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

Multiple locations_3D_Timelapes with PFS

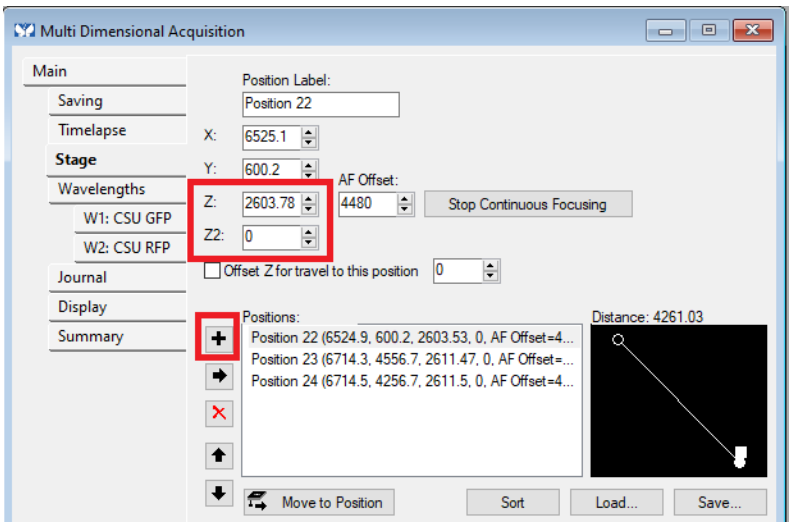
Refer to the main manual of liveSR_CSU for the normal 2D, 3D, time lapse.

The following is for multiple stage location imaging setup in MDA for in time lapse, e.g to image n different locations of cells from a petridish for overnight, engaging PFS to correct the focus drift of the objective.

PFS works for air or water lens for majority of samples, or oil lens for sample in aqueous solution.

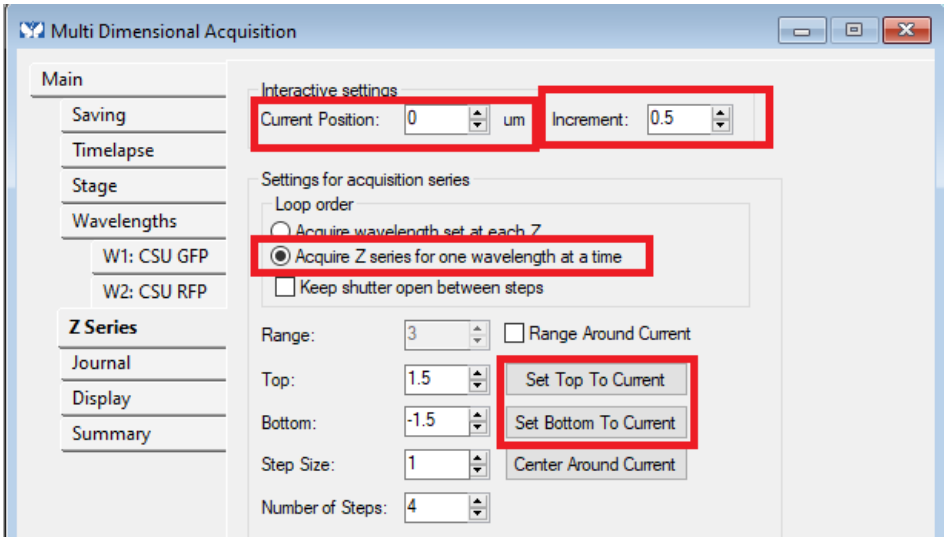
Do remember to off PFS when you define 3D imaging range (top/bottom) and restore PFS before start image acquisition.

1. Start MDA, load state/setup light path, adjust laser and exposure time (refer to the main manual for CSU).
2. MDA-> Stage.
3. Manually move to a ROI, region of interesting by Live view.
4. Activate PFS by pressing “Focus” button on the front of the scope.
5. Add the current position. The Z is the objective position and Z2 is the piezo position. Make suer Z2 is always at “0” but Z normally varies among different positions.



6. Complete registration for all the stage position you would like to image.
7. MDA-> Z series.
8. Define the Z imaging volume following the steps below.
    a. Keep live view.
    b. Stop PFS by pressing “Focus” button on the front of the scope body.
    c. Enter a proper value for “Increment” for the piezo stepper size.

d. Move piezo to top end of the sample signal by pressing it up-arrow. The “current position” will be updated accordingly.
e. Click on “Set Top to Current”.
f. Move the piezo to bottom end of the sample signal by pressing the down-arrow. Click on “Set Bottom to Current”.



9. Choose “acquire Z series for one wavelength at a time” to increase acquisition speed (for single camera).
10. To verify the Z volume is suitable for all different stage location:
    a. MDA -> stage.
    b. Select the next stage point, double click to move to the point.
    c. “Z2” should be automatically updated to “0” as mentioned in step 5 above.
    d. MDA-> Z series.
    e. In “Current position” filed, enter for the Top and Bottom positions and “Snap” to have a quick check of signals respectively. If the signal is not fully covered, move the current position and register “Set Top” and “Set Bottom” to current respectively.
11. Start “PFS”.
12. Start “acquire”.
13. Watch out to make sure PFS is on (focus button LED light is on) when the stage moves to the different positions and during time lapse interval at least for the first two time points of the imaging experiment.