

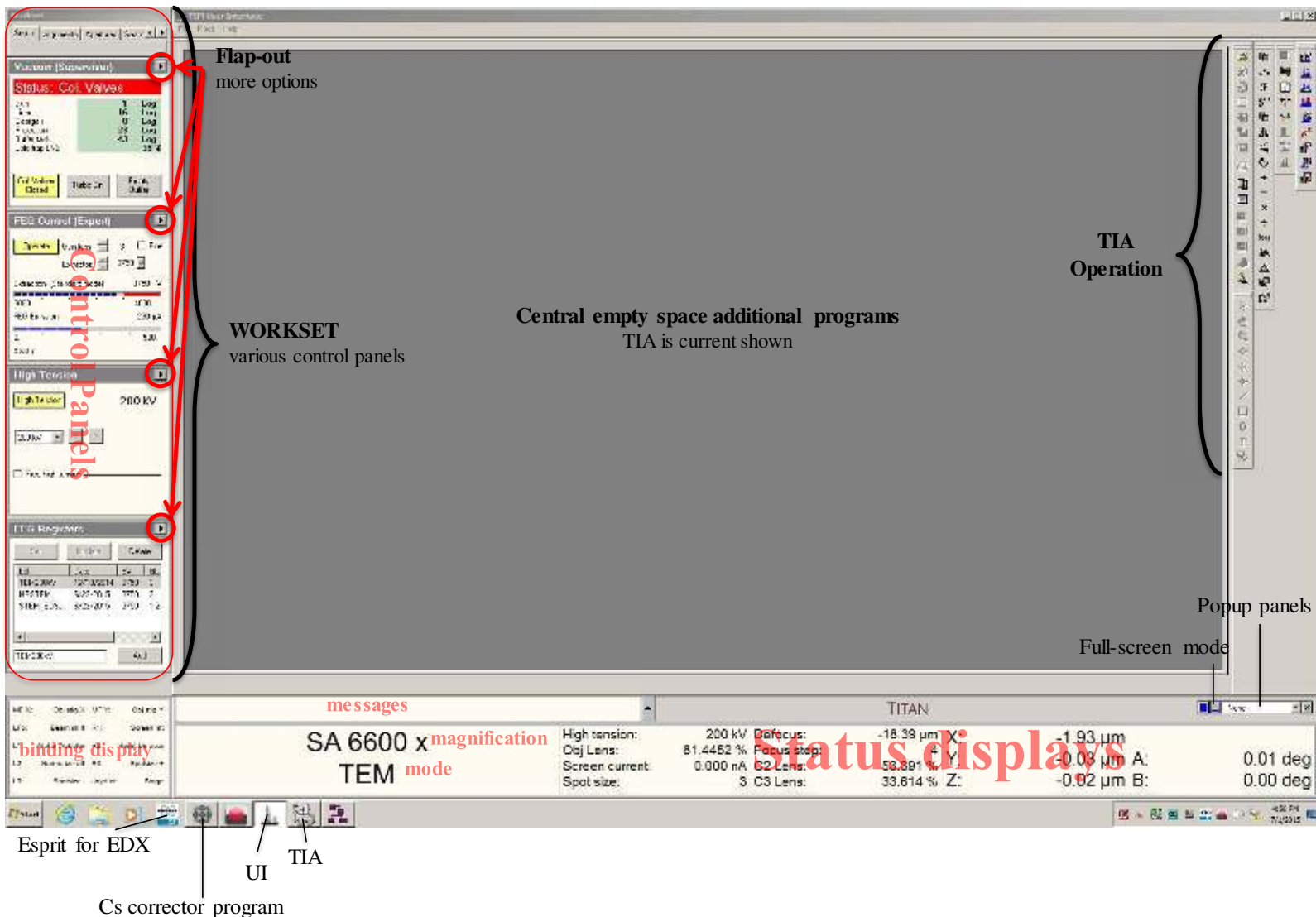
## **200 kV Titan Themis (S)TEM with Cs Image Corrector**



**By Tong Wang, Sheng Zhang  
CUNY ASRC Imaging Facility**



## User Interface Overview



TITAN Themis is 200 kV high resolution TEM, whose interface contains three parts: TEM server, User Interface (UI), and Control pads (left & right). Users interact with TEM server via UI. An overview of UI is shown above. UI contains elements that can be divided into three categories:

- Main program: program title and menu bar.
- Control panels: control panels are grouped in worksets that can be selected via a tab at the top of the area with control panels.
- Information panels: binding, messages, status displays.

The central empty space is reserved for other programs (e.g. TIA, Esprit).

Notes: Position the mouse cursor over a particular panel and press F1 on keyboard to invoke the UI help pages for that particular panel.

## Microscope Control Pads Overview

Control pads (left & right) consist of a set of standard TEM controls:

- Left: Tilt, user beam shift trackball, Intensity (Brightness), Stigmator, Multifunction X (MF X), programmable buttons (L1, L2, L3).
- Right: Diffraction, Wobbler, Eucentric Focus, Multifunction Y (MF Y), Magnification, Focus, Focus Step, Joystick, Z height, programmable buttons (R1, R2, R3).

MF X/Y knobs' default function is "user beam shift X/Y" and can be assigned (bind) to other functions. Programmable buttons are user-defined buttons and can be assigned (bind) to frequently used functions.



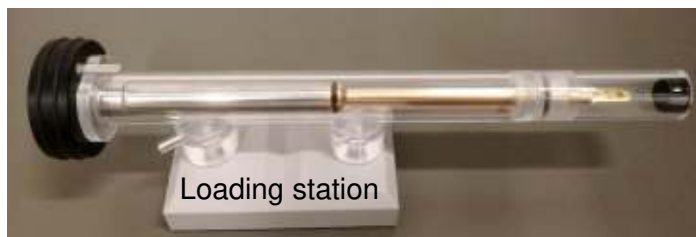
## Preparation & Start-up

1. Log in using supervisor account (Optional). The microscope server control software (Microscope Software Launcher) should automatically start. Click the start icon to start microscope UI. Launch other programs (e.g. TIA, Cs image corrector) if needed. UI software launching progress can be monitored by click **window>message**.
2. Check the liquid nitrogen level (LN2) in cold trap (**Setup>Vacuum**). Please notify Imaging Facility staff if it is below 20%.
3. Make sure that column valves are closed: **Col Valves Closed** button in **Setup>Vacuum** is in yellow color.
4. Make sure that camera is retracted: **Insert** button in **Camera>CCD/TV Camera** is in grey color.
5. Make sure the stage is reset: values for X, Y, Z, A, and B in TEM status window at the lower right corner of UI are zero or close to zero. If not, click **Holder** in **reset** panel of **Search>Stage>Control** page to reset **Holder**.
6. Make sure the objective lens aperture is retracted. Objective lens aperture control can be found in **Apertures**.



## Load Sample

1. Load TEM grid to single or double tilt holder using holder station. Note: Put a washer on top of the grid when using the double-tilt holder. If doing EDX, put the sample side facing down (e.g., the side with sample applied on) using the double tilt holder. After loading, double check the sample and washer won't fall off.



2. For samples prepared by chemical solvent, it's suggested to use plasma etcher in the Imaging Suite to clean the TEM grid within the holder together (70% power, 20 to 30 s).
3. Log in and enable the microscope in **Badger** system.
4. Make sure Turbo pump is off ("Turbo On" is grey). Insert holder into stage half way in by aligning guide pin on holder (see picture below) at 5 o'clock position on the stage ("Close") until you feel some resistance. Push the holder further in for 8 mm with a slight force, sometimes need to twist the holder a little to find the right angle to push further inside. The holder should be firmly inside the hole. This will trigger turbo pump to pump the airlock (turbo pump button will turn orange and then yellow, 3 mins counting down starts). If using double-tilt holder, connect the cable to the CompuStage.



5. Select holder in the message tab of UI (single tilt, double tilt, etc.) and confirm.
6. After 3 min counting down, turn holder **counter-clockwise 150°** and guide holder fully into the column. Note: after turning 150°, apply an outward force to guide the holder into the column, **DO NOT** let the vacuum pulls the holder in freely.
7. Check the vacuum of Octagon and wait until the value is less than **20 log**. Note: the vacuum of Octagon may show value below 10 log after inserting sample holder, you still need to wait at least 3 mins before opening column valves.

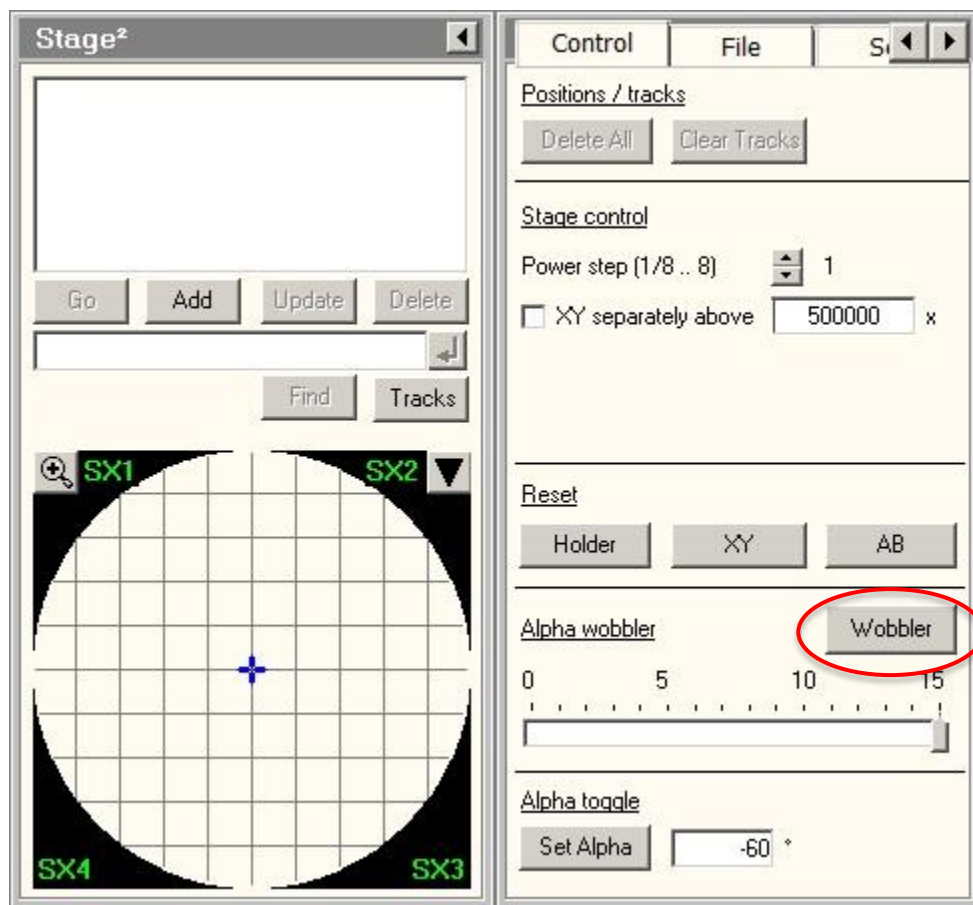
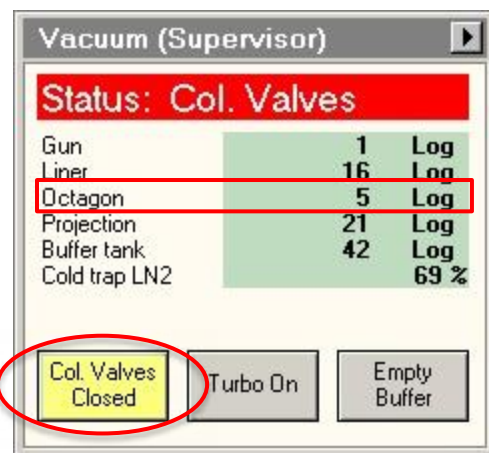
## TEM Alignment

1. Click **Col Valves Closed** button to open column valves, **Column Valves Closed** button should change from yellow to grey and you should observe beam on the fluorescence screen. In case you cannot find the beam, reduce the Magnification and move the stage in X-Y plane using Joystick.

Find a visible feature on the grids (e.g., a cluster of particles) and center it in the view. Check the “Coarse” above **Intensity** on the left control pad is activated.

2. Adjust Eucentric Height by one of the following methods:

Method 1: Center a recognizable feature on the sample, activate **Alpha Wobbler** in **Search>Stage>Control** (see picture below), changing Z height to minimize the movement of sample image, increase the magnification and repeat Z adjustment until above SA 20000 x. Then deactivate **Alpha Wobbler**.



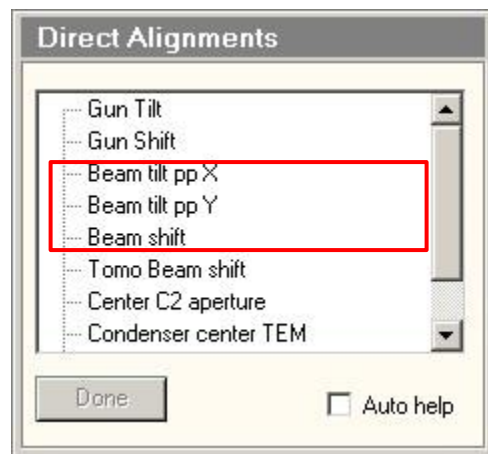


Method 2: Center carbon film region in the view. Press **Eucentric Focus** on right control pad once, change Intensity to make the beam a minimum spot, then adjust Z height to minimize halo around the beam spot at a magnification above SA 20 kx.

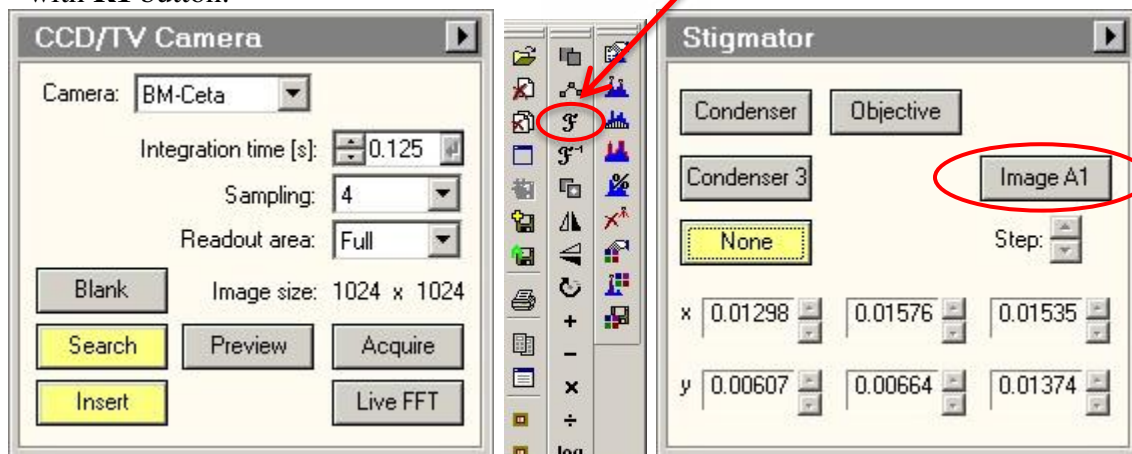
Method 3: Press **Eucentric Focus** once, expand beam to show sample image, adjusting Z height to minimize image contrast.

3. **Direct Alignments** in “Apertures” Tab:

- Change magnification to ~ 310 kx in SA range.
- Press **Eucentric focus** button on right control pad.
- Condense the beam to a spot using **Intensity**. Activate **Beam tilt pp X** in **Aperture>Direct Alignments**, bring the two spots together using **MF X/Y** knobs. Repeat the same procedure to tune **Beam tilt pp Y**. (If beam is out of the view, change to lower magnification, activate **Beam Shift** and use **MF X/Y** to move the beam back to the center.)
- Activate **Beam Shift** and use **MF X/Y** to center the beam. (Do not use the trackball on left control pad to move the beam.)
- Click “Done” to finish **Direct Alignments**. Spread beam **Intensity** to cover full fluorescence screen.

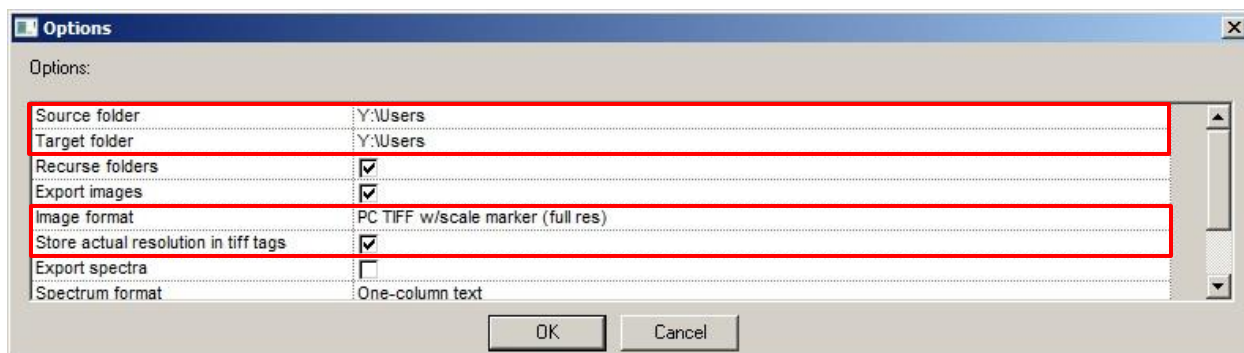
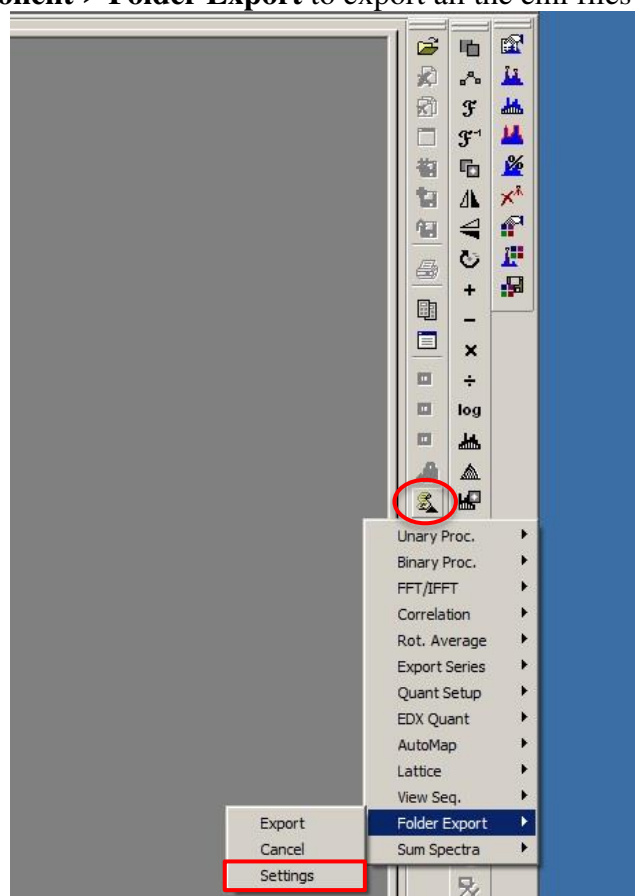


4. In **Camera>CCD/TV Camera**, click **Search** and **FFT** buttons. Lift the fluoresce screen with **R1** button.



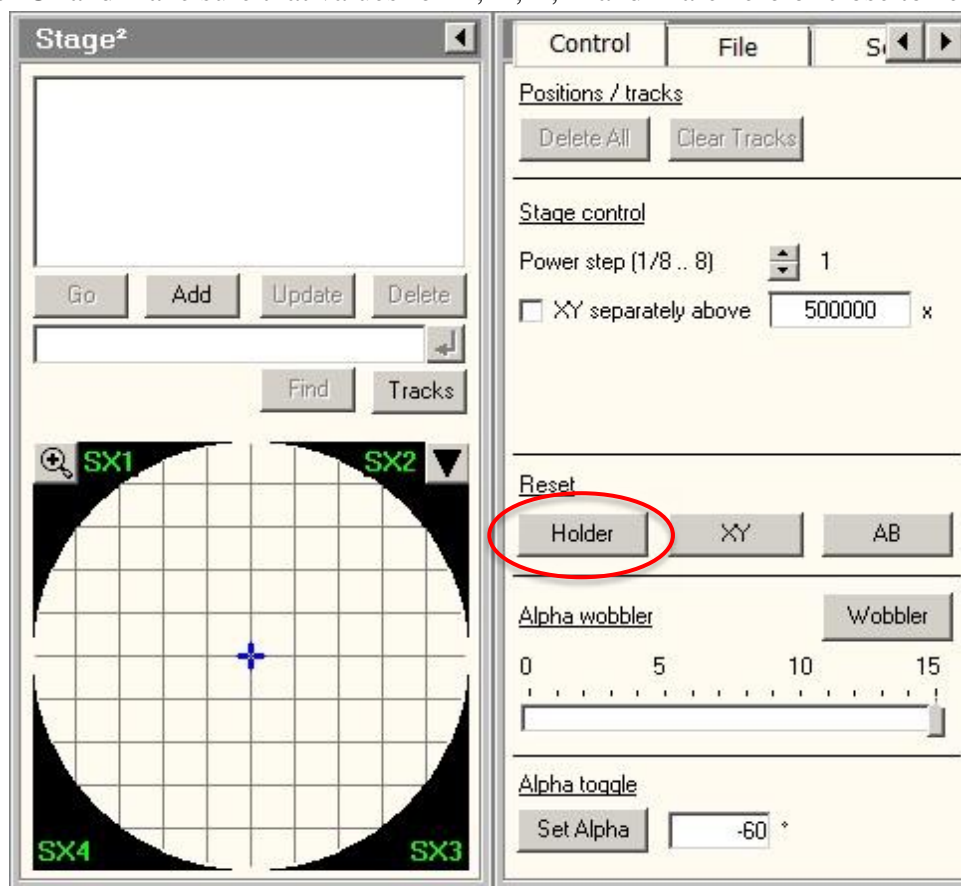
(Optional) Find a pure carbon area (amorphous carbon support film) without any sample of interest. If the FFT rings are not circular, activate **Image A1** in **Stigmator** (**Apertures** tab) of UI and use **MF X/Y** to manually adjust A1 stigmatism by making the rings circular (change focus to make the rings bigger). Click **None** after done. Perform Cs Image Correction if you plan to image HR-TEM at atomic resolution (instruction on the following pages).

5. Find areas of interest, adjust Focus to bring the sample close to focus (no rings on FFT), press **R2** button to reset focus value. Adjust defocus and take images by clicking **Acquire** in **Camera>CCD/TV Camera**. Click **Save as** or **Auto Save** to save the images in \*.emi format. Alternatively, right click on the image and select **Export**, save the image as tif file with scale marker and full resolution.
6. Folder Export all \*.emi files: after capturing all the images on the sample, locate the **Component** icon in the **TIA**. Select **Settings** in **Folder Export**, change both Source folder and Target folder to where you saved \*.emi files (Y:\Users\.....). Then click **Export** in **Component > Folder Export** to export all the emi files to tif files.



## Shutdown Procedure

1. Return to TEM mode in FEG registers if you performed STEM application.
2. Stop **Search** in Camera. Retract CCD (BM-ceta) camera and make sure that the **Insert** button is in grey color.
3. Lower the fluorescence screen (**R1** button). Put the rubber cover on.
4. Click **Col Valves Closed** button in **Setup>Vacuum** to close the column valves and make sure the button is in yellow color.
5. Center the stage: click **Holder** in **Reset** panel of **Search>Stage>Control** page (see picture below) to move stage to center, watch the TEM status window at the lower right corner of UI and make sure that values for X, Y, Z, A and B are zero or close to zero.



6. Retract objective lens aperture and selected area aperture if you ever used one.
7. Leave the magnification in the lower SA range (4kx - 10kx). This is essential to maintain stable objective lens current and prevent thermal drift for the next user
8. Now you can remove the holder out from microscope and unload your grids: Pull out the holder slowly until it stops, turn the holder **clockwise** until it stops, and pull it out with a little force carefully. **Note:** Use two hands to pull the holder out of the station: use two fingers of the left hand to gently push against the station (purple region) and use the right hand to pull out the holder, in order to prevent any damage to the station.
9. If the Turbo pump starts, turn it off. The "Turbo On" should be grey.
10. Repeat from the sample loading process if you have more samples to characterize.
11. When you finish, clean up working area. Log out the facility in Badger system.

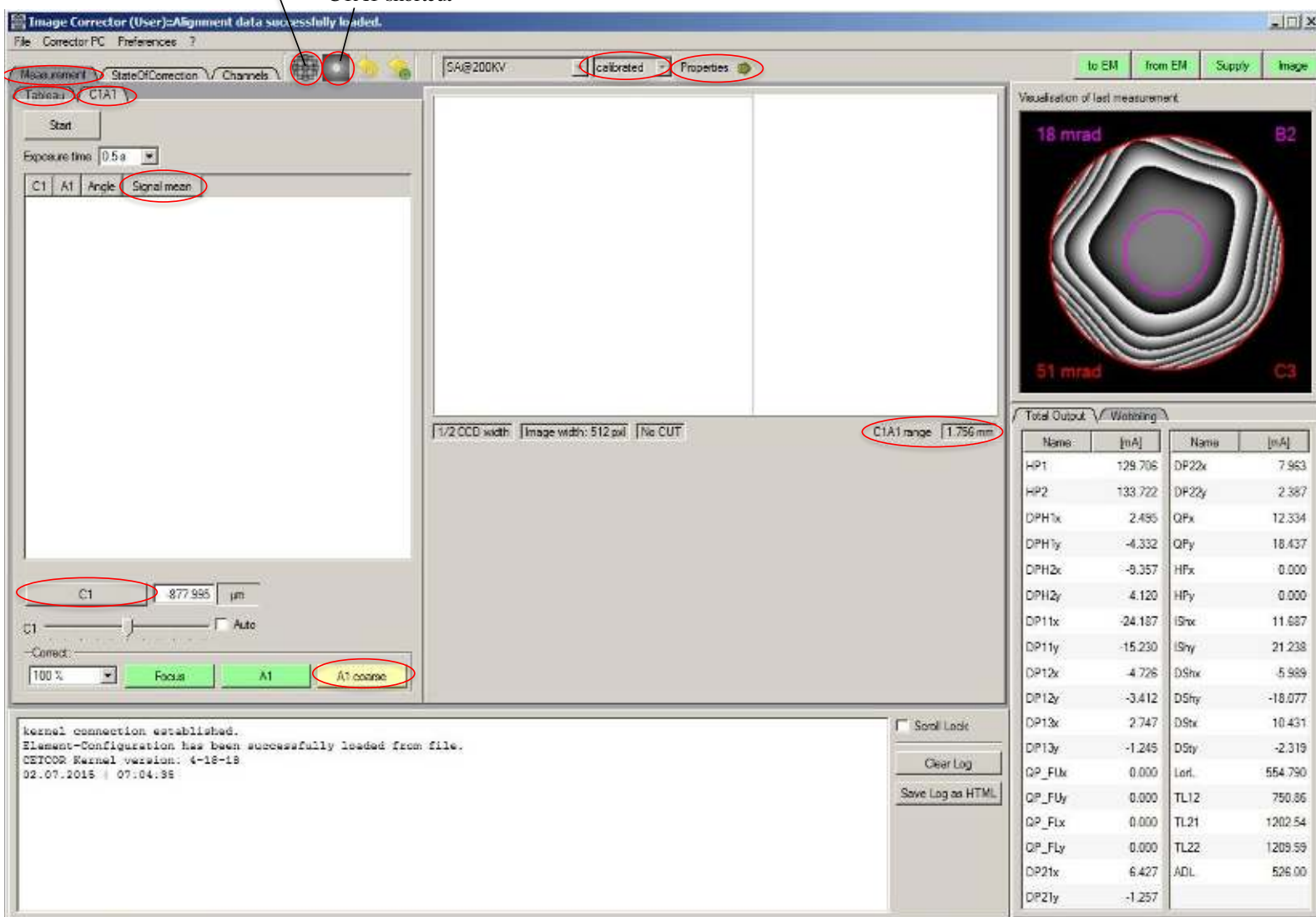


## Tuning Cs Image Corrector

1. Click **Search** and **FFT** buttons in **Camera>CCD/TV Camera**. Find a pure carbon area (amorphous carbon support film) without any sample of interest. Adjust magnification to 310 kx in SA range. This makes sure that the magnification is shown “calibrated” in Image Corrector Tuning program and Nyquist Frequency value (shown in Properties) is about 4~5 (This range of magnification is optimum for tuning corrector). Do **Direct Alignments** following instructions above. Adjust **Image A1** in **Stigmator** tab of UI to manually adjust A1 stigmatism by making the rings circular (do not need to be perfect at this stage). Stop the camera **Search**. Make sure the **Sampling** of **Search** is 4.

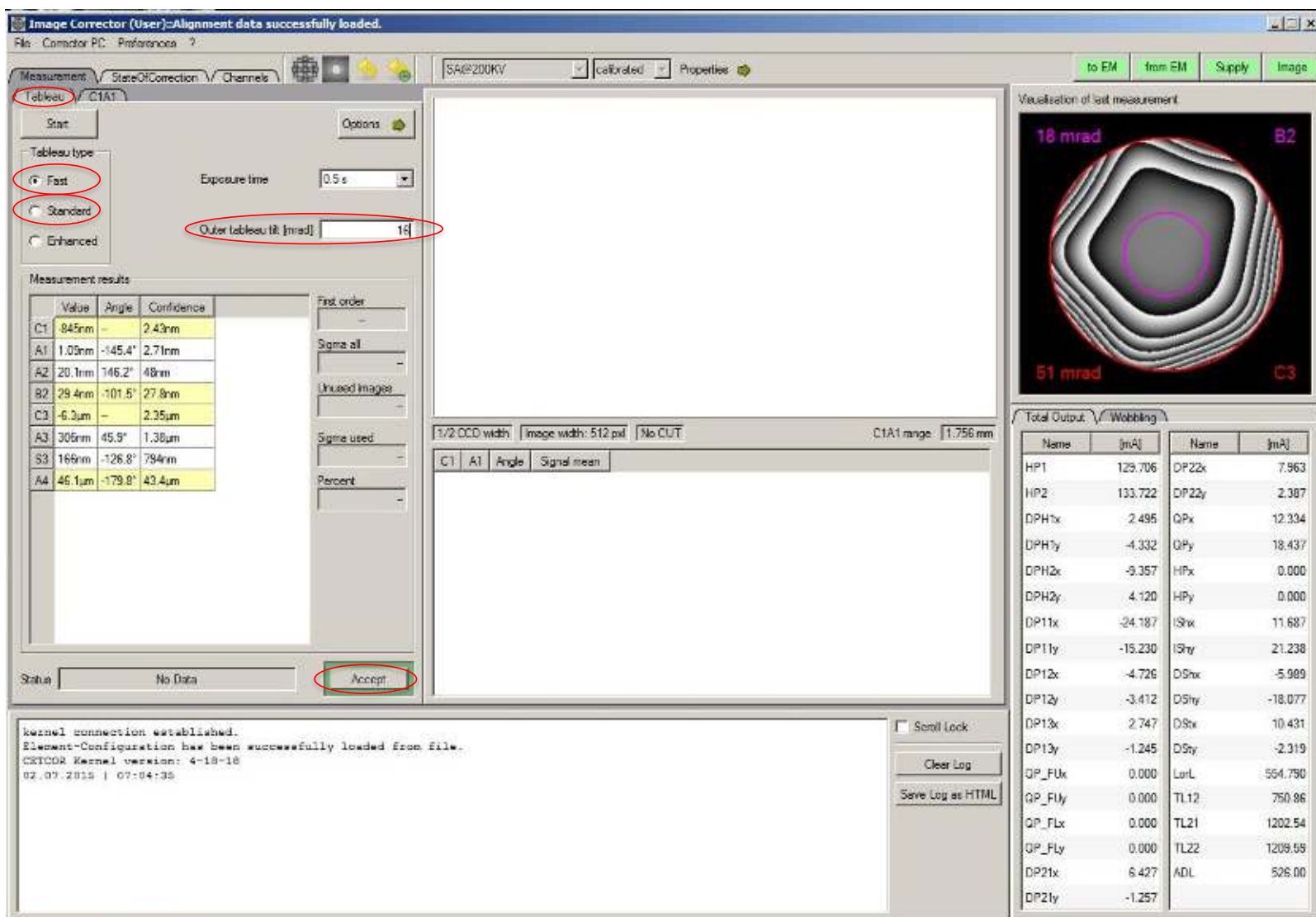
Tableau shortcut

C1A1 shortcut



2. In Image Corrector software, press **Start** in C1A1 tab, adjust **Intensity** to make the Signal mean in the range of 6000 ~ 10000. Adjust **Focus** until C1 value is in the range of -600 nm to -800 nm (underfocus). Alternatively, you can directly apply a negative value (~ 30 % of C1A1 Range) to C1: at the bottom of the measurement window, choose a value for C1 and press **C1** button.

3. Press **A1 coarse** to reduce A1 value (obj stig) to be less than 5 nm.
4. Go to Tableau tab, select Tableau type to “Fast”, Outer Tableau tilt [mrad] to 16 (or 18), and exposure time to 0.5 s. Note: In Tableau, beam is tilted to collect series of images to do measurements, when beam is tilted TEM is in dark field mode.



5. Press **Start** to measure B2 and A2. If A2 is larger than 100 nm, B2 is larger than 50 nm, or either value is larger than Confidence, press **Accept**. Otherwise go to step 7. Note: the parameters highlighted in yellow (except C1) are above Confidence and can be further tuned. Others not highlighted are below Confidence and do not need further tuning.
6. The program will automatically enter correction page. Select correction power (100%), press B2 or A2coarse (not A2) whichever needs to be tuned based on the results of step 6.




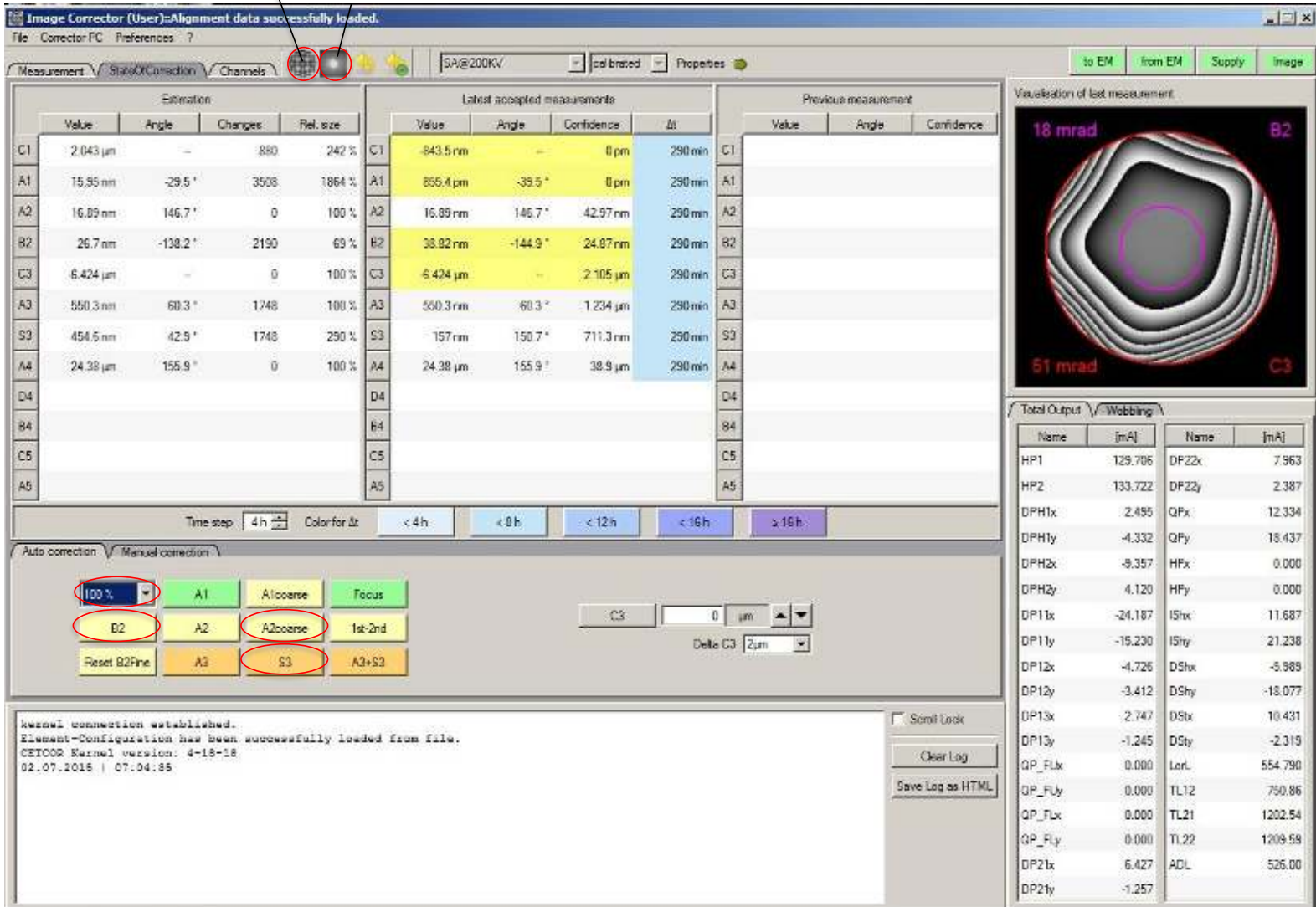
Then press shortcut  to start Tableau measurement again, repeat Step 5 to 6 until the parameters are less than or close to confidence values.


Tableau shortcut

C1A1 shortcut



The screenshot shows the 'Image Corrector' software interface. The top menu bar includes 'File', 'Corrector PC', and 'Preferences'. The main window is divided into several sections:

- Measurement Section:** Contains three tables: 'Estimation', 'Latest accepted measurements', and 'Previous measurement'. Each table has columns for 'Value', 'Angle', 'Changes', 'Rel. size', 'Confidence', and 'Δt'.
- Visualization of test measurement:** A circular image showing concentric rings, with labels '18 mrad', '51 mrad', 'B2', and 'C3'.
- Total Output / Wobbling Section:** A table listing various parameters and their values in [mA].
- Correction Controls:** Includes a 'Time step' dropdown (set to 4h), a 'Color for Δt' dropdown, and buttons for 'Auto correction' and 'Manual correction'. The 'Manual correction' section has buttons for 'A1', 'A2', 'A3', 'B2', 'B3', 'S3', and 'Focus'.
- Log Section:** A text area at the bottom left showing kernel connection status and timestamps.

7. Select Tableau type to “Standard”, change Outer Tableau tilt [mrad] to 22 (or 24). Press **Start**. If the parameters except C1 is larger than Confidence, press **Accept**.
8. The program will automatically enter correction page. Select correction power (50% ~ 100%) and press the parameter (S3, B2, A2) that you would like to correct. Choose 100% correction power for lower order operation, like A2 and B2. Choose 50% correction power for higher order operation, like S3. Then press shortcut  to start Tableau measurement again, repeat Step 7 to 8 until the parameters are less than or close to confidence values.

Note: Order of correction (low order operation to high order of operation) is C1, A1, B2, A2, S3. Note: if after Tableau measurement, the value stays the same but the angle changes, which indicates overshoot. If this happens, lower correction power in correction page and re-measure in Tableau.

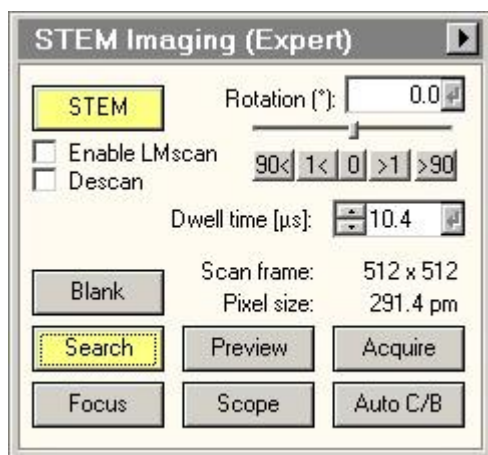
## EDS operation

TITAN Themis has a SuperX detector installed for energy dispersive x-ray spectrum collection. When doing EDS, use the double tilt holder and load the TEM grid with sample side facing down (e.g., the side with sample applied on).

1. Before switching to STEM EDS mode, make sure that the CCD camera is retracted and fluorescence screen is lowered.
2. Load a EDS setup in FEG Registers, e.g. “EDS 200 kV”.



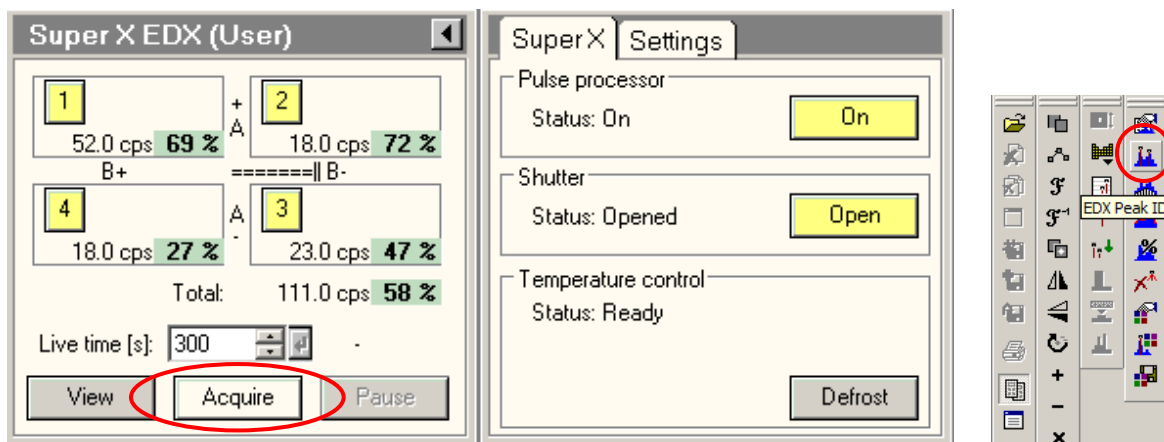
3. Click **Search** in **STEM>STEM imaging** to get an image in TIA. (Insert HAADF detector in **STEM** tab of UI if it's not inserted.) You may need to lower magnification to



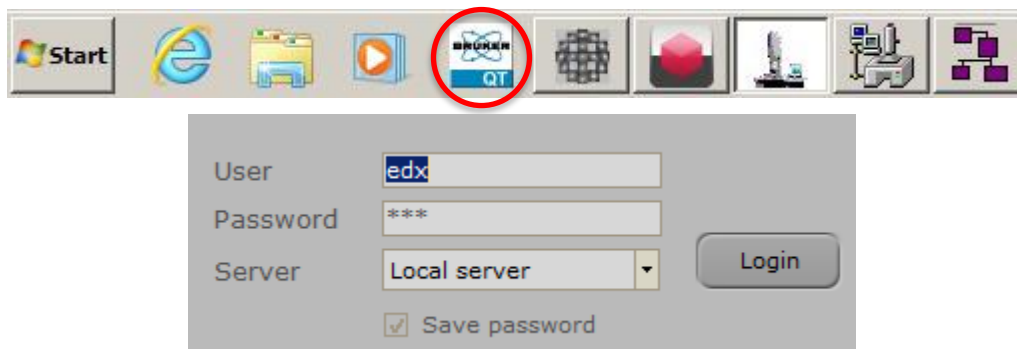


find sample. Adjust **Z** height to bring the image close to focus. Fine adjust **Focus** to make the image more in focus.

4. (Optional) For quick EDS measurement, open Super X EDX (User) in UI, move the beam position marker to where you want to collect EDS spectrum and start Acquire. Press EDX Peak ID to get elemental analysis. Stop Acquire after getting enough counts.

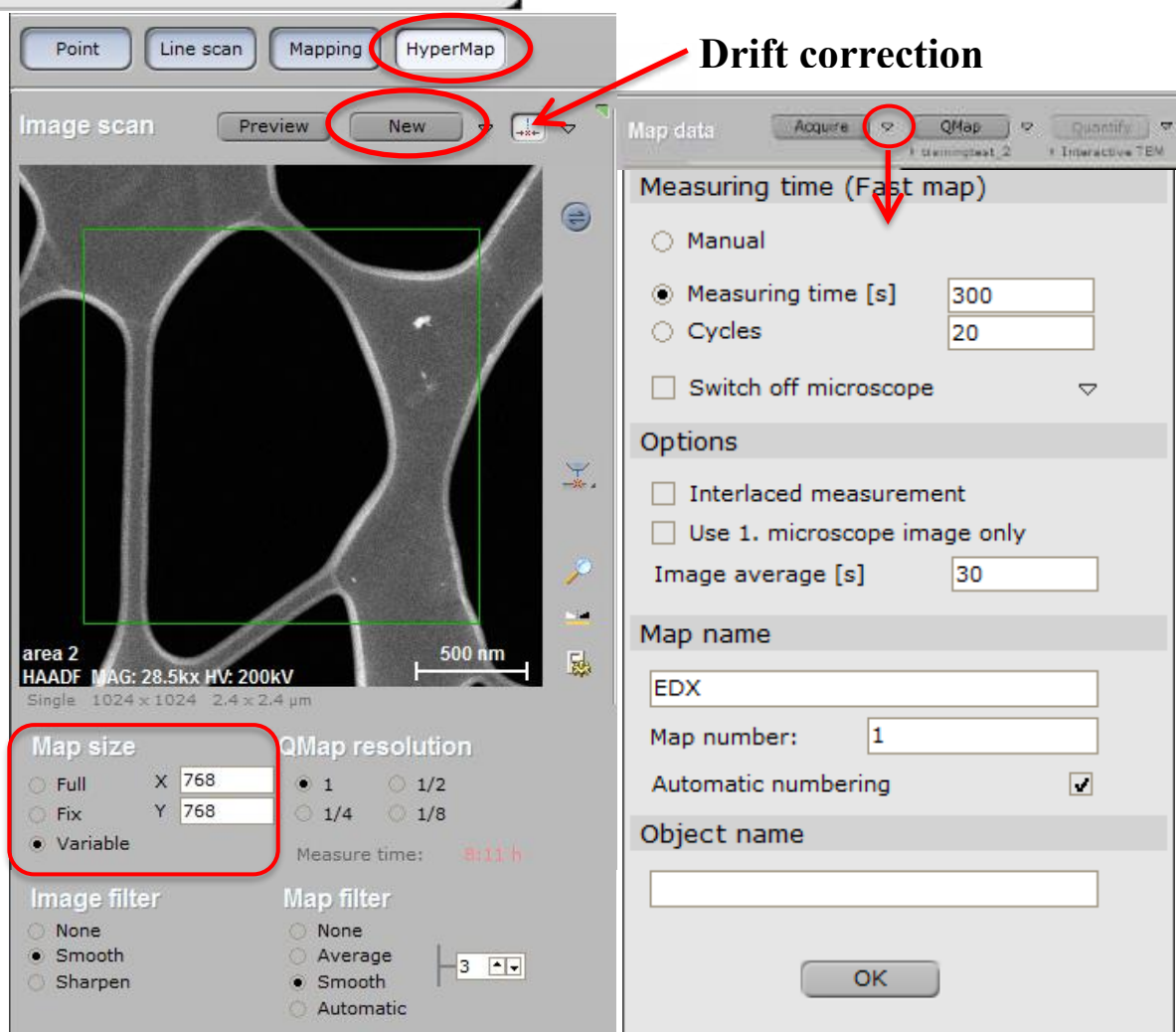
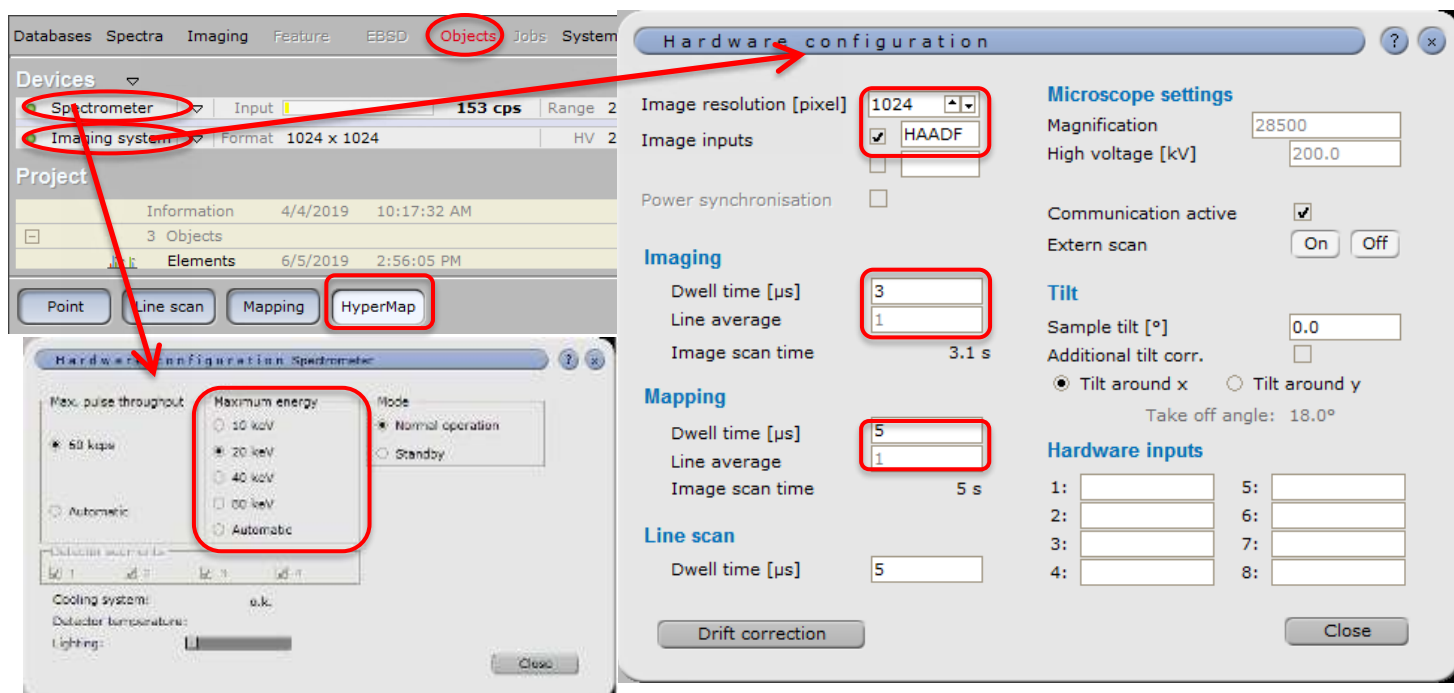


5. For advanced EDS measurement, open Esprit software. Start the Esprit software by double clicking desktop icon or by clicking icon in the Windows quick launch panel, log in with default username and password.

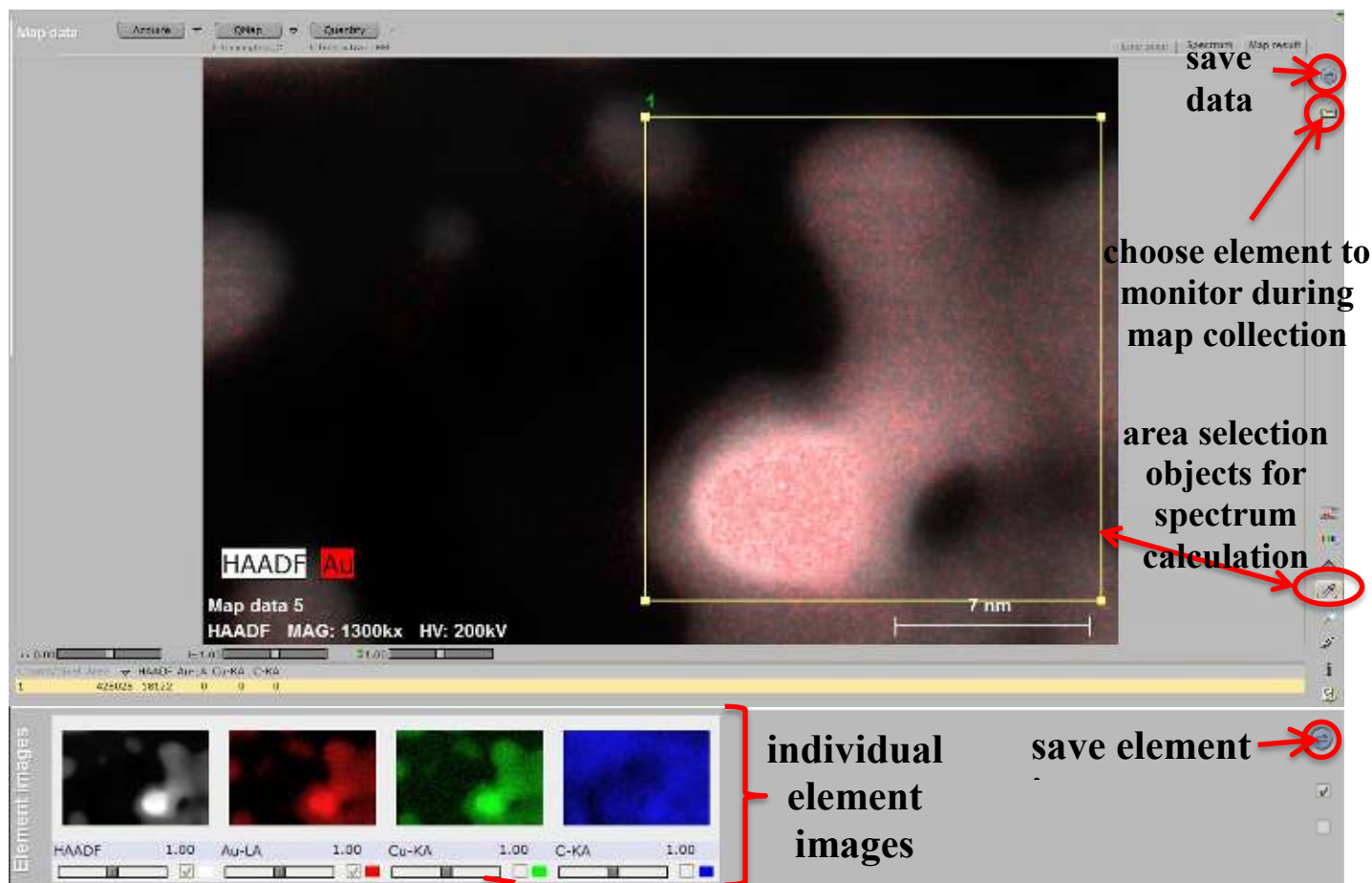


6. Select **Objects>HyperMap** in workspace, set up energy range (20 keV is good for most application) in **Spectrometer** page and resolution (1024), detector (HAADF), Mapping dwell time (5-20 us) in **Imaging system** page.
7. Click **New** in **Image scan** panel to obtain a STEM reference image. Drag the corner of the box to select area to collect fast map and activate Drift correction button.  
Note: there are rare occasions when the reference map cannot be collected but you can still collect map. Restart the computer will solve the problem. Ask EM staff for help.  
Note: there are three methods to define map size.
8. Click the **triangle icon** to the right of **Acquire** button to set up acquisition time.  
Manual: acquire EDS hypermap until user clicks **Stop**.  
Measuring time: set up total acquisition time.  
Cycles: set up number of frames to acquire and integrate for EDS hypermap.  
Note: during data acquisition process, clicking **Stop** once will terminate acquisition after the current frame and double-clicking **Stop** will terminate acquisition immediately.  
Note: reducing the gun lens number and beam spot size number can increase the beam intensity, thus enhances the x-ray counts per second.



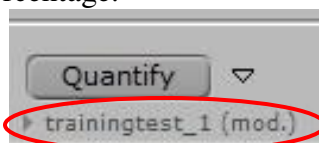


9. Click **Acquire** to collect hypermap of selected area. A status bar with remaining time is displayed in the upper right corner of the software interface. Select elements (multiple selection) from flap-out periodic table to monitor hypermap acquisition progress.
10. Save hypermap database and individual element images.

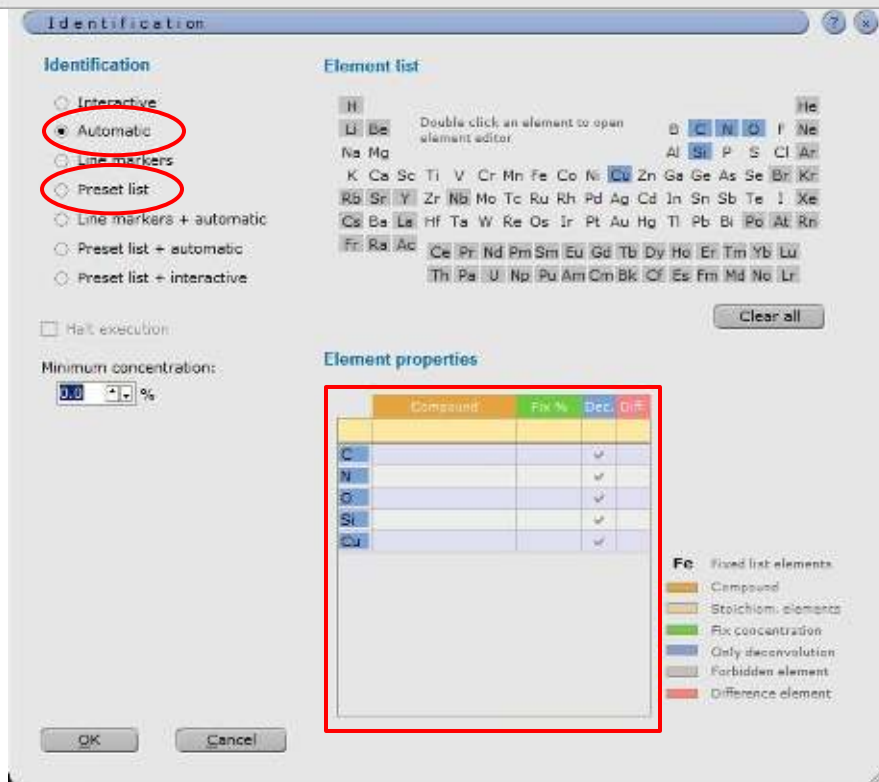
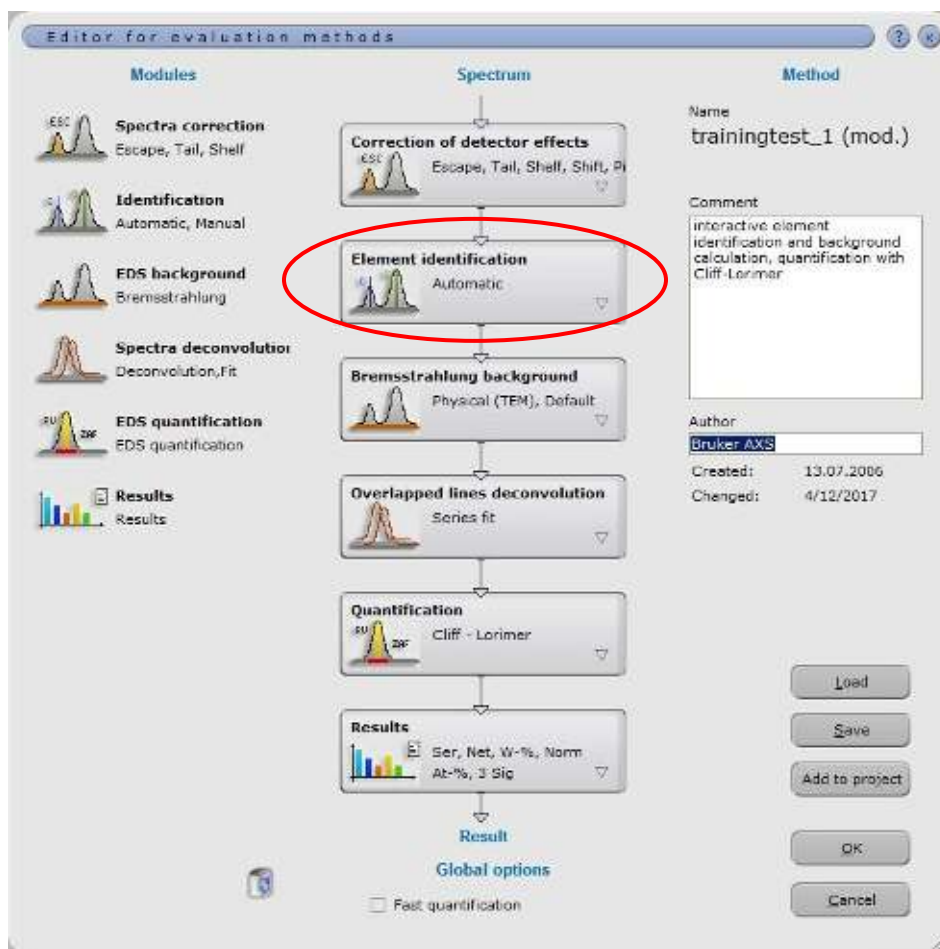


display check box

11. Use area selection objects to select full or partial map for spectrum calculation and click Spectrum tab to show spectrum.
12. (Optional) Use **Quantify** to calculate the weight or atomic percentage of selected elements: click the method in grey under Quantify button, check Element identification, you can choose “Automatic” or select elements using “Preset list”. Click **Quantify** button to calculate the elemental percentage.

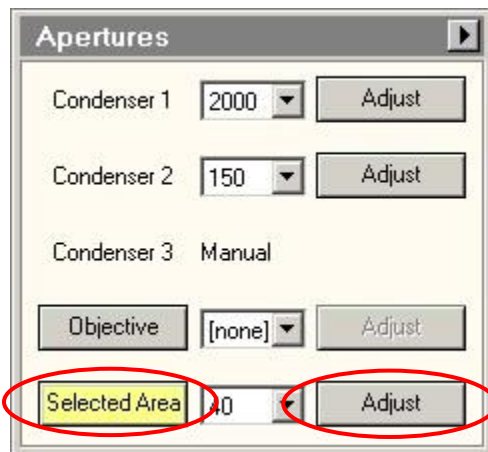


Note: you can check Deconvolution (“Dec.”) of some elements to exclude them in the final calculation.



## Selected Area Diffraction operation

1. In TEM mode, center the area of interest in the CCD image. Insert a Selected Area Aperture with preferred size (e.g. 40 or 10), center the aperture by activate **Adjust** and use **MF X/Y**.



2. Make sure the fluorescence screen is lowered. Press **Diffraction** button on the right control pad to enter diffraction mode. Adjust **Camera length** and **Focus** to get sharp diffraction pattern in proper size.
3. Insert the beam-stop. Move the diffraction pattern by **Direct Alignments>Diffraction alignment** using **MF X/Y** to get the central diffraction spot blocked.
4. Lift the fluorescence screen and use CCD camera to take the diffraction pattern image (exposure time 0.5s or less).

Note: you can tilt the sample in Alpha and Beta angles to align it with desired zone axis, after that, readjust Eucentric Height.

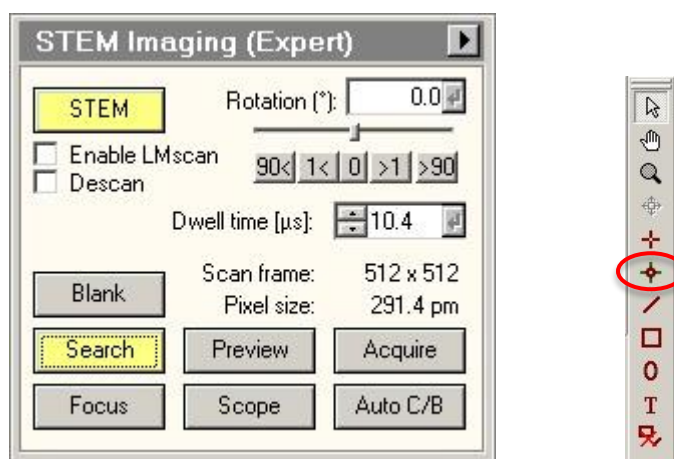
5. When finishing, put down the fluorescence screen, retract the Selected Area Aperture and the beam-stop. Return to TEM mode by pressing **Diffraction** button.

## Nanobeam Diffraction operation

1. In TEM mode, locate the area of interest in the CCD image.
2. Make sure the fluorescence screen is lowered. Load a NBD setup in FEG Registers.

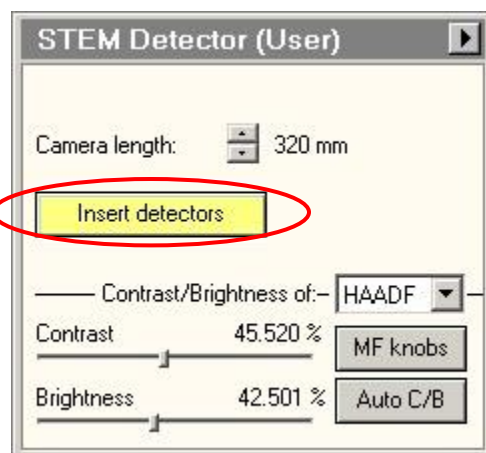


3. Insert HAADF detector in **STEM** tab of UI. Click **Search** in **STEM>STEM imaging** to get an image in TIA. You may need to reduce magnification to find sample. Adjust **Z** height to bring the image in focus. Fine adjust **Focus** to make the image more in focus.



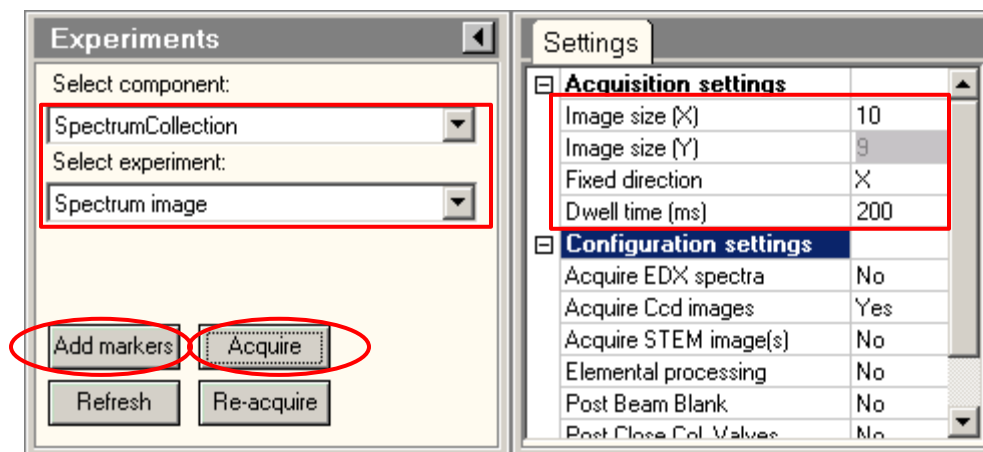
4. Stop the Search in STEM, and put a beam position marker to where you want to take Nanobeam diffraction pattern.
5. Insert the beam-stop. Move the diffraction pattern by **Direct Alignments>Diffraction alignment** using **MF X/Y** to get the central diffraction spot blocked.
6. Retract the HAADF detector in STEM. Lift the fluorescence screen and use CCD camera to take the diffraction image (exposure time 0.5s or less).





Note: you can tilt the sample in Alpha and Beta angles to align it with desired zone axis, after that, readjust Eucentric Height.

Note: you can use **Experiments** to acquire Spectrum image in a matrix. **Add markers** to the region where to take Nanobeam diffraction patterns and press **Acquire** to start. When finish, save the data base. Put an “Image position marker” on the STEM image, press “Ctrl” + “Alt” + “Shift” and drag the diffraction image to the image position marker to link them.



7. When finishing, put down the fluorescence screen and retract the beam-stop. Return to TEM mode by loading a TEM setup in FEG Registers.

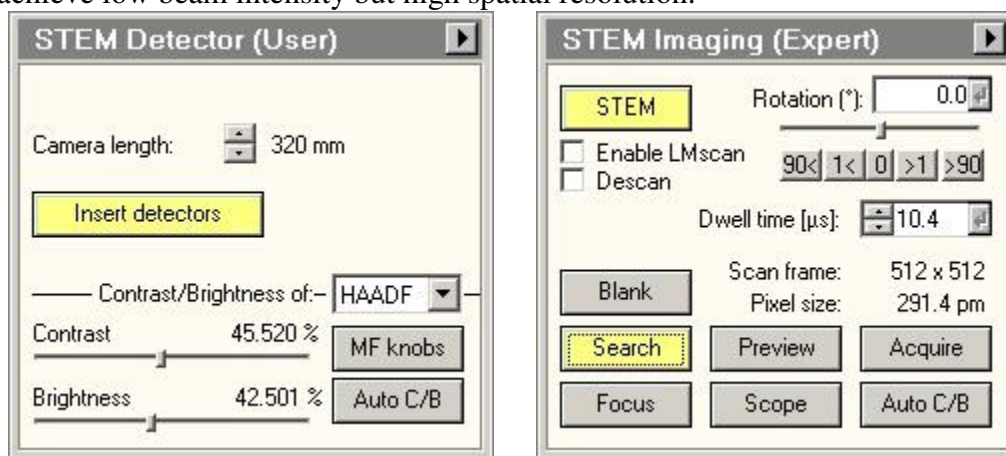
## STEM Alignment

Start from TEM mode (microprobe)

1. Locate area of interest in TEM mode. Finished the TEM alignments. (Cs image corrector is not necessary).
2. Bring the sample to Eucentric height using one of the three methods (step 2 in TEM Alignment).

Alignment in STEM mode (nanoprobe)

3. Make sure the fluorescence screen is lowered. Load a STEM FEG register. Note: default STEM mode is “Diffraction”. Note: Larger spot size number and smaller C2 aperture aim to achieve low beam intensity but high spatial resolution.



4. Choose HAADF detector in STEM tab of UI. Set camera length to 210 mm. Click **Search** in **STEM>STEM imaging** to get an image in TIA. You may need to reduce magnification to find sample. Adjust Z height to bring the image in focus.
5. Center an area that is not region of interest (ROI) but contains some features (This region will be sacrificed in alignment). Check if there is condenser stigmatism: click **Focus** in **STEM>STEM imaging** to focus on a small area, Using Focus knob (you may need to use large focus step, e.g. 5) to wobble on both sides of focus to see whether there is image stretching. If yes, activating **Condenser Stigmatism** in **Stigmator** panel and using **MF X/Y** to correct condenser stigmatism.
6. Stop **Search**. Put a “Beam Position Marker” on the image. Double click the Beam Position Marker and set its position to 0,0 nm (uncheck “display in pixel first”). Park beam at amorphous carbon area to avoid damaging area of interest by beam.
7. Click “Diffraction” on the right control pad to enter Imaging mode.
8. If the beam (probe) is not visible, reduce magnification to find the beam, center it with **Beam Shift** in **Tune>Direct Alignments (MF X/Y)**.
9. Increase magnification to SA ~500 kx and center beam with **Beam Shift (MF X/Y)**. If the probe is not circular. Make it circular by adjusting **Objective Stigmatism (obj stig)** in **Stigmator** panel. Sometime you need to use **Focus** knob to focus beam and then adjust obj stig. Note: this assumes that condenser stigmatism has been corrected in Step 5.
10. Center condenser (C2) aperture (50 um): Expand the beam with **Focus** knob to check whether the beam expands concentrically. If not, adjust condenser aperture (C2) so that

- the beam expands concentrically when changing **Focus** knob. Note: In STEM mode, **Focus** knob is linked to intensity and **Intensity** knob is not functional.
11. Over focus (turn **Focus** knob clockwise to expand beam) the beam (probe) to see caustic image which contain a bright beam dot (hot spot) within halo. Note: You may not see the hot spot in the center, what you normally see is that certain part of halo edge is brighter than other area, this is ok since you will center the hot spot in step 13.
  12. Click **Rotation Center (Intensity)** in **Direct Alignment**. Stop beam wobbling by turning **Focus Step** knob counter-clockwise.
  13. Adjust **MF X/Y** to center the hot spot within the halo. Note: this step assumes that C2 aperture is centered in step 10.
  14. Focus the beam to a spot (a spot with high intensity within the halo). Defocus can be reset by R2 button. Adjust **Beam tilt pp X/Y** in **Tune>Direct Alignments (MF X/Y)**: align the bright tips of the two images.
  15. Center beam with **Beam Shift** in **Tune>Direct Alignments (MF X/Y)** if necessary.
  16. Using **Focus** knob to shrink/expand beam on both sides. While doing so check the following:
    - a. Beam expands concentrically (C2 aperture is centered);
    - b. Hot spot is in the center of the halo (rotation center);
    - c. Objective stigmatism is corrected (probe to be circular).
 If not, repeat step 9 -15.

#### Fine Tuning and Imaging

17. Click “Diffraction” on the right control pad to return to diffraction mode. Park the beam position marker away from ROI (usually at one of the four corners)
18. Click “Search” in STEM imaging, press “Eucentric Focus” and bring the sample to focus using Z height control
19. Watch the STEM image, carefully adjust focus and condenser stigmatism to achieve the sharpest image (no image stretch when changing focus on both sides), see step 5 for details.
20. Acquire STEM images.

#### Extra: Using Ronchigram to Adjust Focus and Condenser Stigmatism

1. After step 18 find an amorphous area and center it in the frame, stop **Search**, and park the Beam Position Marker at the center of the frame. See step 6 for details.
2. Change Camera Length to 510 mm. Watch Ronchigram on the screen. If Ronchigram is not centered and might be partial blocked by HAADF detector, using MF X/Y to center the Ronchigram.
3. Focus and carefully correct condenser astigmatism with condenser stigmator: use **Focus** knob to wobble on both sides of focus and minimize any stretching inside Ronchigram. Note: when showing Ronchigram, Focus knob is to zoom in/out of sample images, zoom in from both sides of focus to blow-up-point to check condenser stigmatism (stretched image change direction from one side to another; bright disk circular in center with dark ring indicates good condenser stigmatism).
4. Change camera length back to 210 mm.
5. Click **Search** in **STEM>STEM imaging** to find the region of interest, focus, and acquire STEM images.