Data collection Using the K3 on the Midlands Krios

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General rules:

- If something is NOT explicitly mentioned in this manual please ask permission before you tweak it or change it.
- Do not overwrite any of the EPU settings files. You can always export a settings file to your directory if needed.
- Obtaining new gain references and GIF tuning are performed by the facility. Please discuss with the facility if you want to do these yourself.

Changes since July 2021:

1. The stage has been moved by ~60um to allow the C2 aperture to be in focus and reduce the number of fringes in the beam (Fringe Free Illumination). As such we can use a smaller beam and accommodate more images per hole.
2. Pixel sizes have been re-calibrated using oriented gold diffraction spots and differ slightly to the pre-FFI upgrade pixel sizes. Refer to Table 1.
3. Titan software upgraded to Version 7.81 (and Windows 10)
4. EPU has been upgraded to Version 2.11.1. Major changes include automatic dark ref acquisition every hour and multi-grid EPU. Please do not use auto Zero-loss. The GIF remains very stable as before.
5. GMS has been updated to Version 3.4.3420
   - GIF tuning now uses spot 1, 150um C2.
   - Prior to a gain acquisition start a counting view in GMS (otherwise it only collects a linear gain ref)
   - The linear gain is acquired at 1500 counts now (previously 1280)
   - Please note the gain ref images have slightly different naming convention now. Use K3-18370212 Gain Ref. x1.m1.kv[300].dm4

6. The most productive setting for SPA is the 105kX_FFI setting (0.835Å pixel). Unless you require a larger pixel size or want to go beyond 1.7Å this will give you the most data in the same amount of time. Using this setting apoferritin went to 1.7Å with 6 hours collection using the settings described in Table 2.

Logging in Remotely to the Krios

A 22” or larger monitor is recommended.

Download and install Team Viewer and create a free account.

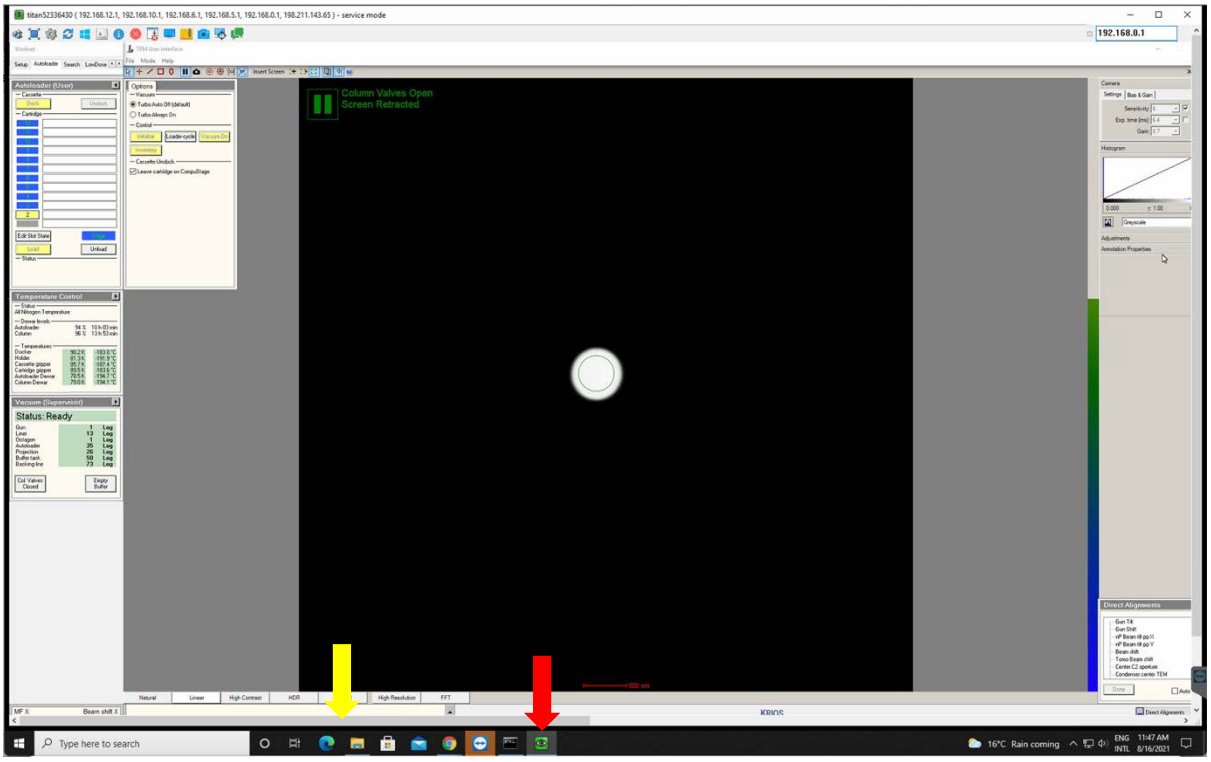
After you create your account go to:

https://login.teamviewer.com/cmd/joincompany

Enter Christos’s email as the company: gwcs1@leicester.ac.uk

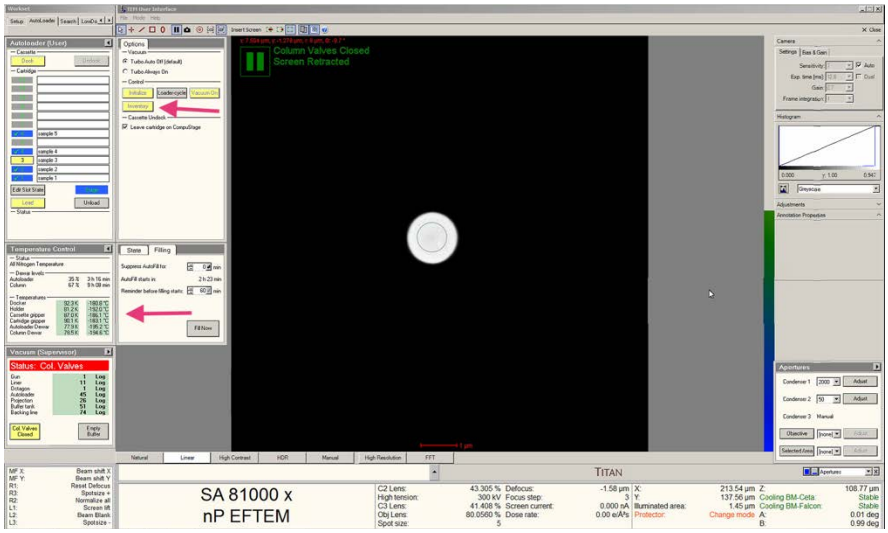
You will be added as a contact of the Cryo-EM facility and given access on a need to basis, i.e. you will not be able to log in if not booked to use the microscope.

In the TeamViewer application enter the ID of the Krios: 263 729 736
The password will be allocated to you before your session.
Connect to the support PC which then uses VNC (Red arrow) to connect to the Krios PC (192.168.0.1) and Gatan PC (192.168.12.2). The VNC password is d3643
You can move between the two screens by scrolling horizontally (Yellow arrow).

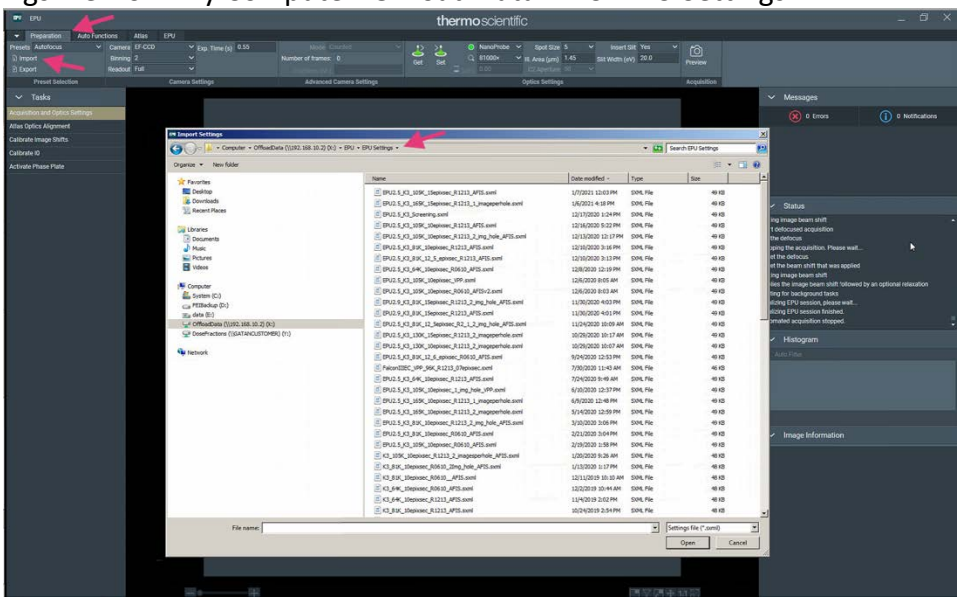


Setting up data collection

1. **Perform an Inventory of the Autoloader:** Under the Autoloader make sure all the temperatures are colder than 110 K or wait until they have reached this point. Then click on Inventory to begin checking the contents of the Autoloader. Occupied slots will turn Blue. Empty slots will turn dark grey. When a grid is loaded in the column the slot is Yellow. Any grids that have been previously loaded into the column will have a green check mark.

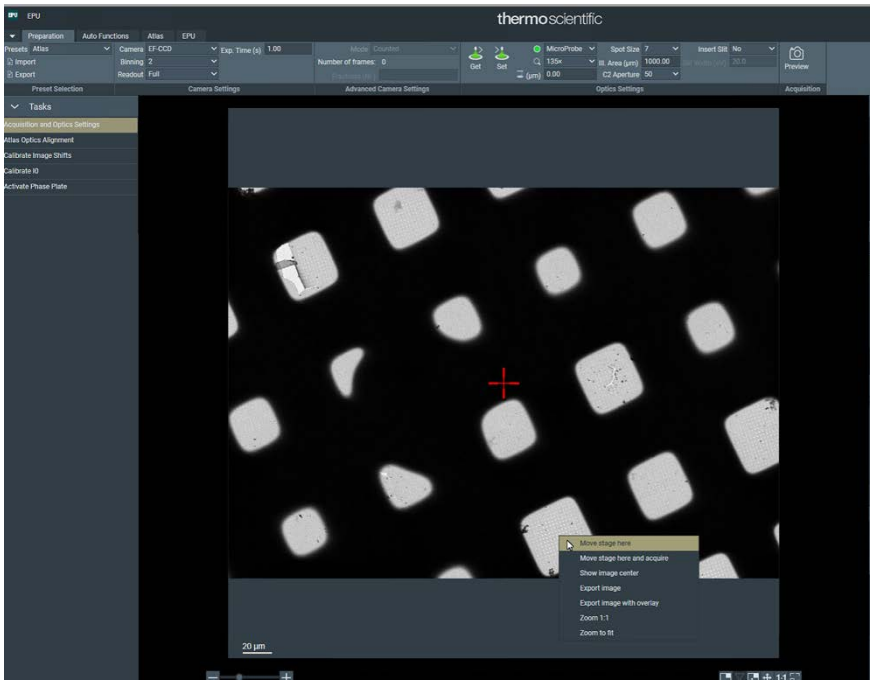


2. **Load the correct EPU setting file:** Under the Preparation tab click on Import and load a settings file from My Computer>Offload Data>EPU>EPU Settings.

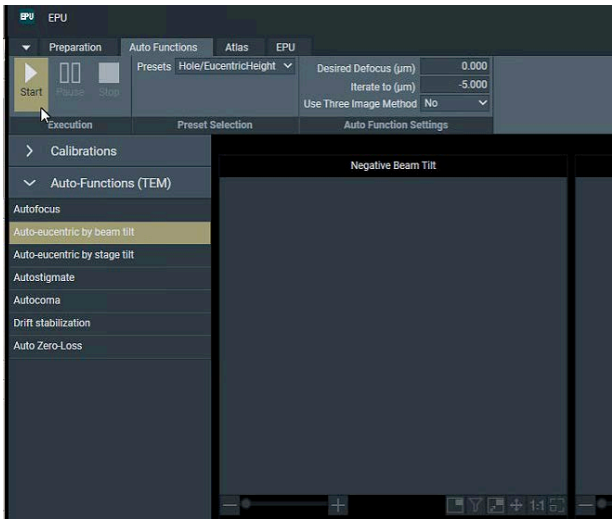


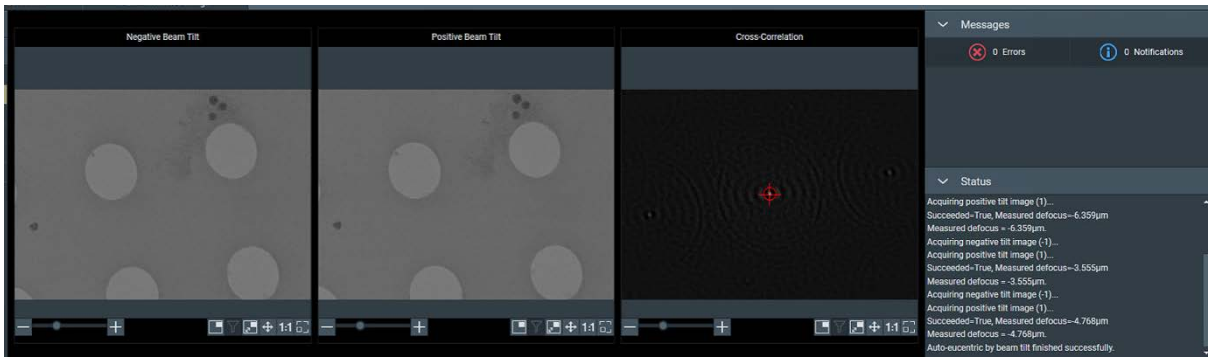
3. **Prepare for an Image Shift Calibration:** The goal here is to find a suitable object to track from the lowest mag up to the highest (data collection) mag. The calibration is then performed from highest to lowest mag.

- Load one grid from the autoloader by selecting the grid in the Autoloader tab and clicking on load.
- Once loaded the slot turns yellow. Open the column valves by clicking open column valves. The button will turn grey.
- Take an Atlas Image (under Preparation tab, Presets) and find a suitable gridsquare with a feature.
- Navigate to the gridsquare: Right click and Move Stage Here

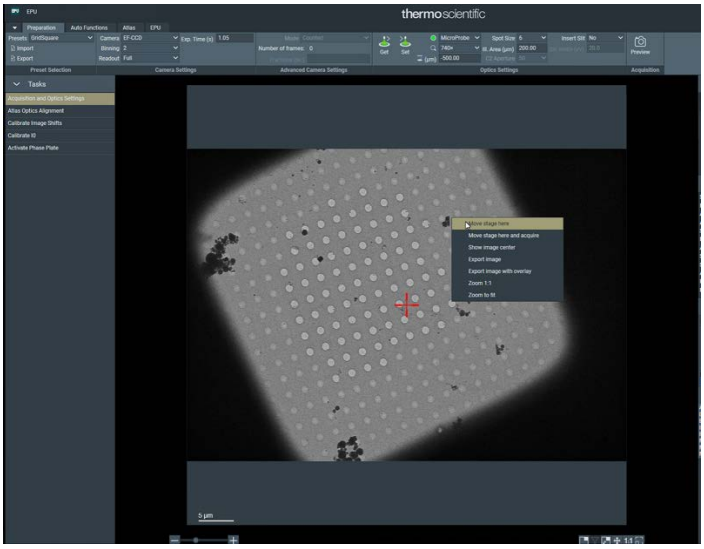


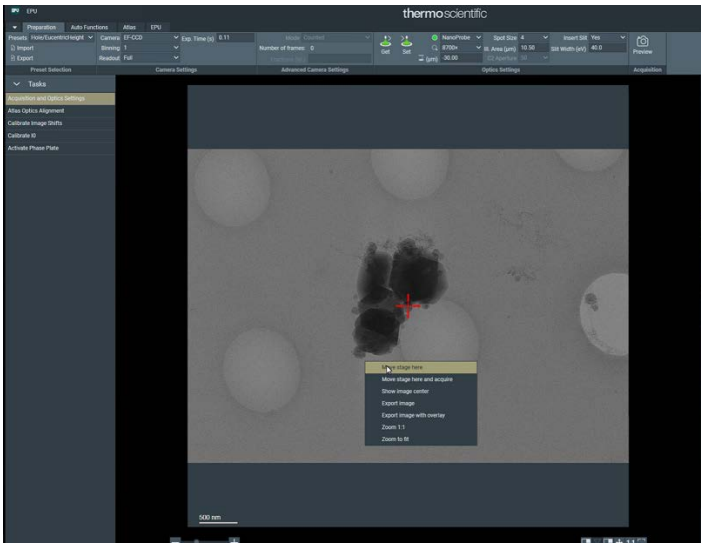
- Perform auto-eucentric height adjustment: Under the Auto Functions tab select the Hole/Eucentric Height preset and the Auto-eucentric by beam tilt function. Then click Start. EPU will use a negative and positive beam tilt image acquisition to determine the defocus and thereby the sample height. You should get a nice bright spot in the cross correlation window.

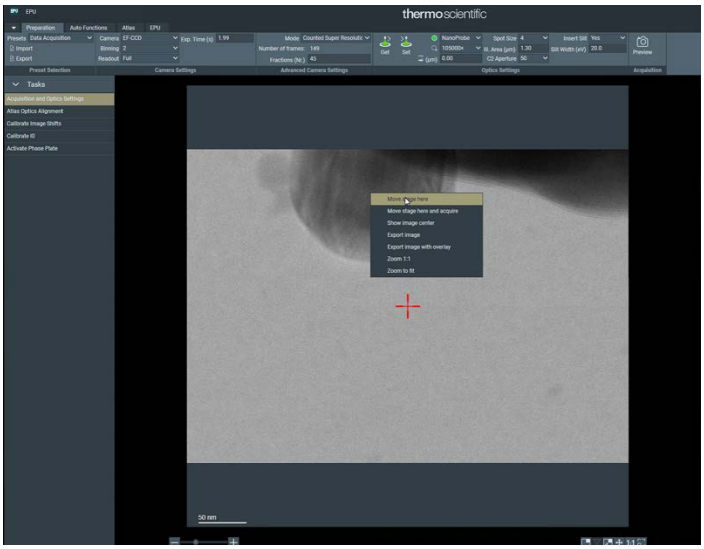




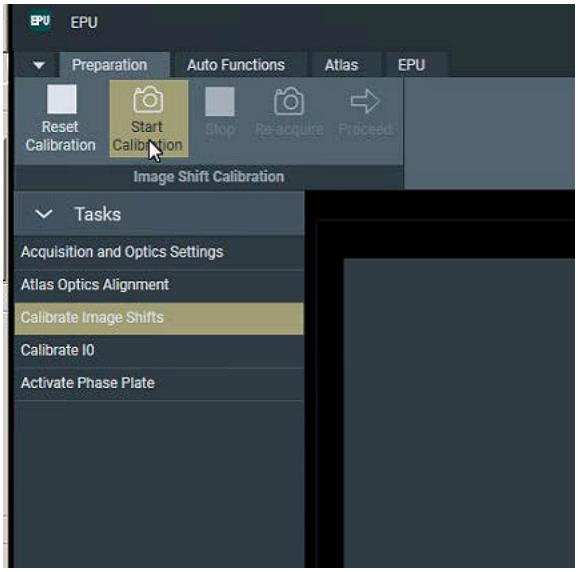
- Starting from a Gridsquare image follow your feature moving up through the presets under the Preparation tab and using right-click> move stage here to keep the feature centered.



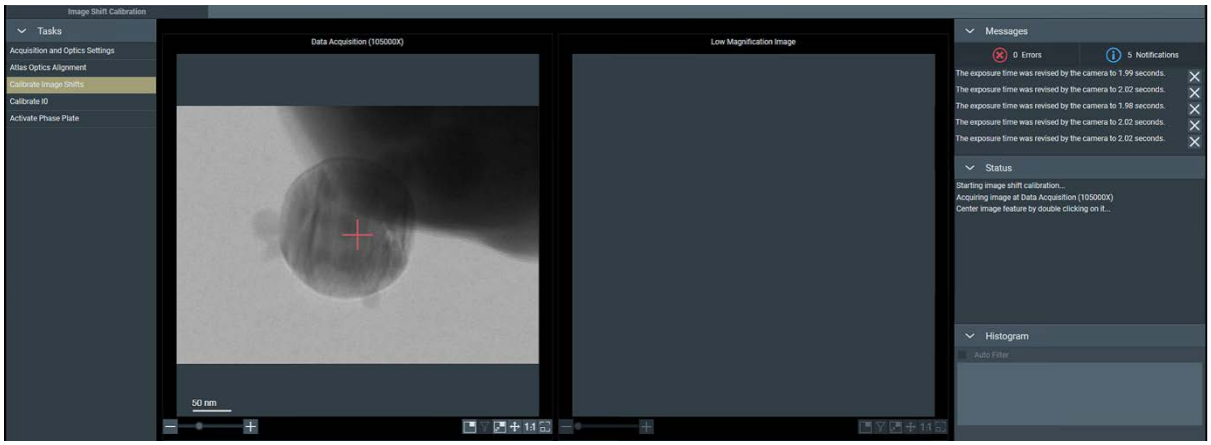




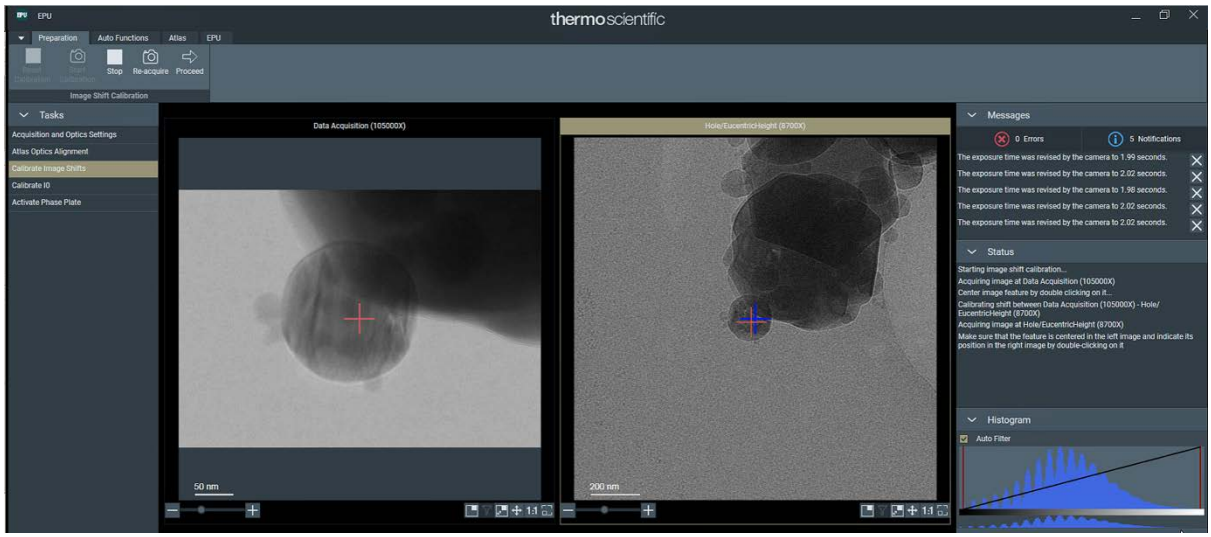
4. **Start the Image Shift Calibration:** Under the Preparation tab click on Calibrate Image Shifts and then Start Calibration.



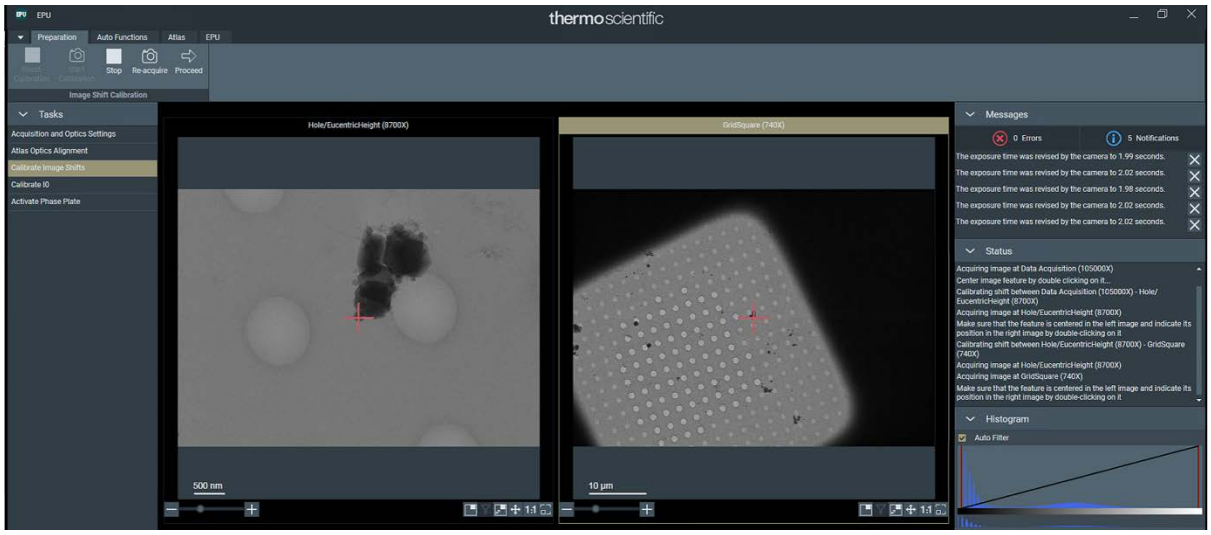
- The procedure will begin from the Data acquisition mag (in which the image shift will be zero) to the lower mags using some image shift to keep the feature centered. Double-click on the feature to re-center if needed. Click on Re-acquire image to ensure it is centered after double-clicking. Click on Proceed to continue onto the next mag.

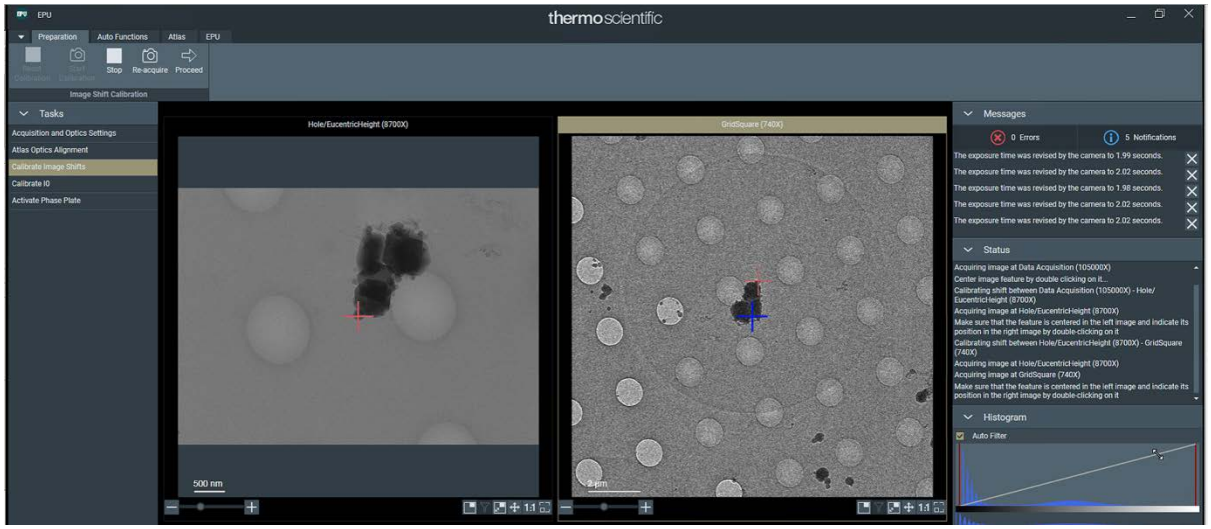


- Zoom in if needed (scroll wheel or slider)



- There is a 180 degree rotation between the Hole/Eucentric mag and the Gridsquare mag.


EPU
thermoscientific
Preparation
Auto Functions
Atlas
EPU
Stop
Re-acquire
Proceed
Image Shift Calibration
Tasks
Acquisition and Optics Settings
Atlas Optics Alignment
Calibrate Image Shifts
Calibrate I0
Activate Phase Plate
Hole/EucentricHeight (8700X)
GridSquare (740X)
500 nm
10 µm
Messages
0 Errors
5 Notifications
The exposure time was revised by the camera to 1.99 seconds.
The exposure time was revised by the camera to 2.02 seconds.
The exposure time was revised by the camera to 1.98 seconds.
The exposure time was revised by the camera to 2.02 seconds.
The exposure time was revised by the camera to 2.02 seconds.
Status
Acquiring image at Data Acquisition (105000X)
Center image feature by double clicking on it...
Calibrating shift between Data Acquisition (105000X) - Hole/EucentricHeight (8700X)
Acquiring image at Hole/EucentricHeight (8700X)
Make sure that the feature is centered in the left image and indicate its position in the right image by double-clicking on it
Calibrating shift between Hole/EucentricHeight (8700X) - GridSquare (740X)
Acquiring image at Hole/EucentricHeight (8700X)
Acquiring image at GridSquare (740X)
Make sure that the feature is centered in the left image and indicate its position in the right image by double-clicking on it
Histogram
Auto Filter


EPU
thermoscientific
Preparation
Auto Functions
Atlas
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Stop
Re-acquire
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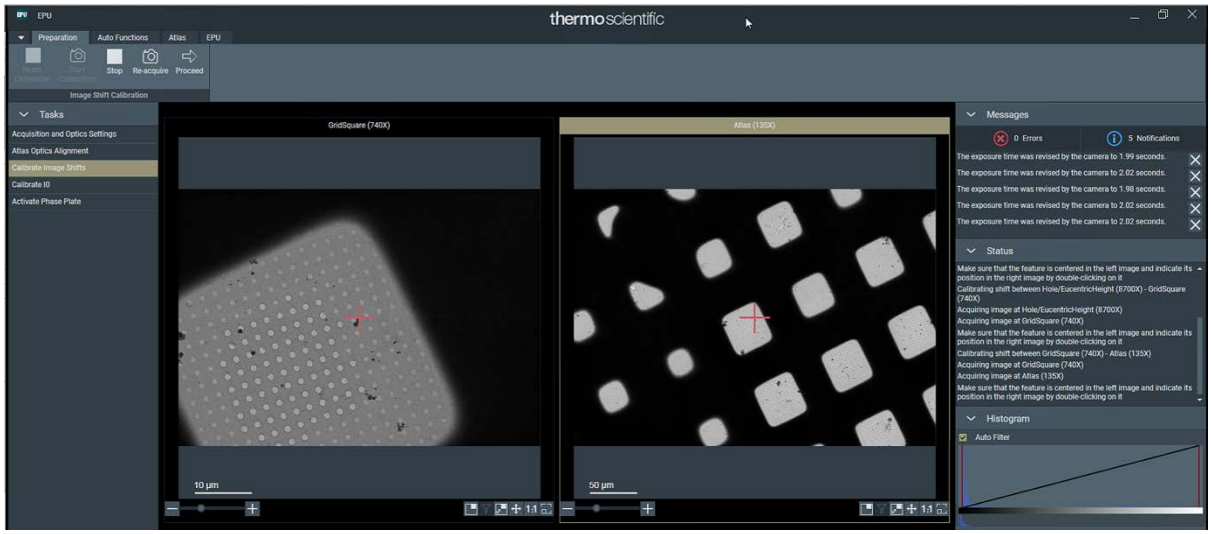
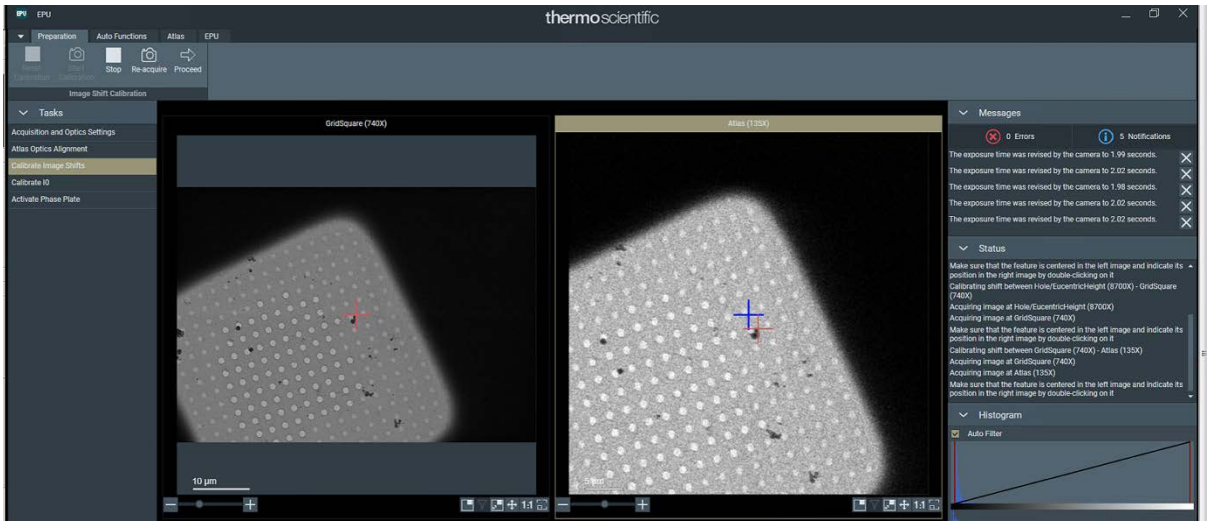
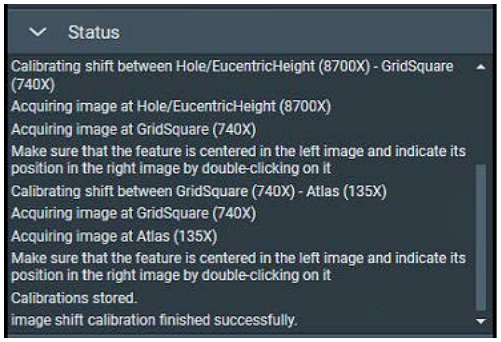
Image Shift Calibration
Tasks
Acquisition and Optics Settings
Atlas Optics Alignment
Calibrate Image Shifts
Calibrate I0
Activate Phase Plate
Hole/EucentricHeight (8700X)
GridSquare (740X)
500 nm
2 µm
Messages
0 Errors
5 Notifications
The exposure time was revised by the camera to 1.99 seconds.
The exposure time was revised by the camera to 2.02 seconds.
The exposure time was revised by the camera to 1.98 seconds.
The exposure time was revised by the camera to 2.02 seconds.
The exposure time was revised by the camera to 2.02 seconds.
Status
Acquiring image at Data Acquisition (105000X)
Center image feature by double clicking on it...
Calibrating shift between Data Acquisition (105000X) - Hole/EucentricHeight (8700X)
Acquiring image at Hole/EucentricHeight (8700X)
Make sure that the feature is centered in the left image and indicate its position in the right image by double-clicking on it
Calibrating shift between Hole/EucentricHeight (8700X) - GridSquare (740X)
Acquiring image at Hole/EucentricHeight (8700X)
Acquiring image at GridSquare (740X)
Make sure that the feature is centered in the left image and indicate its position in the right image by double-clicking on it
Histogram
Auto Filter

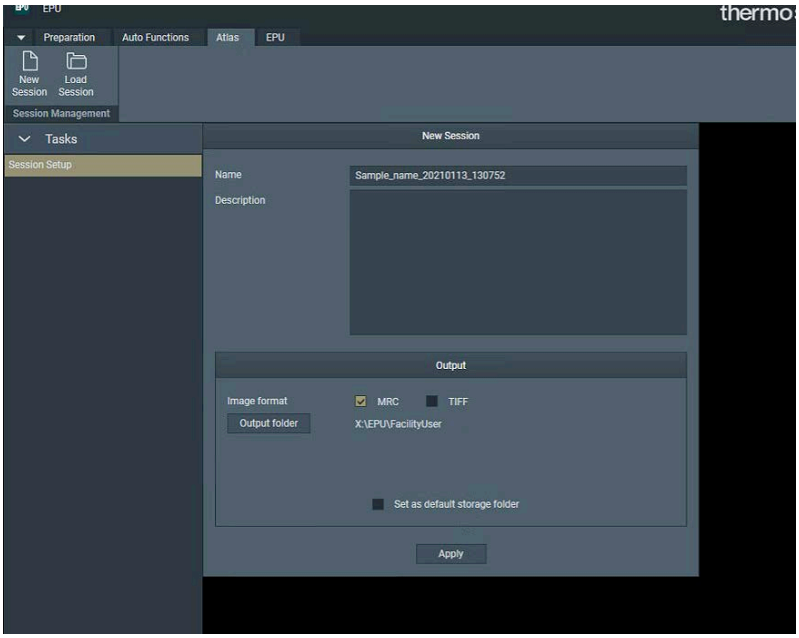

EPU
thermoscientific
Preparation
Auto Functions
Atlas
EPU
Stop
Re-acquire
Proceed
Image Shift Calibration
Tasks
Acquisition and Optics Settings
Atlas Optics Alignment
Calibrate Image Shifts
Calibrate I0
Activate Phase Plate
GridSquare (740X)
Atlas (135X)
10 µm
50 µm
Messages
0 Errors
5 Notifications
The exposure time was revised by the camera to 1.99 seconds.
The exposure time was revised by the camera to 2.02 seconds.
The exposure time was revised by the camera to 1.98 seconds.
The exposure time was revised by the camera to 2.02 seconds.
The exposure time was revised by the camera to 2.02 seconds.
Status
Make sure that the feature is centered in the left image and indicate its position in the right image by double-clicking on it
Calibrating shift between Hole/EucentricHeight (8700X) - GridSquare (740X)
Acquiring image at Hole/EucentricHeight (8700X)
Acquiring image at GridSquare (740X)
Make sure that the feature is centered in the left image and indicate its position in the right image by double-clicking on it
Calibrating shift between GridSquare (740X) - Atlas (135X)
Acquiring image at GridSquare (740X)
Acquiring image at Atlas (135X)
Make sure that the feature is centered in the left image and indicate its position in the right image by double-clicking on it
Histogram
Auto Filter



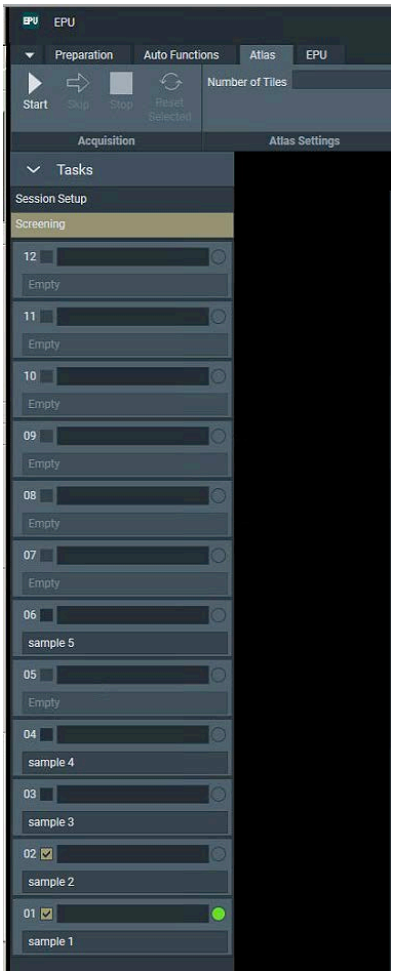
- Once the final mag is done it will give the message “Image Shift Calibration finished successfully”



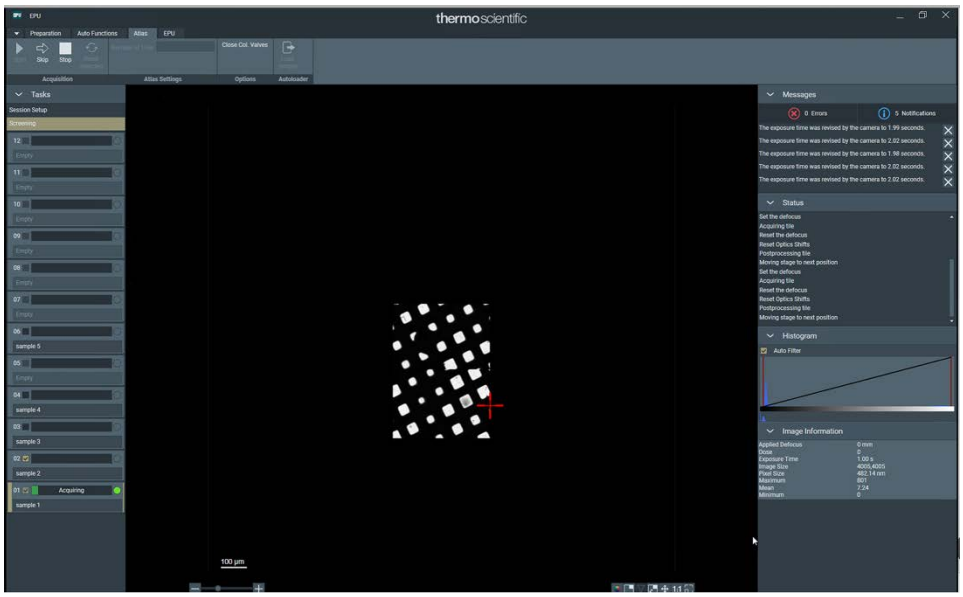
5. **Perform Atlas collections for the grids in the Autloader:** Under the Atlas Tab select a New Session and Save files to the OffLoadData drive (X:) under the EPU folder in a sub-folder of your choice.



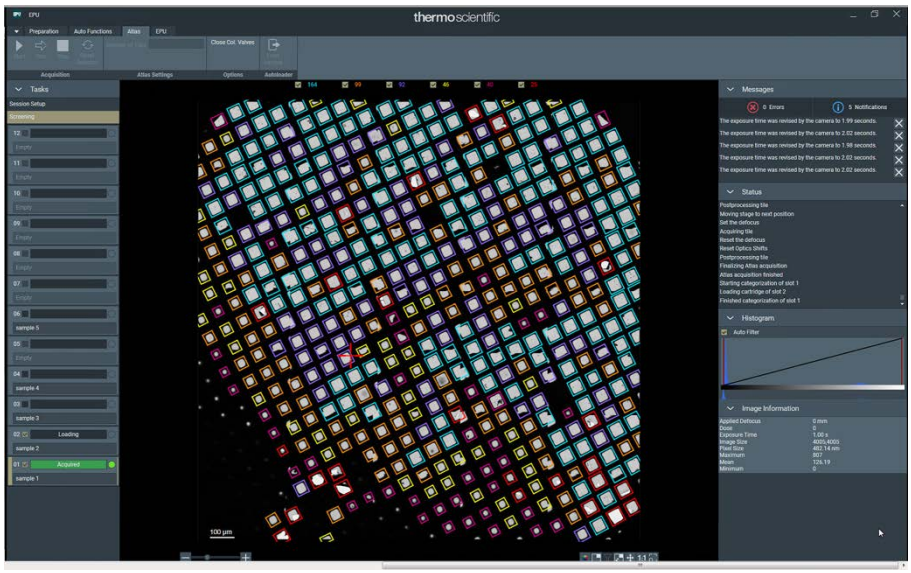
- Once you click Apply a new screening menu appears. Select (check box) for the grids you want an Atlas for and click Start. Each Atlas take about 10 minutes including sample exchange.



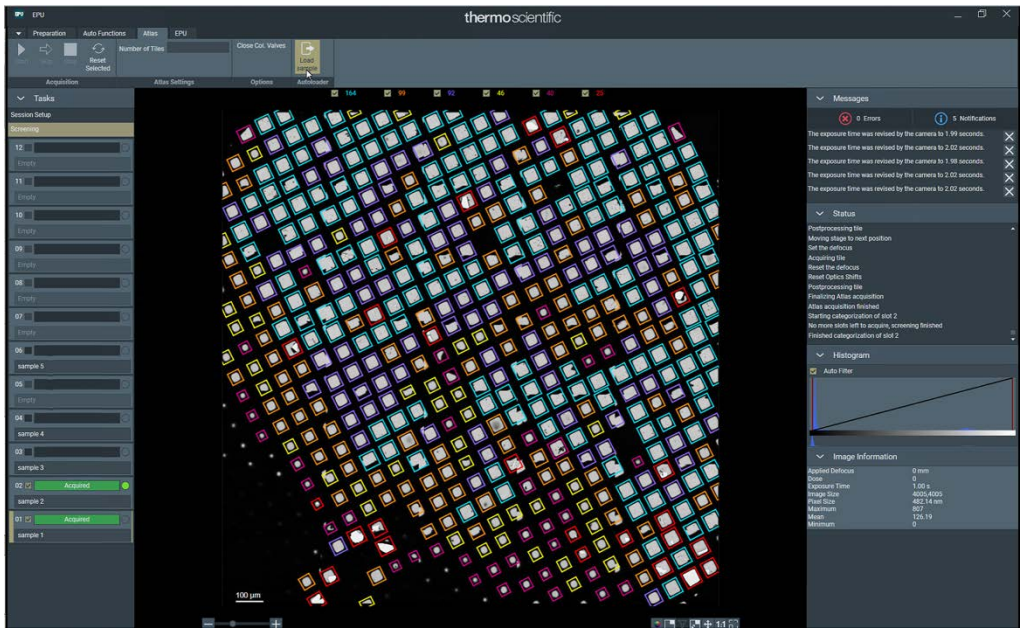
- To view a collecting/collected Atlas click on the slot to highlight it (a vertical yellow line appears).

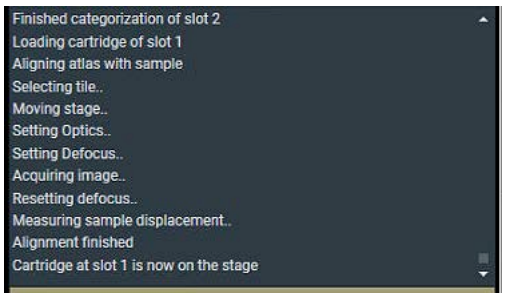


- Once an Atlas is done EPU will categorise the squares. The colours match similar squares. Red means some cracks have been identified.

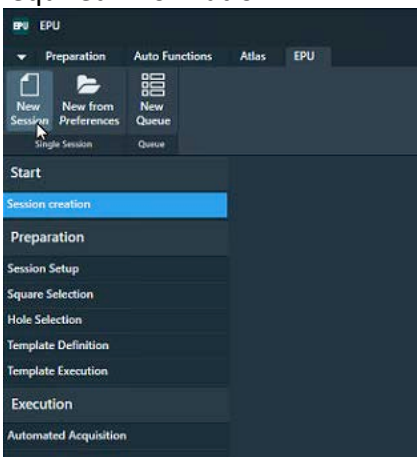


6. **Choose a grid from the screening window and load it:** Using EPU to load a grid allows you to use an existing Atlas of that grid. After the grid is loaded an image is taken of an area and the existing Atlas is re-aligned to the new grid position. Loading a grid from the Autoloader window will not perform this alignment. Highlight a slot and click on Load Sample.



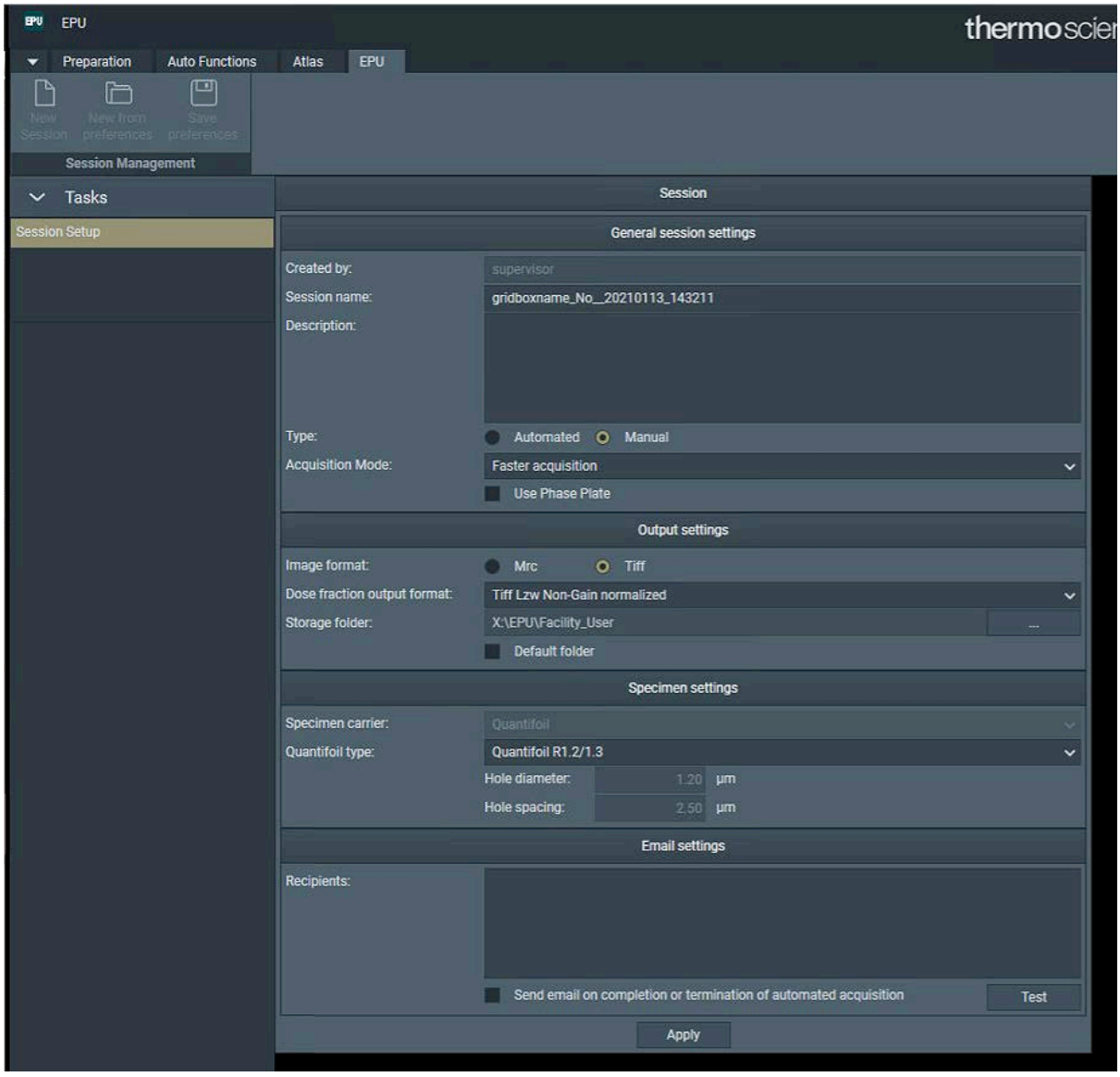


7. **Create a new EPU session:** Under the EPU tab click on Session creation and then New Session. Fill in all the required information.

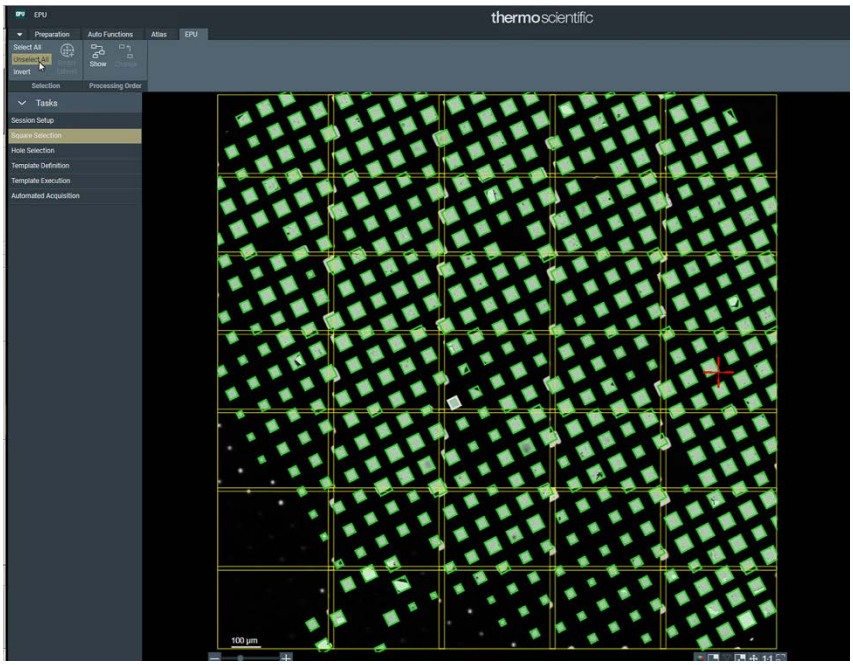


- Name the session: ***Tip:*** *Gridbox name and grid position works well and you can relate this back to your freezing form.*
- Type: Manual
- Acquisition Mode: Fast acquisition if collecting by AFIS
- Image format can be Tiff or MRC (This is for the summed images we will not process anyway)

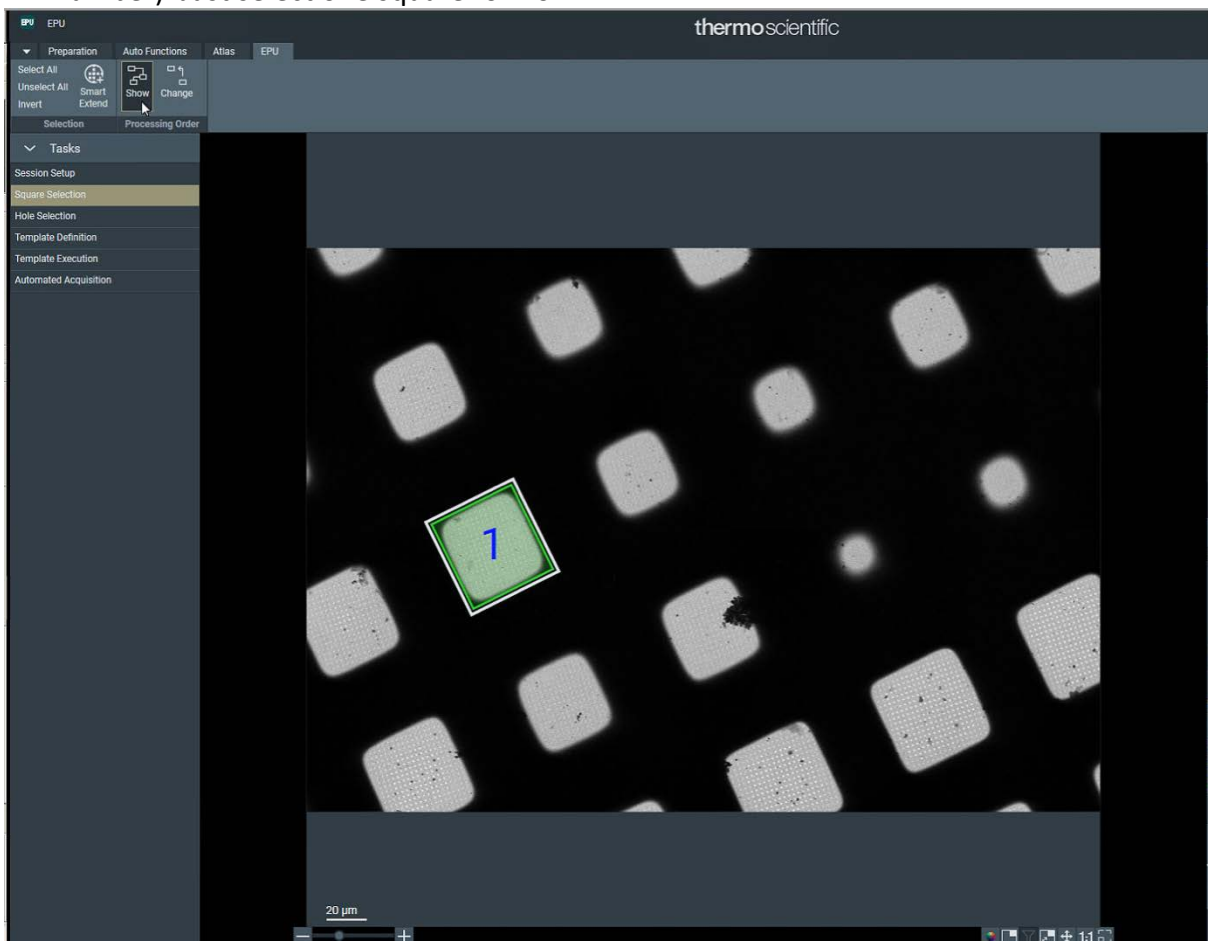
- Dose Fraction output format: Use Tiff lzw non gain-normalised unless there is a reason not to.
- Storage Folder: Save EPU session files on the OffLoadData drive under the EPU folder in a sub-folder of your choice. The movies are automatically saved under DoseFractions in the K3 drive mount under a folder with your session's name. This movies folder gets copied to the cluster automatically as you collect data.
- Specimen carrier: Select the right grid type
- Click Apply



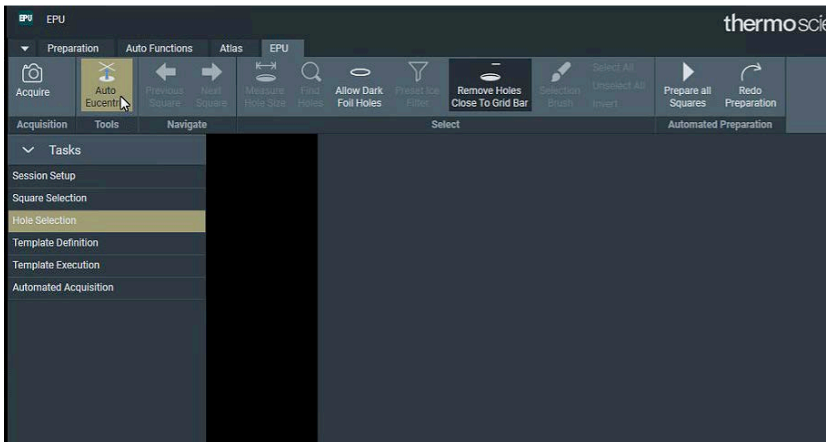
8. **Select and setup the first square to collect on:** Under the EPU tab click on Square selection and select suitable squares for data collection.
   - Initially EPU selects all squares so you have to Unselect All Squares. The Atlas is made out of the tiles it has stitched together (yellow outlines).



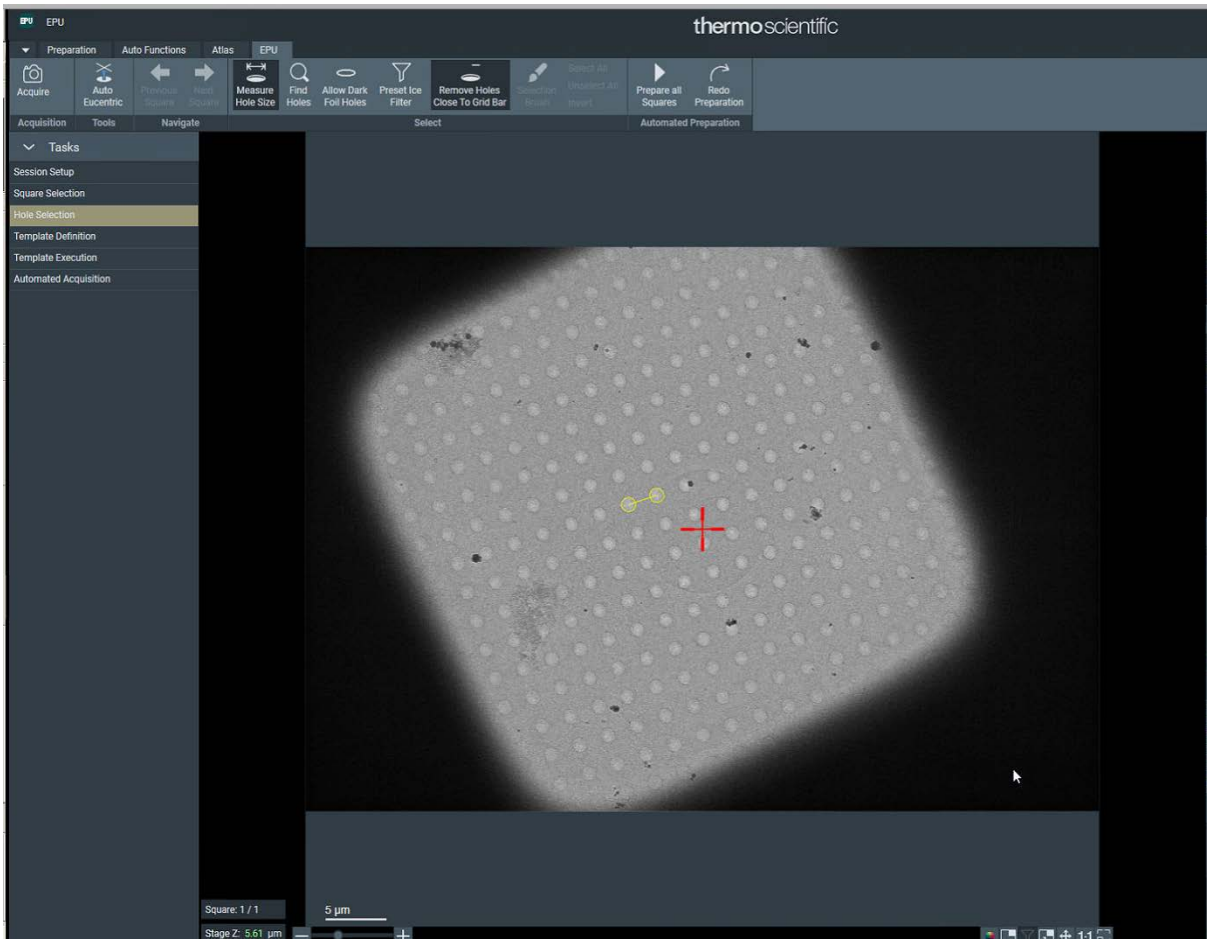
- You can open a tile by double clicking on it. You can select a square for data collection by holding down Control and clicking on the square. You can unselect by using Shift + click. Clicking on Show will indicate the square number (its queue number). Just select one square for now.



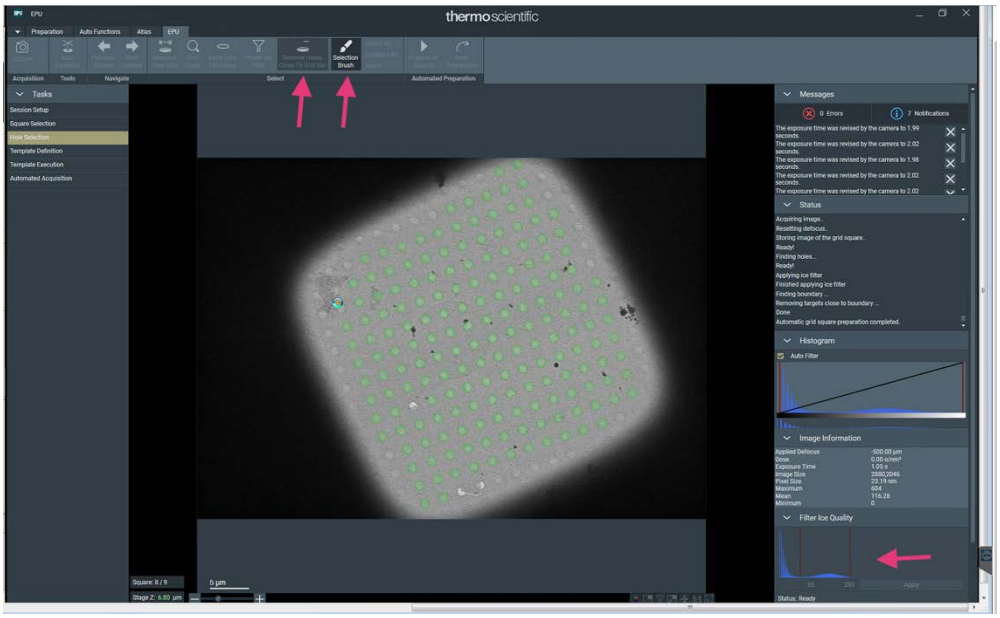
- For this first grid square we setup the height semi-automatically. Click on Hole Selection and then Auto Eucentric.



- Once the height is adjusted EPU will take a Gridsquare image. Click on Measure holes and drag the yellow circles to two orthogonally-related adjacent holes. Adjust the diameter size to match your holes. Zoom in if needed.

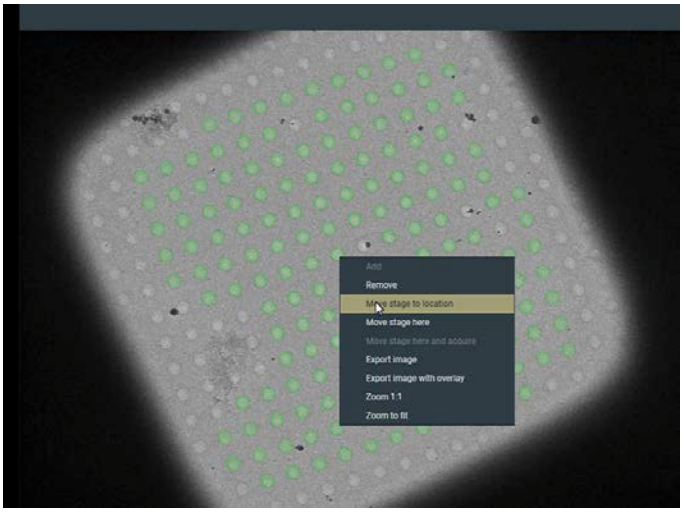


- Click on Find Holes to automatically find the holes.

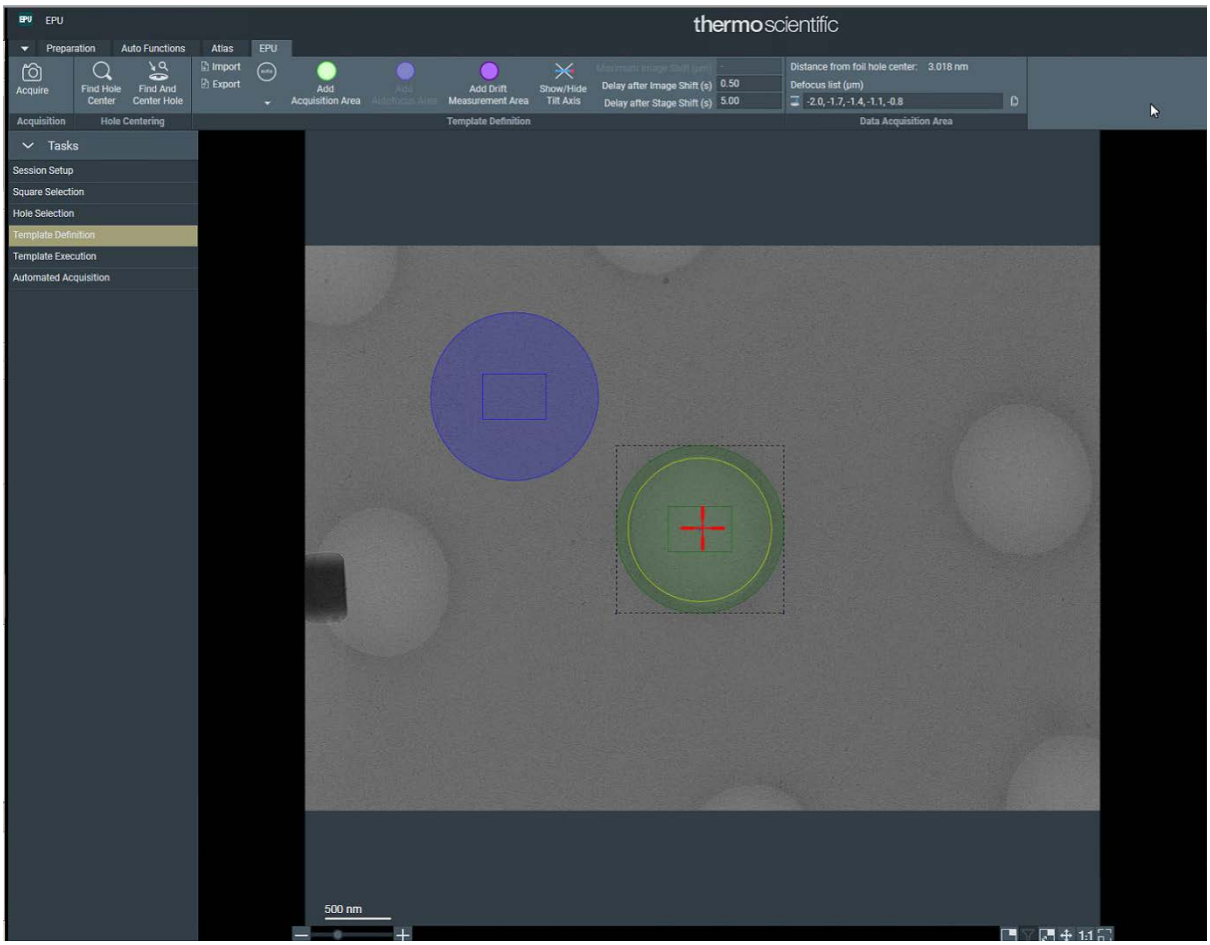


- Use the Ice Filter sliders to select holes of a certain ice thickness. Click on Remove Holes Close to Grid Bars to remove holes near the edge which are likely to have crystalline ice. Finally use the Selection Brush to clean up.

- Right-click on one hole and move stage to location to roughly center a hole. ***Tip:*** *Choose a hole close to a feature so you can double check your image shift* calibrations are good.



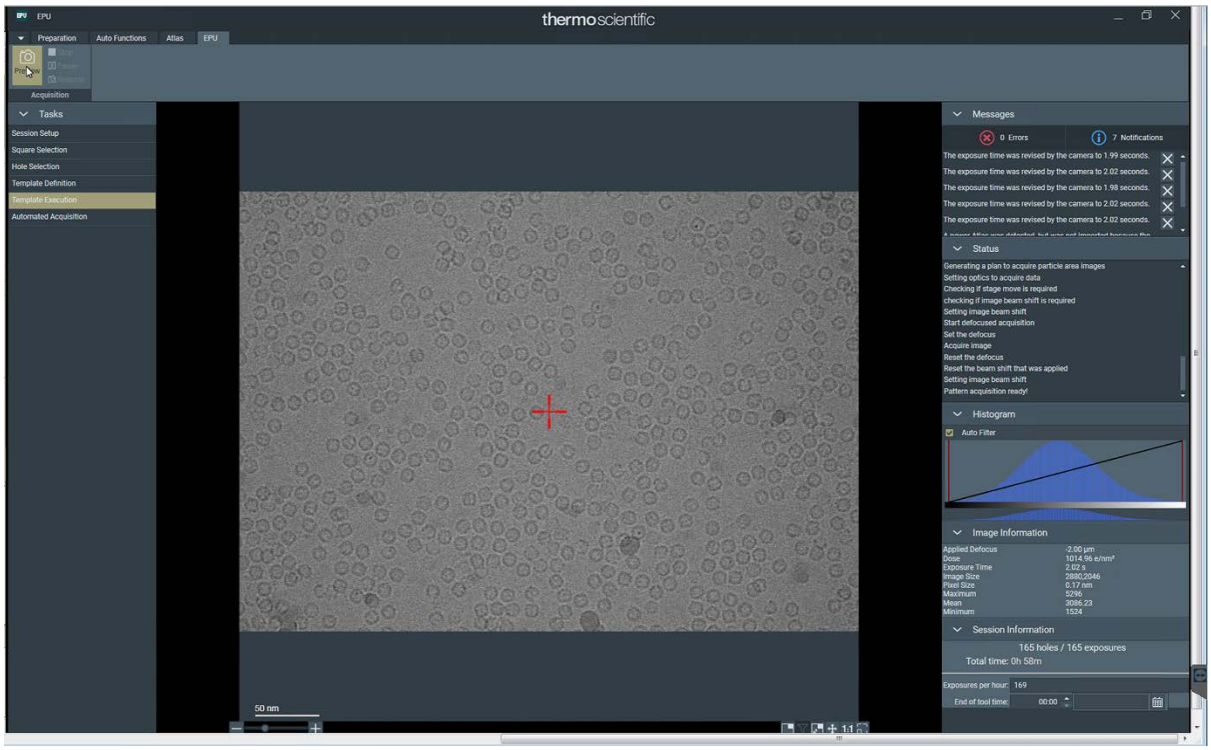
9. **Setup the Template definition:** Next we have to decide how we collect the data. Click on Template Definition.



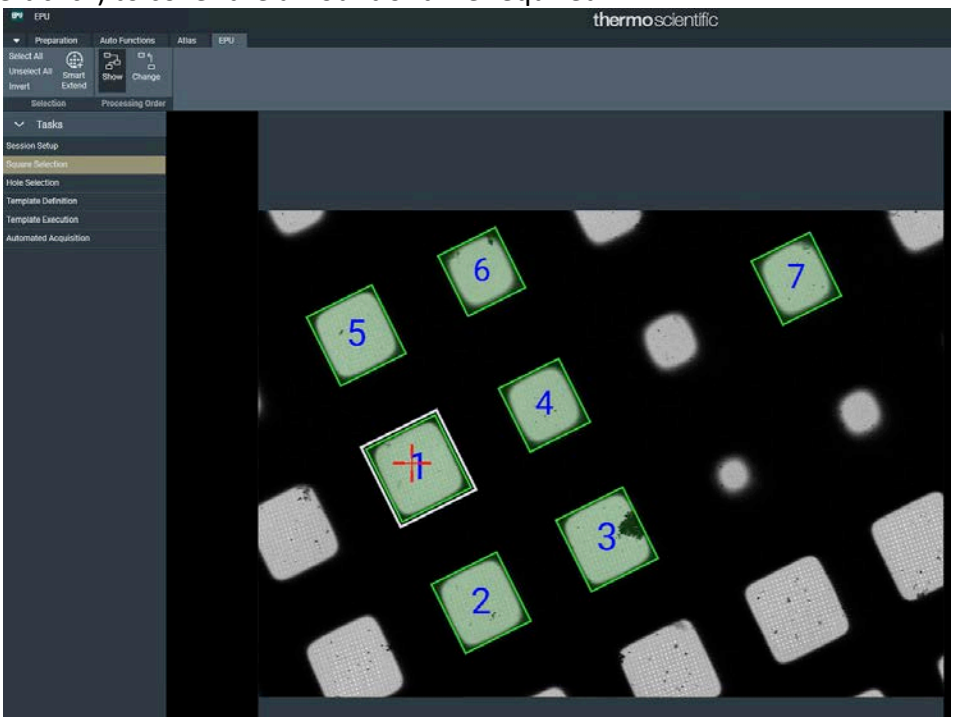
- Click on Acquire to take an image
- Click on Find and Center Hole
- Click on Add Acquisition Area and adjust the green circle (Data acquisition beam illumination area) position in the hole. For 1 image per hole we usually illuminate the hole symmetrically. For 2 or more images per hole ensure the green circles do not infringe on the others field of view (green rectangle) to avoid radiation damage.
- Delay after Image shift to 0.5-1.0 seconds
- Delay after stage shift: 5 seconds minimum.
- Click on the green circle. Enter the desired defocus values in the list separated by commas. Use a minus (-) to denote under-focus. Typical ranges are -2.5 to -1.0 um in 0.3 um intervals. ***Tip:*** *Start with the highest first as this value is used for testing the template and gives the best contrast.*
- Click on Add Acquisition Area to add more acquisition areas to the same hole. The desired defocus vales should transfer automatically.
- Click on the Add Autofocus Area and drag the blue circle in-between the holes. For Autofocus Recurrence choose After Centering for AFIS or every 10-15um for regular stage-shift collection. Always focus using Objective lens.

10. **Decide what mode you want and how you want to fractionate your movies.** Under the Preparation tab select the Data Acquisition preset. Here you can change the exposure time, the way you scale the data (binning) and how you fractionate your movies:
    - **Exp. time:** The preset you have loaded is already set for the correct exposure time to give a total dose of ~45 e/Å^2. If you want a higher total dose you can increase this.
    - **Mode and Binning:** Choose between Super-resolution or Counted mode. We typically use Super-resolution with Binning 1. This leads to the biggest files and slowest speed but the highest DQE on the detector (higher S/N). Alternatively Counting Binning 1 is also a good option for 99% of projects. The file size is half and the speed 10% faster than SR bin 1. Finally Super-resolution Binning 2 gives the fastest speeds (>50% faster than SR bin1) but the lowest DQE. However in our benchmarks a 210 kDa protein with C7 symmetry goes to 2.55Å in this mode (vs 2.3Å with SR bin1).
    - **Fractions:** We have set this up so every frame has ~1 e/Å^2 dose.
    - Everything else can be left as default.

11. **Template Execution:** We next want to make sure the template we setup works well and the microscope is shooting where we want it to. Click on Template Execution and then Preview.



12. **Choose the remaining squares:** Go back to square selection and add more squares (Ctrl-left click) to cover the amount of time required.

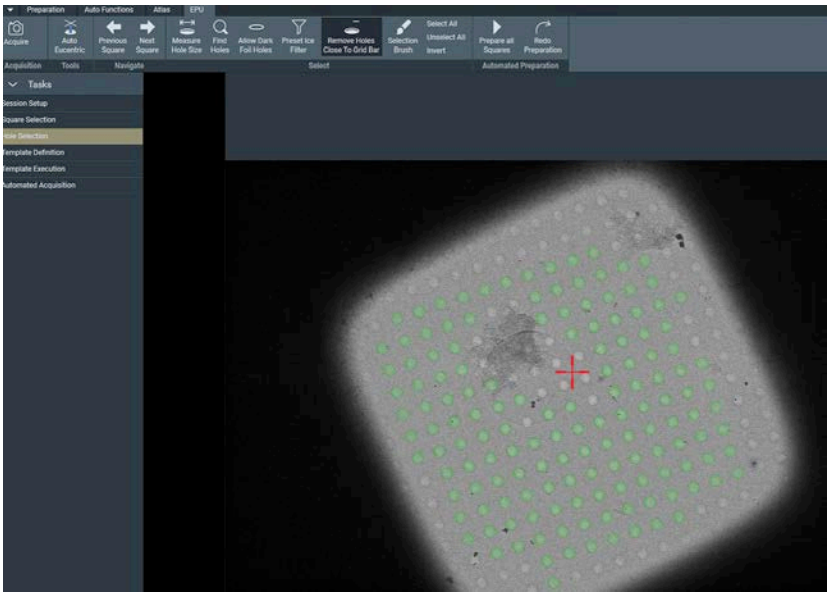


13. **Automatically prepare the remaining squares:** EPU now has the functionality to setup the remaining gridsquares automatically. It will navigate to each square, perform Auto-eucentric and select the holes based on your ice filter selection. Go to Hole Selection and click on Prepare All squares. It takes approx. 45 seconds per square. ***Tip****: Go grab a coffee* ☺

14. **Inspect the hole selection manually and clean as required:** In Hole Selection you may now navigate the squares by using the Previous or Next square buttons and cleaning up using the selection brush. The square number along with the determined Z height are shown on the bottom. If a square is particularly bad simply click the unselect all holes button. ***Tip:*** *When using AFIS collection becomes inefficient if there are few, scattered holes. In this case its best to skip such squares.*

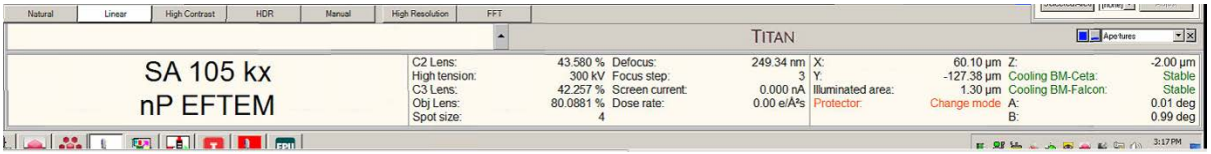
15. Comma-free and objective lens astigmatism corrections: The microscope alignments are incredibly stable but if a new optics setting is used between sessions it is best to check for comma and make sure objective lens astigmatism is a small as possible.

- Navigate to an area with carbon. ***Tip:*** *Use the last gridsquare that was setup using Prepare All Squares as the current height is eucentric for that square.*
- In the Hole selection windows right click and navigate over some carbon.

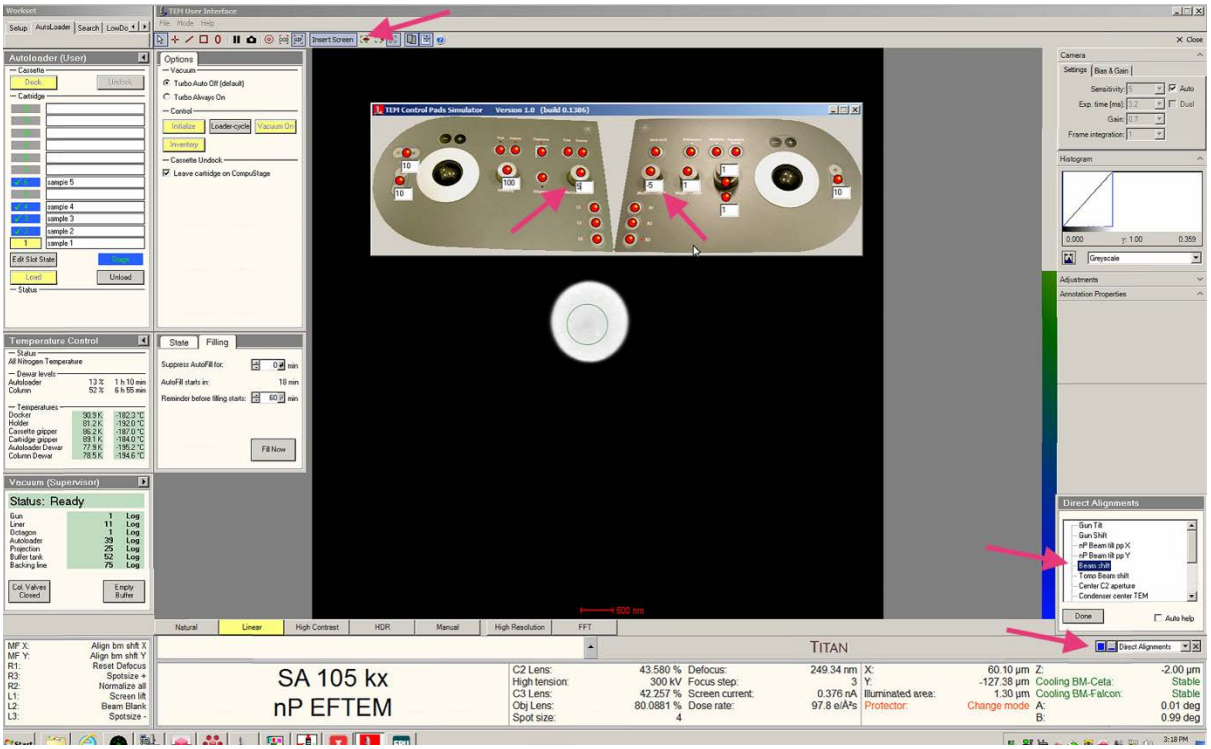


- **Read this carefully!!!** Under Preparation select the Data Acquisition Preset and then click **SET**. (Do not click get)

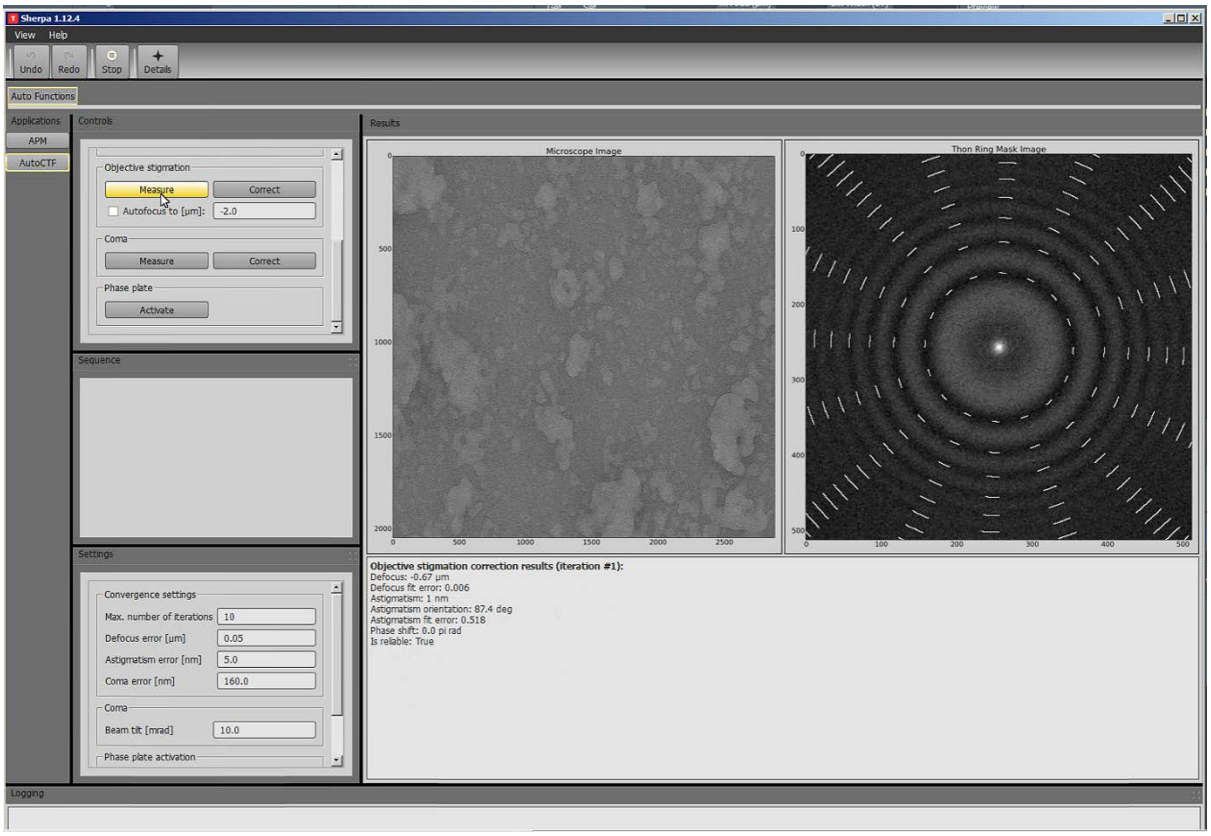
- Clicking SET will set the Data acquisition optics and allow us to check comma with the exact illumination conditions used for data collection (mag, beam diameter, spot size).



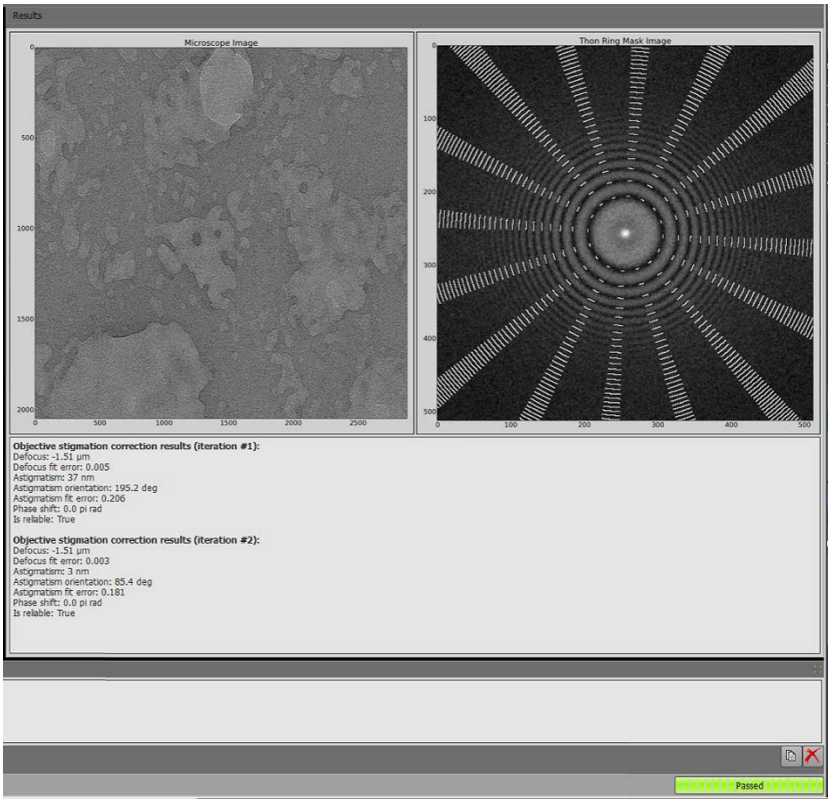
- Let's check if the beam is centered. On the left monitor click on Insert Screen.



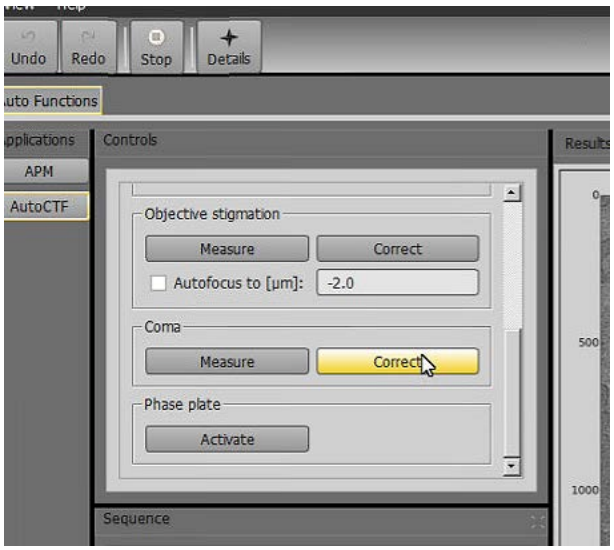
- If the beam requires centering open the Direct Alignments and click on Beam Shift. Use the hand-panel MF X+Y (Or the virtual hand-panel MF X+Y) to center the beam. Change the direction of the virtual hand-panel knob by entering a different sign (- or +).
- Once the beam is centered dial in a defocus of ~-1.5um using the Focus knob (type in a -ve number to underfocus).
- Open Sherpa if not already open (Under Software Launcher>Tools)



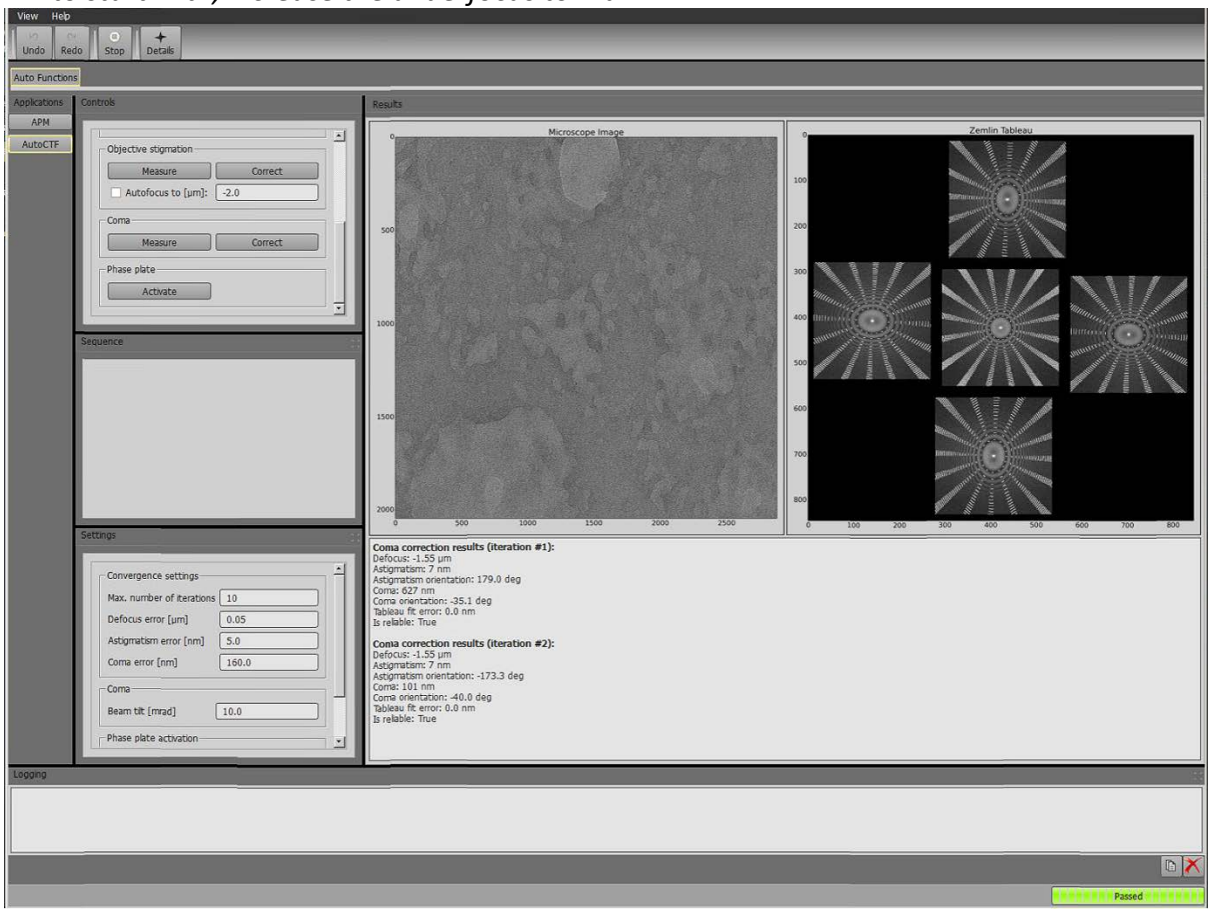
- Make sure EF-CCD is selected and use a 2 sec exposure and bin 2
- Under Objective stigmation click on Measure so we can check the real defocus.
- Adjust the defocus manually if required
- Click on Correct and wait for the Passed message to appear



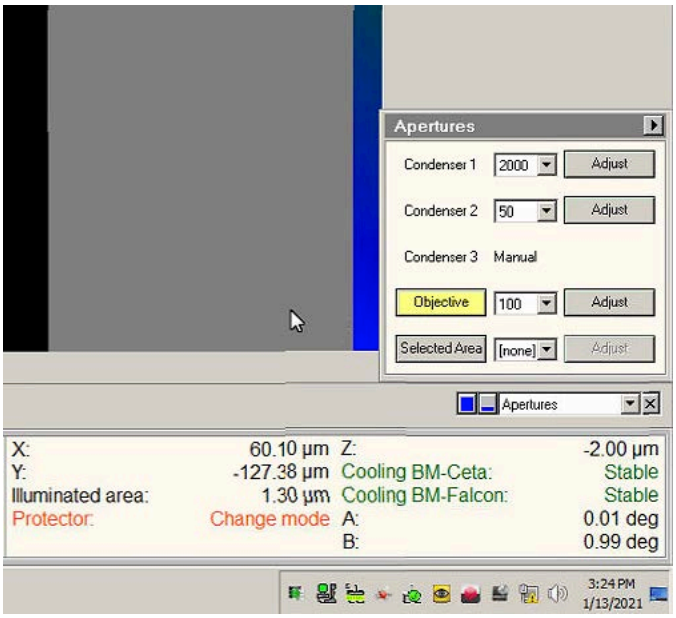
- Next click on Coma Correct to start a Zemlin Tableau



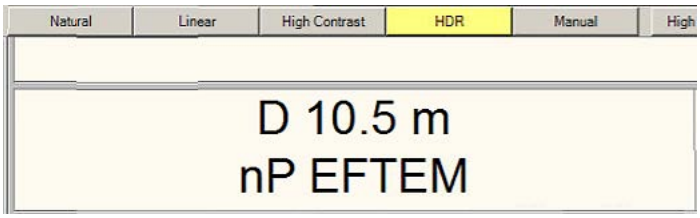
- Wait for the procedure to finish (Passed message). ***Tip:*** *if there is too much coma to start with, increase the underfocus to -2um.*



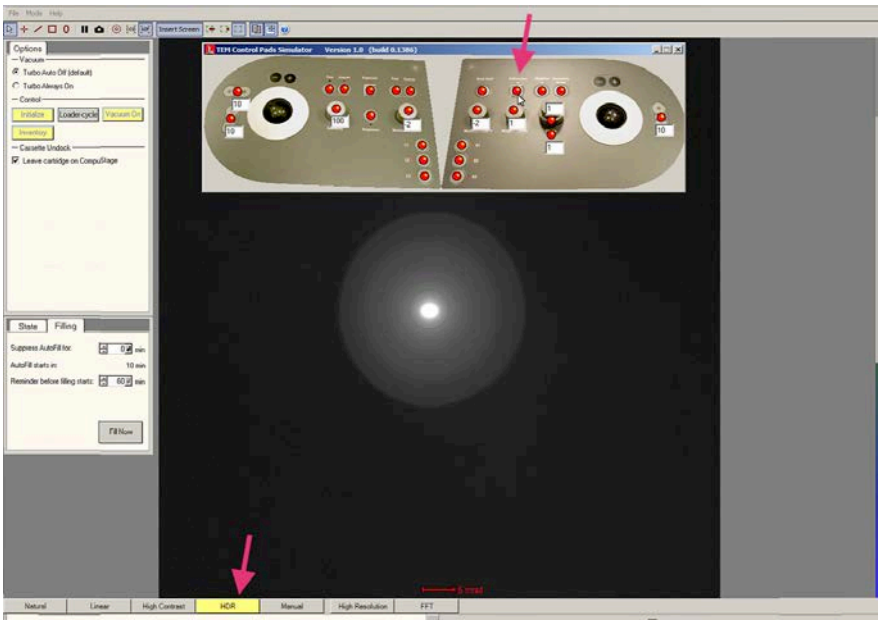
- Next insert the Objective Aperture (we normally use the 100um aperture).

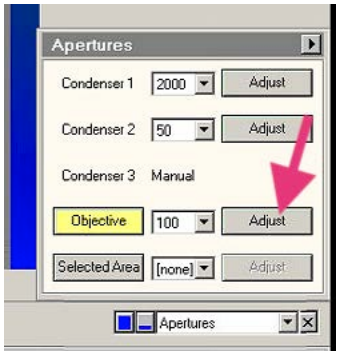


- **Please read this carefully. Never take an image on the K3 in diffraction mode**.
- Insert the flu-screen and make sure you can see the beam
- Click on the Diffraction button on the handpanels and HDR setting on the Flu-screen.
- Make sure the camera length is set to 10.5 meters. Use the magnification knob to set this if not.

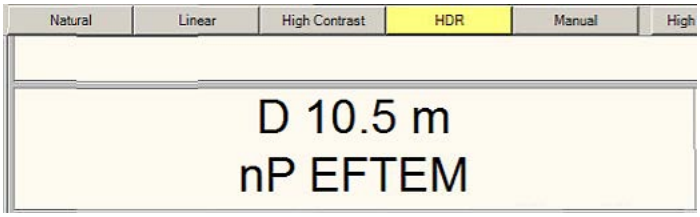


- Check if the aperture is centered around the central spot (un-diffracted beam) and center if needed by clicking on Adjust and centering aperture by MF X+Y. Click Adjust again to exit.

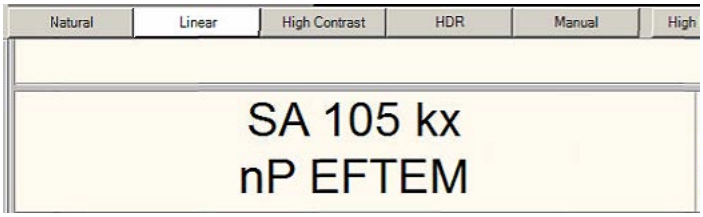




- **Click on Diffraction again to exit Diffraction mode**. Ensure you do not see this anymore:

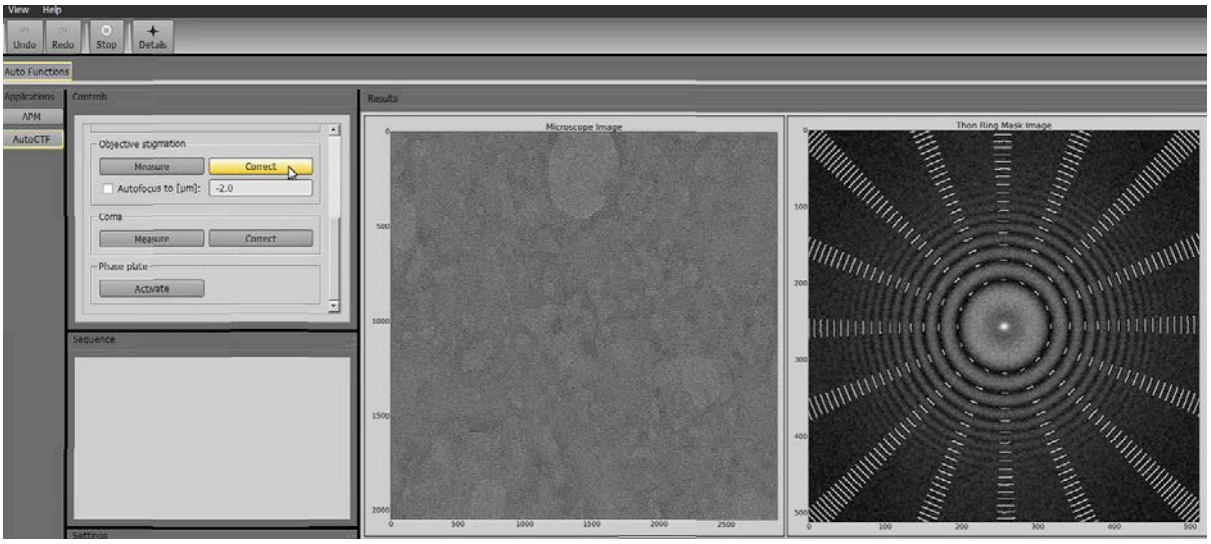


You should now see something like this (Magnification may vary):

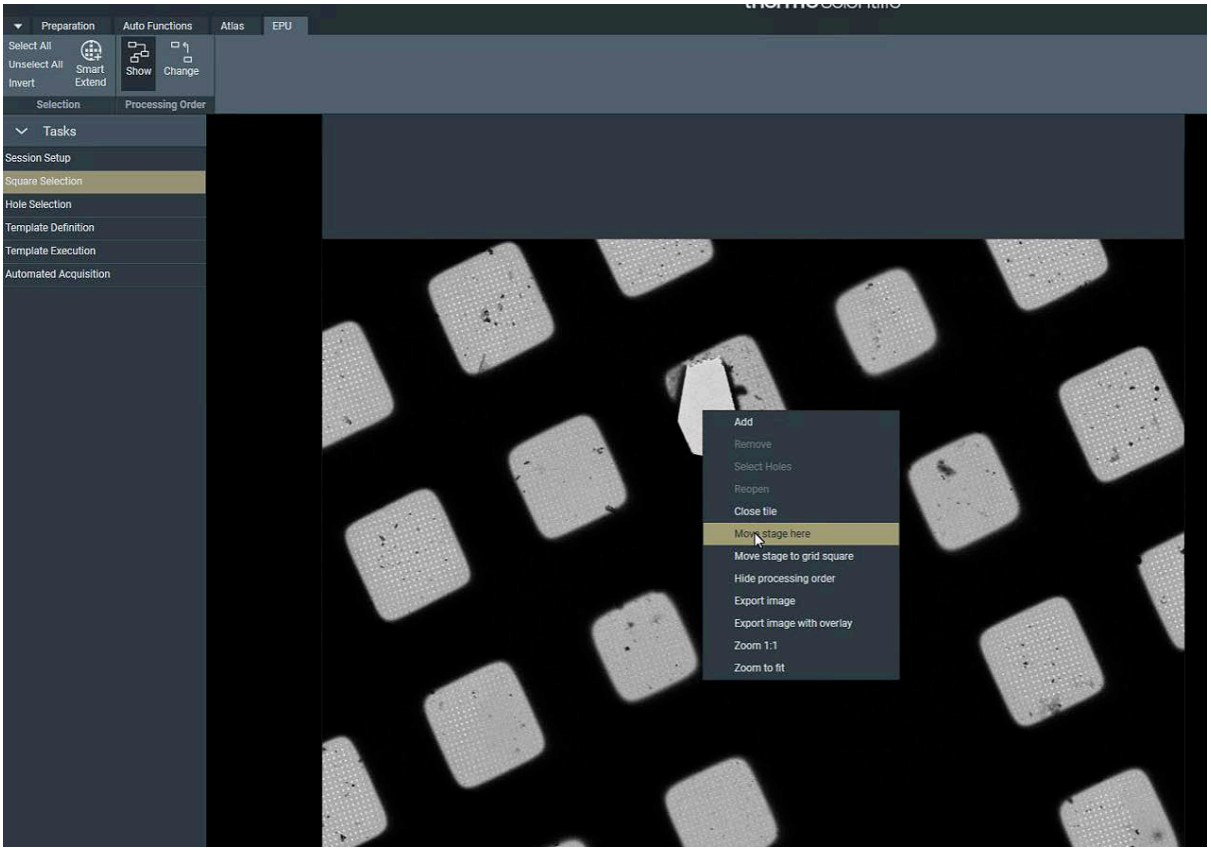


- You will also have to dismiss a message in EPU which has complained about switching to diffraction mode. Click on Yes to continue in EPU.

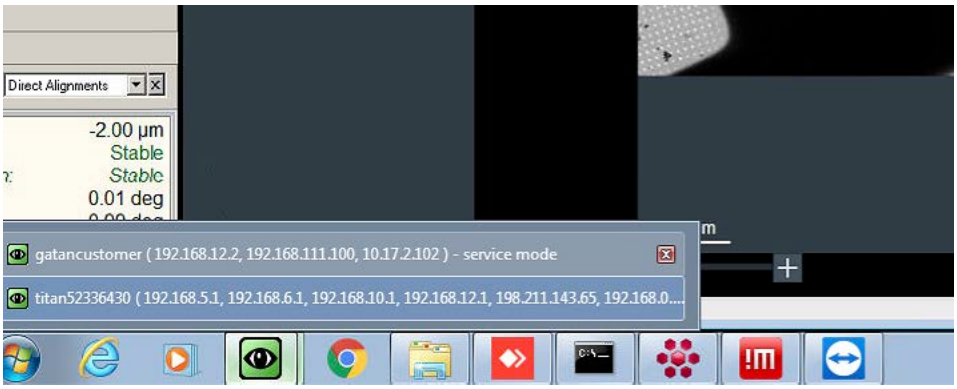
- Back in Sherpa click on Objective stigmation Correct for the final correction with an inserted objective aperture (the latter affects astigmatism).



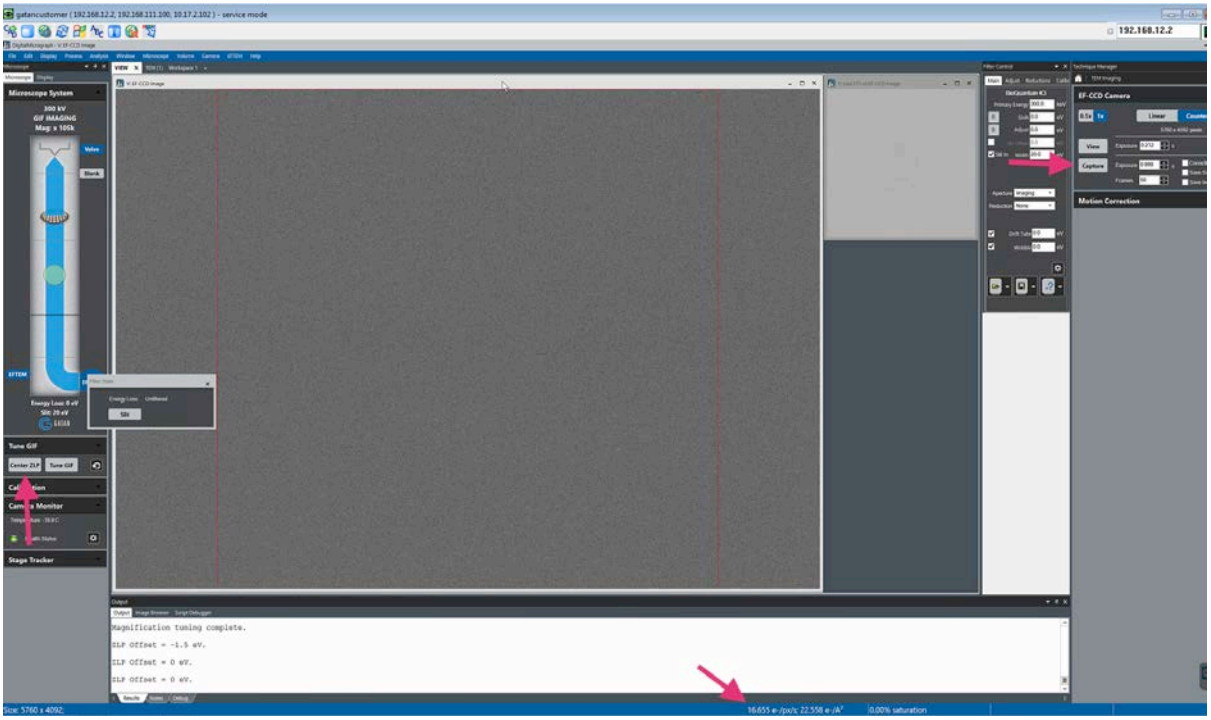
16. **Center the Zero energy loss peak on the GIF:** The MRCEMF Krios has a very stable energy filter that will not deviate more than 1-2 eV over a period of several days. However its always best practise to have a final check before you start collecting. With the microscope optics still on Data acquisition mode navigate using the Square Selection Atlas image to an empty or broken square so the beam is over vacuum.



- Switch to the K3 PC VNC window (gatancustomer)



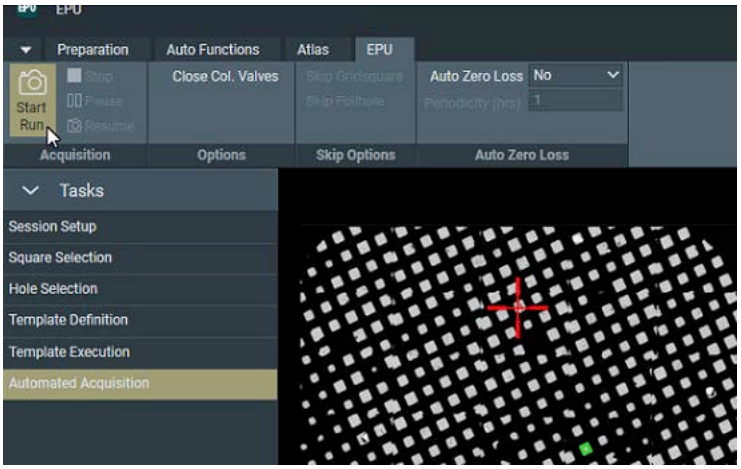
- Click on Center ZLP and wait for the procedure to finish. The energy shift will be indicated and should not be more than 1-2 eV.



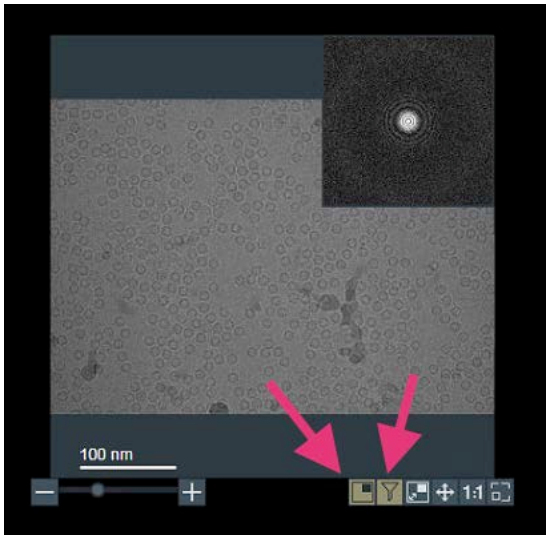
- It is also a good idea to check the dose on the specimen at this point for slight variations from the desired settings. Click on Capture and read the dose rate. Convert this to e/Å^2/sec if needed using the pixel sizes in the Appendix. Remember this is the dose on your specimen as measured over vacuum. The dose on the detector will be slightly less once your sample is in the way. This is taken into account when doing a gain ref. For example, the gain ref is done at 15 e/pix/sec but the dose on the specimen is at ~16.5 e/pix/sec. The final dose on

the detector will vary with your ice thickness but for most samples will be in the region of 15 e/pix/sec to match the gain.

17. **Start Data Collection.** Go back to the Krios PC using the VNC window and in EPU go to Automated acquisition. Click on Start Run to begin data collection. Also click on Close Col. Valves in the same window so when the collection finishes the column valves are left closed for safety. We do not use Auto Zero Loss as it is not required.



- Check the FFT of the data. Click on the first two buttons as indicated below.



Useful checks once data collection has started:

- Can you see your particles at the lowest defocus? Could you go closer?
- Does the FFT look good?
- If you see a big FFT cutoff is the Autoloader turbo pump on? (Autoloader tab on left monitor> Auto-Turbo Off should be checked, see figure on page 1)
- Do you have enough holes setup for the duration of the session?
- Did you remember to insert the objective aperture?

- If you need to adjust any parameters Stop the run and then Start it once you have made the adjustments.

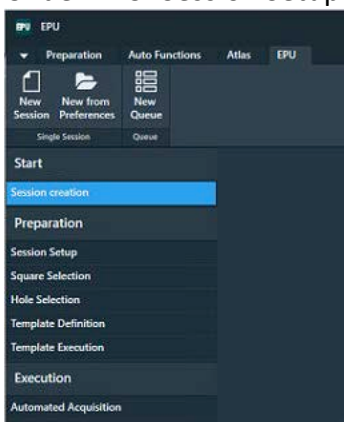
Multi-Grid EPU Setup

It is now possible to setup collection on multiple grids in one session or have multiple collections on one grid. The drawbacks are:

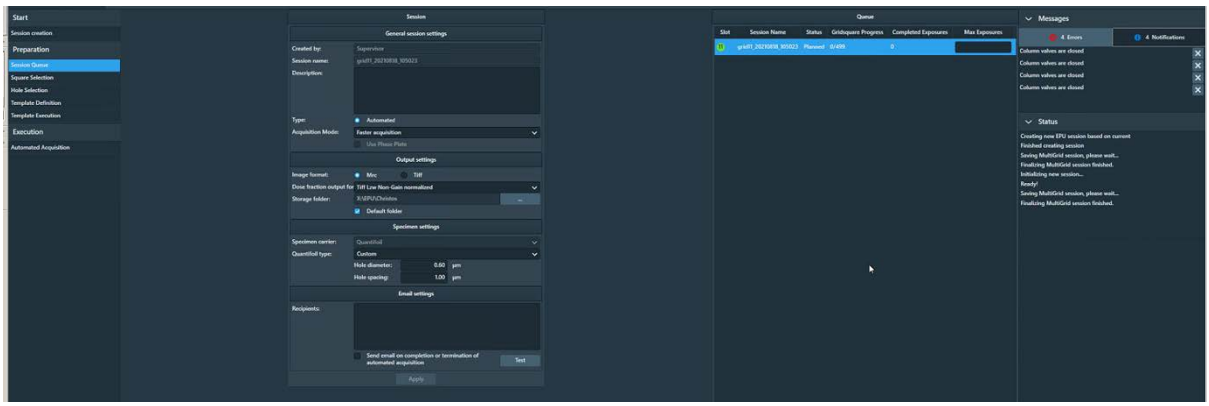
1. that hole selection is automated and you cannot add/remove holes manually or brush away bad areas. Hole selection is done using the Ice Filter.
2. The “Prepare All” functions isn’t available for automatically processing selected squares. Each square must be added and setup manually (using the auto-eucentric and find holes functions).

To start a Multi-Grid session follow the guide below

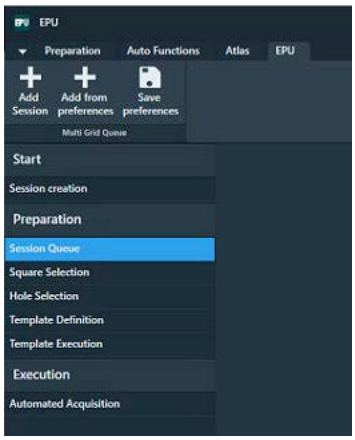
1. Create and Atlas for each grids required (EPU Step 5)
2. Load the 1st grid using the Atlas> Screening window
3. Under EPU>Session Setup click on “New Queue”



4. Fill in all the information as in EPU Step 7 in the Session Queue. Then click Apply. A queue menu will show up on the right.

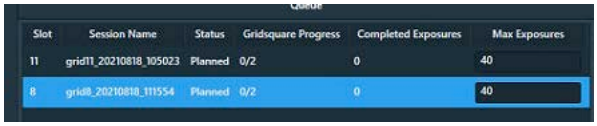


5. Select required squares using Square selection as in EPU Step 12. Each one must be then set to eucentric position using the Auto-Eucentric button in the Hole selection menu. Find the holes as before and adjust the Ice Filter for the desired hole selection.
6. Proceed to add a Template Definition as in EPU Step 9.
7. To setup additional grids first load the next grid from the Screening menu and then click on Add Session in the Session Queue menu.



8. Repeat steps 4-6.
9. To begin collection go to EPU>Automated Acquisition and click on Start Queue.

You can also edit each Session by double-clicking on the actual session queue and set the maximum number of exposures. The session will be highlighted in blue as shown below:



Processing the data on the Leicester cluster

Follow the instructions below to get started with processing your data along with the Relion tutorial.

1. Setup for using a terminal and X-windows on a Mac (using XQuartz) or Windows (using Putty and Xming). For a Mac type this line after installing XQuartz for the 1st time:

$ defaults write org.macosforge.xquartz.X11 enable_iglx -bool true

2. From the terminal login to Spectre2

$ ssh -X username@spectre2.le.ac.uk

3. From Spectre 2 login to the cluster

$ ssh -X username @143.210.183.163

4. You are now in your home directory. Create a new directory for your next project and go into this directory

$ mkdir project1

$ cd project1

5. Create a Micrographs directory and go into this directory

$ mkdir Micrographs

$ cd Micrographs

6. Create symbolic links to your data:

Option1: On the fly processing:

$ screen
$ autolink /net/krios/202X-xx-xx/epu-session-name/

Ctrl plus A, Ctrl plus D to get you out of screen

Option2: Once data is collected:

$ ln -s /net/krios/202X-xx-xx/epu-session-name/Images-Disk1/*/*/*fractions.tiff .

7. Back to project directory

$ cd ..

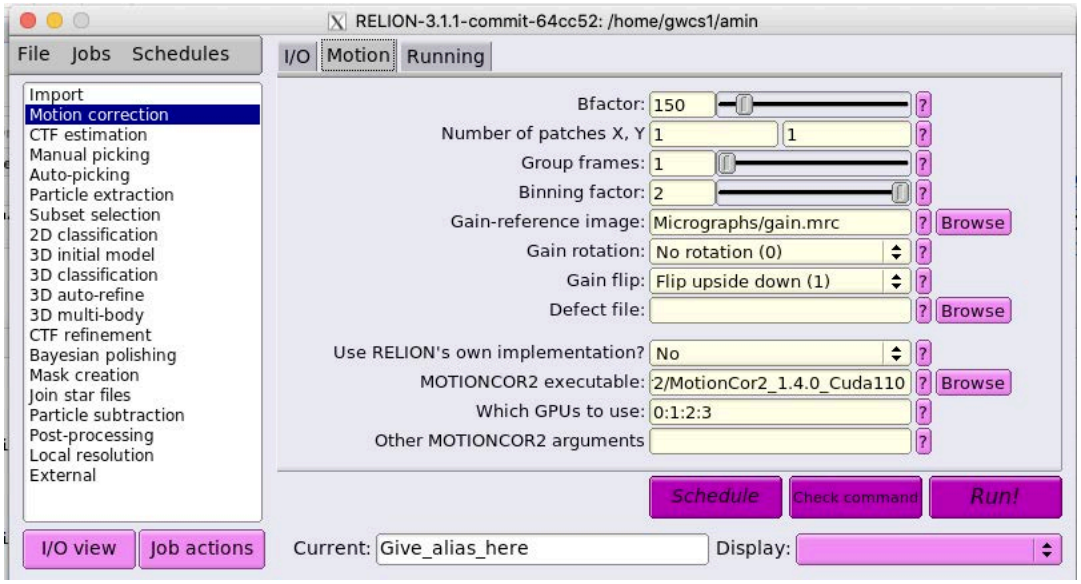
8. Start Relion

$ relion3.1-beta &

Copy the K3 gain reference to your DoseFractions folder

Up to EPU 2.10 the gain reference files generated by EPU are still not usable. We have to copy the K3 gain generated by GMS (Digital Micrograph) in .dm4 format and convert this to .mrc format.

- Use the VNC window to go to the K3 PC and open Windows Explorer. Navigate to C:\Program Data\Gatan\Reference Images\
- Copy the gain file ending in x1.m1.dm4 to X:\Dosefractions\YourEPUsessionName\ (the file will automatically get moved to the data collection folder on the cluster under /net/krios/202x-xx-xx/YourEPUsessionName/)
- Copy this file to your Relion project directory by typing this command from your project directory: cp /net/krios/202x-xx-xx/YourEPUsessionName/ K3-18370212\ Gain\ Ref.\ x1.m1.KV[300].dm4 . (remember the dot at the end)
- Convert to mrc format by typing: dm2mrc K3-18370212\ Gain\ Ref.\ x1.m1.kv[300].dm4 gain.mrc
- This will give you an mrc gain image in Super-resolution size. Use as is for Super-resolution Bin 1 data.
- For Counting or Super-resolution Bin 2 data you have to bin the gain by 2. Type: newstack -in gain.mrc -ou gainbin2.mrc -bi 2
- For Relion there is also an orientation difference between the gain and the recorded images. When using Motioncorr flip the gain file upside down. Remember to also bin Super-resolution Bin1 data by 2 to come back to the physical pixel size. For CryoSparc no orientation flip or rotation is required.


RELION-3.1.1-commit-64cc52: /home/gwcs1/amin
File Jobs Schedules
Import
Motion correction
CTF estimation
Manual picking
Auto-picking
Particle extraction
Subset selection
2D classification
3D initial model
3D classification
3D auto-refine
3D multi-body
CTF refinement
Bayesian polishing
Mask creation
Join star files
Particle subtraction
Post-processing
Local resolution
External
I/O view
Job actions
I/O
Motion
Running
Bfactor: 150
Number of patches X, Y: 1 1
Group frames: 1
Binning factor: 2
Gain-reference image: Micrographs/gain.mrc
Browse
Gain rotation: No rotation (0)
Gain flip: Flip upside down (1)
Defect file:
Browse
Use RELION's own implementation? No
MOTIONCOR2 executable: 2/MotionCor2_1.4.0_Cuda110
Browse
Which GPUs to use: 0:1:2:3
Other MOTIONCOR2 arguments
Schedule
Check command
Run!
Current: Give_alias_here
Display:

Appendix

Table 1: Calibrated Physical Pixel sizes on the K3. (Calibrated using oriented gold @2.35Å and Au/Pd @2.32Å). 105K confirmed using map vs model FSC of ApoF at 1.68Å resolution.
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K3 Mag	Pixel (Å)
64K	1.338
81K	1.066
105K	0.835
130K	0.656
165K	0.514

Note: If you collect Super-resolution Bin1 data then enter half of this value during Import and bin by 2 during Motion correction. For counting and SRbin2 use the values as is with no binning during motion correction.

Table 2: Data collection parameters and approximate throughput (Using R1.2/1.3).

Mag	**Beam** (um)	**Spot**	**C2**	**Exp/ Hole**	**Speed**(#/hr) Counting/ SRbin2	**Speed**(nm^2/hr) Counting/ SRbin2	**Dose Rate** e/pix/s	**Exp. Time** (sec)	**# frames**	**Total Dose** e/Å^2
81K	1.8	5	50	1	180/?	$5.0x10^9$ /?	16.5	4	43	43
105K	0.75	5	50	3	330/530	$5.5x10^9$ /$9.1x10^9$	18	2	50	50
130K	0.57	5	50	5	370/750	$3.9x10^9$/$7.9x10^9$	18	1.25	50	50

Notes:

1. Even though the dose rate on the specimen differs at 81K vs 105/130K the final dose on the detector is not significantly different and is mainly dependent on ice thickness. Therefore and the gain is still acquired at 15 e-/pix/sec.
2. On R2/2 one can accommodate ~5-6 exposures per hole at 105K (0.75um beam). The speed is ~380 images/hour in counting mode.
3. On R0.6/1.0 one can accommodate 2 exposures per hole at 105K (0.75um beam). The speed is ~340 images/hour in counting and ~560 images/hour in SRbin2.