LSM 880 Airyscan Instruction Manual

ZEISS

Seeing beyond

LIÈGE université

GIGA institute

Content

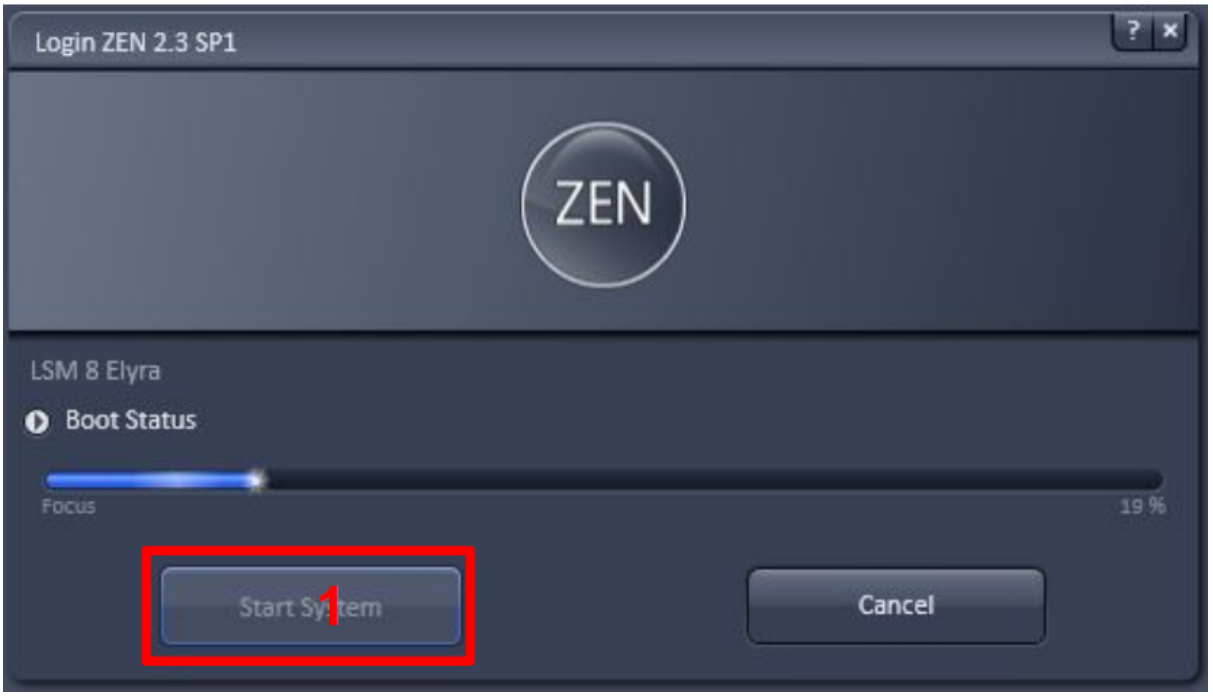
I. Start up procedure

Switch on all the button in the following order : the main switch (1), the systems/PC (2), Switch on the computer with the password : Zeiss*

When the user's session is open switch on the components (3)

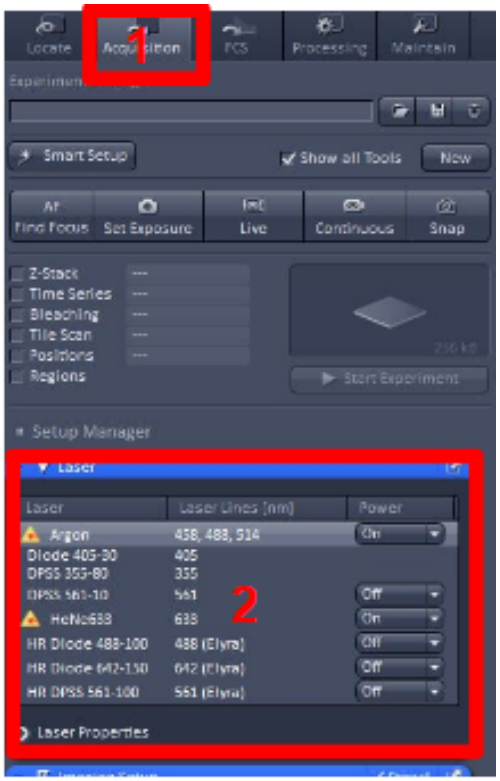
Open Zen Black

Select Start System (1)



Wait for the calibration (Don’t put your sample on the insert before the calibration).

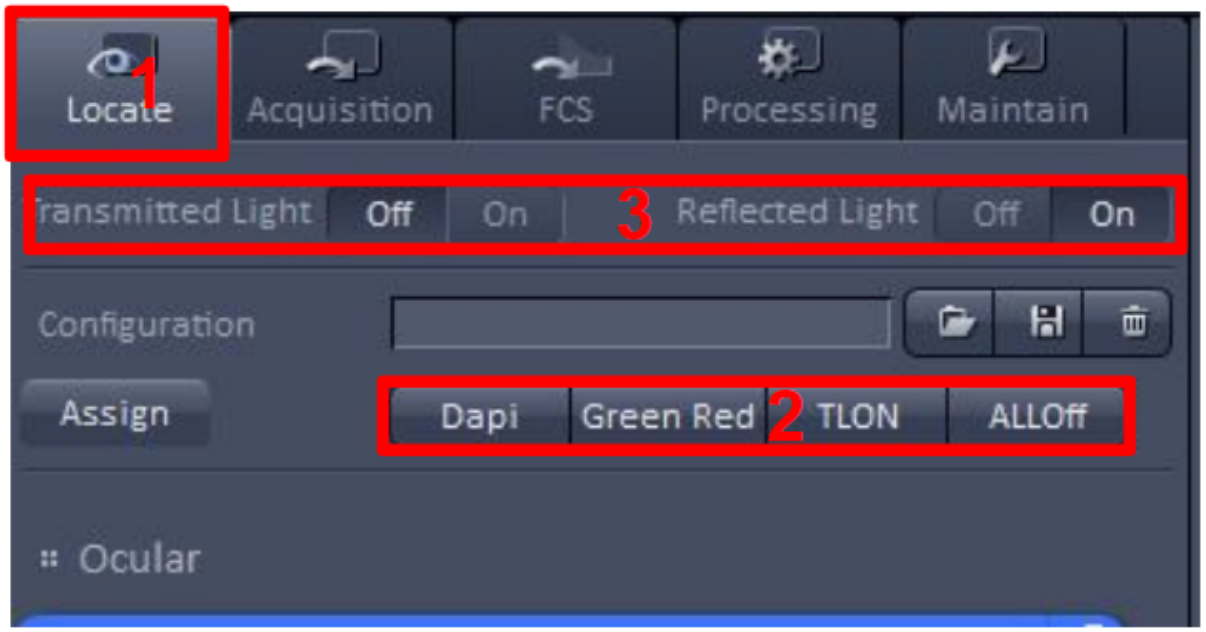
In the acquisition mode (1) switch on the laser (2) NOT ELYRA



II. Software/Interface description

Locate Menu :

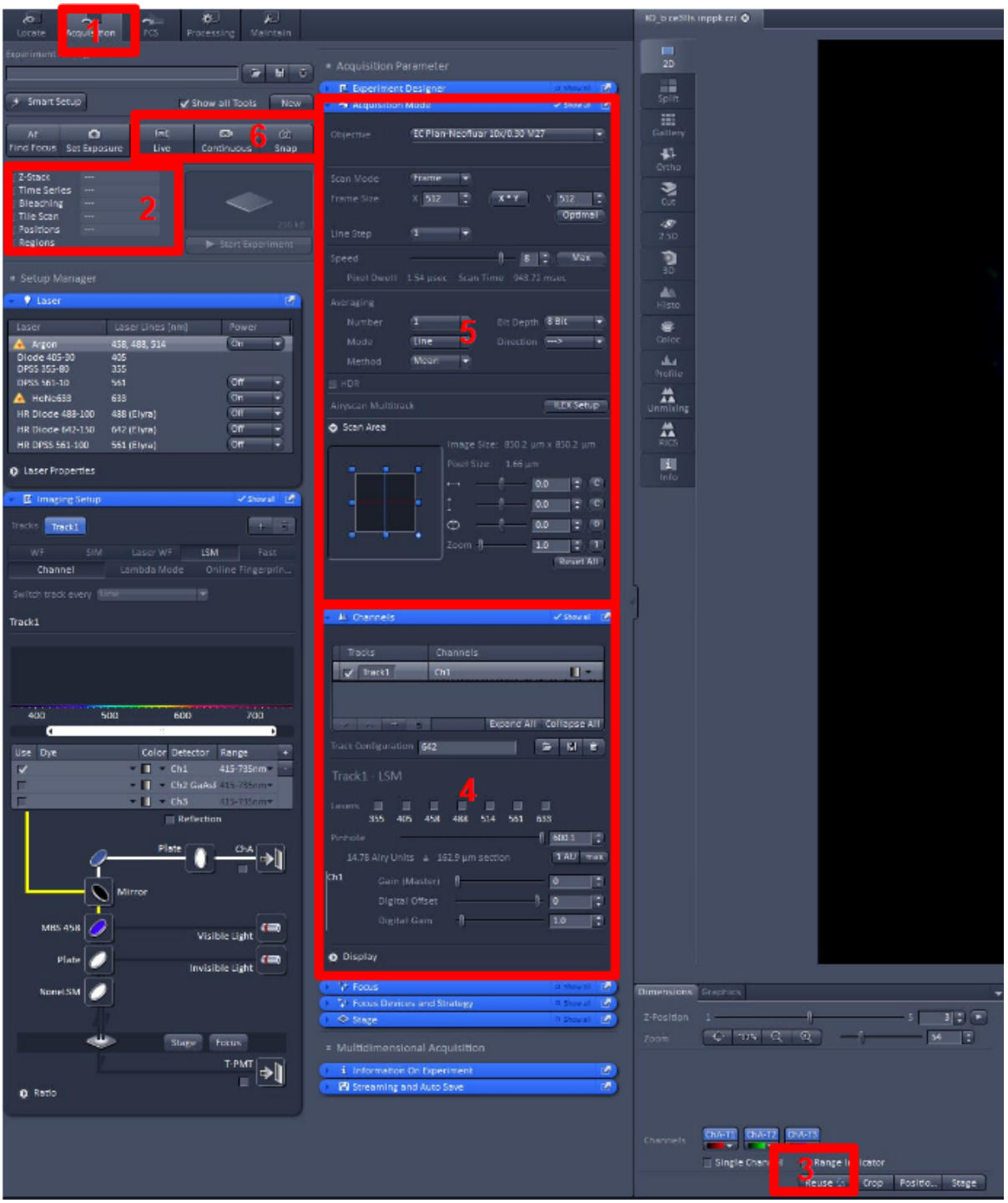
The locate menu (1) allows you to observe the sample, excited by the epifluorescent lamp or illuminated by the reflected light, with the eyes and to adjust the focus on it. Use predefined macros to observe the different fluorophores in your sample (2). Once the focus of the sample is done, don't forget to turn off the transmitted or reflected light (3) depending on the one you have used.



Reminder: Green/Red Allows you to see the Green (ie GFP/A488) and the Red/Orange (RFP/Al555) at the same time.

Acquisition Menu :

The acquisition menu (1) allows you to make 2D, 3D, time lapse, large images or to combine these differents options (2).



If you already have a previous image with good settings, you can open this one and reuse the right settings with the reuse button (3).

In the channel section you can select the fluorophores you want to observe but also change the laser power and the gain of the PMT (laser power between 2 and 7%

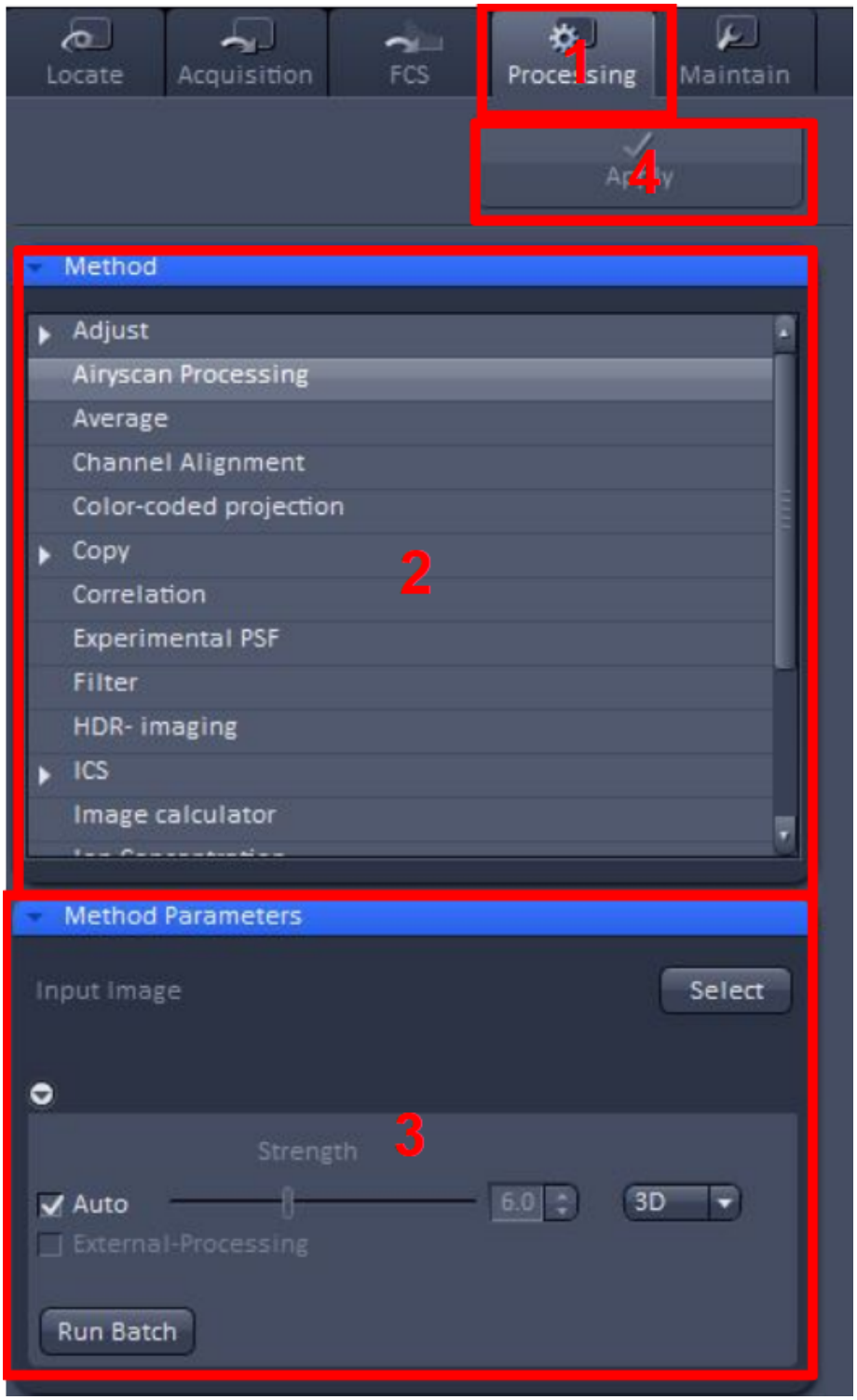
and Gain settings between 500 and 850)(4). In the case of airyscan mode, Fast Airyscan adjust the laser power with the number after the “E” in the laser power bar scale.

Finally you can locate the field of view in the sample and define the parameters with the Live mode button (it is fast but with low resolution). if you want to make a live view that takes into account the final parameters of the acquisition section (5), Select the continuous button : this will use the same parameters as the snap button the Snap button (6). To acquire, click on the snap button (6).

Processing Menu :

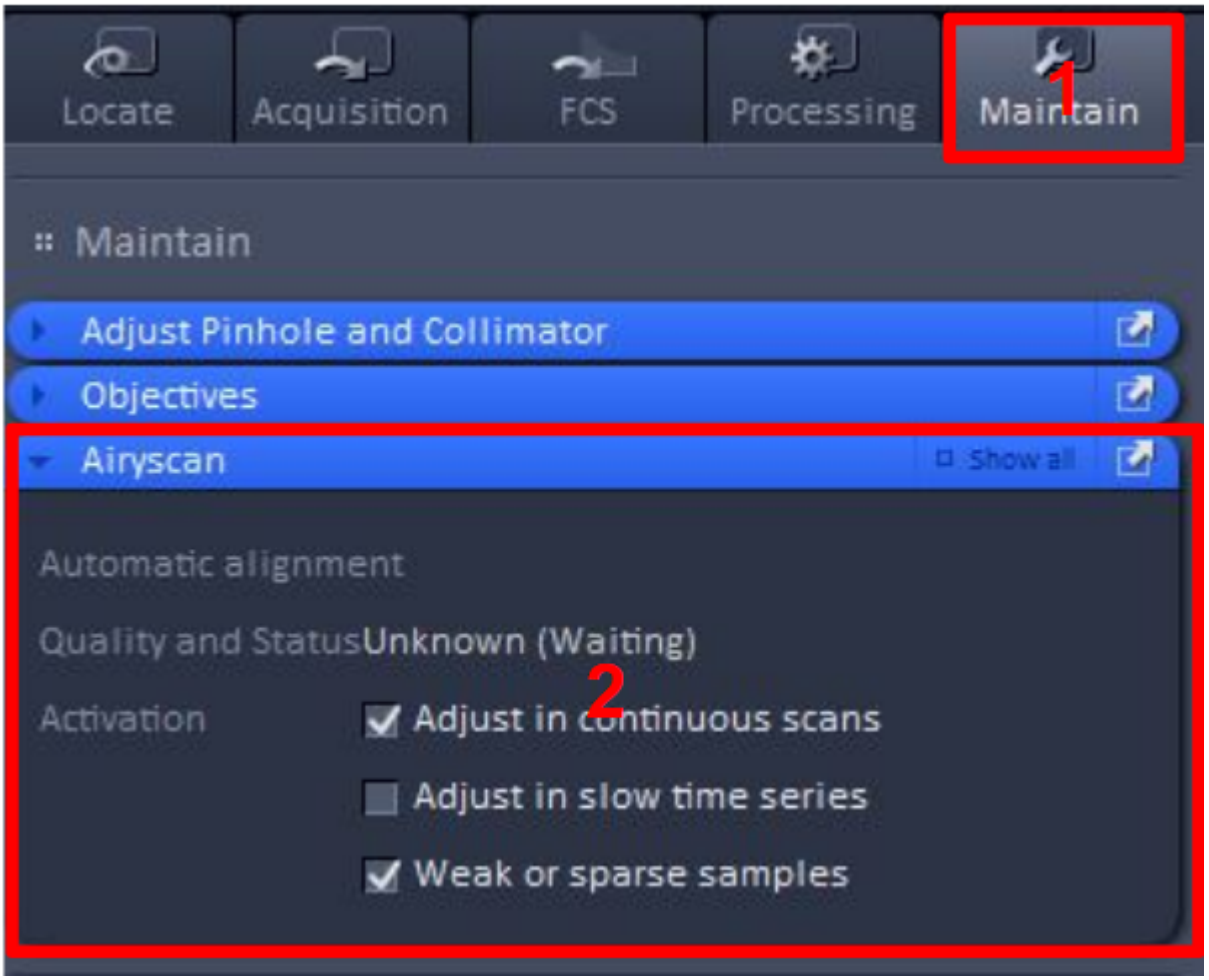
In the processing menu (1) you can perform different processing (2) including airyscan processing on your different type of images by selecting it in the input part (3) then apply to launch your processing method (4)

Attention : For tiles scan, always run airyscan processing before the stitching



Maintain Menu :

In the maintain menu (1) you can access the calibration of the airyscan(2). To execute this calibration go on continuous mode (in the acquisition menu) at the lowest resolution and a high gain on the laser 488, 514 or 561.

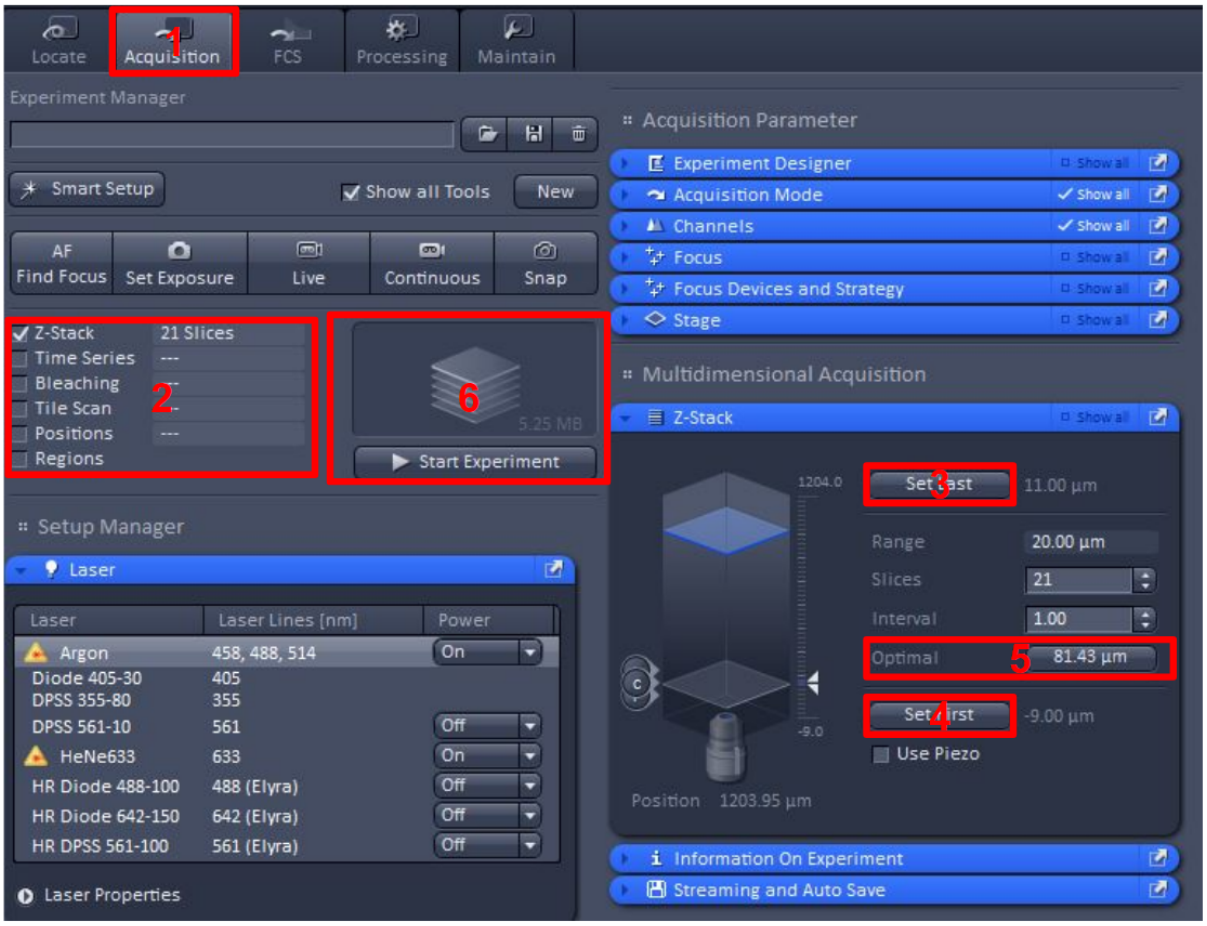


III. 3D Pictures

In the acquisition menu (1) check the Z stack option (2). Then open the Z stack menu then to set up your Z stack: while a 'live' image is acquired, use your fluorescence of interest as a guide.
Focus to the top of your sample and press "Set Last"(3). To define the bottom position, focus to the bottom of the sample and press "Set First"(4). Then stop the live to avoid bleaching of your sample.

Select optimal step size for best Z-resolution (5) or define the number of steps or steps size you need.
To speed-up your acquisition use the Piezo (available only if your sample is less than 100 µm)

Press "Start Experiment"(6) to start the acquisition.

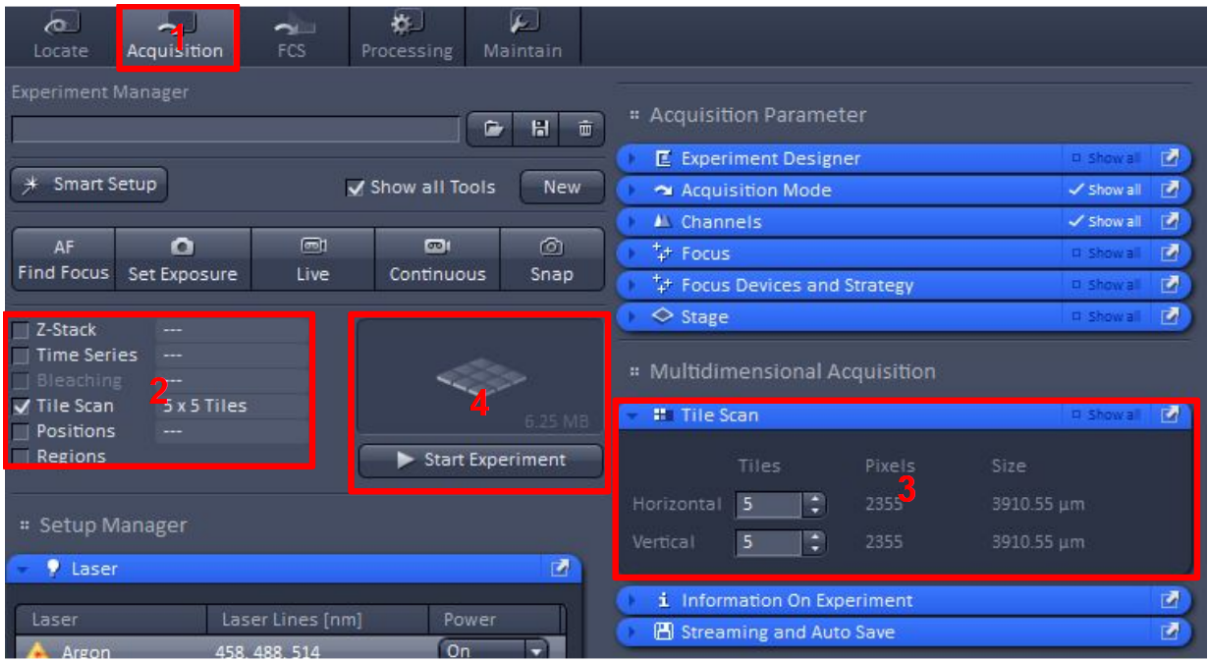


IV. Tiles scan

in the acquisition menu (1) select the tiles scan option (2). In the tiles scan menu you can define tile regions by number (3).

In addition, to facilitate the creation of your scan area, you can activate a “live” to locate your sample.

Press “Start Experiment”(4) to start the acquisition.

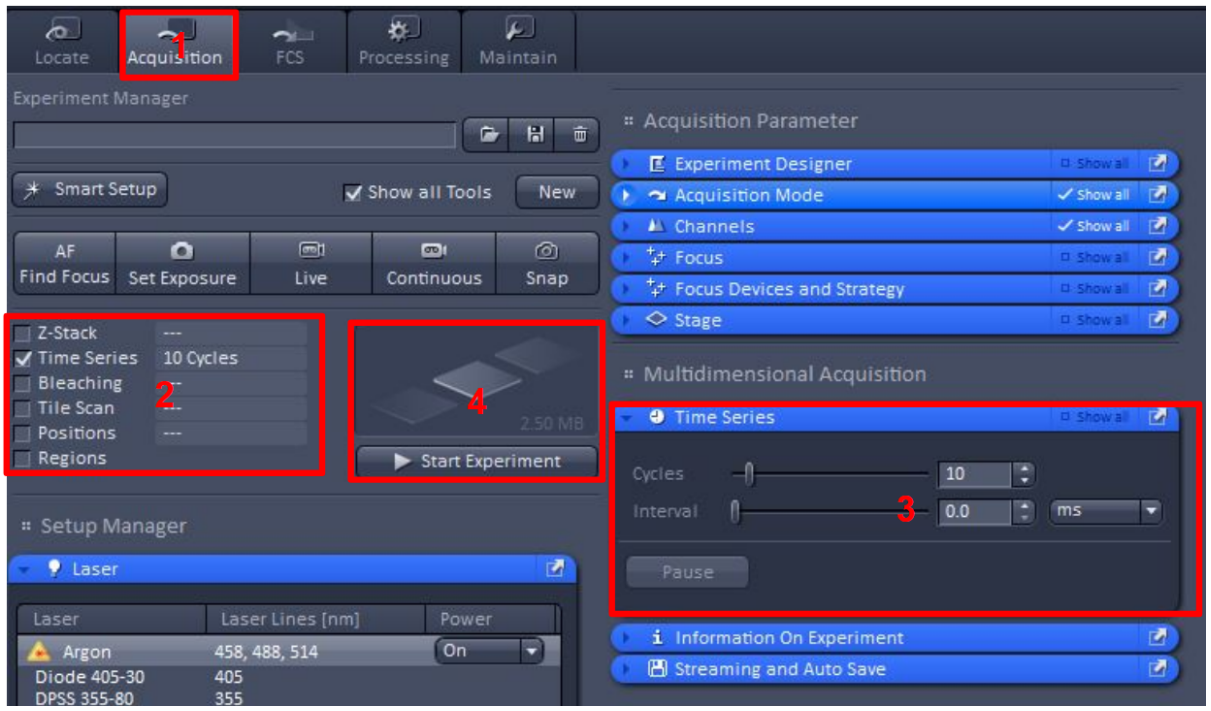


V. Timelapse

Switch on the CO_2 bottle and also turn on the Tokai module to activate the temperature and the CO_2.

Swap to the sample order for timelapse and add water into the grey part of the insert.

To configure your timelapse, go on Acquisition mode (1) select the options you want in addition to the time series(2).



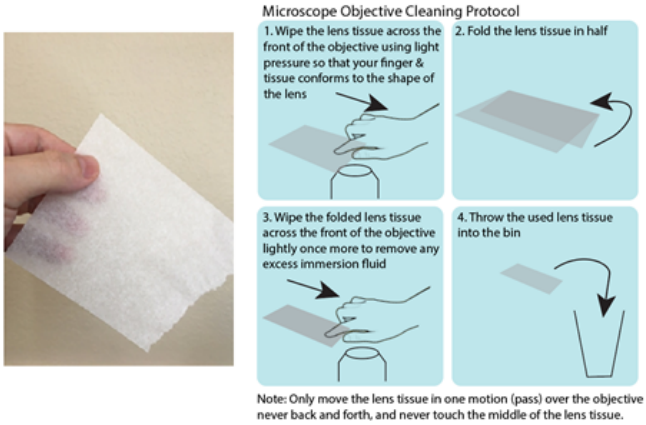
In the time series menu, you can select the number of loops and the time interval between each loop(3)

Press “Start Experiment”(4) to start the acquisition.

At the end of your timelapse don’t forget to remove water of the sample order then remove the sample order, switch off the CO_2 bottle and Tokai Module

VI. Shutdown procedure

Clean up the objective lens before and after your experimentation with special lens paper and 70% ethanol or isopropanol. (Particularly objective lens who need immersion oil or water)



Switch off all the button in the following order : switch off all laser in Zen Black then Switch off Zen Black
Shut down the computer
The components(3)
The systems/pc(2)

Wait 10 to 15min to switch off the main switch or until the ventilation noise decreases(1).