



SPINNING DISK CSU-X1 USER MANUAL

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Starting the temperature controller

1- Light on the temperature controller with the switch on the wall.



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

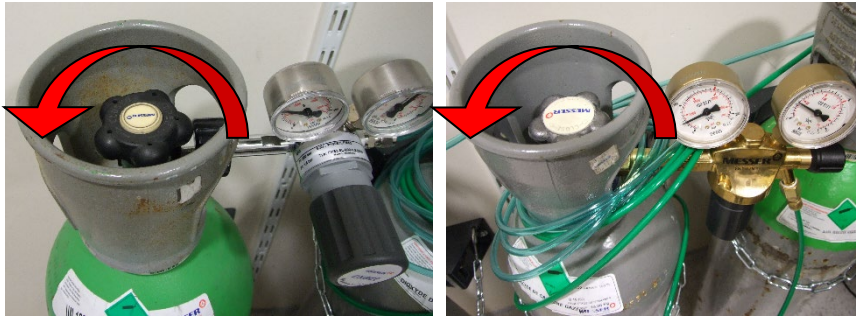


3- Place the sample holder in the incubator.

It is important to switch on the temperature one hour before the beginning of the session. Once your sample installed on the microscope and the positions registered, to avoid an important loss of focus, it is necessary to wait one more hour and to redo the focus of your positions or to use the definite focus of the microscope.

Starting the CO₂ controller

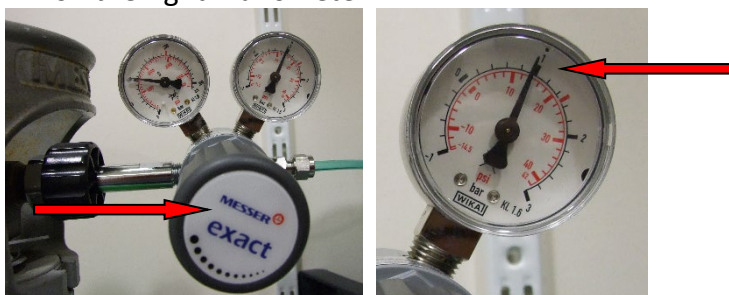
- 1- Open cylinders of compressed air (silvery regulator) and of CO₂ (golden regulator) counterclockwise.



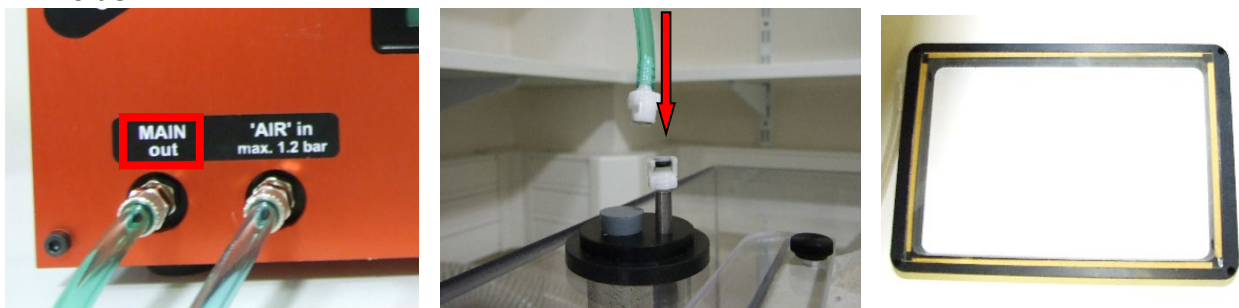
- 2- Switch on the controller of CO₂ (red brick) with the switch on the wall.



- 3- With the thumb wheel, fit the pressure of the air regulator (silvery regulator) to obtain 1 bar on the right manometer.

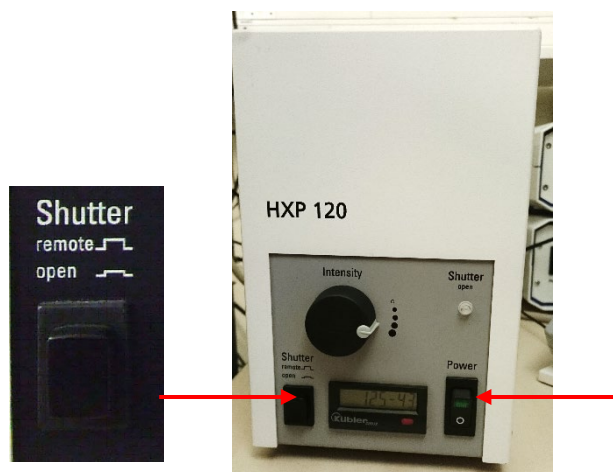


- 4- Connect the green transparent pipe bound with the release of the CO₂ controller (MAIN out) with water supply of the incubator of the microscope. Place the cover for CO₂ on the sample holder.



Start the spinning disk

- 1- If needed, start the fluorescent lamp.
Shutter button should be in remote position.



- 2- Switch ON all the different elements of the system by using the wall switch.



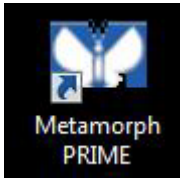
- 3- Start the piezo controller from the rear.



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

Sample observation with the oculars

1- Start the software « *MetaMorph* » with the chosen camera.



1- Click on the type of illumination you want to use Brightfield (grey) or fluorescence (color).



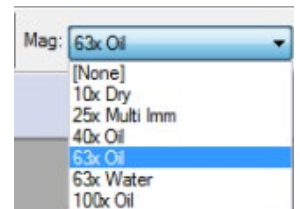
2- On the microscope, check that the button is turn on ocular.



3- Lower the objective with button on the microscope and chose the objective in the software,

4- Put the sample older on the stage gently to avoid damage on the piezo.

5- Put the sample and do the focus.



Spatial sampling, pixel size, dynamics of the image

Pixel size of the cameras

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.

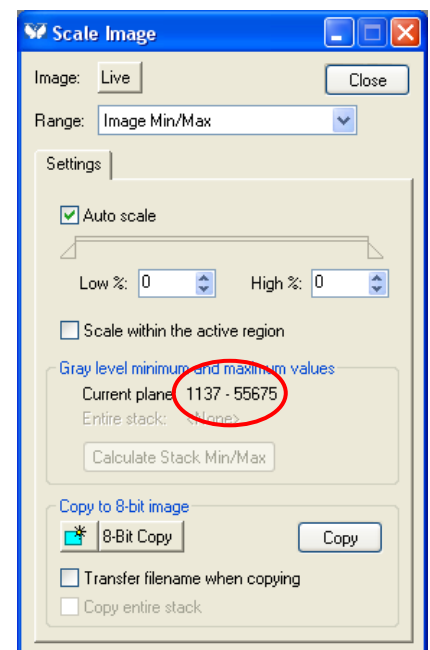
Dynamics of the image

Here, the image dynamics corresponds to the difference between the maximum and the minimum grey level of the image.

The more a labelled sample is illuminated, the more we photo-bleach it. You will have to find the right compromise between the quality of the signal (signal to noise ratio) and the preservation of the sample.

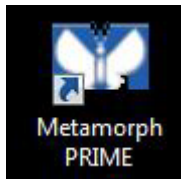
The maximum intensity of the image must never exceed 65 520 to avoid any saturation of the camera.

If the dynamic of the image is too low, increase the exposure time or the laser power.



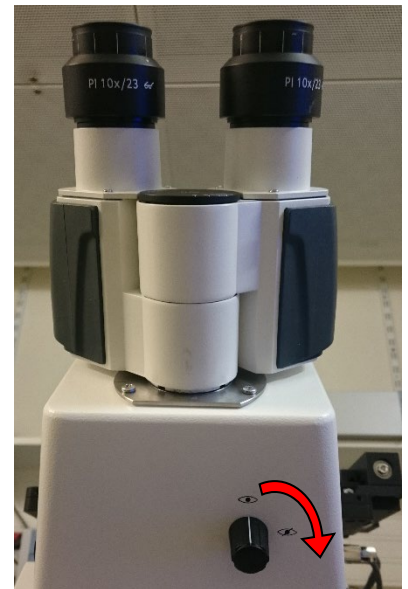
Acquire an image with one camera

- 1- Start the software *MetaMorph* with the PRIME icon.

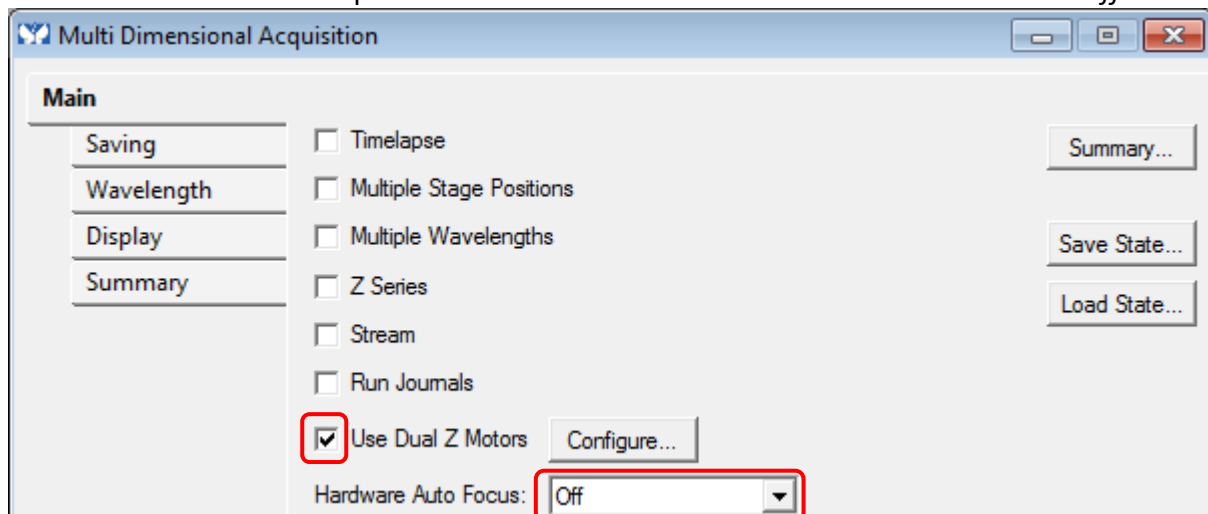


On the microscope, turn the safety catch to close the eyepieces on the front of the microscope

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



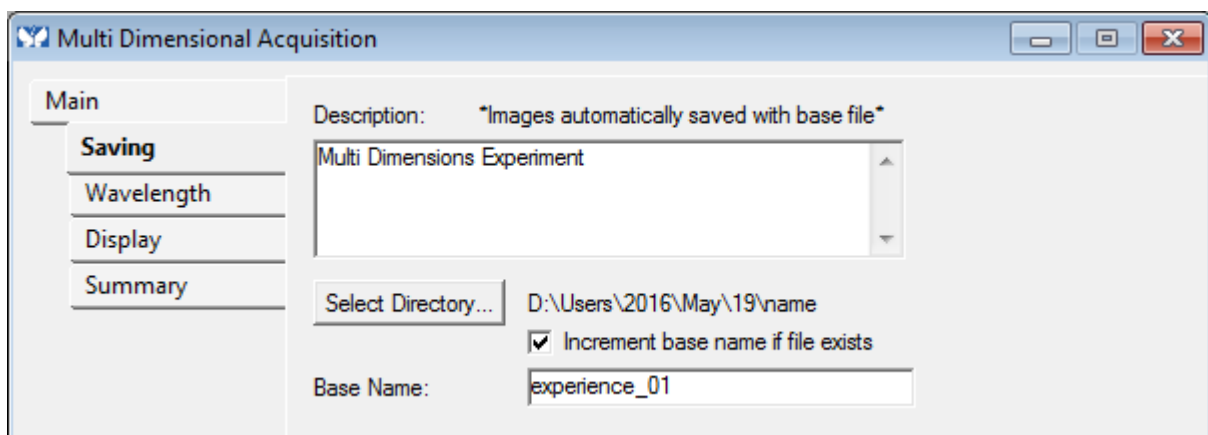
- 3- Open « *Multidimensional Acquisition* » MDA window. In the tab « *Main* ». Verify that only the « *Use Dual Z Motor* » option is selected. « *Hardware Auto Focus* » must be on « *Off* ».



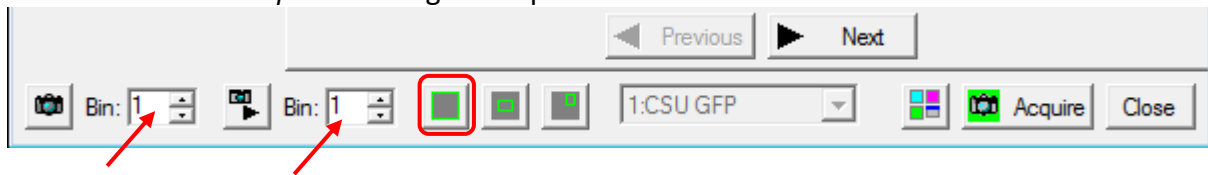
- 4- Click on the « *Saving* » tab in MDA.

With the « *Select Directory...* » button, select your saving pathway:

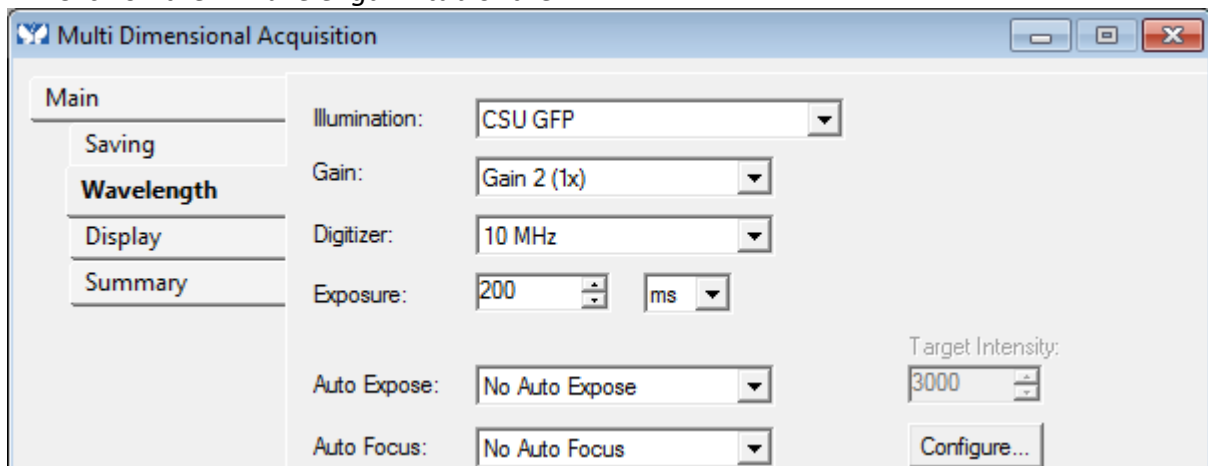
D: (MMUsers / year / current month / day of the experiment / your name) Images saved elsewhere or older than 15 days will automatically be deleted without any notifications.



- 5- In the « *Base Name* » window, add the name of the experience follow-up with -01 (this number will increment automatically). The images are automatically saved at the end of each acquisition.
- 6- Below the window, set a binning of 1 for the camera.
Click on « *Full chip* » the left green square is set to use the entire field of view of the camera.

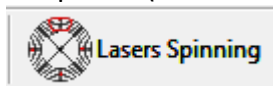


- 7- Click on the « *Wavelength* » tab of the MDA.



- 8- In the « *Illumination* » window, choose the type of transmission illumination (BF; DIC) or the emission filter for the fluorescence (Dapi, GFP, GFP narrow, RFP, Cy5).
- 9- In the « *Exposure* » window, choose the exposure time (start at 100 ms).
- 10- Disable « *Auto-focus* » and « *Auto-exposure* » options (No Auto Expose, No Auto Focus).

- 11- Choose the laser power for each channels.



- 12- Set the focus with the microscope wheel or use the buttons



- 13- Move the stage with the joystick or the buttons



- 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.

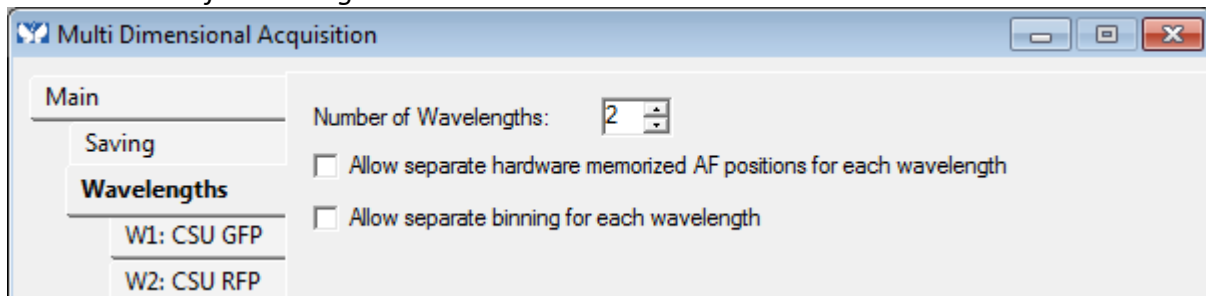
Acquire multiple colors

Refer to the chapter « acquire an image with the camera» but add those steps:

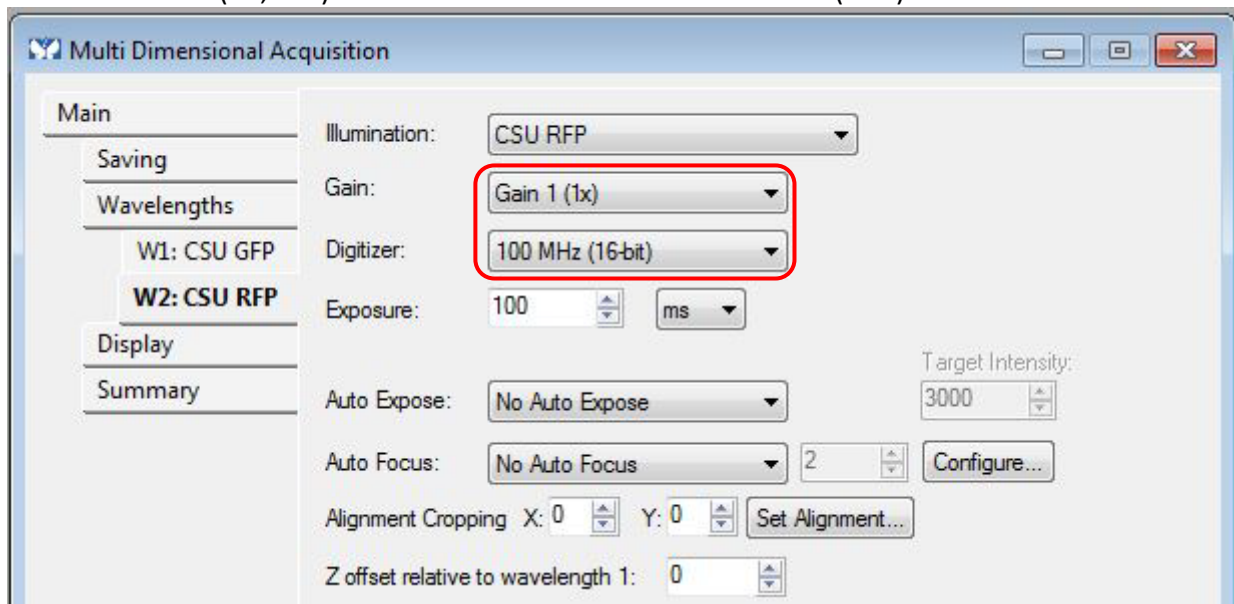
1- In the « *Main* » tab in the MDA window, select « *Multiple Wavelengths* ».



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



3- For each tab (W1, W2,...) created, in the « *Illumination* » window, choose the type of illumination (BF; DIC) or the emission filter for fluorescence (CSU)



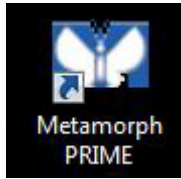
4- For each tab (W1, W2,...) created, in the « *Illumination* » window, choose the type of illumination.

5- Set a laser power and exposure time (which can differ) for each channel.

6- Click on « *Acquire* » down to the right to start the acquisition.

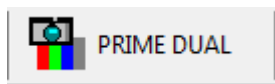
Acquire an image with Dual Cam

- 1- Start the software *MetaMorph* with the PRIME icon.

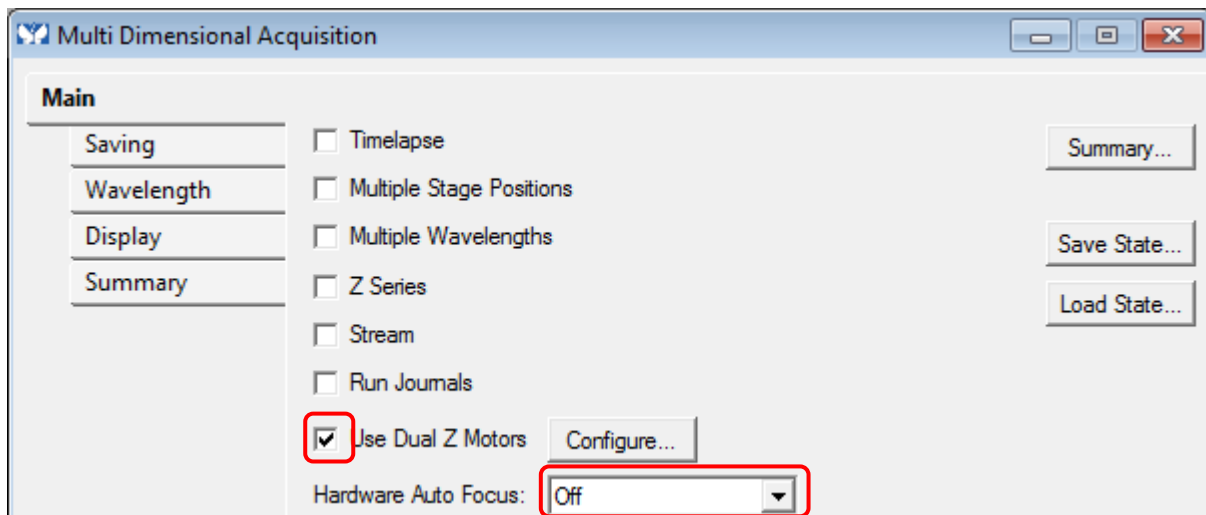


- 2- Pull the mirror on the Spinning Disk.

- 3- In MetaMorph, click on PRIME DUAL icon and choose the dichroic mirror among three.



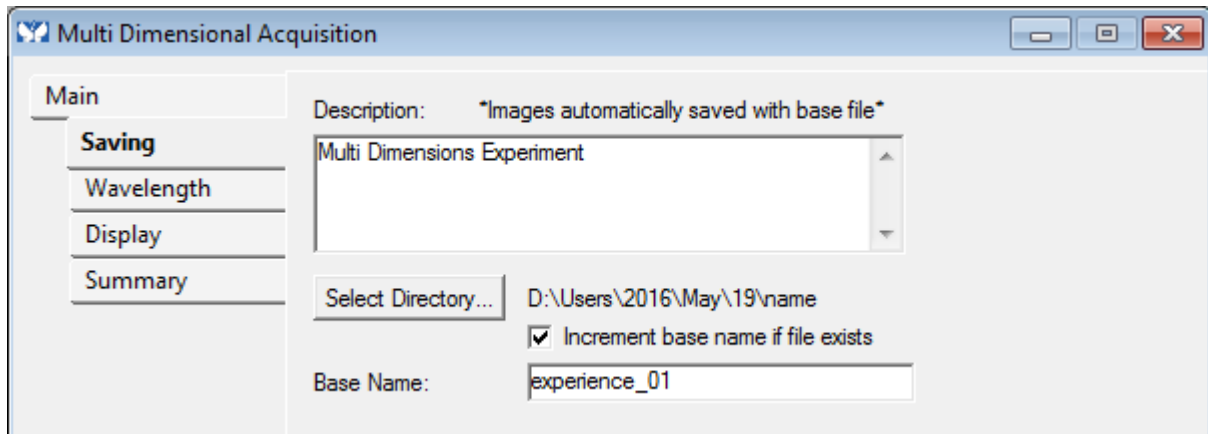
- 4- Open « *Multidimensional Acquisition* » MDA window. In the tab « *Main* ». Verify that only the « *Use Dual Z Motor* » option is selected. « *Hardware Auto Focus* » must be on « *Off* ».



5- Click on the « *Saving* » tab in MDA.

With the « *Select Directory...* » button, select your saving pathway:

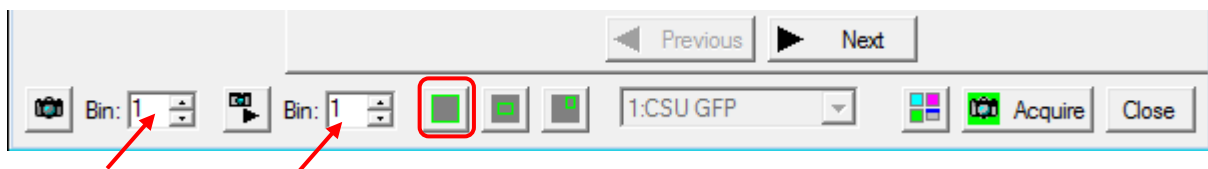
D: (MMUsers / year / current month / day of the experiment / your name) Images saved elsewhere or older than 15 days will automatically be deleted without any notifications.



6- In the « *Base Name* » window, add the name of the experience follow-up with -01 (this number will increment automatically). The images are automatically saved at the end of each acquisition.

7- Below the window, set a binning of 1 for the camera.

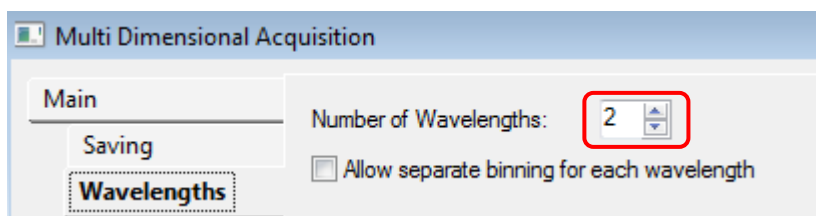
Click on « *Full chip* » the left green square is set to use the entire field of view of the camera.



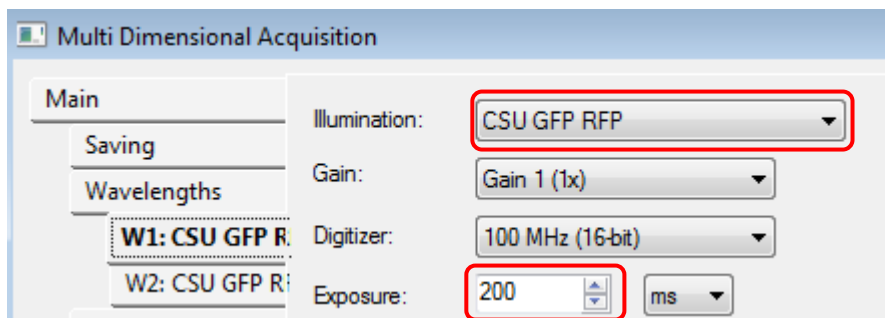
8- In the « *Main* » tab in the MDA window, select « *Multiple Wavelengths* ».



9- Click on the « *Wavelengths* » tab in MDA and select 2 in « *Number of Wavelengths* ».

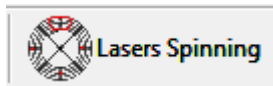


10- For each tab (W1 and W2) created, in the « *Illumination* » window, choose the **same** emission filter for fluorescence (CSU) and the **same** exposure time.



11- Disable « *Auto-focus* » and « *Auto-exposure* » options (No Auto Expose, No Auto Focus).

12- Choose the laser power for each channels.



13- Set the focus with the microscope wheel or use the buttons



14- Move the stage with the joystick or the buttons



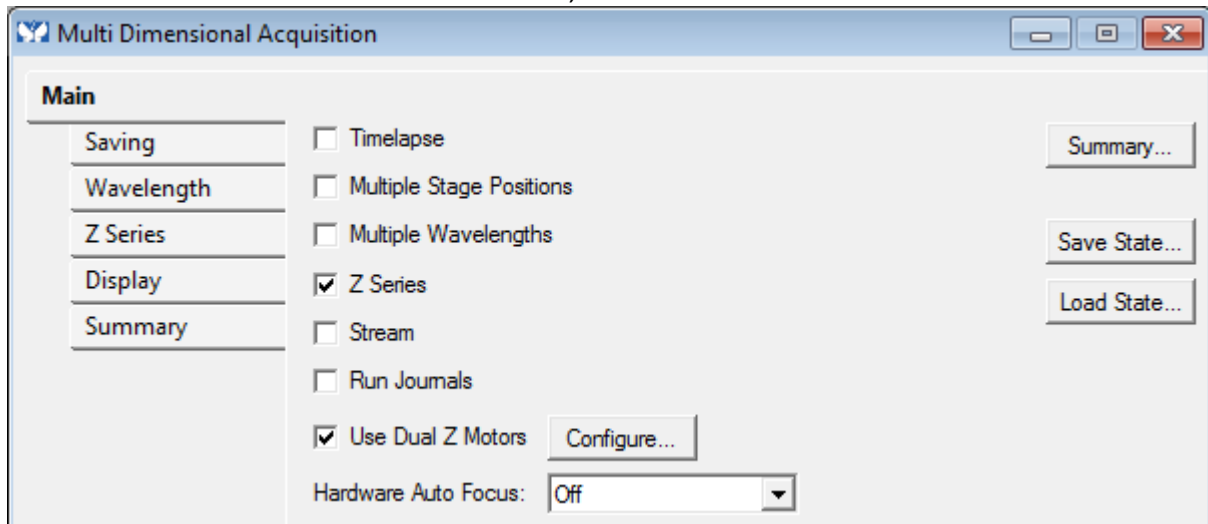
15- Click on « *Snap* » and check the dynamic of the image to adjust the laser power and exposure time.

16- Click on « *Acquire* » to begin the acquisition.

Acquire a Z-stack serie

Refer to the chapter « acquire an image with the camera» but add those steps:

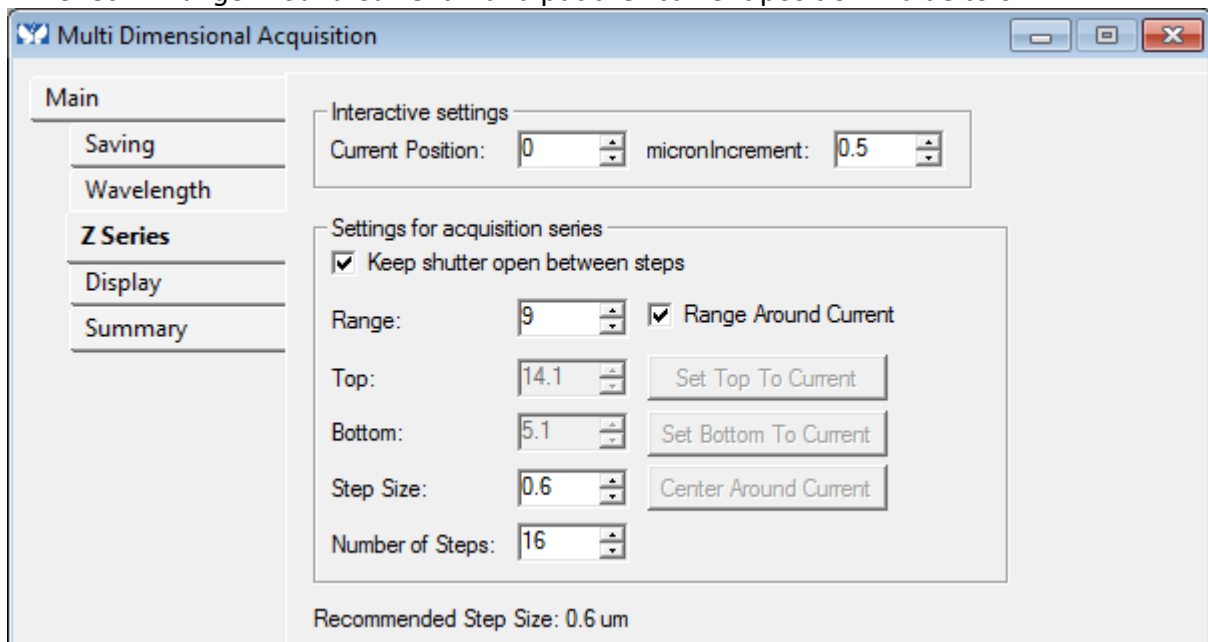
1- In the « *Main* » tab in the MDA window, check « *Z Series* ».



In the « *Z Series* » tab you can define your Z-stack either by setting the center of the stack, or setting the top and bottom.

To set the current focus as the Z-stack center.

2- Check « *Range Around Current* » and put the “current position” value to 0.

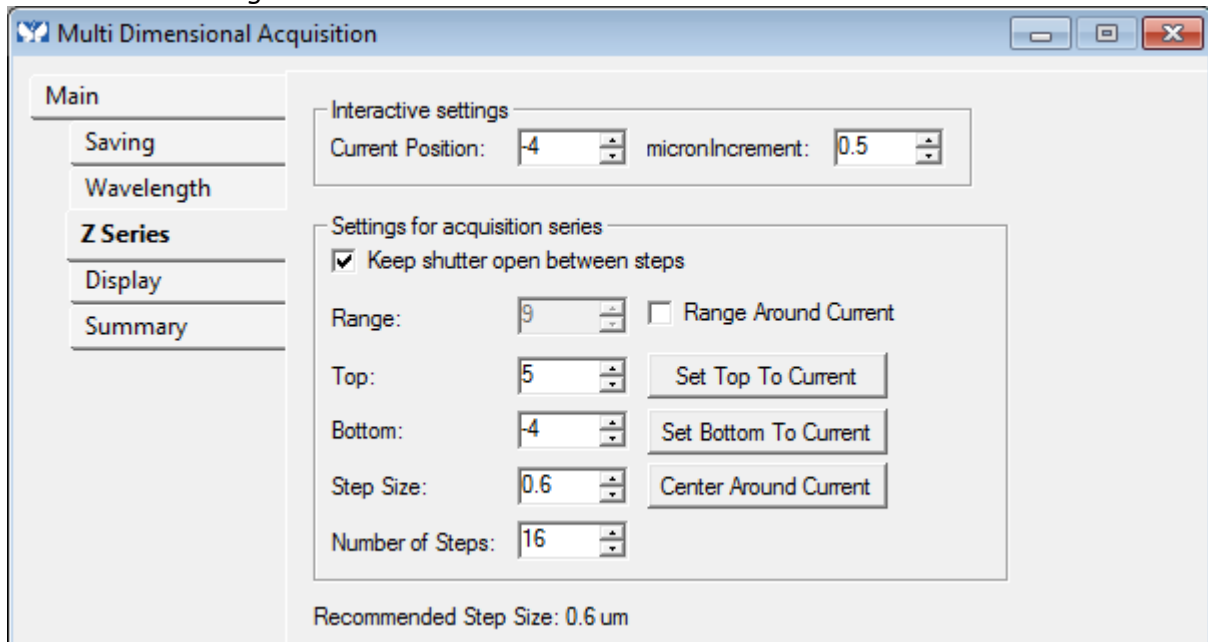


3- Define the distance (μm) between two acquisitions in Z « *Step Size* ».

4- Choose the number of slices which you want to acquire « *Number of Steps* ».

To set the Z-stack top and bottom

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* ».

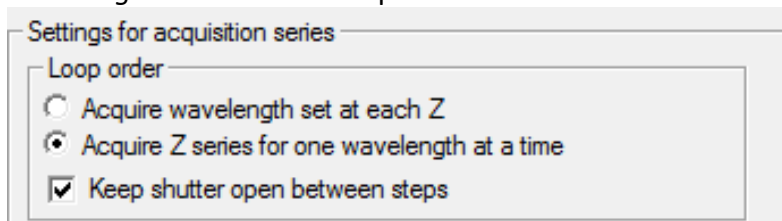
7- Change the focus with the piezo stage until the lower limit of the stack and click on « *Set Bottom to Current* ».

8- Set the distance between two acquisitions « *Step Size* ».

9- Click on « *Acquire* » to start the acquisition.

Notes:

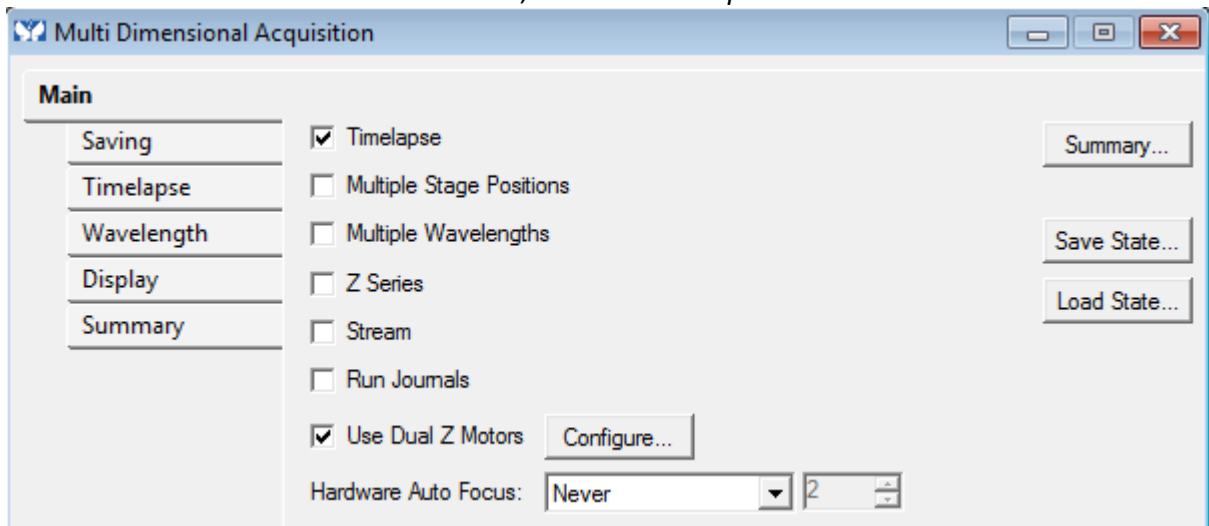
- The laser power and exposure time must be set on the most intense plane of the Z-stack.
- If you are doing acquisition on fixed sample with multiple wavelength, check both the « *Acquire Z series for one wavelength at a time* » and « *Keep shutter open between steps* » options.
- If you are doing acquisition on live sample with multiple wavelength, check the « *Acquire wavelength set at each Z* » option.



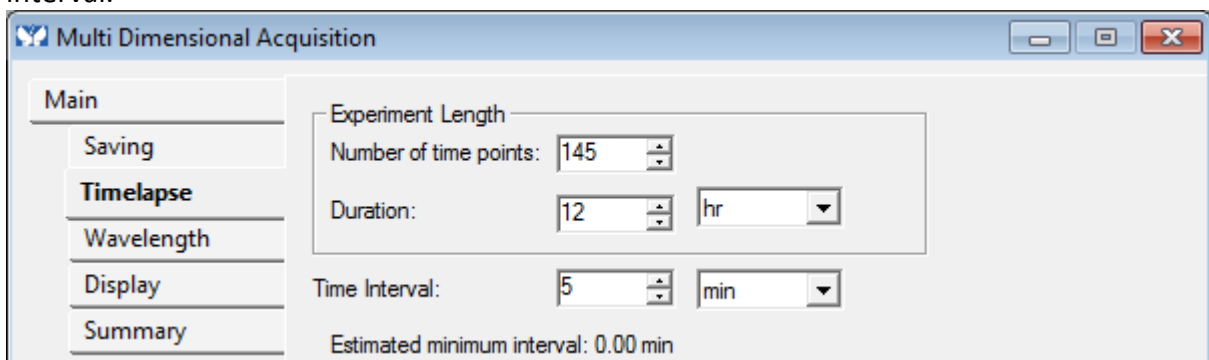
Acquire a time serie

Refer to the chapter « acquire an image with the camera» but add those steps:

- 1- In the « *Main* » tab in the MDA window, check « *Timelapse* ».



- 2- In the « *Timelapse* » tab select the number of desired time points 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 that takes to save the image).

- 3- Click on « *Live* » and set a fine focus using the piezo stage.
- 4- Click on « *Snap* » down to the left and check the dynamic of the image. Adjust the exposure time and laser power accordingly.
- 5- Click on « *Acquire* » down to the right to start the acquisition.

Multiple stage positions

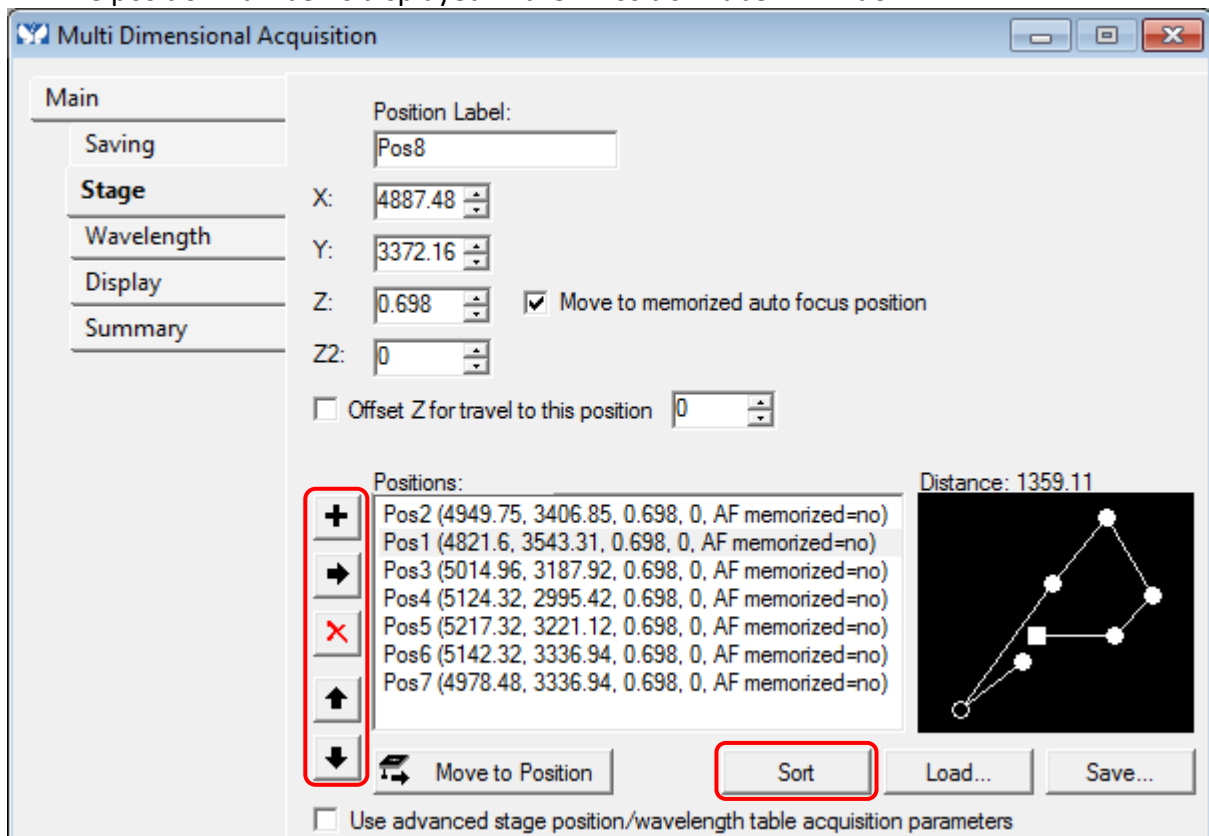
Refer to the chapter « acquire an image with the camera» but add those steps:

1- In the « *Main* » tab of the MDA window, check « *Multiple Stage Positions* ».



2- In the « *Stage* » tab in MDA:

The position number is displayed in the « *Position Label* » window.



3- Click on + to add a new position. You can modify, replace, and delete positions using the graphic tools on the left of the box.

4- To replace a position, select it and click on +.

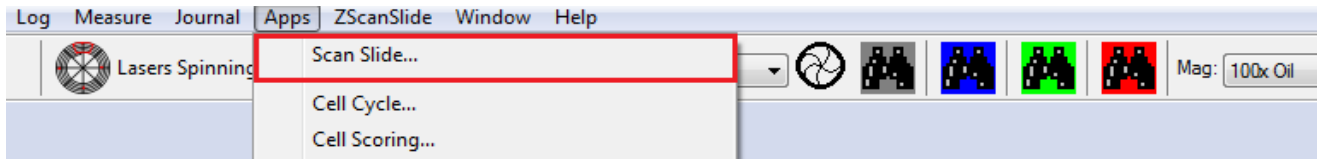
5- The position name and coordinates will appear in « *Positions* ».

6- A graphic display will appear on the right. Click on « *Sort* » to choose the fastest pathway between the positions.

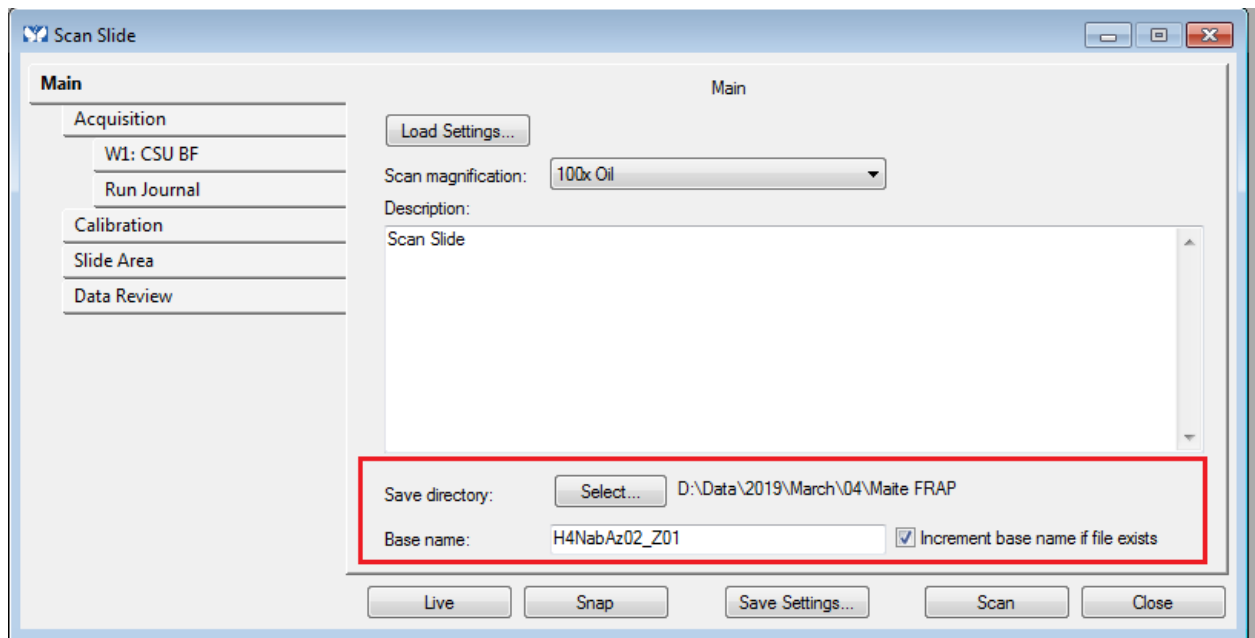
7- Click on « *Acquire* » down to the right to start the acquisition.

Acquire an image mosaic

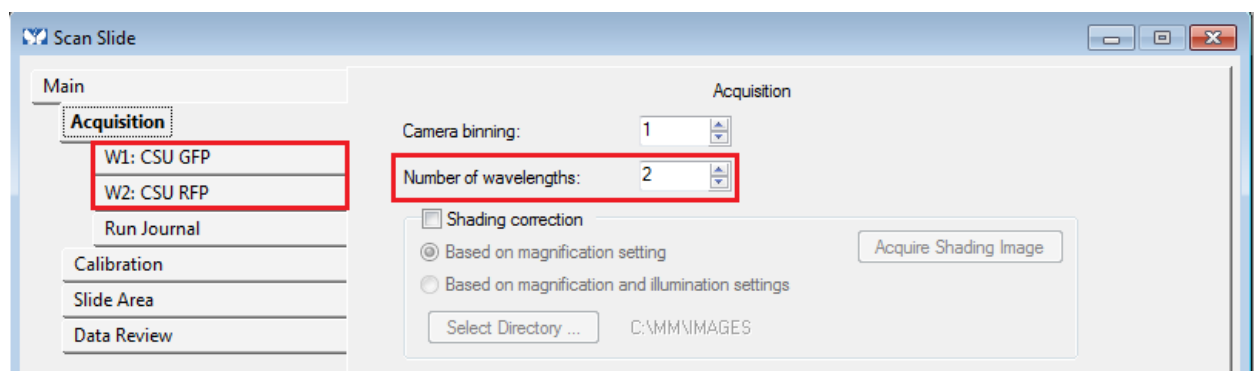
- 1- In the « *Apps* » menu, select « *Scan Slide* »



- 2- In the « *Main* » tab, Select the folder destination and choose the base name for the image.

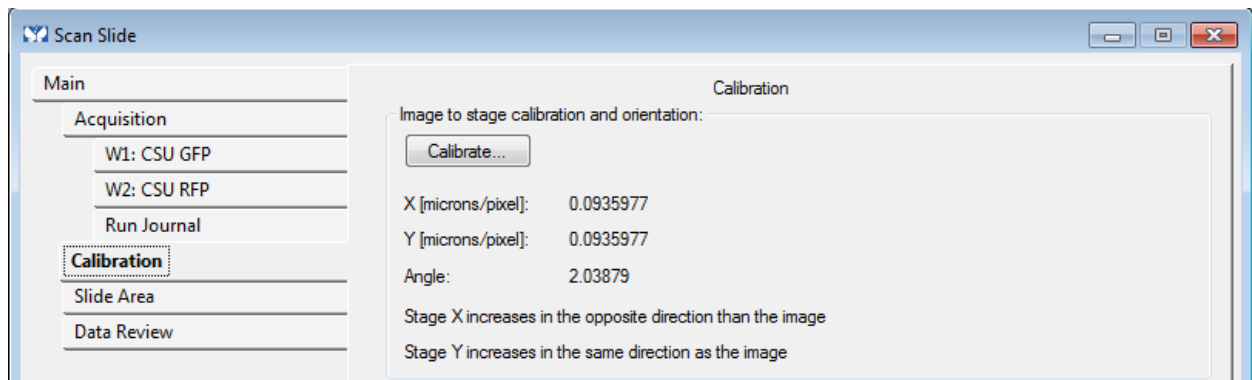


- 3- In the « *Scan slide* » window and in the « *Acquisition* » tab, Select the number of wavelengths and do the settings for each.



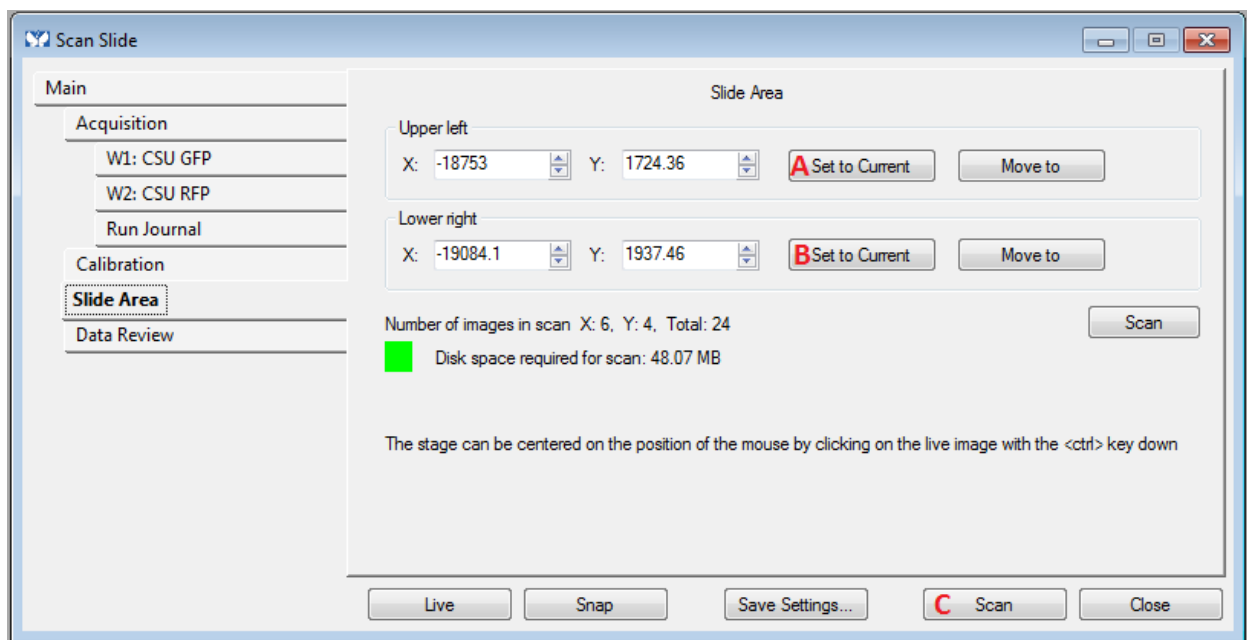
- 4- In the « *Run Journal* » tab, check that there are no journal.

- 5- in the « Calibration » tab, check that the calibration is saved. If not, click « *Calibrate* » and follow the instructions.



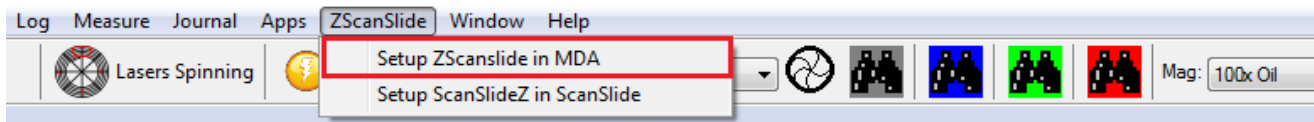
- 6- In the « Slide Area » tab :

- A- Click on « *live* » and shift the sample to the upper left corner of your field of interest.
Click on « *Set to Current* »
- B- Click on « *live* » and shift the sample to the lower right corner of your field of interest.
Click on « *Set to Current* »
- C- Click on « *Scan* »

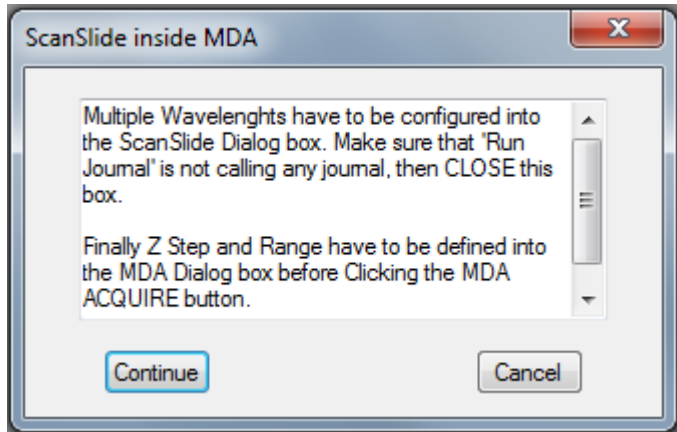


Acquire an image mosaic with series Z

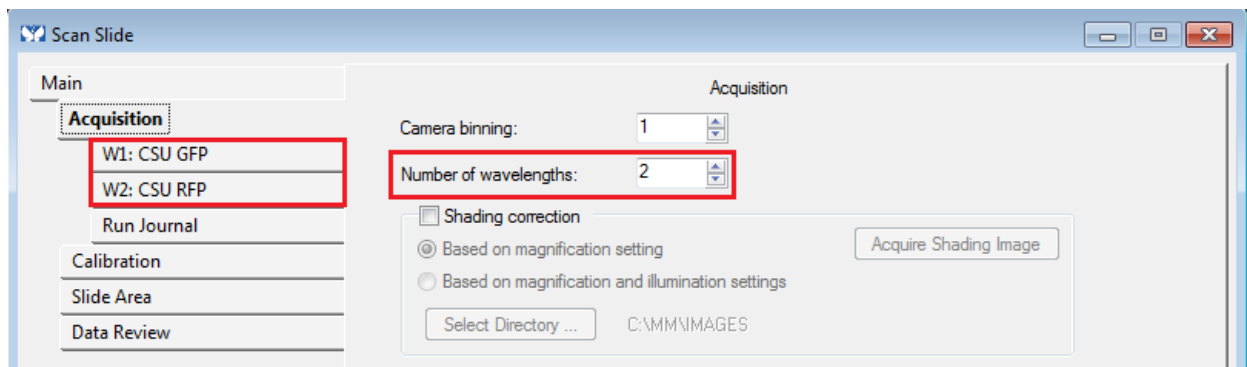
1- Select in the « ZScanSlide » menu : « Setup ZScanslide in MDA »



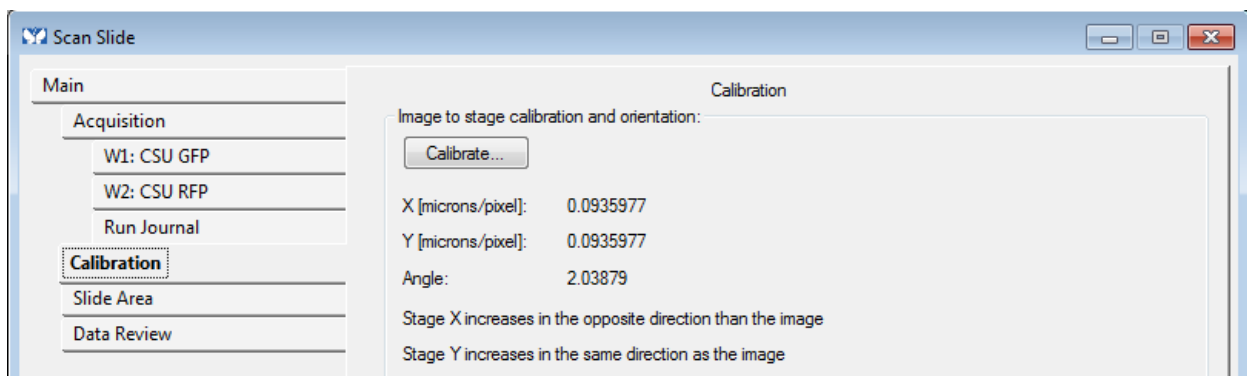
2- An info window appears. Click on « Continue »



3- In the « Scan slide » Window and in the « Acquisition » tab, select the number of wavelength and do the settings for each in the corresponding tab.

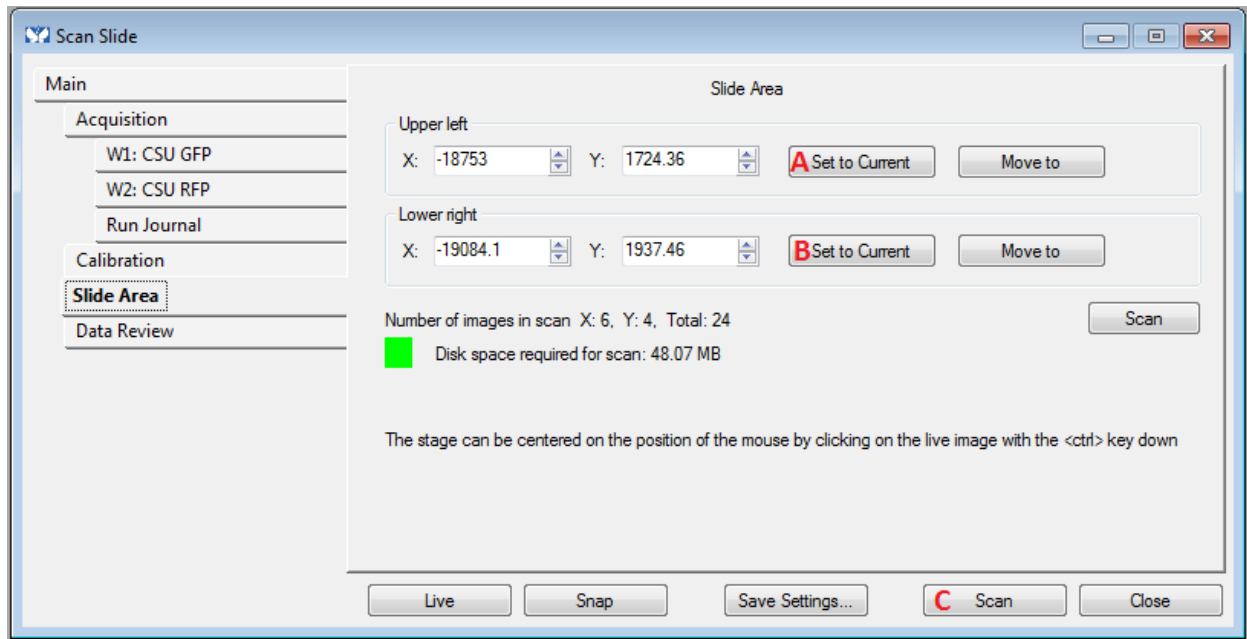


4- In the « Calibration » tab, check that a calibration is saved. If not, click on « calibrate » and follow the instructions.

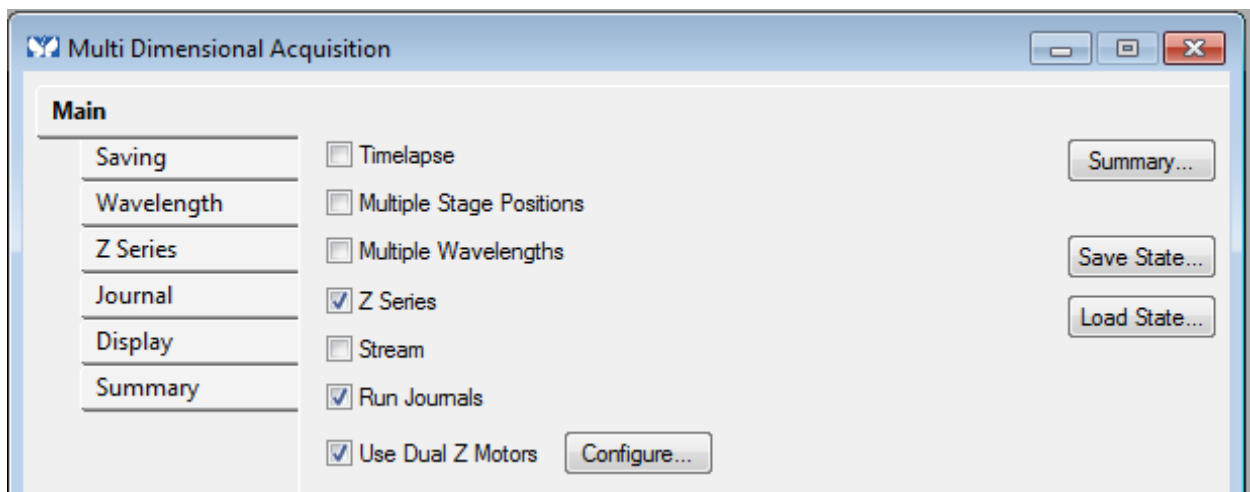


5- In the « *Slide Area* » tab:

- D- Click on « *live* » and shift the sample to the upper left corner of your field of interest.
Click on « *Set to Current* »
- A- Click on « *live* » and shift the sample to the lower right corner of your field of interest.
Click on « *Set to Current* »
- B- Click on « *Scan* »

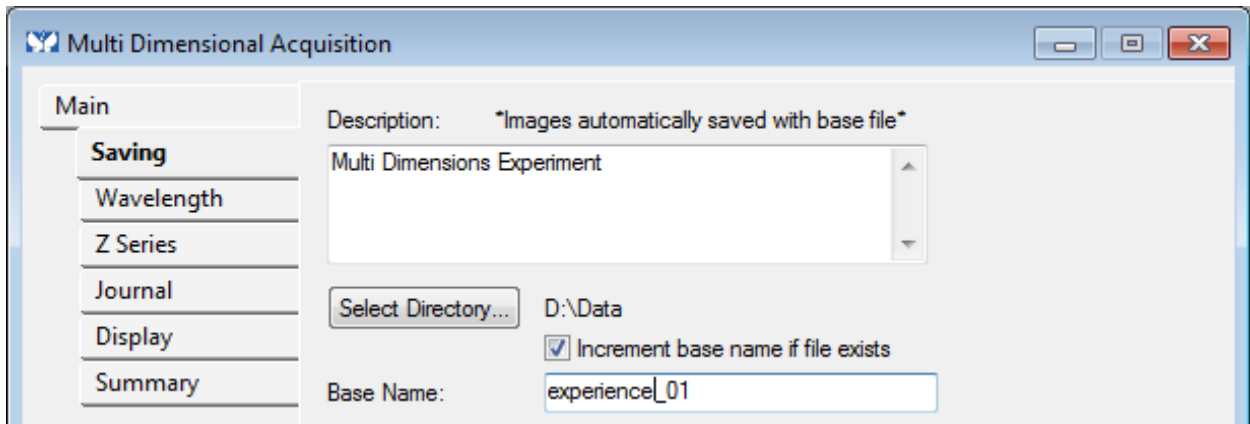


6- In the MDA window, uncheck all except « *Z-Series* », « *Run Journals* » and « *Use Dual Z Motors* ».



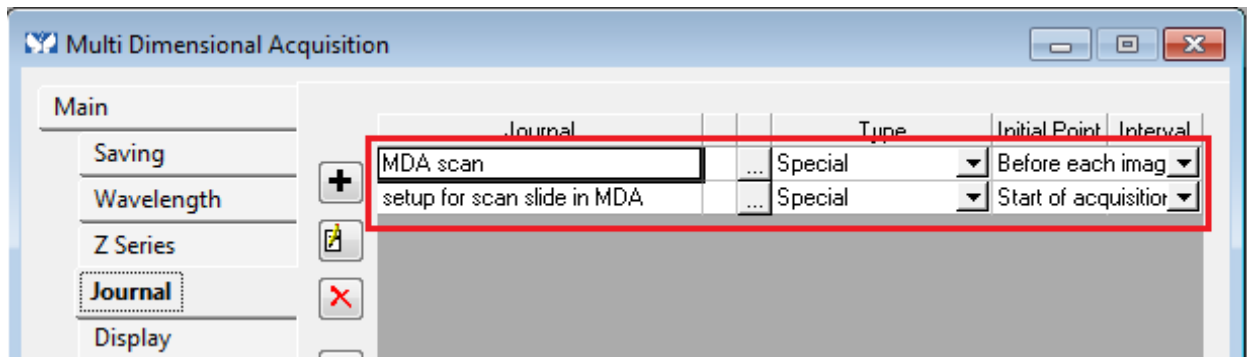
- 7- Click on the tab « *Saving* » in MDA, with the button « Select Directory... », select your saving pathway: **D: (Users /année / mois en cours / jour de la manip / dossier à votre nom)**

Images saved elsewhere or older than 15 days will automatically be deleted without any notifications.



- 8- In the « *Z Series* » tab, define the z-stack parameters (cf p12)

- 9- Check that two journals are saved in the « *Journal* » tab.



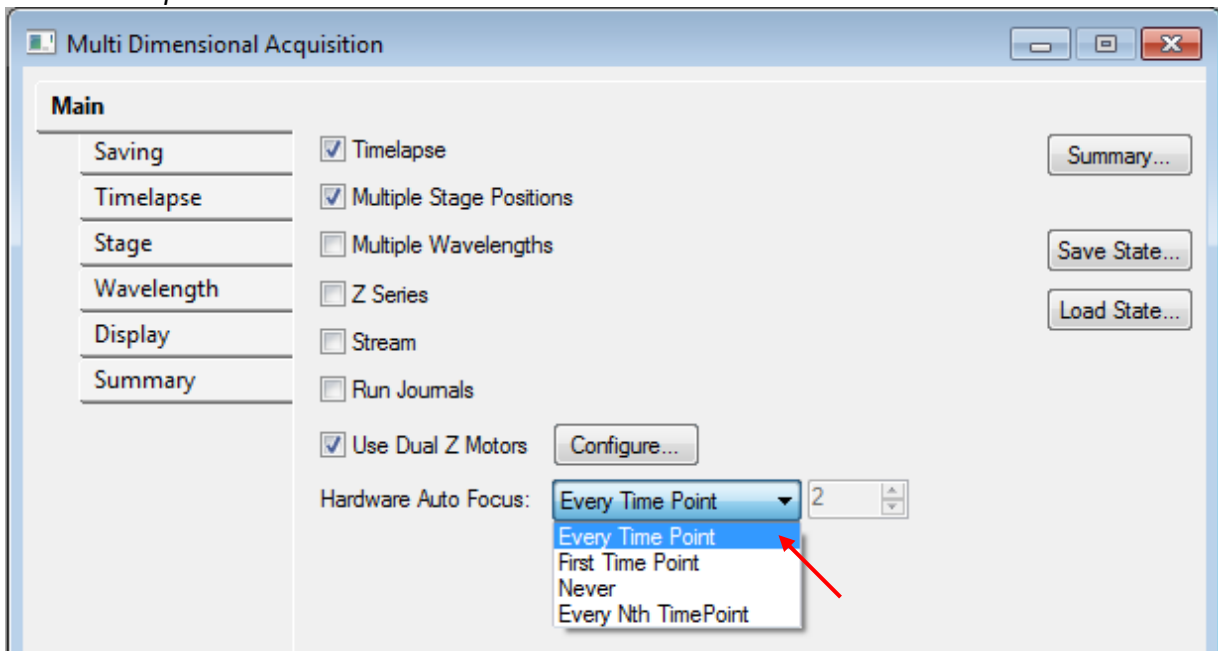
- 10- Click on « Acquire »

Image mosaic finalization

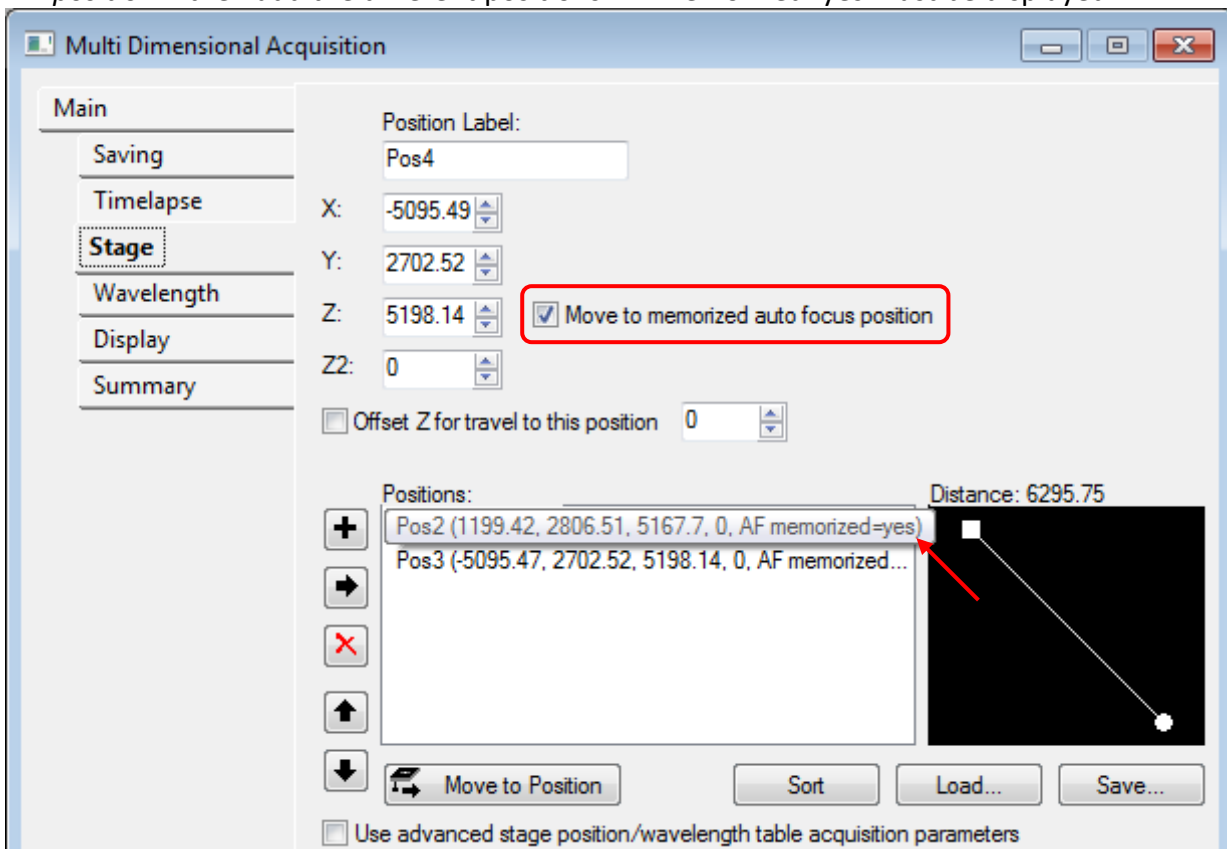
- 1- In the « *Scan Slide* » window :
 - A- Select « *File* »
 - B- Open the image
 - C- Select the wavelengths to display
 - D- Click on « *Show image* »

Use the hardware autofocus

- 1- Set the focus on your sample. In the « Main » tab in MDA, in the « *Hardware Autofocus AFC* » window choose the interval use of the autofocus. This option appears only if « *Timelapse* » is checked.



- 2- If you use the « *Multiple Stage Position* » option, check « *Move to memorized Auto focus position* » then add the different positions. AF memorized=yes must be displayed.

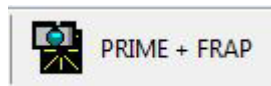
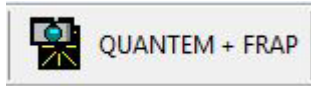


- 3- Click on « *Acquire* » down to the right to start the acquisition.

Use The FRAP system

Start the FRAP system :

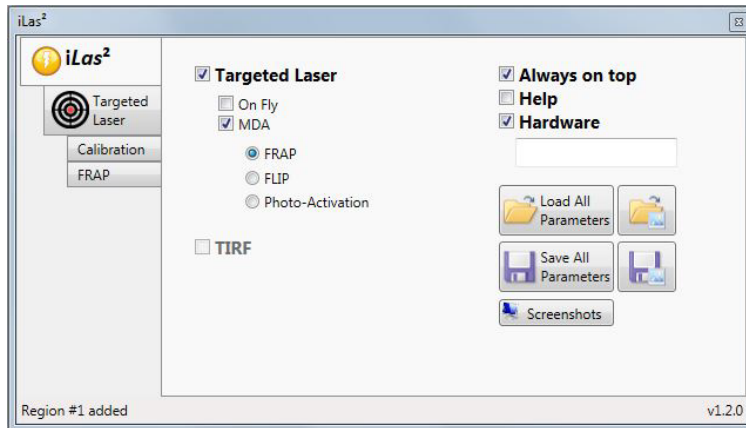
1- In « *MetaMorph* », Select your camera of your choice with FRAP.



2- Click on « *ILAS²* » in the « *MetaMorph* » menu.

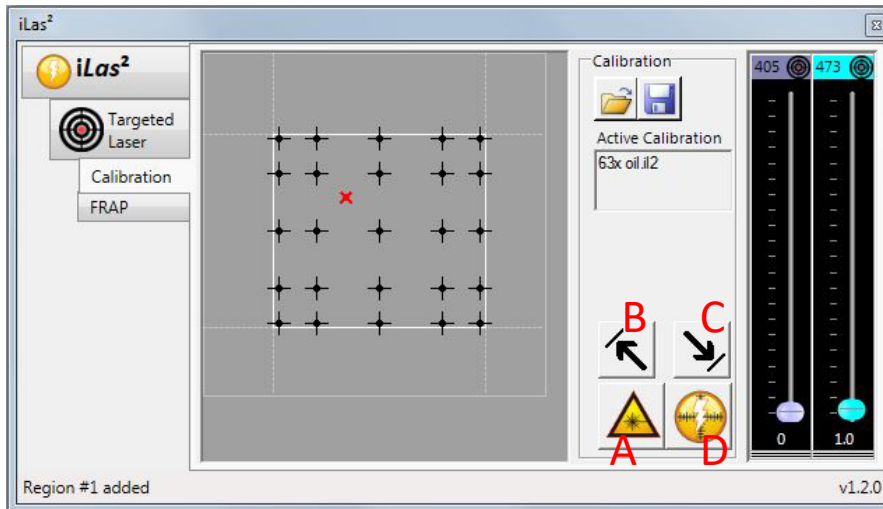


3- In « *ILAS²* » tab, select « *MDA* » and « *FRAP* ».



Laser calibration :

- 4- Use the calibration lamella, with your objective, start « live » and get in focus.
- 5- In « *Calibration* » tab, put FRAP laser intensity at **2%** and activate laser with button (A).
- 6- With the cross, select the upper left corner and click on button with arrow (B). Remake by selecting the lower right corner and click on button with arrow (C).
- 7- Initiate the calibration (D).

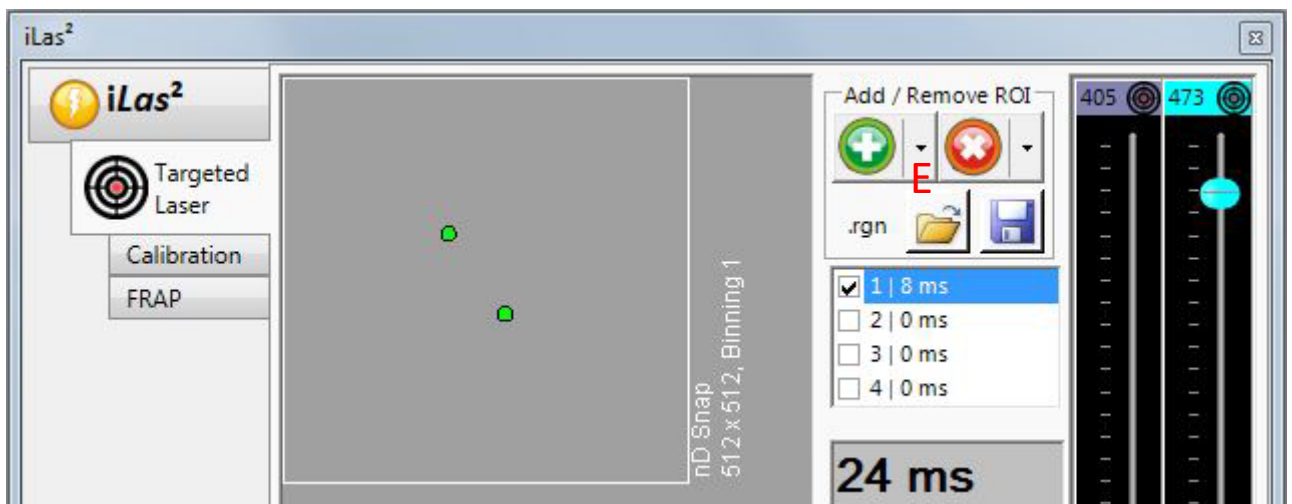


Choose regions of interest for photobleach:

- 8- On your sample make a « *SNAP* » and select regions for photobleach with ROI tool. The last icon is to define a region fixed size.

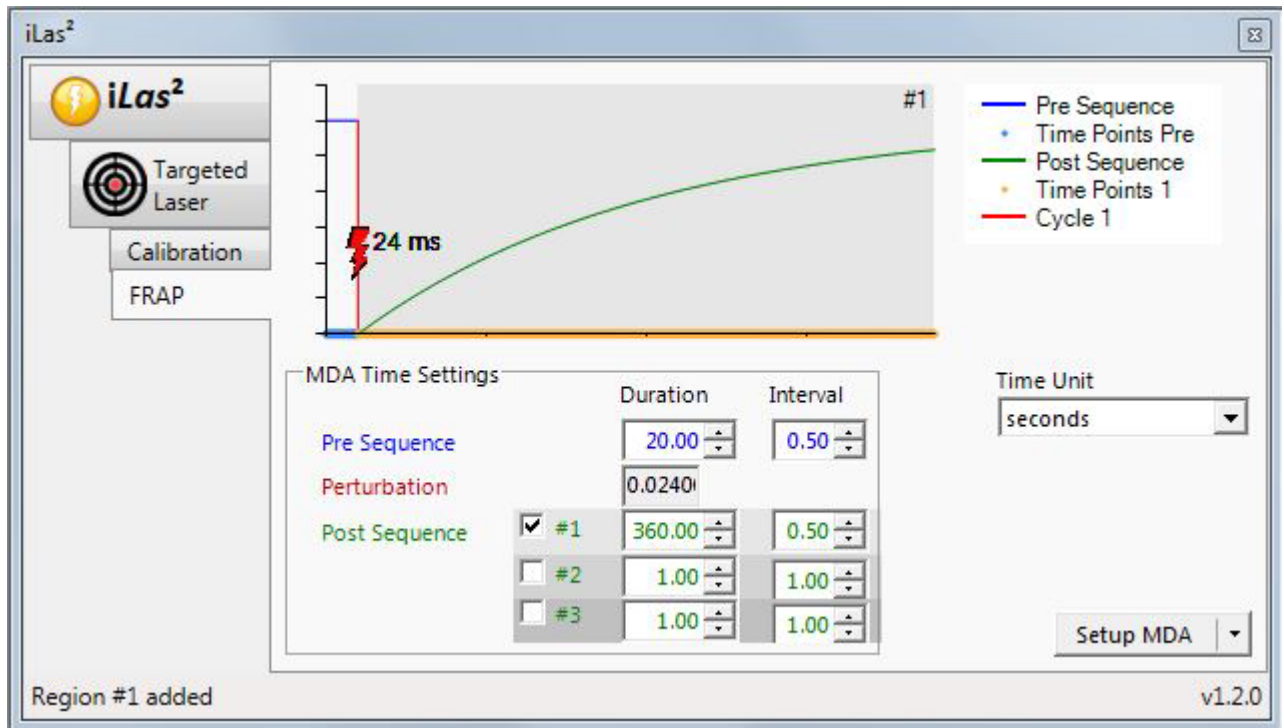


- 9- In « *Targeted Laser* » tab, add all areas that you have traced on the picture by clicking on the arrow beside the green button (E). Choose the laser power and the repetition number(F).

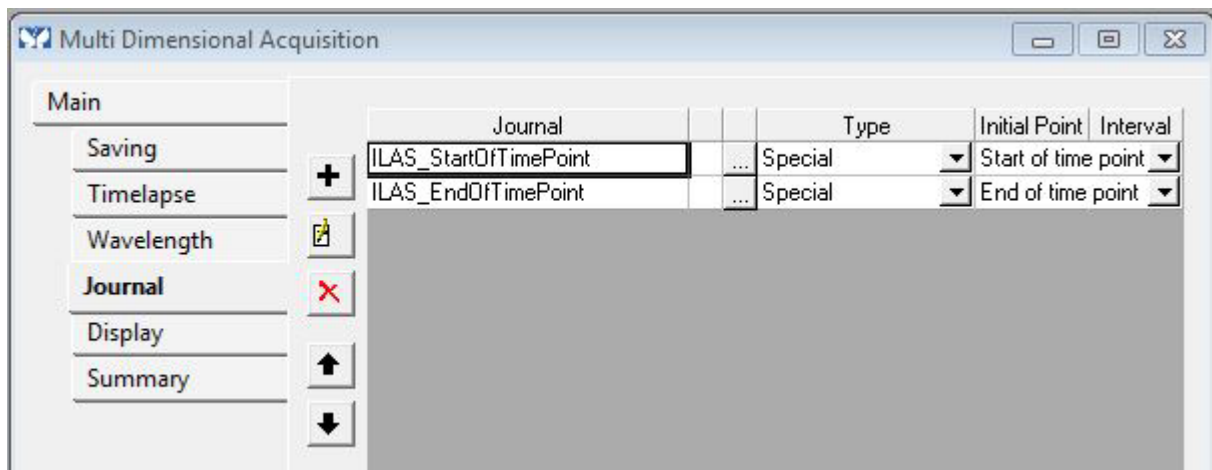


Paramétrer l'expérience de FRAP:

10- In « FRAP » tab, enter a time value and interval between each picture for the sequence before FRAP and after FRAP. Choose the time unit and these values.



11- Click on « Setup MDA » for activate your experience in the « journal » tab of « MetaMorph ».



14- Check that the « Journal » tab in the « Multi-Dimensional Acquisition » window of « MetaMorph » software is activated and contains only two lines.

15- Start your FRAP experience by clicking on the « acquire » button.

Switch off the system

Check on the planning if the system is used after you.

If the system is used:

- 1- Lower the objectives and check that they are cleaned properly (lens + sides).
- 2- Exit MetaMorph (File/Close)
- 3- Transfer your data

If the system is not used:

- 1- Lower the objectives and check that they are cleaned properly (lens + sides).
- 2- Exit MetaMorph (File/Close)
- 3- Transfer your data and switch off the computer
- 4- Stop the piezo controller
- 5- Turn off the switch on the wall



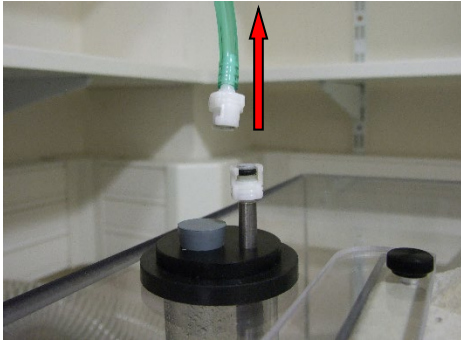
- 6- Turn off the fluorescent lamp

Switch off the temperature and CO₂ controller

1- Remove the CO₂ cap. Be careful not to break the glass cover.



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



3- Switch off the CO₂ and temperature controller with the switch on the wall.



4- Close cylinders of compressed air (silvery regulator) and of CO₂ (golden regulator) clockwise.

