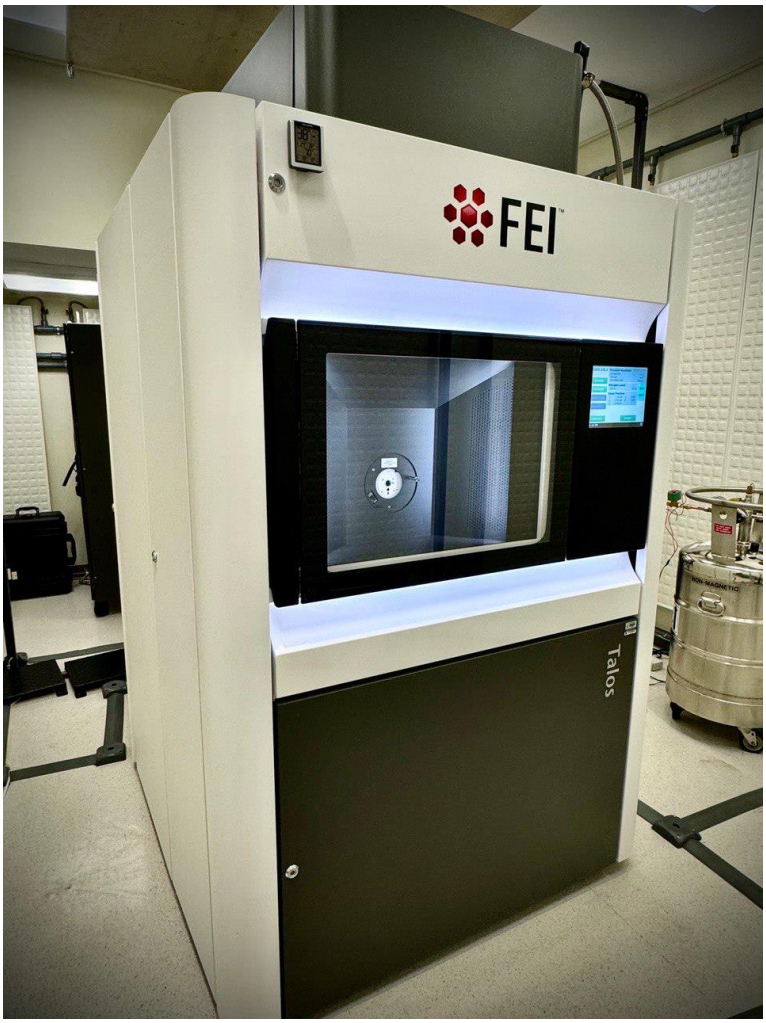
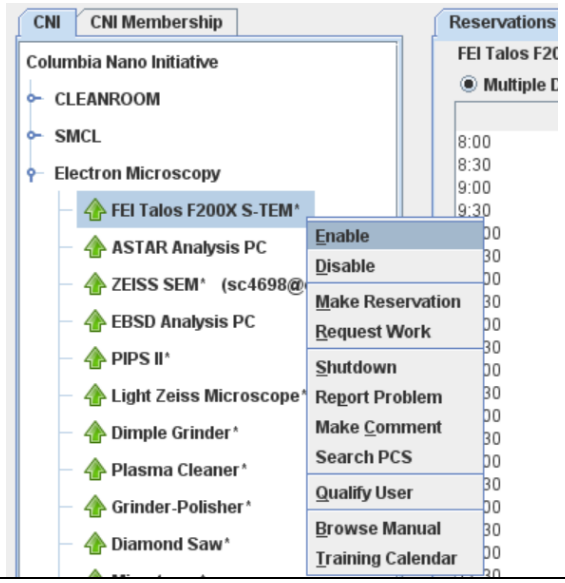
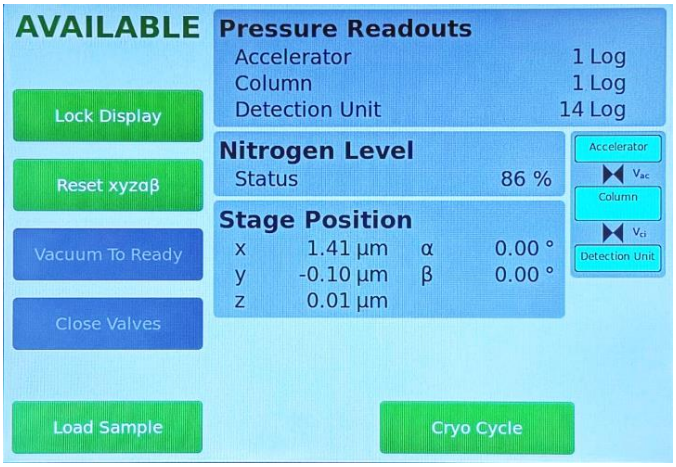
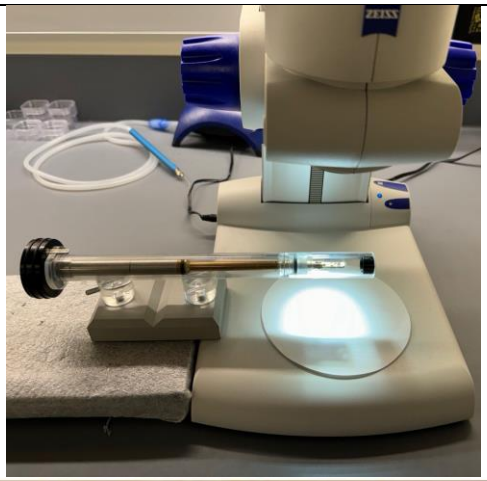
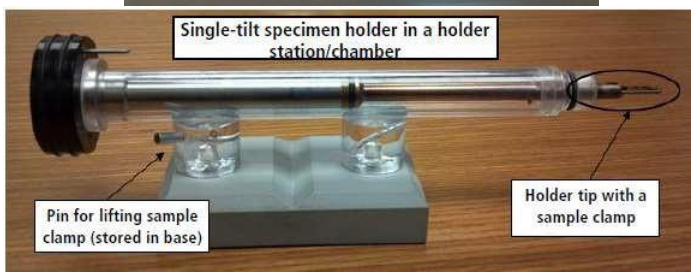
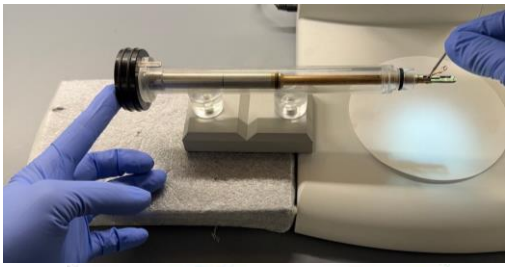
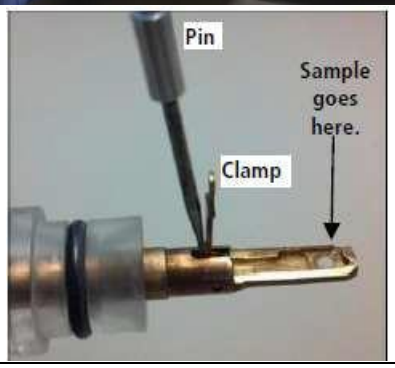
COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK
COLUMBIA NANO INITIATIVE / CENTER FOR INTEGRATED SCIENCE AND ENGINEERING

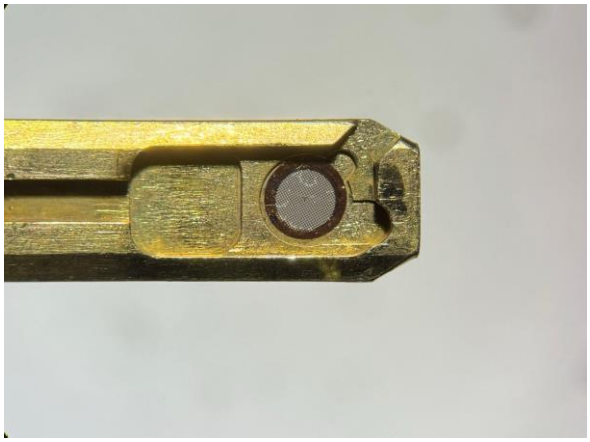
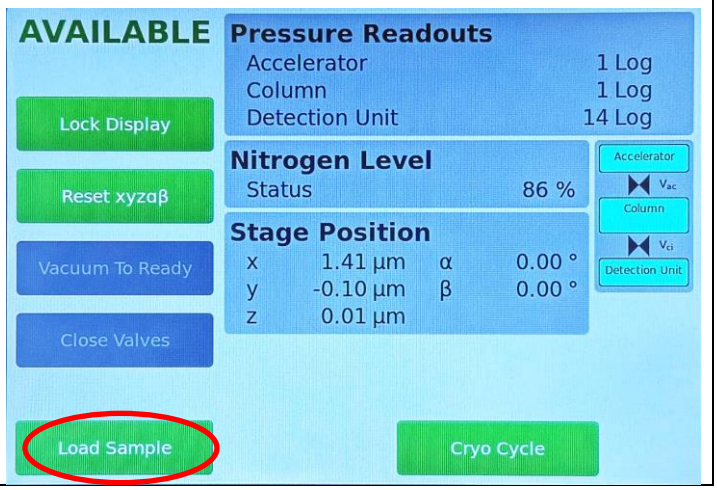
Talos F200X
Standard Operating Procedure



These instructions are intended for reference only, and will *not* replace the thorough training required for proper system operation. Contact electron microscopy staff with questions or to report a problem.

1	Enable the tool in **BADGER**	
2	**Vacuum:** Look at the pressure values for the system. The **Column** reading should be below 20, and the system must be **Available** before starting. Nitrogen level should be above 10%. Otherwise contact EM staff.	

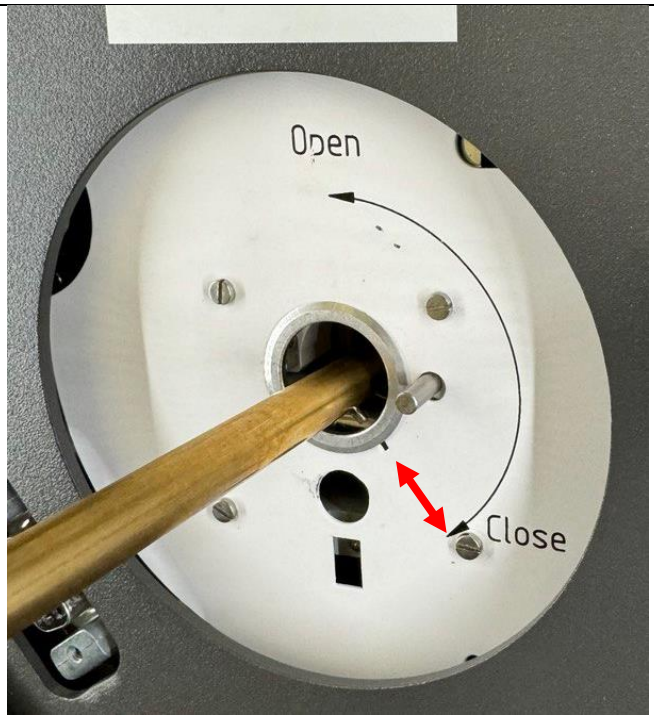
3	**Loading the TEM grid to the holder:** Wear gloves! Turn on the stereoscope light by pressing the blue M button on the right side (pressing it 3 times, turn it off). Place the single tilt holder (located on the second drawer of desiccator) on the table. Remove the plastic cap. Use the pin to lift the sample clamp. Put your left-hand finger on the back to prevent the holder from moving. Place the sample in the middle of the ring. Shown below:	   

	Cont… The TEM grid should lay flat.	
4	Video on holder loading: youtu.be/qDFljfuem3I **Load the Holder:** On the microscope touch screen press “Load Sample”. Wait a few seconds till it stabilizes.	

5 **Insert holder:**

Carefully insert the holder, with the holder pin aligned at the 5 o'clock position, as far as it will go into the CompuStage.

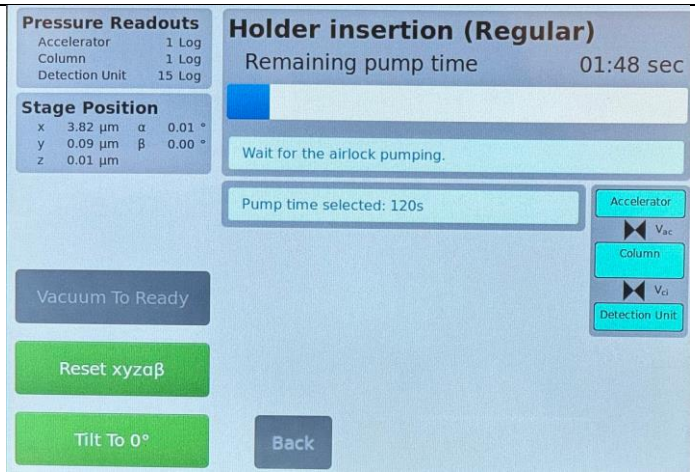
Be careful not to scrape the tip.

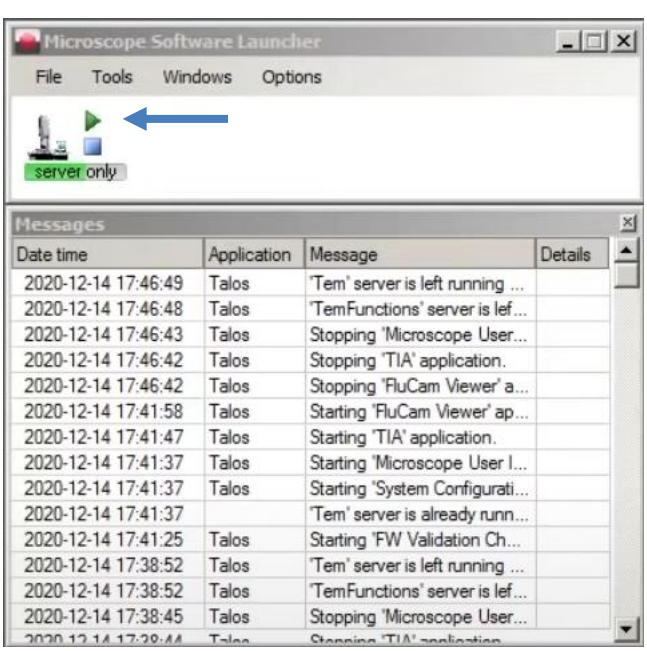


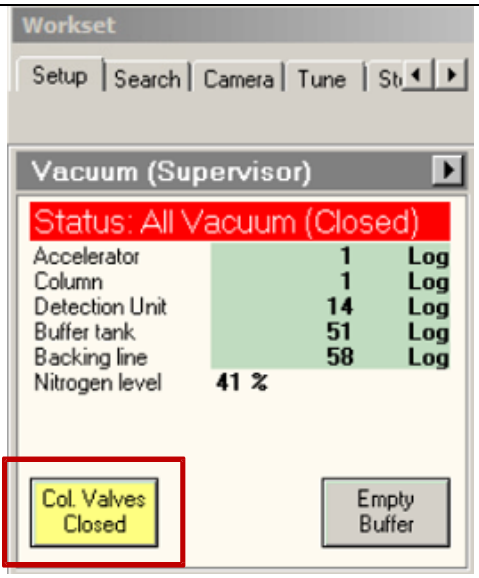
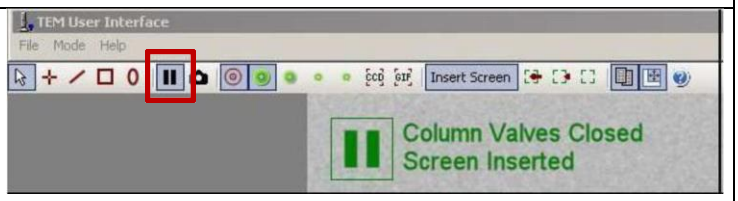
You will feel a slight pressure when the black O-ring hits the mating seal. Push firmly but carefully until the holder goes in about 1 cm further and stops at the final position of the mating seal.

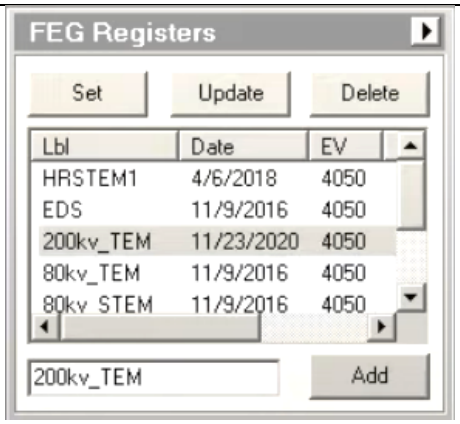
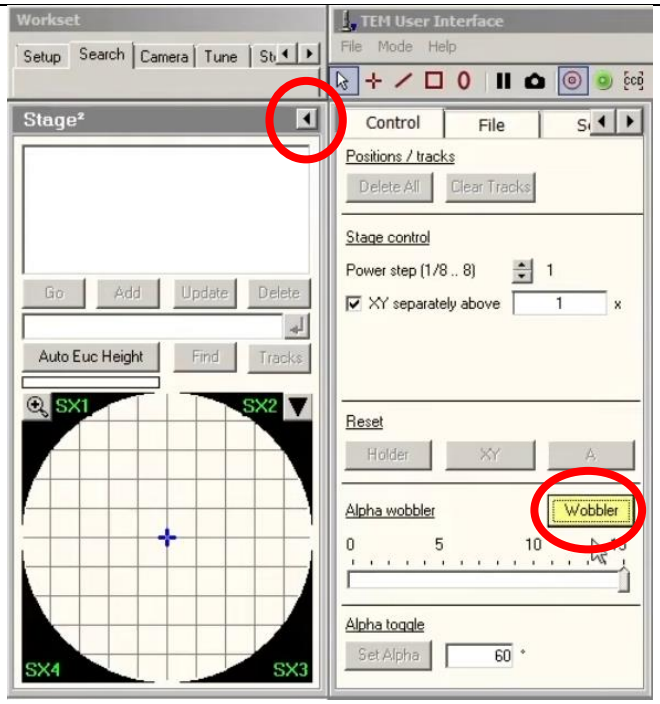
The black line marker on the holder will be aligned with the white panel.

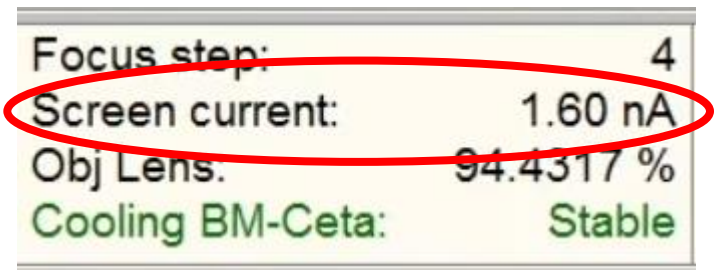
If the holder is misaligned, and can't go till the black line, take it out and try again.
Waiting more than 5s while the holder is misaligned will cause vacuum failure.

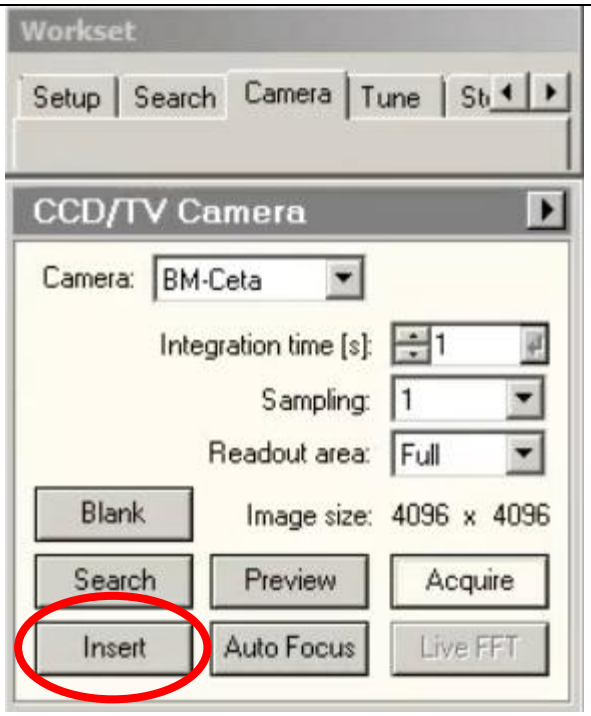
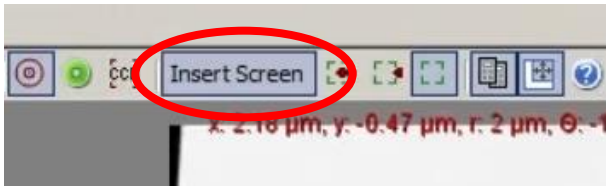
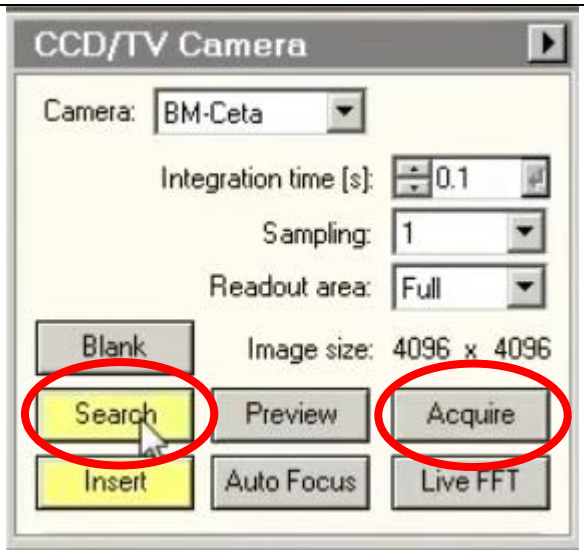
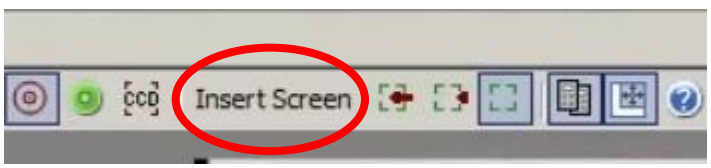
6	**Pumping load lock:** The remaining pumping time for the specimen exchange is shown on the TEM screen. If you are using the double-tilt holder, you need to connect the cable and select double-tilt from the "Select Holder Type" menu.	
7	**Final insertion:** When the pumping cycle ends (red light on the CompuStage turns off), gently rotate the holder pin counter-clockwise from 5 o'clock (close) to 12 o'clock (open) position. Gently guide the holder into the column. Do not let go of the holder at this point! Tap the end of the holder gently to ensure stabilization. (*Note*: For high-resolution work, you may have to wait 30 minutes or more for the O-ring seal to stop drifting.)	

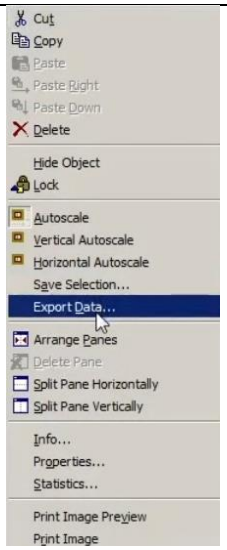
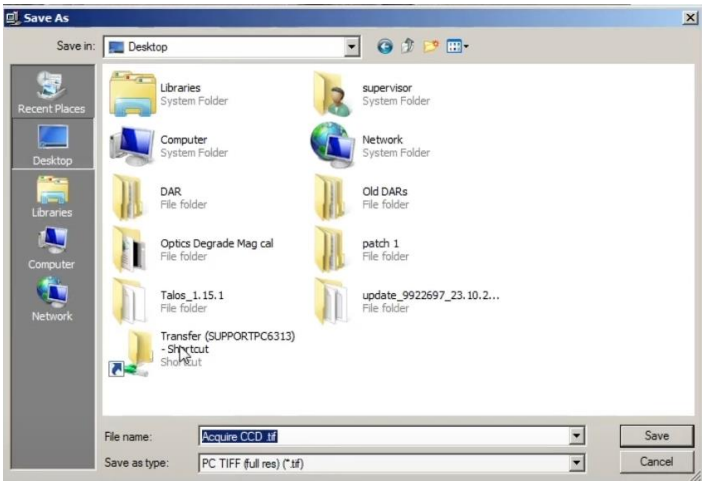
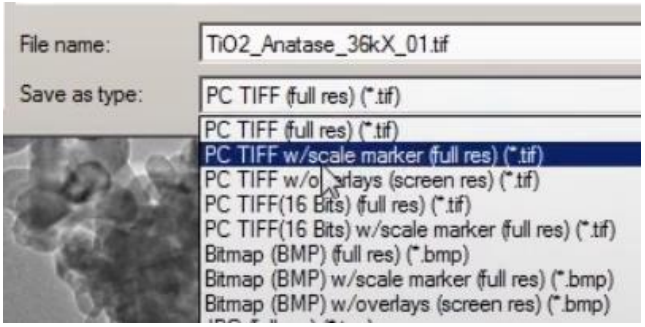
8	In the event of dumping the vacuum contact EM staff member immediately. They will recover the vacuum.	Vacuum recovery may take from half an hour up to several hours. If you need more practice on sample loading, please ask EM staff or an experienced user to be with you.
9	Video on operating the TEM software: youtu.be/NkHjQKrpX2U Click on the 'Start' or triangle button to open the TEM software package: -TEM User Interface -TEM imaging and analysis (TIA) -Flucam Viewer Drag the TIA program to the right monitor and maximize it.	

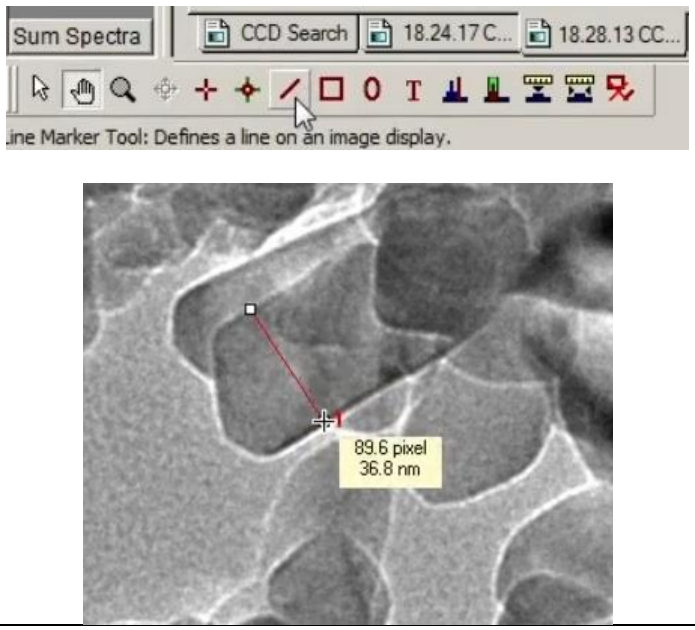
10	**Column valve**: Ensure the Column pressure is below 20 log and press "Col. Valves closed" button to open it. It becomes grey when it is open.	
11	**Flucam viewer:** A live TEM image will automatically appear in the Flucam Viewer. If image does not appear, check that Flucam viewer is in an un-paused position and screen is inserted ("insert screen" is blue). It's easier to find image at low magnification. If the image is too dark (looking at the grid or thick sample) use joystick to move the sample to the carbon film window or to a thinner area.	

12	**Select an alignment:** In the FEG Registers window, select the 200kv_TEM then click "Set" to load optimized working conditions.	
13	**Finding the correct Z height of your sample (eucentric height):** Bring some feature/particle to the center of the FluCam. Then go to Search tab, then click on the small triangle to get to the stage control panel. Click on the "Wobbler" button. You see the feature on the screen goes left to right when the holder tilts. **You can also activate Wobbler by pressing L2 on the panel in front of you.**	
14	Start by pressing the lower Z button and watch if the movement of the sample gets less and less. Currently the focal Z height on our TEM is in the range of -40µm to -100µm. You can also observe the contrast of your sample is getting less and less, becoming whiter. **Stop the Wobbler.**	

15	Video on adjusting mag, intensity and imaging: https://youtu.be/d-F_jV0AFSE **Magnification and Intensity:** Move the stage with the joystick to the area of interest then increase the "Magnification" to a desired value (clockwise). You will see the screen is getting darker. Use the intensity knob to adjust the Screen current between 1-2 nA. Rotate it clockwise to reduce the intensity.	  

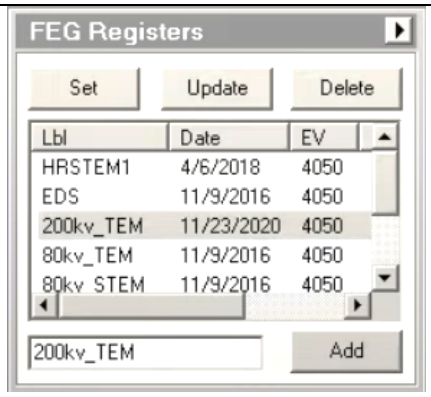
16	**Taking Image:** Go to the Camera tab. Then click "Insert" to bring the high-resolution camera in. The button becomes yellow. Click on the "Insert Screen" to remove the Fluoresce screen. The beam will be able to reach the high-res camera.	 
17	Click on the "Search" button to get an overview of your sample on the right monitor. Click "Acquire" to capture that image. When the image appears, click on the "Insert Screen" to bring back the Flu-screen. This also prevents the camera to be exposed to the beam for too long.	 

18	If you need to change the magnification, do it over the Flu-Screen. Not on the high-res Camera.	
19	**Save Image:** Right click on the image that you have acquired. Then click "Export Data". Go to Transfer folder, find your folder with your name (if you don't have one, create a new folder with your name and UNI). Make sure click on the file type "with scale maker"	  

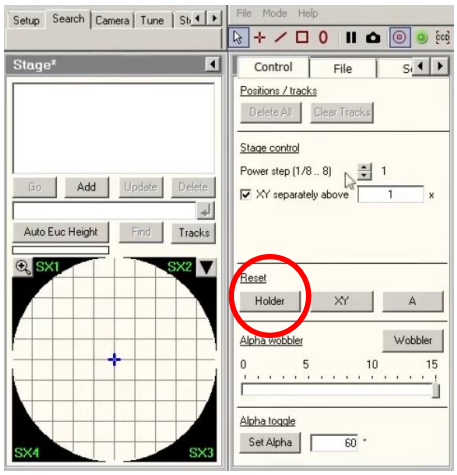
20	**Measurement on your image:** From the tool bar in the TIA program, you can click on the "Line" tool. Click anywhere on your sample and drag the line to see the particle/feature size.	

21 Closing your Session:

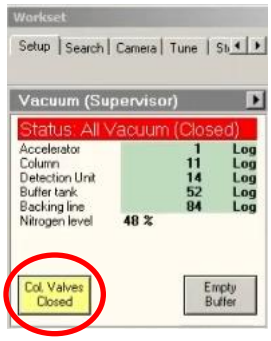
1-From the FEG Registers, click on the “200kv_TEM” and “Set”.



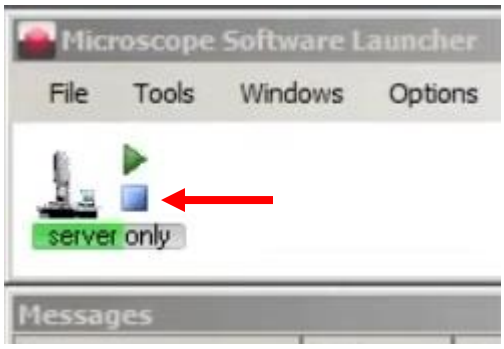
2- Click on “Holder” in the search tab to reset the stage to zero coordinates.

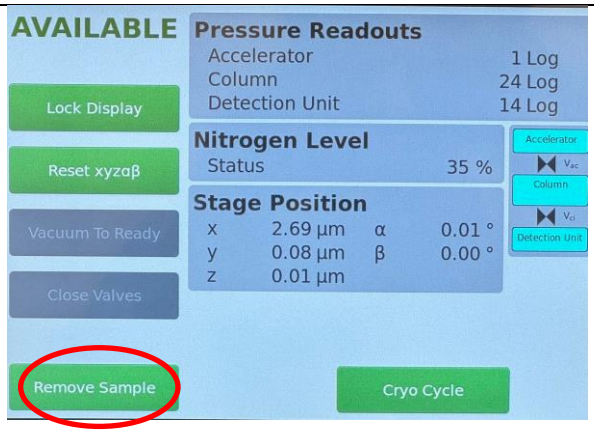
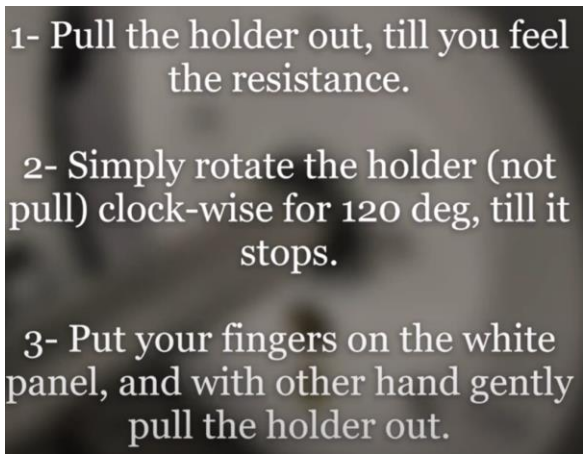
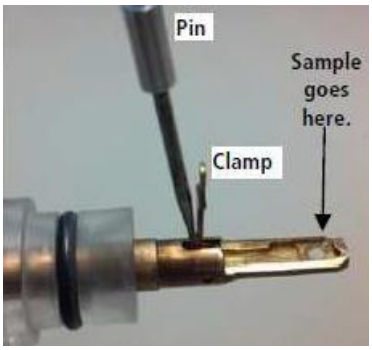


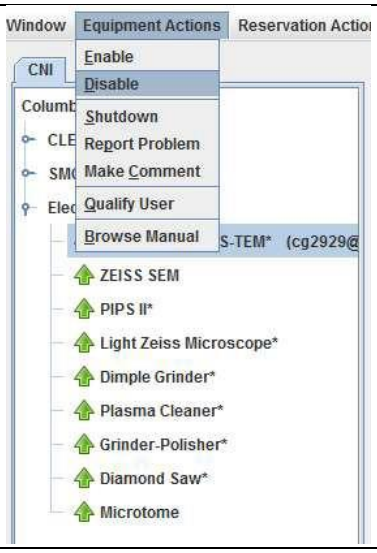
3- Close the “Column Valves”.



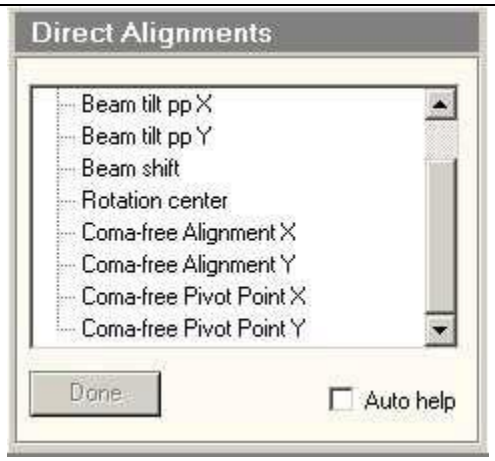
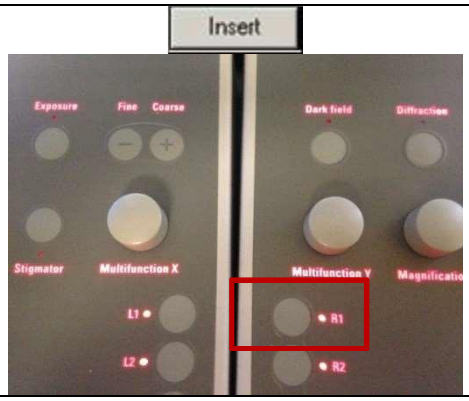
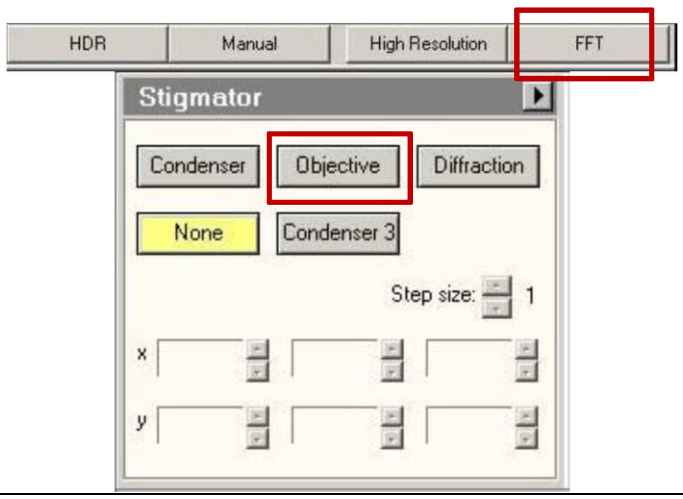
4- Stop the TEM programs by clicking the square/stop button on the TEM software launcher.
TIA software on the right monitor will ask you if you still need to save your data. Click “No to all” and click “Yes”.

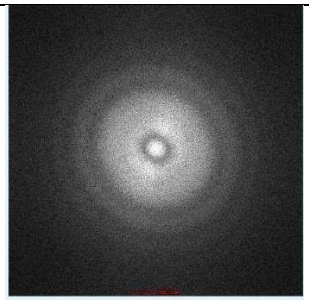


22	**Removing the Holder:** On the TEM screen click “Remove Sample” then wait for a few seconds. Follow the following instructions to remove the holder from the TEM: Video on how to remove the holder youtu.be/qDFljfuem3I?si=XI_6OFtCyJqZiTPZ&t=84	 
23	**Take the grid out:** Remove the clamp by the pin Gently take the holder out of its plastic tube (only 2 mm), then rotate it to drop the grid on the filter paper on the stereoscope. Close the clamp and place the holder in the desiccator.	

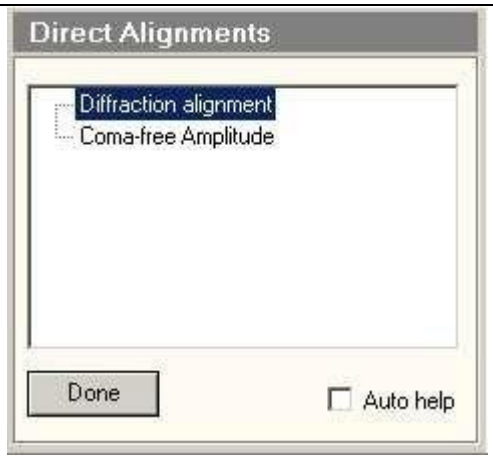
24	Disable the tool in badger.	
25	**Please make sure to keep the table and working area clean and organized.**	

HR TEM set-up

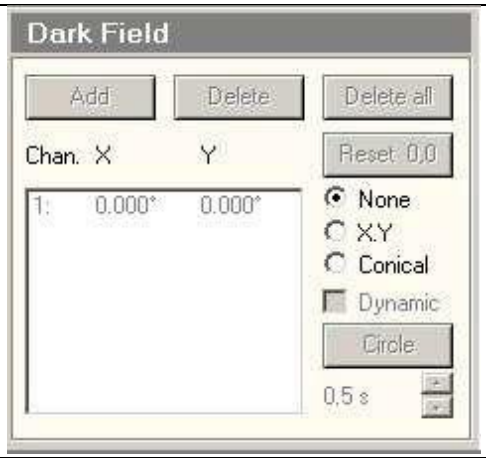
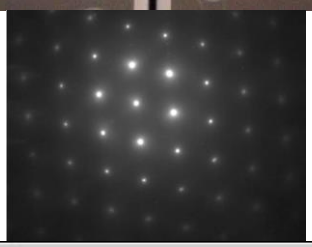
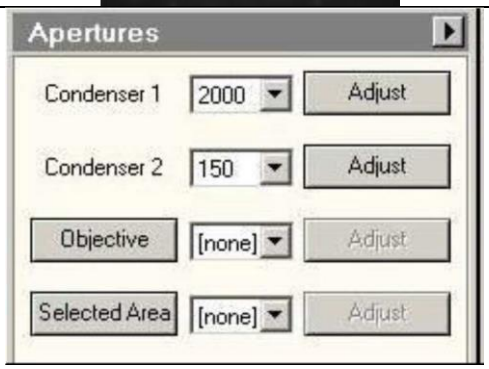
1	**STANDARD TEM ALIGNMENT:** follow the steps necessary for TEM alignment described above. Go to higher mag, e.g. SA 245KX. Click on "Comma Free Pivot Point X" in the Direct Alignments window. Condense the beam using intensity knob and use multifunction X & Y knobs to overlap two spot images. Press "Done". Repeat for Pivot Point Y.	
2	**TEM ALINGMENT CONT.**: Insert Ceta camera. Set the integration time, lift the screen (R1), press "Search". In the camera tab change stage piezo moves to Picometer steps.	
3	**FFT:** Press "Live FFT" in the CCD/TV Camera window. Go out of focus until you see rings. Adjust objective stigmators using the multifunction knobs to round and concenter the rings.	

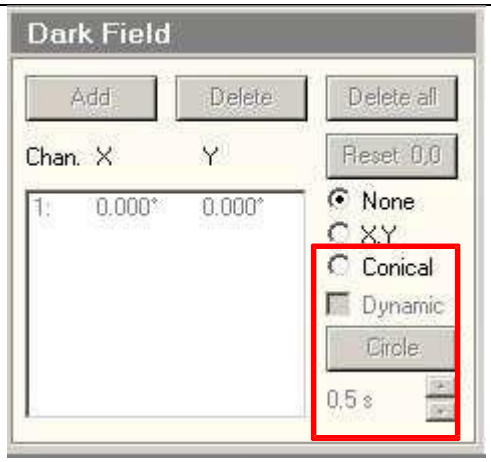
		
4	**COMA-FREE ALIGNMENT:** Align the beam with the optic axis by clicking on "Coma-free Alignment X" and use the multifunction x knob to minimize shape change and movement of FFT rings (should be stable). Repeat for "Coma-free Alignment Y".	
5	**EUCENTRIC FOCUS:** Click "Eucentric focus" and use the Z height to get to optimized focus position at max ring size. Use the focus knob to get to max fine focus (one ring for crystalline material).	

Diffraction set up

1	**DIFFRACTION:** Insert a smaller C2 aperture (e.g. 70) and finish your gun shift on a larger spot size (e.g.6). This will protect the ceta camera from over exposure. Focus the beam and press "Diffraction". Use the intensity knob to sharpen the diffraction (larger spot size will increase sharpness).	
2	In the Direct Alignments window press "Diffraction alignment". Use multifunction knobs to center the diffraction pattern. When inserting the ceta camera put the search and acquire buttons on the lowest integration time and carefully monitor the histogram intensity chart to determine acceptable exposure.	

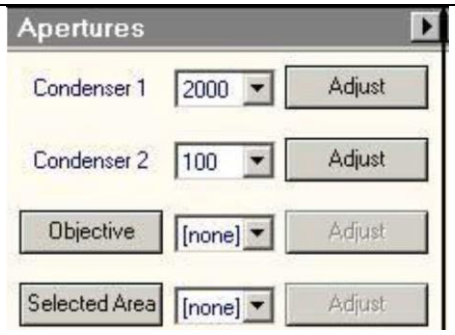
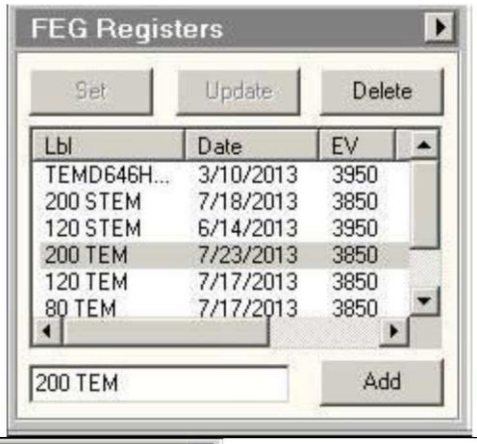
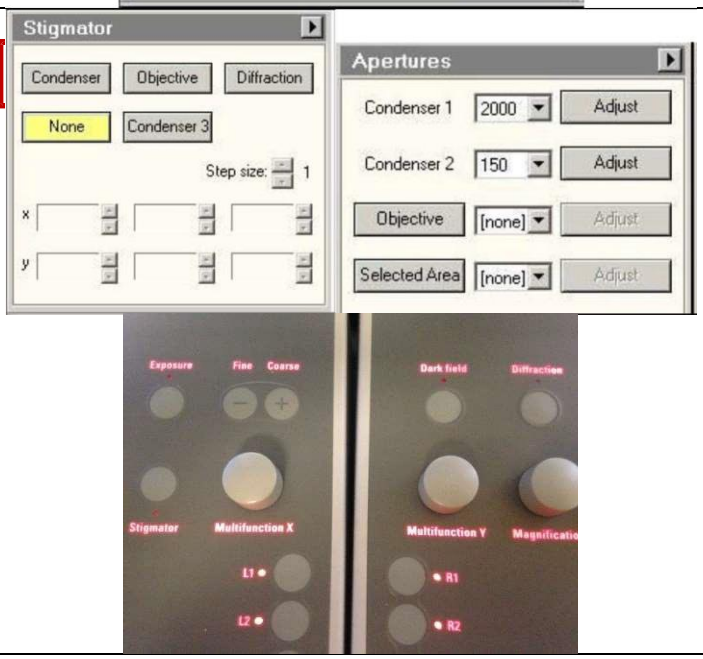
Dark-Field set up

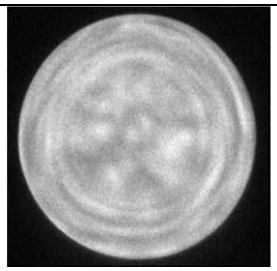
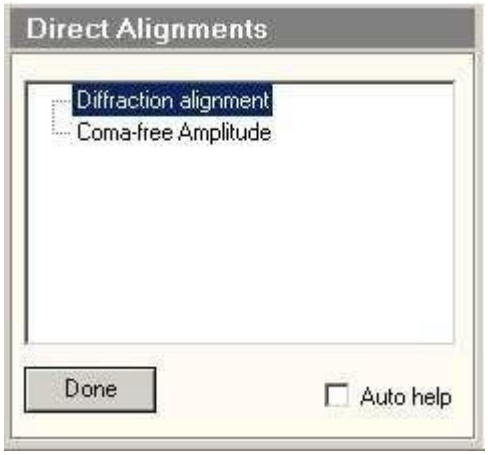
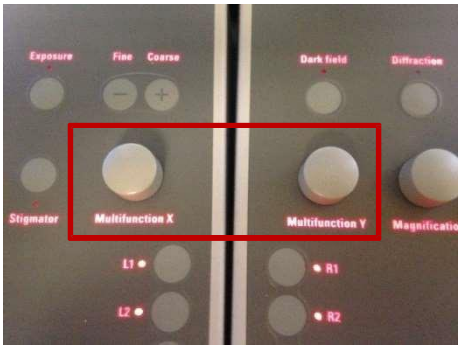
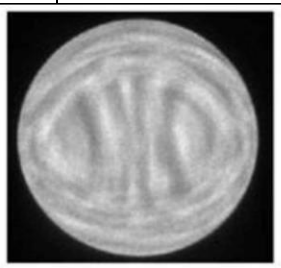
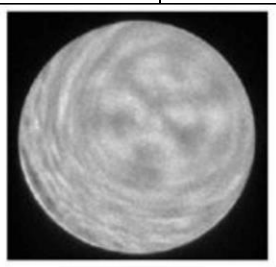
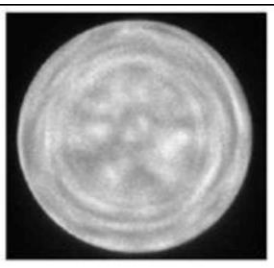
1	**DIFFRACTION ALIGNMENT:** Follow diffraction alignment steps described above. Camera Tab: Select Dark Field.	
2	Click XY button.	
3	**CENTER REFLECTED BEAM:** Use multifunction knobs to move the desired reflected beam to center.	 
4	**OBJECTIVE APERTURE:** Insert small objective aperture (e.g. 20 µm) and select the desired diffraction spot. Press and unpress “dark field” to toggle between BF and DF image.	

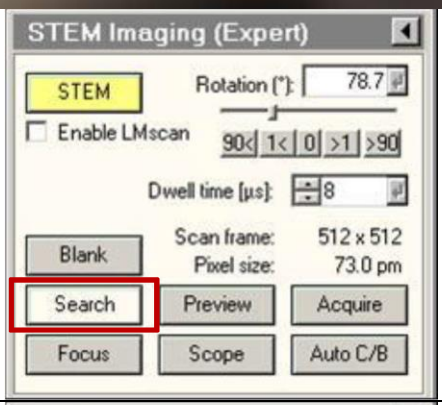
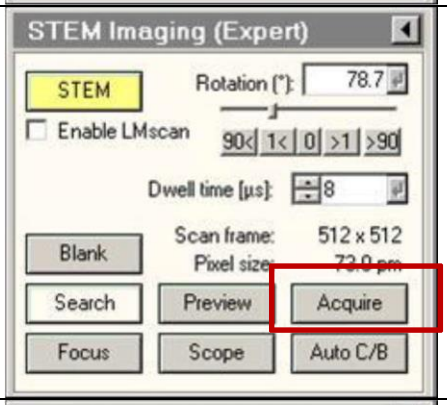
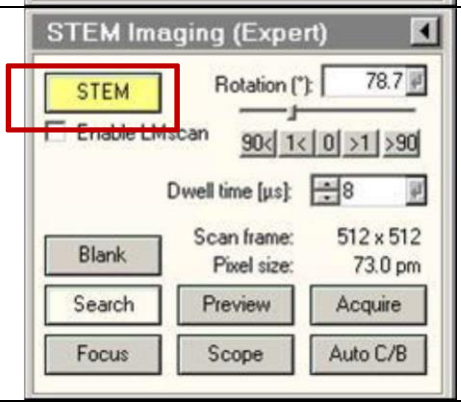
		
5	**Obtaining Hollow Cone Dark-Field:** In this mode you can select a circular region (like a donut) of the diffraction pattern for obtaining the image. In the diffraction mode, center the transmitted beam, then press "dark field" button on the nub and the "Conical" option on the screen. Insert an objective aperture and center it. Then use the X and Y nubs to tilt the beam and move and select a desired part of the diffraction pattern on the center. Now, press "Dynamic" and "Circle" button. Now, the diffraction pattern will rotate around the center and is obtaining a shape of a donut from your diffraction pattern. Now go to the imaging mode. You will see that some grains in respect to their diffraction condition turn bright and dark.	

	By obtaining an image which covers the whole ring, you will get the “Hollow Cone Dark Field” image. You can also change the rotation time of the diffraction pattern in the panel.	

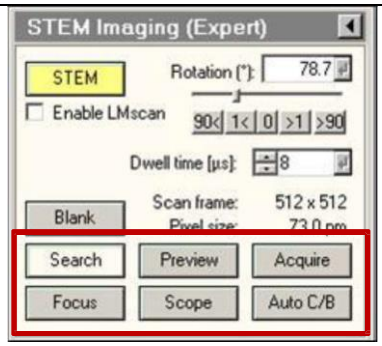
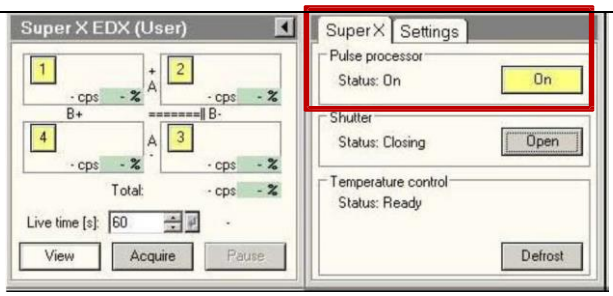
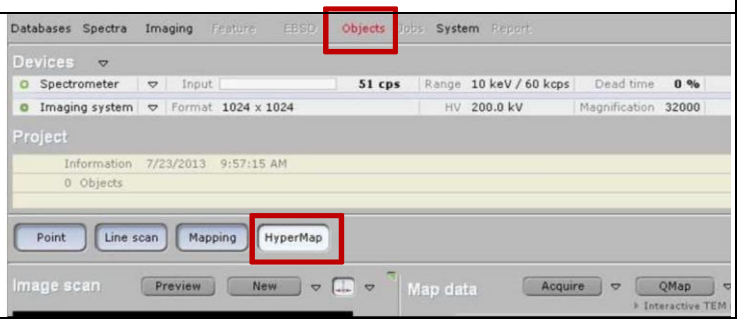
STEM

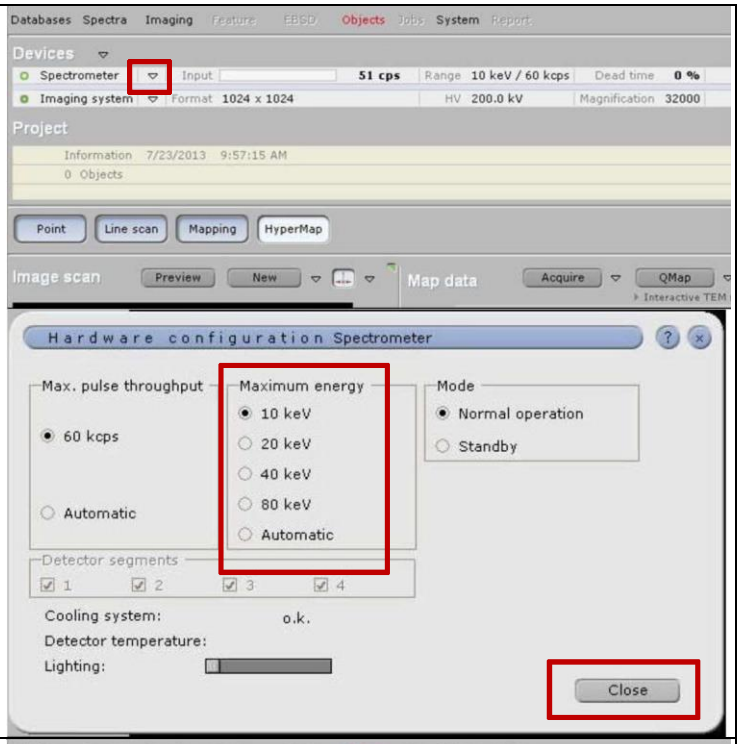
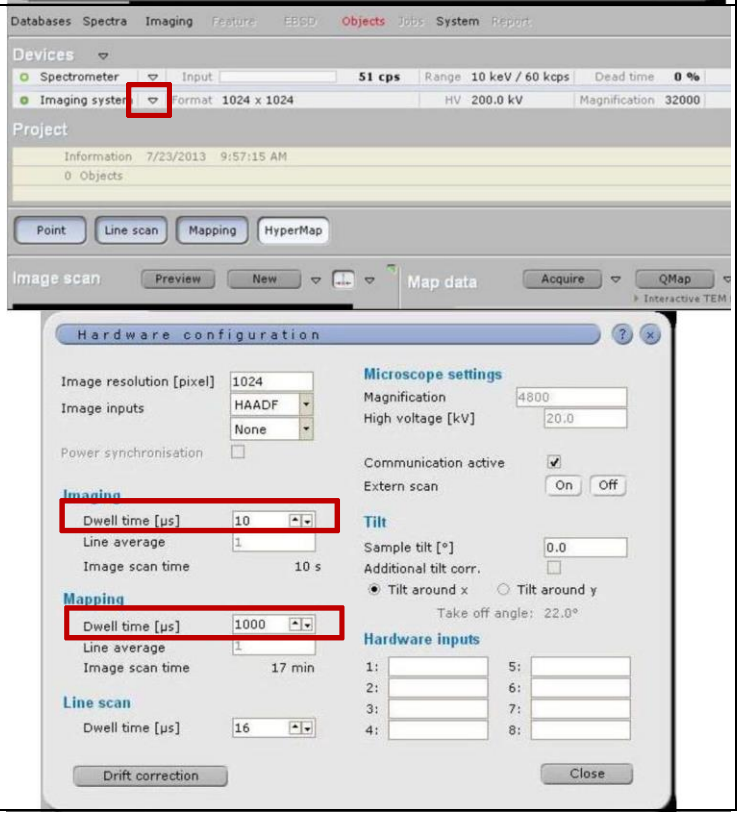
1	**STANDARD TEM ALIGNMENT:** perform alignment, make sure camera, objective, and SA apertures are out ("none" in drop down menu), insert screen and select Condenser aperture (e.g. 70 µm).	
2	**STEM MODE SELECTION:** Switch to STEM mode. In FEG registers window upload previously optimized conditions by pressing "lbl", e.g. 200 STEM, click on "set". For HR-STEM use spot size 6-9, EDX spot size 3, both 6.	
3	**CONDENSER:** Center C2 aperture and correct its stigmation (round the beam after clicking "Condenser" in the stigmator window and then "none" after correction). If the beam doesn't condense and expand concentrically click on "Condenser 2 Adjust" and correct it using multifunction X&Y knobs.	

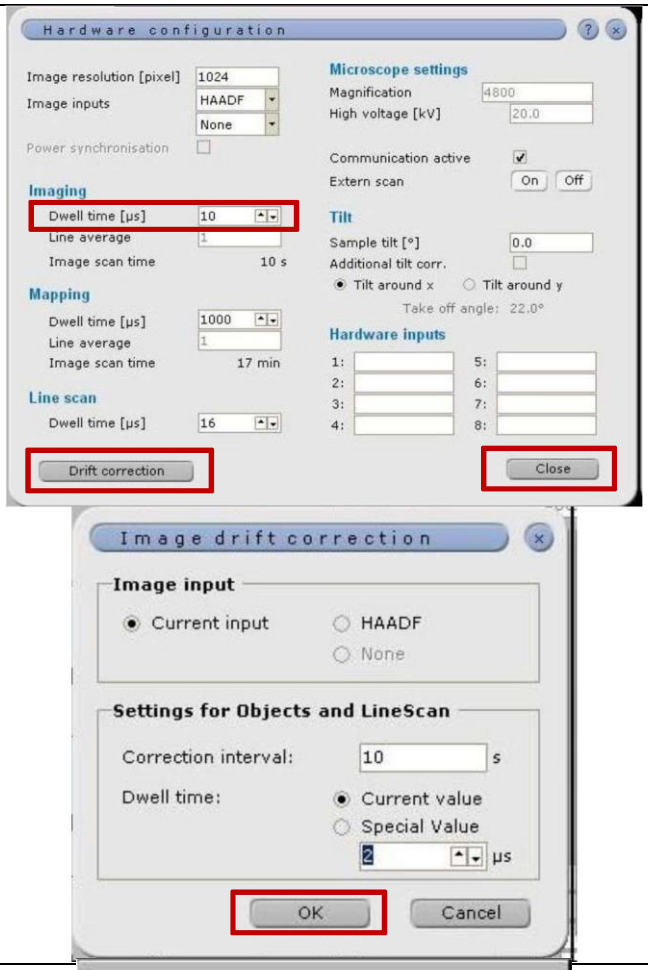
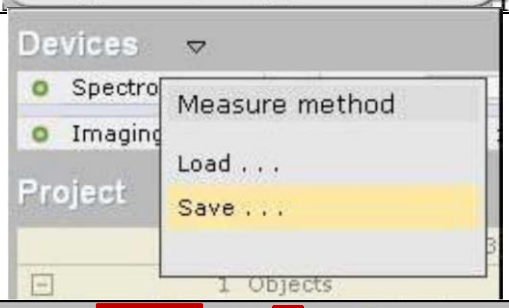
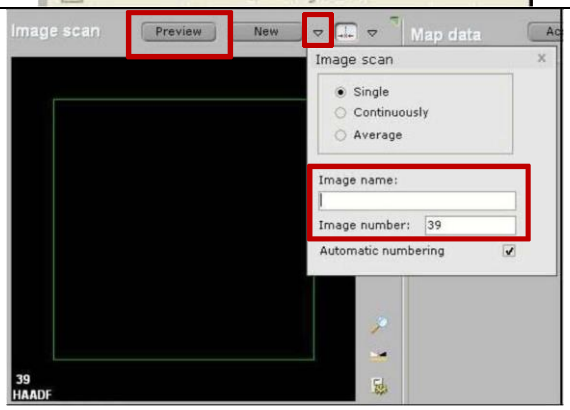
4	**CENTER BEAM and DETECTOR:** Click on "Diffraction", a Ronchigram should be visible in the beam. Move the beam to the center of the HAADF detector (should look like the image to the right). Click on "Diffraction alignment" in the direct alignments window. Click on "HAADF Detector Area" button in the Flu Cam viewer and use the multifunction X&Y knobs to move the beam to the center of the detector. Click "Done" to save. Change the height (Z) of the sample on the nub to make the Ronchigram well aligned and then use the condenser stigmation to make the Ronchigram Astigmated (see images below). An aligned Ronchigram looks like a magnifier with an infinite (in theory) magnification.	 HAADF detector area   
	 Astigmatic  C2-Aperture misaligned  Well aligned and stigmated	
5	**DIRECT ALIGNMENT:** If necessary perform direct alignment as described above.	

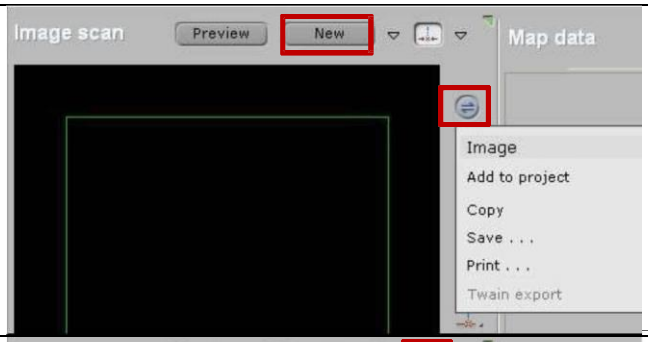
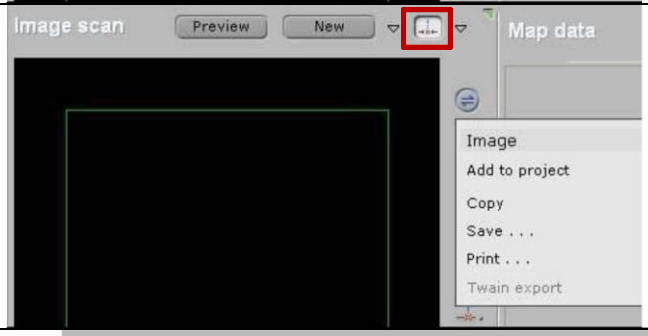
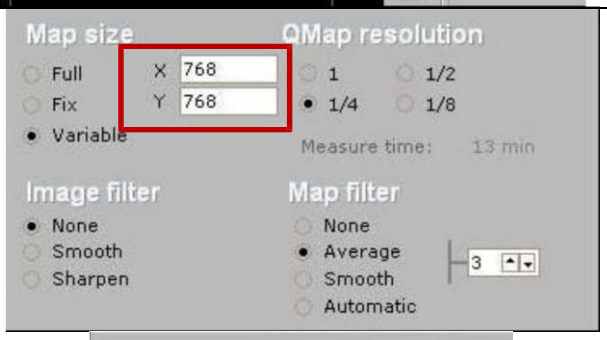
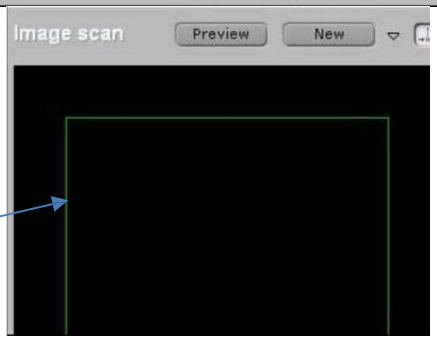
6	**LIVE IMAGE:** press the Z-axis buttons to bring your sample into rough focus. Click "Search" in the STEM imaging window. A live image will appear in the TIA program. If image does not appear check camera length (should be around 220mm depending on the sample), contrast, and brightness (Auto C/B). Fine focus using the Focus knob.	 
7	**ACQUIRE An IMAGE:** Click "Acquire" in the STEM Imaging window to obtain HAADF STEM image. Slow scan rate – higher quality.	
8	**EXIT:** To exit STEM mode click on "STEM" in the STEM Imaging window and the HAADF detector will be auto retracted.	

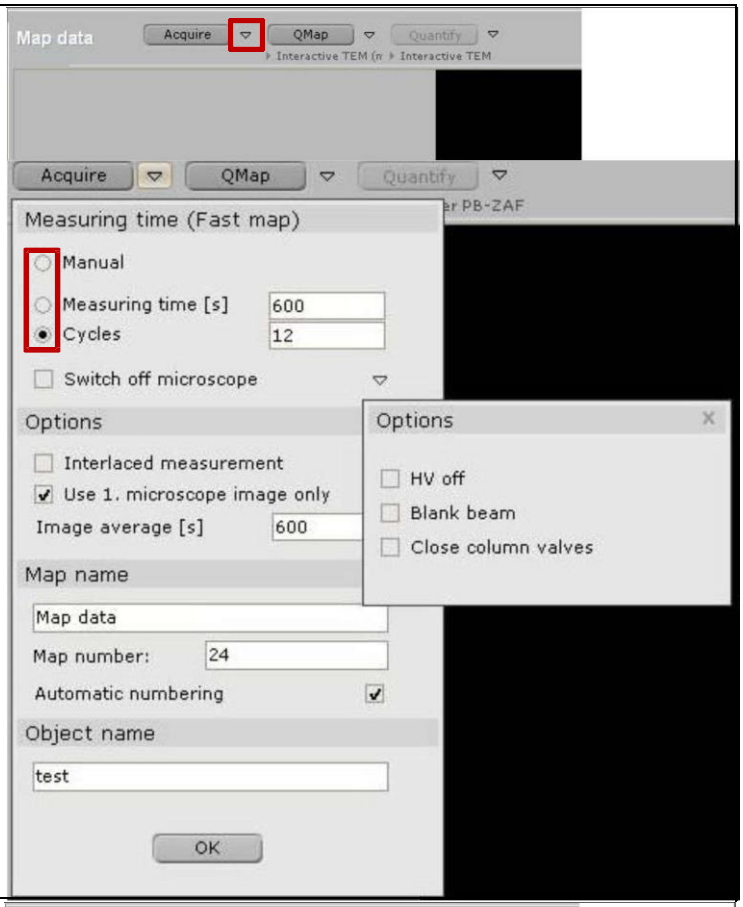
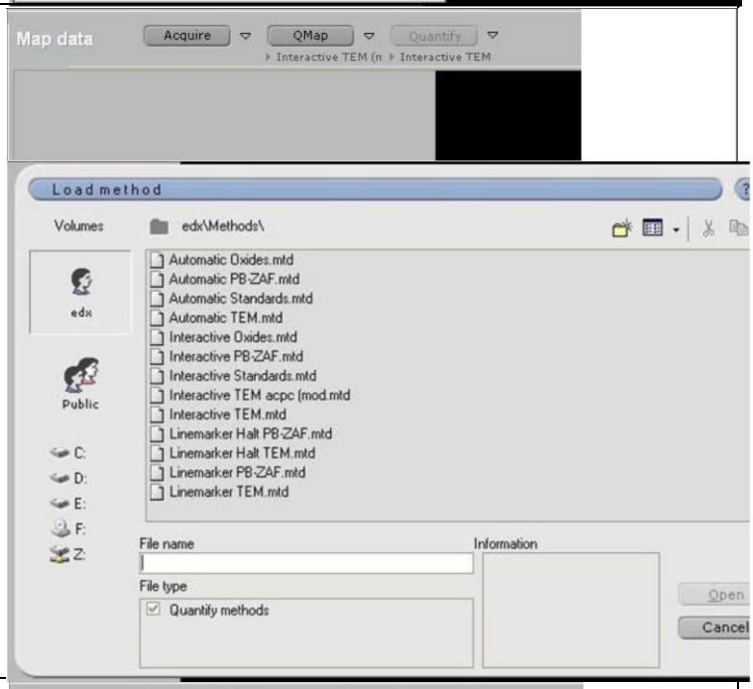
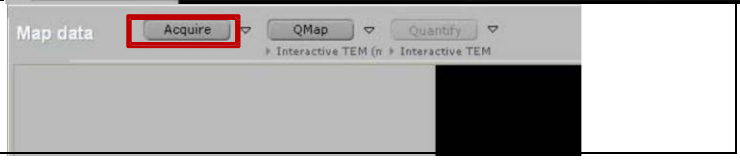
EDX Analysis

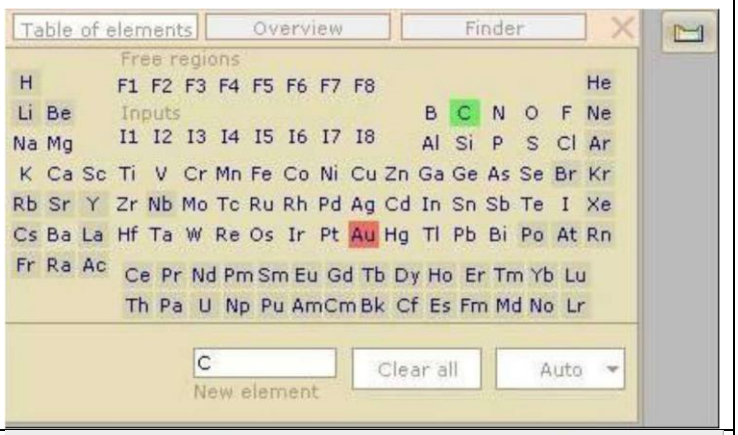
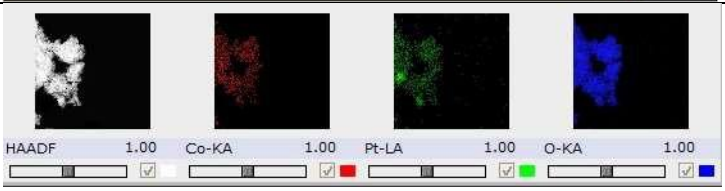
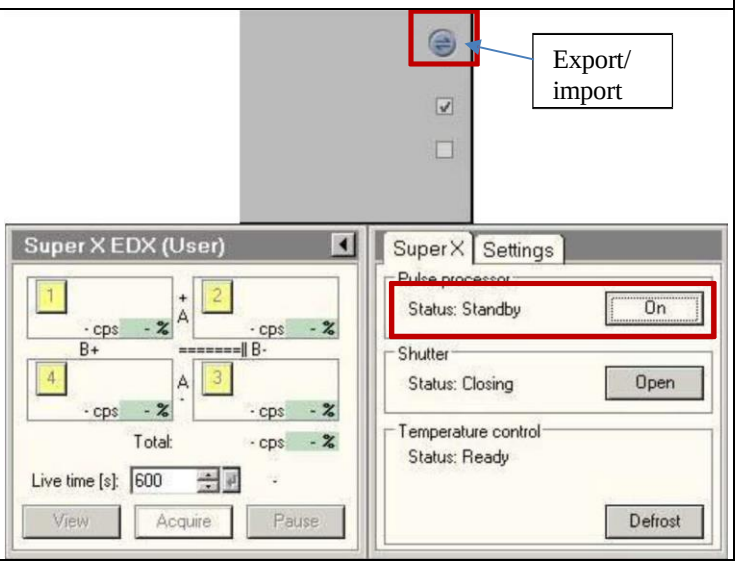
1	**STEM ALIGNMENT:** Perform STEM alignment and HAADF STEM imaging as described above.	
2	Press "Search", "Preview", "Acquire", "Focus", "Scope", and "Auto C/B" in the STEM imaging window. They should be inactive when using the Esprit software. To ensure they are inactivated you can press the buttons, so they no longer appear yellow.	
3	**DETECTOR ON:** Optimum EDX conditions: smallest C1 aperture, 100 µm C2 aperture, Gun Lens 5, and Spot Size 3, giving a probe size of 1-2 nm. Go to the Super X EDX window and click on ►to show the flap-out window. Under the Super X tab if the Pulse processor Status appears Standby, click on the "On" button to start the EDX detectors. For better resolution it should be kept on for about an hour before collecting X-ray data.	
4	**HYPERMAP :**In the Bruker Esprit program choose the "objects" tab and click on "HyperMap" to activate the HyperMap workspace.	

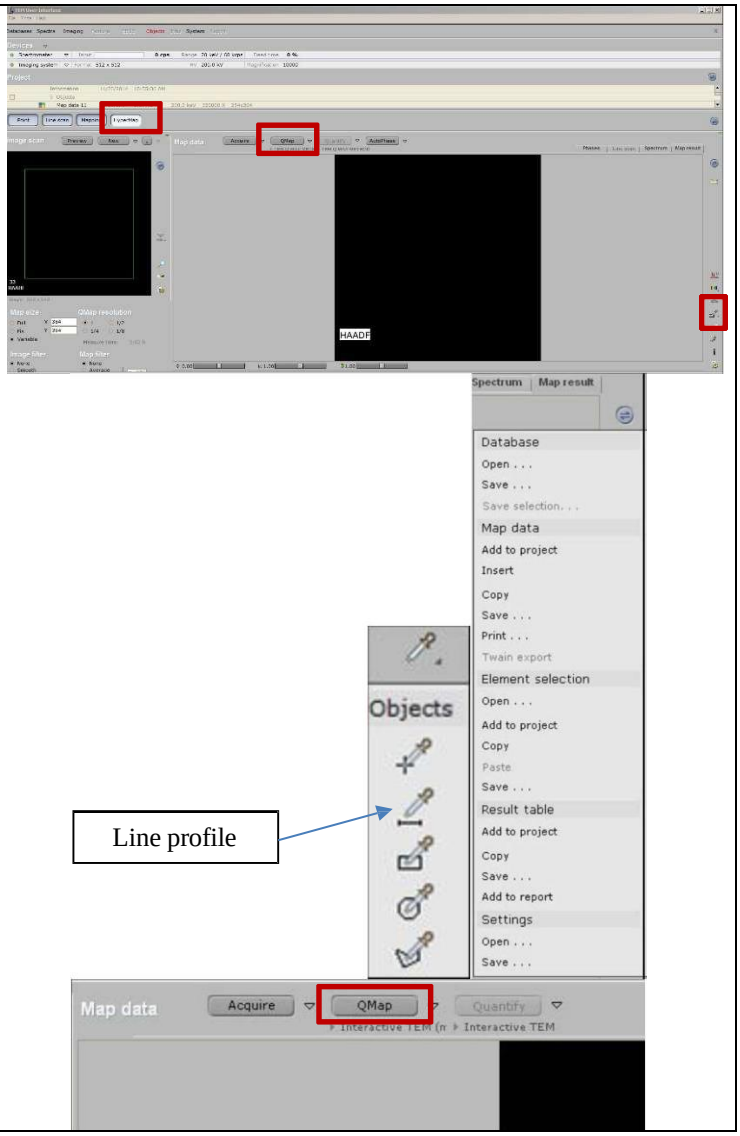
5	**SPECTROMETER SET UP:** Open the Hardware configuration spectrometer window by clicking on the dropdown arrow. Set the maximum energy at a desired value: 10 KeV is recommended for higher elemental resolution and 20 KeV is for better elemental coverage. Click on "Close" after you're done.	
6	**HARDWARE CONFIGURATION:** Open "Imaging system" (drop down arrow) to open the Hardware configuration window. Set Image resolution [pixel] to a desired value, e.g. 1024. Set Dwell time for Imaging (suggested value 20 µm), Mapping (10-50 µm for fast surveys, 1000 µm or more for trace and quantification), and Line scans.	

7	**DRIFT CORRECTION:** click on "Drift correction" from the Hardware configuration window to open the Drift correction window and adjust the correction interval and Dwell time for correction image. Close both windows when you're done by clicking on "OK"and "Close".	
8	**SAVE:** The measurement parameters by clicking: ▽ under "Devices" as a measurement setup that can be loaded (by clicking "Load") next time.	
9	**COLLECT STEM IMAGE:** Open the New Image scan set up windows. Image name and number will appear on image. Click on "Preview" to view the live STEM image. Use the joystick to move your sample to an area of interest, adjust brightness and contrast and click "OK" to close.	

10	Click "New" to acquire a STEM image with predefined conditions (in Hardware configuration and imaging), then click on export/import button  in image scan window to open Image menu and save the image.	
11	**DRIFT CORRECTION** (if needed): can be activated by clicking on:  .	
12	**MAP SET UP:** In the map setup window select desired map size (mapping area of interest in pixels as showed by green frame in Image scan window). Please note that there isn't enough memory for the full size (1024x1024). Image filter is for digital image processing. The QMap resolution allows converting up to 8 pixels of spectrum data to 1 pixel in the final EDX maps during quantified mapping to shorten measurement time. Quantified mapping can be perform using HyperMap workspace post data collection. Map filter changes elemental map digitally.	 

13	MEASUREMENT SET UP: in Map data open the Acquire window (drop down arrow) to adjust measurement set up. If "Manual" is checked, the measurement will require user's second click on the Acquire button to stop. If Measuring time [s] or Cycles is checked, the measurement will be automatically stopped when reaching the set values. **Never** check HV off in the Switch off microscope drop down menu.	
14	**LOAD METHOD:** Open the Load method window (from either the QMap or Qunatify). Linemarker TEM.mtd is the recommended selection for beginners.	
15	**START EDX MAPPING:** Click on "Acquire" to start the mapping. Click again and the Acquire turns	

	to "Stop" and the mapping will stop after finishing the current frame. Click on "Stop" to stop immediately.	
16	**SELECT ELEMENTS:** Click on  to select elements to monitor. The "Auto" will eliminate option that should not be detected.	
17	**IMAGE OVERLAY:** In the Element images window, click on the check box below each image to overlay/no-overlay the image in the Map data window. Click on the color button to change color for an image.	
18	**SAVE DATA:** Once the acquisition is done click on the export/import button in the Map result tab of the Map data window to Save the acquisition result under Database. If this is the last acquisition turn off the super X detectors by changing their status to: "Standby" (the button "On" should be available but not yellow at this status).	

19	**DATA ANALYSIS:** Click on the pipette button to choose the marker you wish to use and use it to select an area on the map. Analyze the elements as described above and obtain composition by clicking on "Quantify" (composition in % will be described at the bottom of the Map data window). Click on "QMap" for concentration maps.	

20	**SAVING OPTIONs:** Element maps can be Saved under Map data to image files, e.g. .bmp format. Various data and processing results can also be added to project and save all together as one Project package.