

A photograph of a Nikon Spinning Disc Confocal microscope setup in a laboratory. The microscope is a white Nikon TE2000 with a black spinning disc unit mounted on top. It is surrounded by various accessories, including a black control box with the 'okolab' logo, a black control box with the 'CrestOptics' logo, and a black control box with the 'KINETIC' logo. The setup is on a black perforated metal table. In the background, there are several white and black electronic devices on a shelf, and a large white speaker on the left. A grey banner with the text 'Nikon Spinning Disc Confocal-User Guide' is overlaid on the top of the image. A diagonal watermark 'Kavli Nanolab Imaging Center' is visible across the center of the image.

Nikon Spinning Disc Confocal-User Guide

Kavli Nanolab Imaging Center

Turn ON- Hardware

5. PC



I. main switch



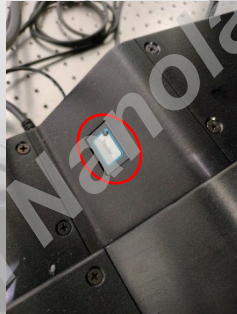
4. FRAP Ctrl



On table,
behind



3. SD unit



2. Camera



Don't forget to turn off the chamber light

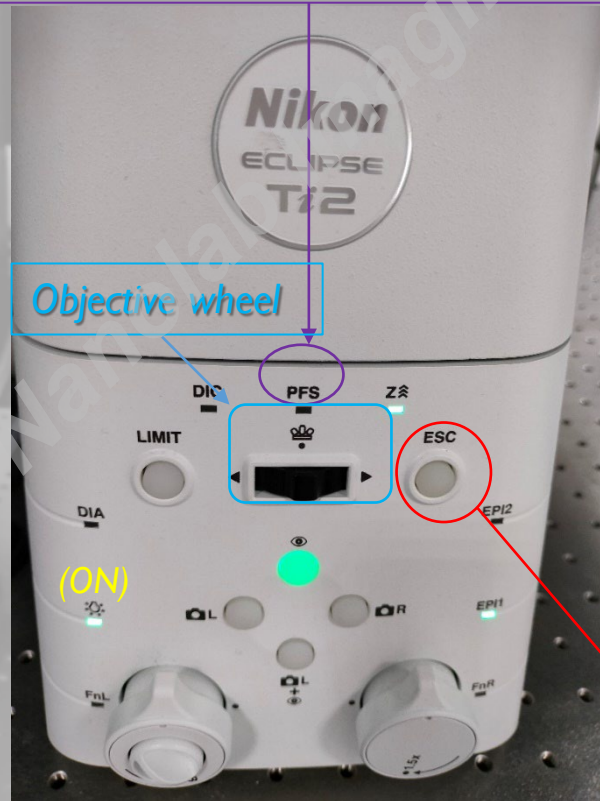
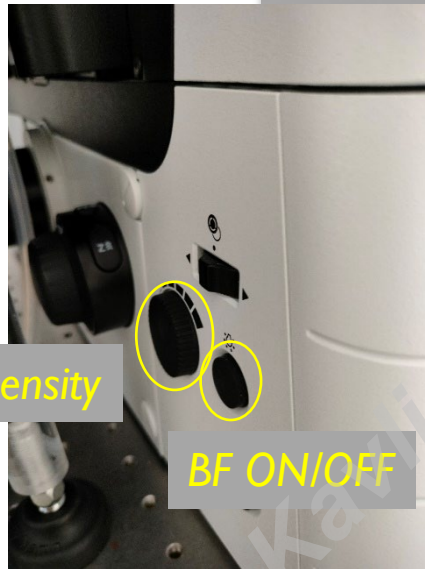


Nikon Ti2- how to



!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



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

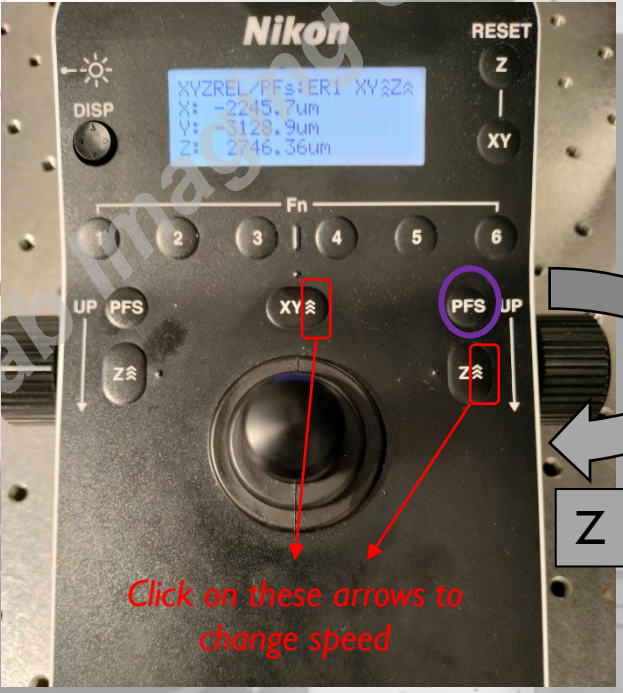
Nikon Ti2- Joystick

1, objective and light path

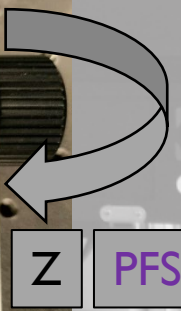
2, Stage positions, PFS



Press from the side to shift info views:



Click on these arrows to change speed



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

Objectives (from Nikon):

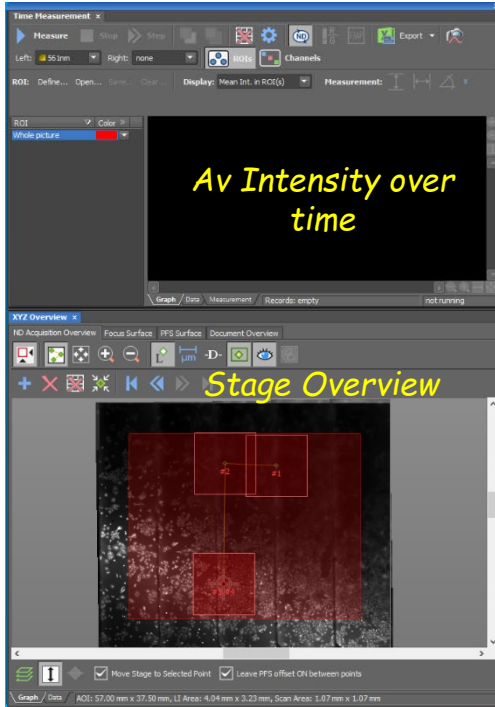
Objective type	Mag/NA	Medium	Contrast	WB (mm)
Plan Achrom	10x / 0.25	Air	Fluo, Ph1	10.5
Plan Apo λ	20x / 0.75	Mimm	Fluo, DIC N2	0.35 (Oil) 0.34 (Gly) 0.33 (W)
Plan Apo λ	60x / 1.4	Oil	Fluo, DIC, Phase	0.13
CFI SR HP Apo λ S	100x / 1.35	Silicon	Fluo, DIC, Phase	0.3

Nis Elements

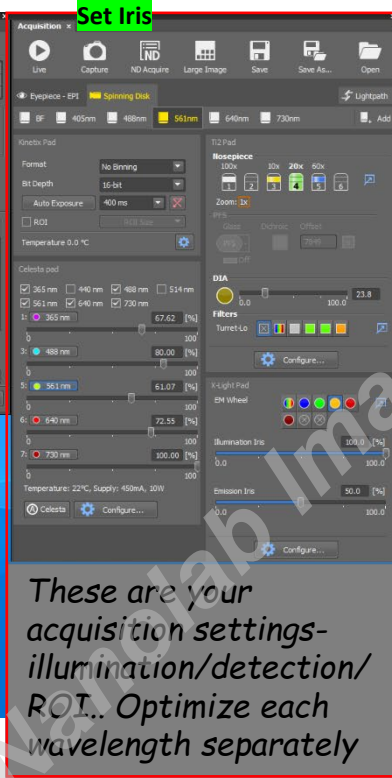
Your Work Interface looks like this:

Acquisition controls

Image controls

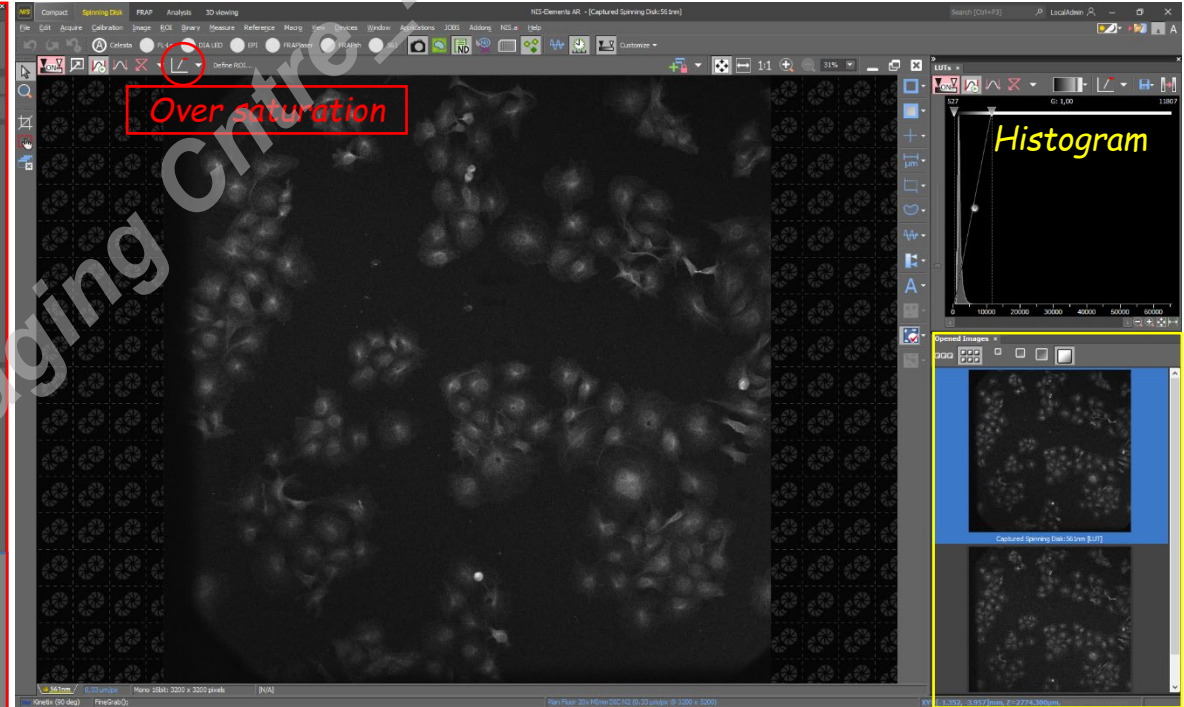


This is your design of experiment:
Time/Z/XY/channels
Save each experiment in your own folder and name it properly



*Set Iris Macro Panel:

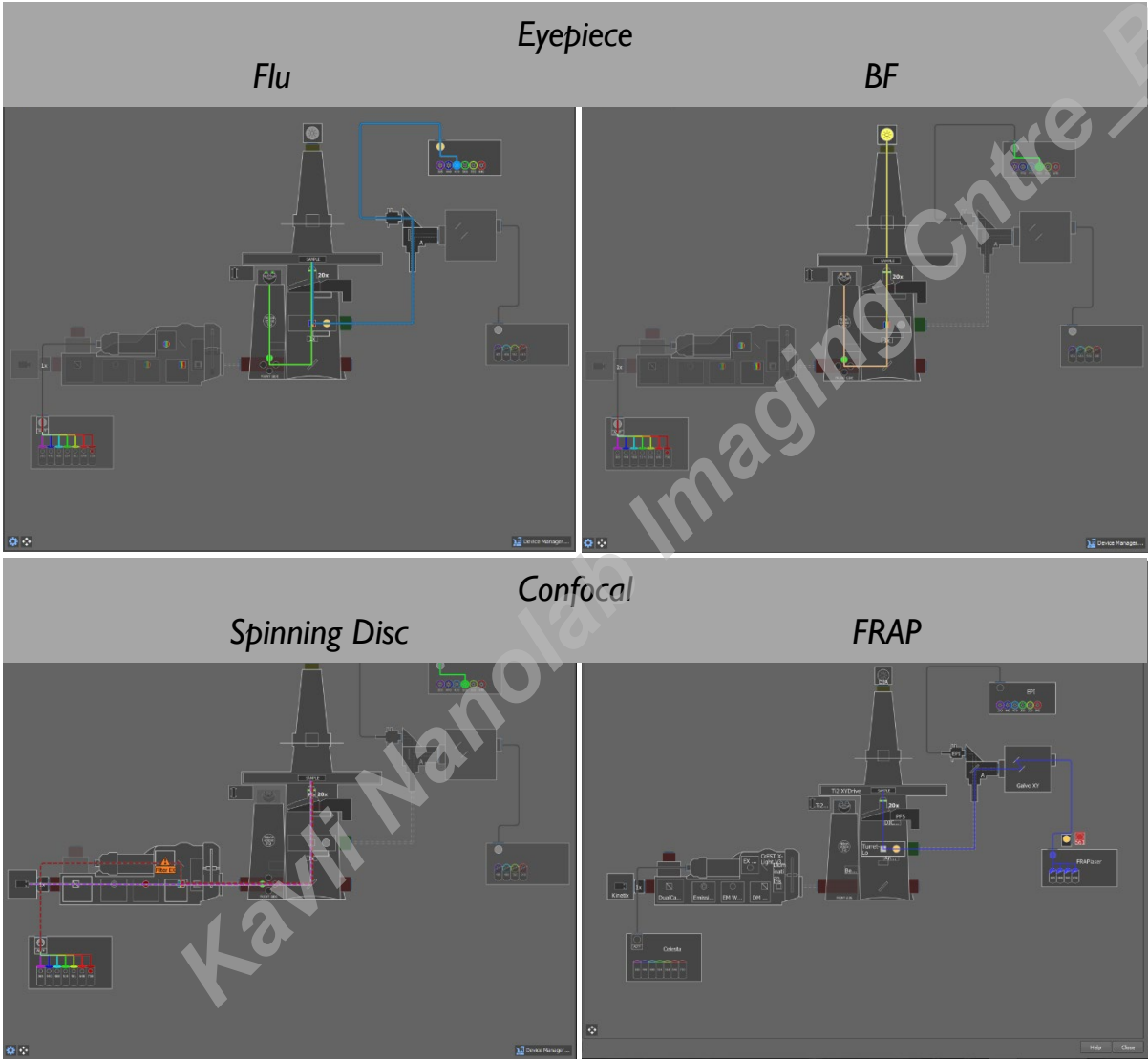
Click this once per objective, to set emission Iris correctly



Here you can toggle between acquired images.
Ctrl "i" shows image information

Nis Elements

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



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).

Acquisition x

Live Capture ND Acquire Large Image Save Save As... Open

Eye-piece - EPI Spinning Disk Lightpath

Brightfield DAPI GFP TxRed Cy5 Add

Kinetix Pad

Format No Binning

Bit Depth 16-bit

Auto Exposure 200 ms

ROI ROI Size

Temperature 0.0 °C

Ti2 Pad

Nosepiece 100x 10x 20x 60x

Zoom: 1x

PFS Glass Dichroic Offset

PFS 78.49

Spectra Pad

395 nm 440 nm 470 nm 508 nm

555 nm 640 nm

1: 395 nm 57 [%]

2: 440 nm 43 [%]

3: 470 nm 52 [%]

4: 508 nm 35 [%]

5: 555 nm 42 [%]

6: 640 nm 9 [%]

DIA Bright Field 0.0 100.0 28.7

Shutters FL-Lo

Filters Turret-Lo Configure...

EPI Configure...

Acquisition x

Live Capture ND Acquire Large Image Save Save As... Open

Eye-piece Eye-piece - DIA Kinetix - EPI Spinning Disk Spinning Disk - HS

Brightfield DAPI GFP TxRed Cy5 Add

Kinetix Pad

Format No Binning

Bit Depth 12-bit

Auto Exposure 500 ms

ROI ROI Size

Temperature 0.0 °C

Ti2 Pad

Nosepiece 100x 10x 20x 60x

Zoom: 1x

PFS Glass Dichroic Offset

PFS 78.49

Spectra Pad

395 nm 440 nm 470 nm 508 nm

555 nm 640 nm

1: 395 nm 18 [%]

2: 440 nm 13 [%]

3: 470 nm 12 [%]

4: 508 nm 24 [%]

5: 555 nm 27 [%]

6: 640 nm 9 [%]

DIA Epi Flu 0.0 100.0 86.4

Shutters FL-Lo

Filters Turret-Lo Configure...

EPI Configure...

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

Nosepiece position	Objective name	Z-Step (Auto Focus)	Z-Step (Slices)	Working distance
1	SR HP Plan Apo Lambda S 100	0.60	0.30	300.00
2	Uncalibrated			
3	Plan 10x	16.00	8.00	10500.00
4	Plan Fluor 20x MImm DIC N2	1.80	0.90	350.00
5	Plan Apo IR 60x WI DIC N2	0.60	0.30	180.00
6	Uncalibrated			

Define optional objectives... OK Cancel

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 exposure time

Missing filters.

Kinetix Pad
Format: No Binning
Bit Depth: 16-bit
Auto Exposure: 400 ms
 ROI (2720x2720) ROI Size
Temperature 0.0 °C

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!

Acquisition x

Live Capture ND Acquire Large Image Save Save As... Open

Eyeiece - EPI Spinning Disk Lightpath

BF 405nm 488nm 561nm 640nm 730nm Add

Kinetix Pad
Format: No Binning
Bit Depth: 16-bit
Auto Exposure: 400 ms
 ROI
Temperature 0.0 °C

Celesta pad
 365 nm 488 nm
 561 nm 640 nm 730 nm
1: 365 nm 67.62 [%]
3: 488 nm 80.00 [%]
5: 561 nm 61.07 [%]
6: 640 nm 72.55 [%]
7: 730 nm 100.00 [%]
Temperature: 22°C, Supply: 450mA, 10W

Nosepiece
100x 10x 20x 60x
Zoom: 1x

PFS
Glass Dichroic Offset
PFS 7849

DIA
0.0 100.0 23.8

Filters
Turret-Lo
Configure...

X-Light Pad
EM Wheel
Illumination Iris 100.0 [%]
0.0 100.0
Emission Iris 50.0 [%]
0.0 100.0

*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!!

Experiment: ND Acquisition

T:
L:
λ:

Save to File
Path: C:\Data\Michal\test capacity PC memory
Filename: timelapse_405no delay 10h003.nd2 ND2

Custom Metadata
Order of Experiment

Time XY Z Large Image λ

Scan Area:
 2 x 2 fields
 6,0 x 6,0 mm
 Pattern

Stitching:
Overlap: 10 % Stitching via: Blending
 Image Registration Use 1.561nm

Close Active Shutter during Stage Movement Use PFS

Time XY Z Large Image λ

Time Schedule

Phase	Interval	Duration	Loops
<input checked="" type="checkbox"/> #1	1 sec	???	1
<input type="checkbox"/>			
<input type="checkbox"/>			
<input type="checkbox"/>			
<input type="checkbox"/>			
<input type="checkbox"/>			

Close Active Shutter when idle Use PFS
 Perform Time Measurement (0 ROIs)
 Switch Transmitted Illuminator off when Idle (0.01 s)

Nis Elements- ND acquisition

Time experiment- use PFS!!

ND Acquisition x

Experiment: ND Acquisition

T:

M:

λ:

Save to File

Path: C:\Data\Michal\test capacity PC memory

Filename: timelapse_405no delay 10h003.nd2 ND2

Custom Metadata

Order of Experiment

Time XY Z Large Image λ

Time Schedule

Phase	Interval	Duration	Loops
<input checked="" type="checkbox"/> #1	1 sec	???	1
<input type="checkbox"/>			

Close Active Shutter when idle

Perform Time Measurement (0 ROIs)

Switch Transmitted Illuminator off when Idle (0.01 s)

Use PFS

1 time loop

Set wavelengths (taken from your acq parameters)

ND Acquisition x

Experiment: ND Acquisition

T:

M:

λ:

Save to File

Path: C:\Data\Michal\test capacity PC memory

Filename: timelapse_405no delay 10h003.nd2 ND2

Custom Metadata

Order of Experiment

Time XY Z Large Image λ

Setup

Opt. Conf.	Name	Comp. Color	T Pos.	Focus Off...
<input checked="" type="checkbox"/> Spin...:561nm	561nm		All	
<input checked="" type="checkbox"/> Spin...:488nm	488nm		All	0
<input checked="" type="checkbox"/> Spin...:405nm	405nm		All	0
<input type="checkbox"/>				

Close Active Shutter during Filter Change

Use Ratio

Use PFS Use Trig. Acq.

1 time loop

Nis Elements- ND acquisition

Z stack

Absolute- Piezo

ND Acquisition x

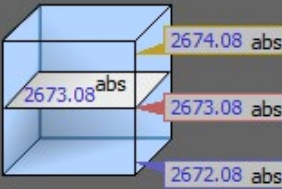
Experiment: ND Acquisition

T:
M:
λ:

Save to File
Path: C:\Data\Michal\test capacity PC memory
Filename: timelapse_405no delay 10h003.nd2 ND2

Custom Metadata
Order of Experiment

Time XY Z Large Image λ



Step: 0,3 μm ← 0,9 μm 8 Steps Range: 2,00 μm
Bottom: 2672.08 μm Top: 2674.08 μm
Z Device: Ti2 ZDrive
Relative Positions:
Top: +1.00 μm
Bottom: -1.00 μm

Close Active Shutter during Z Movement Direction: Bottom to Top Top to Bottom

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

ND Acquisition x

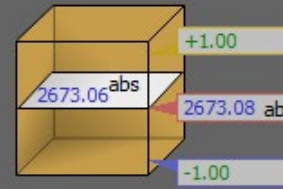
Experiment: ND Acquisition

T:
M:
λ:

Save to File
Path: C:\Data\Michal\test capacity PC memory
Filename: timelapse_405no delay 10h003.nd2 ND2

Custom Metadata
Order of Experiment

Time XY Z Large Image λ



Step: 0,3 μm ← 0,9 μm 9 Steps Range: 2,00 μm
Relative Positions:
Top: +1.02 μm
Bottom: -0.98 μm

Close Active Shutter during Z Movement Direction: Bottom to Top Top to Bottom

Nis Elements- stage overview tools

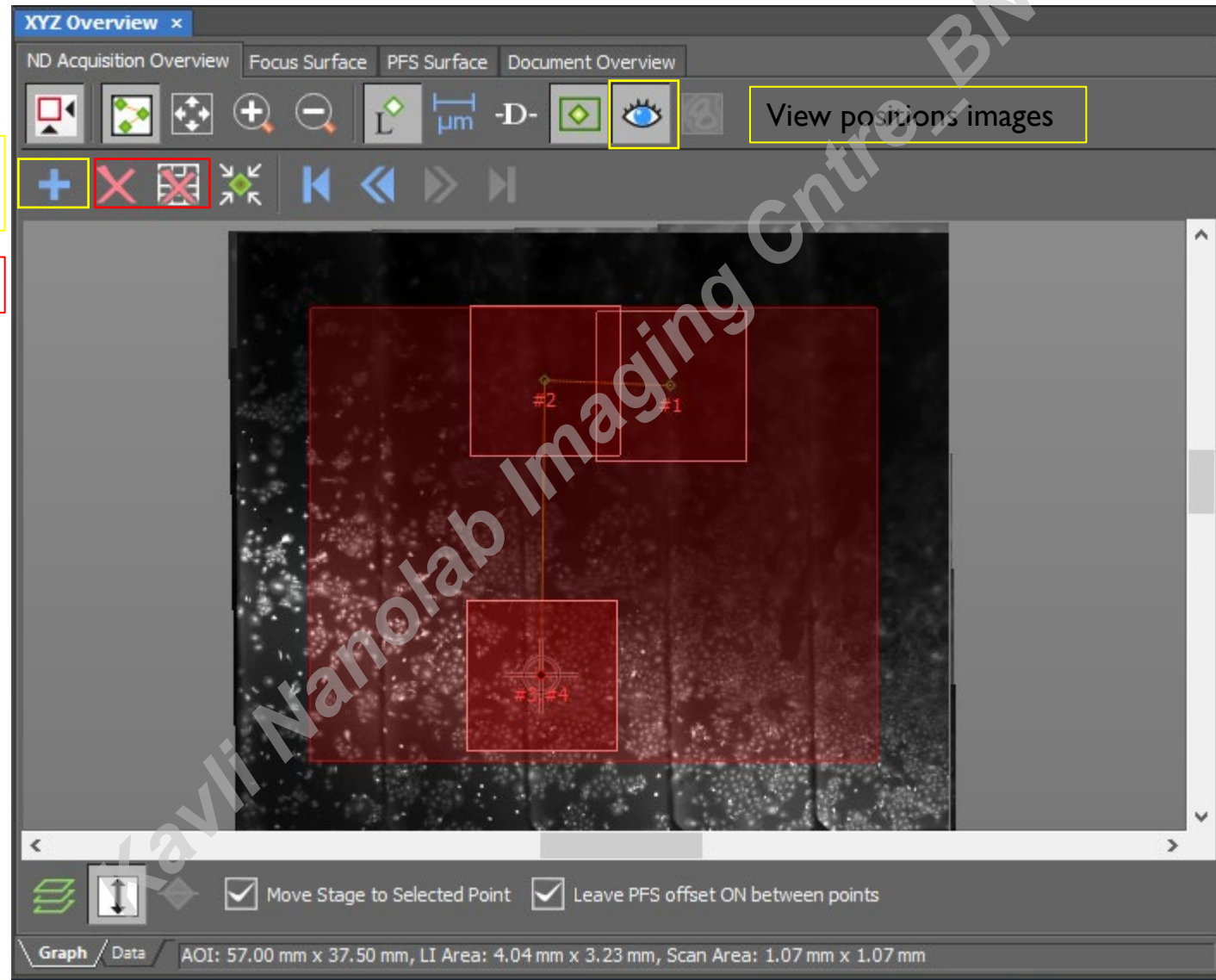
You can run fast preview scan or large image scan

Add new position
(also added to ND acq list)

Delete one point or all

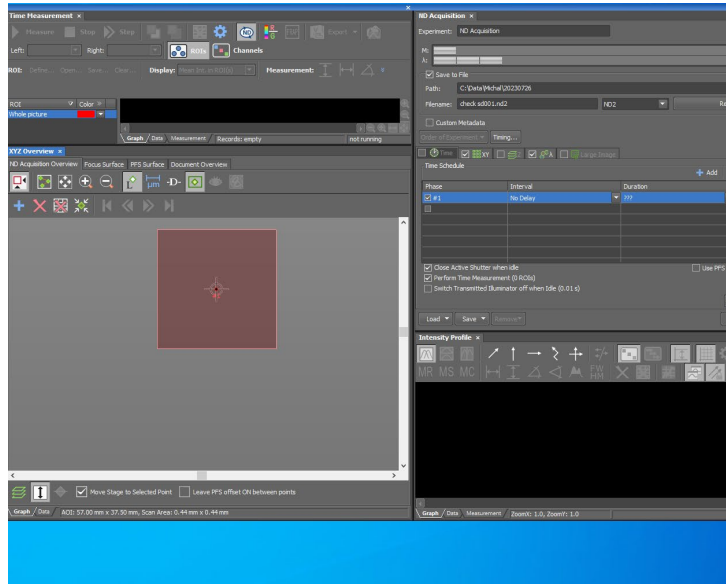
Doble click on position will move
the stage there

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

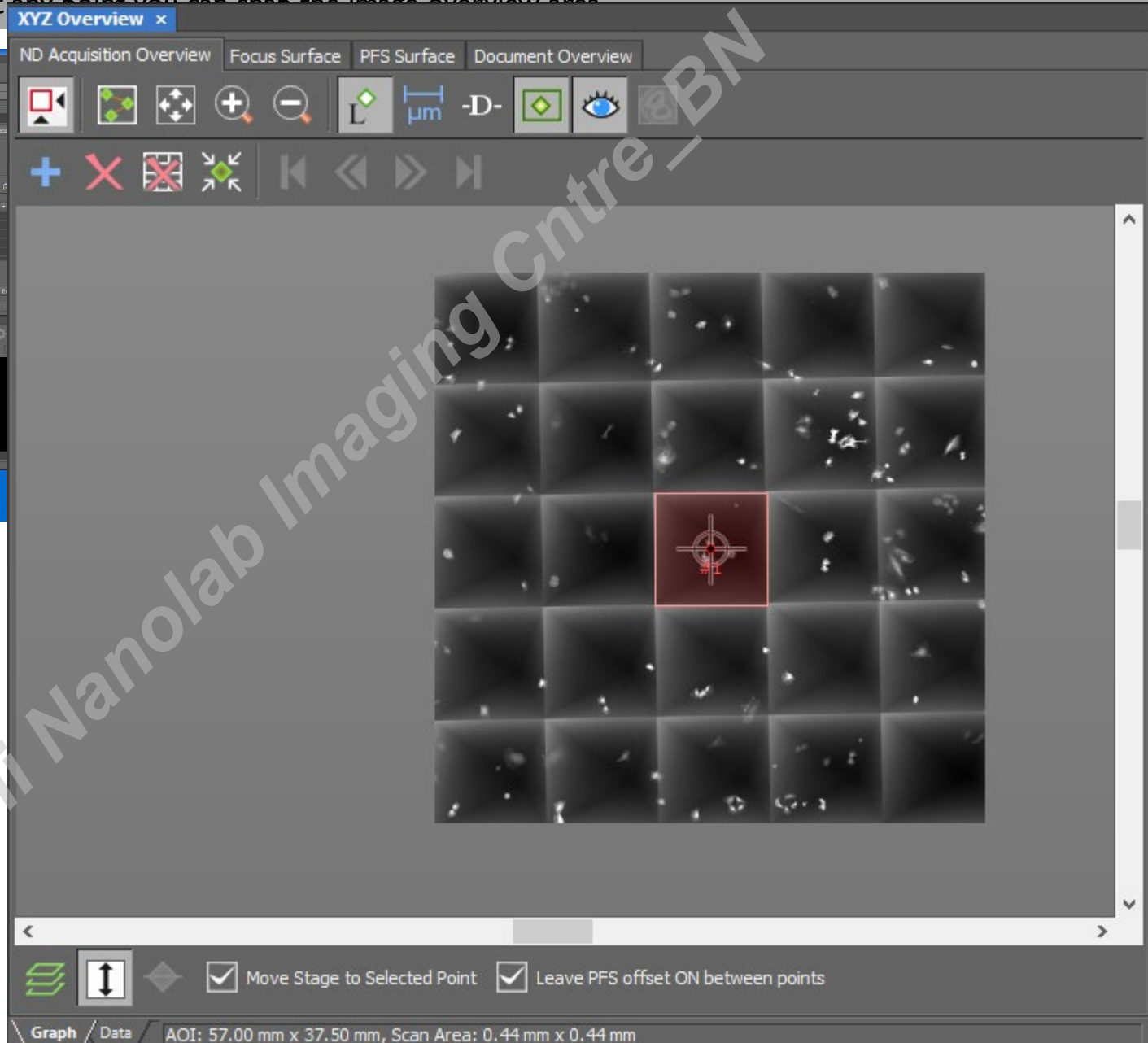


Nis Elements- stage overview tools

At any point you can open the image overview area



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

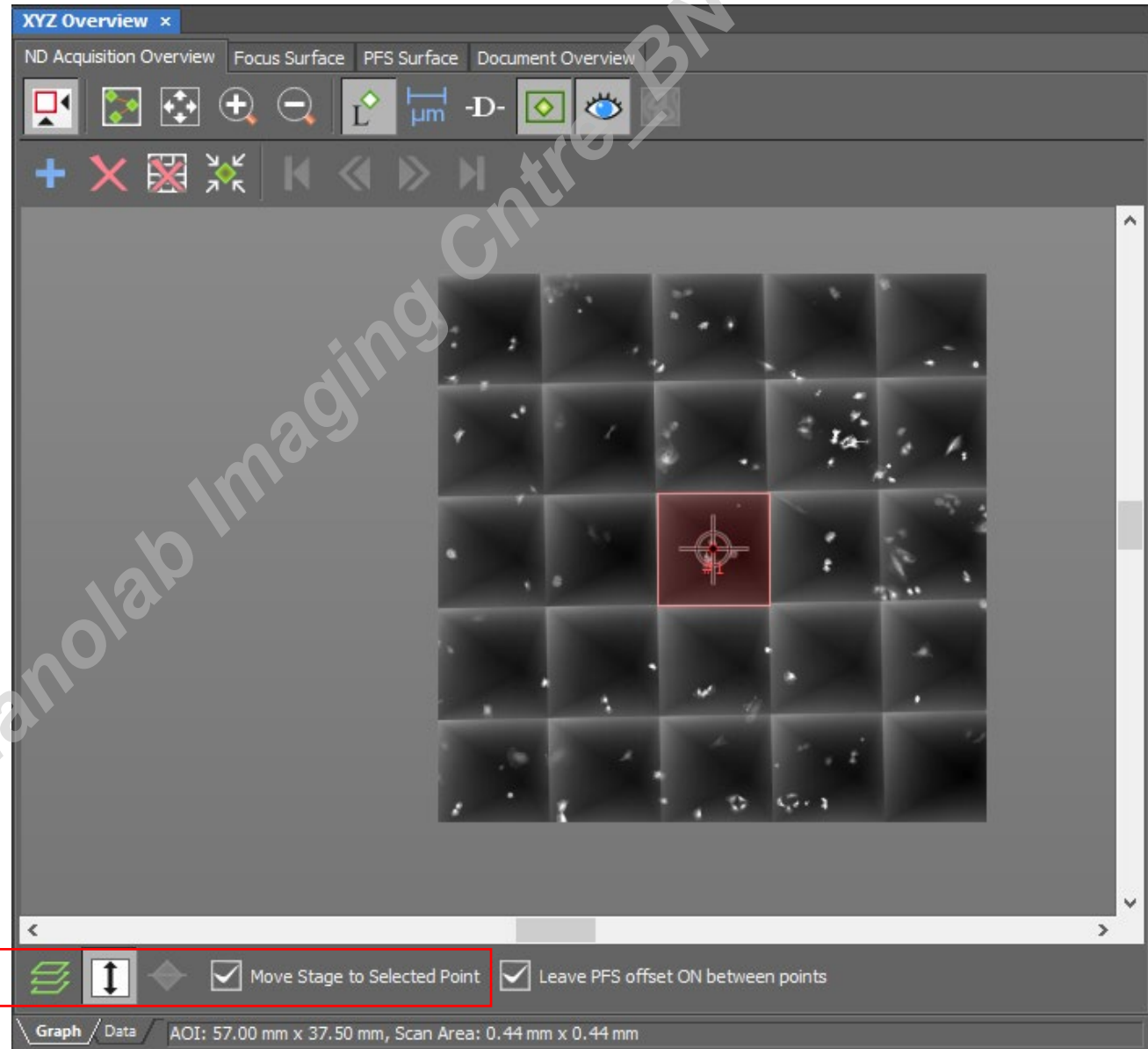


Nis Elements- stage overview tools

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:

Scan Large Image

Capturing
Macro Image
Optical conf.: <current> Objective: 4: 20x
Scanning
Optical conf.: <current> Objective: 2: 40x

Area
Large image area in XYZ Overview
5 x 4 fields, 5744 x 4302 pixels, 1,87 x 1,4 mm, 47 MB of memory

Close active shutter during stage movement
 Correct XY Offset between Macro and Scanning Objective
 Wait after Stage Movement: 0 ms

Stitching
Overlap: 10 %
Stitching via: Blending
 Image registration

Create large image
 Store single images
 Create both

Save large image to file
 Save to Auto capture folder
Format: nd2
Filename: C:\Data\Biond\002-001\A4_large_001.nd2
Storage for single images:
Folder: C:\Data\Michal\test capacity PC memory\Large Image
Format: tif

None
 HDR Steps: 5
 Z Series Count: 5
 Max IP Step: 0,9 µm ± 0,900 µm

EDF:
Z-drive: Ti2 ZDrive
Order: Z series per channel

Opt. Conf.	Name	Comp. ...
<input checked="" type="checkbox"/> Spin...561nm	561nm	

Stitch using channel: 561nm

Focus
 Focus manually at start
 Use Focus Surface
 Use step-by-step focus Setup...
 Focus manually
every 1 field
after 3 mm distance
 Skip frames without sample
 Manually locate sample and background at start

Optical conf.: Spinning Disk:561nm

Scan Close Help

XYZ Overview
ND Acquisition Overview Focus Surface PFS Surface Document Overview

Selected Point Leave PFS offset ON between points
m, LI Area: 1.43 mm x 0.96 mm, Scan Area: 0.44 mm x 0.44 mm

Annotations:
- Define area boundaries
- You can image several Z plans as Z stack or max projection +
- Channel setup
- Define stitching method
- You can keep both raw and stitched images

Nis Elements- stage overview tools

Options to define area

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

The image displays three panels from the NIS Elements software interface, illustrating different methods to define a scan area:

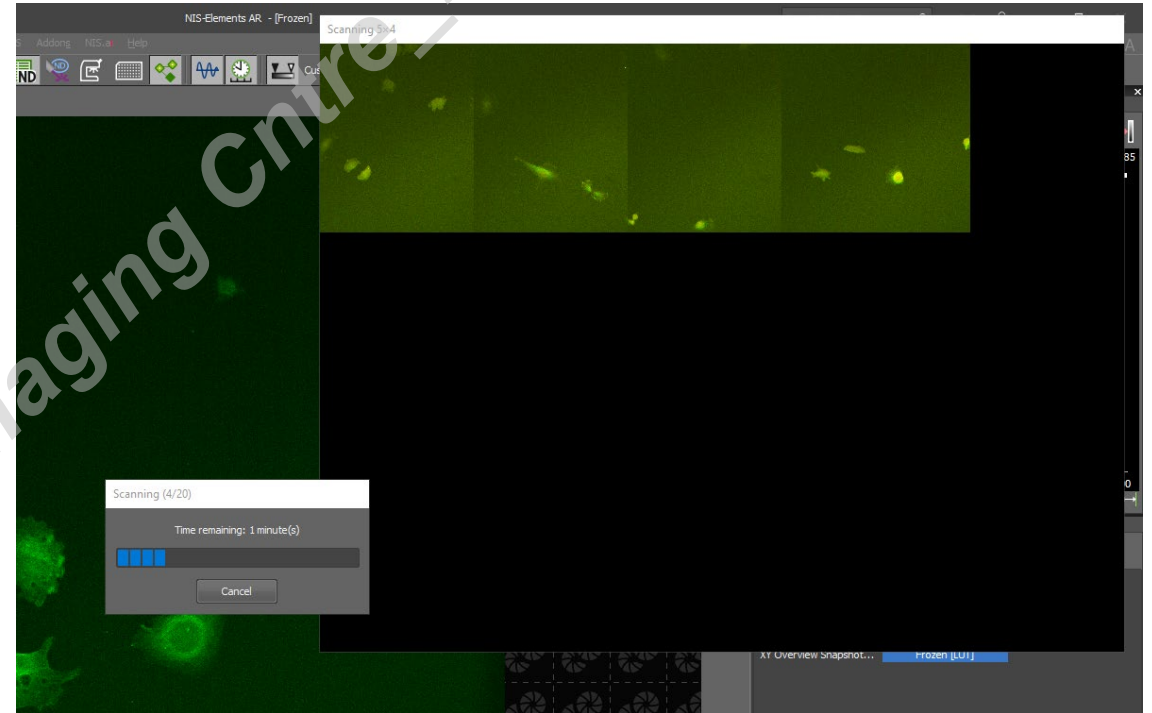
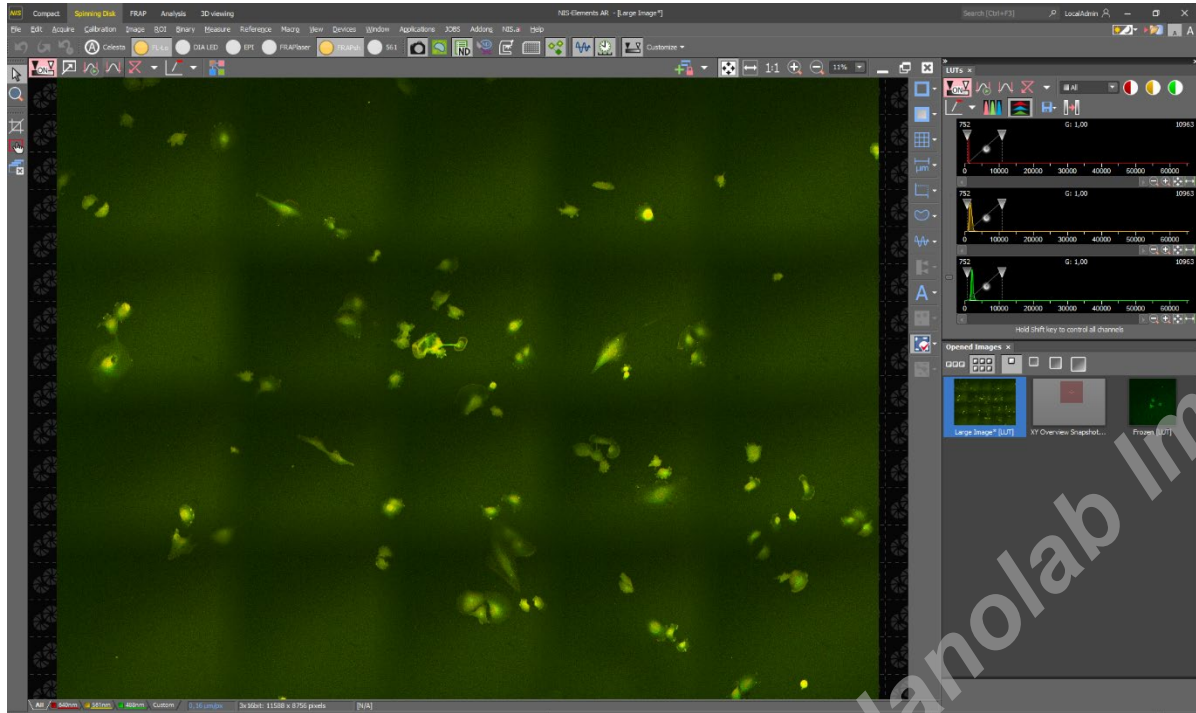
- Panel 1 (Top Left):** Shows the 'Area' settings for a 3x3 grid. The 'Left, top, right and bottom limits' dropdown is highlighted with a red '2'. The area is defined by coordinates: X: [0.7797, -0.1667] and Y: [-0.2125, 0.792]. It specifies 3 x 3 fields, 3052 x 2949 pixels, 0,99 x 0,96 mm, and 17 MB of memory.
- Panel 2 (Bottom Left):** Shows the 'Area' settings for a 4x4 grid. The 'Number of fields in X and Y' dropdown is highlighted with a red '3'. It specifies 4 x 4 fields, 5032 x 5032 pixels, 1,64 x 1,64 mm, and 48 MB of memory. Fields placement options include 'Around the current position' (selected), 'Current position is at top-left corner', and 'Create both'.
- Panel 3 (Center):** Shows the 'Scan Large Image' dialog. The 'Area' dropdown is set to 'Large image area in XYZ Overview' and is highlighted with a red '1'. A red box around this dropdown is labeled 'Define area boundaries'. The dialog also shows a 'Stage Overview with Macro Image' window and various capture and stitching options.

The 'Scan Large Image' dialog includes the following sections:

- Capturing:** Macro Image (Optical conf.: <current>, Objective: 4: 20x), Scanning (Optical conf.: <current>, Objective: 2: 40x).
- Area:** Large image area in XYZ Overview (5 x 4 fields, 5744 x 4302 pixels, 1,87 x 1,4 mm, 47 MB of memory).
- Stitching:** Overlap: 10%, Stitching via: Blending, Image registration (checked).
- Shading Correction:** Off (not available) (selected), Automatic Shading Correction (unchecked).
- Storage:** Create large image (selected), Save large image to file (unchecked), Save to Auto capture folder (selected), Filename: C:\Data\Biond\002-001\A4_large_001.nd2, Format: nd2, Folder: C:\Data\Michal\test capacity PC memory\Large Image, Format: tif.
- Combine with:** Max IP (selected), Steps: 5, Count: 5, Step: 0,9 µm, Z-drive: T12 ZDrive, Order: Z series per channel.
- Focus:** Focus manually (unchecked), Use step-by-step focus (unchecked), Focus manually (unchecked), every 1 field.

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”



Kavli Nanolab Imaging Centre - BN

Nis Elements- Stage Overview tool

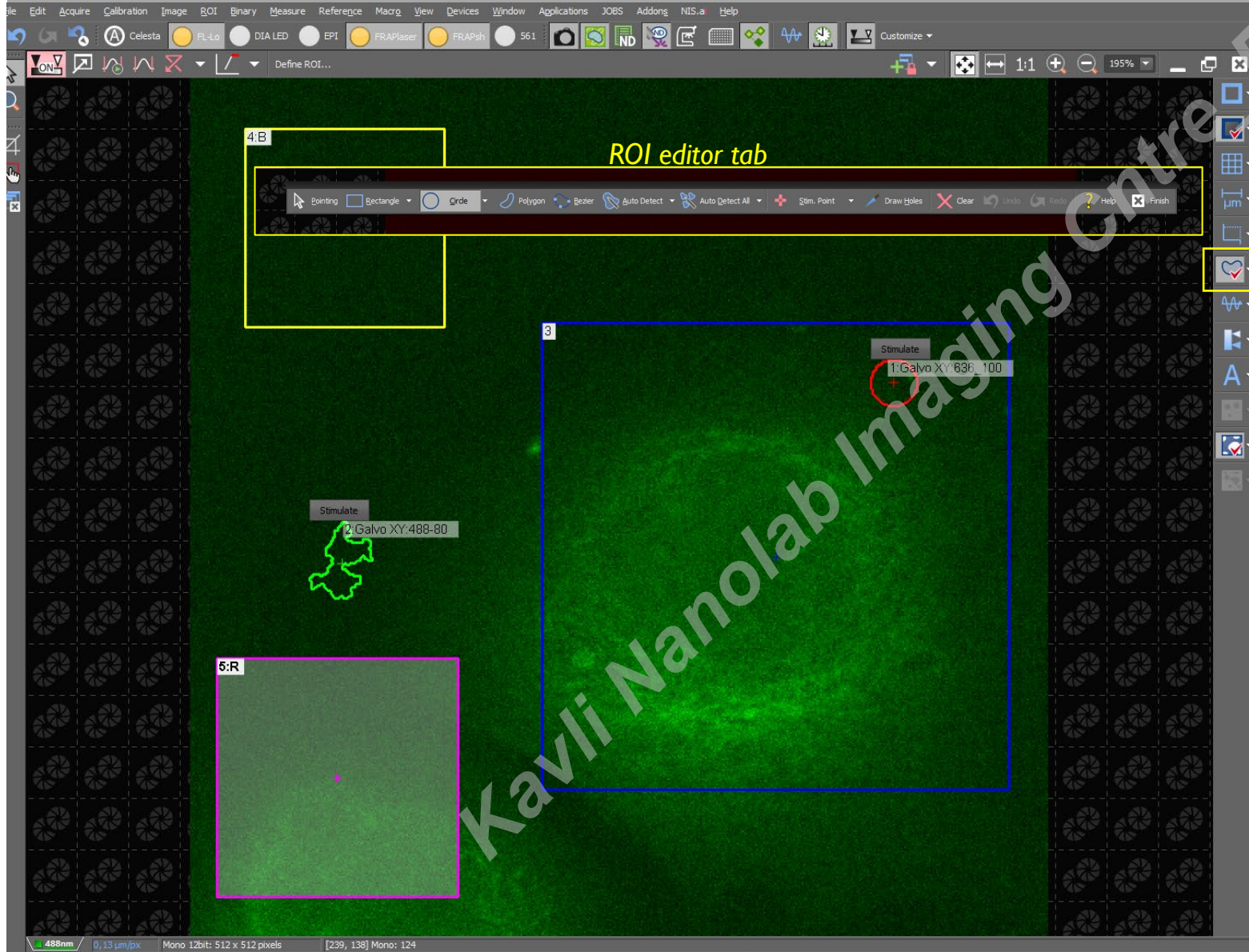
Create focus map

The screenshot shows the NIS Elements software interface. The top panel is 'Time Measurement' with buttons for Measure, Stop, Step, and various icons. Below it are ROI and Channels settings. The 'XYZ Overview' panel is active, showing 'Focus Surface' selected. A color scale on the right indicates Z-height in micrometers, ranging from 3027,2 to 3037,7. A graph at the bottom shows Z-height data for points #1 to #7, with a red dashed line at 3028,1. A text box with a small focus map icon says: 'Create Focus surface- add position with different Z, the software will interpolate trend'.

This screenshot shows a zoomed-in view of the 'XYZ Overview' panel. The 'Focus Surface' tab is highlighted. A yellow box contains the text: 'You can apply the same with PFS'. The main area displays a 3x3 grid of grayscale images with a central focus map. A color scale on the right shows Z-height values from 3027,6 to 3037,7. Below the grid, there are controls for 'Interpolation method' and 'Move Stage to Selected Point'. A graph at the bottom shows Z-height data for points #1 to #10, with a red dashed line at 3032,5.

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)

Nis Elements- FRAP

Acquisition control- Simultaneous Acquisition/Bleaching

Galvo XY

Presets: 405-25, 488-25, 488-80, 561-100

1: 405 nm, 50 [%]
2: 488 nm, 80 [%]
3: 561 nm, 51,3 [%]
4: 636 nm, 52,6 [%]

Dwell Time: 200 µs
Stimulation / Scan Time: 1073,6 / 1478,8 ms

Stimulate ROI 1

Calibrated [Kinetix]

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.

ND Stimulation

Experiment: ND Stimulation

Save to File: Path: C:\Data\Michal\20230726\frap check, Filename: check_roi_488_simultaneous003.nd2, ND2, Record Data...

Time schedule (Kinetix) 1

Interval	Duration	Loops
No Delay	1 min	300

Stimulation/Bleaching: 2

Wait	Position	Duration	Stim. Conf.
500 msec	Static	1 sec	488-80 (Galvo XY)

Perform Time Measurement (0 ROIs, 1 stim./bleaching ROIs)

Enable lasers for acquisition

Load Save Remove 1 time loop Run now

Time Measurement

Measure Stop Step ROIs Channels

ROI: Define... Open... Save... Clear... Display: Mean Int. in ROI(s) Measurement:

ROI: #1 - Stimulation Color: Red

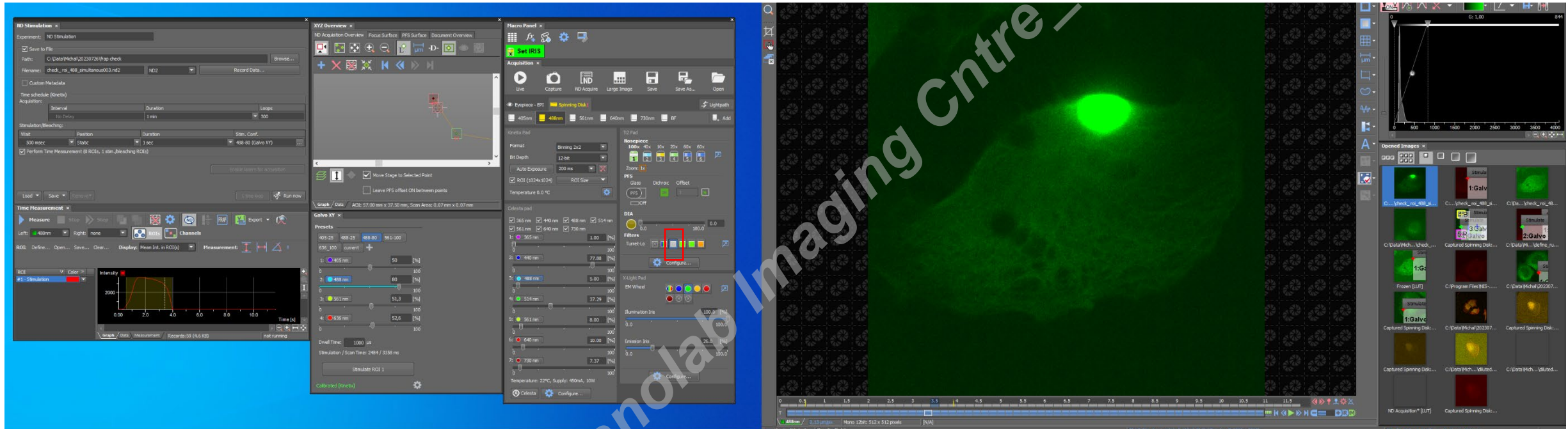
Intensity vs Time [s] graph showing a peak at ~2.5s and a drop at 4.0s.

Graph / Data / Measurement / Records:59 (4,6 KB) not running

Nis Elements- FRAP

Acquisition control- Simultaneous Acquisition/Bleaching

You can Image while Bleaching (Except 56 I)



Nis Elements- FRAP

Acquisition control- Sequential imaging

You Can also set sequential ND experiment:

- Acquisition
- Bleaching
- Acquisition

The screenshot displays the NIS Elements software interface for configuring a sequential ND experiment. The main window is titled "ND Sequence Acquisition" and shows the following details:

- Path:** C:\Data\Michal\20230726\frap check
- Prefix:** define_run_488_636
- Timelapse
- Sequence Definition:** A table with columns "Action" and "Description". The first row is "#1 ND Acquisition" with a description of "Time(1 sec), Lambda(2)".
- Merge ND files if possible
- Buttons: Load, Save, Remove, Run Now

The "Time Measurement" window is also visible, showing:

- Buttons: Measure, Stop, Step
- Left: Cy5, Right: none
- ROI: Define..., Open..., Save..., Clear..., Display: Mean I
- ROI list: #1 - Stimulation (red), #2 - Stimulation (green)
- Buttons: Graph, Data, Measure

The "ND Sequence Acquisition" dialog box is open, showing:

- Experiment: [empty]
- T: [empty], λ: [empty]
- Buttons: Order of Experiment, Timing, Record Data...
- Options: Time, XY, Z, λ, Large Image
- Time Schedule:** A table with columns "Phase", "Interval", "Duration", and "Loops". The first row is "#1" with "No Delay", "1 sec", and "???".
- Options: Close Active Shutter when idle, Use PFS, Switch Transmitted Illuminator off when Idle (0.01 s)
- Buttons: Events..., Advanced >>
- Buttons: Load, Save, Remove, OK, Cancel

ng to Ring Buffer

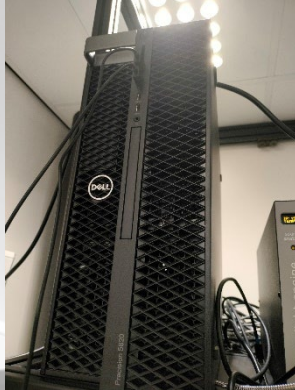
ting and Photoactivating

ulator Setup

Shut down

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

4. PC



8. main switch



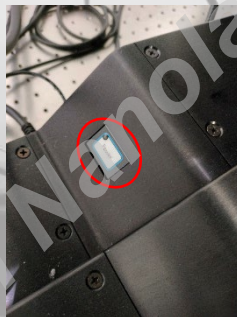
5. FRAP Ctrl



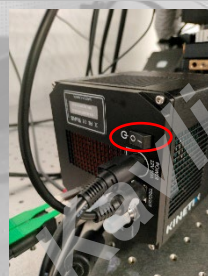
On table,
behind



6. SD unit



7. Camera



Don't forget to turn off the chamber light

