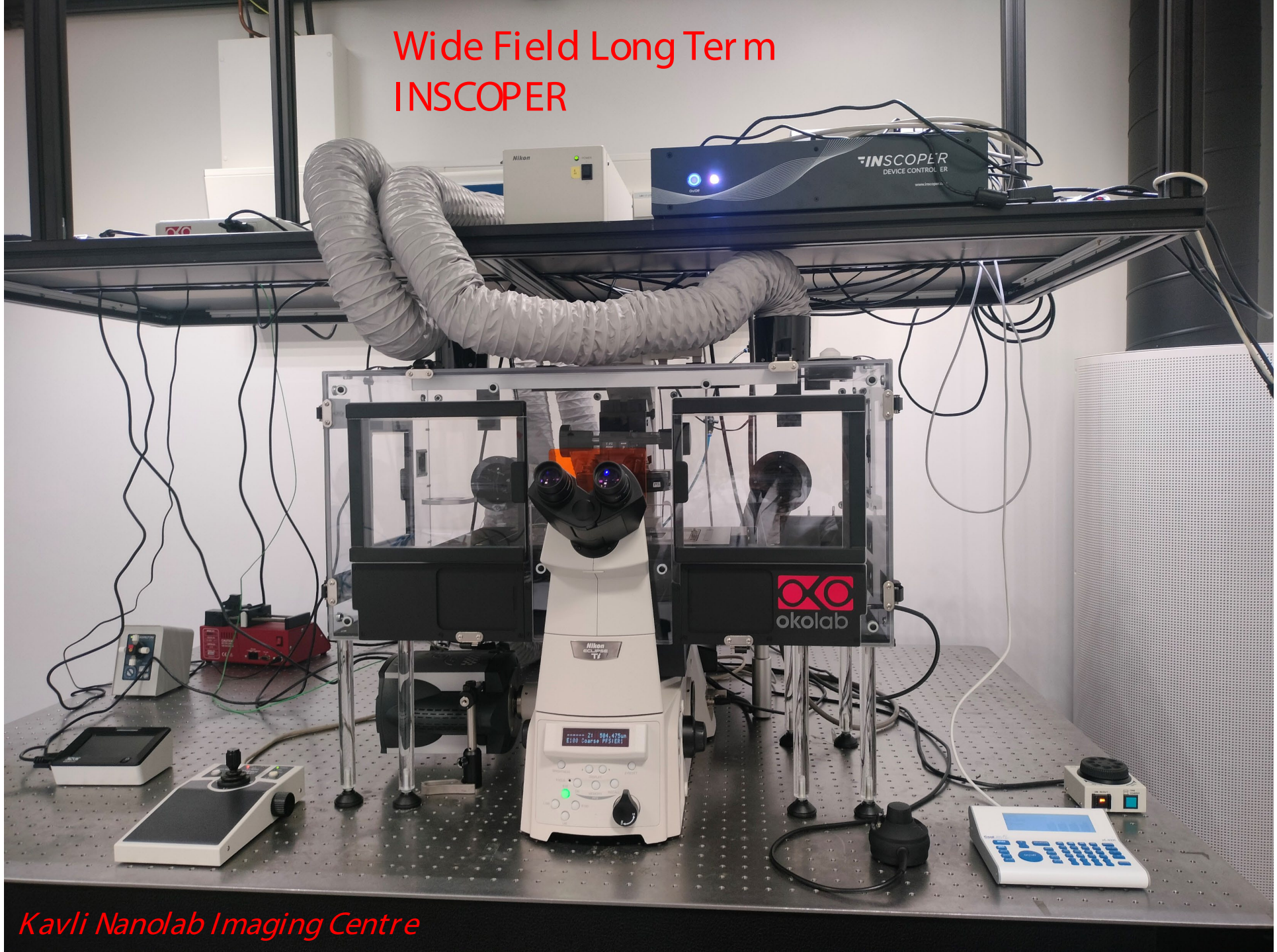


Kavli Nanolab Imaging Centre

Start Up

1, Turn on 2 switches

2, Turn on Ni body

3, Turn on INSCOPER controller

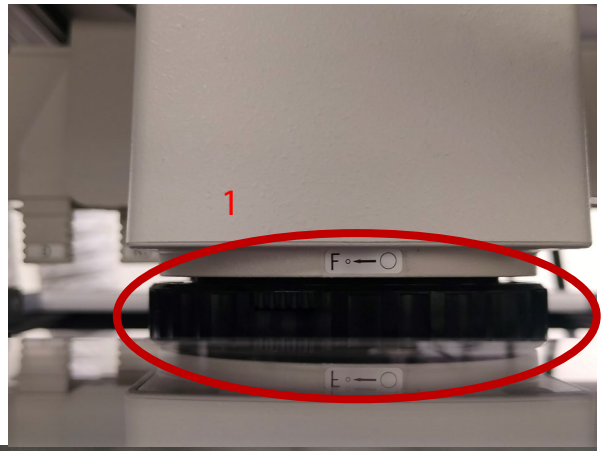
4, Turn on PC and log in with User ID

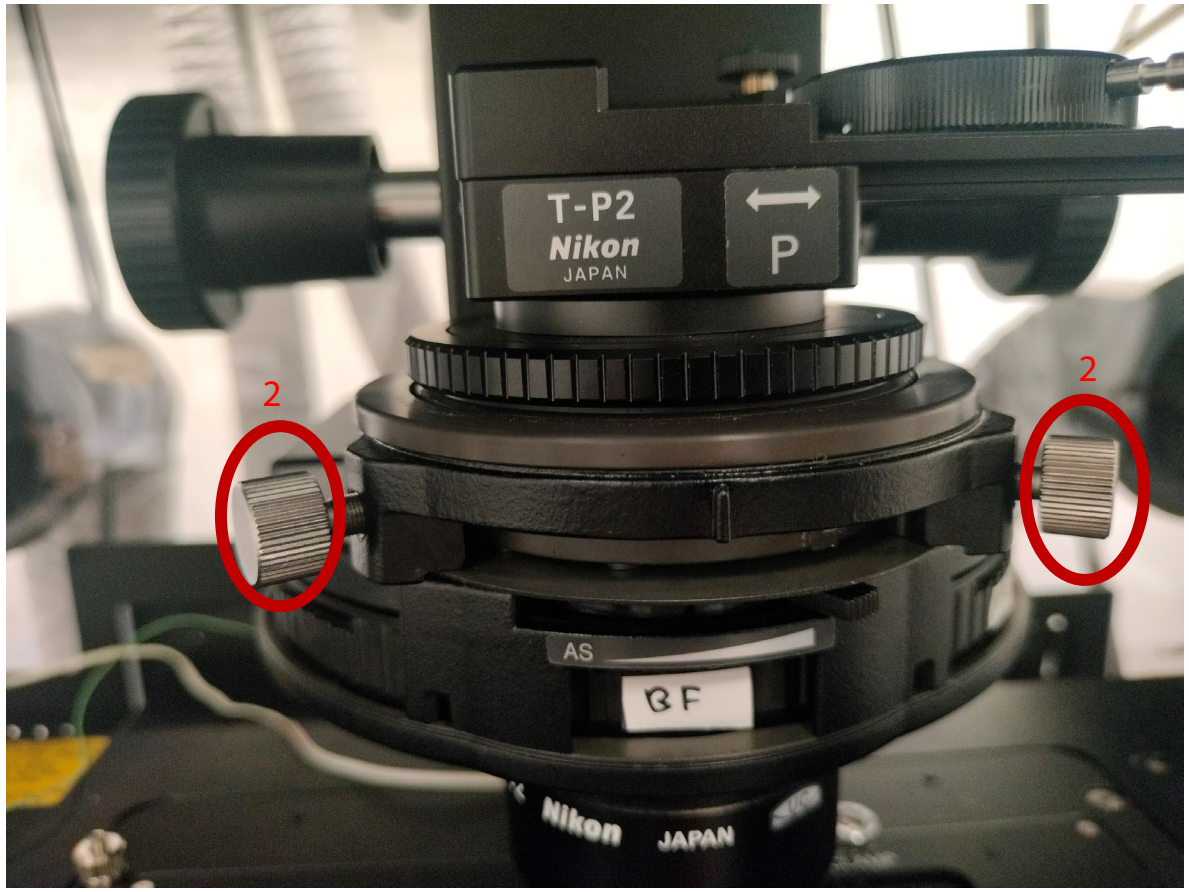
Note: Inscoper Box should show green light before you start the software

For Phase imaging

Suitable Objective: 100X/1,45 Ph3
Take ND filter out to increase light intensity
Move condenser manually to plate ph3

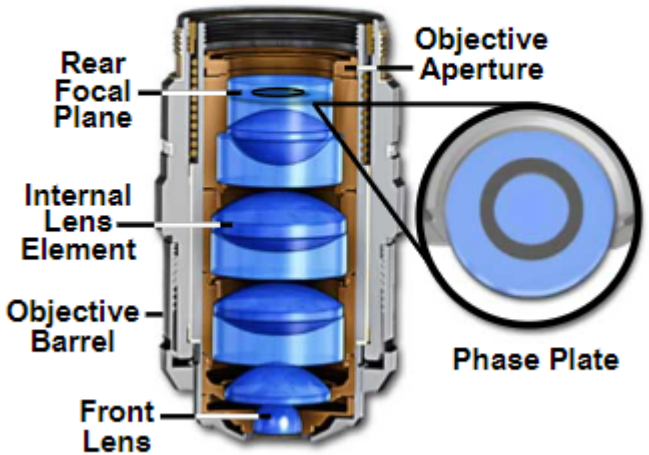
To align: Place 10X objective, close the shutter (#1) until you see the edge
Move the Condenser height until the edge is sharp
Center with the screws (#2)





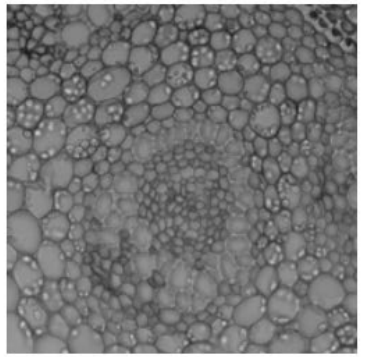
Phase contrast alignment (1/2): Procedure

Figure 4 - Phase Contrast Objective

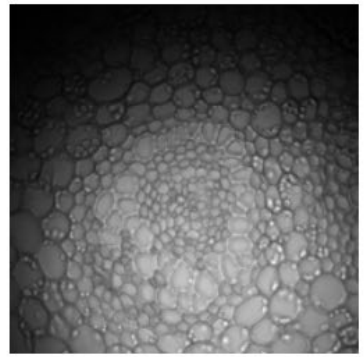


https://www.microscopyu.com/techniques/phase-contrast/phase-contrast-microscope-configuration

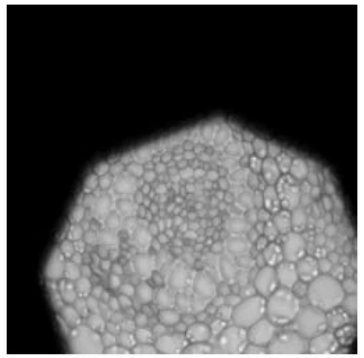
1- Choose either the 10x, 20x or 40x objectives, as this procedure won't work with higher magnification



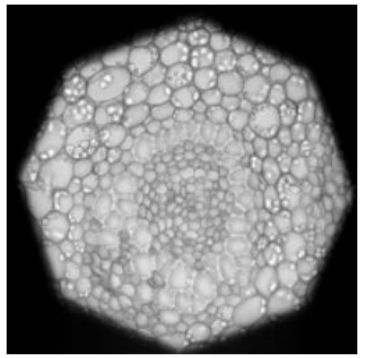
2- Focus on the sample and keep it fixed during the whole procedure



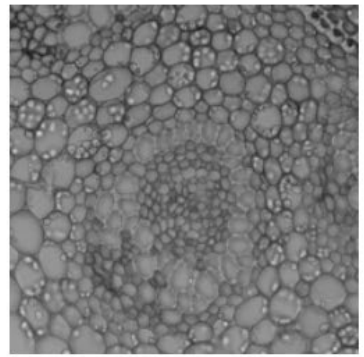
3- Close the stop-field iris



4- Adjust the condenser hight to get a clear image of the iris



5- Centre the iris

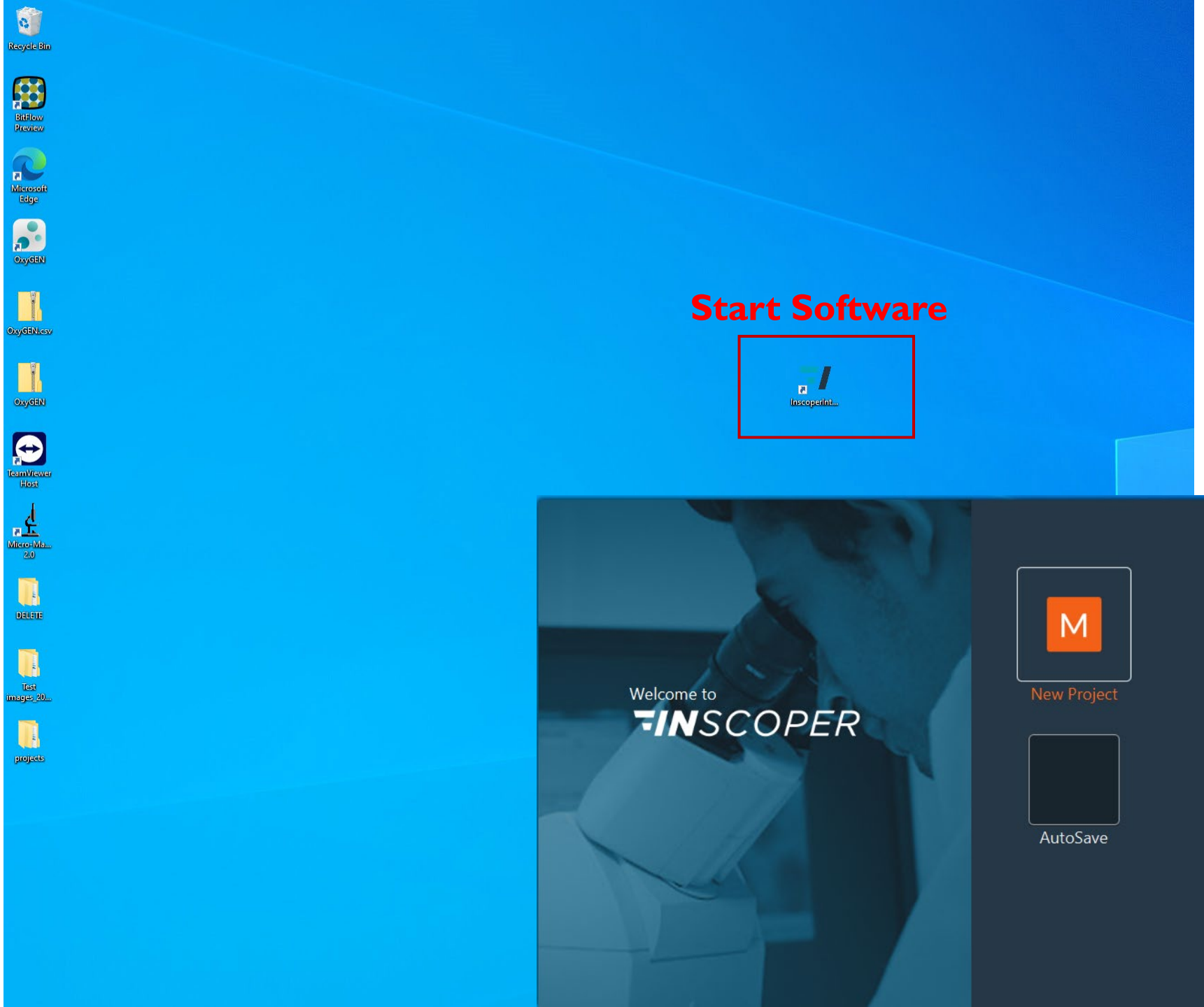


6- Open the iris slightly more than the field-of-view

Jérémie Capoulade

See also:

https://zeiss-campus.magnet.fsu.edu/articles/basics/practical.html


Recycle Bin
BitFlow Preview
Microsoft Edge
OxyGEN
OxyGEN.csv
OxyGEN
TeamViewer Host
Micro-Ma... 2.0
DELETE
Test images_20...
projects
Start Software
InscoperInt...
Welcome to
INSCOPER
M
New Project
AutoSave
Exit
Open project


FINSCOPER
Project
Settings
Support
Expert Mode
Configuration
Configuration window- check Flu:
Project Name
Project 2024-10-31
Camera Settings
Camera
Binning
1x1
Exposure (ms)
50
Advanced
Snap
Stop
Threshold
Contrast
Min : 1487.00 Average : 2631.37 Max : 13573.00
44.33 µm
Calibration
Tiling
Stage
NikonTie
PfsOffset
PfsOffset
158975
Step (nm)
1
PFS
PfsState
Objective
60X Oil
XY
X_Axis (nm)
438700
Y_Axis (nm)
143100
Step (nm)
1
Focus
Focus
1611275
Step (nm)
1
pE4000
A WaveLength
365
B WaveLength
460
C WaveLength
525
D WaveLength
635
Channel
GFP
In the channel, excitation, filter cube and shutters are pre-defined
NikonTie
SidePort
L100
Cube
GFP
TL Off/On
Camera path- left
pE4000
A Digit Off/On
A Intensity (%)
100
B Digit Off/On
B Intensity (%)
50
C Digit Off/On
C Intensity (%)
10
D Digit Off/On
D Intensity (%)
51
You can modify intensity
Channel
BF_GFP
BF_GFP
Add
Remove
Load
Save
NikonTie
SidePort
L100
Cube
GFP
TL Off/On
FPS : 9.46
Edit Chart
Crop Image
Go To Acquisition

Configuration window- check BF/Phase:

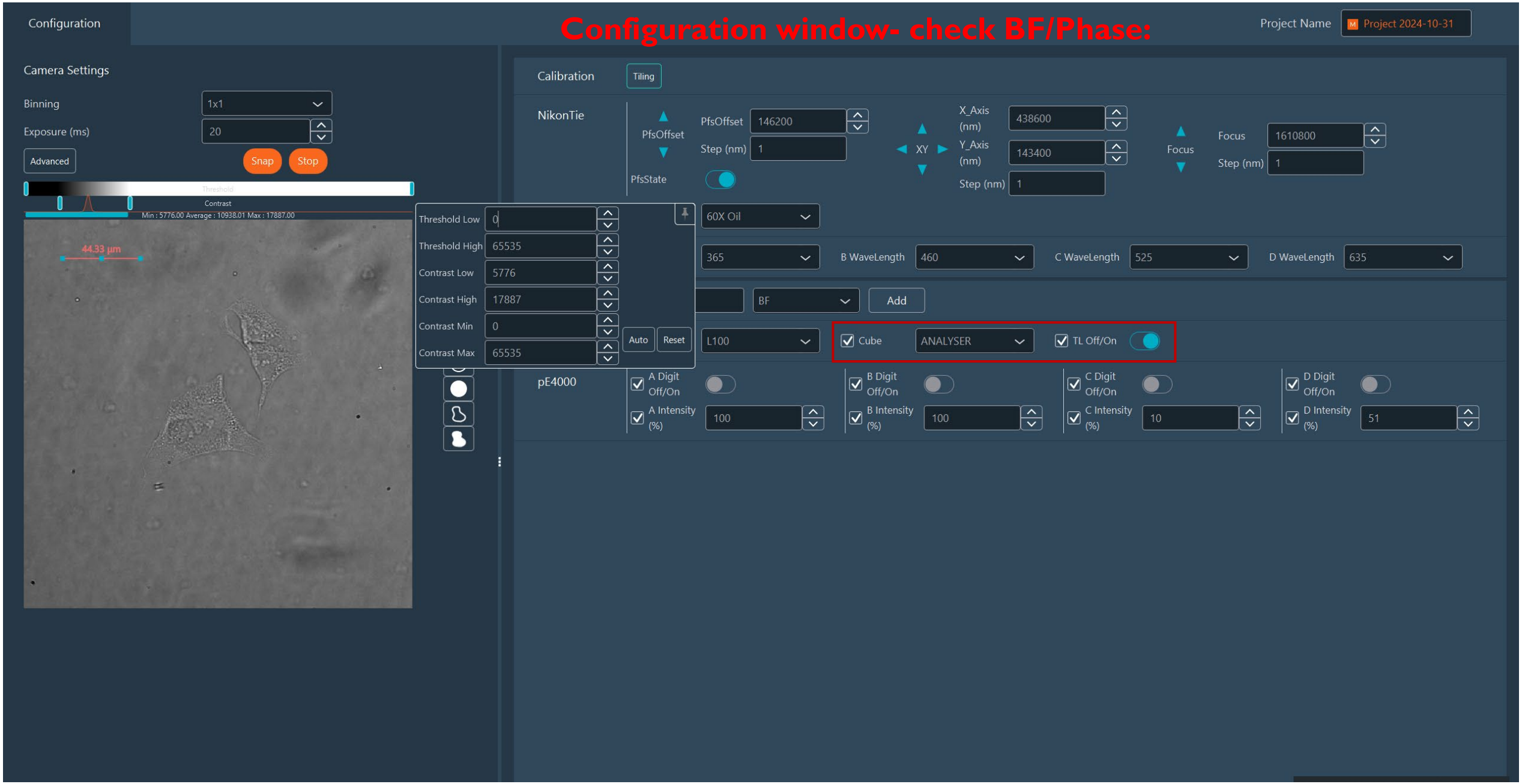


Image window:

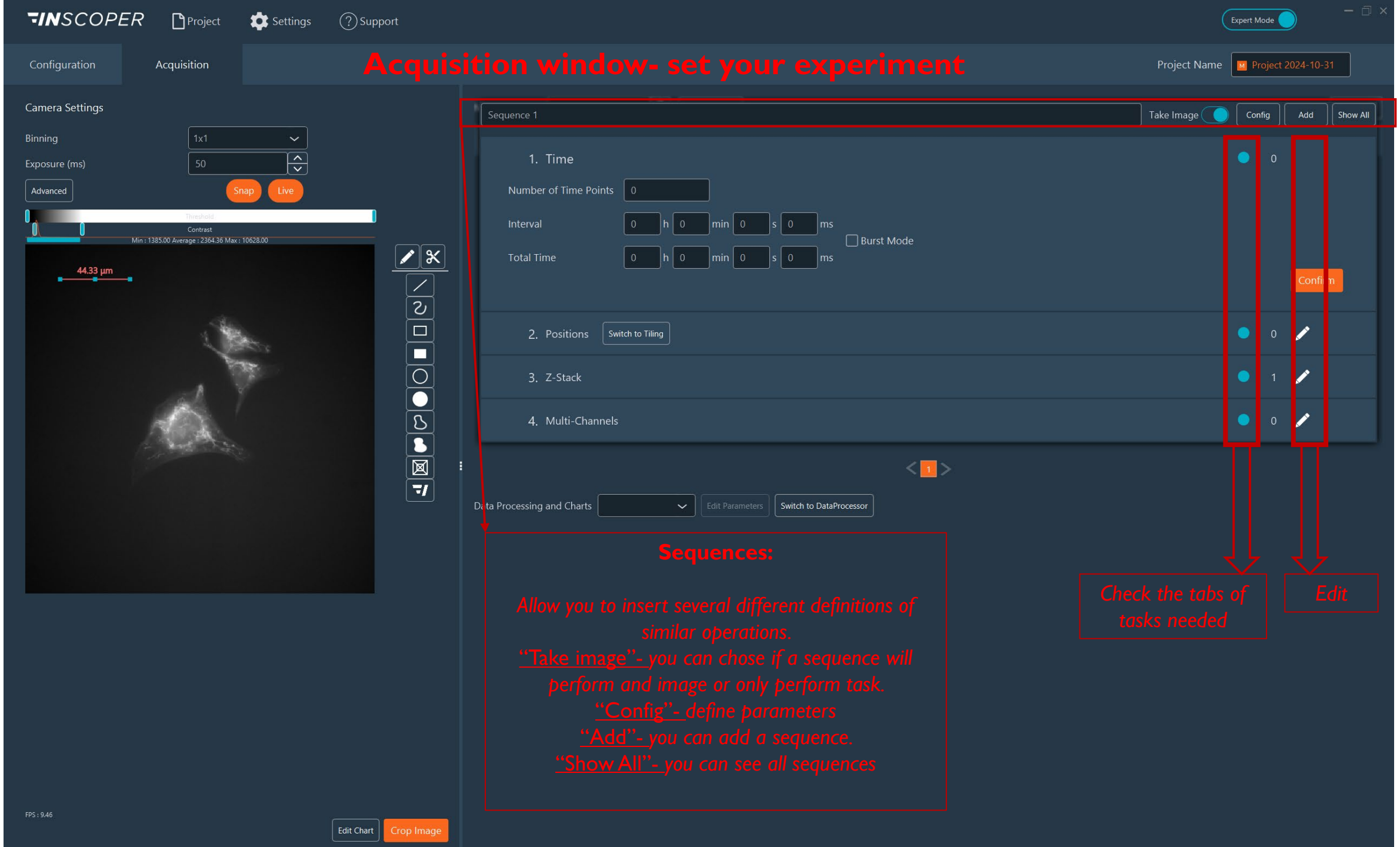
Display tools:

	You can move inside the live image by drag-and-drop
	You can select this option to add some ROI or make some crops on their live images
	You can choose this option to set the contrast automatically or manually. If it is manually, adjust the blue sliders on top of the camera view.
	Press this button to switch to full screen mode. To close this mode, press this button again or click on the cross in the top-right corner.
	You can change the LookUp Table (LUT) in real time using this option. You have 3 options: no LUT LUT with one color Preset LUT

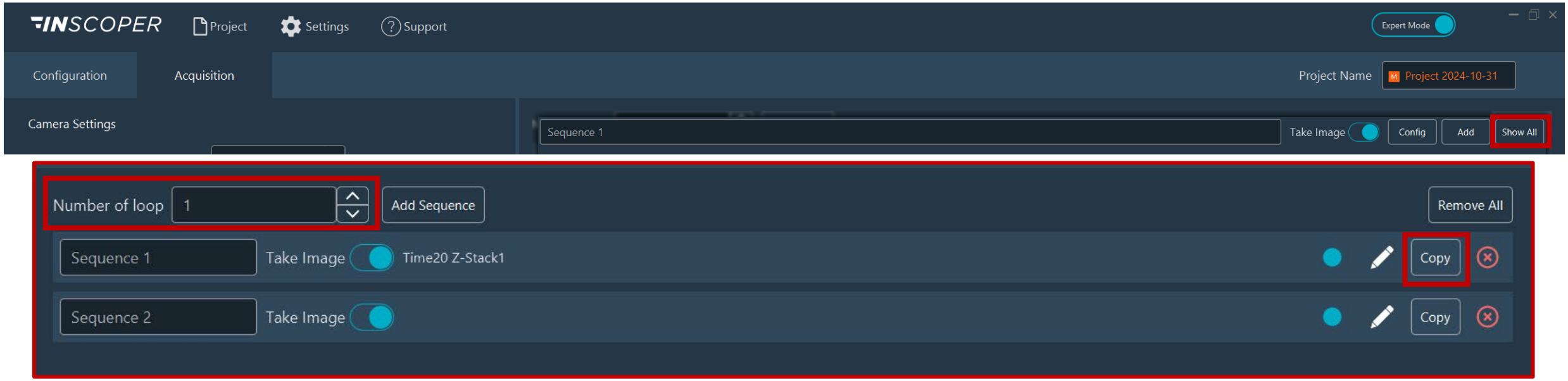
Pixel indicator *showing in red the overloaded pixels*
Inscoper ratiometric *dedicated to ratiometric images visualization*
Conventional multicolor LUT as "fire", "physics", etc...

	The **pen** mode allows you to add one or multiple ROI.
	The scissors mode allows you to cut (remove) into a full shape while retaining the surrounding selected area.

	Draw a straight line.
	Draw a freehand line.
	Draw the edge of a rectangle.
	Draw a filled rectangle.
	Draw the edge of a circle.
	Draw a filled rectangle.
	Draw a free form edge.
	Draw a filled free form.

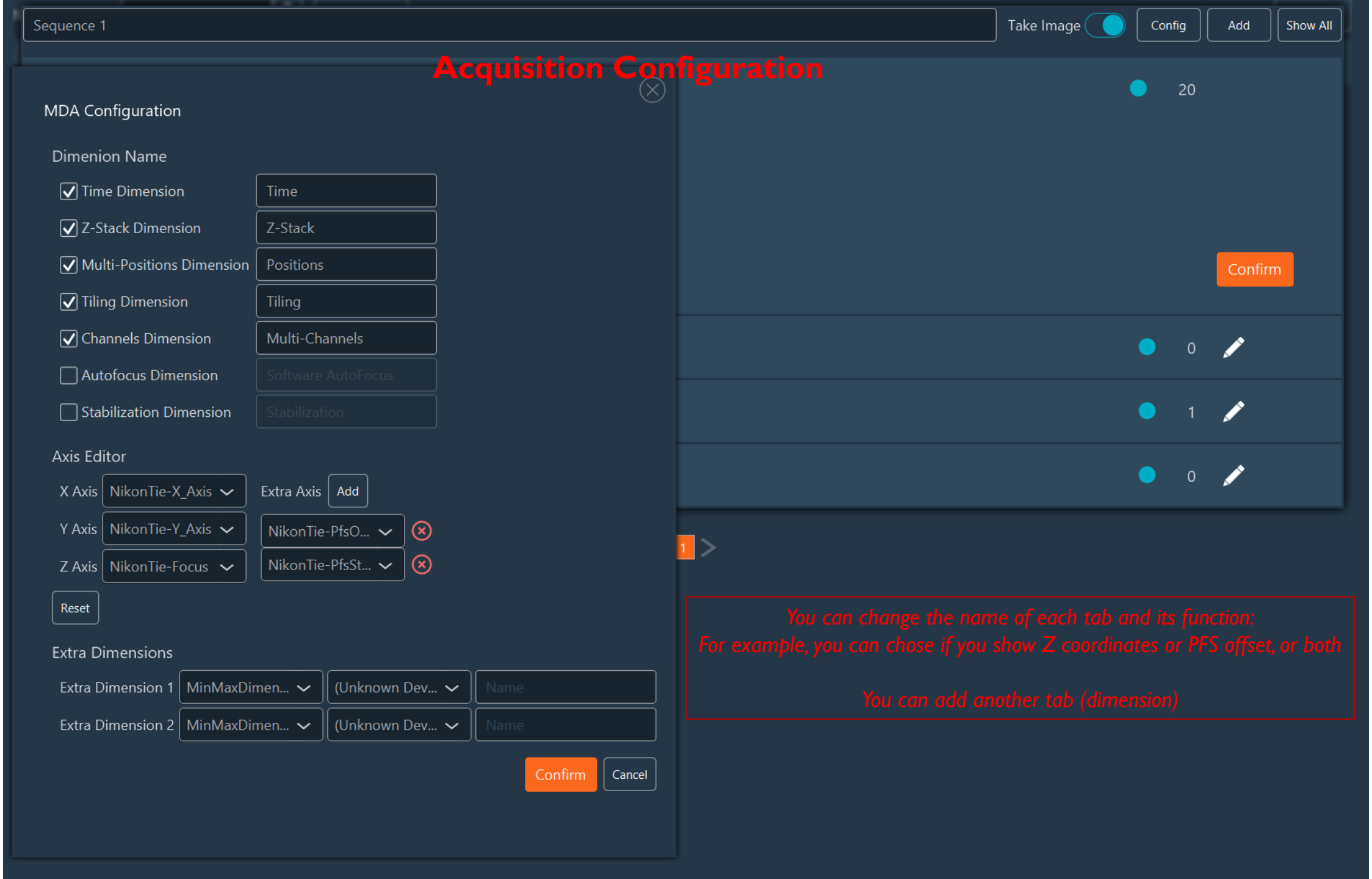

FINSCOPER
Project
Settings
Support
Expert Mode
Configuration
Acquisition
Acquisition window- set your experiment
Project Name
Project 2024-10-31
Camera Settings
Binning
1x1
Exposure (ms)
50
Advanced
Snap
Live
Threshold
Contrast
Min : 1385.00 Average : 2364.36 Max : 10628.00
44.33 µm
FPS : 9.46
Edit Chart
Crop Image
Sequence 1
Take Image
Config
Add
Show All
1. Time
0
Number of Time Points
0
Interval
0 h 0 min 0 s 0 ms
Burst Mode
Total Time
0 h 0 min 0 s 0 ms
Confirm
2. Positions
Switch to Tiling
0
3. Z-Stack
1
4. Multi-Channels
0
1
Data Processing and Charts
Edit Parameters
Switch to DataProcessor
Sequences:
Allow you to insert several different definitions of similar operations.
"Take image"- you can chose if a sequence will perform and image or only perform task.
"Config"- define parameters
"Add"- you can add a sequence.
"Show All"- you can see all sequences
Check the tabs of tasks needed
Edit

Acquisition window- sequences

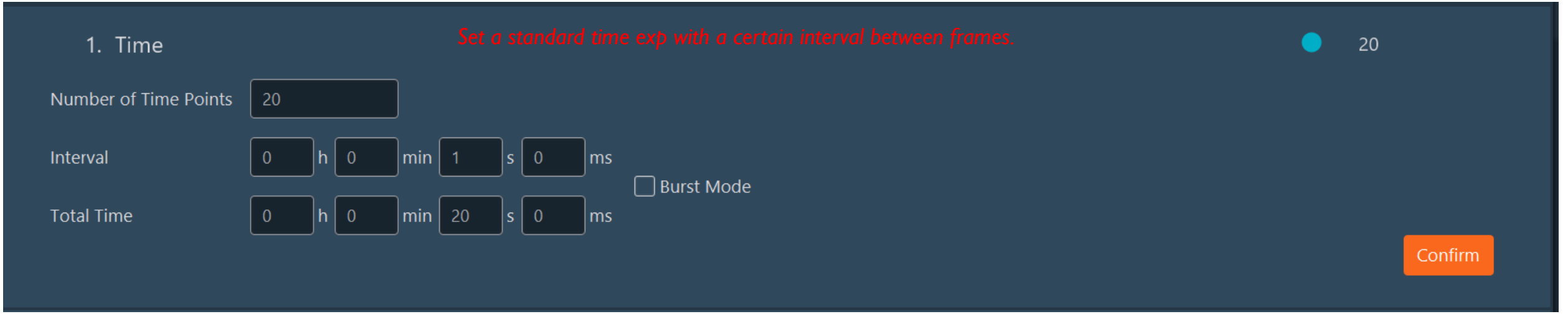


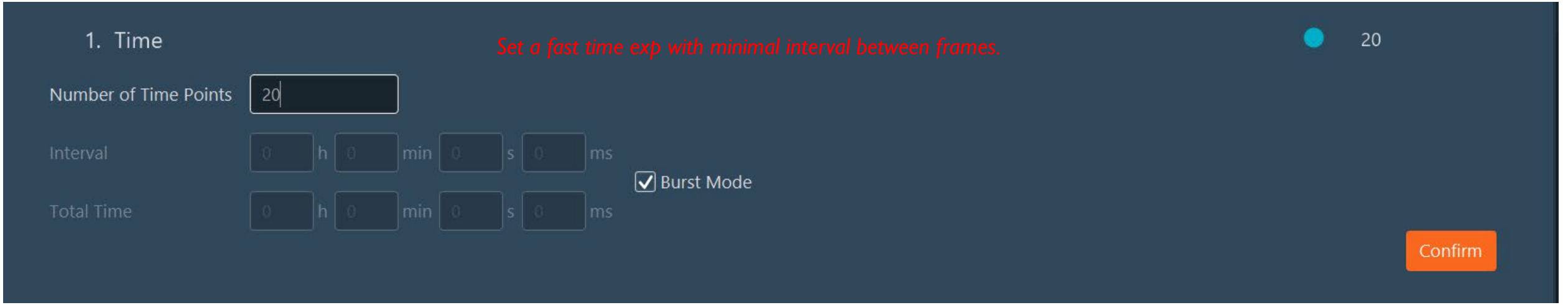
You can define loops to repeat the sequences

You can copy one sequence and only change the parameters modified. Quicker than starting new sequence

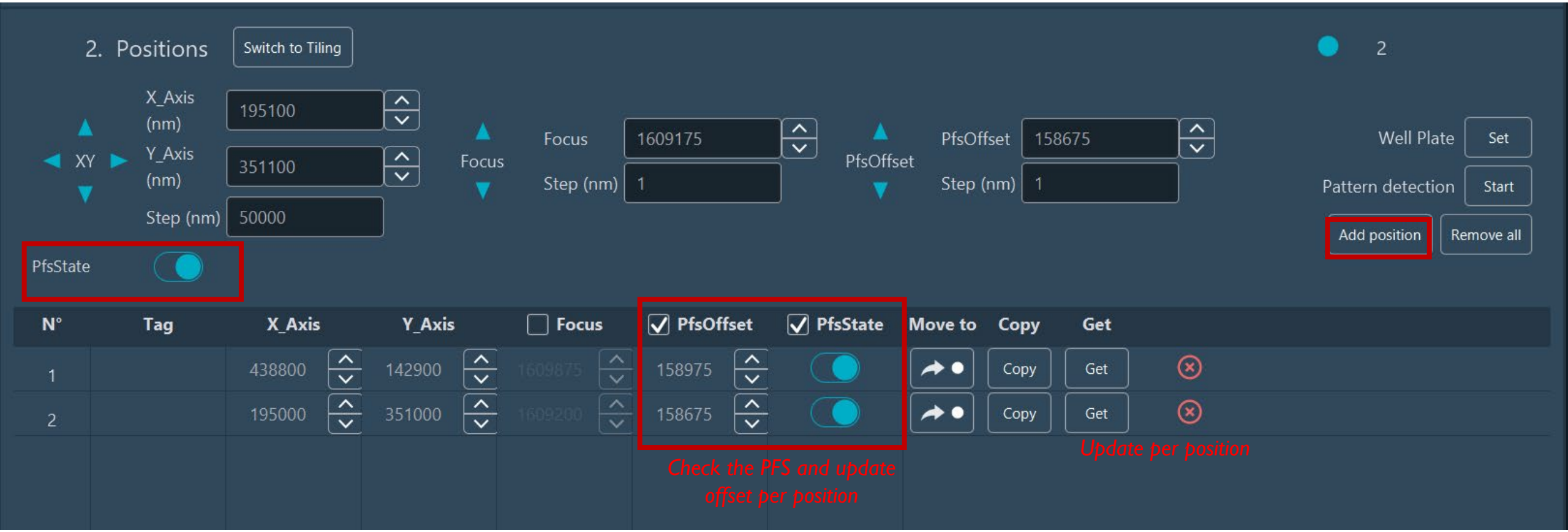

Sequence 1
Take Image
Config
Add
Show All
Acquisition Configuration
MDA Configuration
Dimenion Name
Time Dimension
Time
Z-Stack Dimension
Z-Stack
Multi-Positions Dimension
Positions
Tiling Dimension
Tiling
Channels Dimension
Multi-Channels
Autofocus Dimension
Software AutoFocus
Stabilization Dimension
Stabilization
Axis Editor
X Axis
NikonTie-X_Axis
Extra Axis
Add
Y Axis
NikonTie-Y_Axis
NikonTie-PfsO...
Z Axis
NikonTie-Focus
NikonTie-PfsSt...
Reset
Extra Dimensions
Extra Dimension 1
MinMaxDimen...
(Unknown Dev...
Name
Extra Dimension 2
MinMaxDimen...
(Unknown Dev...
Name
Confirm
Cancel
20
Confirm
0
1
0
1
You can change the name of each tab and its function:
For example, you can chose if you show Z coordinates or PFS offset, or both
You can add another tab (dimension)

Acquisition window- Time

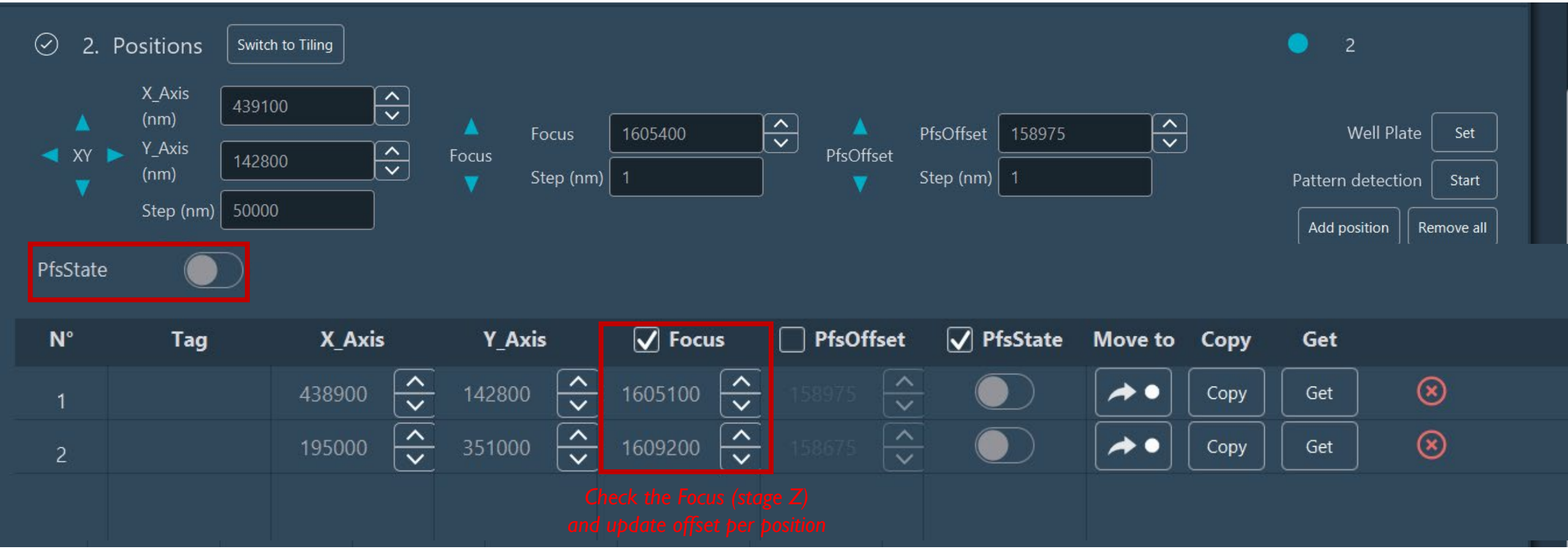




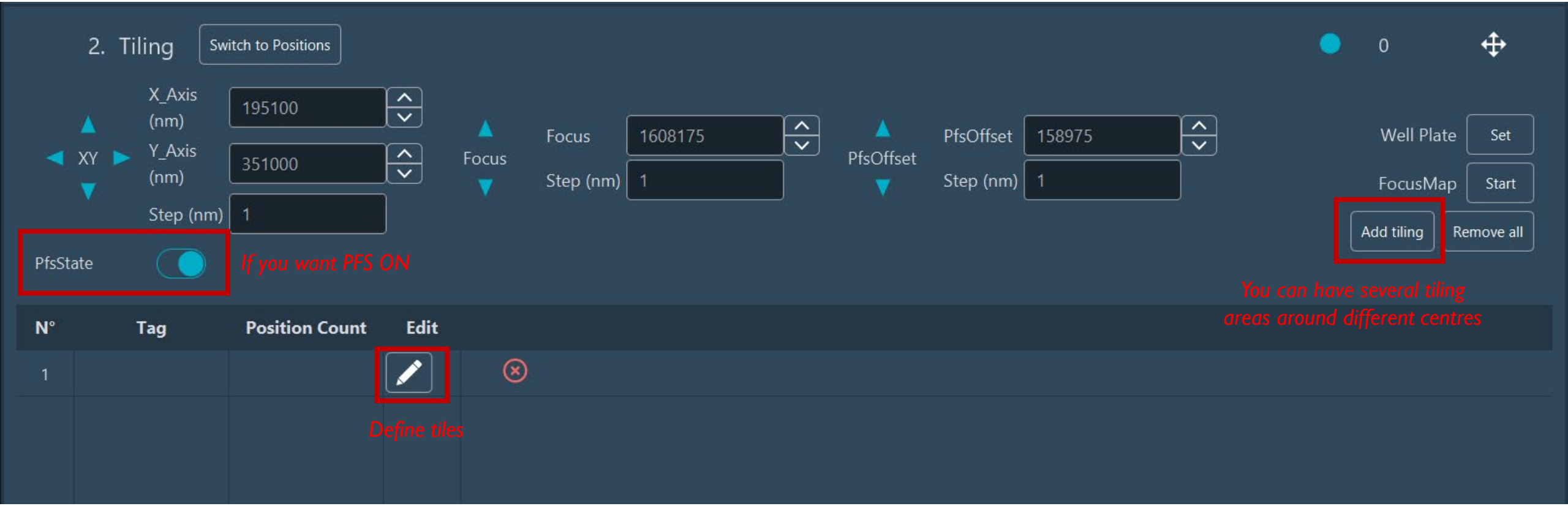
Acquisition – define multi positions, using PFS offset



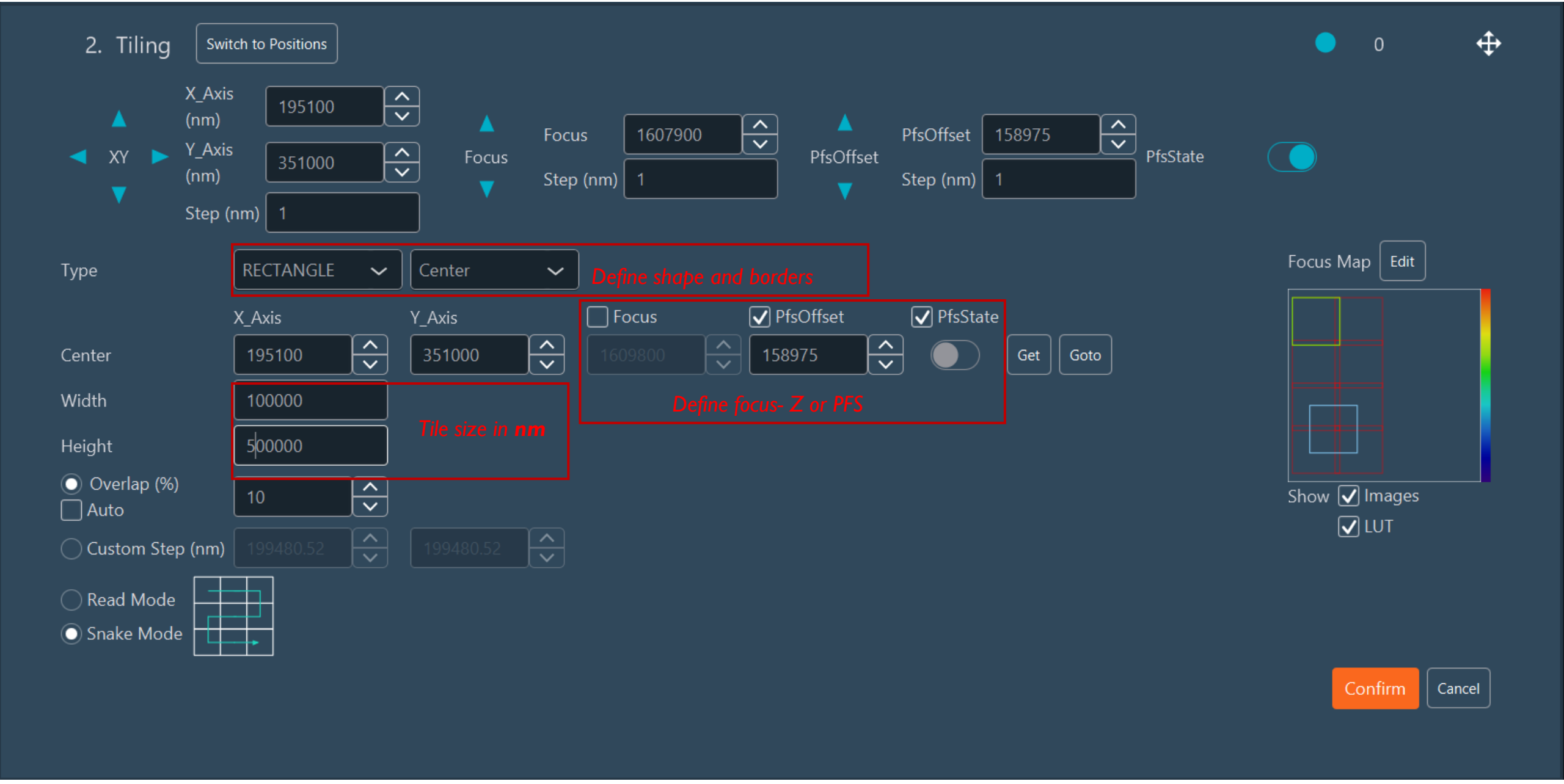
Acquisition – define multi positions, using Abs Z position



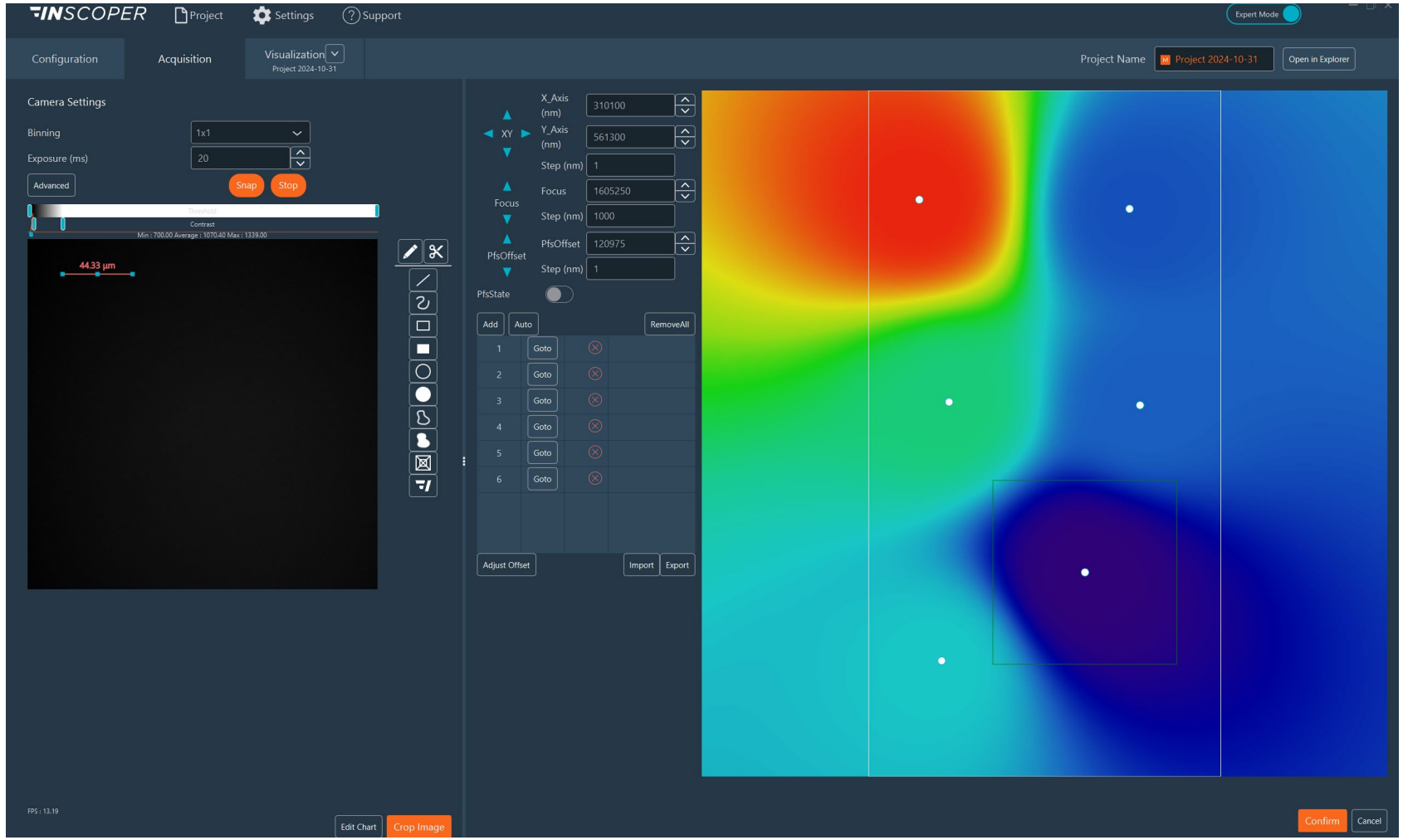
Acquisition – Tiling

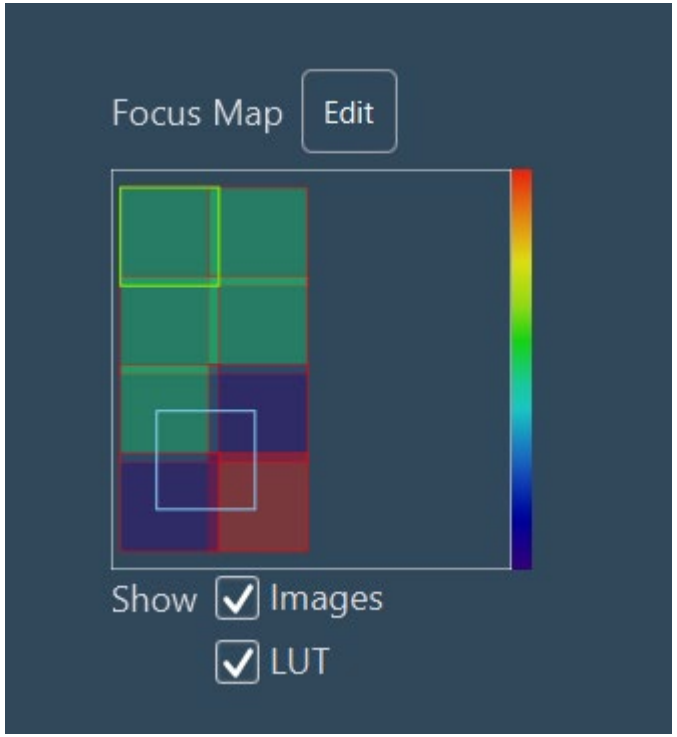


Acquisition – Define Tile



Tiling with Focus map



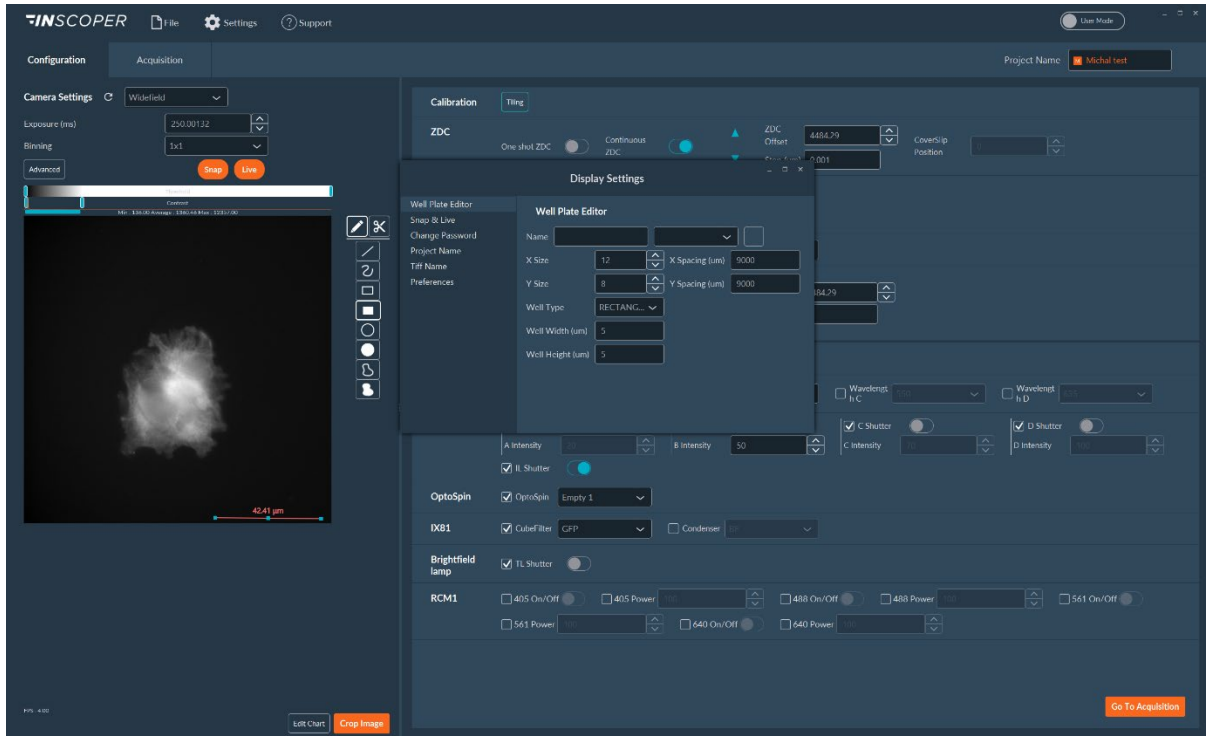


You can edit your focus map to add and redefine Z within positions

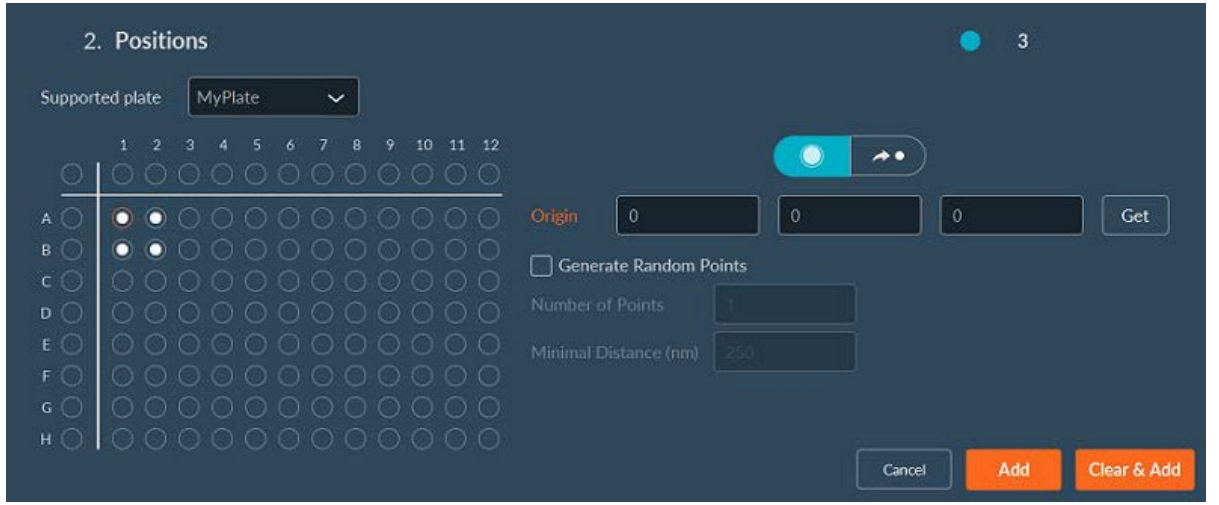
INSCOPER- Acquisition- Stage experiment: Define well plate (Auto position list)

!Only using Air Objectives!

Set	Set	If you have a well-plate, click on this button to select the wells you are interested in.
Add position	Add Position	Add a position manually and enter its coordinates.
Remove All	Remove All	Delete all the positions.
	Move To	Move to the selected position.
Copy	Copy	Copy the required coordinates.
Get	Get	Get the current coordinates of the stage.
	Delete	Delete the selected position.



MyPlate	Choose Your Plate	Select the well-plate you wish to use in the drop-down list.
	Select	Click on this button to select the wells you need.
	Move To	Move to the selected well.
Get	Get	Get the coordinates entered on the well-plate.
Generate Random Points	Generate Random Points	By checking this box, the interface will choose several random points on the well-plate. If you check this box, be sure to enter the following information: • the number of points, • the minimum distance.
Cancel	Cancel	Click on this button to go back to the manual positions.
Add	Add	Add the positions you have chosen to the previous ones.
Clear & Add	Clear & Add	Delete all the previous positions and add the selected ones.



Acquisition – Z stack

3. Z-Stack

11

Focus

Focus (nm) 1607600

Step (nm) 1000

Min/Max Plane

Min (nm) 1607575 Get

Max (nm) 1604500 Get

Absolute- stage

Center Plane

Center (nm) 1606037.5 Get

Volume (nm) 3075

Center First

Stack Step (nm) 253.775 Nyquist

Stack Size 11

Confirm

If you use PFS, your offset is the centre of stack

3. Z-Stack

8

Focus

Focus (nm) 1608850

Step (nm) 1

Min/Max Plane

Min (nm) 1608000 Get

Max (nm) 1610000 Get

Center Plane

Center (nm) 1609000 Get

Volume (nm) 2000

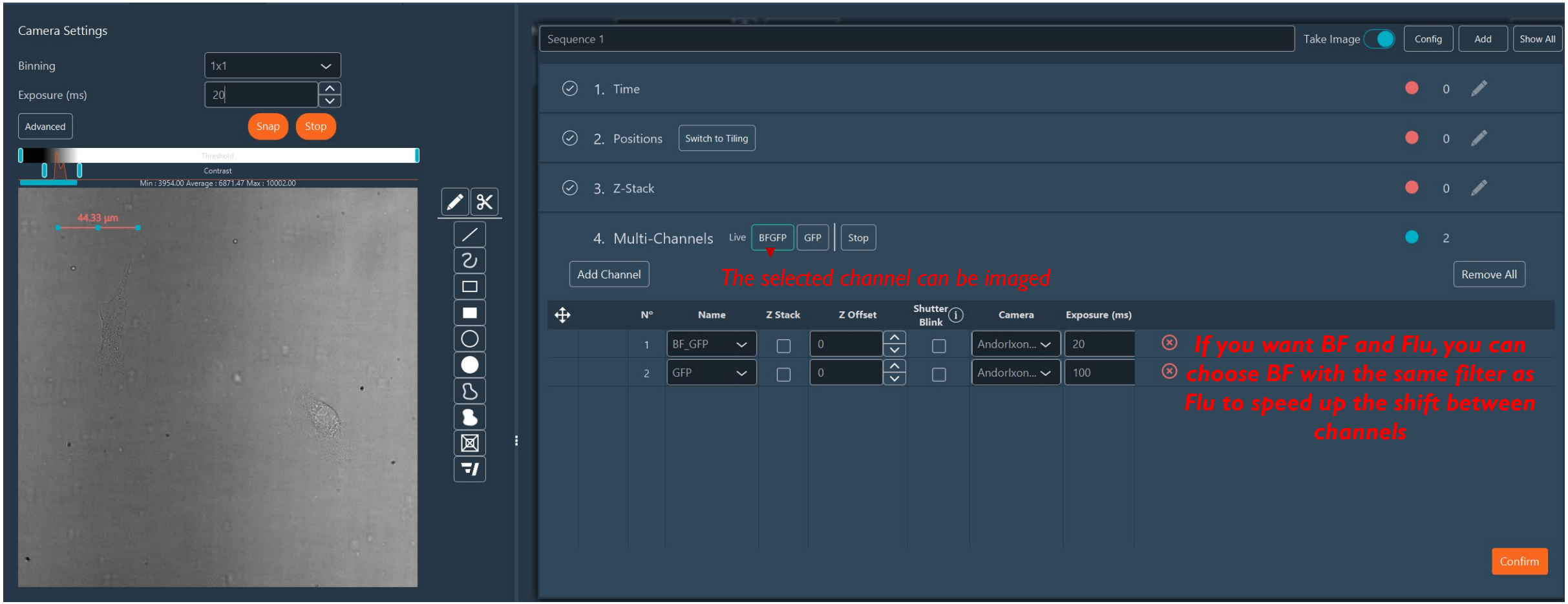
Center First

Stack Step (nm) 253.775 Nyquist

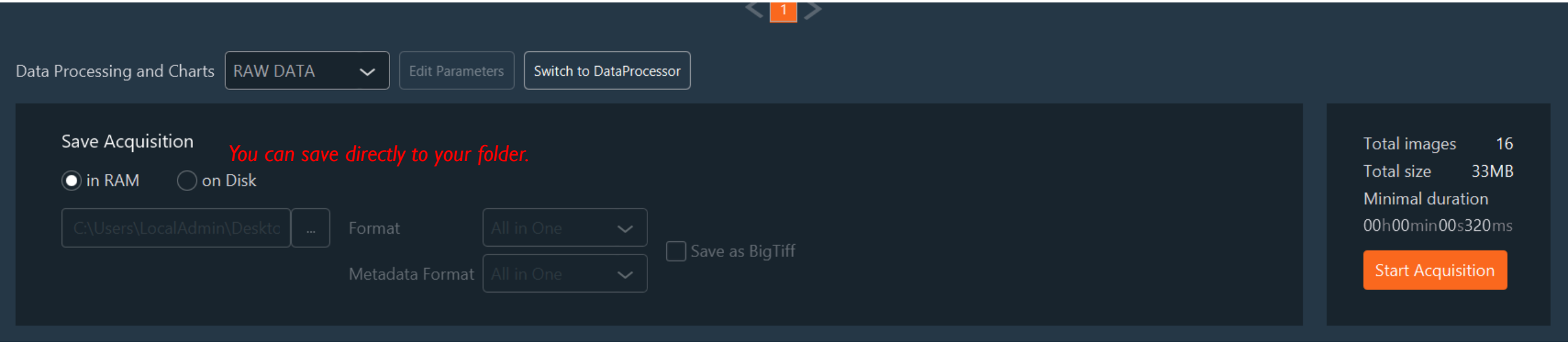
Stack Size 8

Confirm

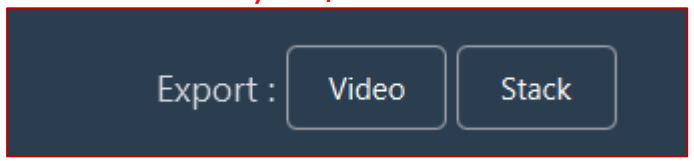
Acquisition – Multi channel



Acquisition – start experiment

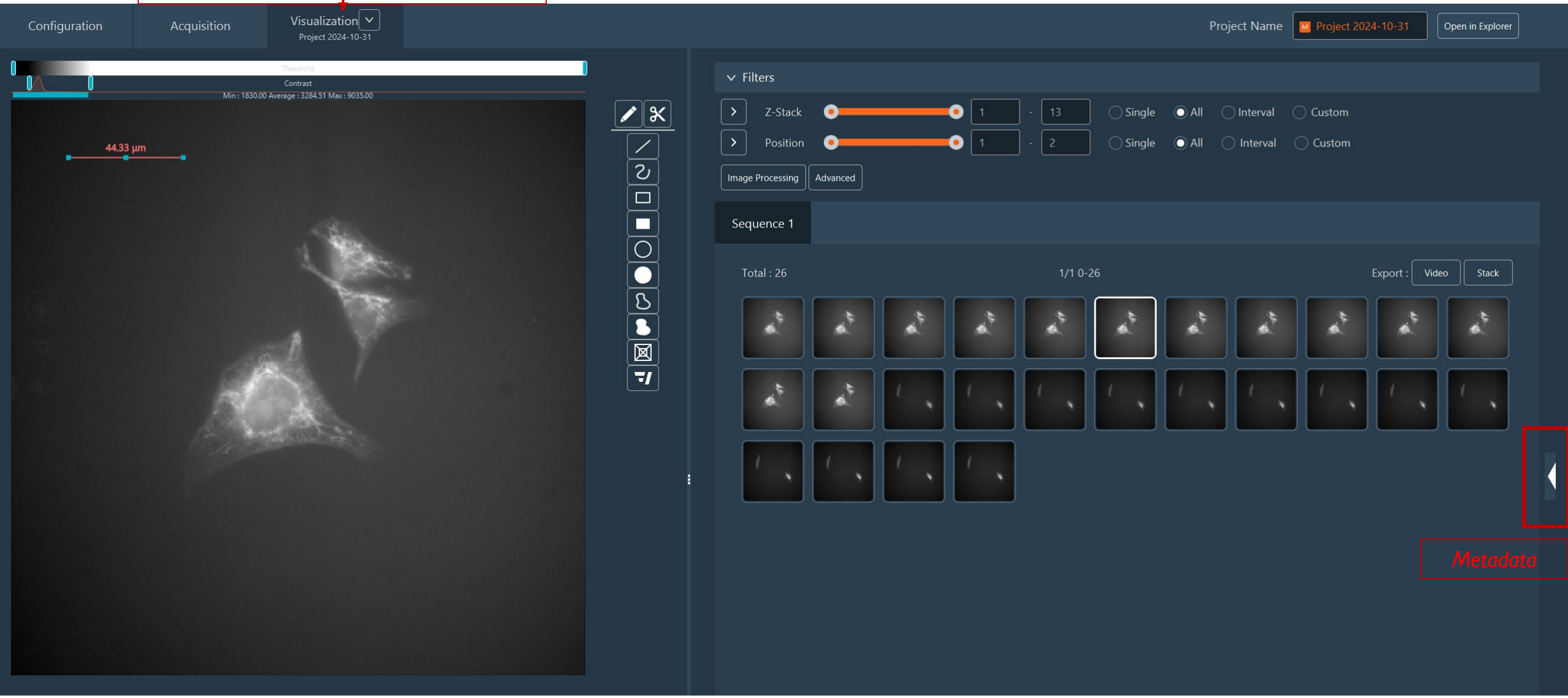


If you choose RAM, remember to export to your folder



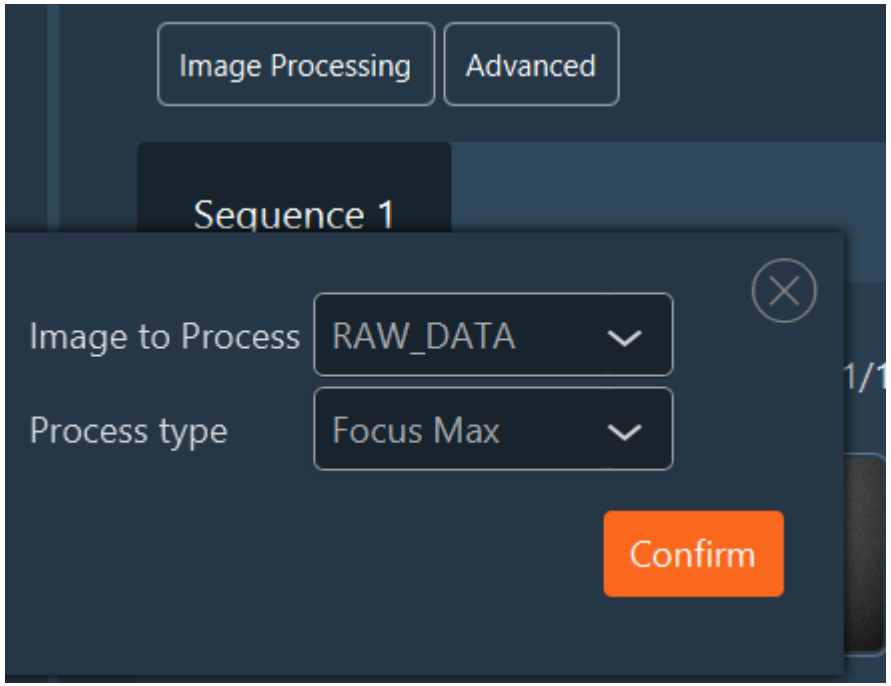
Visualization

You can check all your experiments from this session

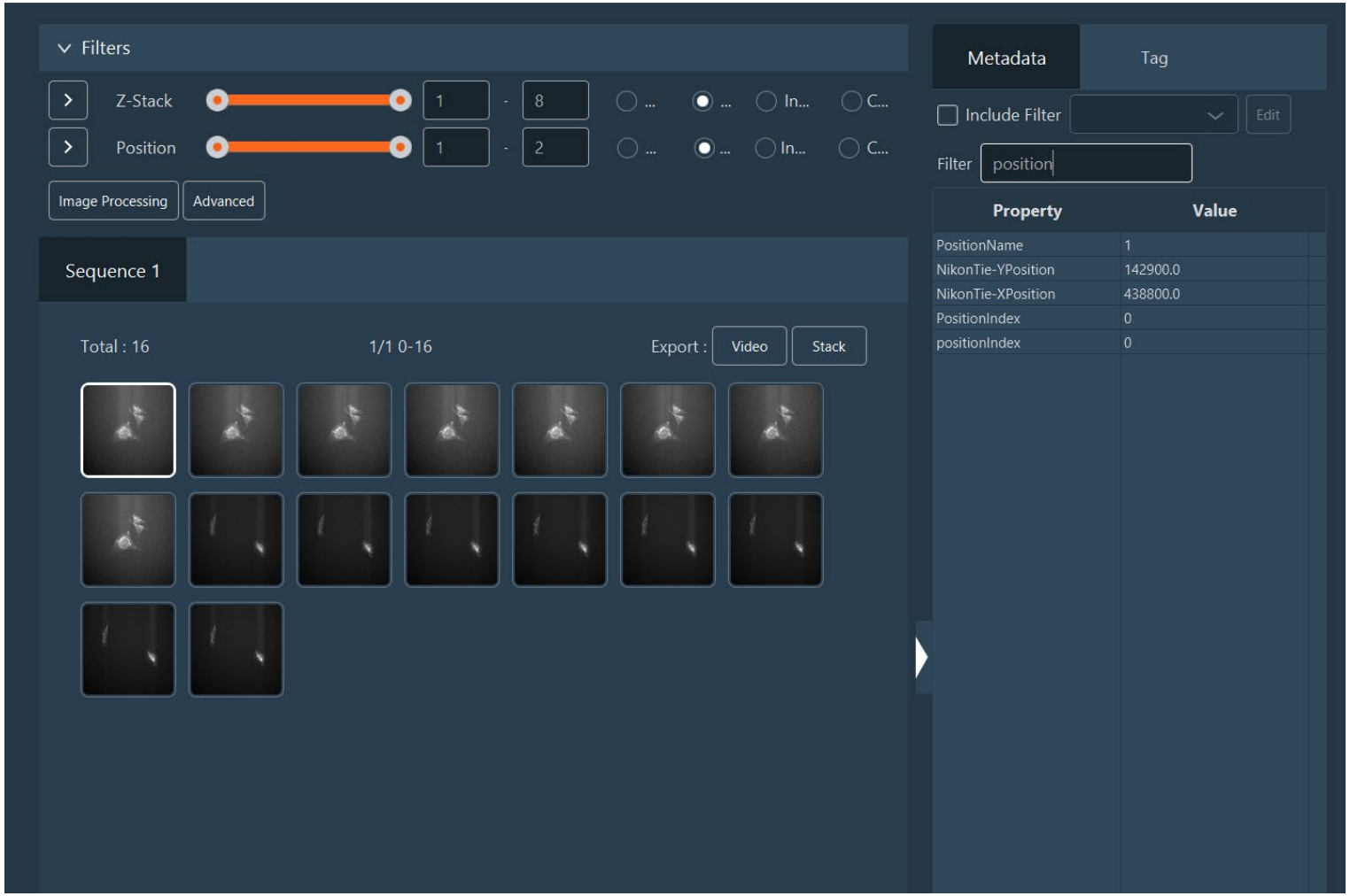


Visualization

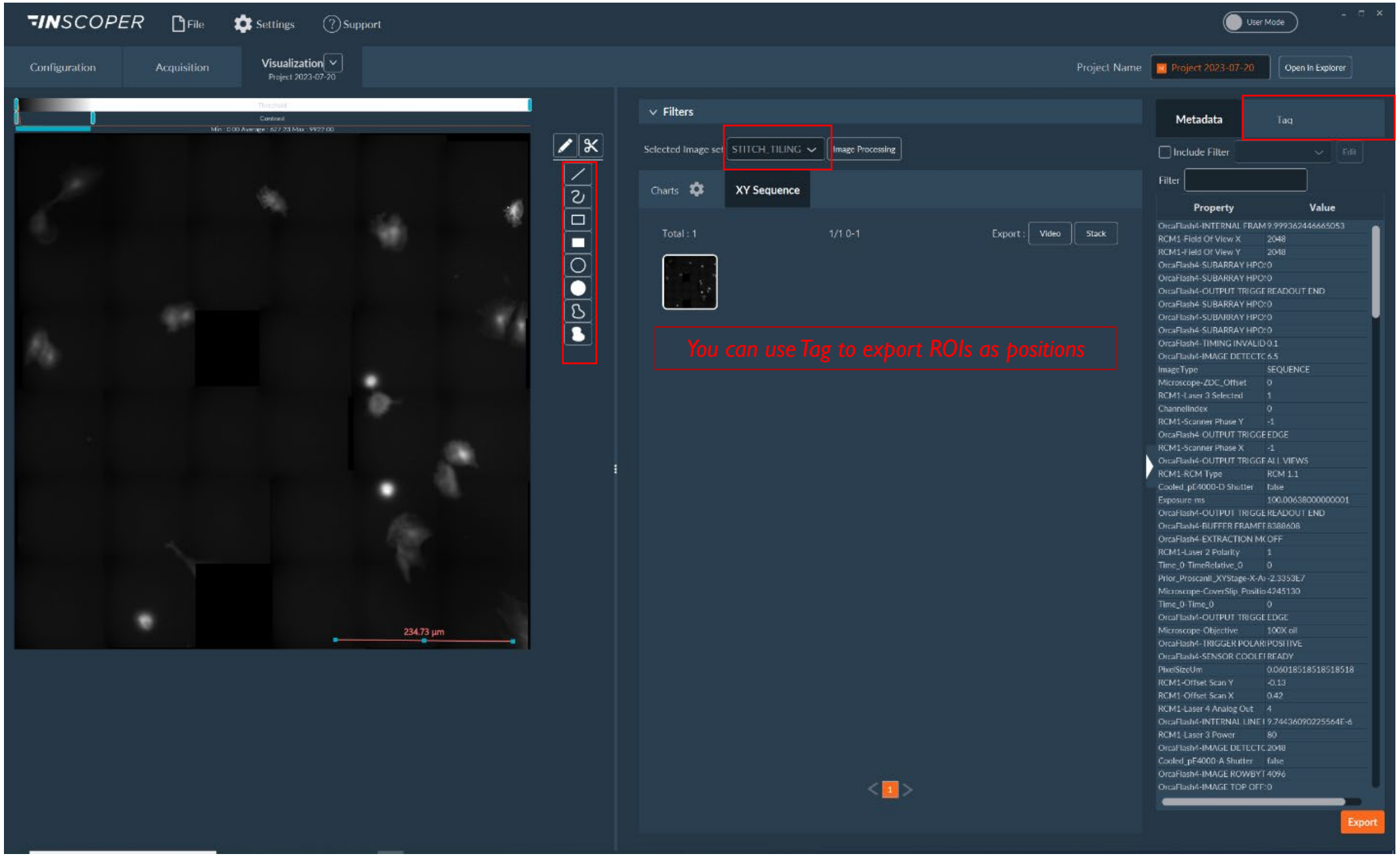
Continue to process your data,
Example: max Z projection



Metadata: you can filter to check specific parameter



Visualization- Tag



Shut down

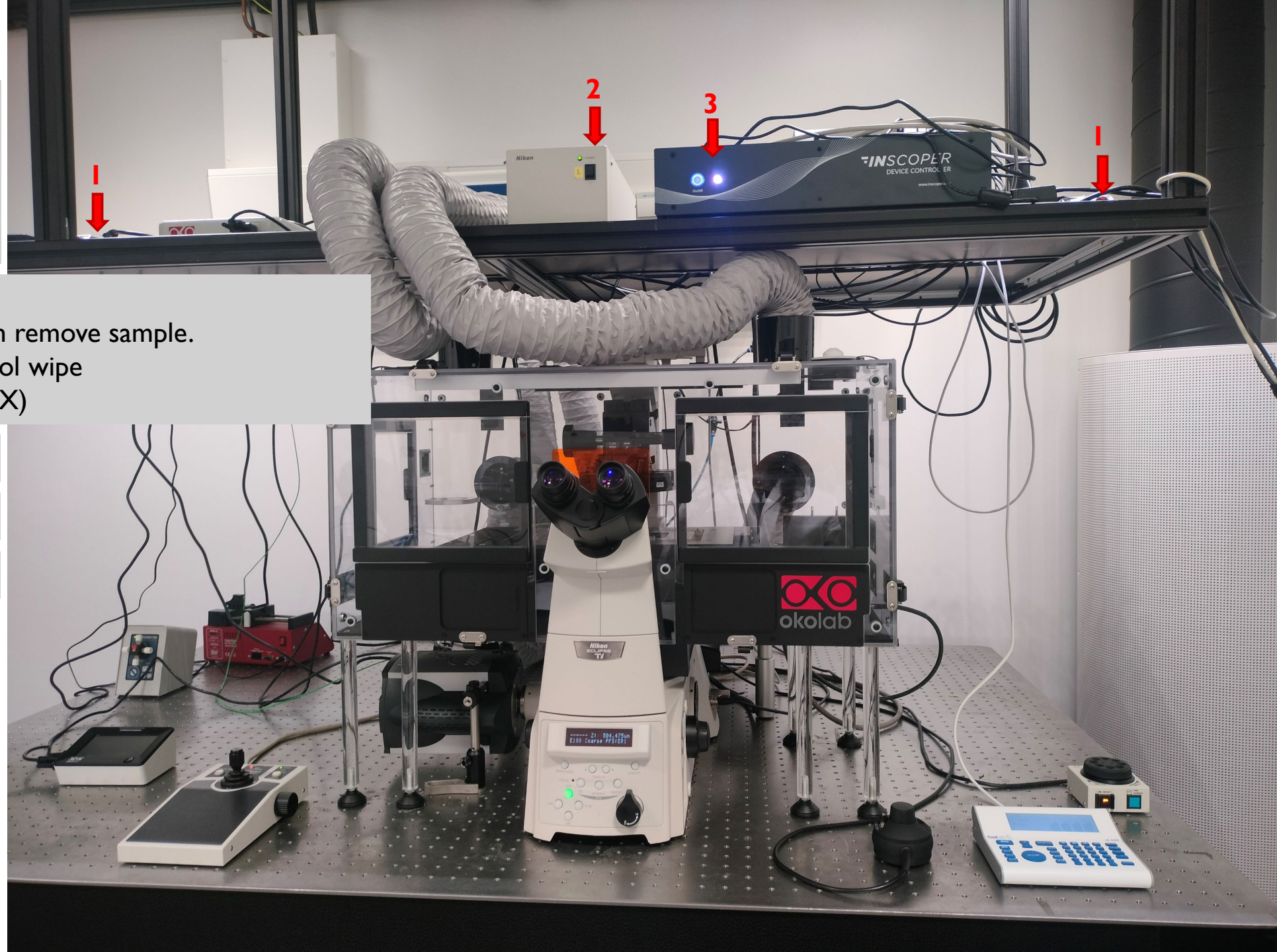
1, Save your data and close software and computer.
!Save data to BULK folder. Data is erased from local folder regularly

2, Clean up:
2,1, lower the objective, only then remove sample.
2,2, clean with dry and isopropanol wipe
2,3, switch to lowest Mag obj (10X)

3, Turn off INSCOPER BOX

4, Turn off Nikon controller

5, Turn off 2 main switches



Need help/advice?

Contact:
Michal: M.Shemesh@tudelft.nl
Wiel: W.H.Evers@tudelft.nl