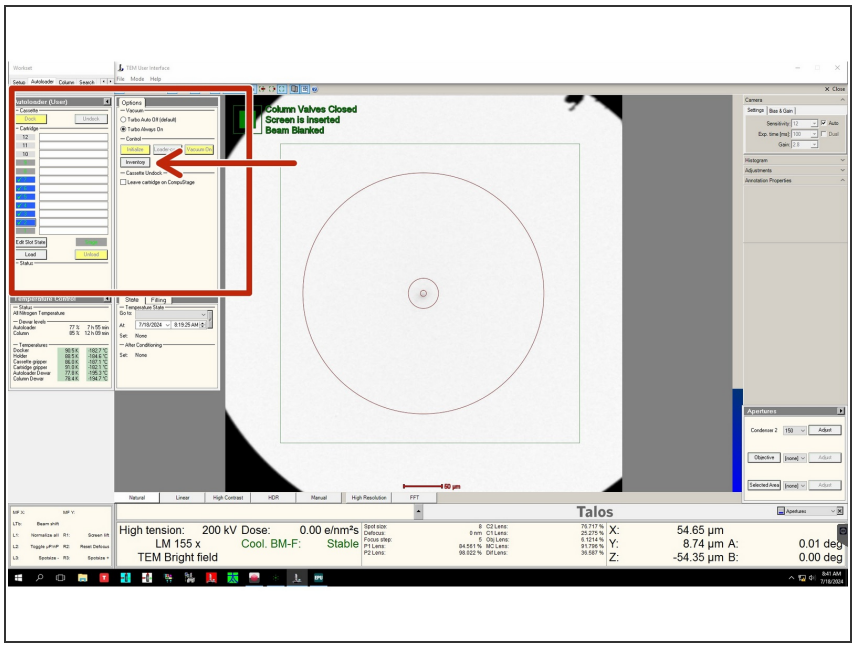
# TEM - TFS Titan Krios G3i: general workflow

Written By: z mbstaff



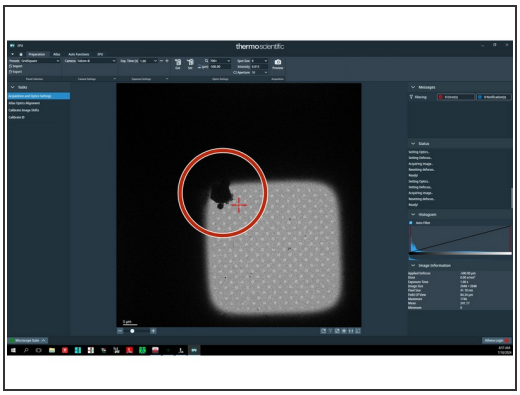
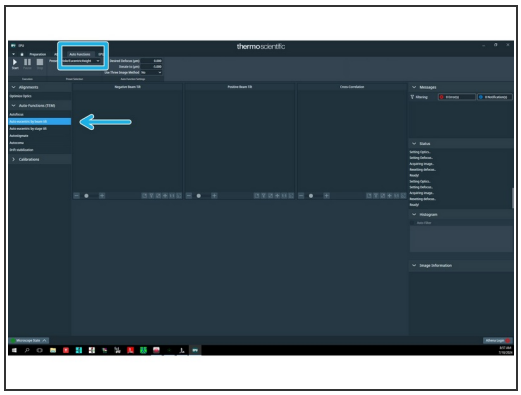
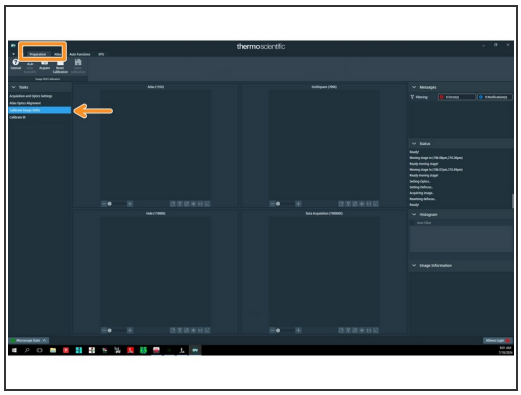
## Step 1 — Autoloader inventory




- After loading a new cassette with your grids perform autoloader inventory.

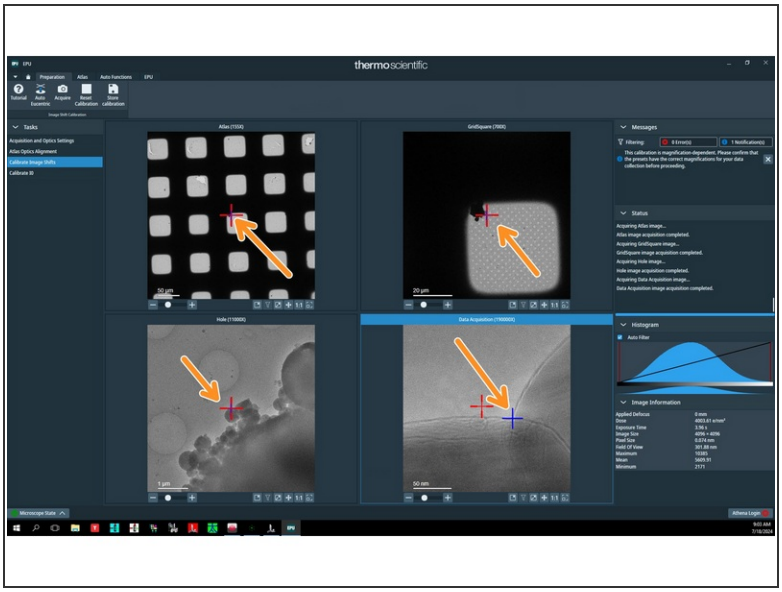

© 2025

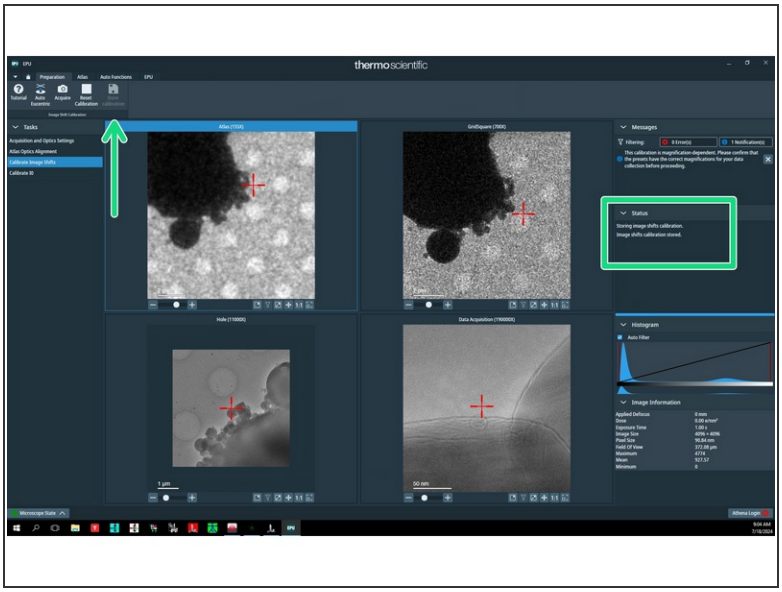
## Step 2 — Prepare for calibrating image shifts

- Load your first grid on stage using the microscope user interface (Autoloader tab). Identify an easily recognisable feature that is visible at every magnification you will use during your EPU session (e.g. piece of ice).
- Go to 'Auto functions' tab and perform the eucentric height routine. It is essential to be at eucentric height for image shifts calibrations.
- ⓘ Make sure that the feature you intend to use for the calibrations is visible using the 'Data acquisition' preset.
- In the 'Preparation' tab go to 'Calibrate image shifts'.

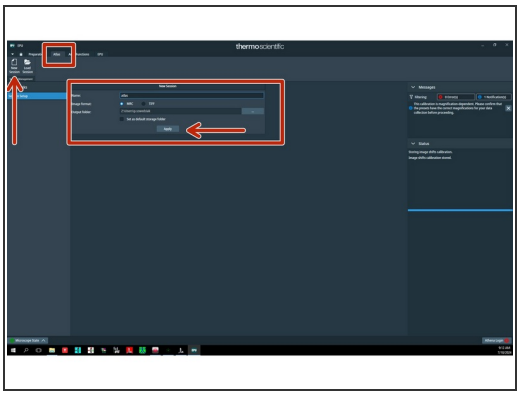
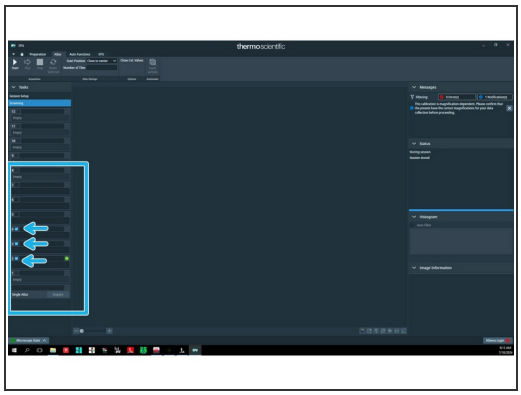
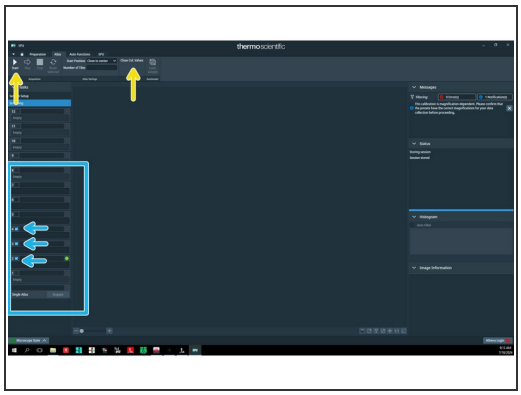
## Step 3 — Calibrate image shifts



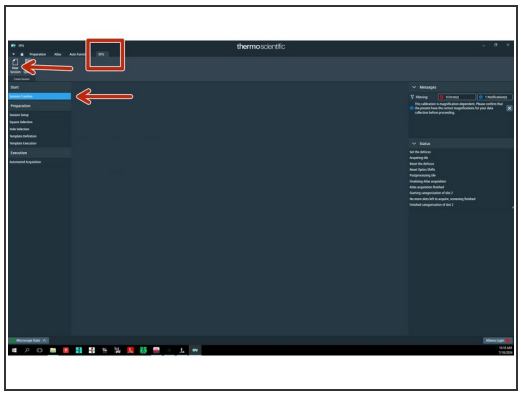
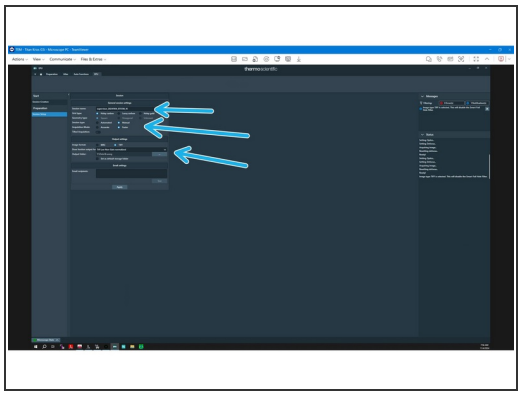
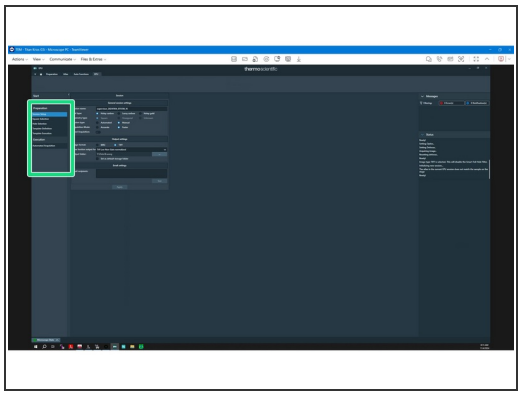

- EPU will automatically acquire four images using Atlas, Grid square, Hole/Eucentric height and Data acquisition presets.
- Identify the same feature in every image and double click on it. Please note that Hole/Eucentric height and Data acquisition images are 180 deg rotated with respect to Atlas and Grid square images.
- Finally, press 'Store calibration'.

## Step 4 — Setup automated atlas acquisition

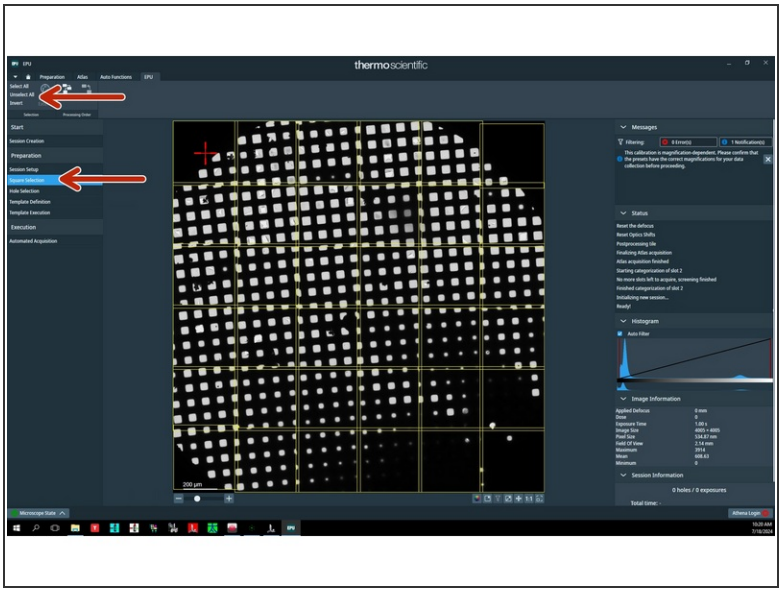
- In 'Atlas' tab click 'Session setup' and 'New session', specify appropriate path and name of the storage folder and click 'Apply'.
- In 'Screening' tick boxes next to the symbols of grids that you want to acquire atlases of.
- Once you are ready click 'Close col. valves' (this will automatically close column valves after the atlas acquisition has completed) and click 'Start'.

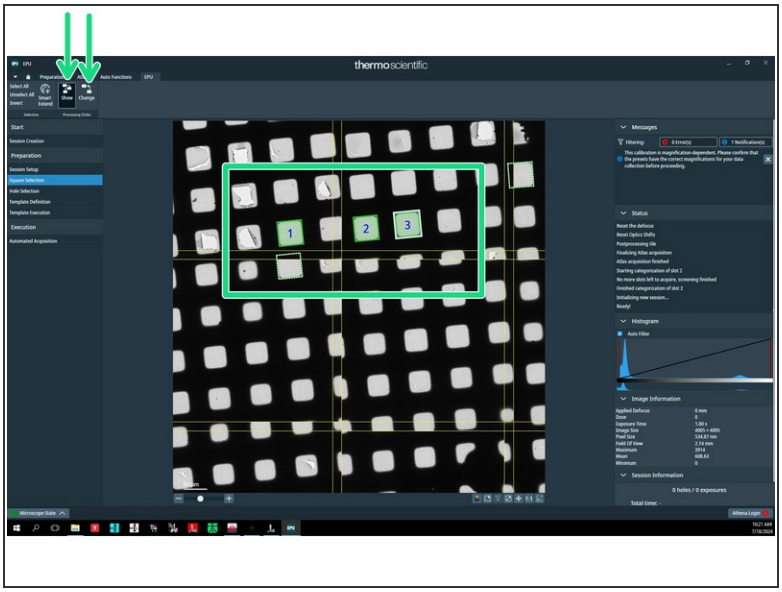
## Step 5 — Start EPU session

- Once you have reviewed the atlases and screened your grids in more detail and selected the best grid, you are ready to setup an EPU session.
- In 'EPU' tab click 'Session creation' and 'New session'.
- Specify session's name and appropriate output storage folder. Select 'Grid type' - 'Holey carbon'; 'Session type' - 'Manual'; 'Acquisition mode' - 'Faster'; 'Dose fraction output format' - 'Tiff Lzw Non-Gain normalized'. Click 'Apply'.
- This will open 'Preparation' steps on the left hand side that will intuitively guide you through the process.

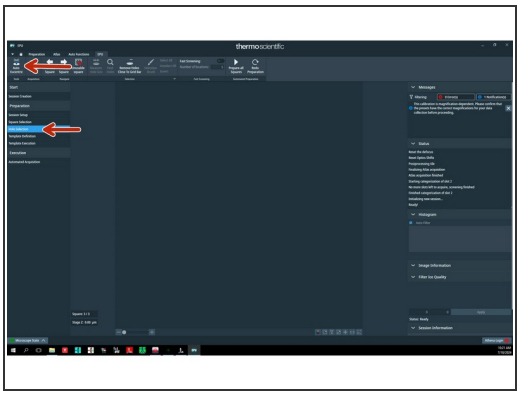
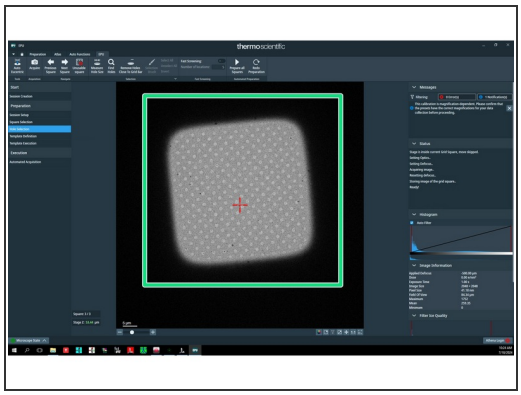
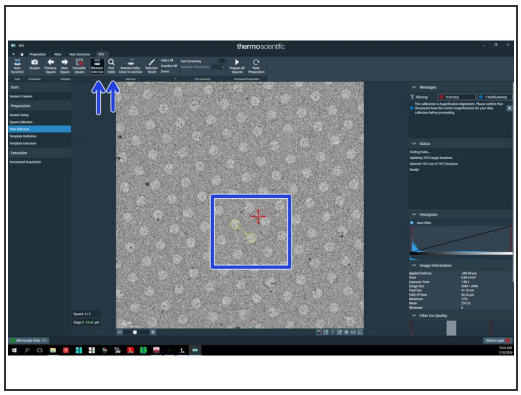
## Step 6 — Select squares



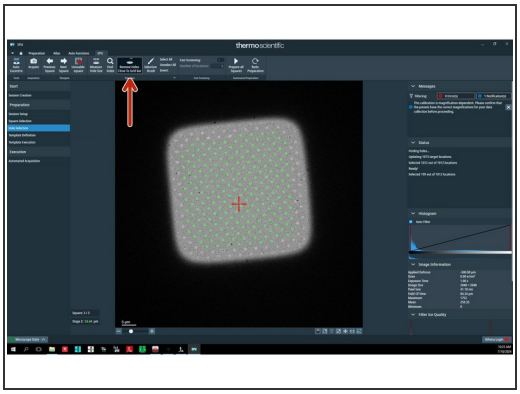
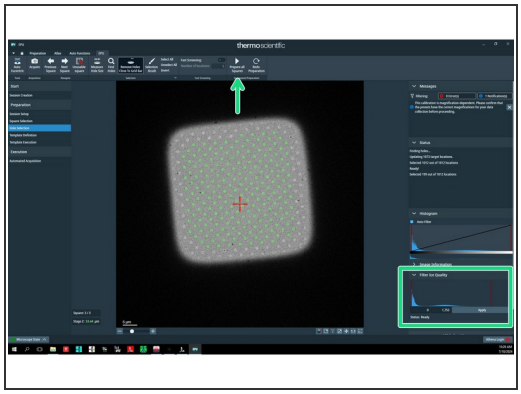

- In 'Square selection' click 'Unselect all'.
- In order to add a square right click on it and choose 'Select'. You can see the order of squares by clicking 'Show' and you can change this order by clicking 'Change' and then clicking on respective grid squares.

## Step 7 — Hole selection - part 1

- Go to 'Hole selection' and press 'Auto eucentric'.
- The auto eucentric routine will run in the background and ultimately a grid square image will appear.
- Click on 'Measure hole size'. Two yellow circles will appear. Adjust their size so that it matches the grid hole size and place the two yellow circles over neighboring holes. Click 'Find holes'.

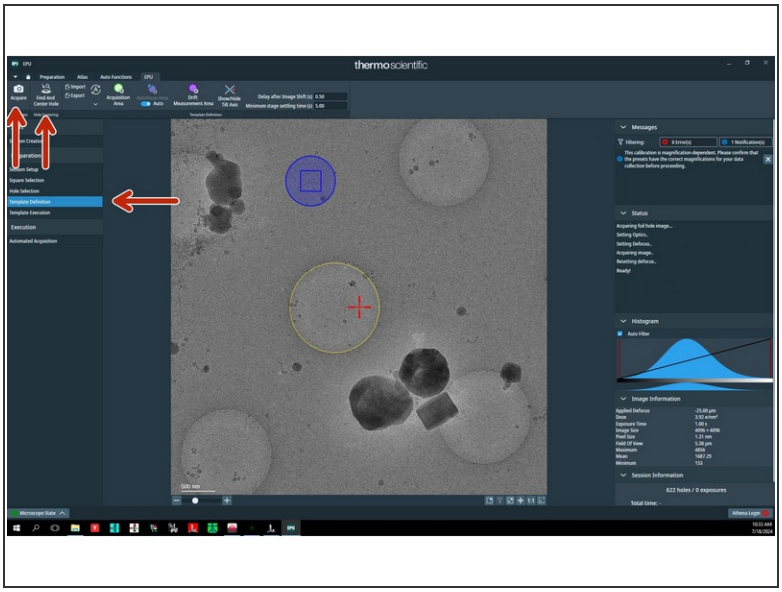
## Step 8 — Hole selection - part 2

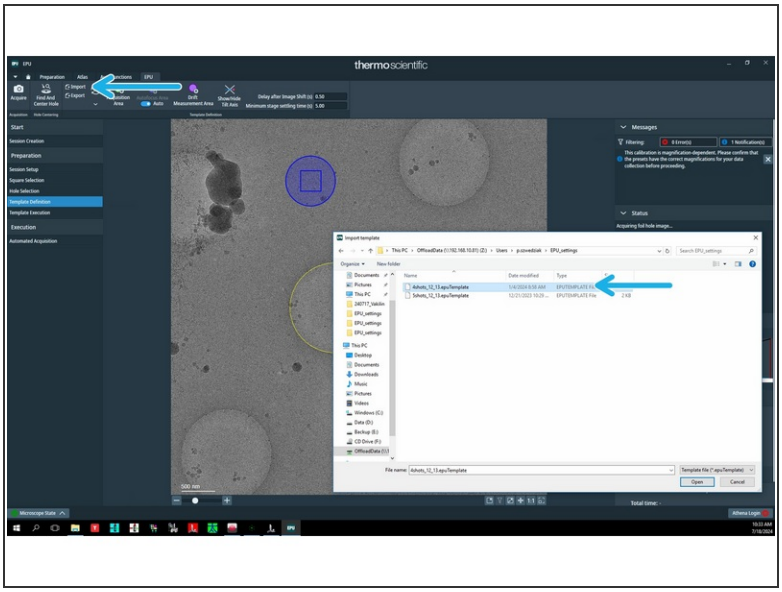




- Click on 'Remove holes close to grid bar' to automatically unselect holes over grid bars and their proximity.
- Play with the ice filter settings to select holes with optimal ice thickness. Once you are happy click on 'Prepare all squares'. Wait until all the grid squares have been processed.
- Click on 'Selection brush' and manually go through all your grid squares and unselect holes with obvious problems (ice, cracks etc.).


© 2025

## Step 9 — Template definition

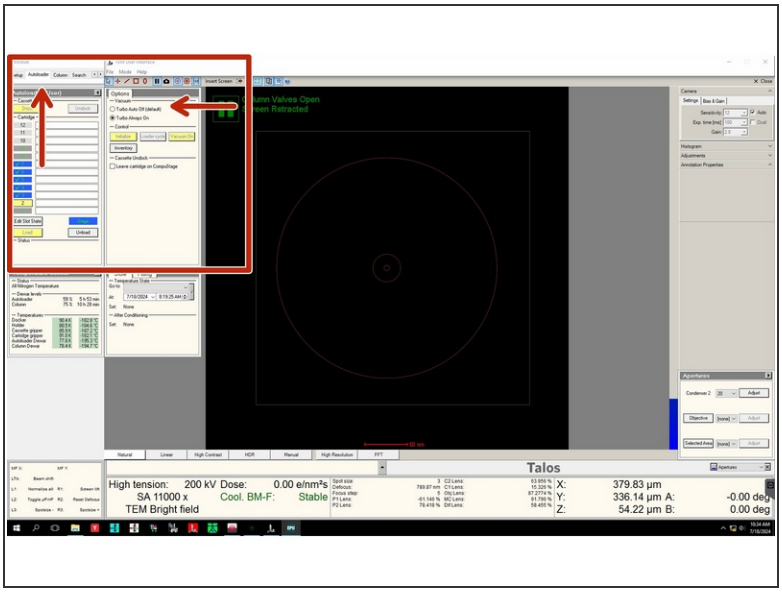


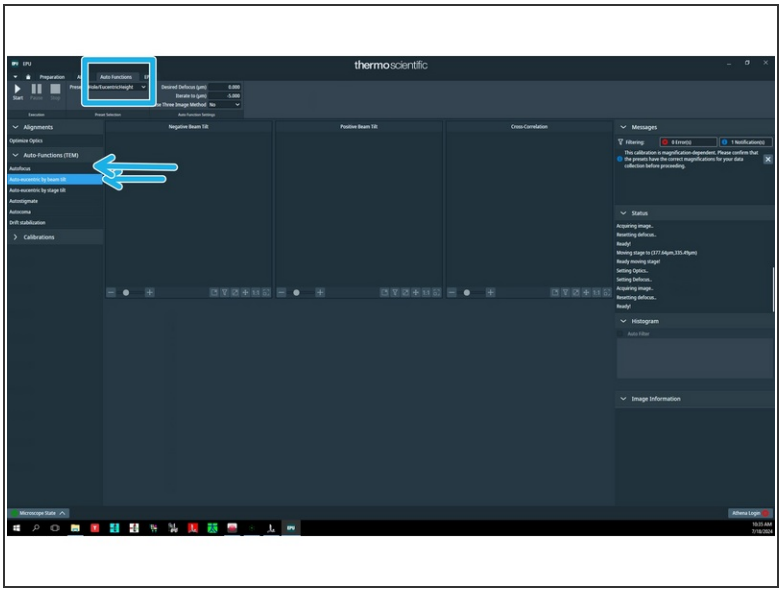


- Go to 'Template definition' and click 'Acquire' and then 'Find and center hole'.
- Click on 'Import' and select your template of choice. Make sure to place the autofocus area on the carbon between the holes. Autofocus recurrence should be set to 'After centering'. Don't forget to specify the defocus list values that will be used for data collection (will show up after clicking on the green circle).


© 2025

## Step 10 — Direct alignments - prep



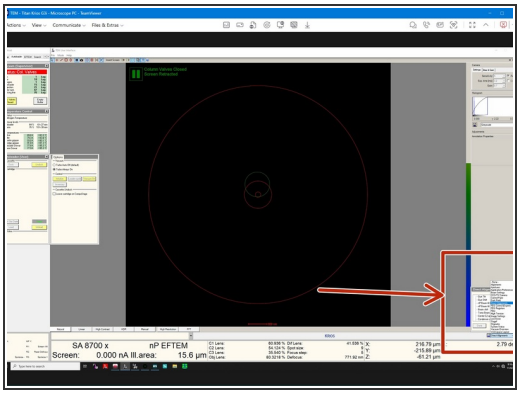

- Switch off turbo pump in the 'Autoloader' tab.
- Navigate to an area that will not be used for data collection. Make sure that you are over the carbon film and not over the hole. Go to 'Auto functions' tab and run 'Auto eucentric' and 'Autofocus' routines.

⚠ It is of crucial importance to be at eucentric height and in focus before proceeding with direct alignments. The next step will help you to make sure this is the case.

- Verify you are in focus by live FFT using the 'View' option in the Digital Micrograph (K3 computer). No Thon rings should be visible.

## Step 11 — Direct alignments - part 1

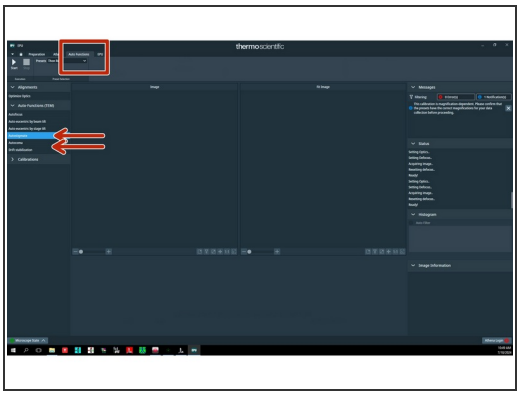
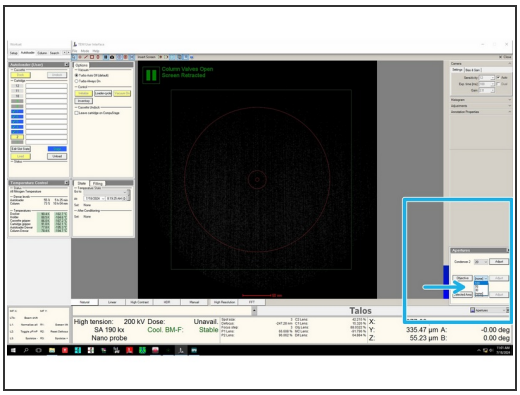
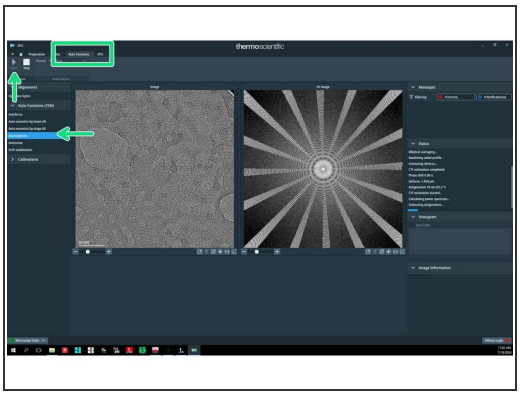




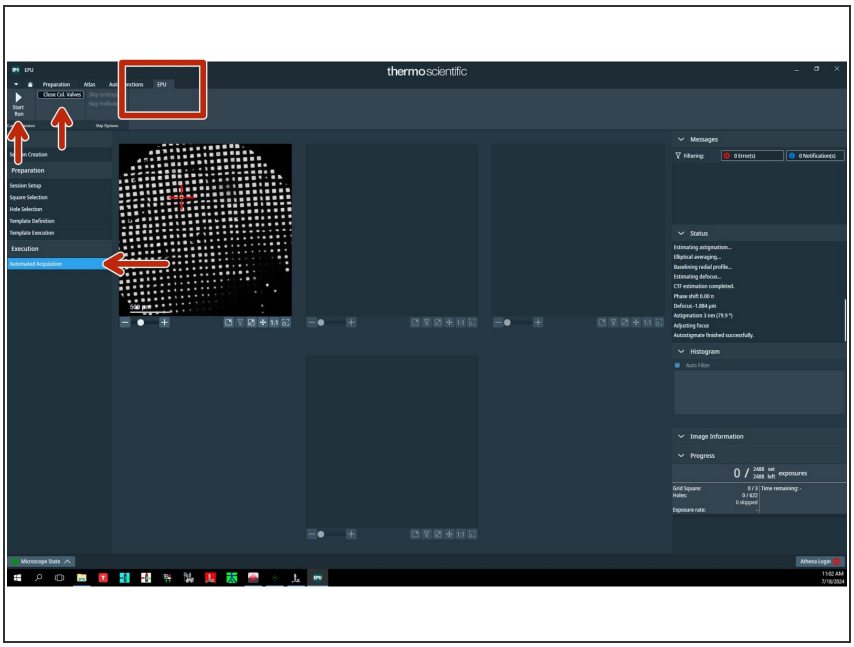

- Drop the FluCam screen by pressing 'R1' button on the handpanel.
- Go to 'Direct alignments' menu (bottom right) and select 'nP Beam tilt ppX'.
- The beam will start oscillating. If the pivot points are off you will see two circular beams with little or no overlap (see the image). By turning the 'Multifunction X' knob minimize the movement until you see a single beam.
- Once you see a single beam (see image) click on 'Done'. Repeat for 'nP Beam tilt ppY' by clicking on it, adjusting with 'Multifunction X' and clicking on 'Done' again.
- Center the beam around the camera (green square on the FluCam) by clicking on 'Beam shift' and adjusting the beam position with both 'Multifunction X' and 'Multifunction Y' knobs on handpanels.

## Step 12 — Direct alignments - part 2

- Go to 'Auto functions' tab and run first 'Autostigmate' routine and then 'Autocoma' routine.
- In 'Aperture' menu select '100' from the drop down menu next to 'Objective'.
- Run the 'Autostigmate' routine in the 'Auto functions' tab again.

## Step 13 — Start EPU run



- Go to 'EPU' tab and 'Automated acquistion', click 'Close col. valves' in the EPU interface and 'Start run'.


© 2025