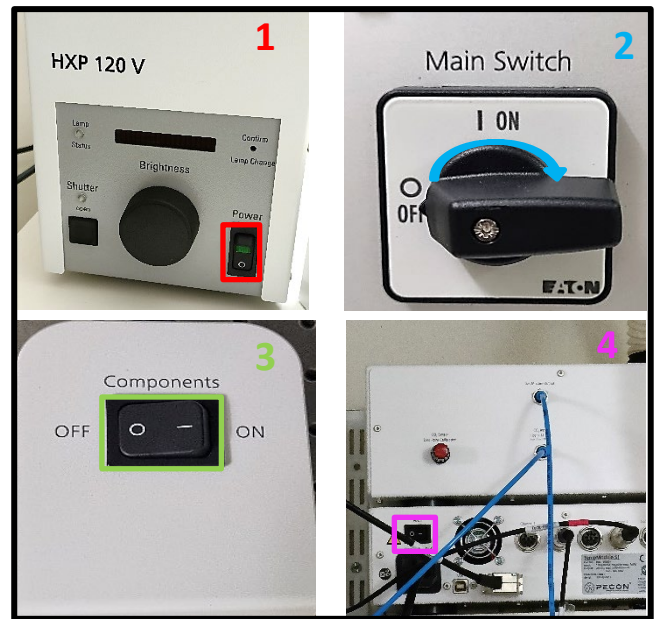


<b>START-UP SYSTEM.....</b>	<b>2</b>
<b>START-UP SOFTWARE.....</b>	<b>2</b>
<b>OCULAR OBSERVATION.....</b>	<b>3</b>
<b>MANUAL COMMAND.....</b>	<b>4</b>
<b>ACQUISITION WIDEFIELD .....</b>	<b>4</b>
<b>ACQUISITION HILO / TIRF .....</b>	<b>6</b>
<b>TWO CAMERAS ALIGNMENT.....</b>	<b>6</b>
<b>Z-STACK ACQUISITION .....</b>	<b>7</b>
<b>TIME SERIE ACQUISITION.....</b>	<b>7</b>
<b>SIM ACQUISITION .....</b>	<b>7</b>
<b>ANALYSE SIM .....</b>	<b>10</b>
<b>ACQUIRED IMAGE OBSERVATION .....</b>	<b>11</b>
<b>IMAGES RECORDING .....</b>	<b>12</b>
<b>SHUTDOWN SYSTEM.....</b>	<b>12</b>

## Start-Up System

1. Start fluorescence lamp
2. Turn the « Main Switch » on ON
3. Turn « components » on ON
4. Optional: Turn on the temperature and CO2 control



## Start-Up Software


1. Switch on the PC and start the « LSM USER » session
2. Start ZEN Software (Black Edition)
3. Choose « Start System » to access the acquisition mode
4. In the « Acquisition » tab, switch on lasers.



# Ocular Observation

1. Select the tab named « Locate »

2. On Microscope Control windows, you can choose the objective that you want and see the information to use it

 Ps: *take care of the utilization if you have to use an immersion liquid uses the adapted one.*

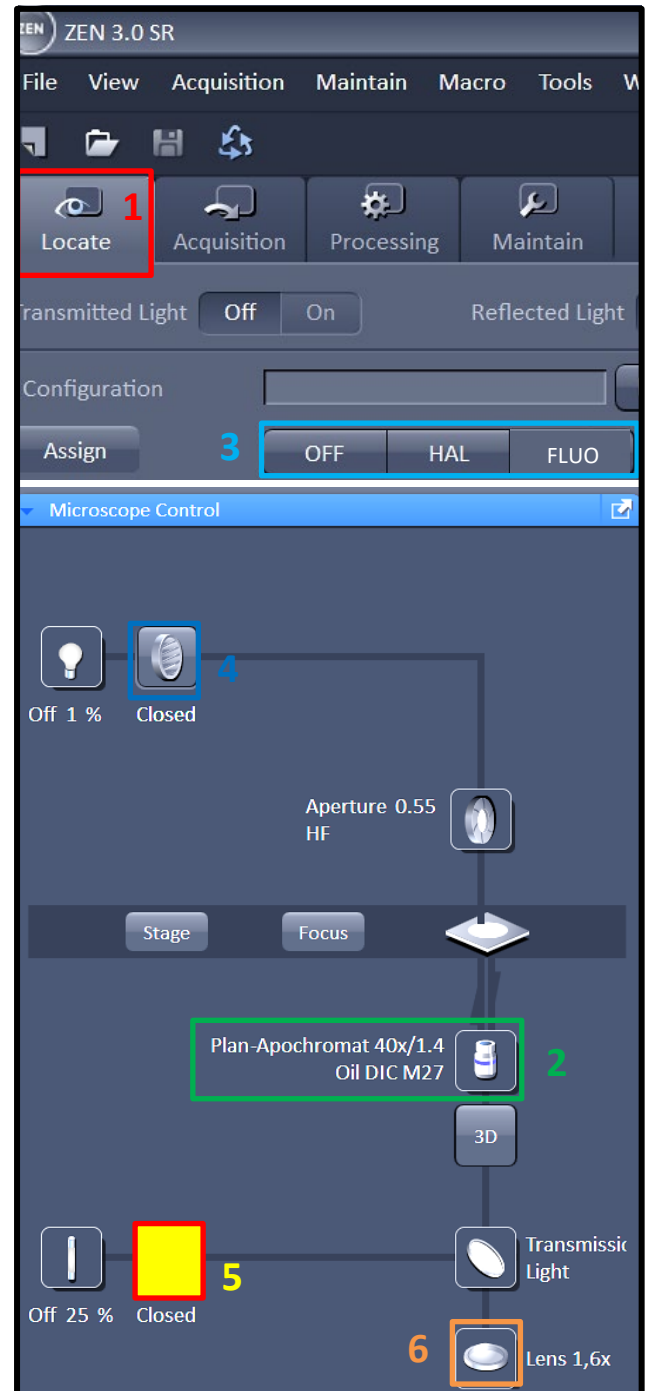
3. Select the illumination that you need.

4. Open/close the shutter for transmission light

5. Open/close the shutter for fluorescence

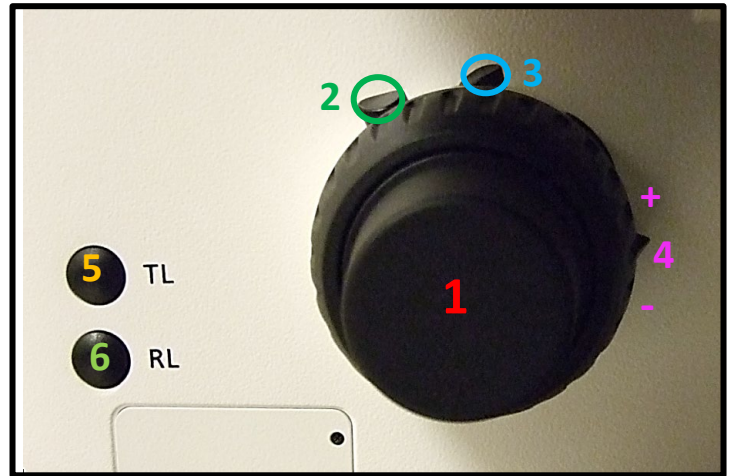
6. Choose a filter for what you need

7. Control fluorescence intensity



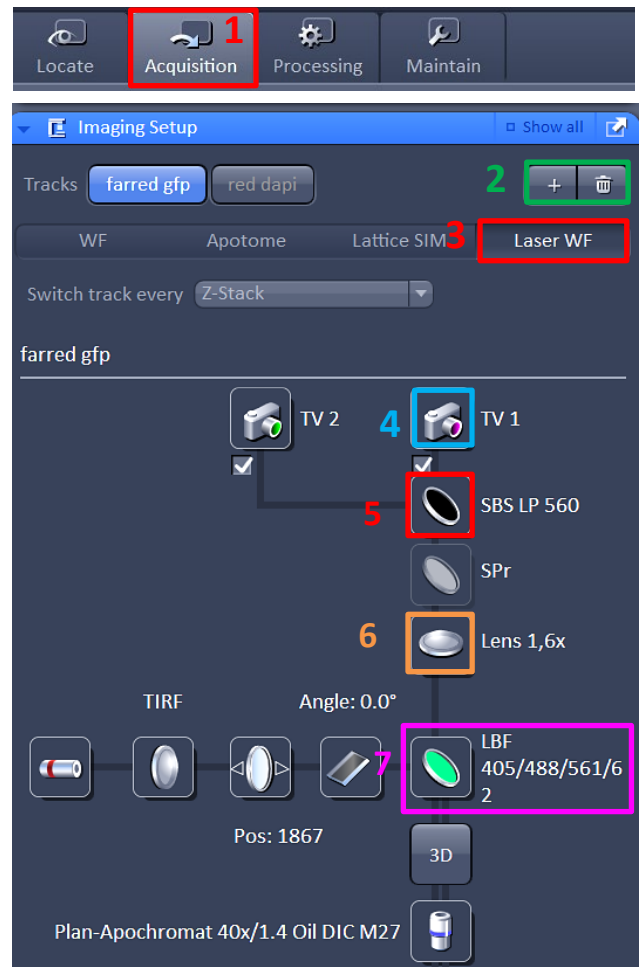
## Manual Command

1. Wheel to adjust the focus  
The big one permit to adjust fast, and the front one permit to adjust with more precision.
2. put down the objective to install your sample
3. Put back the objective at the focus point
4. Change the magnification of the objective
5. Open/Close the shutter of the transmission light
6. Open/Close Fluorescent lamp



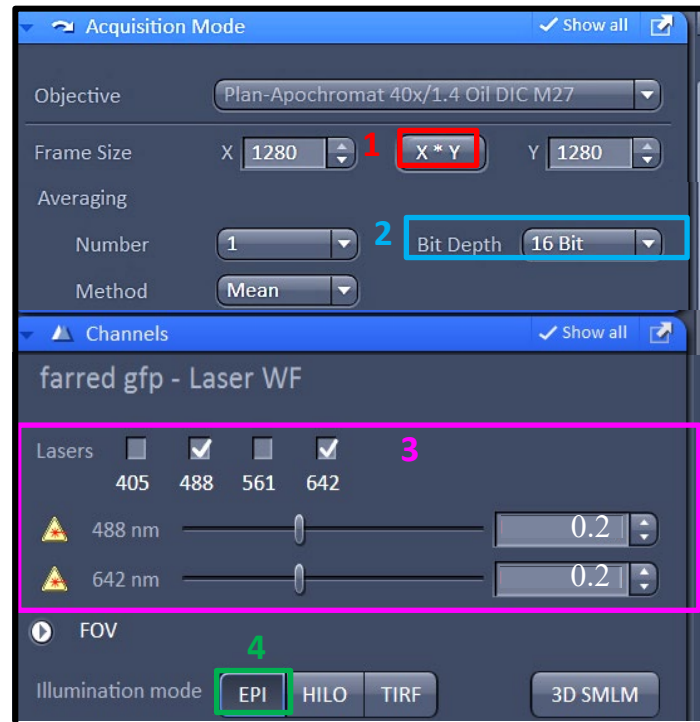
## Acquisition Widefield 1/3

1. Choose the « Acquisition » tab
2. Add Tracks depending on your fluorochrome combination.
3. In « Imaging Setup », Select « laser WF »
4. Select the camera you need  
(Cf look at the system documentation or on every camera).
5. For every track, select the good emission filter and dichroic mirror combination.
6. Select « Lens 1.6x » lens
7. Select the same dichroic mirror



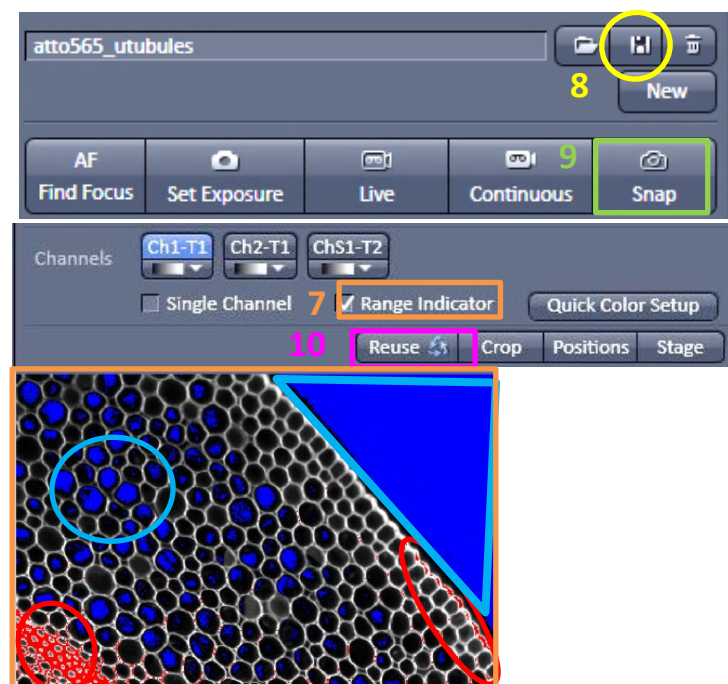
## Acquisition Widefield 2/3

1. In « Acquisition mode », choose the frame size X\*Y full chip or crop the image.
2. The Bit depth permit to increase in the quality of the image. However, an increase also in the weight of the file. I advised you to use 16 bits for the best quality
3. In the *channels* window, you activate the laser(s) adapted at your staining and adjust their power.  
*Ps: Beginning with 0.2% is better to not bleach or make damaged your sample. If the power is too low then you can increase the value*
4. Choose the acquisition mode (EPI, HILO, or TIRF)  
**EPI:** Parallel beam of light is passed directly upwards through the sample, maximizing the amount of illumination



## Acquisition Widefield 3/3

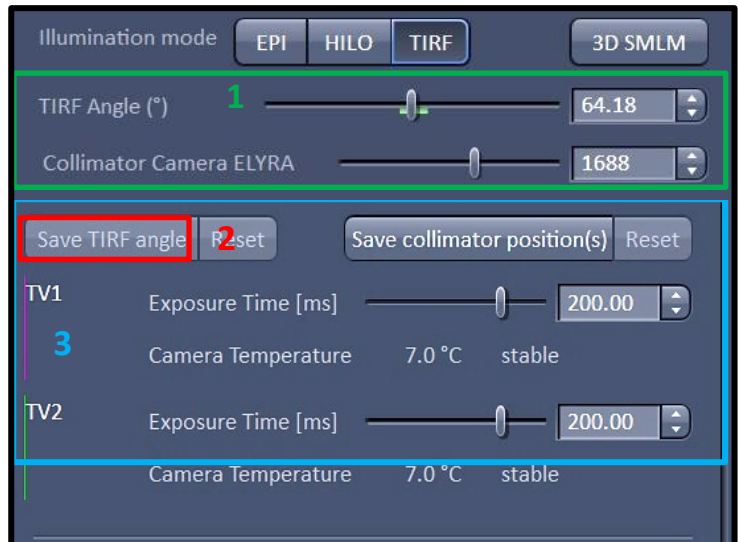
7. Saturation Control: To adjust the gain and laser power, it is recommended to use "Range Indicator, to determine if the pixels are "Saturated" (red pixel) or too "Low Excited" in blues
8. In the "Acquisition Tab" you can save your settings
9. Click on SNAP for image acquisition
10. You can **Reuse** the settings of an acquired image later by selecting the image and clicking on **REUSE**



## Acquisition HILO / TIRF Set Up

**1. TIRF:** Total Internal Refraction Fluorescent  
For TIRF and HILO modes, Choose the best reflection angle. The frame needs to be homogenous in intensity between left and right.

**HILO:** The laser is directed at a sharp angle through the sample. This allows an; imaging depth of up to 10 $\mu$ m, at a signal-to-background noise ratio (SNR) slightly lower than that of TIRF



**!** Ps: Don't modify the collimator value.

**2.** Save your TIRF's angle.

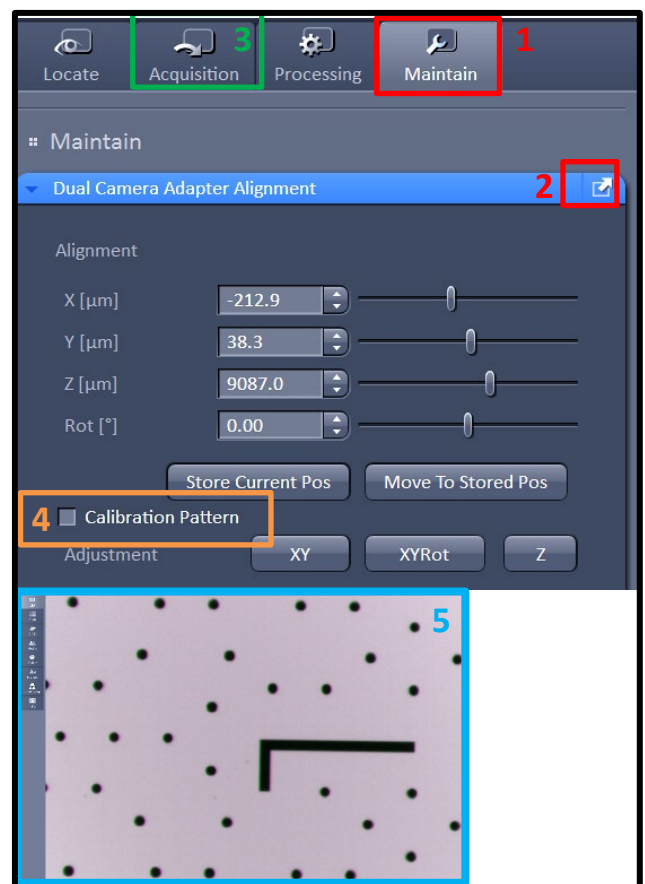
**3.** Adjust exposition time.

**!** Ps: This needs to be the same for all cameras.

## Two Cameras Alignment

If you want to use two cameras, it's necessary to align the X, Y, and Z positions.

- 1.** Choose the « *Maintain* » tab.
- 2.** Isolate « Dual Camera Adapter Alignment » to create an independent window.
- 3.** Go to the « *Acquisition* » tab, then start « live » and move to the focus position
- 4.** Tick « *Calibration Pattern* »
- 5.** Adjust the x, y et z alignment and the rotation et la rotation by using patterns until both colors are completely covered.
- 6.** Untick « *Calibration Pattern* » et close « *Dual Camera Adapter Alignment* »



## Z-Stack Acquisition

In the « *Multidimensional Acquisition* » stick Z-Stack to make appear the window corresponding to the Z-setting acquisition

### Thin Sample

1. Select the « CENTER » tab in the Z-Stack window
2. Make the focus and press on the Center button

### Thick Sample

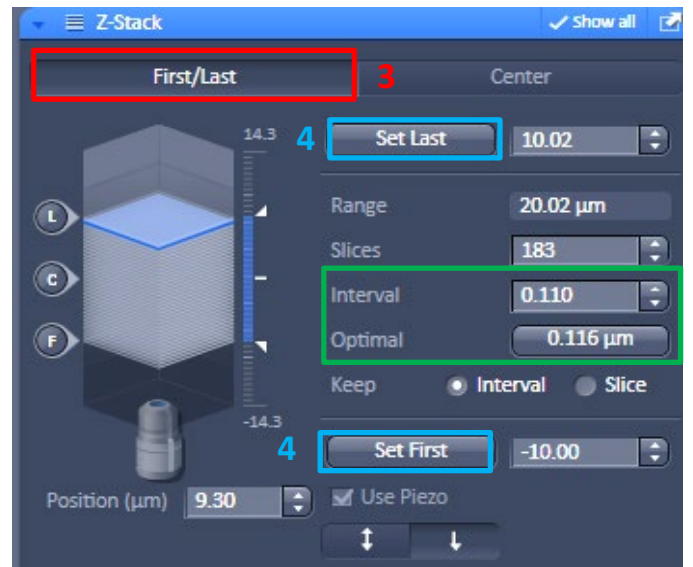
3. Select the « FIRST/LAST » tab in the Z-Stack window
4. Move at the limit position out focus on the slide side extremity and press « SET FIRST ».
4. In the same way find the limit out focus position for the other extremity and press « SET LAST ».

6. To define the space between two Z-plan you have to tick the « *Intervalle* » case and write the space wanted in  $\mu\text{m}$ .

### PS:

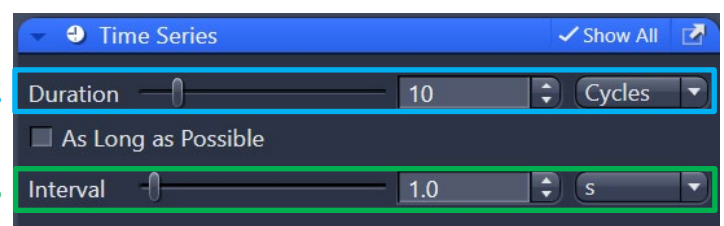
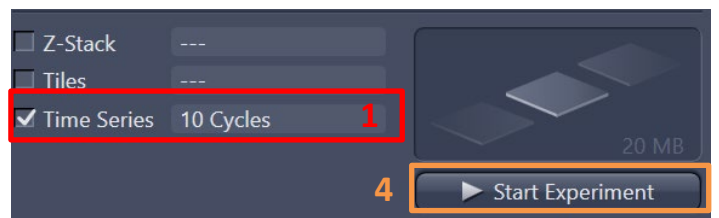
- *In SIM mode, a sample of 110nm is recommended.*
- *The piezo couldn't be unticked*

7. Press « Start Experiment » to begin the acquisition.



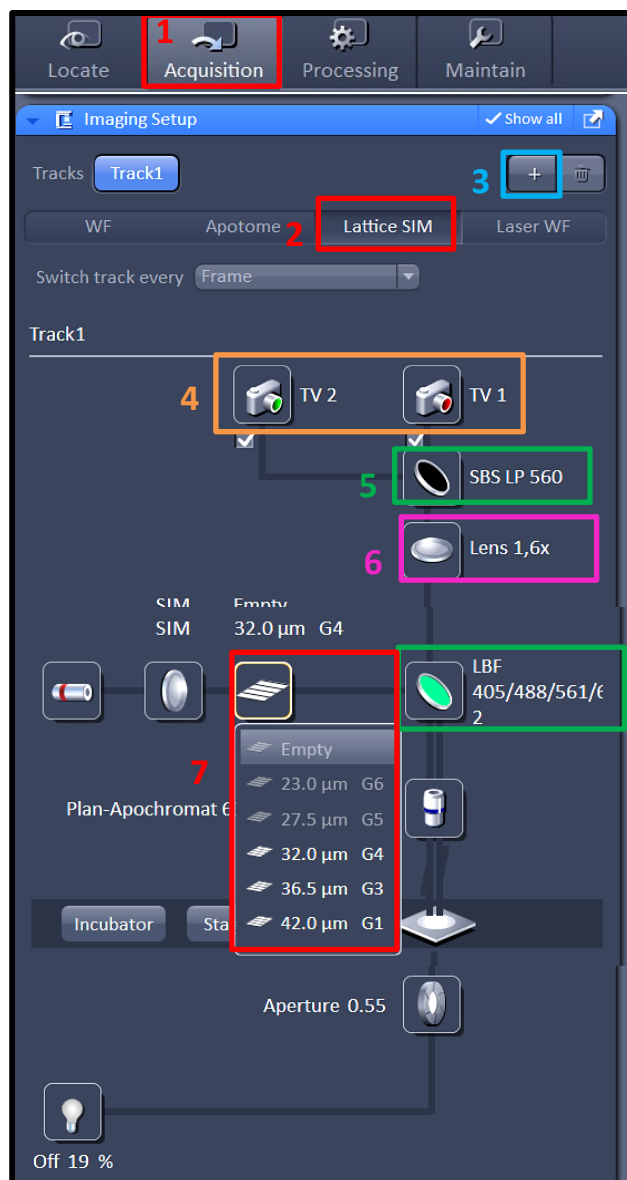
## Time Serie Acquisition

1. Tick « *Times Series* » to make appear the setting window in the « *Multidimensional Acquisition* » menu.
2. Define the number of cycles/ or duration times of the experiment.
3. Define the time-lapse between each acquisition
4. Press « Start Experiment » to begin the acquisition



## SIM Acquisition 1/2

1. Select the « Acquisition » Tab
2. In the « Imaging Setup » window select « Lattice SIM »
3. Add tracks in the function of your fluorochrome combination.
4. Define the adapted camera, with the help of the different documents in the system.
5. For each track select the adequate Emission Filter combination and a good dichroic mirror.
6. Choose the « lens 1.6x »
7. In «Imaging Setup », the good grid will be automatically selected in the function of the laser-activated. If the grid is not visible enough, it is possible to change it to a bigger one. Be care full because that decreased also the resolution.





## SIM Acquisition 2/2

1. In the « Channel » window, activate the laser Needed. Under the laser selection, you can define their power.

*Ps: It is advised to start with low power such as 0.2% to not bleach or damage your sample during your setup.*

2. In the « Channel » you can define the Exposure Time, which increases your signal quality but also the bleaching and the phototoxicity.

*Ps: the exposure time have to be the same between both camera on the same track*

3. In the « Acquisition Mode » window, you can select the frame size that defines your pixels number (image resolution) in each plan Dans « acquisition mode »,

*Ps: If you reduce the pixel number you'll not change the pixel size but you'll reduce the field of you.*

*Les pixels numbers*

4. The bit depth defines the grey shade of your pixel and increases the image quality but also the size of your data. However, It is the best way to use 16 bits

5. Phase number is the number of patterns passing on your sample during the acquisition.

*Ps: Between the different choices (9; 15 or 13) phase 13 is recommended to obtain a good balance between resolution, time acquisition, and bleaching.*

The screenshot shows the SIM Acquisition software interface with several panels and settings highlighted by numbered callouts:

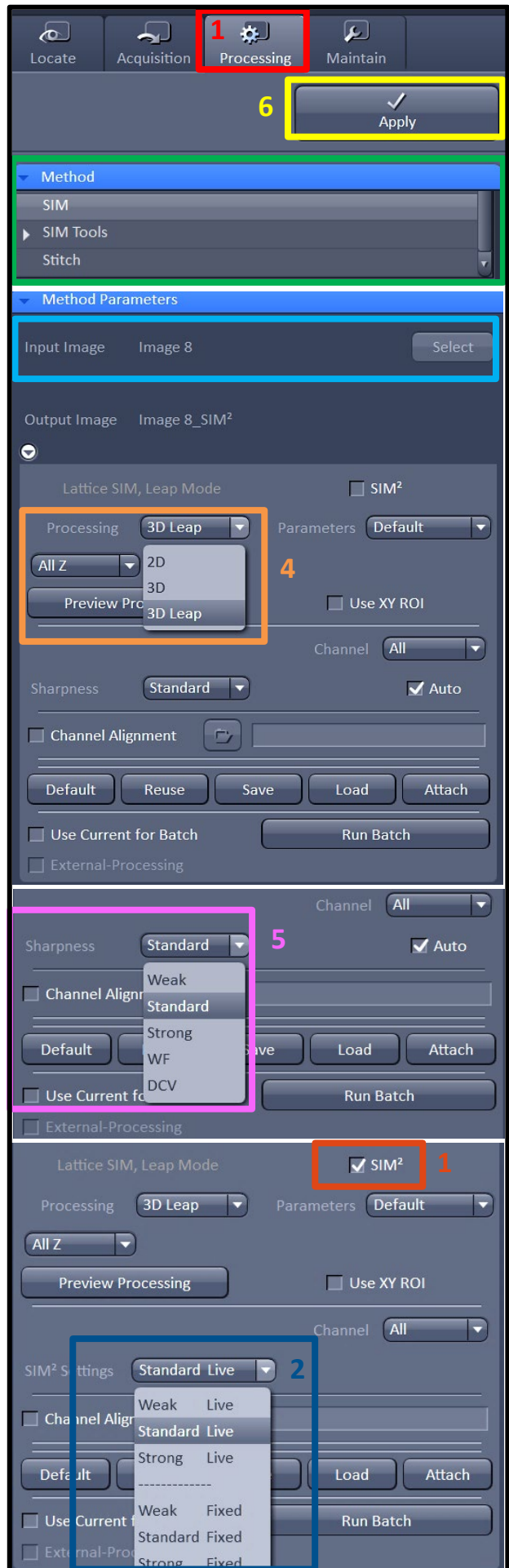
- 1:** The "Lasers" section in the "Track1 - Lattice SIM" panel, showing checkboxes for 405, 488, 561, and 642 nm lasers, with the 642 nm laser selected and its power set to 0.018.
- 2:** The "FOV" section, showing exposure time and camera temperature settings for TV1 and TV2 cameras, both set to 50.00 ms and 7.0 °C.
- 3:** The "Frame Size" section, showing X and Y dimensions set to 1280 pixels.
- 4:** The "Averaging" section, showing "Number" set to 1 and "Bit Depth" set to 16 Bit.
- 5:** The "Grating" section, showing a dropdown menu with "13 Phases" selected.

# ANALYSE SIM

1. Select the « Processing » tab
2. In the « Method » window, select « SIM »
3. In the « Method Parameters » window, choose your image
4. Choose the analysis method in the function of your acquisition types. (2D, 3D, or 3D Leap if you ticked the leap mode Z-stack)
5. Select the analysis power (Weak, Standard, or Strong) in the function of the acquisition quality.
6. Press « apply » to start your analysis.

## SIM^2 UTILISATION

1. Tick « SIM^2 »
2. Select the analysis power in the function of your acquisition type of your sample live or fixed.



# Acquired Image Observation

1. Observe Z plan, a Time Point, or a Position

2. The Native Zoom (Image pixel = Screen pixel); make a 100% Screen Zoom. You can also adjust the zoom with Zoom in / Zoom out buttons

3. tick the name of the channel (blue part) that you want to observe.

*Ps: if you untick the all channels the image will appear black.*

4. Tick on the SINGLE CHANNEL case to see the channel individually.

5. Tick on the RANGE INDICATOR case to observe the saturation levels.

*Ps: The red pixel are saturated and the blues are Too few are excited.*

6. observation of all channels overlapped.

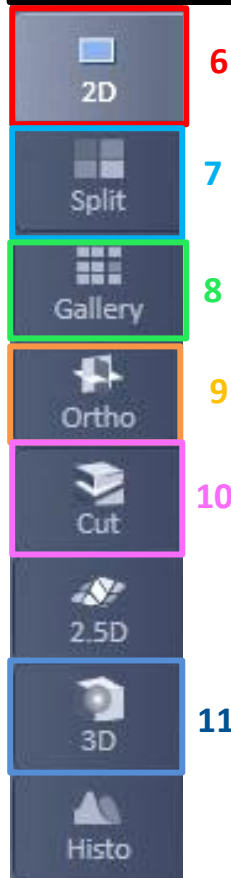
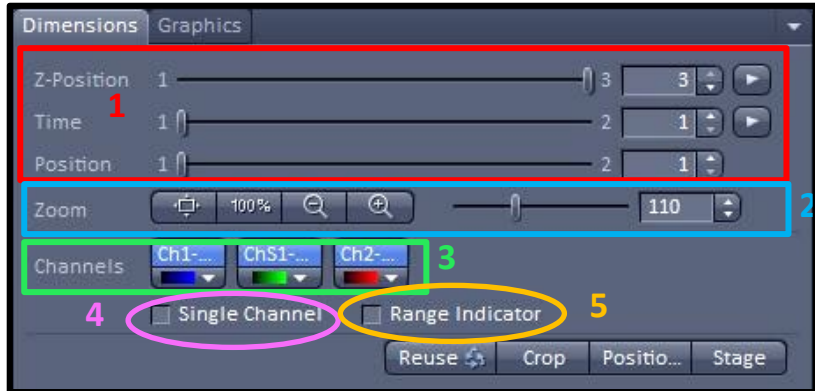
7. Observation of all channels separated.

8. Observation of all images in the function of the Time; Focus Point; or Wavelength.

9. Orhtogonal cut in XZ and YZ.

10. 3D section

11. 3D View



## Images Recording

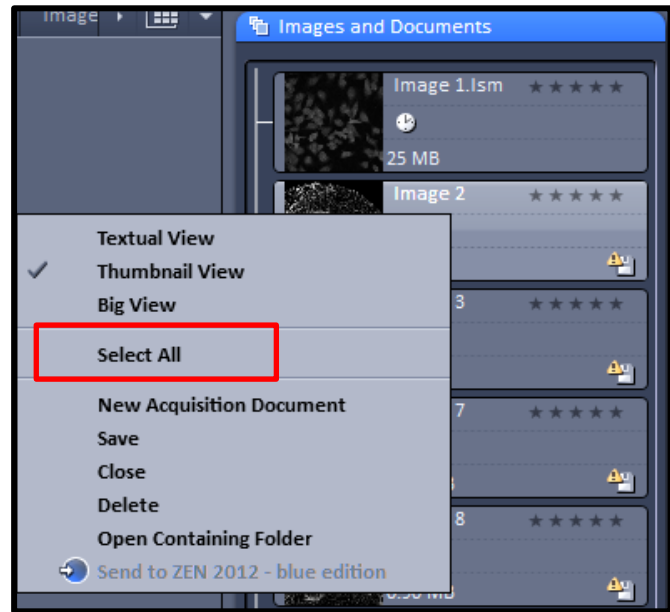
Select the image and save it in CZi or Lsm format.

The file has to be saved in the Folder  
User/Years/Month/days/name

I advise saving in CZi that could be opened by Image J and keeping all information as the scale bar and pixels Information etc...

You can select all your images by right-clicking on the images from top to bottom, then choosing to SELECT ALL.

You can also delete all your series so be careful full



## Shutdown system

The first step check on the planning if the system is booking after your session.

**If the system is booking directly in a timed lap of 4h DON'T shut down the system:**

1. Check that all your data have been saved
2. Verify that all objective has been cleaned and down at the lowest position

**If the system is not used after your session you have to shut down the setup:**

1. Check that all your data have been saved Vérifier que toutes les données sont bien sauvegardées.
2. Close the Zen window menu *File* → *Exit*)
3. ShutDown the computer with the tools bar (START → SHUTDOWN)
4. Check that all objective has been cleaned and down at the lowest position
5. Turn Off the fluorescent Light and the other component.
6. Turn Off the « Main Switch »