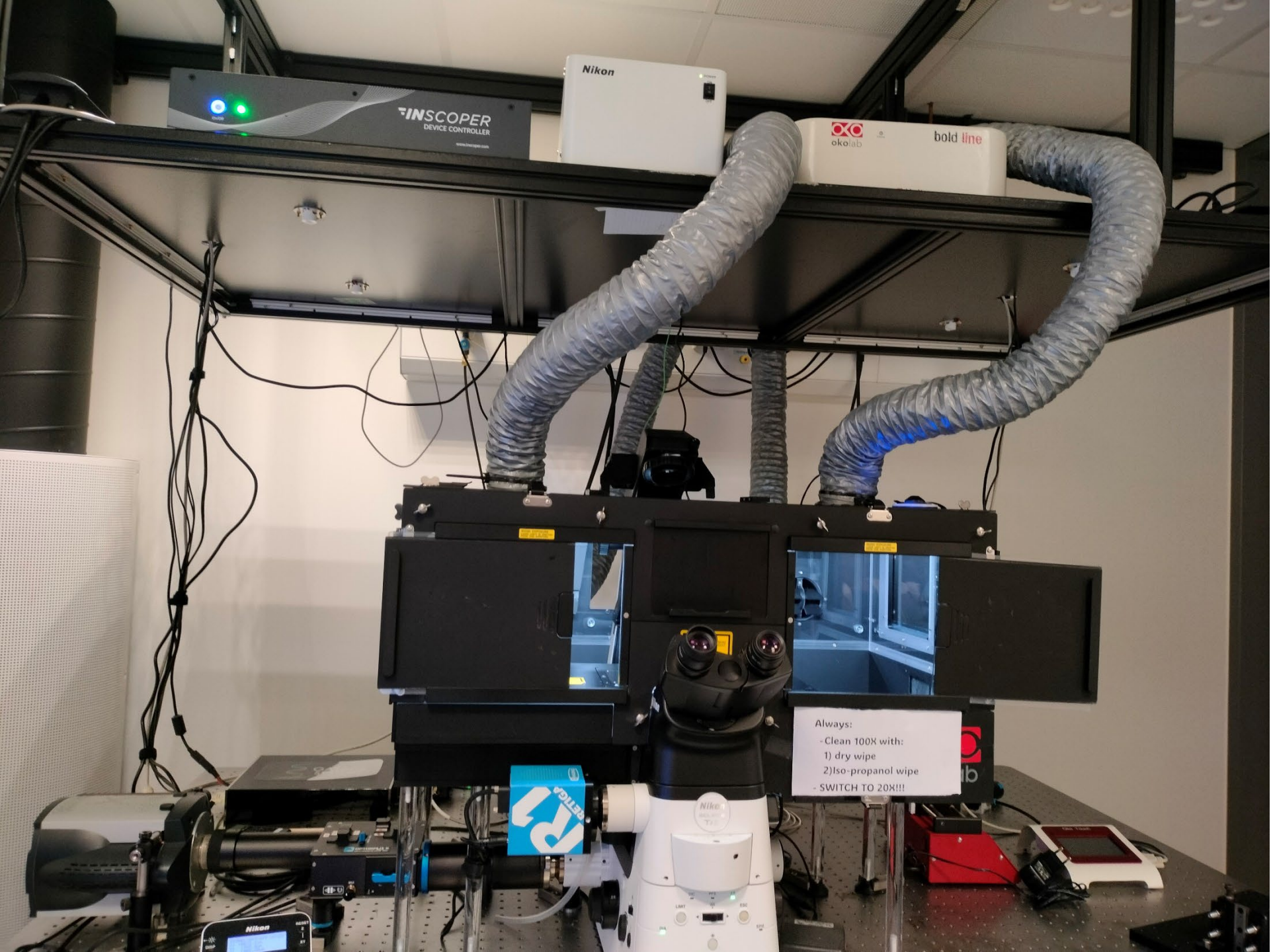


iLAS_TIRF INSCOPER

User guide

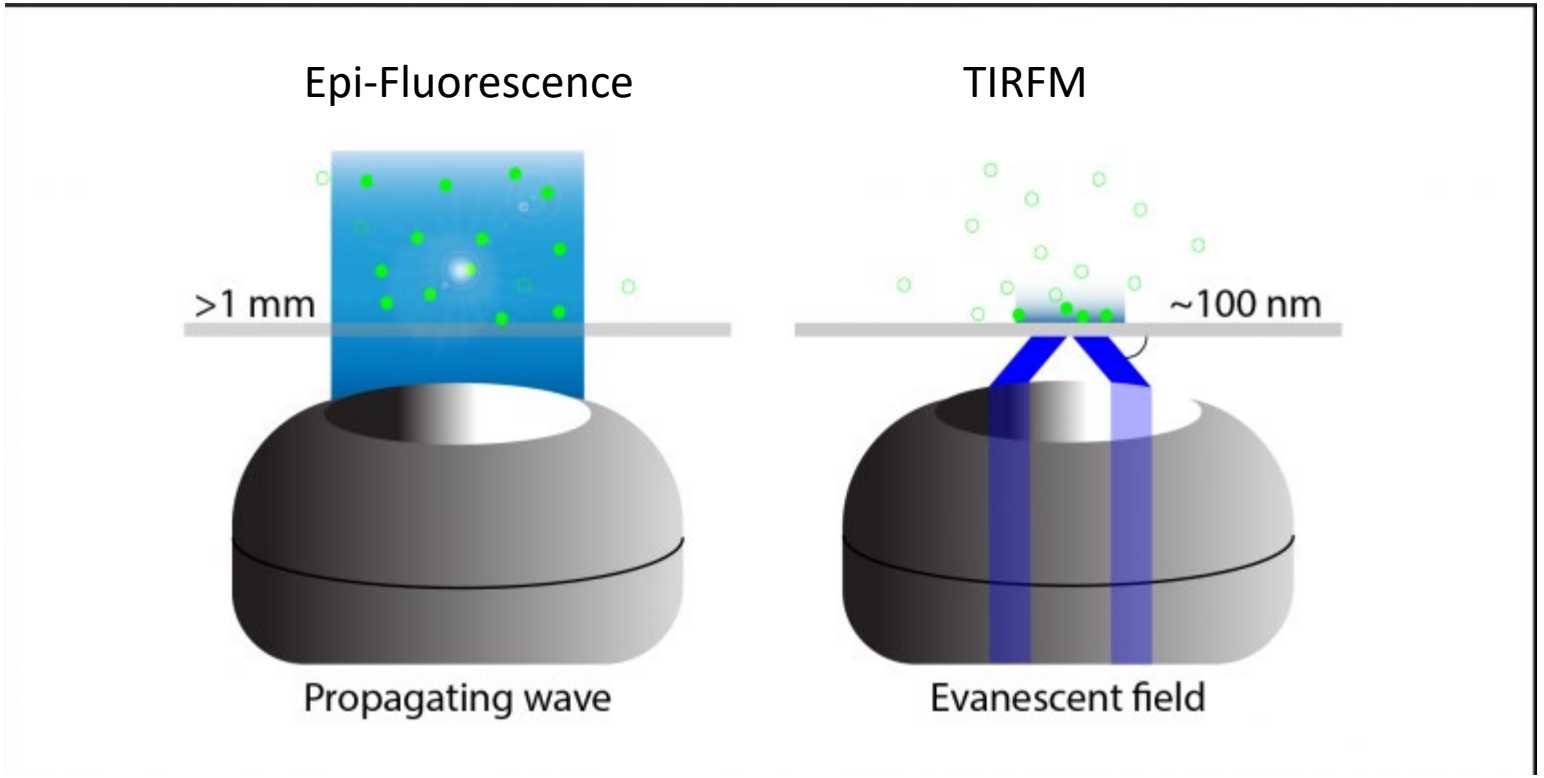
Kavli Nanolab Imaging Centre

Snell's law for the critical angle of light

$$\theta c = \sin^{-1}(n1/n2)$$

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



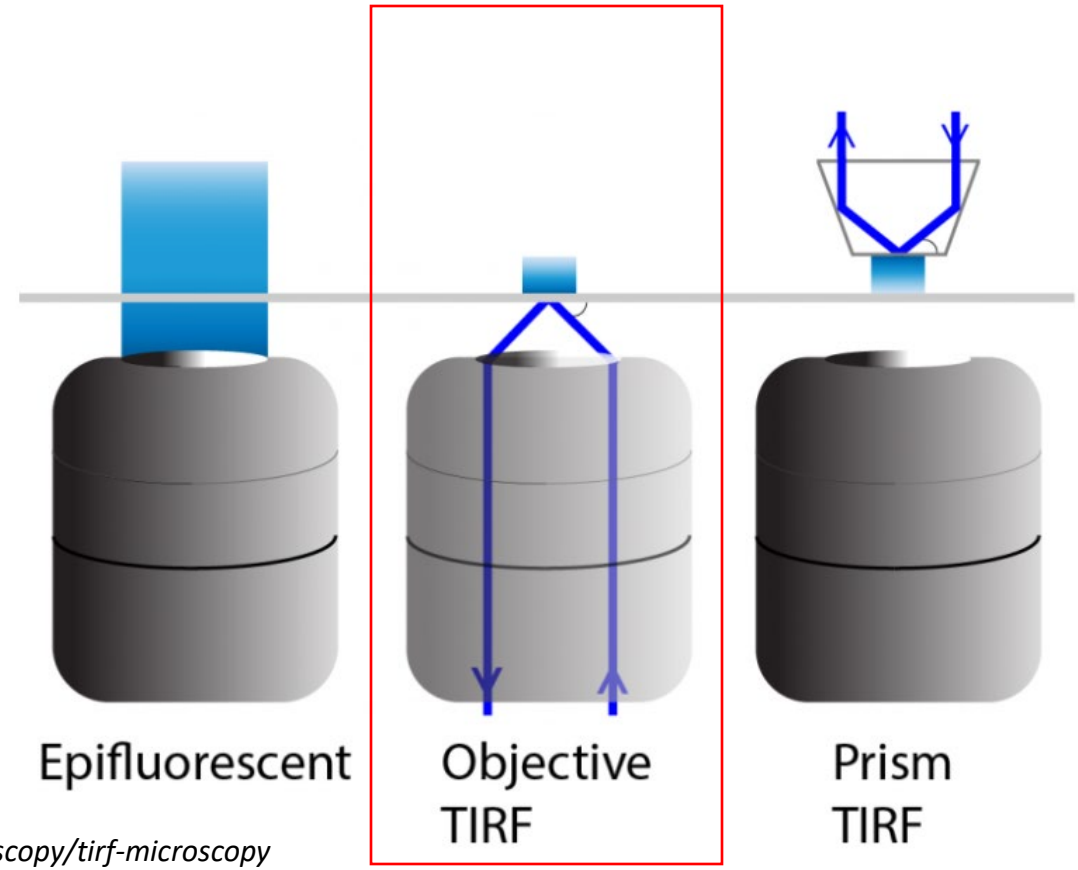
https://www.photometrics.com/learn/single-molecule-microscopy/tirf-microscopy

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



https://www.photometrics.com/learn/single-molecule-microscopy/tirf-microscopy

StartUp:

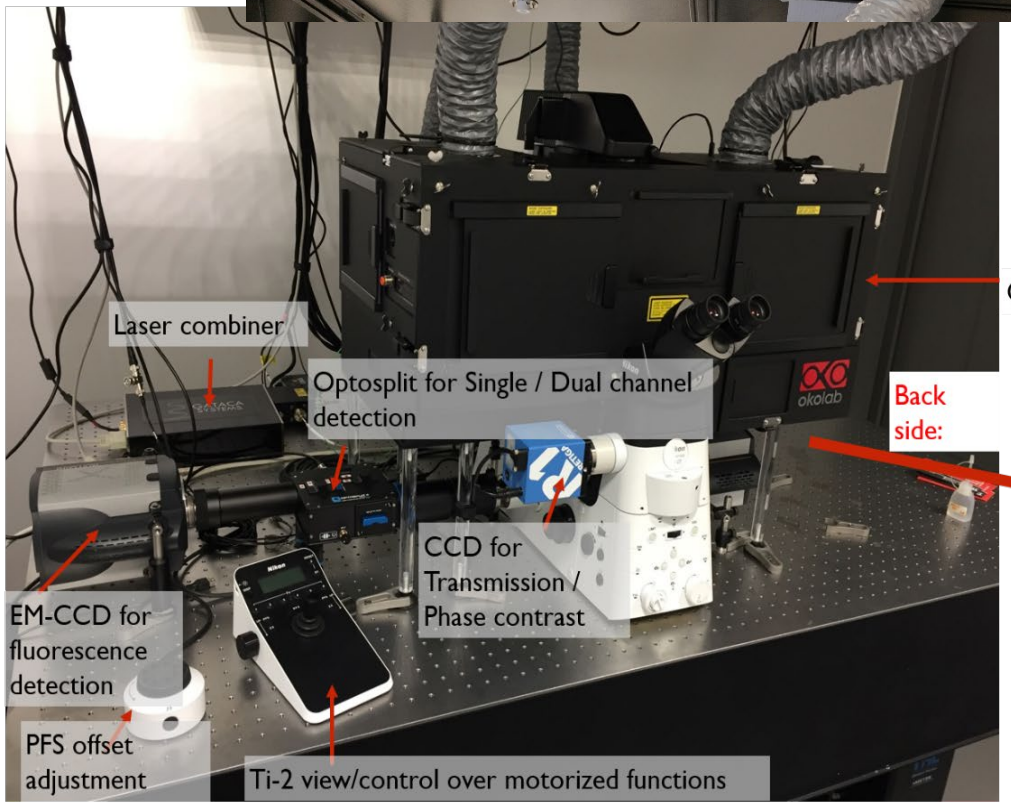
1, Turn ON the 2 main switches
2, Turn ON INSCOPER box
3, Turn ON PC and INSCOPER software
4, Locate your sample (BF only):
    1, place oil on 100X objective, place path to eyepiece
    2. Turn on BF light and turn the focus knob to move objective UP until the oil spreads on the coverglass (Slow!)
    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

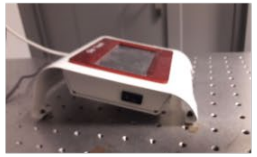
Shut-Down:

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:



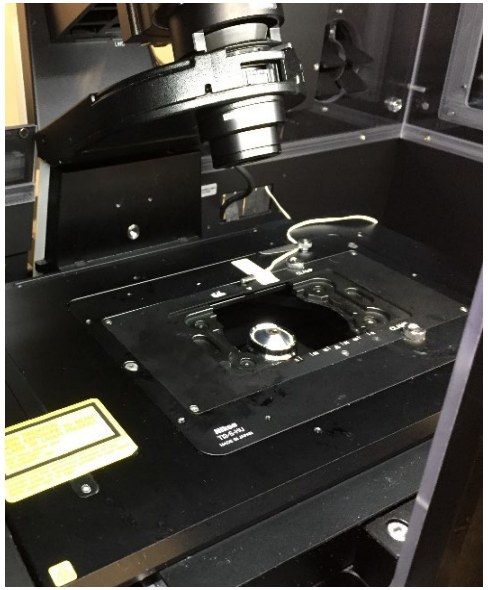


OkoLab incubation box

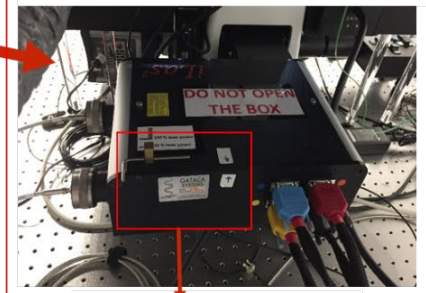
2

PC

3

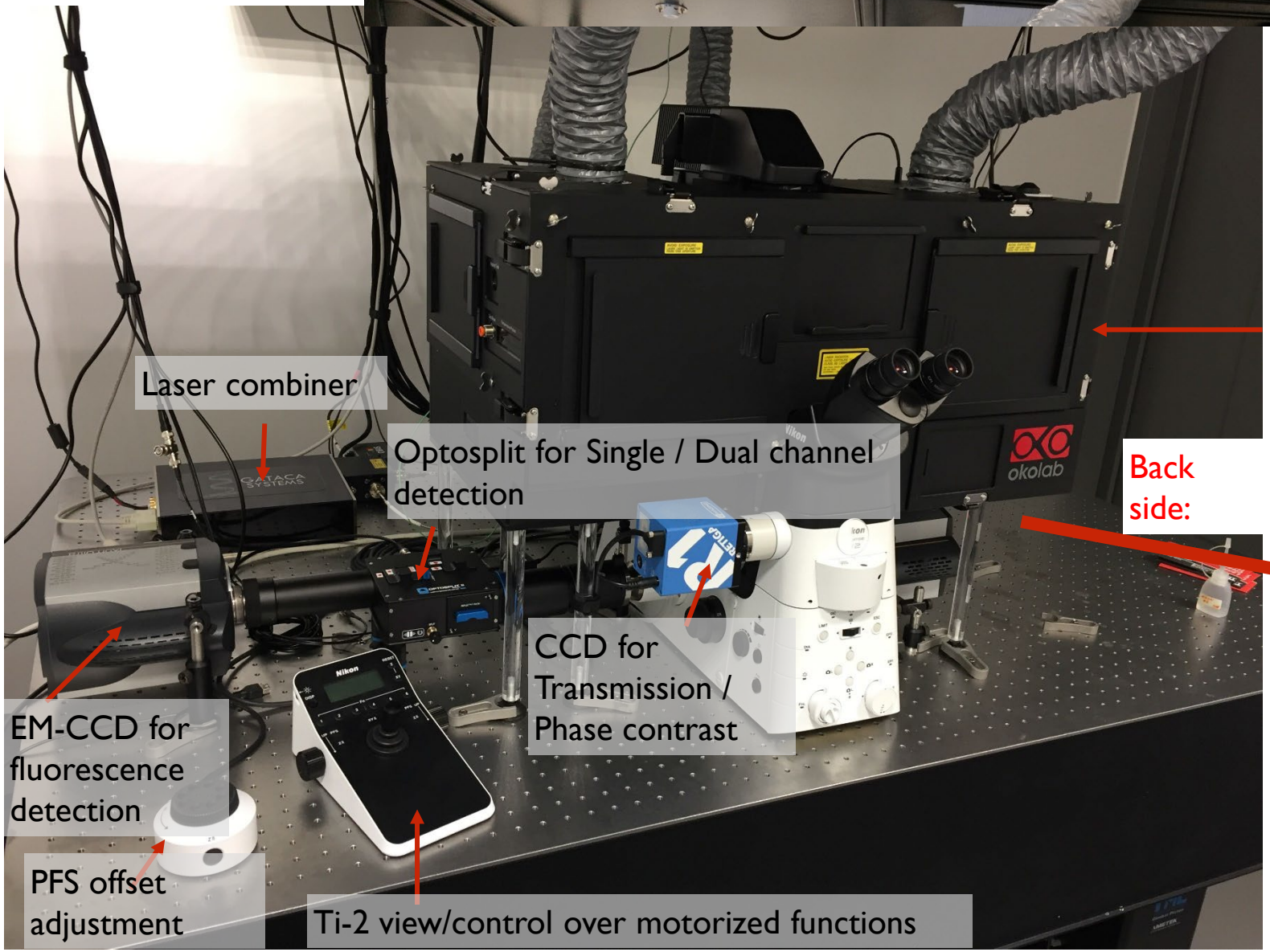


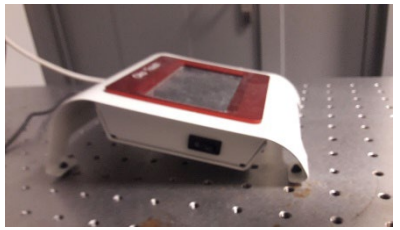
Optical fibers for TIRF and FRAP laser illumination



Laser power range adjustment

Hardware- General:

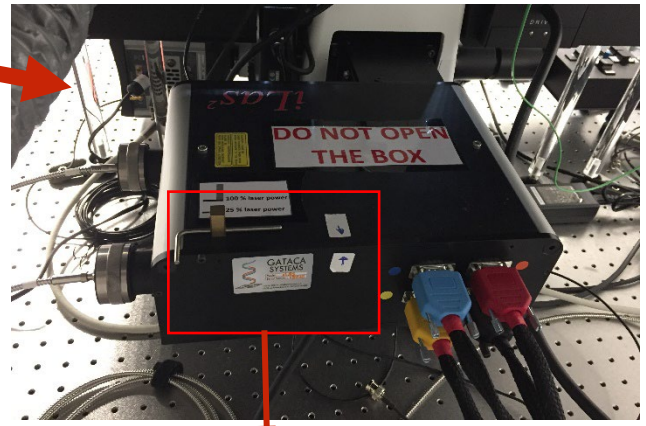




OkoLab incubation box

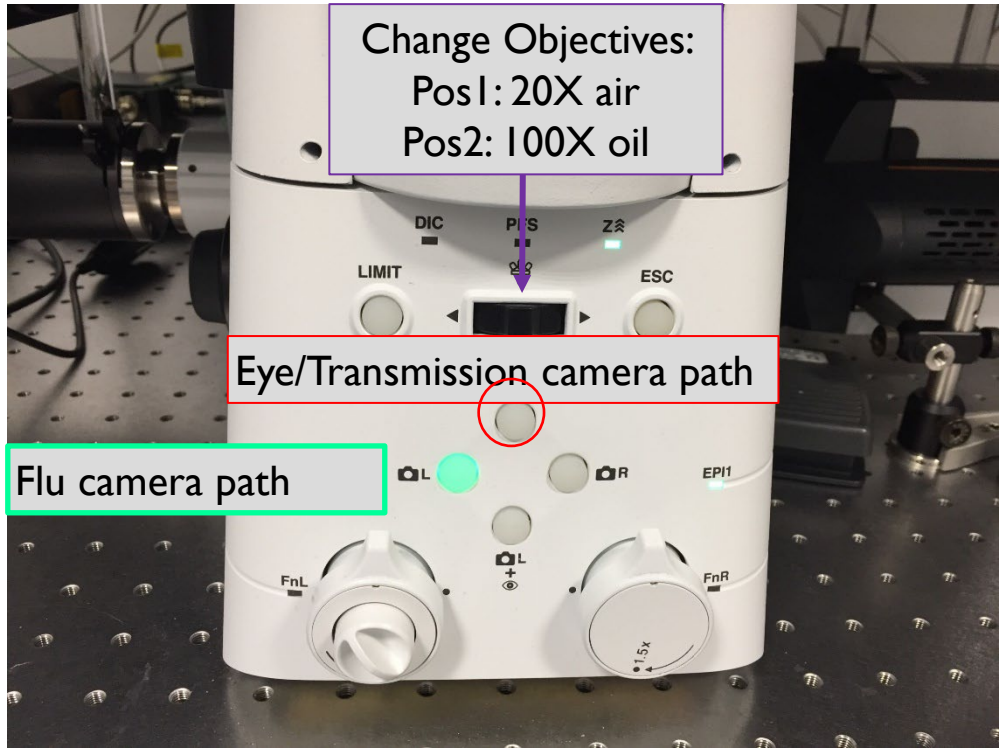
Back side:

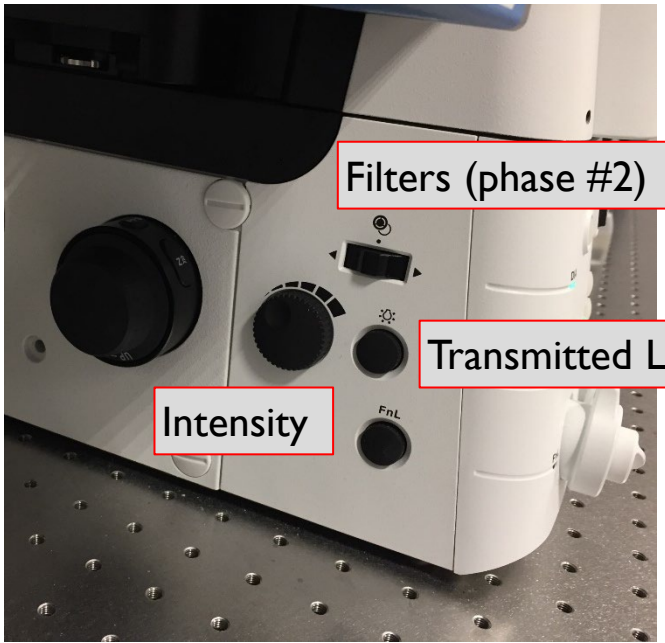
Optical fibers for TIRF and FRAP laser illumination



Laser power range adjustment

Ti-2 body:





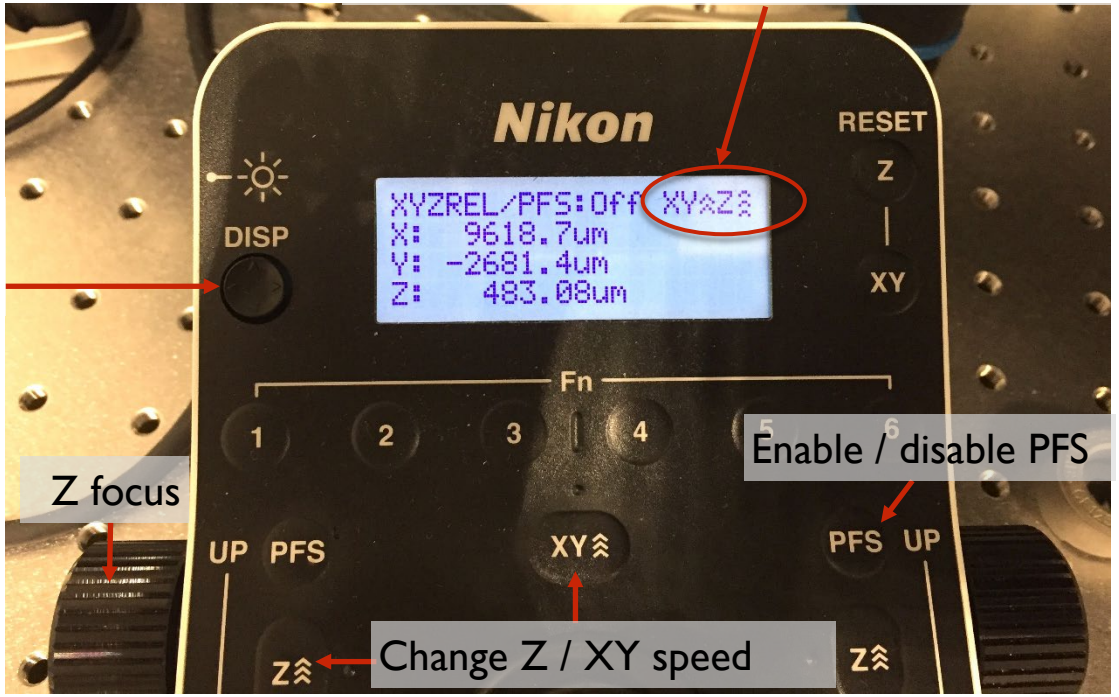
Turn PFS ON

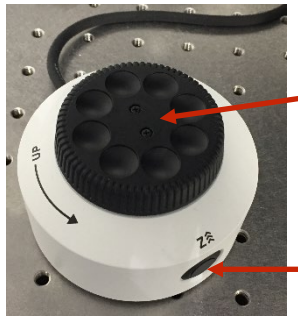
Turn on light in the OKO cage

Ti-2 Joystick

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

Change information display



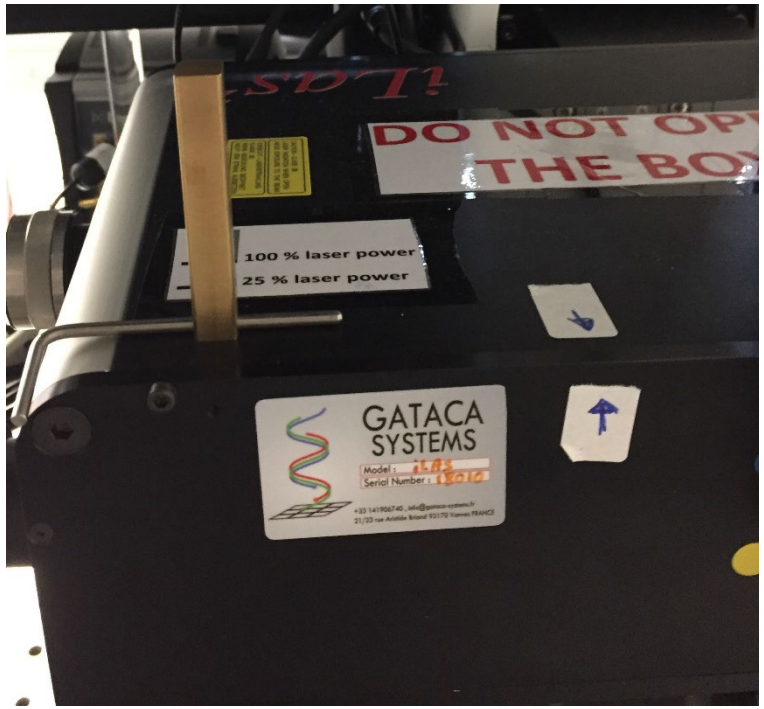


When the PFS is enabled, use this knob to adjust the focus

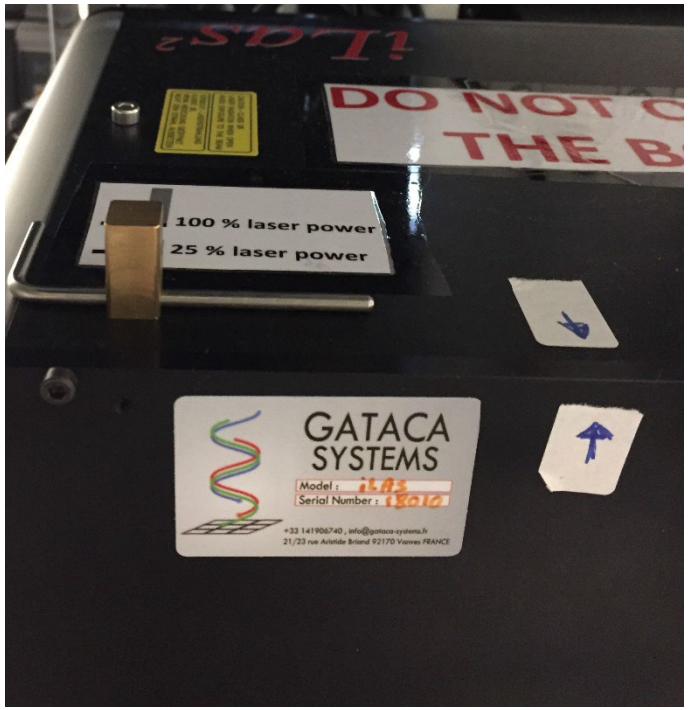
Keep pressing this button to speed up the focus adjustment

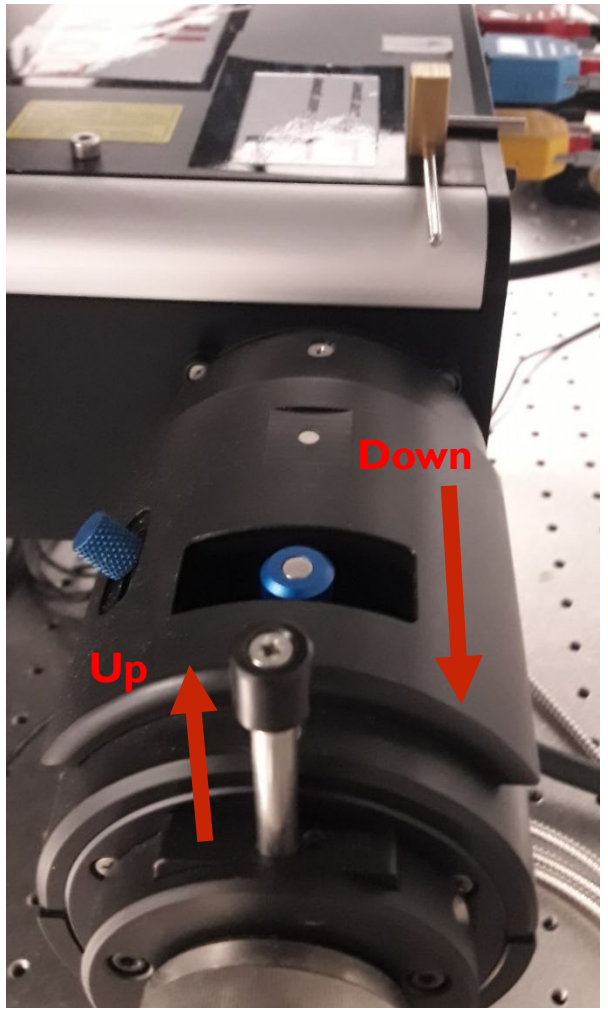
To modify laser intensity- Hardware

High laser Intensity



Low laser Intensity

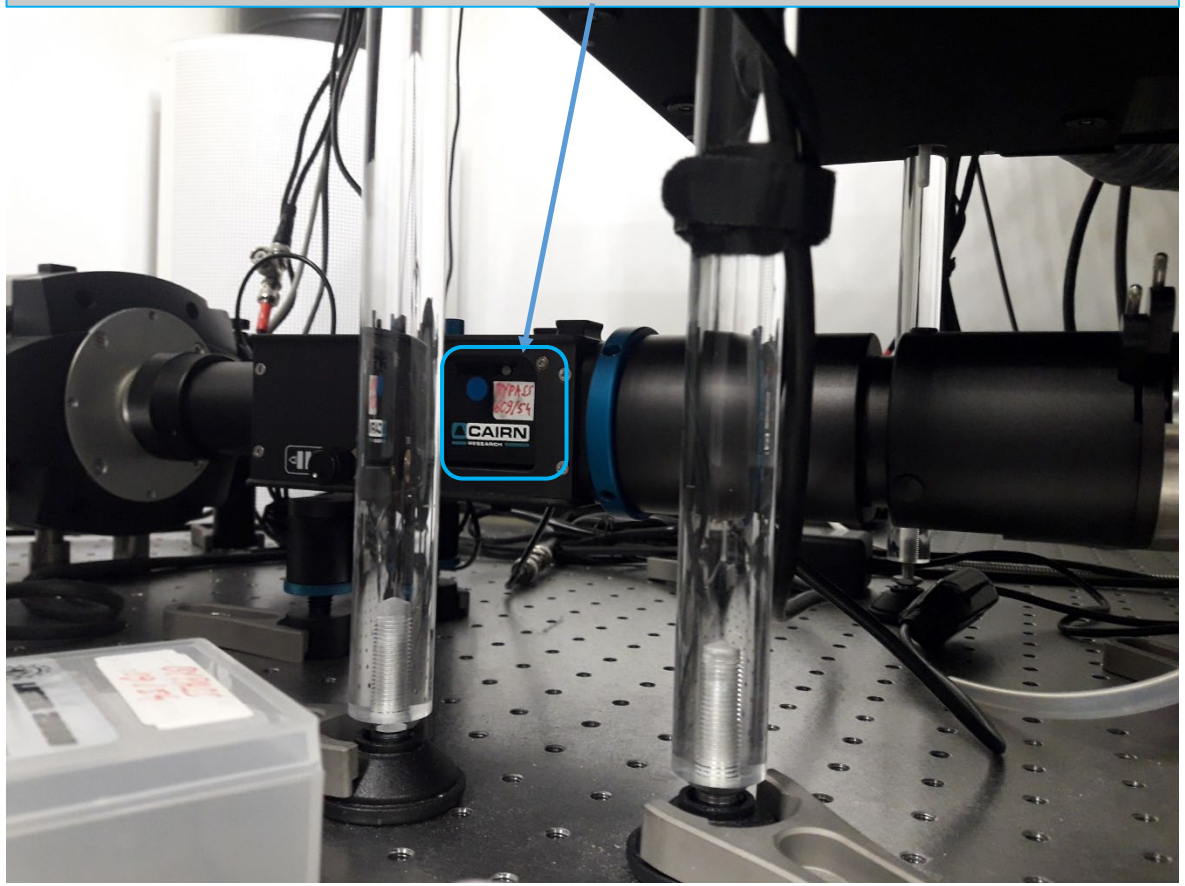




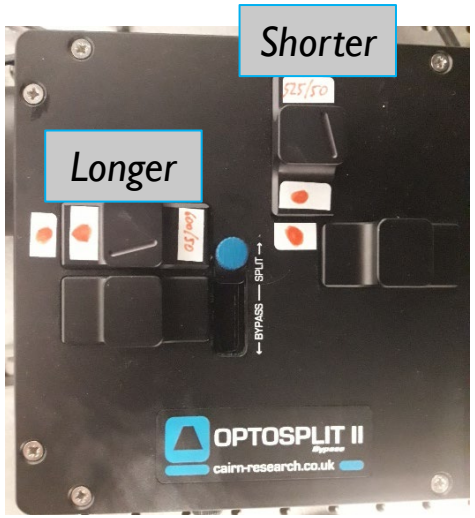
Optosplit for single/dual flu imaging- Emission path

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

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

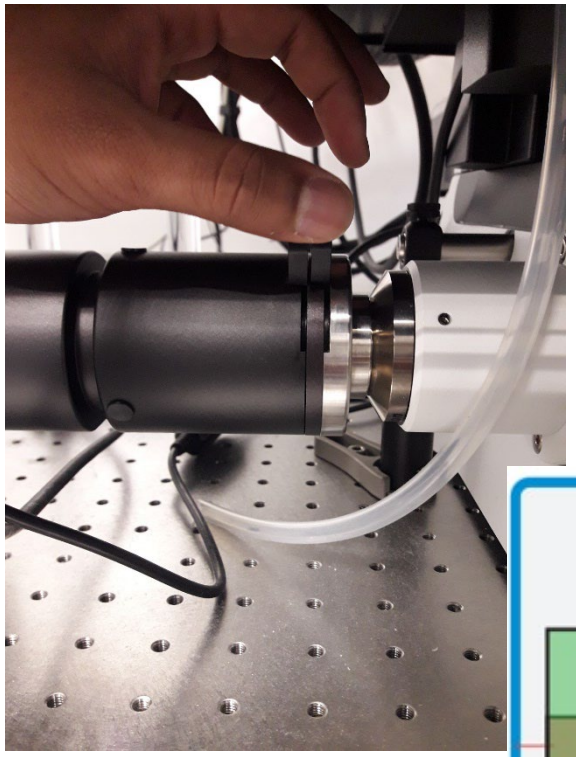


Split filters

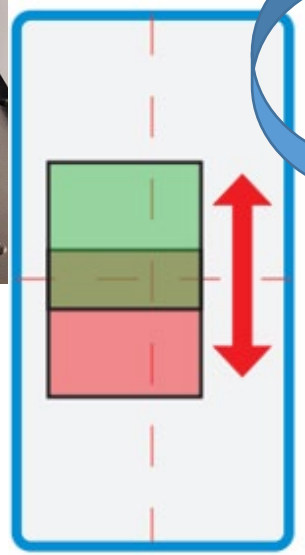


Optosplit for single/dual flu imaging- Alignment

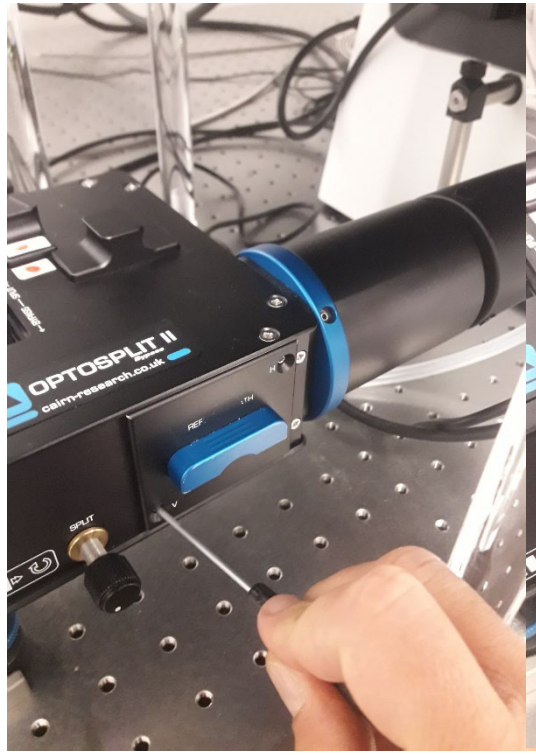
1, Use the knobs to reduce Field size

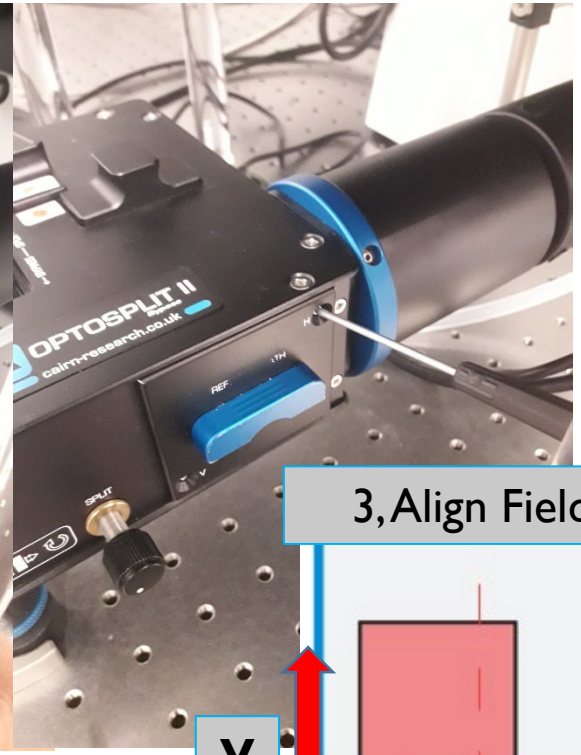


2, Separate Fields (anti-clockwise)

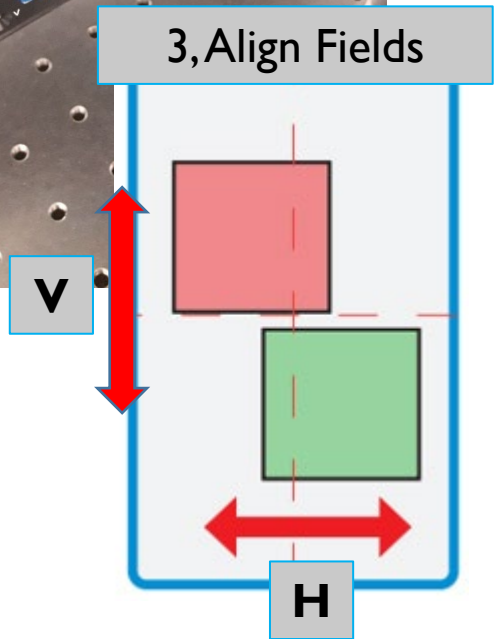


3, Align Fields



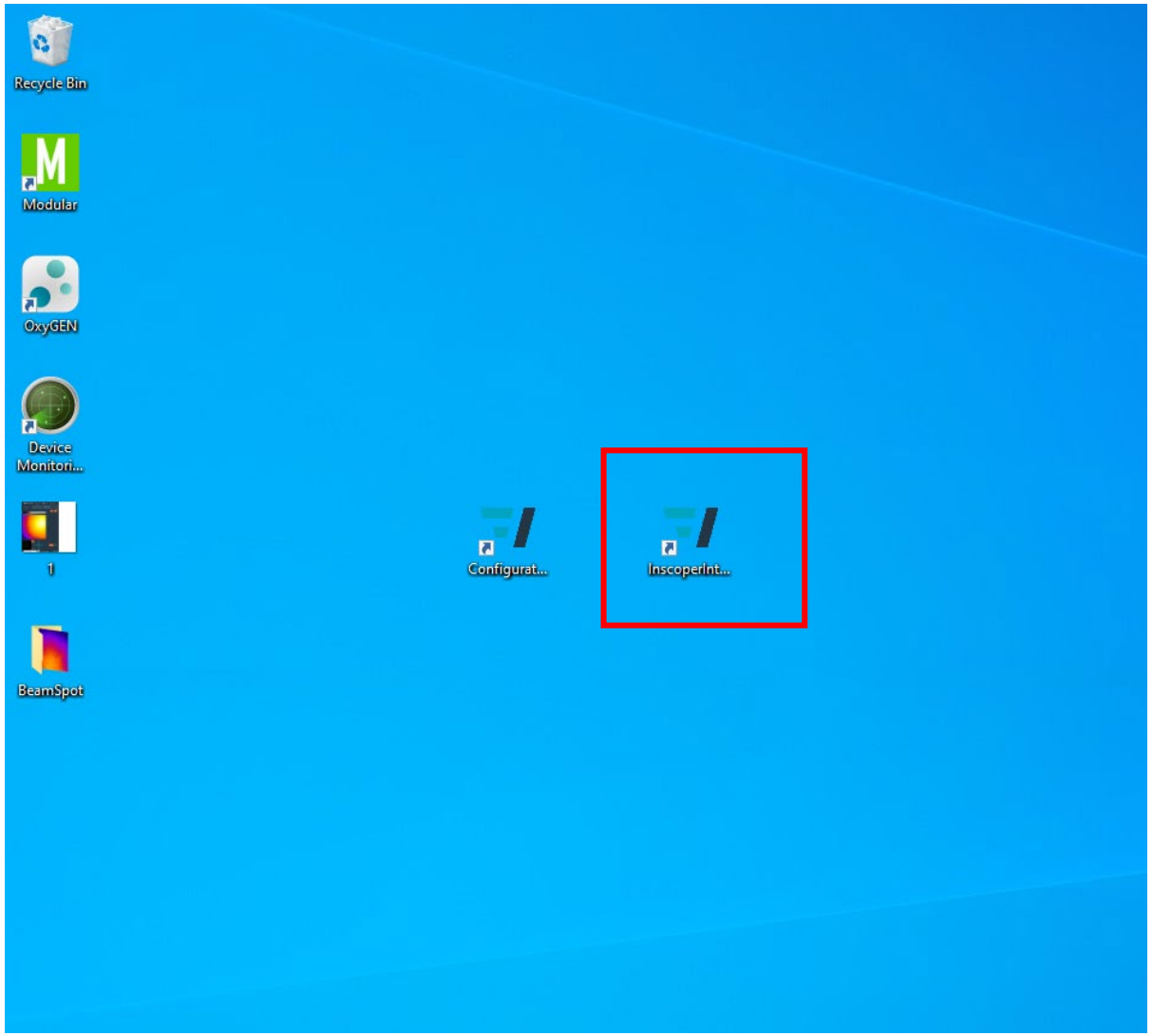


3, Align Fields

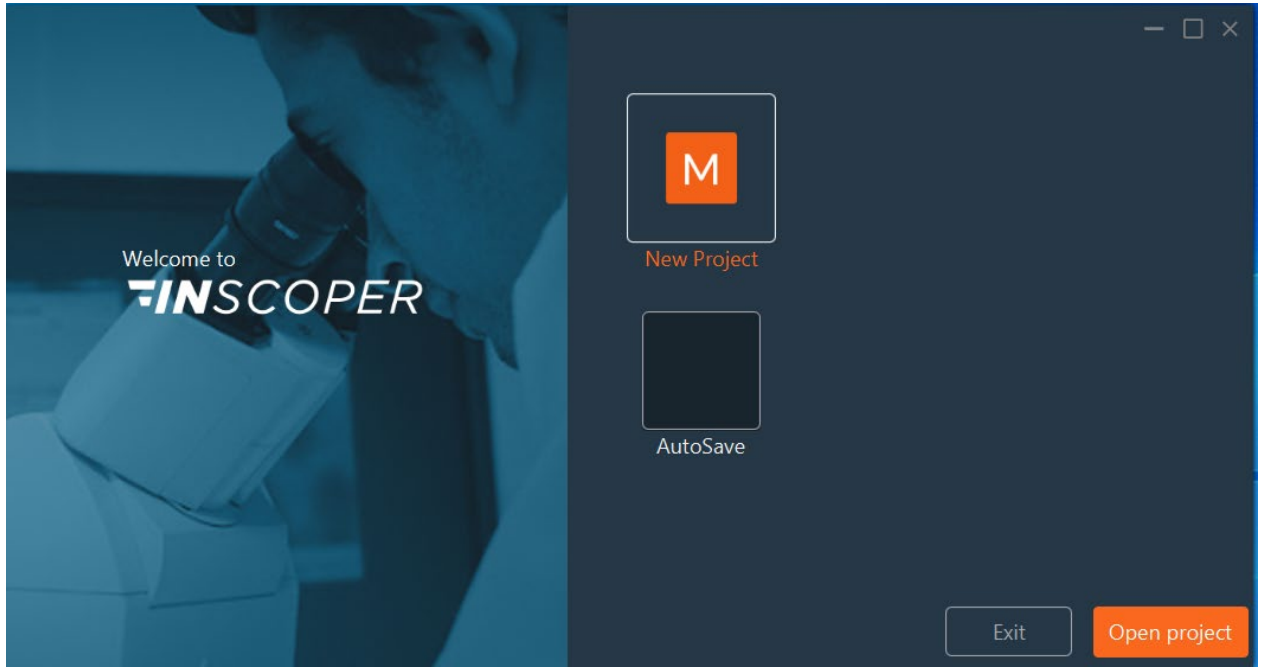


INSCOPER

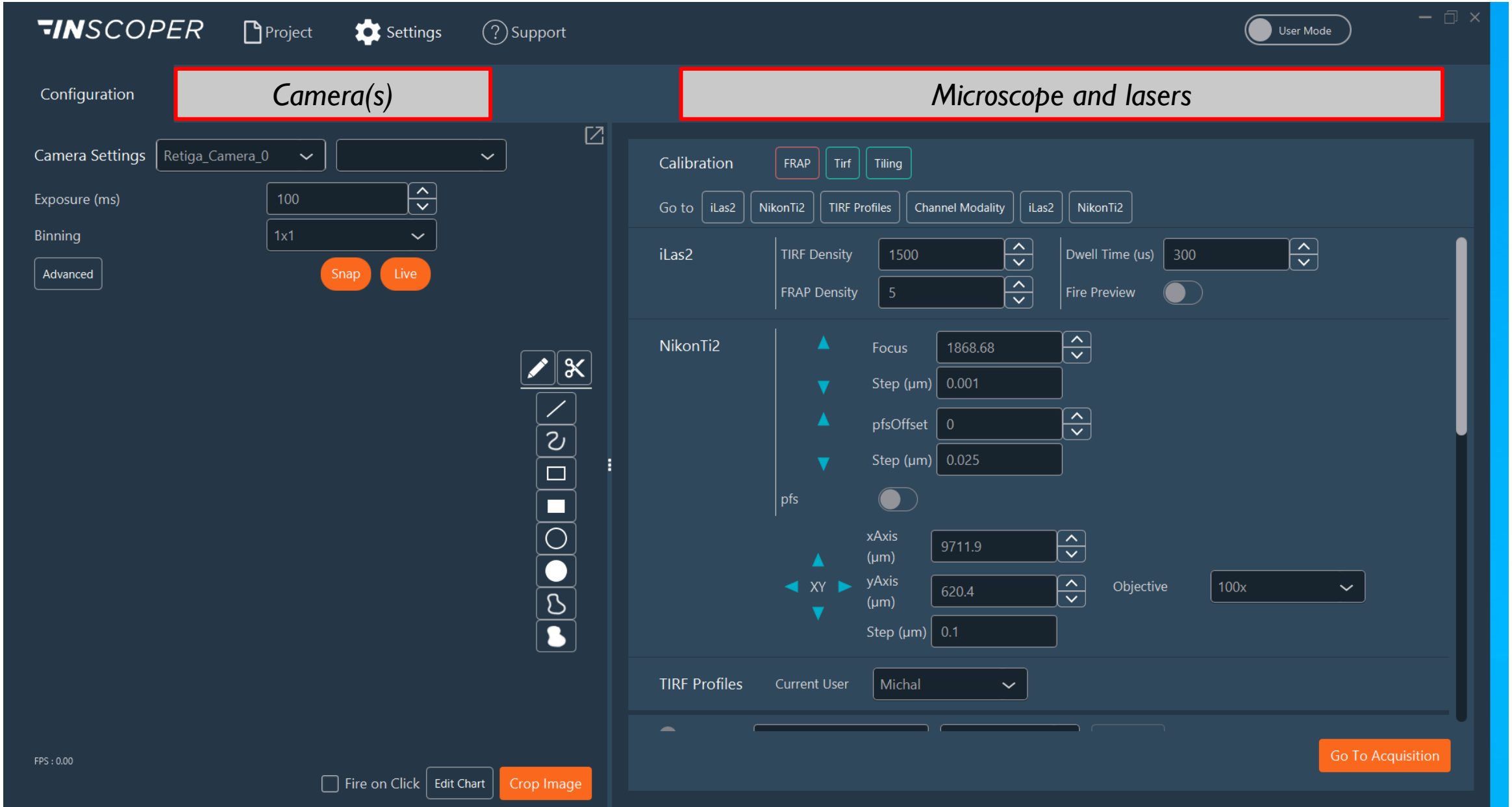
Open the Inscoper interface



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



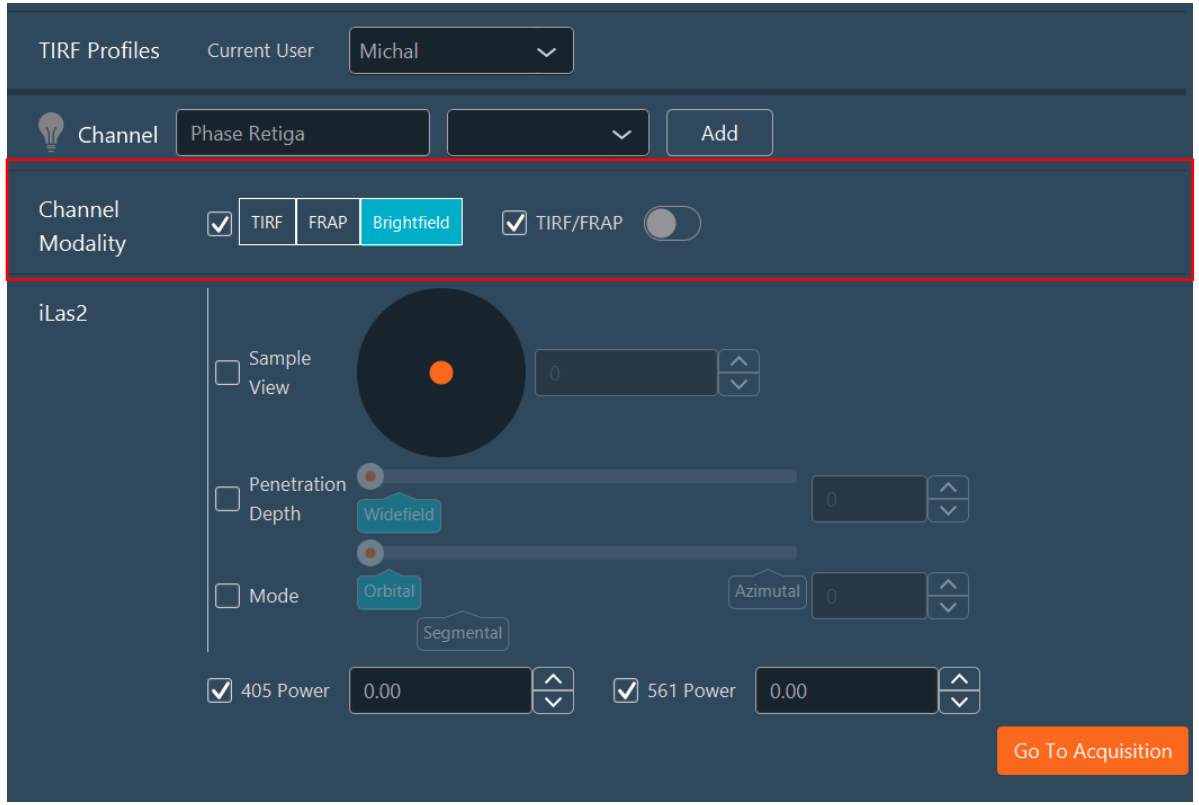
INSCOPER interface- configuration tab
This is used to define and optimize your imaging parameters



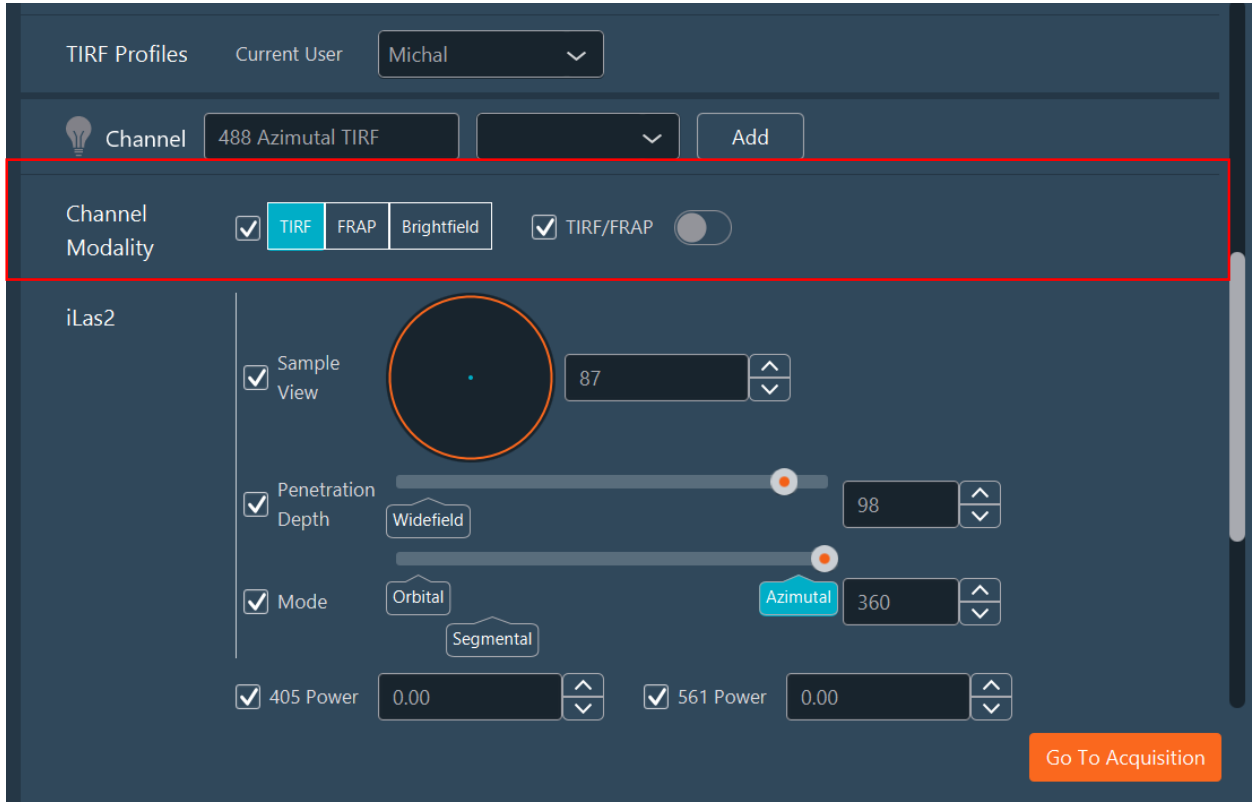
INSCOPER interface- configuration tab

Channel modality:

Chose your optical path- transmitted/TIRF/FRAP

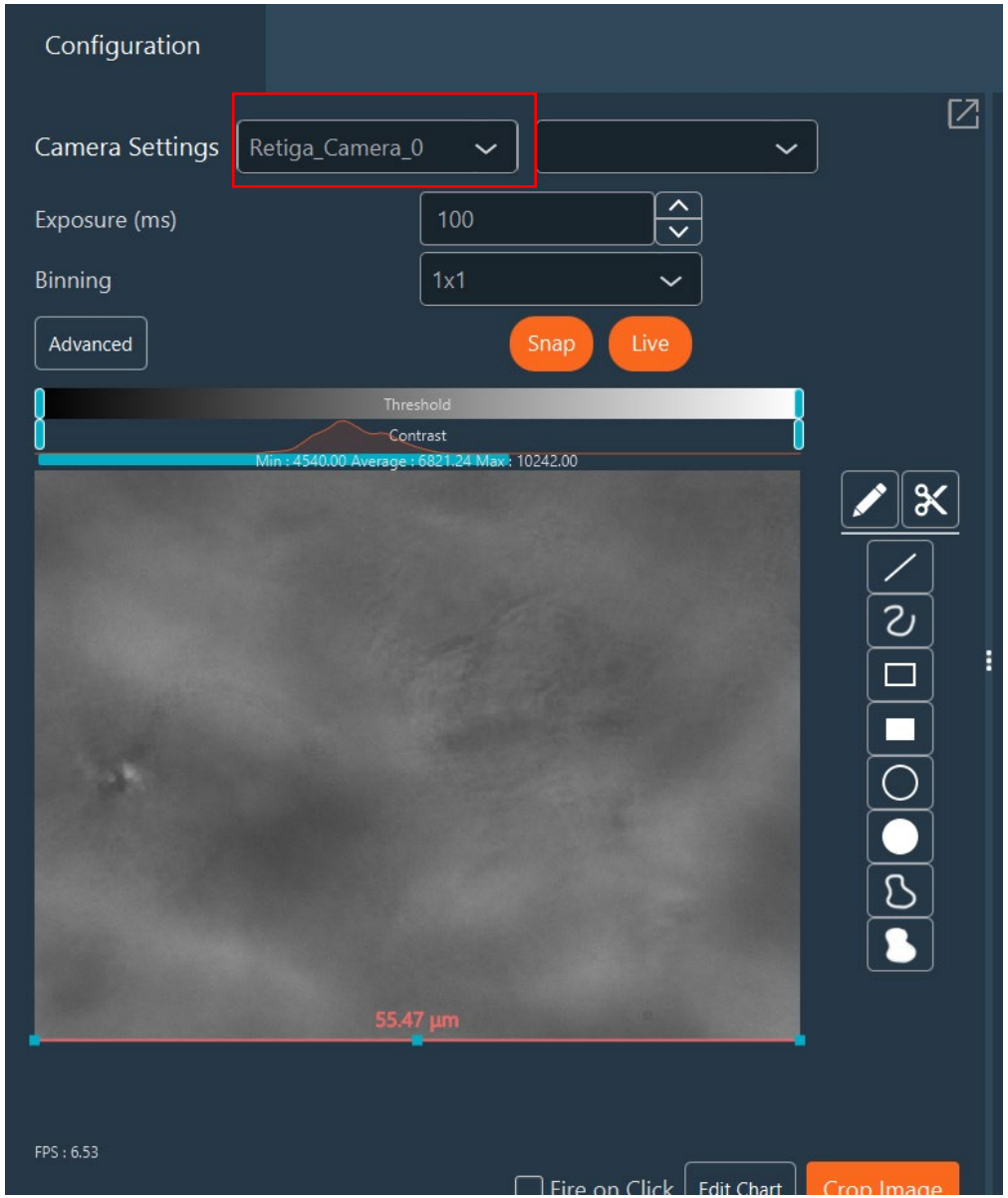


In transmitted light, the shutter for BF is open, and you work in WF mode. All lasers are off and their shutter is closed



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*

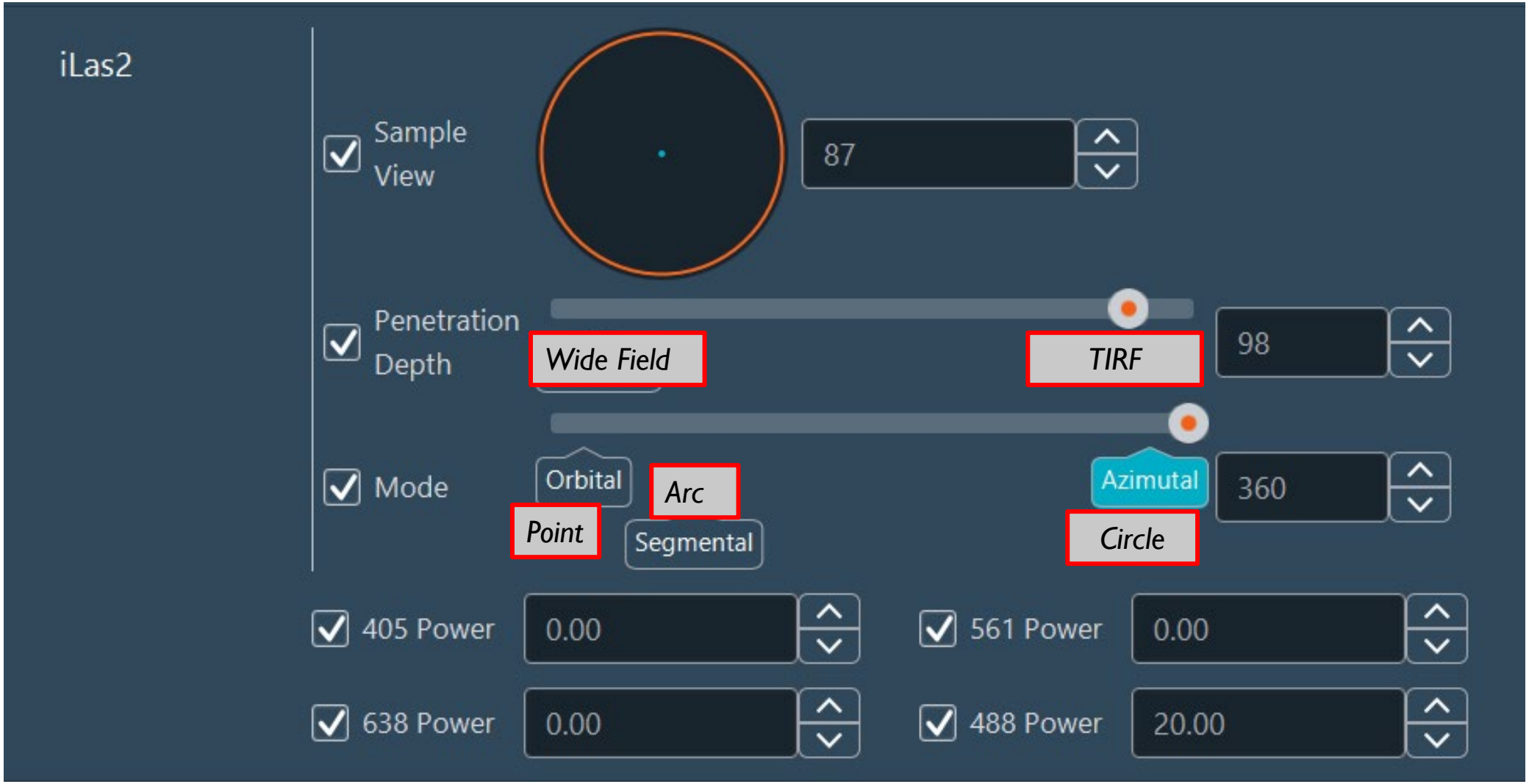


Advanced	**Advanced Settings**	In the **Advanced Settings**, you can adjust the properties of the camera and filter them according to what you are looking for.
Snap Live Stop	**Snap** **Live / Stop**	You can view the sample live to have a video preview or take a snap.
107.56 pixels	**Measuring Tool**	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.
Auto	**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: • select no LUT • choose any color • choose preset colors.
476,251) = 64	**Coordinates**	This function displays the X/Y coordinates with display intensity.

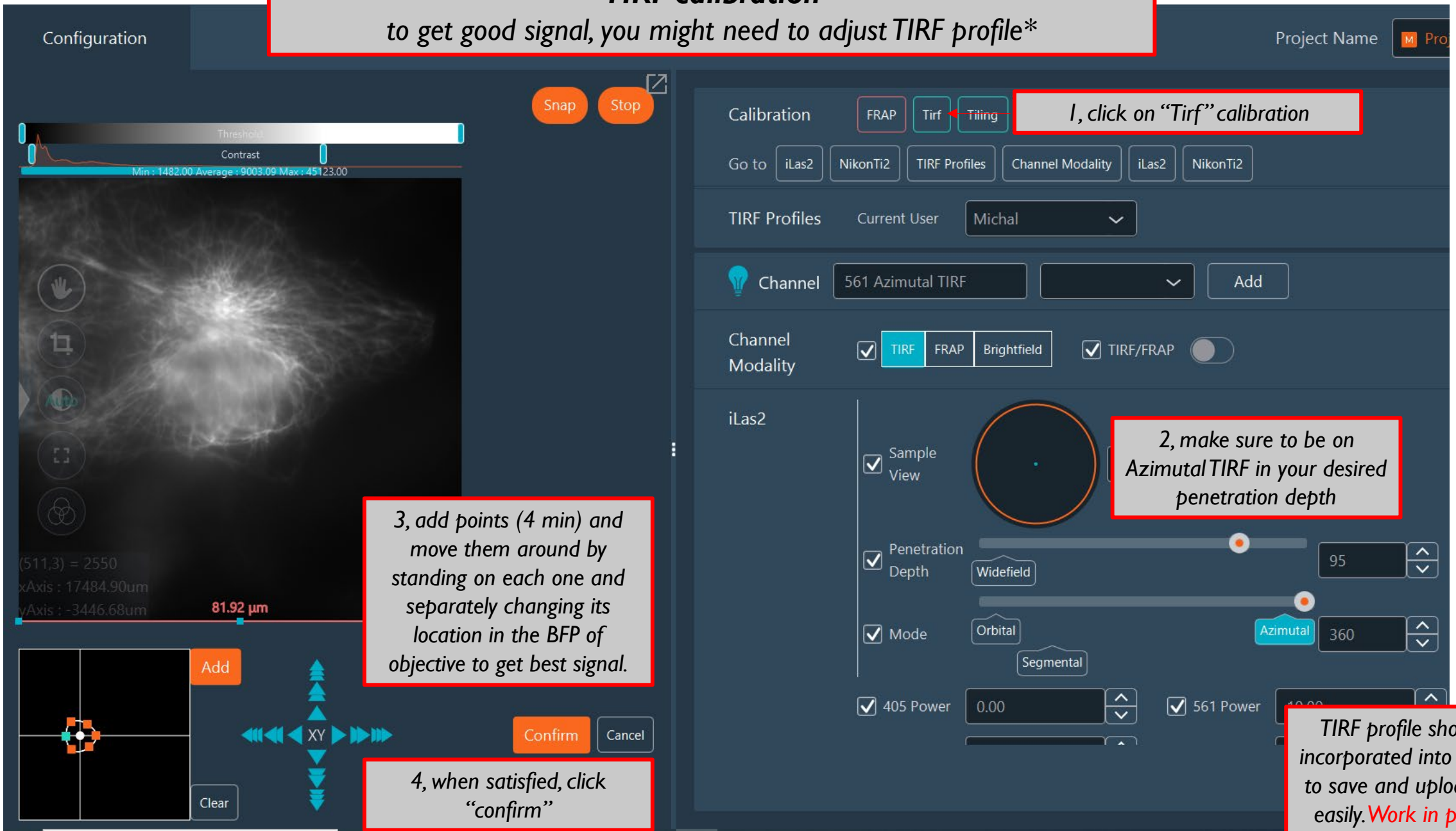
INSCOPER interface- configuration tab

TIRF illumination

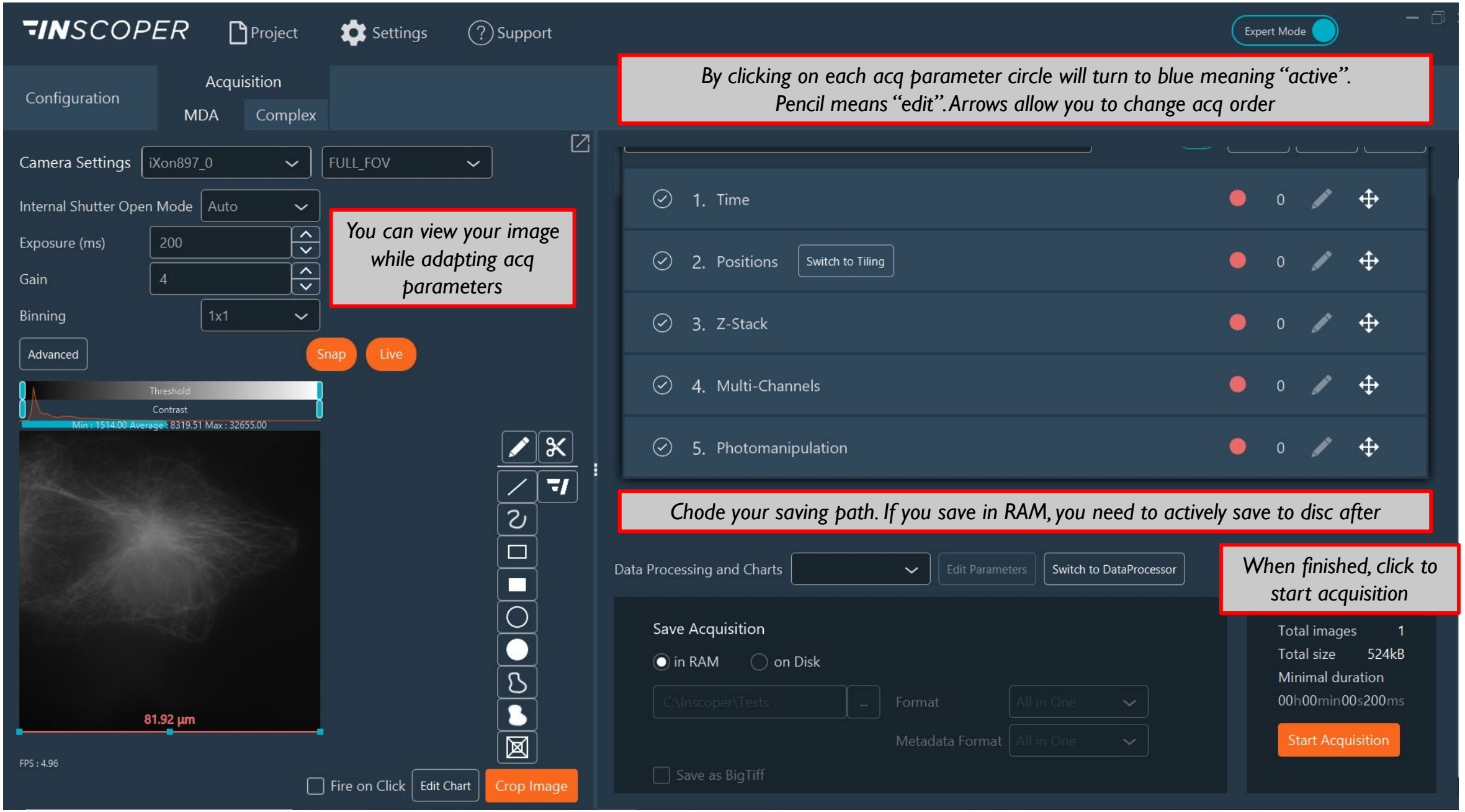
Set desired laser power and penetration depth (when in good focus)



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



INSCOPER interface- acquisition tab

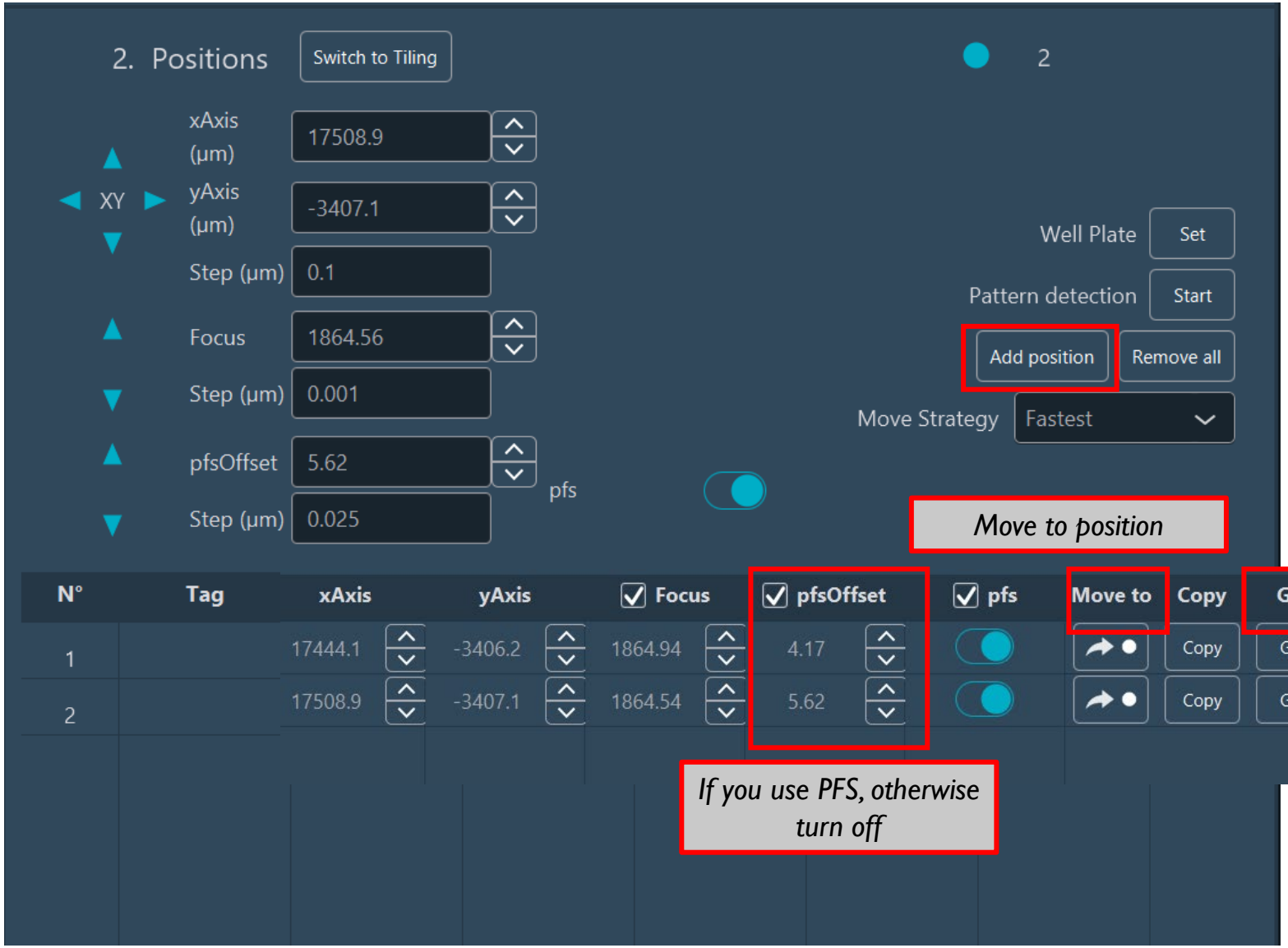


INSCOPER interface- acquisition tab

Time

INSCOPER interface- acquisition tab

Positions



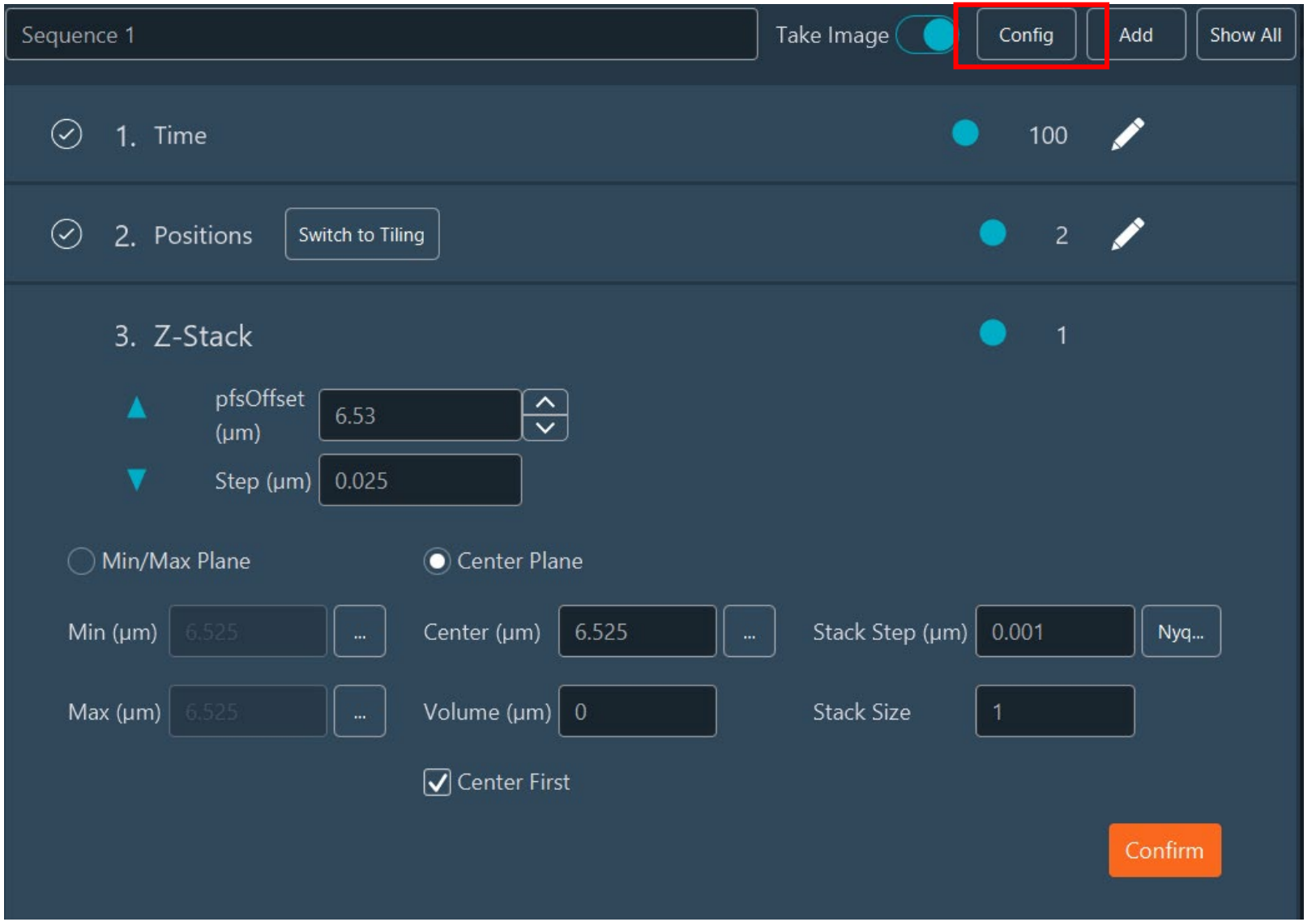
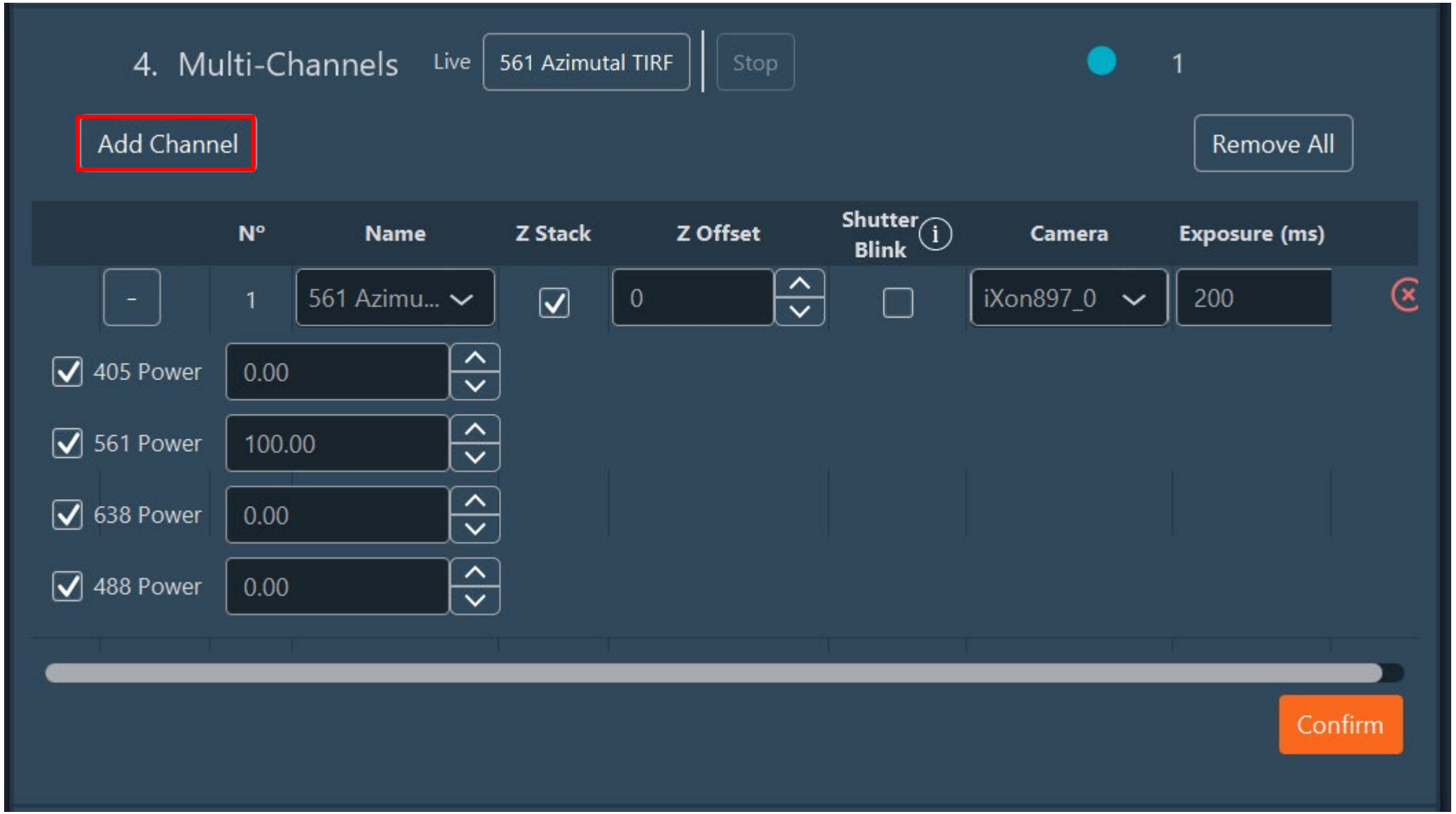
Move to position

Retrieve current coordinates

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



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