

# TROUBLESHOOTING GUIDE (200KV)

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

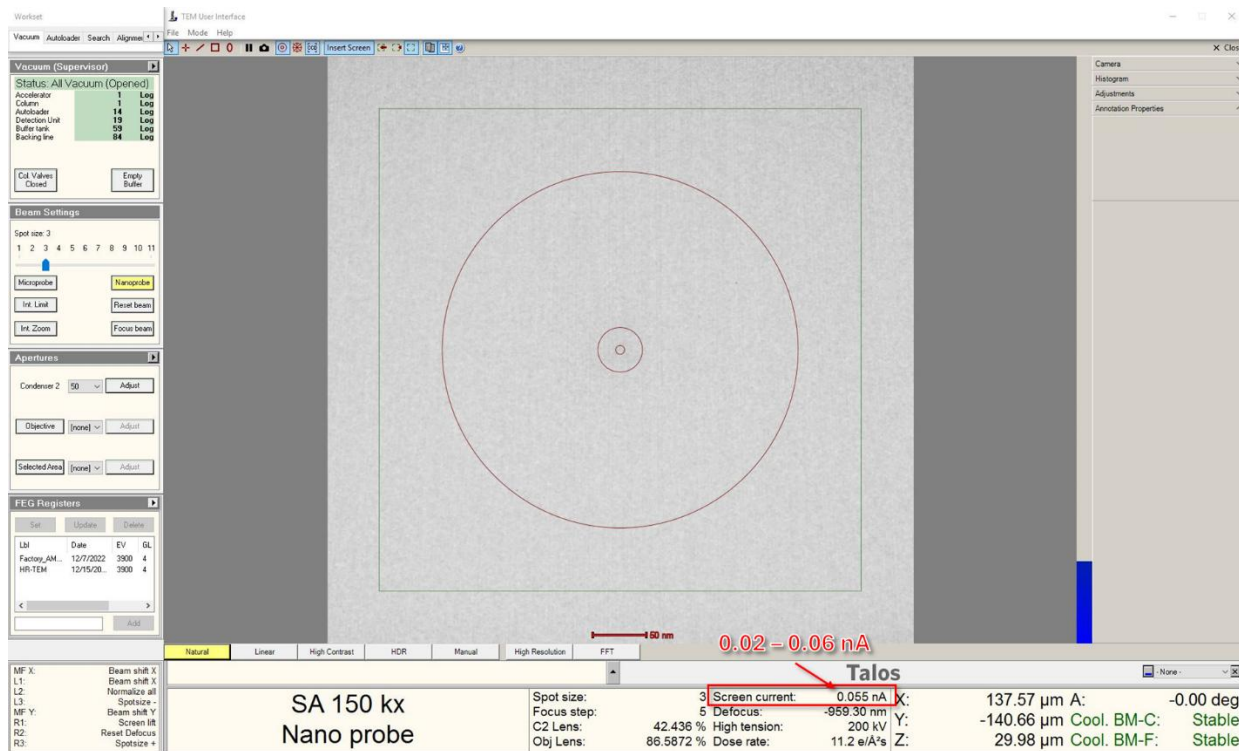
Happy hunting for Cryo-TEM images!

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## PRE-SET FILE SETTING

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

- The primary option would be using another pre-set file directly.
- 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 observable area and check the Screen current is within 0.02 – 0.06nA



- Changing from low magnification to high magnification usually has lower risk.
- When reducing magnification, monitor the Screen Current. Change the intensity to reach appropriate Screen Current (0.02-0.06nA) if necessary.
- Adjust intensity or spot size after changing magnification to maintain a parallel beam (for the magnification of data acquisition).

Spot Size	C2 Value (Nanoprobe)	EPU – Intensity
3	42.438%	0.443
4	41.114%	0.446
5	39.908%	0.448
6	38.964%	0.449
7	38.378%	0.452

- 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.

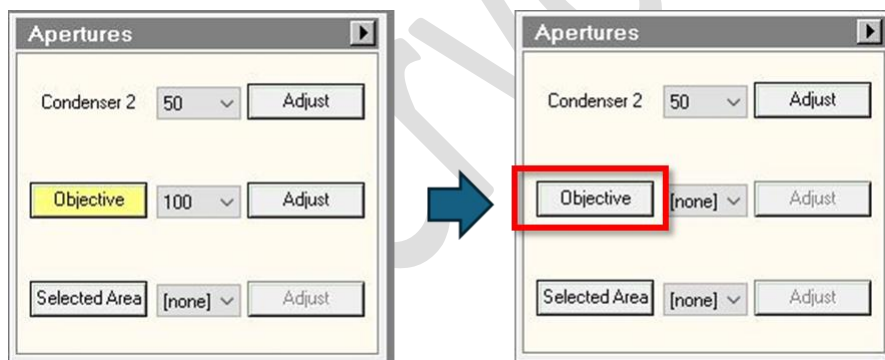
Step 2: Adjust the intensity / spot size and make sure the beam size is covering the whole area. Make sure the Screen Current is within the safe range (0.02-0.06nA).

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.



**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.

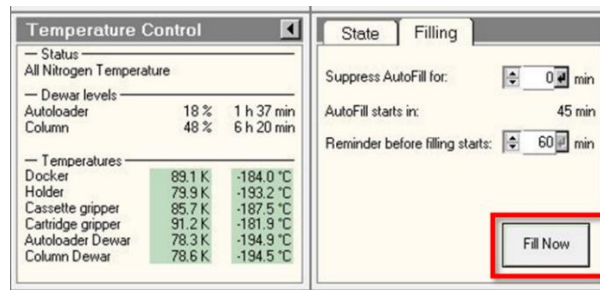
**Q: WHAT IS THE POSSIBLE REASON FOR AUTOFOCUS FAILURE?**

- 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**

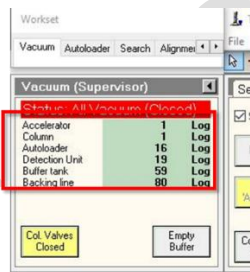
Q: THERE IS AN ERROR AT UI ABOUT NOT HAVING ENOUGH LIQUID NITROGEN.

- Click "Fill now" to refill liquid nitrogen.



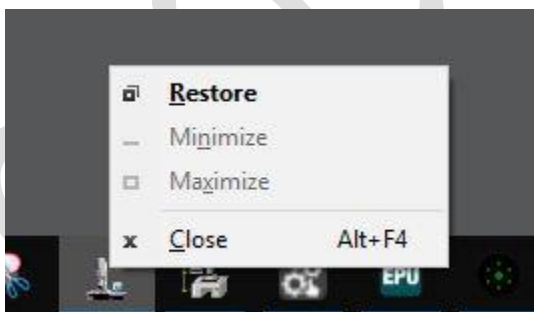
- If the level remains low, inform staff about insufficient LLN in the tank.

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



Q: THE WINDOW OF UI DISAPPERAED. WHAT SHOULD I DO?

- Press **SHIFT** on the keyboard and right-click the icon at the tool bar.
- Click **Restore**.



SETTING UP EPU

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

- Perform image calibration.

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

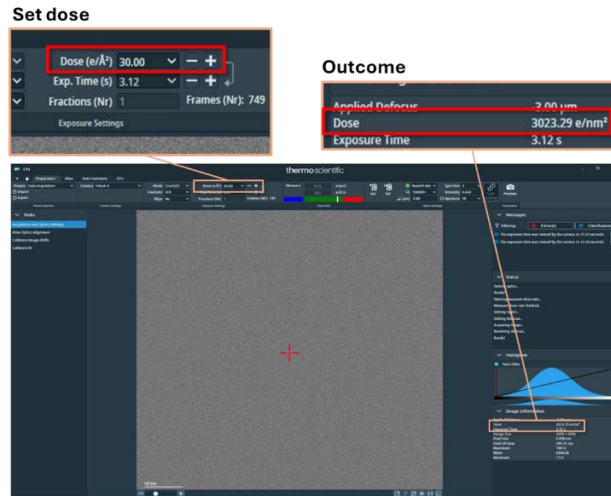


IMAGE DOSE IS LOWER THAN THE EXPECTED VALUE.

- Thick ice/sample may hinder electron passage.

IMAGE DOSE IS HIGHER THAN THE EXPECTED VALUE.

- Redo dose measurement at an empty space.

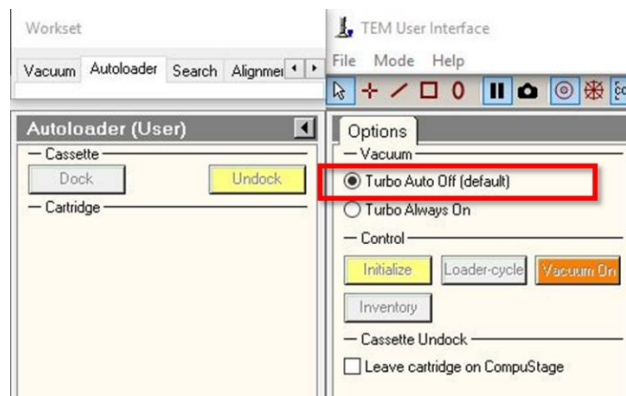
Q: HOLES CANNOT BE ACCURATELY IDENTIFIED WHEN PREPARING SQUARES.

- Adjust hole size and distances, or report software bugs if necessary.

## IMAGE ACQUISITION

Q: IMAGES ARE BLURRY DURING ACQUISITION.

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



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

- Check defocus at the preparation tab.

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.

