Nikon Eclipse

User manual

Cell Imaging & Cytometry

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1. Information

The Cell Imaging and Cytometry Core is responsible for maintenance of this microscope.
Each user must follow the CIC user policy.
More information: https://bioscience.fi/services/cell-imaging

- **Only authorized users may use the CIC instruments.**
- **Users must report any malfunction or problem to the CIC personnel.**
- **The user is responsible for removing their data from the hard drive and should do so immediately.**
- **Files older than 30 days are automatically wiped from the system without prior notice.**

2. Before Imaging

Check your sample with an ordinary fluorescence microscope

It is a good practice to check the quality of your sample before making use of a more expensive instrument.

Clean your slides

Clean the remaining salt and mounting medium off the coverslip. Dirty coverslip compromises the image quality. You can use ethanol to aid the cleaning. Do not use the microscope lens tissue. Clean your slides beforehand in your own lab, as it is impractical to use microscopy time for cleaning.

Check the environment

Switch on the lights and check if the microscope environment is tidy.
If there are oil spills or other issues, please inform CIC personnel.
(microscopy@bioscience.fi)

Start the heating earlier if possible

When doing live cell imaging, it is good to switch on the heating at least two hours in advance. If someone is using the instrument, ask whether it is possible to switch it on. If no one is using the instrument, start the instrument and switch on the heating.

Cancellation

Cancellation must be done 24 hours before the reservation starts. However, if you suddenly cannot use your time, inform the next user and cancel your reservation.
If you are the last user of the day and cannot come, you are responsible for the instrument shutdown.

Unsure?

If you feel that you need support, please contact CIC personnel.

3. Working with immersion objectives

The immersion medium should match the objective.

Oil objectives need oil with a refraction index of 1.515.

The image will be suboptimal when incorrect oil is applied. Mixing immersion oils is prohibited.

A small drop of oil is enough, adding to much can make a mess and damage the instrument.

Start imaging with the objective in the lowest position. Then, focus the objective upwards until the oil touches the coverslip. Next, focus the sample visually through the eyepieces.

After imaging, wipe the oil off from the objective softly with a dry lens tissue. Then, finish the cleaning by wiping softly with a new lens tissue moistened with isopropanol.

Only lens tissue may touch the objective lens.

Buttons on microscope body

Hitting the foot switch will turn on light inside microscope incubator.

Lower the objective when changing the sample to view with an immersion objective

- Press **ESC** on the microscope body or in the software to move the objective down.

- Remove the sample and add oil if needed

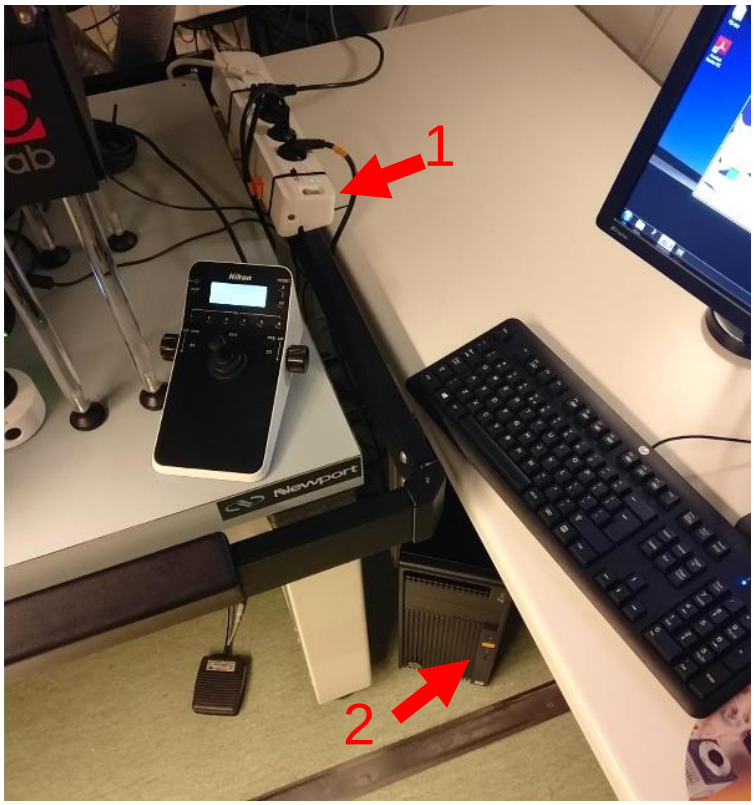
- Place the sample. If you are changing the objective in between, wipe the objective clean with a dry lens tissue. Otherwise, oil might spill into the microscope.

- Pressing **ESC** again will raise the objective to its previous elevated position.

- Pressing **LIMIT** on the microscope body will set the top z-limit of the objective.

4. Starting up and shutting down the instrument

4.1. Start-up



Switch lights on and check that the environment is clean.
If you see some issues, report them to CIC personnel.

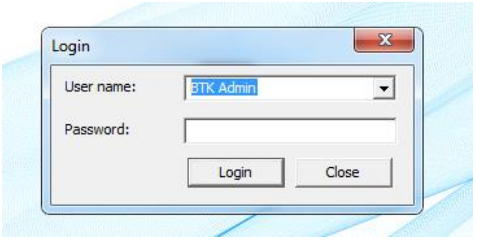
1. Switch the adaptor to start microscope and components.
2. Switch PC on and wait for computer to log in.

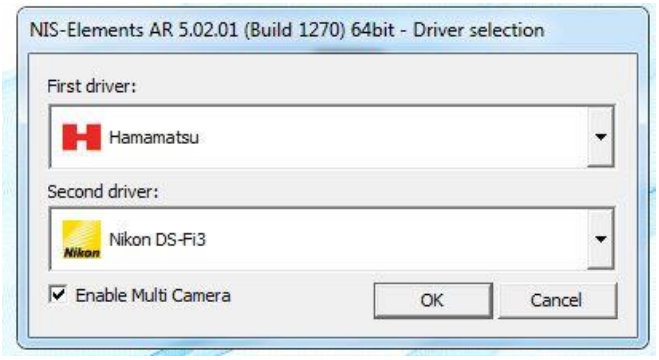
4.2. Shut down

Take your data and shut down the microscope in reverse order than start up

1. Close NIS Elements software and shut down PC
2. Switch both adaptors off to shut down microscope and components.

5. Starting the NIS software





1. Select the software and login with your User name and password
2. Select which cameras you want to use by selecting drivers. Selecting Enable Multi-Camera allows selection of both cameras.
3. Select OK, the software will then take a few minutes to initialise.

Note: The two cameras on this instruments:

1. **Nikon DS-Fi3** colour camera
2. **Hamamatsu Orca flash 4** monochromatic camera.

6. NIS software interface

The Ti2 Pad windows allows selection of numerous setting including objective, light path, z step. Transmitted light lamp and fluorescence filters and shutters.

6.1. Ti2-Pad

Under **Nosepiece** are the available **objectives**. Placing the arrow over each objective will provide information.

Escape Z moves the objective to its lowest position. This needs to be deselected to allow Z movement.

Under **Light Path** are the directions for where the light is directed for visualisation. The upper selection “Camera/eye” directs to the eyepieces. **L100** (currently selected) directs the light to the left side camera, which is the Hamamatsu Orca flash, **R100** directs the light towards the **Nikon DS-Fi3** colour camera found on the right of the microscope.

Z Drive allows incremental control of the z steps while also displaying the current Z-position.

DIA is the **transmitted light**. Selection of the Yellow button will active the light. Light power percentage is also controllable using the slider by typing into the box.

Shutters are responsible for the two sets of filters. The shutter for the selected filter set must be open to view sample. I.e. **FL-Lo** activated when using **Turret-Lo**.

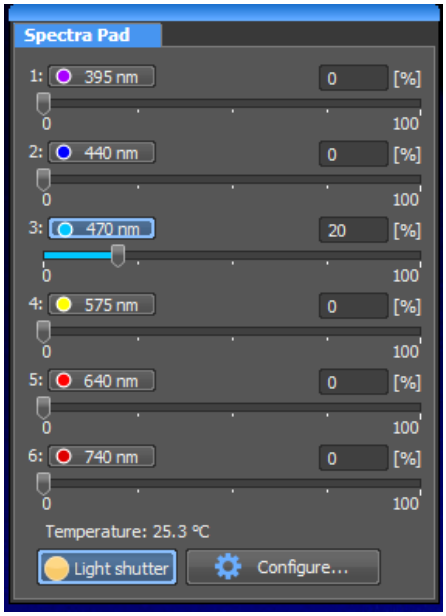
Under **Filters** are the two filters sets. **Turret-Up** is the filter set for the eyepieces and colour camera **Turret-Lo** are the band pass filters for the Orca camera. Hovering over each filter provides more information. Only one filter set can be used at a time, the other must be in the empty position visibly selected for the Turret-Up filters. Otherwise, the light path will be blocked.

The **EM wheel** is an emission filter set for the Orca camera and should match with the appropriate Turret-Lo filter set.

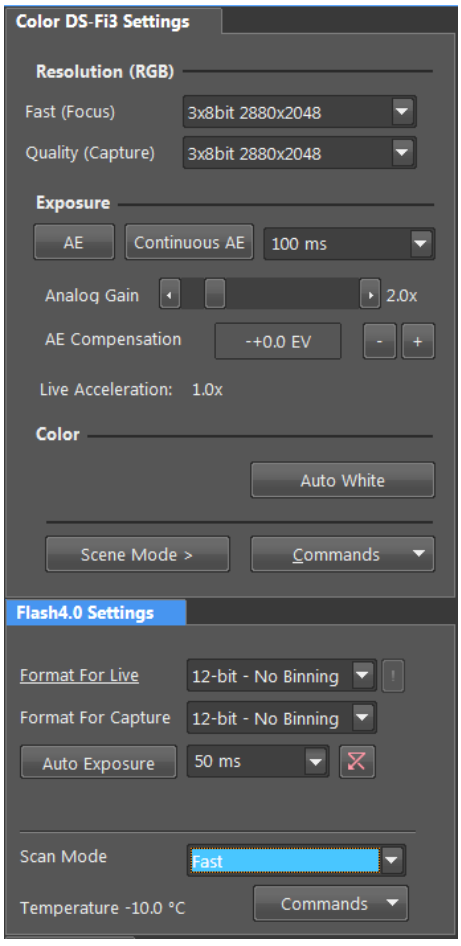
Condenser allows selection of the bright field or contrast prisms of phase ring that must be matches appropriately for each objective. As mentioned before hovering the arrow over the objective will show correct DIC prism/phase ring.

For fluorescence images, the condenser should have the **shutter** position selected.

6.2. Spectra Pad



The **Spectra Pad** controls the LED light source for image acquisition. Selecting the wavelength such as 470 highlights the tract and selecting the **Light shutter** button at the bottom of the tab activates the light source. The light power percentage can be adjusted using the slider of by typing into the box.



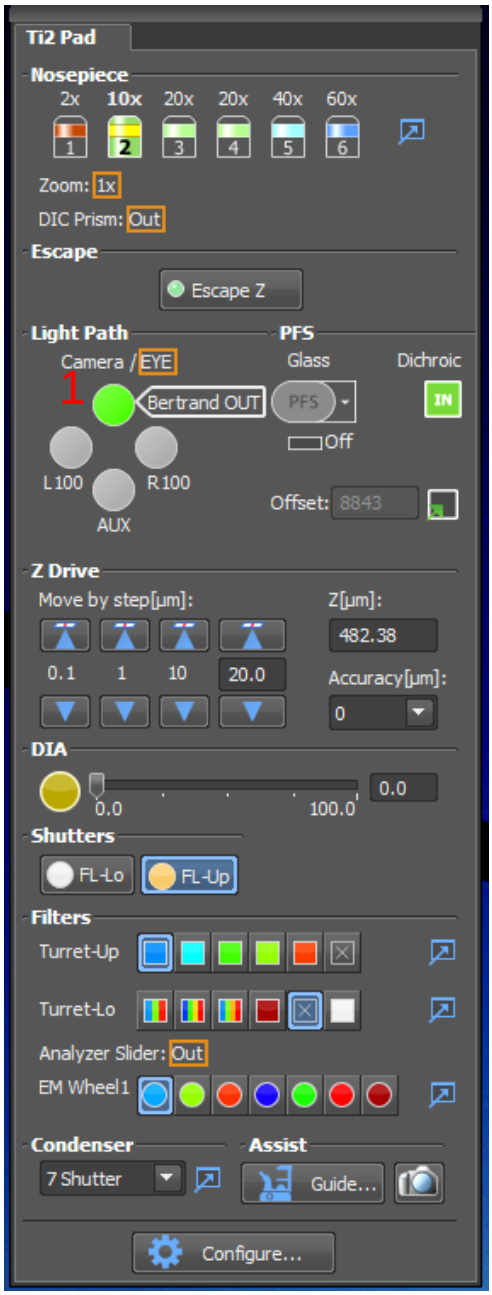
Cameras are controlled in their separate tabs.

Exposure times are selectable from the drop down or by typing the value.

Bit depth and **binning** are adjustable for both live and capture quality of the Orca camera

Note: Make sure you have checked the format for capture setting!

7. Visualising through the eyepieces



Here is an example of how to visualise DAPI in your sample through the eyepieces.

Note: Some light path settings are already saved and can be used.

To visualise the sample the **Camera/EYE** light path along with selecting the appropriate **Turret-Up** filter while having the **FL-UP shutter open.**

For visualising fluorescence through the eyepieces, the only setting that needs to be changed for different channels is the **Turret-Up filter**

For bright field put the Turret-Up and Turret-Lo to empty and active the DIA condenser bulb and adjust the power.

Note: The **EM wheel** setting is irrelevant as this is only for the Hamamatsu Orca Flash camera.

The available fluorescent channels for visualisation through the eyepieces are DAPI, CFP, GFP, YFP and Texas Red.

8. Creating optical configurations for image capture

8.1. Configuring channel set up

Here is an example of how to visualise GFP in your sample with the Orca Flash camera.

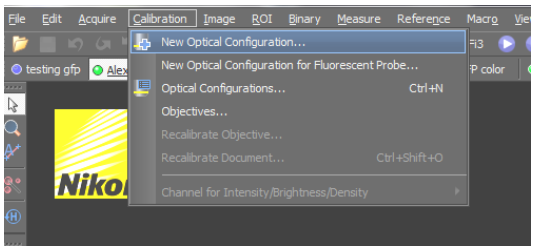
Note the light path, correct open and closed shutters, filter and EM wheel selection.

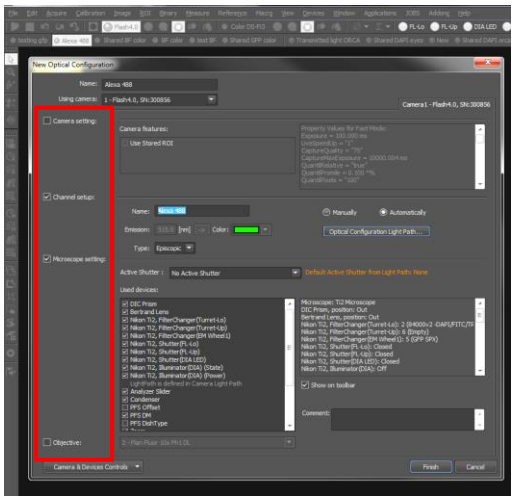
In addition, notice the **correct wavelength** (470nm) selected on the **Spectra Pad**

Compared to the eyepiece setting more changes are required for different channels.

A change of filter (Turret-Lo), EM wheel filter and activated light wavelength will all need to be adjusted.

8.2. Saving new optical configuration





New optical configurations such as shown above can be saved.

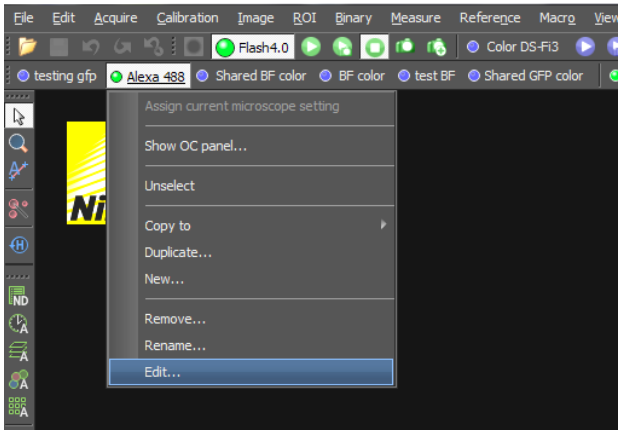
From the **Calibration** menu, select **New Optical Configuration…**

In the new window, rename your new configuration.

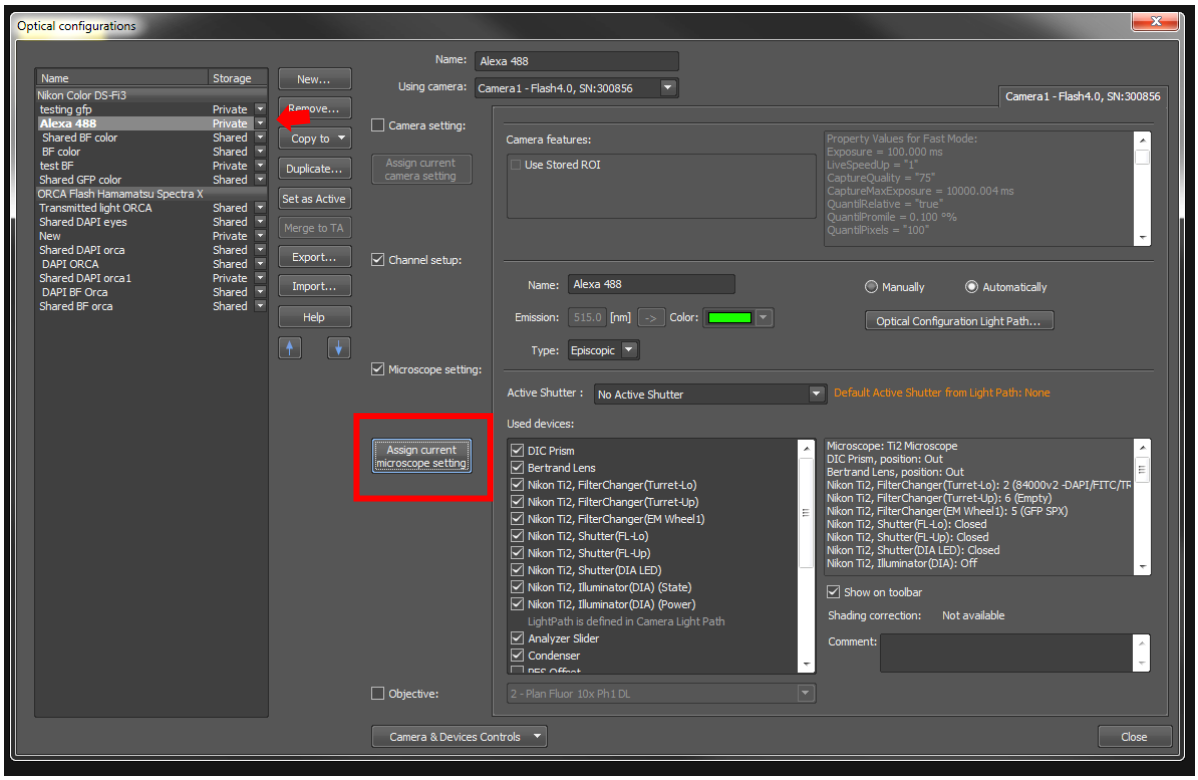
On the left side are titles (red box):

- Camera setting
- Channel setting
- Microscope setting
- Objective

Selection of the boxes means these setting are recorded into the optical configuration.
Modifications to these selected setting will need to be actively saved.



Right-click the optical configuration and select **edit**.

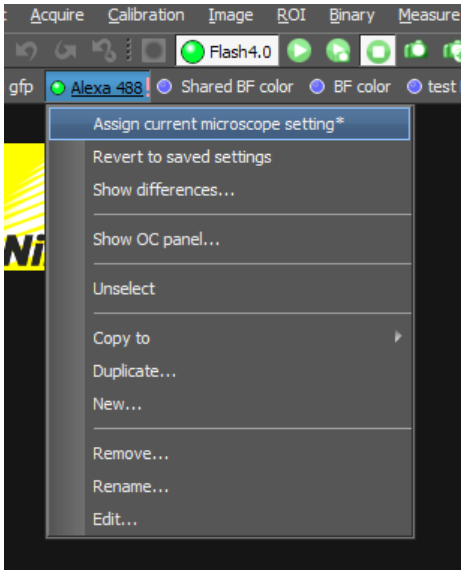


Right-click the optical configuration and select **edit**.

Select **Assign current microscope** settings.

Note 1: Active shutters are also useful to select as their opening and closing can be integrated into ND acquisition.

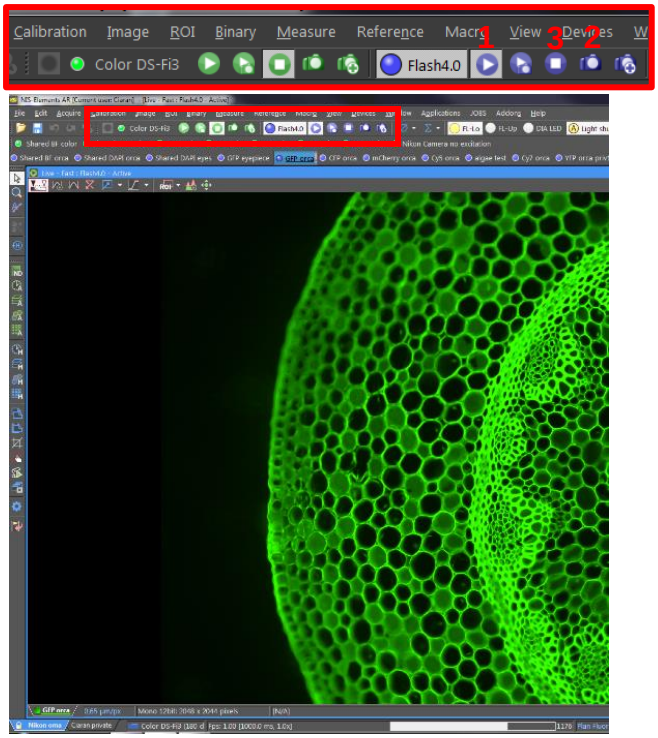
Note 2: Make sure to make your Optical configuration Private (red arrow). Therefore, only you may view and modify its settings.



Any further changes to the optical configuration will create an exclamation point beside the name of the configuration.

The changes can be assigned by right clicking the configuration name and selecting **Assign current microscope settings**.

8.3. Imaging window



The visualisation window is where the camera can be activated to obtain a live image of the sample.

Select your optical configuration of interest.

Pressing the live button **(1)** of the camera used in the optical configuration will bring up the live image.

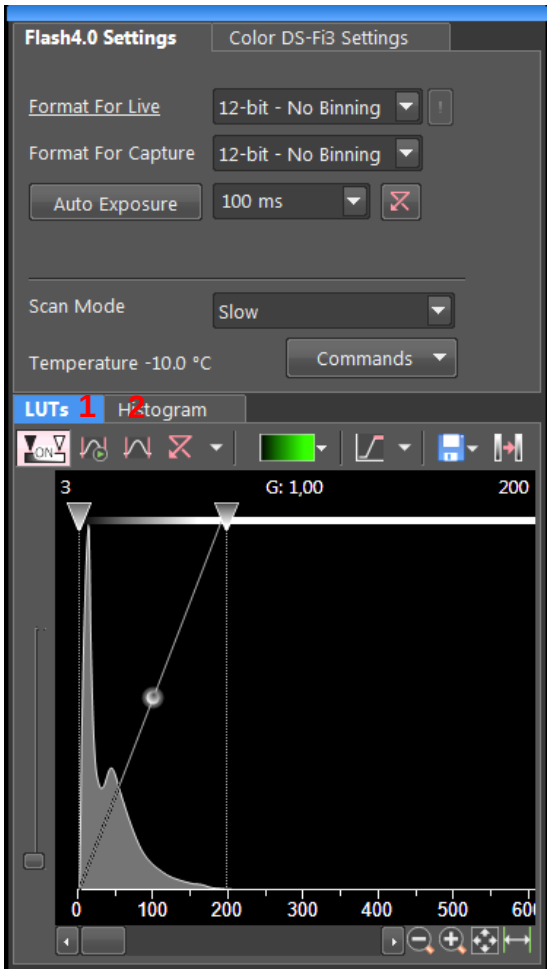
To captures a single frame press the camera capture button **(2)**.

A new window of the captured image opens and can be saved to your folder.

Pressing the stop button stops live camera image **(3)**.

This snap setting only works for single channel images.

8.4. Adjusting exposure time and histogram



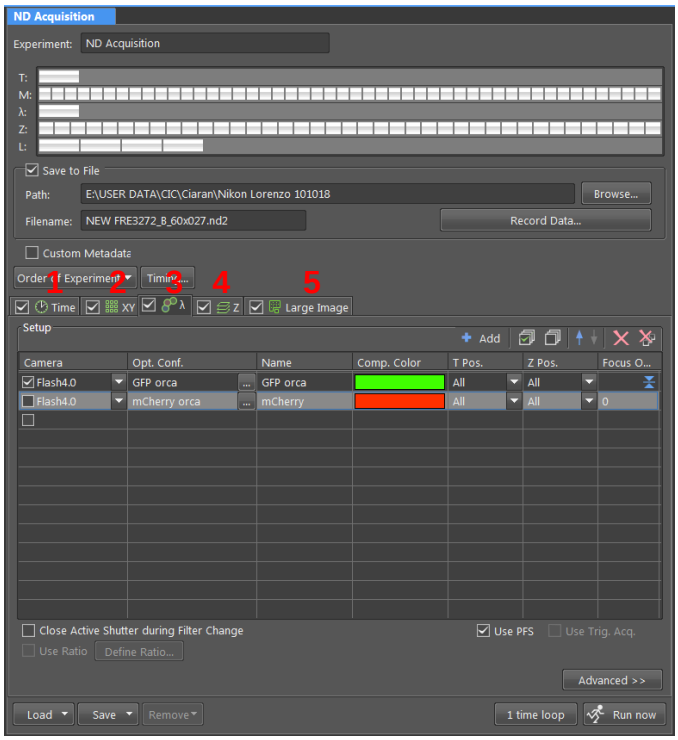
As previously mentioned, exposure time and binning can be modified in the camera setting windows.

Note: Make sure you are editing the correct camera settings

Adjustment of the look up tables (LUTs) may also be needed to better visualise your data. This is done in the LUTs tab.

Adjustment may be done manually by sliding the upper and lower limits, automatically adjusting during live imaging **(1)** or by selecting the max and min button, which performs a one off adjustment to the sliders **(2)**.

9. ND Acquisition tab for multi-parameter experiments



ND Acquisition tab allows capturing:

1. Time points
2. Multi-position
3. Multi-channels
4. Z-stacks
5. Tile images

<u>Active parameters are shown by a tick.</u>

As parameters are specified, the complexity of the experiment is shown by increasing bars for parameters at the top of the acquisition tab.

The displayed acquisition tab has one time point with multiple positions and z slices. There is one channel selected **(GFP Orca)** and the image has a tile that has four images.

Once and experimental set up is ready it can be tested by pressing **1 time loop** to for a test-run.

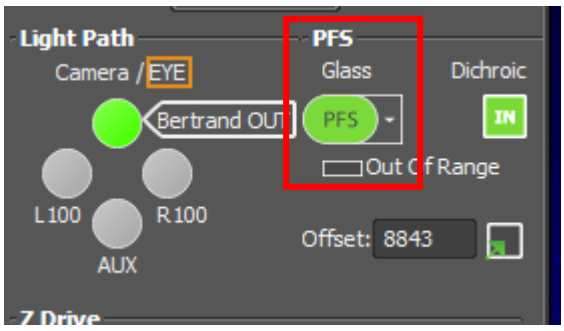
Pressing **Run now** starts the experiment.

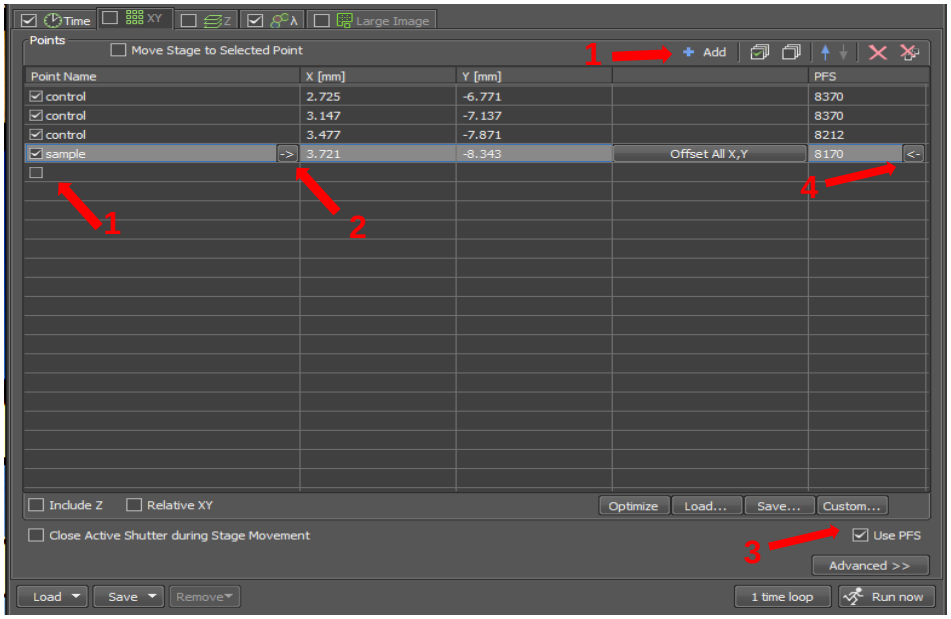
Each tab has multiple parameters. As shown above, the **λ** tab allows selection of saved optical configurations. **T position** allows determined the time points the configuration is used, in this instance at every time point. At what **z positions** may also be, define along with **Focus offset**.

For each tab, there are options on closing active shutters during filter change. These should be checked if needed for all experiments and not selecting them may for example leave transmitted light shutter open between time points.

9.1. Using Perfect focus system for multi-position

The Perfect focus system (PFS) is well suited for long/term imaging. Once the PFS is activated is becomes green, the focus needs to be adjusted with PFS offset controller.





In the **XY** tab, update your XY coordinate.

New coordinates are added by selecting the empty box below previous coordinate or by pressing the add button **(1)**.

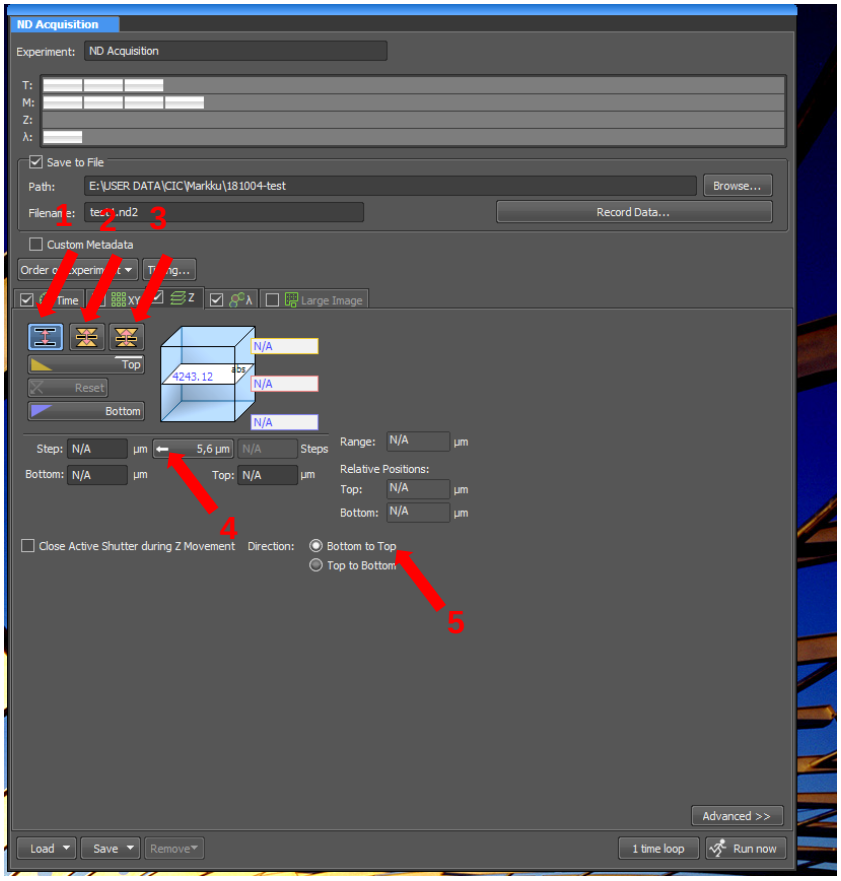
Updating coordinated are done by selecting the arrow **(2)**.

Select the **Use PFS** option **(3)**.

Once the correct focus is found by using the PFS offset controller, update the PFS value by selecting the arrow **(4)**.

Note: Remember to activate the XY tab so it is integrated into the experiment. In this example, it is not active.

9.2. Capturing Z stacks

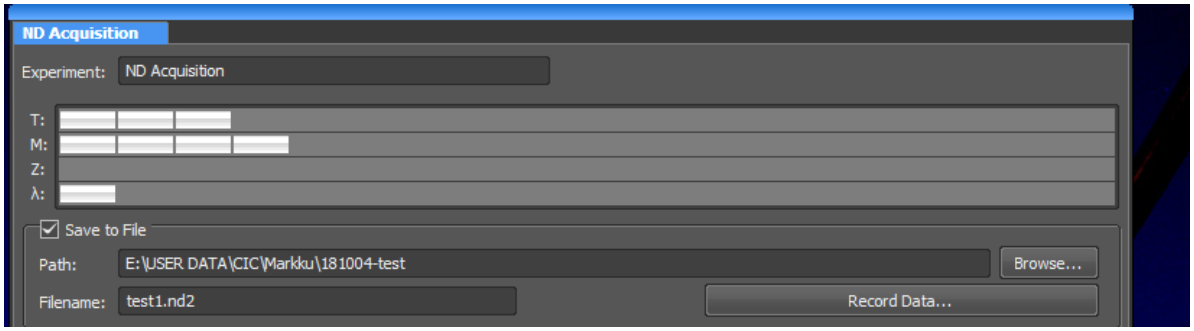


Z stacks can be captured in three ways.

1. Defining top and bottom positions
2. Setting a symmetric range around a point of focus
3. Setting an asymmetric range around a point of focus

The steps size can be manually defined or the recommended step size can be selected **(4)**.

9.3. Saving and renaming data



Always make sure to activate **save to file** to **auto save** your data. The file save location can be chosen by selecting **Browse…**

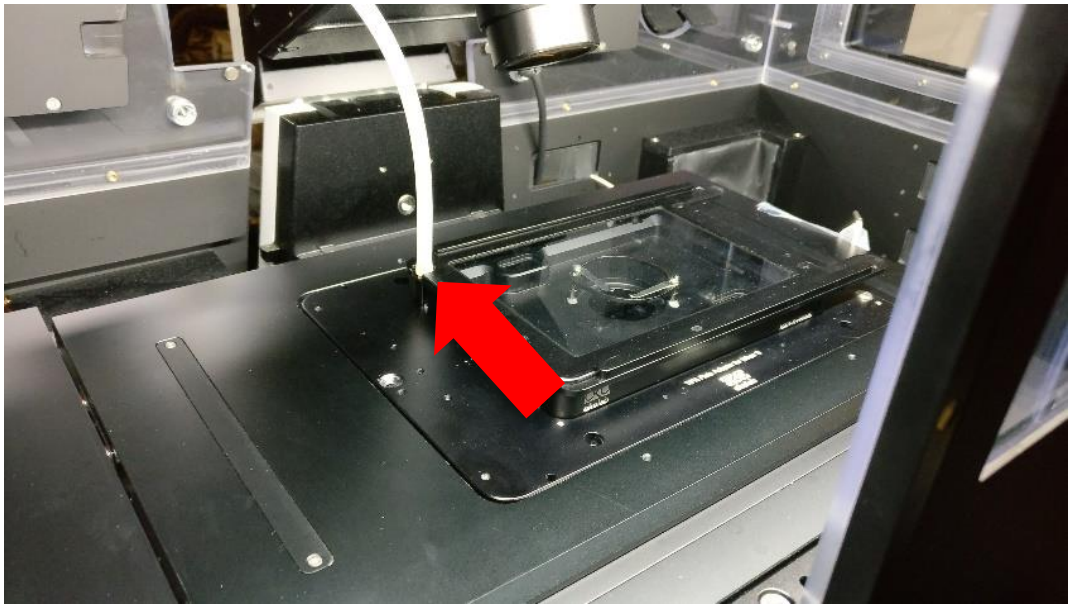
The **filename** can be renamed with the following files having the same name with an increasing number.

10. Long-term imaging

For long-term imaging, the Okolab incubator should be switched on a few hours before the experiment to allow the system to heat and equilibrate.

Just before imaging, switch the CO_2 on (at the wall beside PC) by turning the valve counter-clockwise to vertical position. Then adjust the CO_2 setting on the Okolab controller.

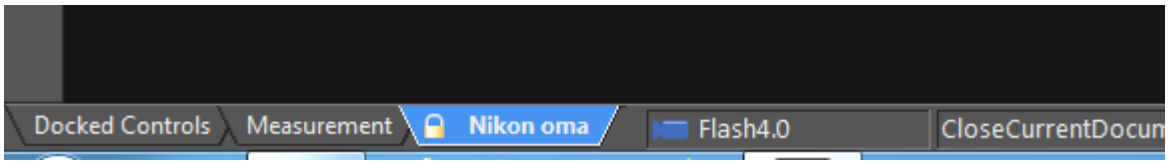
Note: It will take a few minutes for the CO_2 value to stabilise to the desired setting.



Note: Make sure to connect the air tube (arrow) into the imaging chamber so the CO_2 reaches the cells.

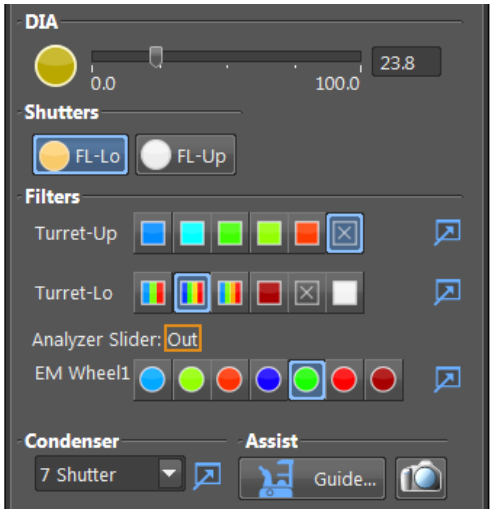
11. Troubleshoot

11.1. Layout has been changed.



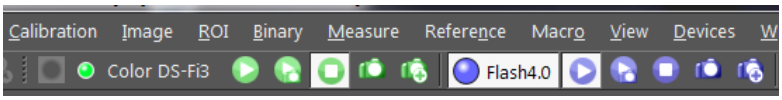
Check the correct layout window is selected.

11.2. Light path blocked



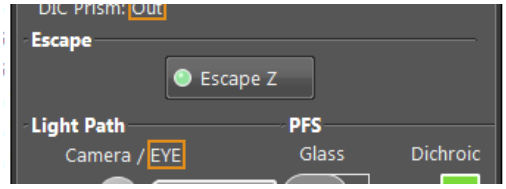
Check that the **correct shutters** are open and that **unused filter** are not in the way and.

11.3. Light hitting sample but cannot see with camera



Check that the **light path** is going to the camera and that the camera is live

11.4. Cannot adjust focus



Check if **Escape Z** is activated.