

TROUBLESHOOTING GUIDE (300KV)

CONTENTS

- Pre-set file setting 2
 - Q: I cannot see the beam at EPU. 2
 - Q: If I want to change the magnification, what should I take note? 2
 - Q: If I want to change spot size / intensity, what should I pay attention to? 3
 - Q: When I use low magnification, the image is incomplete and only appears at the center circle. 3
 - Q: I would like to take images in the tiff format, how should i set?..... 3
- Problems about User Interface..... 4
 - Q: Part of the UI cannot be shown on the screen, what should i do? 4
- Alignment 4
 - Q: If the beam is not at the center of the image, what should I do? 4
 - Q: What is the possible reason for Autofocus failure? 4
- Common issues during the whole preparation 4
 - Q: There is an error at UI about not having enough liquid nitrogen. 4
 - Q: In UI, if any items at the Vacuum are red, please inform our staff. 5
- Setting up EPU 5
 - Q: Images taken are not at the center of the holes. What should I do? 5
 - Q: I have set the does, but the image dose is higher / lower that expected. 5
 - Q: Holes cannot be accurately identified when preparing squares..... 6
- Image Acquisition..... 7
 - Q: Images are blurry during acquisition. 7
 - Q: I have collected several images and they were good at the beginning. However, the images taken after a few hours were out of focus. 7
 - Q: Final image differs from the observed one during preparation. 7
 - Q: Image acquisition time is longer than expected..... 8

If you encounter new problems, welcome to let us know so we can
spice up this Troubleshooting Guide.

Happy hunting for Cryo-TEM images!

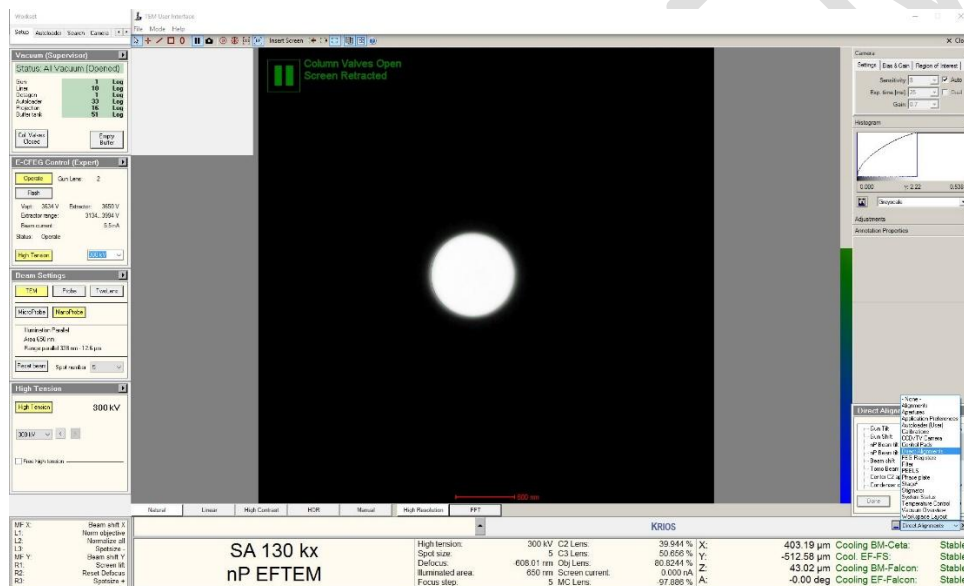
PRE-SET FILE SETTING

Q: I CANNOT SEE THE BEAM AT EPU.

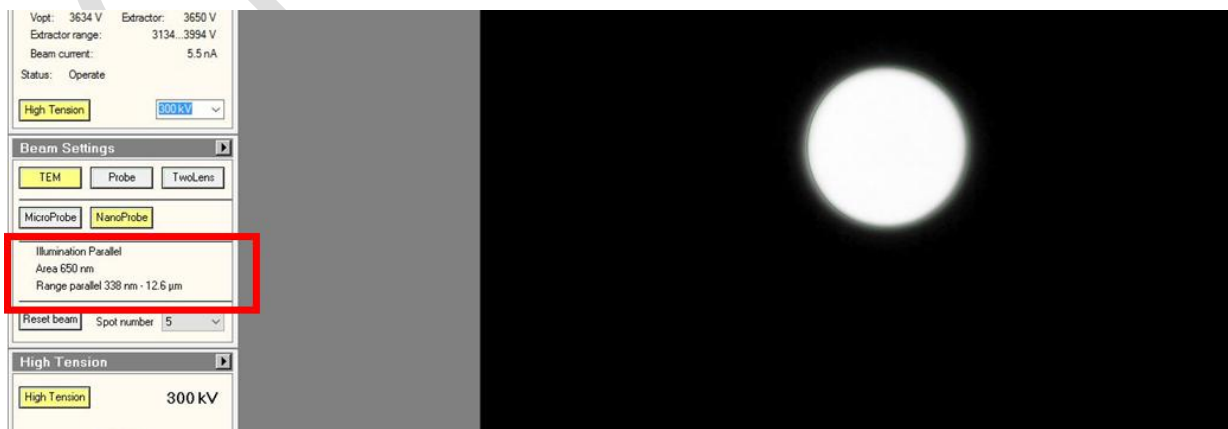
- Close the software **SerialEM** and try again.

Q: IF I WANT TO CHANGE THE MAGNIFICATION, WHAT SHOULD I TAKE NOTE?

- The primary option would be using another pre-set file directly. Changing magnification / spot size without caution may damage the detector. You can consult CPOS staff before making adjustments.
- To adjust the current pre-set file, insert the screen in UI. Move the stage to an empty area. Ensure the beam size is greater than the green circle.



- Changing from low magnification to high magnification usually has lower risk.
- When reducing magnification, monitor illumination parallel area and change the intensity (within the range of parallel beam) with the Intensity knob at the panel.



- At EPU, click “Get” to transfer the parameters to EPU settings.
- Measure dose at an empty area after adjusting new magnification, spot size, or intensity.

Q: IF I WANT TO CHANGE SPOT SIZE / INTENSITY, WHAT SHOULD I PAY ATTENTION TO?

- Similar to changing magnification, focus on ensuring the dose reaches the sample/camera.

Step 1: Insert screen at UI.

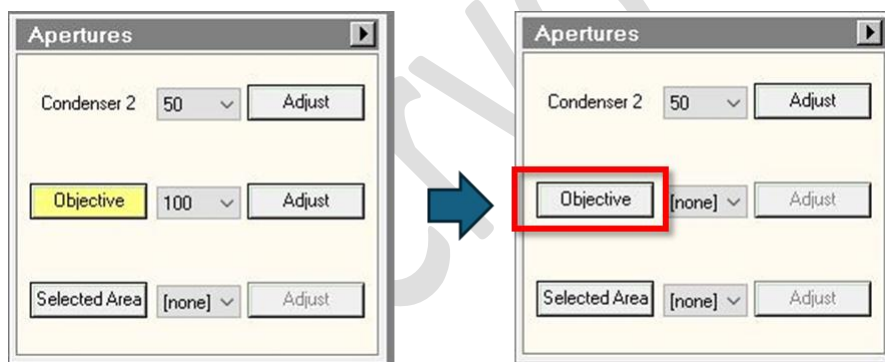
Step 2: Adjust the intensity / spot size and make sure the beam size is bigger than the green circle.

Step 3: At EPU, click “Get” to transfer the parameters to EPU settings.

Step 4: Check dose before acquiring images.

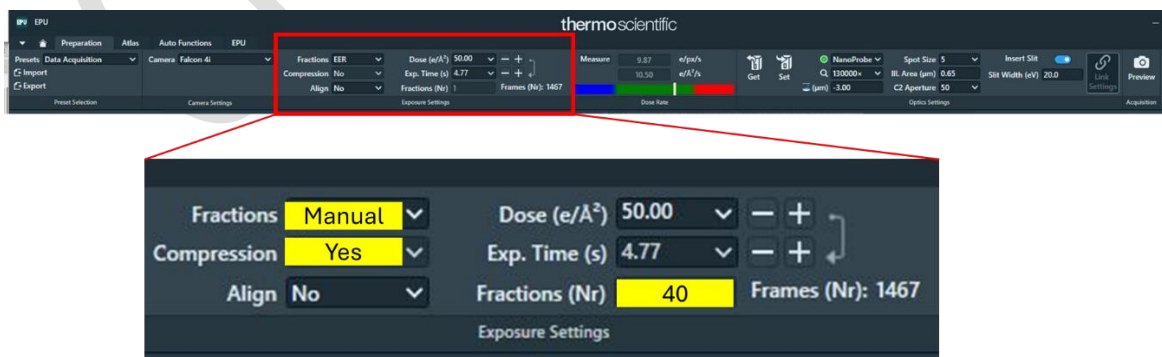
Q: WHEN I USE LOW MAGNIFICATION, THE IMAGE IS INCOMPLETE AND ONLY APPEARS AT THE CENTER CIRCLE.

- At UI, remove the aperture at objective.



Q: I WOULD LIKE TO TAKE IMAGES IN THE TIFF FORMAT, HOW SHOULD I SET?

- In Preparation, change the fractions, compression and Fractions (Nr) as the yellow box below:



- The Fractions must be value between 30 to 40, and it must be smaller than the dose set.

PROBLEMS ABOUT USER INTERFACE

Q: PART OF THE UI CANNOT BE SHOWN ON THE SCREEN, WHAT SHOULD I DO?

- Hold **Shift** and right-click mouse.
- Select **Restore**.

ALIGNMENT

Q: IF THE BEAM IS NOT AT THE CENTER OF THE IMAGE, WHAT SHOULD I DO?

- Align beam shift at UI at eucentric focus.
- Check image calibration at focus image for accurate alignment.

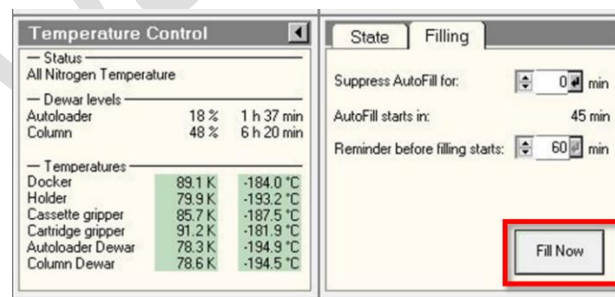
Q: WHAT IS THE POSSIBLE REASON FOR AUTOFOCUS FAILURE?

- Ensure the stage height is appropriate by using AutoEucentric.
- If the sample is too thick, find a region with thinner ice for autofocus.

COMMON ISSUES DURING THE WHOLE PREPARATION

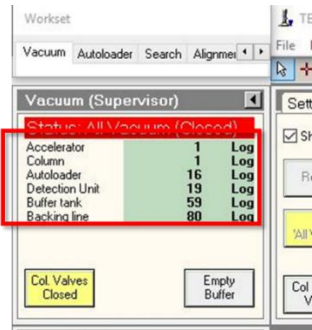
Q: THERE IS AN ERROR AT UI ABOUT NOT HAVING ENOUGH LIQUID NITROGEN.

- Click "Fill now" to refill liquid nitrogen.



- If the level remains low, inform staff about insufficient LN in the tank.

Q: IN UI, IF ANY ITEMS AT THE VACUUM ARE RED, PLEASE INFORM OUR STAFF.



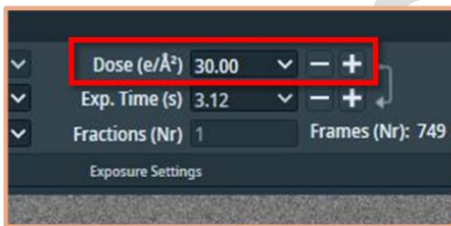
SETTING UP EPU

Q: IMAGES TAKEN ARE NOT AT THE CENTER OF THE HOLES. WHAT SHOULD I DO?

- Perform image shift calibration.

Q: I HAVE SET THE DOES, BUT THE IMAGE DOSE IS HIGHER / LOWER THAT EXPECTED.

Set dose



Outcome

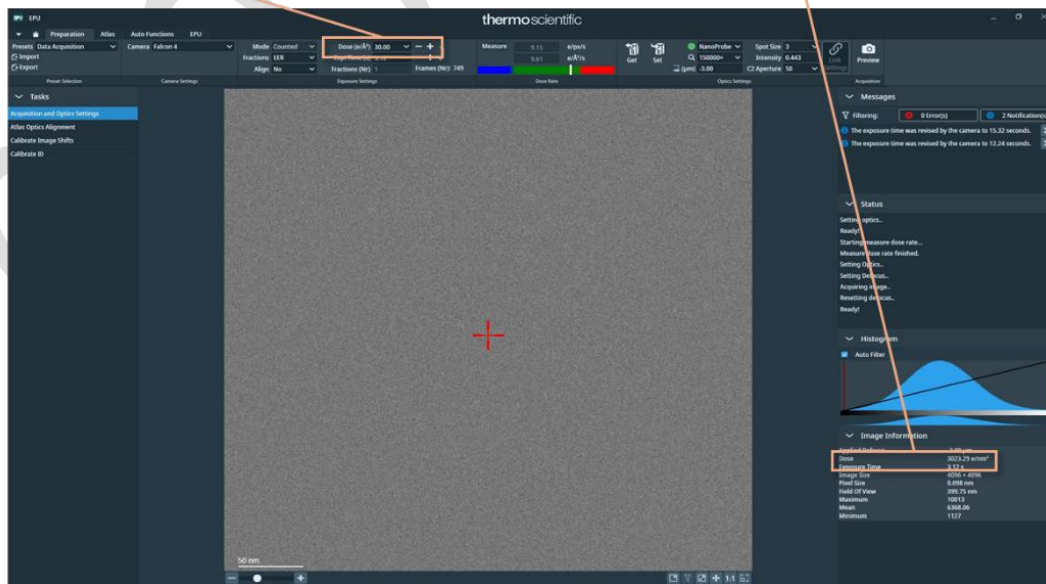
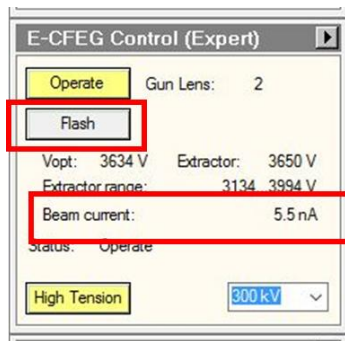


IMAGE DOSE IS LOWER THAN THE EXPECTED VALUE.

- Thick ice/sample may hinder electron passage.

IMAGE DOSE IS HIGHER THAN THE EXPECTED VALUE.

- Redo dose measurement at an empty space.
- If the beam current is below 5 nA when you measure dose, it may miscalculate the dose. Therefore, before measuring dose, check if the “beam current” at UI. If the value is smaller than 5 nA, click “Flash” and then measure dose again.



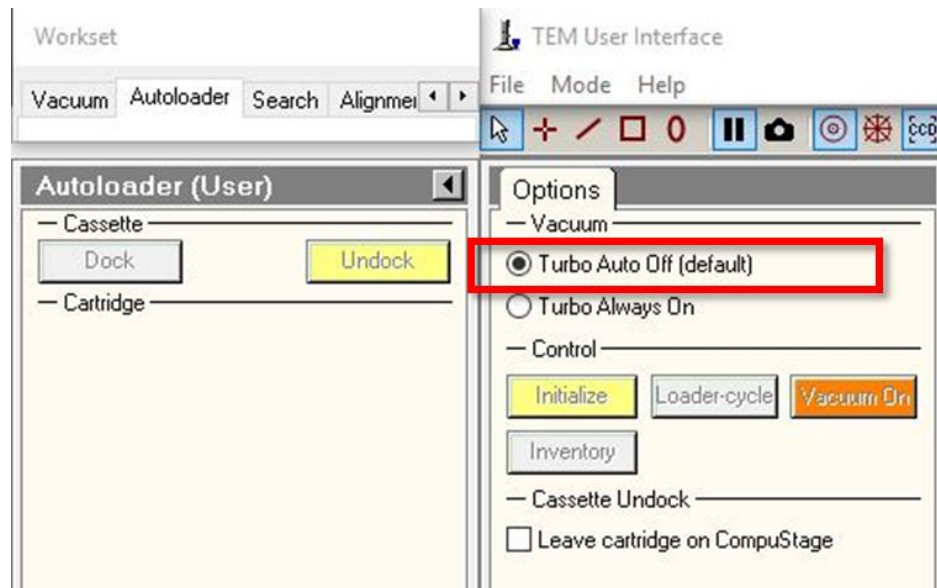
Q: HOLES CANNOT BE ACCURATELY IDENTIFIED WHEN PREPARING SQUARES.

- Adjust hole size and distances,
- It may be due to poor quality of the sample preparation. You can try to increase the exposure time at GridSquare to enhance the signal.
- Report software bugs if necessary.

IMAGE ACQUISITION

Q: IMAGES ARE BLURRY DURING ACQUISITION.

- Check autofocus.
- Check then ring circularity. Ensure “Turbo Auto Off”.



Q: I HAVE COLLECTED SEVERAL IMAGES AND THEY WERE GOOD AT THE BEGINNING. HOWEVER, THE IMAGES TAKEN AFTER A FEW HOURS WERE OUT OF FOCUS.

- The grid may not be smooth enough, causing the eucentric height at that square to be incorrect.
- Therefore, when checking squares in the EPU setting, pay attention to the "z" value at UI. If the Z value has changed significantly compared to the previous square, it may result in incorrect focus.
- You can reposition this square and perform "Autoeucentric" again. Make sure it is successful. Then, move to the EPU square and capture a new image, applying the new eucentric height to this square.

Q: FINAL IMAGE DIFFERS FROM THE OBSERVED ONE DURING PREPARATION.

- The defocus set at DataAcquisition the preparation tab may be different from the defocus set in EPU.

Q: IMAGE ACQUISITION TIME IS LONGER THAN EXPECTED.

- Review exposure time. If the area chosen for measuring dose may not be empty area. Lower dose detected will increase the exposure time. In this case, you can measure the dose again at empty space.
- Review drift measurements area. If you have added drift measurements at the template definition, it will take longer time.
- Review setting at Autofocus area. If the recurrence is not “After centering”, it may take longer time.

