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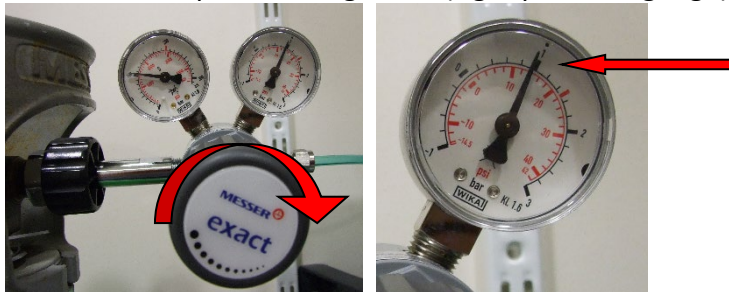
Switch on the temperature controller

1- Open the air compressed bottle (silver pressure regulator) and the CO2 bottle (golden pressure regulator) in the anti-clockwise direction.

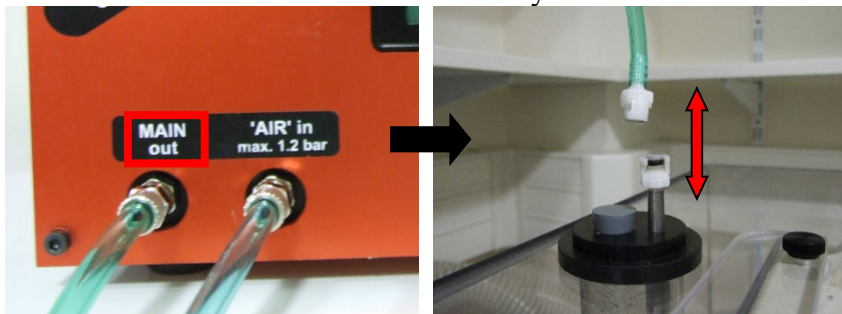


2- Put the wall switch to the ON position to switch on the CO2 controller (red brick) and the temperature controller (blue box).

3- Turn the air compressed bottle valve (silver) clockwise until you obtain a pressure of 1 Bar in the exit of the pressure regulator (right pressure gauge).



4- Take the greenish tube connected to the CO₂ controller ((« MAIN out » exit) and clip the end of the tube to the water reservoir on the system.



5- Put the CO₂ cover on the sample holder.



Switch on the spinning disk

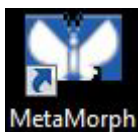
- 1- If necessary, turn on the fluorescent lamp. The Shutter button must be pressed " open ".




- 2- Turn on all the elements of the microscope with the wall switch .

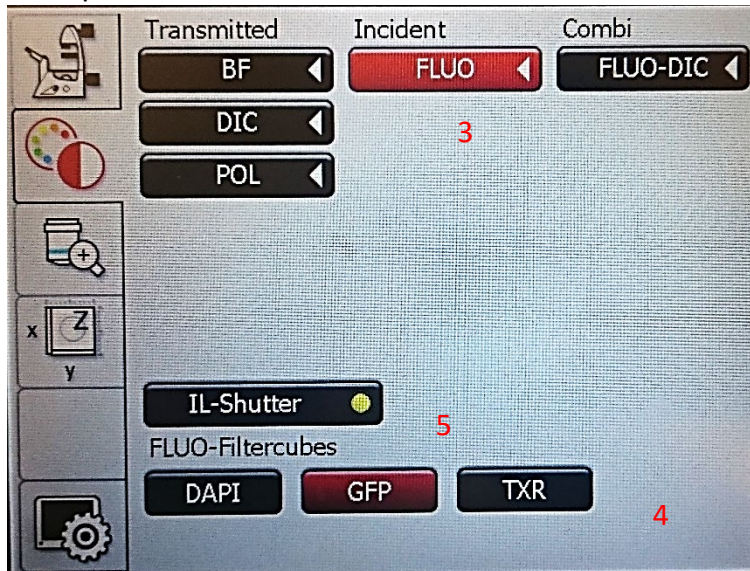


- 3- Wait until all the elements are started and then turn on the PC.
- 4- Start the " Spinning W1 " session.
- 5- Start the "MetaMorph" software.

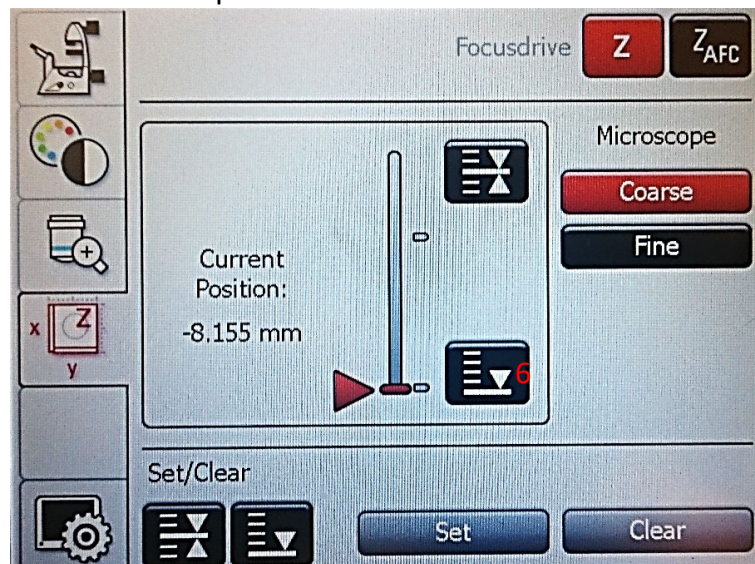


Sample observation with the oculars

- 1- In MetaMorph, choose the lens by clicking on the corresponding icon at the top.
- 2- In the menu on the left, click on the "Eyepieces" icon. 
- 3- On the second tab of the touch screen of the microscope choose an illumination mode Brightfield, DIC or Fluorescence.
- 4- Choose the filter block in fluorescence.
- 5- Open/Close shutter.



- 6- On the third tab of the touch screen of the microscope, lower the objectives to the bottom stop.



- 7- Install the sample holder taking care not to damage the piezo plate. Then place the sample and focus.
- 8- Adjust the light intensity on the touch screen or the small wheel on the left of the microscope.

Spatial sampling, Pixel size, dynamic of the image

Optimal XY sampling

For all the objectives, the best resolution is obtained with a binning of 1.

Objectives	Optimal pixel size	Camera pixel size (binning 1)	Camera pixel size (binning 2)
100x Oil	76 nm	70 nm	140 nm
63x Oil	76 nm	112 nm	224 nm
63x Water	88 nm	112 nm	222 nm
40x Oil	81 nm	175 nm	350 nm
20x Imm	141 nm	350 nm	700 nm
10x Dry	265 nm	707 nm	1414 nm

Optimal Z sampling

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

Dynamic of the image

The dynamic of the image corresponds to the difference between the maximum and the minimum intensity of the image.

The more we illuminate a labelled sample, 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.

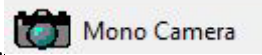
If the dynamic of the image is too low, increase the exposure time or the laser power.

Acquire an image with the camera

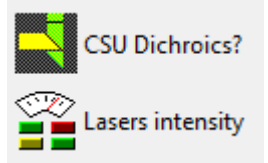


1. Before switching to camera mode, select Brightfield (BF) mode on the microscope touch screen.

2. In the menu on the left click on "Mono Camera",

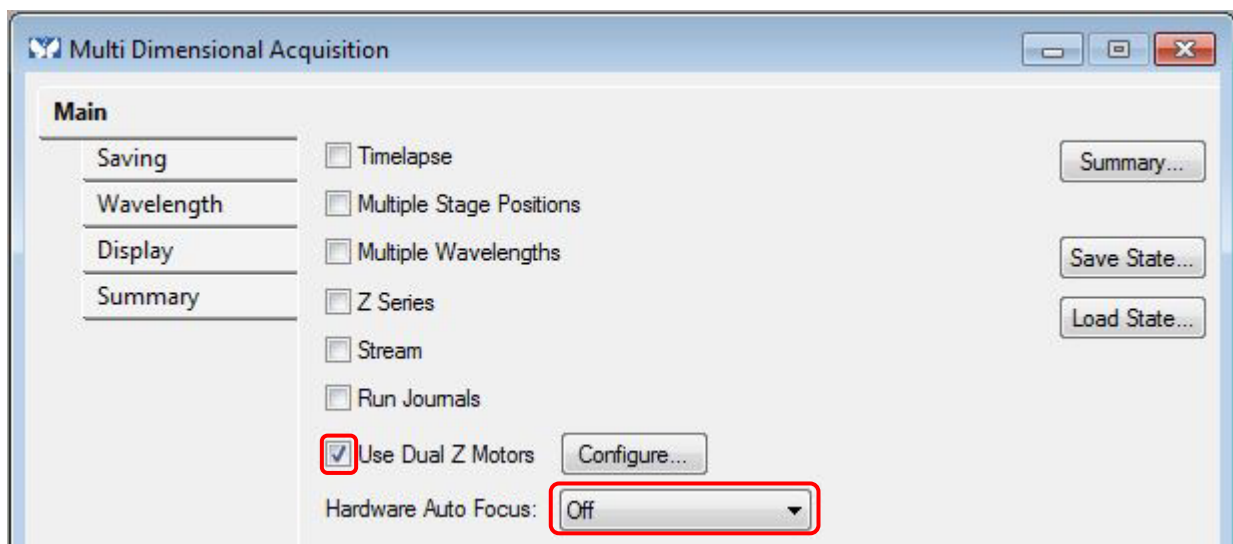


3. Choose the appropriate dichroic mirror among the 3 proposed.



4. Open the "Laser intensity" window, to set the intensity of the lasers.

5. Open the "Multidimensional Acquisition" MDA window. In the "Main" tab. Make sure that only "Use Dual Z Motor" is selected. "Hardware Auto Focus" must be set to « Off».

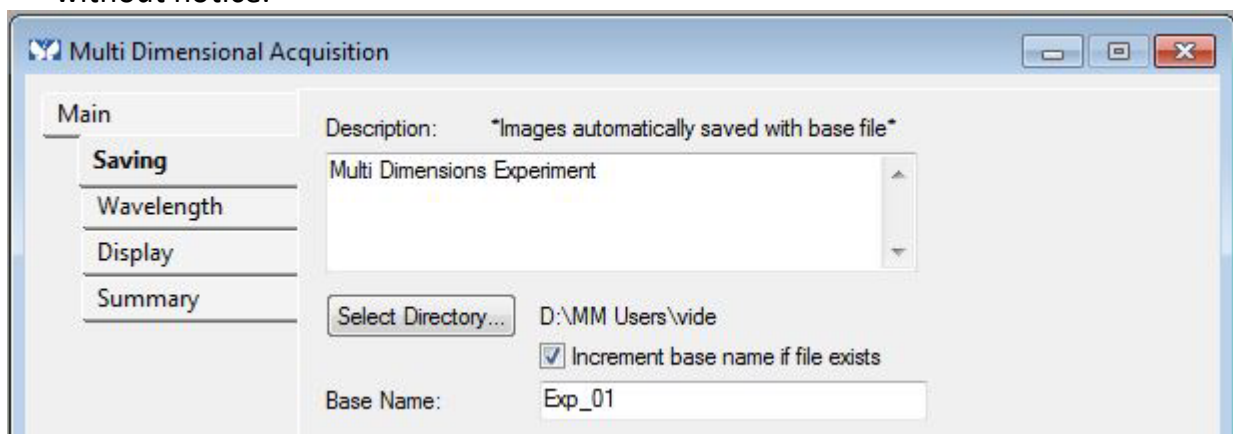


6. Click on the "Saving" tab of the MDA.

Using the "Select Directory..." button, select the following backup path :

D : (MMUsers / year / current month / day of the manipulation / folder with your name)

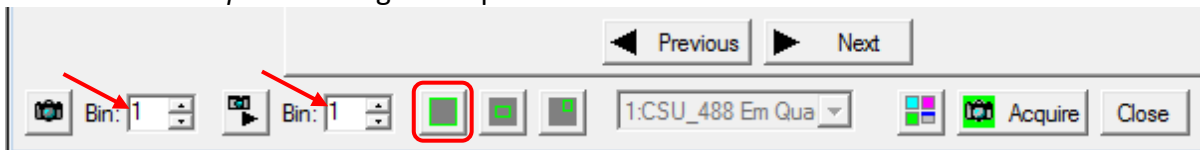
Images saved in another directory or older than 15 days are automatically deleted without notice.



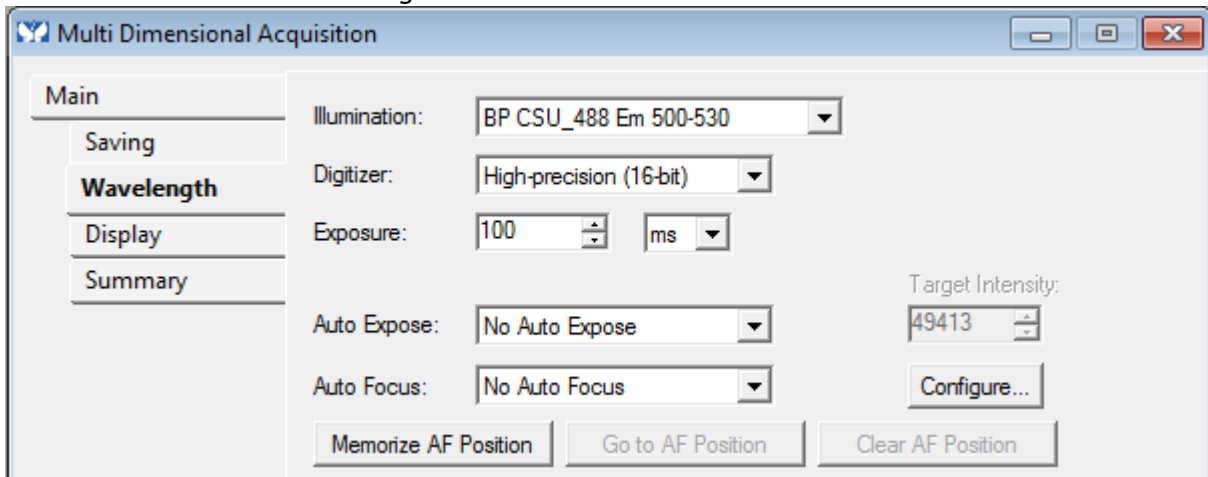
7. In the window « *Base Name* », indicate the name of the image you are about to acquire follow-up with -01 (this number will increment automatically). The images are automatically saved at the end of the acquisition.

8. 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.



9. Click on the tab « *Wavelength* » of the MDA



10. In the window « *Illumination* », choose the type of transmission illumination (OBF; ODIC) or the emission filter for the fluorescence (BP CSU ou Quad).


11. The camera can be used with the « High-precision (16-bit) » mode.

12. In the window « *Exposure* », choose the exposure time (start at 100 ms)

13. Check that the options « *Auto-focus* » and « *Auto-exposure* » are not activate (No Auto Expose, No Auto Focus).

14. Set the laser power that correspond to the chosed filter.

15. Click on « *Live* » and make a fine focus using the piezo stage.

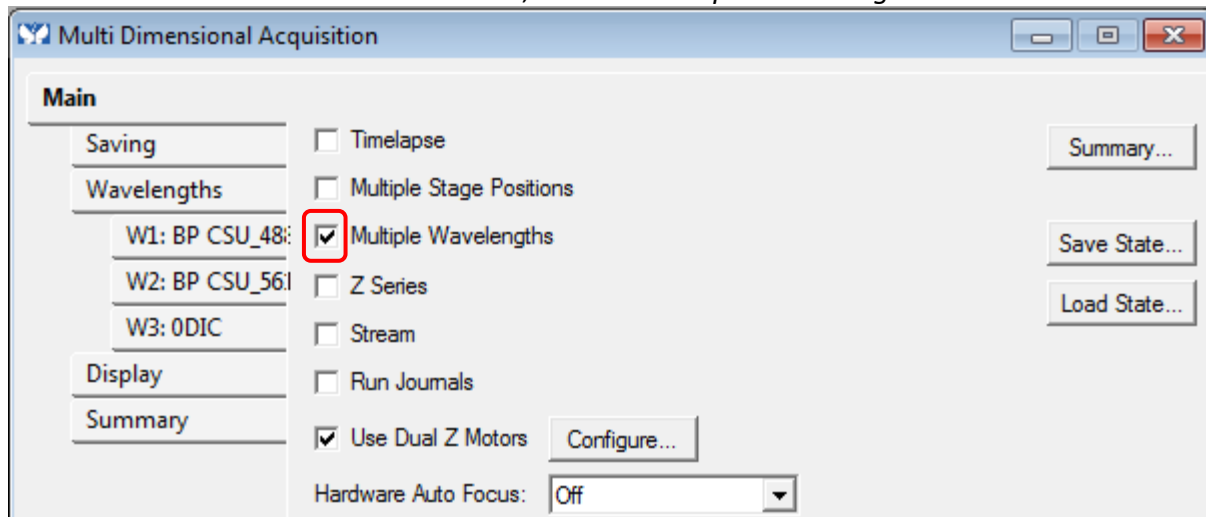
16. Click on « *Snap* » down to the left and  check the dynamic of the image. Adjust the laser power and exposure time accordingly.

17. Click on « *Acquire* » down to the right to begin the acquisition.

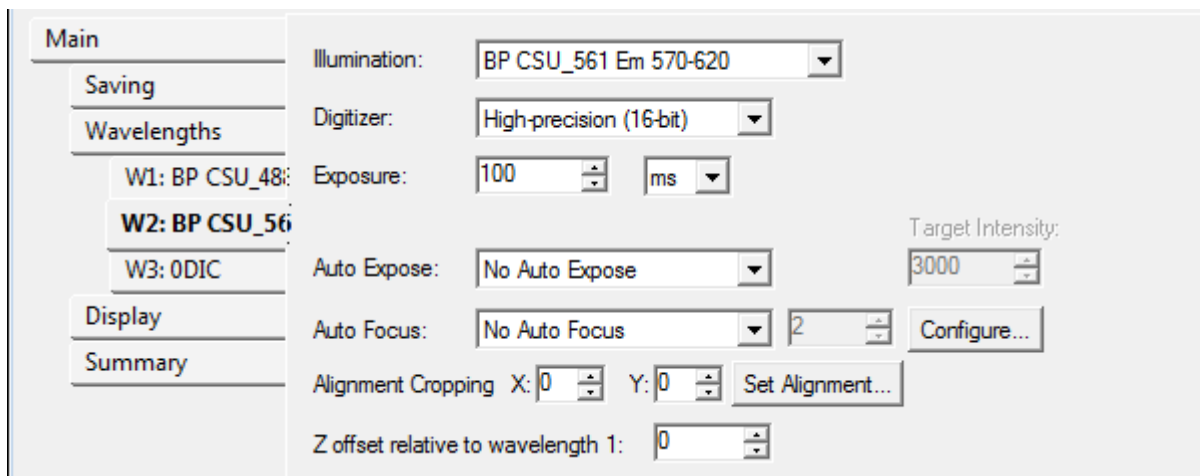
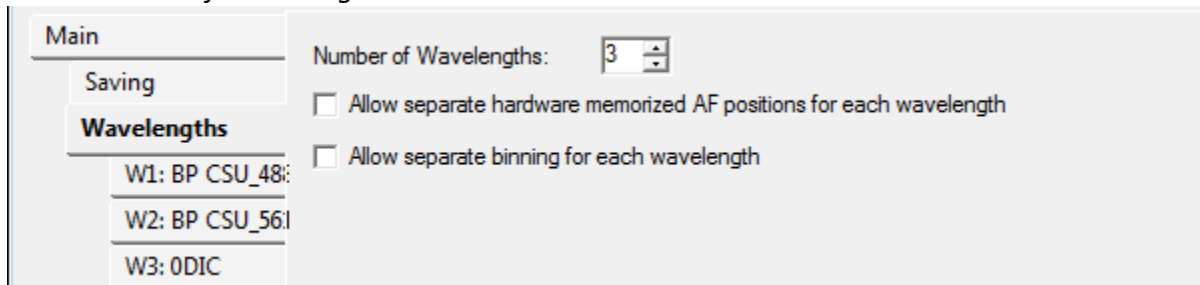
Acquire multiple colors

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

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



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

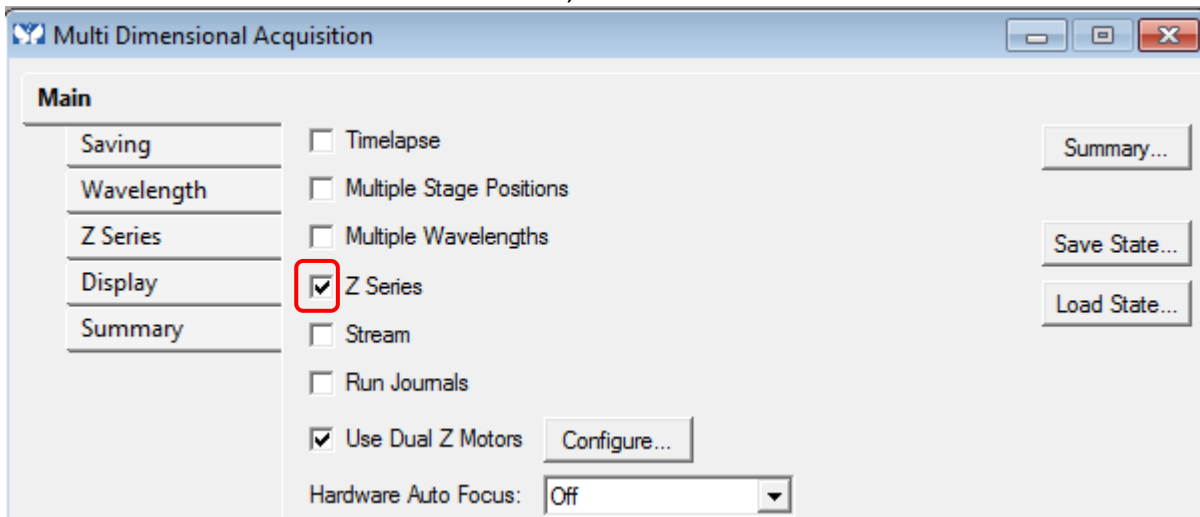


3. For each tab (W1, W2,...) created, in the window « *Illumination* », choose the type of illumination.
4. Set a laser power and exposure time (wich can differ) for each channel.
5. Click on « *Acquire* » down to the right to start the acquisition.

Acquire a Z-stack serie

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

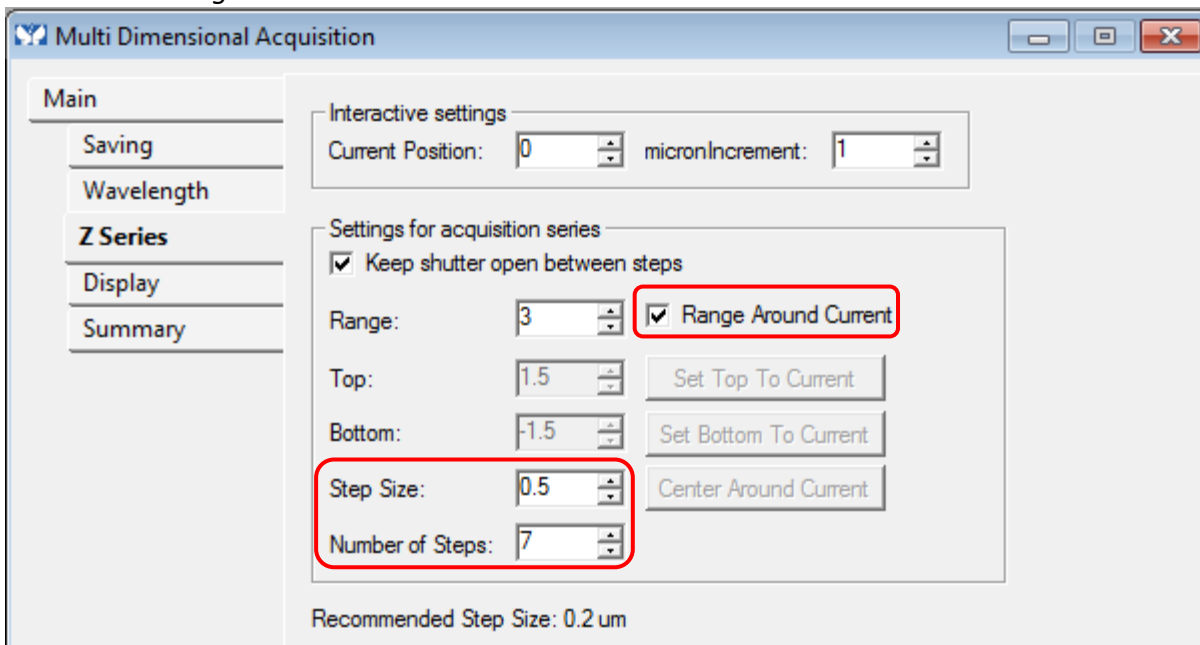
1. In the tab « *Main* » in the MDA window, check « *Z Series* ».



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

To set the current image as the center of the Z-stack.

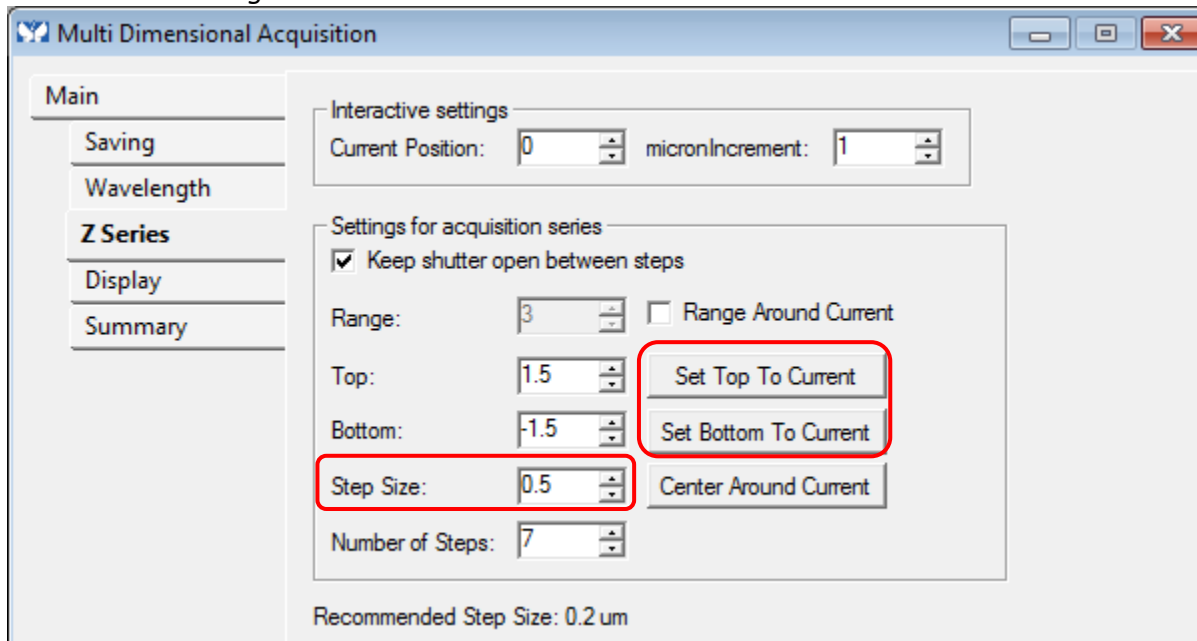
2. Check « *Range Around Current* ».



3. Set the focus using the macrometric and micrometric knobs.
4. Define the distance between 2 optical sections « *Step Size* ».
5. Choose the number of slices you wish to acquire « *Number of Steps* ».

To set the top and bottom of the Z-stack

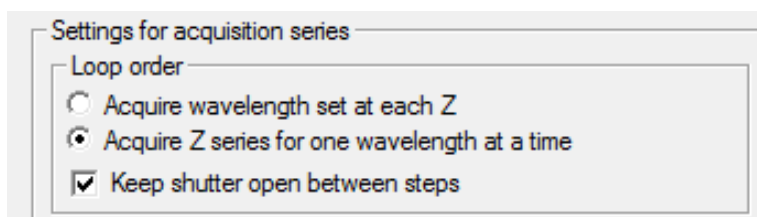
6. Uncheck « Range Around Current ».



7. Change the focus with the piezo stage until the upper limit of the stack and click on « Set Top to Current ».
8. Change the focus with the piezo stage until the lower limit of the stack and click on « Set Bottom to Current ».
9. Define the distance between 2 optical sections « Step Size ». The number of planes is automatically set « Number of Steps ».
6. Click on « Acquire » down to the right to start the acquisition.

Notes :

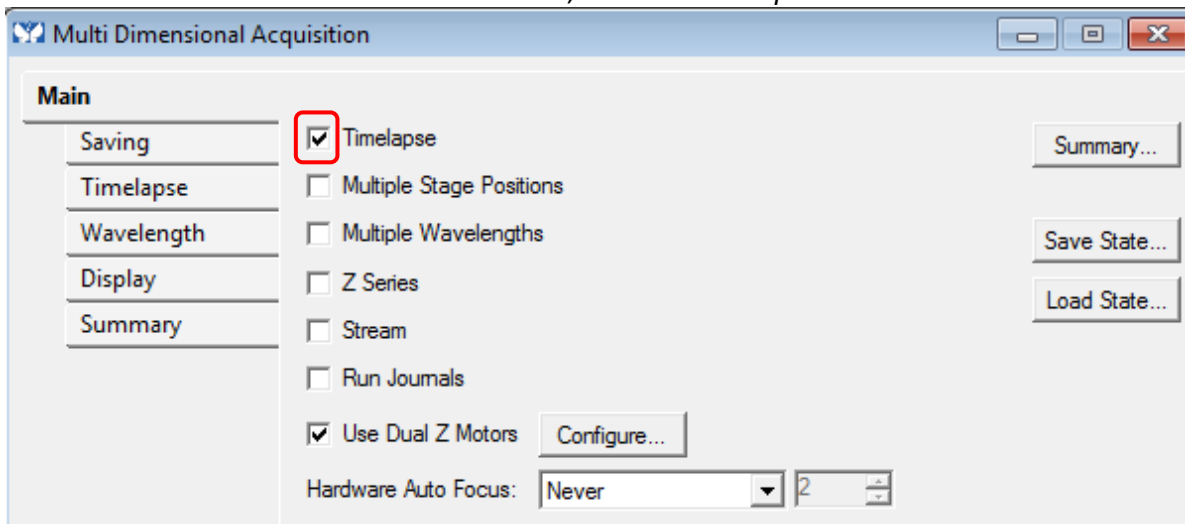
- The laser power and exposure time must be set on the most intense plane of the Z-stack.
- If you are doing acquisition on fixed sample with multiple wavelength, check both the « Acquire Z series for one wavelength at a time » and « Keep shutter open between steps » options.
- If you are doing acquisition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



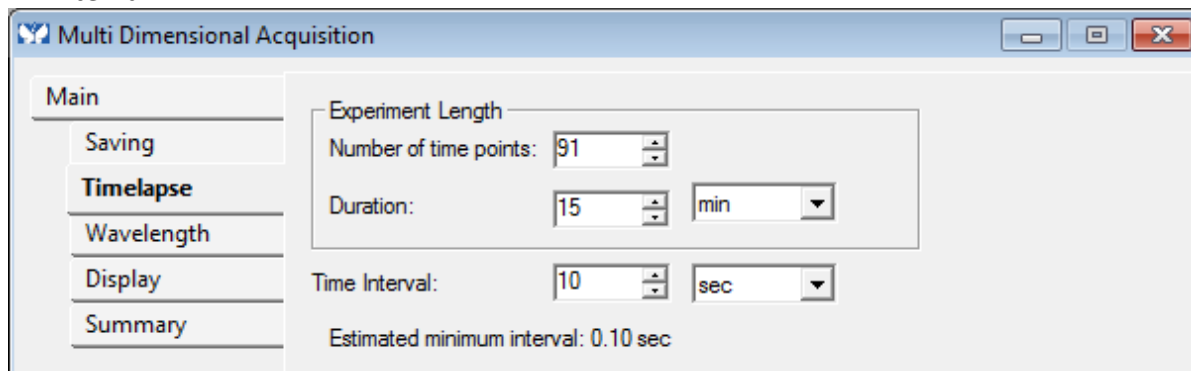
Acquire a time serie

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

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



2. In the tab « *Timelapse* » 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

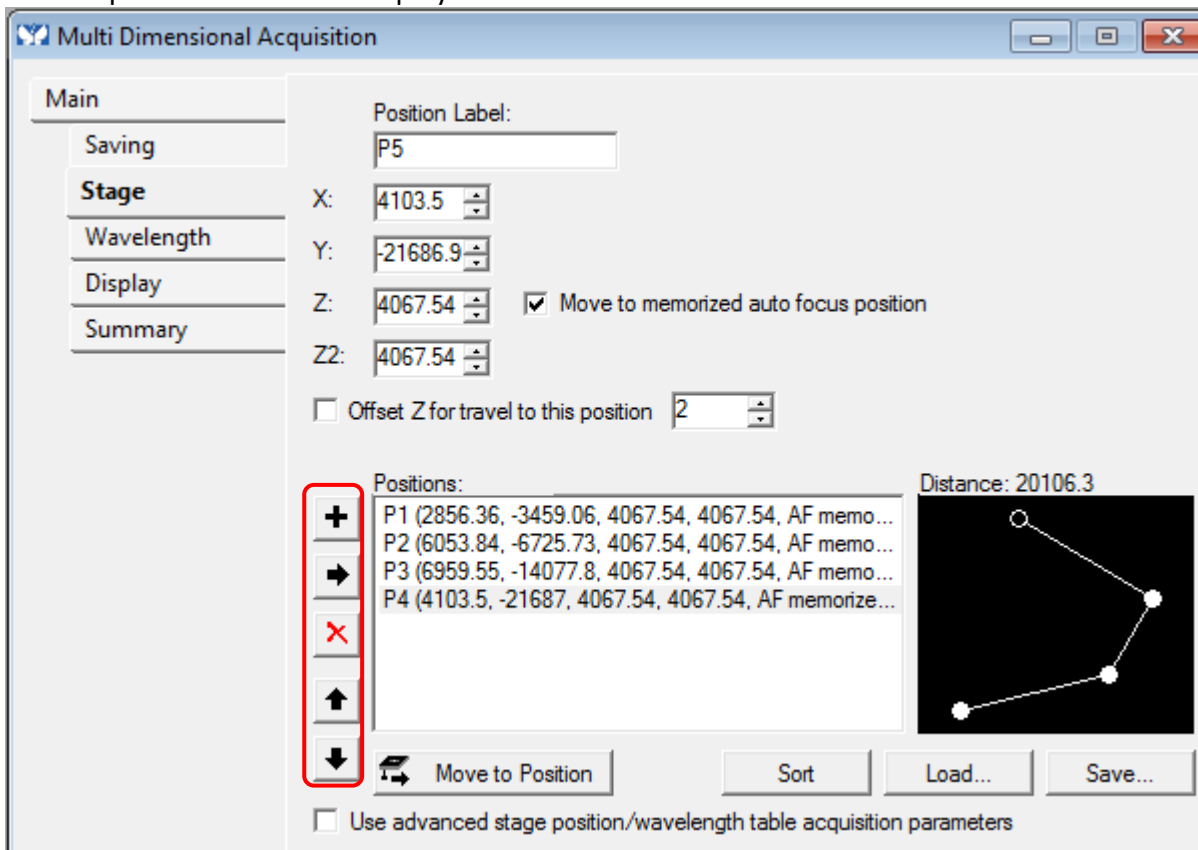
Refer to the chapter « acquire an image with the camera» but add those steps:

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



2. In the tab « *Stage* » in MDA:

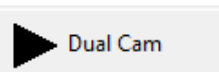
The position number is displayed in the window « *Position Label* ».



3. Click on + to add a new position. You can modify, replace, 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.

Use two cameras simultaneously

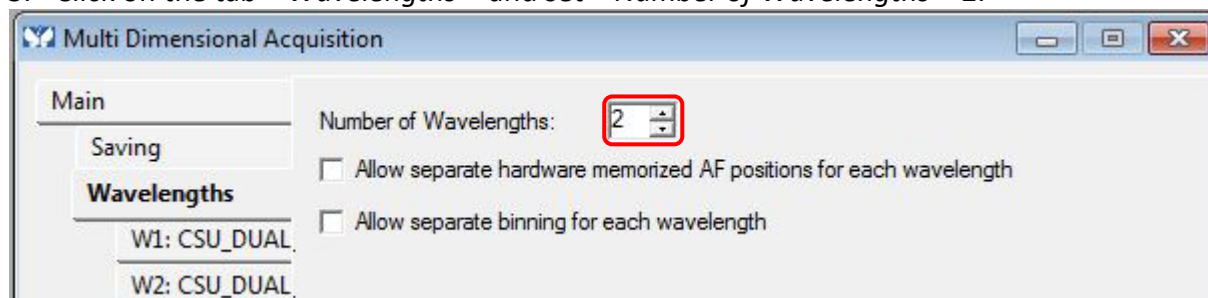
1. In the menu to the left, select the « *Dual Cam* » mode.



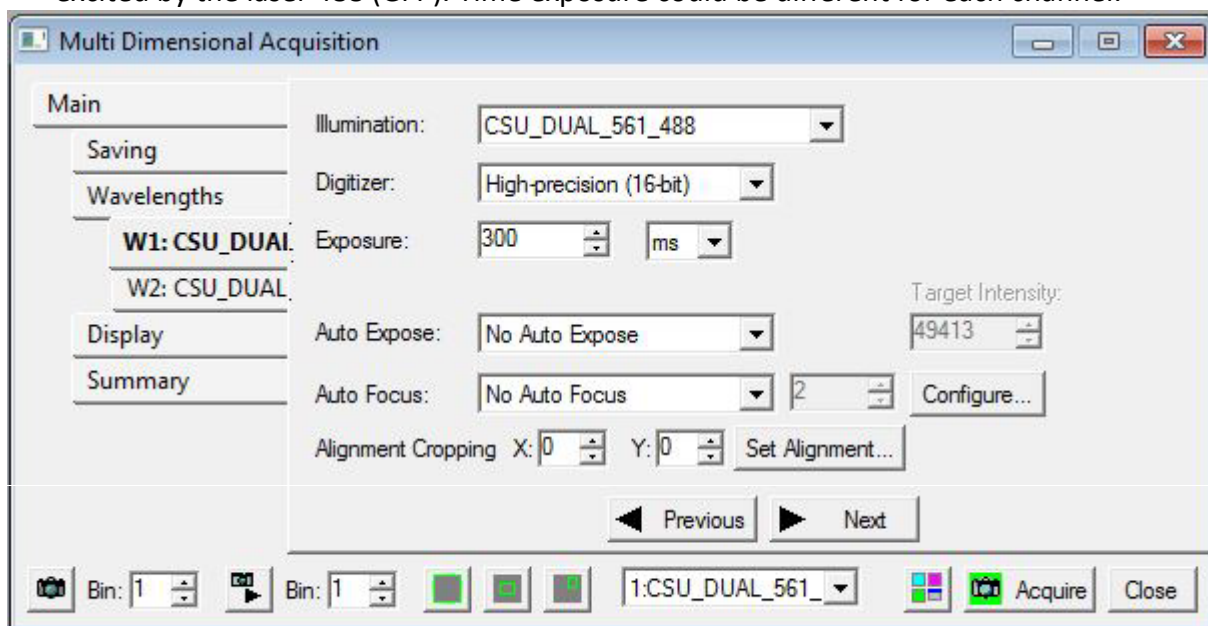
2. In the tab « *Main* » in the MDA window select « *Multiple Wavelengths* ».



3. Click on the tab « *Wavelengths* » and set « *Number of Wavelengths* » 2.



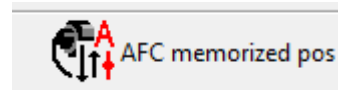
4. In the tab « *Wavelengths* » in the MDA window, choose the same illumination « *CSU-DUAL* » For the two channels. Ex: W1 is the channel excited by the laser 561nm (RFP) and W2 is excited by the laser 488 (GFP). Time exposure could be different for each channel.



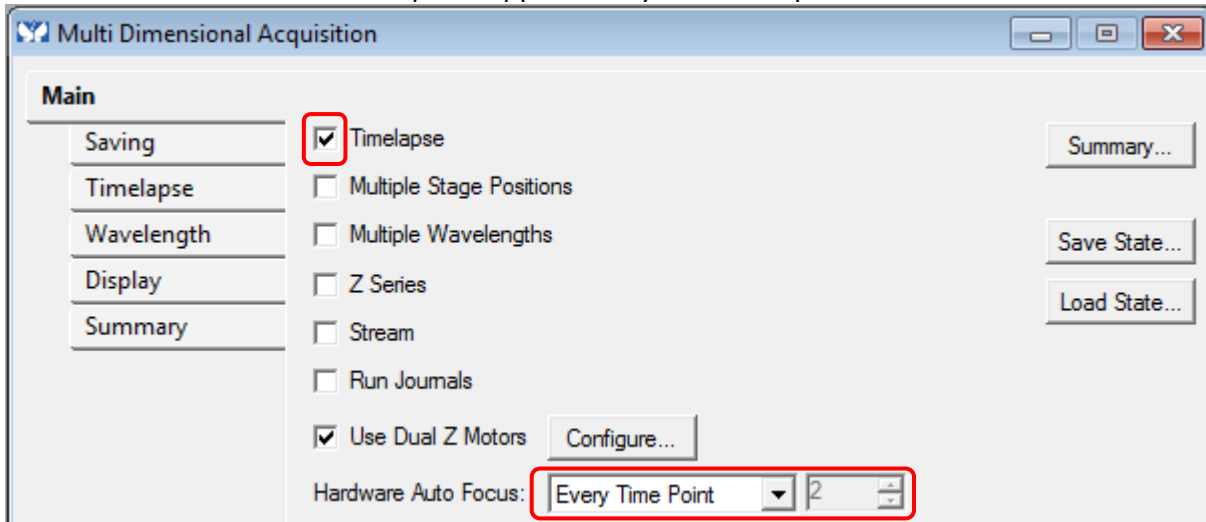
5. Set the laser power for each channels.
6. The two images appear linked in live mode, but will be separate during the acquisition. Click on « *Acquire* » down to the right to start the acquisition.

Use the hardware autofocus

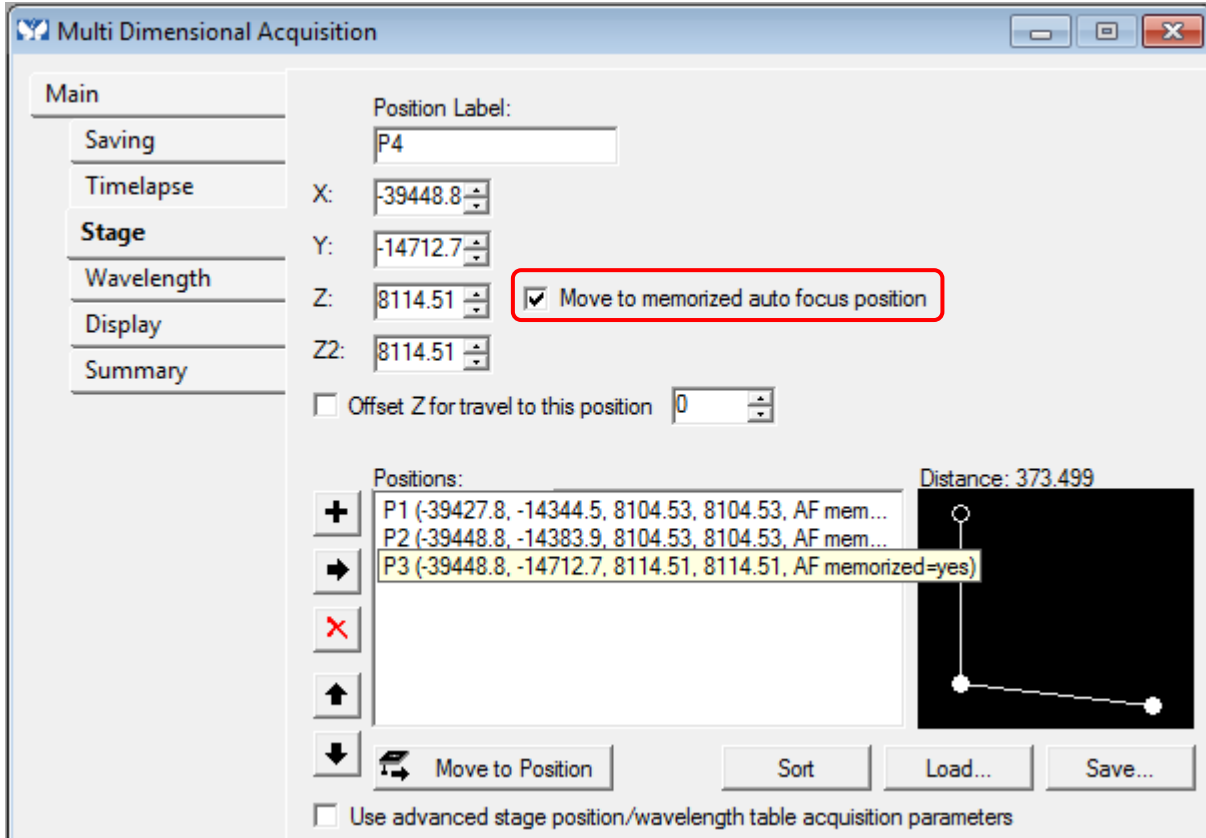
1. Set the focus on your sample.
2. Up to the right, click on « AFC memorized position ».



3. In the tab « Main » in MDA, in the window « Hardware Autofocus AFC » choose the interval use of the autofocus. This option appears only if « Timelapse » is checked.



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



5. Click on « Acquire » down to the right to start the acquisition.

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:

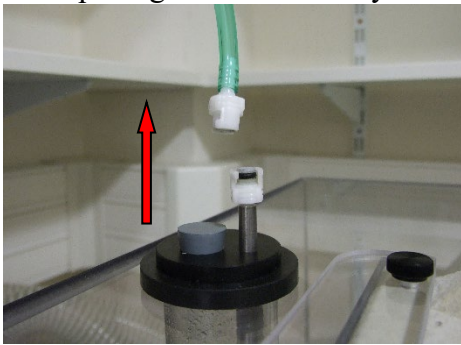
1. Lower the objectives and check that they are cleaned properly (lens + sides).
2. Exit MetaMorph (File/Close)
3. Transfer your data and switch off the computer
4. Turn off the wall switch
5. Turn off the fluorescent lamp

Switch off the temperature and CO2 controller system

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

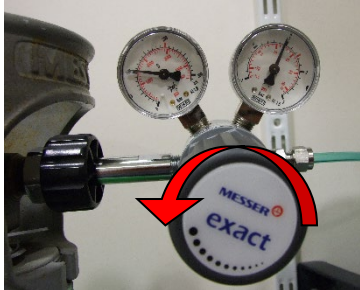


2- Unclip the greenish tub that you will find on the water reservoir on the system.



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

4- Turn the valve of the compressed air pressure regulator (silver) in the clockwise direction.



5- Shut the compressed air bottle (silver pressure regulator) and the CO₂ (golden pressure regulator) by turning them in clockwise direction.

