CONFOCAL MICROSCOPE ZEISS LSM 780 USER MANUAL

START THE SYSTEM

1. Press the « MAIN SWITCH » button.

2. Press the « SYSTEM PC » button.

3. Switch on the PC and
log in to the session « USER ».

4. When Windows has started,
press the « COMPONENT » button.

5. Switch on the fluorescent light.

6. Turn the Power key on « I ».

⚠ Check if the switch is at the « ON » position.
Don't touch it when you turn off the system

7. Push the button up to « LASER RUN ».
Once the green LED is lit

⚠ Wait 5 minutes after turn of the LASER RUN button to turn the potentiometer to the **9 o'clock position**, while taking care of not switching on the red LED.

8. Start Zen Software by a click on its ICON

9. Select Start System to launch the acquisition software
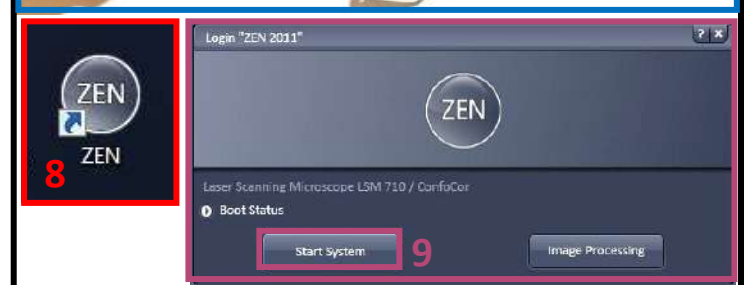
OCULAR OBSERVATION

1. Select the LOCATE tab (first on the left).

2. Select the desired illumination

3. Open/Close the transmission light

4. Open/close the reflected light

5. Adjust the fluorescent lamp intensity

6. Choose your objective
Depending on the type of illumination that you would like to.

Manual Command

1. Wheel to adjust the focus
PS: The big one permits you to adjust fast, and the extremity one permits 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

ACQUIRE ONE OR SEVERAL COLORS

I. Dye Assistant

1. Select the **ACQUISITION tab**

2. Open SMART SETUP

3. Then, in the DYE menu select the different fluorophores and their respective colors

Several methods are suggested:

- **FASTEST:** Allows simultaneous acquisition for all your channels; this is the fastest method, but cross-talk between consecutive channels can be important.
- **BEST SIGNAL:** Each channel is acquired separately. This method is the slowest one but it avoids (as far as it is possible) cross-talk between consecutive channels.
- **SMARTEST:** Compromise between speed and reduction of cross-talk. For example, both blue and red channels are acquired simultaneously, then the green one is acquired separately.
Remarks: These modes are just a base to start a configuration. The software doesn't necessarily choose the best one. You have to check by yourself and then modify appropriately the settings.

III. Acquision SetUp

1. In the "ACQUISITION MODE" window, choose the sampling of your image which has an impact on the resolution.

2. The frame size define the number of pixels of your image.
- You can select Optimal to use the optimal lateral resolution in function of your objective's choice.
- You can also define your personal number.

PS: the acquisition time increase as the lateral resolution increase, and Having pixel size smaller than the resolution is useless (over-sampling).

3. Choose the scan speed (the faster mode has the worst signal/noise ratio).

4. Bidirectional mode allows you to acquire 2 times faster.

5. Select 16 bits to increase your grey level and the quality of images.

**PS:** the bidirectional mod could create phase issues that need correction X in phase settings

6. you can select the "AVERAGING" mode if the signal/noise ratio is not satisfying.
If you have a fixed sample the mode Frame is more appropriate. If you have a living sample the mode Line is preferable. (Method Mean is recommended)

7. Zoom in If necessary, move your zoom zone to see your object of interest. If necessary, rotate your image
PS: Zoom can increase the photobleaching but can decrease the acquisition time

8. Click on SNAP to acquire the image
**PS:** click on "Start Experiment" to begin your all acquisition (Tile; Z-stack etc).
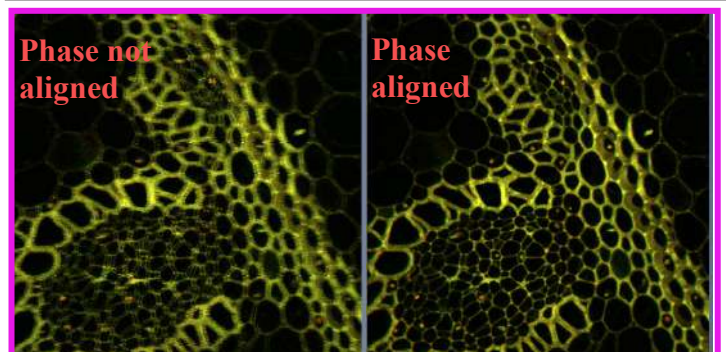
III. Acquision SetUp 2/2

1. You can zoom your region of interest (ROI) with the CROP option. just under the image. Click on CROP. Then you can rotate and process your image cropped

2. Save your settings of image acquisition.

3. You can reuse the parameters of an image previously acquired by selecting the image and then clicking on REUSE.
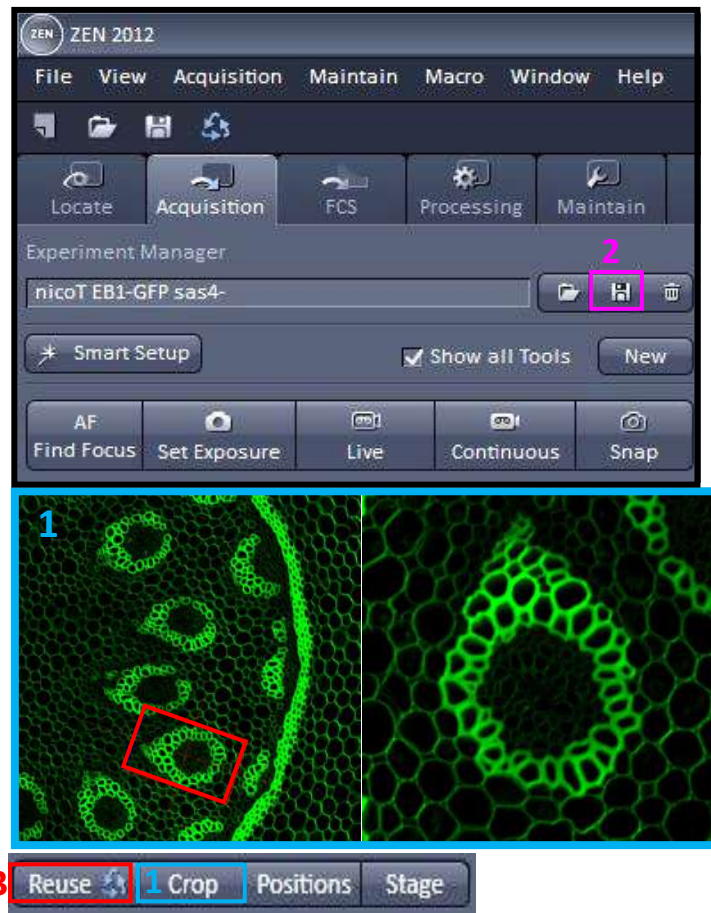
IV. Channels

1. In the toolbox CHANNELS, adjust the pinhole at 1 AU (Airy unit) for each channel to obtain the best resolution/signal ratio.

2. Click on LIVE.

3. Adjust the laser power for each channel

4. You can increase the GAIN (MASTER) to decrease the laser power if it is too high. But the signal/noise ratio will be impacted. **Stay between 650-800**

5. You can adjust the DIGITAL OFFSET to improve the image by removing the background signal

6. Don't increase the DIGITAL GAIN except if the GAIN (MASTER) is high enough

7. To find adapted parameters for gain and laser power, it is recommended to use the « Range Indicator » display.
In this representation, saturated pixels are red *and black pixels are* blue.

Ps: To use the live with only one channel you have to keep check only the tracks box **(8)** corresponding at the channel choose and uncheck the other one.
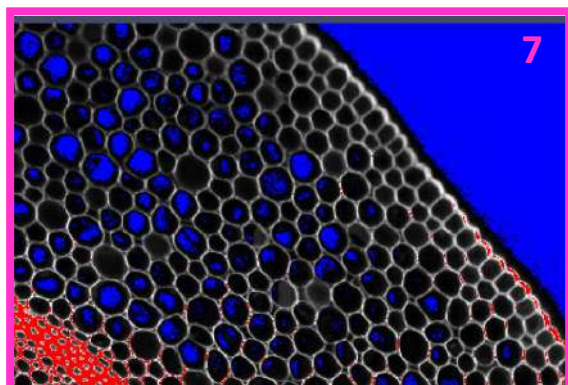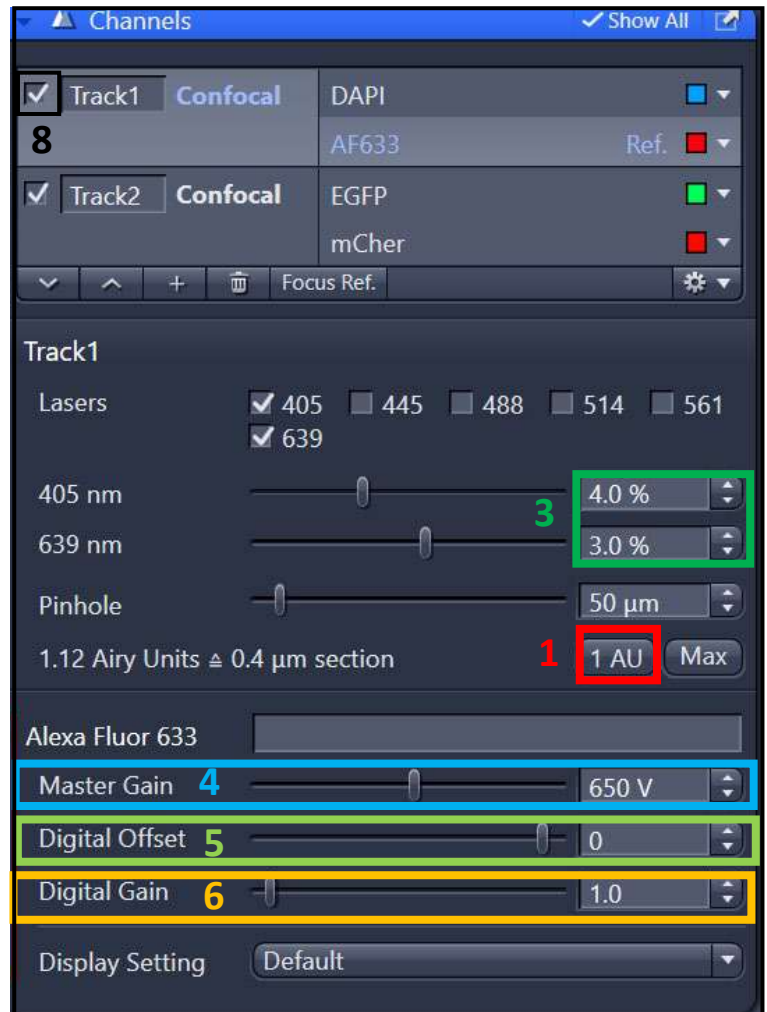
ACQUIRE A Z SERIE 1/2

The Z-Stack acquisition permits to visualize your sample in 3D.
To calibrate your Z, you have to choose your brightest channel or/and you must pertinent.

1. Tick the Z-STACK box to open the window box in the menu Multidimensional Acquisition.

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

3. 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.

6. To choose the optimal resolution, you can use the « *Optimal* » option.
Be careful to not oversampling. The optimal as don't be under 0.5-0.7um, except to reply to specific biologic questions.

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

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

ACQUIRE A Z SERIE 2/2

1. To make a co-localization analysis, click on **Match Pinhole** in order to automatically adjust pinhole sizes and obtain the same voxel size for all channels.

**2.** To adjust the acquisition order, in Light **Path** go to **Switch Track Every**.
Then choose Z-STACK if you want to acquire an entire stack channel by channel. Otherwise, choose FRAME to acquire all channels per frame.
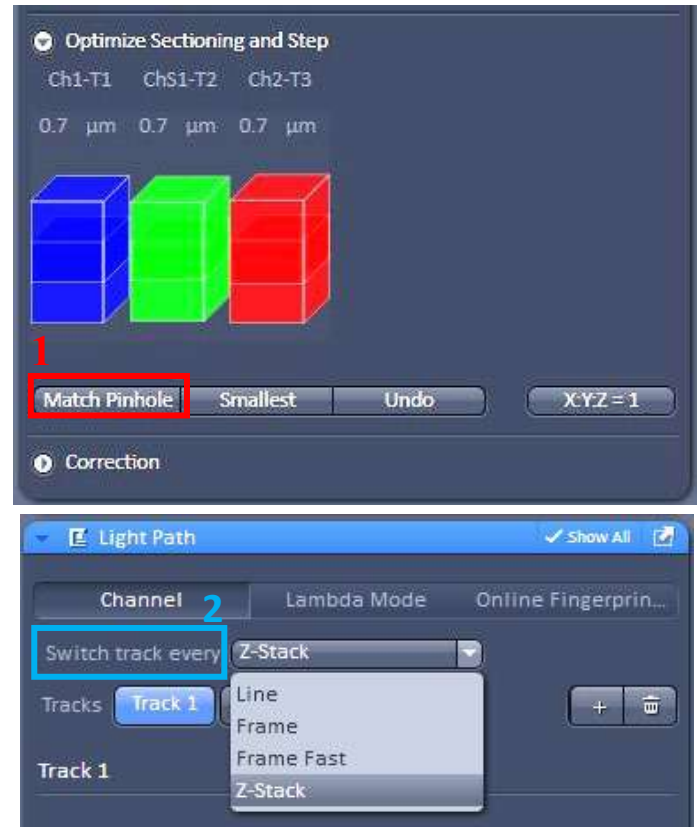
ACQUIRE A TEMPORAL SERIE

1. Proceed in as the same manner as in the previous section. Tick Times Series to open the tool box in the menu Multidimensional Acquisition

2. Choose the number of cycle and the interval between each time point. You can't define a time but only a cycle you need to count the time than your experiment will take
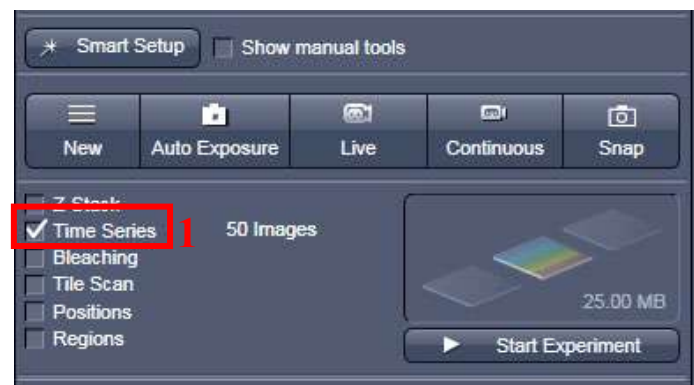
ACQUIRE A MOSAIC OF IMAGES

1. Tick Tile Scan to open the tool box in the menu Multidimensional Acquisition.

PS: There are different modes in Tile Scan.

2. CENTERED GRID:
This mode is to acquire a mosaic **from the center**.

3. Choose the number of lines and columns.

**4**. Let OVERLAP at 10%.

5. Tick BI-DIRECTIONAL to go faster, Tick Online stitching, and let the THRESHOLD at 0.10.

6. BOUNDING GRID,
to make a mosaic including different fields of interest

7. Look for your fields of interest in LIVE and save their coordinates by clicking on ADD. To remove a position, select the coordinates in the list and click on REMOVE. To remove all, click on REMOVE ALL.

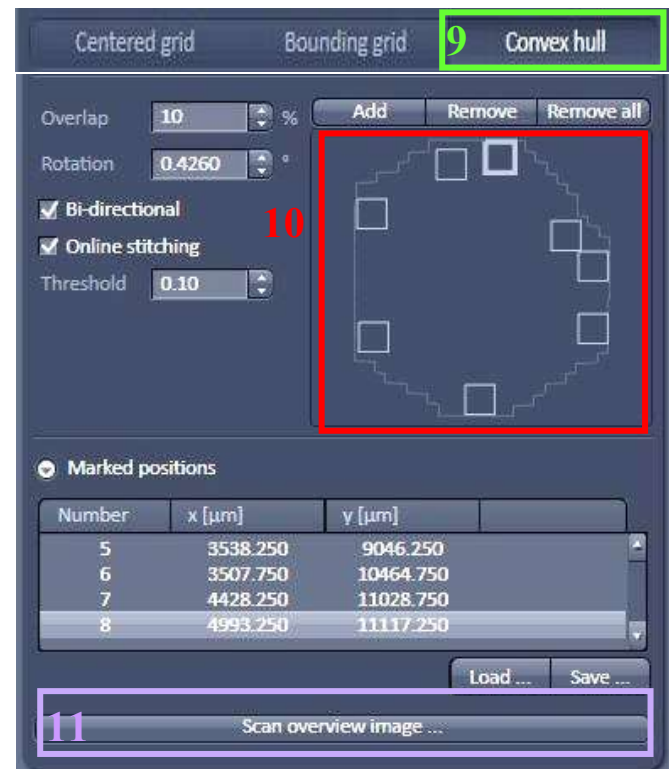**8.** Dimensions of your final mosaic are shown
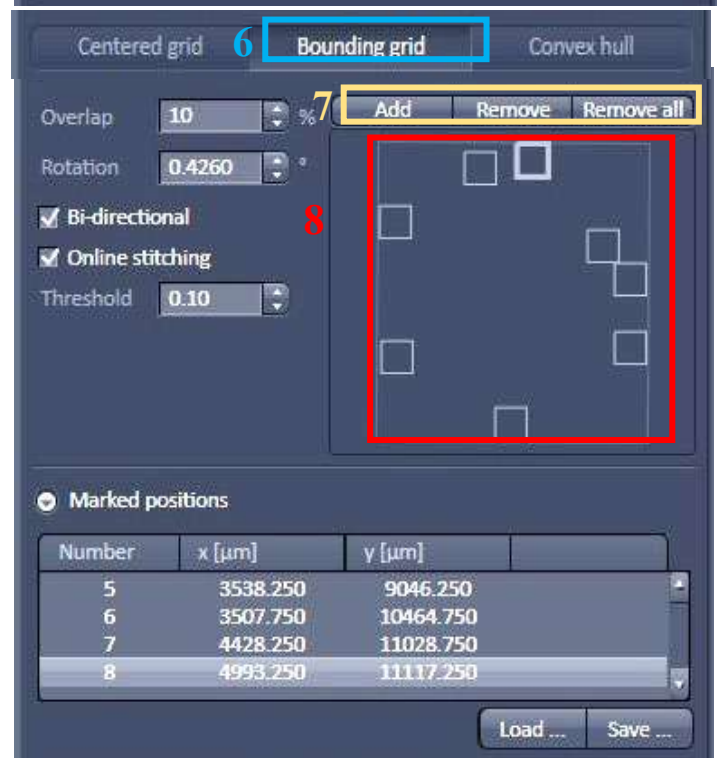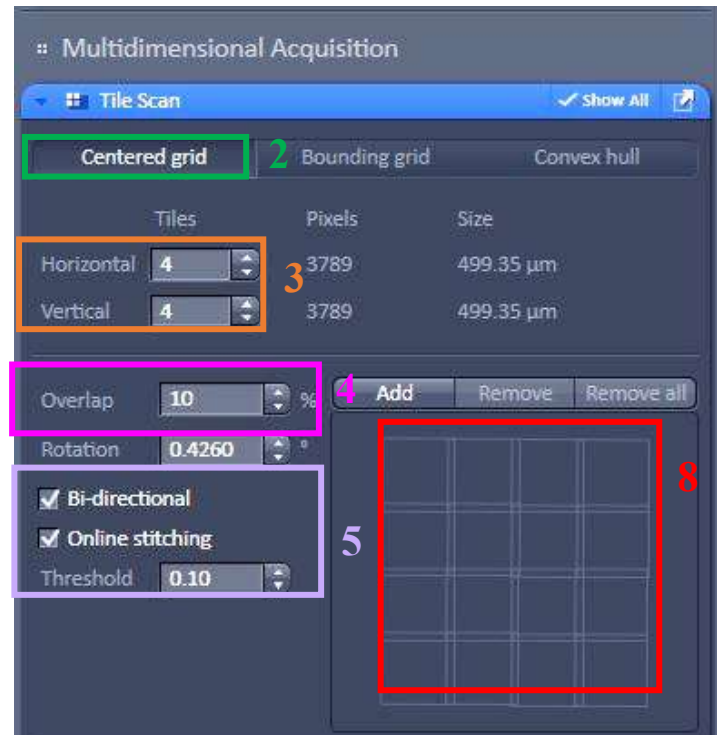
ACQUIRE A MOSAIC OF IMAGES 2/2

9. CONVEX HULL.

To make a mosaic including different fields of interest without the external zones

    10. To make a mosaic including different fields of interest without the external zones, go to the option CONVEX HULL.
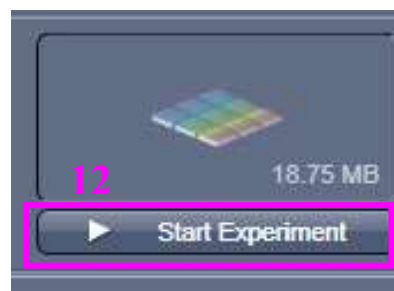
11. OVERVIEW

You can have an overview of your mosaic by clicking on Scan Overview Image. You can choose an objective and a zoom different from the final acquisition (be careful when you change from an oil objective to a dry one or inversely).

12. When all settings are chosen Click on START EXPERIMENT to start the acquisition.

ACQUIRE SEVERAL POSITIONS

1. Tick the POSITIONS box to open its windows in the menu Multidimensional Acquisition.

2. if you want to choose different positions on the same slide, select Position List. You can observe all your position.

3. To choose a position, you need to position yourself on the position of interest and click on ADD in the POSITION LIST tab.
You must ADD each position one by one.
You can delete all or part of a position if you have made a mistake.

PS: Another solution to locate fields of interest is to acquire rapidly a mosaic with a low resolution and spot wanted positions on the image by clicking on POSITIONS.
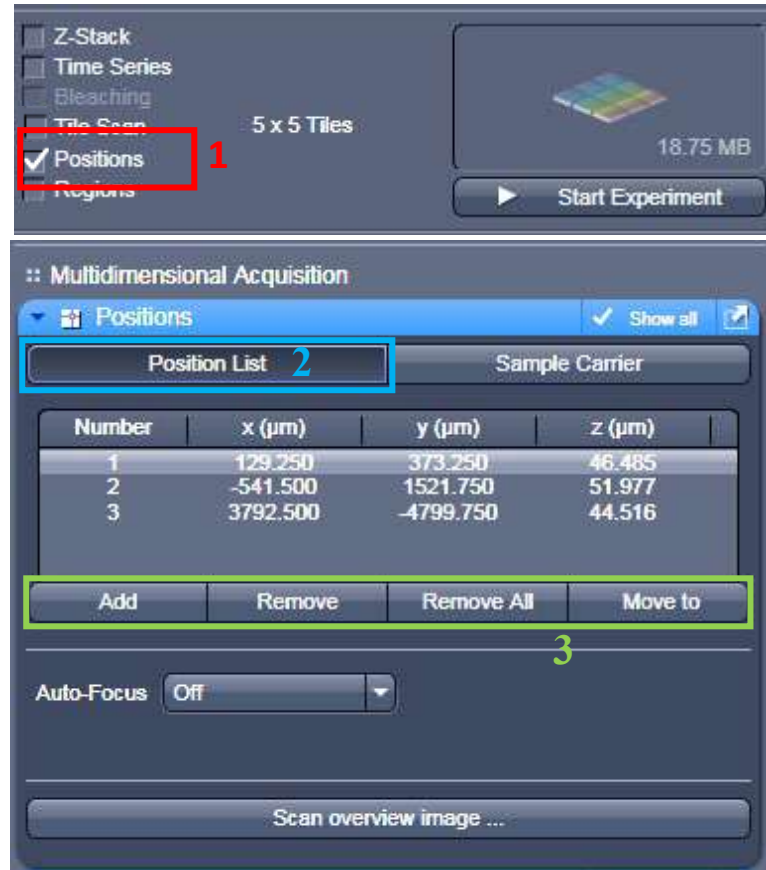
Multi-well Acquisition

3. Select SAMPLE CARRIER.

4. Click on PROPERTIES and load parameters of your multi-well plate.

5. Then click on CALIBRATE to consider these parameters.

6. Then select wells of interest with the buttons SELECT, SELECT ALL, or CLEAR ALL.

7. Tick OBJECTIVE LOWERED WHEN MOVING STAGE if you want to lower the objective for each displacement. (For oil objectives)
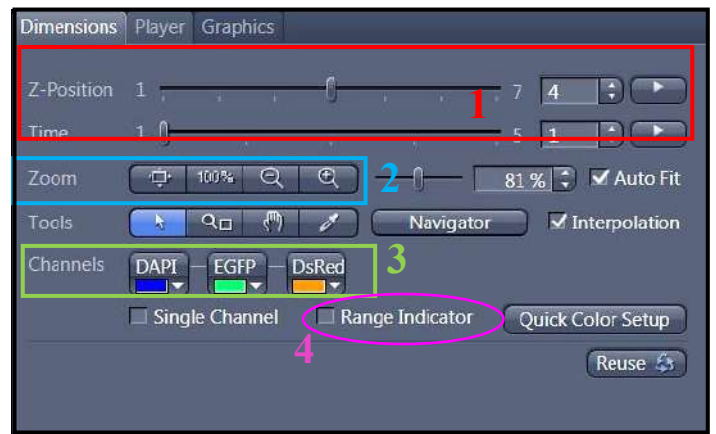
8. Click on START EXPERIMENT to start the acquisition.

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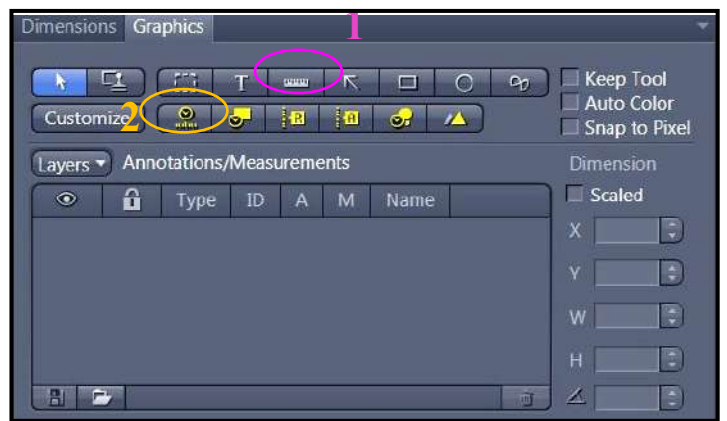4. Saturation observation (Red is the saturated pixel).

In the tab « *Display* »

1. Choose the channel or all to modify the image quality.
2. Contrast adjustment. Check on Reset to reinitialize it.
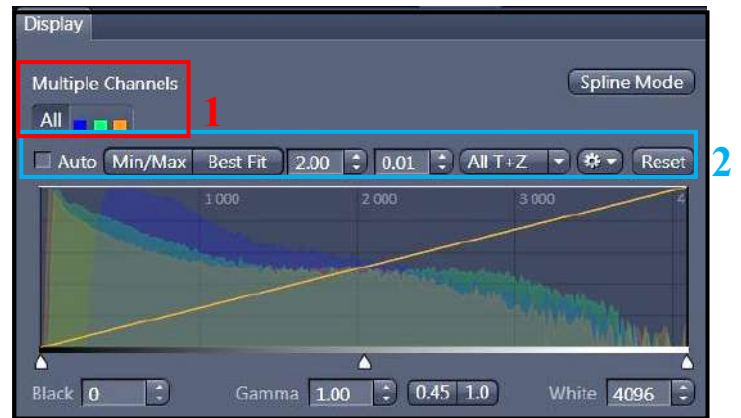
In Tab « *Graphics* »

1. Show the scale bar.
2. Show the time.

SIGNAL QUANTIFICATION

1. To quantify a fluorescence signal, you have to acquire them with the same acquisition parameters (same laser power, same gain, and Offset for PMTs).
2. To don't change the parameter you have to adjust your acquisition's settings with the sample whose fluorescence signal is the brightest. To limit the Over exposition effect.

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.
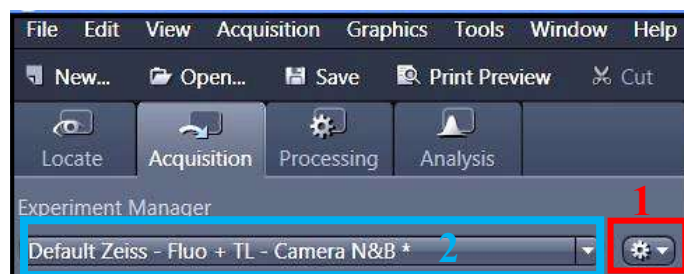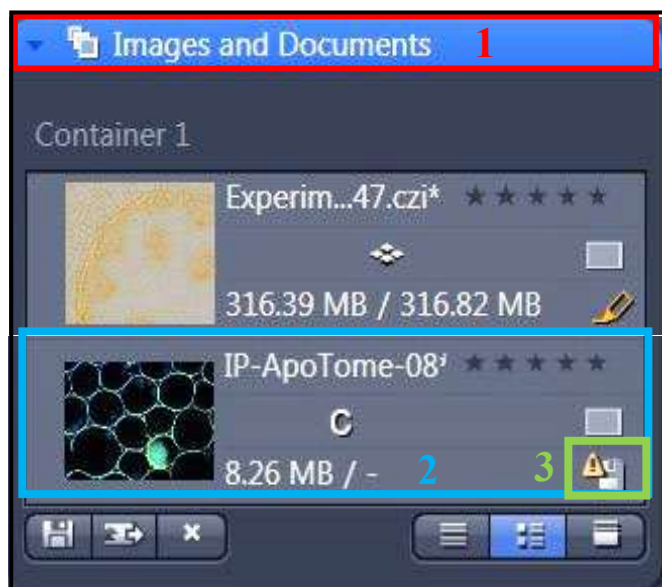

Image Saving

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.

START THE SYSTEM

1. Check that all your acquisitions are saved.

2. Close the software

3. Clean the objectives that you used and put them at the lowest position.

4. Push the button up to « LASER RUN ».
Once the green LED is lit

⚠ Turn the potentiometer to the **0 position**, while taking care of not switching on the red LED. Wait a few moments to turn the power off.

⚠ **5.** Turn the Power key on « OFF ».
Let the switch is at the « ON » position.
Don't touch it when you turn off the system

6. Switch off the fluorescent light.

7. turn of the Computer with "the start" menu

8. turn off the component button,

9. Turn off the PC system

10. Finally wait 5 min and turn off the Main switch