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

## A. Start the instrument and software:

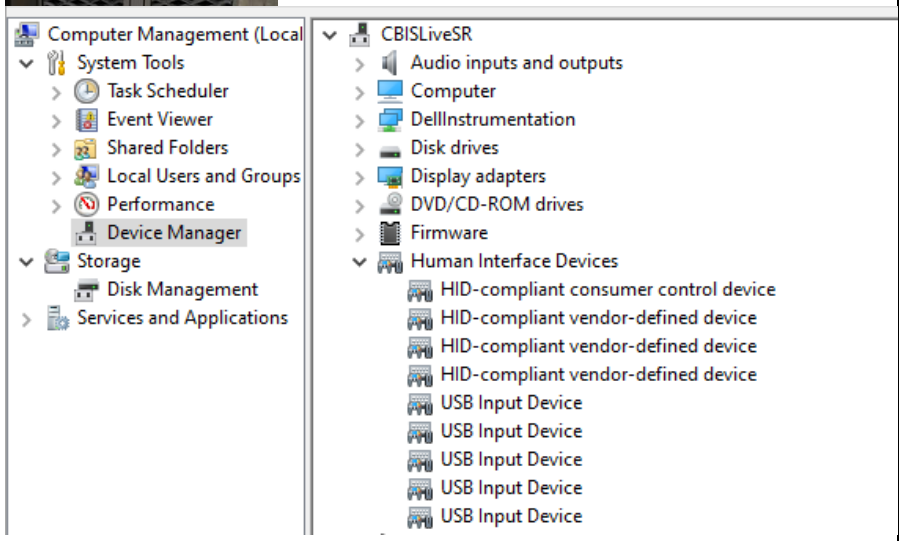
1. Switch on: power supply 1, 2, 3.


2. Wait for two minutes, switch on the computer: 4.

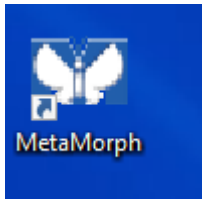
3. Select "Imconfocaluser" account and enter the password for the account.

4. Enter your PPMS account and password to start.

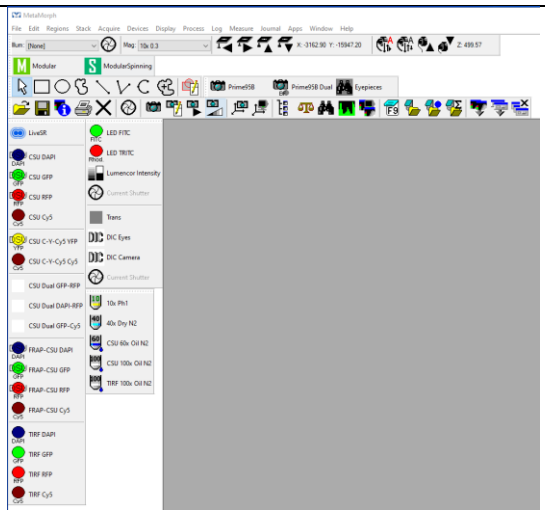
5. This PC-> Manager ->Device Manager-> Human Interface Devices to make sure the list is clean and normal (as shown by the picture on the right). If there is any warning sign on the list, re- connect the USB cable to the computer (on the back of the computer).

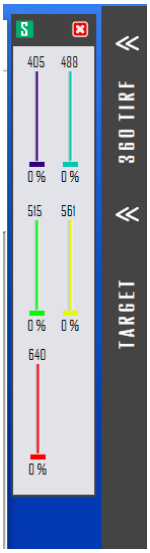



6. Double click on "MetaMorph" icon to start image acquisition software.



7. “MetaMorph” and “Modular” windows present.

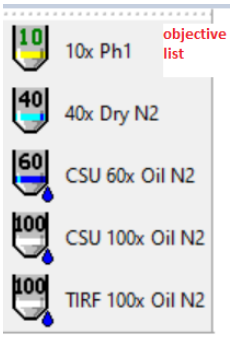


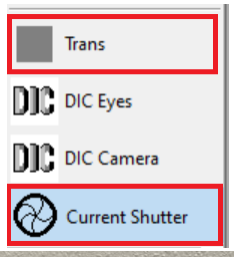


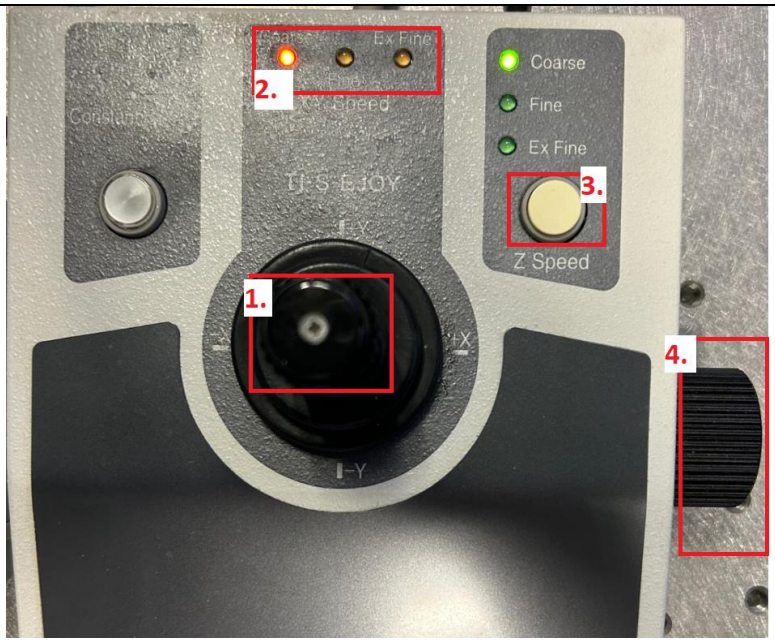

## B. Find and focus sample using transmitted light.

1. Select objective. Apply immersion medium accordingly.
2. Mount sample onto stage.
3. Click on “Trans” and “Current Shutter”. The microscope should be on “eye” mode now (1).
4. You may use PFS, perfect focusing system, find the coverslip of the sample as follows. Otherwise, go to Step 5 directly to find focus without using PFS.

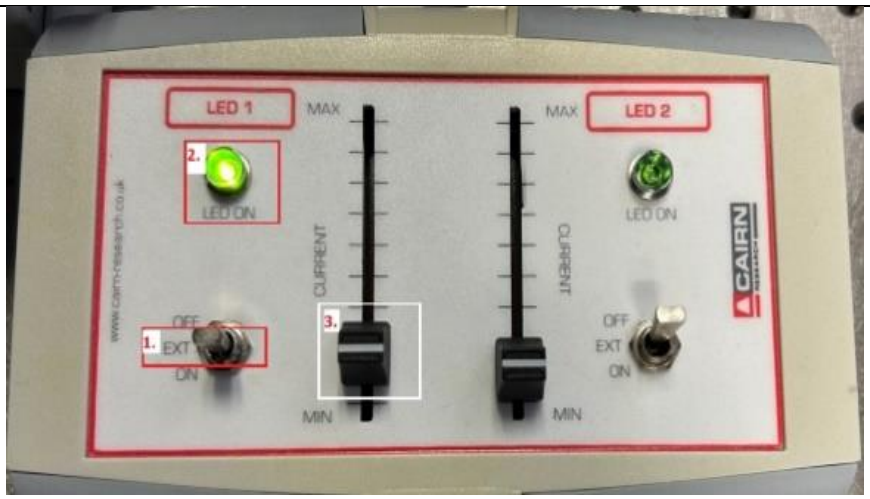
- Press “Focus” (2) button to activate PFS. The button blinks when the sample is far away from focus.
- Move microscope focus knob anti clockwise to move the objective upwards.
- When the objective near focus plane, the “Focus” button stops blinking.




| | |
|---|---|
| • Change Z position by using “PFS” focus (refer to C.5.) to get a clear image of the sample.<br>5. Look at the sample via eyepiece while you turn microscope focus knob until the sample is in focus. | The objective Z position is around 2500-3000um (3) when a typical glass slide sample is in focus by using 10x objective lens. |
| **6.** To view different part of the sample:<br>• For XY: use joystick (1) to move stage. The tip of the joystick can be tuned to different speed movements (2).<br>• For Z (when “PFS “is not engaged): use remote focus drive (4) to move the objective lens at different speeds (3). |  |

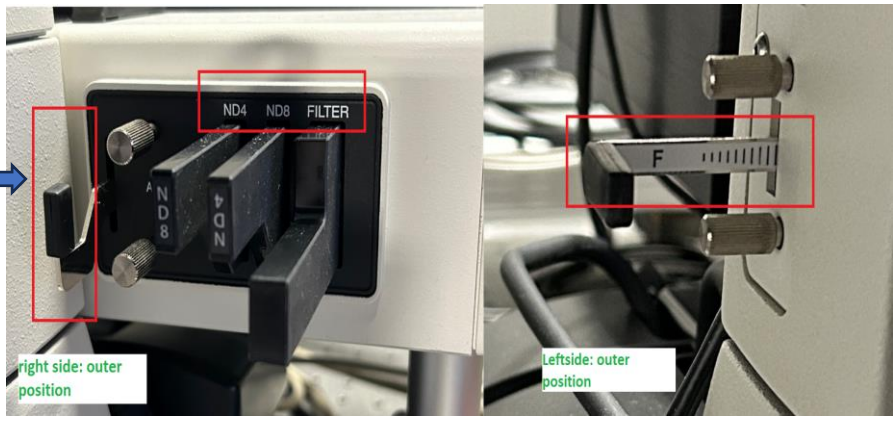
7. Make sure the Transmitted light is on (2) and on the "Ext" (middle) position (1). Change transmitted light intensity (3) when necessary.




## C. Find and focus sample using fluorescent light.

1. Follow and complete section B.


2. Check the accessories of microscope body:
   a. Back: set mirror position to lumencor illumination.
   b. Left side: field diaphragm is on the outer position.
   c. Right-side, all filters are on their outer position.




Push the Apture iris in to reduce the fluorescent light illumination when necessary.

3. Activate "LED FITC" (or TRITC) and "Current Shutter". The remote control will be lit up.

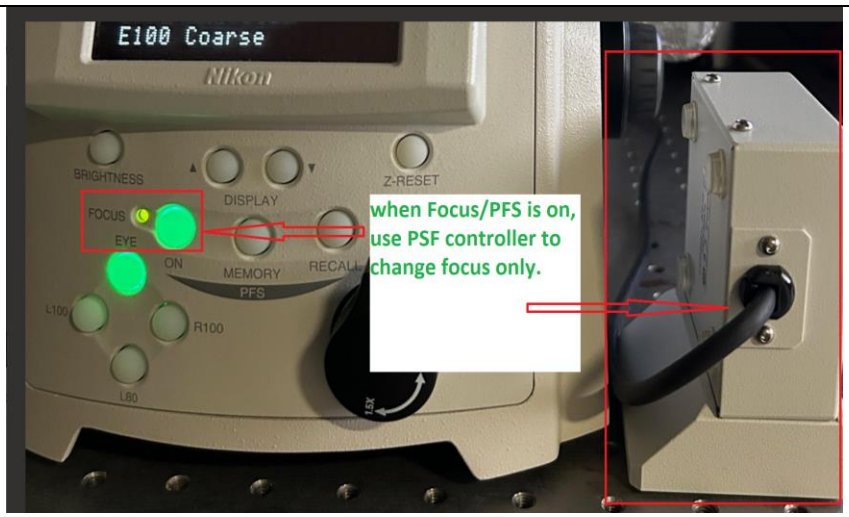

4. Adjust light intensity if necessary:
   - Push the Apture iris in (refer to 2C), or

- Lumencor intensity: change value 0-255.

5. To maintain focus, press “Focus” button on scope body. You need to use PFS to fine tune focus once you activate PFS.

6. Close “Current Shutter” to protect sample from photobleaching.

7. Do Koehler illumination if you need DIC/PH

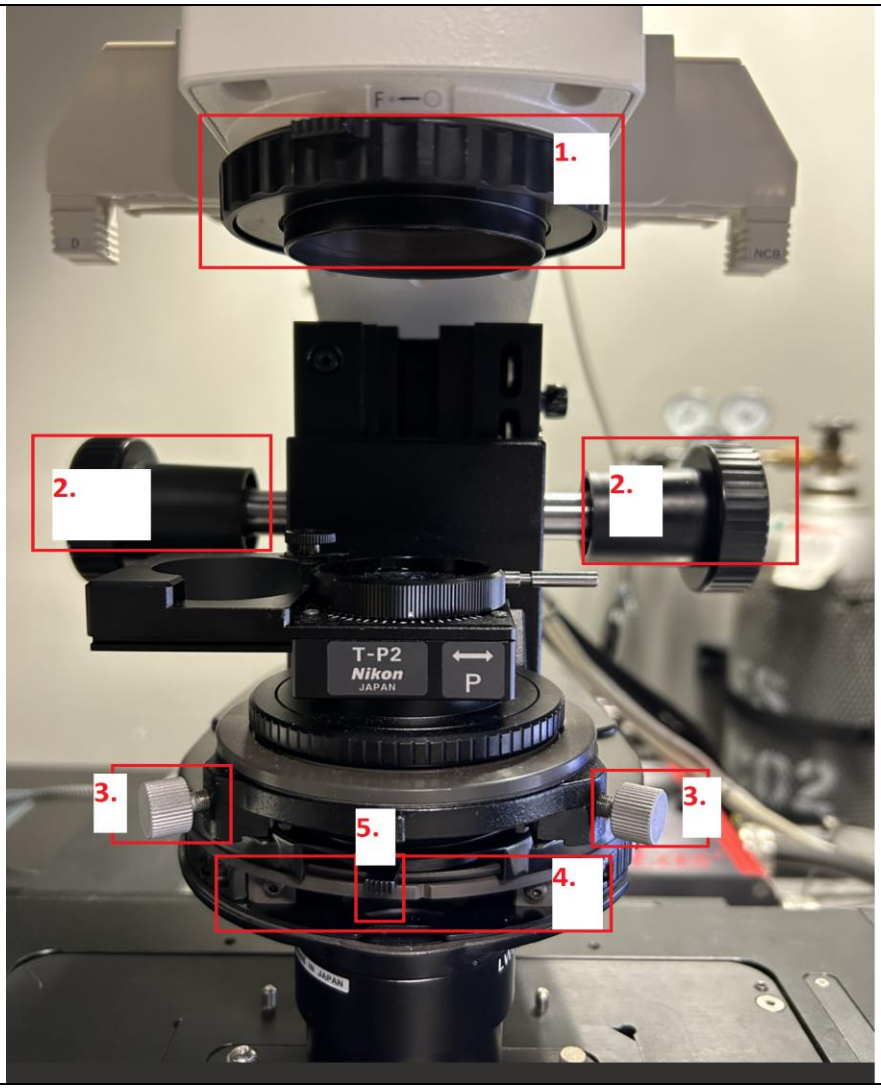



**D. Koehler illumination and DIC**

1. Focus sample.
2. Close down field diaphragm (1), adjust condenser position (2) until image of field diaphragm is sharp under eyepiece, which is super imposed to your sample.
3. Using two centering pins (3) to adjust image of field diaphragm to center of FOV.
4. Open field diaphragm (1) until its edge just disappears.
5. Open condenser aperture (5) to maximum.
6. Based on the objective lens in use, choose the condenser position (4) accordingly.

7. For example, one needs DIC for the 40x/0.95 lens:

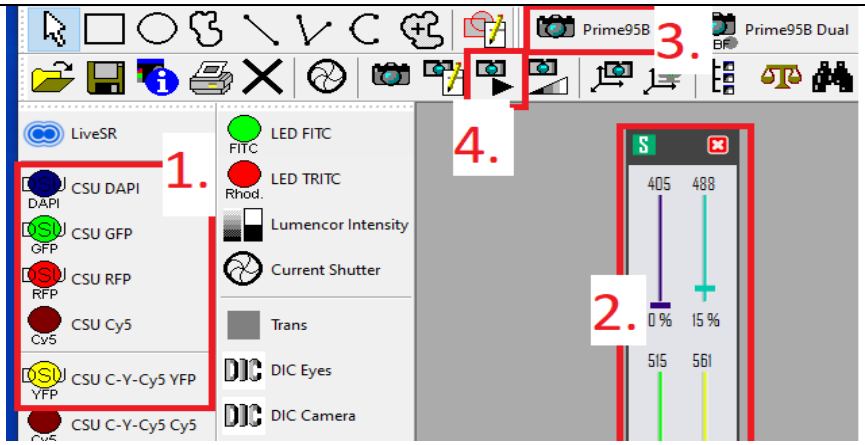
- MM Select DIC Eyes.
- Tune condenser to DICN2 (6).



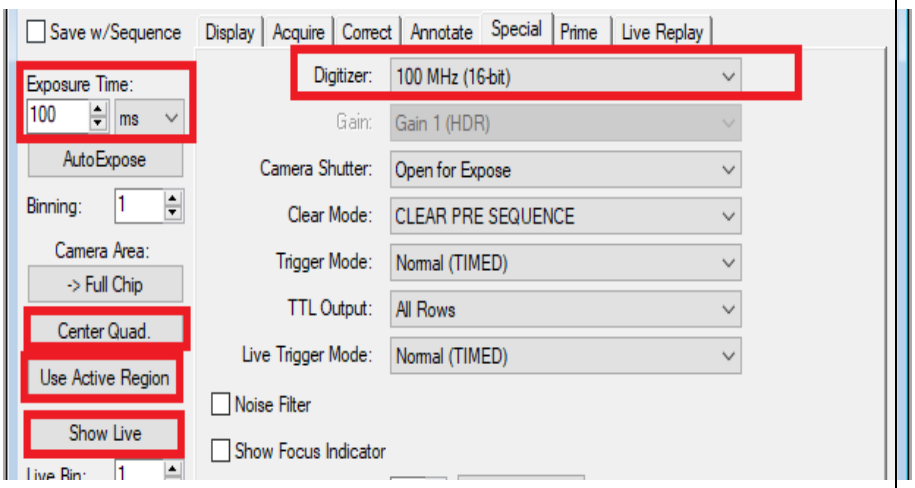
- Loosen screw for polarizer (7).
- Look at the sample through eyepiece while moving polarizer (8) to the position gives best DIC effect.
- Fasten screw for polarizer to the optimal position.


**E. Preview images using camera for spinning disc confocal:**

1. Select the appropriate CSU Light path (1).
2. Adjust laser intensity (2).
3. Choose camera Prim95B (3).
4. Show live (4). (Click one more time to close the live window).

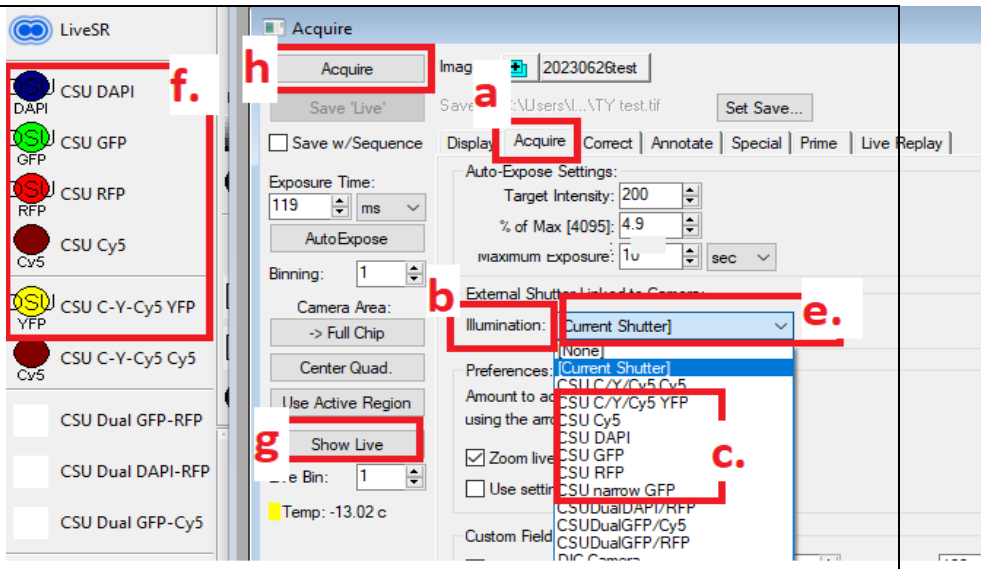



5. Go to “Acquire” -> “Acquire” to open the window. Under “Special” tab, one can change camera settings, such as crop the view (center Quad, Use Active region), change exposure time/autoExpose, and read out speed, etc.

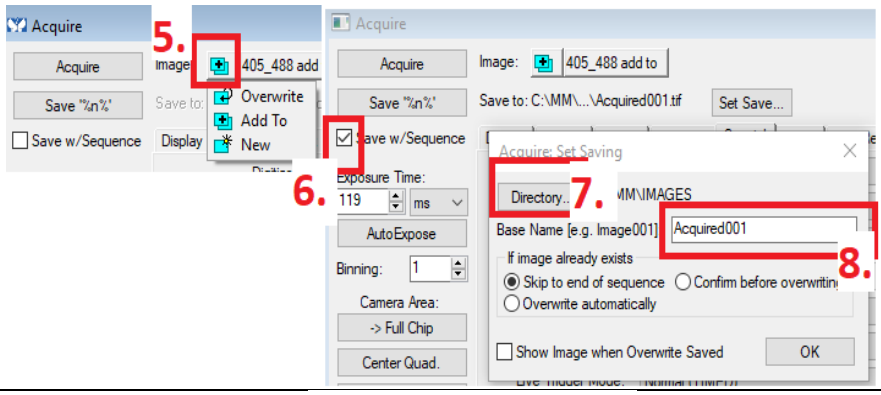



6. Under “Acquire” tab (a.) for illumination (b):

- Choose any light path (C) and “show live” (g) to preview image before acquire image (h).
- Or, if one only need to preview image, just choose “current shutter” (e.) and choose any light path in the main side panel (f.) to preview the image by “show live”.

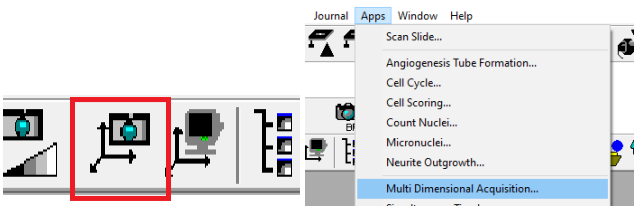



7. MM allows to save acquired image onto a predefined folder (5, 7) and based image name (8).




## F. Setup acquisition using MDA window.

1. Start MDA using the icon. Or MM -> Apps -> Multi Dimensional Acquisition (MDA).




2. Load Lightpath:
   a) Main Window, “Load State” (1).
   b) Select All except for “main: file Name and directory (2).
   c) Select and open the one fits to your application (3). (C:\Users\lmconfocal

user\Desktop\MDA settings)

d) You may save your settings and load next time for your own convenience.

3. **Main** window, check the dimensions accordingly for timelapse, multiple stage position, channels, Z series, etc.

4. **Saving** Tab: to select the folder destination and image name.

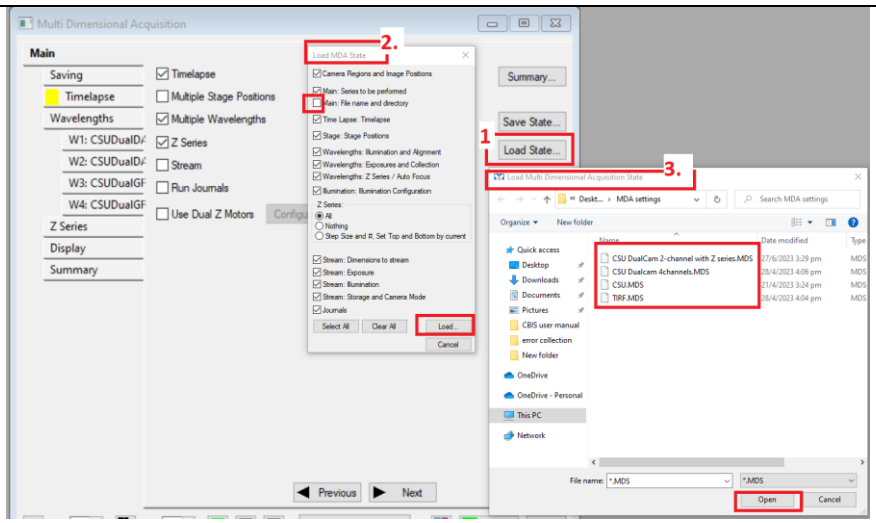
5. **Wavelengths**: You may manually choose a proper one from the dropdown list.

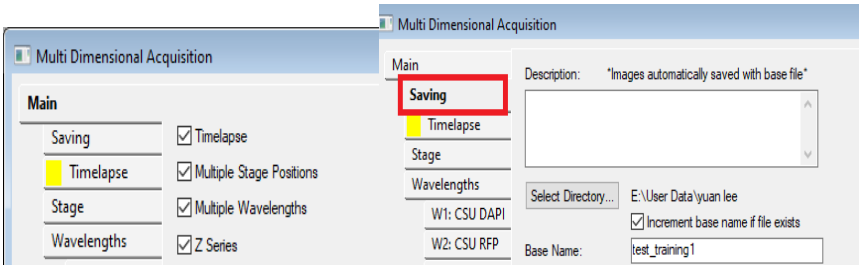
1) Understand the Lightpath name:

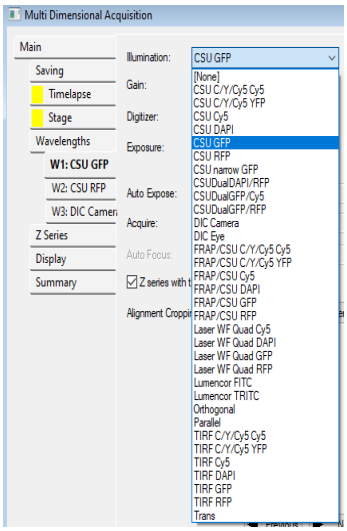
- CSU: spinning disc confocal
- CSU Dual: spinning disc confocal two camera simultaneous scan.
- FRAP/CSU: spinning disc with FRAP
- Laser WF: laser widefield (non-confocal)
- TIRF: Total internal reflect Fluorescent, for membrane signals.

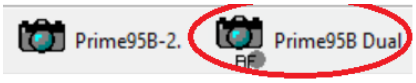
2) If you have loaded DualCam state, choose Prim95B Dual.

3) Keep “live” (3) while change Gain, Digitizer, Exposure (1) and laser settings (4) for signals. Different scale settings (5) gives different contrasts.



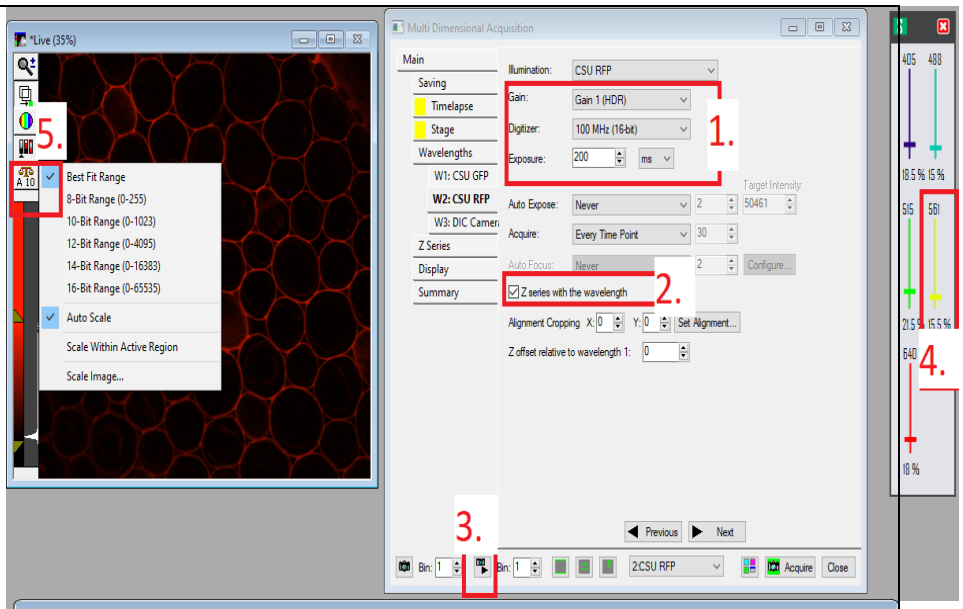



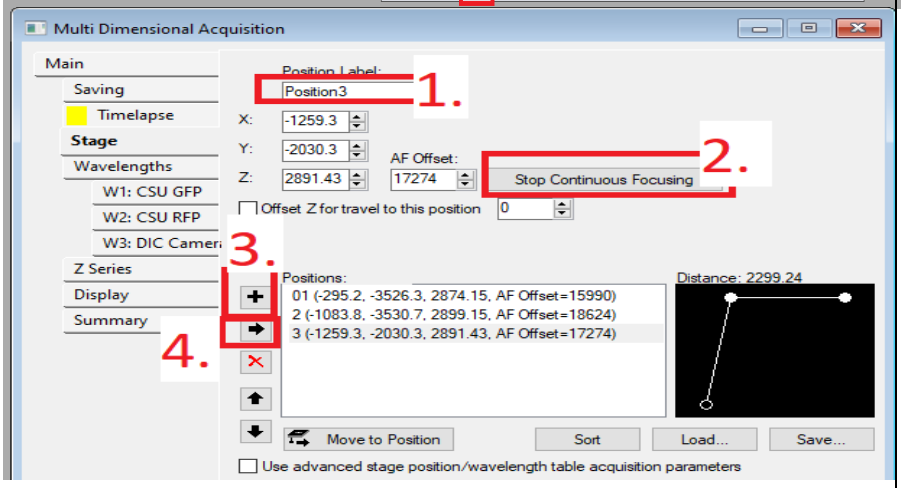
4) Click “live” (3) one more time to stop live view.
5) Repeat step 3) and 4) to optimize signals for all the channels.
6) Check “Z series with the wavelength” for 3D imaging (2).

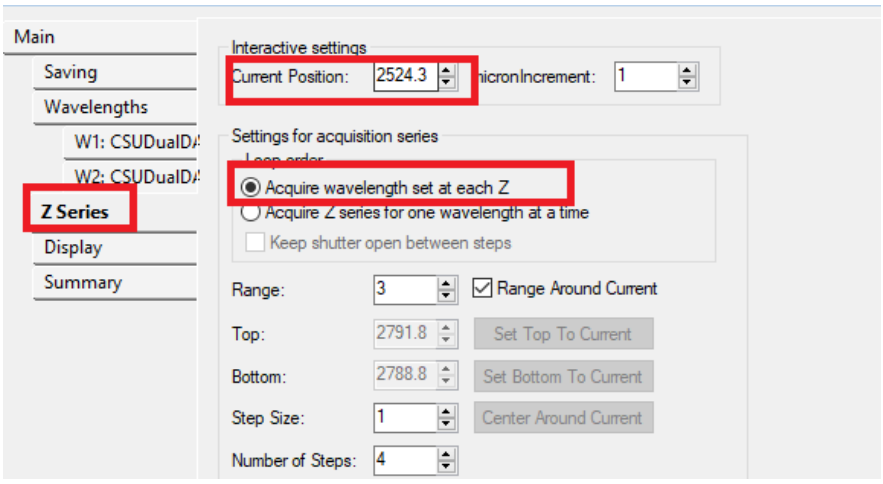
6. **Stage:** name stage position (1), change PFS wheel to find the best focus. “Stop continuous Focusing” (2) is shown when adding (3) position. Otherwise, press “focus” (refer to section C.5.) to engage PFS and add (3) or insert current position (4) when necessary.

7. **Z series:**
   a) For dual camera imaging, “Acquire wavelength set at each Z”.
   b) For single position scan, use “range around current “to set the Z volume. For multiple position scan, use “center around current”.
   c) Make sure the current position is at the bottom of the Z volume before starting acquisition.

8. **Summary:**
   a) Print: to save experiment file as future reference (not for re-use)
9. “Acquire” to start.

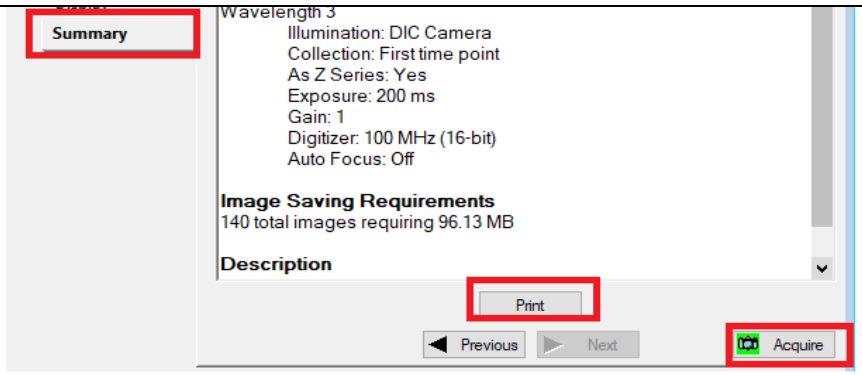


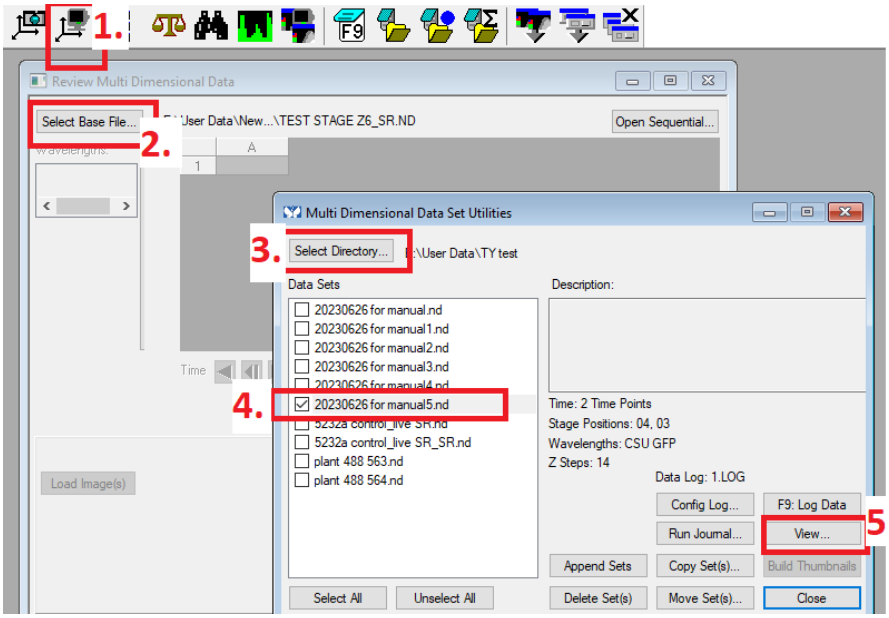



## G. View acquired data using MM.

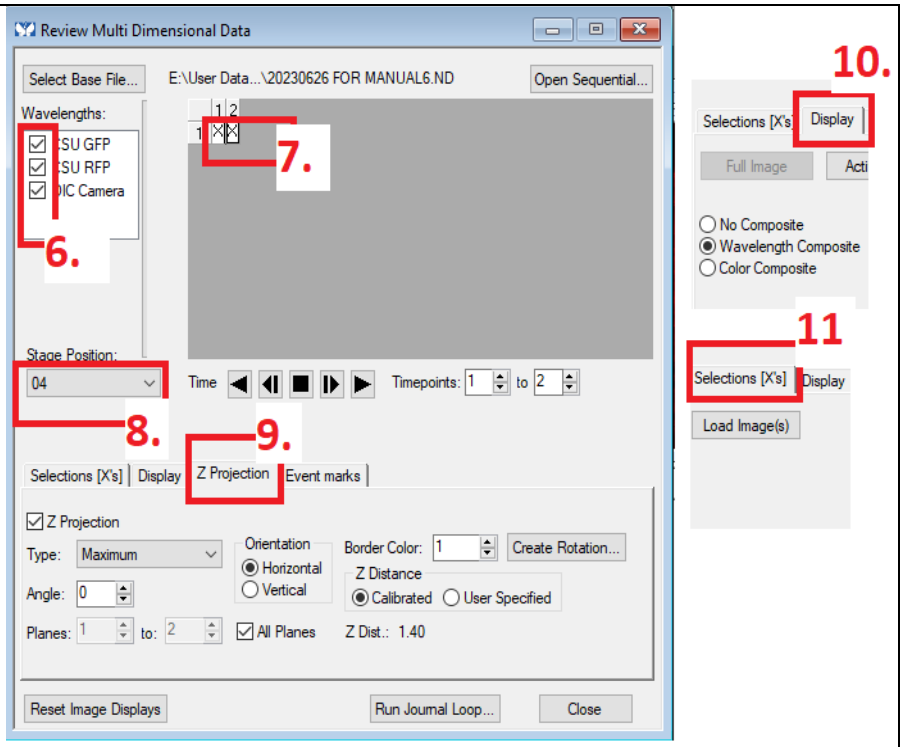
1. Start “Review MDA data” (1).
2. Follow the sequence shown: 1-5 to open the image to be viewed.



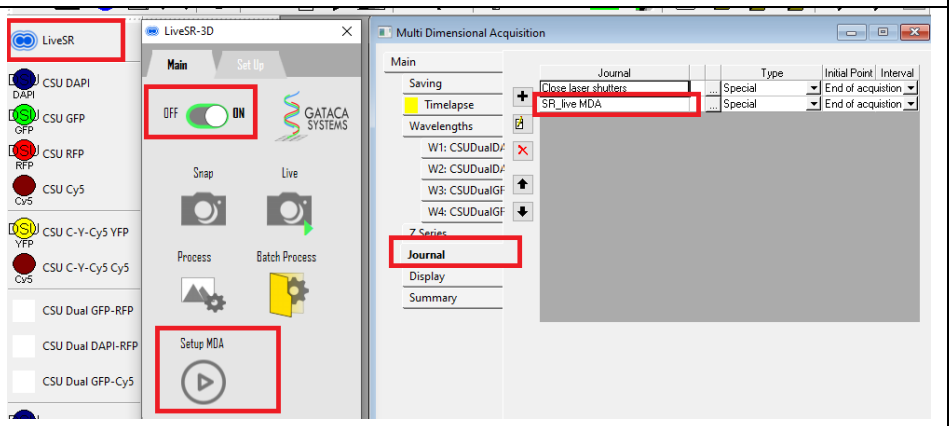

3. Check the boxes under “Wavelengths” to view the image (6).
4. Click the right mouse button at the top left corner to select images to be loaded (selected images are marked with a X) (7).
5. Select the “stage position” you wish (8).
6. If the images are multicolour, both the wavelengths and the In display wavelengths and colour composite should be checked (10).
7. In the “selection [xs]” Press “Load images” to build a stack of images which can then be saved as MetaMorph stack (*.stk) or a multipage tiff (11).
8. You may use “Z projection” to display the

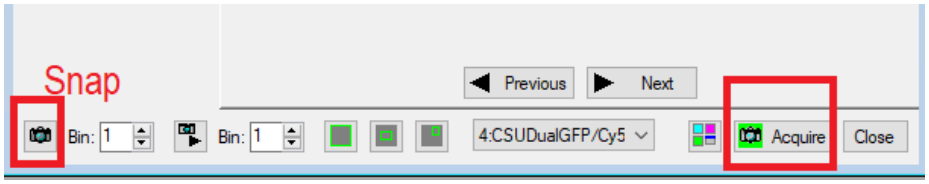
maximum projected images (9).




## G. Acquire super-resolution images (SR) .

1. Capturing a normal CSU image before SR imaging is recommended.
2. Click “LiveSR” to start LiveSR-3D window and toggle “On”
3. “Snap” in liveSR -3D window to have a look at the image quality of the SR image ( SR processed one).
4. Click “Start MDA” to add the Journal of “SR_live MDA” into MDA protocol so that processed SR image will be saved automatically.
5. Snap in MDA until you get image (a software bug).
6. Acquire image. (Refer to section F).



| | |
|---|---|
| **H. Shutdown the instrument and software:**<br>check PPMS for the NUS_CBIS Facility | If there are users coming:<br>I. Remove your sample, clean the lens. Go to 10x lens.<br>II. Exit from MM.<br>III. Transfer data: Confl3 or Onedrive/google driver/CBIS<br>IV. Log out PPMS. Enter logbook.<br>If there are no users coming:<br>I. Refer to above step I-III.<br>II. Shut down workstation. Enter logbook.<br>III. After the monitor is black off, switch off power supply 3, 2. 1. |