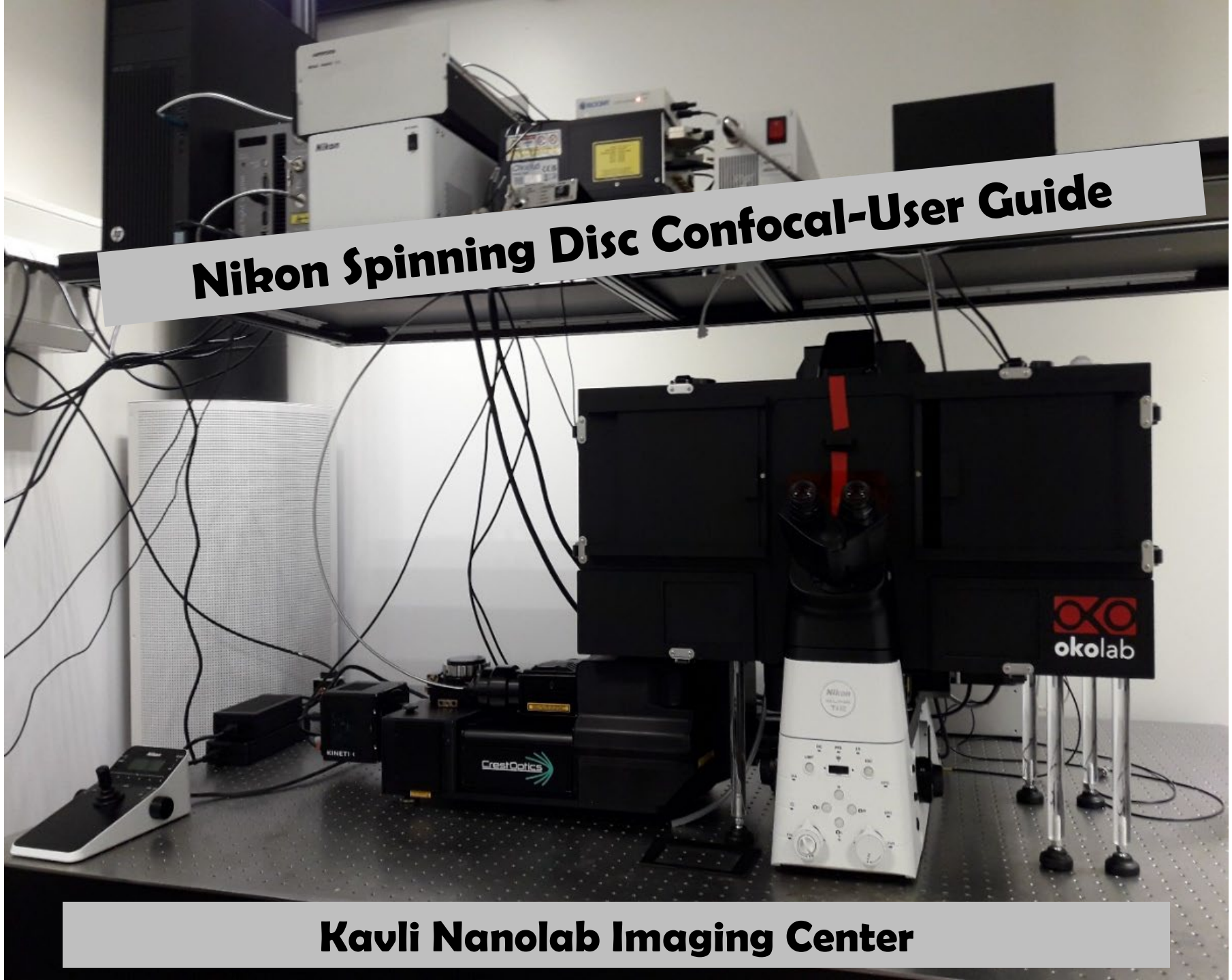
Nikon Spinning Disc Confocal-User Guide

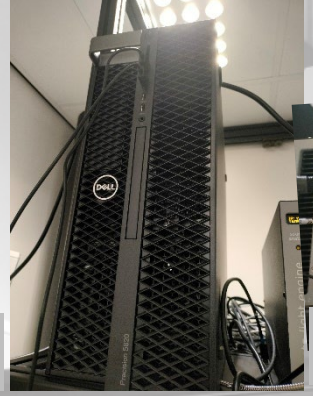


Kavli Nanolab Imaging Center

Turn ON- Hardware

1. main switch

6. PC

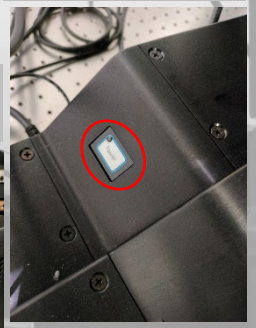


2. Nikon Controller

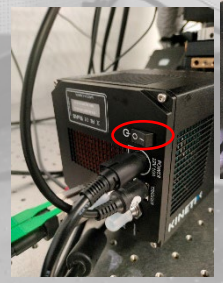
5. FRAP Ctrl

On table, behind

4. SD unit



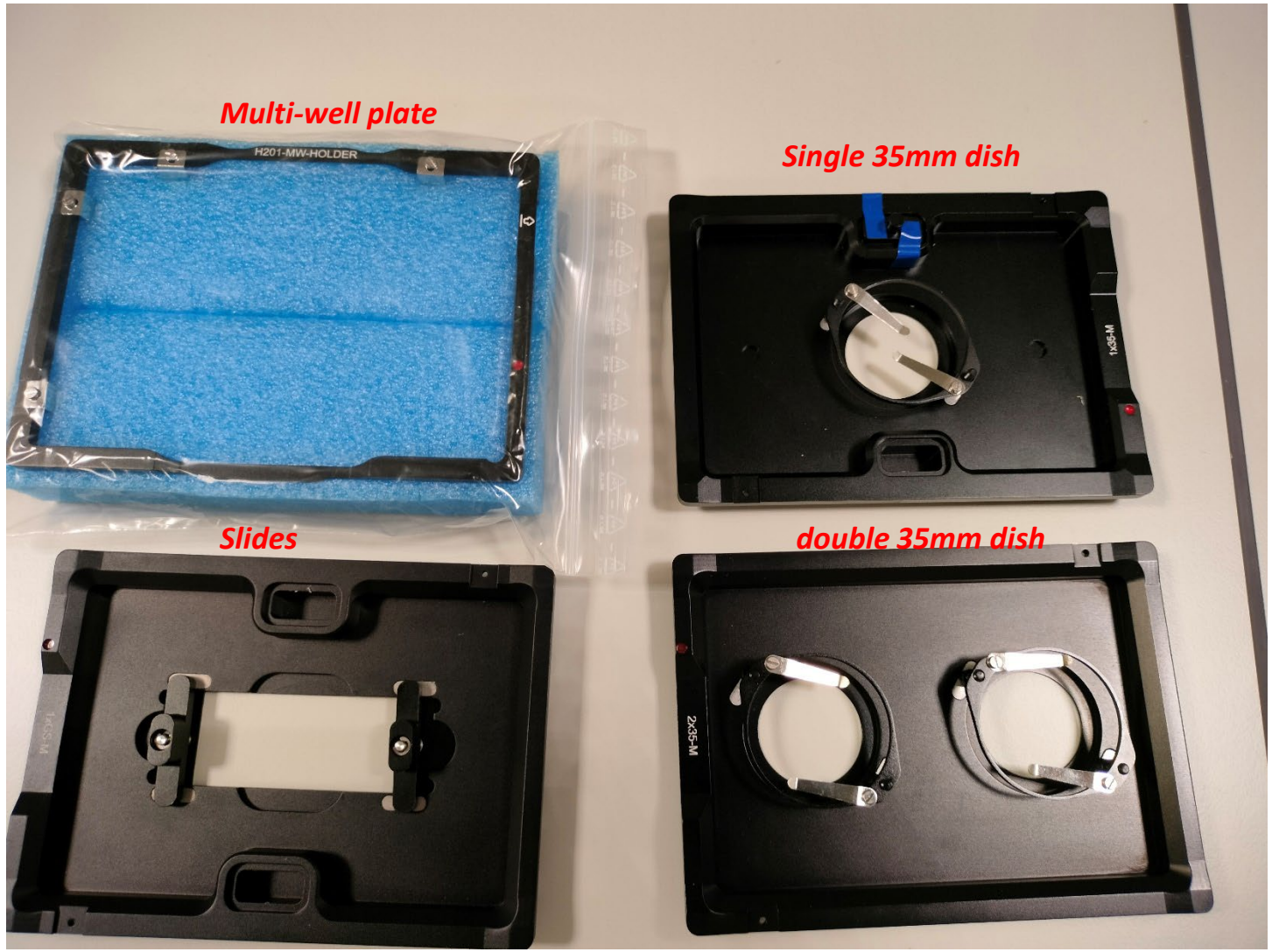
3. Camera

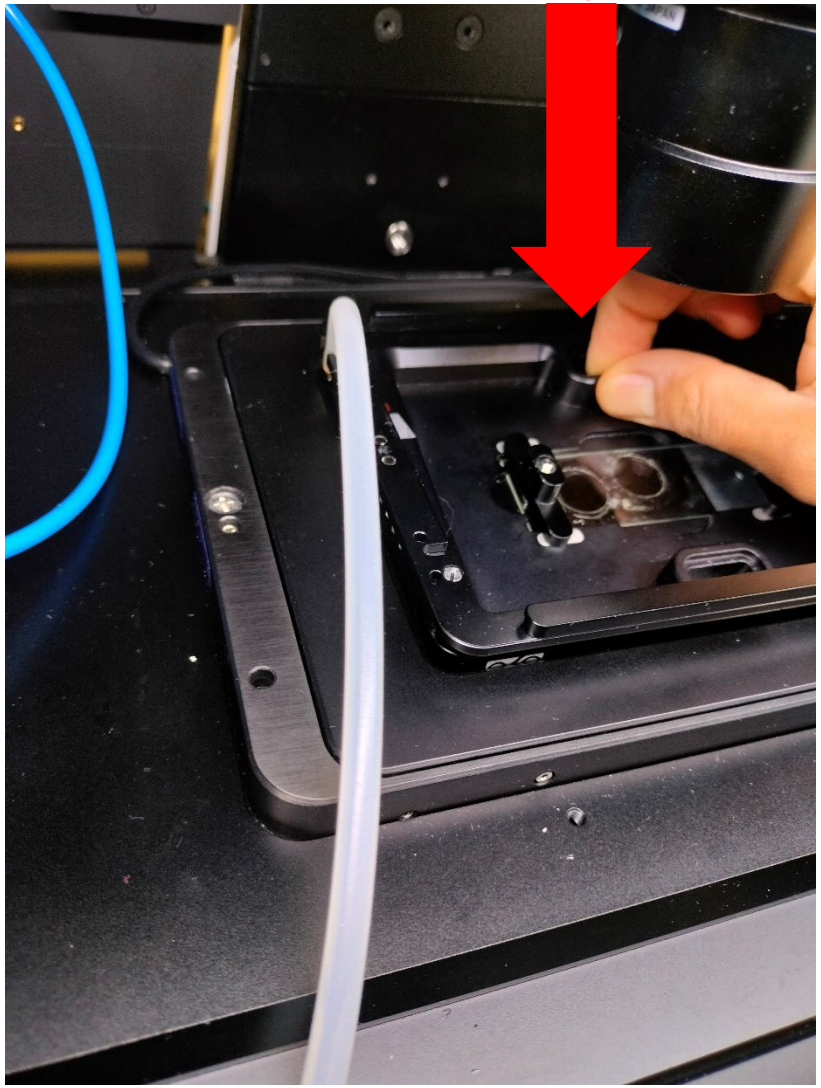


Don't forget to turn off the chamber light

! Make sure the objective is far away (<500) before changing holders

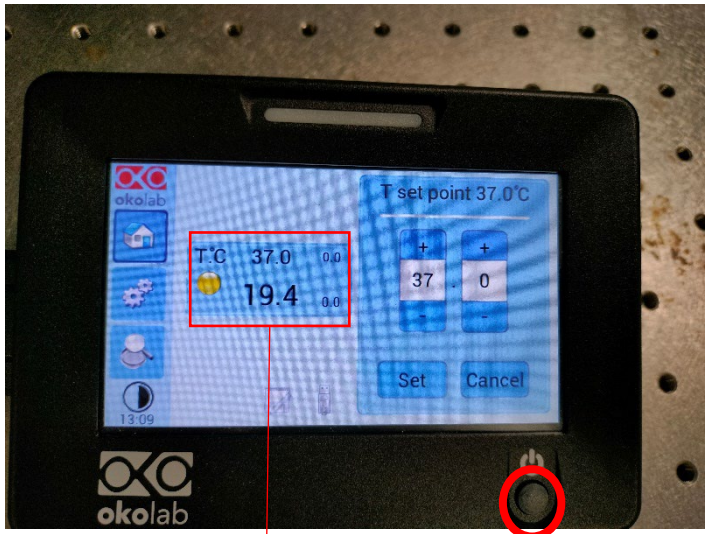
Available stage holders





Environmental control

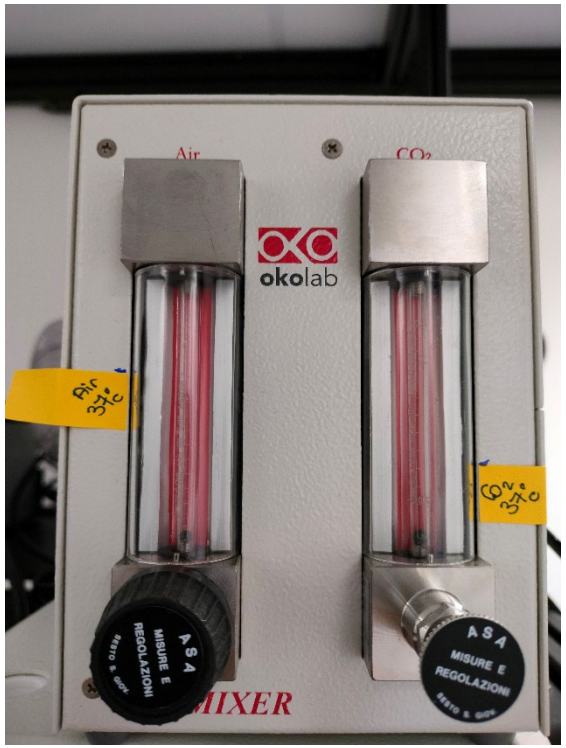
Temp Control



1, Turn on the controller

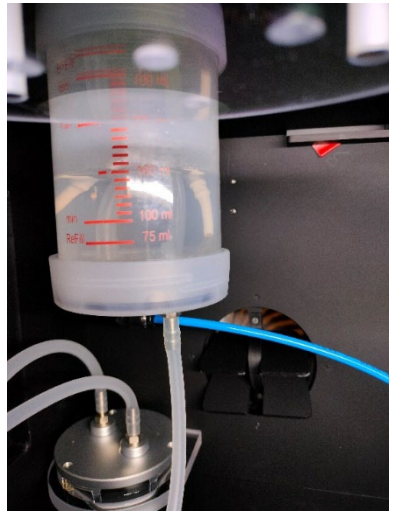
2, click to set desired temperature

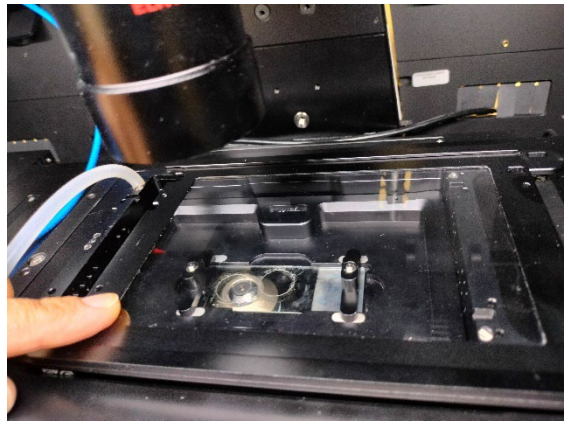
CO_2 Control



1,Open the valves until levels are stabilized to 5% setpoint

2, Check the level in humidifier is sufficient. If needed, fill with DI water



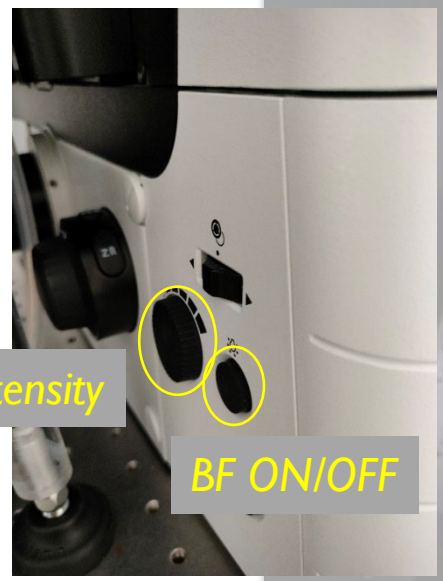


3, Close the lid

Nikon Ti2- how to

!Note- its better to operate all Eyepath channels (BF/Flu) from the software

When PFS finds the surface, you will hear a "beeping" sound.
You can then activate (turns green) it to keep Z position



BF intensity

BF ON/OFF

No need to change filter cubes manually- operate through software!

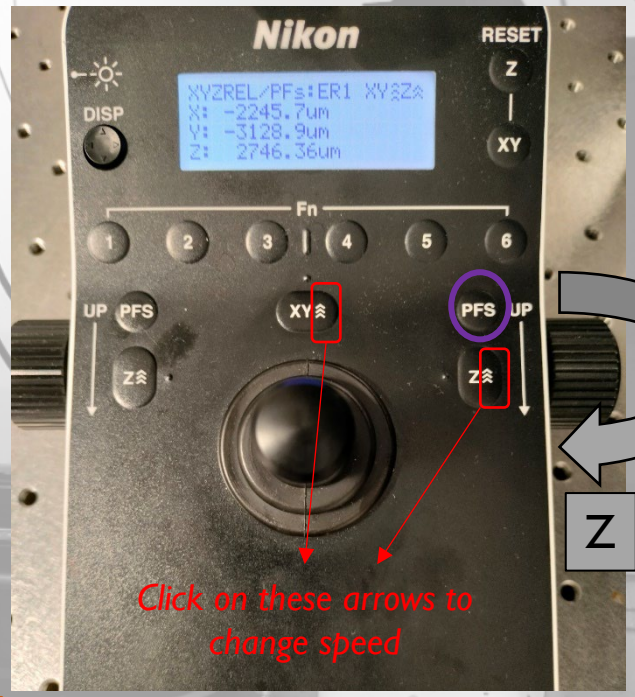
If you used ESC- to release, press and hold to avoid jumping back to position

Nikon Ti2- Joystick

1, objective and light path

2, Stage positions, PFS

Press from the side to shift info views:



Objectives (from Nikon):

Objective type	Mag/NA	Medium	Contrast	WB (mm)
Plan Achro	10x / 0.25	Air	Fluo, Ph1	10.5
Plan Apo λ	20x / 0.75	MImm	Fluo, DIC N2	0.35 (Oil) 0.34 (Gly) 0.33 (W)
Plan Apo λ	60x / 1.4	Oil	Fluo, DIC, Phase	0.13
CFI SR HP Apo λ S	100x / 1.35	Silicon	Fluo, DIC, Phase	0.3

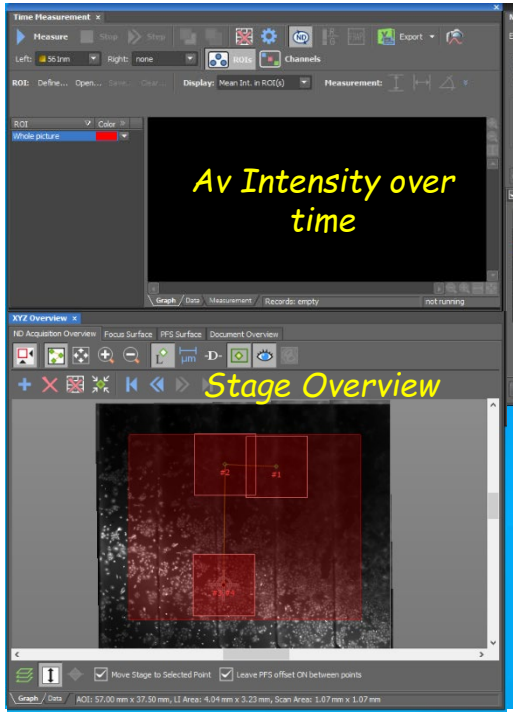
Note- optional objectives exist: 20X/40X air; 60X Water, 100X oil

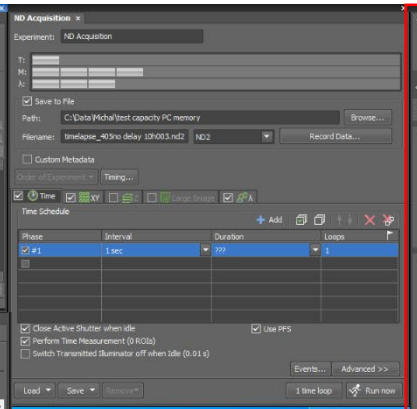
Nis Elements

Your Work Interface looks like this:

Acquisition controls

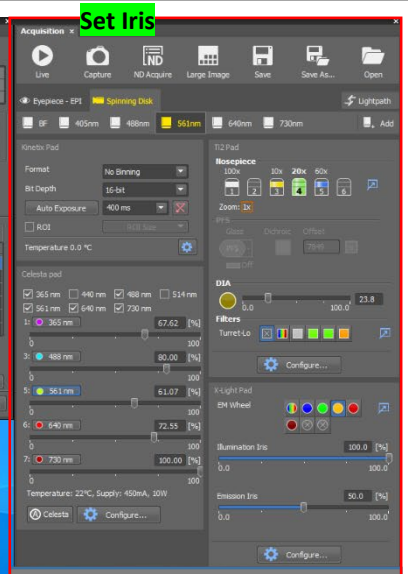
Image controls



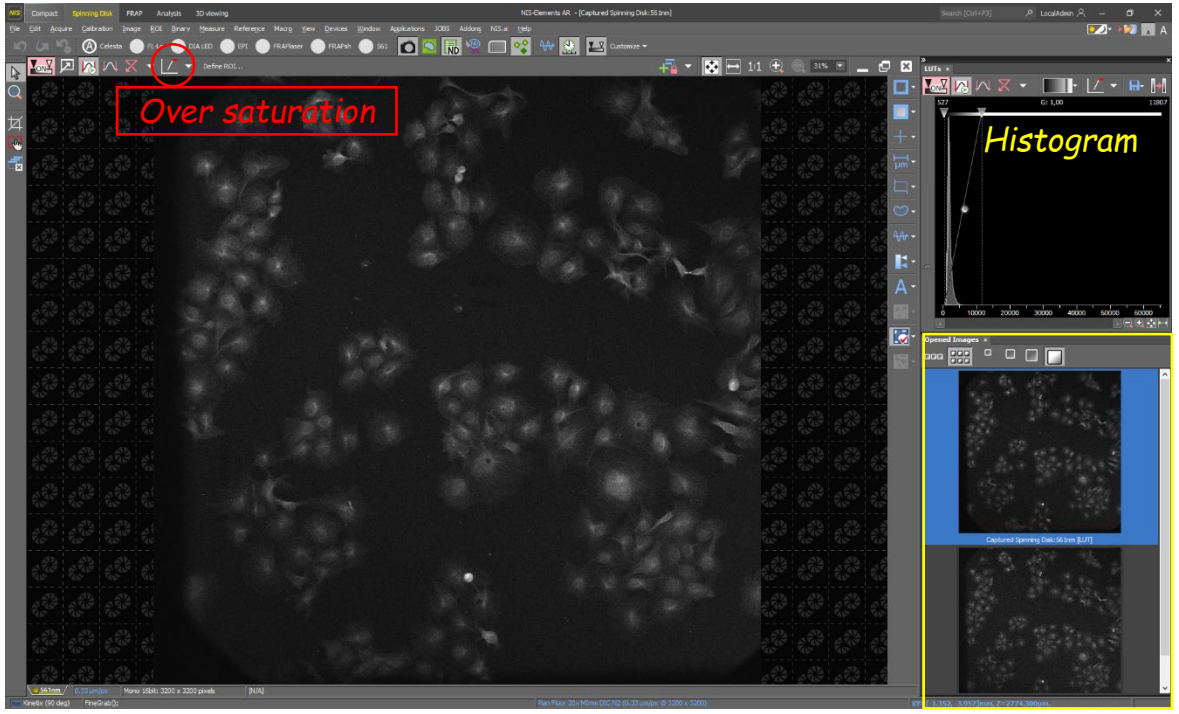


This is your design of experiment: Time/Z/XY/channels

Save each experiment in your own folder and name it properly



These are your acquisition settings- illumination/detection/ ROI.. Optimize each wavelength separately



Here you can toggle between acquired images.

Crl "i" shows image information

***Set Iris** Macro Panel:*

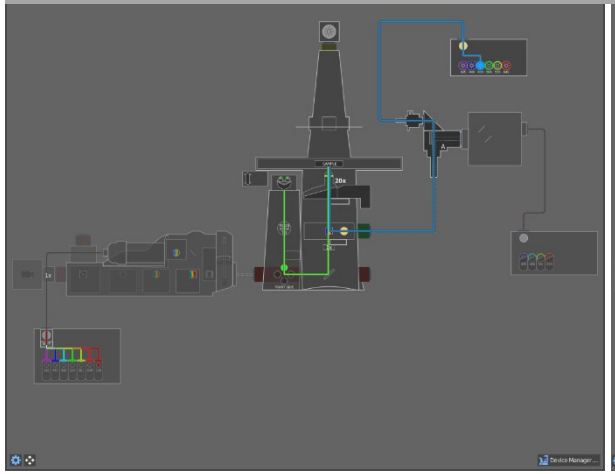
Click this once per objective, to set emission Iris correctly

Nis Elements

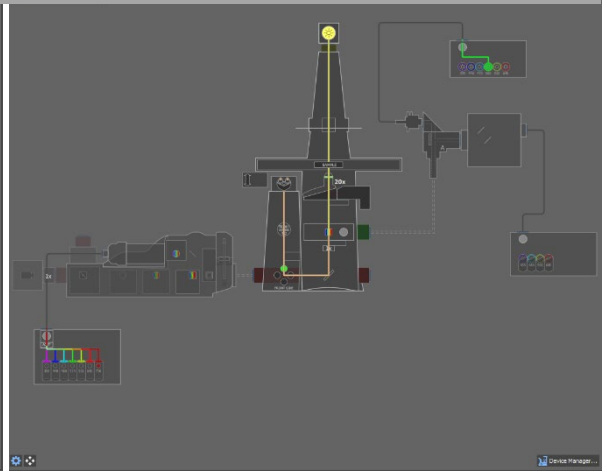
Light Path defines your interface, the illumination source and detection device:

Eyepiece

Flu

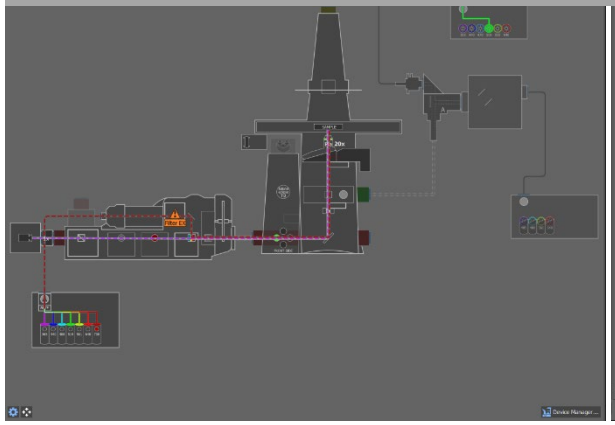


BF

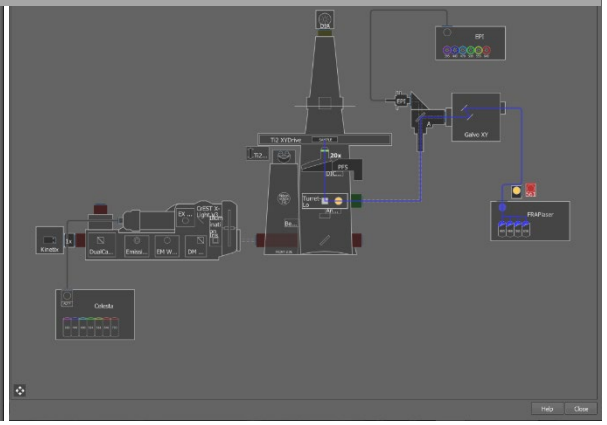


Confocal

Spinning Disc



FRAP

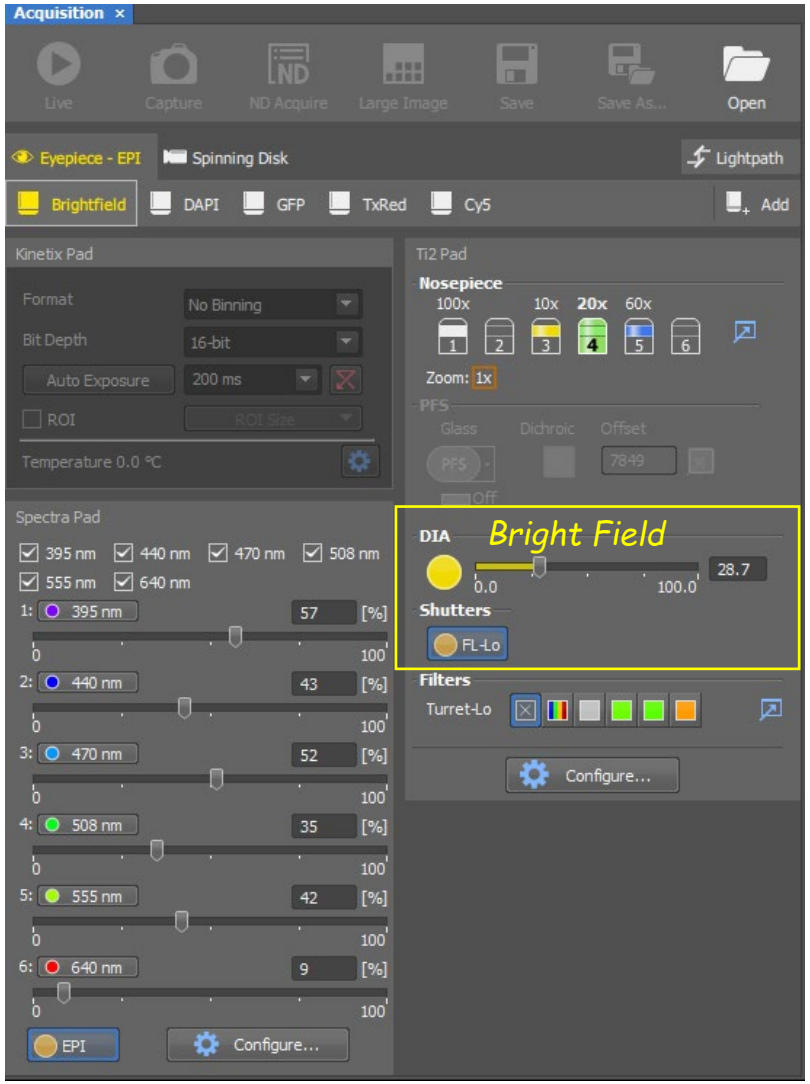


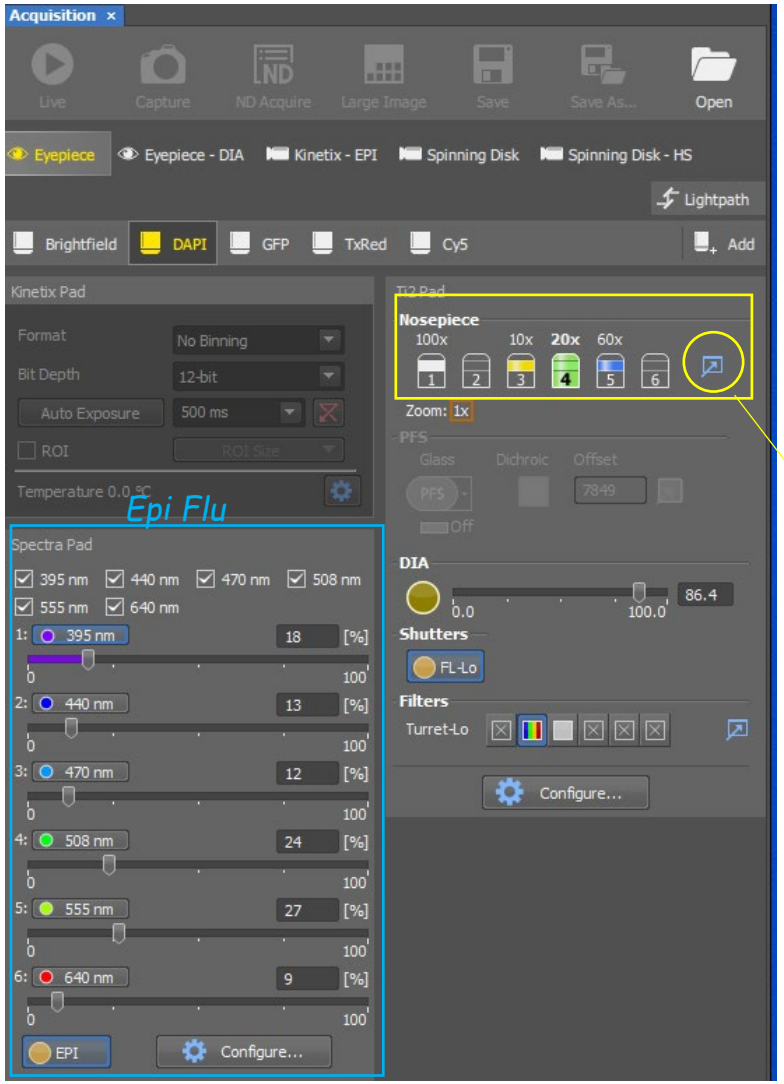
Nis Elements- Eyepiece

You can shift between BF and Epi-Flu

Click on each of the presets to activate the suitable illumination-detection settings

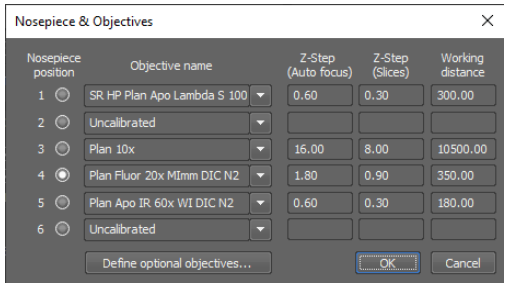
To see BF, make sure the shutter is on "O" (open) position and not "C" (closed).





You can see the objectives here. !Note! only choose from Ti2 body **before mounting sample! Do not click to avoid auto switch!**

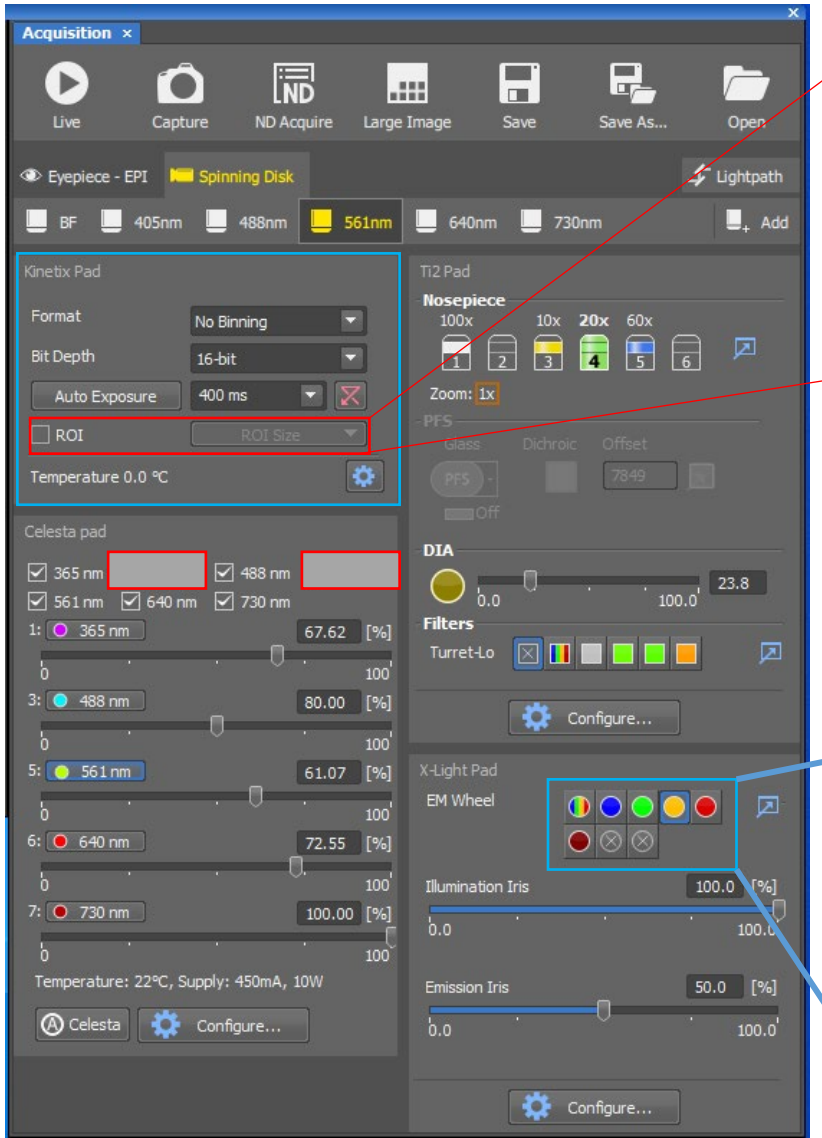
Here you can see full obj info

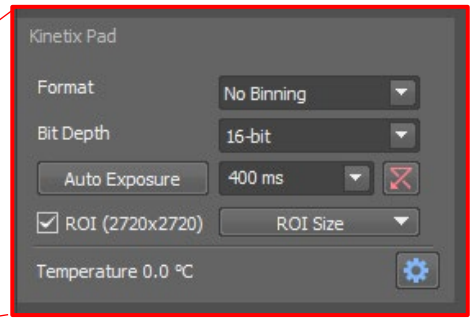


Nis Elements- SD

You can shift between BF (transmitted) and Confocal-Flu

Click on each of the presets to activate the suitable illumination-detection settings





Camera settings.
Recommended:*
No binning
16bit
Minimal exposer time

Missing filters.

ROI size:
If unchecked, you see full FOV- more than illuminated area
Crop to 27mm or lower, or define your own ROI on image

***Emission filters-** matching excitation.*

**for fast imaging- use multiband cube*.*

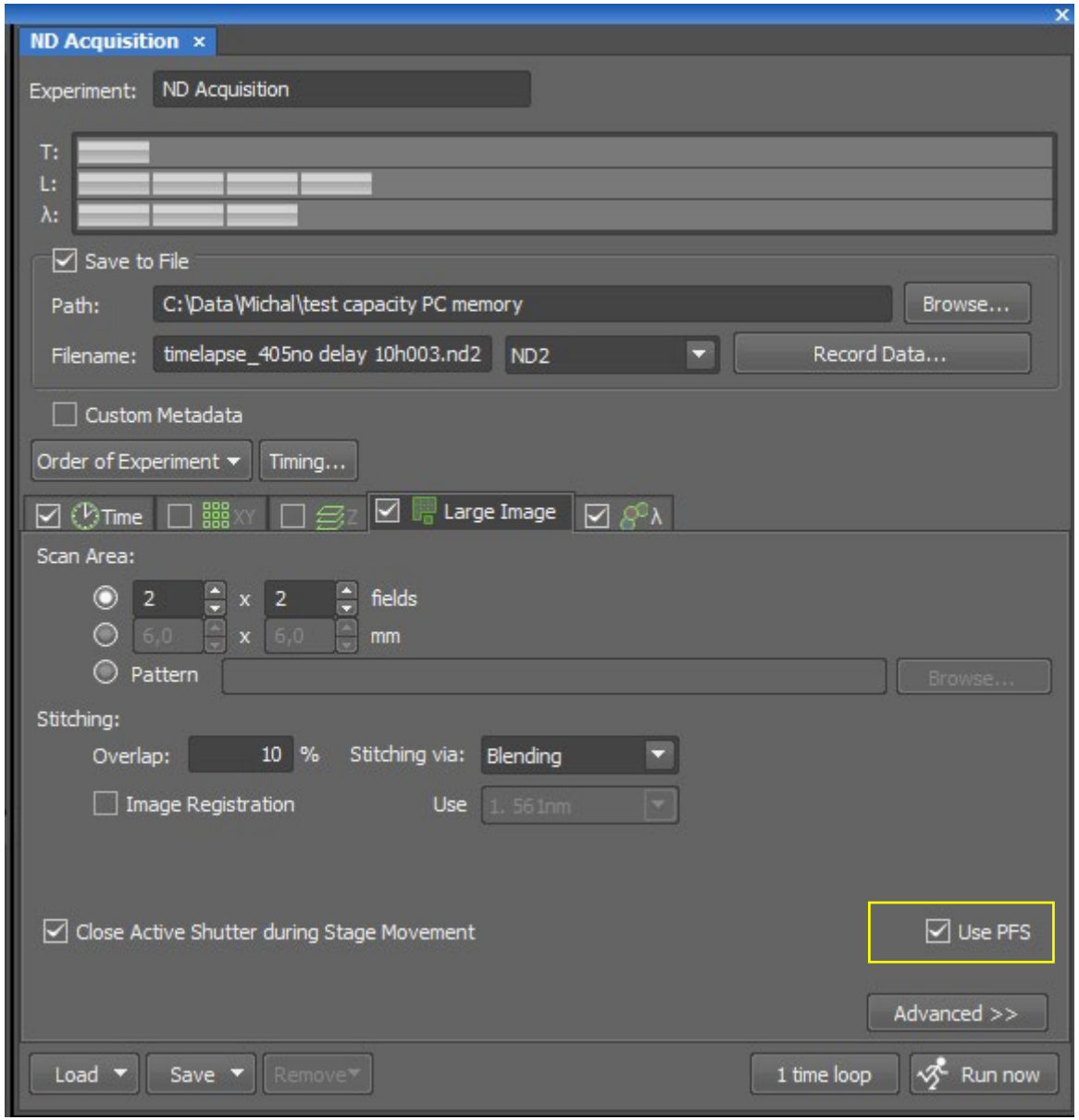
**First check controls for channel leakage!*

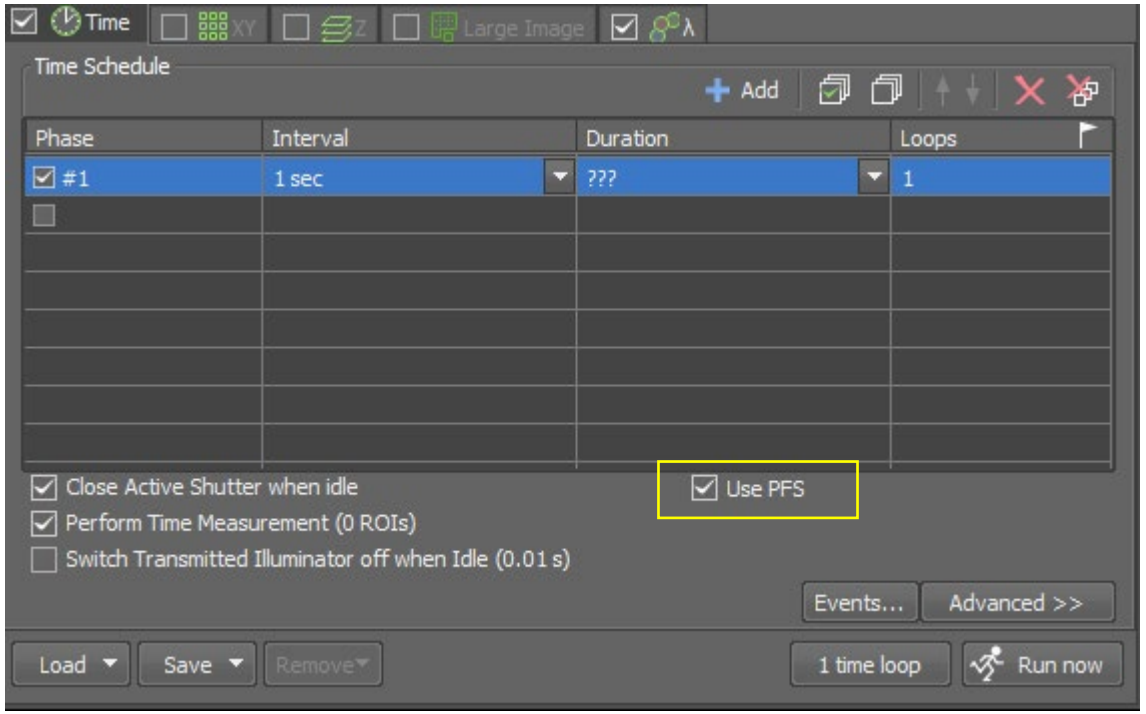
**Note that imaging lasers are strong and can bleach fast sensitive samples. If this is an issue:*

Try to bin (sCMOS pixel size is smaller than EMCCD), lower laser as possible and work in low signal regime, with post-enhancements (Huygens)

Nis Elements- ND acquisition

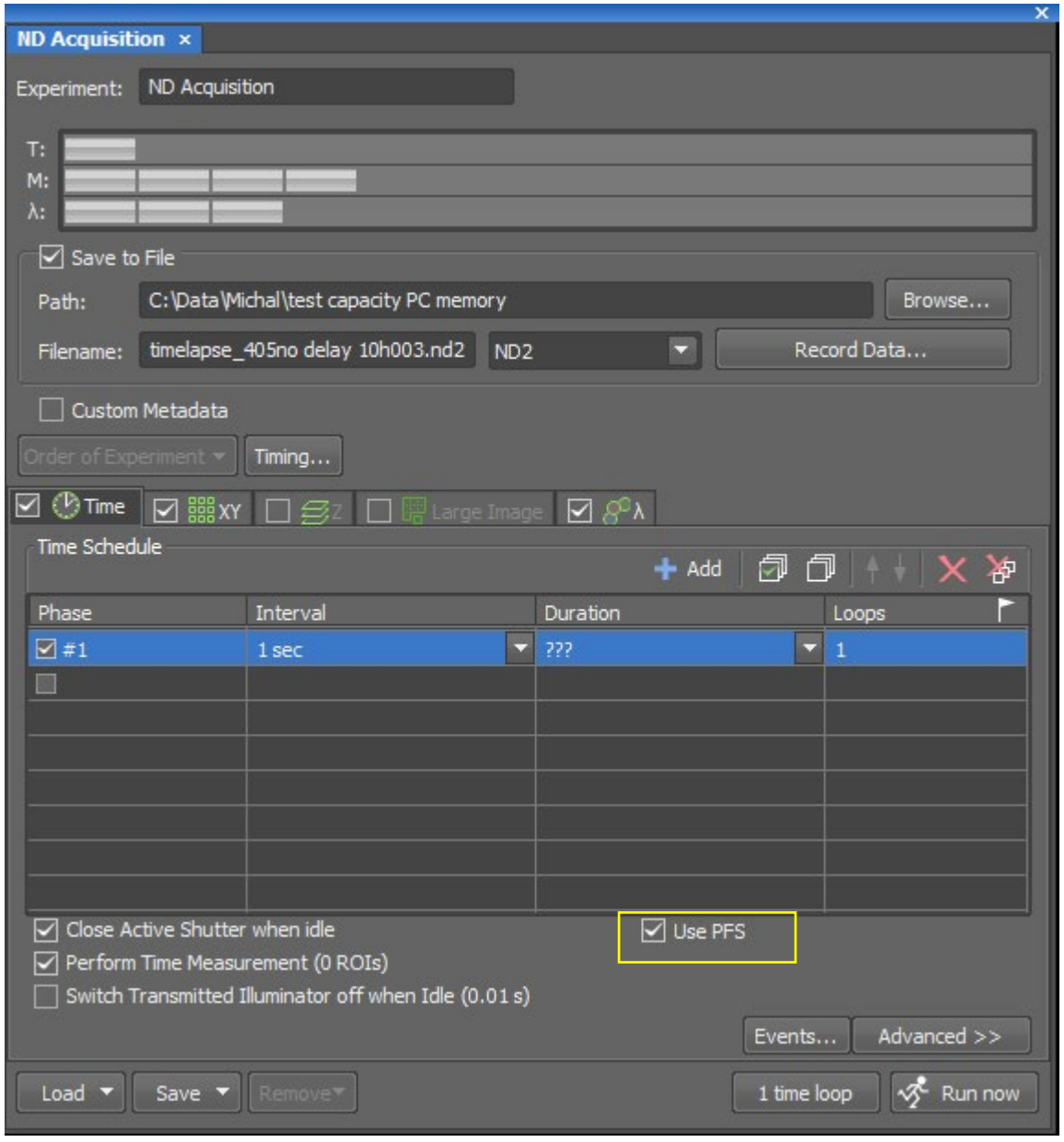
Image large area- better use PFS!!



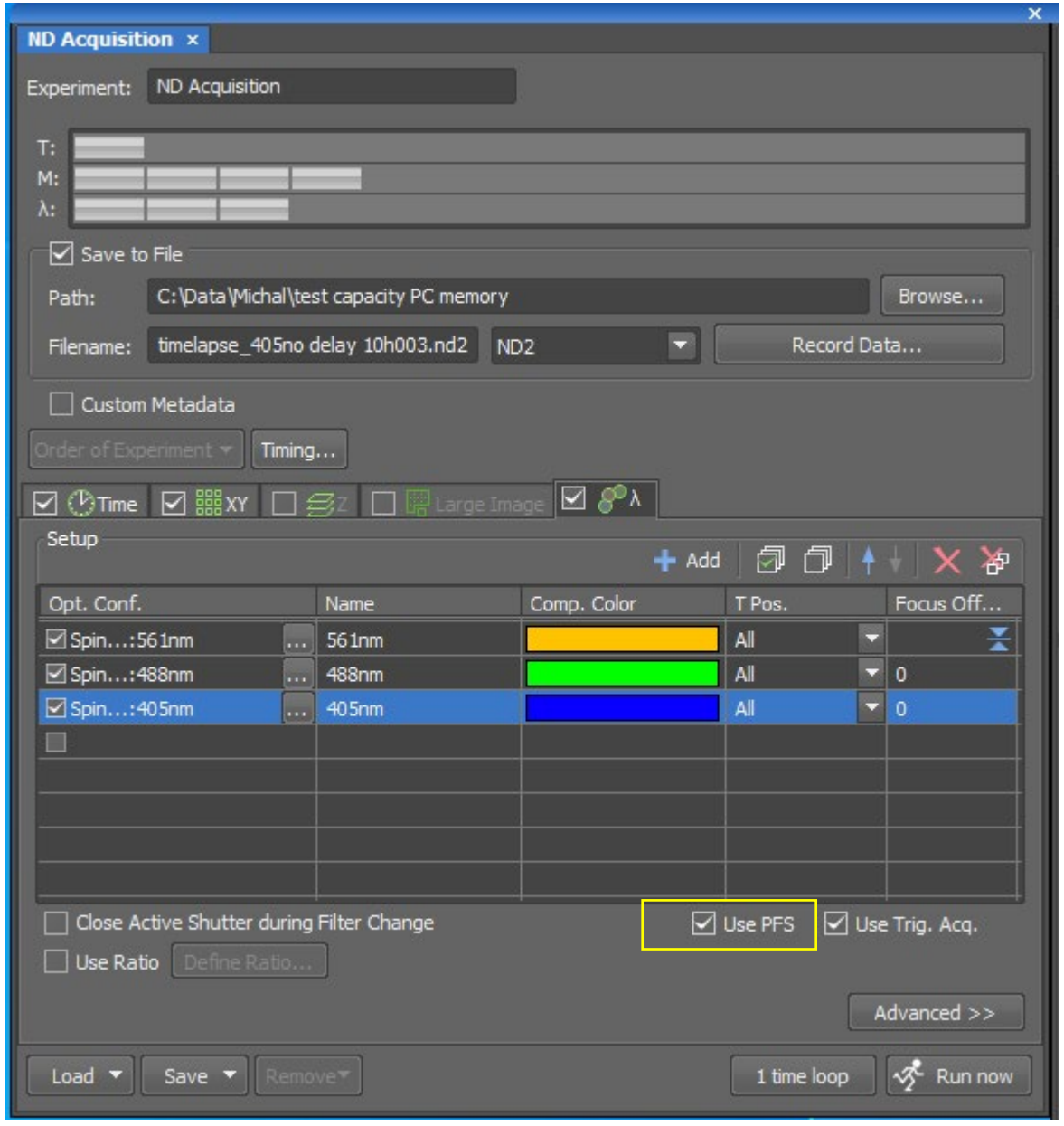


Nis Elements- ND acquisition

Time experiment- use PFS!!



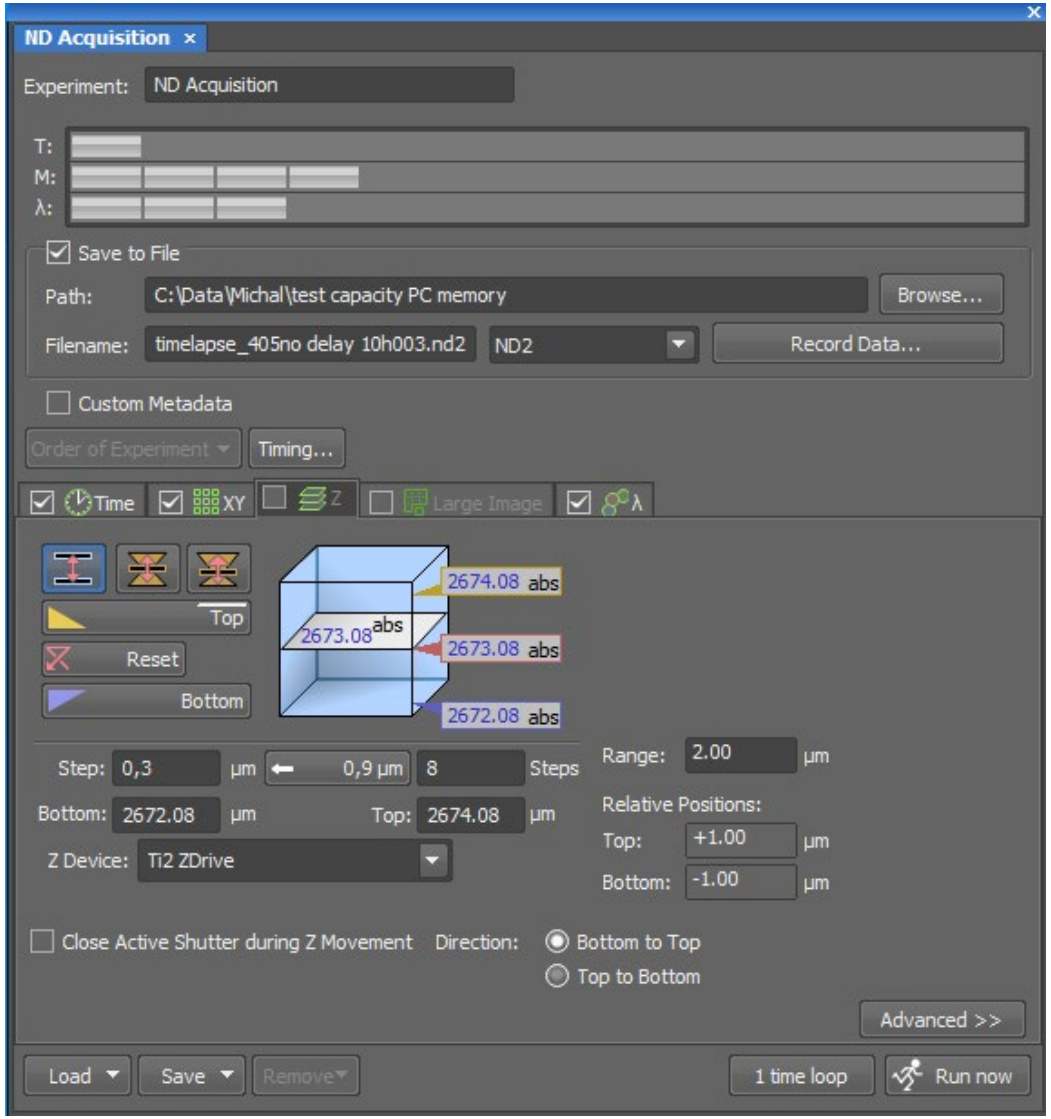
Set wavelengths (taken from your acq parameters)



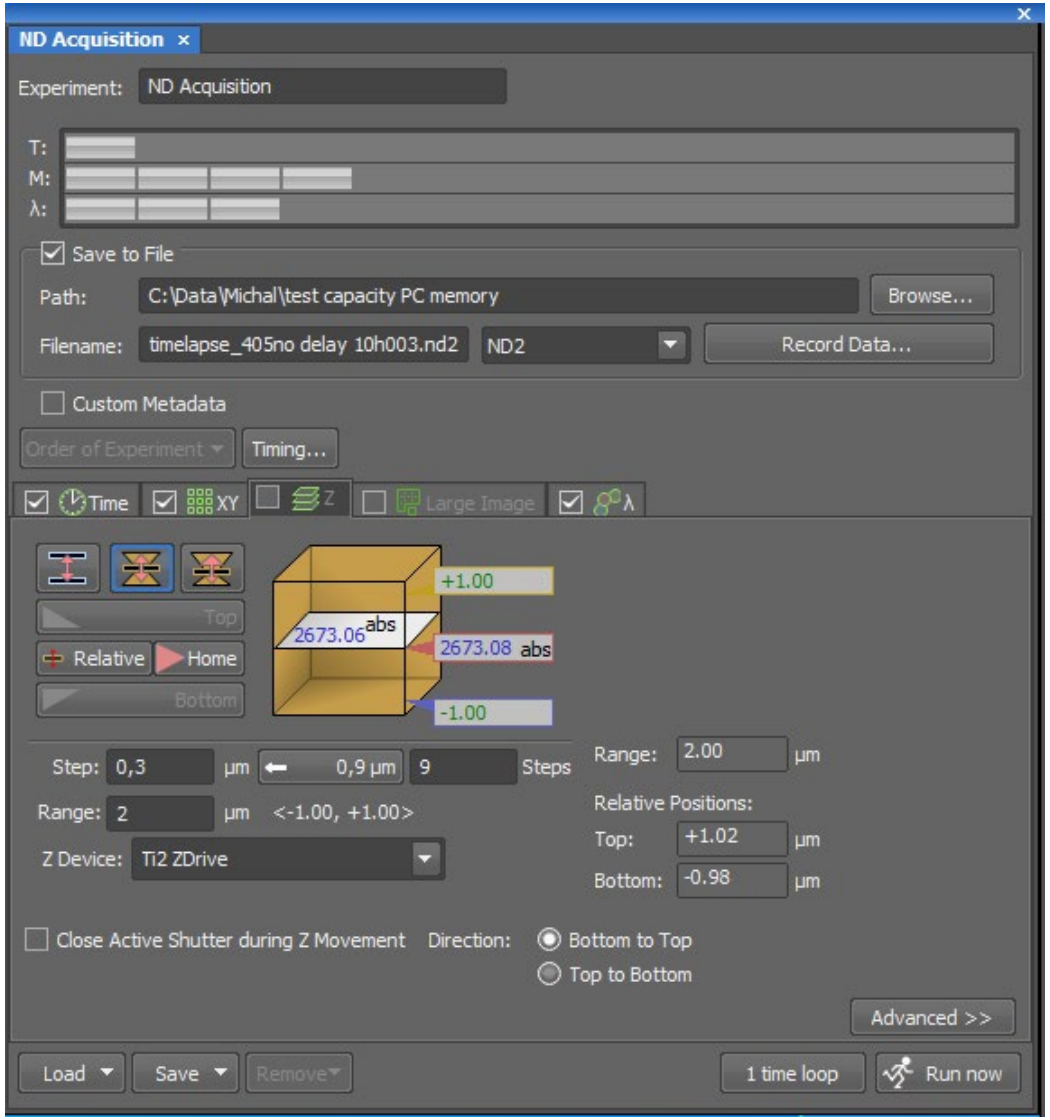
Nis Elements- ND acquisition

Z stack

Absolute- Piezo



Relative- you can use PFS and objective (Ti2) Zdrive



Nis Elements- stage overview tools

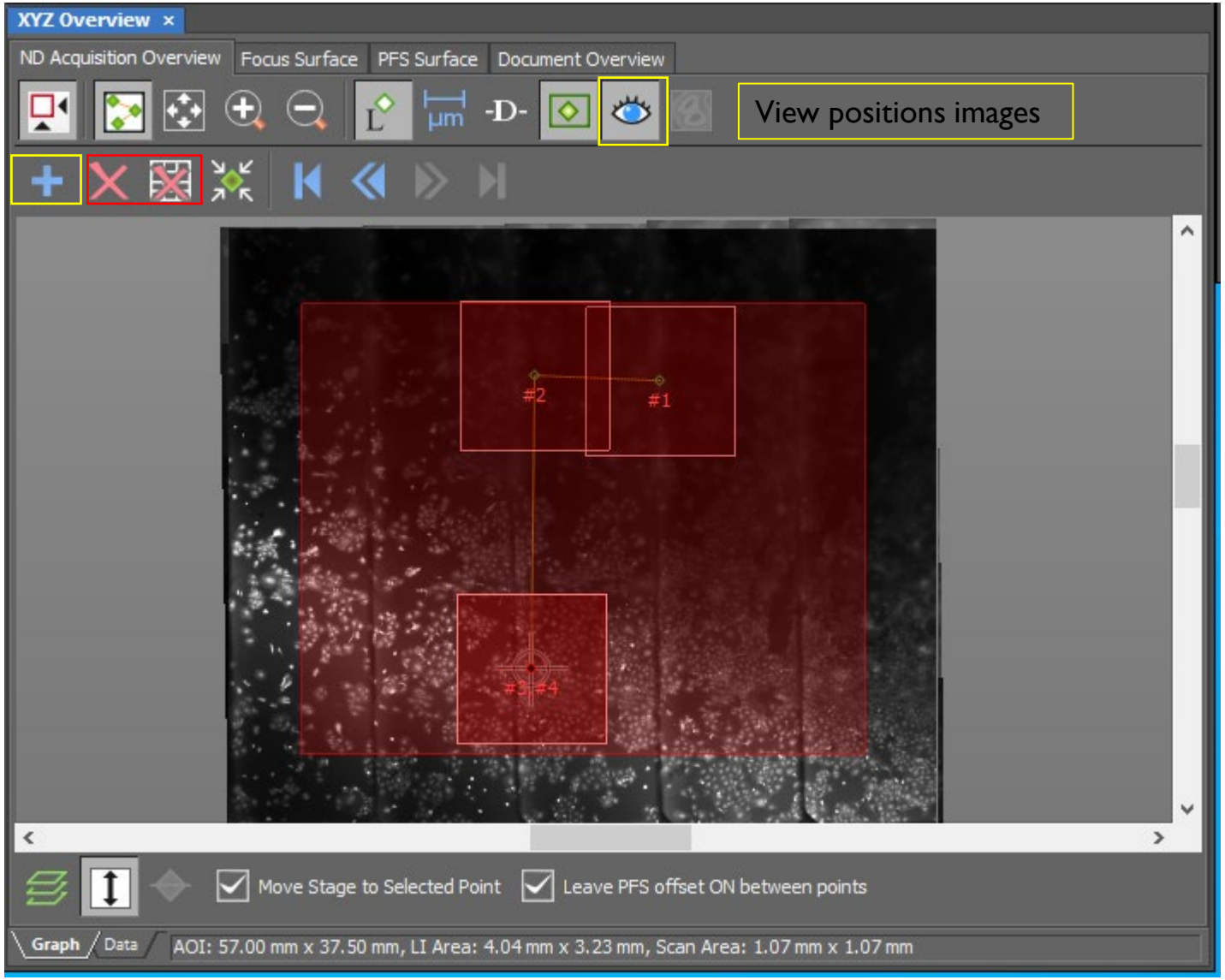
You can run fast preview scan or large image scan

Add new position
(also added to ND acq list)

Delete one point or all

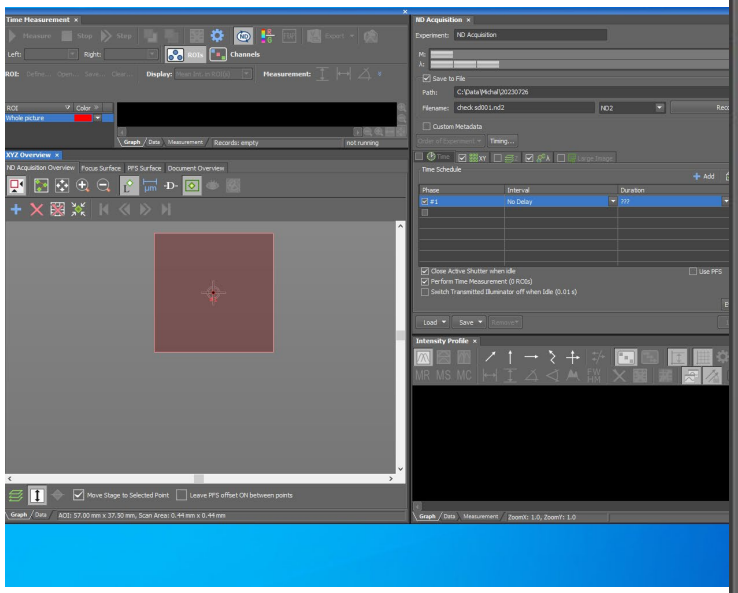
Doble click on position will move the stage there

Right click will allow you to open a dialog box in which you can define large image scan.

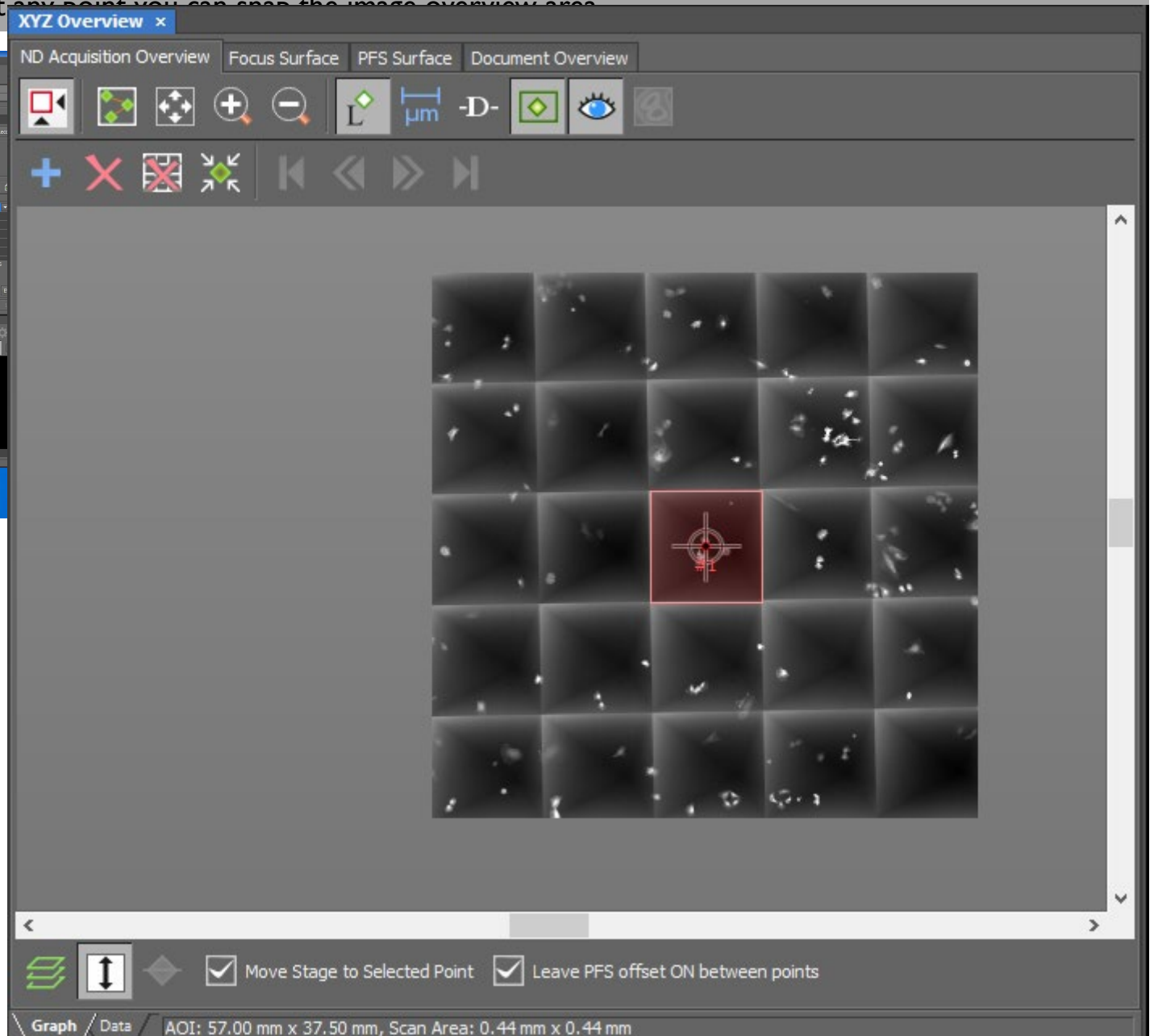


Nis Elements- stage overview tools

At any point you can snap the image overview area



Right click will allow you to open a dialog box in which you can snap the overview and save.



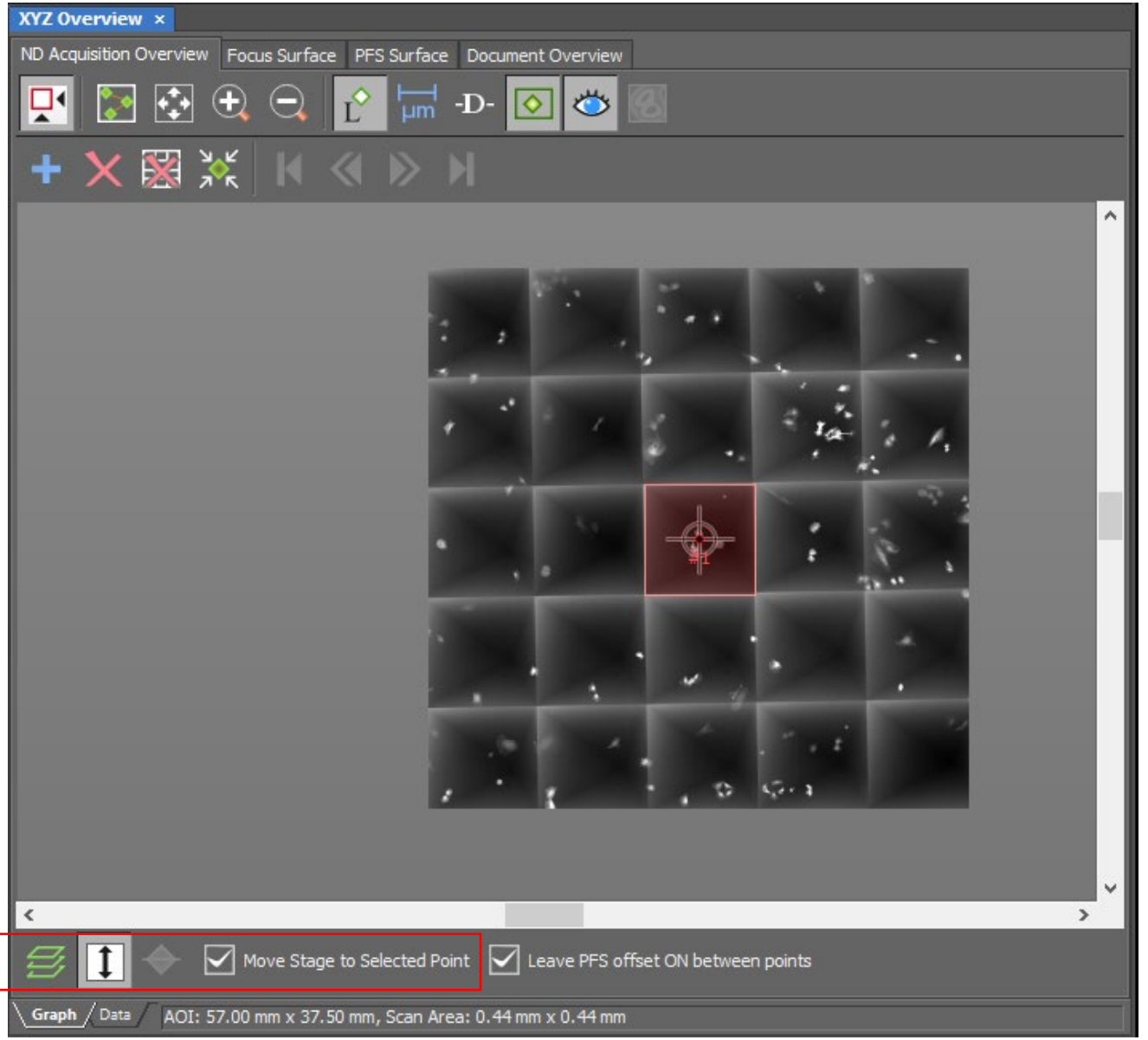
Nis Elements- stage overview tools

Fast Preview

Right click will allow you to open a dialog box in which you can define preview image to be scanned quickly
(one channel, one plan, no stitching).

This preview can be further used as a map for navigation.

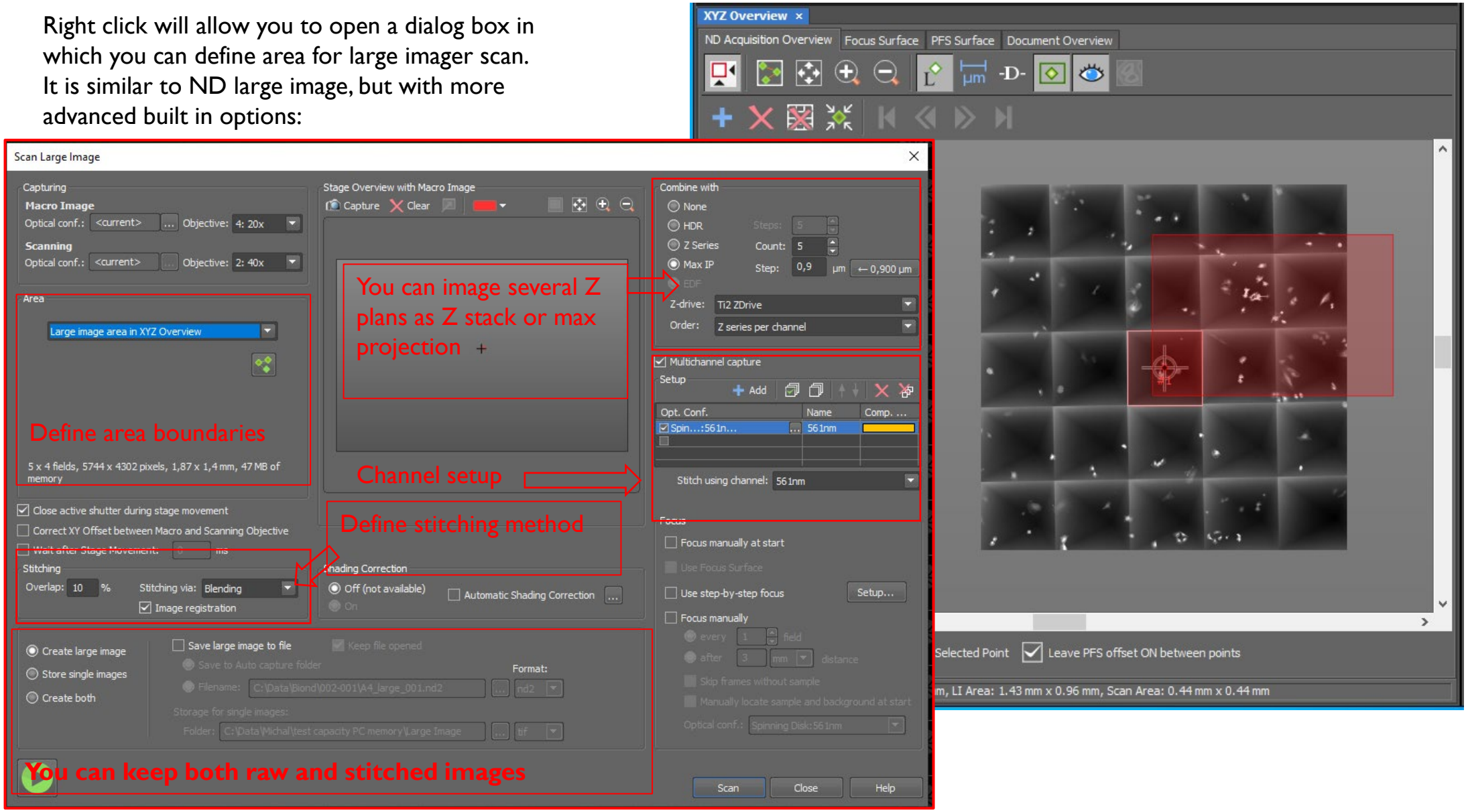
Double click on any area in the preview will move stage



Nis Elements- stage overview tools

Large image scan options

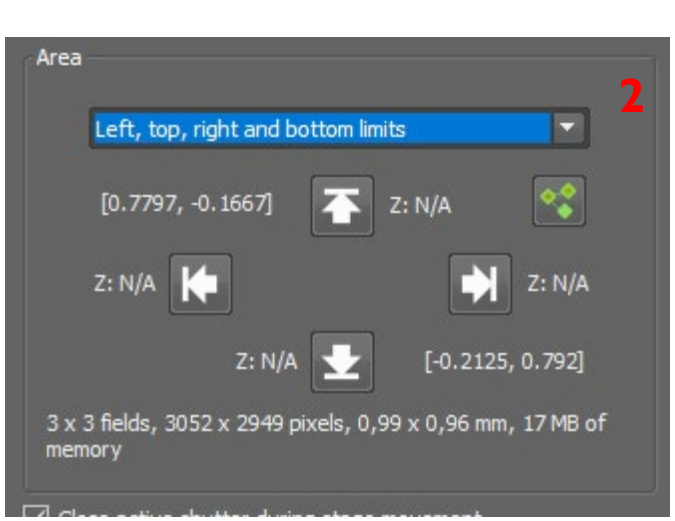
Right click will allow you to open a dialog box in which you can define area for large imager scan.
It is similar to ND large image, but with more advanced built in options:

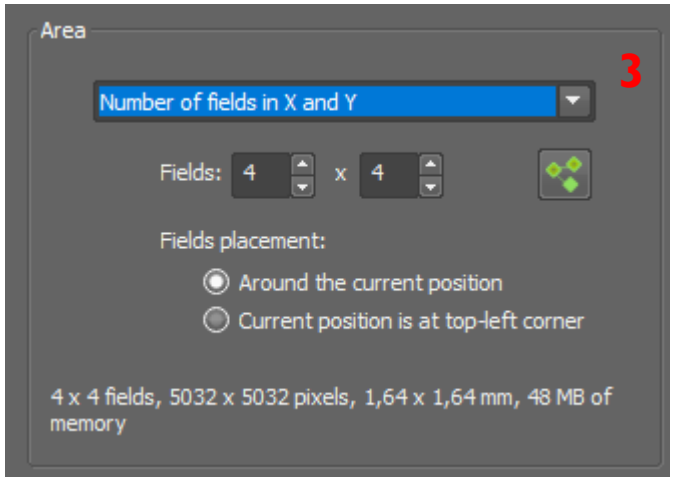


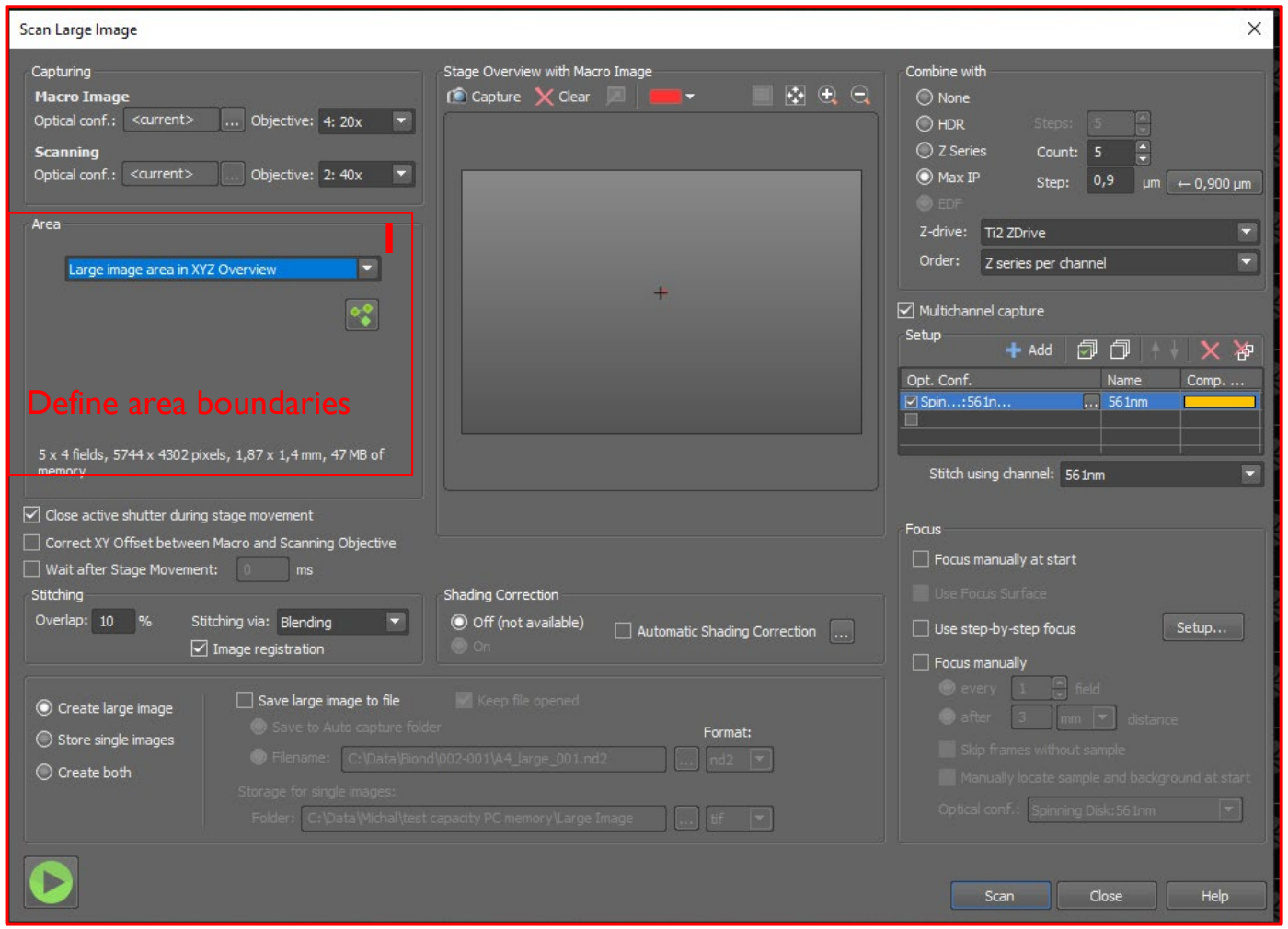
Nis Elements- stage overview tools

Options to define area

You can define your scan are by an ROI (1) ; four border points (2) ; number of FOVs (3)

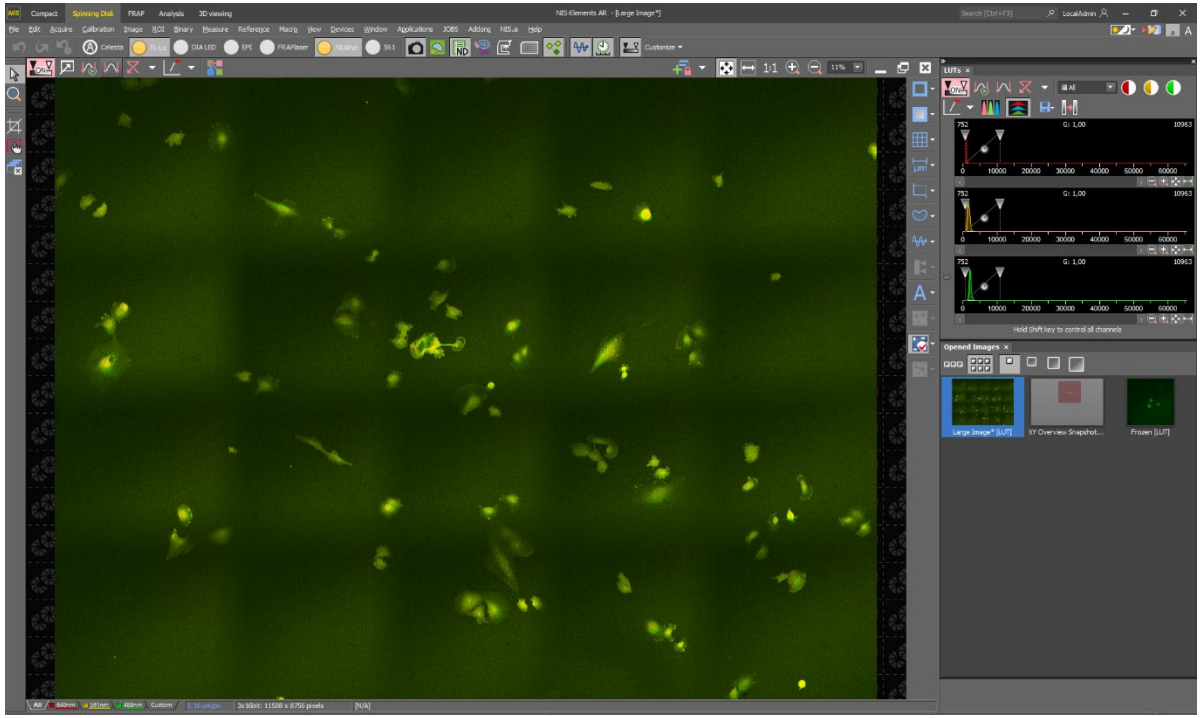


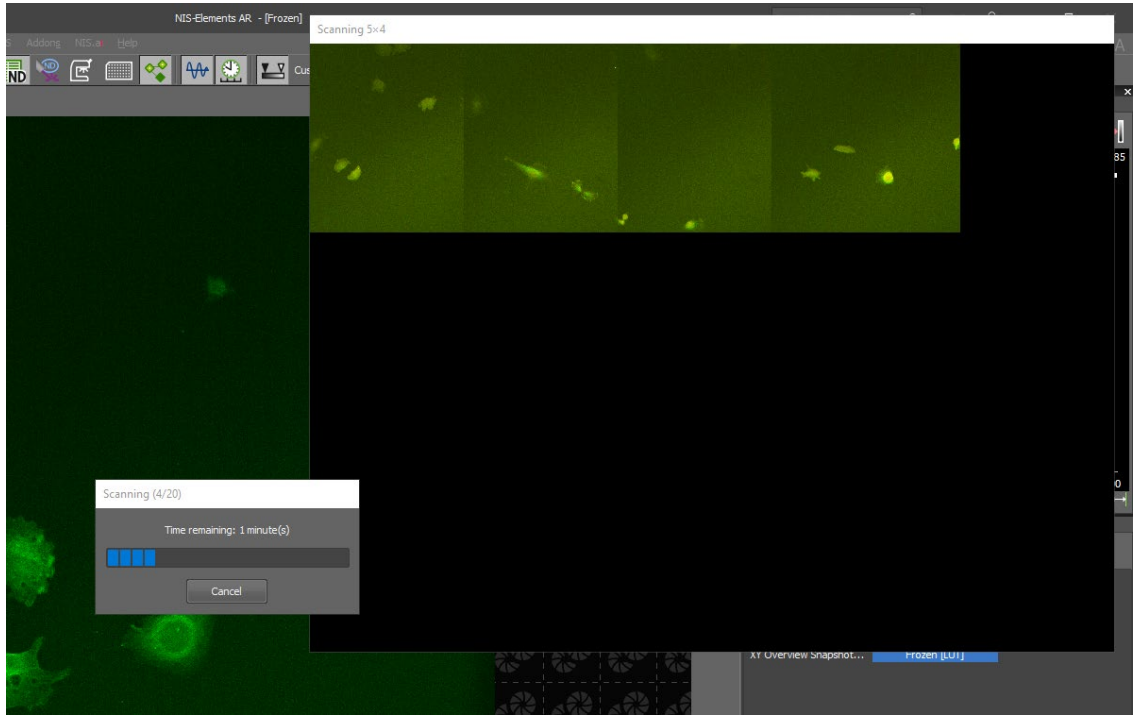




Nis Elements- stage overview tools

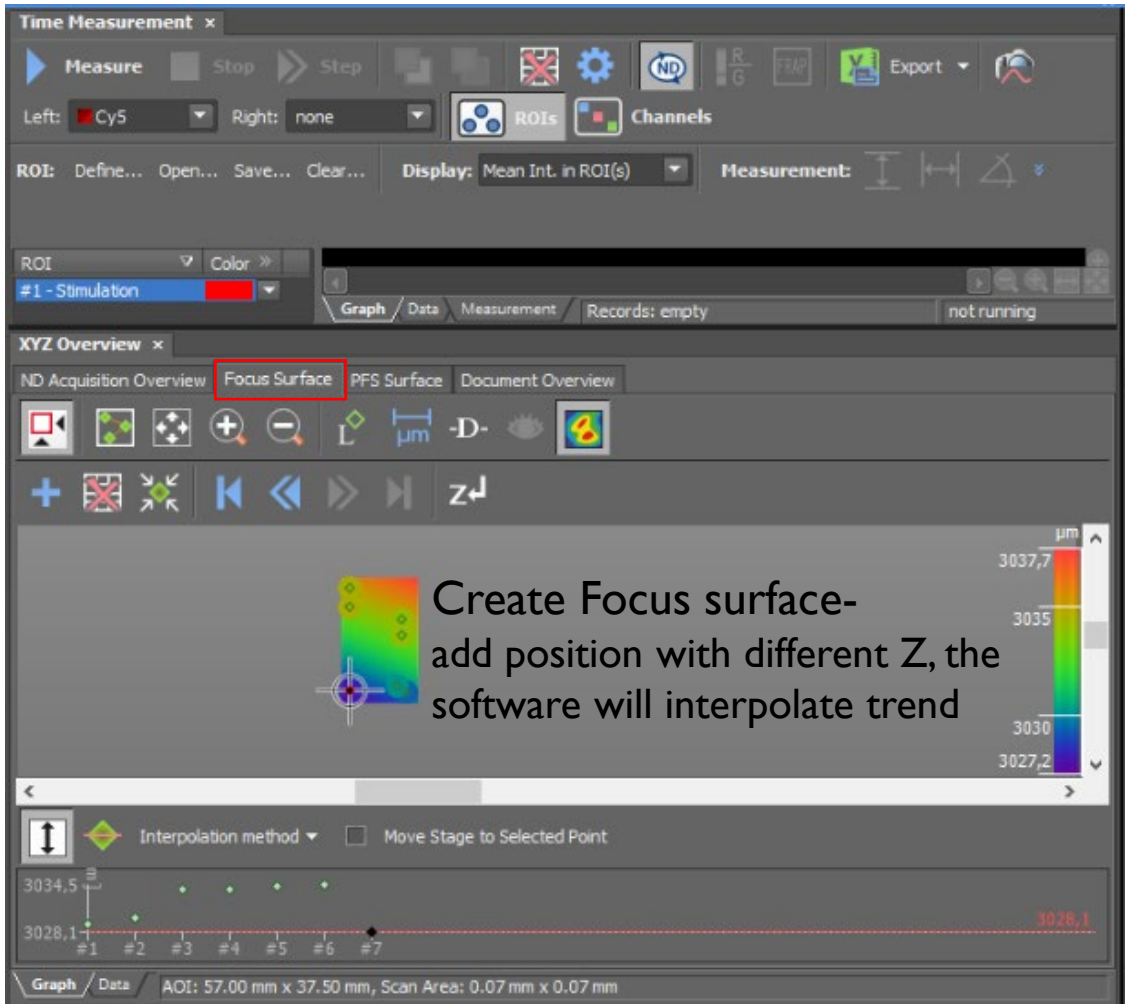
Your scanned large image (here in 3ch) can be further used as a map for navigation- right click and “add this point to ND acq”

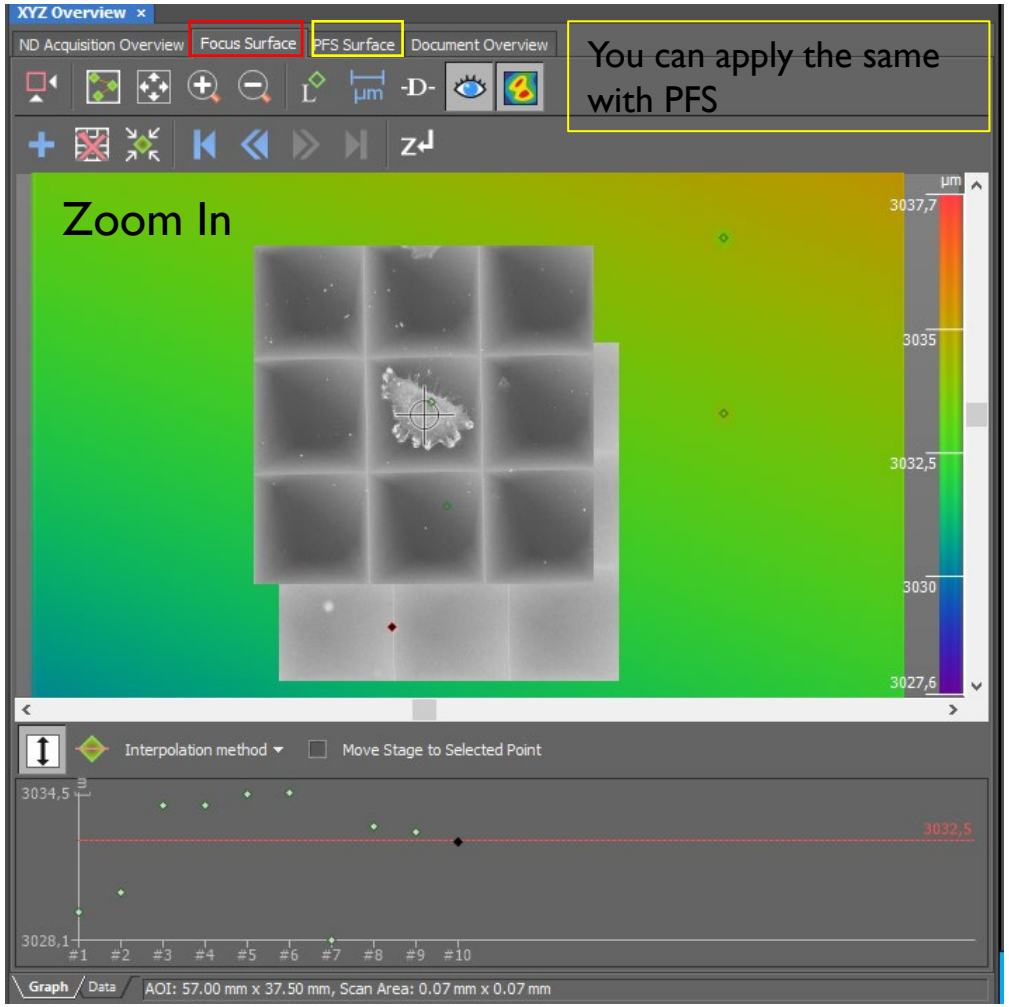




Nis Elements- Stage Overview tool

Create focus map



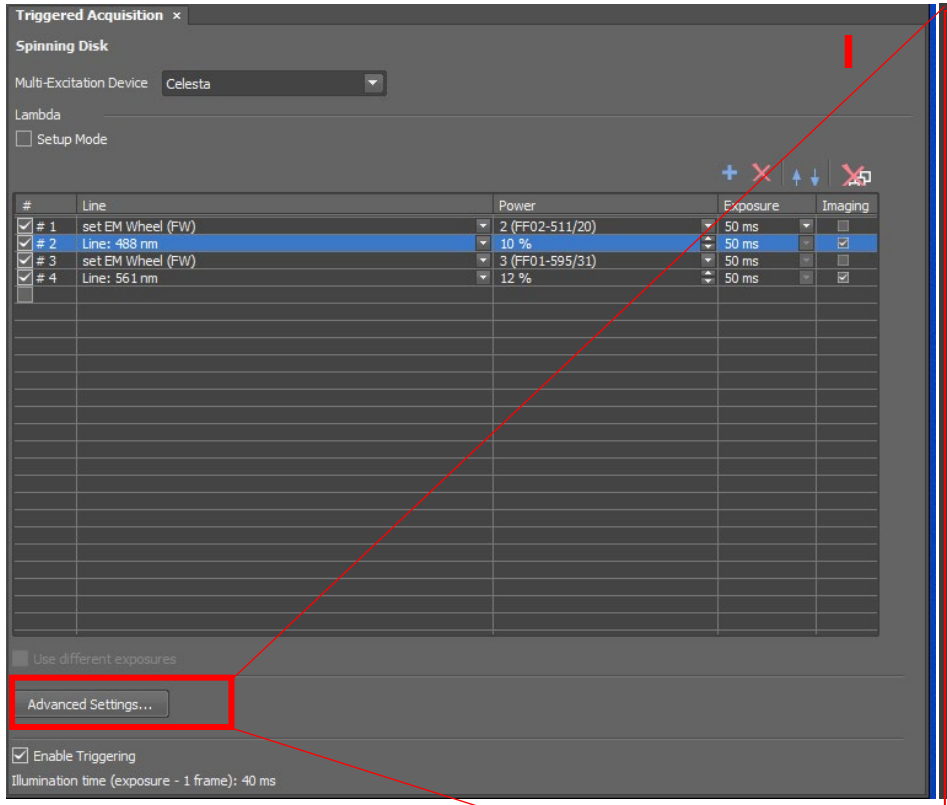


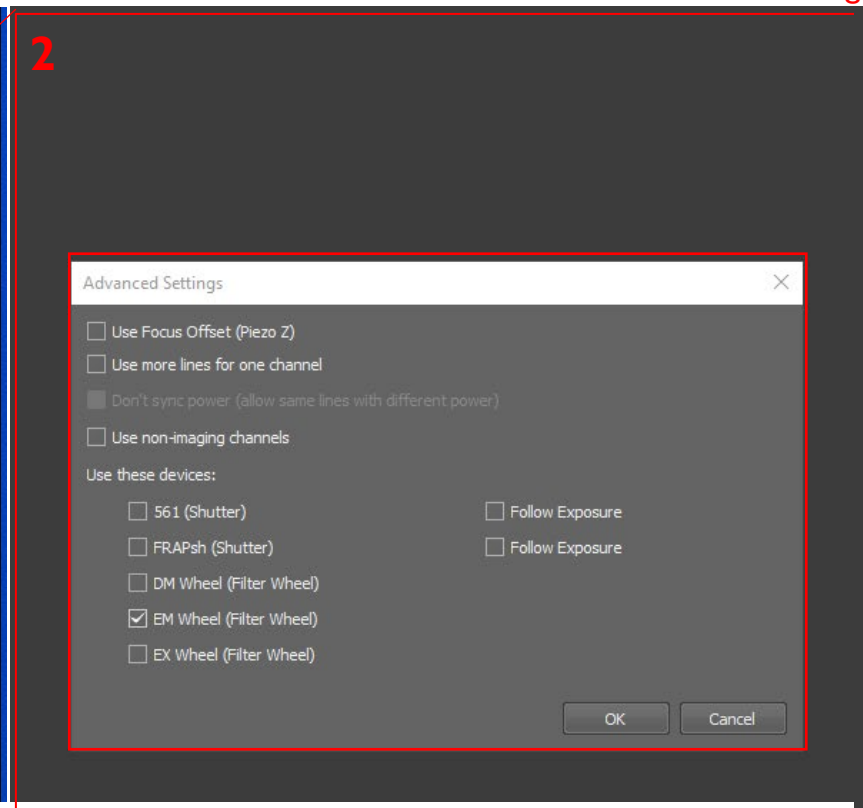
Nis Elements- Triggering

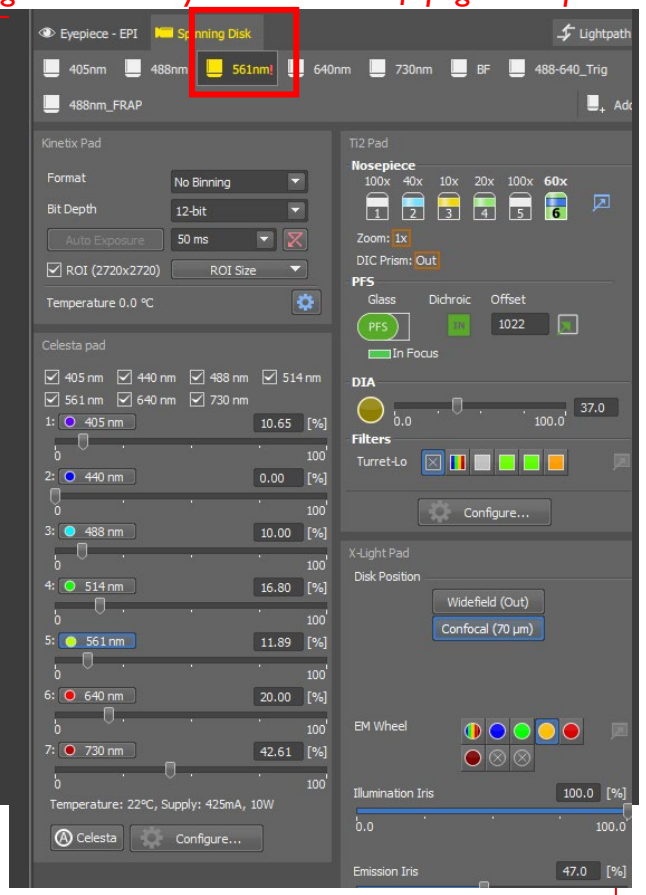
The system is fully triggered: light source-emission filters-camera-piezo stage

You can define your acquisition to be triggered, meaning running very fast acquisition:

Trig is saved to your OC- don't foprget to update





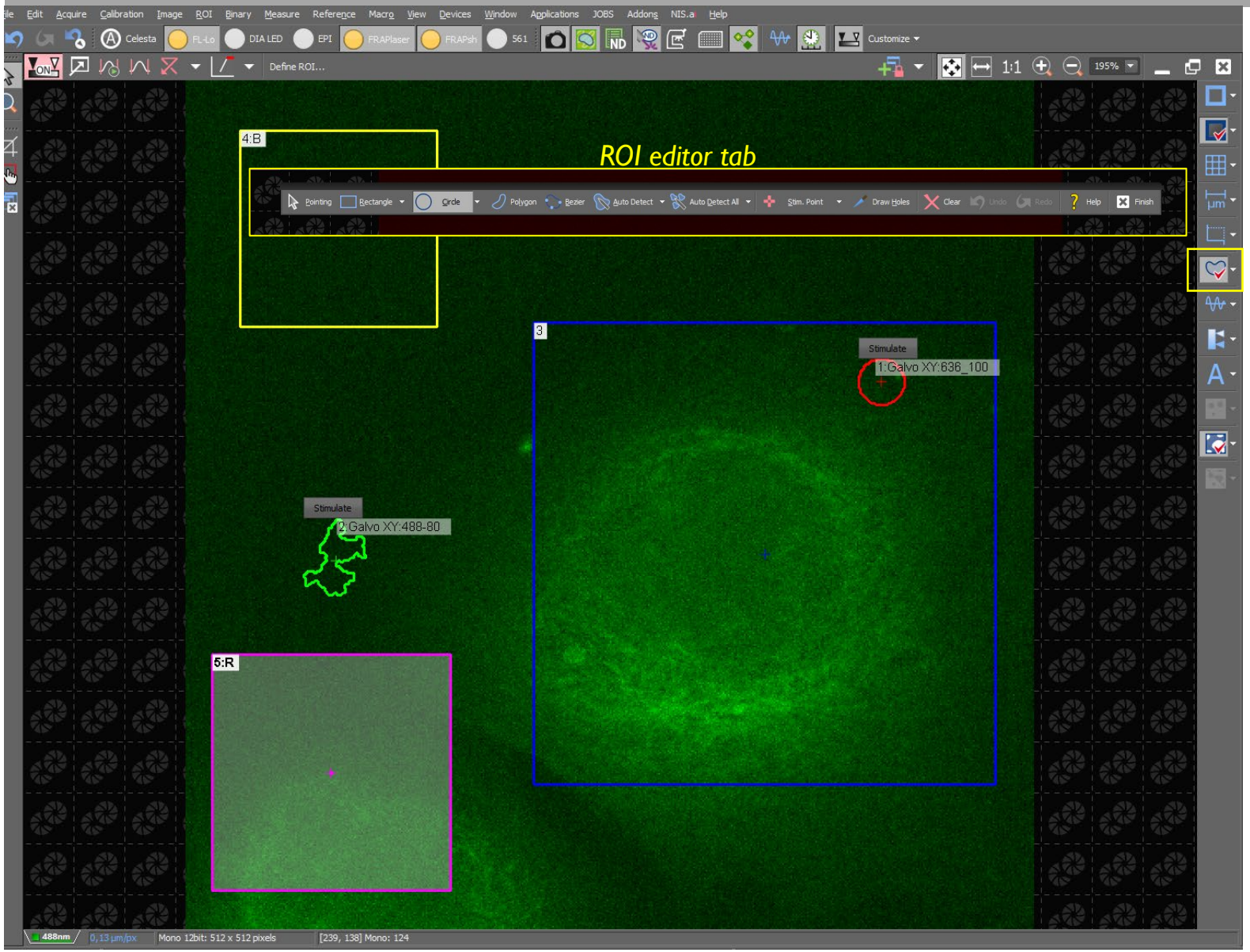


You can set the exposer time and power per line
Make sure to uncheck "imaging" for the filter wheel line

Once you enabled triggering- it will be in your channel when running ND experiment, no need for extra definitions.
If you run a Z stack- chose "triggered piezo" for fast imaging

Nis Elements- FRAP

Image view

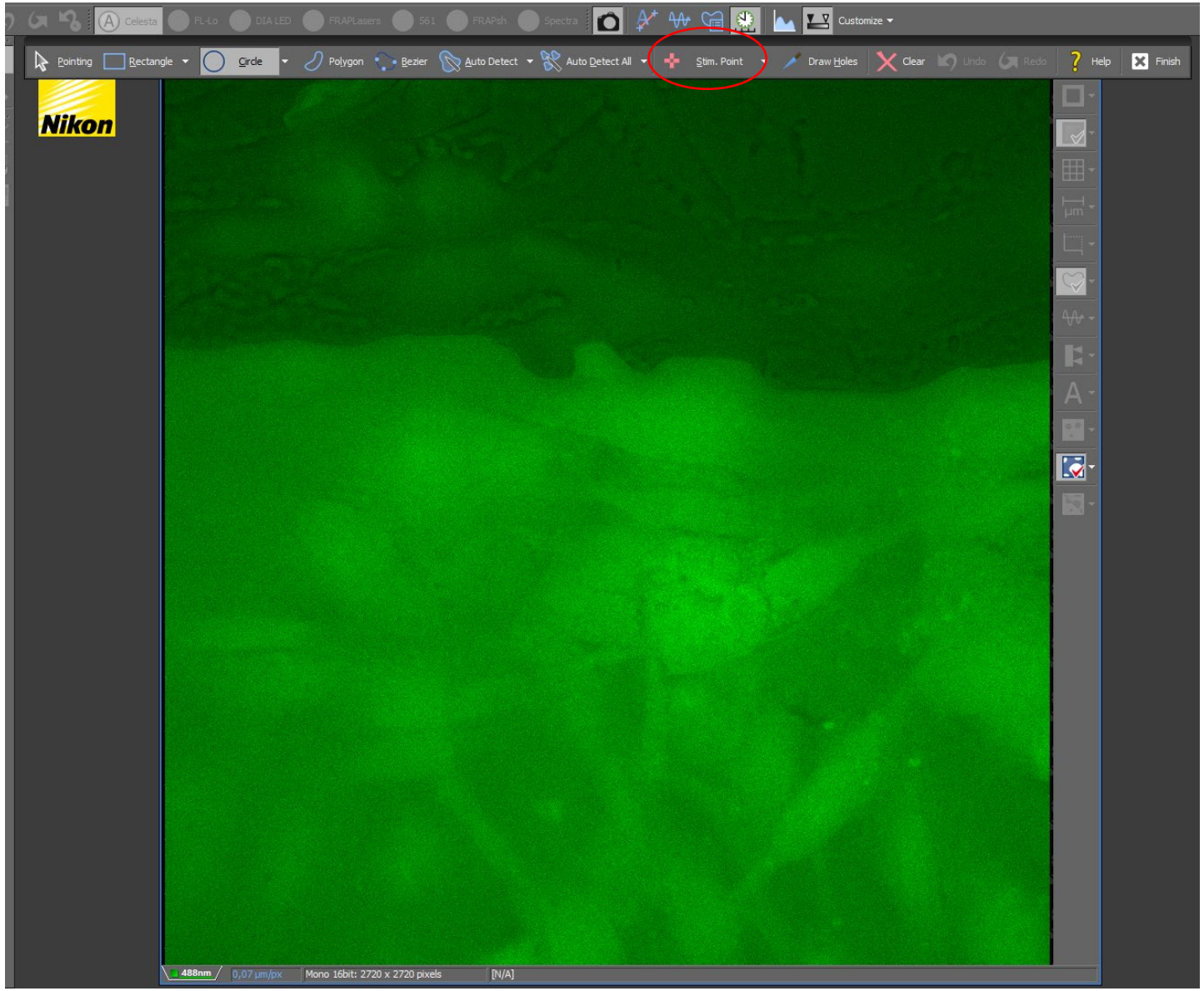


1, **Snap** an image
2, You can **set ROIs** with different sizes/contour:
A fixed shape, auto-detect, or point*

*For point stimulation you need to be on "Live" mode

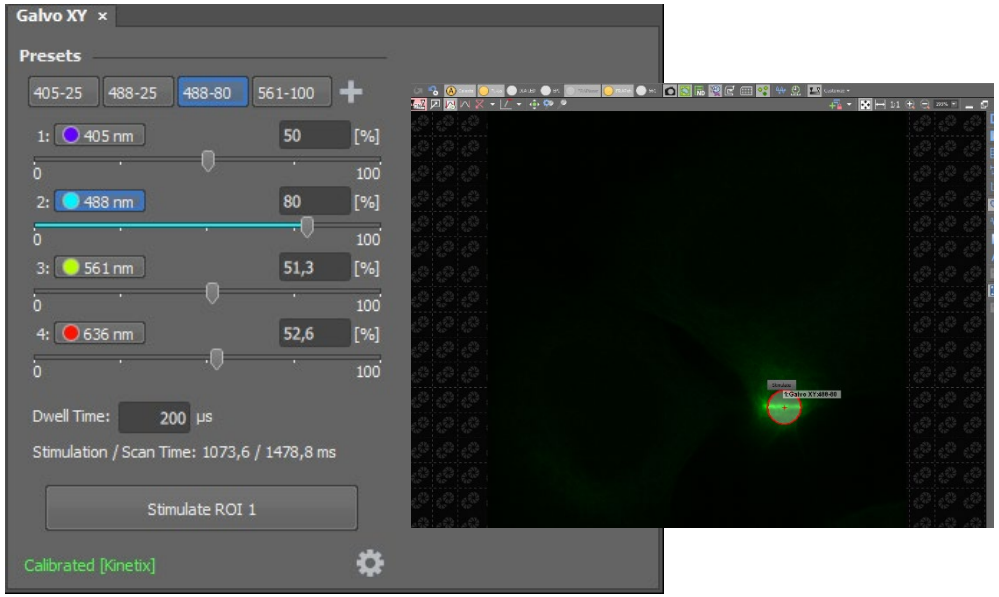
3, **Assign each ROI :**
Stimulation- assign FRAP laser and intensity pre-set
Background
(no signal)
Reference
(signal but not bleached)

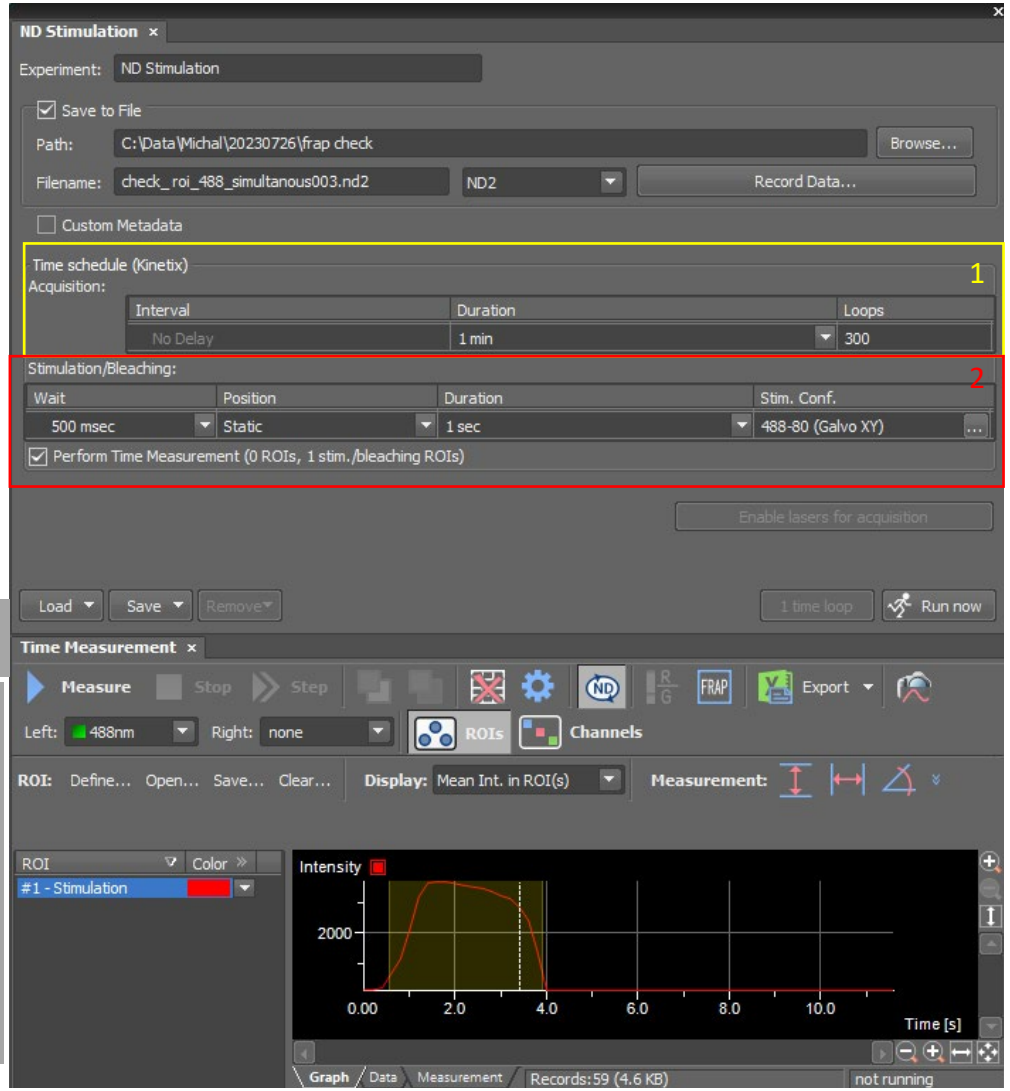
To mark a stimulation point on your sample-
1, Make sure you are in live mode
2, right click on ROI tools and mark "ROI toolbar" to display the bar on top of your image
3. In it you can see the red cross for stim point, place it on the desired location. Clicking "stimulate" on top (without releasing the mouse) can allow you to test the time required for bleaching



Nis Elements- FRAP

Acquisition control- Simultaneous Acquisition/Bleaching





On the Fly

1,Enable the lasers
2,Save pre-sets
3,Optimize dwell time
4, Click on "Stimulate ROI"

ND Stimulation

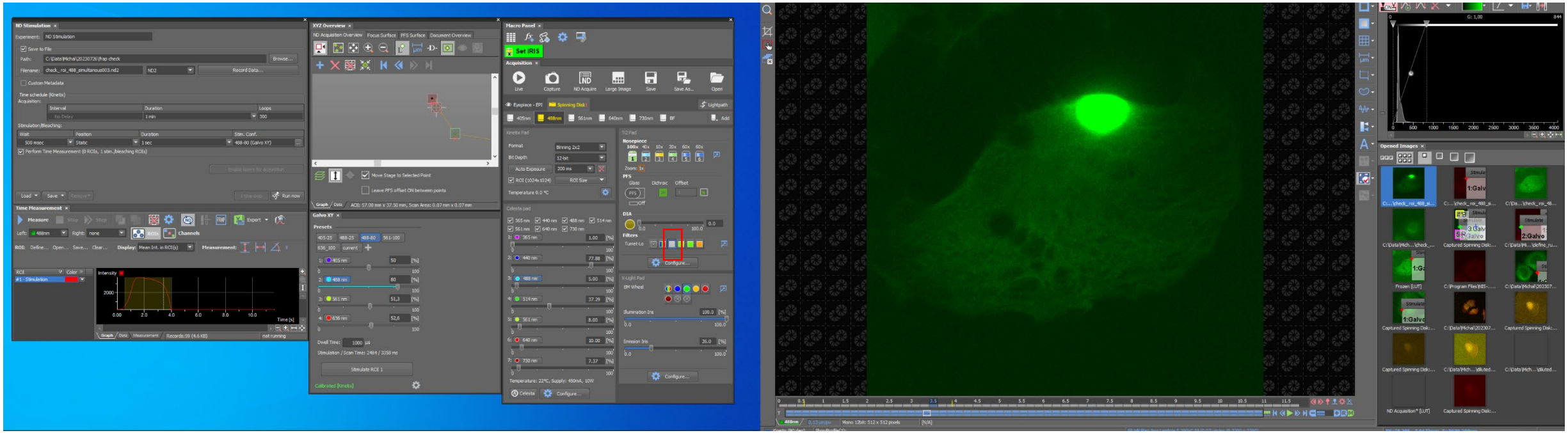
1, Set the total time of acquisition
2, Set bleaching/Stimulation:
Wait : Delay from start of Acq
Position: Static
Duration: Dwell time as optimized
Stim.Conf: your chosen pre-set*

*Make sure it fits your ROI assignement.

Nis Elements- FRAP

Acquisition control- Simultaneous Acquisition/Bleaching

You can Image while Bleaching (Except 561)

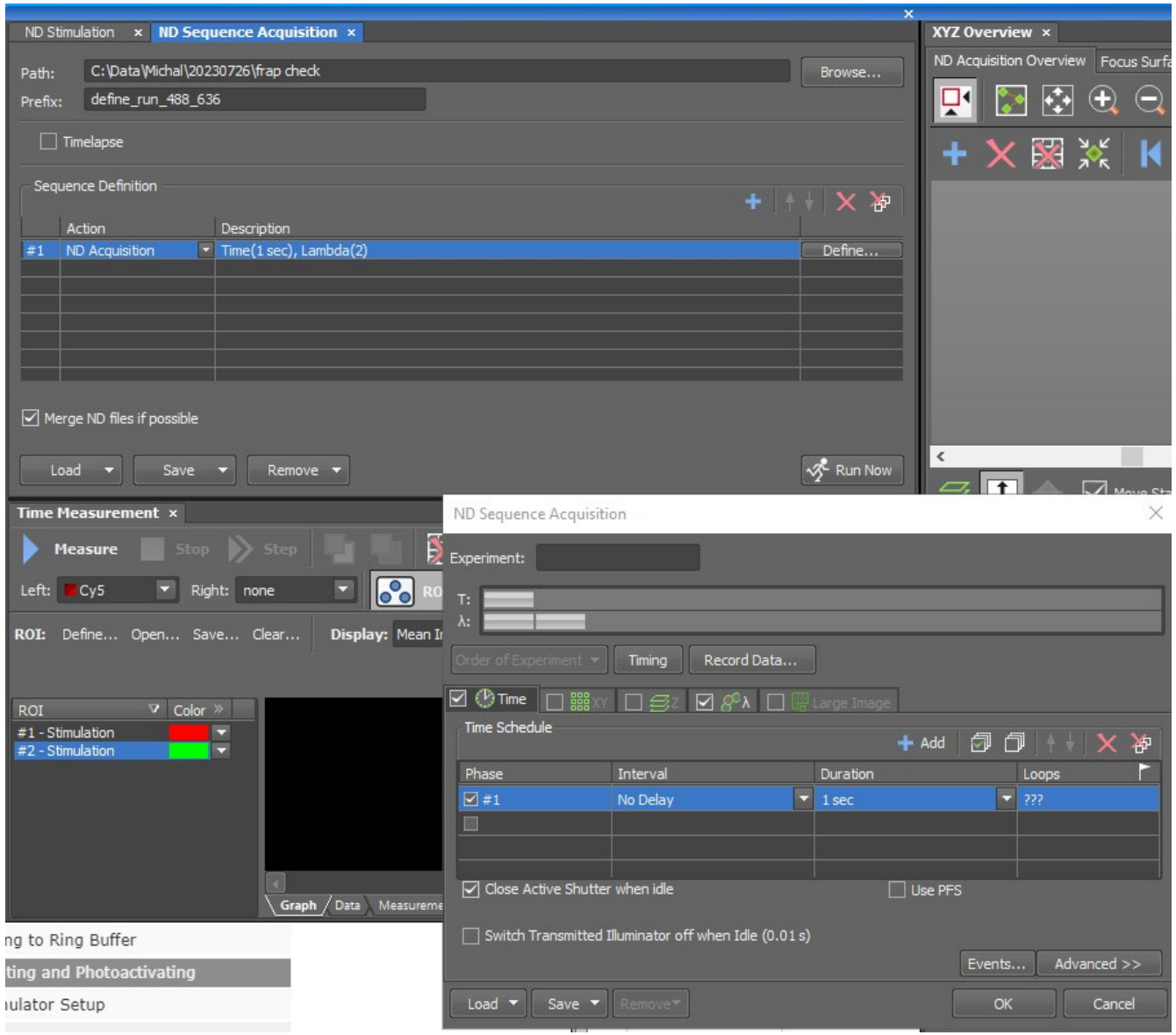


Nis Elements- FRAP

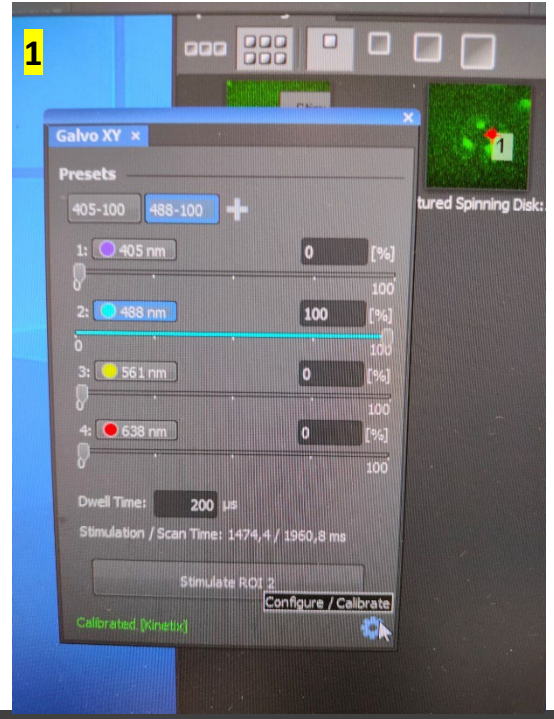
Acquisition control- Sequential imaging

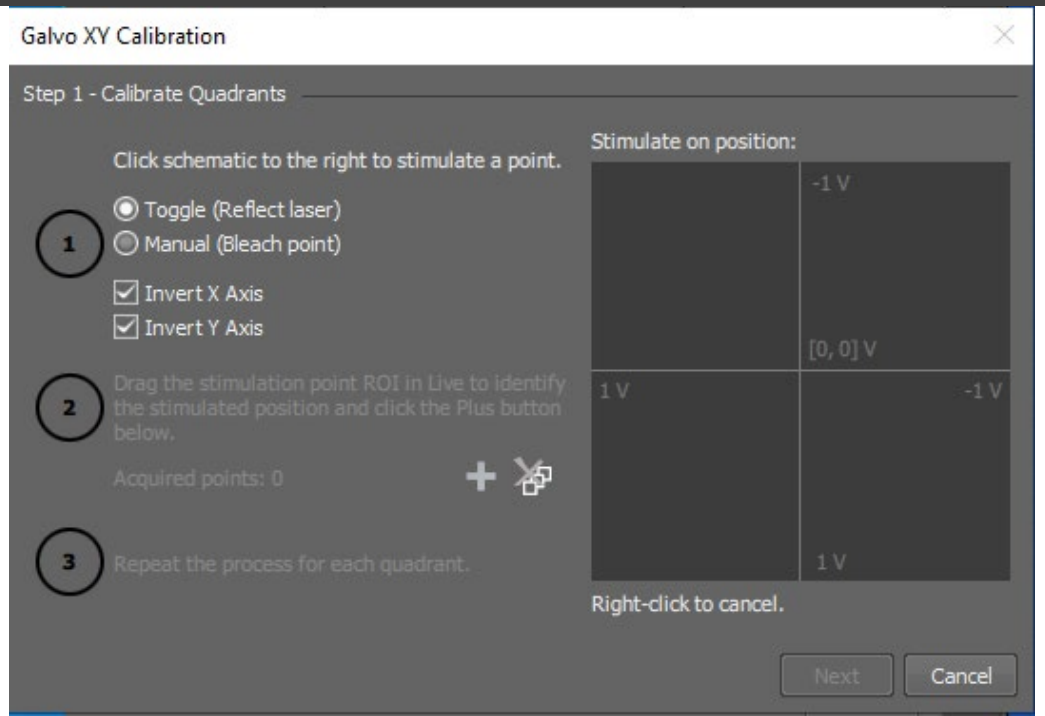
You Can also set sequential ND experiment:

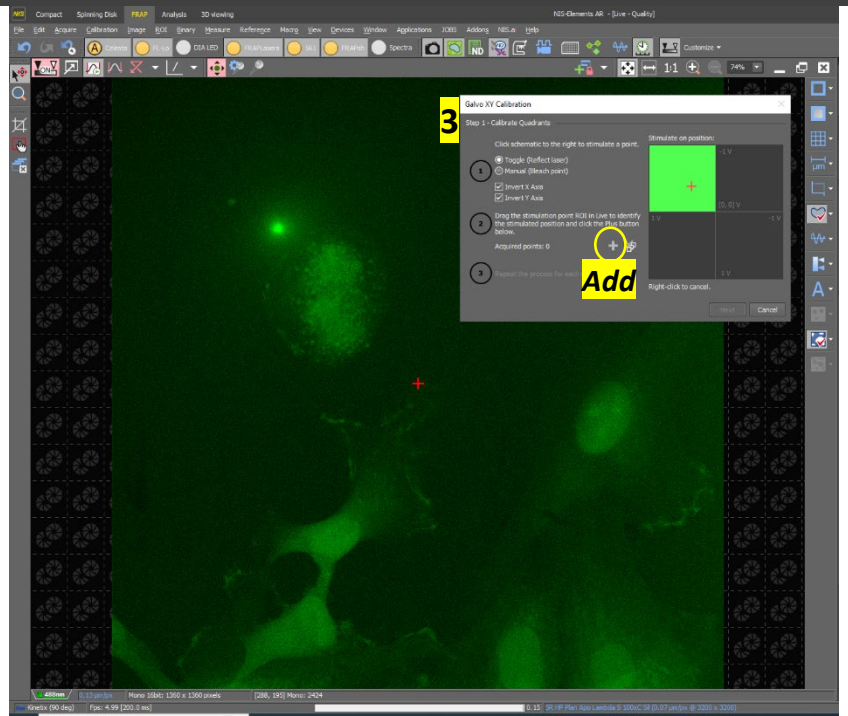
Acquisition
Bleaching
Acquisition



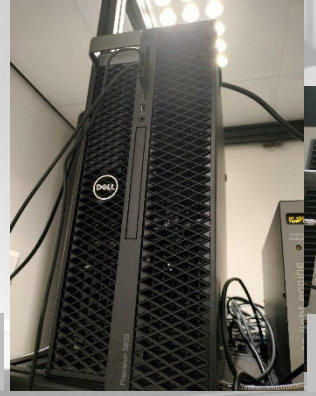
To calibrate FRAP mirrors:
1, go to configure/cal settings in GalvoXY panel
2, set on Invert
3. Click on one of the four quarters to pick a stim location that presents on your live view the stim point. Drag the red cross on it and then click add. Do this for the other quarters as well and repeat for accuracy







Shut down



4. PC

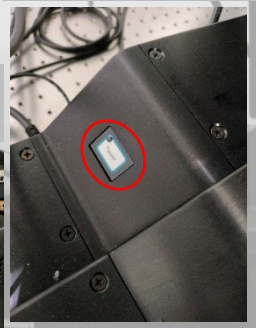
8. Ni Controller

9. main switch

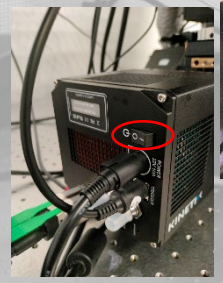
5. FRAP Ctrl

On table, behind

6. SD unit



7. Camera



1. **Save your data** locally and Copy to Bulk folder.
   Note! All local data is erased monthly to keep memory free
2. ESC to minimal Z position
3. Remove your sample; Clean **Objective**; shift to 10X

Don't forget to turn off the chamber light