SPINNING DISK CSU-X1 FRAP USER MANUAL

Table of contents

Start up the incubator

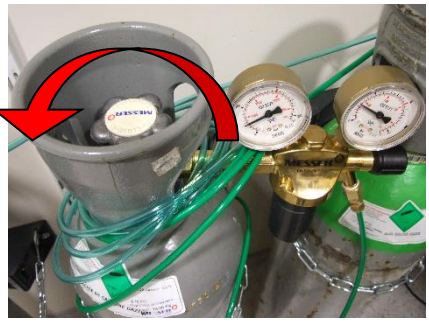
It is essential to switch on the temperature control system at least **one hour** before the start of the experiment. Once your sample has been loaded onto the microscope and the positions have been saved, in order to avoid significant loss of focus, you must wait a further 30 minutes, refocus on your positions and use the microscope's hardware focus function.

1- Switch on the switch on the wall.

2- Choose the temperature to be reached (shown in green) with the arrowed buttons. The current temperature is displayed in red.

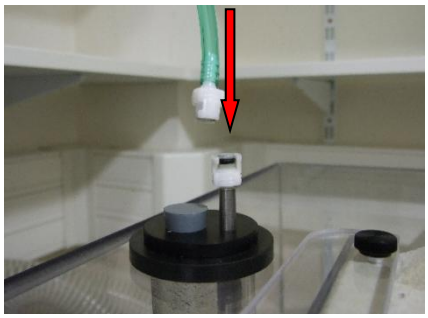
3- Place the sample holder in the incubator.

4- Open cylinders of compressed air (chrome-plated regulator) and of CO_2 (golden regulator) counterclockwise.



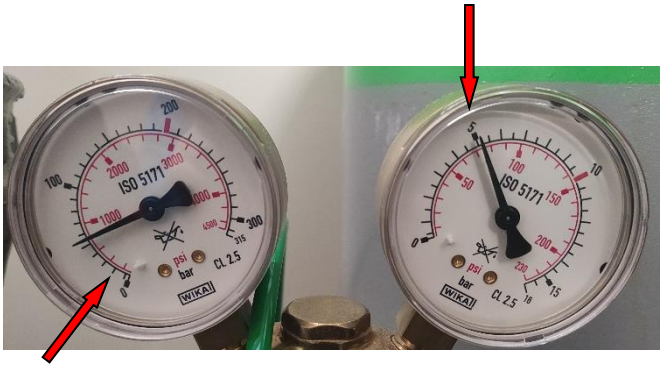
5- Switch on the CO_2 controller (red brick).

6- Connect the transparent green tube marked “MAIN out” to the water tank of the microscope incubator. Check that the water tank is full.



7- Place the CO_2 cover on the sample holder. Be careful not to pinch the pipes.

8- Check that the cylinders have a pressure of at least 10 bar (Pressure gauge on the left). Check that the outlet pressure (Right-hand pressure gauge) is 1 bar for the air and 5 bar for the CO_2. Otherwise, please **do not touch anything** and **contact one of the platform engineers**.



9- After few minutes, check that the CO2 level is at 5% on the brick.

Start up the Spinning Disk system

1- Switch ON the wall switch.

2- Start the piezo controller from the rear.

3- Switch on both cameras and wait a minute until the orange status lights stop blinking.

4- All parts must be started before switching ON the computer.

5- Use the “Utilisateur_X1-FRAP” session.

Image properties

Pixel size in the image with binning 1

Objectives	Pixel size in the image (nm)	Field of view (µm)
100x	91	103 x 76
63x	145	164 x 121
40x	229	259 x 191
25x	366	414 x 305
10x	916	1036 x 763

Optimal Z sampling

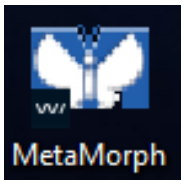
Refer to the table that you will find in the microscope room.

Dynamics of the image

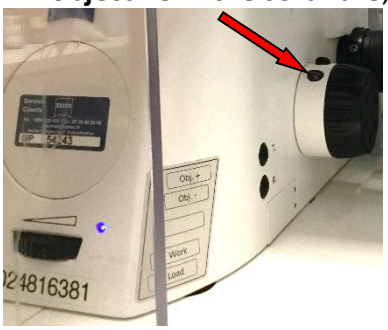
If the dynamic of the image is too low, increase the exposure time or the laser power.
But the maximum intensity of the image must never exceed 65,520 to avoid any saturation of the camera.

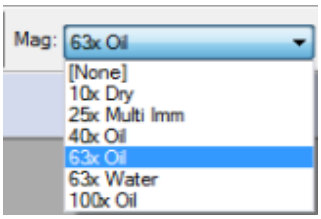
Start up MetaMorph software

1- Start the software “MetaMorph”.



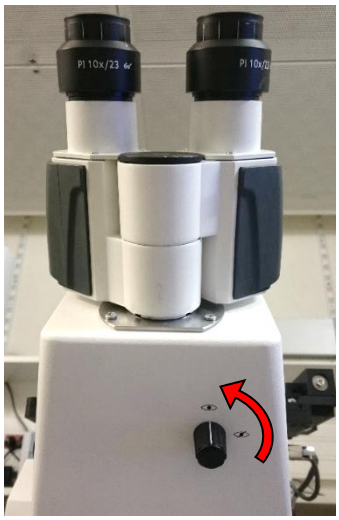
2- Lower the objective using the button on the right-hand side of the microscope and **select the objective in the software**, not on the microscope’s touchscreen.





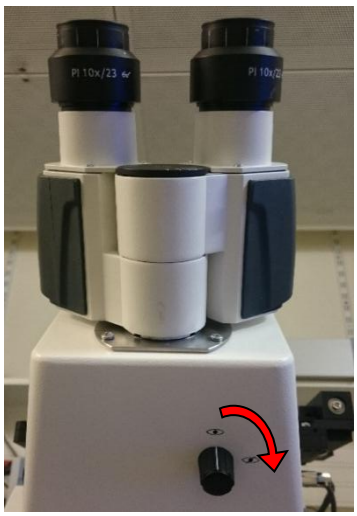
3- Place the sample older on the stage gently to avoid damage to the piezo and place the sample.

4- To look through the eyepieces, on the microscope, check that the safety knob is turned to the ‘eyepieces’ position and click on the binocular’s icon.



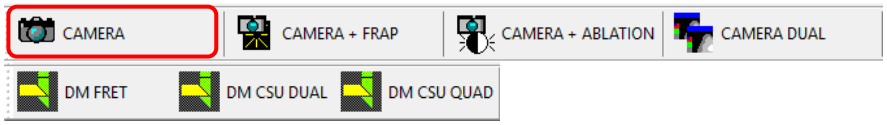
5- Adjust the focus on your sample.

Acquire images with one camera



1- On the microscope, check that the safety knob is turned to the “eyepiece closed” position.

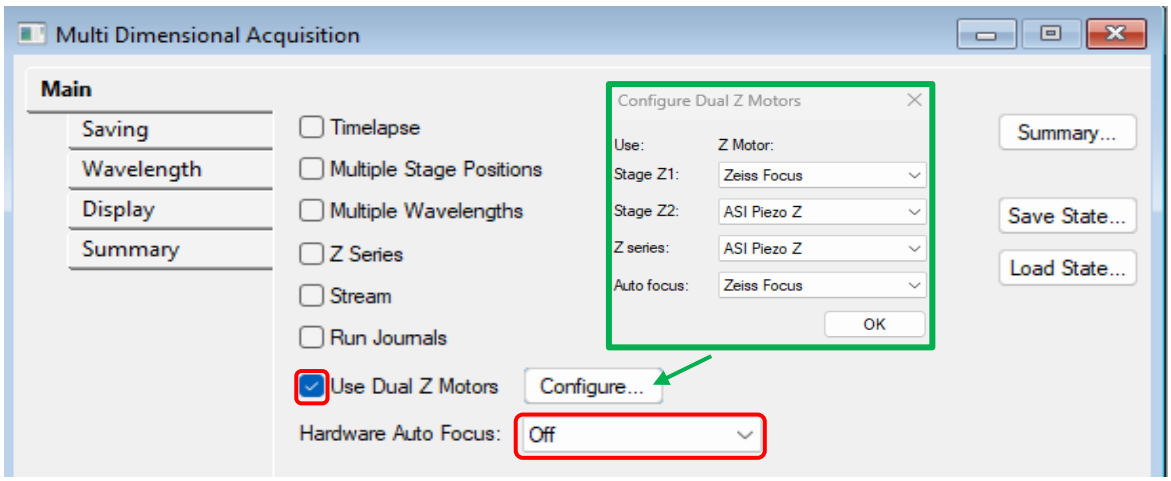
2- In MetaMorph, click on the CAMERA icon and choose the dichroic mirror among the three.



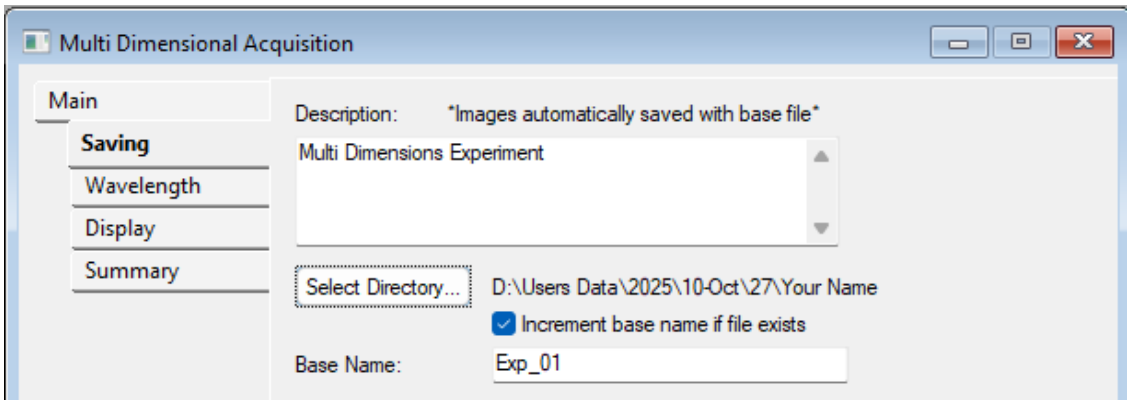
- DM FRET -> Cyan / Yellow for FRET experiment
- DM CSU DUAL -> Green / Red
- DM CSU QUAD (default setting) -> Blue / Green / Red / Far red

3- Open the “Multidimensional Acquisition” MDA window.

In the “Main” tab, verify that the “**Use Dual Z Motor**” option is selected and check its configuration. “**Hardware Auto Focus**” must be on “**Off**”.



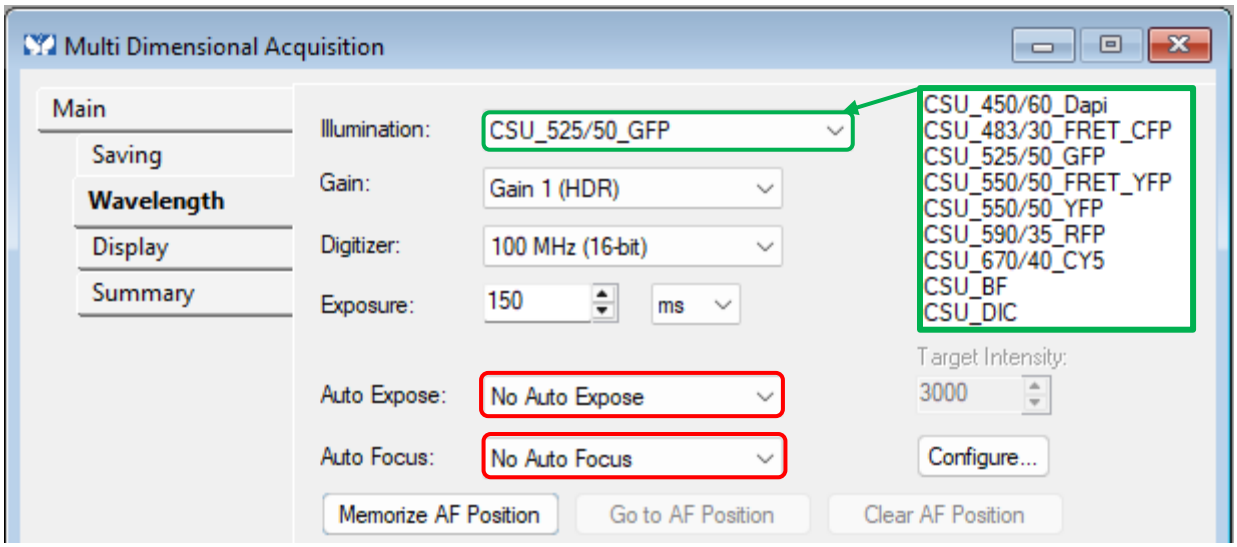
4- Click on the “*Saving*” tab in MDA. With the “*Select Directory…*” button, select your saving pathway: D: (UserData / year / month / day of the experiment / your name)



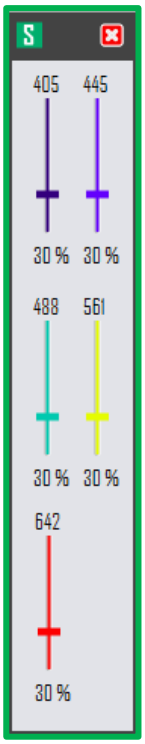
5- In “*Base Name*”, add the name of the experience followed by “_01” (this number will increment automatically). The images are automatically saved at the end of each acquisition.

Images saved elsewhere or older than 15 days will be deleted without notification.

6- Click on the “Wavelength” tab of the MDA.



7- In the “*Illumination*”, choose the type of transmission illumination (BF; DIC) or the emission filter for the fluorescence (DAPI, CFP, GFP, YFP, RFP, Cy5).

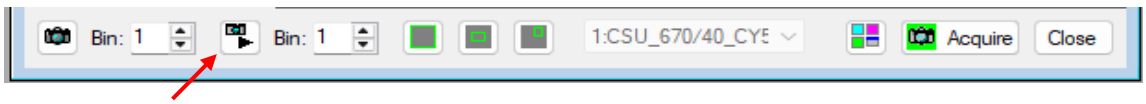


8- In the “*Exposure*”, choose the exposure time (start at 100 ms).

9- Disable “*Auto-focus*” and “*Auto-exposure*” options (No Auto Expose, No Auto Focus).

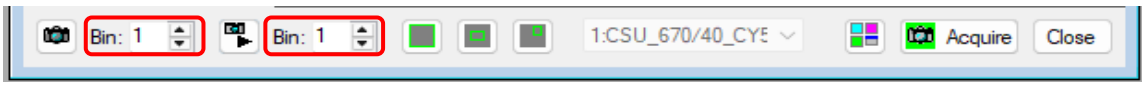
10- Choose the laser power for each channel.

11- Set the focus in Live with the microscope wheel. Check the dynamic of the image at the left and adjust the laser power and exposure time

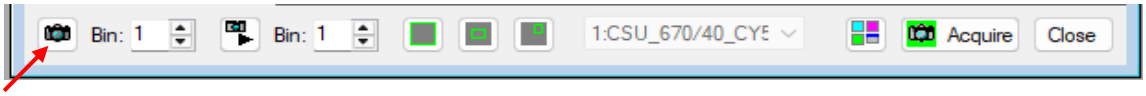


12- Move the stage with the joystick.

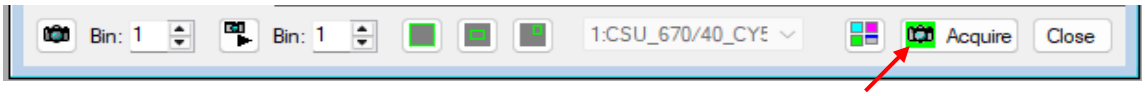
13- At the bottom of the window, set a binning of 1 for the camera.



14- Click on “Snap” and check the dynamic of the image to adjust the laser power and exposure time.



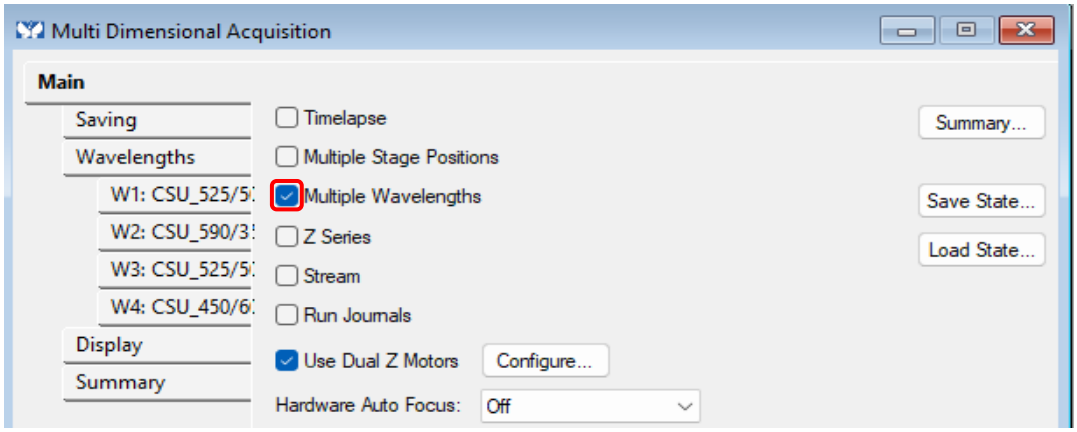
15- Click on “Acquire” to begin the acquisition.



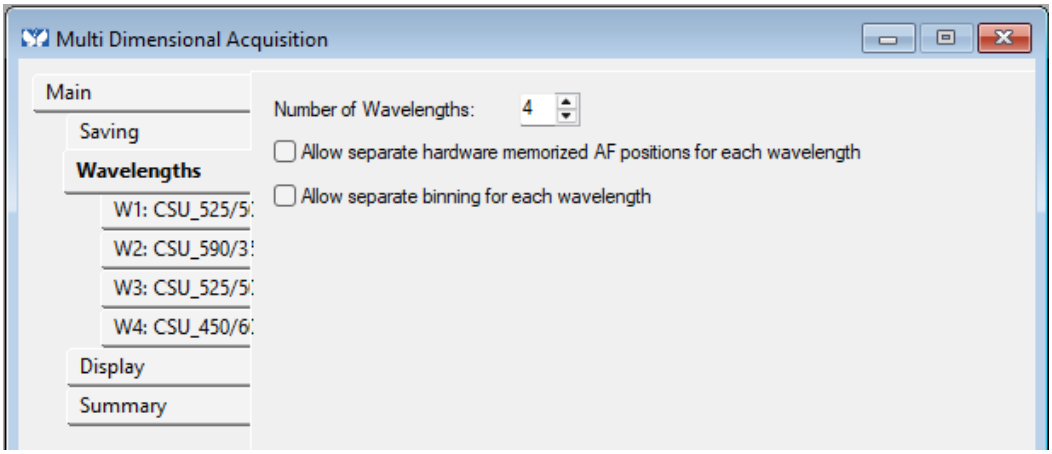
Acquire multiple wavelengths

Refer to the chapter "**Acquire images with one camera**" then:

1- In the "*Main*" tab in the MDA window, check "Multiple Wavelengths".



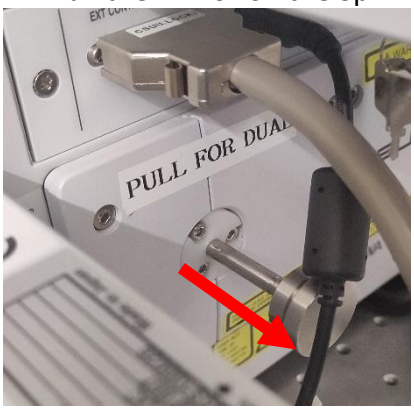
2- Click on the "*Wavelengths*" tab and select the number of colors to observe in "*Number of Wavelengths*".



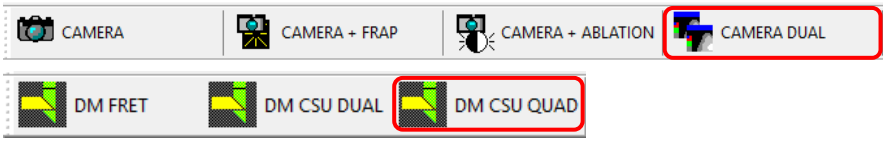
3- For each tab (W1, W2, …) you create, select the filters and then adjust the settings.

Acquire with two cameras (Dual Cam)

1- Pull the mirror on the Spinning Disk.

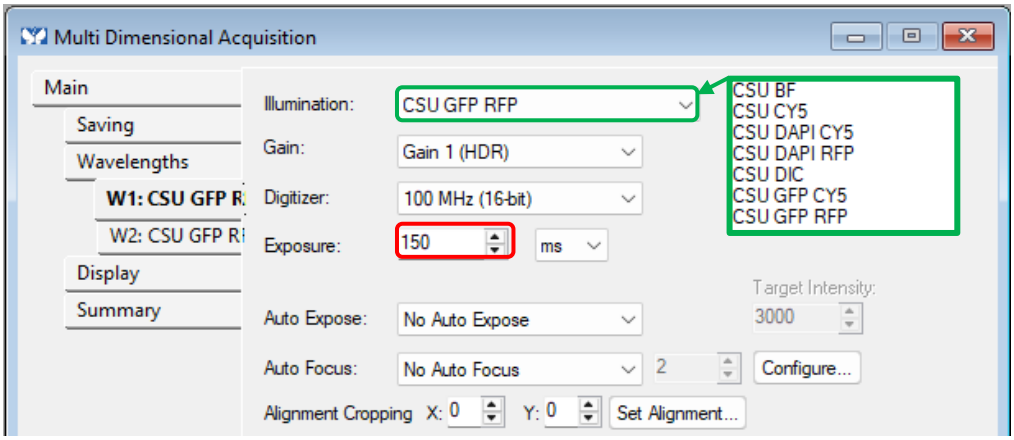


2- Refer to the chapter "**Acquire images with one camera**", but in the second step, click on the "CAMERA DUAL" icon and the "DM CSU QUAD" dichroic mirror.



3- Refer to the chapter "**Acquire with multiple colors**", but select an even number of colors in « Number of Wavelengths ».

4- For each group of colors created, in the « Illumination », choose the same emission filter for fluorescence (CSU) and the same exposure time.



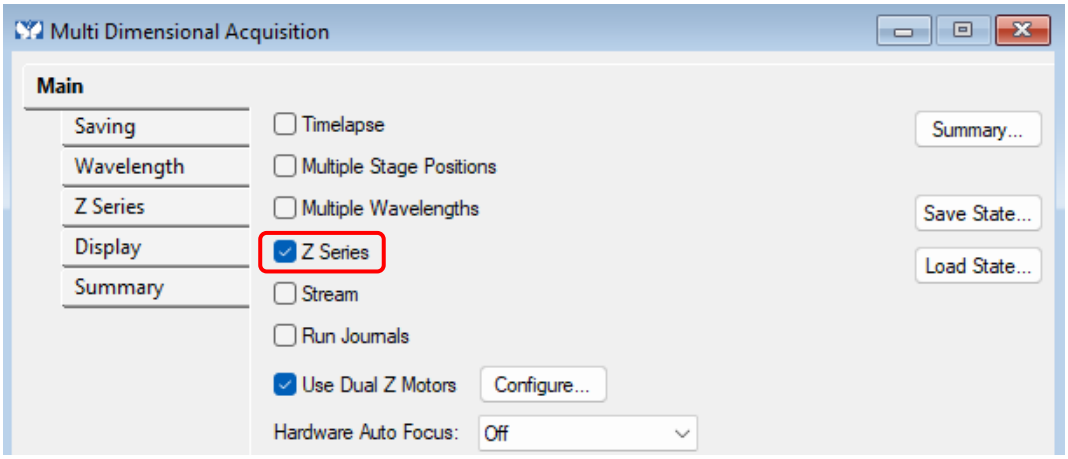
For example, W1 and W2 are for Green and Red with the two cameras, so they have the same illumination (CSU GFP RFP) and exposure time (150 ms).

5- Refer to the end of the chapter "**Acquire images with one camera**" from step 4.

Acquire a Z-stack

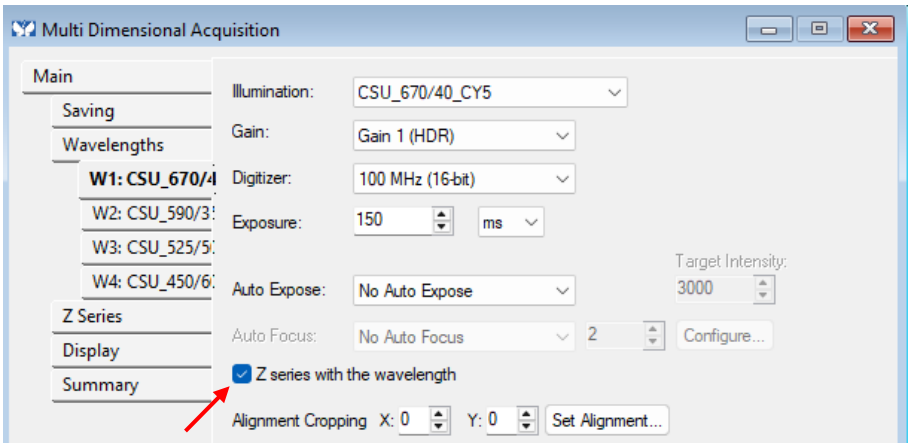
Refer to the chapter "**Acquire images with one camera**" then:

1- In the "Main" tab in the MDA window, check "Z Series".



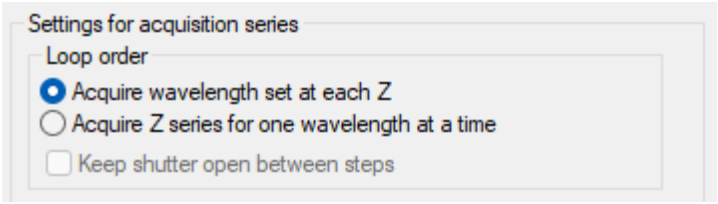
2- Focus on the sample using the microscope's focus knob

3- Check that "Z series with the wavelength" is ticked for each channel. Otherwise, only a single Z-plane image will be acquired for that channel.



The laser power and exposure time must be set on the **most intense plane** of the Z-stack.

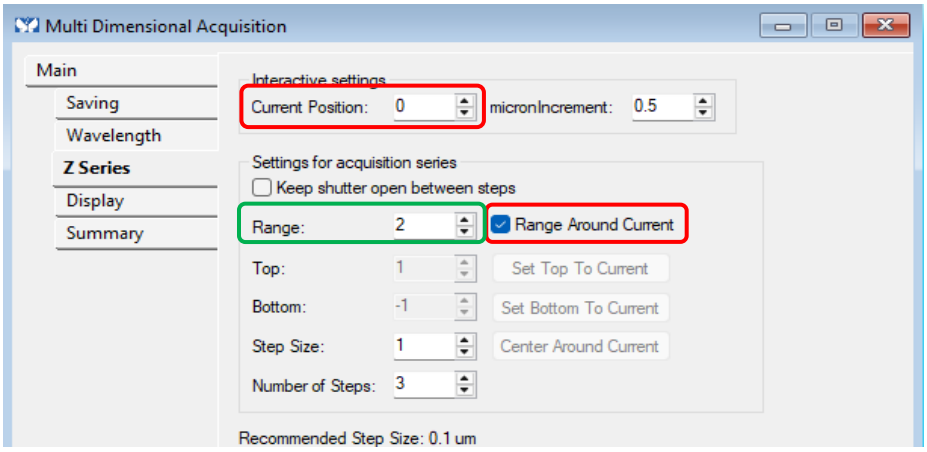
4- If you are using multiple wavelengths, you must choose between two options.
"Acquire a set of wavelengths at each Z position": this prevents any misalignment between wavelengths for samples moving quickly.
"Acquire a Z series for a single wavelength at a time": faster acquisition.



In the “Z-series” tab, you can define your Z-stack in two different ways.

<u>To set the current focus point as the centre of the Z-stack.</u>

5- Check “Range Around Current” and put the “current position” value to 0.



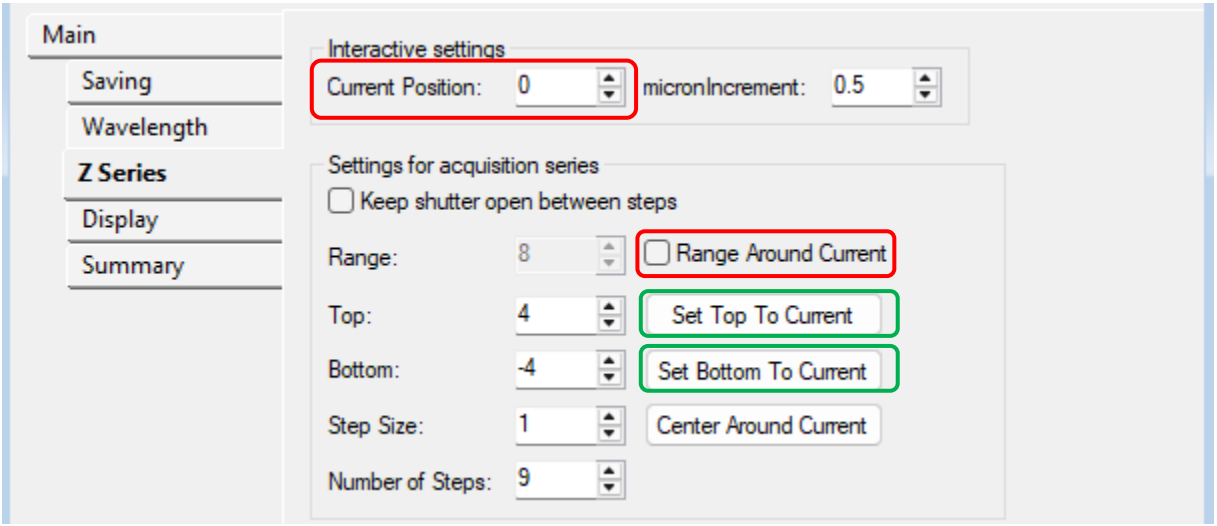
6- Define the total size (µm) of the stack in “Range” and the distance (µm) between two acquisitions in Z « Step Size ».

<u>To set the Z-stack top and bottom</u>

5- Uncheck “Range Around Current”.

6- Change the focus with the piezo stage “Current Position” until the upper limit of the stack and click on “Set Top to Current”.

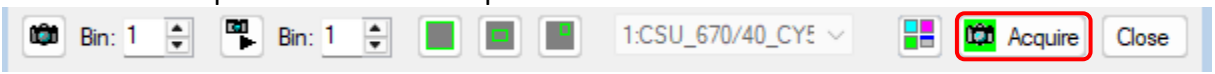
7- Change the focus with the piezo stage until the lower limit of the stack and click on « Set Bottom to Current ».



The piezo stage can only acquire up to 150 µm. The “Bottom” value can’t be lower than -75 µm and the “Top” value can’t be higher than +75 µm.

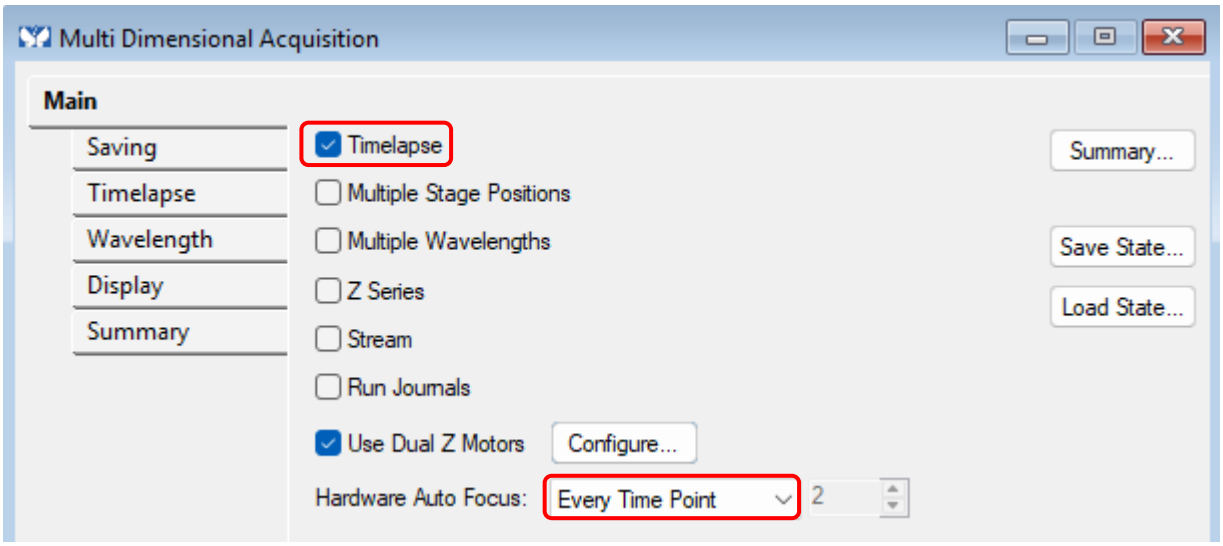
8- Set the distance between two acquisitions “Step Size”.

9- Click on “Acquire” to start the acquisition.

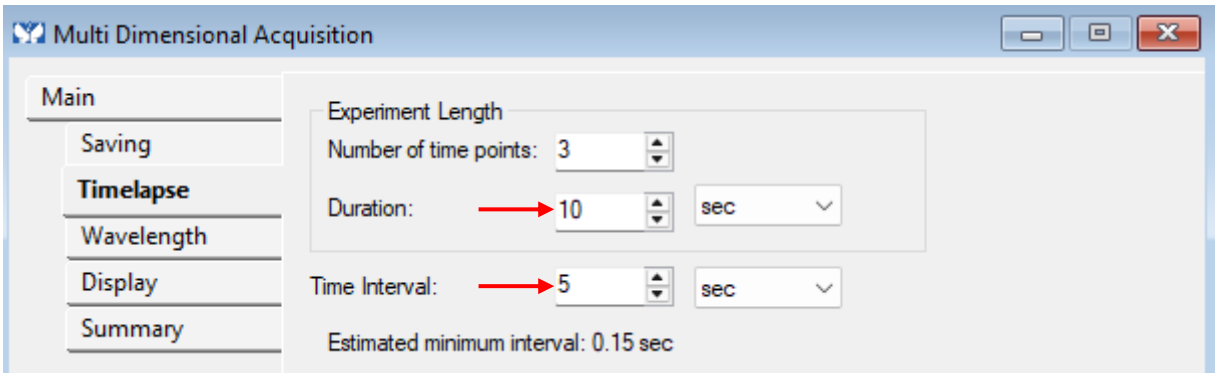


Acquire a time series and use of the auto focus

1- In the « Main » tab in the MDA window, check “Timelapse” and choose “Every Time Point” for the Hardware Auto Focus.

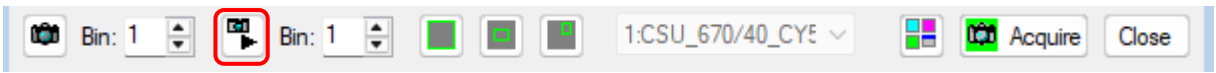


2- In the « ***Timelapse*** » tab, select the duration as well as the time interval.



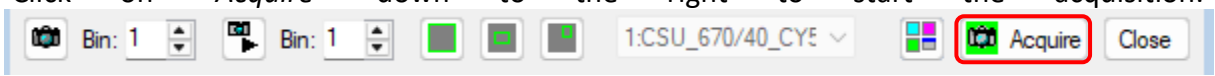
Warning: Be sure that the chosen interval is **enough** to make the entire acquisition (exposure time + readout time + time it takes to save the image). **Don’t trust the “Estimated minimum interval”!**

3- Click on « *Live* » and set a fine focus using the piezo stage.



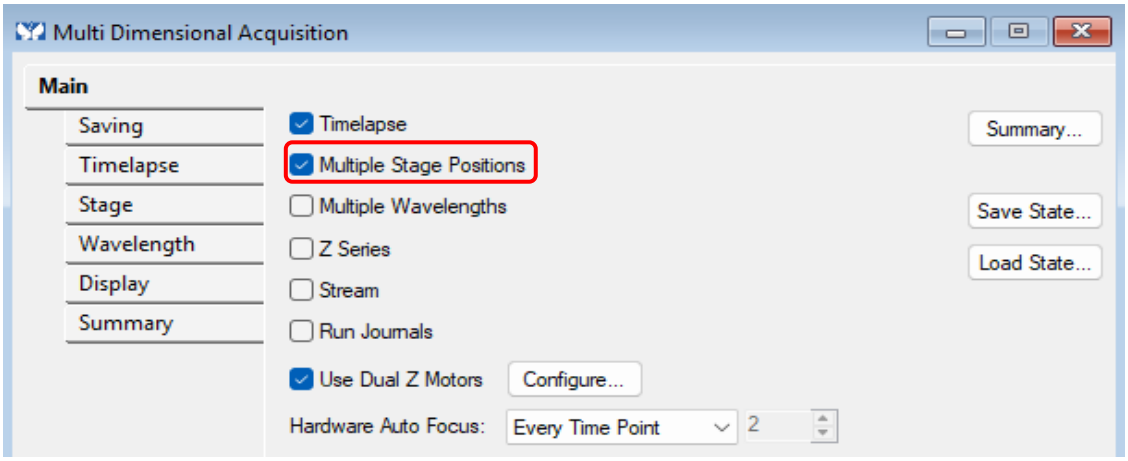
4- Refer to the chapter « **Acquire images with one camera**» or “**Acquire multiple wavelengths**” for the Wavelength tab.

5- Click on “*Acquire*” down to the right to start the acquisition.

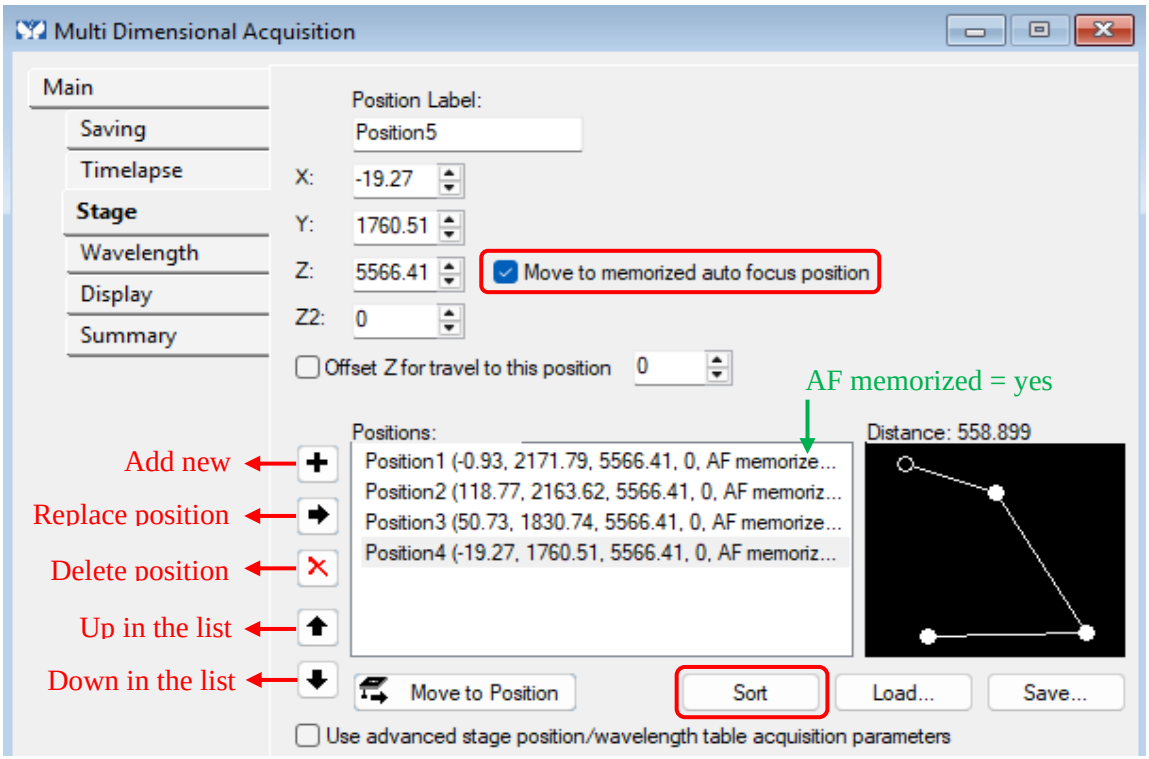


Multiple stage positions and use of the auto focus

1- In the “Main” tab of the MDA window, check “Multiple Stage Positions”and choose “Every Time Point” for the Hardware Auto Focus.



2- In the “Stage” tab, the position number (or name) is displayed in the “Position Label” window. The “Move to memorized auto focus position” must be checked.



3- You can add a new position, replace or delete an existing position, or move a position in the list using the graphic tools on the left.
In the list, you see the **name**, the **coordinates** of the positions, and after “**AF memorized =**”, you need to see “**yes**”.

4- After you select all the positions, click on “Sort” to choose the fastest pathway to do the acquisitions.

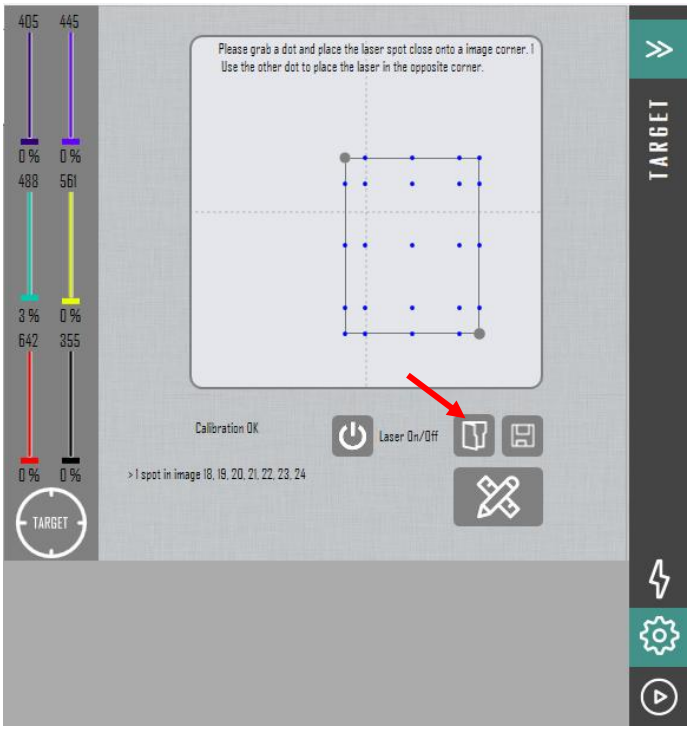
5- Click on “Acquire” down to the right to start the acquisition.

Calibration for FRAP

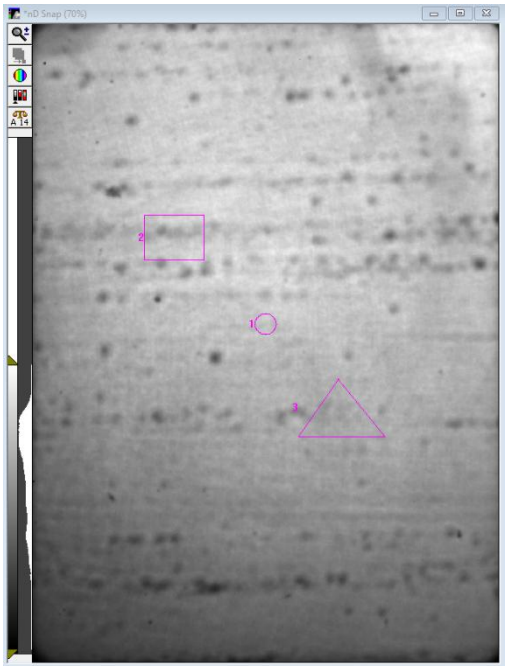
1- Take the yellow fluorescent slide from the shelf, place it in the carrier, and focus on it.

2- In Modular, click on the settings icon 

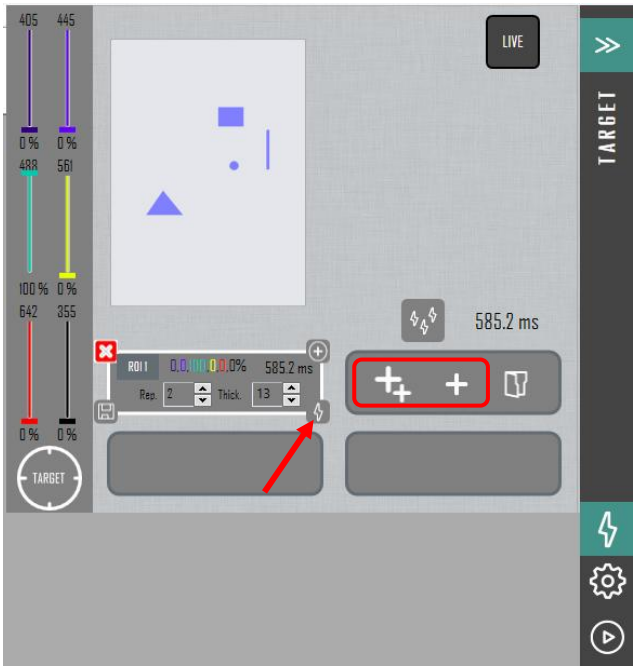
3- Load a previous calibration if it exists (and if it's not old) with the file icon.

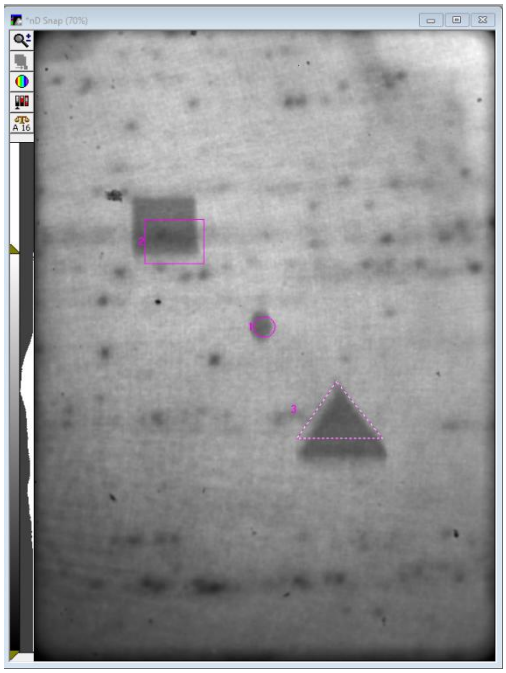


4- Do a snap and draw some areas.



5- Go in the tab, load the areas by clicking on ; then verify the calibration by doing FRAP in the areas, using around 40% or more laser power (**red arrow**). Check the quality of the FRAP. Here is an example of a wrong calibration.

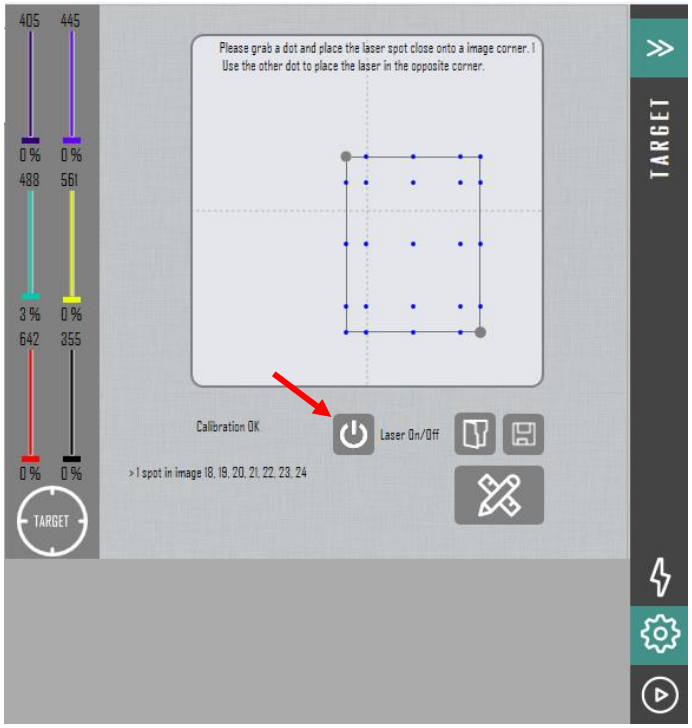




If the calibration loaded is wrong, redo the calibration.

6- Go back to the settings tab

7- With a laser power around 3%, do a Live, then click on the power button in Modular. Do the following steps:

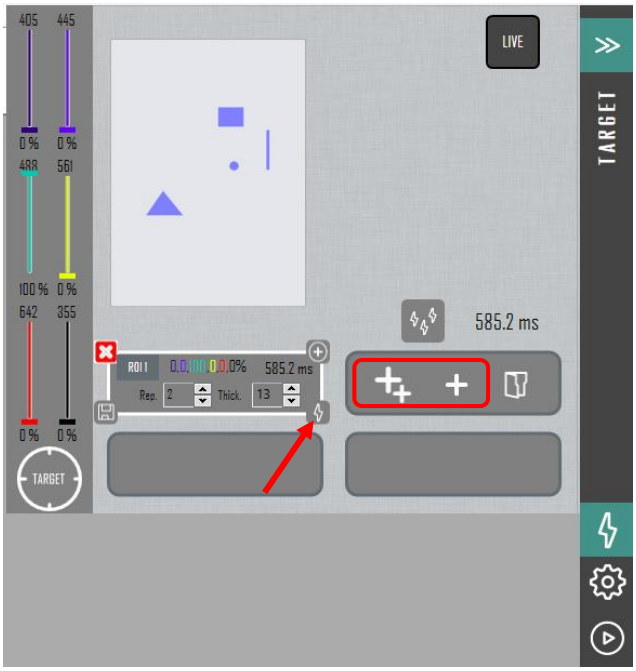


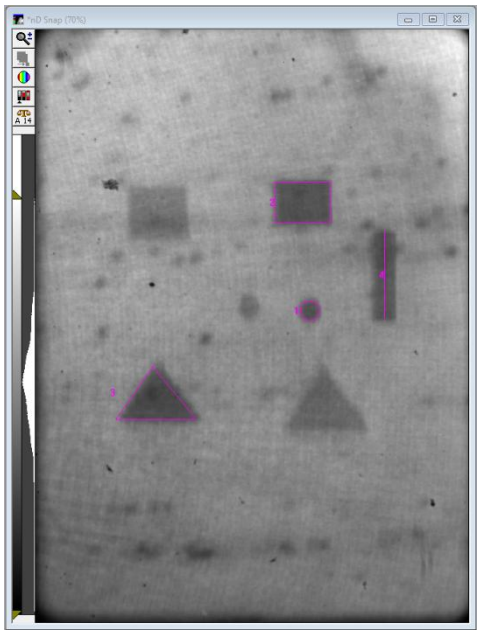
Select the first dot in the upper-left corner and place it close to the border of the image you see on live. Do the same with the dot in the bottom-right corner. It should look like this:

Then click on and start the calibration.

8- Do a new snap and draw some new areas.

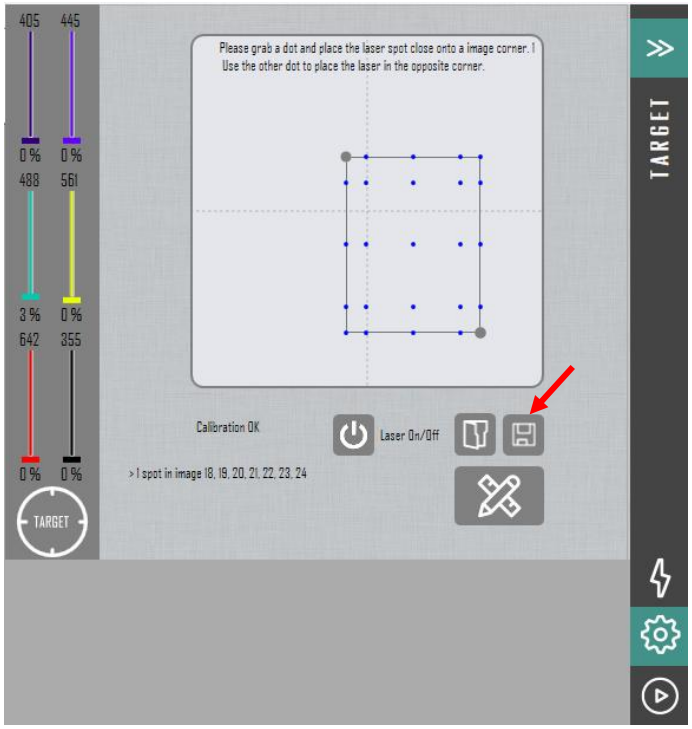
9- Go in the tab, load the areas by clicking on ; then verify the calibration by doing FRAP in the areas, using around 40% or more laser power (**red arrow**). Check the quality of the FRAP. Here is an example of a good calibration.





If the calibration is still not good, ask engineers for help.

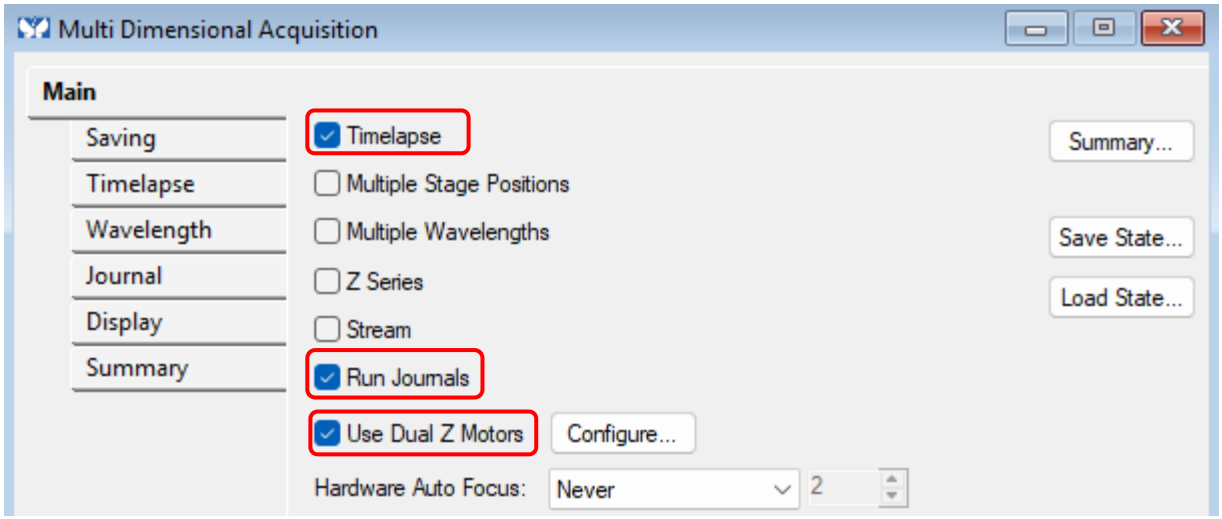
If the calibration is good, you can save the calibration and start your experiment.



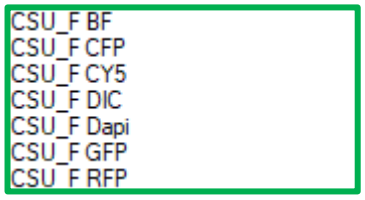
Use the FRAP module

1- In MetaMorph, select CAMERA + FRAP.

2- In the MDA window, check “Timelapse”, “Run Journals”, and “Use dual Z Motors”.

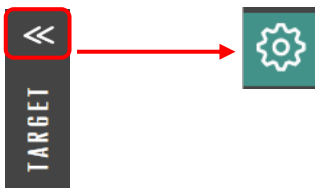


3- In the “Wavelength” tab, select the illumination you want in the list.



4- In the “Journal” tab, the table must be empty.

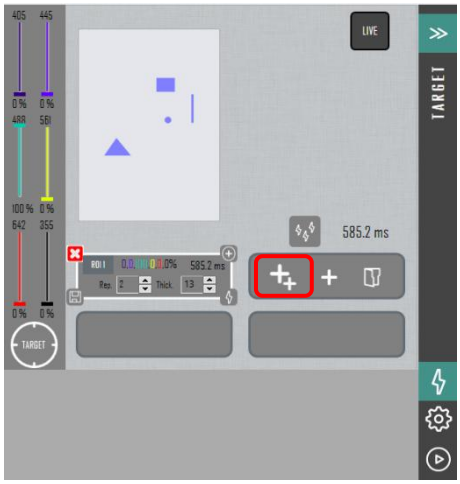
5- In Modular, click on “**<<**” and then the **parameter** icon.



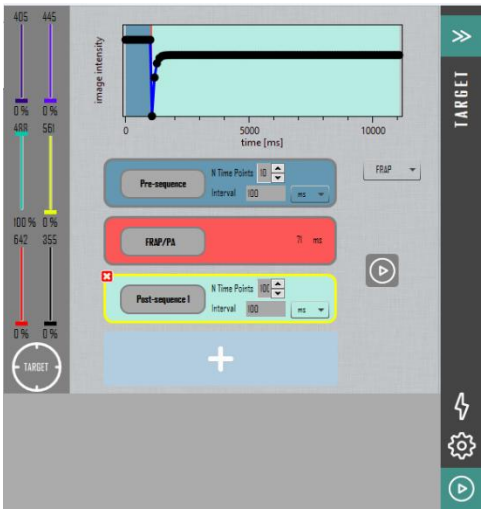
6- This opens a window; you can calibrate the laser now. To do so, refer to the chapter “**Calibration for FRAP**”.

7- When the calibration is done, put your sample and focus where you want.

8- Do a Snap and draw the zone you want to do FRAP.
In Modular, in the tab, click on to place the zone you drew.



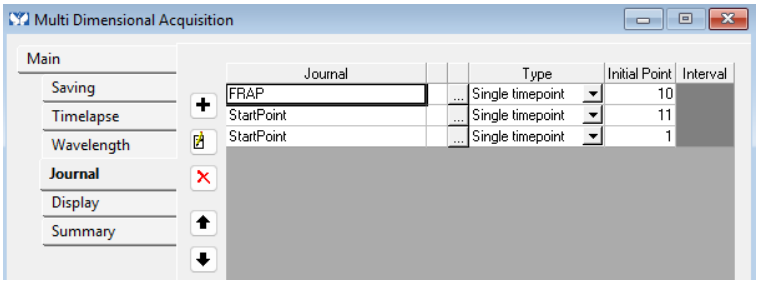
9- In Modular, click on Select the number of time points before and after FRAP. Be careful, you need enough time points to have a good fit of the FRAP curve.



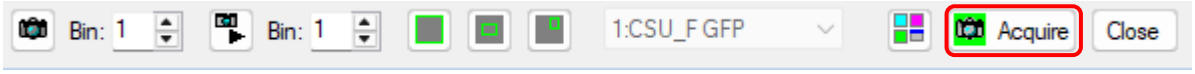
Here we did:

- Before FRAP: 10 images, one every 100 ms.
- After FRAP: 100 images, one every 100 ms.

10- Click on to transfer the information in the MDA.



11- Click on Acquire to start the acquisition.



Switch off the Spinning Disk system

1- **Clean the objectives** and the sample holder.

2- Close Modular software.

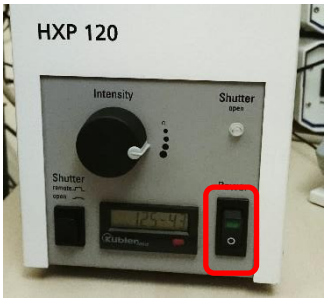
3- Close MetaMorph software.

4- Transfer your data.

5- Shut down the computer.

6- Turn off the piezo controller from the rear.

7- Close the lamp for fluorescence.



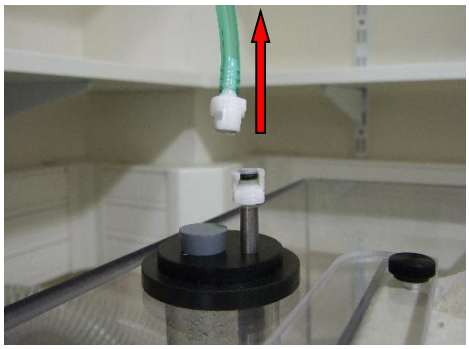
8- Close the two cameras.

9- Switch off the wall switch.

Switch off the incubator

1- Remove the CO_2 cap. Be careful not to break the glass cover.

2- Unclip the greenish pipe that you will find on the water supply in the system.



3- Switch off the red brick and then the switch on the wall**.**

4- Close cylinders of compressed air (silvery regulator) and of CO_2 (golden regulator) clockwise.