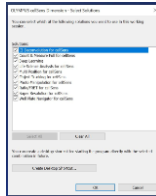9 January 2023

Queensland Brain institute

Olympus SR10 Sora Spinning disk

Getting started

1) If you find the system turned off, follow the start-up procedure in appendix I.
2) If the system is already on;
   a. Log in the computer using User name "olympus" password "olympus" if not already done.
   b. Log into PPMS using your credentials.
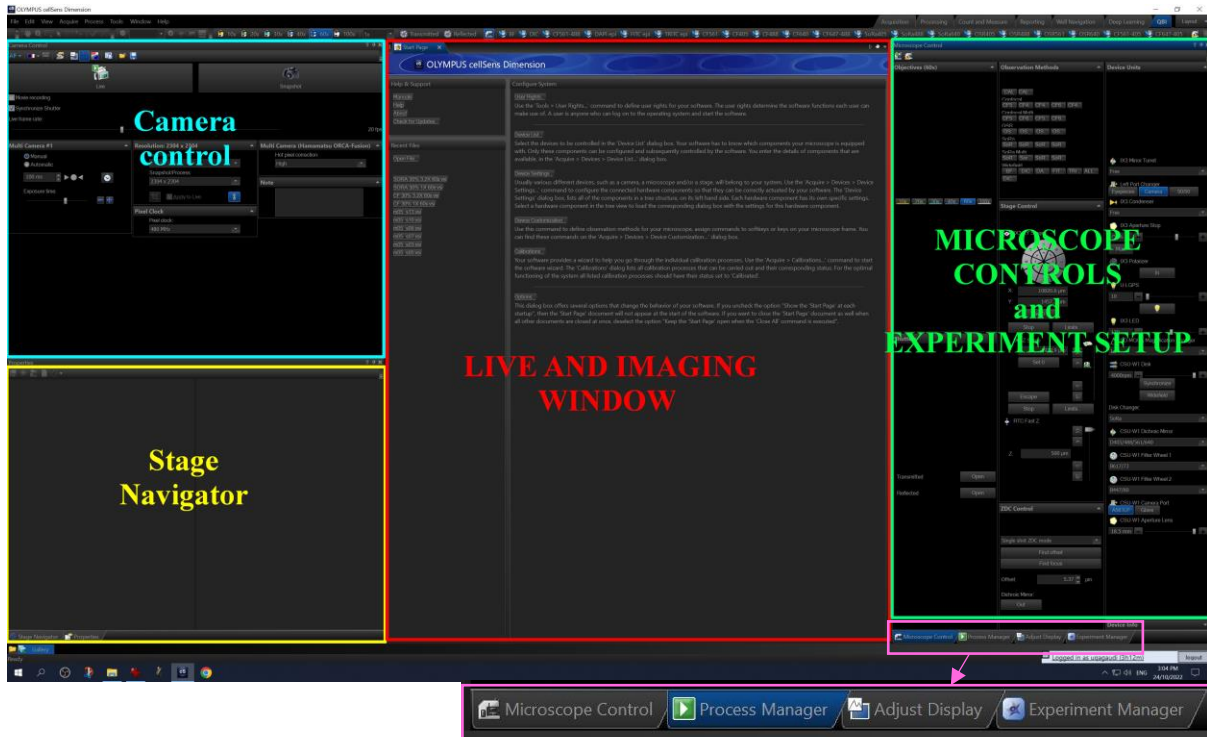   c. Start "cellSens Dimension" using the desktop shortcut or the taskbar shortcut.

   d. Select all options and OK

   e. choose "no" to the cleaning of the stage.
   f. Start imaging.

CellSens overview

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CellSens's GUI consists of 4 main sections:

Camera Control for exposure and camera settings, Stage navigator for creating a large overview of the sample to use as a map to navigate during imaging, the live and imaging window and the microscope controls window to set up experiments. The latter has the specificity of changing the content based on what tab is active: microscope control, process manager, adjust display and experiment manager.

The layout depends on the option selected. By default, we have a QBI option that fits all needs, found at the upper right corner.
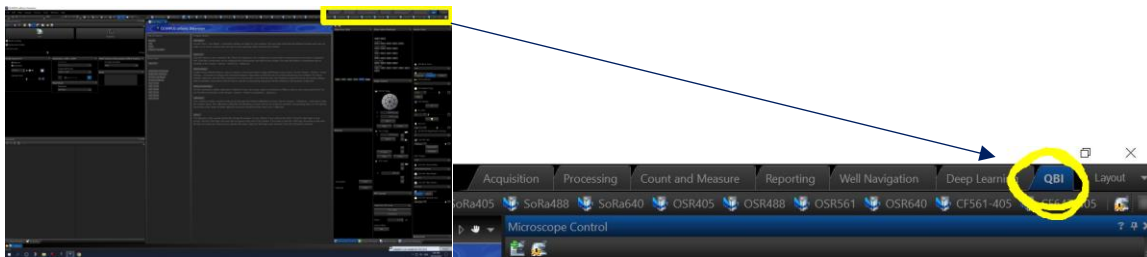

Routine checks before starting

In the start page or in the main menu "acquire/devices/device customization", before any experiment, make sure that the laser configuration you are going to use has the correct laser intensity. The system allows to change the laser power only in the device customization menu which implies that the next user will inherit the laser power from the previous one in the same configuration.

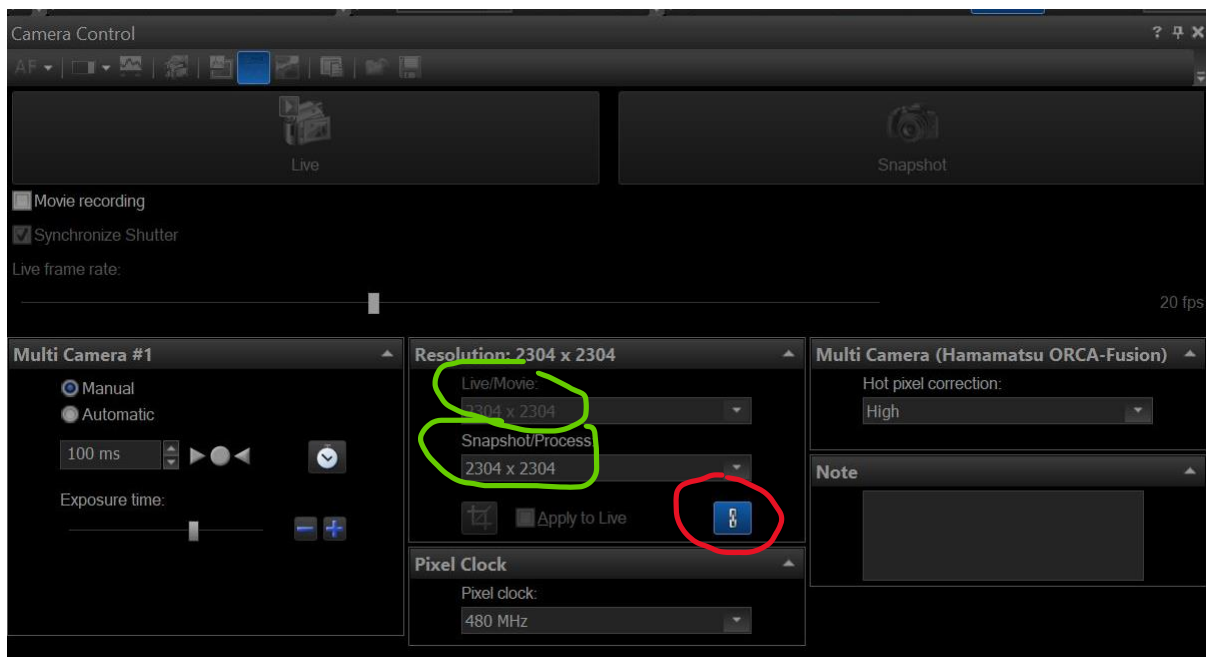Laser#1 = 405nm, Laser#2=488 nm, Laser3#=561 nm, Laser#4=640 nm

Set laser intensity accordingly. Keep in mind the lasers are very bright and anything past 20% will involve some bleaching.
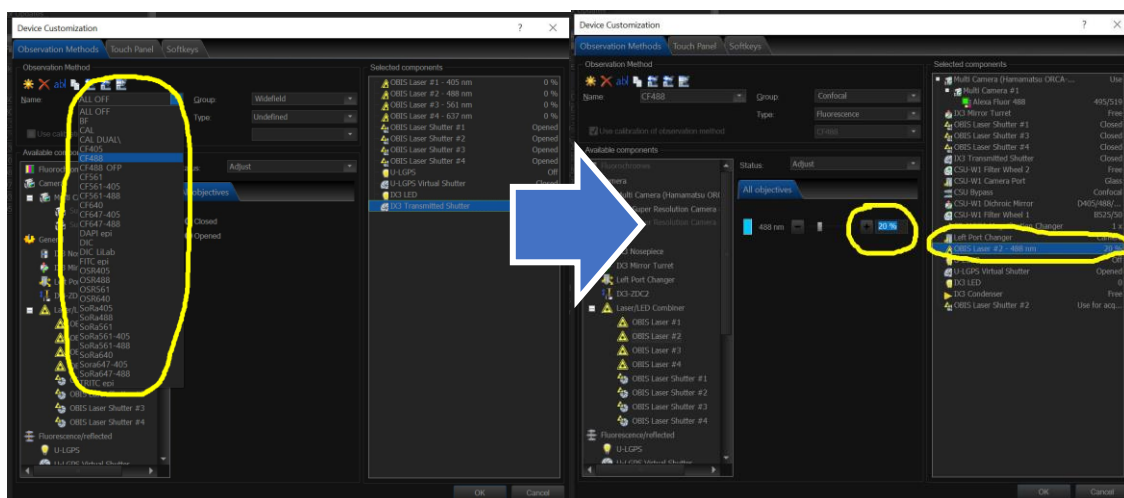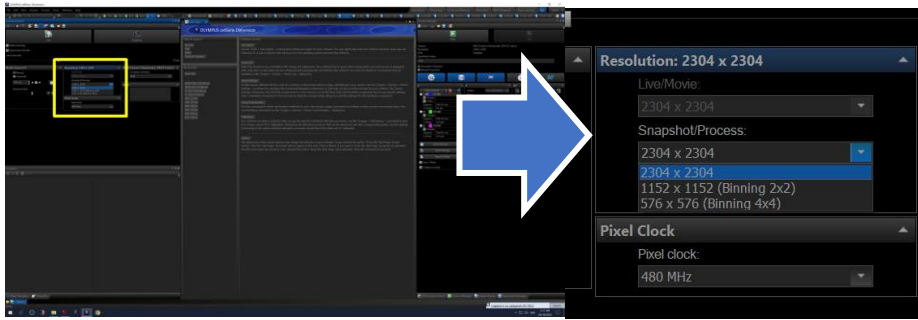
Binning is also retained from previous user, so it is important to double check as this dramatically affects resolution and intensity profiles of acquired images.

Camera control

The system has 2 CMOS Hamamatsu ORCA fusion Cameras that can be used for simultaneous multi-channel acquisition if needed.

Regardless of whether you use single or multi-camera acquisition, the principle remains the same:

1) Set up the exposure time for the live window for each camera.
2) Press the live button to have a continuous image displayed or press snapshot to get a still image on the current active configuration with the set exposure time.
3) Note that live and snapshot/Process can have different binning values when the chain symbol is not highlighted, be mindful of this before acquiring you can set up an exposure on a 2x2 binned live image but having 1x1 binning in process, resulting in a significantly dimmer signal in your final acquisition than in the live view for example (and vice versa).

Microscope control
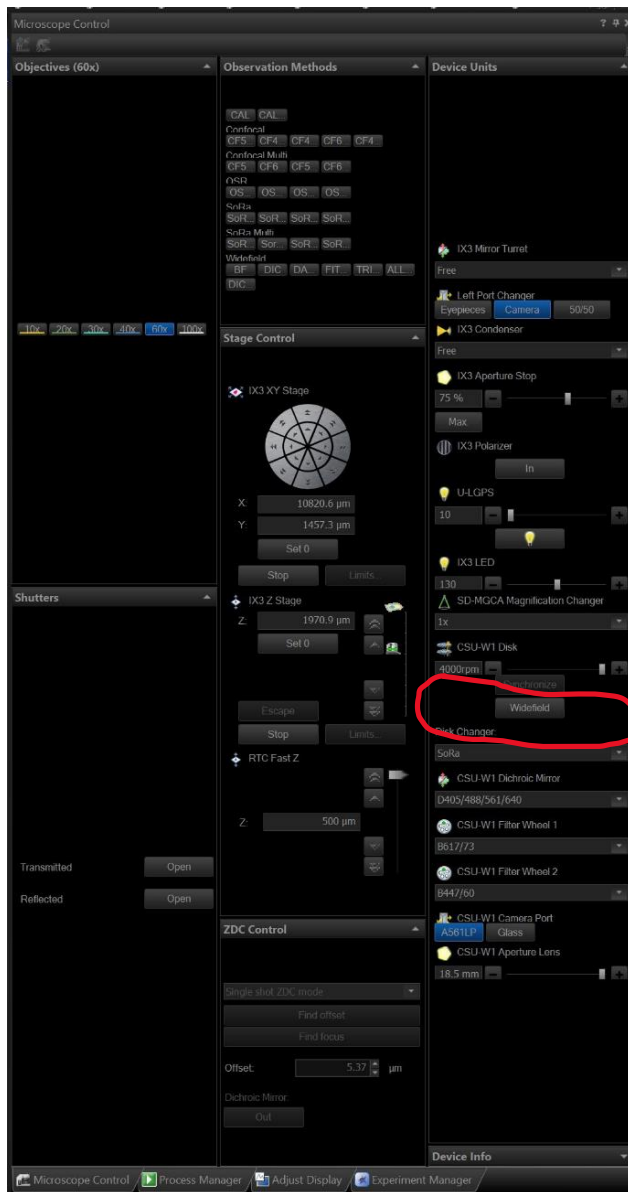
1) Objectives: change the objectives, redundant with the touch screen on the optical table.

2) Shutters: transmitted for brightfield and DIC, reflected for epifluorescence.

3) Observation methods: must hover over the thumbnail to display the full name. changes the optical path to the desired setup:

- **Confocal** uses the normal **50 um** spinning disk and the **1X magnifier** (normal spinning disk configuration)
- **SoRa** uses the **Sora disk AND the 3.2X magnifier** (Super resolution Configuration).
- OSR = SoRa + automatic enhancing filter post processing (currently not recommended).

4) Stage control: controls the XY movement of the magnetic stage and the Z movement of the Z drive (redundant with joystick) as well as the position of the piezo (500 um total range, usable in experiment manager only).

5) ZDC Control = Z drift compensation. A function to correct the Z drift over timelapse acquisitions.

6) Device units: list each piece of the optical path that can be modified. Each observation method sets the optical path to a particular configuration automatically for you. THE EXCEPTION BEING THE DISK CHANGER. **YOU MUST SELECT THE DISK POSITION BEFORE YOUR ACQUISITION.**
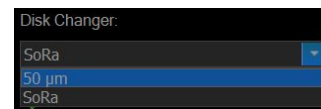

Acquiring via the Process manager

To acquire **multiple channels**, a **Z stack**, a **multiposition/montage** or a **timelapse**, you will need to use the process manager and activate the corresponding tab.


| C | Z | XY | timelapse | Autofocus |

Channel tab (C)

Add a new channel by clicking the "add channel" and selecting the desired observation method from the drop-down menu list. By default, when adding a new channel, the default exposure time is the one that is currently set in the camera control window for the live.
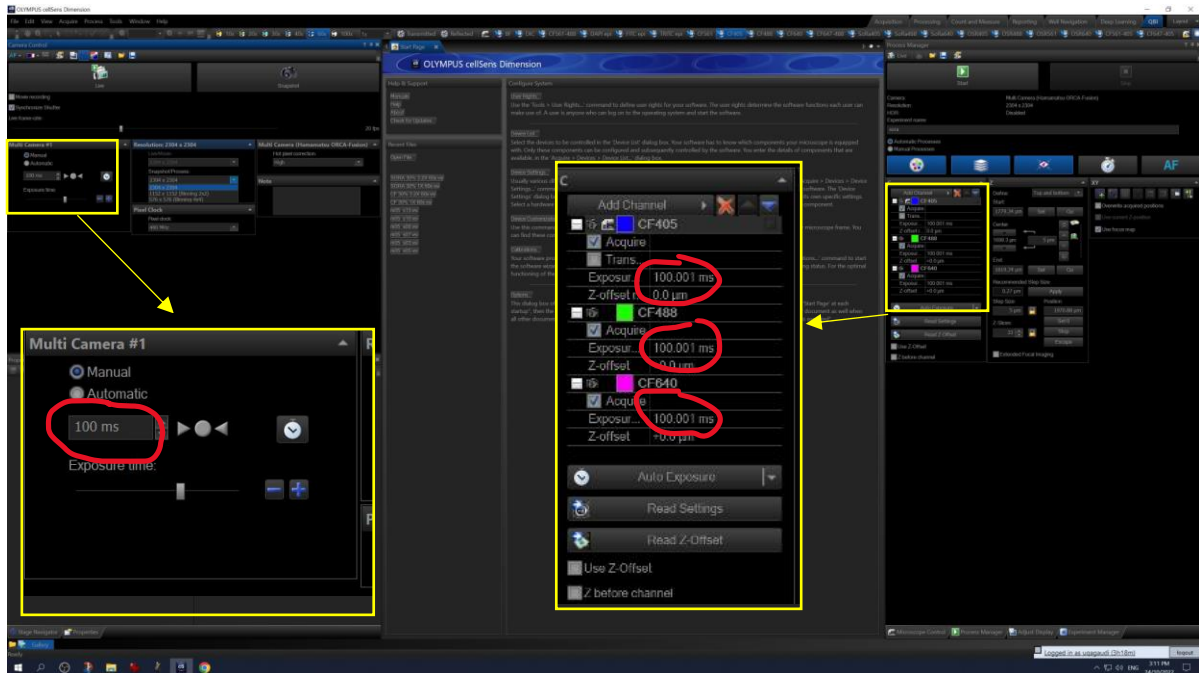

You can also edit the value of the exposure for each individual channel by clicking on the exposure field and enter the value manually. Another possibility is to use the live window while having the channel of interest active (selected and highlighted in the process manager) to adjust the exposure time required for each channel and use the "read settings" button to apply the current exposure time to the highlighted channel:
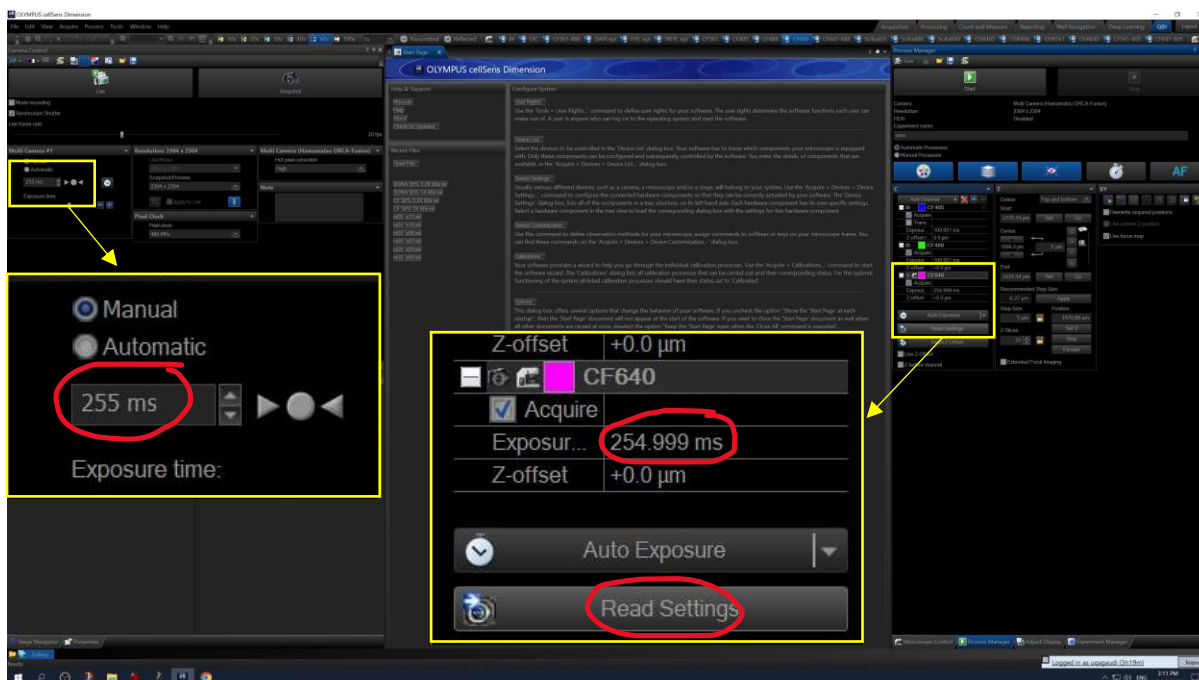
How to use the display to adjust exposure?

At the bottom of the right window, there are tabs to navigate between microscope control, process
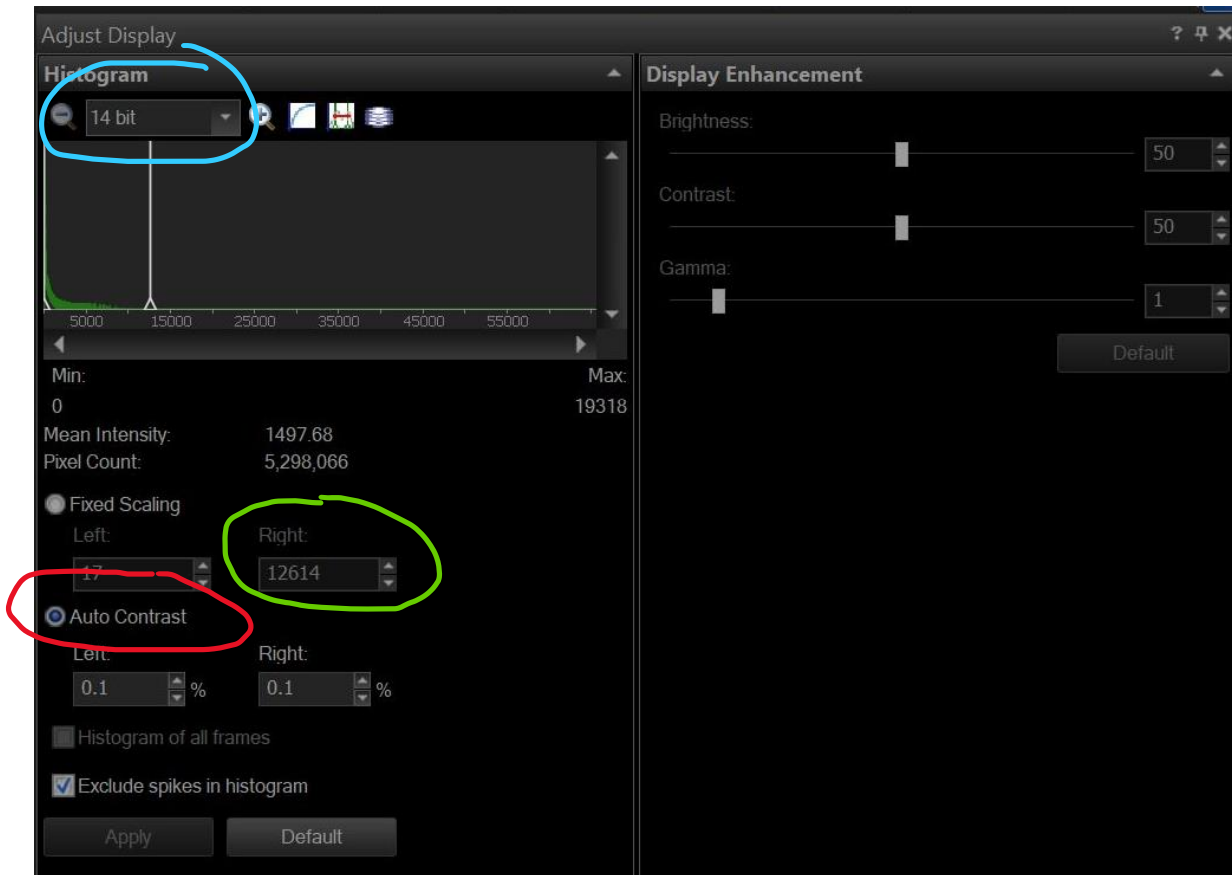
We recommend using the "Auto contrast" option in order to have the display automatically shown as bright as possible. This implies that in order to know the actual intensity of the signal at any given time, you need to check the value "right" which contains the maximum intensity in the field of view currently displayed. A minimum for a decent signal to noise ratio is around 1000. The scaling is displaying the range currently used, not the actual depth of the image being recorded: images are always recorded as 16 bit (65536 grey values) but the range adapts to the maximum values hence in the example the 14bit instead of 16bit.

Using the stage navigator.

The stage navigator is a feature that allows to capture a quick tiled overview of an entire portion of your slide/sample/preparation using the current observation method. Once the tiled overview is acquired, navigating across it is as simple as clicking on the portion that you want to visit. It is especially handy when setting up montage and multi-positions.
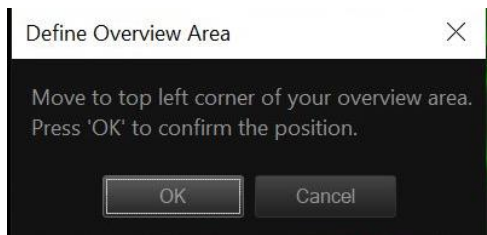
To get started, you need first to click the left most icon in the tool bar of the stage navigator window:

If you are not currently using 10x but any higher magnification, you will get the following prompt:
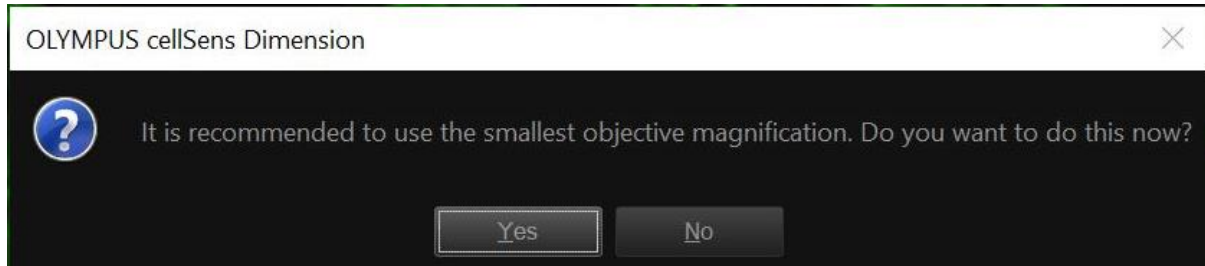

"yes" will change to 10X (even if you are using an immersion lens!!!) so it is not recommended, rather select "no" to keep using the current objective.

The system will then instruct you to move the field of view to define the top left corner of your overview area. This refers to the top left of the image displayed on the screen's live window which is inverted compared to what you see in the occulars in case you are setting this via the occular in BF, DIC or epifluorescence. Use the joystick to navigate and select this position, then press ok. Same goes for the lower rigth corner.
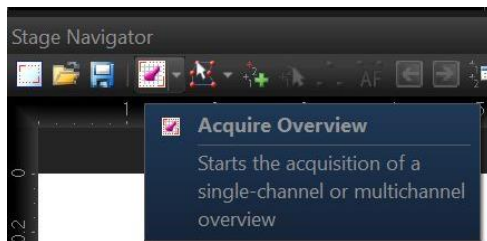
Once bother corners are defined, the range of the prospective oveview is displayed in plain white fields. Click on the acquire overview icon in order to acquire it using the currently active observation method. Again the system will complain that you are not using the 10X if you are on higher magnification; ignore it and press NO to acquire the overview using the current objective.
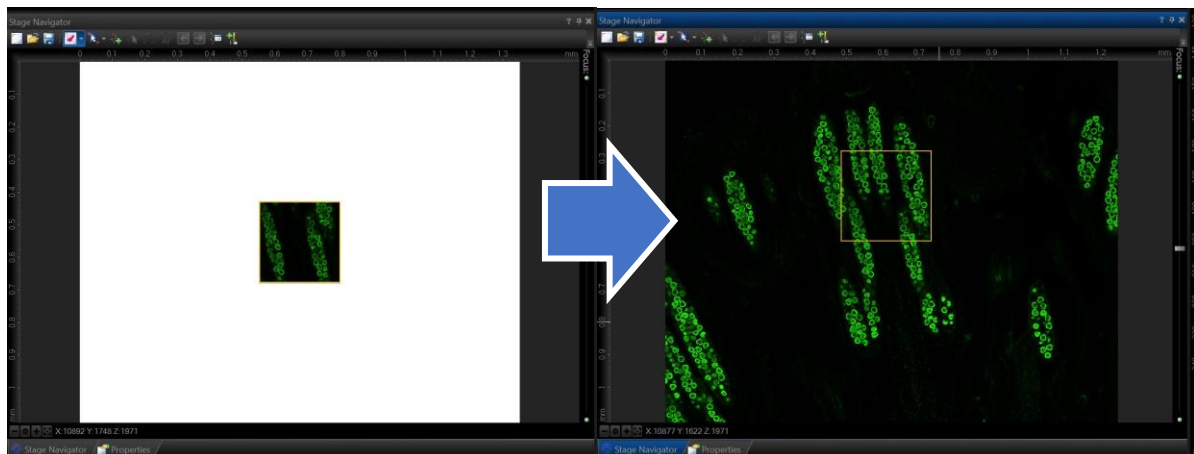

Setting up a Z stack

Like in most systems, you can create a Z stack with either of 2 approaches: Top/bottom or range.

When setting the stack using the top and bottom option, you are going to want to stick to imaging the stack at the current position only. If you want to associate XY to Z acquisition, then using the range is recommended.

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Currently, Cellsens uses only the Z drive of the microscope body in the process manager. The system also possesses a piezo with 500 um range that is only usable in GEM mode. For most acquisitions not involving very fast Z movements (timelapse) the Z drive is sufficient.

Use the Z knob of the multi dimensional joystick on the optical table to navigate your Z position. Note there is a button to switch between coarse and fine Z and XY movements on the joystick. **Clockwise rotation lowers the objective, making you focus on more superficial layers of the sample. Anti-clockwise rotation lifts the objective, making you focus on deeper layers of the sample.**
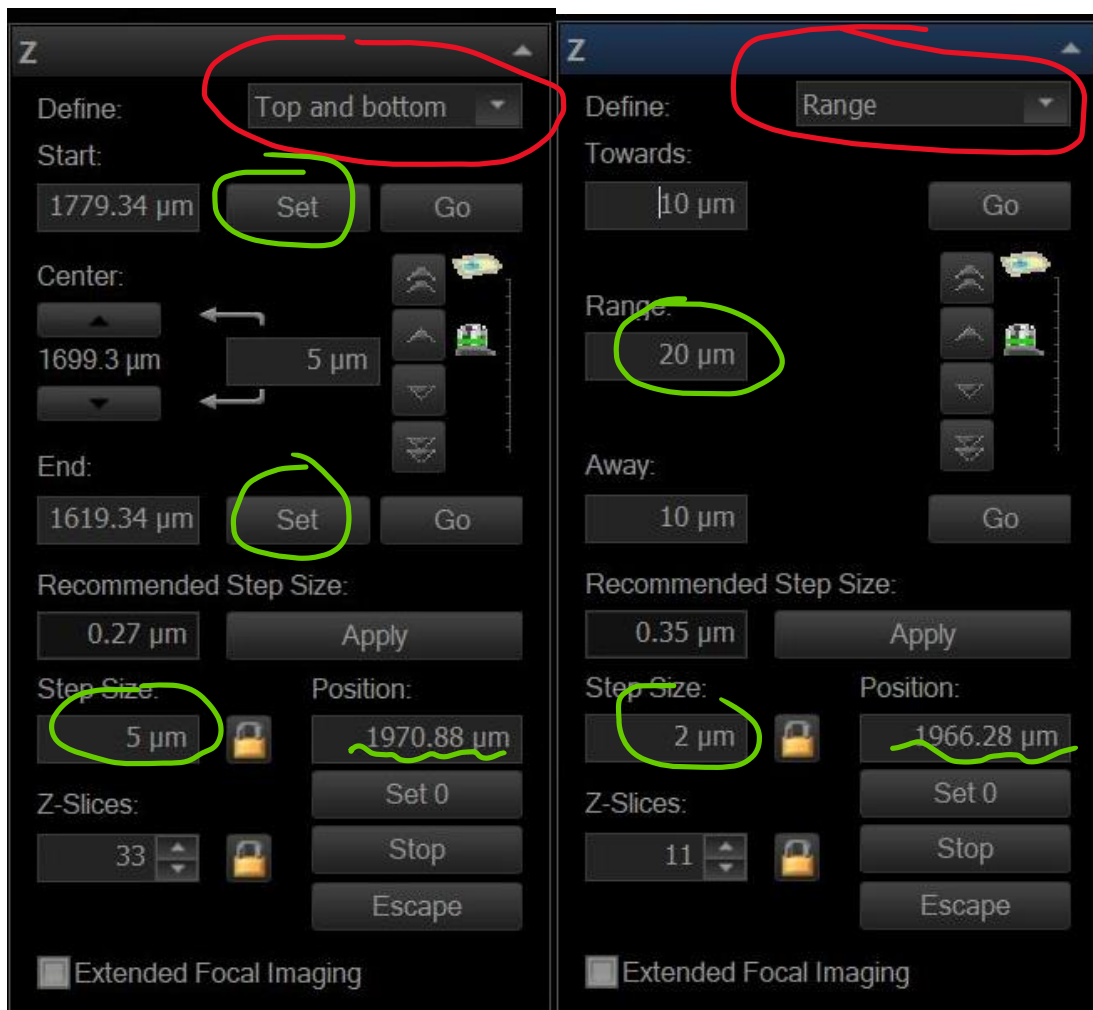
When acquiring a Z stack in the current XY position only, defining top and bottom will return the exact Z stack intended.

But when using XY montage, make sure to set up the top and bottom position, calculate the range it represents by subtracting start and finish values, and to make sure that the reference Z value for the XY position in the position list has the Z value of the middle of the Z stack. Then use the Range option for Z stack acquisition, imputing the range value that you have calculated during your top/bottom set up.

Setting up XY acquisitions.

To integrate multiple XY or montage positions listed in the "position list" in the imaging routine, activate the XY tab.

Setting up your ROI

Once the overview area has been set, you can use it as a canvas to draw your regions of interests for imaging. The ROIs are called "scan area".

You define them by pressing the corresponding icon, revealing a drop down menu and select the option from here:
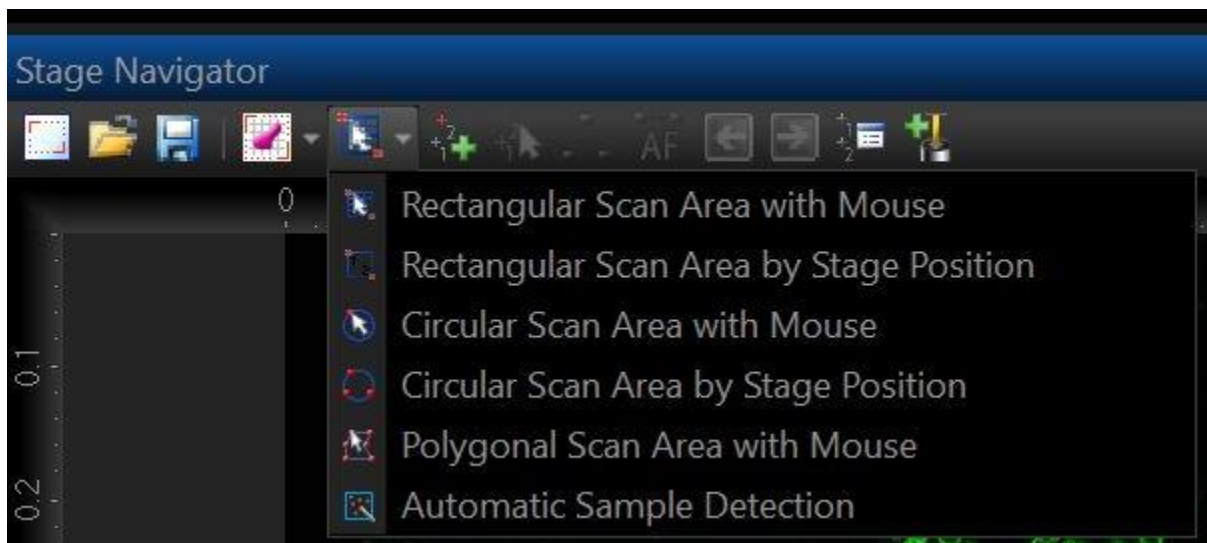
You can essentially draw the ROI using the mouse on the canva of your overview or move the stage. The scan area takes the form that you want, i.e. rectangular, circular or polygonal.

Note that in order to draw a polygon, there is currently a glitch preventing to draw anywhere on your overview area as the system requires at least the width of a 10x field of view of edge buffer before being able to draw on the overview canva. This means that if you acquired a 3x3 overview on
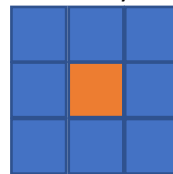

the 10x, you can only draw a polygon in the central square.

But if you drew a 3x3 overview with the 100x, you won't be able to draw a polygon at all.

This is not an issue when using the rectangular or circular scan area though.

Once the ROI have been created, they are listed on the stage navigator in order of creation and can be accessed and edited in the "position list" window by pressing the corresponding icon in the navigator menu bar:
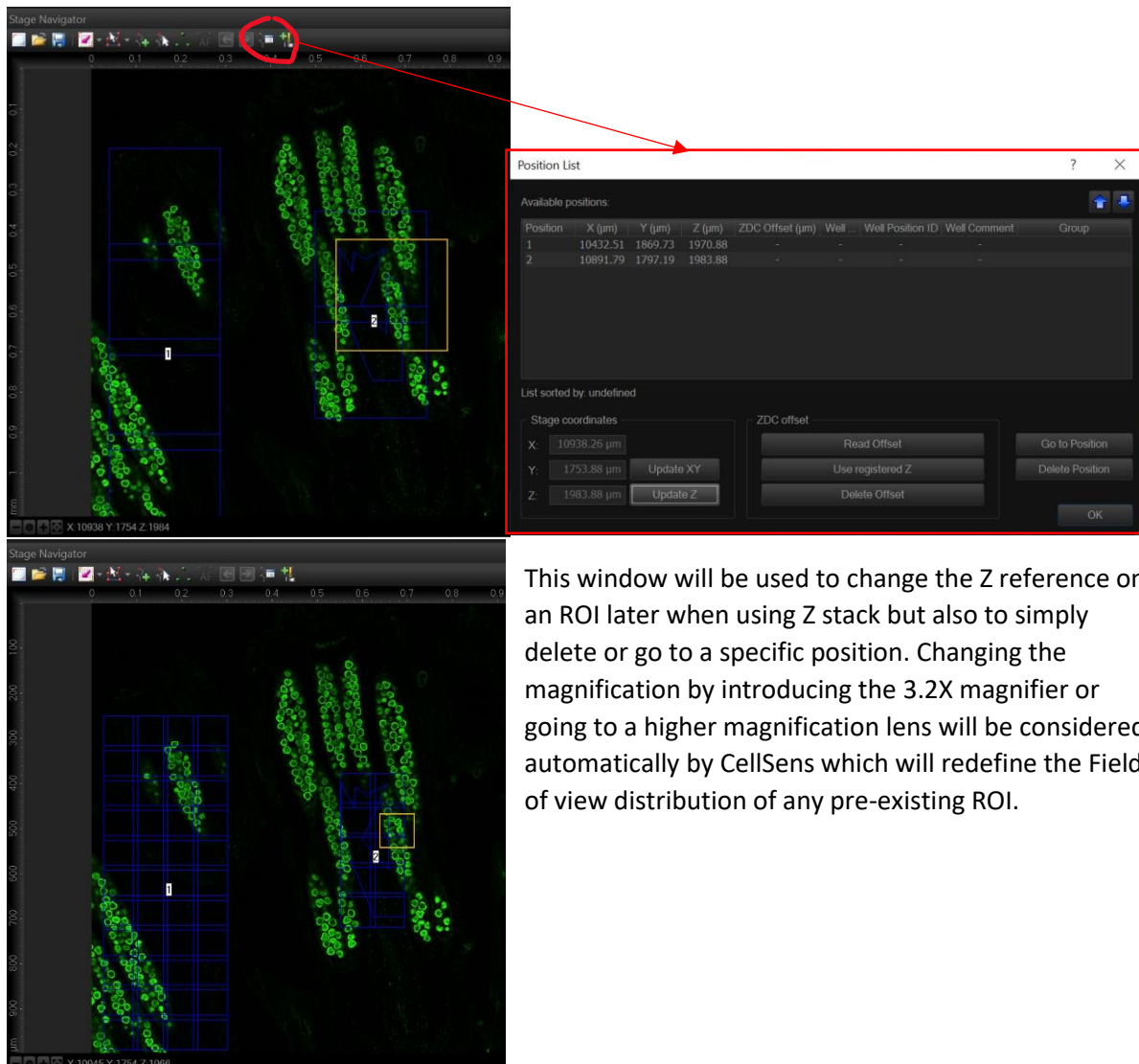
This window will be used to change the Z reference on an ROI later when using Z stack but also to simply delete or go to a specific position. Changing the magnification by introducing the 3.2X magnifier or going to a higher magnification lens will be considered automatically by CellSens which will redefine the Field of view distribution of any pre-existing ROI.

Each position has a Z reference attached to it. This reference will define the position the Z drive is at during the acquisition for the corresponding XY position.

In the following example, le top left corner image was acquired with the Z reference value 2004um in position #1, the top right corner one at 2064 um in position #2.

By ticking "use current position" in the XY tab of the process manager, those Z references for each position are overridden to use only the current Z position for all, as shown in the lower 2 images that have been acquired with this option ticked while the Z drive was at z=2064, allowing to have both of those images in focus.
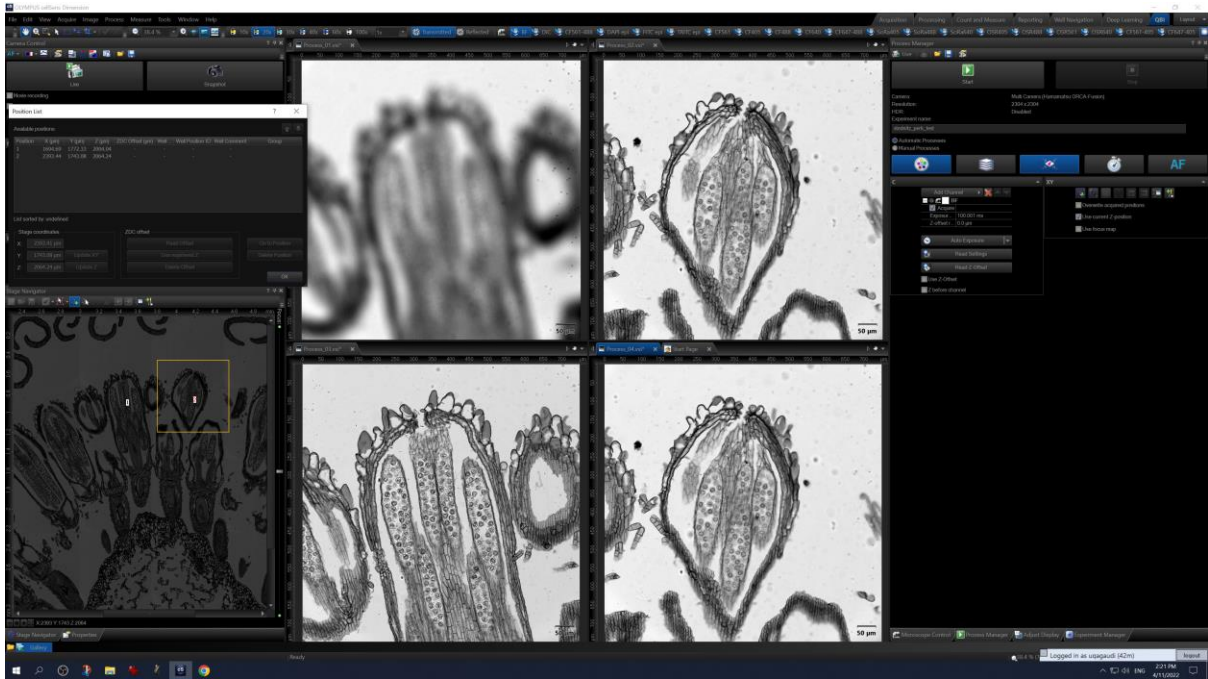
Stitching XY montage

CellSens has been set up to automatically stitch together the XY montages that are being acquired.

Some sample can sometimes present stitching artifacts with part of the image at the junction being duplicated. If this happens a lot with your imaging, then you can set the system so it does not automatically stiches the acquired montage and stitch it later in CellSens or using third party software.
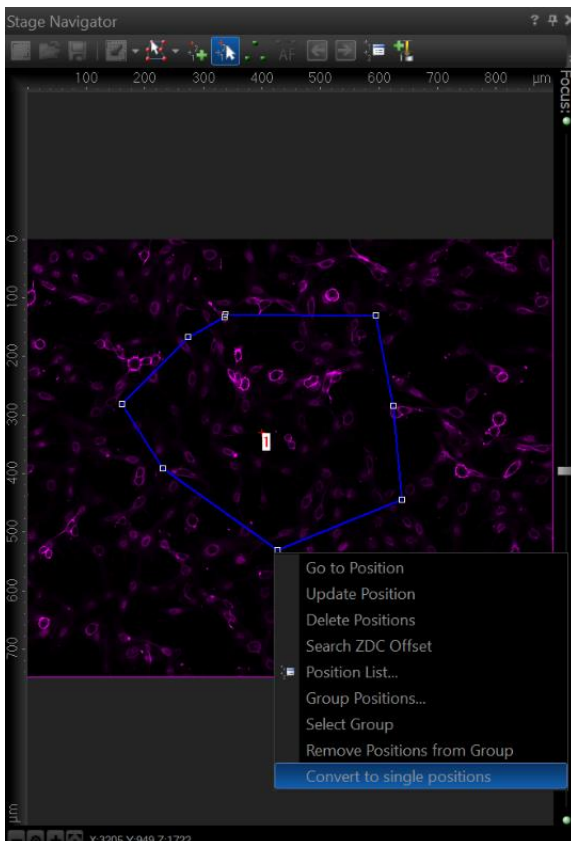

Draw your ROI on the navigator image normally then right click "convert to single positions". cellSens will acquire your tiled montage (including Z stacks, channels and additional dimensions that you have active in the process manager when starting the acquisition.
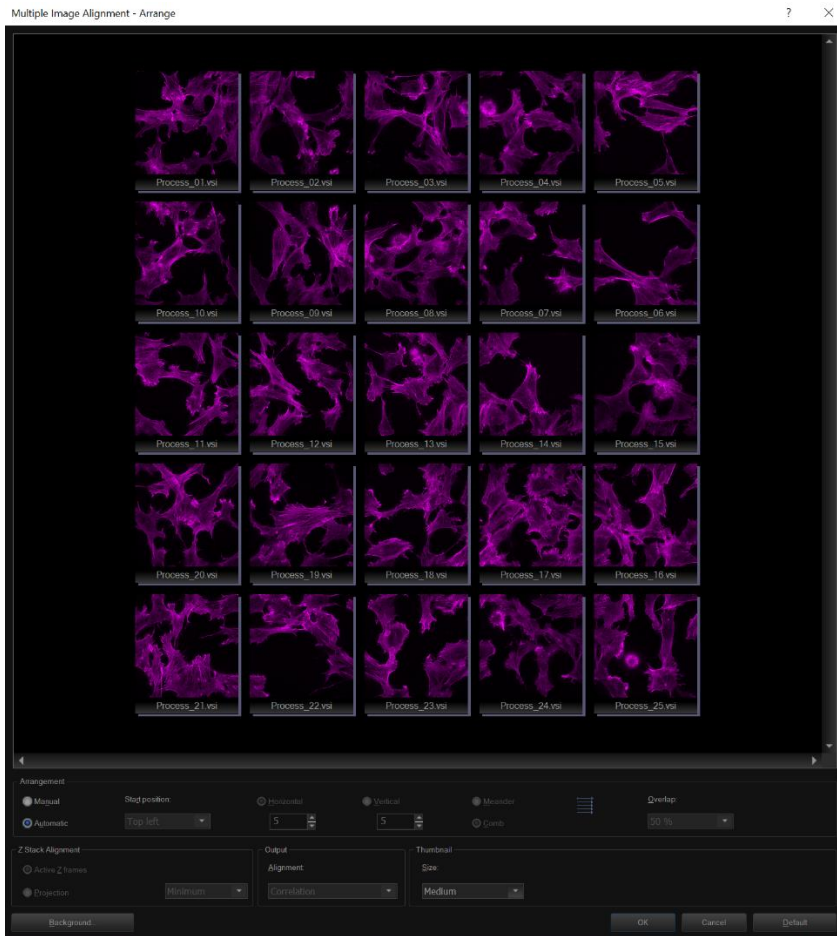
Once the acquisition is finished, go to view / tool windows / documents where you will find the acquired individual vsi files. You can copy those files to another location if you want to stitch them later on a workstation or on a third party software. Otherwise, open the files. Open the MIA via PROCESS > MULTIPLE IMAGE ALIGNMENT alignment. The options for alignment and blending can be adjusted in "acquisition settings" ➔ "automatic MIA". For blending choose Alpha (Gauss) and for Z-stack chose EFI projection.
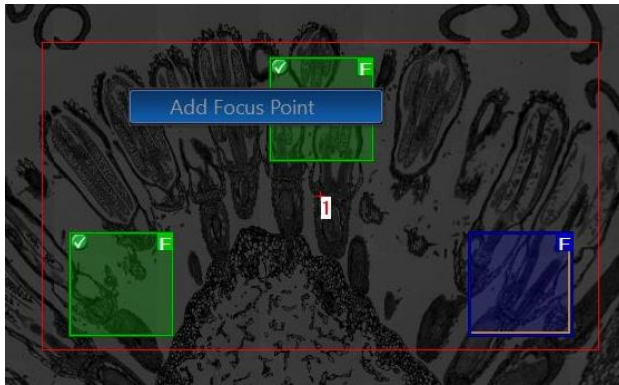
Focus map

When acquiring XY montage on a tilted or a wavy sample, having a single Z reference will prevent to have each tile of the montage in focus.
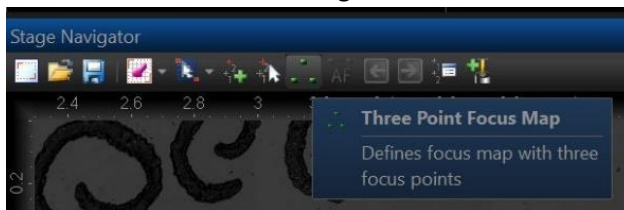

To alleviate this, you can use the function "three point focus map" in the stage navigator toolbar in order to define multiple focus points across the tiles to match the tilt and rescue it.
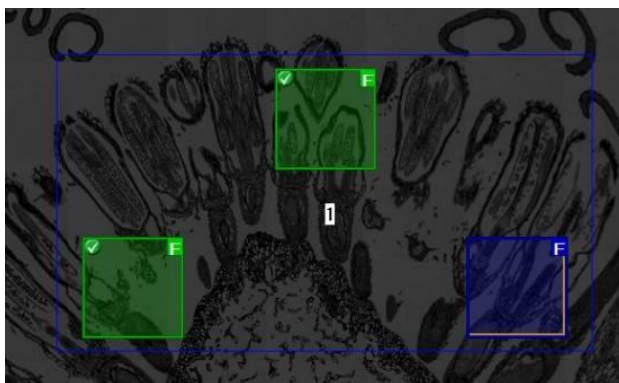

When clicking the option in an ROI drawn previously, you get 3 fields of view distributed across the ROI. You can navigate between the 3 points using the green arrows in the menu or clicking them with the mouse. When a focus has been left, the Z position it has been left at becomes its new value and the focus point is displayed green. The active one is displayed blue.

You can also add as many additional points as you wish by right clicking with the mouse pointer where you want it to be created. You can also drag the focus point to another location within the ROI.
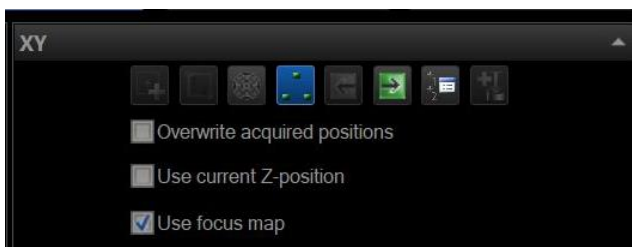

Once the focus map is set, make sure the "use focus map" option is ticked in the XY tab of the process manager.
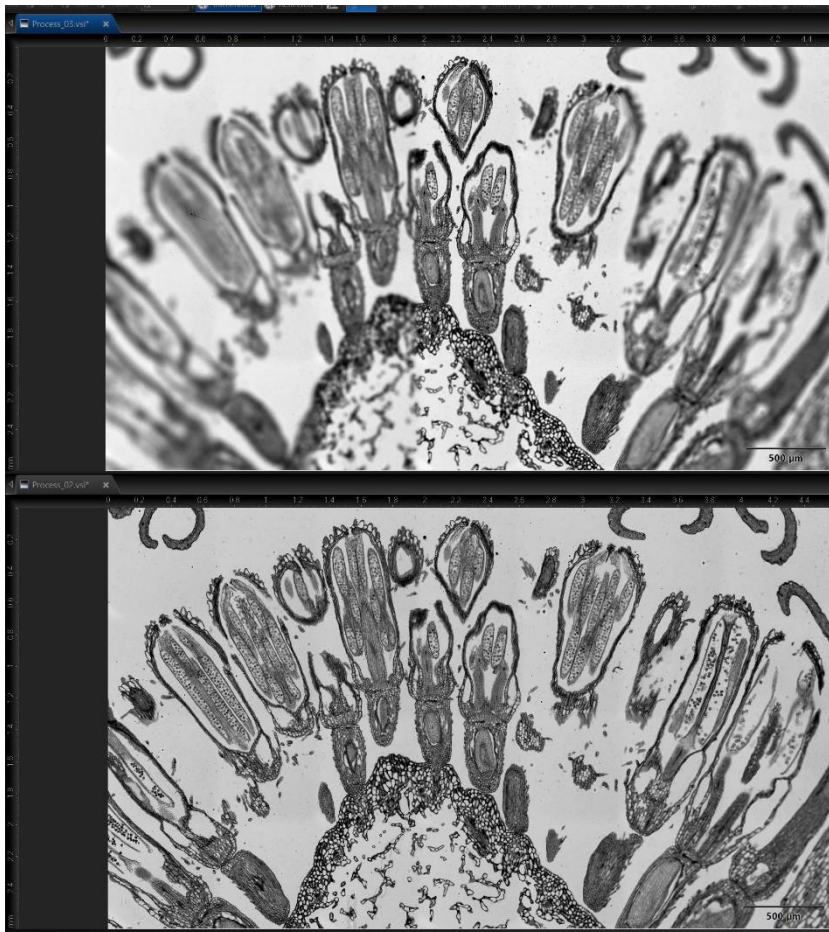
The top image was acquired without Ffocus map on a tilted slide, the focus was made at the center of the ROI, leaving both left and right extremities blurred.
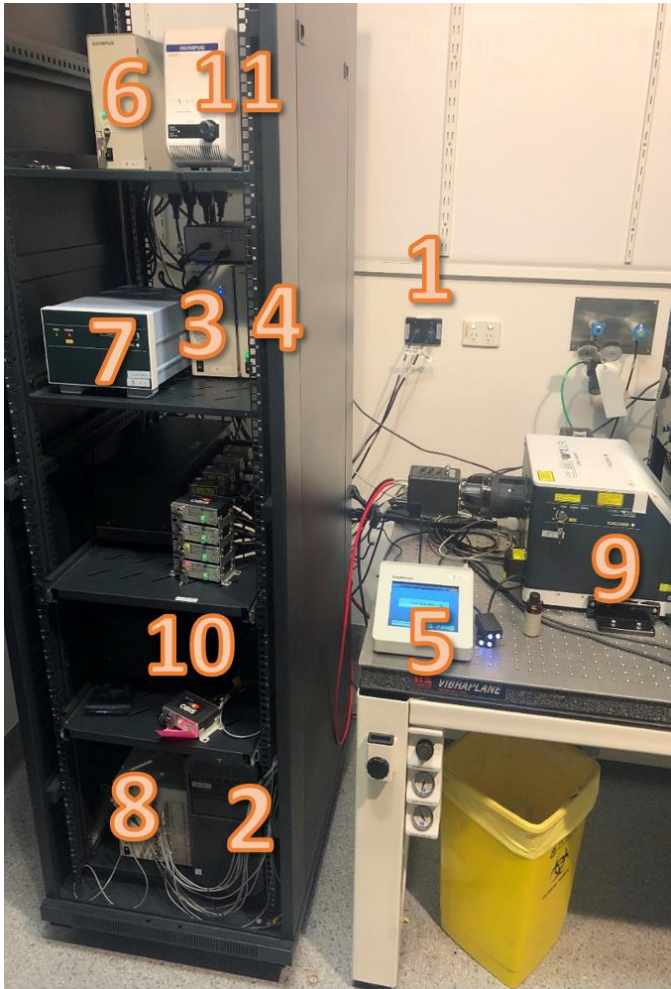
After setting the focus map to correct it and reacquiring the image with the map option activated, all tiles are in focus.

Timelapse and autofocus
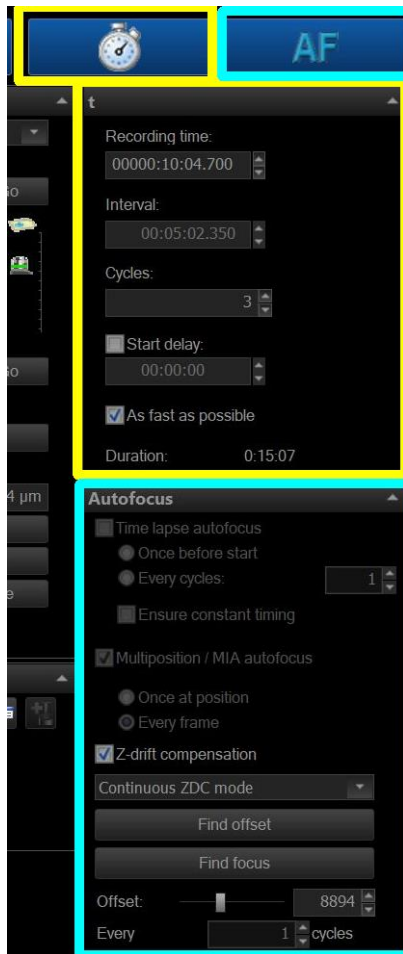
Options for live sample imaging.

Appendix 1: Start-up procedure

Turn on the different components in the following order **with 5 seconds between each step:**

1) Power switch for cameras at the wall

2) PC (front button)

3) CBH

4) Main Power supply

5) LCD screen controller (TPC)

6) Power supply 2

7) CSU-W1 power and control

8) U-RTCE (real time PC)

9) CSU-W1 unit

10) Lasers; first the switches then the keys

11) U-LGPS (epifluorescence)

12) Start CellSens

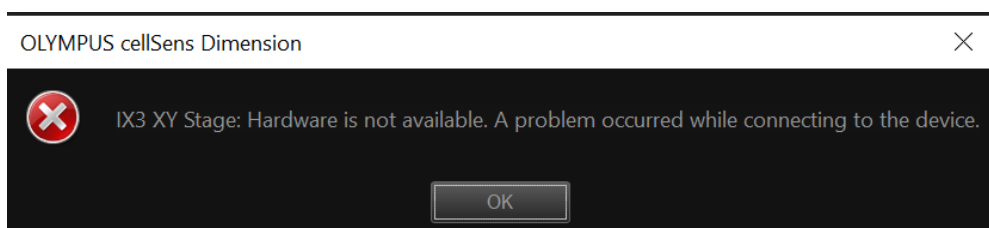13) Incubation brick and cube if needed

Appendix 2: trouble shootings

- <u>XY stage not connected or recognized</u>

If this message appears on the LCD screen controller after booting
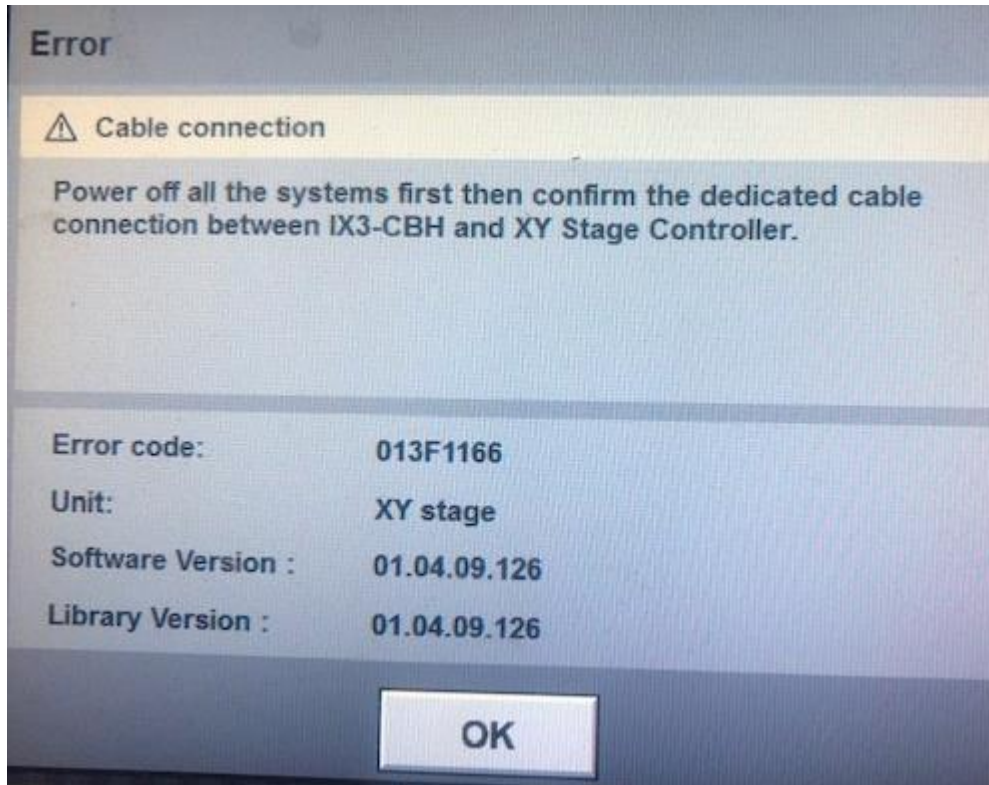
Or this message appears on the computer after launching CellSens,

This error occurs randomly, seemingly because the stage gets stuck and loses its calibration. It usually comes with three loud beeps from the component #5 in quick succession.

Fix: turn OFF components 5-4-3 and move around the stage manually to free it from the gripping that caused to issue. Restart 3-4-5. If this fails, contact the microscopy team.

- <u>Cleaning stage procedure failed</u>

Currently the automatic cleaning procedure for the XY stage does not seem to finish without this error message. The cleaning seems to occur normally though. Ignore.

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- ## <u>Epifluorescence view through the ocular is blinking.</u>

Untick the "synchronize shutter" option in the camera tab (top left part of the CellSens window.