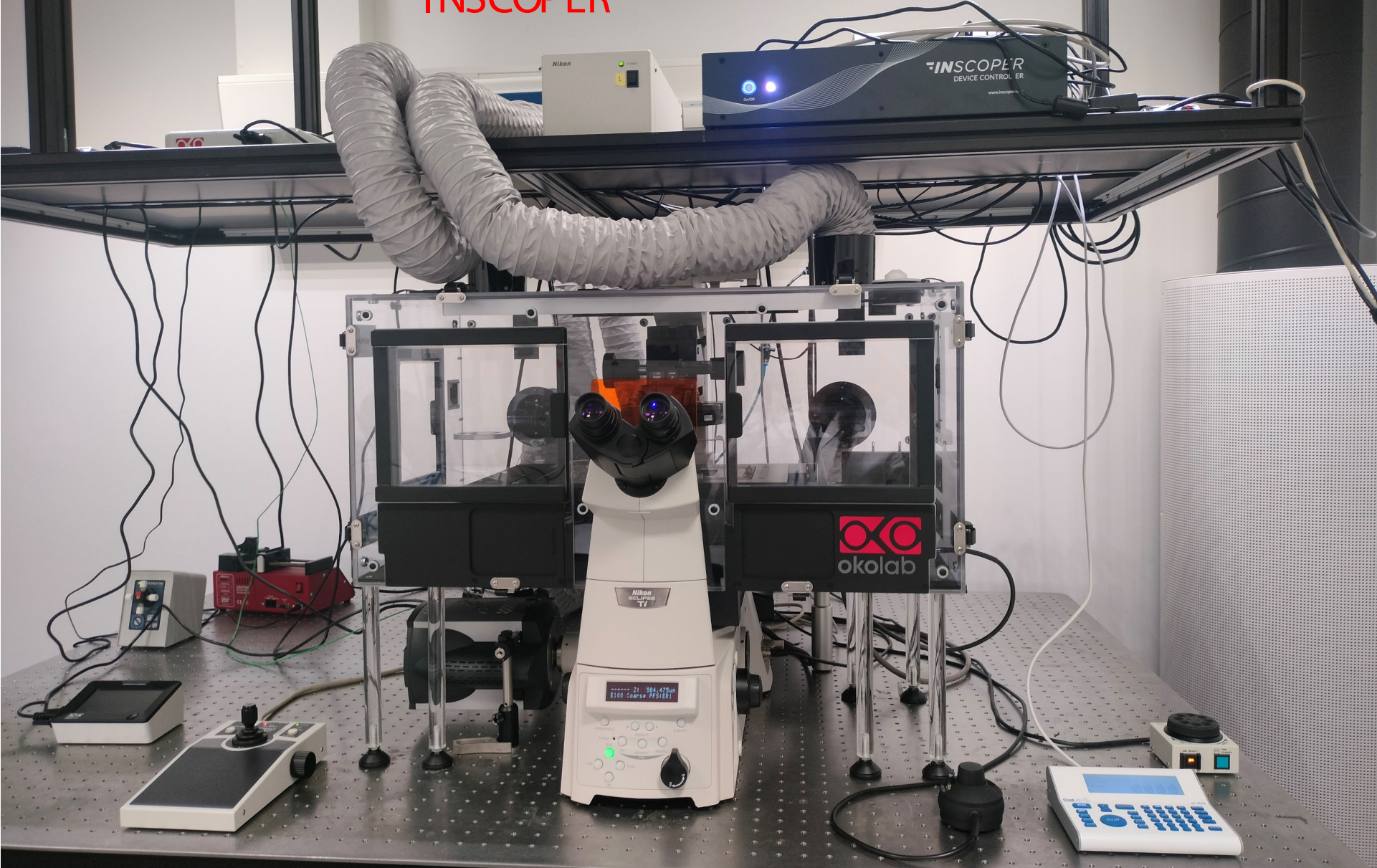
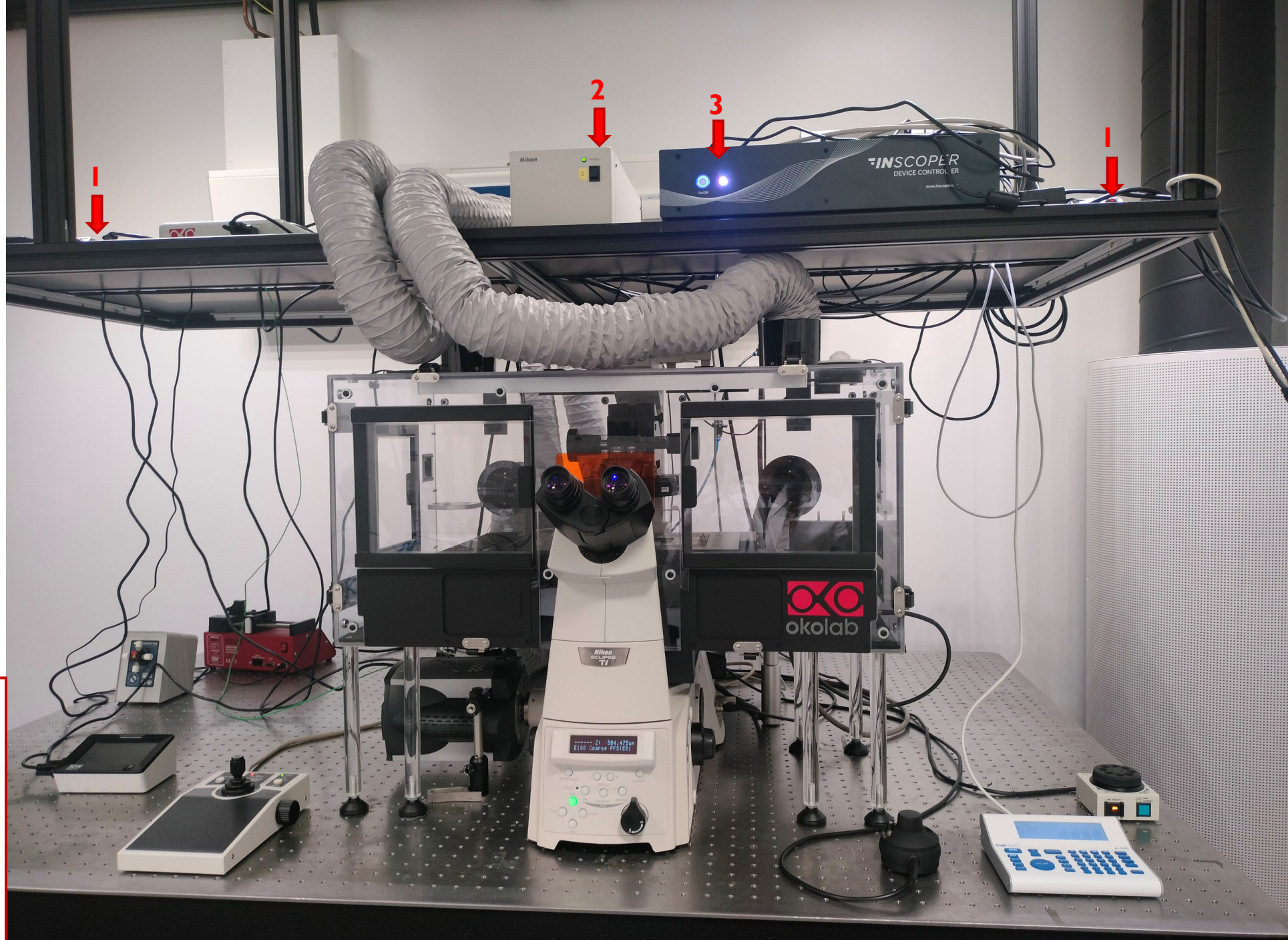


Wide Field Long Term
INSCOPER



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

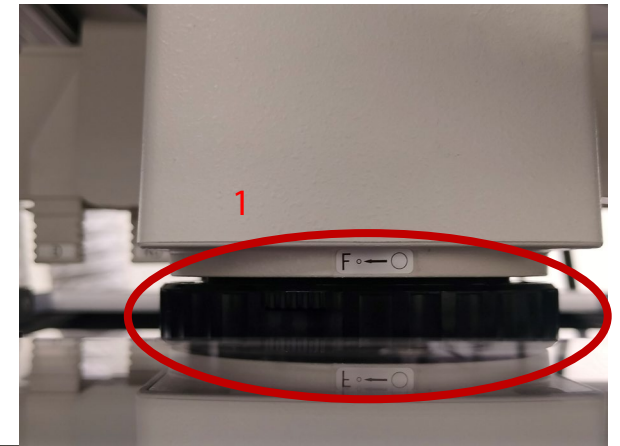
Take ND filter out to increase light intensity

Move condenser manually to plate ph3

To align: Place 10X objective, close the shutter (#1) until you see the edge

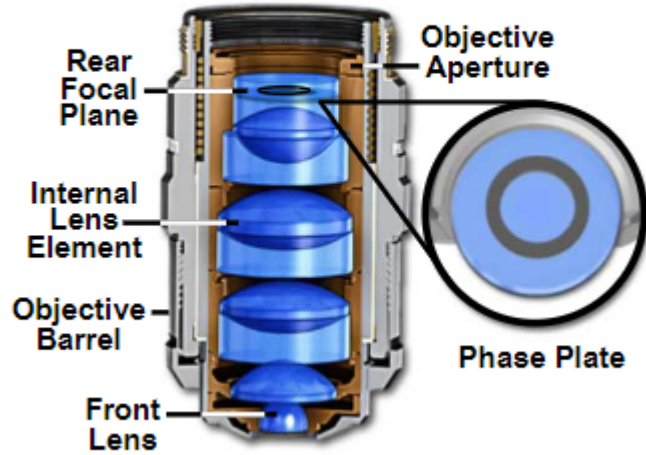
Move the Condenser height until the edge is sharp

Center with the screws (#2)



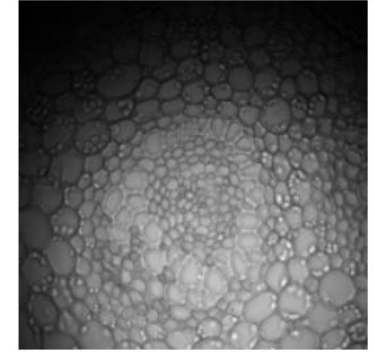
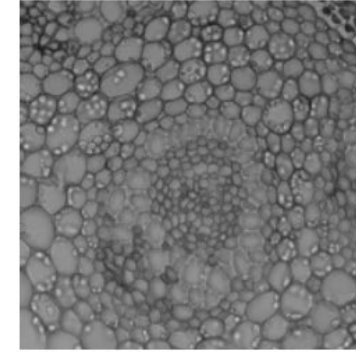
Phase contrast alignment (1/2): Procedure

Figure 4 - Phase Contrast Objective



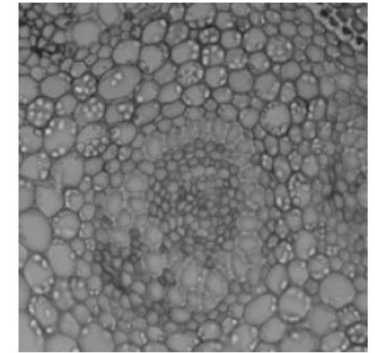
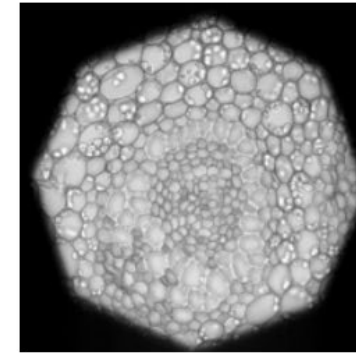
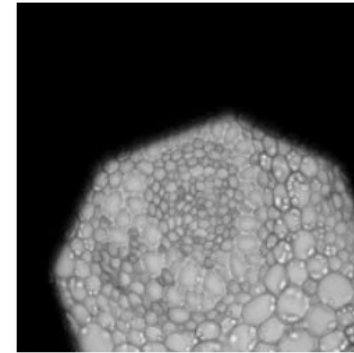
<https://www.microscopyu.com/techniques/phase-contrast/phase-contrast-microscope-configuration>

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 height to get a clear image of the iris

5- Centre the iris

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

See also:

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

Jérémie Capoulade



Recycle Bin



BitFlow Preview



Microsoft Edge



OxyGEN



OxyGEN.csv



OxyGEN



TeamViewer Host



Micro-Ma... 2.0



DELETE

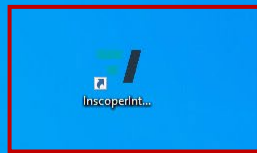


Test images_20...



projects

Start Software



New Project



AutoSave

Exit

Open project

Configuration

Configuration window- check Flu:

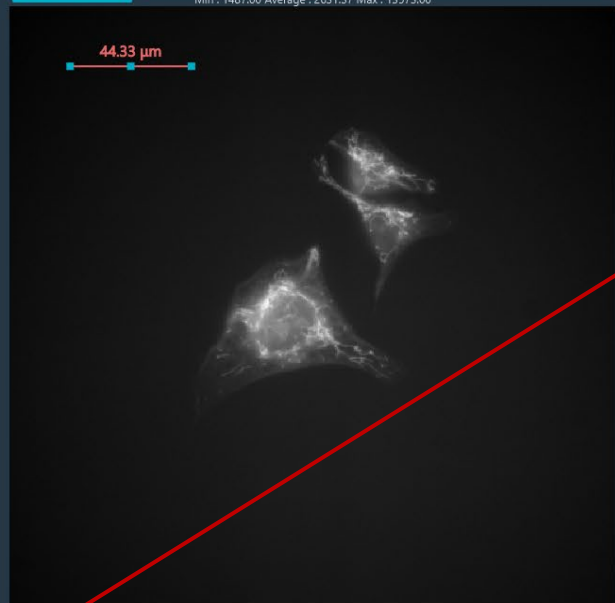
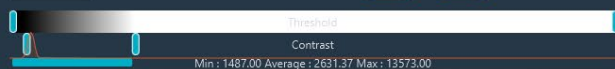
Project Name **M** Project 2024-10-31

Camera Settings

Camera

Binning:

Exposure (ms):



Calibration

Tiling

NikonTie PfsOffset: 158975 Step (nm): 1

PFS

PfsState:

Objective: 60X Oil

Stage

X_Axis (nm): 438700 Y_Axis (nm): 143100 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

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**

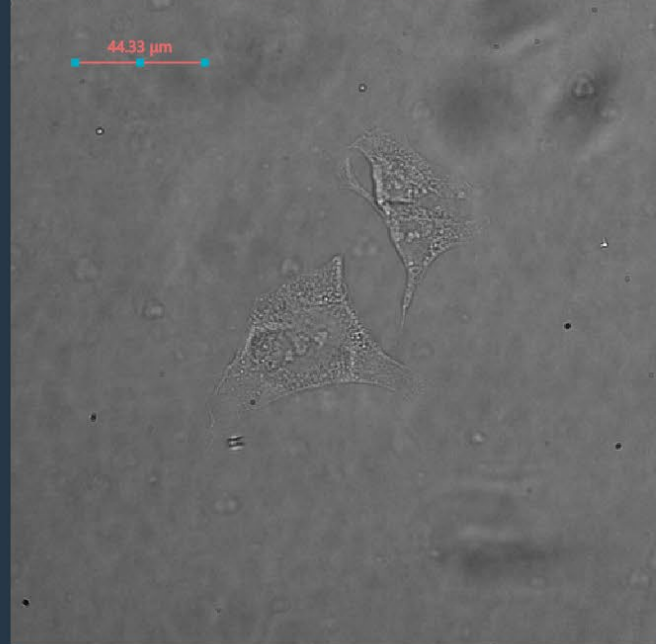
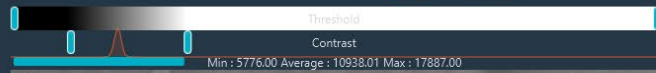
NikonTie SidePort: **L100** Cube: **GFP** TL Off/On:

Configuration window- check BF/Phase:

Camera Settings

Binning:

Exposure (ms):



Threshold Low:

Threshold High:

Contrast Low:

Contrast High:

Contrast Min:

Contrast Max:

Calibration

Tiling

NikonTie

PfsOffset:

Step (nm):

PfsState:

X_Axis (nm):

Y_Axis (nm):

Step (nm):

Focus:

Step (nm):

B WaveLength C WaveLength D WaveLength






Cube TL Off/On

pE4000

| | | | | | | | |
|---|----------------------------------|---|----------------------------------|---|---------------------------------|---|---------------------------------|
| <input checked="" type="checkbox"/> A Digit Off/On | <input type="checkbox"/> | <input checked="" type="checkbox"/> B Digit Off/On | <input type="checkbox"/> | <input checked="" type="checkbox"/> C Digit Off/On | <input type="checkbox"/> | <input checked="" type="checkbox"/> D Digit Off/On | <input type="checkbox"/> |
| <input checked="" type="checkbox"/> A Intensity (%) | <input type="text" value="100"/> | <input checked="" type="checkbox"/> B Intensity (%) | <input type="text" value="100"/> | <input checked="" type="checkbox"/> C Intensity (%) | <input type="text" value="10"/> | <input checked="" type="checkbox"/> D Intensity (%) | <input type="text" value="51"/> |



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. |

Acquisition window- set your experiment

Camera Settings

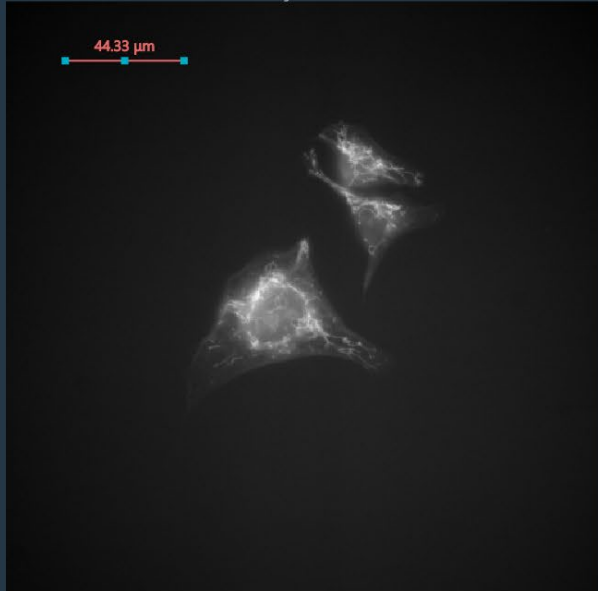
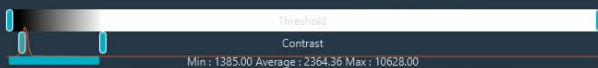
Binning 1x1

Exposure (ms) 50

Advanced

Snap

Live



Sequence 1 Take Image Config Add Show All

1. Time

Number of Time Points 0

Interval 0 h 0 min 0 s 0 ms

Total Time 0 h 0 min 0 s 0 ms

Burst Mode

2. Positions Switch to Tiling

3. Z-Stack

4. Multi-Channels

| | | |
|-------------------------------------|---|-------------------|
| <input checked="" type="checkbox"/> | 0 | |
| <input checked="" type="checkbox"/> | 0 | Edit |
| <input checked="" type="checkbox"/> | 1 | Edit |
| <input checked="" type="checkbox"/> | 0 | Edit |

Confirm

< 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

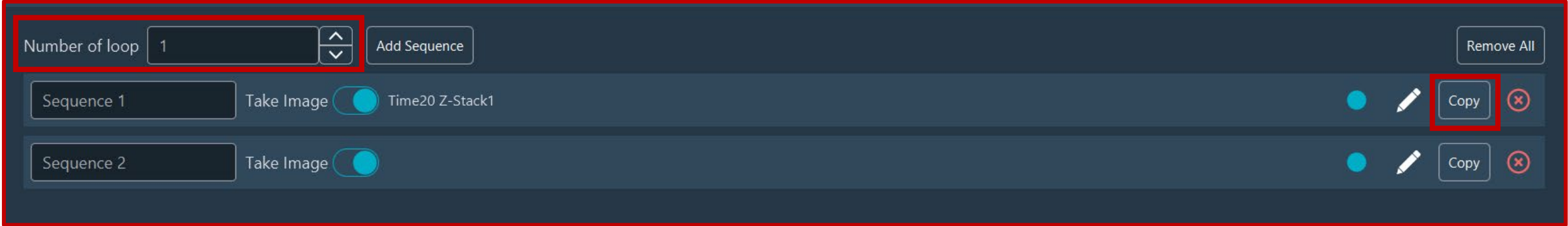
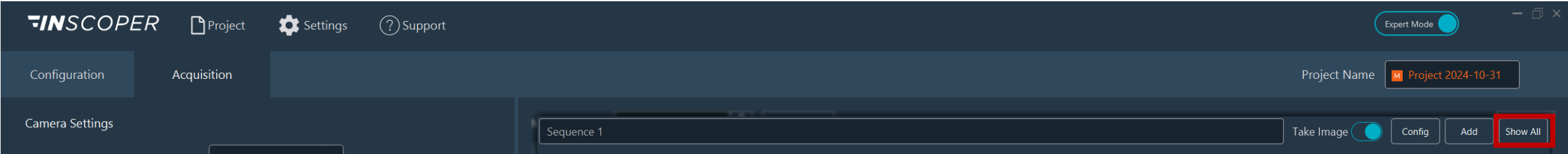
“Add”- you can add a sequence.

“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

Acquisition Configuration

MDA Configuration

Dimension Name

- Time Dimension
- Z-Stack Dimension
- Multi-Positions Dimension
- Tiling Dimension
- Channels Dimension
- Autofocus Dimension
- Stabilization Dimension

Axis Editor

- X Axis Extra Axis
- Y Axis
- Z Axis

Extra Dimensions

- Extra Dimension 1
- Extra Dimension 2

 20 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

1. Time

Set a standard time exp with a certain interval between frames.

● 20

Number of Time Points

20

Interval

0 h 0 min 1 s 0 ms

Burst Mode

Total Time

0 h 0 min 20 s 0 ms

Confirm

1. Time

Set a fast time exp with minimal interval between frames.

● 20

Number of Time Points

20

Interval

0 h 0 min 0 s 0 ms

Burst Mode

Total Time

0 h 0 min 0 s 0 ms

Confirm

Acquisition – define multi positions, using PFS offset

2. Positions Switch to Tiling ● 2

XY
 X_Axis (nm) 195100
 Y_Axis (nm) 351100
 Step (nm) 50000

Focus
 Focus 1609175
 Step (nm) 1

PfsOffset
 PfsOffset 158675
 Step (nm) 1

Well Plate Set
 Pattern detection Start
 Add position Remove all

PfsState

| N° | Tag | X_Axis | Y_Axis | <input type="checkbox"/> Focus | <input checked="" type="checkbox"/> PfsOffset | <input checked="" type="checkbox"/> PfsState | Move to | Copy | Get | |
|----|-----|--------|--------|--------------------------------|---|--|---------|------|-----|--|
| 1 | | 438800 | 142900 | 1609875 | 158975 | <input checked="" type="checkbox"/> | | Copy | Get | |
| 2 | | 195000 | 351000 | 1609200 | 158675 | <input checked="" type="checkbox"/> | | Copy | Get | |

Check the PFS and update offset per position

Update per position

Acquisition – define multi positions, using Abs Z position

2. Positions Switch to Tiling

XY X_Axis (nm) Y_Axis (nm) Step (nm)

Focus Focus Step (nm)

PfsOffset PfsOffset Step (nm)

Well Plate

Pattern detection

PfsState

| N° | Tag | X_Axis | Y_Axis | <input checked="" type="checkbox"/> Focus | <input type="checkbox"/> PfsOffset | <input checked="" type="checkbox"/> PfsState | Move to | Copy | Get |
|----|-----|--------|--------|---|------------------------------------|--|--|-------------------------------------|------------------------------------|
| 1 | | 438900 | 142800 | 1605100 | 158975 | <input type="checkbox"/> | <input type="button" value="Move to"/> | <input type="button" value="Copy"/> | <input type="button" value="Get"/> |
| 2 | | 195000 | 351000 | 1609200 | 158675 | <input type="checkbox"/> | <input type="button" value="Move to"/> | <input type="button" value="Copy"/> | <input type="button" value="Get"/> |

Check the Focus (stage Z)
and update offset per position

Acquisition – Tiling

2. Tiling

Switch to Positions

0



XY

X_Axis (nm) 195100

Y_Axis (nm) 351000

Step (nm) 1

Focus 1608175

Step (nm) 1

PfsOffset 158975

Step (nm) 1

Well Plate Set

FocusMap Start

Add tiling

Remove all

PfsState



If you want PFS ON

You can have several tiling areas around different centres

| N° | Tag | Position Count | Edit |
|----|-----|----------------|---|
| 1 | | |   |

Define tiles

Acquisition – Define Tile

2. Tiling

Switch to Positions

0



XY

X_Axis (nm) 195100

Y_Axis (nm) 351000

Step (nm) 1

Focus

Focus 1607900

Step (nm) 1

PfsOffset

PfsOffset 158975

Step (nm) 1

PfsState



Type

RECTANGLE Center *Define shape and borders*

Center

X_Axis 195100

Y_Axis 351000

Focus PfsOffset PfsState

1609800 158975

Define focus- Z or PFS

Get Goto

Width

100000

Tile size in nm

Height

500000

Overlap (%)

10

Auto

Custom Step (nm)

199480.52

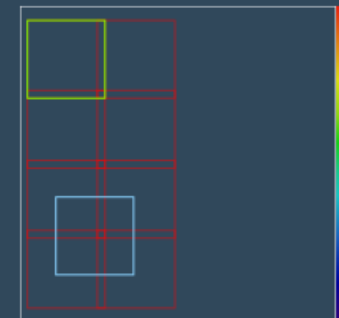
199480.52

Read Mode



Snake Mode

Focus Map Edit



Show Images

LUT

Confirm

Cancel

Tiling with Focus map

The screenshot shows the INSOPER software interface. On the left, the 'Camera Settings' panel includes 'Binning' (1x1) and 'Exposure (ms)' (20). Below this is a 'Contrast' slider and a '44.33 μm' scale bar. The central panel displays a focus map with a color gradient from red (high focus) to blue (low focus). To the right of the focus map is a table of acquisition parameters:

| Step (nm) | Focus (nm) | Step (nm) | PfsOffset (nm) | Step (nm) |
|-----------|------------|-----------|----------------|-----------|
| 1 | 1605250 | 1000 | 120975 | 1 |



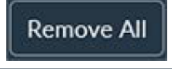




Below the table are buttons for 'Adjust Offset', 'Import', and 'Export'. The top navigation bar includes 'Project Name: Project 2024-10-31' and 'Open in Explorer'.

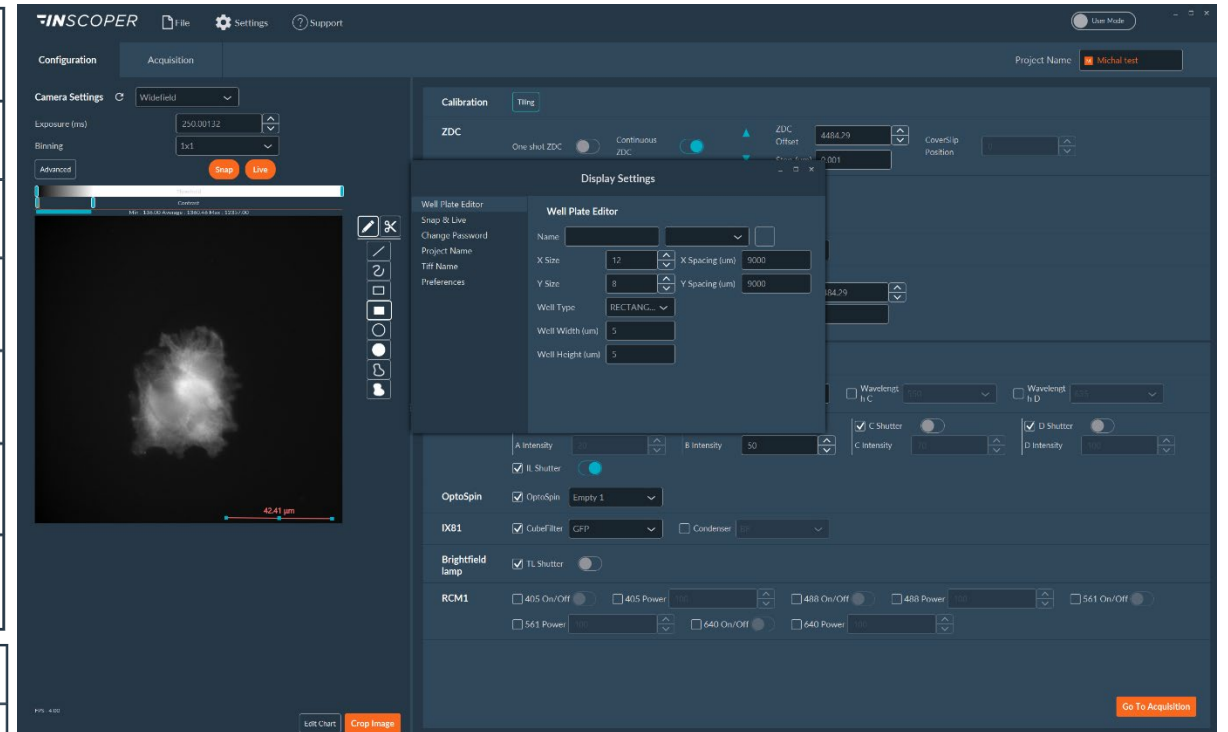
The 'Focus Map Edit' dialog box shows a grid of focus map tiles. A color scale on the right indicates focus levels. Below the grid are two checked checkboxes: 'Show Images' and 'LUT'. An 'Edit' button is located at the top right of the dialog.

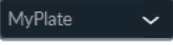


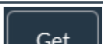


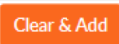
You can edit your focus map to add and redefine Z within positions

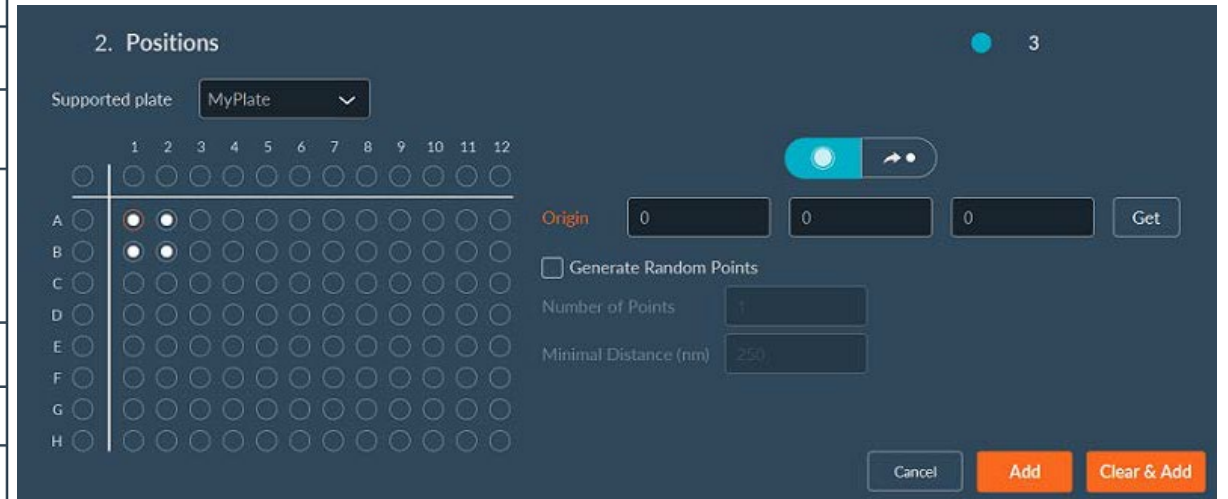
INSCOPER- Acquisition- Stage experiment: Define well plate (Auto position list)

!Only using Air Objectives!

| | | |
|---|--------------|---|
|  | Set | 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. |



3. Z-Stack

Acquisition – Z stack

11

▲ Focus (nm) 1607600 ▲
▼ Focus (nm) 1607600 ▼

▲ Step (nm) 1000 ▲
▼ Step (nm) 1000 ▼

Min/Max Plane

Center Plane

Min (nm) 1607575 Get

Center (nm) 1606037.5 Get

Stack Step (nm) 253.775 Nyquist

Max (nm) 1604500 Get

Volume (nm) 3075

Stack Size 11

Absolute- stage

Center First

Confirm

3. Z-Stack

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

8

▲ Focus (nm) 1608850 ▲
▼ Focus (nm) 1608850 ▼

▲ Step (nm) 1 ▲
▼ Step (nm) 1 ▼

Min/Max Plane

Center Plane

Min (nm) 1608000 Get

Center (nm) 1609000 Get

Stack Step (nm) 253.775 Nyquist

Max (nm) 1610000 Get

Volume (nm) 2000

Stack Size 8

Center First

Confirm

Acquisition – Multi channel

Camera Settings

Binning: 1x1

Exposure (ms): 20

Advanced Snap Stop

Threshold

Contrast

Min: 3954.00 Average: 6871.47 Max: 10002.00

44.33 μ m

Sequence 1

Take Image Config Add Show All

1. Time 0

2. Positions Switch to Tiling 0

3. Z-Stack 0

4. Multi-Channels Live **BFGFP** GFP Stop 2

Add Channel Remove All

The selected channel can be imaged

| N° | Name | Z Stack | Z Offset | Shutter Blink | Camera | Exposure (ms) |
|----|--------|--------------------------|----------|--------------------------|--------------|---------------|
| 1 | BF_GFP | <input type="checkbox"/> | 0 | <input type="checkbox"/> | Andorlxon... | 20 |
| 2 | GFP | <input type="checkbox"/> | 0 | <input type="checkbox"/> | Andorlxon... | 100 |

⊗ If you want BF and Flu, you can choose BF with the same filter as Flu to speed up the shift between channels

Confirm

Acquisition – start experiment

< 1 >

Data Processing and Charts

RAW DATA



Edit Parameters

Switch to DataProcessor

Save Acquisition

You can save directly to your folder.

in RAM

on Disk

C:\Users\LocalAdmin\Desktc



Format

All in One



Save as BigTiff

Metadata Format

All in One



Total images 16

Total size 33MB

Minimal duration

00h00min00s320ms

Start Acquisition

If you choose RAM, remember to export to your folder

Export :

Video

Stack

Visualization

You can check all your experiments from this session

Configuration

Acquisition

Visualization 
Project 2024-10-31

Project Name

 Project 2024-10-31

Open in Explorer

Threshold

Contrast

Min: 1830.00 Average: 3284.51 Max: 9035.00

44.33 μm



Filters

> Z-Stack - Single All Interval Custom

> Position - Single All Interval Custom

Image Processing

Sequence 1

Total: 26

1/1 0-26

Export:



Metadata

Visualization

Metadata: you can filter to check specific parameter

Continue to process your data,
Example: max Z projection

Image Processing Advanced

Sequence 1

Image to Process RAW_DATA ▾

Process type Focus Max ▾

Confirm

Filters

Z-Stack 1 - 8 In... C...

Position 1 - 2 In... C...

Image Processing Advanced

Sequence 1

Total : 16 1/1 0-16 Export : Video Stack

| Property | Value |
|--------------------|----------|
| PositionName | 1 |
| NikonTie-YPosition | 142900.0 |
| NikonTie-XPosition | 438800.0 |
| PositionIndex | 0 |
| positionIndex | 0 |

Visualization-Tag

The screenshot displays the INSCOPER software interface. At the top, there are menu items for File, Settings, and Support. The main navigation bar includes Configuration, Acquisition, and Visualization (selected). The project name is 'Project 2023-07-20'. The interface is divided into several panels:

- Left Panel:** A large image viewer showing a microscopy image with a scale bar of 234.73 μm. A vertical toolbar on the right side of the image viewer contains various tools for annotation and analysis.
- Filters Panel:** Shows 'Selected Image set' as 'STITCH_TILING' and 'Image Processing' options.
- Charts Panel:** Displays 'XY Sequence' with 'Total: 1' and '1/1 0-1'. It includes an 'Export' button with 'Video' and 'Stack' options.
- Right Panel:** A 'Metadata' and 'Tag' section. The 'Tag' tab is active, showing a list of properties and values. A red box highlights the 'Tag' tab and the 'Export' button at the bottom right.

A red box highlights the 'Tag' tab in the right panel, and a red box highlights the 'Export' button at the bottom right. A red box also highlights the 'STITCH_TILING' dropdown in the Filters panel.

You can use Tag to export ROIs as positions

| Property | Value |
|--------------------------------------|---------------------|
| OrcaFlash4-INTERNAL FRAM | 9.999362444665053 |
| 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-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 | 100.006380000000001 |
| 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 COOLEF 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 | |

Shut down

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

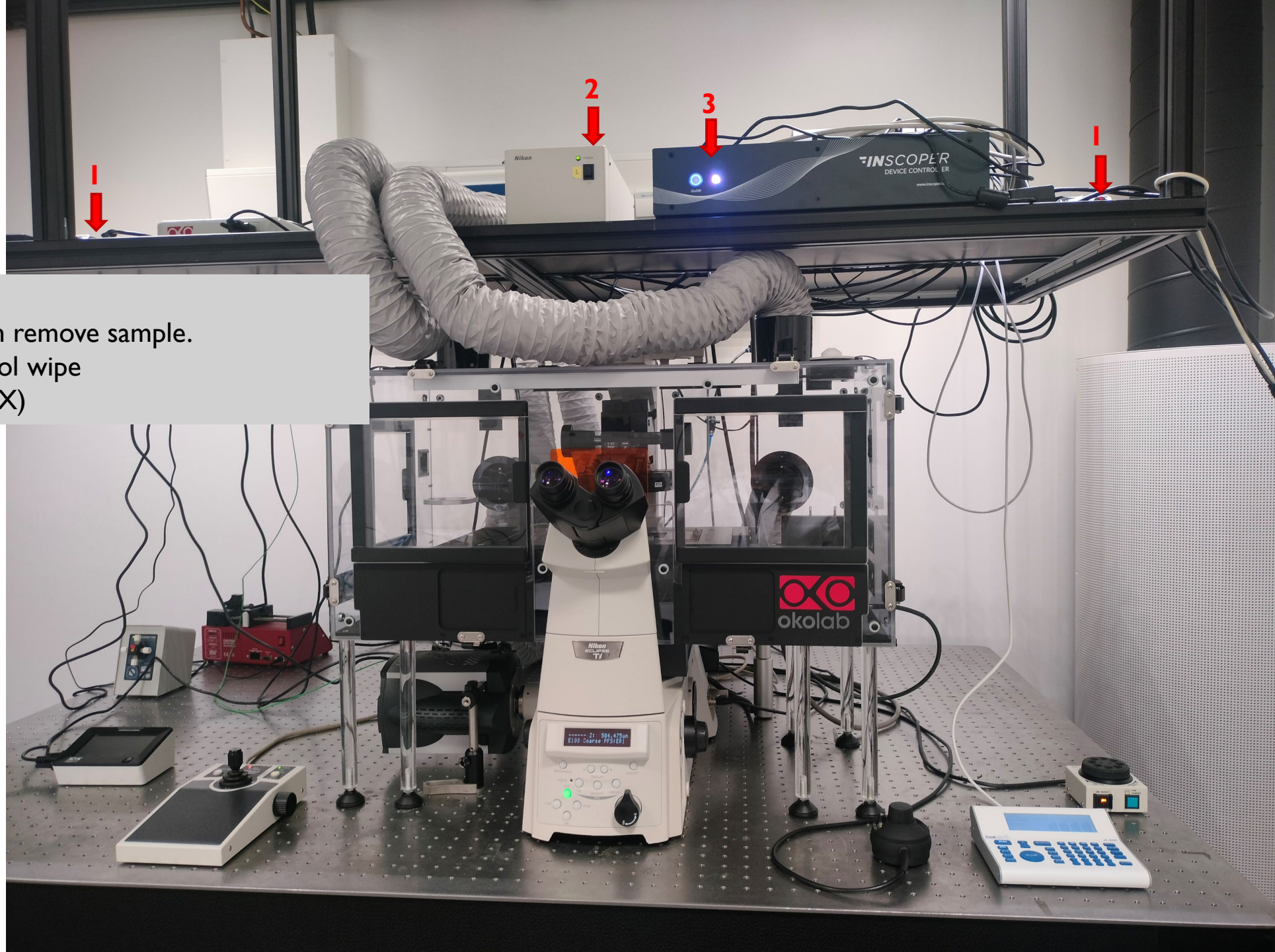
2, Clean up:

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?

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