Usermanual for Nikon Ti2 Eclipse

Table of content

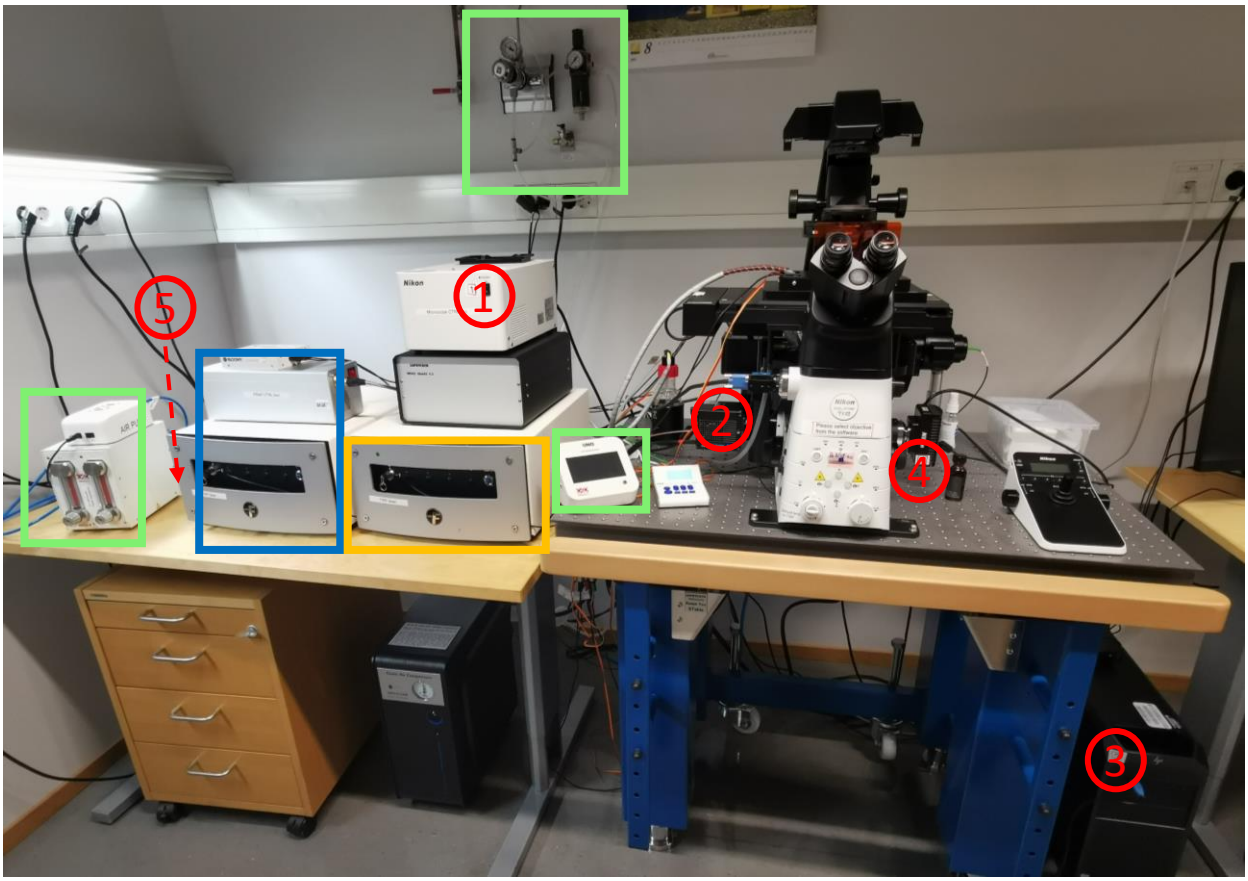
How to turn on the system

1. **Turn on the microscope ctrl box (1).**
2. **Turn on the TIS camera for fluorescence imaging (2) if needed.**
3. **Turn on the computer and log in using "user" and password " ".**
4. **Turn on the Fi3 color camera (4) if needed.**
5. **Turn on the CoolLed for fluorescence (switch on el. pad)**

For live cell imaging, open the CO2 valve, turn on the OKO lab mixer and start the heating (green boxes). See page 7 for more information.

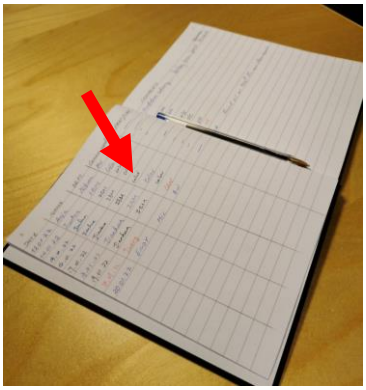
For FRAP imaging, remember to turn on the FRAP laser and the FRAP ctrl box (blue).

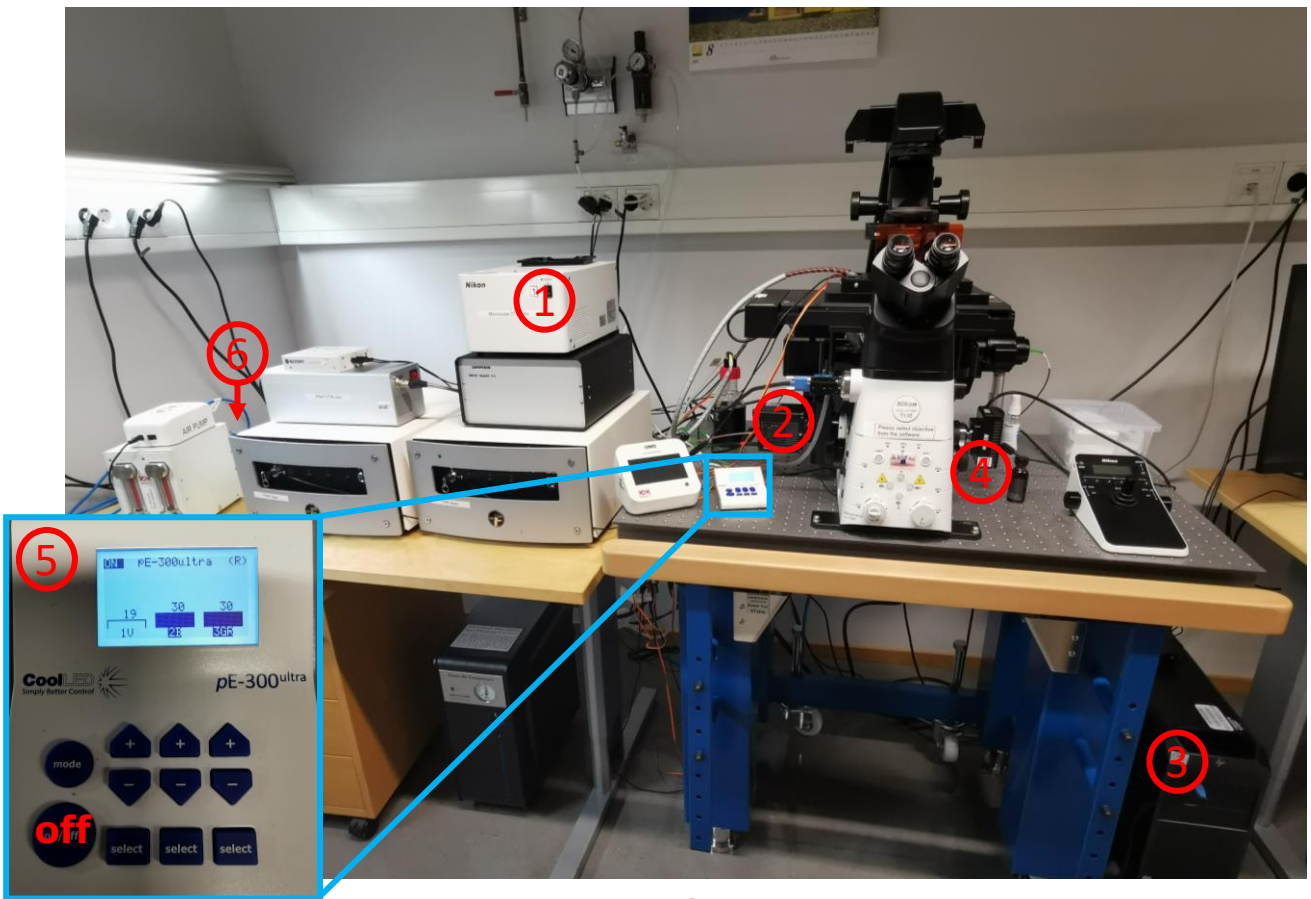
For TIRF, remember to turn on the TIRF lasers (yellow).



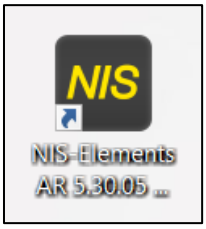
How to turn off the system

1. **Transfer your data (page 22).**
2. **Close the software and start a computer shutdown.**
3. **Turn off the TIS camera (2) and the Fi3 camera (4) (if they were on).**
4. **Turn off the microscope control box (1).**
5. **Turn off the CoolLED (5).**
6. **Switch of the CoolLED on el. pad (6).**
7. **Remember to sign the logbook and report any issues to MIC personnel.**

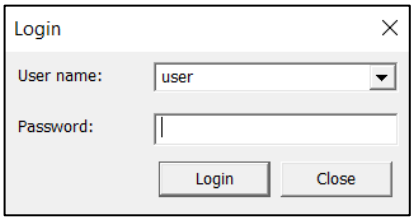


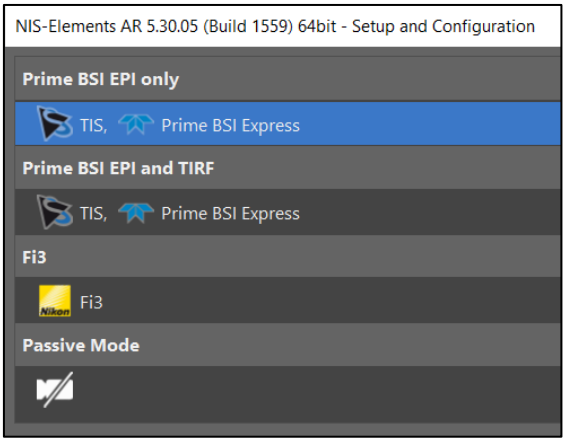


Starting the software?

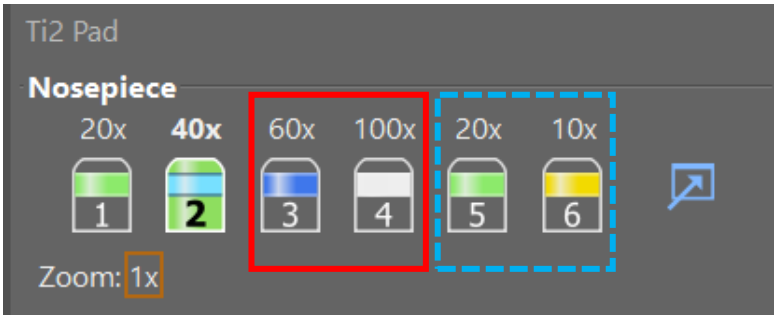


- Log on to the computer with Nikon (password Nikon).
- Start "NIS-ELEMENTS
- Login using the "**user**" account (no password needed).
- Select the camera you would like to use. If you do not want to acquire any images, select "passive mode". For more details about the camera options, see page 6.
- The objectives are the eyes of the system, so make sure you use them correctly. The 60x and 100x must be used with immersion oil. The other objectives are DRY! The two objectives in position 5 and 6 have large working distance. These can be used for cell culture plates and give nice phase contrast images.





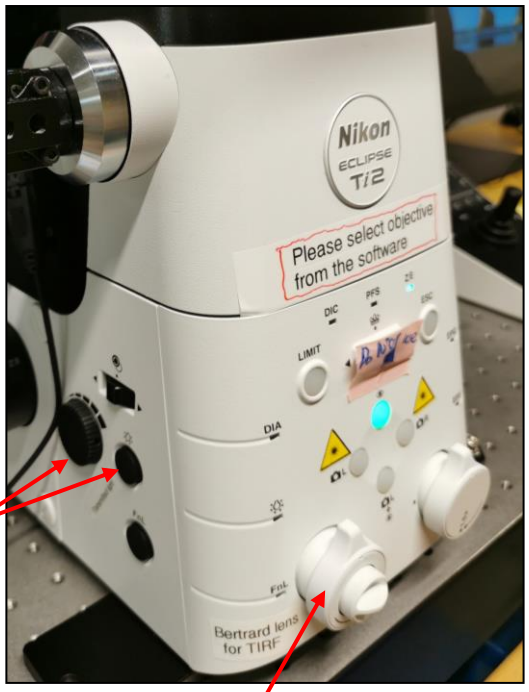
Immersion oil



Oil objectives with small working distance and high NA.

DRY objectives with large working distance.

Viewing through the microscope

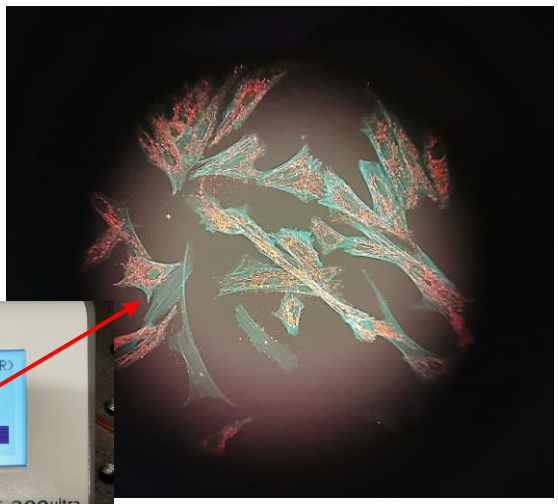


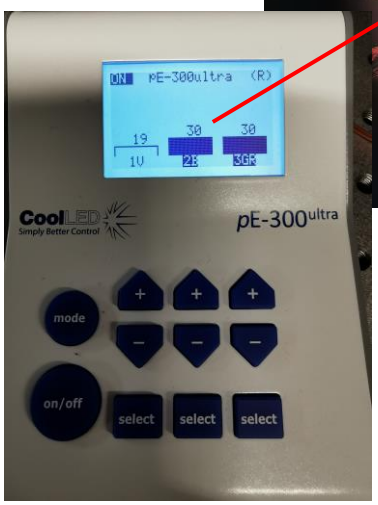
Halogen light

Bertrans lens

Fluorescence filter turret.
Use the multiband filter.
Press the button to open/close the shutter.

Click on a fluo setting in the software in order to get the multiband filter in position.





CoolLed ctrl panel

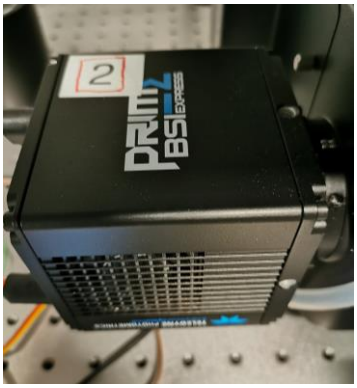
You can leave the 2nd and 3rd channel on to see both green and red!

To change Z speed, press here

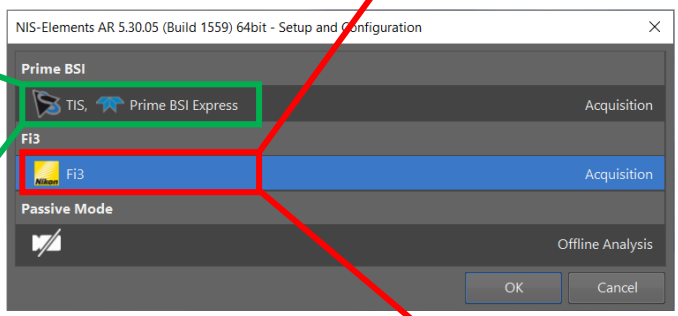
To change the XY speed, press here

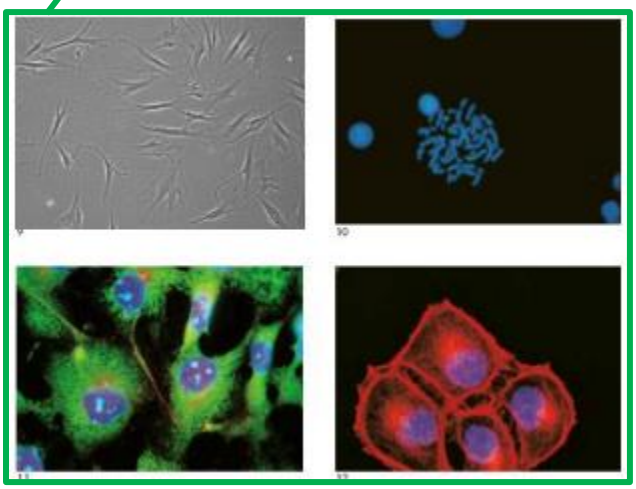
Which camera should I use?

TiS camera (2048 x 2048 pixels)

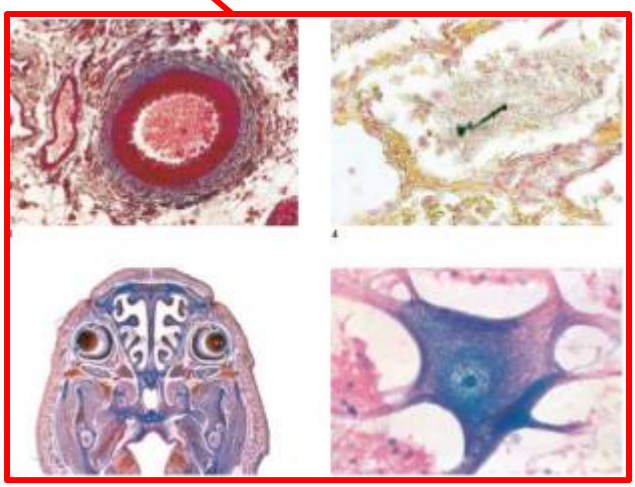


Fi3 camera (2880 x 2048 pixels)





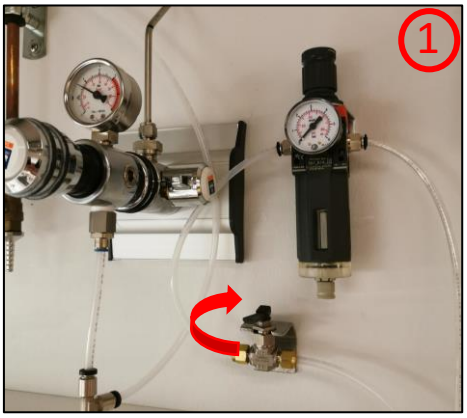
Use this camera for imaging of fluorescent samples.



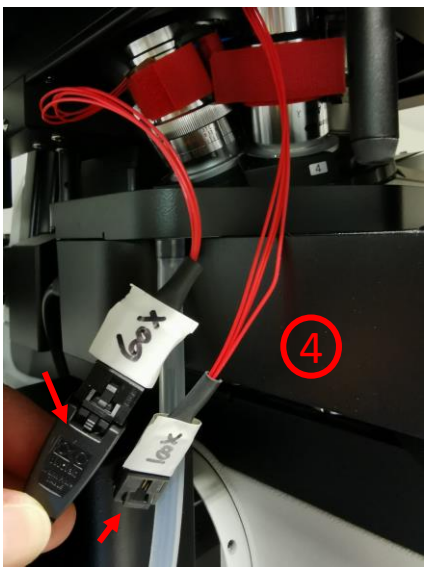
Use this camera for imaging of brightfield, phase contrast and histological samples.

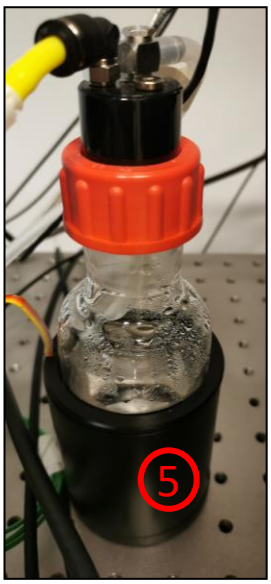
Live cell imaging setup

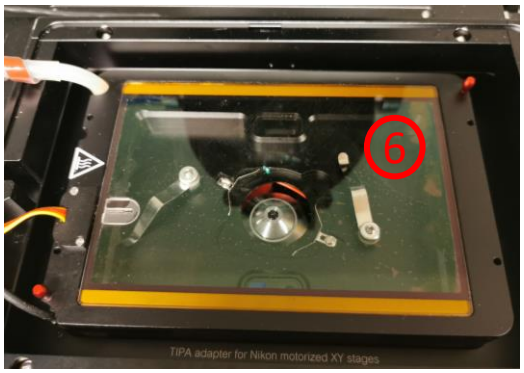
- Flip the small switch upwards to open for the CO2 gas (1). Do Not touch any of the other valves!
- Turn on the air pump (2). Do not touch any of the valves on the box underneath!
- Turn on the small OKO control box (3) to start the heating of the insert.
- Make sure you connect the correct heating cable (4) for the objective you will use (60x or 100x). The red heating element will start to warm up the objective. For good stability allow 0,5-1 h warm up time.
- Make sure there is enough distilled water in the humidifier (5).
- Use the live cell insert and heating lid (6).



No no!





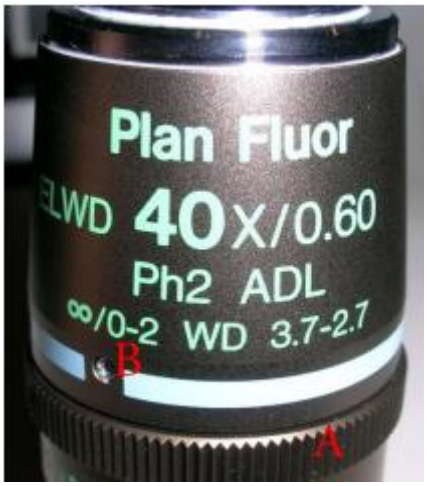


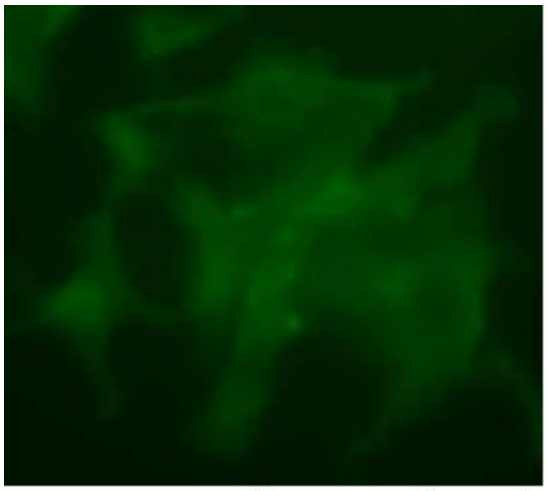
Objectives available on the system

Magnification	Correction	NA	WD (mm)	Contrast method and application
10 x Air	Plan Fluor	0,30	15,2	Ph 1 / phase contrast
20 x Air	Plan Fluor (coverslip correction)	0,45	7,4	Ph 1 / phase contrast
20 x Air	Plan Apochromat lambda	1,20	1,0	fluorescence
40 x Air	Plan Apochromat lambda (coverslip correction)	0,95	0,21	fluorescence
60 x Oil	CFI Apo TIRF (coverslip and temperature correction)	1,49	0,12	TIRF / fluorescence
100 x Oil	CFI Plan Apochromat lambda	1,45	0,13	fluorescence

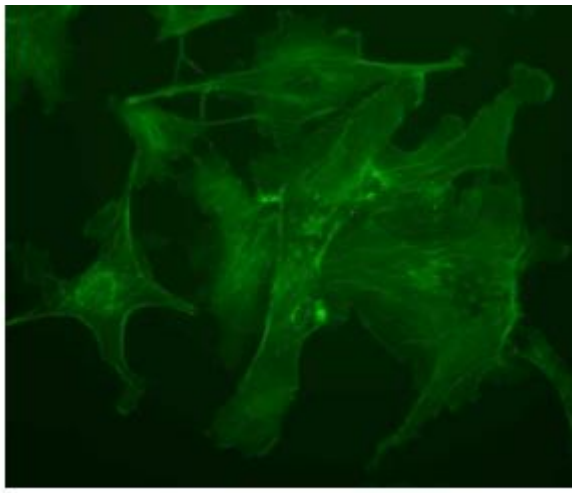
Some objectives have a correction collar (A) to adjust for "cover slip" thickness between 0 and 2 mm (B). In order to adjust:

- Look through the eyepiece at all times.
- Turn the correction collar slightly and refocus. Does it look sharper?
- Turn the correction collar again slightly and refocus.
- Repeat until you get a sharp, crisp image.



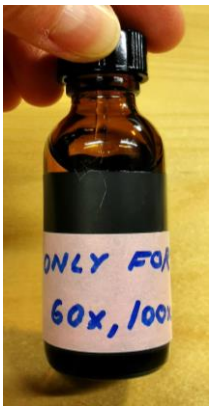


Correction collar not adjusted

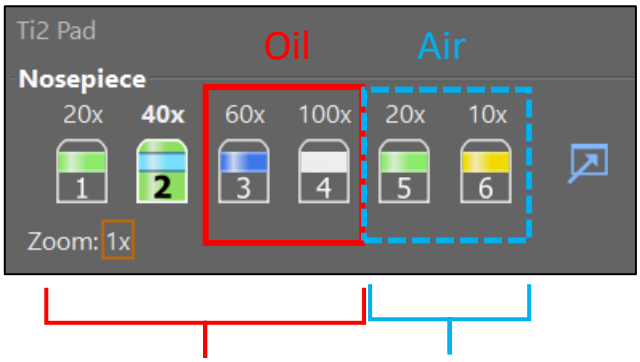


Correction collar adjusted

Objectives available on the system continues…

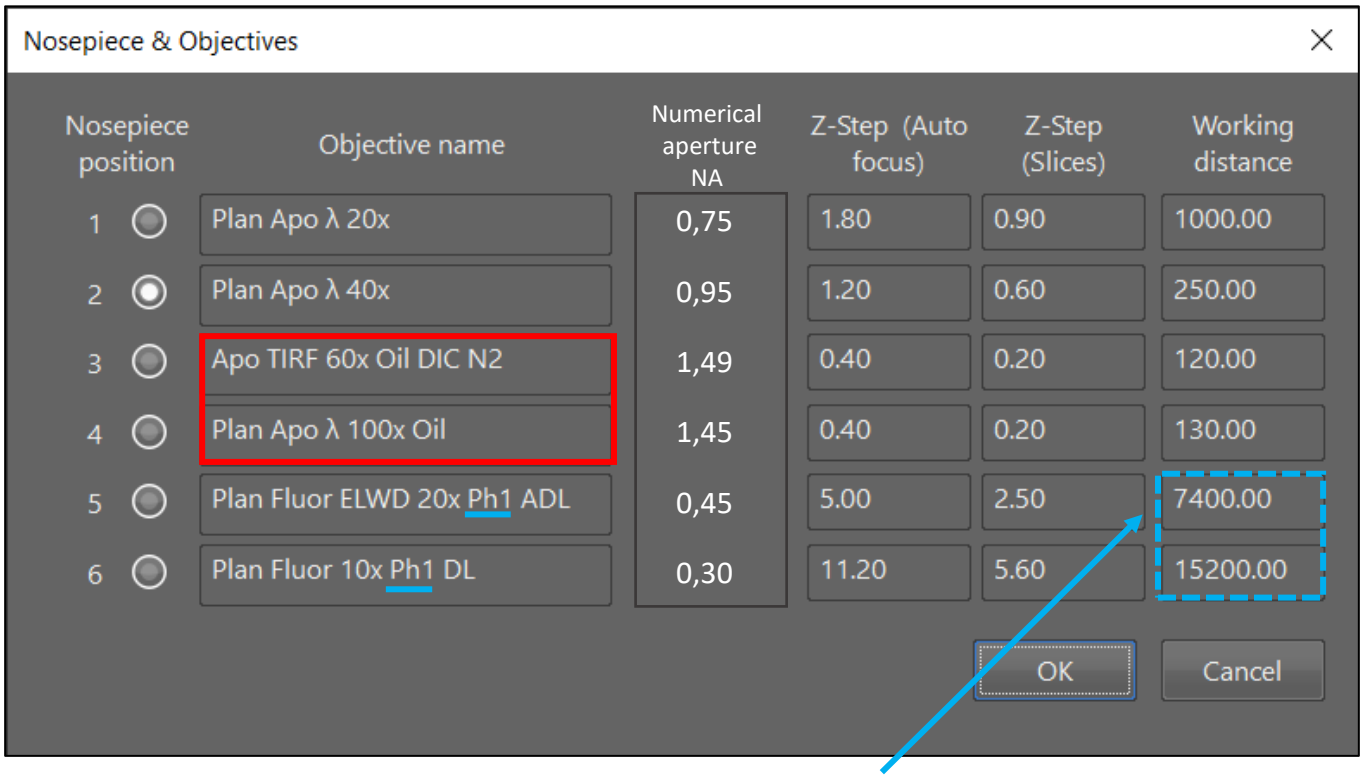


Immersion oil only for 60x and 100x



Objectives suitable for coverslip (170 µm thick)

DRY objectives suitable for coverslip as well as plastic bottom dish (cell culture, spheroids, larger samples)



Before using a high magnification objective, check confluency and fluorescence with 10/20x objective.

Remember to adjust the correction collar (20x) to match the thickness of your bottom dish or coverslip.

Phase contrast acquisition

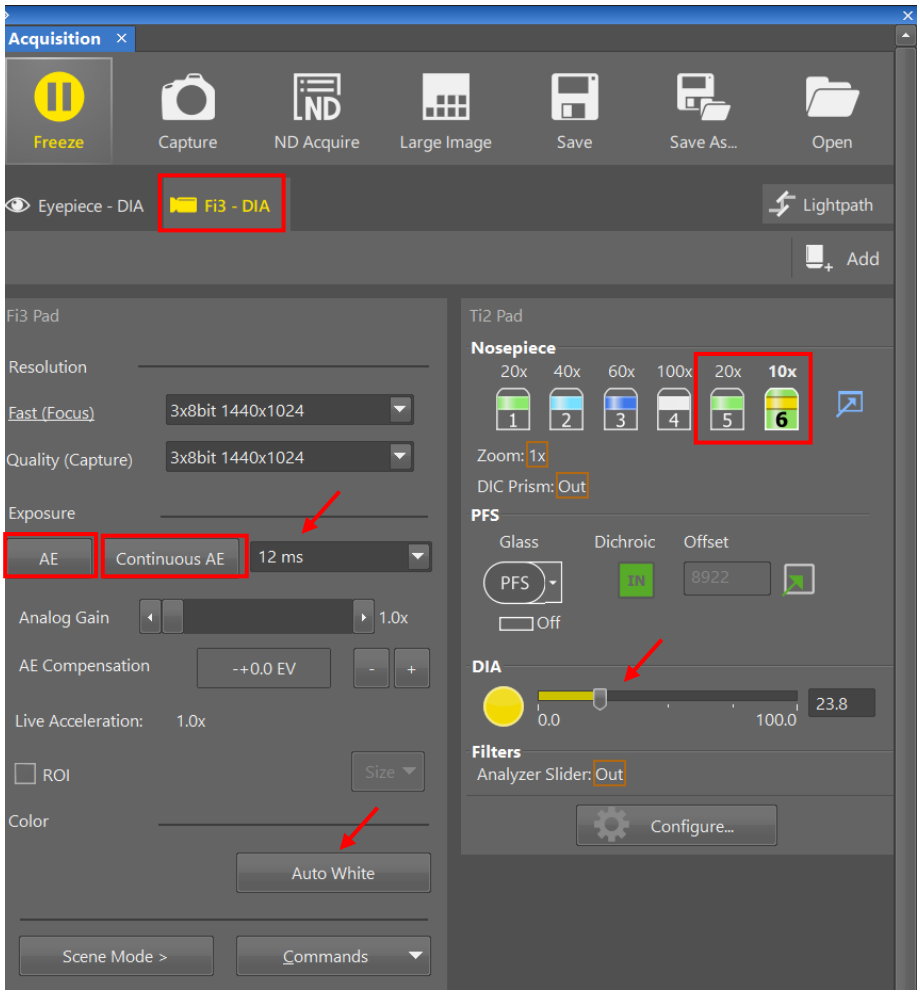
- It is important to adjust Köhler and phase contrast (see page 11) for the objective you want to use (**10x, 20x**). These two objectives are optimal for phase contrast. We also have a 40x objective (0,60 NA, Ph2) but this will only be placed on the system upon demand.

- Select the **Fi3 camera** at startup.

- Press "**+**" on the keyboard to get the live image or press the "**Freeze**" icon. Adjust exposure time and DIA lamp power to get the best result. You can also use the AE (auto exposure) or leave the continuous autofocus on.

- Move outside your sample to adjust for the white balance (**auto White**).

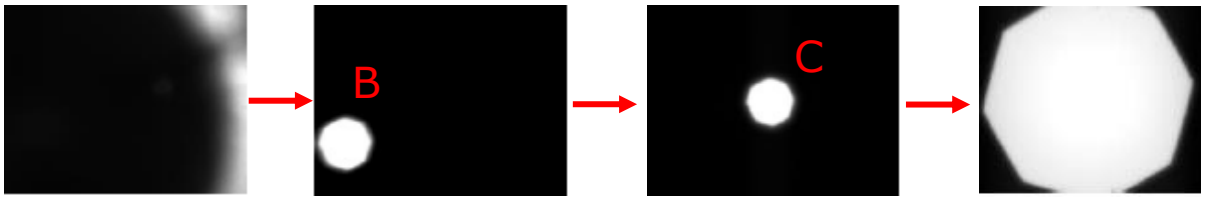
- Acquire the images by pressing "**capture**" at the top.



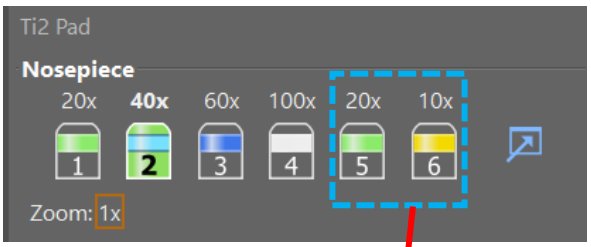
Köhler adjustment and fase contrast

Always adjust Köhler after changing objective or replacing sample.

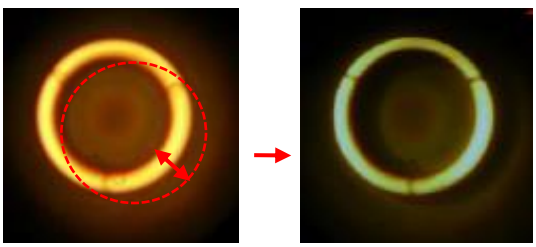
- Close the field stop diaphragm (A) in order to see the edges of the diaphragm.
- Move the condenser up and down (B) until you see a sharp image of the diaphragm.
- Center the diaphragm by using the allen key (C).
- Open the field stop diaphragm (A) until the edges lie just beyond the field of view.
- Increase depth/contrast of field by adjusting the aperture stop (arrow).



The system is equipped with a setup for phase contrast with 10x, 20x and 40x (on request). Select the correct phase matching the objectives inscription. Insert the Bertrans-lens (Bl) and use the eyepieces to align the two rings using allen keys.



Phase contrast lens

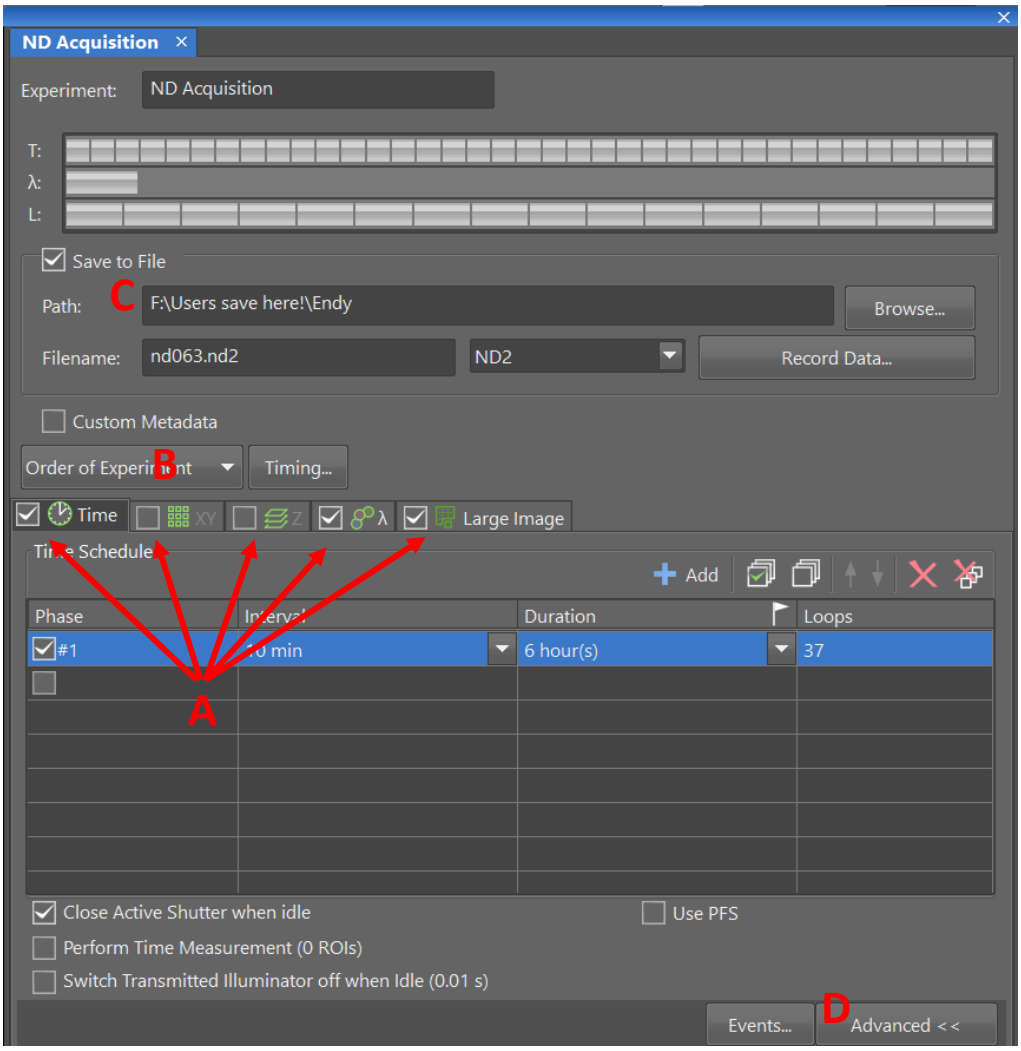


Automated ND acquisitions

Using the ND acquisition, you can set up experiments that include:

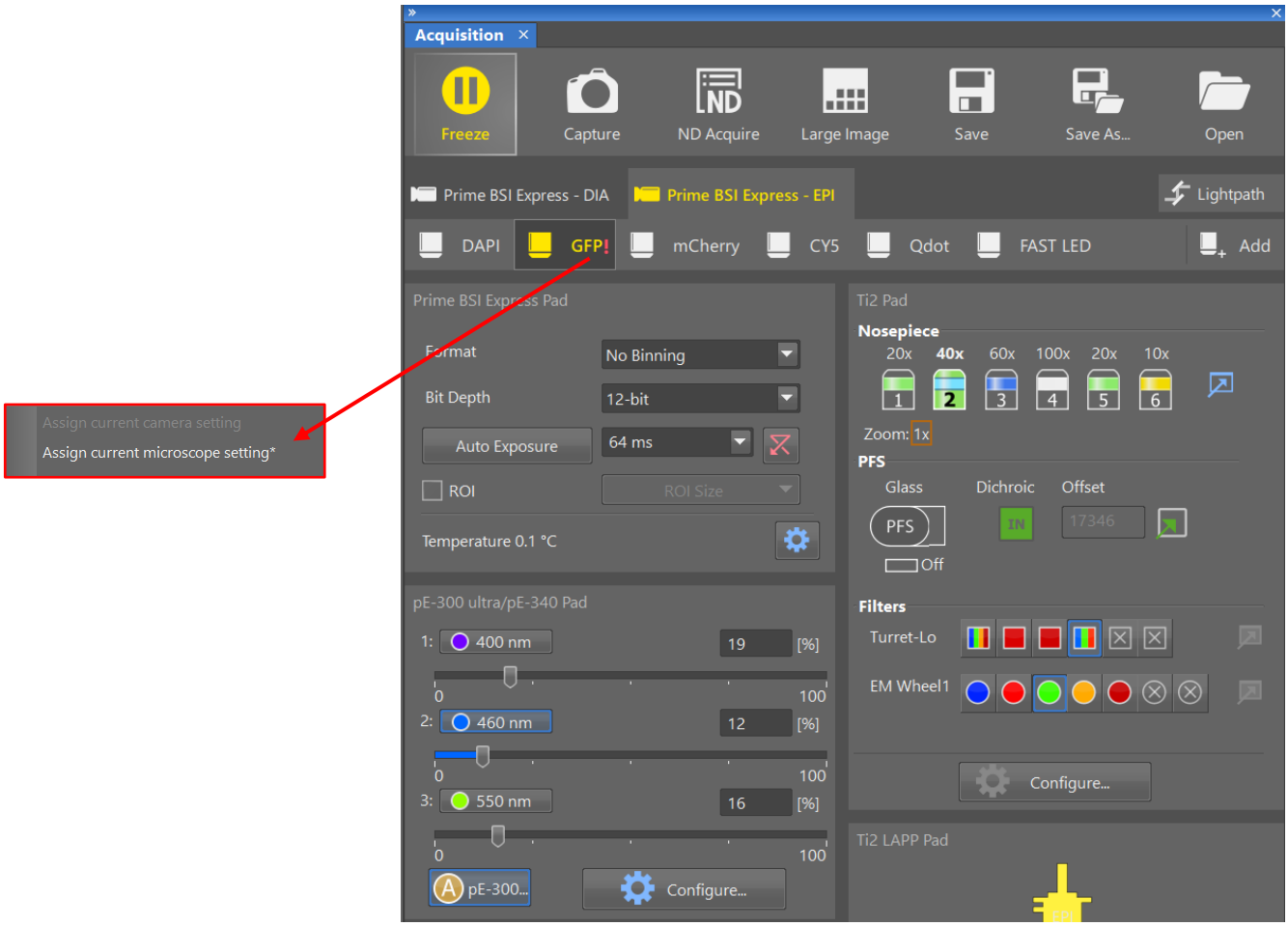
Timelapse, Multipoint, z-series, Multichannel, Large image

- Open the ND acquisition window (icon on the top menu).
- Check the module(s) you need (A) and define each as described on the following pages. Define the order of the experiment (B). Define also the save path (C), preferably Biomic.
- If you need to define autofocus, open "advanced" (D).
- When you are ready, start the experiment by clicking on "Run now".
- It is recommended to heat up the system 0,5-1 hour before starting timelapse.



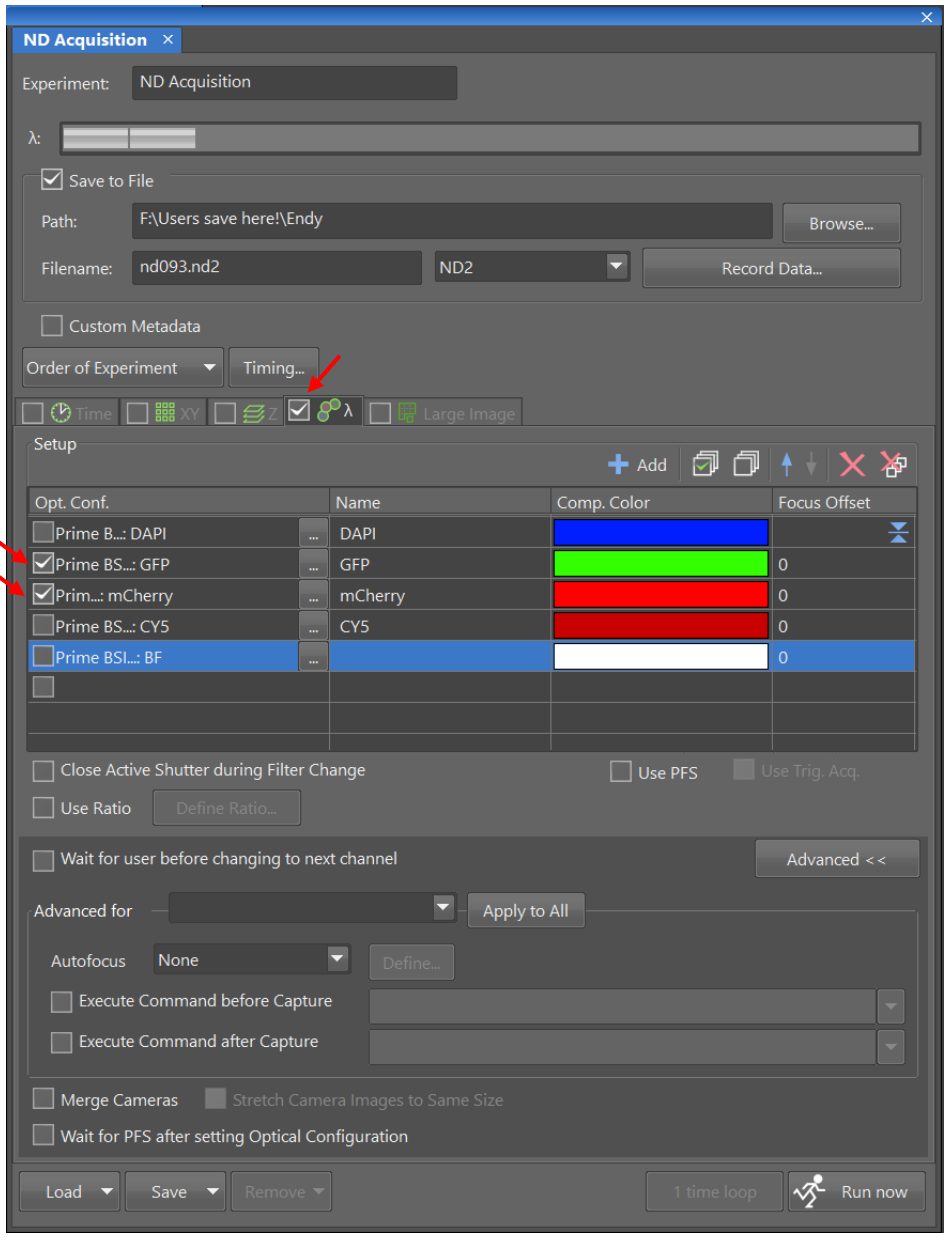
Multichannel acquisition

- Check the fluorescence on your sample using the oculars. Check each fluorescent channel in live view and set the exposure time and coolLED intensity for each channel. Changes with exposure time will automatically be saved. If you see the red exclamation mark "!" behind the name of the channel, it means that you have change the LED setting. To save this new intensity, right click on the "!" and "assign current microscope setting".
- To set up an automatic acquisition, see next page.



Multichannel acquisition continues…

- Open the ND acquisition window (icon on the top menu).
- Check the lambda window and tick the optical configurations you need.

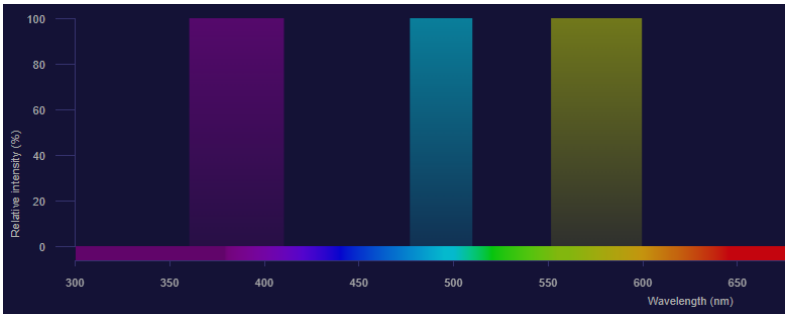


- Use the "M" in the left toolbar to start capturing with the option to focus manually. Click "continue" to record the image.

- Use the "A" to automatically capture all the channels. You might want to define "autofocus" in the ND acquisition.

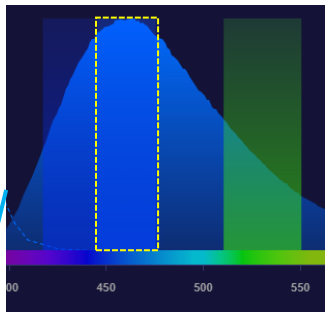
Fluorescence filter setup

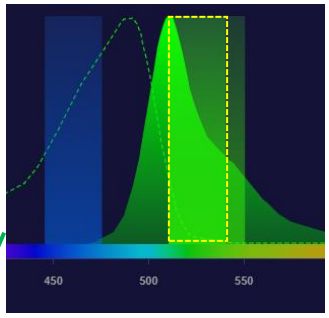
The system comes with two filter turrets and by combining them we get the following setup:

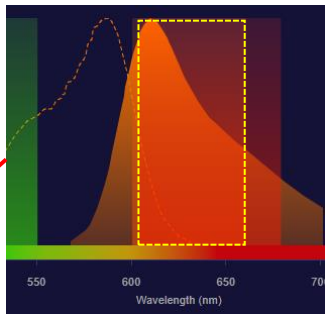


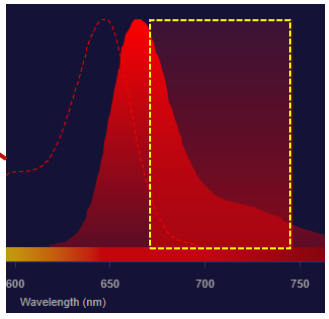
Multi **exitation** filter for coolLED is used for the following filters: dapi, GFP and mCherry.

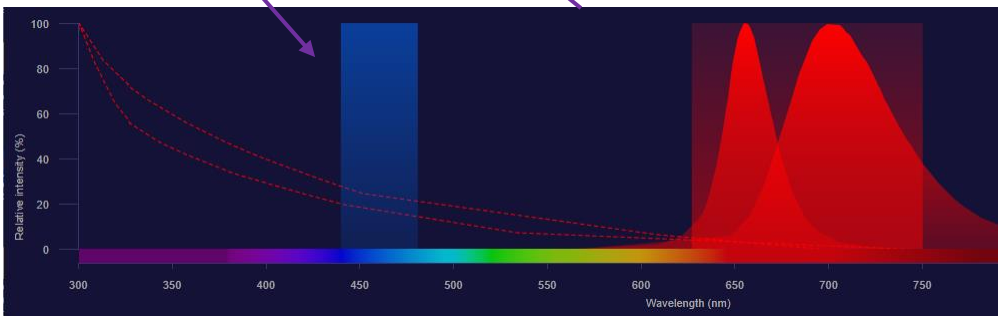
Filter name	Excitation	"Real" emission
Dapi	360 – 365 nm	445 – 475 nm
GFP	477 – 510 nm	510 – 540 nm
mCherry	551 – 599 nm	604 – 660 nm
CY5	590 – 650 nm	670 – 738 nm
Qdot (655 and 705)	440 - 482 nm	626 – 749 nm





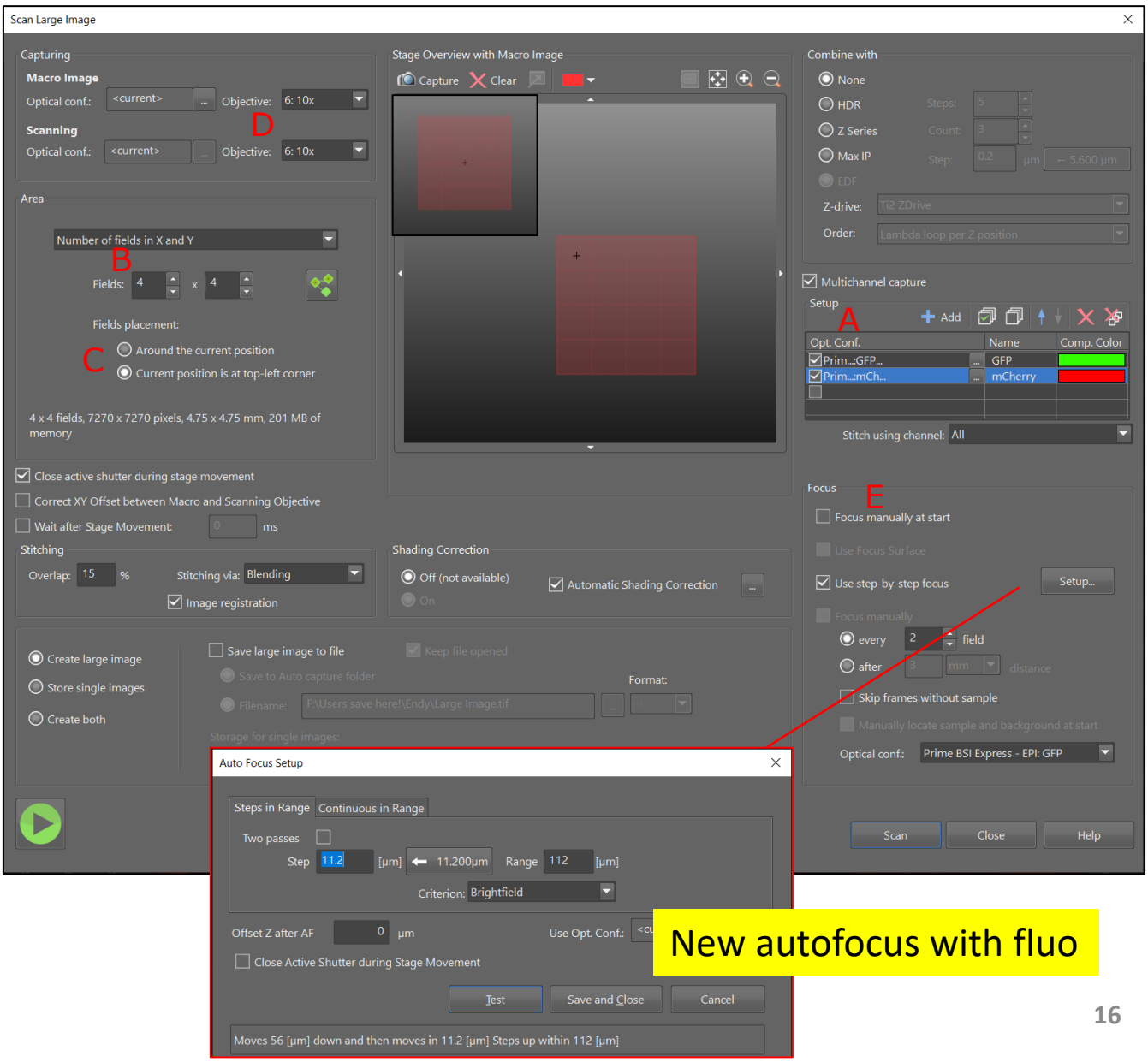






Large image acquisition

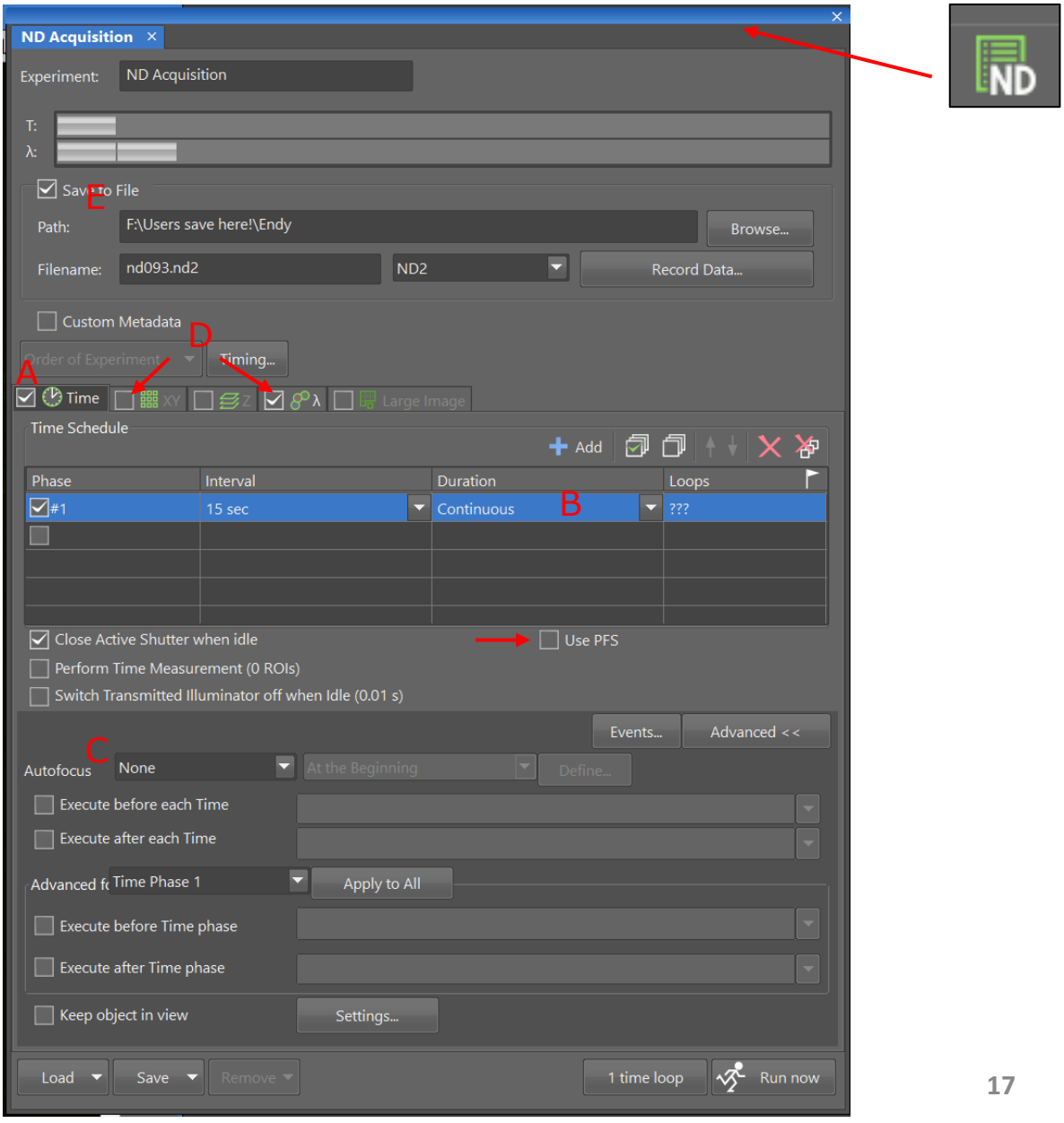
- Before you start an automatic large image acquisition, make sure the exposure time and LED intensity is correct for all the channels you want to acquire (see page 13).
- Find "**scan large image**" under the "**acquire**" menu (this has more options than through the ND acquisition mode. If you need multichannel capture, define your channels (A).
- Define the size of the field (B) and placement of start position (C).
- Choose which objective you want to use (D).
- Define how you want to focus (E).



New autofocus with fluo

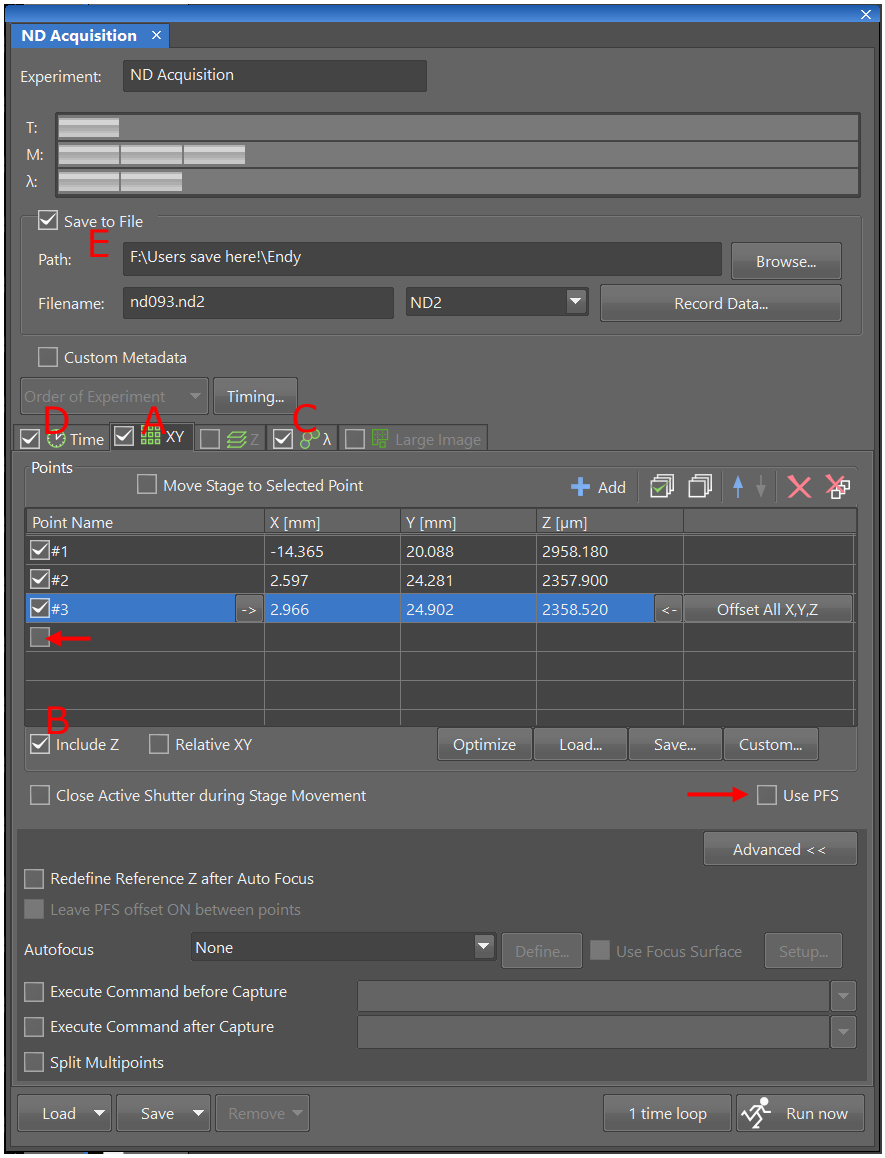
Timelapse acquisition

- Open the ND acquisition window (icon on the top menu).
- Check the "**Time**" flag (A) and define "**intervals**" at which the images should be taken. Define the "**duration**" (B) of the experiment.
- For long term experiments, use **PFA** (perfect focus system) or **autofocus** (remember to start up the heating at least 0,5-1 hour before imaging, se page 7).
- If you want to image multichannels or/and multipositions, remember to check the flag and set up these too (D).
- You can define where the data is going to be saved (E), preferably Biomic.



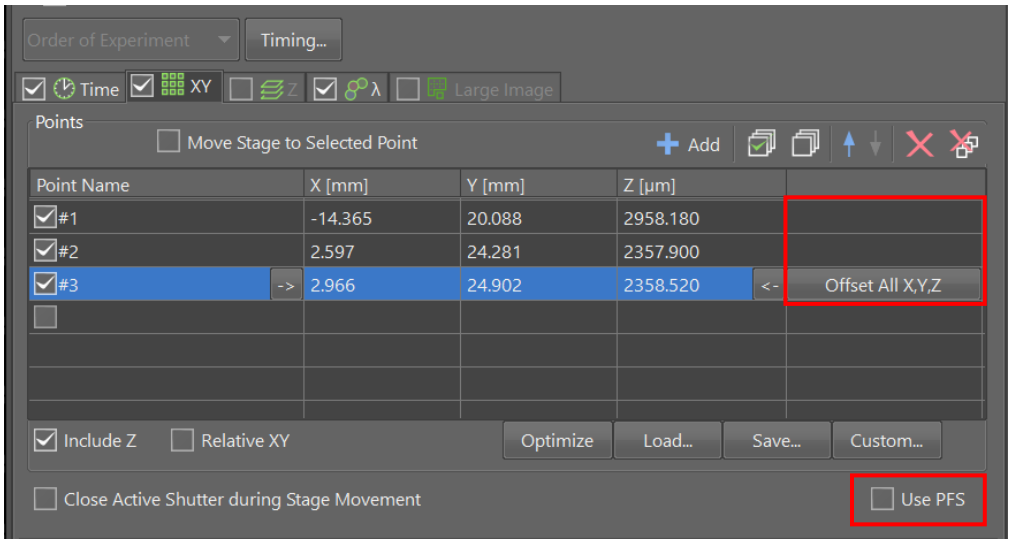
Multi-point acquisition

- Open the ND acquisition window (icon on the top menu).
- Check the "**multipoint**" flag under ND acquisition (A).
- Define your multipoint positions in the XY window with a tick into an empty square (arrow). Remember to check "**include Z**" (B) if your positions are far apart.
- Define your channels (C) and interval (D) of your timelapse.
- For long term experiments, use **PFA** (perfect focus system) or **autofocus**.
- You can define where the data is going to be saved (E), preferably Biomic.
- Remember to start the heating 0,5-1 hour before imaging (see page 7).



How to use autofocus (PFS)

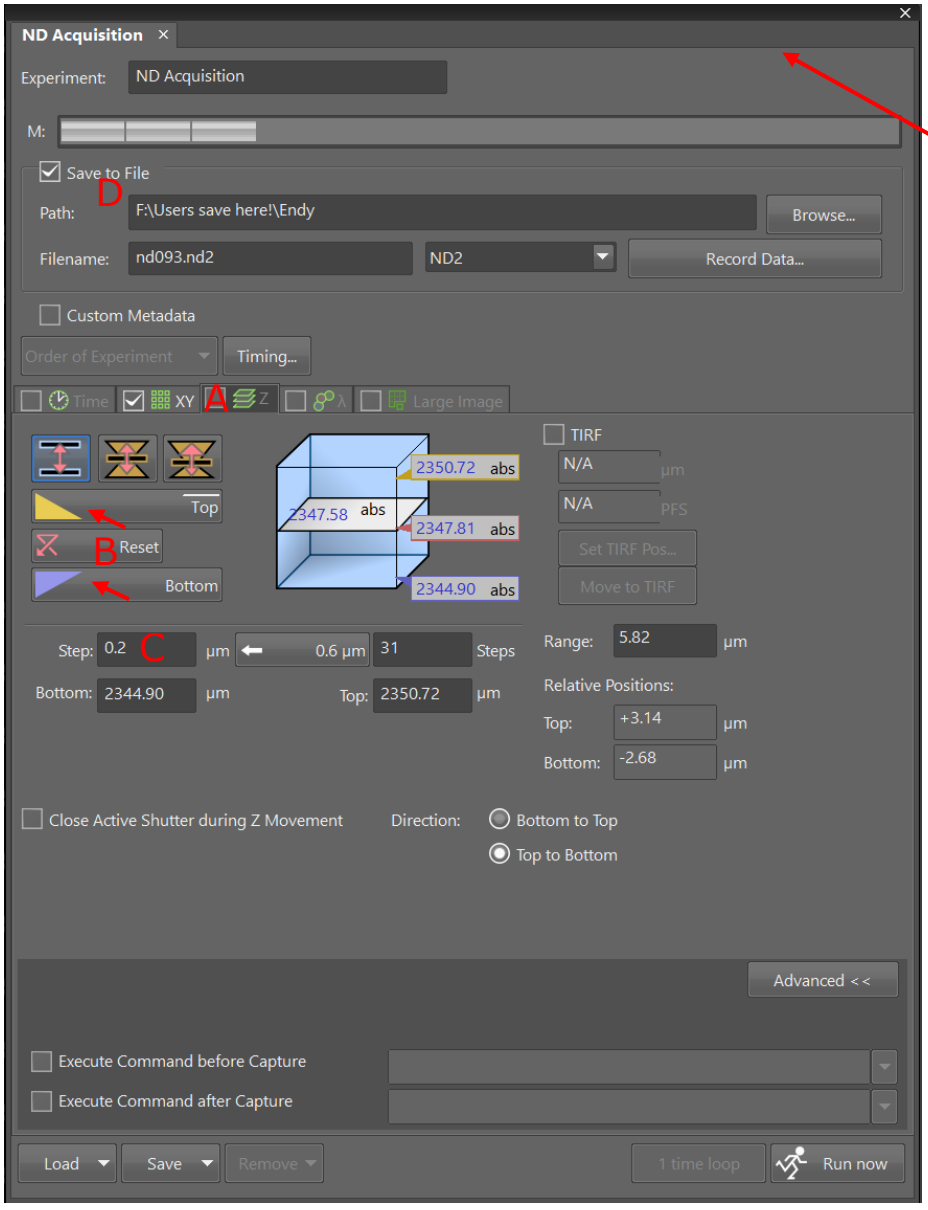
- Open ND acquisition.
- Check “Use PFS”. You should now hear a different noise when changing the z position.
- If you are registering multipoint, make sure the offset comes up in the table.



Z-acquisition

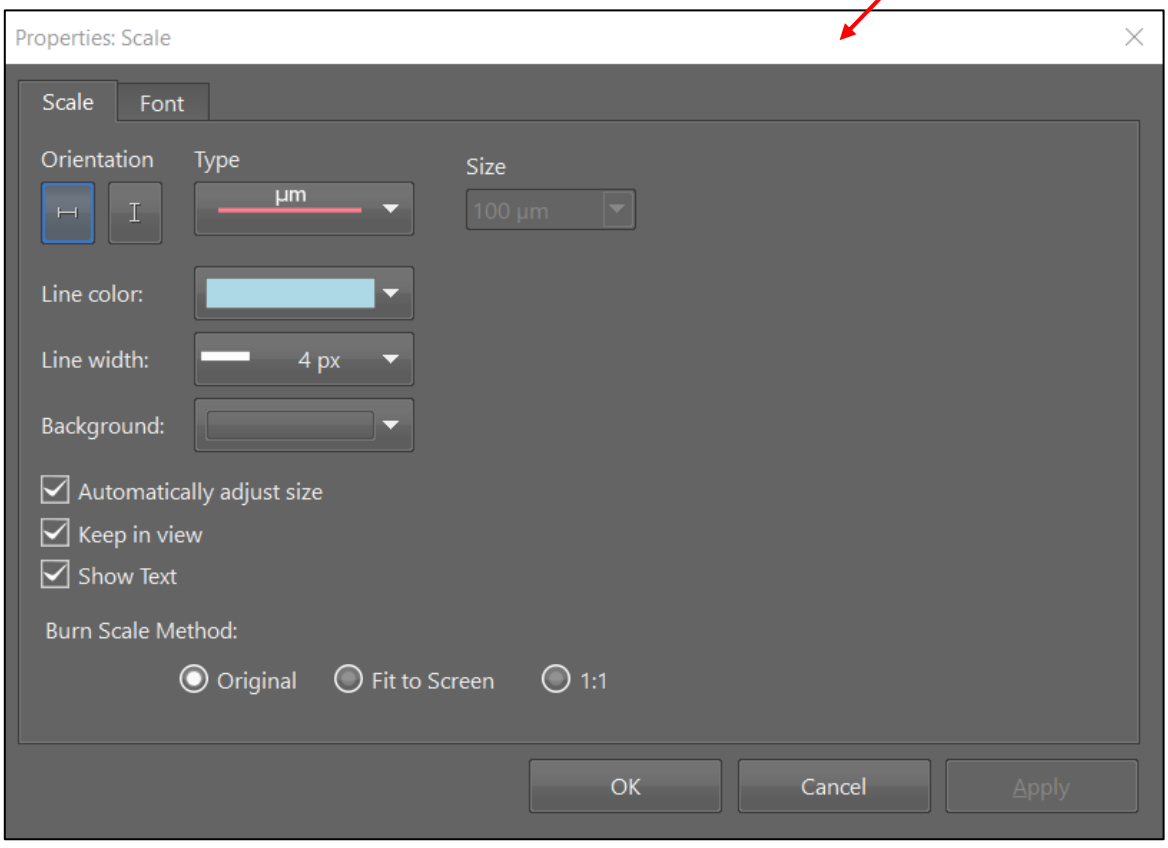
This is not frequently used as it requires an additional module in NIS-Element to handle these images.

- Open the ND acquisition window (icon on the top menu).
- Check the "**Z**" flag under ND acquisition (A).
- Move through your sample in z and define the "**top**" and "**bottom**".
- Define the step size (C) or leave it as default (according to Nyquist sampling).
- You can define where the data is going to be saved (D), preferably Biomic.



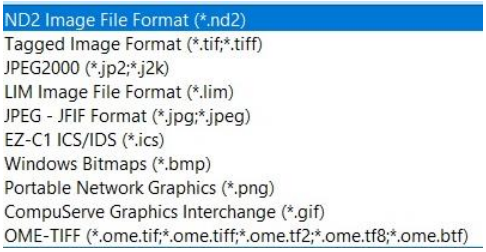
Adding a scale bar

- The images you acquire on the system will have information about the pixel size incorporated into the metadata due to the objective calibration. You can therefore add a scale bar later and in any other software.
- If you need a scale bar burned (it's the only way) into your image, click on the scale bar icon on the left tool bar menu.
- A new window will open where you can customize the design.

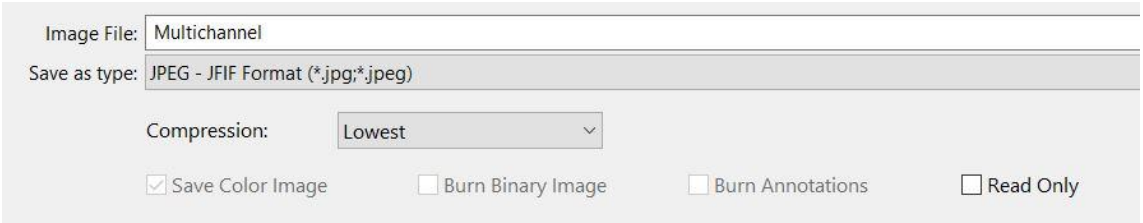


How do I save my data

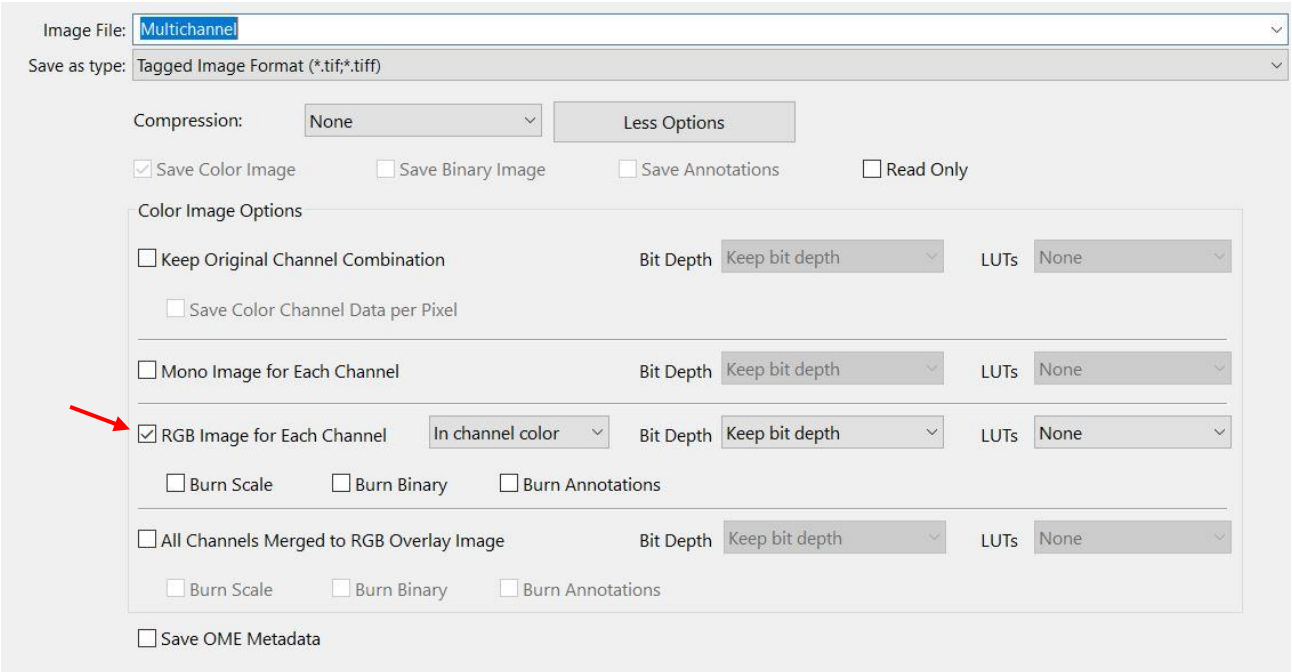
Nd2 format are the raw format from Nikon. You can open these in most software, but you will not be able to visualize these in power point, word etc.



JPG format will remember the changes you did in the LUT, but JPG images have reduced size because the image has gone through a lossy compression (some data from the original image file is eliminated). The human eye will not see the difference. Choose lowest compression.



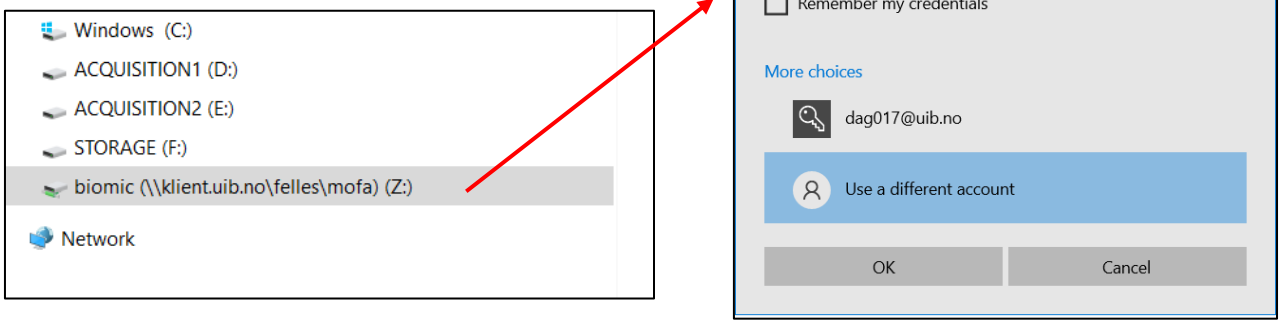
Tif/tiff format is a standard in printing and publishing industry. Tif format will have a bit depth and will use lossless compression. To save each channel separately, check “RGB



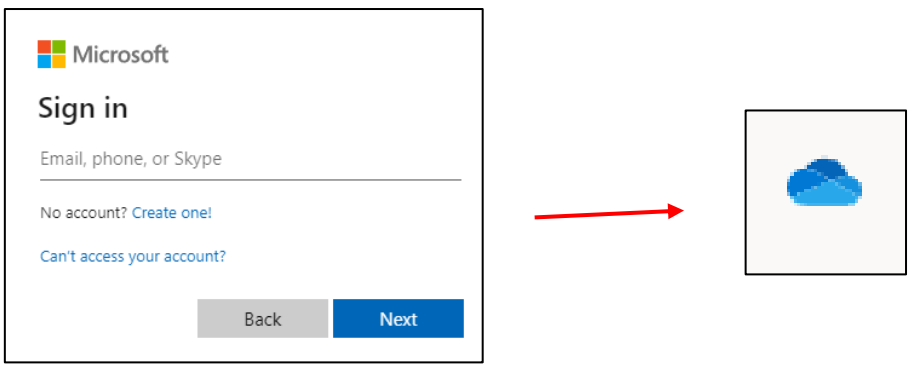
Where should I save my data

We prefer that you **do not** use USB keys and external hard drive on the system. The pc is on the net, so there are a few options.

- Save onto Biomic. (\\klient.uib.no\felles\mofa\biomic). Ask MIC personnel if you would like access.



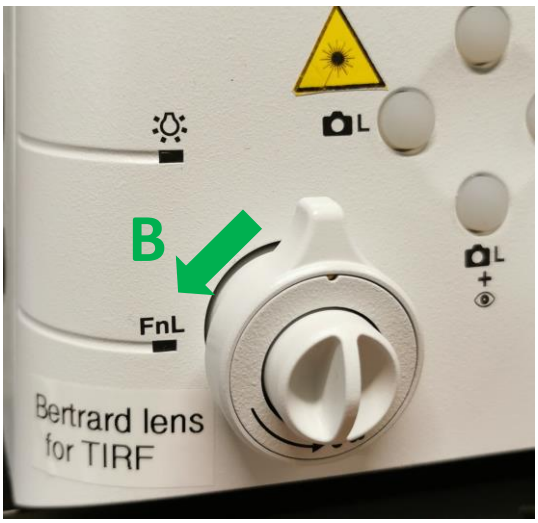
- Save on to your cloud/one drive. Go to any browser and search for "office 365" and sign in using your email. In the left menu you will see the cloud icon. Here you have access to 1 TB.



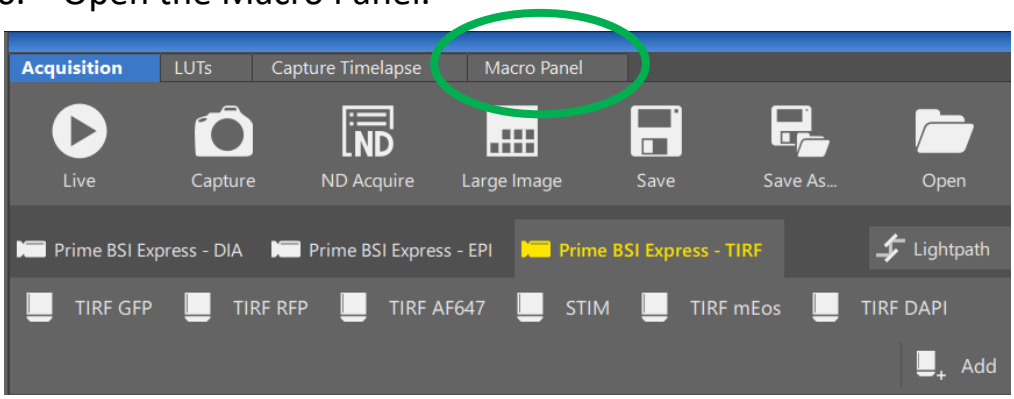
- Save to "users save here" on the hard disk for a limited time. This data must be transferred and deleted asap.

TIRF alignment

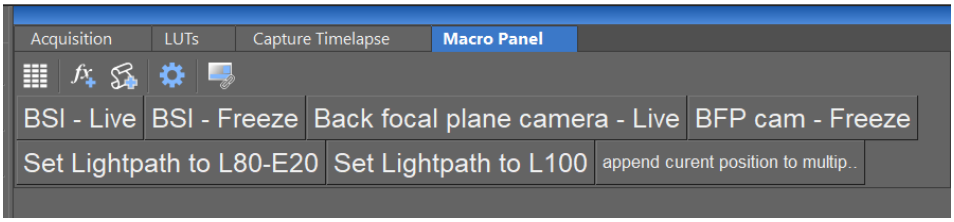
1. Open NIS elements in TIRF mode.
2. Choose the 60x TIRF objective (oil).
3. Find focus in your sample.
4. Add the black lid on top of the stage (laser safety protection). (the box on top of the FRAP-ctrl box should have a green light when software is opened).
5. On the microscope; turn from eye to camera (A) and turn 90^0 left to Bertrand lens (B).



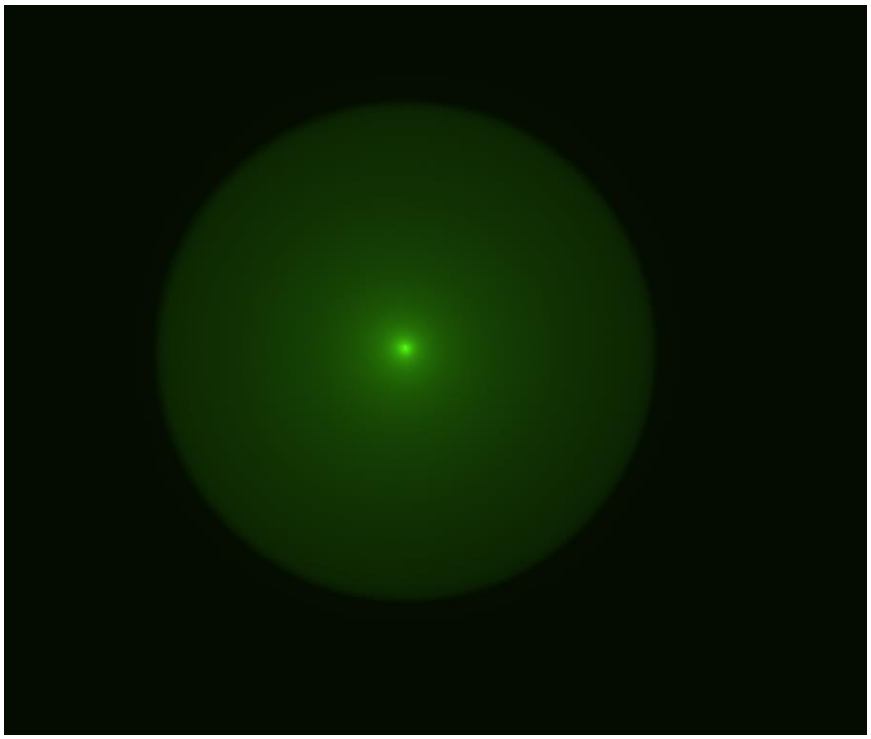
6. Open the Macro Panel.



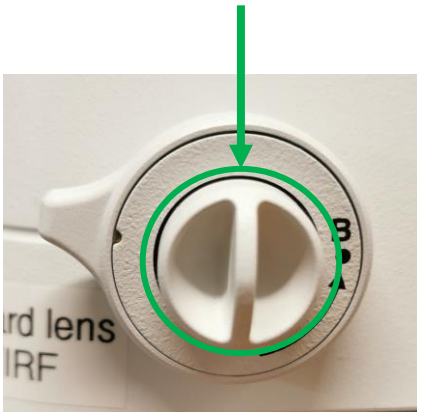
7. Click on Set Lightpath to and L80-E20 and Back focal plane camera – live.

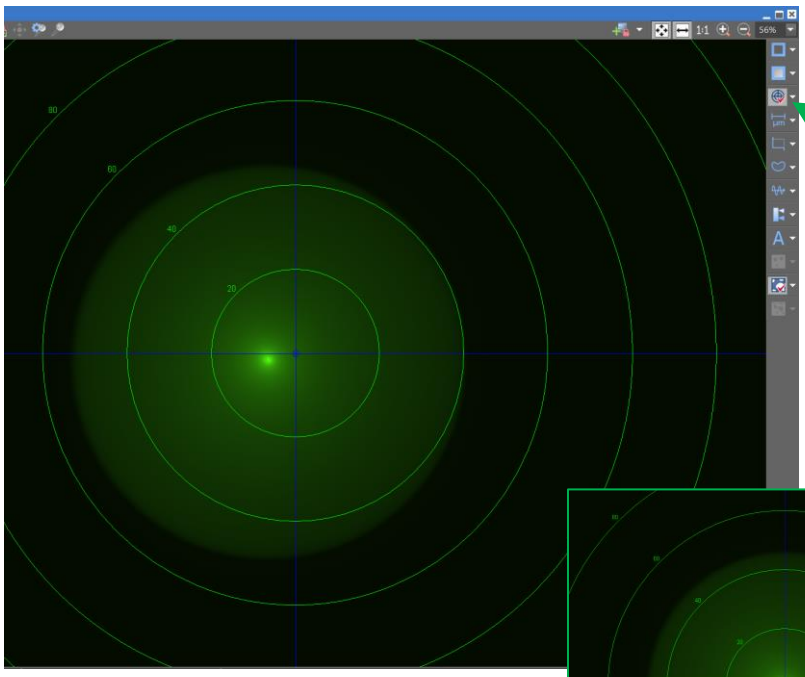


The image in the screen appears something like this.
(1 % laser power is most likely enough)

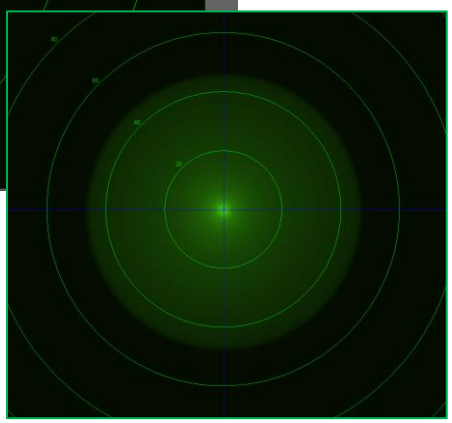


8. Focus the bright spot in the image by turning this nob



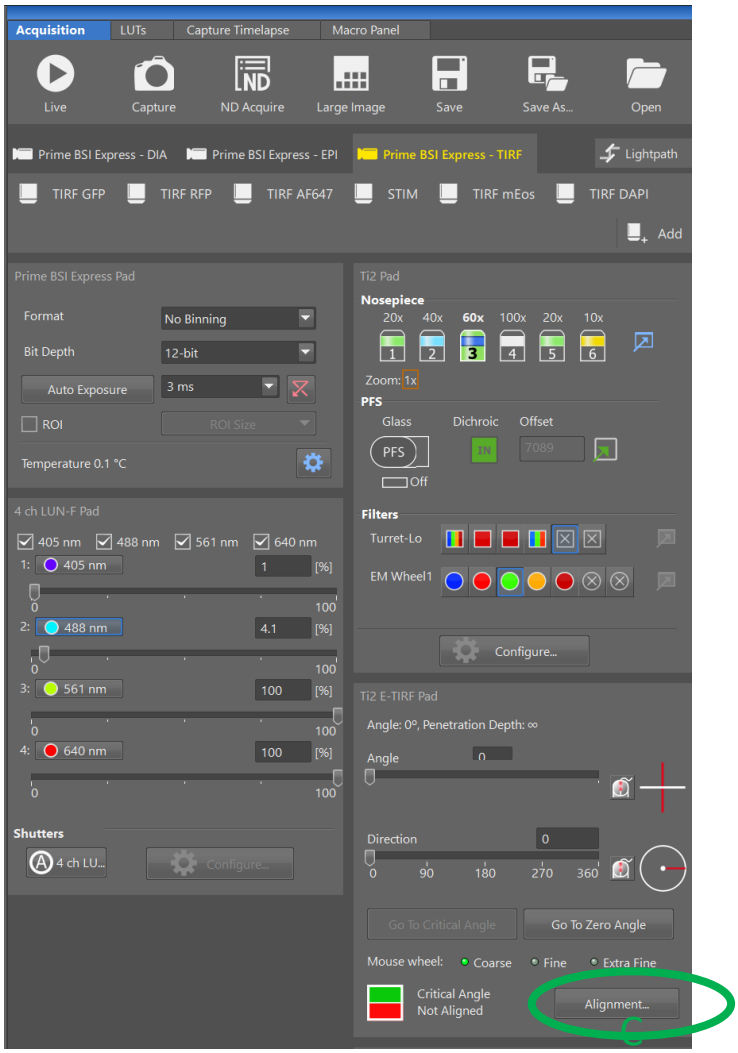


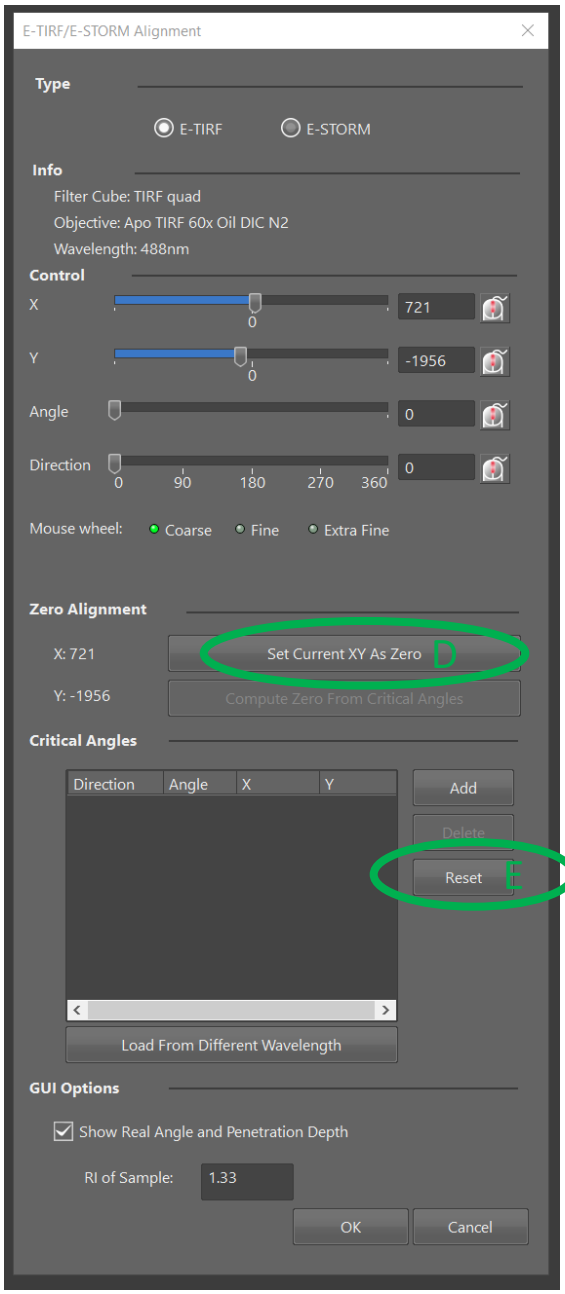
9. Activate this and move the center of the rings over the bright spot.



10. Click Alignment (C) and Set current XY to Zero (D).

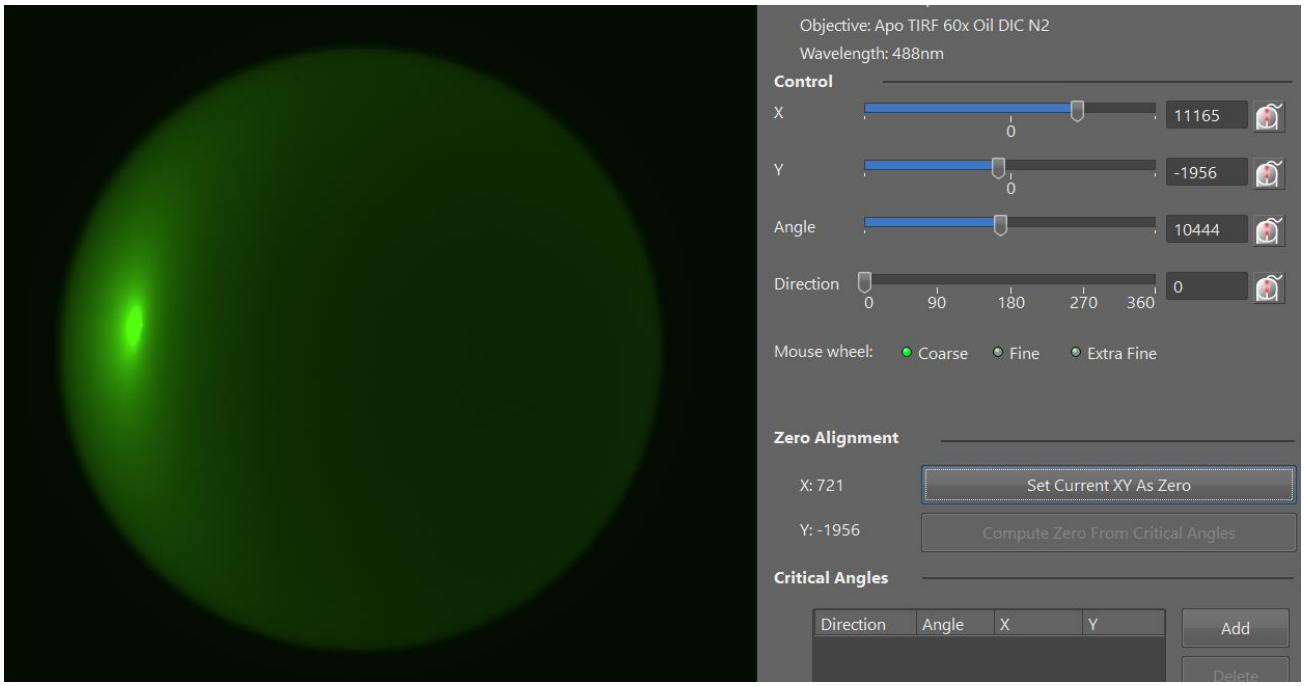
11. If there are any pre-stored coordinates under Critical Angels, press Reset (E).

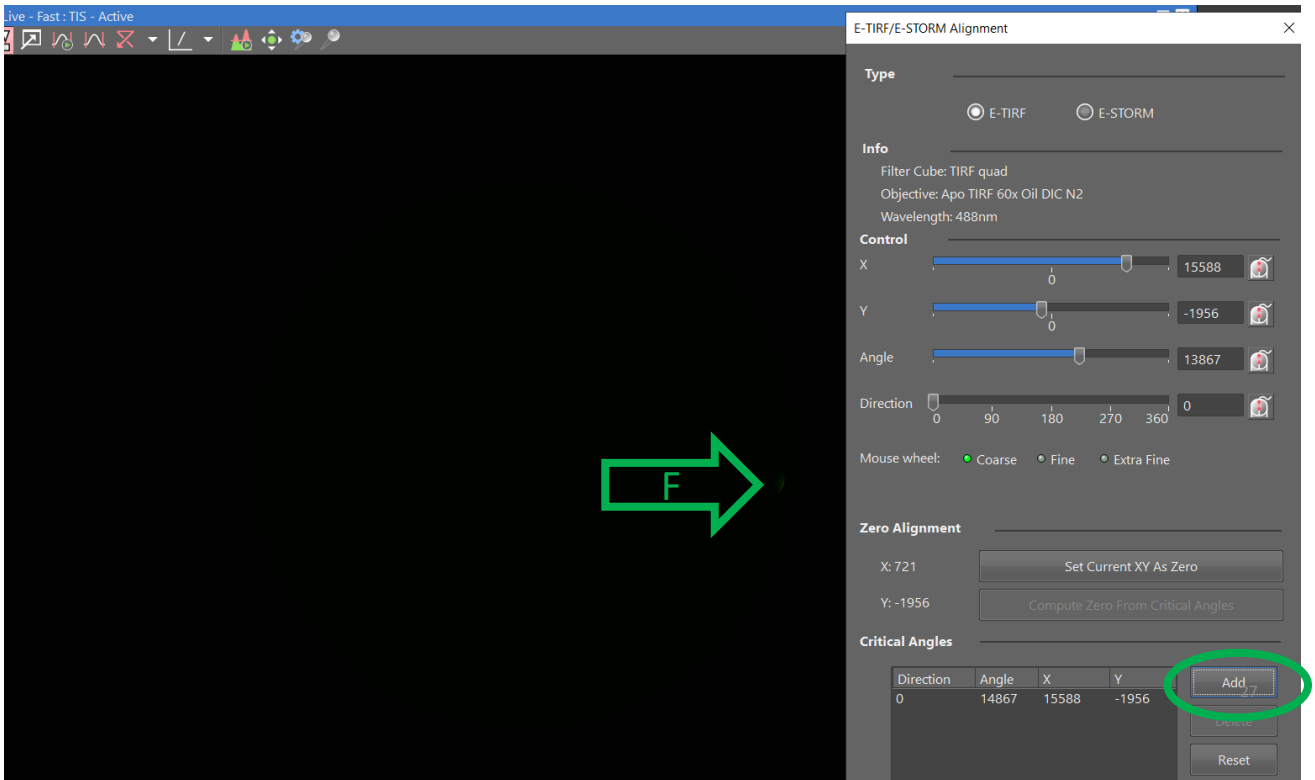




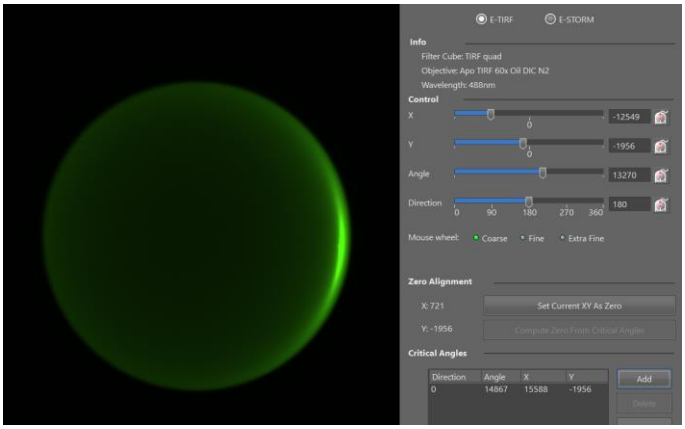
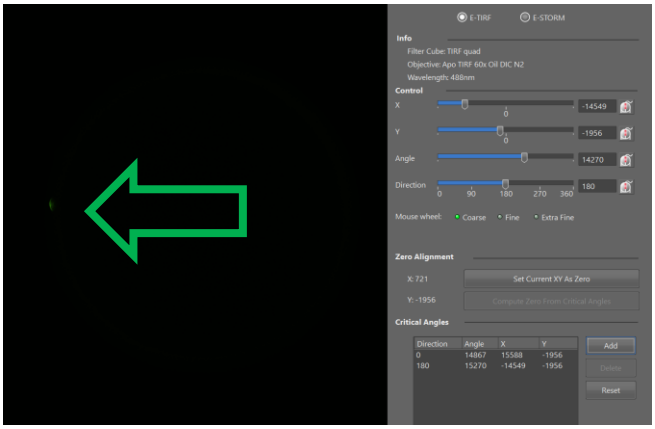
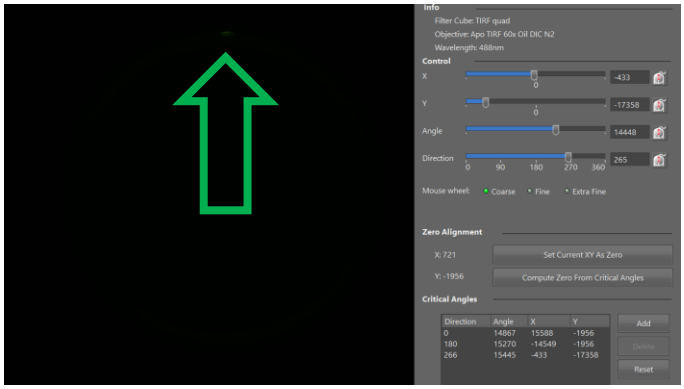
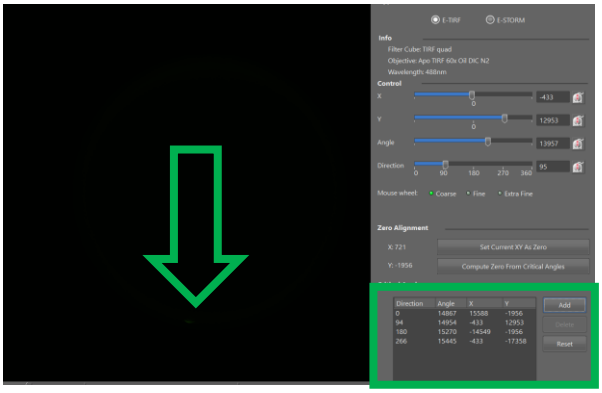
12.

- Start adjusting by moving the X-slider in one direction.
- The bright spot moves accordingly.
- Move it until the bright spot is at the edge and just disappears (put the mouse wheel to “fine” for more precision at the edge).
- At the same time a tiny signal appears on the opposite side of the circle (F).
- Then you stop moving the slider and ADD the coordinates under Critical Angels.

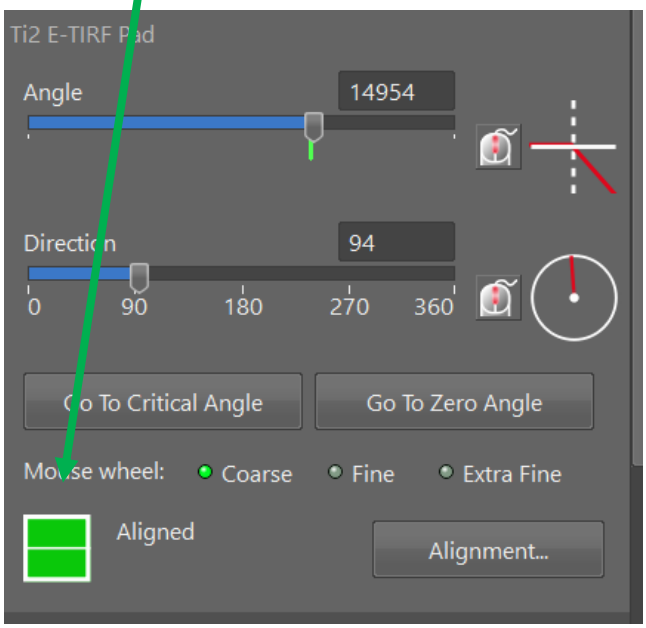




13. Repeat this in the other three directions (opposite X, Y up and down), and ADD coordinates for each direction.
14. Click OK at the bottom of the Alignment window and the Critical Angels are saved.

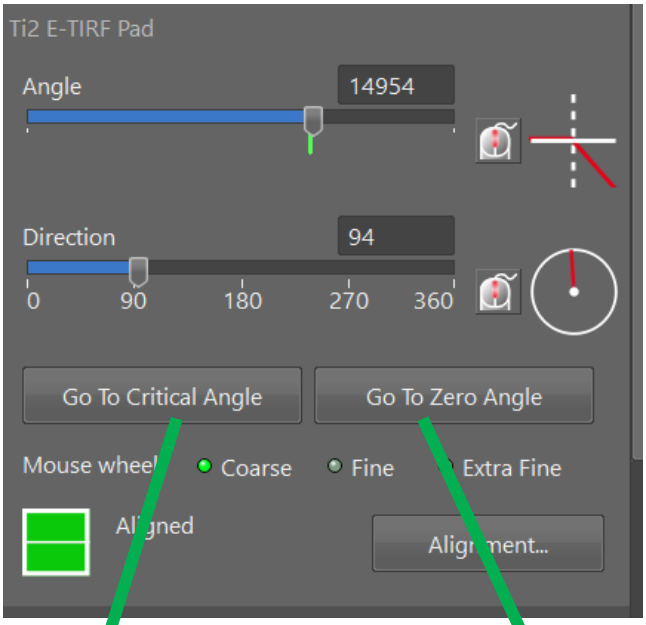
The panel now lights up in green and shows that the TIRF is aligned.

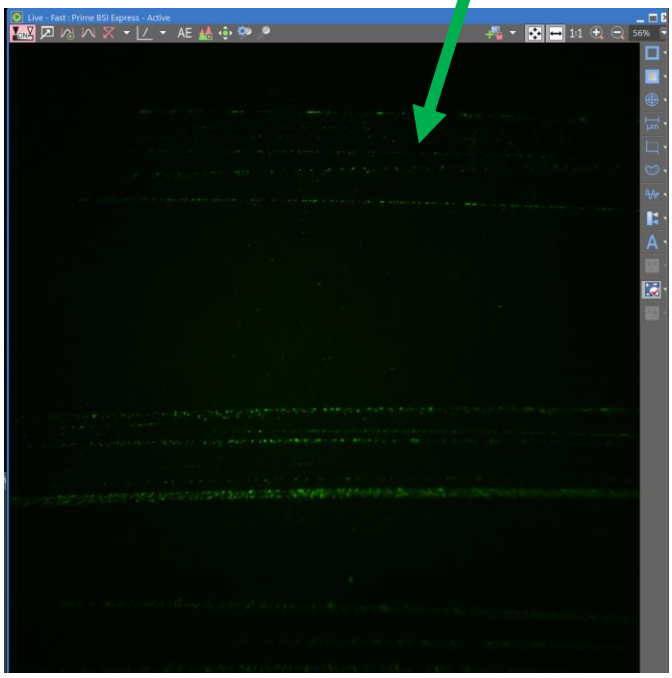


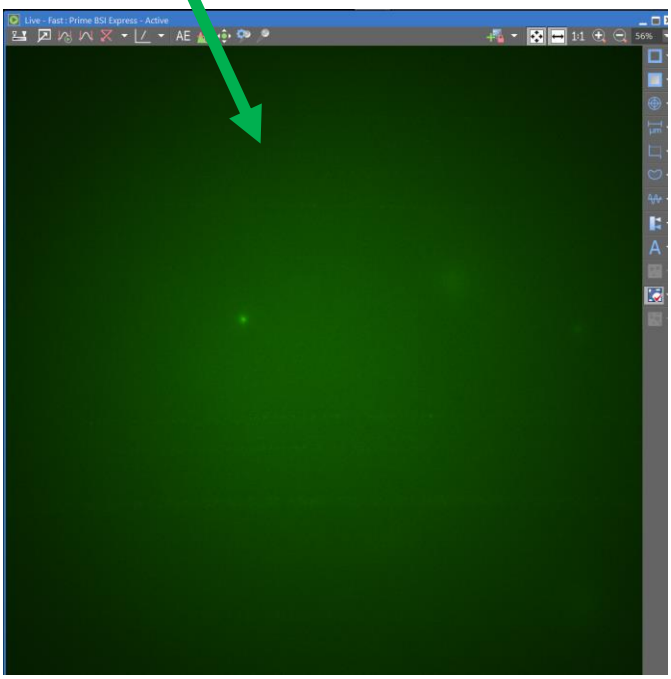
15. On the microscope; turn back the knobs to the starting positions (eye & 90^0 upwards).

16. In the software; in Macro Panel deactivate Back Focal Camera and set Light path to L100.

You’re ready to TIRF







No TIRF

TIRF

REMEMBER:
Imaging parameters (laser, exposure time, adjust display parameters etc.) must be optimized as you do for regular fluorescence imaging.