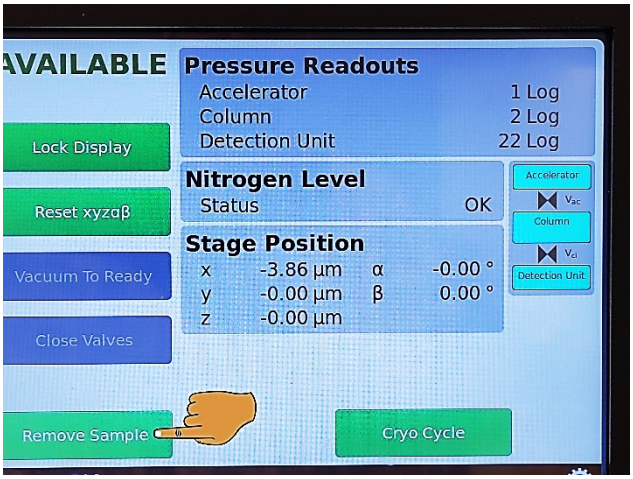
Protocol for microED on Talos F200C (in YH1290) May 16, 2022

Step 1. Book time on the Google Calendar. Open google calendar using the gmail account that you gave Duilio during onboarding. Click the box for TALOS F200C BOOKING CHEMISTRY. Draw a box to enclose the the target time you want to book. Write your name on the box.

Step 2. Cool down the microscope. If your booking is the first of the day, then come in 1.5 hours ahead to fill the nitrogen dewar that cools the microscope. Put on gloves. Fill out the **checklist** on the clipboard. Check water flow. Fill transfer dewar with nitrogen using the giant nitrogen tank by the entrance door. Pour nitrogen from the transfer dewar to the thermos. Pour nitrogen from the thermos into the dewar with the cold fingers on the EM. Wait 1 hour.

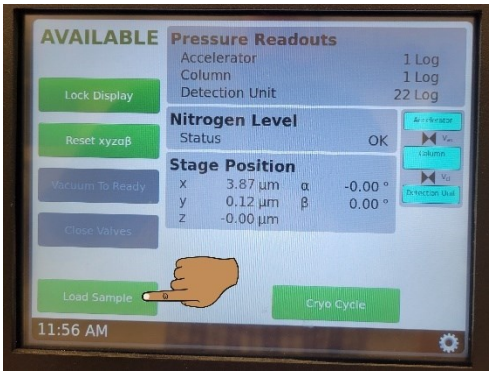
Step 3. Bring supplies. Bring your sample and forceps, notebook, and personal code to YH1290.

Step 4. Top off nitrogen. Put on gloves. Pour more nitrogen on the cold fingers and top off the dewar.



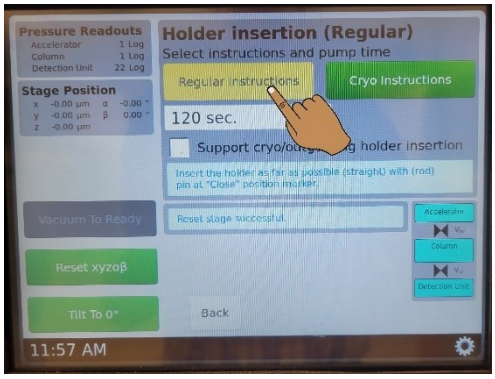
Step 5. Remove the sample holder from the microscope. If the sample holder is inserted in the microscope, you must remove it. Put the sample holder sheath on the cart next to the microscope port. Push the "remove sample" button on the microscope display. Follow directions on display. Pull holder out until resistance is felt. Rotate clockwise until resistance is felt. Pull all the way out. Put sample holder in the sheath that is sitting on the cart. With two hands, carry the holder to the bench.

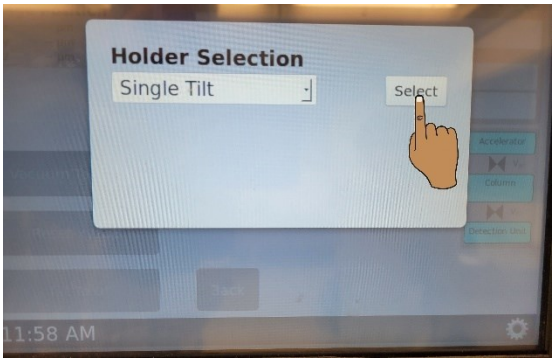
Step 6. **Lay the grid in the sample holder**. Place a Kimwipe on the bench surface, directly under the grid position. The Kimwipe is there to catch the grid if it slips out of the forceps during transfer. Remove the pin from its storage position in the sheath and use it to open the hinge on the sample holder. The pin inserts horizontally into a hole positioned sideways in the specimen clamp. Return the pin to its storage position in the sheath. Turn on the desk lamp to help you see the grid. Open your grid storage box the minimum amount to access your grid. Use forceps to remove the grid and place it in the sample holder. Tap the sample holder to seat the grid in the holder. Use the pin to close the clamp. Return the pin to its storage position in the sheath. Put the cap on the sheath over the sample.



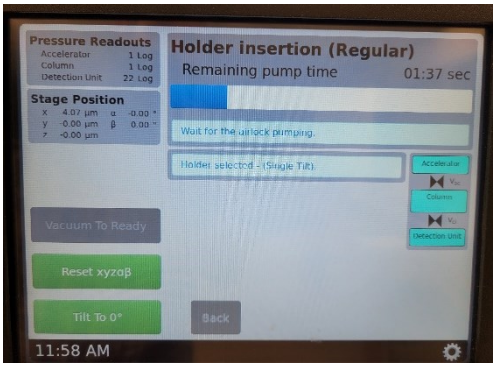
Step 7. Insert the sample holder in the microscope. Use both hands to carry the sheathed sample holder to the cart next to the microscope. Push the "Load Sample" button on the display next to the microscope.

Select "Regular Instructions".

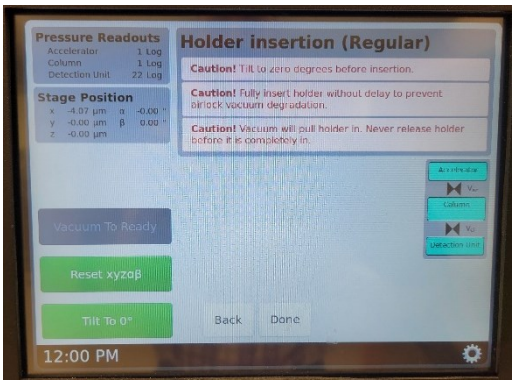




Select "Single Tilt" holder and follow the instructions. "Hold with pin at 5 o'clock (Close) position. Insert the holder straight in as far as possible.



Wait 2 minutes as vacuum pumps.

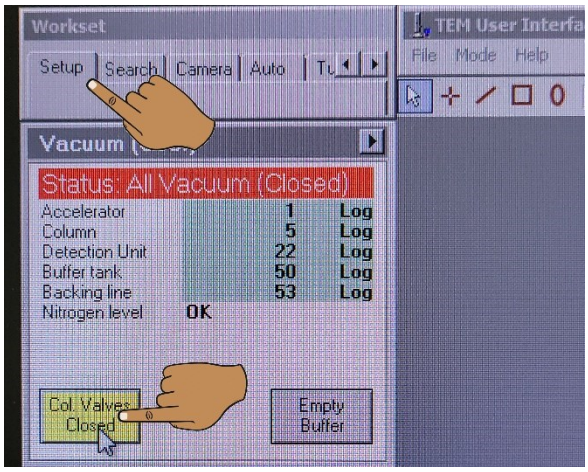


And then rotate sample to 12 o'clock. Fully insert holder.

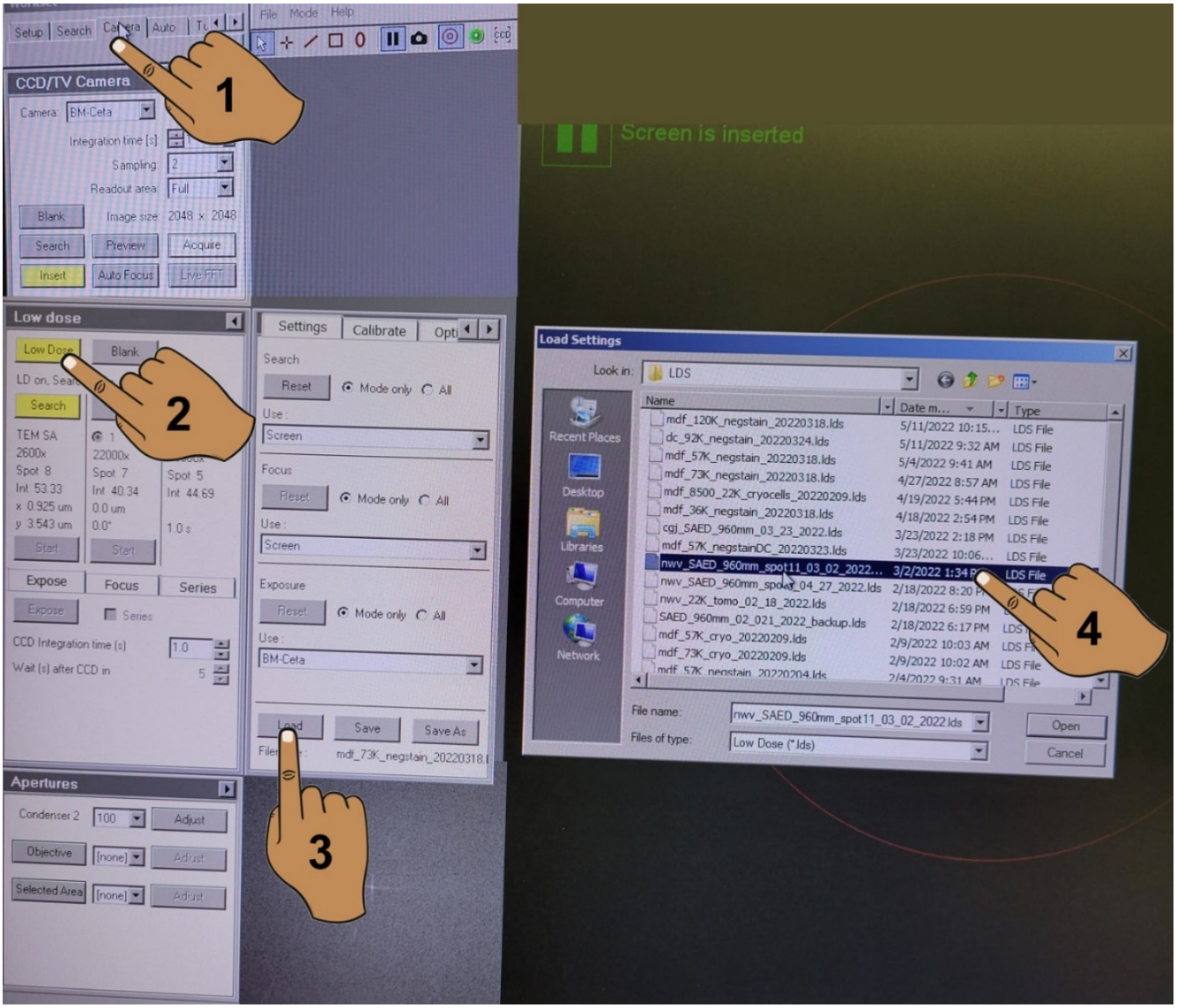
Wait until "column" vacuum decreases to 3. It takes about 6 minutes. Write down all the column pressures in the **checklist**.

Step 7. Start logging your time on the chemistry department computer (south wall) for recharge. Open mobaxterm. Answer questions. Include personal code.

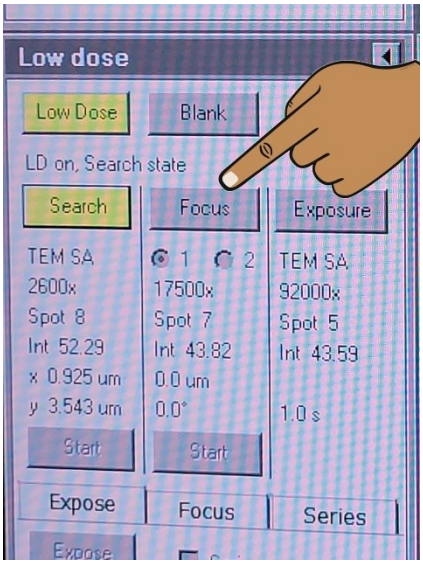
Step 8. Open the Column Valves. Sit at the desk with the computer terminal. Click the yellow button "**Col. Valves Closed**". If you can't find it, go to the "**Setup**" tab and "**Vacuum**" window. It should be in upper left corner of the screen. The button should turn gray to indicate the valves are no longer closed.



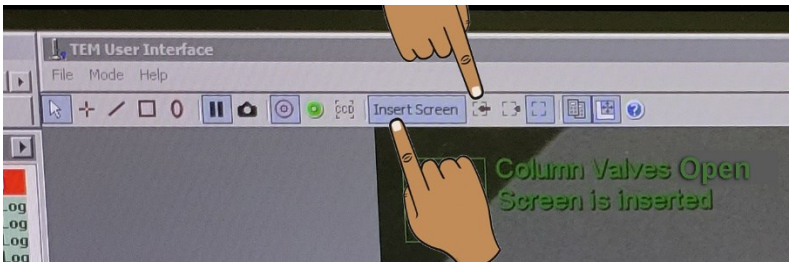
Step 9. Select microscope settings for MicroED. Select "**Camera**" tab. Press the button "**Low Dose**" to make it yellow (active). Press the "**Load**" button select **nwv_SAED_960mm_spot11_03_02_2022.lds** from the options in the popup window.



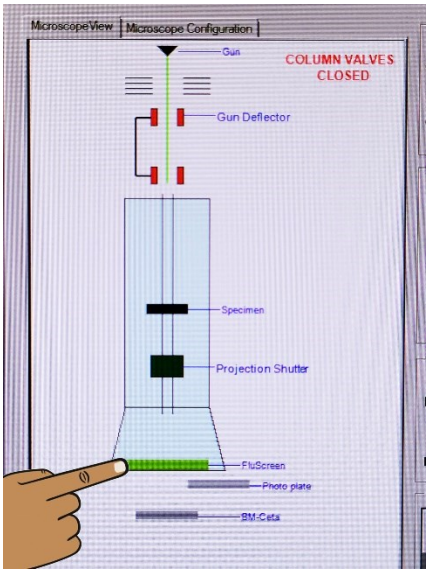
Step 10. Survey the grid for crystals. There are 3 modes: Search, Focus, and Exposure. "Search" and "Focus" modes are for imaging. "Exposure" mode is for taking diffraction images. Go to "Search" mode to quickly survey the grid. Use joy stick to find crystals. Then go to "**Focus**" mode. Beam will blank for a second as settings change over.

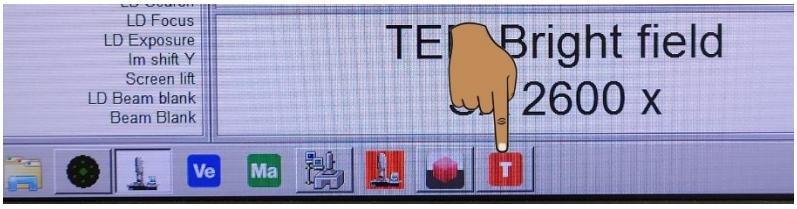


Step 11. Check that the beam is centered behind beamstop. Insert beamstop by pressing the button that looks like a square with a red bar in the middle. Confirm that the fluorescence screen is inserted. The **"Insert Screen**" button should be colored blue. Green lettering will say "Screen is Inserted" on the image display, as shown below.

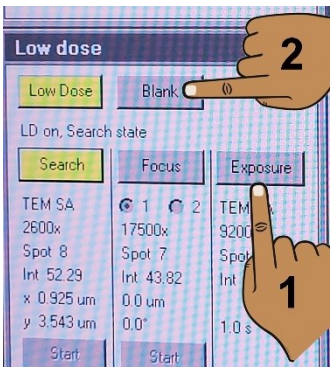


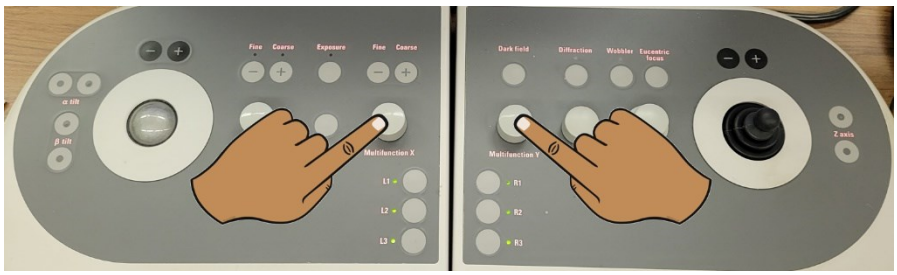
It is imperative to have the fluorescence screen inserted or you risk damaging the CCD detector. If you are unsure about the status of the screen or valves, press the **"T" button** at the bottom of the screen. It will display a schematic that shows the status of the

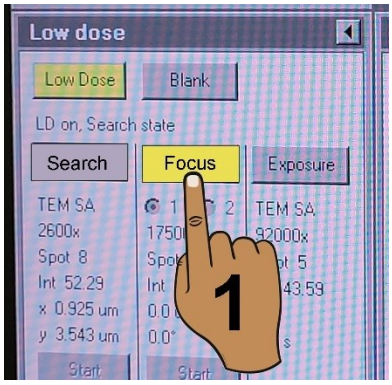




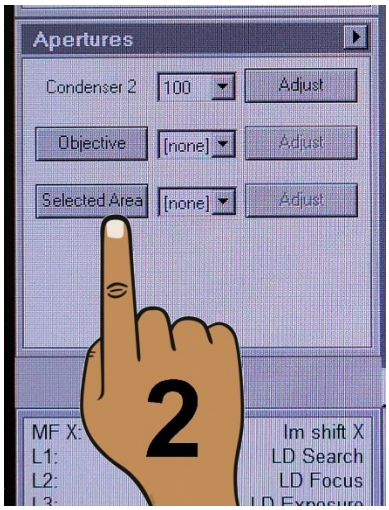
screen and valves and beam (see right panel). (1) Go to "**exposure**" mode to see the beam position. You might have to unblank the beam by (2) pressing the button "**Blank**". The beam will appear as a green dot on the fluorescence screen. It should be hiding behind beam stop. If the beam is not behind the beam stop, then call Duilio. He will move the beam by adjusting the multifunction x and y dials on the left and right control pads.

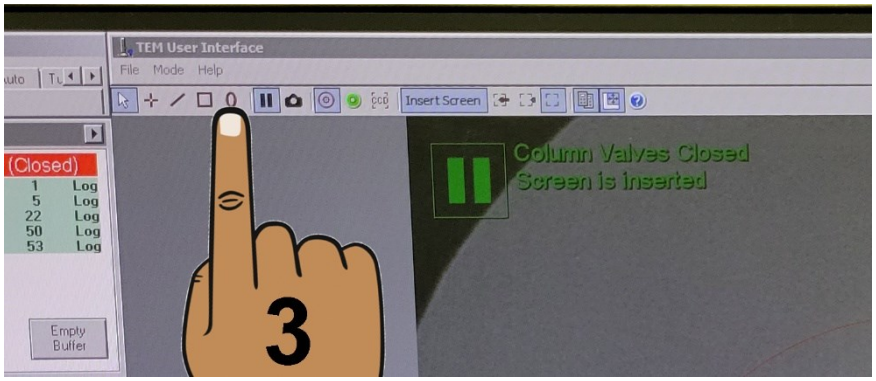




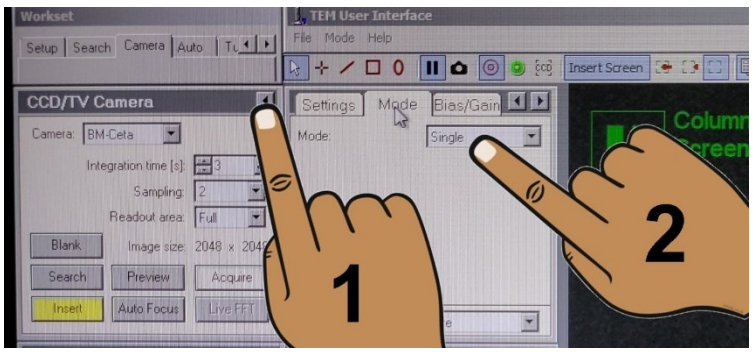


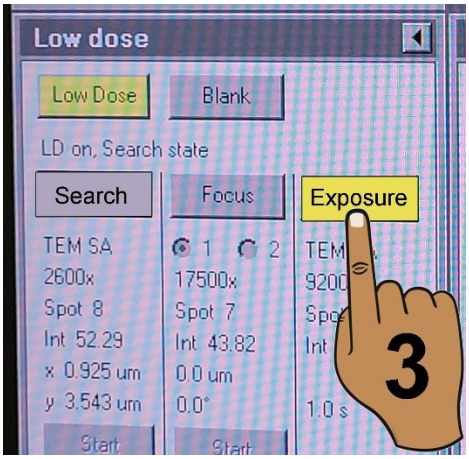
Step 12. Center the crystal in the aperture. (1) Switch to "Focus" mode by pressing "**Focus**" button. (2) Insert the aperture by pressing the button "**Selected Area**", visible in "Camera" tab. Most of the image will turn black, but an illuminated circle will remain. (3) Use the **circle drawing tool** at the top of the screen to draw an outline circle that encompasses this illuminated area. Remove the aperture. Use joystick to translate the crystal into the red circle.



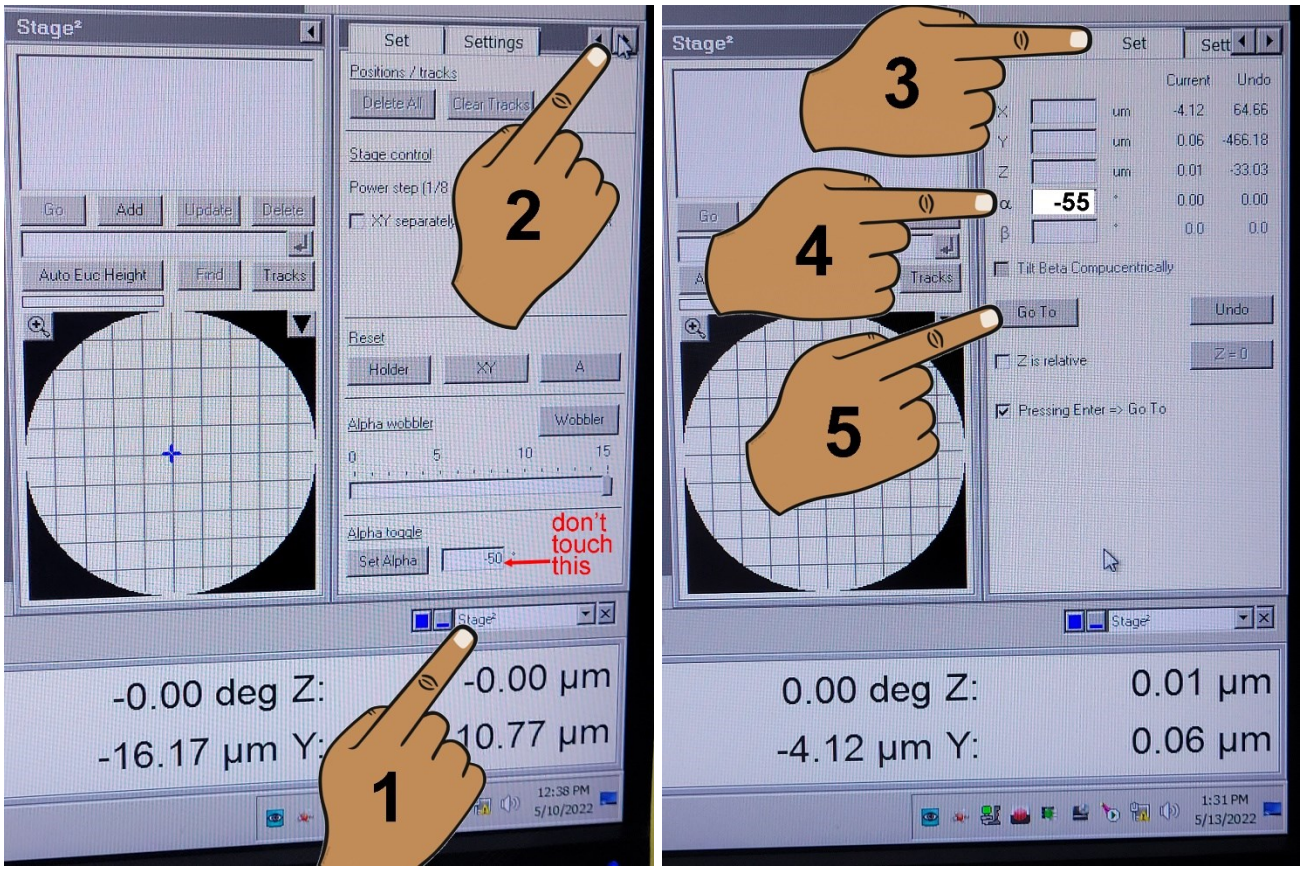


Step 13. Take a diffraction snapshot to asses diffraction quality. In the CCD/TV Camera window check that the integration time is 3 seconds, the Sampling is "2", and the "Readout area is "Full". (1) Click the arrow in the CCD window to get the camera control window to pop out. Select "Mode" tab in the camera control window, and (2) select Mode "**Single**" from menu. Check that the beamstop is inserted and that the screen is retracted. (3) Press "**Exposure**" mode. Press the "**Acquire**" button in the CCD/TV Camera window. Diffraction image will appear. You can adjust the contrast by dragging the "gamma" curve in the popup graph.

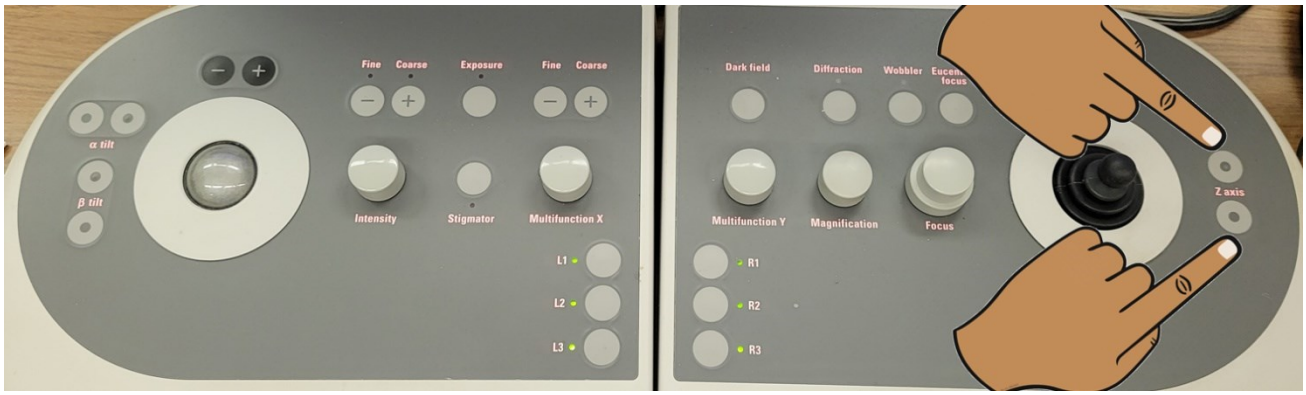




Step 14. Before collecting a diffraction rotation series, find the eucentric height (Z) of the crystal. Return to "**Focus**" mode so you can see the crystal. (1) Open the Stage^2 windows by clicking on "**None**" in the small white rectangular box at the lower right corner of the monitor and selecting "**Stage^2**" from the menu. The Tracks display and wobble control panel will appear in lower right corner of the monitor. (2) Click the arrow box to reveal (3) the "**Set**" tab. (4) Type "**-55**" in the "α" window. (5) Press "Go To" to rotate stage to -55°.



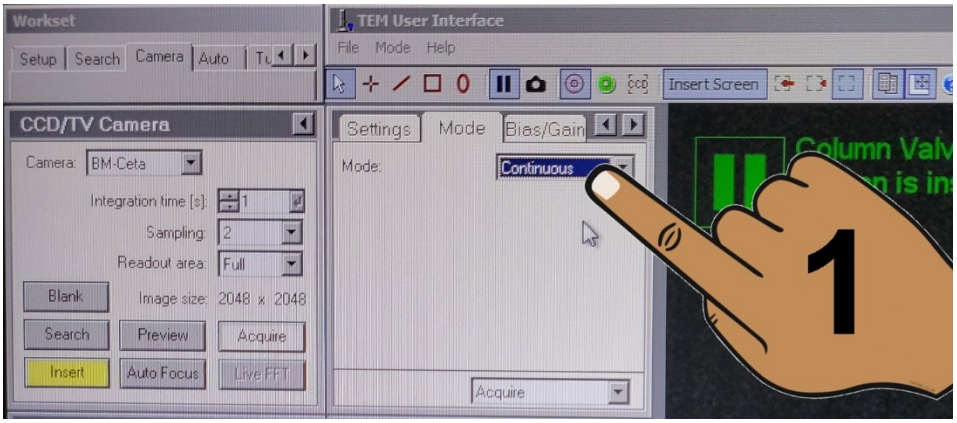
Bring the crystal to the center of the aperture by adjusting the "+**Z**" and/or "**-Z**" using the buttons on the right control pad.

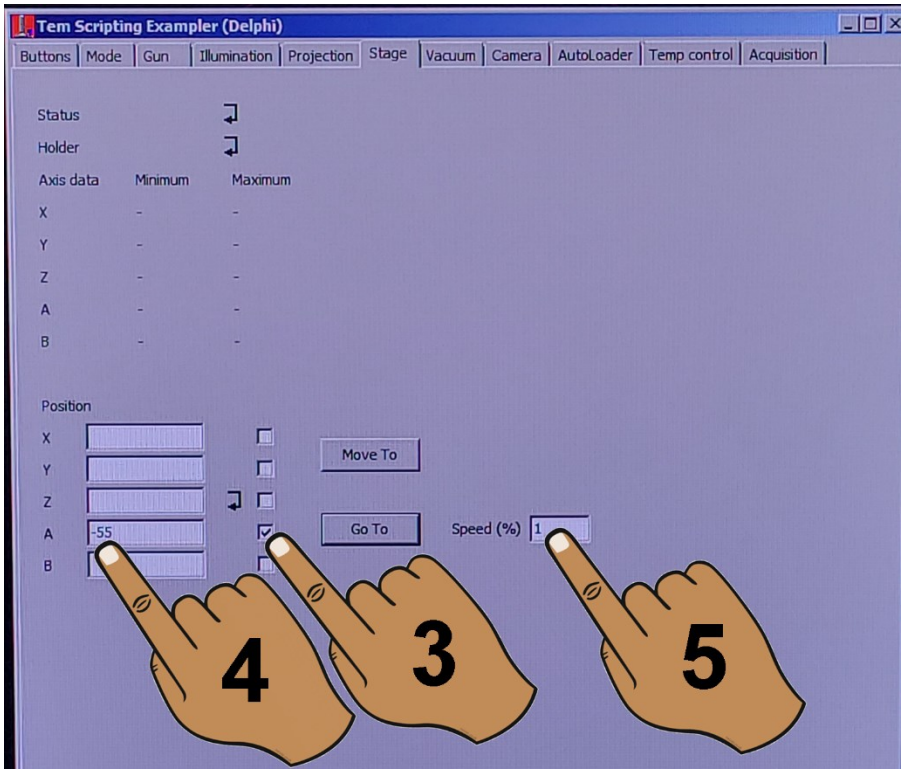


Return the stage to 0°. Check if crystal is centered in the aperture. If not, adjust the Z-axis again. Tilt the stage to +55°. Check that the crystal is centered in the aperture. If not, adjust the Z-axis again. Keep the stage at +55° as the starting point for collecting a diffraction movie. It's convenient to start from positive

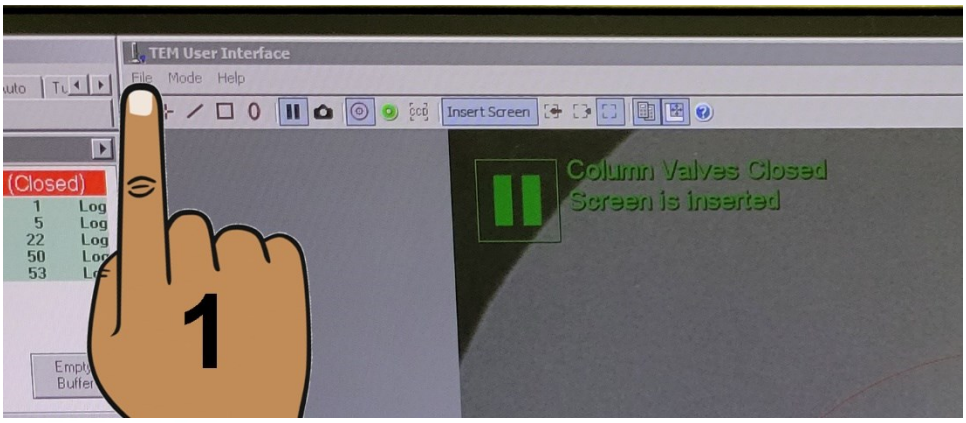
rotation angles because the template XDS scripts were written for this direction of rotation. Take a snapshot at +55° to check the quality of diffraction at this high tilt angle. To do this, press "**Exposure**" button.

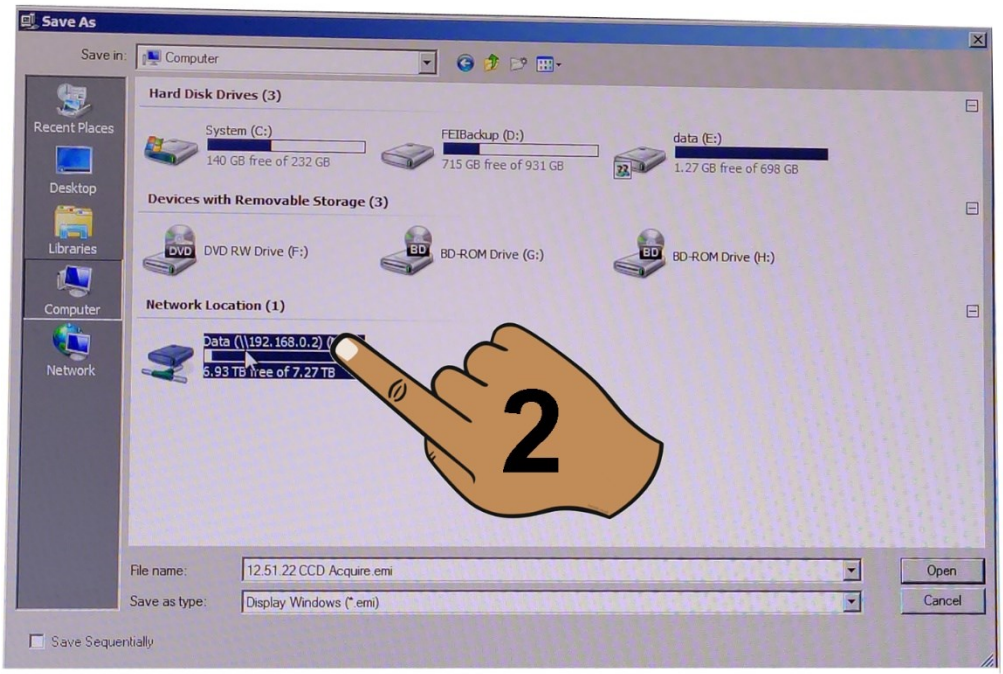
Step 15. Acquire a movie. Verify that the EM is set to "**Exposure**" mode, and **blank** the beam to minimize radiation damage to your crystal while you set up the stage rotation parameters. (1) Set the camera mode from "**Single**" to "**Continuous**" as shown below. (2) Open the TEM Scripting Exampler by clicking the icon of an electron microscope with red background. (3) **Check the box for "A"**, indicating alpha rotation. (4) Type "**-55**" as the start position. (5) Type "**1%**" as the speed. This setting corresponds to rotation of 0.3°/second. **Unblank** beam. Press "**Acquire**" in the CCD/TV Camera window, and when first diffraction image appears press the "**Go To**" button in the Tem Scripting Exampler to start rotation of the crystal. Note: rotation of the stage cannot be stopped once you press "Go To". When rotation reaches **+55°**, press "Acquire" again to stop acquiring images. Set stage to 0° in the "Set" window and "Go To".





Step 16. Save movie file. (1) Click on “File” menu, select “Save As”. (2) In the popup window, Select “Data” drive. Select “Eisenberg”. Select “Mikey”. Type an informative name for the saved movie file. For example, AVAAGA_rt_dropcast_960mm_P55toN55_mov01.emi. A file with a “.ser” extension will automatically be saved in addition to the “.emi” file. The .ser file can be converted to images using /xray/ccduser/ELECTRONDIFFRACTION/UCLA_TALOS/SOFTWARE/ser2smv. To process data, use XDS template files in /xray/ccduser/ ELECTRONDIFFRACTION/ UCLA_F200C_TALOS/ RODRIGUEZ/ DIFFRACTION_ TEMPLATE_FILES/. For data processing, note that 960 mm microscope setting corresponds to 905 mm crystal-to-film distance. If you have acquired an image of the crystal itself, you can save it by right clicking on the image and select “Export Data”. Save as a TIFF file in the same directory as the movie.





Step 16. Transfer images to Sayre/Escher. Move to the Chemistry Department computer (South wall) and start MobaXterm software. Login to Sayre or Escher. Transfer data files to location such as /xray/ccduser/ELECTRONDIFFRACTION/UCLA_F200C_TALOS/PI_name/My_name/Date/movies.

Step 17. Search for more crystals. Return to step 10, above.

Step 18. Remove grid. See step 5.

Step 19. Log out of Chemistry computer to stop time charges.

Step 20. Start Cryo-cycle if it is after 5PM and no one is scheduled for the rest of the day. Remove dewar from Talos and press the “Cryo-Cycle” button on the Talos display. Don’t delay to press this button. Recycle the nitrogen that may be remining in the Talos dewar by pouring it into the transfer dewar. Put the Talos dewar upside down in the storage box on the table next to the microscope.