ZEISS Axio Observer 7 with Apotome - User Guide

Last Updated – January 2026

Purpose: The ZEISS Axio Observer inverted widefield microscope equipped with the Apotome optical sectioning system is a high-performance imaging platform designed for advanced fluorescence microscopy of biological samples. The system enables high-resolution multichannel, time-lapse, and three-dimensional imaging of samples mounted on slides, multiwell plates, and live-cell imaging chambers. Typical applications include cell biology and tissue imaging, protein localization studies, live-cell dynamics, multi-fluorophore experiments, and quantitative fluorescence analysis.

IMPORTANT NOTICE

THIS MICROSCOPE CANNOT BE USED WITHOUT EXTENSIVE TRAINING!

PLEASE BE CAREFUL NOT TO HIT THE OBJECTIVES ON THE STAGE OR ON THE SAMPLE HOLDER WHEN FOCUSING THE SAMPLE!

This happens when you go above the focal position so NEVER move the objectives up without a sample being present and NEVER lift the sample with the objectives!

Contents

Safety instructions and contacts

Safety instructions and first aid procedures

Beam paths are shielded except for a fixed open beam between objective and specimen.

- Never look directly into the beam path.
- Never interfere with the beam path.
- Never change a specimen during acquisition.
- Never change objectives during acquisition.
- Never change filters during acquisition.

Specimens should be sealed before observation under the microscope and handled with care using the appropriate level of protection.

- Leaky or cracked samples must not be examined.
- Any spillages should be cleaned up immediately and the area swabbed with 95% alcohol.
- Glass slides should be disposed of in the contaminated sharps bin – broken fragments of glass should be brushed on to paper and disposed of in a similar fashion.

All biological samples and hazardous waste must be appropriately disposed of by the user. Potential risks associated with the waste material should be addressed by the user in conformity with ITQB-NOVA rules.

Contacts

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Suggestion for description in “Materials and Methods”

Images were acquired using a ZEISS Axio Observer inverted widefield microscope equipped with an Apotome structured illumination module for optical sectioning. A 20 MP monochrome Axiocam 820 camera, (pixel size 2.74 µm) and a [objective model, e.g., Plan-Apochromat 63×/1.4 NA oil] objective were used. Multichannel fluorescence imaging was performed using [single/multiband] filter sets optimized for [fluorophore(s), e.g., DAPI, GFP, Alexa Fluor 594].

The excitation was provided by a Viluma 7 LED light source, using the following channels: [LED wavelengths used, e.g., 385, 469, 555 nm]. Image acquisition parameters — exposure time ([e.g., 100 ms]), LED intensity ([e.g., 15%]), binning ([e.g., 2×2]), and gain ([e.g., 1×]) — were kept constant across all samples to allow quantitative comparisons.

For 3D imaging, Z-stacks were acquired with a step size of [e.g., 0.3 µm], and optical sectioning was performed using the Apotome with [e.g., 5] grid phases. Time-lapse and tile scan acquisitions were controlled using ZEN software (ZEISS), with hardware autofocus enabled and environmental control maintained at [e.g., 37 °C, 5% CO_2, [O_2]%, and humidified atmosphere], where applicable.

Information to be added to the "acknowledgements" section

This work was partially supported by PPBI – Portuguese Platform of BioImaging (PPBI-POCI-01-0145-FEDER-022122), co-funded by national funds from OE – Orçamento de Estado and by European funds from FEDER – Fundo Europeu de Desenvolvimento Regional. The acquisition of the imaging system used in this work was supported by the project "Infraestruturas Integradas de Investigação no ITQB NOVA" (Integrated Research Infrastructures at ITQB NOVA), co-funded by FEDER via the "Programa Operacional Regional Lisboa 2030", with the reference LISBOA2030-FEDER-01318100.

User Pre-requisites, Booking and General Rules

- The equipment cannot be used without official training.
- New users must have a clear understanding of the principles of light microscopy and how fluorescence works.
- Before your first use, **contact Carolina Feliciano** (cfeliciano@itqb.unl.pt) to schedule training.
- Users are responsible for saving their data to personal or institutional storage devices.
- Register your utilization in the logbook.
- For all downstream image analysis, use the available workstations. Alternatively, you can use FIJI.
- Contact the responsible people in case of any doubt or any issue with the equipment.

Booking is done online via Agendo: https://europe.agendoscience.com/

Equipment location, description and technical specifications

- **Manufacturer:** ZEISS
- **Model:** Observer 7 with Apotome
- **Software:** ZEN Blue 3.13
- **Location:** Room 5.25, 5th floor, ITQB NOVA

Equipment Specifications

- Axio Observer 7 inverted microscope
- Definite focus (hardware-based)
- Motorized XY stage, resolution 0.1 µm, reproducibility ± 0.6 µm
- Additional magnification (optovar): 1.25x and 1.6x
- Motorized condenser (NA 0.55), with phase contrast rings and DIC possibility
- Dual motorized filter wheels for up to 6 dichroics and 8 emission filters: 300 ms switching speed
- Illumination: LED transmitted light
- **Viluma 7 (7 independent LEDs):** 385, 423, 469, 555, 591, 631, 735 nm
- **Fluorescence filter sets:** Narrow emission filters and multiband sets for DAPI, GFP, dsRed, Alexa Fluor 594, Cy5 and Cy7

- **Camera:** Monochrome CMOS camera, 20 MP, pixel size 2.74 × 2.74 µm, diagonal chip 17.4 mm
- **Objective lenses**

Objective	Magnification	NA	Working distance (mm)	Comments
EC Plan-Neofluar 10x/0.3 Ph1	10x	0.3	5.2	Especially suitable for overview images. Ok for plastic and thick sample carriers
Plan-Apochromat 20x/0.8	20x	0.8	0.25	Air objective
Plan - Apochromat 40x/0.95 Corr	40x	0.95	0.25	Air objective. To work with CG thickness from 0.13 mm to 0.21 mm. With correction collar to compensate for spherical aberrations caused by different cover glass thickness
Plan - Apochromat 63x/ 1.4 Oil	63x	1.4	0.19	Oil immersion objective
Plan - Apochromat 100x / 1.4 Oil	100x	1.4	0.17	Oil immersion objective
Plan - Apochromat 100x / 1.4 Oil Ph3	100x	1.4	0.17	Oil immersion objective. Phase (Ph3) contrast objective

- Incubation system with temperature, CO2, O2 and humidity regulation, large dark chamber and heating stage.
- Apotome structured illumination module with selectable grid phases (typically 5–15).
- Software ZEN Blue with modules: Multichannel, Z-stack, Time-Lapse, Extended Focus, Autofocus, Mosaics, Multiposition, Experiment Designer, Deconvolution, Online Processing
- High-performance PC: Windows 11 LTSC 2024, 128 GB RAM, NVIDIA GPU with 24 GB VRAM.
- Monitor: 31.5" 4K monitor (3840 × 2160)

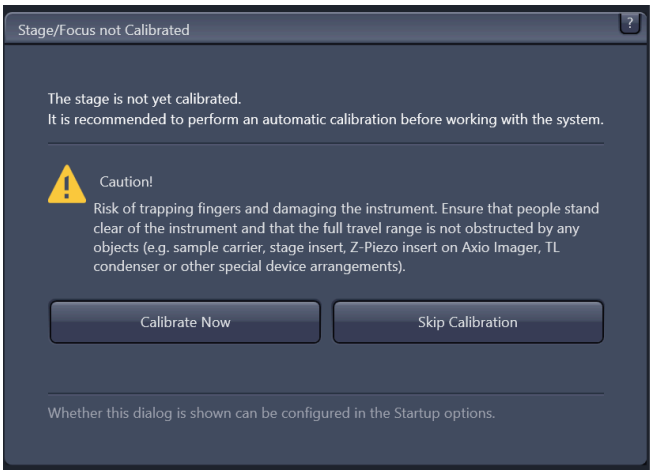
Getting Started

Starting System

1. Turn on the **Computer switch** outlet (1/3);
2. Turn on the **Components switch** outlet (2/3);
3. Turn on the **microscope using the silver power button (3/3)**
4. Start the **Computer**.
5. Launch **ZEISS ZEN 3.13**.

6. On the startup screen, select "**ZEN"**.

7. On the touchscreen, go to **Load Position** > **Set Working Position**. !!! Ensure objective is on "**load position**" !!!
8. Click "**Calibrate now**".



Mounting a sample and acquiring images:

Sample Preparation Notes

- You can use resources such as the FPBase or the Thermofisher Spectra Viewers to explore your fluorophores. Learn their excitation and emission wavelengths, and what are their optimal excitation wavelengths!
- Use #1.5 high precision glass coverslips (#1.5H). Microscope objectives are designed for a specific thickness (typically ~0.17 mm). Deviations cause spherical aberration, leading to image blur, reduced contrast or loss of resolution.
- Confirm the coverslip is secure as this is an inverted microscope.

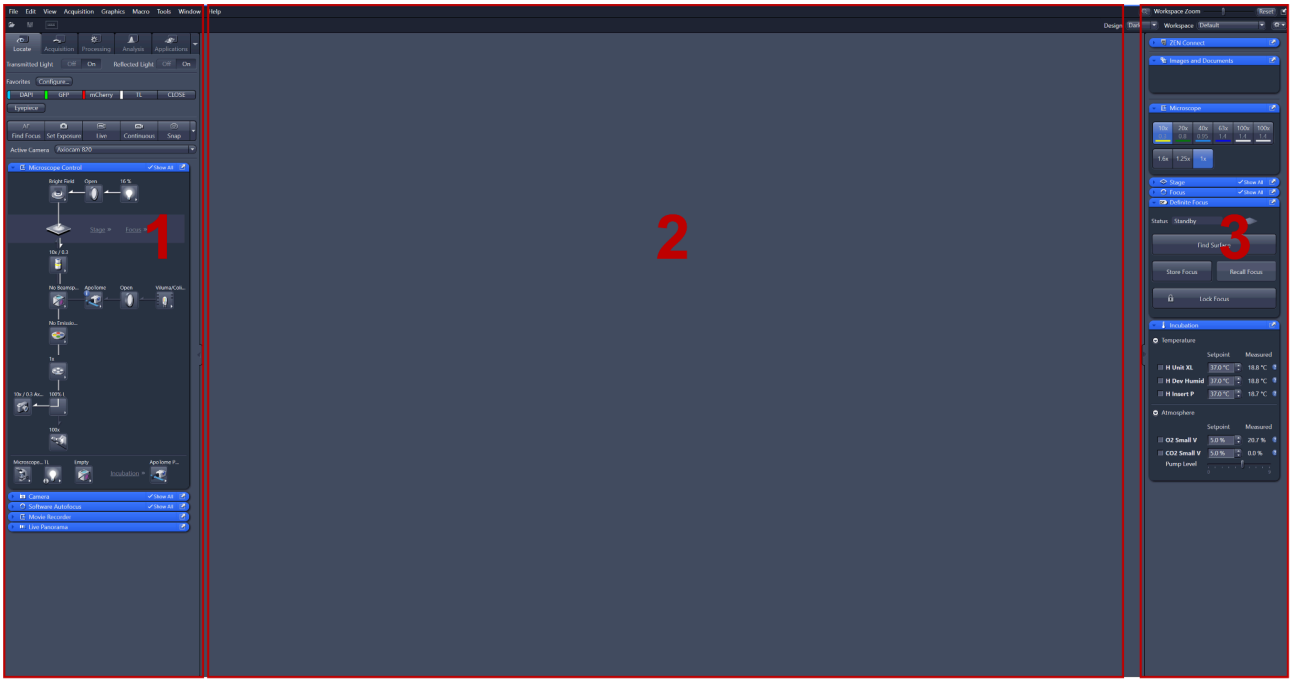
Sample Mounting

The stage is compatible with slides, multiwell plates and Petri dishes.

- Ensure that the 10x objective is selected before mounting or changing samples or stage adapters.
- Place slide so that the sample is on top of the objective (coverslip facing down if your sample is on a microscopy slide).
- Choose the objective you want to use using the touchscreen of the microscope.
- Close all incubator doors. Don’t forget to switch off the incubator light.

Zen Blue General User Interface

The user interface is divided into three main sections, the **Controller Section (1)**, the **Image Display Section (2)**, and the **Image and Document Manager Section (3)**, with a menu bar above and a system information bar below.



The **Image and Document Manager Section** shows all the currently open files and allows you to save and close the currently selected image.
The **Image Display Section** is where your current image is shown. When an image is open it has a tab on the bottom that allows the adjustment of brightness and contrast.
The **Controller Section** is the most important of the three as it allows the user to control the microscope to locate, acquire and process their samples' images.

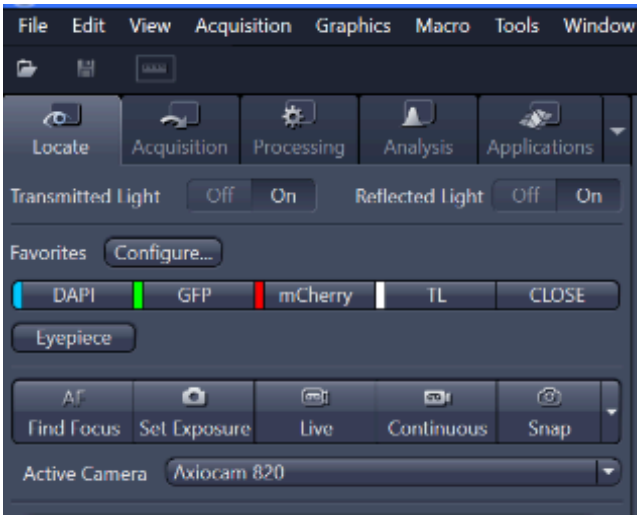
Sample Localization

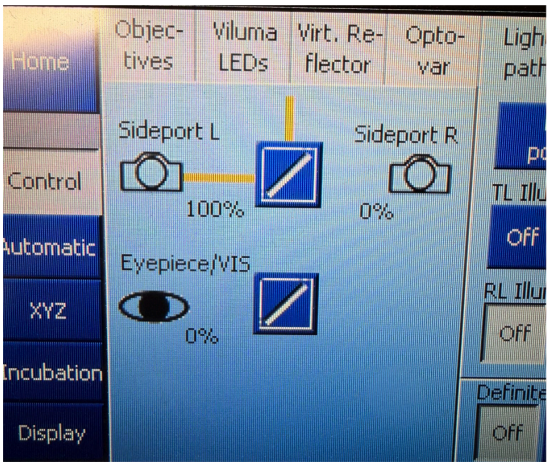
In the **Locate tab**, the user can locate and inspect the sample using either the eyepieces or the camera.

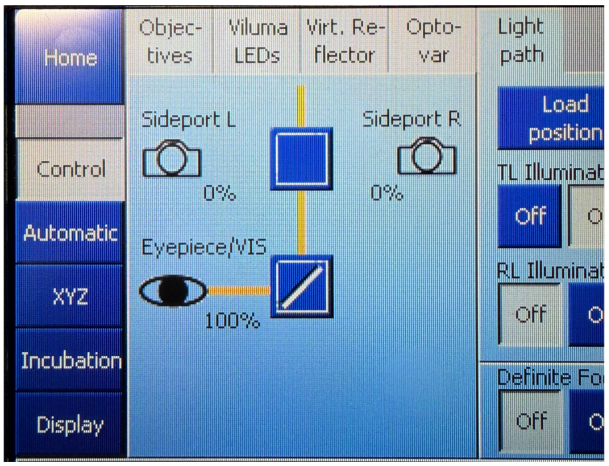
Use the preset buttons to quickly change between the configurations for DAPI, GFP, mCherry fluorescence, BF and Close. The transmitted light setup is used for every brightfield configuration, with the type (brightfield, phase contrast or DIC) being dependent on the condenser setting and

prisms on the light path. On the optical path you need to choose if light goes to the camera or to the eyepieces.

Important: Always press "Close" after inspection to switch off the illumination. Failure to do so will result in continued exposure of the sample to light.







The joystick on the right of the microscope controls the motorized stage and it is used to move the field of view across the sample. On both sides of the microscope body you can find course and fine focus screws to find and focus the sample.

Once the region of interest has been located using the Locate tab, proceed to the **Acquisition tab** to set imaging parameters and acquire images.

Definite Focus

Definite Focus projects a far-red grid onto the plane of focus. When the grid is projected onto the sample carrier surface, it is reflected back into the Definite Focus detector. This method is therefore used to detect the surface of the sample carrier by identifying differences in refractive index at the carrier interface.

1. Click **“FIND SURFACE”**.

2. If the sample is not in focus at the sample carrier surface, adjust the focus manually or use **Software Autofocus**.
   Note: Software Autofocus determines the focal plane within the sample. For thick samples, the focal plane may not be located near the coverslip.

3. Click **“STORE FOCUS”**. Definite Focus will calculate and store the offset between the sample carrier surface and the selected focal plane.

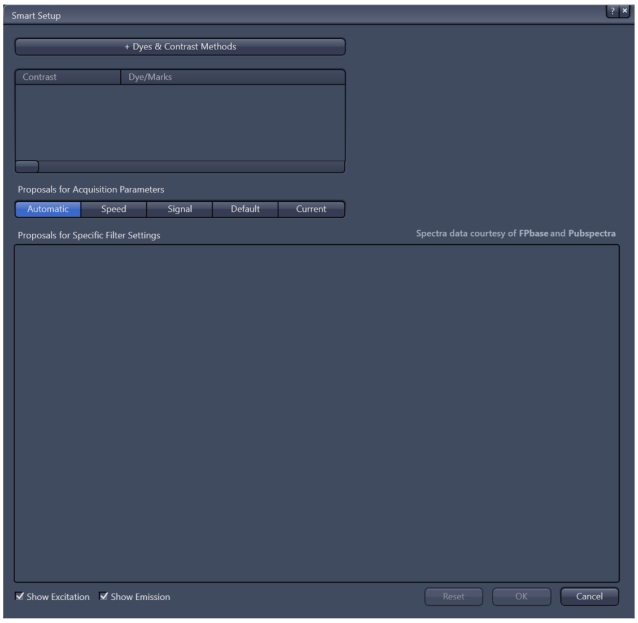
4. Click **“RECALL FOCUS”** to return to the stored focal plane relative to the sample carrier surface.

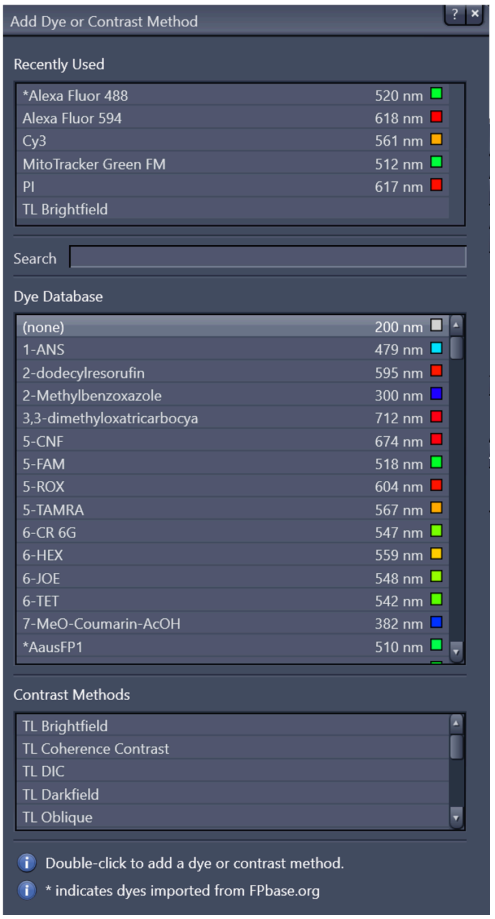
Image Acquisition

Below is a general overview of each section. Further details are provided in the following pages.

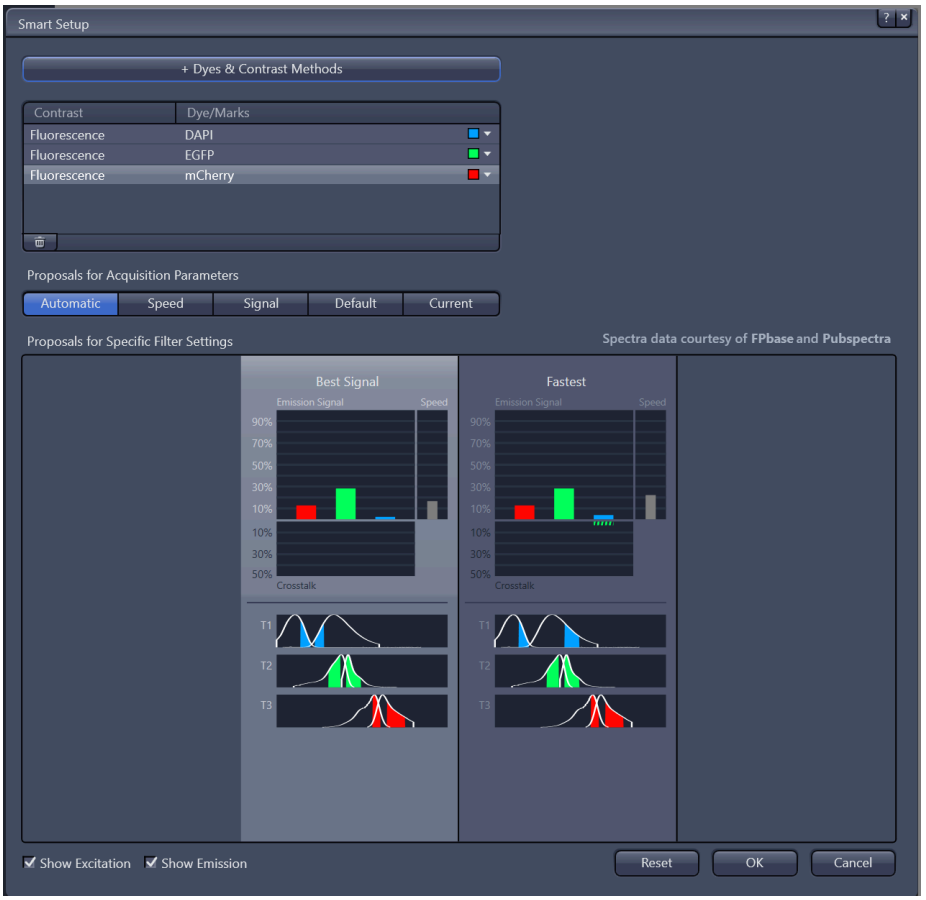
Smart Setup

Clicking **Smart Setup** opens a new window where fluorophores can be selected from a predefined list. If a fluorophore is not available in the list, consult its datasheet to identify the excitation and emission wavelengths and select an equivalent fluorophore from the list. A display colour can be assigned to each fluorophore to facilitate visualization. All acquired images are stored in grayscale; display colours are for visualization purposes only (e.g., GFP may be displayed in purple).





Two acquisition configurations are generated automatically: one optimized for speed (**Fastest**) and one optimized for signal quality (**Best Signal**). The **Best Signal** configuration is recommended unless rapid imaging is required (e.g., for time-lapse experiments). In speed-optimized configurations, a single emission filter may be shared between channels (e.g., DAPI and GFP) to reduce acquisition time.

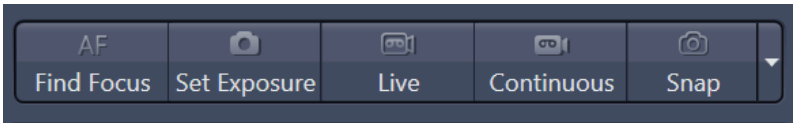


Before starting image acquisition, verify that the light path parameters are correct and that the appropriate LEDs are enabled before proceeding to the **Channels** and **Acquisition Mode** tabs.

⚠️ **IMPORTANT:** Clicking **Apply** will overwrite the current experiment configuration. Do not overwrite configurations created by other users.

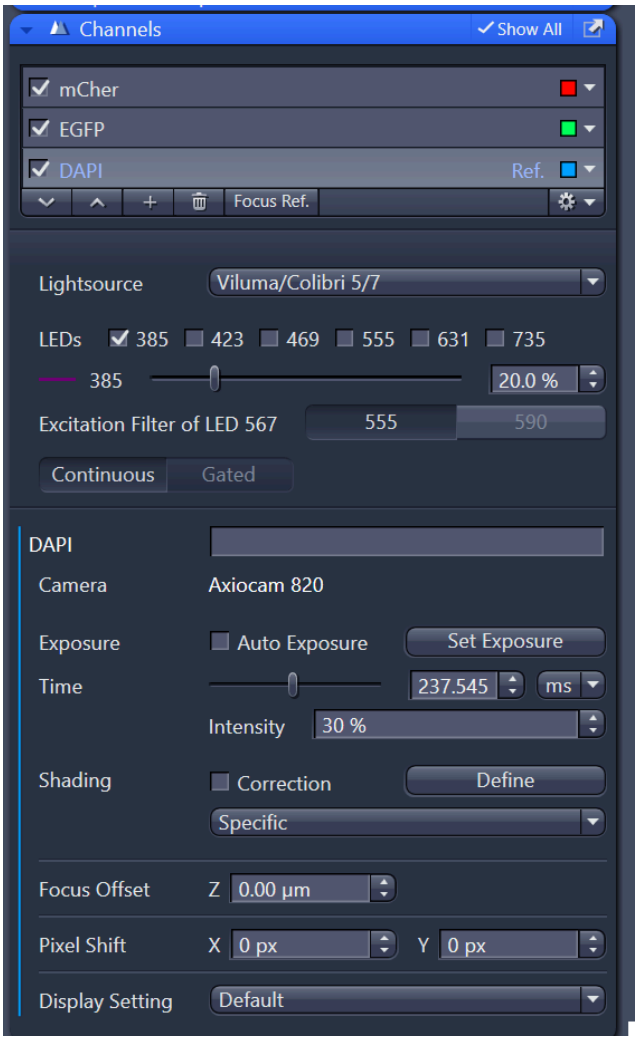
After completing the Smart Setup, click **"Set Exposure"** to automatically determine the exposure times for all channels.

Note: Exposure time defines the duration for which the camera collects signal.



Channels

In this section, users can select which channels (tracks) to acquire and set the **LED intensity**, **exposure time**, and **intensity target** for each channel.



By default, the LED intensity is set to **20%** (e.g., 20% LED with a 100 ms exposure time is generally appropriate).

Tips:

- If the exposure time exceeds **1000 ms**, it is recommended to increase the LED intensity.
- If the exposure time is very short (e.g., **4 ms**), reduce the LED intensity (e.g., to **10%**) and increase the exposure time accordingly.

Intensity target:

Setting the intensity target to **30%** means that the exposure time is calculated to fill 30% of the total dynamic range of the camera. The camera is a **14-bit detector (16,384 gray levels)**, providing a large dynamic range; therefore, it is not necessary to use 100% of the dynamic range. A 30% target is standard, as using 100% may lead to saturation in very bright regions.

For **Apotome imaging**, **deconvolution**, and **quantitative fluorescence analysis**, it is recommended to use **50% of the dynamic range** to increase the number of gray values.
Note: The intensity target can be increased to 50% only for the channel of interest, allowing quantitative imaging without unnecessarily increasing exposure times for other channels.

⚠️ **Important:** For quantitative comparisons, all images must be acquired using the **same exposure time and LED intensity**.

Order of acquisition:

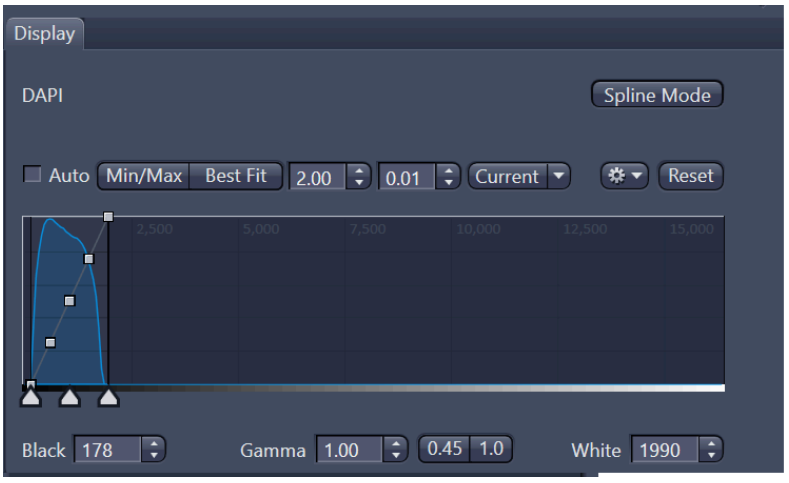
It is recommended to acquire channels from **longer to shorter wavelengths** (e.g., Cy3 → GFP → DAPI) to minimize photobleaching and crosstalk.

Histogram display:

Always evaluate image quality using the **histogram**, not the displayed image. The **Auto**, **Min/Max**, and **Best Fit** options adjust only the display contrast and do not modify acquisition parameters.

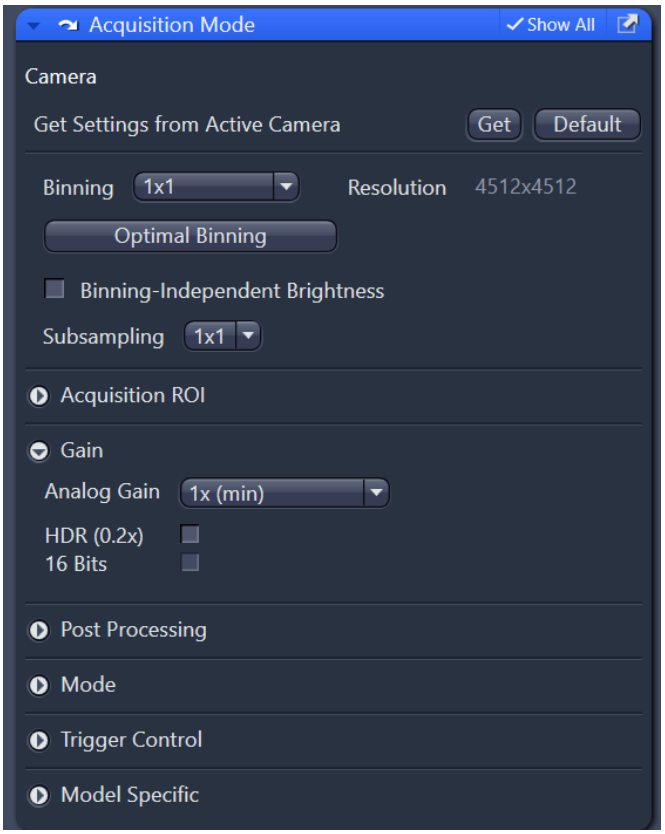
- **Best Fit** readjusts the display limits.

- **Min/Max** sets the display limits to the minimum and maximum pixel intensities.



Acquisition Mode

In this tab, users can adjust **binning** and **gain** settings. These parameters are applied globally to all acquisition channels.



Binning groups adjacent camera pixels, increasing signal intensity at the expense of spatial resolution.

The effective resolution of the microscope depends on:

- **Optical resolution**, determined by the optical pathway and objective
- **Camera resolution**, determined by the effective pixel size (binning increases the pixel size)

To determine the optimal balance between resolution and signal, click **"Optimal Binning"**.

Gain refers to analog amplification of the signal within the camera. Note that gain amplifies all signals equally, including noise.

⚠️ **Important:** Changes to binning and gain affect the required exposure time. Always set **binning and gain first**, and then adjust the **exposure time** accordingly.

Optovar (Additional Magnification)

The system is equipped with an **optovar** providing additional magnification factors of **1.25x** and **1.6x**. The optovar increases the effective magnification at the cost of reduced field of view and photon density per pixel.

The use of the optovar affects:

- Effective pixel size
- Sampling relative to the Nyquist criterion
- Field of view

Examples:

- 20× objective + 1.25× optovar
- 100× objective + 1.6× optovar (recommended for bacterial imaging)

⚠️ **Note:** When using the optovar, exposure time and/or LED intensity may need to be adjusted to compensate for reduced signal per pixel.

For quantitative experiments, changes in optovar settings must be kept consistent across all compared samples.

Image view

- **Live:** Displays a live image of the currently selected (highlighted) channel.
- **Snap:** Acquires a single image of all selected (checked) channels.

Image Display

- **2D:** Displays the current position, time point, and Z-plane for all activated channels as an overlay.
- **Split View:** Displays the current position, time point, and Z-plane for all activated channels as individual channels, as well as an overlay.
- **Gallery:** Displays each Z-slice, tile, or position either as an overlay or as individual channels.
- **Orthogonal:** For the current scene and time point, displays the entire Z-stack with the current optical slice shown in **XY**, and orthogonal views (**XZ** and **YZ**) of all slices. Use the slider bars to navigate through the stack.
- **3D:** Generates a three-dimensional rendering of the Z-stack for the current time point and position, including all activated channels.

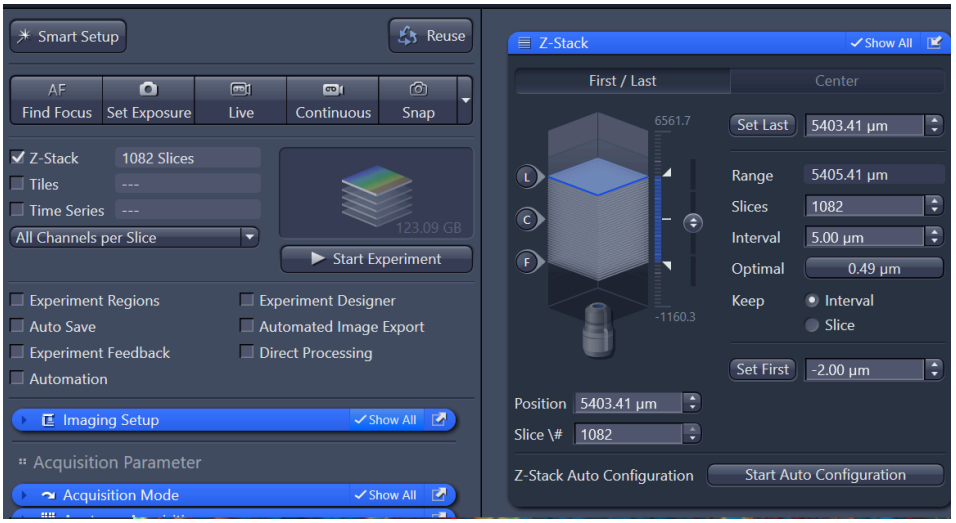
Info

Displays image acquisition metadata, including LED intensity, exposure time, optical path (filters and LEDs), and the number of positions, tiles, time points, and channels. This panel can also be used to annotate images.

Multidimensional Acquisition

All acquisition modes within **Multidimensional Acquisition** can be combined. For example, it is possible to acquire a time-lapse of a Z-stack tile scan at multiple positions within the same sample.

Z-Stack



Using an optimal distance between Z-slices ensures adequate sampling for three-dimensional reconstruction while minimizing photobleaching and phototoxicity (see Nyquist sampling). If the Z-step size is smaller than recommended by the Nyquist sampling theorem, no additional spatial information is gained, and photobleaching and phototoxicity are likely to increase due to repeated imaging of redundant information.

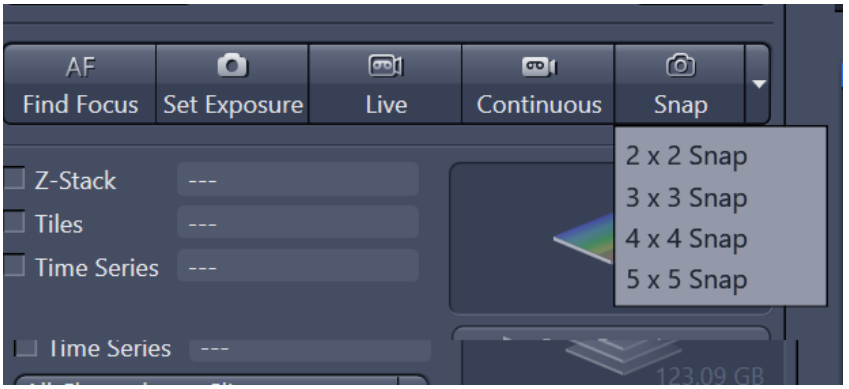
The Z-range can be defined by setting the first and last planes of the stack, or by selecting the current plane as the center of the stack and specifying the total number of slices to acquire. When using **Definite Focus**, the **Center** option is often the preferred approach. In this case, an odd number of slices should be selected to ensure that the current focal plane is included in the acquisition.

For increased accuracy, it is recommended to acquire **all channels at each Z-slice**.

Tile Scan

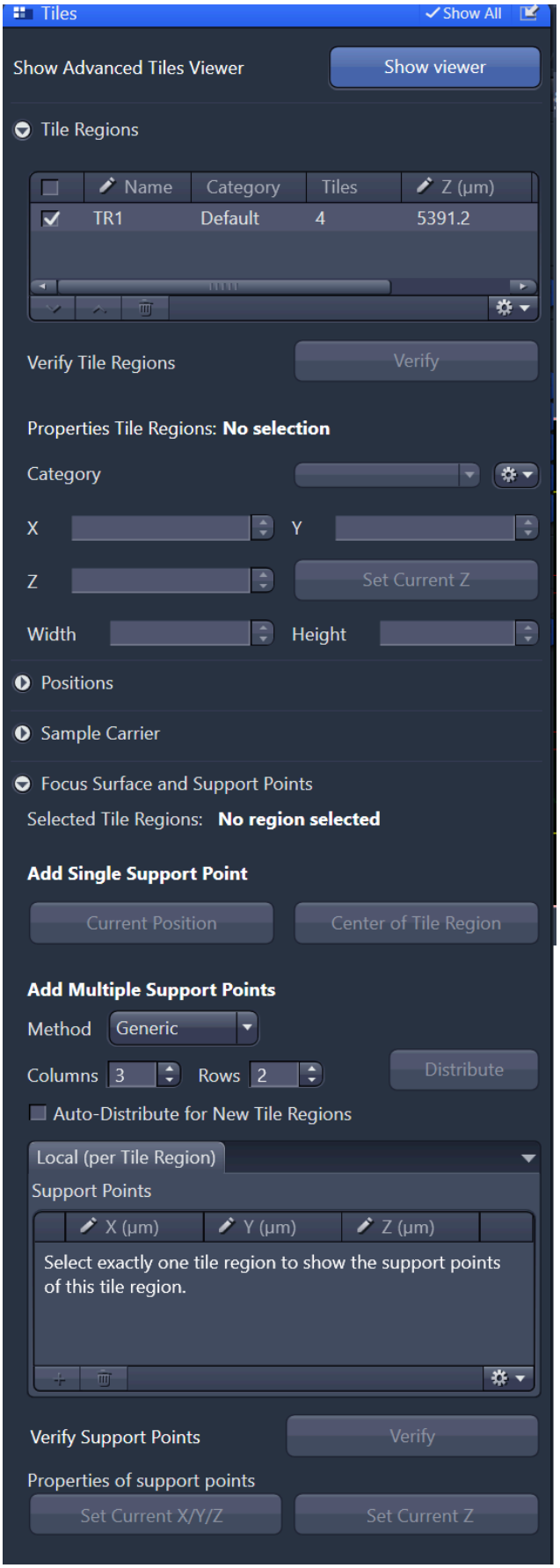
Fast Tile:

By clicking the arrow next to the **"Snap"** button, users can quickly select predefined tile patterns (e.g., **1x1, 2x2, 3x3**) to acquire a fast tile scan.



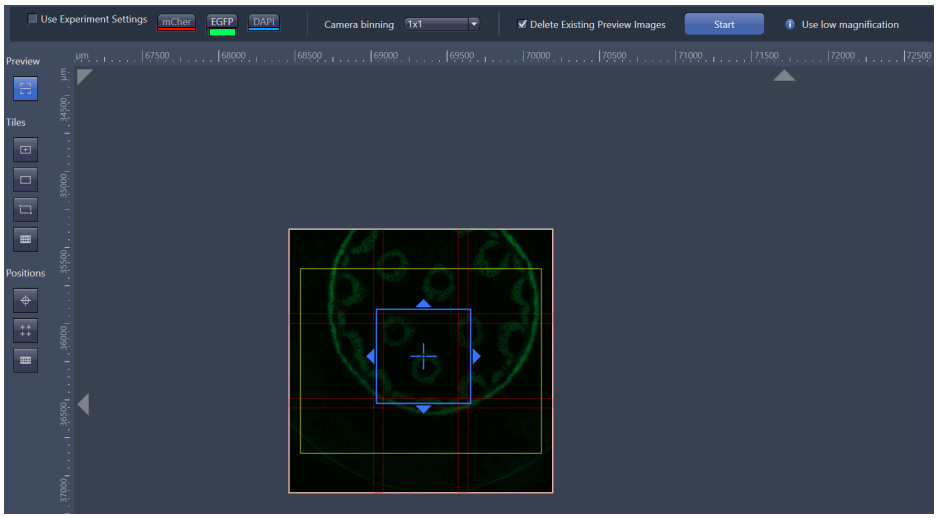
Tile Scan:

Tile scanning allows imaging of large samples that do not fit within a single field of view at high magnification. Multiple images are acquired with overlapping regions (minimum **10%**, optimally **30%**) and subsequently stitched into a single composite image.


Tiles
Show All
Show Advanced Tiles Viewer
Show viewer
Tile Regions
Name
Category
Tiles
Z (µm)
TR1
Default
4
5391.2
Verify Tile Regions
Verify
Properties Tile Regions: No selection
Category
X
Y
Z
Set Current Z
Width
Height
Positions
Sample Carrier
Focus Surface and Support Points
Selected Tile Regions: No region selected
Add Single Support Point
Current Position
Center of Tile Region
Add Multiple Support Points
Method
Generic
Columns
3
Rows
2
Distribute
Auto-Distribute for New Tile Regions
Local (per Tile Region)
Support Points
X (µm)
Y (µm)
Z (µm)
Select exactly one tile region to show the support points of this tile region.
Verify Support Points
Verify
Properties of support points
Set Current X/Y/Z
Set Current Z

Example of Workflow:

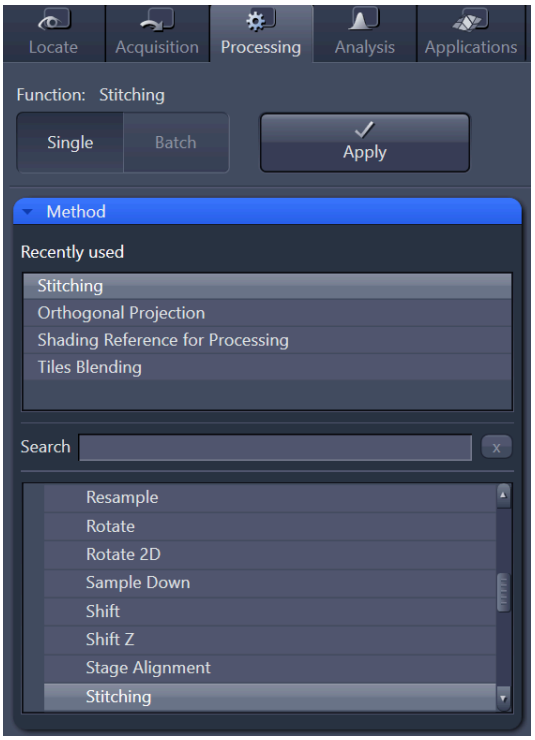
1. Select the **10× objective** and acquire a preview image.
2. Select the desired channel and click **"Start"** to generate the preview.
   The preview image can be saved by right-clicking on the image.



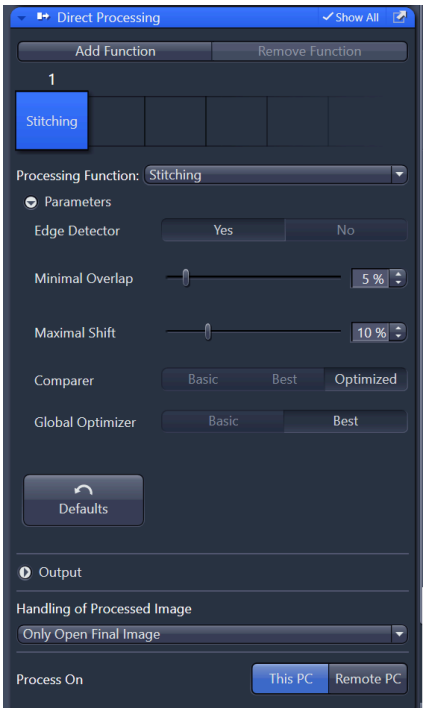
3. Switch to the **20× objective**, draw the region of interest, activate **Live**, and focus the sample.
4. Add **support points** as needed. Support points are particularly useful for large tile scans, as they compensate for Z-position variations across the sample.

Stitching:

- Go to Processing → choose **Stitching.** Select **"All by reference"** and choose the most relevant channel for alignment.



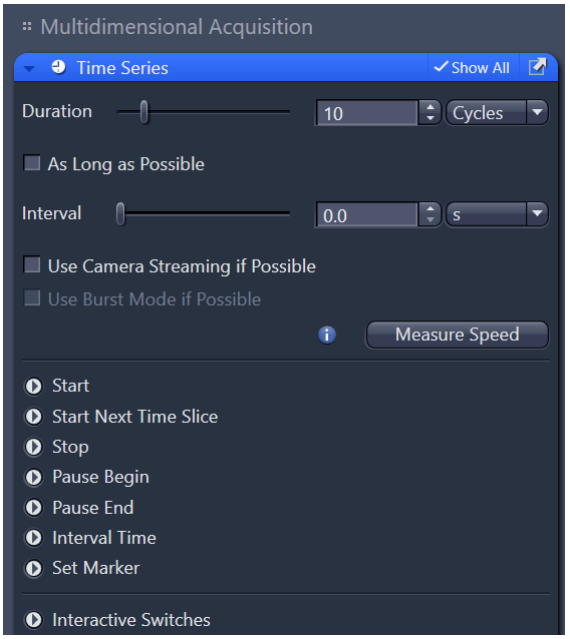
- If stitching will always be performed, it can be included directly in the experiment by enabling **Stitching** under **Direct Processing**.



- **Note:** When acquiring both Z-stacks and tile scans, perform stitching **after acquisition**.

Time Series

This mode allows the acquisition of time-lapse experiments and is particularly effective when used in combination with **Definite Focus**, especially for long-term imaging, to ensure that the sample remains in focus.

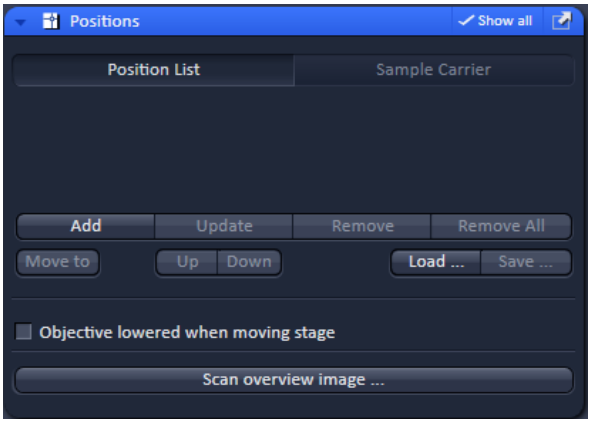


Number of cycles defines the total number of images to be acquired. If set to **0**, acquisition will continue until it is manually stopped.

Interval specifies the time between consecutive acquisition cycles. Note that the interval timer starts when image acquisition begins. If the selected interval is shorter than the acquisition time, the next cycle will start immediately after the previous one finishes.

Positions

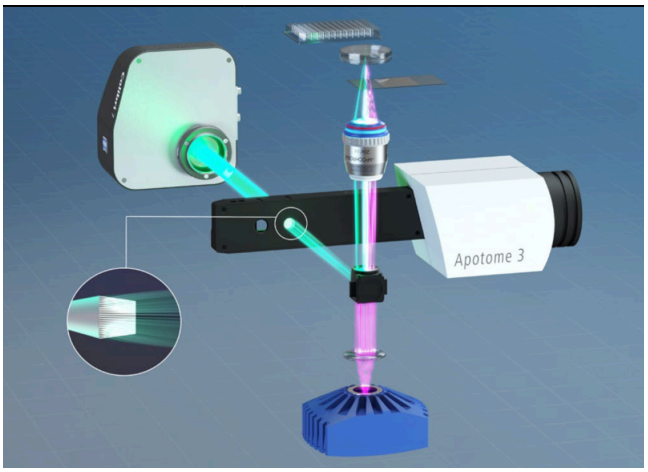
The **Positions** function allows users to save the coordinates of fields of view identified as interesting during sample inspection, enabling their acquisition at a later time.



To store a position, click **“Add”**; the coordinates of the current field of view will be saved.

Apotome: Optical sectioning in fluorescence imaging by structured illumination

The **Apotome** is a movable slider inserted into the excitation light path between the light source and the filter wheel. It enables optical sectioning in fluorescence imaging using structured illumination.

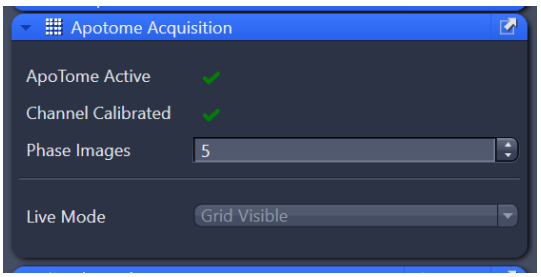


⚠️ IMPORTANT – APOTOME USE

- **Insert and remove the Apotome gently.**
- **Always remove the Apotome from the optical path after use.**

Apotome Acquisition

- Insert the Apotome gently into the optical path of the microscope.
- Switch to **Apotome acquisition** mode in ZEN Blue.



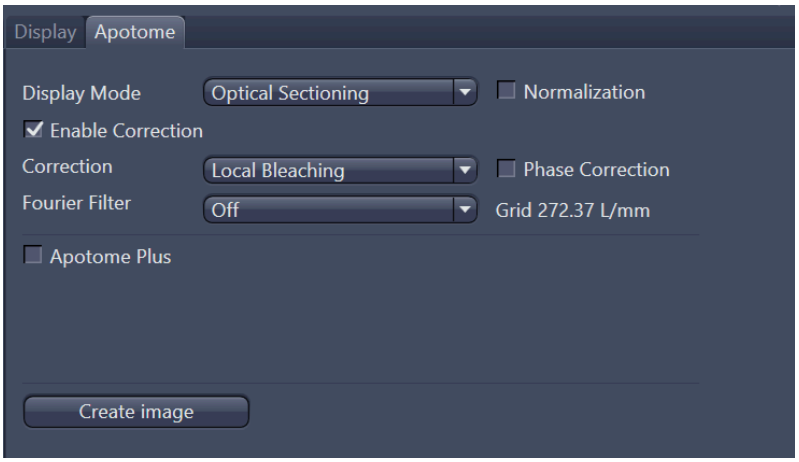
- Verify that the Apotome is **calibrated for the selected objective and excitation wavelength**. If it is not calibrated, contact the facility staff.
- Set the **intensity target to 50%** to use 50% of the camera's dynamic range.

- Click **“Set Exposure”**.

  Note: Longer exposure times are expected in Apotome mode.
- The number of **grid phases** is selected automatically (typically **3–15**).
  - If significant photobleaching is observed with **5 phases**, reduce to **3 phases**.

Processing Apotome Images

1. Select the acquired Apotome image.
2. In the **Display** panel, select the **Apotome** option.



3. Under **Optical Sectioning**, click **“Create Image”**.

   The resulting optically sectioned image is smaller than the raw dataset (e.g., a five-phase acquisition is converted into a single processed image).

Available data outputs include:

- **Optically sectioned image**
- **Conventional (widefield) image**
  Raw data

Apotome Plus – Deconvolution

Apotome Plus enables structured illumination–based optical sectioning combined with deconvolution for improved contrast and axial resolution.

⚠️ **IMPORTANT:** Always leave the Apotome out of the optical path after completing your experiment to ensure proper system configuration for the next user.

Shutting Down System

1. Lower the stage to **Load Position** > **Set Working Position.**
2. Remove your sample.
3. Clean oil objectives with lens tissue with a small amount of absolute ethanol (do NOT use dry lens tissue, do NOT rub the paper forcefully in the objective, after cleaning check the objective is clean by shining light and seeing if there are no reflections - for example with the light from your mobile phone).
4. Leave the microscope with the **10x objective** in place!
5. Make sure Apotome is out!
6. Exit software and backup your data.
7. Switch off computer.
8. Switch off the microscope (indicated as 3/3).
9. Switch off the **Components switch** outlet (indicated as 2/3).
10. Turn off the **Computer Switch** outlet (1/3);