



WIDEFIELD ZEISS AXIO OBSERVER USER MANUAL

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Switch on the temperature controller

1. Turn on the temperature controller with the red switch.

PS: It's important to switch on the temperature at least 30 minutes before the beginning of the session.

To avoid a Z shift, it is advisable to wait 1 hour for your sample to heat up in the system and present a stable temperature.

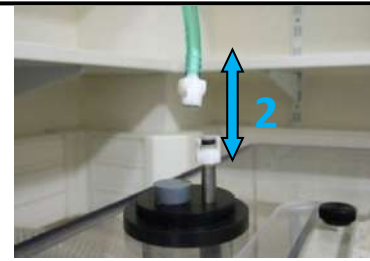
2. Choose the desired temperature (displayed in green) with the buttons up (+) and down (-).

PS: The real temperature is displayed in RED.



Switch on the CO₂ controller

1. Turn on the CO₂ controller (red brick)
2. Take the greenish tube connected to the CO₂ controller (« MAIN out » exit) and clip the end of the tube to the water reservoir on the system.



3. Open the air-compressed bottle (silver pressure regulator) and the CO₂ bottle (golden pressure regulator) in the anti-clockwise direction.



4. Turn the air-compressed bottle valve (silver) clockwise until you obtain a pressure of 1 Bar at the exit of the pressure regulator (right pressure gauge).



Put the CO₂ cover on the sample holder.

Start the system

1. Turn on the multi-socket power.

PS: The fluorescent lamp is switched on at the same time.

2. Switch on the PC, and use the « IJM » session.



Equipment

Available filters:

Excitation	Dichroïque	Emission	Fluo
BP 335 - 383	BS 395	BP 420 - 470	DAPI
BP 424 - 448	BS 455	BP 460 - 500	CFP
BP 450 - 490	BS 495	BP 500 - 550	GFP
BP 538 - 562	BS 570	BP 570 - 640	DsRed
BP 625 - 655	BS 660	BP 665 - 715	CY5

Available Objectives

Widefield ZEISS							
Objectives (ref Zeiss)	Immersion	Numerical Aperture	Cover glass	Free Working Distance (µm)	Relative brightness	DIC	N°
EC Plan-NEOFLUAR 5x/0,16 Ph1 (420331-9911)	Dry	0,16	0,17	18500	7	I	5-1
Plan-Apochromat 10x/0,45 Ph1 (420641-9910)	Dry	0,45	0,17	2000	14	II	10-1
Plan-Apochromat 20x/0,8 (420650- 9901)	Dry	0,8	0,17	550	16	II	20-1
LD Plan-NEOFLUAR 20x/0,4 Ph2 Korr (421351-9970)	Dry	0,4	0 - 1,5	7900 (0,75)	2,8	II	20-2
LD LCI Plan-Apochromat 25x/0,8 Imm Corr DIC (420852-9870)	Oil	0,8	0 - 0,17	570	10	II	25-1
Plan-Neofluar 40x/0,6 LD (421360-9970)	Dry	0,6	0 - 1,5	2900 (0,75)	2,3	II	40-5
LD Plan-NEOFLUAR 40x/0,6 Ph2 Korr (421361-9970)	Dry	0,6	0 - 1,5	2900 (0,75)	1,5	II	40-6
Plan-Apochromat 40x/1,3 Oil DIC (420762-9800)	Oil	1,3	0,17	210	10,5	III	40-1
Plan-Apochromat 63x/1,4 Oil DIC (420782-9900)	Oil	1,4	0,17	190	5	III	63-1
Plan-Apochromat 100x/1,4 Oil DIC (420792-9900)	Oil	1,4	0,17	170	2	III	100-1

Manual Command

1. Wheel to adjust the focus

PS: The big one permit you to adjust fast, and the extremity one permit you to adjust with 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

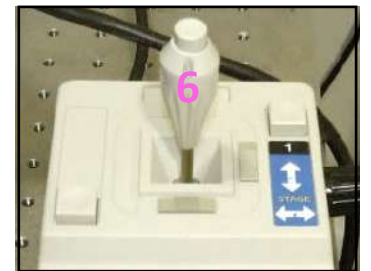


Intensity

5. Light intensity adjustment (wrench adjuster).

Memorization

6. Move the motorized stage in X and Y.
Hold the joystick button to move fast.



TFT screen (touch screen)

1. Open/Close the Brightfield shutter.

2. Open/Close the fluorescence shutter.

In « Microscope », in the « Control » menu, several tabs are allowing you to:

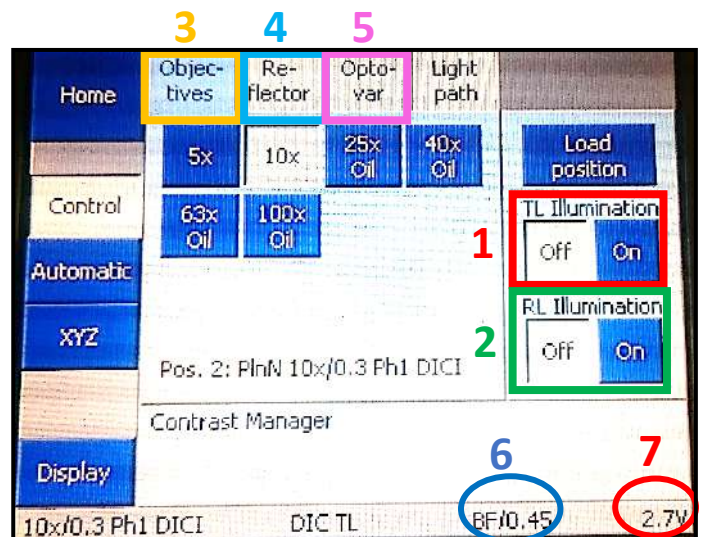
3. Objectives selection

4. Filters selection

5. Add/Remove a 1.5X lens « Optovar ».

6. Observation in brightfield (BF), phase contrast (PH) or Normanski contrast (DIC) / Aperture Diaphragm

7. Power of the halogen lamp



1. The LED control for the fluorescence

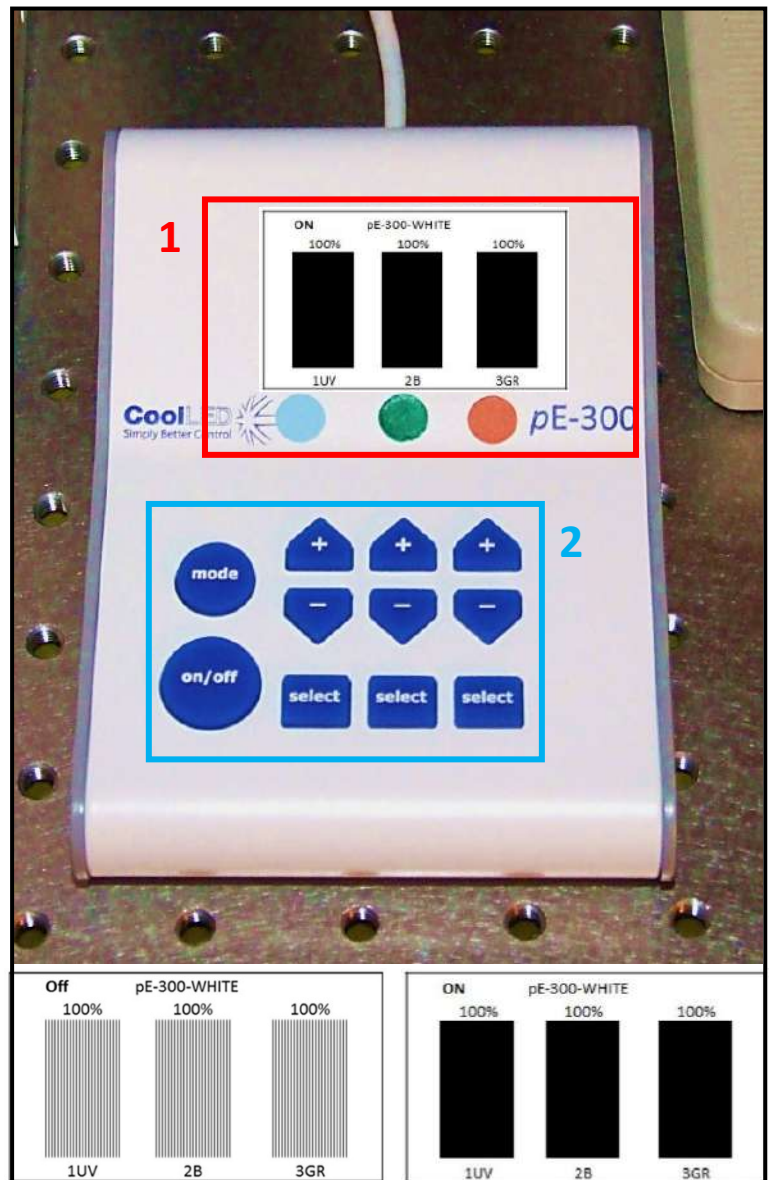
The fluorescent lamp is composed of 3 LEDs.

- The first one is for the DAPI
- The second one is for the GFP
- The third one is for the mCherry and Cy5.

2. LED CONTROL:

- Press the button On/Off to switch it on and off the LEDs.
- Select the wanted LED « select ».
- Adjust the power of each LED « +/- ».

Turn off the LEDs « On/Off » when you don't use them to increase the lifetime of the LEDs



Ocular Observation Set Up

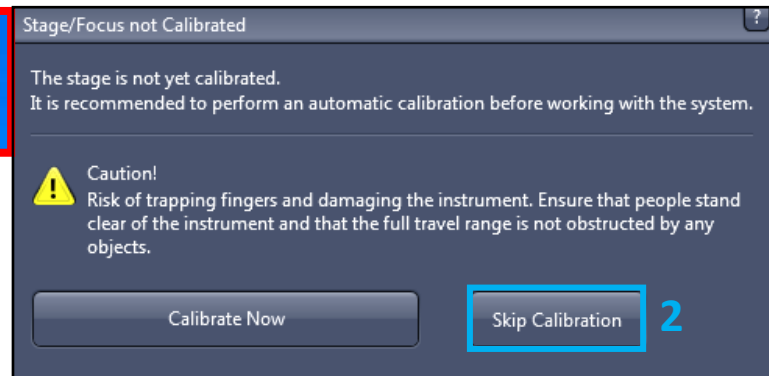
1. Start the « ZEN » software (Icon on the desktop).

PS: Select Zen Pro and not processing



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2. The stage calibration is necessary only if you use multi-well plates or if you want to retrieve positions from a past experiment



3. Select the tab « **Locate** » and then, « **BF** » which allows you to observe your sample in brightfield (transmission).

PS: If you want to observe in-phase contrast or DIC, first select « BF » and then select « PH » or « DIC » on the TFT screen (View previously)

4. Choose the emission light adapted to your staining

DAPI: Observe in Blue

GFP: Observe in Green

Ds Red: Observe in Orange-Red

Cy5: Observe in Far Red

YFP: Observe in Yellow

Light Off: Close the shutter of the transmission light



For transmitted light acquisitions, phase contrast, or DIC, it is important to adjust the microscope (set the Köhler illumination...) Refer to the panels in the room of the microscope.

Acquire a widefield image in transmission mode

Before observing in brightfield, you have to set the **Khöller illumination**, then if you need the phase contrast or DIC (refer to the posters in the microscope room).

Select « *BF* », « *PH* » or « *DIC* » on the TFT screen.

1. Select the tab « *Acquisition* ».

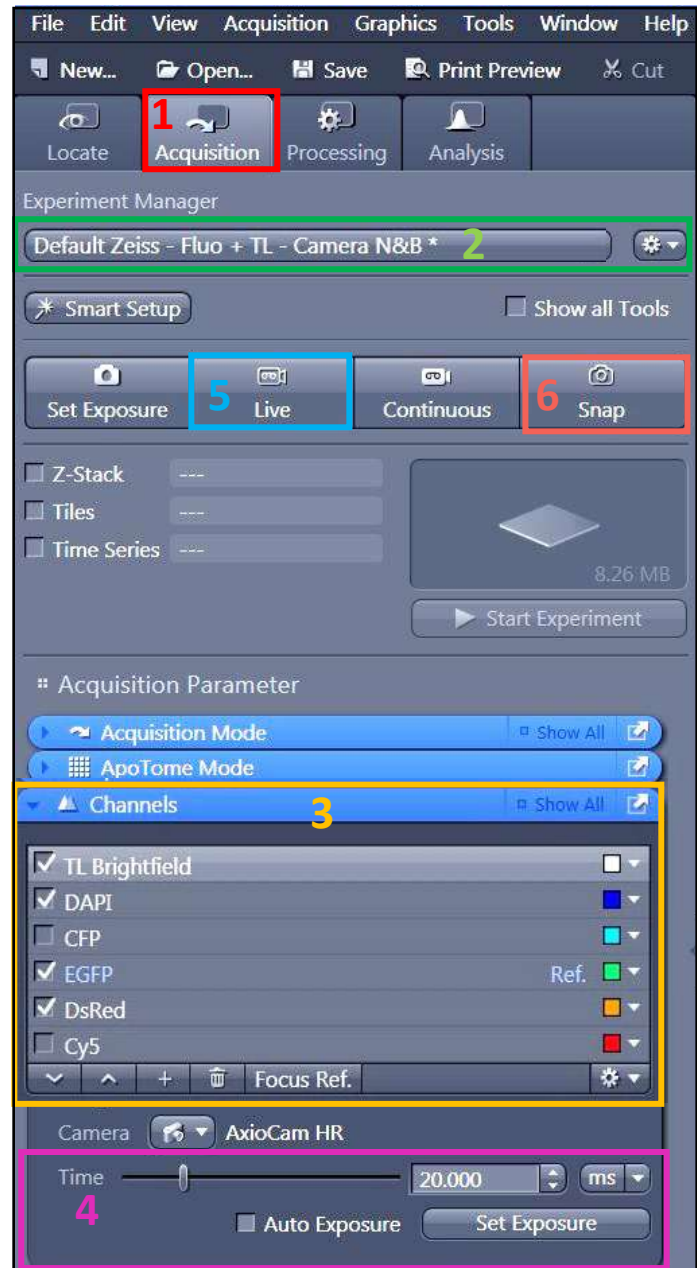
2. the Black and white camera is the only one in this microscope

3. Check the line(s) with your fluorophores of interest. Select one channel (light grey)

4. Make a « *Live* ».

5. Adjust automatically the exposure time by clicking on « *Set Exposure* ». Or Adjust manually the exposure time.

6. Make a « *Snap* » to acquire your image



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** ».

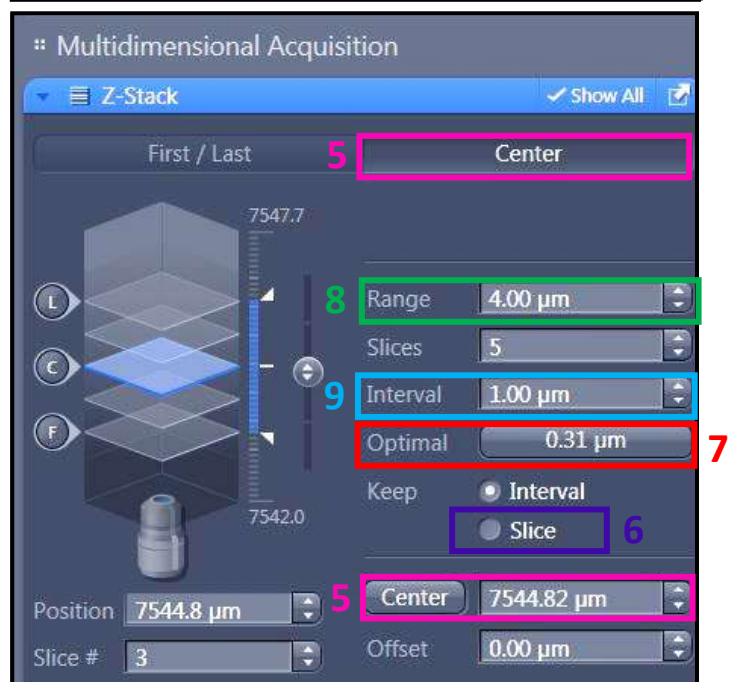
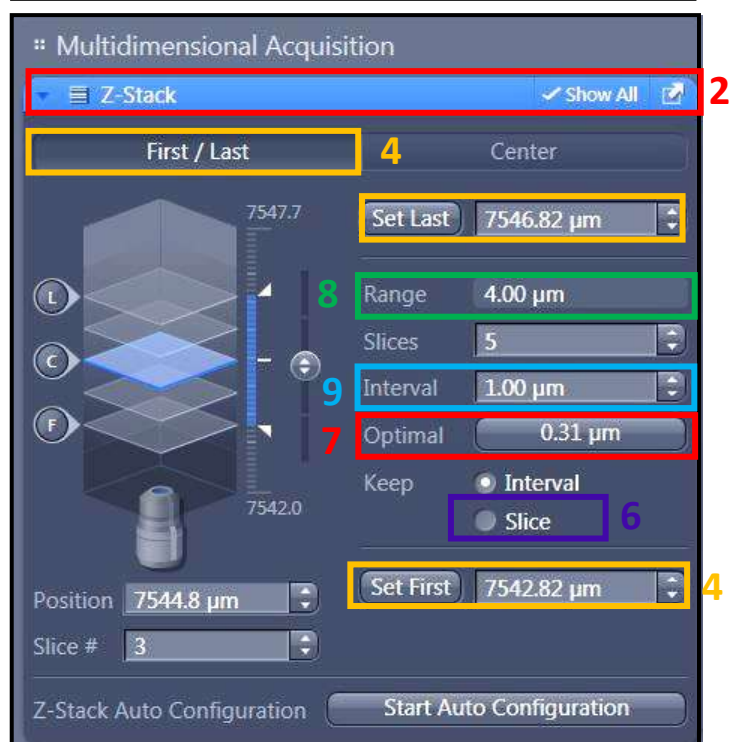
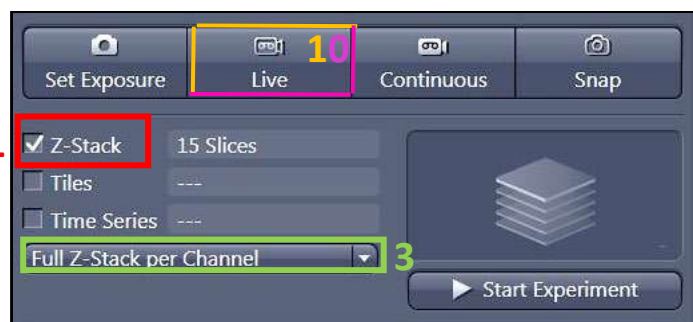
6. « **Interval** » has to be checked to ensure a fixed and chosen interval value in μm .

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** ».



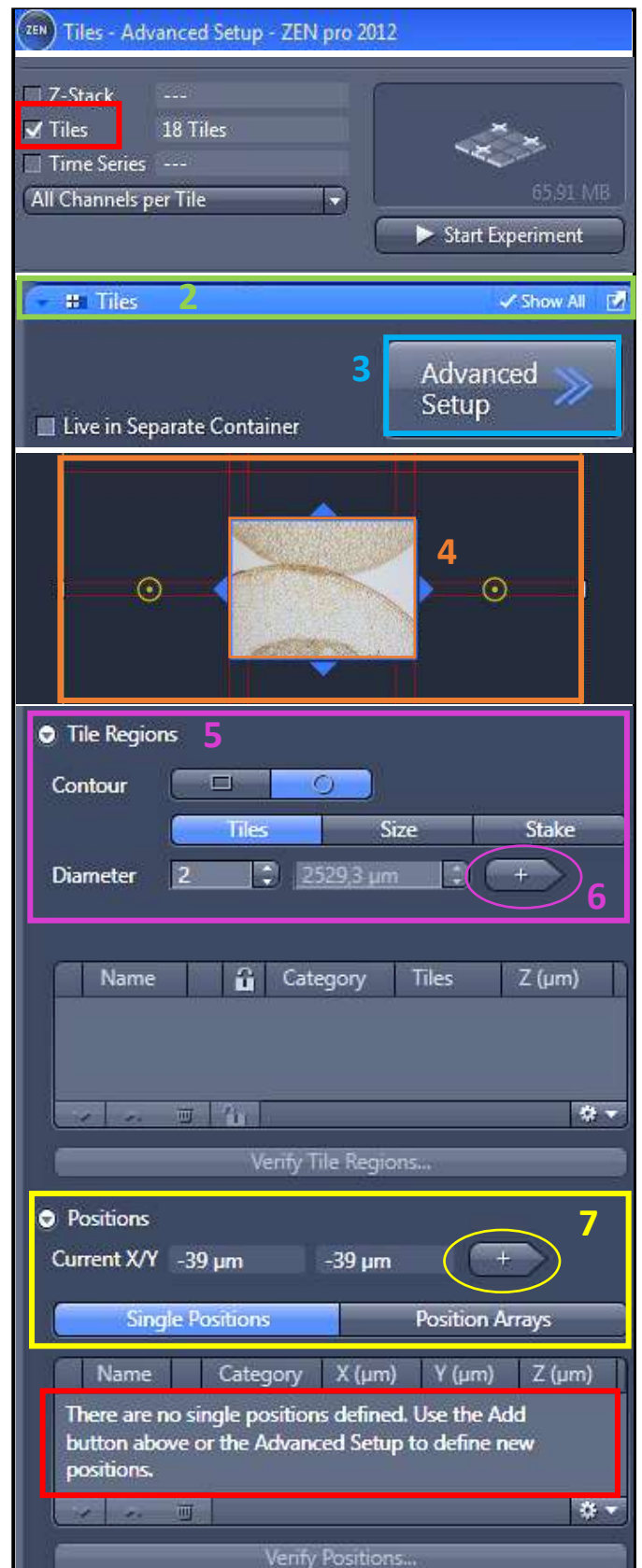
Tile scan acquisition

1. Check « Tiles ».
2. Open the menu « Tiles ».
3. Click on « Advanced Setup ». 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. Click on « + », the tile is then displayed on the navigation space.
7. Tile Position: you can add the position one by one to be more specific in your choice. To do this you have to Click on « + », of the position window to add the position selected on the navigation space
8. When all your positions and tile are selected you can click on « Start Experiment » to start the acquisition.

Focus correction

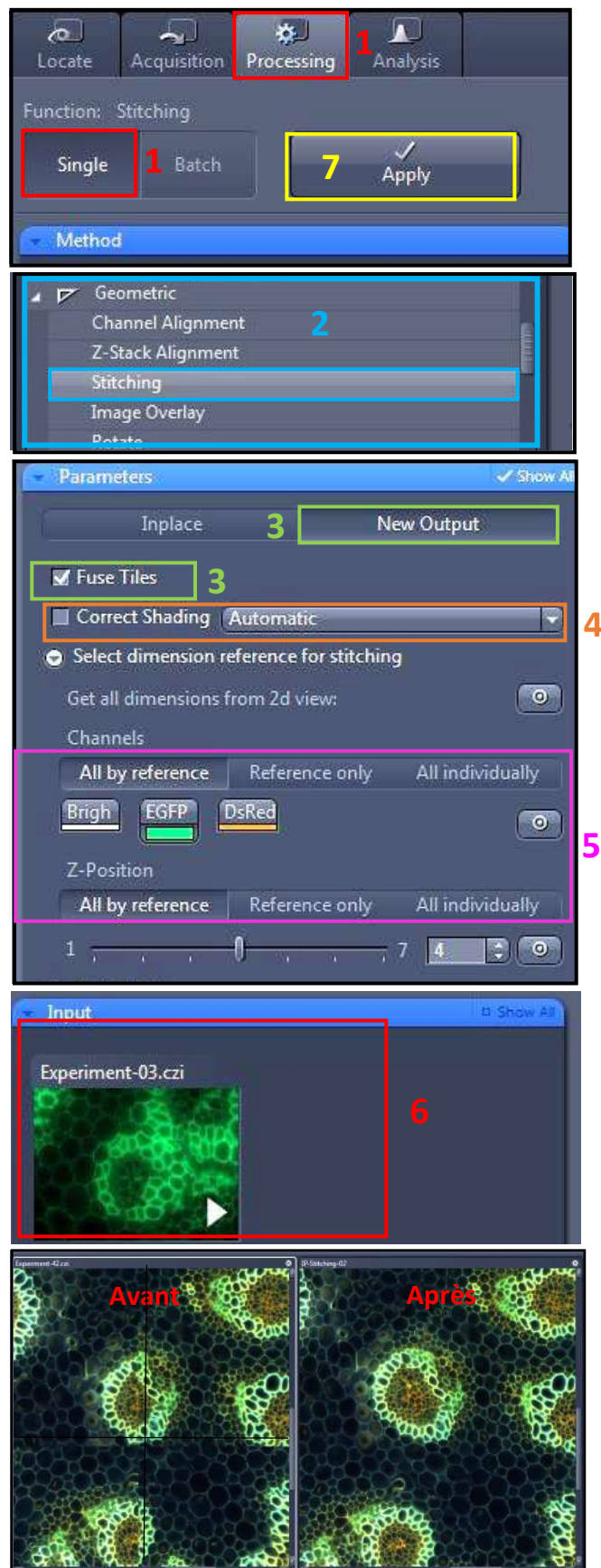
9. Make a right click on the position in the position window (tile window) and select “set current X/Y/Z or Current Z

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



Fluorescence Mosaic Acquisition

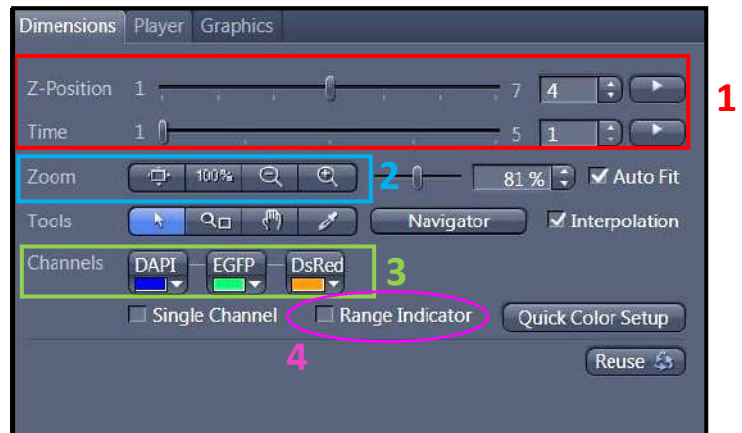
1. Open the window « *Processing* », and « *Single* ».
2. In the « *Method* » menu open the « *Geometric* » window and select the « *Stitching* » function
3. select « *New Output* » in the « *parameter* » menu, then check « *Fuse Tiles* ».
4. « *Correct Shading* » permit you to correct the shading effect between the different position you can select the « *Automatic* » mode.
5. If you acquired multiple channels or Z-series open the menu « *Select dimension reference for stitching* », select « *All by reference* » and choose your reference.
6. Open the menu « *Input* » and select the acquisition that you want to process.
7. Finally press « *Apply* » the acquisition process is called « *IP- Stitching* ».



Graphics Tools

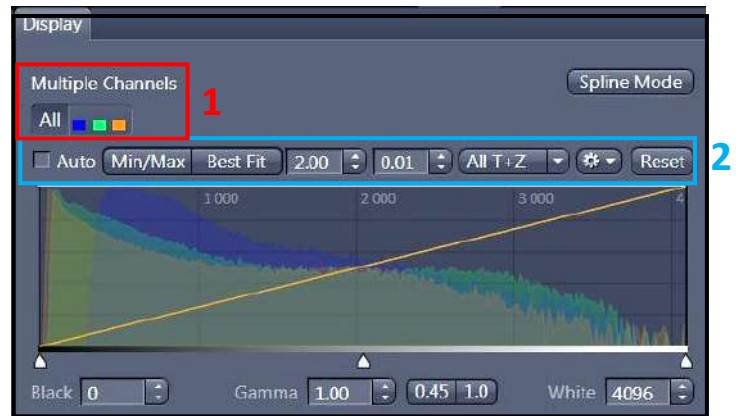
In the « *Dimensions* » Tab

1. Move in the Z axe or in 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.

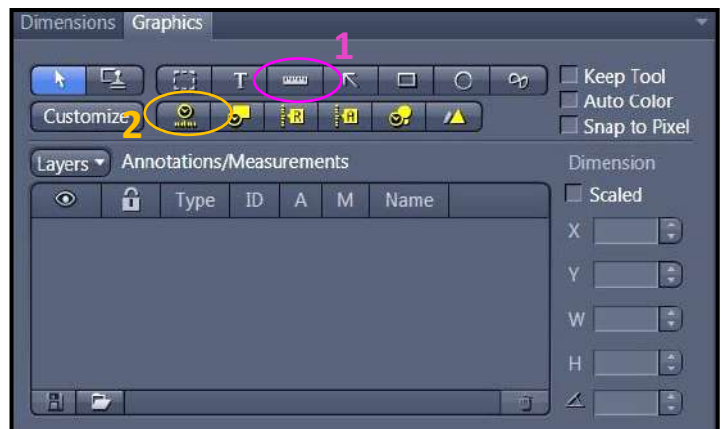


Image and Parameter Imaging

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.

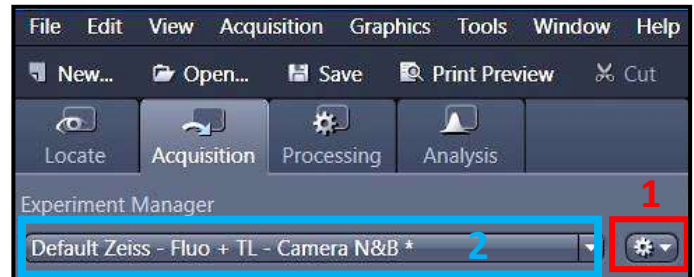
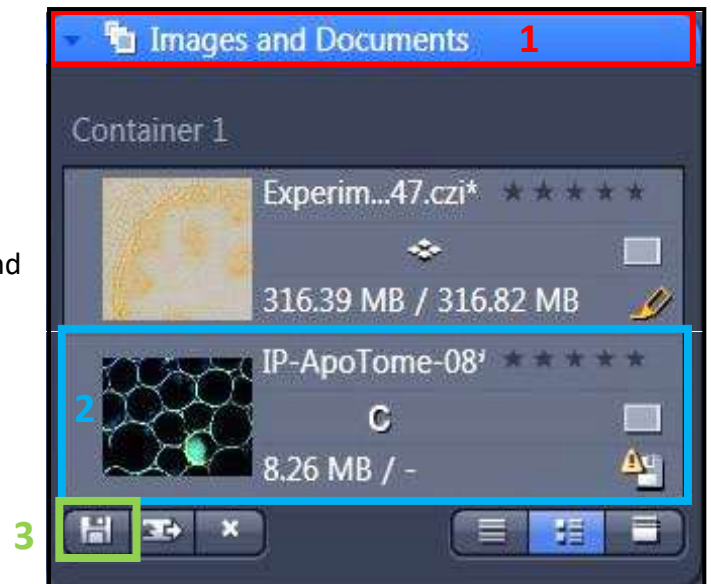


Image Saving

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.



Shutdown system

1. Clean all the objectives used with the paper adapted
2. Put the turret on the lowest magnification objective and put it in the lowest stage.
3. Close Zen.
4. transfer your data to your hard drive.
5. Turn off the PC
6. Turn off the power strip.

PS: if someone book the microscope after don't turn off the system

Turn Off the Temperature and CO2 system

1. Turn off the Temperature system with the red button.
2. remove the link of the CO2 system, of the sample support and put it on the anti-vibration table.
3. Remove the cable of the CO2 manager of the microscope system.
4. Shut down the CO2 manager.
5. Screw in the close way CO2 and Air bottle.

