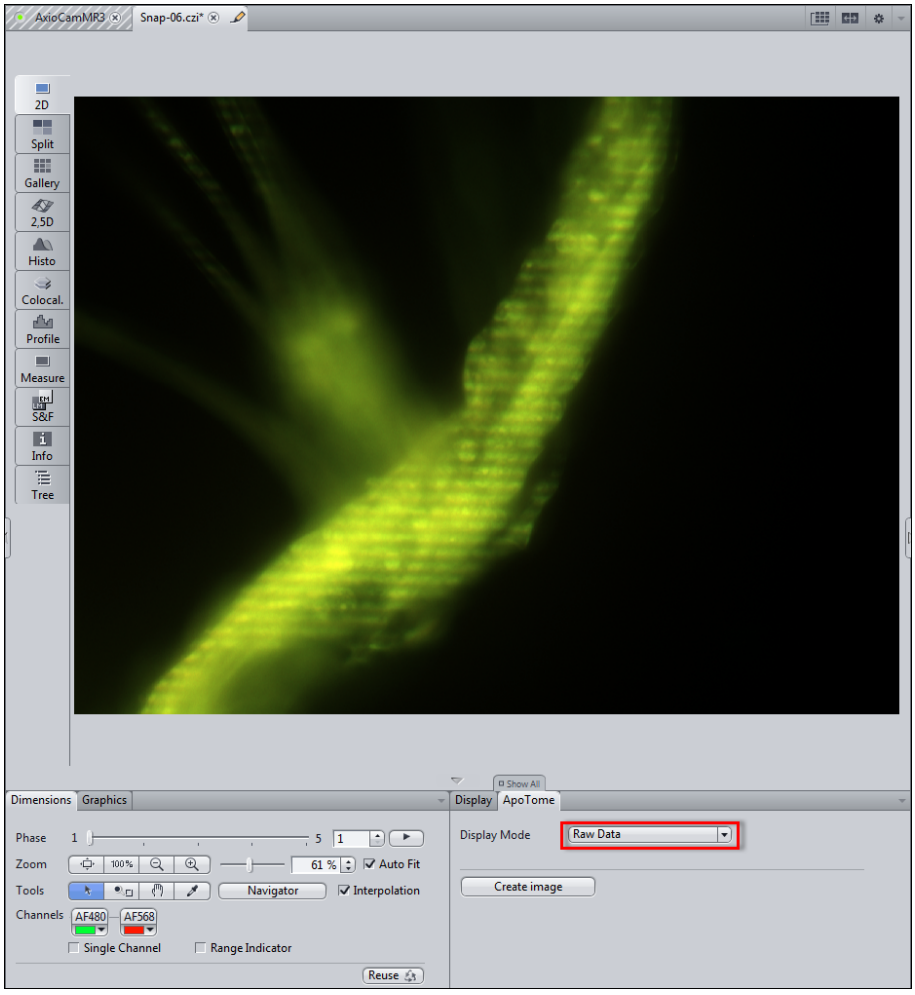
The image of the grid provides the necessary information on the distance of the various specimen structures from the set focal plane (see figure above). Some specimen structures are in focus, while others lie above or below the focal plane, and enter the set focal plane.

The technique of grid projection makes use of the fact that the image of the grid above and below the actual focal plane is blurred, and enters the blurred areas of the specimen. When the grid line is moved, significant brightness differences (= contrast) appear in the focal plane.

Outside the focal plane only minor differences are produced, as the sample and the image of the grid are practically "blurred" together. The brightness differences are detected by the algorithm used to combine the raw images, and are used to remove the parts of the image that are out of focus.

The Raw Data mode

To view the raw data directly switch to the **Raw Data** display mode on the **ApoTome** tab.



You will now see the **Phase** slider on the **Dimensions** tab. Here you can locate the individual grid positions. This view can be useful when looking for errors, e.g. to find out where residual streaks in the processed resulting image originate from.

4.5.1.2 Optimum acquisition conditions

The following requirements should be met in order to produce optimum ApoTome images:

- **Exposure time of the camera**: this should be set so that approx. 80% of the camera's dynamic range is used. The smaller the dynamic range of the images of the lines, the more noise the combined resulting images will contain.
- **Correct calibration**: Good results can only be achieved if calibration has been performed correctly. Ideally you should use your own sample for calibration. If this does not lead to good results, use the calibration slide provided.
- **Sufficient grid contrast in the sample**: Good section image results can only be achieved if the grid lines in the live image can be clearly identified in all object areas. Under certain circumstances samples with very homogeneous staining throughout may not be suitable for ApoTome images.
- **Avoid vibrations** during acquisition, as any movement of the grid position during acquisition can lead to streak artifacts.
- **Number of phases**: Although 3 grid positions (also called phases) can completely cover the object structures that are in focus and are therefore sufficient for creating optical sections, the results are significantly better when 5 or more phases are acquired. For this reason 5 phases are acquired as standard.
- **Selection of the correct grid frequency**: Under normal circumstances, the automatic grid selection yields the best results. In the case of difficult samples, e.g. if the staining is weak, selecting a different grid manually can lead to better results.
- **Avoid electronic interference**: The ApoTome's scanner unit is equipped with highly precise control. Avoid electrical interference, e.g. leaving cell phones close to the ApoTome, to prevent incorrect positioning of the grid.

4.5.1.3 List of recommended objectives

EC Plan-Neofluar

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
			Highgrid	Middlegrid	Lowgrid		
10x	0.3	Air	2.9 / 31.9	1.7 / 18.2	0.9 / 9.9	OK	OK
20x	0.5	Air	2.4 / 9.2	1.4 / 5.3	0.7 / 2.9	OK	OK
40x	0.75	Air	1.6 / 2.8	0.9 / 1.6	0.5 / 0.9	OK	OK
40x	1,3	Oil	2.5 / 2.2	1.4 / 1.2	0.8 / 0.7	OK	OK

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
63x	0,95	Air	1,0 / 1,1	0,6 / 0,7	0,4 / 0,4	OK	No
63x	1,25	Oil	1,6 / 1,5	0,9 / 0,9	0,5 / 0,5	OK	OK
100x	1,3	Oil	1,0 / 0,9	0,6 / 0,5	0,4 / 0,3	OK	OK

LCI Plan-Neofluar

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
			Highgrid	Middlegrid	Lowgrid		
25x	0,8	Oil, water or glycerin	2,9 / 6,6	1,7 / 3,7	0,9 / 2,0	OK	OK
63x	1,3	Water or glycerin	1,5 / 1,3	0,9 / 0,7	0,5 / 0,4	OK	OK

Plan-Apochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
			Highgrid	Middlegrid	Lowgrid		
10x	0,45	Air	4,2 / 20,4	2,4 / 11,5	1,3 / 6,2	OK	OK
20x	0,8	Air	3,2 / 4,9	1,8 / 2,8	1,0 / 1,5	OK	OK
40x	0,95	Air	1,6 / 1,7	0,9 / 1,0	0,5 / 0,5	OK	OK
40x	1,3	Oil	2,5 / 2,2	1,4 / 1,2	0,8 / 0,7	OK	OK
40x	1,4	Oil	2,4 / 1,8	1,4 / 1,0	0,7 / 0,6	OK	OK
63x	1,4	Oil	1,6 / 1,2	0,9 / 0,7	0,5 / 0,4	OK	OK

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
100x	1,4	Oil	1,0 / 0,8	0,6 / 0,5	0,4 / 0,3	OK	OK

LD LCIPlan-Apochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
			Highgrid	Middlegrid	Lowgrid		
25x	0,8	Oi., water or glycerin	2,9 / 6,5	1,7 / 3,8	0,9 / 2,0	OK	OK

CApochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
			Highgrid	Middlegrid	Lowgrid		
10x	0,45	Water	4,2 / 20,2	2,4 / 11,7	1,3 / 6,1	OK	OK
40x	1,2	Water	2,1 / 1,9	1,3 / 1,1	0,7 / 0,6	OK	OK
63x	1,2	Water	1,4 / 1,3	0,8 / 0,7	0,5 / 0,4	OK	OK

LD CApochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
			Highgrid	Middlegrid	Lowgrid		
40x	1,1	Water	2,1 / 2,3	1,3 / 1,4	0,7 / 0,7	OK	OK

a Plan-Apochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
			Highgrid	Middlegrid	Lowgrid		
63x	1,46	Oil	1,5 / 1,0	0,9 / 0,6	0,5 / 0,3	OK	OK
100x	1,46	Oil	1,0 / 0,7	0,6 / 0,4	0,3 / 0,2	OK	No

a Plan-Fluar

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS 34	DAPI with FS 49
			Highgrid	Middlegrid	Lowgrid		
100x	1,45	Oil	1,0 / 0,7	0,6 / 0,4	0,3 / 0,2	No	No

4.5.1.3.1 Axio Observer

EC Plan-Neofluar

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
			Highgrid	Middlegrid	Lowgrid		
10x	0,3	Air	2,9 / 31,5	1,7 / 18,5	0,9 / 9,8	OK	OK
20x	0,5	Air	2,3 / 9,0	1,4 / 5,4	0,7 / 2,9	OK	OK
40x	0,75	Air	1,6 / 2,7	0,9 / 1,6	0,5 / 0,9	OK	No
40x	1,3	Oil	2,4 / 2,1	1,4 / 1,3	0,8 / 0,7	OK	OK
63x	0,95	Air	1,0 / 1,1	0,6 / 0,7	0,4 / 0,4	OK	OK
63x	1,25	Oil	1,6 / 1,5	0,9 / 0,9	0,5 / 0,5	OK	No

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
100x	1,3	Oil	1,0 / 0,9	0,6 / 0,6	0,4 / 0,3	OK	No

LCI Plan-Neofluar

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
			Highgrid	Middlegrid	Lowgrid		
25x	0,8	Oil, water or glycerin	2,9 / 6,5	1,7 / 3,8	0,9 / 2,0	OK	OK
63x	1,3	Water or glycerin	1,5 / 1,3	0,9 / 0,8	0,5 / 0,4	No	No

Plan-Apochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
			Highgrid	Middlegrid	Lowgrid		
10x	0,45	Air	4,2 / 20,2	2,4 / 11,7	1,3 / 6,1	OK	OK
20x	0,8	Air	3,1 / 4,8	1,8 / 2,8	1,0 / 1,5	OK	OK
40x	0,95	Air	1,6 / 1,7	0,9 / 1,0	0,5 / 0,5	OK	OK
40x	1,3	Oil	2,4 / 2,2	1,4 / 1,3	0,8 / 0,7	OK	OK
40x	1,4	Oil	2,4 / 1,8	1,4 / 1,1	0,7 / 0,6	OK	OK
63x	1,4	Oil	1,5 / 1,2	0,9 / 0,7	0,5 / 0,4	OK	OK
100x	1,4	Oil	1,0 / 0,8	0,6 / 0,5	0,4 / 0,3	OK	No

LD LCIPlan-Apochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
			Highgrid	Middlegrid	Lowgrid		
25x	0,8	Oil, water or glycerin	2,9 / 6,5	1,7 / 3,8	0,9 / 2,0	OK	OK

C-Apochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
			Highgrid	Middlegrid	Lowgrid		
10x	0,45	Water	4,2 / 20,2	2,4 / 11,7	1,3 / 6,1	OK	OK
40x	1,2	Water	2,1 / 1,9	1,3 / 1,1	0,7 / 0,6	OK	OK
63x	1,2	Water	1,4 / 1,3	0,8 / 0,7	0,5 / 0,4	OK	OK

LD C-Apochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
			Highgrid	Middlegrid	Lowgrid		
40x	1,1	Water	2,1 / 2,3	1,3 / 1,4	0,7 / 0,7	OK	OK

a Plan-Apochromat

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
			Highgrid	Middlegrid	Lowgrid		
63x	1,46	Oil	1,5 / 1,0	0,9 / 0,6	0,5 / 0,3	OK	OK
100x	1,46	Oil	1,0 / 0,7	0,6 / 0,4	0,3 / 0,2	No	No

A Plan-Fluar

V	NA	Immersion	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
			Highgrid	Middlegrid	Lowgrid		
100x	1,45	Oil	1,0 / 0,7	0,6 / 0,4	0,3 / 0,2	No	No

4.5.1.3.2 Axio Zoom.V16

Plan-Apochromat

V	Zoom	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
		Highgrid	Middlegrid	Lowgrid		
1x	40	1,8 / 82,7	1,22 / 56,7	0,9 / 43,8	OK	OK
1x	80	1,5 / 25,2	1,0 / 17,4	0,8 / 13,5	OK	OK
1x	100	1,2 / 19,7	0,9 / 13,7	0,7 / 10,8	OK	No
1x	112	1,1 / 17,7	0,8 / 12,4	0,6 / 9,8	OK	No

Plan-Neofluar

V	Zoom	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
		Highgrid	Middlegrid	Lowgrid		
1x	40	1,8 / 82,7	1,22 / 56,7	0,9 / 43,8	OK	OK
1x	80	1,5 / 25,2	1,0 / 17,4	0,8 / 13,5	OK	OK
1x	100	1,2 / 19,7	0,9 / 13,7	0,7 / 10,8	OK	OK
1x	112	1,1 / 17,7	0,8 / 12,4	0,6 / 9,8	OK	OK

Plan-Neofluar

V	Zoom	Grid / Section Thickness @ 490nm [RE/µm]			DAPI with FS34	DAPI with FS49
		Highgrid	Middlegrid	Lowgrid		
2,3x	40	2,0 / 72,0	1,4 / 49,3	1,1 / 37,9	OK	OK
2,3x	120	1,7 / 9,1	1,1 / 6,3	0,9 / 4,9	OK	OK
2,3x	150	1,6 / 5,9	1,1 / 4,1	0,8 / 3,1	OK	OK
2,3x	160	1,5 / 5,3	1,0 / 3,6	0,8 / 2,8	OK	No
2,3x	250	1,1 / 3,2	0,7 / 2,2	0,6 / 1,8	No	No

Recommended filter sets : 20, 38, 43.

4.5.2 Preparation: Phase calibration

Before you can use the **ApoTome** for your experiments, the optimum angle of deflection of the scanner unit must be set on the **ApoTome 2**. This fine adjustment of the scanner unit only has to be performed once after the system has been set up. The mirror slide and special reflected light reflector cube, both of which are supplied with the ApoTome 2, are used for this purpose.

Calibration only needs to be performed for one grid and one objective. It is advisable to calibrate the grid for the low magnification range (grid marked with an "L" for "Low magnification") using a 20x objective.

The positioning of the camera is also optimized in the dialog for phase calibration. To achieve optimum performance, the camera horizontal should be aligned parallel to the ApoTome 2 grid lines with as much precision as possible.

The calibration process is supported by a wizard. Start the function by selecting the ApoTome Phase Wizard function from the Acquisition menu.

The wizard guides you through the calibration process in 5 steps. Follow the instructions in the text field of the wizard.

i Note

For phase calibration you will need the mirror slide provided and the calibration filter (424930-9902-000). The filter is designed for use with white light sources such as the **HXP120C** and cannot be used with the LED light source **Colibri**. If your ApoTome system was ordered and supplied exclusively with Colibri, a suitable calibration filter (424930-9000-000) has already been provided. If Colibri has been retrofitted and the calibration filter is not available, it is not possible to perform phase calibration. In this case please contact your ZEISS sales representative.

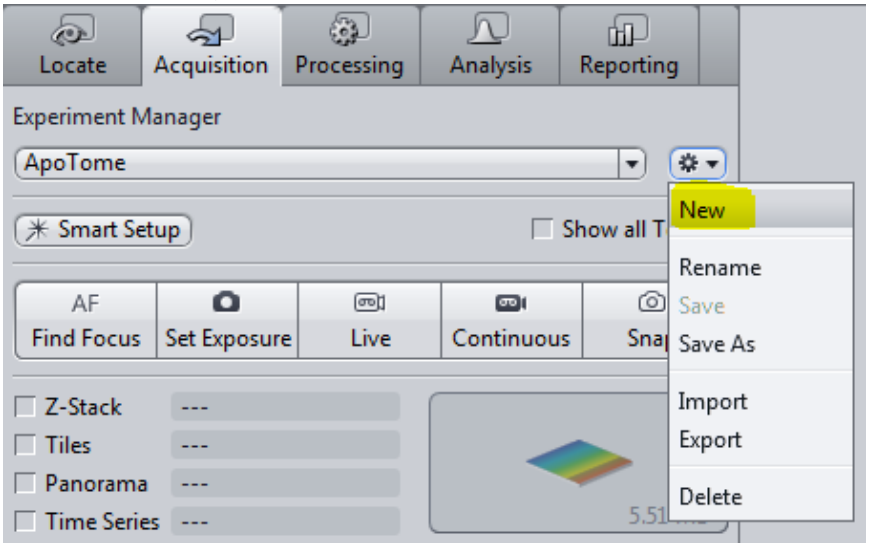
4.5.3 Step 1: Define channels using Smart Setup

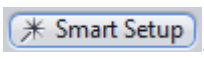
Aim

In this step we will set up a two-channel experiment. To do this, we will use the Smart Setup function. The ApoTome is in the first click-stop position, i.e. in the empty position without a grid.

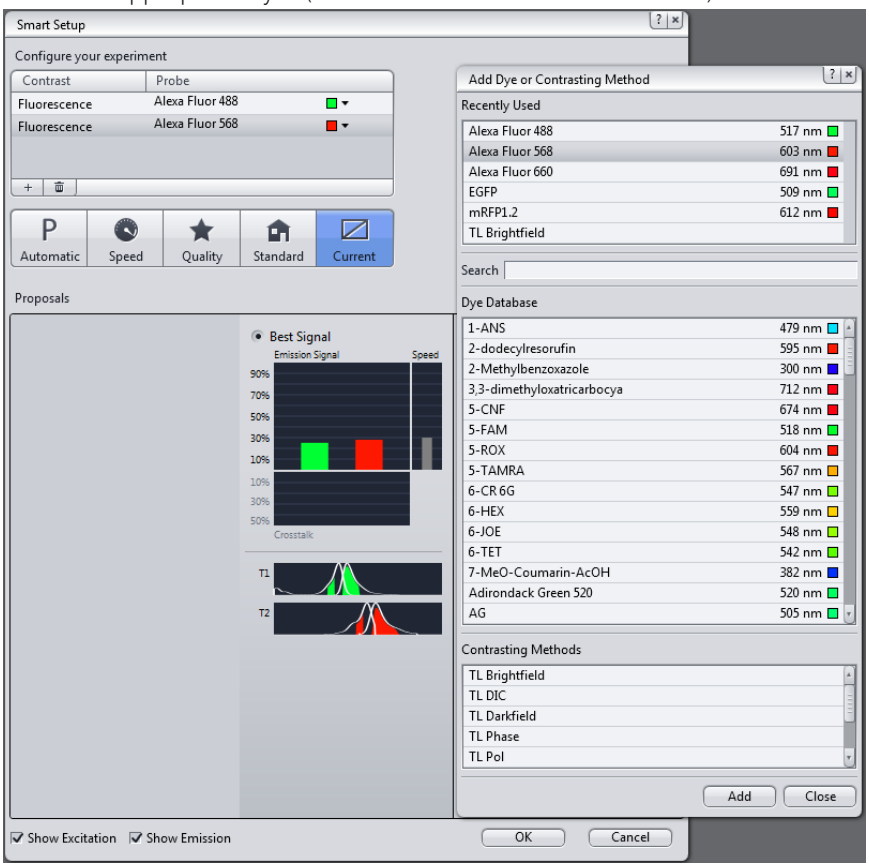
Procedure

1. Place your sample onto the microscope stage, localize it with the help of the functions on the **Locate** tab and bring it into focus.
2. Now go to the **Acquisition** tab and create a new experiment in the **Experiment Manager**:



3. Open the **Smart Setup**  .

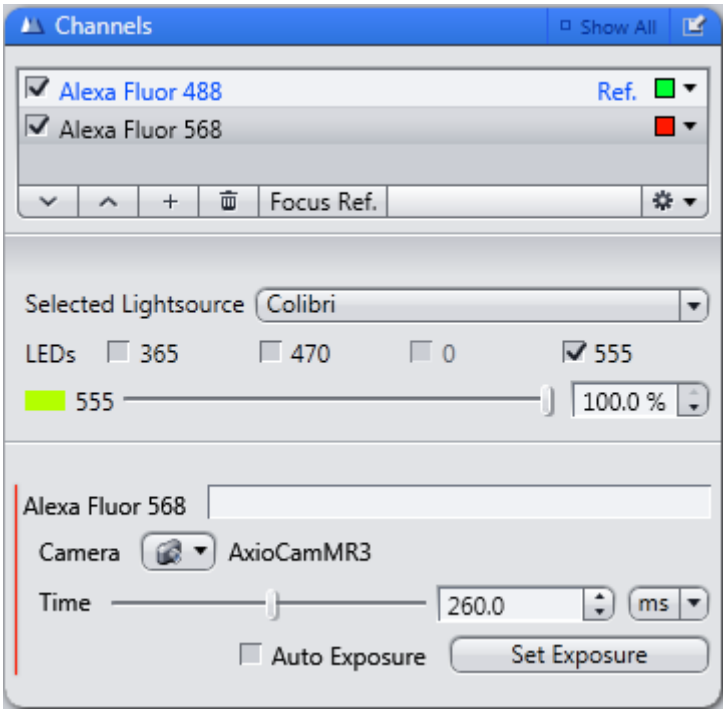
4 Select the appropriate dyes (in our case Alexa 488 and Alexa 568):



5 Close the dialog by clicking on **OK**.

You will now see two channels in the **Channels** tool.

6



You have successfully set up the channels. In the live mode you can now check the focus and exposure time for the two channels.

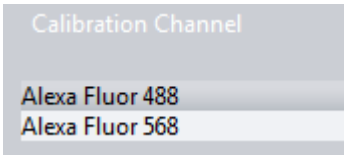
4.5.4 Step 2: Grid focus calibration

Aim

In this step you will calibrate the focus position of the ApoTome grid for the selected channels. This step is essential, as without a valid calibration it is not possible to perform an ApoTome experiment. Provided that no changes are made to the device settings that are important for calibration (objective, filter and illumination source, camera) the calibration remains valid for future experiments. We nevertheless recommend that you repeat the calibration from time to time, especially if the sample type you are analyzing changes. Calibration takes place in a wizard, which guides you through 3 simple steps.

Procedure

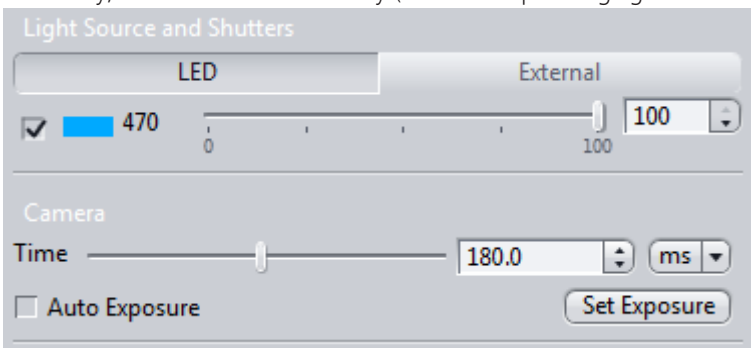
1. Move the ApoTome to the second click-stop position so the grid is positioned in the beam path.
2. From the **Acquisition** menu item open the **ApoTome Focus Calibration Wizard …** entry.
3. In the first step select the channel for which you want to perform calibration.



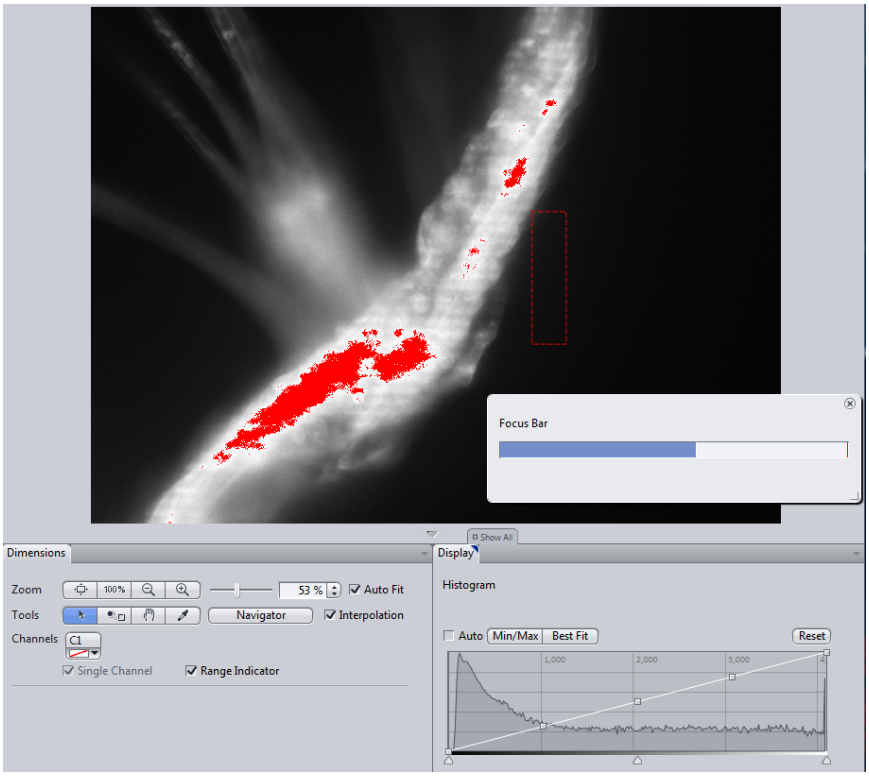
4. Click on **Next** to proceed.

   You will now see the **Live Image**. Bring the sample into focus. The ApoTome grid has been moved to an end position to make it easier for you to focus on the sample. If you can nevertheless see the grid lines, something that can never be avoided entirely if you are using a 100x objective for example, click on one of the two buttons **Grid To Start** or **Grid To End** and use the position at which the fewest grid lines can be seen.

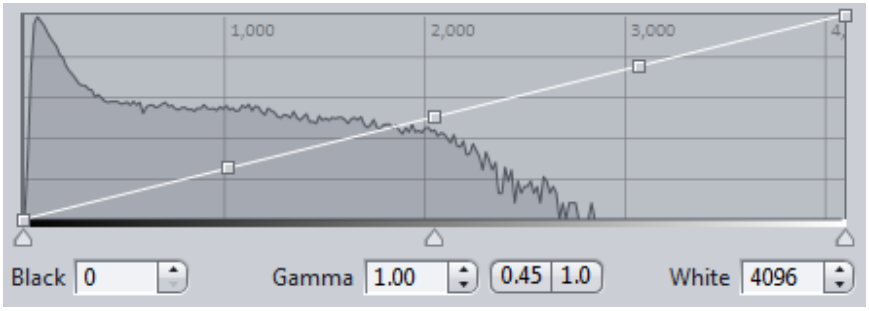
5. On the left you have the option of adjusting the exposure time and, if necessary, the illumination intensity (with corresponding light sources only).



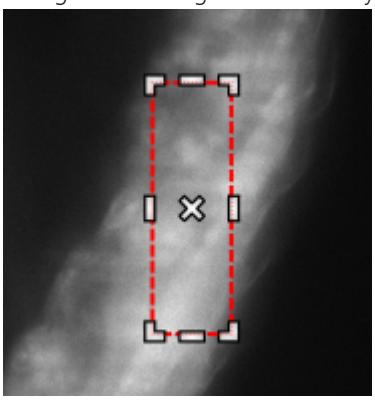
6 To adjust the exposure time correctly, select the saturation display on the **Dimensions** tab. Regions overlaid in red indicate that pixels are saturated.



7 Reduce the exposure time accordingly. The ideal situation is where approx. 70% of the histogram is filled for the brightest region of the sample.



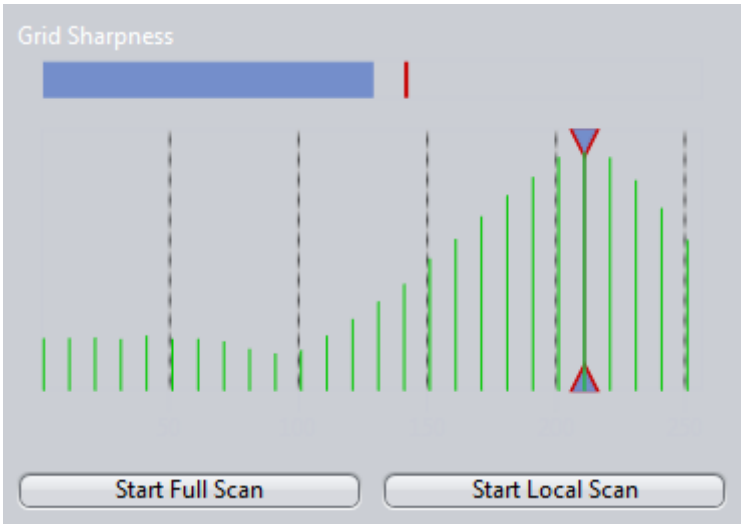
8 Position the rectangle in the live image and adjust its size in such a way that it covers fairly homogeneous fluorescent structures and does not lie over the background. The grid focus is only determined within this rectangle.



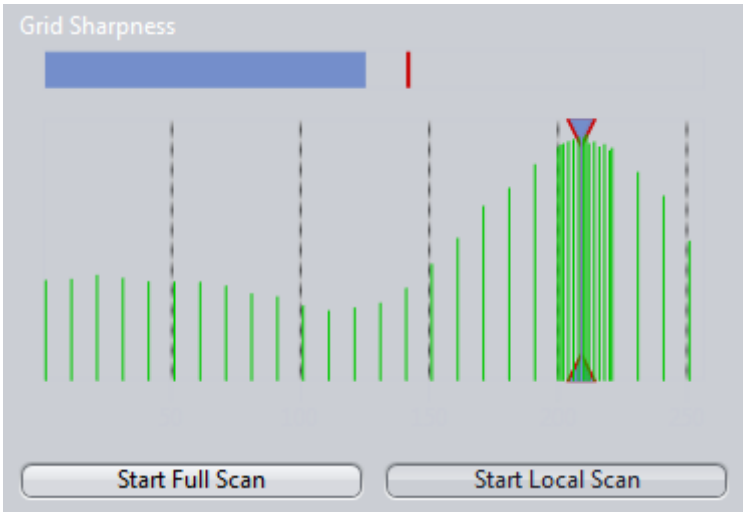
9 Click on **Next** to proceed to the final step.

10 Now click on the **Start Full Scan** button to start the grid focus search.

The grid contrast is displayed in the histogram.



11 More precise grid focusing can be achieved if you click on the **Start Local Scan** button.



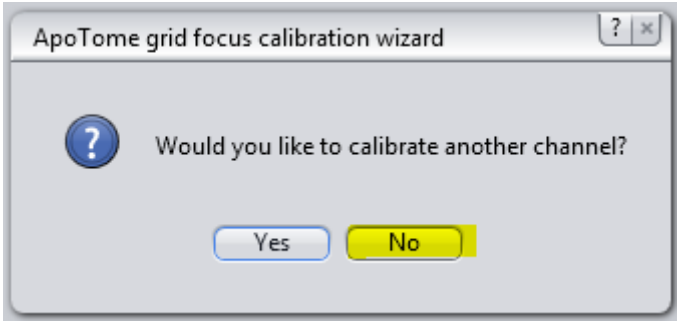
However, this is only recommended for samples that are not particularly prone to bleaching. For samples prone to significant bleaching, the results of the **Local Scan** would measure considerably lower intensities and distort the result.

12 Click on the **Finish** button.

13 To perform calibration for another channel, answer **Yes** to the question "Do you want to calibrate another channel?" in the dialog that is now displayed.

The wizard then begins again from Step 1.

14 As soon as you have calibrated all channels, exit the wizard by clicking on **No**.



You have successfully performed grid focus calibration. Now continue with the next step.

4.5.5 Step 3: Perform ApoTome experiment

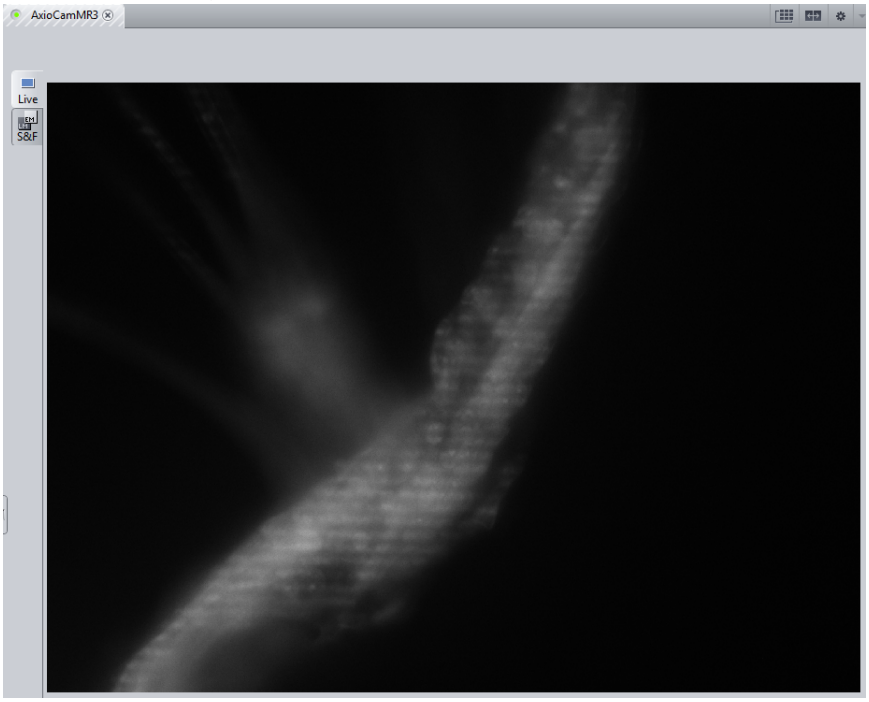
Aim

In this step you will perform acquisition for a two-channel experiment. You will use the same channels that were set up in Step 1. The objective must also be the same one used to calibrate the grid focus.

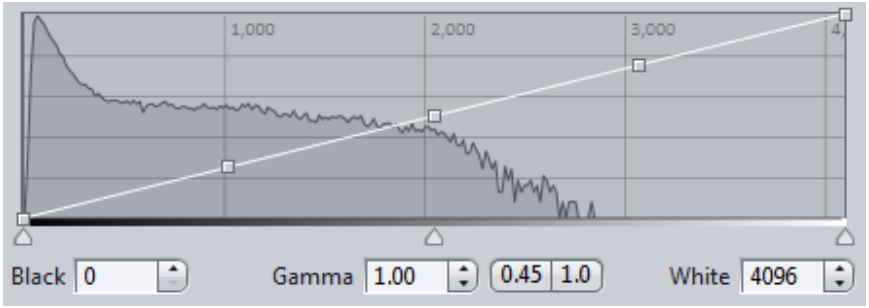
Procedure

1 Insert the desired sample into the slide holder.

2 Open the **Live** Image and focus on the specimen.

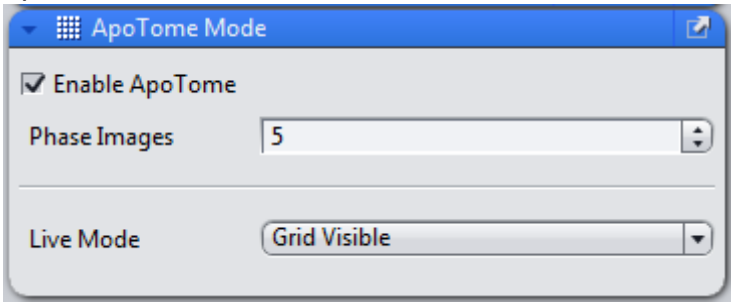


3 Adjust the exposure time for the two channels in such a way that approx. 70% of the **Histogram** is used.



4 Close the Live Image to protect the sample.

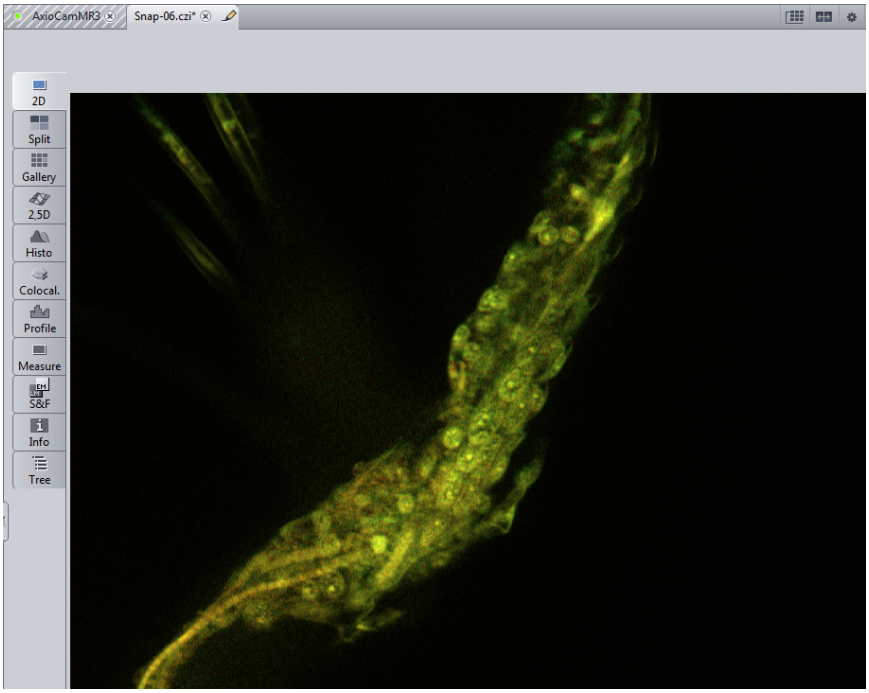
5 Open the **ApoTome Mode** tool on the **Acquisition** tab and enable the **ApoTome**.



6 Start acquisition by clicking on the **Snap** button.

The result is a two-channel image of your specimen.

7



You have successfully performed an ApoTome experiment.

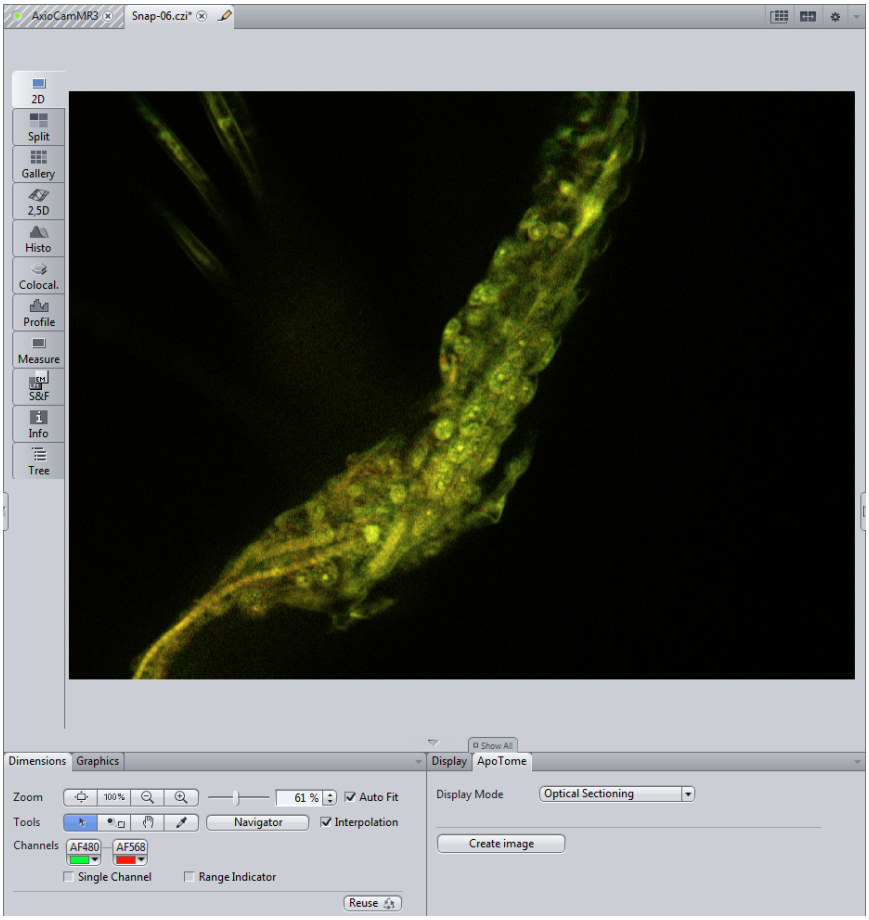
4.5.6 Step 4: Process the resulting image

Aim

ApoTome images that are acquired from the **Acquisition** tab always take the form of raw data. In this step, with the help of the image you acquired in Step 3, we will look at the various display options available for ApoTome raw images. We will also create a processed resulting image, which you can process further as required.

Prerequisites

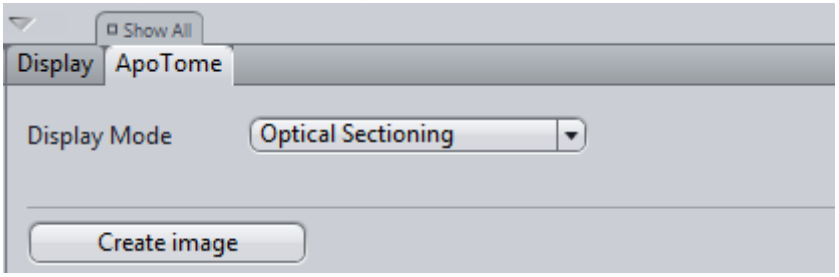
- For this step you need to be in the *2D view* [▶ 501].



Procedure

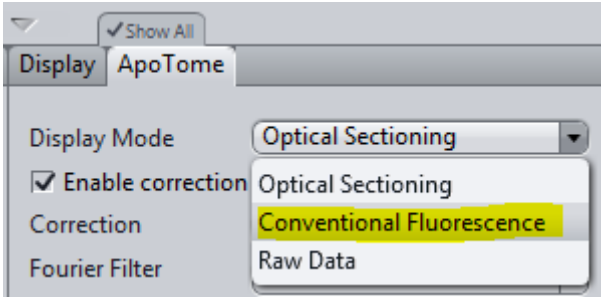
1. In the Center Screen Area go to the **ApoTome** tab. This view option is only displayed for ApoTome raw images.

   If the Show all mode is deactivated you will see two view options: the **Display Mode** settings & the **Create Image** button.

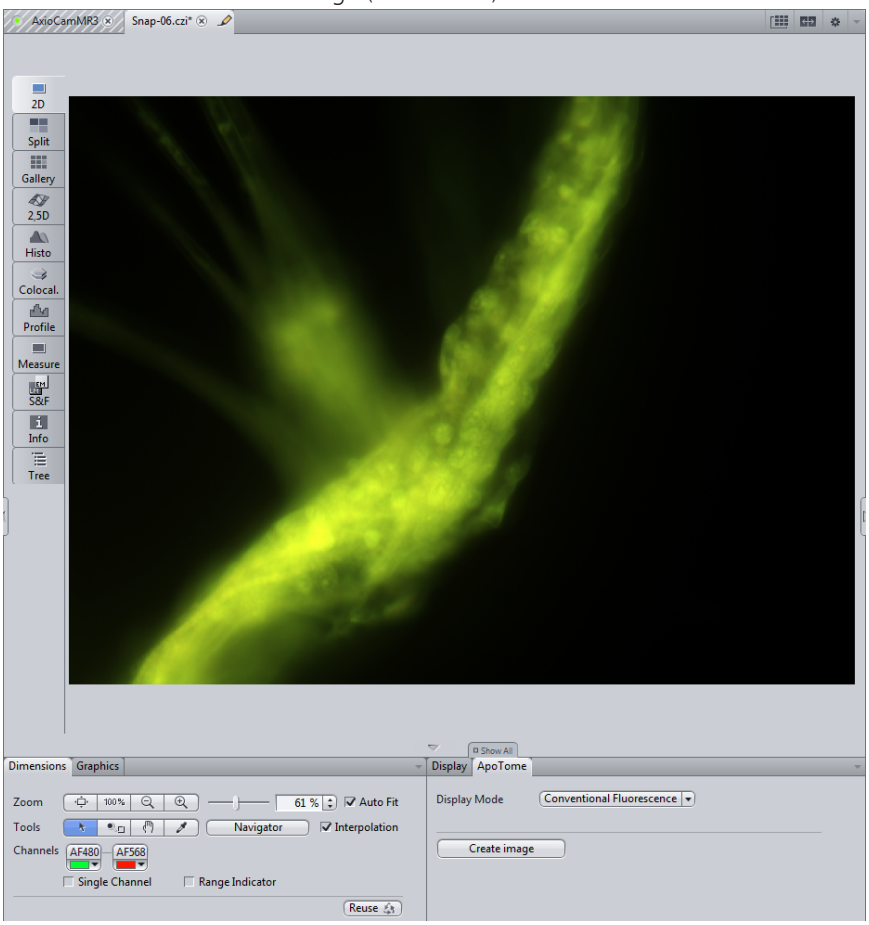


   The default selection for Display Mode is the **Optical Section** view.

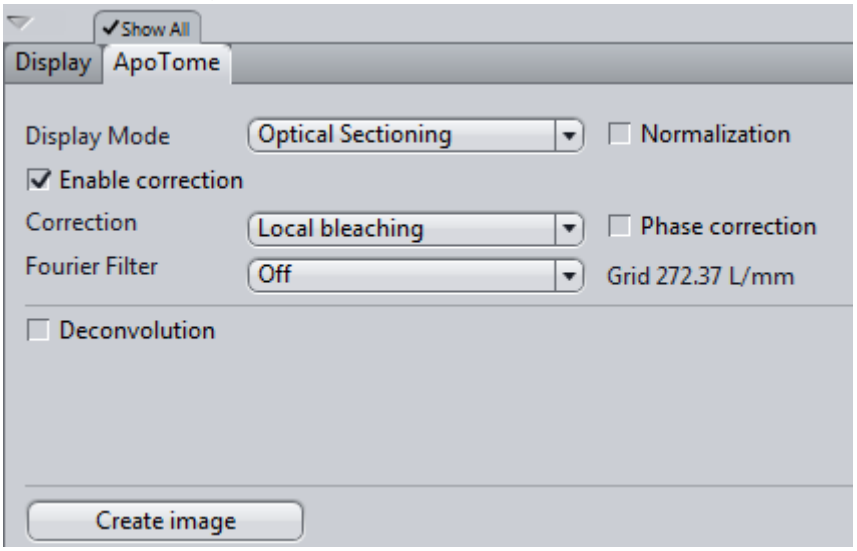
2 Select the **Conventional Fluorescence** option from the **Display Mode** dropdown list.



The image is now no longer displayed as an optical section, but as a conventional fluorescence image ("widefield"):



3 Activate the **Show All** mode to see additional settings for the calculation of the optical section image.



Note

The main cause of streak artifacts is the bleaching of the fluorescence signal during acquisition of the grid images (= phase images). Depending on the degree of bleaching, fine streak artifacts can appear in the resulting image when the grid images are combined. These can be easily corrected, however.

4 To see the difference between a corrected and uncorrected image, deactivate the **Enable Correction** option. Detailed information on the individual options can be found in the online help.

5 If you have not yet done so, enable the correction using the **Local Bleaching** option.

6 Create a new, processed resulting image by clicking on the **Create Image** button.

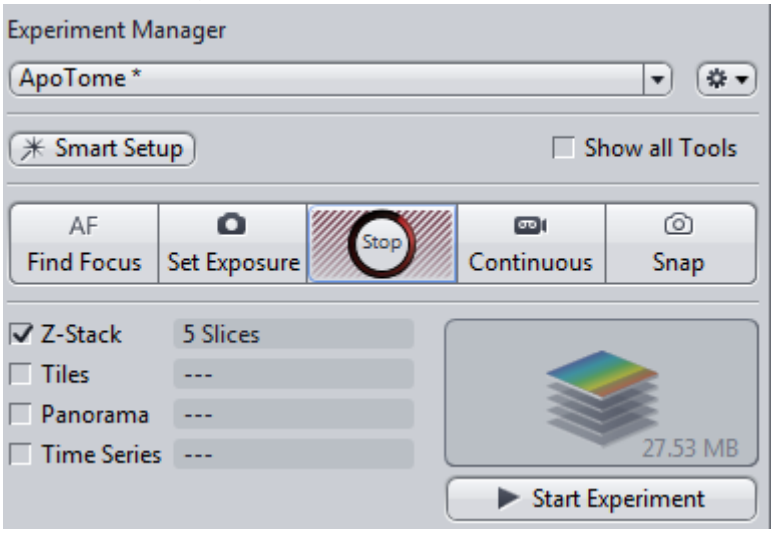
You have successfully processed the ApoTome image and created a resulting image for further processing.

4.5.7 Step 5: Perform Z-stack acquisition

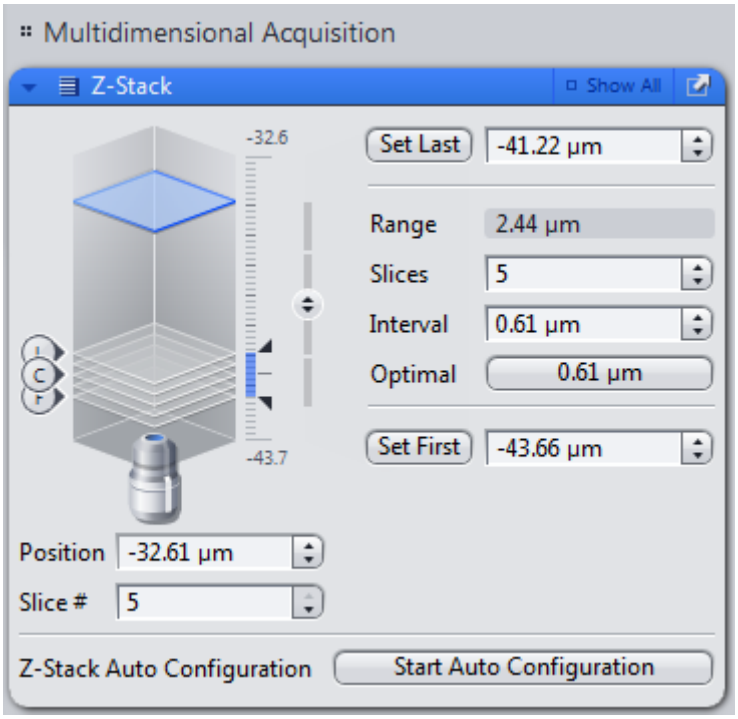
Aim

In this step you will acquire a Z-stack image with the same channel settings as in Step 3.

Procedure **1** On the **Acquisition** tab activate the **Z-Stack** acquisition dimension in the Experiment Manager.

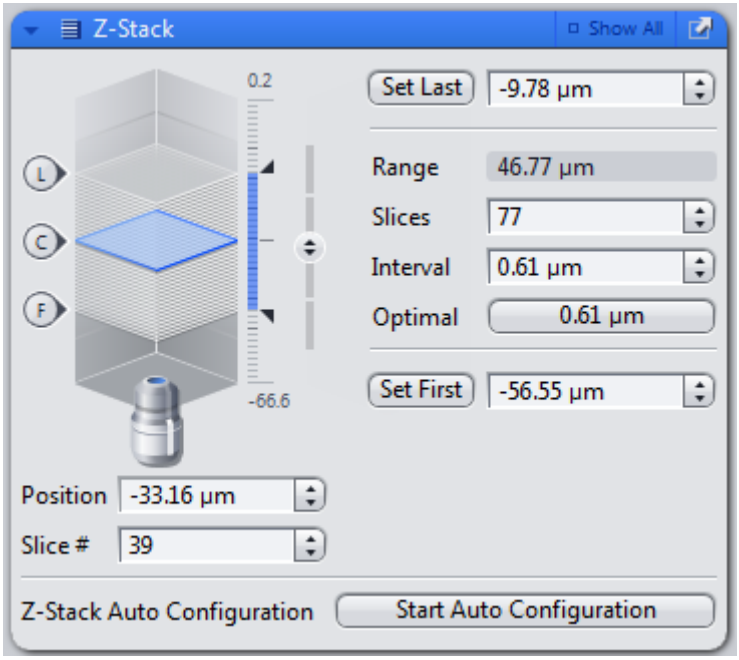


2 Open the Z-Stack tool.



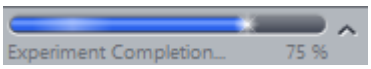
3 Start the **Live Mode** and define the dimensions of the Z-stack for your specimen. To capture the entire object three-dimensionally, you should set the upper and lower limit in such a way that object structures can no longer be

seen in focus. Set the interval between the individual Z-planes using the **Optimal** button:



4 Start acquisition by clicking on the **Start Experiment** button.

You can follow the progress of the experiment in the progress bar on the status bar:



You have successfully acquired a Z-stack image. Save the resulting image under a meaningful name via the **File** menu | **Save** (**Ctrl+S**).

4.5.8 Step 6: Perform ApoTome deconvolution

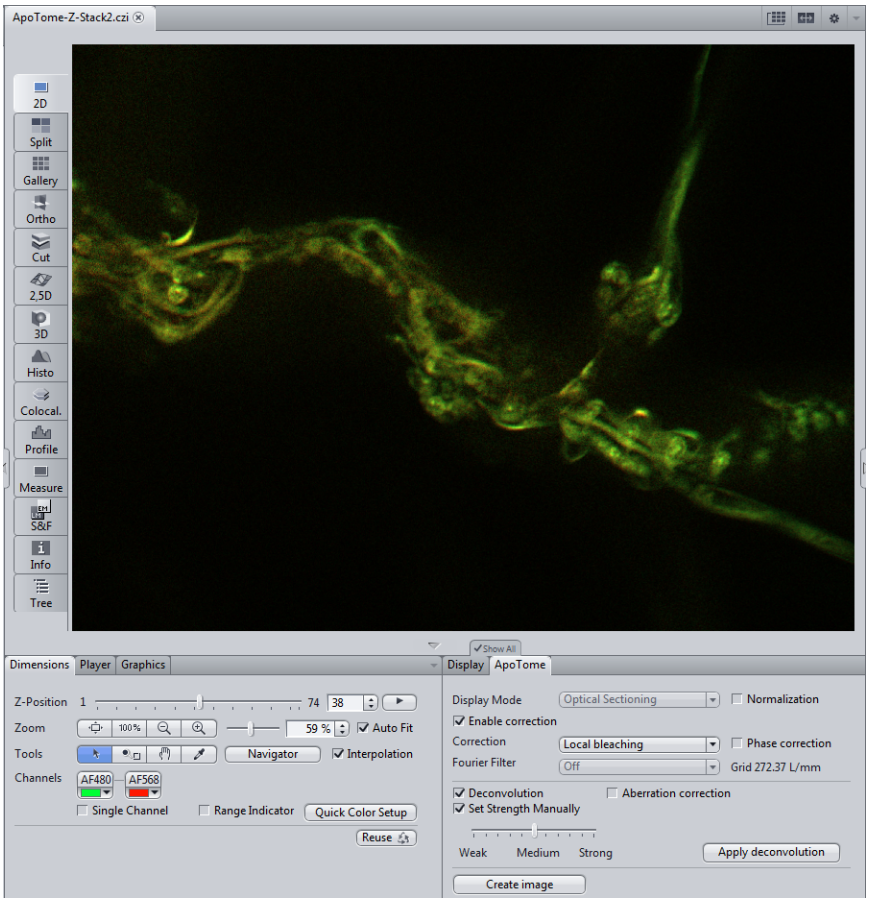
Aim

In this step ApoTome deconvolution will be performed for the Z-stack acquired in Step 5. This enables you to significantly enhance the image, beyond what is possible using the normal **ApoTome** processing functions.

Prerequisites

- The Z-stack image must be in the foreground and in the 2D view. Go to the **ApoTome** tab (view option). Make sure that the **Show All** mode has been activated.

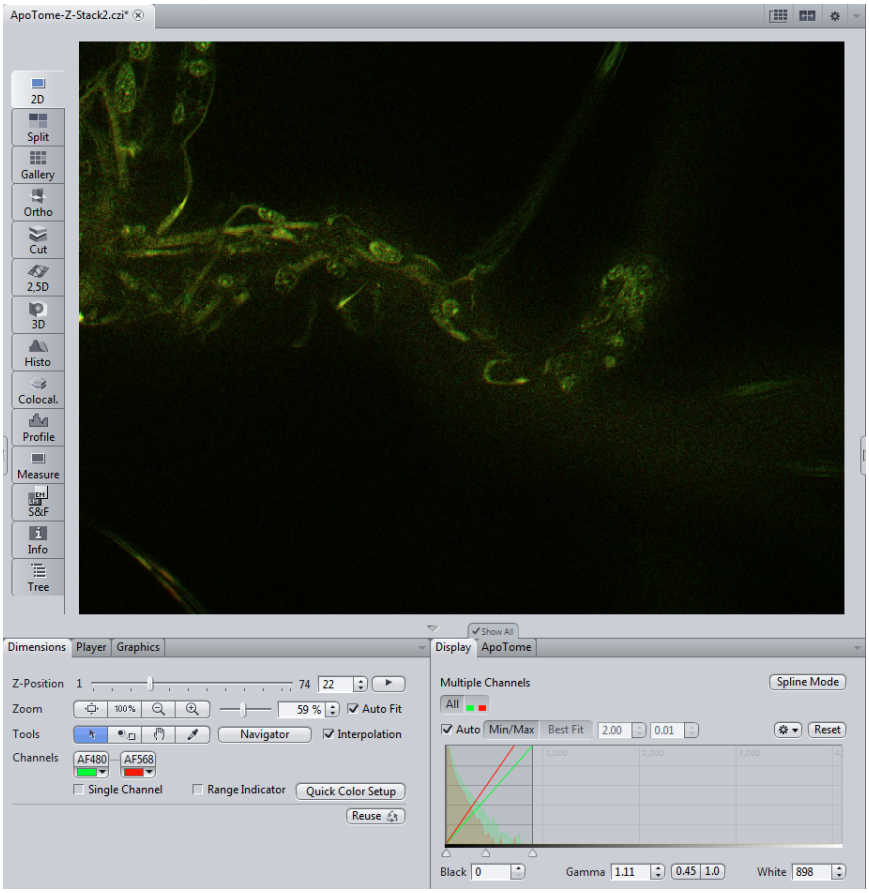
Procedure **1** Activate the **Deconvolution** checkbox.



Make sure that the **Set Strength Manually** option is also activated. The **Strength** slider is set to **Medium** by default. Retain this setting for the time being.

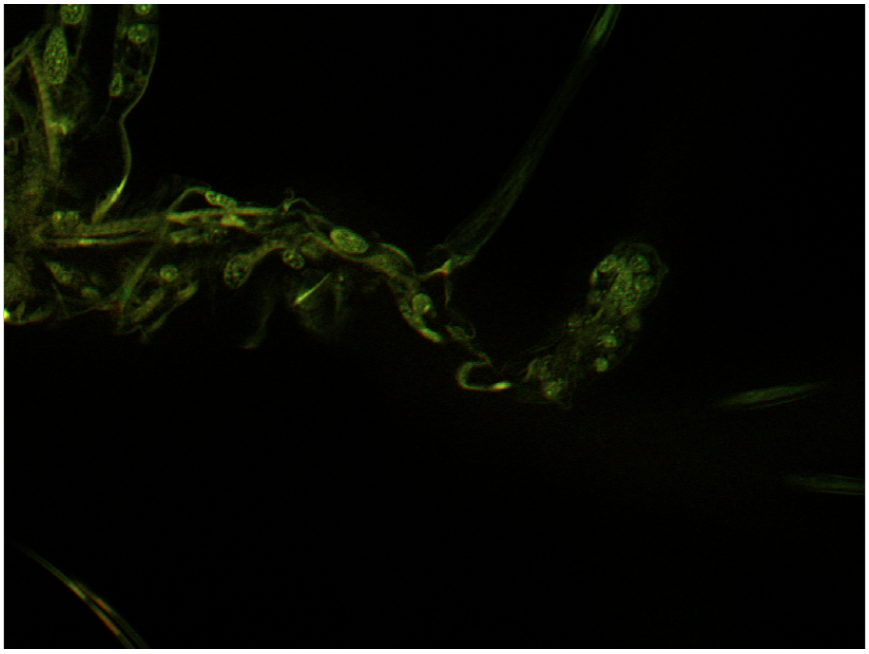
2 Click on the **Apply deconvolution** button.

Depending on the image size and the specifications of the computer, the processing can take anything between a few seconds and a few minutes. Make sure that you also adjust the brightness and contrast using the settings on the **Display** tab (tip: try out the **Min/Max** button).

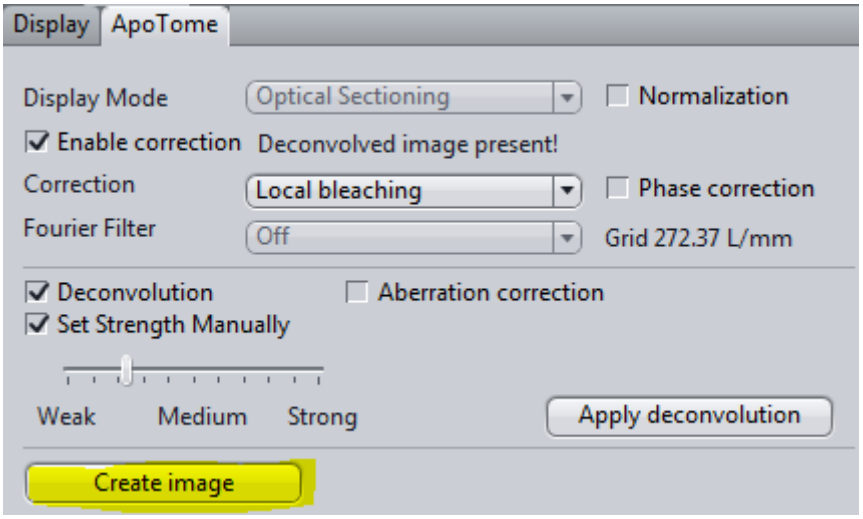


3 Examine the result by navigating through the Z-stack using the **Z-Position** slider on the **Dimensions** tab.

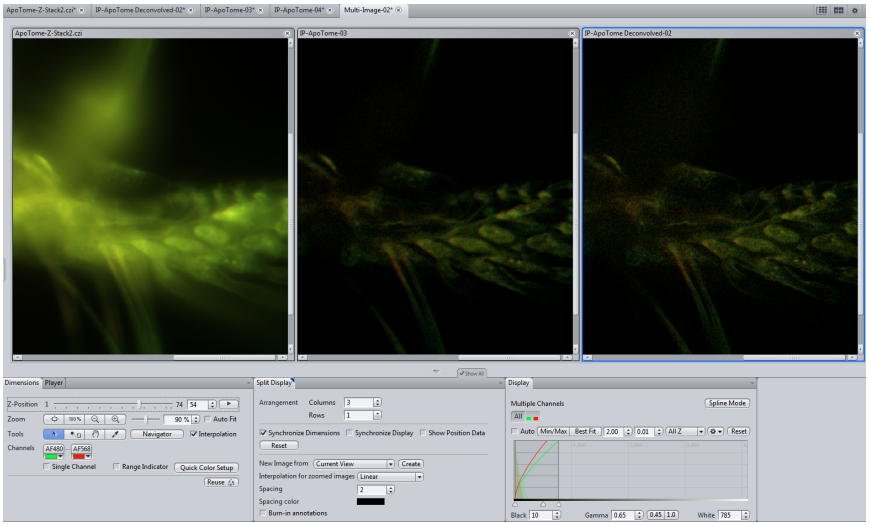
4 Change the **Strength** setting on the **ApoTome** tab until there is an obvious improvement and there is no disruptive background noise.



5 To obtain the result as a separate image document, click on the **Create image** button.



6 Using the *Splitter mode* [▶ 611] you can now compare the resulting image with the widefield and the ApoTome processed version.



You have successfully performed ApoTome deconvolution, created a resulting image and compared different resulting images.

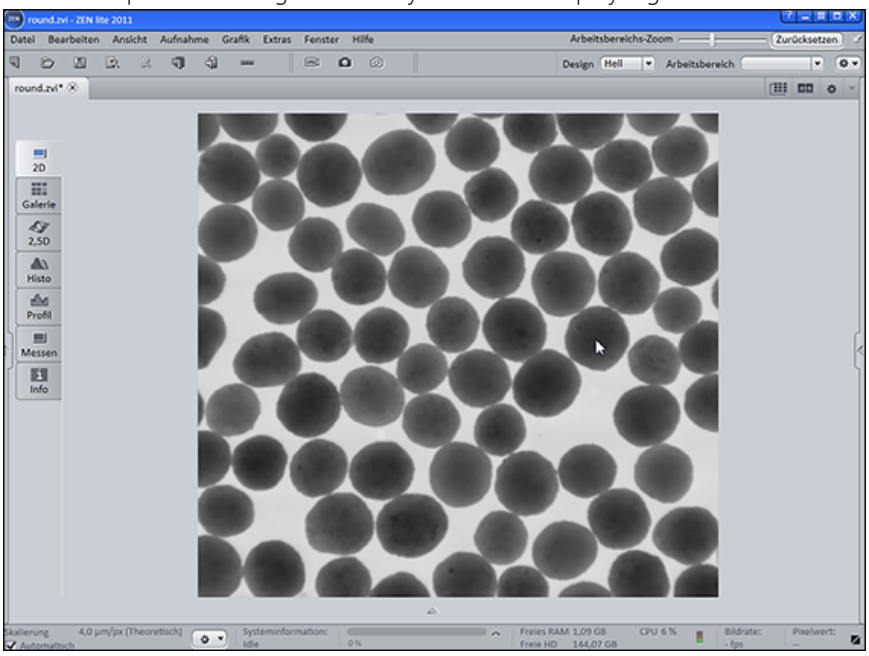
4.6 Displaying and adapting a grid in the image area

In this section you will find out how to display a grid in your images and how to adapt it.

> **Note**
>
> A grid can only be displayed in an acquired image or in a live image in **Continuous** mode.

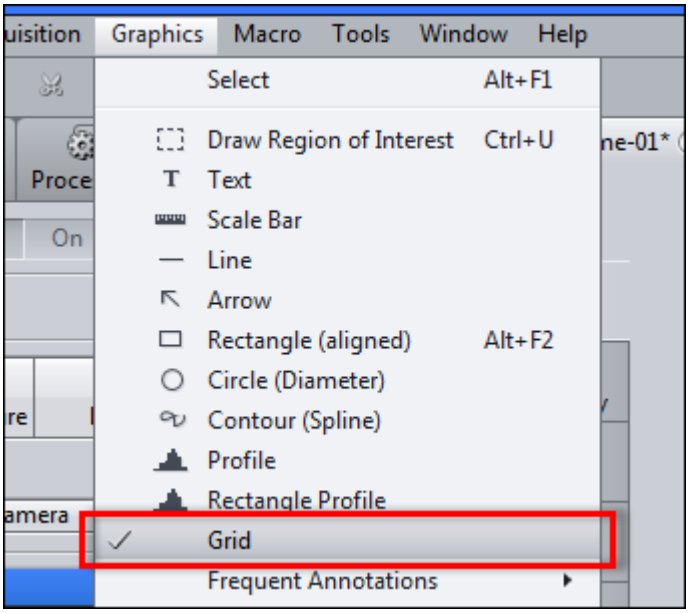
Prerequisites

- You have opened an image in which you want to display a grid.

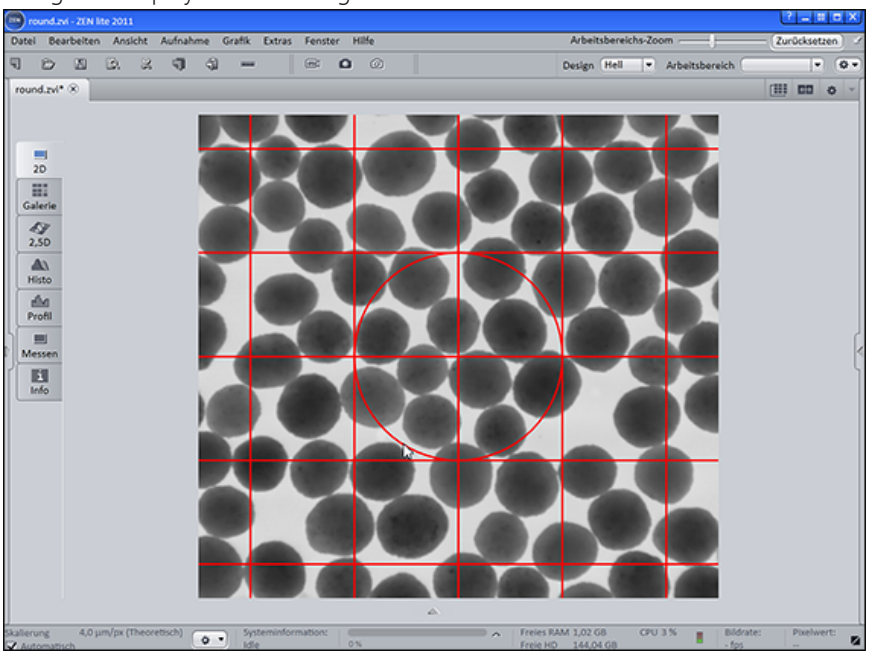


Procedure

1. Click on the **Grid** entry on the **Graphics** menu.



The grid is displayed in the image.

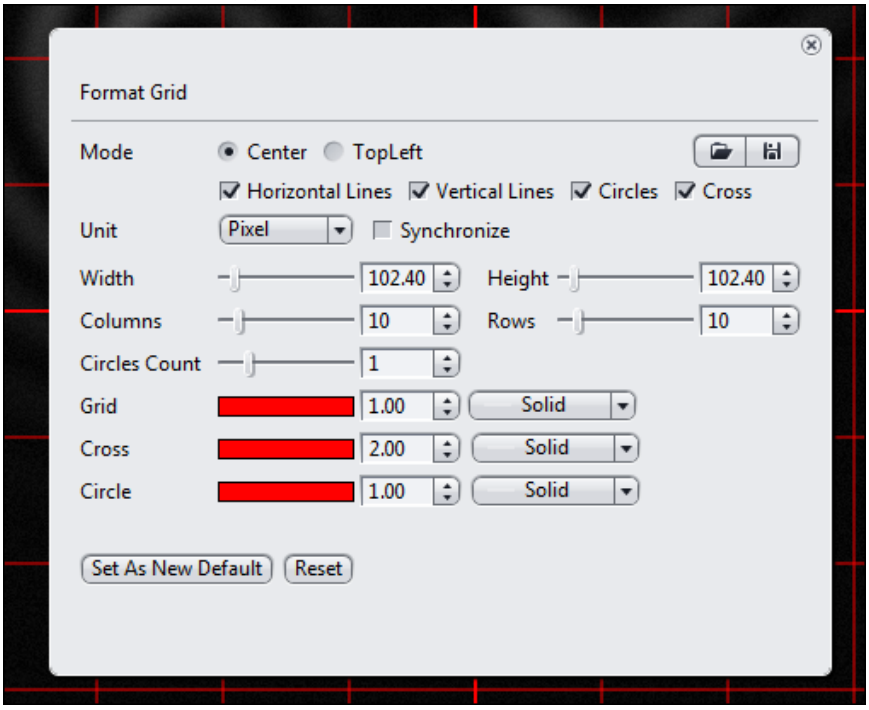


2 Right-click precisely on a grid line.

The shortcut menu opens.

3 Click on the **Format Graphical Elements** entry in the shortcut menu.

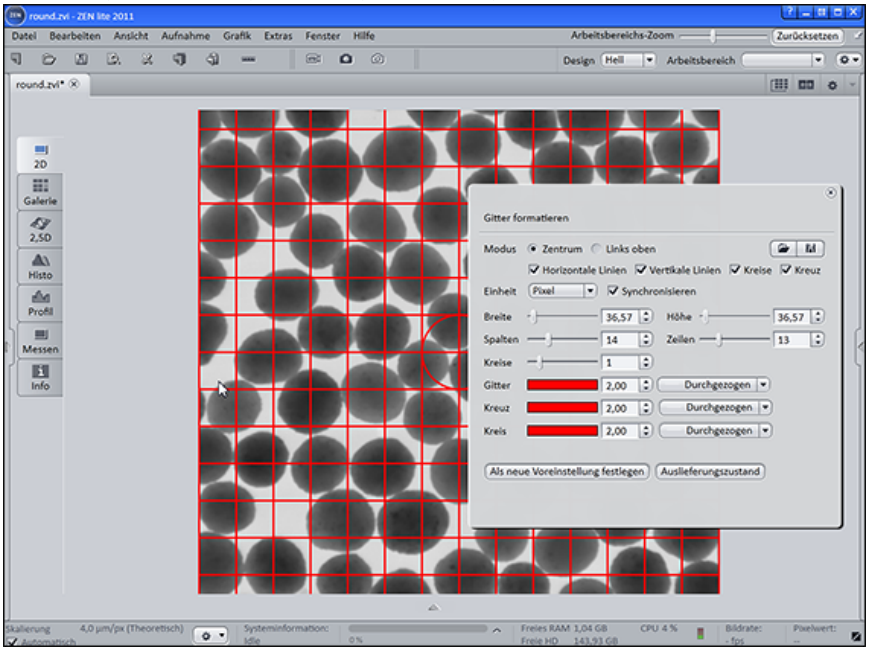
The **Format Grid** dialog window is displayed.



4 Activate the **Synchronize** checkbox. This function means that any changes made, e.g. to the number of columns, are adopted simultaneously for the number of rows. The grid therefore remains square.

5 Set a higher number of columns using the **Columns** slider. Alternatively, you can enter a value in the spin box/input field.

The changes are displayed directly in the image.



6 Click on the **Save** button to save the grid settings.

The Windows dialog for saving settings is opened.

7 Enter a name and click on **Save**.

8 Close the **Format Grid** dialog window.

You have inserted a grid into your image, edited the grid and saved your grid settings.

5 Image Processing

5.1 Performing deconvolution using default values

Successful deconvolution depends on aspects such as image quality and sample type. Numerous parameters are available for deconvolution which allow you to adjust the algorithms accordingly. This wide range of parameters can be overwhelming. With the **Deconvolution (defaults)** method, an attempt is therefore made to provide a good initial result using a preselected set of default parameters. This will not be possible in all situations. In such cases, you should use the **Deconvolution (adjustable)** method.

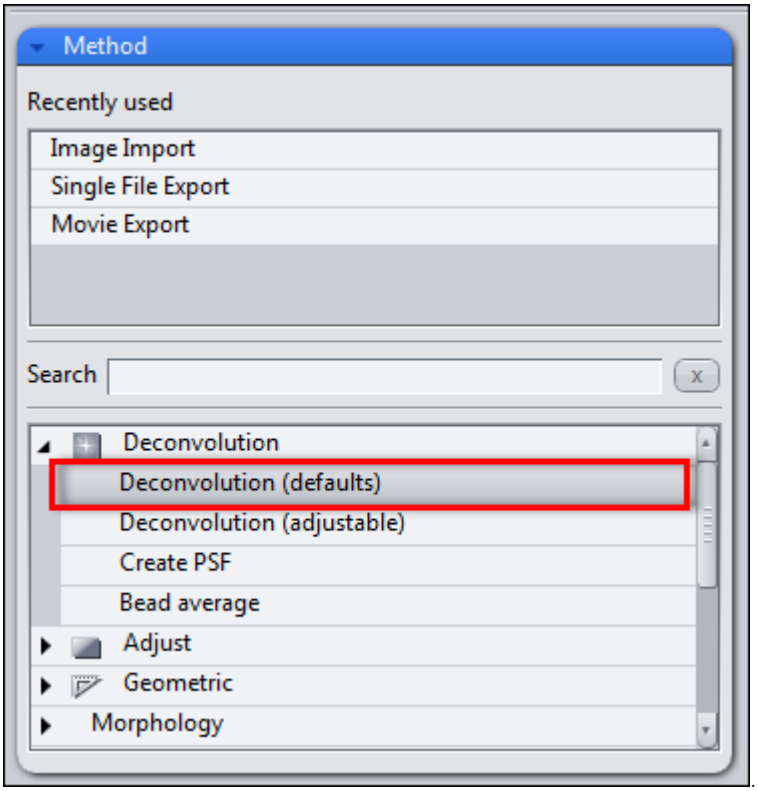
Prerequisites

- You are on the **Processing** tab.
- You have acquired or opened a fluorescence image on which you wish to perform deconvolution.

Procedure

1. Open the **Method** tool under **Method Selection.**
2. Click on the **Deconvolution** group.

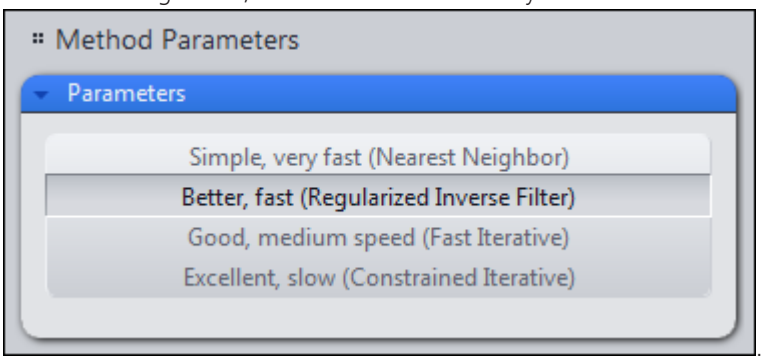
   You will see the methods **Deconvolution (defaults)** and **Deconvolution (adjustable)**.
3. Click on **Deconvolution (defaults).**



4. Open the **Parameters** tool under **Method Parameters**.

Here you will see 4 different algorithms for deconvolution (Nearest Neighbor, Inverse Filter, Fast Iterative, Iterative), which you can apply to your image automatically.

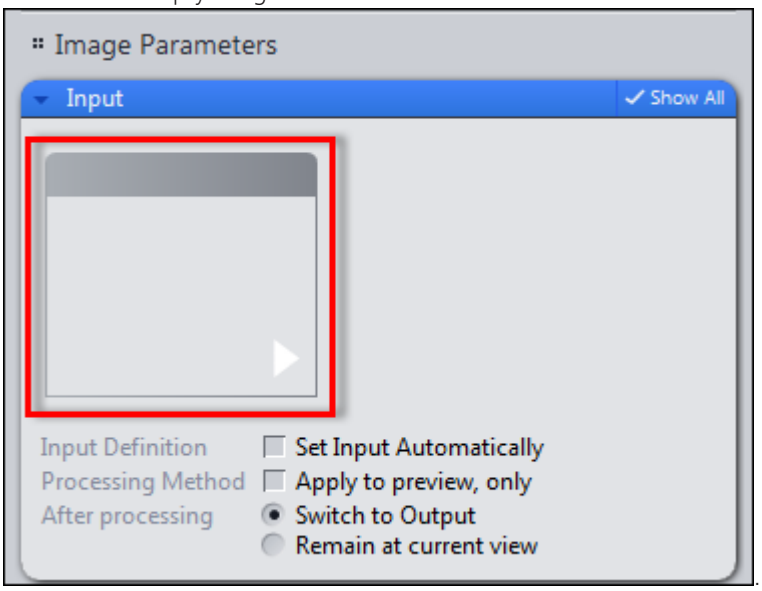
5 To select an algorithm, click on the relevant entry



6 Open the **Input** tool under **Image Parameters.**

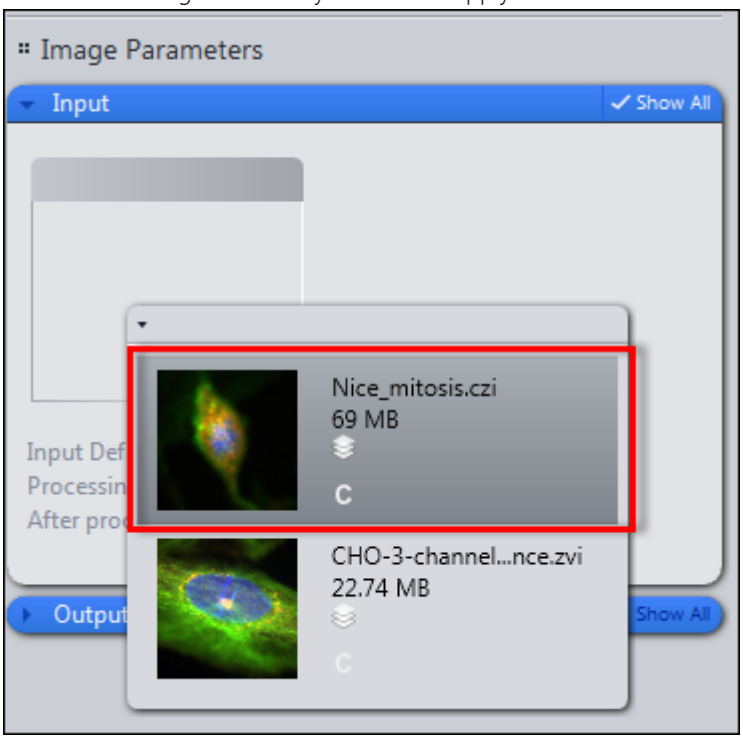
You will see the **Input** tool. The container for the input image is empty.

7 Click on the empty image container.



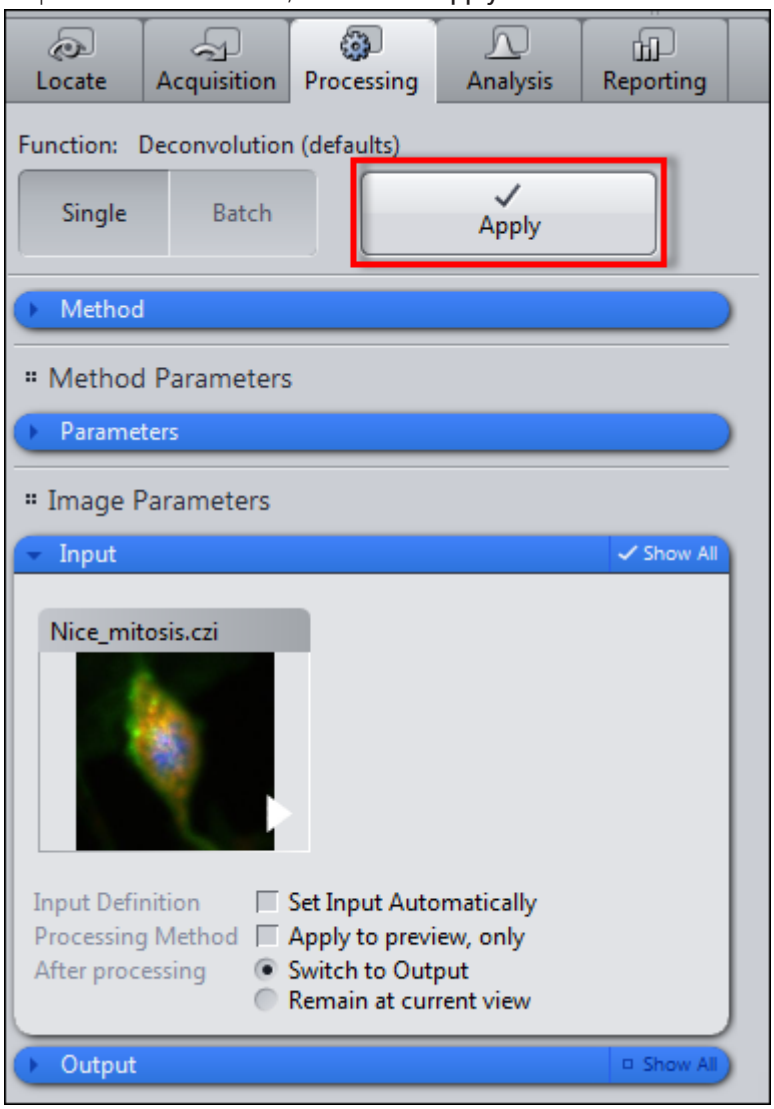
A list opens containing preview images of all images currently open.

8 Click on the image to which you want to apply deconvolution.



The image can now be found in the image container and will be used as the input image for processing.

9 To perform deconvolution, click on the **Apply** button.



Deconvolution is performed. A new image file is generated and is opened automatically in the center screen area after processing. If you are satisfied with the result, save the processed image. Repeat deconvolution using the other default values to obtain different results. If you have expert knowledge, you can configure all the deconvolution settings yourself using the **Deconvolution (adjustable)** method.

5.2 Performing configurable deconvolution

These instructions explain how to deconvolve a Z-stack image correctly step by step.

We will use the best method **Constrained Iterative** and a **measured PSF**.

Preparation

To follow these instructions you will need a Z-stack image of your sample and a Z-stack image of beads acquired on the same system and under the same conditions. You have opened ZEN 2.1 and no images are loaded.

Overview

The following steps are described in these instructions:

- **Step 1: Bead averaging**
  In this section you will find out how to create an averaged bead Z-stack image.
- **Step 2: Create PSF**
  In this section you will find out how to create a PSF from the averaged bead Z-stack image.
- **Step 3: Load input image**
  In this section you will find out how to load an input image in Deconvolution (Configurable).
- **Step 4: Set parameters**
- In this section you will find out how to set the parameters and add a measured PSF.
- **Step 5: Process image**
  In this section you will find out how to process the image and compare it with the input image.

5.2.1 Step 1: Bead averaging

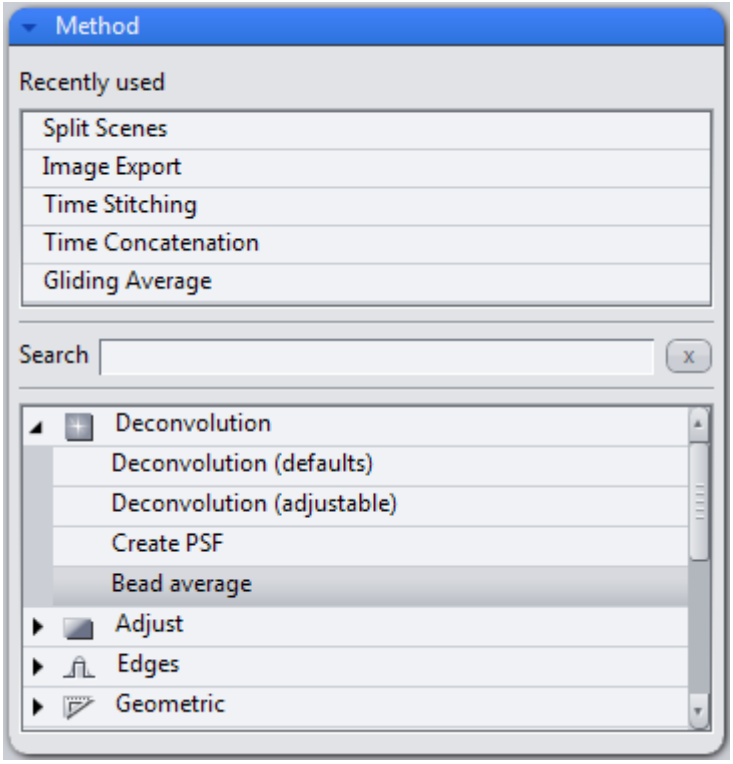
Aim

In this section you will create a Z-stack image of an individual bead, generated from several beads by means of averaging. The averaging reduces image noise and improves the quality of the resulting PSF.

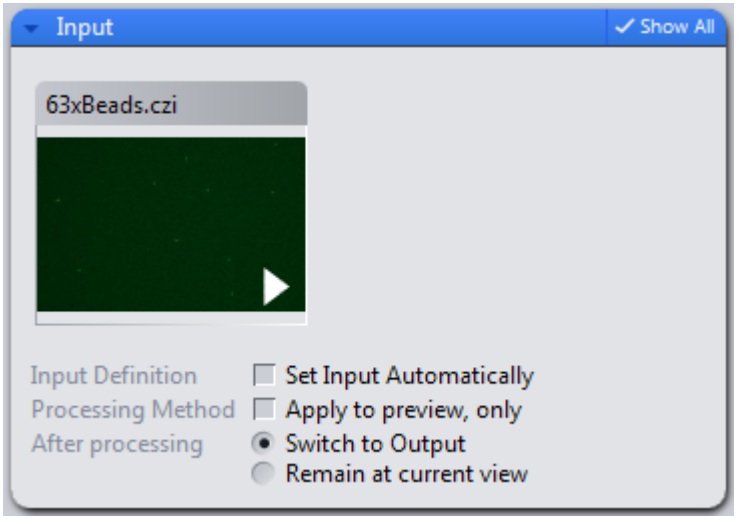
Procedure

1. Open the image containing the acquired bead.
2. Go to the **Processing** tab. Make sure that the **Single** processing mode is selected.

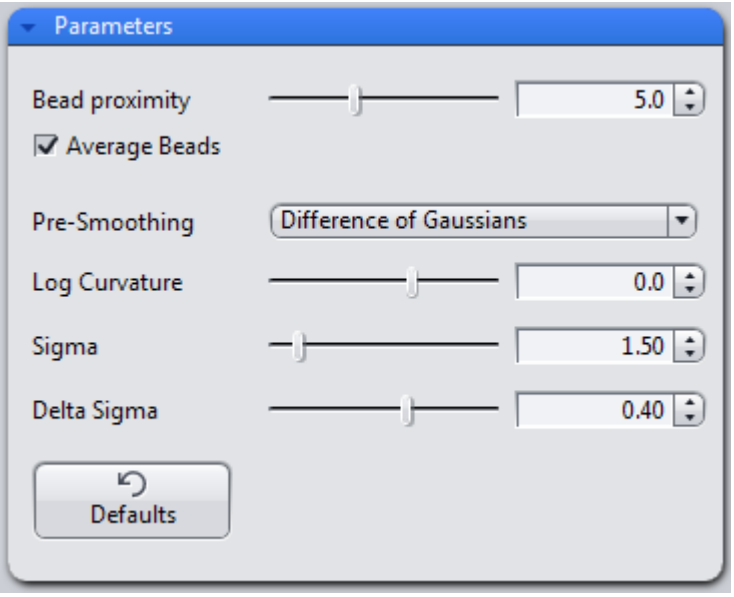
3 In the **Method** tool | **Deconvolution** group select the **Bead Average entry.**



4 In the **Input** tool select the bead image as the input image and deactivate the **Set Input Automatically** option.



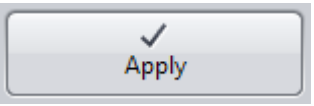
5 Open the **Parameters** tool. With the sample image shown here the default parameters are sufficient:



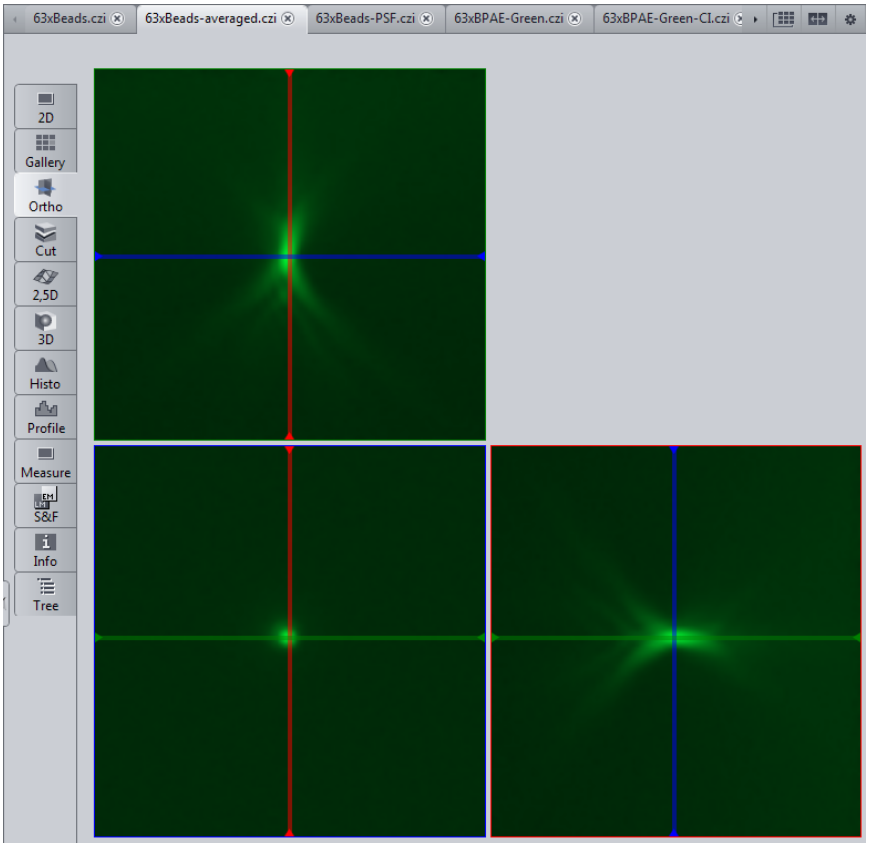
i Note

To allow you to test what effect the settings have, we recommend that you deactivate the **Bead Averaging** option. In the result you can then view the individual beads found to ascertain whether all suitable beads were actually found using the parameters in question. After doing this, reactivate the option and create the final bead image.

6 Click on the **Apply** button.



After a few minutes you will obtain a new image document. In this you will see an individual, centered bead with significantly reduced image noise. It is best to view the result in **Ortho** view.



7 Save the result under a suitable name.

You have successfully performed bead averaging and have created a Z-stack image from the resulting individual averaged bead.

5.2.2 Step 2: Create PSF

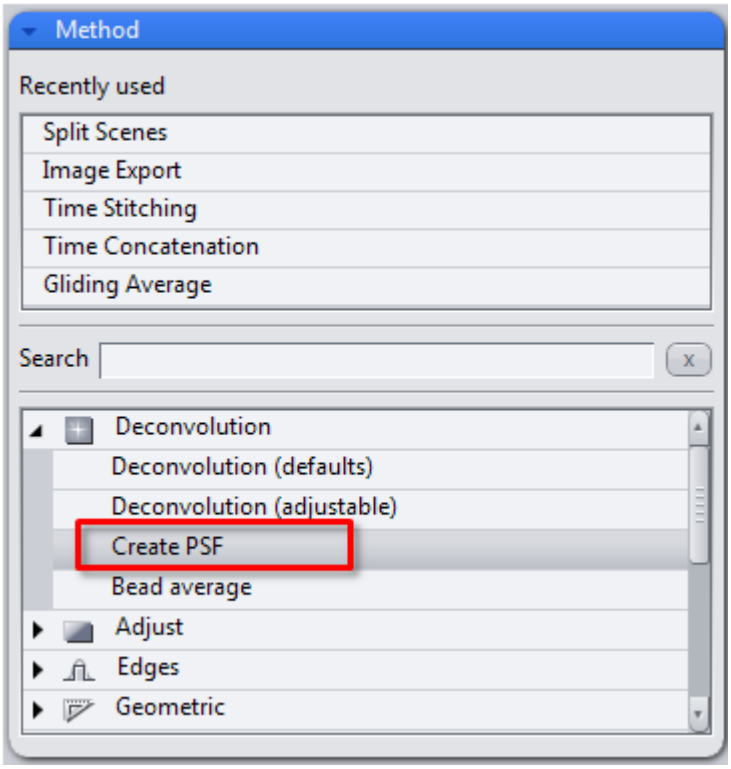
Aim

In this step you will create a PSF from the bead image created in **Step 1**. The image data are converted into a different format, any image noise still present is removed and the PSF parameters are calculated.

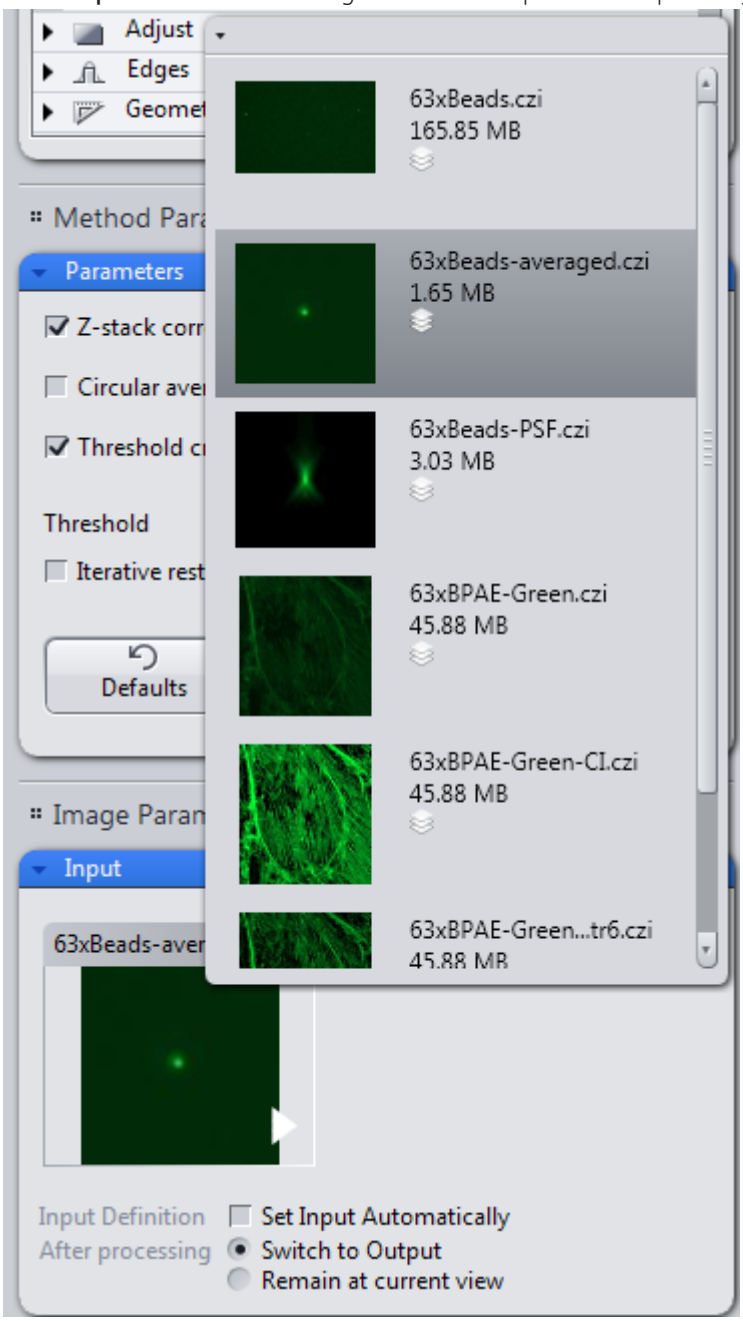
Prerequisites

- The image from Step 1 should still be open.

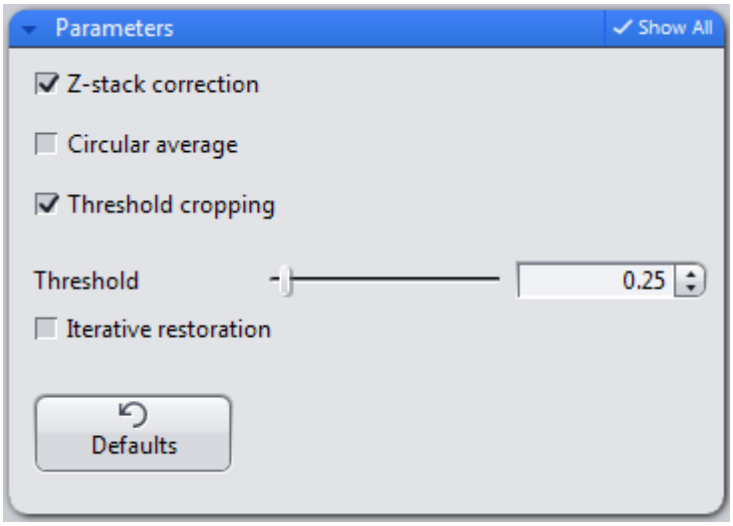
Procedure **1** On the **Processing** tab in the **Deconvolution** group select the **Create PSF** method:



2 In the **Input** tool select the image created in Step 1 as the input image.



3 Open the **Method Parameters** tool.

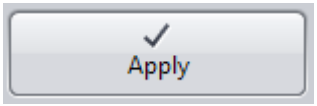


The default parameters generally produce good results and do not need to be changed.

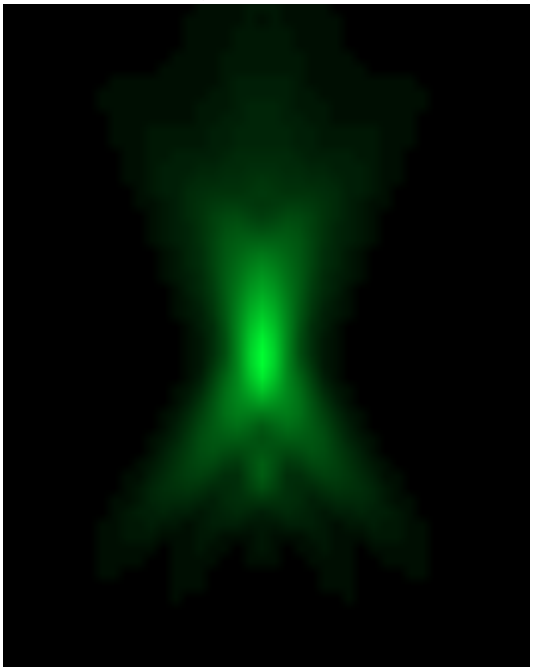
Note

If you have used beads with a diameter greater than the resolution limit of your objective, you must use the Iterative Restoration option here and indicate the diameter of the beads.

4 Execute the function by clicking on the Apply button.



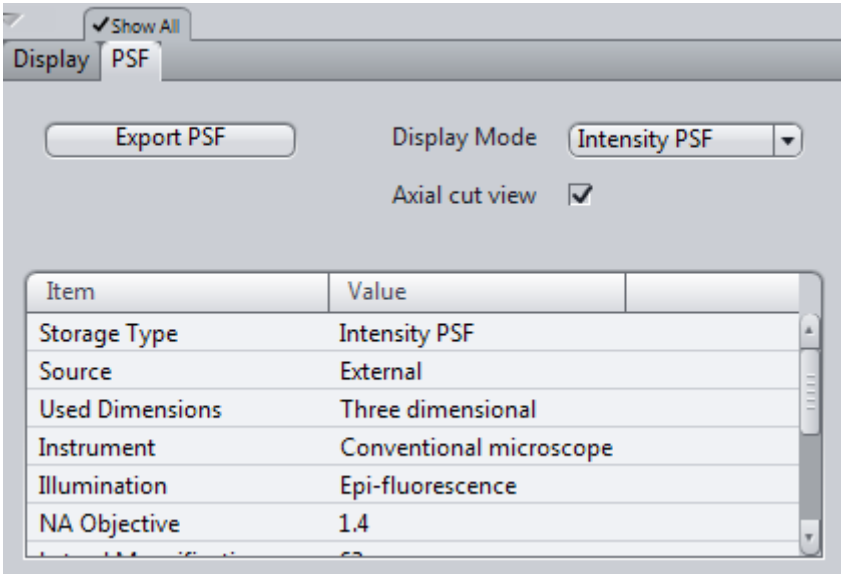
After a short time you will obtain a new image document of the created PSF.



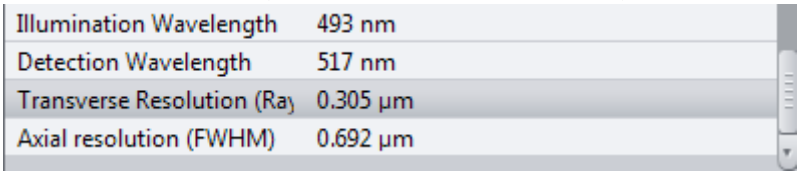
5 Save the document under a suitable name.

You will now see the **PSF** view option tab. This tab is only displayed for PSF documents.

6



The values determined for lateral and axial resolution are of particular interest here. Move down through the contents of the table using the scrollbar:



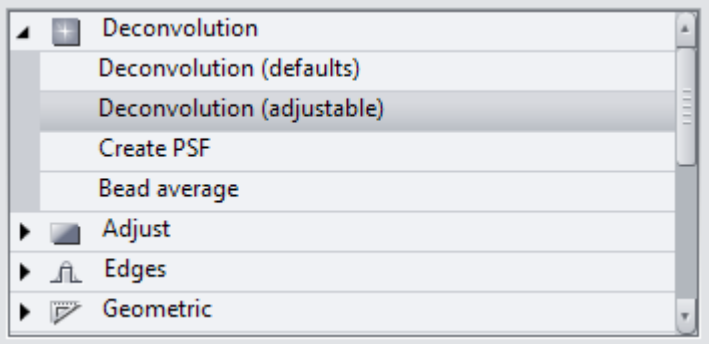
Here you can see how under real conditions the lateral resolution can differ from the design values of the objective. This can only be attributed to the quality of the objective in very few cases, however, and simply describes the actual conditions under which the sample being analyzed was acquired, including all aberrations. Spherical aberration in particular has an adverse impact on resolution.

5.2.3 Step 3: Load input image for deconvolution

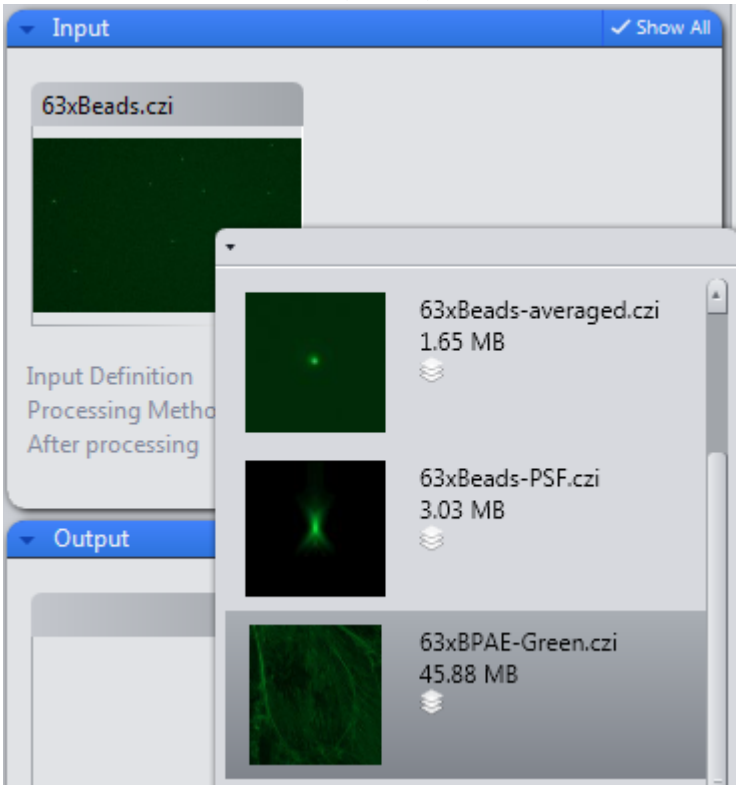
Aim

In this step you will select the image to be processed and load it as an input image for deconvolution. During loading the meta data contained in the image are checked to ensure that all information required for deconvolution is available. If it is not, you will receive a corresponding error message.

Procedure **1** On the **Processing** tab | **Method** tool in the **Deconvolution** group select the **Deconvolution (adjustable)**method



2 In the **Input** tool select the image that you want to deconvolve.



i Note

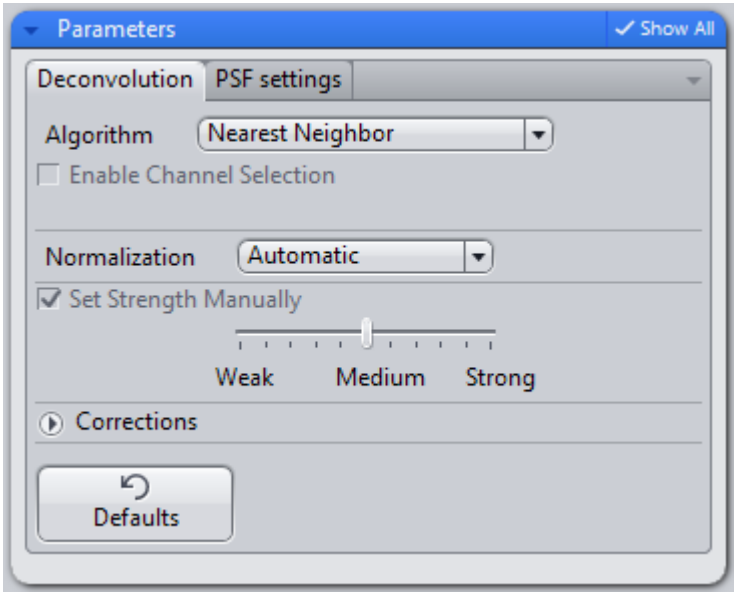
If a warning appears at this point, it is likely that parameters required for deconvolution are missing from the image. You can subsequently enter or change these values in the **Parameters** tool.

5.2.4 Step 4: Set parameters for deconvolution

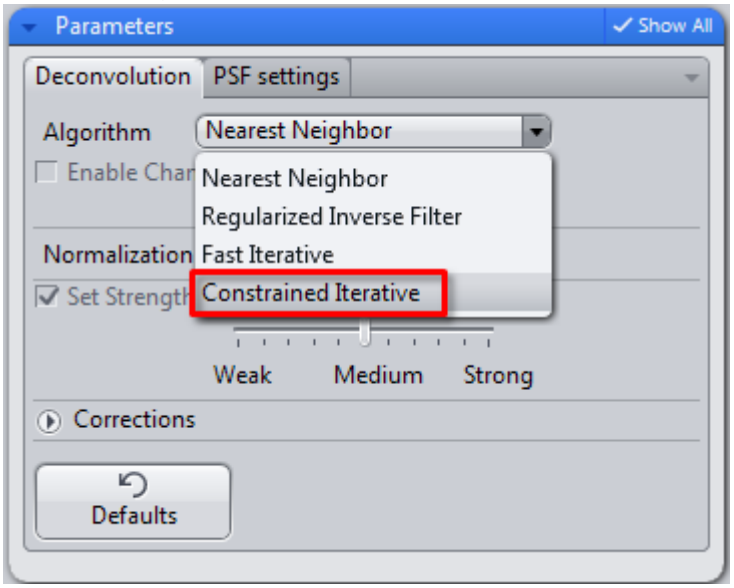
Aim

In this step you will set the desired algorithm and the associated method parameters. You will also assign the PSF created in **Step 2** to the image. Lastly, you will perform deconvolution and save the result.

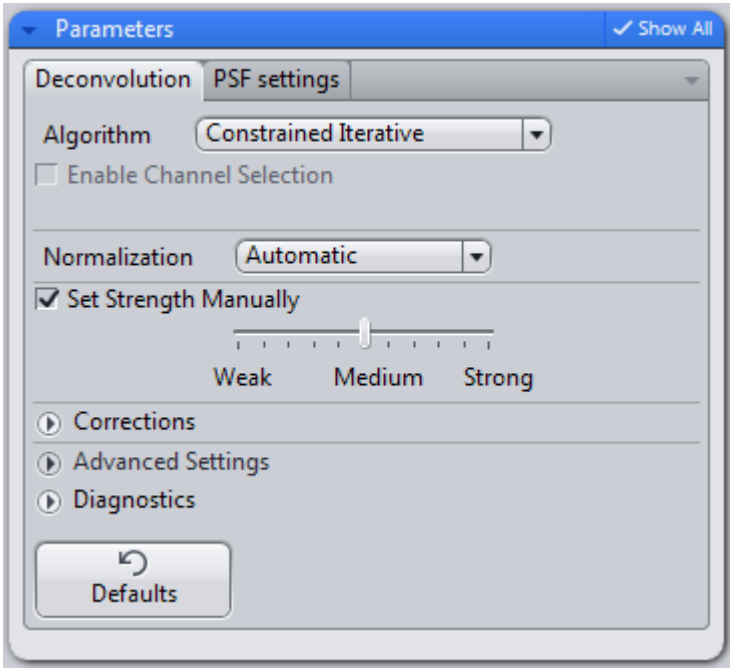
Prerequisites ■ On the **Processing** tab you have selected the **Parameters** tool in the **Method Parameters** group.



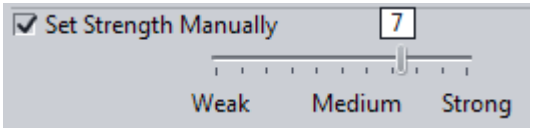
Procedure **1** On the **Deconvolution** tab first set the desired algorithm. In our example we are using Iterative, the most complex algorithm, but the best one to use for wide-field images.



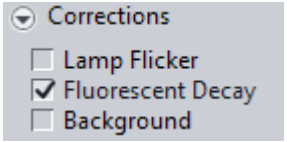
You will now see a number of additional parameters appear on the tab (**Show All** mode).



2 Set the desired image restoration strength. Using this slider you can determine how strong the restorative effect will be. The stronger this is, however, the greater the risk that image noise will also be intensified and that data will appear over-restored. With the Iterative algorithm it is usually possible to set a strength of 6-8 when working with good-quality images before any artifacts are formed.



3 Under **Corrections** select the Bleaching Correction function. It always makes sense to do this if the fluorescent dye bleaches during acquisition of the Z-stack.



The other corrections should only be used when necessary. Details on this can be found in the online help.

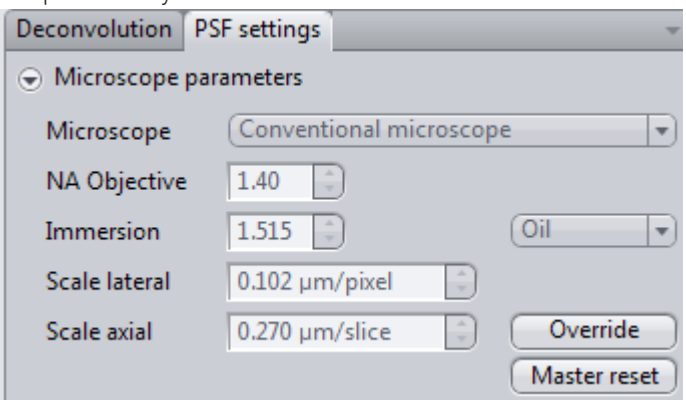
4 Open the **Diagnosis** section (**Show All** mode). Keep this section in the foreground during processing. You will find this useful, as it is the best way to visualize the progress of the processing.

Note

The **Advanced Settings** section contains expert functions that are not normally necessary. Please bear in mind that if incorrect settings are made here, this can lead to unexpected effects, such as extremely long calculation times. Details on the individual parameters can be found in the online help.

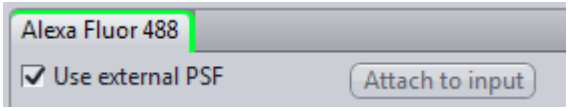
5 Go to the **PSF Settings** tab.

In the **Microscope Parameters** section you will see all the parameters that were used during acquisition and saved with the image. If one of the parameters is missing, the corresponding input field is active and you can enter the parameter yourself.

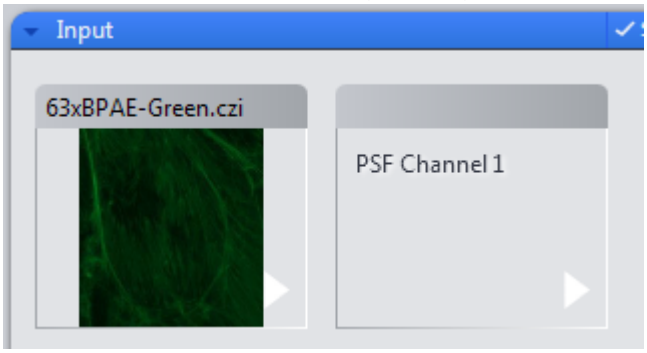


As we want to work with a measured PSF, we will skip the Advanced Settings and aberration correction.

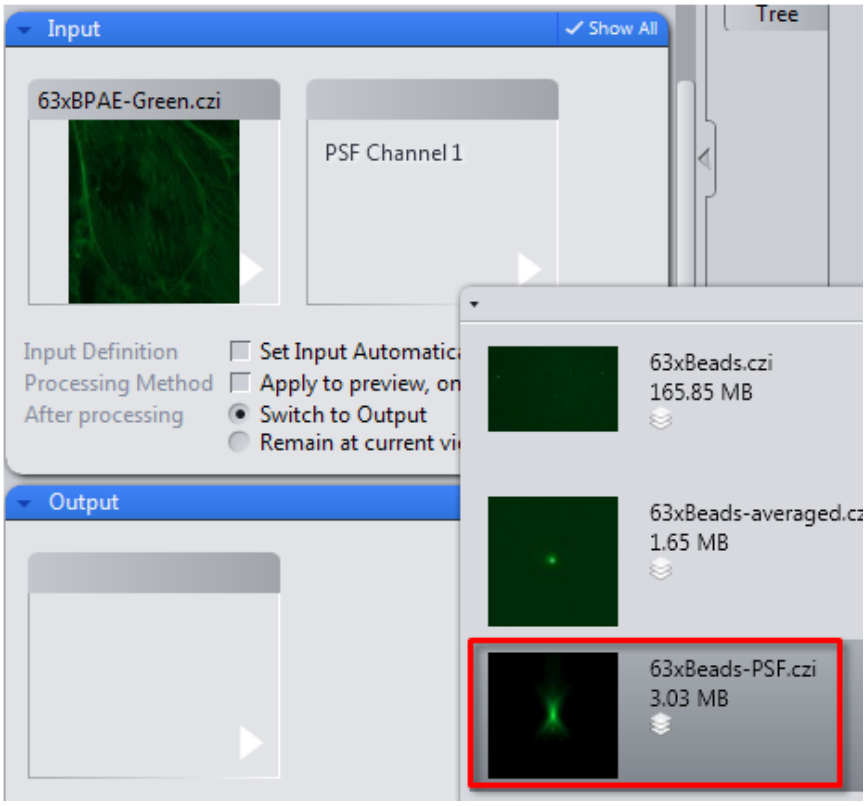
6 Select the **Use External PSF** option here.



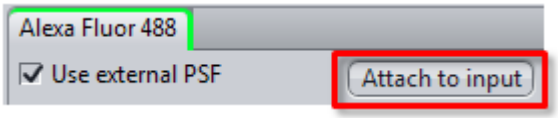
7 Now open the **Input** tool. Here you will see a second input option, **PSF Channel 1**, next to the input image already selected.



8 Select the PSF document here that you created in Step 2.



9 To add the measured PSF permanently to the input image, click on the **Link to Input** button.



10 Now save these changes to the input image. You can now easily process this image on other computers using the correct, measured PSF.

5.2.5 Step 5: Perform deconvolution

Aim

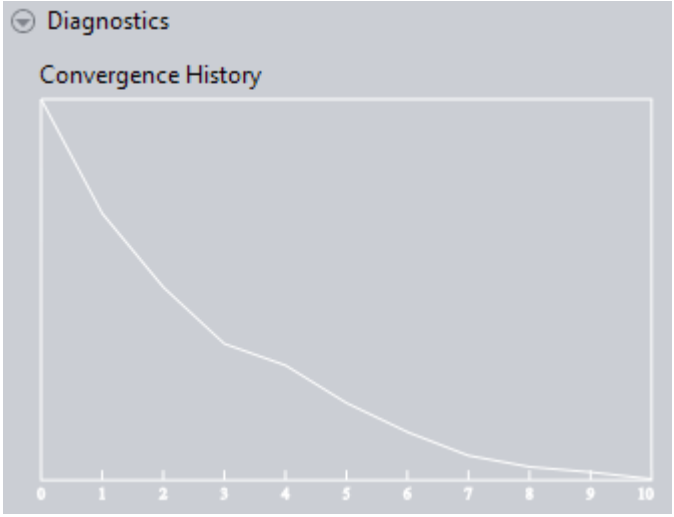
In the last step you will perform deconvolution. The resulting image will be compared with the input image and details relating to the processing procedure will be observed.

Procedure

1 Go to the **Deconvolution** tab in the **Parameters** tool to keep the **Diagnosis** section in the foreground.

2 Click on the **Apply** button.

You can monitor progress in the Diagnosis window, in which the progress of convergence is plotted against the iterations.

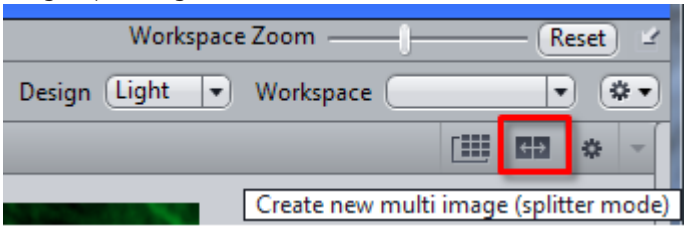


Note

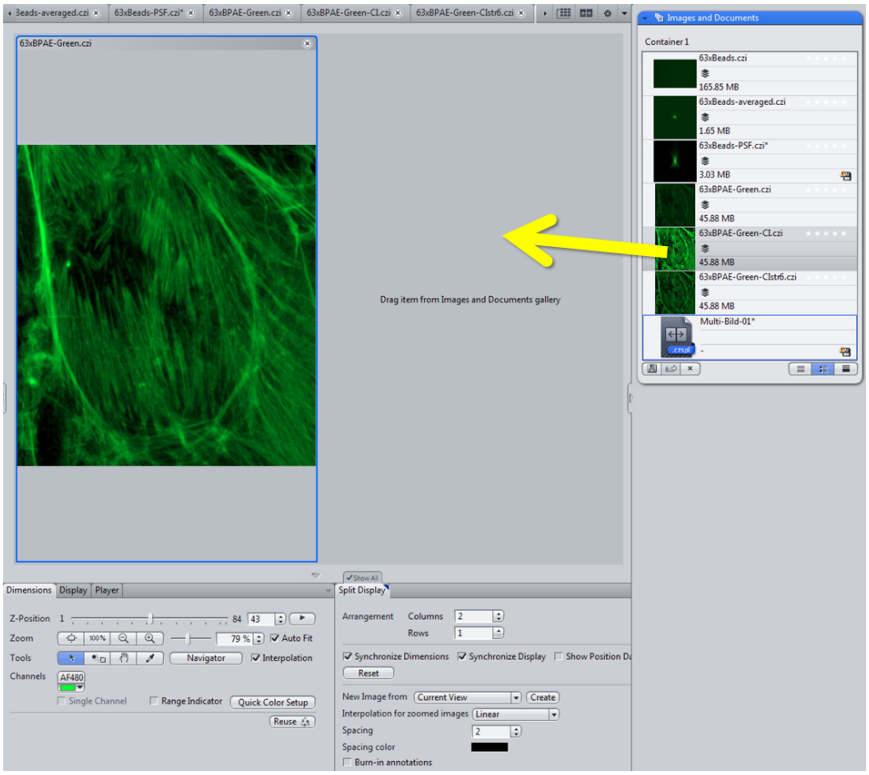
The processing of particularly large Z-stack images or long time series can take some time. During processing we recommend that you do not perform any other complex actions in ZEN or in other programs on the computer, to avoid increasing the processing time unnecessarily.

3 Save the resulting image under a meaningful name.

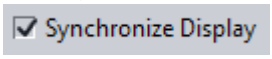
4 Click on the **Split Mode** button in the document bar. This creates a multiple image (split image).



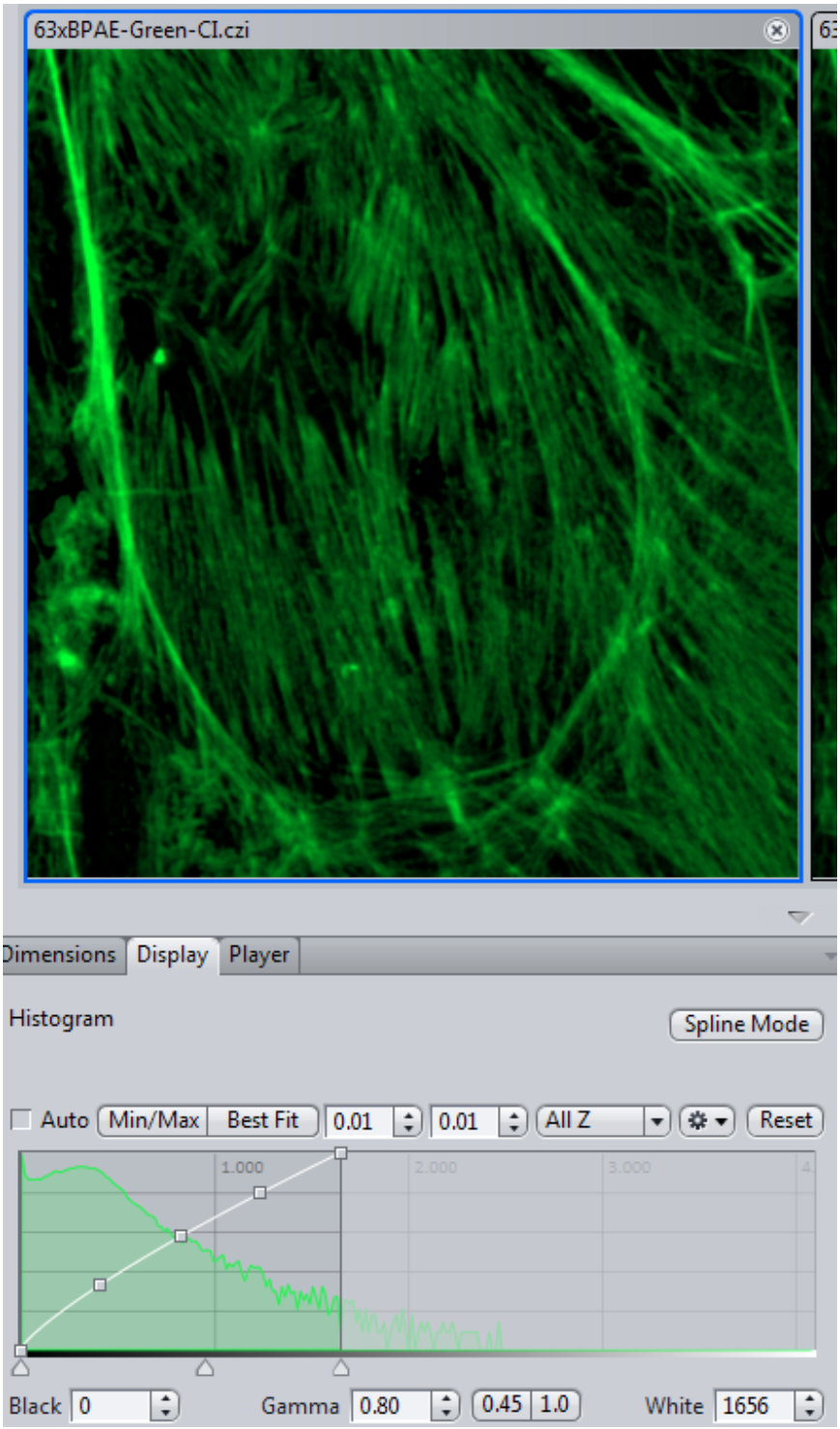
5 In **Splitter** mode the currently active image is loaded automatically. Now drag the input image from the document gallery in the Right Tool Area into the split document.



6 On the **Split Display** tab deactivate the **Synchronize Display** option. This allows you to define a separate histogram setting for each image.



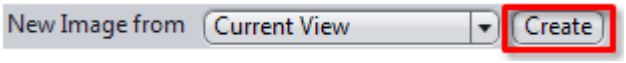
7 Click in the image that you want to change and adjust the display as desired, e.g. **Best Fit** with 0.01 and for black and white value with a **Gamma** of 0.8.



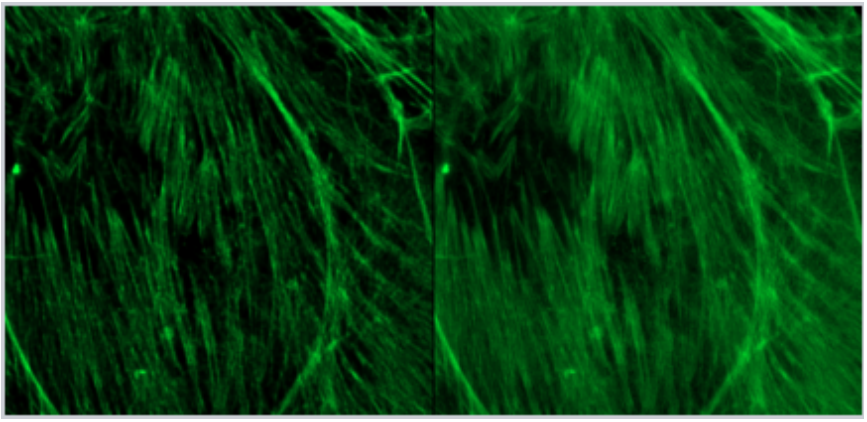
8 Repeat this setting for the other image.

9 If the **Synchronize Dimensions** option is activated, you can now zoom synchronously into the images (mouse wheel) and, with the mouse wheel held down, move the image content as desired to concentrate on regions of interest.

10 To create an image of the desired view click on the **Create** button.



This creates a new output image document with both images shown side by side.

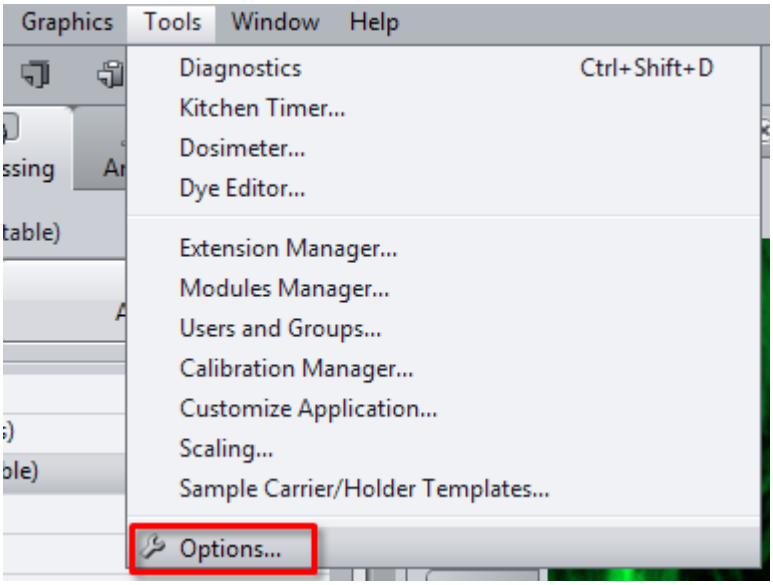


You have successfully performed deconvolution, observed the processing procedure and created an output image to compare processing results.

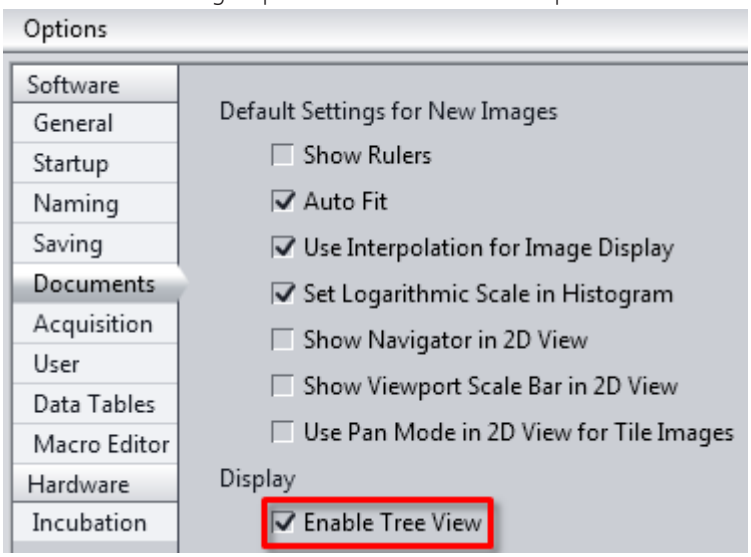
5.2.6 Step 6: Activate and use the Tree View

Lastly, we want to call up information on the duration of the image calculation.

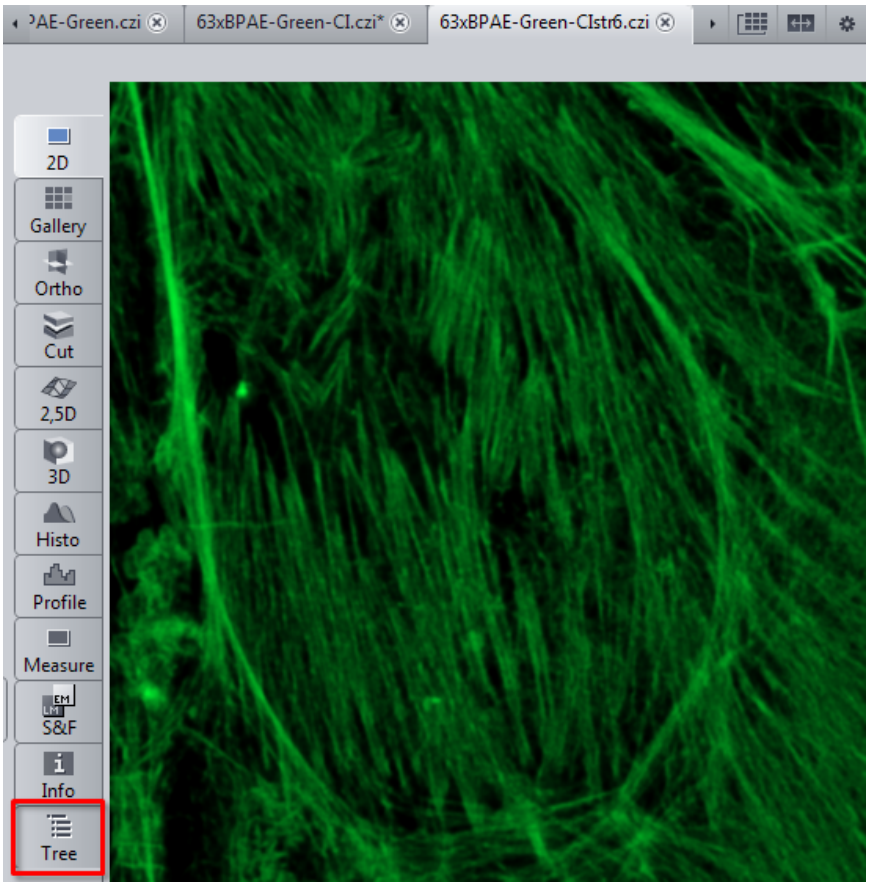
Procedure **1** Open the menu **Extras** | **Options**.



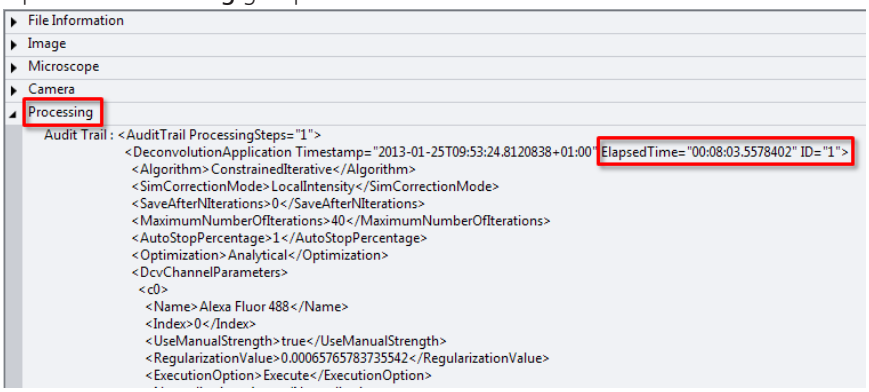
2 In the **Documents** group activate the **Tree View** option.



3 Now bring the processed image into the foreground and switch to the **Tree View**.



4 Open the **Processing** group.



Here, in addition to all the settings that were used to perform deconvolution, you can also see the calculation time that was required. This information is helpful for estimating how long the calculation would take for several images, for example.

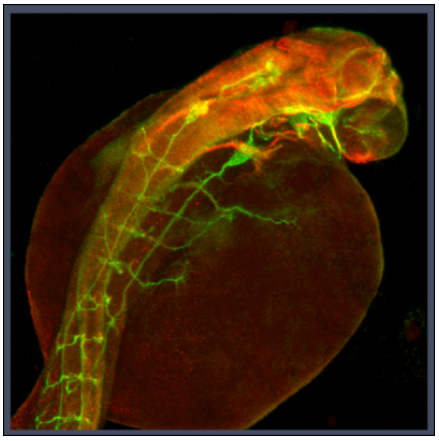
You have successfully displayed advanced information relating to your deconvolved image.

5.3 Extracting individual fluorescence images of a multichannel image

In this topic we will show you how to extract individual fluorescence channel images of a multichannel image.

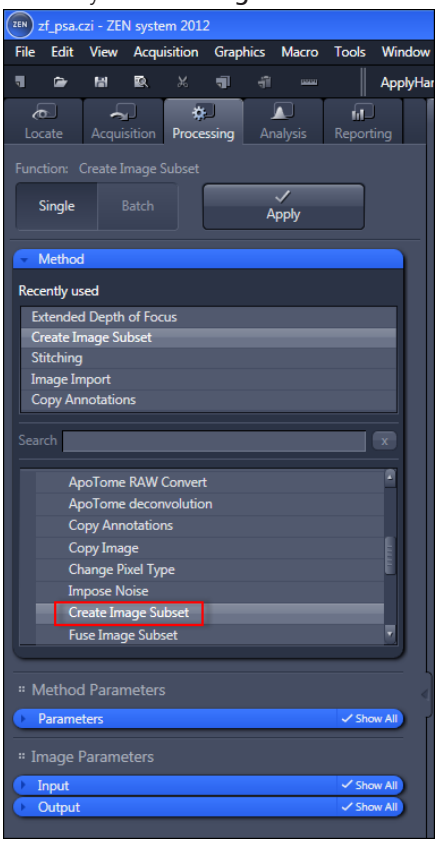
Prerequisites

- You have acquired or opened a multichannel image (for example product DVD 40 (09/2011)-(ZEN)\Example Image Databases\zf_psa.czi).

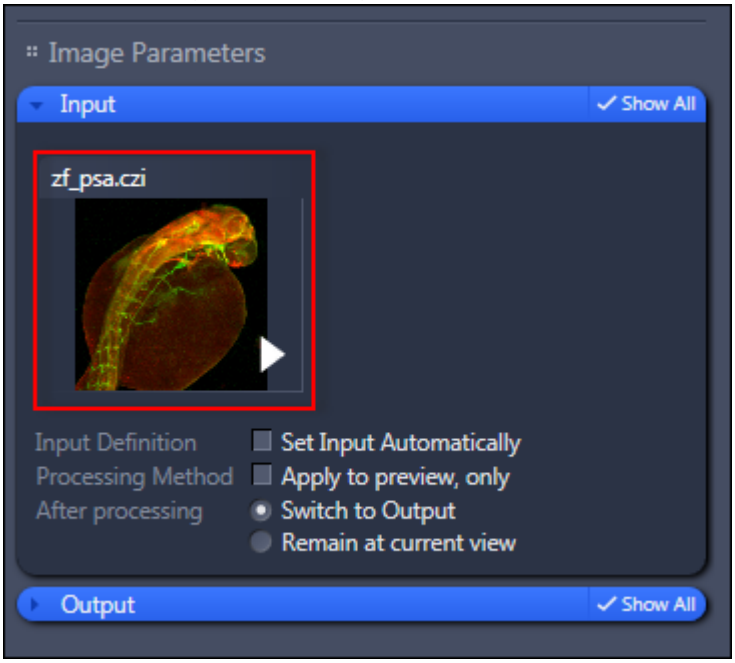


Procedure

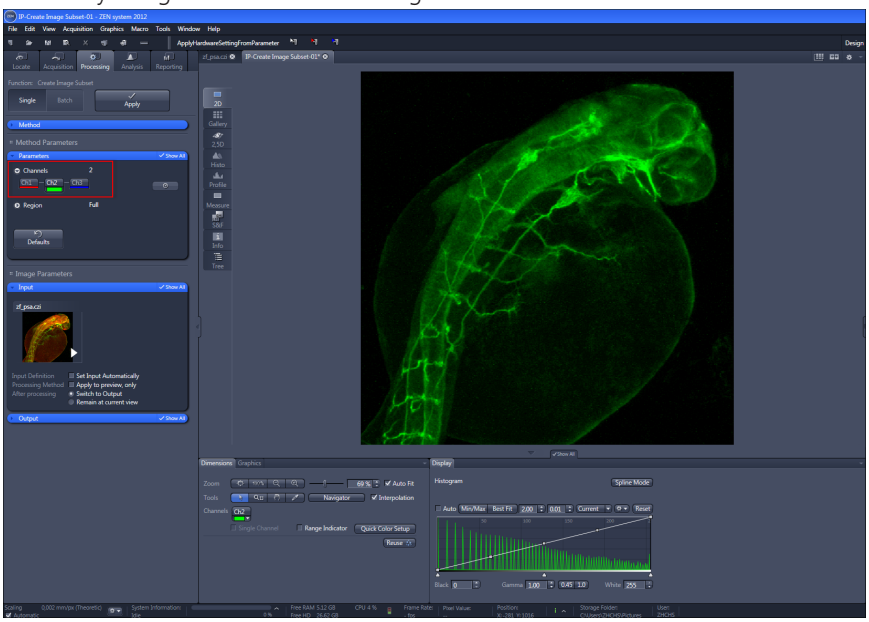
1 Select the **Processing** tab. Open the **Method** tool and select under **Utilities** the entry **Create Image Subset.**



2 In the section **Image Parameters** open the **Input** tool and select the multichannel image as Input image.



3 In the section **Method Parameters** open the **Parameter** tool and the select the entry **Channels**. Deactivate the red and the blue fluorescence channel. Now only the green fluorescence image is selected.



4 Click on the **Apply** button.

You have the green fluorescence channel image extracted of the Multi-Channel image. Activate the corresponding channel and deactivate the other channels to extract further individual fluorescence images. Save the extracted sub images as separate files.

5.4 Using Batch Processing for Image Export

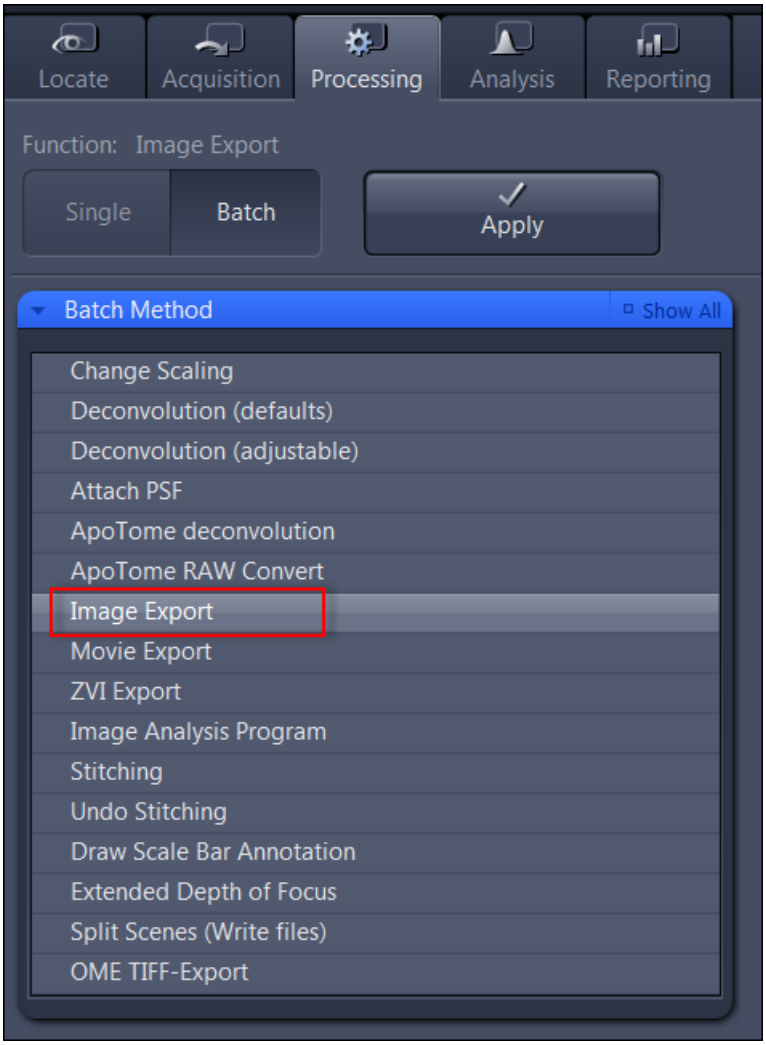
In this topic we will show you how to export all images of a folder as batch. For each image you can use identical or different export settings.

Prerequisites

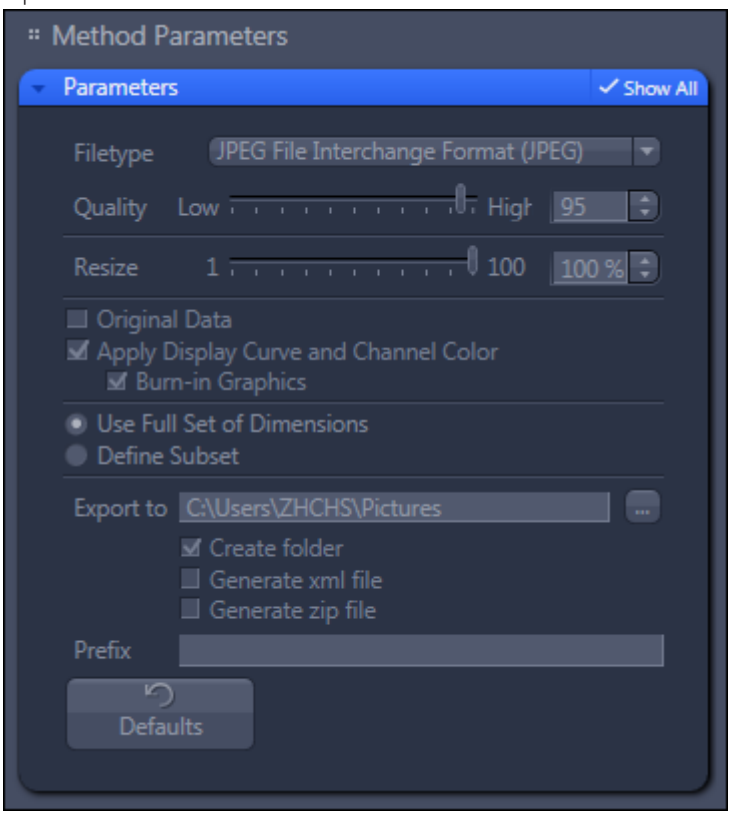
- You have a folder with several CZI-images to be exported in a new image type (ie. TIFF, BMP, JPEG). For example two 2channel-Timelapse images, one 2channel-Z-stack image, one 3Channel image of the product DVD 40 (09/2011)-(ZEN)\Example Image Databases\CaRationSeries.czi, kaede5lsm.czi, ratbrainstack.czi, zf_psa.czi).

Procedure

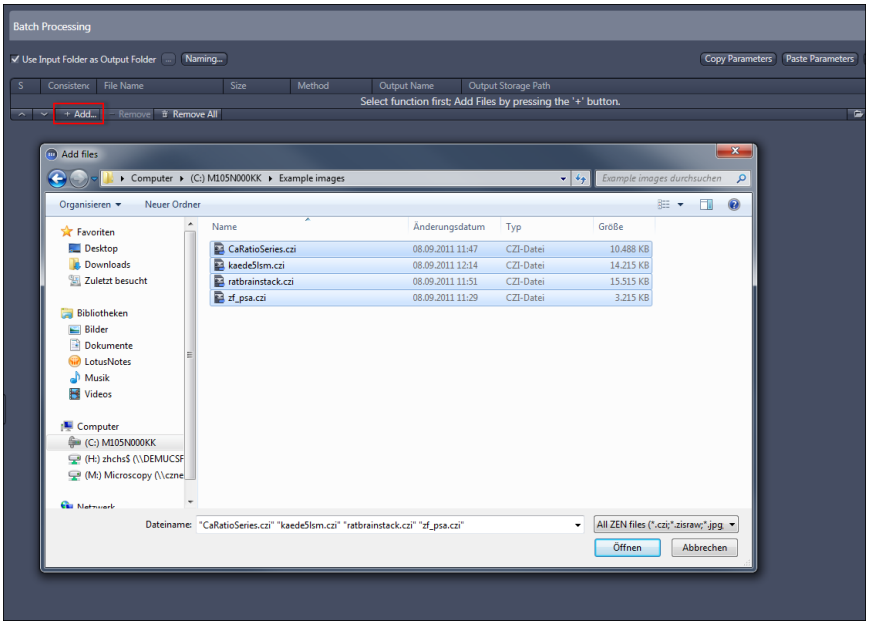
1. Select the **Processing** tab. Click on the **Batch** button.
2. Open the **Method** tool and select the entry **Image Export**.



3 Open in the **Method Parameters** section the **Parameters** tool.

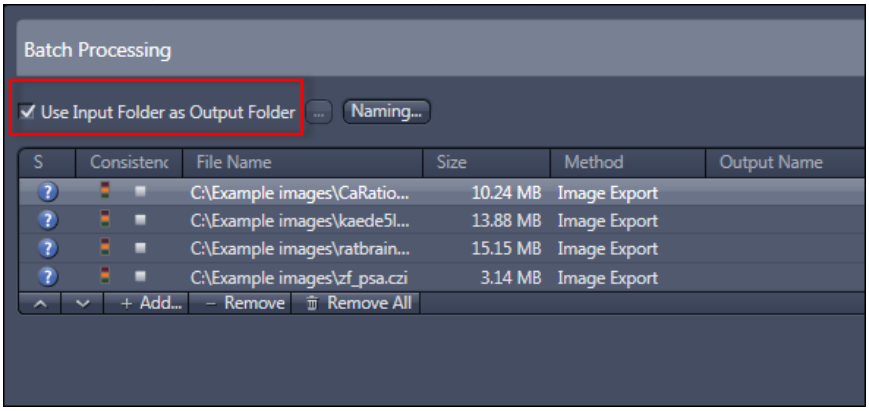


4 Click in the tool **Batch Processing** on the **Add** button. Select the folder and marked all images to be exported and click on the **Open** button.



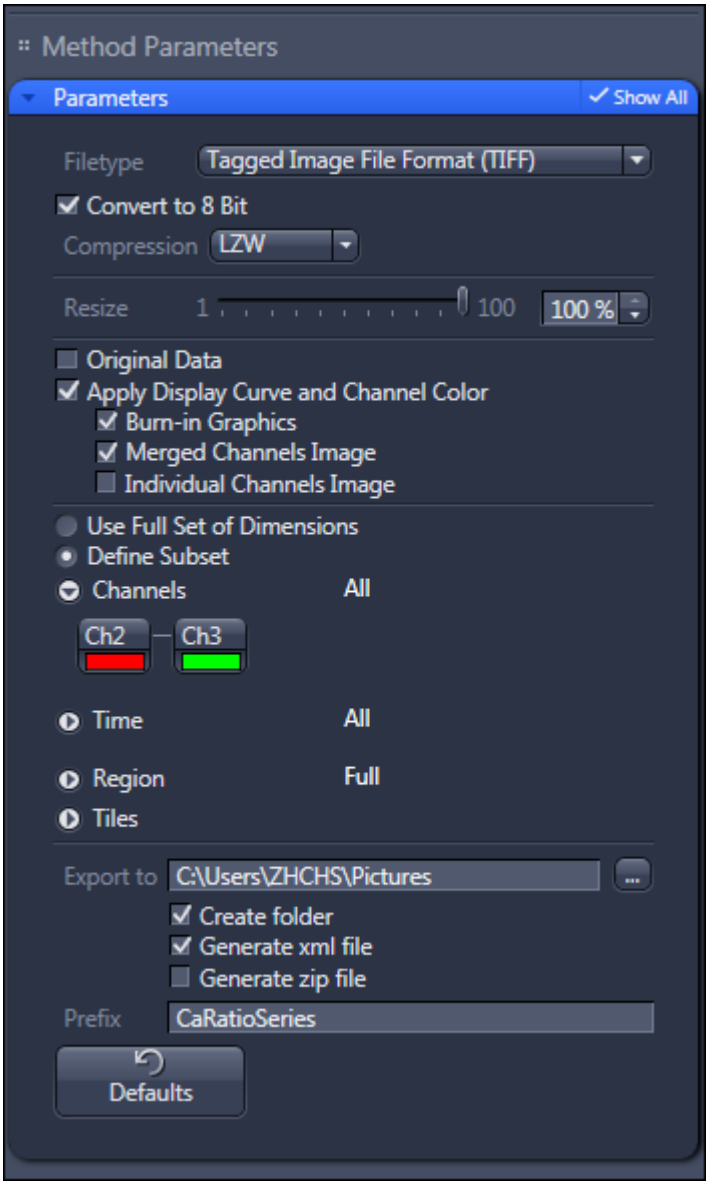
The list of images is displayed in the **Batch Processing**.

5 Activate the checkbox **Use Input Folder as Output Folder** to save the exported images in the folder of the original images.



6 Click on one image in the list and do the export settings in the **Parameters** tool. In the example the TIFF format will be used for all time points and channels. This setting is only valid for the selected image, but can be copied to

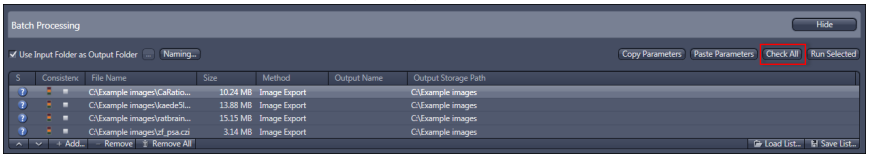
further images in the list with identical dimensions (i.e. 2channel Time-Series image).



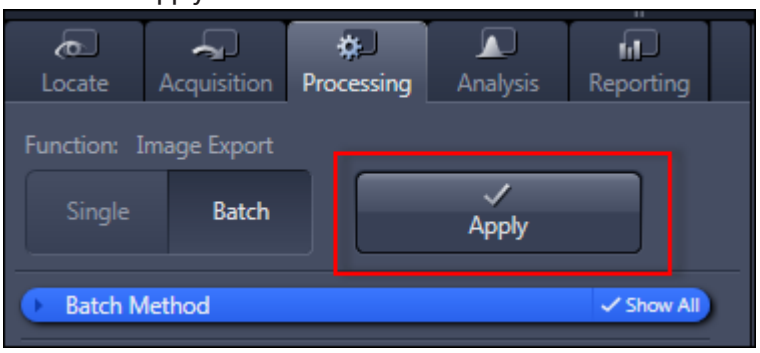
7 Click on the **Copy Parameters** Copy Parameters button. Select the image with identical dimensions in the list and click on the **Paste Parameters** Paste Parameters button.

8 Continue with the other images of the list. Use **Copy Parameters** and **Paste Parameters** or define the export settings for each image individually in the **Parameters** tool.

9 Click on the **Check All** button. All images in the list will be tagged with a green marker, if they have the correct setting.



10 Click on the **Apply** button.



All exported images in the list will be checked and are exported in separate folders in the Input folder.

5.5 Cropping region of interest (ROI)

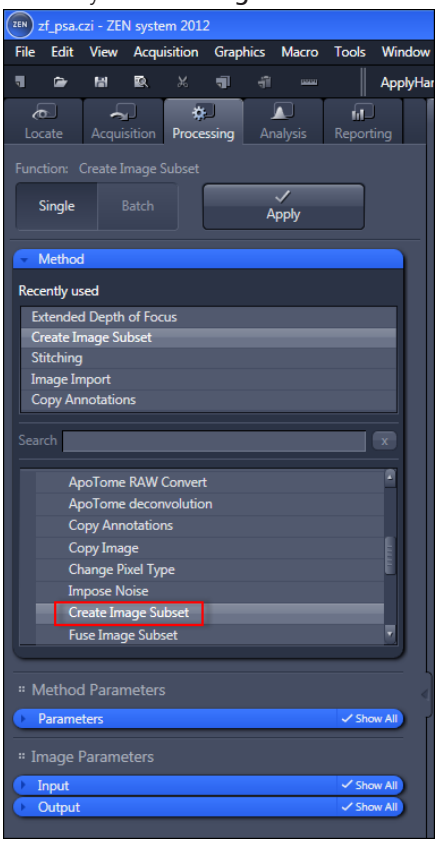
In this topic we will show you how to crop a region of interest (ROI) of a image.

Prerequisites

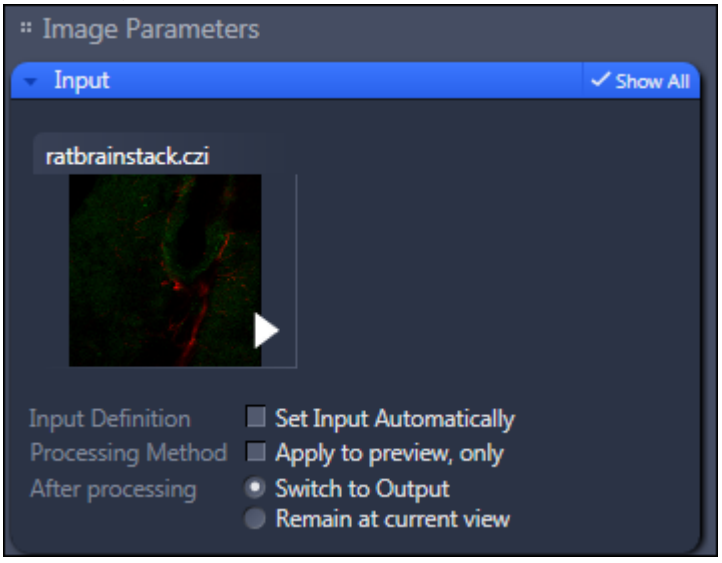
- You have acquired or opened a multichannel image.

Procedure

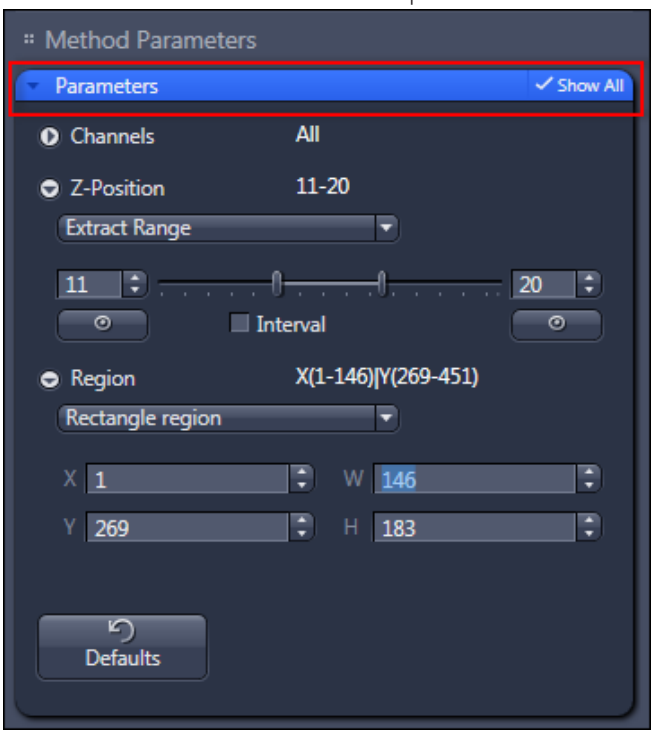
1 Select the **Processing** tab. Open the **Method** tool and select under **Utilities** the entry **Create Image Subset**.



2 In the **Image Parameters** section open the **Input** tool and select the image.



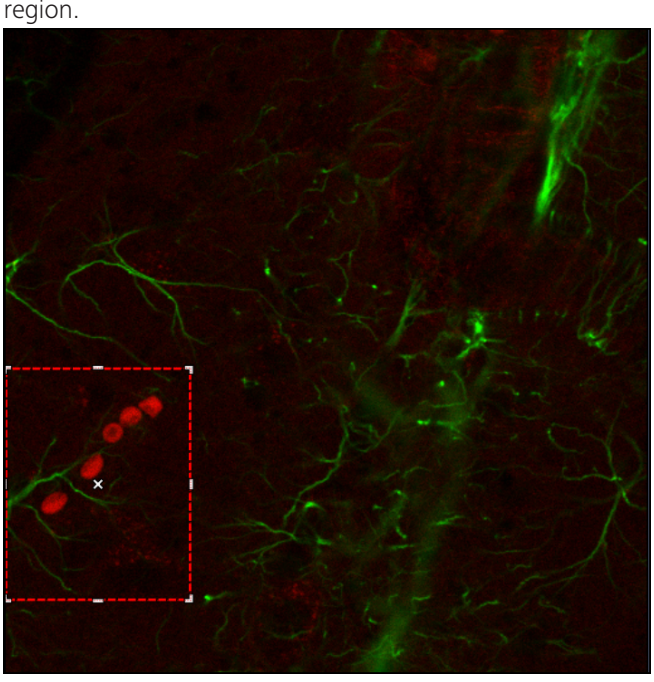
3 In the **Method Parameters** section open the **Parameters** tool.



4 Open the **Z-position** dimension setting and select **Extract Range** of the dropdown list. Set the start position to **11** and the end position to **20**using the slider.

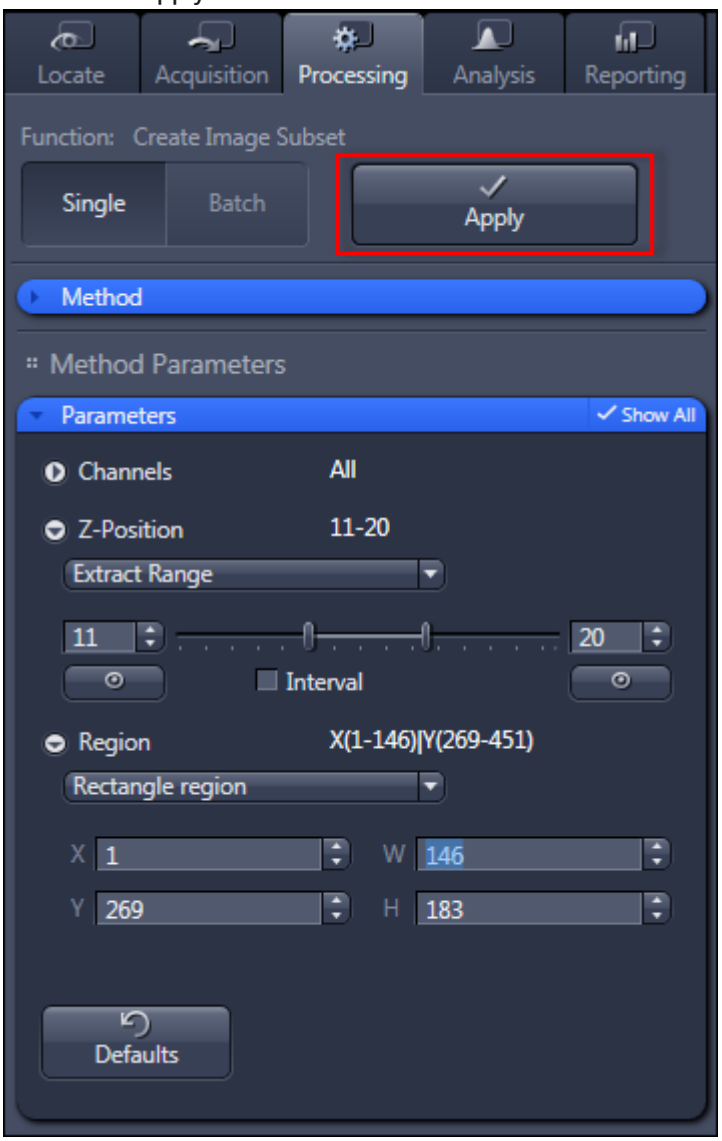
5 Open the **Region** setting and select **Rectangular Region** of the dropdown list.

6 Click in the image on the start position of the ROI and drag out a rectangular region.



You have marked the ROI which should be cropped.

7 Click on the **Apply** button.



The marked ROI with the defined dimensions is extracted of the image. You can now save or process this image.

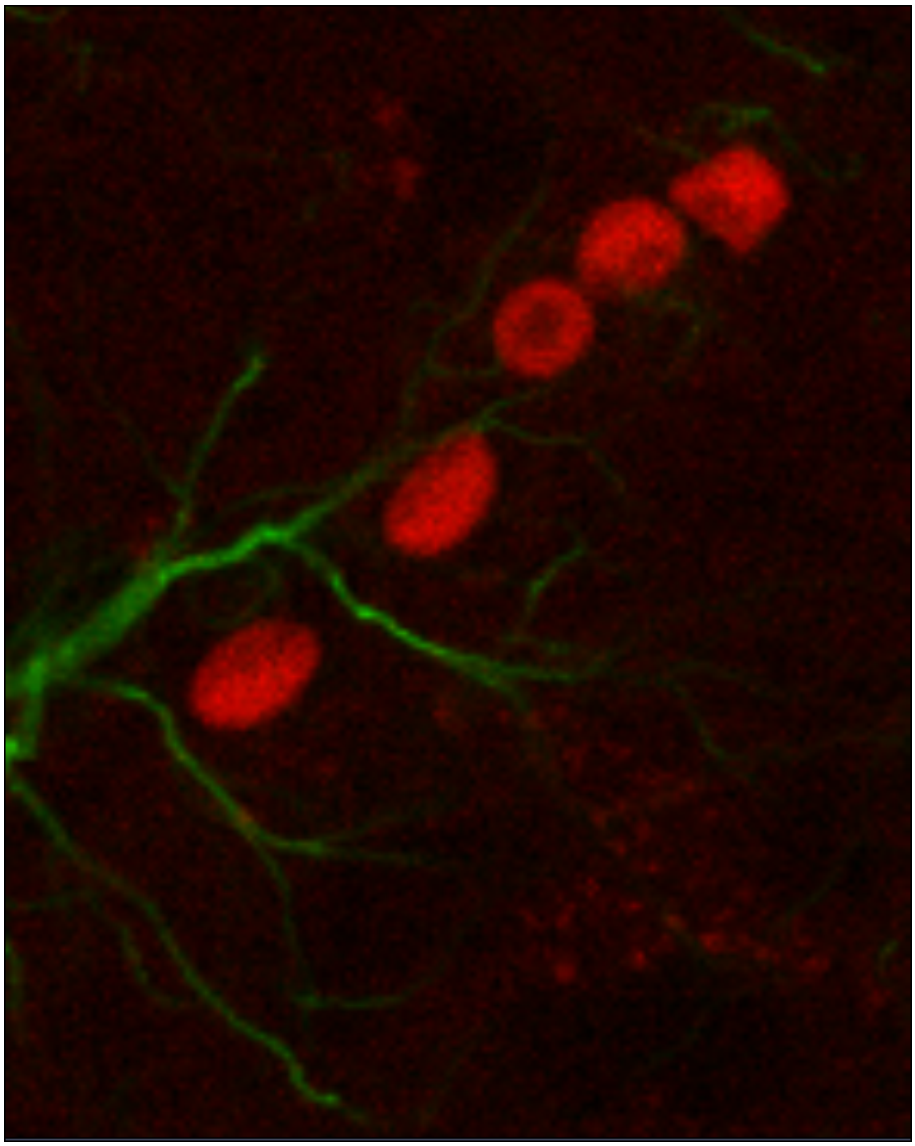


*Fig. 13: Cropped ROI with the dimensions **All channels** and the **Z-Postion 11-20**.*

5.6 Creating an Extended Depth of Focus image of a Z-Stack image

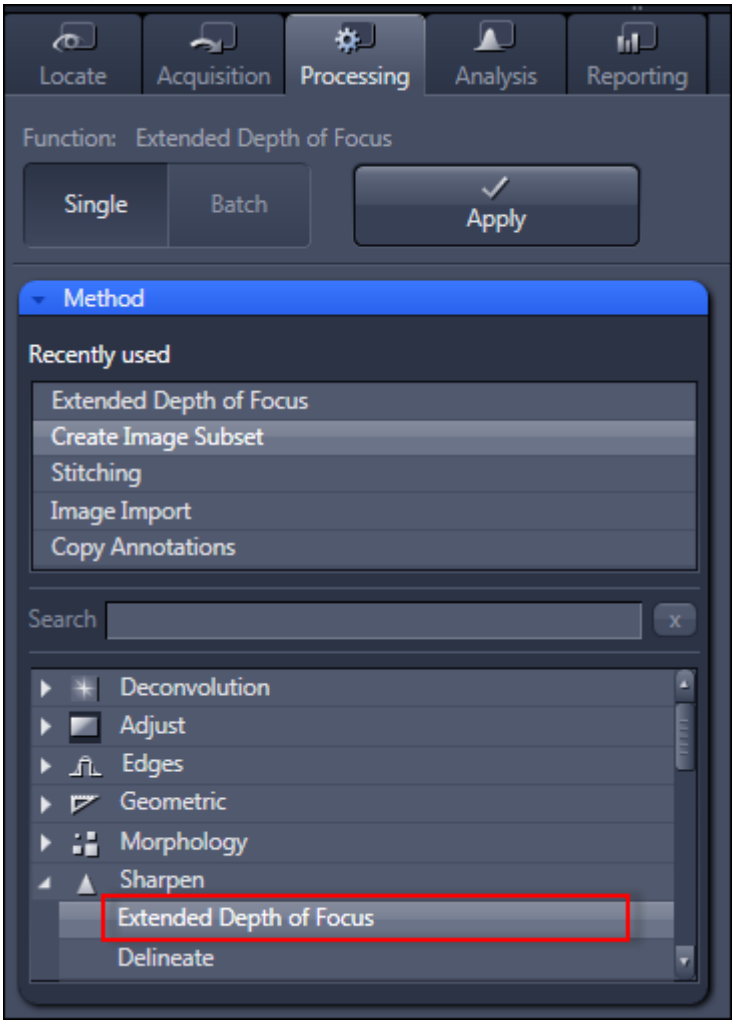
In this topic we will show you how to create an extended depth of focus (EDF) image of a Z-Stack image. The focus planes of all the z-positions will be calculated to one EDF image.

Prerequisites

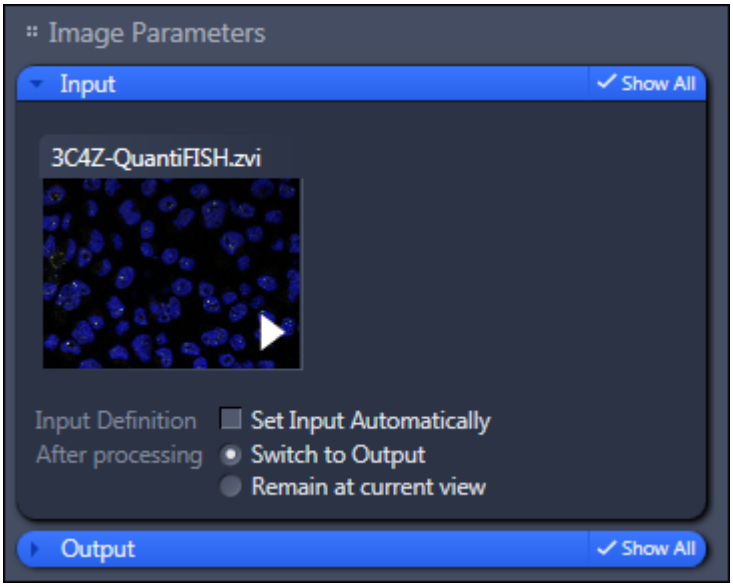
- You have acquired or opened a z-stack image.

Procedure

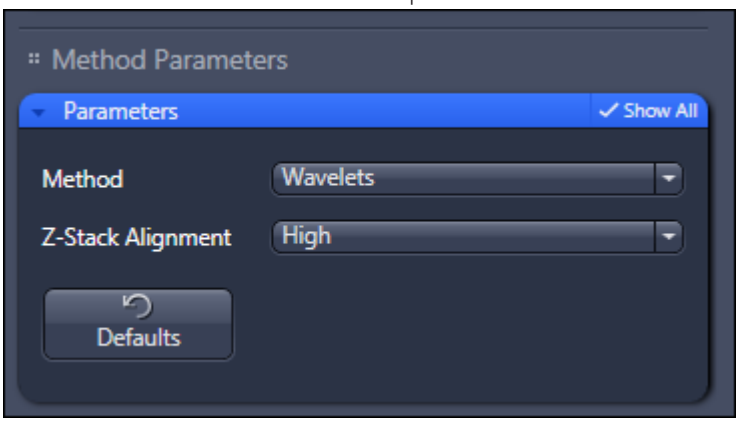
1 Select the **Processing** tab. Open the **Method** tool and select under **Sharpen** the entry **Extended Depth of Focus**.



2 In the **Image Parameters** section open the **Input** tool and select the Z-Stack image as Input image.



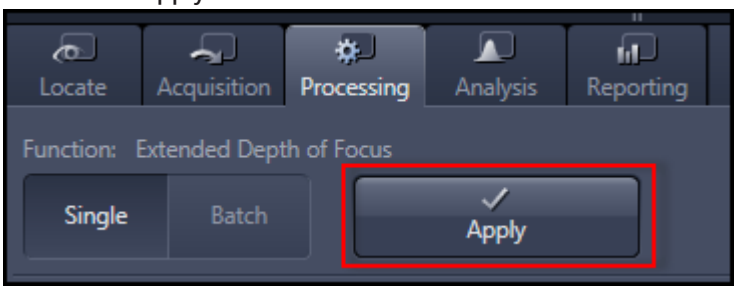
3 In the **Method Parameters** section open the **Parameters** tool.



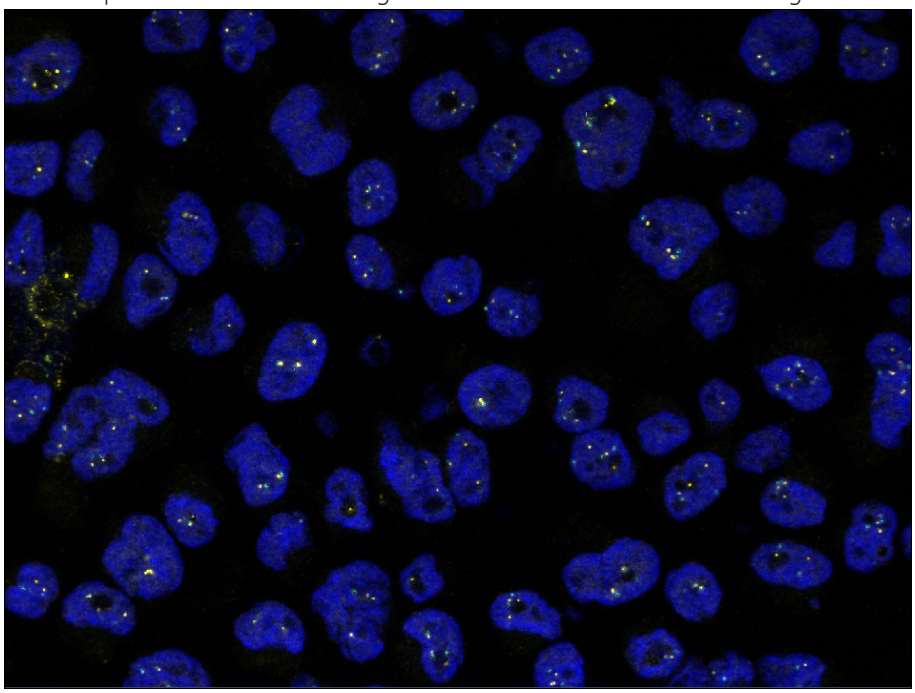
4 Select **Wavelets** of the **Method** dropdown list.

5 Select **High** of the **Z-Stack Alignment** dropdown list.

6 Click on the **Apply** button.



All focus planes of the Z-Stack image are now calculated to one EDF image.



6 Image Analysis

6.1 Measuring fluorescence intensity in a multichannel image

Introduction

This topic will show you how to set-up a measurement program using the Image Analysis Wizard. After this the program will be used to measure fluorescence intensity in a multichannel image. In this example we are using a multichannel image with 2 channels (1st channel blue, DAPI / 2nd channel red, mRFP1) of fluorescence-stained cells. First we detect the blue-stained cell nuclei in the first channel. Then we measure the fluorescence intensity for both channels in this channel.

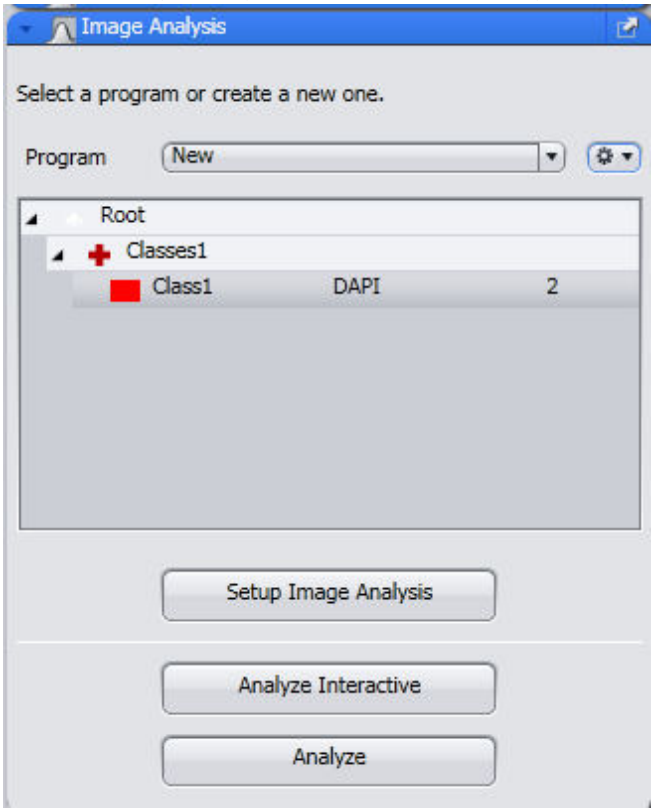
Set-up a measurement program using the Image Analysis Wizard

Prerequisites

- You have opened the multichannel image.
- You have selected the **Analysis** tab.

Procedure

1. Open the **Image Analysis** tool and start the **Image Analysis Wizard**.



2. Open the **Options** menu.
3. Click on the **New** entry and enter a name for your measurement program.

4 Click on the **Save** button to save the program.

5 Click on the **Setup Image Analysis** button.

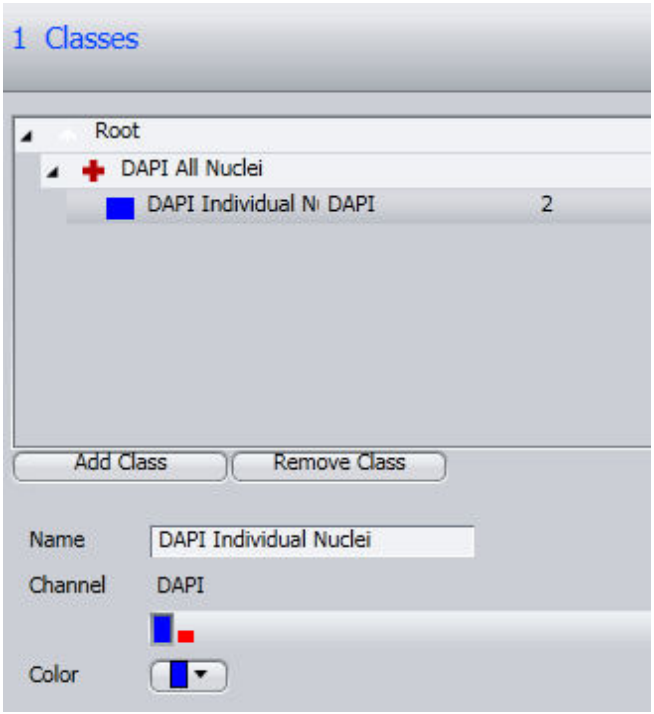
The **Image Analysis Wizard** is opened. In the left-hand area you will see 7 steps which will guide you through the wizard.

Step 1: Classes

Procedure

1 Click on the **Class1** entry in the list and enter **Name DAPI Individual Nuclei** in the input field.

2 Click on **Classes1** in the list and enter **Name DAPI All Nuclei** in the input field.



3 Click on **Next**.

Step 2: Measurement frame

Procedure

1 Deactivate the **Interactive** checkbox.

2 Click on **Next**.

Step 3: Automatic segmentation

Procedure

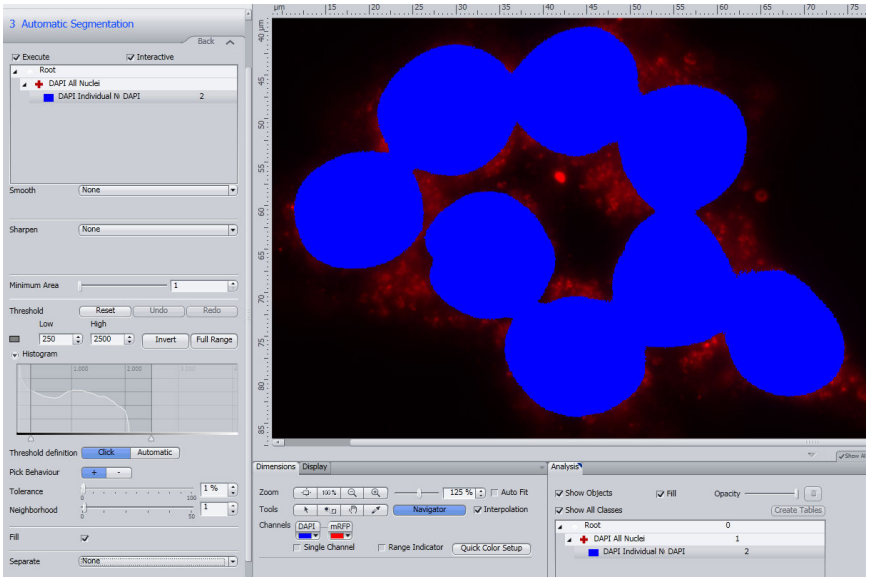
1 Click on the **DAPI Individual Nuclei** entry in the list.

The segmentation parameters (**Smoothing**, **Image Sharpness**, **Minimum Area**, etc.) are displayed below the list.

2 In the **Threshold** section set the **Tolerance** parameter to 1%.

3 Click in the image on the blue-stained cell nuclei.

The detected nuclei are overlaid in blue. The threshold values are displayed in the **Threshold** section in the **Lower** / **Upper** input fields.



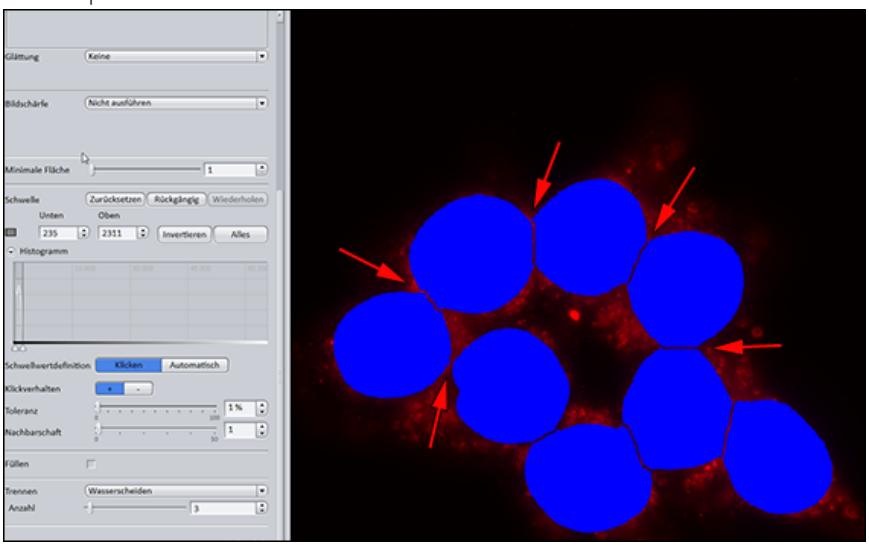
4 Click on the areas of the blue cell nuclei that have not yet been detected until these have been completely overlaid.

5 Activate the **Fill** checkbox.

This fills any holes in the detected cell nuclei.

6 Select the **Watersheds** entry from the dropdown list in the **Separate** section and set the number to 3.

Clear separation lines are now visible between the cell nuclei.



7 Click on **Next**.

Step 4: Condition

Procedure 1 Deactivate the **Run** checkbox.

2 Click on **Next**.

Step 5: Interactive segmentation

Procedure

1 Deactivate the **Interactive** checkbox.

2 Click on **Next**.

Step 6: Measurement features

Procedure

1 Click on the **Edit** button in the **Object Features** section.

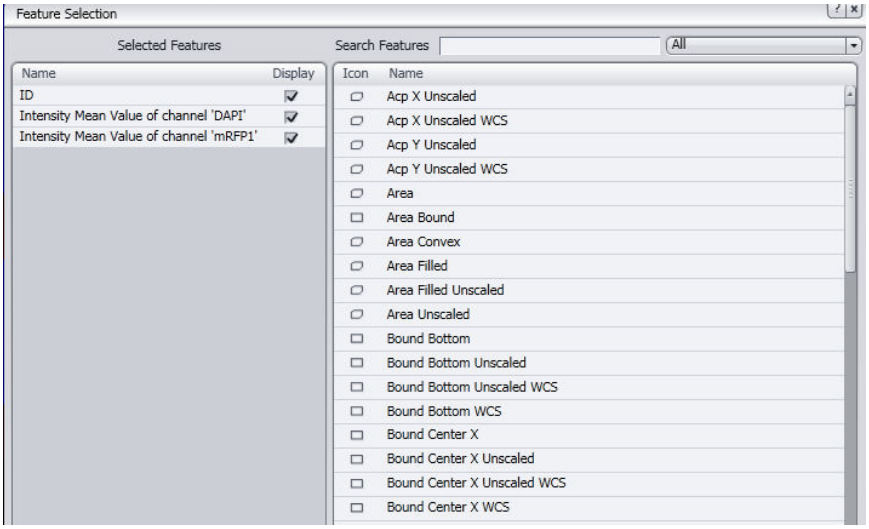
The **Feature Selection** dialog is opened.

2 Double-click in the right-hand list on the **Object ID**, **Average Intensity of 'DAPI' Channel** and **Average Intensity of 'mRFP1' Channel** features, one after another.

The features are displayed in the **Selected Features** list on the left.

3 Remove superfluous features (e.g. Area, Perimeter) from the list. Select the feature and click on the **Delete** button .

4 Activate the **Display** checkbox for the features.



5 Click on the **OK** button.

The selected features are displayed in the **Object Features** section.

6 Click on **Next**.

Step 7: Measurement

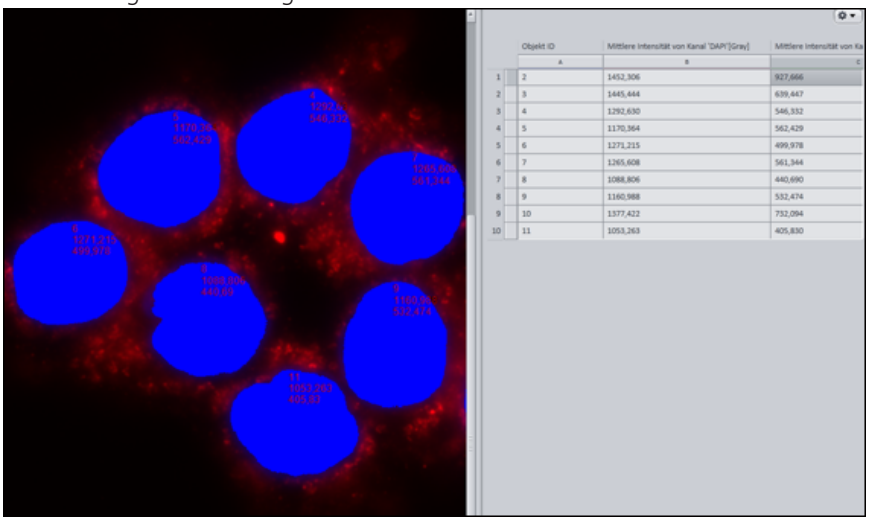
Procedure

1 Click on **DAPI All Nuclei** in the list.

The number of measured cell nuclei is displayed in the data table to the right of the image.

2 Click on **DAPI Individual Nuclei** in the list.

The object ID and the values for the average fluorescence intensities per channel are displayed in the image at the cell nuclei in question and in the data list to the right of the image.



3 Click on the **Finish** button.

This saves the measurement program.

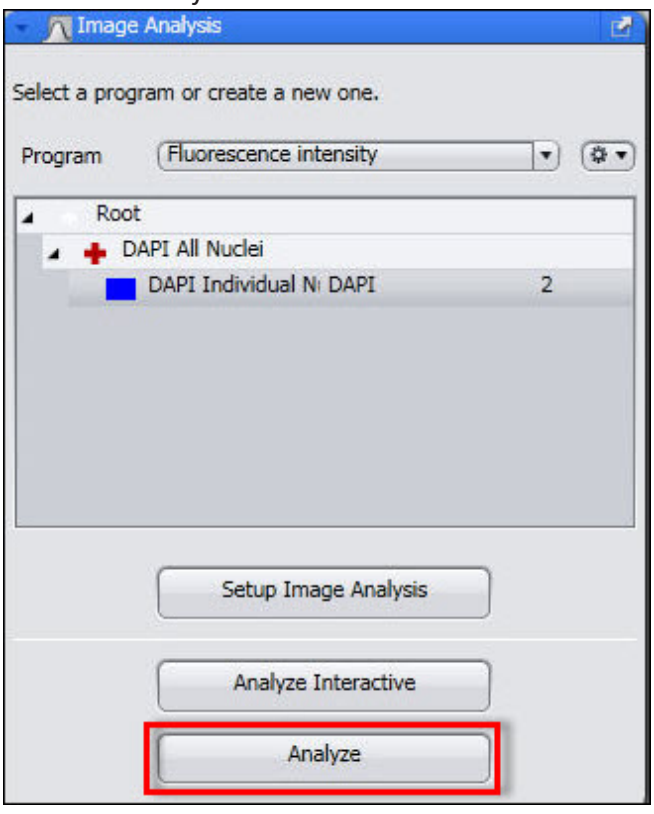
Executing the measurement program

Prerequisites

- You are in the **Image Analysis** tool.
- You have loaded the measurement program that you have generated.

Procedure

1 Click on the **Analyze** button.

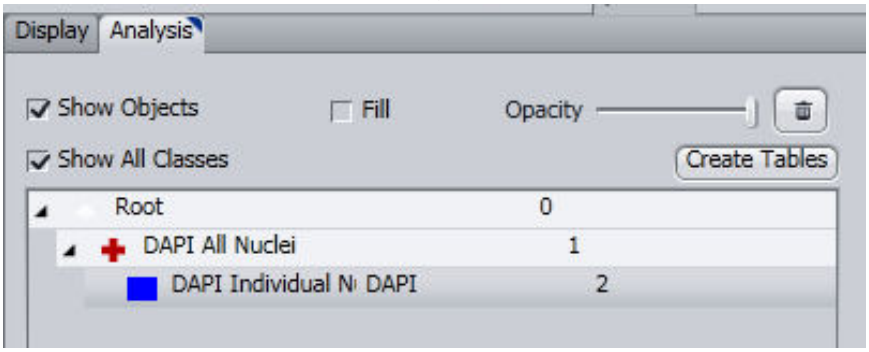


The measurement program is applied to the image.

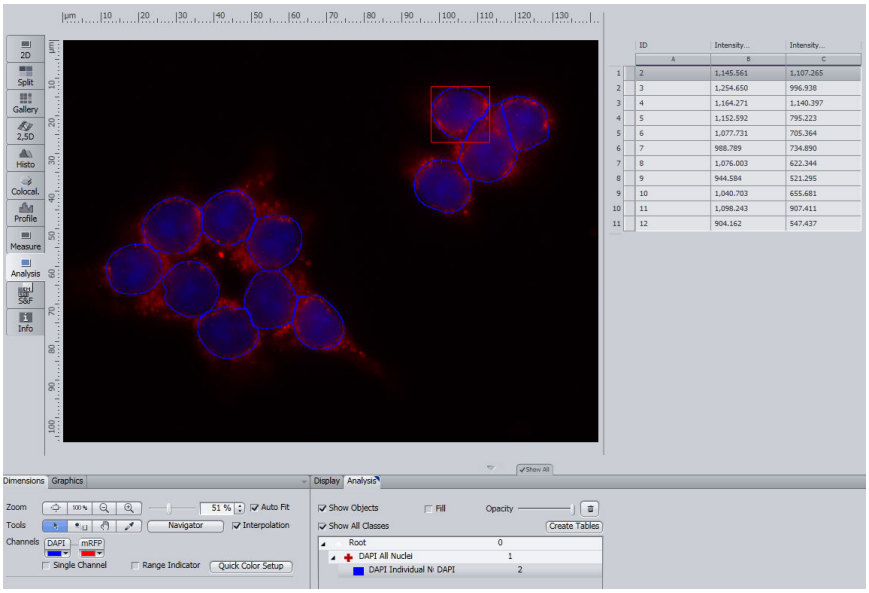
The **Analysis View** now also appears in the **Center Screen Area**.

2 In the **Analysis View** you will see your image with the measured cell nuclei overlaid in blue and, to the right of this, the data list containing the individual measurements.

3 Deactivate the **Fill** checkbox in the **Analysis** control element.



The selected cell nuclei are displayed as contours.



4 Click on a row in the data list or alternatively on a cell nucleus in the image.

The row in the data list containing the measurement values is highlighted. The associated cell nucleus is surrounded by a red rectangle.

Note

There is a direct link between the measured cell nuclei in the image and the measured values in the data table. You can either click on a measured cell nucleus in the image or on a row in the data table.

Creating a measurement data table

Procedure

1. Click on the **Create Measurement Data Table** button on the **Analysis** tab.

   The two data lists are now separate documents and have been removed from the image.

2. Save each of the data lists via the **File** menu | **Save As**. Allocate a name and select .**csv** as the file type.

   The measurement data tables are saved in **CSV** format and can therefore be opened directly in Excel.

3. Click on the image and save it via the **File** menu | **Save As**. Allocate a name and select .**czi** as the file type.

The image is saved with the measurement results. If you open the image, the measurement results can be viewed in the **Analysis View**.

6.2 Counting number of fluorescence signals per nuclei

Introduction

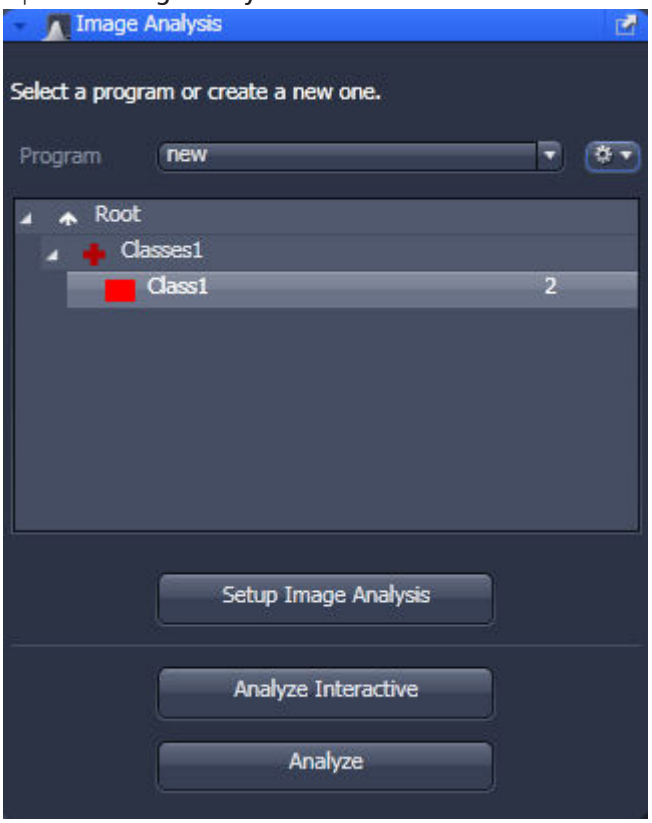
This topic will show you how to set-up a measurement program using the Image Analysis Wizard. After this the program will be used to count the number of fluorescence spots in a multichannel image. In this example we are using a multichannel image with 2 channels (1st channel blue, DAPI / 2nd channel green, GFP) of fluorescence-stained cell nuclei. First we detect the blue-stained cell nuclei in the first channel and then the green stained signals in the second channel. Then we measure the number of green fluorescence signals per nucleus.

Set-up a measurement program using the Image Analysis Wizard

Prerequisites

- You have opened the multichannel image.
- You have selected the **Analysis** tab.

Procedure 1 Open the **Image Analysis** tool .



2 Open the **Options** context menu.

3 Click on the **New** entry and enter a name for your measurement program.

4 Click on the **Save** button to save the program.

5 Click on the **Setup Image Analysis** button.

The **Image Analysis Wizard** is opened. In the left-hand area you will see 7 steps which will guide you through the wizard.

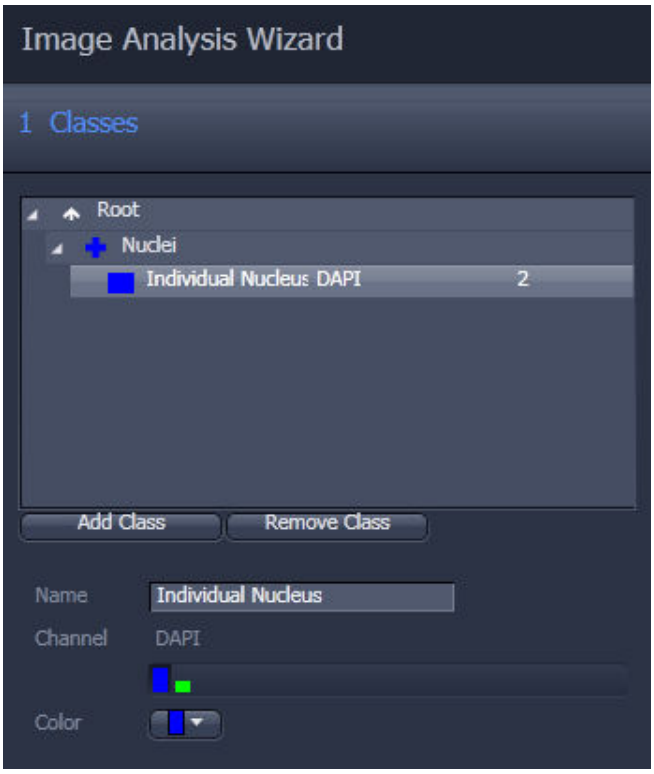
Step 1: Classes

Procedure 1 Click on **Classes1** in the list and enter **Nuclei** in the **Name** input field.

2 Select a blue color from the dropdown list in the **Color section**.

3 Click on the **Class1** entry in the list and enter **Individual Nucleus** in the **Name** input field.

4 Click on the blue channel icon in the **Channel** section .



You have now setup a class pair for the nuclei.

Procedure

1 Click on the button **Add Class**.

2 Click on **Classes3** in the list and enter **Signals** in the **Name** input field.

3 Select a green color from the dropdown in the **Color** section

4 Click on the **Class3** entry in the list and enter **Individual Signal** in the **Name** input field.

5 Click on the green channel icon in the **Channel** section.

You have now setup a class pair (child class) for the signals inside the Individual Nucleus class (parent class).

Procedure 1 Click on **Next**.

Step 2: Measurement frame

Procedure 1 Deactivate the **Interactive** checkbox.

2 Click on **Next**.

Step 3: Automatic segmentation

Procedure 1 Click on the **Individual Nucleus** entry in the list.

The segmentation parameters (**Smooth**, **Sharpen**, **Minimum Area**, etc.) are displayed below the list.

2 In the **Smooth** section select **Gauss** from the dropdown list and set the parameter **Sigma** to **1.5**.

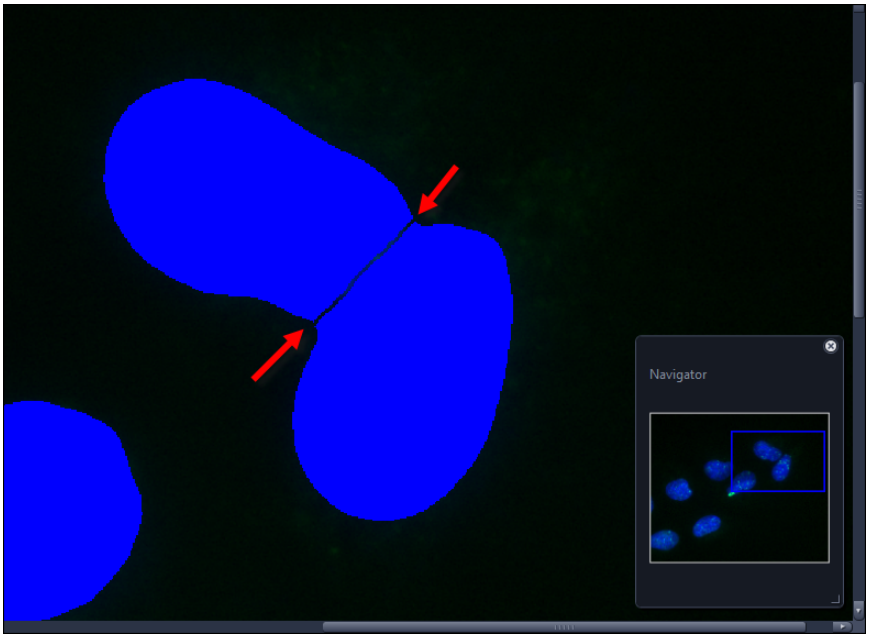
3 Click on the blue-stained cell nuclei in the image.

The detected nuclei are overlaid in blue. The threshold values are displayed in the **Threshold** section in the **Lower** / **Upper** input fields.

4 Click on the areas of the blue cell nuclei that have not yet been detected until these have been completely overlaid.

5 Select the **Watersheds** entry from the dropdown list in the **Separate** section and set the number to 17.

Clear separation lines are now visible between the cell nuclei.



6 Click on the **Individual Signal** entry in the list.

The segmentation parameters (**Smoothing**, **Image Sharpness**, **Minimum Area**, etc.) are displayed below the list.

7 In the **Smoothing** section select **Gauss** from the dropdown list and set the parameter **Sigma** to 1.5.

8 Click in the image on the green-stained signals.

The detected signals are overlaid in green. The threshold values are displayed in the **Threshold** section in the **Lower** / **Upper** input fields.

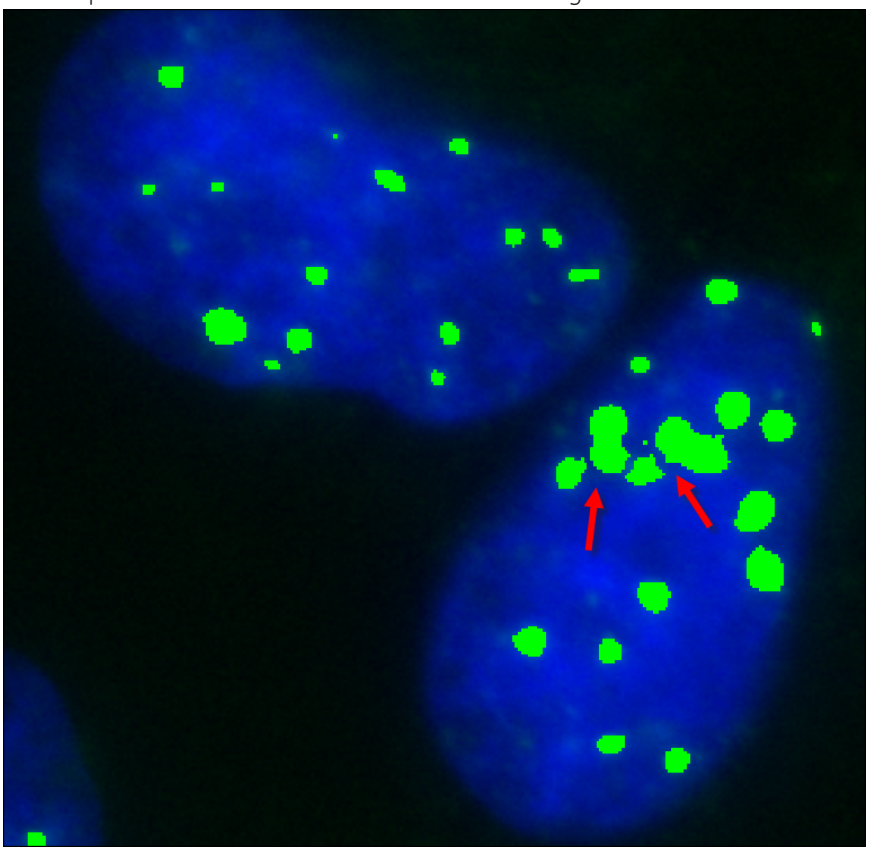
9 Click on the areas of the green signals that have not yet been detected until these have been completely overlaid.

10 Activate the **Fill** checkbox.

11 This fills any holes in the detected signals.

12 In the **Separate** section select the **Watersheds** entry from the dropdown list and set the number to 17.

Clear separation lines are now visible between the signals.



13 Click on **Next**.

Step 4: Condition

Procedure

1 Deactivate the **Run** checkbox.

2 Click on **Next**.

Step 5: Interactive segmentation

Procedure

1 Deactivate the **Interactive** checkbox.

2 Click on **Next**.

Step 6: Measurement features

Procedure

1 Click on the **Nuclei** entry in the list.

2 Click on the **Edit** button in the **Regions Features** section.

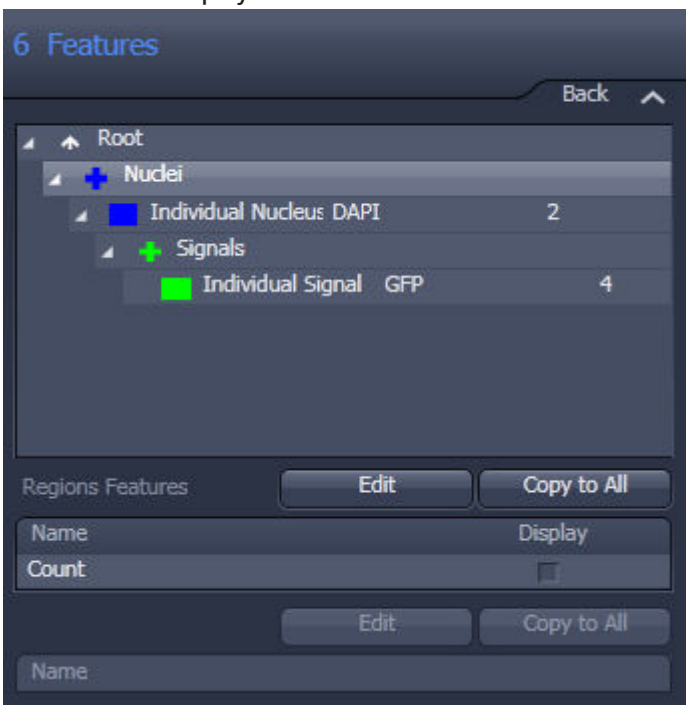
The **Feature Selection** dialog is opened.

3 Double-click in the right-hand list on the **ID**.

The features are displayed in the **Selected Features** list on the left.

4 Remove superfluous features from the list. Select the feature and click on the **Delete** button .

5 Deactivate the **Display** checkbox for the feature.



6 Click on the **Individual Nucleus** entry in the list.

7 Click on the **Select** button in the **Region Features** section.

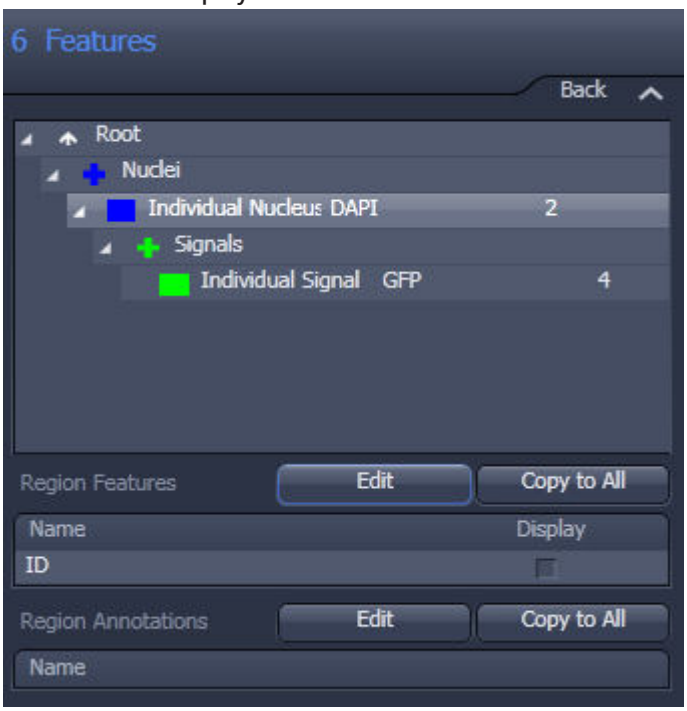
The **Feature Selection** dialog is opened.

8 Double-click in the right-hand list on the **ID of the parent**.

The features are displayed in the **Selected Features** list on the left.

9 Remove superfluous features (e.g. Area, Perimeter) from the list. Select the feature and click on the **Delete** button .

10 Deactivate the **Display** checkbox for the feature.



11 Click on the **Signals** entry in the list.

12 Click on the **Edit** button in the **Regions Features** section.

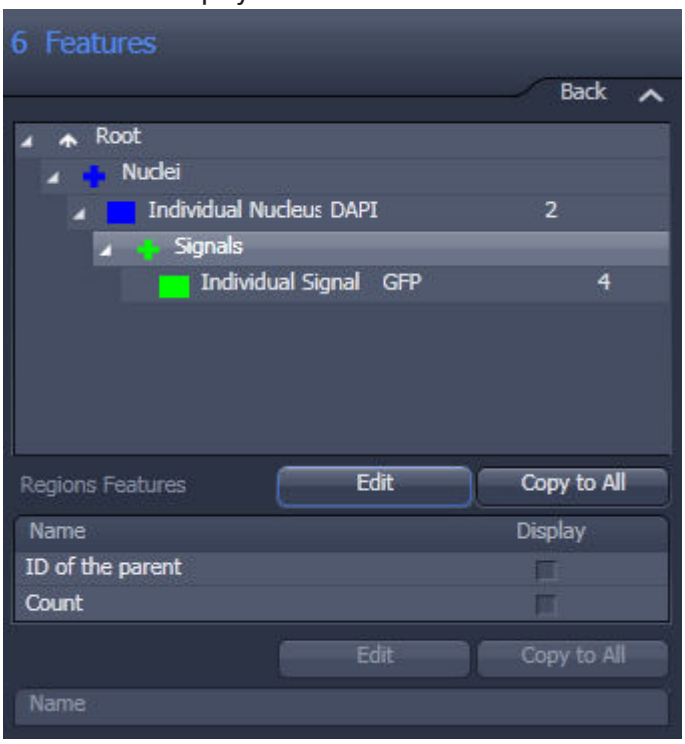
The **Feature Selection** dialog is opened.

13 Double-click in the right-hand list on the **ID, Count**.

The features are displayed in the **Selected Features** list on the left.

14 Remove superfluous features from the list. Select the feature and click on the **Delete** button .

15 Deactivate the **Display** checkbox for the features.



16 Click on the **Individual Signal** entry in the list.

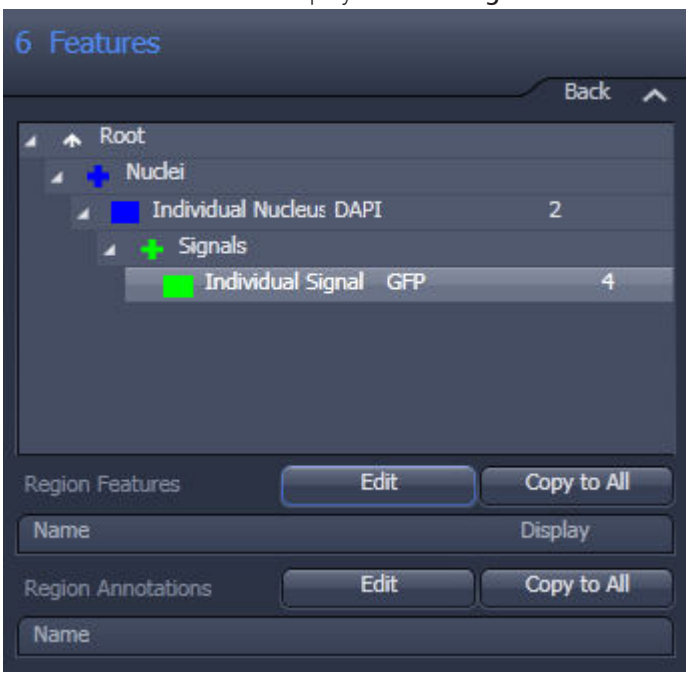
17 Click on the **Select** button in the **Region Features** section.

The **Feature Selection** dialog is opened.

18 Remove superfluous features (e.g. Area, Perimeter) from the list. Select the feature and click on the **Delete** button .

19 Click on the **OK** button.

The selected features are displayed in the **Regions Features** section.

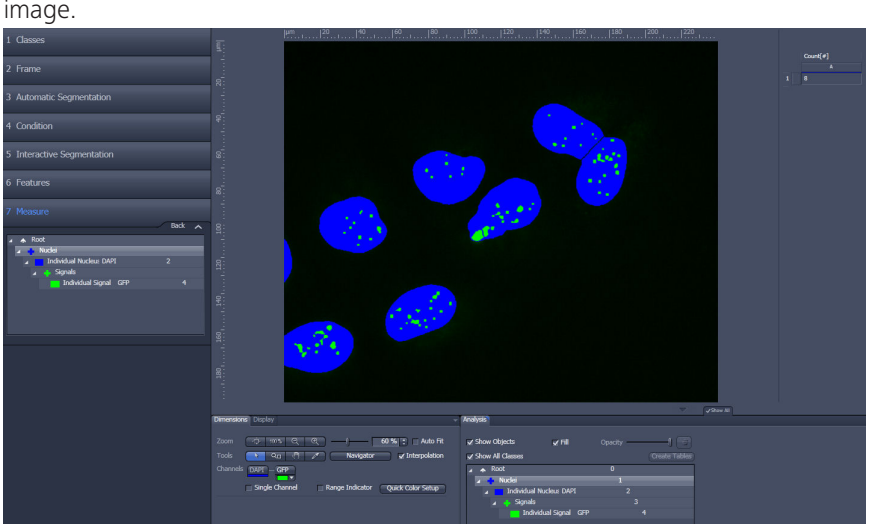


20 Click on **Next**.

Step 7: Measurement

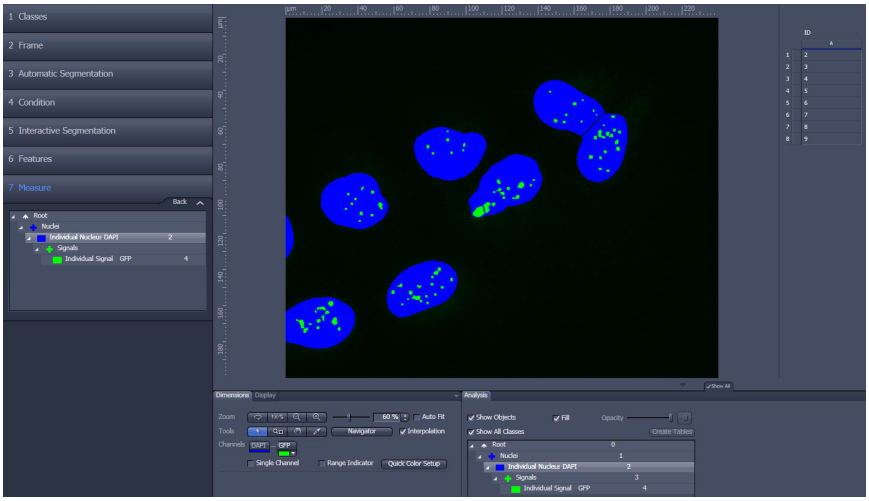
Procedure **1** Click on **Nuclei** in the list.

The number of measured nuclei is displayed in the data table to the right of the image.



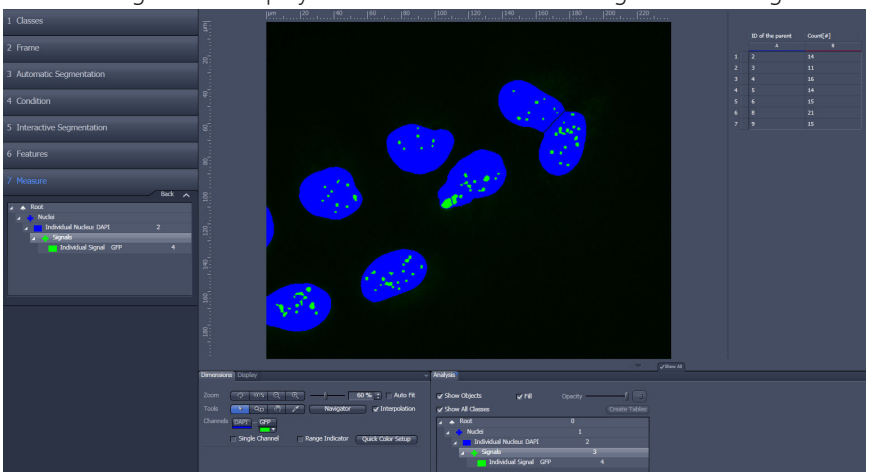
2 Click on **Individual Nucleus** in the list.

The object ID of the measured nuclei is displayed in the data list to the right of the image.



3 Click on **Signals** in the list.

The ID of the parents (corresponds to the ID of the nucleus) and the number of measured signals are displayed in the data table to the right of the image.



4 Click on the **Finish** button.

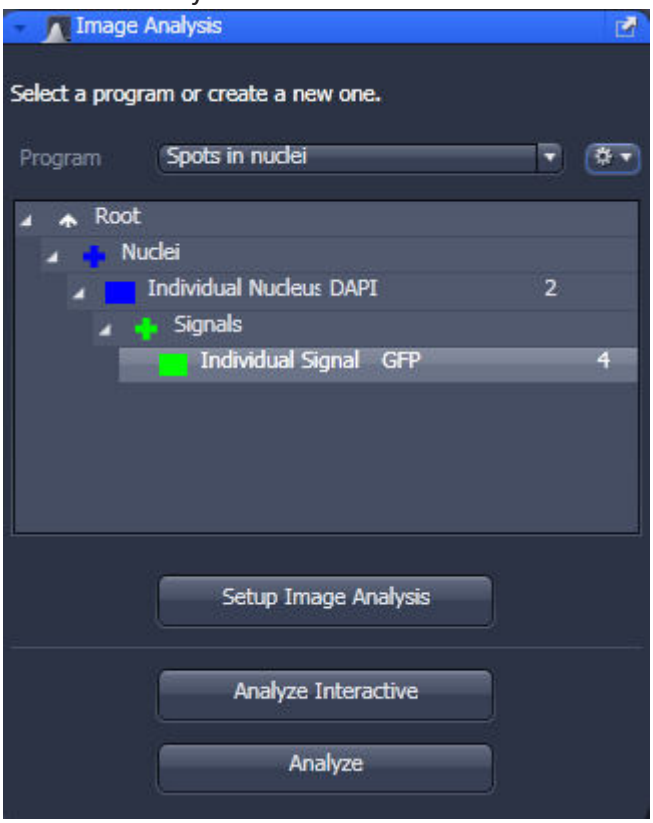
This saves the measurement program.

Executing the measurement program

Prerequisites

- You are in the **Image Analysis** tool.
- You have loaded the measurement program that you have generated.

Procedure **1** Click on the **Analyze** button.

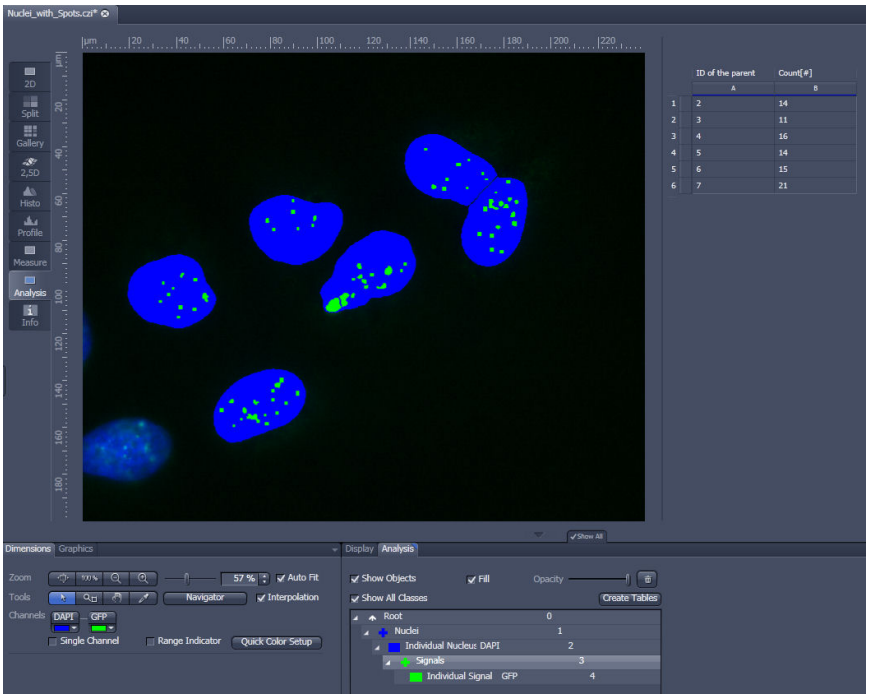


The measurement program is applied to the image.

The **Analysis View** now also appears in the **Center Screen Area**.

In the **Analysis View** you will see your image with the measured cell nuclei overlaid in blue and the signals overlaid in green. Right of this, the data list containing the number of signals per nucleus.

2



Only the number of signals of measured nuclei is displayed. Nuclei touching the frame are not taken into account.

Creating a measurement data table

Procedure

1 Click on the **Create Measurement Data Table** button on the **Analysis** tab.

The two data lists are now separate documents and have been removed from the image.

2 Save each of the data lists via the **File** menu | **Save As**. Allocate a name and select .**csv** as the file type.

The measurement data tables are saved in **CSV** format and can therefore be opened directly in Excel.

3 Click on the image and save it via the **File** menu | **Save As**. Allocate a name and select .**czi** as the file type.

The image is saved with the measurement results. If you open the image, the measurement results can be viewed in the **Analysis View**.

7 Experiment Feedback

7.1 Introduction

The experiment feedback (conditional or adaptive experiments) functionality of ZEN allows the definition of specific rules and actions to be performed during an experiment. E.g. changing the course of an experiment depending on the current system status or the nature of the acquired data on runtime. Moreover, it is possible to integrate certain tasks like data logging or starting an external application, directly into the imaging experiment. Typically, but not exclusively, such an experiment connects the image pickup with an automatic online image analysis.

Feedback experiments can be set up and controlled by the help of the *Experiment Feedback Tool* [▶ 439] and the *Script Editor* [▶ 440]. Please note that experiment feedback functionality is a part of the **Advanced Processing** module. An example workflow for experiment feedback is described in the chapter *Typical Workflow* [▶ 138].

Please note that we will not describe experiment feedback in detail here, as you will find a detailed instruction on how to perform feedback experiments and a lot of tutorials on the latest ZEN DVD in the folder .....**/Other /Experiment Feedback / Example Tutorials**.

7.2 Typical Workflow

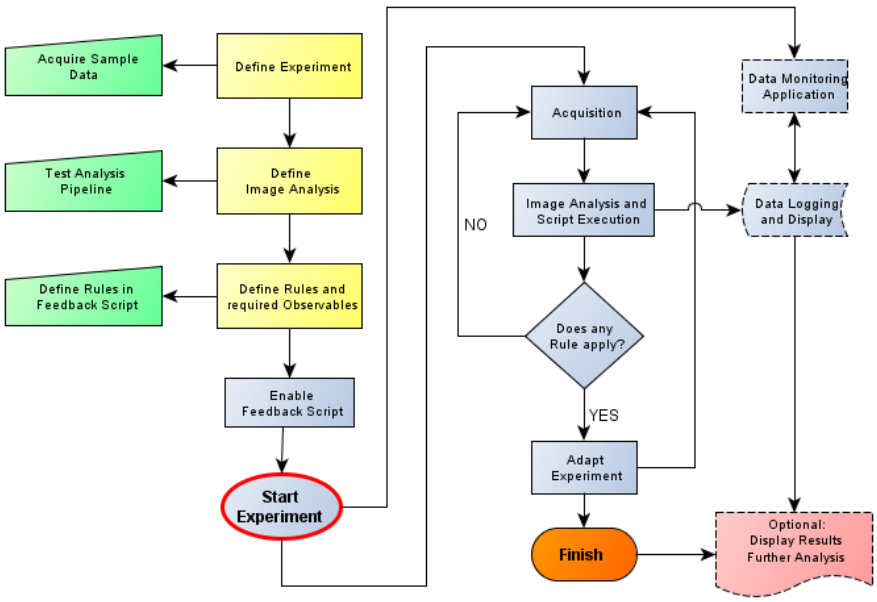


Fig. 14: Workflow Experiment Feedback

This diagram is one possibility to illustrate the typical workflow of feedback experiments. It contains the most important steps:

Step	Description
Define Experiment	The experiment should be well planned including the right parameters (Time Series, Z-stack, Multi-Channel, etc.), that are required to the get the correct data in a study once defined it is recommended to **Acquire Sample Data**, which can be used later on to test the image analysis.
Define Image Analysis	This step is using the image analysis wizard in order to create and image analysis pipeline for the latter use inside the feedback script only the specified parameters can be accessed from with the script later and it is strongly recommended to **Test Image Analysis Pipeline** to ensure the created results are meaningful. Optionally it is also possible to create an image analysis setting programmatically via an OAD macro.
Define Rules and Observables	This step is all about making up one´s mind on how the script should actually work. What must be observed and how should the experiment react upon a certain event once the main idea becomes clear one can start to **Define Rules in Feedback Script**.
Start Experiment	At this point one can start the experiment and watch the output. The general concept behind this workflow can be described as a loop, which is the actual acquisition. Every time “something”, usually an image acquisition took place the script will be executed. The rules will be checked and if required, certain tasks will be carried out. Additionally it is possible to export or log data into a text file and/or start an external application at any time point during the experiment. The best time point for those actions depends on the type of application.

8 Customizing the Application

8.1 Customizing toolbar

Prerequisites

- You are in the menu **Tools | Customize Application** dialog.
- The **Toolbar** tab is selected by default.

Procedure

1. Click on an entry in the **Available Toolbar Items** list.

   You will see a list of all available items in this group.

2. Double-click on an item.

   The item will be added to the **Selected Toolbar Items** list and does appear in the **Toolbar** within the application. Alternatively you can add the items per Drag&Drop.

3. In order to change the order of symbols in the toolbar use the **Up/Down** buttons.

4. If you want to delete an Icon from the toolbar, click on the **Delete** button 
   .

5. Click on **Close** button to close the dialog. The changes will be effective right now.

You have successfully customized the **Toolbar**.

9 Open Application Development (OAD)

9.1 Introduction

The acronym OAD for Open Application Development is a term describing both the OAD platform on ZEN as well as the process of developing applications on it. The platform has been made available for our customers to enhance the functionality of ZEN in a flexible way. With OAD typical microscopy workflows can be integrated into the ZEN software. A short list of OAD highlights: Macro Interface to access the major functionality of ZEN and its objects and the access to external libraries like the .Net Framework to significantly enlarge the field of application.

Our software ZEN 2.1 offers the following components which we regard as main parts for Open Application Development (OAD):

- Macro Runtime Environment (integrated)
- Macro Recorder
- Macro Editor
- Macro Debugger
- Macro Interface (Object Library)
- ImageJ Extension

Basic functionality

All ZEN Products (ZEN lite excluded) come with a basic macro functionality which allows to play existing macros within the software (**Macro tool**).

i Note

Within ZEN you can only run **.czmac** macro files which are aquired or saved in the ZEN macro environment. To run your macros they must be located in the folder: …/**User/Documents/Carl Zeiss/ZEN/Documents/Macros**.

Advanced functionality

The Macro Recorder, Editor and Debugger form the Integrated Development Environment (IDE) comes with the ZEN module **MacroEnvironment**. The IDE for the Macro Environment consists of two parts. There is a reduced IDE in the **Right Tool Area** which you find within the *Macro tool* [▶ 484]. The full blown IDE is available in the *Macro Editor dialog* [▶ 494] and allows users to generate and work with macros similar to Excel/Word macros. The Macro Interface is built into the software and therefore not a separate product. The **ImageJ** Extension is the first extension for ZEN and will be free of charge. It will not be available in ZEN lite.

User forum

A user forum was established to allow users to exchange macros and to discuss solutions. You will find a lot of example macros and further documentation there. The user forum can be reached under www.zeiss.com/ZEN-OAD.

9.2 Running an existing macro

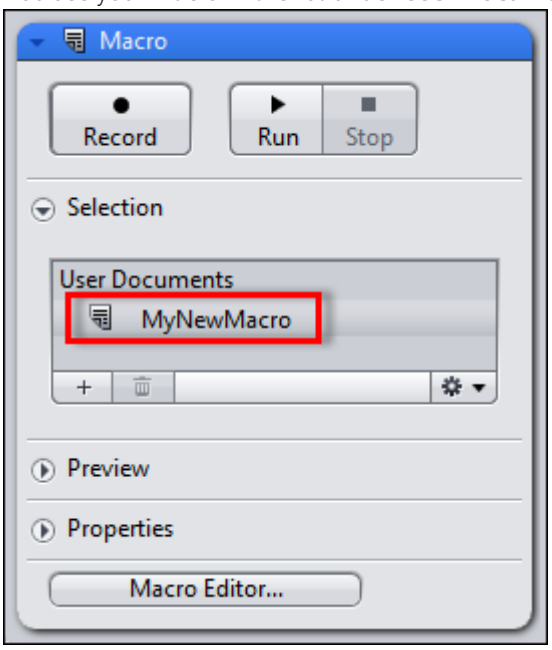
Prerequisites

- You work with a licensed version of ZEN 2.1, e.g. **ZEN pro**, **desk** or **system**. Remember that the macro environment for ZEN lite is not available.
- You have not licensed the **Macro Environment** module.
- You have a macro file available that you want to play in ZEN.

Procedure

1. Copy your macro file in the following folder: **.../User/My Documents/CarlZeiss/ZEN/Documents/Macros**.
2. Start the ZEN software.
3. In the **Right Tool Area** open the **Macro** tool.

   You see your macro in the list under **User Documents**.



4. Select your macro.
5. Click on the **Run** button.

Your macro is executed. You have successfully played a macro in ZEN.

9.3 Recording a macro

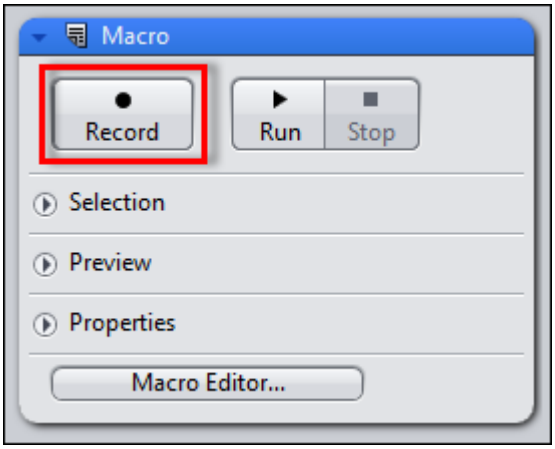
This guide shows how to record a macro of a simple processing workflow.

Prerequisites

- You have licensed the **MacroEnvironment** module.
- You are in the **Right Tool Area** | **Macro tool.**

Procedure

1 Click on the **Record button**.



2 Load a color image via menu **File** | **Open**…

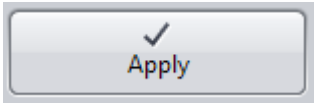
3 Go to the **Processing** tab.

4 Under **Method** select **Edges** | **Sobel**.

5 Under **Method Parameters** | **Normalization** select the entry **Clip.**

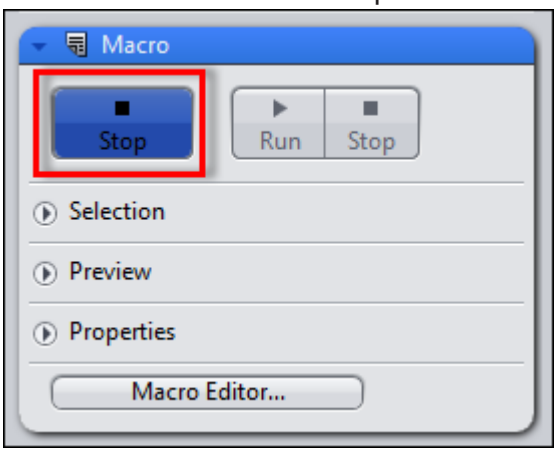
6 Under **Image Parameters** set your color image as **Input** Image.

7 On top of the **Processing** tab click on the **Apply** button .



The Sobel method will be applied to your image. The output image will be generated and opened in a new image container.

8 In the **Macro** tool click on the **Stop** button.



You have successfully recorded a macro for a simple processing workflow. The workflow can now be repeated automatically just by playing the recorded macro file.

9.4 ImageJ Extension

9.4.1 Introduction

Our ZEN software includes the no-charge **ImageJ** extension, which offers following possibilities:

- The extension allows an easy exchange of images, from simple two-dimensional images, to more complex, multidimensional entities, like Z-stacks, time series and so on. The exchange can go both ways, from ZEN to ImageJ, as well as from ImageJ to ZEN.
- The user can execute ImageJ functions on ImageJ, without having to leave the ZEN environment.
- Lastly, the ImageJ extension allows the user to combine the two benefits, introduced above: the users can send a ZEN image to ImageJ, have it processed there, and then return the resulting image back to ZEN in one single step.

Note that different versions and variants of ImageJ and Fiji exist. This document is based on the ImageJ/Fiji version 1.46. See notes for specifics of other versions and variants. For the sake of simplicity, Fiji is implied also, wherever ImageJ is mentioned in the following text.

9.4.2 Preparations

i Note

Note that the extension for ImageJ is not available in ZEN lite.

Procedure

1. Install **ImageJ** on your computer. Make sure that you use the latest version (check for online updates after installation).
2. Download loci_tools.jar and drop it into in the **ImageJ/plugins** folder.

Procedure

1. Note the name of the folder with your preferred alternative. While you can switch freely among them all, it makes sense to stick to one and the same environment, once you have started to add your own programs and macros.
2. The ImageJ/Fiji folder you will eventually decide on, can either belong to you alone or be shared among other users of the system. It is up to you decide, what you prefer: if you are the only user, nobody will meddle with its contents (images, macros etc), but then, you will need to copy and distribute the contents, if they are of interest to others as well.

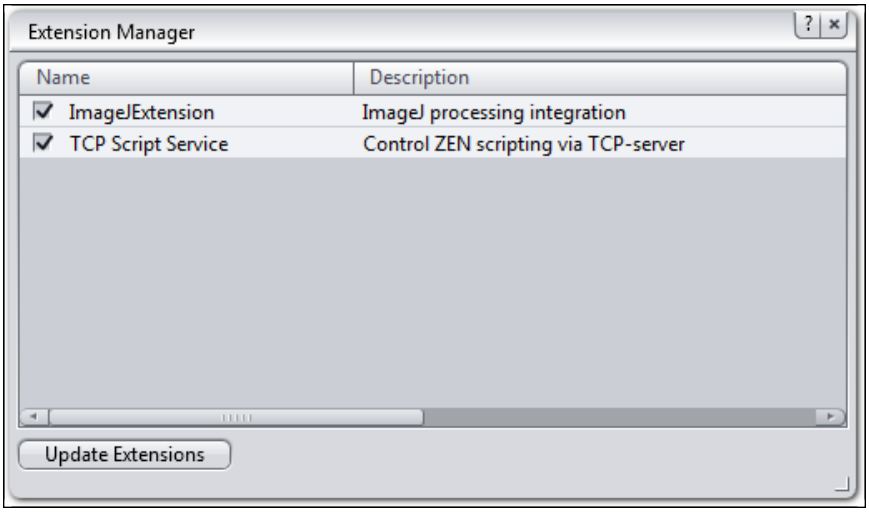
You have successfully fulfilled all prerequisites. You can now continue with setting up ImageJ within ZEN software.

9.4.3 Activate ImageJ extension

The extension is automatically included in the ZEN installation. To set it up, start the software and then proceed as follows:

Procedure

1 For activating the extension go to **Tools | Extension Manager…** and activate the checkbox **ImageJExtension.**

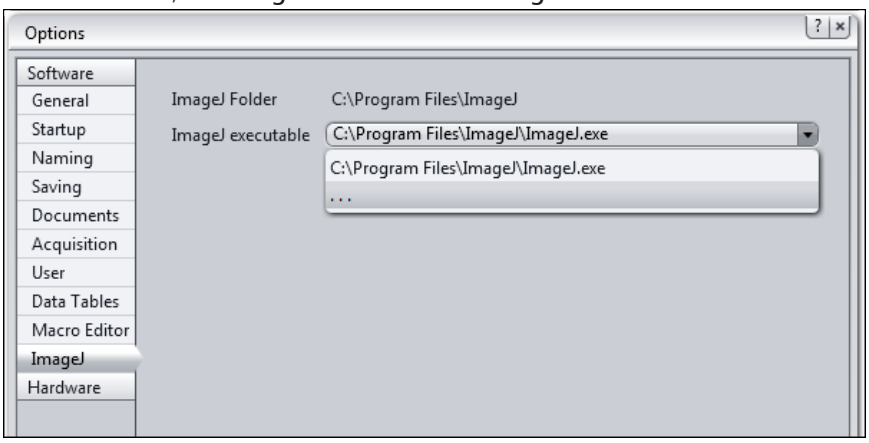


2 Click on **Update Extensions** button.

The extension looks through the usual places, where an existing installation could be found, and makes a sensible suggestion. To select the environment that you would prefer, proceed as follows:

3 Select **Tools | Options | ImageJ** to open the ImageJ options.

The two entries, the **ImageJ Folder** and the **ImageJ executable** will be shown.



Procedure

1 Select **ImageJ.exe** as ImageJ executable. If you prefer another executable or your preferred executable is not in the list click on the last entry ("…") to search for it.

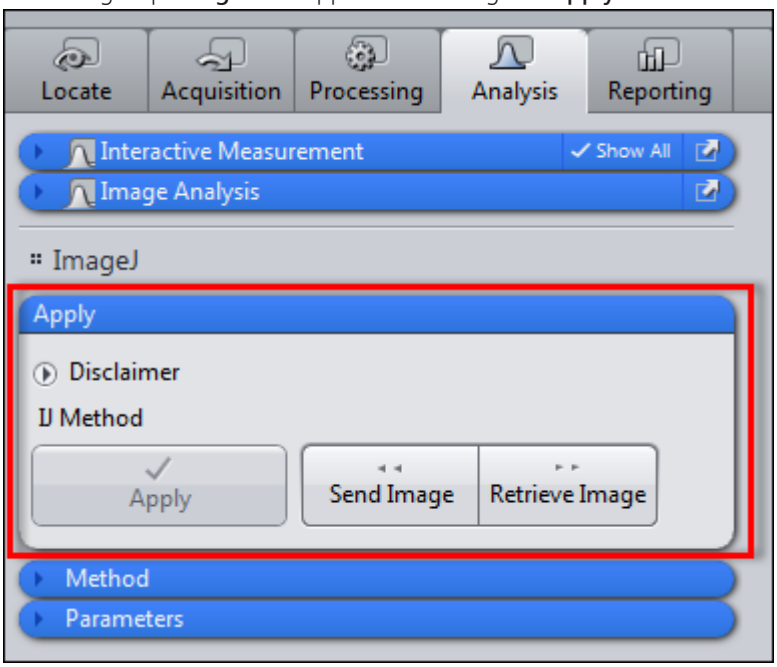
You have successfully set up ImageJ extension within the software. Now you can start working with the extension.

9.4.4 Send and retrieve images

Procedure

1 In the **Left Tool Area** click on the **Analysis** tab.

The tool group **ImageJ** will appear containing the **Apply** tool.



Procedure

1 To send an image from **ZEN** to **ImageJ** select the image in ZEN.

2 Click on **Send Image** button.

The image will be sent to ImageJ and opened within a new image frame. You can now edit the image within the ImageJ application.

3 To retrieve an image from **ImageJ** select the image in ImageJ.

4 Click on **Retrieve Image** button.

The image will be opened in ZEN.

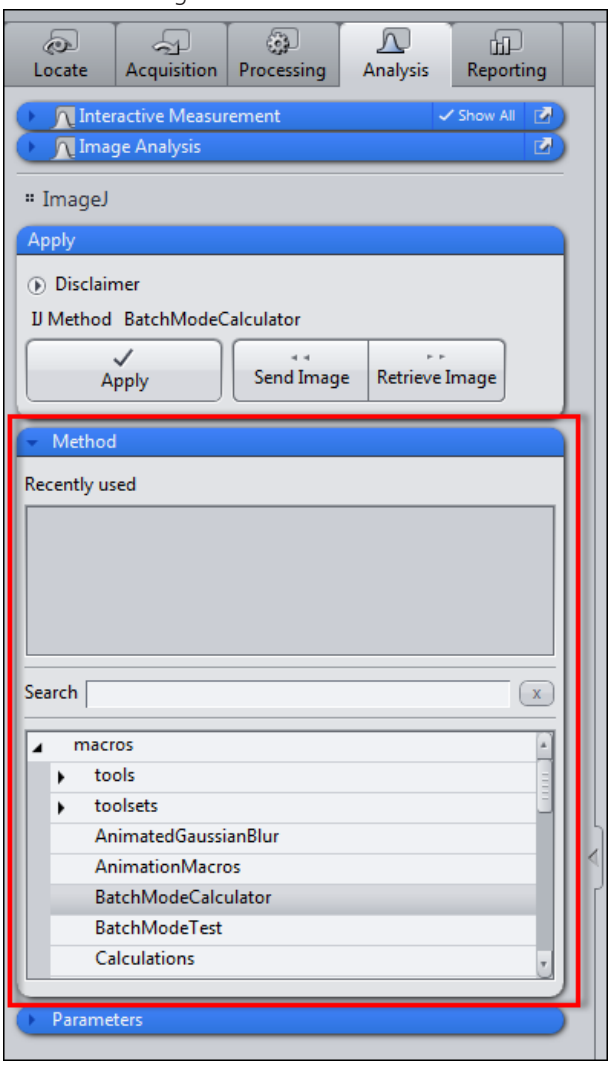
You have successfully sent images from ZEN to ImageJ and retrieved images from ImageJ.

9.4.5 Use ImageJ methods

The extension offers the possibility of sending images to ImageJ to get processed, to retrieve the result of the operation or both. The following instruction will show the basic steps which are necessary to apply ImageJ methods on any images.

Procedure

1 In the **Methods** tool select the command or method to be executed, for instance an ImageJ macro.



2 In the **Parameters** tool you specify if the method selected will need an input image and/ or provide a resulting image.

3 In the **Apply** tool click the **Apply** button to execute the command.

You have successfully applied an method to an image.

9.4.6 Image Type Send/Retrieve Conventions

ZEN to ImageJ

Image Type	Received as…	Comments
.tif, .jpg, .bmp, .png, .gif	Original	
.ome.tif	Original	
2D image B/W .czi	32-bit (RGB)	Convert the image in **ImageJ** to the required pixel type using Image > Type command
2D image 24/48 bit color .czi	32-bit (RGB)	
2D image 36/42 bit color .czi	-	Convert the CZI image to 24/48 bits before sending it or using it in a method
Multi-channel x Z-Stack x T-series	MD image	If necessary reassign the dimensions using Image > Hyperstacks for instance. Channel colors may be different from those set in ZEN
Tiled images	-	Only the first tile gets loaded.
12bit B/W images	Error in ImageJ	Workaround: convert the pixel type of the image to 16 bits in ZEN

ImageJ to ZEN

Image Type	Received as…	Comments
.tif, .jpg, .bmp, .png, .gif	Original	
.ome.tif	Original	
2-D images, B/W and RGB	B/W, RGB TIF	
Multi-channel x Z-Stack x T-series	MD image	Hint: select RGB in Quick Color Setup to get the same colors for channels as in ImageJ
Tiled images	-	Only the first tile gets loaded.

10 Module Tiles

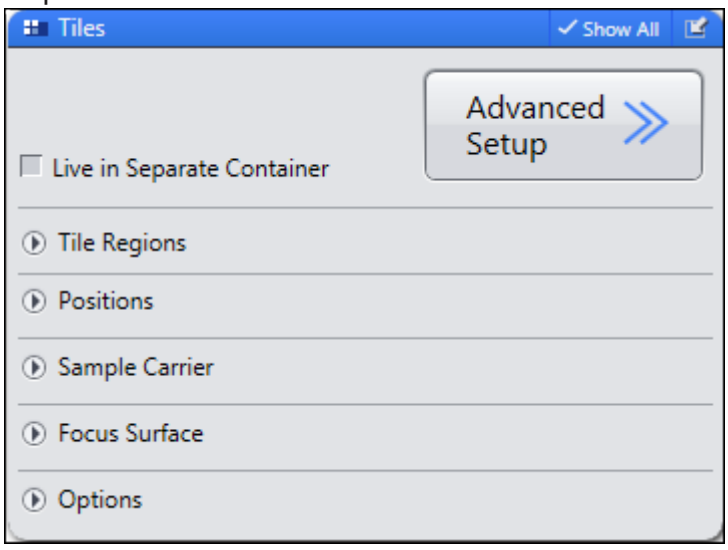
10.1 User Interface and Functions

10.1.1 Tiles tool

> **Note**
>
> This tool is only visible if you have licensed and/or activated the module in the **Modules Manager** and additionally activated it on the **Acquisition** tab in the **Experiment Manager**.

In the **Tiles** tool you configure the acquisition of images that consist of several image fields. Therefore you define Tile Regions or Positions. In addition you can set up Focus Surfaces and Sample Carrier Templates here.

The Tiles tool is located in the **Left Tool Area** under **Multidimensional Acquisition**.



Parameter	Description
Live in Separate Container checkbox	Opens the **Live** mode in a separated window. Note that the option **Automatic Container Layout** has to be activated.
Advanced Setup button	Opens the *Advanced Tiles Setup* [▶ 173] view in the **Center Screen Area**.

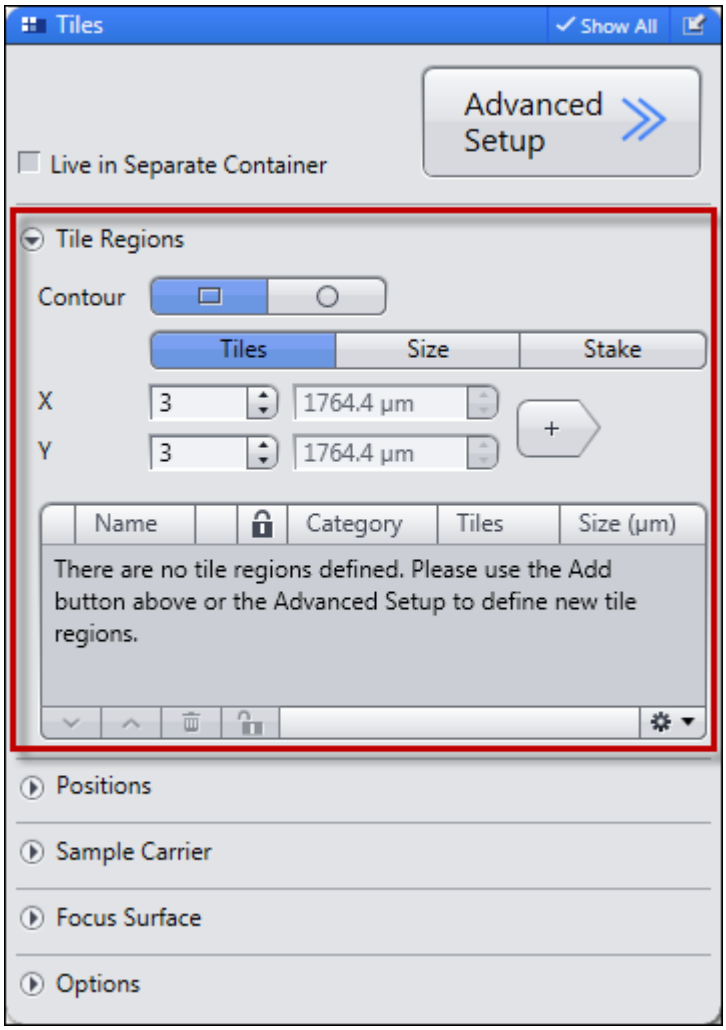
Note

The **Sample Carrier**, **Focus Surface** and **Options** sections are only visible if the **Show All** mode is activated.
If you have no license for the **Tiles** module you will only find **Tile Regions**, **Positions** and **Options** sections here.

Each section is described in the following topics.

10.1.1.1 Tile Regions section

To show the section in full, click on the **arrow** button .



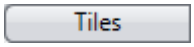
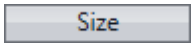
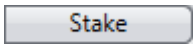
Contour section

Only visible if the **Show All** mode is activated.

Defines the outline of the tile region that you are adding. To learn more about tile regions see glossary "Tile region"

Option	Description
Rectangle button 	Adds a rectangular tile region.
Circle button 	Adds a circular tile region

Mode section

Option	Description
Tiles button 	Using this mode you have to enter the number of tiles as a reference for the size of the tile region. Enter the number of tiles in the **X / Y** input fields. If you are adding a circular tile region, enter the number of tiles for the diameter in the **Diameter** input field.
Size button 	Using this mode you have to enter the size as a reference for the size of the tile region. Enter the size of the tile region in the **X / Y** input fields. If you are adding a circular tile region, enter the diameter of the tile region in the **Diameter** input field.
Stake button 	This mode allows the definition of a tile region by the placement of at least two markers (user defined X/Y stage coordinates). If you want to modify the tile region (expand/ reduce) you have to adjust the tile region to the desired size. To complete the tile region press **Done**. Circular or rectangular tile region can be created in this manner by selection of the appropriate contour.
Add button 	Adds the tile region to the **Tile Regions List** and activates it for acquisition. Added tile regions are displayed in the form of red grids in the stage view of the **Advanced Tiles Setup**.

Tile Regions list

Displays the added tile regions. The list contains the following columns and buttons:

Option	Description
Checkbox column	Activates the relevant list entry for acquisition.
Name column	Allows you to edit the name of the tile region.

Option	Description
Contour column	Displays the contour of the tile region.
Category column	Displays the category of the tile region. Categories can be defined in the view options of the advanced tiles setup on the properties tab.
Tiles column	Displays the number of tiles of the tile region.
Size column	Displays the size of the tile region.
Z column	Displays the Z-position of the tile region.
List Navigation buttons  and 	With the buttons you can shift selected list entry one position up or down in the tile regions list. This allows you to modify the acquisition order.
Delete button 	Deletes the selected list entry.
Lock button 	Unlocks the selected list entries to allow editing.
Options button 	Opens the *Options for editing Tile Regions* [▶ 152].

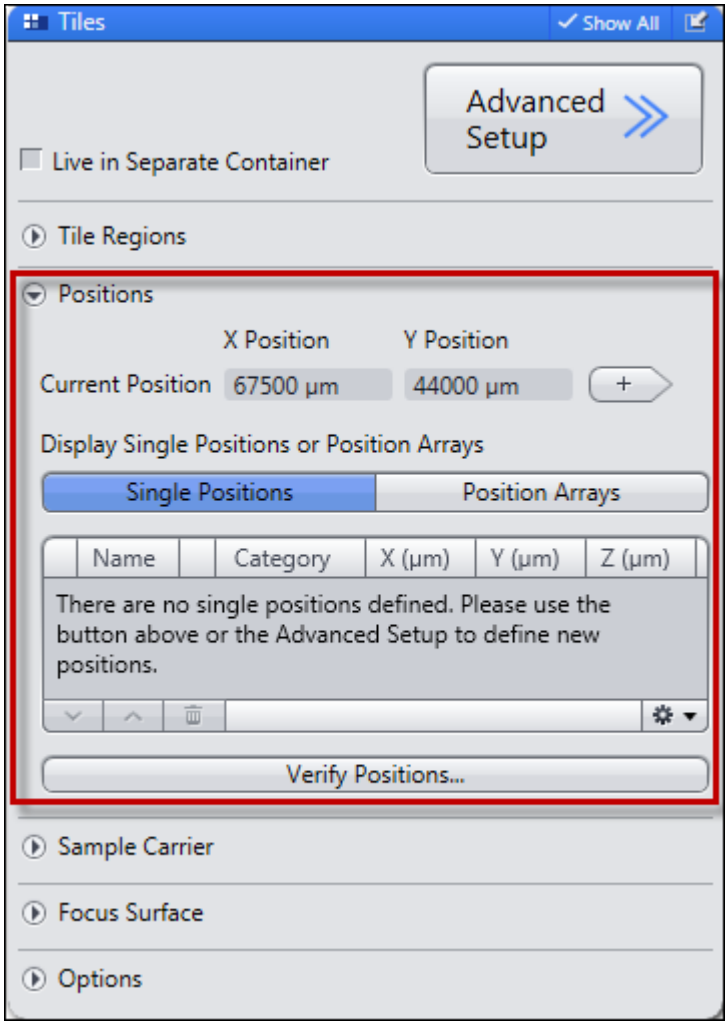
10.1.1.1.1 Options for editing Tile Regions

Option	Description
Set Current Z for Selected Tile Regions	Sets the current Z-Position for all selected tile regions.
Delete	Deletes the current tile region.
Delete All	Deletes all tile regions.
Activate	Activates the current tile region for acquisition.
Deactivate	Deactivates the current tile region for acquisition.
Unlock	Unlocks the current tile region.
Unlock All	Unlocks all locked tile region.

Option	Description
Sort	**By Center Position (Y -> X)** sorts all tile regions according to their overall Y position.
	By Center Position (X -> Y) sorts all tile regions according to their overall X position.
	By Category sorts all tile regions according to their category.
Convert to Positions…	Converts a selected tile region into Positions or a Position Array.

10.1.1.2 Positions section

To show the section in full, click on the **arrow** button .

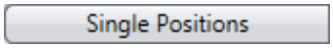
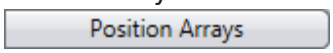


Current Position section

Displays the current stage position.

Option	Description
X Position display field	Displays the X coordinate of the current position.
Y Position display field	Displays the Y coordinate of the current position.
Add button 	Adds the current position to the **Positions List** and activates it for acquisition.

Display mode section

Option	Description
Single Positions button 	Shows the **Single Positions List**. To learn more about single positions see glossary "Position".
Position Arrays button 	Shows the **Position Arrays List** and the **Positions of selected Array List**, that shows a full **Single Positions List** for the selected position array. To learn more about position arrays see glossary "Position".

Single Position List

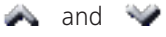
Displays the added positions. The list contains the following columns and buttons:

Option	Description
Checkbox column	Activates the relevant list entry for acquisition.
Name column	Displays the name of the single position.
Category column	Displays the category of the single position. Categories can be defined in the view options of the advanced tiles setup on the properties tab.
X column	Displays the X-position of the single position.
Y column	Displays the Y-position of the single position.
Z column	Displays the Z-position of the single position.

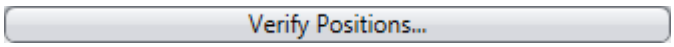
Option	Description
List Navigation buttons 	With the buttons you can shift selected list entry one position up or down in the tile regions list. This allows you to modify the acquisition order. Note that the **Tile Regions/Positions** checkbox have to be deactivated *Tiles Options* [▶ 193].
Delete button 	Deletes the selected list entry.
Lock button 	Unlocks the selected list entries to allow editing.
Options button 	Opens the *Options for editing Single Positions* [▶ 156].

Position Array List

Displays the added position arrays. The list contains the following columns and buttons:

Option	Description
Checkbox column	Activates the relevant list entry for acquisition.
Name column	Allows you to edit the name of the tile region.
Contour column	Displays the contour of the position array.
Positions column	Displays the number of positions of the position array.
Size column	Displays the size of the position array.
List Navigation buttons 	With the buttons you can shift selected list entry one position up or down in the tile regions list. This allows you to modify the acquisition order.
Delete button 	Deletes the selected list entry.
Lock button 	Unlocks the selected list entries to allow editing.
Options button 	Opens the *Options for editing Position Arrays* [▶ 156].

Verify Positions section



Option	Description
Verify Positions... button	Opens the *Verify Z Position dialog* [▶ 163].

10.1.1.2.1 Options for editing Single Positions

Option	Description
Set Current Z for Selected Positions	Sets the current Z-Position for all selected positions.
Set Current XYZ for Selected Position	Sets the current X-Y-Z-Position for the selected position.
Delete	Deletes the current position.
Delete All	Deletes all positions.
Activate	Activates the current position for acquisition.
Deactivate	Deactivates the current position for acquisition.
Sort	Sorts the list entries according to the chosen parameter.
- **By Center Position (Y -> X)**	Sorts all positions according to their overall Y position.
- **By Center Position (X -> Y)**	Sorts all positions according to their overall X position.
- **By Category**	Sorts all positions according to their category.

10.1.1.2.2 Options for editing Position Arrays

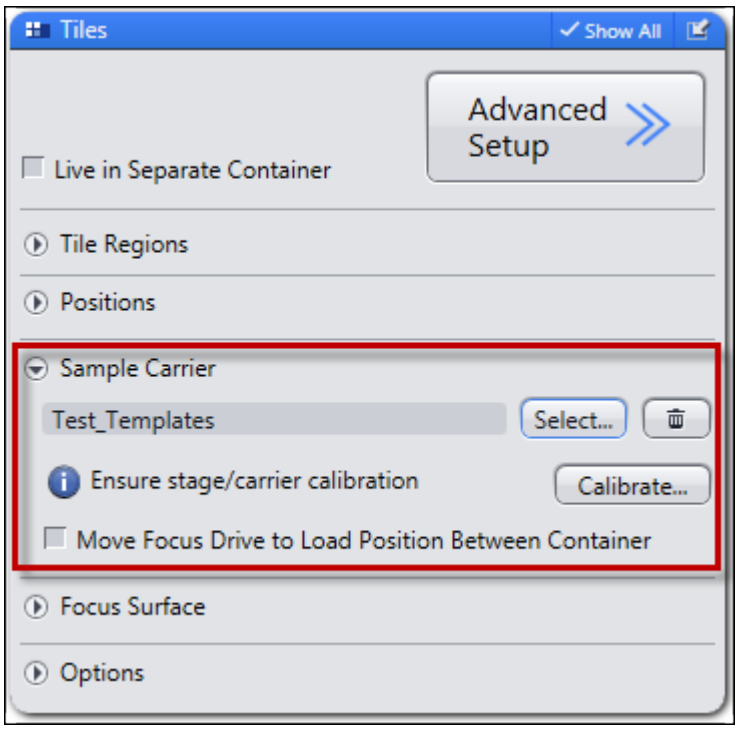
Option	Description
Delete	Deletes the current position array.
Delete All	Deletes all position arrays.
Activate	Activates the current position array for acquisition.
Deactivate	Deactivates the current position array for acquisition.
Unlock	Unlocks the current position array.
Unlock All	Unlocks all locked position arrays.

Option	Description
Sort	Sorts the list entries according to the chosen parameter.
- **By Center Position (Y -> X)**	Sorts all positions according to their overall Y position.
- **By Center Position (X -> Y)**	Sorts all positions according to their overall X position.

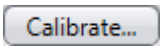
10.1.1.3 Sample Carrier section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button .



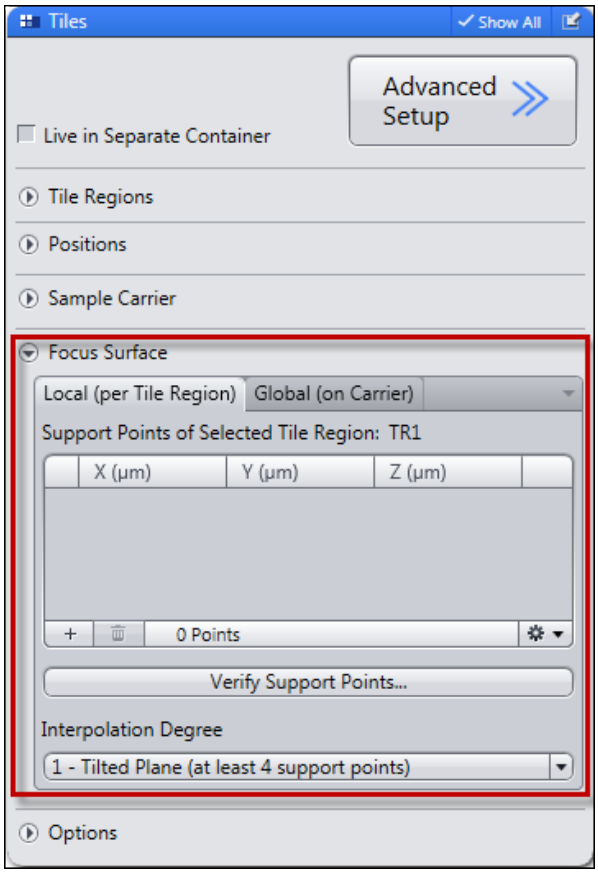
Option	Description
Sample Carrier field	Displays the selected sample carrier template. If no template is selected it will display "None".
Select... button 	Opens the *Select Sample Carrier Template dialog* [▶ 166]. Here you can select the sample carrier template.

Option	Description
Delete button 	Deletes the selected sample carrier from the sample carrier field. The template will still be available in the **Select Sample Carrier Template** dialog.
Calibrate... button 	Opens the **Sample Carrier Calibration Wizard**. Here you will be guided through the sample carrier calibration.
Move Focus Drive to Load Position Between Containers checkbox	**Activated:** Moves the focus drive to the loading position during the movement of the stage to another container of the sample carrier (e.g. a well or slide). This prevents possible damage.

10.1.1.4 Focus Surface section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button  .



Local (per Tile Region) Support Points List

Displays the added local support points of a selected tile region. These can be edited in the view options of the advanced tiles setup on the support points tab. The list contains the following columns and buttons:

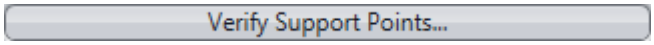
Option	Description
X column	Displays the X coordinate of the focus reference point.
Y column	Displays the Y coordinate of the focus reference point.
Z column	Displays the Z coordinate of the focus reference point.
Add button 	Adds a new support point to the selected tile region at the current stage and focus position.
Delete button 	Deletes the selected list entry.
Options button 	Opens the *Options for editing Support Points* [▶ 160].

Global (on Carrier) Support Points List

Displays the added global support points of the selected sample carrier. These can be edited in the Select Sample Carrier Template dialog.
As the list does not differ much from the **Local Support Points List** only the additional columns are explained in the following:

Option	Description
Container column	Allows you to sort the global support points according to their container on the sample carrier.

Verify Support Points section



Option	Description
Verify Support Points... button	Opens the *Verify Z Position dialog* [▶ 163].

Interpolation Degree section

Option	Description
Interpolation Degree dropdown list	Shows the selected degree of interpolation. To select an other degree of interpolation click on and select it from the dropdown list.

i Note

The more variable the surface of your specimen the higher you should choose the interpolation degree. For higher degrees you will need more support points. The minimum number of support points for each interpolation degree is given in the dropdown list. As an overachievement of this minimum number ensures a solid calculation, we recommend minimizing the interpolation degree even if you added more support points. Increase the interpolation degree only that far as the surface condition of your specimen demands for.

10.1.1.4.1 Options for editing Support Points

Options for editing Local Support Points

Option	Description
Add Support Point at Current Stage and Focus Position	Adds a new support point at the current stage and focus position.
Set Current Z for Selected Support Points	Sets the current Z-Position for all selected support points.
Set Current X/Y/Z for Selected Support Points	Selects the current X-Y-Z-Position for the selected support point.
Delete	Deletes the current support point.
Delete All	Deletes all support points from the current tile region.
Delete all Support Points from Selected Tile Regions	Deletes all support points from the selected tile regions.
Delete all Support Points from all Tile Regions	Deletes all support point from all tile regions.

Options for editing Global Support Points

As the list does not differ much from the **Options for editing Local Support Points** only the additional options are explained in the following:

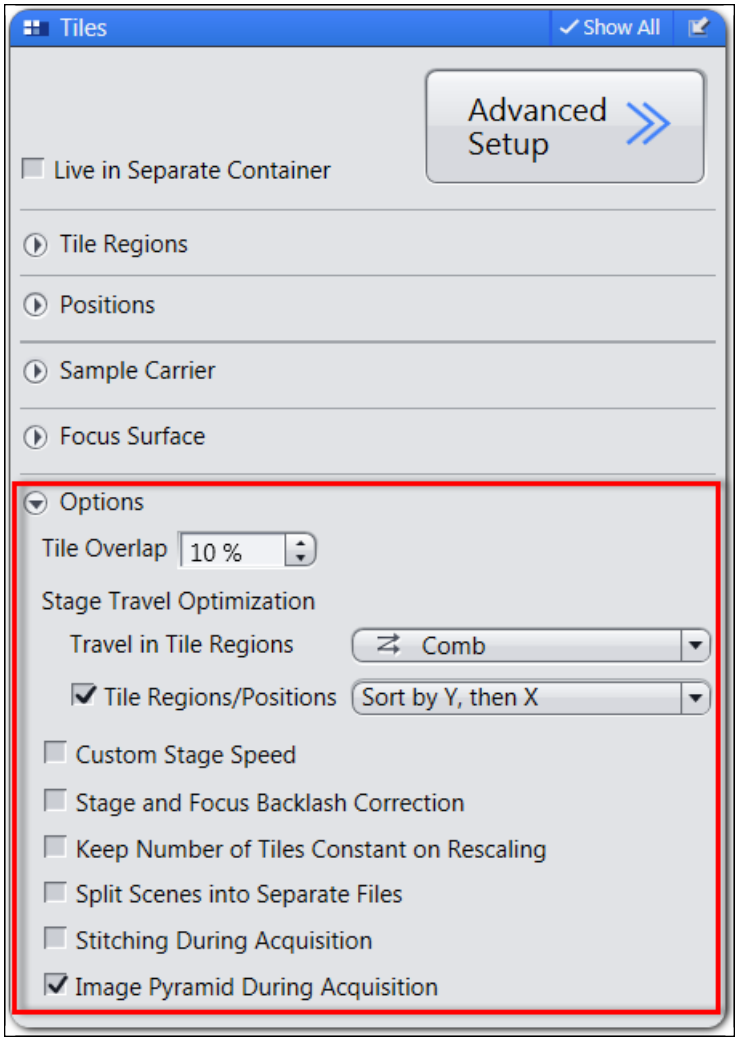
Option	Description
Set Current Z for Selected Support Points	Sets the current Z-Position for all selected support points.

10.1.1.5 Options section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button .

Here you can determine the acquisition and travel behavior during the experiment. Changes in this section of the tool affect all elements, tile acquisitions, positions and position arrays.



Tile Overlap section

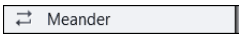
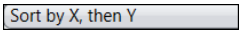
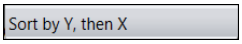
Option	Description
Tile Overlap input field	Defines the overlap in percent of individual tiles of the tile regions here. The value is set to 10 % by default.

i Note

Lower overlap might cause artifacts. No overlap will not allow the images to be stitched correctly.

Stage Travel Optimization section

Adjust settings for stage traveling during an experiment here:

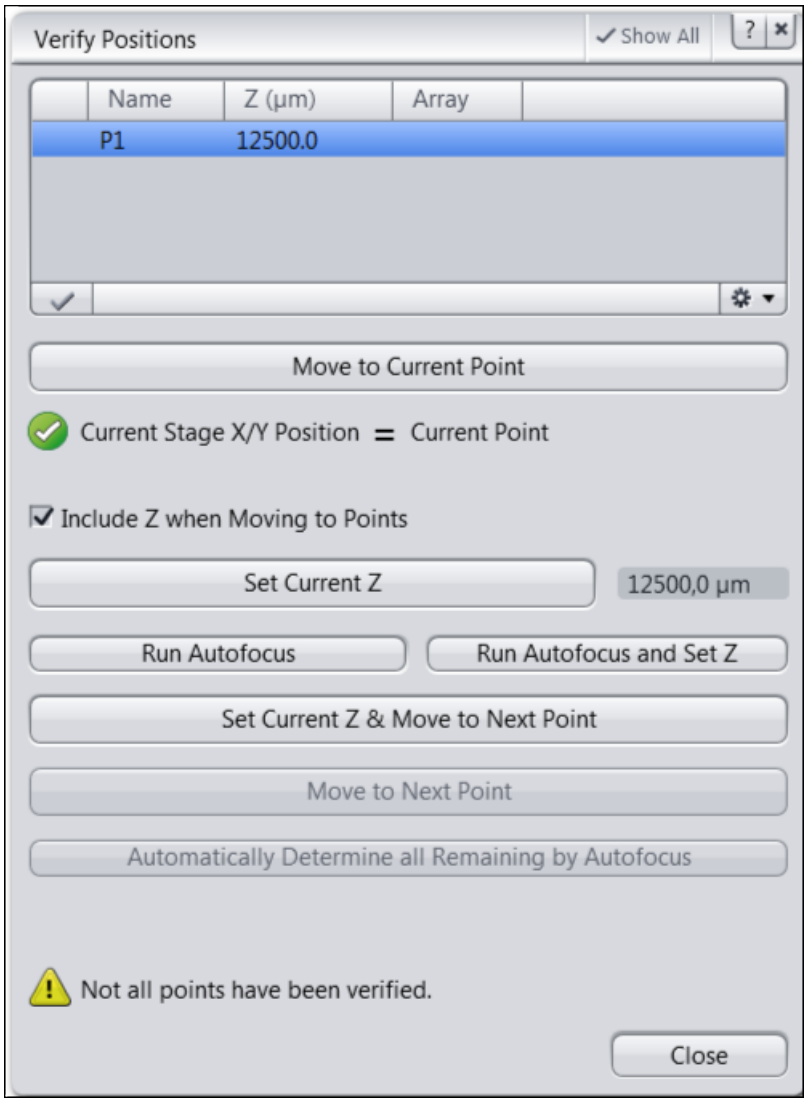
Option	Description
Travel in Tile Regions	Allows you to optimise the stage travel in a tile region.
- **Comb** button 	Acquires tile regions following a comb pattern – always from one travel direction only (left -> right). This scan movement is more precise.
- **Meander** button 	Acquires tile regions following a meander pattern – alternately from both travel directions (left -> right; right -> left). This scan movement is faster.
- **Spiral** button 	Acquires tile regions following a spiral pattern – from the center of the region to the outer bounds in a clockwise motion. This mode works only for regions with rectangular or elliptical contours.
Tile Regions/Positions	**Activated**: Individual positions and tile regions are not acquired in the sequence in which they are defined in the **Tile Regions** list. The stage movement will be automatically adapted to the location of the individual tile regions and positions. If you add or remove tile regions or positions, the sequence of acquisition therefore also changes.
- **Sort by X, then Y** button 	The tile regions and positions are sorted by their absolute position (first x, then y).
- **Sort by Y, then X** button 	The tile regions and positions are sorted by their absolute position (first y, then x).
Carrier Wells/Container	**Activated**: Applies the Comb/Meander patterns (see description above) when acquiring tiles using wells/containers.

Additional Options

Option	Description
Custom Stage Speed checkbox	**Activated**: A custom continual stage speed is used instead of the current possible speed. You can adjust the speed (in percent) if you have activated the checkbox.
Stage and Focus Backlash Correction checkbox	**Activated**: Stage and focus positioning is done with a backlash correction which is more precise but slightly slower.
Keep Number of Tiles Constant on Rescaling checkbox	**Activated**: The number of tiles (columns and rows) remains constant when the scaling changes, e.g. due to an objective change. When this option is activated, the scanned area on the stage does not remain fixed. It depends on the current tile size.
Split Scenes into Separate Files checkbox	**Activated**: The scenes (e.g. tile regions and positions) are stored into separate physical files. They are still combined into one logical image file.
Stitching During Acquisition checkbox	**Activated**: Stitching of tiles is done during the acquisition.
Image pyramid during acquisition checkbox	**Activated**: An image pyramid is generated during the acquisition. This optimizes the image for fast display. If this option is not activated, the acquired image will not be shown and updated in the document area while the acquisition is running. This function is only visible if a carrier has been selected in the sample carrier section.

10.1.1.6 Verify Z Position dialog

The Z positions of positions, local support points and global support points are verified in a seperate dialog. As the dialog contains the same items and options for verifying the Z positions it is described once.



Z Position List

Displays the added positions. The list contains the following columns and buttons:

Option	Description
Status cloumn	Shows if the Z-Position is already verified.
Name column	Only valid for verifying Z positions of positions. Displays the name of the selected point.
X column	Displays the X-position of the point.
Y column	Displays the Y-position of the point.
Z column	Displays the Z-position of the point.
Array column	Only valid for verifying Z positions of positions. Shows if the position is part of an Position Array.

Option	Description
Tile Region column	Only valid for verifying Z positions of local support points. Shows to which tile region the local support point belongs.
Container column	Only valid for verifying Z positions of global support points. Shows to which container the global support point belongs.
Status button 	Changes the status of the selected point to verified. If the selected position is already verified the button looks different  and will set the status of the selected point back to unverified.
Options button 	Opens the options for verifying Z positions.

Options for verifying Z positions

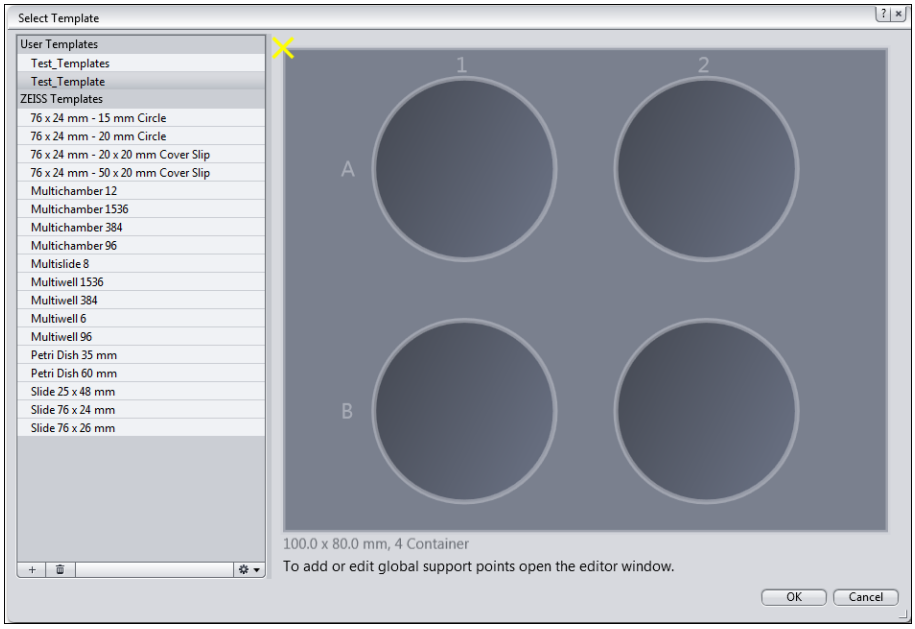
Option	Description
Current Point Verified	Changes the status of the selected point to verified. If the selected point is already verified it will set the status of the selected point back to unverified.
Set all Points as Verified	Changes the status of all points to verified.
Reset the Verification State of all Points	Changes the status of all points to unverified.
Apply Z-Offset...	Opens al dialog to apply a z-offset for all or the selected points.

Verify Z Positions section

Option	Description
Move to Current Point button	Moves the stage to the selected point.
Current Stage position indicator field	Show if the current stage position is the position of the selected point.
Set Current Z button	Sets the current z for the selected point and sets the status to verified.

Option	Description
Run Autofocus button	Runs the software autofocus.
Include Z when Moving to Points checkbox	Runs th software autofocus and sets the determined z value for the point.
Move to Next Point button	Moves the stage to the next point.
Move to Next and Run Autofocus button	Moves the stage to the next point and runs the software autofocus.
Automatically Determine all Remaining by Autofocus button	Automaticcaly moves to the remaining points and determines the z value with the software autofocus for them.
Verification Status idicator field	Shows if all points have been verified.

10.1.1.7 Select Sample Carrier Template dialog



Option	Description
User Templates section	Shows all custom sample carrier templates.

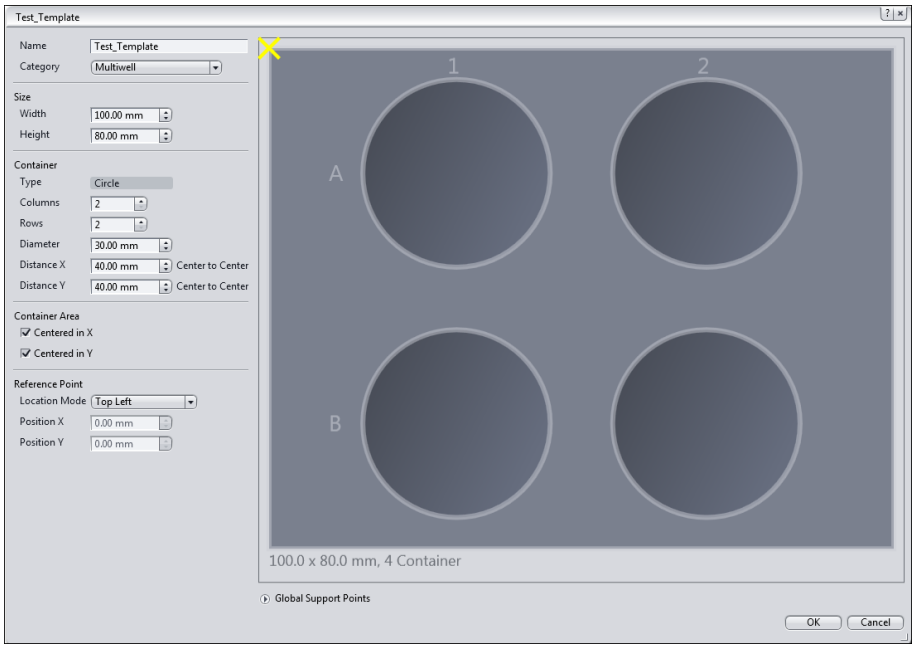
Option	Description
ZEISS Templates section	Shows predefined ZEISS templates for several sample carriers.
Add button 	Opens the *Edit Sample Carrier Template dialog* [▶ 167] to create a new template.
Delete button 	Deletes the selected sample carrier template.
Option button 	Opens the *Options for editing sample carrier templates* [▶ 167].
Preview field	Shows a preview of the selected sample carrier template.

10.1.1.7.1 Options for editing Sample Carrier Templates

Option	Description
New Template...	Opens the *Edit Sample Carrier Template dialog* [▶ 167] to create a new template.
Show/Edit...	Opens the selected template in the **Edit Sample Carrier Template** dialog and allows editing. ZEISS templates are read only. If you want to edit a ZEISS template you have to use the **Copy and Edit...** option.
Copy	Copies the selected template.
Copy and Edit...	Copies the selected template and opens it in the *Edit Sample Carrier Template dialog* [▶ 167] dialog for editing.
Import	Imports an template.
Export	Exports the selected template.
Delete	Deletes the selected template.
Refresh Templates	Refreshes the list of templates after creating a new one.

10.1.1.7.2 Edit Sample Carrier Template dialog

The dialog for editing a template and creating a new one are the same. If you create a new template the dialog fields are empty. On the right you see a live **Template Preview** of the setting for the template and global support points.



Name and Catergory section

Option	Description
Name input field	Shows the name of your template. You can enter a new name for the template here as well.
Category drop down list	Shows which sample carrier category the template uses. You can choose between **Slide**, **Multislide**, **Petri Dish**, **Multiwell**, **Multichamber** and **Custom**. The category defines the overall appearance of the template and affects the further editing possibilities of the template.

Size section

Width input field	Determines the width of the sample carrier template.
Height input field	Determines the height of the sample carrier template.

Container section

Depending on the category of the template you have different options for editing in this section.

Option	Description
Type field	Shows if the containers of the template are rectangles or circles. If the category is **Custom** you can manually set the type of containers.
Columns input field	Shows how many columns of containers the template contains. You can not edit this field if the templates category is **Slide** or **Petri Dish**
Rows input field	Shows how many rows of containers the template contains. You can not edit this field if the templates category is **Slide** or **Petri Dish**
Width and **Height** input fields	Only for **rectangular** containers. Determines the width and height of the containers. Not active for **Slide**.
Diameter input field	Only for **circular** containers. Determines the diameter of the containers. Not active for **Slide**.
Distance X input field	Determines the distance in x direction between the containers from center to center. Not active for **Slide** and **Petri Dish**.
Distance Y input field	Determines the distance in x direction between the containers from center to center. Not active for **Slide** and **Petri Dish**.

Container Area section

Option	Description
Centered in X checkbox	**Activated**: The containers will be positioned centered in X direction on the sample carrier template.
Centered in Y checkbox	**Activated**: The containers will be positioned centered in Y direction on the sample carrier template.

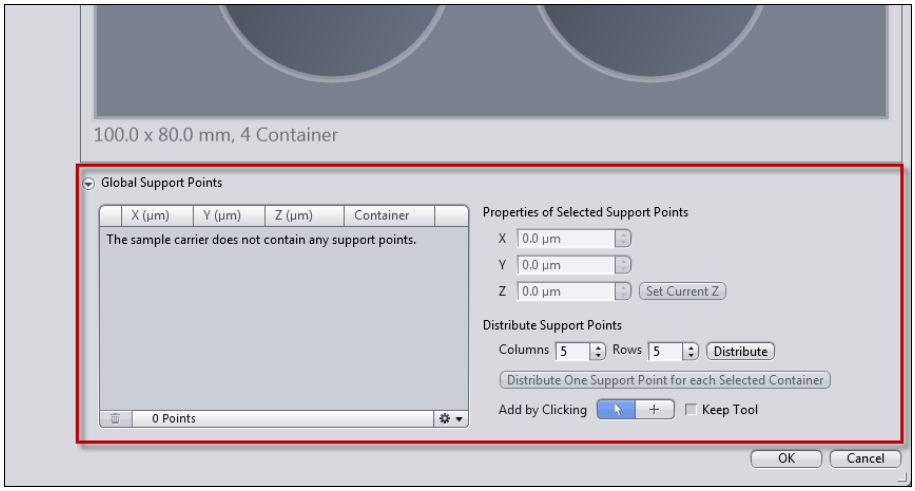
Reference Point section

The reference point is marked by a **Yellow X** .

Option	Description
Location Mode drop down list	Defines the position of the templates reference point. You can choose between **Center**, **Top Left**, **Top Right**, **Bottom Left**, **Bottom Right** and **Custom**. The default position of the reference point varies with the type of carrier.
Position X input field	Only active if you have selected **Custom** location mode. Sets a custom X position for the reference point of the template.
Position Y input field	Only active if you have selected **Custom** location mode. Sets a custom Y position for the reference point of the template.

10.1.1.7.2.1 Global Support Points section

To show the section in full, click on the **arrow** button  .



Global Support Points list

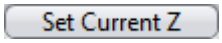
Option	Description
X column	Displays the X coordinate of the support point.
Y column	Displays the Y coordinate of the support point.
Z column	Displays the Z coordinate of the support point.
Container column	Shows the container of the support point.
Delete button 	Deletes the selected list entry.

Option	Description
Options button 	Opens the Options for editing global support points.

Options for editing global support points

Option	Description
Set Current Z for Selected Support Points	Sets the current Z value for the selected support points.
Delete	Deletes the selected support points.
Delete All	Deletes all support points.

Properties of Selected Support Points section

Option	Description
X input field	Sets the X coordinate of the selcted support point.
Y input field	Sets the Y coordinate of the selcted support point.
Z input field	Sets the Z coordinate of the selcted support point.
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

Distribute Support Points section

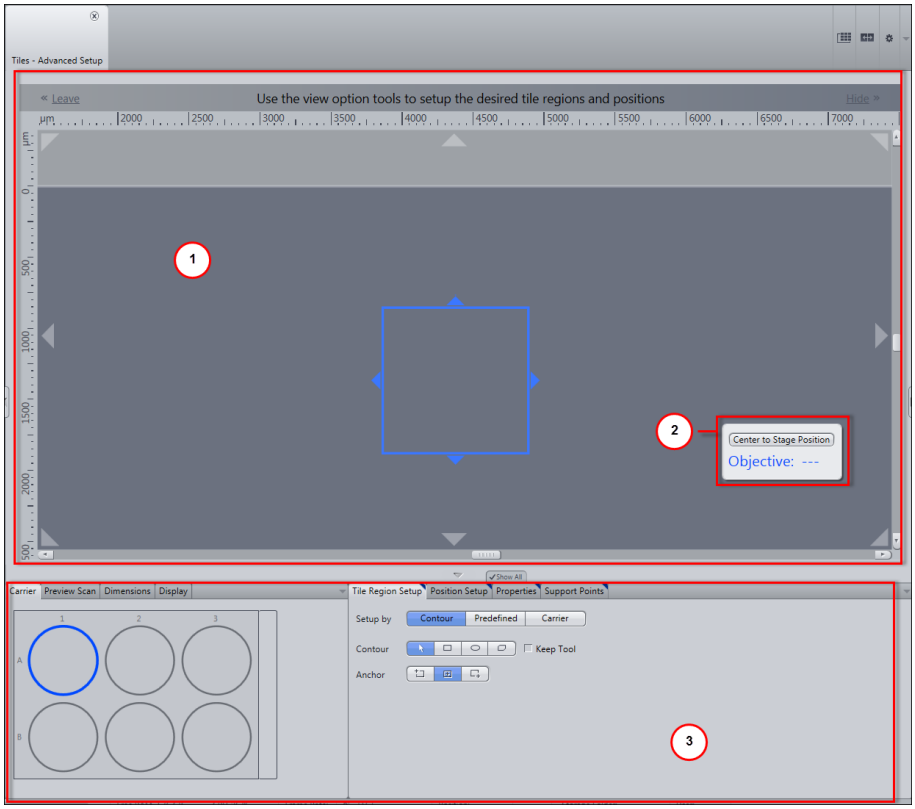
Option	Description
Columns input field	Sets the number of columns of support points within the template.
Rows input field	Sets the number of rows of support points within the template.
Distribute button 	Distributes the entered number of support points defined in the column and row input fields within the template. Previously defined support points will be deleted.
Distribute One Support Point for each Selected Container button	Sets one support point in the center of the selected containers. Previously defined support points will be deleted.

Add by Clicking section

Option	Description
Selection button 	Select a support point to move it. Support points distributed by the **Distribute One Support Point for each Selected Container** button can not be moved.
Add button 	Add a new support point on the template perview.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

10.1.2 Advanced Tiles Setup

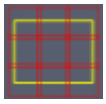
Here you can configure advanced settings and plan your Tiles and Positions experiment. In the Center Screen Area you can see the *Stage view* [▶ 173]. When the Advanced Tiles Setup (ATS) opens the stage view is "zoomed" to a predefined factor. The Zoom can be changed view the Dimensions view control, or by pressing Ctrl + scrolling mouse wheel.
To navigate around one has the following options: In each corner and along each edge the arrowheads can be clicked to move the view in this direction. To re-center the view on the current stage position press the **Center to Stage Position** button located in the lower right-hand corner of the Stage View. Additional settings and tools relating to tile regions or positions can be found under *Specific View options* [▶ 175].



1 *Stage View* [▶ 173] **2** Center to Stage Position
3 *Specific View options* [▶ 175]

10.1.2.1 Stage View

The image area shows the full travel range of the microscope stage, along with the current stage position, the graphical display of sample carriers and your acquired mosaic images. You can control the stage view using the arrow icons at the edges of the image area. The view can be enlarged, reduced or moved using the general control elements.

Symbol	Name	Description
 / 	**Selected / Active** container/ well	The currently **Selected / Active** container / well is represented by a blue border.
	**Live Navigator** tool	In the Live navigator tool the current stage position including the live image is shown as a frame outlined in blue. To move the frame, double-click on the position to which you want to move it. Alternatively hold the left mouse button on the live navigator tool while dragging the mouse. The frame can also be used to control acquisition. If you click on one of the frame's blue arrow icons, an image is acquired. The Live Navigator tool is moved one frame width in the relevant direction. You can create tile images of your sample easily in this way.
	**Tile Region**	Tiles / tile regions are represented in the stage view by a red grid.
	**Positions**	Positions are represented in the stage view by a yellow plus symbol.
	**Position Array**	Position Arrays are represented in the stage view by the corresponding position symbols surrounded by a dashed line.
	**Local Support Point**	Local Support Points are represented in the stage view by a yellow circle with a dot in the middle.
	**Global Support Point**	Global Support Points are represented in the stage view by a white circle with a dot in the middle.

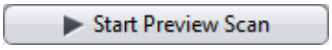
10.1.2.2 Specific View options

10.1.2.2.1 Preview scan tab

Here you can define the settings for a preview scan. Typically a low magnification objective is used, especially when a larger tile is to be acquired, to give the user a low resolution overview of the sample in question.

i Note

The objective setting used in the preview scan is not independent of that found in the experiment settings, but is the same as that set in locate or on the microscope´s TFT.

Option	Description
Use Existing Experiment Settings checkbox	**Activated:** Uses the existing experiment Settings. That is the default setting. **Deactivated:** Additional options **Camera** and **Channels** appear. That allow independent activation/ deactivation of channels and **Use Binning from Experiment** versus the defined experiment settings. If binning is used then the exposure time is automatically compensated to avoid saturation. Changing these parameters does not effect the settings that will be used for the experiment.
Delete Existing Preview Images checkbox	**Activated:** Deletes all existing preview images when the next preview is acquired.
Objective dropdown list	Here you can select the objective which will be used for the preview scan. To acquire an overview of all active tiles and positions images, switch to an objective with a low magnification. Set the channel exposure time and start the acquisition of the overview image. To select an other objective click on  and select it from the dropdown list.
Start Preview Scan button 	Starts the preview scan to acquire the overview images.

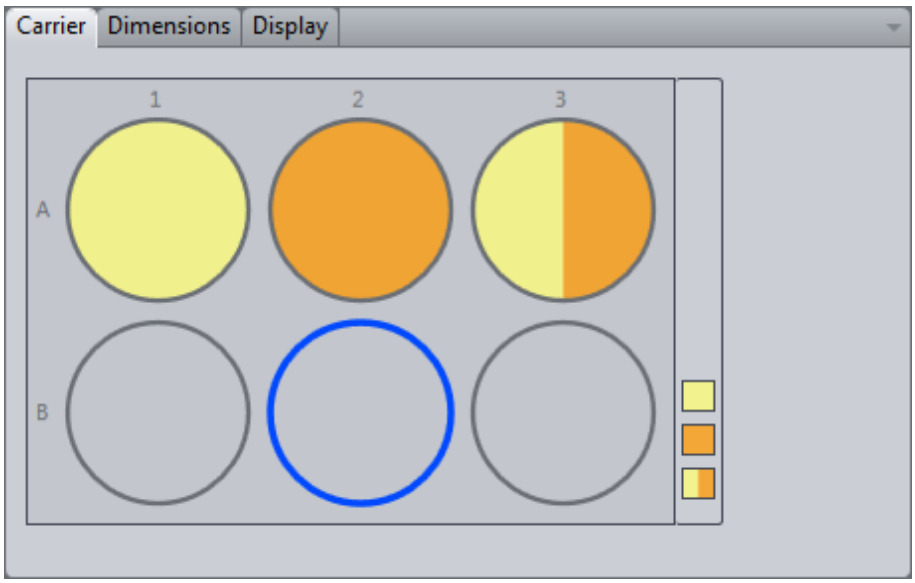
i Note

Note that the selected objective will now be used for any subsequent activities with Locate or Acquisition tab. Thus, you must actively change the objective after the preview scan if you want to use another objective for your experiment.

10.1.2.2.2 Carrier tab

Only visible if a sample carrier was selected.

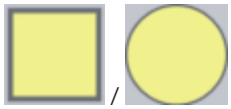
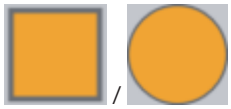
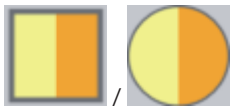
Here you can see a graphical preview of the sample carrier being used. Please note the following features of the display:



i Note

Only the containers / wells whose tile regions and positions were set up with the *Setup by Carrier* [▶ 181] of the **Tile Regions Setup** tab or the *Setup by Carrier* [▶ 185] of the **Positions Setup** tab will be taken into account.

Symbol	Description
/	**Empty** containers / wells, meaning that no tile regions or positions were set up with the Carrier option, are represented by a grey conatiner / well.
/	The currently **Active** container / well is represented by a blue border.

Symbol	Description
	A container / well only filled with **Tile Regions** is represented by a yellow filled conatiner / well.
	A conatiner / well only filled with **Positions** is represented by a orange filled conatiner / well.
	A container / well filled with **Tile Regions** and **Positions** is represented ba a half yellow, half orange filled conatiner / well.

i Note

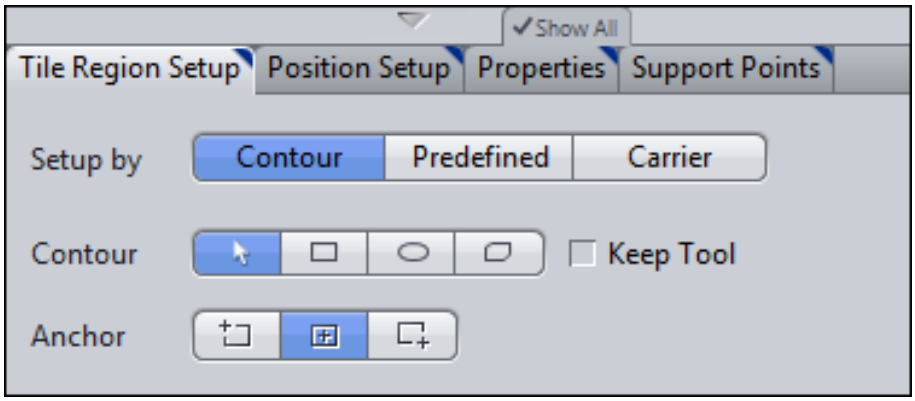
Right click opens a small context menu. Here you can copy the contents of the selected well, or paste the contents to the selected or all wells.

10.1.2.2.3 Tile Region Setup tab

Here you can select which setup you want to be used for the settings of the tile regions. Three setups with different setting options are available:

10.1.2.2.3.1 Setup by Contour

Here you can define the tile regions by means of the contour.



Contour section

Here you can select the contour of your tile region. The following tools are available:

Option	Description
Selection button 	With this tool you can select an already created tile region by clicking on it to move or edit it.
Rectangle button 	With this tool you can draw a rectangle tile region.
Ellipse button 	With this tool you can draw a elliptical tile region.
Polygon button 	With this tool you can draw a polygonal tile region.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

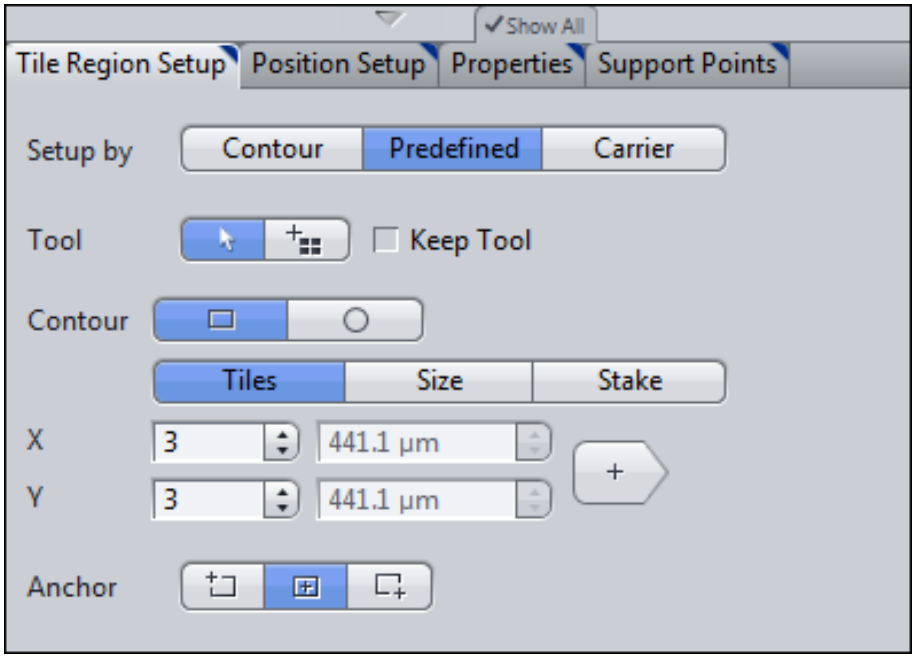
Anchor section

Here you can select the anchor position of the new tile region. The following tools are available:

Option	Description
Anchor Top Left button 	The anchor of the defined shape is at the top left.
Anchor Centered button 	The anchor of the defined shape is centered.
Anchor Bottom Right button 	The anchor of the defined shape is at the bottom right.

10.1.2.2.3.2 Setup by Predefined

Here you can define the tile regions by means of the number or size.



Tool section

Here you can select a tool to work with. The following tools are available:

Option	Description
Selection button 	Select an element in the stage view to edit or move it.
Add Tile Region button 	Adds the current tile definition in the image area.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

Contour section

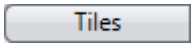
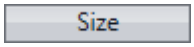
Only visible if the **Show All** mode is activated.

Here you can select the contour of your tile region. The following tools are available:

Option	Description
Rectangle button 	Adds a rectangular tile region.

Option	Description
Circle button 	Adds a circular tile region

Mode section

Option	Description
Tiles button 	Using this mode you have to enter the number of tiles as a reference for the size of the tile region. Enter the number of tiles in the **X / Y** input fields. If you are adding a circular tile region, enter the number of tiles for the diameter in the **Diameter** input field.
Size button 	Using this mode you have to enter the size as a reference for the size of the tile region. Enter the size of the tile region in the **X / Y** input fields. If you are adding a circular tile region, enter the diameter of the tile region in the **Diameter** input field.
Stake button 	This mode allows the definition of a tile region by the placement of at least two markers (user defined X/Y stage coordinates). If you want to modify the tile region (expand/ reduce) you have to adjust the tile region to the desired size. To complete the tile region press **Done**. Circular or rectangular tile region can be created in this manner by selection of the appropriate contour.
Add button 	Adds the tile region to the **Tile Regions List** and activates it for acquisition. Added tile regions are displayed in the form of red grids in the stage view of the **Advanced Tiles Setup**.

Anchor section

Only visible if the **Show All** mode is activated.

Here you can select the anchor position of the new tile region. The following tools are available:

Option	Description
Anchor Top Left button 	The anchor of the defined shape is at the top left.

Option	Description
Anchor Centered button 	The anchor of the defined shape is centered.
Anchor Bottom Right button 	The anchor of the defined shape is at the bottom right.

10.1.2.2.3.3 Setup by Carrier

Here you can define the tile regions automatically by means of the fill factor of the sample carrier.

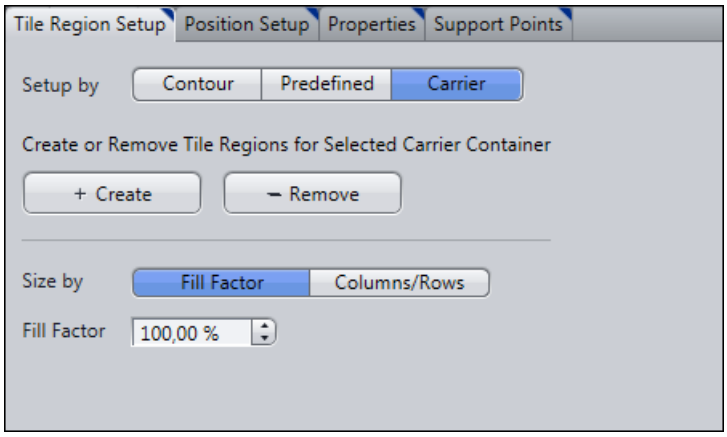
Note

A sample carrier must have been selected in the *Sample Carrier section* [▶ 157] of the **Tiles** tool.

Note

Manually created tile regions and positions (setup by **Contour** and setup by **Predefined**) will be deleted, if you switch to the setup by **Carrier**. If you want to combine manual and automatic setup, first use setup by **Carrier** and then switch to a manual setup.

Tile regions that are created automatically by setup by **Carrier**, are defined to a container and permanently assigned and locked by default, against manual edits. You can unlock the tile regions in the **Tiles** tool by selecting the desired tile region and click on the unlock button.



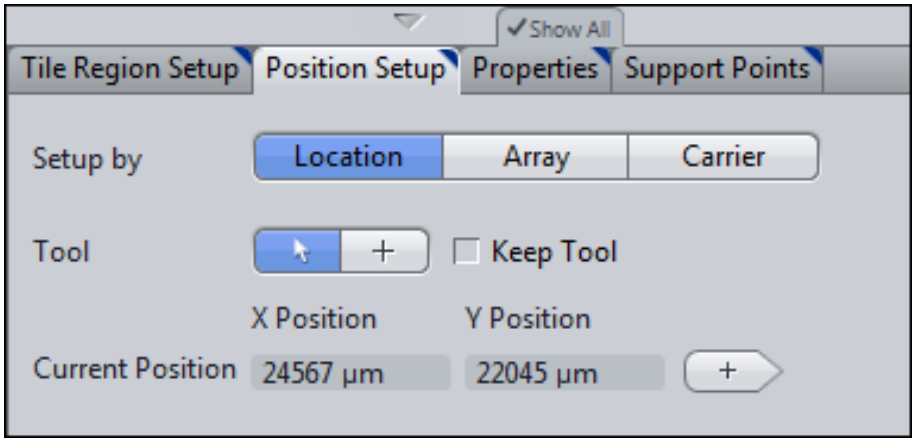
Option	Description
Create button + Create	Only active if you have selected a container on the *Carrier tab* [▶ 176] or in the *Stage View* [▶ 173]. Automatically creates the tile regions with the set fill factor in the selected container of the sample carrier.
Remove button – Remove	Removes all tile regions in the selected container.
Fill Factor input field Fill Factor	Here you can enter the fill factor used to fill the selected container.
Columns/Rows input field Columns/Rows	Here you can add single tile regions to a container by defining the number of columns and rows of the tile. The tile region is always placed at the center of the well container.

10.1.2.2.4 Position Setup tab

Here you can select which setup you want to be used for the settings of the positions. Three setups with different setting options are available:

10.1.2.2.4.1 Setup by Location

Here you can define the positions by means of the location. You can add various positions in the stage view using the mouse.



Tool section

Here you can select a tool to work with. The following tools are available:

Option	Description
Selection button	Select an element in the stage view to edit or move it.
Add button	Add a new position on the stage view.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

Current Position section

Displays the current stage position (X/Y).

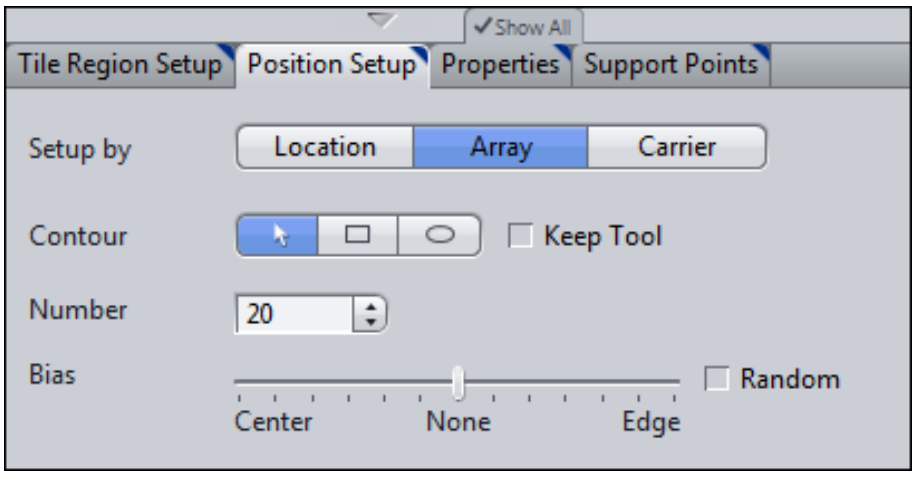
Option	Description
Add button	Adds a new position at the current stage position.

10.1.2.2.4.2 Setup by Array

Here you can define the positions by means of position arrays. You can add various contours for position arrays in the stage view.

Note

Position arrays are groups made up of a number of individual positions. Typically, position arrays contain several hundred individual positions. They make your work easier if you work with regular or evenly distributed samples.



Contour section

Here you can select the contour of your tile region. The following tools are available:

Option	Description
Selection button 	With this tool you can select an already created position array by clicking on it to move or edit it.
Rectangle button 	With this tool you can draw a rectangle position array.
Ellipse button 	With this tool you can draw a elliptical position array.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

Number section

Option	Description
Number input field	Shows the current number of positions that are distributed to newly created position array. Change the number to increase or decrease the number of single positions obtained by a position array.

Bias section

Only visible if the **Show All** mode is activated.

Here you can set the distribution bias of the single positions created for a new position array.

Option	Description
Bias slider	Adjusts the overall position of the single positions in the position array.
- **None**	The single positions of the position array will be distributed evenly within the array.
- **Center**	The single positions of the position array will mainly be distributed near to the center of the position array. Less positions will be at the edges of the array.
- **Edge**	The positions of the position array will be distributed to the edges of the array. Less positions will be in the center of the array.
Random checkbox	**Activated**: The single positions will mainly be distributed randomly within the position array. The overall bias will still be taken into account.

10.1.2.2.4.3 Setup by Carrier

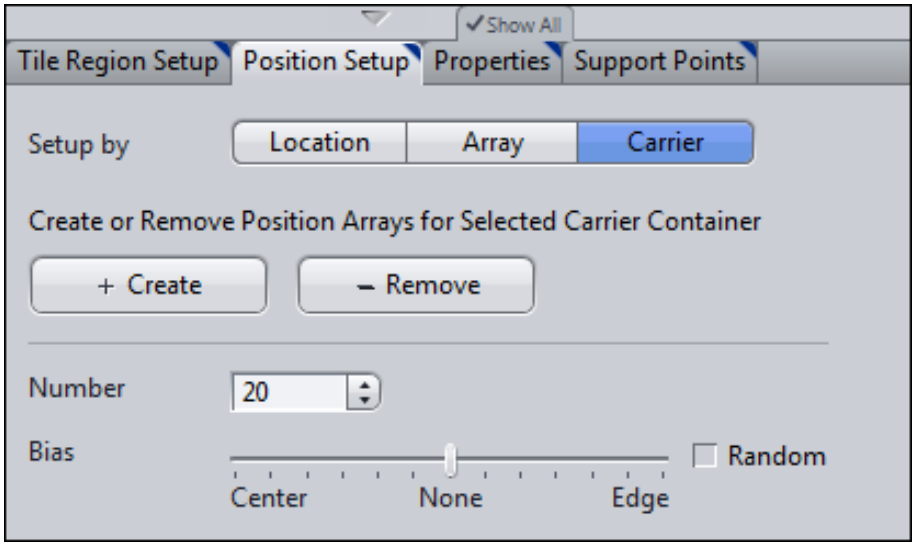
Here you can define the positions automatically by means of the relevant sample carrier.

i Note

A sample carrier must have been selected in the *Sample Carrier section* [▶ 157] of the **Tiles** tool.

i Note

Manually created tile regions and positions (setup by **Contour** and setup by **Predefined**) will be deleted, if you switch to the setup by **Carrier**. If you want to combine manual and automatic setup, first use setup by **Carrier** and then switch to a manual setup.



Option	Description
Create button + Create	Only active if you have selected a container on the *Carrier tab* [▶ 176] or in the *Stage View* [▶ 173]. Automatically creates the tile regions with the set fill factor in the selected container of the sample carrier.
Remove button – Remove	Removes all tile regions in the selected container.

Number section

Option	Description
Number input field	Shows the current number of positions that are distributed to newly created position array. Change the number to increase or decrease the number of single positions obtained by a position array.

Bias section

Only visible if the **Show All** mode is activated.

Here you can set the distribution bias of the single positions created for a new position array.

Option	Description
Bias slider	Adjusts the overall position of the single positions in the position array.
- **None**	The single positions of the position array will be distributed evenly within the array.
- **Center**	The single positions of the position array will mainly be distributed near to the center of the position array. Less positions will be at the edges of the array.
- **Edge**	The positions of the position array will be distributed to the edges of the array. Less positions will be in the center of the array.
Random checkbox	**Activated**: The single positions will mainly be distributed randomly within the position array. The overall bias will still be taken into account.

10.1.2.2.5 Properties tab

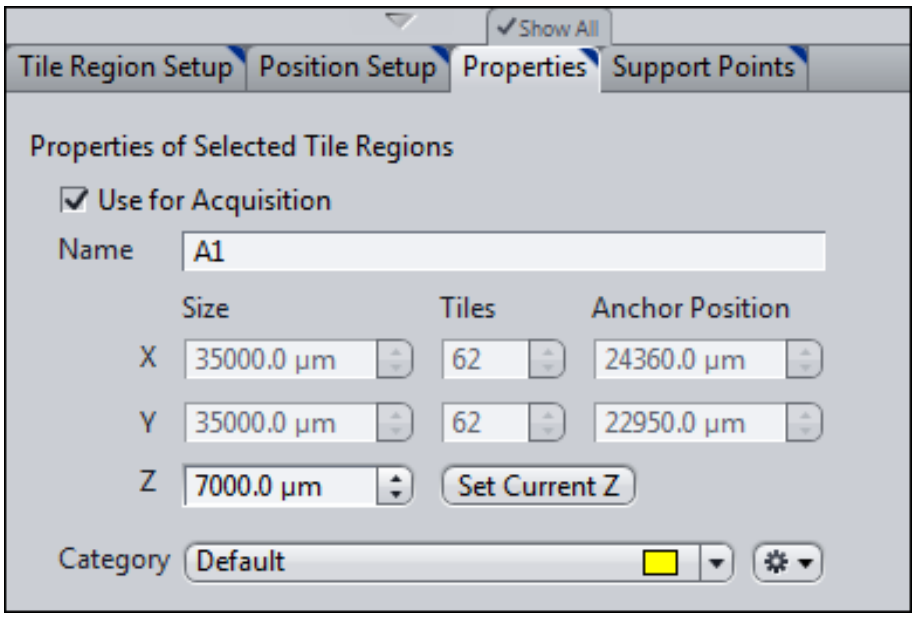
Here you can adjust the properties of a selected tile region or position.

i Note

You have to select a tile region or single position to see the parameters available on this tab. It is not possible to see and adjust the parameters for a whole position array.

10.1.2.2.5.1 Properties of selected Tile Regions

Here you can adjust the properties of the selected tile region.



Option	Description
Use for Acquisition checkbox	**Activated:** Uses the selected tile region for acquisition.
Name input field	Here you can enter a name for the selected tile region.

Properties section

Only visible if the **Show All** mode is activated.

The properties section contains the following columns and buttons:

Option	Description
Size column	Here you can see and edit the size of the tile region in the **X / Y / Z** dimensions. The **X / Y** dimensions of tile regions created with the *Setup by Carrier* [▶ 181] can not be edited as they are fixed by the container / well size.
Tiles column	Here you can see the number of tiles in the **X / Y** dimensions. You can not edit the number of tiles as it is fixed by the size of the tile region.

Option	Description
Anchor Position column	Here you can enter the anchor position of the selected tile region in **X / Y** dimensions. The anchor position of tile regions created with the *Setup by Carrier* [▶ 181] can not be edited as they are fixed by the container / well.
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

Category section

Only visible if the **Show All** mode is activated.

Here you can assign categories to tile regions. Category definitions will be displayed in the appropriate column of the table in the Tiles tool. This value is also written in the image meta-date. Thus, well definition patterns or variables can be created and stored as part of a experiment template.

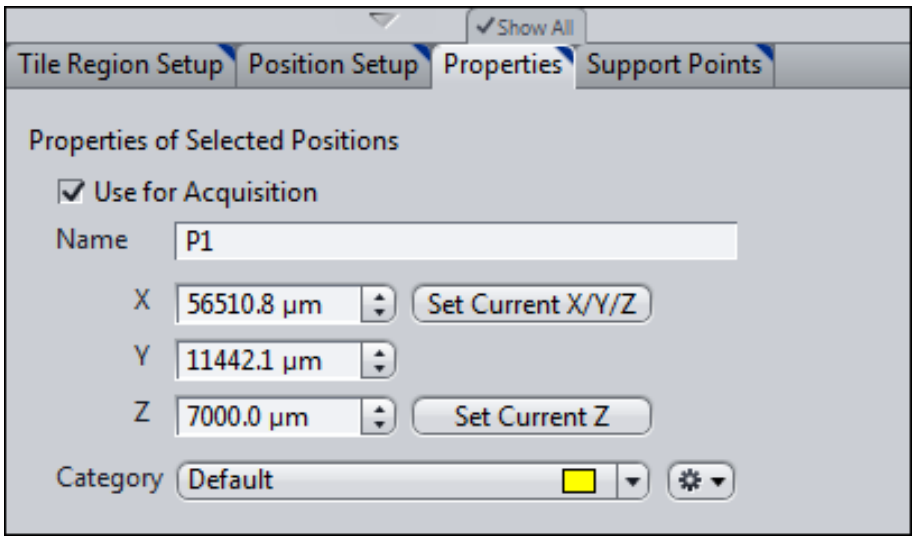
Option	Description
Category dropdown list	Shows the currently assigned category of the selected tile region. The **Default** category is set for all new tile regions. Click on  assign an other category.
Options button 	Opens the options for editing categories.

Options for editing Categories

Option	Description
New...	Opens the **New Category** dialog to create a new category.
Edit…	Opens the **Edit Category** dialog to edit the selected category.
Delete	Deletes the selected category and sets the category of the tile region to **Default**.

10.1.2.2.5.2 Properties of selected Positions

Here you can adjust the properties of the selected position.

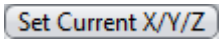
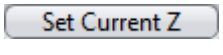


Option	Description
Use for Acquisition checkbox	**Activated:** Uses the selected position for acquisition.
Name input field	Here you can enter a name for the selected position.

Properties section

Only visible if the **Show All** mode is activated.

The properties section contains the following columns and buttons:

Option	Description
Position column	Here you can see and edit the position of the selected position on the stage in **X / Y / Z** dimensions.
Set Current X/Y/Z button 	Sets the **X / Y / Z** dimension at the current **X / Y / Z** position of the stage.
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

Category section

Only visible if the **Show All** mode is activated.

Here you can assign categories to tile regions. Category definitions will be displayed in the appropriate column of the table in the Tiles tool. This value is also written in the image meta-date. Thus, well definition patterns or variables can be created and stored as part of a experiment template.

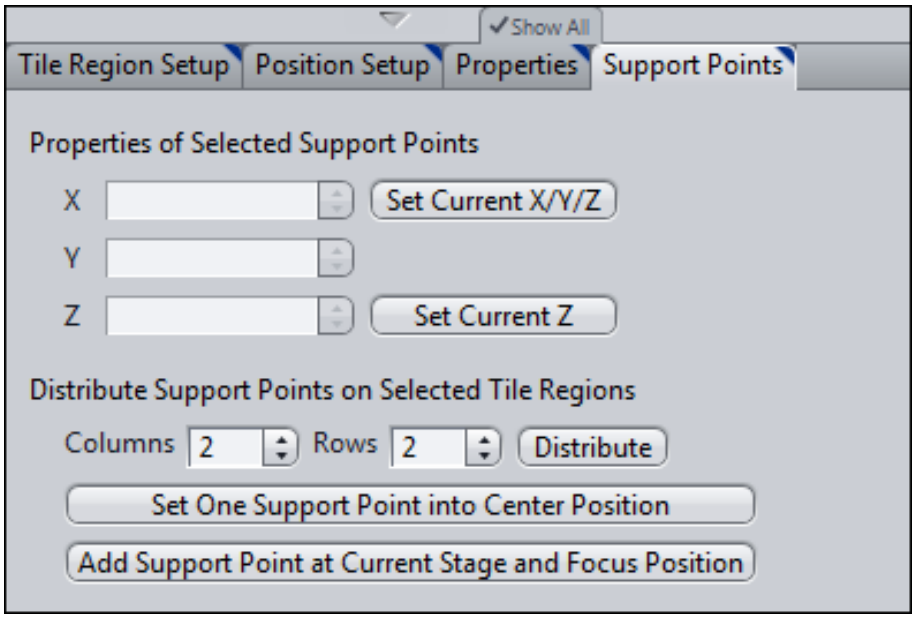
Option	Description
Category dropdown list	Shows the currently assigned category of the selected tile region. The **Default** category is set for all new tile regions. Click on assign an other category.
Options button	Opens the options for editing categories.

Options for editing Categories

Option	Description
New...	Opens the **New Category** dialog to create a new category.
Edit...	Opens the **Edit Category** dialog to edit the selected category.
Delete	Deletes the selected category and sets the category of the tile region to **Default**.

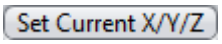
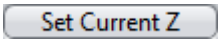
10.1.2.2.6 Support Points tab

Here you can adjust the properties of selected local and global support points.



Properties of Selected Support Point section

The properties of selected support point section contains the following columns and buttons:

Option	Description
Position column	**X / Y** dimensions are only visible if the **Show All** mode is activated. Here you can see and edit the position of the selected position on the stage in **X / Y / Z** dimensions.
Set Current X/Y/Z button 	Only visible if the **Show All** mode is activated. Sets the **X / Y / Z** dimension at the current **X / Y / Z** position of the stage.
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

i Note

Properties of Global Support Points

The properties of a selected global support point slightly differ from those of a local one as you can not edit the **X / Y** dimensions because they are fixed by the sample carrier template you have selected. Therefore there is no **Set Current X/ Y/Z** button for global support points.
Activating / Deactivating the **Show All** mode will not show / hide any additional options for global support points not even the options that are shown / hidden for local support points.

Distribute Support Points on Selected Tile Regions section

i Note

You can see this section only if you have selected a tile region or a local support point of a tile region. Selecting a local support point will prevent editing the section.

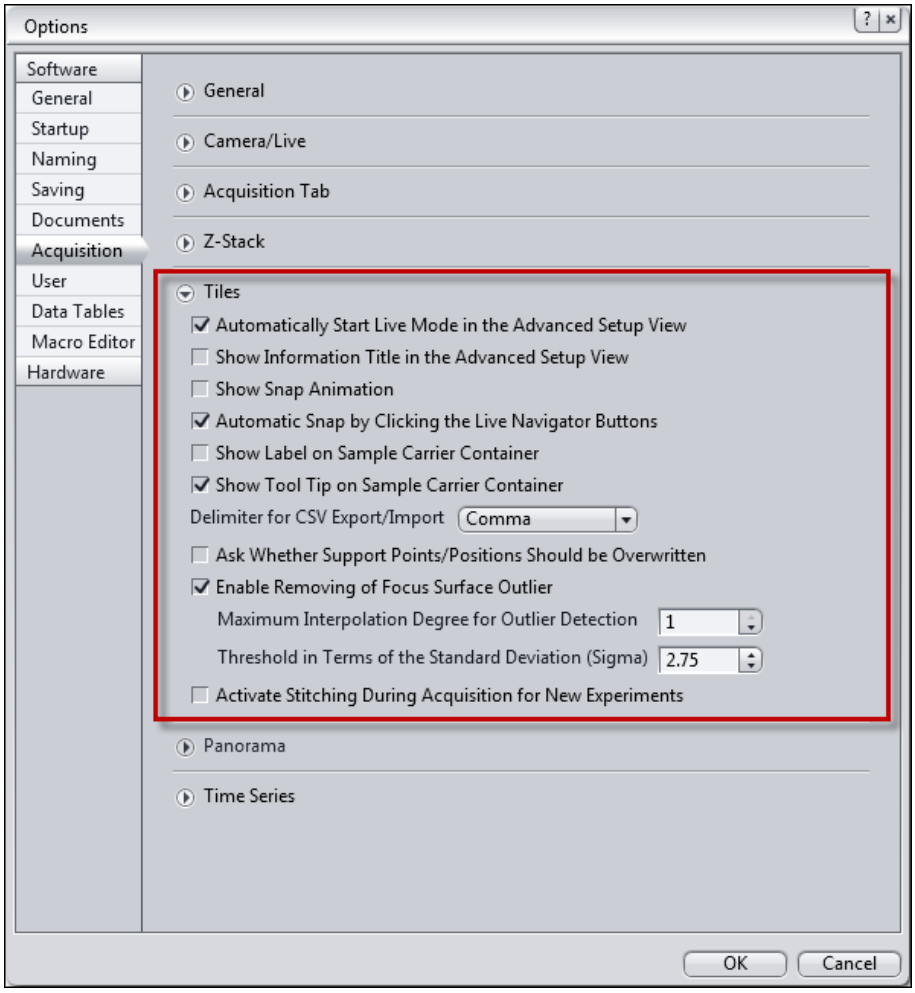
The distribute support points on selected tile regions section contains the following input fields and buttons:

Option	Description
Columns input field	Sets the number of columns of support points within the selected tile region.
Rows input field	Sets the number of rows of support points within the selected tile region.
Distribute button 	Distributes the entered number of support points defined in the column and row input fields within the tile region. Previously defined support points will be deleted.
Set One Support Point into Center Position button	Only visible if the **Show All** mode is activated. Sets one support point in the center of the selected tile region. Previously defined support points will be deleted.
Add Support Point at Current Stage and Focus Position button	Only visible if the **Show All** mode is activated. Adds a support point at the current stage and focus position. Does not affect previously defined support points.

10.1.3 Tiles Options

The additional options for the tiles module allow to set up several options for image acquisition and additional information. The tiles options dialog can be found in the menu bar under **Tools** | **Options...** | **Acquisition** | **Tiles**.

To show the section in full, click on the **arrow** button .



Option	Description
Automatically Start Live Mode in the Advanced Setup View checkbox	**Activated**: Automatically starts the **Live** mode in the live navigator tool when you open the advanced setup. Uncheck this option to prevent unnecessary specimen bleaching.
Additionally Open Snap Images as Separate Documents checkbox	**Activated**: Snap images created in the advanced tiles setup are opened additionally in a separate image containers, not just as a thumb nail in the preview area of the advanced tiles setup.

Option	Description
Show Information Title in the Advanced Setup View checkbox	**Activated**: Displays a bar abbove the Advanced Setup view containing additional information.
Show Snap Animation checkbox	**Activated**: Shows the snap animated when snapping a new image in Advanced Setup.
Automatic Snap by Clicking the Live Navigator Buttons checkbox	**Activated**: A snap will be taken every time the live navigator tool is moved with its navigation buttons.
Enable Stage Moving with Live Navigator Handle checkbox	In the Live navigator tool the current stage position including the live image is shown as a frame outlined in blue. To move the frame, double-click on the position to which you want to move it. The frame can also be used to control acquisition. **Activated**: If you click on one of the frame's blue arrow icons, an image is acquired. The Live Navigator tool is moved one frame width in the relevant direction. You can create tile images of your sample easily in this way.
Show Label on Sample Carrier Container checkbox	**Activated**: Shows a label on every container / well of a selected sample carrier.
Show Tool Tip on Sample Carrier Container checkbox	**Activated**: Shows a tool tip with the name of the container / well when the mouse is over it in the **Carrier** tab.
Delimiter for CSV Export / Import dropdown list	Specifies the delimiter for a CSV export or import. You can choose between **Comma** (default), **Semicolon** and **Tab**.
Ask Whether Support Points / Positions Should be Overwritten checkbox	When the support points and/ or positions are determined by a software autofocus run the existing points can be overwritten with the new **Z** values. **Activated**: Shows a message box asking if the points should be overwritten if there is a autofocus **Z** value.
Enable Removing of Focus Surface Outlier checkbox	**Activated**: Support points that are significantly outside the interpolated focus surface are ignored. You have the following setting options available:

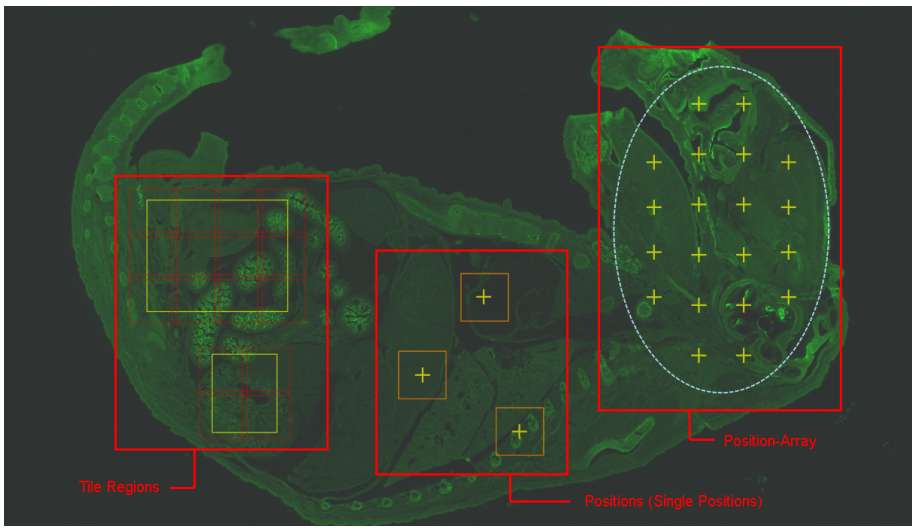
Option	Description
- **Maximum Interpolation Degree for Outlier Detection** input field	This value can be 0 or 1. If 1 then a linear fit is used to detect the outlier support points. This is the default. If 0 a simple average value is used to detect outliers.
- **Treshold in Terms of the Standard Deviation (Sigma)** input field	This parameter defines a threshold value to determine which of the support points are outliers from the fitting process. This is defined by the standard deviation (sigma value) set in the spin box. Support points not meeting this criteria are subsequently ignored when the focus surface is determined.
Activate Stitching During Acquisition for New Experiments checkbox	**Activated**: Stitching during acquisition is active by default for all new experiments. This value is overwritten by the corresponding option in the **Tiles** setup for a new experiment.
Use Local Focus Surface for Preview Scans checkbox	**Activated**: Local focus surface values (z-values of positions, tile regions and if defined interpolated focal surfaces defined by support points) will be used for the acquisition of preview scan images. Note that on activation of the Tiles dimension the appropriate strategy **Use Focus Surface Defined by Tiles Setup** is pre-selected.
Binning Compensation of Exposure Time in Preview Scans input field	Defines the power to which the binning ratio is modified to automatically determine the exposure time value used for a preview scan were the binning setting between the experiment and preview scan differs. The default value is 2.0 i.e. quadratic. Thus, for example the exposure time would be reduced by a factor of four if the experiment binning is 1x1 and the preview scan binning is 2x2. The value can be varied between 1.0 and 2.0 in steps of 0.1.
Live Image in Sample Carrier Calibration Wizard (relevant for systems with cameras)	

Option	Description
- **Use Imaging Device from Selected Channel with "Acquisition" Settings** radio button	**Activated**: Default setting for the live image that allows navigation and focus interaction during the carrier calibration wizard.
- **Use Active Camera with "Locate" Settings** radio button	**Activated**: Allows the user to alternatively apply locate camera settings for use in the carrier calibration wizard (live image). By default the experiment settings for the currently selected channel/ Track will be used. This option is only relevant for systems with a wide field (camera based) detector.

10.2 Working with Tiles and Positions

10.2.1 Introduction

Using the **Tiles** tool you can acquire images that are made up of a number of individual images (tiles). To do this, it is possible to define tile regions and positions. The **Tiles** module supplements the functions of the **Tiles** tool with the **Advanced Setup** feature. This allows you to set up **Tiles** experiments more easily and also to use sample carriers and focus surfaces.



Note

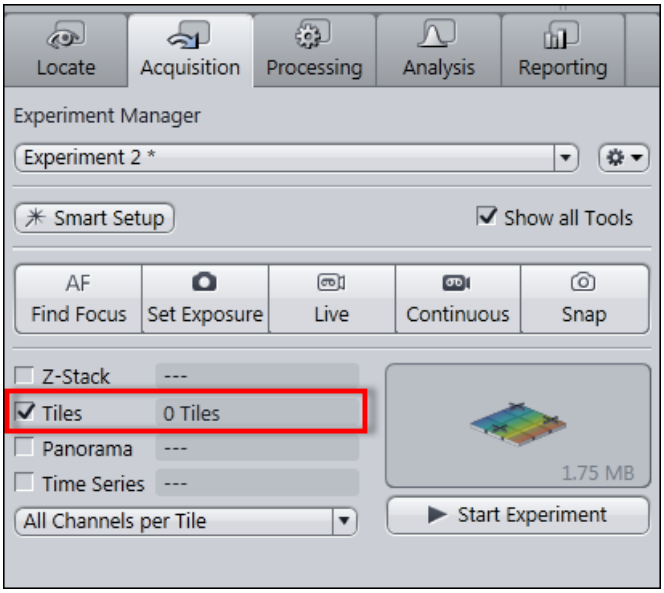
If you want to acquire tile regions or positions with different Z-positions, you need to use a suitable focus strategy. To do this, please first read: *Using focus strategies* [▶ 44].

Prerequisites

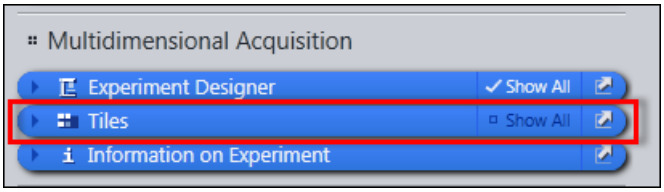
- To set up **Tiles** experiments, you require a motorized stage. This must be configured and calibrated correctly in accordance with the camera orientation. For more information read *Calibrating Stage and Selecting Channel* [▶ 197].
- You have *created a new experiment* [▶ 35], *defined at least one channel* [▶ 34] and correctly set the focus and exposure time.
- You are on the **Acquisition** tab.

Procedure

1 Activate the **Tiles** tool by activating the **Tiles** checkbox in the **Acquisition Dimensions** section.



In the **Left Tool Area** the **Tiles** tool appears under **Multidimensional Acquisition**.



You have successfully completed the general preparations. You can now set up **Tiles** or **Positions** experiments (see *Setting up a simple tiles experiment* [▶ 200].

10.2.1.1 Calibrating Stage and Selecting Channel

On start up of a system with motorized stage and/or focus a request will appear asking if the components should be driven to the end switches **calibrated**. This ensures that you beginning working with absolute coordinates in this session with the microscope. If the microscope power is cycled then this process should be repeated. This function is of particular use if you continually work with a sample carrier e.g. 96 well plate, of the same format mounted in the same manner

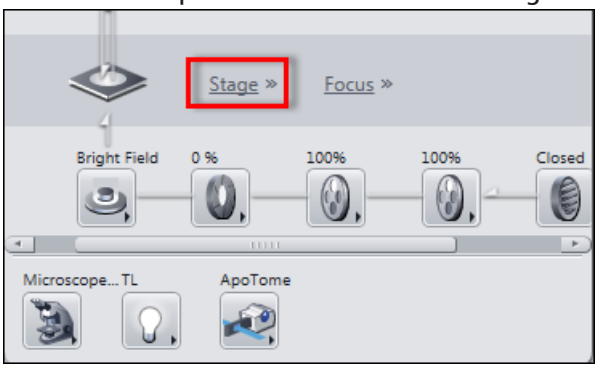
repeatedly with a given experiment template. If you perform a carrier calibration *Sample Carrier section* [▶ 157] once with a calibrated stage then the carrier calibration is essentially always valid.

i Note

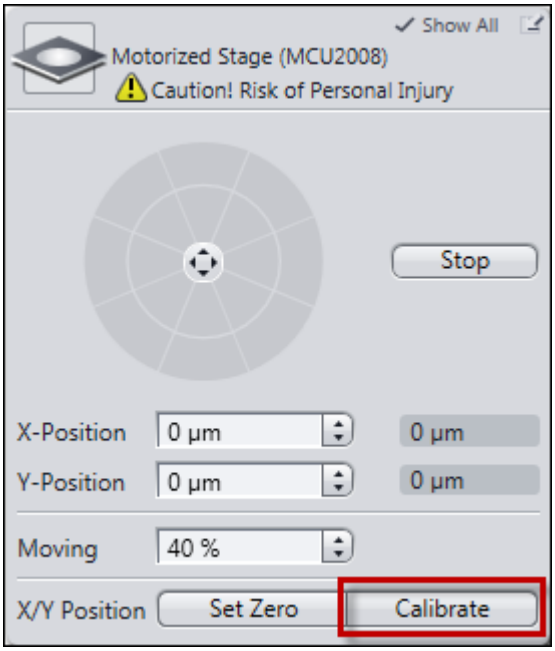
The request to calibrate stage and focus on Startup can be activated/deactivated under **Options** | **Startup** | **Stage/Focus Calibration**.

Procedure

1. Put your Sample Carrier on the stage.
2. Go to the **Locate** tab.
3. Choose a low magnification objective (e.g. 10x).
4. Click on the **Live** button and find your focus area either using transmission or fluorescence light.
5. In the **Microscope Control** tool click on the **Stage** button.



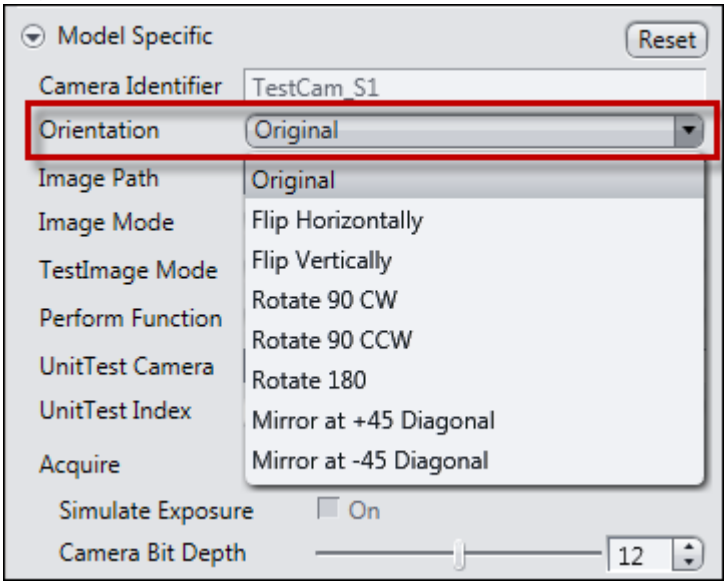
6. Activate the **Show all** mode and then click on the **Calibrate** button.



7 Check if the alignment of your camera and joystick is correct by dragging the software joystick up, down, left and right and observe whether the movement of your image corresponds to movement of the circle. In addition, check whether the image movement also corresponds accordingly when you move the joystick.

8 If the alignment is incorrect, go to the **Camera** tab activate the **Show all** mode and click **Model Specific**.

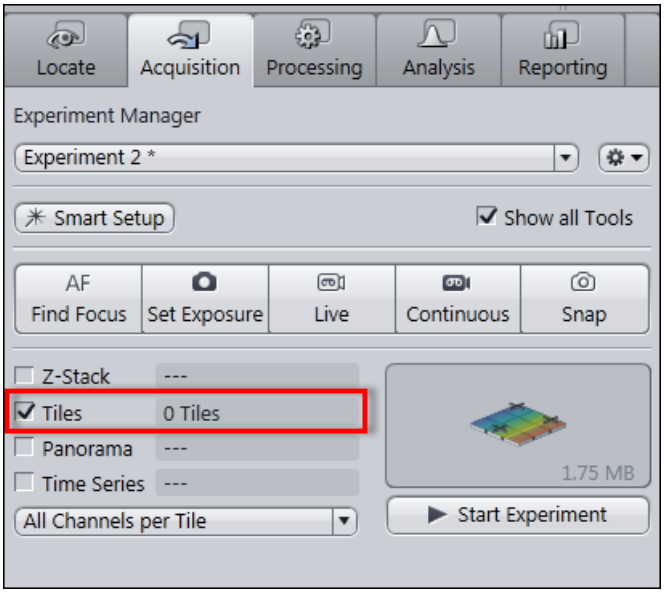
9 In **Orientation** you can now adjust the camera orientation to the joystick orientation. Alternatively, you can and may also invert X- and Y-axis of your stage in the **MTB** in order to align the joystick and the software-controlled stage movement.



10 Go to the **Acquisition tab**.

11 Do all the prerequisites (e.g. channel and camera settings) for a **Tiles & Positions experiment** on your sample. For that use Smart Setup and, if needed, Experiment Designer (not advisable for beginners).

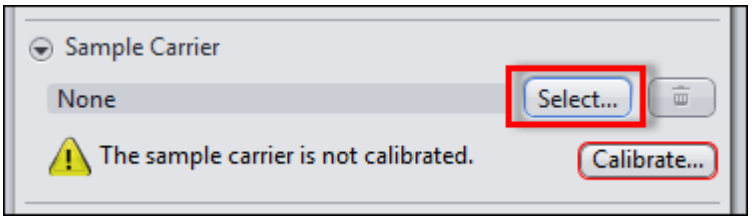
12 After you have defined at least one channel (e.g. EGFP), activate the **Tiles** module checkbox.



13 Open the **Tiles** tool in the **Multidimensional Acquisition** module and activate the **Show all** mode.

14 In the **Tiles** tool open the **Sample Carrier** section.

15 Click on the **Select...** button.



16 Choose a predefined **Sample Carrier template** and click **OK**.

10.2.2 Setting up a simple tiles experiment

Prerequisites

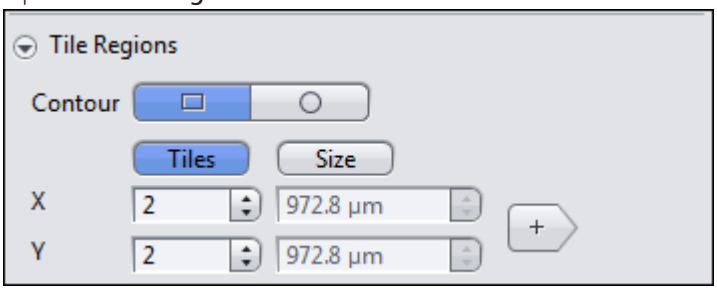
- You have read the chapter *Introduction* [▶ 196].
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1 Start the **Live** mode to use the stage to locate a point that you want to be at the center of your tile region.

2 Bring the specimen into focus using the focus drive.

3 Open the **Tile Regions** section.



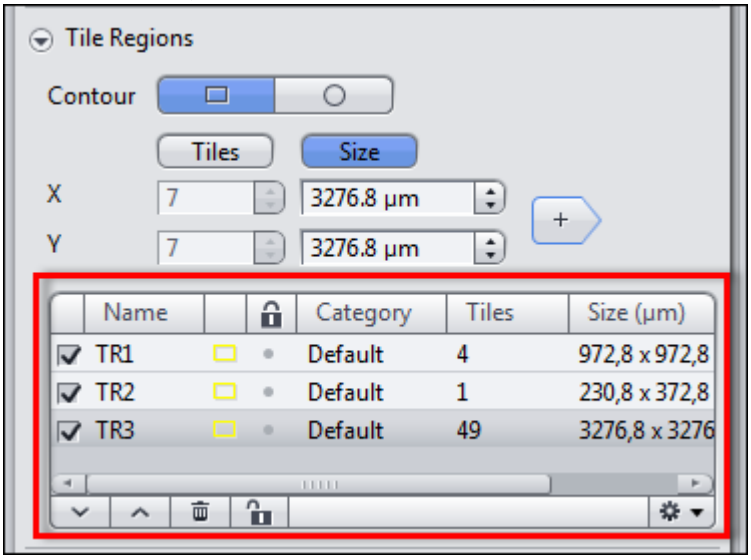
4 The **Tiles** mode is activated by default. In this mode enter the number of tiles you want in the **X** and **Y** input fields, e.g. **X** = 3, **Y** = 3 equals a tiles region containing 9 tiles.
Alternatively, you can enter the size of the tile region that you want to add. To do this, activate the **Size** mode.

5 Click on the **Add** button  .

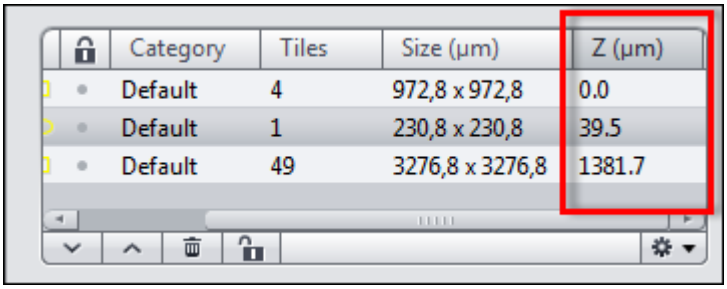
The tile region is added to your experiment. The current stage position determines the center and the Z-position of the tile region.

6 To add further tile regions, move the stage to another position on the sample and repeat the previous steps.

The added tile regions (**TR1**, **TR2**, etc.) are displayed in the tile regions list.



If you scroll to the right in the table, you can read the Z-position of the tile regions.



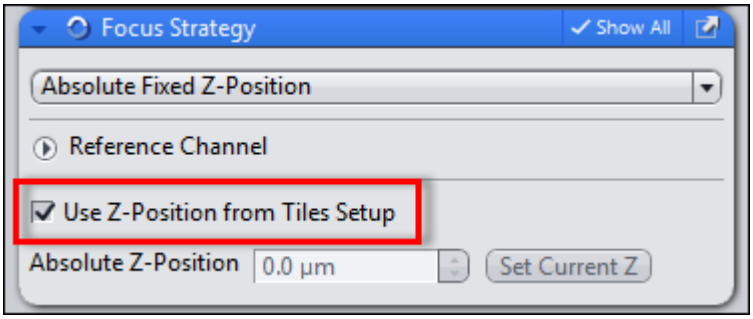
Note

To ensure that the individual Z-positions of the tile regions are taken into account ZEN automatically selects the most appropriate *focus strategy* [▶ 44] when the checkbox **Tiles** is activated. For the experiment described here no further modification needs to be made. If you want to acquire all tile regions at the same Z-position then you must select **None** from the dropdown list in the **Focus Strategy** tool. The individual Z-positions are then ignored and the current Z-position at the time the experiment is started is used for all tile regions.
The steps 7-9 are not necessary then.

7 In the **Left Tool Area** open the **Focus Strategy** tool on the **Acquisition** tab.

8 Select the **Absolute Fixed Z-Position** entry as the focus strategy from the dropdown list.

9 Activate the **Use Z-Position From Tile Setup** checkbox.



10 Save the experiment. To do this, in the **Experiment Manager** click on the **Options** button and select the **Save As** entry. Enter a name for the experiment in the input field (e.g. Simple Tile Experiment).

11 Click on the **Start Experiment** button.

The **Tile Region** experiment is acquired.

The individual tile regions are displayed in the acquired file as scenes and can be selected using the **Scene** slider on the **Dimensions** tab. If you deactivate the **Scene** checkbox, all tile regions are displayed as an overview.

You have successfully set up and acquired a simple **Tile Region** experiment.

10.2.3 Setting up a simple positions experiment

Prerequisites

- You have read the chapter *Introduction* [▶ 196].
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1 Open the **Positions** section.

2 Start the **Live** mode to use the stage to locate a position that you want to acquire.

The X and Y coordinates of the current position are displayed in the **Current Position** display fields.

3 Bring the specimen into focus using the focus drive.

4 Click on the **Add** button  .

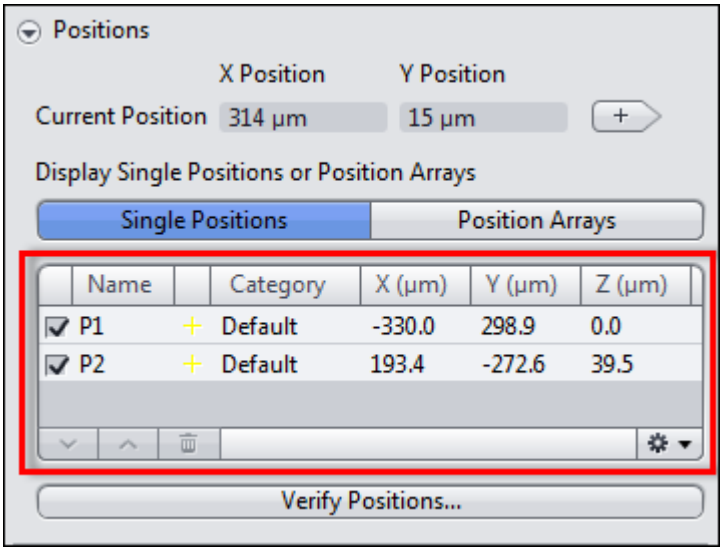
The current position is added to your experiment.

i Note

If you are about at the same position in the sample which has been added, then a message appears if you really want to add the last selected position.

5 To add further positions, move the stage to another position on the sample and repeat the previous steps.

The added positions are shown in the list in the **Single Positions** section with their X, Y and Z-coordinates.



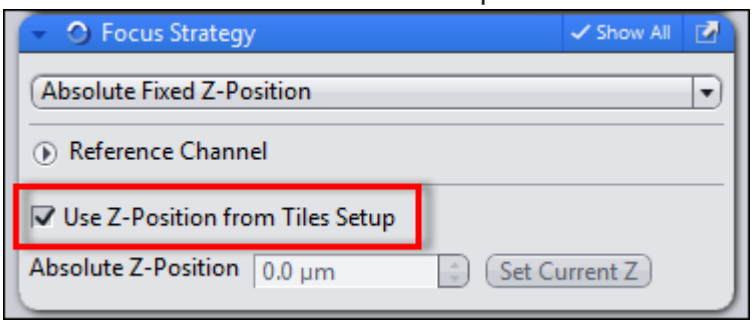
i Note

To ensure that the individual Z-positions of the tile regions are taken into account ZEN automatically selects the most appropriate *focus strategy* [▶ 44] when the checkbox **Tiles** is activated. For the experiment described here no further modification needs to be made. If you want to acquire all tile regions at the same Z-position then you must select **None** from the dropdown list in the **Focus Strategy** tool. The individual Z-positions are then ignored and the current Z-position at the time the experiment is started is used for all tile regions.
The steps 6-8 are not necessary then.

6 In the **Left Tool Area** open the **Focus Strategy** tool under **Acquisition Parameters**.

7 Select the **Absolute Fixed Z-Position** entry as the focus strategy from the dropdown list.

8 Activate the **Use Z-Position From Tile Setup** checkbox.



9 Save the experiment. To do this, in the **Experiment Manager** click on the **Options** button and select the **Save As** entry. Enter a name for the experiment in the input field (e.g. Simple Tile Experiment).

10 Click on the **Start Experiment** button.

The Positions experiment is acquired.

The individual positions are displayed in the acquired file as scenes and can be selected using the **Scene** slider on the **Dimensions** tab. If you deactivate the **Scene** checkbox, all positions are displayed simultaneously as an overview.

You have successfully set up and acquired a Positions experiment.

10.2.4 Tiles & Positions with Advanced Setup

Advanced Setup makes it easier for you to create tile regions and positions by displaying the distribution and dimensions of tile regions and positions in the travel range of the stage. You can generate a **Preview Scan** and drawn in tile regions or positions precisely on the basis of this template. For the preview scan you have the option of using an objective with a lower magnification and/or a different channel (e.g. transmitted light).

i Note

To ensure that the individual Z-positions of the tile regions are taken into account ZEN automatically selects the most appropriate *focus strategy* [▶ 44] when the checkbox **Tiles** is activated. For the experiment described here no further modification needs to be made. If you want to acquire all tile regions at the same Z-position then you must select **None** from the dropdown list in the **Focus Strategy** tool. The individual Z-positions are then ignored and the current Z-position at the time the experiment is started is used for all tile regions.

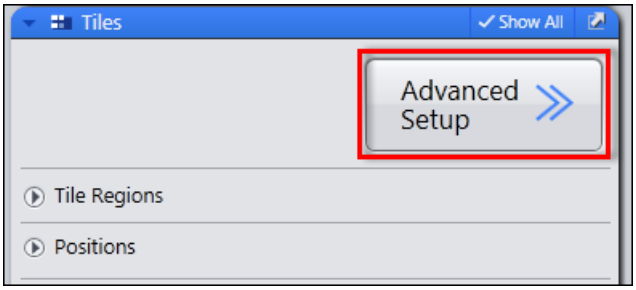
Prerequisites

- To set up tiles experiments in **Advanced Setup**, you need the **Tiles** module.

- You have read the chapter *Introduction* [▶ 196].
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1. Click on the **Advanced Setup** button.



The **Advanced Tiles Setup** view opens.

The Live mode is activated automatically. Deactivate the live mode if you do not need it to prevent bleaching of the sample. To do this, click on the active **Stop** button in the **Left Tool Area**.

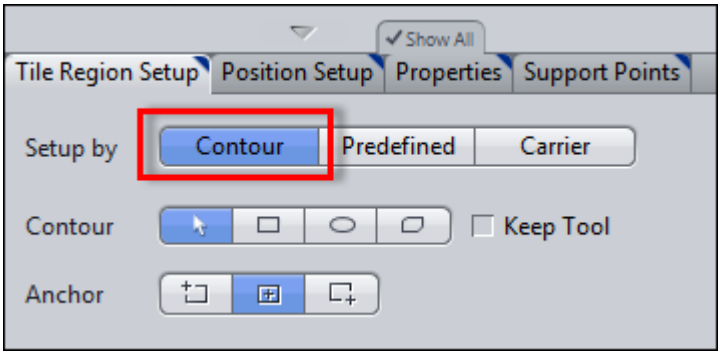
10.2.4.1 Generating a preview scan

Prerequisites

- You are in **Advanced Setup** in the **Tiles** tool.

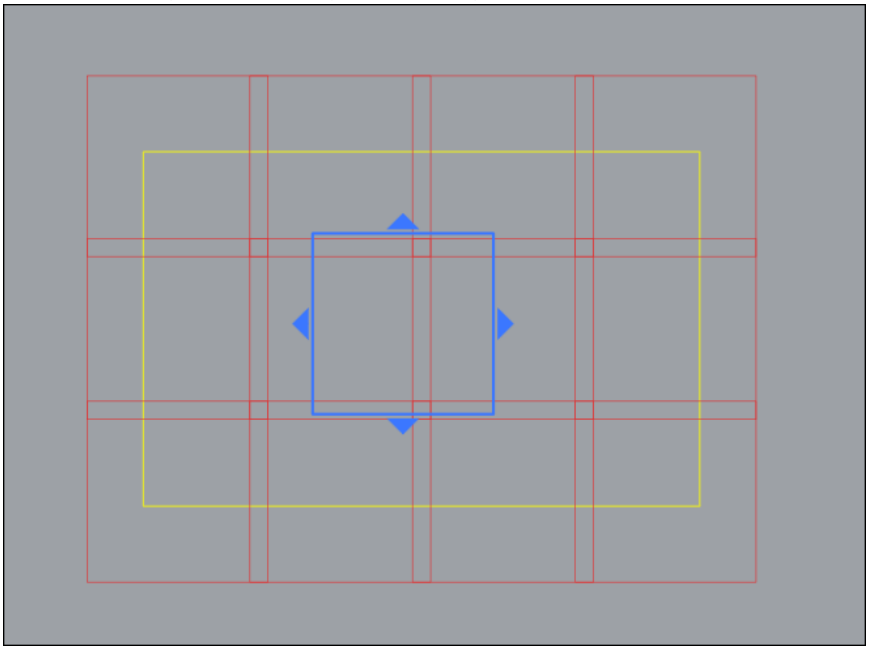
Procedure

1. In the **Specific View options** area open the **Preview Scan** tab.
2. Select an objective with a relatively low magnification.
3. In the **Left Tool Area** select a channel in the *Channels tool* [▶ 407] that you want to use for the preview scan. Deactivate the other configured channels.
4. If necessary, use the Live mode to adjust the focus area and exposure following a change of objective or channel.
5. To obtain a better overview, zoom out of the **Advanced Setup** view slightly.
6. Start the **Live** mode to use the stage to locate approximately the center of the region for which you want to generate a preview scan.
7. Select the **Tile Region Setup** tab from the **Advanced Setup** view options.
8. Under **Setup by** click on the **Contour** button.



9. Under **Contour** select the **Rectangular Contour** tool.

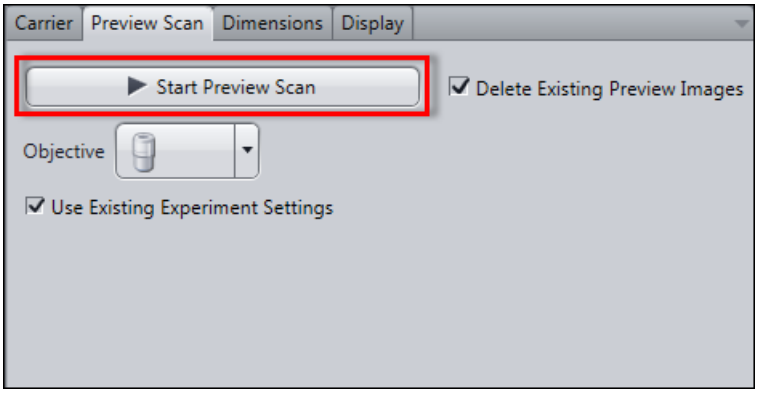
10 In the stage view, use the tool to drag out a rectangle that approximately encloses the region for which you want to generate a preview scan.



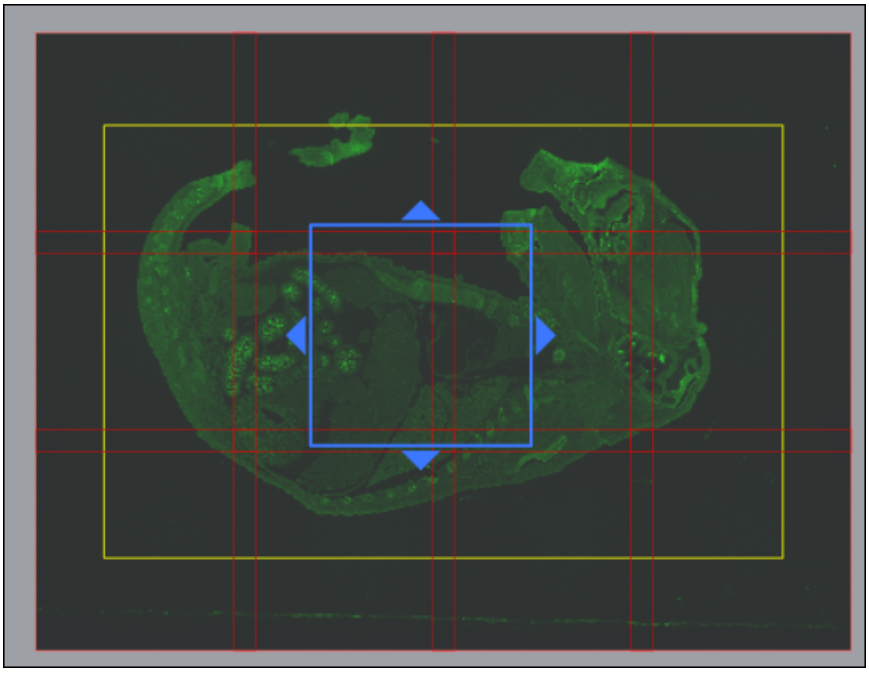
A tile region is created for the marked region and displayed in the list in the **Tile Regions** section of the **Tiles** tool.

11 With the help of the **Live** mode, check whether the desired image region is covered by the tile region. To do this, use the stage to locate the corners and edges of the tile region and increase or reduce the yellow selection frame as necessary.

12 In the **Preview Scan** tab click on the **Start Preview Scan** button.



A series of snap images is acquired to generate a preview of the marked region.

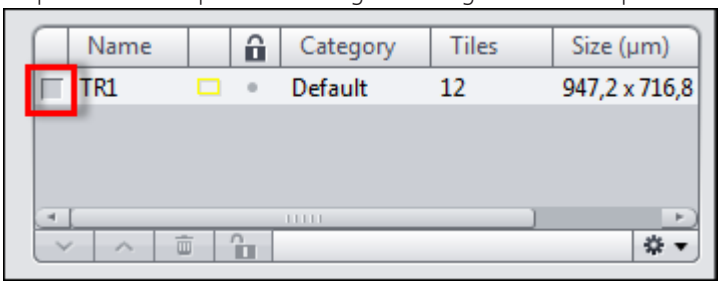


You have successfully generated a preview scan.

Before you continue with the actual experiment, carry out the following steps:

Procedure

1. In the **Tiles Regions** section deactivate the preview tile region (TR 1) by deactivating the checkbox of the corresponding list entry. This prevents the acquisition of the preview tile region during the actual experiment.



2. In the **Preview Scan** tool select the objective you want to use for final acquisition.
3. In the **Channels** tool activate the channels for actual acquisition.
4. Use the **Live** mode to adjust the **focus area** and **exposure** accordingly.

You can now continue setting up the tile experiment.

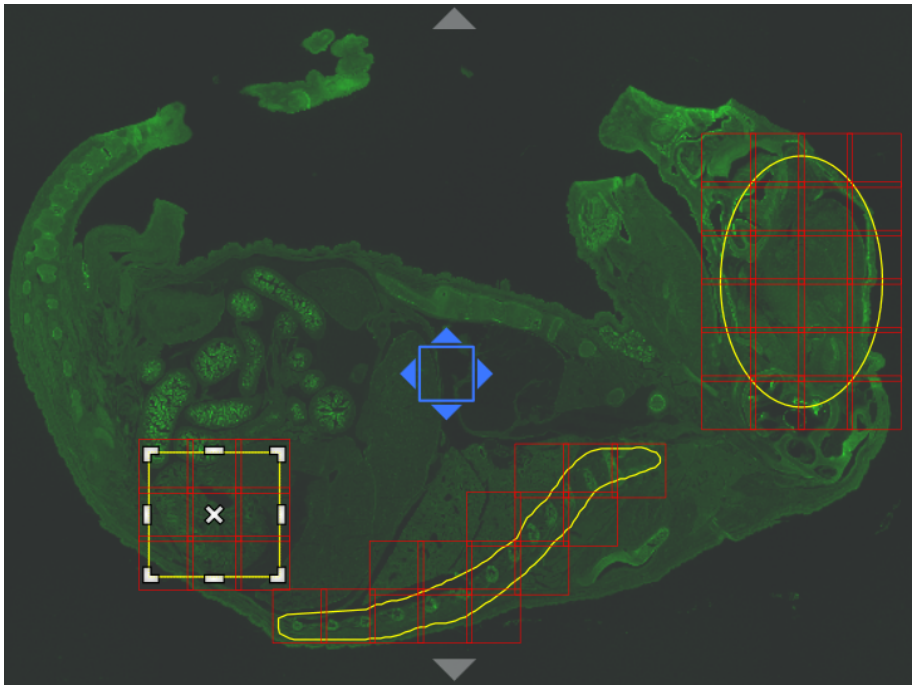
10.2.4.2 Creating tile regions by Contour

Prerequisites

- You have generated a *preview scan* [▶ 205] that will help you to position the tile regions more easily.

Procedure

1. Select the **Tile Region Setup** tab from the **Advanced Setup** view options.
2. Under **Setup by** select **Contour**.
3. In the **Contour** section select the desired contour tool.
4. Use the **Contour** tool in the stage view to draw in the tile regions you want to acquire.



Tile regions are created for each marked region. They are added to the list in the **Tile Regions** section of the **Tiles** tool.

You have successfully created tile regions in **Advanced Setup**.

10.2.4.3 Creating tile regions by Predefined

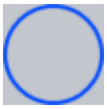
Prerequisites

- You are in **Advanced Setup** in the **Tiles** tool.

Procedure

1. In the **Carrier** tab (lower left side, below the Center Screen area) select the well(s) of interest by holding **Ctrl-key** and clicking on the desired wells.

   The selected wells are now bordered by a blue circle.

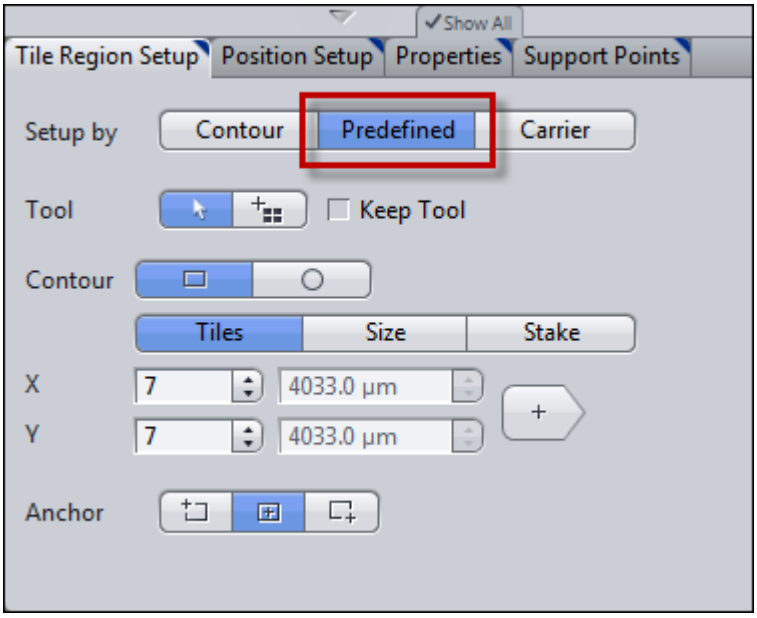


i Note

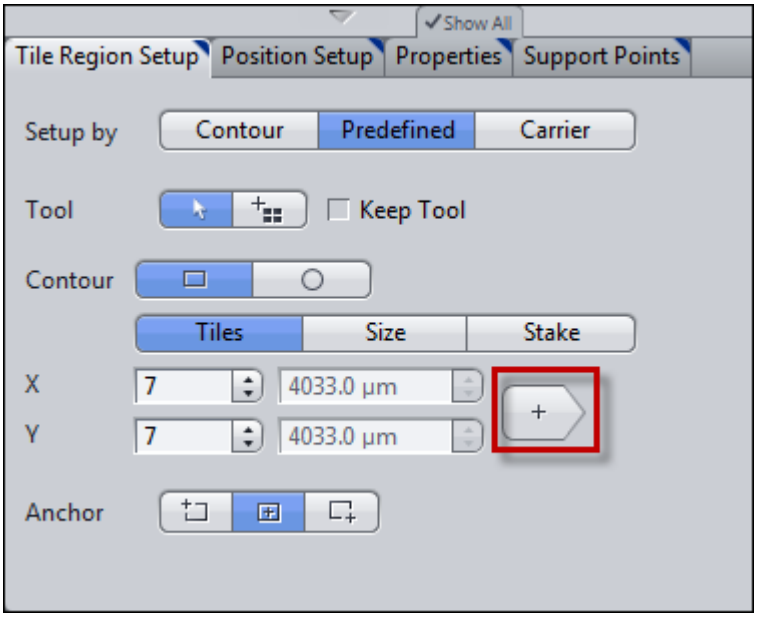
If only one well is double-clicked, the stage will move to the center of that well.

2 Open the **Tile Region Setup** tab below the Center Screen Area and activate the **Show All** mode.

3 In the **Tile Region Setup** tab under **Setup by** select **Predefined**.



4 Choose how many tiles in x and y dimension you want to add and click on the **Add** button + to confirm.



The predefined Tile Region is now created top left, centered or bottom right in the chosen wells depending on the anchor position .

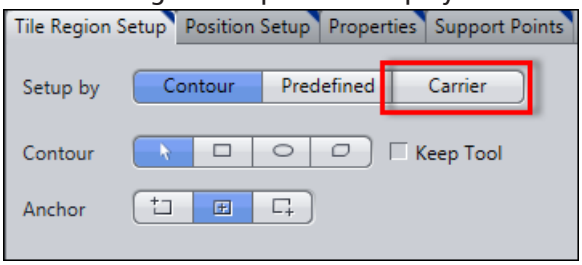
10.2.4.4 Creating tile regions by Carrier

Prerequisites

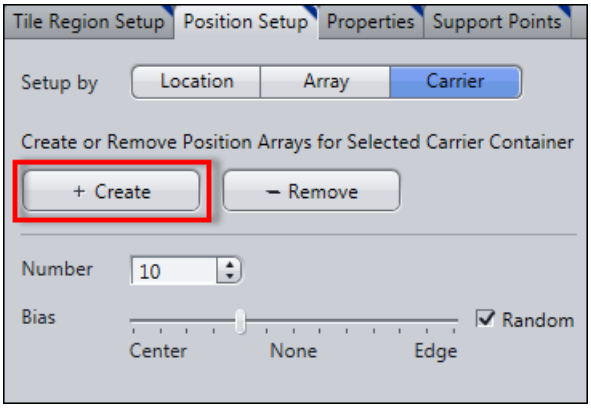
- You are in **Advanced Setup** in the **Tiles** tool.

Procedure

1. Open the **Tile Region Setup** tab below the Center Screen Area and activate the **Show All** mode.
2. In the **Tile Region Setup** under **Setup by** select **Carrier**.



3. In the **Carrier** tab select the individual wells for which you want to create Tile regions by holding **Ctrl-key** and clicking the desired wells.
4. In the **Position Setup** tab enter the size of your desired area per well in the **Fill Factor** input field and click on the **Create** button.



According to the selected **Fill Factor**, the wells will be filled with a calculated number of tiles that are located around the center.

10.2.4.5 Creating positions by Location

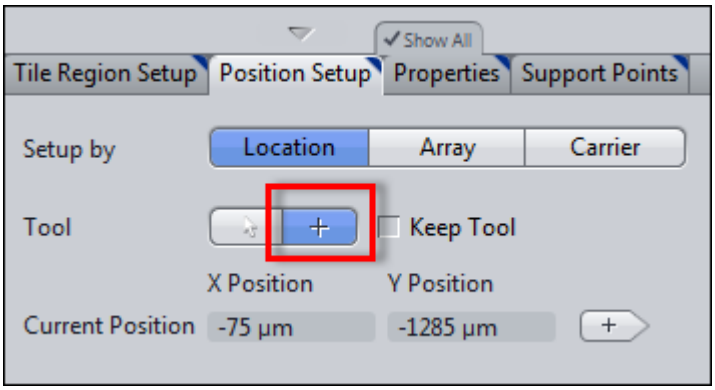
Prerequisites

- You are in **Advanced Setup** in the **Tiles** tool.

Procedure

1. Select the **Position Setup** tab from the **Advanced Setup** view options.
2. Under **Setup by** click on the **Location** button.

3 In the **Tool** section select the **Add** tool.



4 In the stage view click on the location at which you want to add a position.

The added positions are displayed in the **Single Positions** list in the **Positions** section of the **Tiles** tool.

You have successfully created positions in **Advanced Setup**.

10.2.4.6 Creating positions by Array

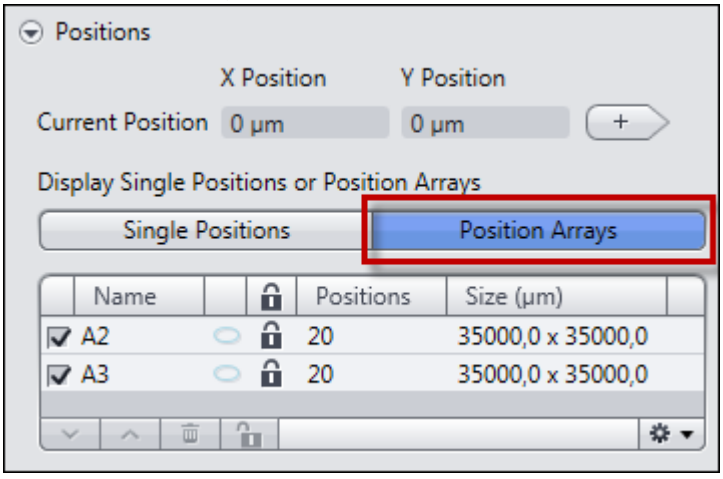
Prerequisites

- You are in **Advanced Setup** in the **Tiles** tool.

Procedure

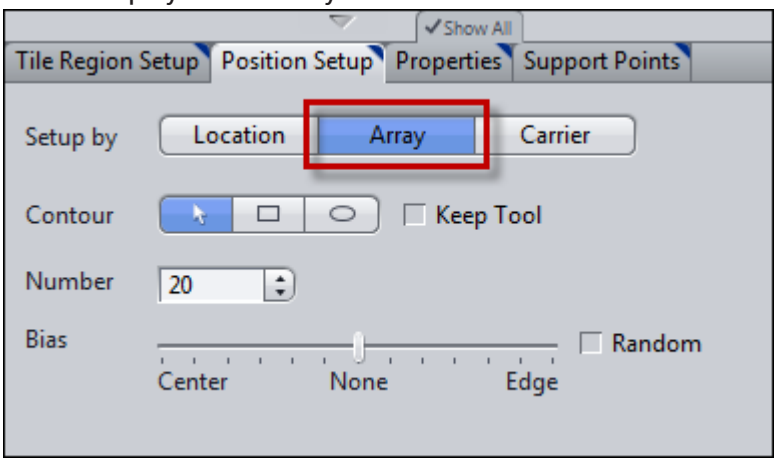
1 In the **Tiles** module go to the **Positions** section.

2 Select **Position Arrays**.



3 Go to the **Position Setup** tab (below the Center Screen Area) and activate the **Show All** mode.

4 Under **Setup by** click on **Array**.



5 Either choose the rectangular or circular **Contour**, adjust the **Number** of required positions and the **Bias** where the positions should be located.

i Note

If the **Random** checkbox is activated the chosen number of positions for the array will be determined randomly within the arrays space.

6 Subsequently, mark the interesting area of the carrier in the Center Screen Area by keeping the left mouse button clicked.

The positions will be automatically generated.

10.2.4.7 Creating positions by Carrier

Prerequisites

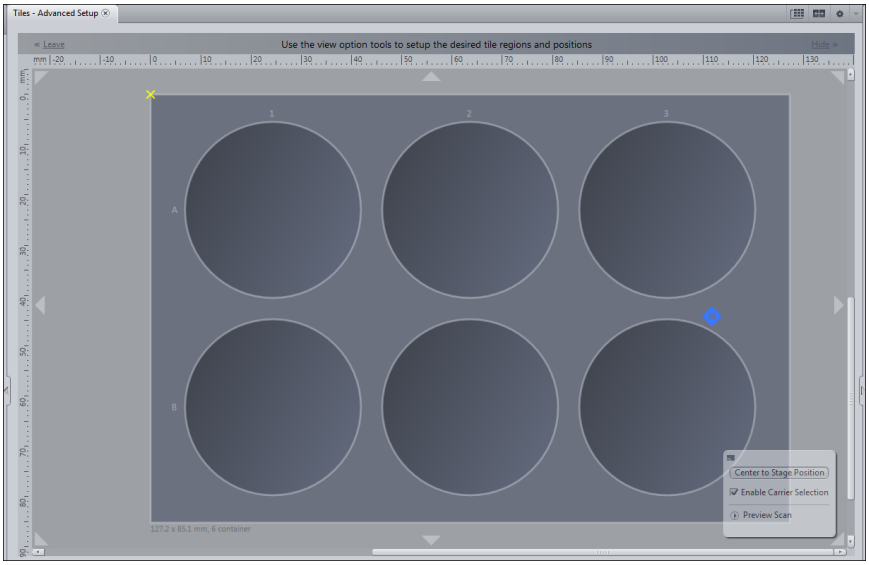
- You have selected and calibrated a *sample carrier template* [▶ 236].
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1. Click on the **Advanced Setup** button.

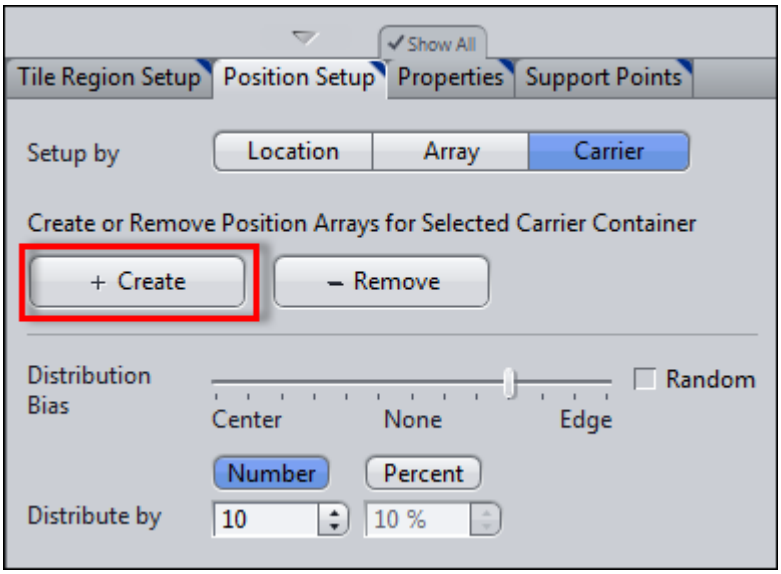
   Advanced setup is opened.

2. To obtain a complete overview of the sample carrier, zoom out of the view (**Crtl** + Mouse wheel).

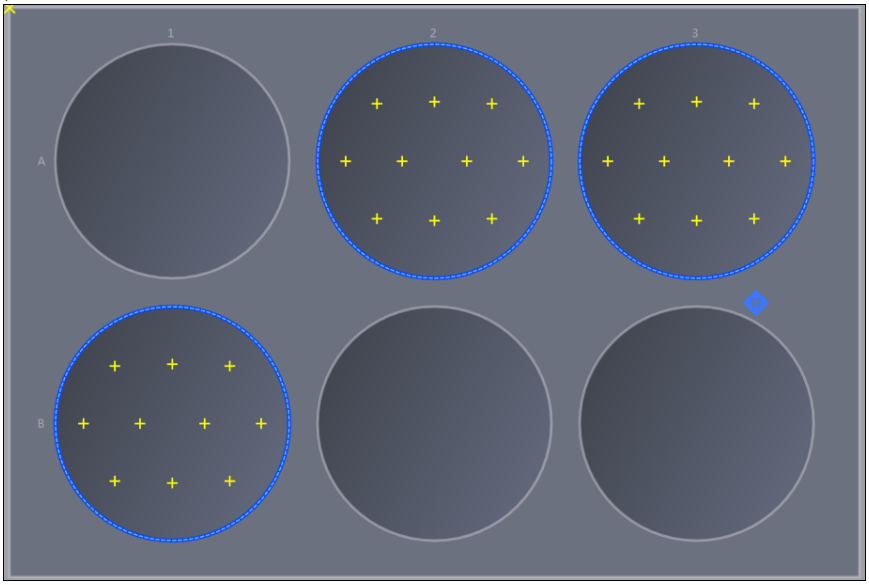


3. In the view options select the **Position Setup** tab.
4. In the **Setup by section** click on the **Carrier** button.
5. Select the **containers** in which you want to distribute **positions** by holding down the **Ctrl** key and clicking on the relevant containers.

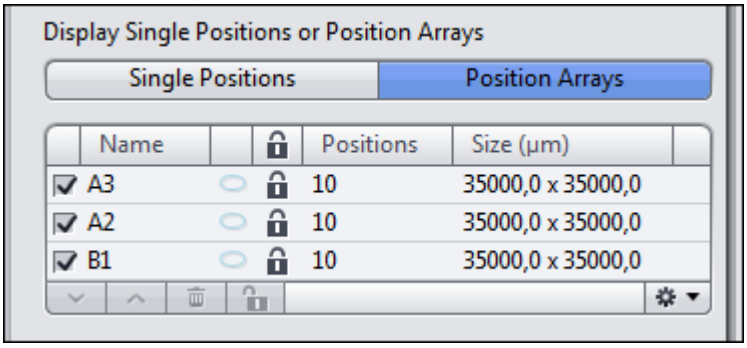
6 Click on the **Create** button.



The selected **containers** are each filled with a **Position Array** (group of positions).



In the **Positions** section of the **Tiles** tool the **Position Arrays** are displayed in the **Position Arrays** list.



You have successfully used a **sample carrier** and the **Setup by Carrier** to create positions.

> **Note**
>
> Analogous to the **Position Arrays** tile regions can also be created on the**Tile Region Setup** tab by using the **Carrier** button. In both cases, you can use the additional functions of the carrier setups to make other useful settings. For example, the patch surface of containers or the number and distribution of positions can be set, see *Setup by carrier (tile region)* [▶ 181] and *Setup by carrier (position)* [▶ 185].

10.2.5 Copying a Tile Region or Position

When you want to copy and paste a Tile Region or Position setting (e.g. a certain arrangement of tiles, positions or local support points) from one well to other wells or even to all containers of a carrier, apply the following workflow.

Procedure

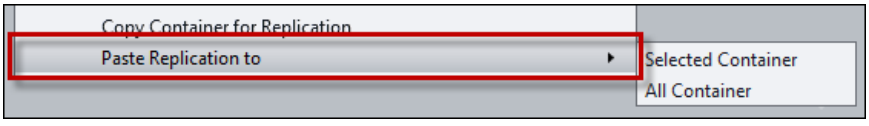
1. Select the well from where the Tile Region/ Position setting should be copied.

   The selected well is now highlighted by a blue border.

2. Right click within the selected well in the Center Screen Area (outside the tile region) to open the context menu.
3. Select **Copy Container for replication**.
4. If you want to choose specific wells and not all use the left mouse button to select the wells into which you want to paste the copied Tile Region/ Position setting.

> **Note**
>
> You can select multiple wells in combination with the **Ctrl**-key.

5. Right click in the Center Screen Area and select the context menu entry **Paste Replication to** and either choose **Selected Container** or **All Container**.



The copied Tile Region/ Position setting is pasted into the selected wells or all the wells of the carrier with the same relative coordinates to the center of each well.

10.2.6 Adjusting Z-positions

If you add positions or tile regions, the current Z-position is automatically adopted for the tile region or position.

- You can read about how to check and change the Z-positions of positions under *Adjusting Z-positions of positions* [▶ 217].
- You can read about how to check and change the Z-positions of tile regions under *Adjusting Z-positions of tile regions* [▶ 216]. Please bear in mind that the Z-positions defined here are valid for all tiles in the tile region in question.

To acquire large tile regions on tilted or uneven specimens, you need to assign individual Z-values to the individual tiles of a tile region. You do this by *Creating a local focus surface* [▶ 219]. Please bear in mind that a **Local Focus Surface** is always associated with precisely one tile region. You therefore need to create a focus area separately for each tile region.

To create a focus area covering the entire sample, create a Global Focus Surface. **Global Focus Surfaces** are based on a sample carrier template (e.g. for slides or multiwell plates) and result in a focus surface that is valid for the entire sample carrier and therefore for all the tile regions and positions it contains. This allows you to compensate for any tilting and bending of the sample carrier.

i Note

To ensure that the individual Z-positions of the tile regions are taken into account ZEN automatically selects the most appropriate *focus strategy* [▶ 44] when the checkbox **Tiles** is activated. For the experiment described here no further modification needs to be made. If you want to acquire all tile regions at the same Z-position then you must select **None** from the dropdown list in the **Focus Strategy** tool. The individual Z-positions are then ignored and the current Z-position at the time the experiment is started is used for all tile regions.

10.2.6.1 Adjusting Z-positions of tile regions

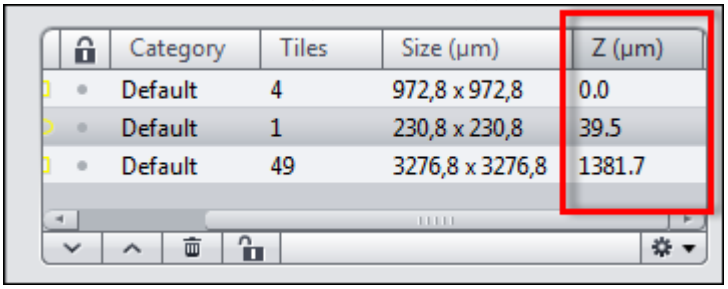
Prerequisites

- You have set up a **Tiles** experiment with at least one tile region. Further information on this can be found under: Set up tiles and positions experiments.

Procedure

1. To check the Z-position of tile regions, open the **Tile Regions** section in the **Tiles** tool.

   The Z-positions of the tile regions are displayed in the last column of the list.



2 Double-click on the list entry of the tile region that you want to check.

The stage automatically locates the center of the tile region and the associated Z-position.

3 Use the Live mode to check the Z-position of the tile region.

4 To adjust the Z-position, set the new Z-position using the focus drive.

5 In the **Tile Regions** list click in the bottom right on the **Options** button and select **Set Current Z For Selected Tile Regions**.

6 To check further tile regions, repeat steps 2 to 4.

You have successfully checked and adjusted the individual Z-positions for **tile regions**.

10.2.6.2 Adjusting Z-positions of positions

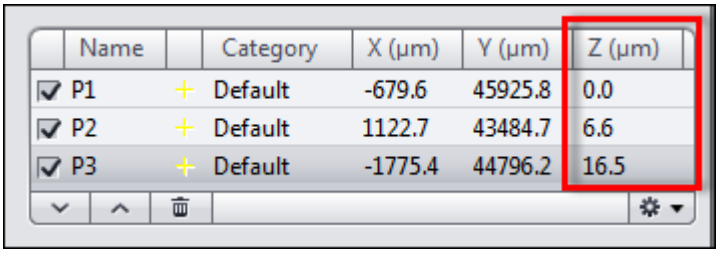
Prerequisites

- You have set up a tile experiment with at least one position. Further information on this can be found under: Set up tiles and positions experiments.
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1 To check and adjust the Z-position of positions, open the **Positions** section.

The Z-positions are displayed in the last column of the **Single Positions** list.



2 Double-click on the list entry of the position that you want to check.

The stage automatically locates the position.

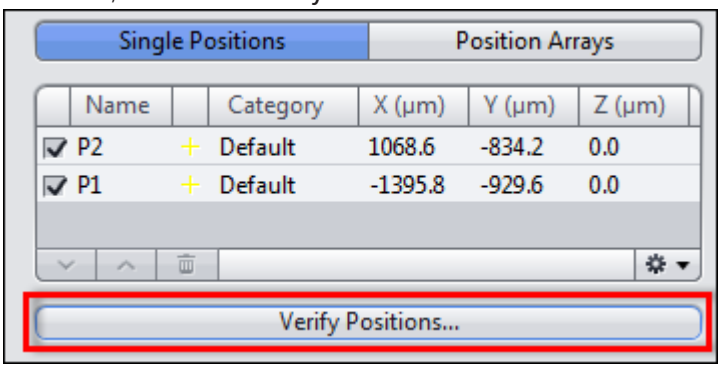
3 Use the Live mode to check the Z-position of the position.

4 To adjust the Z-position, set the desired Z-position using the focus drive.

5 In the **Single Positions** list click in the bottom right on the **Options** button and select **Set Current Z For Selected Positions**.

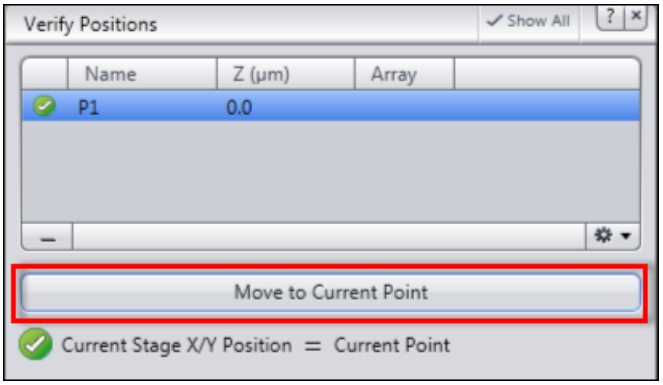
6 To check and adjust a large number of **positions**, use the **Verify Positions** dialog.

7 To do this, click on the **VerifyPositions…** button in the **Positions** section.



The **Verify Positions** dialog opens.

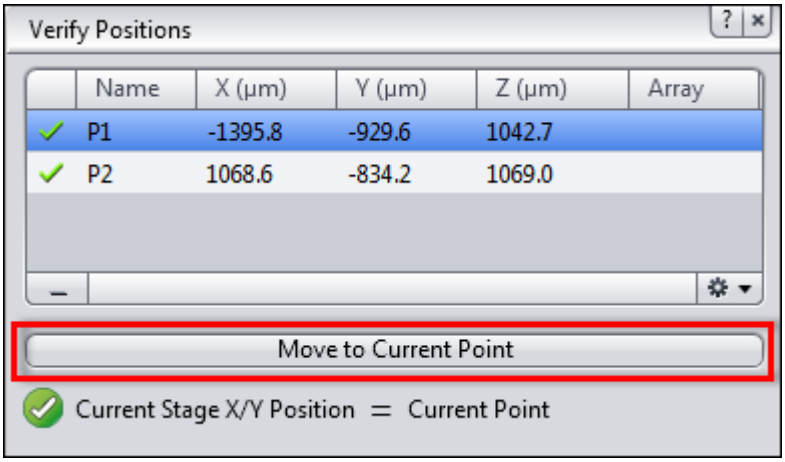
8 Click on the **Move to Current Point** button.



The stage moves automatically to the position in the list that is highlighted in blue. Alternatively, you can double-click on the position in the list that you want to check.

9 Use the **Live** mode to set the desired Z-position using the focus drive. Alternatively, you can have the Z-position of the focal plane determined automatically by clicking on the **Start Autofocus** button.

10 Click on the **Set Current Z** button.



The position is marked with a check mark.

11 Click on the **Move to Next Point** button.

The stage moves automatically to the next position in the list.

12 Repeat the last 3 steps until you have checked all the points in the list.

The message **All points have been verified** appears.

13 Close the **Verify Positions** dialog.

You have successfully verified and adjusted the individual Z-positions for positions.

10.2.6.3 Creating a local focus surface

To create local focus surfaces, you must distribute support points across your tile regions and assign their focus position. Tile-region-specific focus areas are then interpolated from the values of these support points.

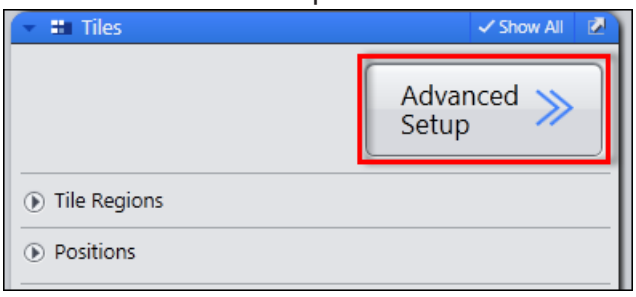
10.2.6.3.1 Distributing Support Points

Prerequisites

- To create a local focus surface you will need the **Tiles** module.
- You have set up a **Tiles** experiment with at least one tile region. Further information on this can be found under: Set up tiles and positions experiments.
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1 Click on the **Advanced Setup** button.



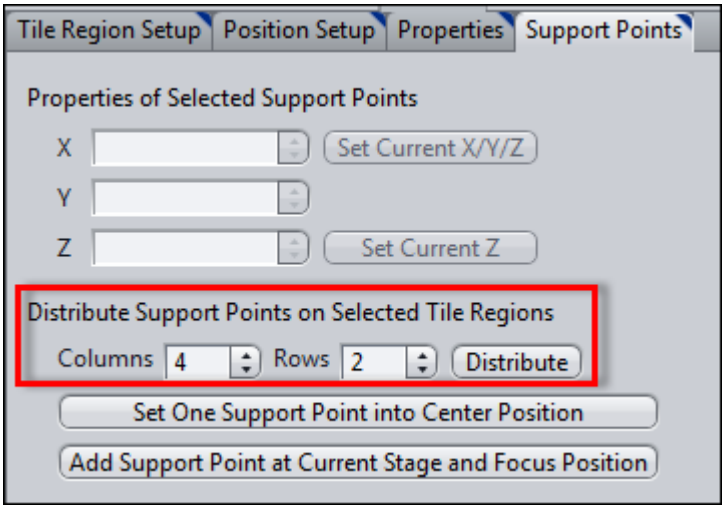
Advanced tile setup is opened.

2 Select a tile region for which you want to create support points. To do this, click on the corresponding tile region in the list in the **Tile Regions** section of the **Tiles** tool.

> **Note**
>
> Alternatively, you can select tile regions by clicking directly on the desired tile region in the **Advanced Setup** view. Both methods allow you to select several tile regions simultaneously by holding down the **Ctrl** key.

3 Select the **Support Points** tab from the **Tiles - Advanced Setup** view options.

4 Under **Distribute Support Points on Selected Tile Regions**, indicate the number of columns and rows for the distribution of the reference points.



5 Click on the **Distribute** button.

The support points are distributed within the tile region selected and shown as yellow points in the  stage view.

The support points of the selected tile region are displayed with their coordinates in the **Local (per Tile Region)** list in the **Focus Surface** section of the **Tiles** tool.

6 If necessary, you can adjust the distribution of the support points manually in the **Advanced Setup**. You can change the position of the support points using drag & drop.

7 Additional, individual support points can be added by using the stage to locate the desired position and clicking on the **Add Support Points At Current Stage and Focus Position** button on the **Support Points** tab.

i Note

Distribute the support points evenly across your **tile region**. The more irregular the surface of your specimen, the more reference points you should set. An even but tilted surface requires at least 4 reference points for a solid calculation, while a simple saddle surface requires at least 9 reference points. A high reference-point density leads to a more precise result, although the maximum useful density is one reference point per tile.

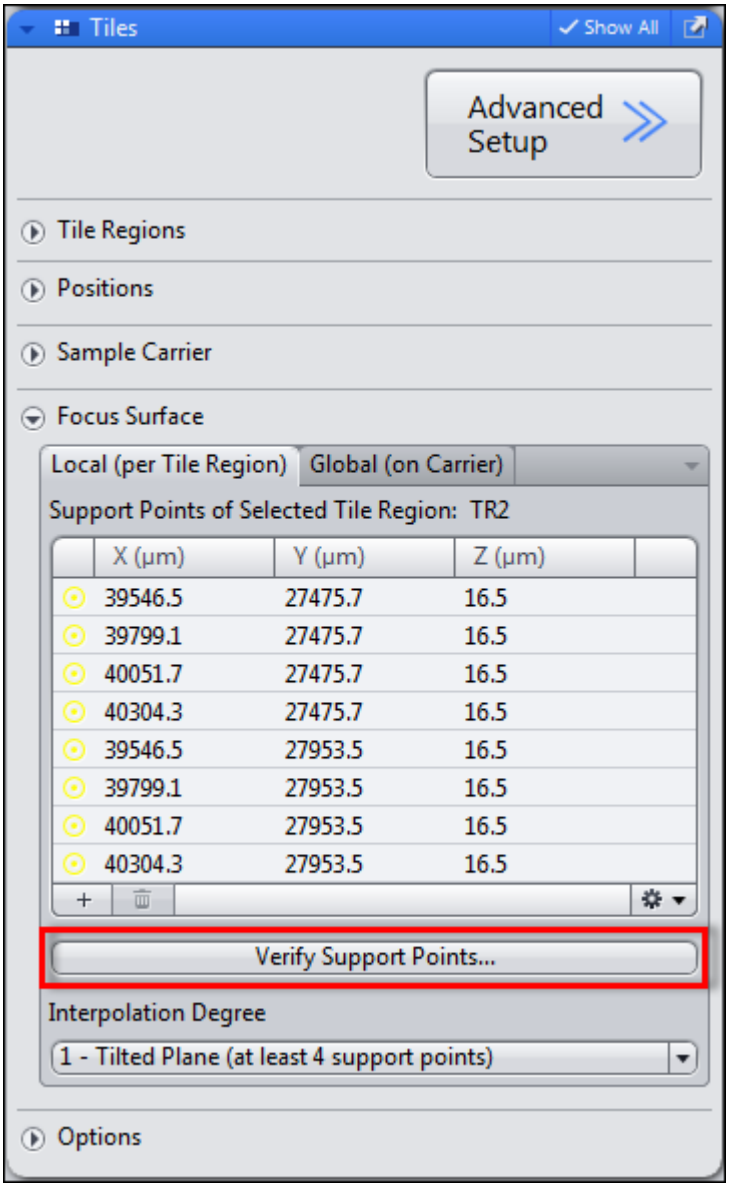
8 Repeat steps 2 to 6 until you have distributed reference points across all desired tile regions.

You have successfully distributed support points across the tile regions.

10.2.6.3.2 Verifying Z-Position of Support Points

Procedure

1 Click on the **Verify Support Points...** button in the **Focus Surface** section of the **Tiles** tool.



The **Verify Local Support Points** dialog opens.

2 Click on the **Move To Current Point** button.

The stage moves automatically to the support point that is highlighted in blue in the reference point list. Alternatively, you can also double-click on the support point in the list that you want to check.

3 Use the Live mode to set the Z-position using the focus drive. Alternatively, you can have the Z-position of the focal plane determined automatically by clicking on the **Run Autofocus** button.

4 Click on the **Set Current Z** button.

The checked reference point is marked with a green check mark.

5 Click on the **Move To Next Point** button.

The stage moves automatically to the next support point in the list.

6 Repeat the last 3 steps until you have checked all the support points.

The message **All points have been verified** appears.

7 Close the **Verify Local Support Points** dialog.

You have successfully verified the Z-positions of the support points.

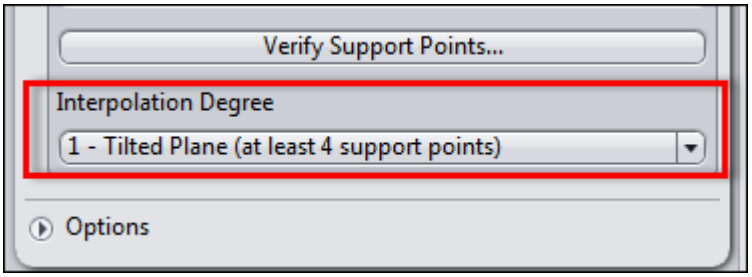
i Note

Positions always have a horizontal local focus surface, which is determined by the Z-value of the position. If you use positions in addition to tile regions, you can verify the Z-values of the positions with the help of a similar dialog. Open this dialog by clicking on the **Verify Positions...** button in the **Positions** section of the **Tiles** tool.

10.2.6.3.3 Selecting Interpolation Degree

Procedure

1 Select the interpolation level in the **Interpolation Degree** dropdown list in the **Focus Surface** section.



i Note

The minimum number of support points necessary per tile region is indicated in the **Interpolation Degree** dropdown list for each entry. The calculation is more solid if the number of support points exceeds this minimum number. We therefore recommend that you only increase the interpolation degree as far as the surface of the sample demands, even if you have set more support points. If the number of support points does not correspond to the minimum number for the selected interpolation degree, the interpolation degree will be reduced automatically.

You have successfully created a local focus surface.

> **Note**
>
> To ensure the Tiles are acquired along the focus surface during the experiment ZEN automatically selects the most appropraite focus strategy in the **Focus Strategy** tool. For information on this please read: *Using Focus Strategies* [▶ 44].

10.2.6.4 Creating a global focus surface

To create a global focus surface, you must distribute support points across your sample carrier and indicate their focus position. A focus area across the sample carrier is then interpolated from the values of these reference points.

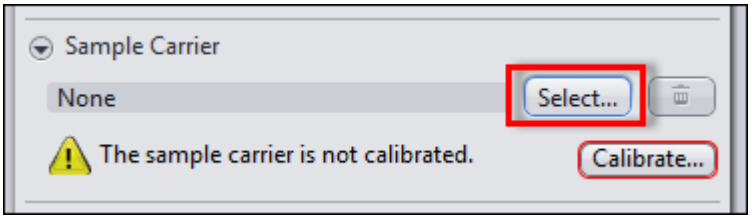
10.2.6.4.1 Distributing Support Points

Prerequisites

- You have configured the general settings for setting up a tile experiment (experiment created, at least one channel defined, Tiles dimension activated).
- To create a global focus surface, you will need the **Tiles** module.
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1. Open the **Sample Carrier** section.
2. Click on the **Select...** button.

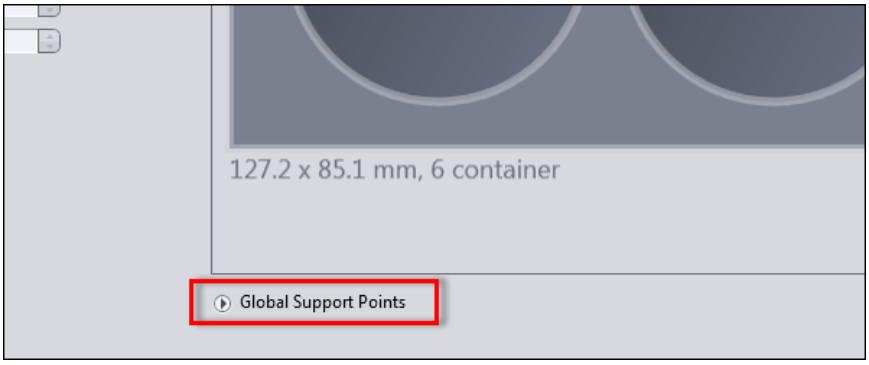


   The **Select Template** dialog opens.

3. Select the sample carrier template that you want to use.
4. Click on the **Options** button and select the **Copy And Edit...** entry.

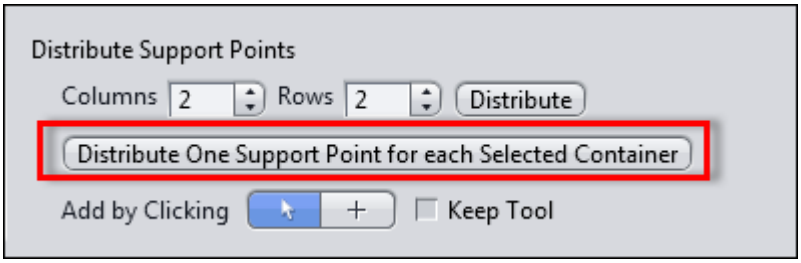
   A copy of the existing template is generated and opened in the Sample Carrier Editor.

5. To distribute support points across the sample carrier template, open the **Global Support Points** section.



6 Select the containers in which you wish to create support points. To do this, hold down the **Ctrl** key and click on the containers.

7 Click on the **Distribute One Support Point For Each Selected Container** button.



One support point is assigned to each container selected.

i Note

If you use a **sample carrier** without containers (e.g. slide), use the **Distribute** button instead, to distribute support points on the basis of columns and rows.

The support points are distributed automatically across the sample carrier.

You can add further support points manually using the **Add** button  .

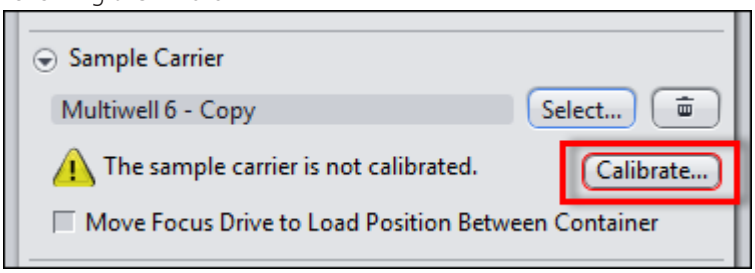
i Note

Only create support points where you can bring the sample into focus (within the containers). This is the only way that you can indicate the Z-position of the support points later. The assignment of container-based support points to the center of the container is fixed and these cannot be moved. If the surface of your **sample carrier** is tilted but even, you will need at least 4 support points for a solid calculation. The more irregular the surface, the more support points you should distribute.

8 To close the **Editor** window, click on the **OK** button.

9 To select the edited **sample carrier template**, click on the **OK** button.

10 Calibrate the sample carrier by clicking on the **Calibrate…** button and following the wizard.



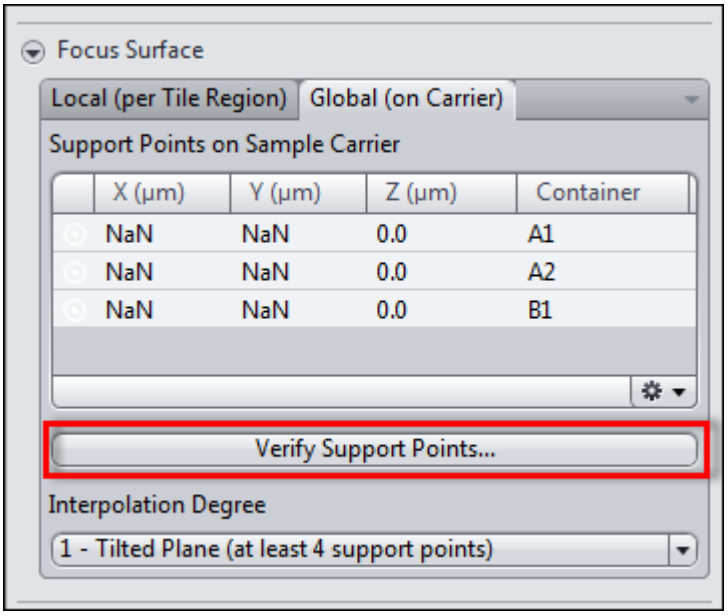
You have successfully distributed support points across a sample carrier template and have selected and calibrated it.

10.2.6.4.2 Verifying Z-Position of Support Points

Procedure

1. In the **Tiles** tool open the **Focus Surface** section.
2. Go to the **Global (on Carrier)** tab.

   All the support points of the selected sample carrier template are displayed in the **Support Points on Sample Carrier** list.
3. Click on the **Verify Support Points…** button.



   The **Verify Global Support Points** dialog opens.
4. Click on the **Move To Current Point** button.

   The stage moves automatically to the support point that is highlighted in blue in the list. Alternatively, you can also double-click on the support point in the list that you want to check.
5. Use the Live mode to set the Z-position using the focus drive. Alternatively, you can have the Z-position of the focus surface determined automatically by clicking on the **Start Autofocus** button.
6. Click on the **Set Current Z** button.

   The support point is marked with a check mark.
7. Click on the **Move To Next Point** button.

   The stage moves automatically to the next support point in the list.
8. Repeat the last 3 steps until you have checked all the support points.

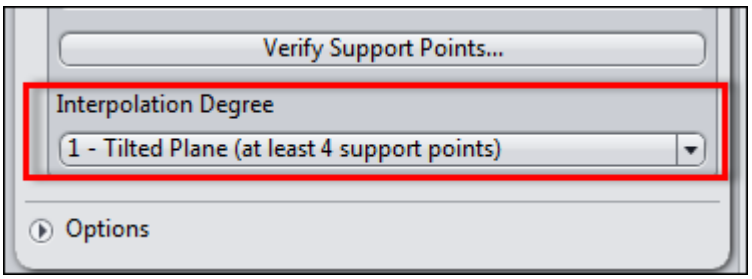
   The message **All points have been verified** appears.

9 Close the **Verify Global Support Points** dialog.

You have assigned a Z-position to all support points.

10.2.6.4.3 Selecting Interpolation Degree

Procedure 1 Select the interpolation degree in the **Interpolation Degree** dropdown list in the **Focus Area** section.



Note

The minimum number of support points necessary is indicated in the **Interpolation Degree** dropdown list for each entry. The calculation is more solid if the number of support points exceeds this minimum number. We therefore recommend that you only increase the interpolation degree as far as the surface of the carrier demands, even if you have created more support points. If the number of support points does not correspond to the minimum number for the selected interpolation degree, the interpolation degree will be reduced automatically. Interpolation degree **1 – Tilted Plane (at least 4 support points)** is sufficient to compensate for any tilting of the sample carrier.

You have successfully created a global focus surface.

You can now set up your tile experiment using the sample carrier. Further information on this can be found under: *Using sample carriers* [▶ 236].

Note

To ensure the Tiles are acquired along the focus surface during the experiment ZEN automatically selects the most appropriate focus strategy in the **Focus Strategy** tool. Make sure that you select the **Global (on Carrier)** tab in the **Focus Surface** section. For information on this please read: *Using Focus Strategies* [▶ 44].

10.2.7 Assigning Categories to Tile Regions and Positions

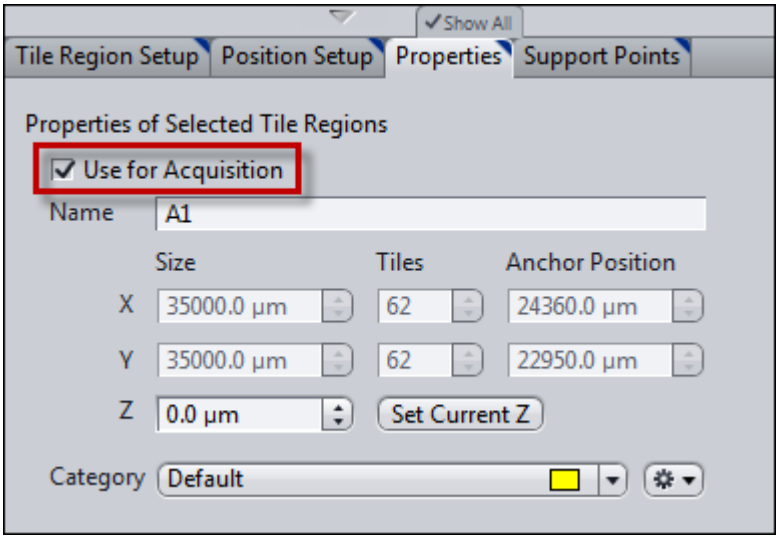
For some customers it could be important to not only display the well number together with the acquired images (Path: Graphics -> Frequent Annotations -> Carrier Container Name) but also create certain additional annotations for different

tile regions or positions, e.g. "control condition" or "experimental condition 1". For that purpose, ZEN 2.1 allows you to add/ edit names and categories to the different Tiles Regions/ Positions that have been generated.

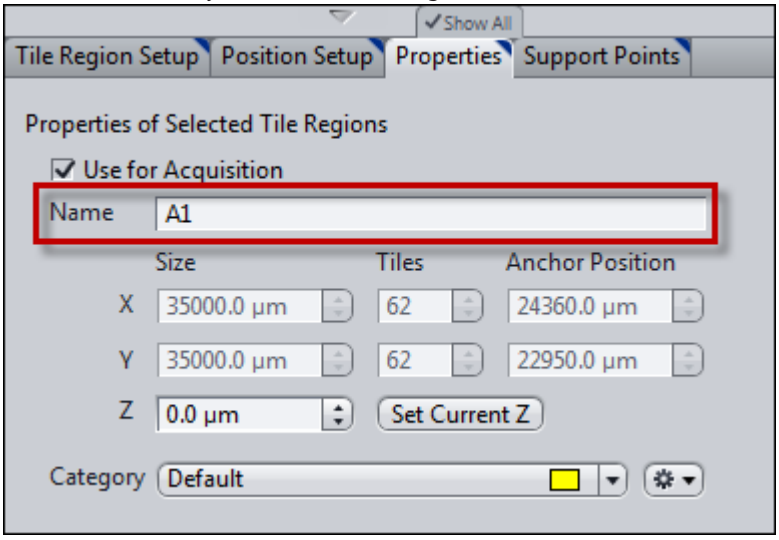
Procedure

1. In order to assign individual names to different individual positions and/or tile regions in a well plate experiment, click on the respective Tile Region or Position and open the **Properties** tab in the Center Screen Area (Tiles – Advanced Setup view).

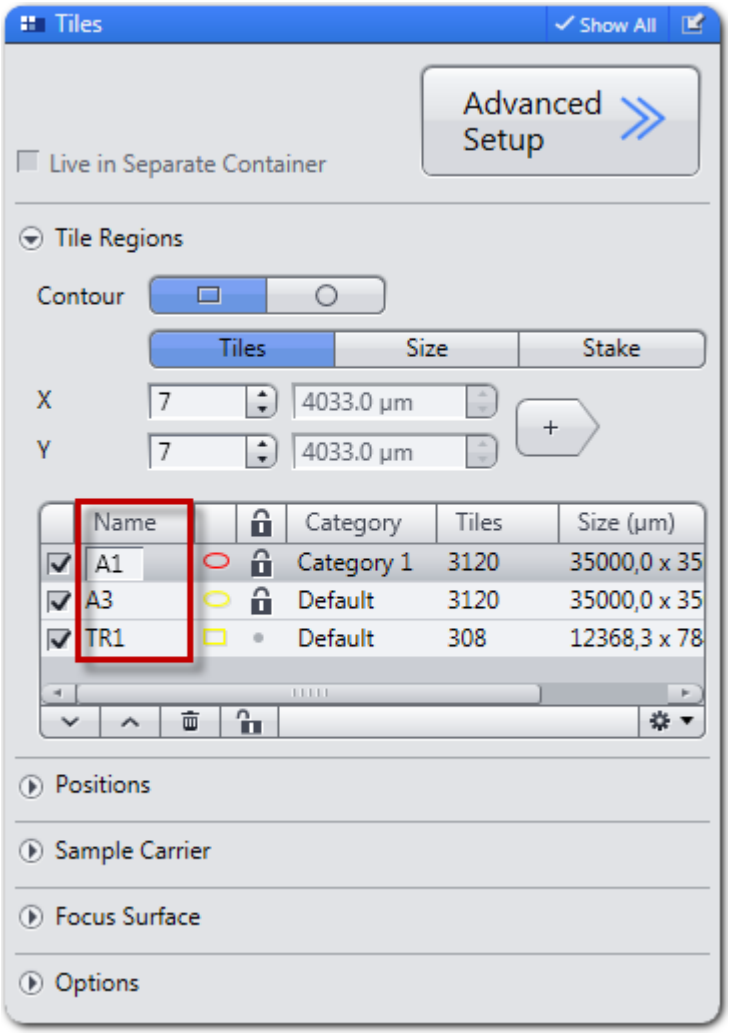
2. Activate the **Use for Acquisition** checkbox.



3. Edit the **Name** for your selected Tile Region/ Position.



4 Alternatively edit the **Name** of a Tile Region/ Position by clicking in **Tiles** module on the respective name under **Tile Regions** or **Positions**.



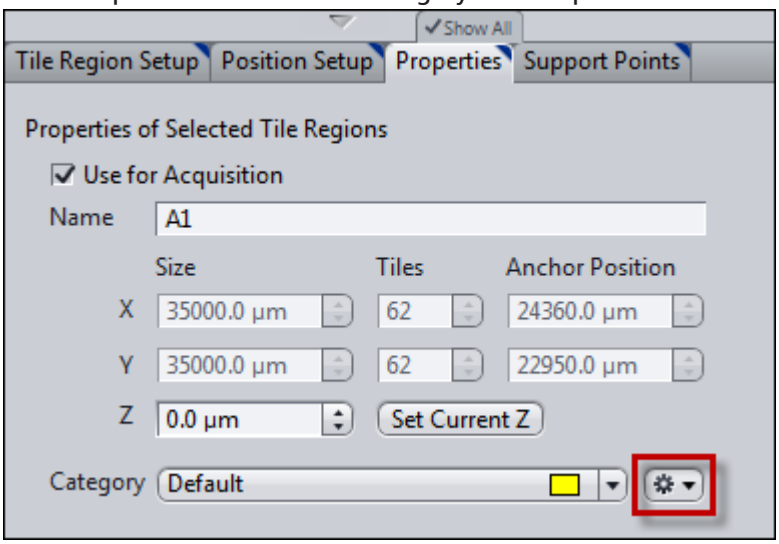
5 Repeat this step when you want to rename different Tile Regions or Positions.

Note

To display the name of your Tile Region/ Position later in your acquired image(s), go to **Graphics**, **Frequent Annotations**, **More…** and select **Image.Scene.Name** from the Metadata list.

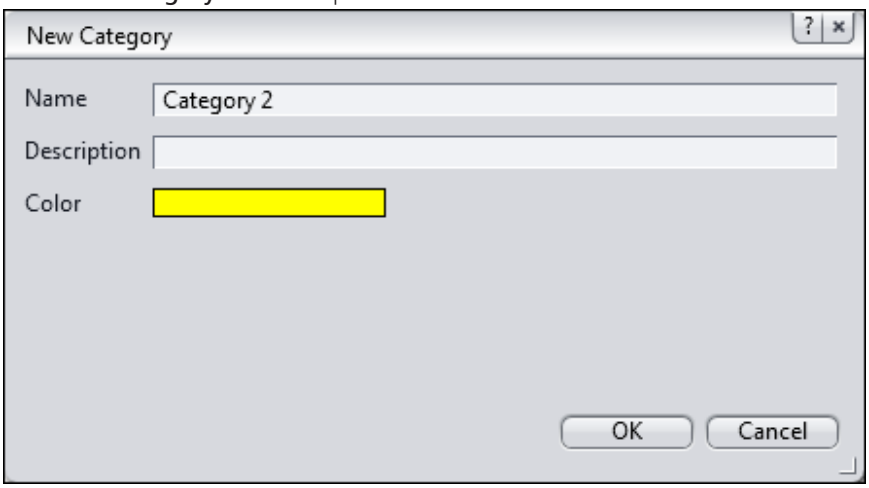
6 To assign or edit categories of your Tile Regions/ Positions, first activate the checkbox of all desired Tile Regions/ Positions that should be grouped in the same category.

7 In the **Properties** tab click under **Category** on the **Options** button.



8 Select **New…** from the dropdown list

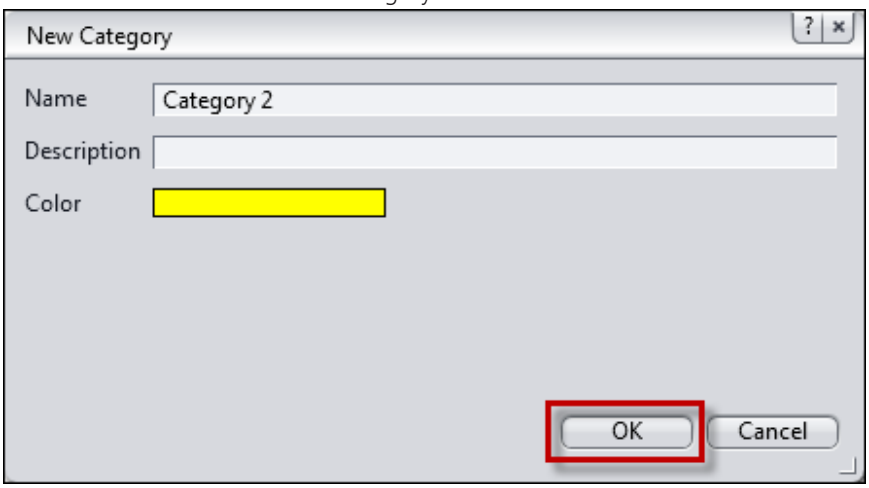
The **New Category** window opens.



9 Enter a **Name** and add a **Description** for the selected Tile Regions/ Positions.

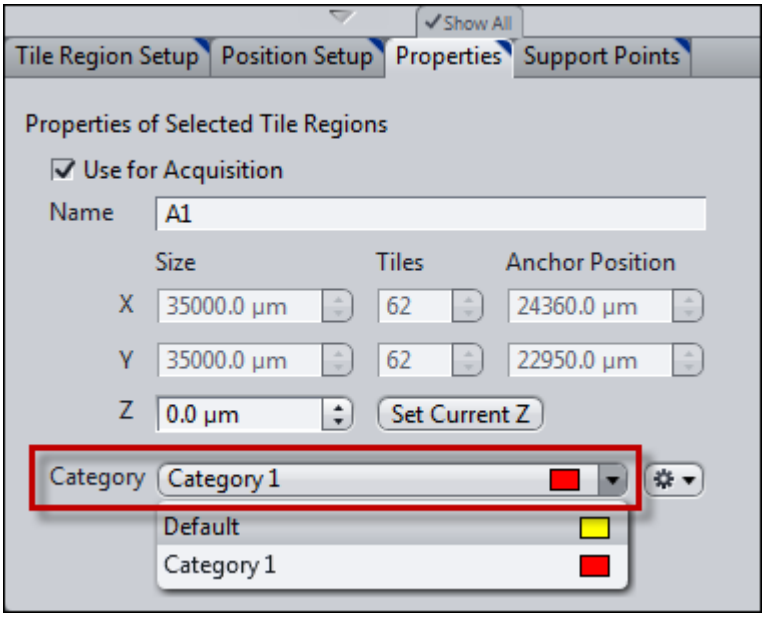
10 Assign a **Color** for the new category by clicking on the color bar and choosing a preferred color.

11 Click on **OK** to create the new category.



The **New Category** window closes and the new categroy is created.

12 Under **Category** choose the desired category for the selected Tile regions/ Positions from the drop down list.



The chosen category is now assigned to the selected Tile Regions/ Positions.

i Note

Note that a predefined category can also be applied to a differentiated selection of Tile Regions/ Positions from more than one well.
Note also, that the assigned color is only used as a feature in the Tiles tab (Left Tool Bar Area).

i Note

To display a Tile Region/ Position Category feature (**Name** and/ or **Description**) in your acquired image, you go to **Graphics**, **Frequent Annotations**, **More**….

Type “category” in the search bar and select the desired feature to be displayed. (Although the option "Color" is given, no reasonable element will be displayed by the software)

i Note

To adjust parameters of your annotations (e.g. font size), right-click on it and go to **Format**, **Graphical Elements**.

Displaying categories in the Tiles/ Positions List (Left Tool Bar Area)

Prerequisites

- You have selected several different positions or tile regions and assigned different categories.

Procedure

1. Under **Positions** or **Tiles** of the Tiles module (Left Tool Bar Area) select a position or tile region.
2. Right-click on the selected position/ tile, choose **Sort** and select **By Category**

The positions/ tiles will be sorted alphabetically according to the assigned categories.

10.2.8 Re-positioning Sample Carrier after Incubation

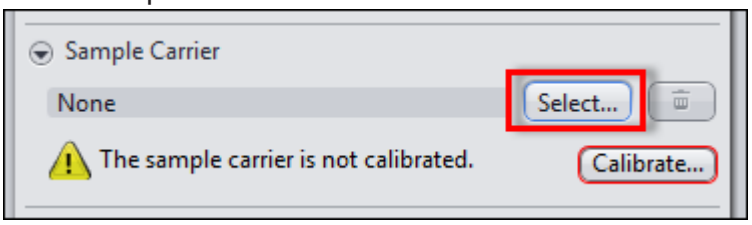
When you want to take images of positions/ tile regions on a sample carrier, that had to be taken off the stage, e.g. for incubation purposes or changes of the immersion medium, proceed as follows to re-position your sample carrier..

Starting the Experiment

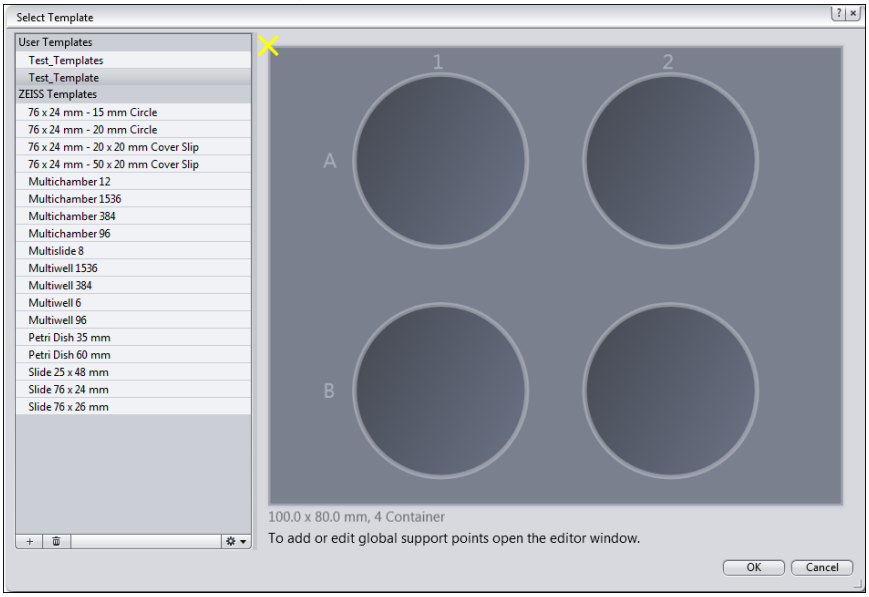
Prerequisites

- You have located you sample under **Locate** and run the **Stage Calibration** see also the chapter *Calibrating Stage and Selecting Channel* [▶ 197].
- You have set up at least one channel and adjusted the light/ camera exposure time.
- You have activated the **Tiles** checkbox and the **Show All** mode

Procedure **1** Click on **Sample Carrier** and then click **Select**.



The **Select Template** dialog opens.



2 Select the template of choice.

i Note

For demo purposes, select a standard slide that can mimic your test sample.

i Note

Regarding calibration of your template, you can customize your own carrier see the chapter *Customizing Sample Carrier* [▶ 237], but for slides with one coverslip or well, there is only the option for Single Reference Point Calibration. For Multi-well plates, you will have the option for 7-point, 4-point, 3-point or 1-point calibration. This becomes important for adjusting for the rotation of the sample.

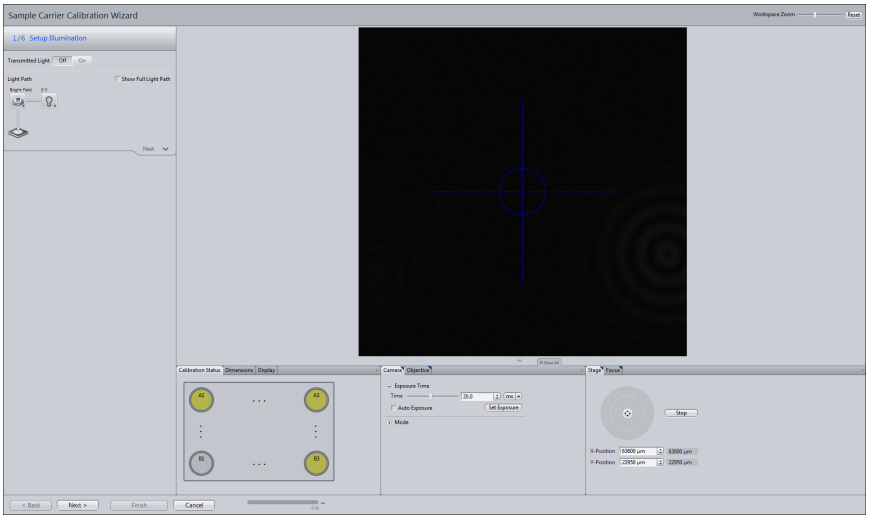
3 Adjust the surface of the sample carrier. Refer to the chapter *Creating a global focus surface* [▶ 223].

The **Select Template** dialog closes.

> **i Note**
>
> In the following it is assumed that you just use a conventional glass slide with some cells or tissue that is positioned in the center

4 Press **Calibrate**.

The **Sample Carrier Callibration Wizard** opens.

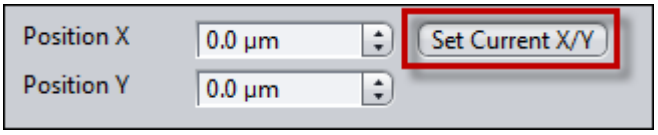


5 Right-click to activate **Crosshairs** in the live image and move a sample reference point into the middle of the crosshairs. This reference point can be any unique identifiable point on the slide and does not have to in the middle of the slide.

6 Under **X/Y Position** click on **Set Zero**.

7 Click **Next** and go to the step **Search Reference Point**.

8 Click on **Set Current X/Y**.

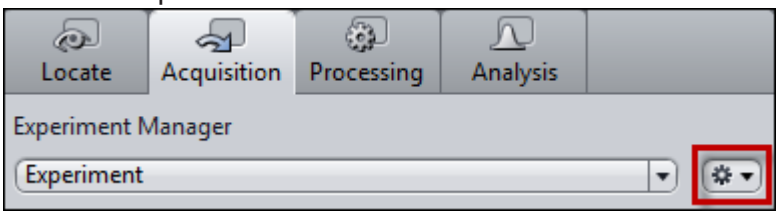


9 In the **Tiles** tool click on **Advanced Setup** and add positions/ tile regions at your locations of interest.

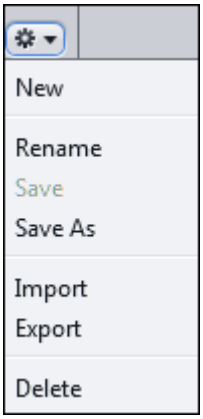
> **i Note**
>
> You can zoom in and out using the mouse scroller, and move the stage in the Center Screen Area to a point of interest with a double-click on the sample carrier.

10 Once you have defined all your positions/ tile regions, go in the **Acquisition** tab to the **Experiment Manager** tool.

11 Click on the **Options** button.



12 Save your experimental settings, including the lists of positions/ tile regions, by using either **Save As** or **Export**.



Note

With **Save As** the settings will be saved directly in the Experiment Manager.
With **Export** the settings will be saved in a folder of your choice.

13 Start your experiment and record images from your selected positions/ tile regions.

14 Remove your sample off the stage and e.g. put it back into the incubation chamber.

15 Close ZEN 2.1 software.

You have done all settings for a successfull re-positioning of your sample carrier after the experiment.

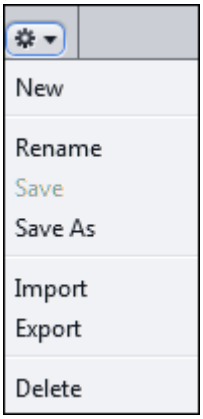
Re-Positioning of the Sample Carrier after the experiment

Note

If you cycle the power on the microscope ZEN will prompt you to calibrate the stage and/or focus drives. Thus, if the calibration of the multi-well plate was performed under the same conditions then the sample carrier calibration will still be valid. You must however, ensure that other parameters like plate orientation and placement on the microscope have not changed.

Procedure

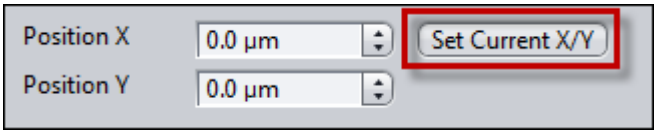
1. Restart the ZEN 2.1 software.
2. In the **Acquisition** tab go to the **Experiment Manager** and Reload or Import your experimental settings including your list of positions/ tile regions.



3. In **Tiles** under **Sample Carrier** click on **Calibrate**.

   The **Sample Carrier Calibration Wizard** opens.
4. Right-click to activate **Crosshairs** in the live image and move your previously chosen sample reference point into the middle of the crosshairs.
5. Under **X/Y Position** click on **Set Zero**.

6. Click **Next** and go to the step **Search Reference Point**.
7. Click on **Set Current X/Y**.



8. Now, you still need to verify the Z-offset of your positions. Therefore, follow the corresponding instructions given in the chapters *Adjusting Z-positions of tile regions* [▶ 216] and *Adjusting Z-positions of positions* [▶ 217].

All of your selected positions/ tile regions are now re-assigned to the correct X/Y/Z-values in relation to your (unique identifiable) reference point.

You can re-start your experiment and record images from your selected positions/ tile regions.

10.2.9 Using sample carriers

Use a sample carrier template to display the size and appearance of your sample carrier (e.g. slide or multiwell plate) in **Advanced Setup**. This allows you to distribute tile regions or positions easily across your sample carrier.

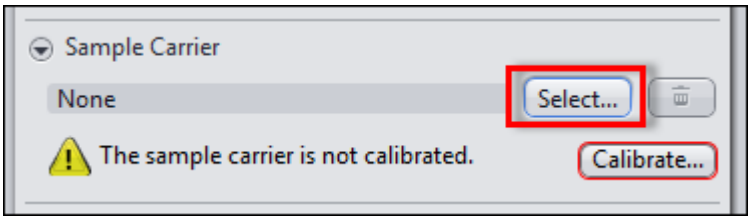
10.2.9.1 Selecting the sample carrier template

Prerequisites

- You have configured the general settings for setting up a tile experiment (experiment created, at least one channel defined, Tiles dimension activated).
- You are on the **Acquisition** tab in the **Tiles** tool.

Procedure

1. Open the **Sample Carrier** section.
2. Click on the **Select...** button.

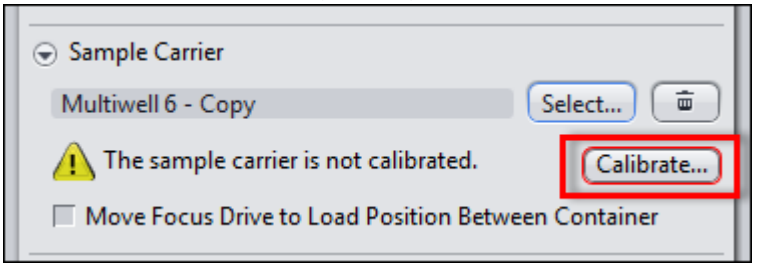


   The **Select Sample Carrier Template** dialog opens.

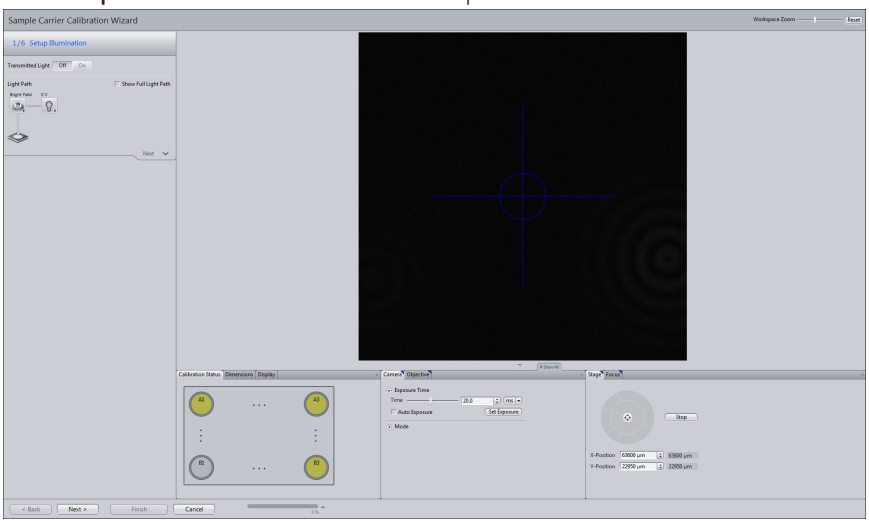
3. Select an existing **sample carrier template** or generate a new template by clicking on the + button.

> **Note**
>
> You can display or edit the **user templates** by clicking on the button and selecting the **Display/Edit…** entry. **Zeiss templates** cannot be edited. To create an editable user copy, select the **Copy And Edit…** entry.

4. To close the dialog, click on the **OK** button.
5. Calibrate the sample carrier by clicking on the **Calibrate…** button.



The **Sample Carrier Calibration Wizard** opens.



6 Follow the wizard until you have fully calibrated the sample carrier.

The information **The sample carrier is calibrated** appears in the **Sample Carrier** section.

Note

Note that the calibration values are stored in the experiment and can be re-used if you work with absolute coordinates by calibrating the end stops of the stage and focus drives and no other changes are made to the hardware set-up. This can save considerable time when you want to work repeatedly with the same sample carrier model and acquisition regime.

You have successfully selected a **sample carrier**.

10.2.9.2 Customizing Sample Carrier

As some customers work with sample carrier that are not listed in the template database of ZEN, you need to apply the following workflow in order to create such a template for the software.

Prerequisites

- You have done all prerequisites for a Tiles & Positions experiment
- You have defined at least one channel.
- You have activated the **Tiles** checkbox.

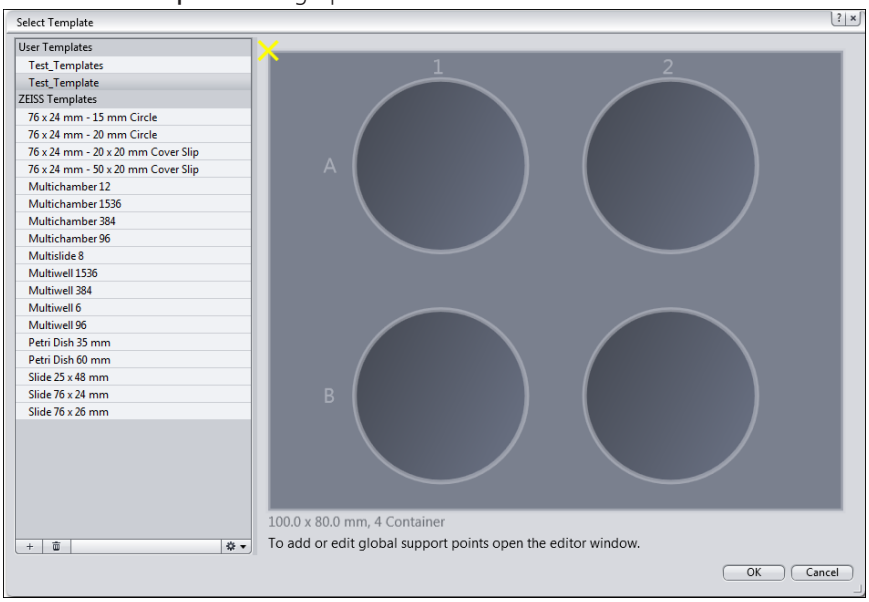
Procedure

1 Go to the **Acquisition** tab.

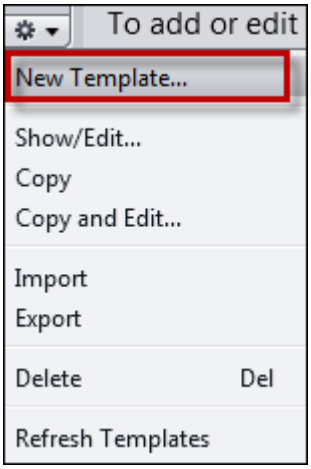
2 Open the **Tiles** tool and activate the **Show All** mode.

3 Open the **Sample Carrier** section and click on the **Select…** button.

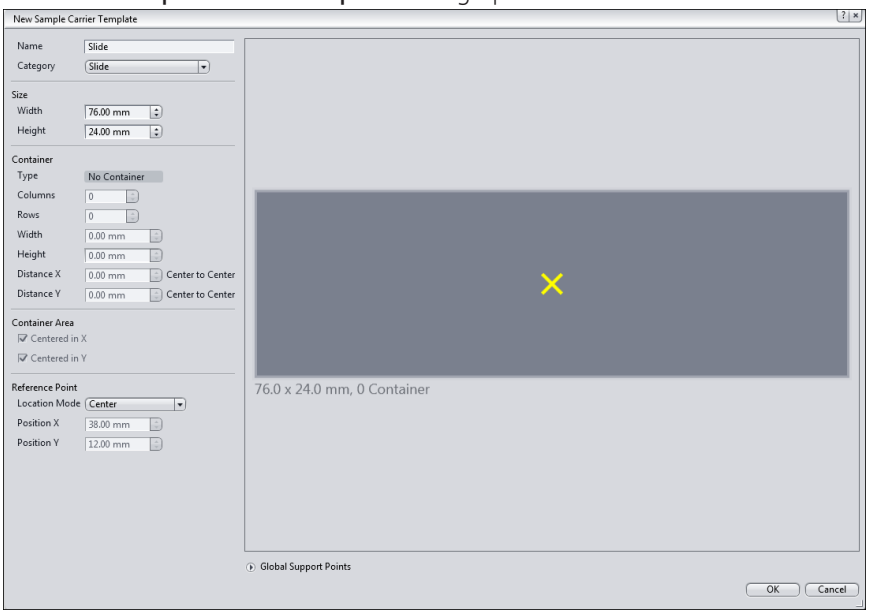
The **Select Template** dialog opens.



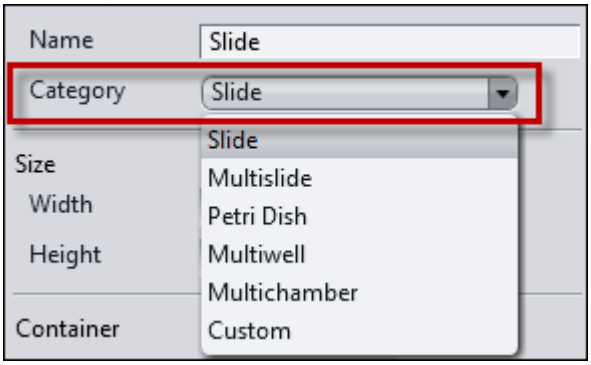
4 Click on the **Options** button and choose **New Template**….



The **New Sample Carrier Template** dialog opens.



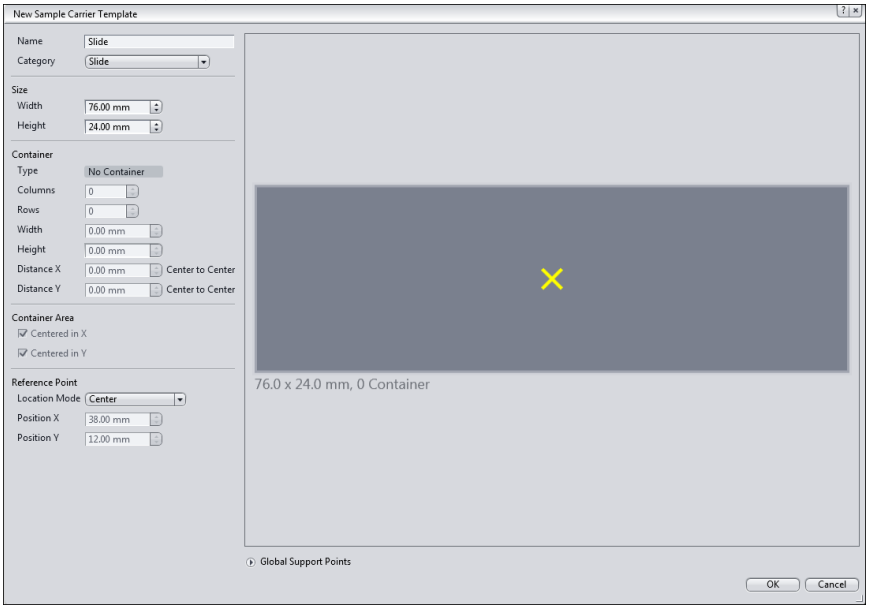
5 Choose a **Category** that corresponds to the type of your carrier and assign a **Name** to your template.



Note

Corresponding to the **Category** you choose xou can define different parameters for the template.

6 For example if you select **Slide**, you can now configure the **Width**and **Height** of the slide and adjust the location of a **Reference Point**.



7 If you select a **Multislide**, **Petri Dish**, **Multiwell** or **Multichamber** template, you can configure and adjust additional parameters of your carrier.

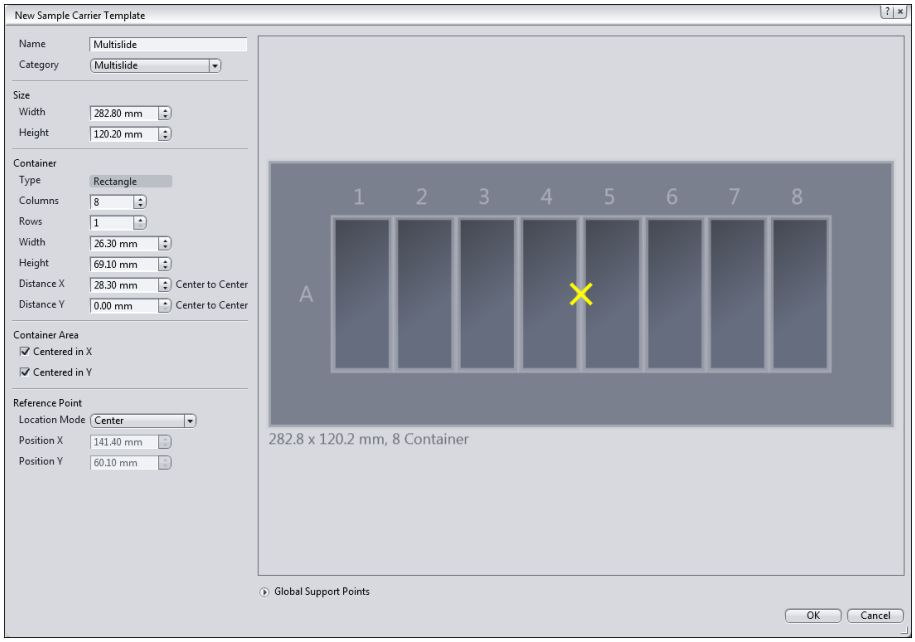


Fig. 15: Multislide template

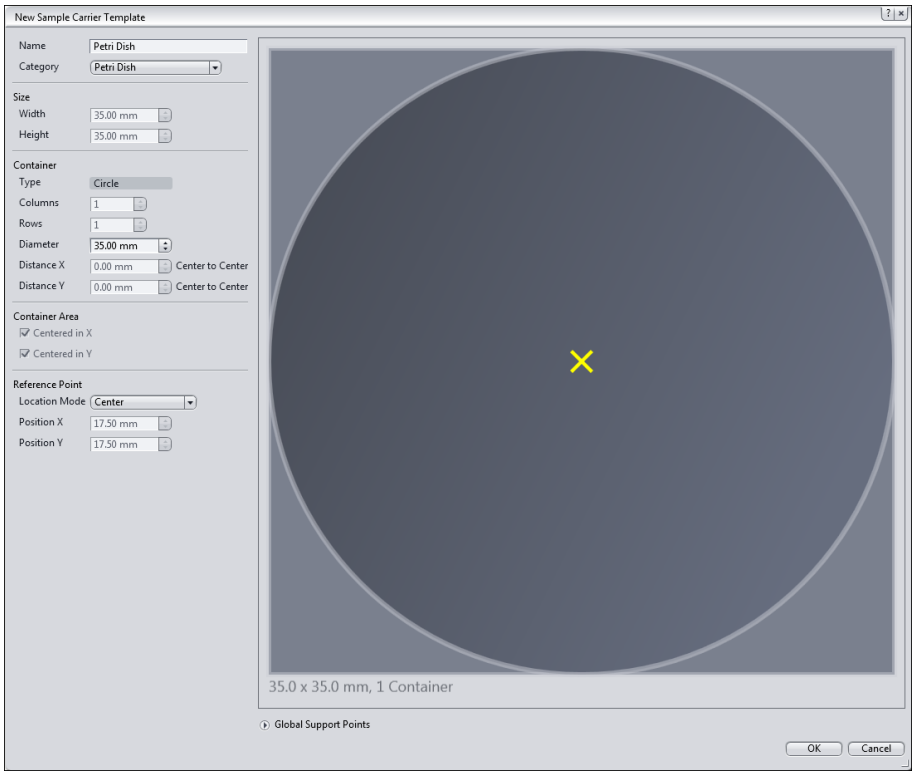


Fig. 16: Petri Dish template

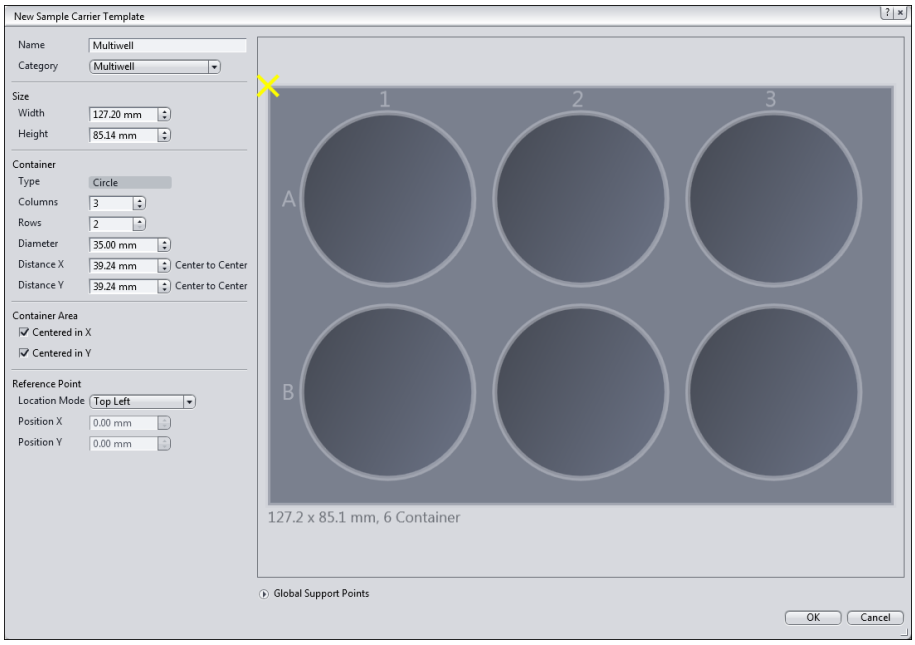


Fig. 17: Multiwell template

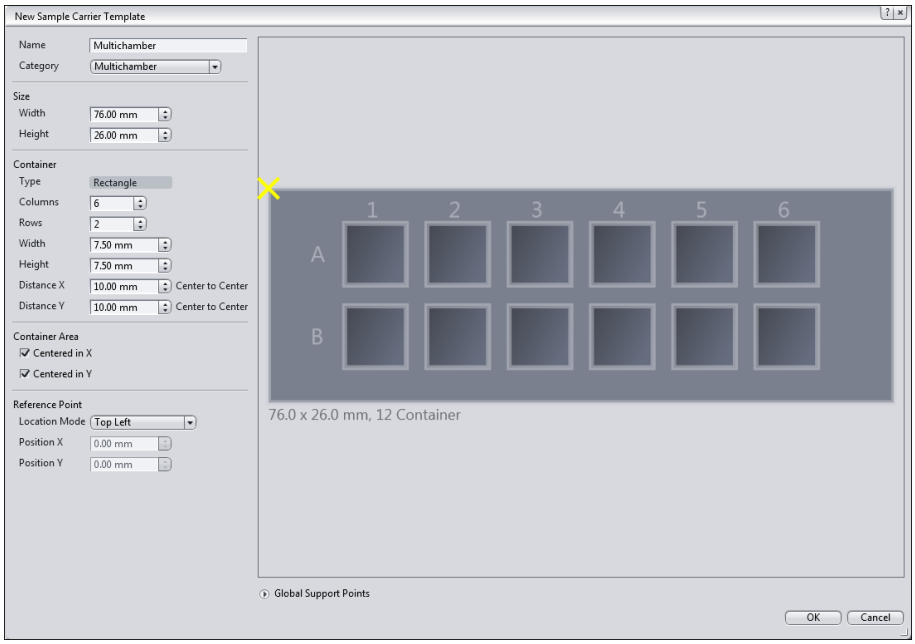


Fig. 18: Multichamber template

8 In case you need to modify one of the above depicted templates even further, first select the **Category** that appears closest to your carrier, go again to the **Category** tab and then choose **Custom**.

i Note

By that, some predefined options of the template will be made accessible for further modifications.

You have customized a sample carrier template or set up a custom one.

11 Module Physiology

11.1 Working with MeanROI View (Offline)

11.1.1 Introduction

Using the **MeanROI** offline functions you can specify user-defined measurement regions (ROIs) following acquisition of your time lapse experiment and analyze their time-dependent changes in intensity. You can display the intensity curves in diagrams or export the values in the form of tables. This basic functionality is available for time series images opend in ZEN 2.1 (excluded ZEN lite).

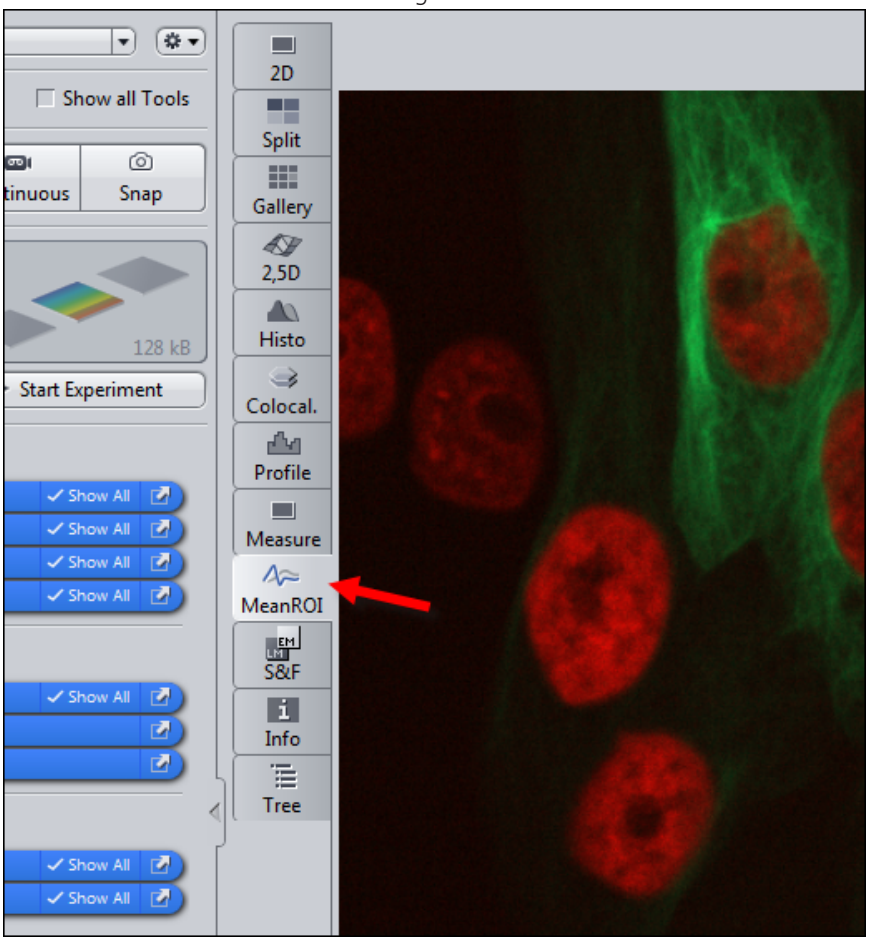
The **Physiology** module expands the MeanROI offline functions to give you the option of calculating online ratios and makes additional display options available for this purpose (**Timeline** view, etc.).

Prerequisites

- You have acquired a *time series experiment* [▶ 43]. The experiment is open and the first time point is displayed in the **2D view**.

Procedure

1 Select the **MeanROI** tab from the image view tabs in the **Center Screen Area**.



The **MeanROI view** opens.

You`re now prepared to start working with the **MeanROI** view. The following chapters will show you the first steps.

11.1.2 Drawing in and adjusting ROIs

Here you will find out how to draw in and edit measurement regions (ROIs) for intensity measurements and how to adjust them for individual time points.

> **Note**
>
> If you save the experiment, the **ROIs** are automatically saved with the experiment. They are available to you once again in the **MeanROI View** the next time it is opened. Click on the **Recalculate** button on the **ROI Tools** tab to perform and display the intensity calculations again for the saved ROIs.

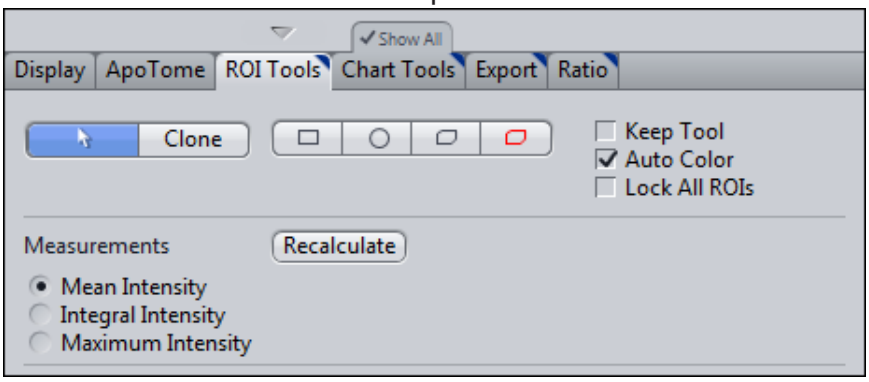
11.1.2.1 Drawing in ROIs

Prerequisites

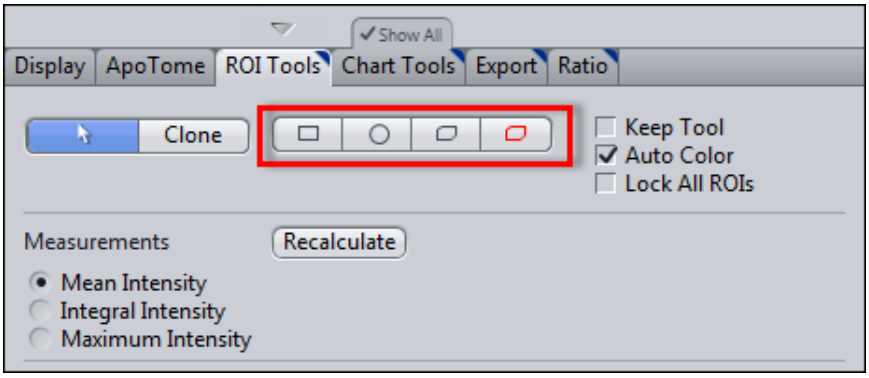
- You are in the **MeanROI View** or **Physiology Setup**.

Procedure

1. Select the **ROI Tools** tab in the **View Options**.



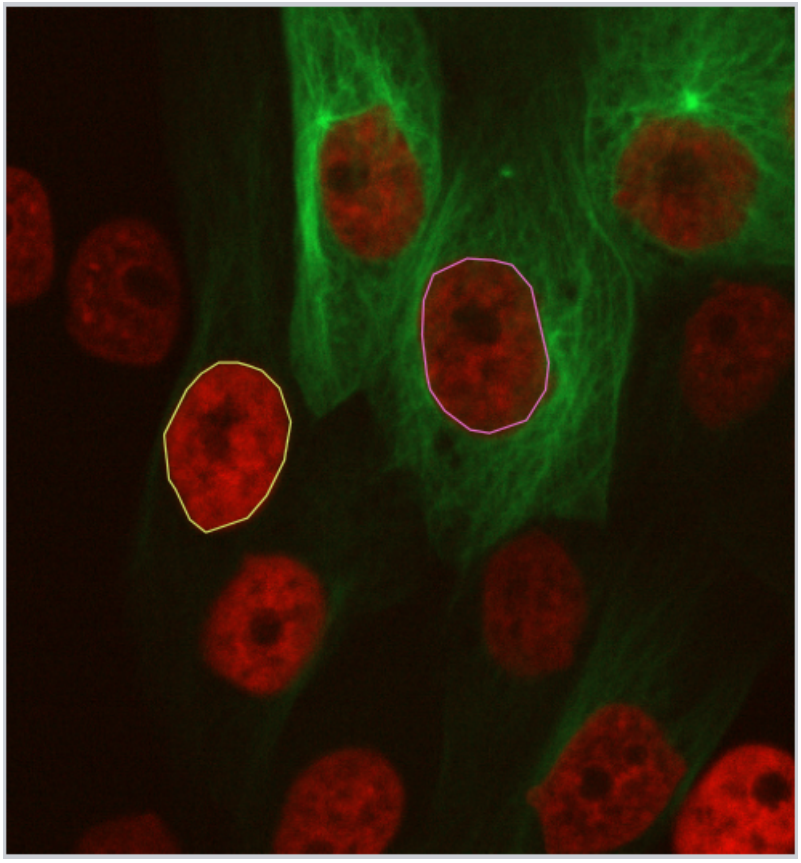
2. Select a tool for drawing in **ROIs**.



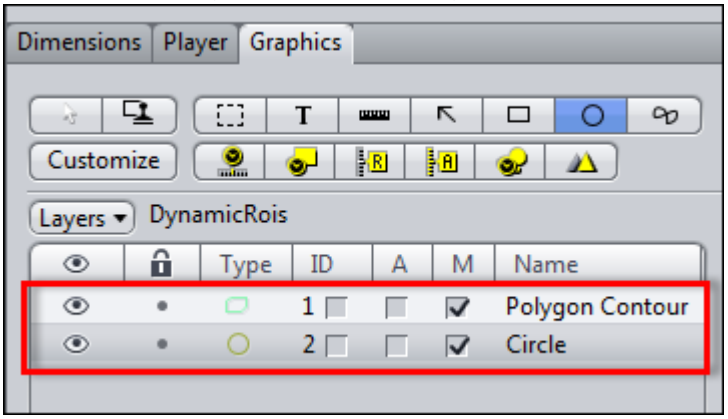
3. Activate the **Keep tool** checkbox.

   The selected tool remains active after you have drawn in an **ROI**. This means you can draw in several ROIs without having to re-select the tool.

4 Using the selected tool, in the image view draw in the objects or regions (ROIs) for which intensity measurements are required.



The **ROIs** are displayed in the list under **DynamicROIs** on the **Graphics** tab.



Intensity measurements are performed for each **ROI** and displayed in the diagram area to the right of the image view.

You have successfully defined measurement regions for the intensity measurement.

11.1.2.2 Adjusting ROIs for time points

If objects move laterally in the course of the time series, you can adjust the **ROIs** at each **Time Point** in order to follow the objects.

Prerequisites ■ You have defined at least one ROI.

- You are in the **MeanROI view**.

Procedure

1. Open the **Dimensions** tab in the general view options.
2. Use the **Time slider** to scroll through the time points. Stop at the first time point at which you want to adjust an **ROI.**
3. Adjust the position of the **ROI** using drag & drop. To do this, select the ROI in the **image area** by left-clicking and hold the mouse button down. Then move the ROI to the new position and release the mouse button.
4. To change the shape of an **ROI**, right-click on an ROI and select the **Edit Points** entry (e.g. for polygon contours).
5. Adjust the shape of the **ROI**, by drag & drop the contour points.

   Changes to the position and shape of the **ROIs** are adopted for all subsequent time points.
6. Repeat the previous steps for all other time points for which you want to adjust an ROI.

You have successfully adjusted the measurement regions to the course of the experiment.

11.1.3 Adjusting the display

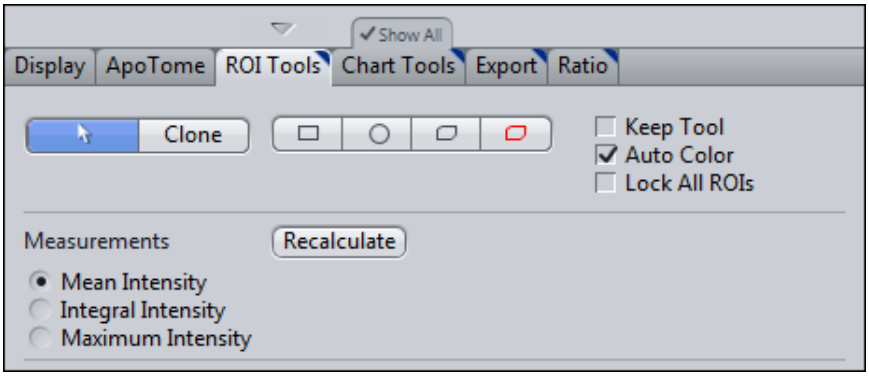
Here you will find out how to adjust the display of the measured intensity values in diagrams and tables according to your wishes.

Prerequisites

- You are in the **MeanROI** view.
- You have defined at least one ROI.

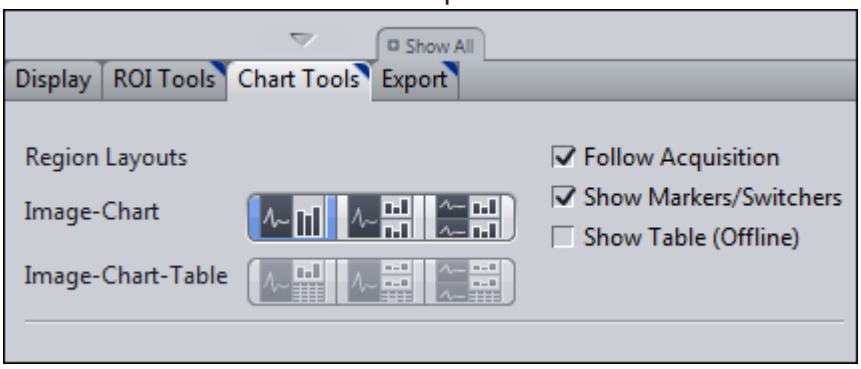
Procedure

1. Select the **ROI Tools** tab in the view options.



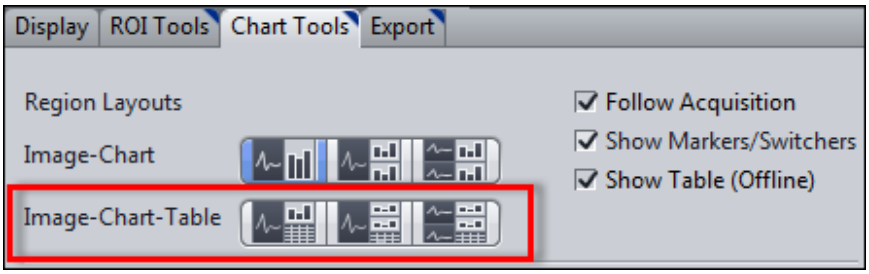
2. In the **Measurements** section select the intensity type (Mean, Integral or Maximum Intensity) that you want to be displayed in the diagrams.

3 Select the **Chart Tools** tab in the **view options**.



4 To adjust the layout of the image and diagram display, select the desired display mode under **Image-Chart**.

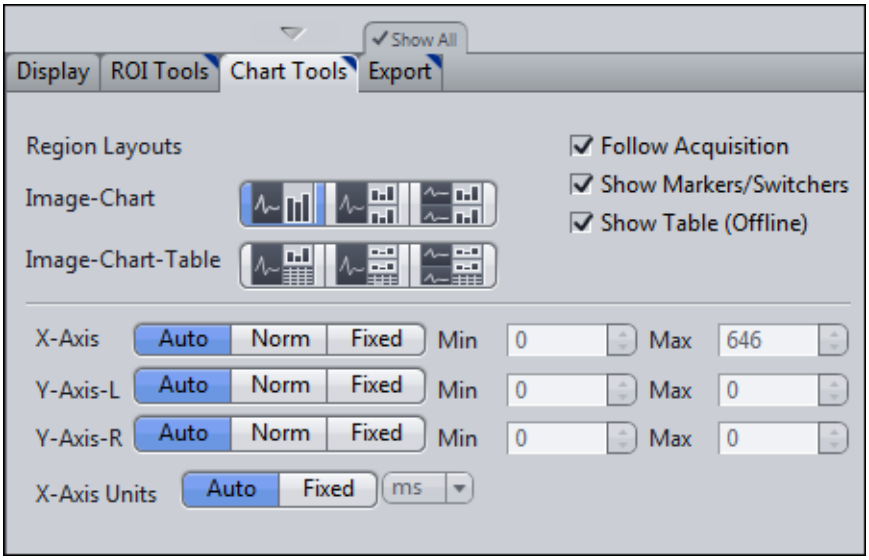
5 If you also want your data to be displayed in table form, activate the **Show Table (Offline)** checkbox.



The Image-Chart-Table modes are activated. You can now select a suitable layout for the image, diagram and table display.

6 If you want to adjust the axis scaling, activate the **Show All** option.

The axis scaling functions become visible.



7 To define the minimum and maximum values of the axes manually, click on the **Fixed** button under **X-/Y-Axis**.

The **Min** and **Max** input fields for the axis are activated.

8 Enter the desired values into the **Min** and **Max** input fields.

The minimum and maximum axis values of the diagrams are adjusted.

9 To change the unit of the X axis, click on the **Fixed** button under **X-Axis Units**.

The dropdown menu for the units is activated. You can now select the desired unit.

You have successfully adjusted the display of the intensity values.

11.1.4 Exporting a data table

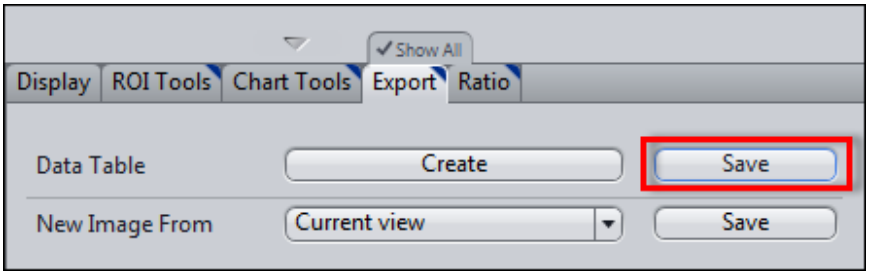
Prerequisites

- You are in the **MeanROI View**.
- You have defined at least one ROI.

Procedure

1 Select the **Export** tab in the View Options.

2 In the **Data Table** section click on the **Save** button.



The **Save As** dialog opens.

3 Enter a suitable file name, navigate to the desired folder and click on **Save**.

All the measurement data are saved as comma-separated values in a csv file. This contains the time information, the area of the ROIs and the values for three types of intensity measurements (Mean, Sum, Maximum) for each channel and each ROI.

11.1.5 Using background correction

Use this function to subtract background values from the measurement values. A background correction will allow you to make a better comparison in the magnitude of any fluorescent intensity changes observed over the time course of an experiment. Determine the background value with the help of a Background ROI or define a fixed value.

Defining a Background ROI

Prerequisites

- You are in the **MeanROI** View or **Physiology Setup**.

Procedure

1 At the first time point of the time series draw an **ROI** into a region of the image that contains only background signal in all channels.

2 Right-click on the **ROI.**

The shortcut menu opens.

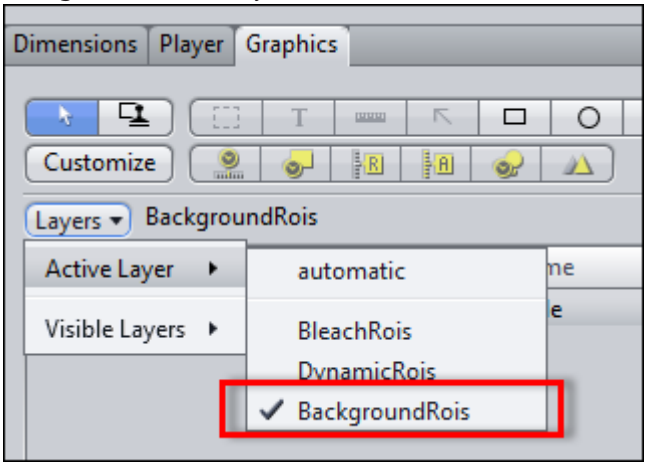
3 Select the **Use as Background ROI** entry.

The **ROI** is defined as a **Background ROI** and displayed with cross-hatching.

The **Background ROI** is assigned to the **Background ROI** layer. This is inactive by default and therefore cannot be edited.

4 To edit the **Background ROI**, activate the **Background ROI** layer. To do this, open the **Graphics** tab.

5 From the **Layers** dropdown menu under **Active Layers** activate the **Background ROIs** entry.



The background ROI is displayed in the list and can be selected and edited there and in the image area.

6 On the **ROI Tools** tab in the **Background Correction** section select the **ROI** option.

7 Click on the **Recalculate** button.

You have successfully defined a **Background ROI**. The mean intensity of the background ROI is subtracted from the measured values of the **ROIs** in a channel-specific and time-point-specific way. The corrected values are adopted into all diagrams and tables.

Defining a fixed background value

Prerequisites
- You are in the **MeanROI View** or Physiology Setup.

Procedure

1 On the **ROI Tools** tab in the **Background Correction** section select the **Constant** option.

The associated input field is activated.

2 Enter a fixed background value into the **Constant** input field.

3 Click on the **Recalculate** button.

The defined background value is subtracted from all measured values of the **ROIs**.

11.1.6 Calculating ratios

11.1.6.1 Calculating a ratio for one wavelength

Prerequisites

- To calculate ratios (quotient of two fluorescence intensities) and display ratio images, you need the **Physiology** module.
- You are in the **MeanROI View** on the **Ratio** tab (view option).

Procedure

1. In the **Method** dropdown list select the **Single Wavelength (F/F_0)** entry.
2. In the **Calculation** dropdown list select the channel for calculating the ratio.
3. In the **Reference image (Ft_0) Set-up**, define the frames of the time series image from which you want the reference value Ft_0 to be calculated.
4. Click on the **Recalculate** button.

   The ratio values are calculated. The ratio image and a diagram for the ratio values are displayed in the MeanROI View.

You have successfully calculated a ratio for a channel.

11.1.6.2 Calculating a ratio for two wavelengths

Prerequisites

- To calculate ratios (quotient of two fluorescence intensities) and display ratio images, you need the **Physiology** module.
- You are in the **MeanROI View** on the **Ratio** tab (view option).

Procedure

1. In the **Method** dropdown list select the **Dual Wavelength** entry.
2. In the **Calculation** dropdown lists select the channels for calculating the ratio.
3. Click on the **Recalculate** button.

   The ratio values are calculated. The ratio image and a ratio diagram are added to the MeanROI View.

You have successfully calculated a ratio for two channels.

11.2 Setting up Physiology Experiments

11.2.1 Introduction

Using the **Physiology** tool you can specify user-defined measurement regions (ROIs) before the acquisition of your time lapse experiment and analyze their time--dependent changes in intensity online during acquisition. Ratios can also be calculated and displayed online.

Before the experiment

A precondition for a physiology experiment is a **Time Series experiment,** which is set up in the **Time Series** tool. In the **Physiology** tool you must activate the physiology functions (online measurements) first. Here you can also activate and

configure settings for the calculation of the **Online Ratio**. In addition, the tool contains the button for opening **Physiology Setup**. There you can draw in ROIs and adjust the display of the measurement results. When Setup is opened a snap is automatically acquired, on the basis of which you can configure the settings for the subsequent experiment. The structure of Physiology Setup is based on the MeanROI View.

During the experiment

After being started, physiology experiments are displayed in the online mode of the **MeanROI View**. This allows you to analyze and influence the experiment during acquisition. The structure and options largely correspond to the offline mode of the **MeanROI View**. We therefore recommend that you familiarize yourself with the *MeanROI View* [▶ 243] (offline) before performing your Physiology experiment.

After the experiment

After you have performed your Physiology experiment the data are displayed in the offline mode of the MeanROI View and can be analyzed, processed and exported there. Further information on this can be found under: *Working with MeanROI View (Offline)* [▶ 243].

Prerequisites

Prerequisites

- To perform **physiology experiments**, you need the **Physiology** module.
- You have *created a new experiment* [▶ 35], *defined at least one channel* [▶ 34] and adjusted the focus and exposure time.
- You are on the central **Acquisition** tab.

Procedure

1. Activate the **Time Series** tool in the **Acquisition Dimensions** section.

   The **Time Series** tool appears in the **Left Tool Area** under **Multidimensional Acquisition**.

   The **Physiology** tool appears in the **Left Tool Area** under **Applications**.

> **Note**
>
> The Physiology tool is not available if the **Z-Stack**, **Tiles** or **Panorama** dimensions are activated. Deactivate these dimensions to make the **Physiology** tool available.

2. Set up a *time series experiment* [▶ 43].
3. Open the **Physiology** tool.
4. Activate the **Enable Physiology** checkbox.

You have completed the general prerequisites for physiology experiments.

11.2.2 Activating online ratio calculation

Prerequisites

- You have read the *Introduction* [▶ 250] chapter.

Procedure

1. In the **Physiology** tool open the **Online Ratio** section.
2. Activate the **Enable** checkbox.
3. If you want to save the ratio images, activate the **Save Ratio Images** check box. Otherwise only the ratio values and not the calculated ratio images are available after the experiment.
4. In the **Method** dropdown list select a method for the ratio calculation.

> **i Note**
>
> If you select the **Single wavelength (F/F0)** method for the calculation of the online ratio, you will be informed that no reference image has been defined. To calculate a reference image, indicate the number of images from which the reference image should be calculated in the input field of **Reference Image Set-up** in the **Online Ratio** section. Then click on the **Define** button to acquire the images and calculate a reference image from them.

5. If you want to use background correction for the calculation of the online ratio, activate the desired entry under **Background Correction.**

   To allow you to indicate a constant background value (**Constant** entry), an input field, in which you can enter the desired value, appears under **Calculate** in the formula for the ratio calculation.

   The **ROI** entry can only be selected once you have defined a background ROI in Physiology Setup.
6. Under **Calculate** complete the formula for calculating the online ratio by selecting the desired entries from the dropdown lists and indicating values in the input fields.
7. Under **Color** define an LUT (Look-Up-Tabelle) for the display of the ratio image.
8. Activate the **Threshold** checkbox, if you want to set a threshold in your experiment.
9. Click on the **Physiology Setup** button.

   Physiology Setup is opened. Snaps of the activated channels are acquired automatically. The ratio image is calculated and displayed with the associated diagram.
10. You can adjust the settings for the ratio calculation in the **Physiology** tool. Then click on the **Snap** acquisition button to update the ratio image.

You have successfully activated the calculation of the online ratio.

11.2.3 Setting up an experiment in Physiology Setup

Prerequisites

- You have read the *Introduction* [▶ 250] chapter.
- You are in the **Physiology** tool.

Procedure

1 Click on the **Physiology Setup** button.

Physiology Setup is opened.

An image is acquired automatically, on the basis of which you can configure your settings. You can click on **Snap** at any time to update the image.

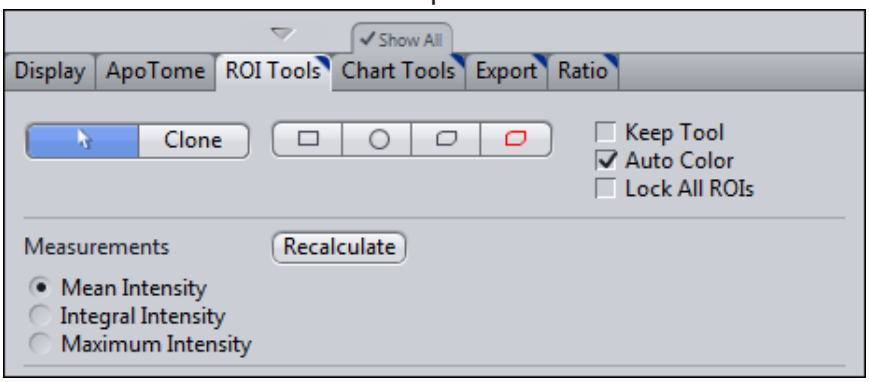
11.2.3.1 Drawing in ROIs

Prerequisites

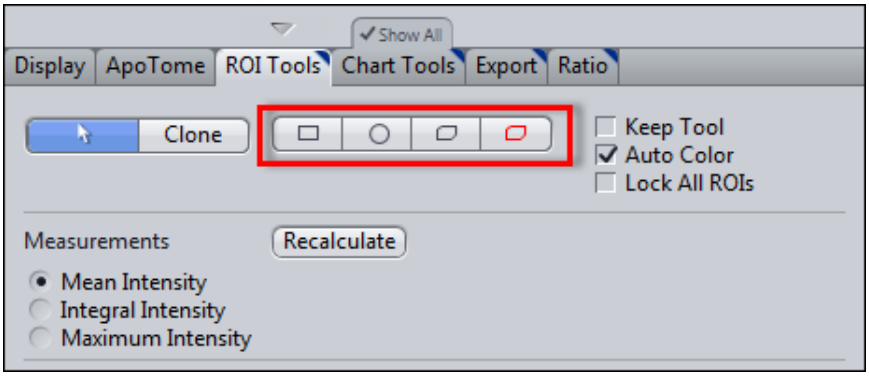
- You are in the **MeanROI View** or **Physiology Setup**.

Procedure

1 Select the **ROI Tools** tab in the **View Options**.



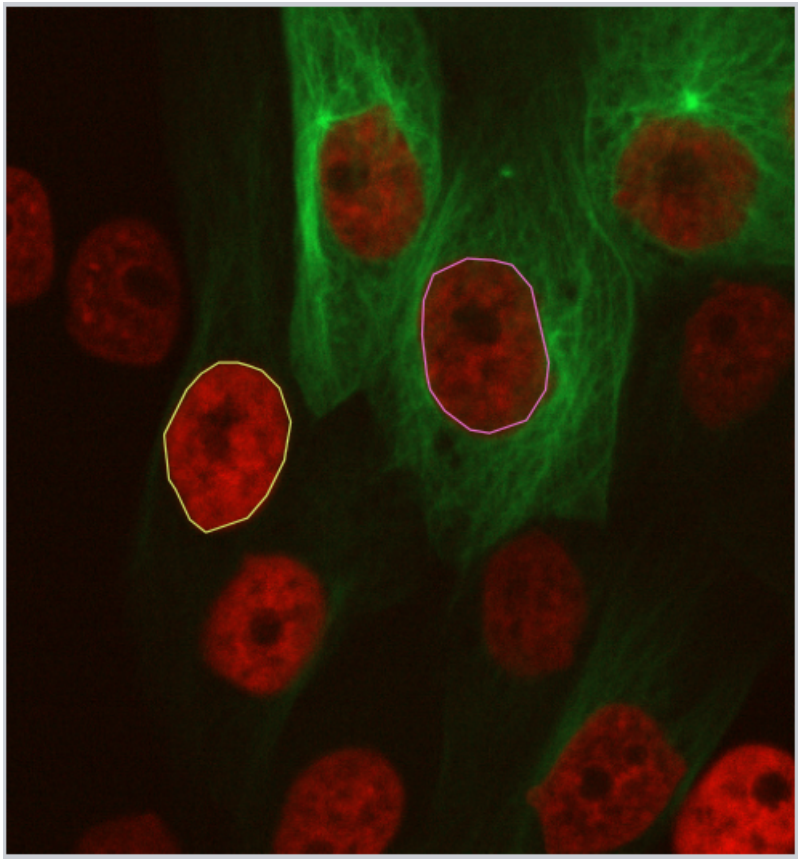
2 Select a tool for drawing in **ROIs**.



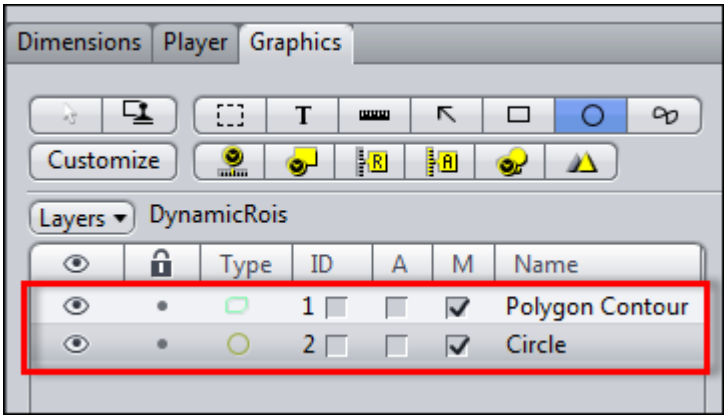
3 Activate the **Keep tool** checkbox.

The selected tool remains active after you have drawn in an **ROI**. This means you can draw in several ROIs without having to re-select the tool.

4 Using the selected tool, in the image view draw in the objects or regions (ROIs) for which intensity measurements are required.



The **ROIs** are displayed in the list under **DynamicROIs** on the **Graphics** tab.



Intensity measurements are performed for each **ROI** and displayed in the diagram area to the right of the image view.

You have successfully defined measurement regions for the intensity measurement.

11.2.3.2 Adjusting the display

Here you will find out how to adjust the display of the measured intensity values in diagrams and tables according to your wishes.

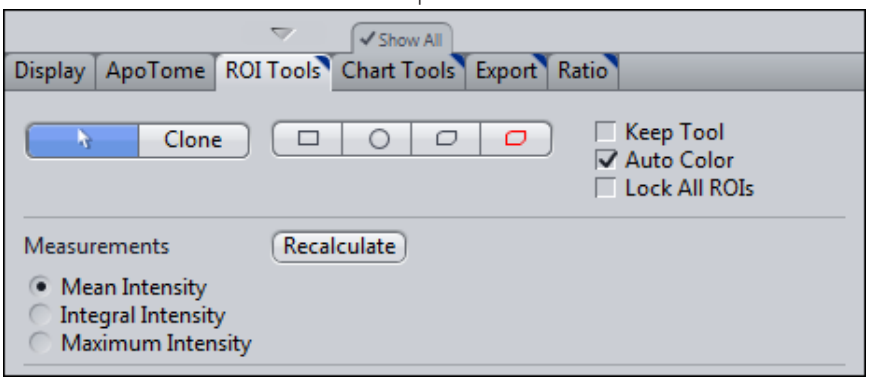
Prerequisites

- You are in the **MeanROI** view.

- You have defined at least one ROI.

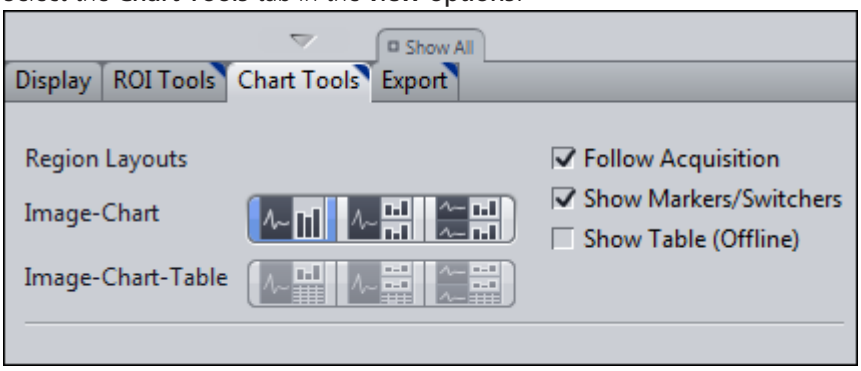
Procedure

1. Select the **ROI Tools** tab in the view options.



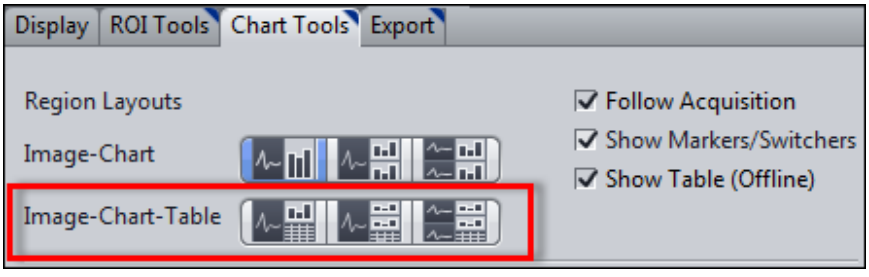
2. In the **Measurements** section select the intensity type (Mean, Integral or Maximum Intensity) that you want to be displayed in the diagrams.

3. Select the **Chart Tools** tab in the **view options**.



4. To adjust the layout of the image and diagram display, select the desired display mode under **Image-Chart**.

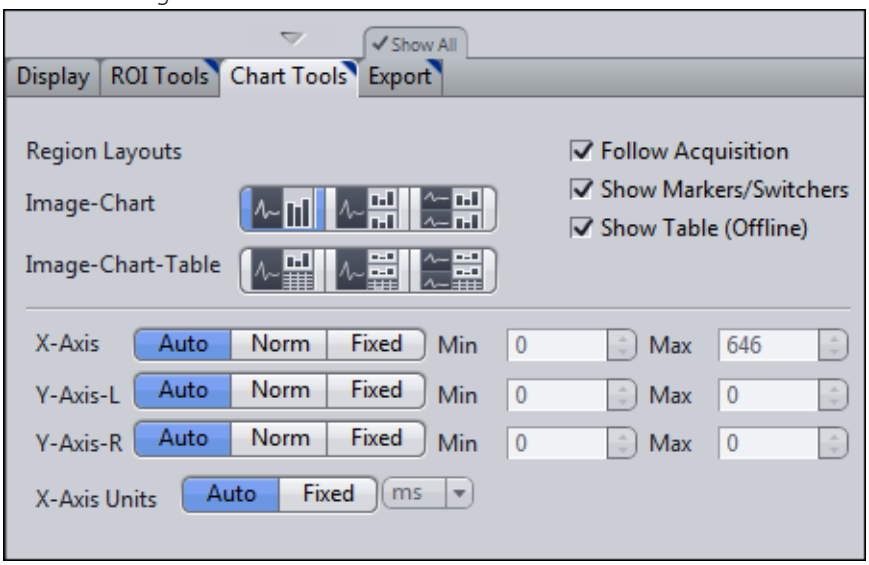
5. If you also want your data to be displayed in table form, activate the **Show Table (Offline)** checkbox.



The Image-Chart-Table modes are activated. You can now select a suitable layout for the image, diagram and table display.

6. If you want to adjust the axis scaling, activate the **Show All** option.

The axis scaling functions become visible.



7 To define the minimum and maximum values of the axes manually, click on the **Fixed** button under **X-/Y-Axis**.

The **Min** and **Max** input fields for the axis are activated.

8 Enter the desired values into the **Min** and **Max** input fields.

The minimum and maximum axis values of the diagrams are adjusted.

9 To change the unit of the X axis, click on the **Fixed** button under **X-Axis Units**.

The dropdown menu for the units is activated. You can now select the desired unit.

You have successfully adjusted the display of the intensity values.

11.2.3.3 Using background correction

Use this function to subtract background values from the measurement values. A background correction will allow you to make a better comparison in the magnitude of any fluorescent intensity changes observed over the time course of an experiment. Determine the background value with the help of a Background ROI or define a fixed value.

Defining a Background ROI

Prerequisites
- You are in the **MeanROI** View or **Physiology Setup**.

Procedure

1 At the first time point of the time series draw an **ROI** into a region of the image that contains only background signal in all channels.

2 Right-click on the **ROI.**

The shortcut menu opens.

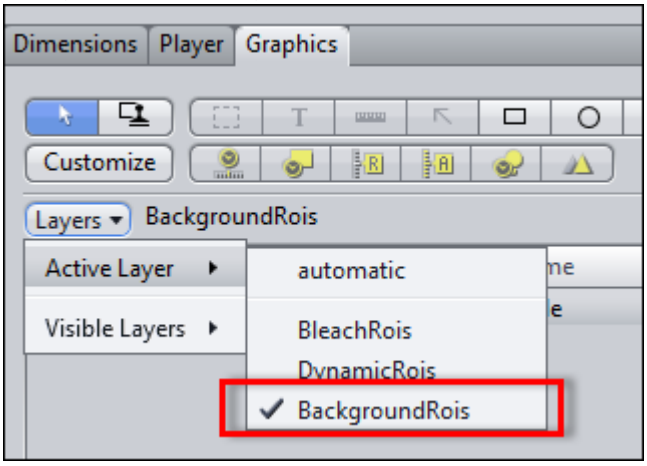
3 Select the **Use as Background ROI** entry.

The **ROI** is defined as a **Background ROI** and displayed with cross-hatching.

The **Background ROI** is assigned to the **Background ROI** layer. This is inactive by default and therefore cannot be edited.

4 To edit the **Background ROI**, activate the **Background ROI** layer. To do this, open the **Graphics** tab.

5 From the **Layers** dropdown menu under **Active Layers** activate the **Background ROIs** entry.



The background ROI is displayed in the list and can be selected and edited there and in the image area.

6 On the **ROI Tools** tab in the **Background Correction** section select the **ROI** option.

7 Click on the **Recalculate** button.

You have successfully defined a **Background ROI**. The mean intensity of the background ROI is subtracted from the measured values of the **ROIs** in a channel-specific and time-point-specific way. The corrected values are adopted into all diagrams and tables.

Defining a fixed background value

Prerequisites

- You are in the **MeanROI View** or Physiology Setup.

Procedure

1 On the **ROI Tools** tab in the **Background Correction** section select the **Constant** option.

The associated input field is activated.

2 Enter a fixed background value into the **Constant** input field.

3 Click on the **Recalculate** button.

The defined background value is subtracted from all measured values of the **ROIs**.

11.2.4 Starting and influencing an experiment

Prerequisites

- You have read the *Introduction* [▶ 250] chapter and set up an experiment in **Physiology Setup**.
- You are on the **Acquisition** tab.

Procedure

1. Start your Physiology experiment by clicking on the **Start Experiment** button.

   The time lapse experiment is started. The MeanROI View (online) opens and displays the current images and the intensity curves for each ROI measured online. The intensity curves are displayed in the Time Line View and in the diagrams.

2. You can pause the experiment at any time by clicking on the **Pause Experiment** button and continue it again by clicking on the **Continue Experiment** button.
3. The **Focus** can be adjusted during the experiment. To prevent images that are not sharp being acquired, pause your experiment and use the **Live** acquisition button to adjust the focus. Then continue the experiment.
4. Adjust the display of the intensity values during the experiment by changing the settings on the **Chart Tools** tab. The unit of the X-axis cannot be changed during the experiment.
5. You can move and change ROIs during acquisition. The changes are adopted for all subsequent time points, see *Drawing in and adjusting ROIs* [▶ 244].
6. Activate **Switches** in the **Time Series** tool during the experiment to perform the corresponding actions.

i Note

Various events, such as the activation of switches or the pausing of the experiment, are labeled in the Time Line view by markers.

7. On the **Chart Tools** tab deactivate the **Follow Acquisition** checkbox to analyze the data acquired up to that point. To do this, select the corresponding time points using the **Time** slider on **Graphics** tab, the diagram sliders or the Time Line view slider in the **MeanROI** view.
8. Change the size of the area marked in blue in the Time Line View to adjust the section displayed in the diagrams.

You have successfully started the experiment, analyzed it online and influenced it.

11.2.4.1 Adjusting ROIs during experiments

If objects move laterally in the course of the experiment, you can adjust the **ROIs** at any time during the experiment in order to follow the objects.

Prerequisites

- You have defined at least one ROI.

- You have started your Physiology experiment.

Procedure

1. In the **Experiment Manger** click on **Pause experiment** button.
2. Adjust the position of the **ROI** using drag & drop. To do this, select the ROI in the **image area** by left-clicking and hold the mouse button down. Then move the ROI to the new position and release the mouse button.
3. To change the shape of an **ROI**, right-click on an ROI and select the **Edit Points** entry (e.g. for polygon contours).
4. Adjust the shape of the **ROI** by dragging and dropping the contour points.

   Changes to the position and shape of the **ROIs** are adopted for all subsequent time points.
5. Repeat the previous steps for all subsequent ROIs that you wish to adjust.

You have successfully adjusted the measurement regions (ROIs) to the course of the experiment.

11.2.5 Sample experiment Fura-2 with DG4/5

11.2.5.1 Step 1: Creating channels

Prerequisites

- To perform the experiment, you need the **Physiology** module.
- You have a **Sutter DG4/5** with appropriate excitation filters for **Fura-2** and a **Fura-2 filter set** in the microscope's reflector wheel.
- You are on the **Acquisition** tab.

Procedure

1. Create a new experiment in the **Experiment Manager**, e.g. "**Physiology Fura-2**".
2. Add the channel Fura-2 using **Smart Setup**.
3. Activate the **Time Series** checkbox in the acquisition dimensions.
4. Open the **Channels** tool.
5. Select the Fura-2 channel from the list.
6. Click on the **Options** button  and select the **Copy** entry.
7. Select the first Fura-2 channel from the list.
8. Click on the **Options** button  and select the **Rename** entry.

   You can now rename the channel, e.g. **Fura-2 340 nm**.
9. Repeat steps 7 and 8 to rename the second channel, e.g. **Fura-2 380 nm**.
10. Select the **Fura-2 380 nm** channel.
11. Select another LUT from the dropdown list, e.g. red.
12. Select the entry **21 HE Ex. FURA 380** from the Excitation dropdown list.

The excitation filter is used for this channel.

13 Adjust the exposure time and focus for both channels.

You have created the channels for your experiment.

11.2.5.2 Step 2: Setting up a time series and creating switches

Procedure

1 Open the **Time Series** tool.

2 Using the **Duration** slider and the dropdown list for the unit, specify the duration of the experiment, e.g. 10 min.

3 Using the **Interval** slider and the dropdown list for the unit, specify the length of the interval between acquisitions, e.g. 1 second.

4 To create buttons open the **Switches** section in the **Time Series** tool. This section is visible only if the **Show All** mode is activated.

5 Click on the **Add** button  .

A new switch is added.

6 Edit the switch by clicking on the black arrow to the right of the switch.

The switch properties are visible.

7 Enter a name, e.g. **Fast**. Activate the **Color** checkbox and select a color, e.g. blue. Define an action to be performed when you activate the button, e.g. **As fast as possible**.

You have successfully set up a time series and created a switch.

11.2.5.3 Step 3: Setting up an online ratio

Procedure

1 Open the **Physiology** tool.

2 Activate the **Enable Physiology** checkbox.

3 Open the **Online Ratio** section.

4 Under **Method** select the **Dual Wavelength** entry from the dropdown list.

5 Under **Calculation** select the **Fura-2 340 nm** entry from the dropdown list in the numerator of the formula.

6 Under **Calculation** select the **Fura-2 380 nm** entry from the dropdown list in the denominator of the formula.

7 Activate the **Activate** checkbox.

You have successfully activated the ratio functions and specified the calculation of the ratio.

11.2.5.4 Step 4: Physiology Setup

Procedure

1 In the **Physiology** tool click on the **Physiology Setup** button.

Physiology Setup is opened. Snaps of the configured channels are acquired automatically and displayed in the Center Screen Area. A preview of the ratio image, which is calculated according to the ratio settings, is also displayed. The diagrams for each image are displayed to the right of this.

2 On the **ROI Tools** tab, select a tool for drawing in ROIs, e.g. Circle.

3 Activate the **Keep Tool** checkbox.

4 Draw your ROIs into one of the images.

5 Deactivate the **Keep Tool** checkbox and select the selection tool (**arrow**) again.

6 Under **Measure** on the **ROI Tools** tab select the type of intensity measurement to be displayed, e.g. Mean Intensity.

7 Under **Region Layouts** on the **Chart Tools** tab select a layout for the image and diagram display, e.g. Multi-Image Multi-Chart.

8 Click on the **Fixed** button under **X-Axis Unit** and select a unit from the dropdown list, e.g. seconds.

9 Click on **Finish** at the top left of **Physiology Setup** to leave Physiology Setup.

You have successfully configured and adjusted the Physiology Setup.

11.2.5.5 Step 5: Starting, analyzing and influencing an experiment

Procedure

1 Start the experiment by clicking on the **Start Experiment** button.

The experiment is started. In our example an image is acquired every second for a period of 10 minutes. The experiment opens in the online mode of the MeanROI View, which displays the current images and measurements.

2 Activate the created switch at the desired time point. To do this, open the **Switches** section in the **Time Series** tool. Click on a switch as soon as you want its action to be performed, e.g. click on the "**Fast**" switch to acquire the subsequent images as quickly as possible one after the other. A marker will mark the time point at which the switch was activated on the X axis in the color of the switch (e.g. blue).

3 Once the time series has been completed you can analyze the experiment in the offline mode of the **MeanROI** view, process it and export its values.

You have successfully performed the experiment.

12 Module Shuttle & Find

12.1 Introduction

Fig. 19: SEM / LM system for correlative microscopy

The **Shuttle & Find** module in ZEN 2.1 is used for the relocation of sample positions in two different microscopes, e.g. a light microscope and a scanning electron microscope (SEM), and the correlation of two images to one merged image. This technique is called correlative microscopy or just "CorrMic". It is used to combine the two worlds of scanning electron microscopy and light microscopy and brings it together in one image.

The samples can be mounted in special designed correlative holder systems (with three correlative calibration markers) from ZEISS. Also user-defined holder systems with three calibration markers can be used. Biological samples are mainly deposited on cover glasses or on TEM grids. In contrast to biological samples, the shape and size of material samples vary strongly. In respect to these requirements, the correlative holders were designed accordingly.

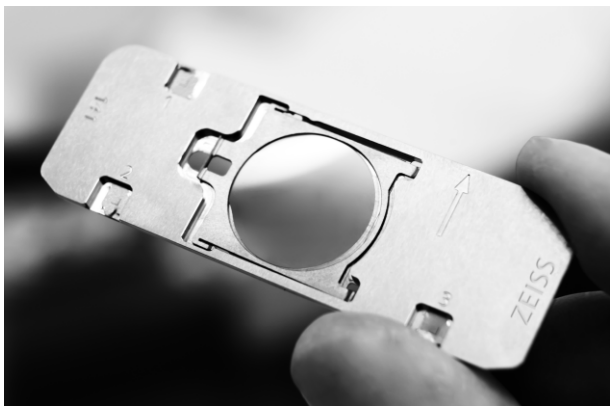


Fig. 20: Example of a correlative ZEISS sample holder

12.2 User Interface and Functions

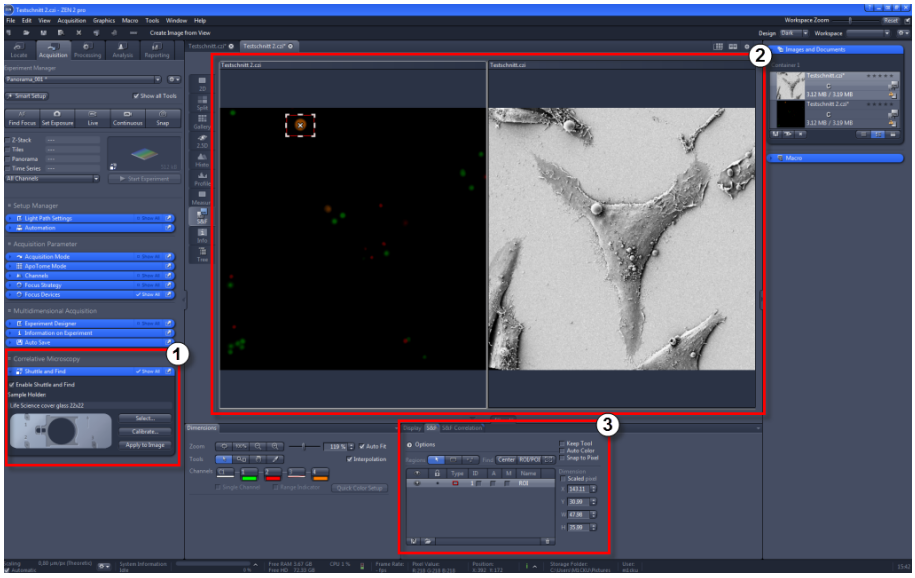


Fig. 21: User Interface Shuttle and Find Module

- **Shuttle and Find tool** in the Left Tool Area **(1)**
- **S&F view** in the Center Screen Area **(2)**
- **S&F** and **S&F Correlation view options** below the Center Screen Area **(3)**

12.2.1 Shuttle and Find Tool

Here you choose and calibrate your sample holders.

Fig. 22: Shuttle and Find tool

Option	Description
Enable Shuttle and Find checkbox	**Activated:** The Shuttle and Find tool is active. You are able to start the correlative workflow by selecting and calibrating the sample holder. Deactivate the checkbox if you don't want to use Shuttle and Find within your experiments.
Sample holder	Here you see the name and preview of the selected sample holder.
Select... button	Opens the **Select Template** dialog. There you select the preferred sample holder or define new holder templates, see *Selecting the sample holder* [▶ 277].
Calibrate... button	Opens the Sample Holder Calibration Wizard. There you can calibrate the selected sample holder.
Apply to Image button	Only visible if the **Show All** mode is activated. **Use this button only, when you forgot to calibrate the holder before you acquire the image.** Applies a calibration to an acquired image. Do not remove the sample out of the correlative holder between image acquisition and calibration.

Shuttle and Find tool for SEM

Only visible if you have started the ZEN 2.1 SEM software.

The tool window is adapted to the requirements of the correlative workflow on a SEM. Therefore three additional buttons are available.

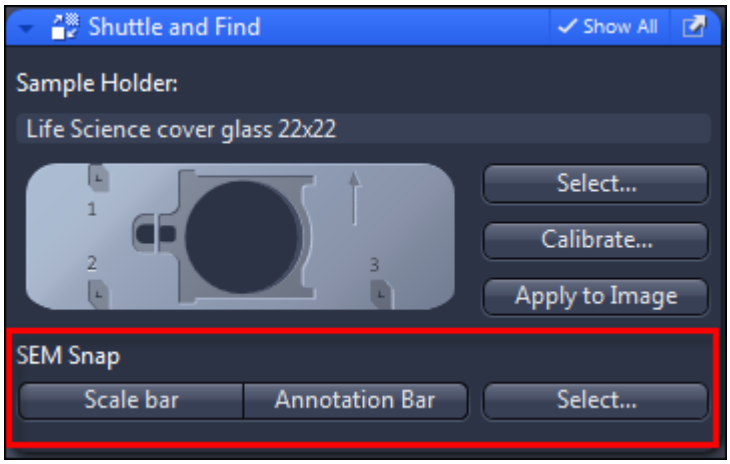


Fig. 23: Shuttle and Find tool in ZEN SEM software

Button	Function
Scale bar	Adds a scale bar to the snapped (acquired) image.
Annotation bar	Adds an annotation bar to the snapped (acquired) image.
Select...	By clicking on this button a dialog opens to select parameters for the annotation bar. You can select max. 9 parameters for the annotation bar.

12.2.2 S&F view

Besides the **Shuttle and Find** tool in the **Left Tool Area**, the **S&F (Shuttle & Find)** view is visible in the **Center Screen Area** of the ZEN software. If the S&F view is selected, the **S&F** tab and **S&F Correlation** tab will appear as specific view options under the image area.

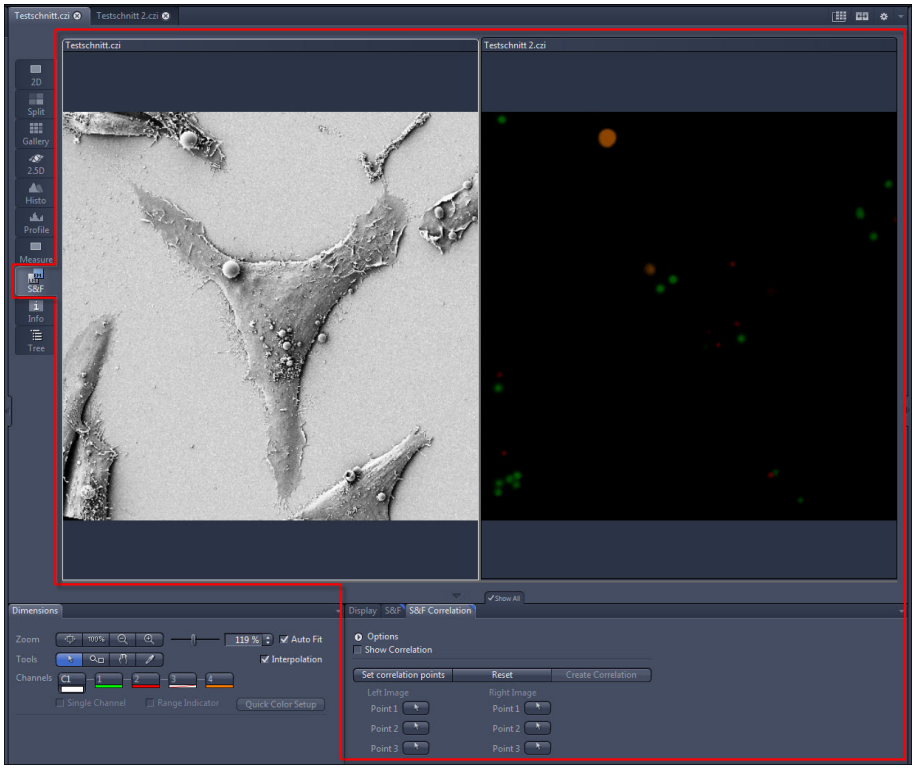


Fig. 24: Shuttle and Find View

12.2.2.1 S&F tab

Here you find helpful options and tools to draw in and relocate regions of interests (ROIs) or points of interest (POIs) within the sample image.

12.2.2.1.1 Options

Options	Description
Mirror Image	Here you can mirror the image horizontally or vertically by using the two buttons at the right. The alignment of the images depends on the microscope (upright/inverted) and orientation of the sample holder.
Keep tool	**Activated:** Keeps the current tool active. That's helpful if you want to draw in more than one ROI/ POI.
Auto color	**Activated:** Uses a new color for each new element which is drawn in.
Snap to Pixel	**Activated:** Draws in graphical elements using the pixel grid.
Use fine calibration value	**Activated:** Uses the measured fine calibration. The precision of relocation and therefore the quality of the overlay image can be improved by determination of an offset value. This value describes the offset between the loaded image and the live image. The defined offset value is only valid for the loaded image which you can see in the container. If another image is loaded or if you close the dialogue, the offset value will be deleted. Determine the offset by identification of a POI (Point Of Interest) within the snapped image. To identify a POI use the buttons in the **Regions** section. By clicking on the **Set Offset** button, the stage moves to the supposed sample position. Compare the sample position within the live image with the set POI and correct the stage in that way that both shown positions are identically. Confirm the fine calibration with the **Ok** button. Now the fine calibration is measured and the checkbox is activated.
Double click in image to move stage	**Activated:** Moves the stage to the position you have double clicked on.
Refocus after stage movement	**Activated:** Adjusts the focus automatically after the stage has moved.
Move stage in z-direction before x/y movement	**Activated:** Moves the stage to the load position before it moves to the next correlative calibration marker.

Options	Description
Show splitter view	**Activated:** Activates **Splitter Mode** in the **Center Screen Area**.

12.2.2.1.2 Regions, Find and Dimensions

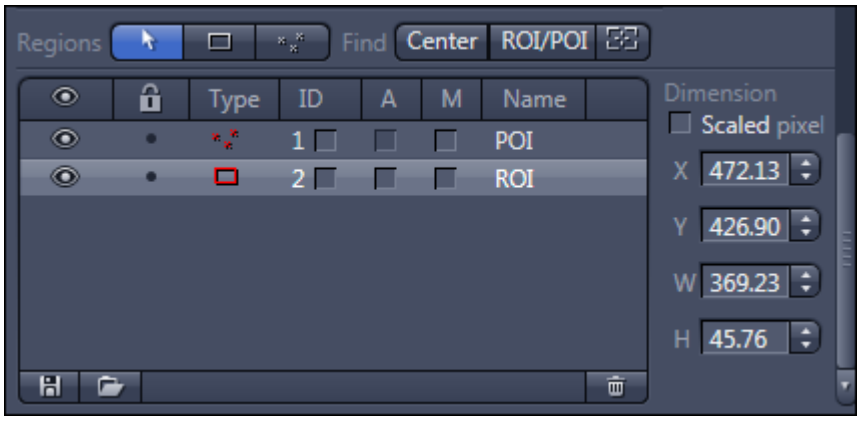


Fig. 25: Regions, Find, Dimension

Regions and Find tool bar

Button	Description
Selection mode	Selects the ROIs or POIs in the image area. If you are currently in another mode, you can switch back to the **Selection** mode using this button.
Draw rectangle	Draws in a rectangle (**Region of Interest (ROI)**) that is always parallel to the edges of the image.
Draw marker	Draws in a marker point (**Point of Interest (POI)**).
Center	Moves the stage to the center of the opened image.
ROI / POI	Moves the stage to the selected **ROI** / **POI**.
Show stage position	Shows the current stage position as a rectangle in the image.

Dimension section

Here you see coordinates and dimensions of the selected graphical element in the list. If the **Scaled** checkbox is activated, the unit is µm, otherwise Pixel.

- Parameter **X**: Shows the horizontal position (x coordinate) of the center of the graphical element.
- Parameter **Y**: Shows the vertical position (v coordinate) of the center of the graphical element.

- Parameter **W**: Shows the width of the graphical element.
- Parameter **H**: Shows the height of the graphical element.

Graphical elements list

Here you see the list of all ROI / POI which are drawn in. The following table describes the list columns and its functions:

List columns	Description
Eye symbol	Shows or hides the ROI / POI in the image.
Lock symbol	Locks a ROI / POI to prevent changes.
Type	Displays the icon for the tool type (ROI/POI). To format a graphic element, double-click on the icon. The **Format Graphic Elements** dialog opens.
ID	Only visible if the **Show All** mode is activated. Displays the ID for the graphic element. To do this, activate the checkbox at the corresponding list entry.
A	Only visible if the **Show All** mode is activated. Displays annotations for a graphic element (ROI). To do this, activate the checkbox at the corresponding list entry. Then double click on the checkbox. The **Format Graphic Elements** dialog opens. Choose an annotation you want to have displayed within the image from the **Annotation** dropdown list.
M	Only visible if the **Show All** mode is activated. Displays measurement data for a graphic element. To do this, activate the checkbox at the corresponding list entry.
Name	Displays the name of the graphic element. To change the name, double-click in the Name field. Then enter the text of your choice.

12.2.2.2 S&F Correlation tab

Here you find all functions to overlay (correlate) two images.

Options

To show the section in full, click on the **arrow** button  .

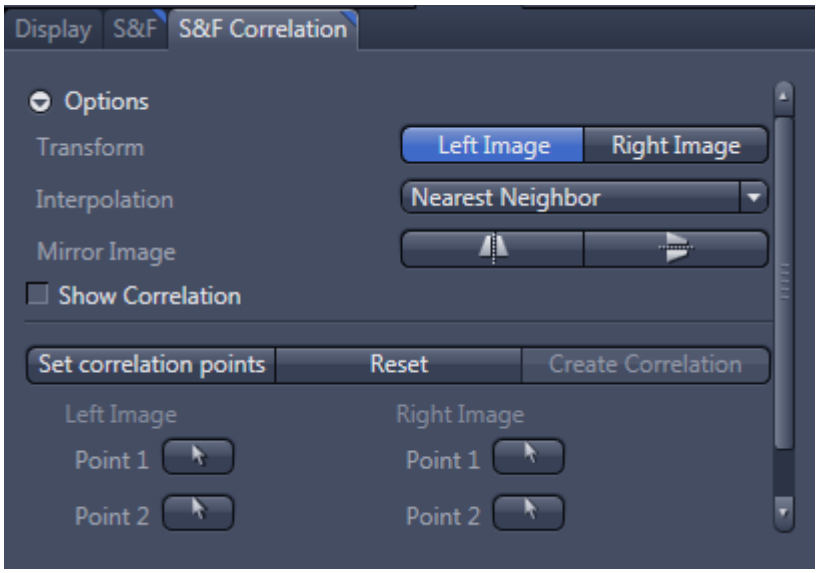
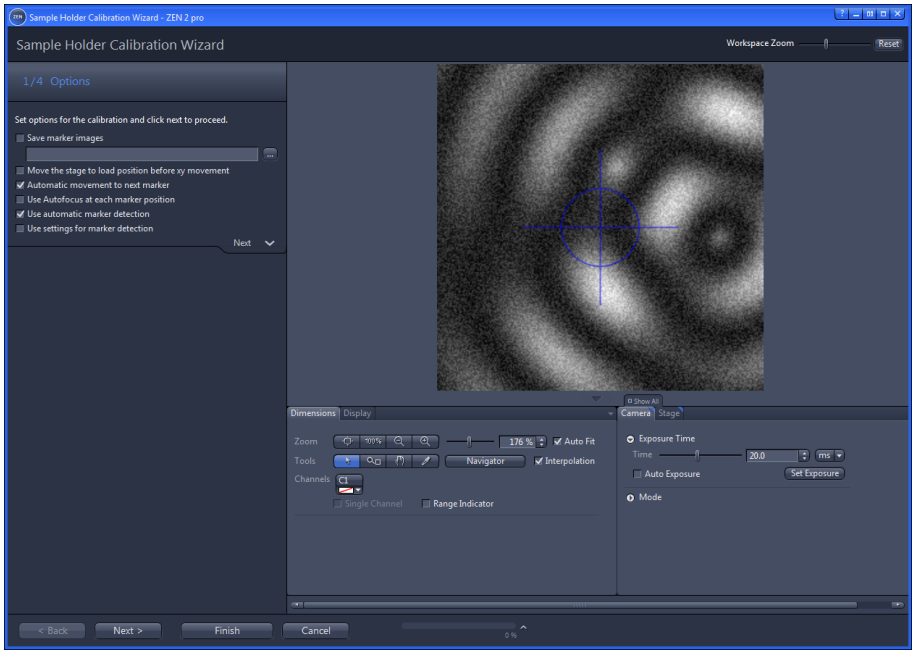


Fig. 26: S&F Correlation tab

Option	Function
Transform	Here you select which image will be transformed. During transformation a pixel in the overlay image is calculated by using pixels of the two original images that shall be overlaid / merged.
Interpolation	Here you can select one of the following interpolation methods: **Nearest Neighbor**: The gray value of the resulting pixel in the overlay image is made of a pixel which is located next. This interpolation method is very fast. **Linear:** The resulting or calculated pixel in the overlay image is assigned to a gray value, which is the result of a linear combination of gray values derive from pixels located nearby (in the original image). **Cubic:** The calculated pixel in the overlay image is assigned to a gray value, which is calculated by means of a polynomial function using gray values of pixels in the original images; these pixels are located nearby the calculated pixel.
Mirror image	Here you can mirror the image horizontally or vertically. Therefore simply click on the corresponding button. Mirroring an image is necessary, when the loaded image shows a different orientation than the live image.
Show Correlation	**Activated:** Opens the correlated image in a new image document / new container.

Option	Function
Set correlation points	Enables you to set 6 points (3 points in each image) as correlation markers in a row, see *Correlating two loaded images* [▶ 290].
Reset	Deletes all correlation points in the images.
Create Correlation	Active only, if all correlation points are set in both images. Creates a correlative overlay image. A third image container with the correlated image will be opened in the **Center Screen Area** and the **Show Correlation** checkbox will be activated automatically.

12.2.3 Sample Holder Calibration Wizard



With the Sample Holder Calibration Wizard you calibrate your selected correlative sample holder. The wizard is opened via the **Shuttle and Find** or **Correlative Array Tomography (CAT)** tool. Make sure that you have selected the desired sample holder, see *Selecting the sample holder* [▶ 277].

12.2.3.1 Step 1: Options

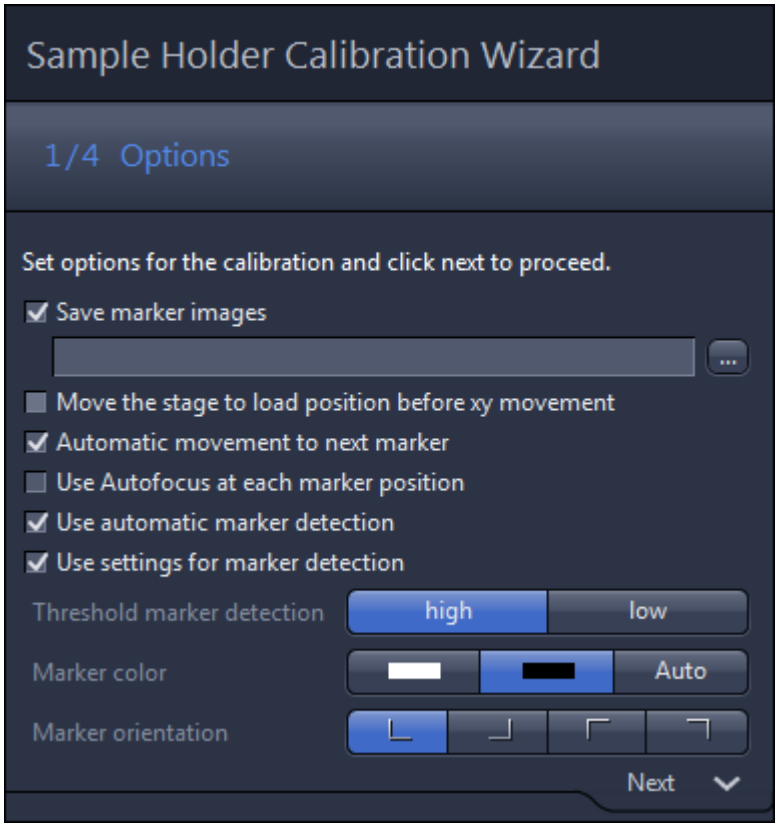


Fig. 27: Sample Holder Calibration Wizard Options

Option	Description
Save marker images	**Activated:** the marker images are saved during the calibration. The images can be used to check the calibration afterwards. Click on the **Select Folder (...)** button to select a storage folder.
Move the stage to load position before x/y movement	**Activated:** the stage will move to load position before moving to the next correlative calibration marker. In case of using an AxioObserver, the objective revolver moves to load position.
Automatic movement to next marker	**Activated:** By clicking on the **Next** button within the wizard the stage moves automatically to the next calibration marker.
Use Autofocus at each marker position	This option is active only if the **Automatic movement to next marker position** checkbox is activated. **Activated:** the focus is adjusted automatically after moving to the next marker position.

Option	Description
Use automatic marker detection	**Activated:** The software will try to detect the small calibration marker automatically.
Use settings for marker detection	This option is active only if the **Use automatic marker detection** checkbox is activated. **Activated:** shows settings for marker detection (see description below). Here you select the properties of the calibration markers.

Settings for marker detection

Only visible if the **Use settings for marker detection** checkbox is activated.

Option	Description
Threshold marker detection: high – low	A low threshold for marker detection is used when the dimensions of the correlative L markers cannot be recognized precisely, e.g. when the sample holder is slightly filthy.
Marker color	Here you select the color of the markers displayed in the live image. **White:** the marker is displayed white on a dark background. **Black:** the marker is displayed dark on light background. **Auto:** the marker color is set automatically.
Marker orientation	Here you need to set the orientation of the L-markers on your sample holder. Click on the corresponding button to select the orientation of the calibration marker which you can see in the live image

If you click on the **Next** button you will move to the next step of the wizard.

12.2.3.2 Step 2-4: Calibration

In steps 2-4 of the wizard you will be guided through the calibration procedure.

Fig. 28: Sample Holder Calibration Wizard

Option	Function
Holder position	**Move to Position 1** button Moves the stage to marker position 1. This is possible only if the first position was set before and x/y coordinates are given. **Current** button Only visible for marker position 2 and 3. Moves the stage to the current marker position. This is possible only if the current position was set before and x/y coordinates are given.
Stage movement to the next marker	Here you can change the movement of the stage in x or y direction. This is necessary if during calibration the stage moves in the wrong direction.
Marker position	By clicking on the **Set** button, the actual marker position will be confirmed.

Click **Finish** to leave the wizard.

12.3 The Shuttle & Find Workflow

12.3.1 Settings and image acquisition with the light microscope

Before acquiring an image with the light microscope and using it for correlative microscopy, it is necessary to make general settings e.g. stage calibration, camera orientation, calibrating objectives and setting the correct scaling. Please notice that we do not describe all theses topics within this guide as we focus on the Shuttle and Find workflow only.

Furthermore we will not describe basic functionality of ZEN 2.1 software in this guide, like program layout or general image acquisition topics. If you want to read more about these topics, please read the online help of the software.

12.3.1.1 Mounting the sample holder to the LM

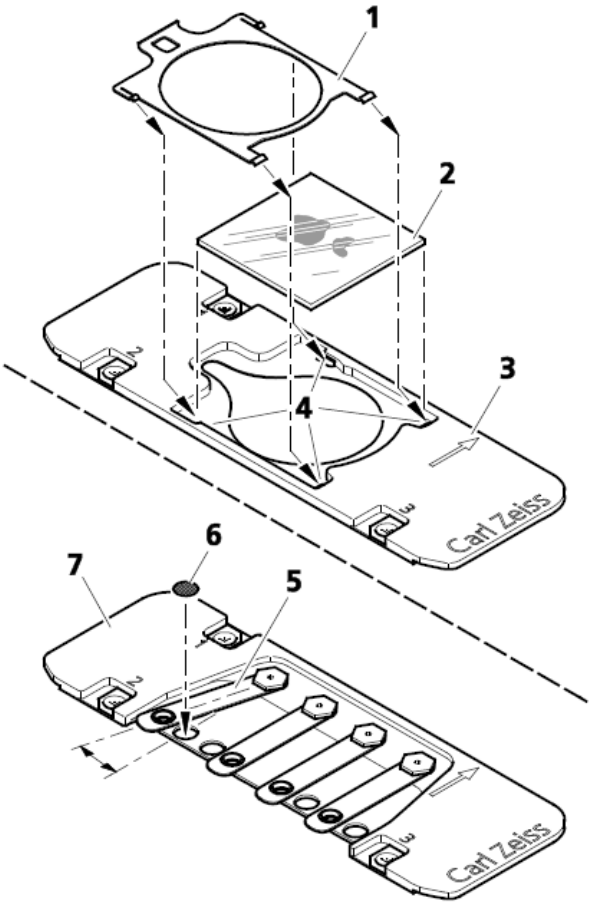


Fig. 29: Mounting of cover glasses or TEM grids

1 Place the cover glass **(2)** in the suitable sample holder and fix it.

In case of using the holder **Life Science Cover Glass 22x22**:

– Remove the clamping frame **(1)** using tweezers.

– Insert the cover glass **(2)** in the sample holder **(3)**.

– Slide in the clamping frame into the sample holder until the clamps are clicking into place **(4)**.

In case of using the holder **Life Science for TEM grids**:

– Lift the spring of the appropriate position and turn it sidewards **(5)**.

– Insert the TEM grid **(6)** into the provided holding spot of the holder and fix it with the spring **(7)**.

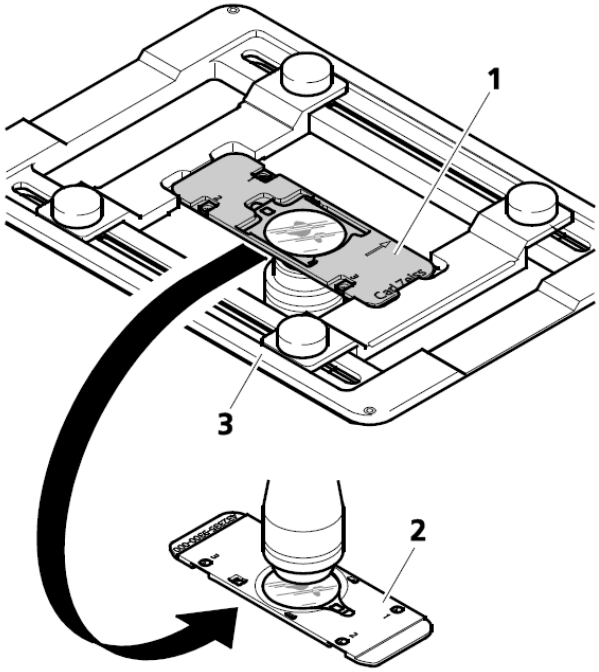


Fig. 30: Inserting a sample holder

2 Insert the sample holder **(1)** into the mounting frame of the microscope stage in the following way:

– For inverted stands, see **(3)**.

– For upright stands, see **(2)**.

12.3.1.2 Starting the LM software

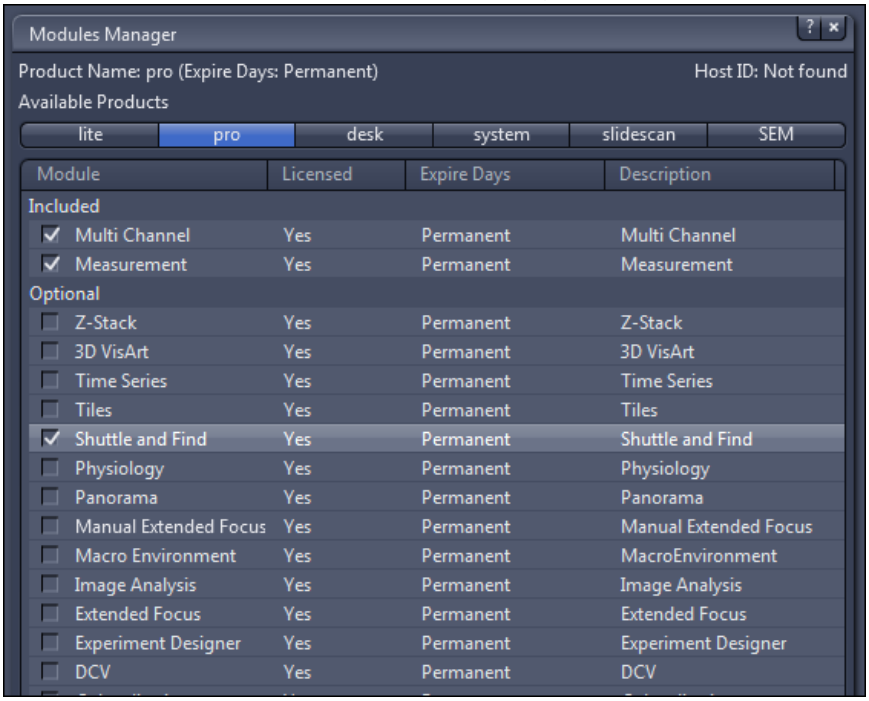
For correlative microscopy with light microscopes ZEN 2.1 software has to be installed. In addition you need to licence the **Shuttle and Find** modul.

Procedure **1** Start ZEN 2.1 by clicking on the corresponding program icon on your desktop.

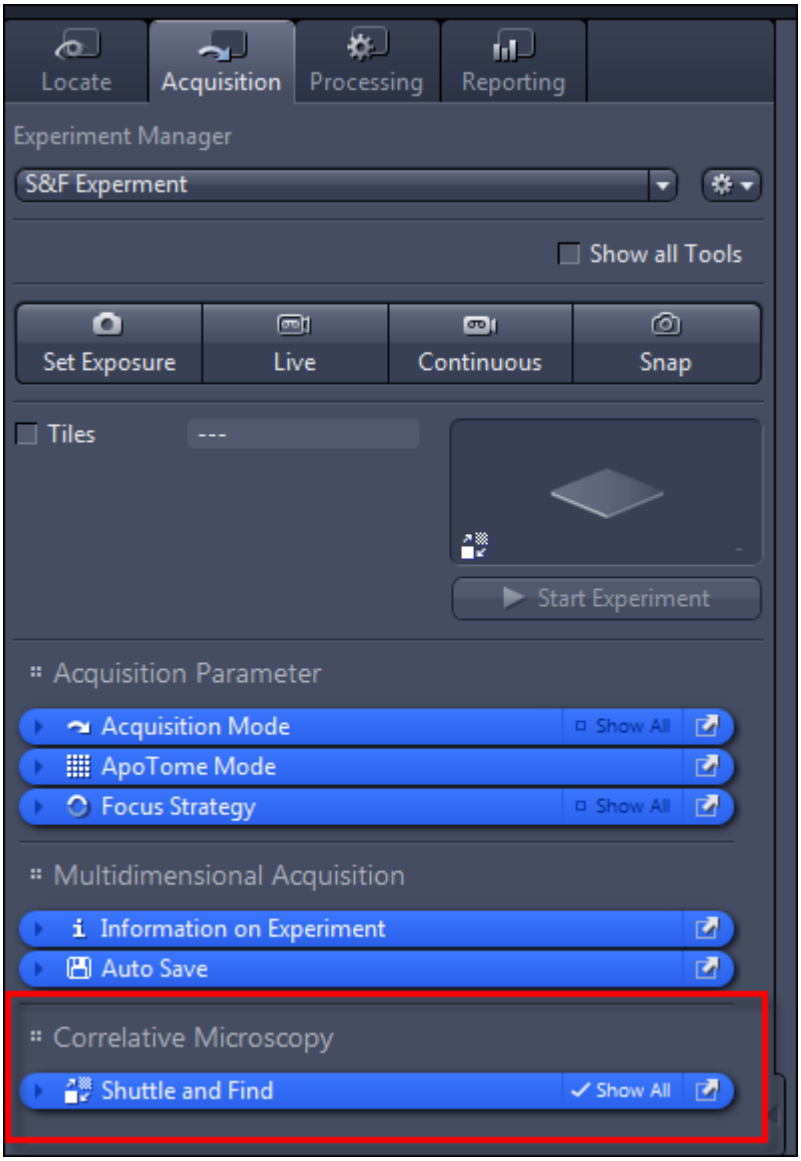
Following window will appear.

2 Click on the **ZEN pro** or **ZEN system** button to start ZEN 2.1.

The software will start now. Make sure that you have activated the Shuttle and Find module in the menu **Tools | Modules Manager ...**



3 In the **Left Tool Area** click on the **Acquisition tab** and open the **Shuttle and find** tool.



You have successfully started the software. Now you can start working with the **Shuttle and Find** module.

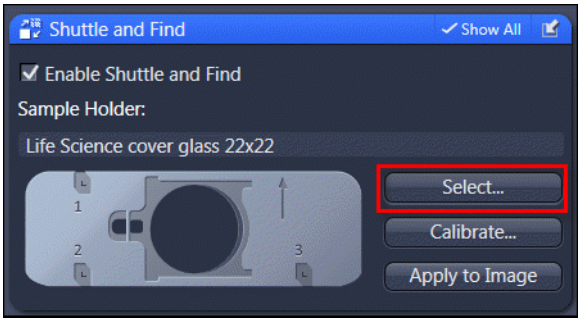
12.3.1.3 Selecting the sample holder

Prerequisites ■ You are in the **Shuttle and Find** tool.

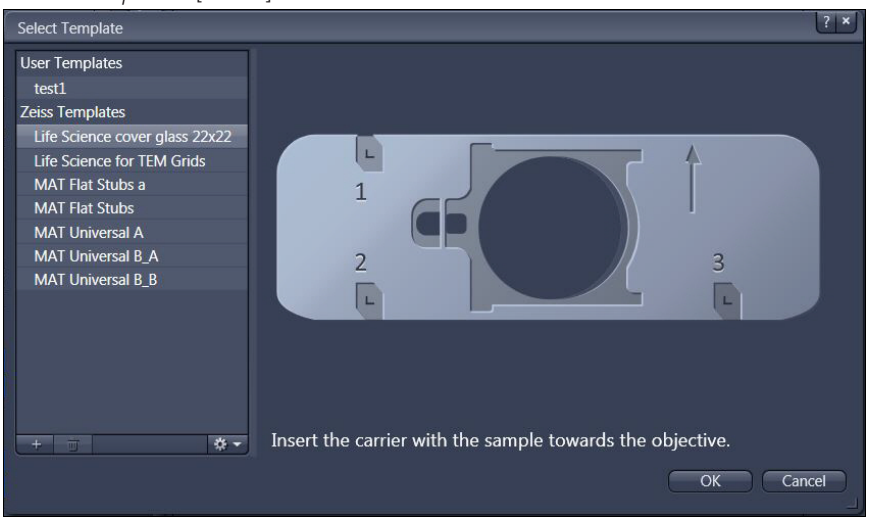
Procedure

1 Within the **Shuttle and Find** tool activate the checkbox.

2 Click on the **Select…** button to open the **Select Template** dialog and to choose the correlative holder you want to use. Different types of correlative holders are available, see Appendix *Correlative Sample Holders* [▶ 292]



3 In the **Select Template** dialog select the correlative holder you want to work with. If you want use your own sample holders, click on the **+ (Add)** button below the list and follow the instructions in the chapter *Defining new sample holder templates* [▶ 279].



4 Click on the **Ok** button to close the dialog.

You can now continue with the calibration of the sample holder, like it is shown in the chapter *Calibrating the sample holder* [▶ 280]. The calibration of the sample holder is mandatory to acquire images.

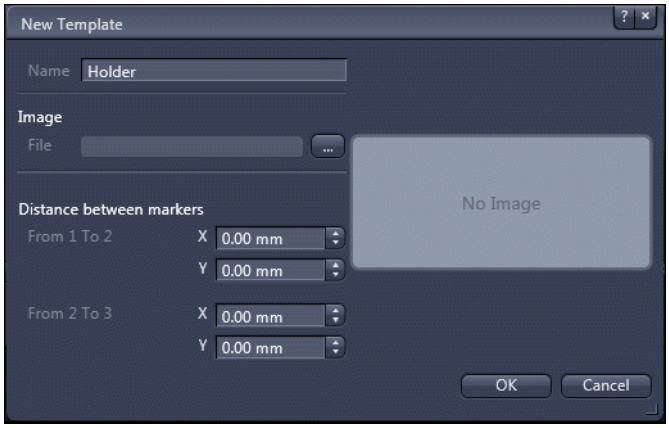
12.3.1.4 Defining new sample holder templates

With this dialog you can define new correlative holders in addition to the existing holder templates. It is not mandatory to use correlative holders from ZEISS. User-defined correlative holders with 3 fiducial markers can be used as well.

Procedure

1 To open the dialog click on the **Add (+)** button in the **Select Template** dialog. This dialog can be opened via the Shuttle and Find tool.

The **New Template** dialog opens.



2 Type in a name for the new holder / sample carrier. An image of the new holder can be loaded as well.

3 Insert the distances (in millimeters) between the first and the second marker and between the second and third marker.

The distances can be determined using the **Stage Control** dialog accessible via the **Ligth Path** tool on **Locate** tab. We recommend to do this before you start the New template dialog. Write down the distances to be prepared to enter them within the New Template dialog.

1 Activate the live view in the Center Screen Area by clicking on the **Live** button in the Locate tab.

2 Navigate the stage manually to the calibration marker on the sample holder by means of the joystick and note the x/y-coordinates of the marker.

3 Repeat this procedure for all three markers and calculate the distances between marker 1 and marker 2 and between marker 2 and marker 3, respectively.

Fig. 31: Stage Control dialog

12.3.1.5 Calibrating the sample holder

The correlative sample holders have three fiducial markers enabling a three point calibration (signed with the numbers 1-2-3) The calibration markers consist of one small (length 50 µm) and a large L-shape marker (length 1 mm). The bigger marker is used for coarse orientation, whereas the smaller marker is used for the calibration.

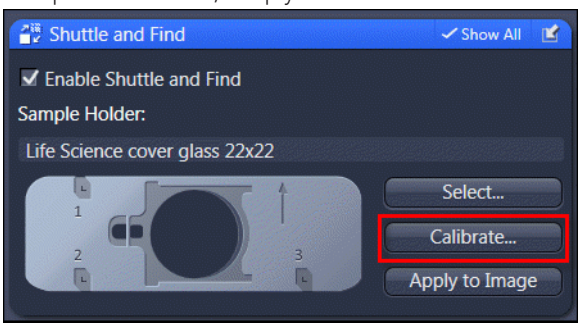
12.3.1.5.1 Preparations

Prerequisites

- Your system needs a motorized stage to perform the calibration.

Procedure

1. Move the stage to marker position 1.
   To locate the marker positions we recommend to use a dry objective with low magnification (5x – 20x).
2. To open the wizard, simply click on the **Calibrate…** button.



NOTICE

Using different objectives for calibration and image acquisition can cause an offset. If a correction is necessary use the option **fine calibration** in the **S&F** tab.

12.3.1.5.2 Step 1: Options

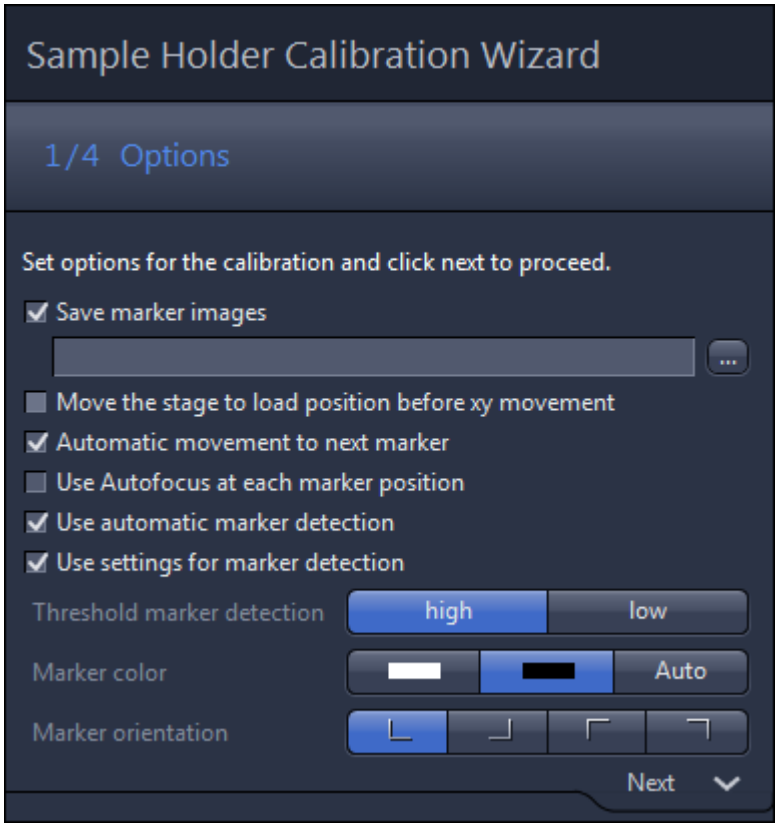


Fig. 32: Sample Holder Calibration Wizard Step 1

Option	Description
Move stage in z-direction before x/y-movement	**Activated:** the stage moves to load position before moving to the next correlative calibration marker.
Automatic movement to next marker	**Activated:** the stage moves automatically to the next marker if the position of the correlative marker has been set manually or confirmed (automatic marker detection).
Use Autofocus at each marker position	This option is only visible if, the **Show All** mode is activated. **Activated:** the focus is adjusted automatically after moving to the next marker.

Option	Description
Save marker detection images	This option is only visible, if the **Show All** mode is activated. **Activated:** the marker images are saved during the calibration. The images can be used to control the calibration after the calibration. Click on the **Select Folder (...)** button to select a folder the images should be saved into.
Stage movement	Here you can invert the movement of the stage. This is helpful if during the calibration the stage is moving in the wrong direction. Activating **Invert X** button inverts the movement of the stage in X-direction. Activating**Invert Y** button inverts the movement of the stage in Y-direction.
Threshold marker detection: high – low.	This function is only visible, if the **Show All** mode is activated. A low threshold for marker detection is used when the dimensions of the correlative L markers cannot be recognized precisely, e.g. when the sample holder is slightly filthy.
Marker color:	Here you select the color of the markers: **White:** the marker is displayed white on a dark background. **Black:** the marker is displayed dark on light background. **Auto:** the marker color is set automatically.
Marker orientation:	Here you select the orientation of the L-markers within the live image.

Click on **Next** to move to the next wizards steps.

12.3.1.5.3 Step 2-4: Calibration

In the steps 2-4 of the wizard you will be advised to set the marker positions.

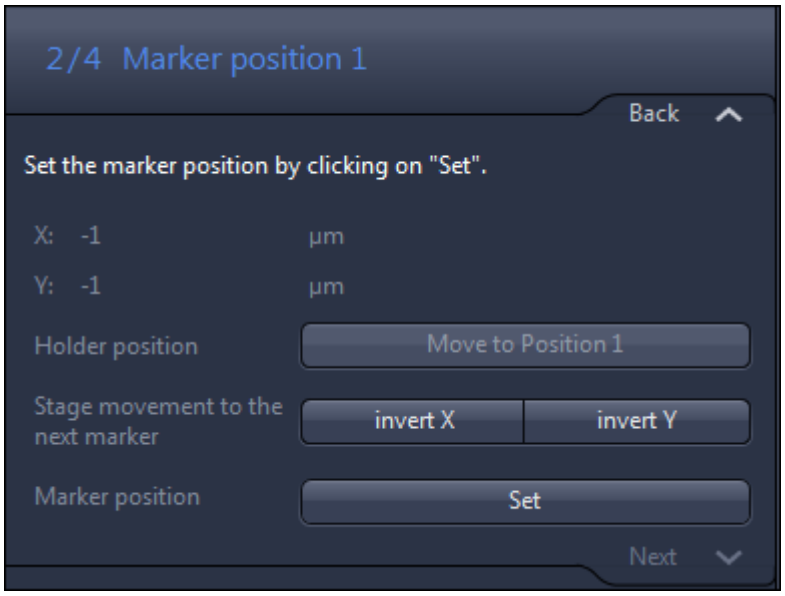


Fig. 33: Sample Holder Calibration Wizard Step 2-4

The marker detection can be done manually or automatically by selecting the buttons **Manual** or **Automatic** within the wizard steps.

Option	Function
Calibration:	**Position 1**: Moves the stage to marker position 1. This is possible only if the position has been detected once and x/y coordinates are given. **Current**: Moves the stage to the current marker position. This is possible only if the position has been detected once and x/y coordinates are given. **Restart**: Restarts the detection of marker positions by jumping back to step 2 of the calibration wizard (= Marker Position 1).
Set Marker Position: Manual	If you want to set marker positions manually, you must move the stage to the respective marker position e.g. using the software joystick. Position the crosshair in that way, that the point of intersection coincides exactly with the intersection point of the small L-marker. Now you must confirm the position of the crosshair by clicking on the **Manual** button. If in **Step 1: Options** the **Automatic movement to next marker** checkbox is activated, the stage moves to the next calibration marker automatically. If this checkbox is not activated, you must move the stage to the next marker using the software joystick.

Option	Function
Set Marker Position: Automatic	The Software suggests a marker position by setting the crosshair on top of the marker. This is only possible, if the marker is visible in the life image and the L-markers are clearly visible (no contamination of the L-markers). After clicking on the **Automatic** button the message *Is the marker position accurately aligned* appears. If this is the case, confirm the alignment by clicking on **Yes**. If in **Step 1: Options** the **Automatic movement to next marker** checkbox is activated, the stage moves to the next calibration marker automatically. If this checkbox is not activated, you must move the stage to the next marker position manually, e.g. using the software joystick.

After confirming a marker position (either manual or automatic) the wizard jumps to the next wizard step. After confirming marker position 3 the wizard closes automatically and the calibration is done.

NOTICE

Do not use the **Next** button within the wizard because then the calibration data will not be saved. The data must be saved for doing correlative microscopy and for the relocation of the sample position in the second microscope. Using the **Back** button within the wizard brings you to the previous step and the microscope stage moves to the correspondent marker position.

12.3.1.6 Acquiring the LM image

Image acquisition can be done as you are used to do it within ZEN software. The file format for Shuttle and Find data is the common ***.czi** file format. Saved images can be loaded in ZEN via the menu **File | Open**.

After image acquisition the next step in the correlative workflow is to define / draw in ROIs / POIs in your image. Therefore you can use the **Region** tools on the **S&F** tab, see *Regions, Find and Dimensions* [▶ 267].

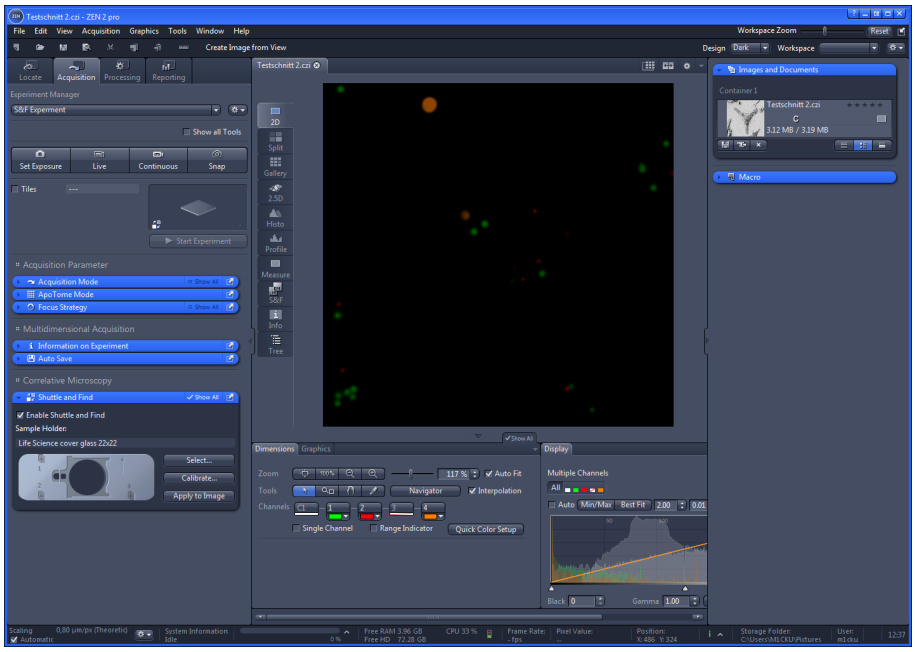


Fig. 34: LM image

12.3.2 Shuttle and Find sample positions at the electron microscope

Now you can transfer (Shuttle) the sample and the LM (Light Microscope) image file (**.czi**) to the SEM (Scanning Electron Microscope). There you can easily relocate (Find) the same sample positions and acquire a corresponding image within the ZEN 2.1 **SEM** software. Therefore exactly the same steps have to be done as for the light microscope.

12.3.2.1 Mounting the sample holder to the SEM

For imaging your sample in the SEM, insert the sample holder **(2)** in the special SEM adapter **(1)** and mount it to the SEM.

Note

The arrow of the sample holder has to face the arrow of the SEM adapter.

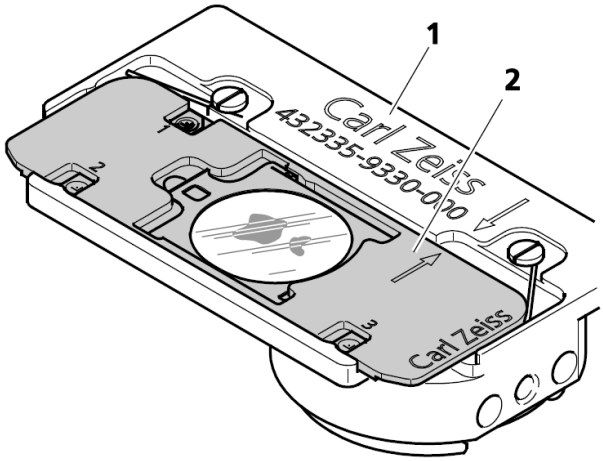


Fig. 35: Sample holder mounted in SEM adapter

12.3.2.2 Starting the ZEN SEM software

For correlative microscopy with scanning electron microscopes **SmartSEM** and ZEN 2.1 SEM have to be installed. SmartSEM is still the control software of the scanning electron microscope. ZEN 2.1 SEM comes as an add-on for SmartSEM to perform correlative microscopy and using Shuttle&Find on a SEM.

Prerequisites

- You have started SmartSEM.

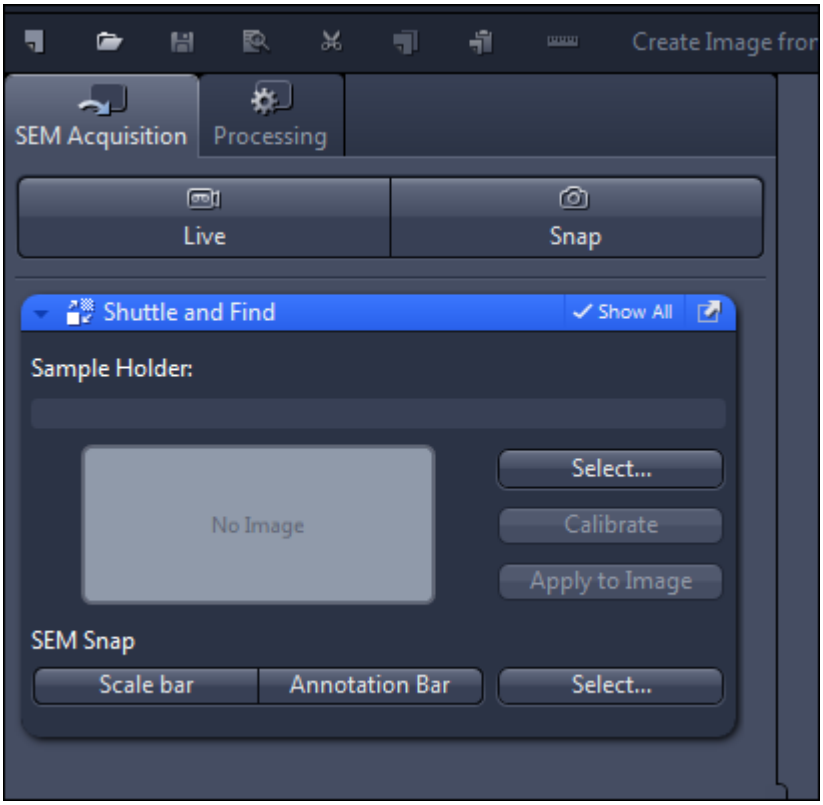
Procedure

1. Start ZEN 2.1 software by clicking on the corresponding program icon on your desktop.

   Following window will appear.

2. Click on the **SEM** button to start.

You will see the program interface with a reduced user interface comparing to ZEN 2.1. In the Left Tool Area the **SEM Acquisition** tab and the **Processing** tab are available only. On the SEM Acquisition tab you will find the **Shuttle and Find** tool which has 3 additional buttons at the lower part of the tool.



12.3.2.3 Selecting the sample holder

This step is exactly the same step like for the light microscope, so please read the chapter *Selecting the sample holder* [▶ 277] if you want to know the exact steps which you have to perform.

12.3.2.4 Calibrating the sample holder

Like the step before this step is exactly the same like for the light microscopy, so please refer to the chapter *Calibrating the sample holder* [▶ 280] for details.

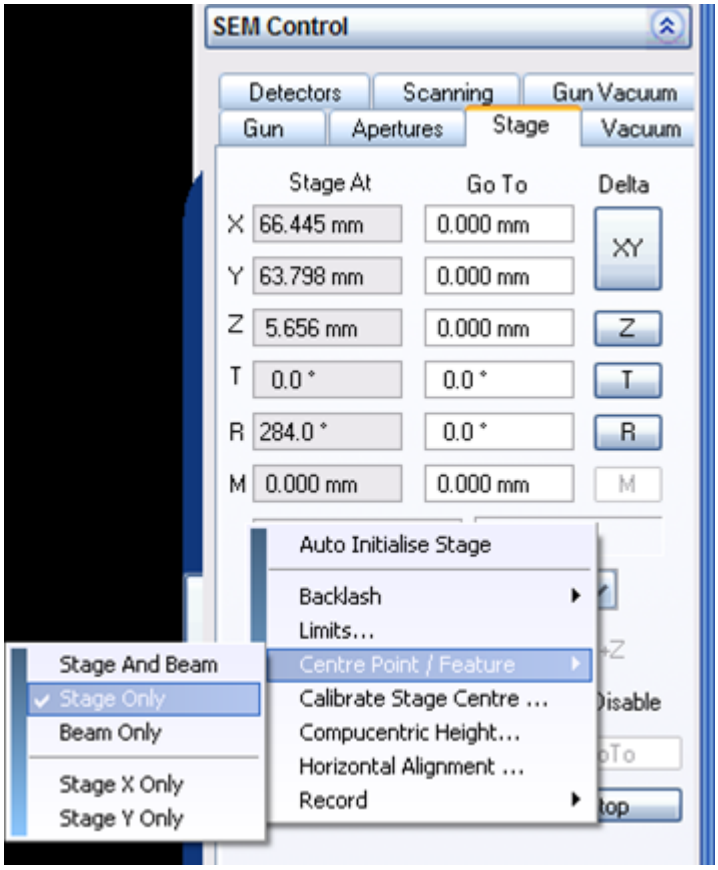
i Note

The calibration of the sample holder has to be done on both systems the LM and the SEM. Otherwise the relocation of your sample positions or ROIs / POIs stored in the image won`t be successful.

Note

Note that for Shuttle & Find the beam shift must be switched off. The beam shift is deactivated in **SmartSEM** as follows:

- Call up the shortcut menu **Center Point / Feature** by right-clicking on the **Stage** property page.
- Select **Center Point / Feature** and select **Stage only**.



12.3.2.5 Acquiring the EM image

Procedure

1. Load your LM image in ZEN 2.1 SEM (.**czi**).

   The image will be displayed in the center screen area.

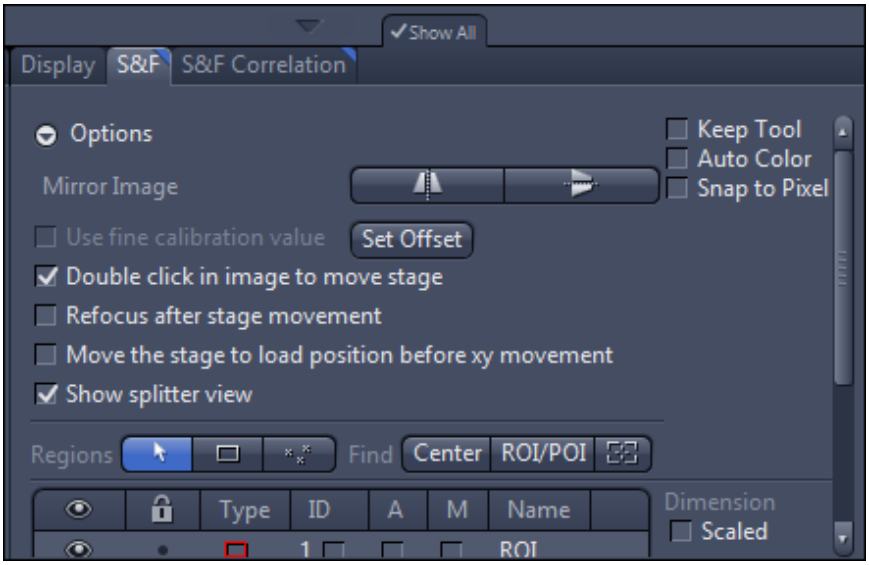
2. Activate the **Live** mode.

   You will see the Live image from the SEM. Notice that all settings for the SEM image have to be done within the SmartSEM software.

3. Activate the **S&F View** in Center Screen Area.

4. Go to the **S&F** tab.

5 Check if the **Double click in image to move stage** and **Show splitter view** checkboxes are activated (default setting).



In the left image container you see the live image from the SEM. The right image container is empty.

6 Drag the loaded LM image from the **Images and Documents** gallery into the empty image container.

Now you can easily relocate sample positions by double clicking within the image or on the ROI/POI button (if ROI / POI are drawn in and selected) on S&F tab.

For image acquisition you have to use the **Snap** button within ZEN 2.1 SEM. Notice that we will not describe setup and image acquisition with the SEM. Please read the online help or user guide for the SEM software.

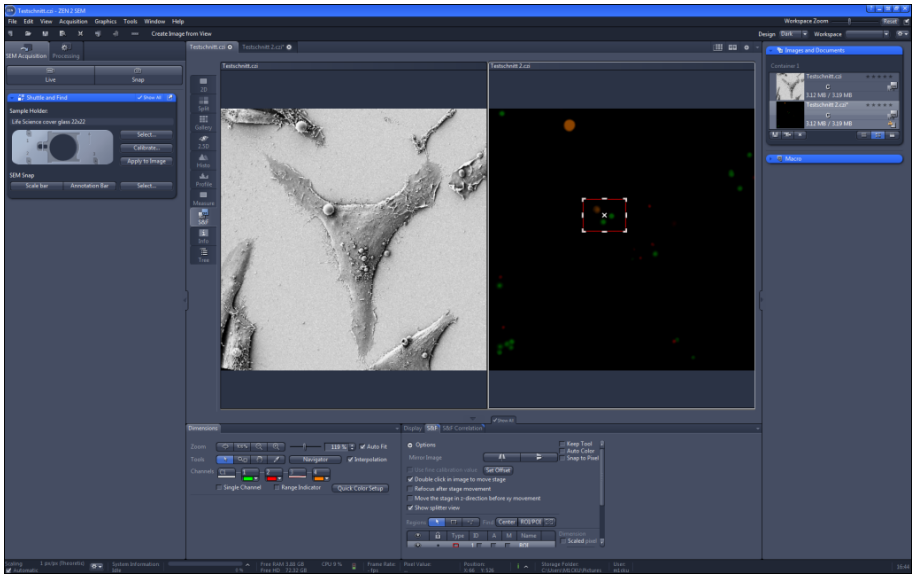


Fig. 36: SEM and LM image

12.3.2.6 Fine Calibration of the sample holder

The precision of relocation can be improved by determination of an offset value. This value describes the position offset between the loaded image and the live image. The defined offset value is only valid for the loaded image. If another image is loaded or if you close the dialogue, the offset value will be deleted.

Prerequisites

- An offset is visible when you try to relocate marker positions on the live image comparing to the LM image.

Procedure

1. Click on the **Set Offset** button.

   The stage moves to the selected marker position. Then a message appears which asks you to move the stage to the correct position.

2. Move the stage manually to the correct position by using the joystick.
3. Confirm the message by clicking on the **OK** button.

Now you can repeat the relocation. The positions should be identical now.

12.3.3 Image Correlation

12.3.3.1 Correlating two loaded images

Prerequisites

- You have acquired and loaded two images containing S&F calibration data (e.g. LM / SEM) to be correlated. If the images are not oriented identically you can use the **Mirror Image** buttons under **Options** on the **S&F Correlation** tab.
- You see the two images next to each other (splitter view) in the center screen area. If not, drag your images from the **Images and Documents** gallery into the center screen area.

Procedure

1. Click on the **Set correlation points** button in the **S&F Correlation** tab.

   The curser will change to a pipette symbol.

2. Click in the left image to set a correlation point. Set all 3 marker points in the left image first, before you set the corresponding 3 markers in the right image. If a correlation point is set, a check mark icon will appear in front of the corresponding point.

   Make sure that the positions in both images are identical. After you have set all 6 points the cursor will be changed backwards from the pipette to the arrow.

3. Click on the **Create Correlation** button.

The correlated image will be generated and opened in a new image container.

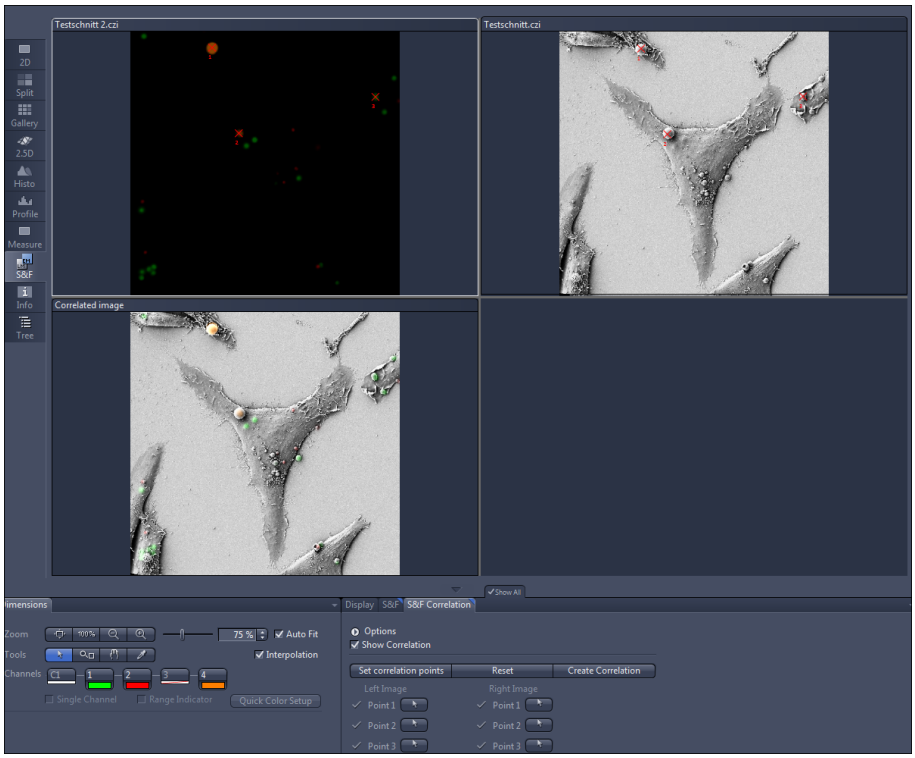


Fig. 37: Correlated image

Tips & Tricks

- It is also possible to set each correlation point individually. Therefore under **Left Image** / **Right Image** click on the **Arrow** button behind a point (e.g. **Point 1**). Then click on the desired position within the image.
- To improve the accuracy of the identification you can zoom into the images by using the mouse wheel.
- To edit/move a point, click on the point you would like to move. When the point is marked with a dashed rectangle you are able to move the point by holding the left mouse button. Alternatively, below **Left Image** / **Right Image** click on the points **Arrow** button you want to move and click on a new position within the image.

12.3.3.2 Correlation of live image and loaded image

This guide shows you how to overlay a live image with a loaded image.

Prerequisites

- You have activated the **Live** mode.

Procedure

1. To activate the live mode click on **Live** button in the **Left tool Area**.
2. Select the **S&F** view in the **Center Screen Area**.

   The splitter view will become visible.
3. Drag an image from the **Documents and Images Gallery** into the Center Screen Area and select the **S&F Correlation** tab.

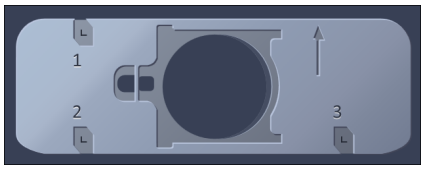
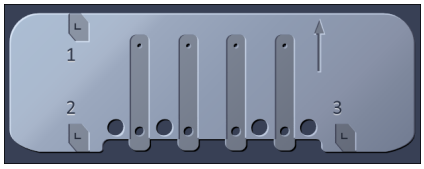
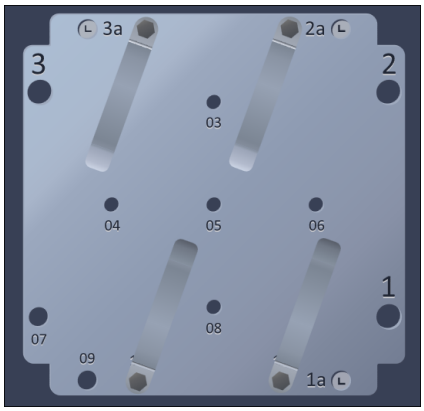
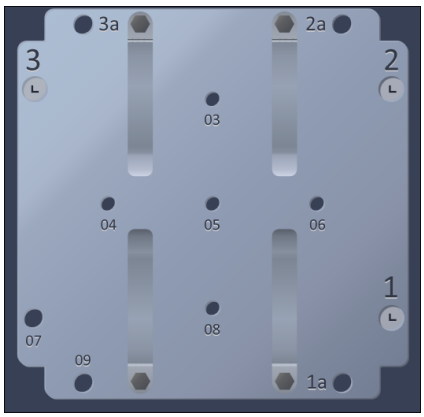
4 Click on **Set All** button to set all correlation points: Proceed setting 3 points within the live image and in the loaded image.

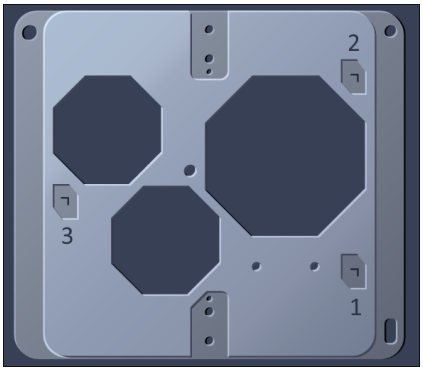
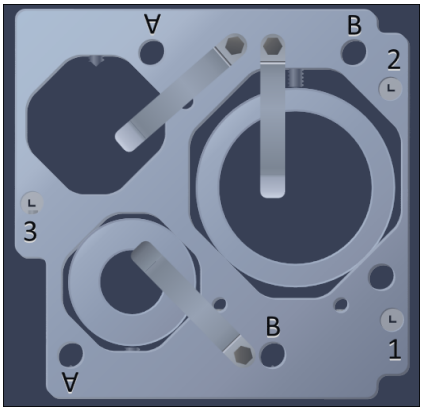
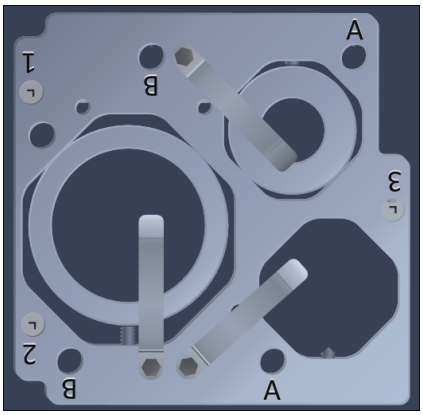
5 Click on **Create Correlation** button.

The correlated image of the live image and the loaded image becomes visible.

12.4 Appendix

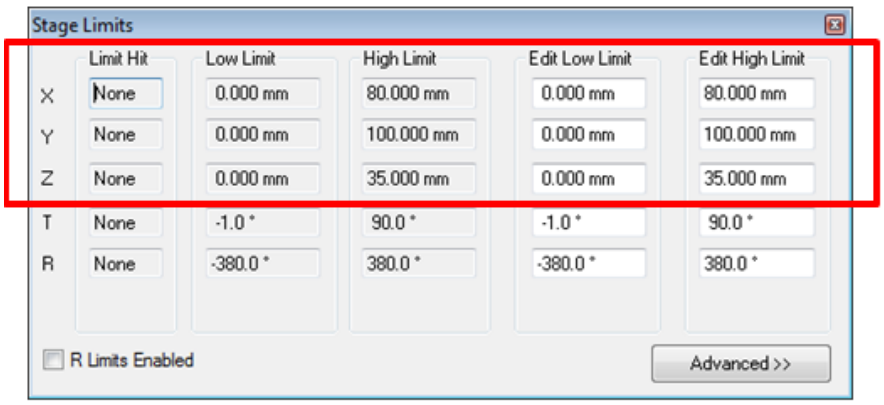
12.4.1 Correlative Sample Holders

Name	Image
Life Science cover glass 22x22	
Life Science for TEM Grids	
MAT Flat Stubs a	
MAT Flat Stubs	

Name	Image
MAT Universal A	
MAT Universal B_A	
MAT Universal B_B	

12.4.2 Shuttle and Find with an EVO 10

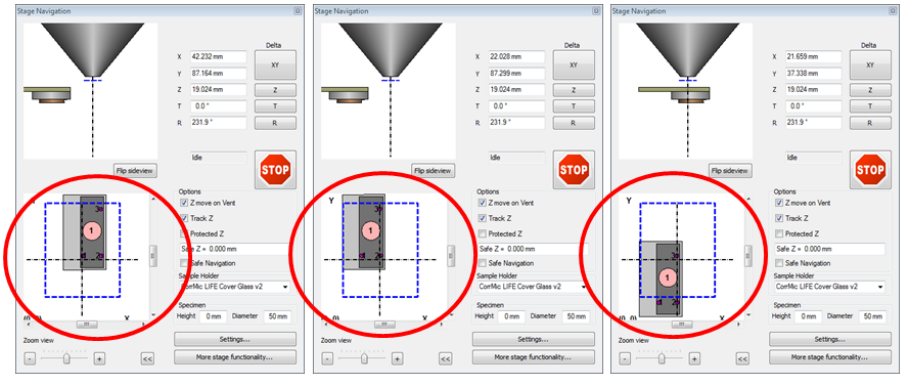
To use Shuttle and Find (SW and correlative holders) with an EVO 10 make sure that the stage limits (for x, y and z) are set as follows:



Holder Positions

The holder positions must be oriented like shown in the images

Position 1 | Position 2 | Position 3

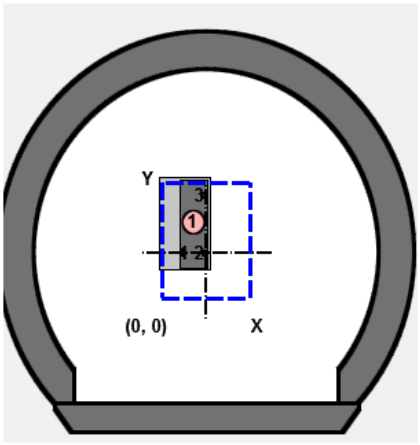


NOTICE

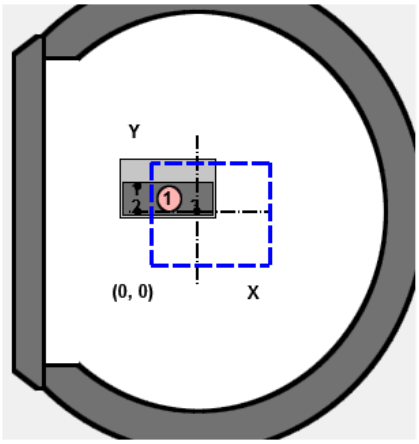
If you set a wrong orientation the stage cannot be moved to all correlative markers because of the stage limits for the EVO 10.

- The holder has to be mounted into the EVO in that the way that the correlative markers (**1**) and (**2**) have to be near the chamber door whereas marker (**3**) is located furthest from the chamber door (see **Mounting A/B**).
- If necessary, the SEM image can be rotated according to the LM image using the option **Scan Rotate** in SmartSEM.

Mounting A:



Mounting B:



13 Module CAT

13.1 Introduction

Array Tomography is a volumetric microscopy method using serial sections. Tissue samples or cells embedded in resin are cut into consecutive sections with an ultramicrotome and collected onto a sample carrier (e.g. cover glasses) afterwards. The sequence of the sections determines the z-position and allows the reconstruction of the 3rd dimension. Therefore the z-resolution of the resulting 3D data set is given by the thickness of the section.

The correlation of SEM data especially with data of a fluorescence LM, enables the visualization of fluorescently labeled proteins in their ultrastructural context not only in 2D but now in 3D with the ZEN Correlative Array Tomography module.

ZEN Correlative Array Tomography (CAT)

The software module ZEN Correlative Array Tomography (CAT) enables automated imaging of ultra-thin serial sections (ribbons) in the light- and scanning electron microscope (LM and SEM). After calibration of the sample carrier and detection of the sections, regions of interest can be defined manually in one section and will be automatically propagated to all following sections. The selected regions of interest can be imaged with different contrast methods and magnifications in the LM.

In the SEM the previously defined regions of interest will then be imaged automatically after loading the calibration information previously acquired at the LM . Depending on the number of sections and the resolution settings, the SEM acquisition process can take hours. The 2D corresponding image sequences recorded in the LM and SEM are aligned into a three-dimensional z-stack using the integrated alignment and correlation algorithms of the ZEN Correlative Array Tomography module. This process results in a correlative three-dimensional data set combining LM and SEM information into one image volume.

The software module can be used with ZEISS widefield microscopes as well as with ZEISS scanning electron microscopes. The module consists mainly of four wizards:

- **Calibration Wizard**, see *Sample Holder Calibration Wizard* [▶ 270].
- **Acquisition Wizard**, see *Acquisition Wizard* [▶ 300].
- **Z-Stack Alignment Wizard**, see *Z-Stack Alignment Wizard* [▶ 307].
- **Correlation Wizard**, see *Correlation Wizard* [▶ 310].

For the correlative workflow, one CAT module has to be installed on the widefield system, a second module hast to be installed on the SEM system. Both parts of the module on both systems are perfectly adapted to each other. The typical workflow is starting on the light microscope to image fluorescence labeled proteins before imaging the ultrastructure of the sample with the scanning electron microscope.

Beside the correlative solution, the CAT module can be used as an array tomography module only and has not be mandatorily used within a correlative workflow.

13.2 CAT Tool

Using this tool you can calibrate and manage the sample holders and start the wizards which are used for acquiring images from serial sections, generating Z-stack images out of the single images and correlate two Z-Stack images from the light microscope (LM) and the scanning electron microscope (SEM).

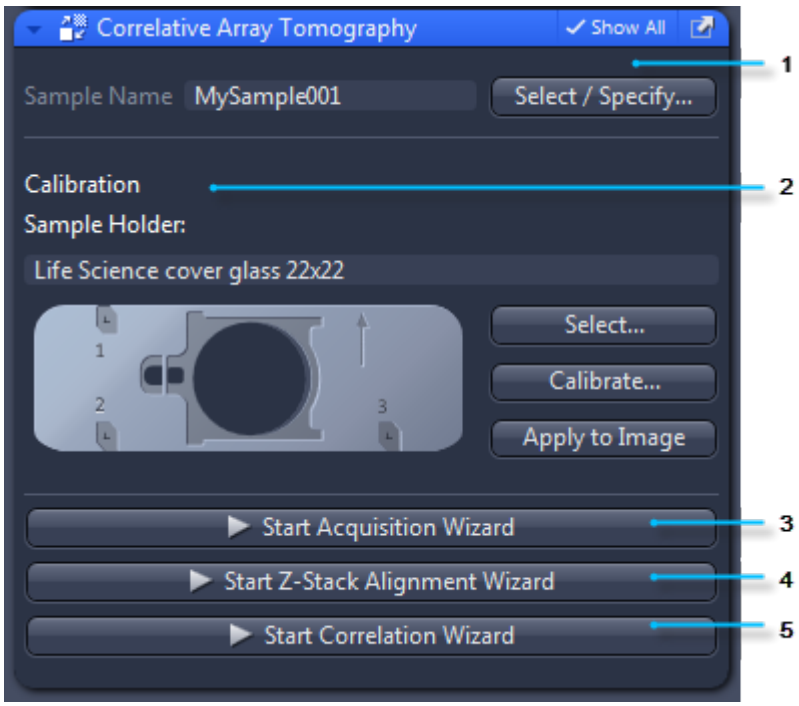


Fig. 38: CAT Tool

Parameter	Description
Sample Definition (1)	
- Select / Specify...	Opens the **Select Sample** dialog, see *Select Sample Dialog* [▶ 299]. There you can select a sample data sheet from the list or specify a new sample with user specific information. The specified sample information will be used for image processing (i.e. Z-stack alignment) or for data management.
Sample Holder Calibration (2)	

Parameter	Description
- Select...	Opens the **Select Template** dialog. There you select the preferred sample holder or define new holder templates, see *Selecting the sample holder* [▶ 277].
- Calibrate... button	Opens the Sample Holder Calibration Wizard. There you can calibrate the selected sample holder.
- Apply to Image button	Only visible if the **Show All** mode is activated. **Use this button only, when you forgot to calibrate the holder before you acquire the image.** Applies a calibration to an acquired image. Do not remove the sample out of the correlative holder between image acquisition and calibration. Exception: correlative markers are on the sample holder.
Start Acquisition Wizard (3)	Starts the **Acquisition Wizard**, see *here* [▶ 300].
Start Z-Stack Alignment Wizard (4)	Starts the **Z-Stack Alignment Wizard**, see *here* [▶ 307].
Start Correlation Wizard (5)	Starts the **Correlation Wizard**, see *here* [▶ 310].

13.2.1 Select Sample Dialog

Here you can select or create a sample data sheet.

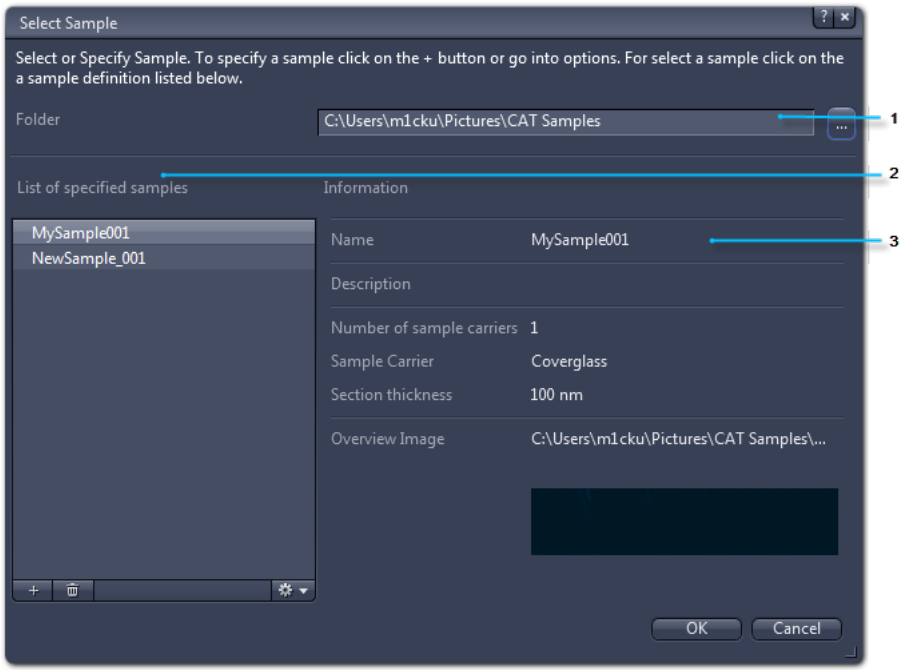


Fig. 39: Select Sample Dialog

Parameter	Description
Folder (1)	Shows the location, where the sample files are saved. If you click on the **...** button you can change the storage location. The default path and folder is *C:\Users\user \Pictures\CAT Samples*. Within this folder, each sample is saved in a sub-folder. Images taken during image acquisition within the CAT Acquisition Wizard will be saved within the sub-folder, automatically.
List of specified samples (2)	Shows the samples which are already specified within the software. If you select a sample in the list and click **OK**, the sample will be used in your experiment. If you click on the Add **+** button, the **New Sample** Dialog opens. There you can create a new sample definition which will be added to the list. If you click on the **Options** button, you will see further options for managing samples like **Show/Edit** or **Refresh Sample List**.

Parameter	Description
Information (3)	Shows the specified sample information, e.g. name, description, number of sample carriers, sample carrier, and section thickness.

13.3 Acquisition Wizard

This wizard is used to image the serial sections or user-defined region of interest within the sections.

The steps **Overview Imaging**, **Ribbon imaging**, **ROI Imaging** and **Re-Shoot** are image acquisition steps. The step **Re-Shoot** gives you the opportunity to image parts of the ROI-series or tiles of a tile image, afterwards.

The wizard consists of 7 steps which are described in the following chapters:

- *1/7 Overview Imaging* [▶ 300]
- *2/7 Ribbon Specification (optional)* [▶ 303]
- *3/7 Ribbon Imaging (optional)* [▶ 303]
- *4/7 Section Specification* [▶ 304]
- *5/7 ROI Definition* [▶ 306]
- *6/7 ROI Imaging* [▶ 306]
- *7/7 ReShoot (optional)* [▶ 307]

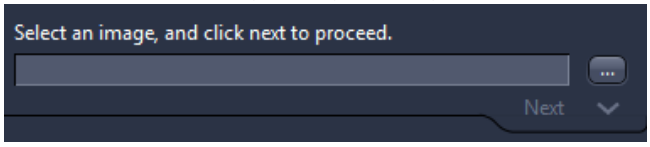
13.3.1 Overview Imaging

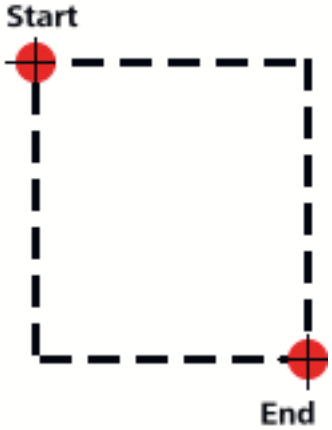
With this step you can acquire an overview image that allows you to navigate on your sample. You will see the positions of the serial sections on the sample carrier. In general, for the overview image an objective with low magnification is used. This makes the acquisition fast due to a large field of view and limited number of tiles.

Image acquisition with objectives with higher magnification is possible. But keep in mind, that the number of tiles will increase due a smaller field of view as well as the acquisition time.

i Note

We recommend to use phase contrast images for the acquisition. The used algorithm for the automatic section specification, see step 4, is most reliable then.

Parameter	Description
Image Acquisition / Load Image	By selecting the corresponding button you can decide whether you would like to acquire an overview image or load an image.
Image Selection	If you have selected **Load Image**, you can select a saved image file from the file system. Therefore simply click on the **...** button and navigate to your image file.  The wizard will jump to the wizard step according to the information saved with the loaded image.
Experiment	If you have selected **Image Acquisition** you have to select an experiment from the **Experiment** list. Note that the experiment has to be set up and saved in advance, before you enter the wizard.
Objective	Here you can select the objective that you want to use for the acquisition of the overview image. As mentioned before, we recommend to use an objective with a low magnification (e.g. 2.5x or 5x).
Channels	Here you can select the channels that you want to use for the acquisition of the overview image. You can use more than one channel in one run, when your microscope is equipped with a motorized condenser.
Selected Light Source	Here you can select the light source that you want to use for the acquisition of the overview image. The light intensity can be adapted if a corresponding light source is selected.
Camera settings	Here you can adapt the camera settings like changing the exposure time or activating / deactivating the shading correction. If a shading correction has been performed and activated in the selected experiment, the checkbox will also be activated automatically in the wizard.

Parameter	Description
Software Autofocus	Here you can activate the Software Autofocus functionality and apply it to the overview image. If activated, you can select the positions for focusing. During focusing no live image will be visible. Note that sensitive fluorescence labels might be bleached during the autofocus process.
Overview Image Definition	Here you can define the size of the overview image by defining a start and end position.  The software will calculate the area by means of the defined start and end position (= overview image). The number of tiles and the used memory will be displayed below the buttons.
- Set start position	Sets the current stage position as start position of the image area.
- Set end position	Sets the current stage position as end position of the image area.
- Move to start position	Moves the stage automatically to the defined start position. Note that the start position has to be defined before.
- Move to end position	Moves the stage automatically to the defined end position. Note that the end position has to be defined before.
No. of sample carrier	Only active, if you use more than one sample carrier for one correlative z-stack. Here you have to select the number of the used sample carrier. The total number of used sample carriers was defined in the CAT tool under **Select / Specify sample**.

Parameter	Description
Move the stage to load position before xy movement	**Activated:** the stage will move to load position before moving to the next correlative calibration marker. In case of using an AxioObserver, the objective revolver moves to load position.
Acquire Overview Image	Starts image acquisition. The acquisition can be stopped in between. Then the button will change to **Restart**. Before you restart the image acquisition, you can modify the settings. The status of the image acquisition is shown in the status bar of the software. After the overview image was taken, the image can be stitched. If you click on the **Apply Stitching** button stitching will be performed.
Next	Brings you to the next wizard step.

13.3.2 Ribbon Definition

This step is an optional step. Here you can mark the outlines of the sample structures on a ribbon. If you can`t see the sample structures, due to the overview image was acquired with a too low magnification, you can image your sample again using a higher magnification.

For marking the outlines in the image use the tools from the **Ribbon Definition** tab (e.g. Rectangle, Circle or Polygon).

13.3.3 Ribbon Imaging

For this step a split view will appear. On the left side you see the Live image. On the right side you see the overview image with the defined ribbons.

i Note

To modify either the Live image or the image with the defined ribbons click on the corresponding container. The activated container will be marked with a white frame.

Again, like in step one for the overview image, you have to select the objective, channel, light source and adapt the exposure time. Additionally you have to generate a focus surface to ensure that your sample will be in focus during the image acquisition.

If you click on the **Create Ribbon Image** button, the ribbon image will be acquired.

13.3.4 Section Specification

To determine the positions of regions of interest (ROIs) within the sections, you have to define the sections. The section lines generate the reference system for the ROI positions. The sections are marked and outlined with a frame.

i Note

Note that in each ribbon one section has to be selected as a reference and a reference line has to be drawn in. Meaning when your sample has three ribbons, one sections on each ribbon have to be drawn in.

We recommend to use phase contrast images for the section specification. The used algorithm for the automatic section specification is most reliable then. When you use bright field images, the algorithm might not work optimal. In that case you have the opportunity to add and to move the section contours manually.

On the **Section Definition** tab you will find tools and options for creating sections in the image.

Parameter	Description
Reference Section	
- Select	Activates the selection mode (default).
- Contour	If selecting this mode you can draw in a contour line of a section.
- Keep	Keeps the selected tool active. You can then use the tool several times without interruption.
- Step Backward	Undo the last step
- Step Forward	Redoes the last step.
- Delete	Deletes a selected graphical element.
Section Index	Here you can determine the starting number of the ribbons. This is important when the ribbons of a sample are deposited on more than one sample carrier.
Selected Channel	Shows the selected channel which is used for the detection.

Parameter	Description
Detection Sensitivity	Here you can adjust the detection sensitivity from **Low** to **High** by using the slider. This will be done by modifying the contrast thresholds for the section detection algorithm. When setting a low sensitivity, sections will be recognized even if the contrast between the section and the substrate is low; disadvantage: sections will be recognized, even in areas where no serial sections are deposited. When setting a high sensitivity, the algorithm only recognizes sections, if there is a high contrast between section and substrate. If not all sections were recognized you have the possibility to copy section contours or to stamp section contours.
Section Detection	
- Apply	Starts the section detection on the sample. The software tries to detect each section within the ribbon.
Contrast Method	
- Auto	Auto is used by default. The system recognizes the contrast method of the image automatically.
- Ph.Contrast	Applies the phase contrast method. Even if you are using a brightfield image, phase contrast will be applied as contrast method.
- Brigthfield	Applies the brightfield contrast method. Even if you are using a phase contrast image, brightfield will be applied as contrast method.
Use Internal Structure	Activate this checkbox only when sample structures are clearly visible within the sections. If activated, sample structures are used for section detection, additional to contrast differences between sections and substrate. In case that sections are not detected properly, you have the possibility to either to stamp section contours or to copy section contours.
Post Definitions	

Parameter		Description
-	Stamp tool	If selecting this tool you can stamp undetected contours after the section detection was finished. Therefore simply select the tool and move the mouse curser in the area nearby the last detected section. The curser will change to a stamp icon and you are able to stamp in the missing section contours.
-	Accept Ref. Section	If you click on this button reference contours are transformed into section contours.

13.3.5 ROI Specification

With this step you can screen your sample for interesting sample regions (ROIs) and mark this area by a graphical element. You can define several regions of interest within in one section.

On the **ROI Definition** tab you can draw either a rectangle, a circle or a freehand polygon/contour. Click on the **Apply** button to identify the region of interest in all other sections, automatically. It is also possible to **Undo** / **Redo** an action using the corresponding buttons. To remove a graphical element select it and click on the **Delete** (bin icon) button.

i Note

With the arrow keys on your keyboard you can jump from one ROI to the next ROI along the ROI series to check if the structure of interest is still within the defined region of interest.

13.3.6 ROI Imaging

With this step you can image the ROIs which are detected and marked in the previous step. The tile images will be generated from all defined region of interests automatically.

i Note

The size of the snapped tile images of a ROI series can change due to the number of tiles which are necessary to image the defined region of interest. The number of tiles will vary due to the bending of the ribbon.

13.3.7 Re-Shoot

This step is helpful, if some tiles or regions of interest are blurry. These tiles/regions can be replaced by repeating the acquisition of the selected tiles or tile images. The procedure is as follows:

- Select all blurry tiles, first.
- Adjust the focus for each tile position.
- Take new images, afterwards.

Parameter		Description
Select Tiles		If this mode is active, you can select the tiles which you want to re-shoot. Use the **Z-Position** slider on **Dimension** tab or the arrows within the Image area to scroll through the acquired images. If you found a tile image that you want to re-shoot, simply click on it. Then the color of the image frame turns from red into green. Note that all tiles or blurry regions have to be defined, before the image acquisition can be repeated.
Acquire		If this mode is active you can acquire the selected tiles again. If you click on this button, the stage will move to the first tile and the following buttons will appear:
-	Snap	Acquires a new image.
-	Replace	Replaces the old tile by the new tile.
-	Correct Brightness	In case the tile is brighter or darker, here you have the possibility to adapt the brightness of the tiles image

Click **Finish** to leave the wizard.

13.4 Z-Stack Alignment Wizard

This wizard is used to align the single images of a Z-Stack image. The wizard consists of 6 steps which are described in the following chapters:

- *1/6 Image Import* [▶ 308]
- *2/6 Pre-Processing* [▶ 308]
- *3/6 Image Review* [▶ 309]
- *4/6 Alignment* [▶ 309]
- *5/6 Manual Correction (optional step)* [▶ 310]
- *6/6 Final Image Creation* [▶ 310]

13.4.1 Image Import

In this step you can load your acquired Z-Stack images which you want to align. Therefore simply click on the **Load** button and select the image file from the file system.

13.4.2 Pre-Processing

In this step you can perform pre-processing functions on the loaded image, e.g. Stitching (only for tile images), Brightness and Contrast Correction (only for SEM images).

Parameter	Description
Apply Stitching button	Only visible if a Tiles image is loaded. If you click on this button, stitching is performed automatically on the image. The stitching can be canceled (Undo) or repeated (Redo) by using the arrow buttons.
Clip Limit	Reduces noise in the image. The higher the Clip Limit , the lower the noise. The clip limit can be adjusted between 0 and 10 %.
Region Size	Defines the region for histogram equalization. The smaller the area, the higher the contrast, but the noise will increase, too. The Region Size can be adjusted from 16 to 1024 px.
Histogram Equalization	If you click on this button, the SEM images are adapted to the selected values. The Histogram Equalization can be canceled (Undo) or repeated (Redo) by using the arrow buttons.

13.4.3 Image Review

This step is used for reviewing the single images of a Z-Stack. This is necessary because certain images might not be useful for 3D reconstruction due to problems during the image acquisition or sample preparation issues (wrinkles or ruptures within the section). These regions can be replaced either by the previous image or by the following image. To review the images, the images can be displayed as single 2D images in the**2D** view or as images series in the **Gallery** view.

Parameter	Description
2D View	If selected, you can review the single images of a Z-Stack image by using the 2D view. You can use the **Z-Position** slider to navigate through the single images. To replace an image click whether on the **Replace with next** or **Replace with previous** button. If you click on the **Undo** button the last action performed will be undone.
Gallery View	If selected, you can review the single images by using the **Gallery** view. The single images of a Z-Stack image are displayed as an image gallery. If you found an image that does not meet your expectations, simply select the image and replace it by the next or previous image.

13.4.4 Alignment

In this step you perform the image alignment. Therefore simply click on the **Start Alignment** button. To cancel the alignment click on the **Stop** button.

i Note

Before you start the alignment, select one channel as reference channel (e.g. DAPI, because DAPI stains the nucleus and the nucleus is a proper structure for doing an alignment.

During alignment a splitter view is visible. In the left container you can see the original images, in the right container you can see the aligned images.

13.4.5 Manual Correction

In this step (optional) you can navigate through the aligned images and check the result of the alignment.

In case you are not happy with the result, you have the possibility to correct the alignment of the images manually. Imprecise alignment can occur, when no characteristic structures are visible in the images.

13.4.6 Final Image Creation

Last but not least, in this step you create the final image.

Parameter	Description
Total	If selected, the complete image will be used for the image creation.
ROI	If selected, only the ROI area will be used for the image creation.
Create final image button	Creates the final aligned Z-Stack image.

Click **Finish** to leave the wizard.

13.5 Correlation Wizard

This wizard is used to correlate a Z-Stack image from the Light Microscope (LM) with the Z-Stack image from the Scanning Electron Microscope (SEM). The wizard consists of 4 steps which are described in the following chapters:

- *1/4 Import Z-Stacks* [▶ 310]
- *2/4 Correlation* [▶ 311]
- *3/4 Manual Correction* [▶ 312]
- *4/4 Create Final Correlation Image* [▶ 312]

13.5.1 Import Z-Stacks

In this step you can import the aligned Z-Stack images from the LM and the SEM, e.g. the Z-Stack image from the LM in the left container and the Z-Stack image from the SEM in the right container.

If you click on the **Left Container** button, the image is opened in the left image container.

If you click on the **Right Container** button, the image is opened in the right image container.

13.5.2 Correlation

In this step you correlate the images.

Parameter	Description
Transform	Here you select which z-stack will be transformed. During transformation a pixel in the overlay image of the Z-Stack is calculated by using pixels of the two original images that shall be overlaid / merged.
Interpolation	Here you can select one of the following interpolation methods:
- Nearest Neighbor	The gray value of the resulting pixel in the overlay image is made of a pixel which is located next. This interpolation method is very fast.
- Linear	The resulting or calculated pixel in the overlay image is assigned to a gray value, which is the result of a linear combination of gray values derive from pixels located nearby (in the original image).
- Cubic	The calculated pixel in the overlay image is assigned to a gray value, which is calculated by means of a polynomial function using gray values of pixels in the original images; these pixels are located nearby the calculated pixel.
Mode	Here you can choose an algorithm mode:
- 3-Points	If selected, this mode enables you to set 6 correlation points after clicking on the **Set Points** button (3 points in each z-stack in each container)
- 4-Points	If selected, this mode enables you to set 8 correlation points after clicking on the **Set Points** button (4 points in each z-stack), 3 points in the first z-section, the last point in the last section

Parameter	Description
Correlation Points	If you click on the **Set Points** buttons you can set the correlation points. The number of correlation points is according to the selected algorithm. The cursor will change to a pipette symbol. Simply click in the image to set the points. Start with setting the first three points in the left container then set the corresponding correlation points in the right container. If a correlation point is set, a check mark icon will appear in front of the corresponding point. When you select the 4-Points-Algorithm the display will move automatically to the last image of the Z-stack. Set the fourth correlation point in both containers. Make sure that the positions in both z-stacks are identical. After you have set all correlation points the cursor will be changed backwards from the pipette to the arrow. **Reset** deletes all correlation points in the image.
Create correlated Z-Stack	If you click on this button, the correlated Z-Stack will be generated and opened in a new image container.

13.5.3 Manual Correction

In this step you can correct the correlation manually by moving and rotating the transformed image.

Therefore simply click on the **Start** button. Then you can interactively move the image by dragging and dropping it with the mouse or rotate the image by clicking on the circle button attached on top of the green image frame or using the **Rotation** slider. You can also change the image opacity by adjusting the corresponding slider.

If you click on the **Accept** button the manual correction will be adopted to the correlated image.

13.5.4 Create Final Correlation Image

In this step create the final correlation image. Therefore simply click on the **Create final Z-Stack** button. Click **Finish** to leave the wizard.

14 Software Functions and Reference

14.1 Menus

14.1.1 File menu

Menu item	Function	Short cut
New	Opens the **New Document** dialog window.	(Ctrl +N)
Open	Opens the **Open Document** dialog window. Here you can select the file you want to open.	(Ctrl +O)
Save	Saves the selected file.	(Ctrl +S)
Save As	Saves the selected file under a new name. In case of an image only .czi file format can be used.	
Save As with Options	Saves the selected file under a new name. Advanced options can be selected: **File type**: czi, jpeg, jpg, png, tif, tiff, bmp, gif, wmp, wdp **Compression** (only for **czi** and **jpg/jpeg**): ■ **Original**: The image keeps the compression of the original image. ■ **Uncompressed**: The image is saved without compression. ■ **Compressed (JPEG XR)**: An uncompressed image will be compressed with the selected quality. A compressed image keeps the compression. ■ **Force Compression (JPEG XR)**: A compressed image will be decompressed and compressed with the selected quality. **Zoom Level** (only for pyramid images): Different zoom levels can be selected, depending on the pyramid. **Set as default**: Sets the selected options as default saving options.	

Menu item	Function	Short cut
Rename	Opens the **Rename** dialog window. Enter a new name for the file. Confirm the entry with **Yes**.	
Delete	Deletes the selected file.	
Export/Import	Opens the relevant parameters on the **Processing** tab | **Method Selection** | **Method** | **Extras** | Export/Import.	
Close	Closes the selected file.	**(Ctrl +F4)**
Save All	Saves all open files.	
New File Browser	Opens the **Browser window** in the **Center Screen Area**.	
Open Containing Folder	Opens the folder in which the selected file is located.	
Recent Files	Opens the **Recent Files** dialog window.	**(Ctrl +R)**
Print Preview	Opens the **Print Preview** dialog window for the selected file.	**(Ctrl +F2)**
Login	Opens the **Login** dialog window.	
Exit	Exits the software.	**(Alt +F4)**

14.1.2 Edit menu

Menu item	Function	Short cut
Undo	Undoes the last action.	**(Ctrl +Z)**
Redo	Redoes the last action.	**(Ctrl +Y)**
Cut	Cuts the selected graphic element out of the image.	**(Ctrl +X)**
Copy	Copies the selected graphic element.	**(Ctrl +C)**

Menu item	Function	Short cut
Paste	Inserts the copied graphic element into the image.	(Ctrl +V)
Delete	Deletes the selected element.	(Del)
Select All	Selects all graphic elements drawn into the image.	(Ctrl +A)
Display	Here you can manage image display settings. Functions include copy, paste, export or import of the display settings.	
ROI (Region of Interest)	Here you can draw a new rectangular selection region (ROI) into the image. It is subsequently possible to create a subset image from the selection.	(Ctrl +U)

14.1.3 View menu

Menu item	Function
Zoom	Here you can configure various zoom settings.
Player	Here you can navigate through a Z-stack or a time series image.
Text View	Displays the text name of a file in the **Document bar**.
Small Thumbnail View	Displays a small preview image and name of a file in the document bar.
Large Thumbnail View	Displays a large preview image and name of a file in the document bar.
1 Container	Displays one image container in the image area.
2 Containers	Displays two image containers in the image area.
3 Containers	Displays three image containers in the image area.
Automatic Container Layout	Uses the predefined container layout.
Shared View Controls	General and specific view controls are shared for all containers and are active for the currently selected image container.

Menu item	Function
Separate View Controls	Each container has its own separate general and specific view controls that become active when the associated image container is selected.
Show All (Global)	Activates **Show All** mode globally.
Show Macro Environment	The Macro Environment is deactivated by default. If you want to activate the Macro Environment and access the controls (e.g. Macro tool) you must activate this option.

14.1.4 Acquisition Menu

Menu item	Function	Shortcut
Locate Snap	On the **Locate tab** this executes a image acquisition. A single image from the active camera will be acquired.	(F2)
Locate Live	On the **Locate tab** this opens **Live mode**. A live image from the active camera will be shown **in Center Screen Area**.	(Shift +F2)
Acquisition Snap	On the **Acquisition tab** this command will execute only if at least one **channel** has been defined. All the defined channels will be acquired and displayed as an overlay.	
Acquisition Live	On the **Acquisition tab Live mode** will execute only if a **channel** has been defined. The currently selected **channel** will be used for the live image.	
Set Exposure	On the **Locate tab** this executes an exposure time calculation for the active camera. On the **Acquisition tab** this command will execute only if at least one **channel** has been defined.	
Set White Balance	Performs an automatic white balance measurement.	(Alt +W)
Find Focus	Starts an autofocus search.	
Start Experiment	Only active, if you have configured an experiment on **Acquisition** tab. Starts a defined experiment.	

Menu item	Function	Shortcut
Stop Experiment	Only active, if you have started an experiment on **Acquisition** tab. Stops the running experiment.	
Pause Experiment	Only active, if you have started an experiment on **Acquisition** tab. Pauses the running experiment.	
Continue Experiment	Only active, if you have paused an experiment on **Acquisition** tab. Continues the paused experiment.	
Action After Snap	Offers the possibility to choose between several actions after your image is acquired.	
- **Select Automatically (Default)**	Systems with an LSM (Laser Scanning Microscope) will always overwrite the currently selected image document. Systems without an LSM always create a new image document. This reflects the typical workflows.	
- **Always Create New Document**	A new image document will be created regardless of system configuration after a snap.	
- **Overwrite Current Document**	The selected image document will be overwritten regardless of system configuration after a Snap.	
Dual Camera Calibration Wizard…	Only visible if a dual camera configuration is active. Starts **Dual Camera Calibration Wizard.**	
ApoTome Phase Calibration Wizard…	Only visible if a ApoTome configuration is active. Starts **ApoTome Phase Calibration Wizard.**	
ApoTome Focus Calibration Wizard…	Only visible if a ApoTome configuration is active. Starts **ApoTome Focus Calibration Wizard.**	

14.1.5 Graphics menu

Menu item	Function	Short cut
Select	Starts selection mode.	Alt+F1
Draw Region of Interest (ROI)	Draws in ROI.	Crtl+U
Text	Adds text field to the image.	
Scale bar	Adds a scale bar to the image.	
Line	Adds a line to the image.	
Arrow	Adds an arrow to the image.	
Rectangle (aligned)	Adds an aligned rectangle to the image.	
Circle (Diameter)	Adds a circle to the image.	
Contour (Spline)	Adds a contour (Spline) to the image.	
Profile	Adds an intensity profile along the drawn in arrow region.	
Rectangle Profile	Adds an intensity profile within the drawn in rectangle region.	
Grid	Adds a grid to the image.	
Frequent Annotations	Adds frequently used annotations to the image, e.g. Relative Time, Channel Name.	
Distance	Adds distance annotations to the image, e.g. Length, Curve length.	
Multiple Distances	Adds annotations for measuring multiple distances.	
Region	Adds region annotations to the image, e.g. Contour, Rectangle.	
Circle	Adds circle annotations to the image.	
Angle	Adds angle annotations to the image.	
Points (POI)	Adds points of interest (POI) to the image such as events or markers.	
Burn-in annotations	Creates a new image with all annotations burned-in to the image.	

Menu item	Function	Short cut
Show Bounding Box	Shows bounding boxes around graphics/annotations.	
Hide Bounding Box	Hides bounding boxes around graphics/ annotations.	
Bring to Front	Brings selected graphic/annotation to the front of the image.	
Send to Back	Sends selected graphic/annotation to the back of the image.	
Bring Forward	Brings selected graphic/annotation one layer forwards.	
Send Backwards	Sends selected graphic/annotation one layer backwards.	

14.1.6 Macro menu

i Note

This menu is visible only if you have licensed the **Macro Environment** module and if you have activated in the **View** menu the **Show Macro Environment** entry.

Menu item	Function
Record a New macro	Starts recording a new macro.
Stop Recording	Stops recording the macro.
Macro-Editor…	Opens the *Macro Editor* [▶ 494] dialog.

14.1.7 Tools menu

Menu item	Function
Axio Scan Calibration…	Only available for Axio Scan systems. Opens the Axio Scan calibration wizard. By the wizard you can calibrate the following functions: ■ mapping of the preview camera to the scan camera, ■ parfocality of the objectives, ■ parcentricity of the objectives. ■ shading correction of the optical system (including fluorescence) and color calibration. It is not necessary to execute all steps, e.g. the shading correction for fluorescence and the color calibration can be skipped.
Diagnostics (Ctrl+Shift +D)	Opens the Diagnostics dialog.
Kitchen Timer…	Opens the Kitchen Timer.
Dosimeter…	Opens the Dosimeter.
Dye Editor…	Opens the *Dye Editor* [▶ 321] dialog.
Extension Manager…	Opens the Extension Manager dialog.
Modules Manager…	Opens the *Modules Manager* [▶ 323] dialog.
Users and Groups	Opens the *Users and Group Management* [▶ 323] dialog.
Settings Editor	Opens the **Settings Editor** dialog. In this dialog you can select from existing hardware settings or adopt the settings from the hardware being used into the software. You can also transfer settings from the software to the hardware that you are using.
System Maintenance and Calibration	Opens the System Maintenance and Calibration dialog. This dialog contains wizards and tools that will help you, to keep your system in perfect working condition.
Calibration Manager…	Opens the **Calibration Manager** dialog.

Menu item	Function
Customize Application…	Opens the *Customize Application* [▶ 323] dialog.
Scaling…	Opens the *Scaling* [▶ 324] dialog.
Sample Carrier Templates…	Opens the Sample Carrier Templates dialog.
Options…	Opens the *Options* [▶ 325] dialog.

14.1.7.1 Dye Editor dialog

Here you can edit and create data sets for fluorescent dyes. You can also see the excitation, emission and extinction curves of the selected dye displayed in a graph. The Dye Editor displays databases that are available in the *.ExEmL file format. ExEmL stands for **Ex**citation and **Em**ission **L**ibrary and is a special variant of the .XML format.

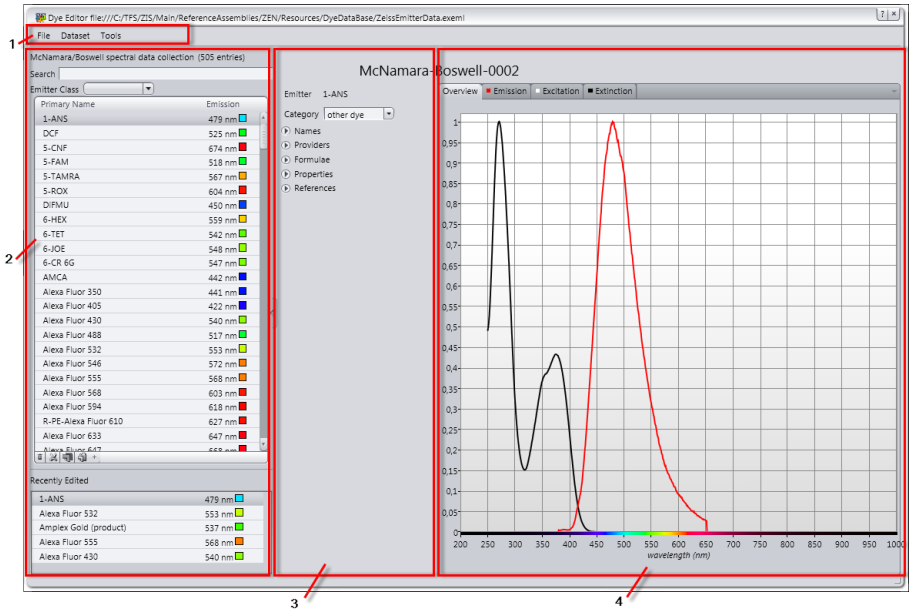


Fig. 40: Dye Editor

No.	Name	Description
1	Menu	Here you can create new data sets and import available dye databases. A detailed description can be found under *Menu* [▶ 322].
2	Dye search & database	The list shows the dyes that are available in the open database. You can search for dyes and see which dyes you edited last.

No.	Name	Description
2	Dye information area	Here you can see all the additional information about a selected dye.
4	Dye spectra display field	Here you can see the available dye spectra. Click on the relevant tabs to display the **emission**, **excitation**, or **extinction** spectra. On the **Overview** tab you can see all spectra at a glance.

14.1.7.1.1 Menu

File menu

Menu item	Function
New (Ctrl+N)	Creates a new ExEml file in which you can create dye data sets.
Open File (Ctrl+O)	Opens a single ExEml file.
Open Folder	Opens several ExEml files that have been saved together in the same folder.
Import File	Imports an ExEml file.
Save (Ctrl+S)	Saves the open ExEml file.
Save As	Saves the open ExEml file under a new name.
Save Folder	Saves all open ExEml files in a folder.
Close	Closes the Dye Editor.

Data Set menu

Menu item	Function
Add Data Set	Creates a new, empty data set.
Copy	Copies the selected data set to the clipboard.
Cut	Cuts the selected data set and copies it to the clipboard.
Delete	Deletes the selected data set.
Paste	Pastes a data set from the clipboard.
Paste Range	Pastes a range of a data set from the clipboard.

Tools menu

Menu item	Function
Create Abbreviations	Creates abbreviations for names of dyes. This helps to avoid duplication.
Calculate Main Emission Wavelength	Calculates the main emission wavelength of the selected dye from spectral data that has either been copied or entered manually.

14.1.7.2 Modules Manager dialog

Here you can activate or deactivate the modules for which you currently own a license. Note that all the changes made here are implemented immediately and the corresponding module(s) are activated / deactivated.

- **Available Products section**
  Here you can see the products available for your license. Click on the relevant button to select the product.
- **Included Modules list**
  In this list you can activate/ deactivate the modules that are included with you product. To do this, activate the checkbox in front of the module.
- **Optional Modules list**
  In this list you can activate the modules that you have licensed as an option for your product. To do this, activate the checkbox in front of the module in question.
- **Optional Hardware list**
  In this list you see the hardware that you have configured.

Using the **Select All** / **Unselect All** buttons you can activate or deactivate all available modules by clicking on the corresponding button.

To save the current selection of modules within a **.txt** file click on the **Save Information...** button.

14.1.7.3 User and Group Management dialog

Here you can create new users and groups and manage their access rights. To learn more about user and group management, read the chapter User rights and user groups.

14.1.7.4 Customize Application dialog

Here you can customize the application layout, e.g. adopt the toolbar or shortcuts. To learn more about how to customize the application, read the chapter *Customizing Application* [▶ 140].

14.1.7.5 Scaling dialog

Here you can specify how your images are scaled.

Option	Description
Active Scaling	Shows the scaling that is set currently.
Units dropdown list	Select the desired unit for the current scaling here.
Select automatically checkbox	If activated, the scaling will be calculated automatically from the microscope and camera configuration.

Available Scaling section

From the dropdown list you can select scalings which are stored on your system. The scaling details will be displayed in the fields below the list. By clicking on the **Options** button  you can perform the following actions :

Option	Description
Activate Scaling	Activates the selected scaling. The scaling will be applied to all images that are acquired from this time point onward.
Assign Scaling to Image	Assigns the selected scaling to the current image.
Import	Opens the **Import Scaling** dialog window. Here you can select a scaling file that you want to import.
Export	Opens the **Export Scaling** dialog window to export the selected scaling. Select the folder in which you want the exported scaling file to be saved and specify a file name.
Delete	Deletes the selected scaling.
Interactive Calibration button	Starts the *Scaling Wizard* [▶ 324].

14.1.7.5.1 Scaling Wizard

Here you can create a new scaling. To do this, draw a reference line with a predefined length in the current image. An image of a calibration slide is best suited for this purpose.

Parameter	Description
You can draw in two types of reference line:	

Parameter	Description
- **Simple Reference Line**	Draw a line along a distance with a known length.
- **Parallel Reference Lines**	Draw two parallel lines along a distance with a known length. The two parallel lines allow errors in the parallel axis resulting from the drawing of the lines to be corrected. A third, corrected line is drawn in automatically from which the scaling is determined.
Automatic Line Detection checkbox	**Activated:** Automatically detects individual lines of the scale bar in the image close to the interactively defined distance. Using this method the centers of the lines are determined exactly, increasing the precision of the scaling.
Length input field	Enter the length of the line you have drawn in the spin box/input field.
Unit dropdown list	Select the scaling unit from the dropdown list.
Name input field	Here you can enter the name for the scaling file that will be created.
Save button	Saves the scaling file that has been created under the specified name.

14.1.7.6 Options dialog

Here you can configure the settings for general software options.

14.1.7.6.1 General

Parameter	Description
Select Automatically checkbox	**Activated**: Automatically selects the user language of the operating system as the user language for the software.
Language Selection dropdown list	Select the language from the dropdown list in which the software will be run next time it is started.

14.1.7.6.2 Startup

Parameter	Description
Show Splash Screen checkbox	**Activated:** Displays the splash screen when program starts.

Parameter	Description
Show Application Selection checkbox	**Activated:** Shows the application selection dialog when the software starts.
Reload Last Used Documents checkbox	**Activated:** When the software is started, all (image) documents, that were open when you last exited the system, will be reloaded.
Reload Last Used Experiments checkbox	**Activated:** When the software is started, all experiments, that were open when you last exited the system, will be reloaded.
Request Stage/ Focus Calibration on Startup checkbox	**Activated:** Shows the message which asks you to perform stage / focus calibration.

14.1.7.6.3 Naming

Here you can specify how images are (automatically) named and indexed.

Parameter	Description
Category	Here you can select the category of an image which should be named automatically, e.g. an image from a Snap or an Experiment. With the next parameters you can configure the image name which is to be generated.
Prefix	Here you can enter a prefix for the image name, e.g. IMG.
Digits	Here you can select how many digits you want the counter used to have. Changes will be stored after the session is ended.
Format	Here you can specify what information are appended after the prefix. From the **Format-IDs** list below you can add the desired attribute to the name. Therefore simply double click on the desired format in the list.
Initial Counter Value	Here you can set the initial value for the counter. Simply enter the desired value in the input field.
Preview	Displays the preview of the naming format that will be allocated next for the current category.
Save/Load Counter Value checkbox	**Activated:** Saves the counter values for the individual categories. If the software is restarted, the values are restored.

Parameter	Description
Format IDs list	Here you see the list containing all the attributes which can be used for the **Format** field.

14.1.7.6.4 Saving

Locate/Camera section

Parameter	Description
Auto Save after Snap checkbox	**Activated:** Automatically saves images that are acquired on the **Locate** (Camera) tab using the **Snap** button.
Don´t Open a Document Window checkbox	Only active if the **Auto Save** checkbox is activated. **Activated:** The automatically saved images are closed immediately after acquisition.
File Type dropdown list	Select the image format from the dropdown list that will be used when an image is saved automatically.

Auto Save Path section

Here you can specify the folder into which the images are saved automatically.

14.1.7.6.5 Documents

Default Settings for New Images section

Parameter	Description
Show Rulers checkbox	**Activated:** Displays rulers at the top and left-hand edge of the image – the units used are according to the scaling settings.
Auto Fit checkbox	**Activated:** Automatically adjusts the zoom factor of the image so that the entire image is visible and the view area is filled.
Use Interpolation for Image Display checkbox	**Activated:** Displays pixels in interpolated form.
Set Logarithmic Scale in Histogram checkbox	**Activated:** On the **Display tab** the frequency distribution (y-axis) of the histogram is plotted using a logarithmic scale.

Parameter	Description
Show Viewport Scalebar in 2D View checkbox	**Activated:** Shows a scalebar within a small window in 2D view.
Show Viewport Scalebar in Live Window checkbox	**Activated:**Shows a scalebar within a small window in the Live window.
Show Navigator in 2D View checkbox	**Activated:**Shows the Navigator window in the image area.
Use Pan Mode in 2D View for Tile Images checkbox	

14.1.7.6.6 Acquisition

General section

Option	Description
Show a Request to Move Manual or Coded Hardware Components	**Activated**: The software shows a dialog which asks you to move manual components. You have to confirm the dialog and move the component by hand. If you don`t want this dialogs to be shown, deactivate this checkbox.
Show a Confirmation Dialog for Channel / Track Deletion	**Activated**: The software shows a dialog which asks you to confirm to delete a channel or a track.

Camera / Live

Option	Description
Close Live Mode After Snap checkbox	**Activated:** The **Live** window will be closed automatically after an acquisition via the **Snap** button.
Enable Stage/Focus Control in Live/ Continuous View checkbox	**Activated:** Enables to navigate the stage and focus in **Live** and **Continuous** view. Here you can also configure the travel speed of the focus by adjusting the values in the corresponding fields (from "Very Sow" = 0,005 to "Very Fast" = 50,0). To reset your adjustments click on the **Default** button.

Option	Description
Automatically Add Scalebar Annotation at Snap checkbox	**Activated:** If an image was acquired via the **Snap** button, a scalbar will be added automatically to the image.
Show Camera Expert Options checkbox	**Activated:** Shows advanced (expert) camera options within the **Camera** tool on **Locate** tab.
Use Centered Camera ROI only checkbox	**Activated:** A camera ROI will always be positioned at the center of the camera chip regardless of its size. i.e. center of Camera ROI = center of camera detector.
Show Crop button checkbox	**Activated:** The **Crop** button is displayed within the **Camera** tool.

Acquisition Tab section

Option	Description
Acquisition Tab without channel support check box	**Activated:** enables the use and set-up of experiments without any channel support in the **Acquisition** tab.
Prevent execution of after channel setting while Live Mode is active checkbox	**Activated:** Prevents execution of after channel setting automatism while **Live** mode is active.
Automatically start Live Mode when exposure measurement was started checkbox	**Activated:** Starts the**Live** mode when the **Set Exposure** button has been pressed such that the live image begins immediately after the **Set Exposure** measurement is complete. The normal function when the check box is unselected is to take a **Snap** subsequent to **Set Exposure**.
Follow Acquisition in displayed images checkbox	**Activated:** The image and slider of the **Dimensions** and **Graphics** tab follow (are updated) the acquisition so that image last collected is displayed as the Experiment progresses.
Switch to next Enabled Acquisition Block in Experiment Designer checkbox	**Activated:** Automatically switched the selection to the next enabled acquisition block in the Experiment Designer.

Option	Description
Enabled Advanced Imaging Setup checkbox	**Activated:** Shows the Advanced Imaging Setup section in the Imaging Setup tool. As this section should be touched by experts only, the default setting did not show this section.

Z-Stack section

Option	Description
Adjust Auto-Z-Stack Focus Match on First Slice	This value determines the degree of match between the image focus of the first image and that determined as the true focus (centre plane of the resulting Z-stack).
Adjust Auto-Z-Stack Focus Match on Last Slice	This value determines the degree of match between the image focus of the last image and that determined as the true focus (centre plane of the resulting Z-stack).

Tiles section

Option	Description
Automatically Start Live Mode in the Advanced Setup View checkbox	**Activated**: Automatically starts the **Live** mode in the live navigator tool when you open the advanced setup. Uncheck this option to prevent unnecessary specimen bleaching.
Additionally Open Snap Images as Separate Documents checkbox	**Activated**: Snap images created in the advanced tiles setup are opened additionally in a separate image containers, not just as a thumb nail in the preview area of the advanced tiles setup.
Show Information Title in the Advanced Setup View checkbox	**Activated**: Displays a bar abbove the Advanced Setup view containing additional information.
Show Snap Animation checkbox	**Activated**: Shows the snap animated when snapping a new image in Advanced Setup.
Automatic Snap by Clicking the Live Navigator Buttons checkbox	**Activated**: A snap will be taken every time the live navigator tool is moved with its navigation buttons.

Option	Description
Enable Stage Moving with Live Navigator Handle checkbox	In the Live navigator tool the current stage position including the live image is shown as a frame outlined in blue. To move the frame, double-click on the position to which you want to move it. The frame can also be used to control acquisition. **Activated**: If you click on one of the frame's blue arrow icons, an image is acquired. The Live Navigator tool is moved one frame width in the relevant direction. You can create tile images of your sample easily in this way.
Show Label on Sample Carrier Container checkbox	**Activated**: Shows a label on every container / well of a selected sample carrier.
Show Tool Tip on Sample Carrier Container checkbox	**Activated**: Shows a tool tip with the name of the container / well when the mouse is over it in the **Carrier** tab.
Delimiter for CSV Export / Import dropdown list	Specifies the delimiter for a CSV export or import. You can choose between **Comma** (default), **Semicolon** and **Tab**.
Ask Whether Support Points / Positions Should be Overwritten checkbox	When the support points and/ or positions are determined by a software autofocus run the existing points can be overwritten with the new **Z** values. **Activated**: Shows a message box asking if the points should be overwritten if there is a autofocus **Z** value.
Enable Removing of Focus Surface Outlier checkbox	**Activated**: Support points that are significantly outside the interpolated focus surface are ignored. You have the following setting options available:
- **Maximum Interpolation Degree for Outlier Detection** input field	This value can be 0 or 1. If 1 then a linear fit is used to detect the outlier support points. This is the default. If 0 a simple average value is used to detect outliers.

Option	Description
- **Treshold in Terms of the Standard Deviation (Sigma)** input field	This parameter defines a threshold value to determine which of the support points are outliers from the fitting process. This is defined by the standard deviation (sigma value) set in the spin box. Support points not meeting this criteria are subsequently ignored when the focus surface is determined.
Activate Stitching During Acquisition for New Experiments checkbox	**Activated**: Stitching during acquisition is active by default for all new experiments. This value is overwritten by the corresponding option in the **Tiles** setup for a new experiment.
Use Local Focus Surface for Preview Scans checkbox	**Activated**: Local focus surface values (z-values of positions, tile regions and if defined interpolated focal surfaces defined by support points) will be used for the acquisition of preview scan images. Note that on activation of the Tiles dimension the appropriate strategy **Use Focus Surface Defined by Tiles Setup** is pre-selected.
Binning Compensation of Exposure Time in Preview Scans input field	Defines the power to which the binning ratio is modified to automatically determine the exposure time value used for a preview scan were the binning setting between the experiment and preview scan differs. The default value is 2.0 i.e. quadratic. Thus, for example the exposure time would be reduced by a factor of four if the experiment binning is 1x1 and the preview scan binning is 2x2. The value can be varied between 1.0 and 2.0 in steps of 0.1.
Live Image in Sample Carrier Calibration Wizard (relevant for systems with cameras)	
- **Use Imaging Device from Selected Channel with "Acquisition" Settings** radio button	**Activated**: Default setting for the live image that allows navigation and focus interaction during the carrier calibration wizard.

Option	Description
- **Use Active Camera with "Locate" Settings** radio button	**Activated**: Allows the user to alternatively apply locate camera settings for use in the carrier calibration wizard (live image). By default the experiment settings for the currently selected channel/ Track will be used. This option is only relevant for systems with a wide field (camera based) detector.

Panorama section

Option	Description
Automatically Start Live Mode in Panorama View	**Activated:** Specifies that the live mode will start running automatically when you begin a panorama experiment.
Show Information Title in Panorama View	**Activated:** Displays an additional information bar above the panorama view.
Show Acquisition Animation	**Activated:** Displays an animation when an image is acquired.
Automatically move Stage / Live after an Acquisition	**Activated:** After acquisition of a snap image the stage automatically moves half a camera frame diagonally. Thus, the snap image can be inspected.
Enable Transparency Effect on selected Tile Image	**Activated:** The selected tile image is displayed with a transparency effect that enables the user to see it relation to the tiles underneath (lower layer = earlier acquisition) and those above (upper layer = more recent acquisition) at the same time.

Focus Strategy section

Option	Description
Show a Dialog to Prepare the Definite Focus Initialization	**Activated:** For experiments using Definite Focus, the user will be reminded to make appropriate adjustment to the focus prior to initialization at the experiment start.

Dynamics section

Option	Description
Show Information Title in the Dynamics Setup View	**Activated:** Displays the information title at the top of the dynamics setup panel.

LSM section

Parameter	Description
Scanner Online Correction	**Activated**: The scanner online correction is enabled. It ensures an optimal image quality at scan speeds > 13.
Airyscan Processing Baseline Shift	**Activated**: Adds an offset of 10.000 to the processed Airyscan images. This allows to display details in the processed Airyscan image that have negative intensity values and are therefore normally cut from the histogram.

14.1.7.6.7 Reuse

Allows the user to specify if the objective or incubator parameters will be including in the new experiment settings generated from an image by the reuse function. By default both instances are set to not selected.

14.1.7.6.8 User

Here you can enter user and company information. These are then written into the image metadata during acquisition. They are also used in reports.

14.1.7.6.9 Data Tables

Data Table Import Options section

Parameter	Description
Start Import in Row No.	Defines the starting row of the data table into which the data will be imported.
Automatic CSV format detection checkbox	**Activated:** Tries to detect the format of the data table automatically when importing the table to the software.

Parameter	Description
Use column, decimal and list separator from Windows regions settings checkbox	**Activated:** Uses the settings which are configured in the Windows regions settings when importing a table to the software.
Column, Decimal and Thousands Separators options	These options are active only if you have deactivated the checkboxes **Automatic CSV format detection** and **Use column, decimal and list separator from Windows regions settings**. Here you can configure the import options according to the format of your data table you want to import, e.g. specify the type of column or decimal separator.

Data Table section

In the **Number of Decimal Places** field you can set the maximum number of decimal places for the numbers imported into the data table.

14.1.7.6.10 Macro Editor

Parameter	Function
Show Inherited Members in Pop-up checkbox	**Activated:** Shows the inherited members of the ZEN class in intellisense pop-up window.
TCP Macro Section checkbox	**Activated:** Enables to enter the TCP Port Number. **Edit Field TCP Port Number** Enter the number of the TCP Port here.
Allow IPv4 Nat Traversal checkbox	
Overwrite Interactive Recording Flag checkbox	**Activated:** Overwrites the parameter for interactive execution of a function during recording with the macro recorder.

14.1.7.6.11 Connected Assistance

This option is visible only if you are using the **ZEN slidescan** application.

Here you can configure the settings for using Axeda remote service. This service is used for tracking statistical data of the system use and submitting the data to the service personnel. During installation of Axeda software, all of these settings can be

entered as well. To change the settings you can use this dialog or open the Axeda software again. We recommend to change these settings only if you know what you are doing.

Parameter	Description
Use Axeda Agent checkbox	If activated, Axeda agent is used for tracking system data. To switch of the service, simply deactivate the checkbox.
Use Proxy Server checkbox	If activated, you can enter / edit data for Proxy Server settings like HTTP or SOCKS settings (see below).
HTTP or SOCKS section	Only active, if the **Use Proxy Server** checkbox is activated. Here you can enter/edit HTTP or SOCKS settings.
Authentication section	Only active, if the **Use Proxy Server** checkbox is activated. If the **Use Authentication** checkbox is activated, a user name and a password can be entered for authentication. You can enter /edit the user name and password in the corresponding input fields.
Auto Configuration section	If the **Use Proxy Auto Configuration** checkbox is activated, all settings will be configured automatically according to the information in the PAC file. The Proxy Server settings will be deactivated, if the auto configuration is used.

14.1.8 Window menu

Menu item	Function	Shortcut
Full Screen	Sets the **Full Screen** mode to maximize the image view area size	**F11**
Next Window	Displays the next open image in the **Center Screen Area** (direction of movement is to the right)	**F6**
Previous Window	Displays the previous open image in the **Center Screen Area** (direction of movement is to the left)	**Ctrl+F6**
Close	Closes the currently selected image.	**Ctrl+F4**
Close All	Closes all the open images.	**Ctrl+Alt+W**

14.1.9 Help menu

Menu item	Function	Shortcut
Contents…	Opens the online help contents page.	**Ctrl+F1**
Index…	Opens the online help index page.	**Ctrl+F2**
About Zen…	Shows notices of the producer.	

14.2 Main tabs

14.2.1 Locate tab

Depending on the system configuration and the licensed modules this tab can have a different appearance. In general you can use the locate tab for finding or "locating" interesting areas on your sample.

For mixed systems (e.g. LSM 800 including a microscope camera) the section **System Mode** is available additionally.

In the **Eyepiece** mode (for confocal systems) this tab contains only functions for controlling the light path and viewing the sample via the eyepiece, see *Microscope Control Tool* [▶ 356]. In the **Camera** mode this tab contains more control elements and tools, see Tools on Locate tab

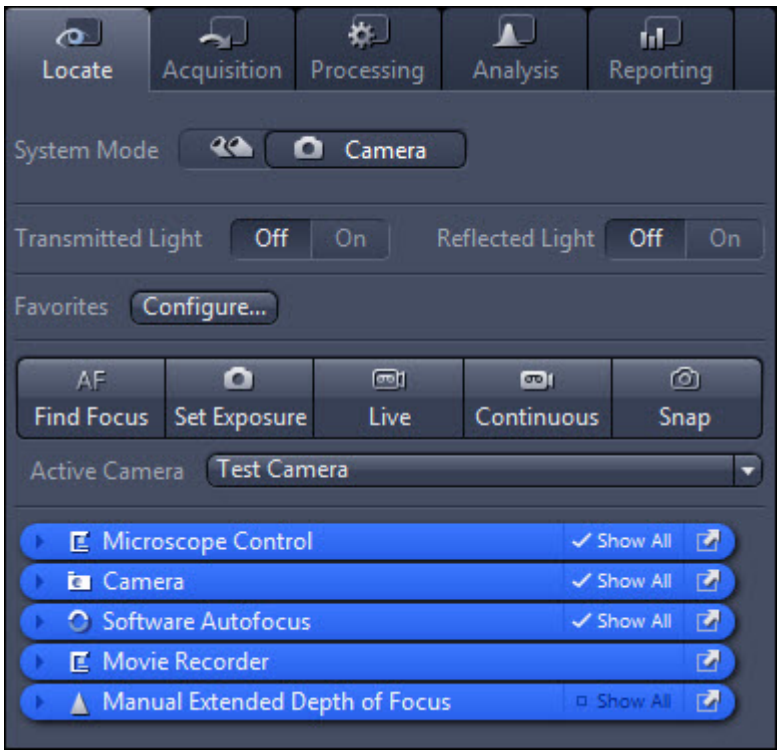


Fig. 41: Locate tab (Camera mode)

System Mode section

This section is only visible if a camera is installed with the system.

Here you can switch the system mode between **Eyepiece** and **Camera** mode. To switch between the 2 modes simply click on the corresponding button. In the table below you find a description of the two modes:

Mode	Description
Eyepiece mode	If you switch to Eyepiece mode the system adjusts the light path automatically to the eyepiece. The following list shows the changes in the ocular mode in detail: ■ All **Action buttons** are hidden ■ Only the **Microscope Control** tool is visible ■ Within the Microscope Control tool only the light path leading to the eyepiece is displayed. ■ All possible light paths to cameras are hidden.
Camera mode	■ All Widefield relevant components are displayed as usual ■ The LSM Scan Head icon is not displayed in the light path

Transmitted Light / Reflected Light section

Only visible if you have configured a motorized TL/RL shutter in MTB (MicroToolBox).

Parameter	Description
Off	Closes the shutter of the transmitted / reflected light source on a motorized microscope.
On	Opens the shutter of the transmitted / reflected light source on a motorized microscope.

Favorites section

Here you can configure further buttons with your favorite hardware setting functions. Click on **Configure...** button to open the *configuration dialog* [▶ 493].

Action buttons

With these buttons you control microscope and camera and acquire your images.

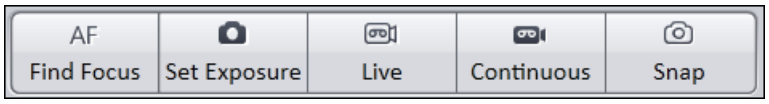


Fig. 42: Action buttons

Icon	Button	Function
AF	Find Focus	Only visible if you have configured a motorized focus (MicroToolBox). Starts an autofocus search using the current settings from the **Software Autofocus** tool.
	Set Exposure	Starts an automatic exposure time measurement with the settings defined in the **Light Path** and **Camera** tool.
	Live	Opens **Live View** and shows the live image from the camera.
	Continuous	Starts a series of **Snaps** using the settings defined in the **Light Path** and **Camera** tool. In contrast to a live image, the exact same camera setting that has been set in the **Camera** tool is used. The result at the end of this mode is a single, acquired image that can be saved.
	Snap	Acquires a single image.
Stop	Stop	Only active if one of the acquisition buttons has been clicked. Stops the function of the relevant acquisition button.

Active Camera section

Link Cameras checkbox

Only active if you have connected two structurally identical cameras to your system.

Activated: Acquires images using two cameras in parallel. This is often the case with 2-channel images for ratio measurements or FRET measurements.

Active Camera dropdown list

Shows the active camera. If you have several cameras connected, you can select the detector to use here.

Blue tools section

Depending on which modules you have purchased you see different tools available in this section, see Tools on Locate tab.

14.2.1.1 Configure Favorites dialog

Here you configure up to 20 new buttons to get quick access to your preferred camera and hardware settings.

Favorite Settings section

If you have not yet defined any buttons, you will see an empty list here. To create a new button, click on the **Add** + button. In the input fields described below you can configure your favorite setting:

Field / Option	Description
Name	Here you can enter a name for the button.
Hardware Setting Ref.	Shows the selected hardware settings.
Camera Setting Ref.	Shows the selected camera settings.
Color	Here you can select a color for the related button. Click on the color dropdown list to choose a color.
Use Color also for Button Text coloring	**Activated:** Uses the selected color as the button text color.

Available hardware settings on disc section

Here you see a list of all hardware settings that are saved on your hard drive. Select the hardware setting that you want to use with the configured button.

Available camera settings on disk section

Here you see a list of all camera settings that are saved on your hard drive. Select the camera setting that you want to use with the configured button.

14.2.2 Acquisition tab

On the **Acquisition** tab you configure and control your acquisition experiments. Note the following for working with this tab:

- The content of the tab changes depending on the configuration of your imaging system and the options that you activated or deactivated.
- Settings that you configure in the top part of the tab have an effect on settings in the bottom part of the tab. I.e. Settings that you configure in the **Acquisition Parameters** tool group in the **Channels** tool also apply to the acquisition of all images that you configure in the **Multidimensional Acquisition** tool group in the **Z-Stack**, **Tiles** or **Time Series** tools.

The area above the blue tools is called the **Experiment Manager (1)**. There you can load and save your experiments, control acquisition and decide which tools will appear in the certain tool groups.

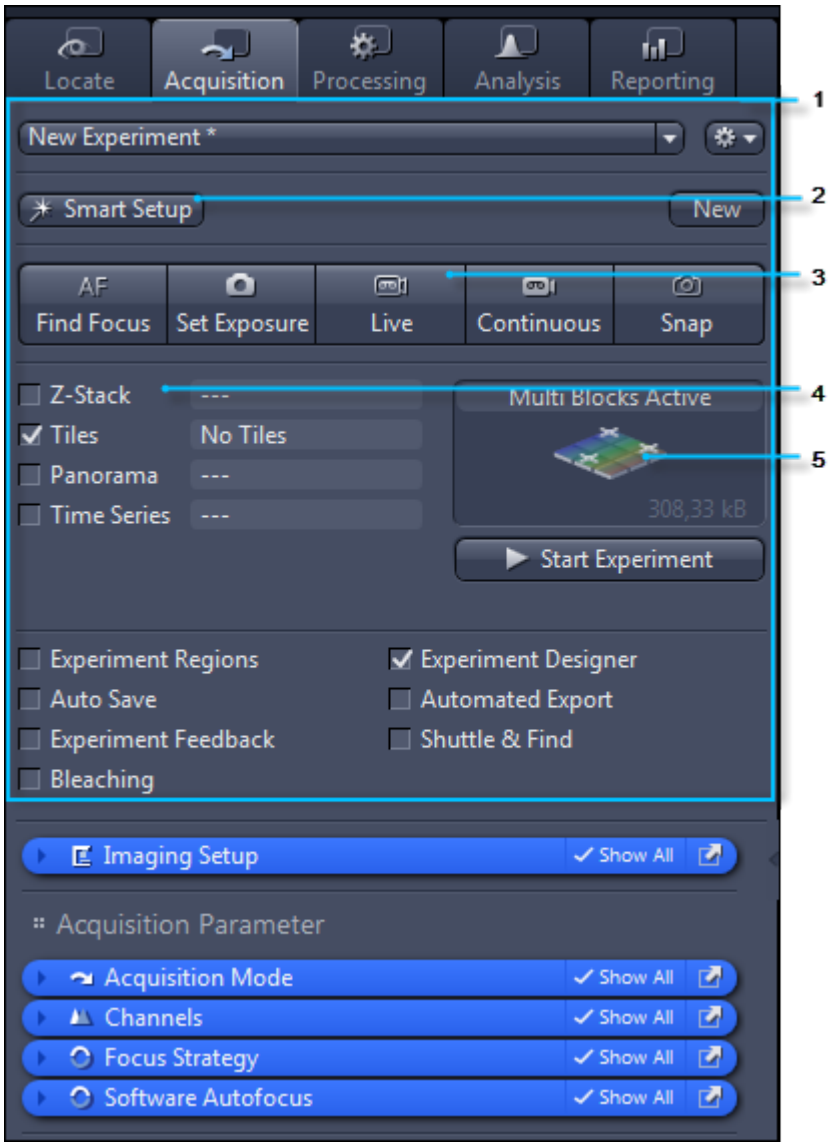
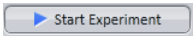


Fig. 43: Acquisition tab

Parameter	Description
Experiment Selection	From the dropdown list you can select saved experiments. If you make changes to an experiment, the name of the experiment is marked with an asterisk (*). If you close the application without saving a changed ("asterisked") experiment, you will be asked whether you want to save the changes.

Parameter	Description
Options button 	Opens the Options shortcut menu.
New button	Creates a new image document for the next "snapped" image.
Smart Setup (2) button	Opens the **Smart Setup** dialog, see *Smart Setup* [▶ 344]
Action buttons (3)	With these buttons you control microscope and camera and acquire your images. The Acquisition buttons on the **Acquisition** tab differ from the Acquisition buttons on the **Locate** tab. The buttons on the Locate tab relate to an individual image. The buttons on the Acquisition tab relate to a multidimensional image with at least one channel.
- Find Focus 	Only visible if you have configured a motorized focus (MicroToolBox). Starts an autofocus search using the settings from the **Focus Devices** tool.The autofocus search is performed for the selected reference channel in the **Channels** tool.
- Set Exposure 	Starts an automatical exposure time measurement with the settings defined in the **Light Path** and **Camera** tool.
- Live 	Starts the **Live mode.** In the **Center Screen Area** a live image from the camera is shown.
- Find Focus 	Starts a series of **Snaps** using the settings defined in the **Light Path** and **Camera** tool. In contrast to a live image, the exact same camera setting that has been set in the **Camera** tool is used. The result at the end of this mode is a single, acquired image that can be saved.
- Snap 	Acquires a single image.
- Stop 	Only active if one of the acquisition buttons has been clicked. Stops the function of the relevant acquisition button.

Parameter	Description
Acquisition Dimensions (4)	In the Acquisition Dimensions section you can activate the acquisition dimensions (e.g. Z-stack, Tiles, Panorama, Time Series) required in your experiment. The field to the right of the acquisition dimension shows how extensive the acquisition will be (e.g. number of Z-Stacks or number of tile images).
Experiment Preview (5)	Here you can see a graphical representation of the configured experiment. The **Disc** icon indicates that you have enabled **Auto Save** function for the experiment.
Start Experiment 	Only active when additional acquisition dimensions were added to the experiment. Starts the experiment with the current configuration.

14.2.2.1 Smart Setup

Smart Setup offers you support when configuring multichannel acquisition experiments. To start it click on the **Smart Setup** button on the **Acquisition** tab.

Select the fluorescent dyes and contrast techniques that you want to include in your experiment from a large dye database. Smart Setup takes the configuration of your microscope hardware and the properties of the selected dyes into account. Based on this information, it makes one or more suggestions for acquisition. You can adopt these into your experiment as required and make further changes to them there.

i Note

If **Smart Setup** is unable to make a proposal, it is not possible to use the selected dyes, contrast techniques, or current microscope hardware to make acquisitions. Select other dyes or another contrast technique or configure your acquisition experiment using the **Acquisition Mode** tool and the **Channels** tool.

For working with Smart Setup please note the following:

- Smart Setup tries to configure the motorized components of your system for the acquisition of multichannel images.
- Smart Setup does not change any parameters of other acquisition dimensions (e.g. Z-stack, Time series, or Multi-position acquisitions).
- For widefield tracks it does not influence any camera parameters (e.g., Exposure time or Resolution).
- For LSM tracks it adjusts parameters within the Imaging Setup, the Acquisition Mode and the Channels tool windows.

Depending on your system you will see two buttons on top of the dialog.

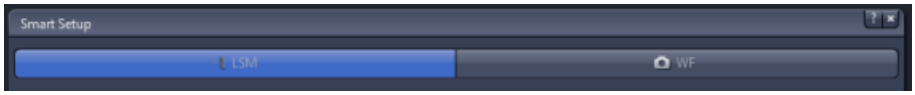


Fig. 44: Smart Setup Modes

If you select the **LSM** button you can use Smart Setup for configuring confocal experiments, see *Smart Setup (LSM)* [▶ 349].

If you select the **WF** button you can use Smart Setup for configuring widefield experiments, see *Smart Setup (WF)* [▶ 345].

14.2.2.1.1 Smart Setup (WF)

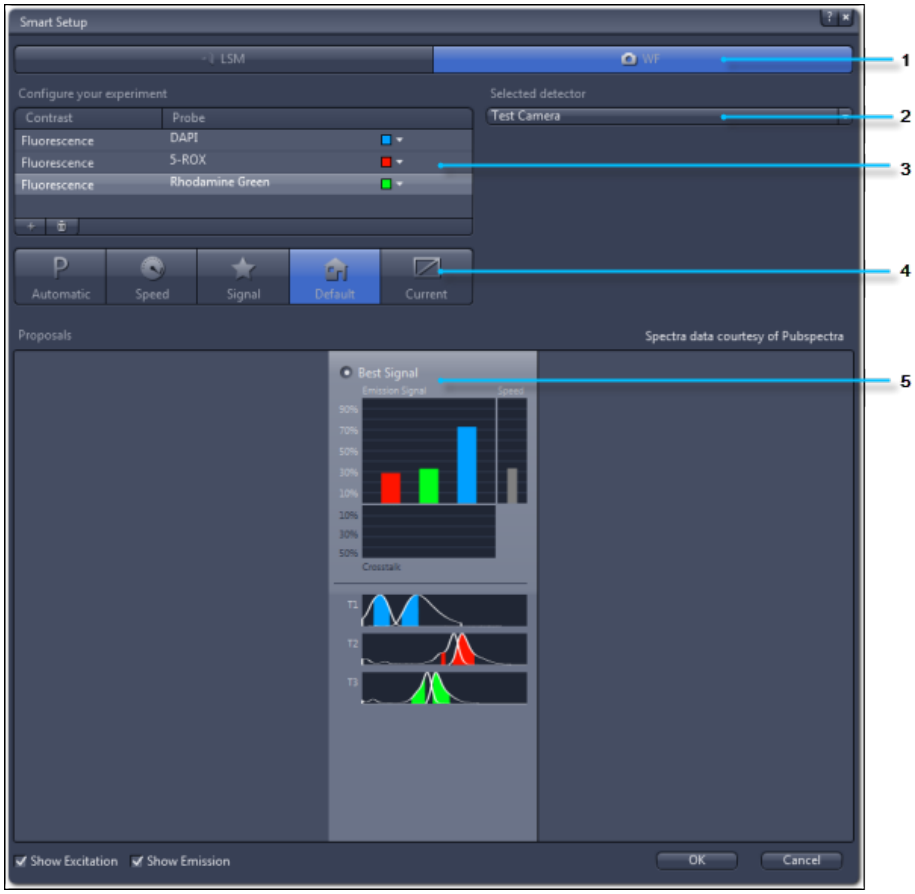


Fig. 45: Smart Setup (WF)

1 Imaging Mode Selection
2 Detector Selection
3 Experiment Configuration
4 Motif Buttons
5 Graphical Display of Proposals

Parameter	Description
Detector Selection (2)	Only visible if two or more cameras are configured for the system. Here you can select the desired camera for the experiment.

Parameter	Description
Configure your experiment (3)	Here you can add up to four reflected light fluorescence channels and one transmitted light contrast technique to your experiment. The added dyes or the contrast technique are shown in the list below. If you click on the **Add** button the *Add Dye or Contrast Technique* [▶ 485] dialog will be opened. There you can select the desired dye or contrast technique from the Dye Database.
Motif buttons (4)	Here you can optimize image acquisition regarding particular requirements like speed or quality. All parameters, e.g. camera resolution or dynamic range in the **Acquisition Mode** or the **Channels** tool, were set automatically. They will essentially influence the camera, detector, and lightning settings.
- Automatic	■ The system will try to set the optimal resolution for the camera in the **Acquisition Mode** tool. The resolution will be calculated from camera parameters and numeric aperture; Microscanning will not be applied even if the camera supports this mode. ■ The dynamic range for all fluorescence channels will be set to 50% or 80% for all transmitted light channels.
- Speed	■ If binning is supported, one binning category will be set for the camera under the optimal resolution in the **Acquisition Mode** tool. ■ Sets dynamic range of all fluorescence channels to 20% or 50% for transmitted light channels. ■ Sets power of all Colibri-LEDs to 100%. ■ Sets EMGain of the camera (if available) to half of Gain max. Sets read mode of camera to fastest. ■ Creates an acquisition configuration which removes all reducers or neutral filters. ■ Changes acquisition sequence for dimensions to fastest. Only effective with 2 or more dimensions.

Parameter	Description
- Signal	■ Sets the optimal resolution for the camera in the **Acquisition Mode** tool. Microscanning will be applied if the camera supports this mode. ■ Sets the dynamic range for all fluorescence channels to 90% or 100% for all transmitted light channels. ■ Sets power of all Colibri-LEDs to 75%. ■ Sets EMGain of the camera (if available) to 10% of Gain max. Sets read mode of camera to slowest.
- Default	Sets all parameters in **Channels** and **Acquisition Mode** tool to the default values. All changes will be overwritten and reset.
- Current	No changes are made. Only the necessary hardware settings for acquisition are applied by **Smart Setup**. **NOTICE** **If you changed hardware settings in the Acquisition Mode tool manually and do not want to lose them, make sure you select the Current button.**
Proposals (5)	Here you can see the proposals made by **Smart Setup** displayed graphically. You can find a detailed description of the graphical display under *Graphical Display of Proposals* [▶ 352]. The proposals change the imaging settings in the **Imaging Setup** tool window accordingly. The number and type of proposals depend on the microscope hardware being used, the selected dyes, and the contrast technique:
- Best Signal	This proposal results in the best signal strength.
- Fastest	This proposal results in the fastest acquisition.
- Best Compromise	This proposal results in the best compromise between signal strength and fastest acquisition.
Show Excitation checkbox	Shows the excitation spectrum of the selected dyes in the graphical display.
Show Emission checkbox	Shows the emission spectrum of the selected dyes in the graphical display.
OK button	Adopts the proposal displayed as the current acquisition experiment. The suggestion overwrites existing experiments on the **Acquisition** tab.

Parameter	Description
Cancel button	Ends **Smart Setup**. The suggestions are not adopted into the experiment.

14.2.2.1.2 Smart Setup (LSM)

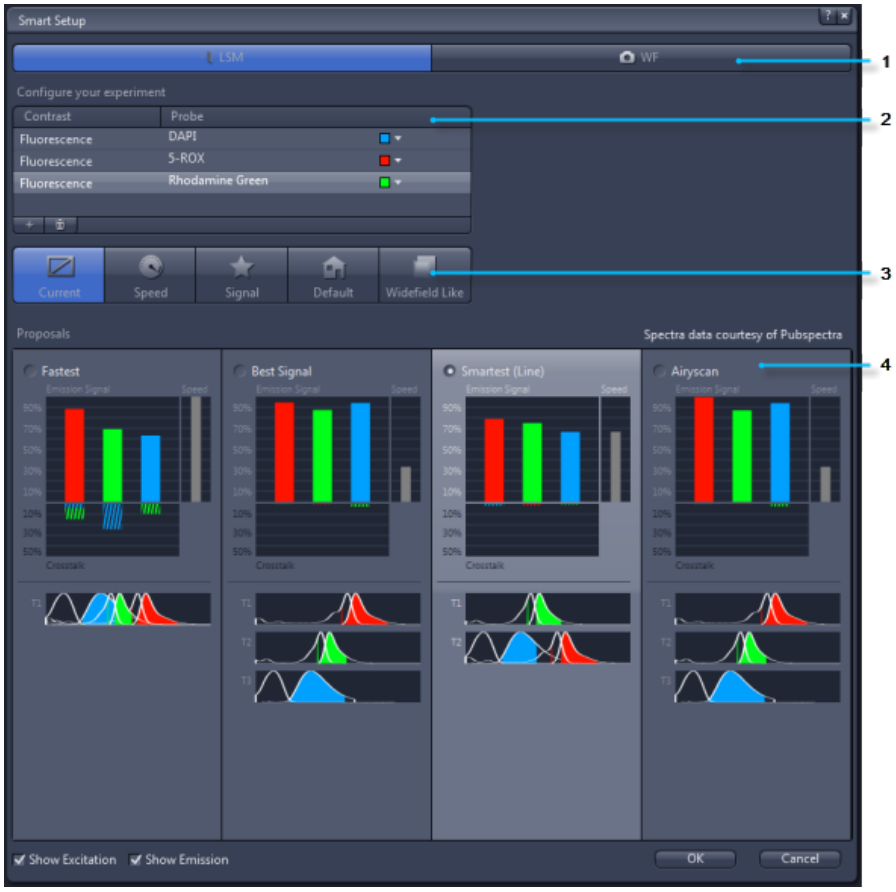


Fig. 46: Smart Setup (LSM)

1 Imaging Mode Selection
2 Experiment Configuration
3 Motif Buttons
4 Proposals

Parameter	Description
Configure your experiment (2)	Here you can add up to 6 reflected light fluorescence channels and one transmitted light contrast technique to your experiment. The added dyes or the contrast technique are shown in the list. If you click on the **Add** button the *Add Dye or Contrast Technique* [▶ 485] dialog will be opened. There you can select the desired dye or contrast technique from the Dye Database.

Parameter		Description
Motif buttons (3)		Here you can optimize image acquisition regarding particular requirements like speed or quality. If clicking on a button different parameters in the **Acquisition Mode** or the **Channels** tool, were set automatically. The automatic settings will influence parameters like Frame Size, Speed, Direction, Bit Depth (in Acquisition Mode tool) and Pinhole Diameter, Gain, Laser Power (in Channels tool), depending on the selected button. Various proposals for further experiment settings are shown in the graphical display below the buttons.
-	Current	No changes are made. Only the necessary hardware settings for acquisition are applied by **Smart Setup**. **NOTICE** **If you changed hardware settings in the Acquisition Mode tool manually and do not want to lose them, make sure you select the Current button.**
-	Speed	■ Sets the frame size to 400x400 pixels ■ Sets the scanning speed to maximum value ■ Sets the scanning direction to bi-directional ■ Opens the pinhole to 2 Airy Units (AU)
-	Signal	■ Aims to provide high quality images with best signal to noise ratio. ■ Sets the frame size to a minimal value that fulfills the Nyquist criterion, but to a maximum of 2048x2048 pixel ■ Sets the scanning speed to 6 ■ Sets the scanning direction to uni-directional ■ Sets the Bit Depth to 16 bit
-	Default	■ Applies the most common imaging settings for the acquisition ■ setting for a compromise between Speed and Signal ■ Sets the frame size to 512x512 ■ Sets the scanning speed to 9 ■ Sets the scanning direction to uni-directional

Parameter	Description
- Widefield Like	Sets parameters in the Acquisition Mode tool for a maximum light efficiency by opening the pinhole to its maximum. This light efficient setting sacrifices optical sectioning.
Proposals (4)	Here you can see the proposals made by **Smart Setup** displayed graphically. You can find a detailed description of the graphical display under *Graphical Display of Proposals* [▶ 352]. The proposals change the imaging settings in the **Imaging Setup** tool window accordingly. The number and type of proposals depend on the microscope hardware being used, the selected dyes, and the contrast technique:
- Fastest	This proposal results in the fastest acquisition.
- Best Signal	This proposal results in the best signal strength and minimizes the level of cross talk.
- Smartest (Line)	Combines the advantages of Fastest and Best Signal. It minimizes the number of tracks as well as cross talk.
- Airyscan	Activates the **Airyscan** mode in the **Imaging Setup** tool and adjusts the settings accordingly, see *Airyscan Mode* [▶ 388].
Show Excitation checkbox	Shows the excitation spectrum of the selected dyes in the graphical display.
Show Emission checkbox	Shows the emission spectrum of the selected dyes in the graphical display.
OK button	Adopts the proposal displayed as the current acquisition experiment. The suggestion overwrites existing experiments on the **Acquisition** tab.
Cancel button	Ends **Smart Setup**. The suggestions are not adopted into the experiment.

14.2.2.1.3 Graphical Display of Proposals

Note

The bars in the graphs only show relative values. The actual strength of the emission signal and the crosstalk in the image can deviate substantially from this estimate, as Smart Setup has no knowledge of the strength with which the sample has been dyed with the individual dye components.

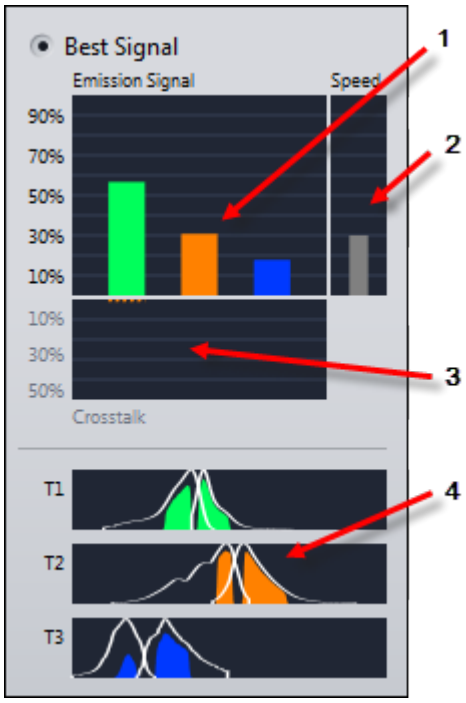


Fig. 47: Emission Signal, Speed, Crosstalk, and Tracks

No.	Name	Description
1	Emission Signal	A filled, colored bar in the **Emission Signal** display field shows the relative emission signal to be expected for the corresponding channel. The channel color corresponds to the color of the selected dye in the **Configure Experiment** section.
2	Speed	A gray bar in the **Speed** display field represents the approximate acquisition speed that can be expected. This is the time required for the movement of microscope hardware during multichannel acquisition. Camera exposure times or parameters for other acquisition dimensions are not taken into account here.

No.	Name	Description
3	Crosstalk	A hatched bar in the **Crosstalk** display field shows the expected relative crosstalk originating from one or more dyes for other channels.
4	**Tracks** display	Only visible if the **Show Excitation** and / or **Show Emission** checkboxes are activated. The various tracks are labeled with **T1**, **T2** etc.. The white lines show the excitation and emission spectra of the dyes schematically. The spectra are filled in color in the places that will be acquired by the acquisition configuration suggested by **Smart Setup**. Transmitted light channels are displayed as a white field.

14.2.3 Processing tab

On the **Processing** tab you will find all the functions you need for image processing using ZEN 2.1.

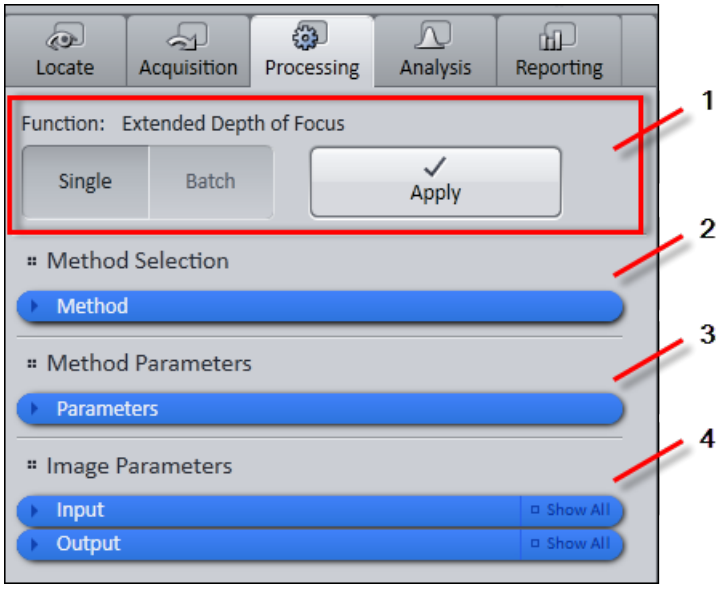


Fig. 48: Processing tab

No.	Name	Description
1	**Single/ Batch** button	Switch between Single and Batch mode here. In **Single Processing mode** you apply a selected processing method, with the relevant method and image parameters, to a single image. In **Batch Processing mode** you apply a selected processing method, with the relevant method and image parameters, to the list of images. In this mode only a limited selection of processing functions is available.
1	**Apply** button	Applies the selected function to the input image.
2	**Method Selection**	Here you select the image processing functions. Click on the blue bar to show the list of IP functions. You can find a detailed description for each processing function under *Image Processing Functions* [▶ 615].
3	**Method Parameters**	Here you configure the parameters of the selected image processing function. Click on the blue bar to show the parameters of the selected IP function.
4	**Image Parameters**	Here you configure the image parameters of the input and output image. Click on the blue bars to open input /output image settings.

14.2.4 Analysis tab

On the **Analysis** tab you will find the tools for image analysis, see *Tools on Analysis tab* [▶ 451].

14.2.5 Reporting tab

Here you can create reports, change the templates or link other images/tables.

i Note

All reports in ZEN are based on templates which are then filled with data. The appearance and content of the report are defined in the template. The creation of templates is not included as a feature in ZEN.

Parameter	Description
New Report button	Only visible if the **Report Preview** is not active. Opens the **Report Preview** in the image area. The appearance of the report is determined by the template, which you can add in the **Favorite Templates** section.
Finish Report button	Only active if a report has been created. Closes the editing of an report.
Print Report button	Only active if a report has been created. Opens the dialog window. Here you can configure the print settings.

i Note

All reports in ZEN are based on templates which are then filled with data. The appearance and content of the report are defined in the templates.

14.2.6 Extensions tab

This tab is visible only if you have activated an extension (e.g. ImageJ) under **Tools | Extension Manager**.

Our extensions concept allows to extend ZEN basic functionality by implementing third party extensions, e.g. ImageJ. The extensions concept is a part of **OAD (Open Application Development)** for ZEN, see Open Application Development (OAD).

Depending on which extension you have activated, you will see the extension´s functions and controls on the extensions tab. Please notice that we will not describe any functions of third party extensions here. Therefore use the third party documentation for each extension.

You can find more information on OAD and the supported extensions under **www.zeiss.com/zen-oad**.

14.3 Tools

14.3.1 Tools on Locate Tab

14.3.1.1 Microscope Control Tool

In the **Microscope Control** tool you can configure the microscope (e.g. light path) and its components for your experiment. The configuration of your system (see MicroToolBox) is shown here in the form of a graphical display.

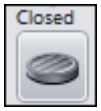
The graphical display of the light path shows all the components in the path from the light source to the specimen and from there to the camera or eyepiece. The icons correspond to the components that are installed on your system. The most important icons are described in the chapter *Reflected/Transmitted Light Path* [▶ 356]. Keep the following points in mind when working with this tool:

- To activate / deactivate a setting, left-click on the relevant icon.
- Icons with an **arrow** icon in the bottom right corner contain dialog windows that allow you to configure additional settings. To open the dialog windows, left-click on the corresponding icon.
- Icons with a **hand** icon in the bottom left corner indicate components that have to be operated manually.

14.3.1.1.1 Reflected/Transmitted Light Path

In the graphical display of the reflected/transmitted light path you will see various optical components, such as the shutter, diaphragms, filters and beam splitters, depending on the microscope and components you are using. All the available components must be configured in advance in the **MTB** (MicroToolBox). The settings for the components can be configured via the icons in the software. Therefore simply left click on the corresponding icon. If you are not using any motorized components, you will have to make the relevant adjustments manually. In the table below you can find descriptions of the most important icons:

Icon	Name	Description
	**Eyepiece** icon	Above the **Eyepiece** icon the total magnification of the selected beam path with all activated intermediate magnifications is displayed. To direct the light path fully to the eyepiece, simply left-click on the icon.

Icon	Name	Description
 / 	**Shutter** icon	To set the shutter to **Open** or **Closed**, left-click on the shutter icon. The status is displayed in text form above the icon.
	**Reflector Turret** icon	All configured filter cubes for reflected light techniques can be found in a list. Select the desired filter cube from the list.
	**Nosepiece / Objective** icon	Select the desired **Objective** from the list.
	**Stage** icon	This icon represents the microscope stage. You will also find the options for *Stage Control* [▶ 359] and *Focus Control* [▶ 359].
	**Aperture Diaphragm** icon	Adjust the diaphragm opening (0% to 100%) using the slider or spin box/input field.
	**Filter Wheel** icon	Here you can enter the first neutral density filter (e.g. 0.4%, 6%, 100%, 100%) that you require.
	**Condenser** icon	The condenser is only available in the **Transmitted Light** path. Select the contrast method from the dropdown list (e.g. brightfield, darkfield, phase contrast ring 1, 2, 3, DIC I, II, III).
	**Camera / Eyepiece Switch**	Select from the list whether you want to direct the light to the camera only (**100% Camera**), to the camera and the eyepiece (**30% Eyepiece/70% Camera**) or to the eyepiece only (**100% Eyepiece**).
	**Camera** icon	Represents a camera. The selected camera is shown above the icon.

Icon	Name	Description
	**Microscope Manager** icon	To open the *Microscope Manager* [▶ 358] dialog window, left-click on the Microscope Manager icon.
	**Reflected Light/ Transmitted Light Switch** icon	If your microscope has a halogen lamp for both reflected and transmitted light illumination, here you can select whether you want to control the halogen lamp for reflected light illumination or the halogen lamp for transmitted light illumination.

14.3.1.1.2 Microscope Manager

Parameter	Description
Contrast Manager	Select the setting for the contrast mode from the **Mode** dropdown list.
- Off	The Contrast Manager is not used. All settings must be made manually or via a settings file.
- On Demand	The function of the Contrast Manager is activated via the touch screen on the microscope.
- Contrast Retaining	If core components (e.g. condenser, reflector, shutter) for a certain contrast technique are changed, dependent components are also changed accordingly. Select one of the available methods for the contrast mode from the **Method** dropdown list.
Light Manager	
- **Enabled** checkbox	**Activated:** Activates the Light Manager. The **Mode** dropdown list in the Light Manager is active. Select a setting for adjusting the brightness of the light from the Mode dropdown list.
- **Objective**	Adjusts the brightness of the light via the lamp voltage. The color temperature changes accordingly.
- **Classic**	Adjusts the brightness on the basis of the available filter wheels. The color temperature is retained. Only if the brightness adjustment cannot be achieved via the filter wheels does adjustment take place via the lamp voltage.

Parameter	Description
Dazzle Protection checkbox	**Activated:** Activates dazzle protection. Dazzle protection prevents light from passing through the eyepiece and dazzling the user, for example when reflector positions are changed. This is mainly achieved by closing the reflected or transmitted light shutter. If no shutters are installed, the lamp voltage is adjusted.
Parafocal Correction checkbox	**Activated:** Activates parafocal correction.

14.3.1.1.3 Opening the stage tool

By clicking on the **Stage** button, the Stage tool will be opened in the Right Tool Area. There you can move the microscope stage virtually with the help of a software joystick or by entering absolute coordinates. You can also calibrate the stage within that tool.

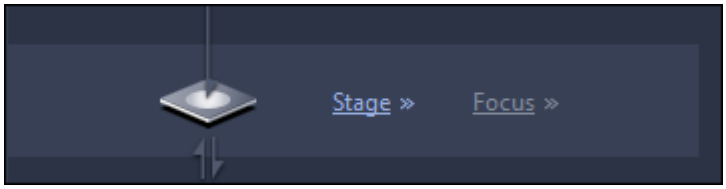


Fig. 49: Stage and Focus

14.3.1.1.4 Opening the focus tool

By clicking on **Focus** the Focus tool will be opened in the Right Tool Area. There you can move the focus drive virtually with the help of a software joystick or by entering absolute coordinates. You can also calibrate the focus drive within that tool.

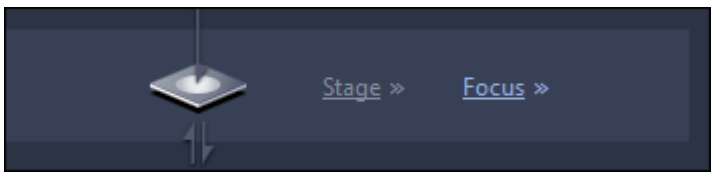


Fig. 50: Link to Focus Tool

14.3.1.2 Camera Tool

In the **Camera** tool you can configure all the settings for the selected camera. Please note that the functions and settings in this tool depend on which camera you are using meaning not all cameras have all the functions described in here.

14.3.1.2.1 Exposure Time Section

The **Exposure Time** section enables you to control the exposure settings of your camera.

If you use automatic exposure, you can select an area on the camera sensor which is used to calculate the exposure time.

Parameter	Description
Time	Specifies the duration of the image acquisition. Select the unit of time (min, ms, s, µs) from the drop-down list on the right and enter the desired value.
Auto Exposure	■ Activated: The exposure time is calculated automatically every time an image is acquired. The exposure time in the corresponding input field fluctuates accordingly. ■ Deactivated: You can set the exposure time manually.
Set Exposure	Starts a one-off measurement of the exposure time, which is then used for all subsequent images. Deactivates **Auto Exposure**. If you are not satisfied with the result, you can adjust the measured exposure time manually.
Auto Exposure Intensity	Enables you to compensate for underexposure or overexposure if you are not content with the auto exposure result ■ 5% - 100%: Darkens the image (compensates for overexposure) ■ 100% - 200%: Brightens the image (compensates for underexposure)
Spot Meter / Focus ROI	Activated: The exposure time and focus measurements use the intensity values within a specified area instead of the entire camera sensor area. This improves the results for the area to be acquired. If the red **Spot Meter / Focus ROI** frame is not visible in the live image, right-click in the live image and select **Spot Meter / Focus ROI** from the context menu.

Parameter	Description
Binning	Binning combines the information of neighboring camera pixels into a single larger pixel. The camera sensitivity is increased by improving the signal-to-noise ratio, but the resolution is decreased by the same factor. For example, if the binning is set to 2 × 2, four pixels are combined to one. The camera sensitivity is increased by a factor of four but the resolution is reduced by a factor of four. Increasing the binning means weaker signals can be detected for a given exposure time.
Resolution	Sets the cameras resolution, e.g. 1024 x 1024 px

14.3.1.2.2 White Balance Section

This section is only visible if you are using a color camera. The section enables you to adjust the color balance to a neutral hue independent of the light source used.

Save suitable white balance settings using the **Settings** section to ensure color reproducibility of images acquired in the future.

Parameter	Description
Auto	Compensates for the color temperature of the light source automatically to yield a neutral hue The entire camera sensor area is measured. If there are no pure white areas on the sample and **Auto** does not yield the desired results, measure and compensate for the color temperature of the light source as follows: ■ Transmitted light: Move the sample such that a clear and transparent region is illuminated or remove the sample from the microscope. Click the **Auto** button to perform the auto white balance. ■ Reflected light: Use a neutral surface (e.g. a piece of white paper) as a sample. Click the **Auto** button to perform the auto white balance. You can now acquire white balanced images of your sample with the above settings.
Pick...	Enables you to select a reference pixel for white balance from the live image The selected pixel should be neutral white.

Parameter	Description
3200 K	Applies a predefined color balance setting to compensate for the color temperature of a halogen light source at approximately 3200 K
5500 K	Applies a predefined color balance setting to compensate for the color temperature of an LED light source at approximately 5500 K
Show Channels	Enables you to set the color balance of each color channel (red/cyan, green/magenta and blue/yellow) individually to make the image appear neutral
Color Temperature	Changes the overall color temperature of the image from cool (blue cast) to warm (red cast) The color channels (red/cyan, green/magenta and blue/yellow) are adjusted automatically. The **Color Temperature** setting can work against the settings applied using **Show Channels**. Use **Color Temperature** for fine tuning in combination with **Pick...** if **Pick...** does not give perfect results.
Saturation	Changes the colorfulness of the image
Reset	Resets any color changes and sets the white balance value to 6500 K.

14.3.1.2.3 Gain

Using this function you can amplify the camera signal so that the image becomes brighter.

14.3.1.2.4 Acquisition ROI section

In this section you can define a Region Of Interest (ROI) on the camera sensor which will be used for acquisition. A smaller ROI can increase the acquisition speed.

The region of interest is indicated by a blue frame in the preview window and can be moved and resized freely. The preview window always shows the entire camera sensor area which can be acquired.

The **Pixel Size** shown below the preview window indicates the size in µm to which a pixel corresponds. This depends on the camera sensor properties and on the binning.

Parameter	Description
Maximize	Selects the entire available image sensor area as the region of interest

Parameter	Description
Center	Positions the region of interest precisely at the center of the image
Size	Sets the width and height of the region of interest in pixels
Offset	Specifies the position of the top left corner of the **Acquisition ROI** (blue frame) with respect to the top left corner of the preview window.
Refresh Overview	An image is acquired and displayed in the preview window with the current ROI settings. This has no effect on the image in the **Center Screen Area**.
Crop button	Only visible if the checkbox **Show Crop Button** under **Tools** | **Options** | **Acquisition** | **Camera/Live** is activated. By clicking on the this button, you can specify a ROI (Region of Interest) in a snapped image. If no image is available in the Center Screen Area the button is not active. The "cropped" area is used as ROI for the next image acquisition.

14.3.1.2.5 Post Processing Section

The **Post Processing** section allows you to apply basic image processing functions while acquiring the image. This can be helpful if certain image processing steps are necessary for any acquired image and saves image processing work later in a job.

Depending on the camera model, different settings are available. The following image processing functions are the most common:

- Noise filter
- Unsharp mask

Parameter	Description
Black Reference	Influences the live image and each image acquired. For the black reference to work, you first need to acquire a reference image. Define a corresponding reference image using the **Define** button. **Activated**: Applies the measured black reference to the image. **Deactivated**: The measured black reference is not used. The reference image is retained. **Define** button: Automatically defines the black reference. The measurement lasts for several seconds. The Black Reference checkbox is then activated automatically.
Shading Correction	**Activated**: Applies the defined shading correction to the image. The applied correction mode is Multiplicative, you`ll find a detailed description of this mode under Shading Correction. **Deactivated**: The measured shading correction is not used. The reference image is retained. Shading correction is used to correct optical effects, such as minor differences in illumination or static contaminants in the beam path, with the help of a reference image. The reference image must be acquired without a sample. You can select between two modes **Global** and **Specific**, see description below. After you have selected the mode simply click on the **Define** button and the shading correction will be calculated.

Parameter	Description
- **Global** mode	Performs an objective specific shading correction. This is the default method for shading correction. The following components will be considered: ■ Magnification: Objective and Optovar ■ Camera bit depth and RGB/BW mode ■ Camera type and port position Fluorescent filters or other fluorescence specific components will not be considered In principle, shading correction is objective specific. A separate reference image has to be created for each objective. Once calibration has been completed, the correction image associated with the objective being used is loaded automatically if shading correction is active. If no correction image is available for an objective, the Shading Correction checkbox is automatically deactivated when the objective in question is swung in. Objective recognition on a motorized or encoded microscope is required for these automatic actions.
- **Specific** mode	Performs channel-specific shading correction. In this case the fluorescence filter block used is saved with the shading file. If the fluorescence channel is changed, a previously created reference image is also loaded. The availability of created reference images can be checked on the menu **Tools** | **Calibration Manager**. Incorrect reference images can also be deleted there. The following components will be considered: ■ Contrasting method and condenser ■ Fluorescence reflector and beam splitter ■ Spinning disc fluorescence filter
Enable Noise Filter	Activated: Noise in the acquired image is filtered according to the adjusted threshold. Affects acquired images only; the live image does not change.

Parameter	Description
– **Threshold**	The noise filter reduces the extent to which individual pixels deviate from the average value of their nearest neighbors. The **Threshold** corresponds to a tolerance value. If the deviation of the middle pixel value from the average value of the pixels immediately surrounding it exceeds the tolerance value (i.e. it is interpreted as noise), it is replaced by the average value. The higher the value, the greater the tolerance for noise. The lower the value, the stronger the noise reduction. This technique reduces the noise of individual pixels that are produced, in particular with EMCCD cameras and CMOS cameras. The selected technique prevents any changes being made to object edges, as in most cases these are larger than individual pixels. This filter is also suitable for removing individual "hot pixels" from an image without having to acquire a reference image in advance.
Enable Unsharp Mask	Enhances contrasts at fine structures and edges. Thus, the resulting image appears clearer and enriched in detail.
– **Strength**	Controls the amount of contrast enhancement applied to fine structures and edges. The higher the strength, the darker or lighter the resulting edges, compared to the original image.
– **Radius**	Determines the size of detail to be enhanced. A small radius enhances smaller details. The radius also affects the appearance of enhanced edges. A large radius leads to a visible halo along enhanced edges. The larger the radius, the broader the halo.

Parameter	Description
– **Color Mode**	Determines the calculation method, which affects the appearance of the output image. ■ **RGB**: – The **Unsharp Mask** filter calculates the sharpness for each color channel individually. – The color saturation and the color of structures may be changed and color noise may occur. ■ **Luminance**: – The **Unsharp Mask** filter calculates the sharpness based on the luminance signal computed from the RGB channels. – This mode avoids possible color noise or shift in color saturation, which could be induced by certain image textures.
– **Auto Contrast**	Activated: You can adjust the **Contrast Tolerance** (0-20). **Auto Contrast** only works in RGB color mode.
– **Contrast Tolerance**	Increasing the contrast during unsharp masking is achieved by broadening the distribution of intensities. This corresponds to a spread of the image histogram. **Contrast Tolerance** controls how much the intensity distribution is spread and thus how strong the contrast is increased. ■ **Contrast Tolerance** = 0: No spread of intensities, no increase of contrast ■ **Contrast Tolerance** = 20: Maximum spread of intensities, maximum increase of contrast
– **Clip To Valid Bits**	■ Activated: The processed image is composed of the same colors as the original image (i.e. the value range of the output image is adjusted to the color range of the input image). ■ Deactivated: Colors not present in the original image may appear in the processed image.

14.3.1.2.6 Mode Section

In this section you can adjust how the software retrieves the camera sensor data.

Parameter	Description
Color Mode	This parameter is available for color cameras only.
– RGB	The image data of a color camera is transmitted unchanged. This corresponds to the standard operating mode of a color camera.
– B/W	The image data of the color channels are treated as grayscale. The data of related color channels are averaged. The saturation of the camera appears reduced as a result. This process does not change the spectral properties of a color camera. The image information of the color sensor still undergoes color interpolation. An infrared filter also restricts the spectral sensitivity of the color camera compared to the spectral sensitivity of a genuine black and white camera.
Live Speed	Specifies the live image update speed. Enables you to focus or to find regions of interest on a sample quickly. A high live image update speed reduces the exposure time of the live image, even at longer exposure times used for image acquisition. To achieve a similar impression of image brightness, however, the image data supplied must be adjusted digitally, which may generate a certain amount of noise or reduce the resolution of the live image.
IP Quality	Only available for **Axiocam 503** color and **Axiocam 506** color. Here you can select the color interpolation quality (IP Quality) for the recorded image. Please notice that this function does not apply to Live mode. **Fast**: Represents the image optimized and requires a shorter computation time. **High**: Represents the image without artifacts and with a higher image quality. This mode is only effective with binining factor 1. If you reset the settings via the **Default** button the IP Quality is set to high.

Parameter	Description
NIR Mode	This function is only available for cameras which offer this feature (e.g. **AxioCam MRm**). **Activated:** Uses the camera in the near infrared range (NIR=Near-Infrared). Sensitivity for signals in the near infrared range (approx. 700nm to 1000nm) is increased. However, the tolerance for overexposure is reduced and, if very bright structures are present, overexposure artifacts (blooming) can result. We recommend that you use this mode mainly for very weak signals.

14.3.1.2.7 Trigger Control Section

Only visible if the selected camera has a trigger input/output.

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button  .

Trigger Out section

Using the trigger output you set how the camera sends a trigger signal to an external component (e.g. shutter).

i Note

Activate both checkboxes if you want the trigger signal to be generated both during the live image and during acquisition.

Parameter	Description
Enable for Snap checkbox	**Activated:** Generates the trigger signal during the acquisition of an image.
Enable for Live checkbox	**Activated:** Generates the trigger signal during the live image.
Control Signal dropdown list	
- **Active High**	The **Control Signal** jumps from 0 Volts to 5 Volts when the camera's exposure begins. Following exposure it returns to 0 Volts.

Parameter	Description
- **Active Low**	The **Control Signal** jumps from 5 Volts to 0 Volts when the camera's exposure begins. Following exposure it returns to 5 Volts.
- **Shutter Open Delay spin** box/ input field	Here you can enter the delay before acquisition.

Trigger In section

The trigger input allows you to trigger acquisition by the camera using an external trigger signal.

i Note

Due to its inertia, a mechanical shutter needs a certain amount of time to change from the closed to the open position after the control signal has been generated. To ensure that this transitional state is not recorded during the exposure of the sensor, the start of actual acquisition can be delayed by an adjustable period of time.

Parameter	Description
Enable for Snap checkbox	**Activated:** Only acquires the image after the trigger signal has been received.
Control Signal dropdown list	
- **Active High**	The **Control Signal** jumps from 0 Volts to 5 Volts when the camera's exposure begins. Following exposure it returns to 0 Volts.
- **Active Low**	The **Control Signal** jumps from 5 Volts to 0 Volts when the camera's exposure begins. Following exposure it returns to 5 Volts.

14.3.1.2.8 Model Specific Section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button  .

In this section you see additional, model-specific camera settings depending on which camera you use on your system.

Reset button

Resets all entries to the original values.

14.3.1.2.8.1 Axiocam 506

Camera Identifier

A clear identifier for the currently active camera is displayed here. It consists of the product name and part of the serial number. This is helpful if you want to identify the camera in question, e.g. when working with a dual camera system.

Orientation dropdown list

Here you can select the orientation of the camera image. This allows you to adjust the camera image to the properties of the different camera ports. The following selection options are available:

- Original
- Flip Horizontally
- Flip Vertically
- Rotate 90 CW
- Rotate 90 CCW
- Rotate 180
- Mirror at +45 Diagonal
- Mirror at -45 Diagonal

Acquire section

Readout Speed (MHz)

Readout speed can be varied between 39 MHz and 13 MHz if the camera is operated on the USB3.0 bus. In case the camera is connected to the slower USB2.0 Bus only the 13 MHz mode is available. At the slower 13 MHz the signal quality is slightly improved due to a reduced noise of the signal transmission.

Cooling

Status information, if camera cooling is active. The Axiocam 506 can be operated without active cooling. Cooling is deactivated if the USB2.0 connector of the camera is not supported to the PC or to a USB compatible power supply.

Readout Port

The Axiocam 506 uses a high performance CCD sensor with four readout ports. It can be adjusted to quadport, dualport, singleport and Auto mode. Maximum speed is reached by using all four ports and short exposure times. When exposure time gets larger than the readout time the benefit for using multiple ports is getting insignificant. By switching the readout mode to single port, the most homogenous

signal quality can be reached as all data is sent through one single processing chain. In Auto mode, the number of used readout ports is selected automatically depending on the exposure time.

Readout time (ms)

The valid camera readout time is given in this status window which is defined by the number of used ports or by defining a sensor sub region window (ROI).

Temperature

The valid CCD sensor temperature is shown here. It is adjusted to 18 C°. It can not be changed. If a black reference is used it should be used at the same sensor temperature when it was created.
If free air circulation for the camera housing is blocked, it may happen that the sensor temperature is increased and the dark current of the sensor may be higher than normal. If the camera is operated without cooling (USB2.0 port of camera not connected) the sensor temperature is increased and dark current will be higher than normal. This should be considered when using the camera at longer exposure times.

Expert section

Abort on missed frames

In case of high speed time lapse acquisition the camera is sending an enormous amount of data to the PC. If the PC is not fast enough in handling the image data it may happen that dropped frames occur. By this parameter the behavior of the acquisition routine can be defined: it is the default mode to abort a acquisition sequence in case of a dropped frame. If unchecking this the event of a dropped frame is ignored and the acquisition is continued without further notification.

Main LED

The LED in the camera lid shows the general operation mode of the camera by showing different colors. In special low light applications it may be desirable to minimize stray light in the microscopy workspace. Therefore the intensitiy of the status LED can be dimmed or switched off completely. The following status informations are available:

Red: after plugging the main USB3.0 cable of the camera to a PC, camera firmware is not yet loaded by driver. As soon as the camera gets loaded with firmware, the color changes one of the following colors:

Yellow: connected to USB2.0 bus, only 13 Mhz clock speed available, no cooling (right USB port connected to USB2.0, left USB port not connected),

Green: connected to USB 2.0 bus, only 13MHz available, cooling active (right USB port connected to USB 2.0 bus, left USB port connected to computer USB port, or separate USB power supply),

Pink: connected to USB3.0 bus, full 39 MHz clock speed available, no cooling (right USB-port connected to USB3.0, left USB-port not connected)

Blue: connected to USB3.0 bus, full 39 MHz clock speed available, cooling active (right USB-port connected to USB3.0, left USB port connected to computer USB port, or separate USB power supply)

Red: wile exposure of sensro is active, in Live mode or acquisition of image series the LED is blinking, therefore.

Trigger LED

A second LED is at the camera back, above the Micro-d connector for the trigger cable. This LED indicated the status of the trigger port. It is only active, in case the trigger port is used.

In special low light applications it may be desirable to minimize stray light in the microscopy workspace. Therefore the intensitiy of the status LED can be dimmed or switched off completely. The following status informations are available:

Green: asynchronous triggering is possible (no jitter in line timing),

Yellow: synchronous triggering is possible (some jitter in line timing),

Red: no trigger accepted,

Blue: external trigger signal active,

Tile Adjustment

Camera expert section shows camera parameters which need deeper understanding of functionality.

The Axiocam 506 camera is using a special CCD sensor with four readout ports in order to read out the image data from the CCD area as fast as possible. It is assured by proper alignment of the electronics that all associated signal paths are absolutely equal and no quadrants get visible as this is very undesirable for good image quality. In order to offer the best quality possible, a special algorithm is activated by default as an additional safety measure to suppress remaining residuals. This correction can be deactivated in order so minimize image processing on the camera raw data.

Acquire (Expert) section

Camera **Expert** section shows camera parameters which need deeper understanding of functionality.

8 bits compression

In case other devices are using bandwidth on the connected USB 3.0 data bus, it is possible to reduce the amount of image data sent by the Axiocam 506 by activating data compression from 14 bit to 8 bit. By converting the data through a square root loaded lookup table the 14 bit values are converted to 8 bits per pixel. This is handled transparently as the data is decompressed automatically by the camera driver in the PC while receiving it. As compression has some slight impact on data quality it is deactivated as default.

Frame time (ms)

In case of fast time lapse acquisition the camera is sending huge amount of image data to the PC within a short time. The data needs to be handled by the PC without delay. There is a certain risk of dropped frames, if the camera is sending data faster, than the PC can handle it, especially at very short exposure times. By setting a frame time larger than zero, an acquisition delay can be defined for continuous acquisitions. Max. value is 5000 ms. Default value is zero.

Readout mode

This is a status information and shows the currently used number of used sensor ports. Values are: quad port, dual port, single port.

Adjust Live Frame Rate

The camera live image can send a lot of image data which need to be processed on the fly. In case of less powerful computers this can overload the PC and cause a slow reaction of the ZEN user interface. By activating this function the slider below gets accessible and a maximum limit for the live frame rate can be adjusted. Abundant image data is then discarded accordingly and not processed to be displayed.

Live Frame Rate Max

Adjustment of maximum accessible frame rate for live display, only. Only accessible if "Adjust Live Frame Rate" checkbox is activated.

Live Frame Rate

This is a display field only. It shows the measured live image speed.

14.3.1.3 Software Autofocus Tool

Parameter	Description
Quality	
- Basic	Selects the simple and fast algorithm to calculate the software autofocus.
- Best	Selects a more complex, optimized algorithm to calculate the software autofocus.
Range Coverage	
- Smart	Sets a part of the travel range as the region for determining the autofocus.
- Full	Sets the full travel range as the region for determining the autofocus.
Sampling	Here you can select the step size of how the search range is sampled.

Parameter	Description
- Default	Uses the default step size (dz = 1/sqrt(2) * 2 * n* lambda/NA).
- Fine	Uses a small Z-distance (0.5 * dz) between the individual focus images that are used to calculate the best focus position.
- Coarse	Uses a medium Z-distance (2 * dz) between the individual focus images that are used to calculate the best focus position.
- Very Coarse	Uses a large Z-distance (4 * dz) between the individual focus images that are used to calculate the best focus position.
Autofocus ROI	
- Spot Meter / Focus ROI checkbox	**Activated:** Only uses the values from the Focus-ROI (Spot Meter) to calculate the focus position. The Focus-ROI can be displayed and adapted in the live mode by right-clicking on the live image and activating the checkbox.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Sharpness Measure	
- Contrast	Calculates the best focus position from the contrast of the focus images.
- Intensity	Calculates the best focus position from the intensity of the focus images.
- Auto	Automatically uses the best method (contrast or intensity) to calculate the focus position depending on the hardware settings.
Autofocus Search Range	
- **Relative Range**	The software autofocus is calculated over a relative range.
Automatic Range checkbox	**Activated:** Calculates the range for the autofocus search automatically depending on the objective set.

	Parameter	Description
	Range input field	Only active if the **Automatic Range** checkbox is not activated. Enter a range here that you want to be used for the autofocus search.
	Step Size display field	Shows the distance between the individual focus images set under **Range**.
-	**Fixed Range**	The software autofocus is calculated over a fixed range.
	Set Last button	Defines the current Z-position as the end (last) point for the software autofocus. Alternatively, you can enter the desired value in the spin box/input field to the left of the button.
	Set First button	Defines the current Z-position as the start (first) point for the software autofocus. Alternatively, you can enter the desired value in the spin box/input field to the left of the button.

14.3.1.4 Movie Recorder Tool

In the **Movie Recorder** tool you can acquire image sequences in the form of videos using the camera's fastest burst mode.

i Note

To play the acquired Movie, use the **Player** tab in the Center Screen Area (Only visible in **show all** mode).

Start Movie button

Starts acquisition. The button changes into the **Pause** button.The animated **Stop** button appears in the window above the button.

Pause Movie button

Pauses acquisition. The button changes into the **Continue** button.

Continue Movie button

Continues acquisition if it has been paused. The button changes into the **Pause** button.

Stop button

Stops acquisition. Save the acquired movies either in the internal CZI format or as a series of individual images via **File** menu | **Export** or as an AVI file via **File** | **Export Film**.

14.3.1.5 Manual Extended Depth of Focus Tool

Mode section

Mode	Function
Timer	Acquires an EDF (Extended Depth of Focus) image automatically after the interval you have set.
F12 Key	Acquires an EDF image when you press F12 key.
Z-Stack	Acquires an EDF image out of a Z-Stack image. If this mode is selected the **Z-Stack** button appears next to the **Start** button. Click on the **Z-Stack** button to acquire the EDF image.

Interval slider

Set interval (in sec.) here, after which the automatic acquisition begins.

Start button

Starts the acquisition of an image serie. Press **Pause** button to pause acquisition. Press **Continue** button, to continue acquisition. Press **Stop** button to stop acquisition. The image with extended focus will be calculated from all single images.

14.3.2 Tools on Acquisition Tab

14.3.2.1 Imaging Setup Tool

Here you can view and adjust the hardware parameters used for confocal (LSM) or camera (WF) experiments. All hardware parameters set to detect one or more specific signals simultaneously are defined as a track.

i Note

Note that the **Microscope Control** tool on the **Locate** tab has a similar appearance and similar control elements but its function differs from the **Imaging Setup** tool described here.

If there is no track available, you will be asked to add a LMS or a WF track to the experiment by clicking on the corresponding button (**+LSM** / **+WF**).

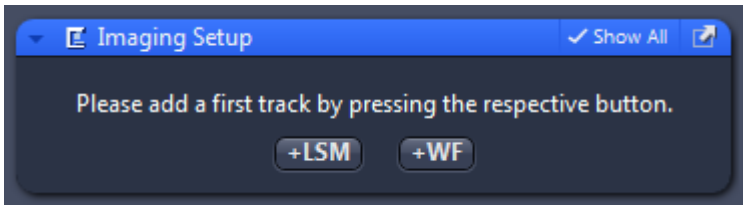


Fig. 51: Imaging Setup without tracks

If there are tracks available you will see buttons with the track name (**Track1**, **Track2**, etc.) on the top of the tool. The active track is always highlighted in blue color. You can switch between the tracks by clicking on the corresponding track button. If you click on the **+LSM** or **+WF** buttons you can add further LSM or Widefield tracks. Using the buttons below the "Add" buttons you can delete tracks or access further **Options** for editing (Add, Delete, Duplicate, Rename).

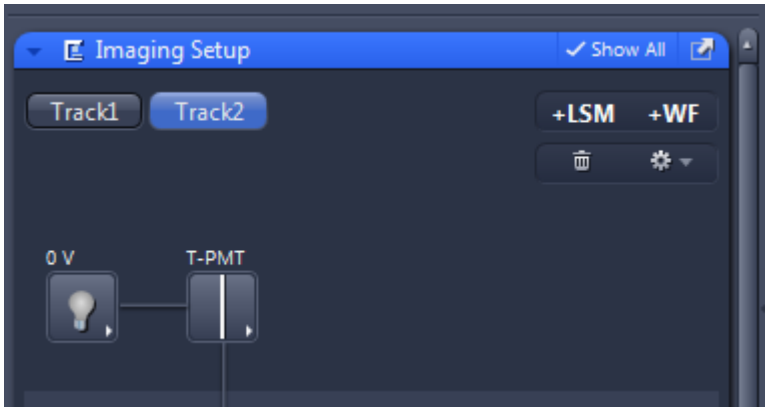


Fig. 52: Imaging Setup with tracks

Depending on what kind of track you have selected (WF or LSM) the tool has a different appearance. For a detailed description of the specific parameters read the chapters *Imaging Setup (WF)* [▶ 379] and/or *Imaging Setup (LSM)* [▶ 380] depending on your information needs.

14.3.2.1.1 Imaging Setup (WF)

Having selected an WF track you can see the graphical display of the acquisition light path with various icons. The arrangement of the icons represents the typical set-up of the microscope components configured on your system. For a description of the most common icons, read *Reflected/Transmitted Light Path* [▶ 356].

The associated hardware settings are shown above the icons and can be changed here. To change the relevant hardware settings, left-click on the icons. In the shortcut menus you will see numerous selection and setting options for adjusting your settings.

i Note

Any change you make is automatically adopted and written to the corresponding hardware setting of the experiment. If you want to undo these changes, do not save the experiment. Instead, reload the experiment in the **Experiment Manager**.

If you change the hardware settings in this section, please bear the following points in mind:

- If the checkbox **Include in this setting** is activated the component is activated. Activated components are included into the hardware settings of the experiment and subsequently applied in the experiment. Activated components are highlighted in blue color.
- Components with a deactivated checkbox are not adopted into the hardware settings of the experiment and are not subsequently applied in the experiment. These components are displayed with a grayed-out icon.
- Components with a filled-in checkbox and a triangle underneath are only partially adopted into the hardware settings of the experiment and subsequently applied in the experiment. To show the sub-components, click on the triangle under the checkbox. To adopt the sub-components into the hardware settings of the experiment and subsequently apply them in the experiment, activate the relevant checkboxes for the sub-components.

14.3.2.1.2 Imaging Setup (LSM)

If setting up an experiment for a LSM application, you first have to choose which mode you want to use. The configuration and parameters for the different modes are described separately.

i Note

If you have already created a LSM track, clicking the **+LSM** button again creates a new track with the same LSM mode (Channel, Airyscan or Lambda) as the selected track.

Parameter	Description
LSM Mode	Here you can select the desired LSM imaging mode:
- Channel	Activates the **Channel** mode, for a detailed description read the chapter *Channel Mode* [▶ 380].
- Airyscan	Activates the **Airyscan** mode, for a detailed description read the chapter *Airyscan Mode* [▶ 388].
- Lambda	Activates the **Lambda** mode, for a detailed description read the chapter *Lambda Mode* [▶ 386].

14.3.2.1.2.1 Channel Mode

In this mode the current hardware settings within the scan head are displayed. This mode uses for each channel one discrete detector. Please note the following:

- On a two channel system, both detectors are either multialali PMTs or GaAsP PMT detectors.
- On a three channel system, Channel 1 and 3 are either multiakali PMT or GaAsP PMT detectors. Channel 2 can be a multiakali or GaAsP PMT or an Airyscan.

Parameter	Description
Switch track every	Here you can adjust how the available tracks are switched:
- Line	Switches the track every line. This setting is used for fast acquisitions. It is especially useful for applications where a high temporal correlation of the different fluorescence signals is required.

Parameter	Description
- Frame	Switches the track every frame. It also allows to change emission filters, VSD positions and detector gain between the tracks.
- Frame Fast	Switches the track every frame (fast). It is not possible to move hardware and to set individual PMT gain for the different tracks.
- Z-Stack	A full Z-Stack is acquired per track. Using this setting the acquisition is faster because the hardware is only moved once per stack. This setting is ideal for fixed specimen.

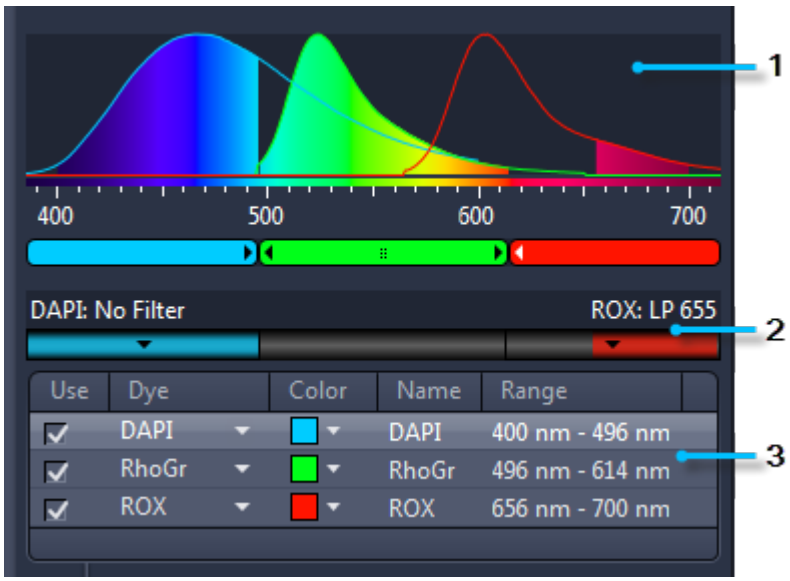
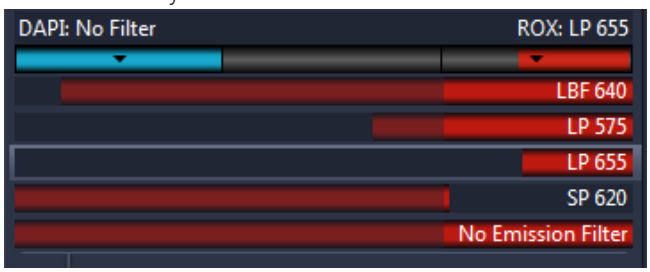
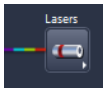
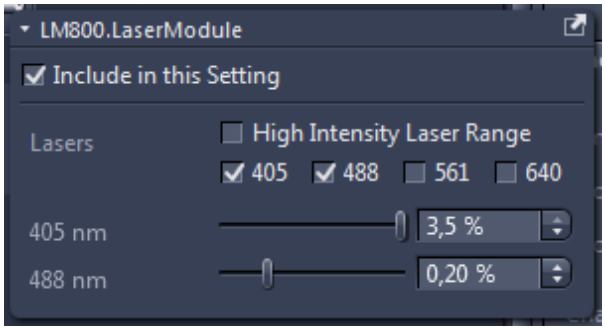


Fig. 53: Imaging Setup Channel Mode

Parameter		Description
Graphical Display (1)		Shows the spectral range of the activated channels in a graphical display. All channels cover a spectral range from 400 to 700 nm. Below the graphical display, a slider is displayed for each activated channel. The slider has the same color that was chosen for the channel. Channel 1 to Channel 3 are always aligned from left to right. These sliders represent the emission range that is covered with the respective channel. When gripping them with the left mouse button, you can change the range and position to the imaging needs. If you point the mouse on a slider, the curser changes to a pen icon. If you click on the slider now a sub-menu is opened. Here you can type in the split positions between channels. The flexible direction of light is done with two Variable Secondary Dichroic Beam Splitters (VSD), positioned between the three Channels (one VSD in a two Channel system prespectively).
-	Two channel system	For a two channel system the sliders are fixed at the left and right side respectively and the middle sides can be moved but cannot be separated. So no gap in between the channels is possible.
-	Three channel system	For a three channel system, the behaviors is similar. The range of the middle channel (Channel 2) is freely selectable between 450 and 650 nm. When you need to image the Range below 450 or above 650 nm with Channel 2, you may not use it in the same track Channel 1 or Channel 3 respectively. To be able to select this range, you have to disable these detectors first.. If adjacent channels are activated, there is never a gap in between them but their spectral range join at their borders.

Parameter	Description
Emission Filters (2)	Shows if filters are used for the relevant dye. If you click on the filter bar with the small arrow button you can select a emission filter for the selected dye. Note that there is no emission filter required for Channel 2 on a three channel system. 
Detectors List (3)	Shows all configured detectors in a list. There you can activate the detectors for the experiment and adjust the channels display.
- Use	If activated, the corresponding detector will be used for the experiment.
- Dye	If you click on the small arrow button you can select a dye from the dye database. The emission spectrum of the selected dye is displayed in the channel display if the **Use** checkbox is activated.
- Color	If you click on the rectangle, you can select a pseudo color for the dye. There are different color schemes available (**Color**, **LUT**, **Custom** or **None**).
- Name	Shows the name of the selected detector channel. If you add a new dye, its name will be adopted to this field. You can change the name of the dye in the channels list of the**Channels** tool.
- Range	Shows the emission range for the selected detector. This range is affected by the VSD position and the emission filter. You can adjust the range by using the corresponding slider under the channel display and by using the emission filters.

Below the dyes list you will find a small graphical representation of the beam path including the configured hardware components. All components can be adjusted and/or controlled via the corresponding icons here. The following list contains a description of the most common icons:

Icon	Name	Description
	**MBS** icon	MBS stands for Main Dichroic Beam Splitter (fixed), that is suitable for all laserlines of the system. No adjustments can be made here. The standard MBS is blocking the excitation light of the 405, 488 and 561nm laser lines completely and the 640nm line by 90%. Thereby, the 640nm line can be used for reflection measurements, including reflection autofocus.
	**Lasers** icon	If you click on this icon you will see the available laser lines of the system.  Activate a laser by clicking on the corresponding icon. Adjust the lasers power with the corresponding slider or enter a value in the input field behind the slider. The laser power can be attenuated in two distinct regimes. In the default setting, the power can be adjusted from **0.01%** to a maximum of **3.5**, **4.5** or **5%**, depending on the laser line. For higher powers, activate the **High Intensity Laser Range** checkbox. All laser lines are now tunable from 0.2 % to 100% power. In multi-track experiments, this setting is the same for all tracks.
	**Stage** icon	Graphical representation of the stage / sample area. No adjustments can be made here.

Icon	Name	Description
	**ESID Detecto r** icon	If you activate the checkboy next to the icon, the detector will be activated and can be configured in the **Channels** tool. Graphical representation of the ESID detector (Transmission Channel). It allows to acquire an image with transmission illumination using any laser for excitation. The image will display the differential interference contrast (DIC) of the specimen if the according optical hardware for this contrast is put into the beam path in front of the detector. Check the manual of the microscope for setting DIC optics. Be aware that the laser light is already polarized and therefore a polarizer device is not necessary for transmission imaging. This imaging mode allows displaying a DIC image simultaneously to the fluorescence signal of the specimen.

14.3.2.1.2.2 Lambda Mode

The Lambda mode is used for imaging heavily overlapping emission signals. If you have activated this mode, the display changes to show the currently active laser line(s) and the detection range for spectral imaging.

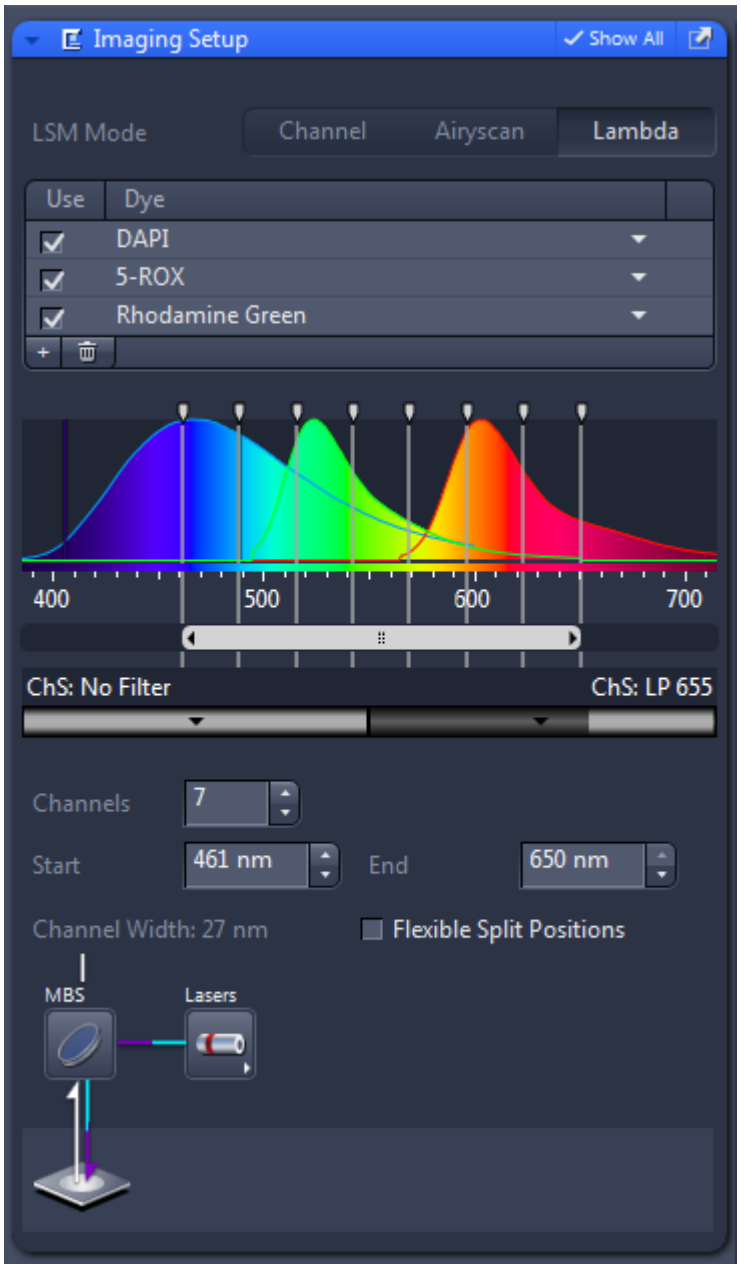


Fig. 54: Imaging Setup Lambda Mode

In this mode the system is recording the overall emission from the sample by moving the VSD (Variable Secondary Dichroic) sequentially to different positions over the selected spectral range. During this movement in steps the lower part and the higher part of the spectrum is detected by PMT 1 and PMT 3, respectively. In a

two channel system PMT 1 and PMT 2 are used. These accumulated signals represent a spectral signature of the dye(s) which can be used for spectral linear unmixing.

With this imaging mode, it is possible to acquire an intensity image which displays the intensity of the fluorochrome(s) with down to 10 nm steps in a spectral range from 450 nm to 650 nm. According to the detection range and the chosen number of steps, a certain number of such images is acquired sequentially, which is called a Lambda stack.

In between the spectral range the number of steps the VSD performs to record the spectral information can be set by typing in a number into the box next to **Channels**. The number displays the actual resulting channels, but the VSD will take one step more and the system will perform one scan more as well.

The image data provide the intensity information within the selected detection range for each pixel. Therefore, the data allow deducing an emission spectrum for each pixel corresponding to the emission spectrum of a specific dye.

This calculation is done by linear unmixing using the acquired Lambda stack and allow to clearly separate even heavily overlapping emission signals.

The images acquired in Lambda mode are displayed in the Lambda View, see *Lambda View* [▶ 590].

Parameter	Description
Channels	Here you can set the number of resulting channels (max. 20). If a new channel is added, you can see it in the graphical display above. Per default the step width will be equally distributed.
Start / End	Sets the starting /ending position of the spectral range. You can also use the slider below the graphical display to adjust the positions.
Channel Width	Displays the current step width (in nm) of a channel.
Flexible Split Positions	If activated, non linear step widths can be adjusted. To adjust the position select a marker in the graphical display. The current value is displayed in the **Current Split** input field. Now you can move the marker by holding the left mouse button down. You can also enter a new value in the Current Split input field.

As the handling of dyes, emission filters and the controls for the beam path are the same like in **Channel Mode** please read the relevant descriptions there.
NOTICE **Using emission filters in Lambda stacks can lead to false results and is considered as an expert setting.**

14.3.2.1.2.3 Airyscan Mode

This mode is only available if Channel 2 is an Airyscan module.

As a result of that most of the parameters and controls of the tool are already described in the chapters *Channel Mode* [▶ 380] and *Lambda Mode* [▶ 386], here`s the description for the Airyscan mode in a nutshell:

- In the graphical display of the spectral range only one channel (Channel 2) remains for editing.
- The detection width and position can be chosen within the range of 400 nm - 700 nm.
- For multi-color Airyscan images, multi-track acquisition must be used.
- Line-wise multi-track acquisition using Airyscan is possible.

14.3.2.1.3 Advanced Imaging Setup

The Advanced Imaging Setup must be activated under **Tools** | **Options** | **Acquisition** | **Acquisition Tab** | **Enable Advanced Imaging Setup**.

If you have activated the checkbox, you will see a switch on top of the tool. By clicking on the button you can switch from the Standard to the Advanced Imaging Setup.

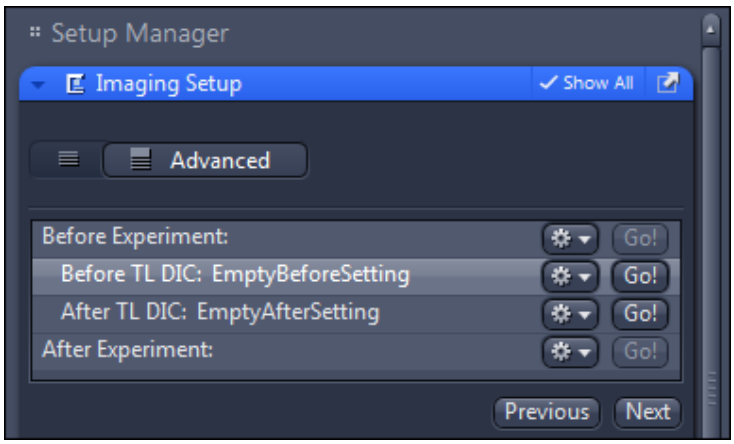


Fig. 55: Advanced Imaging Setup

The Advanced Imaging Setup offers additional options for controlling an experiment. The **Before/After Experiment settings** can be found right under the Standard/Advanced button.

Additionally the **Experiment Settings Pool** can be found at the bottom of the tool. We recommend that you talk to ZEISS Service staff or Imaging Specialists before you try to change settings here, as they should be changed only if you know what you are doing.

Before/After Experiment settings

Parameter	Description
Before / After Experiment entries	Shows the name of the hardware setting that will be applied immediately before or after the experiment.
Options buttons 	Opens the **Options** menu for the specific hardware setting.
Go! buttons	Applies the selected hardware settings.
Previous/Next buttons	The buttons allow you to navigate through the various hardware settings.

Experiment Settings Pool

Parameter	Description
Clear all unused hardware settings from experiment	By clicking on the **Clear** button you can delete all unused hardware settings from your experiment.
All available hardware settings in experiment	All available hardware settings will be shown in the list below.

14.3.2.2 Acquisition Mode Tool

In the **Acquisition Mode** tool you can set the various acquisition parameters that you want to apply for the entire experiment.

> **Note**
>
> If you have created an experiment using the **Experiment Designer** tool, the settings in the **Acquisition Mode** tool only apply to the relevant experiment block and may differ in the next block.

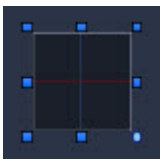
In terms of content and appearance, the Acquisition mode tool is largely dependent on which imaging mode was chosen in the **Imaging Setup tool**, either LSM tracks or widefield channels.

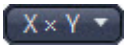
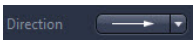
If you have configured LSM tracks, please read the chapter *Parameters for LSM Mode* [▶ 390].

If you have configured widefield channels, please read the chapter *Parameters for Widefield Mode* [▶ 395].

14.3.2.2.1 Parameters for LSM Mode

Here you adjust scanning and acquisition parameters that you want to apply for the entire experiment.

Parameter	Description
Objective	Select the desired objective for acquisition here. ■ **Include in Setting** checkbox Only visible if **Show All** is activated. If activated, the objective information is stored within the experiment. This option is required to change objectives within the Experiment Designer module. For safety reasons, the selection to integrate the objective has to be repeated every time the objective is changed.
Scan Mode	
- Frame	Activates the frame scan mode. If this mode is selected you will see the representation of the scanning frame in the **Scan Area** section at the bottom of the tool. 

Parameter	Description
- Line	Activates the line scan mode. If this mode is selected you will see the representation of the scanning line in the **Scan Area** section at the bottom of the tool. 
Frame Size	Adjust the frame size (in pixel) of the displayed image by entering the desired value in the two input fields.
- X x Y button 	By clicking on this button you can select from a list of default frame sizes (e.g. 128 x 128 or 512 x 512). We recommend to start with 512 x 512 px.
- Optimal button	By clicking on this button the frame size (image resolution) will be set to an optimal value corresponding to the optical magnification (objective), the zoom factor and the emission range detected. This provides an image where no information is lost and no empty data are generated as optimal sampling is achieved. The optimal value is calculated for the given objective and magnification settings matching a 2fold oversampling according to 2 fold Nyquist. Rectangular image dimensions are preserved.
- Bits per Pixel	In the dropdown list you can adjust the color bit depth to 8 Bit or 16 Bit (i.e. 256 or 65536 gray values).
Direction	Following scanning directions can be selected:
- Unidirectional 	The laser scans in one direction only, then moves back with beam blanked and scans the next line.
- Bi-directional 	The laser also scans when moving backwards, i.e. the scan time is halved. Please note that the pixel shift between forward and backward movement (double image) resulting from bi-directional scanning must be corrected. To do that use the **Correction X** / **Correction Y** sliders. By clicking on the **Auto** button an automatic scan correction will be performed. For optimal results this correction should be repeated every time scan parameters like rotation, zoom or speed are changed.

Parameter	Description
Line Step	Only available if the **Frame** Scan Mode is selected. Select the desired line step size (from 1-10). According to your selection only every n-th line is scanned. The lines in between are interpolated. This fast scan mode is called Step Scan. This feature is not available for Airyscan acquisition.
Scan Speed	Set the scan speed by adjusting the slider from 1 (slow) to 16 (very fast). The corresponding values for **Pixel Dwell** and **Scan Time** will be displayed below the slider. Please note that the available maximum scan speed depends on the selected **Frame Size** and zoom factor. Maximum Speed is available with a zoom factor of 6.5x. By clicking on the **Max** button the maximal possible scan speed will be set automatically.
Averaging	
- Number	Select the number of images you want to average (1 - 16).
- Method	Select the method which will be used for averaging: ■ **Mean**: Uses the mean average of all images ■ **Sum**: Uses the sum of all images.
- Mode	Select the mode for averaging : ■ **Frame**: The calculation of the average is based on individual full frames. Each frame is scanned quickly, but there is a higher delay between the individual data sets taken for the averaging. ■ **Line**: The calculation of the average is based upon individual lines, which are acquired sequentially before moving on. By this, the data used for the averaging are is closely connected, but the acquisition of the full frame takes respectively longer.

Parameter	Description
HDR Illumination	This parameter is only available if you have licensed the **HDR Blue Confocal Basic** module. If activated, a HDR effect will be applied to the image. This effect will boost weak structures without saturating bright areas in the image and enable an optimal representation of the morphology of weak and bright objects within the same image. To achieve this, the image will be scanned three times with increasing the excitation intensity. Areas in the image, that displayed overexposure will be excluded in the following scans in order to avoid photobleaching. It is recommended to use 16bit for the acquisition of HDR datasets.
Scan Area	In this section, you can adjust the position of the scan area. The outer frame corresponds to the field of view of the microscope. The inner frame represents the scan area. All changes (Offset, Rotation, Zoom) made in this section will be immediately applied to the scan area. Following functions are available:
- Offset	Adjust the offset by using the **Left** / **Right** or **Up** / **Down** sliders. You can also enter a specific value in the input field. If clicking on the **C** button behind the input filed the offset position will be reset to center position. If you left click on the inner frame and hold down the mouse button you can move the scan area freely. The positions in the input fields will be adopted according to your adjustments.
- Rotation	Adjust the rotation degree by using the **Rotation** slider. You can also enter a specific value in the input field. If clicking on the **O** button behind the input field the rotation degree will be reset to default position (zero degree).

Parameter	Description
- Zoom	Adjust the zoom level (from **0.5x** - **40x**) by using the **Zoom** slider. You can also enter a specific value in the input field. If clicking on the **1** button behind the input field the zoom level will be reset to default (1,0x) for confocal acquisition and 1.3x if an Airyscan track has been configured..
- Reset Scan Area	Resets all adjustment to the system defaults.

14.3.2.2.2 Parameters for Widefield Mode

14.3.2.2.2.1 Camera section

In this section you can adopt camera settings from the active camera to your experiment and adjust basic camera settings.

Parameter	Description
Get Settings from Active Camera	
- Get	By clicking on this button you can apply the settings from the active camera to your experiment.
- Default	By clicking on this button you can reset the camera settings to factory default.

Parameter	Description
Binning	Binning combines the information of neighboring camera pixels into a single larger pixel. The camera sensitivity is increased by improving the signal-to-noise ratio, but the resolution is decreased by the same factor. For example, if the binning is set to 2 × 2, four pixels are combined to one. The camera sensitivity is increased by a factor of four but the resolution is reduced by a factor of four. Increasing the binning means weaker signals can be detected for a given exposure time.
Resolution	Sets the cameras resolution, e.g. 1024 x 1024 px

14.3.2.2.2.2 Acquisition ROI section

In this section you can define a Region Of Interest (ROI) on the camera sensor which will be used for acquisition. A smaller ROI can increase the acquisition speed.

The region of interest is indicated by a blue frame in the preview window and can be moved and resized freely. The preview window always shows the entire camera sensor area which can be acquired.

The **Pixel Size** shown below the preview window indicates the size in µm to which a pixel corresponds. This depends on the camera sensor properties and on the binning.

Parameter	Description
Maximize	Selects the entire available image sensor area as the region of interest
Center	Positions the region of interest precisely at the center of the image
Size	Sets the width and height of the region of interest in pixels
Offset	Specifies the position of the top left corner of the **Acquisition ROI** (blue frame) with respect to the top left corner of the preview window.
Refresh Overview	An image is acquired and displayed in the preview window with the current ROI settings. This has no effect on the image in the **Center Screen Area**.
Crop button	Only visible if the checkbox **Show Crop Button** under **Tools** | **Options** | **Acquisition** | **Camera/Live** is activated. By clicking on the this button, you can specify a ROI (Region of Interest) in a snapped image. If no image is available in the Center Screen Area the button is not active. The "cropped" area is used as ROI for the next image acquisition.

14.3.2.2.2.3 Fast Acquisition section

In this section you can set 3 different modes for acquisition:

Parameter	Description
Interactive mode	Using this mode you can intervene manually at certain points during acquisition. The acquisition is comparatively slow.
Compromise mode	This mode is activated automatically if only individual hardware components, but not the whole system, are compatible with the Triggered mode for acquiring an experiment.
Triggered mode	Fast acquisition via the hardware.
Validate button	To establish whether the system is able to perform an experiment in Triggered mode, click on the **Validate** button. The validation result is displayed in the info box below the button bar.

14.3.2.2.2.4 Post Processing section

In this section you can apply basic image processing functions while acquiring the image. This can be helpful if certain image processing steps are necessary for any acquired image and saves image processing work later in a job. Depending on the camera model, different parameters can be available.

Parameter	Description
Black Reference	Influences the live image and each image acquired. For the black reference to work, you first need to acquire a reference image. Define a corresponding reference image using the **Define** button. Activated: Applies the measured black reference to the image. Deactivated: The measured black reference is not used. The reference image is retained. **Define** button: Automatically defines the black reference. The measurement lasts for several seconds. The Black Reference checkbox is then activated automatically.
Enable Noise Filter	Activated: Noise in the acquired image is filtered according to the adjusted threshold. Affects acquired images only; the live image does not change.
– Threshold	The noise filter reduces the extent to which individual pixels deviate from the average value of their nearest neighbors. The **Threshold** corresponds to a tolerance value. If the deviation of the middle pixel value from the average value of the pixels immediately surrounding it exceeds the tolerance value (i.e. it is interpreted as noise), it is replaced by the average value. The higher the value, the greater the tolerance for noise. The lower the value, the stronger the noise reduction. This technique reduces the noise of individual pixels that are produced, in particular with EMCCD cameras and CMOS cameras. The selected technique prevents any changes being made to object edges, as in most cases these are larger than individual pixels. This filter is also suitable for removing individual "hot pixels" from an image without having to acquire a reference image in advance.
Enable Unsharp Mask	Enhances contrasts at fine structures and edges. Thus, the resulting image appears clearer and enriched in detail.

Parameter	Description
– Strength	Controls the amount of contrast enhancement applied to fine structures and edges. The higher the strength, the darker or lighter the resulting edges, compared to the original image.
– Radius	Determines the size of detail to be enhanced. A small radius enhances smaller details. The radius also affects the appearance of enhanced edges. A large radius leads to a visible halo along enhanced edges. The larger the radius, the broader the halo.
– Color Mode	Determines the calculation method, which affects the appearance of the output image. ■ **RGB**: – The Unsharp Mask filter calculates the sharpness for each color channel individually. – The color saturation and the color of structures may be changed and color noise may occur. ■ **Luminance**: – The Unsharp Mask filter calculates the sharpness based on the luminance signal computed from the RGB channels. – This mode avoids possible color noise or shift in color saturation, which could be induced by certain image textures.
– Auto Contrast	Activated: You can adjust the **Contrast Tolerance** (0-20). Auto Contrast only works in RGB color mode.
– Contrast Tolerance	Increasing the contrast during unsharp masking is achieved by broadening the distribution of intensities. This corresponds to a spread of the image histogram. **Contrast Tolerance** controls how much the intensity distribution is spread and thus how strong the contrast is increased. ■ **Contrast Tolerance** = 0: No spread of intensities, no increase of contrast ■ **Contrast Tolerance** = 20: Maximum spread of intensities, maximum increase of contrast

Parameter	Description
– Clip To Valid Bits	■ Activated: The processed image is composed of the same colors as the original image (i.e. the value range of the output image is adjusted to the color range of the input image). ■ Deactivated: Colors not present in the original image may appear in the processed image.

14.3.2.2.2.5 Mode Section

In this section you can adjust how the software retrieves the camera sensor data.

Parameter	Description
Color Mode	This parameter is available for color cameras only.
– RGB	The image data of a color camera is transmitted unchanged. This corresponds to the standard operating mode of a color camera.
– B/W	The image data of the color channels are treated as grayscale. The data of related color channels are averaged. The saturation of the camera appears reduced as a result. This process does not change the spectral properties of a color camera. The image information of the color sensor still undergoes color interpolation. An infrared filter also restricts the spectral sensitivity of the color camera compared to the spectral sensitivity of a genuine black and white camera.
Live Speed	Specifies the live image update speed. Enables you to focus or to find regions of interest on a sample quickly. A high live image update speed reduces the exposure time of the live image, even at longer exposure times used for image acquisition. To achieve a similar impression of image brightness, however, the image data supplied must be adjusted digitally, which may generate a certain amount of noise or reduce the resolution of the live image.

Parameter	Description
IP Quality	Only available for **Axiocam 503** color and **Axiocam 506** color. Here you can select the color interpolation quality (IP Quality) for the recorded image. Please notice that this function does not apply to Live mode. **Fast**: Represents the image optimized and requires a shorter computation time. **High**: Represents the image without artifacts and with a higher image quality. This mode is only effective with binining factor 1. If you reset the settings via the **Default** button the IP Quality is set to high.
NIR Mode	This function is only available for cameras which offer this feature (e.g. **AxioCam MRm**). **Activated:** Uses the camera in the near infrared range (NIR=Near-Infrared). Sensitivity for signals in the near infrared range (approx. 700nm to 1000nm) is increased. However, the tolerance for overexposure is reduced and, if very bright structures are present, overexposure artifacts (blooming) can result. We recommend that you use this mode mainly for very weak signals.

14.3.2.2.2.6 Model Specific Section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button  .

In this section you see additional, model-specific camera settings depending on which camera you use on your system.

Reset button

Resets all entries to the original values.

14.3.2.2.2.6.1 Axiocam 105

Camera Identifier

A clear identifier for the currently active camera is displayed here. It consists of the product name and part of the serial number. This is helpful if you want to identify the camera in question, e.g. when working with a dual camera system.

Orientation dropdown list

Here you can select the orientation of the camera image. This allows you to adjust the camera image to the properties of the different camera ports. The following selection options are available:

- Original
- Flip Horizontally
- Flip Vertically
- Rotate 180

Acquire section

Gain Boost checkbox
If activated, the image signal is amplified so that the image becomes brighter. The gain factor is 1.7x. This factor is in addition to the standard Gain control.

14.3.2.2.2.6.2 Axiocam 506

Camera Identifier

A clear identifier for the currently active camera is displayed here. It consists of the product name and part of the serial number. This is helpful if you want to identify the camera in question, e.g. when working with a dual camera system.

Orientation dropdown list

Here you can select the orientation of the camera image. This allows you to adjust the camera image to the properties of the different camera ports. The following selection options are available:

- Original
- Flip Horizontally
- Flip Vertically
- Rotate 90 CW
- Rotate 90 CCW
- Rotate 180
- Mirror at +45 Diagonal
- Mirror at -45 Diagonal

Acquire section

Readout Speed (MHz)

Readout speed can be varied between 39 MHz and 13 MHz if the camera is operated on the USB3.0 bus. In case the camera is connected to the slower USB2.0 Bus only the 13 MHz mode is available. At the slower 13 MHz the signal quality is slightly improved due to a reduced noise of the signal transmission.

Cooling

Status information, if camera cooling is active. The Axiocam 506 can be operated without active cooling. Cooling is deactivated if the USB2.0 connector of the camera is not supported to the PC or to a USB compatible power supply.

Readout Port

The Axiocam 506 uses a high performance CCD sensor with four readout ports. It can be adjusted to quadport, dualport, singleport and Auto mode. Maximum speed is reached by using all four ports and short exposure times. When exposure time gets larger than the readout time the benefit for using multiple ports is getting insignificant. By switching the readout mode to single port, the most homogenous signal quality can be reached as all data is sent through one single processing chain. In Auto mode, the number of used readout ports is selected automatically depending on the exposure time.

Readout time (ms)

The valid camera readout time is given in this status window which is defined by the number of used ports or by defining a sensor sub region window (ROI).

Temperature

The valid CCD sensor temperature is shown here. It is adjusted to 18 C°. It can not be changed. If a black reference is used it should be used at the same sensor temperature when it was created.
If free air circulation for the camera housing is blocked, it may happen that the sensor temperature is increased and the dark current of the sensor may be higher than normal. If the camera is operated without cooling (USB2.0 port of camera not connected) the sensor temperature is increased and dark current will be higher than normal. This should be considered when using the camera at longer exposure times.

Expert section

Abort on missed frames

In case of high speed time lapse acquisition the camera is sending an enormous amount of data to the PC. If the PC is not fast enough in handling the image data it may happen that dropped frames occur. By this parameter the behavior of the acquisition routine can be defined: it is the default mode to abort a acquisition sequence in case of a dropped frame. If unchecking this the event of a dropped frame is ignored and the acquisition is continued without further notification.

Main LED

The LED in the camera lid shows the general operation mode of the camera by showing different colors. In special low light applications it may be desirable to minimize stray light in the microscopy workspace. Therefore the intensitiy of the status LED can be dimmed or switched off completely. The following status informations are available:

Red: after plugging the main USB3.0 cable of the camera to a PC, camera firmware is not yet loaded by driver. As soon as the camera gets loaded with firmware, the color changes one of the following colors:

Yellow: connected to USB2.0 bus, only 13 Mhz clock speed available, no cooling (right USB port connected to USB2.0, left USB port not connected),

Green: connected to USB 2.0 bus, only 13MHz available, cooling active (right USB port connected to USB 2.0 bus, left USB port connected to computer USB port, or separate USB power supply),

Pink: connected to USB3.0 bus, full 39 MHz clock speed available, no cooling (right USB-port connected to USB3.0, left USB-port not connected)

Blue: connected to USB3.0 bus, full 39 MHz clock speed available, cooling active (right USB-port connected to USB3.0, left USB port connected to computer USB port, or separate USB power supply)

Red: wile exposure of sensro is active, in Live mode or acquisition of image series the LED is blinking, therefore.

Trigger LED

A second LED is at the camera back, above the Micro-d connector for the trigger cable. This LED indicated the status of the trigger port. It is only active, in case the trigger port is used.

In special low light applications it may be desirable to minimize stray light in the microscopy workspace. Therefore the intensitiy of the status LED can be dimmed or switched off completely. The following status informations are available:

Green: asynchronous triggering is possible (no jitter in line timing),

Yellow: synchronous triggering is possible (some jitter in line timing),

Red: no trigger accepted,

Blue: external trigger signal active,

Tile Adjustment

Camera expert section shows camera parameters which need deeper understanding of functionality.

The Axiocam 506 camera is using a special CCD sensor with four readout ports in order to read out the image data from the CCD area as fast as possible. It is assured by proper alignment of the electronics that all associated signal paths are absolutely equal and no quadrants get visible as this is very undesirable for good image quality. In order to offer the best quality possible, a special algorithm is activated by

default as an additional safety measure to suppress remaining residuals. This correction can be deactivated in order so minimize image processing on the camera raw data.

Acquire (Expert) section

Camera **Expert** section shows camera parameters which need deeper understanding of functionality.

8 bits compression

In case other devices are using bandwidth on the connected USB 3.0 data bus, it is possible to reduce the amount of image data sent by the Axiocam 506 by activating data compression from 14 bit to 8 bit. By converting the data through a square root loaded lookup table the 14 bit values are converted to 8 bits per pixel. This is handled transparently as the data is decompressed automatically by the camera driver in the PC while receiving it. As compression has some slight impact on data quality it is deactivated as default.

Frame time (ms)

In case of fast time lapse acquisition the camera is sending huge amount of image data to the PC within a short time. The data needs to be handled by the PC without delay. There is a certain risk of dropped frames, if the camera is sending data faster, than the PC can handle it, especially at very short exposure times. By setting a frame time larger than zero, an acquisition delay can be defined for continuous acquisitions. Max. value is 5000 ms. Default value is zero.

Readout mode

This is a status information and shows the currently used number of used sensor ports. Values are: quad port, dual port, single port.

Adjust Live Frame Rate

The camera live image can send a lot of image data which need to be processed on the fly. In case of less powerful computers this can overload the PC and cause a slow reaction of the ZEN user interface. By activating this function the slider below gets accessible and a maximum limit for the live frame rate can be adjusted. Abundant image data is then discarded accordingly and not processed to be displayed.

Live Frame Rate Max

Adjustment of maximum accessible frame rate for live display, only. Only accessible if "Adjust Live Frame Rate" checkbox is activated.

Live Frame Rate

This is a display field only. It shows the measured live image speed.

14.3.2.2.2.6.3 AxioCam ERc5s

Camera Identifier

A clear identifier for the currently active camera is displayed here. It consists of the product name and part of the serial number. This is helpful if you want to identify the camera in question, e.g. when working with a dual camera system.

Sharpness

Using this function you can increase the impression of sharpness in an image.

Orientation dropdown list

Here you can select the orientation of the camera image. This allows you to adjust the camera image to the properties of the different camera ports. The following selection options are available:

- Original
- Flip Horizontally
- Flip Vertically
- Rotate 180

14.3.2.2.2.6.4 AxioCam ICc5

Camera Identifier

A clear identifier for the currently active camera is displayed here. It consists of the product name and part of the serial number. This is helpful if you want to identify the camera in question, e.g. when working with a dual camera system.

Orientation dropdown list

Here you can select the orientation of the camera image. This allows you to adjust the camera image to the properties of the different camera ports. The following selection options are available:

- Original
- Flip Horizontally
- Flip Vertically
- Rotate 90 CW
- Rotate 90 CCW
- Rotate 180
- Mirror at +45 Diagonal
- Mirror at -45 Diagonal

14.3.2.2.2.6.5 AxioCam HRc

Camera Identifier

A clear identifier for the currently active camera is displayed here. It consists of the product name and part of the serial number. This is helpful if you want to identify the camera in question, e.g. when working with a dual camera system.

Orientation dropdown list

Here you can select the orientation of the camera image. This allows you to adjust the camera image to the properties of the different camera ports. The following selection options are available:

- Original
- Flip Horizontally
- Flip Vertically
- Rotate 90 CW
- Rotate 90 CCW
- Rotate 180
- Mirror at +45 Diagonal
- Mirror at -45 Diagonal

Readout Speed (MHz)

The **High Speed** mode activates the faster 24 MHz mode, for which the digitization accuracy is set to 12 bits per pixel. This mode offers advantages if sufficient light is available and situations need to be acquired quickly: fast time series or tile images.

In **High Accuracy** mode the readout speed is 12 Mhz and the digitization accuracy 14 bits per pixel. This mode offers advantages if very little light is available and you want the camera to acquire very weak signals just above the camera's noise level.

14.3.2.2.2.6.6 AxioCam MRm

Camera Identifier

A clear identifier for the currently active camera is displayed here. It consists of the product name and part of the serial number. This is helpful if you want to identify the camera in question, e.g. when working with a dual camera system.

Orientation dropdown list

Here you can select the orientation of the camera image. This allows you to adjust the camera image to the properties of the different camera ports. The following selection options are available:

- Original
- Flip Horizontally
- Flip Vertically
- Rotate 90 CW
- Rotate 90 CCW
- Rotate 180
- Mirror at +45 Diagonal
- Mirror at -45 Diagonal

14.3.2.3 ApoTome Mode Tool

Enable ApoTome checkbox

Activated: Uses the ApoTome for acquisition and experiments.

Phase Images

Select here the number of phase images per optical sectioning. 5 phase images are the default value.

Live Mode

Here you set the display of the **Live Mode**. Default value is **Grid Visible**.

14.3.2.4 Channels Tool

i Note

This tool is only visible if you have licensed and/or activated the module in the **Modules Manager** and additionally activated it on the **Acquisition** tab in the **Experiment Manager**.

In the **Channels** tool you can configure channels (also called tracks) for Confocal or Widefield acquisition. The tool offers you the option of entering the hardware settings for acquisition manually or performing the configuration automatically.

If there is no track/channel available, you will be asked to add a LMS or a WF track to the experiment by clicking on the corresponding button (**+LSM** / **+WF**).

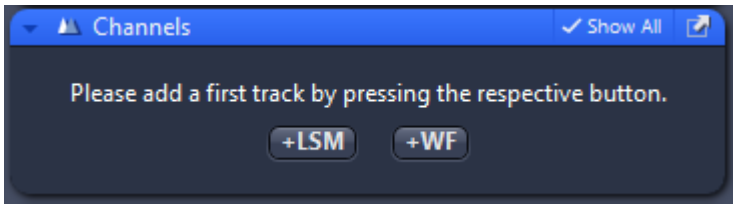


Fig. 56: Channels Tool (without channels)

The following tool appearance will only be visible if you have added one or more tracks/ channels:

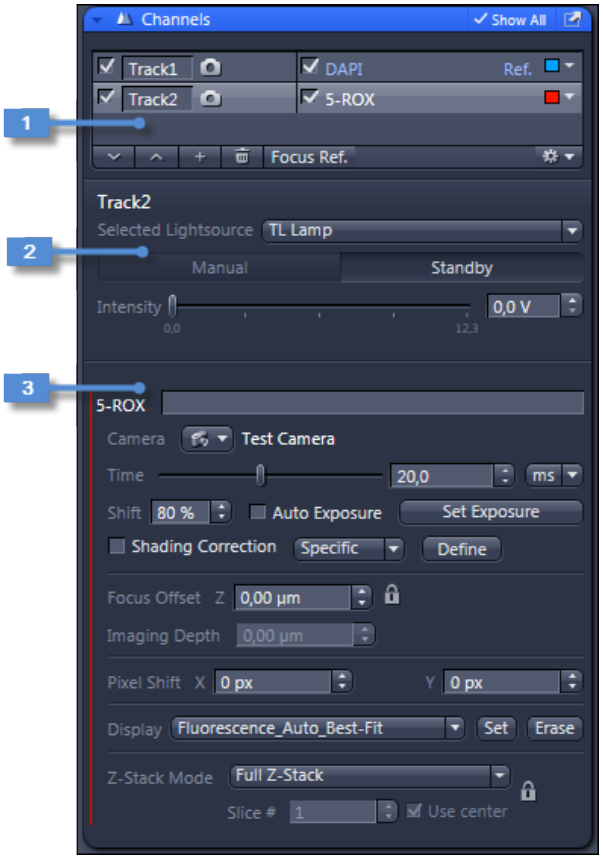


Fig. 57: Channels tool

1. Channels list
2. Light Source section, see *Light Source section (WF)* [▶ 411]
3. Channel specific settings, see *Channel Settings (WF)* [▶ 411]

14.3.2.4.1 Channels list

Please bear the following points in mind for the channels list:

- The selected channel is highlighted by a light gray bar.
- The reference channel for the auto focus is highlighted by a blue font color.

- The preview color (LUT) for the channel is shown on the right side of the list. To change the preview color for the channel, click on the colored rectangle with the arrow icon and select an alternative color from the shortcut menu. The preview color is also shown in the sections for channel-specific hardware settings as a thin line on the left side of the list.
- You can edit the track name directly in the corresponding field. An additional name for the dye can be added in the Dye name input field.

i Note

If you select a dye or contrast technique in the **Add Dye or Contrast Technique** dialog, a suggestion for the hardware settings for the acquisition of this channel is made automatically. If no suggestion can be made, a channel without hardware settings is added. You will then see a corresponding indication in the status area of the program interface.

List control elements

Button	Description
Down	Navigates one row down.
Up	Navigates one row up.
Delete	Deletes the selected channel.
Focus Ref. button Focus Ref.	Sets the selected channel as reference channel for focus actions or stitching during acquisition.
Options	Opens the *Options* [▶ 410] shortcut menu.

Laser Range section

Parameter	Description
High Intensity Laser Range checkbox	If activated, a high intensity laser range will be used, where you can adjust the lasers between 0.2 and 100% of their power. This is especially relevant for bleaching experiments. The setting affects all tracks. While switching, the system is trying to keep the intensities at a similar level. If the currently selected intensity is outside of the overlapping range (0.2% to 3.5-5%) the closest possible value is used. The LSM 800 is working by default in a laser power range between 0.01% and 3.5-5% of the available laser intensity. The available maximum in the default range is depending on the laser wave length.

14.3.2.4.1.1 Options menu

Menu entry	Description
Add New...	If WF channels were added this function will open the *Add Dye or Contrast Technique* [▶ 485] dialog to add more WF tracks. If LSM channels were added this function will add a new LSM track.
Duplicate	Creates a new track with the same settings and dye as the currently selected track.
Rename	Assigns a new name to the channel of the currently selected track. To change the name of the track, you can directly click into the respective field of the channels list.
Reset Color	Resets the color of the selected track(s) to default.
Select All	Selects all tracks of the list.
Delete	Deletes the selected track.
Delete All	Deletes all existing tracks.
Set as Reference Channel	Defines the selected channel as the reference channel for focusing actions.
Compare...	Opens a new window, where you can easily compare and adjust key parameters of the active tracks.

14.3.2.4.2 Channel-specific settings (WF)

The settings always relate to the channel you have selected in the **Channels** list.

To show the settings for all channels, click on the button | **Select All** in the **Channels** list.

14.3.2.4.2.1 Light Source section (WF)

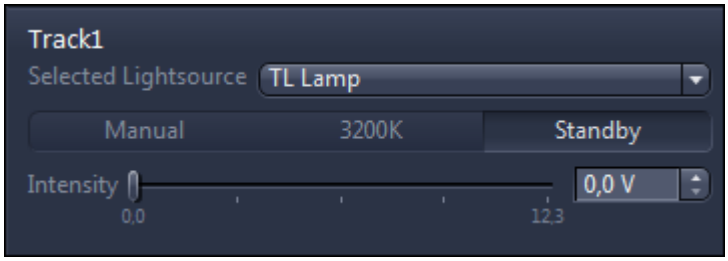


Fig. 58: Light Source Section

In this section you can select the available light sources from the **Selected Lightsource** dropdown list and adjust the corresponding settings. You can adjust the parameters of the light sources without having to save these in the hardware settings. You can therefore adjust the intensity of the laser lines or LEDs, for example, immediately before starting an acquisition.

If your system is equipped with a TIRF slider, the TIRF angle and type of illumination can also be set here.

If you select the **Use Setting** entry, the settings for the light sources disappear. The light source parameters from the hardware settings are used instead for the acquisition of the channel.

14.3.2.4.2.2 Channel Settings (WF)

Paramter	Description
Dye name input field	In the input field after the selected dye you can enter an additional name.
Camera dropdown list	Select the desired camera for the channel from the dropdown list.
Time slider	Adjust the exposure time for the camera using the slider or spin box/input field. Select the unit of time from the dropdown list at the right of the spin box/input field.
Auto Exposure checkbox	**Activated:** Automatically determines the camera's exposure time for the selected channel. The value set manually is ignored.

Paramter	Description
Set Exposure button	Starts an exposure time measurement for the channel. After the measurement the value is adopted as the exposure time setting.
Shading Correction	**Activated:** Uses the calculated shading correction for this channel. To learn more about shading correction read the chapter, *Post Processing Section* [▶ 363].

The following functions are only visible if the **Show All** mode is activated:

Parameter	Description
Shift	Here you can enter the range of the camera's dynamic range that is utilized.
Focus Offset	Here you can enter the focus offset from the channel to the Z-position of the reference channel or to the current position. The**Lock** icon shows that this setting will be synchronized between all channels of this track.
Imaging Depth	Here you can enter the distance from the surface of the coverslip (or bottom material) to the focal plane of interest in micrometer.
Pixel Shift	Here you can define the pixel shift in **X** and **Y**. The defined pixel shifts are applied to images collected with Snap, contentious or in an experiment.
Display	Here you can select an existing predefined display setting. By clicking on the **Set** button the setting will be adopted. By clicking on the **Erase** button the current setting will be deleted.

Parameter		Description
Z-Stack Mode		Only visible if the **Z-Stack** checkbox is activated in the **Experiment Manager**.
-	Full Z-Stack	Acquires the Z-stack as defined in the **Z-Stack** tool.
-	Single slice only	Acquires a single slice of the Z-stack only. Select the single slice in the input box under the list. If the **Use center** checkbox is activated, the center focus plane will be used for acquisition.

Parameter		Description
-	Single slice, rest black	Acquires an image of a single slice of the Z-stack only. All other Z-slices of the stack are filled with black images. Select the single slice in the input box under the list. If the **Use center** checkbox is activated, the center focus plane will be used for acquisition.
-	Fill with single slice	Acquires an image of a single slice of the Z-stack only and fills all other Z-slices with this slice. Select the single slice in the input box under the list. If the **Use center** checkbox is activated, the center focus plane will be used for acquisition.

14.3.2.4.3 Channel-specific settings (LSM)

The settings always relate to the channel you have selected in the **Channels** list.

To show the settings for all channels, click on the  button | **Select All** in the **Channels** list.

14.3.2.4.3.1 Lasers section

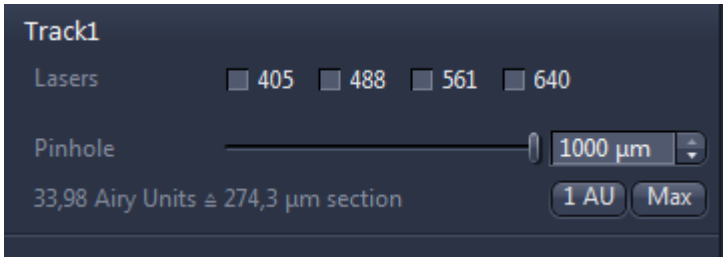


Fig. 59: Lasers Section

Parameter	Description
Lasers	Here you can activate the desired laser line for the selected track. Activate the required lasers by activating the corresponding checkbox. The laser lines along with sliders will appear. Set the required attenuation (%) using the sliders, the arrows, or typing in the input field.

Parameter	Description
Pinhole slider	Adjusts the diameter of the pinhole. The diameter is specified in micrometer. The text below translates this diameter to Airy Units and section thickness for the configured wavelength. Note that this control is not available for Airyscan tracks. Here the physical pinhole is automatically opened and set to the optimal diameter.
- 1 AU button	Sets the pinhole diameter to a value that is corresponding to 1 Airy unit for the configured detection wavelength.
- Max button	Opens the pinhole to its maximum diameter. This can be useful to find the focal plane.

14.3.2.4.3.2 Channel Settings (LSM)

Parameter	Description
Master Gain	Here you can control the voltage of the PMTs. Higher voltage increases the gain of the PMT. The image becomes brighter and you may be able to reduce the laser power. At higher voltage, the noise level in the image increases. The optimum between gain and noise depends on your experimental requirements and on your sample. The maximum available voltage is depending on the type of the detector and is 1200V for multialkali PMTs, 900V for GaAsP PMTs and 1000V for the Airyscan. GaAsP PMTs and the Airyscan have a minimum voltage of 500V.

The following functions are only visible if the **Show All** mode is activated:

Parameter	Description
Digital Offset	This control element is not available for Airyscan tracks. Here you can perform adjustments on the background of the image.
Digital Gain	Here you can digitally amplify the signal.

Parameter	Description
Z-Stack Mode	Only visible if the **Z-Stack** checkbox is activated in the **Experiment Manager**.
- Full Z-Stack	Acquires the Z-stack as defined in the **Z-Stack** tool.

Parameter	Description
- Single slice only	Acquires a single slice of the Z-stack only. Select the single slice in the input box under the list. If the **Use center** checkbox is activated, the center focus plane will be used for acquisition.
- Single slice, rest black	Acquires an image of a single slice of the Z-stack only. All other Z-slices of the stack are filled with black images. Select the single slice in the input box under the list. If the **Use center** checkbox is activated, the center focus plane will be used for acquisition.
- Fill with single slice	Acquires an image of a single slice of the Z-stack only and fills all other Z-slices with this slice. Select the single slice in the input box under the list. If the **Use center** checkbox is activated, the center focus plane will be used for acquisition.

14.3.2.5 Focus Strategy Tool

Here you can select the focus strategy that you want to apply. The strategies that are available depend on the dimensions selected (Z-stack, time series, tiles), the hardware devices present (Definite Focus) and software licenses (e.g. **Autofocus** module).

Focus strategies determine and/or update a Reference Z-Position, which in most cases is used directly for acquisition.

Exceptions

- When Z-stacks are acquired the center of the Z-stack determines the Reference Z-Position.
- Defined offsets for channels and Z-stacks shift acquisition in relation to the Reference Z-Position.
- If two focusing methods are combined, the Reference Z-Position of the first method is used as the starting point for the subsequent method.

Depending on your system, experiment configuration and licensed modules, the following strategies are available:

Strategy	Description
None	No focus strategy has been selected. This is the default setting for all experiments that do not include a tiles dimension. In this case the software automatically selects the **Use Focus surface Defined by Tiles Setup** strategy. The current Z-position at the time the experiment is started is set as the Reference Z-Position and remains unchanged during the experiment. **Exception:** By default, Z-stacks are acquired at the fixed Reference Z-Position that has been defined as the center in the Z-Stack tool. You can change this setting in the **Z-Stack Acquisition** section of the **Focus Strategy** tool.

Strategy	Description
Absolute Fixed Z-Position	Allows you to define a Reference Z-Position. You can choose between two modes: ■ **Fixed Z** (-position) Allows you to define a Reference Z-Position that remains unchanged during the entire experiment. ■ **Current Z** (-position from Tiles Setup) Only used for tile experiments: Uses the individual Reference Z-Positions that can be defined for tile regions and positions in the **Tiles** tool. Use this option if you work with the **Tiles** tool without the optional **Tiles** module.
Software Autofocus	Only available if you have licensed the **Software Autofocus** module. The focus position is determined via the sharpness calculation or intensity calculation of a series of images (Z-stack) and set as the Reference Z-Position. The settings are configured in the **Software Autofocus** tool.
Definite Focus	Only available if your microscope system has attached a **Definite Focus** device. Definite Focus attempts to maintain a certain distance to the cover glass of the sample in order to compensate for mechanical and thermal movements. The Definite Focus is initialized at the start of the experiment by setting the current distance as the reference distance. When the focus is stabilized during the experiment, the current distance is adjusted to the reference distance. This is achieved by moving the focus drive accordingly. The new Z-position resulting from this is used as the Reference Z-Position for acquisition. Two modes are available:
- Standard	This mode uses default settings for stabilization which we are recommend to use if you are not familiar with the Definite Focus device.

Strategy	Description
- Expert	This mode allows advanced settings for using Definite Focus stabilization. Under **Within Image Acquisition Loops** you can select how Definite Focus is used: ■ **Time Series** If activated, this setting repeats Definite Focus at certain predefined points within a image acquisition loop (e.g. every 2nd time point). ■ **Tile Regions / Positions** If activated, this setting repeats Definite Focus at certain **Region / Positions** (e.g. every 2nd Position) ■ **Tiles** If activated, this setting repeats Definite Focus at a certain **Tile** (e.g. every 2nd Tile) Under **Between Image Acquisition Loops** you can enable Periodic Stabilization. **Periodic Stabilization** is available for experiments that include **Time Series** only. If activated, a stabilization with a defined **Period** (e.g. every 10 s) will be performed. This mode is useful if long intervals are needed between image acquisition loops. This mode can be combined with the stabilization events before discrete imaging loops.
Combine Software Autofocus and Definite Focus	This strategy allows you to combine the functions of Definite Focus and Software Autofocus. This can be done in two ways, see descriptions below. Note that in both cases it is possible to modify the time point and frequency at which these events occur. The repetitions and frequency of these events is performed with predefined standard settings. If you wish to adjust these for a particular experiment select the **Expert** mode (only visible in **Show all**).

Strategy	Description
- Software Autofocus as Reference for Definite Focus	Software Autofocus moves the focus drive to the focus position that has been calculated. Taking this as the starting point, a new reference distance is defined for the next distance stabilization performed by Definite Focus. This can reduce the likely-hood of a stabilization failure when the sample is long and elongated and the carrier possibly tilted.
- Definite Focus as Start for Software Autofocus.	The last valid Reference Z-Position defined by Definite Focus is the starting position for the Software Autofocus search. This allows you to optimize the search range and step size of Software Autofocus.
Use Focus Surface Defined by Tile Setup	Only available if you have licensed the **Tiles** module. This strategy is selected automatically when the **Tiles** dimension is activated. In the **Tiles** module (optional module) a focus surface can be defined in two ways: Local (for tile regions and/ or positions) Global (based upon a carrier e.g. petri dish, slide, plate)
- Local (per Region/ Position)	**For Tile Regions:** A local focus surface can be defined for a Tile region in two ways: ■ 1) For large tile regions or samples in which the plane of interest ("focus") is described by a slope or parabolic you can define one or more "Support points" for each tile region. These can be arrange in a regular or irregular manner. Each support point is assigned a discrete z-value ("focus"). From these the software is able to interpolate a local focus surface with a chosen degree of complexity in an attempt to describe the contour of the plane of interest for the Tile region. ■ 2) When no support points are used the z value assigned to the Tile region defines the "focus" for all the tiles it contains. For small tile regions on a suitable sample this might be sufficient. **For Positions:** For positions the local focus surface is defined by the discrete Z-value assigned to it. A position cannot have support points.

Strategy	Description
- Global (Carrier based)	A global focus surface is defined based on a selected carrier template. To create a global focus surface you need to add support points by creating or editing a carrier template from the appropriate section of the Tiles tool. Thus, a group of support points are used to help describe the tilt or curvature of the carrier (again by a process of interpolation). Tile regions or position "placed" upon this global focus surface are mapped onto it accordingly.
- Additional Action section	The Reference Z-Position calculated from the Local Focus Surface is used as the starting point for an additional Software Autofocus search or Definite Focus stabilization, which updates the Reference Z-Position. This allows you to reduce the search range and step size of Software Autofocus (faster). In the case of Definite Focus, minute undulations in at the interface between sample and glass and thermal deformation there of can be quickly corrected for. This method only makes sense in the context of a very thin sample, such as the sections that are used in array tomography, where the sample lies directly on or close to the interface. This also reduces the likely-hood of a stabilization failure when the sample is long and elongated and the carrier possibly tilted.
- Initial Definition of Z-values for Support Points/ Positions section	Normally (default) this is given by the **Fixed Z-Position** values (user defined) in the support points/ positions list but it is optionally possible to use a **Software Autofocus** to initially define these value prior to the start of the image acquisition. The resulting z-values can optionally overwrite the existing listed values in the support points/ positions list.

Strategy	Description
Update Focus Surface Defined by Tiles Setup	This strategy allows a focus surface (local or global) to be updated by the activity of either a Software Autofocus run or a Definite Focus stabilization. The focus surface(s) are regularly adjusted by means of a software autofocus search or a definite focus stabilization, which is performed exclusively at a single defined waiting position. A resulting correction of the Reference Z-Position is adopted for all focus areas. This strategy is only relevant if **Tiles** and **Time Series** experiments are combined. Again the repetition and frequency of the software autofocus or definite focus stabilization can be modulated to meet the experiment needs.

14.3.2.6 Software Autofocus Tool

Parameter	Description
Quality	
- Basic	Selects the simple and fast algorithm to calculate the software autofocus.
- Best	Selects a more complex, optimized algorithm to calculate the software autofocus.
Range Coverage	
- Smart	Sets a part of the travel range as the region for determining the autofocus.
- Full	Sets the full travel range as the region for determining the autofocus.
Sampling	Here you can select the step size of how the search range is sampled.
- Default	Uses the default step size (dz = 1/sqrt(2) * 2 * n* lambda/NA).
- Fine	Uses a small Z-distance (0.5 * dz) between the individual focus images that are used to calculate the best focus position.
- Coarse	Uses a medium Z-distance (2 * dz) between the individual focus images that are used to calculate the best focus position.

Parameter	Description
- Very Coarse	Uses a large Z-distance (4 * dz) between the individual focus images that are used to calculate the best focus position.
Autofocus ROI	
- Spot Meter / Focus ROI checkbox	**Activated:** Only uses the values from the Focus-ROI (Spot Meter) to calculate the focus position. The Focus-ROI can be displayed and adapted in the live mode by right-clicking on the live image and activating the checkbox.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Sharpness Measure	
- Contrast	Calculates the best focus position from the contrast of the focus images.
- Intensity	Calculates the best focus position from the intensity of the focus images.
- Auto	Automatically uses the best method (contrast or intensity) to calculate the focus position depending on the hardware settings.
Autofocus Search Range	
- **Relative Range**	The software autofocus is calculated over a relative range.
Automatic Range checkbox	**Activated:** Calculates the range for the autofocus search automatically depending on the objective set.
Range input field	Only active if the **Automatic Range** checkbox is not activated. Enter a range here that you want to be used for the autofocus search.
Step Size display field	Shows the distance between the individual focus images set under **Range**.
- **Fixed Range**	The software autofocus is calculated over a fixed range.

Parameter	Description
Set Last button	Defines the current Z-position as the end (last) point for the software autofocus. Alternatively, you can enter the desired value in the spin box/input field to the left of the button.
Set First button	Defines the current Z-position as the start (first) point for the software autofocus. Alternatively, you can enter the desired value in the spin box/input field to the left of the button.

14.3.2.7 Experiment Regions Tool

This tool allows to define Regions of Interest (ROIs) which are used for image acquisition, sample manipulation (bleaching) an image analysis.

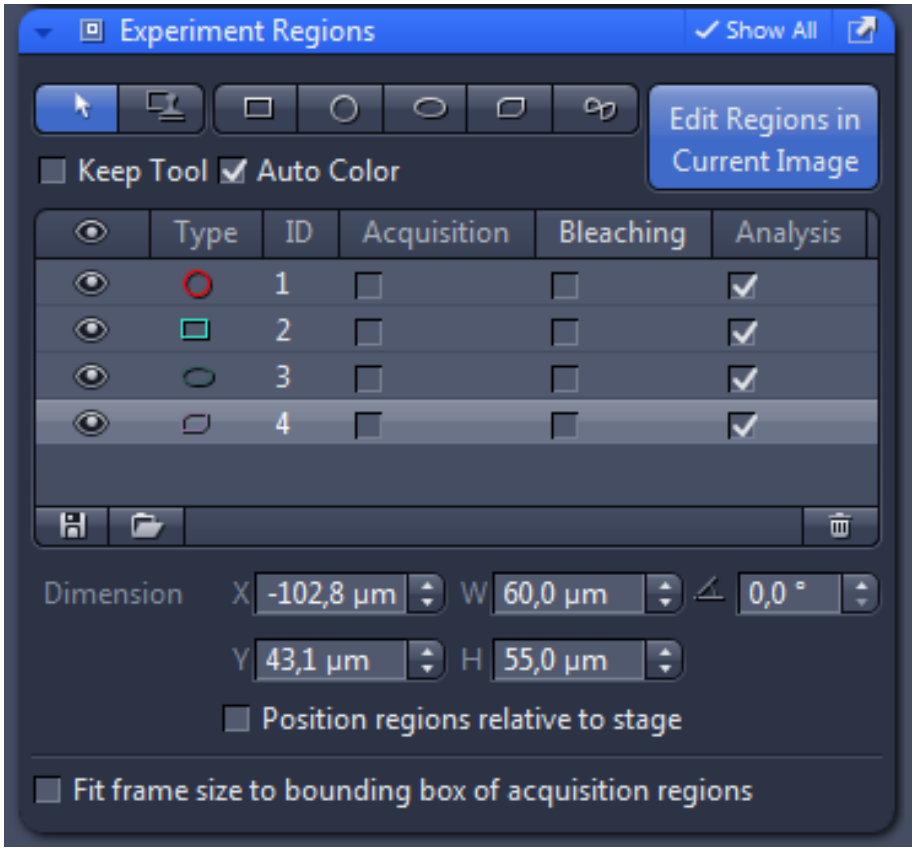


Fig. 60: Experiment Regions Tool (Show All)

Parameter	Description
Toolbar	Use the tools from the toolbar to draw ROIs into the image.

Parameter	Description
Edit Regions in Current Image button	If you draw a ROI into the image this button is active and will be highlighted in blue. By deactivating the button the ROIs are not displayed in the image. By activating the button the ROIs are displayed in the image and can be edited. It is not possible to edit Experiment Regions when the image is displayed in certain viewers. Switch back to the 2D view, in case the button is disabled.
Keep Tool	**Activated**: Keeps the selected graphic tool for multiple actions.
Auto Color	Only available in Show all mode **Activated:** Assigns different colors for each drawn ROI.
ROIs List	This list shows all drawn in ROIs . Their **Type** (form and color), **ID** (number) are listed as identifiers. For each ROI further actions can be chosen by activating the according check box:
- Acquisition	If activated, only within the ROI(s) images will be acquired.
- Bleaching	If activated, a bleaching experiment will be performed within the corresponding ROI(s).
- Analysis	If activated, data from the corresponding ROI(s) will be used for Mean of ROI analysis in the **MeanROI** view.
Save	Saves the selected ROI(s) to the file system.
Load	Loads ROI(s) from the file system.
Delete	Deletes the selected ROI(s) from the ROIs list.
the following parameters are only visible in the Show All mode.	
Dimensions	
- X / Y	Shows the X / Y position of a selected ROI. You can also enter new values in the input fields.
- W / H	Shows the Width (W) and Height (H) of the selected ROI. You can also enter new values in the input fields.
- Angle	Shows the rotation angle of the selected ROI. You can also enter a new value in the input field.

Parameter	Description
- Position regions relative to stage	Does hopefully exactly what the text says it does.
Fit frame size to bounding box of acquisition regions	If activated, the total frame that the scanner will cover fits all ROIs that are marked as Acquisition ROIs in the table. This can decrease imaging time, since the scanner has not to move over the complete frame of the original image, in which the ROIs were drawn.

14.3.2.8 Experiment Designer Tool

i Note

This tool is only visible if you have licensed and/or activated the module in the **Modules Manager** and additionally activated it on the **Acquisition** tab in the **Experiment Manager**.

In the **Experiment Designer** you can create experiments for multidimensional acquisition. Experiments can consist of any number of components. A component is referred to as an experiment block. Each experiment block has a distinct number, which is shown above the block. To create a new experiment block click on the **Create Acquisition** button and select the desired type of block (Acquisition, Delay, Wait, Execute blocks) then click on the button again. The block will appear in the **Timeline** of the experiment which you see below the blocks.

Please note the following when working with experiment blocks:

- Each acquisition block can be seen as its own independent single experiment with its own individual settings.
- Each experiment block can have its own dimensions (e.g. channel settings like exposure time, active camera, camera parameters; Z; T)
- Focus strategies are block specific as well
- You can change the order of experiment blocks by dragging&dropping them in the experiment timeline

Special actions that influence the course of an experiment are performed by means of a special block. In the **Show All** mode you can define Loops and Repetitions and specify the number of image files.

Functions	Description
Import	Imports experiment blocks from another experiment.

Functions	Description
Export	Exports the selected experiment blocks to the file system.
Create Acquisition Block	Adds a new, empty acquisition block to the experiment timeline.
Create Delay Block	Adds a Delay block to the experiment timeline. A delay block pauses the experiment for a predefined period. After that period the experiment continues automatically. You can set the length of the pause in the **Properties** section which will appear when selecting the Delay block. The delay will be shown within the block. If the **Synchronize with preceding blocks** checkbox is activated, the duration of the delay block is reduced by the measured execution time of the preceding blocks.
Create Wait Block	Adds a Wait block to the experiment timeline. A wait block holds the experiment in idle status as long as clicking on **Continue Experiment** in the message box. This can be used for adding a solution or changing the buffer of the specimen. The message box will be shown when a wait block is reached. Enter the message text into the input field in the **Properties** section.
Create Execute Block	Adds an Execute block to the experiment timeline. A execute block executes a selected hardware setting. If the **Sequential** checkbox is activated the experiment will continue while the hardware setting is executed. If it is deactivated the experiment will wait until the hardware setting was executed. By clicking on the **Options** button the options for configuring and selecting the hardware settings will appear, see below. If a hardware setting was selected, the **Go!** button is active. If clicking on this button the selected hardware setting will be applied immediately.
Duplicate	Duplicates the selected block and inserts the newly created block after the last block.
Delete	Deletes the selected block from the timeline.

Options

Option	Description
Edit Setting/ Light Path	Opens the light path dialog in which you can change the relevant hardware setting.
Set to none	Removes the existing hardware setting.
Get Current Hardware	Adopts the current device status.
Experiment Settings Pool	The shortcut menu shows a list of the existing hardware settings.
Harddrive Folder Settings	The shortcut menu shows a list of the saved hardware settings.
From File	Opens the Import Hardware Settings dialog window. Select a ZIS hardware settings file (*.czhws).
Export to User Folder Settings	Exports the current hardware setting.
Separate Image Document for each Acquisition Block	If activated, one separate image file per acquisition block is created.

14.3.2.8.1 Loops and Repetitions section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button  .

In the **Loops and Repetitions** section you can specify which experiment blocks should be repeated during the experiment. You can define as many repetitions as you like for each experiment. An experiment block may only appear once within the repetitions defined.

i Note

If you define several repetitions, the following conditions must be met:

- Repetitions must form a complete unit

- One repetition may not be placed within another

If these conditions are not met, the repetition cannot be performed. In this case a yellow warning symbol appears under the **Active** field.

Parameter	Description
Loops	Enter the number of loops that you want to be performed.
Start	Enter the number of the starting block.
End	Enter the number of the end block.
Active	If the checkbox is activated, this repetition is performed in the experiment.
Add	Adds a new repetition to the experiment.
Delete	Deletes the selected repetition.

14.3.2.9 Z-Stack Tool

i Note

This tool is only visible if you have licensed and/or activated the module in the **Modules Manager** and additionally activated it on the **Acquisition** tab in the **Experiment Manager**.

In the **Z-Stack** tool you can configure acquisitions that comprise several Z-planes of your sample. You can set all the parameters manually using two different modes (see *Manual Configuration* [▶ 430]) or have configuration performed automatically (see *Automatic Configuration* [▶ 429]).

Z-Stack Graphical Display

The graphical display in the left area of the tool represents the configured Z-stack. In the case of inverse microscopes the objective appears in stylized form at the bottom of the Z-stack. In the case of upright systems it appears at the top.

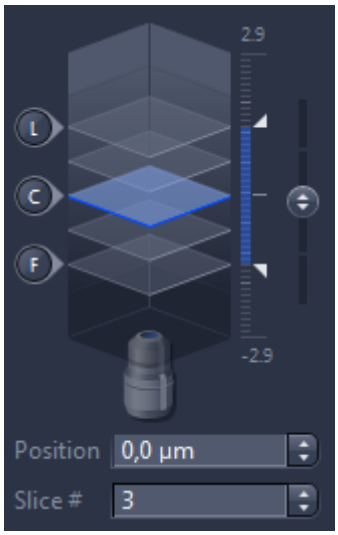


Fig. 61: Graphical Display of the Z-Stack

The blue plane indicates the current section plane. The round **L**, **C** and **F** buttons refer to the corresponding planes (**L** = Last, **C** = Center, **F** = First). To change the current Z-position, click on the relevant buttons. The blue plane then jumps to the desired position.

The values at the top and bottom of the measurement scale on the right-hand side of the graphic indicate the distance to the center of the Z-stack.

The **Position** display field below the graphic indicates the Z-position at which the section plane is located. Here you can navigate precisely to the relevant Z-positions.

The **Slice #** display field below the graphic indicates the number of the current slice.

14.3.2.9.1 Automatic Configuration

i Note

Before you perform automatic configuration, the current focus position must be at the center of the sample. The camera's current field of view must always be at a position on the sample that shows a signal in the selected channel.

If you click on the **Z-Stack Auto Configuration** button at the bottom of the tool the automatic configuration is performed.

Note that automatic Z-stack configuration only works with microscopes and systems that do not use an optical sectioning technique. If you use an **ApoTome**, **VivaTome**, **Spinning Disc (CSU)** or another technique for generating optical sections, the Z-stack must be configured manually.

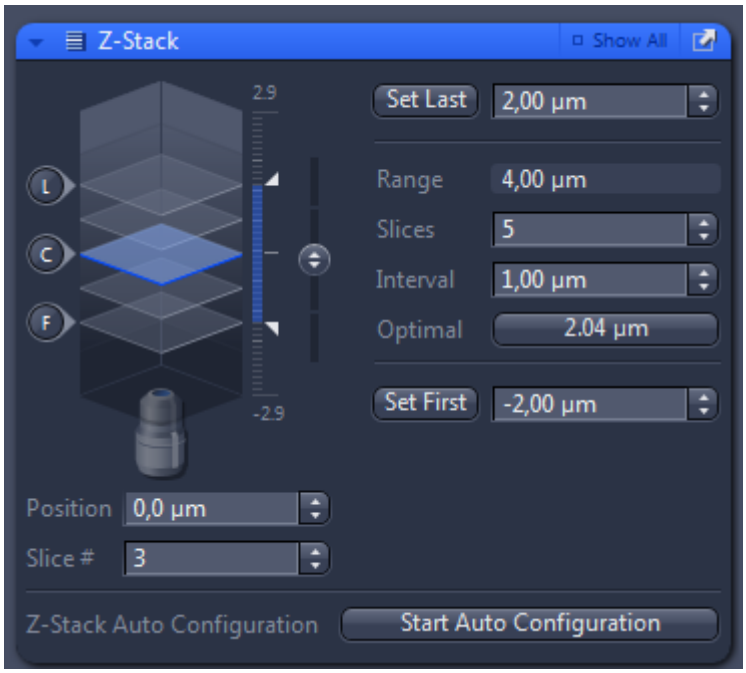


Fig. 62: Z-Stack Tool (Show All deactivated)

The following parameters are set automatically:

- Z-position of the central plane
- Distance between the individual planes
- Number of section planes

Parameter	Description
Start Auto Configuration	Automatically configures the Z-stack using the current sample.
Backlash Correction	Only available if **Show All** is activated. If activated, the positioning is done with a backlash correction. This procedure is more precise but slightly slower.

14.3.2.9.2 Manual Configuration

i Note

Z-stack images are always acquired from bottom to top automatically, irrespective of whether you have defined the top or bottom Z-plane of your stack as the first Z-plane. This acquisition sequence increases the accuracy of the Z-positioning.

For manually configuring Z-Stacks you have two modes available. Please note that these modes are only available if **Show All** is activated.

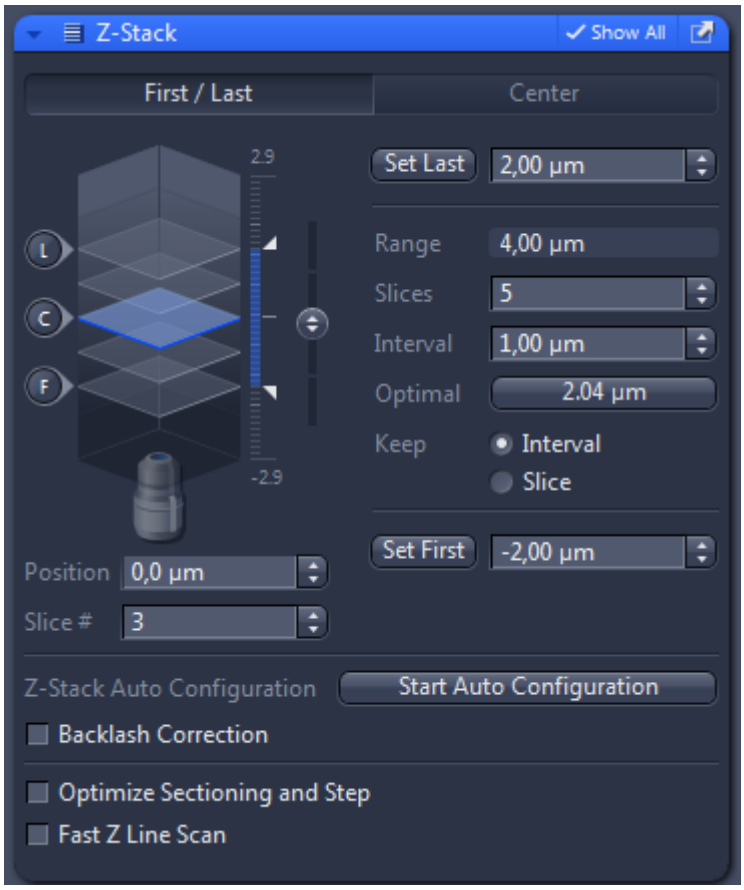


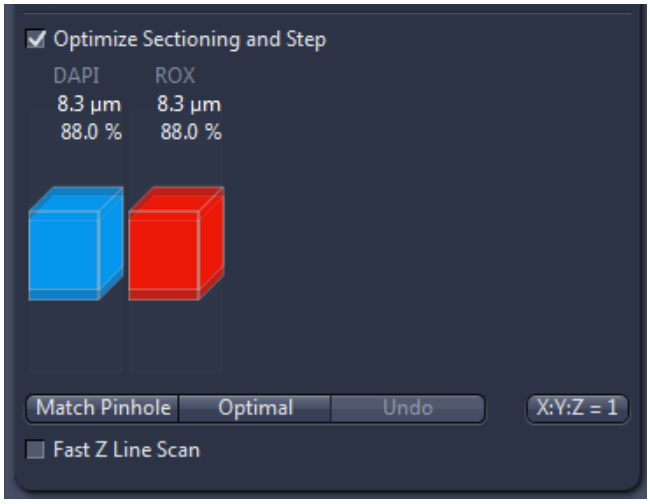
Fig. 63: Z-Stack Tool (Show All activated)

Parameter	Description
First / Last	If activated, you are able to configure the Z-stack via setting the first and the last positions of the Z-stack, see *First/Last mode* [▶ 41].
Center	If activated, you are able to configure the Z-stack via setting the center plane of the Z-stack, see *Center mode* [▶ 42].

Depending on which mode you have activated, you will see the following parameters for configuring the Z-stack:

Parameter	Description
Set Last / **Set First** buttons	Only visible for **First/Last** mode. By clicking on the **Set Last** and on the **Set First** button you determine the current position as last or first position of the Z-stack.

Parameter	Description
Range	Displays the range of the configured Z-stack from the last to the first section plane.
Slices	Here you can enter the number of Z-slices that the Z-stack will have.
Interval	Here you can enter the desired distance between the Z-slices.
Optimal	The number on this button shows the distance calculated for the channels set and the current microscope according to the Nyquist criterion. If you click on the button, this value is automatically adopted into the **Interval** input field.
Keep	■ **Interval**: Keeps the set interval between the section planes constant if you change configuration parameters in the Z-Stack tool. ■ **Slice**: Keeps the set number of Z-slices constant.
Center button	Only visible for **Center** mode. If clicking on this button the current position is set for the central Z-plane. You can also enter the value in the input field to the right of the button.
Offset	Here you can enter a value for an offset if desired.

Parameter		Description
Optimize Sectioning and Step		Here you can set the optimal Interval for more than one channel or track. Each channel is represented by a graphical display of two z-slices. The Channel name, the Z-slice thickness and the current overlap of two z-slices in percent are given above the graphic. 
-	Match Pinhole	Changes the pinhole of the tracks to match the smallest optimal interval of all channels or tracks. If manually a interval was set for all tracks, this value will be used to match the pinhole diameter.
-	Optimal	Sets the interval for all tracks to the optimal value, this might result in different intervals for the tracks. The optimal value is based on the current values for the pinhole diameter of the tracks and therefore the currently valid z-resolution
-	Undo	Resets all parameters to the default values.
-	X:Y:Z = 1	Matches the settings in Z to the settings in X and Y. This produces a cubical voxel. This can be useful for later import into third party software for rendering.
-	Fast Z Line Scan	Only available if the **Line** Scan Mode is chosen in the **Acquisition Mode** tool. If activated, the focus of the microscope will move continuously during a line scan to acquire the Z-stack, hence the Z-stack is acquired with enhanced speed but with no distinct Z-steps. A Z-stack of a line scan will be displayed in the **Ortho** View.

14.3.2.10 Panorama Tool

> **Note**
>
> This tool is only visible if you have licensed and/or activated the module in the **Modules Manager** and additionally activated it on the **Acquisition** tab in the **Experiment Manager**.

In the **Panorama** tool you will find 3 different modes to acquire panorama images:

- **Camera** mode, see *Camera mode* [▶ 435]
- **File** mode, see *File mode* [▶ 435]
- **Gallery** mode, see *Gallery mode* [▶ 435]

Each mode will be described separately in the following topics. For each mode you can choose if stitching (putting together single tile images to a single panorama image) is done automatically after the experiment or if you want to do it manually.

For automatic stitching simply activate the **Perform stitching after the experiment** checkbox. If you want to stitch manually, click on the **Perform Stitching** button, after you have finished the experiment.

The following functions are only visible if the **Show All** mode is activated:

Parameter	Description
Edge Detector	
- **Yes**	An edge detector is applied to localize image edges. This may improve the stitching result.
- **No**	No edge detector is applied.
Minimal Overlap	Sets the extent of the area of minimal overlap (in %) of the individual tiles
Max Shift	Sets the maximal extent of the shift (in %) of the individual tiles to one another.
Comparer	Here you can select how the conformance of the tiles in the overlapping regions is evaluated.
- **Basic**	Basic comparison (faster).
- **Best**	Elaborate comparison (slower).
- **Optimized**	Optimized comparison.
Global Optimizer	Select here which tile overlaps are evaluated.
- **Basic**	Only one overlap per tile is evaluated.

Parameter		Description
-	**Best**	All overlaps of a tile are evaluated.

14.3.2.10.1 Camera mode

With this mode you can use the live image from the camera to acquire your desired panorama image. The acquisition is done directly in the **Panorama** view. If you click on the **Start Experiment** button you will see the camera`s live image. Now you can start acquiring the panorama image using the available controls in the small tool window. By clicking on the **Acquire Tile Image** button the actual position will be acquired. Then move the stage to the next position to get another image. The image acquisition will be stopped by clicking on the **Stop** button.

i Note

If you use a motorized stage, you can use the joystick or the arrow buttons within the Panorama view to move the stage.
If you use a manual stage, you must move the stage manually from position to position to acquire your panorama image.

14.3.2.10.2 File mode

With this mode you can easily create a panorama image from separately acquired single images which you have stored on your file system. By clicking on the **Select Folder** button choose the folder where you have stored the single images (often called tile images). The images will be shown in the list below the Select folder button. After clicking on the **Start Experiment** button you can start putting your panorama image together. Simply drag and drop each image file to the desired position in the Panorama view. By clicking on the **Stop** button, the panorama image will be generated automatically.

If the **Set Grid** checkbox is activated, a grid is shown in the image after you have started the experiment. This can be helpful for adjusting the single tile images. You can easily adopt the grid by changing its **Columns** / **Rows** in the corresponding input fields.

If the **Snap to Grid** checkbox is activated, each image will be arranged automatically in a grid section. If you want to place each image freely in the image area, you must deactivate the checkbox.

14.3.2.10.3 Gallery mode

With this mode you can easily create a panorama image out of separately acquired single images which are opened in the **Images and Documents Gallery** in the Right Tool Area. After clicking on the **Start Experiment** button you can start putting your panorama image together. Simply drag and drop each image file to the desired position in the Panorama view. By clicking on the Stop button, the panorama image will be generated automatically.

If the **Set Grid** checkbox is activated, a grid is shown in the image after you have started the experiment. This can be helpful for adjusting the single tile images. You can easily adopt the grid by changing its **Columns** / **Rows** in the corresponding input fields.

If the **Snap to Grid** checkbox is activated, each image will be arranged automatically in a grid section. If you want to place each image freely in the image area, you must deactivate the checkbox.

14.3.2.11 Time Series Tool

i Note

This tool is only visible if you have licensed and/or activated the module in the **Modules Manager** and additionally activated it on the **Acquisition** tab in the **Experiment Manager**.

In the **Time Series** tool you can configure acquisitions that allow you to acquire an image series consisting of a number of time points. Here you can enter, for example, the acquisition interval, the length of the experiment and other specifications to control the experiment.

Parameter	Description
Duration	Here you can define the duration of your experiment. You can either specify the number of time points (in cycles) or the duration (in milliseconds, seconds, minutes, hours or days).
As Long as Possible	If activated, the acquisition of the time series continues until only 10% of disk space remains. This can impair other programs. To guarantee optimum system performance, make sufficient disk space available before starting an acquisition.
Interval	Here you can define the interval from individual image to individual image in an image series. You can specify the interval to set the gap between individual time points (in milliseconds, minutes, hours or days).
Use Camera Streaming if Possible	Only visible in Camera/ Widefield Mode. If activated, the software tries to use the free running / streaming mode of the active camera.
Use Burst Mode if Possible	Only visible in Camera/ Widefield Mode. If activated, the system buffers acquisition data in the main memory. For more info, see Burst Mode.

Parameter	Description
Measure Speed button	Not available for LSM acquisition. If clicking on this button, the software checks whether the experiment can be performed using the interval which is set. If the interval is too small, the shortest possible value is defined automatically for the interval.

TIP

The shortest possible interval is calculated by performing a blind experiment. The camera exposure time, number of steps of a Z-stack and the number of acquisition channels are taken into consideration in the calculation. Depending on the number of Z-stacks and channels and whether long exposure times have been set, it may take some time to calculate the shortest time interval.

14.3.2.11.1 Experiment Conditions section

The following functions are only visible if the **Show All** mode is activated:

Here you can define the **Start**, **Stop** and **Pause** conditions for your experiment. Select the parameters for the corresponding condition from the dropdown list:

Parameter	Description
Manual	The experiment is started immediately when clicking on the **Start Experiment** button in the **Experiment Manager**.
At Time of Day	The experiment is started, stopped or paused at the entered time. Enter the desired time in the spin box/input field to the right of the dropdown list.
After Delay	The experiment is only started, stopped or paused once the length of time entered has passed.
On Trigger	The experiment is started, stopped or paused once a TTL signal has been received.

i Note

If you define times as start, stop and pause conditions, these apply once for the entire experiment. This also applies to experiments that use the **Experiment Designer**.

14.3.2.11.2 Switches section

The following functions are only visible if the **Show All** mode is activated:

Here you can add and configure switches that can be used to execute certain actions during your experiment. To add a new switch, simply click on the **Add**  button.

To configure a switch, left-click on a new or existing switch. A dialog window will open in which you can configure the button`s parameters:

Parameter	Description
Name	Here you can enter a name for the button.
Description	Here you can enter a description for the button.
Color	**Activated:** Shows a colored line at the left edge of the switch.
Color Selection	Opens the **Color Selection** dialog. Here you can select a color for the line at the left edge of the switch.
Action	Here you can select one of the following actions. This action will be executed when you click on the button: ■ **None** ■ **Set Interval** ■ **As Fast as Possible** ■ **Trigger** ■ **Hardware Setting** ■ **Jump to previous block** ■ **Jump to next block** ■ **Jump to block #**
Delete Switch	Deletes the selected switch.

14.3.2.12 Experiment Information Tool

In the **Experiment Information** tool you can find out various details about your experiment, e.g. the memory requirement of the experiment or its duration.

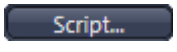
Display field	Function
Required Disk Space	Indicates the calculated memory space that the experiment will take up on your hard drive. All the activated blocks of an experiment created using the **Experiment Designer** are taken into account.

Display field	Function
Duration (Theoretical)	The system adds together all the exposure times arising during acquisition in the experiment and indicates this value. In the case of time series the intervals set are also taken into account. The actual acquisition duration will always turn out longer, however, as switching times for components (diaphragms, reflectors) and positioning times (Z-plane, stage position) also come into play.
Maximum Acquisition Rate	If the **Time Series** acquisition dimension is activated in the **Experiment Manager**, you can measure the maximum possible frame rate of the system in the **Time Series** tool. In that case the frame rate is shown here. Otherwise "not available" is displayed. After any change is made to the experiment the frame rate must be determined again in the **Time Series** tool.
Elapsed Time (Last Experiment)	If you have already run the current experiment before on the system, the duration actually required for it is displayed here. This information disappears again if you change the experiment.
Next Time Point in	Shows duration to next time point.
Tile Size	Shows the X/Y dimensions of your experiment. In the case of a single position this value is identical to the size of the camera field.

14.3.2.13 Experiment Feedback Tool

i Note

This tool is only visible if you have licensed and activated the module **Advanced Processing** in the **Modules Manager** and additionally activated the **Experiment Feedback** checkbox in the **Experiment Manager** on the **Acquisition** tab.

Parameter	Description
Script... button 	Opens the *Script Editor* [▶ 440] dialog. There you can create scripts for an Experiment Feedback.

Parameter	Description
Select script runtime circumstances	
- **Free Observer**	Upon the experiment start the acquisition and the feedback script are started but run from here in an completely unsynchronized manner. The online image analysis or the script run itself will not slow down the actual image acquisition.
- **Determined**	This mode will lead to strictly determined order of events depending on the chosen level of synchronization. The online image analysis and/ or the feedback script will be started after current acquisition is finished. In contrast to the **Free Observer** mode, a synchronized run can slow the whole acquisition down. The big advantage of the mode is, that the synchronized run ensures a predictable workflow.

14.3.2.13.1 Script Editor

Input Windows

The three windows on the left allow you to input scripts based on the programming language Python:

Window	Description
Single Execution on Experiment Start	Script elements in this window are run through and executed once at the beginning of the experiment. This area can be used to define global variables, for example.
Repetitive Execution During Experiment Runtime	Script elements in this window are run through and executed continuously during the experiment. This area contains the instructions for the dynamic control of the experiment.
Single Execution on Experiment Stop	Script elements that are in the lower input window are run through and executed once at the end of the experiment. This area can be used to issue notifications or audible alerts at the end of the experiment, for example.
Accept button	Adopts the script within the experiment
Cancel button	Leaves the dialog without adopting the script.

Specification area

On the right is the specification area for observables, actions and editor tools:

Parameter	Description
Available Observables	Observables are conditions or parameters that can be determined and observed during the course of the experiment. Select observables, actions and tools by clicking on the black triangle at the right-hand edge and dragging the desired action from the list to the desired input area. You can adopt sample scripts by double-clicking. By doing this, however, you will overwrite the content currently available in the script window entirely (after receiving a prompt).The following observables are available:
- Analysis	Analysis observables contain measurement parameters from image analyses that have been previously defined (eg in Analysis Wizard). Only those variables that are specified through the selection of the measurement program (in the list) appear.
- Environment	Environment observables describe the system environment, such as the time or available memory space on the computer.
- Experiment	Experiment observables relate to the experiment currently running and display the current time point or the current Z-stack plane, for example.
- Hardware	Hardware observables include, for example, the status of connected devices, such as trigger devices, or of incubation.
Available Actions	Actions are possible actions and reactions that can be performed during the experiment. These can vary greatly and include, for example, performing hardware actions on the imaging system, changing camera parameters, generating notifications or audible alerts, calling up other programs or canceling the experiment.
- Experiment Actions	This section contains commands that can be used to modify a running experiment on-the-fly. It also includes modifying hardware parameters which are typically part of an acquisition experiment, like exposure times or light source intensities.

Parameter	Description
- **Hardware Actions**	Hardware Actions are modifications of a running experiment concerning the incubation, XYZ positions and the Digital IO ports.
- **Extra Actions**	This section contains commands that can be used to log any kind of data into a text file or to start an external application outside ZEN at any time during a running experiment.
Editor tools	The Editor tools include sample scripts and an option for checking the script syntax.
- **Examples and Templates**	Contains a small collection of python snippets to illustrate some basic ideas of the feedback script itself.
Validate script button	Using the **Validate Script** button you can check your script for errors. If clicking on the button there will appear an info text below the button if your script is ok or not.
Information section	
- **IO Card Port Labels**	Contains the exact naming of the available IO ports for the current system.

14.3.2.14 Timed Bleaching Tool

This tool permits setting the bleach parameters for a bleaching experiment in combination with a time series. Bleaching or photomanipulation is done in between acquisition.

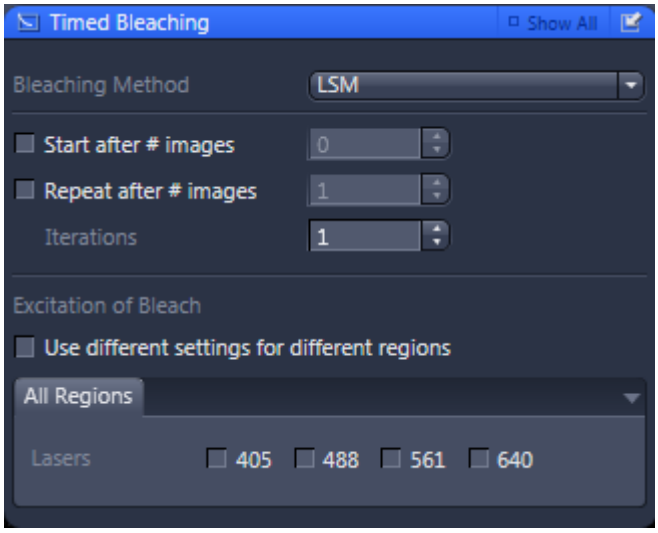


Fig. 64: Timed Bleaching Tool (Show All Deactivated)

Parameter	Description
Bleaching Method	
- LSM	If no other devices are attached, this is the only list entry and cant be changed. If other devices are attached you can select them from the list.
Start after # of images	If activated, you can set the number of frames that are imaged before the bleaching process.
Repeat after # of images	If activated, you can set the number of images that will separate a repetitive bleaching procedure.
- Iterations	Sets the number of images that will be repeated. This indicates the total amount of scans which are performed for bleaching the selected region during each bleaching process.
Excitation of Bleach	
- Use different settings for different regions	If activated, for each previously drawn ROI a tab is present, in which laser line and laser power can be chosen.
All Regions tab	Only visible if the checkbox above is not activated. Activate the according check box for a laser line and use the slider to adjust the power for bleaching / photo-manipulation. Note that for high laser power the **High Intensity Laser Range** must be set in the **Imaging Setup** or **Channels** tool.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Bleaching Settings	Here you can manage bleaching settings, e.g. save, load or delete settings on the hard disk.
Stop on intensity below	If activated, this function allows during an experiment with repetitive bleaching, to bleach the sample to an intensity value calculated as % of the initial intensity within the region to bleach. The intensity value is determined with every taken image.

Parameter	Description
Set Different scan speed	If activated, the scan speed for bleaching can be set differently than the imaging scan speed. A lower speed results in a longer pixel dwell time, which increases the efficiency of bleaching.
Set different Z-Position	If activated, this function allows to perform the bleaching at a different z-level than currently set. Enter the difference in µm into the input field.
Zoom Bleach (fast, less accurate)	If activated, the bleaching process can be accelerated. In this case the scanner movement will be restricted to the bleaching region zooming in onto this region. This may result in a less accurate positioning of the region as the definition of the region has been made in a different zoom in the image. The bleach process will be faster than without this option. The gain in speed is dependent on the speed that is used for bleaching. If a high speed has already been chosen for bleaching the gain in speed will be minimal.
Protect detectors during bleach	If activated, the PMT gain is set to 0V during the bleaching step. Especially for GaAsP detectors it is strongly recommended to use this option in order to avoid physical damage to the detectors.

14.3.2.15 Interactive Bleaching Tool

This tool allows to bleach interactively during a **Continuous** scan or during a **Time Series** experiment while image acquisition is performed. The bleach region is determined by pointing the mouse onto the position in the image.

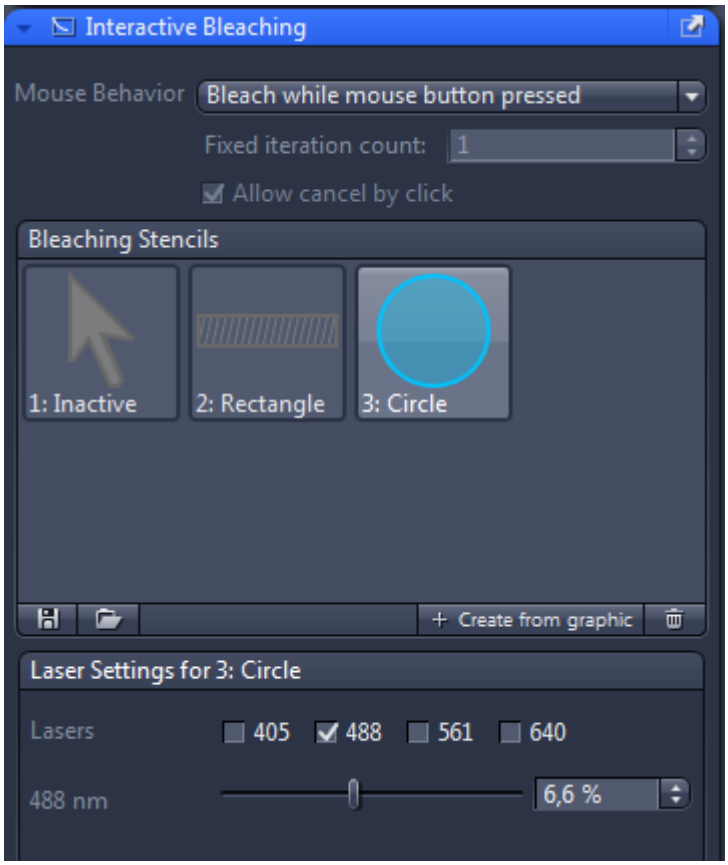


Fig. 65: Interactive Bleaching Tool

Parameter		Description
Mouse Behavior		Change the display of the selected clipping plane using the dropdown list to the right of the **Activate** checkbox. The following settings are available:
-	Bleach while mouse button pressed	The bleaching process is continued while the mouse button is pressed.
-	Bleach fixed number of iterations	The bleaching process is continued for a fixed number of times after the mouse is pressed. The number of iterations can be entered below.
-	Fixed iteration count	Determines the number of iterations.
-	Allow cancel by click	If activated, the bleaching process can be stopped before the fixed number iterations is accomplished. Simply click on the left mouse button again to stop the bleaching process.

Parameter	Description
Bleaching Stencils list	The available bleaching stencils (equivalent to ROIs for bleaching) are listed here. A stencil is displayed in its graphical form, the color displays the laser line that is assigned for bleaching to this stencil. You can add a stencil by clicking the **+ Create from graphic** button. This will import an activated graphical element from the **Graphics** tool tab. A stencil can be saved, loaded or deleted. A stencil is active, when clicked on and highlighted. To use an activted stencil for bleaching, move the mouse curser onto the image and click on the left mouse button.
Laser Settings for section	Here you can set the laser line and laser power for the activated stencil .

14.3.2.16 Dynamics Tool

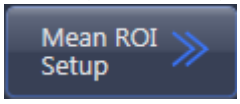
i Note

This tool is only visible if you have licensed and/or activated the module **Physiology** in the **Modules Manager** and additionally activated it on the **Acquisition** tab in the **Experiment Manager**.

The checkbox to activate the tool is only visible if **Time Series** acquisition is active and **Z-Stack** or **Tiles** are not activated.

Using the tool you can open the **Mean ROI Setup** where you can configure dynamics functions for a **Time Series** experiment. Deactivate the **Dynamics** checkbox to deactivate dynamics functions.

If you click on the **Mean ROI Setup** button the setup view will be visible in the **Center Screen Area**. There you will see a snapshot containing all channels of the currently active image.



14.3.2.16.1 Mean ROI Setup

The **Mean ROI Setup** is in essence a modified version of the **MeanROI** view with several minor additions. It allows experiment pre-settings to be made on Snap-shots of the cells/ specimen on which the measurements will be made. These settings include:

- image and chart layouts;
- chart axis settings;
- generation, placement and management of measurement and Background ROIs
- measurement settings

A ratio preview image is also displayed that allows an assessment of the current ratio settings on the Online Ratio tab. To update the images in the Mean ROI Setup a new Snap can be made at any time.

14.3.2.17 Auto Save Tool

i Note

The Auto Save tool is not visible, if you have activated **Panorama** in the Experiment Manager.

When you execute an experiment or click on the **Snap** button, the Auto Save tool is disabled until the experiment is cancelled or finished. The tool also stays disabled when the experiment is paused. This behavior should prevent operation errors during experiments.

If the **Auto Save** checkbox in the **Experiment Manager** is activated, all images which are acquired from the Acquisition tab, are automatically stored as ***.CZI** format during acquisition.

Generally, all images are automatically written to the hard disk during acquisition. This is to prevent data loss in case of technical problems. The folder path for these files is displayed in the status bar under **Storage Folder**. The location can easily be opened by double-clicking on this field. The path can be changed in the **Tools/ Options/Saving** menu. Even though these files are stored physically on the disk, they are indicated with an asterisk and you will be prompted to either rename and store them in a different place, or to delete them. They are maintained only if the software crashes. In case you want to store the files directly in a location of your choice, activate the **Auto Save** checkbox. In this case the files are not written to the temporary folder anymore.

The automatically saved images are contained in the subfolder **temp** within the currently chosen image storage path (default path is: C:\Users\<username>\Pictures \temp). When closing such temporarily saved images you will be asked to specify a document name and a storage location. If you close such an image document without saving it, it will be permanently deleted from the Temp folder in order to prevent the accumulation of unnecessary images.

Parameter	Description
Folder	Shows the directory for the images. The text box is read-only. No values can be entered or pasted by the user. This ensures that the text box contains always a valid directory To change the directory click on the ... button to open the **Browse For Folder** dialog. There you can select a new folder for the auto saved images.
Automatic Sub-Folder checkbox	**Activated:** Creates automatically a top level sub folder in the given directory. The sub folder name is based on the actual date, e. g. 2014-07-04.
Name input field	Here you can specify the image name. An index number is automatically appended to the image name.
Close CZI after acquisition checkbox	**Activated:** Closes the CZI image in the center screen area when the experiment is finished.
File Name Preview	Shows the currently chosen storage path as well as a preview of file name being used next.

14.3.2.18 Automated Image Export Tool

If the **Automated Export** checkbox in the **Experiment Manager** is activated before an experiment is executed, the generated images will be stored in the defined directory with the given parameters and options, provided by the options under the checkbox. This option was developed for automatically exporting images with a user defined format (TIFF or JPEG).

i Note

It is not possible to automatically export images created by the **Snap** function. If you require to use the Auto Export feature for individual images, you must create a Time Series experiment with a single cycle.

When you execute an experiment, the tool is disabled until the experiment is cancelled or finished. The tool also stays disabled when the experiment is paused. This behavior should prevent operation errors during experiments.

For technical reasons images acquired from the Acquisition tab are always auto-saved temporarily as CZI files. If the application requires images to be stored in external common file formats, it is necessary to run the export function. The Automatic Image Export facilitates this in a convenient und automatic way giving the choice of single page TIF or JPEG file formats. It is also possible to automatically close and discard the auto-saved CZI file to streamline the acquisition workflow.

Option / Field	Function
Folder text box	Shows the directory for the images. The text box is read-only. No values can be entered or pasted by the user. This ensures that the text box contains always a valid directory To change the directory click on the ... button to open the **Browse For Folder** dialog. There you can select a new folder for the auto saved images.
Automatic Sub-Folder checkbox	**Activated:** Creates automatically a top level sub folder in the given directory. The sub folder name is based on the actual date, e. g. 2014-07-04.
Prefix input field	Here you can define a prefix for the image file name and a name for the sub folder. If the text box is empty an image gets a localized default prefix (“Untitled”) and a folder gets a localized default name (“New folder”). If an image or folder with a name still exists, the new image or folder gets the same name with an increasing index in accordance to the standard Windows Explorer behavior, e. g. New Folder (1), New Folder (2).
Format dropdown list	Here you select the format for the export images. Two formats are supported: ■ **TIFF**: For the TIFF format (lossless, bigger file size) you can additionally select the **Compression** method. Select **None** for no compression. Or select LZW / ZIP for automatically compressing the files to LWZ/ZIP containers (lossless). ■ **JPEG**: For the JPEG format (lossy, smaller file size) you can set the **Quality** level by adjusting the slider between **Low** (lower quality, smaller images) to **High** (higher quality, bigger file size).
Original data	For **TIFF** format only: **Activated:** Generates an additional raw data TIFF image. Its bit depth depends on the original camera image (Gray 8/16 bit or RGB 24/48 bit)

Option / Field	Function
Gray Scale Linear	For **JPEG** format only: **Activated:** Generates an additional raw data JPEG image. The bit depth depends on the original camera image. In case of 8 bits, the JPEG image has 8 bit gray scale or 24 RGB. In case of 16 bits, the JPEG image is reduced to the "Valid Bits" of the camera. If the camera image is a 16 bit gray scale image, the resulting JPEG image is a 8 bit gray scale image. In case of a 48 bit RGB image the JPEG image has 8 bit RGB. Both additional image types are marked with an **ORG** suffix in their file names.
Apply display curve and channel color	**Activated:** Applies the display curve and channel color to the JPEG or TIFF image
Use channel names	**Activated:** The name of the resulting image contains the name of the defined channel.
Add XML Metadata	**Activated:** Saves an additional xml file with image meta data. Its name has the following nomenclature: Prefix_Metadata(image format).xml Test_Metadata(tif).xml If more than one xml file with the same name exists the file gets an index, e.g. Test-02_Metadata(tif).xml.
Close CZI image after acquisition	**Activated:** Closes the CZI image in the center screen area when the experiment is finished. **NOTICE** **If Auto Save is not activated, this will lead to the loss of the original .czi file for the experiment.**
Dimension / Sub-directory	If you check one of the Channels, Time Series, Z-Stack or Scenes checkboxes, an additional sub-directory will be created if the corresponding dimension exists in the experiment block. The sub-directory will be created in the same image dimension order as the CZI image created, e.g. T-C-Z. The top level folder within the "Dimension" folders is always the B ("Block") folder [new]. Each sub-directory gets a letter that represents its image dimension (T for time series, C for channel, etc.) and an index, if more than one dimension of the same type exists (T=0, T=1).

14.3.3 Tools on Analysis tab

14.3.3.1 Interactive Measurement tool

14.3.3.1.1 Feature Set section

Dropdown list

Here you select and load previously saved feature definitions / feature sets. If you have made changes to a feature definition, the name of the feature selection is marked with an asterisk (*). If you close the application without saving a changed ("asterisked") feature selection, you will be asked whether you want to save the changes.

 **button**

Opens the Options shortcut menu.

Define button

Opens the *Feature Selection* [▶ 452] dialog.

14.3.3.1.2 Feature Subset section

Only visible if the **Show All** mode is activated.

Feature Subset dropdown list

Here you can select and load previously saved definitions of subsets. If you have made changes to a subset definition, the name of the feature subset is marked with an asterisk (*). If you close the application without saving a changed ("asterisked") feature subset, you will be asked whether you want to save the changes.

 **button**

Opens the Options shortcut menu.

Define button

Opens the *Define Feature Subset* [▶ 454] dialog.

14.3.3.1.3 Measurement Sequence section

Only visible if the **Show All** mode is activated.

Dropdown list

Here you can select and load previously saved measurement procedures. If you have made changes to a measurement procedure, the name of the measurement procedure is marked with an asterisk (*). If you close the application without saving a changed ("asterisked") measurement procedure, you will be asked whether you want to save the changes.

 button

Opens the Options shortcut menu.

Define button

Opens the *Define Interactive Measurement Procedure* [▶ 455] dialog.

Run button

Starts the selected *interactive measurement procedure* [▶ 457].

14.3.3.1.4 Create Measurement Table button

Creates a measurement data table. This contains the measurement data from the **Measure** view of the current image.

14.3.3.1.5 Interactive Measurement dialog windows

14.3.3.1.5.1 Feature Selection dialog window

Here you can specify which features are measured with the available graphic elements. This selection is adopted into the current feature definition. The feature definition is then marked ("asterisked") as having been changed.

Available Elements section

In this section you can specify for each available graphic element which features you want to be measured. The graphic elements are ordered by type. The following types are available for selection:

Parameter	Description
Regions (2D)	Here you will find all the graphic elements that define a closed region.
Single Distances	Here you will find all the graphic elements with which you can measure a single distance.
Multiple Distances	Here you will find all the graphic elements with which you can measure several distances at once.
Angle	Here you will find the graphic elements with which you can measure an angle.

Parameter	Description
Point	Here you will find the graphic elements with which you can perform measurements at a pixel.
Events	Here you will find the graphic elements with which you can count various events in an image.

Selected Features section

The features that you have selected for each individual graphic element are listed in this section.

Display checkbox

Activate the **Display** checkbox for each feature to display the value of the measured feature in the graphics plane of the image.

Features section

All the features that you can measure with the graphic element activated in the **Available Elements** section are listed in this section.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered:

Feature	Description
All	If this is selected all features are listed.
Geometric Features	If this is selected all geometric features are listed.
Intensity Features	If this is selected all features that analyze intensity values are listed.
Image Features	If this is selected all features that contain meta information about the measured image are listed.
Position Features / Unscaled Position Features	If this is selected all features that describe the position are listed.

+ button

Click on the + button to select a feature for the measurement.

Wastepaper Bin button

Click on the button to delete the selected feature.

Upwards button

Click on the button to move the selected feature a position higher.

Downwards button

Click on the button to move the selected feature a position lower.

OK button

Click on the **OK** button to end the feature selection.

Cancel button

Click on the **Cancel** button to cancel the feature selection.

14.3.3.1.5.2 Define Feature Subset dialog window

Here you can specify which features are available in the **Feature Selection** dialog window. These features are adopted into the current subset definition. The subset definition is then marked ("asterisked") as having been changed.

Features section

All the features that you can measure with the graphic element activated in the **Available Elements** section are listed in this section.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered:

Feature	Description
All	If this is selected all features are listed.
Geometric Features	If this is selected all geometric features are listed.

Feature	Description
Intensity Features	If this is selected all features that analyze intensity values are listed.
Image Features	If this is selected all features that contain meta information about the measured image are listed.
Position Features / Unscaled Position Features	If this is selected all features that describe the position are listed.

Checkbox

There is a checkbox in front of the name of each of the listed features. Activate the checkbox in front of the features that you want to be offered in the **Feature Selection** dialog.

Shortcut menu

Right-click in the Features section. Select **Select All Features** to activate all checkboxes. Select **Deselect All Features** to deactivate all checkboxes.

OK button

Click on the **OK** button to end the subset definition.

Cancel button

Click on the **Cancel** button to cancel the subset definition.

14.3.3.1.5.3 Define Interactive Measurement Procedure dialog window

Here you can define an interactive measurement procedure. You can specify the order in which you want the individual graphic elements to be drawn in and which measurement parameters you want to have calculated for them. The definition is adopted into the measurement procedure currently selected. The measurement procedure is then marked ("asterisked") as having been changed.

Available Elements section

In this section you can specify for each available graphic element which features you want to be measured. The graphic elements are ordered by type. The following types are available for selection:

Parameter	Description
Regions (2D)	Here you will find all the graphic elements that define a closed region.

Parameter	Description
Single Distances	Here you will find all the graphic elements with which you can measure a single distance.
Multiple Distances	Here you will find all the graphic elements with which you can measure several distances at once.
Angle	Here you will find the graphic elements with which you can measure an angle.
Point	Here you will find the graphic elements with which you can perform measurements at a pixel.
Events	Here you will find the graphic elements with which you can count various events in an image.

Double-click on a graphic element to select it and adopt it into the **Order of the Elements** section.

Order of the Elements list

This list displays the selected graphic elements in the order in which they will be drawn in during the measurement, from top to bottom. To display the value of the measured feature in the image's graphics plane, activate the corresponding checkbox of the graphic elements.

Wastepaper Bin button

Click on the  button to delete the selected feature.

Upwards button

Click on the  button to move the selected feature a position higher.

Downwards button

Click on the  button to move the selected feature a position lower.

Features section

All the features that you can measure with the graphic element activated in the **Available Elements** section are listed in this section.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered:

Feature	Description
All	If this is selected all features are listed.
Geometric Features	If this is selected all geometric features are listed.
Intensity Features	If this is selected all features that analyze intensity values are listed.
Image Features	If this is selected all features that contain meta information about the measured image are listed.
Position Features / Unscaled Position Features	If this is selected all features that describe the position are listed.

+ button

Click on the + button to select a feature for the measurement.

14.3.3.1.5.4 Interactive Measurement Procedure dialog

In this dialog you can run a predefined interactive measurement procedure.

Start button

Click on the **Start** button to begin drawing in the graphic elements in the image.

Pause button

Click on the **Pause** button to interrupt the measurement procedure. This allows you to modify graphic elements that have already been drawn in, for example.

Continue button

If you have interrupted the measurement procedure using the **Pause** button, click on the **Continue** button to continue measuring.

End button

Click on the **End** button to end the current measurement procedure at a position of your choice. To restart the measurement procedure at the first graphic element, click on the **Start** button again.

Graphic Elements section

In this section you will see the graphic elements of the current measurement procedure in the order that you have defined.

Measurement Data section

In this section you will see the graphic elements of the current measurement procedure in the order that you have defined.

OK button

Click on the **OK** button to end the interactive measurement procedure.

Cancel button

Click on the **Cancel** button to cancel the interactive measurement procedure. In this case the graphic elements that have been drawn in are not adopted into the image.

14.3.3.2 Image Analysis tool

Program dropdown list

In the **Program** dropdown list you can select and load previously saved analysis programs.

Set-up Image Analysis button

To define a new analysis program or to change an existing program, click on the *Setup Image Analysis* [▶ 458] button.

Analyze Interactive button

Runs the selected analysis program with all the interactive steps.

Analyze button

Runs the selected analysis program without interruption.

Steps that you have marked as interactive in the **Image Analysis Wizard** are run with the values set in the analysis program. The program does **not** stop to allow you to change these interactively.

14.3.3.2.1 The Image Analysis Wizard

14.3.3.2.1.1 Classes

In this step you can define the classes into which the measured objects in the image are divided.

Back button

Moves back to the previous step of the wizard.

Classes list

The defined classes are listed here. If you create a new measurement program, a class is created automatically. Each class consists of two entries. The first entry concerns all the objects belonging to the class. The second entry represents an individual object.

Add Class button

Adds a new class to the list.

Remove Class button

Deletes the selected class from the list.

Name input field

Here you can enter a name for the selected class in the **Classes** list.

Channel selection

If you create a measurement program for a multichannel image, in this selection field you can select a channel for the selected class in the **Classes** list.

Color button

Opens the **Color Selection** dialog window.Here you can select a color that you want to assign to the selected class.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

14.3.3.2.1.2 Frame

In this step you can define one or more measurement frames. These allow you to specify how objects are dealt with at the edge of the image.

Back button

Moves back to the previous step of the wizard.

Parameter	Description
Interactive checkbox	**Activated:** The measurement frame definition can be changed interactively while the measurement program is running.

Parameter	Description
Tool bar	Use this to select measurement frames that have already been drawn in. To select a measurement frame, click inside it. To select several measurement frames, hold down the **Ctrl** key and click inside the desired measurement frames. Once you have selected a measurement frame, you can change its size.
- Rectangle	Use this to create a rectangle as a measurement frame in the current image.
- Circle	Use this to create a circle as a measurement frame in the current image.
- Contour	Use this to create a contour as a measurement frame in the current image.
- Delete	Deletes all drawn-in measurement frames in the current image.
Maximize Circle checkbox	**Activated:** Maximizes the drawn-in circle to the full image size. In the case of rectangular images the circle is adjusted to the shorter side. Only active if you have defined precisely one circle.
Center Circle checkbox	**Activated:** Centers the drawn-in circle to the full images size. Only active if you have defined precisely one circle.
Mode dropdown list	The following fields are only active if you have selected a drawn-in graphic element: Here you can select how you want the measurement frame to be applied. The following modes are available:
- Inside and touching	All objects are measured that are lying completely within the measurement frame, are touching it or are intersected by it.
- Inside Only	Only those objects are measured that are lying completely within the measurement frame. Objects that are touching the frame or are intersected by it are not measured.
- Cut at Frame	All objects are measured that are lying completely within the measurement frame. Objects that are intersected by the measurement frame are measured precisely up to the measurement frame.
Left spin box/ input field	Here you can enter the start point for the frame on the X axis in pixels.

Parameter	Description
Top spin box/ input field	Here you can enter the start point for the frame on the Y axis in pixels.
Width spin box/ input field	Here you can enter the width of the measurement frame in pixels.
Height spin box/ input field	Here you can enter the height of the measurement frame in pixels.
Next button	Moves on to the next step of the wizard.
Cancel button	Cancels the analysis program.

14.3.3.2.1.3 Automatic Segmentation

In this step you can enter the threshold values for the segmentation of the objects that you want to measure.

Back button

Moves back to the previous step of the wizard.

Execute checkbox

Activated: Sets the defined threshold values when the measurement program is run.

Interactive checkbox

Activated: The defined threshold values can be changed while the measurement program is running.

Classes list

Here you can select the class for which you want to define the threshold values.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

14.3.3.2.1.3.1 Smooth section

Parameter	Description
Smoothing dropdown list	Here you can select how you want to smooth the image before the threshold values are set. The following methods are available:
- None	The image is not smoothed.
- Low Pass	Applies the Low Pass Method.
- Gaussian	Applies the Gaussian Method.
- Median	Applies the Median Method.
Size/Sigma slider	Enter the size of the filter matrix in the X and Y direction or the sigma value using the slider or spin box/input field.

14.3.3.2.1.3.2 Sharpen section

Parameter	Description
Image Sharpness dropdown list	Here you can select how you want to improve the sharpness of the image before the threshold values are set. The following methods are available:
- None	The sharpness of the image is not changed.
- Edge Enhancement	Applies the Edge Enhancement Method.
- Unsharp Masking	Applies the Unsharp Masking Method.
Threshold Value slider	Enter the threshold value for edge detection using the slider or spin box/input field. The threshold value should correspond roughly to the gray value difference between objects and the background.
Size slider	Enter the size of the edge detection filter using the slider or spin box/input field. The value should correspond to the size of the transition area between objects and the background.
Strength slider	Enter the strength of the Unsharp Masking using the slider or spin box/input field. The higher the value selected, the greater the extent to which small structures are enhanced.

14.3.3.2.1.3.3 Minimum Area section

Minimum Area slider

Using the slider or spin box/input field, enter the minimum area in pixels that an object must have in order to be segmented.

14.3.3.2.1.3.4 Threshold section

Here you can define the threshold values for the selected class in the **Classes** list.

Parameter	Description
Reset button	Resets all threshold value settings.
Undo button	Undoes the last change made to the threshold values.
Redo button	Restores the last undone change to the threshold values.
Color Model buttons	Only visible if the image is a color image.
- RGB	In *RGB Mode* [▶ 465] you can define the threshold values for the red, green and blue color channels.
- HLS	In *HLS Mode* [▶ 466] you can define the threshold values for hue, saturation and lightness.
Histogram	In the histogram you can change the lower and upper threshold value for the activated value. Drag the lower or upper adjustment handle or shift the entire highlighted area between the lower and upper threshold value.
Threshold Value Definition button	
- Click	Click in the image on the regions that you want to define as objects.
- Automatic	The threshold values are determined automatically.
Click Behavior buttons	The following parameters are only visible if **Click** has been selected for threshold value definition:
- **+** button	Click on this button to expand the currently segmented regions by the gray values/colors of the objects subsequently clicked on.
- **-** button	Click on this button to reduce the currently segmented regions by the gray values/colors of the objects subsequently clicked on.

Parameter	Description
Tolerance slider	Using the slider or spin box/input field, enter the tolerance range by which the gray/color value read out when you click is expanded to define the threshold value.
Neighborhood slider	Using the slider or spin box/input field, enter a neighborhood range around the pixel clicked on. The threshold value is calculated from the average of the gray/color values in this neighborhood range. The following parameter is only visible if **Automatic** has been selected for threshold value definition:
Method dropdown list	Select the method from the dropdown list that you want to use for the automatic calculation of the threshold values. The following methods are available:
- Otsu	The threshold value is calculated according to the Otsu method.
- Most Frequent Gray Value	The threshold value is the gray/color value that occurs most frequently in the image.
- Iso Data	The threshold value lies in the middle between two maximums in the histogram.
- Triangular Threshold Value	The threshold value is calculated from the sum of the average and three times the sigma value of the histogram distribution.
- 3 Sigma Threshold Value	

i Note

After the automatic calculation of the threshold values you can further modify the threshold values found interactively by selecting **Click** for threshold value definition.

14.3.3.2.1.3.4.1 RGB mode

Red Color Channel threshold values

Red button

Click on the red  button to activate the red channel in the Expander **histogram**.

Lower (Red Channel) input field

Enter the lower threshold value for the red channel using the spin box/input field.

Upper (Red Channel) input field

Enter the upper threshold value for the red channel using the spin box/input field.

Invert (Red Channel) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the red channel.

All (Red Channel) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible gray value for the red channel.

Green Color Channel threshold values

Green button

Click on the green  button to activate the green channel in the Expander **histogram**.

Lower (Green Channel) input field

Enter the lower threshold value for the green channel using the spin box/input field.

Upper (Green Channel) input field

Enter the upper threshold value for the green channel using the spin box/input field.

Invert (Green Channel) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the green channel.

All (Green Channel) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible gray value for the green channel.

Blue Color Channel threshold values

Blue button

Click on the blue  button to activate the blue channel in the Expander **histogram**.

Lower (Blue Channel) input field

Enter the lower threshold value for the blue channel using the spin box/input field.

Upper (Blue Channel) input field

Enter the upper threshold value for the blue channel using the spin box/input field.

Invert (Blue Channel) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the blue channel.

All (Blue Channel) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible gray value for the blue channel.

14.3.3.2.1.3.4.2 HLS mode

Hue threshold values

H button

Click on the  button to activate the hue in the Expander **histogram**.

Lower (Hue) input field

Enter the lower threshold value for the hue using the spin box/input field.

Upper (Hue) input field

Enter the upper threshold value for the hue using the spin box/input field.

Invert (Hue) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the hue.

All (Hue) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible value for the hue.

Lightness threshold values

L button

Click on the  button to activate the lightness in the Expander **histogram**.

Lower (Lightness) input field

Enter the lower threshold value for the lightness using the spin box/input field.

Upper (Lightness) input field

Enter the upper threshold value for the lightness using the spin box/input field.

Invert (Lightness) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the lightness.

All (Lightness) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible value for the lightness.

Saturation threshold values

S button

Click on the  button to activate the saturation in the Expander **histogram**.

Lower (Saturation) input field

Enter the lower threshold value for the saturation using the spin box/input field.

Upper (Saturation) input field

Enter the upper threshold value for the saturation using the spin box/input field.

Invert (Saturation) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the saturation.

All (Saturation) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible value for the saturation.

14.3.3.2.1.3.5 Fill section

Fill Holes checkbox

Activated: Fills holes in segmented objects.

14.3.3.2.1.3.6 Separate section

Parameter		Description
Separate dropdown list		Here you can select whether you want to process the image further after segmentation. Objects that are touching one another can be separated using different methods.
-	None	Objects are not separated.
-	Watersheds	Using this method you can separate objects that are roughly the same shape. This method may however result in the splitting of elongated objects.
-	Morphology	This method separates objects by first reducing and then enlarging them, making sure that once objects have been separated they do not merge together again.
Number slider		Using the slider or spin box/input field, enter how often the method is applied successively to the result at the location of the separation.

14.3.3.2.1.4 Condition

In this step you can define the conditions under which you want an object to be measured.

Back button

Moves back to the previous step of the wizard.

Execute checkbox

Activated: Uses the measurement conditions when the measurement program is run.

Interactive checkbox

Activated: The measurement conditions can be changed while the measurement program is running.

Classes list

Here you can select the class for which you want to define the conditions.

Edit button

Opens the *Conditions Editor* [▶ 469] dialog window.

Conditions list

If you have defined one or more blocks with conditions in the **Conditions Editor** dialog window, here you can select the block for which you want to set the condition. To do this, click on the relevant block and then on the objects in the image that you want to measure.

Undo button

Undoes the last change made to the condition.

Redo button

Restores the last undone change to the condition.

Reset button

Resets all settings for the conditions.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

14.3.3.2.1.4.1 Conditions Editor dialog window

Here you can add features to a block with measurement conditions.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered:

Feature	Description
All	If this is selected all features are listed.
Geometric Features	If this is selected all geometric features are listed.
Intensity Features	If this is selected all features that analyze intensity values are listed.

Feature	Description
Image Features	If this is selected all features that contain meta information about the measured image are listed.
Position Features / Unscaled Position Features	If this is selected all features that describe the position are listed.

+ button

Click on the + button to select a feature for the measurement.

Selected Features for the Condition list

In this list, the features that you have selected for the condition are displayed block by block. All features in a block are "And"-linked for the condition, i.e. an object is only measured if the values of each individual feature fall within the defined range.

Add button

Adds an "Or" block. If several "Or" blocks have been defined, an object is measured if it meets the condition in at least one block.

Empty button

Deletes all features in an "Or" block.

Remove button

Deletes the selected "Or" block.

Remove All button

Deletes all "Or" blocks.

14.3.3.2.1.5 Interactive Segmentation

In this step you can postprocess the segmented objects interactively.

Back button

Moves back to the previous step of the wizard.

Interactive checkbox

Activated: The segmented objects can be postprocessed interactively while the measurement program is running.

Classes list

Here you can select the class whose objects you want to process.

Remove button

Using this button you can remove parts of an object. Holding down the left mouse button, outline the parts of the object that you want to remove. Right-click to remove these parts of the object.

Separate button

Use this button to separate connected objects. Holding down the left mouse button, draw in the separation line between the objects. Right-click to separate the objects.

Connect button

Use this button to connect objects. Holding down the left mouse button, outline the parts of the object that you want to connect. Right-click to connect the objects.

Delete button

Click on the **Delete** button to delete an object by subsequently clicking on it.

Tool bar

Selection Switches the mouse pointer to Selection mode.

Rectangle

Use this button to add a rectangular object or cut a rectangular region from an object.

Circle

Use this button to add a circular object or cut a circular region from an object.

Draw Contour

Use this button to add an object or cut a region from an object.

Contour (Spline)

Use this button to add an object or cut a region from an object.

Active Contour

Use this button to add an object or cut a region from an object.

Delete

Deletes all objects.

Keep checkbox

Activated: The selected graphic element remains selected until you deselect it.

Fill Hole button

Fills a hole. To fill a hole, left-click on the hole.

Region Growth button

Activates the Region Growth mode. Then click on objects in the image that you want to segment. The object "grows" around the point that has been clicked on.

Intensity slider

Enter a tolerance value for the intensity using the slider or spin box/input field. The tolerance value specifies how much the intensity of a pixel may deviate from the average intensity of the object in order to still "grow" to become part of the object.

Color slider

Only active if your input image is a color image.

Enter a tolerance value for the color using the slider or spin box/input field. The tolerance value specifies how much the color value of a pixel may deviate from the average color value of the object in order to still "grow" to become part of the object.

Undo button

Undoes the last action.

Redo button

Restores the last undone action.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

14.3.3.2.1.6 Features

In this step you can select the measurement features that you want to measure.

Back button

Moves back to the previous step of the wizard.

Classes list

Here you can select the class for which you want to define measurement features. For each class there are two entries for which you can define features. The first entry concerns all the objects belonging to the class. The second entry represents an individual object.

Field Features section

Only visible if a "parent" class is active.

The list shows all Field Features that are measured for the current class.

Object Features section

Only visible if a "child" class is active.

The list shows all Object Features that are measured for the current class.

Annotations section

Only visible if a "child" class is active.

The list shows all annotations that are drawn in for the current class.

Select button

Opens the *Feature Selection* [▶ 473] dialog window for the current class.

Copy button

Copies the selected features of the current class to all other classes.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

14.3.3.2.1.6.1 Feature Selection dialog window

Here you can specify which features you want to measure.

Selected Features section

The features that you have selected are listed in this section.

Wastepaper Bin button

Click on the  button to delete the selected feature.

Upwards button

Click on the  button to move the selected feature a position higher.

Downwards button

Click on the  button to move the selected feature a position lower.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered:

Feature	Description
All	If this is selected all features are listed.
Geometric Features	If this is selected all geometric features are listed.
Intensity Features	If this is selected all features that analyze intensity values are listed.
Image Features	If this is selected all features that contain meta information about the measured image are listed.
Position Features / Unscaled Position Features	If this is selected all features that describe the position are listed.

+ button

Click on the  button to select a feature for the measurement.

OK button

Click on the **OK** button to end the feature selection.

Cancel button

Click on the **Cancel** button to cancel the feature selection.

14.3.3.2.1.7 Measure

In this step you will see the result of the measurement. The measured image is displayed in the *Analysis View* [▶ 554].

Back button

Moves back to the previous step of the wizard.

Classes list

Here you can select the class for which you want to see the measured features. For each class there are two entries: The "parent" class, which shows the features for all objects together, and the "child" class, which shows the features for each individual object.

End button

Saves the analysis program created and ends the wizard.

Cancel button

Cancels the analysis program.

14.3.4 Tools in Right Tool Area

14.3.4.1 Images and Documents Tool

In the Images and Documents tool, also called Images and Documents Gallery, you find a list of all images and documents which are currently opened in the **Center Screen Area**. The disk symbol with a small warning sign  means, that you changed and/or have not saved the chosen image or document.

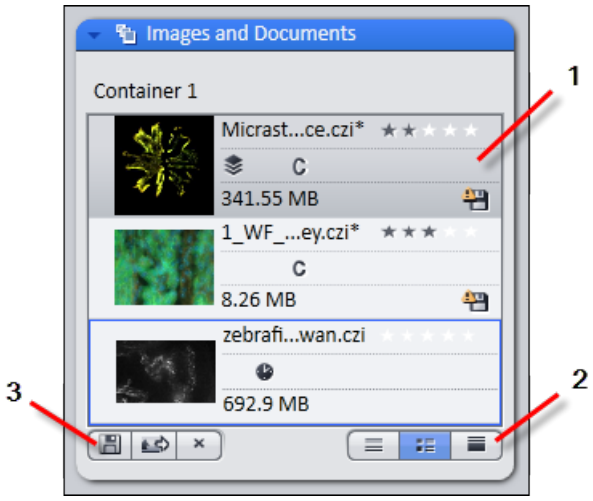


Fig. 66: Images and Documents Gallery

1 List of opened images and documents

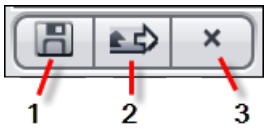
2 Button bar with buttons to change the current view (i.e. Text view or Thumbnail view)

3 Button bar with Save, Quick Export and Close buttons

Button bar (2)

No.	Button	Function
1	Text view	Shows text of file only.
2	Thumbnail view	Shows a small preview image (thumbnail) of file.
3	Image view	Shows a preview image of file.

Button bar (3)



No.	Button	Function
1	Save	Saves he chosen file. **Save as** dialog will open if you haven't saved the file yet.
2	Quick Export	Automatically exports the active image with the default settings of **Single File Export** method to…/**user/pictures** (Windows default folder for images). Images of time series or z-stacks will be automatically exported with the default settings of **Movie Export** method to…**/user/movies** (Windows default folder for movies).
3	Close	Closes the active image or document.

14.3.4.2 Stage Tool

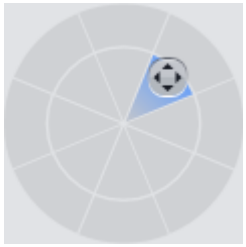
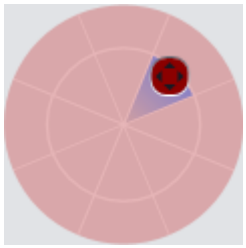
⚠ CAUTION

Risk of crushing fingers.

The drives for a motorized horizontal stage axis (stage drive) are strong enough to crush fingers or objects between the stage and nearby objects (e.g. a wall).

- Remove your fingers or any objects from the danger area before moving the stage.
- Do not touch the stage while it is moving.
- Do not attach or remove the stage while the microscope stand is on.
- Release the joystick immediately to stop the movement.

This tool enables you to navigate the sample in a microscope equipped with a motorized stage. You can use the **Navigation Circle** (software joystick) to move the stage or enter the coordinates directly.

Parameter	Description
Navigation Circle	Enables you to move the stage freely in the X and Y direction and in both diagonal directions. To move the stage, drag the **Navigation Circle** icon in the desired direction. If released, the icon snaps back to the **Navigation Circle** center and the stage stops. The **Navigation Circle** allows four speeds: ■ Normal modes: – Inner segments: Slow – Outer segments: Medium  ■ High-speed modes: – Inner segments: Fast – Outer segments: Very Fast  To enter the high-speed mode, right-click the **Navigation Circle** icon. The **Navigation Circle** turns red. To return to normal speed, right-click the **Navigation Circle** icon again.
Stop	Stops any stage movement immediately. Use this button if you entered **X-Position** and/or **Y-Position** and wish to interrupt the stage movement immediately (e.g. to prevent a collision).
X-Position, Y-Position	Specifies the target coordinates. The stage starts moving immediately after the coordinates have been entered and confirmed; either by pressing the **Return** key or by clicking anywhere outside the current input field.

TIP

You can also control the **Navigation Circle** and thus the motorized stage with the keyboard. To activate keyboard control left-click anywhere inside the segmented **Navigation Circle**. To change between the two speed modes, right-click the central**Navigation Circle** icon.

- To move the stage at the lower speed, use the arrow keys (diagonal movements are also possible).
- To move the stage at the higher speed, use **Shift** + arrow keys.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Moving	Sets the moving speed of the stage in percent (100%= maximum possible speed).
X/Y-Position	
- Set Zero	Sets the current position as the new zero point for the x/y coordinates.
- Calibrate	⚠ **CAUTION** **Risk of Crushing Fingers.** Performs an automatic stage calibration. For this the stage moves to the limit switches to determine the zero points in the x and y direction and then returns to its starting position, which is now defined with its absolute coordinates.
Stage Area	If you click on the **Show Stage** button, a representation of the stage area will be displayed in the image area. It is displayed by a rectangular, dashed white frame.

14.3.4.2.1 Marks section

This section shows a list where you can define **X** / **Y** positions (optional z value), so called marks. It is also possible to import a list of positions from the marks list into an experiment including the Tiles tool.

14.3.4.3 Focus Tool

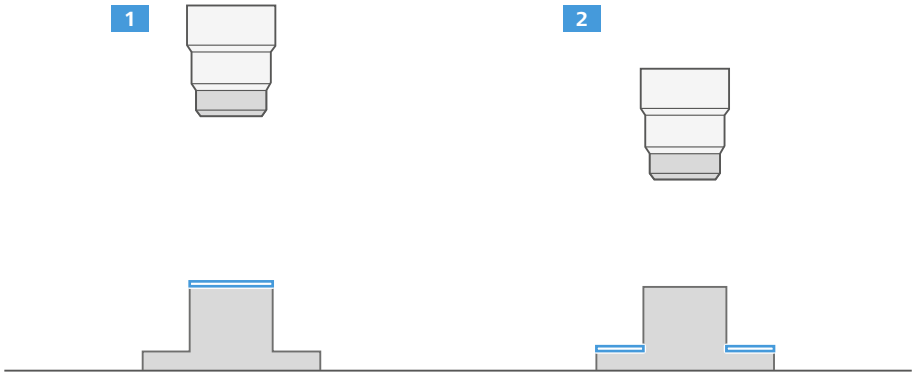
CAUTION

Risk of crushing fingers.

The drives for a motorized vertical axis (focus drive) are strong enough to crush fingers or objects between the stage and the microscope stand.

- Remove your fingers or any objects from the danger area before moving the focus.
- Release the joystick immediately to stop the movement.

This tool changes the vertical distance (i.e. Z direction) between stage and objective. This enables you to focus the sample, or, for a sample with an uneven surface, to focus the area of interest.



1 Higher objective position, higher sample features in focus
2 Lower objective position, lower sample features in focus

Parameter	Description
Current	Displays the stage position in µm Initially, when you use the **Focus** tool for the first time after switching on the microscope, the exact position of the stage is not known. Therefore, the position indicated by **Current** is initially set to zero. If you enter a value, the stage moves by the entered amount relative to the current position. If you want to move the focus to an absolute position, you must first click **Home** to move the focus to one of the end positions. The value of **Current** is set to this known position. You can then enter an absolute position.
Position control	Enables you to set the stage position. You can either use the **Navigation Bar** to move the stage up or down or you can enter the target position in the **Current** input field.

Parameter	Description
– **Navigation Bar**	Enables you to move the stage freely in Z direction To move the stage, drag the **Navigation Bar** button in the desired direction. If released, the **Navigation Bar** button snaps back to the center and the stage stops. The **Navigation Bar** allows four speeds.  Normal modes: ◆ Inner segments: Slow ◆ Outer segments: Medium  High-speed modes: ◆ Inner segments: Fast ◆ Outer segments: Very Fast To enter the high-speed mode, right-click the **Navigation Bar** button. The **Navigation Bar** turns red. To return to normal speed, right-click the **Navigation Bar** again.
– **Current** input field	Defines the target position of the stage in µm. The stage starts moving immediately after the coordinates have been entered and confirmed by pressing the *Enter* key or by clicking anywhere outside the **Current** input field.
– **Stop**	Stops any stage movement immediately.
Backlash Correction	Activated: Enhances the positional accuracy by performing an extra movement. When activated the focusing takes slightly longer
Handwheel on	■ Activated: Turning the handwheel also adjusts the focus ■ Deactivated: The handwheel is deactivated: turning it does not affect the focus
Step Size	Defines the difference in µm by which the stage moves at each step. Indirectly this defines the speed of the stage movement. The **Step Size** also determines the accuracy of the focus position.

Parameter	Description
Home	Moves the focus to one of the end positions. The value of **Current** is set to this known position. This ensures that the position shown as **Current** corresponds to the actual stage position.
Work	Moves the stage back to the position it was in before using the **Load** button (i.e. the work position) If you have moved the stage (e.g. using the **Navigation Bar**) after moving it into the load position, the work position is lost and the **Work** button will not work.
Load	Increases the distance between objective and stage by 8,000 µm This aids you in exchanging the sample. After exchanging the sample, you can move the stage back into its work position by using the **Work** button. Make sure not to move the stage (e.g. using the **Navigation Bar**) after moving it into the load position. Otherwise, the previous position is lost and the **Work** button will not work.
Measure	This function allows to measure distance in Z direction.
Z-Position	Specifies which position of the motorized z drive is used as the origin (zero value)
– **Set Zero**	Sets the current focus position as the origin (zero value)
– **Calibrate**	Performs an automatic calibration

14.3.4.4 Incubation Tool

In the Incubation Tool you can define and control parameters for temperature, atmosphere and the Y-Module. The available parameters depend on which components you have configured on your system.

i Note

The symbols behind measured values indicate if the measured and the set values are the same = green check mark, different = red or blue triangle with exclamation point or not activated = blue circle with question mark.

Parameter	Description
Temperature	Here you can control up to 4 independent heating channels that are linked to certain devices (e.g. incubator XL, heating insert P, objective heater etc.). The devices are assigned to different channels in the Micro Tool Box (MTB).
- Channel (1-4)	**Activated:** The channel will be used for the experiment. Under **Setpoint** you can set the temperature of the channel in °C. Under **Measured** you see the currently measured value.
- Sensor	Shows the current temperature inside the incubation chamber.
Atmosphere	Here you can define the O2 and CO2 concentration, as well as the temperature for an Air Heater module. Note that the meaning of the symbols behind the measurement values is the same like described above.
- O2 Channel	**Activated:** The O2 channel will be used for the experiment. Under **Setpoint** you can set the O2 concentration of the chamber in percent (1-100%). Under **Measured** you see the currently measured value.
- CO2 Channel	**Activated:** The CO2 channel will be used for the experiment. Under **Setpoint** you can set the CO2 concentration of the chamber in percent (1-100%). Under **Measured** you see the currently measured value. ■ **Fan Speed**: Sets the rotation speed of the fan.
- Air Heater	**Activated:** The air heater will be used for the experiment. Under **Setpoint** you can set the temperature of the air heater in °C. Under **Measured** you see the currently measured value. ■ **Fan Speed**: Sets the rotation speed of the fan.
Y-Module	The Y-Module panel allows setting the temperature for two independent modules (thermostats).

Parameter		Description
-	Selected	Here you can select which module you want to control (**Module 1** or **Module 2**).
-	Circulator 1-2	**Activated:** The channel will be used for the experiment. Under **Setpoint** you can set the temperature of the channel in °C. Under **Measured** you see the currently measured value. For each module two circulator channels can be activated.

14.3.4.5 Macro Tool

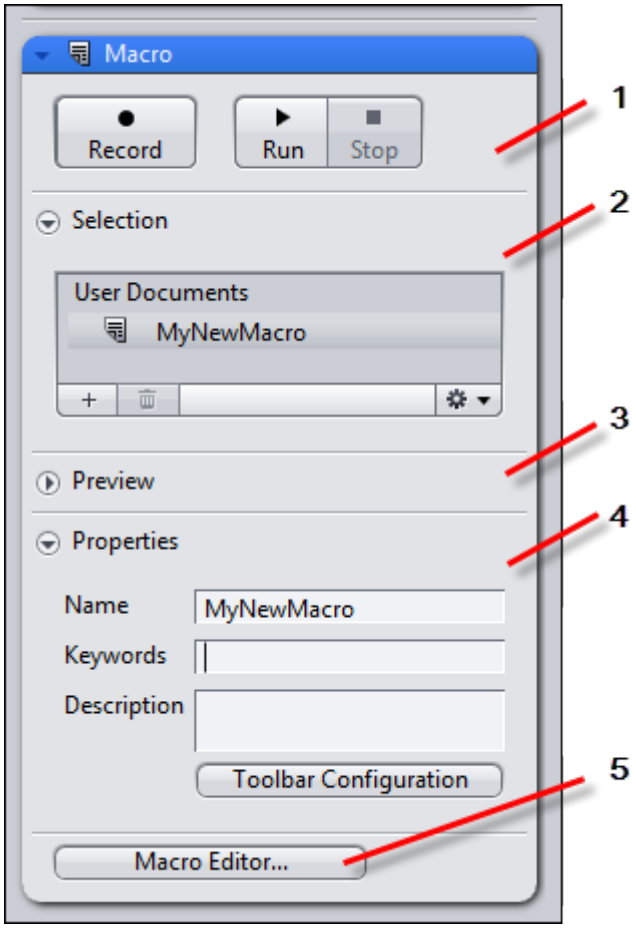


Fig. 67: Macro tool

Button bar (1)

Button	Function
Record/ Stop	Records a new macro. The record button will change to a stop button. Click the **Stop** button to stop the macro recording.

Button	Function
Run	Executes the selected macro.
Stop	Stops the running macro at the active command.

Selection section (2)

Here you find a list of all existing macros. Via the **Options** menu  you can create, duplicate, rename and safe new macro files or delete existing macros.

Preview section (3)

Here you see a preview to the macro program code of the selected macro. Editing the macro here is not possible.

Properties section (4)

Here you see information of the selected macro. You can edit the fields **keywords** and **description** in here.

Toolbar Configuration button

When you click on the button you will enter the **Customize Toolbar** dialog. There you can add macro buttons or functions to the toolbar for a quick access. How you can configure the toolbar is described under *Customizing toolbar* [▶ 140].

Macro Editor… button (5)

Opens the *Macro Editor dialog* [▶ 494].

14.4 Dialogs

14.4.1 Add Dye or Contrasting Method dialog

Here you add dyes and contrast techniques to your experiment. The dyes in the database contain important information that is saved in the image document (e.g. spectral characteristics). This information can be used later during image processing (e.g. deconvolution).

i Note

You can add additional dyes to the database via the **Dye Editor** under **Tools | Dye Editor**.

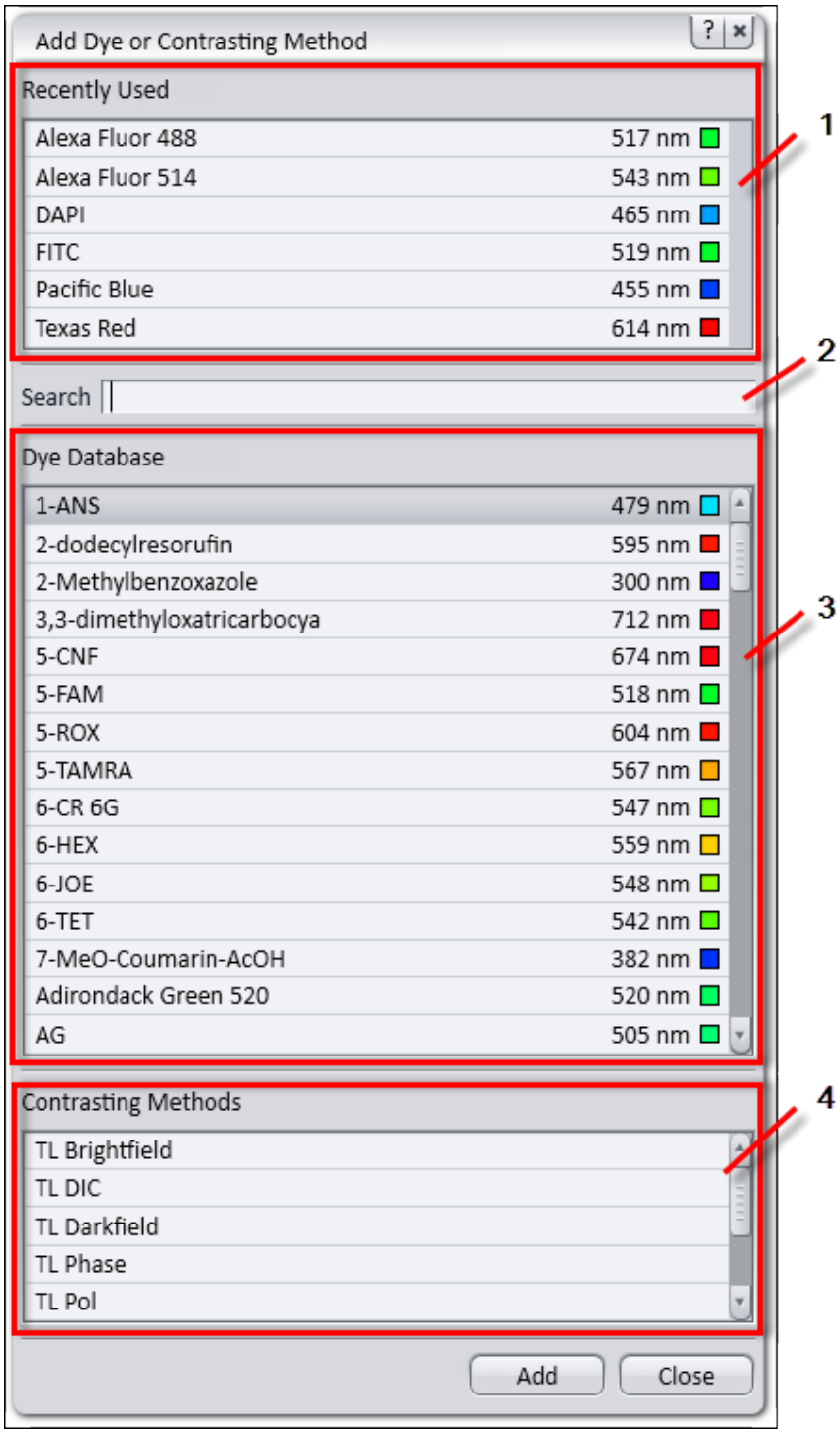


Fig. 68: Add dye dialog

Parameter	Description
Recently used list (1)	Shows the six recently used dyes or contrasting methods in a list. This ensures that you have quick access to the dyes or contrasting methods that you frequently use.

Parameter	Description
Search input field (2)	Enter the name or initial letters of the dye or contrasting method that you want to search for. The search results are displayed immediately in the **Dye Database** list or the **Contrasting Methods** list. If no search filter is active, the lists of dyes or contrast techniques are arranged in alphabetical order. If you cannot find a certain dye, try using a related dye name or a general name.
Dye Database list (3)	Choose fluorescent dyes here. Double click on the dye or click on **Add** button to add it to the experiment. The left column shows the name of the dye. The right column contains its color and main emission wavelength. The "**Custom**" entry adds a channel to your experiment without any additional information. This means that the resulting image cannot be used for certain processing operations.
Contrasting Methods list (4)	This is only available for WF tracks. Choose contrasting method to be used here. Double click on the contrasting method or click on **Add** button to add it to the experiment.
Add/ **Close** button	Adds the selected dye to the experiment. If you click on the **Close** button the dialog closes without adding a dye.

14.4.2 Import experiment blocks dialog

Here you can import experiment blocks from existing experiments.

Choose experiment dropdown list

Here you select the experiment you want to import experiment blocks from. The experiment must have been saved on your computer before.

Select desired blocks section

Here you can select the experiment blocks you want to import. Simply click on an experiment block to select it. Then click on **Import** button. This will import the selected experiment block or blocks (if you have selected more than one) to the experiment timeline. If you want to import all blocks of an experiment don't select a block but continue by clicking on the **Import** button directly. This will import all experiment blocks at once.

Import button

Imports the selected experiment blocks.

Cancel button

Cancels the import.

14.4.3 Dye Editor dialog

Here you can edit and create data sets for fluorescent dyes. You can also see the excitation, emission and extinction curves of the selected dye displayed in a graph. The Dye Editor displays databases that are available in the *.ExEmL file format. ExEmL stands for **Ex**citation and **Em**ission **L**ibrary and is a special variant of the .XML format.

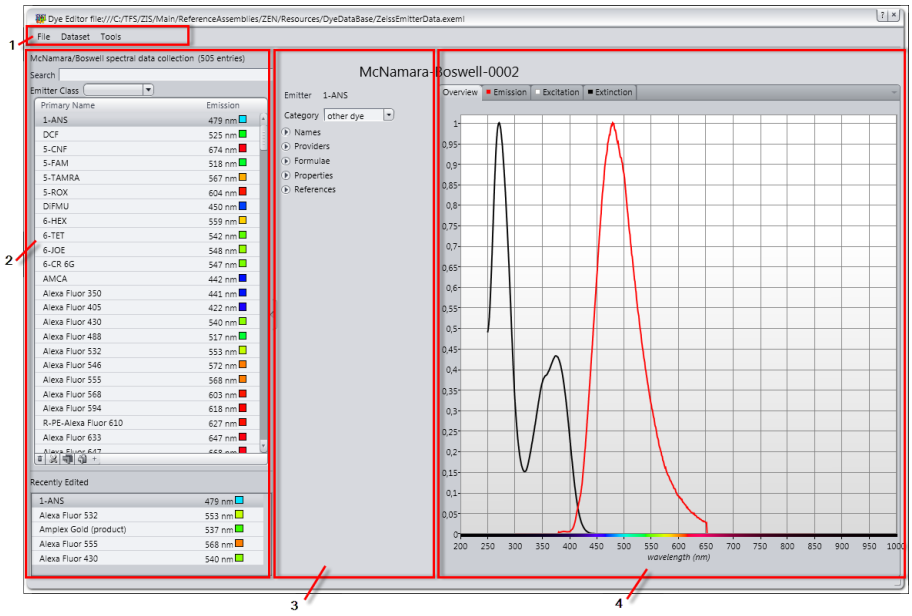


Fig. 69: Dye Editor

No.	Name	Description
1	Menu	Here you can create new data sets and import available dye databases. A detailed description can be found under *Menu* [▶ 489].
2	Dye search & database	The list shows the dyes that are available in the open database. You can search for dyes and see which dyes you edited last.
2	Dye information area	Here you can see all the additional information about a selected dye.

No.	Name	Description
4	Dye spectra display field	Here you can see the available dye spectra. Click on the relevant tabs to display the **emission**, **excitation**, or **extinction** spectra. On the **Overview** tab you can see all spectra at a glance.

14.4.3.1 Menu

File menu

Menu item	Function
New (Ctrl+N)	Creates a new ExEml file in which you can create dye data sets.
Open File (Ctrl+O)	Opens a single ExEml file.
Open Folder	Opens several ExEml files that have been saved together in the same folder.
Import File	Imports an ExEml file.
Save (Ctrl+S)	Saves the open ExEml file.
Save As	Saves the open ExEml file under a new name.
Save Folder	Saves all open ExEml files in a folder.
Close	Closes the Dye Editor.

Data Set menu

Menu item	Function
Add Data Set	Creates a new, empty data set.
Copy	Copies the selected data set to the clipboard.
Cut	Cuts the selected data set and copies it to the clipboard.
Delete	Deletes the selected data set.
Paste	Pastes a data set from the clipboard.
Paste Range	Pastes a range of a data set from the clipboard.

Tools menu

Menu item	Function
Create Abbreviations	Creates abbreviations for names of dyes. This helps to avoid duplication.
Calculate Main Emission Wavelength	Calculates the main emission wavelength of the selected dye from spectral data that has either been copied or entered manually.

14.4.4 Modules Manager dialog

Here you can activate or deactivate the modules for which you currently own a license. Note that all the changes made here are implemented immediately and the corresponding module(s) are activated / deactivated.

- **Available Products section**
  Here you can see the products available for your license. Click on the relevant button to select the product.
- **Included Modules list**
  In this list you can activate/ deactivate the modules that are included with you product. To do this, activate the checkbox in front of the module.
- **Optional Modules list**
  In this list you can activate the modules that you have licensed as an option for your product. To do this, activate the checkbox in front of the module in question.
- **Optional Hardware list**
  In this list you see the hardware that you have configured.

Using the **Select All** / **Unselect All** buttons you can activate or deactivate all available modules by clicking on the corresponding button.

To save the current selection of modules within a **.txt** file click on the **Save Information...** button.

14.4.5 Scaling dialog

Here you can specify how your images are scaled.

Option	Description
Active Scaling	Shows the scaling that is set currently.
Units dropdown list	Select the desired unit for the current scaling here.
Select automatically checkbox	If activated, the scaling will be calculated automatically from the microscope and camera configuration.

Available Scaling section

From the dropdown list you can select scalings which are stored on your system. The scaling details will be displayed in the fields below the list. By clicking on the **Options** button  you can perform the following actions :

Option	Description
Activate Scaling	Activates the selected scaling. The scaling will be applied to all images that are acquired from this time point onward.
Assign Scaling to Image	Assigns the selected scaling to the current image.
Import	Opens the **Import Scaling** dialog window. Here you can select a scaling file that you want to import.
Export	Opens the **Export Scaling** dialog window to export the selected scaling. Select the folder in which you want the exported scaling file to be saved and specify a file name.
Delete	Deletes the selected scaling.
Interactive Calibration button	Starts the *Scaling Wizard* [▶ 491].

14.4.5.1 Scaling Wizard

Here you can create a new scaling. To do this, draw a reference line with a predefined length in the current image. An image of a calibration slide is best suited for this purpose.

Parameter	Description
You can draw in two types of reference line:	
- **Simple Reference Line**	Draw a line along a distance with a known length.
- **Parallel Reference Lines**	Draw two parallel lines along a distance with a known length. The two parallel lines allow errors in the parallel axis resulting from the drawing of the lines to be corrected. A third, corrected line is drawn in automatically from which the scaling is determined.

Parameter	Description
Automatic Line Detection checkbox	**Activated:** Automatically detects individual lines of the scale bar in the image close to the interactively defined distance. Using this method the centers of the lines are determined exactly, increasing the precision of the scaling.
Length input field	Enter the length of the line you have drawn in the spin box/input field.
Unit dropdown list	Select the scaling unit from the dropdown list.
Name input field	Here you can enter the name for the scaling file that will be created.
Save button	Saves the scaling file that has been created under the specified name.

14.4.6 User and Group Management dialog

Here you can create new users and groups and manage their access rights. To learn more about user and group management, read the chapter User rights and user groups.

14.4.7 Customize Application dialog

Here you can customize the application layout, e.g. adopt the toolbar or shortcuts. To learn more about how to customize the application, read the chapter *Customizing Application* [▶ 140].

14.4.7.1 Toolbar tab

Here you can add menu items to the **Tool bar** as buttons for a quick access.

Available Toolbar items list

Here you will find all menu items that you can add to the **Tool bar**.

Add button 

Adds a selected item to the tool bar. It then appears in the **Selected Tool bar items** list.

Selected Toolbar items list

The items that you have added to the tool bar are listed here. Select the items here in order to sort them, for example.

Delete button 

Deletes a selected item from the **Selected Tool bar items** list.

Up button

Moves a selected item up a position in the **Selected Tool bar items** list..

Down button

Moves a selected item down a position in the **Selected Tool bar items** list..

Separator button

Inserts a vertical separator bar into the tool bar after the currently selected item of the **Selected Tool bar items** list.

Close button

Closes the dialog window.

14.4.8 Configure Favorites

Here you configure up to 20 new buttons to get quick access to your preferred camera and hardware settings.

Favorite Settings section

If you have not yet defined any buttons, you will see an empty list here. To create a new button, click on the **Add** button. In the input fields described below you can configure your favorite setting:

Field / Option	Description
Name	Here you can enter a name for the button.
Hardware Setting Ref.	Shows the selected hardware settings.
Camera Setting Ref.	Shows the selected camera settings.
Color	Here you can select a color for the related button. Click on the color dropdown list to choose a color.
Use Color also for Button Text coloring	**Activated:** Uses the selected color as the button text color.

Available hardware settings on disc section

Here you see a list of all hardware settings that are saved on your hard drive. Select the hardware setting that you want to use with the configured button.

Available camera settings on disk section

Here you see a list of all camera settings that are saved on your hard drive. Select the camera setting that you want to use with the configured button.

14.4.9 Select Sample Holder dialog

Here you can select the sample holder from a list. You can also create or import/ export new sample holder templates.

- EVO - Life Science cover glass 22x22
- Life Science cover glass 22x22
- Life Science for TEM Grids
- MAT Universal A
- MAT Universal B_A
- MAT Universal B_B
- MAT Flat stups
- MAT Flat stups A

If you want to select a sample holder, select an entry in the list and click on **Ok** button. The dialog closes and you see the selected sample holder in the preview area of the **S&F** tool.

14.4.10 Macro Editor dialog

The Macro Editor represents the IDE (Integrated Developer Environment) to edit, execute, debug and manage macros.

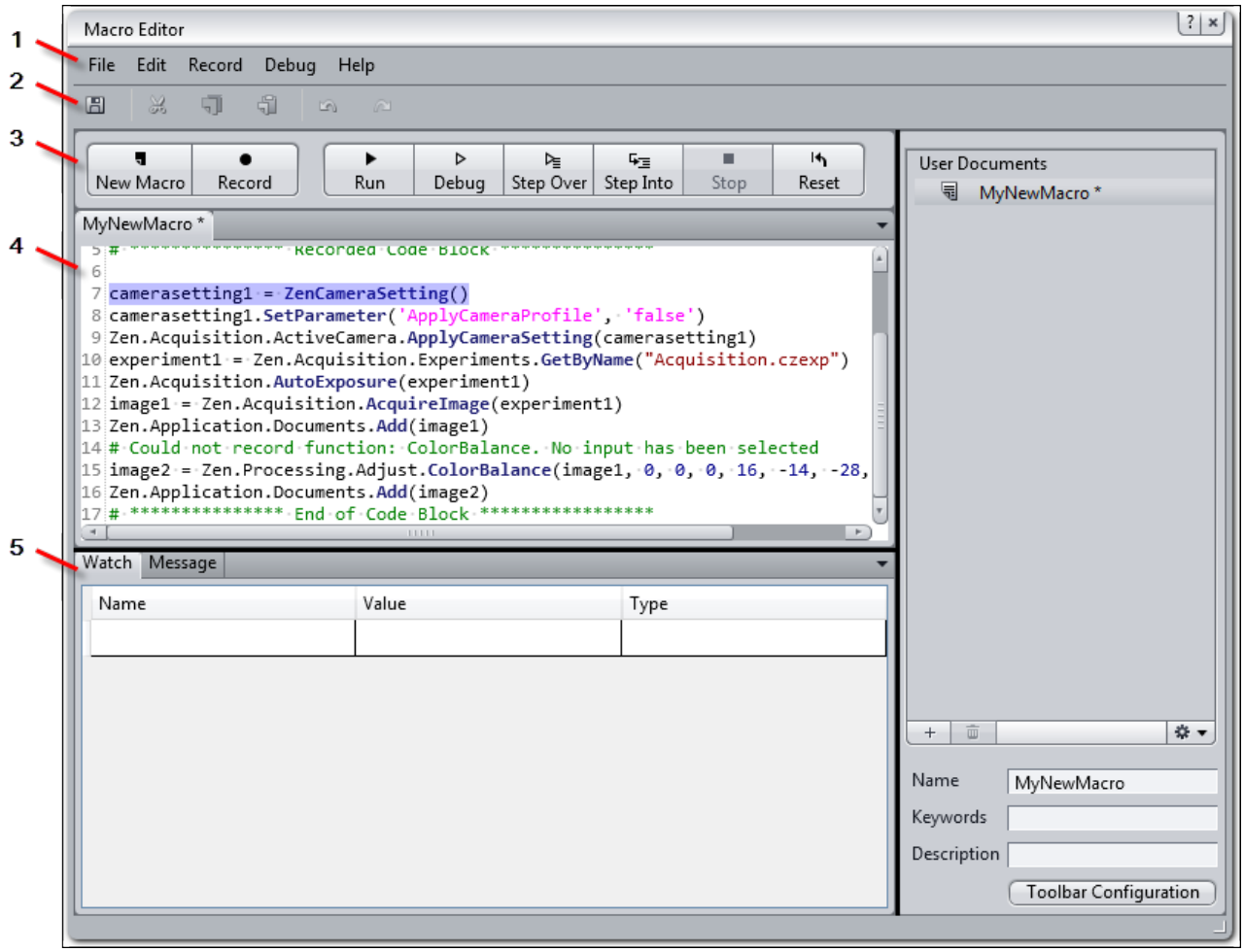


Fig. 70: Macro editor dialog

Menu bar (1)

Here you find the menus **File**, **Edit**, **Record**, **Debug** and **Help**. You'll find a detailed description under *Macro Editor menus* [▶ 496].

Tool bar (2)

Here you have a quick access to the most important functions like **Save** or **Edit** macros.

Button bar (3)

Here you find all the important buttons for controlling your macros, e.g. **Record** and **Run** button.

Button	Function
New Macro	Creates a new empty macro.
Record	Starts macro recording.

Button	Function
Run (Ctrl F5)	Executes the active macro completely.
Debug (F5)	Starts the debugger and executes the macro up to a breakpoint or error.
Step over (F10)	Starts the debugger stepwise, command by command, without stepping into function blocks.
Step into(F11)	Starts the debugger stepwise, command by command, and steps into function blocks.
Stop	Stops the running macro at the active command.
Reset	Resets all variables of the Python interpreter.
Breakpoint (F9)	Sets/removes a breakpoint in the active line, to stop/ continue the macro in debug mode, in the active line.
Set line (F8)	Sets the pointer in the next active command line.

Code Window (4)

The central area of the Macro Editor shows the program code of the selected macro. Edit and write your macros in here. You can either use the **Record** button or type in the program code directly. Also a multi-document view is available, meaning that you can open several code windows at once.

Watch Window (5)

Observe variables of the macro program code here. Enter the variable directly in the column **Name**. You can also mark the variable in the macro and add it using AddWatch of the right mouse key context menu.

Message Window

Display messages here using the print command in your macro.

14.4.10.1 Macro Editor menus

14.4.10.1.1 File menu

Menu item	Function	Short cut
New Macro	Opens the **New Macro** in the Macro programming area.	
Duplicate	Duplicates the selected macro to be saved under a new name.	

Menu item	Function	Short cut
Save	Saves the selected macro.	**(Ctrl +S)**
Save As…	Saves the macro under a new name.	
Rename…	Opens the **Rename** dialog window. Enter a new name for the macro. Confirm the entry with **Yes**.	
Delete	Deletes the selected macro.	

14.4.10.1.2 Edit menu

Menu item	Function	Short cut
Cut	Cuts the selected line out of the macro.	**(Ctrl+X, Shift +Del)**
Copy	Copies the selected line in the macro.	**(Ctrl+C, Ctrl +Ins)**
Paste	Inserts the copied line into the macro.	**(Ctrl+V, Shift +Ins)**
Find	Finds the entered text.	**(Ctrl+F)**
Replace	Replaces the detected text with the new text..	**(Ctrl+H)**
Undo	Undoes the last action.	**(Ctrl+Z)**
Redo	Redoes the last action.	**(Ctrl+Y)**

14.4.10.1.3 Record menu

Menu item	Function
Record	Starts recording..
Stop Recording	Stops recording of the active macro.

14.4.10.1.4 Debug menu

Menu item	Function	Short cut
Debug	Starts the debugger and executes the macro up to a breakpoint or error.	(F5)
Run	Executes the macro completely.	(Ctrl +S)
Stop	Stops the running macro at the active command.	
Step Into	Starts the debugger stepwise, command by command, without stepping into function blocks.	(F10)
Step Over	Starts the debugger stepwise, command by command, and steps into function blocks.	(F11)
Breakpoint	Sets/removes a breakpoint in the active line to stop/continue the macro in debug mode.	(F9)
Set Line	Sets the pointer in the next active command line.	(F8)
Reset	Resets all variables of the Python interpreter.	

14.4.10.1.5 File menu

Menu item	Function	Short cut
New Macro	Opens the **New Macro** in the Macro programming area.	
Duplicate	Duplicates the selected macro to be saved under a new name.	
Save	Saves the selected macro.	(Ctrl +S)
Save As…	Saves the macro under a new name.	
Rename…	Opens the **Rename** dialog window. Enter a new name for the macro. Confirm the entry with **Yes**.	
Delete	Deletes the selected macro.	

14.4.11 ApoTome dialog

Recommended Grid section

In this section you can set the grid with which you want the ApoTome to be operated.

Automatic Grid Control is activated by default. The appropriate grid for the selected objective is selected automatically (in the case of ApoTome.2). If you deactivate the checkbox, it is possible to select another grid from the dropdown list that is now active, e.g. to create a thicker optical section thickness. A list of recommended objectives and suitable wavelengths can be found here.

Calibration Status section

Here you can see whether your ApoTome has been calibrated successfully or whether calibration needs to be performed.

Theoretical Thickness section

The theoretical section thickness for the selected filter set and the objective used is displayed here.

14.4.12 ApoTome Settings dialog

Camera section

Here you can select the camera you wish to use to acquire your **ApoTome** images. As soon as you have selected a camera, **ApoTome** images are generated automatically during acquisition (**Snap**). The selected camera also applies to the **Acquisition** tab.

Live Mode section

Here you can choose between the **No Combination**, **Optical Section** and **Conventional Fluorescence** modes for the live image.

Acquisition Mode section

Here you can choose between the **No Combination**, **Optical Section** and **Conventional Fluorescence** modes for acquired images.

Phase Images section

Here you can choose between no fewer than 3 and no more than 15 phases. Each phase corresponds to a grid position. By default, 5 phases are acquired.

Filter section

Here you can set a filter which can be used to filter out residual streaks from the image. You have a choice between no filtering (**Off**) and three strength levels.

Image Normalization checkbox

Activated: The gray values are extended to the maximum available dynamic range following the calculation, see Normalization .

14.4.13 Stage/Focus not calibrated dialog

If you see this dialog, after you have started the software and the hardware was initialized, you should consider to calibrate the stage and focus drive immediately.

The calibration is necessary, if

- a motorized stage and/or focus drive are used, and
- the stage and focus drive are not calibrated.

To start the calibration procedure, simply click on the **Calibrate Now** button.

CAUTION

Risk of Crushing Fingers !

Before starting the calibration procedure, ensure that people stand clear of the instrument and that the full travel range is not obstructed by any objects.

If you skip the calibration, you can calibrate the stage and focus drive afterwards within the **Stage Control** and **Focus Control** dialogs accessible via the **Lightpath** tool, see *Opening the stage tool* [▶ 359] and *Opening the focus tool* [▶ 359]. Make sure that the **Show All** mode is activated, to see the **Calibrate** button within the dialogs.

Note that for fully automated system like Axio Scan the axes are calibrated automatically. The calibration is not necessary in that case.

14.5 Image views

ZEN 2.1 offers a lot of different image views. The **general image views** are visible in each image. The **specific image views** are visible only if the image has the appropriate characteristics (eg multiple channels, Z-stack, etc.) or you have licensed the view (e.g. 3D View). For image views are **general controls** and **specific controls** available.

14.5.1 General image views

These image views are available with any image. Depending on the type of image in question, the **general control elements** may have additional or more limited functions.

14.5.1.1 2D View

This view is the default view for images in ZEN 2.1. For this view the *General View Controls* [▶ 591] are available to you. To open the view's *context menu* [▶ 503], right-click in the *Center Screen Area* [▶ 20].

i Note

For a better overview and to navigate through enlarged images open the *2D Navigator window* [▶ 501]. Therefore simply click on Navigator entry in the 2D view context menu.

14.5.1.1.1 Navigator window

Fig. 71: Navigator window 2D View

Here you see a preview of the current image and the enlarged image area (blue frame). With the blue frame you're able to navigate through your enlarged image.

Additionally in the **Zoom** section you can set different zoom levels for the active image by clicking on the corresponding objective button. The toolbar below the objectives bar contains a few essential functions from the toolbar on the **Dimensions** tab, see Dimensions tab

i Note

If you are viewing a multidimensional image, you can use the **Navigator** to navigate through the available dimensions. These are represented by thin lines before or after the preview image. To navigate through the dimensions, left-click on the thin lines or use the mouse wheel.

In the case of time lapse images the time points present are displayed to the left and right of the preview image. To navigate through the time lapse images, click on the areas to the left or right of the preview image.

14.5.1.1.1.1 Enlarging the image area

Prerequisites

- You have activated the **Navigator** button on the **Dimensions** tab. The button is highlighted in blue.
- You can see the **Navigator window** in the image area.

Procedure

1. Move the mouse pointer over the blue frame in the Navigator window.

   The mouse pointer will appear as a double-headed arrow.
2. Hold down the left mouse button and move the mouse.

The size of the frame changes and the image area is enlarged.

14.5.1.1.1.2 Moving the region of interest

Prerequisites

- You have enlarged the image area.
- You have activated the **Navigator** button on the **Dimensions** tab. The button is highlighted in blue.
- In the Navigator window you can see the full image and the region of interest. The region of interest is indicated by a blue frame.

Procedure

1. To move the region of interest, move the mouse pointer inside the blue frame.

   The mouse pointer will appear as a four-headed arrow.
2. Hold down the left mouse button and move the mouse.

   The region of interest moves.
3. Release the left mouse button again.

You have moved the region of interest with the help of the Navigator. Alternatively, it is also possible to move the region of interest using the **Move** tool on the **Dimensions** tab.

14.5.1.1.1.3 Enlarging the Navigator window

Prerequisites

- The Navigator window is open in the image area.

Procedure

1. Move the mouse pointer to the adjustment handle in the bottom right corner of the Navigator window.

   The mouse pointer will appear as a double-headed arrow.

2. Hold down the left mouse button and move the mouse.

The size of the Navigator window changes.

14.5.1.1.1.4 Moving the Navigator window

Prerequisites

- The Navigator window is open.
- The image area has been enlarged.

Procedure

1. Move the mouse pointer inside the blue frame.

   The mouse pointer will appear as a four-headed arrow.

2. Hold down the left mouse button and move the mouse.

You can position the Navigator window freely within the image.

14.5.1.1.2 2D View context menu

Menu entry	Function
Full Screen (F11)	Switches to full screen mode. To exit full-screen mode, press **F11** again or **ESC**.
Zoom Group	Here you have access to the main zoom functions (Dimensions tab | *Zoom section* [▶ 592]).
Rulers	Shows rulers at the top and left edge of the image.
Navigator	Shows the **2D view** Navigator window.
Spot Measurement / Focus ROI	This function is only active in the live image or during **Continuous** mode. Shows a region in which the exposure time is measured and the software autofocus is focused.
Graphics	This function is activated by default. Shows graphic elements that have been drawn into the image, e.g. annotations or scale bars.

Menu entry	Function
Show Bleach ROI	This function is only visible with FRAP images. Shows graphic elements that were used during acquisition for bleaching (FRAP).
Copy Display Settings	Copies the display settings from an image (Display tab).
Paste Display Settings	Inserts copied display settings into an image (Display tab).
ROI | Draw ROI	Draw a certain region that particularly interests you into the image. You can draw several regions into an image.
ROI | Create Subset Images From ROI	Creates new image documents from the selection regions you have drawn in. All dimensions of the image are taken into account here.
Paste	Inserts a graphic element into the current image from the clipboard.

14.5.1.2 2,5D View

In the **2.5D view** intensity values in a two-dimensional image are converted into a height map. Here the highest intensity values are represented by the greatest extension in the Z-direction. Overall this results in a so-called 2.5D or pseudo-3D image.

i Note

Type and source of risk

If you are viewing a multichannel image, you can have the intensity values of the individual channels displayed. To do this, activate or deactivate the desired channels on the **Dimensions** tab.

14.5.1.2.1 2.5D View tool bars

The tool bars are arranged to the left of and underneath the image area. You can use the tools to control the display of the 2.5D volumes in the image area.

14.5.1.2.1.1 Left tool bar

Parameter	Description
Top thumb wheel	Enlarges or reduces the image area.
Tool bar	
- Rotate	Use this to rotate the 2.5D volume in any way you wish within the space. This is the default mode when you switch to **2.5D view** for the first time.
- Zoom	Use this to increase the zoom factor of the image area.
- Move	Use this to move the 2.5D volume laterally.
Bottom thumb wheel	Rotates the 2.5D volume around the horizontal (X) axis.

14.5.1.2.1.2 Bottom tool bar

Parameter	Description
Left thumb wheel	Use this to rotate the 2.5D volume around the vertical (Y) axis.
Tool bar	
- Bounding Box	Use this to show or hide a bounding box around the 2.5D volume.
- Show X/Y Axis	Use this to show or hide the X/Y axis.
- Show Z Axis	Use this to show or hide the Z axis.
- Start View	Use this to switch back to the start view. A top view of the 2.5D volume is displayed. Lateral movements and the zoom factor are adjusted so that the 2.5D volume can be seen at the center of the image area.
Right thumb wheel	Use this to compress the 2.5D volume on the (Z) axis perpendicular to the screen plane.

14.5.1.2.2 View specific controls

14.5.1.2.2.1 2.5D Display tab

On the **2.5D Display** tab you have 4 options for displaying your 2.5D image.

Profiles display

Displays the relief divided into a number of profiles with an equal distance.

Set the number of profiles using the **Fineness** slider.

Grid display

Displays the relief overlaid with a grid. This view supports gray levels only.

Make the grid more closely or more coarsely meshed using the **Fineness** slider.

Blocks display

Displays the relief by means of discrete, upwardly protruding columns of differing heights.

Surface display

Displays the relief as a continuous, flowing landscape.

Make the surface coarser or finer using the **Fineness** slider.

Invert Z checkbox

Activated: Displays the lowest intensity values by means of the greatest extension in the Z direction.

Use this function for images that contain many large, bright regions.

Apply Palette checkbox

Activated: Overlays the relief with the pseudo colors that have been set on the **Dimensions** tab.

Show Sides checkbox

Only available in the **Surface** display.

Activated: Closes the sides of the relief.

Show Planes checkbox

Activated: Shows two blue, transparent planes in the 2.5D volume.

Set the position of the planes using the **X/Y** sliders.

Extract Image section

To save an individual image in the current view, click on the **Save As** button.

14.5.1.2.2.2 Series tab

On the **Series** tab you can create a series of images in the 2.5D view. These series can be played back later as a video clip, for example.

Parameter	Description
Render Series dropdown list	Here you can select the desired series mode:
- Rotate Around X	Here you can define the start/stop angle and the rotation direction around the X axis.
- Rotate Around Y	Only visible in the 3D view. Here you can define the start/stop angle and the rotation direction around the Y axis.
- Rotate Around Z	Here you can define the start/stop angle and the rotation direction around the Z axis.
- Start/Stop	Here you can define the angle and zoom settings for the start and end position of your series. The intermediate positions are interpolated evenly.
- **Position** List	Here you can define any number of positions. The positions can each have completely different rotation, zoom and illumination settings.
- Over Time	Only visible in the 2,5D view. Here you can define the start time point and end time point for a series. All other settings (rotation, zoom, etc.) remain unchanged.
Apply button	Creates a series image with the current settings.
Preview section	To obtain a preview of the series, click on the **Play** button. To end the preview, click on the **Stop** button.
Number of Images section	Select the number of individual images in the series from the dropdown list.

14.5.1.2.2.3 2.5D Display Options tab

Only visible if the **Show All** mode is activated.

Parameter	Description
Shape and Position section	
- Angle X	Enter the rotation angle in the X direction with a precision of 1 degree using the slider or spin box/input field.

Parameter	Description
- Angle Y	Enter the rotation angle in the Y direction with a precision of 1 degree using the slider or spin box/input field.
- Z Scaling	Enter the Z scaling using the slider or spin box/input field.
Lighting Parameters section	
- Ambient	Reduces or increases the intensity of the ambient lighting in the 2.5D view.
- Reflection	Reduces or increases the proportion of the ambient light reflected on the relief.
- Shine	Reduces or increases the effect of the ambient light shining on the relief.
- Light Intensity	Reduces or increases the intensity of the lighting in the 2.5D view. A small distance means a circular light source at the center, while a large distance illuminates the scene evenly.
Reset button	Resets all settings to the default values.

14.5.1.3 Gallery View

In the **Gallery View** you see an overview of your multidimensional images. The individual images of the images concerned are presented in a gallery. It is possible to show any combination of dimensions, e.g. channels against time. When you view images for the first time in the **Gallery View**, they are displayed as follows:

Image type	Dispalyed as
Multichannel image	All the channels present in an image are shown, including the mixed color image.
Time lapse image	All the time points present in an image are shown.
Z-stack image	All Z-planes are shown.
Multichannel & time lapse image	All the time points present in an image are shown. All channels are shown as a mixed color image.
Multichannel & Z-stack image	All Z-planes are shown. All channels are shown as a mixed color image.
Time lapse & Z-stack image	All Z-planes are shown.

Image type	Dispalyed as
Time lapse, Z-stack & multichannel image	All Z-planes are shown. All channels are shown as a mixed color image.

14.5.1.3.1 View specific controls

14.5.1.3.1.1 Gallery Tools tab

Here you can specify which dimension you want to be displayed on which axis of the Gallery view. To do this, click on the corresponding dimension´s button.

Displayed Dimensions section

Each of the buttons is only visible if the current image contains the corresponding dimension.

Button	Function
Channels	Shows the channels present as individual images.
Z-Stack	Shows the Z-Planes present as individual images.
Time Series	Shows the time points present as individual images.
Chann.& Z (Channels and Z-Stack)	Shows the channels present in relation to the Z-stack images present.
Chann.& Time (Channels and Time Series)	Shows the channels present in relation to the time lapse images present.
Z&Time (Z-Stack and Time Series)	Shows the Z-Stack images present in relation to the time lapse images present.

Options section

Checkbox	Function
Show Dimension Labels	Inserts annotations into each individual image that provide information on the time point or Z-plane.
Invert X/Y axis	This checkbox is only available if the **Show All** mode is deactivated. It is active only if two dimensions are shown in relation to each other (Chann.&Z, Chann.&Time, Z&Time). If activated, this function inverts the X and Y axis of the view.
Show Graphics	Shows graphics / annotations within the images (in case if graphics / annotations are drawn in).

Checkbox	Function
Show Merged	Only visible for multichannel images. Only active if the channels present are shown. Shows the pseudo colored (mixed) images of all channels in addition to the individual images.

Advanced Functions

The following functions are only visible if the **Show All** mode is activated:

X Axis / Y Axis settings

From the first 2 dropdown list you can select which dimension (depending on which dimensions are available in the active image, e.g. channels, z-stack, etc.) will be shown on the X or Y axis (X axis = horizontal direction, Y axis = vertical direction).

In the second dropdown lists you can select wether you want to display all images of each dimension or if you want to display a certain range of images on the X or Y axis. Therefore you find the following options :

Option	Description
All	Displays all images of the active image in the Gallery view.
Subset by Step	If selected, you can enter a step size in the **Step** input field. If 2 steps are entered, only every second image will be shown. In the **Max.** input field you can enter the desired number of images which will be shown. The step size will be calculated automatically.
Subset by Range	If selected, you can adjust a range of images (e.g. from image 4-10) which is displayed in the view. Use the slider or the input fields to enter the desired range.

Image Creation

Here you can directly create images out of the Gallery view. Select the type of image that you want to create from the **Create image from** dropdown list. If you click on the **Create** button the image will be generated and opened in a new image document. The resulting image contains all the information of the input image; the pixel data are not changed. Following options are available:

Option	Function
Gallery View	Creates an image of the current Gallery view. If this option is selected, the option **Gallery Image from** is available. Here you can additionally select a dimension that is not currently displayed (e.g. Single Image will export each single image additionally). The resulting image is always a 24 bit RGB color image. The pixel data of the original image are changed. If the **Burn in graphics** checkbox is activated, all graphics or annotations will be burned into the output images.
Selection Subset	Creates an image from the images that have been selected in the current view. To select an image simply click on the image in the Gallery view. Hold **Crtl**-Key while clicking to select more images at once.
Range Subset	Creates an image from the defined selection range. If this entry is selected, sliders for the selected dimensions appear (**Start**, **End** and **Interval**). Use the sliders to set the selection range you want.

14.5.1.3.1.2 Gallery Appearance tab

Only visible if the **Show All** mode is activated.

Dimension Labels section

Here you can define the font and the style, color, position and size of the text for the dimension details that are shown.

Layout section

Here you can set the background color of the **Gallery view** and the distance between the individual images (from 1-10 pixels).

14.5.1.4 Histo View

The **Histo** (Histogram) view shows you the gray value histogram of your image. In the right image area you can see your current image and in the left image area you can see the **Histogram window**. At the side you will also find four **data tables**:

- In the first table from the left you will find all the **raw data** for each channel.
- In the second table from the left you will find all the **limits** for each channel of the image next to the image name.
- In the third table from the left you will find the **statistical values** for the gray value distribution, e.g. average, standard deviation, minimum and maximum value.

- The fourth table shows the values of measurements in the histogram. The results (Integral) show the percentaged fractions of the occurences.

14.5.1.4.1 View specific controls

14.5.1.4.1.1 Histo Definition tab

Parameter	Description
Select	Changes the mouse pointer to Selection mode. You can use this to select graphic elements in the image.
Clone	Use this to copy the last selected element and insert it at another position in the image.
Rectangle	Use this to insert a rectangular measurement region.
Circle	Use this to insert a circular measurement region.
Freehand	Use this to insert a measurement region with a shape of your choice. The line is closed automatically.
Polygon	Use this to insert a polygonal measurement region in the original image.
Keep Tool checkbox	**Activated:** Keeps the last selected tool active.
Normal	Switches the Profile window back to the view display.
CaliperX	Use this to perform a measurement of a region in X direction in the histogram display. To adopt the value into the measurement data table, click on the **Add to Table** button.
Add to Table button	Only active if a measurement (using CaliperX mode) was drawn into the histogram. Adds the current measurement into a measurement data table below the original image.
Reset Table button	Deletes the measurement data table below the original image.
Bin count slider	Enter the Bin count using the slider.

Parameter	Description
Lower Threshold slider	Enter the lower threshold value for the gray value distribution using the slider or spin box/input field. All regions in the image with gray values below the lower threshold value are overlaid in blue and all those with gray values above the upper threshold value are overlaid in red. **Skip Black** checkbox: **Activated:** Automatically subtracts the lowest value of the gray distribution. If activated, the settings for the lower threshold value are deactivated.
Upper Threshold slider	Enter the upper threshold value for the gray value distribution using the slider or spin box/input field. All regions in the image with gray values below the lower threshold value are overlaid in blue and all those with gray values above the upper threshold value are overlaid in red. **Skip White** checkbox: **Activated:** Automatically subtracts the highest value of the gray distribution. The settings for the upper threshold value are deactivated.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Auto color	**Activated:** Highlights each drawn-in measurement region with a random color.
Histo Table	Select the type of gray value distribution from the dropdown list. The following types are available:
- Frequency	ooops....no help text available
- SumUp	ooops....no help text available
- SumDown	ooops....no help text available
- Relative Frequency	**Activated:** Displays the histogram according to the relative frequency of the gray values in percent. Using the relevant sliders, you can adjust the precise Bin count, Bin size and Lower / Upper Threshold values.
Bin size slider	Enter the Bin size using the slider.
Logarithmic binning checkbox	**Activated:** Switches from the linear to a logarithmic class width. The class size is calculated automatically.

Parameter	Description
Show Thresholds checkbox	**Activated**: Shows the threshold values as colored overlays in the original image.

14.5.1.4.1.2 Histo View tab

Here you can configure the display for the Histo view.

Show section

Statistical Values checkbox

Activated: Shows the table containing the statistical values in the image area.

Measurement Data Table checkbox

Activated: Shows the measurement data table below the original image.

Frequency Distribution checkbox

Only visible if the **Show All** mode is activated.

Activated: Shows the table containing the raw data for each channel.

Image checkbox

Only visible if the **Show All** mode is activated.

Activated: Shows the original image in the image area.

Channel section

Here you can activate or deactivate the histograms for each channel.

X/Y Axis section

Only visible if the **Show All** mode is activated.

Here you can determine the limits for the **X axis** and **Y axis**.

Parameter	Description
Auto button	Sets the limits for the axes automatically.
Norm button	Normalizes the histogram display to the maximum values of the distribution.
Fixed button	Enter the min/max values for the histogram display in the **Min/Max** spin boxes/input fields.

Data Table section

Click on the **Create** button to create a data table from all the measured values displayed. To save the table, click on the **Save As** button.

New Image From section

Here you can create a new image document. Select the type of image from the dropdown list. To save the image, click on the **Save As** button.

14.5.1.5 Profile View

In the **Profile** view you can create intensity profiles of certain regions in your image. In the right image area you can see your image. In the left image area you can see the **Profile window**. The raw data for each channel are displayed in the **Profile table** below the **Profile window**. The measured values of measurements in the profile are shown in the **measurement data table** below the original image.

i Note

To create an intensity profile of a certain region, select a tool on the **Profile Definition** tab. Use this to highlight a region in your image. An intensity profile of the region is generated automatically and displayed in the **Profile window**. To enlarge the view in the Profile window, drag out a rectangular frame using the left mouse button in the Profile window. The selected region is displayed in enlarged form. Right-click to return to the original view.

14.5.1.5.1 View specific controls

14.5.1.5.1.1 Profile Definition tab

Tool bar

Using the tools you can add certain measurement lines to your image. The intensity profile of each line is shown in the Profile window.

Parameter	Description
Selection	Changes the mouse pointer to Selection mode. You can use this to select graphic elements in the image.
Copy	Use this to copy the last selected element and insert it at another position in the image.
Arrow	Use this to insert a measurement line in the original image. The measurement is shown in the **Profile window** in the direction of the arrow.
Polygon	Use this to insert a polygonal measurement line in the original image.
Freehand	Use this to insert a measurement line with a shape of your choice.
Rectangle	Use this to insert a rectangular measurement region.

Keep checkbox

Activated: Keeps the last selected tool active.

Color checkbox

Only visible if the **Show All** mode is activated.

Activated: Highlights each drawn-in measurement line with a random color.

Line Width spin box/input field

Only visible if the **Show All** mode is activated.

Here you can enter the line width of the measurement line.

Display Profile in Image checkbox

Activated: Also displays the profile of a measurement line in the original image.

Profile Measurements section

Parameter	Description
Normal button	Switches the Profile window back to the view display.
Measurement button	Use this to perform a point measurement in the profile. To adopt the value into the measurement data table, click on the **Insert Values** button.
X Measurement button	Use this to perform a measurement of a region in the X direction in the profile. . To adopt the value into the measurement data table, click on the **Insert Values** button.
Y Measurement button	Use this to perform a measurement of a region in the Y direction in the relevant profile. To adopt the value into the measurement data table, click on the **Insert Values** button.

Empty Table button

Empties the measurement data table below the original image.

Insert Values button

Adds the current measurement in the Profile window to the measurement data table below the original image.

Grid Distance section

Only visible if the **Show All** mode is activated.

Enter the grid distance of the measurement line using the slider or spin box/input field.

14.5.1.5.1.2 Profile View tab

Here you can configure the display for the Profile view.

Show section

Profile Table checkbox

Activated: Shows the profile table.

Measurement Data Table checkbox

Activated: Shows the measurement data table below the original image.

Image checkbox

Only visible if the **Show All** mode is activated.

Activated: Shows the original image in the image area.

Channel section

Here you can activate or deactivate the profiles for each channel.

X/Y Axis section

Only visible if the **Show All** mode is activated.

Here you can determine the limits for the **X axis** and **Y axis**.

Parameter	Description
Auto button	Sets the limits for the axes automatically.
Norm button	Normalizes the profile display to the maximum values of the distribution.
Fixed button	Enter the min/max values for the profile display in the **Min**/**Max** spin boxes/input fields.

Data Table section

Click on the **Create** button to create a data table from all the measured values displayed. To save the table, click on the **Save As** button.

New Image From section

Here you can create a new image document. Select the type of image from the dropdown list. To save the image, click on the **Save As** button.

14.5.1.6 Measure View

In this view measured values from images are displayed in a table. The table is only visible if there are annotations/measured values in the image. To highlight the row of the table containing the measured values of a graphic element, click on a graphic element in the image. To highlight a graphic element in the image, click on the measured value in the row of the table.

14.5.1.6.1 View specific controls

14.5.1.6.1.1 Measure tab

Here you can specify how to draw the graphic elements for measurements into an image and how the measurement data are displayed. You can also add user-specific features to individual graphic elements.

####### 14.5.1.6.1.1.1 Graphic Elements section

Only visible if the current image is a multidimensional image.

Here you can decide, for multidimensional images, whether to draw a graphic element "globally" into all channels, Z-positions, time points, etc., or whether to draw in separate elements for the view currently displayed.

Channel checkbox

Activated: Activates the Single Channel mode. Only draws graphic elements into the channel currently displayed.

Time checkbox

Activated: Only draws graphic elements into the time point currently displayed.

Z-Position checkbox

Activated: Only draws graphic elements into the Z-position currently displayed.

Copy in All Following checkbox

Activated: Draws a new graphic element into the view currently displayed and into all subsequent time points or Z-positions.

####### 14.5.1.6.1.1.2 New Feature section

Here you can add a defined feature to the selected graphic element.

Name input field

Here you can enter the name you want to give the feature.

Value input field

Here you can enter the value that you want the feature to have for the current graphic element.

Unit input field

Here you can enter the unit you want for the feature.

Add button

Adds the feature. The measurement data table is expanded to include this feature.

Remove button

Removes the selected feature.

14.5.1.6.1.1.3 Measurement Data section

Here you can specify how you want the measured values for the drawn-in graphic elements to be displayed.

Table button

Displays the measured values in a row of a table. As you can specify the features individually for each graphic element, the number of columns containing measured values may differ from graphic element to graphic element (i.e. from row to row).

List button

Displays each measured value in a separate row. The measurement data table then has the following defined columns:

Parameter	Description
Name	Name of the graphic element (e.g. line)
Feature	Name of the feature (e.g. distance)
Value	Value of the feature
Unit	Unit of the feature (e.g. µm)

Current View button

Only displays the measured values of the current view.

All Views button

Displays all measured values contained in the image.

Create Measurement Data Table button

Creates a measurement data table from the measured values displayed. The table may be saved as a separate document.

14.5.1.7 Info View

The **Info View** allows you to display extensive information about your image. Using the  buttons in each of the sections you can show additional fields in the sections or hide fields that are currently showing. To show or hide individual sections, click on the  button to the left of the headings for each of the sections.

i Note

The **Info View** only shows the fields that actually contain data. Using the  buttons in each of the sections you can show additional fields. To do this, activate the corresponding checkboxes in the shortcut menu.

14.5.1.7.1 General section

Field	Description
Title	Here you can enter a title for your image.
Description	Here you can enter a description for your image.
Comment	Here you can enter a comment.
Keywords	Here you can enter keywords for your image.
Rating	Here you can enter a rating for your image. To enter a rating, click on the star icons.

14.5.1.7.2 File section

Field	Description
Name	Displays the file name of the image without file extension.
File Type	Displays the file type of the image.
File Path	Displays the location where the image is saved in your file system.
File Size	Displays the file size of the image.
Created	Displays when the image was created.
Last Modified	Displays when the image was last changed.

Field	Description
User	Displays the name of the user. You can enter the user name in the **Extras** menu | **Options** | *User Management* [▶ 334].

14.5.1.7.3 Image Dimensions section

Field	Description
Time Series	Displays how many time points the image contains. The value in brackets shows the full duration of acquisition.
Z-Stack	Displays how many Z-planes the image contains. The value in brackets shows the full size of the Z-stack.
Channels	Displays how many channels the image contains.
Tiles	Displays how many individual images (tiles) the image is composed of.
Scaling (per Pixel)	Displays the scaling per pixel. **Edit** button: Opens the *Edit Scaling dialog* [▶ 524].
Image Size (Pixels)	Displays the image size in pixels. The first number indicates the horizontal dimension and the second the vertical dimension.
Image Size (Scaled)	Displays the scaled image size. The first number indicates the horizontal dimension and the second the vertical dimension.
Bit Depth	Displays the bit depth of the active image, e.g. 24 Bit. The bit depth depends on the camera settings when acquiring the image.
Stage Position	Displays the stage position. Within the image this is the center point. In the case of tile images this is the center point of the first tile.
Scanning Mode	Displays the scanning mode. This can either be the image field, an image line or a pixel.
Scanner Zoom	Displays the zoom factor. The value 1 corresponds to the standardized image field of all confocal systems.
Rotation	Displays the rotation of the image field around the optical axis.

Field	Description
Crop Offset	Displays the shift of the scanned region from the center of the image.
Pixel Time	Displays for how long the emission signal is collected per pixel. This is the so-called integration time.
Line Time	Displays how long the system needs to scan an image line.
Frame Time	Displays how long the system needs to scan the image field displayed in X and Y in full.
Averaging	Displays the number of individual measurements per image or line. The average of the individual measurements produces the pixel intensity values for the image.

14.5.1.7.4 Acquisition Information section

Field	Description
Acquisition Start	Displays the date and time when the acquisition of the image took place.
Microscope	Displays which microscope was used to acquire the image.
Objective	Displays which objective was used to acquire the image.
Optovar	Displays which Optovar was used to acquire the image.
Reflector	Displays which reflector cube was used to acquire the image.
Beam Splitter	Displays which beam splitter was used to acquire the channel.
Emission Wavelength	Displays the main emission wavelength of the channel or dye used.
Excitation Wavelength	Displays the main excitation wavelength of the channel or dye used.
Contrast Method	Displays the contrast technique. In transmitted light this is the condenser setting, while in reflected light it corresponds to the selected reflector cube.
Ligth Source Intensity	Displays the lamp intensity with which the image was acquired.
Pinhole	Displays the diameter of the pinhole.

Field	Description
Laser Power Percent	Displays the percentage of laser power used for acquisition.
Laser Blanking	Blanking of the laser during scanner movement without acquisition.
Laser Atten. Bleach.	Displays the laser power used for bleaching.
Channel Name	Displays the name of the channel.
Channel Description	Here you can enter a description of the channel. Describe the exact use of the channel or what can be seen in this channel.
Dye Name	Displays the name of the dye.
Channel Color	Displays the pseudo color allocated to the channel.
Camera	Displays which camera was used to acquire the image.
Camera Adapter	Displays which camera adapter was used to acquire the image.
EM Gain	Displays the factor by which the camera signal was increased.
Exposure Time	Displays the exposure time with which the image was acquired.
Depth of Focus	Displays the depth of focus. This is calculated according to the following formula: Depth of field = (2 * n * λ) / $(NA)^2$ = (2 * refractive index * emission wavelength) / $(\text{numerical aperture})^2$
Section Thickness	Displays the thickness of the optical section.
Binning Mode	Displays whether binning was applied during acquisition and how much.
Detector	Displays which detector was used for acquisition.
Detector Gain	Displays the gain setting of the detector for acquisition.
Detector Digital Gain	Displays the digital gain of the detector during acquisition.
Detector Offset	Displays the offset settings of the detector during acquisition.

i Note

In the case of multichannel images the channel-dependent information is saved in a table. Here the sorting of the individual information fields may differ.

14.5.1.7.5 Info View dialog window

14.5.1.7.5.1 Edit Scaling dialog

The **Edit Scaling** dialog window is divided up into table form. The columns contain the **Scaling Factor** and **Scaling Unit** and the rows the dimensions.

Scale Factor column

Enter the desired scaling factor in the spin boxes/input fields.

Scale Unit column

Select the desired scaling unit from the dropdown list. The metric units **Meter**, **Centimeter**, **Millimeter**, **Micrometer** and **Nanometer** are available as options, as well as the imperial units **Inch** and **Mil**.

Row X

Shows the scaling in the horizontal direction.

Row Y

Shows the scaling in the vertical direction.

Row Z

Shows the scaling in the 3rd dimension. This is usually the focus direction.

i Note

Row **Z** for the third dimension is only displayed if the image has a third dimension.

OK button

Adopts the settings into the current image.

Cancel button

Closes the dialog window without adopting the settings.

14.5.1.8 Tree View

The **Tree View** is visible only if you have activated the **Enable Tree view** checkbox under **Tools** | **Options** | **Documents**. The checkbox is deactivated by default.

The tree view shows a detailed list containing all meta data of the selected image.

14.5.2 Specific image views

These image views are only visible if the image has corresponding features. The **3D view**, for example, is only visible for Z-stack images.

14.5.2.1 Split View

Only visible for multichannel images and not during the acquisition of LSM images.

In this view you see all channels of a multichannel image. The channels are displayed side by side, in the channel colors that have been assigned to them. You also see the mixed image view in which all the channels are overlaid.

i Note

By double-clicking on an acquired multi-channel image, you can switch quickly to the **2D** view.

Double-clicking on the image in the **2D** view switches you back to the **Split** view. If you double-click on one of the displayed channels, only this channel will be shown in 2D View. If you double-click on the merge view, it will be shown in 2D View.

14.5.2.2 Ortho View

Only visible for Z-stack images.

In the **Ortho** (orthogonal section) view you can analyze your Z-stack images. Here, in addition to the top view (X/Y axis), you will also see the section views of the X/Z axes (top) and Y/Z axes (right).

14.5.2.2.1 Specific view options

14.5.2.2.1.1 Ortho Display tab

Section Lines section

Enter the positions (pixel values) for the section lines using the **X/Y/Z** sliders or spin boxes/input fields.

i Note

Alternatively you can also adjust the positions directly in the image area. To adjust the positions, move the mouse over a section line in the image. Hold down the left mouse button and move the mouse.

To position the relevant slider at the center of the view, click on the **Center** button.

Line Thickness section

Only visible if the **Show All** mode is activated.

Enter the thickness of the section lines in pixels using the sliders or spin boxes/input fields. This results in a maximum intensity projection being displayed over the selected pixel width.

Line Opacity slider

Only visible if the **Show All** mode is activated.

Here you can enter the degree of opacity of the section lines from 0% (invisible) to 100% (completely opaque).

Maximum Intensity Projection checkbox

Activated: Displays a maximum intensity projection (MIP) across all planes for all 3 views. The section lines are hidden and the control elements that are not relevant in this view are deactivated.

3D Distance Measurement checkbox

Only visible if the **Show All** mode is activated.

Activated: Activates the 3D distance measurement. The **Set Start** and **Set End** buttons, the coordinate displays and the **Distance** display window are visible. To set a starting point for the measurement, navigate the cutlines to the desired starting point and click on the **Set Start** button. The **Set End** button will become active. To set an end point for the measurement, navigate the cutlines to the desired end point and click on the **Set End** button. The pixel coordinates of the measurement points are displayed next to the buttons. The measured distance is displayed in the **Distance** display field.

New Image section

Here you can create a new image document. Select the desired view from the dropdown list (only in **Show All** mode). To save the image, click on the **Save As** button.

i Note

The resulting image contains the image data in the same dynamics (bit depth) as the original image and consists of the same number of channels (in the case of multichannel images) or time points (in the case of time lapse images) as the original image, but only contains the Z-plane currently displayed.

14.5.2.3 Cut View

Only visible for Z-Stack images.

This view allows looking from a different x/y/z-position/pitch/yaw to the slices of your Z-Stack image.

14.5.2.3.1 Specific view options

14.5.2.3.1.1 Cut Display tab

Parameter	Description
X/Y/Z slider	Moves view in X/Y/Z-direction.
Pitch slider	Adjusts view pitch.
Yaw slider	Adjusts view yaw.
Navigator button	Shows **Navigator**-Window in Center Screen Area. Adjust settings directly in graphical illustration.
Reset button	Resets each setting to default.
Reset all button	Resets all settings to default.

14.5.2.4 3D View

This view is only available if:

- you have licensed and activated the **3D Visualization** module.
- you have loaded or acquired a Z-Stack image.

The 3D view displays Z-Stack images three-dimensionally as a 3D volume. Using the toolbars on the left, right and bottom of the image area (**1**) you can directly control and move the 3D volume, see *Tool bars* [▶ 528]. With the specific view options (**2**) you find a lot of parameters to adjust the appearance and further settings of the 3D volume, see *Specific View Options* [▶ 533].

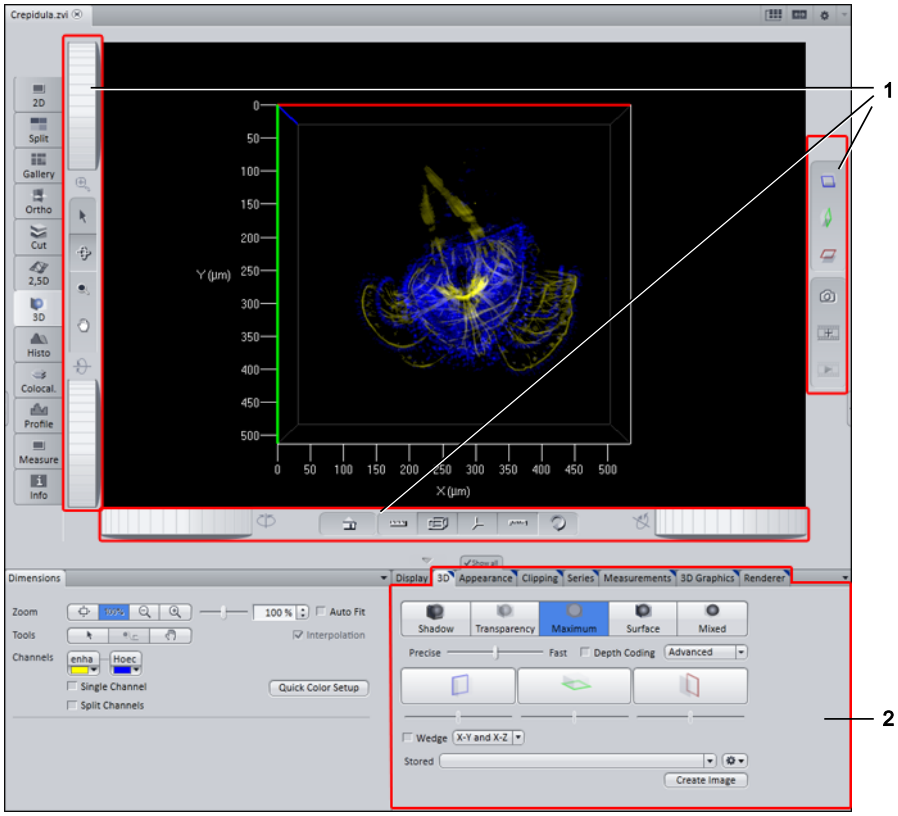


Fig. 72: 3D View

14.5.2.4.1 Tool bars

The tool bars are arranged to the left and right of the image area and underneath it. You can use the tools to control and adjust the display of the 3D volumes in the image area.

14.5.2.4.1.1 Tool Bar (Left)

Control element	Description
Top thumb wheel 	Zooms in or out of the 3d image.
Arrow button 	Use this to select end points of measurement tools that have been drawn into the 3d image (**Measure** tab). You can then edit the position of the end points.
Rotate button 	Use this to rotate the 3d image in any way you wish within the space. This is the default mode when you switch to **3D view** for the first time.
Zoom button 	Use this to increase or reduce the zoom factor of the image area.
Move button 	Use this to move the 3d image laterally.
Fly button 	Clicking on this button enables the flight mode. This mode allows to fly virtually through the 3d image. Use the keys from the list below to control your flight.
Bottom thumb wheel 	Rotates the 3d image around the horizontal (X) axis.

Flight Mode Key Layout / Controls

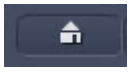
Key	Function
W	Forward
S	Backward
A	Left
D	Right
Space	Up
C	Down
E	Rotate (clockwise)
Q	Rotate (counter-clockwise)
X	Precision Mode; enables slower movement

14.5.2.4.1.2 Tool Bar (Right)

Control element	Description
Toggle X/Y clipping plane (blue) button 	Hides the X/Y clipping plane.
Toggle X/Z clipping plane (green) button 	Hides the X/Z clipping plane.
Toggle Y/Z clipping plane (red) button 	Hides the Y/Z clipping plane.
Snap button 	Creates a 2D image of the current view. The image is a 24 bit color image. All annotations are burnt in automatically.

Control element	Description
Add button 	Adds the current view to a position list as a new position. With the help of position lists you can have your view calculated as a series of individual images. This series can then be exported as a movie, for example.
Play button 	Only active if a position list containing at least two saved positions exists. Plays back a preview of the series that is calculated. To stop the preview, click on the button again.

14.5.2.4.1.3 Tool Bar (Bottom)

Control element	Description
Left thumb wheel 	Rotates the 3D volume around the vertical (Y) axis.
Home view button 	Switches back to the start view from any view. A top view of the 3D volume is displayed. Lateral movements and the zoom factor are adjusted so that the 3D volume can be seen at the center of the image area.
Show measurements button 	Shows or hides drawn-in measurements. If measurements are drawn-in, a table of the measurements appears at the right side of the image area.
Show bounding box button 	Shows or hides a bounding box around the 3D volume.
Show coordinate axes in color button 	Shows or hides the coordinate axes. ■ X axis = red ■ Y axis = blue ■ Z axis = green

Control element	Description
Show scaling button 	Shows or hides the scaling on each axis.
Spin Mode button 	Enables the spin mode. This allows to set the 3D volume in continuous motion. You will find a short description on how to use the spin mode below.
Right thumb wheel 	Rotates the 3D volume around the (Z) axis perpendicular to the screen plane.

14.5.2.4.1.4 Animating the 3D Volume

Prerequisites

- The **Rotation** mode in the left tool bar is selected.

Procedure

1. Click on the **Spin mode** icon.

2. Move the mouse inside the image area.
3. Hold down the left mouse button and move the mouse slightly to the left or right.
4. Release the left mouse button again.

The 3D volume rotates continuously in the direction in which you moved the mouse. If you move the mouse quickly, the 3D volume rotates quickly. If you move the mouse slowly, the 3D volume rotates slowly.

To stop the animation, left-click again in the image area.

14.5.2.4.2 Specific View Options

14.5.2.4.2.1 3D tab

Here you can specify which projection/rendering mode you want to use to display the 3D volume. There are 5 view modes available. To activate the desired view mode, click on the corresponding button. An activated button (respectively the mode) appears in blue color.

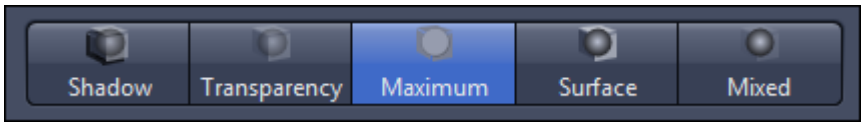


Fig. 73: Mode Buttons

Parameter	Description
Shadow button	Activates Shadow projection mode.
Transparency button	Activates Transparency rendering mode.
Maximum button	Activates Maximum intensity projection mode.
Surface button	Activates Surface reconstruction mode
Mixed button	Activates combination of transparency rendering and surface reconstruction mode

Parameter	Description
Precise/Fast slider	Adjust the level of detail of the 3d volume here. ■ If you select the **Precise** setting, all the information present in the image is used to achieve the best possible display. The calculation time can increase accordingly. ■ If you select the **Fast** setting, the image data are significantly reduced before the calculation. The calculation is fast, but only a very coarse 3D display of the volume is achieved.
Depth coding checkbox	Only active if **Transparency**, **Maximum** or **Mixed** mode is activated. **Activated:** Replaces the channel colors of the volume with a rainbow color table. This is also shown as a palette with an indication of the depth (in scaled units).

Parameter	Description
Toggle Clipping Planes buttons	By activating or deactivating the buttons you can show or hide the corresponding clipping planes in the 3D volume. If you right-click on an activated button, a shortcut menu opens. Here you can select whether you want the back (**Clip Back**), front (**Clip Front**) or both sides of the 3D volume to be clipped. You can also specify the **Style** of the clipping plane . Under each button is a slider. You can use this to move the relevant clipping plane within the volume.
Wedge checkbox	**Activated:** Activates two texture planes. Only the sector between the planes is cut out. You can select which planes you want to be used for the wedge function from the dropdown list. The selection is also visible in the relevant buttons.
Stored dropdown list	Here you can select saved 3D settings.
Options button 	Opens the options menu, see list below.
Create Image button	Creates a new image from the current view. This image is a 24 bit RGB color image. All graphic elements, such as annotations, are burnt in.

Options

Option	Description
New	Creates a new settings file that is given a name automatically and has the file extension *.cz3dr. The settings file can be found in the user path under \My Documents\Carl Zeiss\ZEN\Documents\3D render settings
Delete	Deletes the selected settings file from the hard drive.
Rename	Renames the selected settings file. Enter a new name in the input field and confirm with **OK**.
Save As	Saves the selected settings file under a different name.
Import	Imports a *.cz3dr file and applies it to the current image.
Export	Exports a *.cz3dr file to a different location.

14.5.2.4.2.2 Appearance tab

Here you can define the appearance of the 3D volume. On the tabs available on this tab, select the setting that you want to change (e.g. Transparency). Depending on which mode you have activated (on **3D** tab), different tabs and parameters are available.

14.5.2.4.2.2.1 Transparency tab

Parameter	Description
Channel selection 	Here you can select the channel of a multichannel image for which you want to set the transparency.
Threshold slider	Sets the lower threshold value in percent of the gray levels displayed. With this setting you specify the gray value range for the relevant channel that you want to be included in the rendered image.
The following parameters are only available in **Shadow** and **Transparency** projection mode:	
Ramp slider	Sets the extent of the transition from completely transparent to completely opaque (0-100 percent).
Maximum slider	Sets the level of opacity (0-100 percent).
Histogram display	Displays the settings that you enter using the sliders schematically. The X axis represents the gray level values and the Y axis the opacity. You can also change the position of the curve using the mouse.
Reset	Resets all parameters to the original values.

14.5.2.4.2.2.2 Surface tab

Only visible if **Surface** or **Mixed** view mode is activated.

Parameter	Description
Channel selection 	Here you can select the channel of a multichannel image for which you want to adjust the surface settings.
Threshold slider	Sets the lower threshold value in percent of the gray levels displayed. With this setting you specify the gray value range for the relevant channel that you want to be included in the rendered image.
Ambient Light slider	Sets the ambient light on a scale from 0 to 100%.
Spectacular Light slider	Sets the spectacular light from 0 to 100%. This value influences the differences between bright and dark structures.
Shininess slider	Sets the surface shininess.
Reset	Resets all parameters to the original values.

14.5.2.4.2.2.3 Channels tab

Only visible if **Mixed** view mode is activated.

Here you can specify how **Transparency** and **Surface** settings are mixed. In the case of multichannel images you can also configure these settings differently for each channel.

Activate the corresponding checkboxes for Transparency and Surface in the list.

14.5.2.4.2.2.4 Background tab

Parameter	Description
Background Color	Sets the background color for the 3D view. To do this, click on the color field and select the desired color.

14.5.2.4.2.2.5 Light tab

Parameter	Description
Brightness slider	Sets the brightness of the light source (from 0 - 100 %).

Parameter	Description
Azimuth slider	Here you can enter the angle of the light source above the virtual horizon.
Elongation slider	Here you can enter the light source's horizontal angle of incidence.
Light source display	As an alternative to the slider or input field, you can set the **Azimuth** and **Elongation** together by using the mouse to move the point within the light source display.
Reset	Resets all parameters to the original values.

14.5.2.4.2.2.6 Projection tab

Parameter	Description
View angle slider	Sets the projection angle at which you want to view the scene freely between 0° and 80°. The effect of this on the perspective display is as if you are viewing the 3D image through a telephoto or wide-angle lens.
Scale Z slider	Here you can set the scaling of the volume in the Z direction (value range 10% - 600%).
Stereo anaglyph checkbox	**Activated**: Displays the 3D volume as anaglyphs. You can choose between a ■ **Red/Green** display, or a ■ **Red/Cyan** display.
Camera separation slider	Sets the distance between the two virtual cameras (0-20%).
Parallax shift slider	Sets the degree of movement that is necessary to bring the two camera images back into line (-100 to +100%).
Reset	Resets all parameters to the original values.

14.5.2.4.2.3 Clipping tab

Only visible if the **Show All** mode is activated.

Here you can edit clipping planes. To select a clipping plane, click on the corresponding button. The editing functions which you can use to modify the selected clipping plane become visible when you activate the specific clipping plane.

i Note

On the **Clipping** tab you can edit the clipping planes. On the **3D** tab you can activate or deactivate the relevant clipping planes in the 3D volume.

Parameter	Description
Show All Clipping Planes checkbox	**Activated:** Automatically inserts all 3 clipping planes into the 3D volume. Additionally the editing functions for each clipping plane were activated automatically.
X/Y button 	Activates the editing functions for the X/Y clipping plane.
X/Z button 	Activates the editing functions for the X/Z clipping plane.
Y/Z button 	Activates the editing functions for the Y/Z clipping plane.
Activate checkbox	**Activated:** Activates the selected clipping plane in the 3D volume. The corresponding settings become visible. You will find a detailed description of the settings in the list below. Note: Each plane is positioned at the center of the 3D volume and aligned orthogonally (in the X/Y, X/Z, Y/Z direction).
Reset All	Resets all parameters to the original values.

The following parameters are only visible if the **Activate** checkbox is activated and a clipping plane has been selected.

Parameter	Description
Clipping Plane Style	Change the display of the selected clipping plane using the dropdown list to the right of the **Activate** checkbox. The following settings are available:
- Invisible	The plane is invisible.
- Colored	The plane is displayed in color. The frame color is used with 50% transparency here.

Parameter	Description
- Binary	The data above the threshold value that are touched by the clipping plane are displayed in binary form as a white area. Black pixels are non-transparent.
- Transparent	The data that are touched by the clipping plane are displayed as they are in **Transparent** view mode, but in 2 dimensions. The ramp for the transparency is linear here. Black pixels are transparent.
- Textured opaque	The display appears as it does with the **Textured** setting. Black pixels do not let any light through, however, meaning that the render data behind them are not displayed.
Outline checkbox	**Activated:** Displays the frame of the selected clipping plane. Enter the frame color via the color field.
Clip Front checkbox	**Activated:** Clips the front of the 3D volume.
Clip Back checkbox	**Activated:** Clips the back of the 3D volume.
Clip Transparency checkbox	Only active if **Mixed** view mode is activated. **Activated:** In addition to the surface data, also clips the transparency data.
Clip Surface Channels	Only visible if **Surface** or **Mixed** view mode is activated. Here you can enter which channel you want to be clipped using the channel buttons.
Position	Here you can enter the position of the selected clipping plane.
<X (X Angle)	Here you can enter the X angle for the selected clipping plane.
<Y (Y Angle)	Here you can enter the Y angle for the selected clipping plane.
Reset Orientation button	Resets the selected clipping plane to the original position.

14.5.2.4.2.4 Series tab

Here you can create render series of individual views, which you can later view and export as a movie. The tab contains different control elements depending on the Render Series type. The following parameters are the same for all render series types: **Render Series** section, **Stored** section, **Apply** button and **Fixed Resolution** checkbox.

Parameter	Description
Render Series	
- Rotate Around X	Define the start/stop angle and the rotation direction around the X axis.
- Rotate Around Y	Only visible in the **3D** view. Define the start/stop angle and the rotation direction around the Y axis.
- Rotate Around Z	Define the start/stop angle and the rotation direction around the Z axis.
- Start/Stop	Define the angle and zoom settings for the start and end position of your series. The intermediate positions are interpolated evenly.
- Position List	Define any number of positions. The positions can each have completely different rotation, zoom and illumination settings.
- Over Time	Only visible in the **2,5D** view. Define the start time point and end time point for a series. All other settings (rotation, zoom, etc.) remain unchanged.
Apply button	If clicking on this button the series will be calculated. A new image document will be opened in the **Center Screen Area**. You can view the series by clicking on the **Play** button in the **Dimensions** tab.
Stored section	Only visible if **Show All** is activated. Here you manage your series settings. Via the dropdown list you can select a saved settings file. Clicking on the **Options** button  opens a shortcut menu with the following options:
- New	Creates a new settings file (***.czsht**). This file can be found in the user's local document path (e.g. \My Documents\Carl Zeiss\ZEN\Documents, in a corresponding subfolder).
- Delete	Deletes the selected settings file.
- Rename	Opens a dialog to rename the selected settings file.
- Save As	Saves a copy of the currently selected settings file under a different name.
- Save	Saves changes to a currently selected settings file.

Parameter	Description
- Import	Imports a settings files from the hard disk.
- Export	Exports a settings files to the hard disk.
Preview section	Shows a preview of the series to be created. Use the **Start** / **Stop** button to start or stop the preview.
No. of Frames input field	Sets the number of individual frames that the series consist of after the calculation. The more individual images that you specify here, the more fluidly the scene transitions will be displayed later. Click on the dropdown button to select predefined values from a list (e.g. **20** or **100** frames).
Fixed Resolution	Only visible if **Show All** is activated. As a rule, the image series is calculated using the current screen resolution. If you want to set a different format for the series, activate the checkbox. In the input fields that are now visible you can enter the width and height in pixels with which you want the series to be created.

The following parameters are only available if you have selected **Turn Around X / Y / Z** under **Render Series**:

i Note

The X rotation, Y rotation and Z render series types all have the same control elements and differ only in the axis around which the rotation is calculated.

The preview function is not available for these types of series.

Parameter	Description
360° Panorama	Select 360° panorama, if you want to generate a complete rotation series.
Partial Panorama	If you select partial panorama, you can specify the starting angle and stopping angle that you want to use for the series. To do this, enter the desired values in the input fields or adjust it in the graphical representation of the rotation circle at the right of the input fields.
- Start Angle	Determines the starting angle.
- Stop angle	Determines the stop angle.
- Direction	Determines the direction of rotation.

Parameter	Description
- Angle Definition (graphical representation)	When you are configuring a partial panorama, the desired angles can also be determined easily using the circular control element: Grab the white start/stop points with the mouse and position these accordingly on the circle. The number of individual images is also displayed here.

The following parameters are only available if you have selected **Start / Stop** under **Render Series**:

Parameter	Description
Start Position	You can position the volume in the image area as required using the mouse. The geometric parameters are displayed in the input fields. You can also determine the **Camera Position** and the **Look At** parameters for X, Y or Z and the angle directly using the input fields or the slider. All changes are displayed immediately in the image area.
Stop Position	You can position the volume in the image area as required using the mouse. The geometric parameters are displayed in the input fields. You can also determine the **Camera Position** and the **Look At** parameters for X, Y or Z and the angle directly using the input fields or the slider. All changes are displayed immediately in the image area.

The following parameters are only available if you have selected **Position list** under **Render Series**:

Parameter	Description
Add	Adds the current position to the position list.
Insert	Inserts a new position between two existing positions.

Parameter		Description
Position List		Each position is displayed in the list with its X, Y, Z angle and zoom level.  Using the control elements at the bottom of the list you can change the order of the positions (**Arrow** buttons), cut positions (**Scissors** icon) or copy and paste them again at another position (**Copy / Paste** icon). If you want to delete all positions, click on the **Clear List** button.
Further Parameters		You can determine which of the following parameters you want to be taken into consideration when the series is calculated. To do this, activate the corresponding checkbox:
-	Light	Includes illumination parameters.
-	Transparency	Includes transparency settings (not in Surface mode).
-	Background	Includes color and distance of the background.
-	Time	Includes time series parameters (only for time series images).
-	Camera	Includes camera settings, e.g. viewing angle (from the 3d / virtual camera).
-	Planes	Includes planes settings (not in Shadow mode).
-	Surfaces	Includes surface settings (only in Surface and Mixed mode).

14.5.2.4.2.5 Measurements tab

Only visible if the **Show All** mode is activated.

Here you can perform interactive measurements in the 3D volume. Note that measurements are not possible in **Shadow** projection mode. The measurements can be drawn in directly in the 3D volume using different tools. The measurement results are displayed in a list at the right of the image area.

Parameter		Description
Tool bar		 Using the tools you can perform interactive measurements in the 3D volume. The following tools are available:
-	Select	Changes the mouse pointer to Selection mode. Use this to select measurements in the 3D volume in order to change them.
-	Line	Use this to measure the length of a line in µm. Click once on the starting point and hold down the mouse button. Then drag the mouse to the end point and release the mouse button again. The measurement is complete. The result of the measurement is displayed in the list to the right of the image area.
-	Angle	Use this to measure the angle between two connected legs. First define the starting point. Then use the mouse to drag the first leg to the desired first end point. Define the second leg by clicking on the second end point. The angle measurement ends with a display of the angle measured (in degrees). The result of the measurement is displayed in the list to the right of the image area.
-	Polygon Curve	Use this to measure along a line with any number of segments. Click from corner point to corner point. Complete the measurement by right-clicking. The result of the measurement is displayed in the list to the right of the image area.
-	Color selection	Here you can select a color for the tool you want to draw in. Simply click on the colored rectangle and choose a color from the list.
-	Keep Tool	**Activated:** Keeps the selected tool active.
-	Auto Color	**Activated:** Automatically changes the color of the drawn-in tool.
Show Measurements checkbox		**Activated:** Shows the measurements in the 3D volume or in the list of measured values at the right of the image area. ■ **On top**: If activated, all drawn-in measurement tools appear in the foreground, even if these are in fact obscured by image structures.
Display Values		

Parameter	Description
- on the objects	**Activated:** Displays the measured values in the 3D volume.
- as list	**Activated:** Displays the measured values in the measurement data table.
Delete Selected button	Only active if a measurement tool has been selected in the 3D volume. Deletes selected measurement tools from the 3D volume.
Delete All button	Deletes all measurement tools from the 3D volume.

14.5.2.4.2.6 3D Graphics

Only visible if the **Show All** mode is activated.

Parameter		Description
Tool bar		 Using the tools you can perform interactive measurements in the 3D volume. The following tools are available:
-	Select	Changes the mouse pointer to Selection mode. Use this to select measurements in the 3D volume in order to change them.
-	Line	Use this to measure the length of a line in µm. Click once on the starting point and hold down the mouse button. Then drag the mouse to the end point and release the mouse button again. The measurement is complete. The result of the measurement is displayed in the list to the right of the image area.
-	Angle	Use this to measure the angle between two connected legs. First define the starting point. Then use the mouse to drag the first leg to the desired first end point. Define the second leg by clicking on the second end point. The angle measurement ends with a display of the angle measured (in degrees). The result of the measurement is displayed in the list to the right of the image area.
-	Polygon Curve	Use this to measure along a line with any number of segments. Click from corner point to corner point. Complete the measurement by right-clicking. The result of the measurement is displayed in the list to the right of the image area.
-	Color selection	Here you can select a color for the tool you want to draw in. Simply click on the colored rectangle and choose a color from the list.
-	Keep Tool	**Activated:** Keeps the selected tool active.
-	Auto Color	**Activated:** Automatically changes the color of the drawn-in tool.
3D Measurements list		All measurement contained in the 3D volume are displayed here. The list contains the following columns:
-	Eye icon	Here you can select whether or not a measurement tool is displayed in the image. If you click in the title field of the column, the setting is made simultaneously for all entries.

Parameter		Description
-	Lock icon	Not activated for the 3d view.
-	Type	Displays the type of a tool. If you click on the icon, you can change the color of the tool.
-	ID	Displays the unique identification number of the measurement tool.
-	A	No function
-	M checkbox	**Activated:** Displays the measurement data in the image. If you click in the title field of the column, the setting is made simultaneously for all entries.
-	Name	Displays the name of the tool. To change the name, double-click on the entry. Then enter a new name. Confirm the entry with the Enter key.

14.5.2.5 Colocalization View

Only visible for multichannel fluorescence images.

In the **Colocal.** (Colocalization) view, you can analyze the extent of colocalization quantitatively in two fluorescence channels. The view consists of two main areas: the **X/Y scatter plot** on the left and the actual image (2 channels are displayed) in the right image area. Using the **Coloc. Tools** specific view control, you can also display the **Colocalization table** in the lower image area. To do this activate the **Table** checkbox in the **Extract** section.

i Note

The channels that you are comparing with one another are displayed in the image area in the form of a color overlay. The channel color of the image is used here. If the images have more than 2 channels, you can add additional channels on the **Dimensions** tab. This temporary selection is deactivated, however, when you select the channels to be compared on the **Coloc. Tools** tab.

14.5.2.5.1 X/Y Scatter Plot

The pixel intensities of two channels are plotted against one another in the diagram and each pixel pair with the same X/Y image coordinates is displayed as a point. The frequency with which pixels of a certain brightness occur is visualized by means of a color palette that is displayed at the bottom of the diagram. The relative value range lies between 0-255.

The vertical and horizontal axes show the gray value range that applies for the relevant channel.

The diagram is overlaid with two lines that subdivide it into 4 quadrants, numbered from 1-4. Using the mouse you can position the lines freely and therefore adjust the threshold values to the data.

The quadrants have the following meanings:

- 1: Non-colocalizing pixels from channel 1
- 2: Non-colocalizing pixels from channel 2
- 3: Colocalizing pixels
- 4: Background

14.5.2.5.2 Colocalization table

Only visible if the **Table** checkbox is activated on the **Colocalization** tab.

For each quadrant of the scatter plot there is a correspondingly labeled row in the table. The **Global** row contains the values for the entire image. The table contains columns for the following measured values:

14.5.2.5.2.1 Region

Once a region has been selected it has a number assigned to it. This number appears in the image and in the table.

14.5.2.5.2.2 Quadrant

Indicates the measured values for the four quadrants of the scatter plot.

14.5.2.5.2.3 Pixel Number

Shows the total number of pixels of each quadrant. The sum of all pixels in this column for all 4 quadrants corresponds to the product of the height x width of the original image.

14.5.2.5.2.4 Area (µm²)

Area = number of pixels x scaling factor for X/Y
If there is no scaling for the original image, the following applies: 1 pixel = 1 µm.

14.5.2.5.2.5 Relative Area (%)

Relative area = area of quadrant/total area

14.5.2.5.2.6 Pearson's Correlation Coefficient

Provides information on the intensity distribution within the colocalization region. Value range: -1 to 1.

1: All pixels are on a straight line in the scatter plot from bottom left to top right (if, for example, you have used the same channel twice for the colocalization, you will find the value 1 in this column).

0: The pixels in the scatter plot are distributed in a cloud without a preferred direction.

-1: The pixels do not overlap. The scatter plot stretches from top left to bottom right. This situation can be described as negative colocalization and means "exclusion".

The calculation formula is as follows:

$$\frac{\sum((GreyCh1_i - MeanCh1) \times (GreyCh2_i - MeanCh2))}{\sqrt{\sum(GreyCh1_i - MeanCh1)^2 \times \sum(GreyCh2_i - MeanCh2)^2}}$$

GV: Gray Value; AV: Average Gray Value; C: Channel

14.5.2.5.2.7 Manders' Correlation Coefficient

Insensitive to differences in the signal intensity between the two channels and bleaching.

Value range: 0 to 1

The calculation formula is as follows:

$$\frac{\sum GreyCh1_i \times GreyCh2_i}{\sqrt{\sum GreyCh1_i{}^2 \times \sum GreyCh2_i{}^2}}$$

Fig. 74: C: Channel

14.5.2.5.2.8 Coloc. Coefficient 1

This coefficient indicates the relative number of colocalized pixels in channel 1 in relation to the total number of pixels above the threshold value:

$$\frac{\sum PixelsCh_{1,colocalized}}{\sum PixelsCh_{1,total}}$$

The values range between 0 and 1, with 0 indicating no colocalization and 1 indicating full colocalization.

Numerator = Number of pixels in quadrant 3

Denominator = Number of pixels in quadrant 3 + number of pixels in quadrant 1

14.5.2.5.2.9 Coloc. Coefficient 2

This coefficient indicates the relative number of colocalized pixels in channel 2 in relation to the total number of pixels above the threshold value:

$$\frac{\sum PixelsCh_{2,colocalized}}{\sum PixelsCh_{2,total}}$$

The values range between 0 and 1, with 0 indicating no colocalization and 1 indicating full colocalization.

Numerator = Number of pixels in quadrant 3

Denominator = Number of pixels in quadrant 3 + number of pixels in quadrant 2

14.5.2.5.2.10 CC (weighted) 1

Weighted correlation coefficient channel 1. Calculated like the simple colocalization coefficient, but using the sum of the gray value intensity rather than the number of pixels.

$$\frac{\sum SumGreyCh_{1,colocalized}}{\sum SumGreyCh_{1,total}}$$

The values range between 0 and 1, with 0 indicating no colocalization and 1 indicating full colocalization.

Numerator = Sum of intensity of all pixels in quadrant 3

Denominator = Sum of intensity of all pixels above the threshold value

14.5.2.5.2.11 CC (weighted) 2

Weighted correlation coefficient channel 2. Calculated like the simple colocalization coefficient, but using the sum of the gray value intensity rather than the number of pixels.

$$\frac{\sum SumGreyCh_{2,colocalized}}{\sum SumGreyCh_{2,total}}$$

The values range between 0 and 1, with 0 indicating no colocalization and 1 indicating full colocalization.

Numerator = Sum of intensity of all pixels in quadrant 3

Denominator = Sum of intensity of all pixels above the threshold value

14.5.2.5.2.12 Average Intensity 1

The sum of all gray values from channel 1, divided by the total number of pixels in this channel:

$$\frac{\sum GreyCh1_i}{AreaCh_1}$$

14.5.2.5.2.13 Average Intensity 2

The sum of all gray values from channel 2, divided by the total number of pixels in this channel:

$$\frac{\sum GreyCh2_i}{AreaCh_2}$$

14.5.2.5.2.14 Standard Deviation 1

Displays the standard deviation of the gray values in channel 1:

$$\sqrt{\frac{\sum (GreyCh1_i - MeanIntensityCh_1)^2}{AreaCh_1 - 1}}$$

14.5.2.5.2.15 Standard Deviation 2

Displays the standard deviation of the gray values in channel 2:

$$\sqrt{\frac{\sum (GreyCh2_i - MeanIntensityCh_2)^2}{AreaCh_2 - 1}}$$

14.5.2.5.2.16 Z Index

Displays the Z index for Z-stack images.

14.5.2.5.2.17 T Index

Displays the time index for time lapse images.

14.5.2.5.2.18 Relative Time

Displays the time of acquisition for all dimensions of a multidimensional image, beginning at 0h:00min:00sec:00msec.

14.5.2.5.2.19 Relative Focus

Displays the relative focus position at which an image has been acquired.

14.5.2.5.3 Specific view options

14.5.2.5.3.1 Coloc. Tools tab

Here you will find all the control elements that you need to perform a colocalization analysis.

14.5.2.5.3.1.1 Tool Bar section

Use the tools to draw regions into the image in which you want the analysis to be performed. A description of the tools can be found on the Graphics tab.

Once a region has been drawn in, it is automatically treated as an active region. The scatter plot shows the pixel value frequencies for this region.

The **Colocalization table** displays the data for the entire image and for the selected region. To select several regions, hold down the **Ctrl** key and click on the desired regions.

Apart from drawing regions into the image, you can also draw them into the **X/Y scatter plot**. If you have used the function in the *Regions section* [▶ 554], only those pixels that are framed by a region in the scatter plot are taken into consideration. This means that you can correlate interesting point clouds quickly with the corresponding pixels in the image.

If you have drawn regions into the scatter plot, the ROI (region of interest) button will also appear in the tool bar. As long as this button is activated (highlighted in blue), you can select, move and change the regions in the scatter plot using the **Selection** tool. If you want to change the quadrant lines again, you will need to deselect the ROI button beforehand.

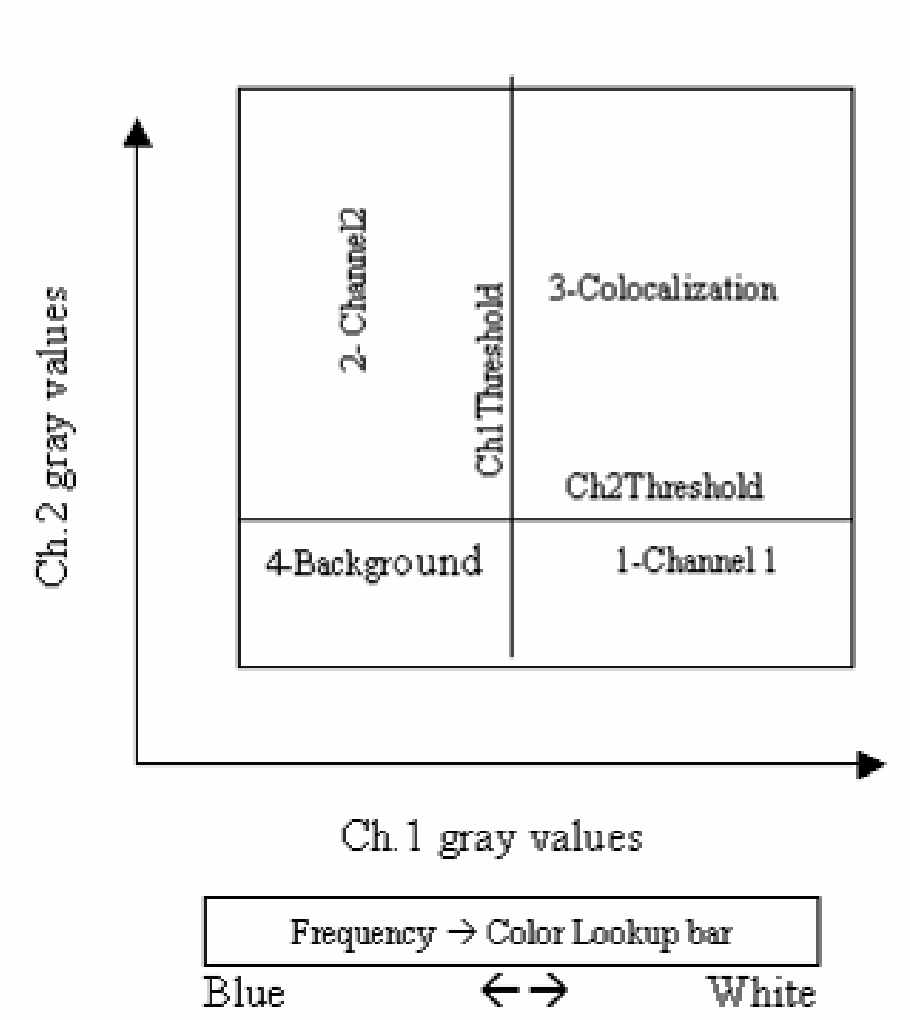
14.5.2.5.3.1.2 Channels and scatter plot control section

Channels dropdown lists

Here you can select which channels of a multichannel image are compared with one another. Select a channel for both the horizontal and vertical diagram axis from the **Channels** dropdown list. The first and second channel are always selected by default. As soon as you have made a selection, all other channels are automatically removed from the image display. You can, however, add other channels temporarily on the **Dimensions** tab.

Threshold sliders

Using the two Threshold sliders and the two spin boxes/input fields, you can set the threshold value (in gray levels) for both channels.



Range dropdown lists

Only visible if the **Show All** mode is activated.

Here you can define the gray value range that the scatter plot axes will display. **Auto** is selected here by default, which means that the range is automatically set to the brightest pixel in the image. You can, however, enter a fixed gray value range between 256 (8 bits) and 65535 (16 bits). If the image is a time lapse or Z-stack image, **Auto** has been selected, you can select the dimension for which you want the gray value range to be automatically determined from another dropdown list. In this way you can easily determine a valid diagram setting for an entire time series, for example, without having to analyze each individual time point.

Costes button

Calculates the optimal threshold value according to Costes et al.

14.5.2.5.3.1.3 Regions section

Channels buttons

Here you can mask pixels in the image according to which one of the four quadrants they belong to. The numbers on the buttons correspond to the numbering of the quadrants in the **X/Y scatter plot**. The color selection window is accessed by clicking on the **color field**. Using the **Opacity** slider you can determine the degree of transparency of the masking.

Cut Mask button

Only active if a quadrant has been masked.

Creates a new image exclusively containing the masked pixels.

14.5.2.5.3.1.4 Extract section

Scatter Plot button

Creates a new image document from the X/Y scatter plot. In the case of time series or Z-stacks the dimensions are also created automatically.

The following functions are only visible if the **Show All** mode is activated:

Table button

Creates a new table document. The document contains all measurement data from the colocalization analysis. All dimensions, such as T and Z, are also taken into account. This table can be saved as a *.csv document for further processing in other programs.

Table checkbox

Activated: Displays the colocalization table in the image area.

14.5.2.6 Analysis View

Only visible if an interactive measurement has been performed.

In the **Analysis** view the image from the interactive measurement and the table containing the measurement results are displayed.

i Note

To highlight the row of the table containing the measured values of an object, click on a measured object in the image.

To highlight the corresponding measured object in the image, click on a row in the table.

14.5.2.6.1 Specific view options

14.5.2.6.1.1 Analysis tab

On the Analysis tab you can define how the measured objects are displayed in an image.

Show Objects checkbox

Activated: Displays the measured objects in the graphics plane.

Fill checkbox

Activated: Displays the objects in filled form.

Deactivated: Displays only the contours of the objects.

Opacity slider

Here you can set the opacity with which the measured objects are displayed in the graphics plane.

Delete Measurement Data button

Deletes all objects and measurement data from the image.

Show All Classes checkbox

Activated: Displays the objects of all classes.

Deactivated: Displays the objects of the selected class.

Create Measurement Data Tables button

Creates two measurement data tables. One measurement data table contains the field features for all classes and the other the object features for all classes.

Classes section

In the Classes section select the class whose measurement features you want to be displayed in the measurement data table. For each class there are two entries: the first entry concerns all the objects belonging to the class (field features) and the second represents an individual object (object features).

14.5.2.7 Panorama View

In the **Panorama** view you can see the complete overview of the microscope stage. The tools that allow you to control acquisition are arranged in the tools window in the bottom right corner of the image (available in Camera mode only). There you can control the exposure time, determine the focus position and acquire an individual or Z-stack image.

14.5.2.7.1 Stage View

In the image area the full travel range of the microscope stage is displayed. You can control the stage view using the arrow icons at the edges of the image area. The view can be enlarged, reduced or moved using the general control elements.

Navigator frame

The current stage position is shown as a tile outlined in blue, the Navigator frame. In the Navigator frame you can see the camera's live image.

To move the frame, double-click on the position on the microscope stage to which you want to move it.

To acquire images, use the **Acquisition** buttons in the **Tools window.**

14.5.2.7.2 Tools window

The tool window for Panorama view is normally visible in the lower right corner of the center screen area. It becomes active, if you move the cursor over it. You can use it to set acquisition parameters and acquire tile images for your panorama image.

Center to Live Navigator button

Centers the stage view at the current position of the Navigator frame.

Action buttons

With the action buttons you`re able to control acquisition parameters (e.g. focus, exposure time) like you are used to do it on the Acquisition tab.

Acquire Tile Image button

Acquires a tile image. This comprises all activated channels as well as Z-stacks. After the acquisition the tile image is placed in the corresponding location in the stage view.

14.5.2.8 S&F view

Besides the **Shuttle and Find** tool in the **Left Tool Area**, the **S&F (Shuttle & Find)** view is visible in the **Center Screen Area** of the ZEN software. If the S&F view is selected, the **S&F** tab and **S&F Correlation** tab will appear as specific view options under the image area.

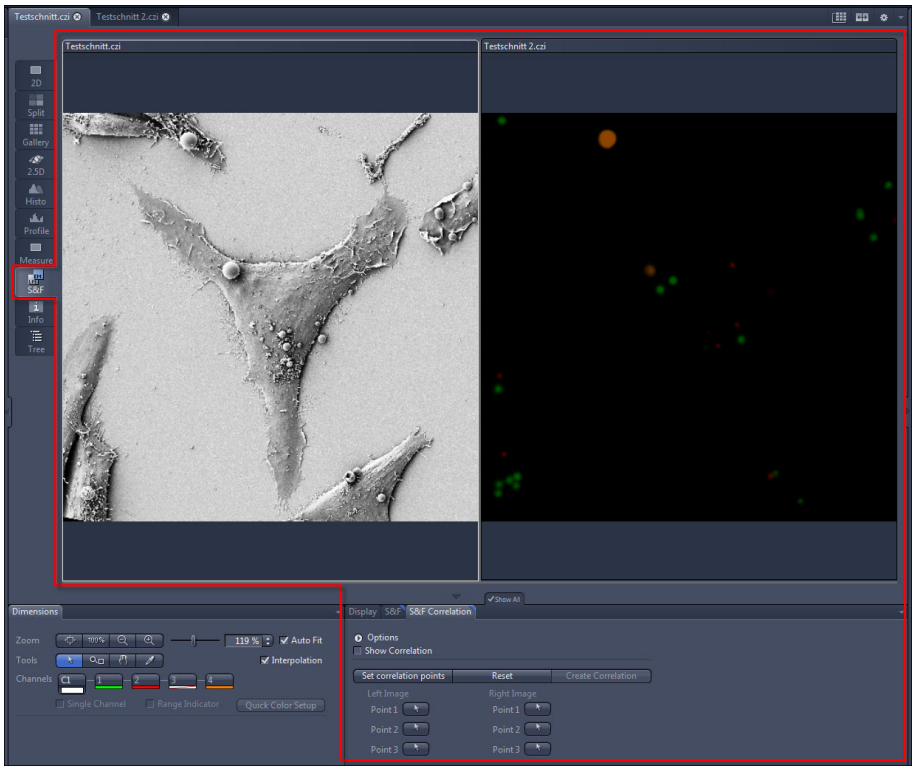


Fig. 75: Shuttle and Find View

14.5.2.8.1 Specific view options

14.5.2.8.1.1 S&F tab

Here you find helpful options and tools to draw in and relocate regions of interests (ROIs) or points of interest (POIs) within the sample image.

14.5.2.8.1.1.1 Options

Options	Description
Mirror Image	Here you can mirror the image horizontally or vertically by using the two buttons at the right. The alignment of the images depends on the microscope (upright/inverted) and orientation of the sample holder.
Keep tool	**Activated:** Keeps the current tool active. That's helpful if you want to draw in more than one ROI/ POI.
Auto color	**Activated:** Uses a new color for each new element which is drawn in.

Options	Description
Snap to Pixel	**Activated:** Draws in graphical elements using the pixel grid.
Use fine calibration value	**Activated:** Uses the measured fine calibration. The precision of relocation and therefore the quality of the overlay image can be improved by determination of an offset value. This value describes the offset between the loaded image and the live image. The defined offset value is only valid for the loaded image which you can see in the container. If another image is loaded or if you close the dialogue, the offset value will be deleted. Determine the offset by identification of a POI (Point Of Interest) within the snapped image. To identify a POI use the buttons in the **Regions** section. By clicking on the **Set Offset** button, the stage moves to the supposed sample position. Compare the sample position within the live image with the set POI and correct the stage in that way that both shown positions are identically. Confirm the fine calibration with the **Ok** button. Now the fine calibration is measured and the checkbox is activated.
Double click in image to move stage	**Activated:** Moves the stage to the position you have double clicked on.
Refocus after stage movement	**Activated:** Adjusts the focus automatically after the stage has moved.
Move stage in z-direction before x/y movement	**Activated:** Moves the stage to the load position before it moves to the next correlative calibration marker.
Show splitter view	**Activated:** Activates **Splitter Mode** in the **Center Screen Area**.

14.5.2.8.1.1.2 Regions, Find and Dimensions

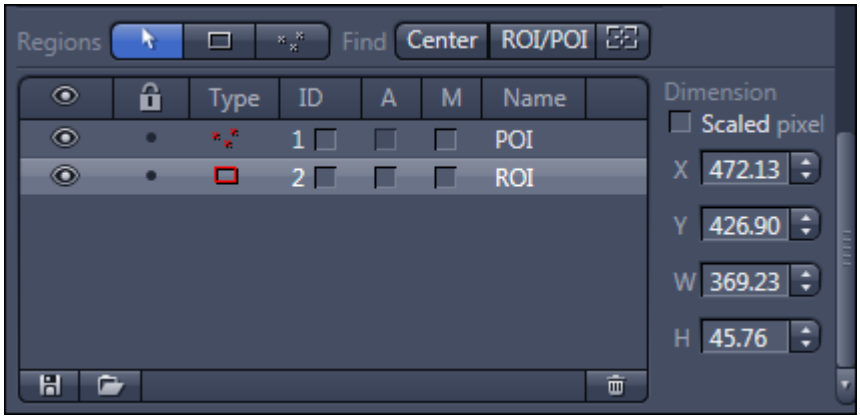


Fig. 76: Regions, Find, Dimension

Regions and Find tool bar

Button	Description
Selection mode	Selects the ROIs or POIs in the image area. If you are currently in another mode, you can switch back to the **Selection** mode using this button.
Draw rectangle	Draws in a rectangle (**Region of Interest (ROI)**) that is always parallel to the edges of the image.
Draw marker	Draws in a marker point (**Point of Interest (POI)**).
Center	Moves the stage to the center of the opened image.
ROI / POI	Moves the stage to the selected **ROI** / **POI**.
Show stage position	Shows the current stage position as a rectangle in the image.

Dimension section

Here you see coordinates and dimensions of the selected graphical element in the list. If the **Scaled** checkbox is activated, the unit is µm, otherwise Pixel.

- Parameter **X**: Shows the horizontal position (x coordinate) of the center of the graphical element.
- Parameter **Y**: Shows the vertical position (v coordinate) of the center of the graphical element.
- Parameter **W**: Shows the width of the graphical element.
- Parameter **H**: Shows the height of the graphical element.

Graphical elements list

Here you see the list of all ROI / POI which are drawn in. The following table describes the list columns and its functions:

List columns	Description
Eye symbol	Shows or hides the ROI / POI in the image.
Lock symbol	Locks a ROI / POI to prevent changes.
Type	Displays the icon for the tool type (ROI/POI). To format a graphic element, double-click on the icon. The **Format Graphic Elements** dialog opens.
ID	Only visible if the **Show All** mode is activated. Displays the ID for the graphic element. To do this, activate the checkbox at the corresponding list entry.
A	Only visible if the **Show All** mode is activated. Displays annotations for a graphic element (ROI). To do this, activate the checkbox at the corresponding list entry. Then double click on the checkbox. The **Format Graphic Elements** dialog opens. Choose an annotation you want to have displayed within the image from the **Annotation** dropdown list.
M	Only visible if the **Show All** mode is activated. Displays measurement data for a graphic element. To do this, activate the checkbox at the corresponding list entry.
Name	Displays the name of the graphic element. To change the name, double-click in the Name field. Then enter the text of your choice.

14.5.2.8.1.2 S&F Correlation tab

Here you find all functions to overlay (correlate) two images.

Options

To show the section in full, click on the **arrow** button  .

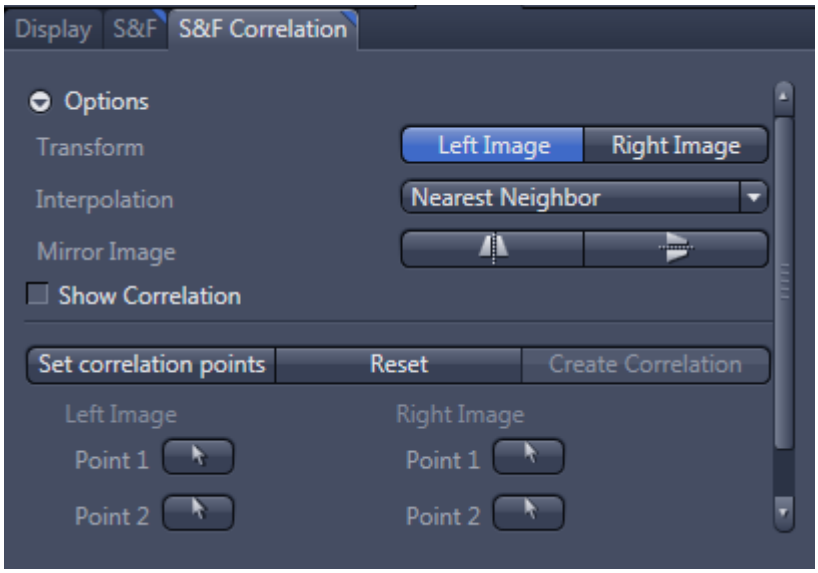


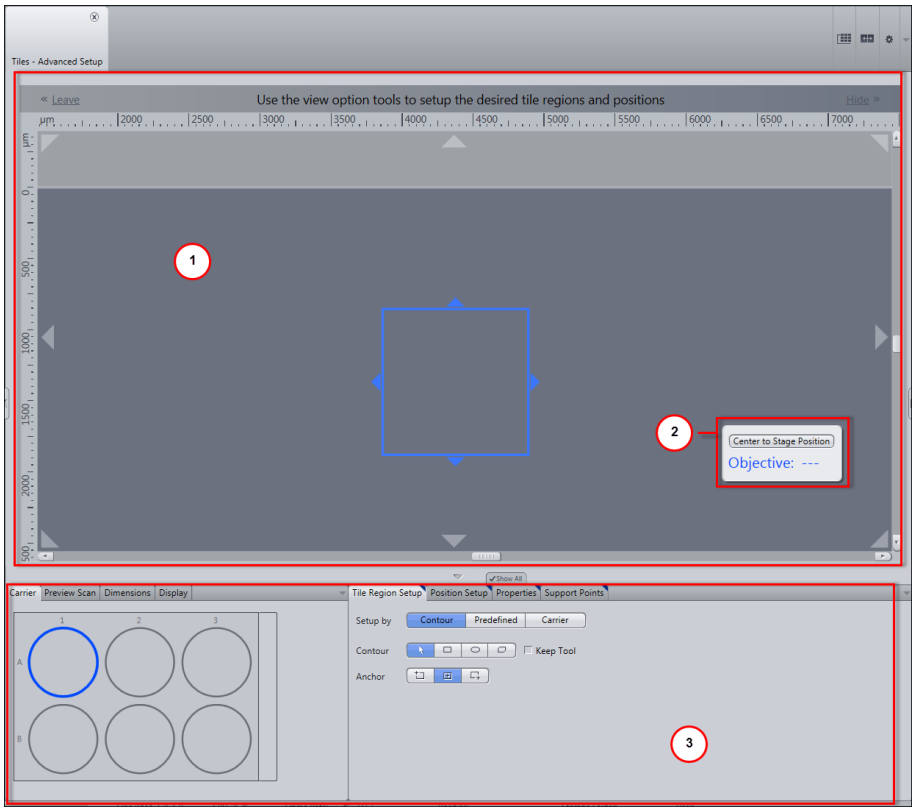
Fig. 77: S&F Correlation tab

Option	Function
Transform	Here you select which image will be transformed. During transformation a pixel in the overlay image is calculated by using pixels of the two original images that shall be overlaid / merged.
Interpolation	Here you can select one of the following interpolation methods: **Nearest Neighbor**: The gray value of the resulting pixel in the overlay image is made of a pixel which is located next. This interpolation method is very fast. **Linear:** The resulting or calculated pixel in the overlay image is assigned to a gray value, which is the result of a linear combination of gray values derive from pixels located nearby (in the original image). **Cubic:** The calculated pixel in the overlay image is assigned to a gray value, which is calculated by means of a polynomial function using gray values of pixels in the original images; these pixels are located nearby the calculated pixel.
Mirror image	Here you can mirror the image horizontally or vertically. Therefore simply click on the corresponding button. Mirroring an image is necessary, when the loaded image shows a different orientation than the live image.
Show Correlation	**Activated:** Opens the correlated image in a new image document / new container.

Option	Function
Set correlation points	Enables you to set 6 points (3 points in each image) as correlation markers in a row, see *Correlating two loaded images* [▶ 290].
Reset	Deletes all correlation points in the images.
Create Correlation	Active only, if all correlation points are set in both images. Creates a correlative overlay image. A third image container with the correlated image will be opened in the **Center Screen Area** and the **Show Correlation** checkbox will be activated automatically.

14.5.2.9 Advanced Tiles Setup

Here you can configure advanced settings and plan your Tiles and Positions experiment. In the Center Screen Area you can see the *Stage view* [▶ 563]. When the Advanced Tiles Setup (ATS) opens the stage view is "zoomed" to a predefined factor. The Zoom can be changed view the Dimensions view control, or by pressing Ctrl + scrolling mouse wheel.
To navigate around one has the following options: In each corner and along each edge the arrowheads can be clicked to move the view in this direction. To re-center the view on the current stage position press the **Center to Stage Position** button located in the lower right-hand corner of the Stage View. Additional settings and tools relating to tile regions or positions can be found under *Specific View options* [▶ 565].



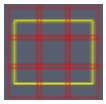
1 *Stage View* [▶ 563] **2** Center to Stage Position

3 *Specific View options* [▶ 565]

14.5.2.9.1 Stage View

The image area shows the full travel range of the microscope stage, along with the current stage position, the graphical display of sample carriers and your acquired mosaic images. You can control the stage view using the arrow icons at the edges of the image area. The view can be enlarged, reduced or moved using the general control elements.

Symbol	Name	Description
 / 	**Selected / Active** container/ well	The currently **Selected / Active** container / well is represented by a blue border.

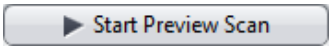
Symbol	Name	Description
	**Live Navigator** tool	In the Live navigator tool the current stage position including the live image is shown as a frame outlined in blue. To move the frame, double-click on the position to which you want to move it. Alternatively hold the left mouse button on the live navigator tool while dragging the mouse. The frame can also be used to control acquisition. If you click on one of the frame's blue arrow icons, an image is acquired. The Live Navigator tool is moved one frame width in the relevant direction. You can create tile images of your sample easily in this way.
	**Tile Region**	Tiles / tile regions are represented in the stage view by a red grid.
	**Positions**	Positions are represented in the stage view by a yellow plus symbol.
	**Position Array**	Position Arrays are represented in the stage view by the corresponding position symbols surrounded by a dashed line.
	**Local Support Point**	Local Support Points are represented in the stage view by a yellow circle with a dot in the middle.
	**Global Support Point**	Global Support Points are represented in the stage view by a white circle with a dot in the middle.

14.5.2.9.2 Preview scan tab

Here you can define the settings for a preview scan. Typically a low magnification objective is used, especially when a larger tile is to be acquired, to give the user a low resolution overview of the sample in question.

i Note

The objective setting used in the preview scan is not independent of that found in the experiment settings, but is the same as that set in locate or on the microscope´s TFT.

Option	Description
Use Existing Experiment Settings checkbox	**Activated:** Uses the existing experiment Settings. That is the default setting. **Deactivated:** Additional options **Camera** and **Channels** appear. That allow independent activation/ deactivation of channels and **Use Binning from Experiment** versus the defined experiment settings. If binning is used then the exposure time is automatically compensated to avoid saturation. Changing these parameters does not effect the settings that will be used for the experiment.
Delete Existing Preview Images checkbox	**Activated:** Deletes all existing preview images when the next preview is acquired.
Objective dropdown list	Here you can select the objective which will be used for the preview scan. To acquire an overview of all active tiles and positions images, switch to an objective with a low magnification. Set the channel exposure time and start the acquisition of the overview image. To select an other objective click on  and select it from the dropdown list.
Start Preview Scan button 	Starts the preview scan to acquire the overview images.

i Note

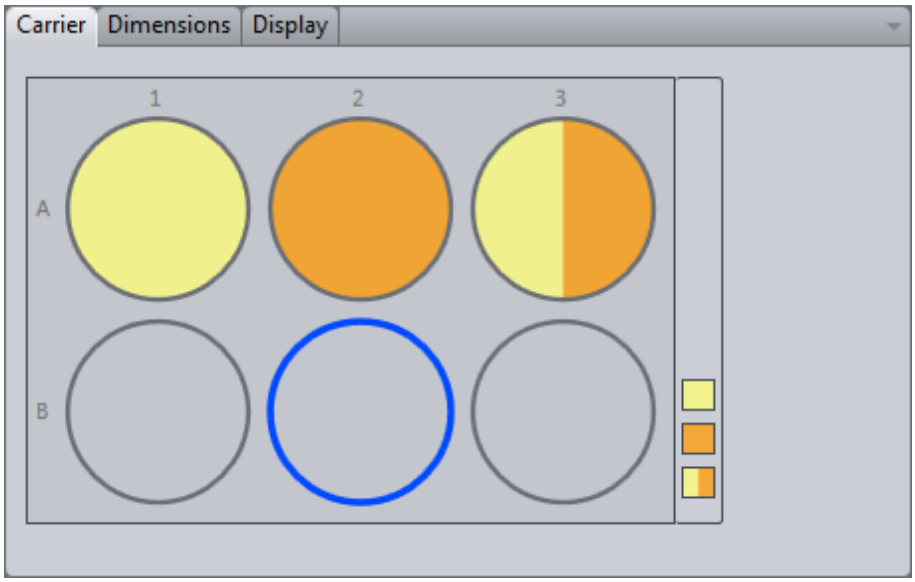
Note that the selected objective will now be used for any subsequent activities with Locate or Acquisition tab. Thus, you must actively change the objective after the preview scan if you want to use another objective for your experiment.

14.5.2.9.3 Specific View options

14.5.2.9.3.1 Carrier tab

Only visible if a sample carrier was selected.

Here you can see a graphical preview of the sample carrier being used. Please note the following features of the display:



Note

Only the containers / wells whose tile regions and positions were set up with the *Setup by Carrier* [▶ 570] of the **Tile Regions Setup** tab or the *Setup by Carrier* [▶ 578] of the **Positions Setup** tab will be taken into account.

Symbol	Description
/	**Empty** containers / wells, meaning that no tile regions or positions were set up with the Carrier option, are represented by a grey conatiner / well.
/	The currently **Active** container / well is represented by a blue border.
/	A container / well only filled with **Tile Regions** is represented by a yellow filled conatiner / well.
/	A conatiner / well only filled with **Positions** is represented by a orange filled conatiner / well.
/	A container / well filled with **Tile Regions** and **Positions** is represented ba a half yellow, half orange filled conatiner / well.

i Note

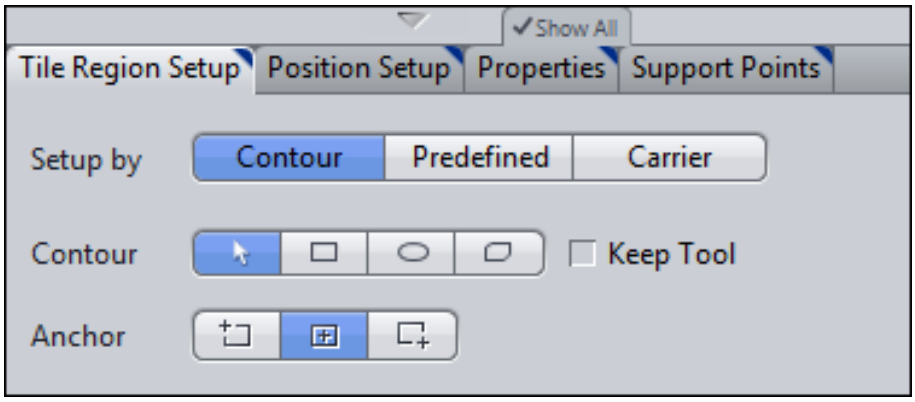
Right click opens a small context menu. Here you can copy the contents of the selected well, or paste the contents to the selected or all wells.

14.5.2.9.3.2 Tile Region Setup tab

Here you can select which setup you want to be used for the settings of the tile regions. Three setups with different setting options are available:

14.5.2.9.3.2.1 Setup by Contour

Here you can define the tile regions by means of the contour.



Contour section

Here you can select the contour of your tile region. The following tools are available:

Option	Description
Selection button	With this tool you can select an already created tile region by clicking on it to move or edit it.
Rectangle button	With this tool you can draw a rectangle tile region.
Ellipse button	With this tool you can draw a elliptical tile region.
Polygon button	With this tool you can draw a polygonal tile region.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

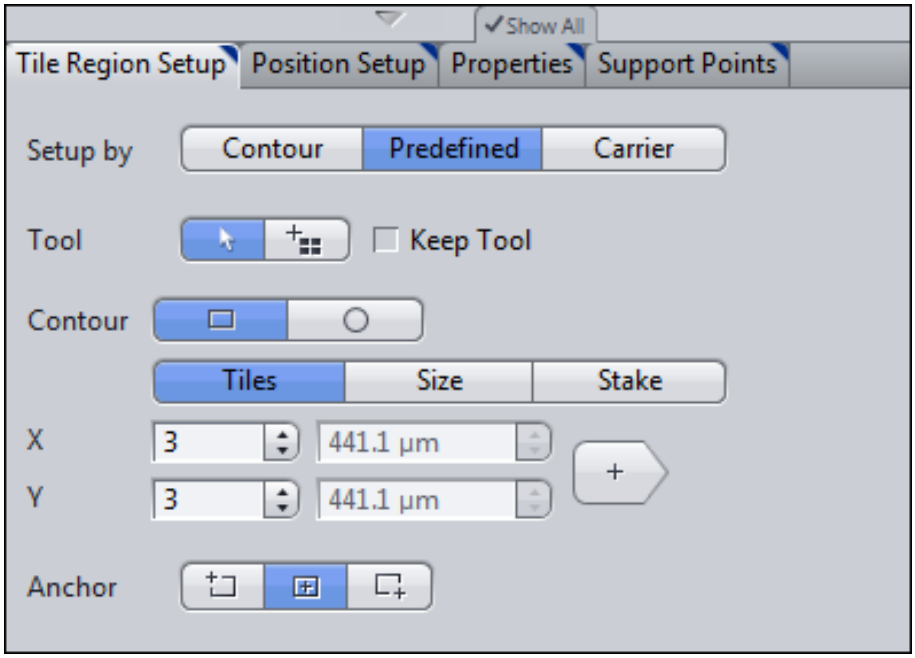
Anchor section

Here you can select the anchor position of the new tile region. The following tools are available:

Option	Description
Anchor Top Left button 	The anchor of the defined shape is at the top left.
Anchor Centered button 	The anchor of the defined shape is centered.
Anchor Bottom Right button 	The anchor of the defined shape is at the bottom right.

14.5.2.9.3.2.2 Setup by Predefined

Here you can define the tile regions by means of the number or size.



Tool section

Here you can select a tool to work with. The following tools are available:

Option	Description
Selection button 	Select an element in the stage view to edit or move it.

Option	Description
Add Tile Region button 	Adds the current tile definition in the image area.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

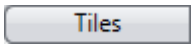
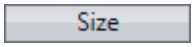
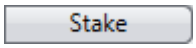
Contour section

Only visible if the **Show All** mode is activated.

Here you can select the contour of your tile region. The following tools are available:

Option	Description
Rectangle button 	Adds a rectangular tile region.
Circle button 	Adds a circular tile region

Mode section

Option	Description
Tiles button 	Using this mode you have to enter the number of tiles as a reference for the size of the tile region. Enter the number of tiles in the **X / Y** input fields. If you are adding a circular tile region, enter the number of tiles for the diameter in the **Diameter** input field.
Size button 	Using this mode you have to enter the size as a reference for the size of the tile region. Enter the size of the tile region in the **X / Y** input fields. If you are adding a circular tile region, enter the diameter of the tile region in the **Diameter** input field.
Stake button 	This mode allows the definition of a tile region by the placement of at least two markers (user defined X/Y stage coordinates). If you want to modify the tile region (expand/ reduce) you have to adjust the tile region to the desired size. To complete the tile region press **Done**. Circular or rectangular tile region can be created in this manner by selection of the appropriate contour.

Option	Description
Add button 	Adds the tile region to the **Tile Regions List** and activates it for acquisition. Added tile regions are displayed in the form of red grids in the stage view of the **Advanced Tiles Setup**.

Anchor section

Only visible if the **Show All** mode is activated.

Here you can select the anchor position of the new tile region. The following tools are available:

Option	Description
Anchor Top Left button 	The anchor of the defined shape is at the top left.
Anchor Centered button 	The anchor of the defined shape is centered.
Anchor Bottom Right button 	The anchor of the defined shape is at the bottom right.

14.5.2.9.3.2.3 Setup by Carrier

Here you can define the tile regions automatically by means of the fill factor of the sample carrier.

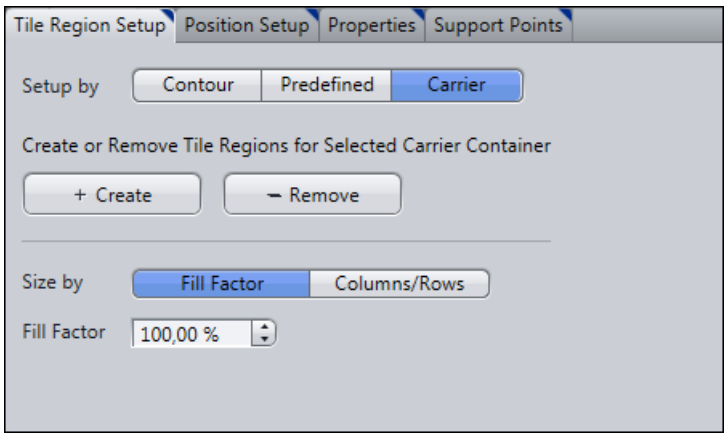
i Note

A sample carrier must have been selected in the *Sample Carrier section* [▶ 157] of the **Tiles** tool.

i Note

Manually created tile regions and positions (setup by **Contour** and setup by **Predefined**) will be deleted, if you switch to the setup by **Carrier**. If you want to combine manual and automatic setup, first use setup by **Carrier** and then switch to a manual setup.

Tile regions that are created automatically by setup by **Carrier**, are defined to a container and permanently assigned and locked by default, against manual edits. You can unlock the tile regions in the **Tiles** tool by selecting the desired tile region and click on the unlock button.



Option	Description
Create button + Create	Only active if you have selected a container on the *Carrier tab* [▶ 565] or in the *Stage View* [▶ 563]. Automatically creates the tile regions with the set fill factor in the selected container of the sample carrier.
Remove button – Remove	Removes all tile regions in the selected container.
Fill Factor input field Fill Factor	Here you can enter the fill factor used to fill the selected container.
Columns/Rows input field Columns/Rows	Here you can add single tile regions to a container by defining the number of columns and rows of the tile. The tile region is always placed at the center of the well container.

14.5.2.9.3.3 Properties tab

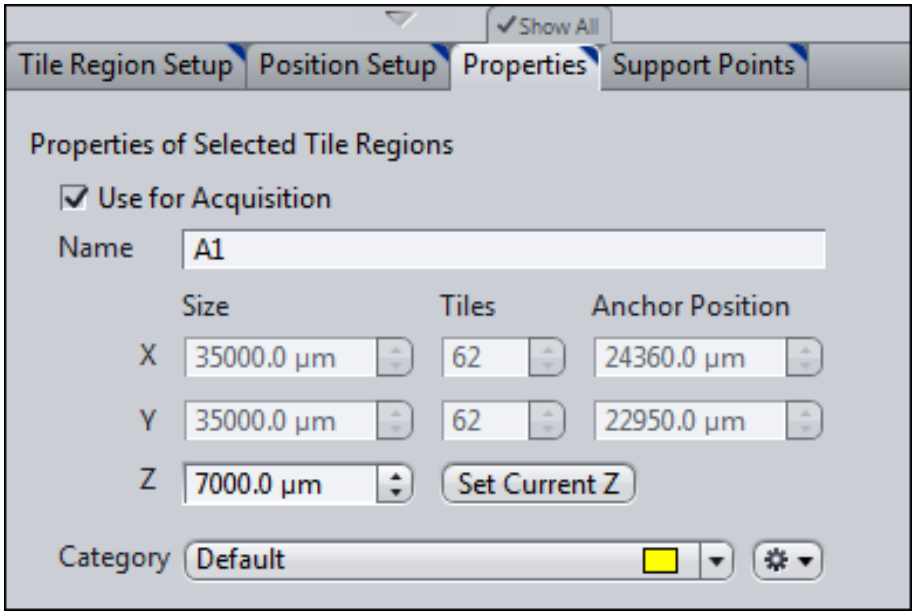
Here you can adjust the properties of a selected tile region or position.

i Note

You have to select a tile region or single position to see the parameters available on this tab. It is not possible to see and adjust the parameters for a whole position array.

14.5.2.9.3.3.1 Properties of selected Tile Regions

Here you can adjust the properties of the selected tile region.

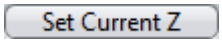


Option	Description
Use for Acquisition checkbox	**Activated:** Uses the selected tile region for acquisition.
Name input field	Here you can enter a name for the selected tile region.

Properties section

Only visible if the **Show All** mode is activated.

The properties section contains the following columns and buttons:

Option	Description
Size column	Here you can see and edit the size of the tile region in the **X / Y / Z** dimensions. The **X / Y** dimensions of tile regions created with the *Setup by Carrier* [▶ 570] can not be edited as they are fixed by the container / well size.
Tiles column	Here you can see the number of tiles in the **X / Y** dimensions. You can not edit the number of tiles as it is fixed by the size of the tile region.
Anchor Position column	Here you can enter the anchor position of the selected tile region in **X / Y** dimensions. The anchor position of tile regions created with the *Setup by Carrier* [▶ 570] can not be edited as they are fixed by the container / well.
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

Category section

Only visible if the **Show All** mode is activated.

Here you can assign categories to tile regions. Category definitions will be displayed in the appropriate column of the table in the Tiles tool. This value is also written in the image meta-date. Thus, well definition patterns or variables can be created and stored as part of a experiment template.

Option	Description
Category dropdown list	Shows the currently assigned category of the selected tile region. The **Default** category is set for all new tile regions. Click on  assign an other category.
Options button 	Opens the options for editing categories.

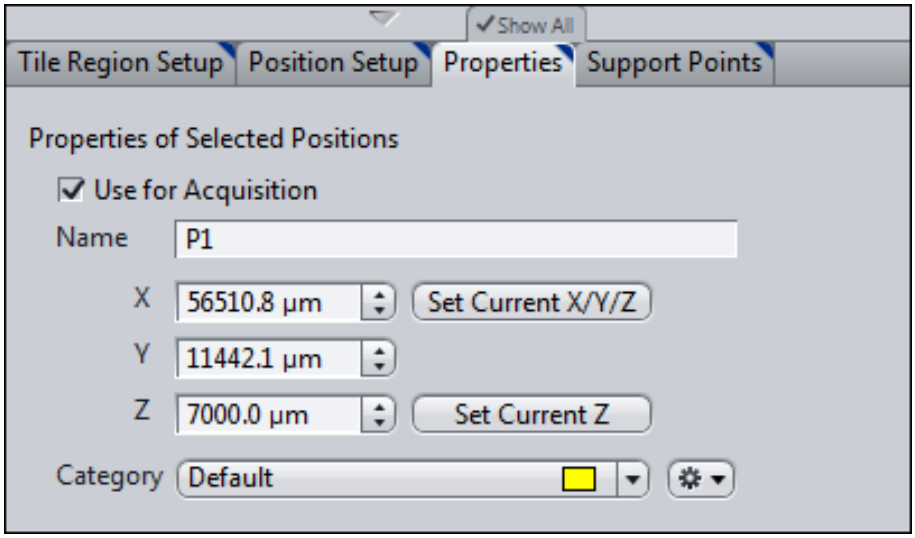
Options for editing Categories

Option	Description
New...	Opens the **New Category** dialog to create a new category.

Option	Description
Edit…	Opens the **Edit Category** dialog to edit the selected category.
Delete	Deletes the selected category and sets the category of the tile region to **Default**.

14.5.2.9.3.3.2 Properties of selected Positions

Here you can adjust the properties of the selected position.

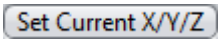


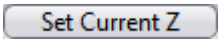
Option	Description
Use for Acquisition checkbox	**Activated:** Uses the selected position for acquisition.
Name input field	Here you can enter a name for the selected position.

Properties section

Only visible if the **Show All** mode is activated.

The properties section contains the following columns and buttons:

Option	Description
Position column	Here you can see and edit the position of the selected position on the stage in **X / Y / Z** dimensions.
Set Current X/Y/Z button 	Sets the **X / Y / Z** dimension at the current **X / Y / Z** position of the stage.

Option	Description
Set Current Z button 	Sets the **Z** dimension at the current **Z** position of the stage.

Category section

Only visible if the **Show All** mode is activated.

Here you can assign categories to tile regions. Category definitions will be displayed in the appropriate column of the table in the Tiles tool. This value is also written in the image meta-date. Thus, well definition patterns or variables can be created and stored as part of a experiment template.

Option	Description
Category dropdown list	Shows the currently assigned category of the selected tile region. The **Default** category is set for all new tile regions. Click on  assign an other category.
Options button 	Opens the options for editing categories.

Options for editing Categories

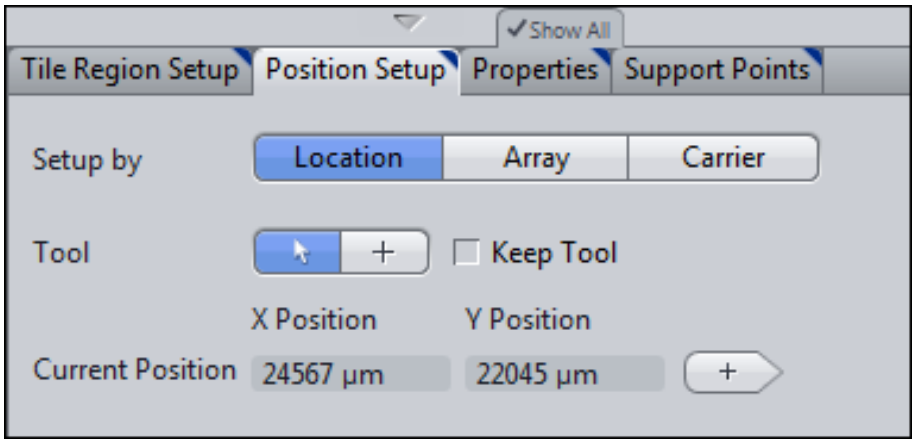
Option	Description
New...	Opens the **New Category** dialog to create a new category.
Edit…	Opens the **Edit Category** dialog to edit the selected category.
Delete	Deletes the selected category and sets the category of the tile region to **Default**.

14.5.2.9.3.4 Position Setup tab

Here you can select which setup you want to be used for the settings of the positions. Three setups with different setting options are available:

14.5.2.9.3.4.1 Setup by Location

Here you can define the positions by means of the location. You can add various positions in the stage view using the mouse.



Tool section

Here you can select a tool to work with. The following tools are available:

Option	Description
Selection button	Select an element in the stage view to edit or move it.
Add button	Add a new position on the stage view.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

Current Position section

Displays the current stage position (X/Y).

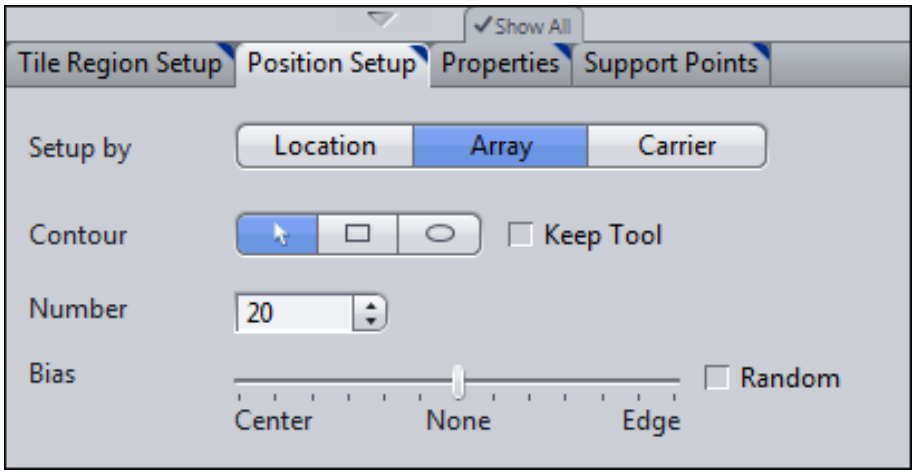
Option	Description
Add button	Adds a new position at the current stage position.

14.5.2.9.3.4.2 Setup by Array

Here you can define the positions by means of position arrays. You can add various contours for position arrays in the stage view.

Note

Position arrays are groups made up of a number of individual positions. Typically, position arrays contain several hundred individual positions. They make your work easier if you work with regular or evenly distributed samples.



Contour section

Here you can select the contour of your tile region. The following tools are available:

Option	Description
Selection button	With this tool you can select an already created position array by clicking on it to move or edit it.
Rectangle button	With this tool you can draw a rectangle position array.
Ellipse button	With this tool you can draw a elliptical position array.
Keep Tool checkbox	**Activated:** Keeps the selected tool active.

Number section

Option	Description
Number input field	Shows the current number of positions that are distributed to newly created position array. Change the number to increase or decrease the number of single positions obtained by a position array.

Bias section

Only visible if the **Show All** mode is activated.

Here you can set the distribution bias of the single positions created for a new position array.

Option	Description
Bias slider	Adjusts the overall position of the single positions in the position array.
- **None**	The single positions of the position array will be distributed evenly within the array.
- **Center**	The single positions of the position array will mainly be distributed near to the center of the position array. Less positions will be at the edges of the array.
- **Edge**	The positions of the position array will be distributed to the edges of the array. Less positions will be in the center of the array.
Random checkbox	**Activated**: The single positions will mainly be distributed randomly within the position array. The overall bias will still be taken into account.

14.5.2.9.3.4.3 Setup by Carrier

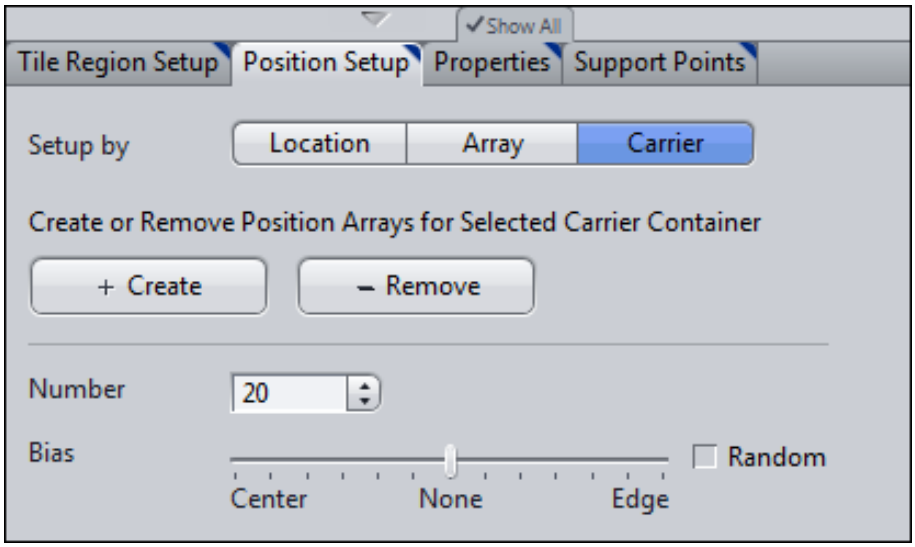
Here you can define the positions automatically by means of the relevant sample carrier.

i Note

A sample carrier must have been selected in the *Sample Carrier section* [▶ 157] of the **Tiles** tool.

i Note

Manually created tile regions and positions (setup by **Contour** and setup by **Predefined**) will be deleted, if you switch to the setup by **Carrier**. If you want to combine manual and automatic setup, first use setup by **Carrier** and then switch to a manual setup.



Option	Description
Create button + Create	Only active if you have selected a container on the *Carrier tab* [▶ 565] or in the *Stage View* [▶ 563]. Automatically creates the tile regions with the set fill factor in the selected container of the sample carrier.
Remove button – Remove	Removes all tile regions in the selected container.

Number section

Option	Description
Number input field	Shows the current number of positions that are distributed to newly created position array. Change the number to increase or decrease the number of single positions obtained by a position array.

Bias section

Only visible if the **Show All** mode is activated.

Here you can set the distribution bias of the single positions created for a new position array.

Option	Description
Bias slider	Adjusts the overall position of the single positions in the position array.

Option	Description
- **None**	The single positions of the position array will be distributed evenly within the array.
- **Center**	The single positions of the position array will mainly be distributed near to the center of the position array. Less positions will be at the edges of the array.
- **Edge**	The positions of the position array will be distributed to the edges of the array. Less positions will be in the center of the array.
Random checkbox	**Activated**: The single positions will mainly be distributed randomly within the position array. The overall bias will still be taken into account.

14.5.2.10 MeanROI View

i Note

The **Physiology** module activates addition features to those of MeanROI for the offline analysis of dynamics (physiology) experiments, e.g. the **Timline View Panel**.

i Note

In this view the Image area is always to the left, charting area always to the right. Depending on which Region layout you have selected in **Layout** tab the MeanRoi view can have a different appearance.

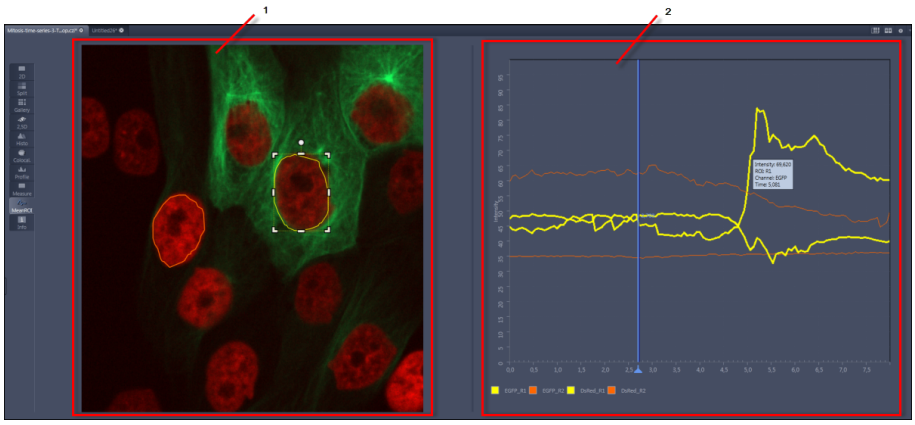


Fig. 78: MeanROI view

1 Image area

2 Charting area (default regions layout)

Charting Area (2)

Playhead (blue line)

Indicates the current frame of the time series visible in the image panel(s). The position of the playhead is synchronised with the displayed image frame number and vice versa. The Playhead can be dragged. Hover over the Playhead with the mouse to reveal a horizontal double headed arrow. Press and hold the left mouse and drag the Playhead in the desired direction. The current time point of the visible frame is displayed to right in the middle of the playhead line in the same time units as the chart x-axis.

Data intensity plot of correspondingly coloured ROI (yellow graph line)
Note that this ROI is selected and therefore the plot line thickness is increased as a highlight.

Data intensity plot of correspondingly coloured ROI (orange graph line).

Note that the this ROI is un-selected and therefore the plot line thickness is normal.

i Note

Tool tip info box

Hover with the mouse (crosshair) over the plot. A tool tip appears with details of the intensity value at this position, ROI ID #, Channel, and time point (in currently set time unit of x-axis). Note these values (intensity and time) are interpolated.

14.5.2.10.1 Time Line View panel

Only visible if you have licensed the **Physiology** module.

This chart supports that same functions as detailed for charts in **Mean ROI** e.g. zoom, data selection and tool tips.

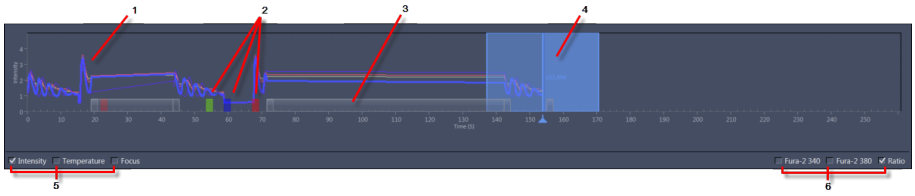


Fig. 79: Time Line View panel

1 Intensity data traces from measurement ROIs

4 Zoom tool control

2 Switch markers with corresponding color code.

3 Experiment pause marker

5 Chart data parameter selection check boxes

5 Intensity parameter selection check boxes

Playhead / Zoom tool (4)

The center line (blue line) displays the playhead. The transparent area (blue) is the user definable zoom range. The zoom range translates into the display range of the x-axis of the other charts displayed above the time line view. To edit the zoom range hover over the bounding lines at either end. A double headed arrow will appear. Click and hold the left mouse to drag and resize the area width.

Chart data parameter selection check boxes (5)

Depending on the available metadata different check boxes are available. Only two parameters can be displayed at a given moment. The combination can be chosen as required.

Intensity parameter selection check boxes (6)

When intensity parameters are displayed, it is possible to further select which of the available channels and or ratio. Channel intensity and ratio intensity can be displayed in a mutually exclusive manner.

14.5.2.10.2 Specific view options

14.5.2.10.2.1 Mean ROI tab

Parameter	Description
Measurements	Change the display of the selected clipping plane using the dropdown list to the right of the **Activate** checkbox. The following settings are available:
- Mean Intensity	If selected, the ROI value represents the mean intensity of the pixels within it.
- Integral Intensity	If selected, the ROI value represents the sum of all pixel intensities within it.
- Maximum Intensity	If selected, the ROI value represents the value of most intense pixel within it.
Recalculate button	Starts a recalculation of the selected measurement. For example when loading a data set that contains ROIs from a previous session.

Parameter	Description
Background Correction	A background correction can be performed on a channel by channel basis. Selection of a background correction method modifies the ratio set-up formula accordingly. Following modes are available:
- None	No background correction is performed.
- Constant	Allows a user defined numeric value to be entered for each channel in the appropriate spin box.
- ROI	Allows to select the background ROI defined in the **Mean ROI Setup**. Note that for dual wavelength protocols the same ROI is used in each case, but its channel specific values are applied for the correction.

14.5.2.10.2.2 Layout tab

Parameter	Description
MeanROI View Layouts	Here you can adjust how the image, chart and table will be displayed.
- Image and Chart	Select one of three different layouts of how an image together with a chart will be displayed. If you click on one of the buttons the layout will be changed.
- Image and Chart with Table	Select one of three different layouts of how an image and a chart together with a table will be displayed. If you click on one of the buttons the layout will be changed.
Follow Acquisition checkbox	**Activated:** The software displays the last acquired image during a Regions on-going time series acquisition procedure. The playhead of the chart display follows the acquisition.
Show Markers/ Switches checkbox	**Activated:** The temporal position of any switches and markers are always displayed on the charts both during acquisition or post-acquisition.

Parameter	Description
Show Time Line View checkbox	Only visible if you have licensed the **Physiology** module. **Activated:** The Time Line View panel is displayed below the other image chart panels of the **Center Screen Area**. The Time Line View panel is designed to provide an overview of the experiment whilst allowing the user to define the detail displayed in the other chart panels by means of an integrated zoom tool. The Time Line View can be hidden by unselecting the check box as required both during or after an experiment.
Show View Captions checkbox	**Activated:** Displays the channel name clearly with the image of each channel in the multichannel view layout.
Show Ratio Viewcheckbox	Only visible if you have licensed the **Physiology** module. **Activated:** The ratio image and ratio chart panel is visible below channel image and chart panel of the **Center Screen Area**. The ratio image and ratio chart panel can be hidden by unselecting the check box as required.

14.5.2.10.2.3 Charts tab

Parameter	Description
Chart Settings (X- / Y-Axis)	Note that a function is active when the button is highlighted in blue. The settings for **X-** and **Y-Axis** (only if Show All is activated) are the same, see description below:
- Auto	The scaling of the respective axis is automatic, allowing for an optimal, and appropriate adjusting display of the all values.
- Norm	The scaling of the axis is normalized to fit the maximum value in the data set.
- Fixed	The upper and lower limit of the axis can be defined using the min and max spin boxes.
X-Units	
- Auto	The units are selected automatically.
- Fixed	You can select the desired unit for the x-axis from the dropdown list.

Parameter	Description
Show Tick Marks	Shows tick marks in the chart. If activated, you can set the **Form** and **Size** of the tick marks.
Show Legend	Shows the chart legend.
Show Axis Captions	Shows captions of the axis.

14.5.2.10.2.4 Export tab

Parameter	Description
Data Table	
- Create	Opens the measurement data table in a new document tab. The table displays all measurement values (Mean, Integral, and Maximum intensity) and area for all ROIs in each channel. If event markers are present these are also listed here at the appropriate time points. This function provides access to the ZEN Blue charting functions.
- Save	Opens the Save As dialogue and allows the measurement data to be exported as a comma separated value (*.csv) file. The following values are exported for each ROI and channel: Intensity (Mean, Integral, and Maximum intensity), area and if present event markers.
New Image From	The menu contains three options for saving a screenshot of the MeanROI view.
- Current view	Both image and chart layouts will be saved in the screenshot.
- Only Chart	Only the chart layout will be saved as a screenshot.
- Only Chart (transparent)	Only the chart layout will be saved as a screenshot where the background color of the chart is removed and only gridlines ,x- and y-axis and data plots are kept.
- Save	Opens the Save As dialogue to save the selected screenshot. Optional image formats include: *.bmp, *.jpg, *.tif, *.png and *.xps.
Ratio image	
- Create from all	
- Save	Opens the **Save As** dialogue to save the ratio image as a ***.czi** file.

14.5.2.10.2.5 Ratio tab

This view option is almost identical to the **Online Ratio** tab in the **MeanROI Setup**, see *Online Ratio tab* [▶ 586]. In fact when an experiment is finished the exact same values used for the display of the online ratio are transferred to the offline ratio tool of the **MeanROI** view.
For offline ratio assessment the settings can be changed and applied to recalculate the ratio image and measurements. The following descriptions will describe the differences on the Ratio tab:

Reference image (Ft_0) Set-up

This difference only applies if the Single wavelength method is selected:

- **Range** spin boxes:
  The numbers in the spin boxes refer to the frame number of the experiment currently been viewed in MeanROI view. The desired frame numbers can be entered by the spin buttons or directly by typing a number into the box.
- **Set** buttons:
  Each **Set** button belongs to the corresponding spin box above it. Use the set buttons as follows: Scroll to the frame in the image sequence that should be the start of the range used to calculate the average. Then press Set. Repeat this process for the other Set button to determine the end frame of the range used to calculate the reference image. The **Recalculate** button will be highlighted then. Click it to recalculate the ratio with the new Ft_0 value.

14.5.2.10.2.6 Online Ratio tab

Only visible in **MeanROI Setup**.

Parameter		Description
Method		Select the ratiometric method you want to use. **Single** or **Dual wavelength** dyes are supported. The ratio set-up will change in accordance with your selection.
Calculation		
-	Single Wavelength Method	Select the channel in the dropdown menu. The Ft_0 value is the averaged fluorescence from the specified number of image frames. The number of frames to average is defined in the spin box of the reference image set-up (see 10). The spin box at the far left is a multiplication factor
-	Dual Wavelength Method	Select the channels in the dropdown list required to calculate the ratio values/image e.g. for Fura-2, a dual excitation dye, the numerator is the 340 nm image the denominator the 380 nm image. For dual emission dyes the function is identical. The spin box at the far right is a multiplication factor.

Parameter		Description
Color		Select the color (LUT) used to display the ratio image during the online measurement. Per default the Rainbow LUT is used as it allows intensity changes to be followed easily.
Background Correction		A background correction can be performed on a channel by channel basis. Selection of a background correction method modifies the ratio set-up formula accordingly. Following modes are available:
-	None	No background correction is performed.
-	Constant	Allows a user defined numeric value to be entered for each channel in the appropriate spin box.
-	ROI	Allows to select the background ROI defined in the **Mean ROI Setup**. Note that for dual wavelength protocols the same ROI is used in each case, but its channel specific values are applied for the correction.
Threshold		
-	Enable	**Activated:** Allows the threshold values to be set for the ratio calculation.
-	Channel / Threshold	A threshold value can be applied in the form of a constant integer value for each channel individually. Thresholds help to reduce noise anomalies that are cause by pixel to pixel variations in areas between cells or near cell boarders during the ratio calculation. Enter the desired threshold value for each channel into the spin boxes provided.

14.5.2.11 Unmix View

This view is only visible for multi-channel or lambda stack images. It is used for:

- display the spectra corresponding to defined ROIs (mean ROI intensity over Lambda),
- show the intensity values in table form, copy the table to clipboard or save the table as a text file and
- generate linear unmixed multi-channel images.

In this view you will see 2 areas as default. The intensity-over-lambda diagram **(1)** to the left and the image display **(1)** to the right. The specific view options **(3)** below the image area are described in the following topics.

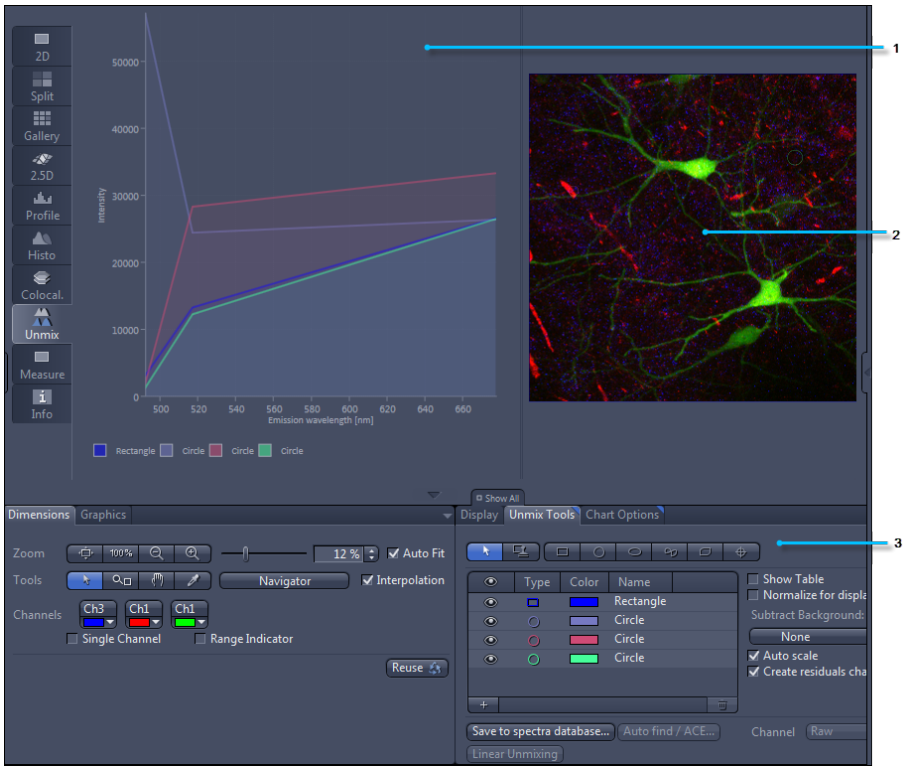


Fig. 80: Unmix View

14.5.2.11.1 Unmix Tools tab

Here you can select various tools and use these to draw graphic elements into your images, similar to the tool bar on the **Graphics** tab. You can also obtain an overview of the graphic elements that you are using in your image.

The following list will describe the specific parameters for this tab:

Parameter	Description
Toolbar	Using the tools you can draw in certain regions of interest which are then displayed in the intensity-over-lambda diagram and will be used for linear unmixing.
List of Spectral Data	The list gives you an overview of the spectral data in the image, which will be used for linear unmixing. The names indicates the origin, e.g. if manually or automatically picked by **ACE** (see below) or loaded from the spectra database. To load a spectrum from the spectral database, press the Add **+** button for a new row. Click into the Name column and select the according name for the needed spectrum.
Save to spectra database	If you click on this button you can save the selected entry to the spectra database.

Parameter	Description
Auto find / ACE...	ACE stands for Automatic Component Extraction. If you click on this button the software automatically searches for regions with distinct spectral signatures and tries to find the defined number of spectra.
Linear Unmixing	Performs the linear unmixing processing of the image with the selected spectra. Note: The channels of the Lambda stack which are de-selected in the **Dimensions** tab are not included in the calculation.
Show Table	**Activated**: Displays a table of intensity values over Lambda below the default image area.
Normalize for display	**Activated:** Normalizes the graphs of the spectra to 1.
Subtract background	Here you can select the list entry of a marked spectrum that should be subtracted before linear unmixing.
Auto scale	**Activated:** Automatically balances the intensity of unmixed channels to equal levels.
Create residuals channel	**Activated:** Generates an additional channel in which the intensity value represent the difference between the acquired spectral data and the fitted linear combination of the reference spectra for each pixel. In essence, the residual value is the biggest remaining "residual" from the least square fit routine. The residuals are a general measure for how good the fit of the algorithm has performed.
Channel	
- Raw	The raw data acquired during a lambda stack is used as channels and for spectral display.
- Spectral	The intensity data of the lambda stack is calculated into channels for each detector (Channel 1 and Channel 2).

14.5.2.11.2 Chart Options tab

This tab provides several parameters to change the appearance and contents of the spectral graph. For the beginning we recommend to use the default settings here.

14.5.2.12 Lambda View

In this view you can display images that are acquired in Lambda mode, see *Lambda Mode* [▶ 386]. the resulting images are called Lambda stacks. For that type of image the **2D** View is not available.

Instead the Lambda View displays a Lambda Stack in a wavelength-coded color view as default. A color palette, mimicking the emission wavelength of the channel, is automatically assigned to the individual lambda images which are then displayed in a merge-type display.

On **Display** tab, the channel-specific settings of brightness, contrast and gamma can be handled as described for channels in the **2D** View.

In order to use other views (e.g. Split or Gallery view) or to view lambda stack data sets in ZEN black, convert the data set using the **Convert to Lambda** image processing function.

The general view options on **Dimension** tab are adapted to the Lambda View with the following changes:

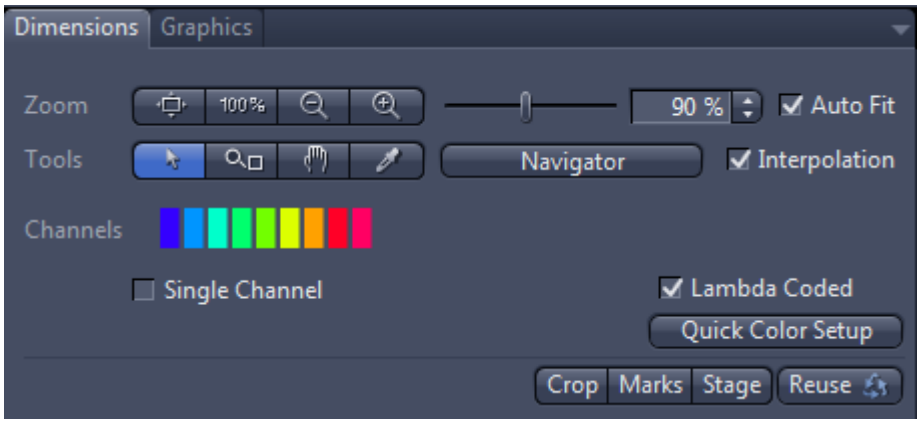


Fig. 81: Dimensions tab changes for Lambda view

Parameter	Description
Channels section	Displays the single channels of a Lambda stack image as a colored button. You can handle the channels like in the 2D View. E.g. if you click on a channel button you can show or hide the channel in the image area.
Lambda Coded checkbox	If activated, all channels are displayed as a merged image. Each channel is assigned to a channel color that represents the recorded emission wavelength in the lambda stack. If deactivated, only one channel of the Lambda Stack is displayed without pseudo coloring. Additionally the **Single Channel** checkbox is activated and cannot be changed. To display a different channel of the Lambda Stack, click on the according channel. This will display the chosen channel and deactivate the previously displayed channel.

14.5.3 General View Options

This view options are visible in any view. Some of the view options are only visible when you open a particular file type. E.G. see the **ApoTome** view option only if you have opened a **ApoTome** picture.

14.5.3.1 Dimensions tab

Here you configure the settings for how the image will be displayed on the screen. You can select the size of the display and call up information about the content of the image. In the case of multidimensional images you can select here which dimension is displayed. The dimension sliders (e.g. time, channels) help you to navigate through the single images of an experiment.

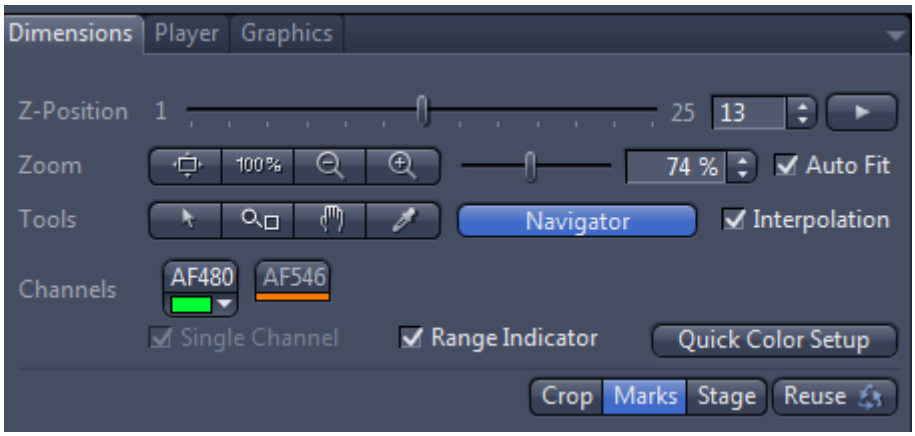


Fig. 82: Dimensions Tab (Show All)

14.5.3.1.1 Slider section

Depending on how many dimensions your image contains, several sliders can be available in this section. Using the sliders you can adjust the position that you want for each dimension available in the image. You will see the current position number in the input field to the right of the relevant slider. You can also enter the position number directly here.

Fig. 83: Slider Section (showing the Time slider only)

The **Play**  buttons to the right of the input fields enable you to play back the dimension automatically. This takes place at a rate of 5 images per second by default. You can change the speed on the Player tab.

i Note

For images with more than 3 dimensions a scrollbar is displayed which you can use to access the other sliders.

Depending on the available dimensions, the following sliders can be visible:

Parameter	Description
Z-Position	Only visible in the case of Z-stack images. Here you can adjust the desired Z-position.
X-Position	Only visible for images acquired in Line scan mode. Here you can adjust the desired X-position.
Time	Only visible in the case of time series images. Here you can adjust the desired time point.
Phase	Only visible when using the Airyscan for imaging. Here you can adjust which of the 32 channels (phase) from the Airycan detector is displayed.
Scene	Only visible if the image contains different scenes. Here you can adjust the desired scene. If you deactivate the **Scene** checkbox, all scenes are displayed in an overview.
Block	Only visible if you have used the **Experiment Designer** and created several experiment blocks. Here you can adjust the desired experiment block.
Total Time	Only visible if you have used the **Experiment Designer**. Here you can adjust the duration across all blocks.

14.5.3.1.2 Zoom section

This section contains tools that you can use to adjust the size of the image region displayed.

Fig. 84: Zoom section

Note

If you hold down the **Ctrl**-key, you can zoom in and out of the image using the mouse wheel.

Parameter	Name	Description
	Fit to View	Automatically sets a zoom factor at which the entire image can be displayed visibly on the screen.
	Normal View (100%)	Shows the image without increasing or decreasing the zoom factor. One pixel of the image corresponds to one pixel on your screen.
	Decrease Zoom (F8)	Decreases the zoom factor.
	Increase Zoom (F7)	Increases the zoom factor.
	Zoom factor	Here you can set the display size steplessly. The desired zoom factor can be entered in the spin/input box in percent.
	Auto-Fit checkbox	**Activated:** Adjusts the display size automatically and continuously to the size of the window.

14.5.3.1.3 Tools section

This section contains tools that you can use to interact with the image. An active tool is highlighted in blue color.

Fig. 85: Tools section

Parameter	Description
Selection	Activates the selection mode (default).

Parameter	Description
Zoom Rectangle	Activates the zoom mode. The mouse pointer appears in the form of a cross hair. Hold down the left mouse button and drag out a selection rectangle. When you release the left mouse button, the region within the rectangle is displayed in enlarged form. If you have a mouse with a mouse wheel, you can also use this to move enlarged image regions. Position the mouse pointer in **Selection** mode inside the enlarged image region. Hold down the mouse wheel. The mouse pointer will then appear as a hand icon. You can now move the image region. When you release the mouse wheel, the mouse pointer changes back to the default icon.
Move	Activates the move mode. The mouse pointer appears then in the form of a hand. Click inside an enlarged image and hold down the left mouse button. You can now move the zoomed region.
Inner Zoom	Only visible in Gallery view. This function allows you to set a new zoom level for all images simultaneously using the mouse wheel. The size of the **Gallery View** does not change here. This allows you to limit the view to one interesting image region. Use the **Move** tool to move the view.
Show Values	Activates the show values mode. If you move the mouse pointer into the image region, a vertical arrow and a display field will appear. The pixel values of the position to which the arrow is pointing are displayed in the display field. In the first line of the display field the X/Y coordinates are shown. The second line shows the X/Y coordinates in scaled units. In the other lines the gray values for each channel are shown.
Navigator	Opens the **Navigator** window in the image area. There you will see an overview of your image and you can navigate to different positions using a rectangular window.
Interpolation	**Activated:** The pixel elements of the image are shown in an interpolated display. This makes it possible to avoid the pixelated display of small or greatly enlarged images. **Deactivated:** The pixel elements of the image are displayed as they are. This function is activated by default.

14.5.3.1.4 Channels section

This section contains all channels that you are using in your image. You can switch the display of channels in images on or off and change the channel colors (pseudo color assignment).

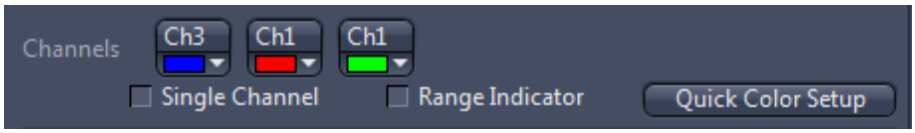


Fig. 86: Channels section

A button is displayed for each channel. Each button has two functions:

1 The channel name is displayed in the top section. To switch a channel off/on again, left-click on this section of the button.

2 The bottom section of the button shows the channel color. The display changes depending on the status of the button:

- When switched off, you will see a colored line below the button.
- When switched on, you will see a color field with a dropdown list below the button. Clicking on the dropdown list opens the color selection, see Color Selection dialog.

Note

For images with 8 or more channels, the channel buttons are reduced in size. In this case it is no longer possible to change the color channel by channel.

Parameter	Description
Single-Channel checkbox	**Activated:** Only a single channel is displayed.
Show Channel Colors checkbox	Only visible in **Split** view. **Activated:** Displays the individual channels of multichannel images with the assignment of pseudo colors.

Parameter	Description
Range Indicator checkbox	**Activated:** Changes the display to single channel mode. The channel is displayed in monochrome. At the same time you will see pixels that are saturated (displayed in red) and pixels that have no signal (values = 0; displayed in blue). Note that with camera systems it is normally not possible to achieve pixel values of 0. The blue indicator is therefore normally not displayed. This function helps you to set your acquisition settings, camera exposure or detector gain, so that saturation of the detector is avoided. The range indicator function is not available for the sum channel of the Airyscan ChA#.
Quick Color Setup button	Opens a dialog that allows you to select a color quickly for all channels of a multichannel image. The following options can be set:
- None	All channels are displayed without a pseudo color.
- Grayscale	All channels are displayed in monochrome (this applies in particular to multichannel images that have been acquired using color cameras).
- BGR	Channel 1: blue, channel 2: green, channel 3: red, no color assigned to any other channels.
- GRB	Channel 1: green, channel 2: red, channel 3: blue, no color assigned to any other channels.
- RGB	Channel 1: red, channel 2: green, channel 3: blue, no color assigned to any other channels.
- Via LUT	: Colors for all channels are selected using a reference look-up table. The LUT is divided up into as many sections as there are channels, with the channel color being used at the separation point. You can select the reference LUT using the **Reference LUT...** button.
- Custom	The colors defined by the user are restored.
- Dye	The color of the dye used during the experiment is restored

On the bottom of the tab further controls are available:

Parameter	Description
Crop	If activated, you can select a user defined acquisition region in the image area.

Parameter	Description
Marks	If activated, stage coordinates (Marks) can be defined by clicking into the image. The coordinates are written into the Marks table in the **Stage** tool . The Marks button stays available during image acquisition
Stage	If activated, you can move the stage with the mouse during a continuous acquisition by clicking and dragging the displayed red cross in the image container.

14.5.3.1.4.1 Color Selection Dialog

Here you can select a pseudo color for the selected channel. In the lower area of the dialog you will see four buttons that offer various methods of color selection. The selected button is highlighted in blue. To change the method, simply click on the appropriate button.

Fig. 87: Color Selection dialog

Parameter	Description
Weight slider	Sets the weighting of one channel to another channel. This is only possible with multi-channel recordings.
Color	Here you can choose the desired color from a default color chart. The selected color is displayed on the color button.
LUT	Here you can choose the desired color from a more complex color look-up table (LUT).
Cust...	Cust... stands for Custom Here you can define an own color and assign it to a color field.
None	Assigns no color to the channel. Images of monochrome cameras are black / white display. images of color cameras are displayed in real colors.

14.5.3.1.5 Reuse

The Reuse functionality is only available if you have loaded an image in *.CZI image format. This is the only image format that is compatible with this function. The **Reuse** button will then appear on the Dimensions tab

With this function you can apply the experiment setup of the acquired image to the current experiment. This will help you to reproduce the acquisition conditions for the image easily. The function only works correctly if the system configuration at the time of acquisition is identical to the system configuration at the time when you execute the function.

Removing components (e.g. filter cubes, LEDs, cameras, etc.) can result in an experiment being created incorrectly. It is therefore essential that you check after executing the Reuse function whether the configuration of the experiment is in line with your expectations.

i Note

Clicking on the **Reuse** button overwrites the current experiment without a prompt and marks it as having been modified. This can be seen from the appearance of an asterisk after the file name. If you want to keep the experiment in its previous form, you must save the modified experiment with a new file name under **Experiment Manager** | **Options** | **Save As**.

If you acquire images and save them in *.CZI image format, the following acquisition conditions are saved together with the image:

- Information on the type and status of your imaging system
- Time of acquisition

- Parameters set in the software.

14.5.3.2 Graphics tab

Here you can select various tools and use these to draw graphic elements into your images. You can also obtain an overview here of the graphic elements that you are using in your image.

A selection of default tools to work with are available to you here. You can also customize this toolbar to your requirements. Customized tools are added under the default toolbar and are highlighted in yellow color.

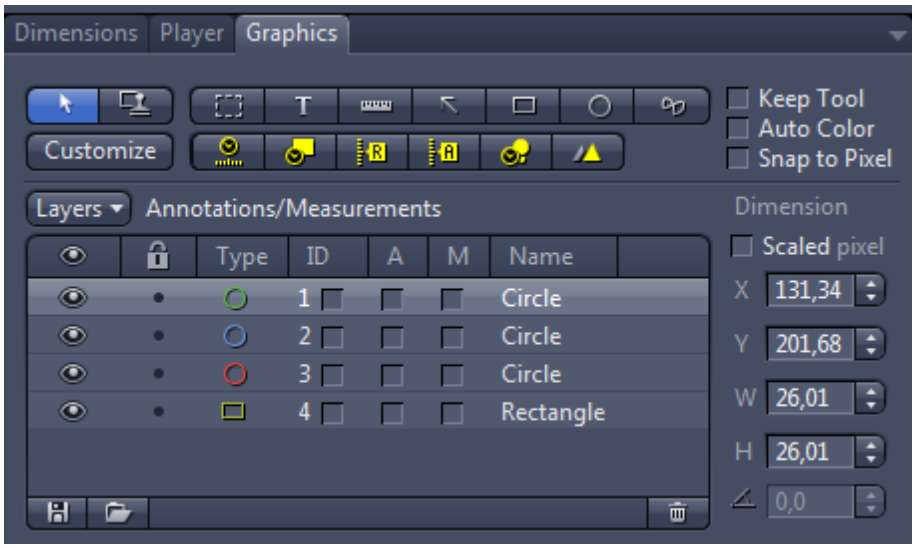


Fig. 88: Graphics Tab (Show All)

Parameter	Description
Selection 	Use this to select the graphic elements in the image area. If you are currently in another mode, you can switch back to the Selection mode using this button.
Clone 	Use this to create an identical copy of the last graphic element drawn in. To exit this mode, either switch back to the Selection mode or press the ESC key.
Region of Interest 	Use this to draw in a Region of Interest (ROI) into the image.
Text 	Use this to insert a text field into the image.
Scale Bar 	Inserts a scale bar into the bottom right corner of the image.

Parameter	Description
Arrow 	Use this to draw in an arrow.
Rectangle 	Use this to draw in a rectangle that is always parallel to the edges of the image.
Circle 	Use this to draw in a circle.
Contour (Spline) 	Use this to draw in a freely selectable contour. Close this contour by right-clicking. Corners are always rounded with this tool.
Keep Tool	**Activated:** Keeps the selected tool active. This allows you to draw in a number of the same elements one after the other.
Customize	Opens the Customize Tools dialog.
The following parameters are only visible if **Show All** is activated.	
Auto Color	**Activated:** Uses a new color for each element drawn in.
Snap to Pixel	**Activated:** Draws in the graphic elements with pixel precision. If this option is not active, the graphic elements are drawn into the graphics layer independently of the actual pixel resolution.

Annotations / Measurements List

In the list you can see the graphic elements that you are using in your image. You can also control the behavior of the graphic elements here, e.g. block or hide them. You can format each graphic element as you wish.

To format a graphic element simply double-click on the list entry or right click on the graphical element in the image and select the **Format Graphical Element** entry. The Format Graphic Element dialog opens. There you can format the graphic element in question according to your requirements.

i Note

In the list you will only see the graphic elements relating to the active graphics plane. To change the active graphics plane, click on the **Layers** button. This button is only visible in **Show All** mode. Select the layer that you want to display under **Active Layer**.

The columns of the list contain the following entries:

Parameter	Description
Eye	Shows or hides a graphic element.
Lock	Locks a graphic element to prevent changes being made.
Type	Displays the icon for the tool type. To format a graphic element, double-click on the icon. The Format Graphic Elements dialog then opens.
Name	Displays the name of the graphic element. To change the name, double-click in the Name field. Then enter the text of your choice.
Save	Saves the selected graphic element.
Load	Loads an existing graphic element.
Delete	Deletes the selected graphic element.
The following parameters are only visible if **Show All** is activated.	
ID	Displays the ID for the graphic element. To do this, activate the checkbox at the corresponding list entry.
A	Displays annotations for a graphic element. To do this, activate the checkbox at the corresponding list entry.
M	Displays measurement data for a graphic element. To do this, activate the checkbox at the corresponding list entry.
Dimension	The coordinates and dimensions of the selected graphic element (standard unit = pixels) are displayed in the corresponding input fields (**X**, **Y**, **W**, **H**, **Angle**)
- Scaled pixel	If activated, the dimensions are shown in scaled unit.

14.5.3.3 Display tab

Here you can adjust the image display. This function is particularly important if you want to display images with a very high dynamic range on the screen.

The histogram shows the brightness distribution of the pixels that are present from all channels simultaneously. The Y axis represents the relative frequency and the X axis indicates the brightness. A curve showing the corresponding distribution, the so-called display characteristic curve, is displayed for each channel.

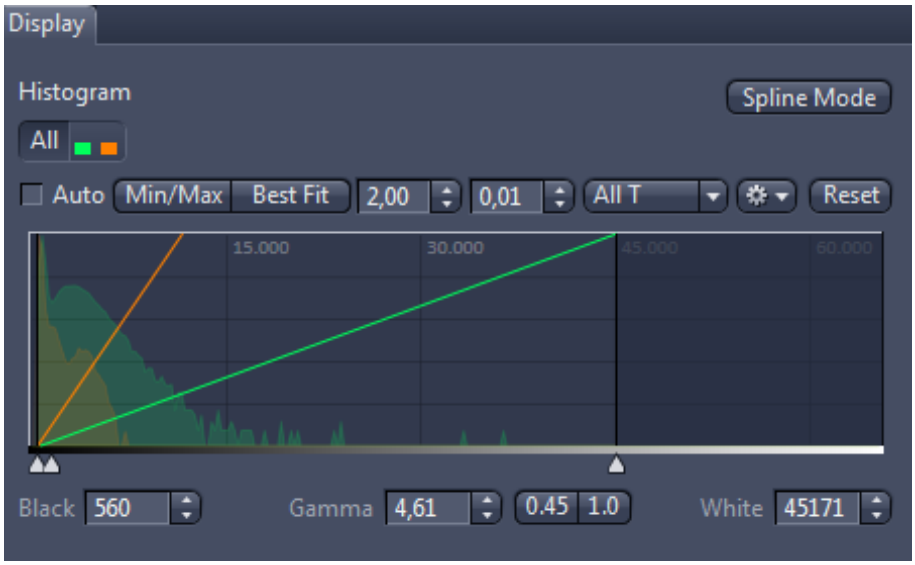


Fig. 89: Display Tab (Show All)

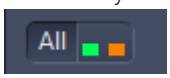
If you want to adjust the histogram for an individual channel, activate this via the relevant color field in the **Channel Selection**. Alternatively you can also click on the corresponding distribution curve directly in the histogram.

Display characteristic curve

Each channel has a display characteristic curve. Using the display characteristic curve you can set the limit for the black value (left) and the limit for the white value (right). This allows you to influence the contrast in the image. Move the mouse pointer over the corresponding adjustment handles at the bottom edge of the display histogram or to the small rectangles on the display characteristic curve. Hold down the left mouse button and move the adjustment handles or rectangles as required.

The curvature of the display characteristic curve influences the gamma value. To change the curvature, move the mouse pointer to the second or fourth small rectangle on the display characteristic curve. Hold down the left mouse button and move the rectangles up or down. The setting is used immediately for the display. Using the middle rectangle you can move the whole display curve. This changes the brightness of the image.

Parameters Description

Parameter	Description
Channel Selection	Here you can select the channel of a multichannel image for which you want to adjust the display on the screen.  To select all channels, click on the **All** button. To select a certain channel, click on the corresponding channel field. Hovering the mouse pointer over a color field displays the relevant channel name. If the image consists of more than 29 channels, a scrollbar will be displayed which you can use to switch to the desired channel. Note that in case of images containing only one channel the selection fields are hidden.
Auto checkbox	Adjusts the image display automatically. This is particularly useful in the case of a live image, in **Continuous** mode or if you play back a time series image that contains changing brightnesses. The automatic adjustment is not available during the acquisition of LSM experiments.
Min/Max button	Adjusts the display characteristic curve so that the darkest pixel is black and the brightest pixel is white in the display.
Best Fit button	Adjusts the display characteristic curve so that 0.1% of the darkest pixels contained in the image are black and 0.1% of the brightest pixels are white in the display.
Reset	Resets all parameters to the default values.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Spline Mode button	Clicking on this button allows you to add up to 8 points to the display characteristic curve. You can then bend the curve around these points. To do this, click on the desired section of the display curve and move it as required. Clicking on the display curve again adds another point. You can delete points by moving them along the display curve until they lie on top of another point. In this way, even in difficult situations you can adjust the display curve so that all important image regions can be displayed well.
Input fields	By the two input fields to the right of the **BestFit** button you can adjust the black/white values from 0.1% to values from 0 to 90% according to your requirements.
Dimension Selection dropdown list	If your images contain time series, Z-stacks or both, here you can select the aspect of an image for which the display settings should be applied. Note that with all settings other than **Current** there may be several seconds of calculation time until the setting is applied, depending on the number of time points/Z-planes. The following options are available:
- Current	Adjusts the display for the current image and keeps this setting for all other time points or Z-planes.
- All T	Collects the intensity values from all time points and adjusts the display according to the brightest and darkest pixels within the entire time series.
- All Z	Collects the intensity values from all Z-planes and adjusts the display according to the brightest and darkest pixels within the entire Z-stack.
- All T+Z	Collects the intensity values from all Z-planes and time points and adjusts the display according to the brightest and darkest pixels within the entire Z+T series.
Options 	Here you can copy display settings to the clipboard, insert them into other images from there or save and reload settings. This allows you to apply identical display settings to several images in order to produce comparable display conditions.

Parameter	Description
Black input field	Displays the gray value currently set up to which all pixels are shown as black. You can also enter a certain value here.
Gamma input field	Displays the gamma value currently set. You can also enter a certain value here.
0.45 button	Sets a linear display characteristic curve with a gamma value of 1.0. Sets a gamma value of 0.45. This is the recommended setting for most color images.
0.45 button	Sets a gamma value of 0.45. This is the recommended setting for most color images.
1.0 button	Sets a linear display characteristic curve with a gamma value of 1.0.
White input field	Displays the gray value currently set from which all pixels are shown as white. You can also enter a certain value here.

14.5.3.4 Player tab

Only visible if the **Show All** mode is activated.

Using the functions on this tab you can play back multidimensional images. The functions largely correspond to the functions for playing back films.

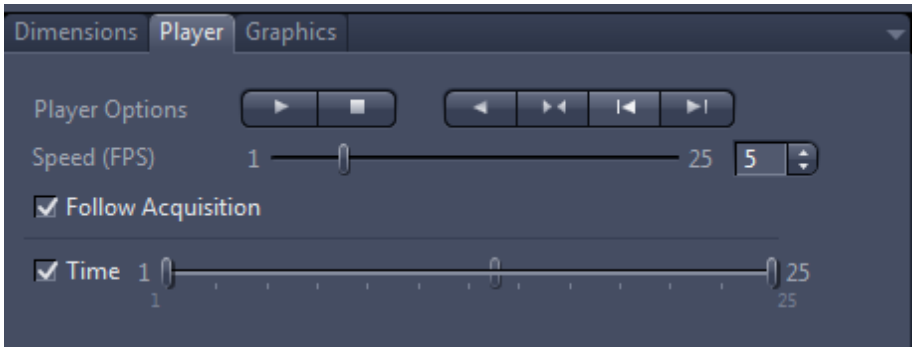


Fig. 90: Player Tab

Parameter	Description
Player Options	The following control elements are available:
- Play (First to Last) 	Plays back the image series forwards from first to last image. The dimensions are played back one after the other in the sequence specified.

Parameter	Description
- Stop 	Stops the play-back of the image series.
- Play (Last to First) 	Plays back the image series backwards from last to first image. The dimensions are played back one after the other in the sequence specified.
- Play alternately 	Plays back the image series forwards and backwards alternately.
- Jump to first 	Jumps to the start of the image series.
- Jump to last 	Jumps to the end of the image series.
Speed (FPS) slider	Here you can adjust the speed at which an image series is played back. The set speed is displayed in frames per second (FPS) in the input field. You can also enter the desired speed directly field. The maximum play-back speed is 25 FPS.
Follow Acquisition checkbox	**Activated:** Always displays the last acquired image during an ongoing acquisition procedure, as well as the slider for the corresponding dimension.
Dimensions sliders	Depending on the available dimensions (e.g. Time, Z-Stack, etc.) in the active image a slider is displayed here for each dimension. The sliders have each two adjustment handles, which you can use to define the start and end point of the playback. If there are several dimensions, you can determine, by activating the corresponding checkbox, if you want the dimension to be taken into account during the play-back. Each slider offers as many steps as there are individual positions in the specified dimension. A third adjustment handle indicates the current position and cannot be controlled directly.

14.5.3.5 PSF tab

In most image views you will see the **PSF Display** tab as soon as a PSF image has been loaded. PSF images differ from the data types of normal images. They are saved, for example, in the high-precision floating point format. A series of important values that allow conclusions to be drawn about the microscope system and sample conditions can also be read from PSF images.

Export PSF button

Generates a new PSF document in 16 bit gray level format, which can be processed as required to allow it to be used in other programs.

Display Mode settings

Choose between three display options from the dropdown list:

- **Intensity PSF**: The PSF is displayed in the position space, gray values are displayed in floating point format.
- **Intensity OTF**: The optical transfer function (OTF) displays the 3D PSF in the frequency space following a 3D Fourier transformation. Gray values are displayed in floating point format.
- **Intensity Slice OTF**: Displays the 2D Fourier transformation of each individual Z-plane.

Axial cut view checkbox
Activated: Displays the PSF in axial section view.

Deactivated: A slider for Z appears on the Dimensions tab. This allows you to move through the various Z-planes.

List of PSF values

A series of important values relating to the PSF are displayed here in a table:

- **Storage type:** Format in which the PSF is saved
- **Source:** Shows whether the PSF has been generated by measuring a bead stack (External) or from the theoretical calculation (Internal).
- **Used Dimensions:** shows whether the PSF is 3D or 2D.
- **Instrument:** shows the type of microscope used.
- **Illumination:** shows the illumination conditions that applied.
- **NA Objective:** Numerical aperture of the objective
- **Lateral Magnification:** shows the objective magnification.
- **Working Distance:** shows the working distance of the objective.

- **Illumination Wavelength:** shows the wavelength of the excitation light; in the case of multichannel PSFs the values for all channels are shown here.
- **Detection Wavelength:** shows the wavelength of the detected emission light; in the case of multichannel PSFs the values for all channels are shown here.
- **Transverse Resolution (Rayleigh)**: shows the actual lateral resolution achieved according to Rayleigh; in the case of multichannel PSFs the values for all channels are shown here.
- **Axial Resolution:** shows the actual axial resolution achieved, determined according to Full Width Half Maximum (FWHM); in the case of multichannel PSFs the values for all channels are shown here.

i Note

Please note that the resolution values for measured PSFs show the performance of the entire system, consisting of all optical and electronic components. The sample, with its optical properties and possible aberrations, therefore has a significant impact on the resolution. This means that these values are not suitable for making statements about the quality of the objective.

14.5.3.6 ApoTome tab

Here you will find various settings for displaying the resulting image following ApoTome acquisition.

Display mode

Here you can select which combination mode is used for the image view. This impacts on how the image is displayed.

If you have selected the Raw Data combination mode, the **Phases** slider will also appear on the **Dimensions** tab. This enables you to select the various phases of the raw images.

Create Image button

Creates a new image document. The available settings are taken into consideration here.

The following functions are only visible if the **Show All** mode is activated:

Normalization checkbox

Activated: The resulting images always fill the entire 16 bit dynamic range of the image histogram, see normalization .

Apply Correction checkbox

Activated: Applies streak correction to the resulting image. Here an attempt is made to remove streak artifacts which may be caused by bleaching of the sample during acquisition or by slight deviations in the grid phase position. Select one of the following corrections from the **Correction** dropdown list:

- Local Bleaching : Corrects the bleaching for each pixel individually (default setting). This is usually the best method.
- Global Bleaching : Corrects bleaching by means of global bleaching correction, which applies equally to the entire image.
- Phase Errors : Corrects phase errors in the image without additional bleaching correction.

Phase Correction checkbox

Only visible if you have selected one of the two bleaching corrections as the correction method.

Activated: Performs a correction of any phase deviations present in addition to the selected bleaching correction.

Fourier filter

The Fourier filter attempts to remove residual streaks. You can choose between **Off**, **Weak**, **Medium** and **Strong**.

Grid

Here you can see the grid frequency used for the image in lines/mm.

Deconvolution checkbox

Activated: Access to the key parameters for **ApoTome** deconvolution is activated.

Adjust Strength checkbox

Activated: Enter the degree of restoration using the slider.

To achieve strong image restoration, move the slider towards **Strong**.

To achieve less image restoration, move the slider towards **Weak**.

If the setting is too strong, image noise may be intensified and other artifacts, such as "ringing", may appear.

Deactivated: The restoration strength for optimum image quality is determined automatically.

The restoration strength is inversely proportional to the strength of so-called regularization. This is determined automatically with the help of Generalized Cross Validation (GCV).

Aberration Correction checkbox

Activated: The entries for aberration correction are taken into account when the image is created.

- **Index of Embedding:**
  Here you can enter the refractive index of the embedding medium used.
- **Distance to Cover Slip:**
  Here you can enter the distance of the acquired structure from the side of the cover slip facing the embedding medium.

Apply Deconvolution

Applies the deconvolution to the ApoTome image. The result is displayed directly without a new document being created. If you wish to create a separate document, click on the **Create Image** button.

14.6 View modes

14.6.1 Full Screen mode

In this mode the image will be displayed in the full monitor size.

To start the full screen mode, position the cursor on the image area and open the context menu via right mouse click. Click on **Full Screen**. You can also press **F11** or click on menu **Window** | **Full screen** as an alternative.

Toolbar

In the toolbar at the bottom you find several buttons for general and image specific functions, like zoom function (**Zoom** button) or image informations (**Info** button). When you open a multidimensional image, you find buttons for specific functions, etc. **Z-Stack**, **Channels**. To open the functions, click on the button.

Previous button

Displays the previous document in full screen mode. You can page step by step backwards through all open documents.

Next button

Displays the next document in full screen mode. You can page step by step forwards through all open documents.

Exit Fullscr. button

Closes the full screen mode.

14.6.2 Exposé mode

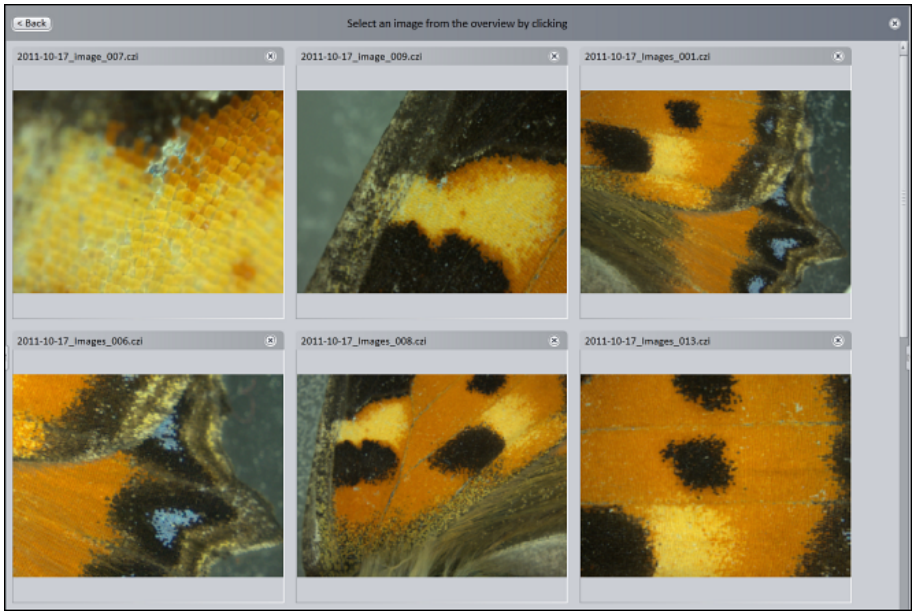


Fig. 91: Expose mode

To open the **Exposé** mode click on the expose mode icon in the *document bar* [▶ 21].

In this mode all open documents will be displayed in an overview.

When you click on an image in the overview the **Exposé** mode will be closed and the image will be displayed in standard view.

Back button

Jumps back to the standard image view.

Close button

Closes the den **Exposé** mode.

14.6.3 Splitter mode

In this mode you can generate a multi image of one or several images in order to compare them. Drag an image of the **Images and Documents** gallery in **Right Tool Area** and drop it in a splitter position. The standard setting for the splitter are 2 columns and 1 row. You can modify these setting in the **Split-View**.

Proceed similarly with further images to be displayed in the multi image. The same image can be dropped several times in the splitter view, i.e. to compare different image scenes.

The multi image can be saved as **CZSPL** (Zeiss Multi Image Files) image type in the menu **File | Save As**. The stored multi image is no image document, but rather a reference of the images displayed in the splitter mode.

Use the **Split Display tab** for further adjustments (i.e. arrangement) of the splitter mode. Here you can create a single image of the multi image to be saved as **CZI** image type.

14.6.3.1 Split Display tab

Arrangement section

Here you can set how much columns and rows the splitter image should have. Therefore simply enter the desired number in the **Columns** / **Rows** input fields.

Dimensions / Display settings

Option	Function
Synchronize Dimensions	**Activated:** The settings of the **Dimensions** tab (i.e. Zoom) will be applied synchronously to all images in splitter mode.
Synchronize Display	**Activated:** The settings of the **Display** tab (i.e. Gamma) will be applied synchronously to all images in splitter mode.
Show Position Data	**Activated:** The cursor changes to an arrow symbol and a cross marker in the image. The **X/Ycoordinates** with scaling unit and **gray value** of the current cursor position are displayed below the image. Furthermore additional information is displayed for multidimensional images: i.e. the **gray value for each channel** of a multichannel image, the **time of each time point** of a time lapse image, the **focus position of each Z position** of a Z-Stack.
Reset button	By clicking on this button you can reset all adjustments applied to the images in splitter view.

Image Generation

New Image From dropdown list

Here you can select the type of image to be generated. The available options are depending on the dimensions of the displayed image.

Selection	Description
Current View	Creates a 2-dimensional image of all opened images visible in the splitter mode.
Time Series	Creates a multi-dimensional image containing each time point of a time series image.

Selection	Description
Z-Stack	Creates a multi-dimensional image containing each Z-plane of a Z-stack image.
Rotation	Creates a multi-dimensional image containing each rotation plane of a rotation series image.

Create button

Generates an single image of the multi image displayed in splitter mode with the corresponding settings. The image can be saved in the **CZI** format in **File | Save as** menu.

Advanced Functions

The following functions are only visible if the **Show All** mode is activated:

Interpolation for zoomed images dropdown list

Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.

Selection	Description
Nearest Neighbor	The output pixel is given the gray value of the input pixel that is closest to it.
Linear	The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.
Cubic	The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

Spacing

Here you can adapt the size of the distance between the single images to values 0 up to 20. The background (distance) will be displayed in black color and appears, if the splitter image is generated by the **Create** button.

Spacing color

Here you can change the background color of the splitter image.

Burn-in annotations

If activated, all existing annotations and graphical elements within the images will be burned into the resulting splitter image.

14.7 File Browser

Here you see an overview of all image or data files stored on your computer. In the left column you see a file structure which is associated with the common image or data containing folders in your file system (Images and documents). In the right area you see the preview to the selected folder.

i Note

The ZEN folders contain automatically the **Auto Save** folder. Here you see all auto-saved images from ZEN. Set Auto Save path in **Tools** | **Options** | **Saving**.

Gallery View

Here you see all files of a folder as small preview images (thumbnails). Use **Tool** tab to adjust preview images size, sorting, etc..

Info View

Here you see a detailed list with all data the selected image contains. Find a detailed description of all possible data under *Info View* [▶ 520].

Table View

Here you see all files of a folder well-arranged in a table. This view is perfect for folders which contain many files.

14.7.1 Tools tab

Parameter	Description
Icon size	Set size of thumbnail images here.
Text Rows	Select entries which you want to have displayed as additional text row under the thumbnail image.
Record	Switch from file to file in the selected folder by using the slider.
Sorting	Arrange your files to certain properties (i.e. file name, type, etc.)
Folders	Manage selected file folders here (i.e. new folder, rename folder, etc.).
Selection	Manage selected files here (i.e. copy file, or delete file).

14.8 Image Processing Functions

14.8.1 Settings concept for IP functions

With the new settings concept for IP functions, you are able to save and reload your personal settings of IP functions. Therefore we introduced the **Settings** section on top of each function`s **Parameters** tool. If you have adjusted the parameters for a function, simply click on **Options** | **New** to save your setting under a new name.

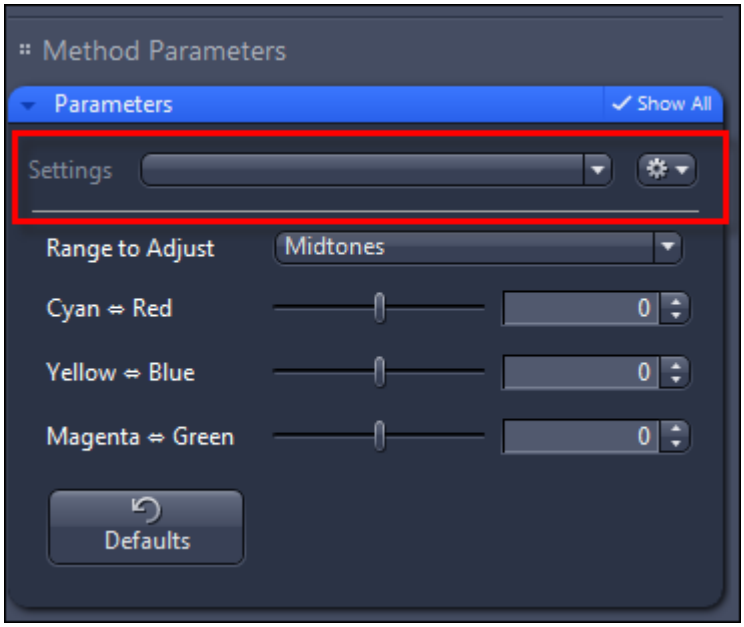


Fig. 92: Settings section in the parameters tool of an IP function

14.8.2 Deconvolution group

14.8.2.1 Deconvolution (defaults)

This method allows you to use 4 different algorithms for deconvolution, without any further settings. The following algorithms are provided in the **Parameter** tool:

Button	Function
Simple, very fast (Nearest Neighbor)	Executes the fast Nearest Neighbor method using default parameters.
Better, fast (Regularized Inverse Filter)	Executes the Regularized Inverse Filter algorithm for image enhancement.
Good, medium speed (Fast Iterative)	Executes the Fast Iterative restoration method.

Button	Function
Excellent, slow (Constrained Iterative)	Executes the Constrained Iterative quantitative restoration method.

14.8.2.2 Deconvolution (adjustable)

This method allows you to use and individually configure 4 different algorithms for deconvolution.

14.8.2.2.1 Deconvolution (adjustable) parameters

Two tabs are available for detailed configuration:

On the **Deconvolution** tab you can select the desired algorithm and define the precise settings for it.

On the **PSF Settings** tab you can see and change all key parameters for generating a theoretically calculated Point Spread Function ("PSF").

14.8.2.2.1.1 Deconvolution tab

Algorithm dropdown list

Here you can select the algorithm that you want to use. The following algorithms are available:

- Nearest Neighbor
- Regularized Inverse Filter
- Fast Iterative
- Constrained Iterative

i Note

Expert knowledge is required for some of the settings. If you are in doubt, leave the settings unchanged.

Channel-Specific Settings checkbox

Activated: Applies the settings on a channel-specific basis. This allows you to set parameters in a targeted way for each channel. You will see a separate, colored tab for each of the channels.

Deactivated: Applies the settings to all channels of a multichannel image.

14.8.2.2.1.1.1 Normalization section

Normalization dropdown list

Here you can specify how the data of the resulting image are handled:

Parameter	Description
Clip	Sets negative values to 0 (black). If the values exceed the maximum possible gray value of 65636 when the calculation is performed, they are limited to 65636 (pixel is 100% white).
Automatic	Normalizes the output image automatically. In this case the lowest value is 0 and the highest value is the maximum possible gray value in the image (gray value of 65636). The maximum available gray value range is always utilized fully in the resulting image.

14.8.2.2.1.1.2 Adjust Strength section

Adjust Strength checkbox

If you have selected the **Nearest Neighbor** algorithm, the checkbox is always activated.

i Note

If you have selected the **Fast Iterative** algorithm, the checkbox is also always activated. Using the slider you can then enter the number of iterations used directly, as, in contrast to the other methods, no regularization is performed.

Activated: Enter the degree of restoration using the slider.

To achieve strong contrast enhancement, move the slider towards **Strong**.

To achieve less contrast enhancement, move the slider towards **Weak**.

If the setting is too strong, image noise may be intensified and other artifacts, such as "ringing", may appear.

Deactivated: Determines the restoration strength for optimum image quality automatically.

The restoration strength is inversely proportional to the strength of so-called regularization. This is determined automatically with the help of Generalized Cross Validation (GCV).

14.8.2.2.1.1.3 Z-Stack Correction section

To show the section in full, click on the **arrow** button  .

Lamp Flicker checkbox

Activated: Analyzes the total brightness of each Z-plane. In the event of non-constant deviations in the total brightness between neighboring planes, a compensation factor is taken into account.

Activate this function if you have acquired your images using an old fluorescent lamp that exhibits strong fluctuations in brightness.

Bleaching Correction checkbox

Activated: Corrects bleaching of the sample during acquisition of the Z-stack.

This function should only be activated for widefield images. Use it if your sample undergoes strong bleaching during acquisition.

Background Correction checkbox

Activated: Analyzes the background component in the image and removes it before the DCV calculation.

This can prevent background noise being intensified during DCV.

14.8.2.2.1.1.4 Advanced Settings section

Only visible if the **Show All** mode is activated.

This section is only visible if you have selected the **Inverse Filter**, **Iterative (Fast)** or **Iterative** algorithm.

To show the section in full, click on the **arrow** button  .

Depending on which algorithm you have selected, different advanced setting options are available. The relevant settings are described in the following sections for each algorithm:

14.8.2.2.1.1.4.1 Advanced settings for Inverse Filter algorithm

Regularization

Here you can select which frequencies in the image are taken into account during regularization:

Parameter	Description
Zero Order	Regularization based on G-difference, modeled on Tikhonov, but accelerated.

Parameter	Description
First Order	Regularization based on Good's roughness. Under certain circumstances, more details are extracted from noisy data. May be better suited to the processing of confocal data sets.

14.8.2.2.1.1.4.2 Advanced settings for Fast Iterative algorithm

Likelihood

Here you can decide which likelihood calculation you want to work with:

Parameter	Description
Poisson (Meinel)	The calculation according to Meinel works with one fold per iteration and converges significantly faster, normally in 4-5 iterations. This method can also produce artifacts, however.
Poisson (Richardson-Lucy)	The calculation according to Richardson-Lucy, on the other hand, normally requires hundreds of iterations and therefore takes considerably longer. This method is, however, somewhat more robust.

Regularization

i Note

For the **Poisson (Meinel)** calculation it is also possible to perform **zero order** (G-difference) regularization here as an option. This means, however, that the calculation will take considerably longer and the main advantage of the greater speed of Meinel is lost.

Optimization

Parameter	Description
Numerical Gradient	If you select this option, an attempt is made to determine the trend of the iterations in advance and extrapolate this to the entire calculation. This can significantly speed up the calculation.

First Estimate

Parameter	Description
Input Image	The input image is used for the first estimate of the target structure (default).
Last Output Image	The result of the last calculation is used to estimate the next calculation. This can speed up a calculation that is repeated using slightly different parameters.
Average of Input Image	No estimate is made. This is the most rigid application of deconvolution, but can take a long time.

Maximum Number of Iterations

Here you can indicate the maximum permitted number of iterations that you want. In the case of Richardson-Lucy, you should allow significantly more iterations here.

Quality Threshold

Defines the quality level at which you want the calculation to be stopped. The percentage describes the difference in enhancement between the last and next-to-last iteration compared with the greatest difference since the start of the calculation. 1% is the default value. Lowering this can bring about small improvements in quality.

14.8.2.2.1.1.4.3 Advanced settings for Iterative algorithm

Likelihood

Here you can decide which likelihood calculation you want to work with:

- Calculation according to Poisson
- Calculation according to Gauss

Regularization

Here you can enter which frequencies in the image are taken into account during regularization:

Parameter	Description
Zero Order	Regularization based on G-difference, modeled on Tikhonov, but accelerated.
First Order	Regularization based on Good's roughness. Under certain circumstances, more details are extracted from noisy data. This regularization can sometimes produce better results for the processing of confocal data sets.

Parameter	Description
Second Order	Regularization according to Tikhonov-Miller. Here higher frequencies are penalized more than in the case of Good's roughness.

Optimization

Parameter	Description
Analytical (Newton Raphson)	Here an attempt is made to optimize the iterations analytically. This option is usually faster but may also be somewhat less precise.
Line Search	Searches rigorously and comprehensively for the minimum. It is therefore more robust, but the calculation takes longer.

First Estimate

Parameter	Description
Input Image	The input image is used for the first estimate of the target structure (default).
Last Output Image	The result of the last calculation is used to estimate the next calculation. This can speed up a calculation that is repeated using slightly different parameters.
Average of Input Image	No estimate is made for the next iteration. This is the most rigid application of deconvolution, but can take a long time.

Maximum Number of Iterations

Here you can indicate the maximum permitted number of iterations that you want.

Quality Threshold

Defines the quality level at which you want the calculation to be stopped. The percentage describes the difference in enhancement between the last and next-to-last iteration compared with the greatest difference since the start of the calculation. 1% is the default value. Lowering this can bring about small improvements in quality.

14.8.2.2.1.1.5 Diagnosis section

Only visible if the **Show All** mode is activated.

This section is only visible if you have selected the **Fast Iterative** or **Iterative** algorithm.

To show the section in full, click on the **arrow** button  .

The course of the calculation is displayed here in a graphical display. Various parameters are shown and the aim of the calculation is to minimize these. This display allows you to observe directly how the iterative method affects the available data.

14.8.2.2.1.2 PSF Settings tab

All key parameters for generating a theoretically calculated Point Spread Function ("PSF") are displayed here.

i Note

Ordinarily, images that have been acquired using **ZEN** (of the *.czi type) automatically contain all microscope parameters, meaning that you do not have to configure any settings on this page. Most parameters are therefore grayed out in the display. It is possible, however, that as a result of an incorrect microscope configuration values may not be present or may be incorrect. You can change these here. The correction of spherical aberration can also be set here.

14.8.2.2.1.2.1 Microscope Parameters section

The most important microscope parameters for PSF generation that are not channel-specific are displayed in this section.

Microscope dropdown list

Displays which type of microscope has been used. There are two options: conventional microscope (also known as a widefield microscope) and confocal microscope, for which the additional pinhole diameter parameter applies.

NA Obj. input field

Displays the numerical aperture of the objective.

Immersion input field

Displays the refractive index of the immersion medium. Please note that this can never be smaller than the numerical aperture of the objective. You can make a selection from typical immersion media in the dropdown list next to the input field.

Lateral Scaling input field

Displays the geometric scaling in the X/Y direction.

Axial Scaling input field

Displays the geometric scaling in the Z direction.

Change button

To change the input fields that are normally grayed out, click on the **Change** button. The input fields and dropdown lists are now active.

The text on the button changes to **Reset**. To restore the original values saved in the image, click on the **Reset** button.

> **i Note**
>
> If you enter incorrect values, this can lead to incorrect calculations. If the values here are obviously wrong or values are missing, check the configuration of your microscope system.

14.8.2.2.1.2.2 Advanced Settings section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the **arrow** button  .

Phase Ring dropdown list

If you have acquired a fluorescence image using a phase contrast objective, the phase ring present in the objective is entered here. This setting has significant effects on the theoretical Point Spread Function ("PSF").

Origin dropdown list

There are two models for calculating the PSF:

Parameter	Description
Scalar Theory	The wave vectors of the light are interpreted as electrical field = intensity and simply added. This method is fast and is sufficient in most cases (default setting).
Vectorial Theory	The wave vectors are added geometrically. However, the calculation takes considerably longer.

Z-Stack text field

This field can only be changed if it was not possible to define this parameter during acquisition, e.g. because the microscope type was unknown. It describes the direction in which the Z-stack was acquired:

Parameter	Description
Downwards	The Z-stack "grows" away from the front lens of the objective.
Upwards	The Z-stack "grows" towards the objective. This setting only applies if you are working with a correction of the spherical aberration.

14.8.2.2.1.2.3 Aberration Correction section

Only visible if the **Show All** mode is activated.

Here you can select whether you want spherical aberration to be taken into account and corrected during the calculation of the PSF. As with the other PSF parameters, most values are extracted automatically from the information about the microscope that is saved with the image during acquisition. The input option is therefore inactive. To make changes manually, click on the **Change** button.

Use Correction checkbox

Activated: Uses the correction function. All options are active and can be edited.

Embedding Medium dropdown list

Here you can select the embedding medium used from the list.

Refractive Index input field

Displays the refractive index of the selected embedding medium. Enter the appropriate refractive index if you are using a different embedding medium.

Manufacturer

Displays the manufacturer, if known.

Distance to Cover Slip

Displays the distance of the acquired structure from the side of the cover slip facing the embedding medium. Half the height of the Z-stack is assumed as the initial value for the distance from the cover slip. The value can be corrected if this distance is known. If possible, this distance should be measured.

Cover Slip Thickness input field and dropdown list

Commercially available cover slips are divided into different groups depending on their thickness (0, 1, 1.5 and 2), which you can select from the dropdown list. Cover slips of the 1.5 type have an average thickness of 170 µm. In some cases, however, the actual values can vary greatly depending on the manufacturer. For best results the use of cover slips with a guaranteed thickness of 170 µm is recommended. Values that deviate from this can be entered directly in the **input field**.

Cover Slip Index input field and dropdown list

Select the material that the cover slip is made of from the dropdown list. The corresponding refractive index is displayed in the input field next to it.

Working Distance input field

Displays the working distance of the objective (i.e. the distance between the front lens and the side of the cover slip facing the objective). The working distance of the objective is determined automatically from the objective information, provided that the objective was selected correctly in the MTB 2004 Configuration program. You can, however, also enter the value manually.

Change/Reset button

Only active if the **Use Correction** checkbox is activated.

To reset the values, click on the **Reset** button.

14.8.2.2.1.2.4 Channel-Specific section

In this section you will find all settings that are channel-specific. This means that they may be configured differently for each channel.

Use external PSF checkbox

Activated: Uses an external measured PSF. You`ll find an additional input window under **Image Parameters | Input** where you can choose the external PSF file. The software will check if the PSFs microscope parameters match with the input image. Aberrations (10nm aberration in wavelength will be accepted) will make the software use a theoretical PSF.

Attach to input button

If an external PSF was selected you can attach the file to the input image. The saved input image will then contain the correct measured PSF. Usage of a theoretical PSF is possible as well for such an image. Just deactivate the **Use external PSF** checkbox.

Illumination display field

Displays the excitation wavelength for the channel dye [in nm]. The maximum is assumed. The color field corresponds to the wavelength (as far as possible).

Detection display field

Displays the maximum of the emission wavelength for the channel dye. The color field corresponds to the wavelength (as far as possible).

Sampling lateral display field

Depends on the geometric pixel scaling in the X/Y direction and displays the extent of the oversampling according to the Nyquist criterion. The value should be close to 2 or greater in order to achieve good results during DCV. As, in the case of widefield microscopes, this value is generally determined by the objective, the camera adapter used and the camera itself, it can only be influenced by the use of an Optovar.

Sampling axial display field

Depends on the geometric pixel scaling in the Z direction and displays the extent of the oversampling according to the Nyquist criterion. The value should be at least 2 or greater in order to achieve good results during DCV. This value is determined by the increment of the focus drive during acquisition of Z-stacks and can therefore be changed easily.

Pinhole display field

Only available if a confocal microscope has been entered under the microscope parameters.

Displays the size of the confocal pinhole in Airy units (AU).

NA Cond. display field

Only visible if the microscope is a **Conventional Microscope** and **Transmitted Light** has been selected as the illumination type.

Displays the numerical aperture of the condenser with which transmitted-light acquisition was performed.

14.8.2.2.1.2.4.1 Microscope Info section

Displays advanced microscope information that influences the form of the PSF in a channel-dependent way:

Illumination dropdown list

Here you can select the illumination method with which the data set has been acquired. In the event that a **Conventional Microscope** has been entered under the microscope parameters, the following options are available here: **Epifluorescence**, **Multiphoton Excitation** and **Transmitted Light**. In the case of confocal microscopes, **Epifluorescence** is the only option.

Imaging

Displays whether the imaging was incoherent (**Conventional Microscope**) or coherent (**Laser Scanning Microscope**).

Lateral Resolution

Displays the lateral resolution of the calculated PSF.

Axial Resolution

Displays the **full width half maximum** as a measure of the axial resolution of the PSF.

14.8.2.2.1.2.4.2 PSF View section

This tool shows you the PSF that is calculated for a channel based on the current settings. If you select the **Update Automatically** checkbox, all changes made to the PSF parameters are applied immediately to the PSF view. This makes it possible to check quickly whether the settings made meet your expectations.

14.8.2.2.2 List of deconvolution methods in ZEN blue

ZEN Method (common name)	Sub-method (general ZEN name)	Reference	Settings	Comments
Nearest Neighbor		K. Castleman, "Digital image processing", Prentice Hall 1997	Algorithm (default): **Nearest Neighbor**	Ad-hoc "2D de-blurring algorithm" focuses on subtraction of out of focus blur.
Regularized Inverse also known as: Linear Least Squares	Regularization: Zero order	For zero order g-difference: Schaefer et al. (2001)	Algorithm (default): **Regularized Inverse** Advanced settings: Regularization: Zero order	Uses difference of observation and estimate as regularization term.

ZEN Method (common name)	Sub-method (general ZEN name)	Reference	Settings	Comments
Regularized Inverse also known as: Linear Least Squares	Regularization: First order	For first order regularization, or *Good's roughness*: Verveer et al. (1997)	Algorithm: **Regularized Inverse** Advanced settings: Regularization: First order	Uses *Good's roughness* first derivative of estimate as regularization term.
Regularized Inverse also known as: Linear Least Squares	In conjunction with structured illumination microscopy (ApoTome)	Schaefer et al. (2006) Schaefer et al. (tbs)	Algorithm: **Regularized Inverse** Advanced settings: Regularization: Zero / First order	Patented method for maximum exploitation of ApoTome raw images
Fast Iterative Also known as: Gold Meinel	Meinel Algorithm	Meinel (1986)	Algorithm (default): **Fast Iterative** Advanced settings: Likelihood: Poisson (Meinel)	Classic, non regularized Meinel algorithm.

ZEN Method (common name)	Sub-method (general ZEN name)	Reference	Settings	Comments
Fast Iterative	Meinel Algorithm + Regularization: Zero order	Meinel (1986), For zero order g-difference: Schaefer et al. (2001)	Algorithm: **Fast Iterative** Advanced settings: Likelihood: Poisson (Meinel) Regularization: Zero order	Regularized Meinel algorithm using g-difference (difference of observation and estimate) term.
Fast Iterative	Meinel Algorithm + Optimization: Numerical Gradient	Meinel (1986), Biggs (1998)	Algorithm: **Fast Iterative** Advanced settings: Likelihood: **Poisson (Meinel)** Regularization: None / Zero order Optimization: Numerical Gradient	Meinel algorithm using a numerical gradient estimator as proposed by D. Biggs.
Fast Iterative Also known as: Richardson Lucy	Richardson Lucy (RL) Algorithm	Richardson (1972) Lucy (1974)	Algorithm: **Fast Iterative** Advanced settings: Likelihood: Poisson (Richardson, Lucy)	Classic, original non-regularized Richardson Lucy algorithm. May need many more iterations than any other algorithm.

ZEN Method (common name)	Sub-method (general ZEN name)	Reference	Settings	Comments
Fast Iterative Also known as: Richardson Lucy	Richardson Lucy Algorithm + Optimization: Numerical Gradient	Richardson (1972) Lucy (1974) Biggs (1998)	Algorithm: **Fast Iterative** Advanced settings: Likelihood: Poisson (Richardson, Lucy) Optimization: Numerical Gradient	Classic, original non-regularized Richardson Lucy algorithm. Improved rate of convergence. About a factor of 10 faster than RL using a numerical gradient estimator as proposed by D. Biggs.
Constrained Iterative	Likelihood: Poisson Regularization: Zero order	Verveer et al. (1997) Schaefer et al. (2001)	Algorithm (default): **Constrained Iterative** Advanced settings: Likelihood: Poisson Regularization: Zero order	Generic conjugate gradient restoration using squared estimate to impose positivity. Uses difference of observation and estimate as regularization term.

ZEN Method (common name)	Sub-method (general ZEN name)	Reference	Settings	Comments
Constrained Iterative	Likelihood: Poisson Regularization: First order	Verveer et al. (1997) Schaefer et al. (2001)	Algorithm: **Constrained Iterative** Advanced settings: Likelihood: Poisson Regularization: First order	Generic conjugate gradient restoration using squared estimate to impose positivity. Uses *Good's roughness* derivative operator as regularization term.
Constrained Iterative	Likelihood: Poisson Regularization: Second order	Tikhonov (1977) Verveer et al. (1997) Schaefer et al. (2001)	Algorithm: **Constrained Iterative** Advanced settings: Likelihood: Poisson Regularization: Second order	Generic conjugate gradient restoration using squared estimate to impose positivity. Uses *Tikhonov Miller Phillips* second derivative operator as regularization term.

ZEN Method (common name)	Sub-method (general ZEN name)	Reference	Settings	Comments
Constrained Iterative	Likelihood: Gauss Regularization: Zero order	Verveer et al. (1997) Schaefer et al. (2001)	Algorithm: **Constrained Iterative** Advanced settings: Likelihood: Gauss Regularization: Zero order	Generic conjugate gradient restoration using squared estimate to impose positivity. Uses difference of observation and estimate as regularization term.
Constrained Iterative	Likelihood: Gauss Regularization: First order	Verveer et al. (1997) Schaefer et al. (2001)	Algorithm: **Constrained Iterative** Advanced settings: Likelihood: Gauss Regularization: First order	Generic conjugate gradient restoration using squared estimate to impose positivity. Uses *Good's roughness* derivative operator as regularization term.

ZEN Method (common name)	Sub-method (general ZEN name)	Reference	Settings	Comments
Constrained Iterative Also known as: ICTM Iterative constrained Tikhonov Miller	Likelihood: Gauss Regularization: Second order	van der Voort et al. (1995) Verveer et al. (1997) Schaefer et al. (2001)	Algorithm: **Constrained Iterative** Advanced settings: Likelihood: Gauss Regularization: Second order	Generic conjugate gradient restoration using squared estimate to impose positivity. Uses *Tikhonov Miller Phillips* second derivative operator as regularization term.
Constrained Iterative	Likelihood: Poisson / Gauss Regularization: Zero/First/ Second order Optimization: Line search/ Analytical	Verveer et al. (1997) Schaefer et al. (2001)	Algorithm: **Constrained Iterative** Advanced settings: Likelihood: Poisson/Gauss Regularization: 0/1/2nd order Optimization: Line search / Analytical	Generic conjugate gradient restoration using squared estimate to impose positivity. **Default for optimization** is the fast analytical (Newton Raphson) method. Line search may be more accurate but is also much slower

Bibliography

Schaefer, L.H., Schuster, D. & Herz, H. *Generalized accelerated maximum likelihood based image restoration approach applied to three-dimensional fluorescence microscopy*, Journal of Microscopy, **204** (2001), Pt. 2, 99-107 (PubMed)

Verveer, P.J. & Jovin, T.M. (1997) *Efficient superresolution restoration algorithms using maximum a posteriori estimations with application to fluorescence microscopy. J. Opt. Soc. Am. A, 14, 1696-1706.*

Meinel E.S., *Origins of linear and nonlinear recursive restoration algorithms*, *J. Opt. Soc. Am. A.*, 1986, 3 (6): 787-799.

Biggs, D.S.C. 1998. *Accelerated Iterative Blind Deconvolution*. Ph.D. Thesis, University of Auckland, New Zealand.

Schaefer, L.H. & Schuster, D. *Structured illumination microscopy: improved spatial resolution using regularized inverse filtering*, Proceedings of the FOM 2006, Perth, Australia

Lucy L.B., *An iterative technique for the rectification of observed distributions*, *Astron. J.*, 1974, 79: 745-754.

Richardson W.H., *Bayesian-based iterative method of image restoration*, *J. Opt. Soc. Am.*, 1972, 62 (6): 55-59.

van der Voort, H. T. M. and Strasters, K. C. (1995) *Restoration of confocal images for quantitative image analysis*. *J. Microsc.*, **178**, 165–181.

Tikhonov, A.N. & Arsenin, V.Y. (1977) *Solutions of Ill Posed Problems*. Wiley, New York.

14.8.2.3 Create PSF

This method creates a PSF image from a Z-stack image of a bead acquired for PSF measurement. Please observe the instructions for optimal acquisition here: Using beads for PSF measurement.

The best results are achieved if you create an averaged Z-stack image from several acquired beads using the *Bead Average* [▶ 635] function.

The result is a so-called PSF image. For advanced settings and options, please use the specific control elements on the *PSF Display* [▶ 607] tab.

You can use two types of beads:

- Beads with diameters below the microscope's resolution limit. In this case the image is converted directly into the appropriate PSF format.
- Beads with diameters greater than the resolution limit. In this case iterative processing reconstructs a PSF taking the known bead diameter into account. The main advantage is that larger beads can be visualized more easily and a better signal-to-noise distance can be achieved during acquisition. The use of beads with a diameter of 1.0 µm is recommended.

Parameters

Z-Stack Correction checkbox
Activated: Performs background correction of the Z-stack before the processing.

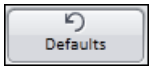
Circular Average checkbox
Activated: Forces a PSF with lateral symmetry. This option should not usually be activated as lateral asymmetries correspond better to the real situation. Circular averaging is only recommended when a measured PSF is used with the Fast Iterative method.

Threshold cropping checkbox
Activated: The PSF is restricted to gray value ranges up to 0.25% of the brightest voxel present. If the value is reduced or the option is deactivated, the PSF may be greater. This increases the calculation time. However, it is also possible to achieve slightly better results in this case. This option is activated by default.

Threshold slider
Using this slider and input field you can set the percentage from which the PSF is clipped if the Volume Clipping option has been selected.

Iterative Restoration checkbox
Activated: If Z-stack images of beads with diameters greater than the microscope's resolution limit are used to generate the PSF, this option must be selected. The bead diameter used can be entered using the slider and input field.

Defaults button



Resets all settings to the default values.

14.8.2.4 Bead Average

This method determines the position of fluorescent beads in a Z-stack image. If these beads are too close to one another they are excluded from the calculation. Beads which are far enough apart from one another are combined into a single bead, from which it is then possible to calculate a PSF using the Create PSF function.

Description of the algorithm

This function consists of a series of steps before and after processing. The aim is to find beads that are far enough apart from one another. The processing steps are as follows:

- Select input image
- Image smoothing
- Segmentation
- Bead selection
- Centering of the beads found
- Averaging of the beads found.

Parameters

Bead proximity slider

Defines the distance between two neighboring beads in µm. A bead is excluded from the averaging if the distance to a neighboring bead is greater than the minimum distance set. A smaller value leads to the detection of fewer beads, albeit ones that are further apart, while a larger value leads to the detection of more beads, but with the risk that beads will partially overlap.

Average Beads checkbox

Activated: An image of an individual, averaged bead is produced.

Deactivated: An image is produced in which each bead found is centered, but saved in the R dimension. A slider for Rotation (R dimension) then appears on the **Dimensions** tab.

Pre-Smoothing dropdown list

Mathematically speaking, image segmentation is an "ill-posed problem". To achieve more robust results, the image can be smoothed before segmentation using the following parameters:

- **None**: no image smoothing is performed; image noise can lead to incorrect results.
- **Gaussian**: performs three-dimensional Gaussian filtering with an isotropic sigma kernel. This filter is not edge preserving.
- **Difference of Gaussians**: The image is smoothed using two different sigma kernels (sigma and delta sigma). The difference image between the two images is used for the segmentation. This filter is edge preserving.

Log Curvature slider

Here you can set the gradient of the curve which can be used to find local intensity maximums (i.e. the beads). A small value leads to the detection of more beads, but makes the function more sensitive to image noise. If a higher value is used, the bead detection is disrupted less by image noise, but fewer beads may be detected.

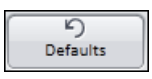
Sigma slider

Here you can set the strength of the effect of the Gaussian kernel, see *Sigma function* [▶ 669] for further details.

Delta Sigma slider

Here you can set the difference between the sigma for the first and second Gaussian filtering. Sigma for the second filtering is sigma + delta sigma.

Defaults button



Resets all settings to the default values.

14.8.3 Adjust group

14.8.3.1 Color Balance

This method allows you to adjust the weighting of the individual color channels of a true color image.

Parameters

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Range to Adjust dropdown list

Here you can select the adjustment range for the color balance. There are 3 ranges available:

Parameter	Description
Shadows	The settings relate to tones in the dark color range.
Midtones	The settings relate to tones in the mid color range.
Lights	The settings relate to tones in the light color range.

Cyan/Red slider

Enter the desired balance between cyan and red tones using the **Cyan/Red** slider or spin box/input field.

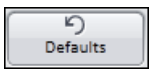
Yellow/Blue slider

Enter the desired balance between yellow and blue tones using the **Yellow/Blue** slider or spin box/input field.

Magenta/Green slider

Enter the desired balance between magenta and green tones using the **Magenta/Green** slider or spin box/input field.

Defaults button



Resets all settings to the default values.

14.8.3.2 Hue/Saturation/Lightness

This method allows you to adjust the hue, saturation and brightness of a true color image.

Parameters

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Hue slider

Enter the desired shift in the color tone using the **Hue** slider or spin box/input field.

The value of the shift represents an angle on the color wheel. The values -180 and +180 therefore have an identical effect. Negative angles shift the color tone towards blue and positive ones shift it towards red.

Saturation slider

Enter the desired saturation using the **Saturation** slider or spin box/input field.

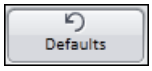
Saturation describes how intense the color of a pixel is. "Chromatic" is the maximum saturation, while "achromatic" describes colors that do not leave a color impression.

Lightness slider

Enter the desired brightness using the **Lightness** slider or spin box/input field.

Lightness describes how light or dark a pixel appears. The greatest difference is between black and white or between violet and yellow.

Defaults button



Resets all settings to the default values.

14.8.3.3 White Balance

This method allows you to adjust the white balance of an image.

Parameters

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Automatic checkbox

If the checkbox is activated, the white point is calculated automatically from the image data.

Pick button

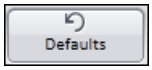
Only visible if the **Automatic** checkbox is deactivated.

To define the white point, click on the **Select** button. The mouse pointer then changes to a selection arrow. Use the selection arrow to click on a white region of your input image. The coordinates and color values of the selected white point are displayed under the button.

Temperature Delta slider

Enter the difference that will be added on to the newly calculated color values using the **Temperature Delta** slider or spin box/input field. Negative values reduce the color temperature, while positive values increase it. A value of 1 corresponds to 10 kelvin.

Defaults button



Resets all settings to the default values.

14.8.3.4 Color Temperature

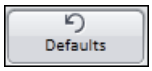
This method allows you to adjust the color temperature of a true color image.

Parameters

Temperature Delta slider

Enter the difference that will be added on to the newly calculated color values using the **Temperature Delta** slider or spin box/input field. Negative values reduce the color temperature, while positive values increase it. A value of 1 corresponds to 10 kelvin.

Defaults button



Resets all settings to the default values.

14.8.3.5 Brightness/Contrast/Gamma

This method allows you to adjust the brightness, contrast and gamma value of an image.

i Note

Unlike the adjustments that can be made on the **Display** tab, here the pixel values of the image are changed.

Parameters

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Brightness slider

Enter the desired brightness using the **Brightness** slider or spin box/input field.

Changing the brightness means that each gray or color value is increased or decreased by the same value. The difference between the biggest and smallest gray or color value in the image remains the same, however.

Contrast slider

Enter the desired contrast using the **Contrast** slider or spin box/input field.

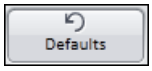
Changing the contrast means that the gray or color values are multiplied by a factor. The difference between the biggest and smallest gray or color value changes.

Gamma slider

Enter the desired gamma value using the **Gamma** slider or spin box/input field.

Changing the gamma value means that the gray or color values are multiplied by individual factors.

Defaults button



Resets all settings to the default values.

14.8.3.6 Stack Correction

This method allows you to improve the quality of Z-stack images that have been affected by bleaching effects during acquisition.

Parameters

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

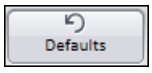
Activated: You can adjust the parameters for each channel individually.

Correction Mode dropdown list

Here you can select the desired correction mode.

Parameter	Description
Bleaching	The **Bleaching** correction mode compensates for the bleaching effect.
Flicker	The **Flicker** correction mode compensates for the flicker of the lamp voltage.
Background	The **Background** correction mode reduces background noise.

Defaults button



Resets all settings to the default values.

14.8.3.7 Shading Correction

This method allows you to improve images in which the quality has been impaired by uneven illumination or vignetting.

Parameters

Tiles shading mode

Mode	Description
Camera	Applies the Shading correction to each tile of a tile image.

Mode	Description
Global	Applies the Shading correction to the whole tile image. Requires a shading reference image with the same size as the tile image.

Automatic checkbox

If the checkbox is activated, the function automatically calculates the reference image for shading correction from the input image.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Mode dropdown list

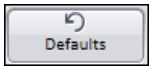
Here you can select the desired correction mode.

Mode	Description
Additive	In this mode the normalized reference image is subtracted from each camera frame. This influences the brightness of the image.
Multiplicative	In this mode each camera frame is divided by the normalized reference image. This influences the contrast of the image. This is the default setting. The simulated/auto reference image is created by averaging up to 20 camera frames in the input image and running a lowpass filter on them.

Offset slider

Enter the gray value that will be added on to the newly calculated gray values using the **Offset** slider or spin box/input field. If this results in negative values, these are set to 0. Values that exceed the maximum gray value are set to the maximum gray value.

Defaults button



Resets all settings to the default values.

14.8.3.8 Histogram Equalization

This function enhances the contrast by linearizing the histogram of the image to equal area fractions in the histogram. The areas (pixel count multiplied by gray value range) of all gray values in the histogram of the result image are the same.

Parameter	Description
High Threshold slider	The fraction of pixels that will be mapped to the highest gray value of the output image.
Lower Threshold slider	The fraction of pixels that will be mapped to gray value 0.

14.8.4 Edges group

14.8.4.1 Highpass

This method performs high-pass filtering. The high pass filter is defined as the difference between the original image and the low-pass filtered original.

Parameter

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Count slider

Here you set the number of repetitions. I.e. the number of times the function is applied sequentially to the respective result of the filtering. The effect is increased correspondingly.

Kernel Size slider

You can set the filter size in the x-, y-and z-direction, symmetrically around the subject pixel. This should be the size of the transition region between objects and background match.

14.8.4.2 Roberts

This method calculates a gradient image using the Roberts filter matrix. Large gray value differences between neighbors are shown as light gray values. No changes are indicated by a value of 0 (black). Edges are thinner than with the **Sobel** method.

14.8.4.3 Gradient Max

This method performs a gradient filtering. Based on the sum of a 2x2 matrix in the X-and Y-direction, a gradient image is calculated and using the larger of the two components. The edges are darker than that of the method **Gradient Sum**.

14.8.4.4 Gradient Sum

This method performs a gradient filtering. Based on the sum of a 2x2 matrix in the X-and Y-direction, a gradient image is calculated. The edges are brighter than that of the method **Gradient Max**.

14.8.4.5 Sobel

Sobel calculates a gradient image using a Sobel filter.

This function indicates gray value changes in the image. Large differences between neighbors are displayed as bright gray values, no changes are indicated by a value of 0 (zero). The pixels in the output image are calculated with the Sobel differential operator on the basis of a 3x3x3 fold of the input image.

Parameter

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Third Dimension dropdown list

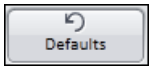
Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Parameter	Description
Normalization dropdown list	Depending on the IP function you have selected not all choices are available in the list.
- **Clip**	Gray levels that exceed or fall below the specified gray value range are automatically set to the lowest/highest gray value (black or white). The effect corresponds to underexposure or overexposure. This means that in some cases information is lost.
- **Automatic**	Automatic normalization of gray values to the available gray value range.
- **Wrap**	If the result is greater than the maximum gray value of the image, the value maximum gray value +1 is subtracted from it.
- **Shift**	Normalizes the output to the value gray value + max. gray value/2.

Defaults button



Resets all settings to the default values.

14.8.4.6 Laplace

This function performs a Laplace highpass filter.

The calculation is based on a 3x3x3 Laplace operator in all directions. The function does not show smooth gray value changes very well.

Parameter

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Third Dimension dropdown list

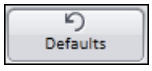
Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Parameter	Description
Normalization dropdown list	Depending on the IP function you have selected not all choices are available in the list.
- **Clip**	Gray levels that exceed or fall below the specified gray value range are automatically set to the lowest/highest gray value (black or white). The effect corresponds to underexposure or overexposure. This means that in some cases information is lost.
- **Automatic**	Automatic normalization of gray values to the available gray value range.
- **Wrap**	If the result is greater than the maximum gray value of the image, the value maximum gray value +1 is subtracted from it.
- **Shift**	Normalizes the output to the value gray value + max. gray value/2.

Defaults button



Resets all settings to the default values.

14.8.4.7 Local variance

This method is an edge filter, which calculates the variance of each pixel with its neighboring pixels by the lateral filter size.

Parameter

Kernel Size in X/Y

Here you set the matrix size in X / Y symmetrically around the pixel. This determines the degree of smoothing effect in the X / Y direction.

Parameter	Description
Normalization dropdown list	Depending on the IP function you have selected not all choices are available in the list.
- **Clip**	Gray levels that exceed or fall below the specified gray value range are automatically set to the lowest/highest gray value (black or white). The effect corresponds to underexposure or overexposure. This means that in some cases information is lost.
- **Automatic**	Automatic normalization of gray values to the available gray value range.
- **Wrap**	If the result is greater than the maximum gray value of the image, the value maximum gray value +1 is subtracted from it.
- **Shift**	Normalizes the output to the value gray value + max. gray value/2.

14.8.5 Geometric group

14.8.5.1 Channel Alignment

Using this method it is possible to automatically align the individual channels of a multi-channel image correctly to one another.

Parameters

Parameter	Description
Registration Method	Here you can select the method (or a combination of these) to be used to align the images.
- Translation	The neighboring sections of the Z-stack image are shifted in relation to each other in the X and Y direction.
- Rotation	The neighboring sections of the Z-stack image are rotated in relation to each other.
- Iso Scaling	
- Skew Scaling	

Parameter	Description
- Affine	The neighboring sections of the Z-stack image are shifted in the X and Y direction, rotated and the magnification is adjusted from section to section.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Quality	Here you can select the quality level that you want the function to work with.
- Low	Highest speed with low image quality.
- Medium	High speed with medium image quality.
- High	Low speed with high image quality.
- Highest	Lowest speed with highest image quality.
Interpolation	Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.
- Nearest Neighbor	The output pixel is given the gray value of the input pixel that is closest to it.
- Linear	The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.
- Cubic	The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

14.8.5.2 Z-Stack Alignment

This method allows you to bring the individual planes of a Z-stack image into line if these are not positioned precisely one above the other. This is the case, for example, when you acquire Z-stacks at an oblique angle using a stereo microscope.

Parameter	Description
Registration Method	Here you can select the method (or a combination of these) to be used to align the images.
- Translation	The neighboring sections of the Z-stack image are shifted in relation to each other in the X and Y direction.
- Rotation	The neighboring sections of the Z-stack image are rotated in relation to each other.
- Iso Scaling	

Parameter	Description
- Skew Scaling	
- Affine	The neighboring sections of the Z-stack image are shifted in the X and Y direction, rotated and the magnification is adjusted from section to section.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Quality	Here you can select the quality level that you want the function to work with.
- Low	Highest speed with low image quality.
- Medium	High speed with medium image quality.
- High	Low speed with high image quality.
- Highest	Lowest speed with highest image quality.
Interpolation	Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.
- Nearest Neighbor	The output pixel is given the gray value of the input pixel that is closest to it.
- Linear	The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.
- Cubic	The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

14.8.5.3 Stitching

This method allows you to align the individual tiles of a tile image with one another automatically and correctly.

Parameters

Parameter	Description
Inplace button	The stitching is applied to the original image.
New Output button	A new image is generated as a result of the stitching process. The original image is not modified.
Fuse tiles checkbox	Only visible when the button **New Output** is selected.

Parameter	Description
- **Activated:**	All individual tile images are fused together after alignment.
- **Deactivated:**	The individual tile images are aligned but not fused.
Correct shading checkbox	Only visible when the button **New Output** is selected. **Activated:** Applies a shading correction (Multiplicative mode, see *Shading Correction* [▶ 641]) to each image of prior to stitching.
Correct Shading dropdown list	Select here which reference should be used for shading correction.
- **Automatic:**	The function automatically calculates a reference image from the input image.
- **Reference:**	The function uses an existing reference image. This must be selected in addition to the input image in the input tool of the image parameters section.

Select 2D image for Stitching section

Only visible for multidimensional input images. Select here a 2D image (one channel, one z-position, one time point) from your multidimensional data set. This 2D image is either stitched exclusively (no other planes of the dimensions are stitched) or serves as reference when stitching all planes of the dimensions.

Entry	Function
Get all dimensions from 2d view	Reads the current planes of the dimensions from the 2D view.
Z-Position	Select here a z-position for the 2D image.
Time	Select here a time point for the 2D image.
Channels	Select here a channel for the 2D image.

Stitch multiple dimensions section

Only visible for multidimensional input images. Select here which planes of the dimensions should be considered for the stitch.

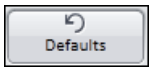
Button	Function
Yes	All planes of this dimension are stitched and appear in the output image. The stitch is calculated individually for each plane.

Button	Function
Reference	Only the reference plane (2D image) for this dimension is taken into consideration for calculating the stitch. All other planes are stitched accordingly and appear in the output image.
No	Only the selected reference plane (2D image) of this dimension is stitched. No other planes appear in the output image.

Parameters section

Parameter	Description
Edge Detector	
- Yes	An edge detector is applied to localize image edges. This may improve the stitching result.
- No	No edge detector is applied.
Minimal Overlap	Sets the extent of the area of minimal overlap (in %) of the individual tiles
Max Shift	Sets the maximal extent of the shift (in %) of the individual tiles to one another.
Comparer	Here you can select how the conformance of the tiles in the overlapping regions is evaluated.
- Basic	Basic comparison (faster).
- Best	Elaborate comparison (slower).
- Optimized	Optimized comparison.
Global Optimizer	Select here which tile overlaps are evaluated.
- Basic	Only one overlap per tile is evaluated.
- Best	All overlaps of a tile are evaluated.

Defaults button



Resets all settings to the default values.

Reset Button

Allows the user to return the output image back to its original form (input) after applying the stitch.

Redo button

Allows the user to return to the output form by reapplying the desired stitch settings.

14.8.5.4 Rotate

With this method you can rotate images by defined angles. This function was especially developed for rotating complex (multi-dimensional) images in the available image dimensions. Therefore the function can be a little bit slower but offers more settings for the rotation. For simple, 2-dimensional rotations we recommend to use the **Rotate 2D** function which is usually lots of faster.

Parameters

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adapt sizes checkbox

Only visible if the **Show All** mode is activated.

Activated: The size of the output image is adjusted in such a way that the entire input image is also visible after the rotation.

Deactivated: The output image is the same size as the input image. Depending on the image size and rotation angle, parts of the input image may not be visible in the output image.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Interpolation

Only visible if the **Show All** mode is activated.

Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.

Parameter	Description
Nearest Neighbor	The output pixel is given the gray value of the input pixel that is closest to it.
Linear	The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.
Cubic	The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

Angle slider

Enter the angle by which you want the input image to be rotated using the slider or spin box/input field. Positive angles rotate the image clockwise.

The following parameters are only visible if the **2D Sections** value is selected in the **Third Dimension** dropdown list:

Angle X slider

Enter the angle by which you want the input image to be rotated on the X axis using the slider or spin box/input field.

Angle Y slider

Enter the angle by which you want the input image to be rotated on the Y axis using the slider or spin box/input field.

Angle Z slider

Enter the angle by which you want the input image to be rotated on the Z axis using the slider or spin box/input field.

The following parameters are only visible if the **Adjust Size** checkbox is deactivated:

Center X slider

Enter the X coordinate of the center of the rotation using the slider or spin box/input field.

The value 0 means that the image is rotated around its center point. Negative values mean that the center of the rotation in the image is shifted to the left in relation to the image's center point. Positive values shift the center to the right.

Center Y slider

Enter the Y coordinate of the center of the rotation using the slider or spin box/input field.

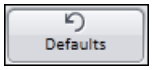
The value 0 means that the image is rotated around its center point. Negative values mean that the center of the rotation in the image is shifted downwards in relation to the image's center point. Positive values shift the center upwards.

Center Z slider

Enter the Z coordinate of the center of the rotation using the slider or spin box/ input field.

The value 0 means that the image is rotated around its center point. Negative values mean that the center of the rotation in the image is shifted forwards in relation to the image's center point. Positive values shift the center backwards.

Defaults button



Resets all settings to the default values.

14.8.5.5 Rotate 2D

With this method you can easily rotate an image clockwise around its center axis. Simply set the desired angle with the slider. Of course you can enter the angle value in the input field directly. To perform the rotation click on the **Apply** button on top of the Processing tab.

14.8.5.6 Mirror

This method allows you to flip an image horizontally or vertically. In the case of multidimensional images, such as Z-stack or time lapse images, you can also use the mirror method to reverse the sequence of the relevant dimension.

Parameters

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adjust per Channel checkbox

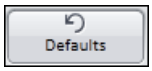
Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Mode dropdown list

Choice	Function
Horizontal	Flips image horizontal
Vertical	Flips image vertical
T/ Z	Only visible if input image is a multichannel image. Reverses sequence of the sections (Z) or time points (T)

Defaults button



Resets all settings to the default values.

14.8.5.7 Resample

This method allows you to change the size of an image in every dimension. You can either enlarge or reduce the image size.

Parameters

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adapt sizes checkbox

Only visible if the **Show All** mode is activated.

Activated: The size of the output image is adjusted in such a way that the entire input image is also visible after the rotation.

Deactivated: The output image is the same size as the input image. Depending on the image size and rotation angle, parts of the input image may not be visible in the output image.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Interpolation

Only visible if the **Show All** mode is activated.

Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.

Parameter	Description
Nearest Neighbor	The output pixel is given the gray value of the input pixel that is closest to it.
Linear	The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.
Cubic	The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

Scaling in X slider

Enter the desired scaling for X using the slider or spin box/input field.

Scaling in Y slider

Enter the desired scaling for Y using the slider or spin box/input field.

Scaling in Z slider

Enter the desired scaling for Z using the slider or spin box/input field.

The following parameters are only visible if the **Adjust Size** checkbox is deactivated:

Shift in X slider

Enter the shift in the X direction using the slider or spin box/input field.

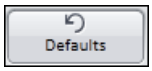
Shift in Y slider

Enter the shift in the Y direction using the slider or spin box/input field.

Shift in Z slider

Enter the shift in the Z direction using the slider or spin box/input field.

Defaults button



Resets all settings to the default values.

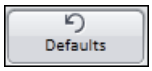
14.8.5.8 Orthogonal Projection

With this method you can extract specific parts of the image of three-dimensional images. This is accomplished with three alternative projection planes, frontal in the XY direction, sagittal in YZ direction or transverse in XZ direction as seen from the observer of the image.
You can choose between different projection methods, all have in common is that the pixels are analyzed by the observer along an imaginary projection beam.
You can also determine the thickness of the projection planes, and thus the projection depth.

Parameters

- **Projection Plane**
  Here you choose the type of the projection plane (Frontal X/Y, Transverse (X/Z), Sagittal (Y/Z).
- **Method**
  Here you choose a method for the projection:
  - **Maximum**: Uses the brightest pixel along the projection beam.
  - **Minimum**: Uses the darkest pixel along the projection beam.
  - **Average**: Calculates the average of all pixel along the projection beam.
  - **Weighted average**: This method is related to the calculation of the extended depth of focus. It prefers structures with more lateral sharpness along the projection beam. The output image contains more significant details.
  - **Standard deviation**: Calculates the standard deviation of pixel grey values along the projection beam.
- **Start position**
  Here you adjust the starting position of the project plane (in pixel units or z-stack positions depending on the chosen projection plane). The maximum range results automatically of the size of the input image.
- **Thickness**
  Here you adjust the thickness of the cutting plane (in pixel or z-stacks depending on the chosen projection plane). The maximum range results automatically of the size of the input image.

Defaults button



Resets all settings to the default values.

14.8.5.9 Shift

This method allows you to shift the content of an image in the direction of the 3 axes X, Y and Z.

Parameters

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Shift in X slider

Enter the shift in the X direction using the slider or spin box/input field.

Shift in Y slider

Enter the shift in the Y direction using the slider or spin box/input field.

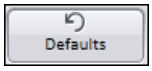
Shift in Z slider

Enter the shift in the Z direction using the slider or spin box/input field.

Shift in T slider

Enter the shift in the time points using the slider or spin box/input field.

Defaults button



Resets all settings to the default values.

14.8.5.10 Channel Alignment (Extended)

Using this method it is possible to automatically align the individual channels of a multi-channel image correctly to one another.

Parameters

Parameter	Description
Load transformation	If activated, the result of a previous transformation can be loaded. Click the **...** button to select an according *.xml file.
Save transformation	If activated, the result of the transformation process is saved in an *.xml file for later use.

Parameter	Description
Registration Method	Here you can select the method (or a combination of these) to be used to align the images.
- Translation	The neighboring sections of the Z-stack image are shifted in relation to each other in the X and Y direction.
- Rotation	The neighboring sections of the Z-stack image are rotated in relation to each other.
- Iso Scaling	
- Skew Scaling	
- Affine	The neighboring sections of the Z-stack image are shifted in the X and Y direction, rotated and the magnification is adjusted from section to section.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Quality	Here you can select the quality level that you want the function to work with.
- Low	Highest speed with low image quality.
- Medium	High speed with medium image quality.
- High	Low speed with high image quality.
- Highest	Lowest speed with highest image quality.

Parameter		Description
Interpolation		Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.
-	Nearest Neighbor	The output pixel is given the gray value of the input pixel that is closest to it.
-	Linear	The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.
-	Cubic	The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

14.8.6 Morphology group

Morphology operators apply structure elements to images. A structure element is like a stencil with holes. When the stencil is placed on an image the only some pixels are visible through the holes. The gray values of these pixels are collected and their external gray value (minimum or maximum) is computed. This external gray value is assigned to that pixel of the result image which corresponds to the place of the origin of the stencil on the input image. When the stencil is placed at all positions of the input image, all pixels of the result image are thus assigned. When bigger structure elements are required than those provided, these can be achieved by iterating the small elements using the 'Count' parameter.

14.8.6.1 Erode

Shrinks bright structures on a darker background in the input image. Thin connections between structures and small structures itself will disappear.

14.8.6.2 Dilate

Expands bright structures on a darker background in the input image. Small gaps between structures are filled and those structures become connected.

14.8.6.3 Open

First erodes (Erode class) the bright structures on a darker background in the input image, then it dilates (Dilate class) the result by the same number of steps. Thus it separates bright structures on a darker background, but approximately keeps the size of the structures.

14.8.6.4 Close

It first dilates (Dilate class) the bright structures on a darker background in the input image, then it erodes (Erode class) the result by the same number of steps. Thus it connects bright structures on a darker background, but approximately keeps the size of the structures.

14.8.6.5 Top Hat (White)

It computes the difference between the original image and the image produced by an open operation (Open class). Bright structures which were flattened by the opening are strengthened in the result. This is like putting a top hat with the size of the open operation upon the structure and keep only the part inside the hat.

14.8.6.6 Top Hat (Black)

It computes the difference between the original image and the image produced by a close operation (Close class). Dark structures which were flattened by the closing are strengthened in the result. This is like lifting a top hat with the size of the close operation beneath the structure from the dark side and keep only the part inside the hat.

14.8.6.7 Gradient

It computes the difference between the dilated image and the eroded image (Dilate and Erode class). Since a point in the dilated image has the maximum gray value and the corresponding point in the eroded image has the minimum gray value within the structure element the difference is zero for regions of constant gray values and gets bigger for steeper gray value ramps or edges.

14.8.6.8 Watersheds

It computes the barriers between catchment basins of local minima in the gray valued input image. A local minimum is a connected plateau of points from which it is impossible to reach a point of lower gray value without first climbing up to higher gray values. A catchment basin of a local minimum is a connected component which contains that minimum and all downstream points to it. A downstream is a path of points along which gray values are monotonically descending. Thus all catchment basins of local minima are expanded until they collide with another catchment basins. At those point barriers (watersheds) are built up. The output image is binary and contains all watersheds. If the 'Basins' flag is set, the catchment basins themselves are in the output image as uniquely labeled connected components without any border lines.

14.8.6.9 Grey Reconstruction

Works mainly as an iterated dilation (Dilate class) of the image, but with a constraint image as a second input image. After every dilation step the pixel wise minimum of the dilated image and the constraint image is computed and gives the next image to be dilated. The computation stops automatically when all the just dilated pixels are bigger than the corresponding ones in the constraint image.

14.8.6.10 Morphology parameters

Structure Element dropdown list

Here you select the structure element. The appearance of the structure element will be represented by an “X” and an “O”. The “O” stands for a hole in the pattern:

Selection	Schematic Representation
Horizontal	x x x x x x x x x x x **o o o** x x x x x x x x x x x
Diagonal 45°	x x x x x x x x **o** x x x **o** x x x **o** x x x x x x x x
Vertical	x x x x x x x **o** x x x x **o** x x x x **o** x x x x x x x
Diagonal 135°	x x x x x x **o** x x x x x **o** x x x x x **o** x x x x x x
Cross	x x x x x x x **o** x x x o o o x x x **o** x x x x x x x
Square	x x x x x x **o o o** x x **o o o** x x **o o o** x x x x x x

Selection	Schematic Representation
Octagon	x o o o x o o o o o o o o o o o o o o o x o o o x

Count slider

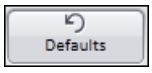
Here you can adjust the number of repetitions to define the size of the structure element.

Binary checkbox

Only available for **Erode**, **Dilate**, **Open** and **Close** function.

Activated: Creates a binary image. The calculation will be faster.

Defaults button



Resets all settings to the default values.

14.8.7 Sharpen group

14.8.7.1 Extended Depth of Focus

Using this method you can combine the sharp regions from the individual sections of a Z-stack image to form a single image. This enables you to display a considerably larger depth of field than is possible on a microscope.

Parameters

Method

Method	Description
Wavelets	A wavelet transform is used to detect the sharpest areas in the images.
Contrast	

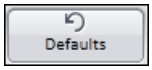
Method	Description
Projection	Images with the brightest and darkest pixels are generated first. Of these images the image with the higher variance is used as the resulting image.
Variance	

Z-Stack Alignment dropdown list

Here you can select whether you want the Z-stack image to be aligned before the calculation and with what quality level. If you want to acquire images with a stereo microscope, the images are displaced against each other. This displacement can be corrected. The higher the quality of alignment is selected, the longer is the calculation. Select **No Alignment**, if you want to acquire images with a compound microscope.

Selection	Description
No Alignment	The Z-stack image is not aligned before the calculation. You should select this setting if the Z-stack image has not been acquired using a stereo microscope.
Normal	High speed with normal image quality.
High	Low speed with high image quality.
Highest	Lowest speed with best image quality.

Defaults button



Resets all settings to the default values.

14.8.7.2 Delineate

This method enhances the edges of individual regions in an image. It corrects the halo effect and only affects edges.

Parameters

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

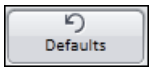
Threshold slider

Enter the threshold value for edge detection using the slider or spin box/input field. The threshold value should correspond roughly to the gray value difference between objects and the background.

Size slider

Enter the size of the edge detection filter using the slider or spin box/input field. The value should correspond to the size of the transition area between objects and the background.

Defaults button



Resets all settings to the default values.

14.8.7.3 Enhance Contour

This method allows you to enhance contours in an image and emphasize regions in which gray values change. The function is suitable for visually emphasizing fine structures in an image.

Parameters

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Strength slider

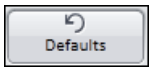
Enter the factor for increasing edge enhancement using the slider or spin box/input field.

Normalization dropdown list

Here you can select how the gray/color values that exceed or fall short of the value range should be dealt with.

Parameter	Description
Clip	Automatically sets the gray levels that exceed or fall short of the predefined gray value range to the lowest or highest gray value (black or white). The effect corresponds to underexposure or overexposure. In certain circumstances some information may therefore be lost.
Automatic	Normalizes the gray values automatically to the available gray value range.
Wrap	If the result is larger than the maximum gray value of the image, the maximum gray value + 1 is deducted from this value.
Shift and Clip	Normalizes the output to the value "gray value + maximum gray value/2".
Absolute	Converts negative gray levels into positive values.

Defaults button



Resets all settings to the default values.

14.8.7.4 Unsharp Mask

Using this method you can increase the impression of sharpness in an image and consequently obtain an image display that is richer in detail. The function allows contrasts at small structures and edges to be enhanced in a targeted way.

Parameters

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Strength slider

Enter the strength of the Unsharp Masking using the slider or spin box/input field. The higher the value selected, the greater the extent to which small structures are enhanced.

The following parameters are only visible if the **Show All** mode is activated:

Radius slider

Enter the radius using the slider or spin box/input field. **Radius** defines the width of structures that you want to appear sharper. A small radius enhances smaller details. If the radius is too big, halo effects can occur at edges.

Parameter	Description
Color Mode dropdown list	Select the desired color mode from the dropdown list.
- **RGB**	Calculates the sharpness for each color channel individually. The color saturation and the color of structures may be changed and color noise may occur.
- **Luminance**	Only calculates the sharpness on the basis of the brightness signal detected. This mode does not show any color noise and changes the color saturation accordingly.
Threshold Mode dropdown list	Here you can select a setting from the dropdown list for calculating the boundary between the sharpened image regions. It is only effective if the value for the **Lower Threshold Value** parameter is not equal to 0 or the value for the **Upper Threshold Value** parameter is not equal to 100.
None	No adjustment takes place.
Binary	The transition follows the threshold values.
Linear	Calculates a linear course.

Threshold Low slider

Enter the lower threshold value using the slider or spin box/input field. This determines the lower limit from which existing contrast structures are changed.

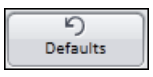
Threshold High slider

Enter the upper threshold value using the slider or spin box/input field. This prevents the existing strong contrasts in the image from being increased further unnecessarily.

Clip To Valid Bits checkbox

Activated: Thevalue range of the gray/color values of the output image is adjusted to the value range of the input image.

Defaults button



Resets all settings to the default values.

14.8.8 Smooth group

14.8.8.1 Median

This method allows you to reduce noise in an image. Each pixel is replaced by the median of its neighbors. The size of the area of the neighboring pixels considered is defined by a quadratic filter matrix. The modified pixel is the central pixel of the filter matrix. The median is the middle value of the gray values of the pixel and its neighbors sorted in ascending order.

Parameter

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Filter Size slider

Enter the size of the filter matrix in the X and Y direction using the slider or spin box/input field.

The following parameters are only visible if the **Show All** mode is activated:

Filter Size in X slider

Enter the size of the filter matrix in the X direction using the slider or spin box/input field.

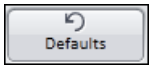
Filter Size in Y slider

Enter the size of the filter matrix in the Y direction using the slider or spin box/input field.

Filter Size in Z slider

Enter the size of the filter matrix in the Z direction using the slider or spin box/input field.

Defaults button



Resets all settings to the default values.

14.8.8.2 Sigma

This method allows you to reduce noise in an image. Each pixel is replaced by the average of its neighbors. The size of the area of the neighboring pixels considered is defined by a quadratic filter matrix. The modified pixel is the central pixel of the filter matrix. To calculate the average, only the gray values that lie within a defined range (+/- sigma) around the gray value of the central pixel are taken into consideration. As a result, fine object structures are not blurred; only the gray levels in image regions that belong together are adjusted.

Parameter

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Sigma slider

Enter the sigma value using the slider or spin box/input field.

Filter Size slider

Enter the size of the filter matrix in the X and Y direction using the slider or spin box/input field.

The following parameters are only visible if the **Show All** mode is activated:

Filter Size in X slider

Enter the size of the filter matrix in the X direction using the slider or spin box/input field.

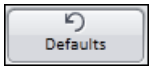
Filter Size in Y slider

Enter the size of the filter matrix in the Y direction using the slider or spin box/input field.

Filter Size in Z slider

Enter the size of the filter matrix in the Z direction using the slider or spin box/input field.

Defaults button



Resets all settings to the default values.

14.8.8.3 Lowpass

This method allows you to reduce noise in an image. Each pixel is replaced by the average of its neighbors. The size of the area of the neighboring pixels considered is defined by a quadratic filter matrix. The modified pixel is the central pixel of the filter matrix.

Parameter

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Repetitions slider

Enter the number of repetitions using the slider or spin box/input field. The function can be applied several times in succession to the result of the filtering. This intensifies the effect accordingly.

Filter Size slider

Enter the size of the filter matrix in the X and Y direction using the slider or spin box/input field.

The following parameters are only visible if the **Show All** mode is activated:

Filter Size in X slider

Enter the size of the filter matrix in the X direction using the slider or spin box/input field.

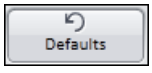
Filter Size in Y slider

Enter the size of the filter matrix in the Y direction using the slider or spin box/input field.

Filter Size in Z slider

Enter the size of the filter matrix in the Z direction using the slider or spin box/input field.

Defaults button



Resets all settings to the default values.

14.8.8.4 Binomial Filter

This method allows you to reduce noise in an image. Each pixel is replaced by a weighted average of its neighbors. The weighting factors are calculated from the binomial coefficients in accordance with the filter size. The binomial filter is very similar to a Gaussian filter in its effect.

Parameter

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

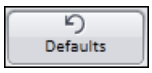
Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Filter Size slider

Enter the size of the filter matrix using the slider or spin box/input field.

Defaults button



Resets all settings to the default values.

14.8.8.5 Gauss

This method allows you to reduce noise in an image. Each pixel is replaced by a weighted average of its neighbors. The neighboring pixels are weighted in accordance with a two-dimensional Gauss bell curve.

Parameter

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Adjust per Channel checkbox

Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Sigma slider

Enter the sigma value using the slider or spin box/input field.

Sigma in X slider

Enter the sigma value in the X direction using the slider or spin box/input field.

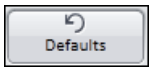
Sigma in Y slider

Enter the sigma value in the Y direction using the slider or spin box/input field.

Sigma in Z slider

Enter the sigma value in the Z direction using the slider or spin box/input field.

Defaults button



Resets all settings to the default values.

14.8.8.6 Single Pixel Filter

This method allows you to remove single pixel phenomena, such as those that occur in the case of clocking induced charge with EMCCDs and as radio telegraph signal noise with CMOS sensors. It can also be used to remove hot pixels.

Parameter

Adjust per Channel checkbox

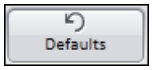
Only visible if your input image is a multichannel image.

Activated: You can adjust the parameters for each channel individually.

Threshold Value slider

Enter the threshold value using the slider or spin box/input field.

Defaults button



Resets all settings to the default values.

14.8.8.7 Rank

This method performs a rank order filtering. The gray levels of the resulting image is determined by calculating the ranking within the matrix of the filter size in the X and Y directions. Even numbers are automatically set to the next odd number. A low value for the rank value enlarges dark areas, a higher value will increase bright areas of the image.

14.8.8.8 Denoise

This method removes noise from images using a real or a complex wavelet transformations. The process of denoising an image can be broken down into the following three parts:

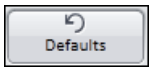
- **Calculate the wavelet transform of the noisy image.**
  The wavelet transformation can be calculated by the method **Real Wavelets** and **Complex Wavelets** (see below).
- **Modify the noisy wavelet coefficients**
  This is done by using bivariate shrinkage with local variance estimation (thresholding). [Bivariate Shrinkage with Local Variance Estimator, Levent Sendur and Ivan W. Selesnick, IEEE Signal Processing Letters, Vol. 9, No. 12, December 2002]
- **Compute the inverse transform using the thresholded coefficients.**

Parameters

Method dropdown list

- **Complex wavelets**
  The Dual Tree Complex Wavelet transform provides better results due to the fact that it is nearly direction invariant and makes more directional sub bands available. The results will be less prone to block-artefacts. However, this method is computationally more intense and therefore takes longer.
- **Real wavelets**
  The real wavelet transform only considers three sides (XYZ) and is therefore faster. However, the result can show block artefacts.

Defaults button



Resets all settings to the default values.

14.8.9 Time Series

This group of image processing functions is a collection of functions which are especially relevant for time series images.

14.8.9.1 Time Alignment

Using this function you can automatically align individual time points in order to compensate for shifts between time points.

i Note

For the alignment function to work the presence of immobile and clearly distinguishable object structures in the time series is required. Also, when aligning z-stacks over time, you should always use the z dimension from the Third Dimension drop down list. Otherwise each z-plane would be aligned over time potentially leading to z-stack artefacts.

Parameter

Third Dimension dropdown list

Only visible, if there is a third dimension in the input image and/or **Show all** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

Choice	Function
2D Slices	The function is used in a 2-dimensional fashion only for multidimensional images such as time lapse or z-stacks.
Z, T or C	Here you can select to which additional dimension the functions should be applied to.

Quality

Only visible if the **Show All** mode is activated.

Here you can select the quality level that you want the function to work with.

Choice	Function
Low	Highest speed with low image quality.
Medium	High speed with medium image quality.
High	Low speed with high image quality.
Very high	Lowest speed with best image quality.

Registration Method

Here you can select the method (or a combination of these) to be used to align the images.

Method	Function
Translation	The neighboring sections of the Z-stack image are shifted in relation to each other in the X and Y direction.
Rotation	The neighboring sections of the Z-stack image are rotated in relation to each other.
Iso Scaling	The magnification is adjusted from section to section.
Skew Scaling	The magnification is adjusted from section to section.
Affine	The neighboring sections of the Z-stack image are shifted in the X and Y direction, rotated and the magnification is adjusted from section to section.

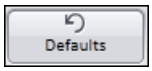
Interpolation

Only visible if the **Show All** mode is activated.

Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.

Parameter	Description
Nearest Neighbor	The output pixel is given the gray value of the input pixel that is closest to it.
Linear	The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.
Cubic	The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

Defaults button



Resets all settings to the default values.

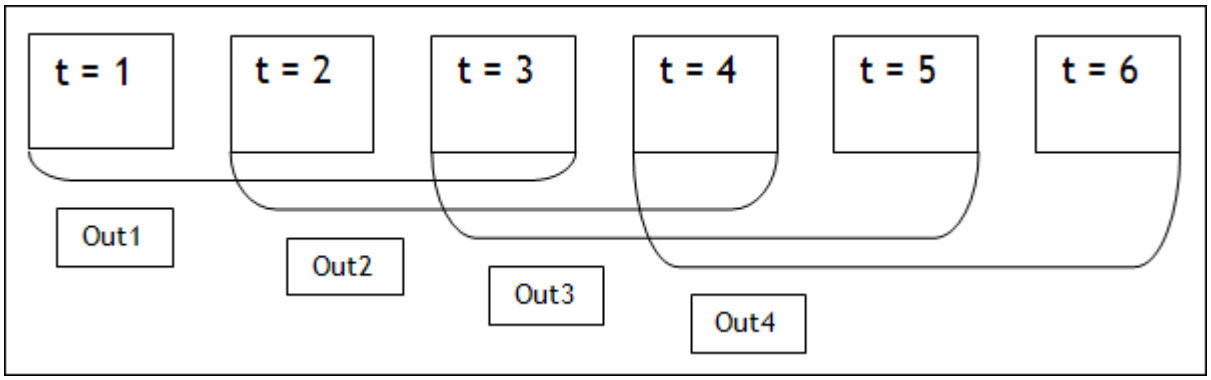
14.8.9.2 Gliding Average

This function calculates the gliding average of a time lapse image, taking into account the defined number of timepoints according to the following schematic:

Input Image: SizeT = 6

Averaging Length: AvL = 3

Output image: SizeT(output) = SizeT – AvL + 1 = 6 -3 +1 = 4



With this function a smoothing effect can be achieved due to averaging out noise.

Parameter

Average Length
Specifies the number of images used to determine the mean value. The maximum value correlates with the number of time points.

Scaling Factor
The preset value is 1. Values > 1 can be applied for images with low intensity. In this case all pixel values are multiplied by the specified factor.

14.8.9.3 Time Differential

This function calculates the first and second order differential of a time lapse image according to the following formula and schematic:

First Order Differential:

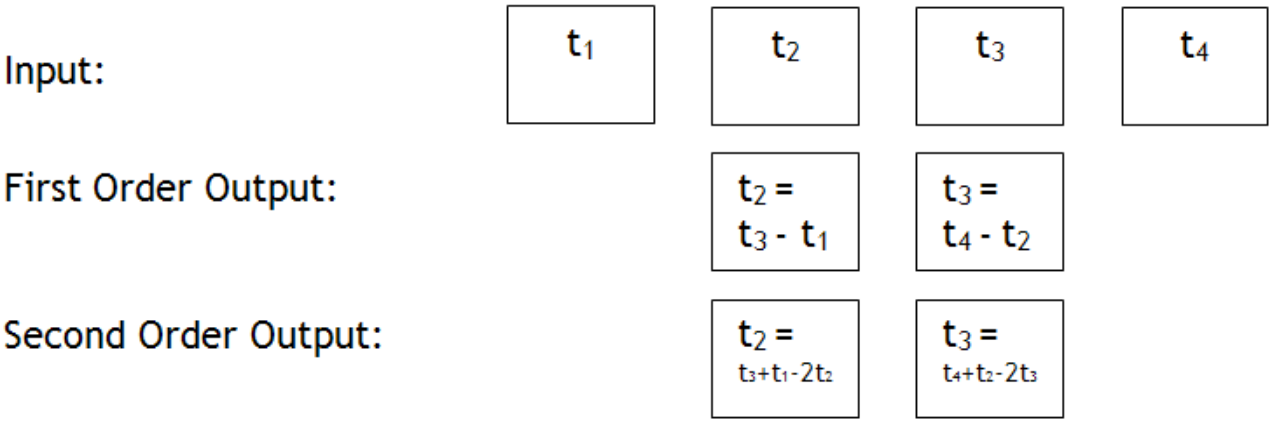
Output[t] = Input[t+1] – Input[t-1]

-> the difference between consecutive pixels is not calculated so that the output is not directional. The first order differential represents the **Speed**.

Second Order Differential:

Output[t] = Input[t-1] + Input[t+1] – 2 x Input[t]

->Second order differential is also known as the "Laplacian" and represents the **Acceleration**. It enhances the fine details in the image (including noise). The smoothing kernel helps reduce this noise.



Parameter

- **Derivative**
  Here you can select whether to calculate the first (speed) or second (acceleration) order differential.
- **Smoothing**
  Indicates the iterative, binominal smoothing filter. This reduces noise in the differential images, whilst retaining maximums and minimums. Value range: 0 – 50
- **Normalization**
  Defines what to do with negative values resulting from the calculation.
  Clip: negative values are set to 0.
  Absolute: negative values are used positively.

14.8.9.4 Time Concatenation

This function joins two images to form a new time series image. Select the desired images in the input tool and click **Apply**.

i Note

Images with varying dimensions will be put into different blocks by this function. The resulting image document shows a **Block** slider in the **Dimensions** view block.

14.8.9.5 Time Stitching

This function stitches heterogeneous **CZI** image together to create a new, single homogeneous time series containing all dimensions and time points in their proper order. This differs from the Time Concatenate function, which simply pastes one time lapse series to the end of another without regard for the proper time order or channel content.

Missing images can either be filled with copies of the previous valid image in the series or filled with black images.

When combining Z-stack time series with non-Z-stack time series a choice can be made between either using only the center plane of the Z-stack or creating an extended focus projection of the Z-stack before stitching the images together.

Parameter

Fill Missing with

Selection	Description
Previous	Fills a missing dimension index with a copy of the last existing image from that index.
Black	Fills a missing dimension index with a black image.

Z-Stacks

Selection	Description
Collapse (EDF)	Reduces a z-stack with an extended focus function to a single plane image which is then added to the output.
Collapse (Center Plane)	Only uses the center plane from a z-stack for the output image.
Expand	Copies the z-stack to the output unchanged, fills the missing indices according to the setting in the **Fill Missing with** drop down list.

14.8.9.6 Kymograph

This method creates a Kymograph. The input image has to be a time series image containing a graphical element (e.g. a line, arrow, curve or polygon) which is not closed.

Parameter

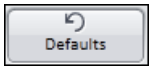
Graphic tool dropdown list

Here you see all graphic tools which can be used. Choose the desired graphic tool from the list. Note that the tool must be selected in the image as well.

Width slider

Here you adjust the graphic tools width (in pixel).

Defaults button



Resets all settings to the default values.

14.8.10 Arithmetics group

14.8.10.1 Add

This function adds the two images **Input1** and **Input2** pixel by pixel and generates the **Output** image. Note that a resulting gray value may be greater than the maximum gray value of the image.

Parameter

Parameter	Description
Normalization dropdown list	Depending on the IP function you have selected not all choices are available in the list.
- **Clip**	Gray levels that exceed or fall below the specified gray value range are automatically set to the lowest/highest gray value (black or white). The effect corresponds to underexposure or overexposure. This means that in some cases information is lost.
- **Automatic**	Automatic normalization of gray values to the available gray value range.
- **Wrap**	If the result is greater than the maximum gray value of the image, the value maximum gray value +1 is subtracted from it.
- **Shift**	Normalizes the output to the value gray value + max. gray value/2.

14.8.10.2 Add Constant

This function adds the factor **Addend** to each pixel of the **Input**image and generates the **Output** image.

To work useful with this function, the pixel values of the input image must be in float format. If images of type 8 bit, 16 bit, 24 bit and 48 bit are used as input image, the output images are of the same type, i.e. the output value will be clipped to an integer value. Negative values are set to 0, values higher than the maximum gray value are set to the maximum possible gray value of the image.

Parameter

Addend

Here you adjust the addend.

Value range: -255...+255

14.8.10.3 Subtract

This function subtracts the two images **Input1** and **Input2** pixel by pixel and generates the **Output** image. Note that a resulting gray value may be less than 0.

Parameter

Parameter	Description
Normalization dropdown list	Depending on the IP function you have selected not all choices are available in the list.
- **Clip**	Gray levels that exceed or fall below the specified gray value range are automatically set to the lowest/highest gray value (black or white). The effect corresponds to underexposure or overexposure. This means that in some cases information is lost.
- **Automatic**	Automatic normalization of gray values to the available gray value range.
- **Wrap**	If the result is greater than the maximum gray value of the image, the value maximum gray value +1 is subtracted from it.
- **Shift**	Normalizes the output to the value gray value + max. gray value/2.

14.8.10.4 Multiply

This function multiplies the two images **Input1** and **Input2** pixel by pixel.

Parameter

Factor

Here you adjust the scaling factor by which the result of the multiplication is divided. Using this factor it is possible to keep the gray values of the **Output** image within the range of 0 to the maximum gray value. Values that are greater than the maximum gray value are in any case limited to the maximum gray value. Negative values are set to 0.

Value range: -20,000 ... +20,000

14.8.10.5 Multiply Constant

This function multiplies each pixel of the **Input** image with a adjustable **Factor** and generates the **Output** image.

To work useful with this function, the pixel values of the input image must be in float format. If images of type 8 bit, 16 bit, 24 bit and 48 bit are used as input image, the output images are of the same type, i.e. the output value will be clipped to an integer value. Negative values are set to 0, values higher than the maximum gray value are set to the maximum possible gray value of the image.

Parameter

Factor

Here you adjust the factor to be multiplied.

Value range: 0 …255,00

14.8.10.6 Divide

This function divides the images **Input1** by **Input2** pixel by pixel.

Parameter

Factor

Here you adjust the scaling factor by which the result of the division is multiplied. Using this factor it is possible to keep the gray values of the output image within the range of 0 to the maximum gray value. Values that are greater than the maximum gray value are in any case limited to the maximum gray value. Negative values are set to 0.

Value range: -20.00 ... +20.00

14.8.10.7 Square

This function calculates the square of the **Input** image pixel by pixel and generates the **Output**image.

To work useful with this function, the pixel values of the input image must be in float format. If images of type 8 bit, 16 bit, 24 bit and 48 bit are used as input image, the output images are of the same type, i.e. the output value will be clipped to an integer value. Negative values are set to 0, values higher than the maximum gray value are set to the maximum possible gray value of the image.

14.8.10.8 Square Root

This function calculates the square root of the **Input** image pixel by pixel and generates the **Output** image.

To work useful with this function, the pixel values of the input image must be in float format. If images of type 8 bit, 16 bit, 24 bit and 48 bit are used as input image, the output images are of the same type, i.e. the output value will be clipped to an integer value. Negative values are set to 0, values higher than the maximum gray value are set to the maximum possible gray value of the image.

14.8.10.9 Logarithm

This function calculates the logarithm of the **Input** image pixel by pixel and generates the **Output**image.

To work useful with this function, the pixel values of the input image must be in float format. If images of type 8 bit, 16 bit, 24 bit and 48 bit are used as input image, the output images are of the same type, i.e. the output value will be clipped to an integer value. Negative values are set to 0, values higher than the maximum gray value are set to the maximum possible gray value of the image.

14.8.10.10 Exponential

This function calculates the exponential function of the **Input** image pixel by pixel and generates the **Output** image.

To work useful with this function, the pixel values of the input image must be in float format. If images of type 8 bit, 16 bit, 24 bit and 48 bit are used as input image, the output images are of the same type, i.e. the output value will be clipped to an integer value. Negative values are set to 0, values higher than the maximum gray value are set to the maximum possible gray value of the image.

14.8.10.11 Invert

This function additively inverts the gray values of the input image into the output image. Bright pixels will become darker and vice versa. To adjust the output range the parameter **Operand** is used. The actual mathematical operation is then: output-gray value = constant - input-gray value. Negative results are clipped to 0 and overflow results are clipped to the maximum possible gray value.

14.8.10.12 Reciprocal

This function computes the reciprocals of the gray values in the input image into the output image. Bright pixels will become darker and vice versa. To adjust the output range the parameter "factor" is used. The actual mathematical operation is then: output-gray value = factor / input-gray value. Negative results are clipped to 0 and overflow results are clipped to the maximum possible gray value.

14.8.10.13 Average

The function calculates the average of the two images **Input1** and **Input2** pixel by pixel.

14.8.10.14 Combine

This function calculates the linear combination of two images on a pixel basis.

Both **Input** images are first multiplied by the specified factor and then added together. The brightness of the **Output** image can then be adjusted. The combination of two images can be used to reduce noise, for example. This is achieved by acquiring several images of the same scene and subsequently combining them.

14.8.10.15 Minimum

The function calculates the maximum value of the two images **Input1** and **Input2** pixel by pixel.

14.8.10.16 Maximum

The function calculates the minimum values of the two images **Input1** and **Input2** pixel by pixel.

14.8.11 Segmentation group

14.8.11.1 Threshold

This function performs a segmentation based on the definition of a brightness range (separated according to color channels (red, green, blue)) for the regions to be segmented. All pixels whose color values lie within the defined color range are marked as region pixels in the resulting image. All the pixels whose color values lie outside the defined color range are marked as background pixels (black).

In the resulting image, the color values of the region pixels can either be set permanently to white or adopted unchanged. If you set the region pixels permanently to white, the result is a binary image, which can then be used as a mask image for a subsequent automatic measurement.

Parameter	Description
Level Low slider	Determines the lower brightness threshold for the regions to be segmented. All the pixels whose gray values lie below this threshold value are marked as background pixels (black).
Level High slider	Determines the upper brightness threshold for the regions to be segmented. All the pixels whose gray values lie above this threshold value are marked as background pixels (black). **Deactivated:** The segmentation result is not inverted.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Create binary checkbox	**Activated:** The resulting image is a binary image. Pixels within the calculated gray level range are set to the maximum gray value (white), whilst pixels outside it are set to the gray value 0. **Deactivated:** The resulting image is of the same type as the input image. Pixels within the calculated gray level range are set to the original gray value. Pixels outside it are set to 0.
Invert result checkbox	**Activated:** Inverts the effect of the function. The segmented regions will be given the value 0, and all other pixels the gray value white or the gray value/color of the input image. See also the description under **Binary**. **Deactivated:** The segmentation result is not inverted.
Defaults button	Resets all settings to the default values.

14.8.11.2 Threshold (auto)

This method performs an automatic gray value segmentation. The function calculates the two minimums in the individual channels in the gray value histogram of the input image (**Input**) and uses these for the segmentation.

Parameter	Description
Create binary checkbox	**Activated:** The resulting image is a binary image. Pixels within the calculated gray level range are set to the maximum gray value (white), whilst pixels outside it are set to the gray value 0. **Deactivated:** The resulting image is of the same type as the input image. Pixels within the calculated gray level range are set to the original gray value. Pixels outside it are set to 0.
Invert result checkbox	**Activated:** Inverts the effect of the function. The segmented regions will be given the value 0, and all other pixels the gray value white or the gray value/color of the input image. See also the description under **Binary**. **Deactivated:** The segmentation result is not inverted.
Defaults button	Resets all settings to the default values.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Method drop down list	
- **Otsu**	
- **Maximum Peak**	
- **Iso Data**	
- **Triangle Threshold**	
- **Three Sigma Threshold**	
Process tiles separately checkbox	

14.8.11.3 Threshold (dynamic)

This method performs an adaptive gray value segmentation. This procedure is particularly well suited to the segmentation of small structures against a varying background.

The function initially applies a low pass filter and then subtracts this low-pass-filtered image from the input image. The effect of this function mainly depends on the size of the filter matrix: Select a low value for **Size** to segment small regions or regions with low gray value contrast from the background. Select a higher value for **Size** to segment larger regions from the background.

Parameter	Description
Kernel Size slider	Matrix size of the low pass filter in x- and y-direction symmetrically around the pixel in question. Determines the extent of the smoothing effect. As the affected pixel is at the center, the edge length of the filter matrix is always an odd number. If an even number is entered via the keyboard, the value is always set to the next highest odd number.
Threshold slider	This value defines the gray value difference between the regions to be detected and the background. Segmented pixels are set to the maximum gray value (white), whilst other pixels are set to the gray value 0.

The following parameters are only visible if the **Show All** mode is activated:

Parameter	Description
Create binary checkbox	**Activated:** The resulting image is a binary image. Pixels within the calculated gray level range are set to the maximum gray value (white), whilst pixels outside it are set to the gray value 0. **Deactivated:** The resulting image is of the same type as the input image. Pixels within the calculated gray level range are set to the original gray value. Pixels outside it are set to 0.
Invert result checkbox	**Activated:** Inverts the effect of the function. The segmented regions will be given the value 0, and all other pixels the gray value white or the gray value/color of the input image. See also the description under **Binary**. **Deactivated:** The segmentation result is not inverted.
Defaults button	Resets all settings to the default values.

14.8.11.4 Valleys

This method detects dark lines (gray value valleys) on a bright background and contours between bright regions.

Parameter

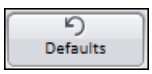
Sigma slider

Degree of smoothing of input image before detection of valleys. The smoothing factor can be used to influence the sensitivity of recognition. If a low value is set, lots of valleys are detected. Fewer valleys are detected with a high value.

Threshold slider

Curvature at the valley bottom high/low. Depending on the setting made, weakly pronounced valleys are also detected, or only ones that are strongly pronounced.

Defaults button



Resets all settings to the default values.

14.8.11.5 Canny

Canny detects edges in an image. This function detects relatively thick contours at the edge of bright regions.

Parameter

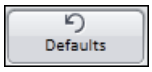
Sigma slider

Degree of smoothing of input image before detection of edges. A Gauss filter is used as a smoothing function. The smoothing factor can be used to influence the sensitivity of recognition. If a low value is set, lots of edges are detected. Fewer edges are detected with a high value. If the value 0 is set, no smoothing is performed.

Threshold slider

Steepness of the edges to be detected. Low values mean "flat" edges with a wide transition area between two regions. In this case lots of edges are detected. If high values are used, fewer edges are detected, as only steep transition areas are interpreted as edges.

Defaults button



Resets all settings to the default values.

14.8.11.6 Marr

This mehtod detects edges or regions in an image. In contrast to **Valleys** and **Canny**, here a **Laplace** filter is calculated, followed by smoothing using a **Gauss** filter, and the edges (Mode - Edges) or regions (Mode - Regions) are detected.

Parameter

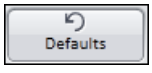
Sigma slider

Degree of smoothing of input image before detection of edges or regions. A Gauss filter is used as a smoothing function. The smoothing factor can be used to influence the sensitivity of recognition. If a low value is set, lots of edges are detected. Fewer edges are detected with a high value. If the value 0 is set, no smoothing is performed.

Display Mode dropdown list

- **Edges:** Region edges are detected.
- **Regions:** The regions, not just their edges, are detected.

Defaults button



Resets all settings to the default values.

14.8.12 Binary group

14.8.12.1 Fill Holes

This method fills holes in regions. Holes are structures that have the gray scale value 0, and is completely surrounded by pixels with a gray value equal to 0. Of regions outside of the image, it is assumed that they are black. Black areas that touch the edge of the image are preserved, therefore, even if they are surrounded by a contour.

14.8.12.2 Scrap

This method removes regions within a interval.

14.8.12.3 Mark Regions

This function marks binary regions of the input image. For each region in the input image, a check is performed to establish whether a pixel has been set in the marker image. Depending on the value of the Select regions parameter, either the marked region or the unmarked region is copied into the output image.

14.8.12.4 And

This method performs a bit-by-bit AND calculation for the input images (**Input1** and **Input2**). This function is particularly useful for the masking of images. All the pixels that are white in input image 1 AND input image 2 are set to white in the resulting image. Pixels that are white in only one of the two input images become black.

14.8.12.5 Or

Or performs a bit-by-bit OR calculation for the **Input1** and **Input2** images. This function can be used to combine binary masks or regions. All the pixels that are white in input image 1 OR input image 2 are set to white in the resulting image. This means that all the white pixels in the two input images are white in the resulting image.

14.8.12.6 Xor

This method performs a bit-by-bit Xor calculation for the **Input1** and **Input2** images. This function can be used to combine binary masks or regions. All the pixels that are white in input image 1 or input image 2 are set to white in the resulting image. Pixels that are white in both input images are set to black.

14.8.12.7 Not

This function performs a binary "not" operation on all bits of the binary representation of an input pixel's gray value. A 0-bit in the input pixel results in an 1-bit in the corresponding output pixel an 1-bit in the input gets a 0-bit in the

output. For integral image types the resulting output gray value is the difference of the maximum possible gray value minus the input gray value, but for float image type the results are strange due to the inhomogeneous float format.

14.8.12.8 Ultimate Erode

This function works in the same way as normal erosion. Structures in the input image are reduced. Thin connections between regions are separated. The difference between this function and normal erosion is that structures are eroded until they would be deleted by the next erosion step. With erosion, the pixel in question is set to the gray value 0 (black) in the resulting image. For regions (pixels) at the image edge, the assumption is that the pixels outside the image are white.

Parameter

Structure Element

Here you select the preferred direction of morphological change.

Count slider

Here you set the number of repetitions. This means that the function is applied a number of times in succession to the filtering result. This increases the effect accordingly. If the number of repetitions is set to 0, the function is automatically repeated until all regions would be deleted by the next erosion step.

14.8.12.9 Distance

This method creates a distance-transformed image (distance map, distance image) from a binary image. The Euclidean distance to the next background pixel (gray value 0) is calculated for each pixel within the white regions of the binary image (input image), and coded as a gray value. Bright pixels (high gray values) indicate a long distance to the background.

14.8.12.10 Thinning

This method thins objects to a line of single pixel thickness.

Parameter	Description
Thinning Element	Select the desired thinning method here.
- Arcelli	Applies thinning in accordance with the Arcelli method.
- Levialdi	Applies thinning in accordance with the Levialdi method.

Parameter	Description
Count	Sets the number of repetitions. This means that the function is applied a number of times in succession to the filtering result. This increases the effect accordingly. If the number of repetitions is set to 0, the function is automatically repeated until all regions have been thinned as far as possible. The value range is from 0...256.
Prune	Cuts off the ends of the thinned lines.
Converge	Stops thinning if no further changes can be achieved.
AxioVision Compatibility	Performs the function exactly like in AxioVision to achieve identical results.

14.8.12.11 Exoskeleton

This method generates an image with the skeleton of the influence zone of regions. The background in the **Input** image is analyzed, and the skeleton of the influence zones of the objects is determined. This is then saved as a binary image in the **Output** image.

14.8.12.12 Separation

Using this function you can attempt to separate objects that are touching (and that you have been unable to separate using segmentation) automatically.

14.8.12.13 Label Image

Assigns a gray value to each object in a binary image.

Label Background checkbox

Activated: Assigns gray values to the background objects with connectivity 4. Connectivity 8 will be used if the checkbox is deactivated.

14.8.13 Utilities group

14.8.13.1 ApoTome RAW convert

This method accepts ApoTome raw data only.

You can configure the same settings like you have on **ApoTome** tab (view option for ApoTome images).

Parameter

Find the description of the parameters here: *ApoTome tab* [▶ 608]. For ApoTome raw data you`re able to apply following correction methods:

- No correction
- Local bleaching
- Global bleaching
- Phase errors
- Phase errors and global bleaching
- Phase errors and local bleaching

The function is available for batch processing as well. This makes it easy to convert a series of ApoTome RAW data images into deconvolved images.

14.8.13.2 ApoTome deconvolution

This method accepts **ApoTome** raw data only.

It was derived from the **Deconvoltuion** modul and is available in every licensed version of ZEN 2.1. It contains settings and parameters which make sense for an Apotome deconvolution only.

Parameter

Find the description of the parameters under: *Deconvolution (adjustable) parameters* [▶ 616] . This method is available for batch processing as well.

14.8.13.3 Copy Annotations

This method copies the annotations of one image into another image.

14.8.13.4 Copy Image

This method creates a copy of an image.

14.8.13.5 Change Pixel Type

This method allows you to change the pixel type of an image. This can be useful if you want to compare or combine images that have different pixel types.

Parameters

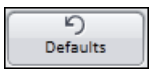
Pixel Format dropdown list

Select the desired pixel format from the dropdown list.

Choice	Function
8 Bit B/W	The output image is a monochrome image, the whole-number gray values of which can lie in the range from 0 to 255.

Choice	Function
16 Bit B/W	The output image is a monochrome image, the whole-number gray values of which can lie in the range from 0 to 65535.
32 Bit B/W Float	The output image is a monochrome image with real numbers as pixel values.
2x32 Bit Complex	The output image is a monochrome image with complex numbers (real part and imaginary part) as pixel values. Such images are generally created by means of transformation into the Fourier space.
24 Bit RGB	The output image is a color image, the whole-number color values of which in the red, green and blue channels can lie in the range from 0 to 255.
48 Bit RGB	The output image is a color image, the whole-number color values of which in the red, green and blue channels can lie in the range from 0 to 65535.
2x32 Bit RGB Float	The output image is a color image with real numbers as color values in the red, green and blue channels.
3x64Bit RGB Complex	The output image is a color image with complex numbers (real part and imaginary part) in the red, green and blue channels. Such images are generally created by means of transformation into the Fourier space.

Defaults button



Resets all settings to the default values.

14.8.13.6 Impose Noise

This function imposes an image with a defined noise for testing purposes.

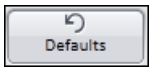
Parameter	Description
Signal to Noise Ratio slider	Adjusts the signal to noise ratio. Range 0.10 - 100.00.
Distribution	
- Poisson	Imposes a Poisson distributed noise.
- Gauss	Imposes a Gauss distributed noise.

14.8.13.7 Create Image Subset

This method allows you to extract parts from one image and use these to create a new image. You can select these parts freely from the individual dimensions of the image.

14.8.13.7.1 Create Image Subset parameters

Defaults button



Resets all settings to the default values.

i Note

Each of the sections described below is only visible if the corresponding dimension is present in the input image.

14.8.13.7.1.1 Channels section

To show the section in full, click on the **arrow** button  .

In the **Channels** section you can select which channels of the input image you want to be used. All channels are selected by default. To deselect a channel, click on the relevant channel button.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

14.8.13.7.1.2 Z-Position section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Z-Stack dropdown list	You can select which sections of the input image you want to be used from the dropdown list.
- **All**	Selects all sections of the input image.
- **Select Individual**	Selects an individual section.
- **Select Range**	Selects a continuous range of sections.
- **Select Several**	Selects several continuous ranges and individual sections.
Select Individual slider	Enter the section that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last section that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more sections that you want to select in the input field. To do this, enter the first section, followed by a minus sign, and then the last section. If you want to define an interval, after the last section enter a colon and then the interval. The entry "2-10:2" means that every second section is selected from section 2 to section 10. Enter a comma after the first section if you want to define another section. You can also select individual sections separated by commas. By entering "2-10:2,14-18,20,23", you select every second section from section 2 to section 10, followed by sections 14 to 18, as well as sections 20 and 23.

14.8.13.7.1.3 Time section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Time Points dropdown list	You can select which time points of the input image you want to be used from the **Time Points** dropdown list. The following selection options are available:
- **All**	Selects all time points of the input image.
- **Select Individual**	Selects an individual time point.
- **Select Range**	Selects a continuous range of time points.
- **Select Several**	Selects several continuous ranges and individual time points.
Select Individual slider	Enter the time point that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last time point that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more time points that you want to select in the input field. To do this, enter the first time point, followed by a minus sign, and then the last time point. If you want to define an interval, after the last time point enter a colon and then the interval. The entry "2-10:2" means that every second time point is selected from time point 2 to time point 10. Enter a comma after the first time point if you want to define another time point. You can also select individual time points separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second time point from time point 2 to time point 10, followed by time points 14 to 18, as well as time points 20 and 23.

14.8.13.7.1.4 Scene section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Scene dropdown list	You can select which scenes of the input image you want to be used from the **Scene** dropdown list.
- **All**	Selects all scenes of the input image.
- **Select Individual**	Selects an individual scene.
- **Select Range**	Selects a continuous range of scenes.
- **Select Several**	Selects several continuous scenes and individual scenes.
Select Individual slider	Enter the scene that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last scene that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more scenes that you want to select in the input field. To do this, enter the first scene, followed by a minus sign, and then the last scene. If you want to define an interval, after the last scene enter a colon and then the interval. The entry "2-10:2" means that every second scene is selected from scene 2 to scene 10. Enter a comma after the first scene if you want to define another scene. You can also select individual scenes separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second scene from scene 2 to scene 10, followed by scenes 14 to 18, as well as scenes 20 and 23.

14.8.13.7.1.5 Block section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Block dropdown list	You can select which blocks of the input image you want to be used from the **Block** dropdown list. The following selection options are available:
- **All**	Selects all blocks of the input image.
- **Select Individual**	Selects an individual block.
- **Select Range**	Selects a continuous block of time points.
- **Select Several**	Selects several continuous blocks and individual blocks.
Select Individual slider	Enter the block that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last block that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more blocks that you want to select in the input field. To do this, enter the first block, followed by a minus sign, and then the last block. If you want to define an interval, after the last block enter a colon and then the interval. The entry "2-10:2" means that every second block is selected from block 2 to block 10. Enter a comma after the first block if you want to define another block. You can also select individual blocks separated by commas. By entering "2-10:2,14-18,20,23", you select every second block from block 2 to block 10, followed by blocks 14 to 18, as well as blocks 20 and 23.

14.8.13.7.1.6 Region section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Region dropdown list	Here you can select if you want to use the entire image or just a region (ROI) of the input image.
- **Full**	If selected this option, the full image is used for the new image.
- **Rectangle Region (ROI)**	If selected this option, you can draw in a rectangle region of interest which will be used for creating a new image. If a rectangle region was drawn in you can see and change its coordinates by editing the **X/Y/W/H** input fields.
Keep tiles checkbox	Has only an effect, if a region (ROI) is defined. **Activated:** Extracts the drawn in region including the complete tiles. This setting is recommend when you want to apply DCV processing functions on the resulting image.

14.8.13.8 Fuse Image Subset

This method allows you to insert an image subset back into the original image. Its contents are replaced by the contents of the image subset. Using this method you can process a previously created image subset using image processing functions and copy the result back into the original image.

14.8.13.9 Image Generator

This function creates a synthetic image where the dimensions can be defined.

Parameter	Description
Width	Width in x of the image
Height	Height in y of the image
Z Slices	Number of z slices of the image. If the value is > 1, it will become a Z-stack image.
Channels	number of channels of the image, if value is > 1, it will become a multi channel image
Time Slices	Number of time slices, if value is > 1, it will become a time series image.
Min. Gray Value	Minimum Gray Value for generation.
Max Gray Value	Maximum Gray Value for generation.

Parameter	Description
Pixel Type	Specifies the pixel type of the image.
Pattern	
- Uniform	all pixels of the image have identical Min. Gray Value
- 2D Gray Scale Vertical	the image shows a gray scale with values between **Min. Gray Value** and **Max. Gray Value** from top to bottom.
- 2D Gray Scale Horizontal	the image shows a gray scale with values between **Min. Gray Value** and **Max. Gray Value** from left to right.
- Ramp	the image shows a ramp with values between **Min. Gray Value** and **Max. Gray Value** starting from each corner of the image to the center.
- Gaussian	the image shows a Gaussian shaped grayscale with values between **Min. Gray Value** and **Max. Gray Value** starting from the borders of the image to the center.
- Checkerboard	the image shows a checkerboard where the “dark” fields have **Min. Gray Value** and the “bright” fields have **Max. Gray Value**.
- Cosine Checkerboard	the image shows a checkerboard where the “dark” fields have **Min. Gray Value** and the “bright” fields have **Max. Gray Value** overlaid with a cosine modulation.
- Chirp Cosine	the image shows a cosine pattern where the “dark” fields have **Min. Gray Value** and the “bright” fields have **Max. Gray Value** overlaid with a chirp modulation.
- Chirp Checker	the image shows a checkerboard where the “dark” fields have **Min. Gray Value** and the “bright” fields have **Max. Gray Value** overlaid with a chirp modulation.
- Random Spheres	a 3D (Z stack) image is created which contains **Number of Spheres** spheres with **Sphere Diameter** diameter which are randomly distributed in the image.
- Sphere Array	a 3D (Z stack) image is created which contains **Number of Spheres** spheres with **Sphere Diameter** diameter which are equally distributed in the image.
- Single Sphere	a 3D (Z stack) image is created which contains a single sphere with **Sphere Diameter** diameter which is positioned in the center of the image.
Sphere Diameter	Diameter of the created spheres.

Parameter	Description
Number of Spheres	Number of spheres which are generated in the 3D image.

14.8.13.10 Create Gray Scale image

This method allows you to create a gray scale image.

14.8.13.11 Image Calculator

This method allows you to apply arithmetic operations to images in the form of a calculator.

You can process a single image or combine two images.

All operations are performed pixel by pixel.

14.8.13.12 Add Channels

This method allows you to combine two input images that have different channels but otherwise have the same dimension (Z-stack, time series, tile, scene). An image is produced that contains all the channels of the input images.

If the two input images differ from one another in the dimensions Z-stack, time series, tiles or scene, input image 1 and input image 2 are copied into the output image as two separate blocks.

14.8.13.13 Generate Image Pyramid

This method allows you to calculate a resolution pyramid for an image. Using the resolution pyramid you can navigate extremely efficiently even in very large tile images and display individual regions in the image window.

Parameters

Option	Description
Background	This option will influence how the background of the image pyramid will look like.
– Auto	The region outside the scenes keeps the grey value of the image, i.e. white for brightfield images and black for fluorescence images.
– Black	The region outside the scenes will be displayed black.
– White	The region outside the scenes will be displayed white.

14.8.13.14 Split into HLS

This method generates the individual color extractions for a HLS input image. The resulting images for hue, lightness and saturation take the form of gray images.

14.8.13.15 Combine HLS

With this method a HLS image can be generated of the single color extractions H, L, S.

14.8.13.16 Split into RGB

This method generates the individual color extractions for red, green and blue from the RGB input image. The resulting images for red, green and blue take the form of gray images.

Parameters

Output Pixel type

Here you choose the desired output image format, e.g. 8 Bit B/W.

14.8.13.17 Combine RGB

With this method a color image can be generated of the single color extractions InputR, InputG, InputB.

Parameters

Output Pixel type

Here you choose the desired output image format, e.g. 8 Bit B/W.

14.8.13.18 Calculate Histogram

This method calculates a histogram distribution for selected measurement parameters of a measurement data table.

Parameter	Description
Columns	Defines the measurement parameters for classification. Clicking on the "…" button opens the **Select columns** dialog window. Here the column names of the data list are displayed. These can be selected or deselected by clicking on the relevant check box. It is also possible to enter the column numbers freely, e.g. 1,3,5, or 1-6 or 1,3-7,8.
Class Boundaries	Defines the mode used to divide up the classes, like >=, …,< or >,…,=<

Parameter		Description
-	Automatic Classification	The class boundaries are calculated according to the **Class Count**. Example: Minimum value is 0 Maximum value is 10000 Range is 10000 units Class Count is 4 Then the class boundaries are as follows: Class 1: 0 .. 2500 Class 2: 2501 .. 5000 Class 3 : 5001 .. 7500 Class 4: 7501 .. 10000
-	Logarithmic	The class boundaries are scaled logarithmic. Example: Minimum value is 0 Maximum value is 10000 Range is 10000 units Number of classes is 4 Then the class boundaries are as follows: Class 1: 0 .. 10 Class 2: 11 .. 100 Class 3: 101 .. 1000 Class 4: 1001 .. 10000
Class Count		Specifies the number of classes that shall be created.
Display Mode		
-	Count	Counts the number of values which belong to each class.
-	Count Cumulative	Cumulates the counts of values in each class, i.e. first class contains the number of values which belong to the first class, the second class contains the number of values of the first and the second class, etc..
-	Percentage	Calculates the percentage of the count of values in each class.
-	Percentage Cumulative	Cumulates the percentage of the count of values in each class.
-	Sum	Calculates the sum of the values of all data points which belong to the class.

Parameter		Description
-	Sum Cumulative	Cumulates the sums of the values in each class.
-	Percentage Sum	Lists the percentage of the sums of values of all data points which belong to the class.
-	Percentage Sum Cumulative	Cumulates the percentage of the sums of values of all data points which belong to the class.

14.8.13.19 Split Scenes (Write files)

This method saves the single scenes (tiles or positions) of a multiscene image (i.e. image of a multiwell plate) as single images in a folder in **CZI** format.

Parameter

Display field

To open the **Browse for folder** dialog window, click on the  button to the right of the display field. Here you can select the folder to which you want to save the single scene images. The path of the destination folder is displayed automatically in the display field.

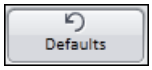
Include Scene Information checkbox

Activated: Includes the scene information in the file name of the separate image.

Compression dropdown list

Choose the type of compression here, eg **None**, **JPEG** or **JPEG XR**.

Defaults button



Resets all settings to the default values.

14.8.13.20 Split Scenes

This method separates scenes from a tiles or positions image. The individual images are displayed in the **Center Screen Area**. Note that the images in this method, in contrast to the method **Split Scenes (write files)**, are not automatically stored in a folder.

14.8.14 Export/Import group

14.8.14.1 Image Export

Using this method you can export single images into various file types so that you can continue to use them in other programs. Multidimensional images (multichannel, Z-stack, time lapse, tile images) are exported as individual images.

14.8.14.1.1 Image Export parameters

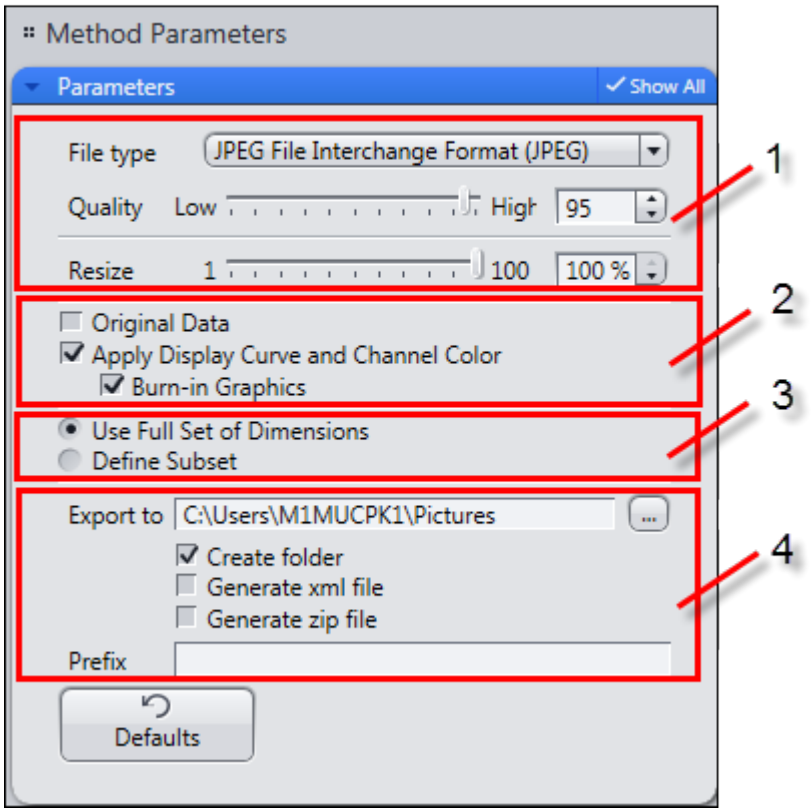


Fig. 93: Parameter picture export

No.	Description
1	Here you define the settings for the file type, eg Format or compression. An exact description of the functions, see the *File Type section* [▶ 706]. Depending on the file type you selected for the image export, the settings of the other parameters change
2	Here you define, how to deal with the image data during the export, eg whether you want to burn graphics firmly in the picture. An exact description of the functions, see *image data section* [▶ 708].

No.	Description
3	Specify here how to proceed with the dimensions, eg whether you want to export all the dimensions of an image, or only certain. An exact description of the functions, see dimensions section.
4	Insert here other export settings, eg the export folder. An exact description of the functions can be found by *export to section* [▶ 714]

14.8.14.1.1.1 File Type section

Name	Function
File Type dropdown list	Select the desired file type from the dropdown list: ■ **JPEG** (File Interchange Format) ■ **BMP** (Windows Bitmap) ■ **TIFF** (Tagged Image File Format) ■ **Big TIFF** (64 bit) ■ **PNG** (Portable Network Graphics) ■ **JPEG XR** (JPEG extended range) **Notice!** Various options are available depending on the file type you have selected.
Quality slider	Only available for the file types **JPEG** and **JPEG XR**. Enter the image quality using the slider or spin box/input field to influence the size of the file. Although low values result in very small files, image quality may be considerably reduced.
Resize slider	Adjust the image size in percent using the slider or spin box/input field.
Convert to 8 Bit checkbox	Only available for the file types **TIFF**, **BigTIFF**, **PNG** and **JPEG XR**. **Activated:** Converts a 16 bit gray level image into an 8 bit gray level image, or a 48 bit color image into a 24 bit color image.

Name	Function
Compression dropdown list	Only available for the file type **TIFF** and **BigTIFF**. Select the compression method for reducing the data volume from the dropdown list: ■ **LZW:** Performs lossless compression in accordance with the Lempel-Ziv-Welch algorithm (LZW). Only available for the file type **TIFF.** ■ **None:** Retains the data volume of the original image. No compression is performed. ■ **ZIP**: Performs lossless compression in accordance with the ZIP method. Only available for the file type **TIFF.** ■ **Loss less**: Performs lossless compression in accordance with the Lempel-Ziv-Welch algorithm (LZW). Only available for the file type **BigTIFF.** ■ **Lossy**: Performs lossy compression in accordance with the JPEG XR (extended range) method. Only available for the file type **BigTIFF.**
BigTIFF checkbox	Only available for the file type **BigTIFF**. **Activated:** Generates a BigTIFF image. The maximum image size is larger than 4GByte. **Deactivated:** Generates a TIFF image with maximum size of 4GByte.
TIFF Tiles checkbox	Only available for the file type **BigTIFF**. **Activated:** Generates new rectangle tiles for internal data handling. **Deactivated:** Combines tiles as stripes for internal data handling..
Pyramid checkbox	Only available for the file type **BigTIFF**. **Activated**: Calculates an image pyramid.
Merge All Scenes checkbox	Only available for the file type **BigTIFF**. **Activated:** Generates one image including all scenes. Single scene images will be generated, if the checkbox is deactivated.

Name	Function
Shift Pixel checkbox	Only available for the file type **BigTIFF**. **Activated:** Shifts the grey value of a 10-bit or 12-bit image to 16-bit

14.8.14.1.1.2 Image Data section

The following functions are only visible if the **Show All** mode is activated:

Original Data checkbox

Activated: Exports the image with the original channel colors and the original display characteristic curve.

Apply Display Characteristic Curve and Channel Color checkbox

Activated: Exports the image with the changed channel color and display characteristic curve settings. These settings are applied to the pixel values of the exported images. They are particularly important if you want to use dark images with a dynamic range of more than 8 bits in other programs.

i Note

At least one of the two checkboxes must be activated. If both checkboxes are activated, you can export the original images and the images with the changed settings in a single step.

Burn In Graphic checkbox

Activated: Burns the graphic elements into the image. The pixels under the graphic element (e.g. scale bars) are overwritten. The burnt-in graphic elements cannot be subsequently modified.

Merged channels image checkbox

Only visible for multichannel images.

Activated: Exports the pseudo color image of all selected channels.

Individual Channels image checkbox

Only visible for multichannel images.

Activated: Exports the individual colored image of all selected channels.

> **i Note**
>
> At least one of the three checkboxes must be activated. If the **Merged channels Image** and **Individual Channel image** checkboxes are activated, you can export the individual colored images and the pseudo color images in a single step.

Use channel names

Only visible for multi-channel images.

Activated: Integrates the channel name in the name of the exported image.

14.8.14.1.1.3 Dimensions section

The following functions are only visible if the **Show All** mode is activated:

Use Full Set of Dimensions radio button

Select this option if you want to export all dimensions without changing them.

Define Subset radio button

Select this option if you only want to export individual dimensions or subsets of individual dimensions.

> **i Note**
>
> Each of the sections described below is only visible if the corresponding dimension is present in the input image.

14.8.14.1.1.3.1 Channels section

To show the section in full, click on the **arrow** button  .

In the **Channels** section you can select which channels of the input image you want to be used. All channels are selected by default. To deselect a channel, click on the relevant channel button.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

14.8.14.1.1.3.2 Z-Position section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Z-Stack dropdown list	You can select which sections of the input image you want to be used from the dropdown list.
- **All**	Selects all sections of the input image.
- **Select Individual**	Selects an individual section.
- **Select Range**	Selects a continuous range of sections.
- **Select Several**	Selects several continuous ranges and individual sections.
Select Individual slider	Enter the section that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last section that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more sections that you want to select in the input field. To do this, enter the first section, followed by a minus sign, and then the last section. If you want to define an interval, after the last section enter a colon and then the interval. The entry "2-10:2" means that every second section is selected from section 2 to section 10. Enter a comma after the first section if you want to define another section. You can also select individual sections separated by commas. By entering "2-10:2,14-18,20,23", you select every second section from section 2 to section 10, followed by sections 14 to 18, as well as sections 20 and 23.

14.8.14.1.1.3.3 Time section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Time Points dropdown list	You can select which time points of the input image you want to be used from the **Time Points** dropdown list. The following selection options are available:
- **All**	Selects all time points of the input image.
- **Select Individual**	Selects an individual time point.
- **Select Range**	Selects a continuous range of time points.
- **Select Several**	Selects several continuous ranges and individual time points.
Select Individual slider	Enter the time point that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last time point that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more time points that you want to select in the input field. To do this, enter the first time point, followed by a minus sign, and then the last time point. If you want to define an interval, after the last time point enter a colon and then the interval. The entry "2-10:2" means that every second time point is selected from time point 2 to time point 10. Enter a comma after the first time point if you want to define another time point. You can also select individual time points separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second time point from time point 2 to time point 10, followed by time points 14 to 18, as well as time points 20 and 23.

14.8.14.1.1.3.4 Scene section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Scene dropdown list	You can select which scenes of the input image you want to be used from the **Scene** dropdown list.
- **All**	Selects all scenes of the input image.
- **Select Individual**	Selects an individual scene.
- **Select Range**	Selects a continuous range of scenes.
- **Select Several**	Selects several continuous scenes and individual scenes.
Select Individual slider	Enter the scene that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last scene that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more scenes that you want to select in the input field. To do this, enter the first scene, followed by a minus sign, and then the last scene. If you want to define an interval, after the last scene enter a colon and then the interval. The entry "2-10:2" means that every second scene is selected from scene 2 to scene 10. Enter a comma after the first scene if you want to define another scene. You can also select individual scenes separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second scene from scene 2 to scene 10, followed by scenes 14 to 18, as well as scenes 20 and 23.

14.8.14.1.1.3.5 Region section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Region dropdown list	Here you can select if you want to use the entire image or just a region (ROI) of the input image.
- **Full**	If selected this option, the full image is used for the new image.
- **Rectangle Region (ROI)**	If selected this option, you can draw in a rectangle region of interest which will be used for creating a new image. If a rectangle region was drawn in you can see and change its coordinates by editing the **X/Y/W/H** input fields.
Keep tiles checkbox	Has only an effect, if a region (ROI) is defined. **Activated:** Extracts the drawn in region including the complete tiles. This setting is recommend when you want to apply DCV processing functions on the resulting image.

14.8.14.1.1.3.6 Tiles section

To show the section in full, click on the **arrow** button  .

Use Existing Tiles radio button

Activated: Exports the image with an unchanged number of tiles and unchanged overlap.

New Tiles radio button

Configure the column, row and overlap settings using the spin boxes/input fields if you want to change the number of tiles and the overlap.

Columns spin box/input field

Only active if the **New Tiles** radio button is activated.

Enter the number of columns using the spin box/input field.

Rows spin box/input field

Only active if the **New Tiles** radio button is activated.

Enter the number of rows using the spin box/input field.

Overlap spin box/input field

Only active if the **New Tiles** radio button is activated.

Enter the percentage for the overlap using the spin box/input field.

14.8.14.1.1.4 Export to section

To open the **Browse For Folder** dialog window, click on the  button to the right of the display field. Here you can select the folder to which you want to save the image that is being exported. The path of the export folder is displayed automatically in the display field.

The following functions are only visible if the **Show All** mode is activated:

Create Subfolder checkbox

Activated: Creates a separate folder with the name of the input image.

Create XML Files checkbox

Activated: Creates two XML files containing the meta information relating to the input image.

- **Meta.xml** contains all additional information relating to the input image (e.g. hardware settings, dimensions).
- **Info.xml** only contains additional information relating to the exported individual images (e.g. names, dimensions, sizes).

Create ZIP Archive checkbox

Activated: Creates a ZIP file in which all exported individual images, including the XML files, are saved.

Prefix input field

Here you can edit the prefix specified or enter a new name. The name of the original image is specified by default.

14.8.14.2 Movie Export

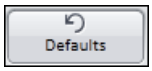
Using this function you can export multidimensional images (e.g. Time Series or Z-Stack images) into various file types in the form of film sequences so that you can continue to use them in other programs.

i Note

If you want to export **MOV** files (H264 or MPEG4 codec) successfully, download the application **FFmpeg** (e.g. on http://www.ffmpeg.org/ oder hier http://ffmpeg.zeranoe.com/blog/?p=178). Copy **ffmpeg.exe** in to the same folder where **ZEN.exe** is located.

14.8.14.2.1 Movie Export parameters

Defaults button



Resets all settings to the default values.

14.8.14.2.1.1 File Type section

i Note

AVI (MS-Video1) mode is available for 32-bit Windows operating systems only.

Parameter	Description
Mode dropdown list	Select the desired file type from the dropdown list. Various setting options are available to you depending on the file type selected (*.avi,*.wmf).
Format dropdown list	Select the desired format from the dropdown list.
- **Original Size**	Not available for the file type AVI (DV). Uses the height and width of the input image and sets the frame rate to 5 frames per second.
- **User-Defined**	Not available for the file type AVI (DV). Enter the values in the Width, Height and Frame Rate input fields.
- **720x576/25 fps (PAL 576p/25)**	Uses the PAL (Phase Alternating Line) video resolution with 25 frames per second.
- **720x480/29 .97fps (NTSC)**	Uses the NTSC (National Television Systems Committee) video resolution with 29.97 frames per second.
- **1280x720/5 0fps (HD 720p/50)**	Uses the HD (High Definition 720) video resolution with 25 frames per second. Not available for the file types AVI (DV) and AVI (MS-Video 1).
- **1920x1080/ 25fps (HD 1080p/25)**	Uses the HD (High Definition 1080) video resolution with 25 frames per second. Not available for the file types AVI (DV) and AVI (MS-Video 1).

Parameter	Description
- **1920x1080/ 29.97fps (HD 1080p/29.97)**	Uses the HD (High Definition 1080) video resolution with 29.97 frames per second. Not available for the file types AVI (DV) and AVI (MS-Video 1).
Width input field	Only active if you have selected the **User-Defined** entry in the **Format** dropdown list. Here you can indicate the width of the image in pixels (px).
Height input field	Only active if you have selected the **User-Defined** entry in the **Format** dropdown list. Here you can indicate the height of the image in pixels (px).
Frame Rate input field	Only active if you have selected the **User-Defined** entry in the **Format** dropdown list. Here you can enter the frame rate in frames per second (fps).
Quality slider	Enter the image quality using the slider or spin box/input field to influence the size of the file. Although low values result in very small files, image quality may be considerably reduced. Only visible if you have selected the **AVI (M-JPEG compression)** entry in the **File Type** dropdown list.

14.8.14.2.1.2 Image Data section

The following functions are only visible if the **Show All** mode is activated:

Burn In Graphic checkbox

Activated: Burns the graphic elements into the image. The pixels under the graphic element (e.g. scale bars) are overwritten. The burnt-in graphic elements cannot be subsequently modified.

Merged channels image checkbox

Only visible for multichannel images.

Activated: Exports the pseudo color image of all selected channels.

Individual Channels image checkbox

Only visible for multichannel images.

Activated: Exports the individual colored image of all selected channels.

i Note

At least one of the three checkboxes must be activated. If the **Merged channels Image** and **Individual Channel image** checkboxes are activated, you can export the individual colored images and the pseudo color images in a single step.

14.8.14.2.1.3 Fitting section

The following functions are only visible if the **Show All** mode is activated:

Parameter	Description
Fitting dropdown list	Select the desired type of fitting from the dropdown list.
- **Fit All (Uniform)**	Fits the image to the selected resolution. The original aspect ratio is retained.
- **Fit and Crop (Uniform to Fill)**	Fits the image to the selected resolution and clips it. The original aspect ratio is not retained.
- **Fit and Stretch (Fill)**	Stretches the image to the selected resolution. The original aspect ratio is not retained.
- **Crop (None)**	Crops the image to the selected resolution. The original aspect ratio is retained.

14.8.14.2.1.4 Sequence section

The following functions are only visible if the **Show All** mode is activated:

Change the sequence of the dimensions in which you want the movie to be created.

 **button**

Shifts the selected dimension up a line.

 **button**

Shifts the selected dimension down a line.

14.8.14.2.1.5 Mapping section

Parameter	Description
Mapping dropdown list	Select how you want the images to be assigned.

Parameter	Description
- **Fixed Duration**	Enter the time per image in seconds using the spin box/ input field. The total length is displayed in the **Final Movie Length** display field.
- **1 Frame per Image**	Assigns one frame per image.
Image count display field	Indicates the number of images in the input image.
Final Movie Length display field	Indicates the total length of the resulting movie, depending on the selected image sequence and the time.

14.8.14.2.1.6 Dimensions section

The following functions are only visible if the **Show All** mode is activated:

Use Full Set of Dimensions radio button

Select this option if you want to export all dimensions without changing them.

Define Subset radio button

Select this option if you only want to export individual dimensions or subsets of individual dimensions.

i Note

Each of the sections described below is only visible if the corresponding dimension is present in the input image.

14.8.14.2.1.6.1 Channels section

To show the section in full, click on the **arrow** button  .

In the **Channels** section you can select which channels of the input image you want to be used. All channels are selected by default. To deselect a channel, click on the relevant channel button.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

14.8.14.2.1.6.2 Z-Position section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Z-Stack dropdown list	You can select which sections of the input image you want to be used from the dropdown list.
- **All**	Selects all sections of the input image.
- **Select Individual**	Selects an individual section.
- **Select Range**	Selects a continuous range of sections.
- **Select Several**	Selects several continuous ranges and individual sections.
Select Individual slider	Enter the section that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last section that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more sections that you want to select in the input field. To do this, enter the first section, followed by a minus sign, and then the last section. If you want to define an interval, after the last section enter a colon and then the interval. The entry "2-10:2" means that every second section is selected from section 2 to section 10. Enter a comma after the first section if you want to define another section. You can also select individual sections separated by commas. By entering "2-10:2,14-18,20,23", you select every second section from section 2 to section 10, followed by sections 14 to 18, as well as sections 20 and 23.

14.8.14.2.1.6.3 Time section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Time Points dropdown list	You can select which time points of the input image you want to be used from the **Time Points** dropdown list. The following selection options are available:
- **All**	Selects all time points of the input image.
- **Select Individual**	Selects an individual time point.
- **Select Range**	Selects a continuous range of time points.
- **Select Several**	Selects several continuous ranges and individual time points.
Select Individual slider	Enter the time point that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last time point that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more time points that you want to select in the input field. To do this, enter the first time point, followed by a minus sign, and then the last time point. If you want to define an interval, after the last time point enter a colon and then the interval. The entry "2-10:2" means that every second time point is selected from time point 2 to time point 10. Enter a comma after the first time point if you want to define another time point. You can also select individual time points separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second time point from time point 2 to time point 10, followed by time points 14 to 18, as well as time points 20 and 23.

14.8.14.2.1.6.4 Scene section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Scene dropdown list	You can select which scenes of the input image you want to be used from the **Scene** dropdown list.
- **All**	Selects all scenes of the input image.
- **Select Individual**	Selects an individual scene.
- **Select Range**	Selects a continuous range of scenes.
- **Select Several**	Selects several continuous scenes and individual scenes.
Select Individual slider	Enter the scene that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last scene that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more scenes that you want to select in the input field. To do this, enter the first scene, followed by a minus sign, and then the last scene. If you want to define an interval, after the last scene enter a colon and then the interval. The entry "2-10:2" means that every second scene is selected from scene 2 to scene 10. Enter a comma after the first scene if you want to define another scene. You can also select individual scenes separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second scene from scene 2 to scene 10, followed by scenes 14 to 18, as well as scenes 20 and 23.

14.8.14.2.1.6.5 Region section

To show the section in full, click on the **arrow** button .

Parameter	Description
Region dropdown list	Here you can select if you want to use the entire image or just a region (ROI) of the input image.
- **Full**	If selected this option, the full image is used for the new image.
- **Rectangle Region (ROI)**	If selected this option, you can draw in a rectangle region of interest which will be used for creating a new image. If a rectangle region was drawn in you can see and change its coordinates by editing the **X/Y/W/H** input fields.
Keep tiles checkbox	Has only an effect, if a region (ROI) is defined. **Activated:** Extracts the drawn in region including the complete tiles. This setting is recommend when you want to apply DCV processing functions on the resulting image.

14.8.14.2.1.7 Export to section

To open the **Browse For Folder** dialog window, click on the button to the right of the display field. Here you can select the folder to which you want to save the image that is being exported. The path of the export folder is displayed automatically in the display field.

Prefix input field

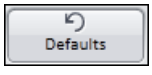
Here you can edit the prefix specified or enter a new name. The name of the original image is specified by default.

14.8.14.3 OME TIFF Export

Using the **OME TIFF Export** function you can export your images into OME (Open Microscopy Environment) TIFF format so that you can continue to use them in other programs. The images are then available as a multipage TIFF file.

14.8.14.3.1 OME TIFF Export parameters

Defaults button



Resets all settings to the default values.

14.8.14.3.1.1 Image Format section

Size slider

Enter the image size in percent using the slider or spin box/input field.

BigTIFF checkbox

Only visible if the **Show All** mode is activated.

Activated: Creates a BigTIFF image that can be bigger than 4 gigabytes and uses 64-bit offset format.

14.8.14.3.1.2 Image Data section

The following functions are only visible if the **Show All** mode is activated:

Original Data radio button

Activated: Exports the image with the original channel colors and the original display characteristic curve.

Apply Display Characteristic Curve and Channel Color radio button

Activated: Exports the image with the changed channel color and display characteristic curve settings. These settings are applied to the pixel values of the exported images. They are particularly important if you want to use dark images with a dynamic range of more than 8 bits in other programs.

Burn In Graphic checkbox

Activated: Burns the graphic elements into the image. The pixels under the graphic element (e.g. scale bars) are overwritten. The burnt-in graphic elements cannot be subsequently modified.

14.8.14.3.1.3 Dimensions section

The following functions are only visible if the **Show All** mode is activated:

Use Full Set of Dimensions radio button

Select this option if you want to export all dimensions without changing them.

Define Subset radio button

Select this option if you only want to export individual dimensions or subsets of individual dimensions.

i Note

Each of the sections described below is only visible if the corresponding dimension is present in the input image.

14.8.14.3.1.3.1 Channels section

To show the section in full, click on the **arrow** button .

In the **Channels** section you can select which channels of the input image you want to be used. All channels are selected by default. To deselect a channel, click on the relevant channel button.

button

Adopts the values from the current display. These values can also be found on the Dimensions tab.

14.8.14.3.1.3.2 Z-Position section

To show the section in full, click on the **arrow** button .

Parameter	Description
Z-Stack dropdown list	You can select which sections of the input image you want to be used from the dropdown list.
- **All**	Selects all sections of the input image.
- **Select Individual**	Selects an individual section.
- **Select Range**	Selects a continuous range of sections.
- **Select Several**	Selects several continuous ranges and individual sections.
Select Individual slider	Enter the section that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last section that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Parameter	Description
Select Several input field	Enter one or more sections that you want to select in the input field. To do this, enter the first section, followed by a minus sign, and then the last section. If you want to define an interval, after the last section enter a colon and then the interval. The entry "2-10:2" means that every second section is selected from section 2 to section 10. Enter a comma after the first section if you want to define another section. You can also select individual sections separated by commas. By entering "2-10:2,14-18,20,23", you select every second section from section 2 to section 10, followed by sections 14 to 18, as well as sections 20 and 23.

14.8.14.3.1.3.3 Time section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Time Points dropdown list	You can select which time points of the input image you want to be used from the **Time Points** dropdown list. The following selection options are available:
- **All**	Selects all time points of the input image.
- **Select Individual**	Selects an individual time point.
- **Select Range**	Selects a continuous range of time points.
- **Select Several**	Selects several continuous ranges and individual time points.
Select Individual slider	Enter the time point that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last time point that you want to select using the sliders or spin boxes/input fields.
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.

Parameter	Description
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more time points that you want to select in the input field. To do this, enter the first time point, followed by a minus sign, and then the last time point. If you want to define an interval, after the last time point enter a colon and then the interval. The entry "2-10:2" means that every second time point is selected from time point 2 to time point 10. Enter a comma after the first time point if you want to define another time point. You can also select individual time points separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second time point from time point 2 to time point 10, followed by time points 14 to 18, as well as time points 20 and 23.

14.8.14.3.1.3.4 Scene section

To show the section in full, click on the **arrow** button  .

Parameter	Description
Scene dropdown list	You can select which scenes of the input image you want to be used from the **Scene** dropdown list.
- **All**	Selects all scenes of the input image.
- **Select Individual**	Selects an individual scene.
- **Select Range**	Selects a continuous range of scenes.
- **Select Several**	Selects several continuous scenes and individual scenes.
Select Individual slider	Enter the scene that you want to select using the slider or spin box/input field.
Adopt button	Adopts the values from the current display. These values can also be found on the Dimensions tab.
Select Range slider	Enter the first and last scene that you want to select using the sliders or spin boxes/input fields.

Parameter	Description
Interval checkbox	**Activated**: Interval mode is active. The Interval spin box/ input field appears.
Interval spin input field	Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.
Select Several input field	Enter one or more scenes that you want to select in the input field. To do this, enter the first scene, followed by a minus sign, and then the last scene. If you want to define an interval, after the last scene enter a colon and then the interval. The entry "2-10:2" means that every second scene is selected from scene 2 to scene 10. Enter a comma after the first scene if you want to define another scene. You can also select individual scenes separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second scene from scene 2 to scene 10, followed by scenes 14 to 18, as well as scenes 20 and 23.

14.8.14.3.1.3.5 Region section

To show the section in full, click on the **arrow** button .

Parameter	Description
Region dropdown list	Here you can select if you want to use the entire image or just a region (ROI) of the input image.
- **Full**	If selected this option, the full image is used for the new image.
- **Rectangle Region (ROI)**	If selected this option, you can draw in a rectangle region of interest which will be used for creating a new image. If a rectangle region was drawn in you can see and change its coordinates by editing the **X/Y/W/H** input fields.
Keep tiles checkbox	Has only an effect, if a region (ROI) is defined. **Activated:** Extracts the drawn in region including the complete tiles. This setting is recommend when you want to apply DCV processing functions on the resulting image.

14.8.14.3.1.4 Export to section

To open the **Browse For Folder** dialog window, click on the button to the right of the display field. Here you can select the folder to which you want to save the image that is being exported. The path of the export folder is displayed automatically in the display field.

14.8.14.4 ZVI Export

Using the **ZVI Export** function you can export your images into ZVI format so that you can continue to use them in AxioVision.

14.8.14.4.1 ZVI Export parameters

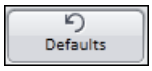
Export to display field

To open the **Browse For Folder** dialog window, click on the  button to the right of the display field. Here you can select the folder to which you want to save the image that is being exported. The path of the export folder is displayed automatically in the display field.

Prefix input field

Here you can edit the prefix specified or enter a new name. The name of the original image is specified by default.

Defaults button



Resets all settings to the default values.

14.8.14.5 Image Import

Using the **Image Import** function you can create a multidimensional image (multichannel, Z-stack, time lapse, tile, position image) from individual images. The individual images may be in any of the external formats supported by ZEN (e.g. .bmp, .jpg, .tiff). The resulting image can then be saved in CZI format and processed further using the functions available in ZEN.

Supported file types

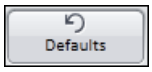
- JPG
- BMP
- TIFF
- PNG
- GIF
- DeltaVision-Bilder
- MetaFluor-Bilder
- Multipage-TIFF

14.8.14.5.1 Image Import Parameters

In the **Image Import Parameters** tool you can enter all the settings you need to import multidimensional images. To select the desired dimension, activate the checkbox in front of the relevant **Section**. At least one dimension must be selected. You can enter the settings for each dimension in the individual **Sections**.

You will find the settings for the import mode, import folder and for identifying your import images at the bottom of the tool.

Defaults button



Resets all settings to the default values.

14.8.14.5.1.1 Multichannel Image section

In the **Multichannel Image** section you can enter all the settings you need to import multichannel images.

Multichannel Image checkbox

Activates the settings to import multichannel images. The **C** dimension (for multichannel images) is displayed automatically in the list in the **Specify Name** section.

No. column

Displays the channel number.

Dye column

Click on the dropdown list to select a dye. If the desired dye is not available, select the **Empty** entry.

Color column

Displays the pseudo color for the dye. To change the color, click on the color field. Select the desired color from the list.

Name column

Here you can enter a name for the dye. If you have selected a dye from the dropdown list, the name of the dye will appear automatically in the input field.

 **button**

Shifts the selected channel down a line.

button

Shifts the selected channel up a line.

button

Adds a new channel.

button

Deletes the selected channel.

button

Opens the **Options** shortcut menu.

- **Add**
  Adds a new channel at the end of the list.
- **Copy**
  Copies the selected channel.
- **Reset Color**
  Resets the changed color of the selected channel to the default setting.
- **Delete**
  Deletes the selected channel.
- **Delete All**
  Deletes all channels and resets the first channel to the default settings.

Use Channel Name as Name checkbox

Only visible if the **Show All** mode is activated.

Uses the name specified in the **Name** column to identify the channel. The channel name will appear in the **Preview** display field in the **Specify Name** section.This deactivates the **Name**, **Start Index** and **Increment** columns in the list.

14.8.14.5.1.2 Z-Stack section

In the **Z-Stack** section you can enter all the settings you need to import Z-stack images.

Z-Stack checkbox

Activates the settings to import Z-stack images. The **Z** dimension (for Z-stacks) is displayed automatically in the list in the **Specify Name** section.

Interval spin box/input field

Here you can enter the value in µm for the distance between the individual Z-sections. The total height of the Z-stack is calculated automatically from this value and the number of sections.

Sections spin box/input field

Here you can enter the number of sections.

The following functions are only visible if the **Show All** mode is activated:

Interval radio button

Activated: The **Interval** spin box/input field and the **Range** display field are visible.

Here you can enter the value in µm for the distance between the individual Z-sections. The total height of the Z-stack is calculated automatically from this value and the number of sections and displayed in the **Range** display field.

Range radio button

Activated: The **Range** spin box/input field and the **Interval** display field are visible.

Here you can enter the total height of the Z-stack in µm. The distance between the individual Z-stacks is calculated automatically from this value and the number of sections and displayed in the **Interval** display field.

Advanced Parameters checkbox

Activates additional parameters that are necessary for further processing of the imported image (e.g. for deconvolution).

Magnification dropdown list

Select the objective magnification that was used for acquisition from the dropdown list.

Immersion dropdown list

Select the immersion medium that was used for acquisition from the dropdown list.

Aperture spin box/input field

Here you can enter the value of the numerical aperture of the objective that was used for acquisition.

14.8.14.5.1.3 Time Series section

In the **Time Series** section you can enter all the settings you need to import time lapse images.

Time Series checkbox

Activates the settings to import time lapse images. The **T** dimension (for time series) is displayed automatically in the list in the **Specify Name** section.

Interval spin box/input field

Here you can enter the value for the **Interval** between the individual time points. Select the unit of time from the dropdown list to the right of the spin box/input field.

Time Points spin box/input field

Here you can enter the number of time points.

The duration of the entire time series is calculated automatically from the interval and time points values.

The following functions are only visible if the **Show All** mode is activated:

Interval radio button

Activated: The **Interval** and **Time Points** spin boxes/input fields and the **Duration** display field are visible.

Here you can enter the value for the interval between the individual time points. Select the unit of time from the dropdown list to the right of the spin box/input field. Enter the number of time points in the **Time Points** spin box/input field.

The duration of the entire time series is calculated automatically from the interval and time points values and displayed in the **Duration** display field.

Duration radio button

Activated: The **Duration and Time Points** spin boxes/input fields and the **Interval** display field are visible.

Here you can enter the value for the duration of the entire time series. Select the unit of time from the dropdown list to the right of the spin box/input field. Enter the number of time points in the **Time Points** spin box/input field.

The interval between the individual time points is calculated automatically from the duration and time points values and displayed in the **Interval** display field.

14.8.14.5.1.4 Tiles section

Tiles checkbox

Activates the settings to import tile images. The **M** dimension (for MosaiX/tiles) is displayed automatically in the list in the **Specify Name** section.

Columns spin box/input field

Here you can enter the number of columns of the tile image.

Overlap spin box/input field

Here you can enter the percentage by which the tiles overlap.

Rows spin box/input field

Here you can enter the number of rows of the tile image.

Meander radio button

Select this option if the images to be imported were acquired in the Meander acquisition/travel mode.

Comb radio button

Select this option if the images to be imported were acquired in the Comb acquisition/travel mode.

14.8.14.5.1.5 Positions section

In the **Positions** section you can enter all the settings you need to import scenes.

Positions checkbox

Activates the settings to import images of individual scenes/motorized stage positions. The **S** dimension (for Scene) is displayed automatically in the **Specify Name** field.

No. column

Displays the position number.

X column

Enter the X coordinate of the position in the spin box/input field.

Y column

Enter the Y coordinate of the position in the spin box/input field.

button

Shifts the selected position down a line.

button

Shifts the selected position up a line.

button

Adds a new position.

button

Deletes the selected position.

button

Opens the **Options** shortcut menu.

- **Add**
  Adds a new position.
- **Delete**
  Deletes the selected position.
- **Delete All**
  Deletes all positions with the exception of the first position.

14.8.14.5.1.6 Scaling section

To show the section in full, click on the **arrow** button  .

Lateral Scaling (X) spin box/input field

Here you can enter the geometric scaling value for the X dimension of the image to be imported. Select the unit for the scaling value from the dropdown list to the right of the spin box/input field.

The following functions are only visible if the **Show All** mode is activated:

Define button

Enter the desired values in the **Lateral Scaling (X)** spin box/input field and in the **Lateral Scaling (Y)** spin box/input field. Select the unit for the scaling value from the dropdown list to the right of each spin box/input field.

Use Current button

Uses the geometric scaling currently selected and displays the values for **Lateral Scaling (X)** and **Lateral Scaling (Y)** with the corresponding unit in the relevant display field.

14.8.14.5.1.7 Import method section

Automatic button

Allows you to import all the images that are available in an import folder automatically.

Sequential button

Allows you to import an image sequence in a certain order (e.g. image dimensions that are encoded by means of numbers in the image name).

Import From display field

Displays the path of the import folder. To select a folder, click on the folder button  to the right of the display field. The names of the images are displayed in the **File Name** list below the display field.

Folder radio button

Only visible if the **Sequential** button is activated.

To select a folder, click on the folder button  to the right of the display field. The names of the images are displayed in the **File Name** list below the display field.

Multipage TIFF Image radio button

Only visible if the **Sequential** button is activated.

To select a multipage TIFF image, click on the folder button  to the right of the display field. The names of the images are displayed in the **File Name** list below the display field.

14.8.14.5.1.8 Specify Name section

In the **Specify Name** section you can enter all the settings you need to identify your images.

Prefix input field

Not available if the **Sequential** button is activated and the **Multipage TIFF Image** radio button has been selected.

Here you can edit the prefix specified or enter a new name. The name of the original image is specified by default.

Suffix input field

Not available if the **Sequential** button is activated and the **Multipage TIFF Image** radio button has been selected.

Here you have the option of entering a suffix.

Name column

In the input field you can enter the name of the relevant dimension from the original image name. A capital letter is displayed by default for the image dimension selected in each case (e.g. **C** for Channel, **T** for Time).

Start Index column

Enter the number of the first image in the spin box/input field.

End Index column

Only available if the **Sequential** button is activated.

Enter the number of the last image in the spin box/input field.

Increment column

Enter the increment in the spin box/input field. This allows you to specify whether you want every image or every xth image to be imported.

Preview display field

Shows the full name of the image (prefix, dimensions, suffix).

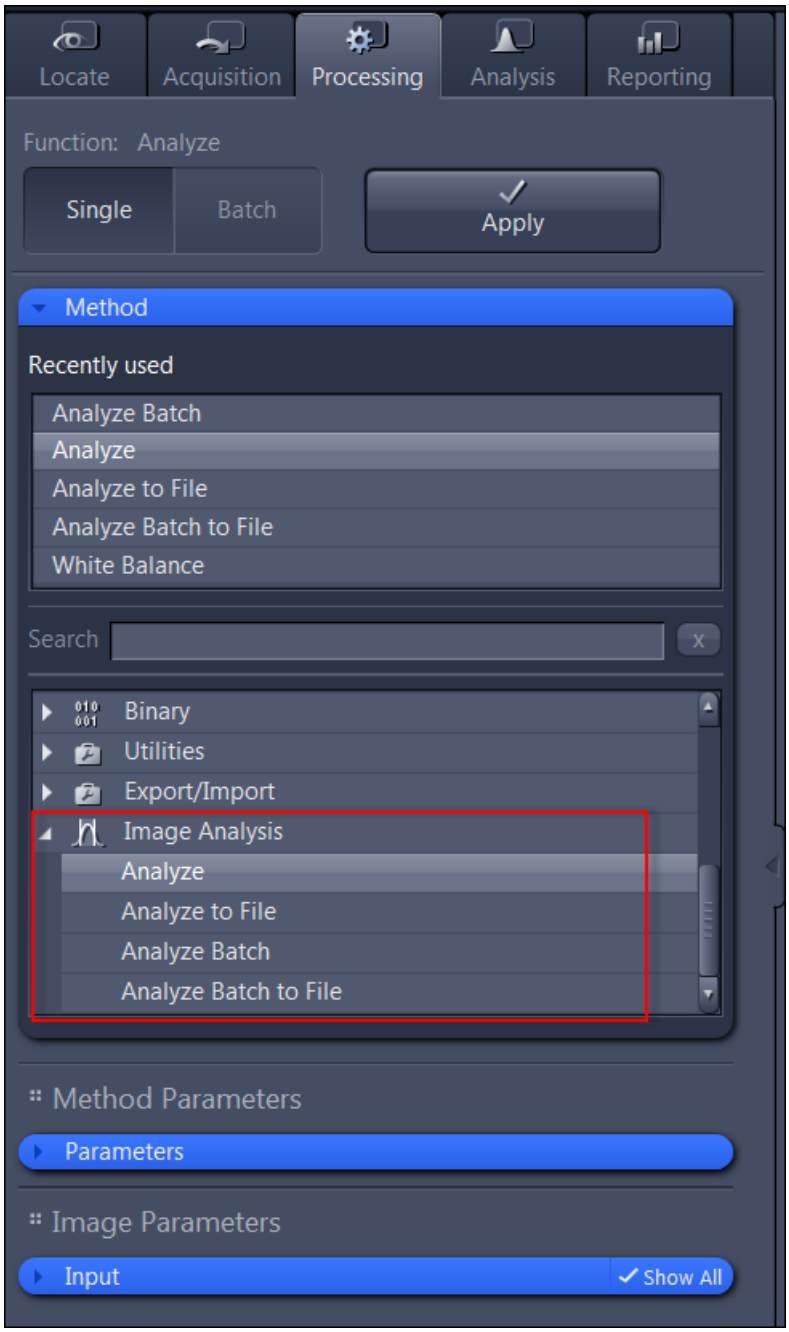
Check Consistency button

Not available if the **Sequential** button is activated and the **Multipage TIFF Image** radio button has been selected.

Checks the consistency of the selected settings and marks these in the **File Name** list.

14.8.15 Group Image Analysis

The Group **Image Analysis** provides four different options to analyze images in single or batch mode. The measurement data can be embedded in the image or save in a ***csv**-datalist.



Analyze

This function is applicable only for one picture: This must be selected in the **Input** tool. The measurement data are embedded in the image.

Analyze to File

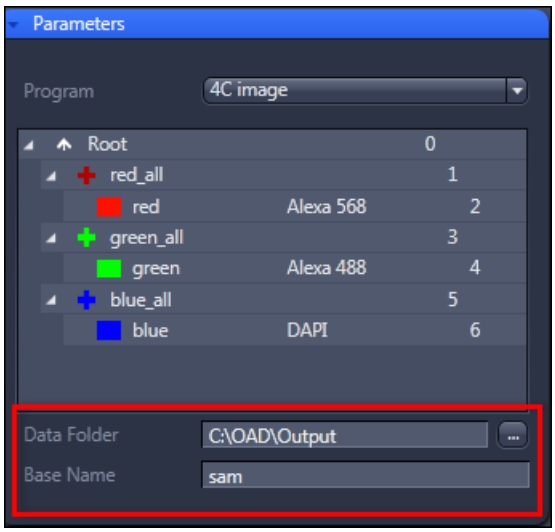
This function is applicable only for one picture: This must be selected in the **Input** tool. The measurement data is stored in a ***csv** list and not embedded into the image.

The following file types are supported:

- CZI
- ZVI
- BMP
- TIF
- JPG

Data Folder: Folder where the ***csv**-data lists will be stored.

Base Name: Name of the data list.



Analyze to Batch

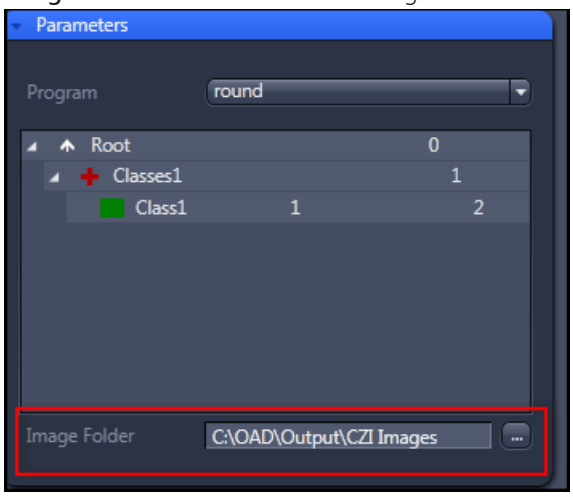
This function allows the analysis of all images in a folder.

Note

The function **Analyze Batch** can only be applied to images with the file type ***czi**.

The measured data are embedded in each original image.

Image Folder: Folder of the ***czi** images to be measured.



Analyze Batch to File

This function allows an analysis of all images in a folder. The measured data are stored in a ***csv** list and not embedded into the image.

The following file types are supported:

- CZI
- ZVI
- BMP
- TIF
- JPG

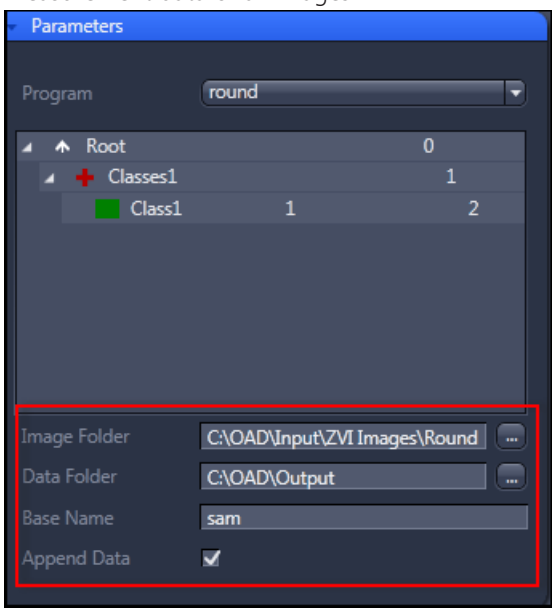
Image Folder: Folder of the images to be measured.

Data Folder: Folder where the ***csv**-data lists will be stored

Base Name: Name of the data list.

Append Data OFF: For each image, one data list is stored per class.

Append Data ON: For each class, an accumulated csv-data list is stored with the measurement data of all images.

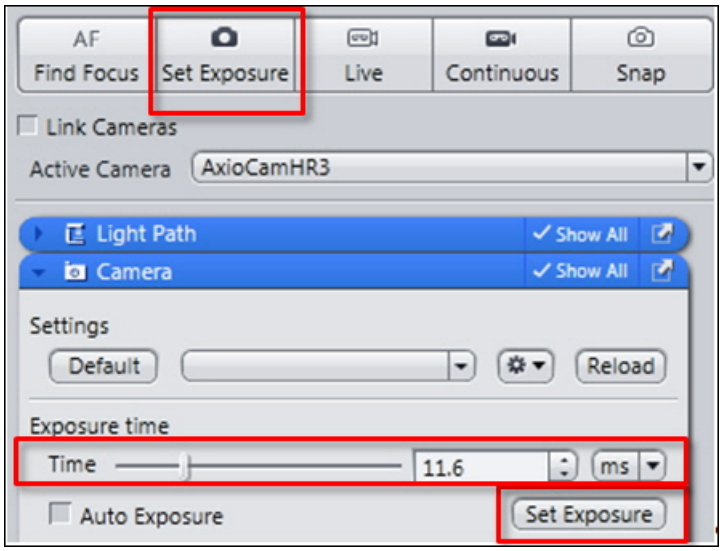


15 FAQ

15.1 What can I do If my image is too dark?

Try to increase the exposure time by performing the following settings:

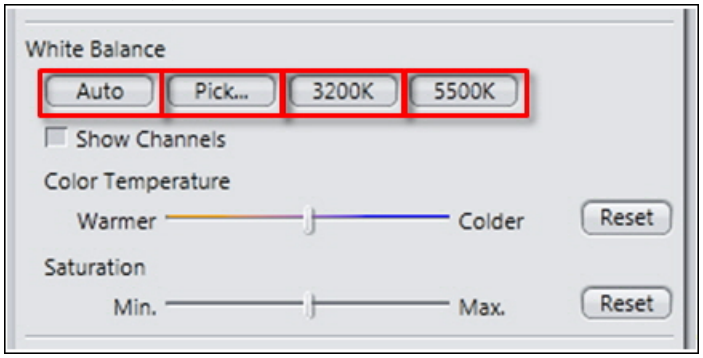
- in the **Locate** tab click on the **Set Exposure** button. This will calculated the correct exposure time automatically.
- in the **Camera** tool manually adjust the **Time** slider until you achieve the desired result.



- in the **General View Options** | **Display tab** adjust the display curve, see chapter *Optimize live image settings* [▶ 30].

15.2 How can I balance my images color?

To perform an automatic or manual white balance you must use a color camera. In the **Left Tool Area** | **Locate tab** | **Camera tool** the **White Balance** section appears. There you can perform a white balance with one of these methods:



Auto **Method:**

Procedure

1. Move the sample out of the **Live** window's field of view, so that you only see the background (essentially the light source).
2. Click on the **Auto** button.
3. The white balance will be calculated automatically. Afterwards move your specimen back into the field of view.

Interactive/Pick… **Method:**

Procedure

1. Click on the **Pick…** button.
2. Click on a white area of the **Live** window which should be represented as white.

This area will be used as a reference for the white balance.

3200K **Method:**

Use this method if working with a halogen bulb.

Procedure

1. If available set the light source to 3200K by pressing the **3200K** button located on the body of the microscope.
2. Click on the **3200K** button.

This method is also largely depending on the quality/age of your bulb. If the color rendition is not as desired, try the **Auto** or the **Interactive/Pick**… methods above.

5500K **Method:**

Use this method if working with a LED.

Procedure

1. Click on the **5500K** button.
2. If the color rendition is not as desired, try the **Auto** or the **Interactive/Pick**… methods above.

If none of the above methods produce a satisfactory result, one can additionally manually adjust the **Color Temperature** slider.

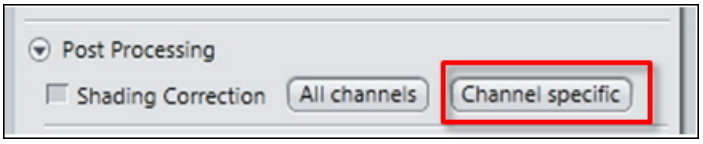
15.3 How can it be that my image has dust or a shadow, although my specimen is clean?

If the dust is not on your specimen, then the best method is to clean the optical elements that lay in the imaging pathway of your microscope. However if that poses a problem, alternatively, you can perform a Shading Correction as shown below. This solution has some limitations, especially if the dust is very dark or thick.

Procedure

1. Move your sample out of the Field of View until you see nothing but the light source/dust.

2 Click in the **Camera** Tool in the **Post Processing** section on the **Channel Specific** button.



3 Move your sample back into the Field of View.

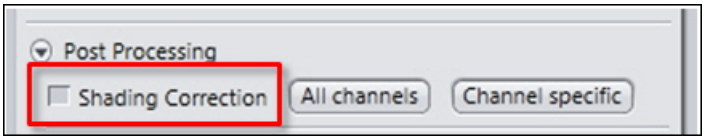
15.4 Why my image seems to look that something have burned in? (i.e. a shadow of a previous specimen?)

Check that The Shading Correction of the previous experiment is **not** already adjusted.

Procedure

1 In the **Camera** Tool open the **Post Processing** section

2 Deactivate the **Shading Correction** checkbox.



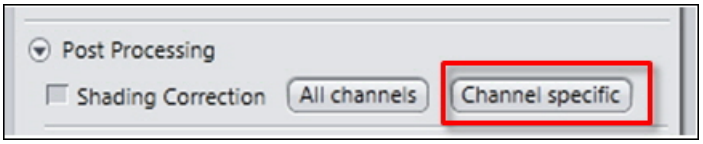
15.5 How can I fix a color gradient cast?

To fix a color gradient cast, you can try the following:

Procedure

1 Move your sample out of the Field of View until you see nothing but the light source.

2 Click in the **Camera** Tool in the **Post Processing** section on the **Channel Specific** button.



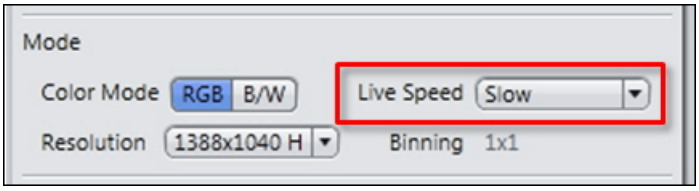
3 Move your sample back into the Field of View.

4 Perform a **White Balance,** see chapter *How can I balance my images color?* [▶ 741]

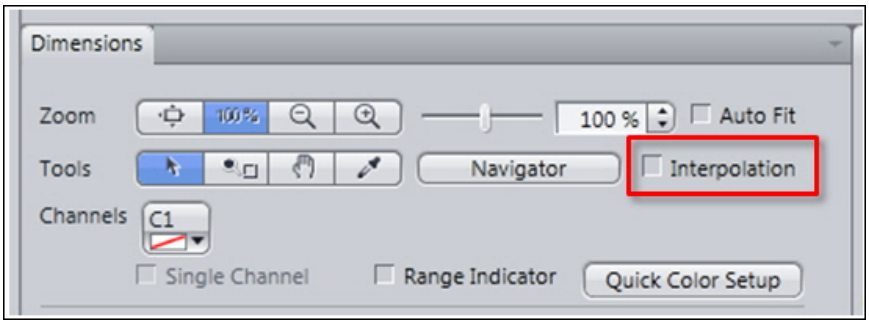
15.6 What can I do if my live image is of a low quality and looks pixelated?

Procedure

1. In the **Camera** Tool | **Mode** section in the **Live Speed** dropdown list select the entry **Slow**.



2. Right-click on the live image and select the **Fit to View** entry.
3. You can also optionally, in the **Dimensions** tab, activate the **Interpolation** checkbox.



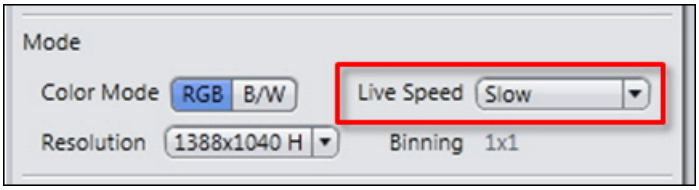
15.7 What can I do if my live image is slow?

Solution A

The live speed of your image is possibly set too slow. Increase the Live Speed.

Procedure

1. In the **Camera** tool in the **Mode** section, select a faster speed from the **Live Speed** dropdown menu. There are at most three choices, depending on the camera: **Slow**, **Medium**, **Fast**.



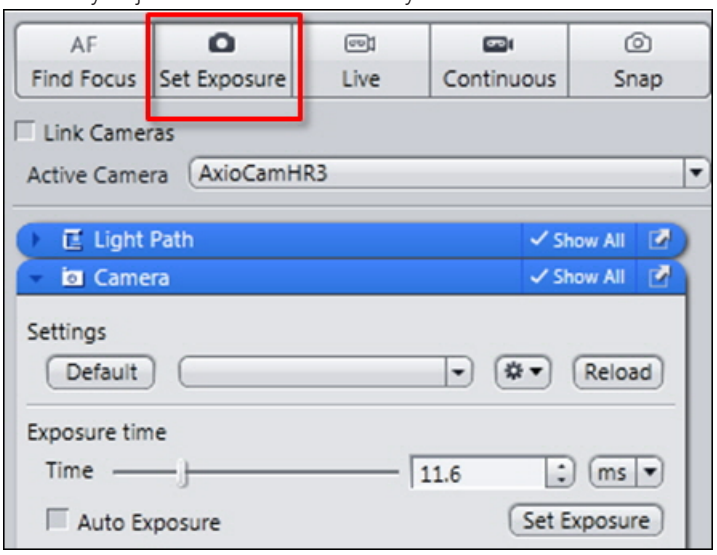
Solution B

The exposure time is possibly set to high, respectively improper. Optimize your settings in the **Camera** tool, in the **Exposure time** section

Procedure

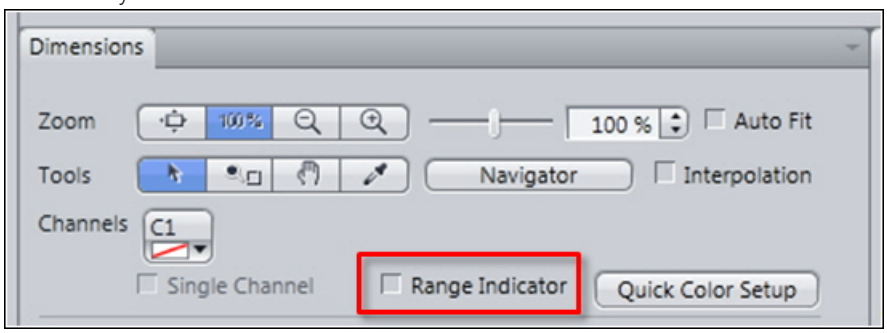
1. On the **Locate** tab click on the **Set Exposure** button.
2. Alternatively in the **Camera** tool click on the **Set Exposure** button.

3 Manually adjust the **Time** slider until you achieve the desired result.



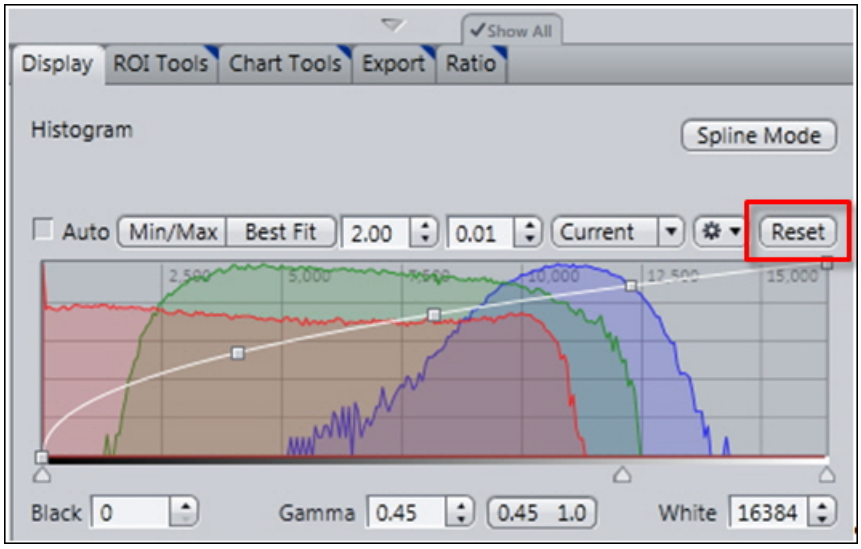
15.8 What can I do if my live image is mostly red/blue?

Check whether the checkbox **Range Indicator** is activated. If this is the case, the display switches to the **Single Channel** mode. The channel will be displayed monochrome. Simultaneously you see areas where the camera sensor is saturated, shown in red. Areas in which the pixel values = 0, are shown in blue. If this is not needed anymore deactivate the checkbox.



15.9 What can I do if my live image is still black or white after setting the exposure?

Check to see that your display curve is not set all the way to the left/right. Try to reset the display curve by clicking in the **Display** tab on the **Reset** button to achieve the default setting.

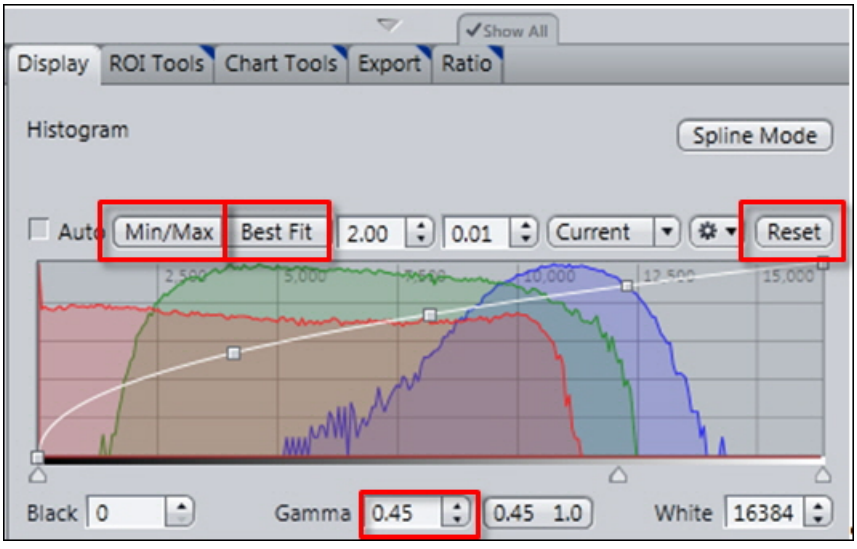


15.10 Why my live image shows extreme colors in comparison to what I see in the eyepieces?

The reason could be, that your **display curve** is not adjusted.

Procedure

1. Click on the **Reset** button.
2. Click on the **0.45** button or set Gamma 0.45.
3. You can additionally click on **Min/Max** or **Best Fit** button.

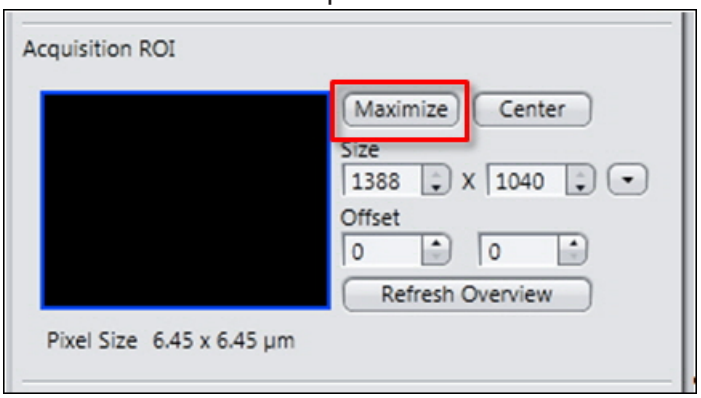


15.11 Why is my image resolution lower than the given camera specification?

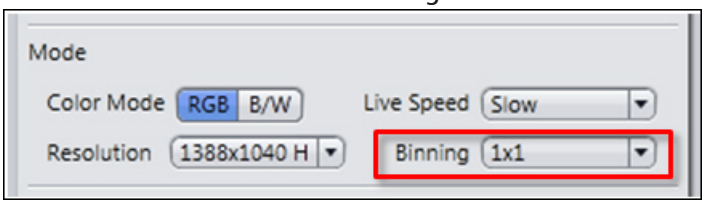
Because you chose a wrong or improper setting.

Procedure

1 In the **Camera** tool in the **AcquisitionROI** section click the **Maximize** button.



2 In the **Mode** section check that **Binning** is set to **1x1.**



15.12 What can I do if I do not see a focused live image?

Refocus the specimen on the microscope. You may activate the **Focus Bar** as an additional aid.

Procedure

1 Open the context menu via right-click in the **Live image.**

2 Select the entry **Focus Bar**.

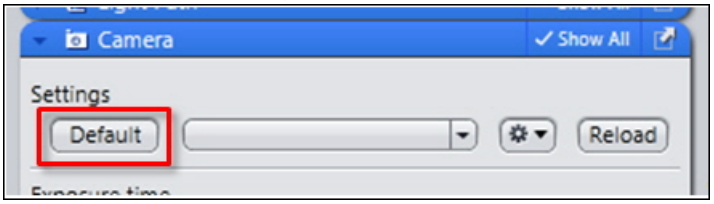
The **Focus Bar** will be shown within the **Live image** now.

15.13 Why is my image color not the same that I see through the eye pieces?

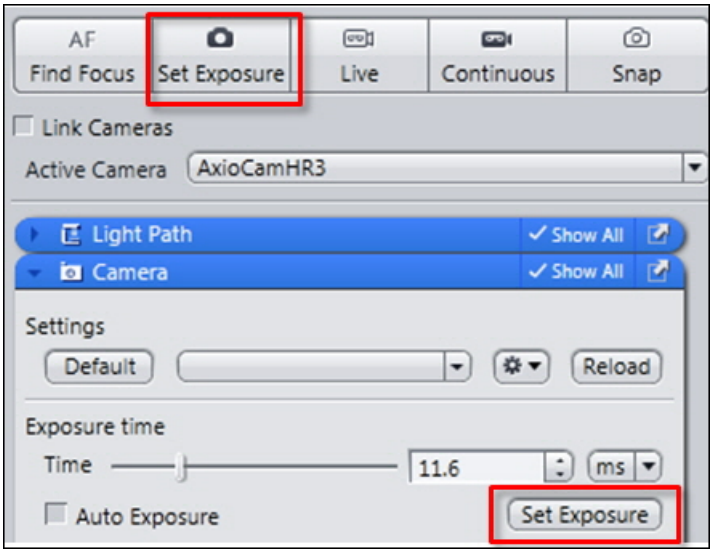
This is largely dependent on the color of your light source. The following instruction assumes that your light source is set to white.

Procedure

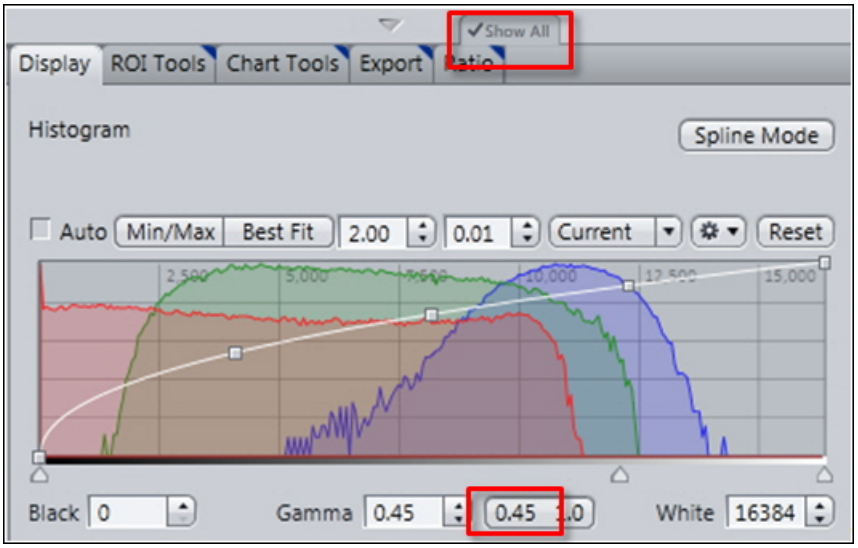
1 Click in the **Camera** Tool in the **Settings** section on the **Default** button, to set the camera back to factory default.



2 Click on the **Set Exposure** button.



3 In the **Display** tab, click the **0.45** button. If you do not see this button, activate the Show All mode.



16 Shortcuts list

Shortcut	Command	Description
Alt+Arrow Down	Previous Z-Stack image	
Alt+Arrow Up	Next Z-Stack image	
Alt+Arrow Left	Previous Time Series image	
Alt+Arrow Right	Next Time Series image	
Alt+F1	Select	Changes courser to Selection mode.
Alt+F2	Rectangle (aligned)	Draws in an aligned rectangle.
Alt+F4	Exit	Exits application.
Ctrl+0	Fit to View	Fits image size to actual window size.
Ctrl +1	Normal View (100 %)	Shows image in original size.
Ctrl +6	Export	Opens **Image Export** on **Processing** tab.
Ctrl +A	Select All	Selects all graphical elements in **Center Screen Area**.
Ctrl +Alt+W	Close All	Closes all open windows in **Center Screen Area**.
Ctrl +C, Ctrl +Ins	Copy	
Ctrl +F	New File Browser	
Ctrl +F2	Print Preview	
Ctrl +F4	Close	
Ctrl +F6	Previous Window	
Ctrl +N	New...	
Ctrl +O	Open...	
Ctrl +R	Recent Files...	
Ctrl +S	Save	
Ctrl +Shift+C	Create Subset Images from ROI	

Shortcut	Command	Description
Ctrl +Shift+D	Diagnostics	
Ctrl +U	Draw Region of Interest	
Ctrl +V, Umschalt +Ins	Paste	
Ctrl +X, Shift+Del	Cut	
Ctrl +Y	Redo	
Ctrl +Z	Undo	
F1	Help	
F2	Snap	
F6	Next Window	
F7, Ctrl +F7	Increase Zoom	
F8, Ctrl +F8	Decrease Zoom	
F11	Full screen mode	
Shift+F2	Live	

A

ApoTome - Fourier Filter

The processed result ApoTome image can sometimes still show fine residual line artifacts. This is due to sample, staining, dynamic range during acquisition or exposure time used. These artifacts can be removed using the Fourier Filter option. This function makes use of the fact, that stripe artifacts appear as a group of dots in frequency space. The filter masks those dots and can in this way remove stripe artifacts from the image. It only works however, if the camera had been aligned parallel to the grid lines during phase calibration. Also, it is quite a crude method and should not be needed if all other preconditions for using the ApoTome have been met.

ApoTome - Global bleaching correction

For each raw image the total (sum) intensity is measured and a global decay curve determined. This decay factor is used to correct the brightness of all pixels in the raw image. This method is suitable for samples with only one fluorescent dye present.

ApoTome - image normalization

The processing algorithm for ApoTome raw images acts in a subtractive manner effectively reducing the gray value range of the output image. Since all calculations are done internally in the high precision 32 bit floating point image format the normalize option can help to generate better output data. The floating point numbers are back-converted into 16 bit integer numbers using the full 16 bit dynamic range normalized to the brightest pixel. This option makes it impossible however to make quantitative comparisons between images.

ApoTome - Local bleaching correction

If more than one fluorescent dye is present in the sample, a single decay curve cannot be used to correct bleaching. This is the case for most biological samples especially when considering the contribution of autofluorescent substances to the total signal detected. The solution is to use a local bleaching correction determining a decay factor for each individual pixel position in each raw image which effectivel removes artifacts also for complex dye combinations.

ApoTome Bleaching correction

The main reason for residual stripe artifacts in ApoTome images is the fact, that acquiring at least 3 grid images for one resulting processed image leads to bleaching of the fluorescent dyes. This bleaching leads to brightness differences in the raw images and to artifacts when processing the data uncorrected. The principle for correcting bleaching in widefield data is the fact, that no matter, how far away from the focal plane the detector is placed, the sum intensity emitted from the sample remains the same. This is also true for the grid images. This fact is being used for the ApoTome. Two methods exist, both of which are patented.

ApoTome Phase correction

The ApoTome grid is moved in precise steps during acquisition in order to cover the sample fully for one section. The used steps are stored in the image metadata and used for all subsequent processing steps. In most cases this will work very well. However, there can be cases e.g. caused by vibrations, when the actual grid position deviates from the reported position causing artifacts during processing. Phase correction analyses the actual grid positions in the raw images and uses the determined values instead for processing. This option will add a bit of processing time however.

ApoTome raw data mode

The ApoTome combines the advantages of widefield imaging systems with the advantages of optical sectioning. Images acquired from the Acquisition tab always contain all images acquired from the grid. These grid images are also called phase- or raw-images. This principle offers several advantages: 1) all informations acquired are kept and not discarded; 2) the acquisition itself is not slowed down by processing overhead; 3) you get access to various correction methods giving you flexibility in treating your sample in the right way after acquisition; 4) Phase (=grid-position) errors occuring during acquisition such as caused by vibrations of the microscope can be likely corrected using the phase correction option without having to redo the acquisition; 5) you can achieve a marked improvement in resolution and contrast by using the specially adapted ApoTome deconvolution option bundled with all systems; 6) the raw mode facilitates easy analysis of images which show errors or artifacts in the sectioned image which would otherwise remain obscure.

B

Backlash Correction

Eliminates small errors inherent in all mechanical drives capable of reverse motion. It only makes sense to use backlash correction when using objectives with a very small depth of field e.g. 63x 1.4 oil immersion. In this case the benefits of the backlash correction will visible. Keep in mind that this extra precision costs a little more time when running the Z-Stack.

Binning

Binning is understood to mean the combination of neighboring image elements (pixels) on the image sensor itself, e.g., the CCD sensor in a digital camera. Source: Wikipedia

Bleaching Correction

The characteristics of a widefield fluorescence microscope are based on the assumption that all Z-planes have the same total brightness, irrespective of the focus position. Use is made of bleaching correction by applying a correction factor to each Z-plane. However, this assumption does not apply to techniques that result in the generation of optical sections, such as confocal images.

Burst Mode

Burst Mode is some kind of optimization to enable the recording of the fastest framerates, which can be achieved by the used camera hardware. This mode requires some compromises for the sake of speed: it supports only single channel time lapse acquisition, an update of the image display is suppressed while recording and the maximum time lapse duration is depending from the size of available main memory (minus some space to breathe for the operating system). If a multi channel image needs to be acquired, Burst Mode will be disabled and maximum frame rate can be slower then specified in the camera hardware performance documentation.

C

Clipping planes

The purpose of clipping planes is to cut open the calculated 3D image so that elements on the inside can be visualized. Clipping planes can cut the volume in such a way that either the front, back or both sides of the volume data are no longer visible. In addition, the clipping plane itself can be given various textures. This is a very important modeling option for analyzing 3D data.

Colocalization Acquiring fluorescence images in several channels makes it possible to visualize the relationship between biological structures. A combined display of two channels in color overlay mode makes it easier to assert whether the components are "colocalized", i.e. whether they are located at the same position. Conventionally, two fluorescence channels are displayed in the form of a color-coded overlay. The most common form is the red/green overlay. Regions in which both fluorescent dyes are present at the same place are displayed in yellow. It is not possible, however, to make quantitative statements concerning the extent of colocalization on the basis of this display. At best, a qualitative statement is possible with regard to whether or not two dyes are colocalizing. The Colocalization module is able to fill this gap and presents the user with a tool that enables colocalization to be determined quantitatively. Principle: It is always the colocalization of two channels that is analyzed. Colocalization results from the pixel-by-pixel comparison of intensities for each channel.

Constrained Iterative The best image quality is achieved using the iterative maximum likelihood algorithm (see Schaefer et al.: "Generalized approach for accelerated maximum likelihood based image restoration applied to three-dimensional fluorescence microscopy", J. of Microscopy, Vol. 204, Pt 2, November 2001, pp. 99ff.). This algorithm is able to calculate light from various focal planes back to its place of origin. Consequently, with this method it is possible to derive the 3D structure from fluorescence images with the correct brightness distribution and to visualize optical sections. It is also possible for missing information to be partially restored from neighboring voxels. The spatial resolution can be increased without artifacts up to a theoretical limit (one voxel). It is essential for Z-stacks to have been acquired in accordance with Nyquist. Acquiring sufficient planes above and below the structure of interest is also imperative for achieving good results. As this is a complex mathematical method, the calculation can take longer, depending on the image size and the PC being used.

Costes Costes et al. (Biophysical Journal, 2004, vol. 86, pp 3993-4003) have published a statistical method with the help of which an attempt is made to determine an optimal colocalization threshold automatically. This takes place by initially maximizing the threshold for both channels and then gradually reducing it. With each step Pearson's Correlation Coefficient is determined for all pixels below the set value. These steps are repeated until the Pearson value is minimized (ideally a value of 0 for perfectly colocalizing channels). See the publication for further details. This method has been implemented in Colocalization. Clicking on Auto initiates the above iterative process, which, depending on the sample, can take several seconds. The threshold now set corresponds to the confidence criterion calculated. This method works very well with large, diffusely stained structures such as nucleoplasm or diffuse cytoplasmic structures. Under certain circumstances it does not function so well for small structures (e.g. nuclear speckles or vesicular structures), particularly in the case of widefield images, where the signal to background ratio is not as good as it is with methods that involve the generation of optical sections (e.g. LSM, TIRF or ApoTome). The Regions button becomes active as soon as a region is inserted into the scatter plot. It remains active as long as regions are selected or moved there. Activating and deactivating the button makes it possible to switch

between threshold selection using the mouse and the selection/moving of selected regions in the scatter plot image. If regions are defined in the scatter plot, the corresponding data appear in the table in addition to the overall image.

D

Deconvolution

Deconvolution is a method that is used to improve fluorescence images in particular. Image information acquired using a microscope system can never fully reproduce the structures of the actual object. This is because unavoidable distortions occur during acquisition due to the optics and electronics. In addition, particularly in the case of fluorescence microscopes that do not offer any methods for generating optical sections, light from areas of the object outside the objective's focal plane is also always acquired. This covers the structures that the user actually wants to see to a varying degree and therefore leads to a reduction in the contrast and consequently in the visible resolution. These optoelectronic effects can be described mathematically in the form of the point spread function (PSF). If the PSF is known, it is possible to correct the negative effects to a large extent using deconvolution. This produces a completely sharp image of the object that is richer in contrast. Deconvolution is usually performed on Z-stacks, i.e. it is used as a 3D method. However, it can also be used to a limited extent to improve 2D images. A good review of deconvolution can be found in Wallace et al., 2001: A Workingperson's guide to deconvolution in light microscopy; Biotechniques 31: 1076-1097.

Display characteristic curve

The display characteristic curve allows you to define the range of the gray value histogram of an image that you want to display on the screen. The limit on the left defines the gray value up to which all pixels are displayed as pure black (black value), while the limit on the right defines the gray value from which all pixels are displayed as pure white (white value). The curvature of the curve defines the so-called gamma value.

Drag&Drop

Literally translates to "drag and drop". Does the moving of objects (eg, files, icons, etc.) on the screen as from one folder to another. Clicking the object with the left mouse button, holding down these, the object moves with the mouse to the desired location.

Dynamic range

The dynamic range describes the number of brightness gradations that a camera or another detector is able to distinguish. Modern, scientific digital CCD cameras, for example, have a dynamic range of up to 2^16 gray levels. In this case we talk of 16 bit cameras.

E

Experiment Feedback

Experiment feedback allows the definition of specific rules and actions to be performed during an experiment. This allows changing the course of the course of an experiment depending on the current system status or the nature of the acquired data on runtime. Moreover, it is possible to integrate certain tasks like data logging or starting an external application, directly into the imaging experiment. Typically, but not exclusively, such an experiment connects the image pickup with an automatic image analysis.

F

Fast Iterative

The "Fast Iterative" method is an iterative restoration method that uses only one iteration per convolution step (see Meinel, E. S.: Origins of linear and nonlinear recursive restoration algorithms. J.Opt.Soc.Am, 3 (6), 1986, 787-799). No regularization is used in this case. Due to the fast processing and convergence after just a few iterations, this method is suitable in particular for the processing of larger time lapse images. The results of the method can quickly lead to good results and remove most of the out-of-focus light. They do not, however, create quantitative brightness conditions in the image. If undersampled images are present, artifact formation may also result.

Field Feature

A field feature is calculated for all segmented objects of a class. The geometric or intensity parameters of all objects of the class, e.g. the area or the average intensity, are added together. In addition, all objects can be counted, for example, or the area of the objects in relation to the total image area can be calculated as a percentage.

Fluorescent beads

Fluorescent beads are often used to measure the point spread function (PSF). The diameter of these beads is usually significantly below the resolution limit of the objective used. Based on the known shape and size, various optical parameters of the microscope system can be determined with the help of such objects. To measure the PSF when using an objective with a numerical aperture of 1.4, beads with a diameter of 50-170 nm should be used.

G

Gamma value

The gamma value makes it possible to correct the display of images on computer screens which do not allow the linear display of gray value curves. By changing the gamma value you can emphasize certain intensity ranges within your image when it is displayed on the screen. A value <1 emphasizes the ranges of medium pixel intensity (medium gray values), while a value >1 emphasizes the dark and bright pixel intensities and therefore increases the contrast. The recommended settings are 0.8 for fluorescence images, 1.2 for phase contrast or DIC images and 0.45 for true color images. Please bear in mind that a "correct" gamma value setting depends on numerous parameters, such as screen settings, ambient brightness, etc., and a universal setting cannot therefore be given.

Gaussian Distribution

The emission of fluorescent light in fact follows a Poisson distribution. If, however, detector noise predominates during imaging, or the image data are only just above the camera noise and therefore very dark, a normal distribution according to Gauss tends to apply to such images.

Generalized Cross Validation (GCV)

Regularization, which lessens the influence of noise during restoration, is normally controlled by a parameter that in most cases is determined heuristically via trial and error. The "generalized cross validation" (GCV) method makes it possible to estimate this parameter even under the complex conditions of Poisson maximum likelihood minimization.

H

Halo effect A halo is a (usually unwanted) effect in digital image processing ("halo" around the image object).

I

Image Analysis Wizard Using the Image Analysis Wizard you can create automatic measurement routines very easily. The wizard delivers precise results without any need to spend time on programming. This allows you to complete even complex measurement tasks in just a few minutes.

Image display A maximum range of 256 gray levels (black and white image) or 16 million colors can normally be displayed on a screen. Modern digital cameras capture a much larger range: black and white cameras up to 65536 values and color cameras theoretically up to (65536)3 colors (281 billion). The display of these gray values/ colors therefore needs to be adjusted for the monitor by the user. For this adjustment an upper and a lower gray/color value are defined. All gray/color values between these limits are displayed on the monitor within the 256 gray values/16 million colors that can be represented.

K

Kymograph A Kymograph is a twodimensional representation of a moving object over time. The movement of the object is traced using a line or curve of a given thickness and an intensity plot is then generated over time. The kymograph image displays the intensities along the line in X direction and the time points are plotted in the Y direction. With this method one can visualize and analyze speed and acceleration of moving objects with a simple 2D representation.

L

Lamp Flicker This phenomenon mainly occurs if fluorescent arc lamps are operated for a long period of time. Under certain circumstances alternating darker and brighter layers can then appear in the Z direction in Z-stacks. This effect may prevent 3D deconvolution from being usefully applied, for example.

M

Maximum mode In the case of a maximum intensity projection, only the pixels with the highest intensity are displayed along the observation axis. This view is well suited to the two-dimensional display of three-dimensional images, e.g. in publications, one reason being that a maximum transparency effect is only visible in this mode.

Microscanning Microscanning is a technological process for the production of high-resolution images using a CCD or CMOS sensor. For a sequence of images, the sensor is moved in two dimensions by micro-mechanics in very small intervals between acquisitions.The distances are smaller than a pixel dimension and allow the inclusion of detailed information that would otherwise not be seen by the sensor.

Mixed mode In Mixed mode, a volume can be displayed in both Surface mode and Transparency mode. In the case of multichannel images, for example, structures inside a cell, such as FISH signals or nucleoli, can be displayed in Surface mode and the cytoplasm around these structures can be displayed transparently in another channel. This means that even highly complex spatial relationships can be shown convincingly.

Motif buttons With the Motif buttons you can optimize image acquisition regarding particular requirements like speed or quality. All parameters e.g. camera resolution or dynamic range in Acquisition Mode or Channels tool were set automatically. They will influence basically camera, detector and lightning settings.

MTB The software MicroToolBox (MTB) is used to generate and manage microscope configurations. Information about microscope components (e.g. nosepieces, reflector turrets, shutters etc.) and, if necessary, additional external units (e.g. motorized xy stages, external light sources etc.) is stored in these configurations. Furthermore, the software can also be used to enter information about microscope components, such as objectives, fluorescence filter cubes etc., in a simple way and to save this information in the microscope (depending on the type of microscope in question). In this case, the information is saved directly in the microscope, allowing it to be displayed on the microscope's TFT screen, for example. Various configurations can be created, of which only one is activated at any time. The active configuration is used by imaging software such as ZEN to provide graphic control dialogs for the configured microscope units (e.g. lightpath or microscope components control).

N

Nearest Neighbor The Nearest Neighbor method uses the simplest and fastest algorithm (Castleman, K.R., Digital Image Processing, Prentice-Hall, 1979). Its function is based on subtraction of the out-of-focus information in each plane of a stack, taking the neighboring sections above and below the corrected Z-plane into account. This method is applied sequentially to each plane of the entire 3D stack. It allows you to enhance contrast quickly, even if image stacks have not been put together optimally.

Nyquist Criterion The Nyquist criterion states that a signal must be detected with at least double precision in order to reliably acquire all the frequencies in the signal. In the case of images acquired with coarser resolution, undesired effects such as aliasing may otherwise result. For the deconvolution of microscope images, this means, in practical terms, that images should be acquired with a pixel resolution that is at least double the optical resolution, both in the lateral and axial direction.

O

Object Feature An object feature is calculated for an individual segmented object. It describes a geometric or intensity property of the object, e.g. its area or its average intensity.

P

Point Spread Function ("PSF")

All optoelectronic effects that influence the creation of a microscope image can be described mathematically in the form of the point spread function (PSF). If the PSF is known, deconvolution can be used to largely remove the negative effects from microscope images. There are three possible ways to determine the PSF: theoretically through knowledge of the key optical parameters, experimentally through measurement using fluorescent beads of a known diameter, or blindly using a method that works with less prior knowledge. In ZEN the theoretical model according to Lanni and Gibson has been implemented, which also models asymmetries like those that can arise due to spherical aberrations (see S. F. Gibson, F. Lanni, "Experimental test of an analytical model of aberration in an oil-immersion objective lens used in three-dimensional light microscopy", J. Opt. Soc. Am. A, vol. 8, no. 10, pp. 1601-1613, October 1991).

Poisson Distribution

The emission of photons by fluorochromes follows a statistical distribution, known as a Poisson distribution. This is the preferred model taken as the basis for the deconvolution calculation. It applies if the predominant proportion of image noise is caused by shot noise ("salt and pepper noise"). This assumption applies to images that have been acquired using good, low-noise detectors, the dynamic range of which has been utilized to a certain extent.

Position

In a tile experiment positions refer to independent individual image fields (tiles) that are localized at various places on the sample. A position corresponds to a tile region consisting of just one tile. Each position is based on an X and Y coordinate of the stage and a Z coordinate of the focus drive. Individual positions or position arrays (grouped individual positions) are defined using the Tiles tool. After acquisition the individual positions are displayed as scenes.

Pseudo color assignment

In fluorescence microscopy, pseudo color assignment describes the assignment of any artificially selected color to the channel of a multichannel fluorescence image. As it is mostly monochrome cameras (which produce black and white images rather than "true colors") that are used in this area of application, we talk of pseudo coloring.

R

Reference Z-Position

By default the current Z-position at the time the experiment is started is set as the Reference Z-Position for acquisition. Z-stack experiments, for which the center of the defined Z-stack is set by default as the fixed Reference Z-Position, form an exception to this. Offsets for channels and Z-stacks shift acquisition in relation to the Reference Z-Position. If a focus strategy is used, this determines and updates the Reference Z-Position during the experiment.

Regularization

Working with real microscope images that are affected by noise leads to considerable difficulties with the practical application of deconvolution methods, which is why regularization (e.g. according to Tikhonov-Miller-Phillips) is essential. Regularization is a method that lessens the influence of noise by means of various penalty terms. Stronger regularization leads to weaker restoration and weaker regularization to stronger restoration, although in this case noise is also intensified.

Regularized Inverse Filter The inverse filter is a genuine 3D method and generally achieves better results than the Nearest Neighbor algorithm. It essentially involves dividing the Fourier transformation ("FT") of the volume by the FT of the PSF, which can be performed very quickly. In the real space this corresponds to deconvolution. In addition, a statistical method ("General Cross Validation – GCV") is applied, which determines the noise component of the image and automatically sets the restoration strength to the optimum level in line with this. This process is also known as regularization. The method is very well suited to the processing of several image stacks in order to preselect images for the application of the iterative "high-end" method. Z-stacks must, however, have been acquired at the correct (Nyquist) distance. The additional acquisition of Z-planes above and below the structure of interest is recommended.

Render Series To display a 3D volume on the screen, each image must be recalculated. This takes time and, in the case of large images, cannot be done interactively. You can, however, have a series of individual images calculated which represent the animation that you want. Such an image series can be displayed considerably faster and more fluidly than is possible interactively on the screen as, in this case, the views no longer have to be rerendered. Furthermore, an image series like this lends itself extremely well to being exported as a film.

S

Shading Correction The Brightness of microscopic images often declines to the edges. This is caused for example, by misaligned, or inhomogeneous lighting, inconstant light conditions or dirty optics. ZEN is able to correct this interference with the so called Shading Correction. First you need a white image. This functions as a reference for the background of your image, which shall be corrected.

Shadow mode In Shadow mode the structures in the image are illuminated by means of a virtual light source. The image stack is viewed from above, as if through the microscope's eyepiece, and a shadow is projected onto a virtual base (in the image background). This gives the data a reference in relation to the space, which makes visualization easier. The impression of a three-dimensional structure is created from the combination of light being reflected and opacity (degree of impenetrability to light) and the casting of a shadow.

Shuttle and Find Shuttle and find is used in the correlative microscopy for reproducible positioning of the sample in the light and scanning electron microscopes. The samples are mounted in a specially developed for this purpose, correlative holder systems. Biological samples are mainly applied to coverslips and TEM grids, while samples from the materials science and industry can have different forms. Accordingly, the sample holder for samples from the materials science have been designed. The sample holder used is selected before the calibration. Calibration is required to retrieve the same sample position in different microscopes.

Smart Setup Smart Setup is the intelligent and convenient control center for your fluorescence images. Simply select a fluorochrome from the more than 500 dyes stored and ZEN will automatically provide the optimal filter combinations and acquisition settings for your experiment.

Spherical Aberration

Every objective requires the use of a defined immersion medium to deliver the best optical resolution. In microscopy practice, particularly in the area of biosciences, it is not always possible, however, to embed the sample in a medium with the correct refractive index. When light enters the embedding medium with the wrong refractive index this results in "spherical aberration". The PSF becomes more asymmetrical the further away from the cover slip it is measured. In practical terms this becomes noticeable in the form of an increasing loss of brightness as the distance from the cover slip increases. It is possible to compensate for spherical aberration either by using objectives with correction rings or objectives that have been calculated for certain embedding media (e.g. aqueous solutions). Within certain limits, however, spherical aberration can also be compensated for during deconvolution, by taking the parameters responsible for this effect into consideration when calculating the theoretical PSF. For further details see S. F. Gibson, F. Lanni, "Experimental test of an analytical model of aberration in an oil-immersion objective lens used in three-dimensional light microscopy", J. Opt. Soc. Am. A, vol. 8, no. 10, pp. 1601-1613, October 1991.

Surface mode

The two modes previously described display the data with soft transitions or with a transparent character, depending on the setting. In Surface mode, the program calculates solid surfaces ("isosurfaces") from the gray values, which emphasizes particularly flat structures (e.g. cell walls of plant cells). This display can be used if you want to draw attention to certain structures, while other, internal structures are hidden.

T

Threshold

Which threshold is the correct one is a question that is frequently asked. Unfortunately it is not possible to give a definitive answer to this question, particularly because this often depends on the problem and the properties of the sample. Generally speaking it can only be said that the best approach is to determine the thresholds using appropriate control samples, e.g. samples without colocalization as a negative control and samples with biologically relevant colocalization as a positive control. Thresholds determined in this way can, under certain circumstances, be transferred to the sample of interest.

Tile region

In a tile experiment a tile region refers to a group of individual image fields (tiles) that belong together and are arranged in the form of a grid. With the help of tile regions it is possible to acquire areas with dimensions that exceed the size of an individual image field. Within an experiment a number of tile regions can be acquired at various positions on the sample. Each tile region is based on an X and Y coordinate of the stage and a Z coordinate of the focus drive. Tile regions are defined using the Tiles tool. After acquisition the individual tile regions are displayed as scenes.

Transparency mode

In Transparency mode a three-dimensional image is calculated with a transparency effect. At least two 2D texture stacks are calculated for this from different views, which are used depending on the position angle. In contrast to Shadow mode, in this case the scene is illuminated from behind by diffuse, white light. Using the setting options, in this mode you can "mix" several channels with one another and

also make information visible inside a structure. This view is therefore particularly well suited to visualizing the spatial relationship between structures within the image.

W

Widefield Classical microscopes frequently are called "widefield" microscopes in order to distinguish them from microscope systems with optical sectioning capability such as laser scanning microscopes. In contrast to such systems widefield microscopes do not possess the ability to discriminate betwee image information in the axial (=Z) direction leading to blurred images and therefore are only poor 3D imaging systems per se. There are methods to add this missing axial sectioning ability to widefield microscoped such as 3D deconvolution or structured illumination (ApoTome, Elyra-S)

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