

**LaVision Light Sheet Microscope Protocol for DBE-cleared Sample. Part I. Locate ROI, Set up Imaging Parameters and Z-stack.**

**1) System Startup**

- ☞ Please note sign-up policy. You must inform the facility at least 24 hours beforehand if you can't come; otherwise, you will receive a charge for unused time. The facility will allow for extenuating circumstances (cells dying, sick day, etc.) if you inform us in a timely fashion.
- ☞ Follow each step of the startup poster and wait for the microscope software to fully load.
- ☞ Turn on the Camera (red button on top), Turn on the Microscope power switch (the right side of the system). Turn on the laser power supply (box under the stage) and turn on the computer.
  - If nothing is turning on check if the red button on extension cord its on
- ☞ Log in into your account and turn on the ImSpector software.

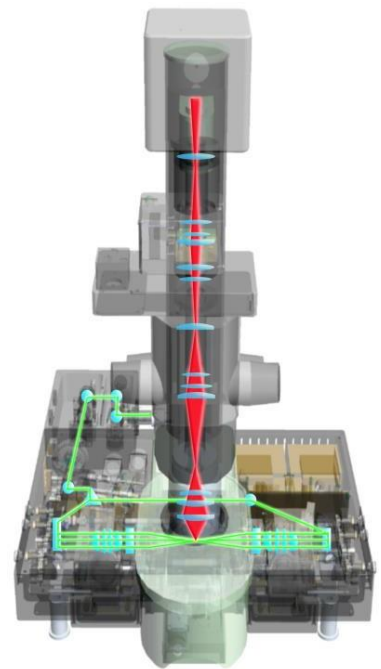
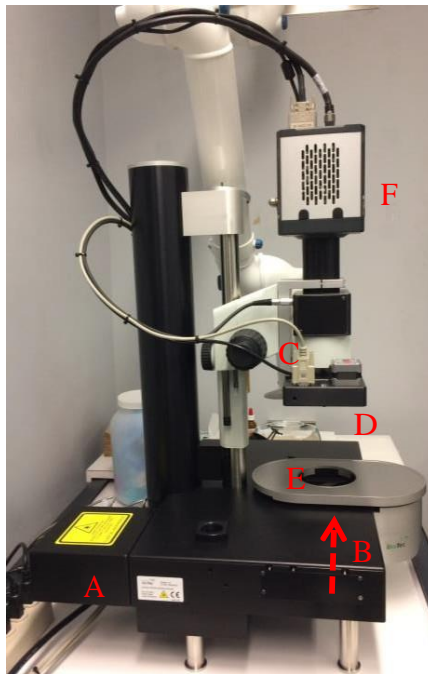
**2) Lens Cleaning**

- ☞ Please **clean the lenses before and after** your session. Use methanol for organic solvent dipping lens. Refer to the lens cleaning poster if you need any help recalling the rules and steps.

**3) Microscope Control**

- ☞ Take note of the microscope light path:

- A. Light source
- B. Light sheet beam path
- C. Focus knob
- D. Lens position
- E. Sample cuvette
- F. Camera



☞ Sample stage control

- Use the dial to find your region of interest
- Use the highlighted button to toggle between control speeds. Press first light from the right on the joystick until is blinking rapidly and you hear “beep beep” sound, to set the XYZ stage movement to coarse mode.



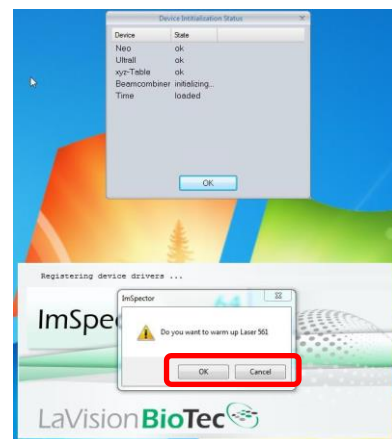
☞ Focus Control

- Course and fine focus control are located on both sides of the microscope.
- Focus on the top of your sample using focus knob. Once in focus, check ROI with different Z-position by dialing the Z-wheel (**not the focus knob**). Check all channels before imaging.

4) Software Control

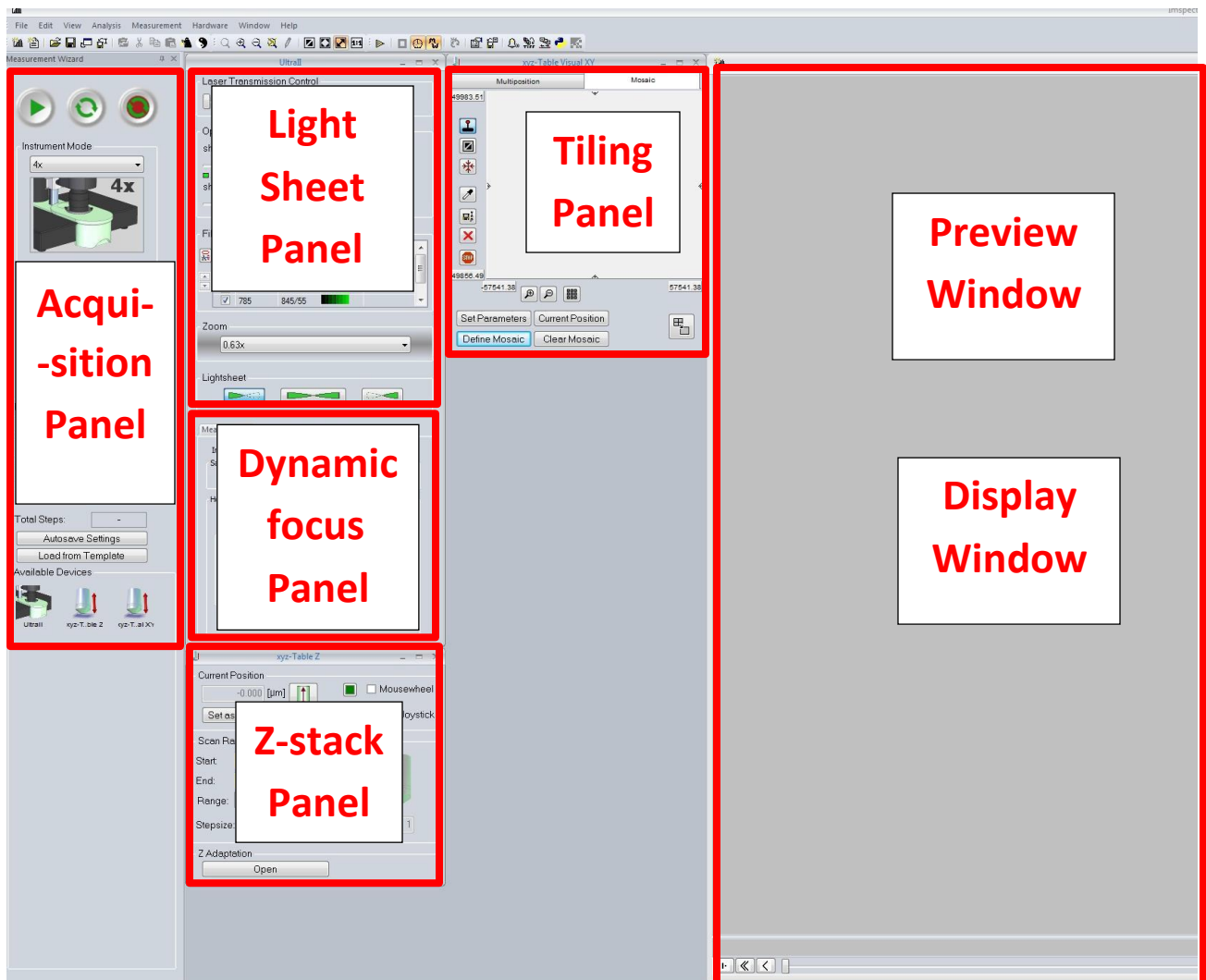
☞ Open the InspectorPro Software

- Click “OK” or “Cancel” to turn on the 561nm laser or not.



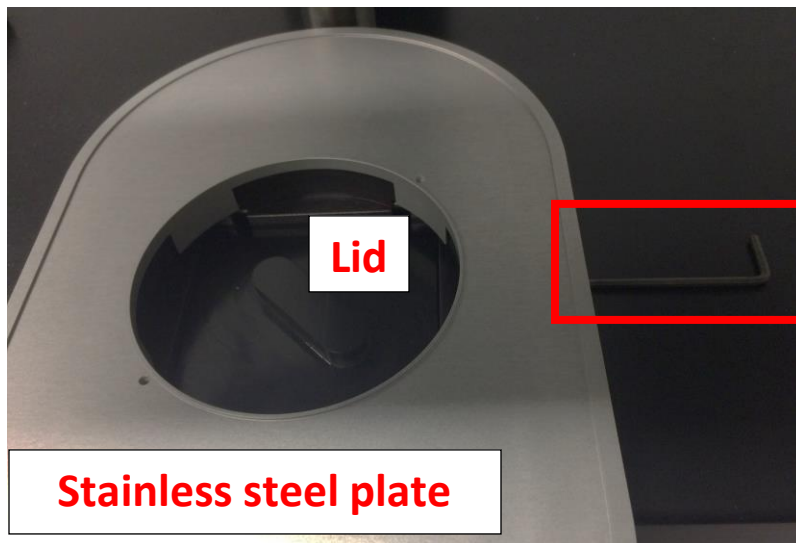
☞ Upon completed startup, please note the 6 divisions of the interface:

- Left: Acquisition control panel
- Middle:
  - Top: Light sheet control panel and tiling setup panel
  - Middle: Horizontal dynamic focus setup panel
  - Bottom: Z-stack setup panel
- Right: image preview and acquired image



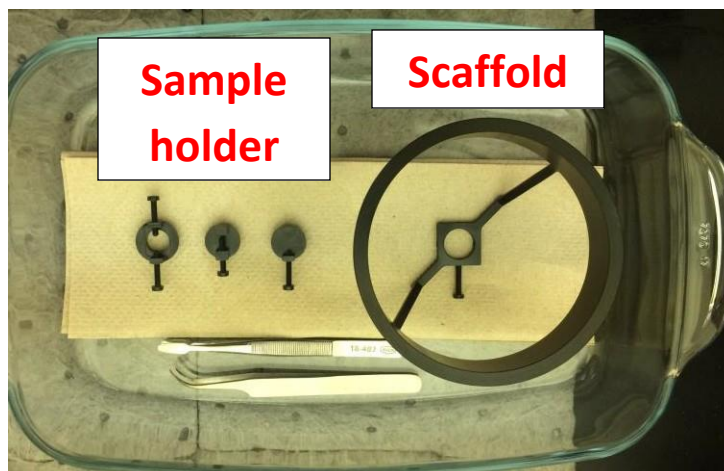
### 5) Mount your sample (DBE protocol)

- ☞ Make sure the hex screw on the right side of silver stage is loosen, use the Alan key
- ☞ Carefully lift off the gray stage plate.
- ☞ Pick up the cuvette lid and rest it on paper towel in the pyrex tray. **Use caution to prevent DBE spill or dripping. Note: Only in one direction does the lid go into the cuvette.**
- ☞ Put back the gray stage plate and tighten the hex screw on the right side.



Under the hood:

- ☞ Put on fresh gloves
- ☞ Sample holder and scaffold sit on clean paper towel in the Pyrex tray.



- ☞ Transfer DBE-cleared sample onto sample holder using tweezers. Finger-tighten the screw to restrain sample.
- ☞ Put the sample holder into the scaffold and transfer the Pyrex tray next to the microscope.

One Gustave Levy Place | Annenberg 18-250 | New York, New York 10029

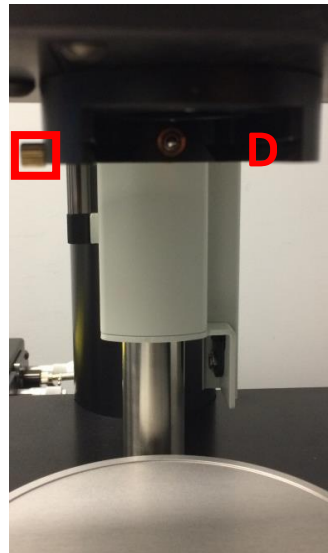
[microscopy.core@msm.edu](mailto:microscopy.core@msm.edu) 212-241-0400

- ☞ Place the sample holder in the cuvette such that the sample is oriented perpendicular to the light path.
- ☞ **Use caution to prevent DBE spill or dripping.**
- ☞ Remove gloves and trash them in the DBE waste container. **Proceed without gloves.**



#### 6) Ask staff to mount the lens

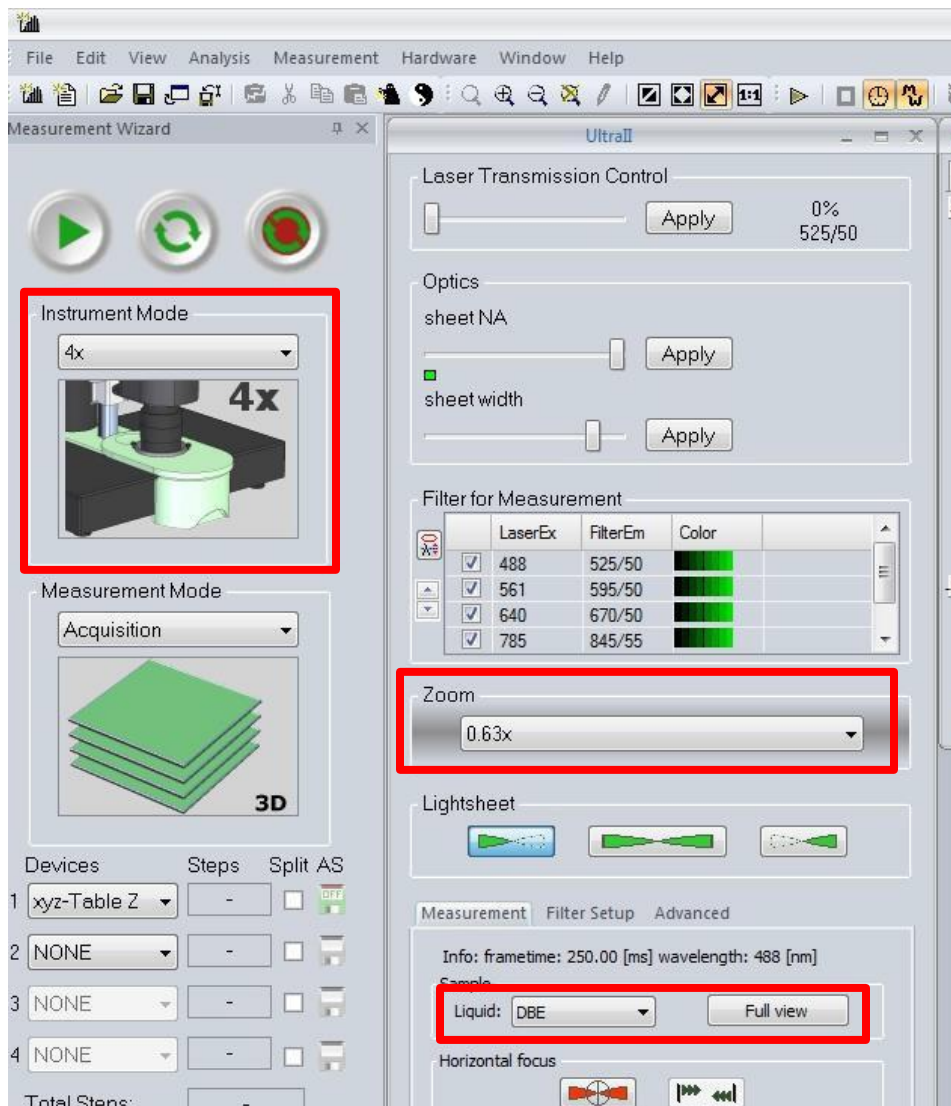
- ☞ Contact staff for the use of specific lens. (1.3x lens is dry while 4x and 12x are dipping lenses.)
- ☞ Lens will be removed from the plastic housing.
- ☞ Adapter will be attached to the base of the lens. Pay attention to the “front side”. 12x lens has a different adapter



- ☞ Adapter and lens slide into lens position (D) and are locked with a screw. **Mounting and dismounting lenses will be handled by staff.**
- ☞ **For the 4x lens remember to put the correction collar on the right RI for you solution in the cuvette !**

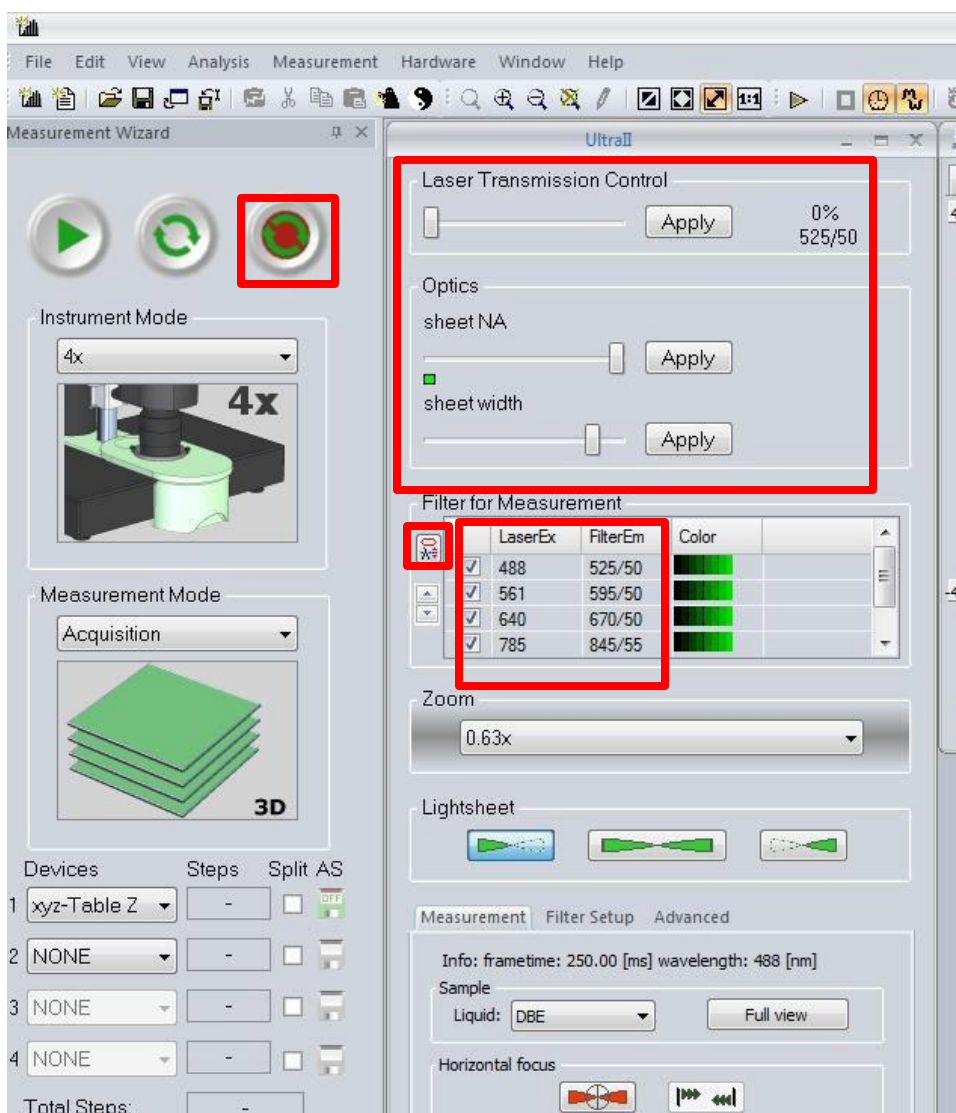
## 7) Find ROI

- ☞ Set the correct lens (e.g. 1.3 x), zoom (e.g. 1x), and liquid (e.g. DBE) and click **“Full View”** in InspectorPro.



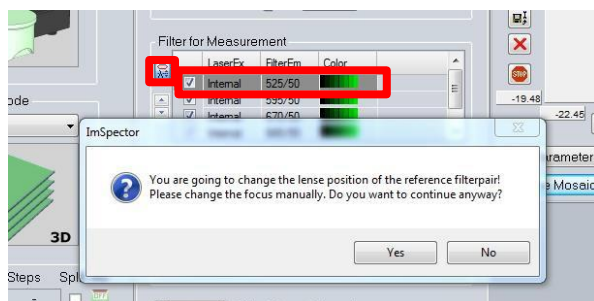
- ☞ Choose the laser wavelength for imaging by checking the box.
- ☞ Activate the laser line one at a time by click/highlighting specific wavelength. Set the power, light sheet width and NA in the Light sheet control panel. E.g. 6% transmission, sheet NA = 0.2, 50% sheet width.

- ☞ Click “Live”.
- ☞ Look into the reservoir: you should see the light sheet hitting the sample holder and your sample. If it is not, maneuver the sample in XYZ until you can see the light sheet going through your sample.
- ☞ Draw ROI anywhere on the Live window and hit the min/max display icon (sun and moon with stars icon on the top right side of the live window) or Toggle fast between F9 and F10 key to reset display brightness.
- ☞ Start lowering the objective, constantly monitoring the image and adjusting the display to avoid hitting your sample ( important for 4x lens).
- ☞ Center the sample in the field of view by using XY joystick.
- ☞ Focus on the top surface of your sample using focus knob. Once in focus, check ROI with different Z-position by dialing the Z-wheel (**not the focus knob**).
- ☞ Check all channels before imaging. Set **chromatic offset** if needed.

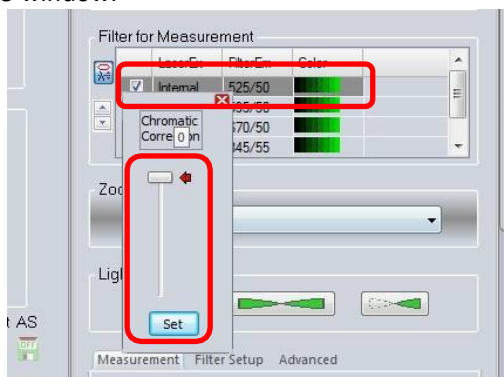


## 8) Chromatic correction

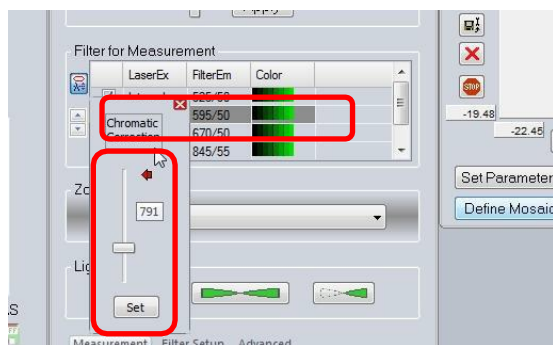
- ☞ For higher magnification lens (4x and up), use “**chromatic correction**” function to correct difference in focus due to different emission wavelengths.
- ☞ 488 laser is the default reference channel. Activate the 488 laser and adjust focus in liveview.
- ☞ Activate the “**chromatic correction**” panel by clicking the top left button in the laser controlpanel.



- ☞ Click “Yes” in the popup dialogue window.



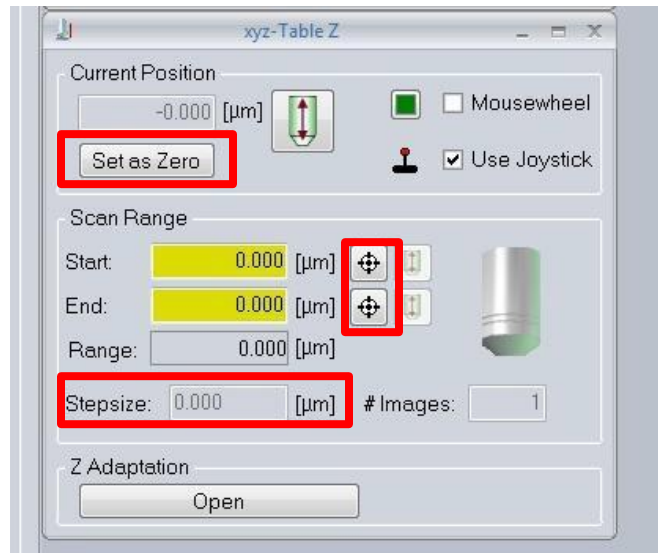
- ☞ Use the scroll bar to fine tune the default focus in the 488 reference channel. This functional also gives extra room for Z-focus when reaching the lower limit of the focus knob.
- ☞ Click “Set” and then close the scroll bar.
- ☞ Once an optimal Z is determined for reference channel (488 as the default), proceed to determine the chromatic offset for other channels. Highlight other laser channels and activate the “**chromatic correction**” panel. Use the scroll bar to fine tune the focus in other channels. Click “Set” and close the scroll bar window.





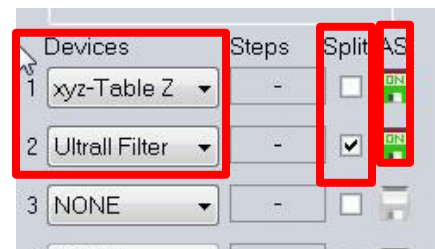
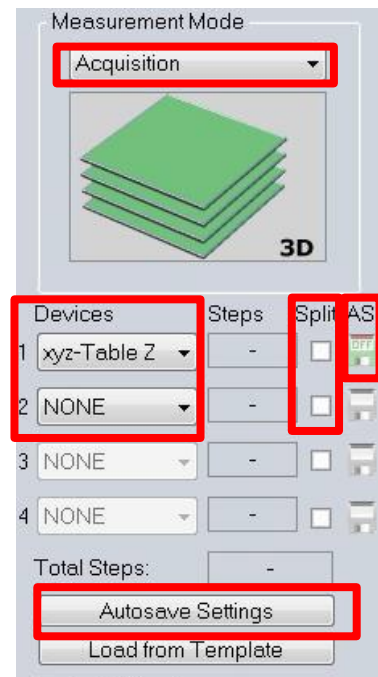
## 9) Set up Z-stack

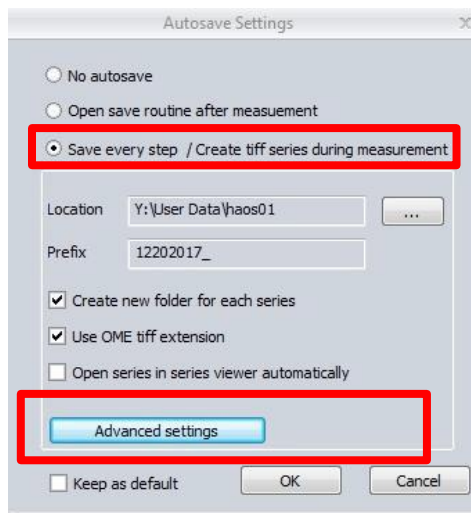
- ☞ Once in focus, use sample stage dial (Z) to check ROI with different Z-position.
- ☞ Set zero position.
- ☞ Rotate the top knob (Z) of joystick clockwise, move to the bottom of your sample. Set as Start.
- ☞ Then move counterclockwise to the top of your sample end set as End.
- ☞ Set z-step size ( to obtain the best possible z resolution of the system set your step size corresponds to Nyquist). Or larger step size for quick 3D overview of large sample (E.g. lowlight sheet NA and large width).
- ☞ Click “STOP”, to end live view.



## 10) Acquisition and Autosave option.

- ☞ Select “Multicolor 3D” from Measurement Mode dropdown.
- ☞ Under “Devices”, select 1) xyz-Table Z, 2) Ultrafilter.
- ☞ Click autosave button (click the first icon under AS).
- ☞ Make sure the “Split” is checked only for Device 2 “Ultrafilter”
- ☞ Click “Autosave Settings”.
- ☞ In dialogue window, enter folder path to save data. Enter name in “Prefix”. Keep other options as shown.
- ☞ Click “Advanced settings”.
- ☞ In dialogue window, check “Add meta data only to first image of series”, uncheck “Use fast autosave with streaming”.
- ☞ Click “OK”, then click “OK”.
- ☞ Click “Start” to initiate multicolor 3D imaging.





## 11) System Shutdown

- ☞ Check the result folder for acquired images once finished. The total number of images should match the total steps calculated based on your imaging sequence.
- ☞ Return the sample position to the center of the cuvette using the stage controller.
- ☞ Raise the lens



- ☞ **Only in the case of DBE dipping lens**, wear glove and use methanol/lens paper to clean the lens. DO NOT TOUCH other part of lens with DBE-contaminated glove during cleaning. Do not remove the lens
- ☞ Remove the scaffold from DBE cuvette. **Use caution to prevent DBE spill or dripping.**
- \*Inform staff if the presence of the lens prevents sample removal. Only staff can remove or replace lenses.**
- ☞ Reverse the sequence to dismount sample from the sample holder.

- ☞ Clean the sample holder and scaffold bottom with a few drops of methanol. Wipe clean using Kimwipes. Dispose the waste and glove in DBE waste container in the fume hood.  
Notify staff when the containers are full.
- ☞ Proceed without gloves. Unlock and remove the plate to allow access to the DBE cuvette. Put the lid back on the cuvette. **Note: Only in one direction does the lid fit into the cuvette.**
- ☞ Put back the plate, you do not have to lock with Allen key