Spinning disk LEICA CSU W1:

Room: E1036 CBI

Description

It's possible to use this Spinning disk to realize acquisition with 1 camera, 2 symultaneouse cameras, Tirf, Frap, Flip,

- **Microscope :** Inverted Leica DMI8 for brightfield and fluorescence only (not DIC and Ph contrast)
- **AFC:** Adaptative Focus Control, to maintain focus for a long time
- **Spinning Head: CSU W1**
- **Atmosphere control :** temperature and Co2 control
- **Stage :** XY mot stage for multiposition recordings
  Z Piezo stage for fast z stack (Range 300µm)
- **Camera :** Evolve 512:
  512X512 pixels ; pixels size :16µm

  Orca Flash 4.0:
  2048X2018 pixels; pixel size: 6.5µm
- **Software** : Metamorph

1. Objectives:

Magnification	Objective Type	Aperture	Immersion	Coverglass	Working Distance
10X	HC PL APO	0,45	DRY	0,17	2,8mm
20X	HC PL APO CS2	0,75	Imm	Corr	0,66mm
40X	HC PL APO	1,3	oil	0,17	0,22mm
63X	HCX PL APO Lambda blue	1,4	oil	0,17	0,1mm
100X	HC PL APO	1,47	oil	0,17	0,1mm

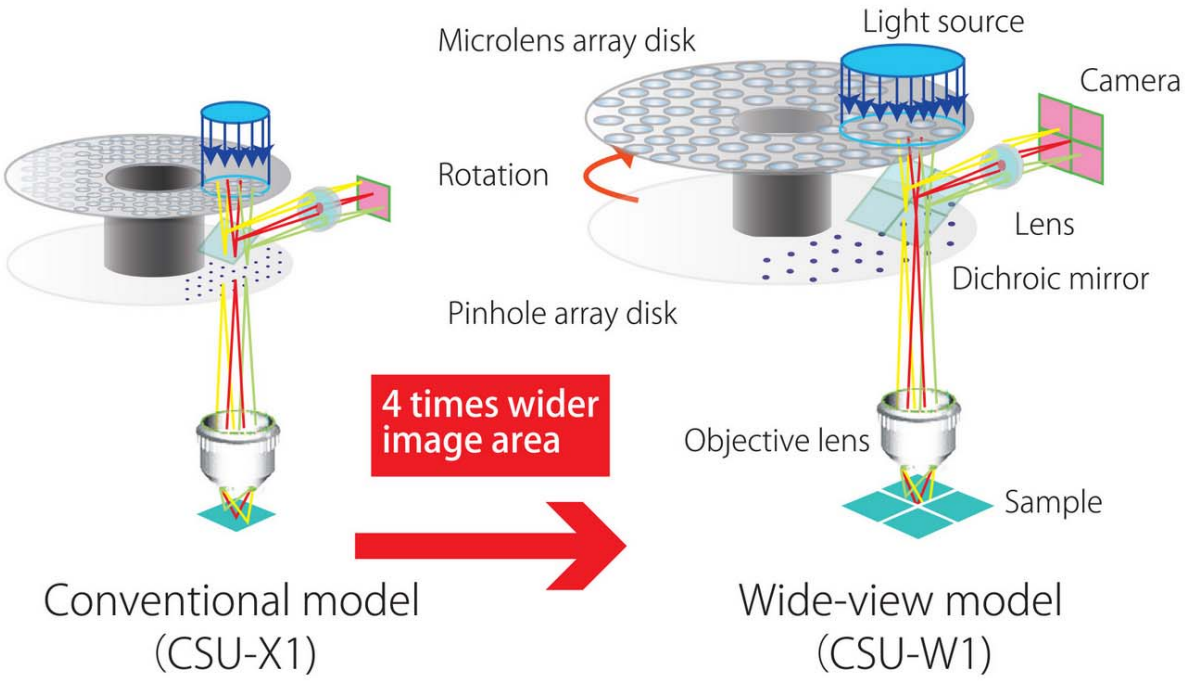
2. Fluorescence filter

Fluorochrome	Excitation nm	Emission nm	Dichroic mirror nm
DAPI	350/50	460/50	400
CFP	436/20	480/40	455
GFP	470/40	525/50	495
TXR	560/40	630/75	585
FRAPCSU			100%
Empty	-	-	-

3. Contrast Method:

Brightfield TL	☒	
Phase Contrats Ph	☐	Not possible
DIC	☐	Not possible
Polarization	☐	Not possible
Darkfield	☐	Not possible
Fluorescence	☒	DAPI, CFP, GFP TEXAS RED

4. CSU-W1:
   - Principe :



- System ILAS²

The iLas MODULAR system is a unique multi-application device that offers complete control over any laser illumination. Its evolutive design allows researchers to choose and simultaneously combine :

- 360° TIRF imaging
- Single molecule imaging
- oblique illumination imaging
- FRAP
- Photo-activation
- Fast spinning disk confocal imaging
- Widefield imaging
- DUAL CAM

- Light Source

405 nm – 445 nm – 488 nm – 561 nm – 642 nm
DAPI – CFP – GFP – Alexa 561 – Alexa 644

- Détection camera 1

1 - 452/45 → DAPI/ Hoechst…
2 - 480/25 → CFP…
3 - 525/50 → Alexa 488/GFP…
4 - 609/54 → Alexa 561/ Rhodamine …
5 - 708/75 → Alexa 644 …

- Detection Camera 2 (only for dual cam)

1 - 452/45 → DAPI/ Hoechst…
2 - 480/25 → CFP…
3 - 525/50 → Alexa 488/GFP…

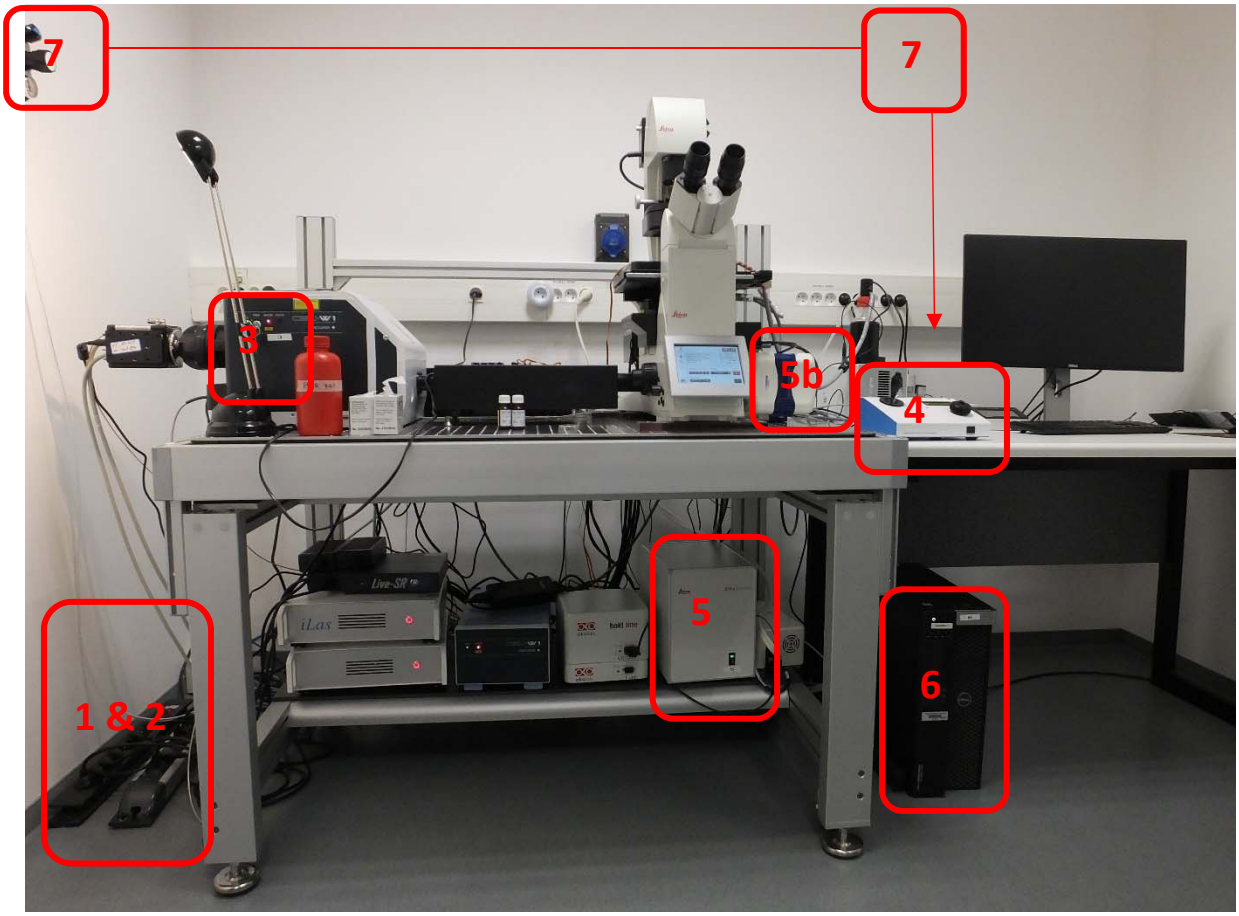
IGBMC CBI

For BOOKING System :

http://ici-grr.u-strasbg.fr/login.php

For any informations : groupe-mic-photon@igbmc.fr

Procedures of starting spinning disk



1 & 2 – Turn on the system (multiple plug X2)

3 – turn the key of the spinning head (CSU W1) on the position « ON »

4 – Switch on the stage controler

5 – Switch on the Microscope controler

5b – Switch on the EMCCD camera if you use the TIRF system

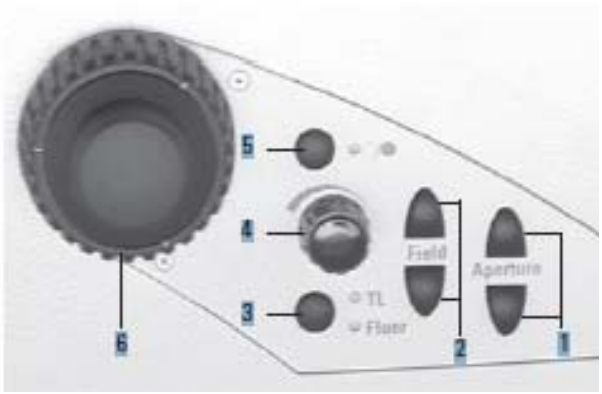
6 – Turn on the computer

7 – If you use the atmosphere control, turn On the Co^2 Valve und the multiple plug

The Microscope: Leica DMI 8

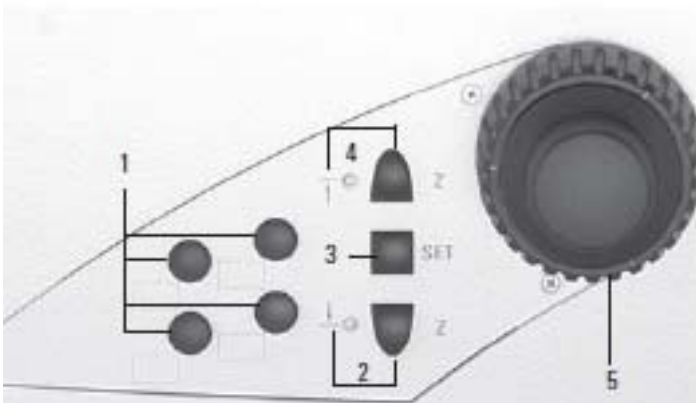
Control panel field on the left microscope side

1 Motorized aperture diaphragm adjustment
2 Motorized illuminated field diaphragm adjustment
3 Motorized toggling between the TL/Fluor illumination axes
4 Adjusting the Transmitted Light brightness of the intensity levels for the automated FIM (fluorescence intensity manager)
An LED indicates the illumination method which is currently affected by the brightness adjustment.
5 Opening/closing the motorized shutter
or during manual shutter light control
An LED displays the state of the shutter:
LED on = shutter opened
LED off = shutter closed.
6 Focusing



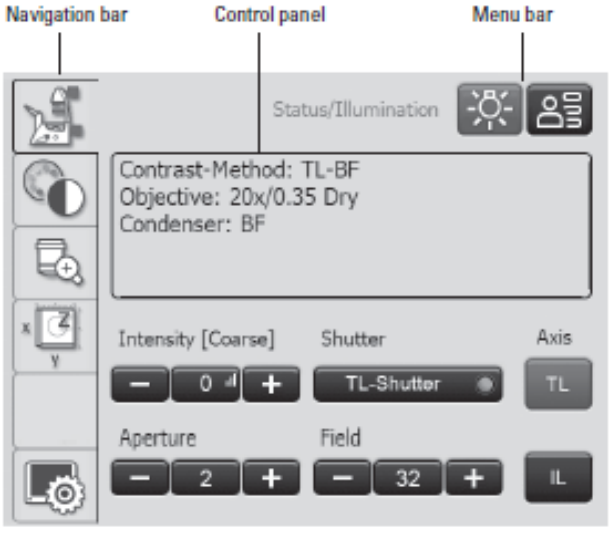
Control panel field on the right microscope side

1 Fonction Keys to move the objective turret and filter Cube turret
2 Focus threshold (the LED illuminates when the focus threshold is set)
3 Setting the focus position and focus threshold
4 Focus position (the LED illuminates when
the focus position is set)
5 Focusing



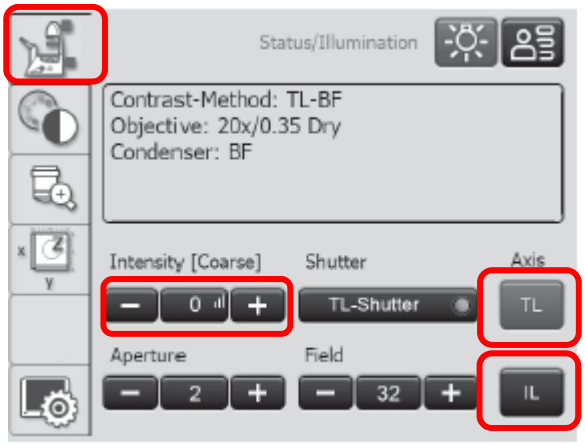
The Touch Screen

The Touch Screen adjusts all motorized components
Of the Leica DMi8 automatically via buttons.
After the device is switched on, the display shows
the current microscopestatus.
The display and functions that can be operated using
the Touch Screen depend on the features of the indivic



Touch Screen after initialization

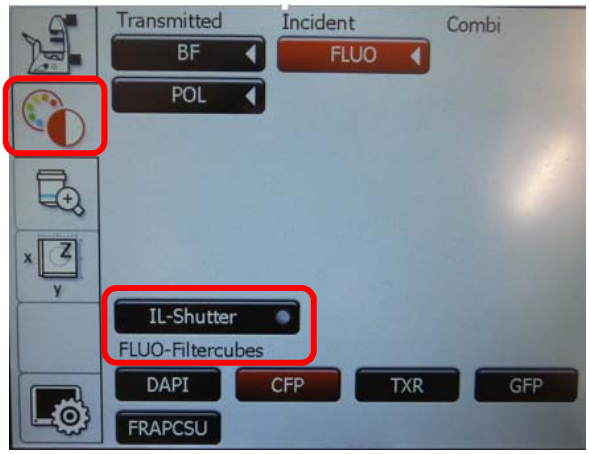
Basic microscope settings



Adjusting the Transmitted Light Brightness

Adjusting the Fluorescence Light Brightness

Contrast methods

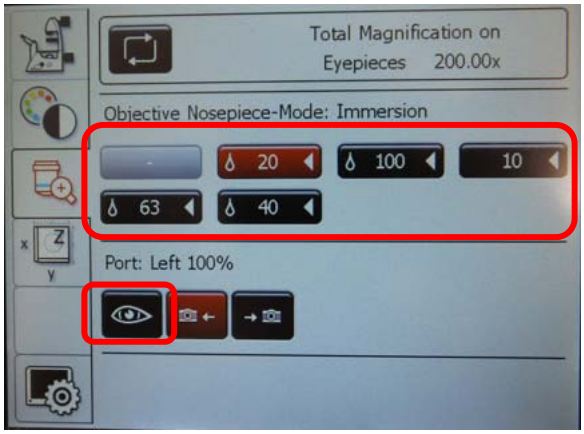


Select the contrast method

Select the filter cube if it's necessary

Switch on the lamp with the shutter button

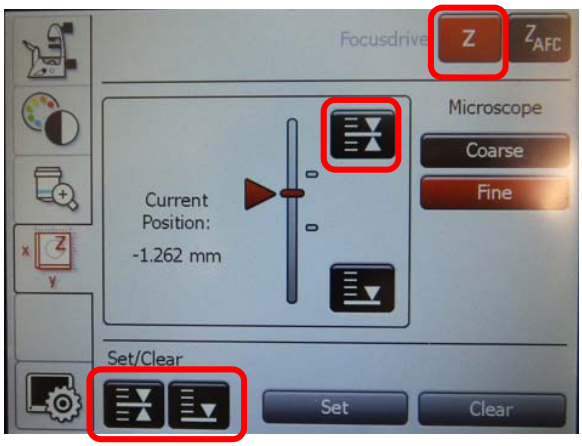
Magnification



Select the magnification (all objectives are immersion oil except the 10X is dry)

Select the right or left side port using the corresponding key or point 100% of the light at the eyepiece.

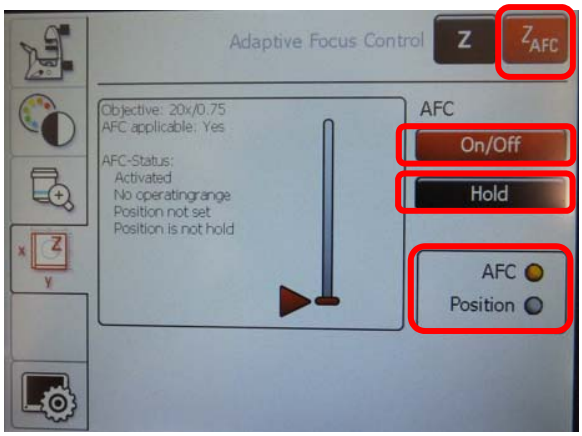
Stage and focus controls



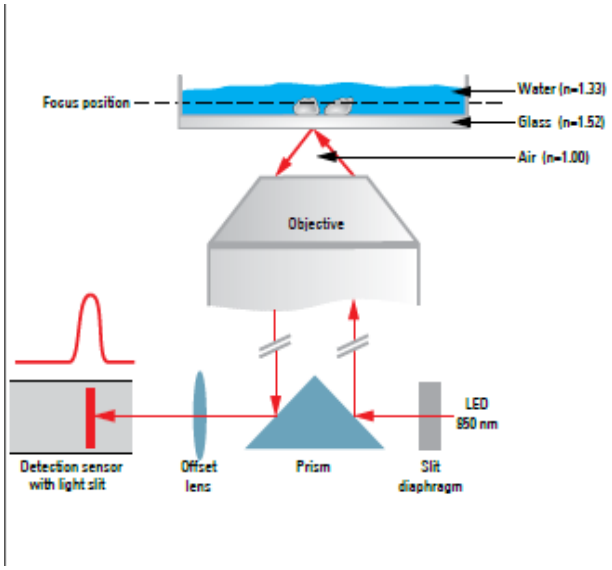
Select the **Focus Drive** menu using the key.
The current Z position is shown in the information field.

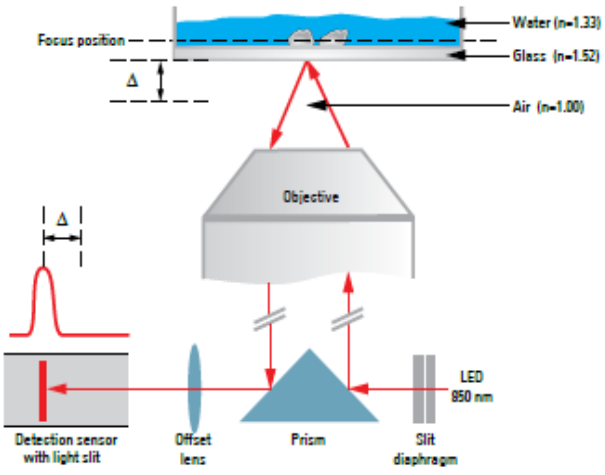
When the focus position is set, it can approached directly using the key
To set or clear the focus position or the lower threshold,select the corresponding position under **Set/Delete**, then press the **Set** or **Delete** key.

Leica **A**daptive **F**ocus **C**ontrol



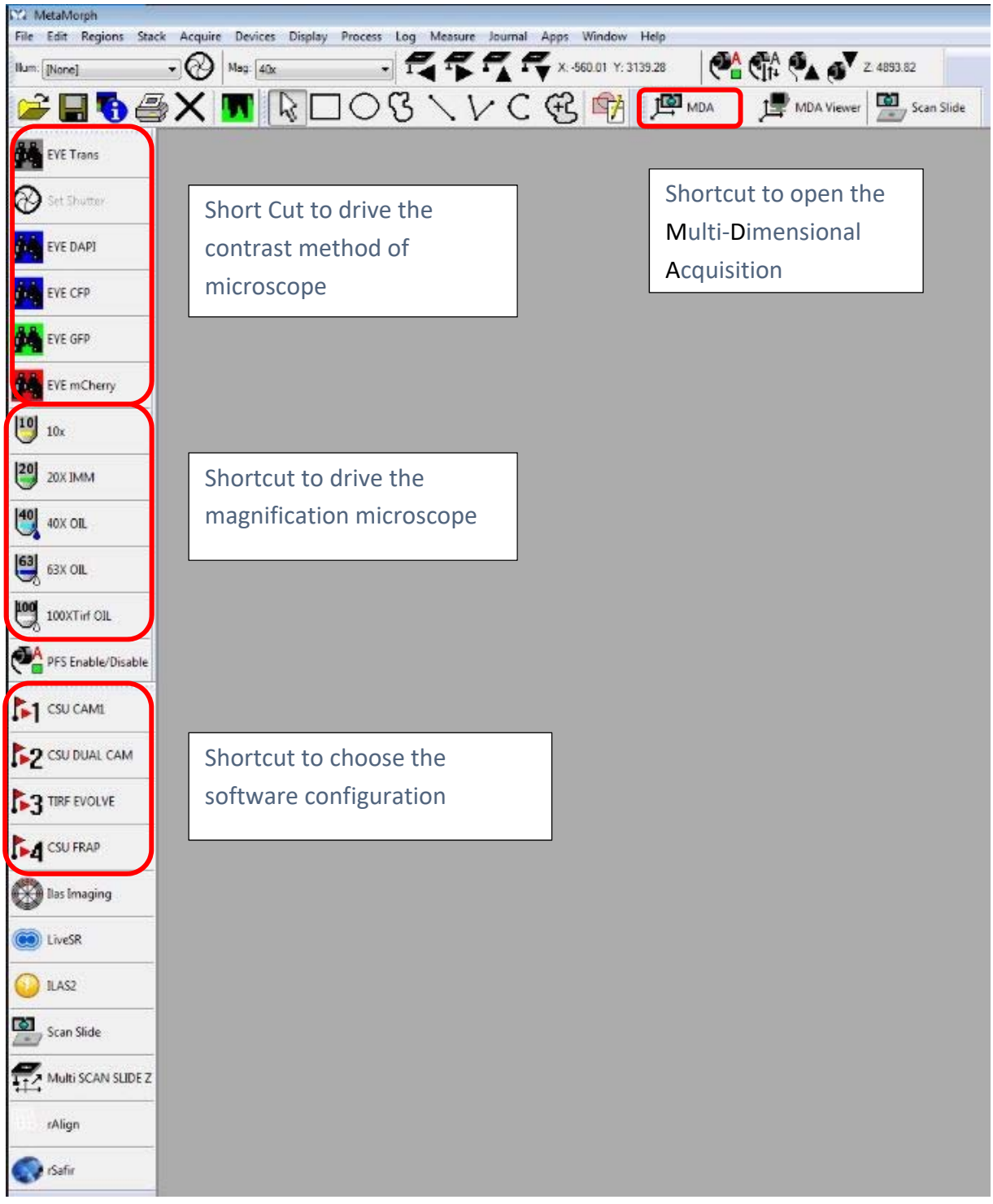
The AFC can be used to actively hold a defined focus position and automatically readjust this position regularly. This is useful if, for example, a temperature change is caused by opening the climate chamber during living cell experiments at 37°C.
The AFC is activated, then the specimen on the stand is focused using the handwheel and the stopping position is saved.
Activate the AFC using the **On/Off** key. The knob behind **AFC** turns green.
Focus the specimen ansd save the position using the **Hold key**.
If the position is held, the knob behind **Position** will turn green.



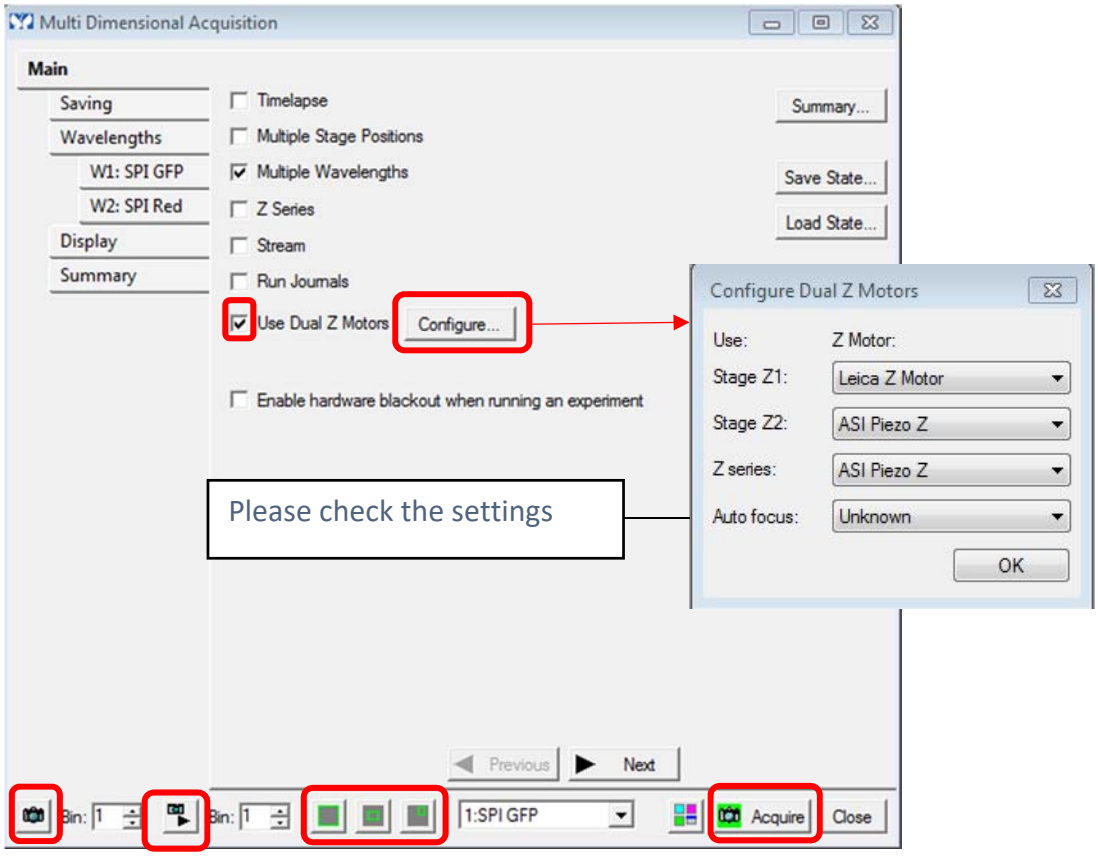


Software: METAMORPH

Double clics on your METAMORPH ICON

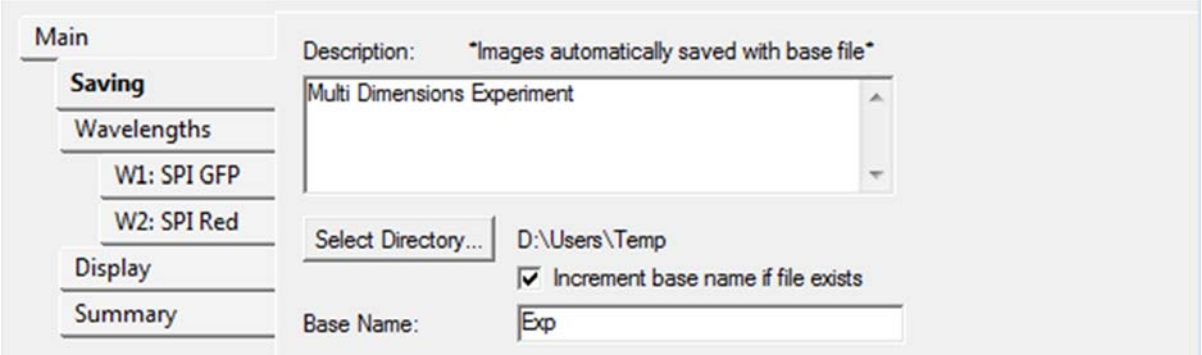


1 – Clic on MDA Icon, a new windows opens. In this interface, you can choose what you want to do.



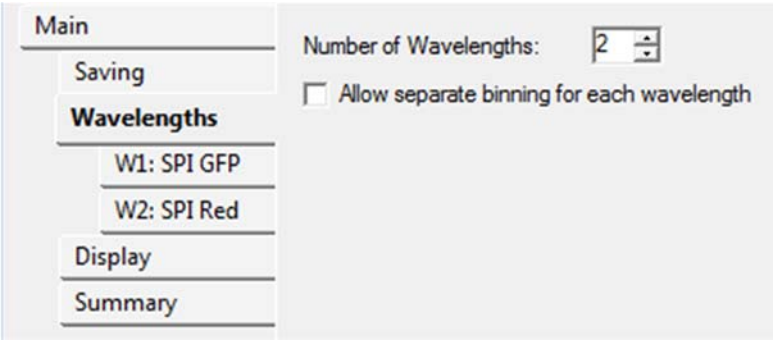
	SNAP	Acquire an image using the current settings specified in the Acquire main dialog box
	LIVE	Showing a live image
	FULL CHIP	Initialiez the full chip of your camera 512x512
	Center CHIP	To use center chip of your camera 256x256
	ROI CHIP	To use only one ROI chip of your camera
Acquire	ACQUIRE	Start MDA experiment

1.1 Saving :

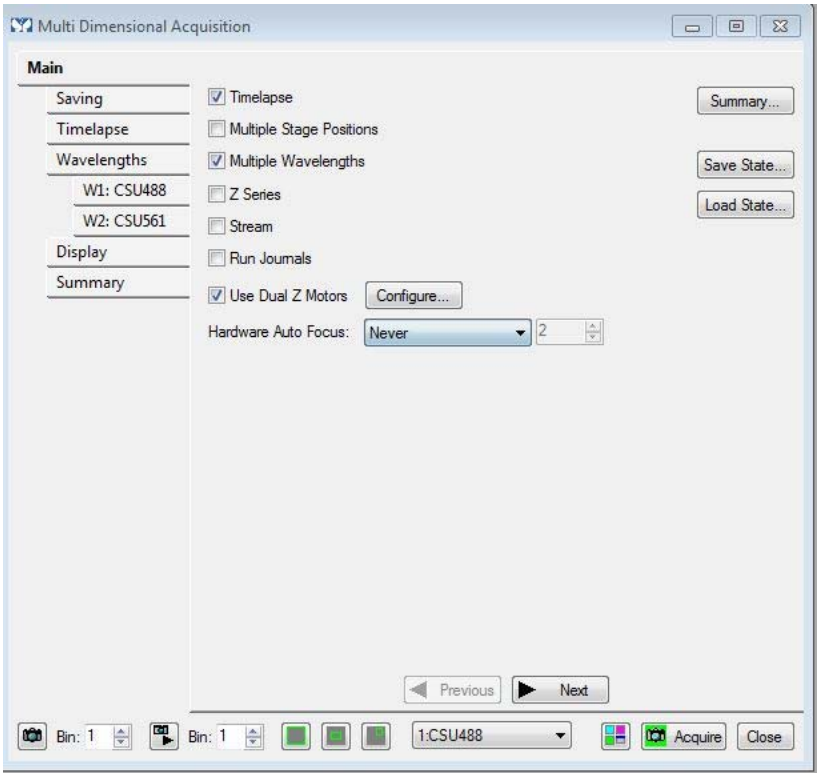


In this windows, select direcly the Save folder by clic on Selct directory icon, it ‘s possible to use the base name and the software automaticly increment the number of the image.

1.2 Multiple Wavelengths :

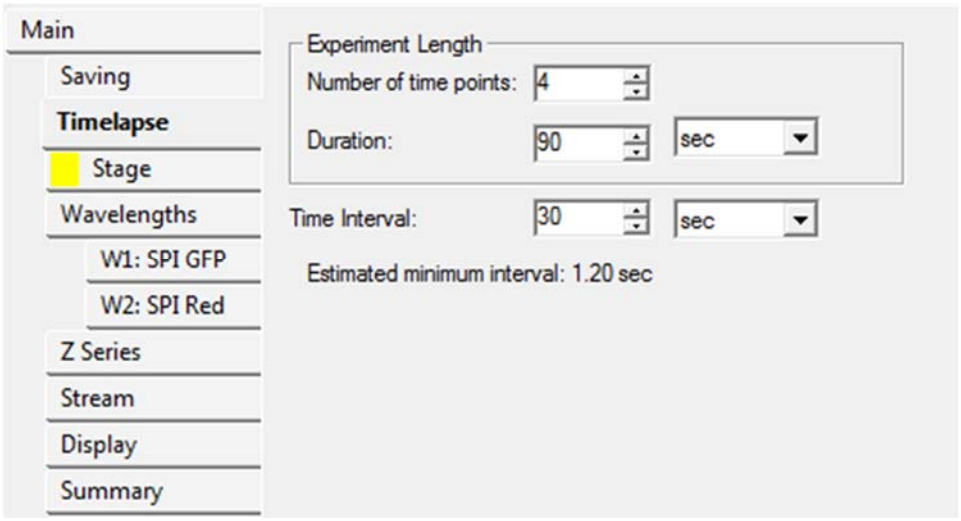


In this windows you select the number of channel (wavelengths).



- After select the illumination you want to use
- You adjust the exposure time (you open the live image for parameter this value)

1.3 Timelapse

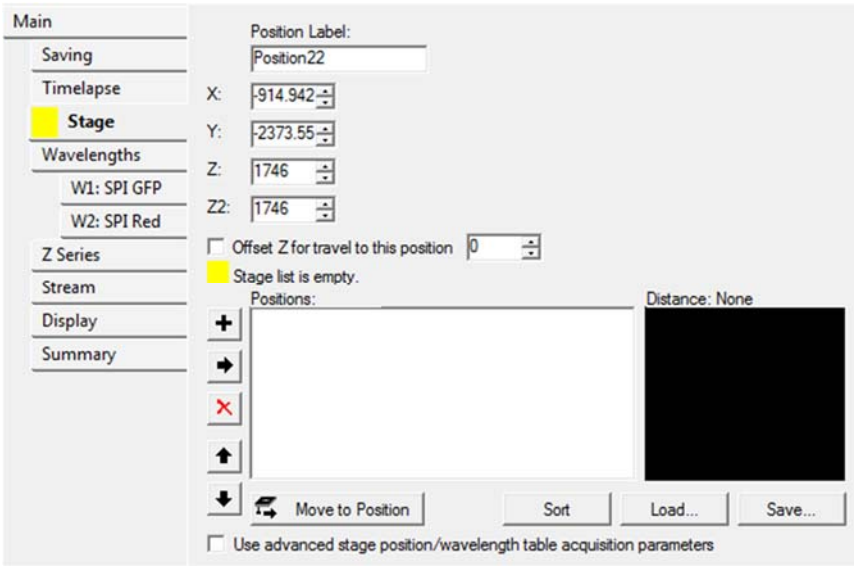


Enter your acquisition parameters.

Be careful, the « Estimated minimum interval » is wrong value. The system doen't consider mechanical movements.

Please test your timelapse with 2 timepoints for estimate the good minimum interval.

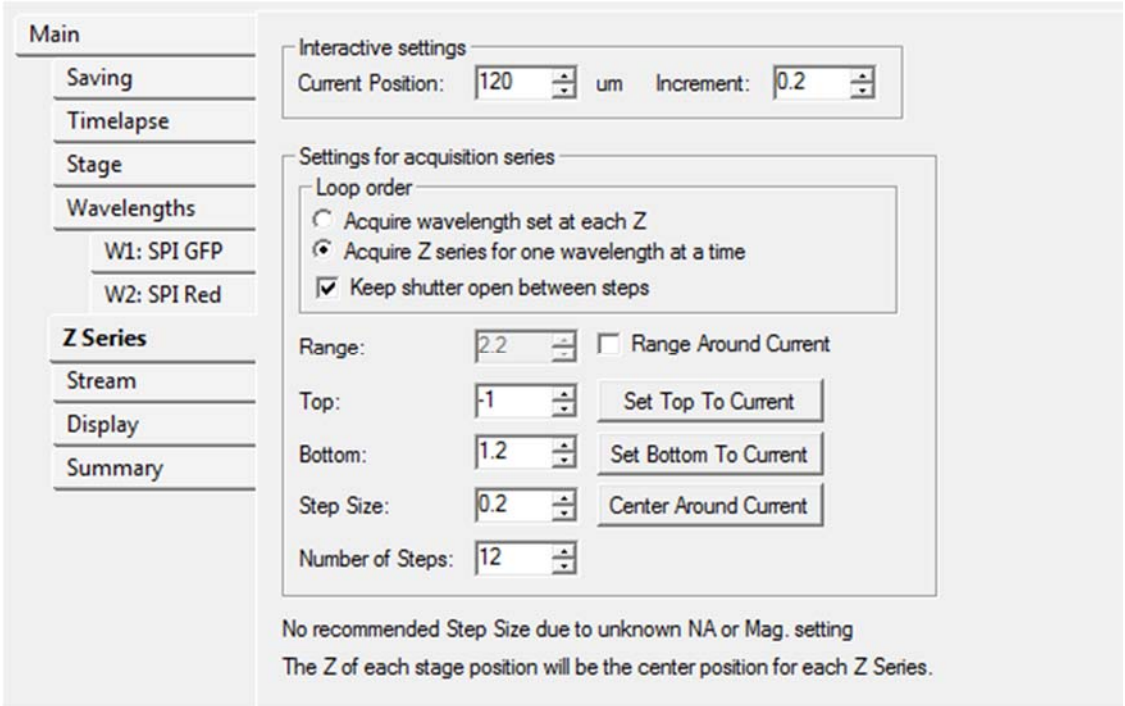
1.4 Stage



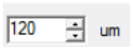
Select your sample area and clic on + bouton, the software consider the x,y and z position.

You can name diferents positions on Position Label, the software increase automaticly.

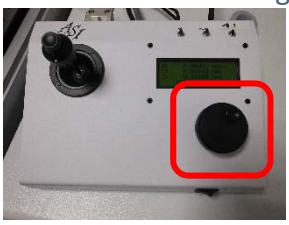
1.5 Z Series



There are two modes for the z stack acquisition

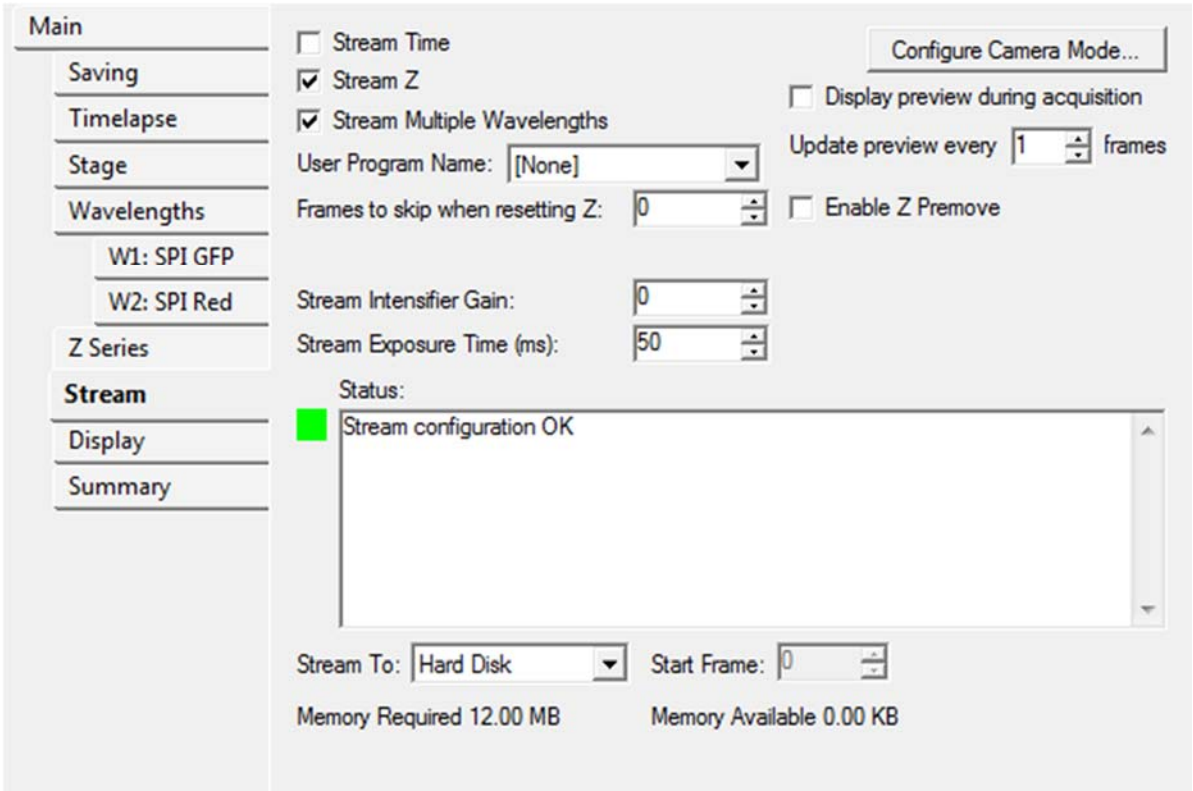
- The **Top and Bottom** mode : for this mode you open the live image and you change the z position with the knob of the stage controler or the cursor “Current Position” 

on the Interactive settings windows.



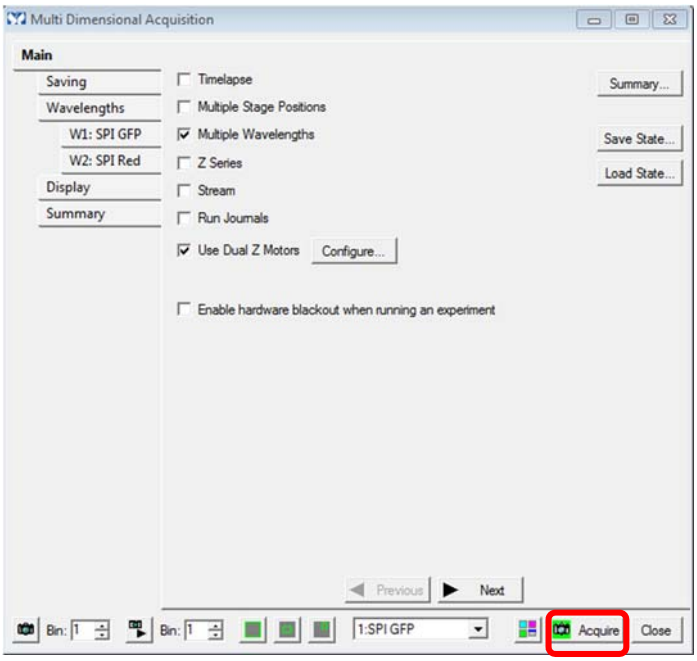
- The second mode is if you know the thickness of your sample (or it’s possible to estimate by the « top-bottom » mode) you chek the « Range Around Current » box and you indicate the range value. This mode is recommanded if you want to combine multipositions and z series.

1.6 stream : Rapidly acquires

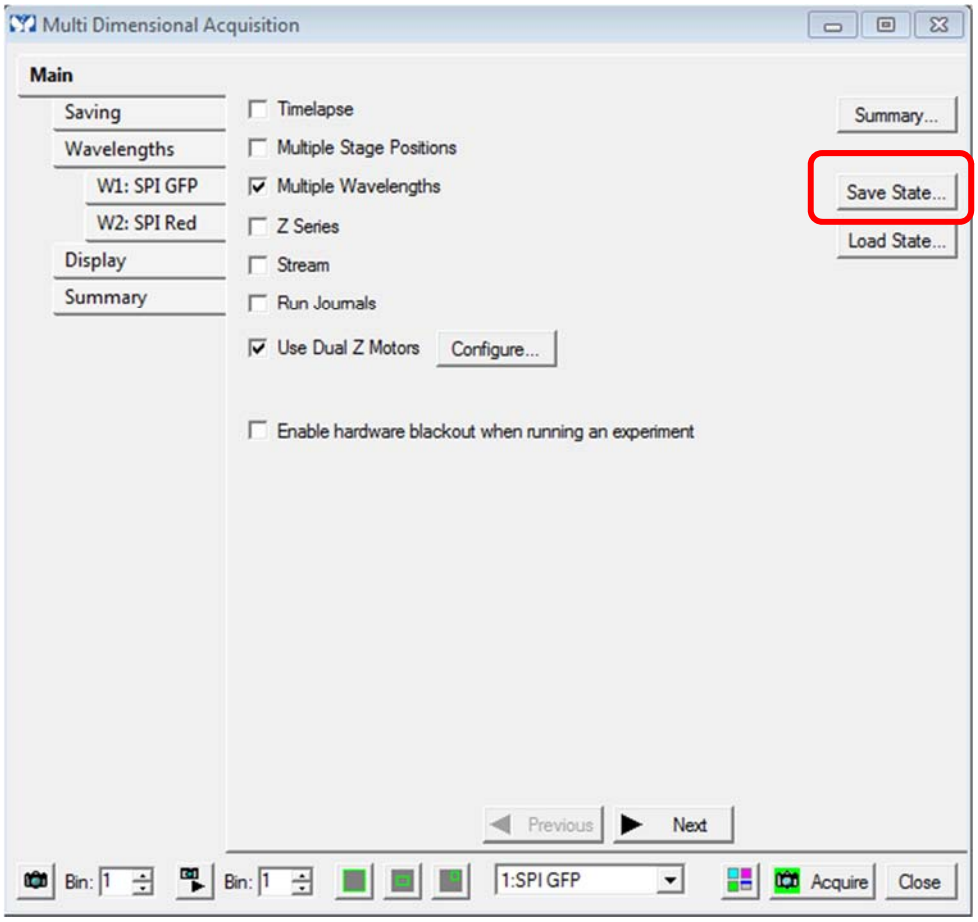


Select what you want to do and set in the different tabs (Timeapse, Zseries, Wavelengths).

2- Acquire:

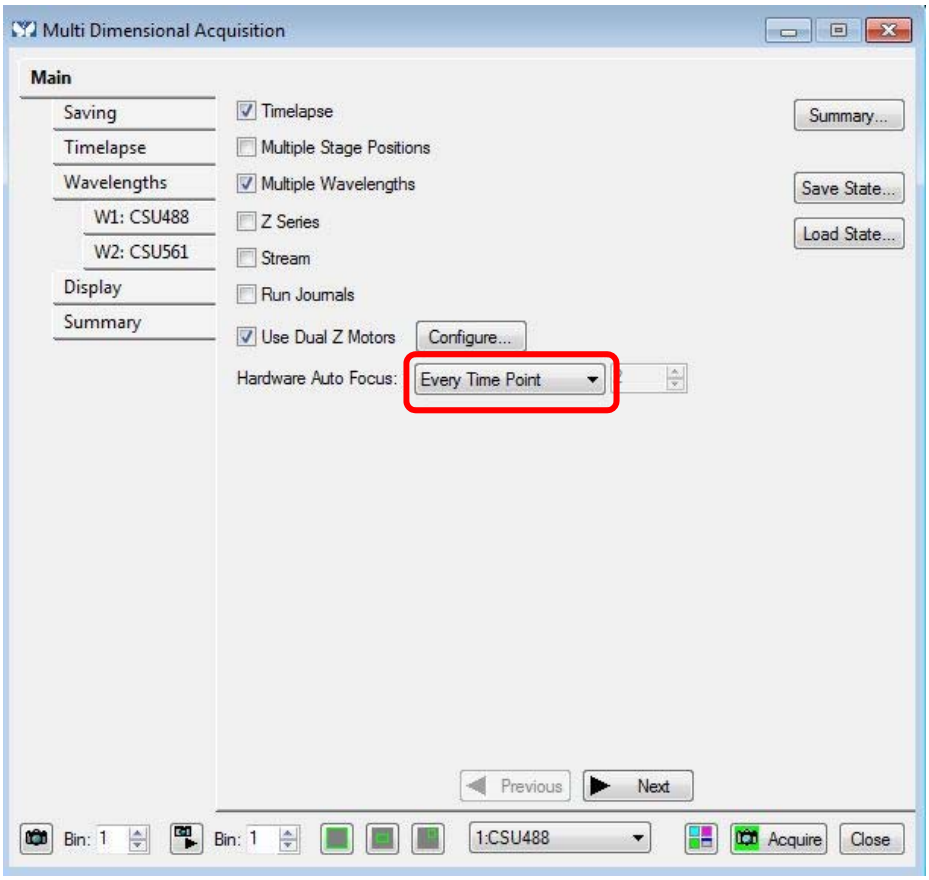


When the settings are completed, clic on the acquire Icon



It’s possible to save the complet experiment for the next session by the “Save state” Icon.

1.7 AFC to maintain the Focus during an time-lapse acquisition:



When you set your experimence, if you select « Timelapse », there is a new part on your « Main » menu : « Hardware Auto Focus ». there are differents options but in the majority of case, « Every Time Point » is a good compromise.
If « Never » is selected, the AFC is disable.