User manual for Olympus SD-OSR spinning disk confocal microscope

Olympus SD-OSR: Spinning Disk Confocal Microscope designed for high speed live cell imaging

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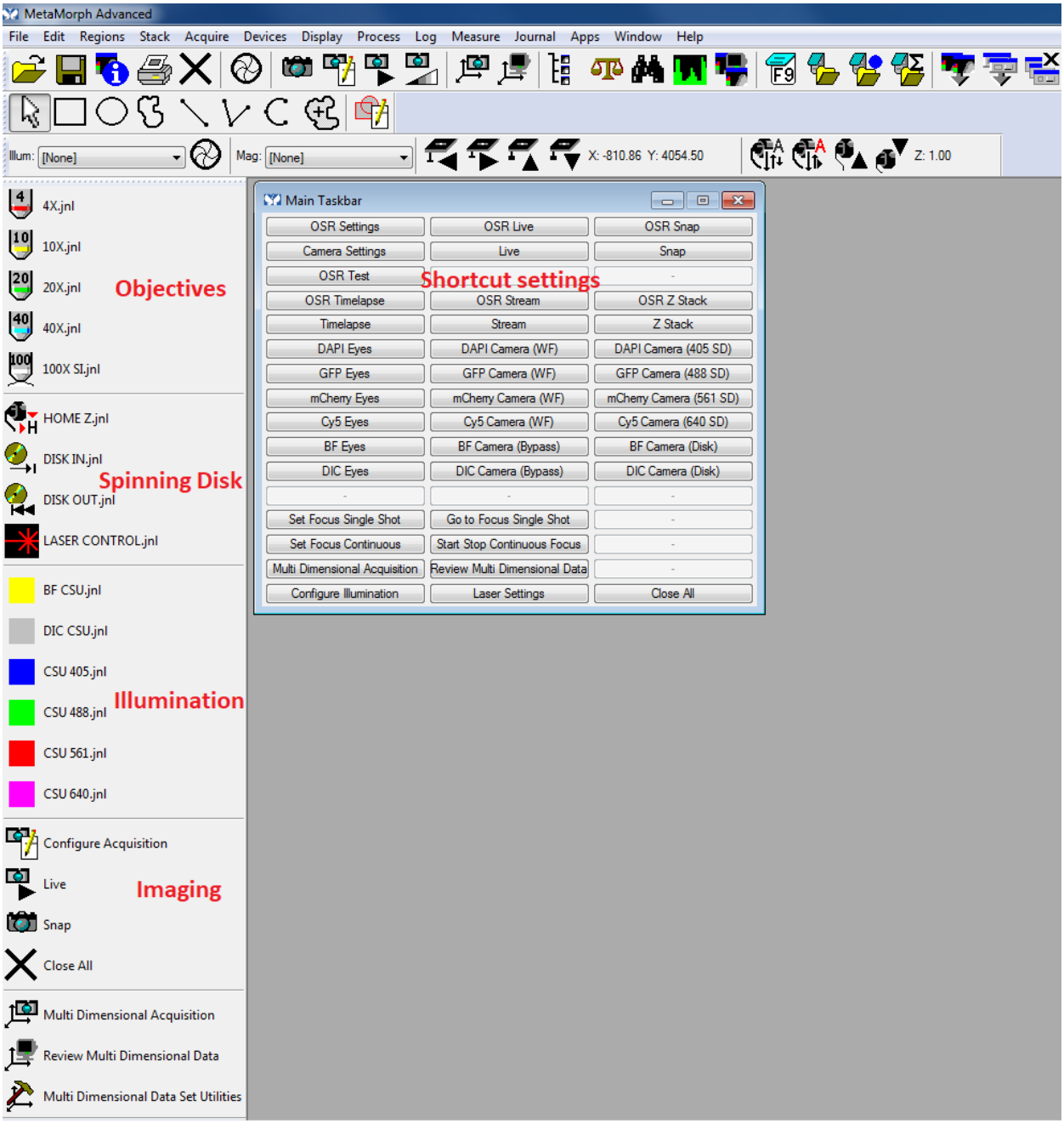
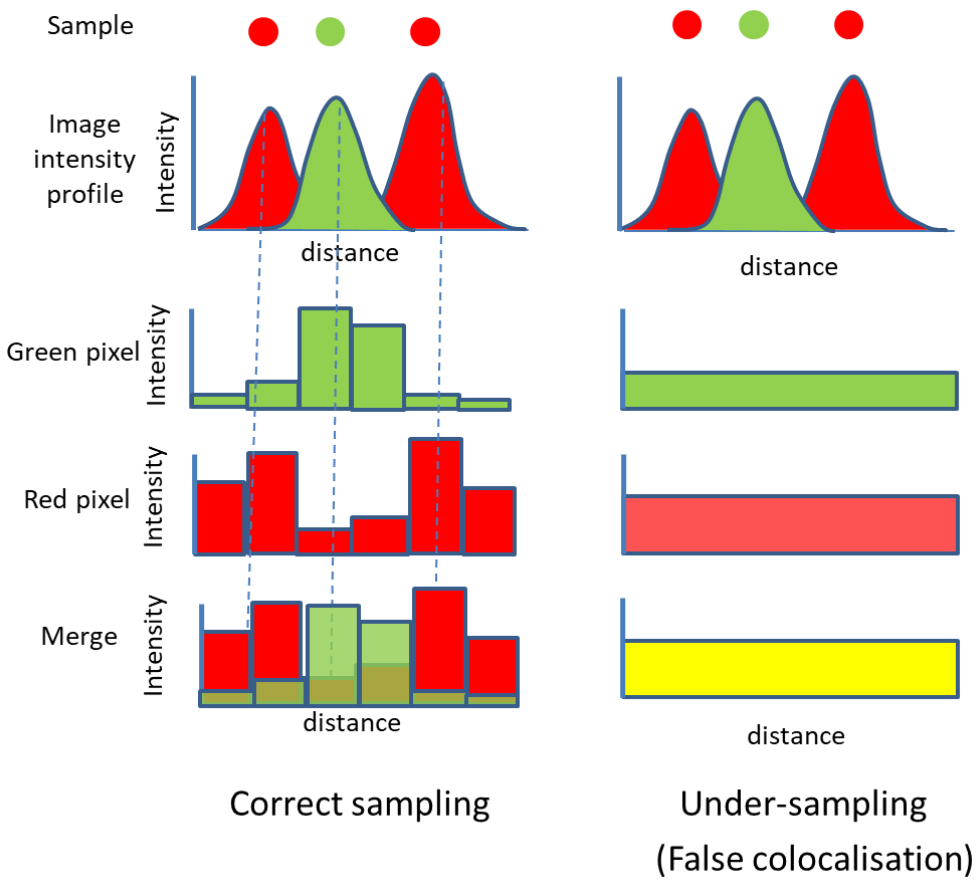
Once you open Metamorph software, a window like this will open.



Selecting the right objective:

Choose the objective depending upon optical resolution you want to achieve. This could be very important parameter especially when you are carrying out quantitative imaging like colocalisation. Inappropriate selection of microscope objective can lead to under-sampling which may give you false results as shown below.



Here is an example calculation of optical resolution that can be achieved at a magnification of 40X with a binning of 2x2.

XY optical resolution of objective at wavelength of 500 nm = 0.61λ/NA = 0.61 x 500/0.95 = 321 nm = 0.321 µm

Pixel size required to match with this optical resolution = 0.321 µm2.3 µm/pixel = 0.139 µm/pixel

(some calculations use 2 instead of 2.3 as Nyquist sampling for analog signal digitization requires minimum sampling of 2 data points per cycle, on the other hand for optical microscopy, we need to oversample a little bit with 2.3 data points per cycle to adequately sample features oriented along diagonal)

Pixel size = (camera pixel size x binning)/magnification

Pixel size for 40X objective at 2x2 binning = 6.5 µm/pixel x 2/40 = 0.325 µm/pixel

Thus, we are undersampling at this magnification and imaging setting. If we want to resolve a structure which is 0.5 µm, above objective would give the necessary resolution but magnification and binning settings would not let us get the results that we are looking for. We might have to either go for 1x1 binning or 100X magnification to get closer to the desired results.

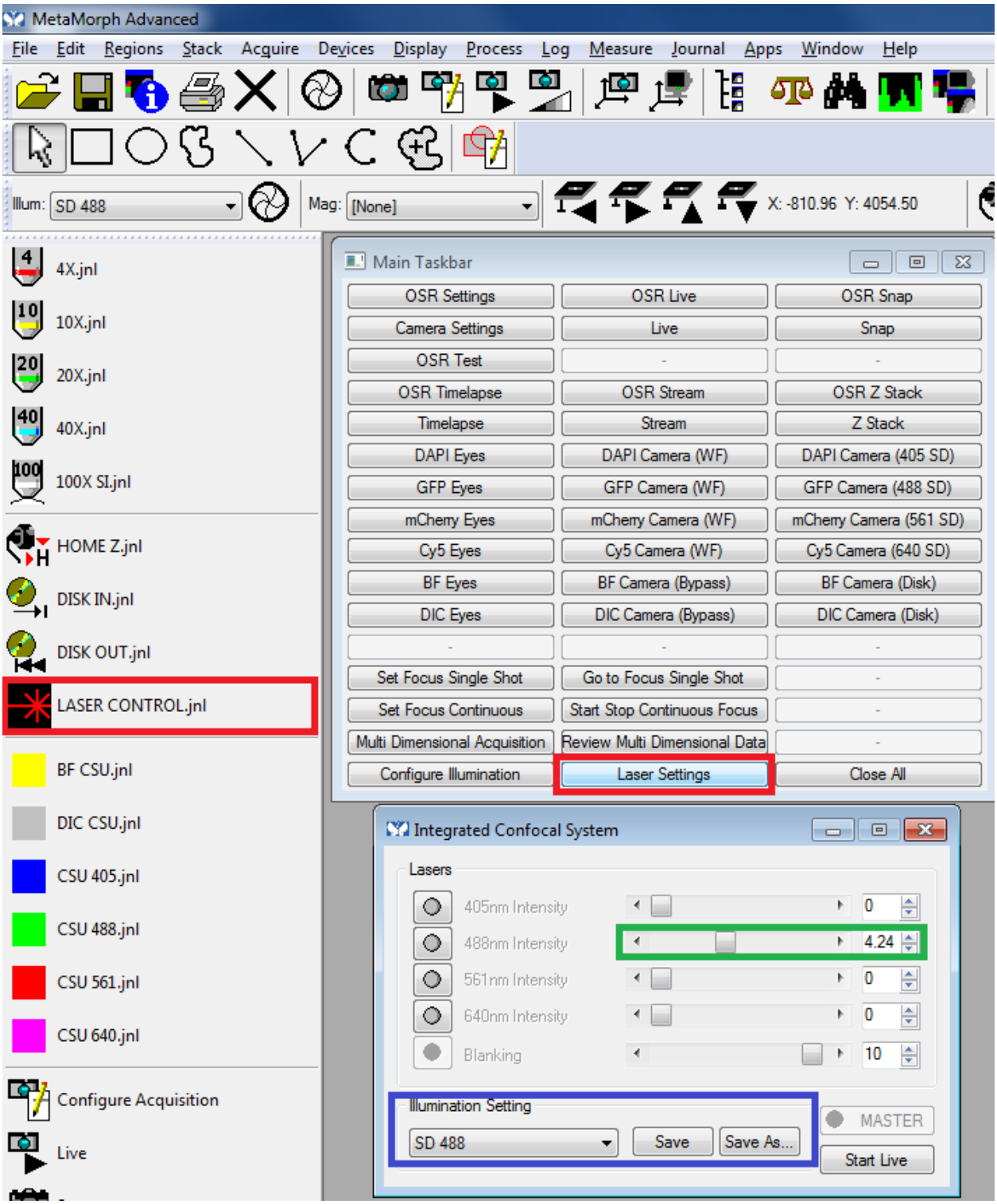
Illumination settings:

Choose the appropriate illumination setting according to fluorescence spectra of fluorescent labels in your sample and the filter settings for that particular illumination setting. Pre-configured illumination may or may not work for your system, so it is good idea to always check before you proceed with

imaging. This is very important if fluorescence spectra of two fluorescent labels are spectrally not that well separated and it may lead to false signal due to "cross talk" or "bleed through".

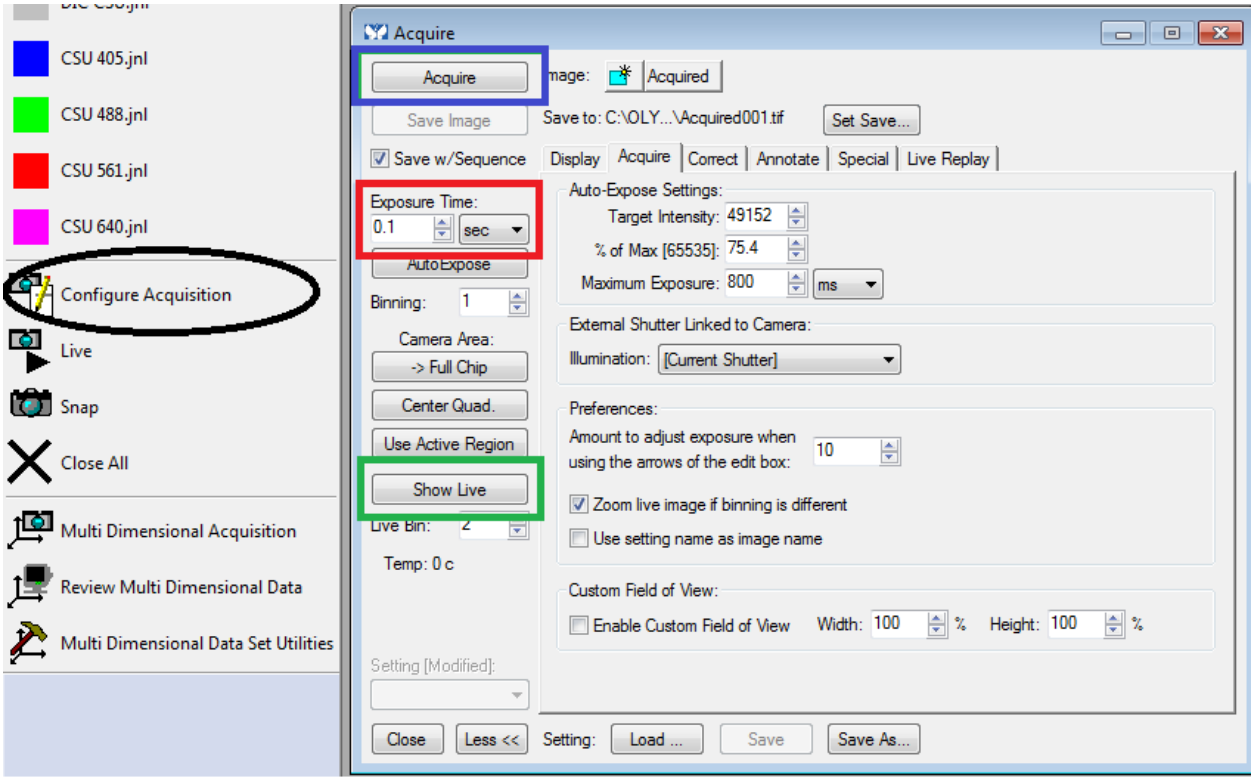
Laser settings

To adjust the laser power, click "LASER CONTROL.jnl" or "Laser Settings", choose appropriate "illumination setting" and adjust laser power between the range "0-10%"



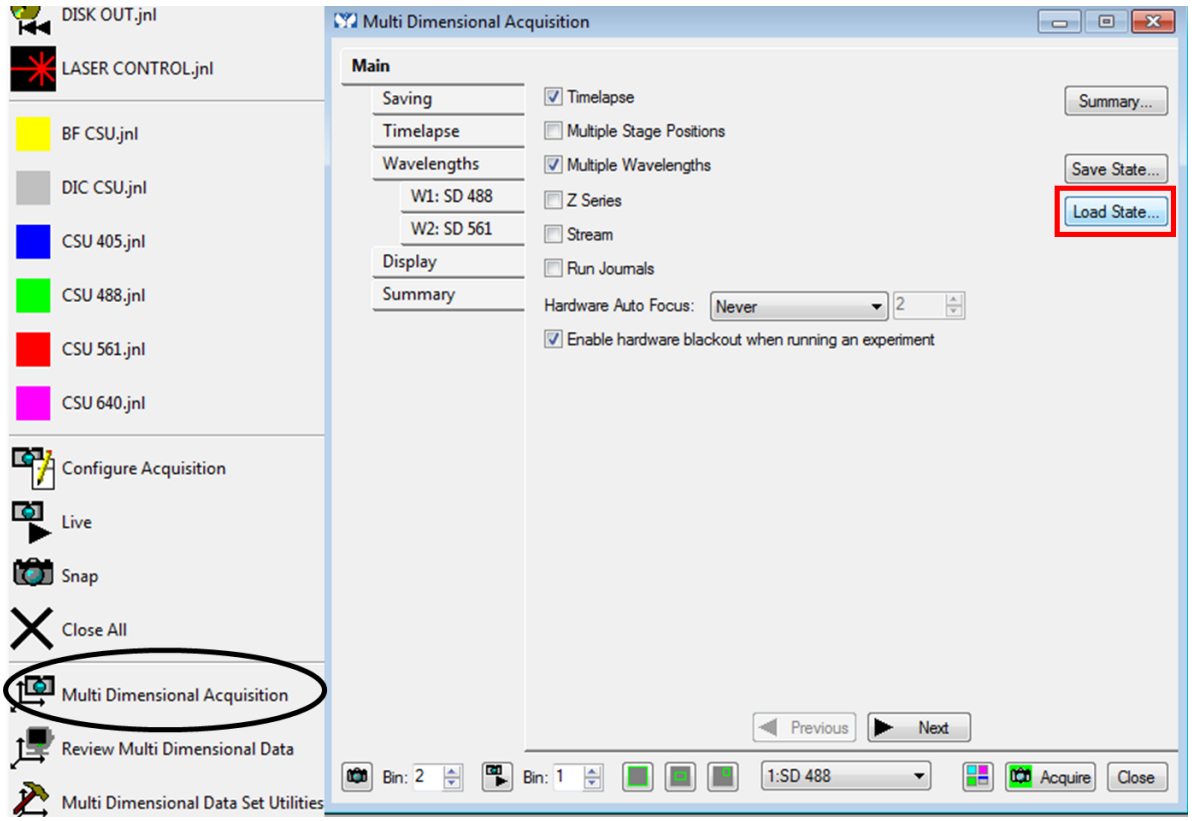
Configure acquisition:

You can quickly acquire an image by using the configure acquisition tab (highlighted in black circle). Click "show live" and set the "exposure time" such that highest intensity in image is below 80% of detector limit (about 50,000 for a 16 bit camera) and then hit "acquire".

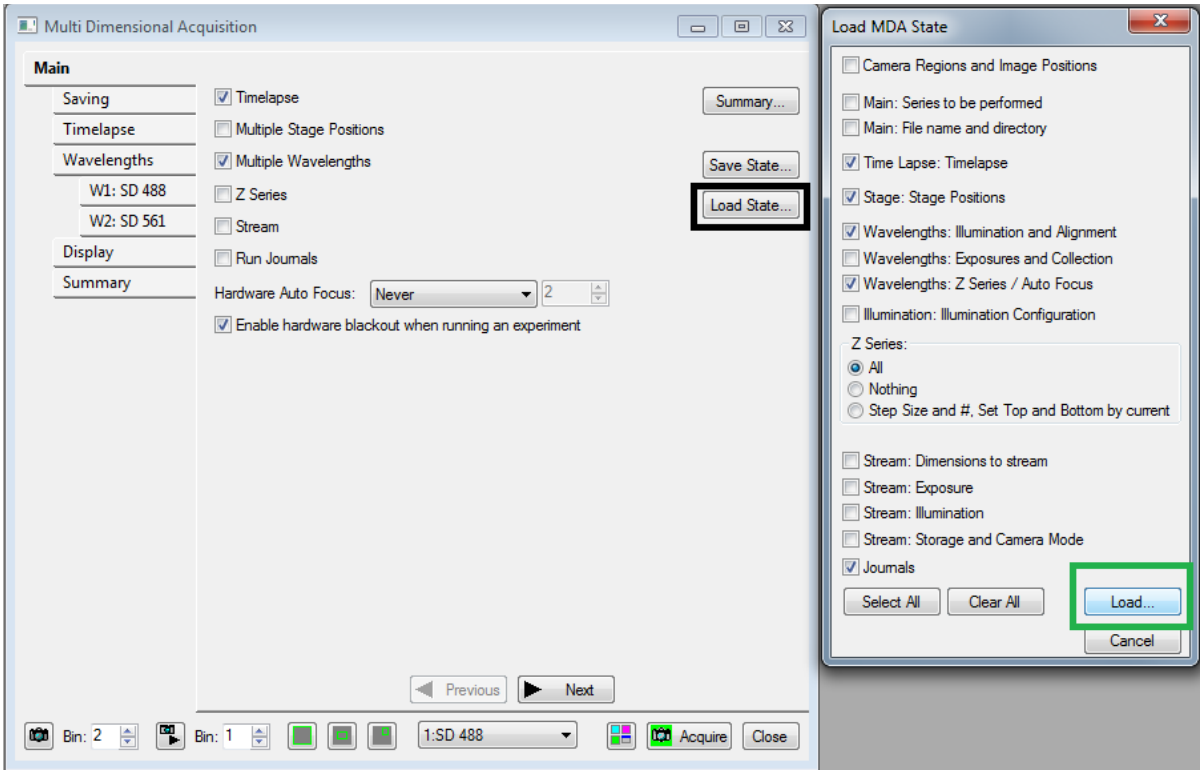


Multi Dimensional Acquisition (MDA)

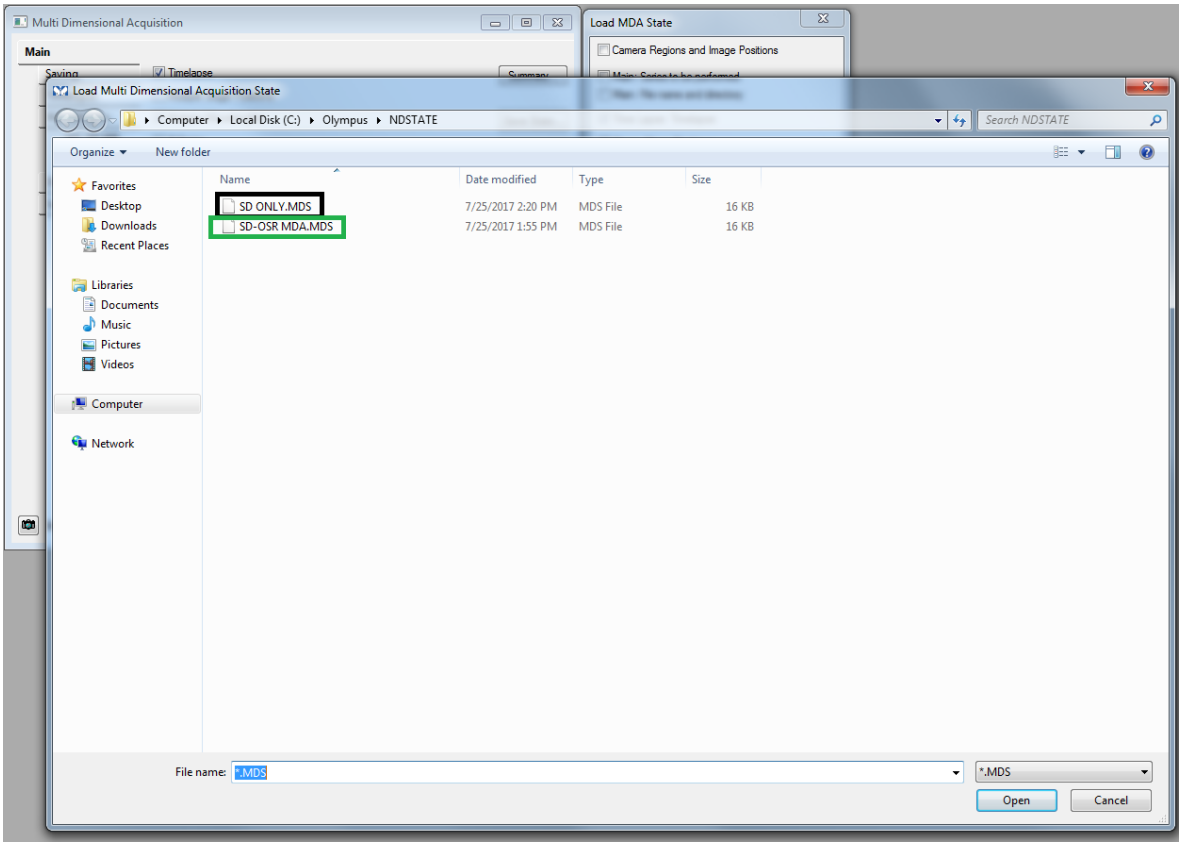
Click on "Multi Dimensional Acquisition" It will open a window where you can set up parameters like saving location, time interval, stage position, wavelengths, exposure time, z-stacks etc.



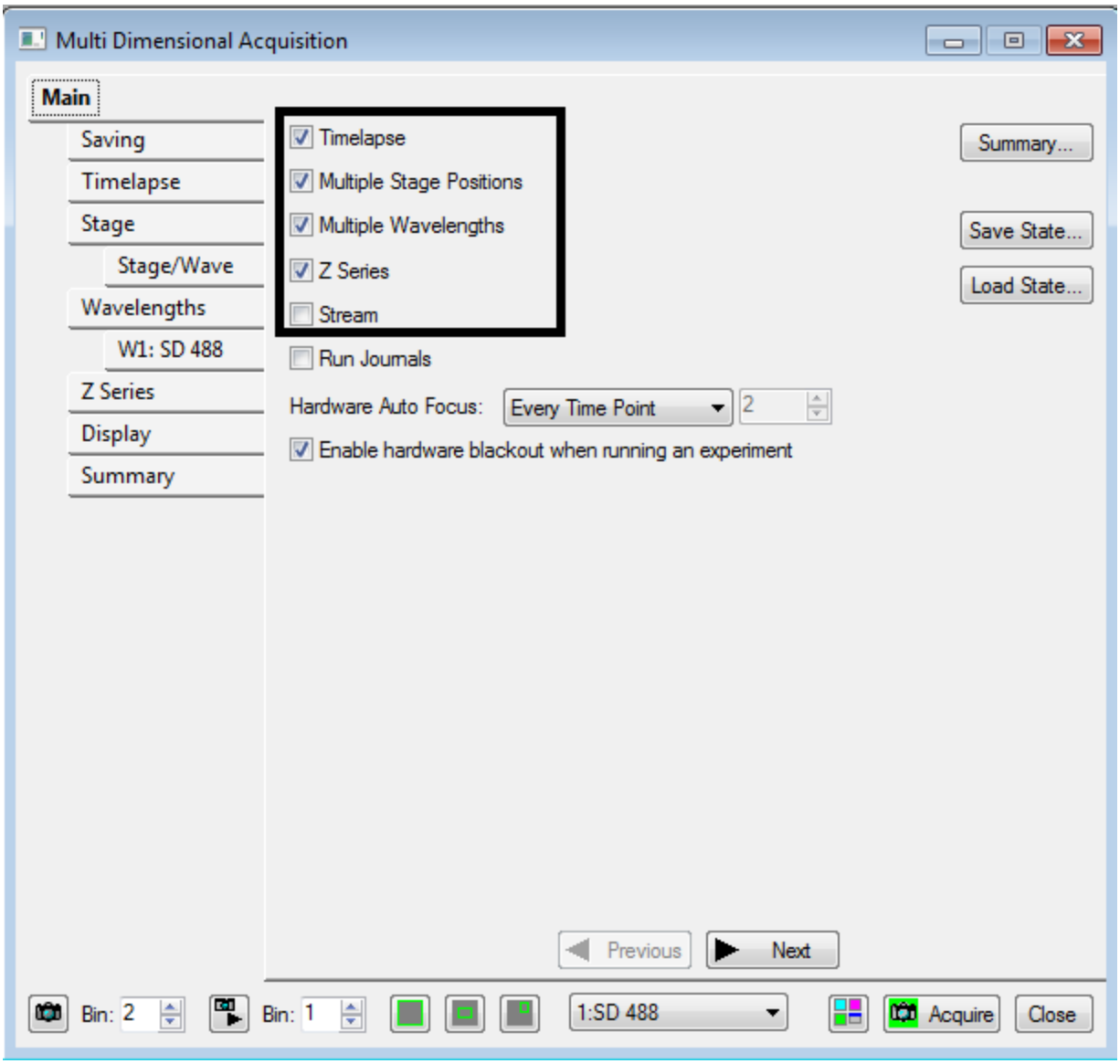
To initiate use of MDA, click "Load State". It will open a new window "Load MDA state" and then click "Load…".



Select appropriate file depending upon the imaging you are planning on carrying out. Use “SD ONLY.MDS” for using spinning disk only or use “SD-OSR MDA.MDS” if you want to carry out super resolution imaging.

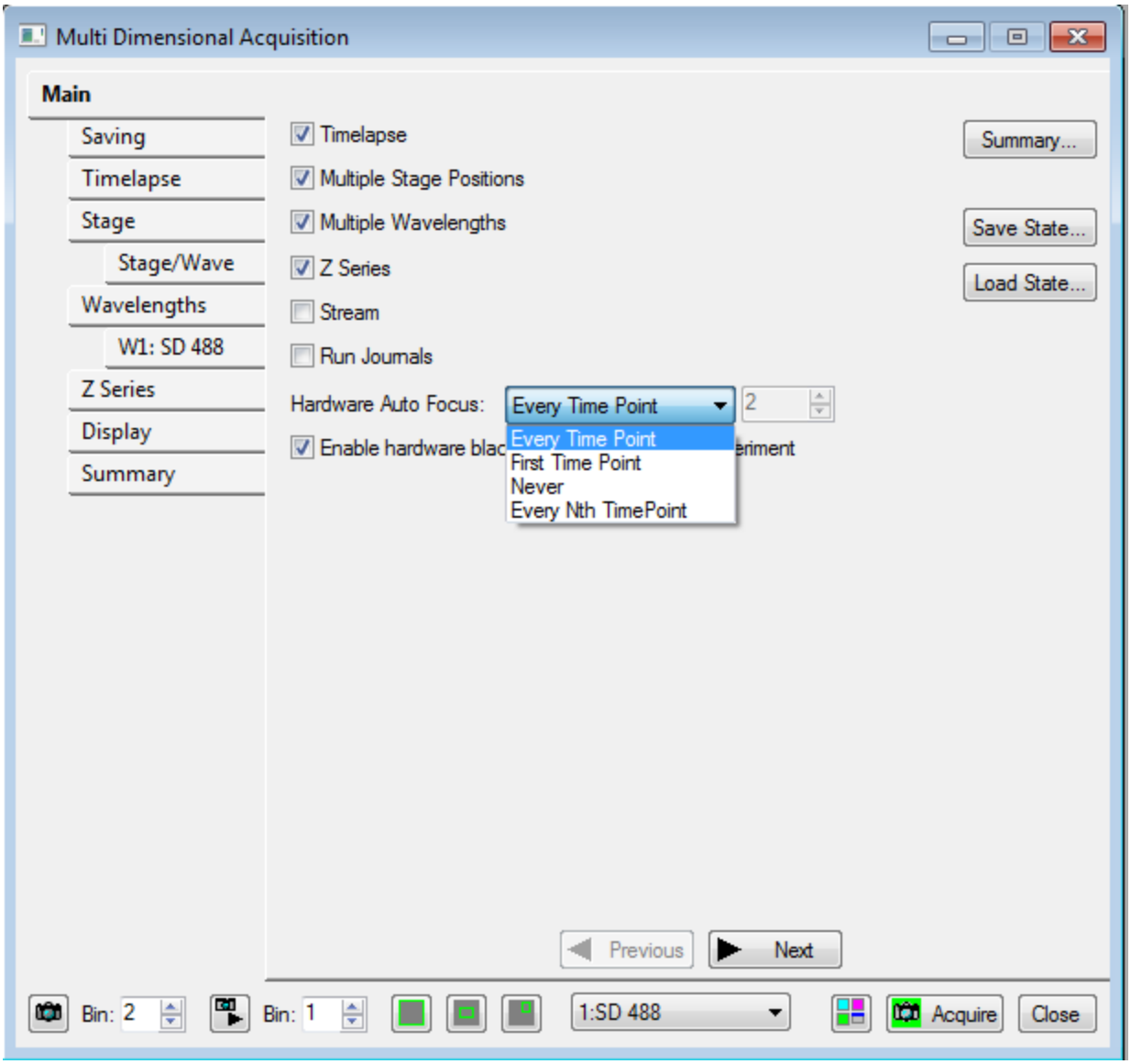


Once the state is loaded, then check the options which you want to use during imaging.



Hardware auto focus

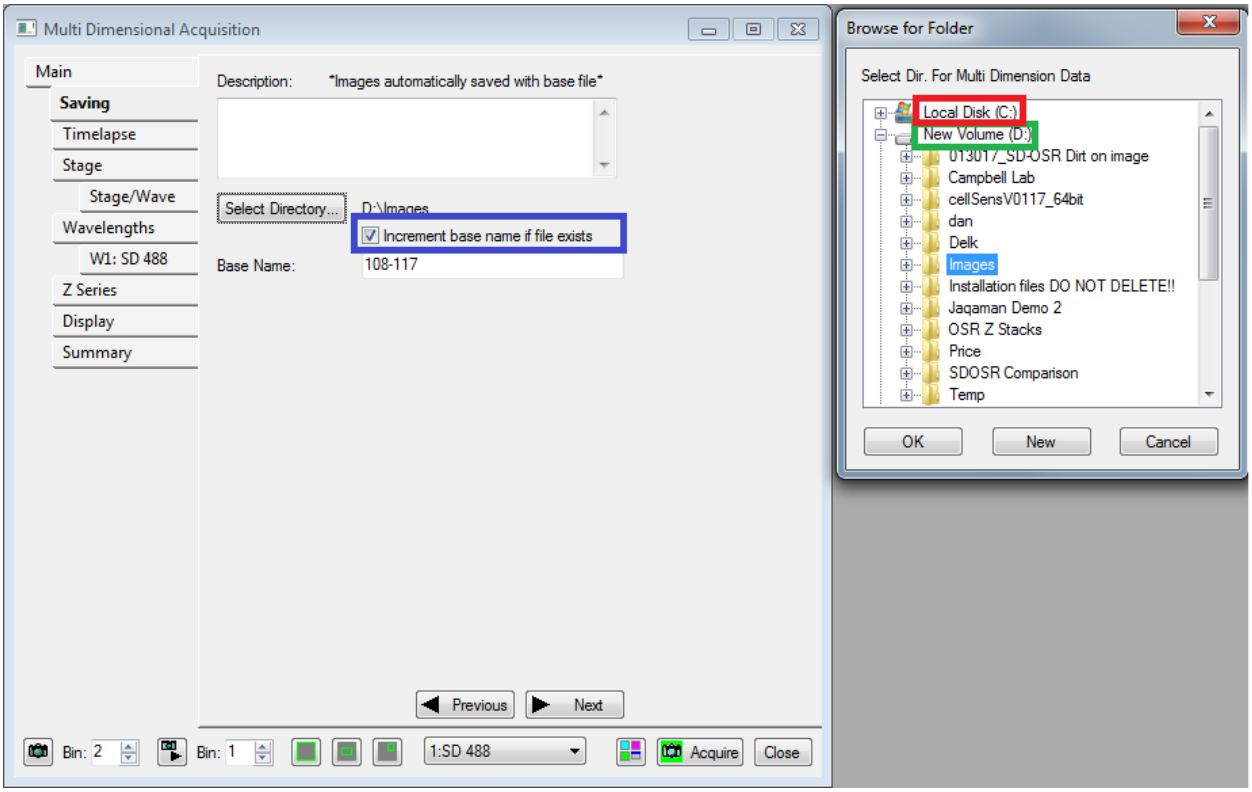
This could be a useful option if you care carrying out long term imaging. Choose the appropriate option from dropdown menu.



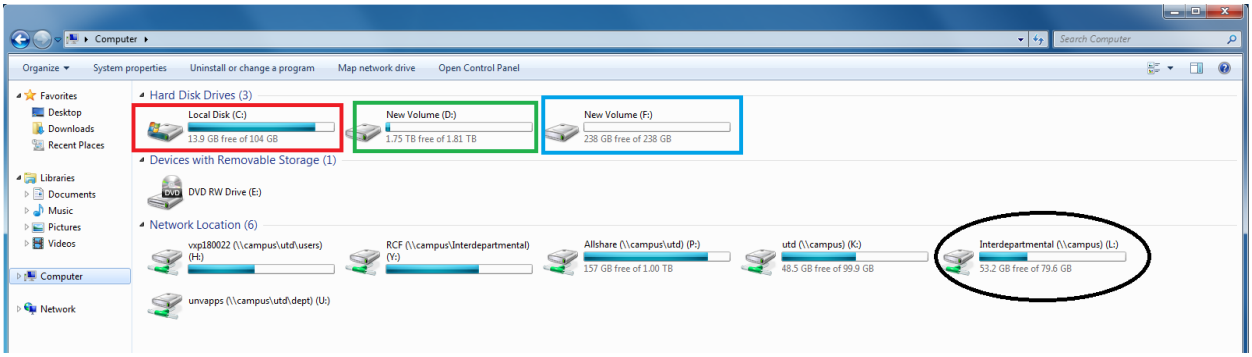
Saving

Click "Select Directory…" and it will open a new window where you can browse for destination folder. DO NOT store any file on C-drive as it is meant for storage of system files only. If you are carrying out regular imaging, D-drive has good enough speed to store the data. If you are acquiring "Streaming" data which requires high speed data transfer, you can use E-drive as it is a SSD memory with double Read-Write speed.

Make sure you click "Increment base file name if file exists" to prevent from automatically overwriting the files.

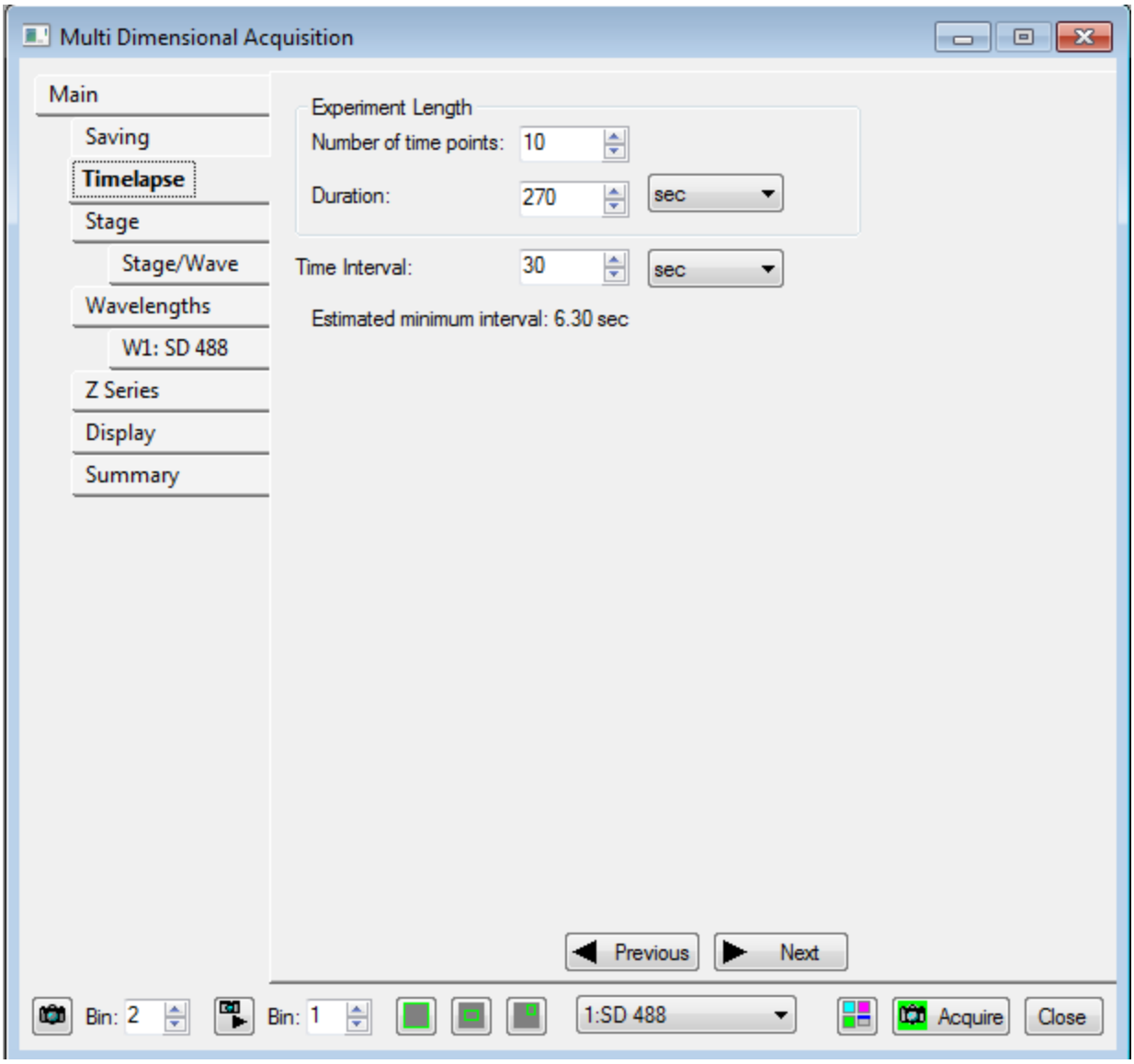


Once you are done with imaging, cut your data folder and save it on L:\RCF\users\<netID> folder. Data stored on D-drive for more than 30 days will automatically be deleted without notice.



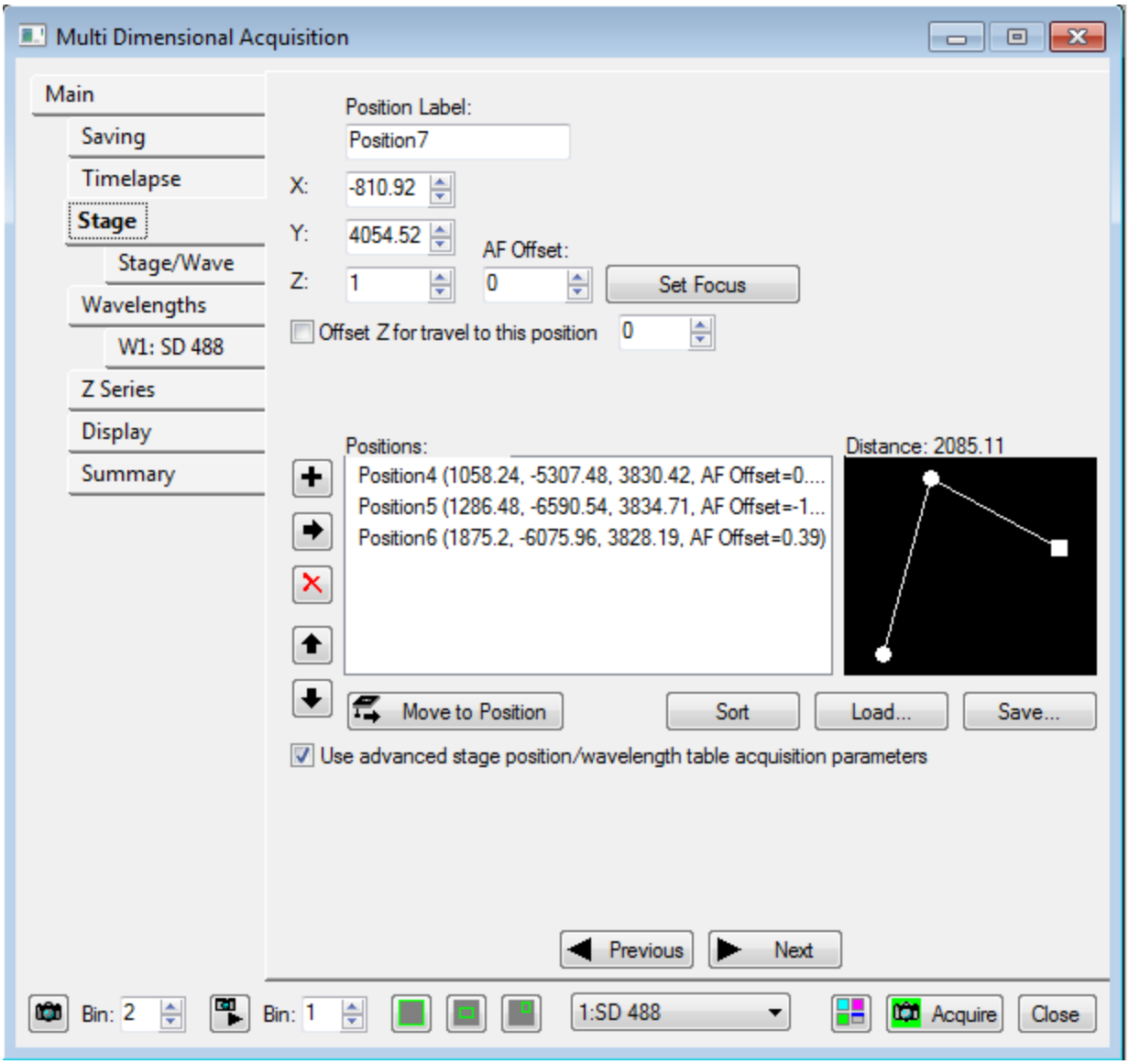
Timelapse

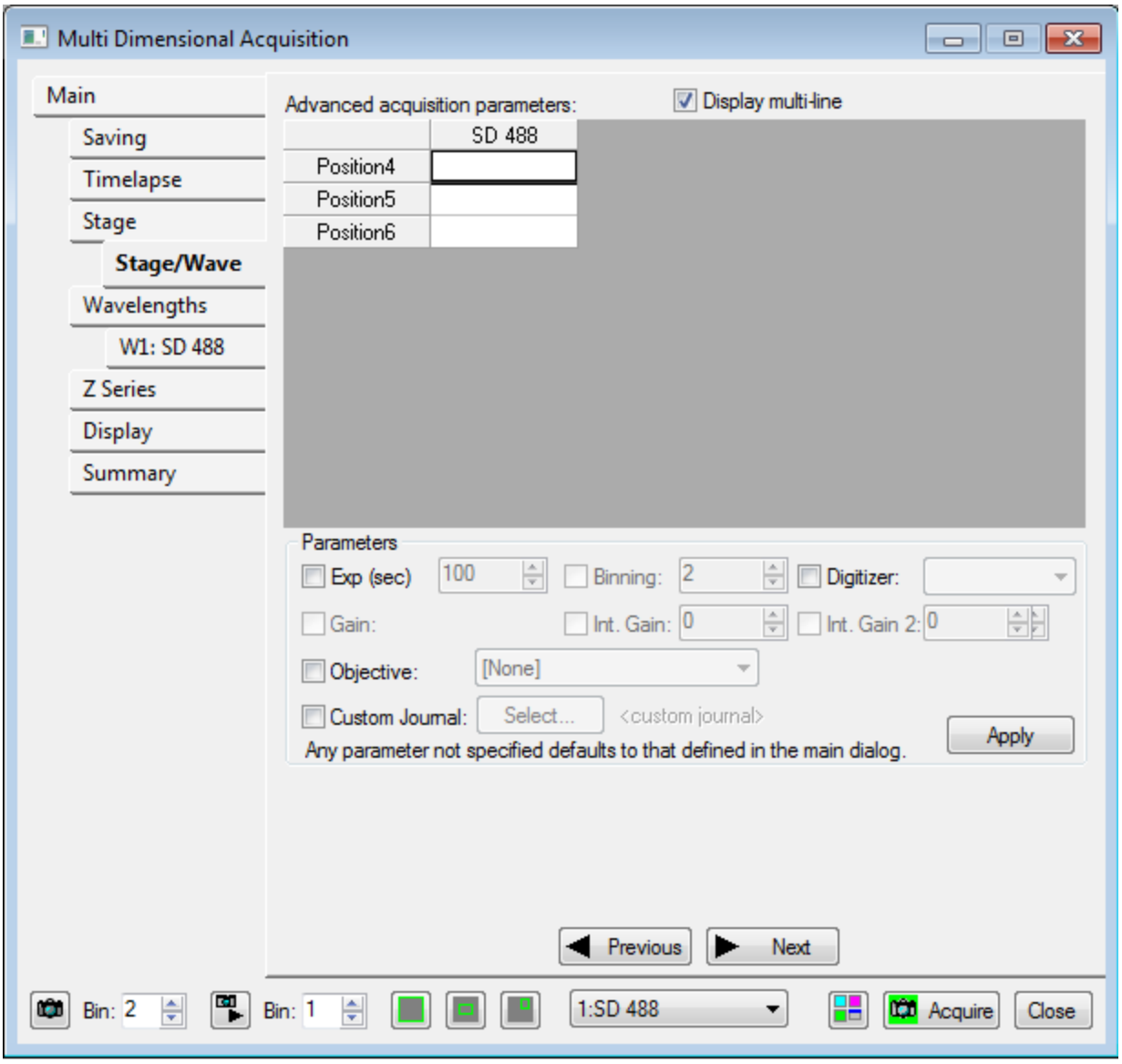
In order to acquire a time series of images, set up the "time interval" and "number of time points" that you want to acquire. Make sure that the "time interval" is greater than "estimated minimum interval" (determined by number of channels, z-stacks, exposure times etc)



Stage position

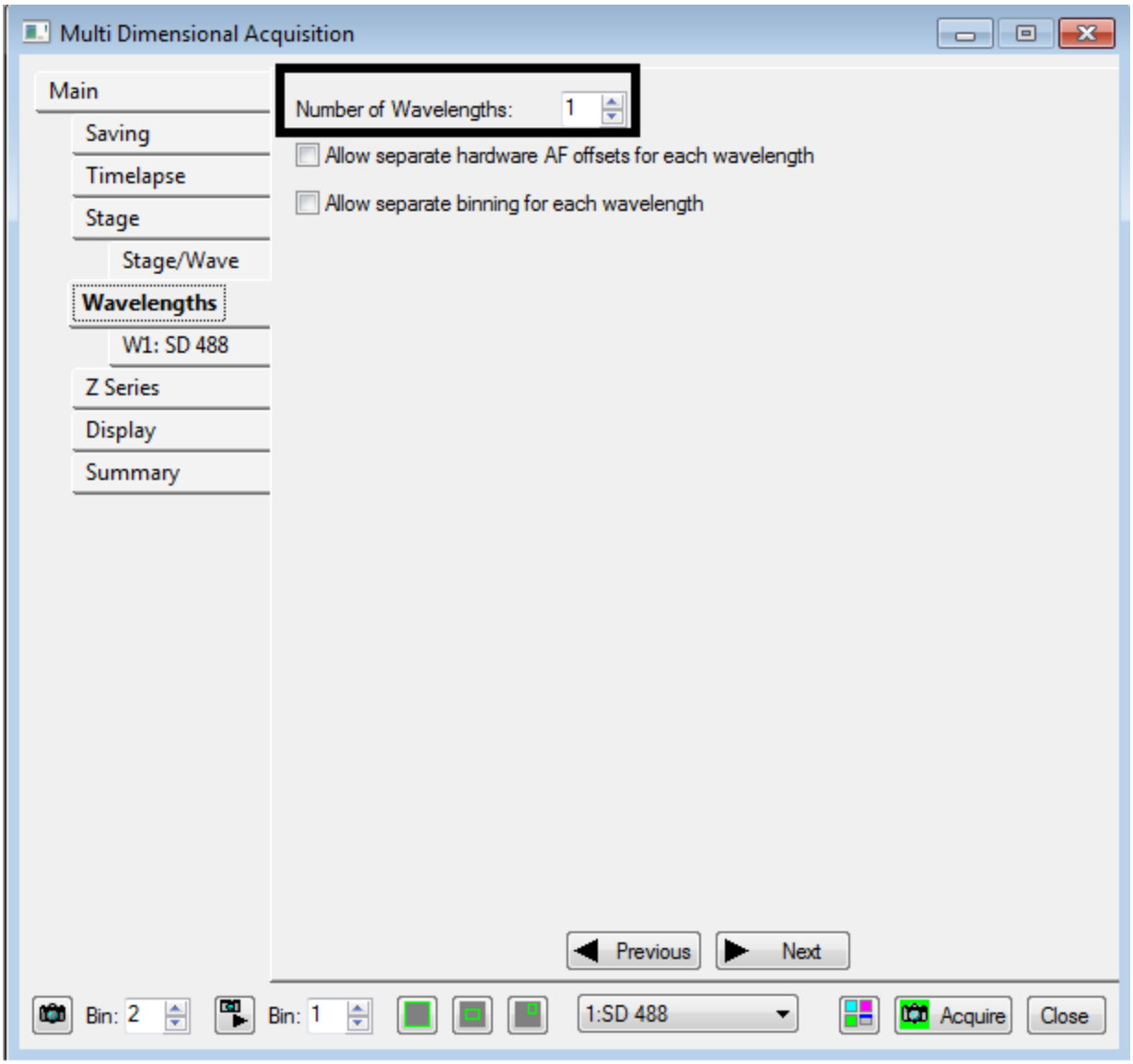
This is very useful feature if you want to acquire images from different positions of stage either as a time series or simply as a stage position series. You can go to stage position of choice and click "+". If you want to delete the entry, click "X". Check in the box "Use advanced stage position/wavelength table acquisition parameters" if you want control on individual parameters like exposure, wavelengths, magnification etc.


Multi Dimensional Acquisition
Main
Saving
Timelapse
Stage
Stage/Wave
Wavelengths
W1: SD 488
Z Series
Display
Summary
Position Label:
Position7
X:
-810.92
Y:
4054.52
AF Offset:
Z:
1
0
Set Focus
Offset Z for travel to this position
0
Positions:
Position4 (1058.24, -5307.48, 3830.42, AF Offset=0....
Position5 (1286.48, -6590.54, 3834.71, AF Offset=-1...
Position6 (1875.2, -6075.96, 3828.19, AF Offset=0.39)
Distance: 2085.11
Move to Position
Sort
Load...
Save...
Use advanced stage position/wavelength table acquisition parameters
Previous
Next
Bin: 2
Bin: 1
1:SD 488
Acquire
Close

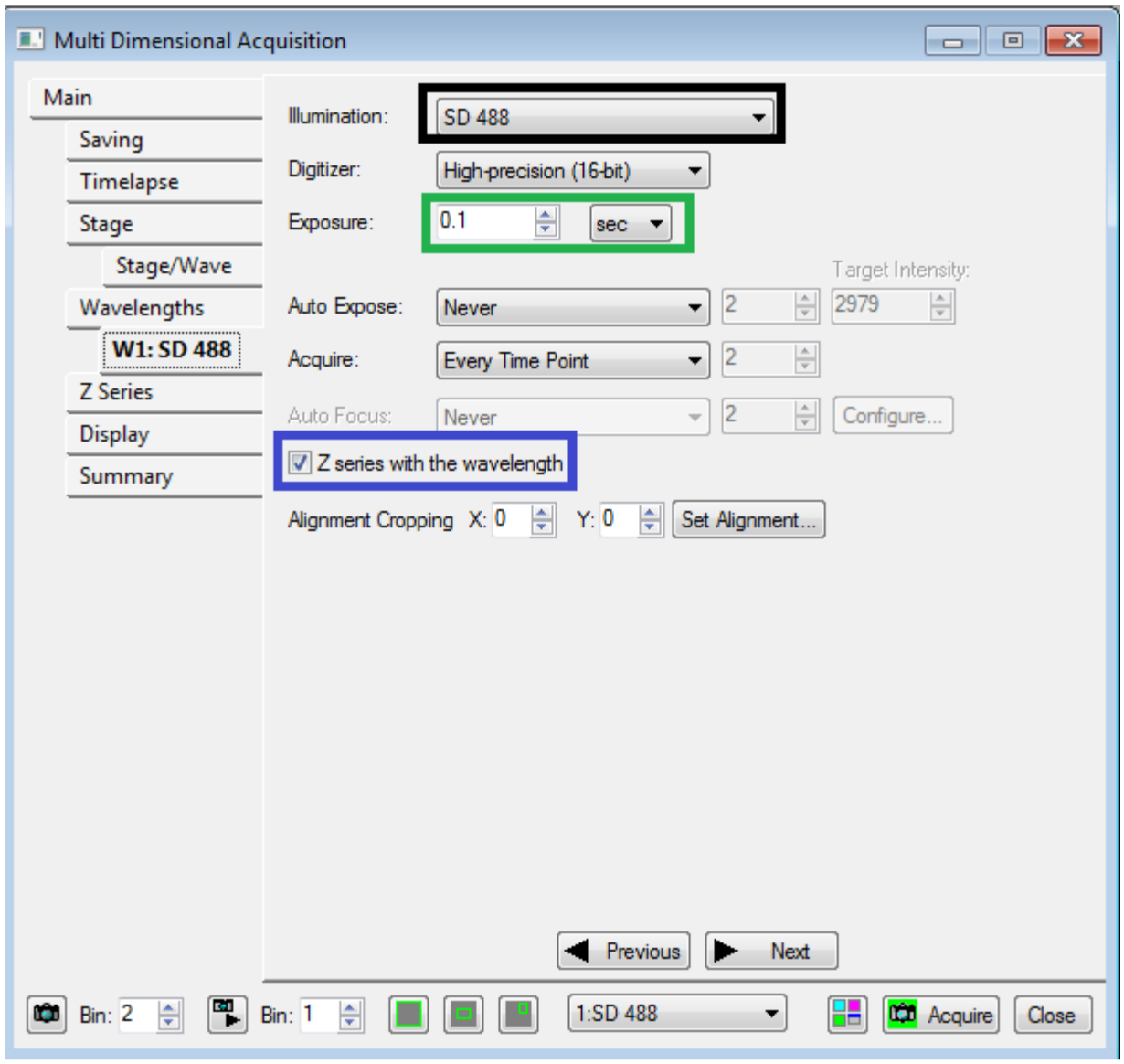


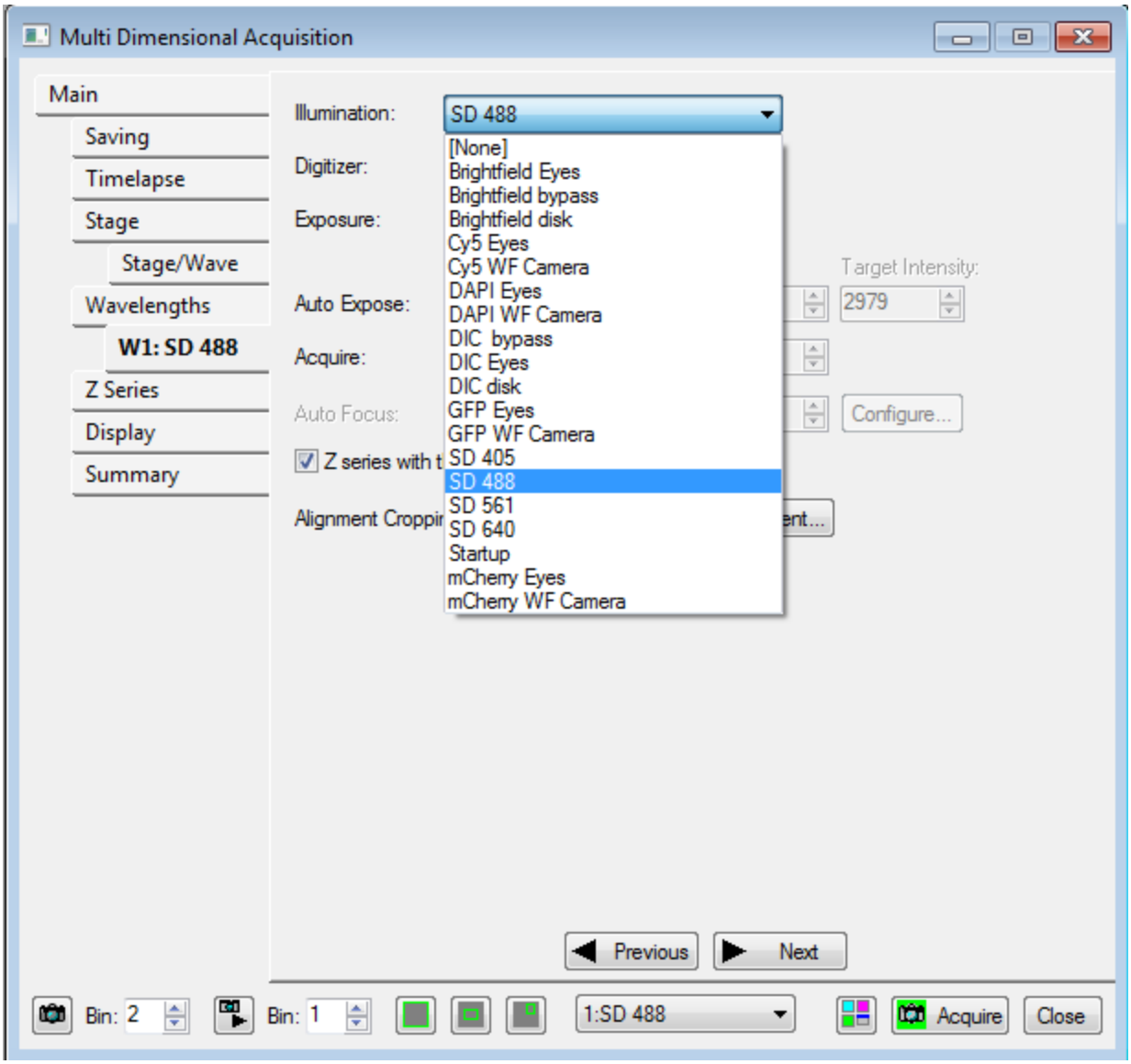
Wavelengths

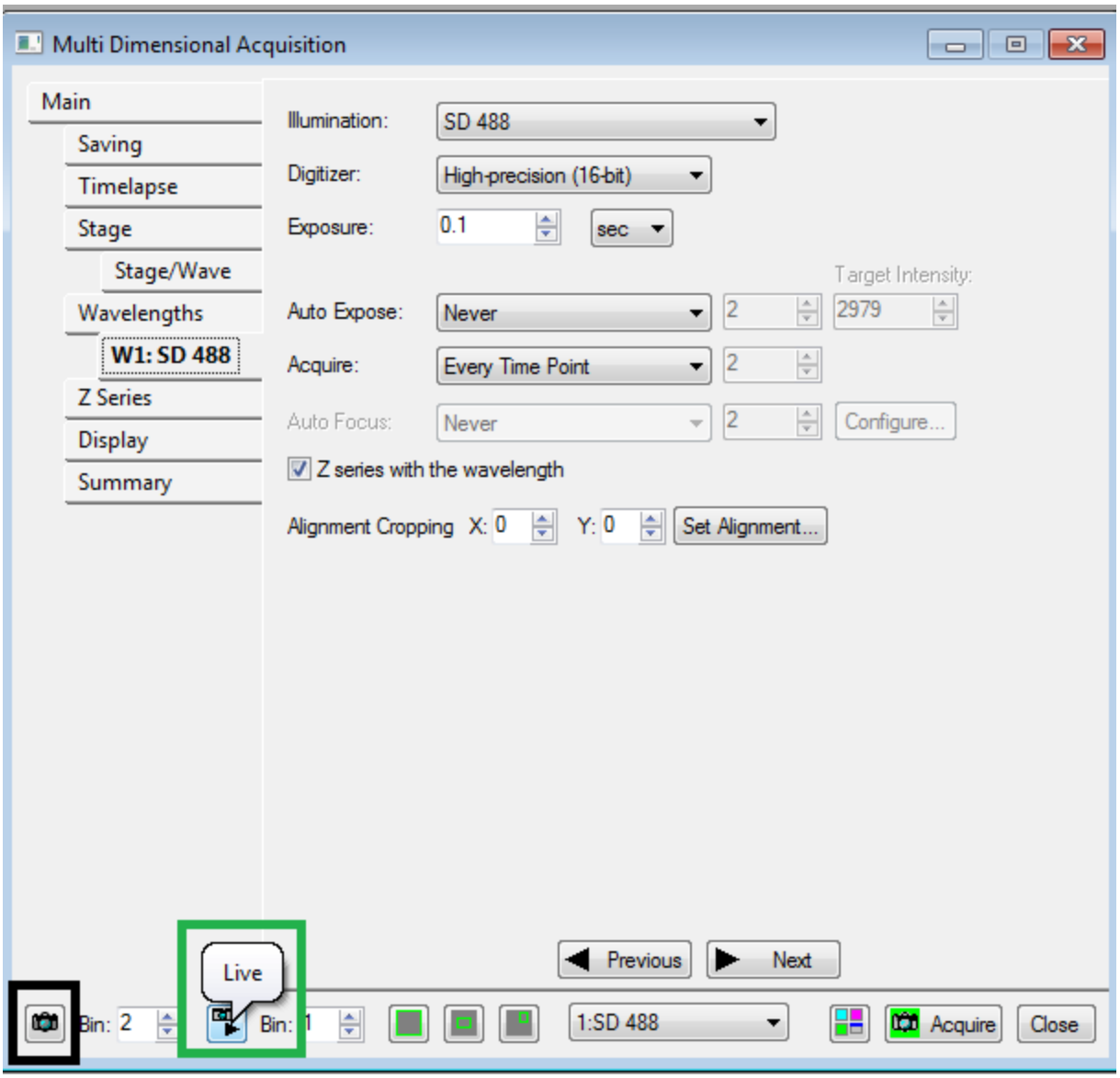
Set up number of wavelengths you want to image.



For each wavelength, choose the "Illumination" from the dropdown menu and "exposure". If you want to acquire z-series with this wavelength, check the box "Z series with the wavelength". If you uncheck this option, software will take an image at the center of z-series that has been set for other wavelengths.


Multi Dimensional Acquisition
Main
Saving
Timelapse
Stage
Stage/Wave
Wavelengths
W1: SD 488
Z Series
Display
Summary
Illumination:
SD 488
Digitizer:
High-precision (16-bit)
Exposure:
0.1
sec
Target Intensity:
Auto Expose:
Never
2
2979
Acquire:
Every Time Point
2
Auto Focus:
Never
2
Configure...
Z series with the wavelength
Alignment Cropping X: 0 Y: 0
Set Alignment...
Previous
Next
Bin: 2
Bin: 1
1:SD 488
Acquire
Close


Multi Dimensional Acquisition
Main
Saving
Timelapse
Stage
Stage/Wave
Wavelengths
W1: SD 488
Z Series
Display
Summary
Illumination:
SD 488
Digitizer:
Exposure:
Target Intensity:
2979
Auto Expose:
Acquire:
Auto Focus:
Configure...
Z series with t
Alignment Croppir
ent...
[None]
Brightfield Eyes
Brightfield bypass
Brightfield disk
Cy5 Eyes
Cy5 WF Camera
DAPI Eyes
DAPI WF Camera
DIC bypass
DIC Eyes
DIC disk
GFP Eyes
GFP WF Camera
SD 405
SD 488
SD 561
SD 640
Startup
mCherry Eyes
mCherry WF Camera
Previous
Next
Bin: 2
Bin: 1
1:SD 488
Acquire
Close



You can click "live" or click "camera button" to get a quick preview of how the image looks like at that particular setting.

Z series

In order to set up this parameter, click live in the brightest channel and move focus to the topmost area of your sample and click "Set top to current". Similarly, move focus to lowest focal plane and click "Set bottom to current". Set up "step size" depending upon the z-resolution for the objective that you are using.

Resolutionz = $2\lambda\eta / NA^2$

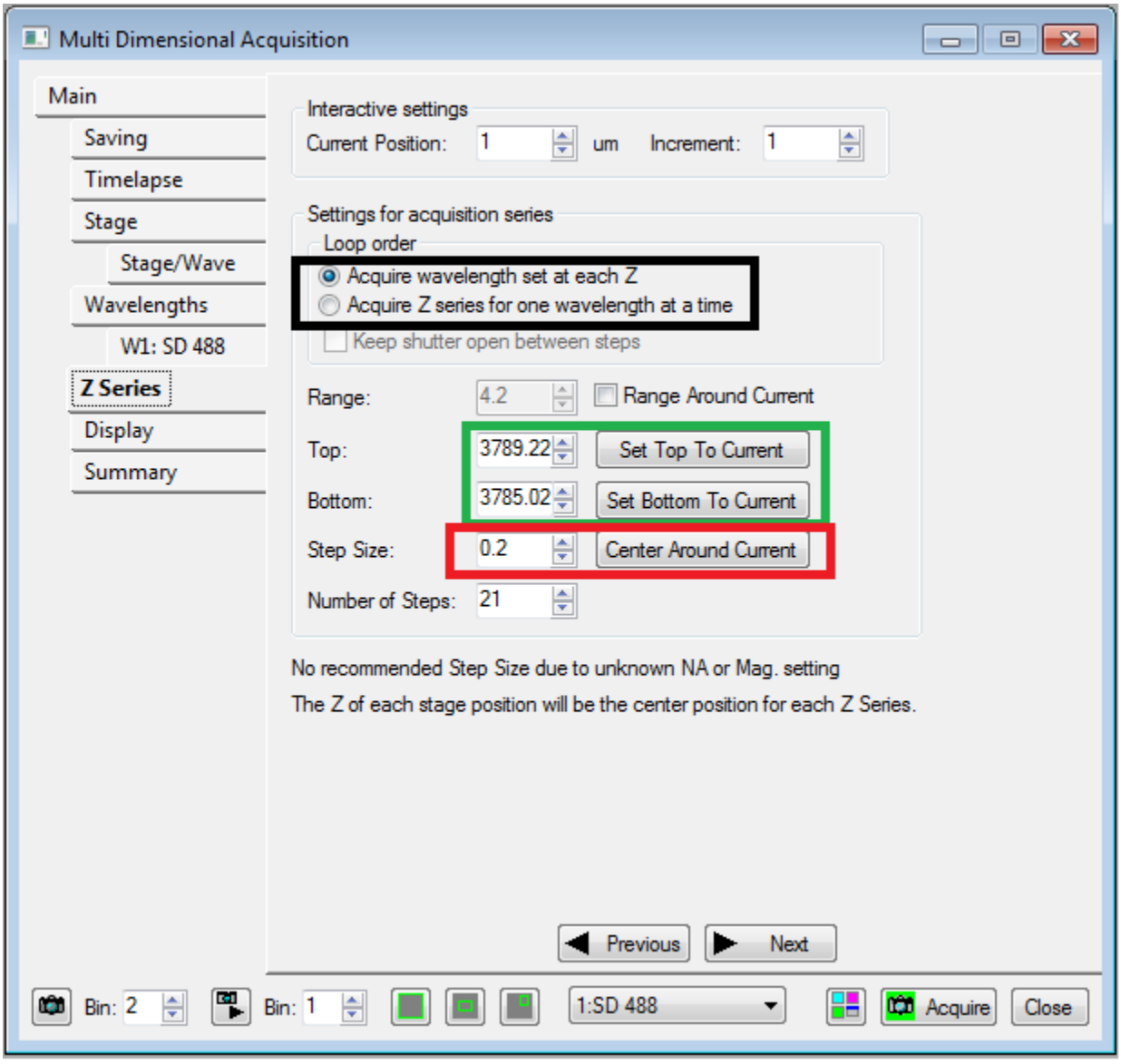
Where λ = wavelength, η = refractive index, NA = numerical aperture.

For λ =500 nm and 40 X (NA = 0.95) air objective (η = 1)

Z-resolution = 1100 nm. This would require a z-step of size 1100/2.3 = 480 nm.

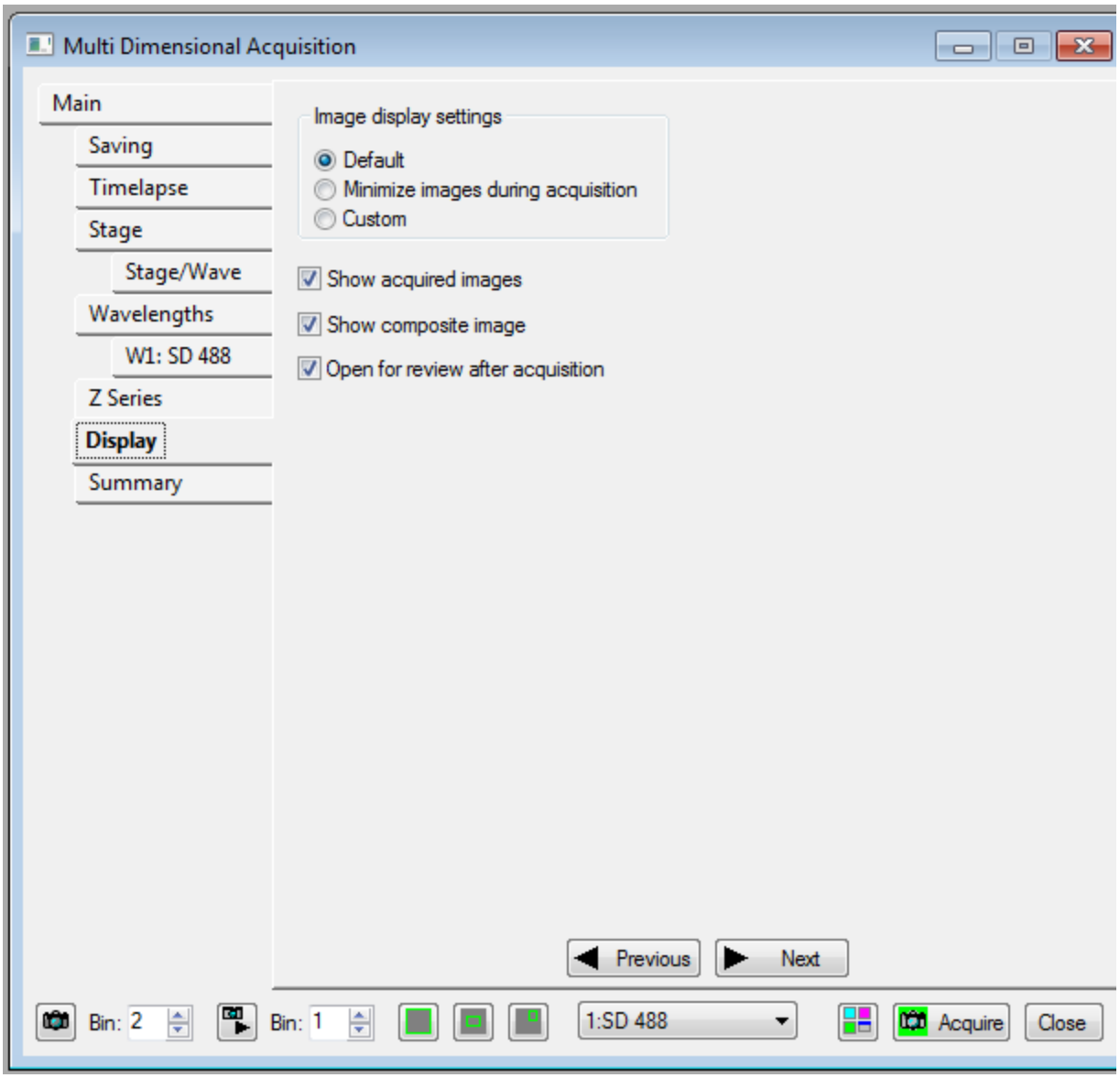
Depending upon the imaging need, you can either of the two loop orders:

1. "Acquire wavelength set at each z". This option is useful is you are carrying out multicolor imaging for a live rapidly moving sample and you want least possible time interval between the various colors.
2. "Acquire series for one wavelength at a time". This is helpful when sample is fixed/stationary and you want highest possible speed for acquiring entire image set.



Display

Quite often it becomes annoying to close all the windows once acquisition is done. You can choose what images you want to be displayed and which ones you don't want to be displayed.



Once you are done with setting up the parameters, click “acquire” and go for a coffee break. Your images will be acquired and saved automatically to the destination folder.