

Zeiss Axio Zoom V16 - Users' Guide

Author(s): Fernando Cruz; Mariana Ferreira

Date: June 2020

Purpose: Zeiss Zoom V16 is a zoom microscope that successfully combines a large field of view, zoom, and working distance. Its 1x objective has a 0.25 numerical aperture (NA), a considerable NA for a medium zoom range allowing for superior fluorescence brightness in large object fields. The Axio Zoom V16 is the fluorescence zoom microscope fully automated for large field visualization having a motorized zoom from 7x to 112x, motorized Z focus and easy-to-use system control panel.

Contents

Safety instructions and contacts.....	2
Equipment location, description and technical specifications	3
Sugestion for description in “Materials and Methods”.	6
Information to be added to the “Acknowledgements” section.....	6
User pre-requisites, booking and general rules.....	7
Usage charges	Error! Bookmark not defined.
System Overview	8
System Protocols.....	9
Start Up Procedure.....	9
Check Before Use	10
Shutdown Procedure	11
Mounting a sample and acquiring images:	12
Sample Preparation Notes	12
Zen 2.1 blue edition General User Interface	12
Multidimensional Image Acquisition (Multi-Channel Images)	13
Channel Combine or Image Overlay.....	21
Extracting Single Fluorescence Images of a Multichannel Image	25
Closing ZEN 2.1	26
Downstream Image Processing and Analysis.....	27

Safety instructions and contacts

Safety instructions and first aid procedures

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

Mariana Ferreira, Bacterial Imaging Cluster (mariana.g.ferreira@itqb.unl.pt)

Adriano O. Henriques, Microbial Development Group (aoh@itqb.unl.pt)

Mónica Serrano, Microbial Development Group (serrano@itqb.unl.pt)

Pedro Matos Pereira, Bacterial Cell Biology Group (pmat@itqb.unl.pt)

Phone +351 214 469 524 Extension 1522/4 or 1566/7

Online information: <http://www.itqb.unl.pt/bic>

Equipment location, description and technical specifications

Location: Room 5.25 (Bacterial Imaging Cluster), 5th floor, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA)

Manufacturer: Zeiss

Model: Zeiss Axio Zoom.V16

Year Installed: 2016

Potential Techniques

- Large Sample Observations
- Time-lapse
- Dark Field Microscopy

Features and Specifications

- Focus Motor 3 with Central Profile Stand
- Transillumination Base 300
- Controller EMS 3
- Sycop System Control Panel
- Motorized Reflector Module Z FL w/ ACR (Automatic Component Recognition)
- Camera: AxioCam 503 Mono
- Eyepieces: PL 10x/23 Br. Foc. (High eyepoint and Focusing)

Transmitted Light Techniques

- Dark Field
- Bright Field

Incident Light

- Fluorescence: HXP 200C Illuminator (w/ 200 W mercury short-arc reflector lamp)

Objectives

Magnification ¹	1x
ZEISS System	Plan-Neofluar Z
Class	Colour and Chromatic Correction
Numerical Aperture (NA)²	0.25
Immersion	Air
Free Working Distance (mm)	5.6

¹Notice that the microscope is equipped with two Eyepieces: PL 10x/23 Br. Foc

² NA - Numerical Aperture. For more information, follow this [link](#).

- Remember to choose the proper calibration if doing montages/size measurements.
- The pixel size in microns of your picture is saved automatically (if you've chosen the correct calibration). Use [LOCI](#) to recognize it with ImageJ.
- The pixel size in microns of your picture is saved automatically when using Zen. Use [Bio-formats](#) to recognize it with ImageJ/Fiji (already installed in the Fiji package).

Filter-Sets for Incident Light

Position	Filter Cube	Excitation Filter	Dichroic Mirror	Emission Filter	Emission Colour
1	HE GFP	BP 470/40	495	BP 525/50	Green
2	HE YFP	BP 500/25	515	BP 535/30	Green/Yellow
3	HE mRFP	BP 565/30	585	BP 620/60	Red
4	Empty	-	-	-	-

BP - Bandpass Filter

HE – High Efficiency Filter

Computer

- Type: Desktop
- Brand: ASUS®
- Model: i9S
- Features: Intel i5 4460 up to 3.2 GHz (Quad-Core); 8GB RAM; 1TB HDD
- OS: Windows 7 Professional
- Available Software: **Zeiss Zen 2.1**

Monitor

- Brand: Samsung®
- Model: S24C250B
- Diagonal: 24"

Zen 2.1 (blue edition)

- The ZEN 2.1 (blue edition) can be equipped with several modules, applications and licenses. This guideline is strictly specific for the **ZEN 2.1 (blue edition)** version available on the microscope's computer.

Suggestion for description in “Materials and Methods”.

Images were acquired on a Zeiss Axio Zoom.V16 stereo microscope equipped with a Zeiss Axiocam 503 mono CCD camera and controlled with the Zeiss Zen 2.1 (blue edition) software, using the 1x 0.25 NA objective, the fluorescence filter sets HE GFP + HE mRFP and the Bright Field optics .

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".

User pre-requisites, booking and general rules.

- The equipment cannot be used without official training.
- Before your first use contact Mariana Ferreira to schedule training.
- New users must have a clear understanding of the principles of light microscopy and how fluorescence works.
- Save all data into your own storage device as we routinely clean the computer's data
- Register your utilization in the logbook
- For all downstream image analysis image analysis a free software license is available for ZEN lite at ZEISS [website](#). Alternatively, you can use [FIJI](#).
- Contact the responsible people in case of any doubt or any issue with the equipment.
- Booking is done online at the [ITQB intranet](#). Please mind the following rules when booking the zoom microscope.

System Overview



System Protocols

Start Up Procedure

If you plan to use fluorescence and the lamp is turned off, ensure it has been off for over one hour and when you touch the right side of the lamp case it should be cool to the touch.

1. Switch on the components in the following order:

1.1. Incident Light Source HXP 200 C (Fluorescence Lamp) IF you need fluorescence

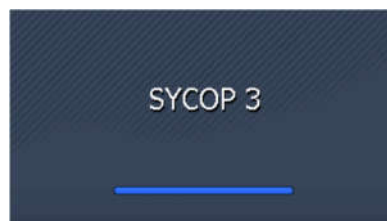


1.2. Electronic Module EMS 3



1.3. Computer

2. Wait for the SYCOP 3 control panel to initialize



3. Run ZEN (blue edition) on the computer and select Zen Pro



Check Before Use

1. Pull the red STOP button on the left side to remove the warning message on the SYCOP 3 screen and unlock system movement.



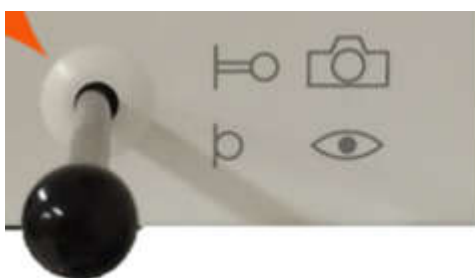
2. Ensure the aperture diaphragm is completely open by turning the rotary knob, on the right-hand side of the microscope, up. You can check on the ZEN Blue software the open percentage.



3. Open the slider and turn on the Transmitted Light source on the right side of the base and choose between the Bright Field (BF) and Dark Field (DF) condensers on the left side.



4. On the right of the eyepieces check if the light is going to the eyepieces or the camera and adjust accordingly.



Shutdown Procedure

1. **Before** starting the shutdown procedure check whether there are any users booked within an hour if so, leave the system on otherwise follow the shutdown procedure.
2. Confirm you have saved all your images and close the ZEN (blue edition) software.
3. Copy your data to a flash drive or external hard drive.
4. Turn off the computer.
5. Press the STOP button to lock the system movement.
6. Turn off the transmitted light source if you turned it on.
7. On the SYCOP 3 touchscreen Home tab press the shutdown button on the top right corner, select Yes and wait for the system to shut down.



8. Finally turn off the Incident Light Source HXP 200 C (Fluorescence Lamp) if it was on.

Mounting a sample and acquiring images:

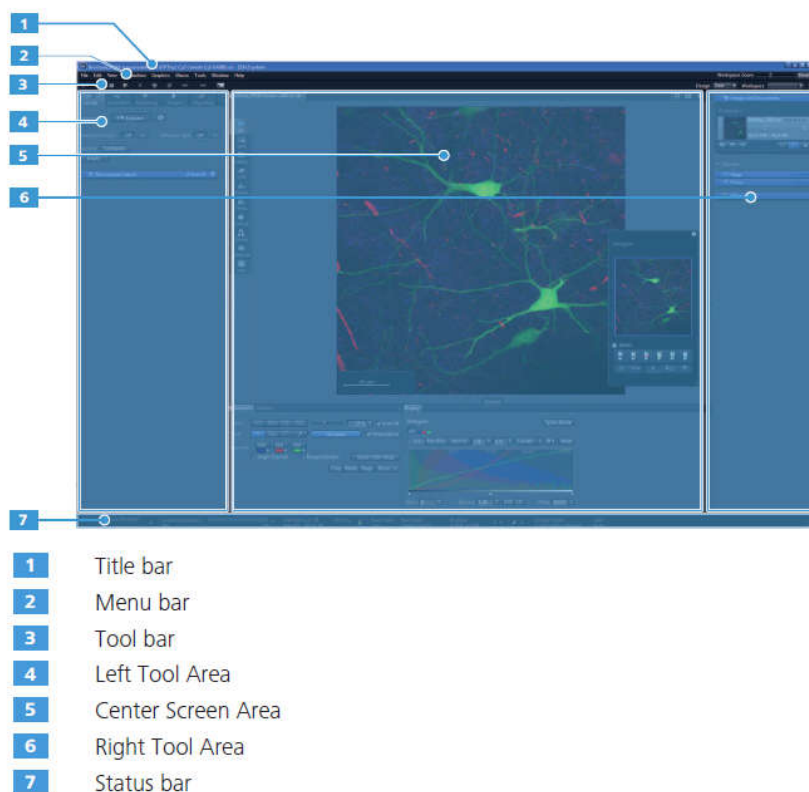
Sample Preparation Notes

- You can use resources such as the [FPBase](#) or the [Thermofisher](#) SpectraViewers to explore your fluorophores. Learn their excitation and emission wavelengths, and what are their optimal filters!
- This system is optimized for larger samples like bacterial colonies or seedlings.

Zen 2.1 blue edition General User Interface

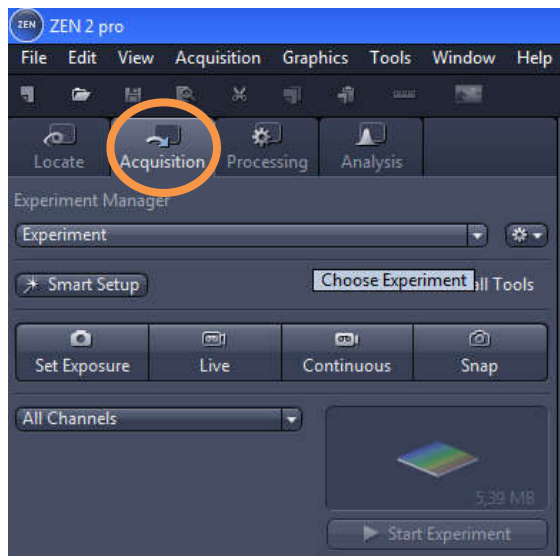
The ZEN 2.1 (blue edition) can be equipped with several modules, applications and licenses. This guideline is strictly specific for the ZEN 2.1 (blue edition) version available on the microscope's computer.

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**) and image analysis (**Analysis tab**). The **Centre 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.




Multidimensional Image Acquisition (Multi-Channel Images)

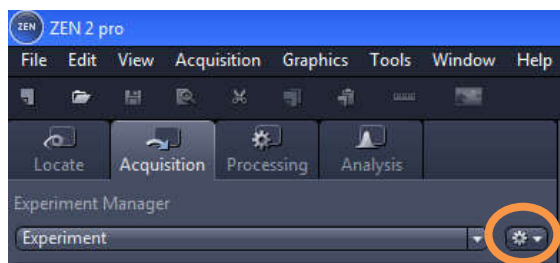
1. Go to the **Acquisition** tab




You can create a new blank experiment. Alternatively, you can use one of the pre-set experiments. Try to follow the protocol until the end and then look at the pre-set experiments that may be useful for your experiment. In this way, we can keep the workspace organized and without redundancy.

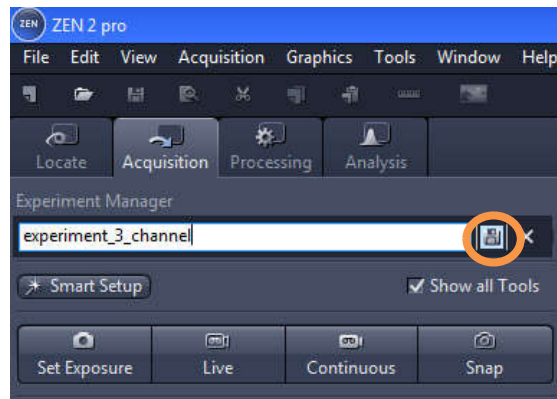
You can use and alter the available pre-set experiments as long as those alterations are not saved at the end! We advise this option for short-term experiments

2. To create a new, "empty" experiment, click in the **Options**  button of the **Experiment Manager** section. The **Options** dropdown list will open.



3. Click on the **New** entry.
4. Enter a name for the experiment, e.g. "*experiment_number_channel*"

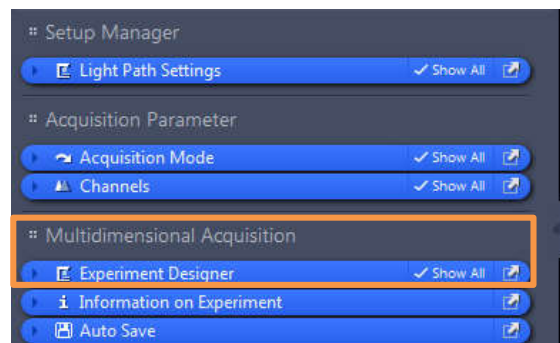
5. To create the experiment, click in the **Save**  button



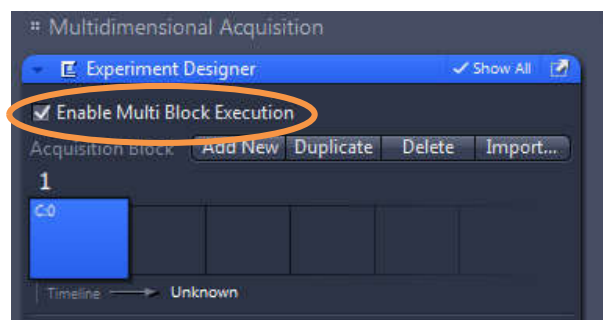
You have created a new blank experiment. For any change in the experiment, an asterisk (*) will appear after the file name. This means that the experiment was modified and not saved. Save your experiments from time to time to ensure that your settings are not lost.

6. Start focusing the sample (See above **Error! Reference source not found. -> Error! Reference source not found.**)

7. Now in the **Multidimensional Acquisition** group, click in the blue header of the **Experiment Designer** tool

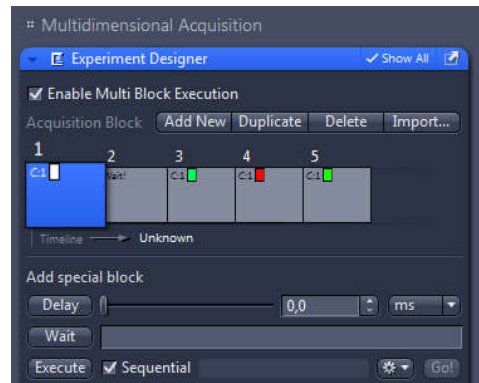


8. Make sure to box the **Enable Multi Block Execution** option



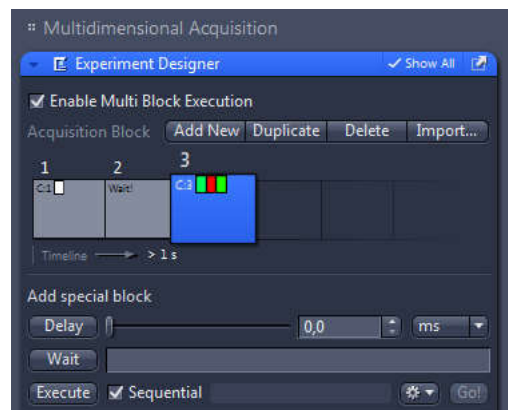
9. In the **Acquisition Block** section, click in the **Add New** **Add New** button.

a) Add as many blocks as contrast methods/filter sets you would like to acquire. For instance, to acquire images of Bright Field, GFP, mRFP and YFP, four blocks must be added. This approach will generate one image for each contrast method and filter set.



Later, you will be able to combine two images at a time into one using ZEN 2.1 (See **Error! Reference source not found.** -> Channel Combine or Image Overlay)

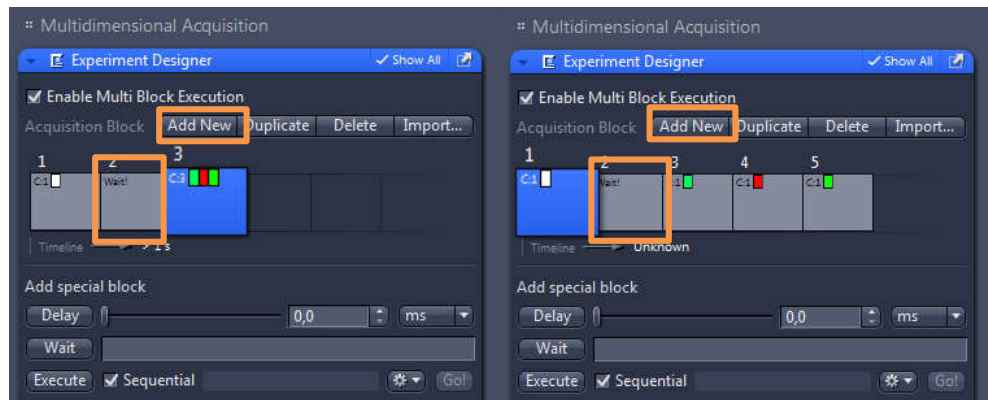
b) Alternatively, you can add only one block. This approach will generate a multichannel image of all selected filter sets. Combine only filter sets into a single block though, as it is required one block for each single contrast method.



In the end, you can extract individual fluorescence images of a multichannel image (See **Error! Reference source not found.** -> Extracting Single Fluorescence Images of a Multichannel Image)

c) If you have selected multiple contrast methods that require distinct illumination methods (e.g. Bright Field and Fluorescence), you must use the **Wait** block. To set a **Wait** block,

just click in the **Wait**  button.

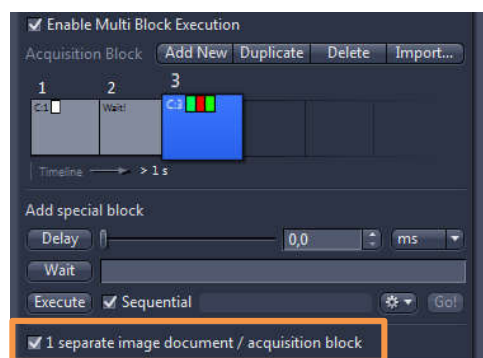


As it is strictly required to operate the diaphragm slide between transitions of transmitted and incident light (e.g. from bright field to fluorescence GFP), the **Wait** block will make a pause during the experiment.

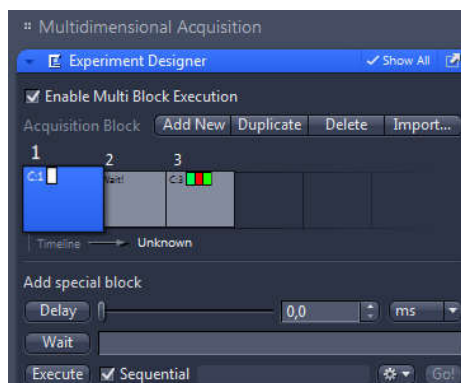
At the pause, you should set the diaphragm slide in agreement with the next contrast method.

To switch the order among blocks, just click in a block and move it into the desired position of the timeline of events.

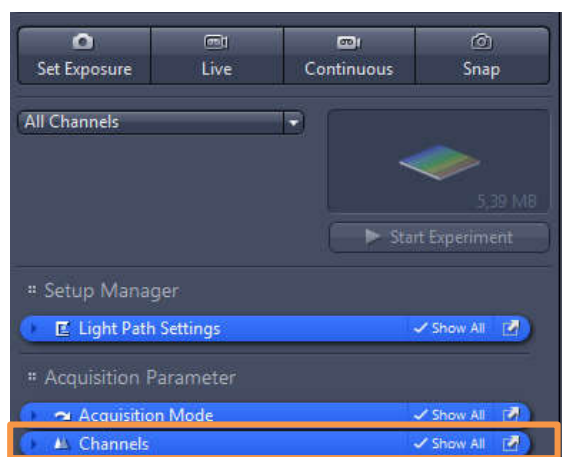
10. Box the **1 separate image document / acquisition block** option to create an image for each block of the timeline of events




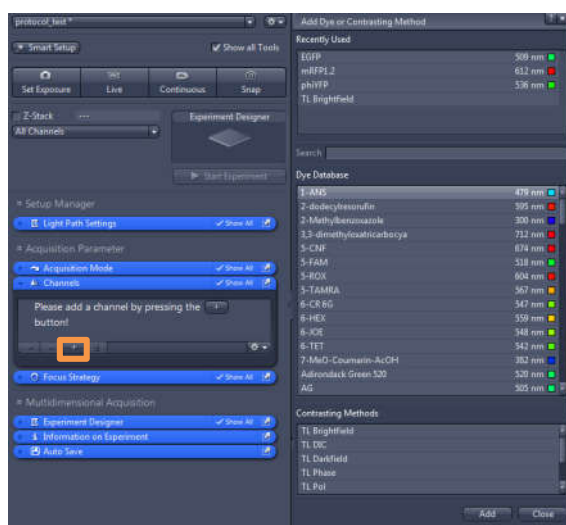
11. Select the first block in the timeline of events



12. Now in the **Acquisition Parameter** group, click in the blue header of the **Channels** tool

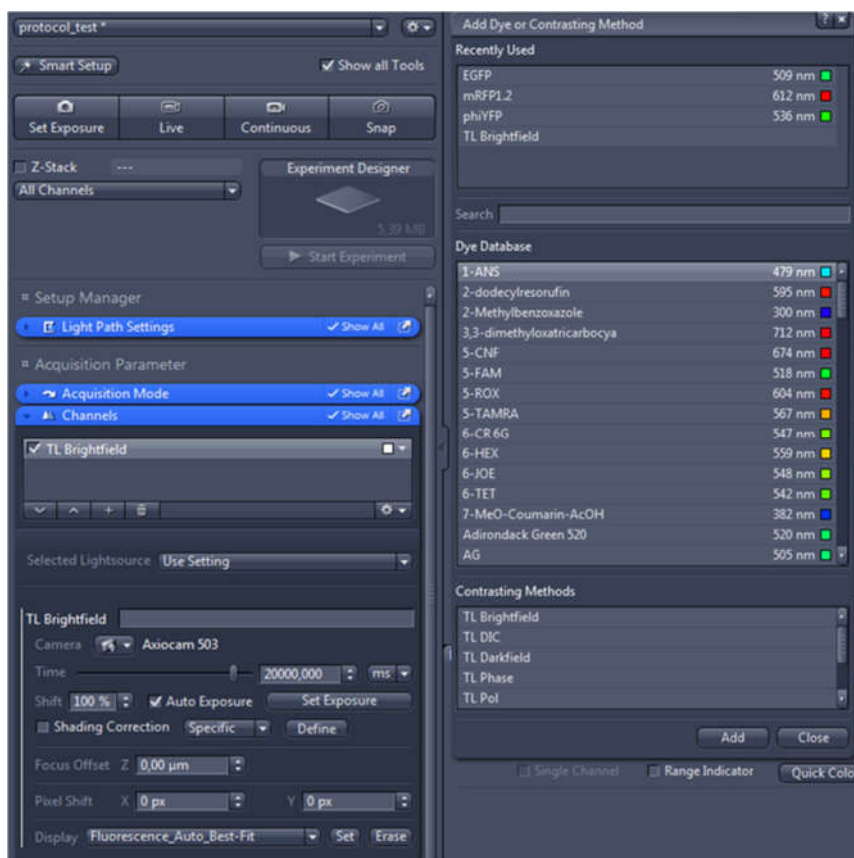


13. Click in the +  button to open the **Add Dye or Contrast Method** dialog



14. Select the desired filter set (from the **Dye Database**) or contrast method (from the **Contrasting Methods**). You can search for a dye by entering its name (or starting letter) in the **Search** input field (See above the available **Filter Sets**).

15. Click in the **Add** button at the bottom of the dialog or simply double-click an entry



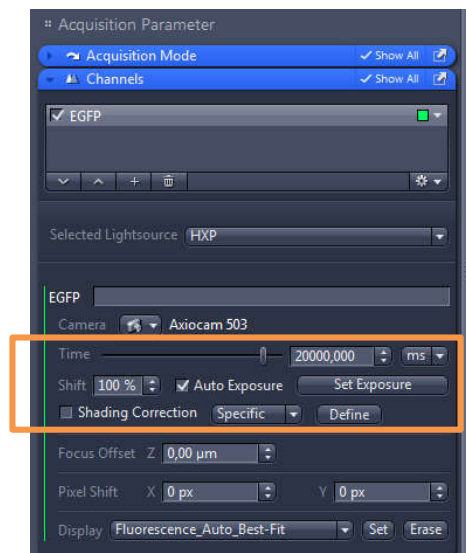
IF you have previously selected one single channel image per block, click in the **Close** button of the **Add Dye or Contrast Method** dialog and move to the next step.

IF you have previously selected a multichannel image by setting only one block for acquiring multiple filter sets (multichannel image), repeat this step to add the remain channels. Close the dialog at the end.

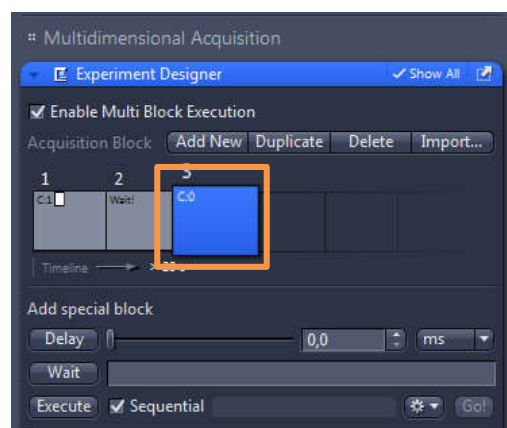
16. Once you have selected a dye(s) or contrast method, one will be displayed in the **Channels** tool




17. Set the **Exposure Time** for each channel, just right beneath the channels list. There will be more than one channel in a multichannel block. Thus, the **Exposure Time** should be set one channel at a time

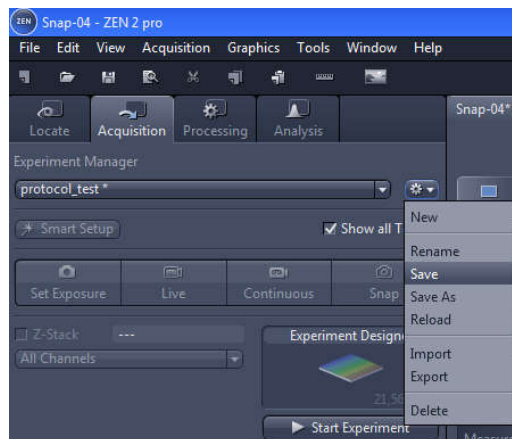


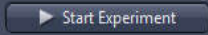
18. Repeat the steps 12-17 for the remaining blocks. Once you have finished assigning all channels and contrast methods, move to the next step

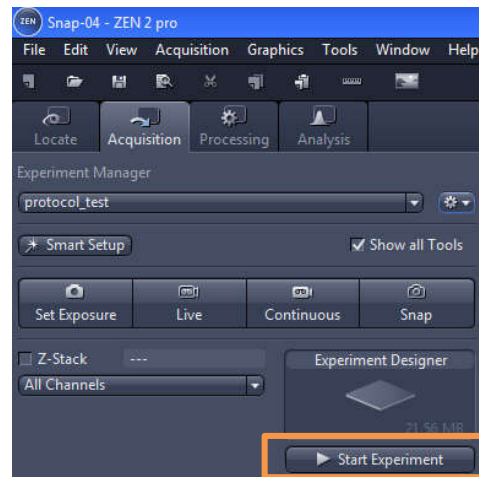
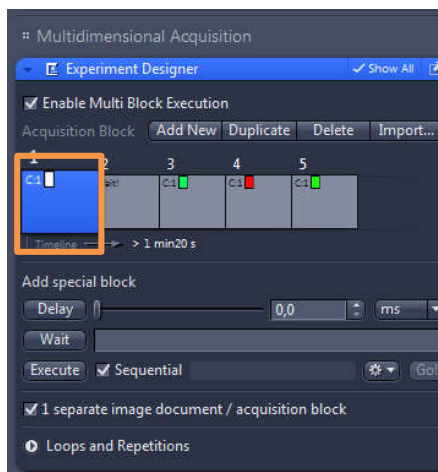


19. To save the experiment together with all the settings, click in the **Options**  button of the **Experiment Manager** section and select the **Save** entry

!!! Save ONLY self-made-experiments!!! **Do not save** previously pre-set experiments, unless they are yours



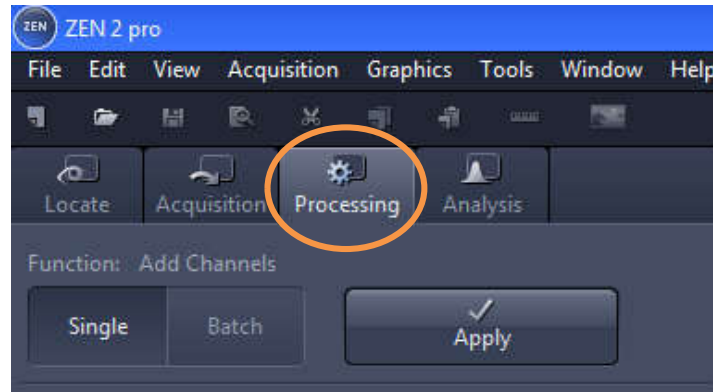
20. Finally, select the first block of the timeline of events (in the **Experiment Designer** tool) and click in the **Start Experiment**  button



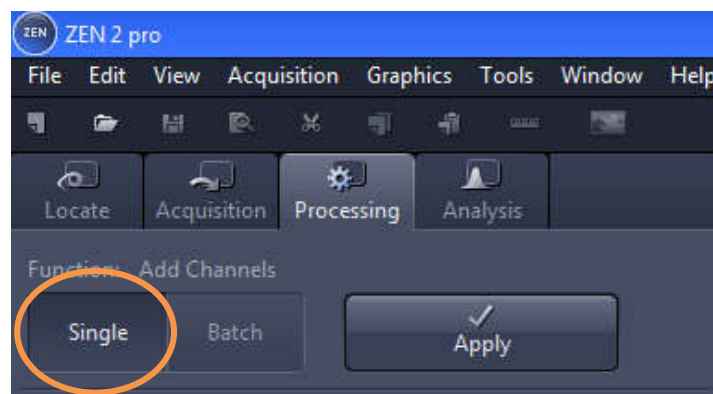
21. The last image to be acquired will be displayed in the **Centre Screen Area**. The remain images will appear in the right-side **Right Tool Area**

Channel Combine or Image Overlay

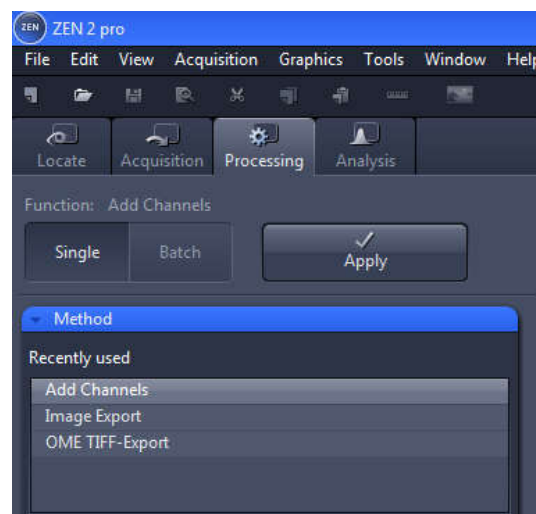
1. Go to the **Processing** tab



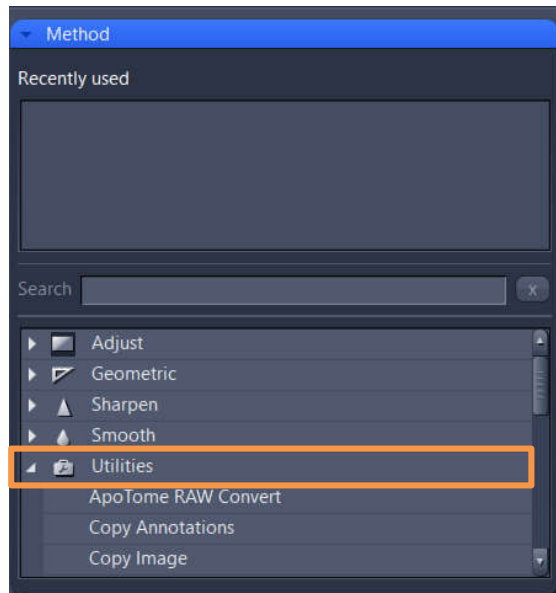
2. Click in the **Single** button



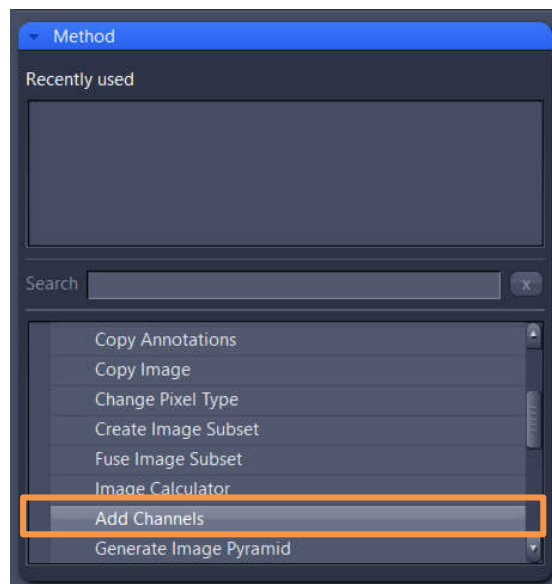
3. Click in the blue header of the **Method** tool



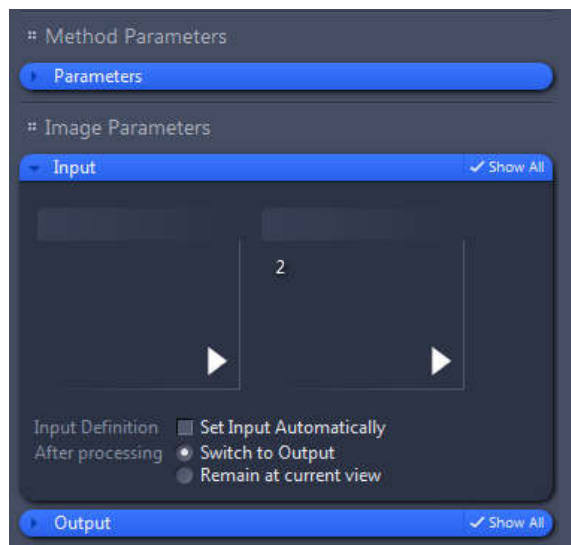
- Click in the **Utilities** group to display the dropdown list of tools included in this category



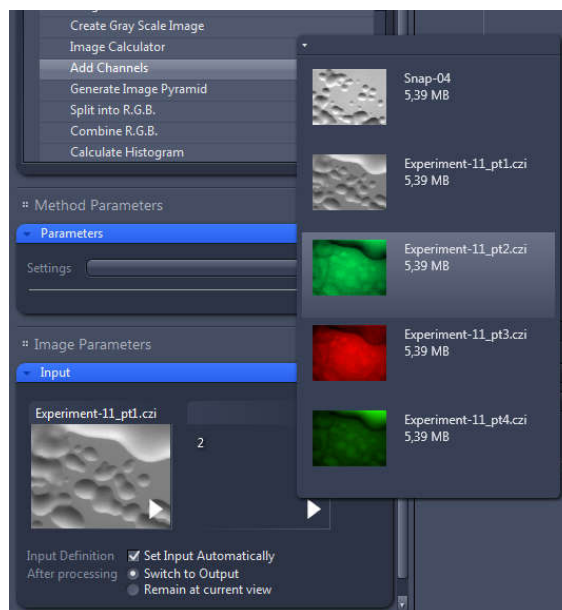
- Search for the **Add Channel** tool and click in it



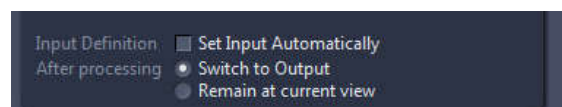
6. Click in the blue header of the **Input** tool



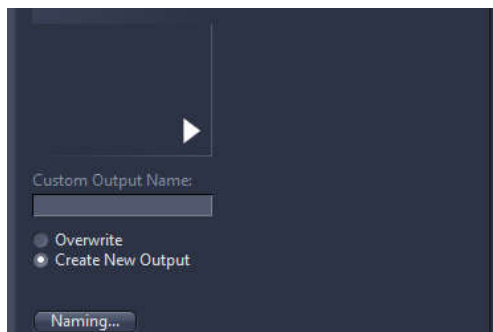
7. Select images of channels you would like to combine. To do so, click in the small preview image within the **Input** tool. You will see a preview of all open images. To select an image, just click in it



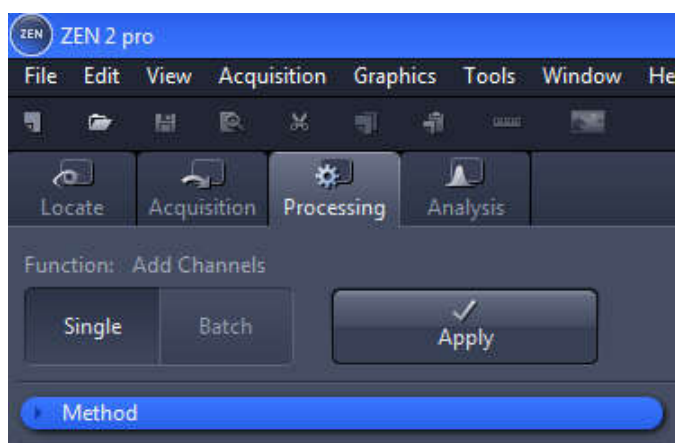
8. Yet in the **Input** tool, select the **Switch to Output** option only



9. Click in the blue header of the **Output** tool and select the **Create New Output** option only. Additionally, you can name the new image as you like by clicking in the **Naming** button

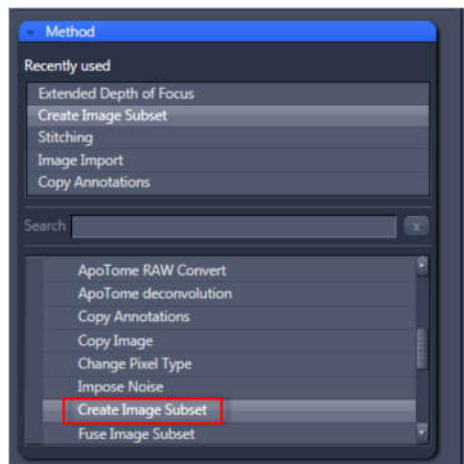


10. Finally, click in the **Apply** button. The image combined will be displayed in the **Centre Screen Area**.

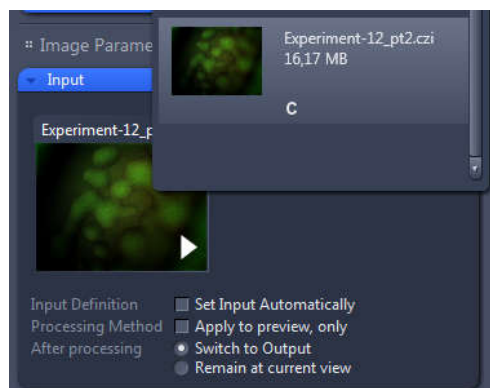


Extracting Single Fluorescence Images of a Multichannel Image

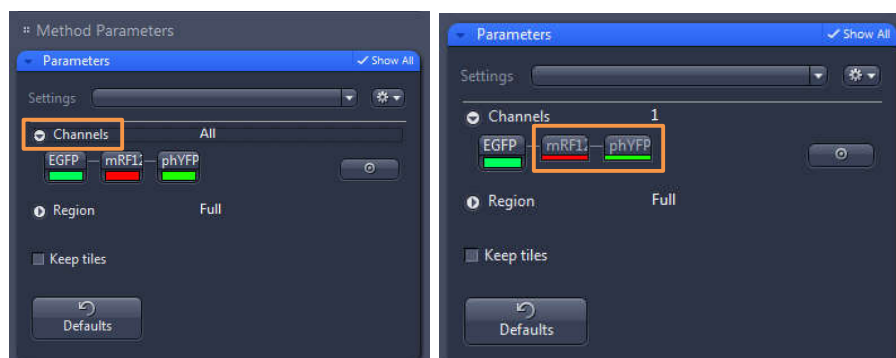
1. Select the **Processing** tab. Open the **Method** tool and under **Utilities** select the entry **Create Image Subset**

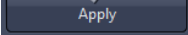


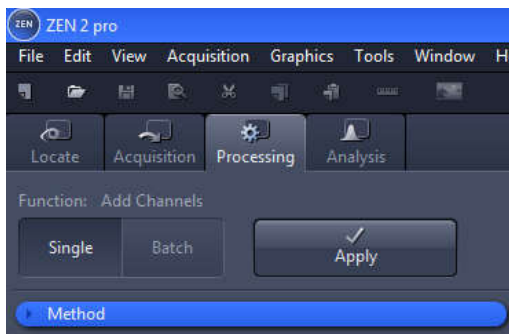
2. In the **Image Parameters** group, open the **Input** tool and select the multichannel image as input



3. In the **Method Parameters** group, open the **Parameters** tool and click in the **Channels** entry. For instance, deactivate the red and green/yellow fluorescence channels by clicking in the corresponding channels to extract only a single green fluorescence image

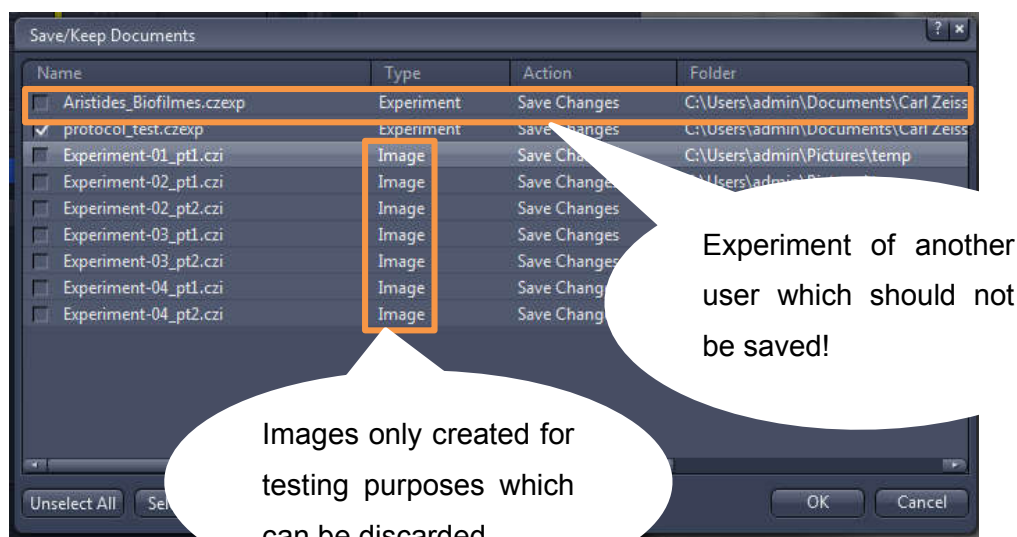


4. Finally, click in the **Apply**  button. The single channel image will be displayed in the **Centre Screen Area**



Closing ZEN 2.1

1. To end ZEN 2.1 software, go to **File -> Exit**. Alternatively, you can press **ALT + F4** on your keyboard or click in the **Close** icon of the program bar.



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.

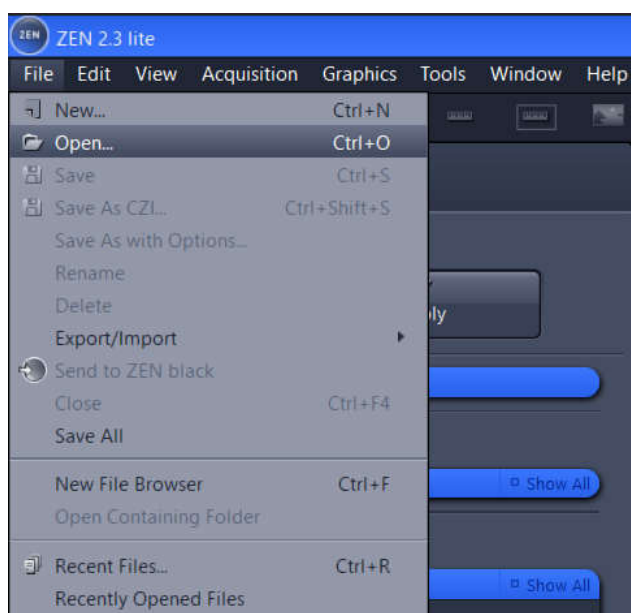
Save only documents that are actually yours, and not pre-set experiments that belong to others or light path settings of the favorites filter sets.

Downstream Image Processing and Analysis

A free software license is available for ZEN lite at ZEISS website (<https://www.zeiss.com/microscopy/int/products/microscope-software/zen-lite.html>). Install and use such version in your own personal computer for downstream image analysis

Open

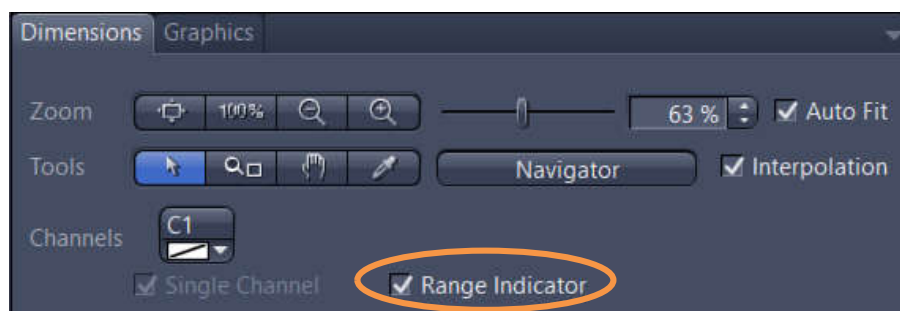
1. Go to **File -> Open**



2. Search for the .dzi files previously saved in the directory dialog
3. The selected files will be displayed in the **Centre Screen Area** and **Right Tool Area**

Display Adjustments

1. In the **Dimensions** tab of the **Centre Screen Area**, activate the **Range Indicator** checkbox. This will mark in red or blue overexposed (too bright) or underexposed (too dark) areas, respectively



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



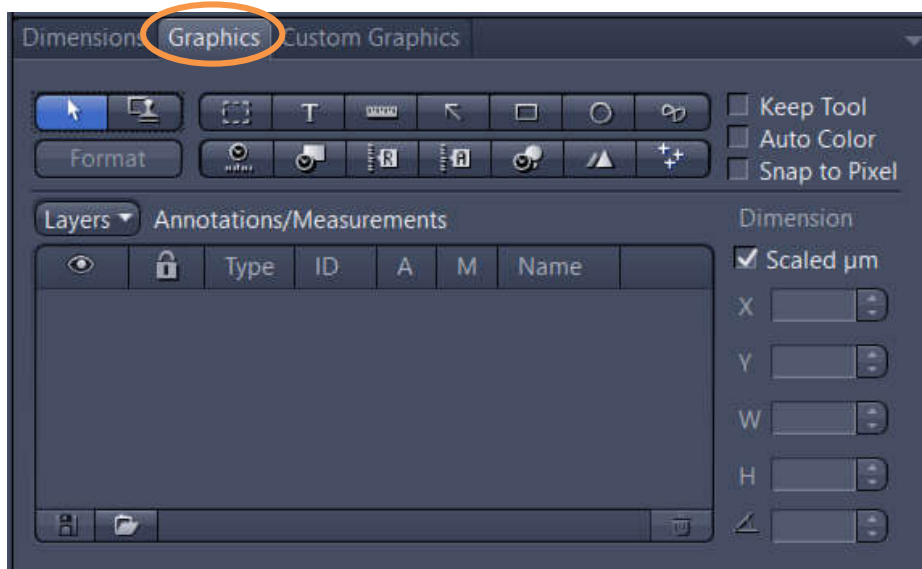
3. Move the controls left and right under the multiple curves in order to directly adjust the values for **Brightness (White)**, **Gamma**, and **Contrast (Black)** in the live image





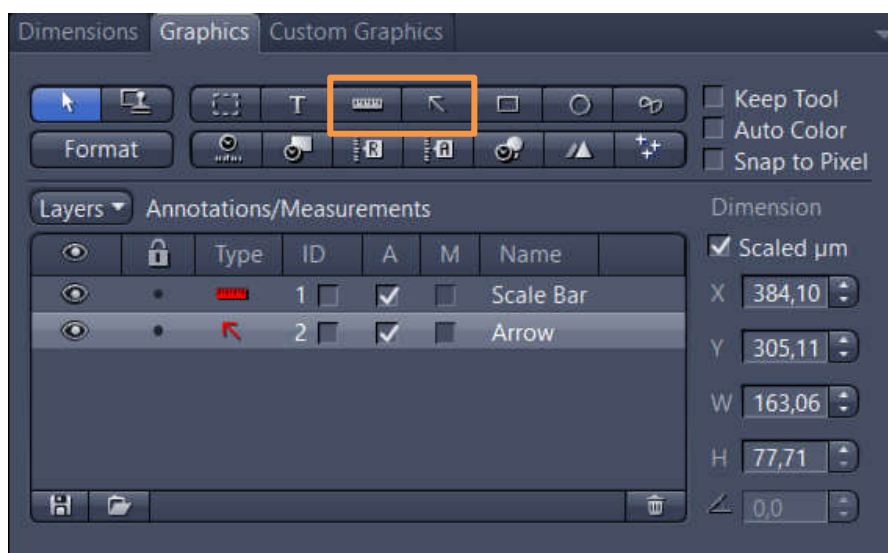
- 1 - Contrast (black point) control
- 2 - Gamma control
- 3 - Brightness (white point) control

Add Scale Bar or Arrows

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



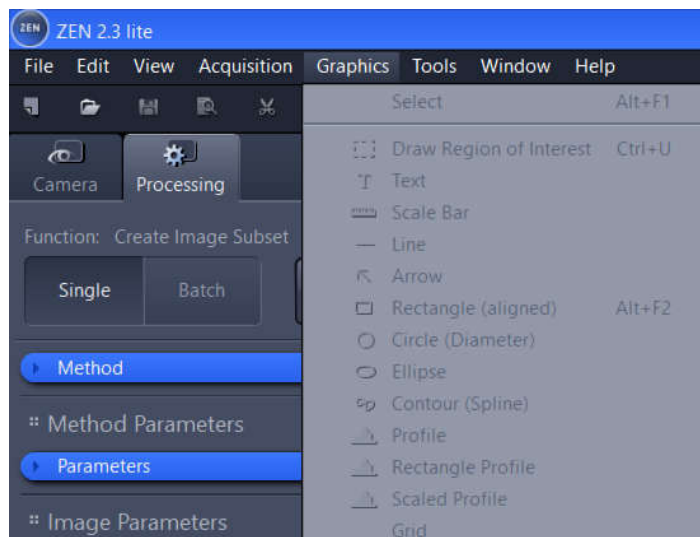
2. Click in the **Scale Bar**  or **Draw Arrow**  buttons. The scale bar will appear directly in the image and in the **Annotations/Measurements** as an entry. To set an arrow, the button will turn blue and then you can draw an arrow over the image



You can access to numerous formatting possibilities by right-clicking in the annotation (scale bar, arrow, etc). This will open the context menu. Select **Format Graphical Elements...** in this dialog

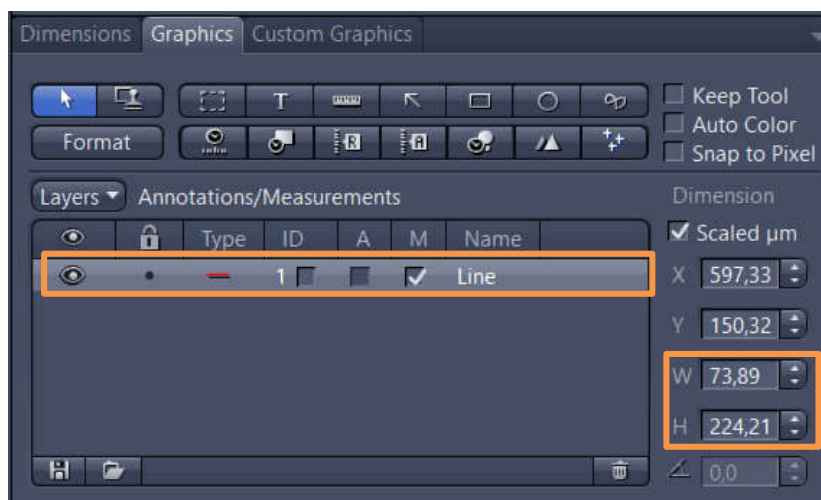
Distance Measurements

1. Go to **Graphics** -> **Line**



2. Draw the line over the object you would like to measure

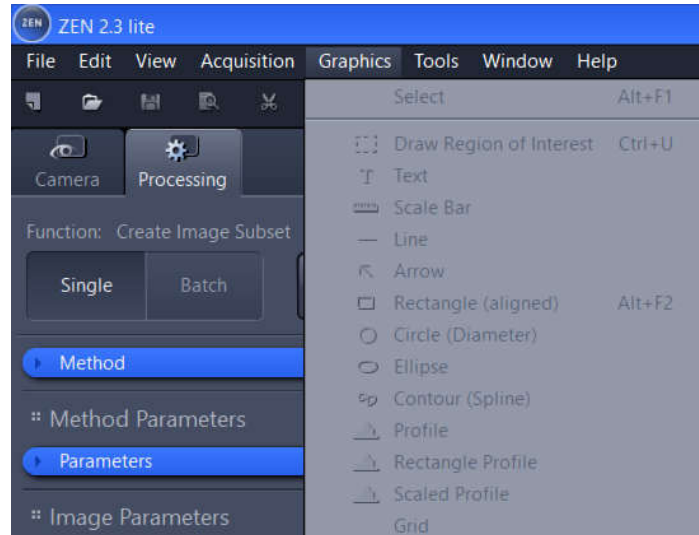
3. In the **Center Screen Area**, select the **Graphics** tab. An entry of the **Line** type will appear together with the positions and sizes (these measurements are not the distance length between the two-point edges of the line)



4. To display the distance length, box the **M** column and the measurement will be displayed in the image under the line

Cropping Region of Interest (ROI)

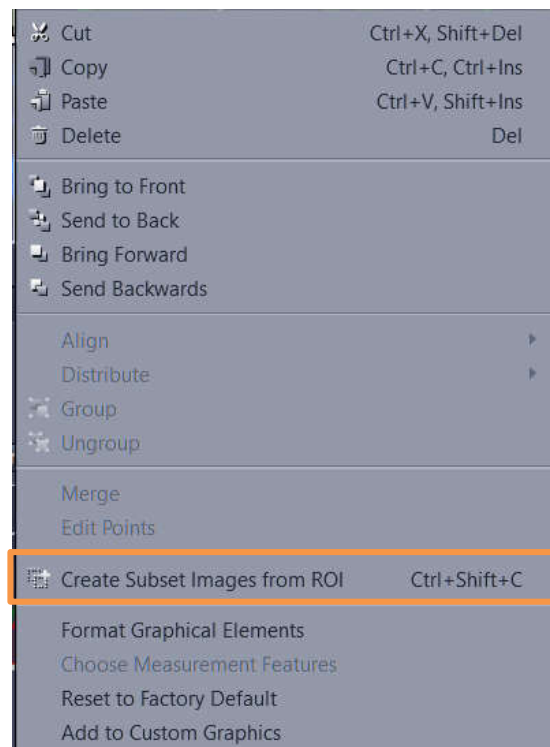
1. Go to **Graphics** -> **Draw Region of Interest**



2. Draw a square or rectangular region over the region of interest

3. Right-click in the drawn region. This will open the context menu. Select **Create Subset**

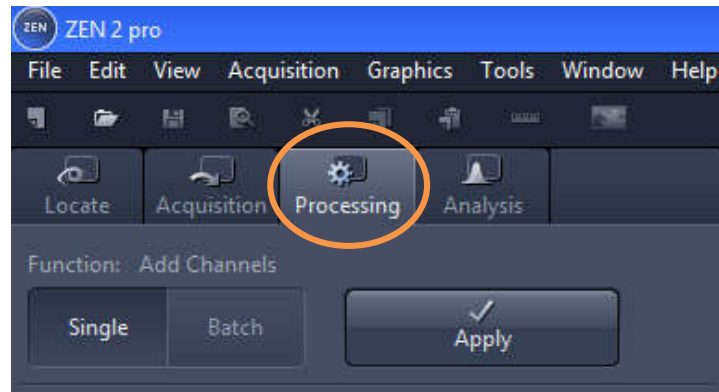
Images from ROI in this dialog



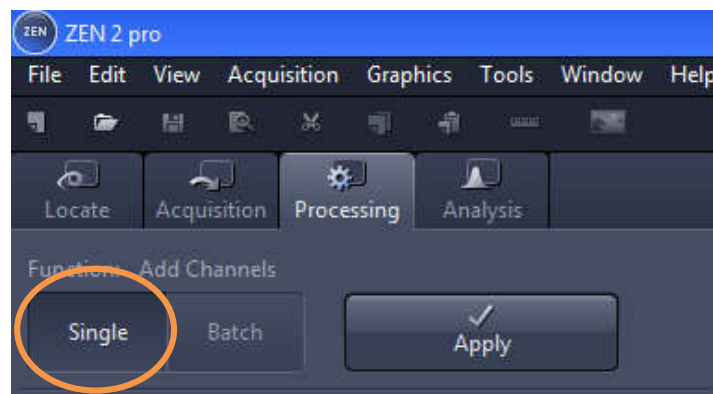
4. The selected region will be displayed as a new image in the **Centre Screen Area**. Besides, the image document will appear in the right-side **Right Tool Area**

Export

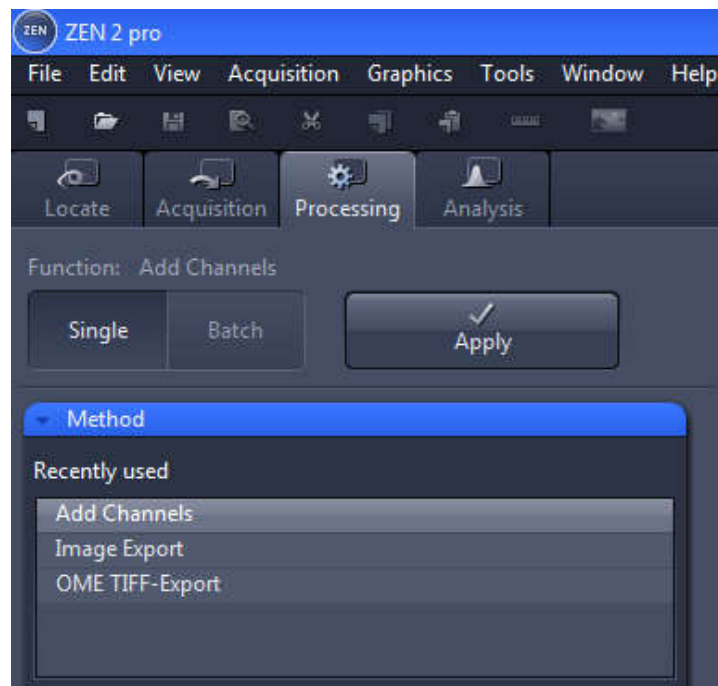
1. Go to the **Processing** tab



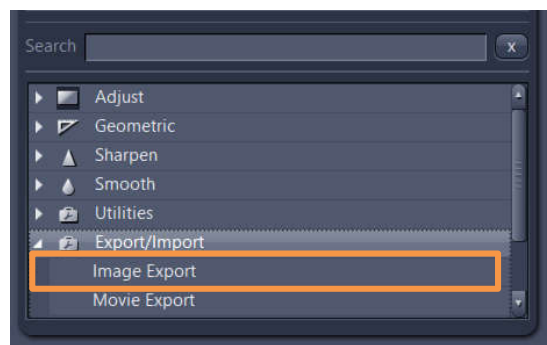
2. Click in the **Single** button



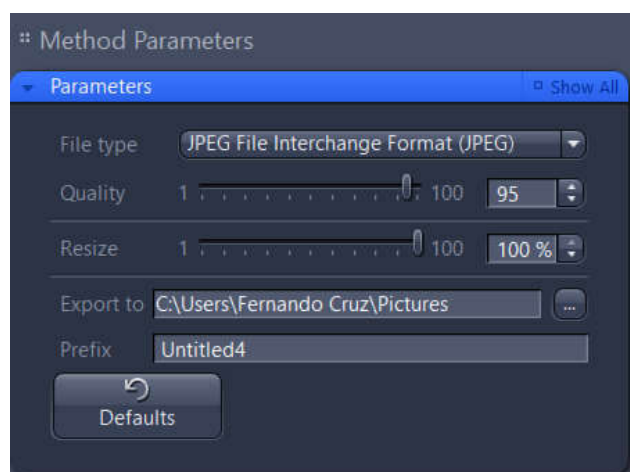
- Click in the blue header of the **Method** tool



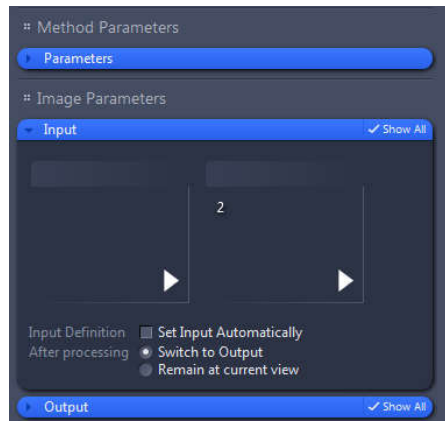
- In the **Method** tool, open the **Export/Import** group and select the **Image Export** method



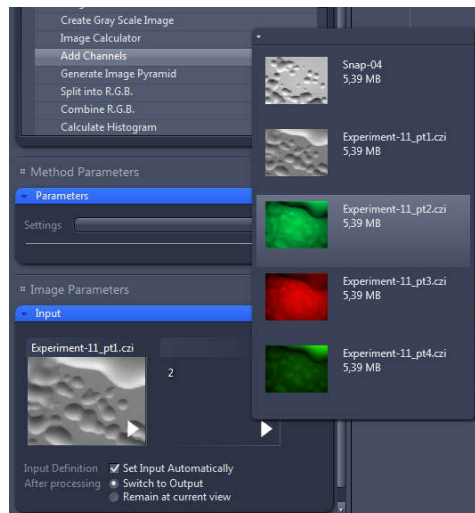
- Under **Method Parameters** group, select the **Parameters** tool, and set the desired export settings, such as file type, quality, export folder, ...



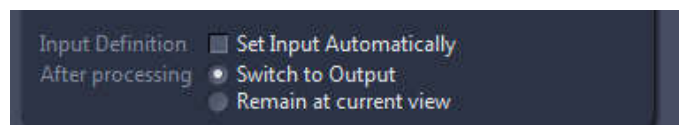
6. Click in the blue header of the **Input** tool



7. Select the images you would like to export. To do so, click in the small preview image within the **Input** tool. You will see a preview of all open images. To select an image, just click in it



8. Yet in the **Input** tool, select only the **Switch to Output** option



9. Finally, click in the **Apply** button.

