

LSM 980 Airyscan 2 Instruction Manual



Seeing beyond

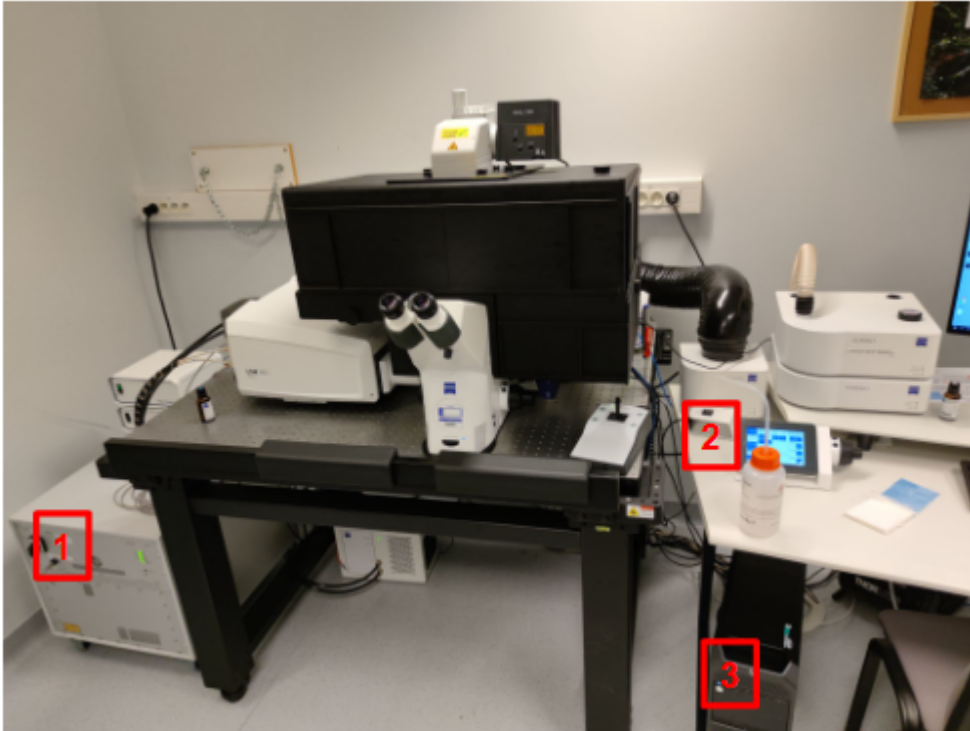


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I. Start up procedure

Switch on all the button in the following order : the laser (1), the components (2), the computer (3)



Password is Zeiss*

Open Zen Blue

Select Zen Systems

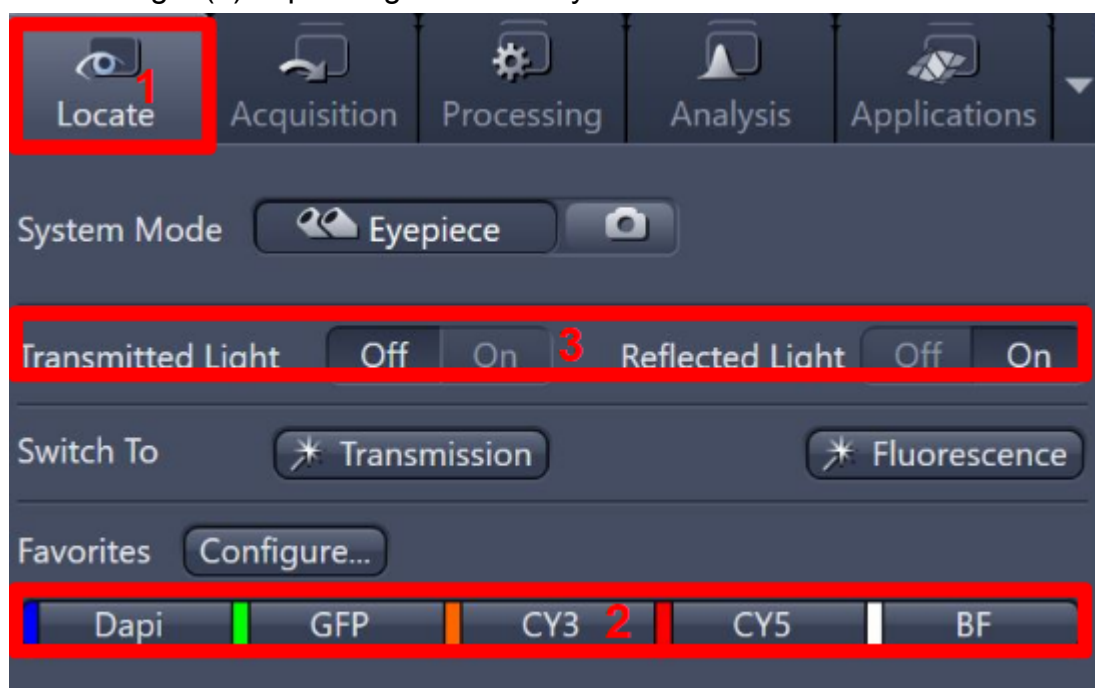
Select start Calibration

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

II. Software/Interface description

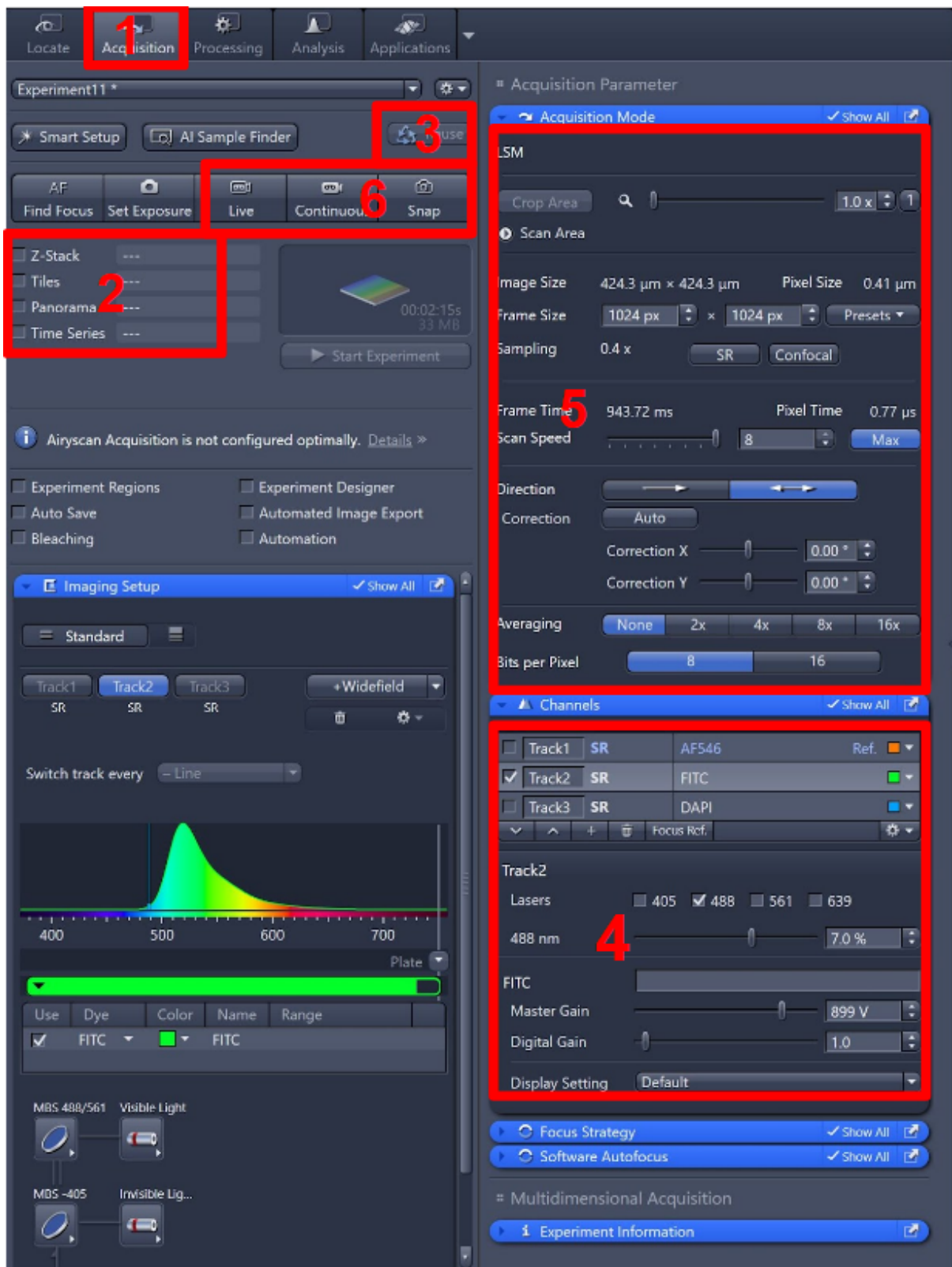
Locate Menu :

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.



Acquisition Menu :

The acquisition menu (1) allows you to make 2D, 3D, time lapse, large images or to combine these different 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 0 and 5% and Gain settings between 500 and 850)(4). In the case of airyscan mode, Multiplex 4Y or Multiplex 8Y adjust the laser power via “Equivalent laser power in confocal mode” under the laser power setting bar (a).

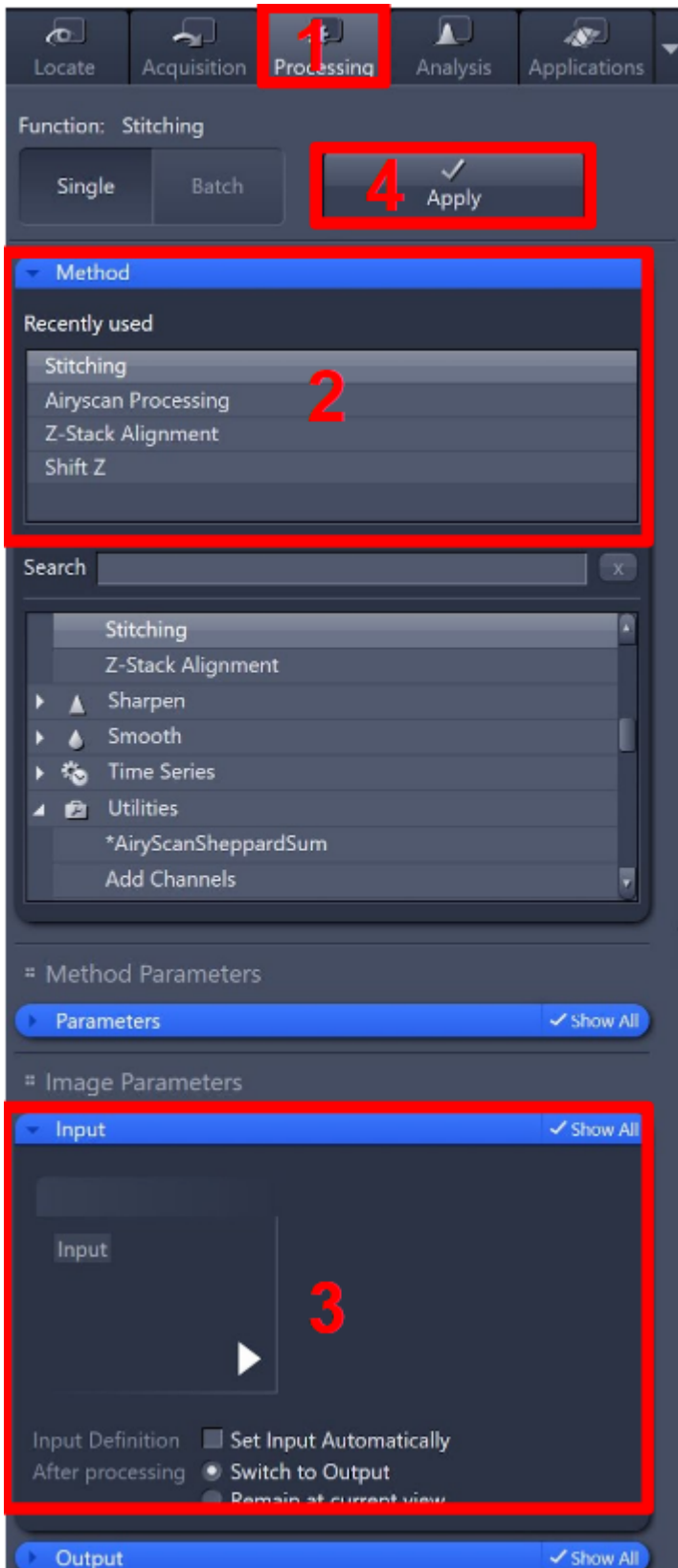


Finally you can locate the field of view in the sample and define the parameters with the Live mode button (it is fast but with low resolution). if you want to make a live view that takes into account the final parameters of the acquisition section (5), Select the continuous button : this will use the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

Processing Menu :

In the proccessing 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



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.

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

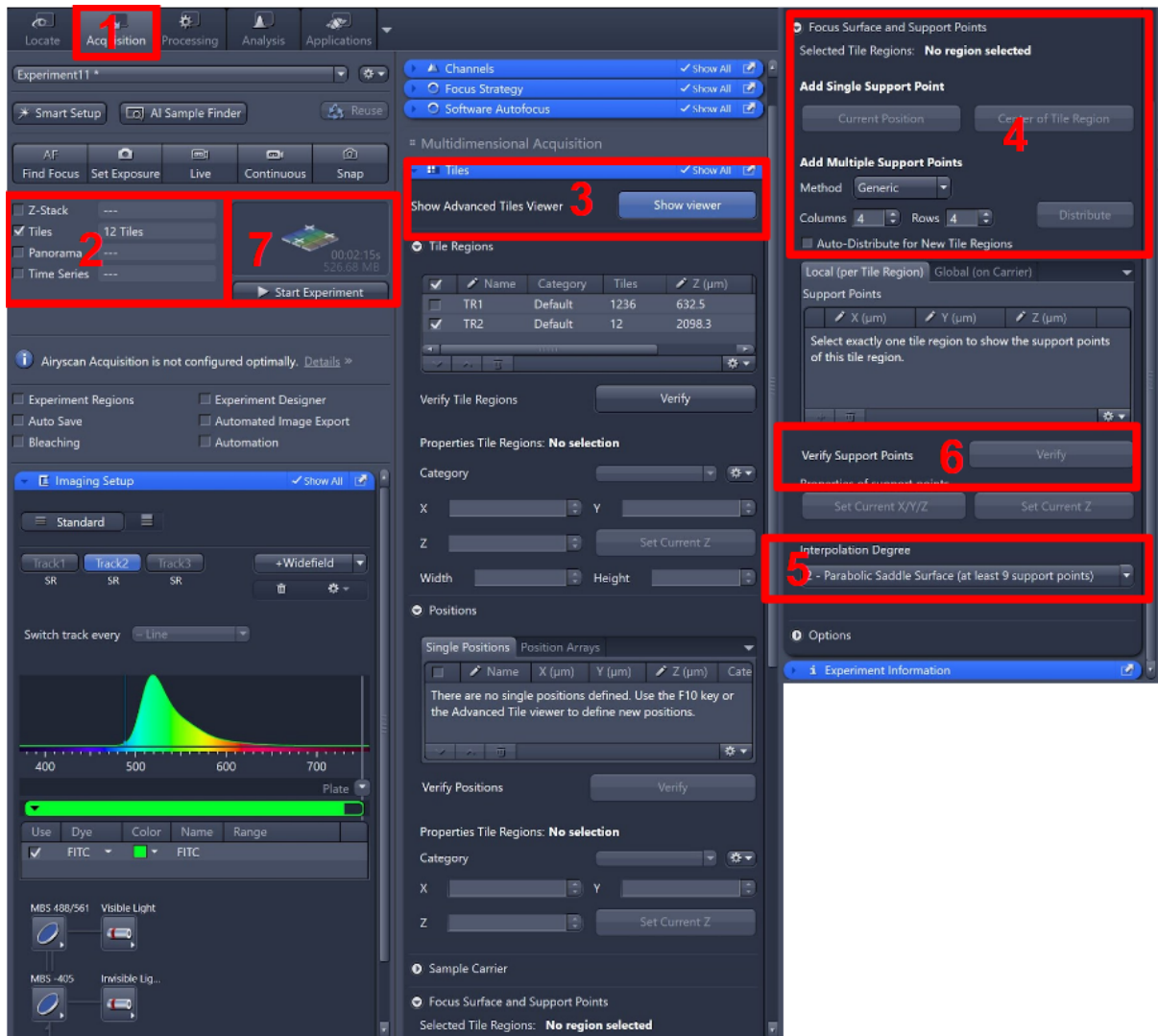
The image shows a software interface for microscope acquisition, divided into several panels. Red boxes and numbers highlight specific features:

- 1:** The **Acquisition** tab in the top navigation bar.
- 2:** A group of checkboxes including **Z-Stack** (checked), **Time Series**, and **Time Series**.
- 3:** The **Set Last** button in the Z-Stack acquisition parameter section.
- 4:** The **Set First** button in the Z-Stack acquisition parameter section.
- 5:** The **Optimal** button in the Z-Stack acquisition parameter section.
- 6:** The **Start Experiment** button.

The interface includes the following sections:

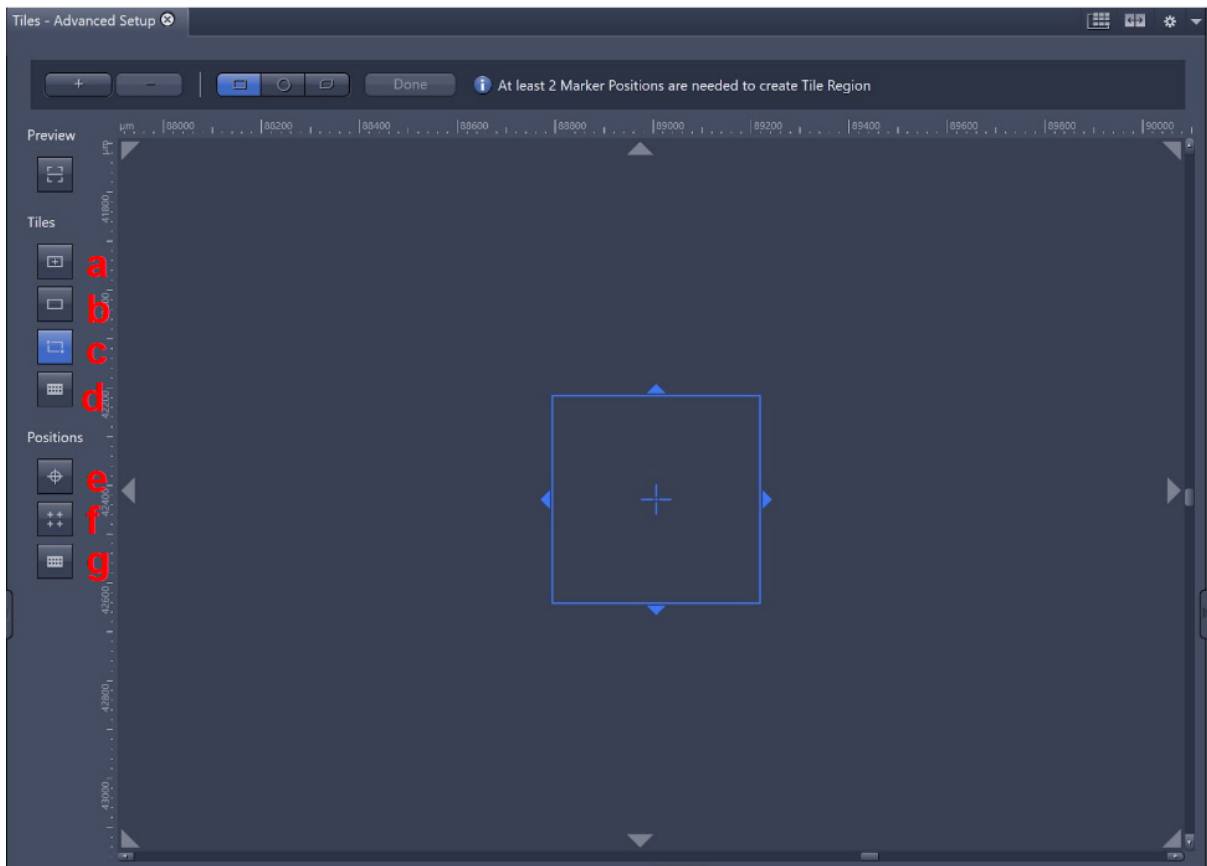
- Top Bar:** Locate, Acquisition, Processing, Analysis, Applications.
- Experiment11 *:** Smart Setup, AI Sample Finder, Reuse.
- AF:** Find Focus, Set Exposure, Live, Continuous, Snap.
- Z-Stack:** 11 Slices, Start Experiment.
- Acquisition Parameter:** Acquisition Mode, Channels, Focus Strategy, Software Autofocus.
- Multidimensional Acquisition:** Z-Stack (First/Last, Center, Range, Slices, Optimal, Keep).
- Imaging Setup:** Standard, Track1, Track2, Track3, +Widefield, Switch track every, FITC.
- Light Sources:** MBS 488/561 Visible Light, MBS -405 Invisible Lig...

IV. Tiles scan



in the acquisition menu (1) select the tiles scan option (2). In the tiles scan menu open the "show viewer" menu (3). In this menu, you can choose different options to create your tiles area:

- Define tile regions by number or size
- Setup new tile regions by drawing a contour
- Setup new tile regions by specifying two or more markers positions
- Setup new tile regions from an underlying sample carrier
- Setup new positions by selecting the location with the mouse cursor
- Setup new positions by defining an array of position within a drawing shape
- Setup new positions from an underlying sample carrier



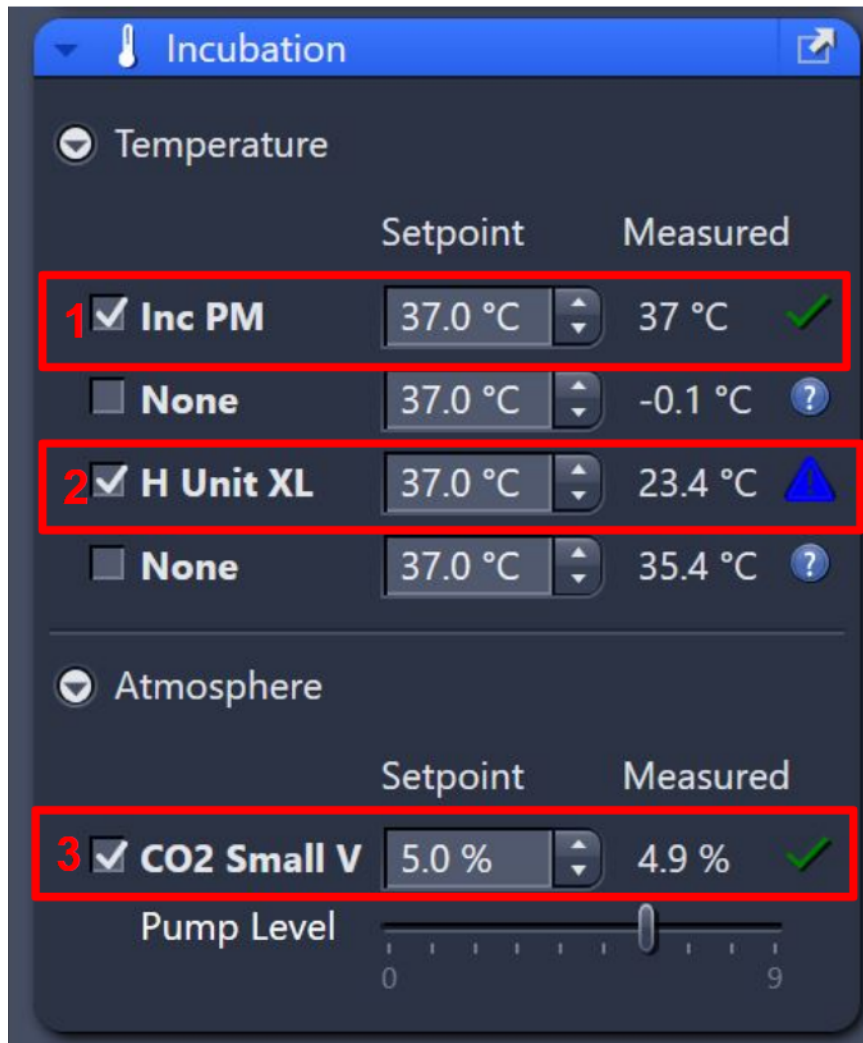
In addition, to facilitate the creation of your scan area, you can activate a “live” to locate your sample.

Once you have defined your tiles, you can insert focus points to make sure you get a tile that is sharp and in focus (Submenu “Focus Surface and Support Points”)(4). To do this, you need to define the number of support points you want to place on your tile, then according to this number of support points, apply an interpolation method (5) and finally check the focus of each point via a live and a Z height validation (6).

Press “Start Experiment”(7) to start the acquisition.

V. Timelapse

Turn on the CO₂ and temperature box systems to launch a timelapse **before** the opening of Zen blue. If it is not the case you must restart Zen blue.

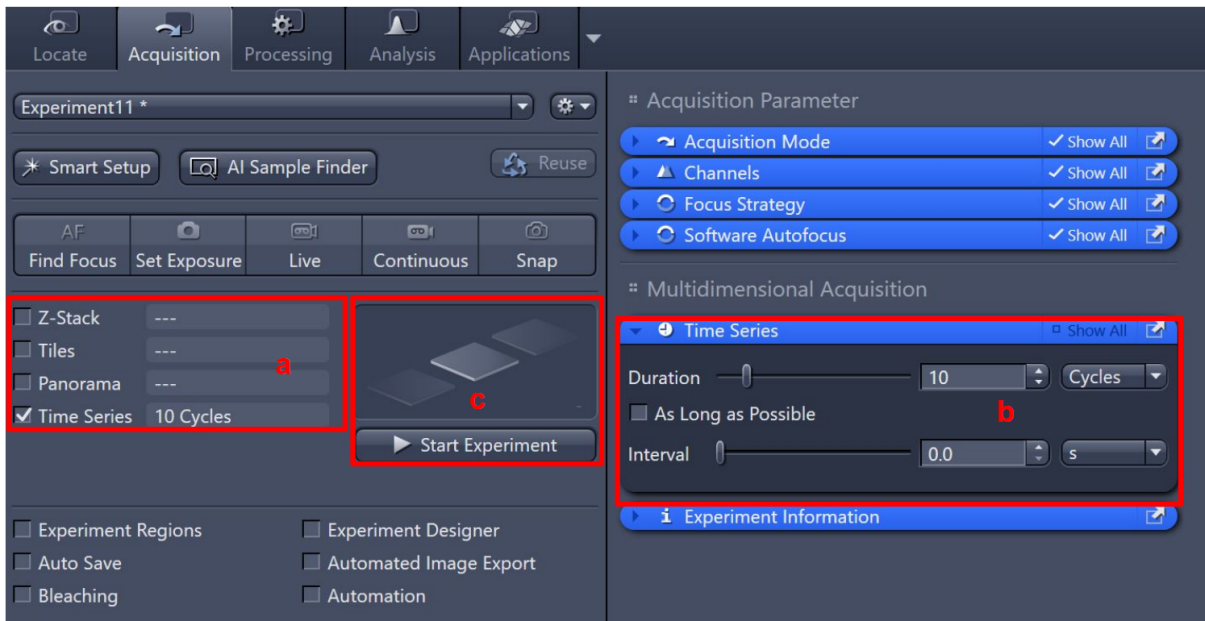


To activate the temperature, select the following two options in the incubation menu :

- 1) Inc PM
- 2) H Unit XL

and for CO₂ select the option "CO₂ Small V" (3) in the menu Incubation and the desired value.

To configure your timelapse, select the options you want in addition to the time series(a).



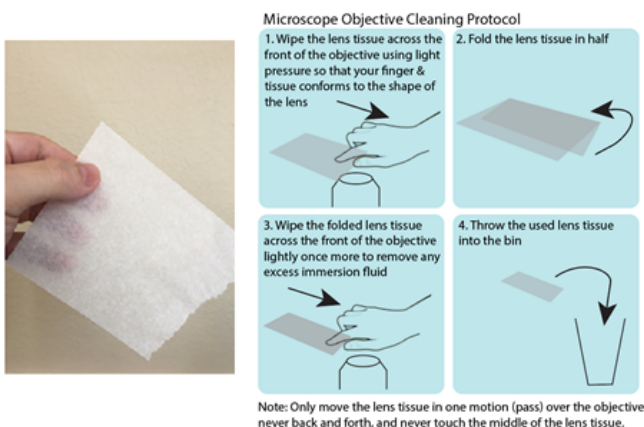
In the time series menu, you can select the number of loops and the time interval between each loop(b)

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

At the end of your timelapse don't forget to remove sample order, switch off the CO₂ bottle, CO₂ module and temperature 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)



Shutdown in the following order : Zen blue, the computer (3) ,the components (2) and the lasers (1)

