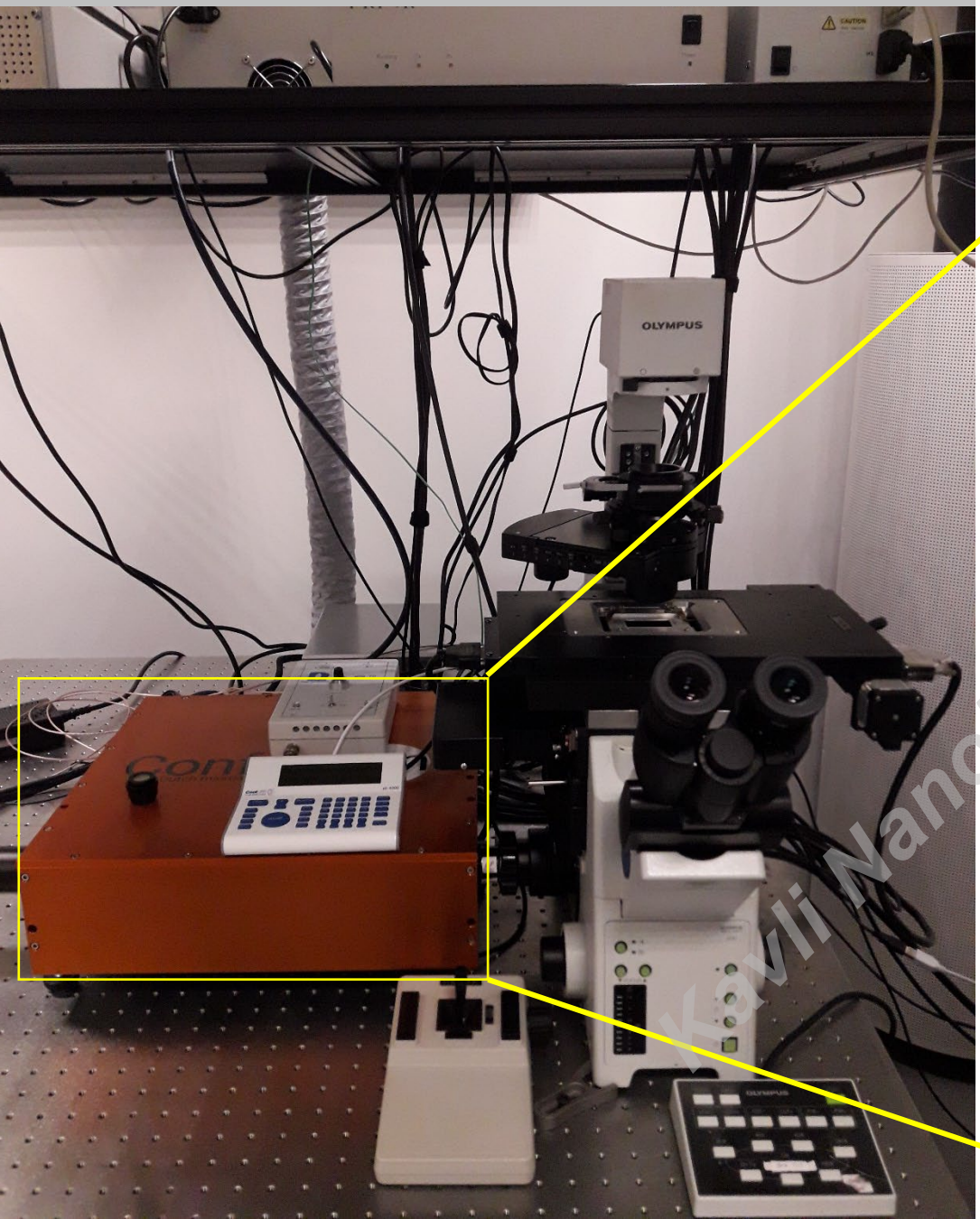
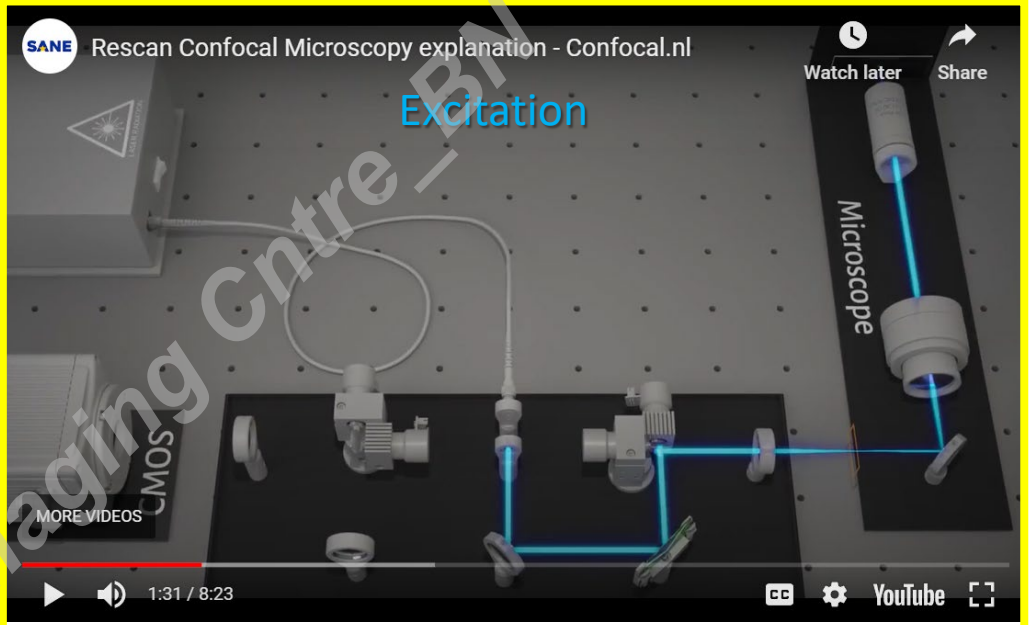
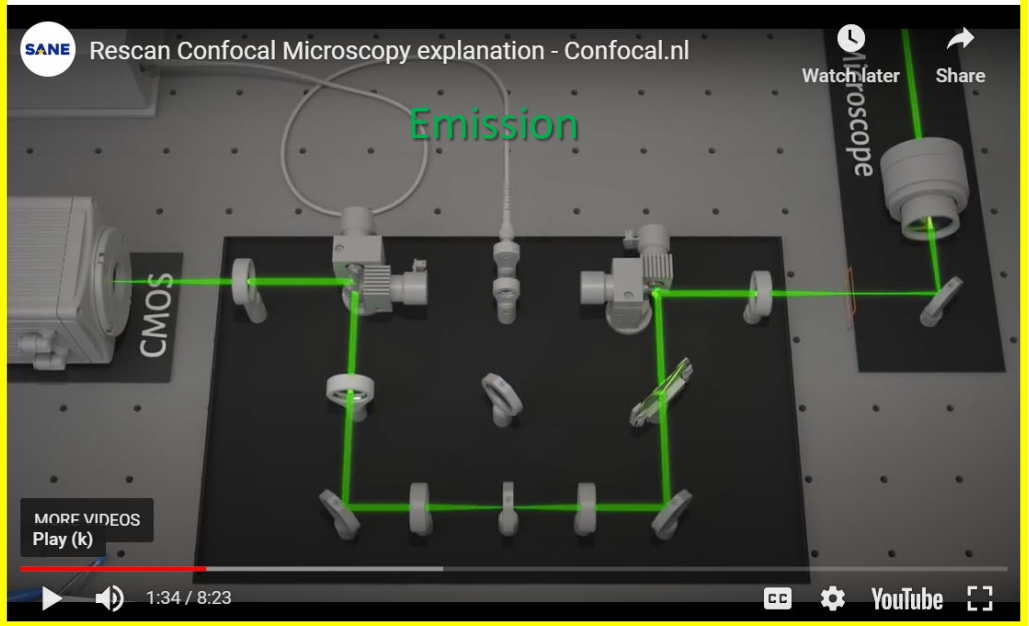
Kavli Nanolab Imaging Center

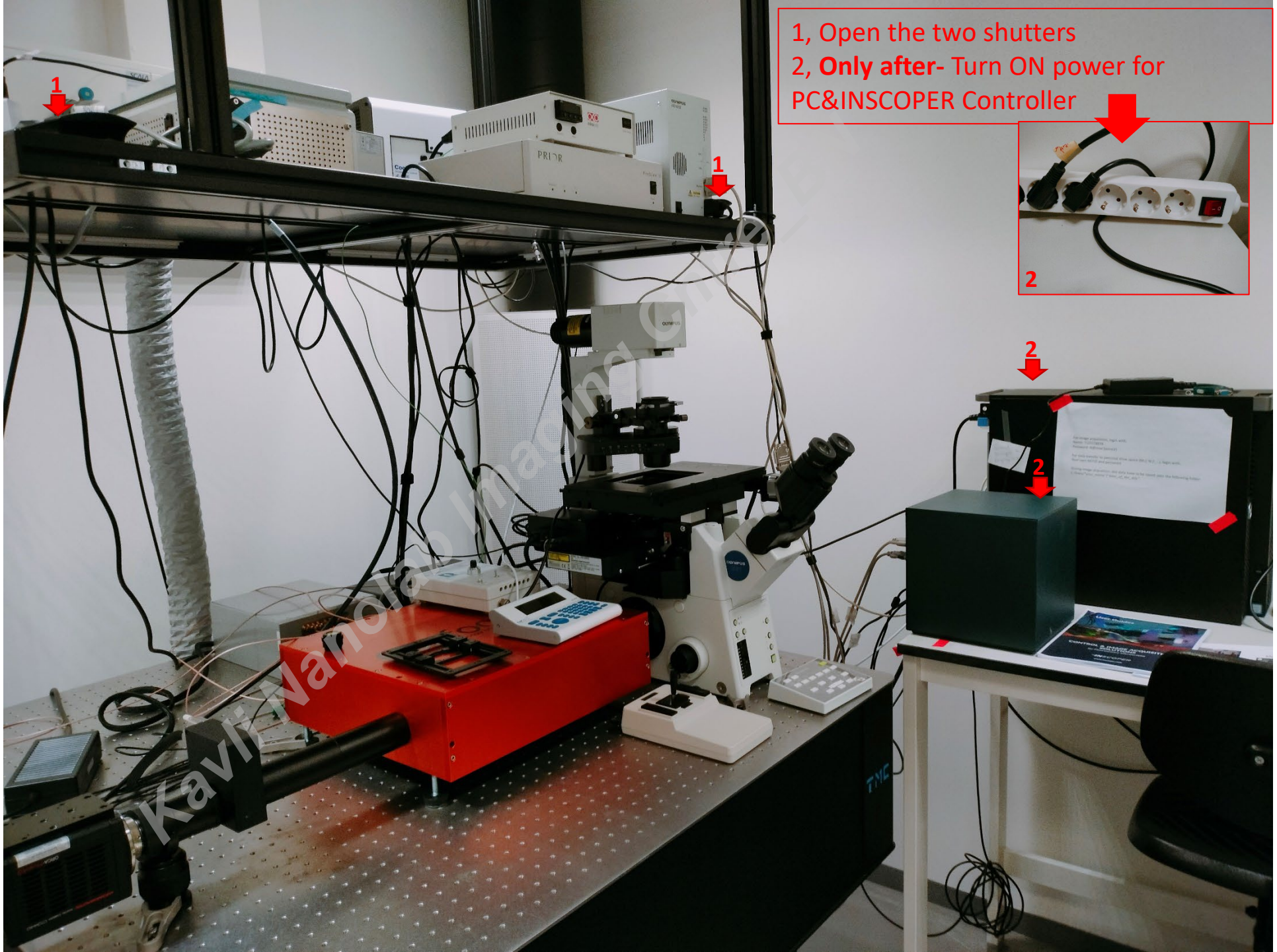


User manual WF+RCM



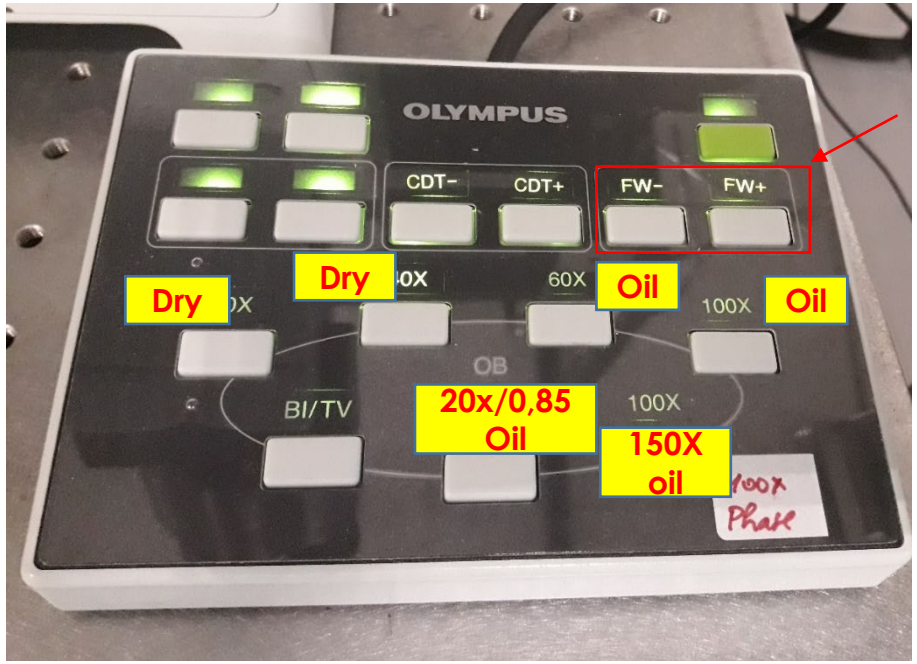


Startup- Hardware



Startup- Hardware

3, locate your sample (focus, BF/CoolLed) using 20x air objective and the eye piece



Filter Wheel

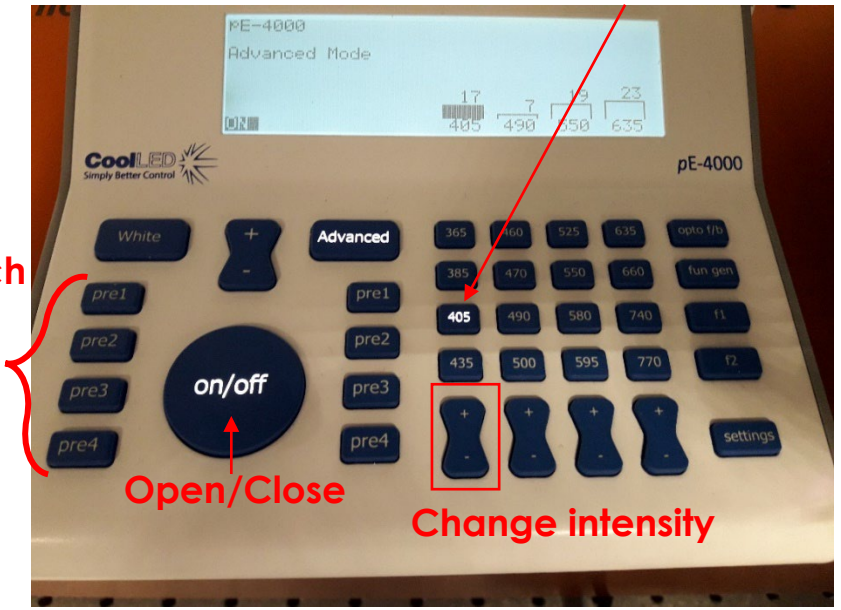
Eye path

Focus

Make sure they match

CoolLed light source:

Choose your excitation



You have each channel separate at presets 1-4

Open/Close

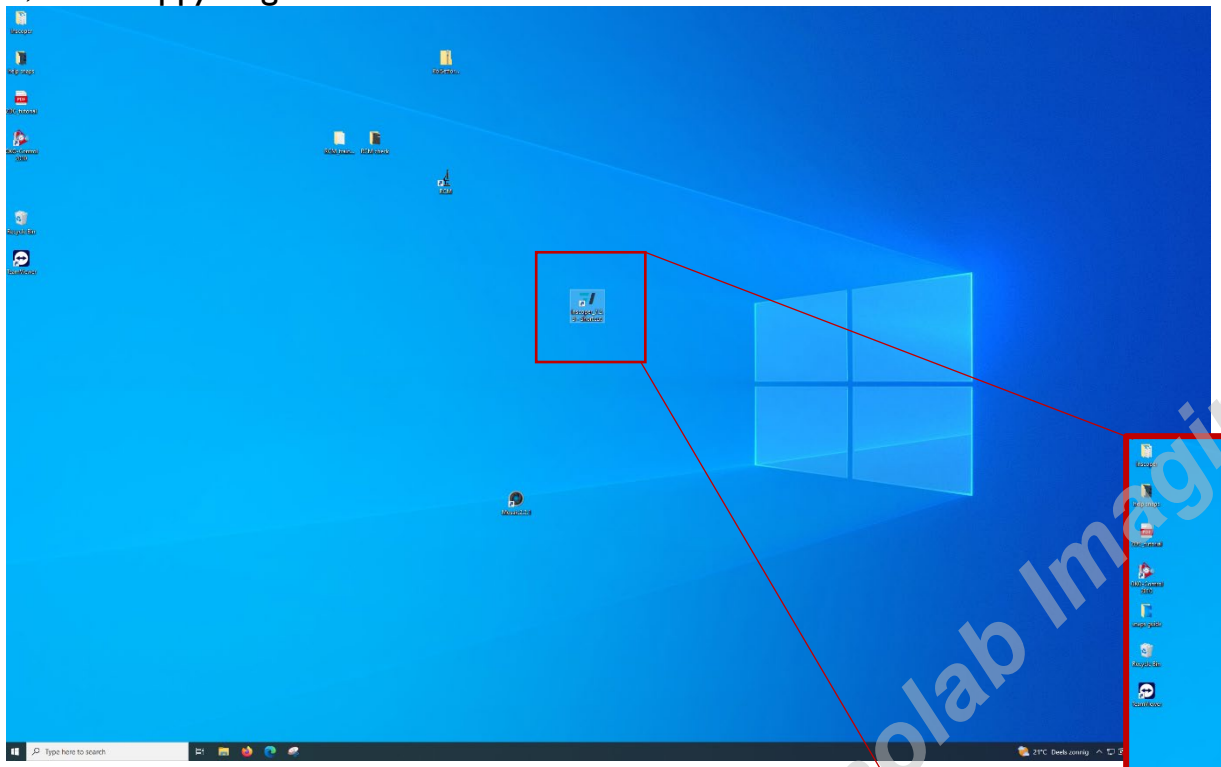
Change intensity

Bright light controller:

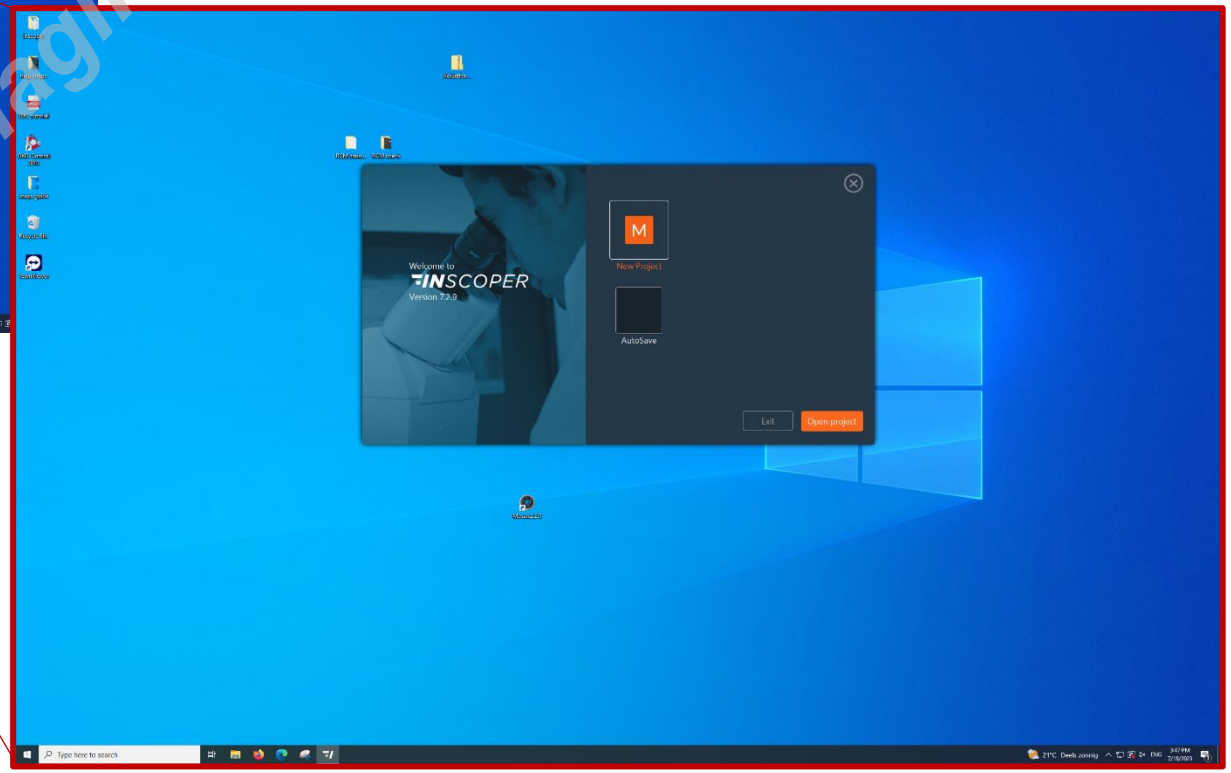
TTL: *Computer control (when starting software)*
0: *light OFF*
Cont: *Continous illumination (for navigation with eye piece)*

Startup- software

3, once happy- log into PC- double click on INSCOPER icon



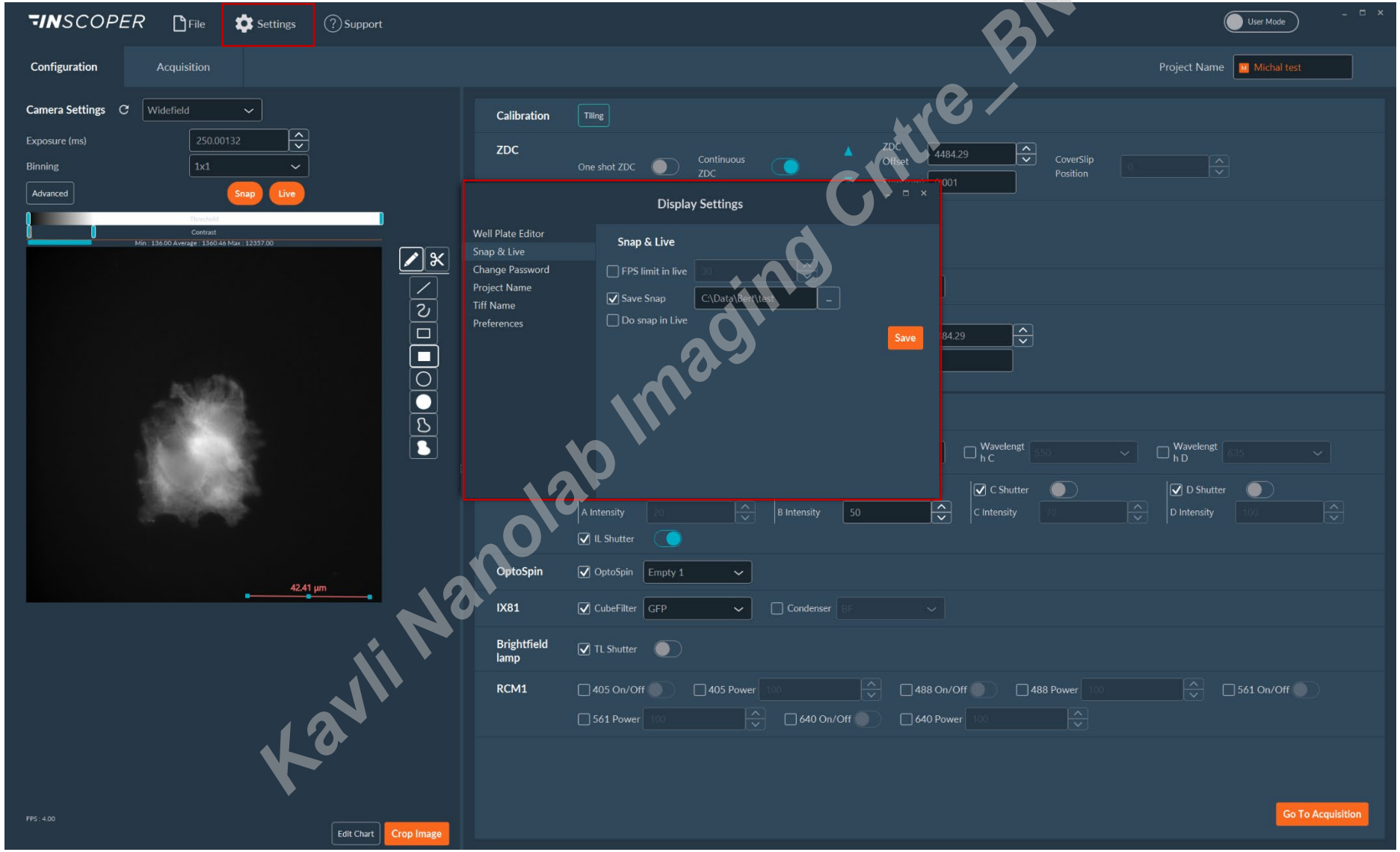
4, you can open a new project, save it to keep parameters and re-load



4, Name your project

INSCOPER- Configuration

Click on “Settings” to pre-define your saving path for snaps



INSCOPER- Configuration tab: set your channels one by one

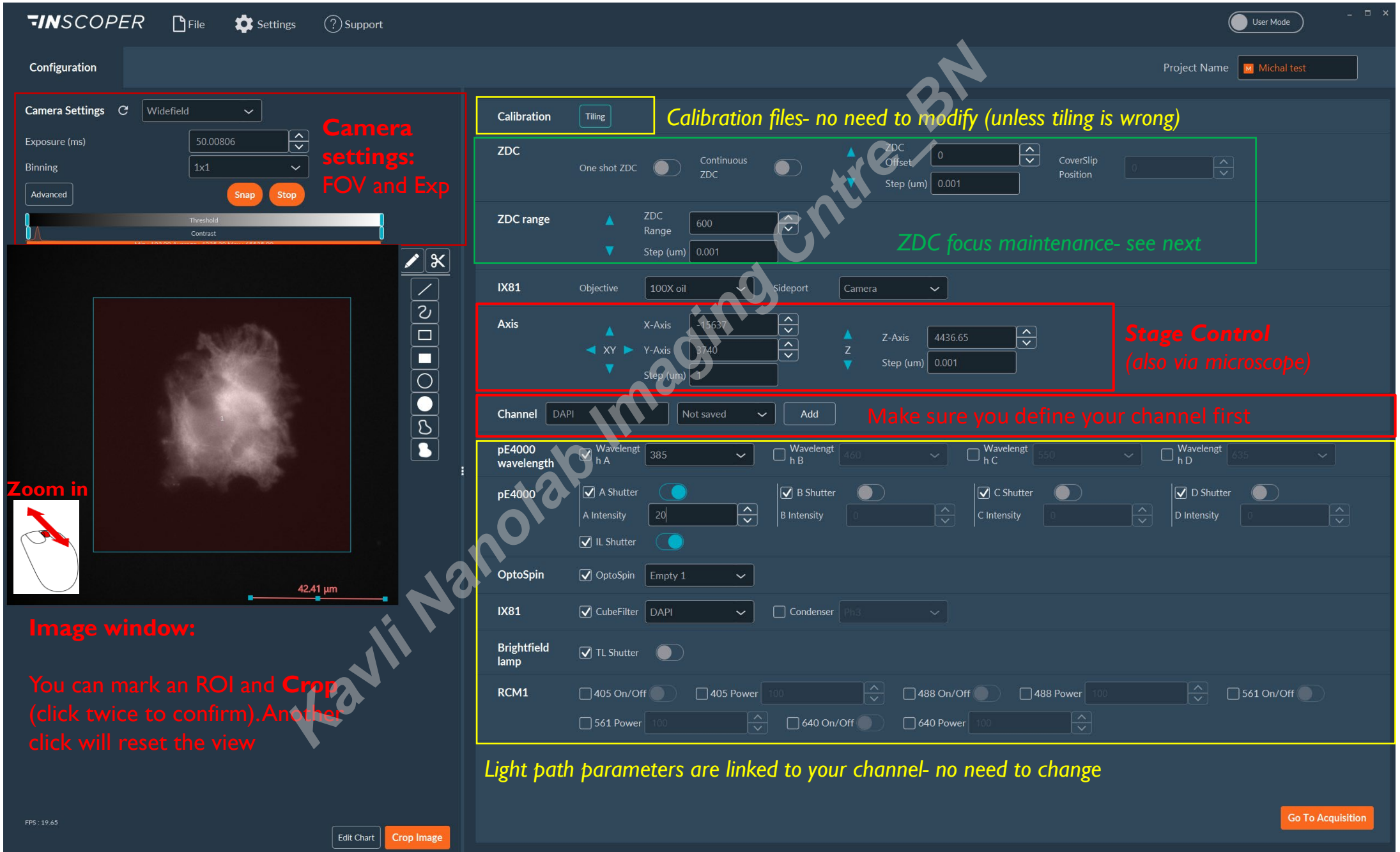


Image window:

Display tools:

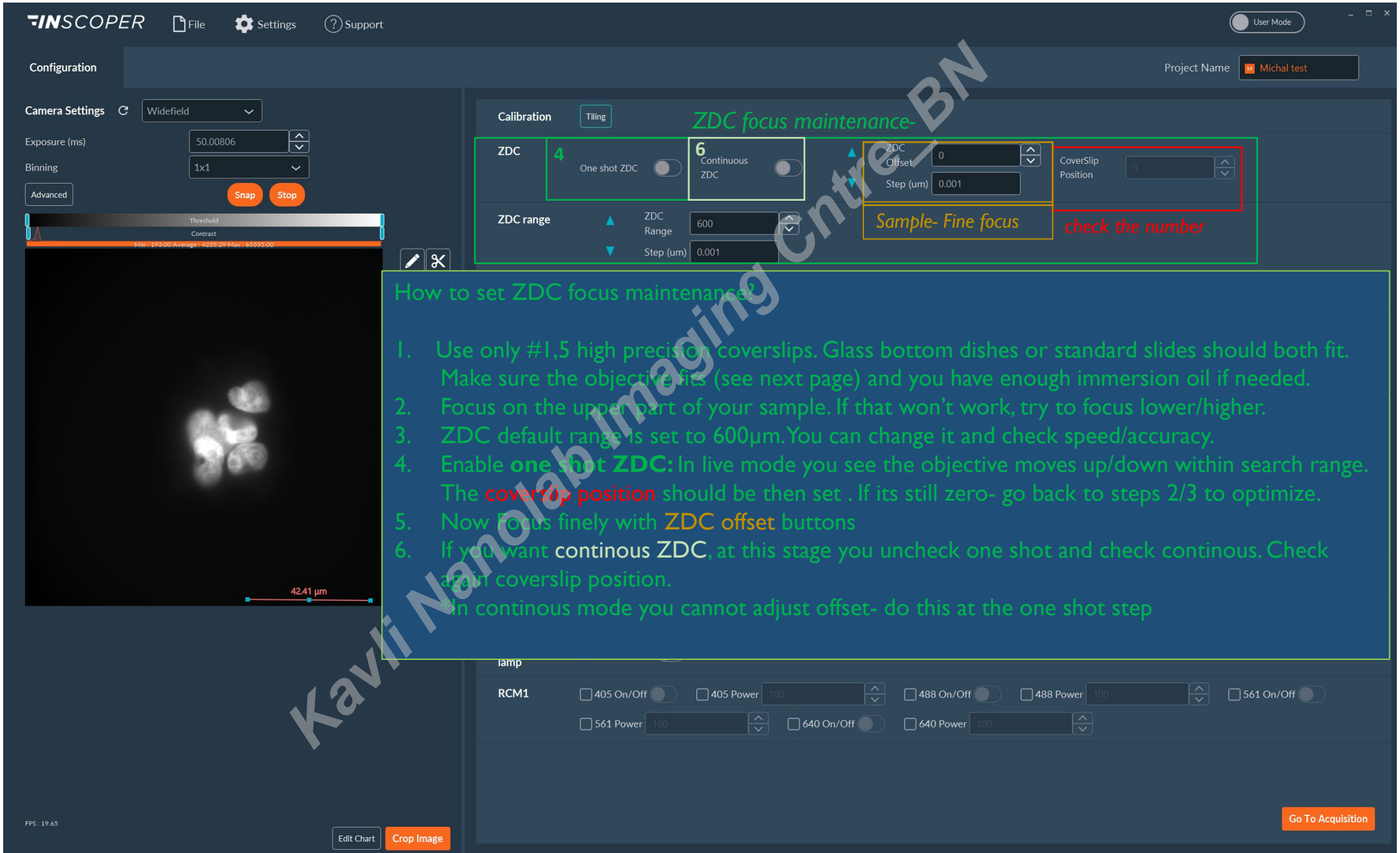
	You can move inside the live image by drag-and-drop
	You can select this option to add some ROI or make some crops on their live images
	You can choose this option to set the contrast automatically or manually. If it is manually, adjust the blue sliders on top of the camera view.
	Press this button to 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.

INSCOPER- Configuration tab: Focus maintenance



Objectives compatible with ZDC-
Usually one shot and flu mode works best

UIS2 Series

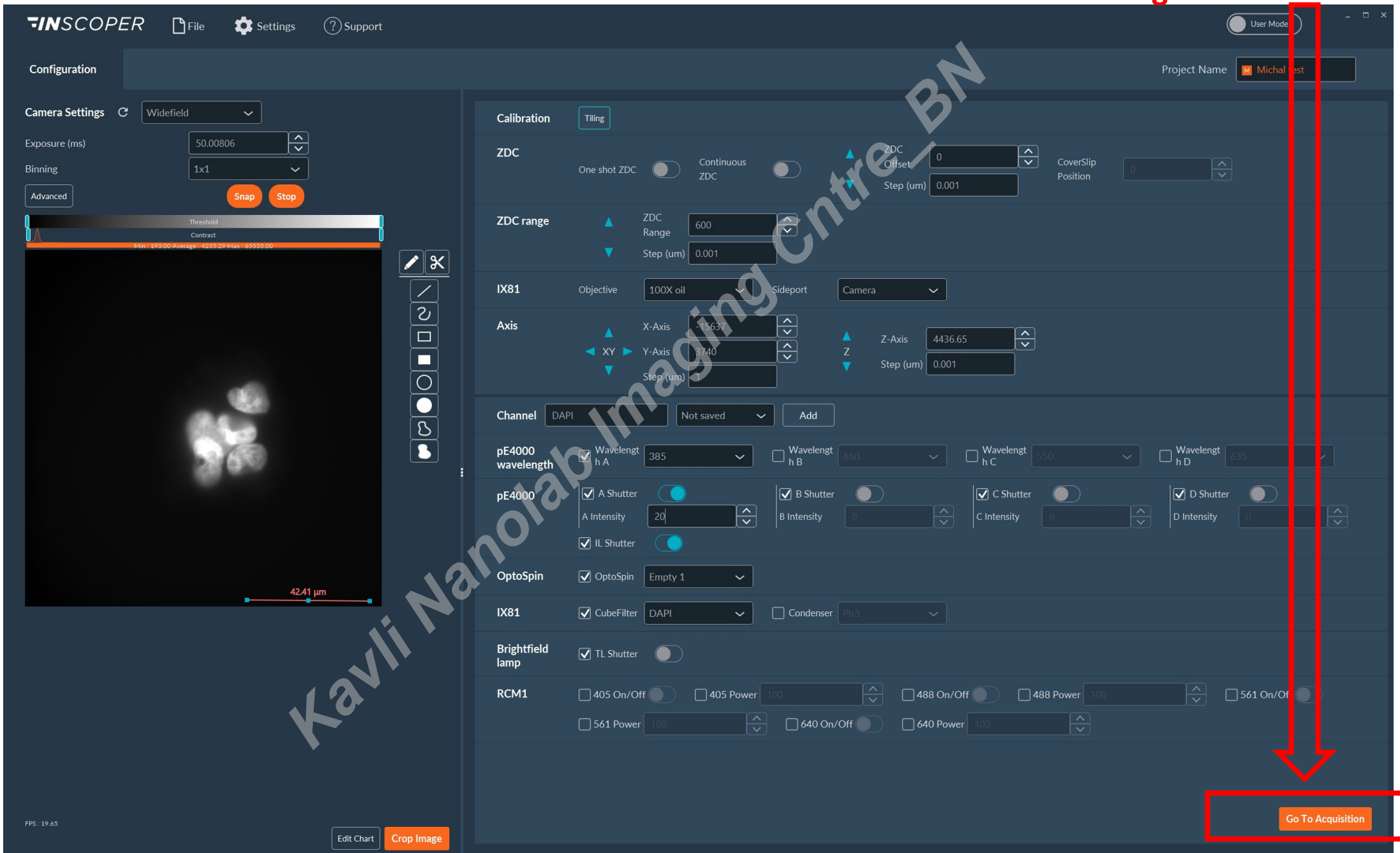
Objective Name	NA	WD (mm)
PlanApoN 60XO	1.42	0.15
UPlanSApo 20X	0.75	0.60
UPlanSApo 40X	0.90	0.18
UPlanSApo 40X2	0.95	0.18
UPlanSApo 60X O	1.35	0.15
UPlanSApo 60X W	1.20	0.28
UPlanSApo 100X O	1.40	0.13
UPlanFLN 20X	0.50	2.10
UPlanFLN 40X	0.75	0.51
UPlanFLN 60X	0.90	0.20
UPlanFLN 100X O/O2	1.30	0.20
UPlanFLN 100X OI/OI2	1.3-0.6	0.20
LUCPlanFLN 20X	0.45	6.6-7.8
LUCPlanFLN 40X	0.60	2.7-4.0
LUCPlanFLN 60X	0.70	1.5-2.2

UIS Series

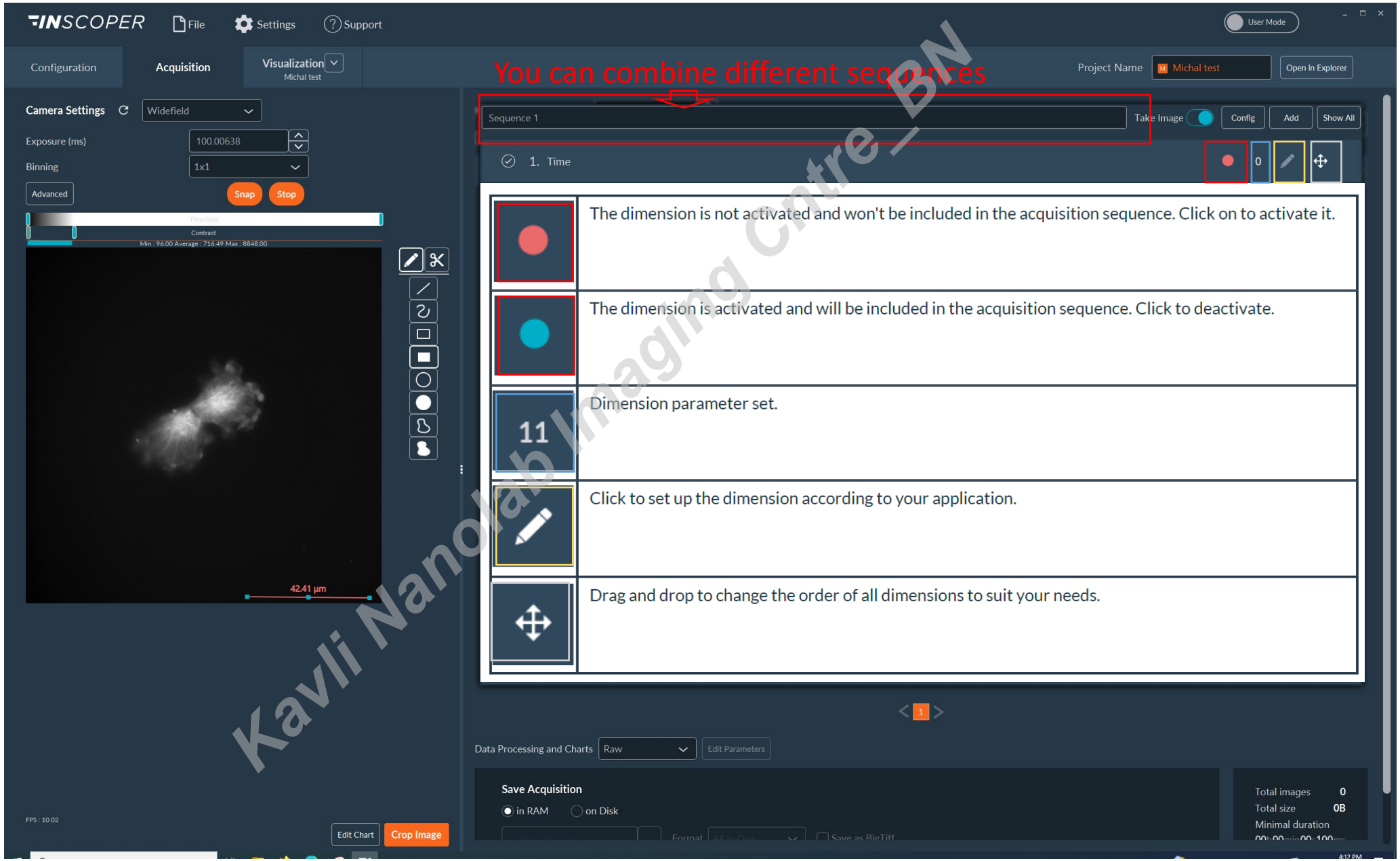
Objective Name*	NA	WD (mm)	
PlanApo 60X O3	1.40	0.15	
PlanApo 100X O3	1.40	0.10	
UPlanApo 60X	0.90	0.20	
UPlanApo 60X W3	1.20	0.25	
UPlanApo 60X W3/IR	1.20	0.28	
UPlanApo 100X OI3	1.35	0.10	
UApo 20X 3/340	0.75	0.55	
UApo 40X 3/340	0.90	0.20	
UApo 40X W3/340	0.70	0.40	
UPlanFl 20X	0.50	1.60	
UPlanFl 40X	0.75	0.51	
UPlanFl 100X O3	1.30	0.10	
PlanApo 40X W/LSM	0.90	0.16	
PlanApo 60X O/LSM	1.10	0.13	
PlanApo 60X O TIRFM-SP	1.45	0.15	(Special order)
UPlanFl 40X O-SP	1.30	0.12	(Special order)
LCPlanFl 40X	0.60	2.60	
SLCPlanFl 40X	0.55	6.4 - 8.3	
LUCPlanFl 40X	0.60	3.40	
LCPlanFl 60X	0.70	1.70	

INSCOPER- Acquisition tab: set your experiment

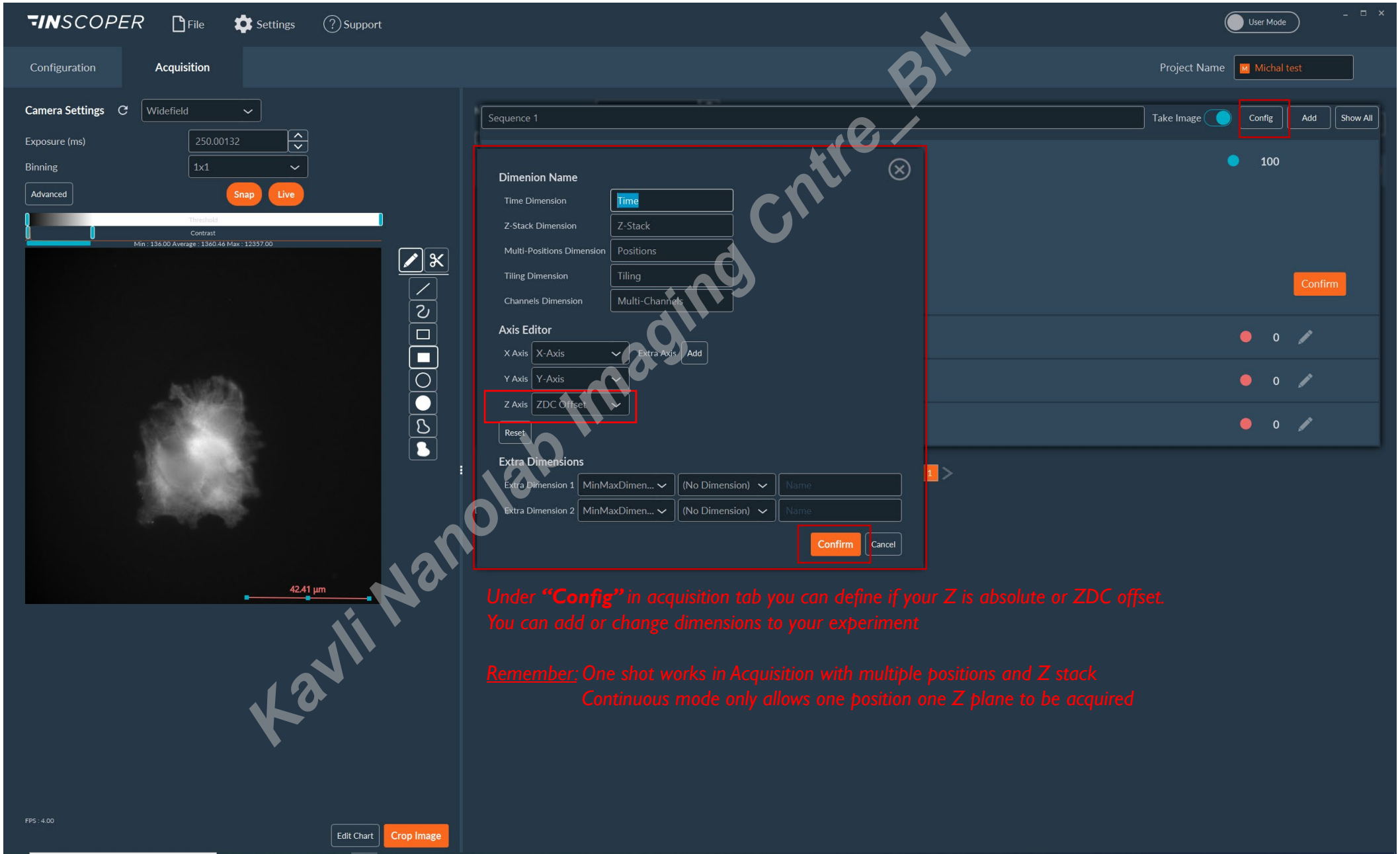
From Configuration window



INSCOPER- Acquisition tab: set your experiment type



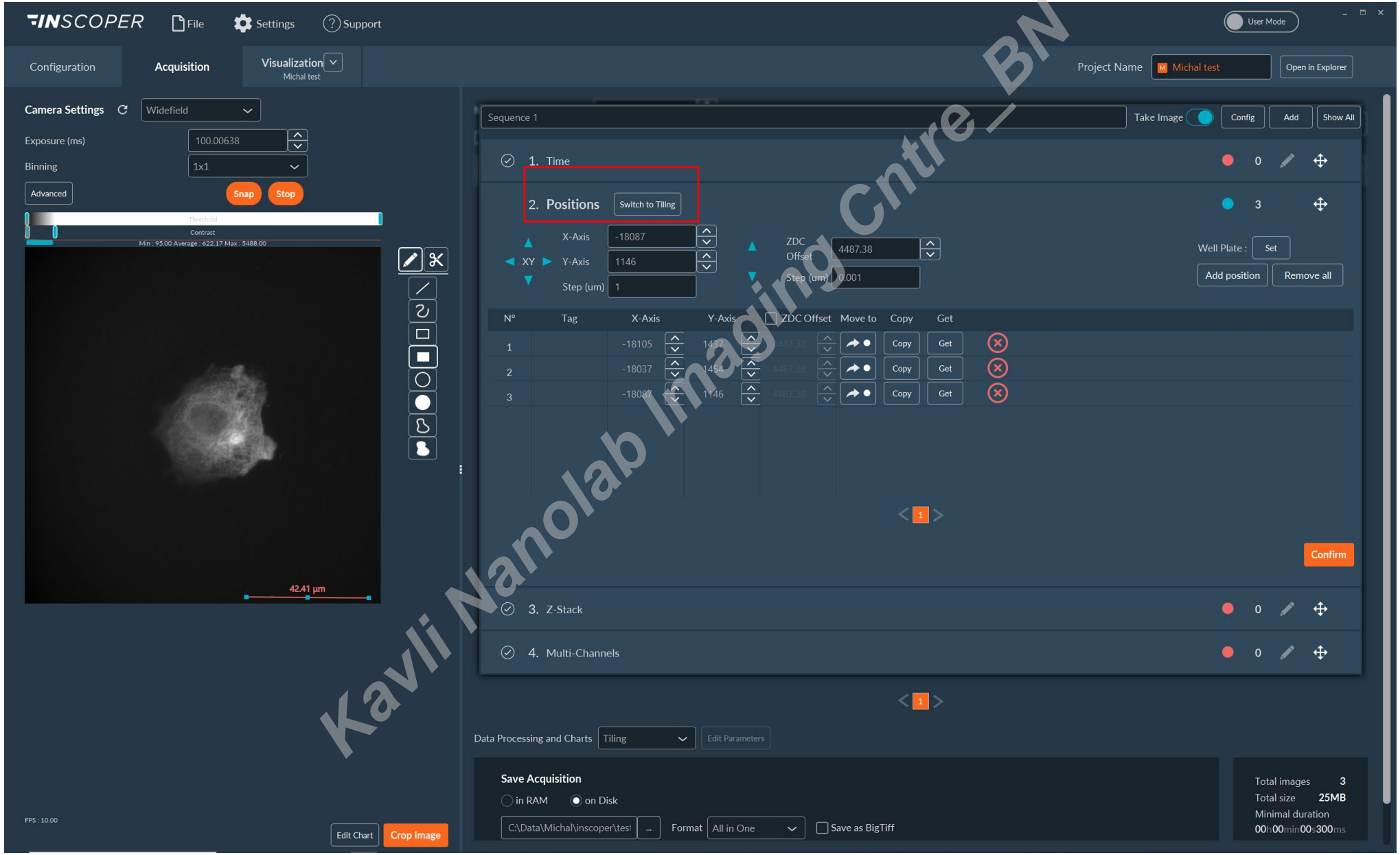
INSCOPER- Acquisition: use ZDC offset / use standard Z



*Under **"Config"** in acquisition tab you can define if your Z is absolute or ZDC offset.*
You can add or change dimensions to your experiment

Remember: One shot works in Acquisition with multiple positions and Z stack
Continuous mode only allows one position one Z plane to be acquired

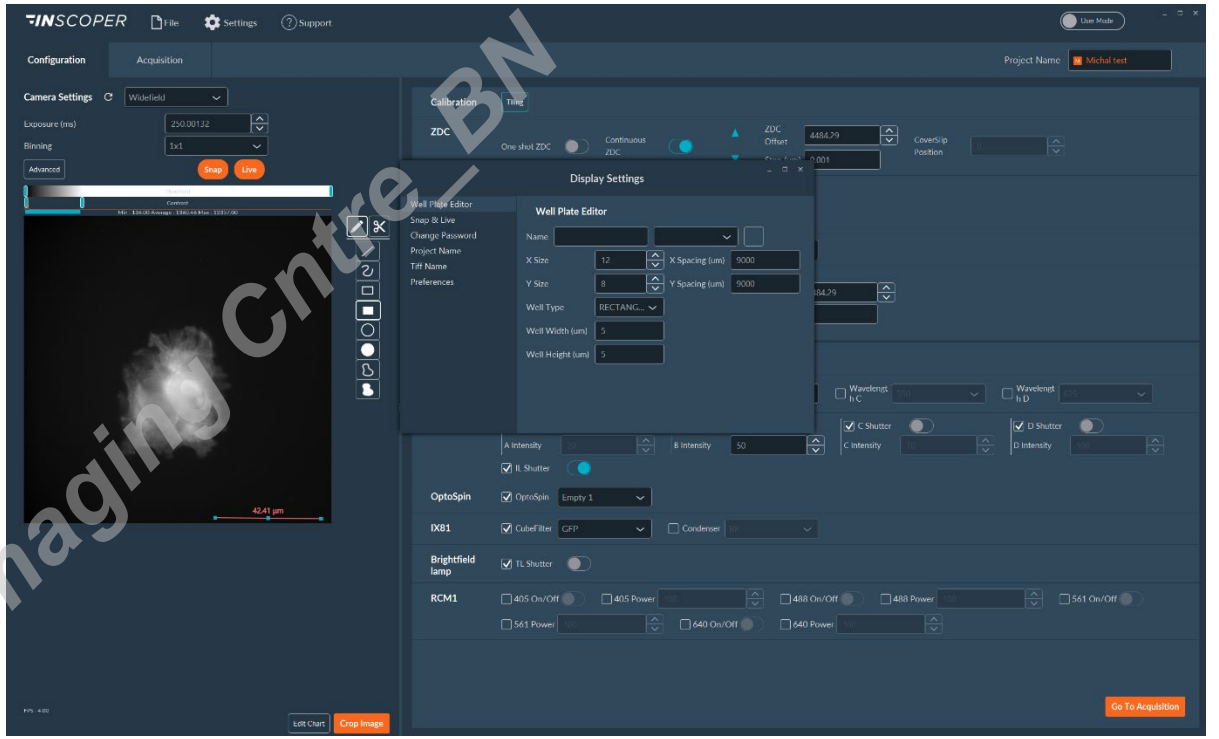
INSCOPER- Acquisition- Stage experiment: position list



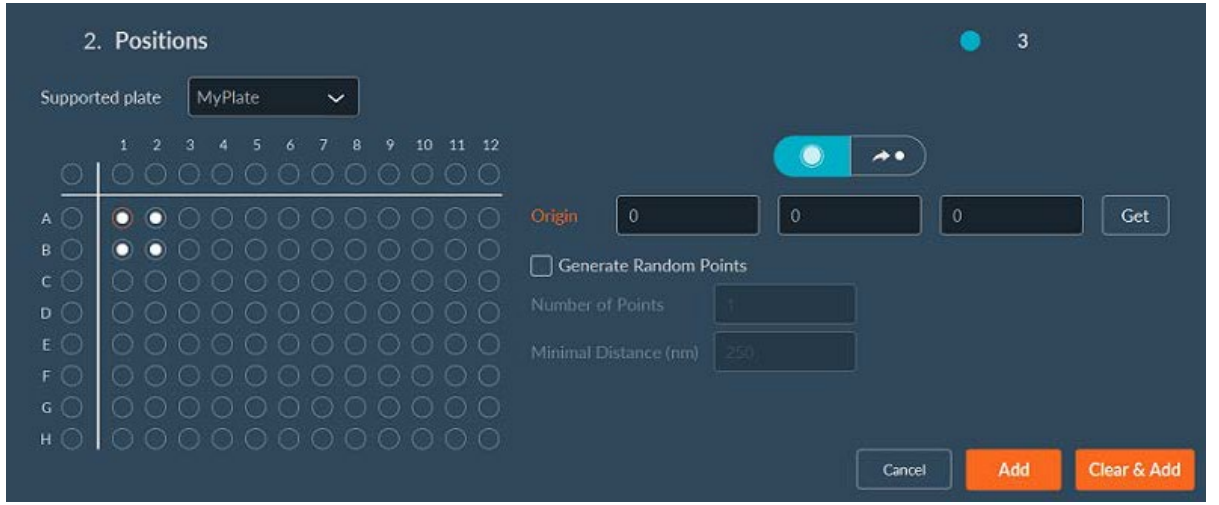
INSCOPER- Acquisition- Stage experiment: Define well plate (Auto position list)

!Only using Air Objectives!

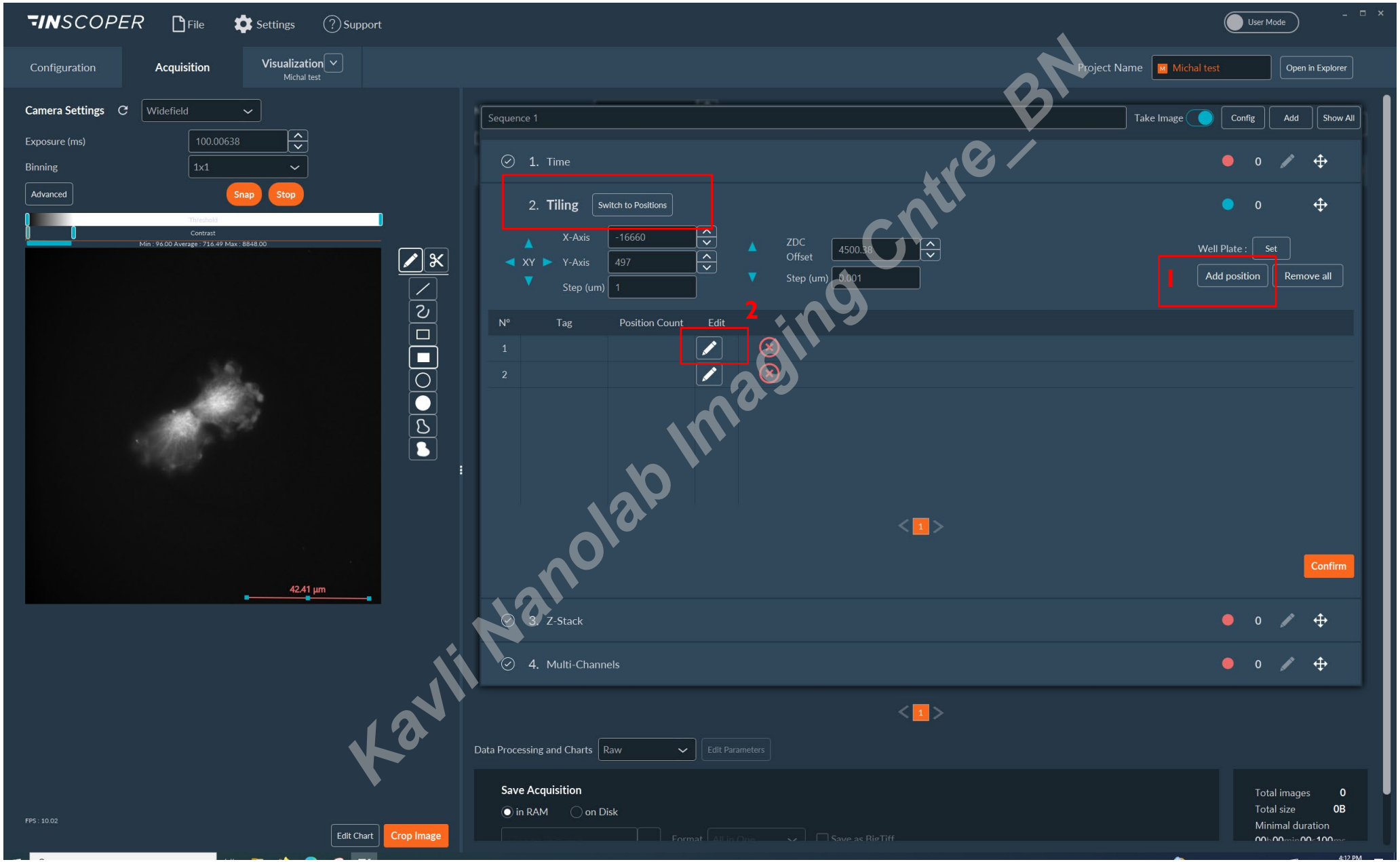
Set	Set	If you have a well-plate, click on this button to select the wells you are interested in.
Add position	Add Position	Add a position manually and enter its coordinates.
Remove All	Remove All	Delete all the positions.
	Move To	Move to the selected position.
Copy	Copy	Copy the required coordinates.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

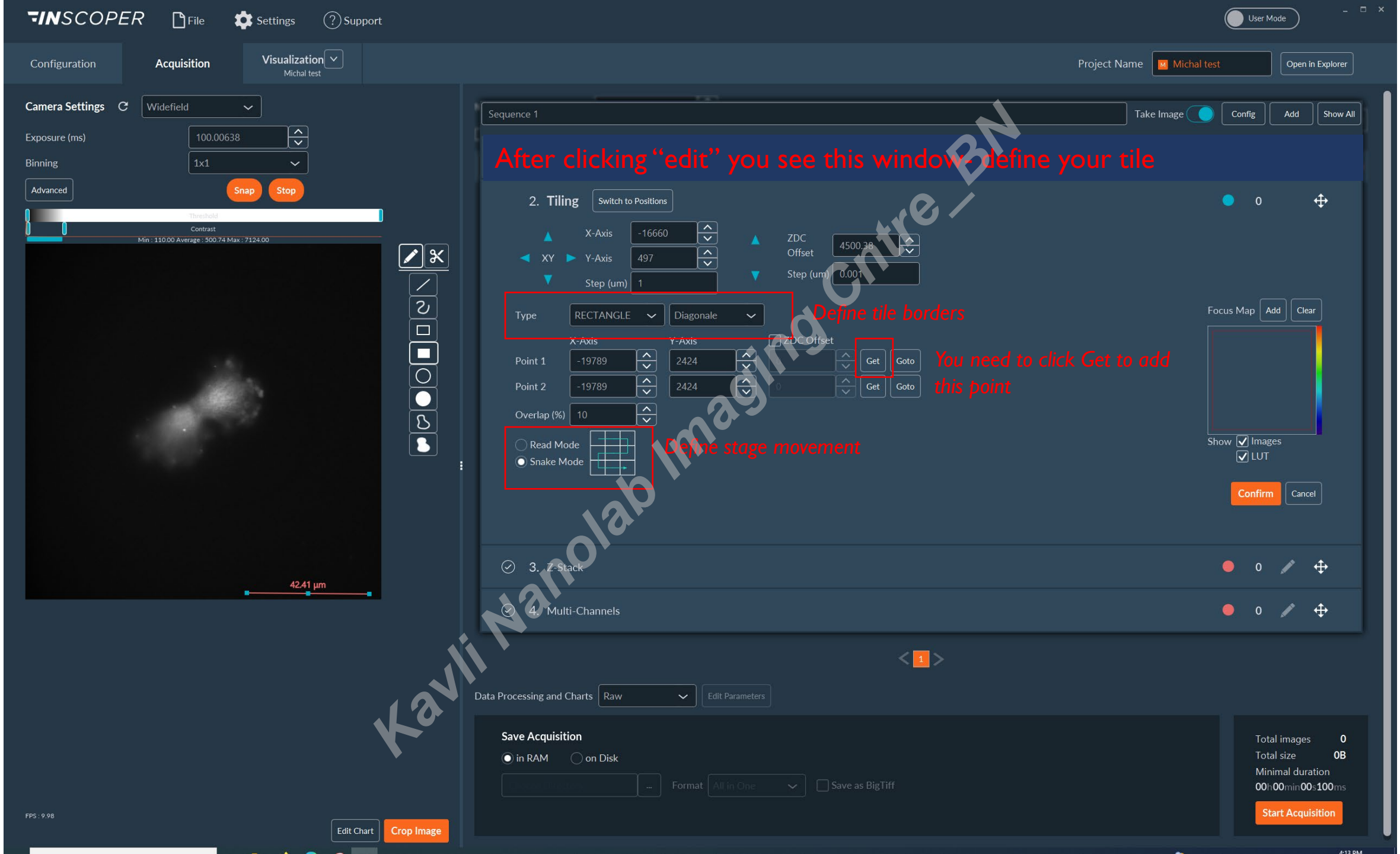


MyPlate	Choose Your Plate	Select the well-plate you wish to use in the drop-down list.
	Select	Click on this button to select the wells you need.
	Move To	Move to the selected well.
Get	Get	Get the coordinates entered on the well-plate.
Generate Random Points	Generate Random Points	By checking this box, 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.



INSCOPER- Acquisition- Stage experiment:Tiling




INSCOPER
File
Settings
Support
User Mode
Configuration
Acquisition
Visualization
Michal test
Project Name
Michal test
Open in Explorer
Camera Settings
Widefield
Exposure (ms)
100.00638
Binning
1x1
Advanced
Snap
Stop
Threshold
Contrast
Min : 110.00 Average : 500.74 Max : 7124.00
42.41 µm
FPS : 9.98
Edit Chart
Crop Image
Sequence 1
Take Image
Config
Add
Show All
After clicking "edit" you see this window- define your tile
2. Tiling
Switch to Positions
X-Axis
-16660
XY
Y-Axis
497
Step (um)
1
ZDC Offset
4500.38
Step (um)
0.001
Type
RECTANGLE
Diagonale
Define tile borders
X-Axis
Y-Axis
ZDC Offset
Point 1
-19789
2424
Get
Goto
Point 2
-19789
2424
Get
Goto
You need to click Get to add this point
Overlap (%)
10
Read Mode
Snake Mode
Define stage movement
Focus Map
Add
Clear
Show
Images
LUT
Confirm
Cancel
3. Z-Stack
4. Multi-Channels
Data Processing and Charts
Raw
Edit Parameters
Save Acquisition
in RAM
on Disk
Format
All in One
Save as BigTiff
Total images
0
Total size
0B
Minimal duration
00h00min00s100ms
Start Acquisition
Kavli Nanolab Imaging Centre_BN

Tiling of ares with large Z differences- Focus Map

Take Image | Config | Add | Show All

1. Time

0

527

3, Move to next positon and redo steps 1-2

XY

X-Axis: -18185

Y-Axis: 1439

Step (um): 1

ZDC Offset: 4441.88

Step (um): 0.001

Type: RECTANGLE | Center

	X-Axis	Y-Axis	ZDC Offset	
Center	-18185	1439	4441.88	Get / Goto

1, click "get" to add position

Width: 100

Height: 100

Overlap (%): 10

Read Mode

Snake Mode

2, click "Add" for focus map

Focus Map | Add | Clear

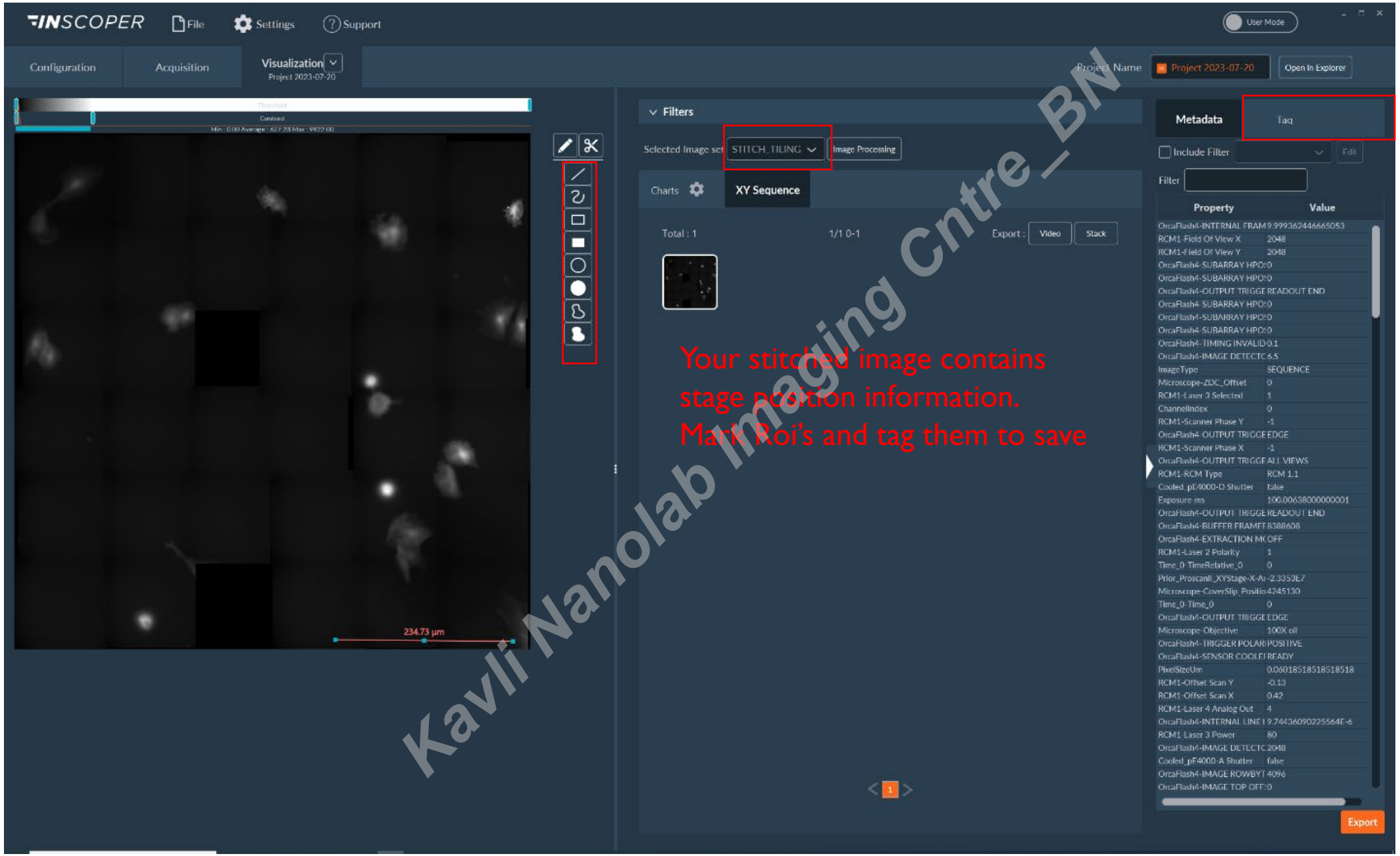
Show Images

LUT

Confirm | Cancel

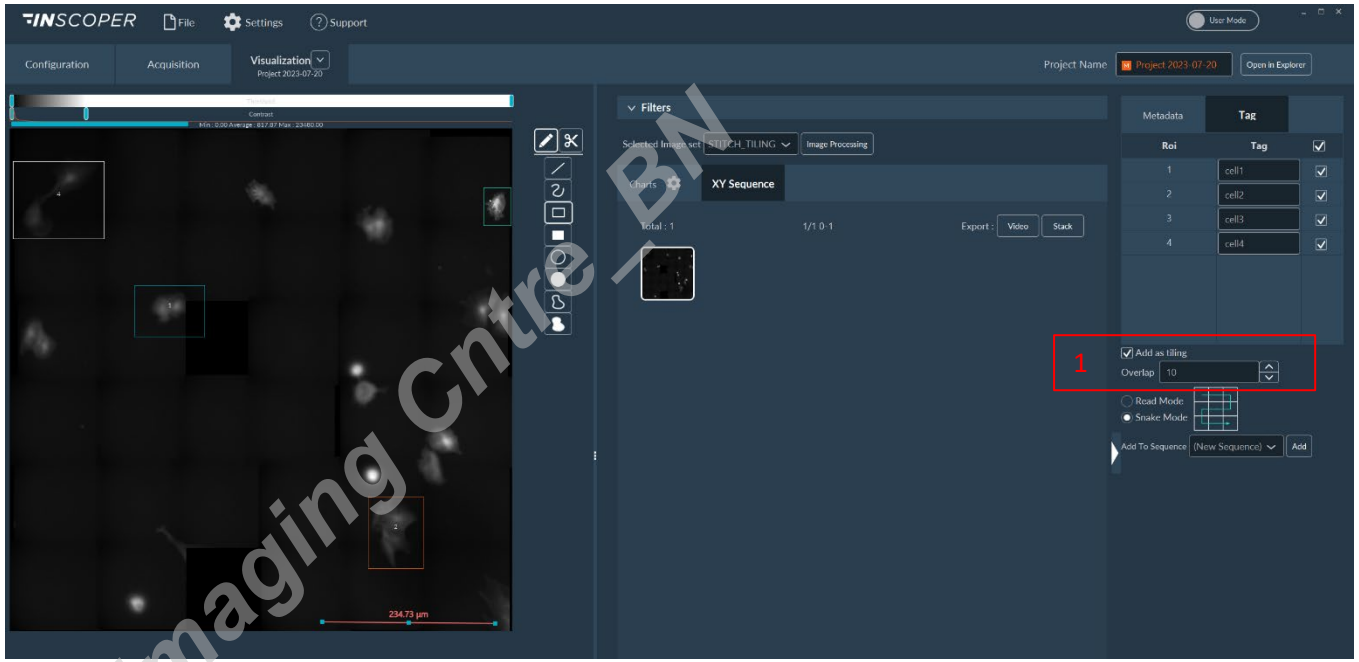
Tiling of ares with ZDC focus maintenance – If you have very different offset (otherwise not needed! ZDC keeps close to coverslip)

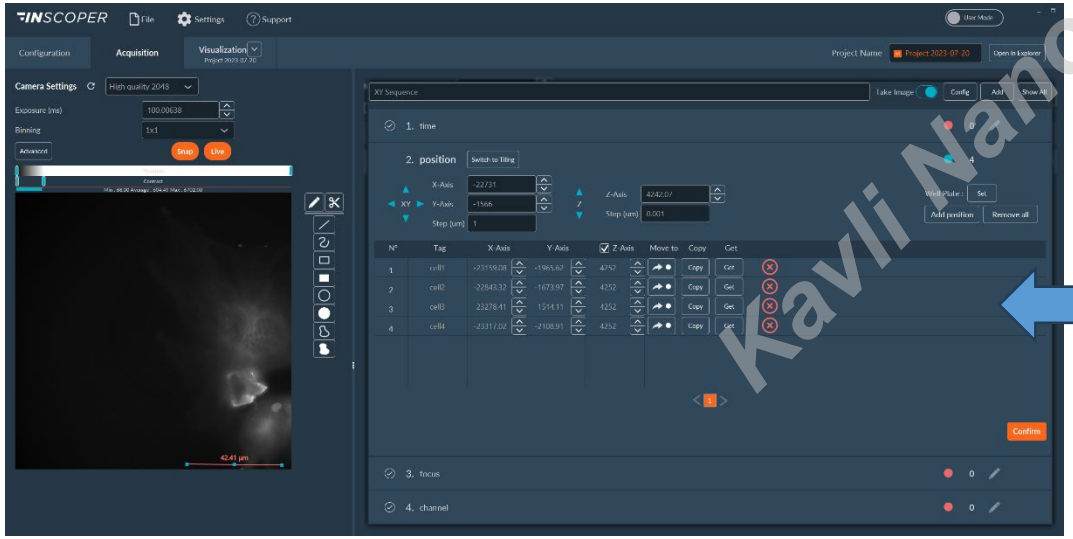
INSCOPER- Visualization tab- Use your tile as a map

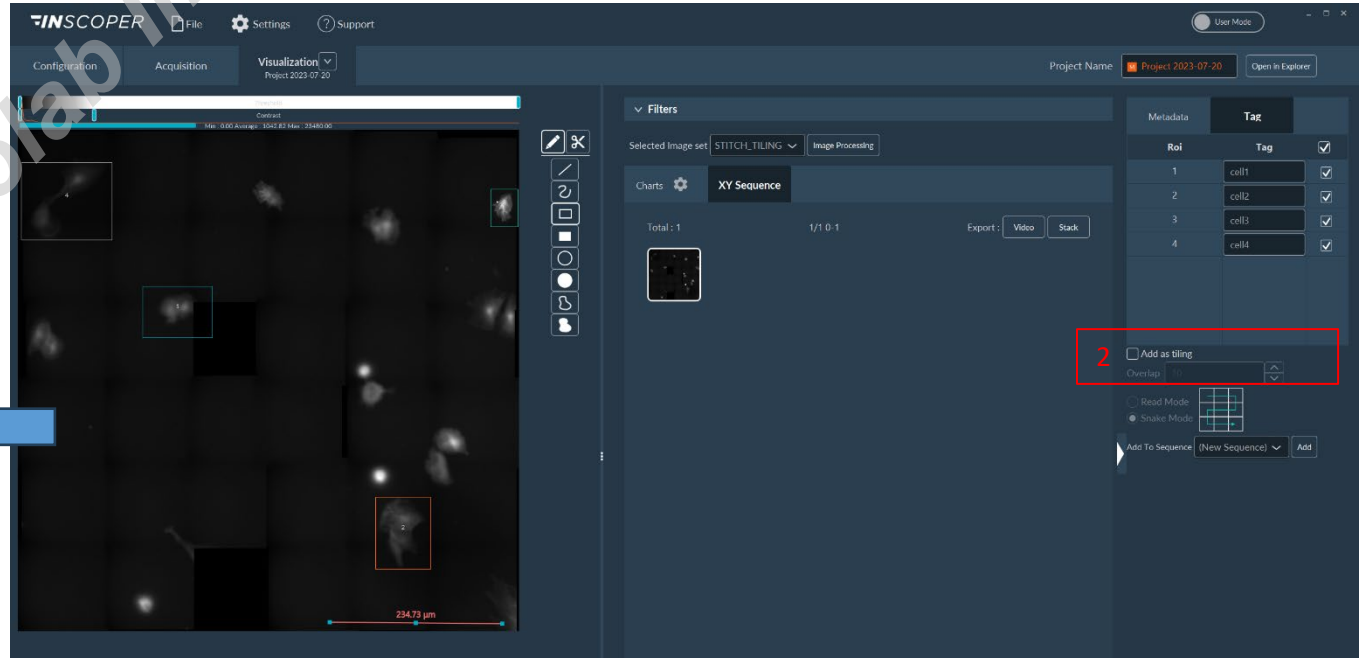


INSCOPER- Visualization tab- Use your tile as a map

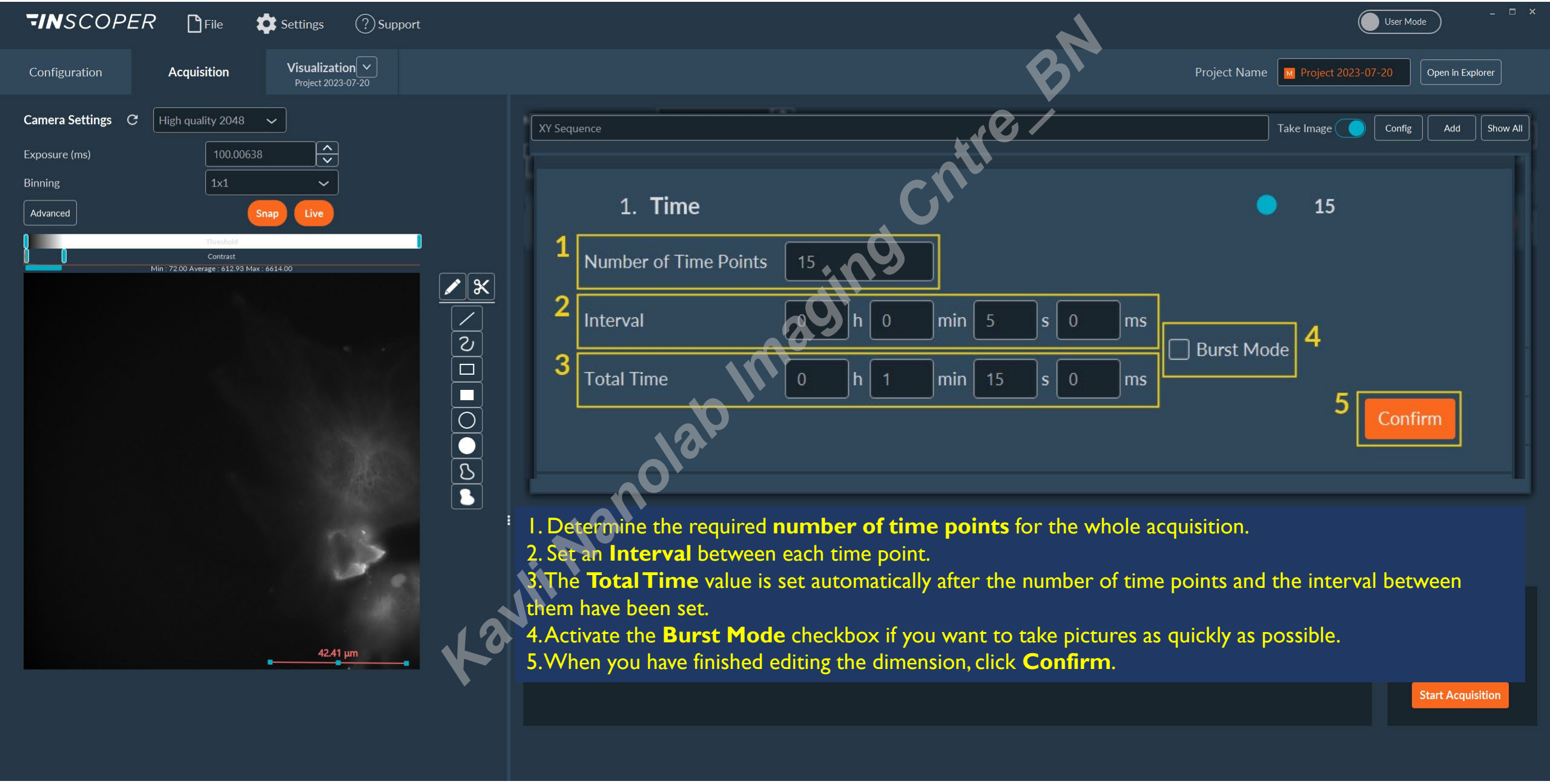
After building your position list, you can use each for a new tile (1) or keep (uncheck "add as tiling"-2) as separate positions



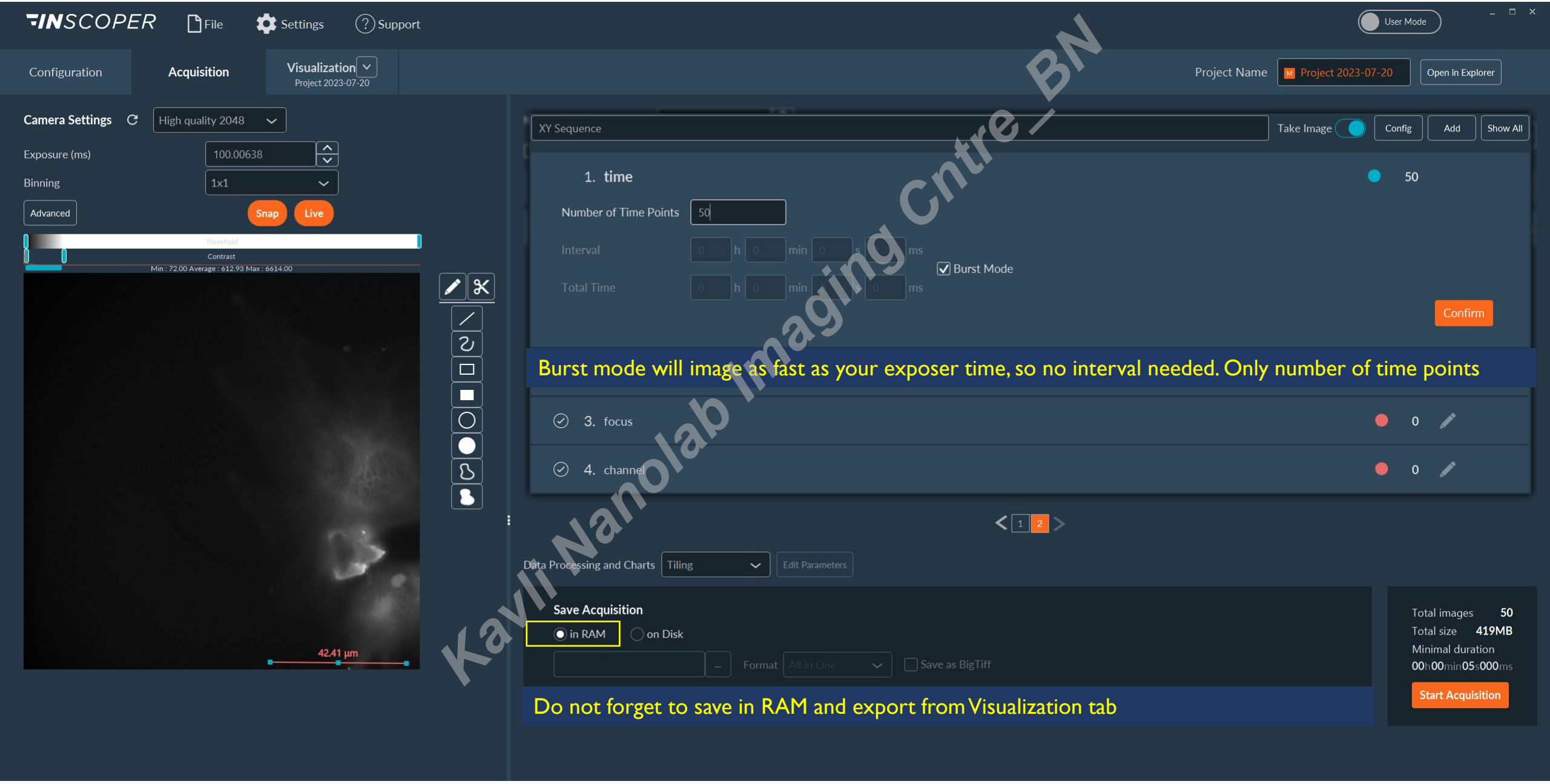




INSCOPER- Acquisition- Time experiment

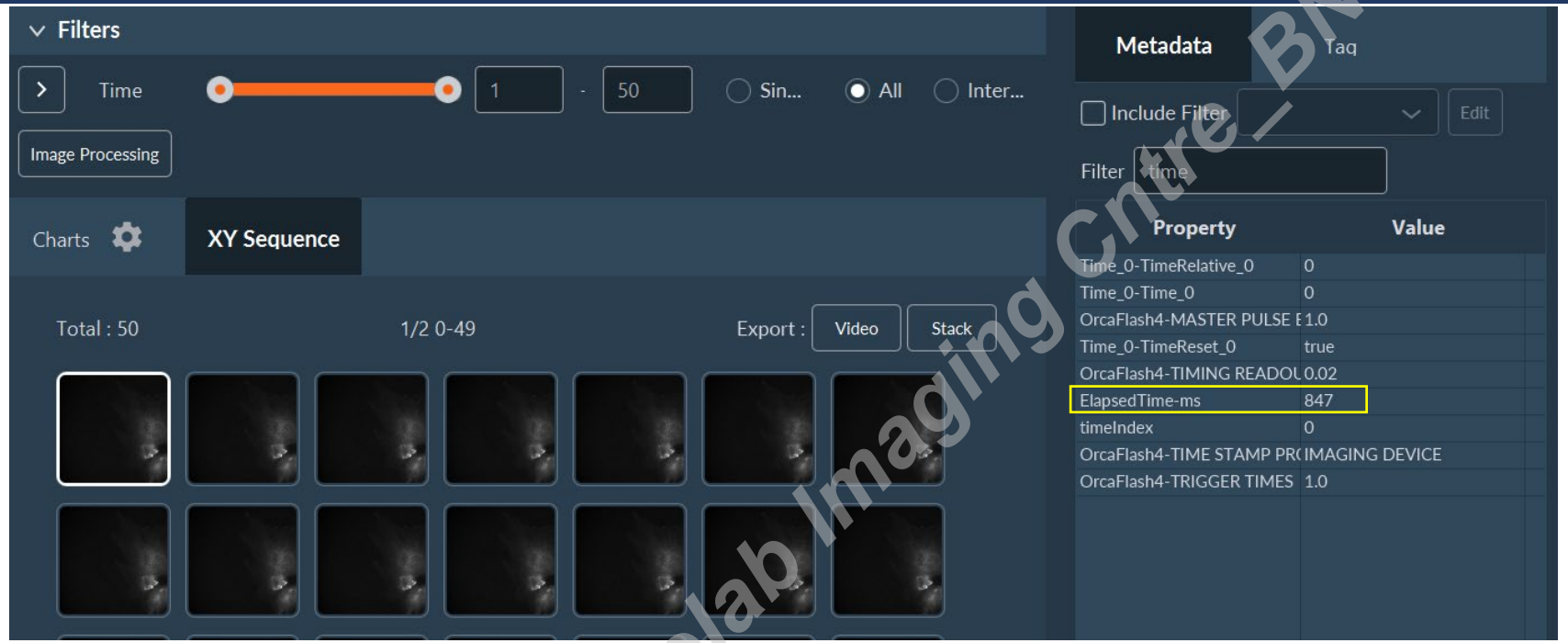


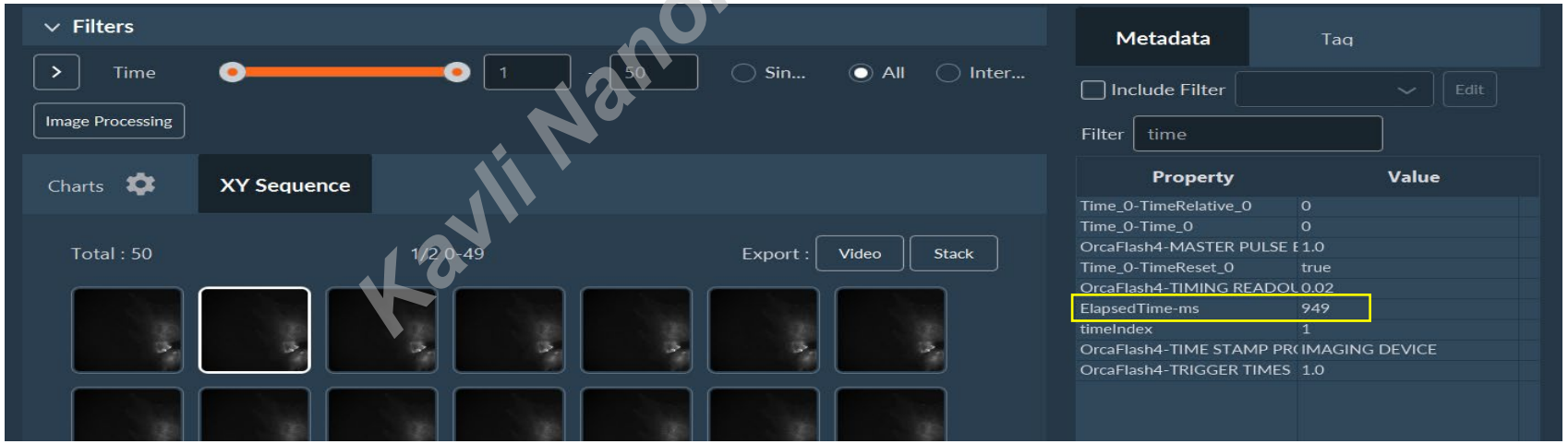
INSCOPER- Acquisition- Time experiment- Burst mode



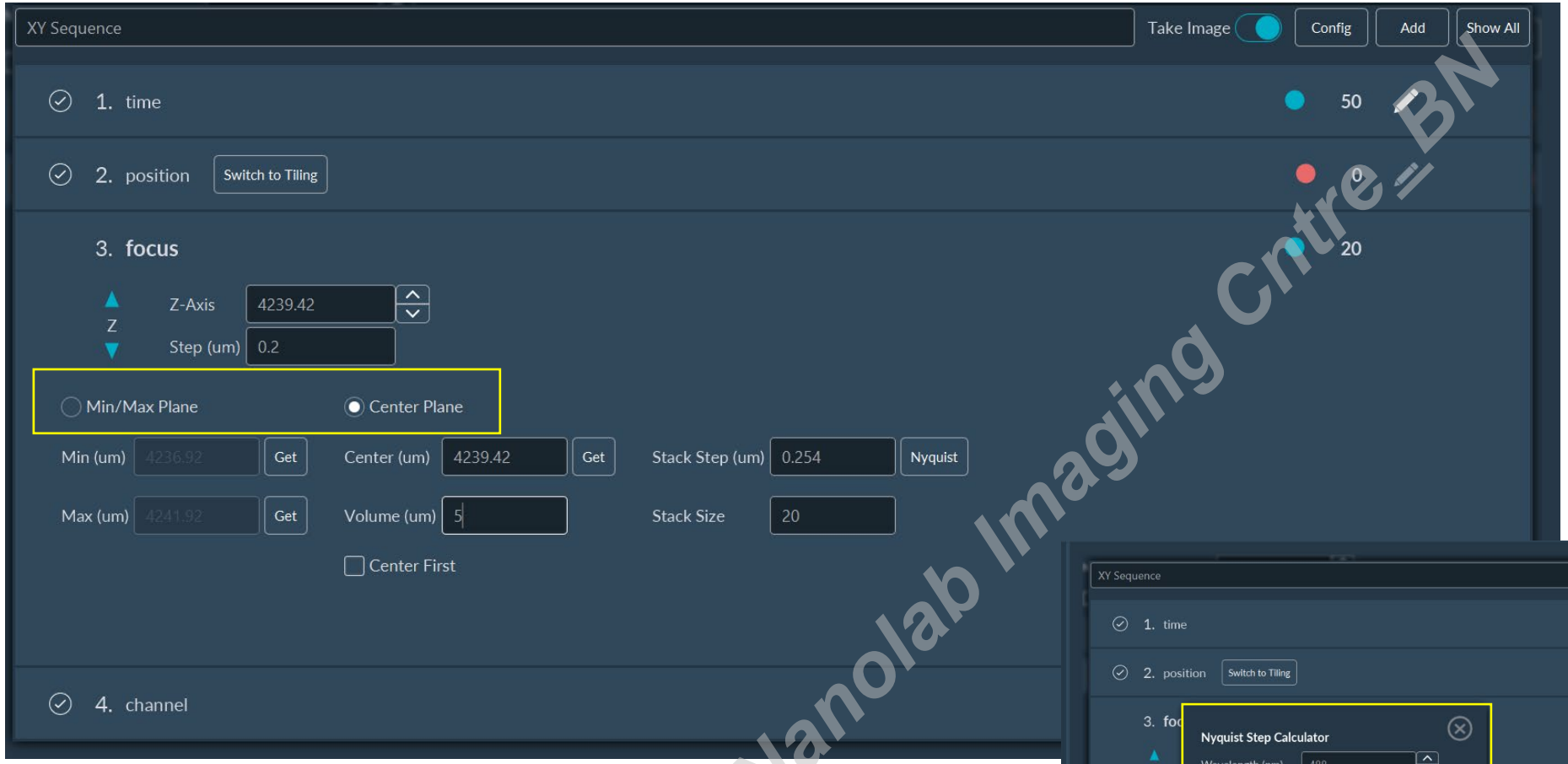
INSCOPER- Visualization- Time experiment- Burst mode

To check the actual time interval in burst mode, filter from metadata elapsed time per image and compare (the 1st image starts with small delay, after it time interval should equal exposer time per channel)

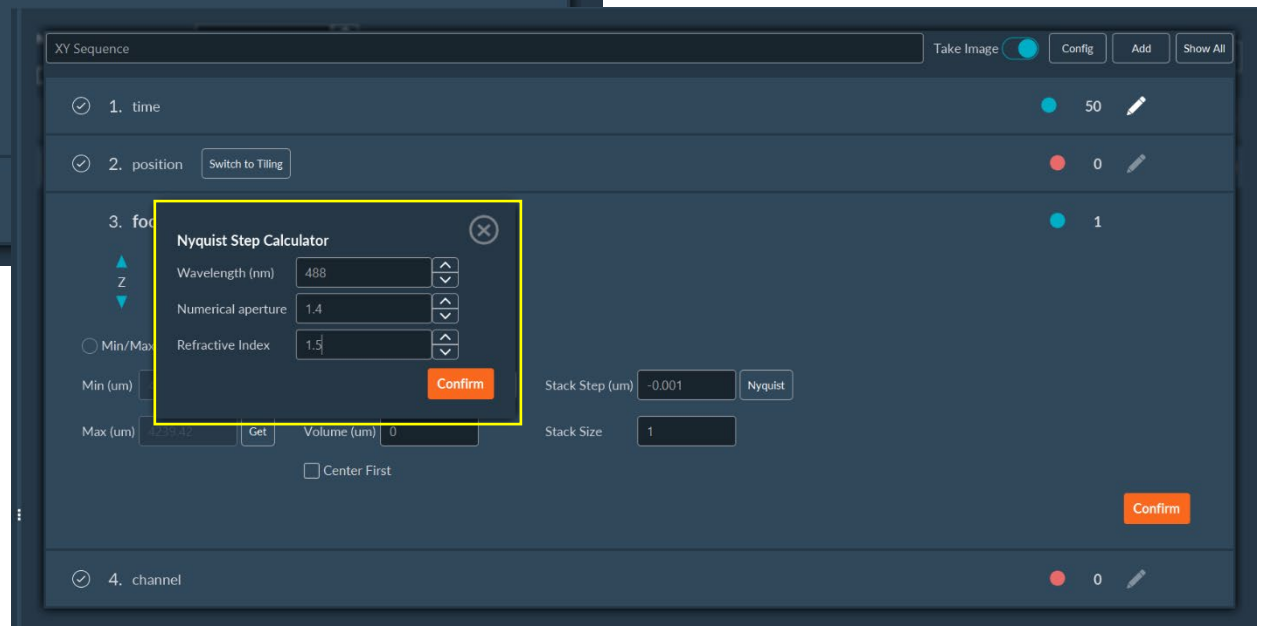




INSCOPER- Acquisition- Z stack

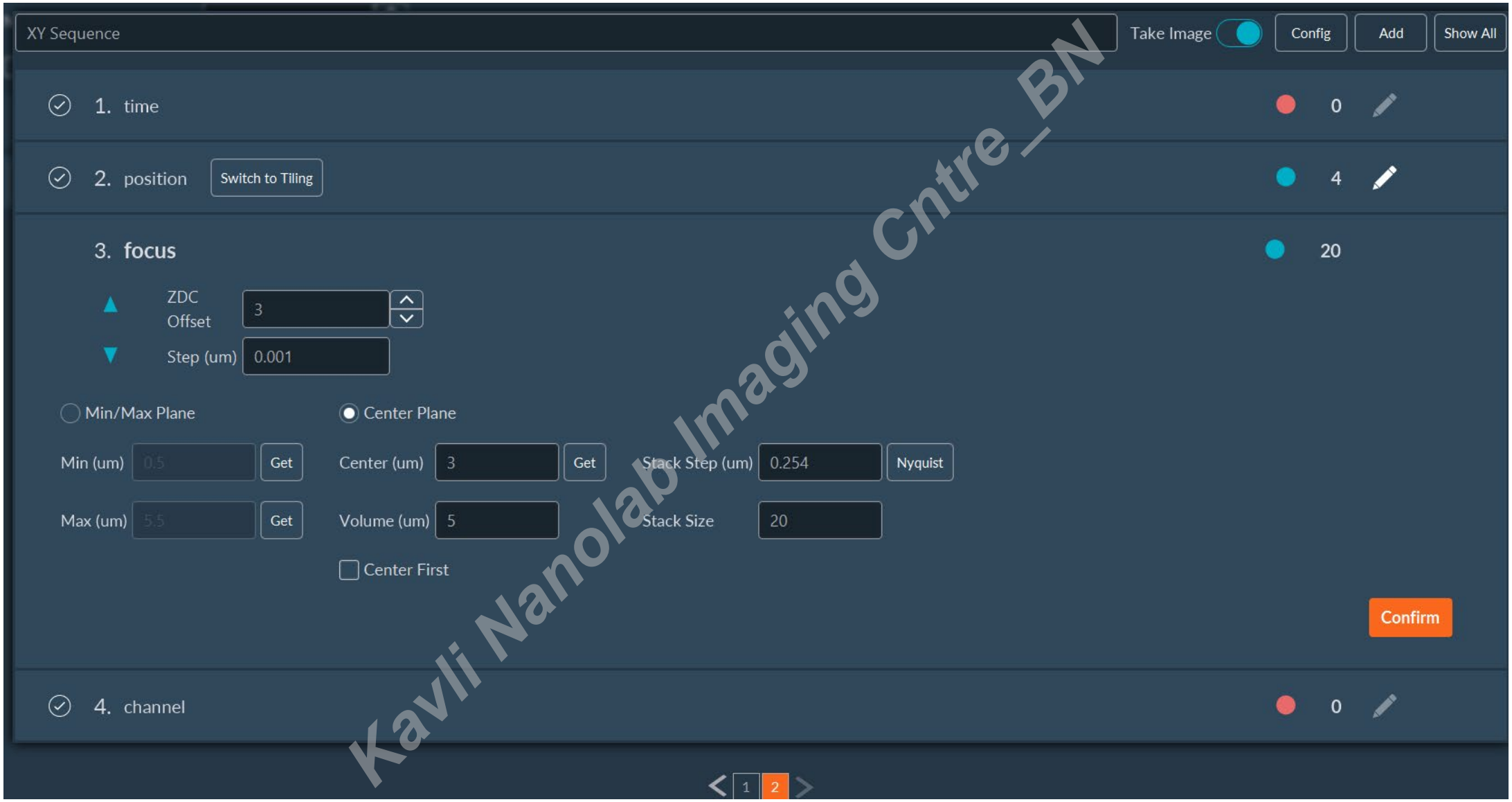


You can define Z stack absolute or around the center

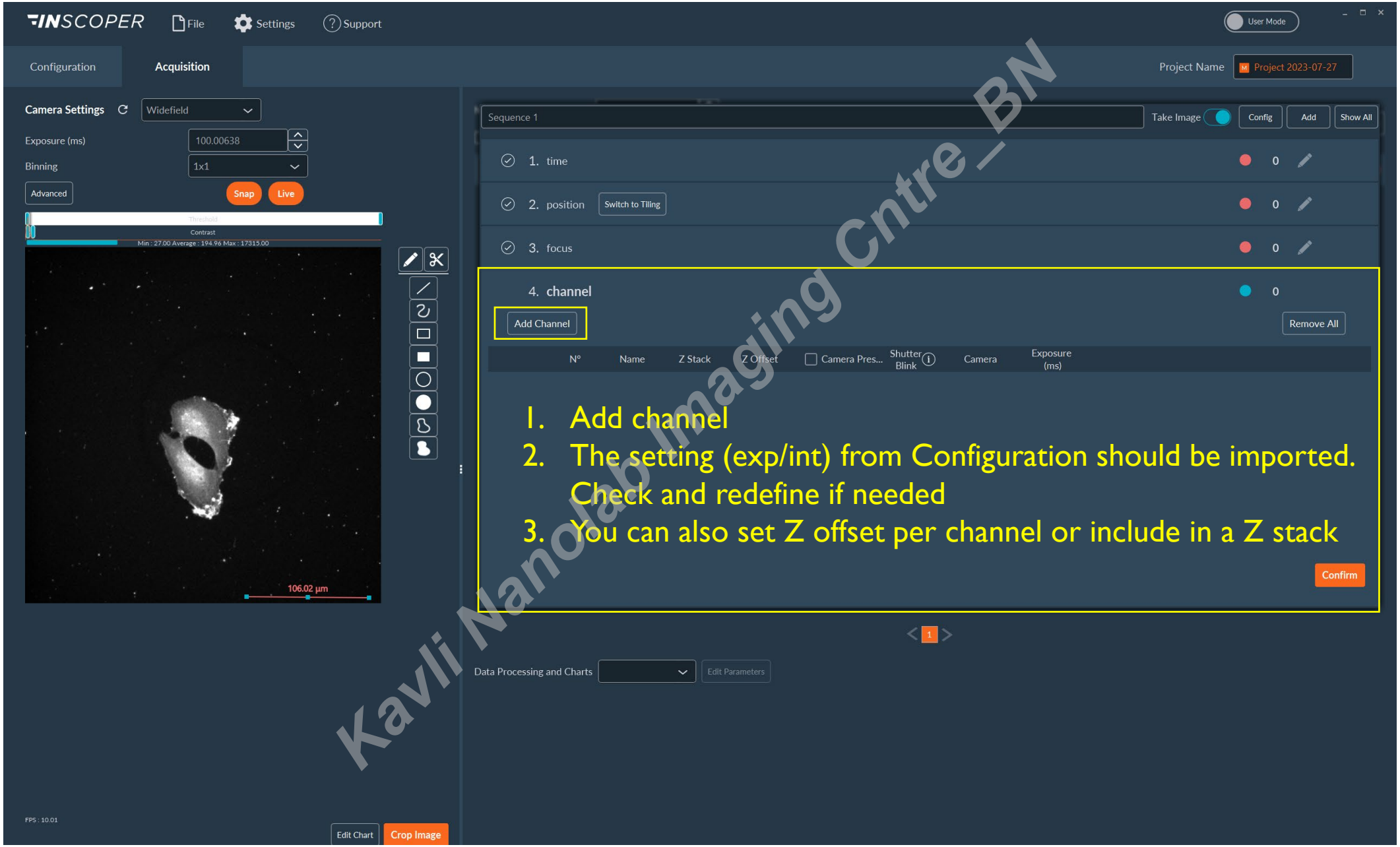


You can calculte recommended Z step from Objective info

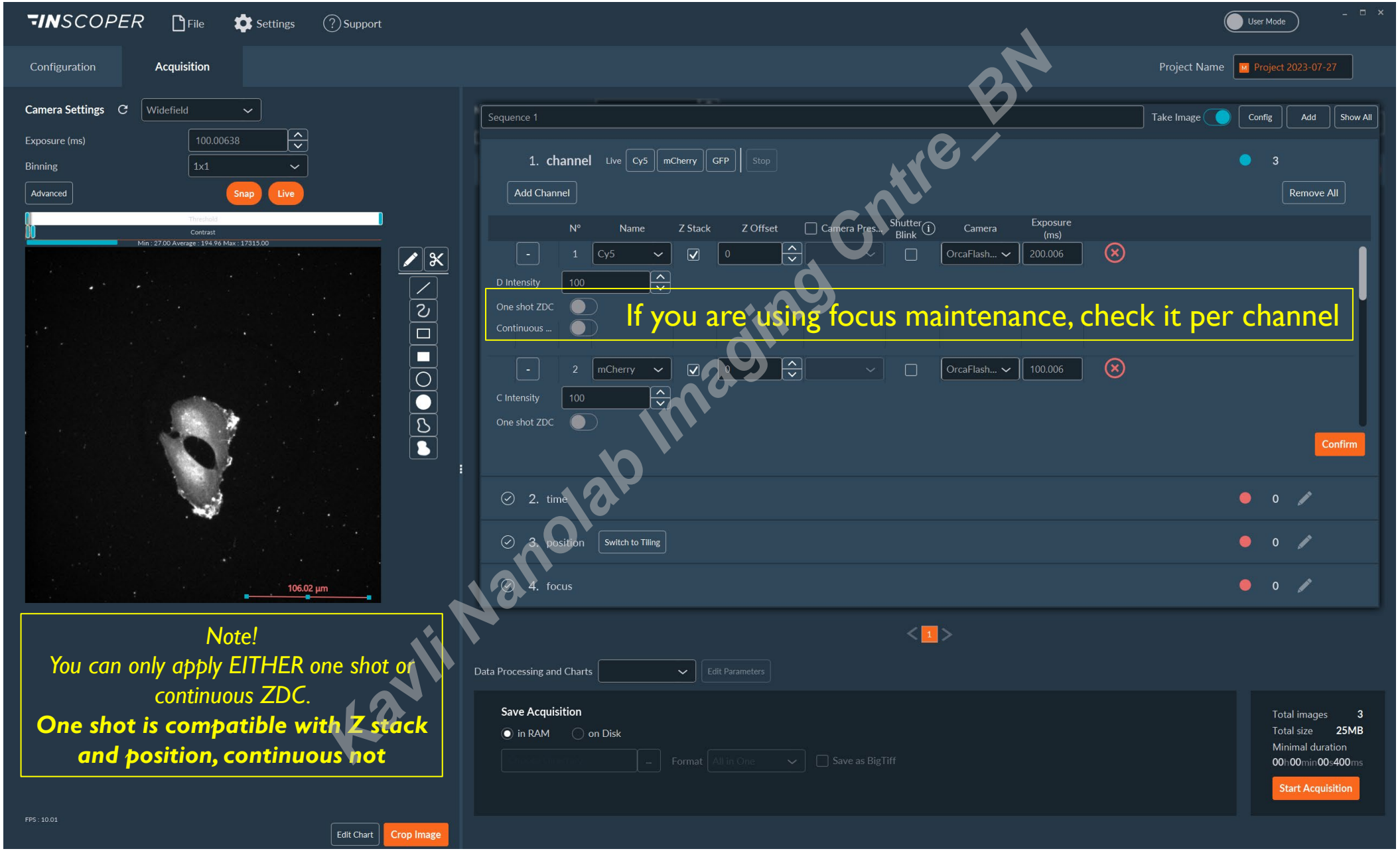
INSCOPER- Acquisition- Z stack around ZDC offset defined center



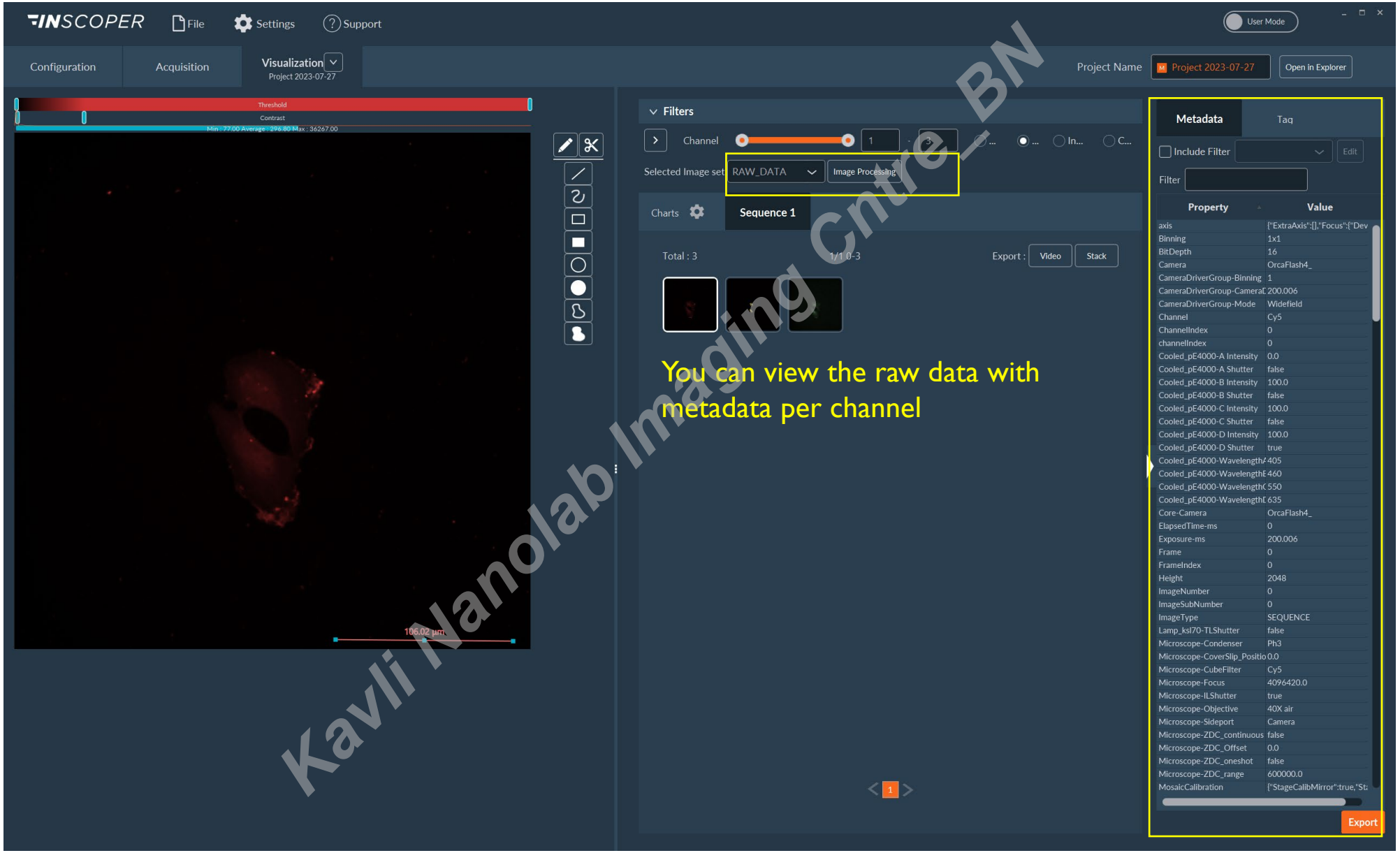
INSCOPER- Acquisition- Channels



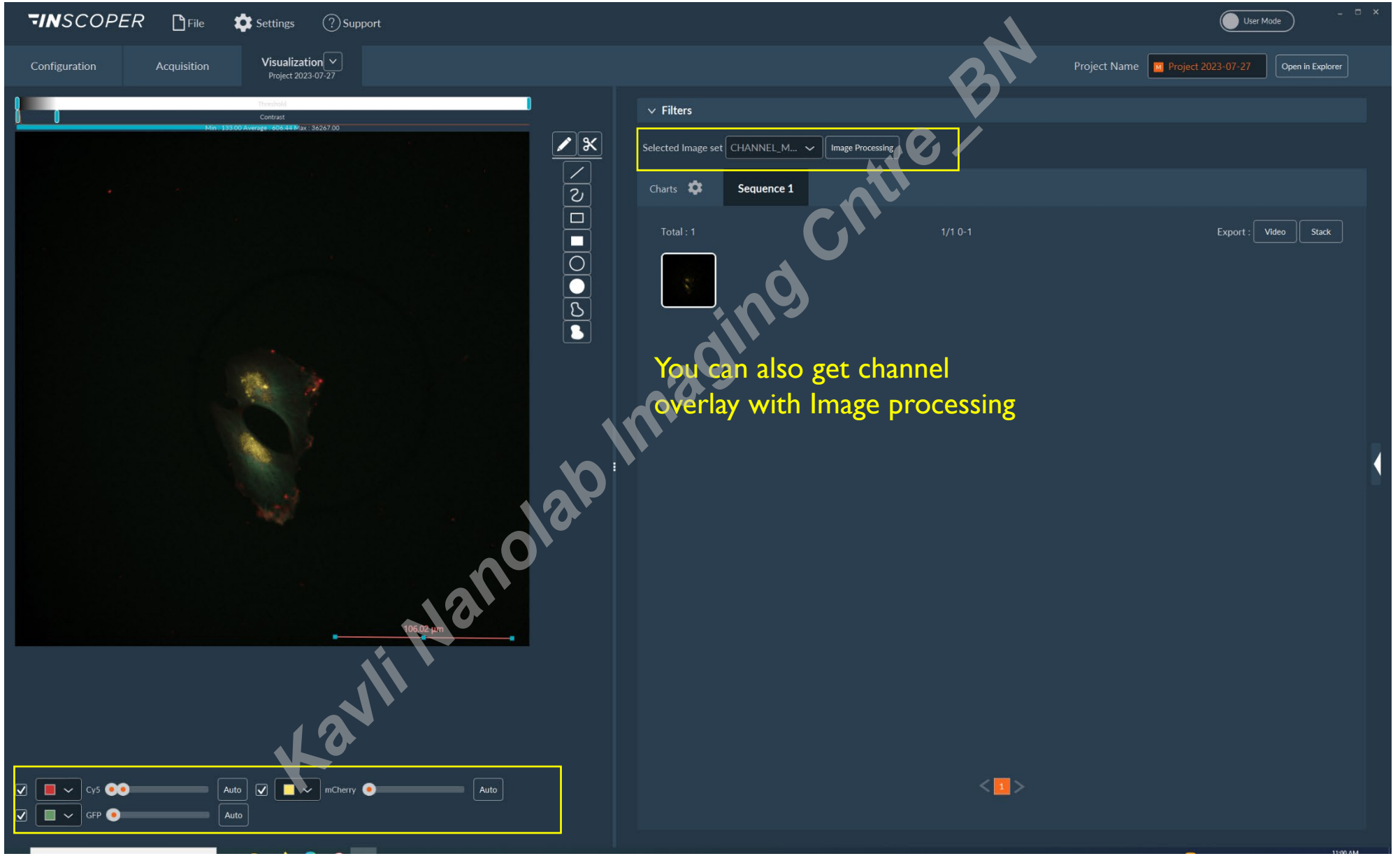
INSCOPER- Acquisition- Channels with ZDC



INSCOPER- Visualization- Channels

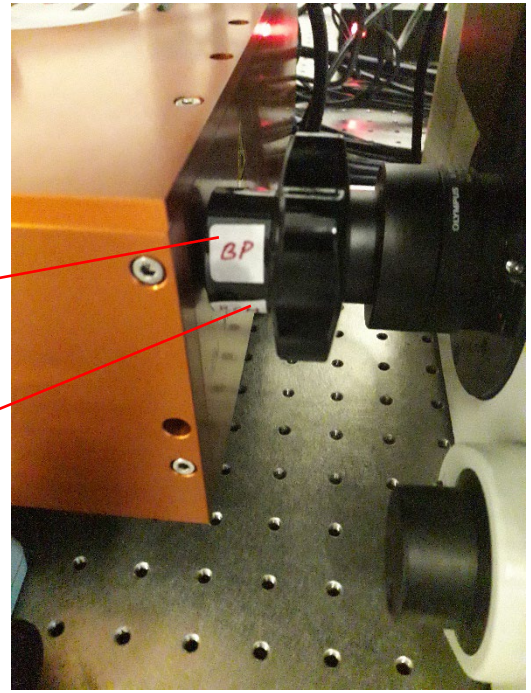


INSCOPER- Visualization- Channels



Shifting to high resolution confocal imaging (Pinhole is constant at 15µm)

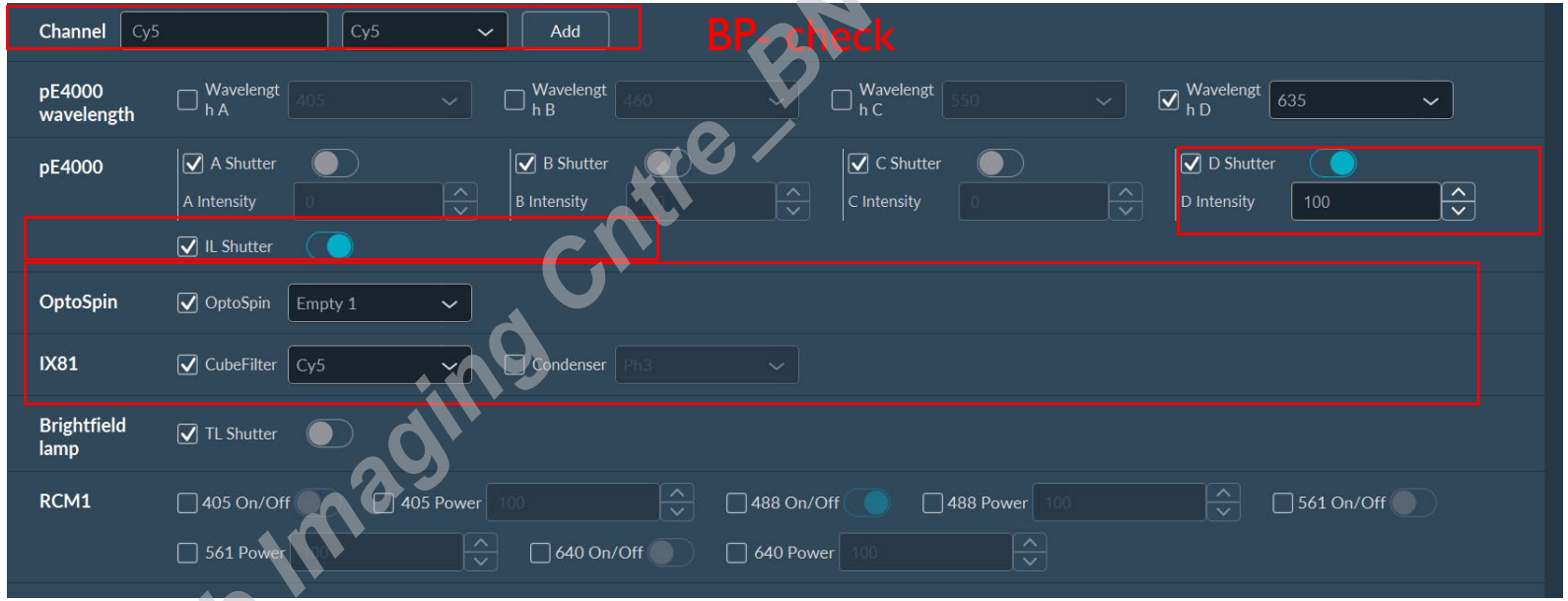
1, Shift knob to RCM

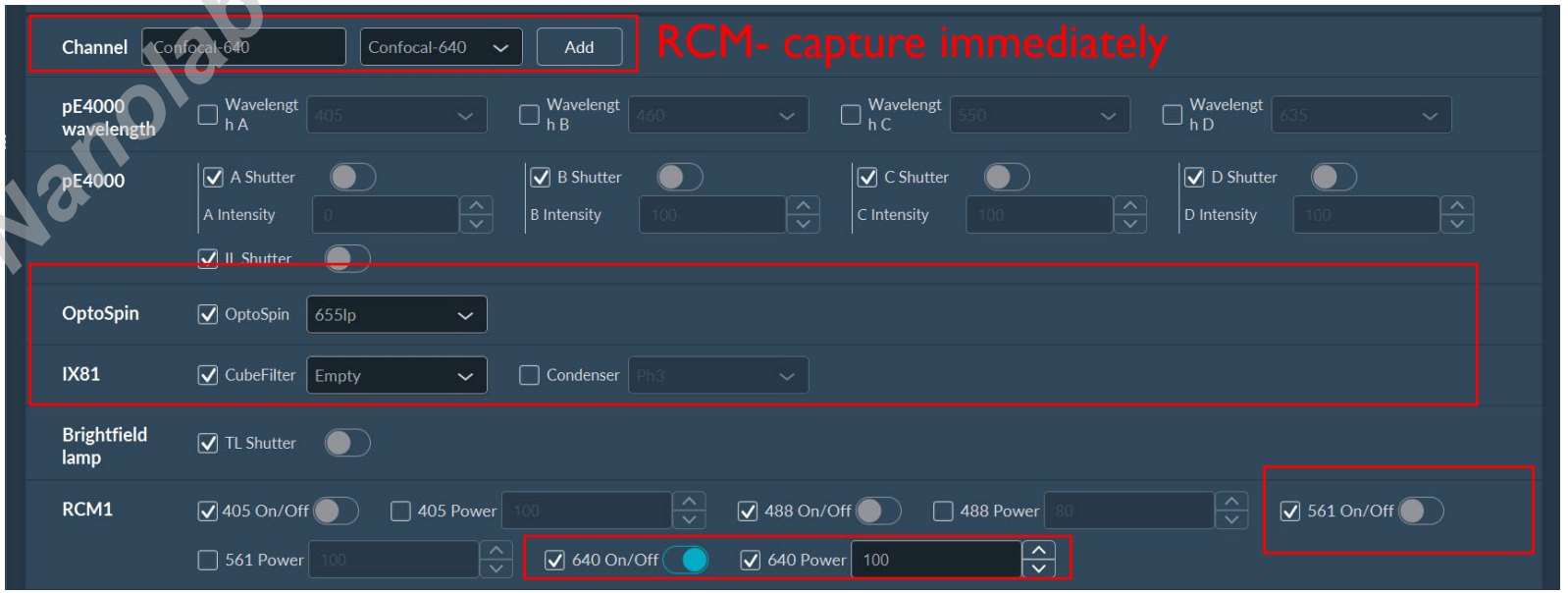


ByPass:
Wide Field

RCM:
Confocal path

*Note that bleaching occurs very fast, optimize first with BP (wide field. LED) on small area aside. Check signal stability in Z stack or over time





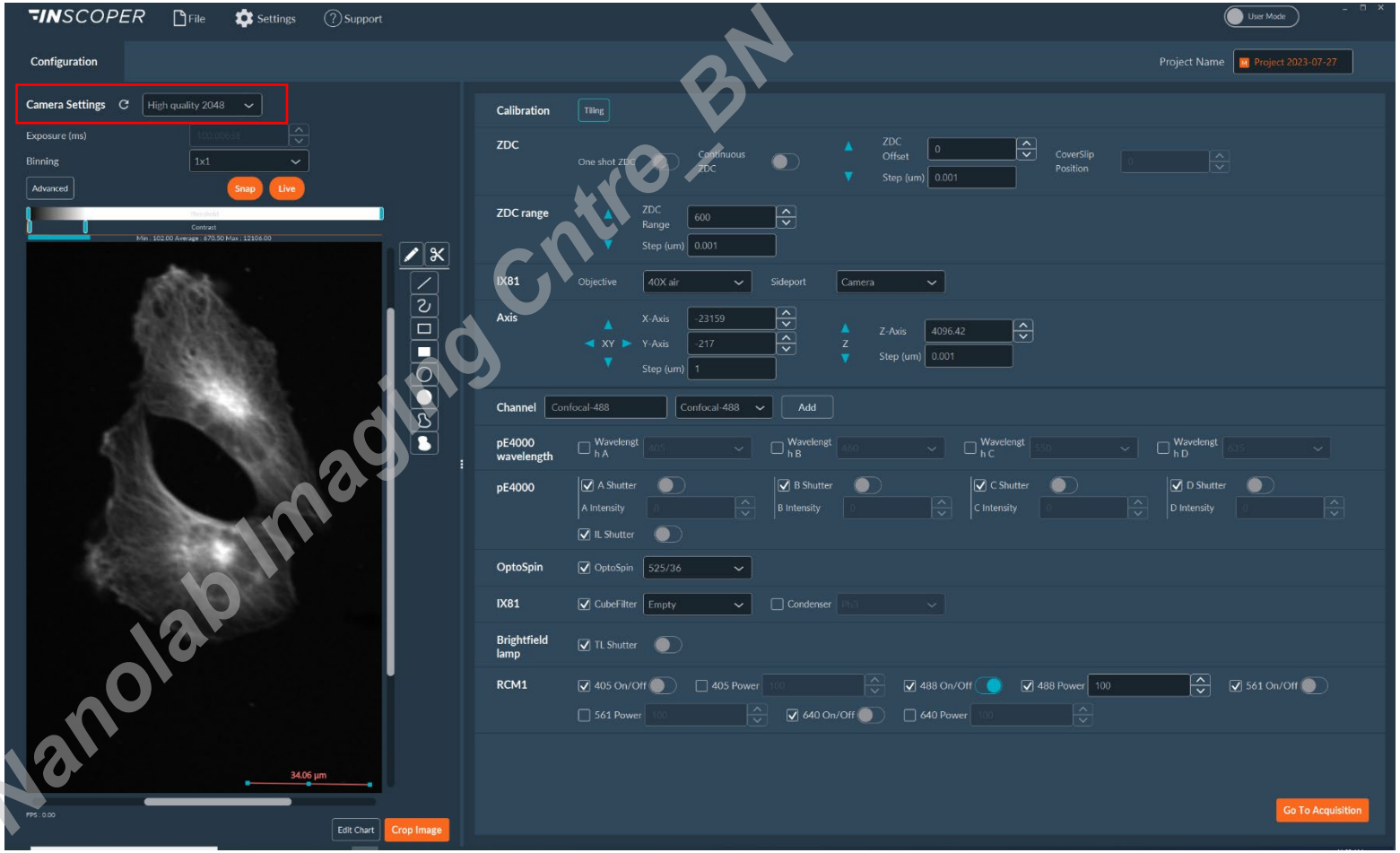
Shifting to high resolution confocal imaging (Pinhole is constant at 15µm)

2 choose your channel and scanning mode:

**2048=full chip*
1024=1/4 chip

**Sweep factor 2= smaller FOV, higher resolution*

**Line skipping is in Y axis*



When you are in RCM mode, exposer time has no meaning. Rather it's the acquisition FieldOfView:

You have several options for FOV: High quality (Sweep factor=2 for highest resolution/400Hz/- line skipping)
High speed (Sweep factor=2 for highest resolution/800Hz/+ line skipping)
Large FOV (Sweep factor=1 for standard confocal/400Hz/- line skipping)

Shutdown-

1, Save your data locally and Copy to Bulk folder!
*All local data is erased monthly
2, First- close computer& **Shut Down power** for PC& INSCOPER Controller
3, Only after- close 2 shutters for hardware
4, Clean up

Need help/advice?

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