

# TimeLapse Leica DMI6000:

Room: E1038 CBI

## Description

- ❖ Software : Metamorph
- ❖ **Microscope** : Inverted Leica DMI 6000
- ❖ **Stage** : XY mot stage for multiposition recordings  
Z Piezo stage for fast z stack
- ❖ **Camera** : **Roper Coolsnp HQ<sup>2</sup>**  
**1392 X 1040 Pixels**  
**6.45 X 6.45-µm/Pixels**
- ❖ **Objectives** :
  - 10x/0.3 PI Fluotar **DRY**
  - 20x/0.7 HC PL APO **DRY** 0.17mm
  - 40x/1.25-0.75 OIL 0.17mm
  - 63x/1.4-0.6 HCX APO **OIL** 0.17mm
  - 100x/1.4-0.7 HCX APO **OIL** 0.17mm
- ❖ **Atmospher control**
  - Temperature and CO2 controller.  
If you need to use the temperature control for long acquisition, 30 Minutes are needed to stabilize the systeme
- ❖ **Filtre cubes** for EPI fluorescence observation :
  - A4 : BP 360/40 - 470-40 : **DAPI** IGBMC
  - L1 : BP 470/40 - 525/50 : **GFP- Alexa 488**
  - N1 : BP 546/12 - 605/75 : **CY3- Txred**

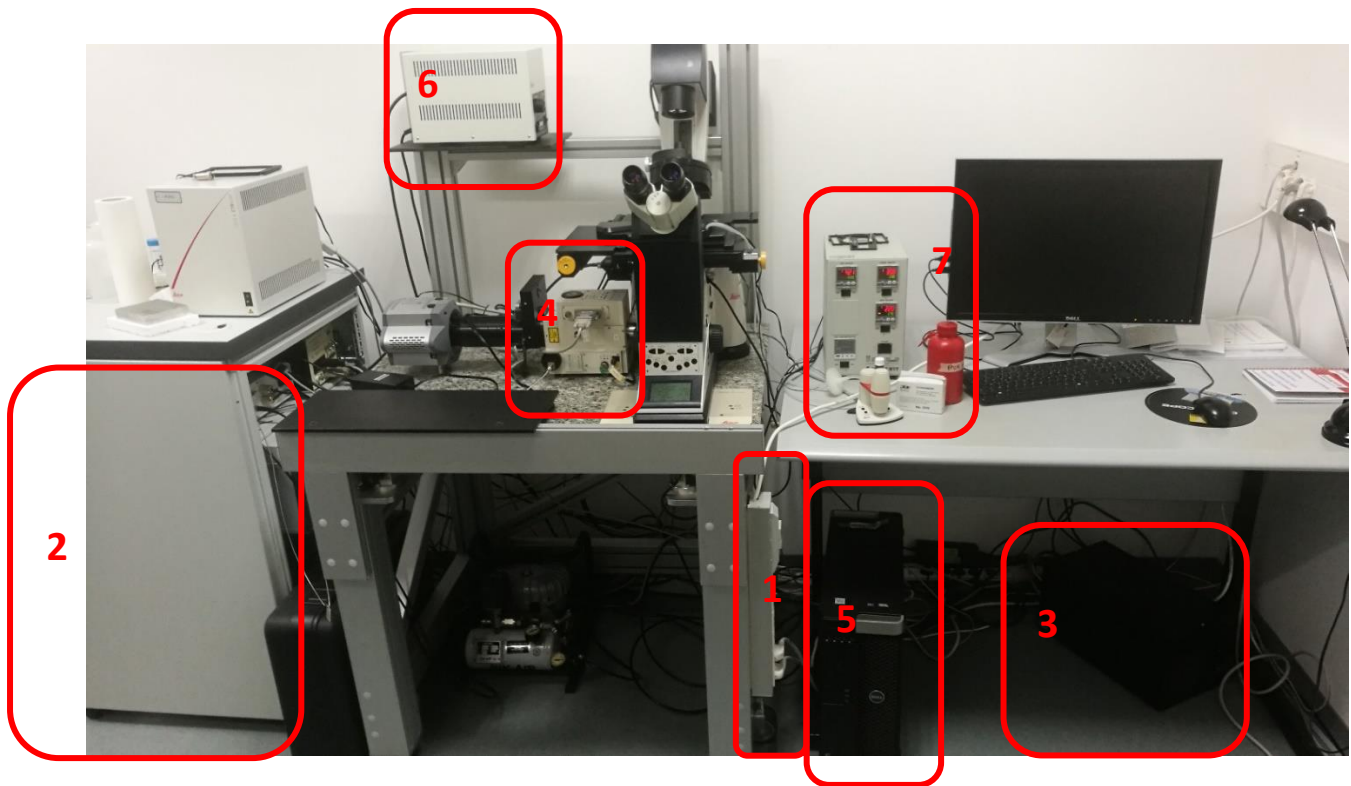
IGBMC CBI

For BOOKING System :

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

For any informations : [groupe-mic-photon@igbmc.fr](mailto:groupe-mic-photon@igbmc.fr)

## Procedures of starting spinning disk



1 – Turn on the system (multiple plug)

2- Open cupboard (left side): press on the lock and then pull the handle.



Switch ON the control unit  
super Z

Turn the key of the  
ANDOR Laser controller

3 – Switch on the camera controller

5 – turn the key of the spinning head on the position « RUN »

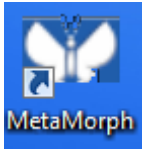
5 – Turn on the computer

6 – Switch on the fluorescence lamp if it's necessary (EL 6000)

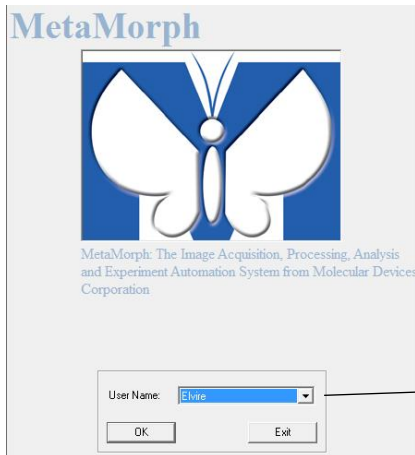
Note: to protect the optical fiber, press on the shutter as soon as you don't use the fluorescence.

7 – Switch on the atmosphere control if it's necessary.

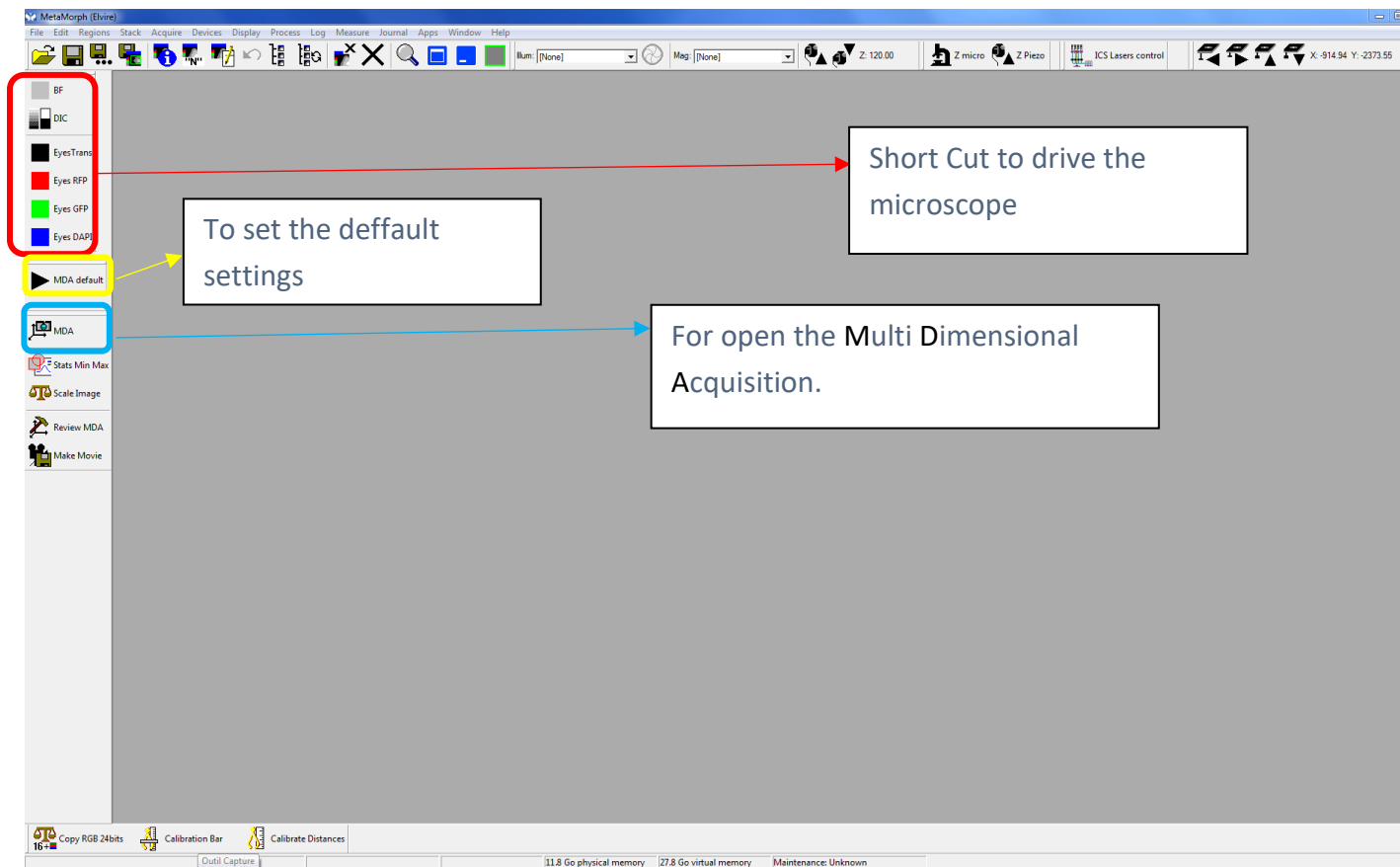
# Software: METAMORPH



Double clics on your METAMORPH ICON



Select User and clic OK

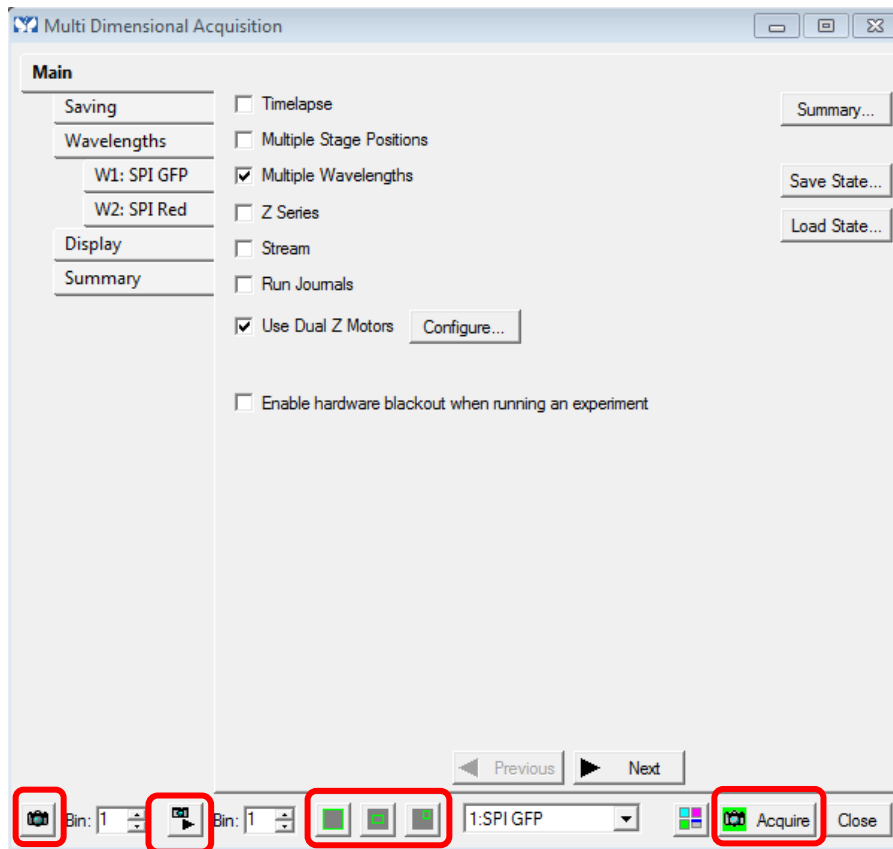







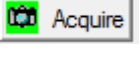
To set the default settings

Short Cut to drive the microscope

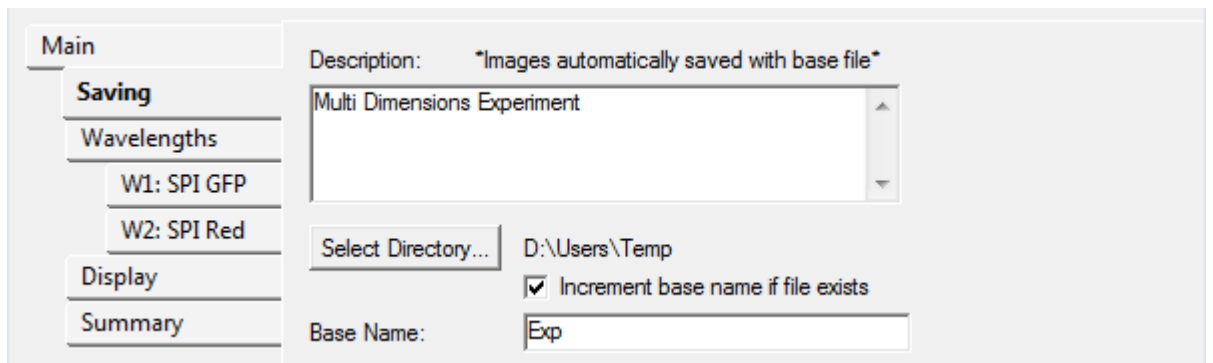
For open the Multi Dimensional Acquisition.

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



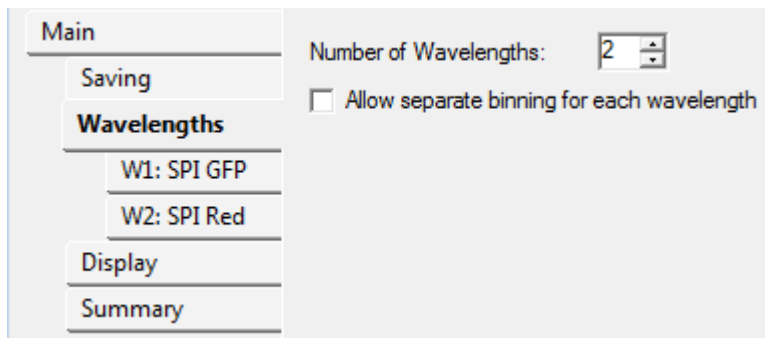
	SNAP	Acquire an image using the current settings specified in the Acquire main dialog box
	LIVE	Showing a live image
	FULL CHIP	Initialiez the full chip of your camera 512x512
	Center CHIP	To use center chip of your camera 256x256
	ROI CHIP	To use only one ROI chip of your camera
	ACQUIRE	Start MDA experiment

### 1.1 Saving :

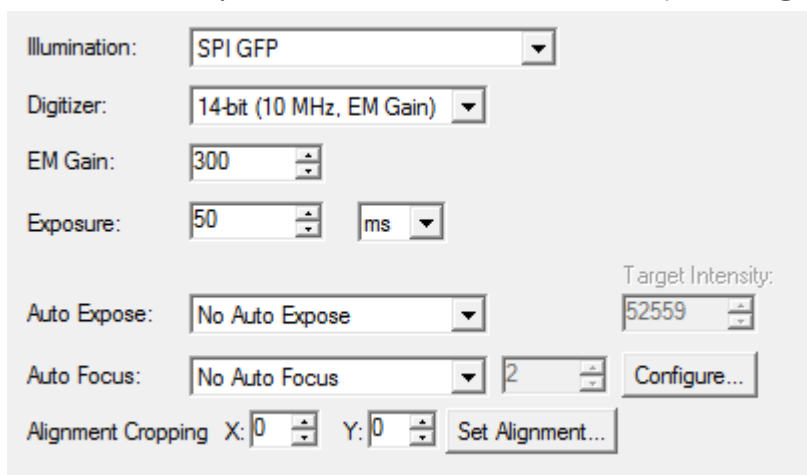


In this windows, select directly the Save folder by clic on Selct directory icon, it 's possible to use the base name and the software automaticly increment the number of the image.

## 1.2 Multiple Wavelengths :

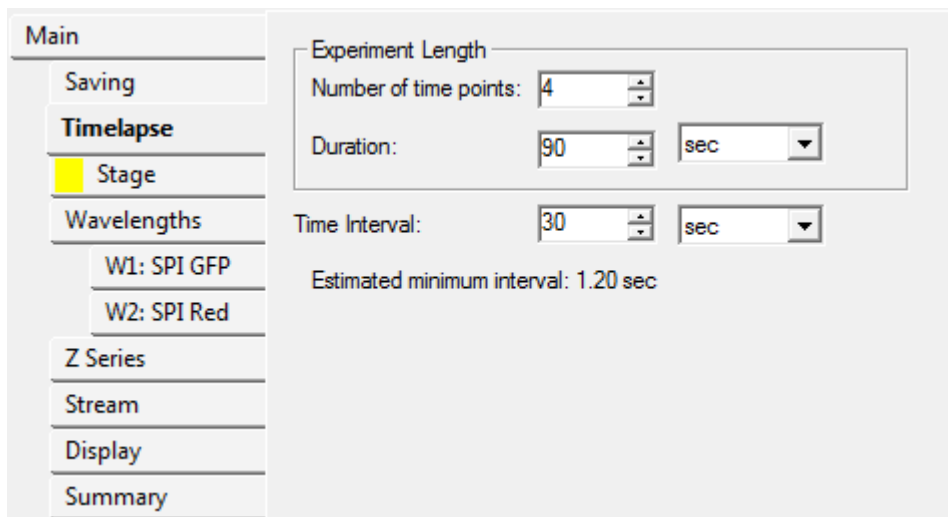


In this windows you select the number of channel (wavelengths).



- After select the illumination you want to use
- Generaly by default we use 14bit ( 10 Mhz, EM Gain) and EM Gain 300
- You adjust the exposure time (you open the live image for parameter this value)

## 1.3 Timelapse

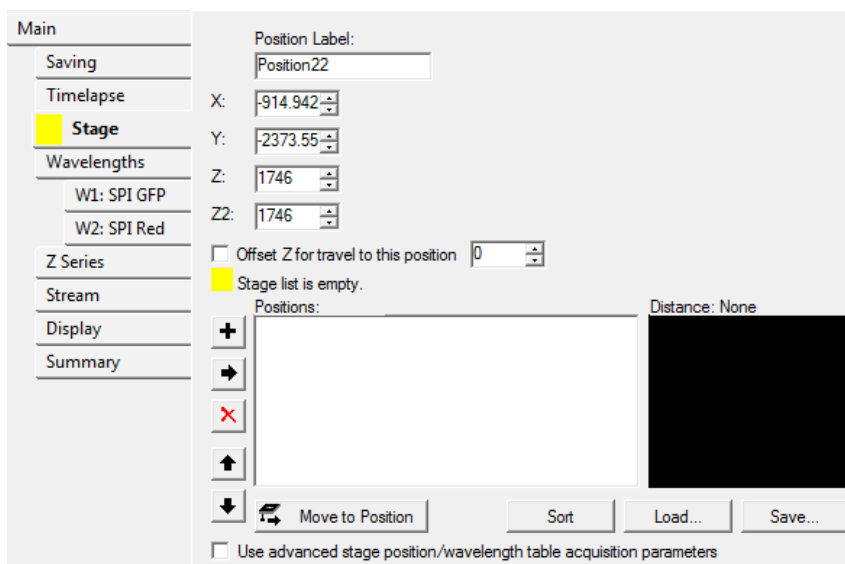



Enter your acquisition parameters.

Be careful, the « Estimated minimum interval » is wrong value. The system doesn'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

The screenshot shows a software interface for Z Series acquisition. On the left is a navigation menu with the following items: Main, Saving, Timelapse, Stage, Wavelengths (with sub-items W1: SPI GFP and W2: SPI Red), Z Series (highlighted), Stream, Display, and Summary. The main panel is titled 'Interactive settings' and contains the following controls:

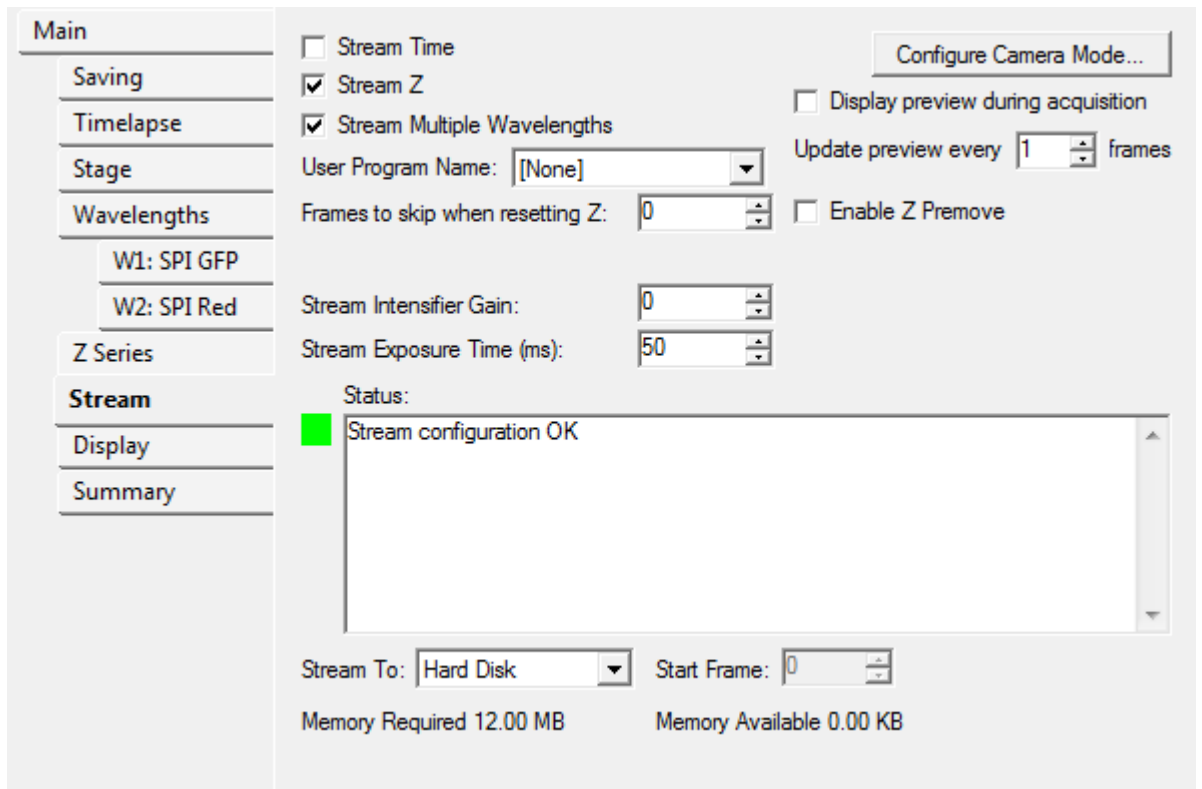
- Interactive settings:** Current Position: 120 um, Increment: 0.2
- Settings for acquisition series:**
  - Loop order:**
    - Acquire wavelength set at each Z
    - Acquire Z series for one wavelength at a time
    - Keep shutter open between steps
  - Range:** 2.2  Range Around Current
  - Top:** -1
  - Bottom:** 1.2
  - Step Size:** 0.2
  - Number of Steps:** 12

At the bottom of the main panel, there is a warning message: "No recommended Step Size due to unknown NA or Mag. setting. The Z of each stage position will be the center position for each Z Series."

There are two modes for the z stack acquisition

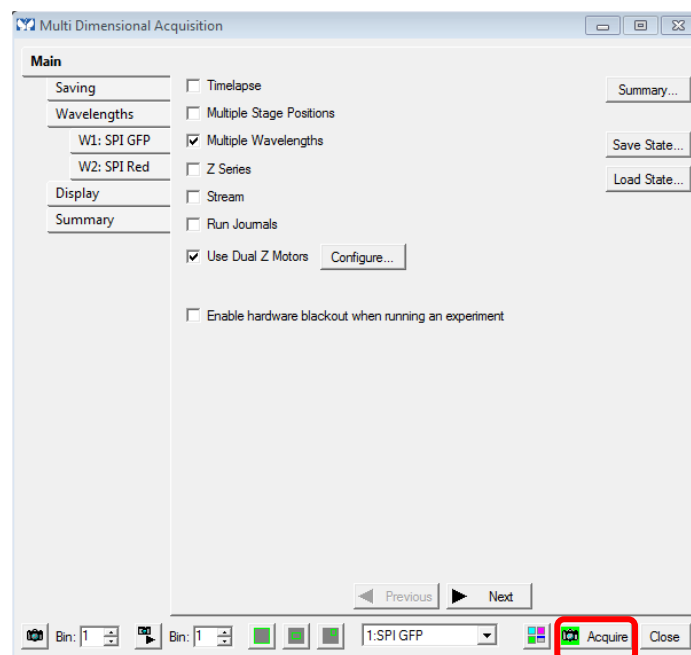
- The **Top and Bottom** mode : for this mode you open the live image and you change the z position with the cursor "Current Position"  um on the Interactive settings windows.
- The second mode is if you know the thickness of your sample (or it's possible to estimate by the « top-bottom » mode) you check the « Range Around Current » box and you indicate the range value. This mode is recommended if you want to combine multipositions and z series.

## 1.6 stream : Rapidly acquires



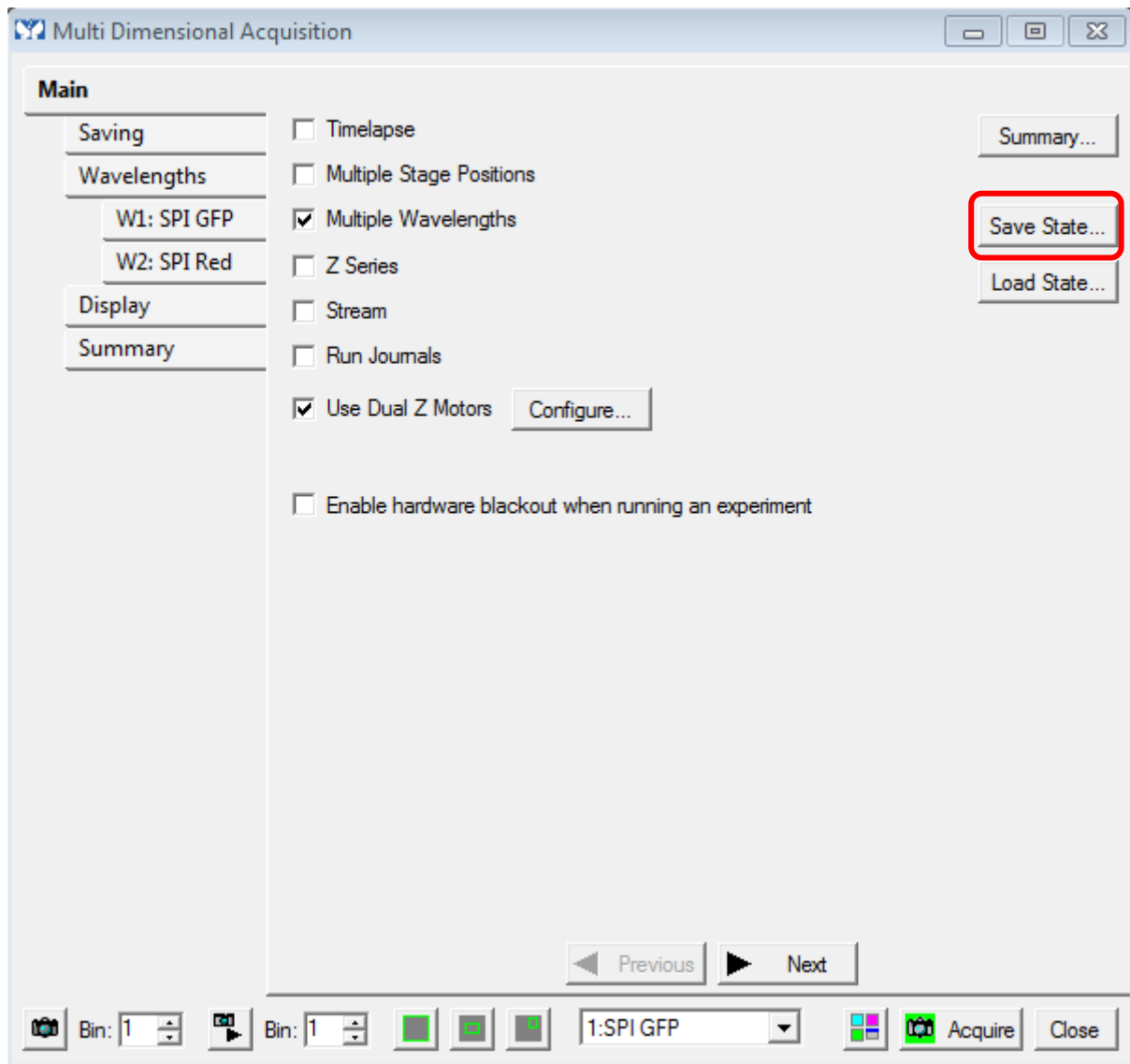
Select what you want to do and set in the different tabs (Timeapse, Zseries, Wavelengths).

## 2- Acquire:



When the settings are completed, clic on the acquire Icon





It's possible to save the complete experiment for the next session by the "Save state" icon.