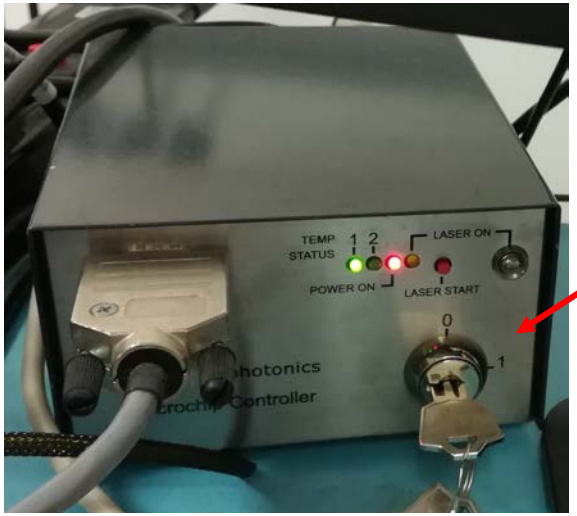
Photoablation UV / DNA damage user guide
Spinning disk « Nikon – CSU-X1 »

Start the system as usually. Consult the Nikon Spinning disk user guide if necessary.

Switch ON the additional UV 355nm laser of photoablation UV / DNA damage

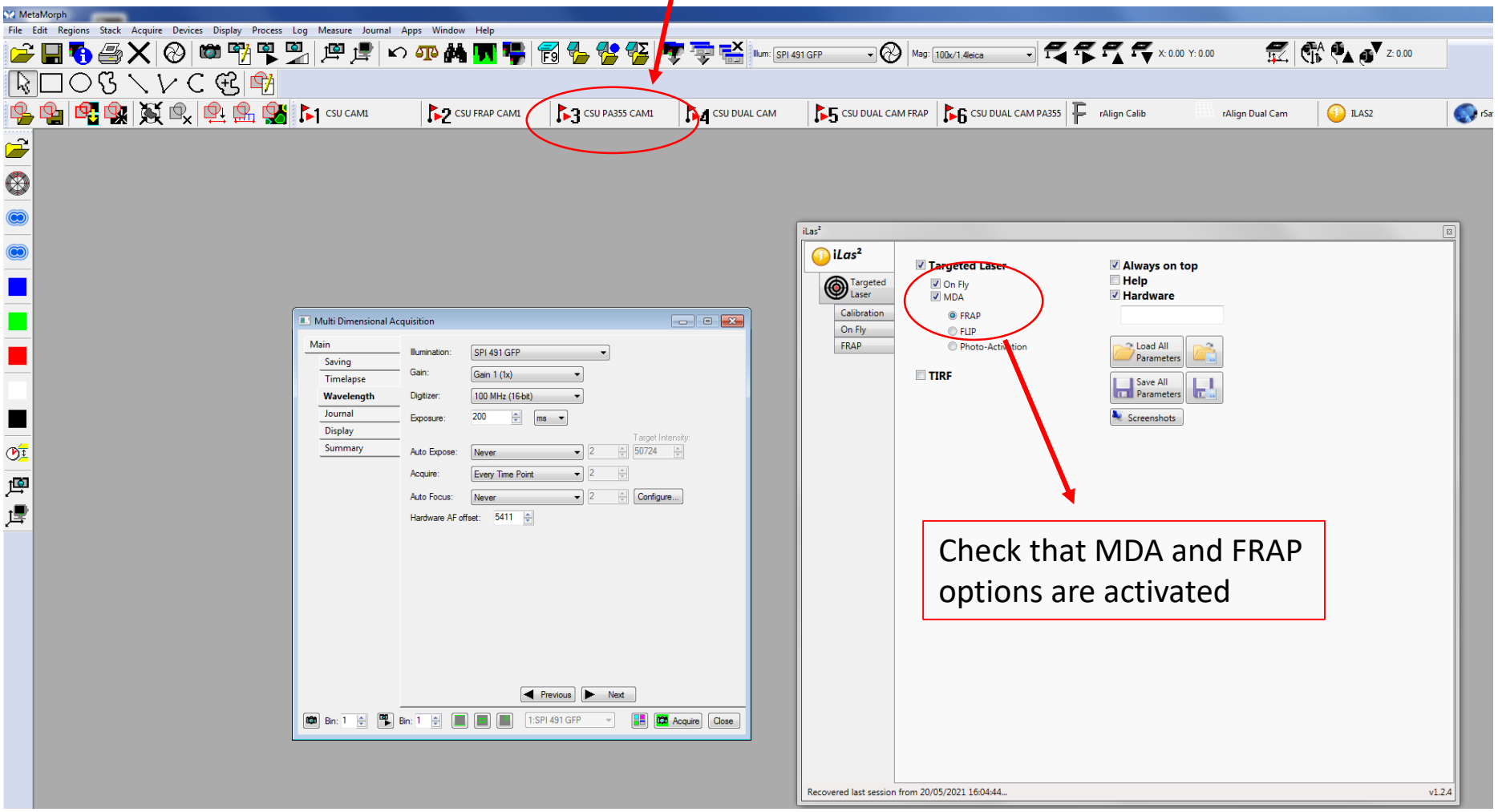


Turn the Key On
Wait for the laser to init

Select the PA355 nm setting

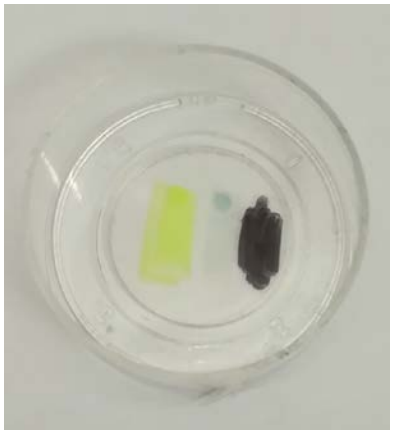
Select the 3 – **CSU PA 355 CAM1** setting

This will set up the correct filter inside the microscope to use the 355nm UV laser and open the iLas^2 window

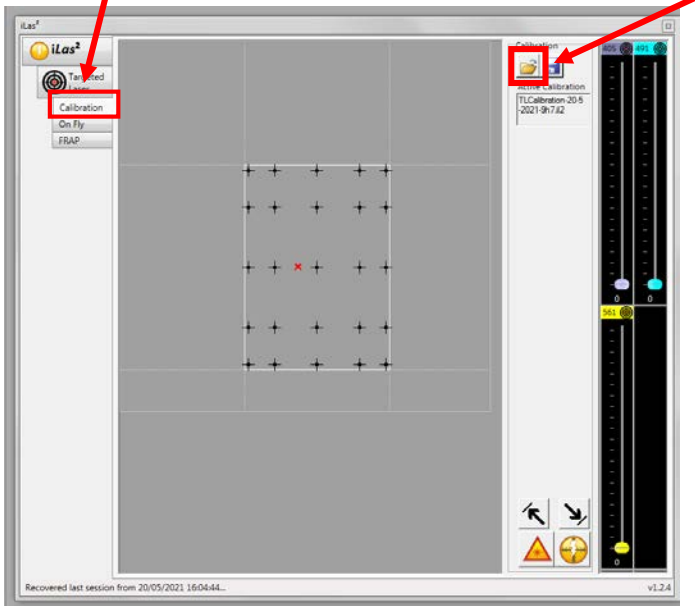


Load the calibration setting

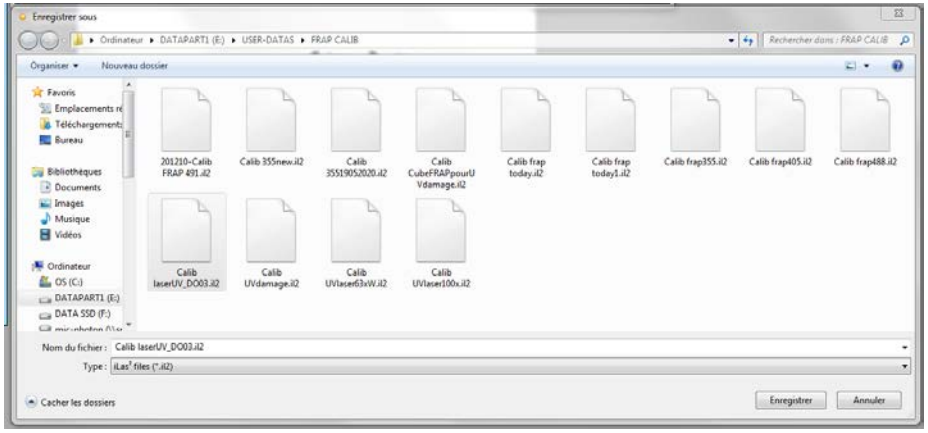
Check if the calibration of the 355nmUV laser position in the acquisition field of view is correct.
You can use a coverglass or glass bottom dish with Stabilo marqueur



Go in the iLas² Calibration menu



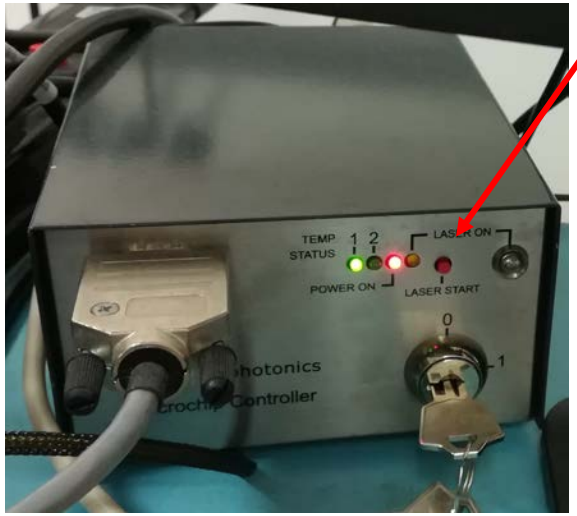
Load your previous .il2 calibration setting



By default, the system load the last setting used

Start the UV laser before running the experiment

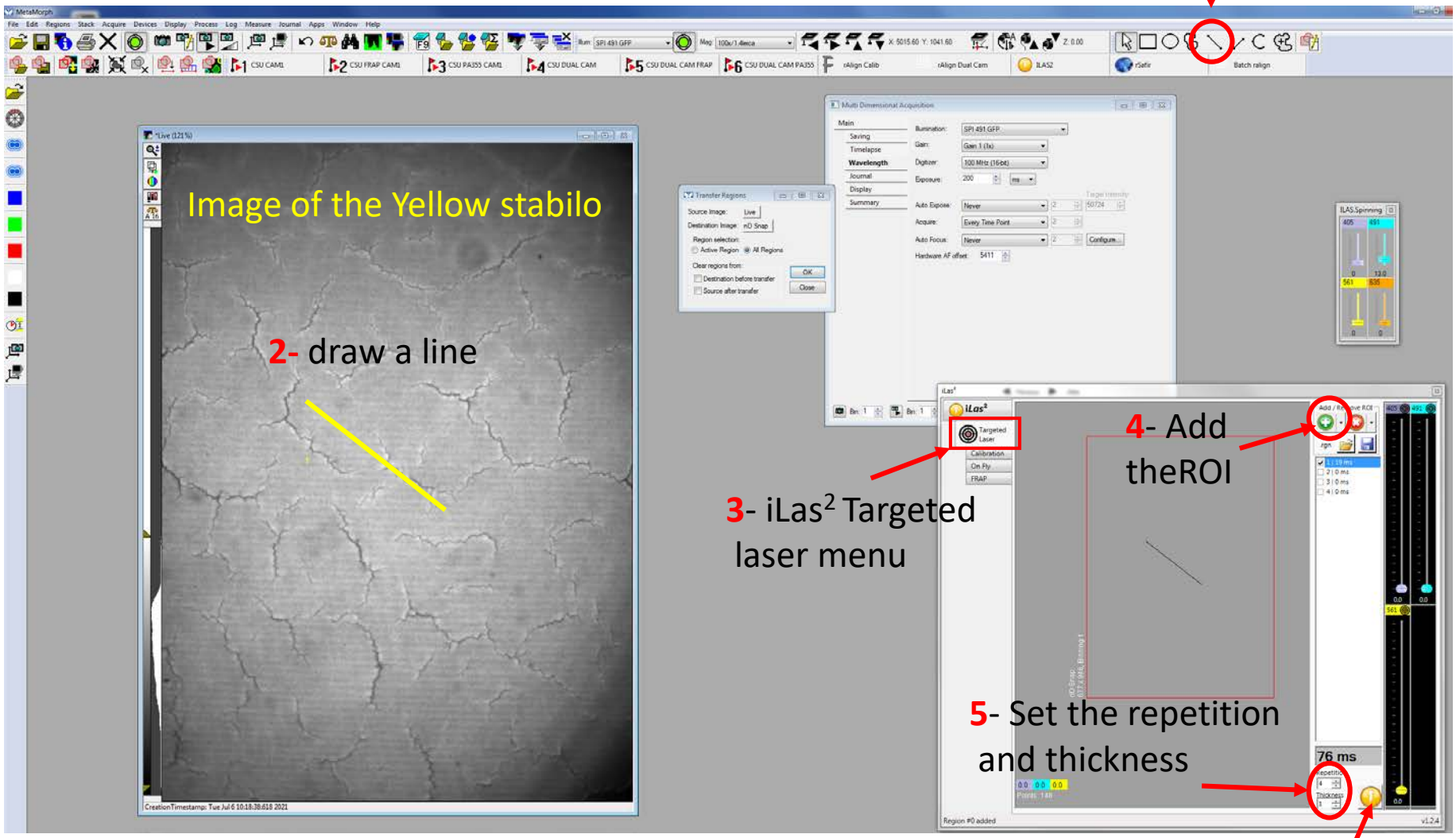
Press the start button for laser emission



The LED lights up when laser is ON
If the LED blinks, press the start button again

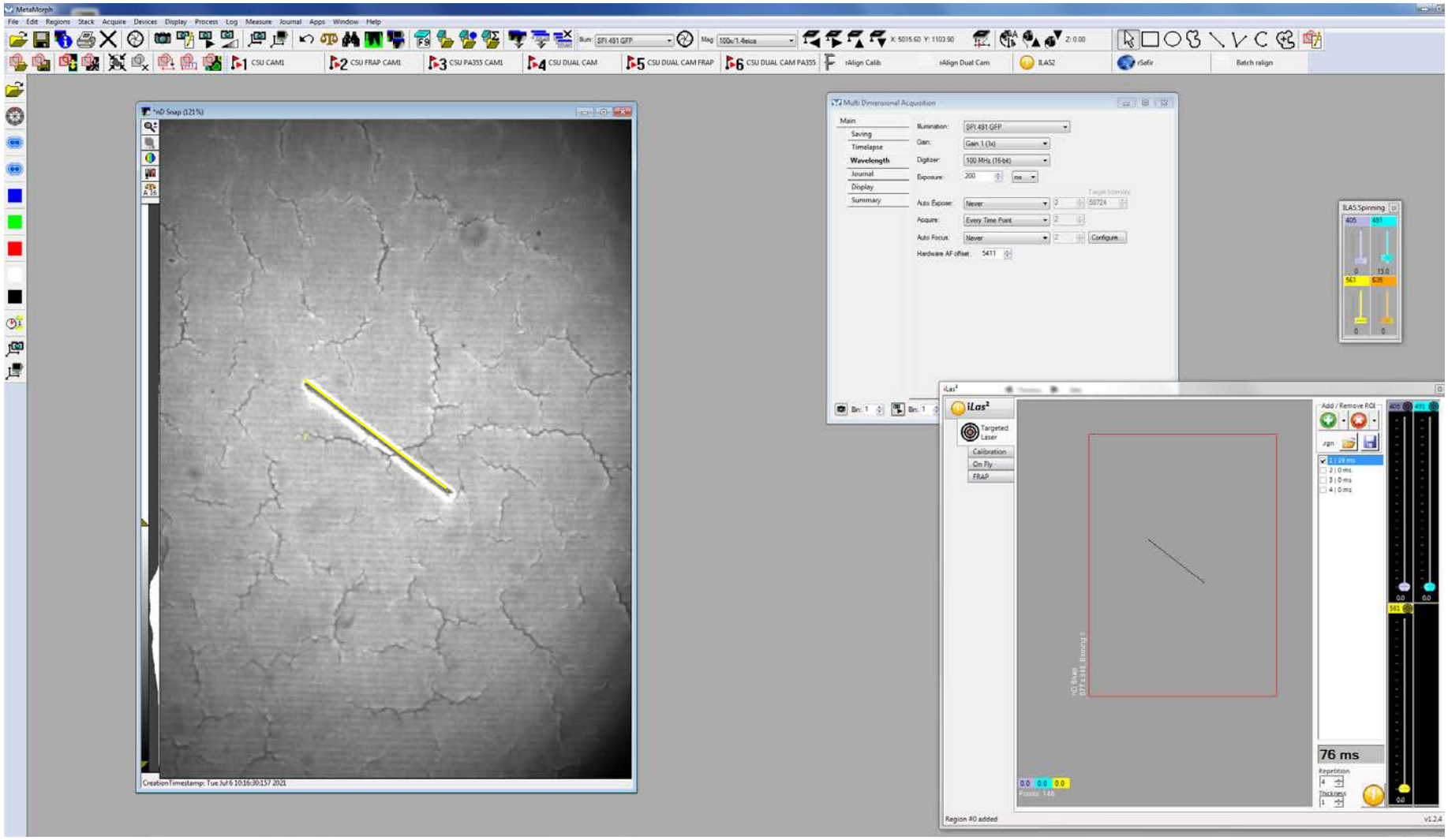
Check if the calibration setting is OK

1- select the line tool



Calibration test

If the calibration setting is OK, the damage occurs exactly at the position of the ROI line.

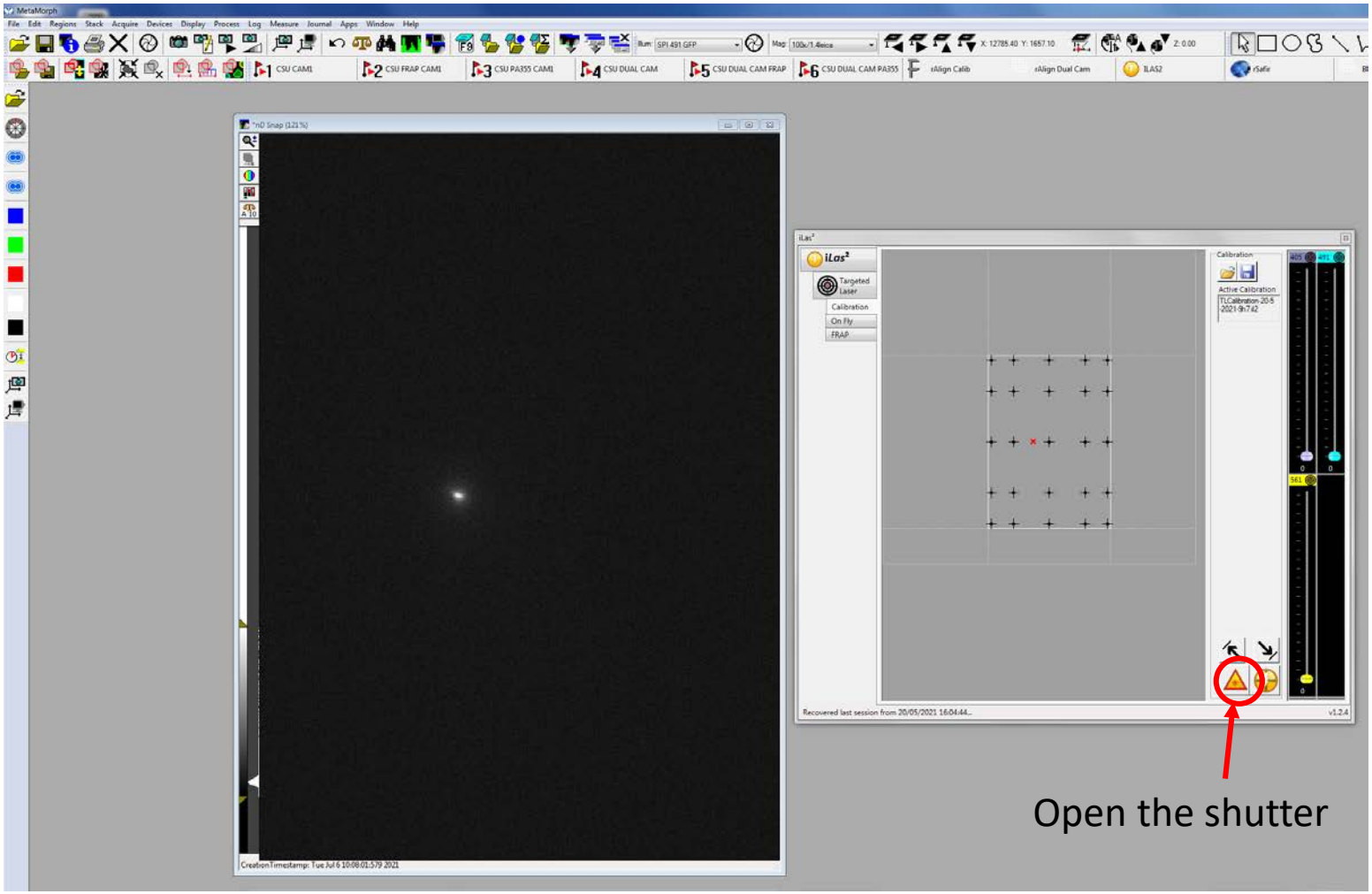


If a shift appears between the damage and the ROI line, run the calibration again.

How to run the calibration – step1

Do the focus on a sample (the coverglass with stabilo), then mode the stage to reach a position with no fluorescence.

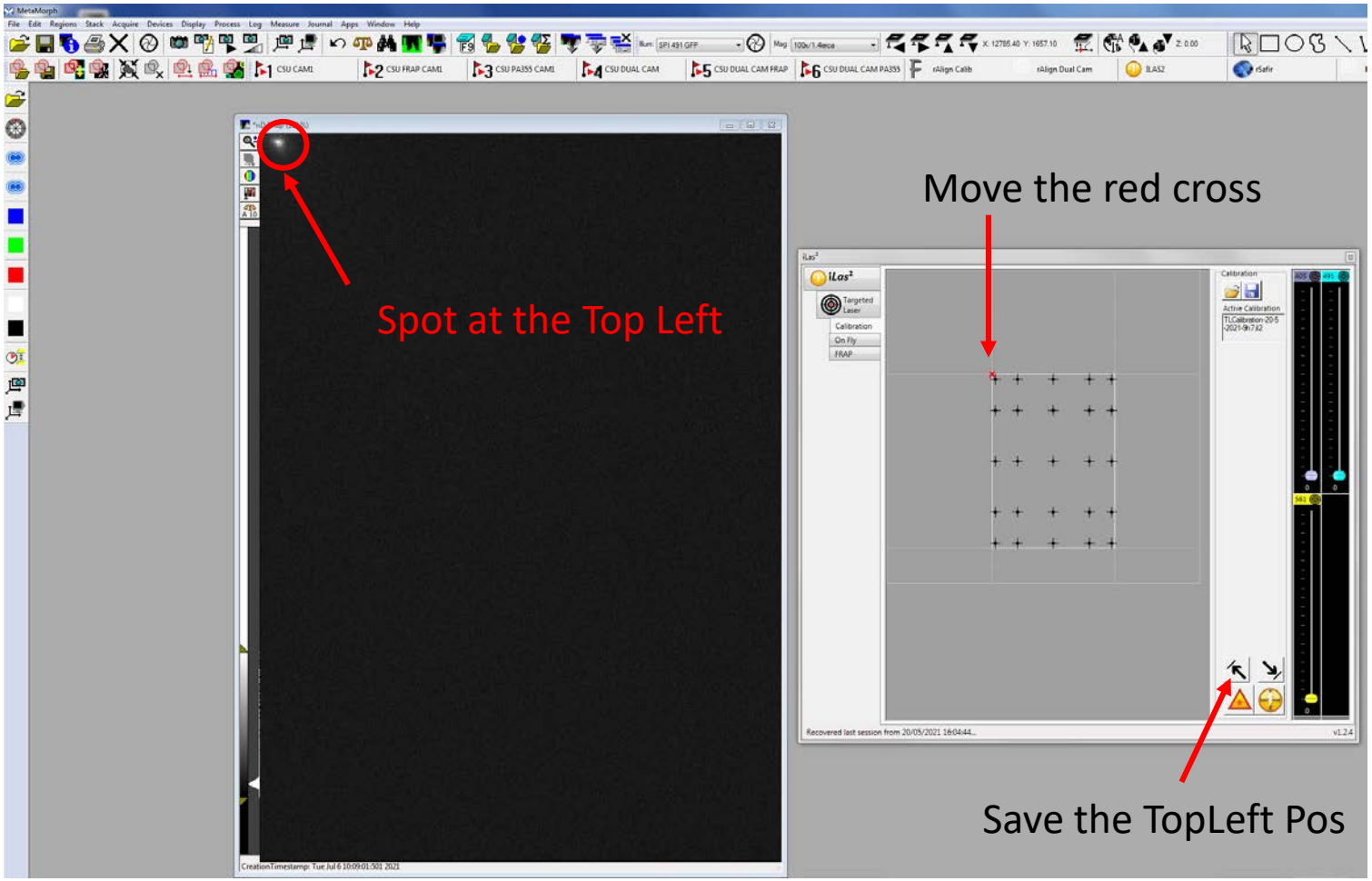
Open the shutter and adjust the focus to observe a thin UV spot



How to run the calibration – step2

Move the Red cross in the iLas2 window to put the laser sopt at the Top Left corner of the field of view

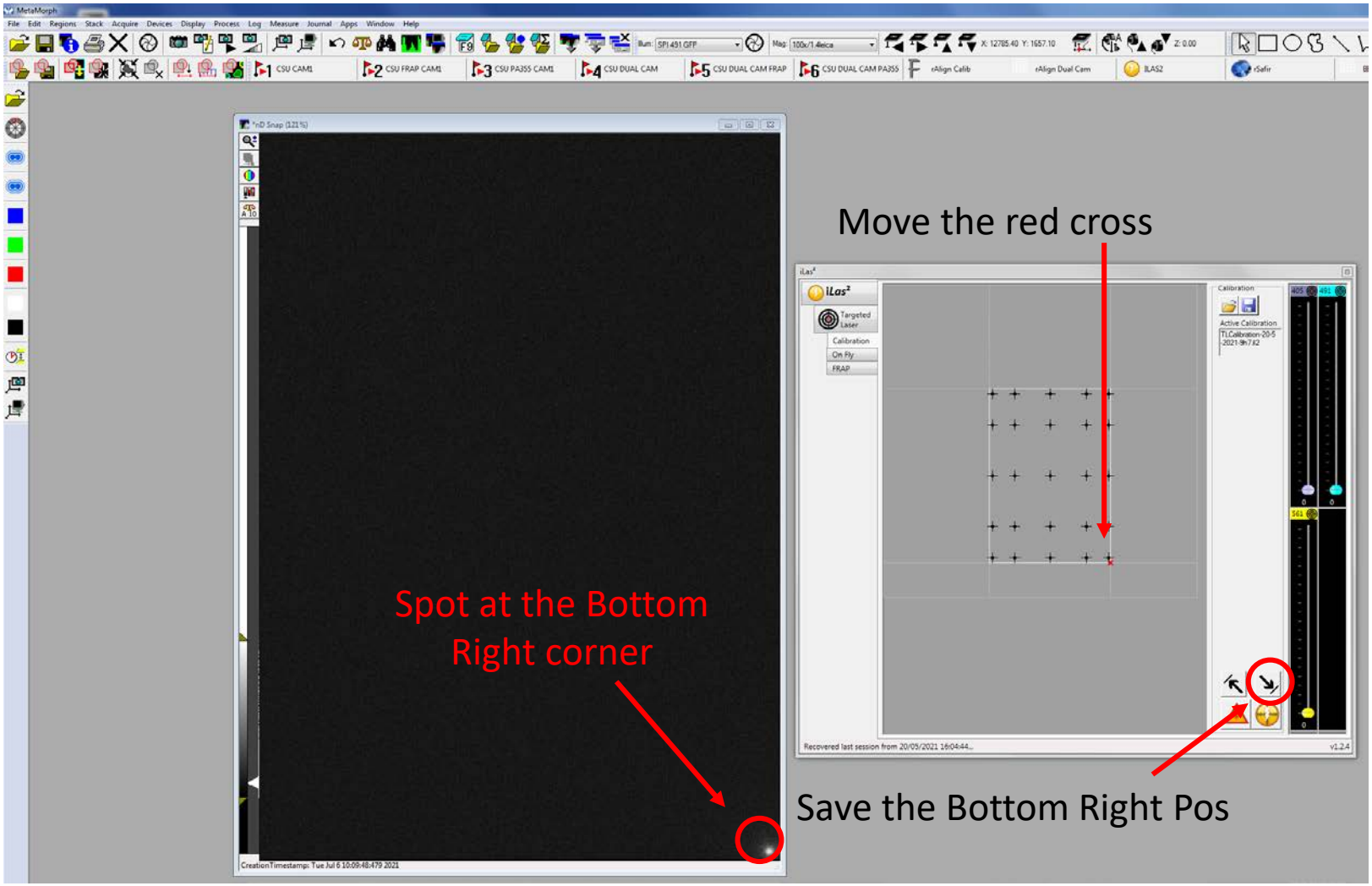
When done, click to save the Top left coordinate.



How to run the calibration – step3

Move the Red cross in the iLas2 window to put the laser sopt at the Bottom Right corner of the field of view

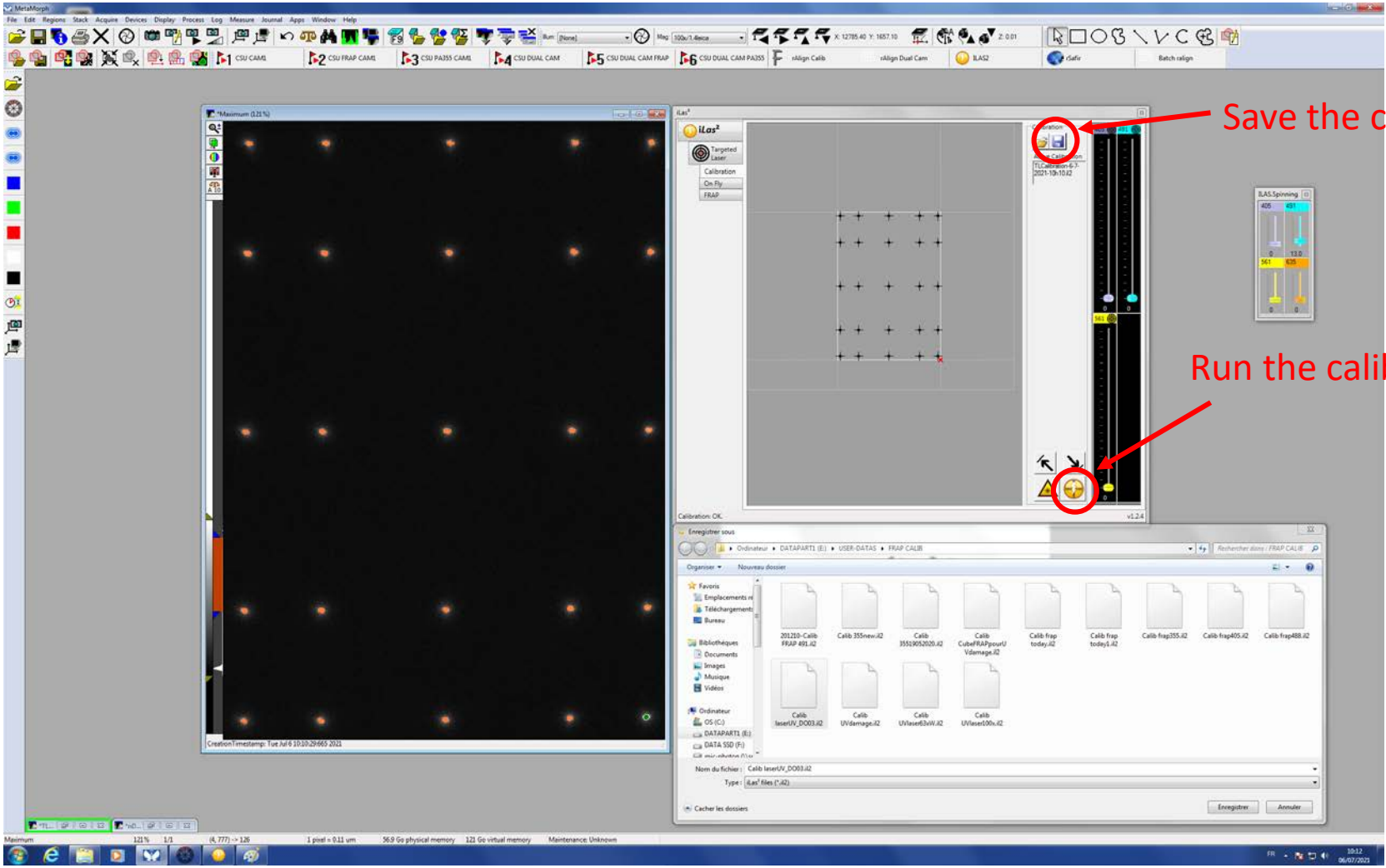
When done, click to save the Bottom Right coordinate.



How to run the calibration – step4

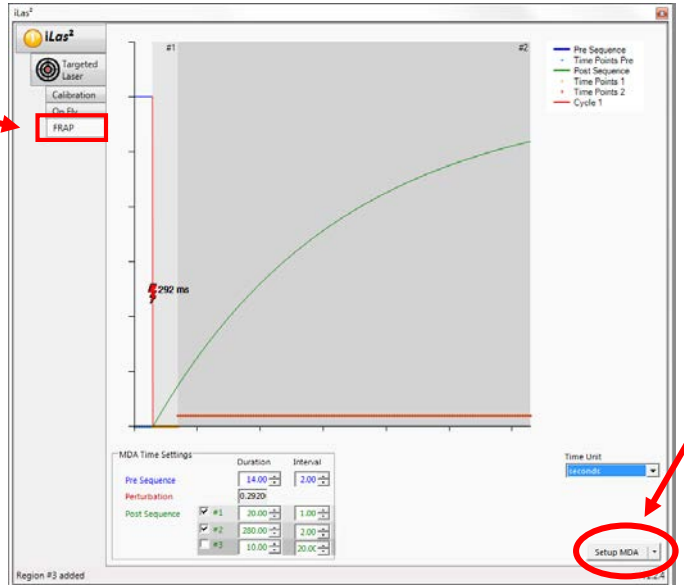
When done, Run the calibration. The sopt will automatically move to scan the entire field of view.

When done, save the Calibration.

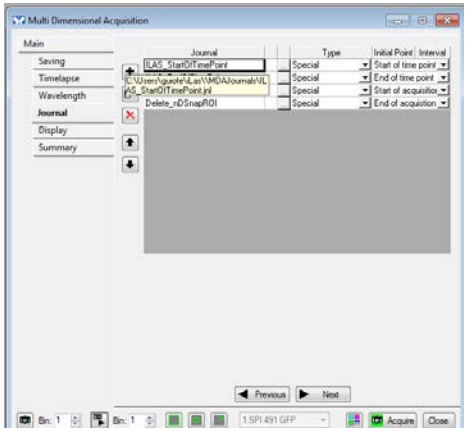


Set up your Frap Sequence

iLas^2 FRAP menu



Setup MDA



Using the iLas2 FRAP menu, you can set your complete sequence:

- duration and time interval before the FRAP
- duration and time interval after the FRAP

Don’t forget to “Setup MDA” after setting. This will call specific journals and transfer the defined parameters in the MDA window.

Ready to Run!

Everything is ready to run!

The only thing to do is to Delete old Roi and Add the new ones before run a new acquisition…