

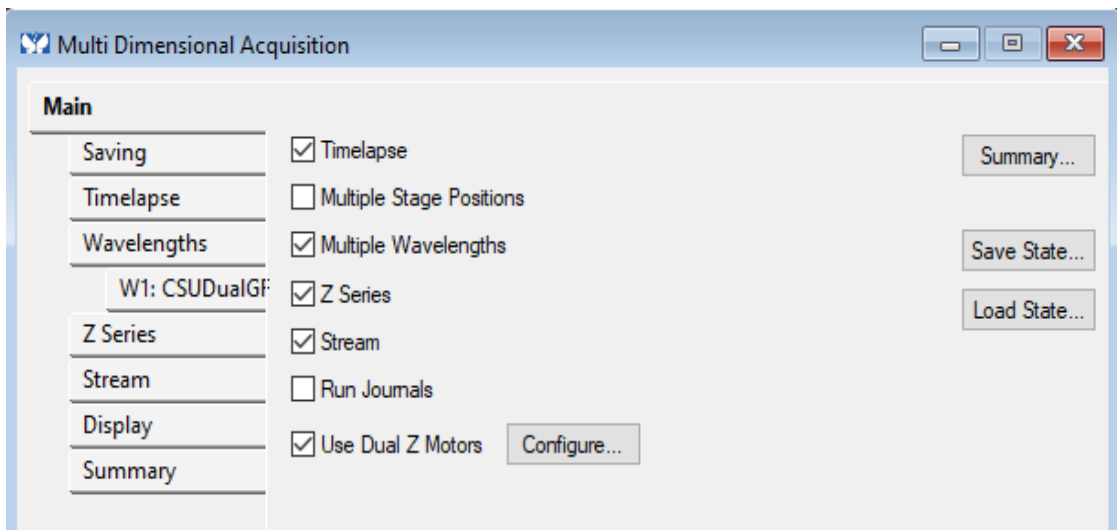
## Live-SR spinning disk confocal using MetaMorph (MM)

### MDA Stream Imaging

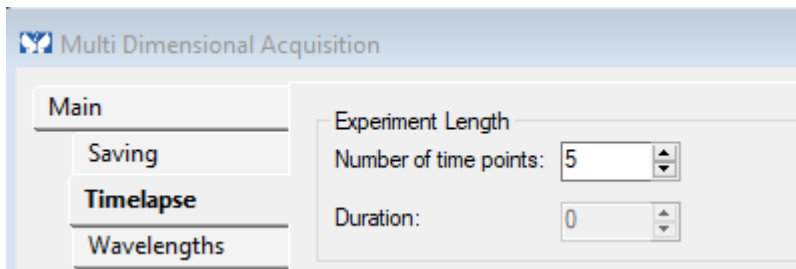
Refer to the main manual of liveSR\_CSU for the normal 2D, 3D, time lapse.

The following is for stream imaging in MDA for 2 channels by dual cameras, 3D time lapse, to reach the acquisition speed limited only by the exposure time. The imaging acquisition is carried out continuously with “0” interval.

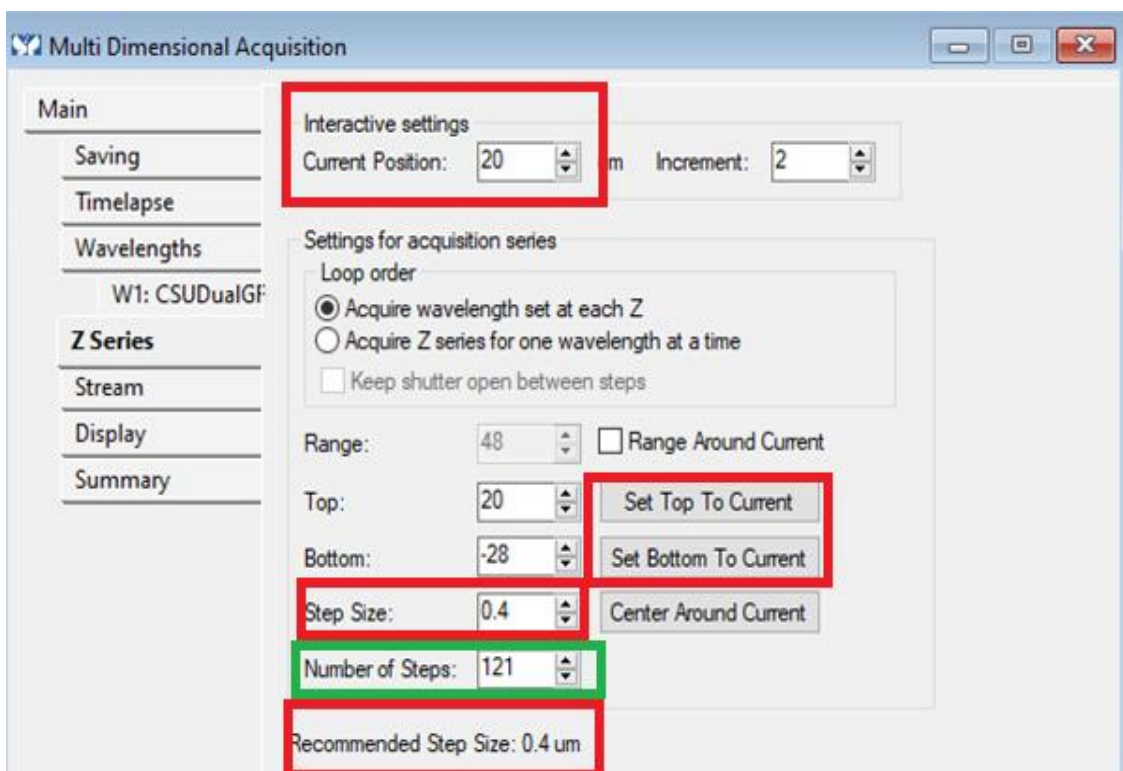
1. Start MDA. Check the boxes as followings:



- A. Timelapse: to capture live events over a certain time.
  - B. multiple wavelength: all the time, regardless of numbers of light path to be selected.
  - C. Z series: to carry out 3D volume imaging
  - D. Stream: to achieve the maximum speed limited by exposure time only
  - E. use dual Z motors: to engage piezo stage to fast imaging.
  - F. Click on “save state” to save current setting of MDA and “Load State” next time to reuse. Laser settings are not saved. So snipping the window of laser settings is recommended.
2. SAVING: TO SSD(D).
  3. “Timelapse”: enter a number for “Number of time points” you need. The time required for one 3D volume imaging is based on the Z slice numbers and camera exposure time. For example, for a 50ms exposure and 120Z slices imaging, you need to spend about  $120 \times 0.05 = 6$ sec to complete one 3D (for one time point) imaging. In this case, if you enter “5” for “Number of time points”, you will have an image acquisition lasting for 30sec.

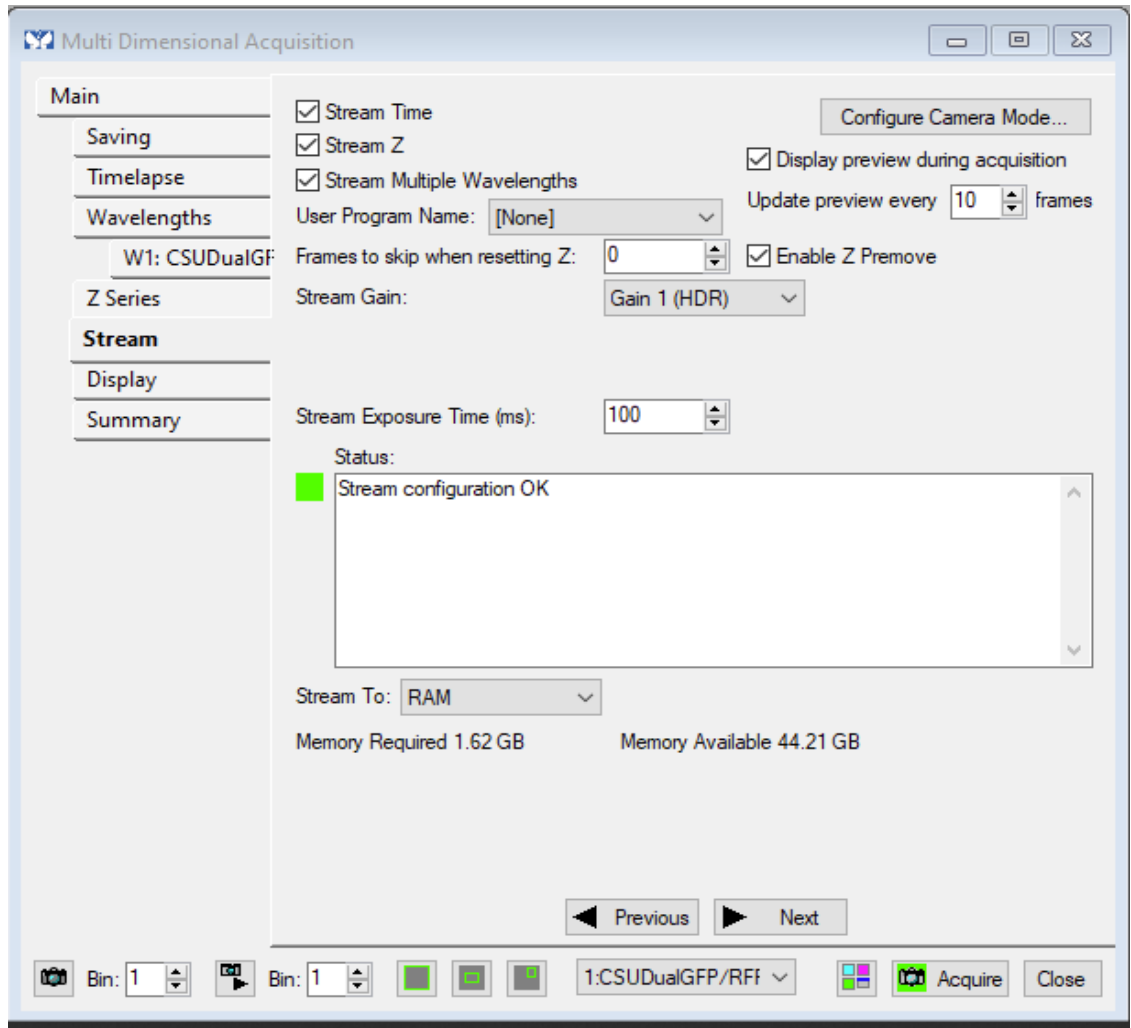


4. "Wavelengths": Choose 1 and select the light path such as Dual\_CSU\_DAPI\_Cy5.
5. "Z Series": "current position" is the piezo stage position. Move the current position to top of the 3D signal, "Set Top to Current"; Move the current to bottom of the 3D signal, click on "Set Bottom to current". Enter recommended step size for "Step Size". Now you can find out the volume/range you are going to image and No. of slice for the 3D imaging.



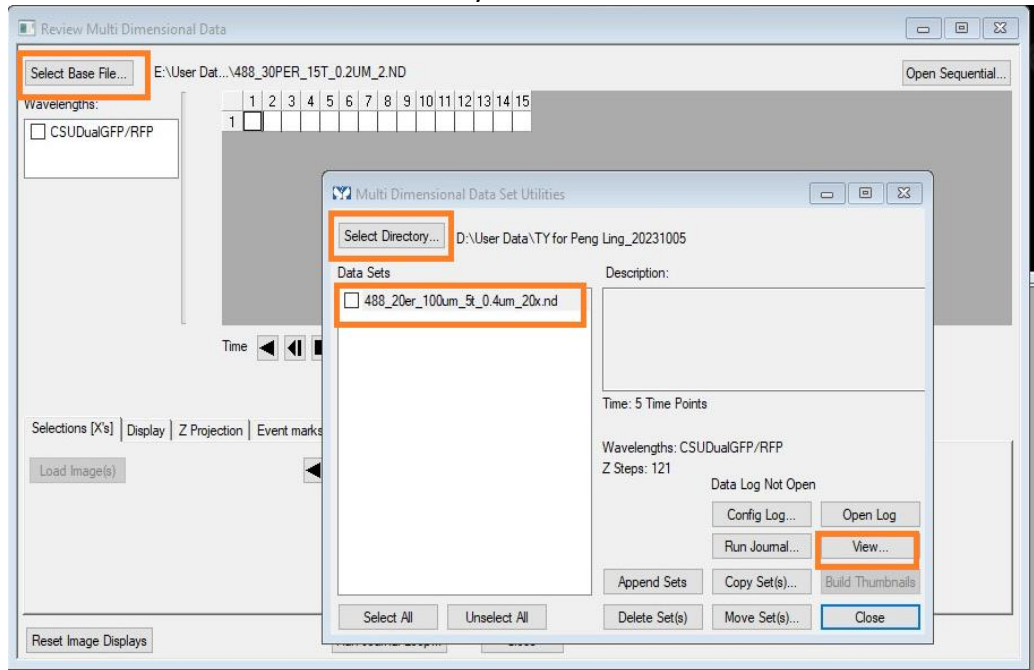
6. "Stream":
  - A. Check the boxes for all. "update preview every 10 frames" will allow the screen to display an image after every 10 frames acquired after you start image acquisition.
  - B. Enter exposure time: this setting will overwrite the exposure time on the wavelengths tab.
  - C. Stream to "RAM" (software will automatically save images onto location you have defined during step 1 after the imaging experiment is completed).
  - D. Take note that "Memory Required" should not exceed "Memory available".
  - E. Frames to skip when resetting Z: enter 1-2 to skip the first 1-2 blurred frames of the 3D stack images resulting from acquisition done before the piezo Z settled.

- F. Enable Z Premove: check the box to reduce the chance of blurring image resulting from moving piezo Z.



7. "Display": you may check the boxes for "Default" and "show acquired images" only.
8. Start "Acquire".
9. To check acquired images, click on "review MDA" to open "Review Multi Dimension Data" dialog window.

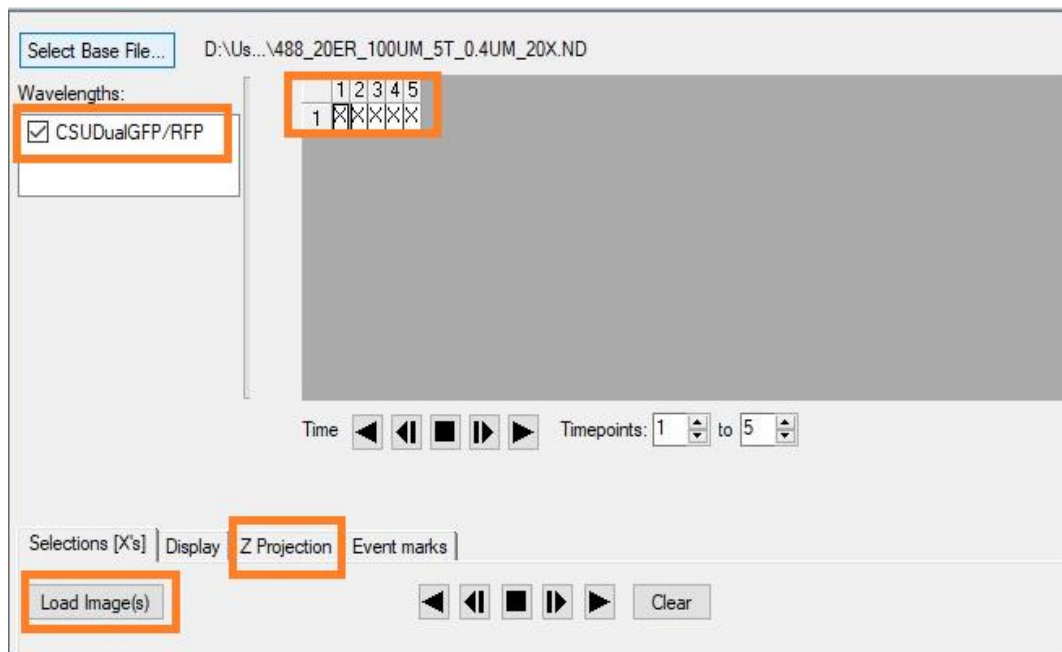
A. "Select Base File" -> "Select Directory" -> select data -> "View".



B. Check the file in the "Wavelength" field.

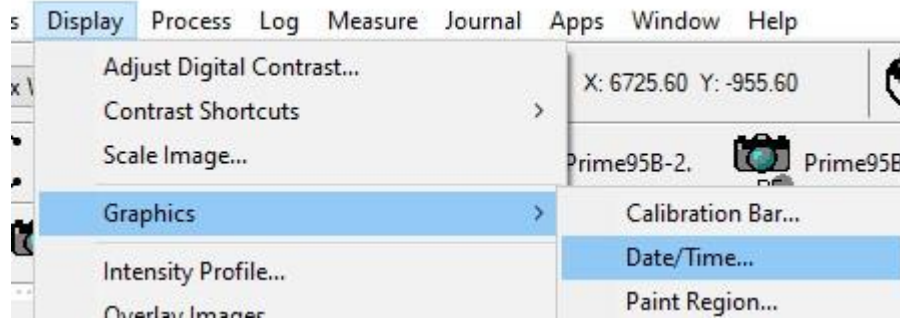
C. Right click on the time frame to select all the time points. For 3D time lapse data set, if only time frames are shown without 3D information, it means the maximum projection has been selected under "Z Projection" already.

D. "Load Image(s)".

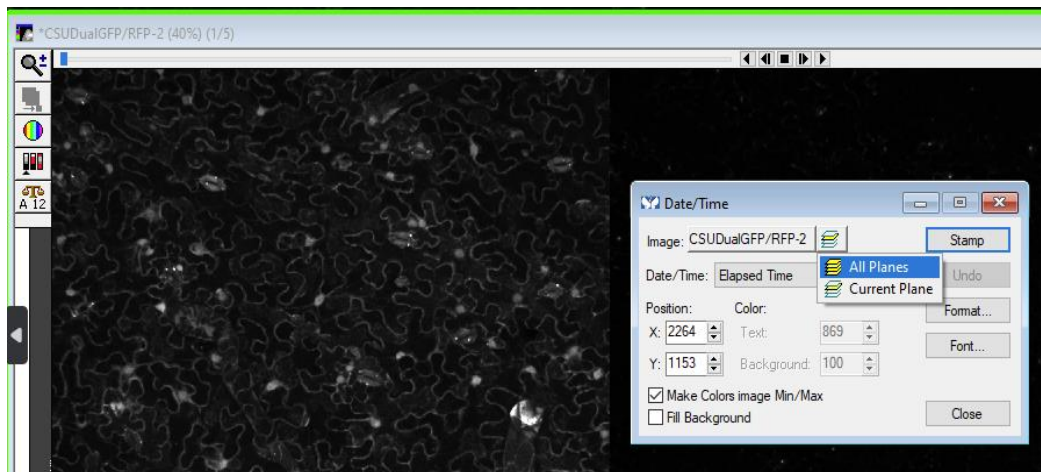


10. To check the actual interval of time lapse acquisition:

A. Display -> Graphics -> Date/Time.

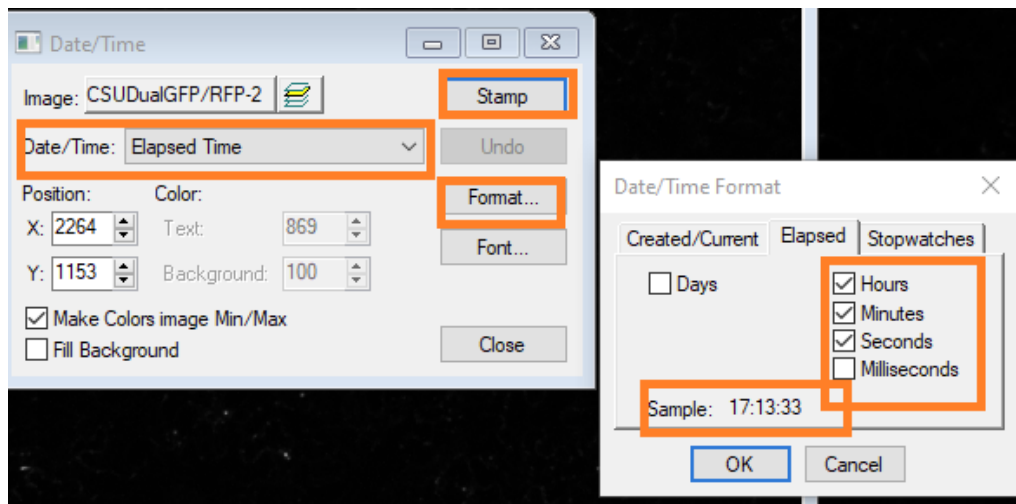


B. Image: Select “all planes”



C. Date/Time: Elapsed Time

D. Format: Check for “Hours”, “Minutes”, “Seconds”. -> Ok. “Stamp”.



E. Move slide back and forth at one time frame step to find out the interval.

