



- 1) Load the cassette/into the microscope. In the 'Autoloader' tab in TEM User Interface (TUI), wait for all the temps to go green, click on 'Inventory', and name the grids accordingly in the meantime.

**>> Grid switching for data collection/screening:**

- 2) Stop the data collection if it is ongoing.
- 3) On the EPU terminal go to the Presets of 'Atlas' magnification ('Preparation' Tab) and click 'Set' to send the presets to TUI in the Microscope (Scope)
- 4) In the TUI, insert the fluorescent screen (Fluo-screen), Reset Holder, and close the column valve
- 5) In the Autoloader tab, select the grid of choice and click **load** (takes 1-2 mins to load a new grid)
- 6) Once the grid is loaded, open the column valve
- 7) Move the stage around each quadrant to check the grid for ice thickness on grid squares quickly on the Fullscreen
- 8) Move around the center of the grid and retract the Fullscreen
- 9) On the EPU terminal click **Preview** at the Presets of 'Atlas' magnification
- 10) Change to the Presets 'Grid-square' magnification on the EPU terminal and click **Preview** to acquire the image
- 11) In the same image, move stage to the center position of the image, change Presets 'Hole/ EucentricHeight' magnification and click **Preview**
- 12) At the same Presets 'Hole/ EucentricHeight' magnification perform **Auto-functions:**
  - i) **Auto Eucentric by beam tilt and ii) Autofocus**, alignments by selecting and clicking start. Repeat them if they fail.
- 13) Move stage to the carbon region (between 4 good ice holes) and acquire it
- 14) In the 'Preparation' tab in the EPU, select the Presets 'Data Acquisition' magnification and send it to the Scope, by clicking 'Set'
- 15) **Press Eccentric Focus** on the microscope control panel. Perform **Direct Alignments** on TUI (TEM User Interface): Beam shift and Beam tilt ppX and Beam tilt ppY. Perform Beam Shift again if the beam needs to be centered  
Note: Perform Center ZLP and tune GIF, if you notice the beam being blocked by the slit
- 16) In TUI, retract the Flu screen, and do **Sherpa alignments (Auto CTF)**: Objective stigmatism /Coma (measure and correct). Finally, measure **objective stigmatism** to check for reasonable defocus values. Minimize the Sherpa window. (Optional: **get** values from scope to EPU for Data Acquisition, Auto Focus, and Drift measurement (all at data magnifications), if needed)
- 17) In the EPU terminal, find detectable ice contamination (**IC**) (in grid square magnification) for **Image shift Calibration**. Adjust the crosshair position w.r.t. **IC** in Atlas, GridSquare, Hole Eucentric, and Data Acquisition Mags. Previewing at each magnification.
- 18) In the Preparation tab: Select '**Calibrate image shifts**' and then at the Data Acquisition magnification, **start calibration**. Double-check the exact position on the ice, **adjust (double click), Reacquire, and Proceed**.
- 19) Under the 'Preparation' tab go back to 'Acquisition and Optics settings'
- 20) **Reset XY on TUI**

- 21) **Acquiring Atlas:** Click on the 'Atlas' tab: **Session setup** → **Start a new session then click on screening. Check the box for the grid (indicated by a green dot under the screening section)** to enable 'Start'. Click on 'Start' to collect Atlas tiles (It takes ~ 10 minutes to collect 5\*8 tiles). Note: Wait till colored tiles appear and unselect boxes for all the colors
- 22) Find and move the stage to the broken square at the 'Grid Square' magnification, change the Presets to 'Data Acquisition' mag, and click 'Set'. On the Gatan terminal, click on '**Tune GIF**'. After the successful tune GIF, make sure CDS is checked. In case **Tune GIF** fails, reduce the spot size (to a lower spot size of 3 from the usual spot size of 5) and redo **Tune GIF**.
- 23) Gatan Terminal: Under the 'Camera' Tab, click on '**Prepare Gain Reference**'. Change the spot size in the TUI to match 760 e-/pix/s (spot size ~2) in the first step and 7.5 e-/pix/s (spot size 5/6) in the second step and adjust the illumination to match 760e- or 7.5e-, respectively. This is for C1, C3= 2000, C2= 50 settings. Adjust the illumination area with the intensity knob (Note: illumination area **SHOULD NOT** be smaller than the green circle in the Fluscreen).

**Q. How to ensure that Gain Reference is all good?**

Once you finish the Gain collection, select 'Gain normalized' in the GMS setting instead of 'Unprocessed' and capture the image at 'Data magnification' in the broken grid square. Then, hit Ctrl+F to get the FFT image. This image should not have any lines on it. Remember to revert back to select 'Unprocessed' before the data collection session.

- 24) 'EPU' tab in EPU terminal: Session creation, square selection, run AutoEucentric (for each grid square), holes selection, template definition and template execution, and finally automated acquisition.
- 25) Check the defocus values in template selection (EPU window): Usual set values: -0.75, -1.0, -1.25, -1.5, -1.75, -2.0, -2.25, -2.5 (You can change the defocus values, as needed)
- 26) (Optional or when needed) To adjust the slits manually, in the 'Filter Control section, click "adjust" on the Gatan monitor and use the up/down arrow keys on the (Gatan) keyboard. Find a median value between two dark regions and set it to zero.

\*\* Monitor for 1hr after starting the automated acquisition for any problem with slits moving and any possible errors that might come up.

Good luck 🍀

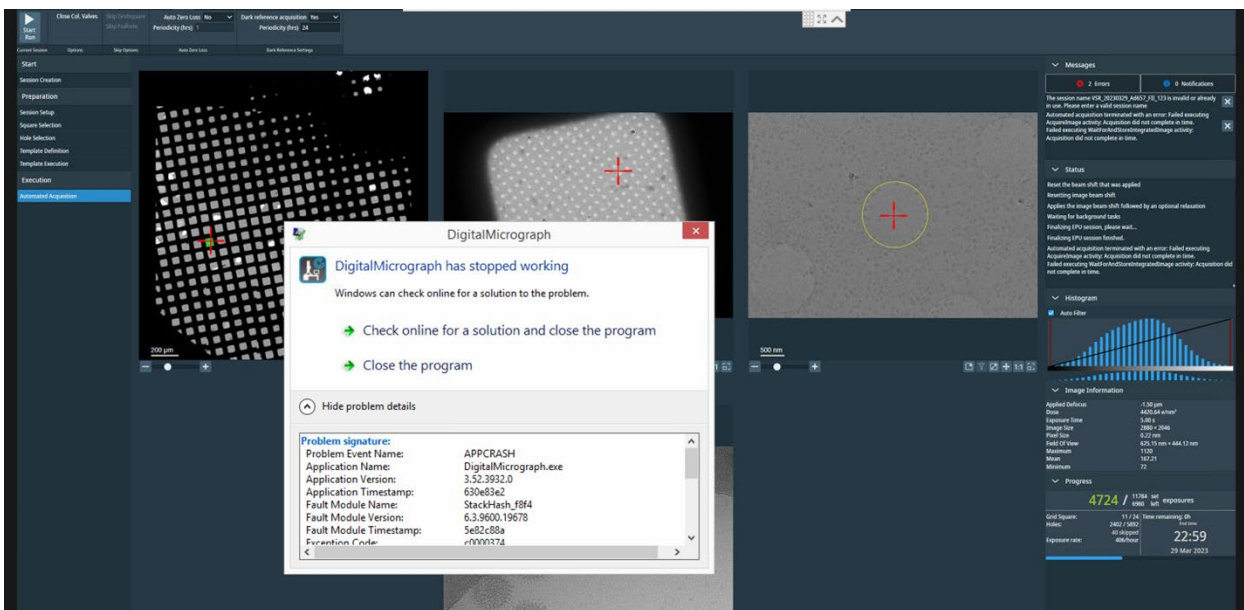
Checklist:

- Load the grid
- Perform Autofunctions (Autofocus & Autoeucentric by beam tilt)
- Check for Direct Alignments
- Run Sherpa (Auto-CTF corrections)
- Calibrate Image shift
- Atlas tiles acquisition

- Tune GIF and Gain Reference Collection
- EPU session setup for automated data collection
- Optional: Adjust the slits when needed.

## Troubleshooting

### 1.1 GMS (Gatan Microscopy Suite) crashes with DigitalMicrograph has stopped working due to a timeout error on EPU OR GMS stopped responding but showed no error message in EPU/TUI



- 1) Close the column valves
- 2) In the Gatan terminal, if the system allows, retract the K3 camera. Close the Digital micrograph program, which results in the closing of the GMS program
- 3) Wait for 3-4 minutes to allow sufficient time for GMS to close completely (GMS is a heavy program and needs a few minutes to close completely)
- 4) Restart the GMS3 program by double-clicking on the GMS3 icon on the Desktop of the Gatan terminal. Patiently, wait for it to open (takes 3-4 minutes to open completely)
- 5) Once the GMS3 is started, click/open the TEM program on GMS3/DigitalMicrograph (Gatan terminal)
- 6) Insert the K3 camera
- 7) Open the Column Valves (TUI terminal) and on the EPU window (select and move the stage to a square in the Atlas (EPU terminal)).
- 8) Make sure the stage is centered on a broken film square at various mags. by previewing. The previews would be clear/white except in the preset of Data Acquisition

magnification (**DA\_mag**), in which the preview might result in a dark image because of the slits blocking the beam.

- 9) When in **DA\_mag**, capture an image on the Gatan terminal. This also would be dark as the slits will be blocking the beam.
- 10) To make sure that there is the beam in the center of the green circle, in the broken film, in the TUI, go to Direct Alignment and perform beam shift and ppX and ppY correction
- 11) Then do "Tune GIF" to adjust the slits to the correct position
- 12) Once the "Tune Gif" is passed, check and make sure the CDS mode and unprocessed images are selected on the Gatan window.
- 13) Then on the EPU window/tab restart the automatic data collection and make sure that the images are acquired correctly
- 14) In case, you have to close the EPU completely, no worries. Relaunch the EPU. In the EPU terminal, go to the EPU tab and click 'Automated Acquisition'. It should recall all the conditions before it was closed. You can click the play icon for 'Start Run' to resume automated data collection.