SPINNING DISK CSU-X1 ZEISS

Start the System

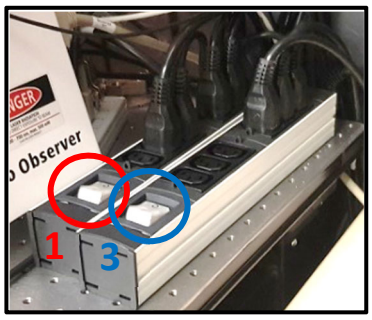
Turn on the 3 power strips on the microscope table.

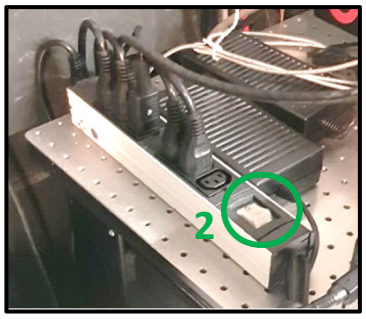
⚠ Follow the order

1. Microscope

2. Spinning

3. Computer (don't forget to start also, the computer with his button)



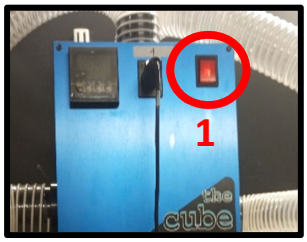


Start the Temperature and CO2

1. Temperature controller: It is already calibrated for 37°C. It is advised to let 1h before your acquisition to stabilize the temperature

2. CO2 controller: it is already calibrated for 5%

⚠ *Don't forget of open the Gas Bottle. But please just touch the main screw*



Manual Command

the extremity one permits you to adjust with

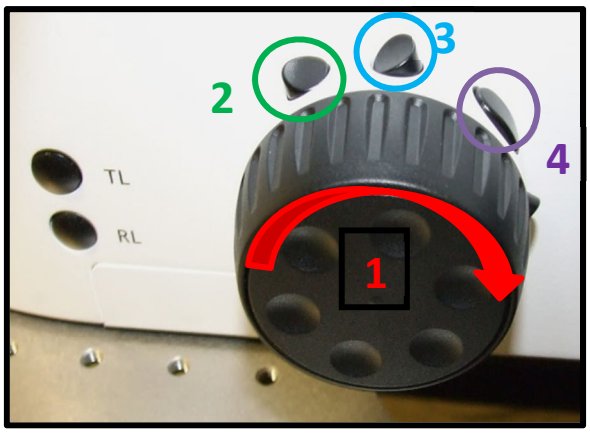
1. Wheel to adjust the focus

PS: The big one permits you to adjust fast, and more precision.

2. put down the objective to install your sample

3. Put back the objective at the focus point

4. Open/Close the shutter of the transmission light



Microscope Control and LAMP

1. Laser sécurity:

⚠ you should deactivate it when observing your sample through the eyepiece. However, you must activate it when you want to use "Live" or make an acquisition.

2. Lamp intensity control

3. Stage controller: move your stage in X and Y directions

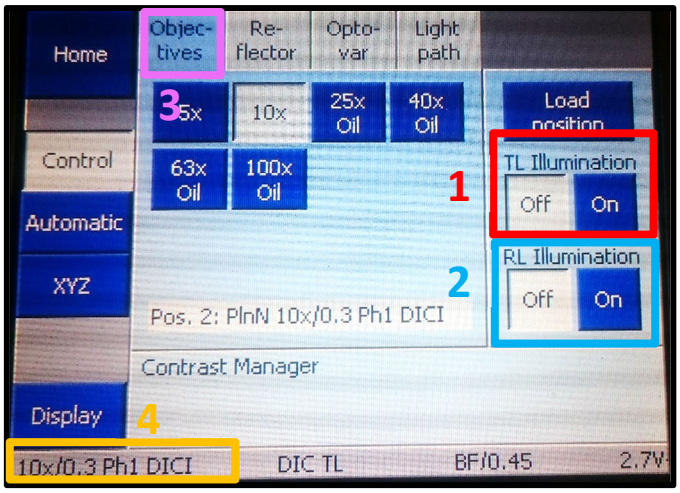
TFT screen (touch screen)

1. Open/Close the Brightfield shutter.

2. Open/Close the fluorescence shutter.

3. Choose the objective « *Objectives* ».

4. Observation in brightfield « *BF* », phase contrast « *PH* » or Normanski contrast « *DIC* ».

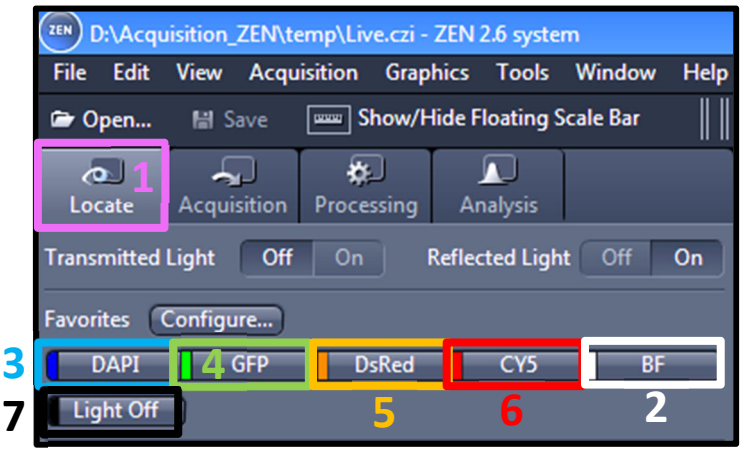


Localize your sample and made the Focus

1. Select the tab « *Locate* ».

2. « *BF*» allows you to observe your sample in brightfield (transmission).

If you want to observe in-phase contrast or DIC, after selecting « BF » we need to choose « PH » or « DIC » in the TFT screen.

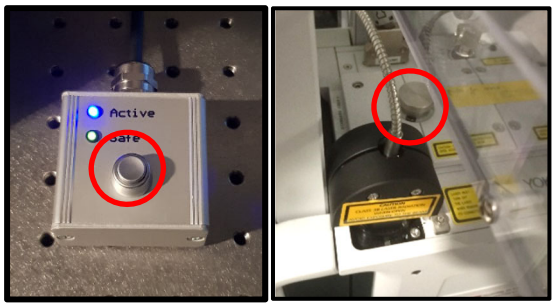


3. « *DAPI* » to observe in blue (405nm).
4. « *GFP* » to observe in green (488nm).
5. « *DsRed* » to observe in orange-red (568nm).
6. « *Cy5* » to observe far red (647nm).
7. « *Lights off* » close the shutter for the transmission and the fluorescent light

For transmitted light acquisitions, phase contrast or DIC, it is important to make adjustments on the microscope (set the Khöller illumination…)
Refer to the panels in the room of the microscope.

Acquire an image in single camera

Activate Safety and Push the Screw on left



1. Select the tab « *Acquisition* ».

2. Choose « single cam ».

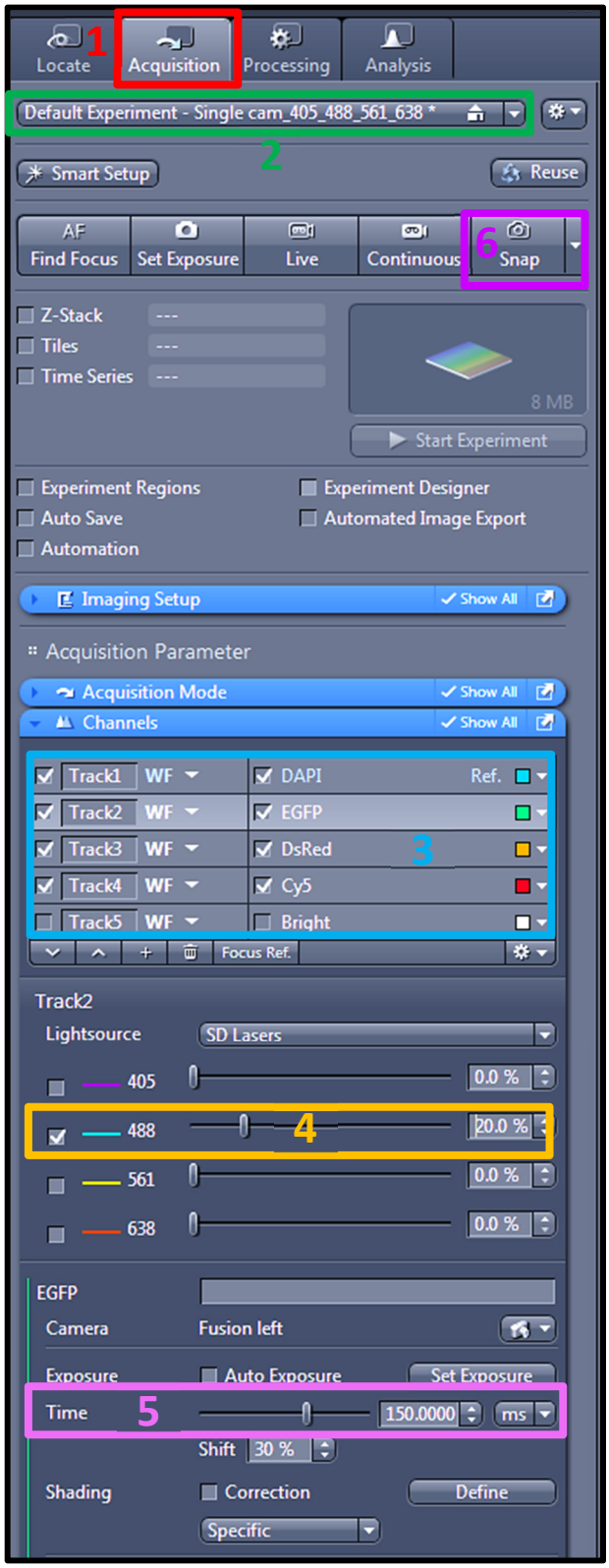
In the “Channel” window, check the line(s) with your fluorophores of interest

3. **Select** one channel (light grey) and **do « *Live* ».**

4. Adjust the laser power (careful if you increase to much you increase the photobleaching and the photoxicity.

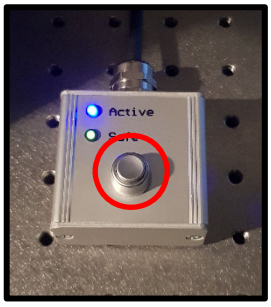
5. Adjust the exposure time (careful if you increase to much the time of your acquisition will too high in compare the cells displacement)

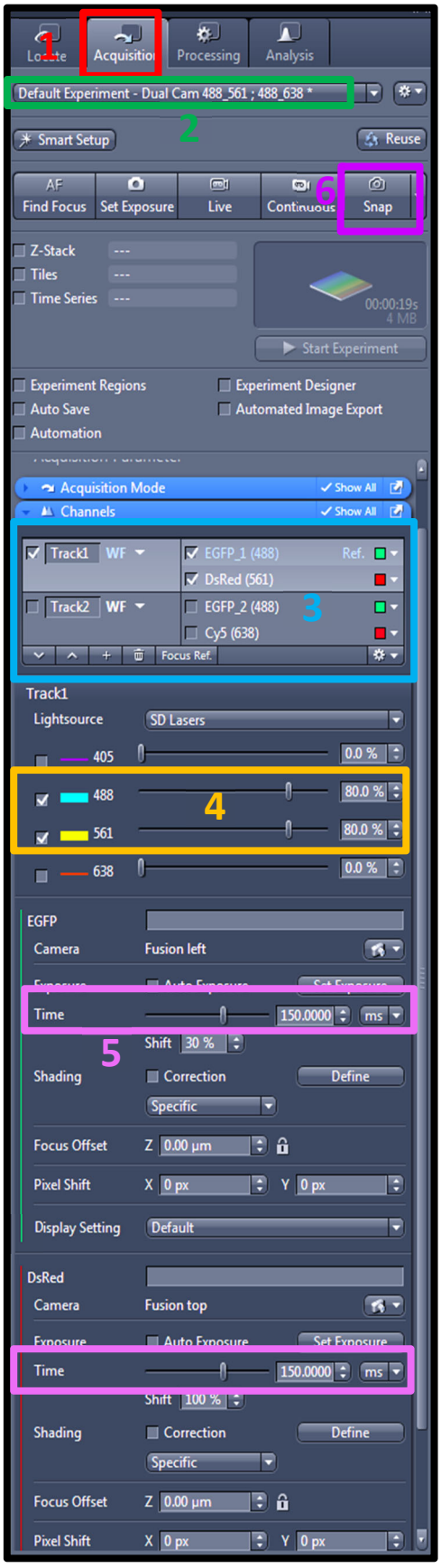
6. When you done for all channels, make a « *Snap* » to have the final result of your settings.



Acquire an image in Dual Camera

Activate safety and Pull the Screw for Dual Cam





1. Select the tab « Acquisition ».

2. Choose « Dual Cam ».

Open the menu « *Channels* », then check the line with your fluorophores of interest

3. Select the channels (light grey) and **do « *Live* ».**

4. Adjust the laser power (careful if you increase to much you increase the photobleaching and the photoxicity.

5. Adjust the exposure time (careful if you increase to much the time of your acquisition will too high in compare the cells displacement)

6. When you done for all channels, make a « *Snap* » to have the final result of your settings.

Acquire a Z-stack series

1. Check the « *Z-Stack* » option.

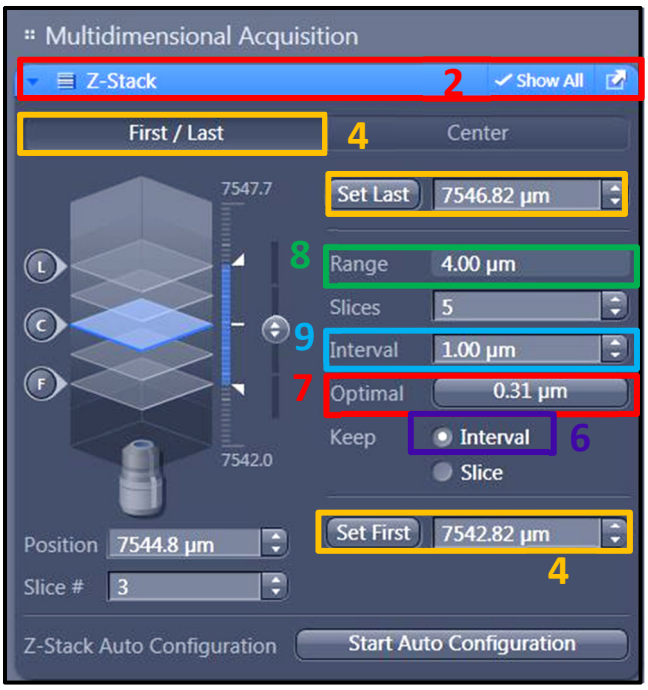
2. Check « *Show all Tools* ».

3. For multicolor acquisitions, make the entire z-stack of one channel, then switch to the other one « Full Z-Stack per Channel » or all channels per plane « All Channels per Slices ».

Two acquisition mode exists: you can define the top and the bottom of your stack, or define only the center of your stack.

4. First /Last Mode

- Check the tab « ***First/Last*** »
- Make a « *Live* ».
- « *Set First* » defines one extremity of your stack and « *Set Last* » the other one



5. Define Center Mode

- Check the tab « ***Center*** ».
- Make a « ***Live*** ».
- Define the center of your stack with « ***Center*** ».

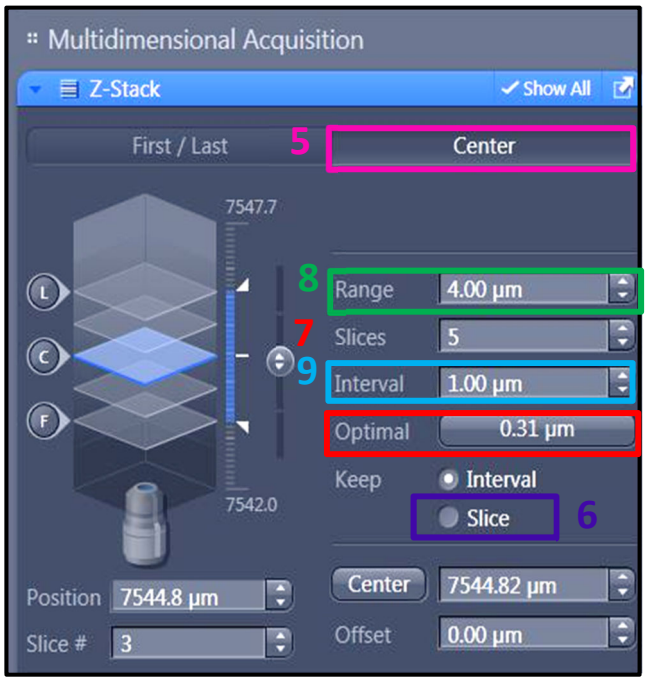
6. « *Interval* » has to be checked to ensure a fixed and chosen interval value in µm.

7. To choose the optimal resolution, you can use the « *Optimal* » option.

8. « *Range* » indicates the thickness of your stack.

9. The number of planes can be changed in « *Slices* ».

10. Start the acquisition by using « *Start Experiment* ».



Display the Stack

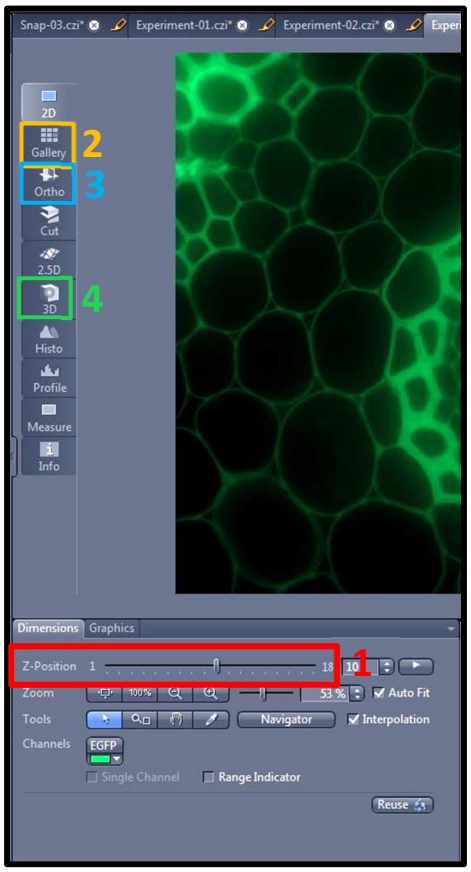
Your stack is displayed in the ZEN interface at the end of the acquisition.
In the tab « *Dimensions* »:

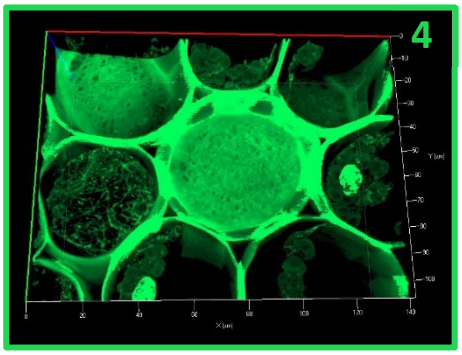
1. « *Z-Position* » allows you to see the different planes of the stack.

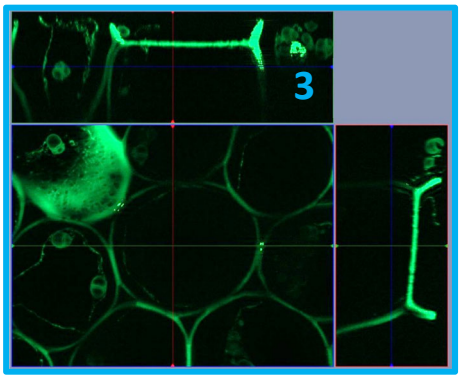
2. « *Gallery* » displays a set of all the images composing the stack.

3. « *Ortho* » allows an orthogonal view of your stack.

4. « *3D* » allows a 3D reconstruction of your stack.







Time Serie

1. Select the **Time Series** box in the Acquisition Tab.

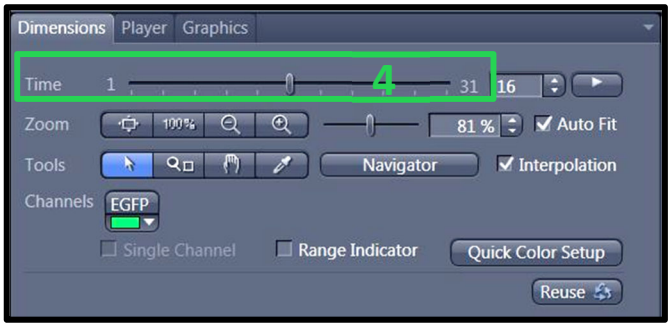
2. Choose the number of **cycles** and the **Interval** between each time point.

3. Click on START EXPERIMENT to start the acquisition

PS: Warning: The time the microscope make to acquire a z-stack (or tile) has to be taken in account in the chosen interval. You can measure it by clicking on « Measure Speed», the value will be shown in the « Interval » rubric

Your time serie is displayed in the Zen interface at the end of the acquisition.

4. In the « *Dimensions* » tab, you can look the different time point of your acquisition « *Time* »



Tile scan/Multi-Position acquisition

1. Check « *Tiles* ».

2. Open the menu « *Tiles* ».

3. Click on « *Show Viewer* ». Make a « *Live* ».

4. The navigation space is displayed in the center of the software interface. Double-click where you want to acquire an image or use the joystick to move the stage

5. In the menu « *Tile Regions* », click on « *Tiles* » and double-click on your position. Your current position will correspond to the center of the tile.

6. Tile Position: you can add the position one by one to be more specific in your choice. For this, you have to Click on « + », of the position window to add the position selected on the navigation space

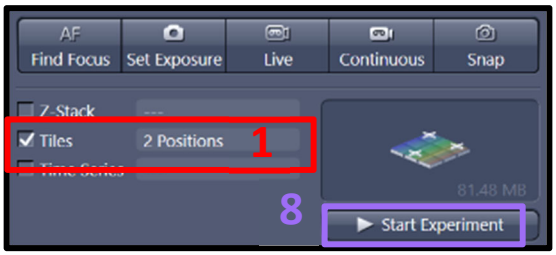
7. Click on « + or Add position/Tile», to validate the choice of your Region of Interest.

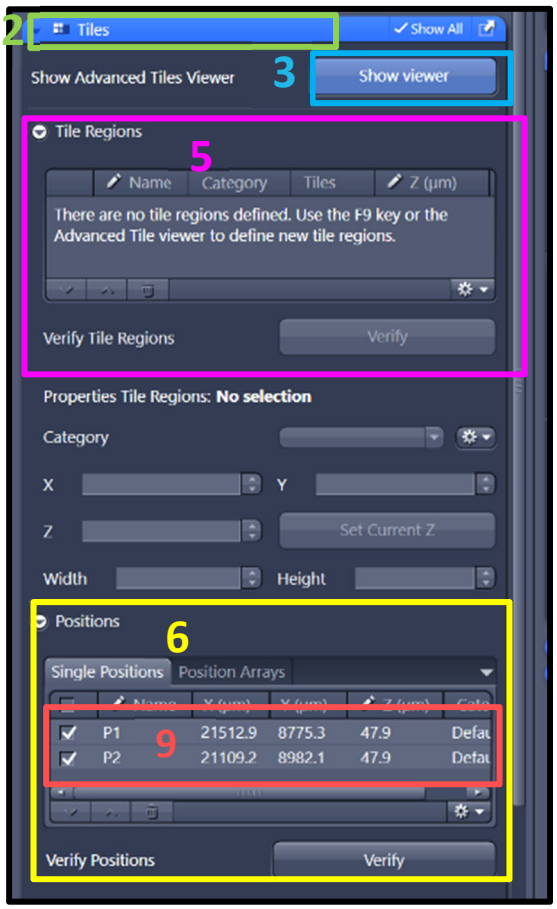
8. When all your positions and tile are selected you can click on « *Start Experiment* » to start the acquisition.

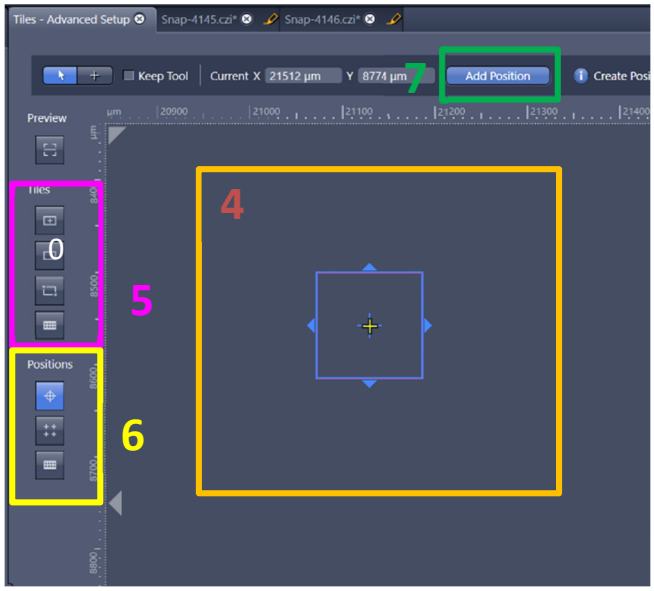
9. Make a right click on the position in the position window (tile window) and select “set current X/Y/Z or Current Z

PS: when you define the Z-stack it is important that you define with the Center mode as described previously to permit a z-stack adapted at the different Z set up of your different position.

For specific operations, you can ask the engineers of the platform or ask for assistance hour on the microscope







Focus Correction

There are two ways to correct the focus on the entire tile. It has to be done before any acquisition.

1. Click on « *Support Points* » below the navigation space.

2. Enter the number of positions than you would correct the focus.

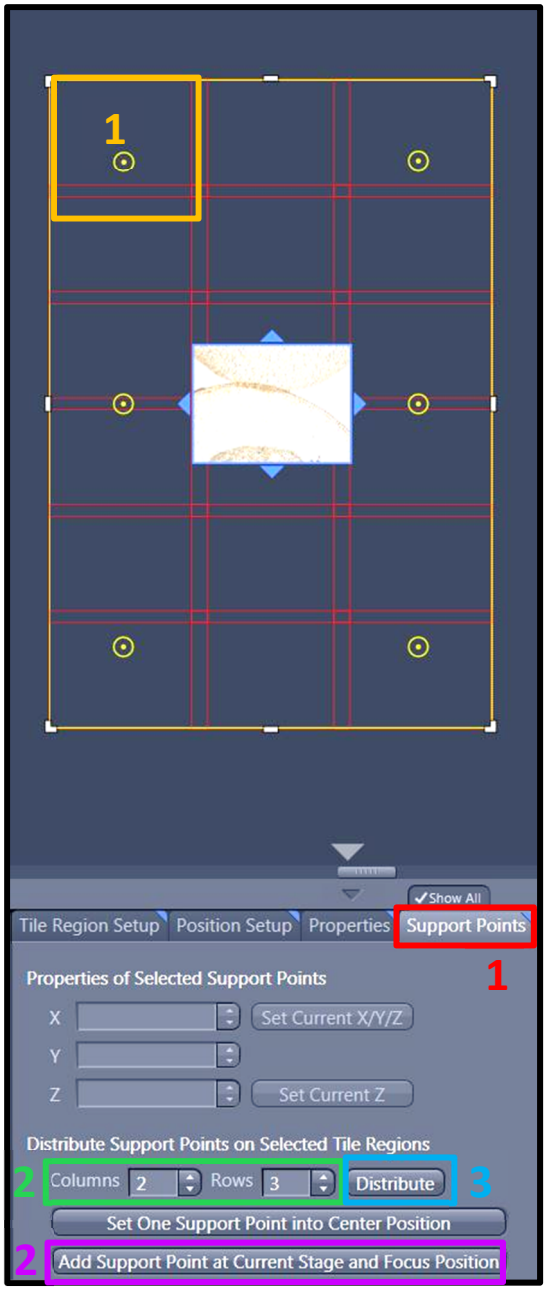
3. Click on « *Distribute* ». Yellow circles will be distributed in the tile. Double click on a yellow circle, make a « *Live* », set the focus and click on « *Set Current Z* » to save it. Repeat for each yellow circle.

OR

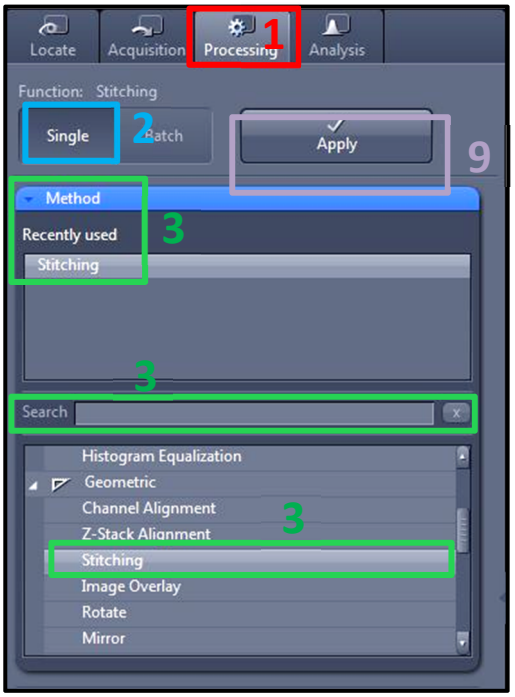
1. Double click on the position where you wish to correct the focus. Adjust the focus.

2. Click on « *Add Support Point at Current Stage and Focus position*», a yellow circle will appear.
Repeat the process on at least 5 positions.

3. Click « *Start Experiment* » to start the acquisition.



Tile scan finalization in fluorescence after the acquisition



For this option, the checkbox “online stitching” has to be unselected during the acquisition (see above).

1. Click on the tab « *Processing* ».

2. Click on « *Single* ».

3. Open the menu « *Method* ». on recently used or in the search bar select « *Stiching* ».

4. Open the menu « *Input* » and select the tile you want to stitch.

5. In the « *Parameters* » window, Click on « *New Output* ».

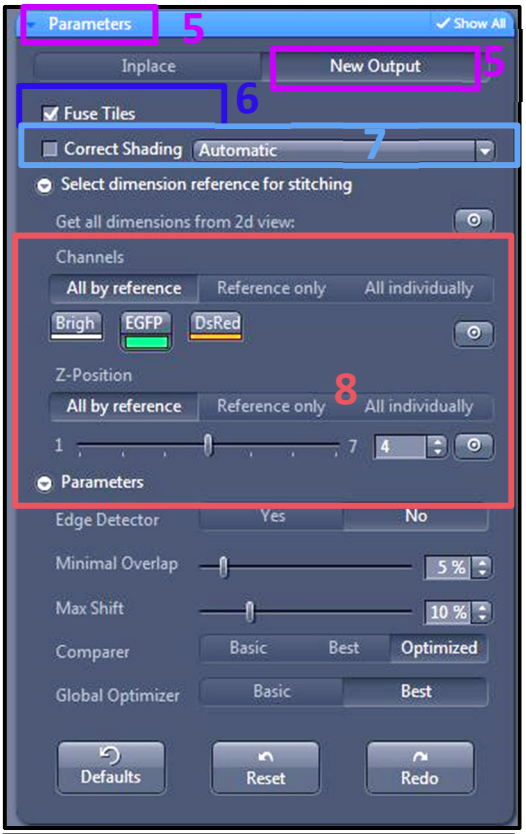
6. Check « *Fuse Tiles* ».

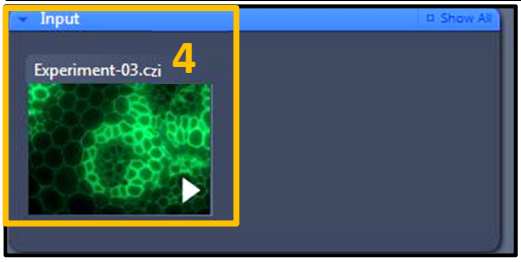
7. If the shading is not correct, click on « *Correct Shading* » and select the « *Automatic* » mode.

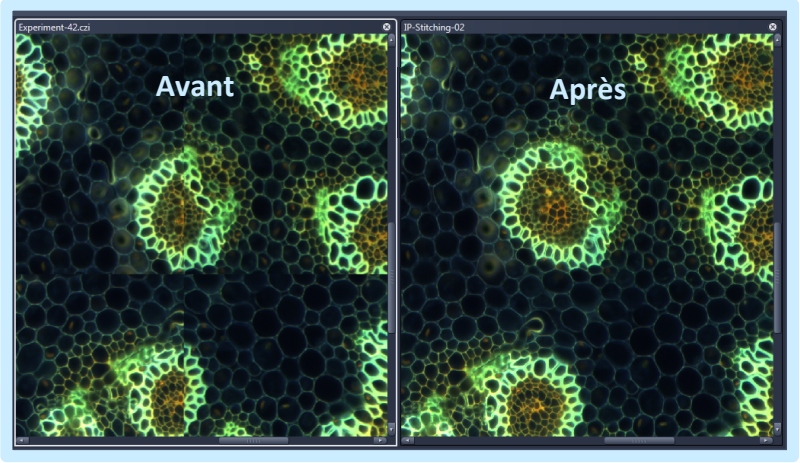
If you have several colors (channels) or a z-stack: Open the menu « *Select dimension reference for stitching* ».

8. Click on « *All by reference* » and select a channel that will be considered as the reference. Choose the z that will be considered the reference.

9. Click on « *Apply* ». The adjusted image is called « *IP-Stitching* ».

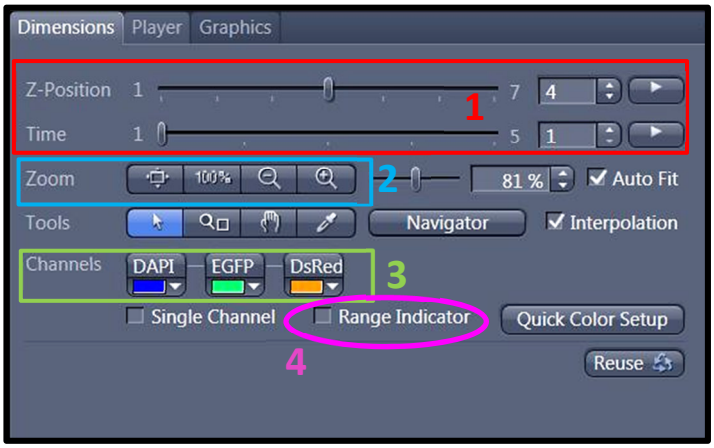






Graphics Tools

In the « *Dimensions* » Tab



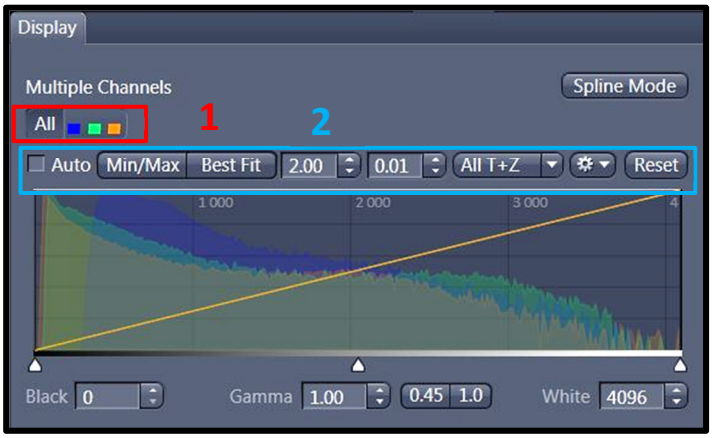
1. Move in the Z axe or in the Time series by the displacement of the adapted.

2. Image size adjustment. With 100% 1 pixel in image acquisition corresponding to 1 pixel of the image obtained

3. Show / Hide the channel on the screen.

4. Saturation observation (Red is the saturated pixel).

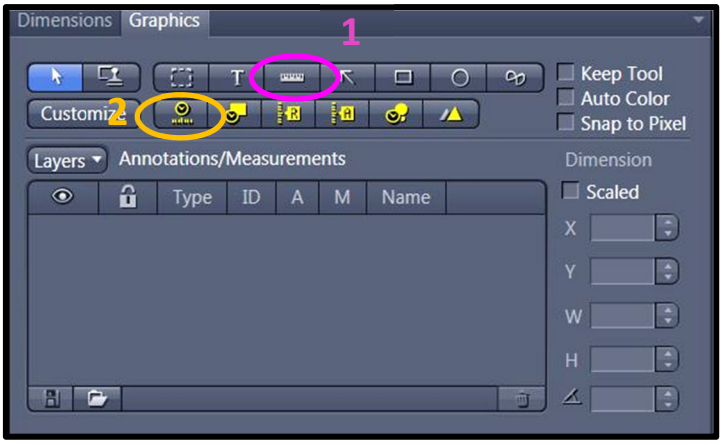
In the tab « *Display* »



1. Choose the channel or all to modify the image quality.

2. Contrast adjustment. Check on Reset to reinitialize it.

In Tab « *Graphics* »

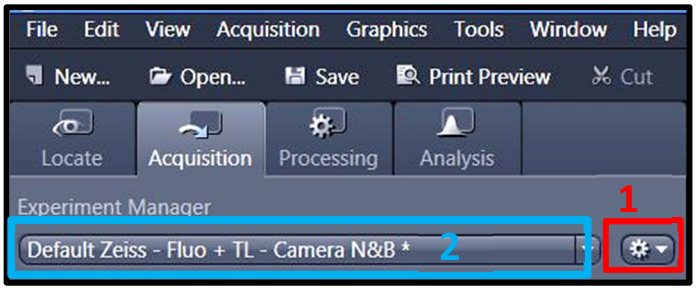


1. Show the scale bar.

2. Show the time.

Image and Parameter Imaging

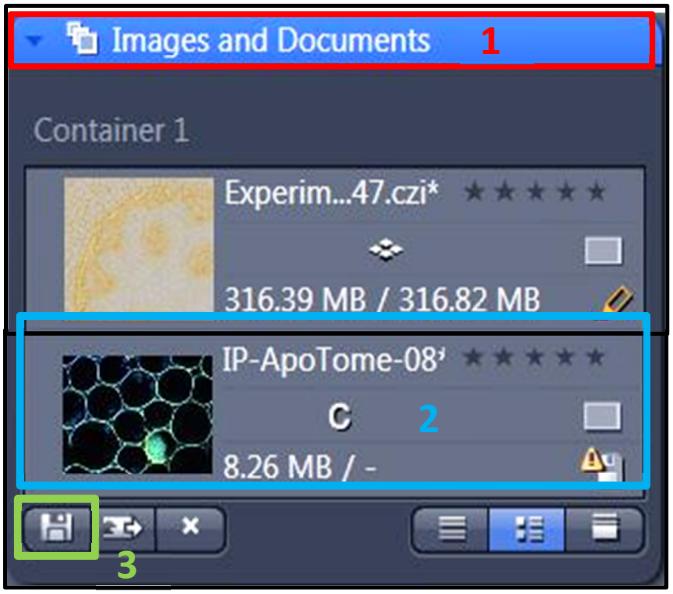
Settings Saving



1. select « *Save As* » in the « Parameter » logo. Name your settings and register its

2. to charge your parameter, open the menu « *Experiment Manager* » and choose your configuration.

Image Saving



1. All images are visible on the right side called « *Images and Documents* ».

2. to save double-click on the images.

3. Click on the diskette logo, name your images, and save them in the folder « *Users* ».

Ps: create a folder with the date and your name.

Stop the System

Turn on the 3 power strips on the microscope table.

⚠ Follow the order

1. Computer (don't forget to start
2. Spinning
3. Microscope
4. shut down the temperature system

⚠ Don't forget to let the objectives clean and at the Lowest position