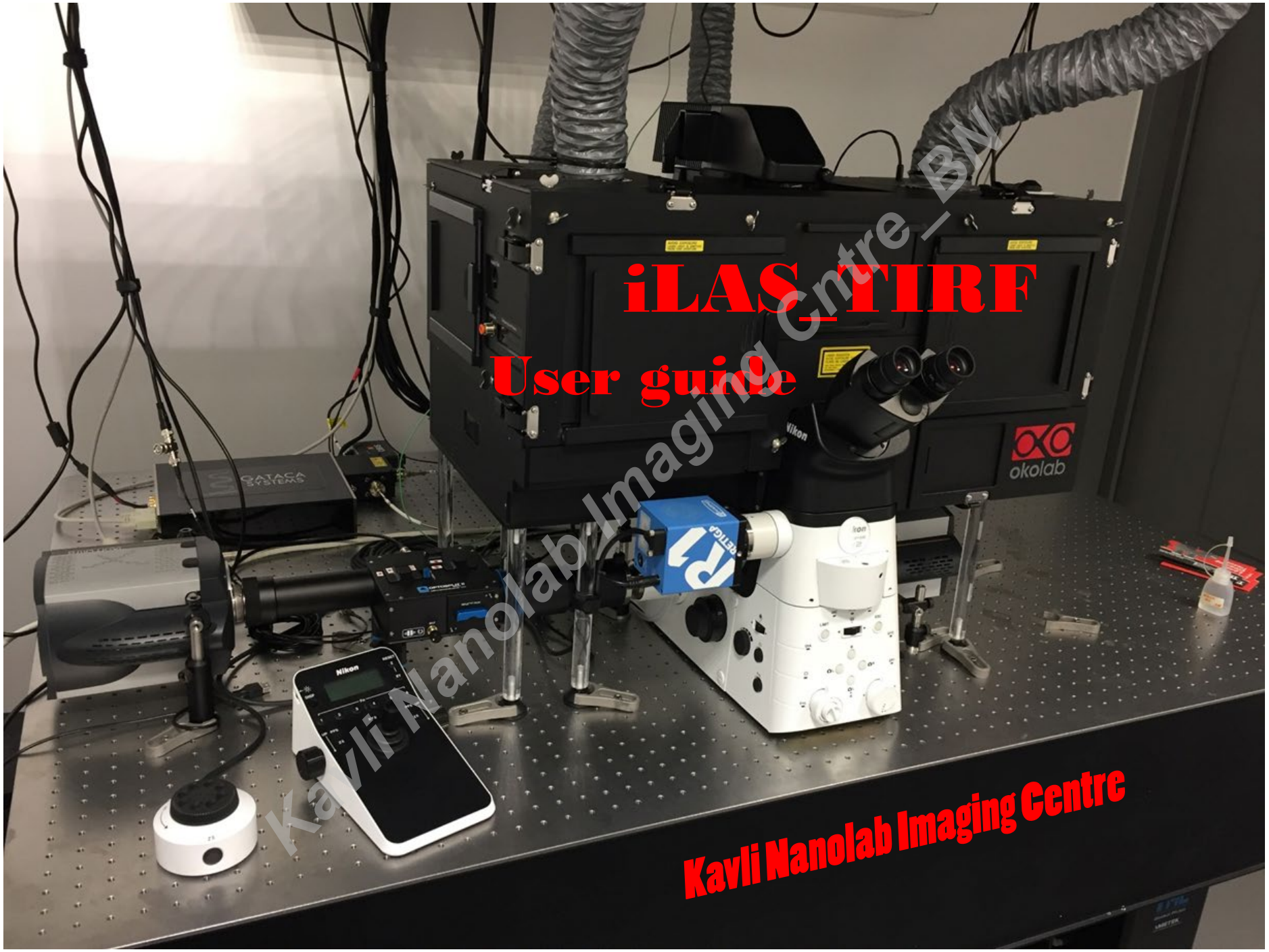
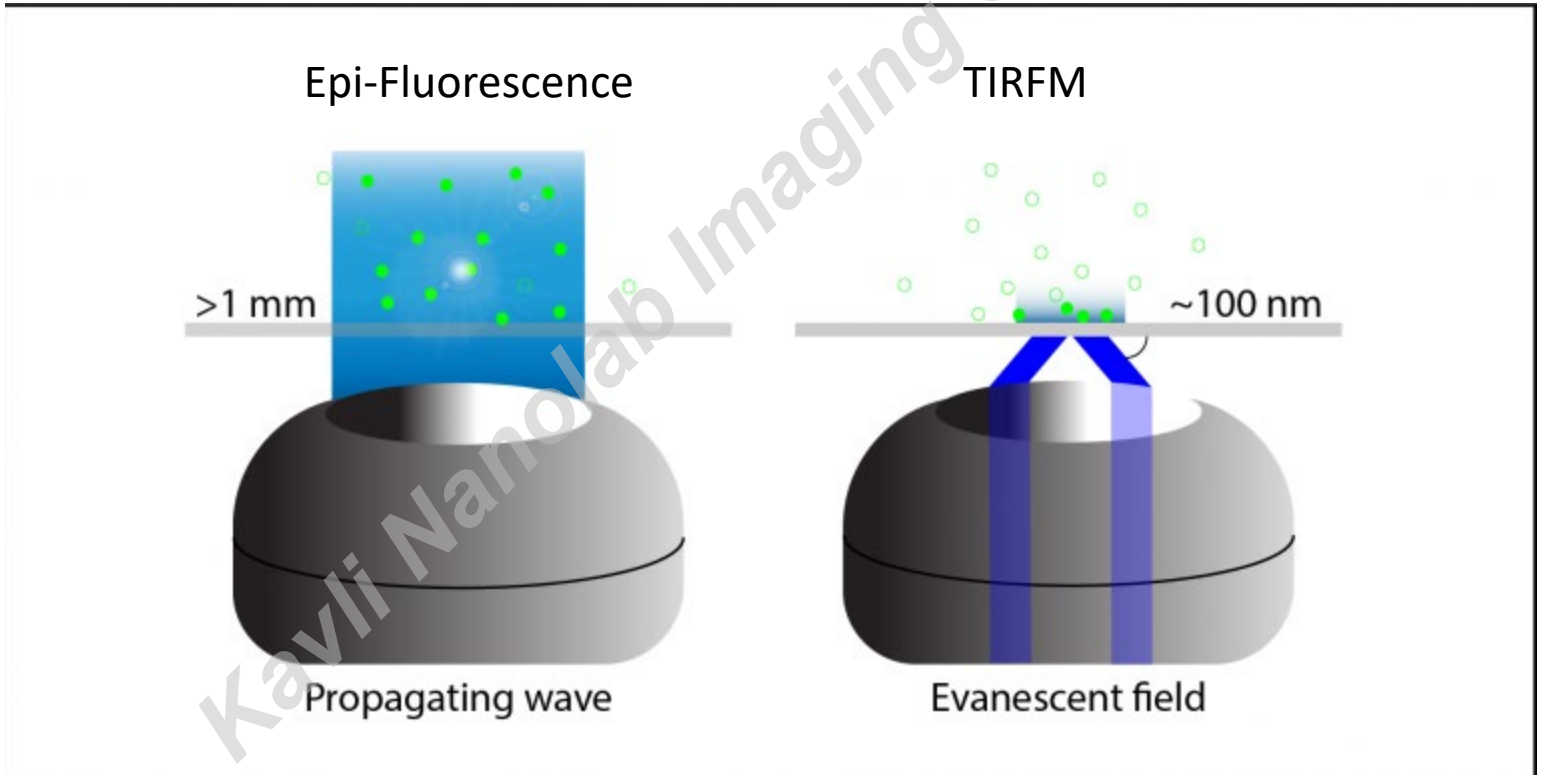

iLAS_TIRF
User guide
Kavli Nanolab Imaging Centre_BV
okolab
Nikon
Kavli Nanolab Imaging Centre

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



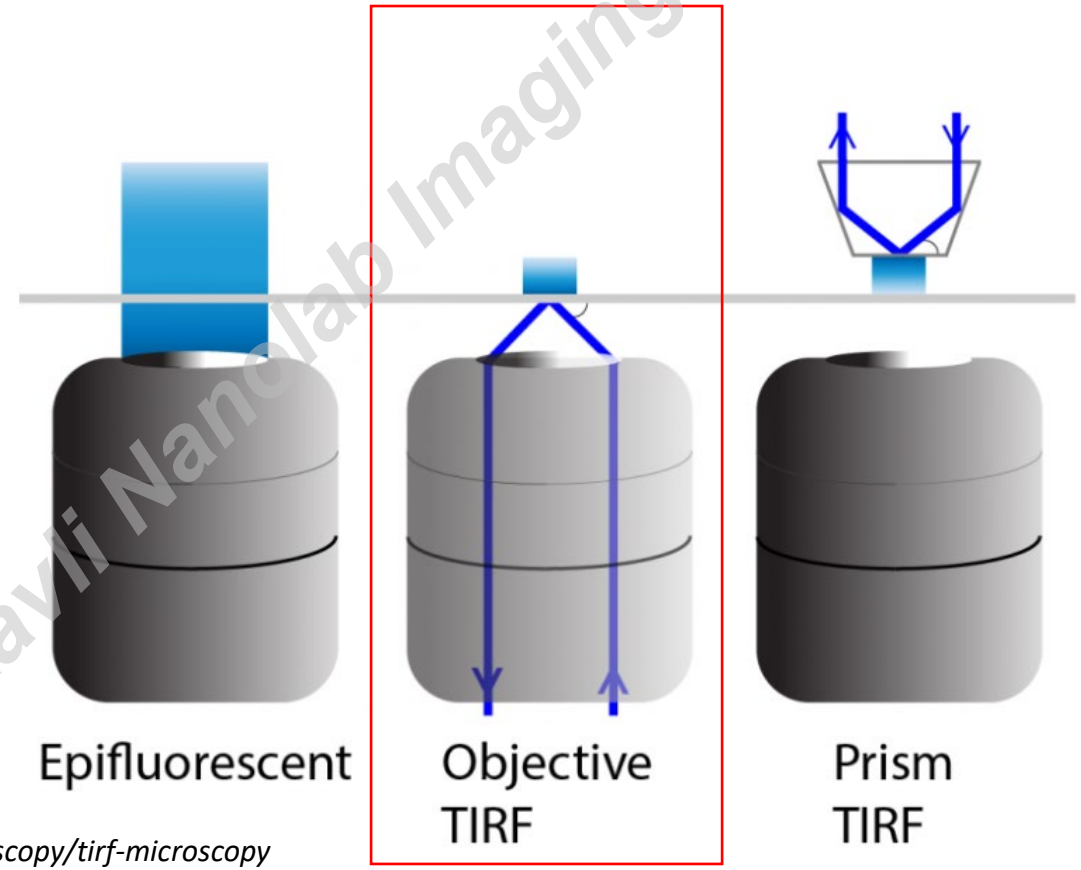
https://www.photometrics.com/learn/single-molecule-microscopy/tirf-microscopy

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

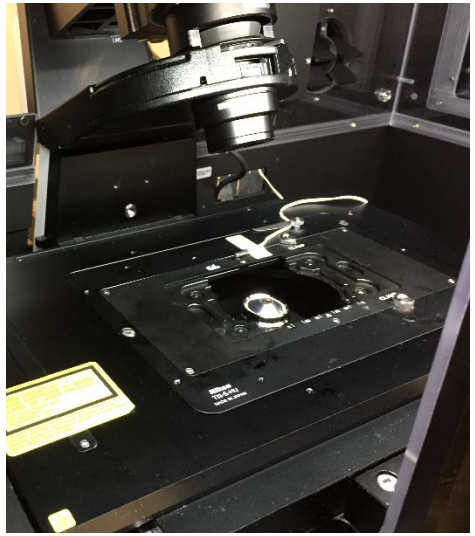


https://www.photometrics.com/learn/single-molecule-microscopy/tirf-microscopy

StartUp:

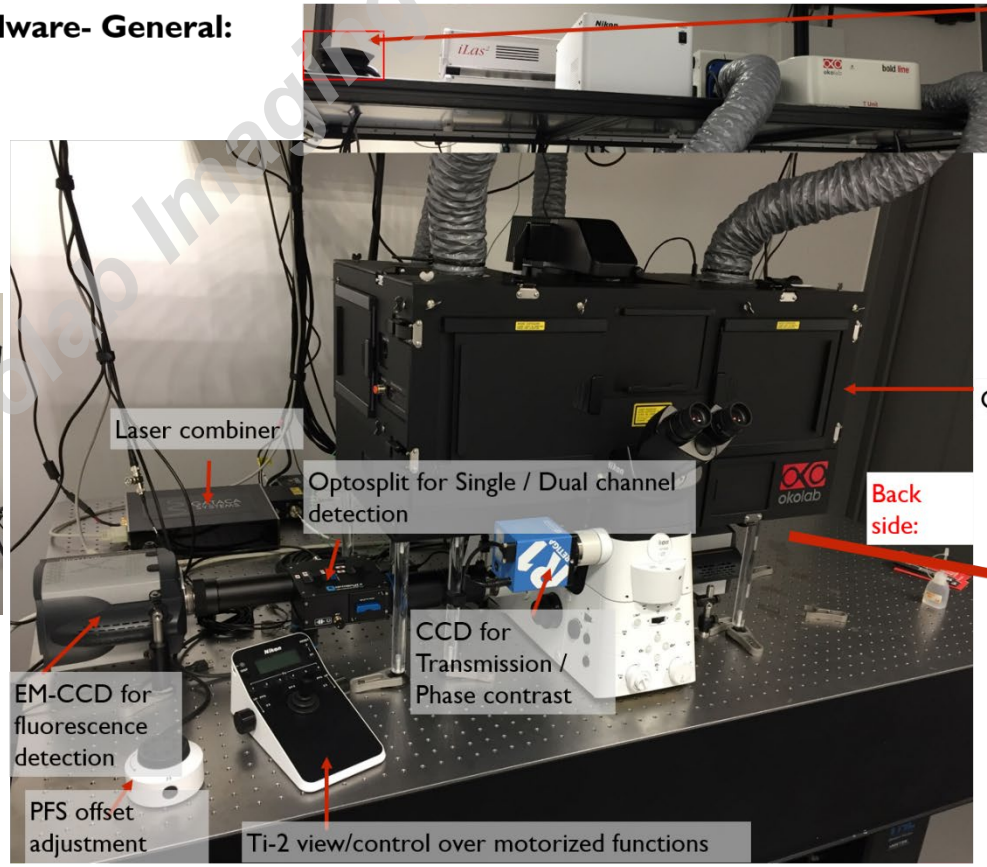
1, Turn ON the 2 main switches
2, Turn ON PC and Metamorph software
3, Locate your sample (BF only):
    1, place oil on 100X objective, place path to eyepiece
    2. Turn on BF light and turn the focus knob to move objective UP until the oil spreads on the coverglass (Slow!)
    3, Pay attention- when 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
4, View with "Live" button

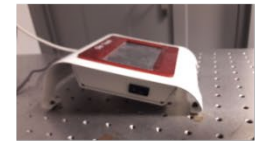
3



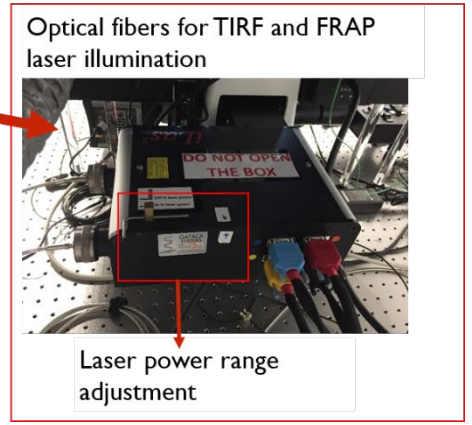
2
PC

Hardware- General:





OkoLab incubation box

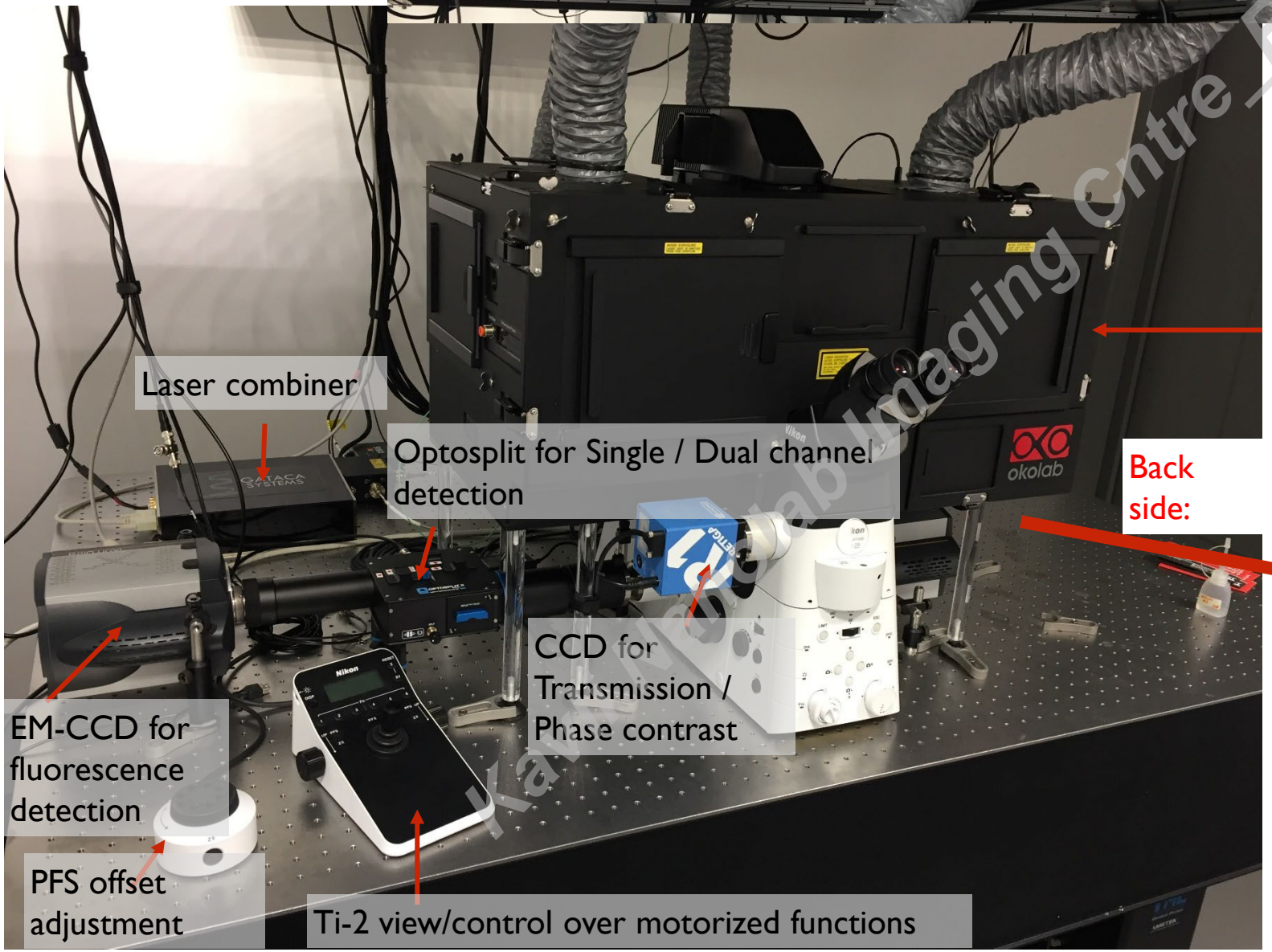


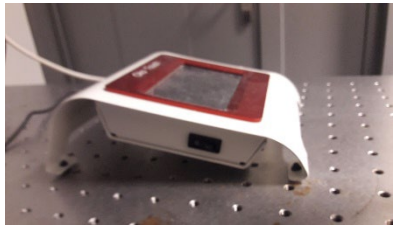
Shut-Down:

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

Hardware- General:

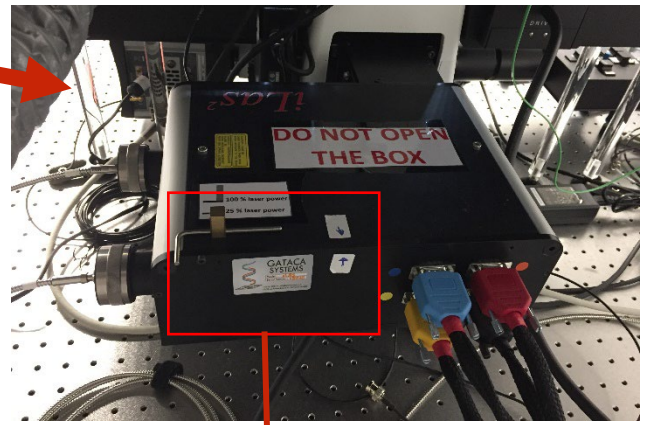
2 main switches





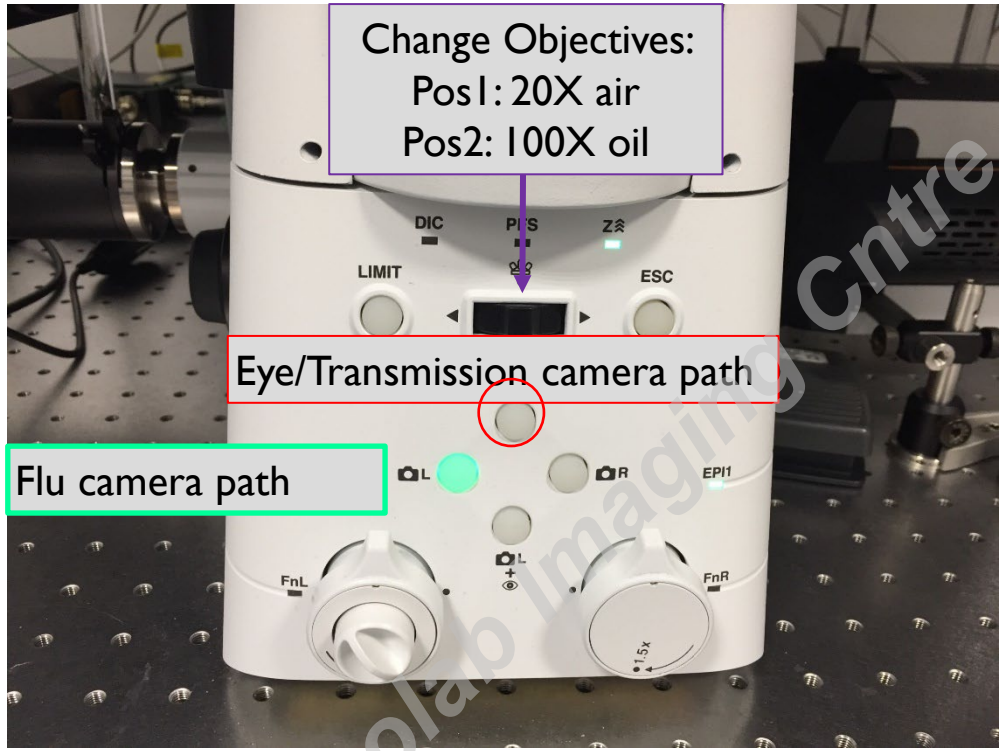
OkoLab incubation box

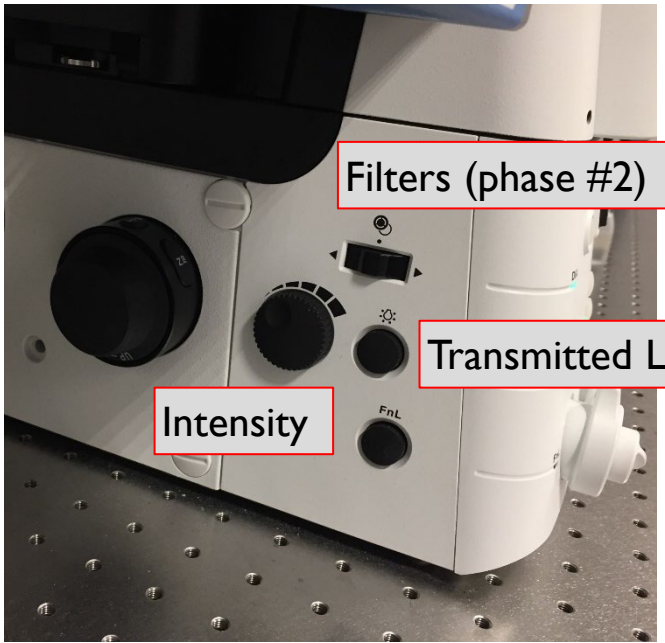
Optical fibers for TIRF and FRAP laser illumination



Laser power range adjustment

Ti-2 body:

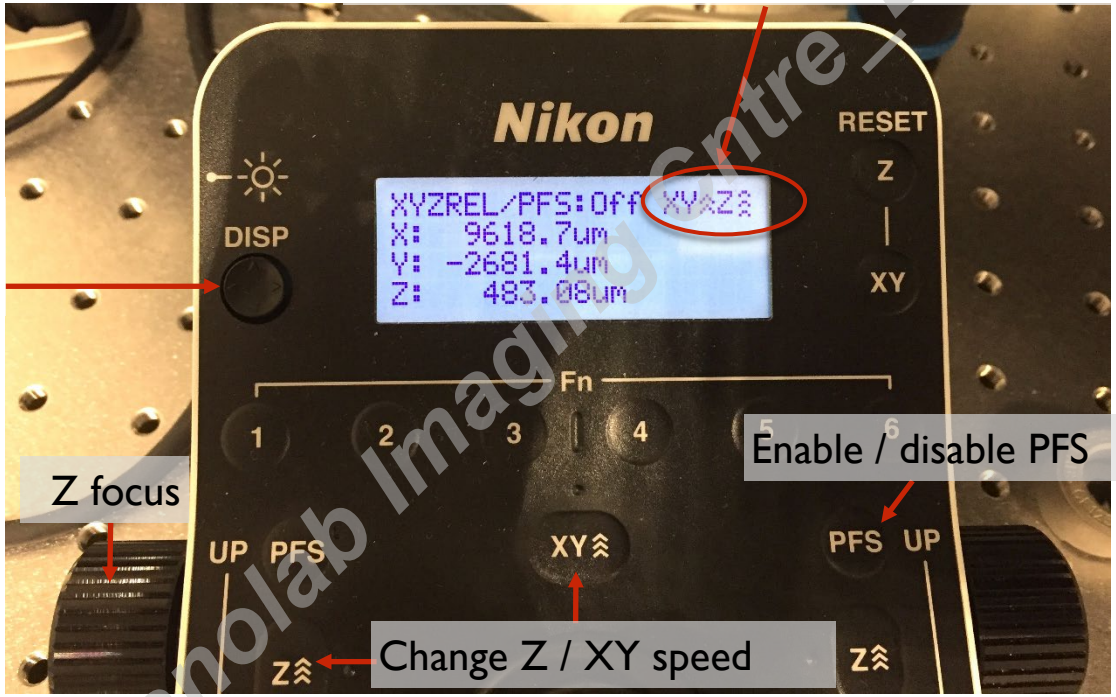


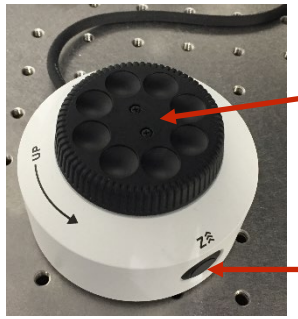


Ti-2 Joystick

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

Change information display





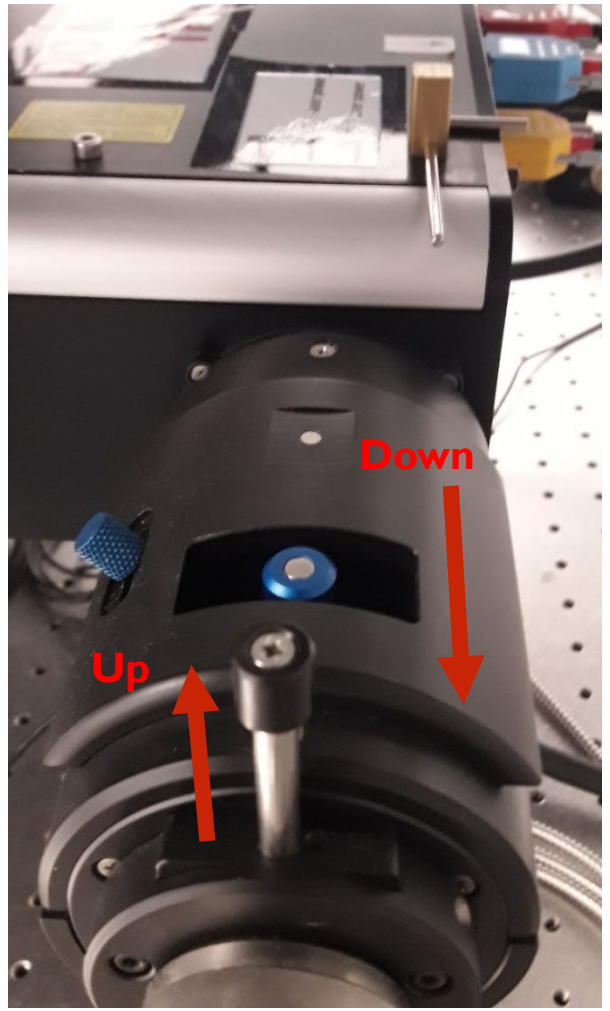
When the PFS is enabled, use this knob to adjust the focus

Keep pressing this button to speed up the focus adjustment

To modify laser intensity- Hardware

High laser Intensity

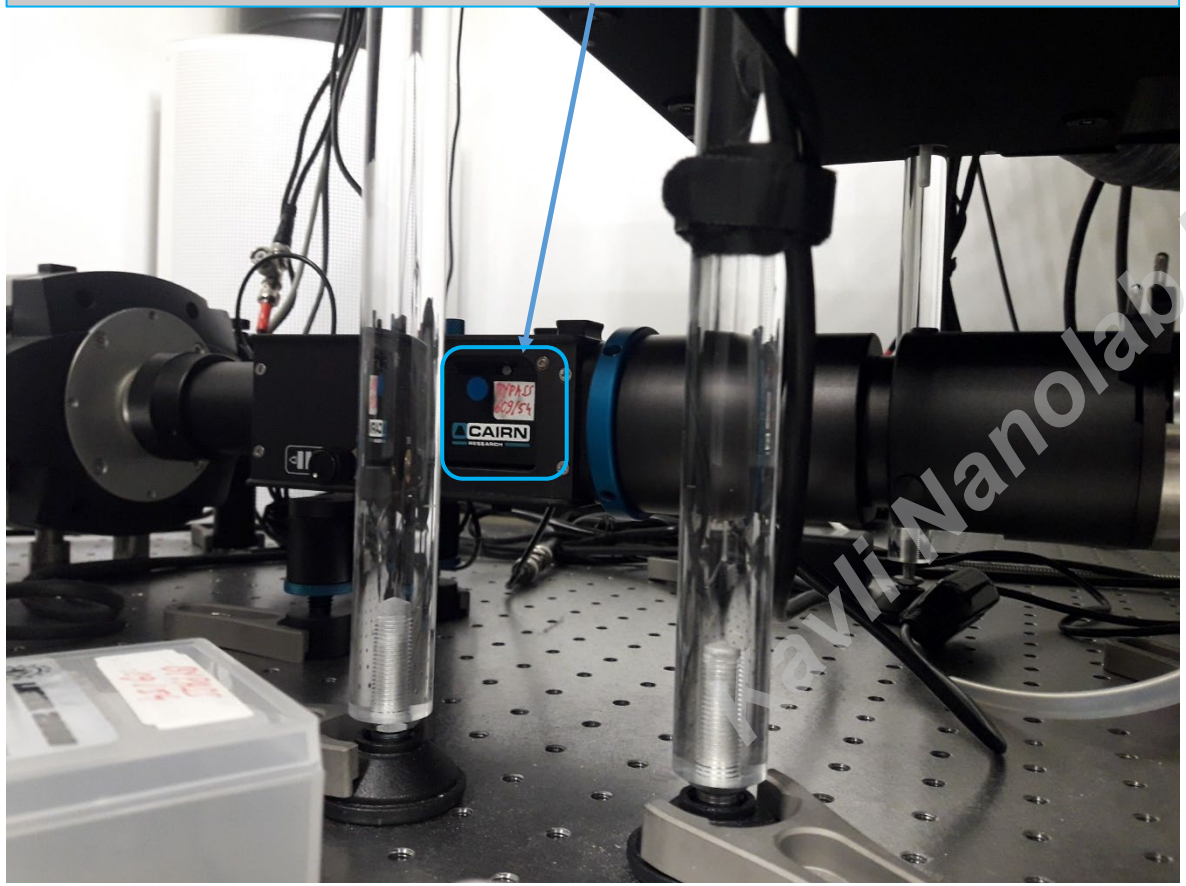
Low laser Intensity



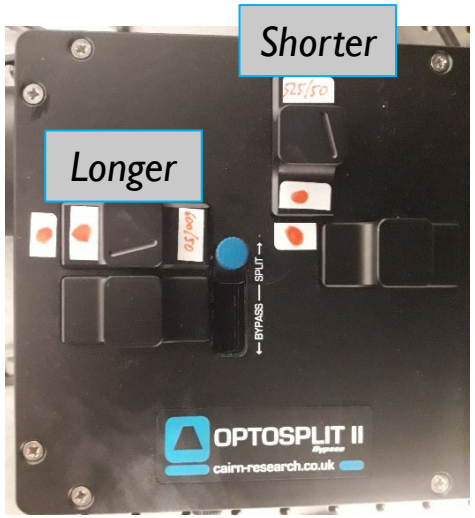
Optosplit for single/dual flu imaging- Emission path

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)*

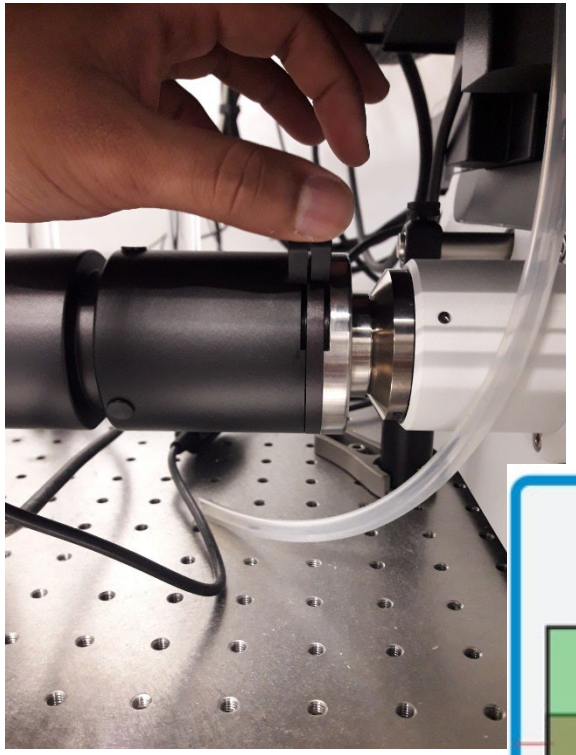


Split filters

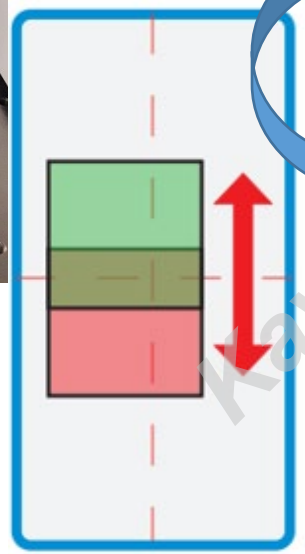


Optosplit for single/dual flu imaging- Alignment

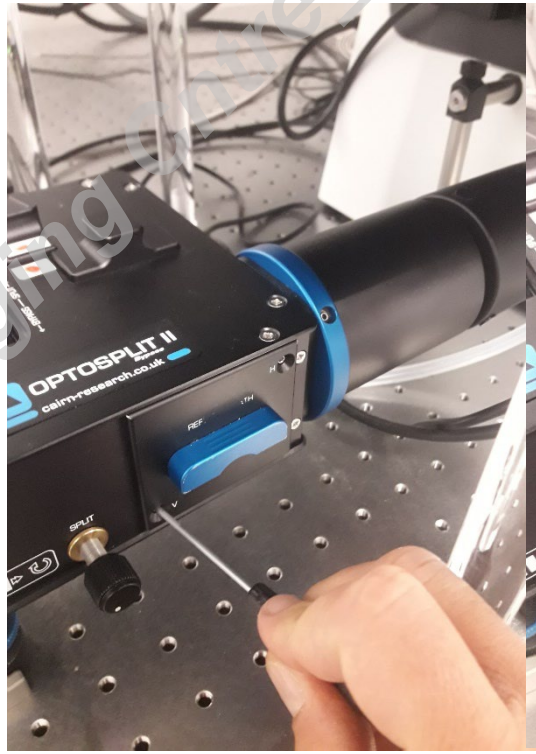
1, Use the knobs to reduce Field size

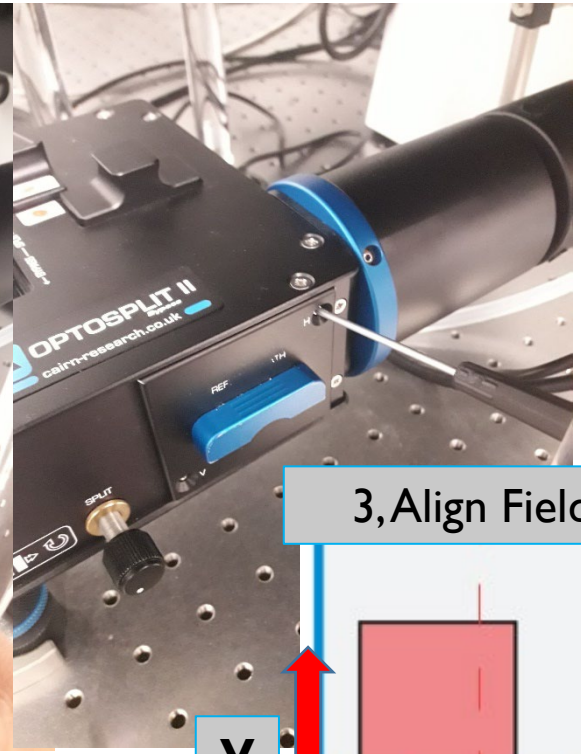


2, Separate Fields (anti-clockwise)

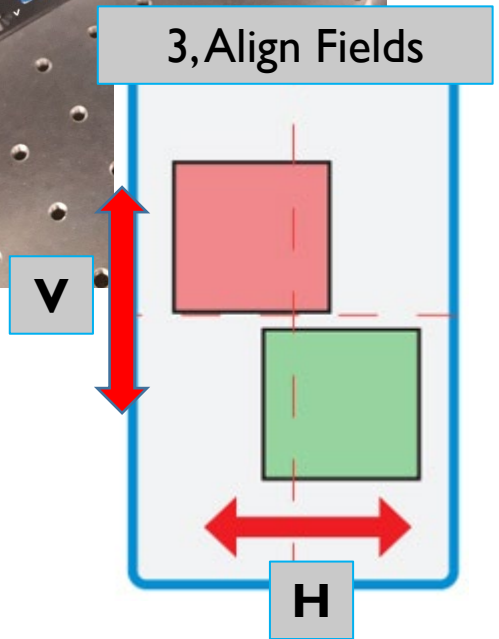


3, Align Fields



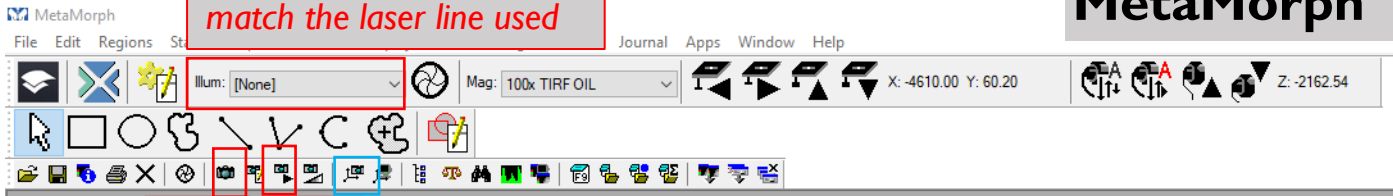


3, Align Fields



MetaMorph

Your illumination should match the laser line used

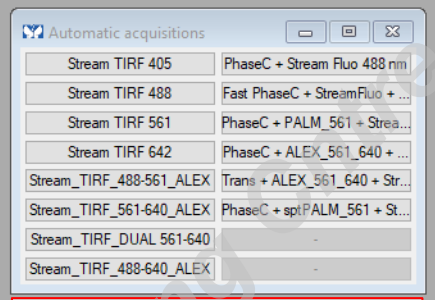


Snap

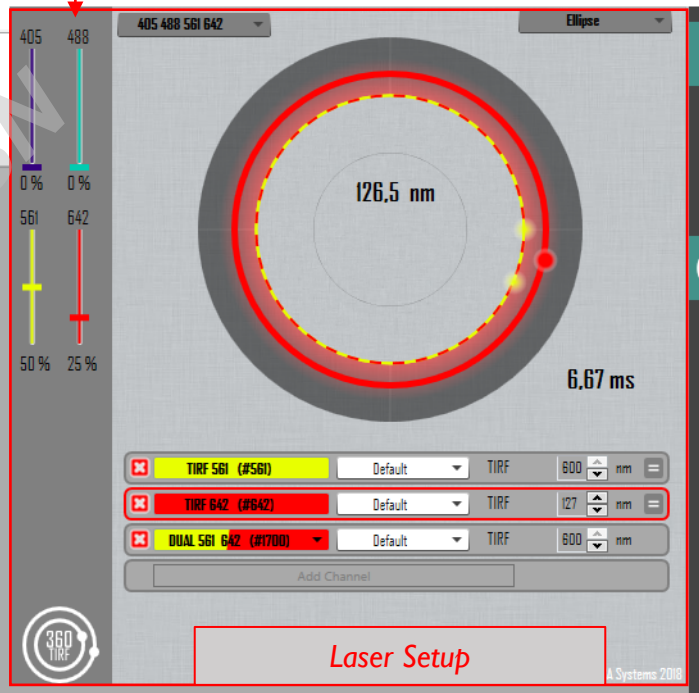
Live

Multi Dimensional Acquisition

In Metamorph, you can work with:
***Journals** (Acquisition scripts)*
or
Multi-Dimensional Acquisition



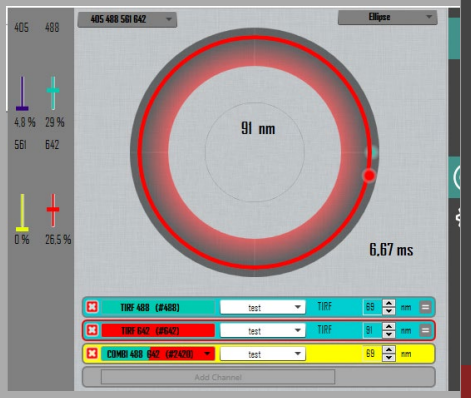
Quick Setups



Laser Setup

TIRF line (#nm1)- illuminate 1 wavelength at a time

Dual (#nm1 #nm2)- illuminate 2 wavelengths simultaneously.
Both are restricted to the same TIRF angle

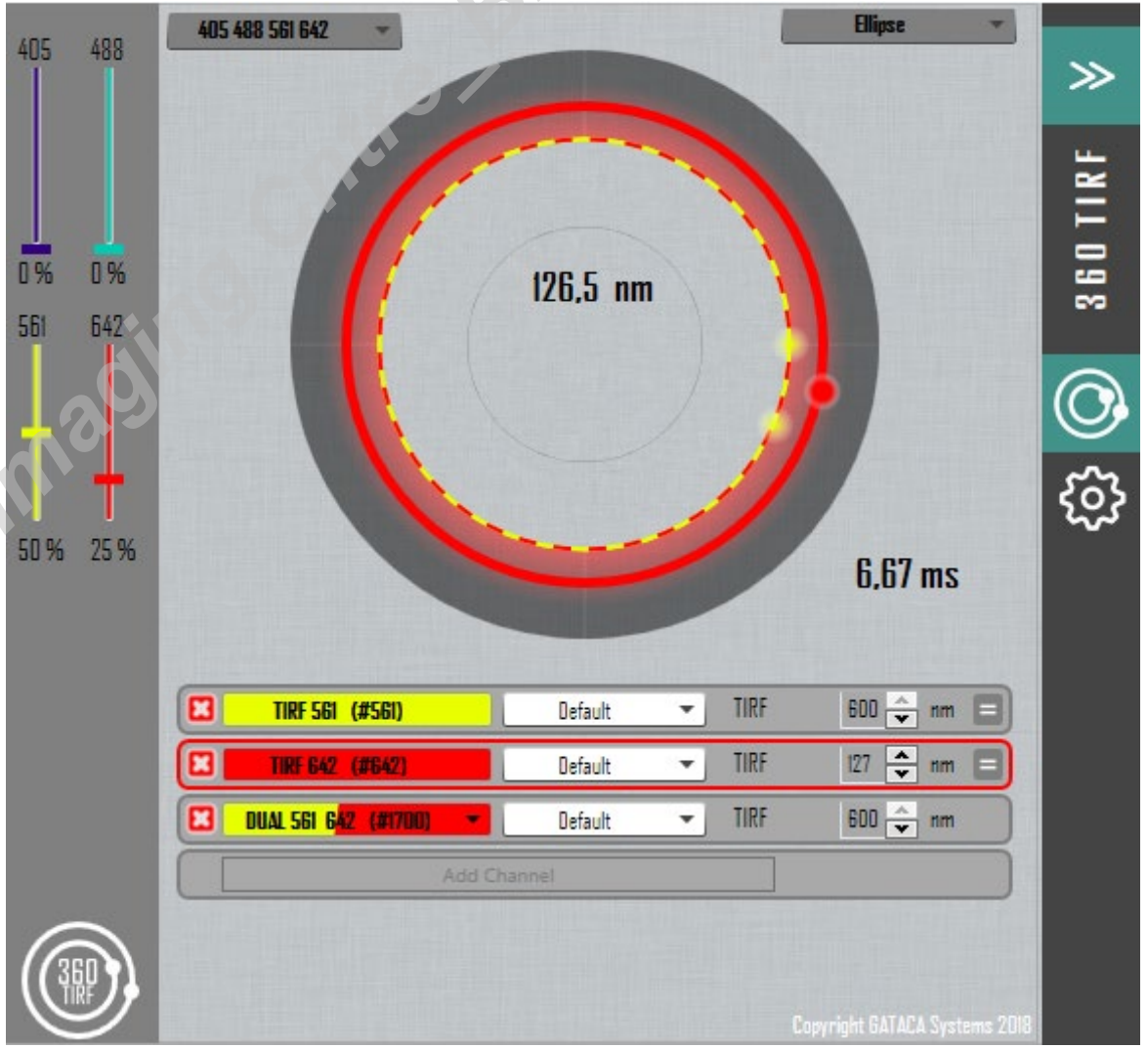


Combi (#nm1 #nm2)- illuminate 2 wavelengths sequentially, within the exposer time set (faster than acquisition).
Both can be set to different TIRF angles
!You need the two individual lasers defined above the combi setup

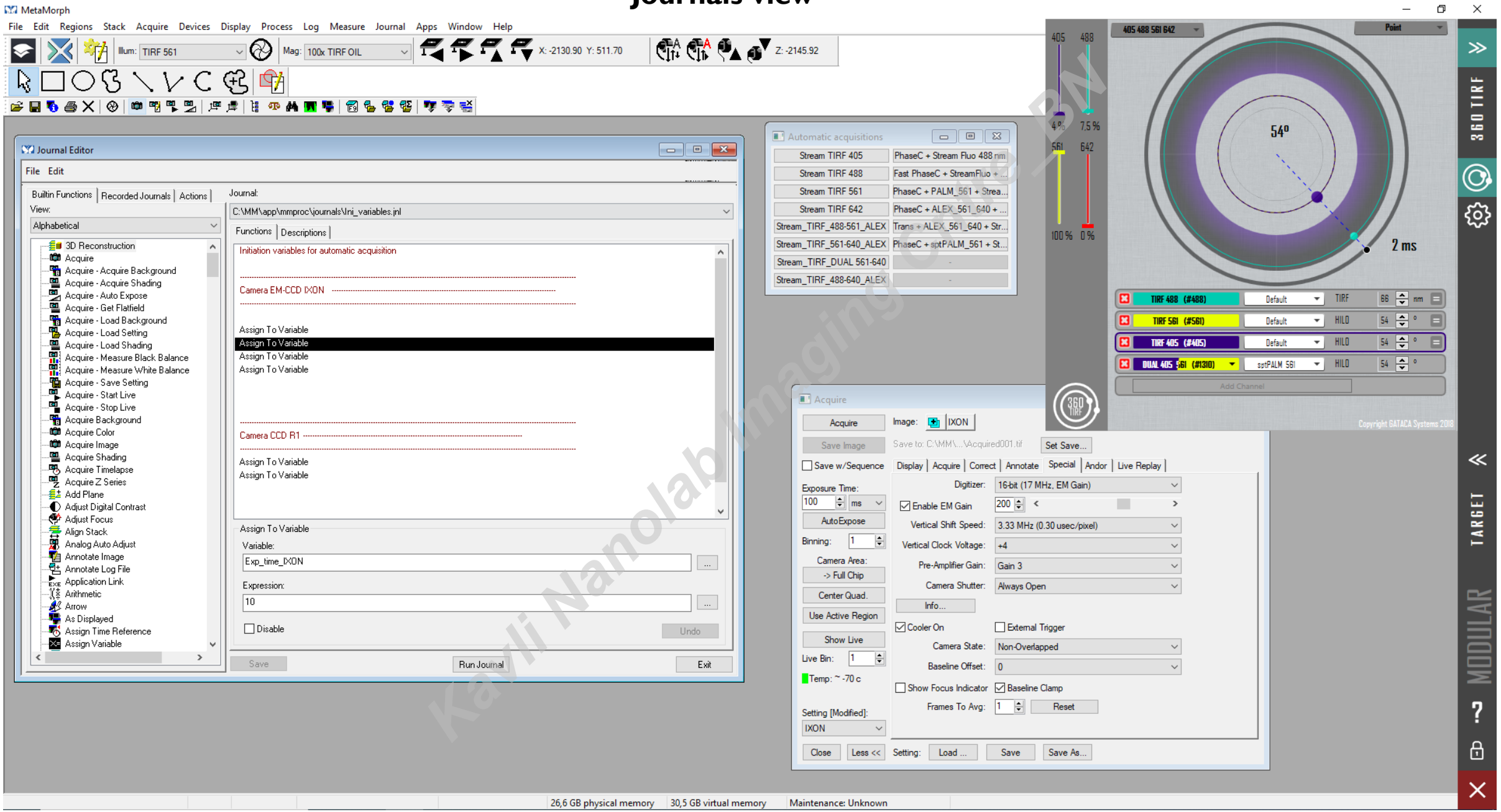
Adjust TIRF illumination

point

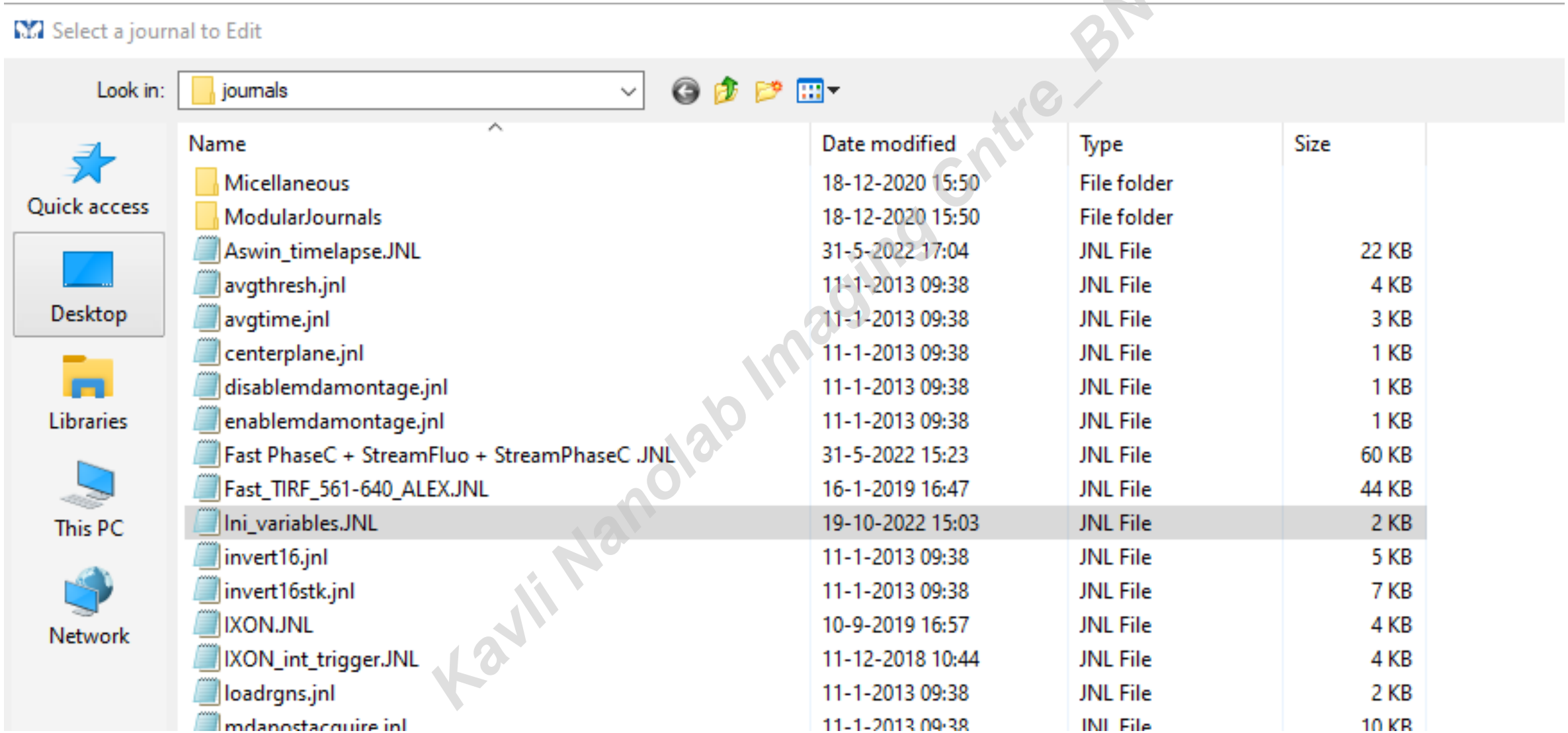
Ellipse



Journals view

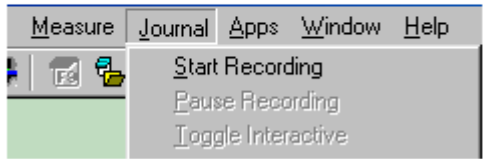


Work on Ini_Variables Journal



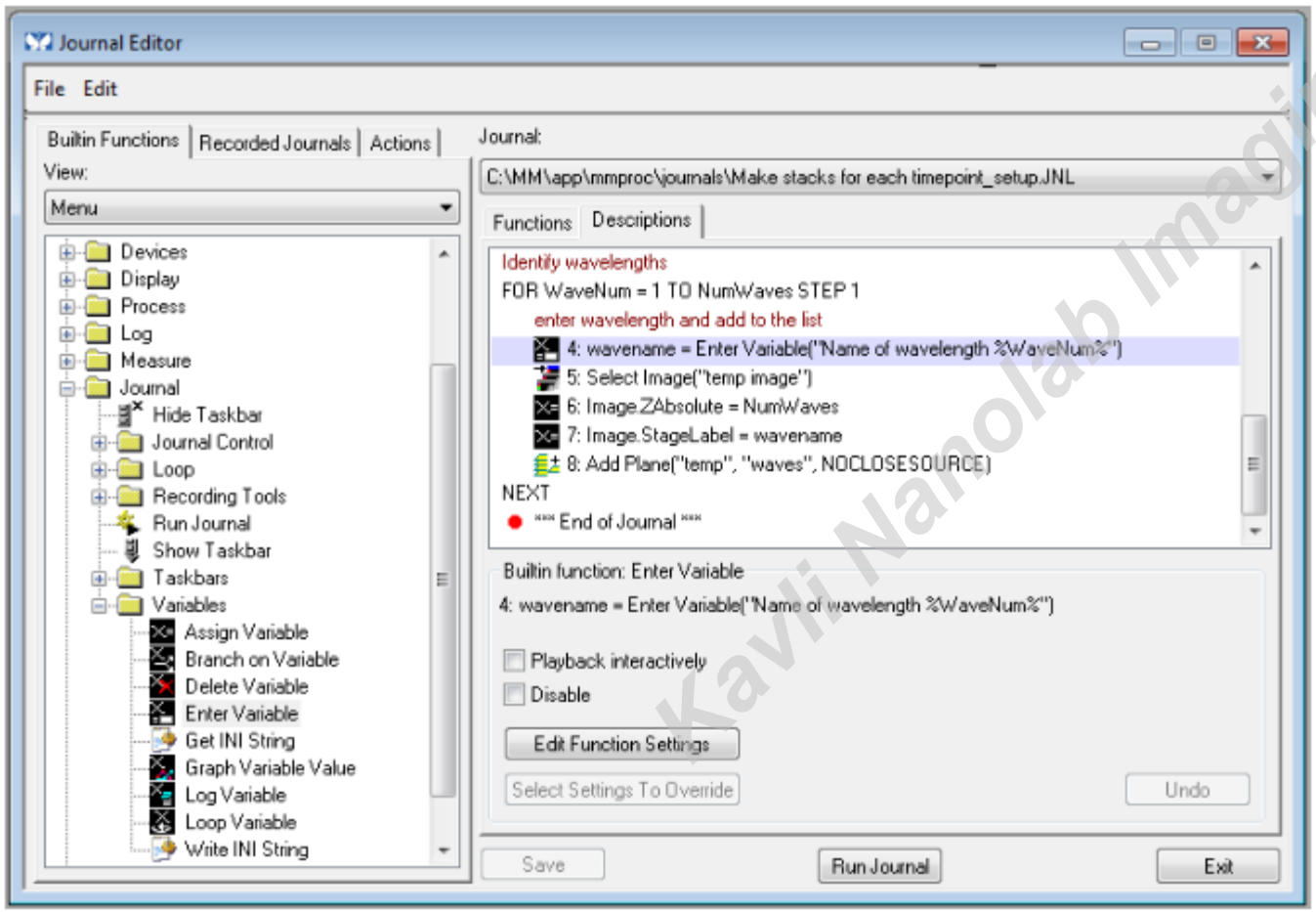
How do I create and edit a journal?

To record a journal, select **Start Recording** from the **Journal** menu.



The Journal Editor

To edit a journal or create a new journal, select **Edit Journal** from the **Journal** menu, .

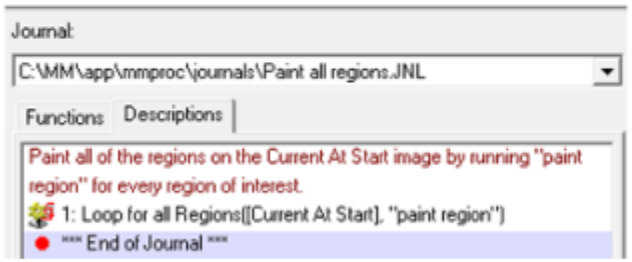


Running sub-journals and looping journals

A sub-journal is a journal that is run within another journal.

Sub-journals

If several steps in a procedure need to be repeated, you can reduce the complexity of a journal by placing the repeated steps into a separate journal that is executed multiple times from the main journal. For example, suppose we are writing a journal in which we want to paint every region on an image. This journal could be written to select region 1, paint the image, select region 2, paint the image, and so on for as many regions as needed. It is much simpler to use the built-in journal function **Loop for all Regions** to run a second journal. The second journal paints the active region of interest.



Sub-journals can also simplify the use of image selectors. If the journal asks the user to select an image interactively or to open an image file, you cannot predict the name of the result active image. In this case, you could request that the user make the image active once, and then run a sub-journal. When you run the sub-journal, the active image window becomes “Current At Start” while it is running.

Types of loops

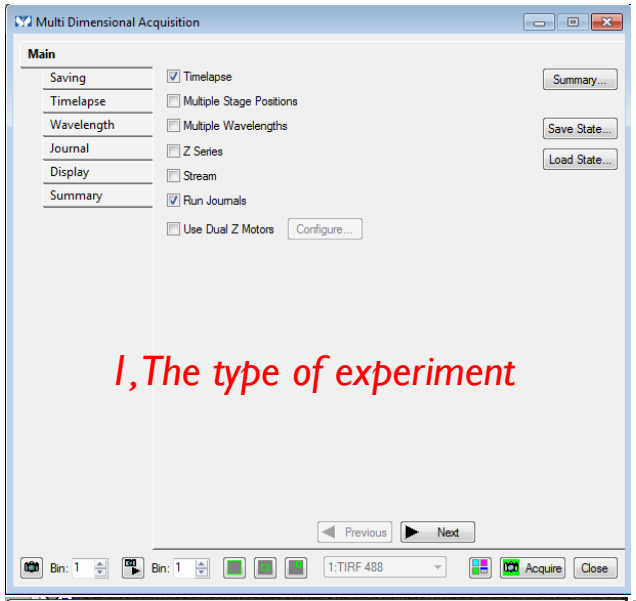
The MetaMorph Software provides a variety of ways to loop and run a journal. The loop functions all execute a selected journal a specified number of times. Some of the loop functions include:

- **Loop a Journal:** Runs a journal a specified number of times and can include a time interval between loops.
- **Loop for all Regions:** Makes each region of interest on the image active and runs a journal.
- **Loop for all Planes:** Makes each plane of a stack active and runs a journal.
- **Loop for all Images**: Makes each image window active and runs a journal
- **Loop for all Images in Directory:** Loads each image in a directory and runs a journal.
- **Run Journal for Multi Dimensional Data:** Loads each time point, Z position, and/or stage position, and runs a journal.

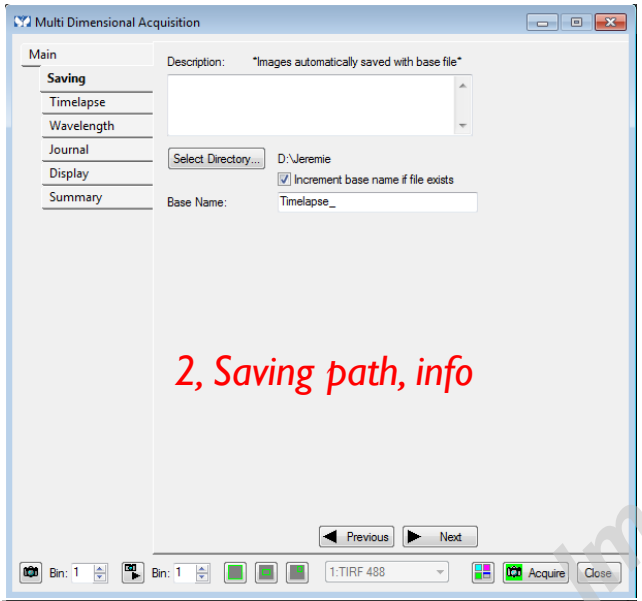
https://support.moleculardevices.com/s/article/Introduction-to-writing-journals-in-the-MetaMorph-Software

Multi Dimensional Acquisition view- Step by Step

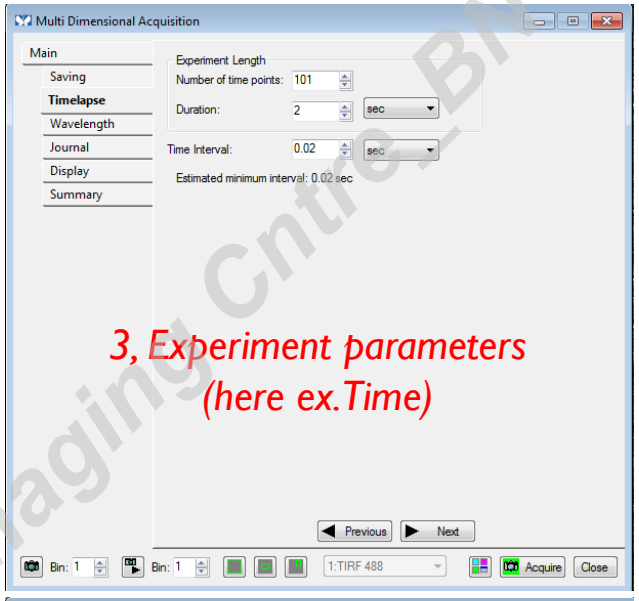
Choose:



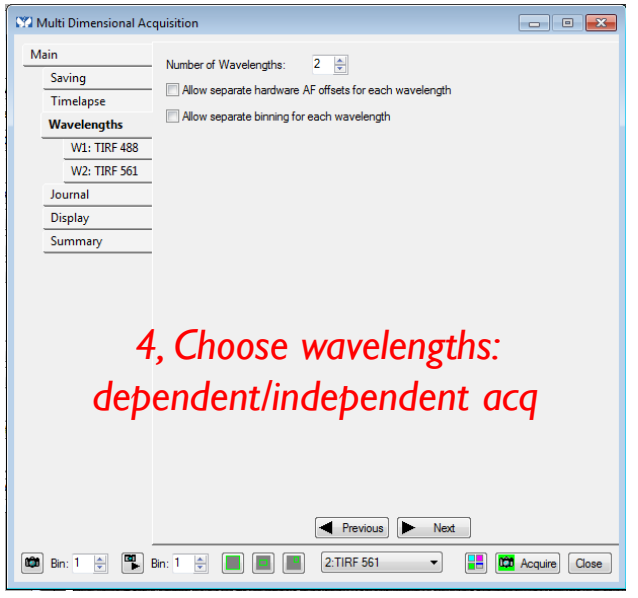
1, The type of experiment



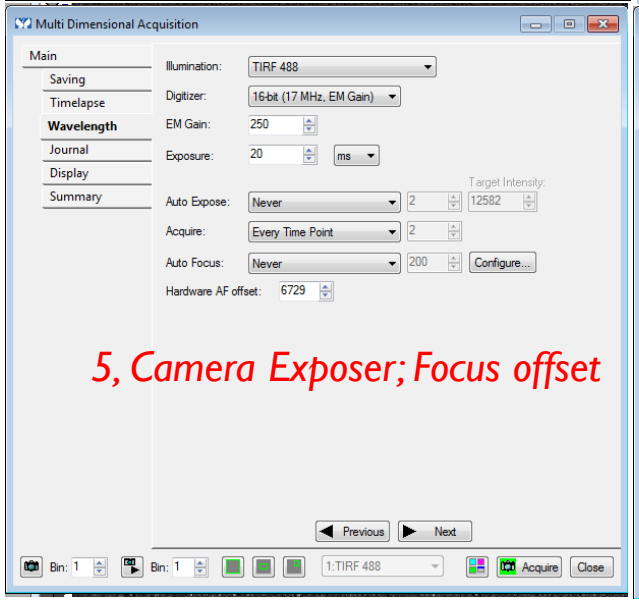
2, Saving path, info



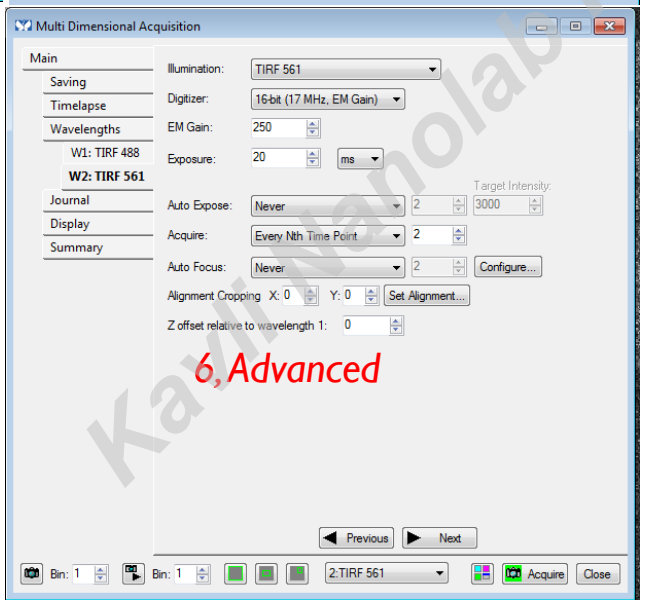
3, Experiment parameters (here ex.Time)



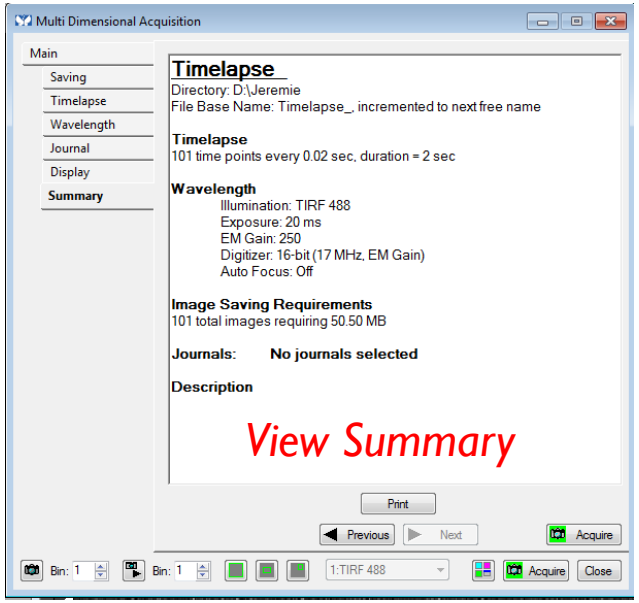
4, Choose wavelengths: dependent/independent acq



5, Camera Exposer; Focus offset



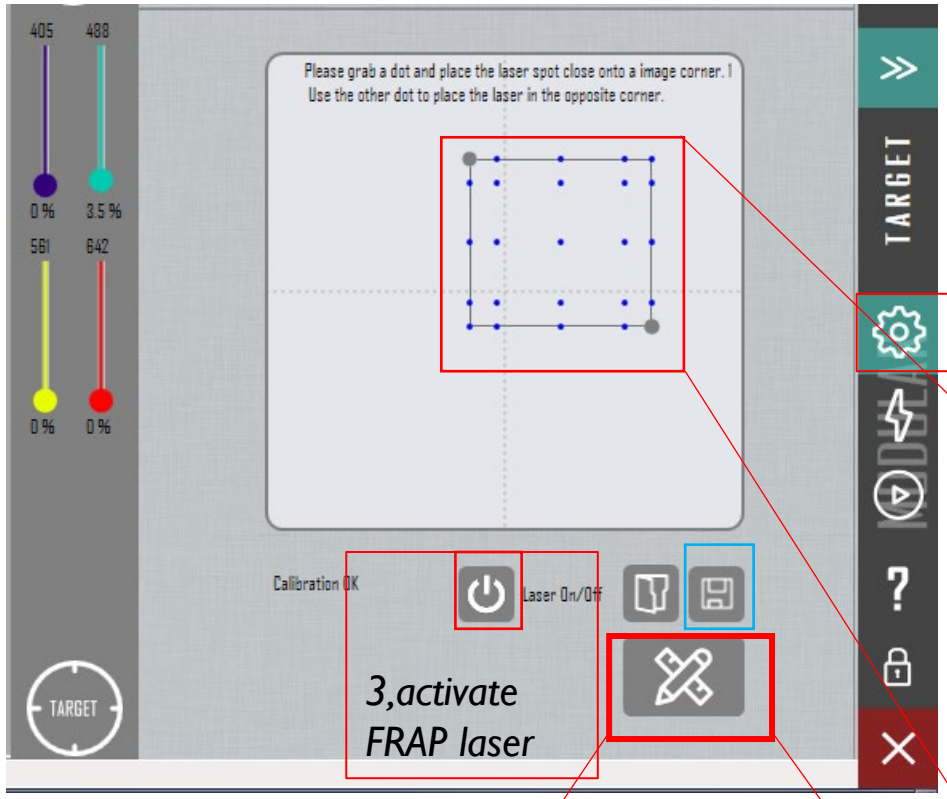
6, Advanced



View Summary

FRAP calibration

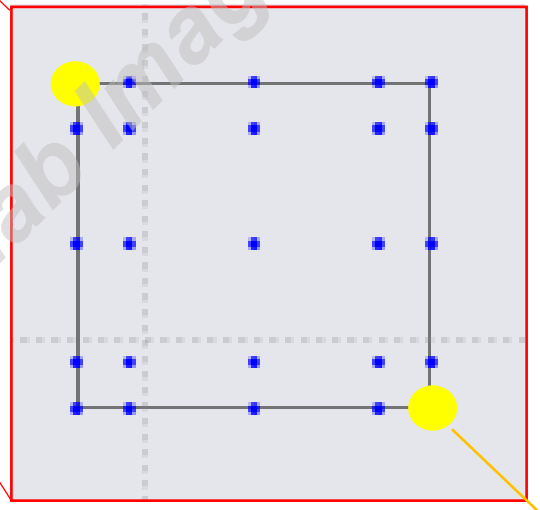
To calibrate the FRAP illumination with your field of view, you need to place a calibration slide (Ask us for it) or an empty position on your own sample (no signal)



1, Find the sample's focus, adjust laser intensity and camera exposer

2, Click on the calibration tab

3,activate FRAP laser



4, click on the top left circle- you should see a bright laser spot : place it on the top left corner in your view.
Then do the same for the bottom right circle

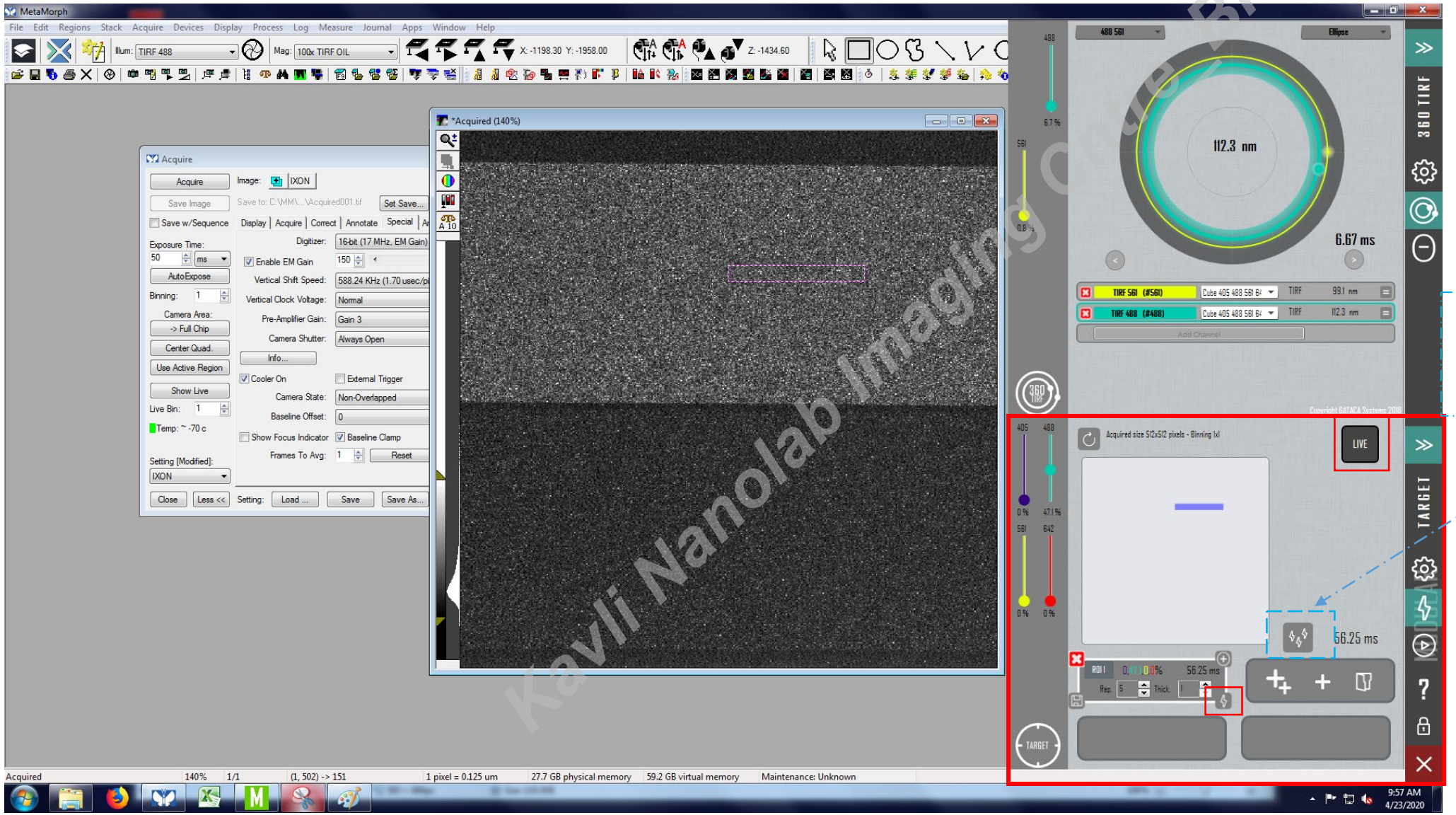
By this you define the area to FRAP.

5, Click on "calibrate" to illuminate a matrix of laser points in your defined area.

Make sure the laser spot is seen round and small size (lower laser intensity if needed)

You can save and load your calibration settings

FRAP on the fly*- check your calibration



*Useful when you want to bleach without setting a full experiment

To verify calibration:
Increase number of repetitions and laser intensity, press “live” and check if the laser spot is on your ROI

FRAP experiment- setup

Use this to record pre and post imaging (camera is OFF while bleaching)

1, Preview and adjust your sample's view. Snap an image at good SNR

2, Mark your ROI(s) on the snap 

3, Adjust bleaching parameters: #repetitions; laser power (On the fly*)

4, Click on laser activation to test it

5, Setup interval and duration for pre and post FRAP acquisitions.

**the bleaching parameters correspond to your "FRAP on the fly" settings*