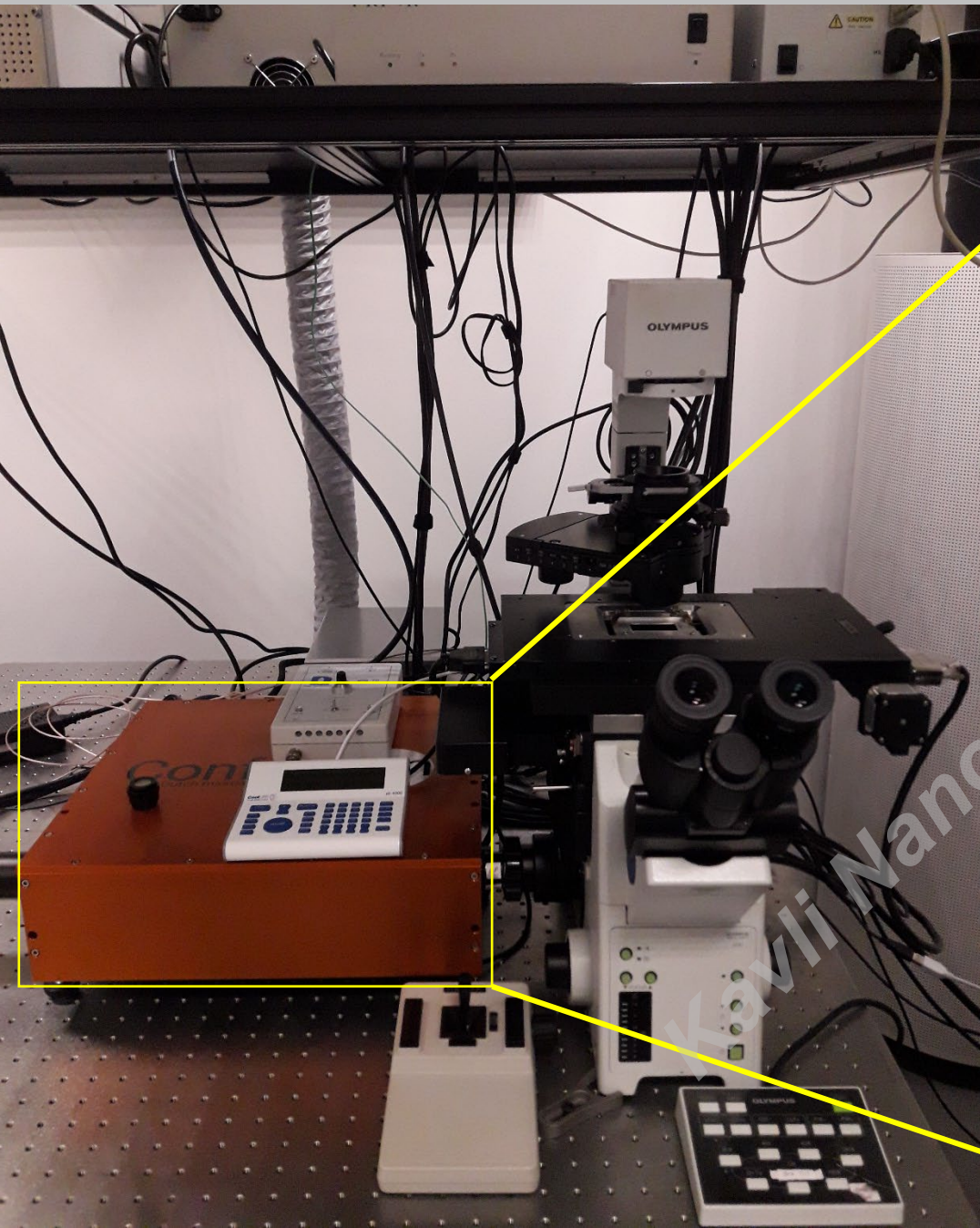
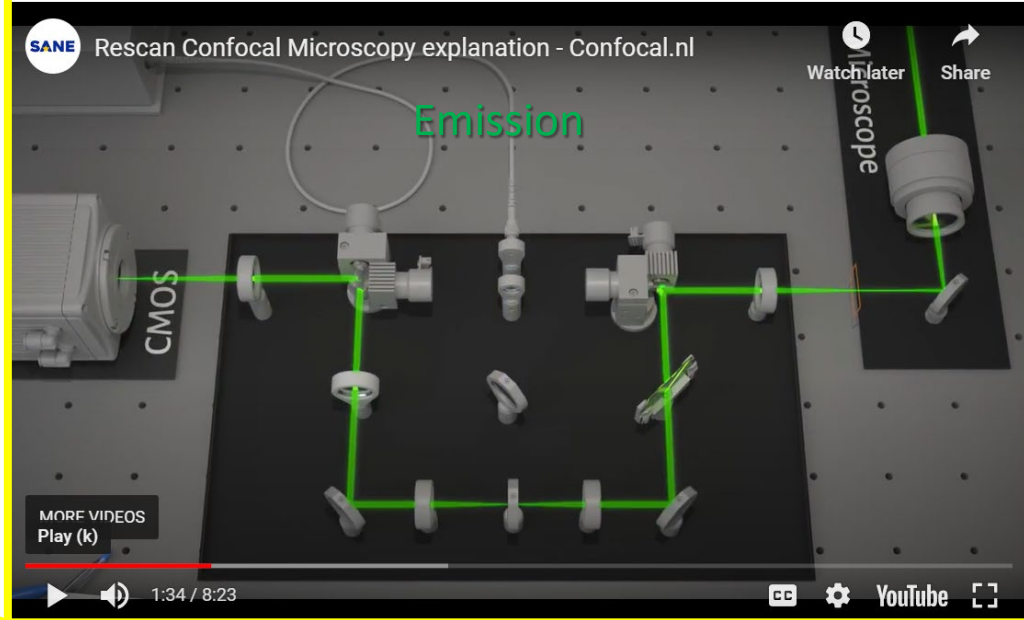
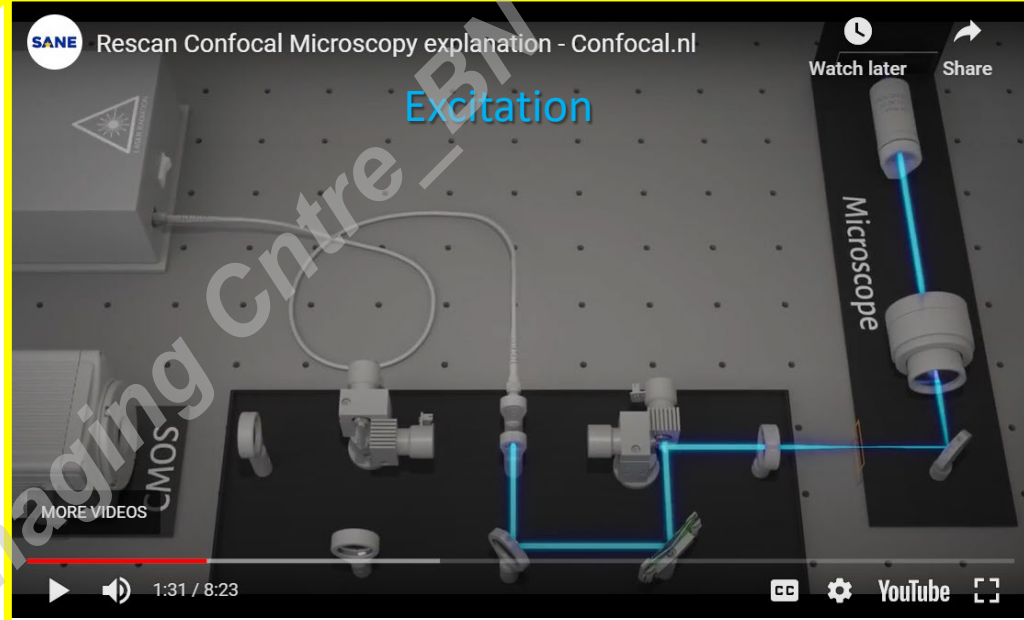


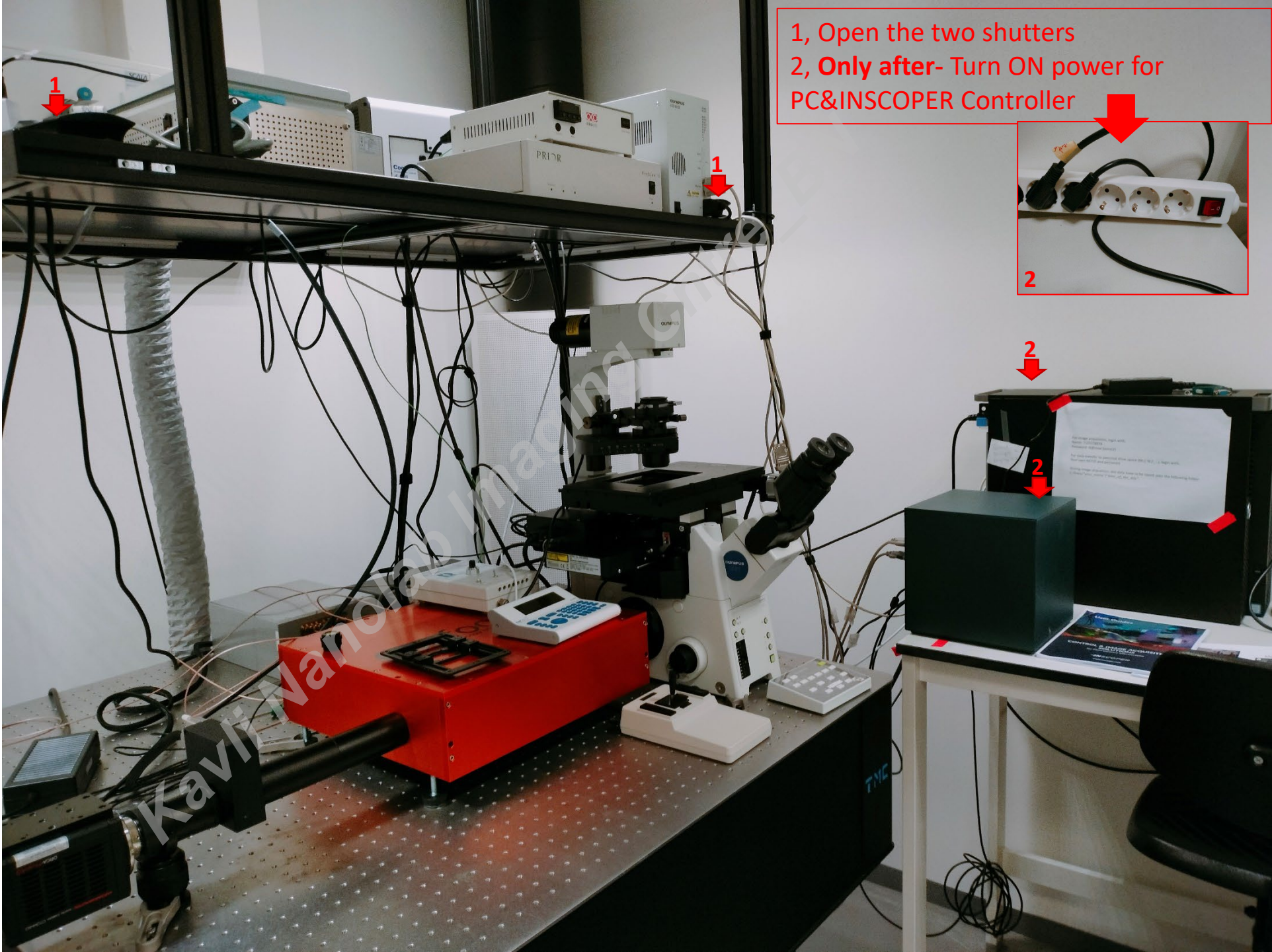
Kavli Nanolab Imaging Center



User manual WF+RCM

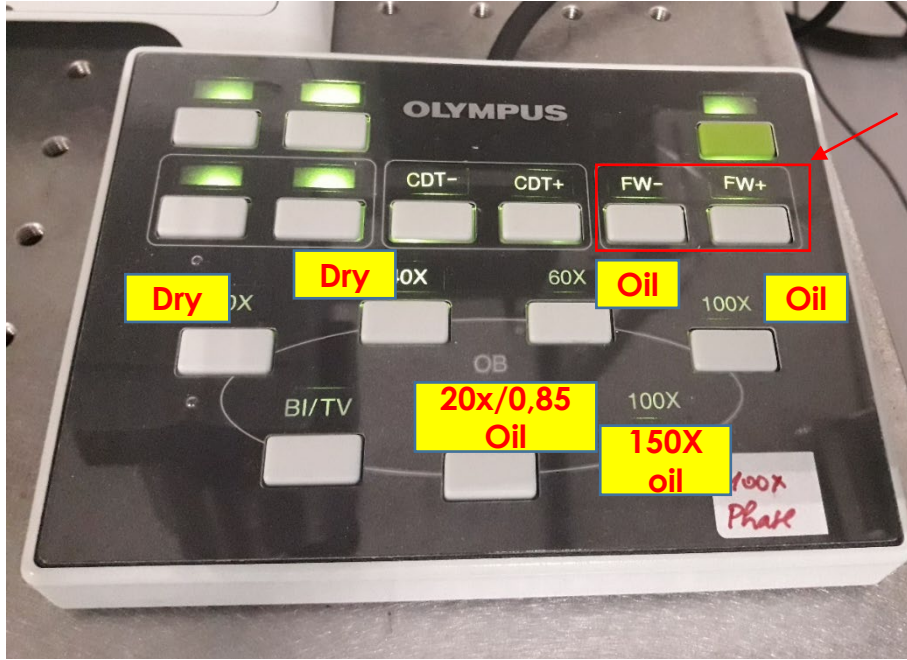


Startup- Hardware



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

Cooled light source:

Choose your excitation



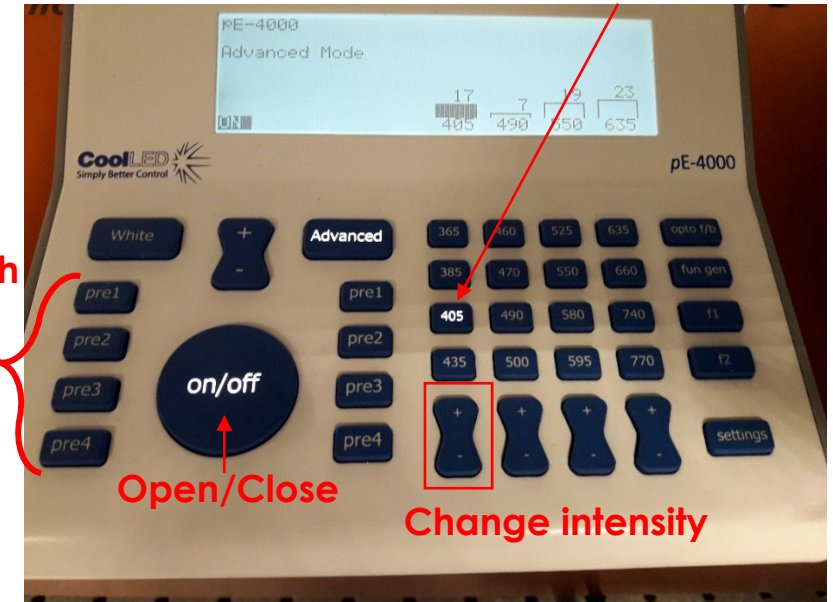
Bright light controller:

TTL: Computer control (when starting software)

0: light OFF

Cont: Continuous illumination (for navigation with eye piece)

You have each channel separate at presets 1-4

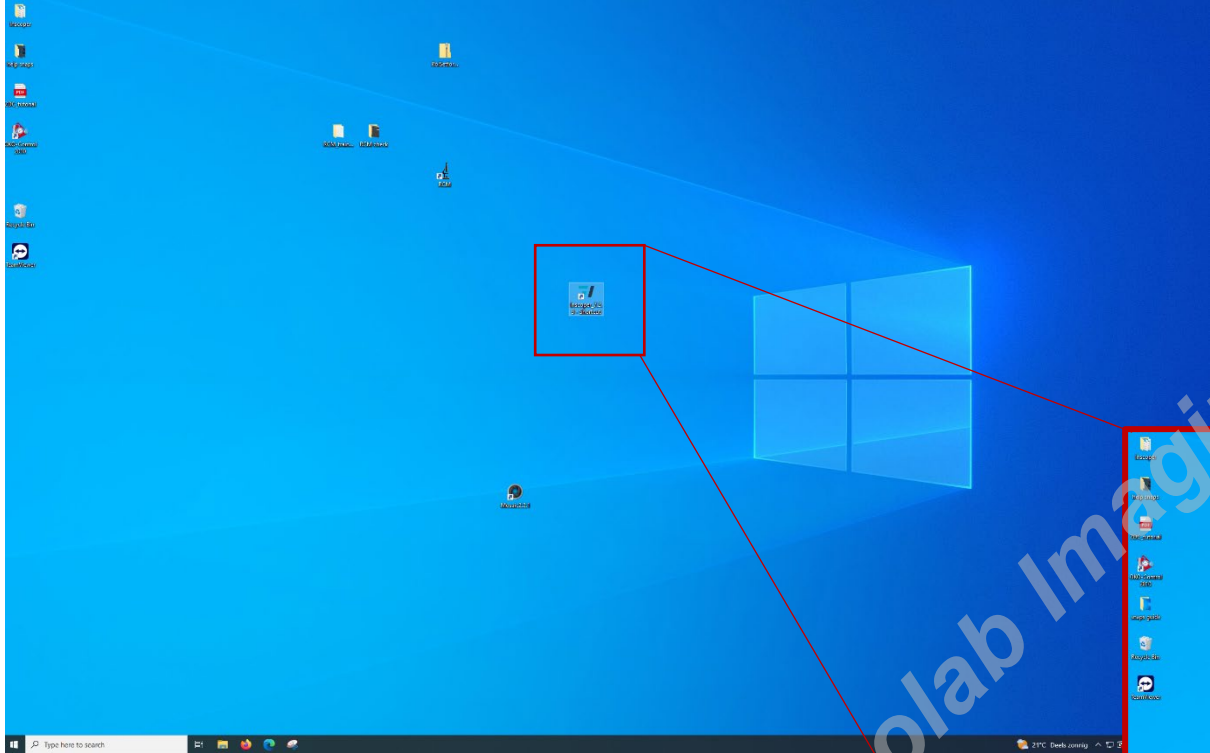


Open/Close

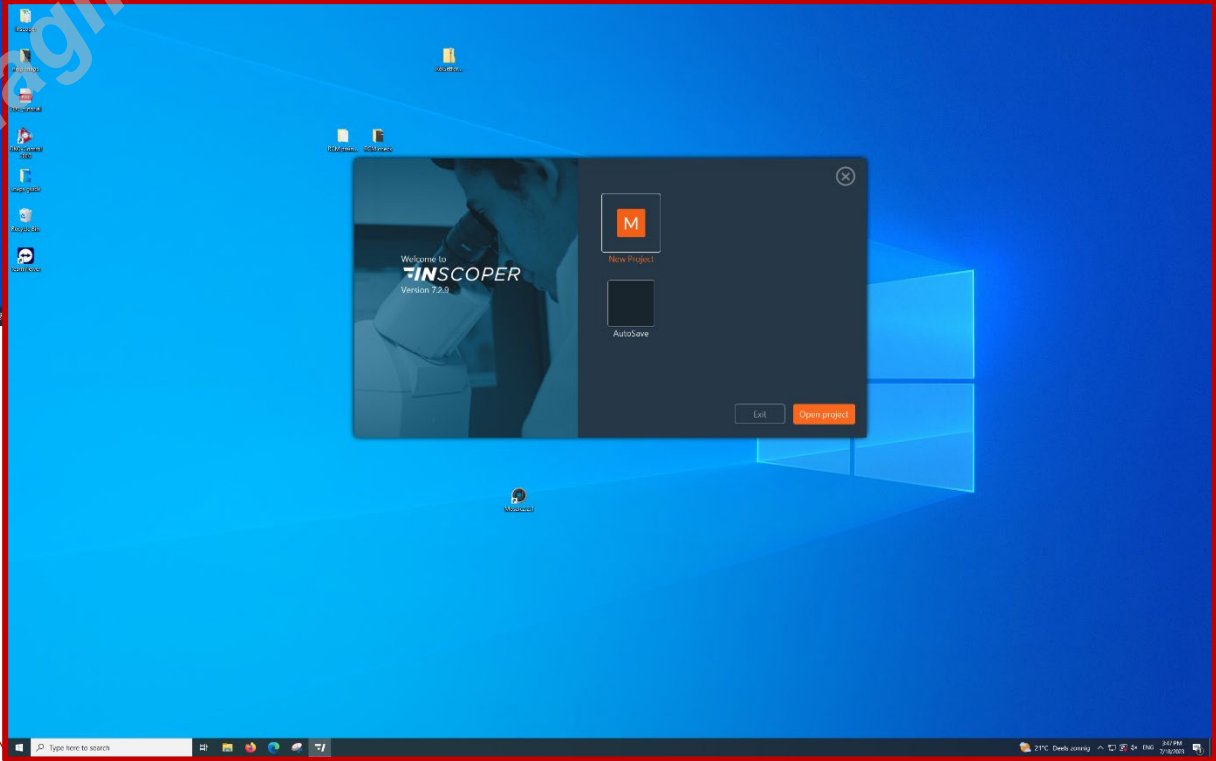
Change intensity

Startup- software

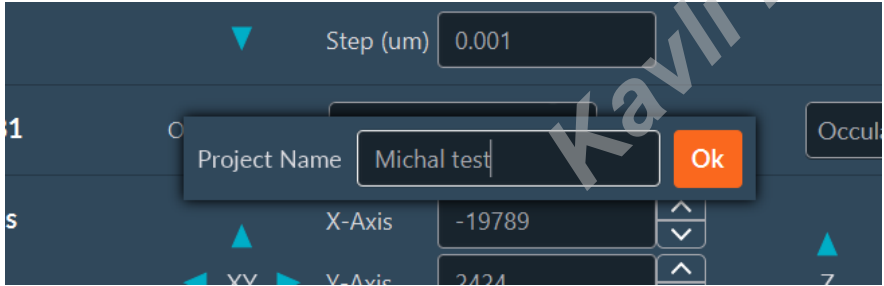
3, once happy- log into PC- double click on INSCOPER icon



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

The screenshot displays the INSCOPER software interface. At the top, the 'Settings' menu item is highlighted with a red box. A 'Display Settings' dialog box is open, showing the 'Snap & Live' section. In this section, the 'Save Snap' checkbox is checked, and the 'Project Name' field is set to 'C:\Data\Bert\last'. A 'Save' button is visible at the bottom right of the dialog box. The background interface shows various configuration panels such as 'Camera Settings', 'Calibration', and 'ZDC', along with a live image of a cell and a 'Go To Acquisition' button at the bottom right.

INSCOPER- Configuration tab: set your channels one by one

The screenshot shows the INSCOPER software configuration window. The interface is dark-themed and includes a top menu bar with 'File', 'Settings', and 'Support' options. The 'Configuration' tab is active, showing a 'Project Name' field with 'Michal test'.

Camera Settings: Includes 'Widefield' mode, 'Exposure (ms)' set to 50.00806, 'Binning' set to 1x1, and 'Snap'/'Stop' buttons. A 'Threshold Contrast' slider is also visible.

Calibration: A yellow box highlights the 'Calibration' and 'Tiling' options. A note states: 'Calibration files- no need to modify (unless tiling is wrong)'. Below this, 'ZDC' settings include 'One shot ZDC' (off), 'Continuous ZDC' (off), 'ZDC Offset' (0), 'CoverSlip Position' (0), and 'ZDC range' (600, Step (um) 0.001). A green box highlights the ZDC range settings with the note: 'ZDC focus maintenance- see next'.

IX81: Objective is '100X oil', Sideport is 'Camera'. The 'Axis' section is highlighted with a red box and labeled 'Stage Control (also via microscope)'. It shows 'X-Axis' (-15637), 'Y-Axis' (3740), 'Z-Axis' (4436.65), and 'Step (um)' (1).

Channel: A red box highlights the 'Channel' section, which is set to 'DAPI' and 'Not saved'. A note says: 'Make sure you define your channel first'. Below this, 'pE4000 wavelength' settings are shown for channels A (385), B (460), C (550), and D (635). 'pE4000' shutter and intensity settings are also visible.






Image window: A large window shows a grayscale image of a cell. A red box highlights the 'Zoom in' button (a magnifying glass icon) and a red arrow pointing to the image. A scale bar at the bottom right indicates '42.41 μm'. A note says: 'Image window: You can mark an ROI and Crop (click twice to confirm). Another click will reset the view'.

Light path parameters: A yellow box highlights the bottom section of the configuration, including 'OptoSpin' (Empty 1), 'IX81' (CubeFilter: DAPI, Condenser: Ph3), 'Brightfield lamp' (TL Shutter: off), and 'RCM1' (405, 488, 561, 640 On/Off and Power settings).

A 'Go To Acquisition' button is located at the bottom right. The bottom left corner shows 'FPS: 19.65' and 'Edit Chart'/'Crop Image' buttons.


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...



<input checked="" type="checkbox"/>	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

Configuration Project Name: Michal test

Camera Settings Widefield

Exposure (ms): 50.00806

Binning: 1x1

Advanced Snap Stop

Calibration Tiling

ZDC focus maintenance

ZDC: 4 One shot ZDC 6 Continuous ZDC

ZDC range: ZDC Range: 600 Step (um): 0.001

ZDC Offset: 0 Step (um): 0.001

CoverSlip Position: 0

Sample- Fine focus check the number

How to set ZDC focus maintenance?

1. Use only #1,5 high precision coverslips. Glass bottom dishes or standard slides should both fit. Make sure the objective fits (see next page) and you have enough immersion oil if needed.
2. Focus on the upper part of your sample. If that won't work, try to focus lower/higher.
3. ZDC default range is set to 600µm. You can change it and check speed/accuracy.
4. Enable **one shot ZDC**: In live mode you see the objective moves up/down within search range. The **coverslip position** should be then set. If its still zero- go back to steps 2/3 to optimize.
5. Now focus finely with **ZDC offset** buttons
6. If you want **continous ZDC**, at this stage you uncheck one shot and check continous. Check **coverslip position**.
In continous mode you cannot adjust offset- do this at the one shot step

lamp

RCM1 405 On/Off 405 Power: 100 488 On/Off 488 Power: 100 561 On/Off 561 Power: 100 640 On/Off 640 Power: 100

FPS: 19.65 Edit Chart Crop Image Go To Acquisition

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
UPlanFI 20X	0.50	1.60
UPlanFI 40X	0.75	0.51
UPlanFI 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
UPlanFI 40X O-SP	1.30	0.12
LCPlanFI 40X	0.60	2.60
SLCPlanFI 40X	0.55	6.4 - 8.3
LUCPlanFI 40X	0.60	3.40
LCPlanFI 60X	0.70	1.70

(Special order)
(Special order)

INSCOPER- Acquisition tab: set your experiment

From Configuration window

The screenshot displays the INSCOPER software interface in the Configuration window. The top menu bar includes File, Settings, and Support. The Project Name is set to 'Michal Test'. The left sidebar shows Camera Settings (Widefield) with Exposure (ms) at 50.00806 and Binning at 1x1. The main area is divided into several configuration sections:

- Calibration**: Tiling is selected. ZDC settings include One shot ZDC (off), Continuous ZDC (off), ZDC Offset (0), and Step (um) (0.001). ZDC range is set to 600 and Step (um) is 0.001.
- IX81**: Objective is 100X oil, Sideport is Camera.
- Axis**: X-Axis is -15637, Y-Axis is 3740, Z-Axis is 4436.65, and Step (um) is 1.
- Channel**: DAPI is selected, Not saved.
- pE4000 wavelength**: Wavelength h A is 385, h B is 460, h C is 550, and h D is 635.
- pE4000**: A Shutter is on (Intensity 20), B Shutter is off (Intensity 0), C Shutter is off (Intensity 0), and D Shutter is off (Intensity 0). IL Shutter is on.
- OptoSpin**: OptoSpin is Empty 1.
- IX81**: CubeFilter is DAPI, Condenser is Ph3.
- Brightfield lamp**: TL Shutter is off.
- RCM1**: 405 On/Off, 488 On/Off, and 561 On/Off are all off. Powers are set to 100.

A red arrow points from the 'From Configuration window' text to the 'Go To Acquisition' button at the bottom right. The bottom left shows FPS: 19.65 and buttons for Edit Chart and Crop Image.

INSCOPER- Acquisition tab: set your experiment type

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

Min: 95.00 Average: 716.49 Max: 8848.00

42.41 μm

Sequence 1 Take Image Config Add Show All

1. Time

	The dimension is not activated and won't be included in the acquisition sequence. Click on to activate it.
	The dimension is activated and will be included in the acquisition sequence. Click to deactivate.
	Dimension parameter set.
	Click to set up the dimension according to your application.
	Drag and drop to change the order of all dimensions to suit your needs.

Data Processing and Charts Raw Edit Parameters

Save Acquisition

in RAM on Disk

Total images 0

Total size 0B

Minimal duration 00:00:00-100:00:00

FPS: 10.02 Edit Chart Crop Image

4:12 PM

INSCOPER- Acquisition: use ZDC offset / use standard Z

The screenshot displays the INSCOPER software interface. The top navigation bar includes 'File', 'Settings', and 'Support' menus, along with a 'User Mode' toggle. The main window is divided into several sections:

- Configuration:** Includes 'Camera Settings' (Widefield, Exposure: 250.00132 ms, Binning: 1x1) and a live image view showing a bright, diffuse spot with a 42.41 μm scale bar.
- Acquisition:** Features a 'Sequence 1' panel with 'Take Image' (checked), 'Config', 'Add', and 'Show All' buttons. A '100' value is displayed next to a blue indicator.
- Dimension Editor:** A central dialog box with the following settings:
 - Dimension Name: Time
 - Z-Stack Dimension: Z-Stack
 - Multi-Positions Dimension: Positions
 - Tiling Dimension: Tiling
 - Channels Dimension: Multi-Channels
 - Axis Editor:
 - X Axis: X-Axis
 - Y Axis: Y-Axis
 - Z Axis: ZDC Offset (highlighted with a red box)
 - Extra Dimensions: Two slots, both set to '(No Dimension)'.

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

The screenshot displays the INSCOPER software interface. On the left, the 'Camera Settings' panel is visible, showing 'Widefield' mode, an exposure of 100.00638 ms, and 1x1 binning. The main acquisition area shows 'Sequence 1' with a 'Take Image' button and a 'Config' button. The 'Positions' section is highlighted with a red box, showing a 'Switch to Tiling' button and parameters for X-Axis (-18087), Y-Axis (1146), Step (um) (1), ZDC Offset (4487.38), and Step (um) (0.001). Below this is a table with 3 rows of positions. At the bottom, the 'Save Acquisition' section shows options for saving to RAM or on Disk, and a 'Crop Image' button.

Camera Settings

- Widefield
- Exposure (ms): 100.00638
- Binning: 1x1
- Advanced: Snap, Stop

Acquisition

Sequence 1

Take Image: [On] Config Add Show All

1. Time 0

2. Positions Switch to Tiling 3

X-Axis: -18087 ZDC Offset: 4487.38
Y-Axis: 1146 Step (um): 0.001
Step (um): 1

Well Plate: Set
Add position Remove all

N°	Tag	X-Axis	Y-Axis	ZDC Offset	Move to	Copy	Get
1		-18105	1437	4487.38	[Move]	Copy	Get [X]
2		-18037	1454	4487.38	[Move]	Copy	Get [X]
3		-18087	1146	4487.38	[Move]	Copy	Get [X]

3. Z-Stack 0

4. Multi-Channels 0

Data Processing and Charts: Tiling Edit Parameters

Save Acquisition

in RAM on Disk








C:\Data\Michal\inscoper\test ... Format: All in One Save as BigTiff

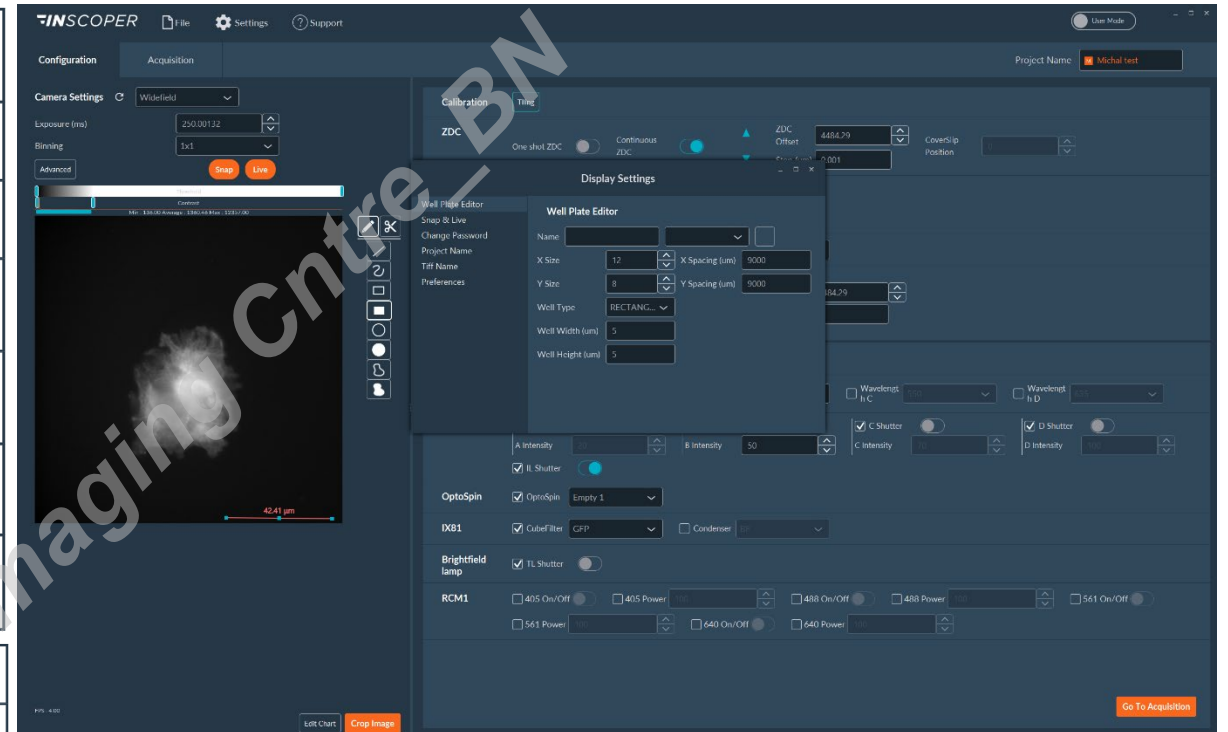
Total images: 3
Total size: 25MB
Minimal duration: 00:00min00:300ms

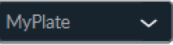




FPS: 10.00

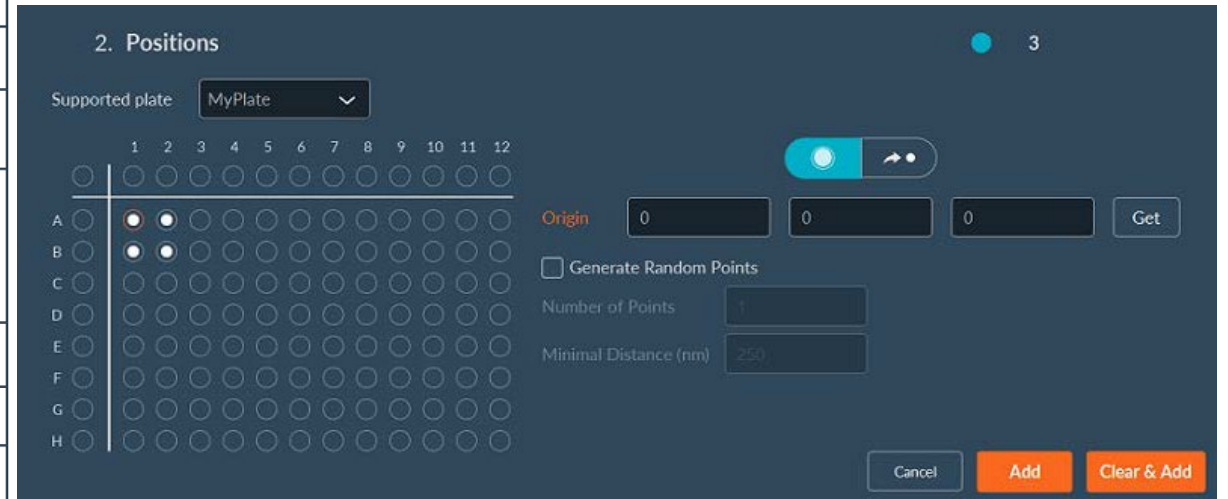
INSCOPER- Acquisition- Stage experiment: Define well plate (Auto position list)

!Only using Air Objectives!

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



	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 the coordinates entered on the well-plate.
<input type="checkbox"/> 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	Click on this button to go back to the manual positions.
	Add	Add the positions you have chosen to the previous ones.
	Clear & Add	Delete all the previous positions and add the selected ones.



INSCOPER- Acquisition- Stage experiment: Tiling

Camera Settings

Widefield

Exposure (ms): 100.00638

Binning: 1x1

Advanced Snap Stop

Threshold Contrast: Min: 96.00 Average: 716.49 Max: 8848.00

42.41 μm

Sequence 1

Take Image Config Add Show All

1. Time 0

2. Tiling Switch to Positions

X-Axis: -16660

Y-Axis: 497

Step (um): 1

ZDC Offset: 4500.38

Step (um): 0.001

Well Plate: Set

Add position Remove all

N°	Tag	Position Count	Edit
1			
2			

Confirm

3. Z-Stack 0

4. Multi-Channels 0

Data Processing and Charts Raw Edit Parameters

Save Acquisition

in RAM on Disk

Total images: 0

Total size: 0B

Minimal duration: 00:00:00

FPS: 10.02

Camera Settings

Widefield

Exposure (ms) 100.00638

Binning 1x1

Advanced

Snap

Stop



Min: 110.00 Average: 500.74 Max: 7124.00



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, Y-Axis: 497, Step (um): 1, ZDC Offset: 4500.38, Step (um): 0.001

Type RECTANGLE Diagonale

Define tile borders

Point 1: X-Axis -19789, Y-Axis 2424, ZDC Offset 0. Get Goto buttons

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 0
4. Multi-Channels 0

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

Tiling of ares with large Z differences- Focus Map

1. Time

0

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

527

XY

X-Axis: -18185
Y-Axis: 1439
Step (um): 1

ZDC Offset: 4441.88
Step (um): 0.001

Type: RECTANGLE Center

Center: X-Axis: -18185 Y-Axis: 1439 ZDC Offset: 4441.88

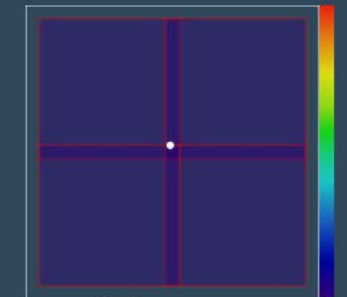
Width: 100 Height: 100 Overlap (%): 10

Read Mode Snake Mode

1, click "get" to add position

2, click "Add" for focus map

Focus Map Add Clear



Show Images LUT

Confirm Cancel

Kavli Nanolab Imaging Centre - BN

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

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

XY

X-Axis: -22844

Y-Axis: -1651

Step (um): 10

ZDC Offset: 2

Step (um): 1

Type: RECTANGLE

Diagonale

Point 1: X-Axis: -22789, Y-Axis: -1936

Point 2: X-Axis: -22844, Y-Axis: -1651

Overlap (%): 10

Read Mode

Snake Mode

ZDC Offset

ZDC Offset: 2

Get Goto

ZDC Offset: 2

Get Goto

1, click "get" to add offset

2, click "Add" for focus map

Focus Map Add Clear



Show Images LUT

Confirm Cancel

3. Z-Stack

4. Multi-Channels

Kavli Nanolab Imaging Centre_BN

INSCOPER-Visualization tab- Use your tile as a map

INSCOPER File Settings Support User Mode

Configuration Acquisition Visualization Project 2023-07-20 Project Name Project 2023-07-20 Open In Explorer

Filters Selected Image set: STITCH_TILING Image Processing

Charts XY Sequence Total: 1 1/1 0-1 Export: Video Stack

234.73 μm

Metadata Tag

Include Filter: Filter

Property	Value
OrcaFlash4-INTERNAL FRAM	9.999362446665053
RCM1-Field Of View X	2048
RCM1-Field Of View Y	2048
OrcaFlash4-SUBARRAY HPO:0	
OrcaFlash4-SUBARRAY HPO:0	
OrcaFlash4-OUTPUT TRIGGE READOUT END	
OrcaFlash4-SUBARRAY HPO:0	
OrcaFlash4-SUBARRAY HPO:0	
OrcaFlash4-SUBARRAY HPO:0	
OrcaFlash4-TIMING INVALID 0.1	
OrcaFlash4-IMAGE DETECTC 6.5	
ImageType	SEQUENCE
Microscope-ZDC_Offset	0
RCM1-Laser 3 Selected	1
ChannelIndex	0
RCM1-Scanner Phase Y	-1
OrcaFlash4-OUTPUT TRIGGE EDGE	
RCM1-Scanner Phase X	-1
OrcaFlash4-OUTPUT TRIGGE ALL VIEWS	
RCM1-RCM Type	RCM 1.1
Cooled_pE4000-D Shutter	false
Exposure.ms	100.00638000000001
OrcaFlash4-OUTPUT TRIGGE READOUT END	
OrcaFlash4-BUFFER FRAMFF	8388608
OrcaFlash4-EXTRACTION MCOFF	
RCM1-Laser 2 Polarity	1
Time_0-TimeRelative_0	0
Prior_ProscanII_XYStage-X-Av	-2.3353E7
Microscope-CoverSlip_Positio	4745130
Time_0-Time_0	0
OrcaFlash4-OUTPUT TRIGGE EDGE	
Microscope-Objective	100X oil
OrcaFlash4-TRIGGER POLARI POSITIVE	
OrcaFlash4-SENSOR COOLET READY	
PixelSizeUm	0.06018518518518518
RCM1-Offset Scan Y	-0.13
RCM1-Offset Scan X	0.42
RCM1-Laser 4 Analog Out	4
OrcaFlash4-INTERNAL LINE I	9.74436090225564E-6
RCM1-Laser 3 Power	80
OrcaFlash4-IMAGE DETECTC	2048
Cooled_pE4000-A Shutter	false
OrcaFlash4-IMAGE ROWBYT	4096
OrcaFlash4-IMAGE TOP OFF:0	

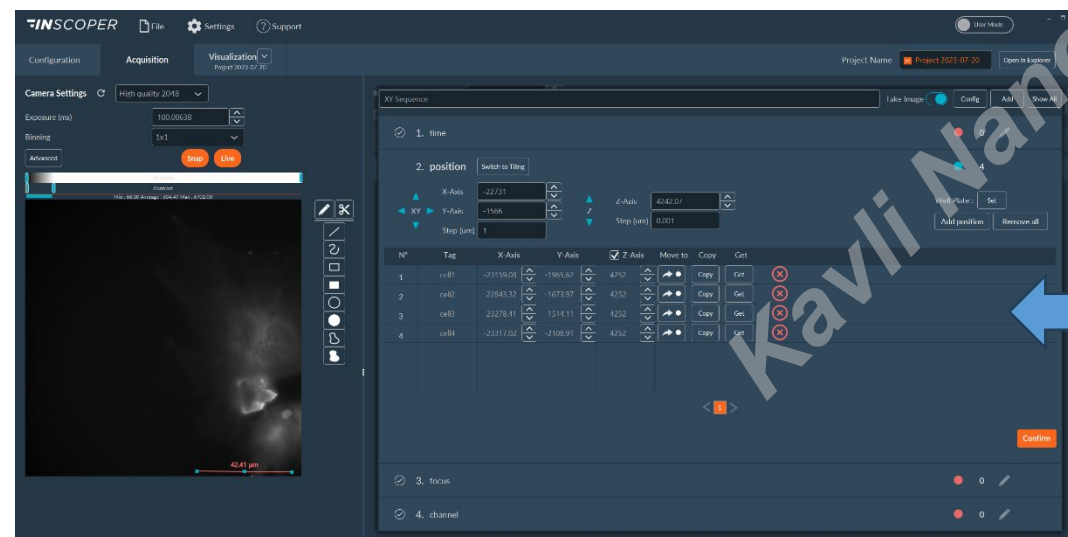
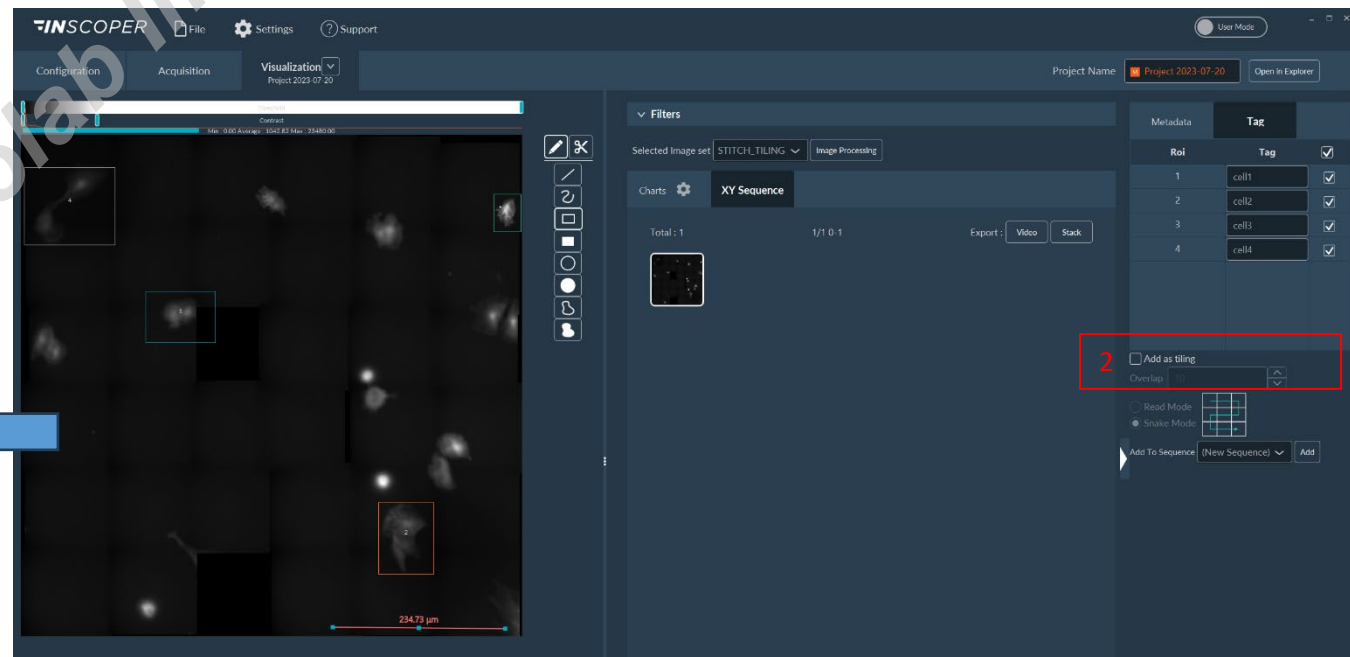
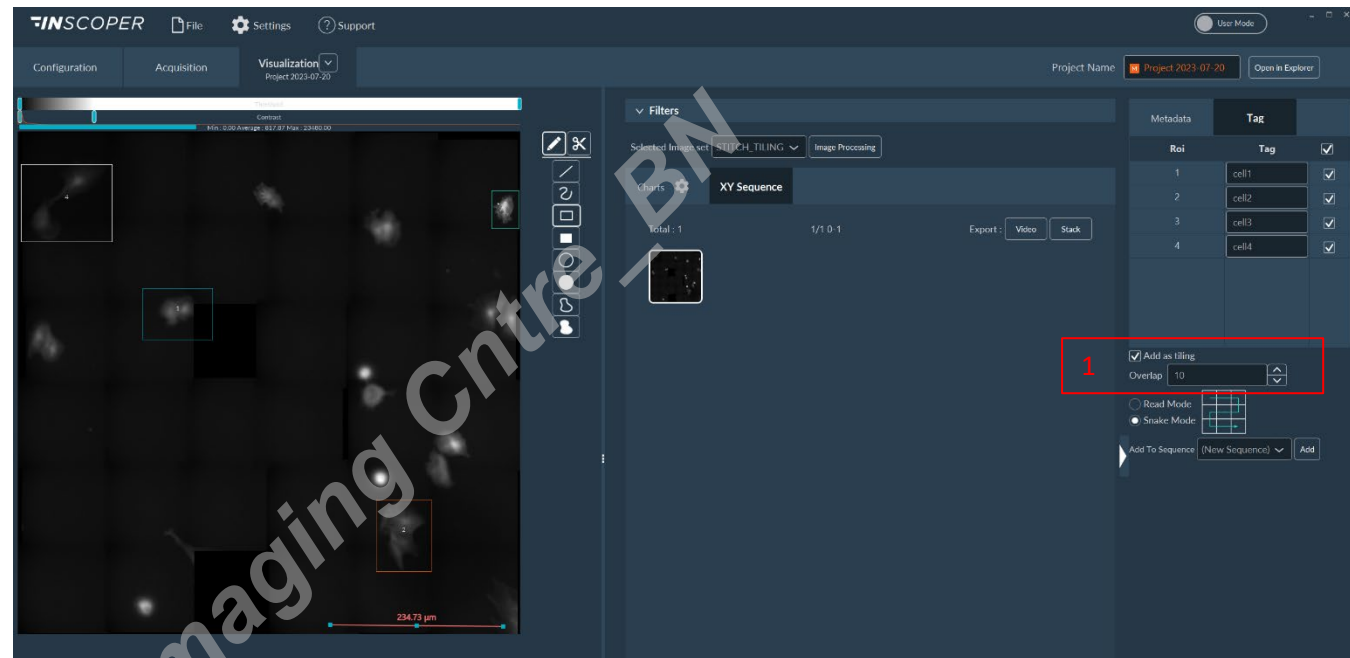
Export

Your stitched image contains stage position information. Mark Roi's and tag them to save

Kavli Nanolab Imaging Centre - BN

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 File Settings Support User Mode

Configuration Acquisition Visualization Project 2023-07-20 Project Name Project 2023-07-20 Open In Explorer

Camera Settings High quality 2048

Exposure (ms) 100.00638

Binning 1x1

Advanced Snap Live

Threshold Contrast Min: 72.00 Average: 612.93 Max: 6614.00

42.41 μm

XY Sequence Take Image Config Add Show All

1. Time 15

- 1 Number of Time Points 15
- 2 Interval 0 h 0 min 5 s 0 ms
- 3 Total Time 0 h 1 min 15 s 0 ms
- 4 Burst Mode
- 5 Confirm

Start Acquisition

1. Determine the required number of time points for the whole acquisition.

2. Set an Interval between each time point.

3. The Total Time value is set automatically after the number of time points and the interval between them have been set.

4. Activate the Burst Mode checkbox if you want to take pictures as quickly as possible.

5. When you have finished editing the dimension, click Confirm.

INSCOPER- Acquisition- Time experiment- Burst mode

INSCOPER File Settings Support User Mode

Configuration Acquisition Visualization Project 2023-07-20 Project Name Project 2023-07-20 Open In Explorer

Camera Settings High quality 2048

Exposure (ms) 100.00638

Binning 1x1

Advanced Snap Live

Threshold Contrast
Min: 72.00 Average: 612.93 Max: 6614.00

XY Sequence Take Image Config Add Show All

1. time 50

Number of Time Points 50

Interval 0 h 0 min 0 s ms

Total Time 0 h 0 min 0 s ms Burst Mode Confirm

Burst mode will image as fast as your exposer time, so no interval needed. Only number of time points

3. focus 0 ✎

4. channel 0 ✎

< 1 2 >

Data Processing and Charts Tiling Edit Parameters

Save Acquisition

in RAM on Disk

Format All in One Save as BigTiff

Do not forget to save in RAM and export from Visualization tab

Total images 50
Total size 419MB
Minimal duration 00h00min05s000ms
Start Acquisition

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)

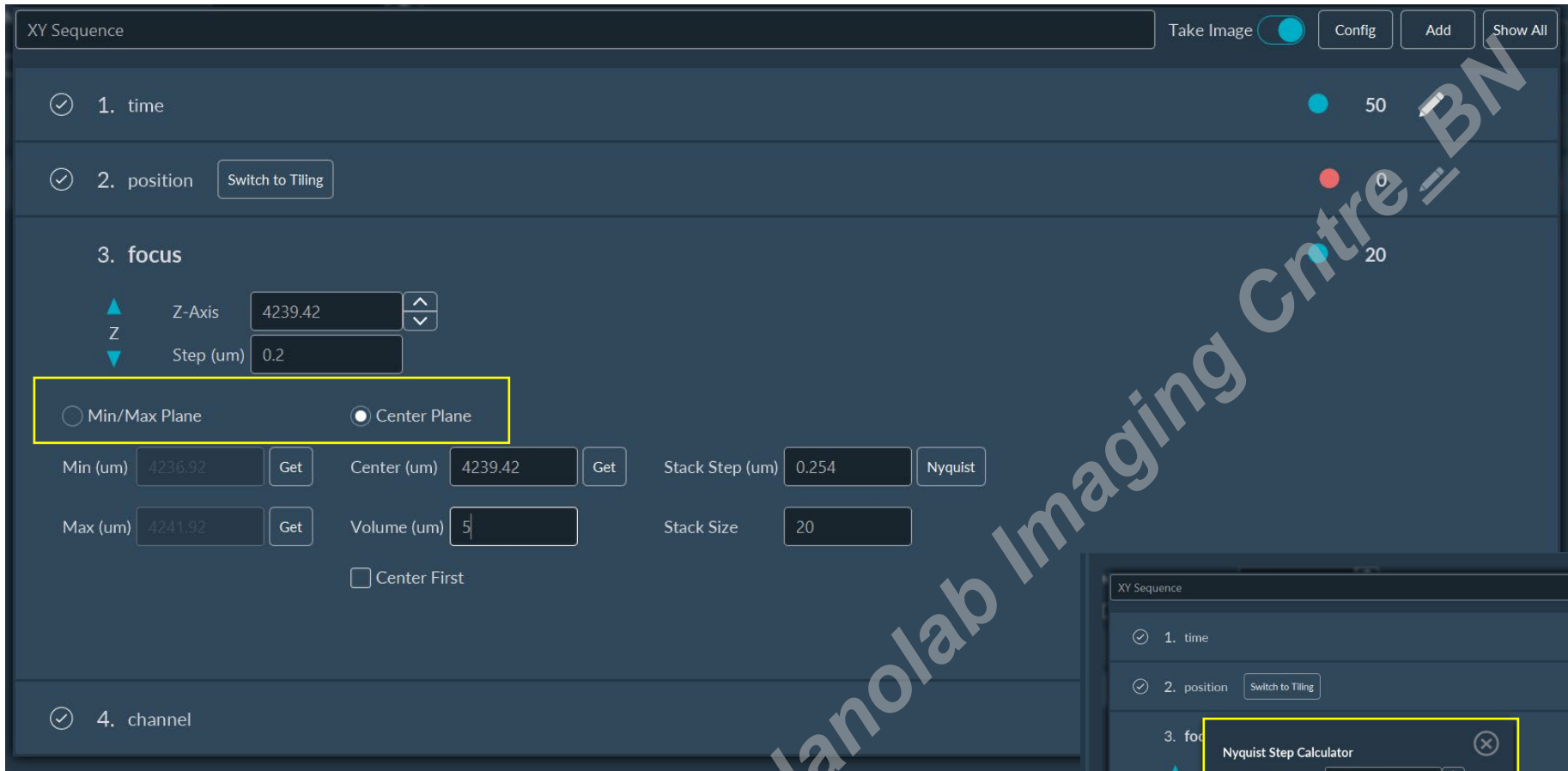
The screenshot shows the INSCOPER software interface. The 'Filters' section has 'Time' set to 1-50, 'All' selected, and 'Image Processing' active. The 'Charts' section shows 'XY Sequence' with 'Total : 50' and '1/2 0-49'. The 'Export' buttons are 'Video' and 'Stack'. The main display shows a grid of 10 image thumbnails, with the first one highlighted. The 'Metadata' panel on the right shows a table with the following data:

Property	Value
Time_0-TimeRelative_0	0
Time_0-Time_0	0
OrcaFlash4-MASTER PULSE E	1.0
Time_0-TimeReset_0	true
OrcaFlash4-TIMING READOL	0.02
ElapsedTime-ms	847
timeIndex	0
OrcaFlash4-TIME STAMP PR	IMAGING DEVICE
OrcaFlash4-TRIGGER TIMES	1.0

The screenshot shows the INSCOPER software interface. The 'Filters' section has 'Time' set to 1-50, 'All' selected, and 'Image Processing' active. The 'Charts' section shows 'XY Sequence' with 'Total : 50' and '1/2 0-49'. The 'Export' buttons are 'Video' and 'Stack'. The main display shows a grid of 10 image thumbnails, with the second one highlighted. The 'Metadata' panel on the right shows a table with the following data:

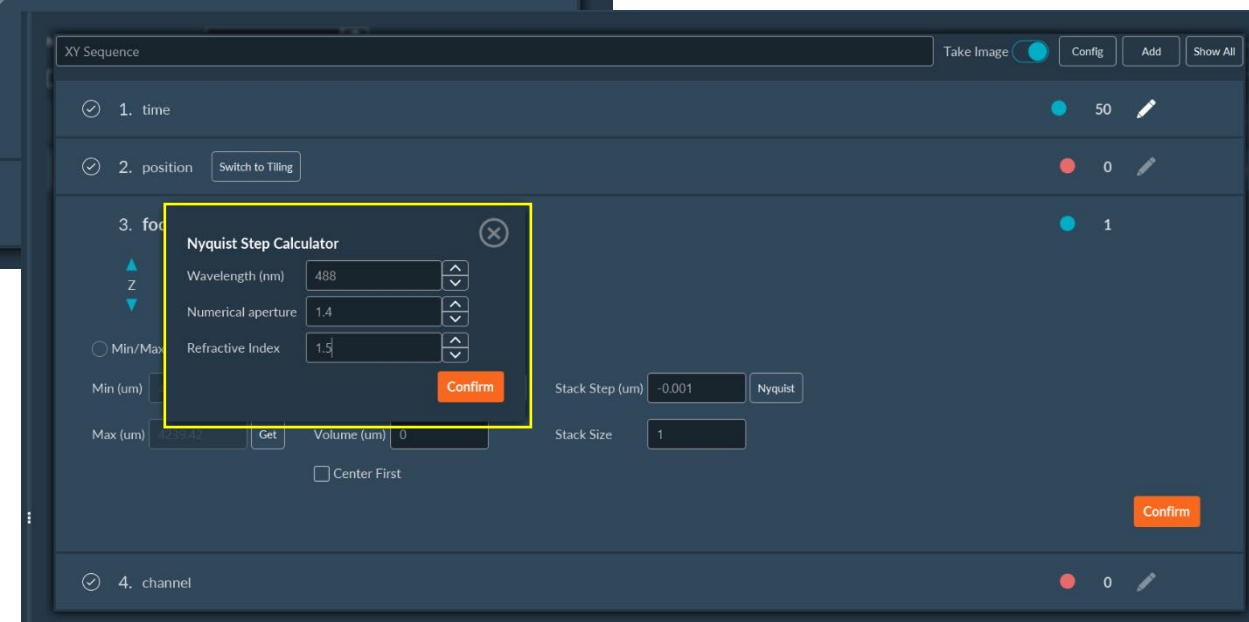
Property	Value
Time_0-TimeRelative_0	0
Time_0-Time_0	0
OrcaFlash4-MASTER PULSE E	1.0
Time_0-TimeReset_0	true
OrcaFlash4-TIMING READOL	0.02
ElapsedTime-ms	949
timeIndex	1
OrcaFlash4-TIME STAMP PR	IMAGING DEVICE
OrcaFlash4-TRIGGER TIMES	1.0

INSCOPER- Acquisition- Z stack



You can define Z stack absolute or around the center

You can calculate recommended Z step from Objective info



INSCOPER- Acquisition- Z stack around ZDC offset defined center

XY Sequence

Take Image Config Add Show All

1. time 0

2. position 4

3. focus 20

▲ ZDC Offset 3 ▲▼

▼ Step (um) 0.001

Min/Max Plane Center Plane

Min (um) 0.5 Center (um) 3 Stack Step (um) 0.254

Max (um) 5.5 Volume (um) 5 Stack Size 20

Center First

4. channel 0

< 1 2 >

INSCOPER- Acquisition- Channels

INSCOPER File Settings Support User Mode

Configuration Acquisition Project Name Project 2023-07-27

Camera Settings Widefield

Exposure (ms) 100.00638

Binning 1x1

Advanced Snap Live

Threshold

Contrast

Min: 27.00 Average: 194.96 Max: 17315.00

106.02 μm

Sequence 1 Take Image Config Add Show All

- 1. time 0
- 2. position Switch to Tiling 0
- 3. focus 0
- 4. channel 0

Add Channel Remove All

N°	Name	Z Stack	Z Offset	Camera Pres...	Shutter Blink	Camera	Exposure (ms)
----	------	---------	----------	----------------	---------------	--------	---------------

1. Add channel
2. The setting (exp/int) from Configuration should be imported. Check and redefine if needed
3. You can also set Z offset per channel or include in a Z stack

Confirm

Data Processing and Charts Edit Parameters

FPS: 10.01 Edit Chart Crop Image

INSCOPER- Acquisition- Channels with ZDC

The screenshot displays the INSCOPER software interface. On the left, the 'Acquisition' tab is active, showing 'Camera Settings' for 'Widefield' with an exposure of 100.00638 ms and 1x1 binning. Below this is a live image of a cell with a scale bar of 106.02 μm. The main panel shows 'Sequence 1' with two channels: '1. channel' (Cy5) and '2. channel' (mCherry). Both channels have 'Z Stack' checked and 'Z Offset' set to 0. The 'One shot ZDC' option is selected for both channels. A yellow box highlights the 'One shot ZDC' and 'Continuous ...' options with the text: 'If you are using focus maintenance, check it per channel'. At the bottom, the 'Save Acquisition' section shows 'in RAM' selected, and the 'Start Acquisition' button is visible.

Note!
You can only apply EITHER one shot or continuous ZDC.
One shot is compatible with Z stack and position, continuous not

N°	Name	Z Stack	Z Offset	Camera Pres...	Shutter Blink	Camera	Exposure (ms)
1	Cy5	<input checked="" type="checkbox"/>	0	<input type="checkbox"/>	<input type="checkbox"/>	OrcaFlash...	200.006
2	mCherry	<input checked="" type="checkbox"/>	0	<input type="checkbox"/>	<input type="checkbox"/>	OrcaFlash...	100.006

Total images: 3
Total size: 25MB
Minimal duration: 00:00min00:400ms

INSCOPER- Visualization- Channels

The screenshot displays the INSCOPER software interface. The top navigation bar includes 'File', 'Settings', and 'Support' menus, along with a 'User Mode' toggle. The main interface is divided into several sections:

- Configuration:** Shows 'Visualization' for 'Project 2023-07-27'. A 'Threshold' slider is set to 0, and a 'Contrast' slider is set to 296.80. The contrast range is 'Min: 77.00 Average: 296.80 Max: 36267.00'.
- Filters:** A 'Channel' slider is set to 1. The 'Selected Image set' is 'RAW_DATA' with 'Image Processing' selected.
- Charts:** Shows 'Sequence 1' with 'Total: 3' and '1/1 0-3'. 'Export' buttons for 'Video' and 'Stack' are present.
- Visualization:** A large central window shows a red fluorescence image of a cell. A scale bar at the bottom right indicates '106.02 μm'.
- Metadata:** A panel on the right displays a list of properties and their values. The 'Export' button is highlighted in orange.

Metadata Table:

Property	Value
axis	["ExtraAxis":{},"Focus":{},"Dev
Binning	1x1
BitDepth	16
Camera	OrcaFlash4_
CameraDriverGroup-Binning	1
CameraDriverGroup-Camera	200.006
CameraDriverGroup-Mode	Widefield
Channel	Cy5
ChannelIndex	0
channelIndex	0
Cooled_pE4000-A Intensity	0.0
Cooled_pE4000-A Shutter	false
Cooled_pE4000-B Intensity	100.0
Cooled_pE4000-B Shutter	false
Cooled_pE4000-C Intensity	100.0
Cooled_pE4000-C Shutter	false
Cooled_pE4000-D Intensity	100.0
Cooled_pE4000-D Shutter	true
Cooled_pE4000-Wavelength	405
Cooled_pE4000-Wavelength	460
Cooled_pE4000-Wavelength	550
Cooled_pE4000-Wavelength	635
Core-Camera	OrcaFlash4_
ElapsedTime-ms	0
Exposure-ms	200.006
Frame	0
FrameIndex	0
Height	2048
ImageNumber	0
ImageSubNumber	0
ImageType	SEQUENCE
Lamp_Is170-TLShutter	false
Microscope-Condenser	Ph3
Microscope-CoverSlip_Positio	0.0
Microscope-CubeFilter	Cy5
Microscope-Focus	4096420.0
Microscope-ILShutter	true
Microscope-Objective	40X air
Microscope-Slideport	Camera
Microscope-ZDC_continuous	false
Microscope-ZDC_Offset	0.0
Microscope-ZDC_oneshot	false
Microscope-ZDC_range	600000.0
MosaicCalibration	["StageCalibMirror":true,"Stu

You can view the raw data with metadata per channel

INSCOPER-Visualization- Channels

The screenshot displays the INSCOPER software interface. At the top, there are menu items for File, Settings, and Support, along with a User Mode toggle. Below this, navigation tabs for Configuration, Acquisition, and Visualization are visible. The main window is divided into several sections:

- Top Left:** A contrast slider and a large image viewer showing a cell with green and red fluorescence. A scale bar at the bottom right of the image indicates 106.02 μm.
- Top Right:** Project Name: Project 2023-07-27, with an Open in Explorer button.
- Right Panel:** A Filters section with a dropdown for Selected Image set (CHANNEL_M...) and a button for Image Processing. Below this is a Charts section for Sequence 1, showing a thumbnail of the image and export options for Video and Stack.
- Bottom Left:** Channel selection controls for Cy5, GFP, and mCherry, each with a color swatch, a checkmark, and an Auto button.

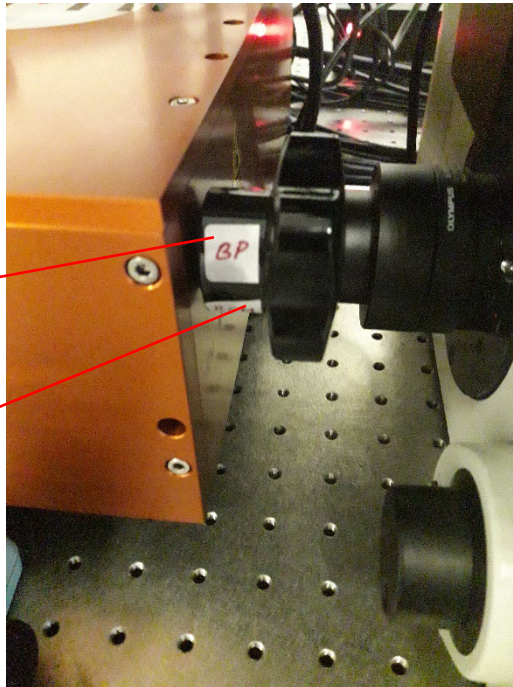
A yellow box highlights the 'Selected Image set' dropdown and the 'Image Processing' button in the Filters section. Another yellow box highlights the channel selection controls at the bottom left. A yellow text overlay in the center-right of the image reads: "You can also get channel overlay with Image processing".

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

I, Shift knob to RCM

ByPass:
Wide Field

RCM:
Confocal path



Channel Cy5 Cy5 Add **BP check**

pE4000 wavelength Wavelength h A 405 Wavelength h B 460 Wavelength h C 550 Wavelength h D 635

pE4000 A Shutter A Intensity 0 B Shutter B Intensity 0 C Shutter C Intensity 0 D Shutter D Intensity 100

IL Shutter

OptoSpin OptoSpin Empty 1

IX81 CubeFilter Cy5 Condenser Ph3

Brightfield lamp TL Shutter

RCM1 405 On/Off 405 Power 100 488 On/Off 488 Power 100 561 On/Off 561 Power 100 640 On/Off 640 Power 100

*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

Channel Confocal-640 Confocal-640 Add **RCM- capture immediately**

pE4000 wavelength Wavelength h A 405 Wavelength h B 460 Wavelength h C 550 Wavelength h D 635

pE4000 A Shutter A Intensity 0 B Shutter B Intensity 100 C Shutter C Intensity 100 D Shutter D Intensity 100

IL Shutter

OptoSpin OptoSpin 655lp

IX81 CubeFilter Empty Condenser Ph3

Brightfield lamp TL Shutter

RCM1 405 On/Off 405 Power 100 488 On/Off 488 Power 80 561 On/Off 561 Power 100 640 On/Off 640 Power 100

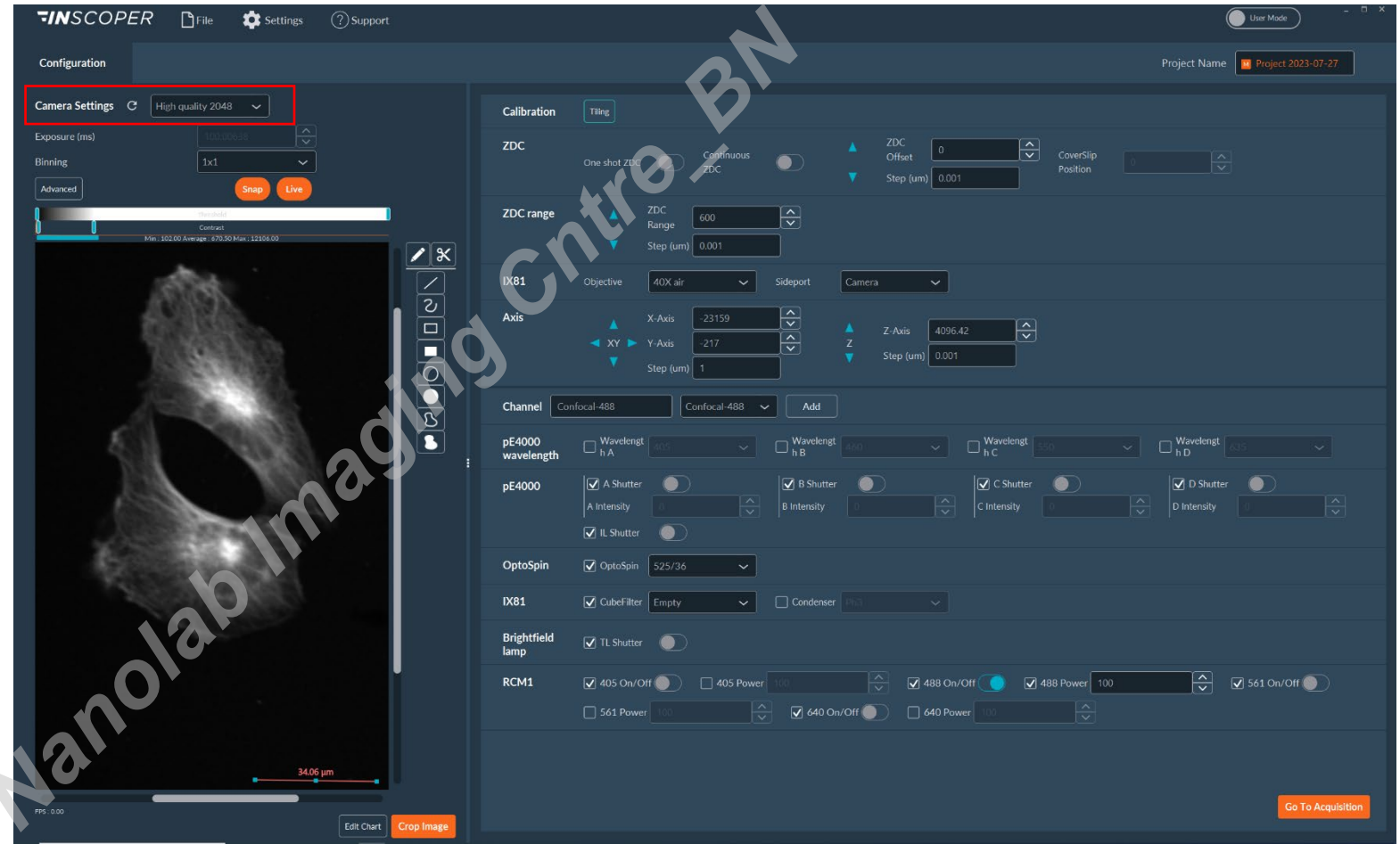
Shifting to high resolution confocal imaging (Pinhole is constant at 15 μ m)

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, exposure 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