Aurox Clarity Manual

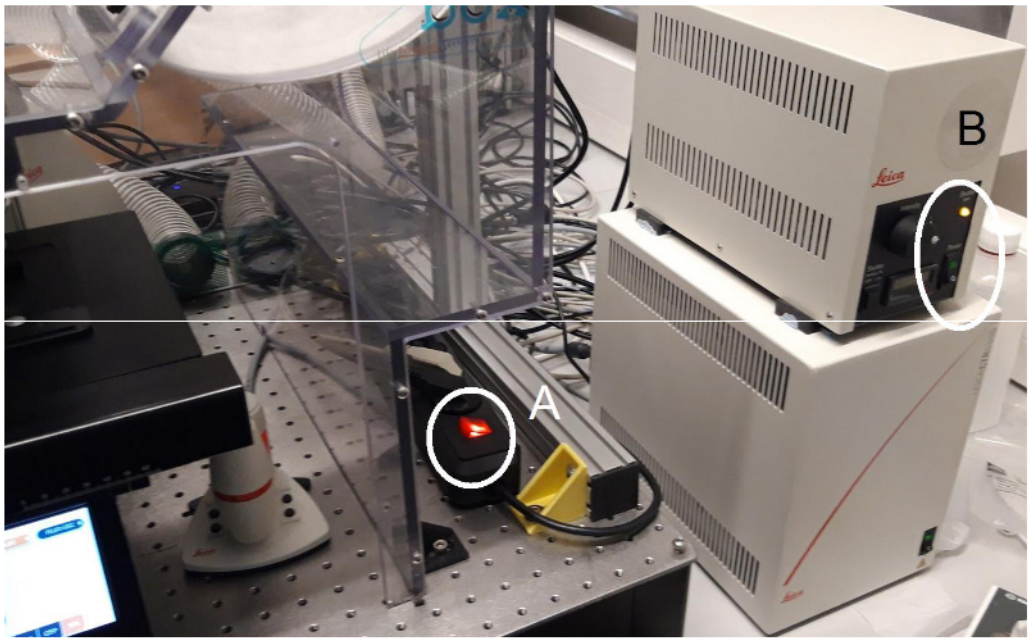
Version 1.0

"To see a world in a grain of sand and a heaven in a wild flower, hold infinity in the palm of your hand and eternity in an hour."

- William Blake

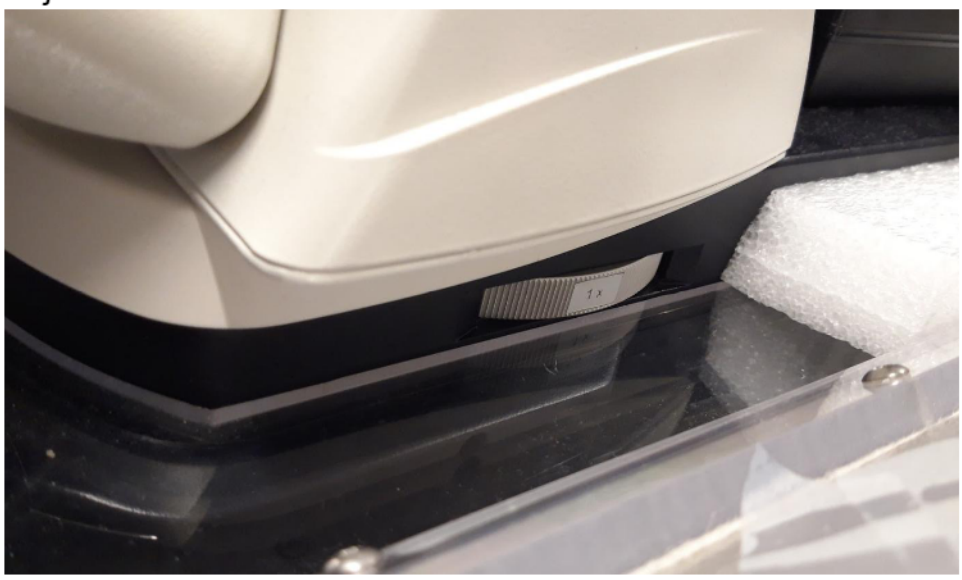
Starting the system

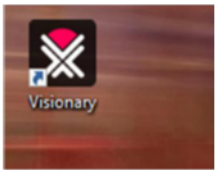
Start the computer and turn on the system. To turn on everything (except the computer) press the switch A in the image below.



You might also want to check that the lamp B is turned on – if the lamp is not on, you cannot see the sample with your eyes. The lamp is coupled with switch A and should be turned on when you flick the switch on. However if someone has turned off the lamp you need to turn it on.

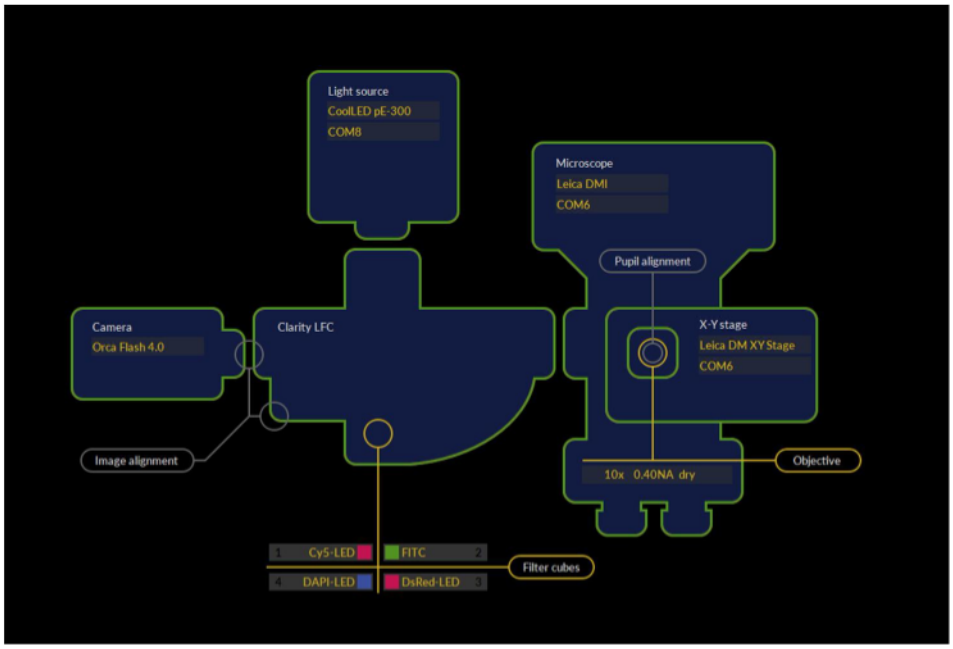
Next check that the magnification changer is in position 1x. You can change it to 1.25x or 1.6x if you want but you must take this into account when you chose the objective.



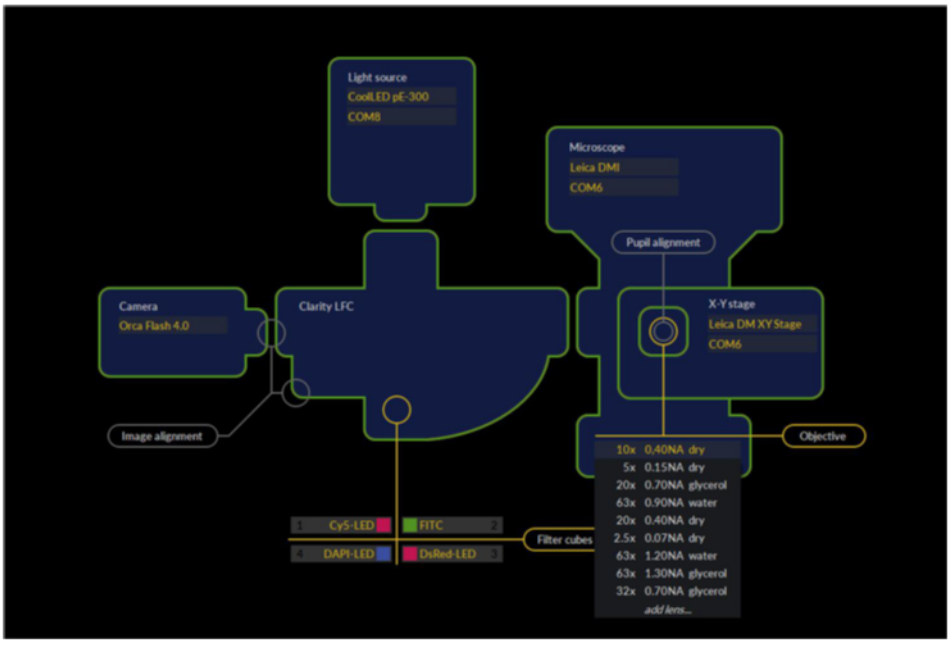


Now you can start the software.

The software should look like this.



Notice how all the boxes have green borders. This is what should happen during the software set-up. If one or more boxes have red borders the software is still loading.



Next you can chose the objective from the drop down menu.

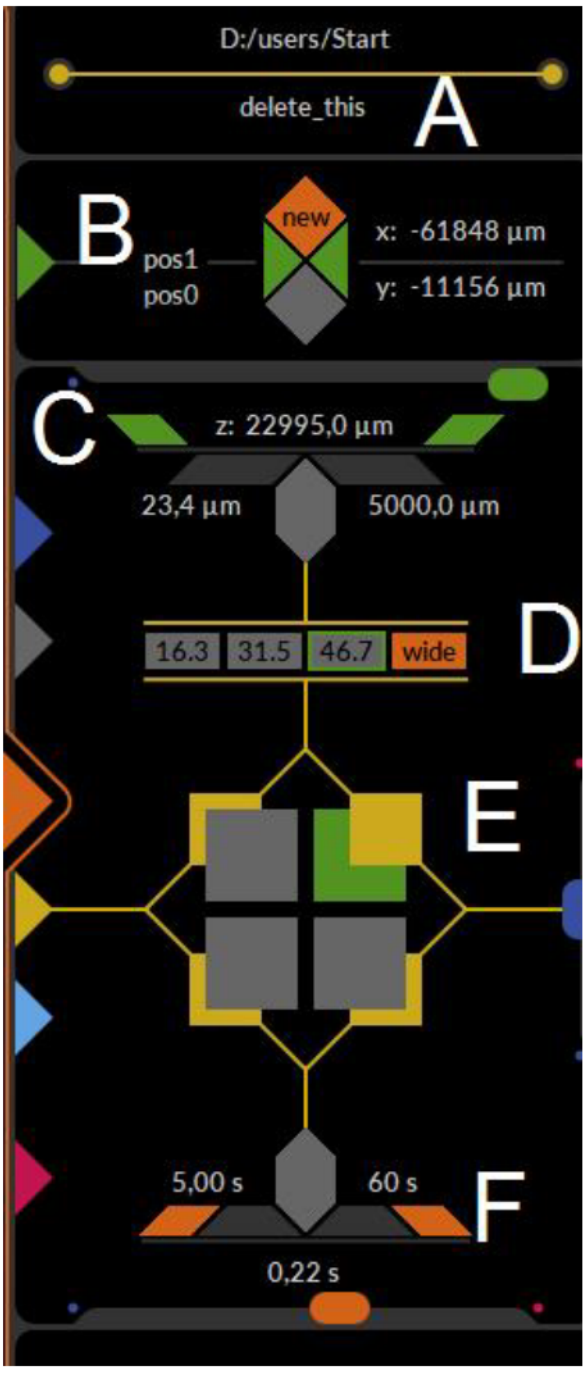
Use the table computer to adjust the physical objective to match your choice within the software. Note that this does not happen automatically.

IF you chose to change the magnification from magnification changer, you must adjust the magnification of the objective within the software. For example, if you have 1.6x magnification chose from the changer and you are using 20x, you must replace the 20x by 32x in the objective menu – otherwise the things go wonky within

the software.

If you are using 1x magnification you do not need to worry about modifying the objective magnification.

Navigating Clarity UI



A
This section is where you determine where your images should be saved. This should be changed to your own folder on the D drive.

Green bits in the UI have to do with location within the sample.

B
Tiling/mosaic options are here. This is explained in detail later.

C
Stacking options are here. This is explained in detail later

D
This is where you adjust confocality of the system. Three options are provided in addition to the widefield option. The numbers shown are the thickness of the optical section.

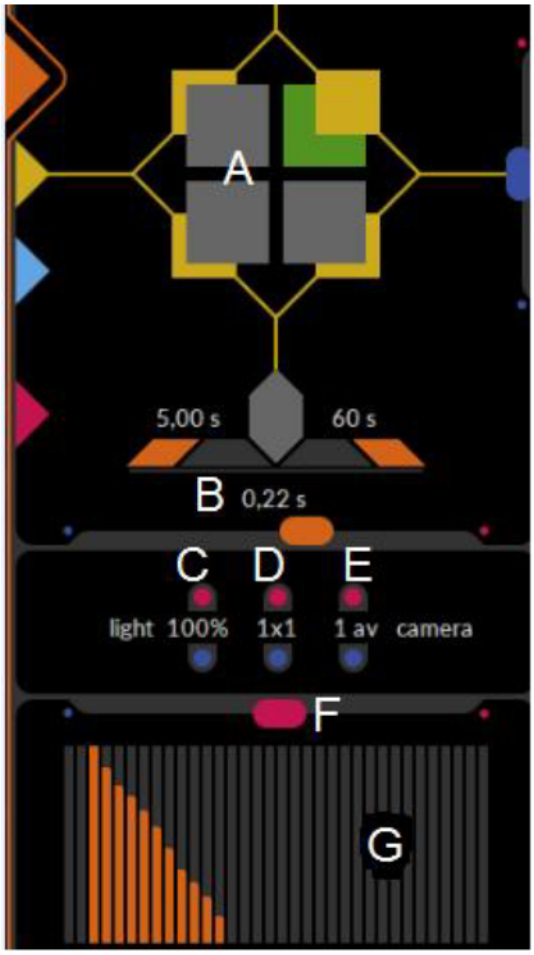
E
This is where you chose the channel to be show and the channels to be captured.

F
This section is where you set up timelapse. Note that at the moment there is no way to stop timelapse experiment.

You should sort out the save path in section A when you start imaging. However after taking an image, if the light blue square is active, clicking A will open the image you have most recently acquired.

Channels and exposure time

To figure out settings for capture click the orange triangle (live mode).



The active channel is chosen by clicking the inner squares A. The slider B selects exposure time for each channel in A separately.

The C changes the amount of light going to the sample, again individually for each channel. **Note that this should be set to 12% for blue channel unless your signal is weak**

Button D adjusts binning – allowing you to measure signal from really dim samples. If you have hard time seeing anything at all, try 2x2 binning

Average is adjusted from button E. Usually increasing exposure time does the same as average. But if you have really, really noisy signal setting E to 2 or 4 might help – however this increases the effective exposure time proportionally.

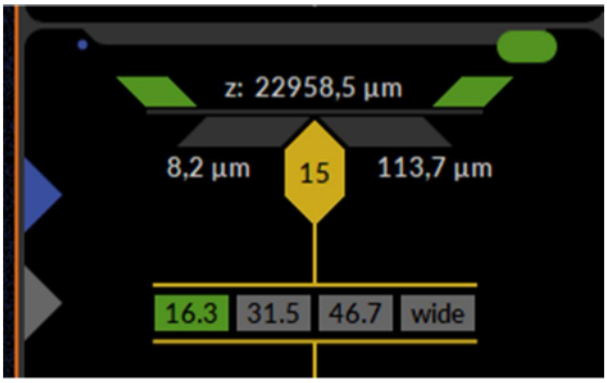
Histogram at G is the most important metric for the incoming data. If the rightmost orange bar is touching the right edge of the panel G you have too much light coming from your sample. Either reduce the amount of light (C) or the exposure time (B). This is true even for confocal images – if the image is over exposed, i.e. the orange bars run all the way to the right, the mathematical confocality will not work

Capturing images

To choose which channels you wish to capture, you must click the yellow boxes on the corners of the grey boxes, each corresponding to a different channel. Not that changing channels will undo the selection of the channels. So basically pick any one channel you want to capture and then click the yellow blocks corresponding to the other channels you want to capture.

Now pressing the large yellow square connected to the channel selection by line will capture an image with current settings, including timelapse, stacking and mosaic if active.

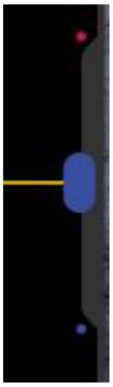
Confocality



The confocality of the acquisition is adjusted from the four step bar on the left.

If ‘wide’ is selected, the images will be recorded as is, corresponding to widefield images.

If any of the three ‘confocal’ modes is chose, the system will deduct off-focus information from the widefield image, creating a faux confocal image. The numbers shown correspond to the thickness of the optical section. This is important and relevant as optimal z-sampling is half of the optical section thickness.



The blue widget next to the channel selection is used to adjust how much of the off-focus information will be deducted from the image.

The final image is calculated as follows:

Final image = widefield image – A*off-focus light.

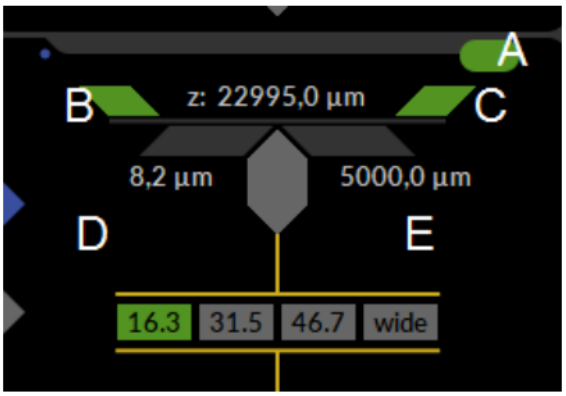
The blue knob adjusts the value of A. Higher the knob, the smaller the A.

The blue knob should be adjusted so that your background is ~mostly blue. This means that you get zero light from the background in your confocal image. However

sometimes, if your sample is dim, you might have to adjust the blue knob in such a way that your signal does not disappear.

Taking z-stacks

Stacking options can be seen in the part of the UI shown below.



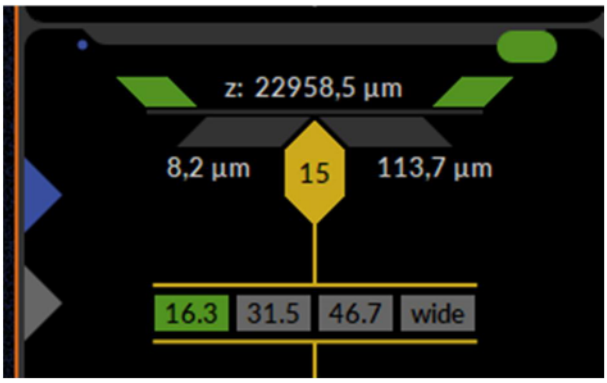
Knob A moves your focus within the stack. Before you set the stack the stack is the movement range of the objective. DO NOT TOUCH KNOB A BEFORE YOU HAVE SET THE STACK.

To set the stack, move the focus below the sample. Press the widget B twice. No idea why you need to press it twice – but stacks tend to get messed if you don't. Then move the focus above the sample (or where you want to set it) and press the widget C once. Now the stack thickness should be shown in the part E.

You can click the number above the D to adjust the capture interval, i.e. how often in depth the images are taken. Ideally this should be one half of the optical section thickness as shown on image above. However this is not enough to activate the stack. That would make too much sense.

To activate the stack you must click the grey hexagon beneath the stacking options. When clicked, it will turn yellow and show you the number of slices in your stack.

Like so:



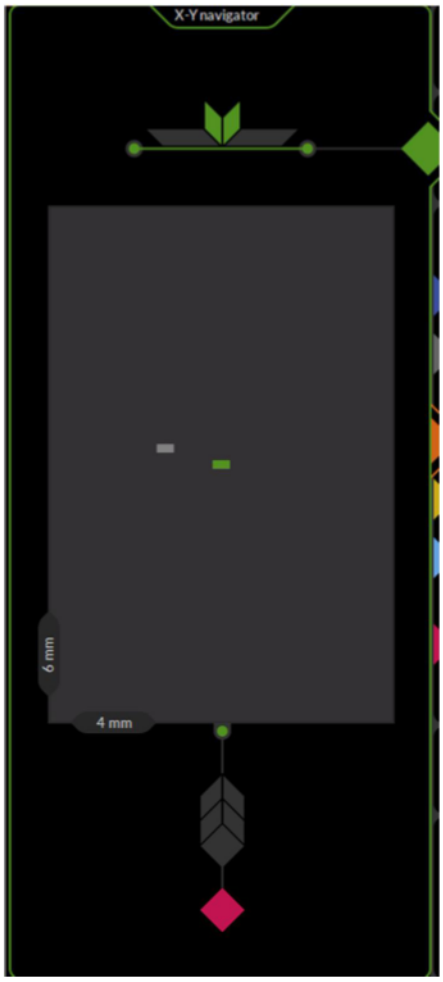
If and if that hexagon is yellow, everything you capture will be a stack. To deactivate he stack press the hexagon again.

If you have multipoint (mosaic) capture, all the stacks will be the same for each point.

Taking mosaic images

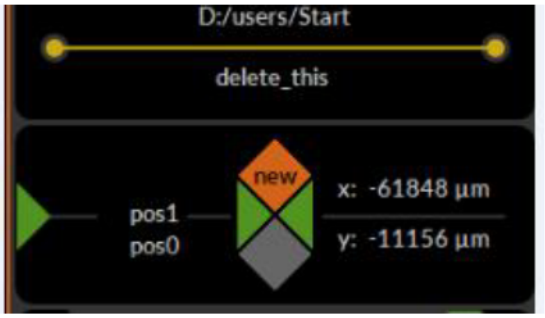
As of now there is no way to stop a mosaic experiment besides killing the software. This might change in the future.

Clarity mosaic tool was programmed by an orangutan on acid. So prepare to be frustrated. The mosaic panel can be opened from the green triangle and looks something like this:



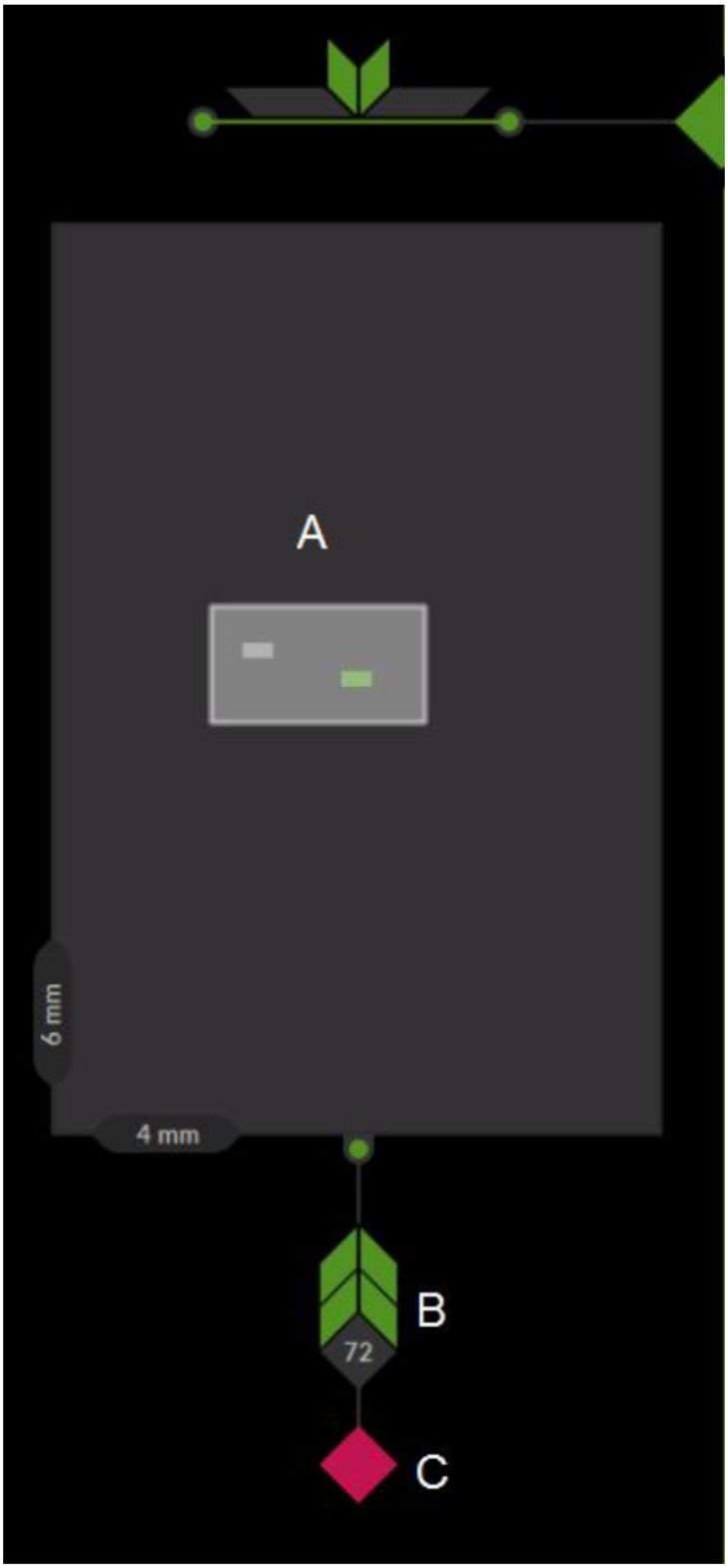
Moving the stage moves the green rectangle around. Marked positions appear grey.

Clicking the ‘new’ on the orange square shown below will mark these positions. In theory this would let you do a multipoint capture, but this is rarely good idea.



It is easier to mark two positions corresponding to the left upper corner and right lower corner of the area you want to capture.

Then use mouse to draw a rectangle around the two marked spots as shown on the next page (A).



Once you have your square set, you can press the green arrow (B).

Now the positions indicating the mosaic will appear in the position list.

If you are not happy with your selection you can press the red square C to remove the selected positions.

Just as before – selecting multiple positions does not mean that the machine will record anything.

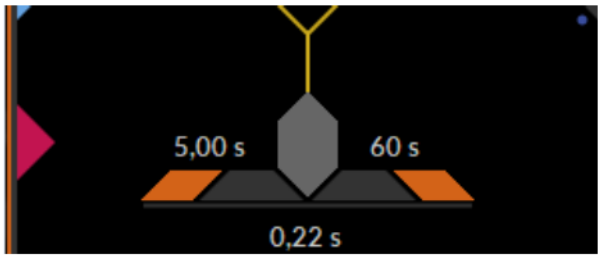
To get your mosaic image the mosaic mode (multipoint mode) has to be active. To activate multipoint mode press the grey square under the orange 'new' button.

When active, this icon will turn yellow and will show you how many images your mosaic has.

Time-lapse experiments

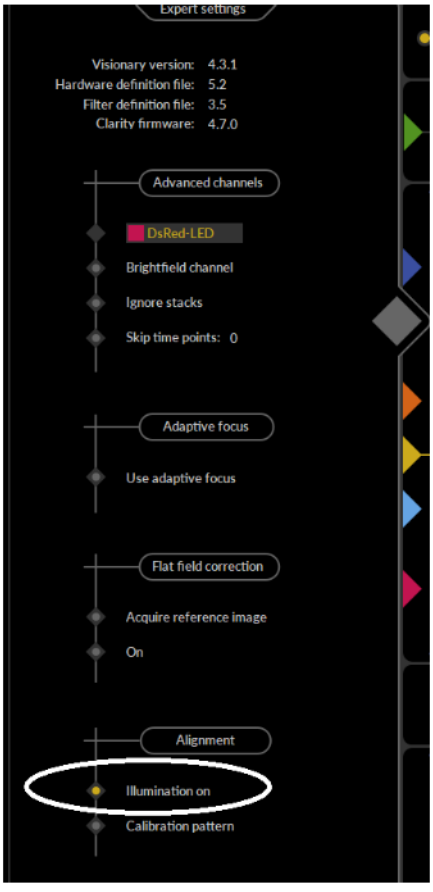
As of now there is no way to stop time-lapse experiment besides killing the software. This might change in the future.

Time-lapse experiments are controlled from the orange widget under the channel selection. The number on the left is the frequency in which the images are taken. The number on the right is the duration of the time-lapse. Both of these numbers can be edited simply by clicking them.



However, this is not enough. To activate time-lapse you need to click the hexagonal grey widget between the two orange widgets. When active, the grey box turns yellow and shows how many time points your time-lapse experiment will have.

Coverslip correction



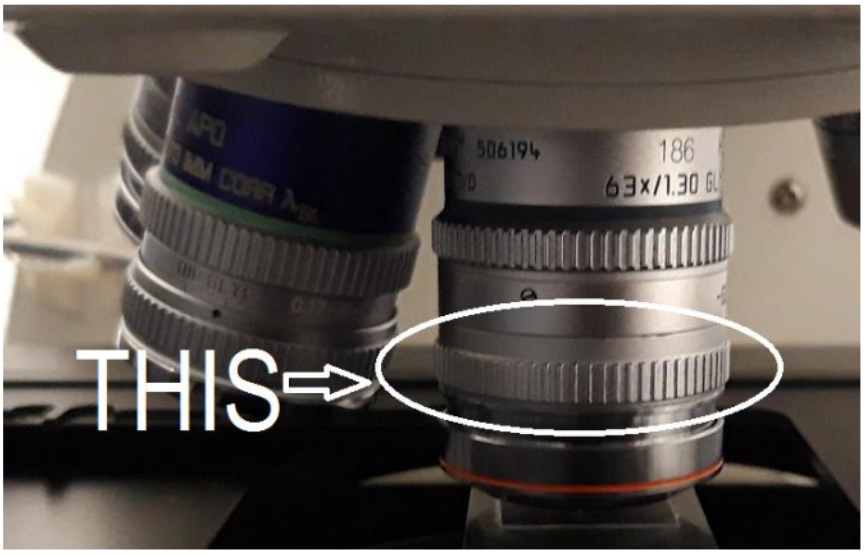
You can correct for coverslip when using 63x objectives. To do so, click the grey triangle between the blue and the orange triangles – so called expert settings.

Make sure you can see the sample somehow when you click the orange square.

Now go to the expert setting by clicking the grey triangle. Under the alignment you can find the ‘Illumination on’ tab. Turn it on.

Now you should see two images of your sample on the screen. One on the left is your ‘in focus’ information and the one on the right is your ‘off focus’ information.

Now you can correct for the coverslip thickness.



Turn the correction collar all the way to the left (or right) and observe how the image quality changes. Your in-focus image should get darker.

Now turn the collar to the other direction until you have a nice and bright image on the in-focus side (left). However you should take into account that turning the correction collar affects the focus which might increase or decrease the intensity of the image. This means that you need to keep re-focusing your sample to properly assess the effects of the correction collar.