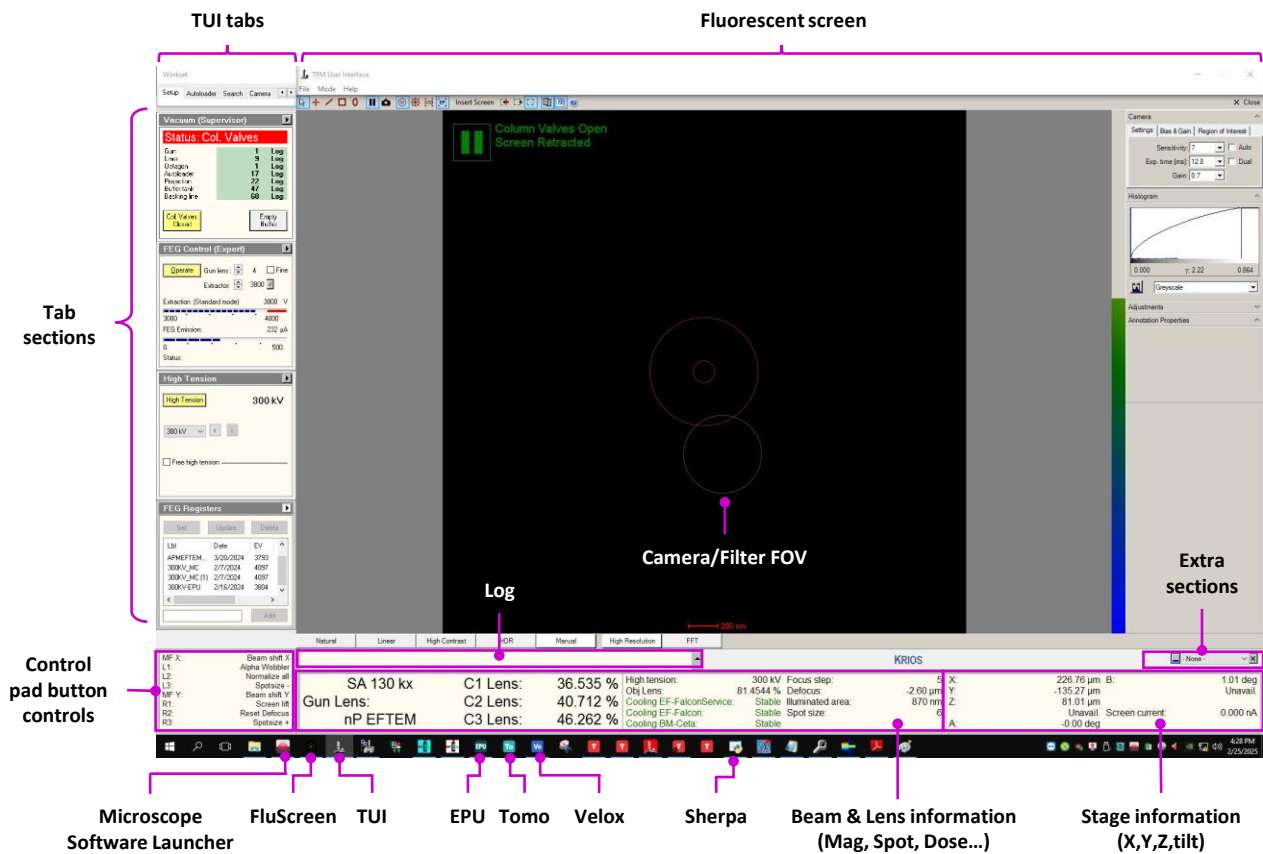


TEM4 Basic Protocol

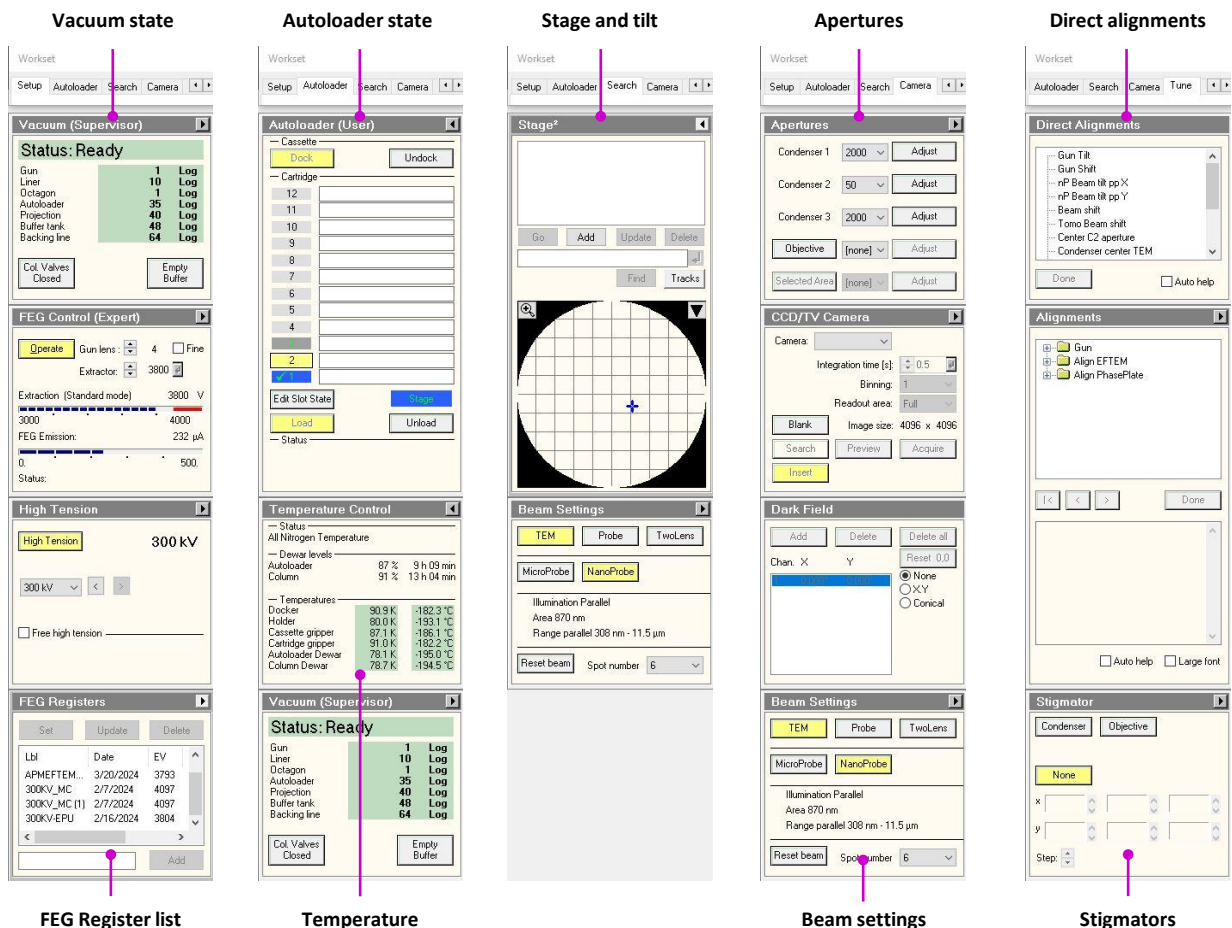
Pictographic Version
TFS Titan Krios G2 + SelectrisX + Falcon4i

David Fernandez-Martinez, PhD
Stanford CryoEM Center (CEMC)
2025

The TEM User Interface (TUI)



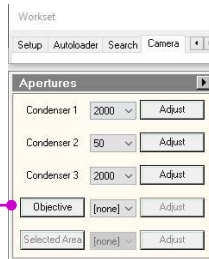
TUI Tabs and sections, in order



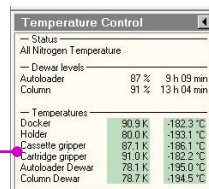
Initial Microscope Setup

1 Preliminary checks

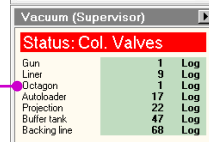
Objective aperture should be out



Temperatures should be around -180C



Optimal vacuum values



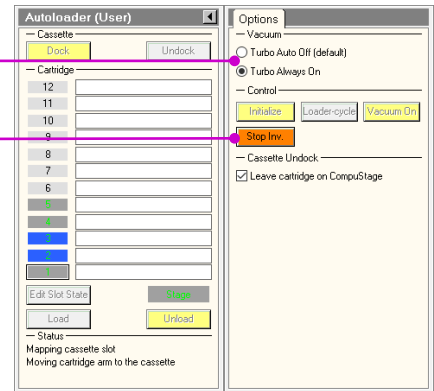
Column valves closed



2 Loading grids

Turn Turbo ON for Inventory and OFF after

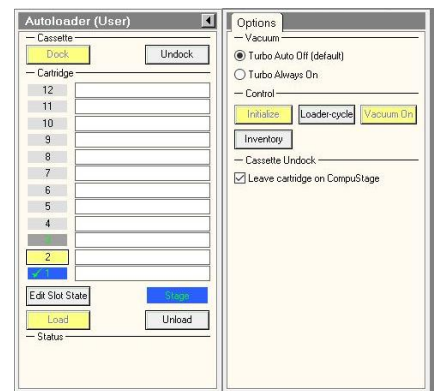
Inventory will recognize loaded grids



Grids should be recognized after Inventory

Blue: Slot with Grid
Gray: Slot without grid
Yellow: Grid on column

Load a grid (Preferably X-grating or carbon) to start setting up your session



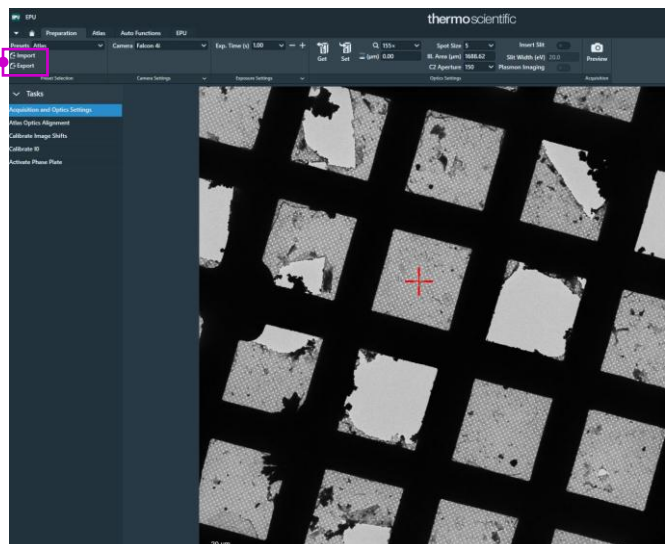
Initial EPU setup

1 Establish the magnification presets for your specific grid

- Import presets from Preset folder before moving forward

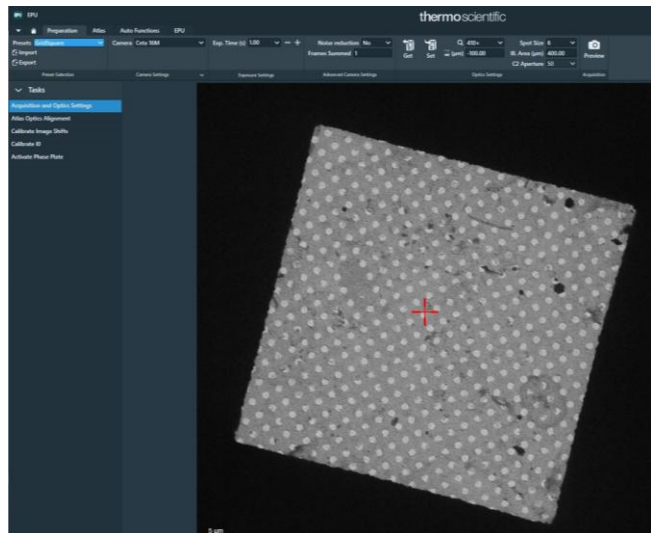
Atlas preset

- Lowest magnification
- Make sure beam fully covers camera
- ~16 squares on FOV



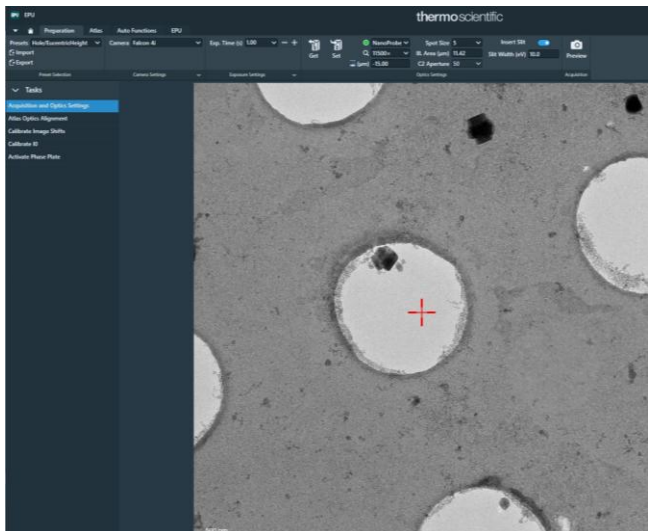
Gridsquare preset

- LM preset
- Auto-eucentric before taking image
- Make sure beam fully covers camera
- One full square on FOV when centered
- Sufficient contrast to clearly identify foil holes



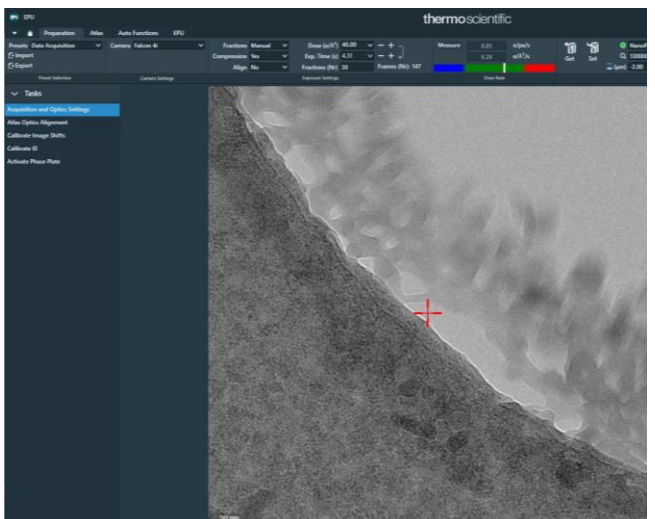
Hole/Eucentric preset

- Hole Centering, Auto-eucentric Height and Template Definition preset
- Contrast should allow to clearly see holes, use spot size and defocus
- Ideally 1 hole in FOV, with parts of surrounding 8 holes

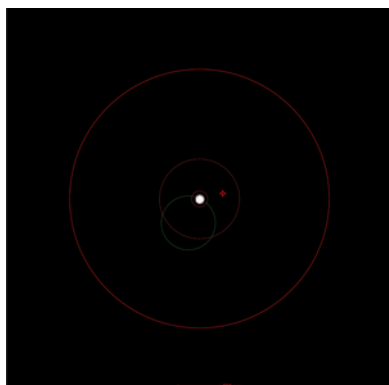
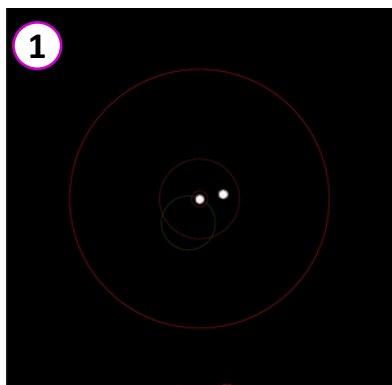


Data acquisition preset

- Magnification used for collection and focus
- Sample dependent, make sure you see sufficient particles per FOV
- Dose rate of 40-60 e should allow for sufficient contrast
- Adjust Spot Size and Intensity to place dose in green section of dose rate bar
- Link remaining presets to this one

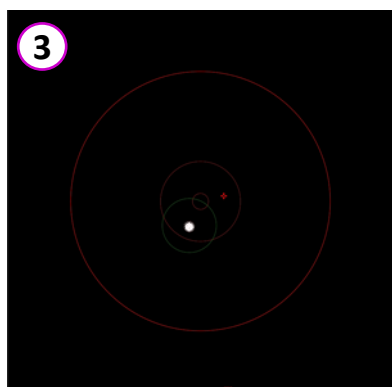
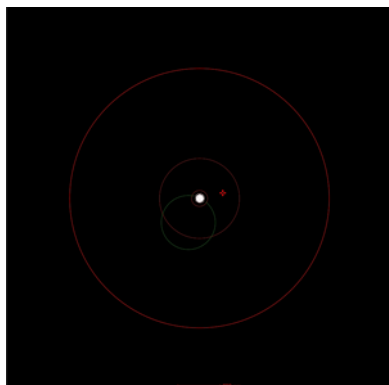
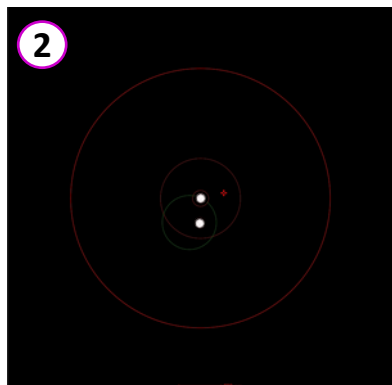


- Press “Eucentric Focus” on Control Pad, center the beam and cover camera with beam beforehand
- Use fluorescent screen with screen down (Press “Insert Screen” or R1 in control pad)



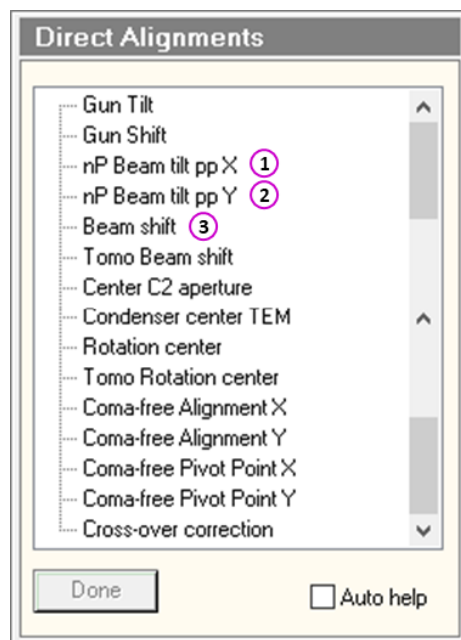
nP Pivot points X/Y

- Smallest beam size
- Correct with Multifunction X
- Make beam 1 spot



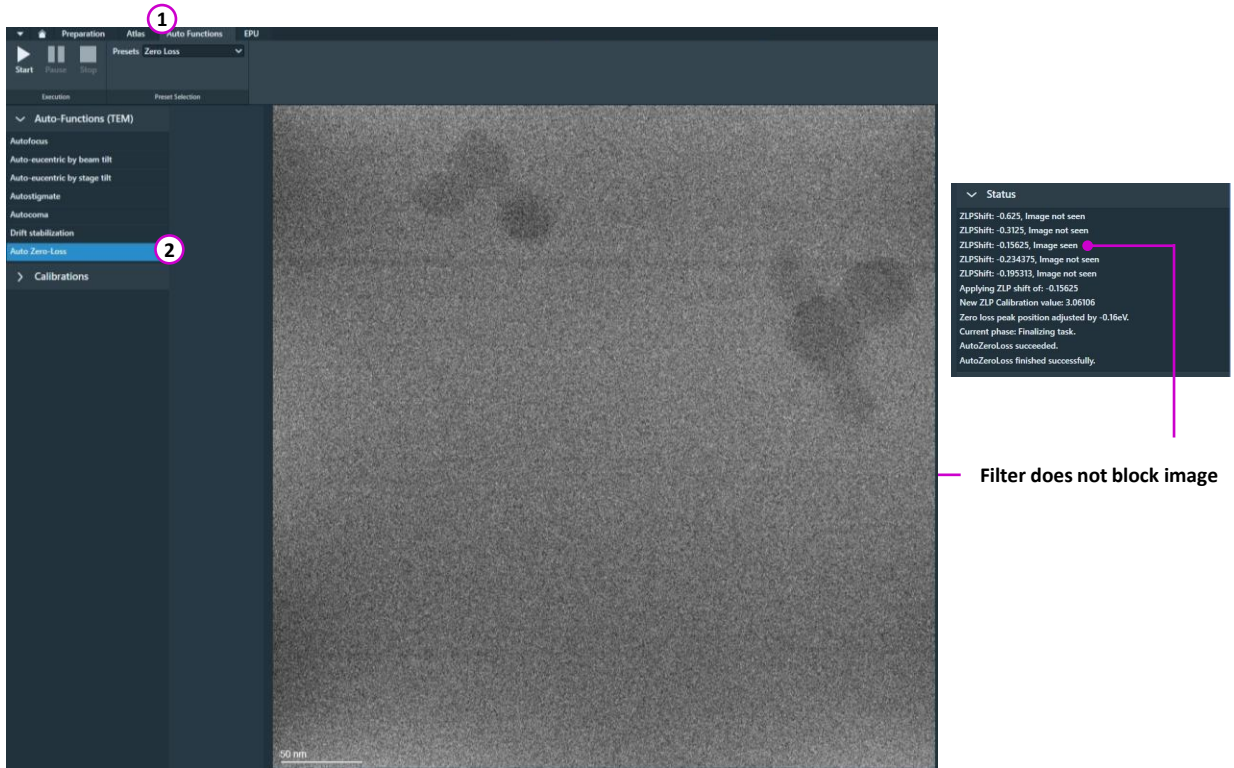
Beam Shift

- Small spot
- Center on filter
- Do after each step



3 Zero-loss peak centering

- While still in the empty square, center the zero-loss peak of the SelectrisX energy
- Make sure the beam is centered and covers the green circle on the fluorescent screen
- If it does not work or filter needs calibration, contact staff to use Sherpa

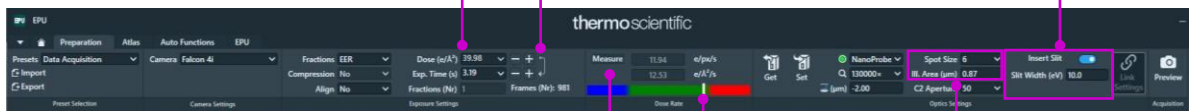


4 Adjust Dose in Data Acquisition Mode

- In Data Acquisition preset, make sure you are on an empty area
- Ensure beam is centered on camera using Fluorescent Screen

1. Make sure Filter is set to 10 eV

2. Set target dose (40-60 $e^-/\text{\AA}^2$) with arrow pointing down

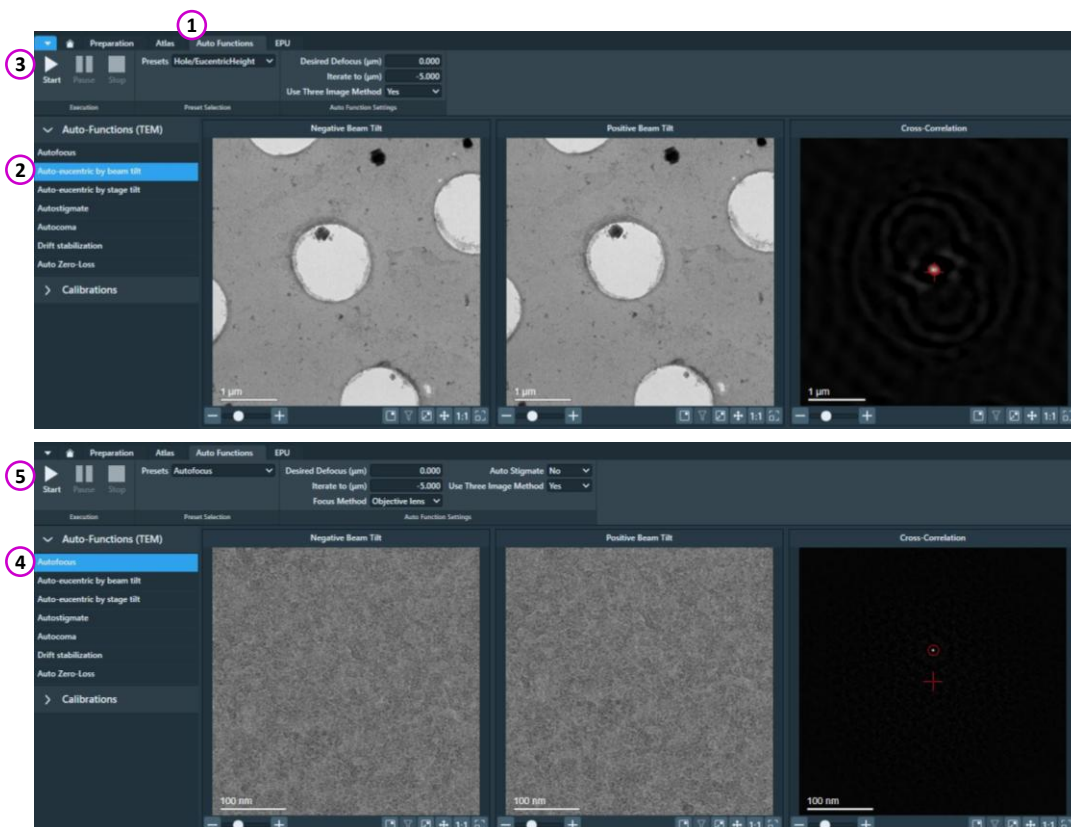


3*. Change beam diameter or spot size Repeat Measure if needed



5 Set Optics Settings For Image Shift Calibration

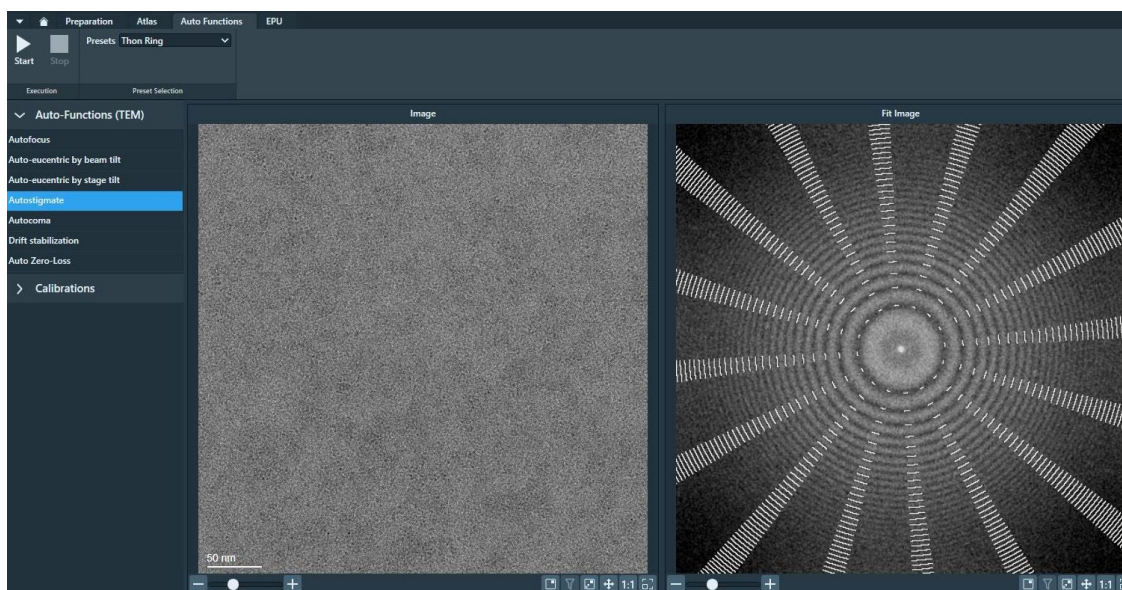
- Navigate to a square with a feature that can be identified at all Presets, then run Auto-eucentric and Autofocus jobs



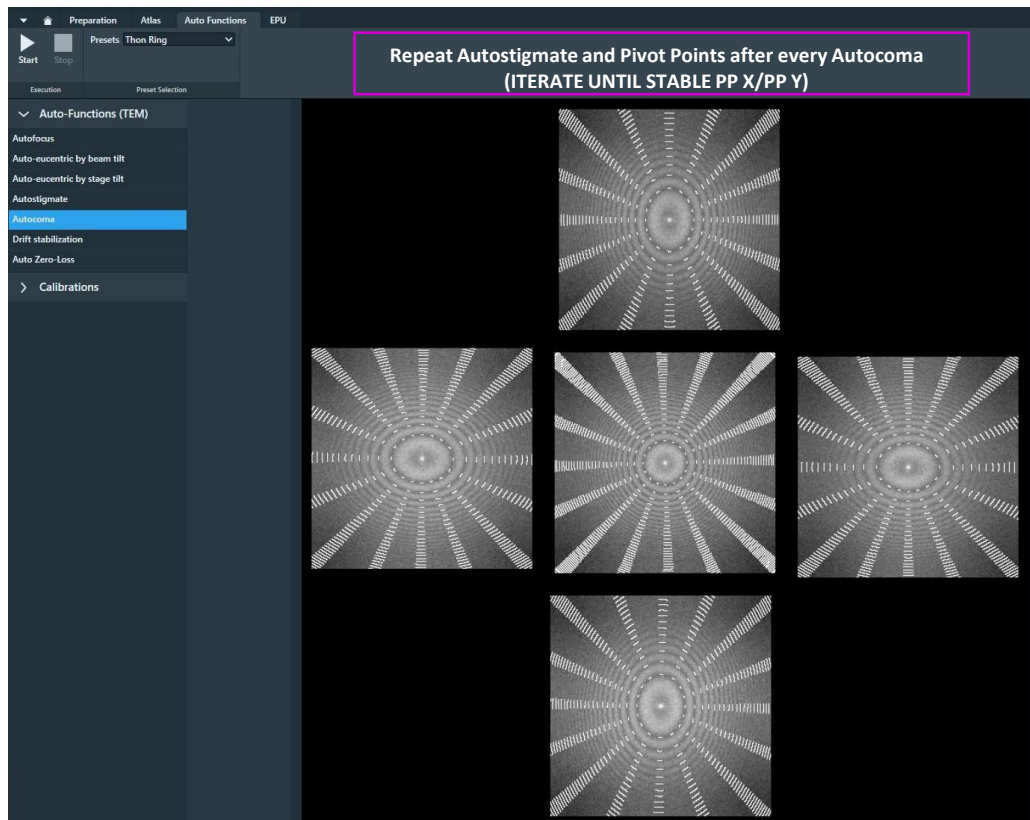
6 CTF correction: Objective Stigmatism and Coma-Free Alignment

- Corrections must be performed on carbon
- Only try Sherpa (Objective Stigmatism/Coma) if EPU fails. Ask staff if you need help.

Objective Stigmatism



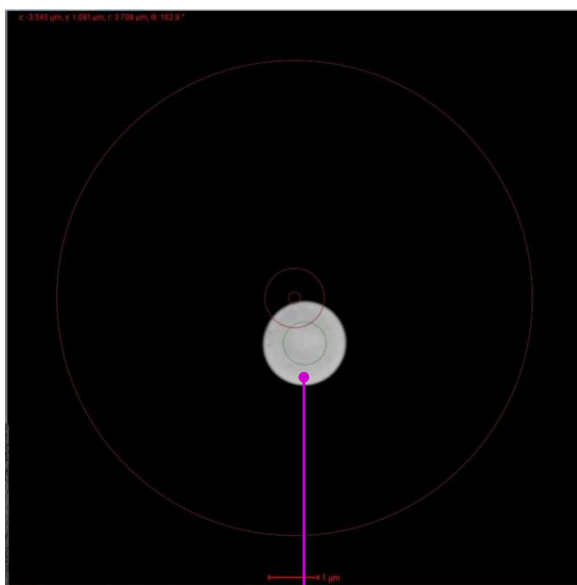
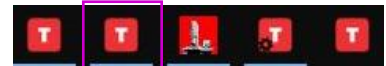
Coma-Free Alignment/Coma Correction



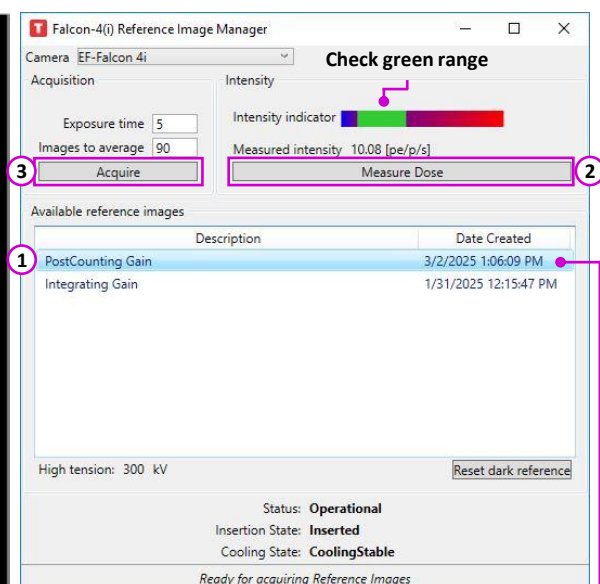
7 Gain Reference Acquisition

- Ensure that the beam is on empty square and at Data Acquisition magnification

Falcon 4i Reference Image Manager



Center beam on energy filter (green circle)

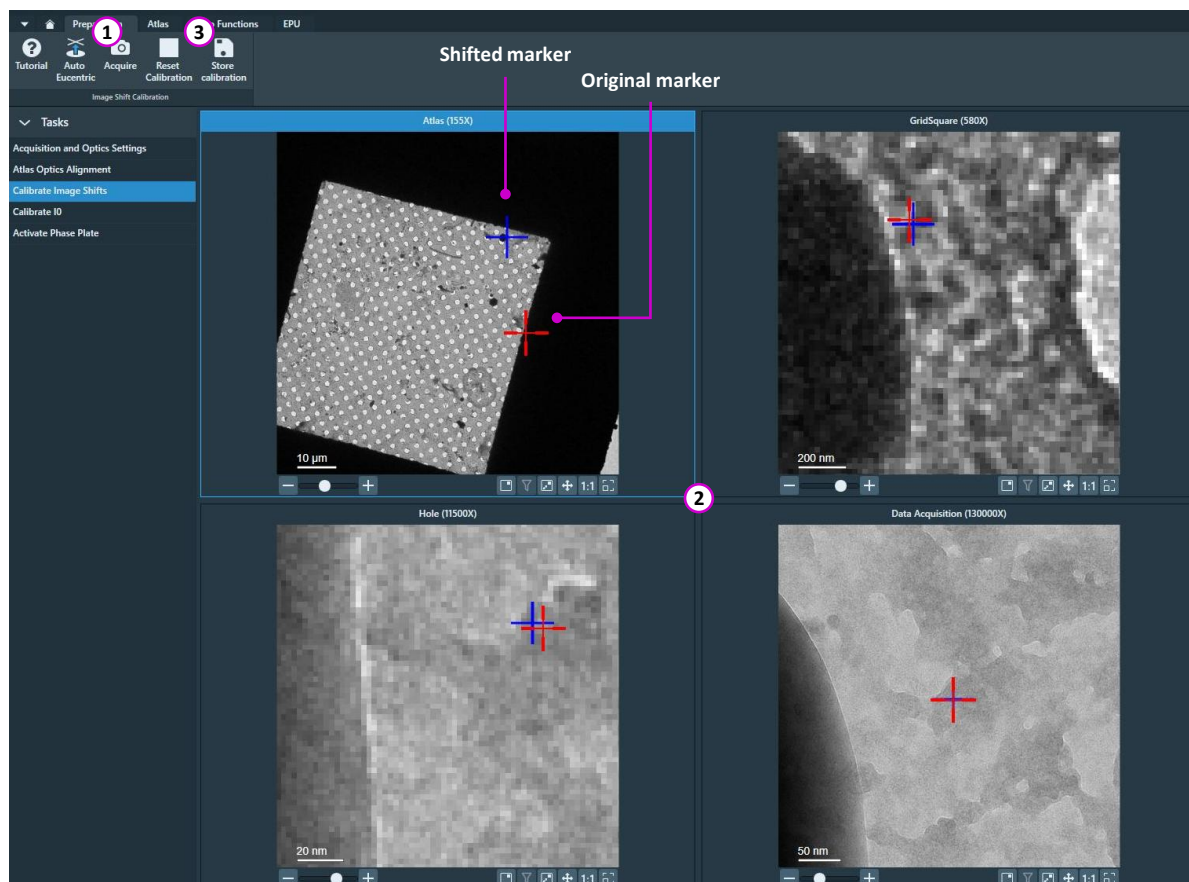


Gain is Stable for around 1 month

Gain Reference File Path: C:\Titan\Data\EF-Falcon4\Reference Images\300kV_EER_GainReference.gain

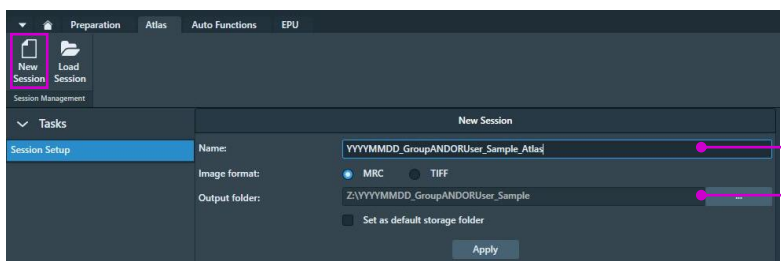
8 Image Shift Calibration

- Find a recognizable feature at all magnifications and center it at Data Acquisition magnification
- Double-click on same feature in every magnification



Atlas Acquisition

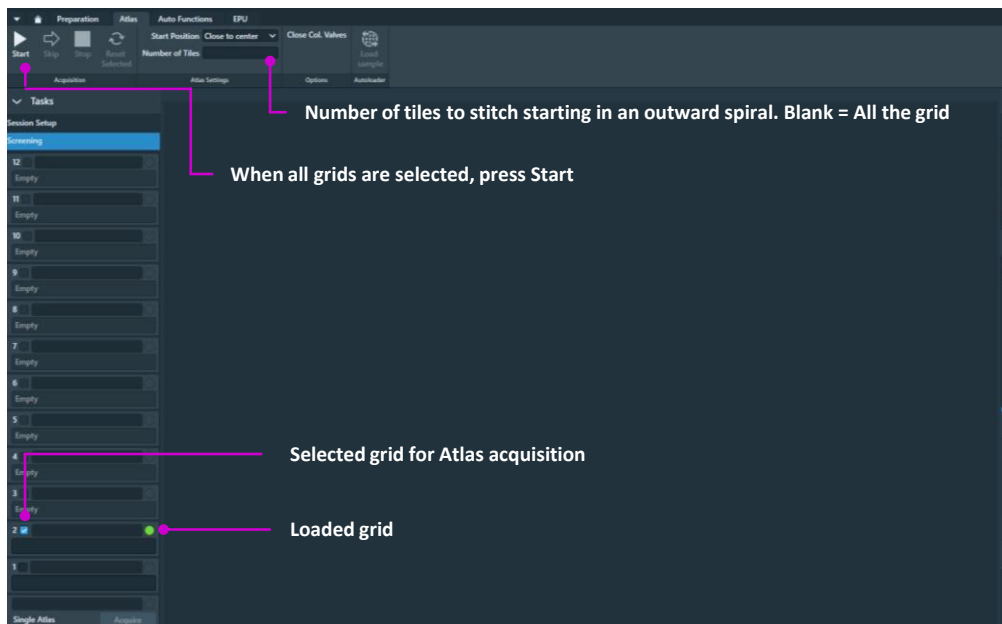
1



Follow the proposed naming convention for ease of search:

YYYYMMDD_CSXX_userInitials_sample

2



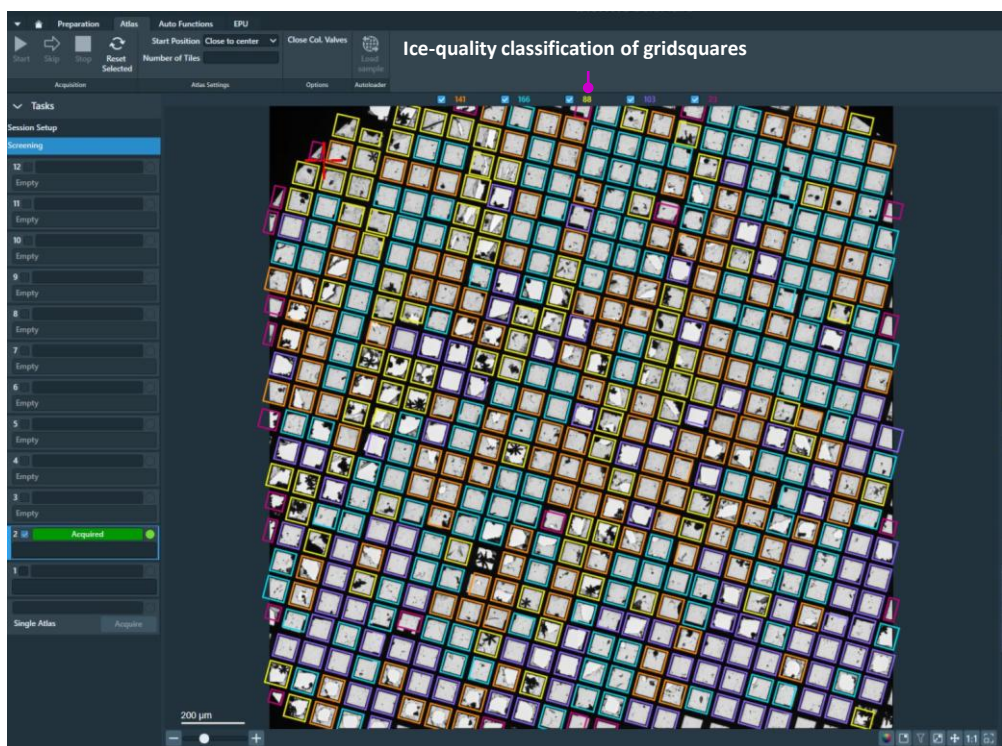
Number of tiles to stitch starting in an outward spiral. Blank = All the grid

When all grids are selected, press Start

Selected grid for Atlas acquisition

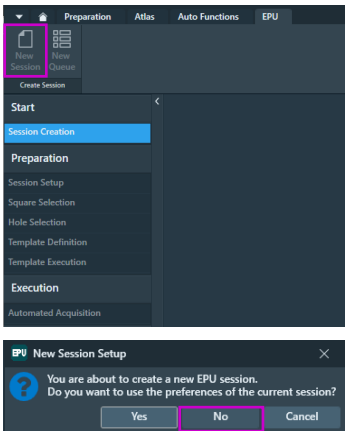
Loaded grid

3

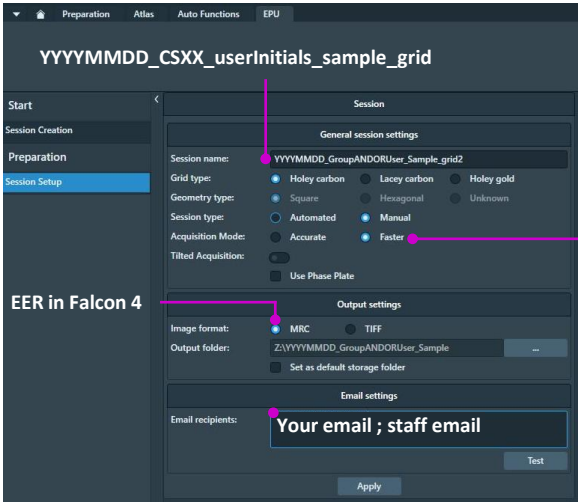


Setting an EPU session

1 Create a session

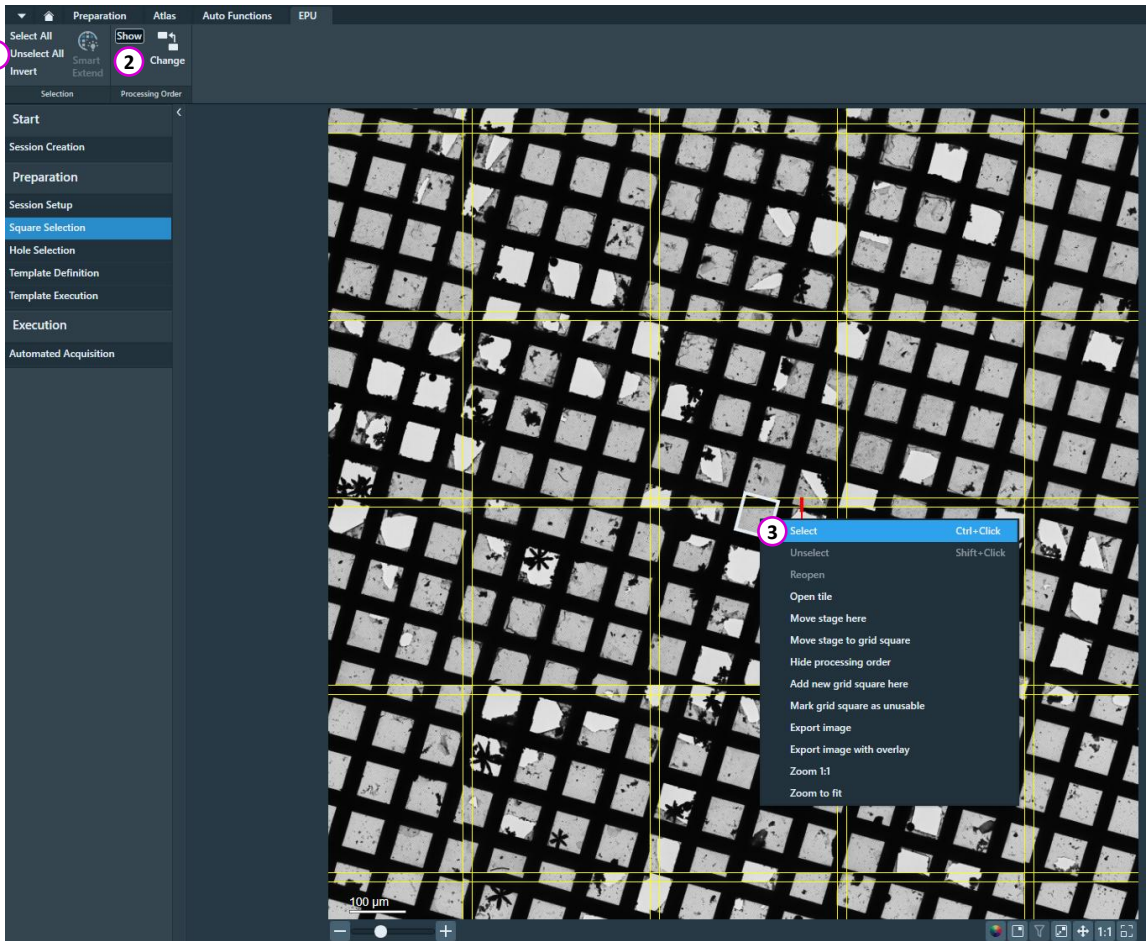


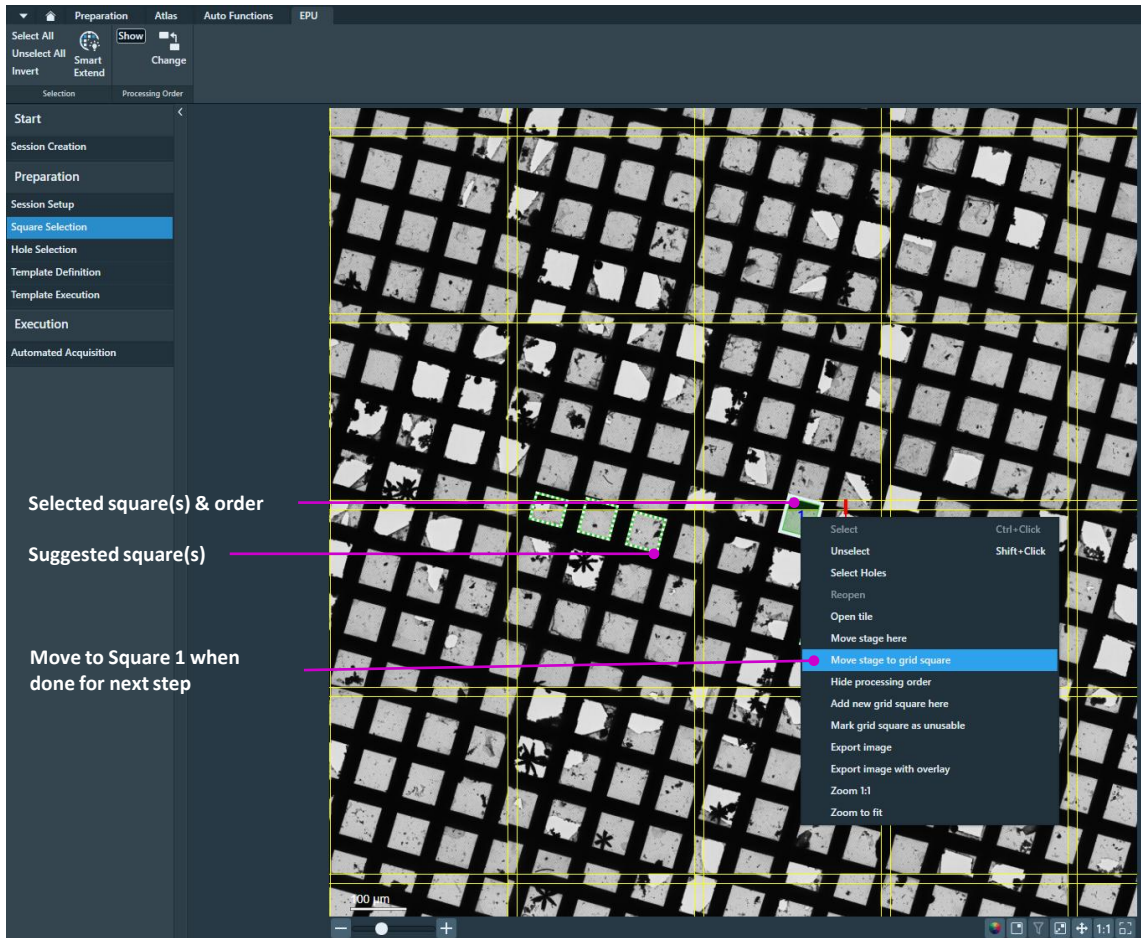
2 Determine session settings



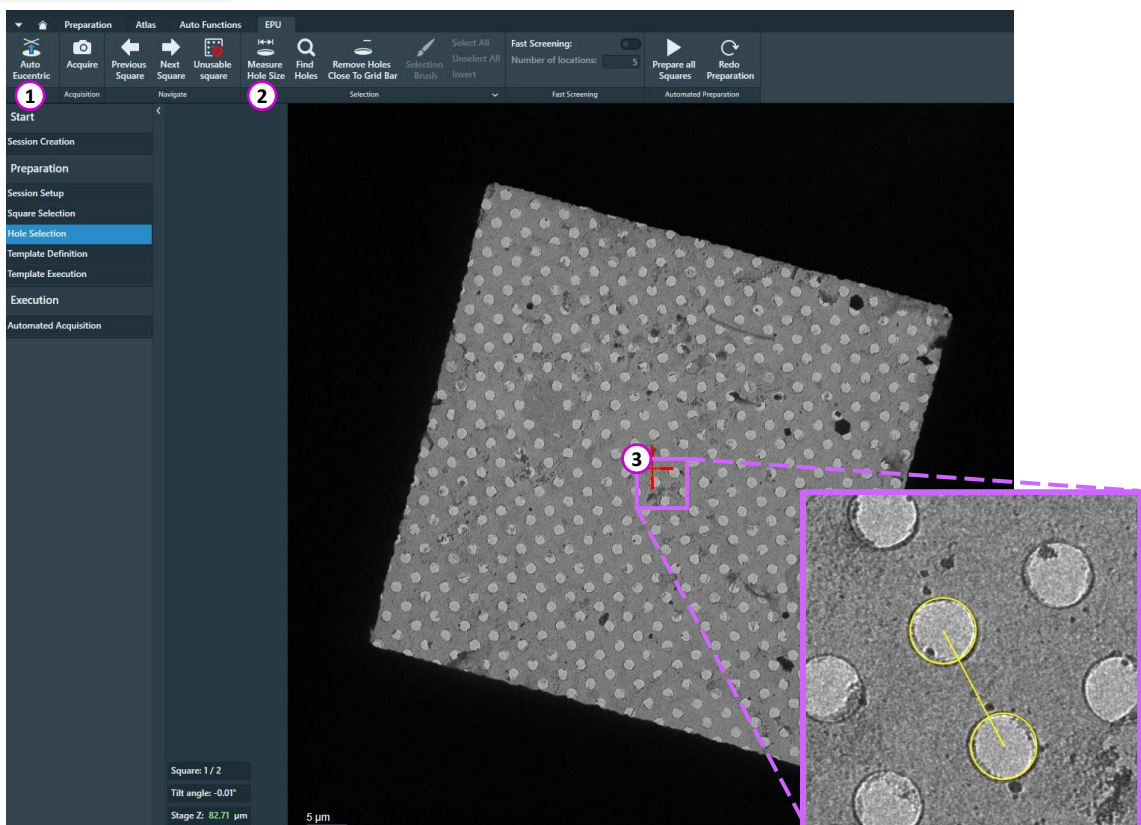
AFIS mode (300-500 exposure/hr)
Requires good alignments

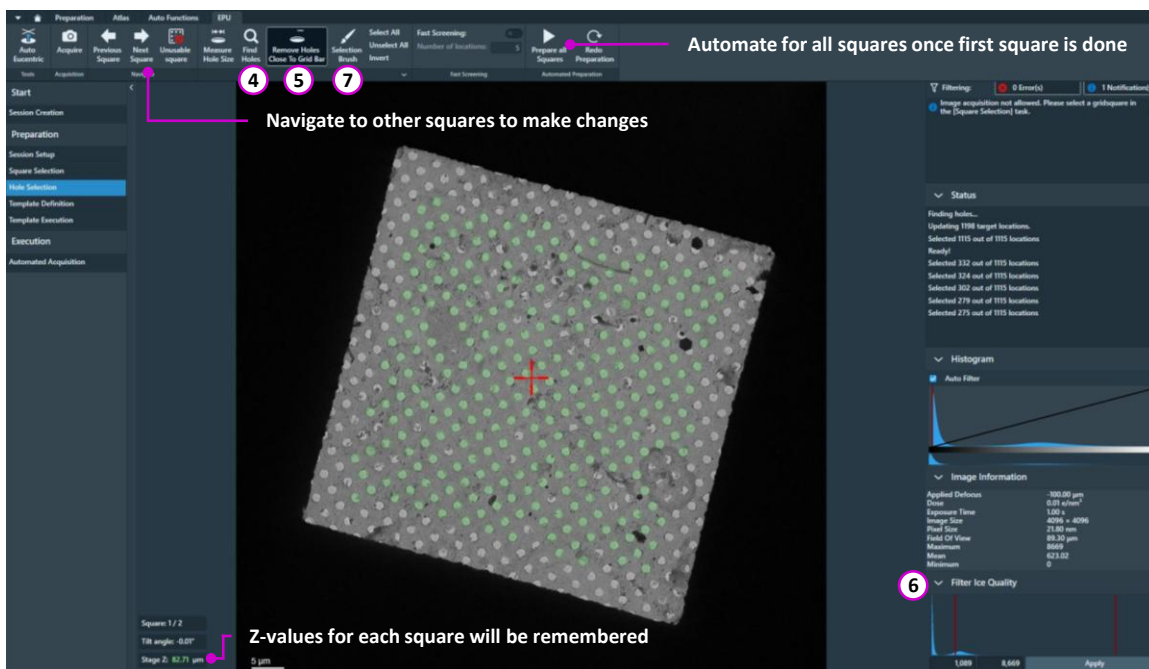
3 Square selection





4 Hole selection





5 Template Definition

Add defocus values separated by comma

Copy to all exposures

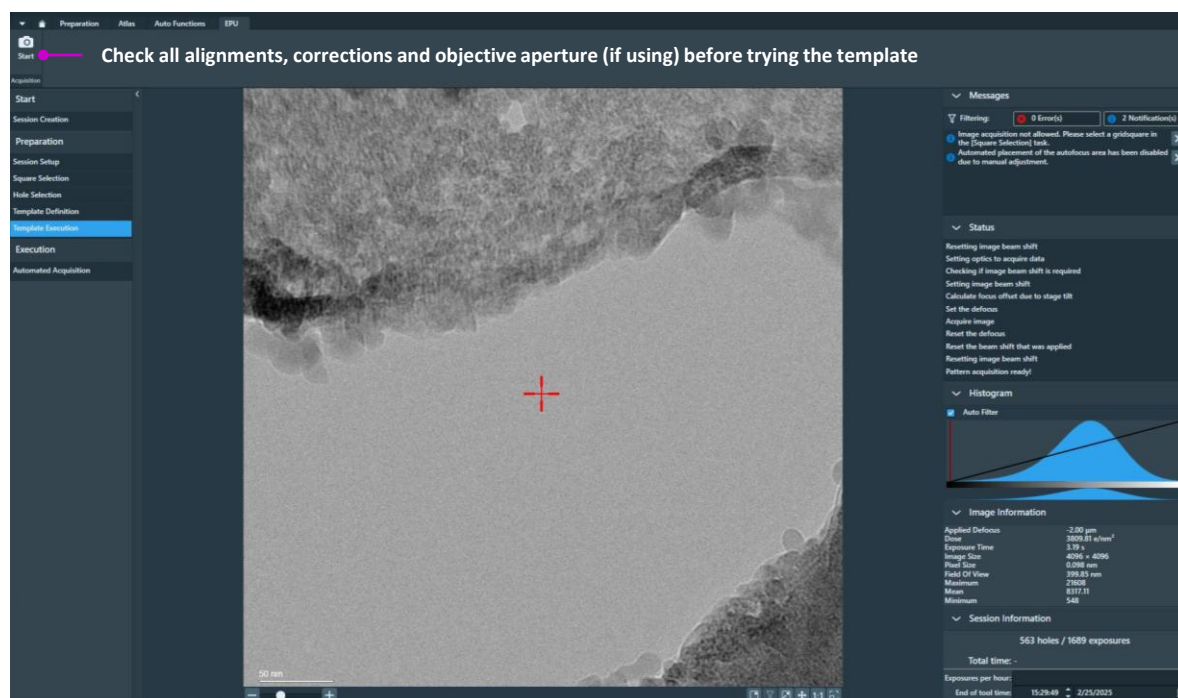
1 Acquire 2 Find And Center Hole 3 Acquisition Area

Recurrence Always
Drift Threshold (nm/s) 0.20
Drift Measurement Area

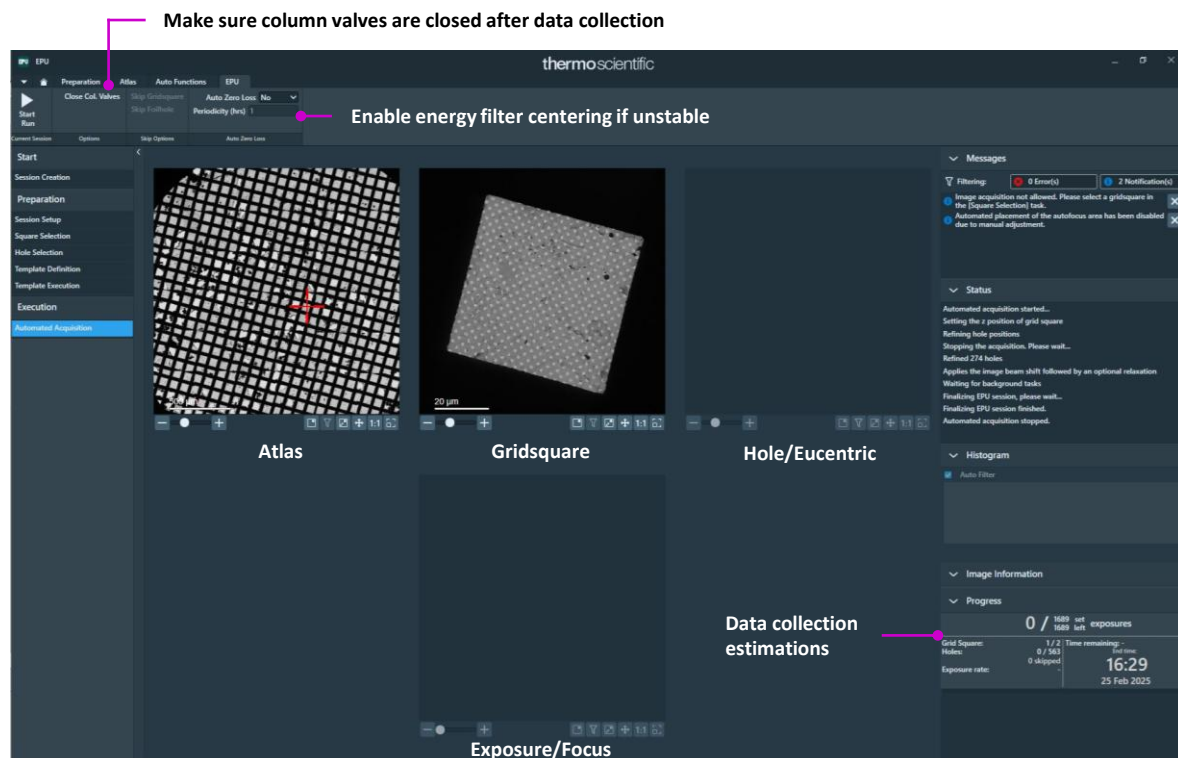
Recurrence After Centering
Distance (µm) 8.00
Focus using Objective lens
Autofocus Area

- If stable beam/stage, use "Once Per Gridsquare"
- Recurrence can also be set up to "After Distance"

6 Template Execution



7 Automated Acquisition Setup



SPA with EPU

Krios TEM4: Falcon4i and Selectris x

by Bharti Singal, PhD

Initial Microscope setup (TEM User Interface aka TUI)

1. Under the Vacuum tab check the pressure readings as follows in log
 - a. Gun and octagon should be ~1
 - b. Liner would be around 9 and autoloader should be ~15
2. Under the Setup tab, check to make sure column valves are closed.
3. Under the Autoloader tab, check the temperature readings. Temperatures for Docker, Holder, Cassette gripper, Autoloader dewar, and Column dewar should all be below -180°C. The temperature for devitrification is ~ -137 °C. Do not start inventory unless the temperature is below -160°C.
4. Under the Tune tab or click dropdown tab on bottom right corner and click “apertures”, check to make sure the Objective aperture is not inserted in the column (greyed).
5. Under the Setup tab, select the Col Valves Closed button to open the column valves. Press R1 (to insert screen) button on hand panel if you do not see beam in the Flu-Cam window of TEM user interface. The R1 button inserts and retracts the Flu screen. Close column valves. This test ensures that beam is not blocked with any aperture or fallen grid/material in the vacuum.
6. Load grids into the cassette and place the cassette into the Krios. Do inventory by clicking expanding the autoloader flap and clicking inventory once the temperature and vacuum recover after loading. If there were grids in the machine, unload, undock and save/trash those as per instructions in the Notepad on the screen.

Quick Steps

- Make sure the turbo is auto-off in the autoloader flap
- Load a reference grid (carbon/ NiTi foil or crossgrating) if you don't have imaging preset file
- Check microscope settings – imaging presets and [direct alignments for data acquisition preset](#) your reference grid
- Dose Check at the broken square/vacuum (if you don't load grid) and adjust the preset for data acquisition; center ZLP only in autofunctions tab; Filter tuning is done once per week. If needed, do it after fixing CTF on vacuum.
- Move stage to carbon or NiTi foil, do auto-eucentricity by beam tilt, autofocus, and perform autostigmat, autocomma, autostigmat in Autofunctions tab or AutoCTF in Sherpa only with staff.
- Move to the broken square and do the Energy filter tuning through sherpa
- Collect gain reference in broken square/vacuum

- Center a feature in the data acquisition mag and start Image shift calibrations
- Take atlas for the selected grids and finally load the sample grid of choice
- Select the color class of squares to roughly use in EPU data collection tab
- Start an EPU session in EPU software
- Select squares and renumber to set priority, perform hole selection and prepare all squares
- Insert the objective aperture if necessary
- Define template, test template and Start acquisition

Preparing for data collection

1. Open the EPU software. Load your old image settings file or one from /thisPC/Data(D:)/Bharti to use as the starting point.
2. Load one grid on stage. It can be a crossgrating (latex sphere) or a grid with carbon foil or NiTi foil grid. Do not load gold ultrafoils for the following steps. Gold sample grids do not perform well with the Auto CTF function.
3. Test all presets, Atlas, Squares, Hole/eucentric and Data Acquisition Mag and link them rings, autofocus, drift measurement, and zero loss to data acquisition present by clicking chain/link symbol in later presets, if not already linked.
4. To get the desired dose rate in the green range on the data acquisition preset, move stage to a broken square (vacuum).
 1. Adjust illumination area (enough to cover the EF-Falcon4i, green ring on flu screen) or adjust the spot size (changing from 4 to 5 or 6 or higher reduces beam strength by half with each number). Check and center, beam shift under TUI [direct alignments](#). Never do beam shift if the option is not active in direct alignment. The alignment doesn't save.
 2. Confirm that the measurement is in the green region of the meter. Middle is better for less coincidence loss.
5. Adjust the total dose as desired (40-60 e- /Å²) and check Exposure time. Check the number of EER frames is divisible by 9 (adjust the exposure time to get a number divisible by 9). This will change the total dose a little.
6. Record the measurement values and the exposure time in your own notes.
7. There are more details in Thermo's [EPU user manual](#) on page 30.
8. Once adjusted, take data acquisition image without energy filter slit.
9. Now insert the slit, top right toggle button, and in Autofuctions tab, run auto-zero loss operation. Check if it finishes successfully.
10. Go back to preparation>Data acquisition, measure the dose again in the vacuum, and take a preview image. Nothing should change.

Note: Critical DO NOT TAKE ATLAS UNLESS at EUCENTRIC HEIGHT AND IMAGE SHIFT CALIBRATIONS ARE DONE.

1. Move stage to an intact square after taking an image in the atlas preset.
2. Check the grid square preset if you are still centered on the square.

Eucentric Height and AutoFocus : EPU Auto Functions Tab

Select the Autofunctions tab

1. Select Auto Eucentric Height by Beam Tilt. Click the play button at the top of the screen to start the sequence. Wait until the sequence has successfully completed
2. Select Autofocus. (for this you must be on foil, carbon/NiTi.)

Click the play button at the top of the screen to start the sequence. You can target defocus as zero or -2 micron.

Image Shift calibration

Image shift calibrations determine the shifts when changing between defined optical settings (the beam presets). They are done for a defined set of magnifications, so it's important you have decided on the magnifications to use in your beam presets. It particularly differs between 200 and 300 mesh size for square map mag.

1. At low mag (atlas/grid square), navigate to an area with a big (microns) feature such as ice crystal or other surface contamination or a broken edge.
2. Carefully set the eucentric height and re-centre the feature.
3. Go to data acquisition mag and make sure the feature is still centered. It is normally easiest to do this by taking preview images in EPU, iterating from lower to higher mags. More sharp/edgy the feature, the better the interpretation will be in all mags. Alternatively, if comfortable, you can also quickly center the feature on flu-screen for data acquisition preset using joy stick.
4. In EPU software, go preparation > calibrate image shifts > start calibration.
5. An image at data acquisition mag will appear with a marker in the center. double-click the left mouse button to position the blue cursor on a recognizable feature in all four quadrants. Click store calibration and acquire again to check. Make changes as required and store calibration in the end.

CTF correction: Objective Stigmatism and Coma Free Alignments

1. Use the joystick in the hand panel to center over a carbon region of the sample while looking at the flu-screen. If using the cross-grating, center in the middle of a square
2. Go to autofunctions and perform autofocus with a target defocus value of -2 microns. (This defocus will help in getting enough signal for auto CTF operation.)
3. In autofunctions, click autostigmatism and hit start
4. In autofunctions, click autocoma and hit start
5. Again, in autofunctions, click autostigmatism and hit start. If using [objective aperture](#), center aperture in diffraction mode first before doing autostigmatism.

SHERPA (if required, perform with staff):

If any of the above operations in 3 to 5 fails, go to sherpa software and check/adjust settings and perform autoCTF operation using sherpa.

1. Go to Microscope software launcher>tools>sherpa
2. Select the Auto CTF tab
3. Under the Camera panel the settings should read:i. Type: EF-Falcon ii. Exposure Time (s): 2.0 iii. Binning: 2 iv. Readout Area: full v. Electron counting: checked
4. Autofocus -2 checked, max number of iterations 10, defocus error μm 0.05, astigmatism error nm 5.0, coma error (nm) 160.0, beam tilt (mrad) 10.0
5. Objective Stigmatism - Select Measure in Objective Stigmatism panel i. Want stigmatism to be close to 0nm ii. Defocus to be -1 to -2
6. Adjust defocus if needed, then select Correct in Stigmatism and ensure stigmatism is close to 0 (less than 2 is acceptable)
7. Coma Free - Select Measure in Coma Free panel to measure initial coma value
8. Select Correct in Coma Free panel i. Should pass after a few iterations (will do a max of 10) ii. If doesn't pass, try adjusting defocus more negative
9. Remeasure (and re-correct if needed) Objective Stigmatism with objective inserted and centered

Selectris X Energy Filter tuning (collect with a 10 eV slit, only with staff)

Measure dose on the data acquisition mag in EPU , if it doesn't make sense with energy filter in, take dose rate without it and then insert and do center zlp

1. Generally, this is performed once a week at a higher mag. Check with staff if you really need to perform tuning.
2. Use EPU and move stage to broken grid square - vacuum
3. Select Data Acquisition and click Set, insert Flu Screen to make sure beam is centered on green circle (energy filter aperture), press eucentric focus button then retract screen. Do not use track ball to center the beam, use Direct Alignment beam shift
4. Open Sherpa and go to Energy Filter section
 1. Click Center under Zero Loss heading. When it is finished, expect to see an absolute value of the calibration offset $\sim 0.5 \pm 0.2$.
 2. Click Measure under Isochromaticity heading. Click Tune if it's outside the indicated spec of 1.00.
 3. Click Measure Geometric Distortions. Click Tune Magnification if it's outside spec of 0.50%. If Geometric Distortion is severe (unlikely), Tune Magnification may not fully resolve the issue, and the tuning will be completed in the Chromatic Distortions step below.
 4. Click Measure Chromatic Distortions. Click Tune Distortions if it's outside spec. Max distortion spec should indicate 0.50%. Max chromatic distortion should indicate 0.40%.

Gain Reference- via reference image manager

1. Click the Falcon-4(i) Reference Image Manager icon that is pinned to the Task Bar. If this icon is missing, find the program shortcut in Desktop > Titan Tools or in Microscope Software launcher > Tools> Camera and Detector> Falcon4i reference image manager
2. Insert Flu Screen and check that the beam illuminates the entire energy filter aperture and is centered (by auto zero loss in EPU), then retract the Flu Screen.
3. In the Reference Image Manager, click Measure Dose. The dose rate should be within the green range of the intensity indicator and closely match the value measured earlier in EPU.
4. For the Post counting gain, Use the default exposure time (5) and images to average (90) and click Acquire.
5. The EER gain reference file is: C:\Titan\Data\EF-Falcon4\Reference Images\300kV_EER_GainReference.gain.
6. This gain format should be directly usable in cryoSPARC. It may require conversion to mrc for RELION
7. The lifetime of a gain reference is about 1 month. Don't worry about dark references, they are acquired automatically (every hour).

Starting a session

1. Switch to the EPU tab. The order of the tasks in the EPU navigation pane indicates the workflow for setting up a data acquisition.

Define a Session

1. Under Session Settings, specify name of the session as YYYYMMDD_CSXX_userInitials_samplename,
2. Specify grid type – holey carbon or holey gold, for lacey grids check with staff (set up is different)
3. Session type “Manual” . Use Automated if you have nice and clean grids which won't require brushing of dirty holes on squares.
4. Acquisition mode “Faster”, Click tilted acquisition “on” if you would like to collect certain squares at a specific angle.
Note: "Faster acquisition" will use beam and image shift to speed up the data acquisition (to acquire ~300 images per hour for one shot per hole and 500 for two shots per hole).
5. Under Output, decide where the data has to be stored (Z: drive) and in what format (this can also be changed between runs).
6. Under Email settings, enter the email addresses separated by commas, semicolons or spaces. Always add one of the staff members email. Click the check box to enable email notifications to these recipients at the end of a run. Use the ‘Test’ button to confirm if the required email settings have been configured. These settings can be updated even after a run is started.

Square Selection

1. Navigate to "Square Selection" to choose the grid squares for data acquisition.
2. The program imports the grid squares selection automatically from the atlas map. Right click to unselect a particular square or click unselect all on the top ribbon to clear all selection.
3. Right mouse click to select the grid squares you would like to acquire.
4. For preselected squares, click "show" in the ribbon to see labels on each square and click "change" in the ribbon to change the order of selected squares by clicking selected squares in a preferred order.

Hole Selection (Quantifoil Only)

1. Navigate to the grid square of your choice in the Square Selection task.
2. Click Hole selection in the navigation pane on the left.
3. Select Auto Eucentric to perform Eucentricity by Beam Tilt. After the Eucentric height is determined, the Z value will be stored, and a Grid Square image will be acquired automatically.
4. When the image displays, click Measure hole size in the Measurements ribbon bar. The yellow circles must be resized and moved to match the hole size and spacing exactly. Zoom into the image to position the measure tool more accurately.
5. Once set, click on Find Holes, and Remove Holes Close to Grid Bar to only have holes that are visible inside the grid square.
6. Adjust the ice filter histogram to be more specific in which holes are selected in the grid square (if necessary)
7. To prepare all the grid Squares, click "Prepare All Squares" function. It will automatically perform eucentric height estimation and find holes as per selections in previous square.
8. Observe which holes were selected in all the grid squares and remove non-desirable holes with the selection brush.
9. The grid square assignment is shown in the lower left of the EPU window. You can navigate between the grid squares with the "Previous Square" and "Next Squares options".

Template Definition

1. Click on the "Template Definition" button.
2. "Acquire" an image (it will be with the Eucentric height presets). The "Acquire" button is active only when at least 1 hole is selected on the current grid square.
3. To check that the hole measuring from the step before is correct, click, "Find and Center Hole". You can also import or export this template.
4. If you don't have a template and you need to prepare one, select "Add Acquisition Area", and then click on the image to add the acquisition area (add as many you like per hole with no beam overlap between square detector area).

5. To add/move the focusing Area, select the "Add Autofocus Area" and add it by clicking on the Foil Hole Image. For the autofocus to be efficient, the Autofocus area should be on carbon/gold/NiTi.
6. Add drift measurement area on top of focus area.
7. Add as many Acquisition Area as they fit in the hole. Be sure to irradiate the Carbon a bit, so that you don't have charging during Acquisition.
8. To be able to define the defocus list, select one of the acquisition areas by clicking on it.
9. Define defocus list in the ribbon. The defocus value should be defined in um and separated by a comma. An example of the format is: "-0.6,-0.8,-1.0, -1.2, -1.4".
10. To apply the defocus list to all the acquisition areas, click on the small copy page icon close to the defocus list.
11. To visualize the method selected for autofocus, activate the autofocus by clicking on the autofocus area.
12. Autofocus should be performed with the "Objective lens". You can select also how often autofocus would be performed during data acquisition. Select after centering or "After Distance" 9 um usually works. Drift measurement can be done once per gridaquare.
13. Select how long the program should wait to acquire an image after Image Shift and after Stage Shift. I prefer 1 sec "Delay after Image Shift" and 10 sec "Delay after Stage Shift" usually works.
14. In the "Template Execution" tab, select "Preview". The program will execute the template in the closest Foil Hole. It will do an autofocus and acquire the defined positions.

Automated Acquisition

1. Select "Close Col. Valves" so that the column valves close automatically when the data acquisition is done.
2. If you want, you can select the energy filter to be centered during data acquisition. In that case change the default value "No" to "Yes" and set the interval.

Manual alignments: extra information

Beam Tilt Pivot Points and Beam Shift

1. Reduce the size of the beam to a small spot using the intensity knob
2. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
 1. Select Beam Shift under Direct Alignments
 2. Use the Multifunction X and Y knobs to center the beam
 3. Once centered, click Done at the bottom of the Direct Alignments menu
3. In the Direct Alignments menu, select "nP Beam tilt pp X"
 1. Use the Multifunction X knob to align the two pulsing spots as closely together as possible
 2. When complete, click Done at the bottom of the Direct Alignments menu

4. In the Direct Alignments menu, select “nP Beam tilt pp Y”
 1. Use the Multifunction X knob to align the two pulsing spots as closely together as possible
 2. When complete, click Done at the bottom of the Direct Alignments menu
5. In the Direct Alignments menu, select Beam Shift
 1. Use the Multifunction X and Y knobs to center the beam
 2. Once centered, click Done at the bottom of the Direct Alignments menu

Manual Eucentric Height

1. Take an image at the atlas mag by pressing preview. 1.5x and 150 x
2. In EPU, select Atlas under the Preparation tab and click Set
3. Press Eucentric Focus on control pad
4. Insert Flu screen by pressing R1.
5. Open column valves in Vacuum tab.
6. Use the joystick to center a prominent feature (e.g. dirt spot). Make sure that the grid square that contains this feature is intact.
7. Go to the Stage+ tab in the TEM software
8. Ensure alpha angle is -20 (or -15), then select alpha (this will tilt the sample by -20 degrees)
9. Use the Z-axis +/- buttons on the control panel to shift the prominent feature back to the center
10. Select alpha again to return to 0 degree tilt and verify that prominent feature remains in about the same place. Repeat steps 4 and 5 if needed.
11. Sometimes just aligning energy filter also fix the issue if you are not able to identify the same object at all different beam settings from low to high mag.

C2 Aperture Alignment (inform staff before doing this)

1. In EPU, select Hole Eucentric Height under the Preparation tab and then click Set
2. In the TEM software go to the Column tab, and the Beam Settings panel.
 1. Select Free Ctrl (button will turn yellow)
 2. Select C3 off in the Free Ctrl tab.
3. Reduce the size of the beam to the diameter of the small circle on the screen using the intensity knob on the control panel
4. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
 1. Select Beam Shift under Direct Alignments
 2. Use the Multifunction X and Y knobs to center the beam
 3. Once centered, click Done at the bottom of the Direct Alignments menu
5. Increase the size of the beam to the diameter of the large circle on the screen using the intensity knob on the control panel
6. In the Column tab of the TEM software, select Condenser 2 Adjust in the Apertures panel
 1. Center the beam over the large circle in the display using the Multifunction X and Y knobs

2. Repeat steps 3-7 until the beam is centered on the small circle when beam size is decreased and centered on the large circle when beam size is increased
3. De-select Condenser 2 adjust in the Apertures panel
7. In the beam settings panel, select TEM and then select Nanoprobe (this reactivates the C3 lens)
8. Reduce the size of the beam to a small spot on the screen using the intensity knob on the control panel
9. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
 1. Select Beam Shift under Direct Alignments
 2. Use the Multifunction X and Y knobs to center the beam
 3. Once centered, click Done at the bottom of the Direct Alignments menu

C3 Condenser Alignment (inform staff before doing this)

1. Using the magnification knob, slowly increase the magnification to 18,000x
2. Adjust the size of the beam to the diameter of the small circle on the screen using the intensity knob
3. Select Direct Alignments, select Beam Shift
 1. Use the Multifunction X and Y knobs to center the beam
 2. Once centered, click Done
4. In the Direct Alignments menu, select Condenser Center TEM. The beam will start pulsing between small and large diameters
 1. Use the Multifunction X and Y knobs to center the largest beam with the smallest beam
 2. Once aligned, click Done
5. Verify that the condenser is centered by adjusting the intensity knob from small to large beam diameters. If any shift is observed while adjusting the beam size, repeat step 4.

Condenser Astigmatism (inform staff before doing this)

1. In EPU, select Data Acquisition under the Preparation tab and then click Set
2. Spot size should be 4 and exposure set to 5 seconds (Ask Staff)
3. Select the FFT button at the bottom of the TEM display screen to view the FFT (Thon rings)
4. Reduce the size of the beam to a small spot using the intensity knob on the control panel
5. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
 1. Select Beam Shift under Direct Alignments
 2. Use the Multifunction X and Y knobs to center the beam
 3. Once centered, click Done at the bottom of the Direct Alignments menu
6. In the Column tab of the TEM software, select Condenser in the Stigmator panel
 1. Use the Multifunction X and Y knobs to make the beam as round as possible. The Thon rings in the FFT view should be perfectly circular.
 2. Once complete, select the None button in the Stigmator panel

Objective aperture centering (we do not recommend using it)

1. Go into record parameters by selecting Rec. in the SerialEM Low Dose Control box
2. Move to an area that has carbon on it
3. Insert the flu screen and check that the beam is centered. If it is not, adjust it with Beam Shift under the TUI Tune tab.
4. Click the Diffraction button on the console.
5. Click the “Adjust” button corresponding to the Objective aperture under the TUI Tune tab. The button will turn yellow.
6. Use the multifunction X and Y knobs to center the Objective aperture on the center of the diffraction pattern. The aperture will cast a shadow onto the pattern; make sure that it does so symmetrically around the center spot.
7. Once done, click the yellow “Adjust” button corresponding to the Objective aperture to release the controls.
8. Click the “Diffraction” button on the console to exit diffraction mode.

Documents to refer:

1. [EPU User Manual](#)
2. [Falcon4i Application notes](#)