20241209（ver. 1.4）

Okayama University

Cryo-Electron Microscope Manual

Titan Krios G4 and Falcon 4i

This manual is based on the KEK Cryo-Electron Microscope Initial Training Text for External Users (ver.3)
https://www2.kek.jp/imss/sbrc/230203_KEKTitan_TrainingText_v3.pdf
and has been created by extracting only the sections related to daily adjustments and routine data measurement, adding screen captures, and making some slight changes to the content.

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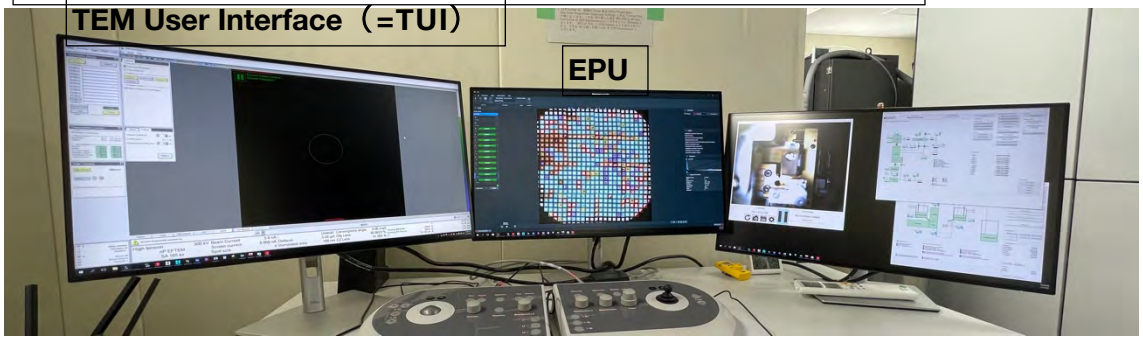
I. From sample exchange to Atlas acquisition

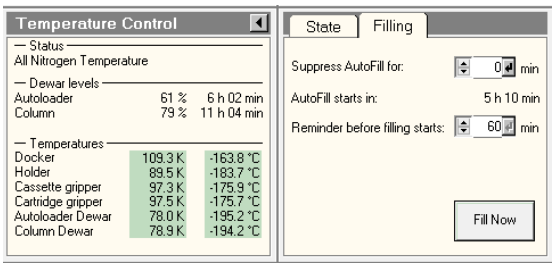
*TEM User Interface is version 3.16.1, EPU is version 3.4.0.5704REL



1. Inventory

Use TEM User Interface (=TUI)

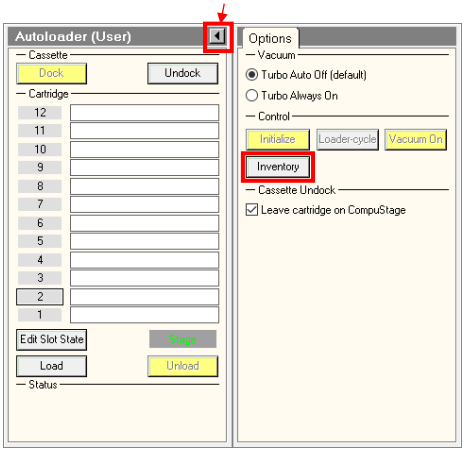
・ TUI > Autoloader tab > Temperature Control



☐ When you're done with the NanoCab Dock (don't forget to take out the NanoCab!) Wait until the temperature display is all green, and then wait until everything drops below -160°C (a few minutes)

If the Option window is not open, click here.

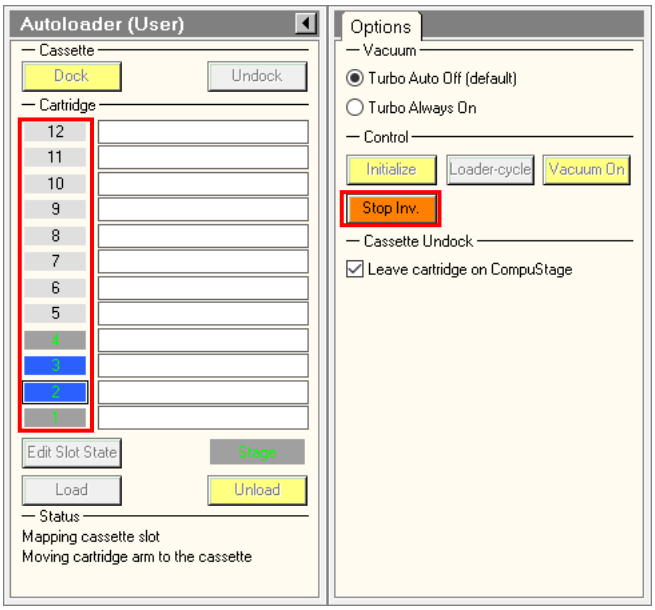
・ TUI > Autoloader tab > Autoloader > Option



☐ Press the Inventory button.

(After the Dock, it takes a while to become active)

□ If all the grids you put in are recognized correctly, OK



Dark gray: Judged to be missing a grid
Blue: Grid is determined to be present
Light gray: Recognition work has not yet been done

(If it is not recognized correctly, Inventory again, and
if there is still a discrepancy, take out the Cassette once and check it.)
Is the grid falling?
If you're still wrong, contact FEI.)

□ When the number of grids you put in +2 is checked, press the Stop Inventory button to stop.

□ The name of the grid is entered after the Inventory is finished (or Unload if there are any grids left in the stage)

* It's not good to have a name in an empty slot

* This name will not be reflected in the folder name of the result. Leave a note.

2. Approximate alignment

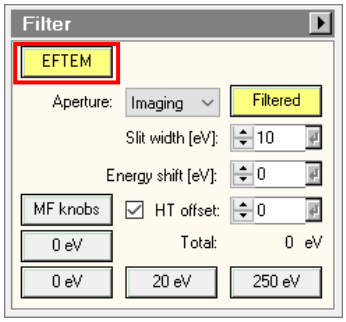
☐ EPU Reboot

(Press the × in the upper right corner to drop it.) Wait about 30 seconds. Launched from the pin icon)

☐ View the TUI > EFTEM tab > Filter

☐ Verify that the EFTEM button is yellow.

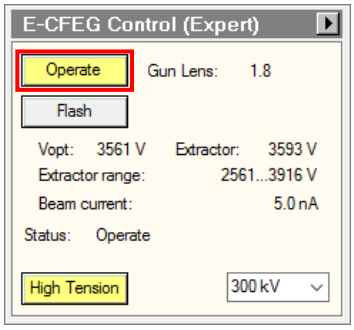
(If it is not yellow, press the EFTEM button to turn it yellow.)



☐ TUI > Setup > E-CFEG

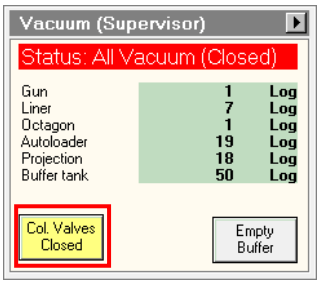
☐ Verify that the Operate button is yellow.

(If it is not yellow, press the Operate button to turn yellow.)

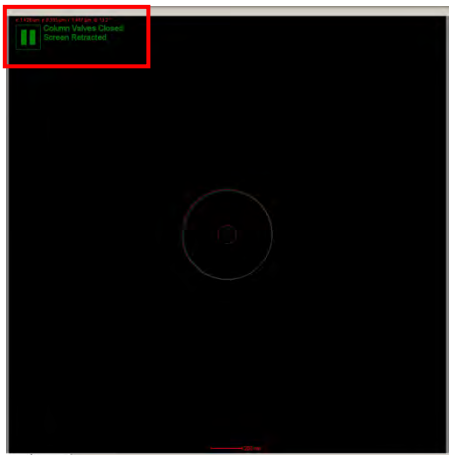


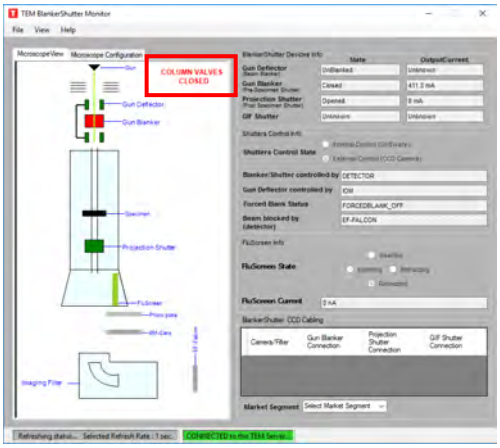
□ TUI > Setup tab > Vacuum

□ Press the Col. Valves Closed button to open the Column valve.



The open/closed status of the column valve can be checked on the screen of the TUI or with the "TEM BlankerShutter Monitor" on the right monitor

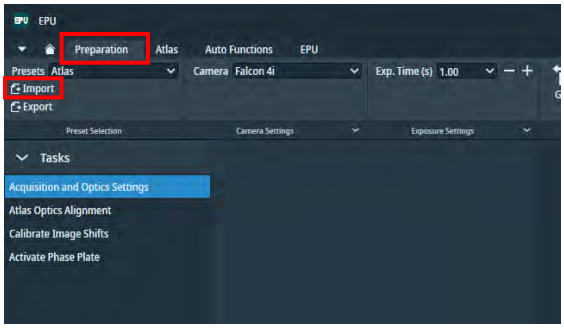


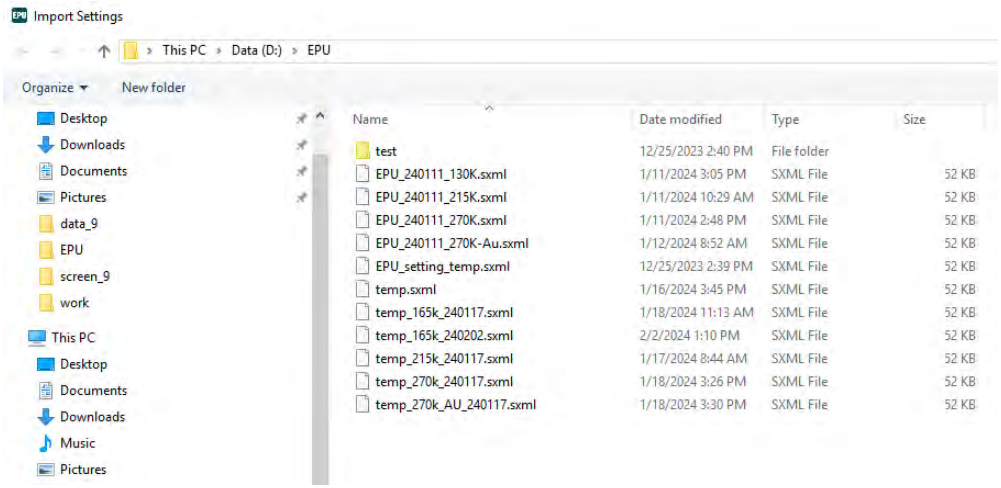


□ EPU > Preparation tab , press Import and open the following .sxml file

D:/EPU/temp_xxxk_YYMMDD.sxml

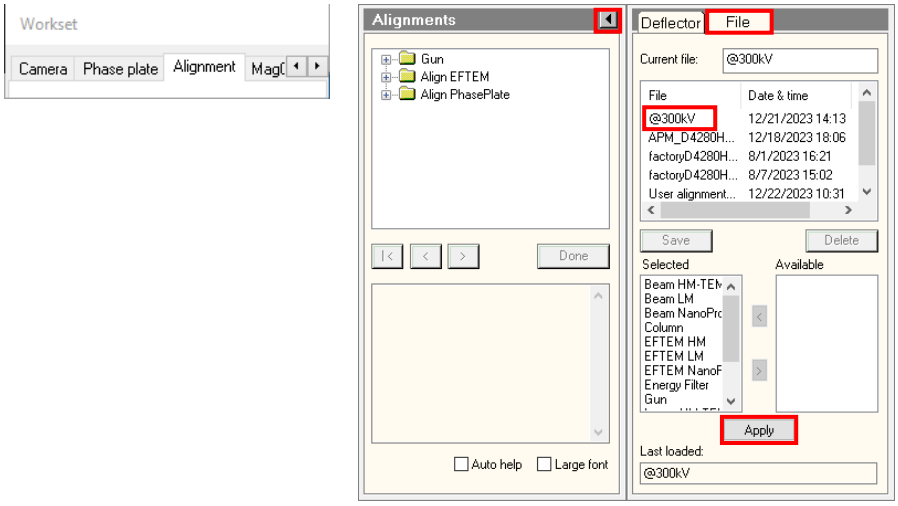
(xxx = magnification. Select the latest one of the date with the magnification you want to use)





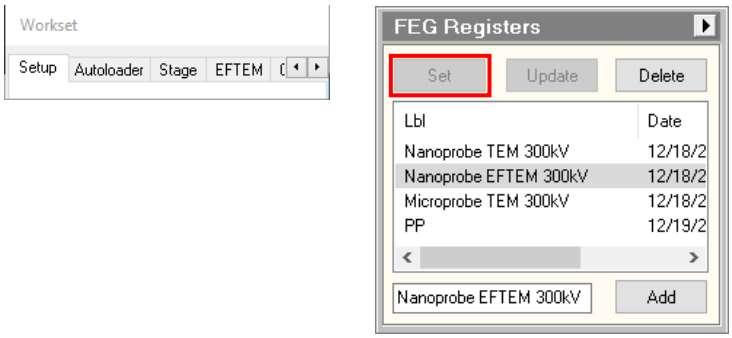
□ TUI > Alignment tab > Alignments > Option > File tab Select 300kV (latest created on 11/27/2024) and select

everything in Available and click < button to move to Selected, then press Apply



□ TUI > Setup tab > Select Nanoprobe EFTEM 300kV (Latest updated on 11/27/2024) from FEG registers and Set

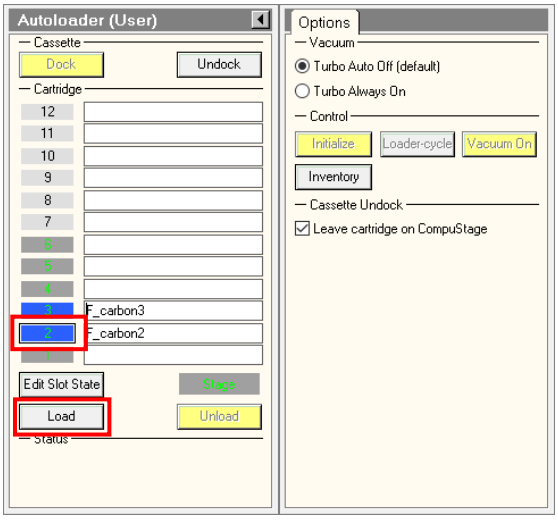
(It is also effective when the beam is lost.) However, once you have done this, you will have to start the adjustment from scratch.)



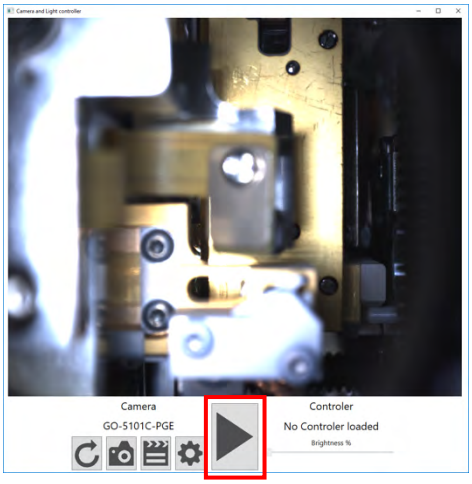
3. Grid load and preview, Atlas retrieval

- TUI > Autoloader tab > Autoloader

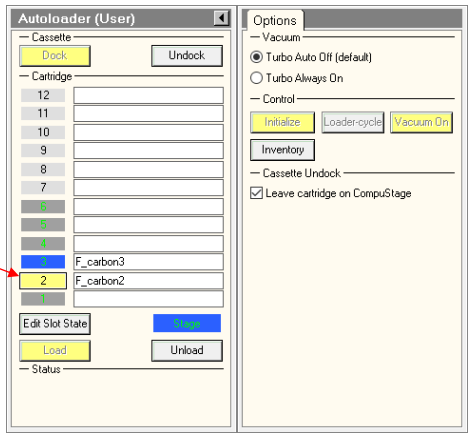
  □ Click on the number in the grid you want to see (highlighted with a black line) Press the Load button.



If you press the play button of "Camera and Light controller" on the right monitor, you can see the live view of Autoloader.
This screen automatically stops after 15 minutes. If you want to watch it again, press the play button.



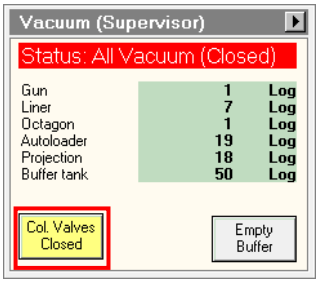
Correctly loaded grids turn yellow



<This page can be omitted entirely>

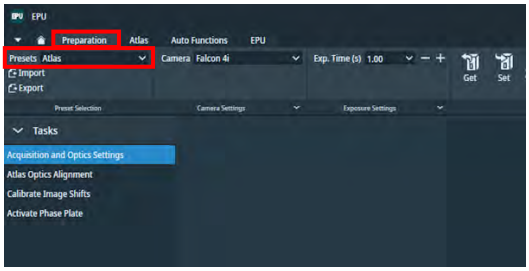
- TUI > Setup tab > Vacuum

  □ Press the Col. Valves Closed button to open the Column valve.



- EPU > Preparation tab

  □ Select Atlas from the Presets pull-down menu in the upper left corner.

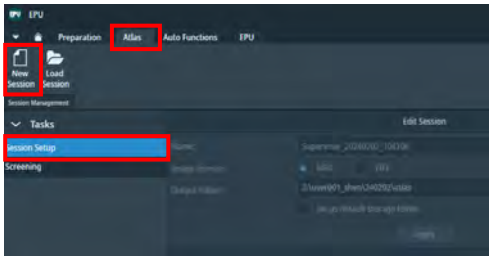


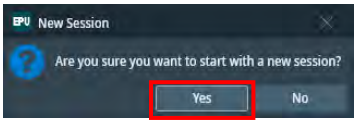
  □ In the EPU > Preparation tab, press Preview on the upper right edge to take a picture.

  * If it is completely dark at this stage, check again that the column valve is open and that the E-CFEG is set to Operate.
  *If the ice is too thick, it will be almost pitch black, but you should be able to see the grid square faintly.

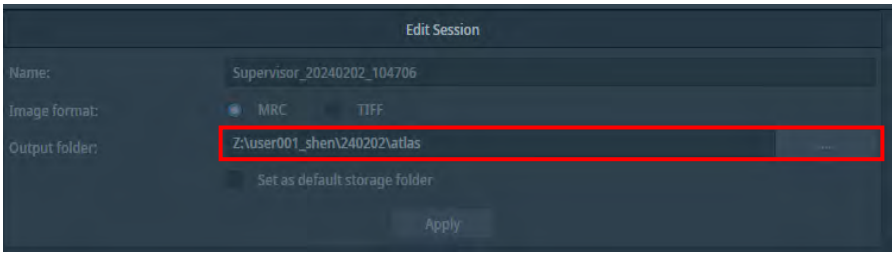
- EPU > Atlas tab

  □ Session Setup > New Session > Yes

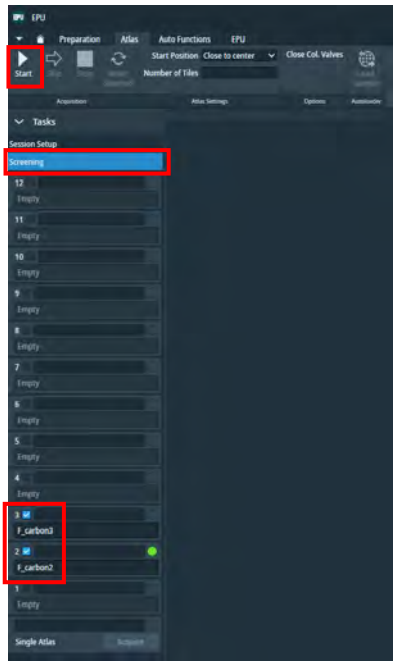




  □ Specify the Output folder (save destination: Z:/userxxx/yymmdd/atlas/) and Apply

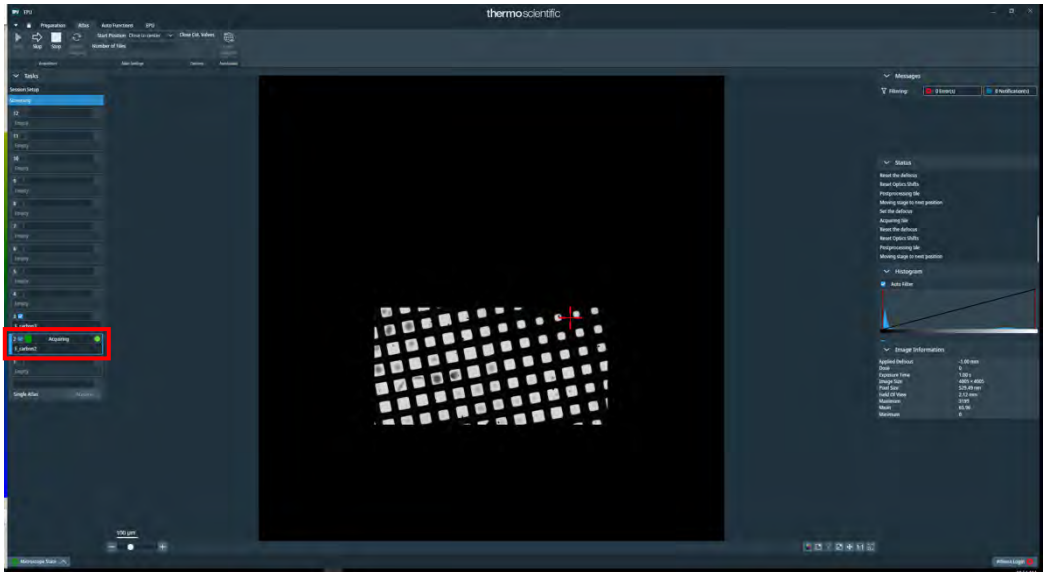


  □ Press Screening, check the number of Grid (green light) you want to shoot, and press the Start button to start shooting as Atlas



□ Click Acquiring to view the image.

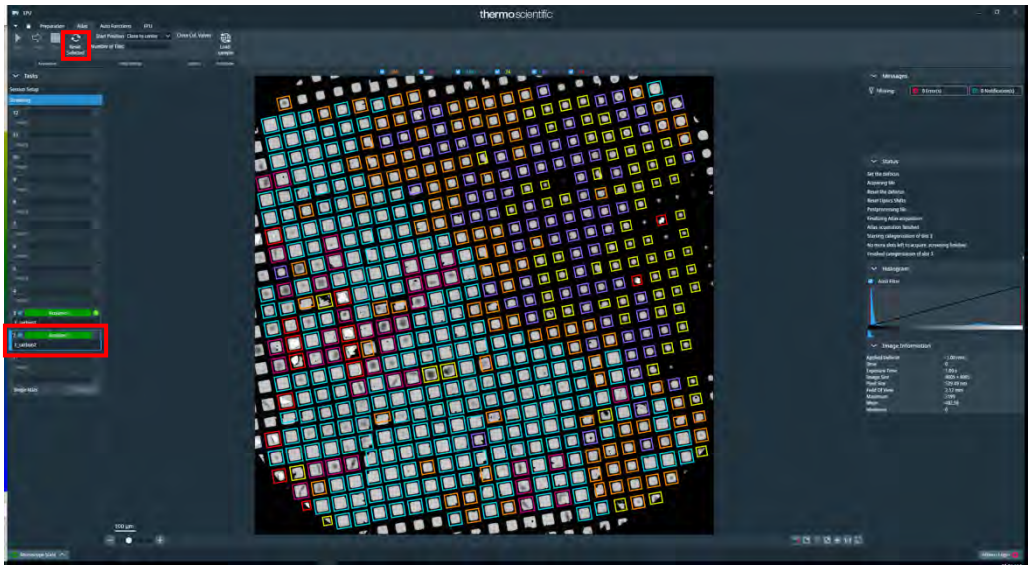
(* Wait a while to shoot the whole picture with 4 x 4.) Including the time it takes to replace the grid, it takes less than 10 minutes per sheet? From 2024/11/29, the recommended magnification for Atlas is 155x)



* If, for some reason, you want to retake Atlas for a specific grid

Select the grid in the Atlas tab and press the Reset Selected button in the upper left corner.

The data will disappear, so check that grid and Start



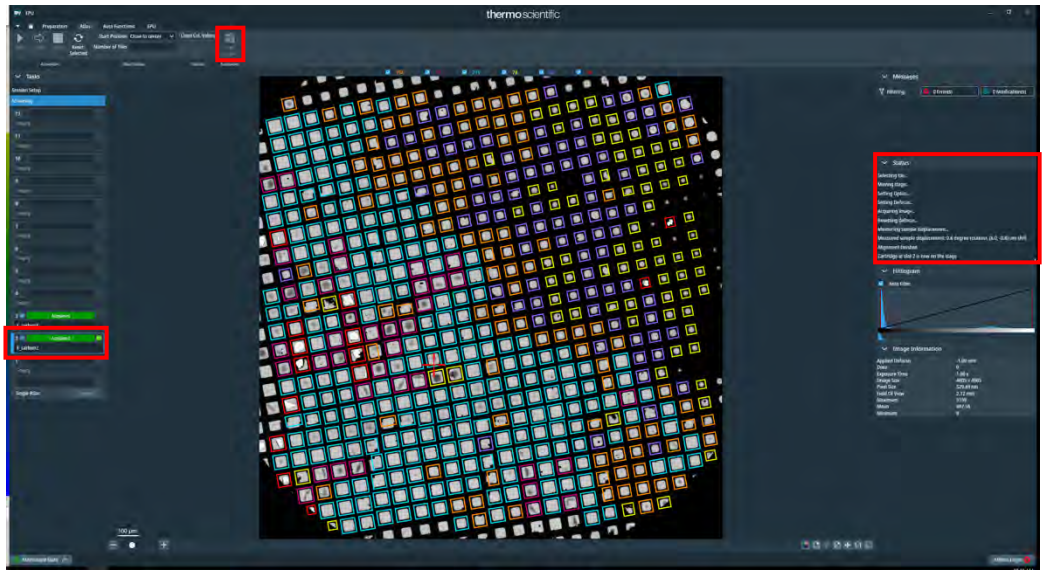
II. Beam adjustment (daily)

(Even when using UltrAufoil**, 6.** can only be done with a grid of carbon film)

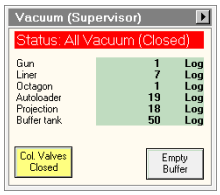
1. Move to the broken square in the grid

☐ EPU > Select the desired grid on the Atlas tab and press Load Sample.

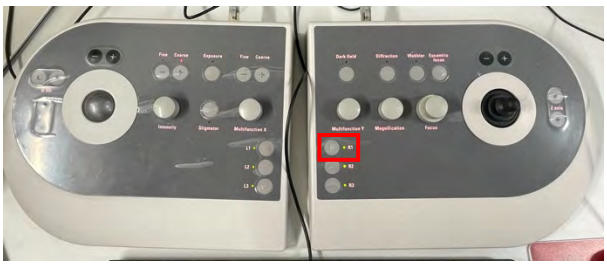
Verify that the EPU > Status displays "Cartridge at slot x is now on the stage"



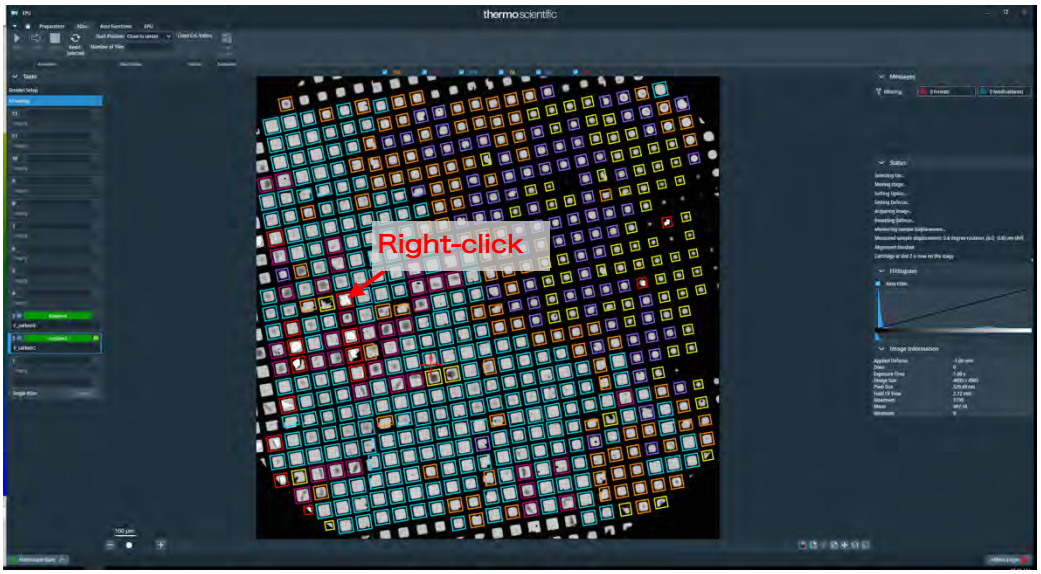
☐ If the Column valve is closed, Press the Col. Valves Closed button to open the Column valve.



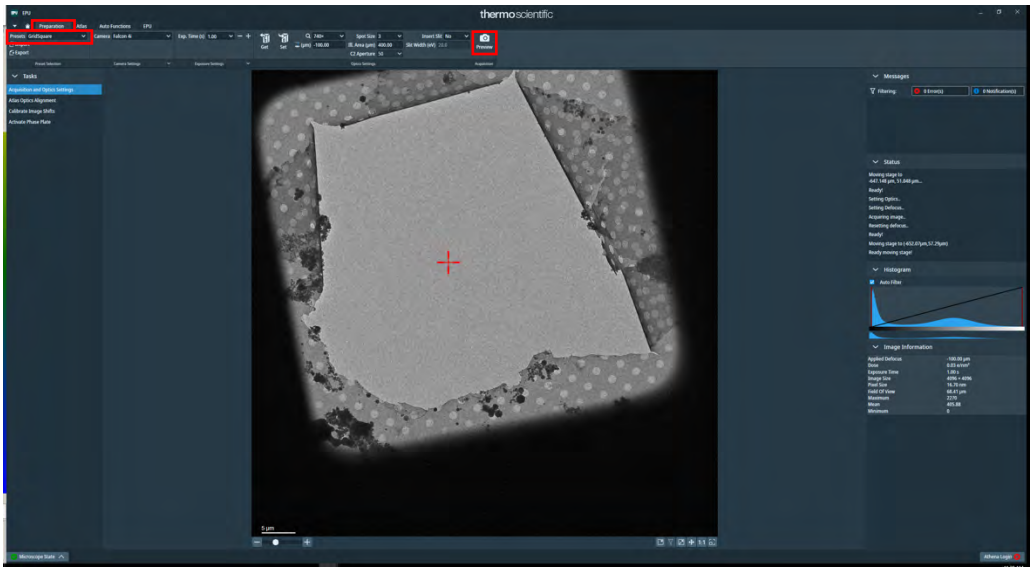
☐ If the fluorescent plate is not raised, press R1 on the operation plate to raise the plate (= Screen Retracted).



☐ EPU > Right-click on a broken square in the Grid in the Atlas tab > Move stage here



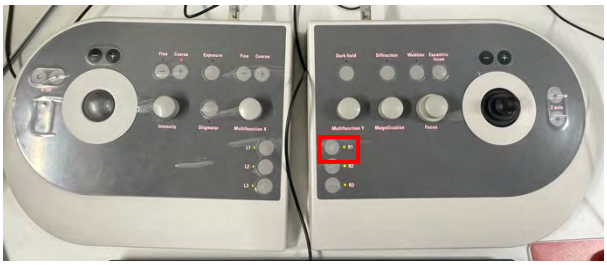
☐ From EPU > Preparation tab > Presets, select Grid Square and Preview



☐ Make sure you're in a broken square

* If the position is off, right-click again on the square image > Move stage here

□ Press R1 to lower the fluorescent plate.



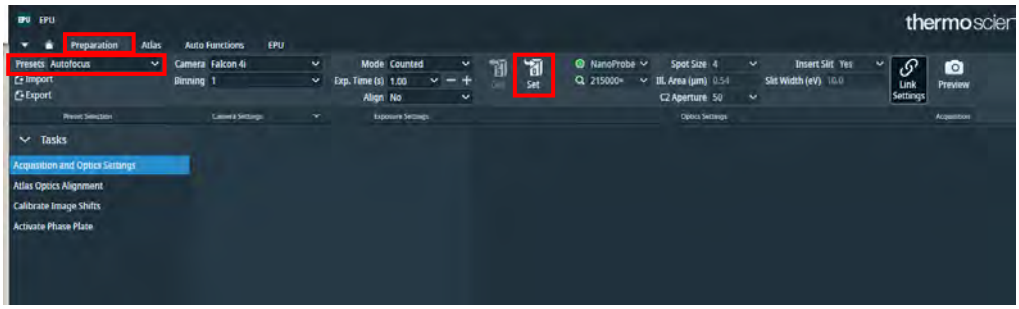
□ Flucam Viewer is now in EF (=EF mode is displayed as a green circle),

Confirmed that it is Natural, High Resolution

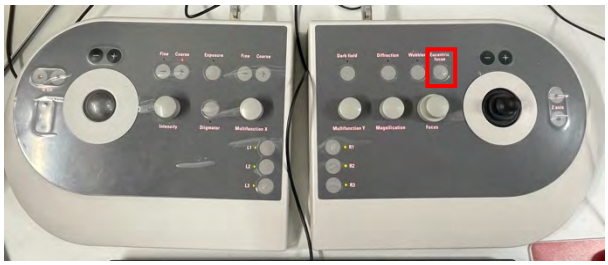
2. **Adjusting the Pivot Point** (KEK Manual 3.)

☐ Be in the broken square in the grid and make sure the fluorescent plate is down

☐ Select Autofocus (Data Acquisition) from EPU > Preparation tab > Presets and set

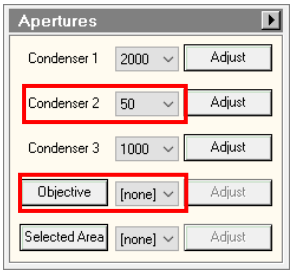


☐ Press the Eucentric Focus on the control board.

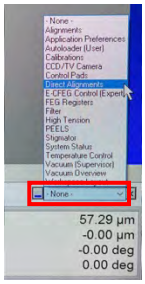


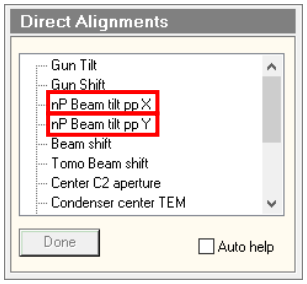
☐ TUI > Stage tab > Apertures and see that C2=50, Obj=none

(*C1=2000, C3=1000 do not need to be touched at all in the future)

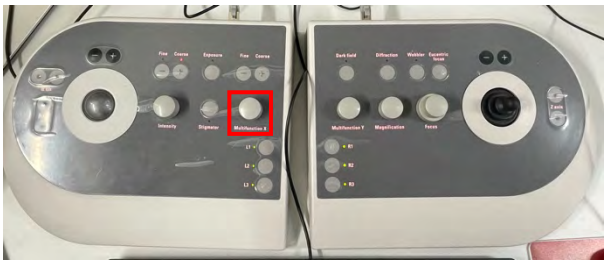


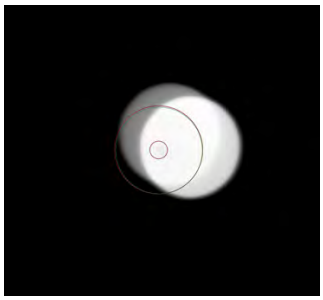
☐ The lower right of TUI > Direct Alignment > Select nP Beamtilt pp X.





☐ Use only Multifunction-X on the control panel to adjust the two flashing lights so that they overlap.

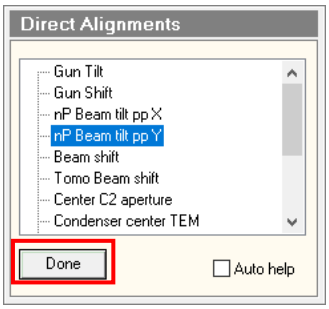




☐ The lower right of TUI > Direct Alignment > Select nP Beamtilt pp Y.

☐ Use only Multifunction-X on the control panel to adjust the two flashing lights so that they overlap.

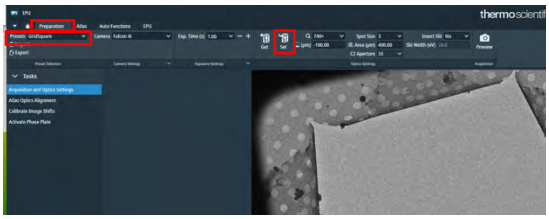
☐ Press Done



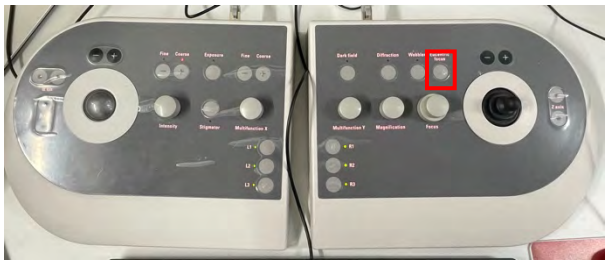
3. C2 Aperture Centering (KEK Manual 2.)

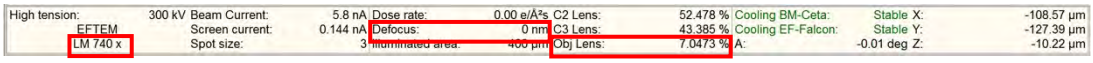
☐ Be in the broken square in the grid and make sure the fluorescent plate is down

☐ From EPU > Preparation tab > Presets, select Grid Square and Set



☐ Press the Eucentric Focus button on the control panel (*Obj lens = around 7% on x740)



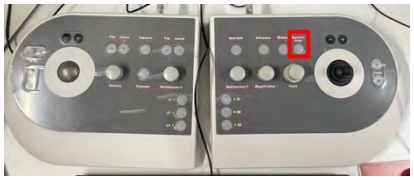


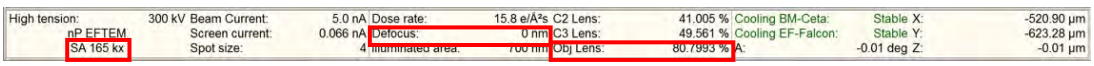
At the bottom of the TUI, x740, Obj lens=7%
If the defocus is not 0, set it to 0 using operation panel R2 (Reset Defocus).

☐ From the EPU > Preparation tab > Presets, select Data Acquisition and Set

☐ Press the Eucentric Focus button on the control panel (*Obj lens = around 80% on x215k)

(*If the defocus is not 0, set it to 0 using operation panel R2 (Reset Defocus).)

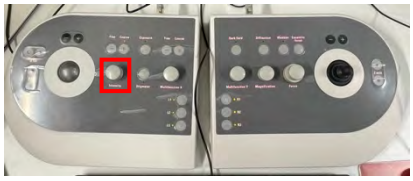




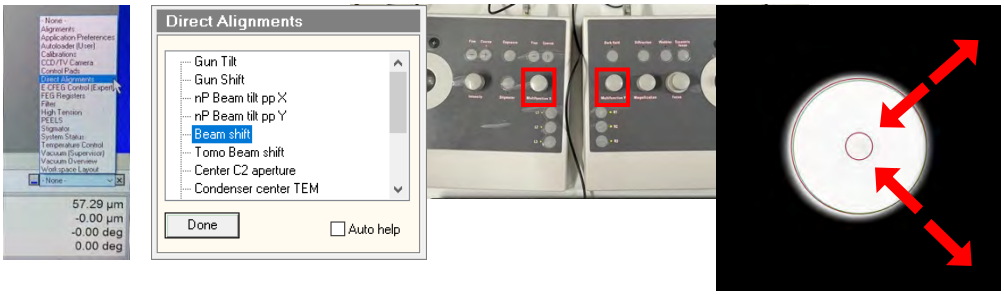
☐ TUI > Stage tab > Apertures and see that C2=50, Obj=none

1st time

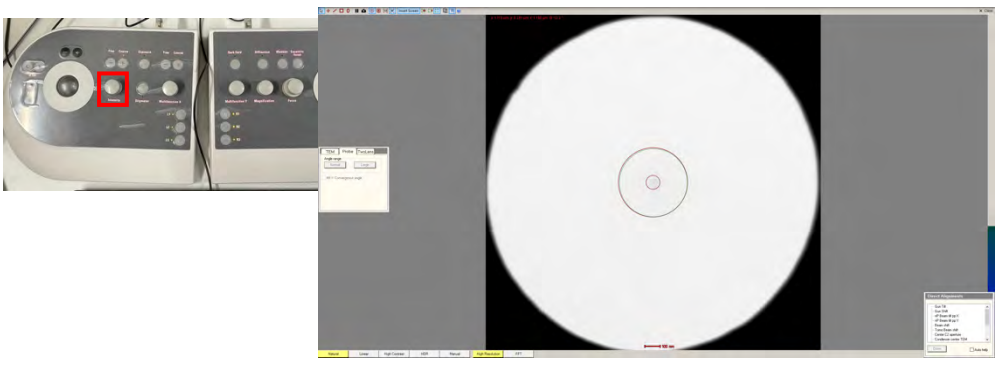
☐ Turn the Intensity dial to the left to squeeze the light so that it is about the size of the larger circle in the center of the Flucam Viewer surface.



☐ TUI Bottom right> Select Direct Alignment > Beam shift and use the Multifunction dial to center the light.

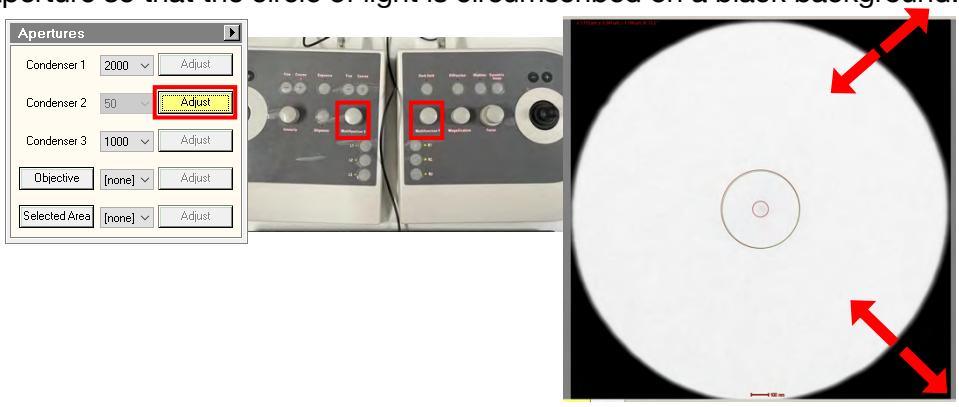


☐ Turn the Intensity dial to the right until the light is the size of the black background



The contrast of the light can be adjusted with the

☐ Press Adjust on the right side of C2 and use the Multifunction dial to move the aperture so that the circle of light is circumscribed on a black background.



☐ Press Adjust again to the right of C2

2nd time

☐ Squeeze the light again to about the larger circle in the center, and move the light to the center with the Multifunction dial.

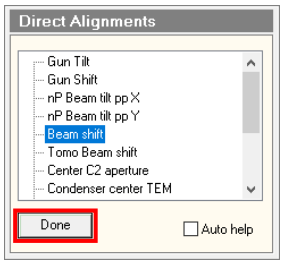
☐ Turn the Intensity dial to the right until the light is the size of the black background

☐ Press Adjust on the right side of C2 and use the Multifunction dial to move the aperture so that the circle of light is circumscribed on a black background.

☐ Press Adjust again to the right of C2

☐ It is OK if the circle of light spreads in almost concentric circles. Repeat the previous operation

☐ If there is no problem, Done the Beam shift of Direct Alignment



* C2 = 150 is used for Atlas, so if you feel a problem with atlas, you may want to make the same adjustment with C2 = 150.

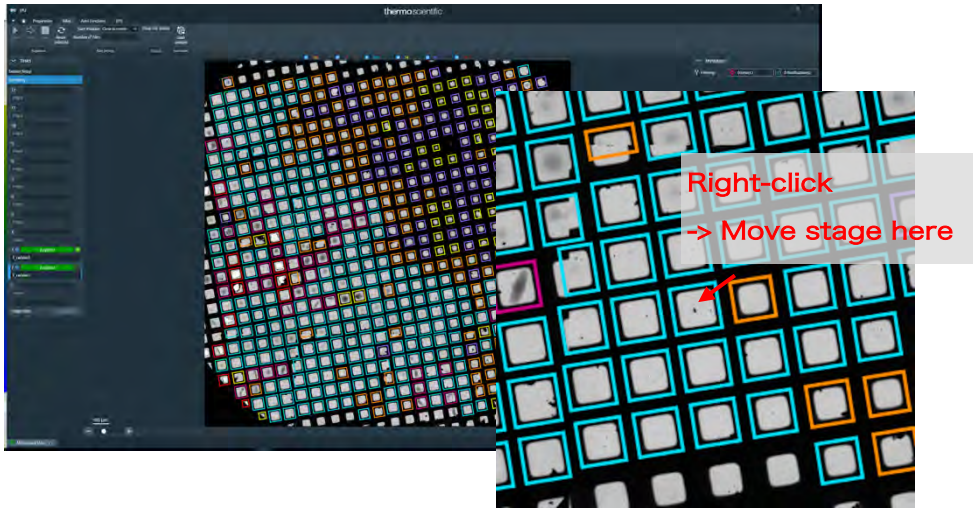
4. **Rotation Center (**KEK Manual 4.)

*This item can be omitted if you will do coma correction later with AutoCTF using Sherpa.

☐ (*Confirm, you are in the broken square, and the fluorescent plate is down.)

☐ Raising the fluorescent plate (control plate R1)

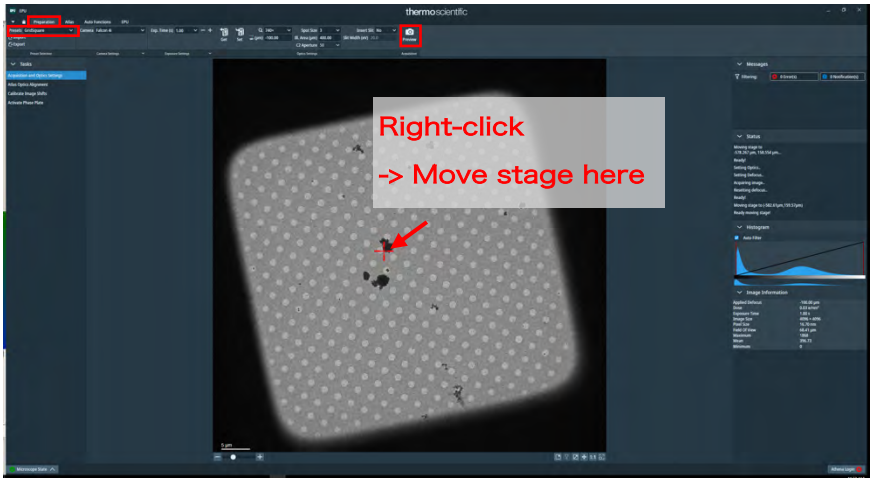
☐ EPU > Atlas tab, where there is conspicuous trash -> Move stage here



* Choose a litter that is not too large. You can control the zoom in/out of the image with the mouse wheel.
A relatively clean square is easier to make the following adjustments.
It is better to bring the center on the boundary between the garbage and the hole

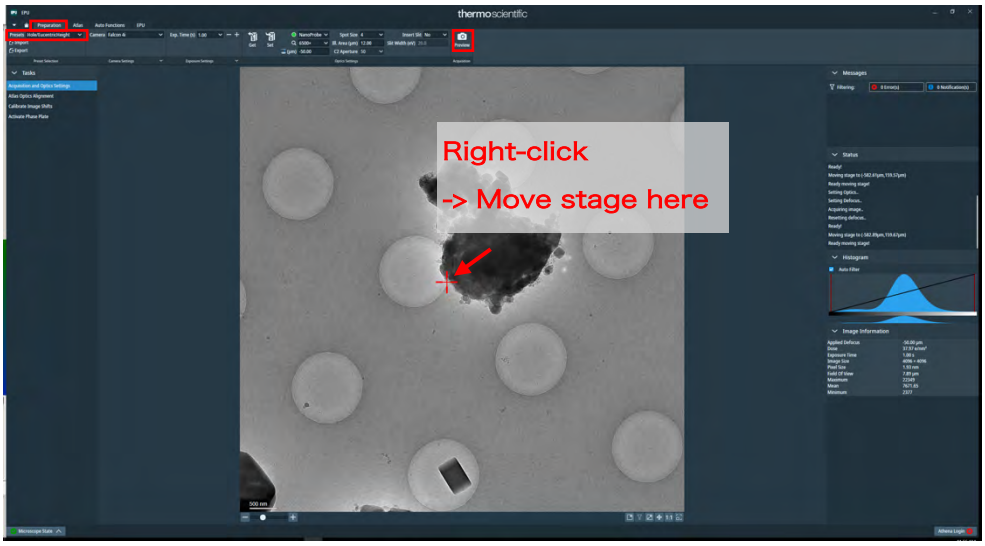
☐ EPU > Preparation tab > Presets > Grid Square > Preview

☐ Right click where there is noticeable garbage - > Move stage here



☐ EPU > Preparation tab > Presets > Hole/Eucentric > Preview

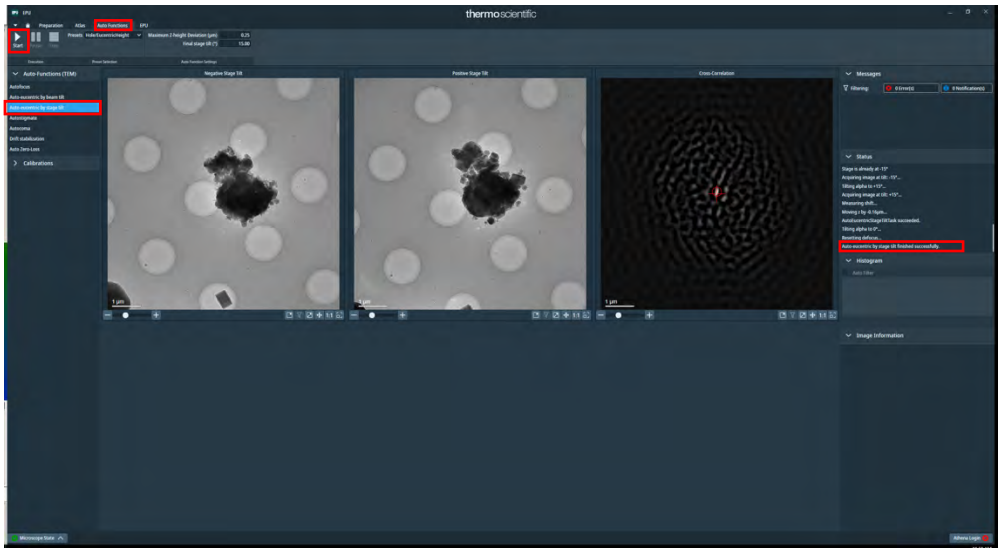
☐ Right click where there is noticeable garbage - > Move stage here



☐ EPU > Auto Functions tab > Auto-eucentric by stage tilt

☐ Set Presets to Hole/Eucentric and Start

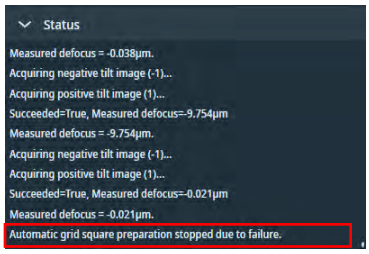
☐ Check the Status to see "Auto-eucentric by stage tilt finished successfully"



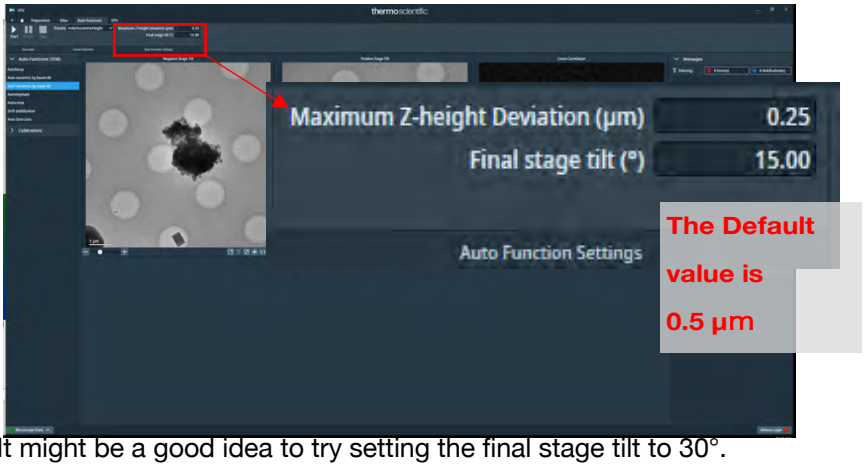
* The tilt axis runs sideways with respect to the screen

Once you have successfully determined the eucentric height, go to p. 23.

*When Auto-eucentric failed



Check the value of the Auto Function Settings (EPU > Auto Functions tab)

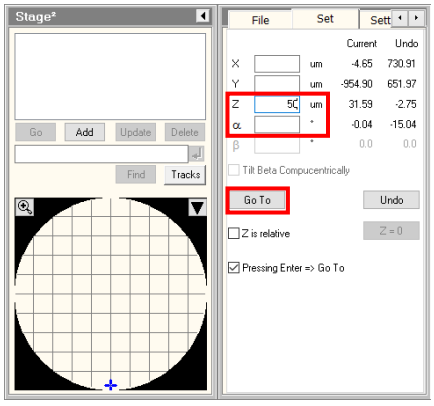


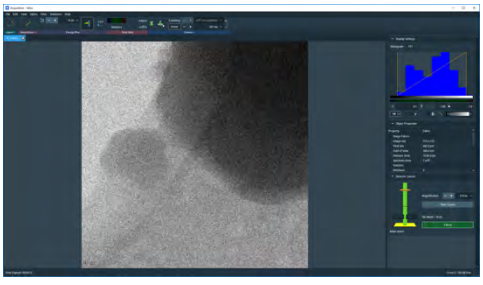
*It might be a good idea to try setting the final stage tilt to 30°.

* If that doesn't work, try using Auto-eucentric by beam tilt
(if you succeed with either one, you're OK).

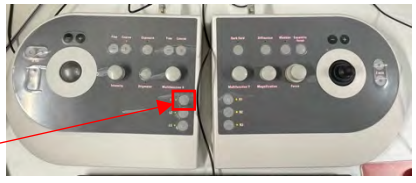
* If that doesn't work, change places.

* If you really want to determine the eucentric height at that location, check it with Velox (p. 35) and manually tilt angle (α) in Stage^2, and move the Z axis to the eucentric hight.

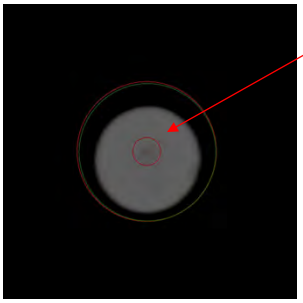




The α wobbler of the L1 control board is also enabled.

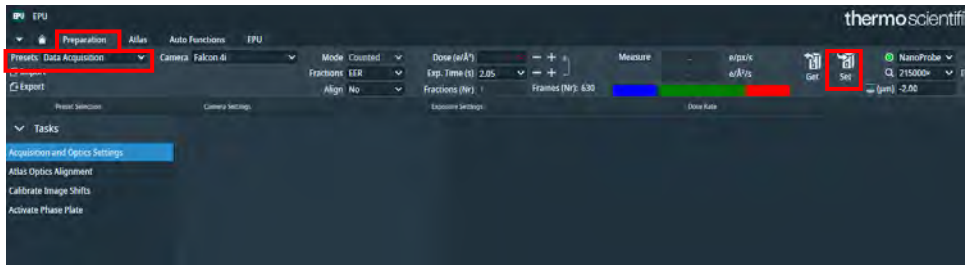


□ Press R1 to lower the fluorescent plate (if the dust is not in the center of the green circle, move it to the center with a joystick)



At this magnification, it is difficult to see with a fluorescent plate, but you can adjust the contrast with the mouse wheel and see a faint black shadow

□ EPU > Preparation tab > Presets > Data Acquisition, and Set



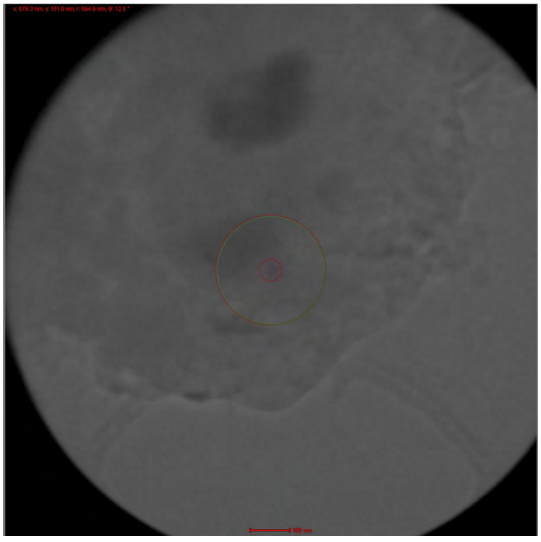
□ TUI > Stage tab > Apertures > select **C2=150 (expand field of view), Obj=none**

*The direction of the image is different between the EPU and the fluorescent plate, so look at it with that intention (is it rotated 90˚?). ）

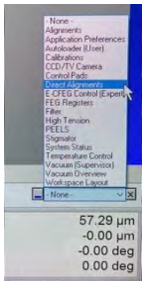
□ **Turn the Magnification dial a few clicks to the right to increase the magnification to x350k**

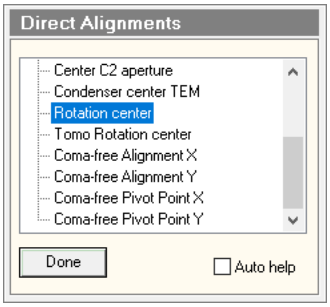
Make sure it's a 350 K-x

* If the garbage is difficult to see, Press "Natural"

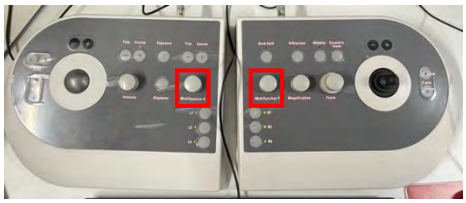


☐ TUI lower right > Direct Alignment > Rotation center



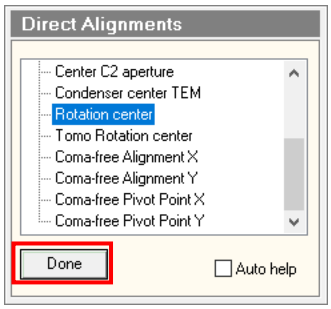


☐ Use the Multifunction dial to fine-tune the debris so that it doesn't move up, down, left, or right



* After this, AutoCTF will remove coma aberration, so it seems that it is good if there is a certain amount

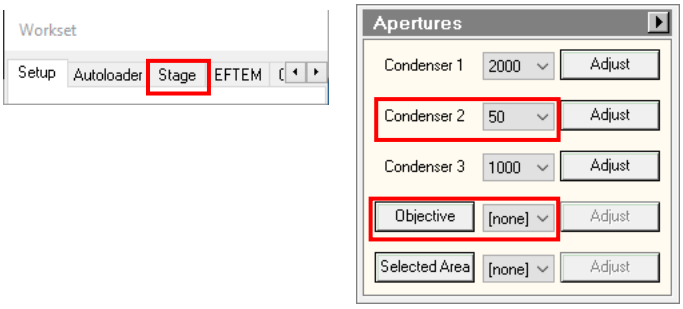
☐ Press Done



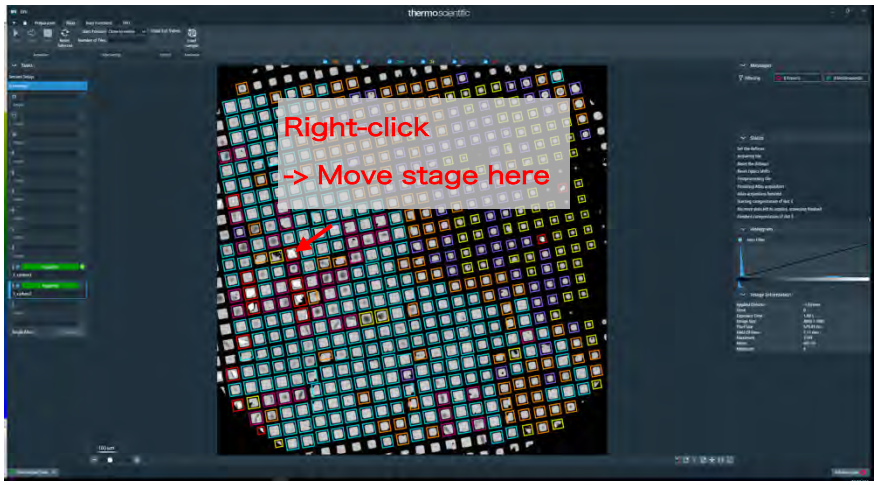
5. **Energy filter related** (KEK Manual 7.)

☐ Press R1 to raise the fluorescent plate.

☐ TUI > Stage tab > Apertures and select, **C2=50, Obj=none**

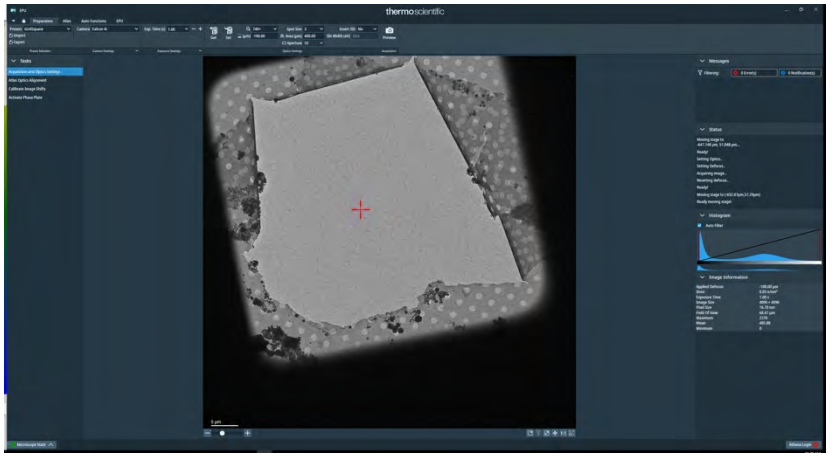


☐ EPU > Right-click on the hole in the Grid in the Atlas tab -> Move stage here

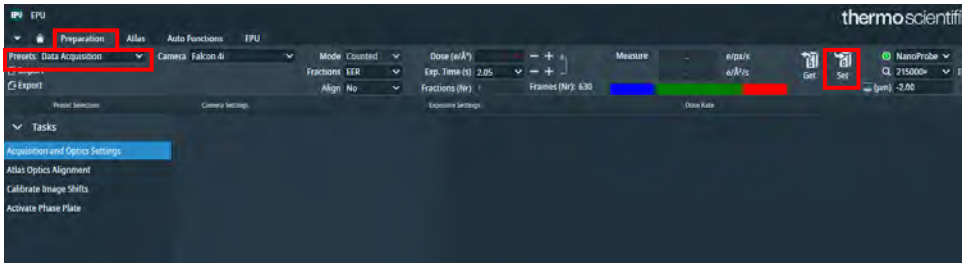


☐ EPU > Preparation tab > Presets > Grid Square, and click Preview

(Confirm that you are in the hole)



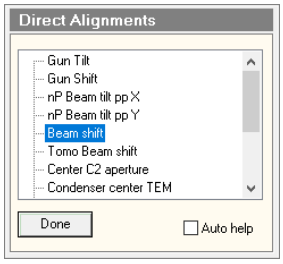
☐ EPU > Preparation tab > Presets > Data Acquisition, and click Set

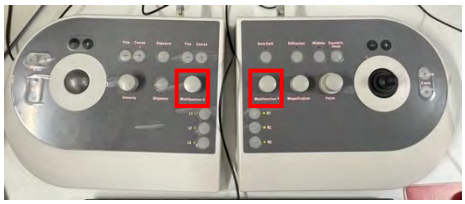


☐ Press R1 to lower the fluorescent plate

☐ If the beam is not centered, center it with Direct Alignment > Beam Shift

☐ Then, Done

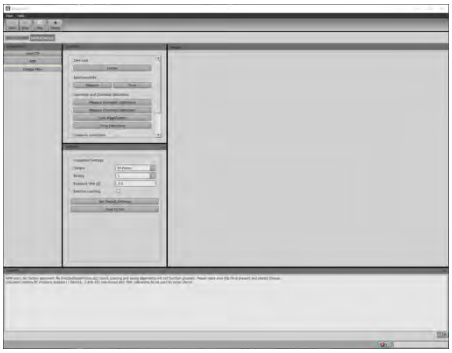




☐ Make sure the beam is hitting the entire green circle.

☐ Press R1 to raise the fluorescent plate.

☐ Normally, Sherpa should already be launched, so put the window on the surface



(If it is not launched, start Sherpa from Tools of the Microscope Software Launcher.)

☐ Press the Energy Filter button on the left side of the Sherpa Window.

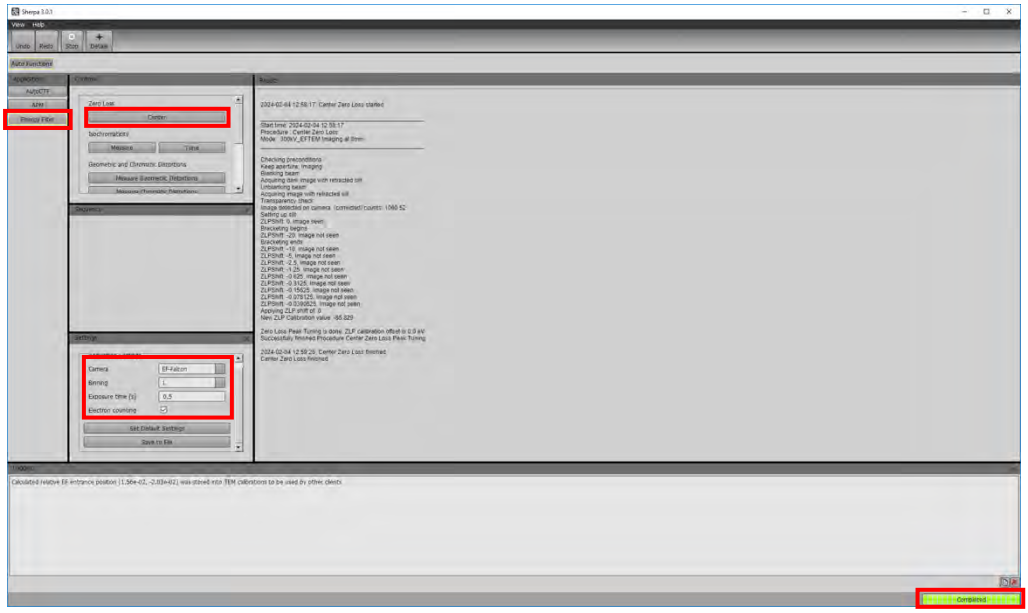
☐ Look at the Settings square at the bottom left of the Sherpa Window

☐ Check EF-Falcon, Bin=1, Exp time=0.5sec, Electron counting

☐ Look at the Controls square in the upper left corner of the Sherpa Window

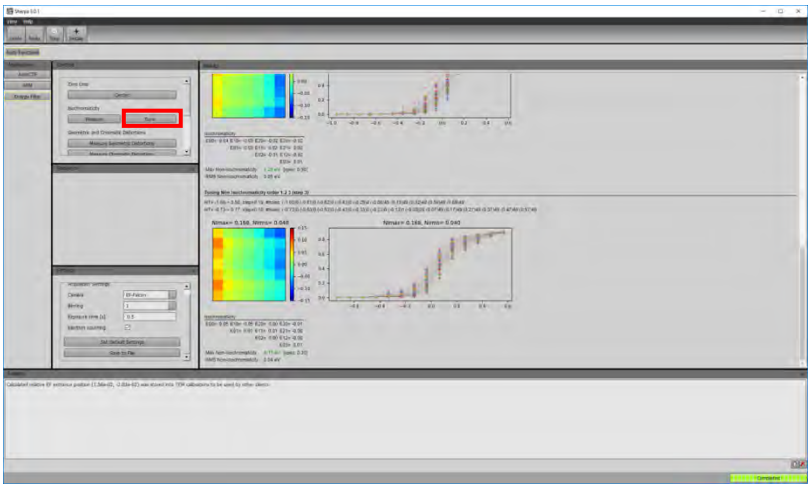
☐ Zero loss: Press the Center button to pass the beam through the center of slit.

☐ Confirm that completed is displayed at the bottom right of the screen (the same applies below).

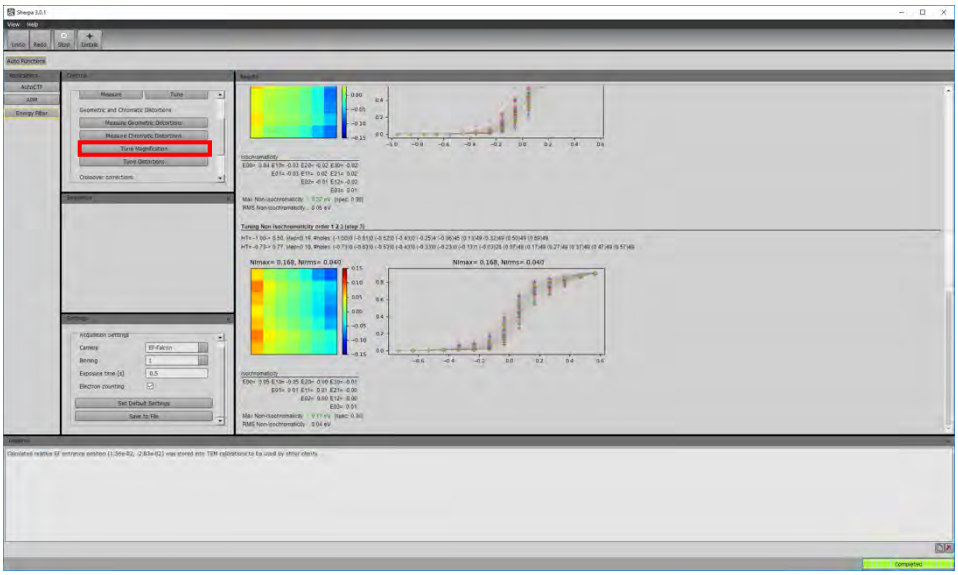


☐ Isochromaticity: Press Tune to equalize the energy hitting the camera.

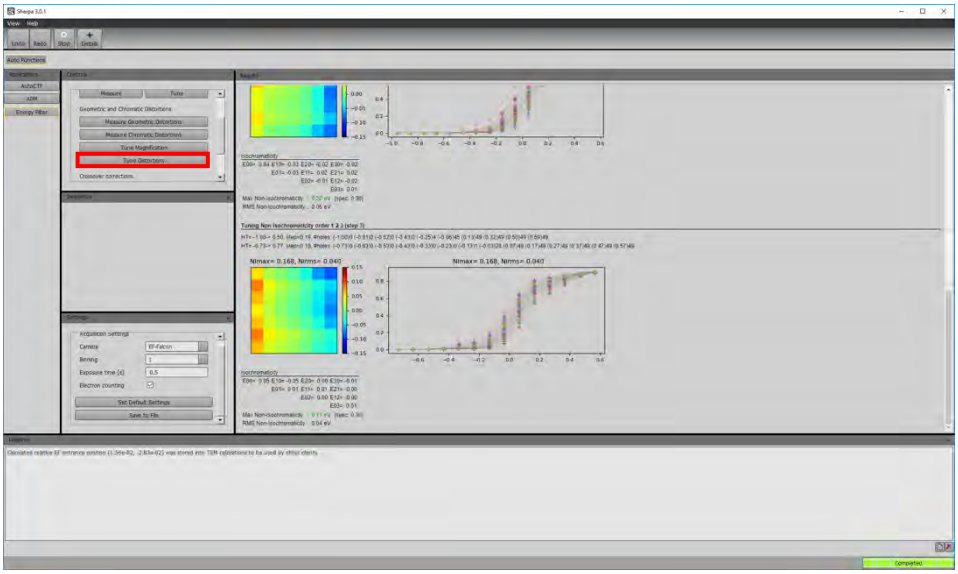
After a while, a red-green image appears. Wait a few minutes for the color of this screen to be uniformly green



☐ Geometric and Chromatic Distortions: Press the Tune Magnification button



☐ Geometric and Chromatic Distortions: Press the Tune Distortion button.

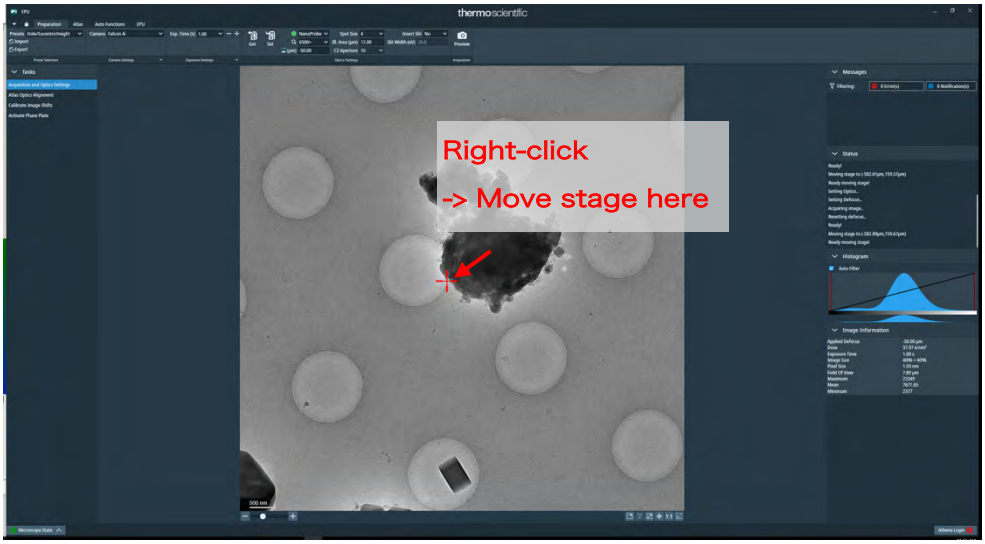


☐ Zero loss: Press the Center button again to adjust the turn.

6. **AutoCTF (Correction of astigmatism and comaration)** (KEK Manual 8.)

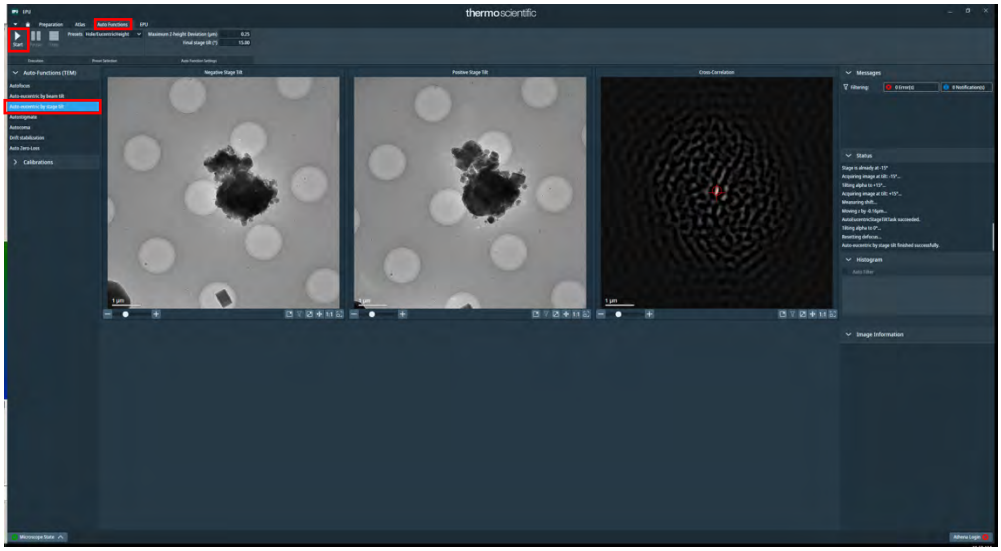
* This correction can only be done with a carbon film grid.

☐ EPU > Preparation tab > Presets > Hole/Eucentric, right-click on the garbage image -> Move stage here to move to the vicinity of the debris.



☐ EPU > Auto Functions tab > Auto-eucentric by stage tilt

☐ Presets > Hole/Eucentric, and click Start



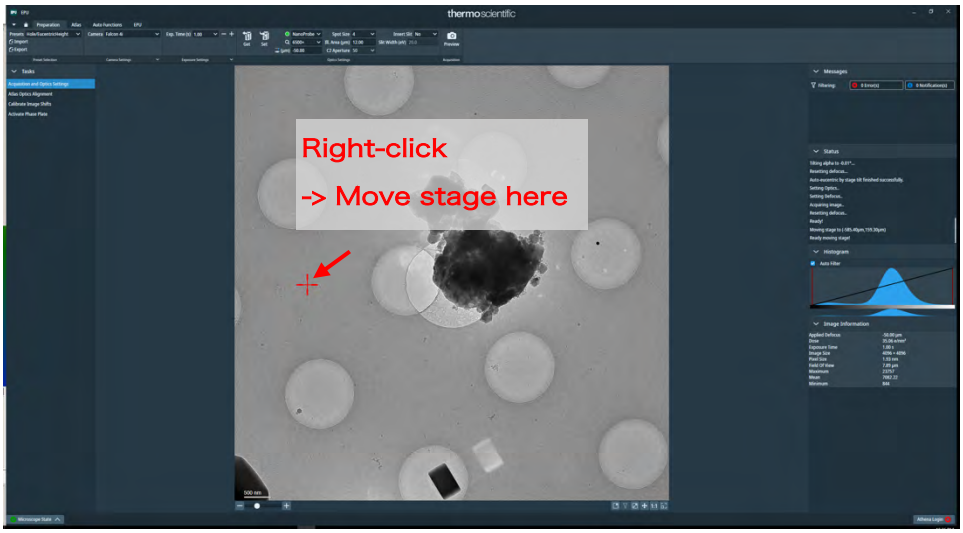
* If it fails, try again with Auto-eucentirc by beam tilt (see also p. 22).

☐ Verify Defocus is 0 um

*It doesn't have to be 0

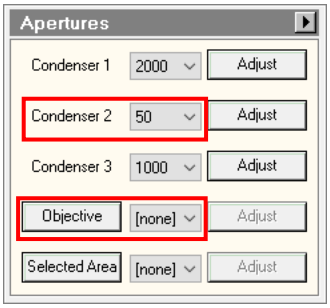
☐ EPU > Preparation tab > Presets > Hole/Eucentric, click Preview

☐ Right-click on the carbon surface **without garbage** and move stage here



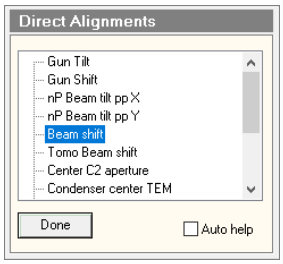
☐ EPU > Preparation tab > Presets > Data Acquisition, and click Set

☐ TUI > Autoloader tab > Apertures and confirm that C2=50, Obj=none

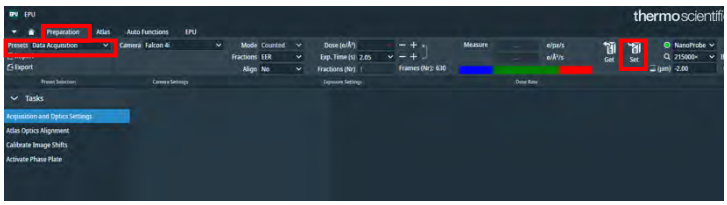


☐ Press R1 to lower the fluorescent plate

If the beam is not centered, center it with Direct Alignment > Beam Shift, and then, click Done



☐ Again, Data Acquisition > Set. Press R1 to raise the fluorescent plate.



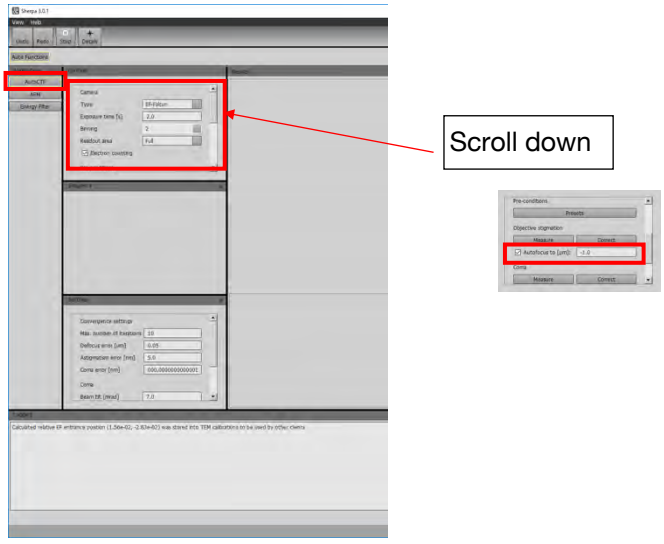
☐ Sherpa should be standing up, so look at the Sherpa screen

☐ Press the Auto CTF button on the left side of the Sherpa Window.

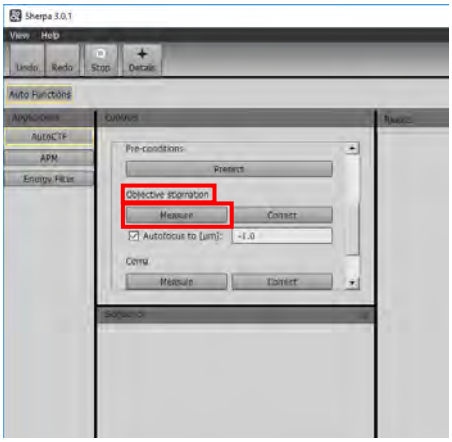
Camera > Type> EF-Falcon

☐ Since it is EC mode, check the box for Electron counting.

☐ Exp. Time=2, Binning=2, Readout=Full, Auto-focus-to=Check-1.0



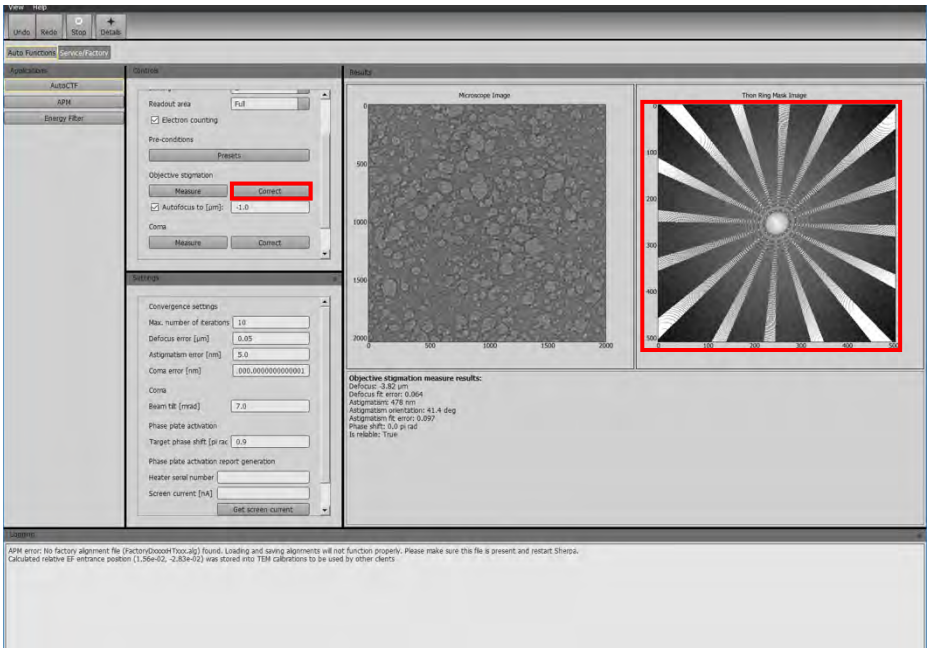
□ Press Mesure for Objective Stigmation



* If there is a mark on the grid, move it a little.

* If you can't pick up the tone ring, turn the Focus dial and set Defocus to –800 nm.

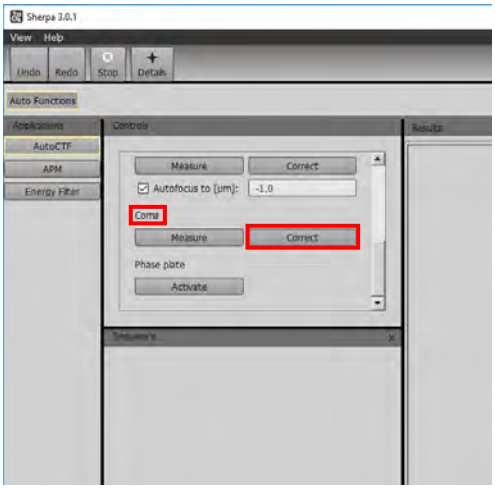
□ When you pick up the tone ring, press Correct.



(It takes astigmatism from the objective lens. If the light is too dim, it won't pick it up.)

*Do not stop in the middle. If you stop, it will stop in a strange state on the way.

□ When "Completed" appears in the lower right corner, press "Correct" on Coma (remove coma).



□ When the word "Completed" appears in the lower right corner, press Correct for Objective Stigmation again.

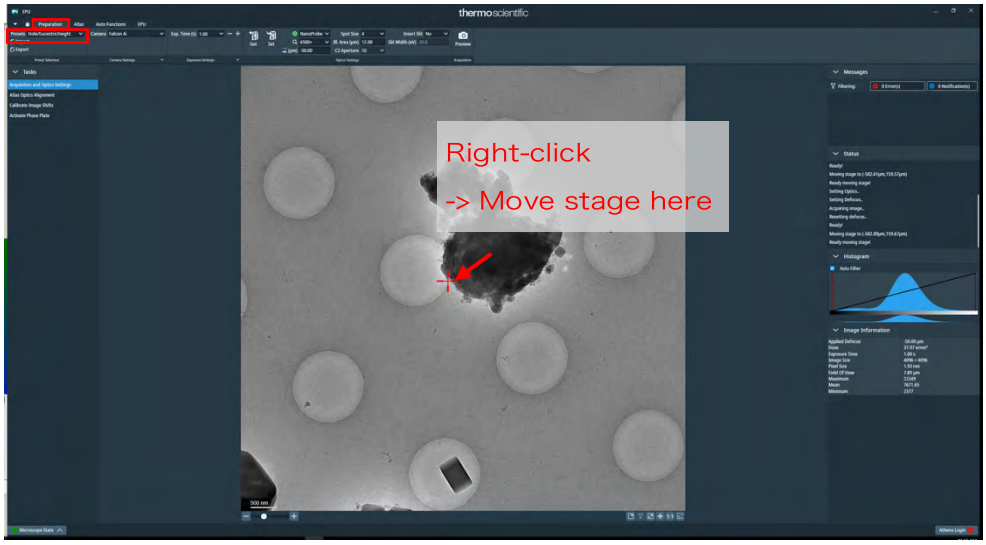
*If AutoCTF doesn't work at all, something is wrong. Let's start over

□ When you're done, keep the Sherpa window minimized.

7. **Center alignment between EPU magnifications** (KEK Manual 11.)

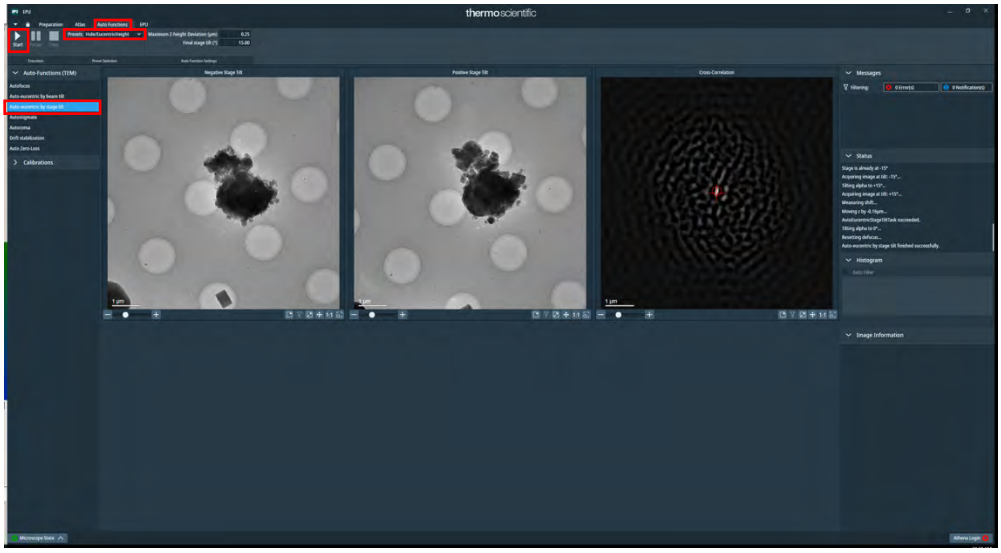
(*When using UltrAufoil, it is better to make this adjustment with UltrAufoil)

☐ EPU > Preparation tab > There should be a garbage image in the Hole/Eucentric of the Presets, so move the corner of the garbage or the intersection of the garbage and the Hole with Move stage here.



☐ EPU > Auto Functions tab > Auto-eucentric by stage tilt

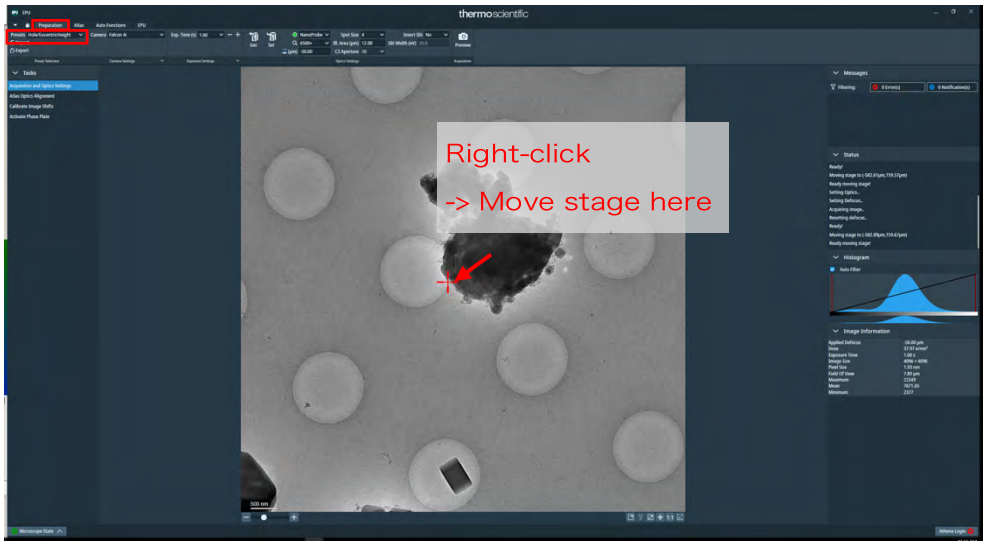
☐ Presets > Hole/Eucentric, and click Start



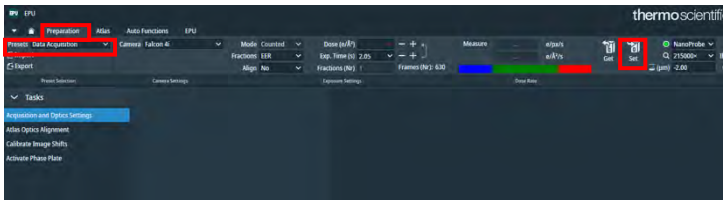
* If it fails, try again with Auto-eucentric by beam tilt (see also p. 22).

☐ EPU > Preparation tab > Presets > Hole/Eucentric, and click Preview

☐ Move stage here at the corner of the garbage or at the intersection of the garbage and the hole



☐ EPU > Preparation tab > Presets > Data Acquisition, and click Set



☐ Launch Velox (there are two windows, but only Acquisition is used)

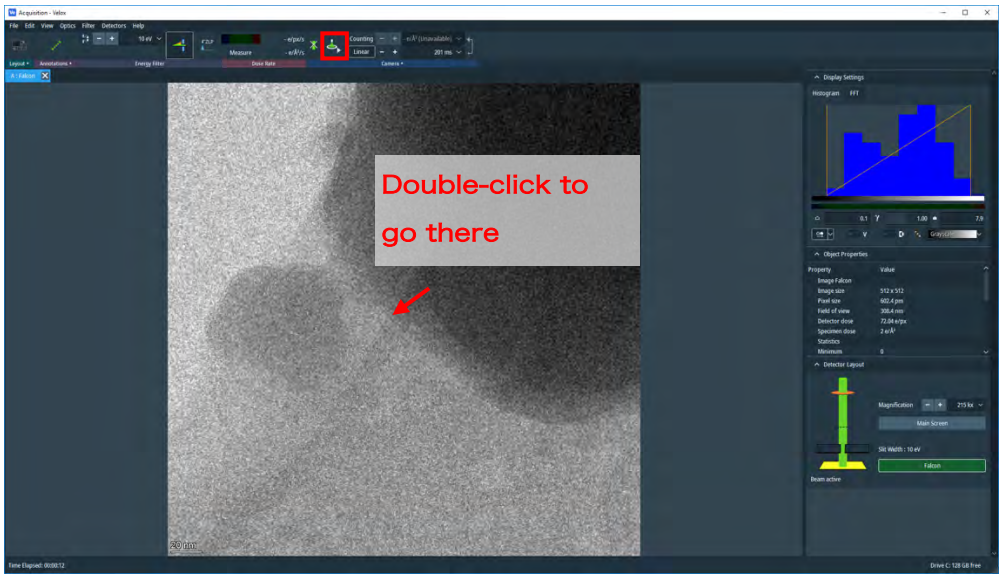
☐ Click the  icon. The beam irradiation begins, and an image is captured.

* Continue irradiation until manually stopped

☐ Double-click to move where you want to go in the image.

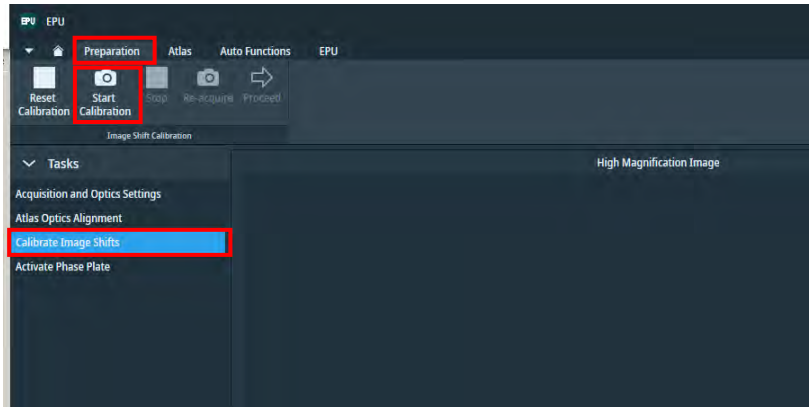
☐ Bring it to the corner of the garbage or the intersection of the garbage and the hole

* Don't go to the center you thought you would do without repeating a few double-clicks?



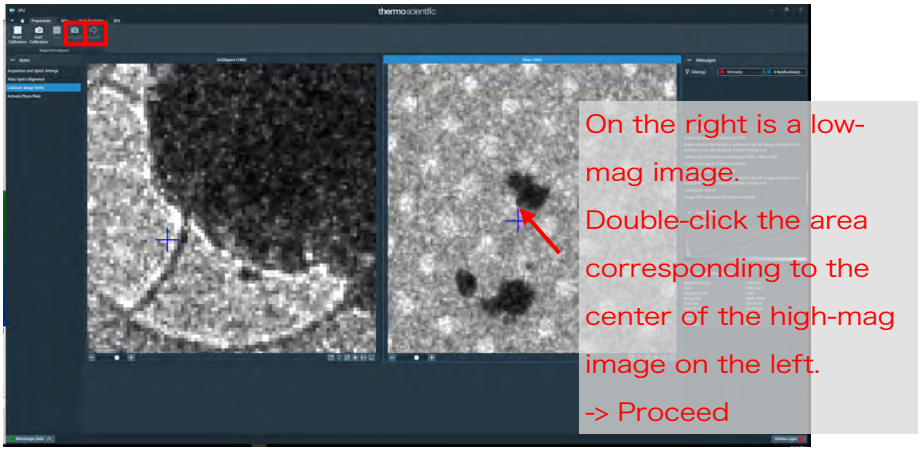
☐ Click the  icon again to end beam irradiation.

☐ EPU > Preparation tab > Calibrate Image Shift, and click Start Calibration



□ View the x215k (or magnification you want to take) image (on the left side of the screen) > Proceed

* If the center is off, double-click to match it and then click Re-aquire
Should I not do it unless it is very off?



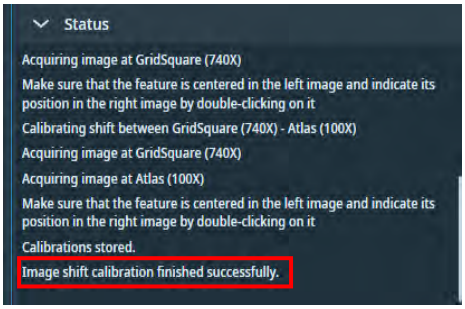
□ Double-click on the x6500 image, the center of x215k and the corresponding location and proceed

□ The x6500 image changes to the left

□ Double-click on the x740 image, the center of the X6500 and the corresponding location and proceed

□ Double-click on the x155 image, the center of the X740 and the corresponding location and proceed

□ Finished when the Status says finished successfully



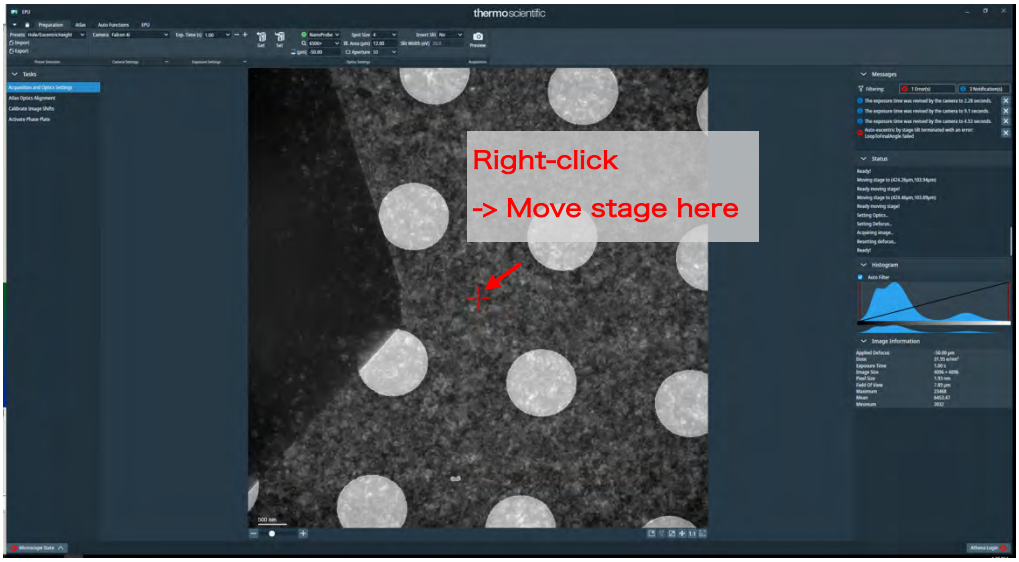
* If you change the magnification, try again.

That's all for daily beam adjustments. (in the case of carbon film grid)

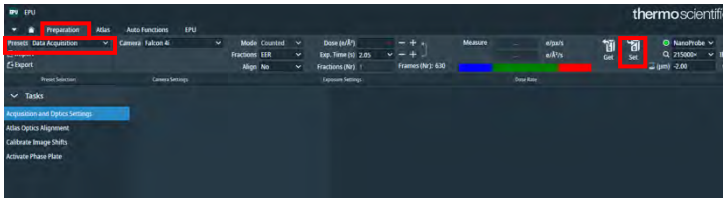
8. **Alignment of the objective diaphragm** (KEK Manual 6.)

(*When using UltrAufoil, make this adjustment with UltrAufoil)

- ☐ Check the fluorescent plate. If it is in, press R1 to raise the fluorescent plate.
- ☐ EPU > Preparation tab > There should be a garbage image in the Hole/Eucentric of Presets, so move it to the vicinity with Move stage here.
- ☐ EPU > Auto Functions tab > Auto-eucentric by stage tilt
- ☐ Presets > Hole/Eucentric, and click Start
- ☐ EPU > Preparation tab > Presets > Hole/Eucentric, and click Preview
- ☐ Right-click between holes and holes where there is no garbage -> Move stage here



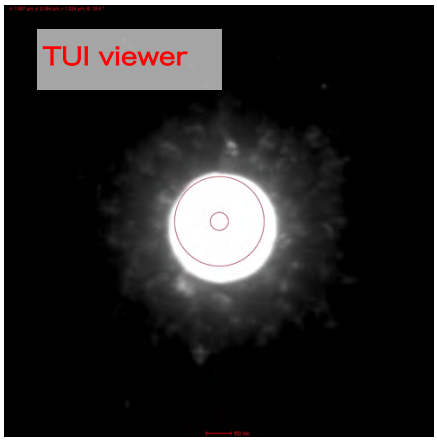
- ☐ EPU > Preparation tab > Presets > Data Acquisition, and click Set

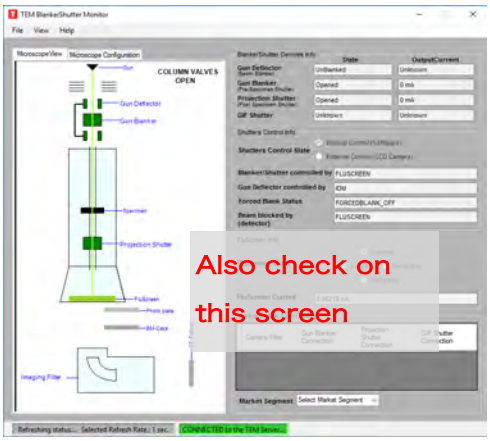


☐ TUI > Autoloader tab > Apertures, confirm that **C2=50, Obj=none**

☐ **Be sure to press R1 to lower the fluorescent plate!**

*** Pressing the Diffraction button without lowering the fluorescent plate will break the camera!**



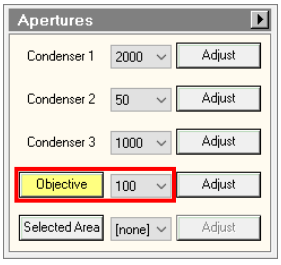


☐ Press the Diffraction button on the control board.

☐ Look under the TUI and see that D = xx m etc.

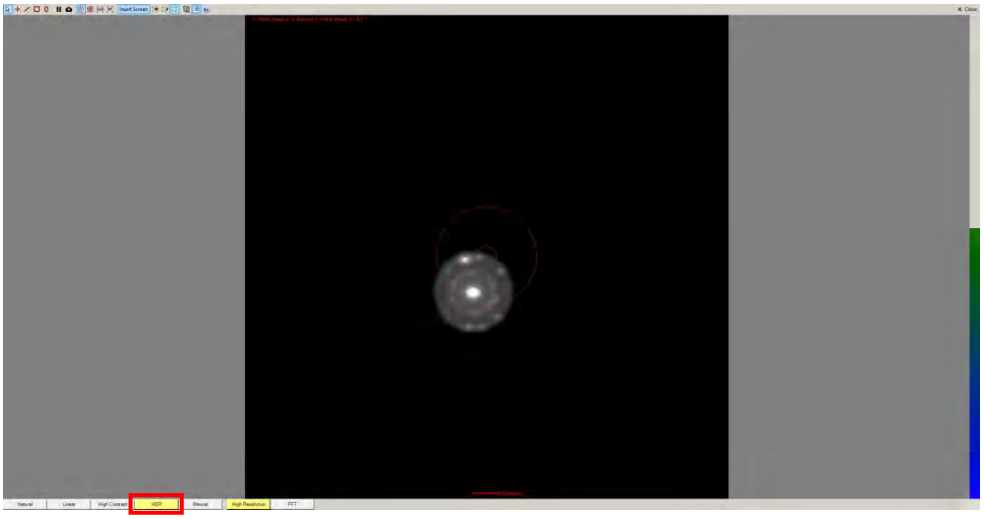
High tension:	300 kV	Beam Current:	5.3 nA
nP EFTEM		Screen current:	0.065 nA
D 2.5 m		Spot size:	4

☐ TUI > Autoloader tab > Apertures, change it to **Obj=100**

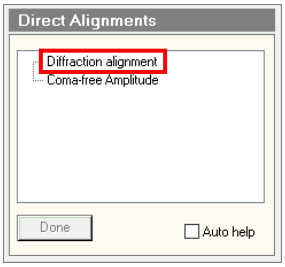


□ TUI in HDR mode

(*If it is difficult to see the shadow of the objective aperture, adjust it with the mouse wheel.)

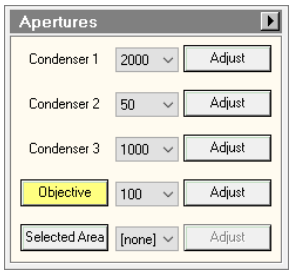


□ If the beam is not centered, it should be centered with the Direct Alignment > Diffraction Alignment > Multifunction dial

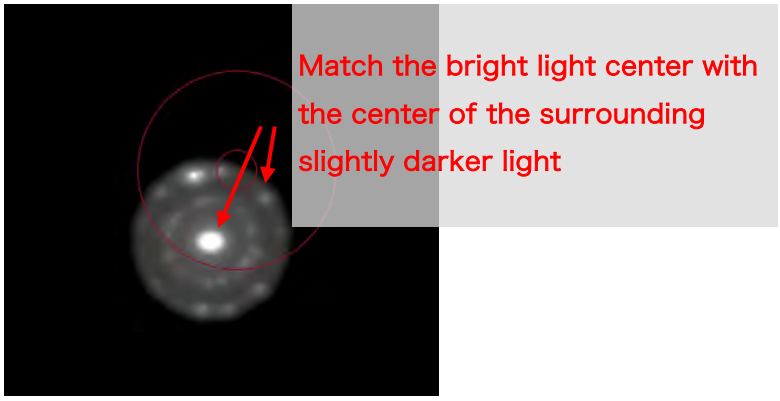


☐ (Is it safer not to do in the following frame unless they are very far off?)

☐ Press the Adjust button next to Objective.



☐ Use the Multifunction dial to align the bright light center with the ambient blurry light center



☐ Press the Diffraction button on the control board again to exit diffraction mode.

*The FluCam Viewer should automatically return to Natural.

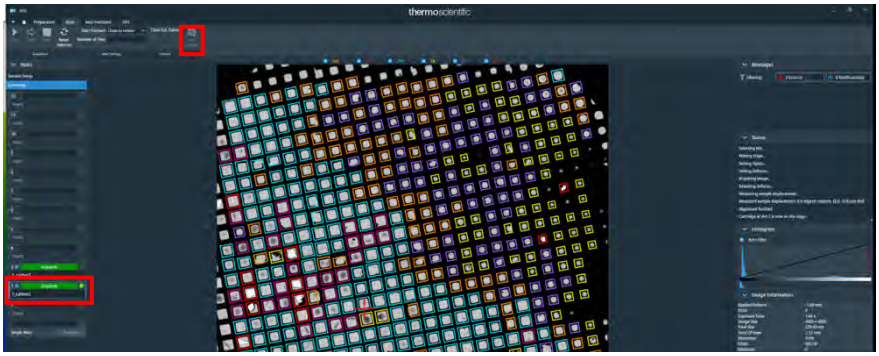
Beam adjustment is over

Observation with UltrAufoil may be performed with Obj = 100

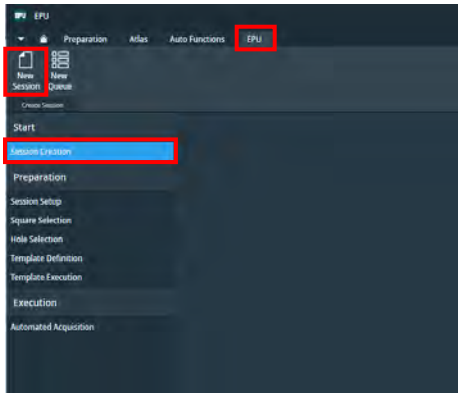
III. Data Measurement

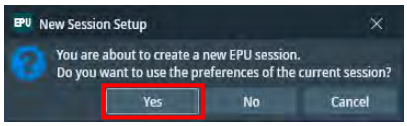
1. Grid Screening

☐ EPU > Atlas tab, select the desired grid and Load Sample



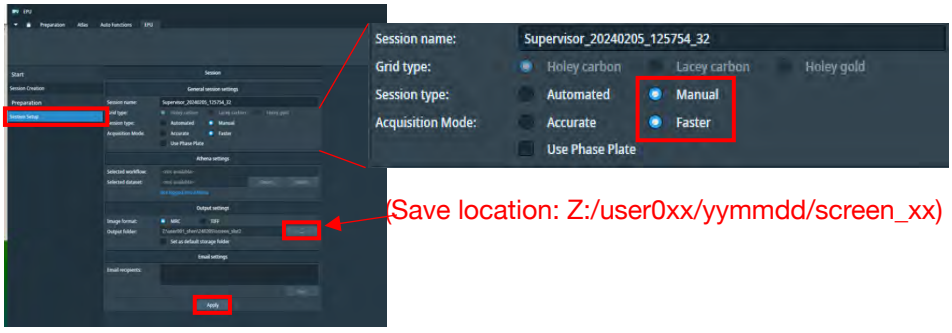
☐ EPU > EPU tab > Session Creation > New Session > Yes





☐ Press Session Setup.

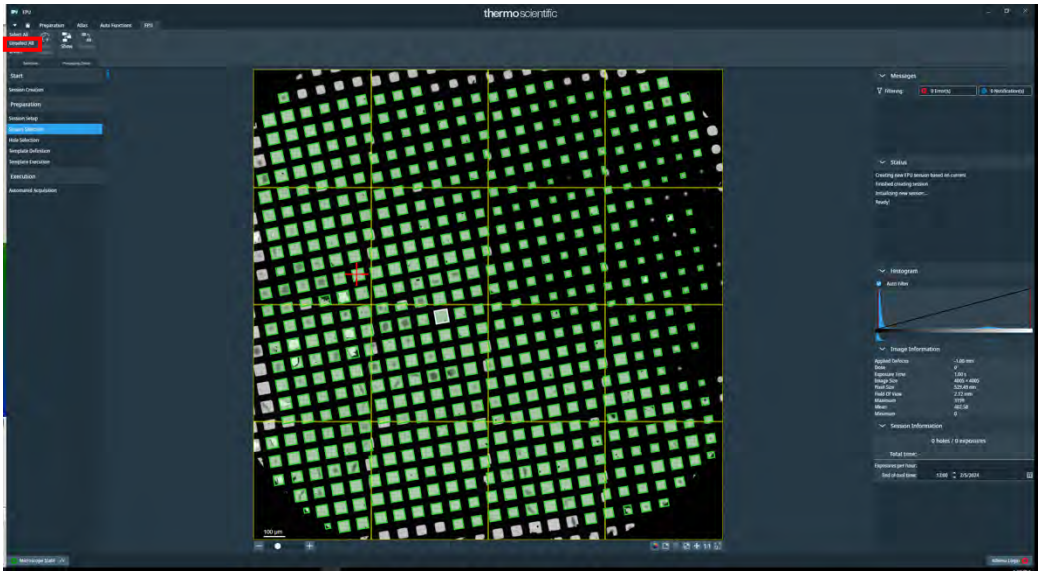
(Select Session type: Manual, Acquisition mode: Faster)



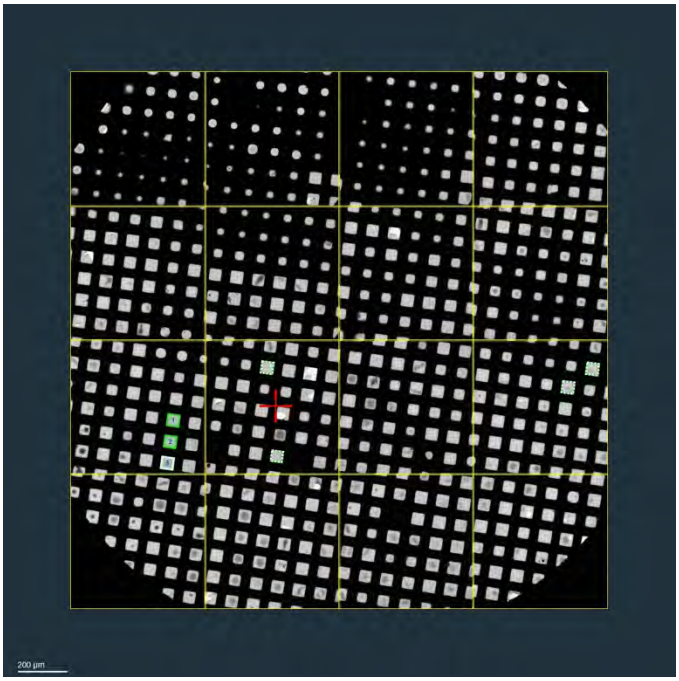
(Save location: Z:/user0xx/yymmdd/screen_xx)

☐ Press Apply.

□ Square Selection, squares are auto-selected, but Unselect All



□ Select 2-3 representative squares (Ctl + left-click)



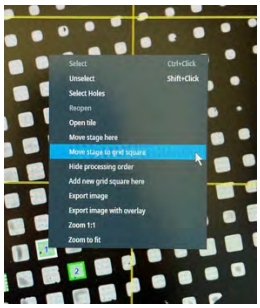
* It is good to vary the ice from thin, a little thick, thick, etc.

* If you want to number the selected square, right-click and show processing order

* Take a screenshot Right-click -> Export image with overlay

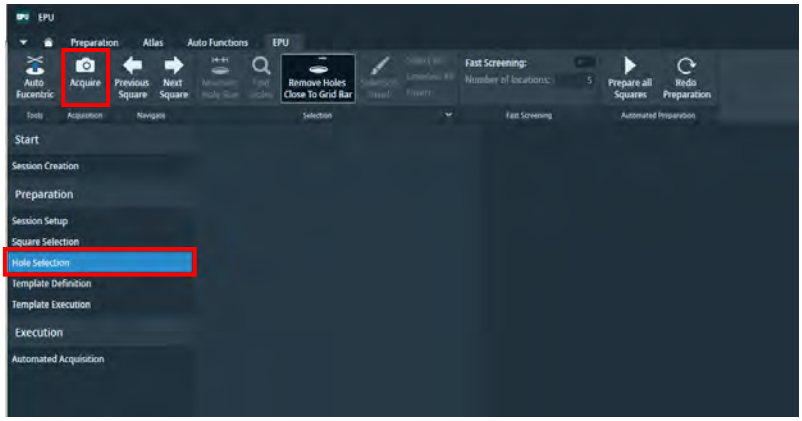
Please note that if you do the above while zooming, the zoom image will be saved.

□ Right click on the first square -> Move stage to grid square

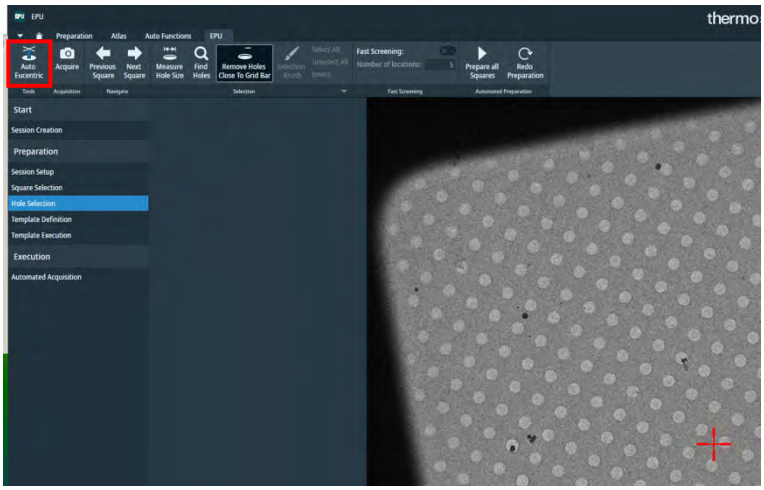


□ Press Hole selection.

□ Take a picture with Acquire and see if it's a good square. If you like, go to the following



□ Press the Auto Eucentric button in the upper left corner.



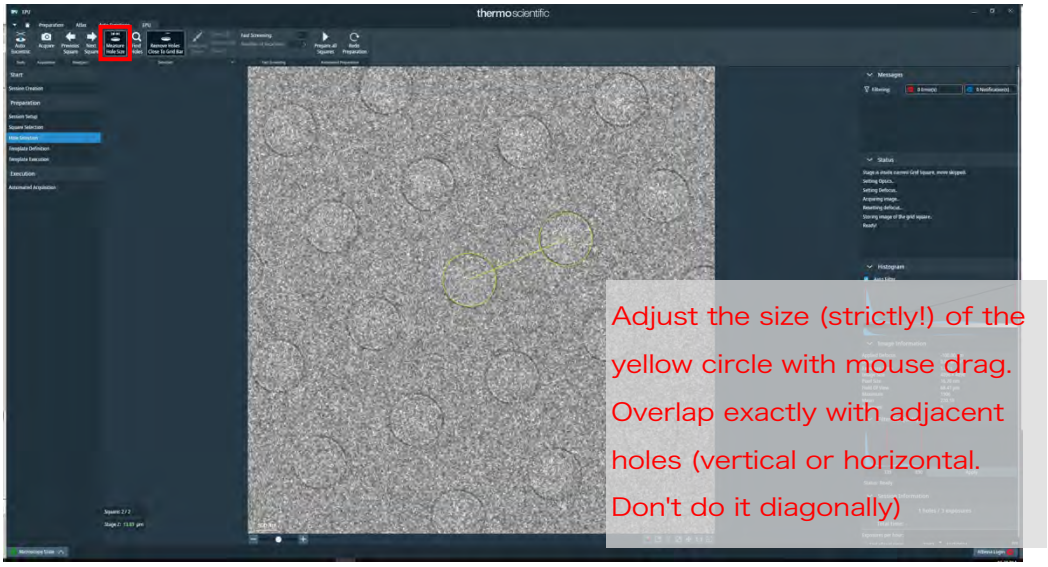
* If Auto Eucentric fails, try Auto-eucentric by stage tilt from the Auto Functions tab.

* If it still fails, try Auto-eucentric by beam tilt

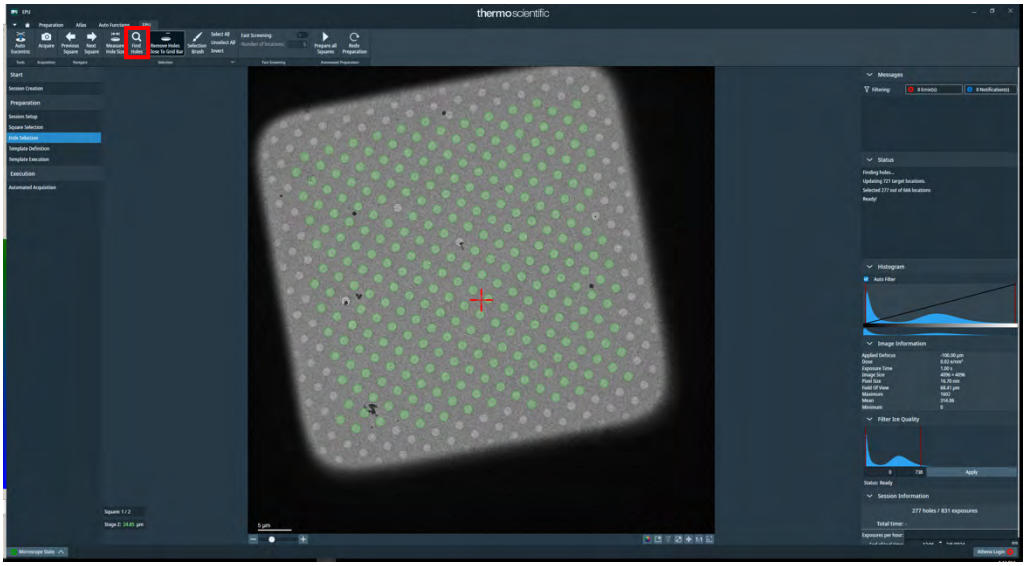
It's fine if either one works (See also p. 22).

*Once the Eucentric height is determined, go back to Hole selection and click Acquire (The new eucentric height is reflected)

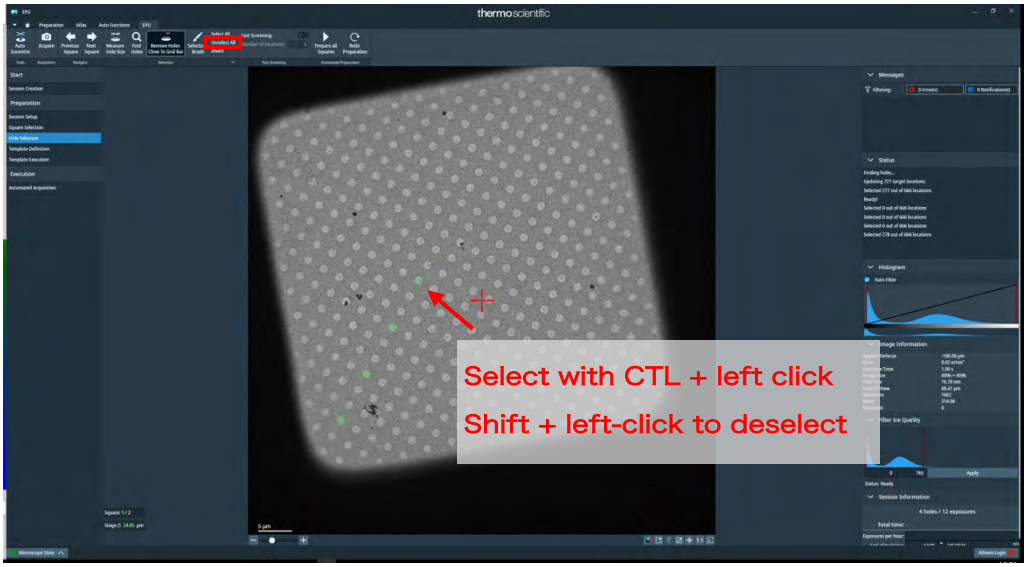
□ Once you have eucentric height, press Measure Hole Size to define the size of the hole and the interval between the adjacent holes.



□ Automatically recognize holes with Find Holes

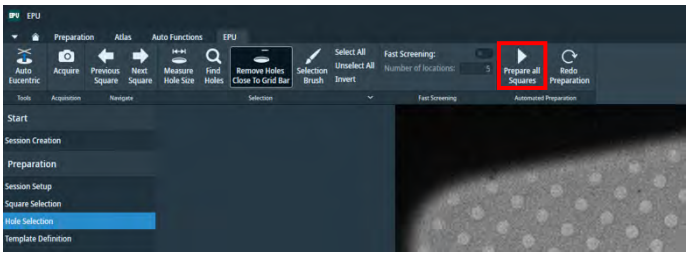


□ If you can pick up the hole successfully, press Unselect All, and select only the representative number of holes (Ctl + left-click).



* It is a good idea to consider the thickness of the ice in appearance, such as the center and periphery of the square.

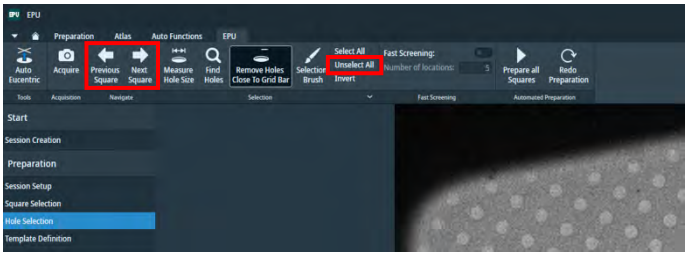
□ Press Prepare all Squares to do the same for all selected squares



* If you fail in the middle, select the square again or see p. 52

□ Move the squares by Next Square/Previous Square to check the recognition of holes' by

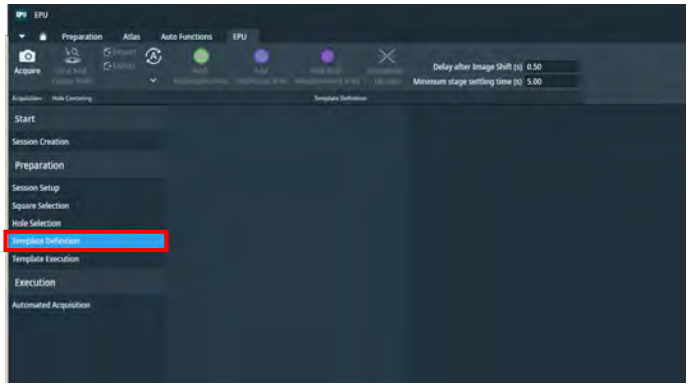
'Unselect All' the holes recognized in each square and select a representative number of holes.



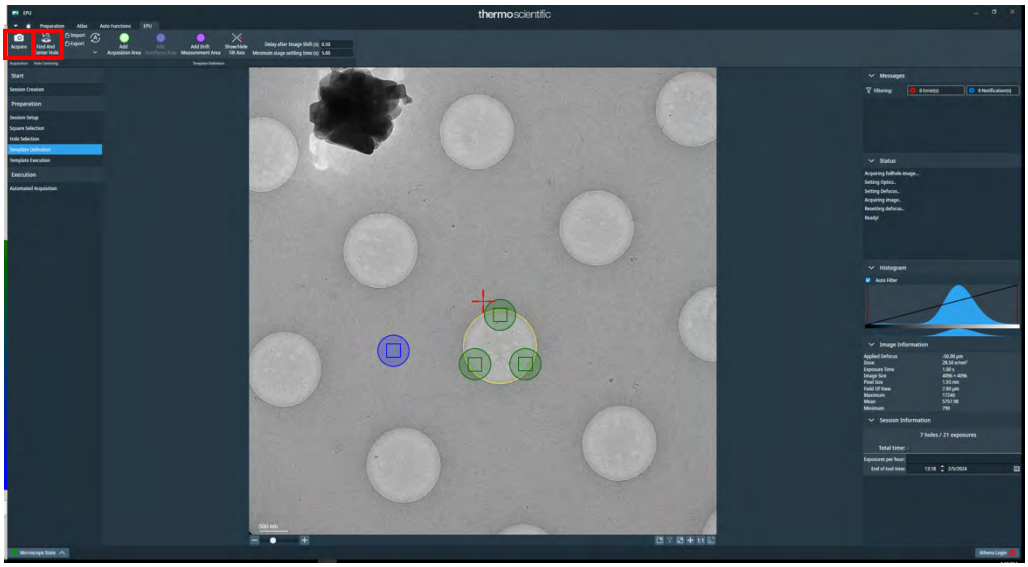
* For each square, take a screenshot of the selected hole.

Right-click -> Export image with overlay

☐ Press Template Definition.



☐ Acquire -> Find and Center Holes



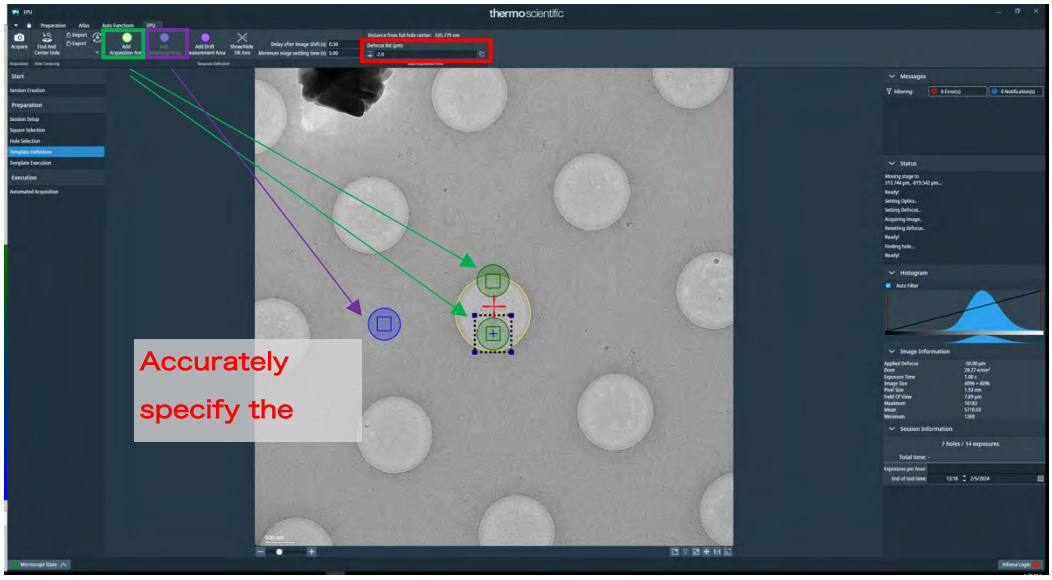
☐ Specify where in the hole to be photographed by Add Acquisition Area.

Multiple locations are possible (it is good to change the conditions such as center and edge).

☐ Specify the Defocus value. When screening, a little larger, like -2.0 um, is fine.

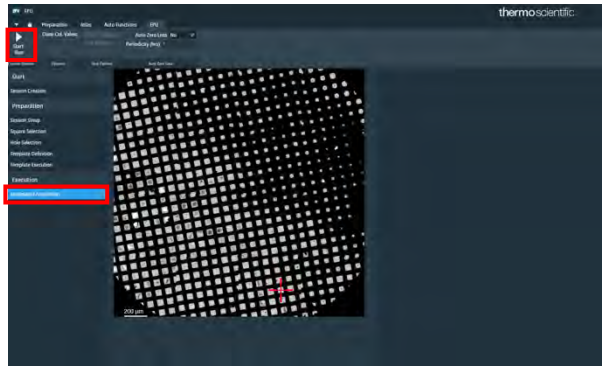
If you specify multiple shooting locations, you can set them individually.

☐ Specify the irradiation position for focus alignment by Add Autofocus Area.



Take a screenshot (take it with the app to take the entire screen including the defocus value)

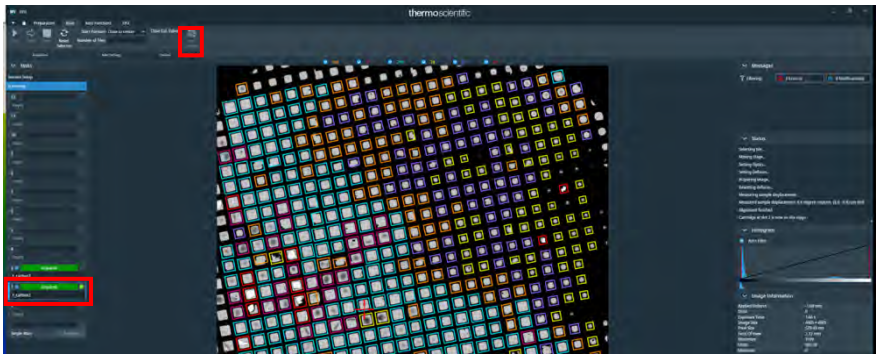
☐ Automated Acquisition > Start



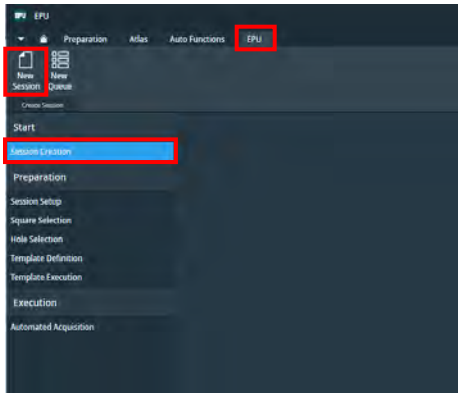
☐ After screening one grid, swap the grids and repeat the process

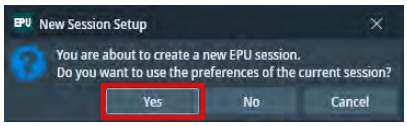
2. Automated measurement of data

☐ EPU > Atlas tab, select the desired grid and Load Sample



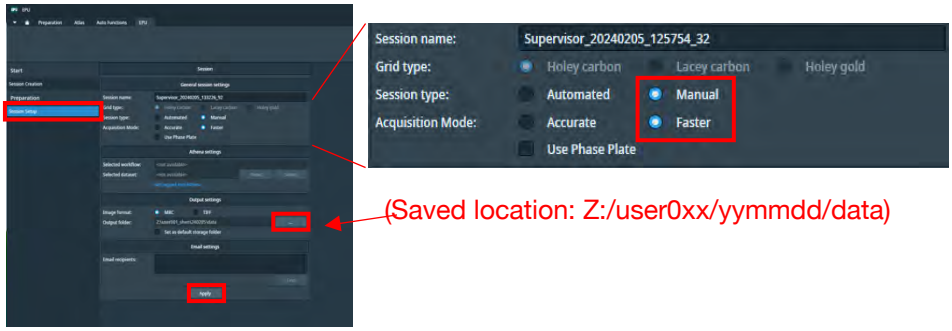
☐ EPU > EPU tab > Session Creation > New Session > Yes





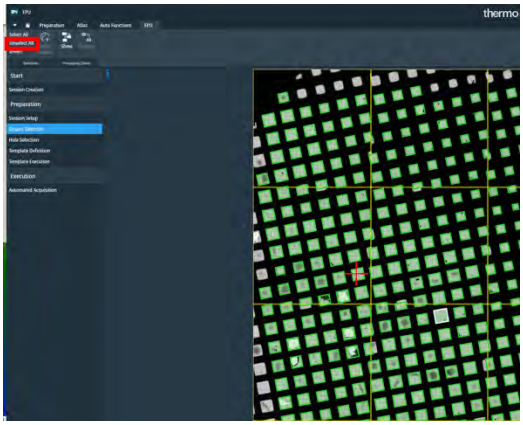
☐ Press Session Setup.

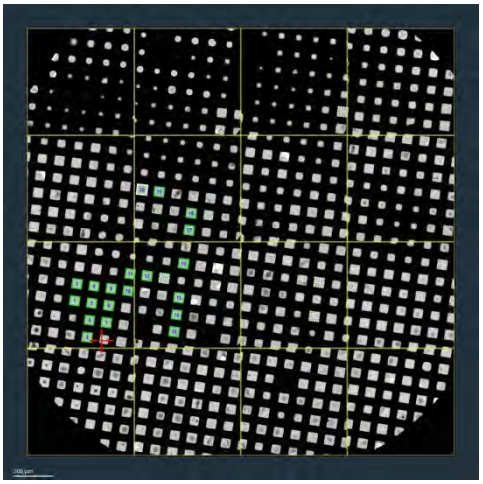
(Select Session type: Manual, Acquisition mode: Faster)



☐ Press Apply.

□ Press Square Selection. Squares are automatically selected, but Unselect All to clear them and manually select 20-30 squares

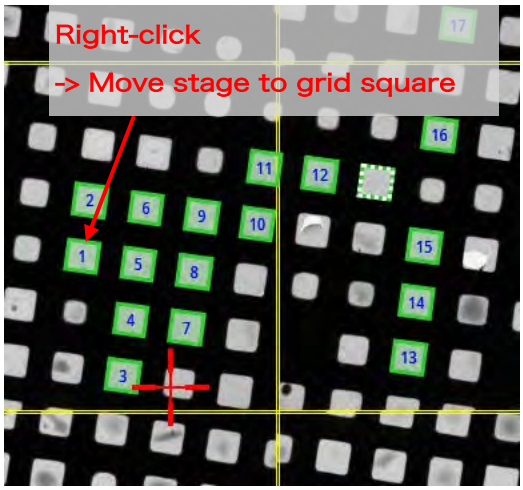




* If the distance traveled between squares is too large, the measurement time will be lost.

* Choose while following the neighboring squares sequentially as much as possible.

□ Right click on the first square (number 1) -> Move stage to grid square



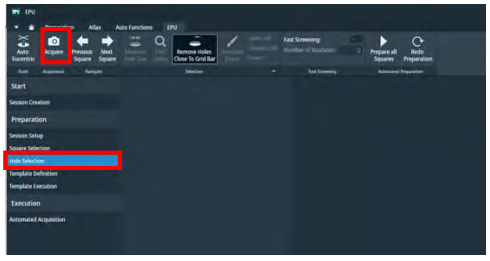
Move stage here:
Go to the place you clicked

Move stage to grid square:
Move to the center of the square you clicked

□ Press Hole selection.

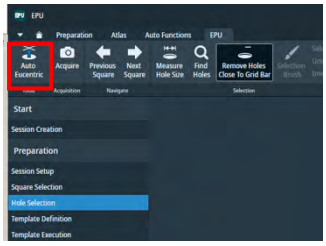
□ Click Acquire to shoot. Make sure it's a good square.

If you like, go to the following



* Suddenly Auto Eucentric may be used.

☐ Press the Auto Eucentric button in the upper left corner.



*If Auto Eucentric is mossed, go to the Auto Functions tab

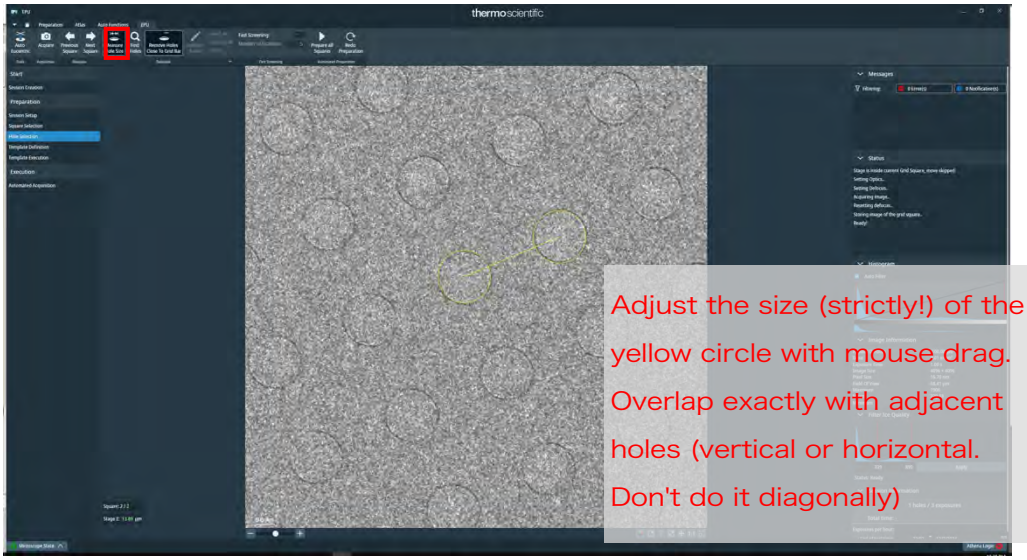
Auto-eucentric by stage tilt or

Auto-eucentric by beam tilt -> Start may work.

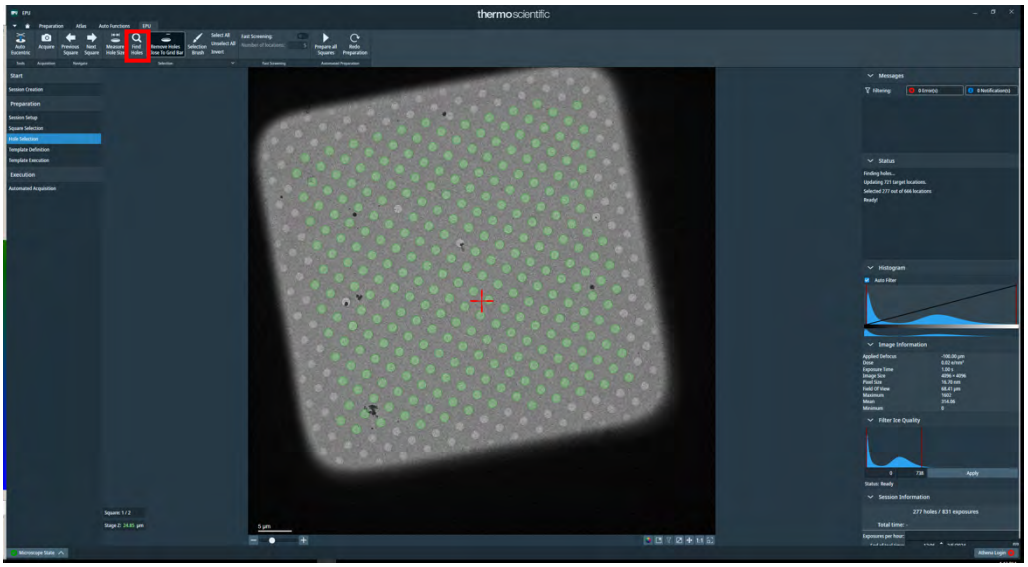
(See also p. 22)

*Once the Eucentric height is determined, go back to Hole selection and click Acquire

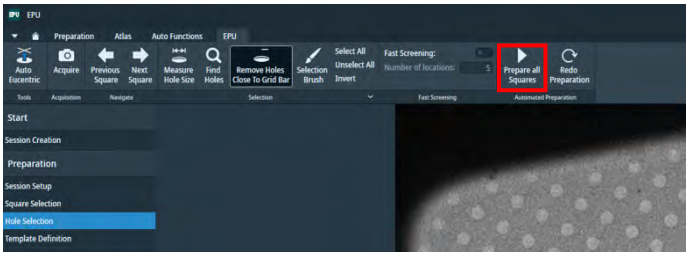
☐ Once you have eucentric height, define the hole with Measure Hole Size.



☐ Automatically recognize holes with Find Holes

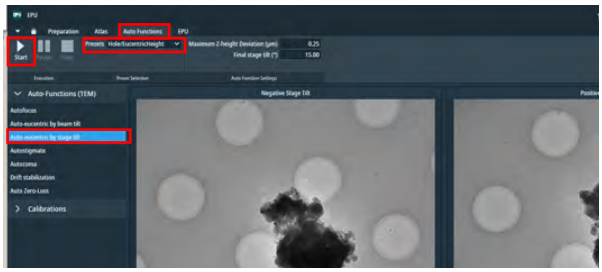


☐ If you successfully pick up the hole, press Prepare all Squares (it will do the same for all selected squares)



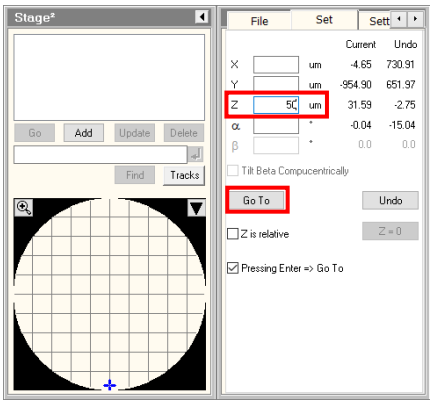
* About 20-30 minutes until all the squares are finished (about 1 minute per square?)

* If eucentric moss and stops in the middle, click Auto Functions tab,
Click Auto-eucentric by stage tilt, set preset to Hole/Eucentric and Start



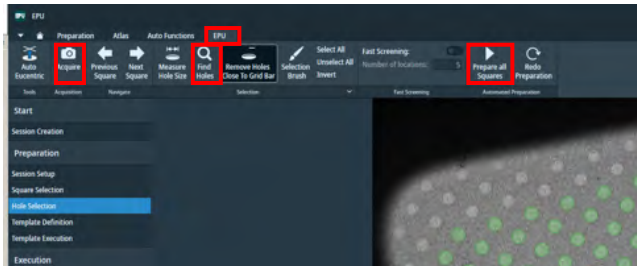
Or Start with Auto-eucentric by beam tilt

If that doesn't work, go to TUI -> Stage tab -> Stage^2 -> option and enter Z=50um (a value that should be close to eucentric height) and Go To

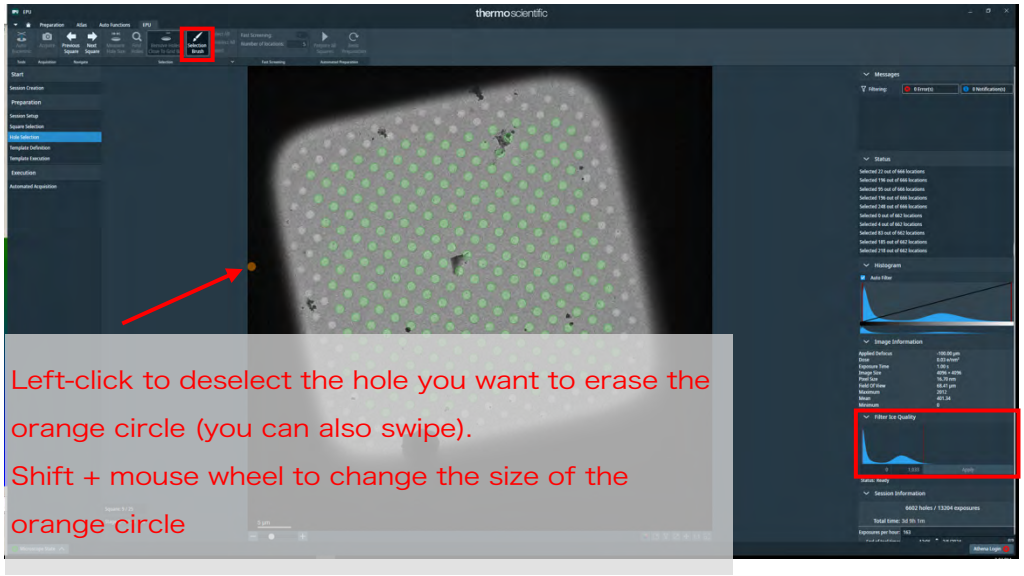


Then Auto Functions tab ->
Auto-eucentric by stage tilt -> Start
(see also p. 22)

If you decide on one of them, go back to the EPU tab and click Acquire -> Find Holes
If the Hole is recognized, Prepare all Squares

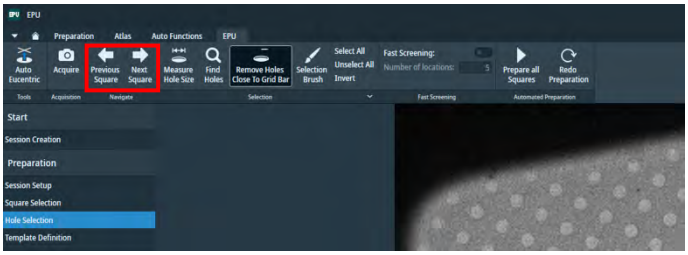


☐ Check the automatically recognized hole. If there is debris or cracks, manually remove it with a selection brush. You may adjust the two bars of "Filter Ice Quality" at the bottom right



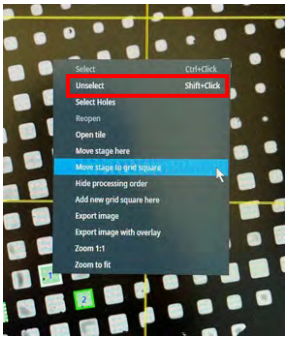
* It is safer not to choose a square with a crack (it is easy to shake when hit by electrons)

☐ Move the square by Next Square/Previous Square to confirm and modify the above hole selection.



☐ If you want to delete an entire square, go back to Square Selection and right-click on the square you want to delete -> Unselect

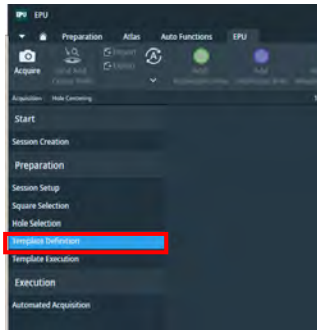
If you don't erase the big number, the numbers won't match!

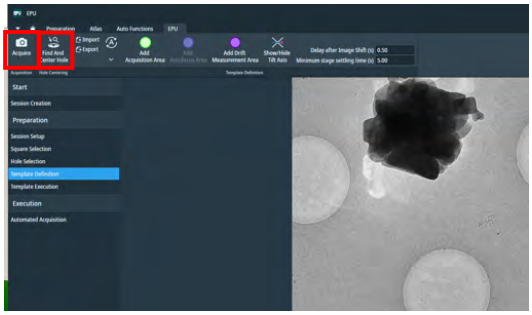


☐ Square Selection -> Move stage to grid square

☐ Press Template Definition.

☐ Acquire -> Find and Center Hole





☐ Specify where in the hole to be photographed by Add Acquisition Area (green).

Multiple locations are allowed (center and edge, etc.).

☐ Specify the Defocus value.

-0.6, -0.8, -1.0, -1.2, -1.4, -1.6, -1.8 um etc. are standard?

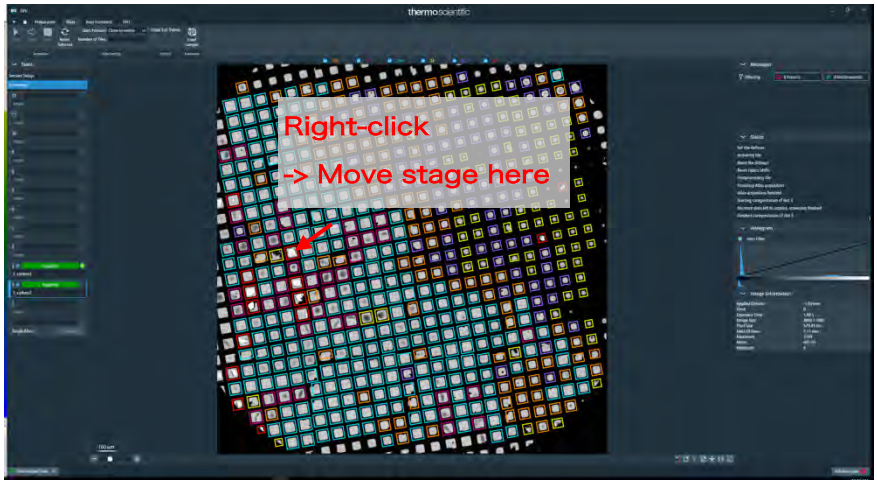
Press the button on the right side to reflect on all areas

☐ Specify the irradiation position for focus alignment by Add Autofocus Area (purple).

Take a screenshot (take it with the app to take the entire screen including the defocus value)

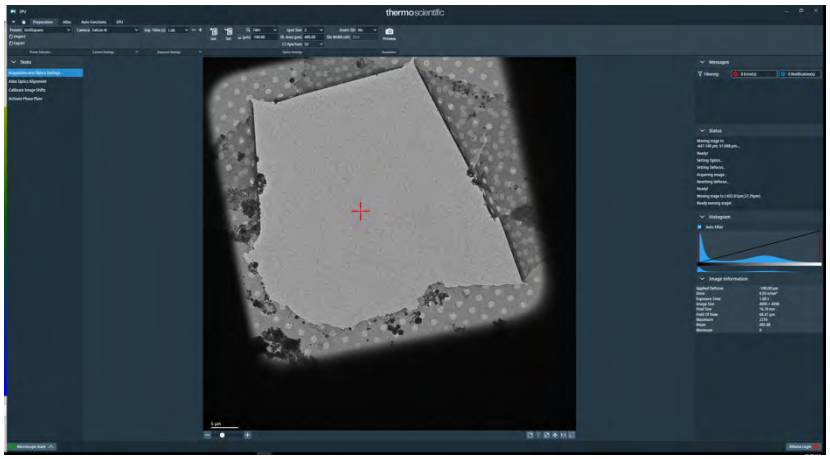
☐ Move to the broken square in the grid to estimate the dose

EPU > Atlas tab, right-click on the broken square in the Grid -> Move stage here



☐ EPU > Preparation tab > Presets > Grid Square > Preview

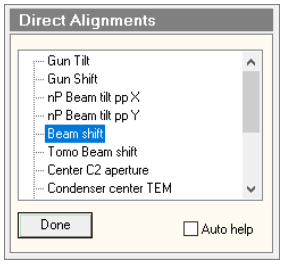
(Confirm that you are in the hole)



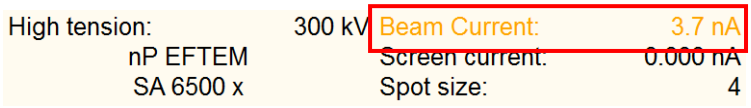
☐ EPU > Preparation tab > Presets > Data Acquisition > Set

☐ Press R1 to lower the fluorescent plate

☐ If the beam is not centered, center it with Direct Alignment > Beam Shift and Done



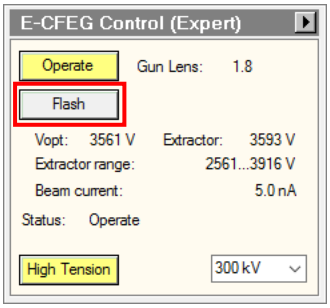
☐ Checked the Beam Current in TUI.



☐ **If it is below 5, Flash**

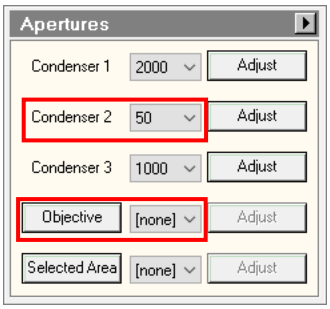
(TUI -> Set up tab -> E-CFEG Control)

*Close the column valve when flushing



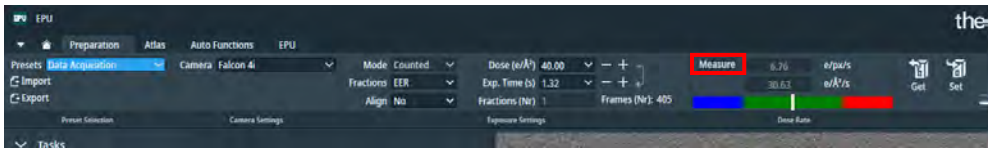
☐ EPU > Preparation tab > Presets > Data Acquisition > Set

☐ TUI > Autoloader tab > Apertures,confirm that C2=50, Obj=none



☐ EER, No, Dose: ~50, select your preferred magnification (around x165k/215k/270k is common?))

□ Press Measure.

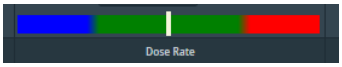


Based on the measurement result, the irradiation time that becomes the specified dose is calculated

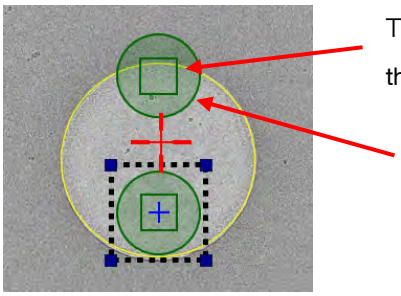
*Irradiation time correlates with spot size and illuminated area.

*If the spot size and illuminated area are narrowed, the irradiation time will be shorter (= the number of images will increase), but the dose rate will be higher because the electrons are concentrated in a narrow area.

* If the dose rate is too high, there will be many adverse effects such as low DQE and damage to the sample, so set it so that it does not go to the red of the bar.



Adjust the value to an appropriate value while comparing it with the detector size in the Template Definition.



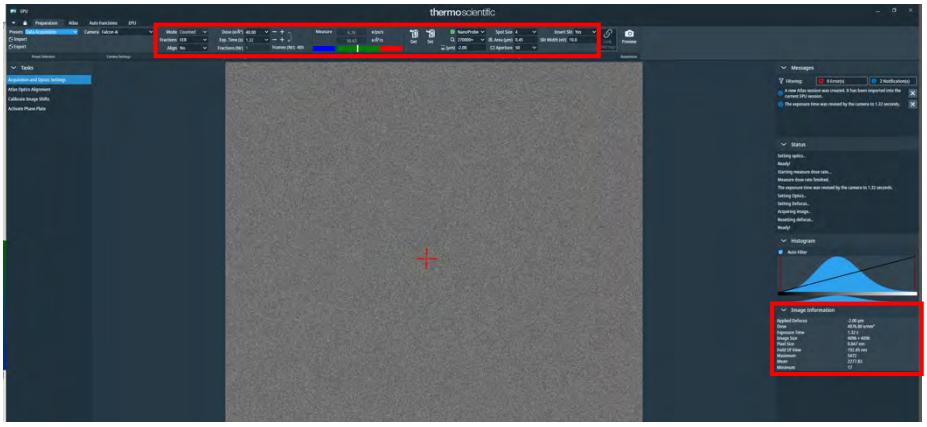
The square is the field of view (FOV) of the detector

Circle illuminated area

It should be wide enough for the field of view.

□ Finally, Preview and then get a screenshot

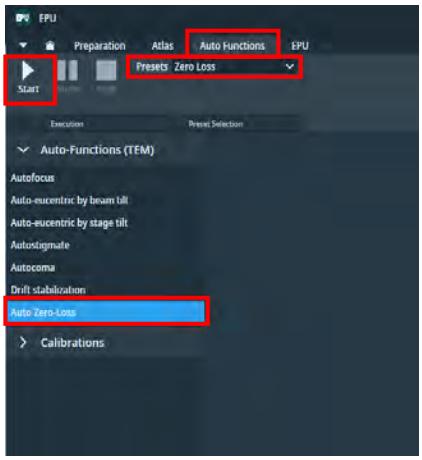
*When previewing, information such as pixel size will appear at the bottom right.



(Take up the entire screen with the app)

□ EPU > Auto Functions tab > Auto Zero-loss

Presets > Zero loss, and Start

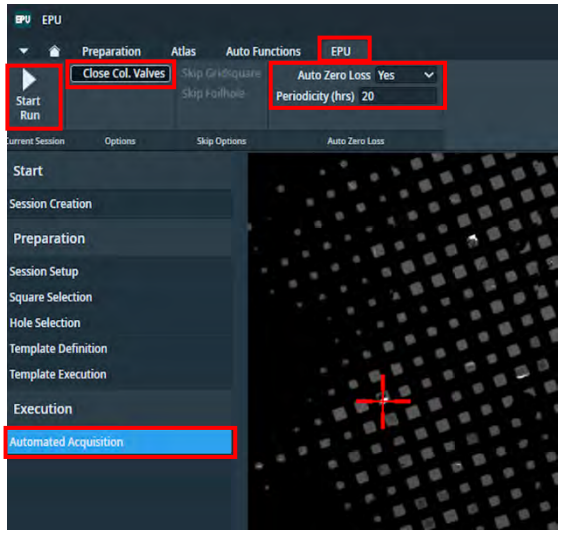


□ EPU > EPU tab > Automated Acquisition

Auto Zero loss = Yes, Periodicity (hrs) = 20 hrs (Do you need it if the measurement is less than 20 h?) ）

Enable Close Col. Valves to close the column valve after measurement

Start measurement with Start



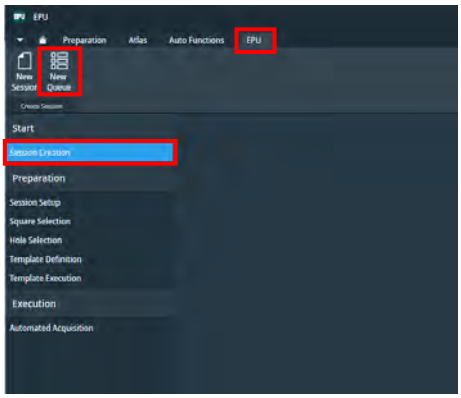
That’s all

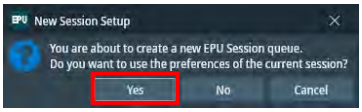
3. Data measurement with Multi Grid Session

☐ In the EPU > Atlas tab, select the first grid and click Load Sample

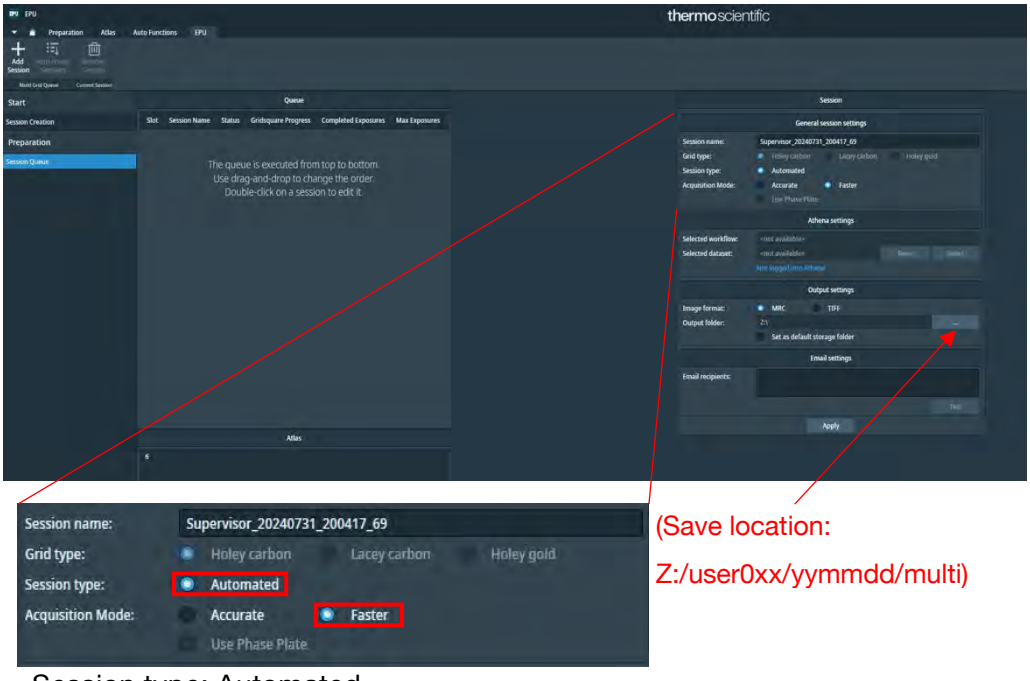
☐ Follow p. 55-57 to estimate the dose.

☐ EPU > EPU tab > Session Creation > New Queue > Yes





☐ Input in the window on the right side of the Session Queue



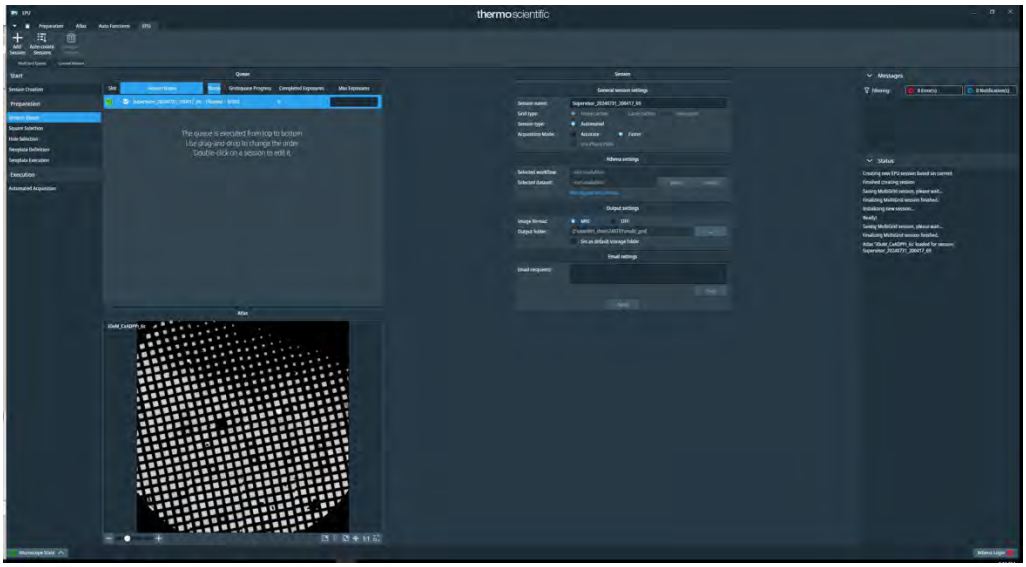
(Save location: Z:/user0xx/yymmdd/multi)

Session type: Automated

Select Acquisition mode: Faster

☐ Press Apply.

□ The first grid enters the Queue and displays the Atlas



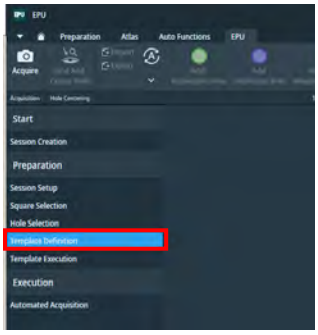
□ According to p. 50-51, select squares and holes.

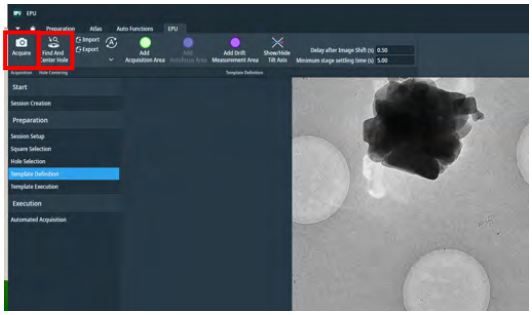
*Automated, so only the first square can be selected.

*Holes cannot be sorted (sorting can be done by setting the Ice filter).

□ Press Template Definition.

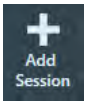
□ Acquire -> Find and Center Hole





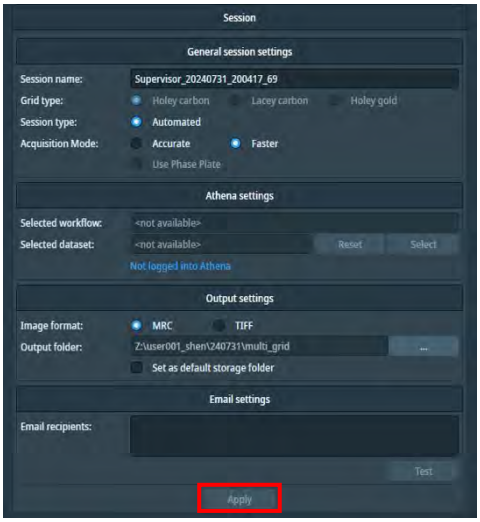
□ In accordance with p. 54, set the auto focus position, irradiation position, defocus value, and the like.

☐ Replace Grid (Load Sample in Atlas tab)

☐ EPU tab > Session Queue -> Add Session 

-> Yes

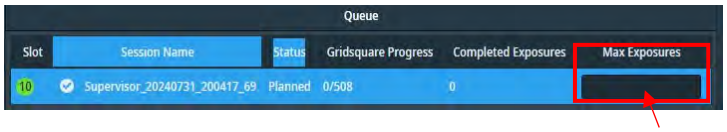
☐ Enter the destination in the new Session window and apply



☐ The current grid is added to the queue. As with the first grid, select squares, select holes, and define a template.

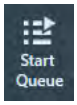
☐ Repeat for all desired grids.

☐ Set Max Exposures as needed



When the number of sheets set here is reached,

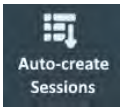
☐ According to p. 55-58, estimate the dose and set Auto Zero-loss.

☐ Automated Acquisition -> Start Queue 

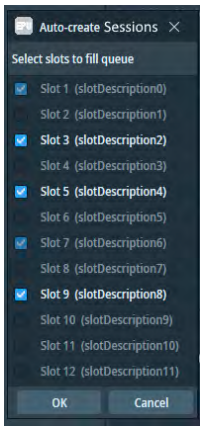
***All at once configuration with Auto-create Sessions**

☐ Complete the dose estimate (pp. 55-57).

☐ Once you have set up the first grid, select it in the Session Queue and

Auto-create Sessions 

☐ Choose which grid to put in the queue.



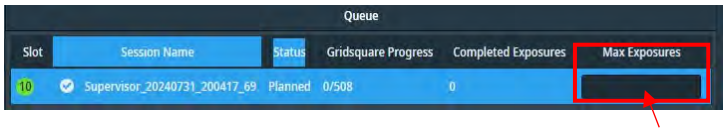
☐ The grid selected by OK and entered into the Queue.

☐ Since the save destination of the new queue is directly under the Z drive, enter the save destination in the Session window on the right and apply

☐ Select squares from Square Selection.

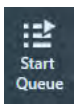
* Since the grid is not on the stage, it is not possible to set the holes and Template Definition (will it be the same setting as the first grid?).

☐ Set Max Exposures as needed



When the number of sheets set here is reached,

☐ According to p. 58, perform Auto Zero-loss.

☐ Automated Acquisition > Start Queue 

That's all