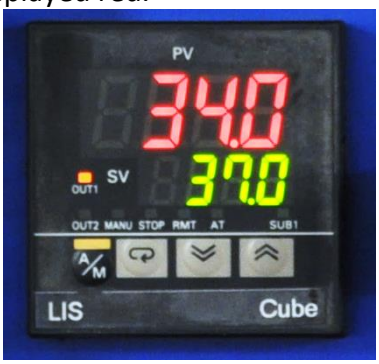
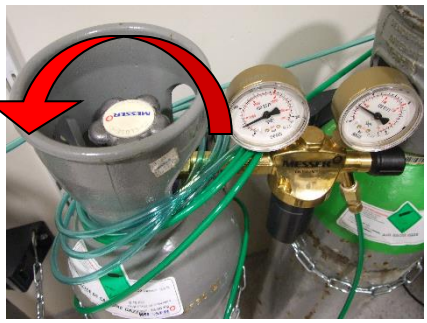
WIDEFIELD LEICA
USER MANUAL

Switch on the CO_2 and temperature controller

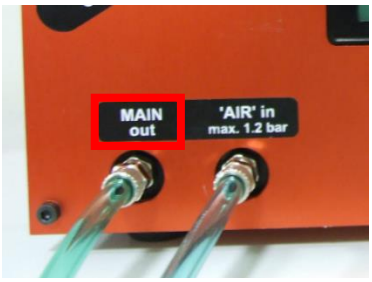
1- Light on the temperature controller with the orange button on the right.
Adjust the temperature to be reached (shown in green) with the arrowed buttons.
The current temperature is displayed red.

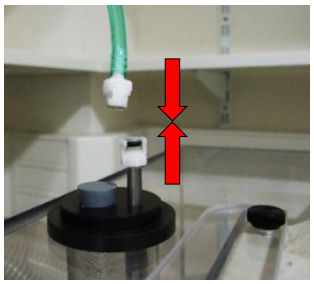


2- Open the air bottle (silver pressure regulator) and the CO_2 bottle (golden pressure regulator) in the anti-clockwise direction.



3- Switch on the CO_2 controller (red brick).
Take the greenish tube connected to the (MAIN out) exit and clip the end of the tube to the water reservoir on the system.

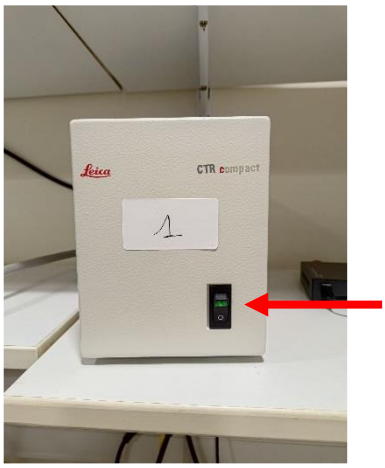




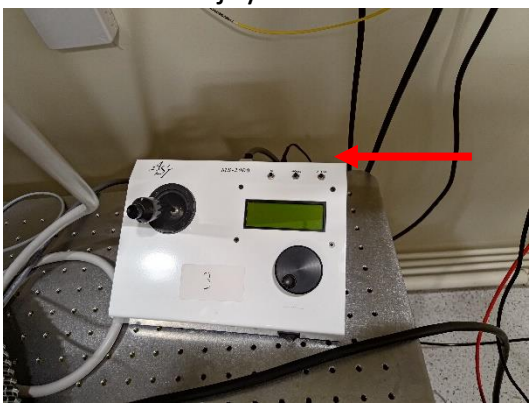
4- Put the CO_2 cover on the sample holder.

Switch on the Widefield Leica

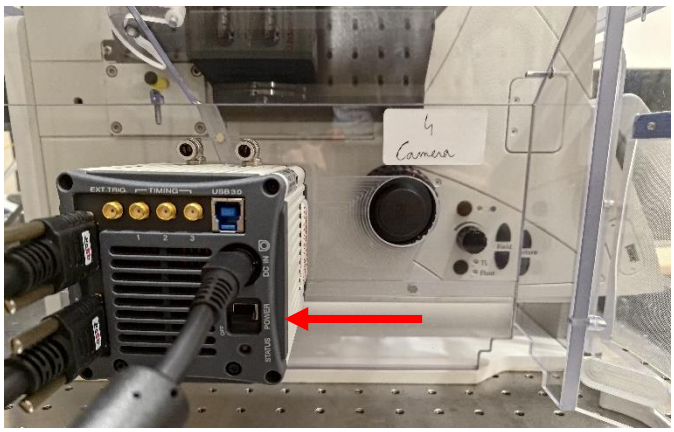
1- Turn on the lamp and the microscope controller.



2- Turn on the joystick. The switch is on the back.



3- Turn on the camera



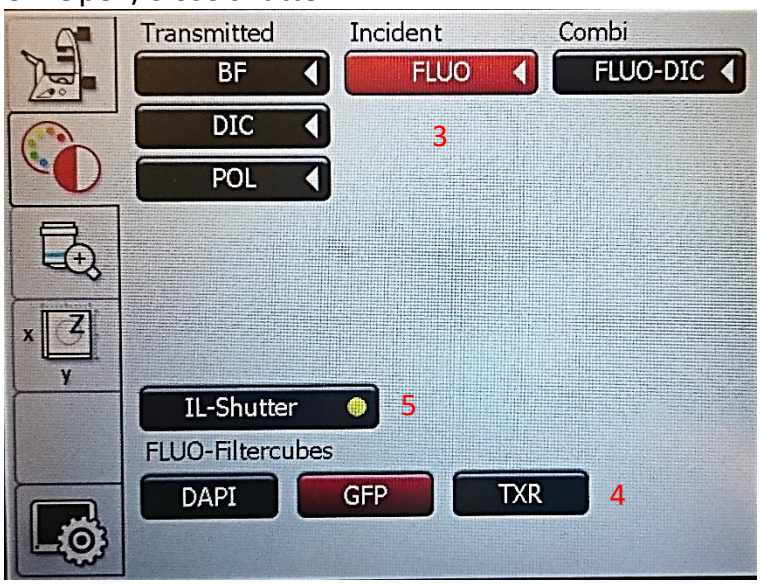
4- Turn on the computer

5- Start the "MetaMorph" software.

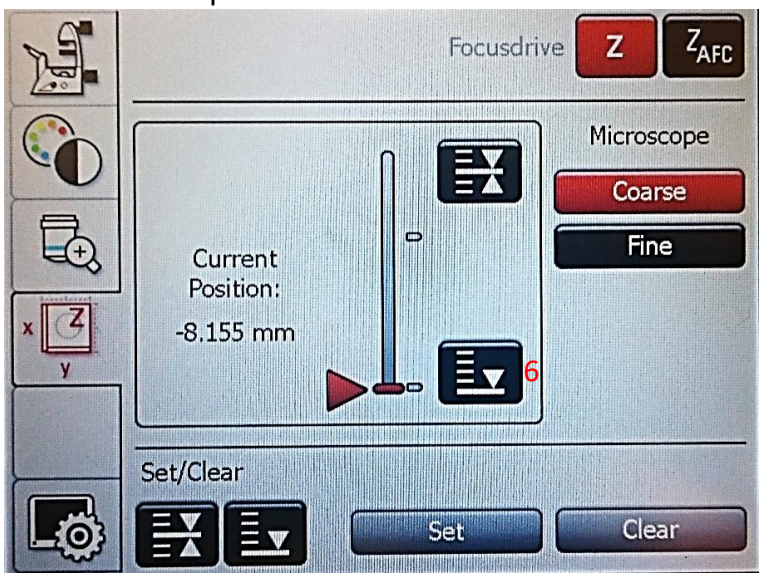
Sample observation with the oculars

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: Brightfiled, 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. (Optimal pixel size calculated with λ_{em} = 520 nm).

Objectives	Optimal pixel size	Camera pixel size (binning 1)	Camera pixel size (binning 2)
100x Oil	99 nm	65 nm	130 nm
63x Oil	99 nm	103 nm	206 nm
40x Oil	106 nm	162 nm	324 nm
40x Dry	230 nm	162 nm	324 nm
20x Imm	423 nm	325 nm	650 nm
10x Dry	460 nm	650 nm	1300 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.

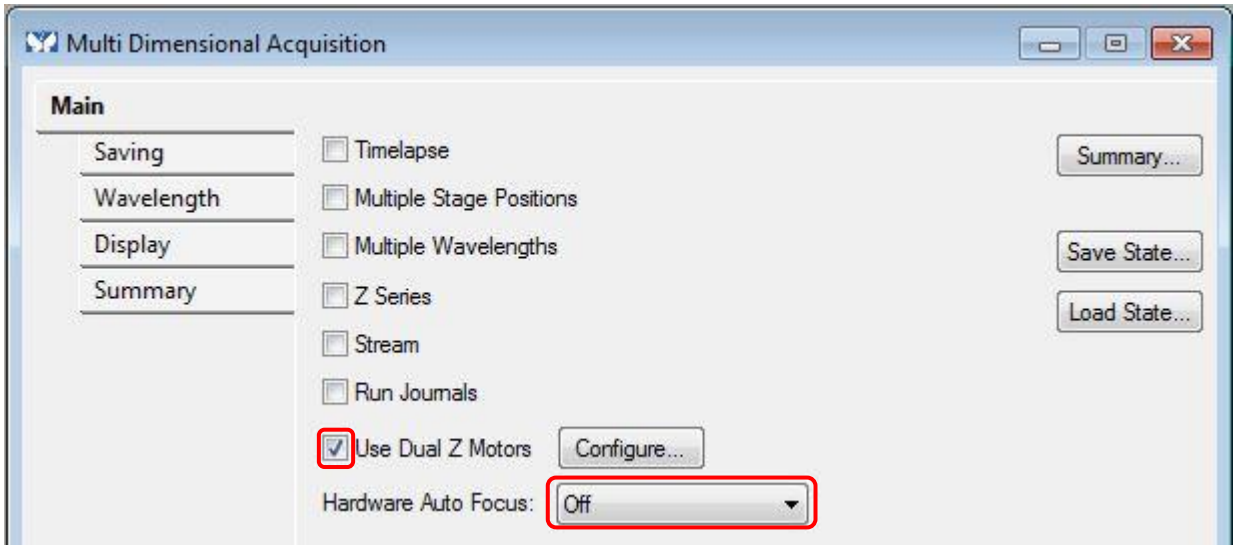
The maximum intensity of the image must never exceed 65 520 to avoid any saturation of the camera.

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

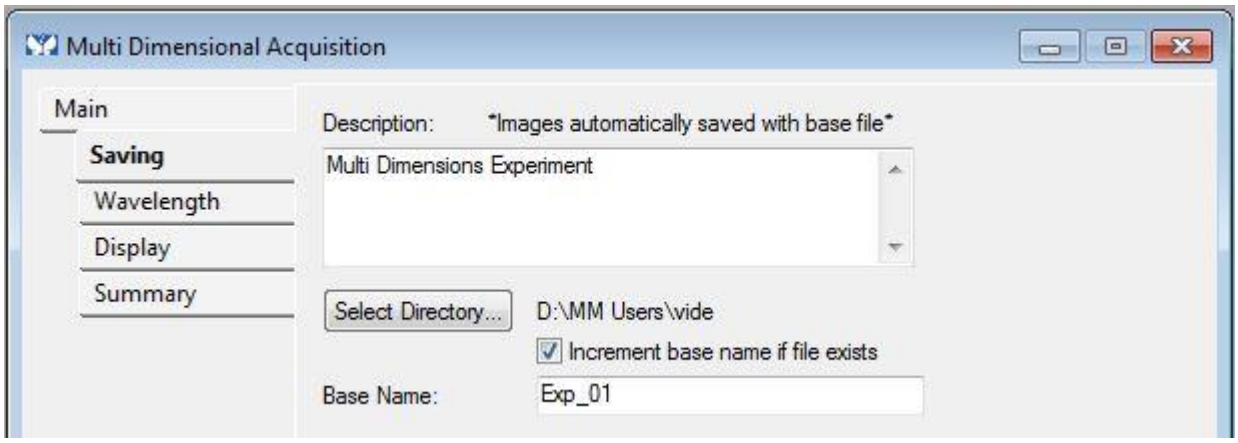
Acquire an image with the camera

1. In the menu on the left click on "MDA". 

2. 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».



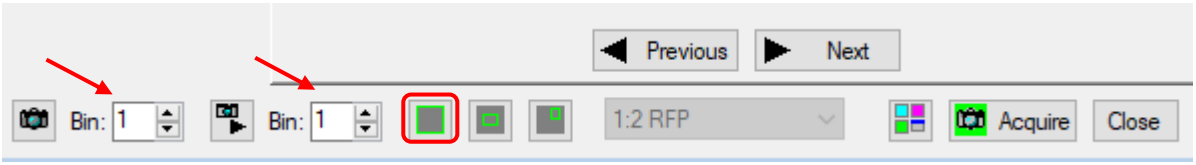
3. Click on the "Saving" tab of the MDA.
   Using the "Select Directory..." button, select the following backup path :
   D : (MMUsers / year / current month / day of the manipulation / folder with your name)
   Images saved in another directory or older than 15 days are automatically deleted without notice.



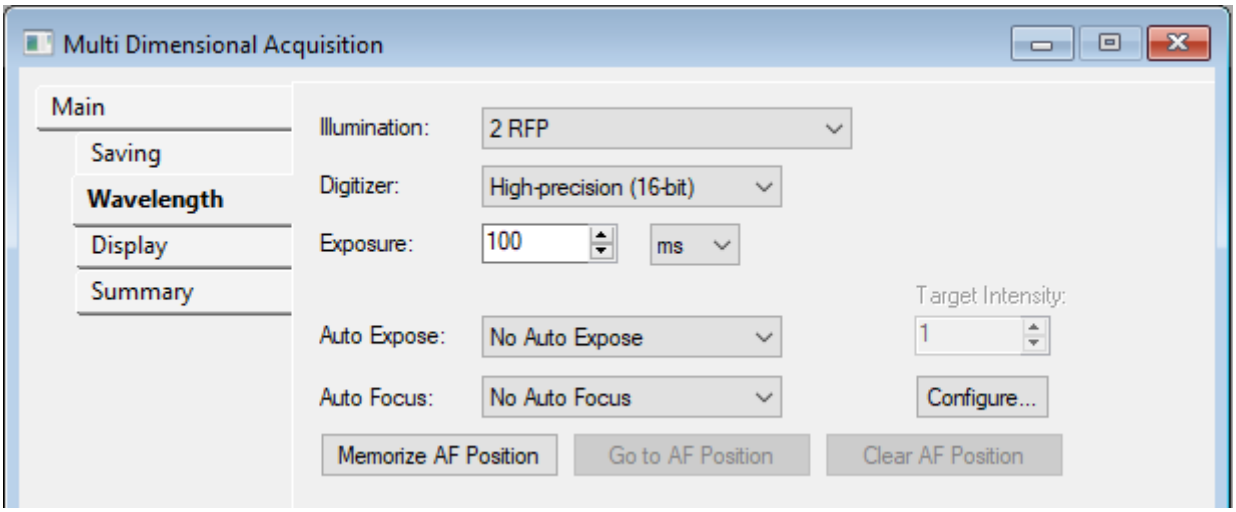
4. In the window « *Base Name* », indicate the name of the image you are about to acquire follow-up with -01 (this number will increment automatically). The images are automatically saved at the end of the acquisition.

5. 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.



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



7. In the window « *Illumination* », choose the type of transmission illumination or the emission filter for the fluorescence.

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

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

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

11. Set the lamp power that correspond to the chosed filter.

12. Click on « *Live* » and make the focus.

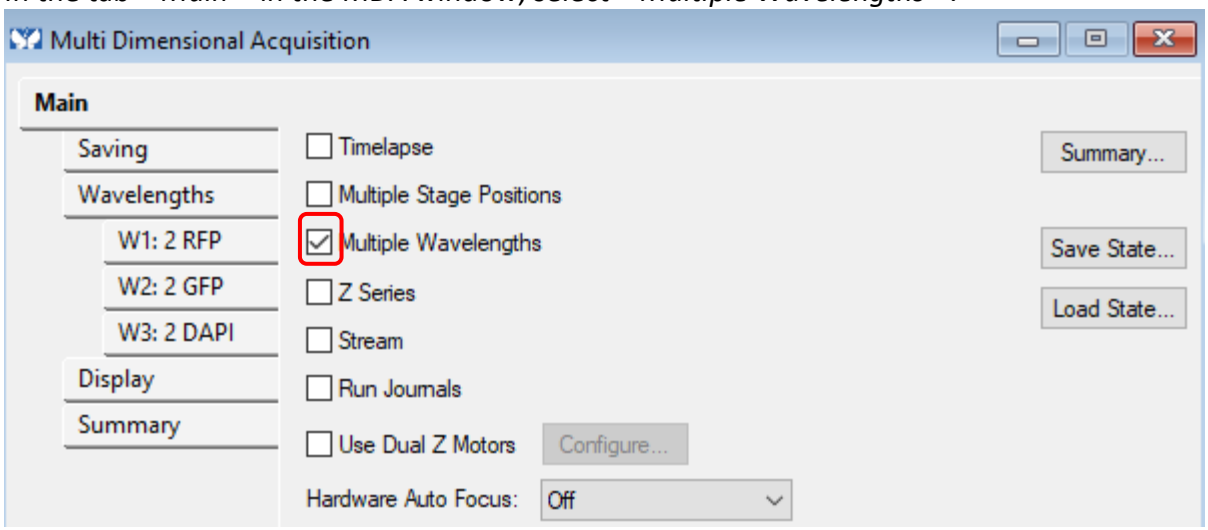
13. Click on « *Snap* » down to the left and  check the dynamic of the image. Adjust the lamp power and exposure time accordingly.

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

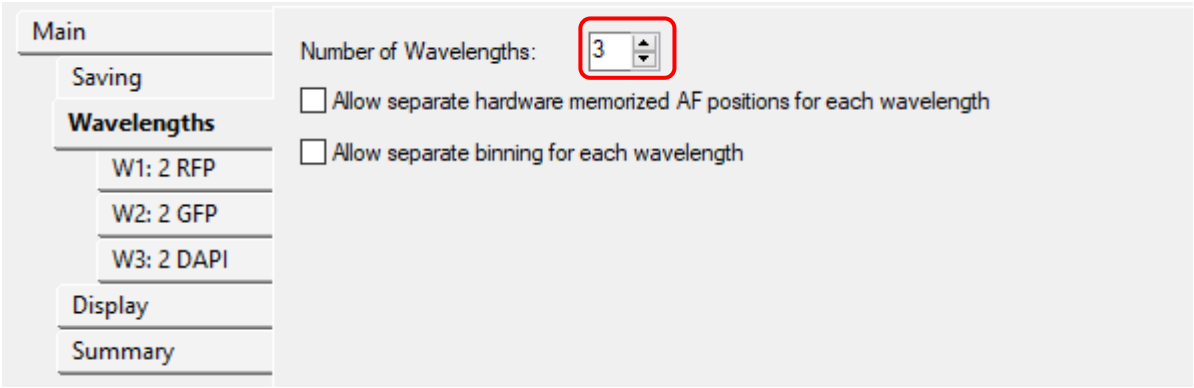
Acquire multiple colors

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

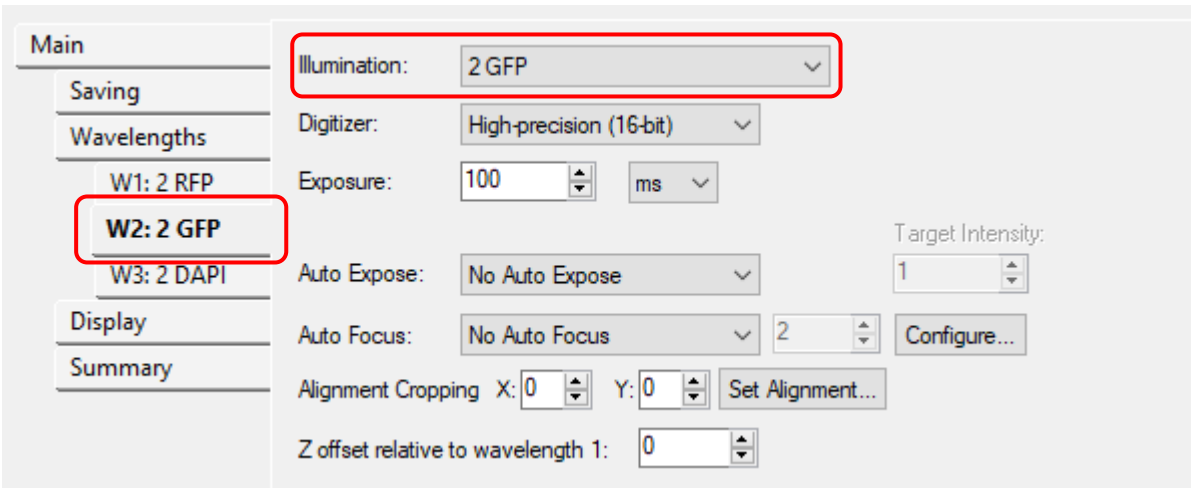
1. In the tab « *Main* » in the MDA window, select « *Multiple Wavelengths* ».



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.



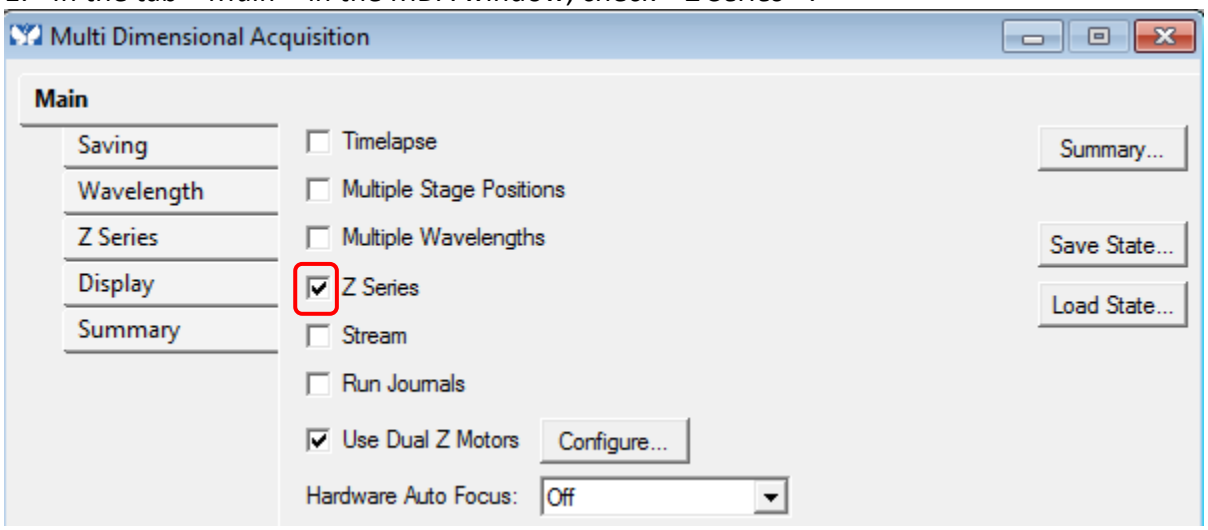
4. Set a lamp power and exposure time (which can differ) for each channel.

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:

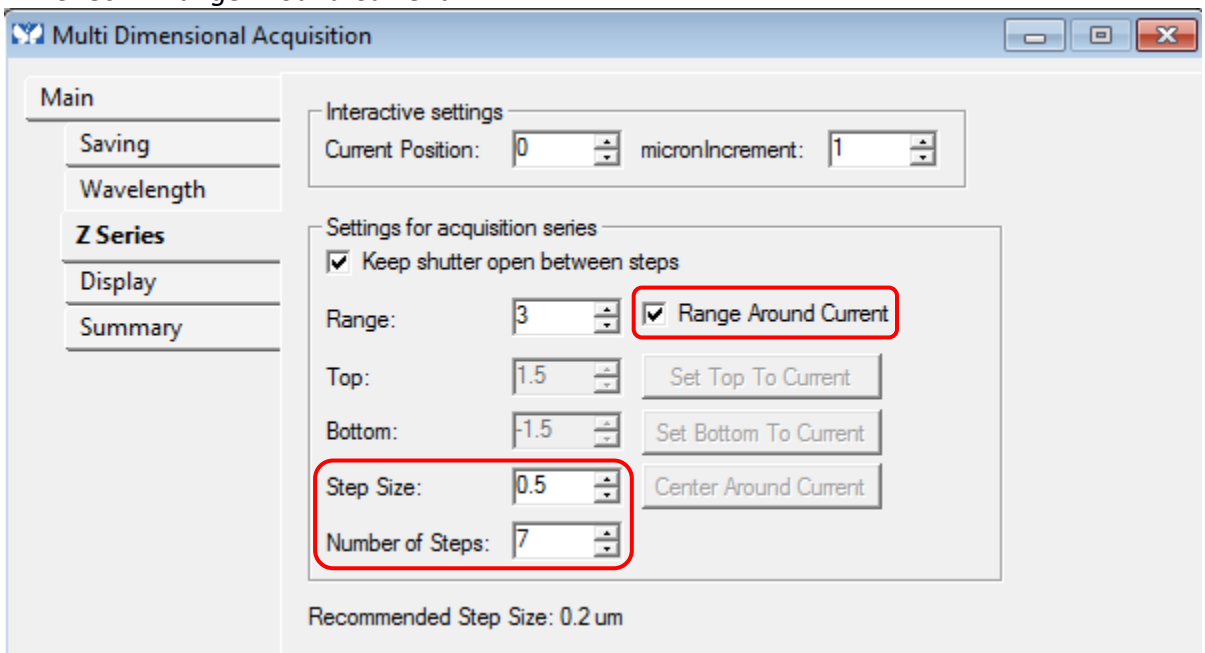
1. In the tab « *Main* » in the MDA window, check « *Z Series* ».



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.

<u>To set the current image as the center of the Z-stack.</u>

2. Check « *Range Around Current* ».



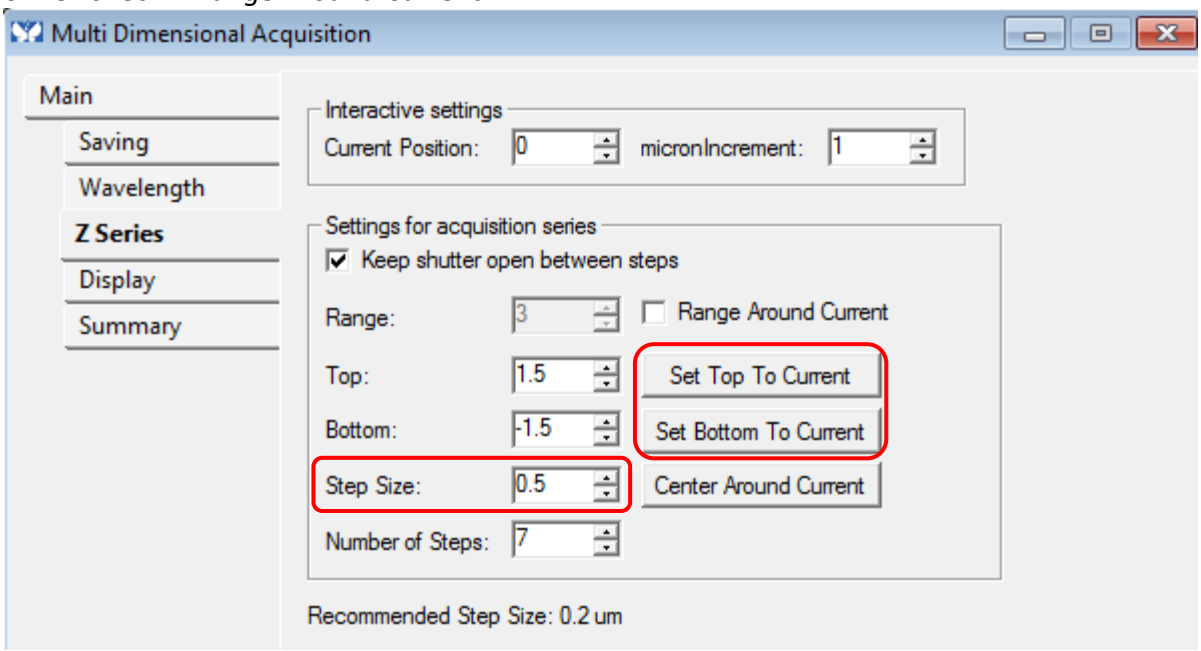
3. Set the focus using the micrometric knobs.

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* ».



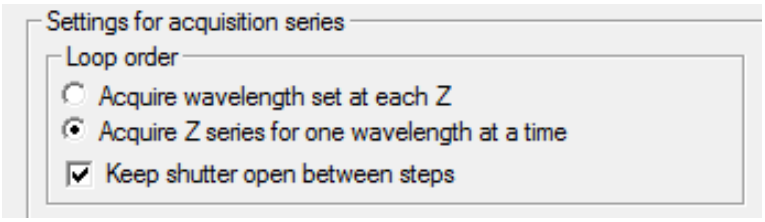
7. Change the focus with the piezo stage until the upper limit of the stack and click on « *Set Top to Current* ».

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.

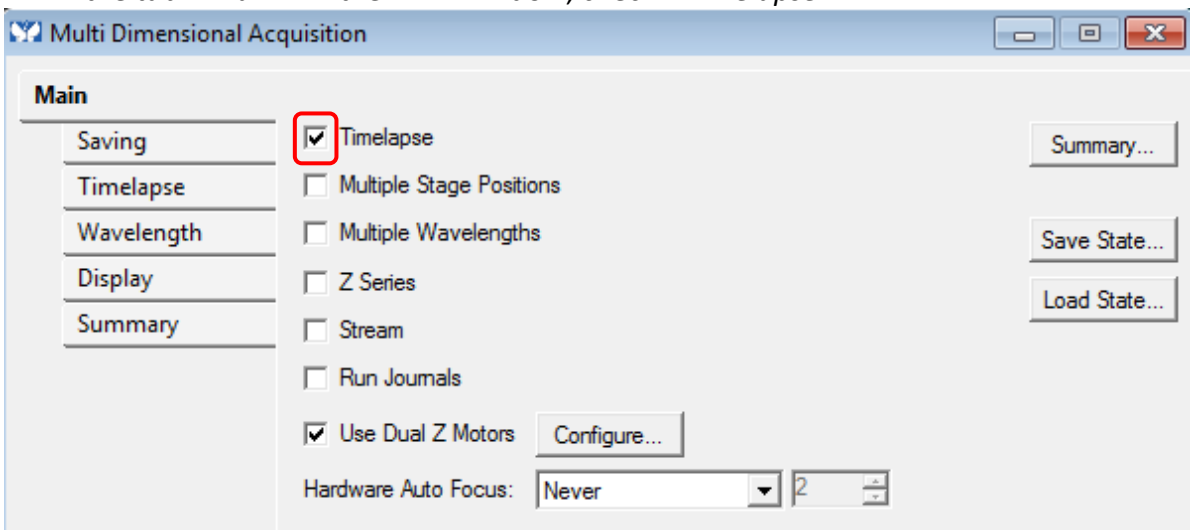
Notes :
- The lamp power and exposure time must be set on the most intense plane of the Z-stack.
- If you are doing acquisition on fixed sample with multiple wavelength, check both the « *Acquire Z series for one wavelength at a time* » and « *Keep shutter open between steps* » options.
- If you are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



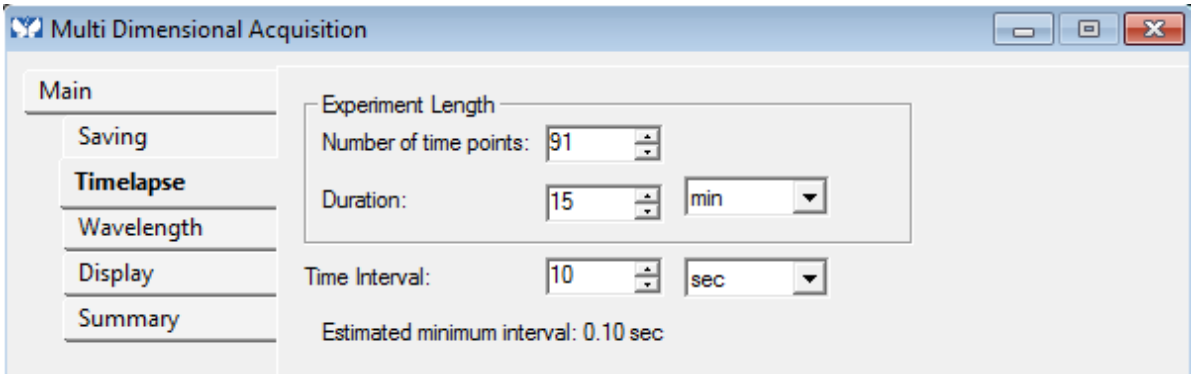
Acquire a time serie

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 lamp power accordingly.

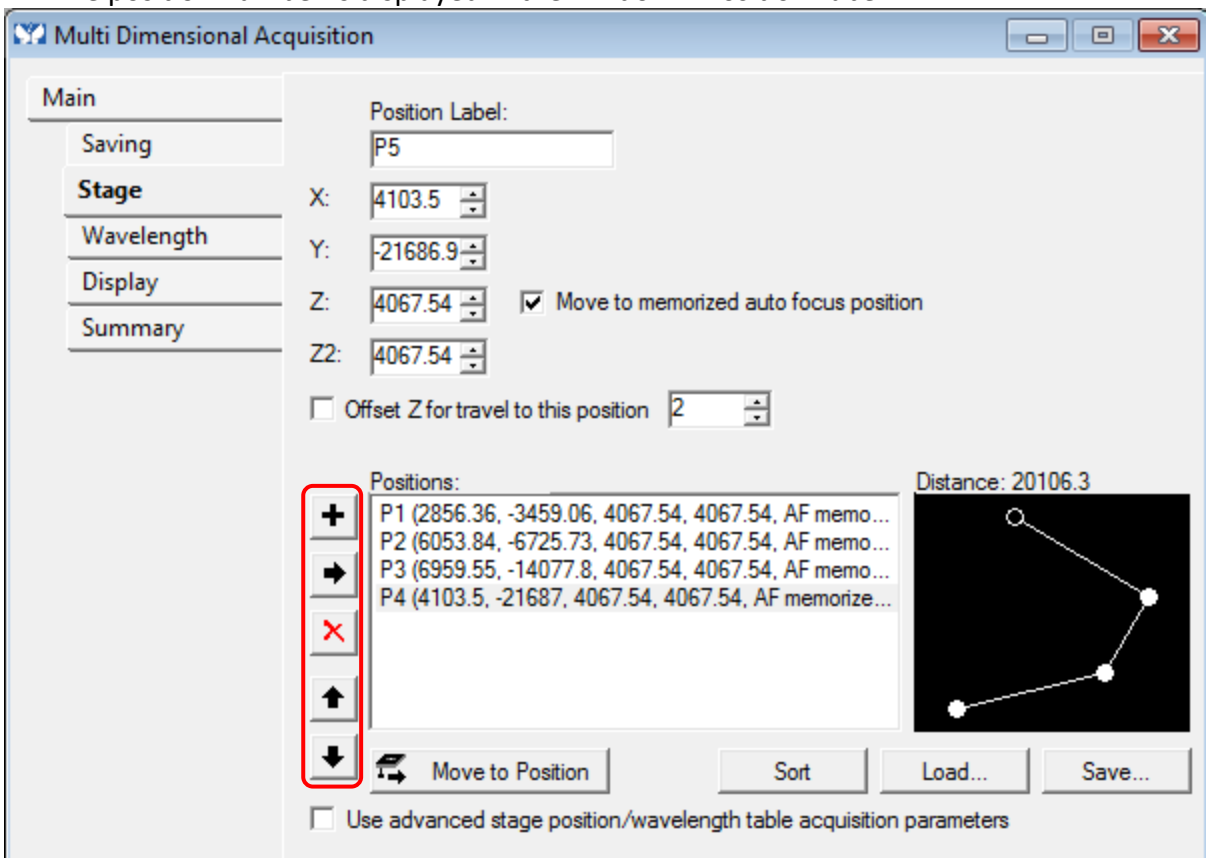
5. Click on « *Acquire* » down to the right to start the acquisition.

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 +.

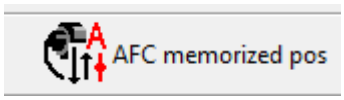
5. The position name and coordinates will appear in « Positions ».

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

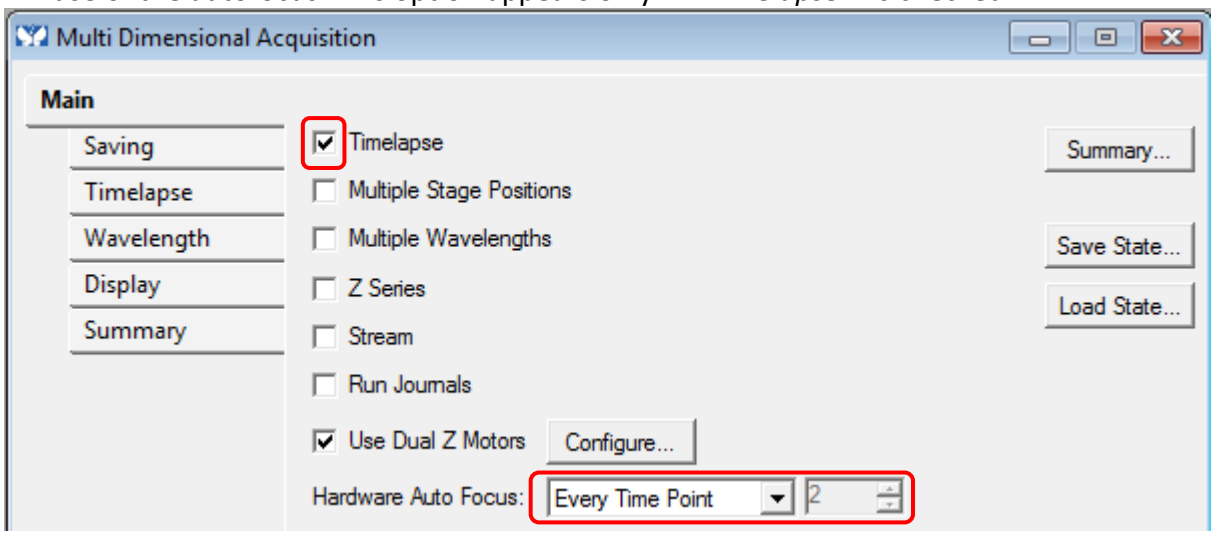
7. Click on « *Acquire* » down to the right to start the acquisition.

Use the hardware autofocus

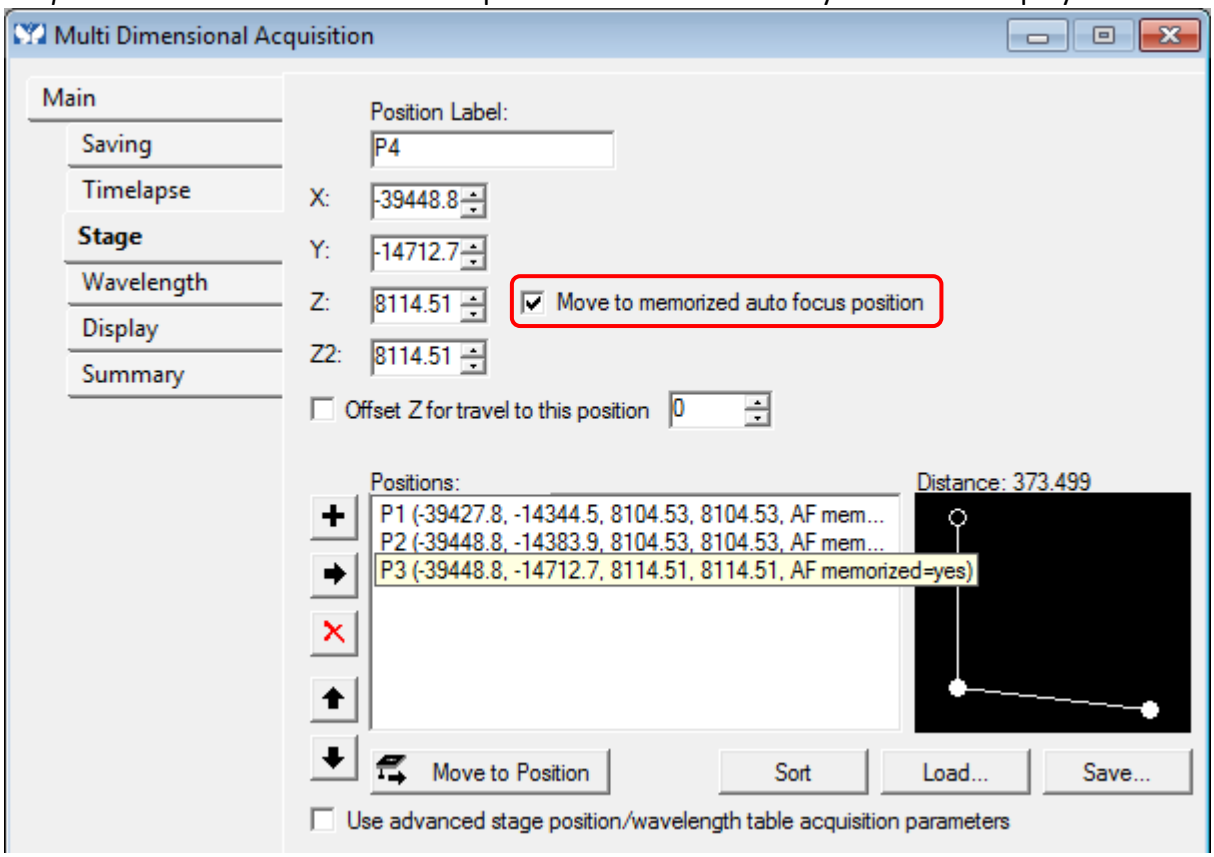
1. Set the focus on your sample.
2. Up to the right, click on « *AFC memorized position* ».



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.

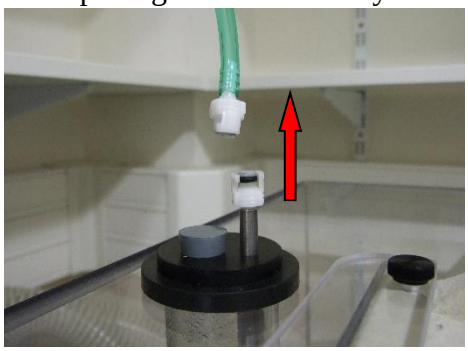
If the system is used:
1. Lower the objectives and check that they are cleaned properly (lens + sides).
2. Exit MetaMorph (File/Close)
3. Transfer your data

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. Turn off the camera
4. Turn off the ASI Joystick
5. Turn off the controller Leica
6. Turn off the fluorescent lamp
7. Transfer your data and switch off the computer

Switch off the temperature and CO2 controller system

1- Remove the CO_2 cap. Be carefull 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 CO_2 and temperature controller.

4- Shut the air bottle and the CO_2 bottle by turning them in clockwise direction.