

iLAS_TIRF INSCOPER

User guide

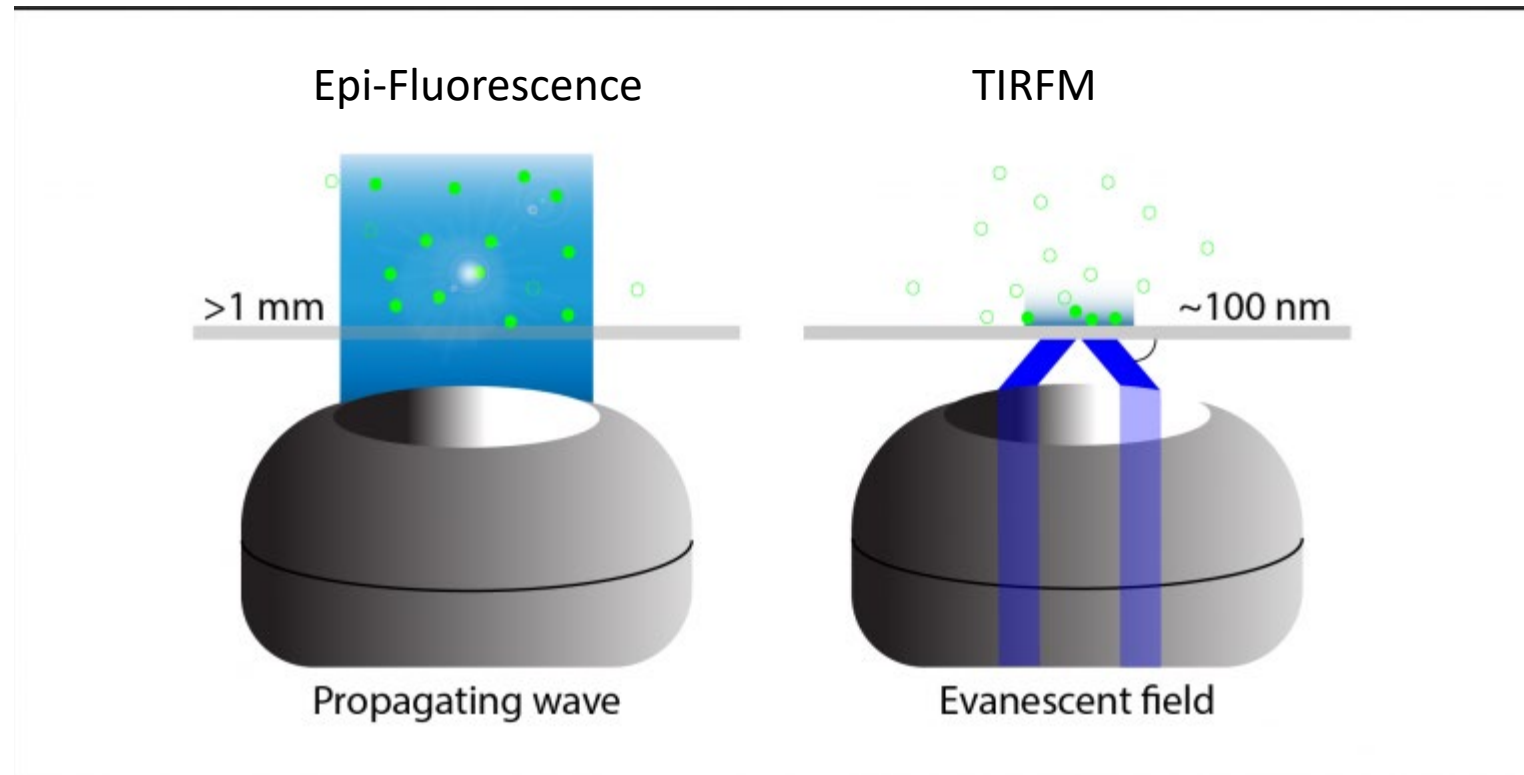
Kavli Nanolab Imaging Centre

Snell's law for the critical angle of light

$$\theta_c = \sin^{-1}(n_1/n_2)$$

When light hits an interface beyond the critical angle it is completely reflected, this is called Total Internal Reflection:

In TIRF microscopy, the light is presented to the slide-sample interface beyond this critical angle, either through the **objective** or using prisms. This produces an electromagnetic field at the interface called **the evanescent field** or wave

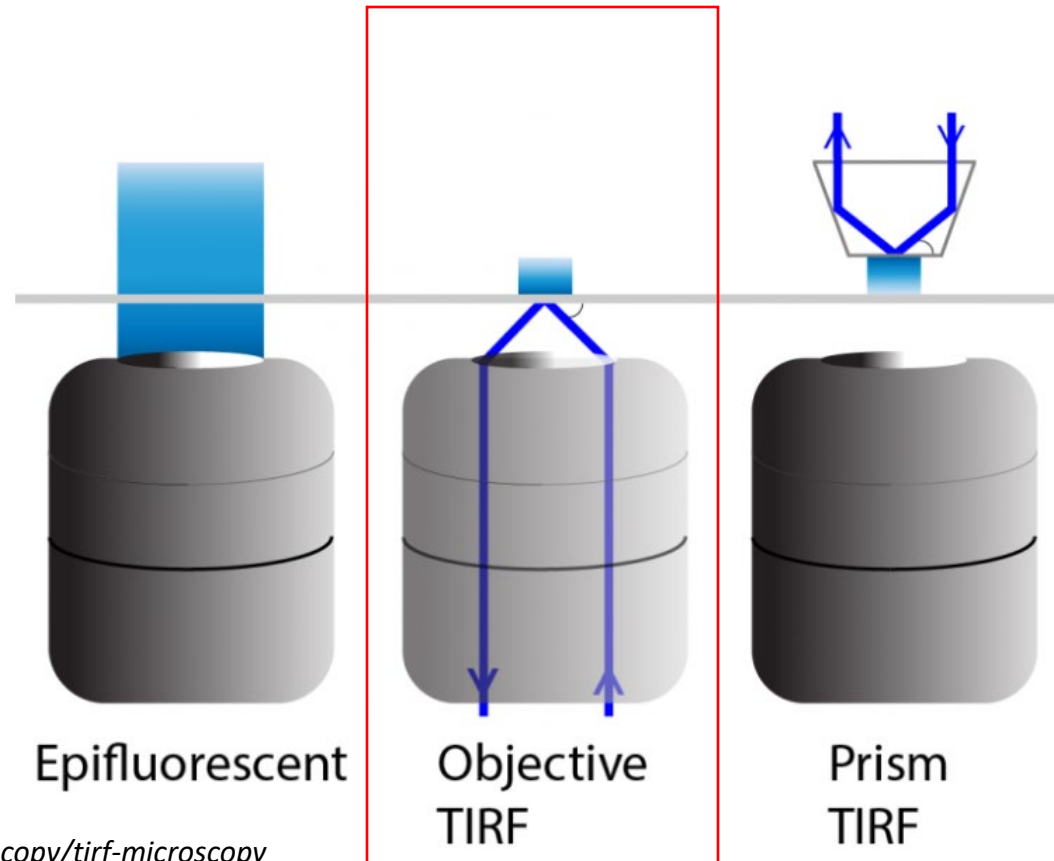


Objective based TIRF: the laser light source entering the back-pupil of the objective off-axis. The angle of incidence leaving the lens correlates with the extent of off-axis input light.

Pay attention- your objective should have:

High NA (> 1.49) to provide shallow field

Correction collar for temperature- compensates for change in oil RI



StartUp:

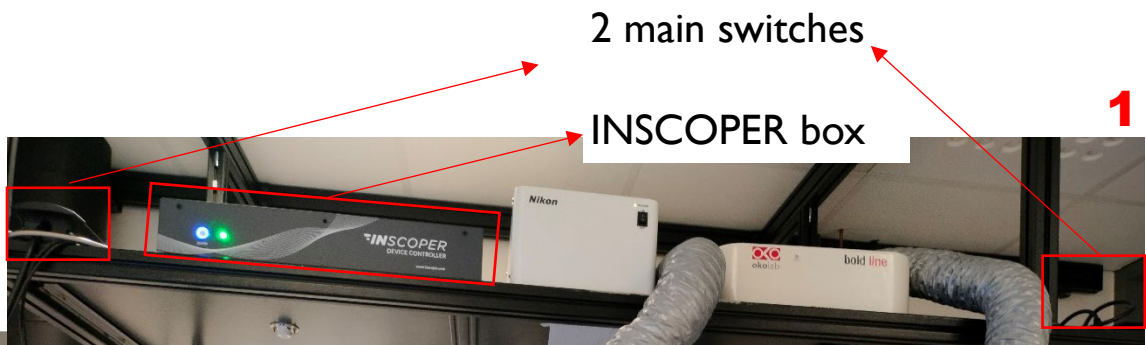
- 1, Turn ON the 2 main switches
- 2, Turn ON INSCOPER box
- 3, Turn ON PC and INSCOPER software
- 4, Locate your sample (BF only):
 - 1, place oil on 100X objective, place path to eyepiece
 2. Turn on BF light and turn the focus knob to move objective UP until the oil spreads on the coverglass (Slow!)
 - 3, Pay attention- when reaching focus the PFS beeps- view with eyepiece (you always see the glass if moving stage). Activate PFS
 - 4, Change to Camera port (left)- choose Bypass/Split, place filters and align if needed
- 5, move to software

Shut-Down:

- 1, Save your data to Bulk folder
- 2, Turn OFF PC and INSCOPER software
- 3, Remove your sample:
 - 1, **Lower** the objective height to minimum
 2. Remove your sample and **clean the objective** with 2-propanol (see instructions)
 - 3, Change back to **20X air** objective
 - 4, Close Oko-cage doors (and chamber light) and **clean** your environment.
- 4, Turn OFF the INSCOPER box
- 5, Turn OFF 2 main switches

StartUp:

Hardware- General:

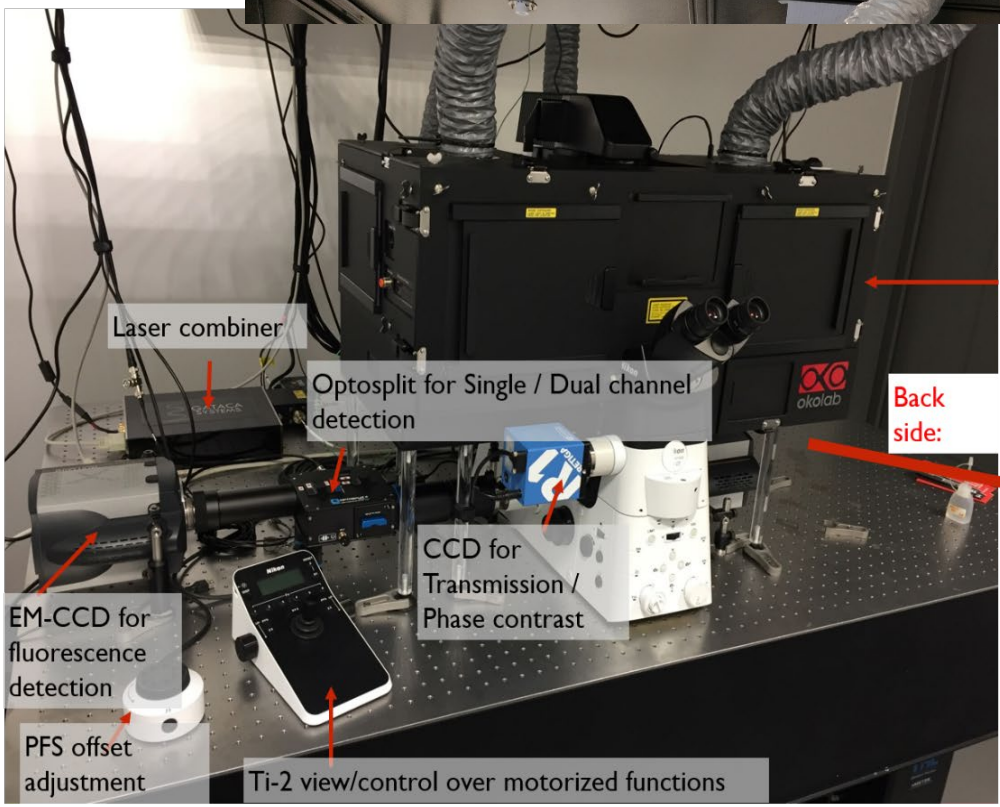


3

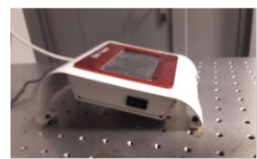


2

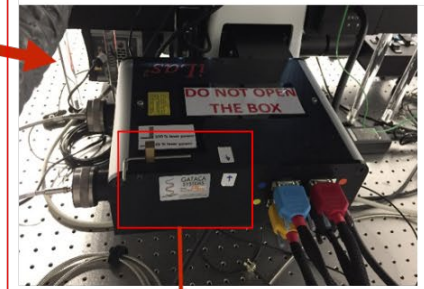
PC



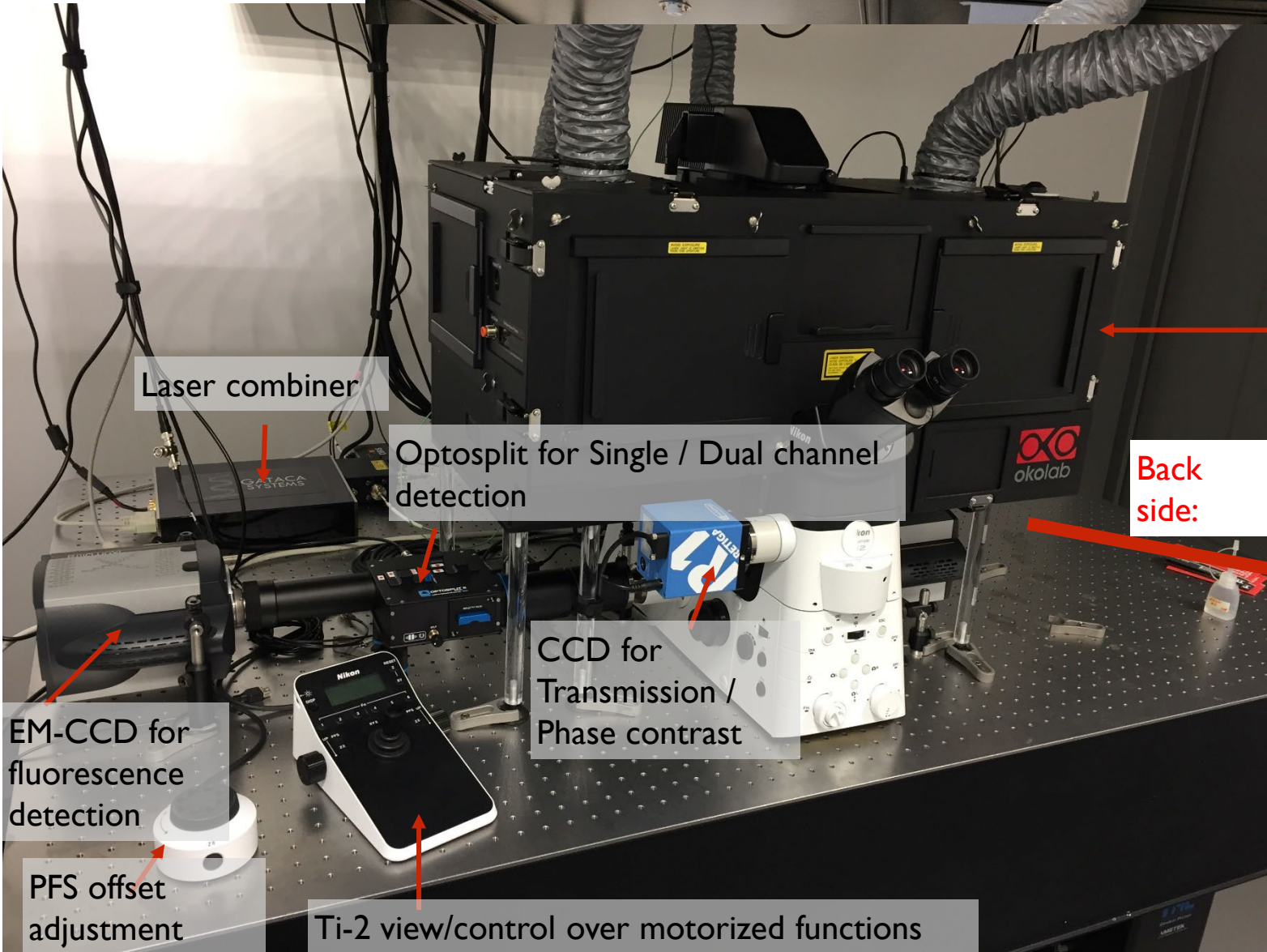
Okolab incubation box



Optical fibers for TIRF and FRAP laser illumination



Hardware- General:



Laser combiner

Optosplit for Single / Dual channel detection

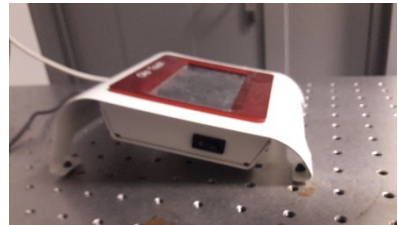
CCD for Transmission / Phase contrast

EM-CCD for fluorescence detection

PFS offset adjustment

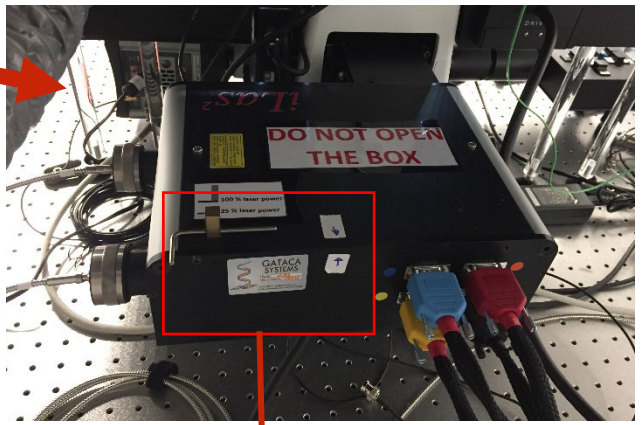
Ti-2 view/control over motorized functions

Back side:



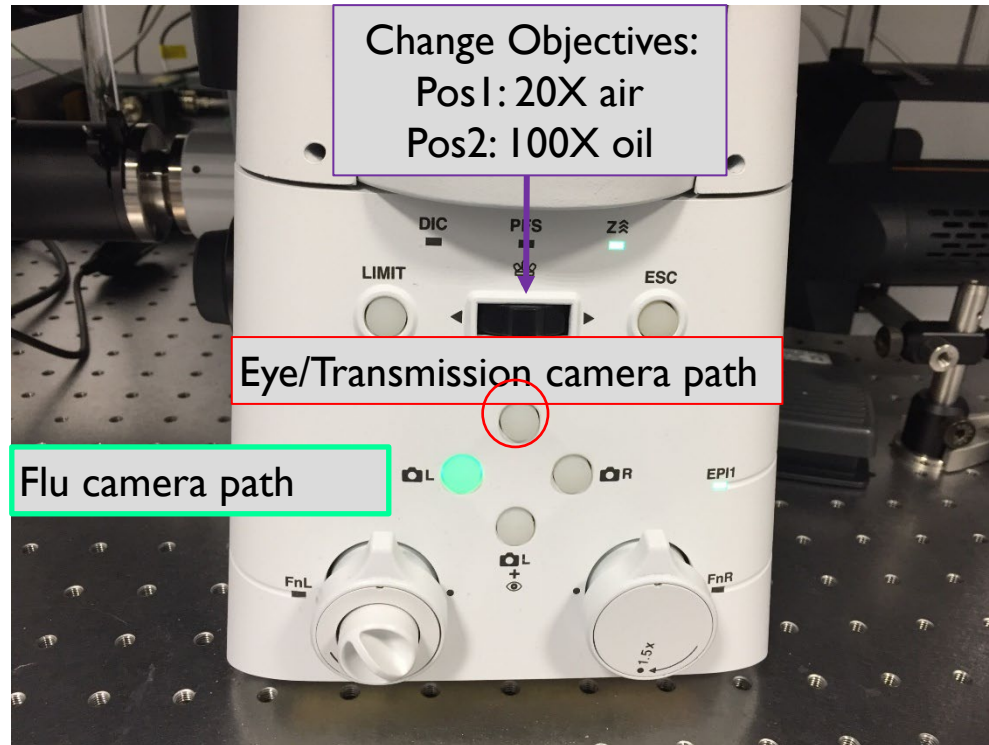
OkoLab incubation box

Optical fibers for TIRF and FRAP laser illumination



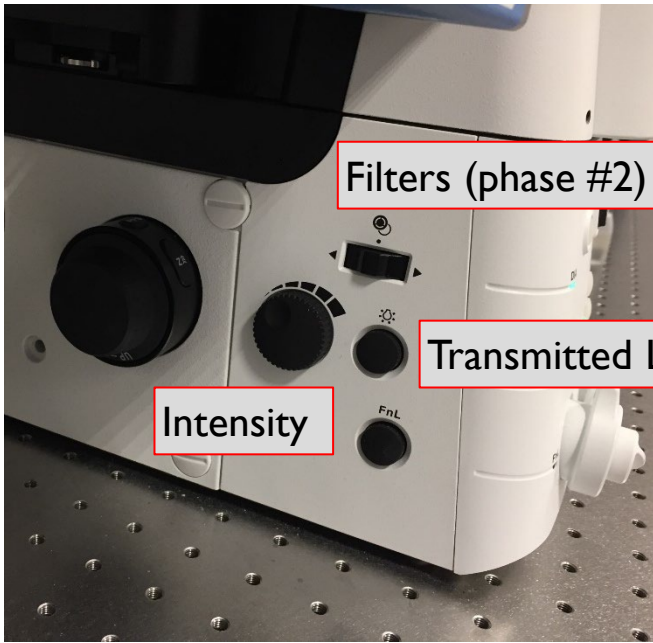
Laser power range adjustment

Ti-2 body:



Turn PFS ON

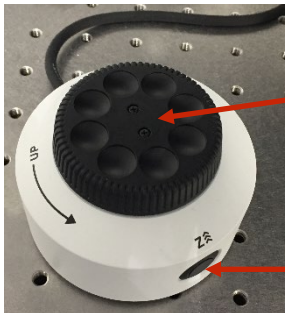
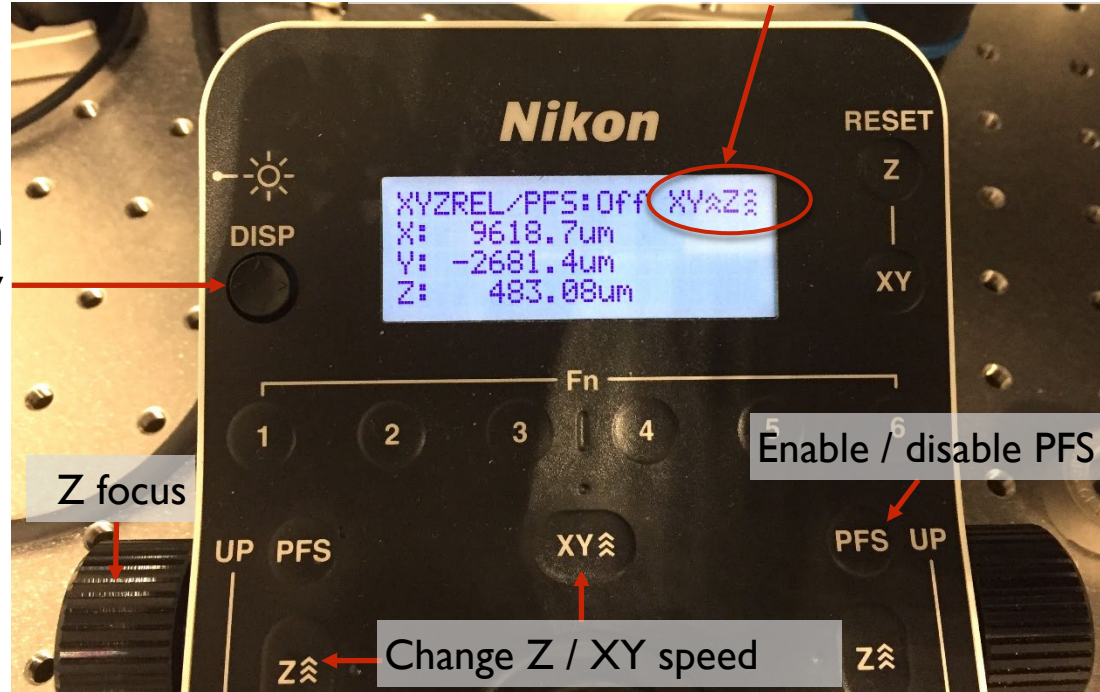
Turn on light in the OKO cage



Ti-2 Joystick

View speed:
XY: ^^ = fast / ^ = slow
Z: ^^^ = fast / ^^ = fine / ^ = extra-fine

Change information display

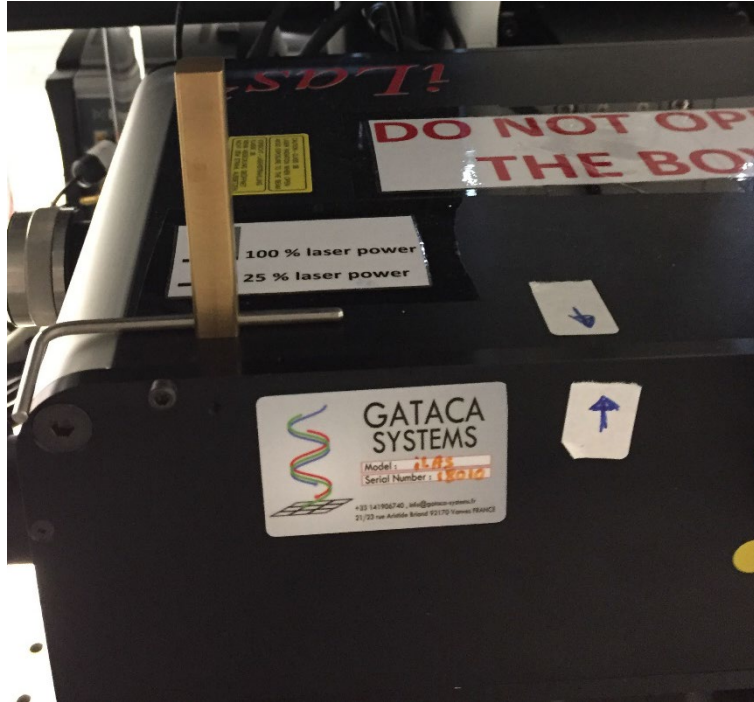


When the PFS is enabled, use this knob to adjust the focus

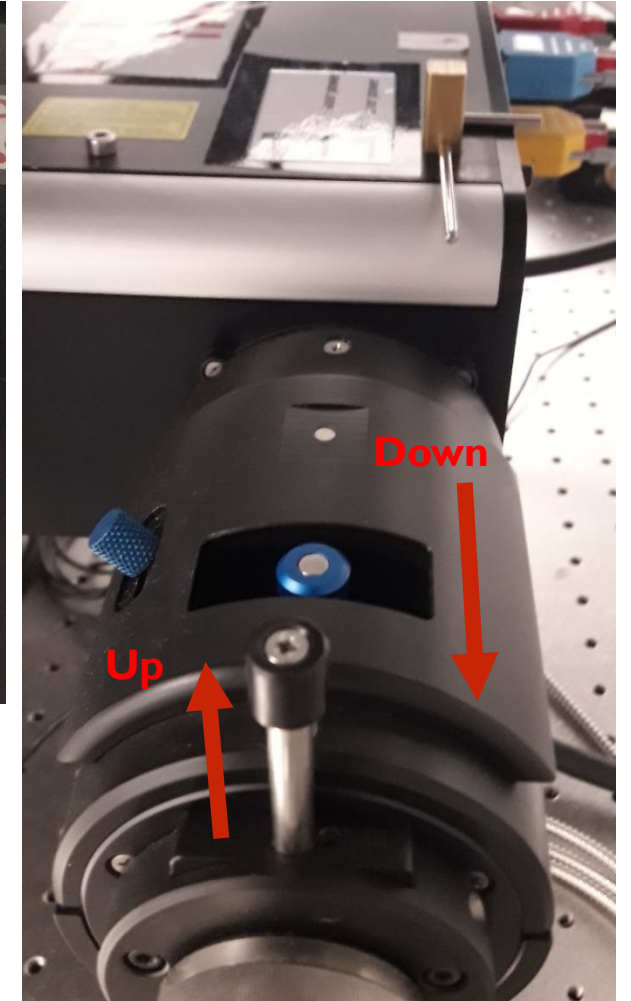
Keep pressing this button to speed up the focus adjustment

To modify laser intensity- Hardware

High laser Intensity

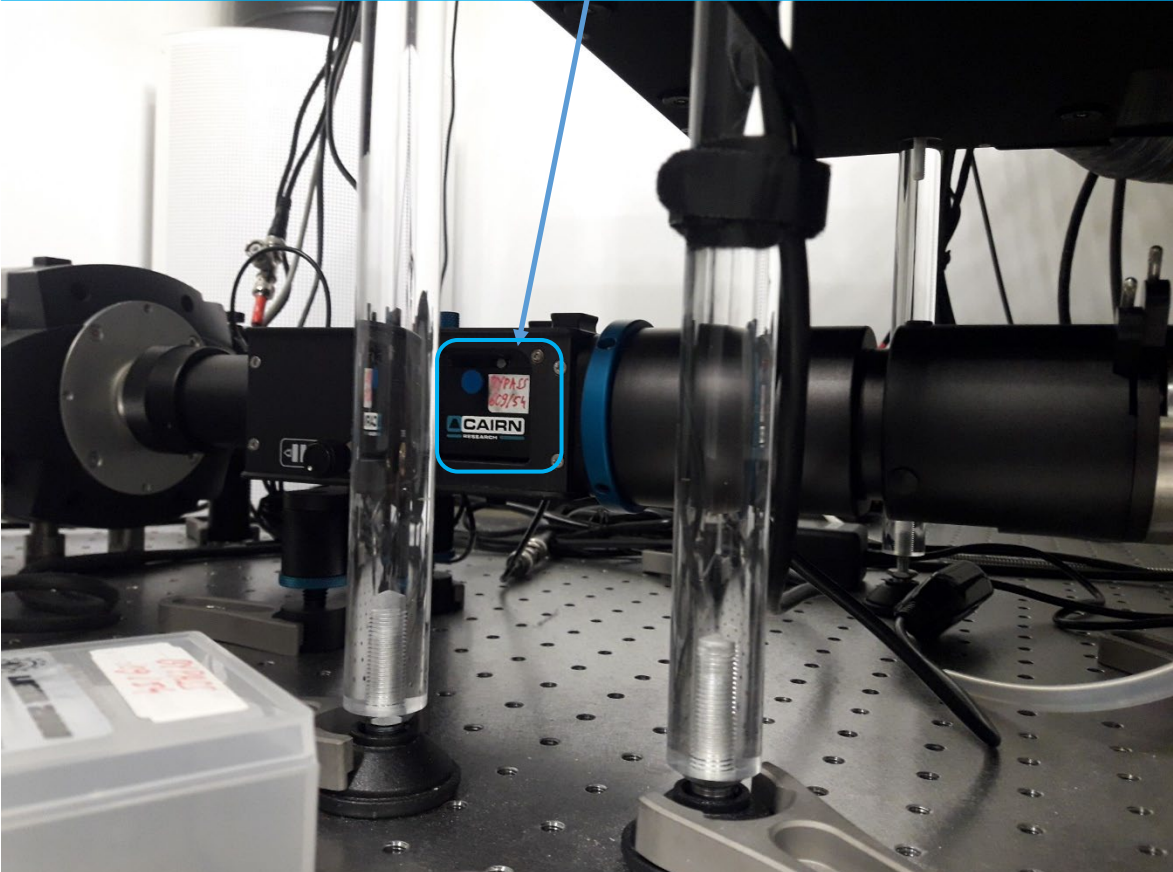


Low laser Intensity



Optosplit for single/dual flu imaging- Emission path

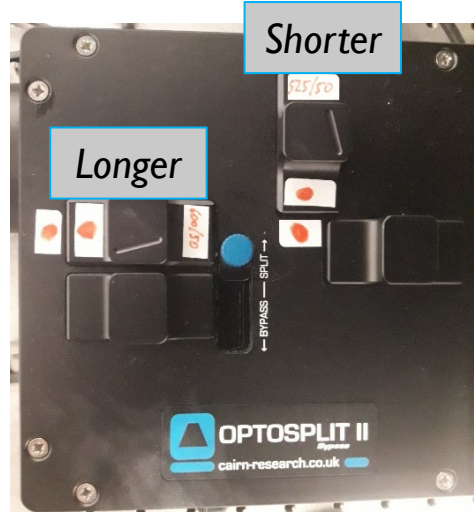
2, Make sure you have the **filter cube** inserted for **Bypass/Split** with desired emission band (*2nd drawer left*)



1, Use Bypass path to image only one channel
Shift knob to split path to image 2 channels

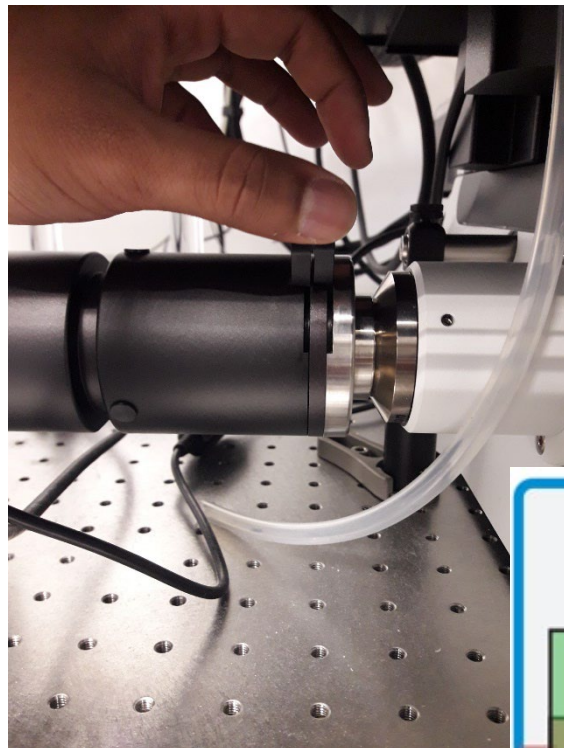


Split filters



Optosplit for single/dual flu imaging- Alignment

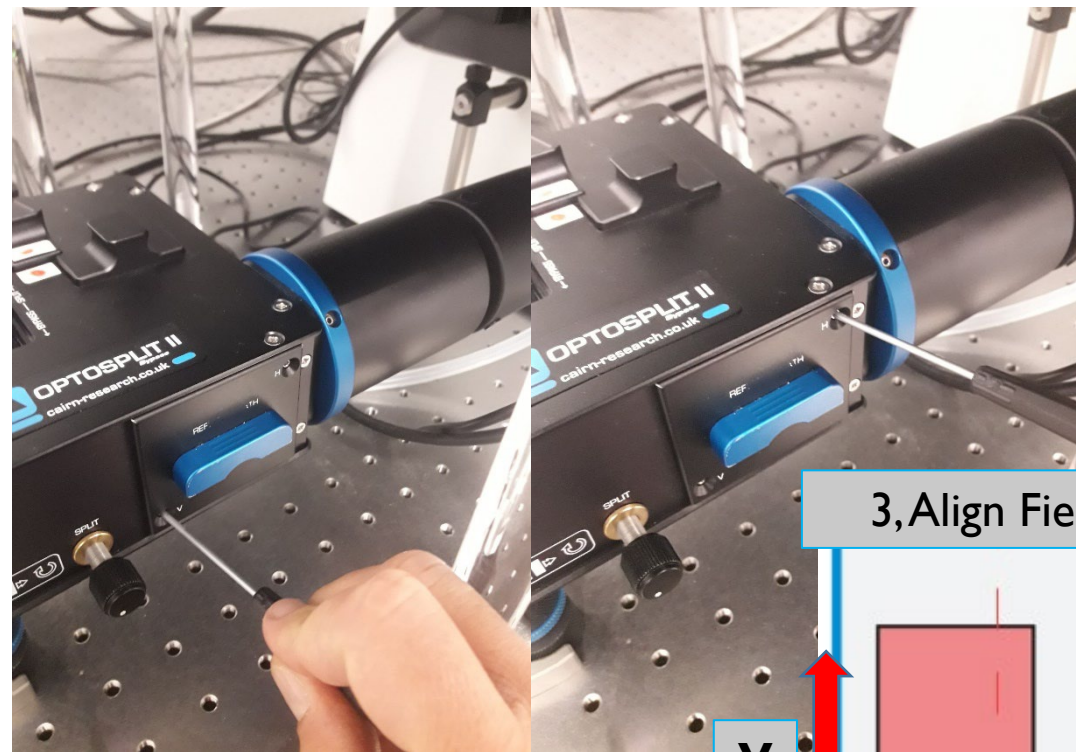
1, Use the knobs to reduce Field size



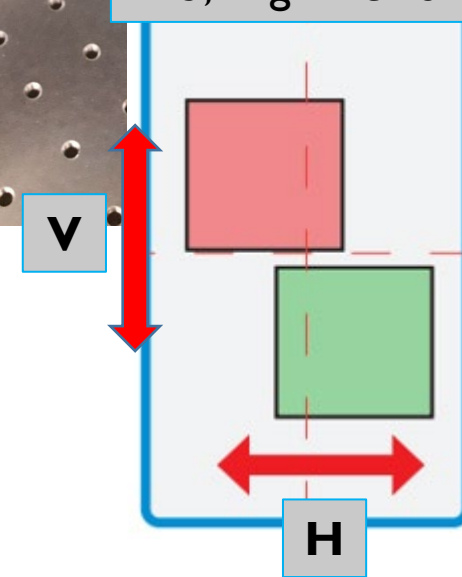
2, Separate Fields (anti-clockwise)



3, Align Fields

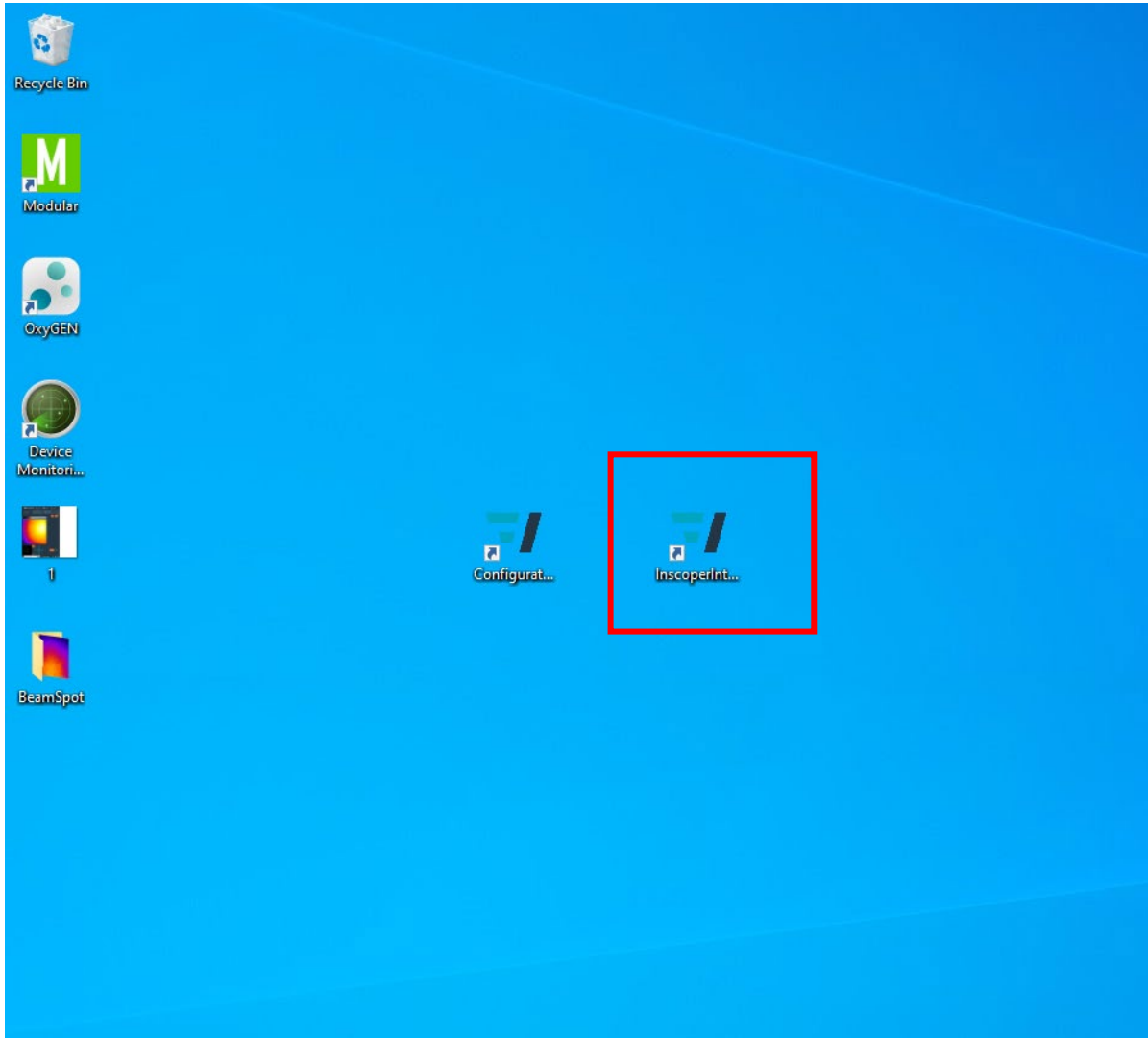


3, Align Fields

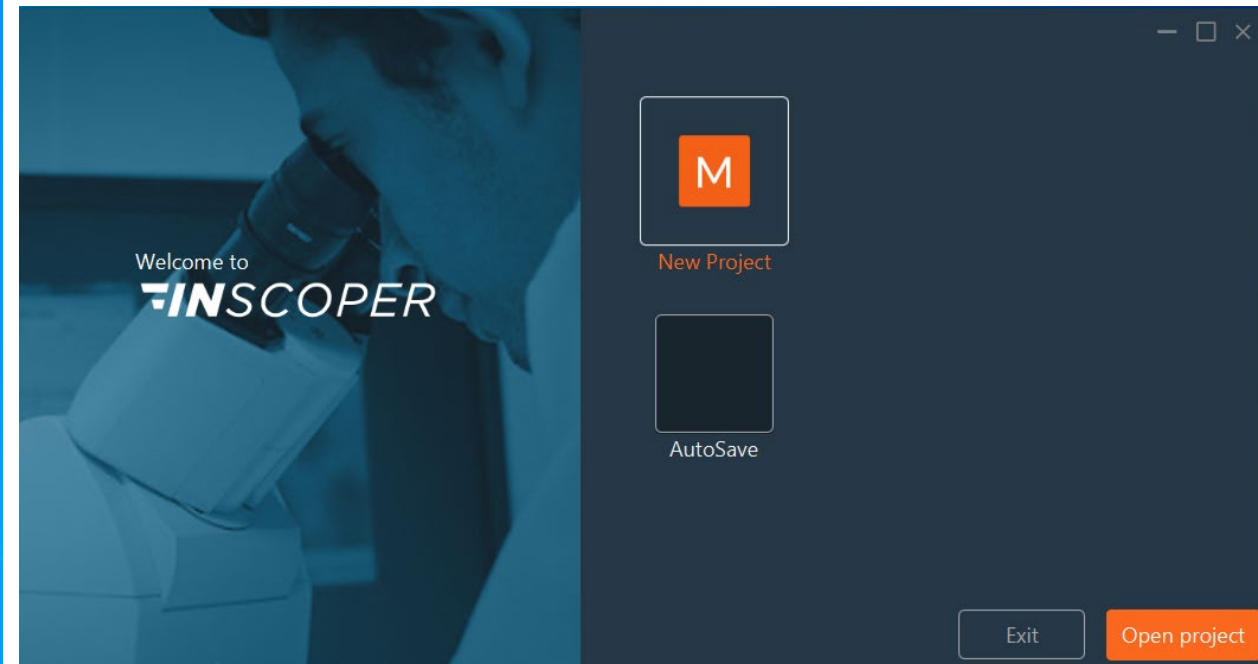


INSCOPER

Open the Inscoper interface



Chose “new project” and name it as you want. You can recall this later



INSCOPER interface- configuration tab
This is used to define and optimize your imaging parameters

INSCOPER

Project

Settings

Support

User Mode

Configuration

Camera(s)

Microscope and lasers

Camera Settings

Retiga_Camera_0

Exposure (ms)

100

Binning

1x1

Advanced

Snap

Live



Calibration

FRAP

Tirf

Tiling

Go to

iLas2

NikonTi2

TIRF Profiles

Channel Modality

iLas2

NikonTi2

iLas2

TIRF Density

1500

FRAP Density

5

Dwell Time (us)

300

Fire Preview

NikonTi2

Focus

1868.68

Step (μm)

0.001

pfsOffset

0

Step (μm)

0.025

pfs

xAxis (μm)

9711.9

yAxis (μm)

620.4

Step (μm)

0.1

Objective

100x

TIRF Profiles

Current User

Michal

FPS : 0.00

Fire on Click

Edit Chart

Crop Image

Go To Acquisition

INSCOPER interface- configuration tab
Channel modality:
Chose your optical path- transmitted/TIRF/FRAP

TIRF Profiles Current User Michal

Channel Phase Retiga Add

Channel Modality TIRF FRAP Brightfield TIRF/FRAP

iLas2

Sample View 0

Penetration Depth 0 Widefield

Mode Orbital Azimutal 0 Segmental

405 Power 0.00 561 Power 0.00

Go To Acquisition

In transmitted light, the shutter for BF is open, and you work in WF mode. All lasers are off and their shutter is closed

TIRF Profiles Current User Michal

Channel 488 Azimutal TIRF Add

Channel Modality TIRF FRAP Brightfield TIRF/FRAP

iLas2

Sample View 87

Penetration Depth 98 Widefield

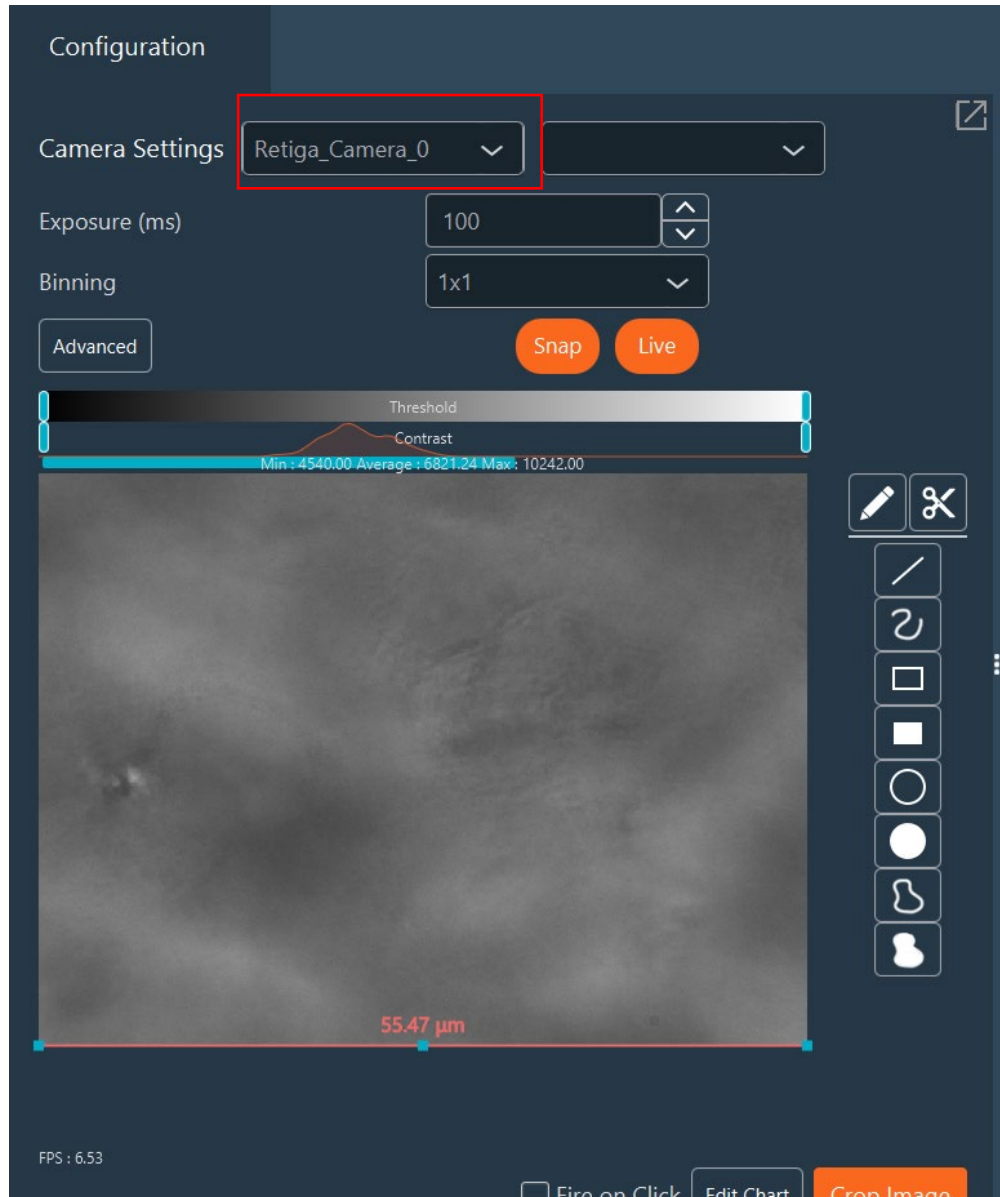
Mode Orbital Azimutal 360 Segmental

405 Power 0.00 561 Power 0.00

Go To Acquisition

In TIRF mode, the shutter for BF is closed, the iLAS shutter is open and you can chose your excitation line (next slides). FRAP does not pass through (separate explanation later)

INSCOPER interface- configuration tab
*You need to actively chose the **camera** and adjust exposer time*



	Advanced Settings	In the Advanced Settings , you can adjust the properties of the camera and filter them according to what you are looking for.
	Snap	You can view the sample live to have a video preview or take a snap.
	Live / Stop	
	Measuring Tool	You can use this tool to see the scale of the picture and measure anything you want in your sample by moving the blue squares.
	Hand Tool	You can move the X/Y axis of the camera by using the Hand Tool to click and drag to reach the observation area.
	Crop The Screen	You can use this function to keep only what you need in your acquisition.
	Contrast	This button allows you to choose whether you wish to set the contrast automatically or manually. If you wish to set it manually, adjust the blue sliders on top of the camera view.
	Full Screen Mode	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.
	LUT	You have three LUT options: <ul style="list-style-type: none"> • select no LUT • choose any color • choose preset colors.
	Coordinates	This function displays the X/Y coordinates with display intensity.


INSCOPER interface- configuration tab


TIRF illumination

Set desired laser power and penetration depth (when in good focus)

iLas2

Sample View  87

Penetration Depth  **Wide Field** **TIRF** 98

Mode  **Point** **Circle** 360

405 Power 0.00 561 Power 0.00

638 Power 0.00 488 Power 20.00

INSCOPER interface- configuration tab
TIRF calibration
to get good signal, you might need to adjust TIRF profile*

1, click on "Tirf" calibration

2, make sure to be on Azimutal TIRF in your desired penetration depth

3, add points (4 min) and move them around by standing on each one and separately changing its location in the BFP of objective to get best signal.

4, when satisfied, click "confirm"

TIRF profile should be incorporated into software to save and upload more easily. Work in progress

INSCOPER interface- acquisition tab

The screenshot displays the INSCOPER software interface. At the top, there are navigation icons for Project, Settings, and Support, along with an Expert Mode toggle. The main interface is divided into several sections:

- Configuration:** Includes Camera Settings (iXon897_0, FULL_FOV), Internal Shutter Open Mode (Auto), Exposure (ms) (200), Gain (4), and Binning (1x1). There are also Snap and Live buttons.
- Advanced:** A section with a Threshold and Contrast slider, and a graph showing Min: 1514.00, Average: 8319.51, and Max: 32655.00.
- Acquisition List:** A list of five steps: 1. Time, 2. Positions (with a Switch to Tiling button), 3. Z-Stack, 4. Multi-Channels, and 5. Photomanipulation. Each step has a red circle, a '0', a pencil icon, and a crosshair icon.
- Data Processing and Charts:** Includes a dropdown menu, Edit Parameters, and Switch to DataProcessor buttons.
- Save Acquisition:** Options for saving in RAM or on Disk, a file path field (C:\Inscoper\Tests), Format (All in One), and Metadata Format (All in One) dropdowns. There is also a checkbox for Save as BigTiff.
- Summary:** Total images: 1, Total size: 524kB, Minimal duration: 00h00min00s200ms. A Start Acquisition button is present.

By clicking on each acq parameter circle will turn to blue meaning "active". Pencil means "edit". Arrows allow you to change acq order

You can view your image while adapting acq parameters

Chode your saving path. If you save in RAM, you need to actively save to disc after

When finished, click to start acquisition

INSCOPER interface- acquisition tab

Time

1. Time

● 0

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 allows you to image continuously as fast as exposur time

Burst Mode

Confirm

INSCOPER interface- acquisition tab

Positions

2. Positions Switch to Tiling ● 2

XY ▲ xAxis (μm) ▲
▼ yAxis (μm) ▲
Step (μm)

▲ Focus ▲
▼ Step (μm)

▲ pfsOffset ▲ pfs
▼ Step (μm)

Well Plate
Pattern detection

Move Strategy ▼

N°	Tag	xAxis	yAxis	<input checked="" type="checkbox"/> Focus	<input checked="" type="checkbox"/> pfsOffset	<input checked="" type="checkbox"/> pfs	Move to	Copy	Get	
1		17444.1	-3406.2	<input checked="" type="checkbox"/>	4.17	<input checked="" type="checkbox"/>	<input type="button" value="Move to"/>	<input type="button" value="Copy"/>	<input type="button" value="Get"/>	<input type="button" value="X"/>
2		17508.9	-3407.1	<input checked="" type="checkbox"/>	5.62	<input checked="" type="checkbox"/>	<input type="button" value="Move to"/>	<input type="button" value="Copy"/>	<input type="button" value="Get"/>	<input type="button" value="X"/>

If you use PFS, otherwise turn off

*INSCOPER interface- acquisition tab
Z stack*

You can either use Z or PFS. Define in “Config” sub tab with acq tab

Sequence 1 Take Image Config Add Show All

1. Time ● 100

2. Positions Switch to Tiling ● 2

3. Z-Stack ● 1

▲ pfsOffset (μm) ▲
▼ Step (μm)

Min/Max Plane Center Plane

Min (μm) ... Center (μm) ... Stack Step (μm) Nyq...

Max (μm) ... Volume (μm) Stack Size

Center First

Confirm

*INSCOPER interface- acquisition tab
Multi channel
Add channels to sequential acquisition*

4. Multi-Channels

Live

561 Azimutal TIRF

Stop



1

Add Channel

Remove All

	N°	Name	Z Stack	Z Offset	Shutter Blink ⁱ	Camera	Exposure (ms)	
-	1	561 Azimu... ^v	<input checked="" type="checkbox"/>	0 [^] ^v	<input type="checkbox"/>	iXon897_0 ^v	200	^x
<input checked="" type="checkbox"/>		405 Power		0.00 [^] ^v				
<input checked="" type="checkbox"/>		561 Power		100.00 [^] ^v				
<input checked="" type="checkbox"/>		638 Power		0.00 [^] ^v				
<input checked="" type="checkbox"/>		488 Power		0.00 [^] ^v				

Confirm

