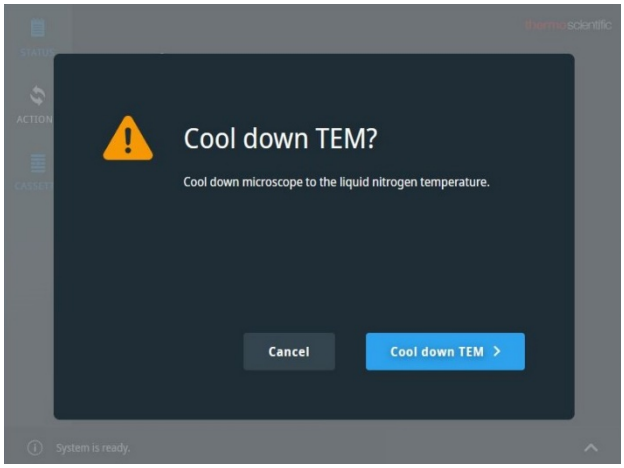
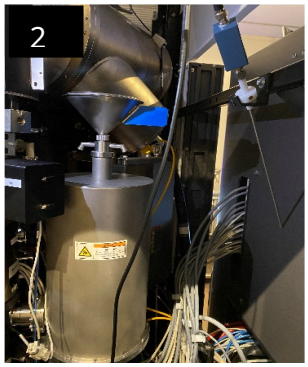
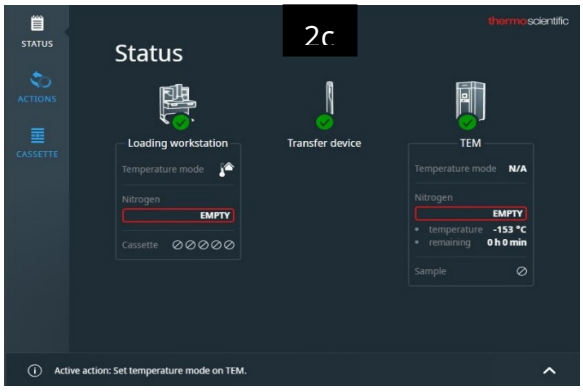
Tundra Quick Start

Sample Loading and Unloading



Once the cryocycle is finished, prepare the Microscope for use.
On System Display (OSD) ⊳ Actions ⊳ Cool down TEM

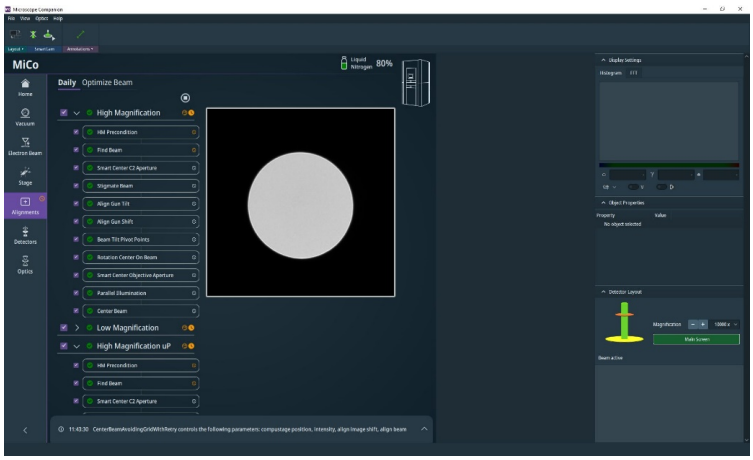




Start filling the TEM Dewar (this takes about 20 minutes). User alignments can be initiated before completion of the fill.
Temperature drop can be monitored:
TEM OSD ⊳ Status ⊳ TEM ⊳ Temperature

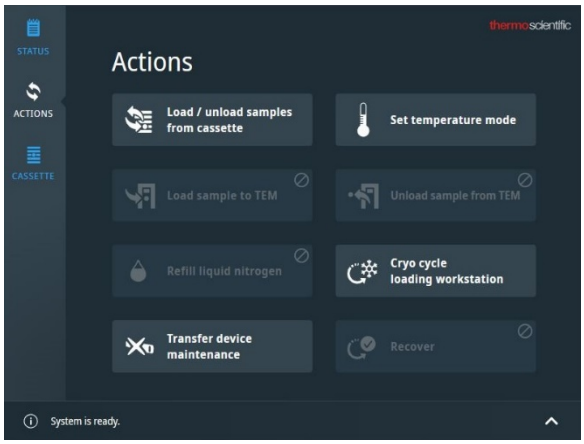
Five minutes into the filling procedure, one can start the “User Alignments” protocol from the Microscope Companion software (MiCO).

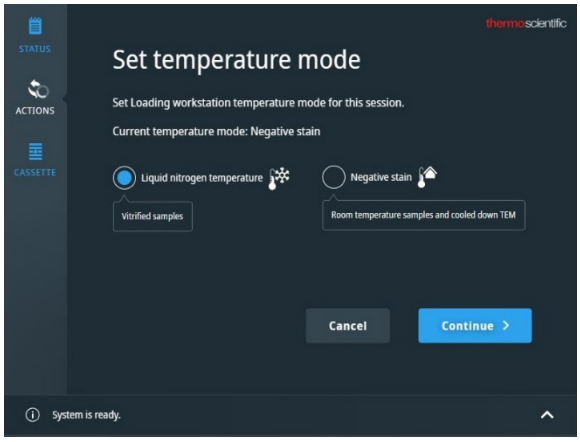
MiCO ⊳ Alignments ⊳ User Alignments.



The three sets of alignments: High Magnifications—µP (microprobe) and nP (nanoprobe), and Atlas/GridSquare—LM (low magnification) will be completed automatically. This procedure takes about 30 minutes.

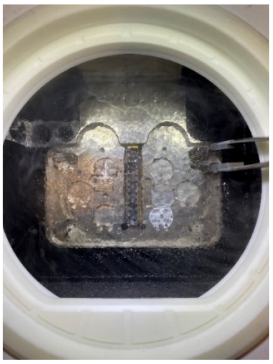
Once the Dewar is full insert the dry sensor (if need be use a hair dryer and wipe it clean) back into the Dewar gradually. When the sensor is returned MiCO will display the percent fill level of the Dewar.

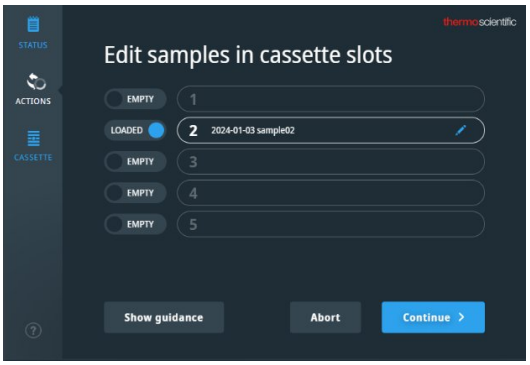




In CryoLoading Station (CLS) OSD ⊳ Actions ⊳ Set Temperature Mode ⊳ Liquid Nitrogen Temperature ⊳ Continue.

Open the valve on liquid nitrogen (LN_2) tank and start to fill the CLS (or one may fill manually by pouring LN_2 into the CLS chamber directly). Fill it to the sample manipulation line for better visibility.

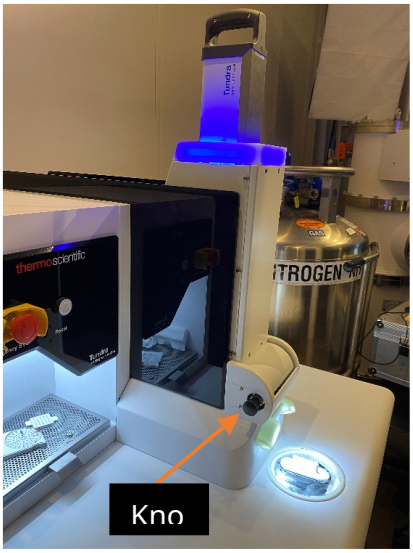




Now load the grids.

CLS OSD ⊳ Load/unload samples from cassette.

The cassette moves out into the loading chamber. Transfer the autogrid(s) to the cassette so that the flat side of the C-clip ring faces away from you. Slots 1 through 5 are accessible to TD. Edit the grid information and click continue. The cassette will retract to its home position.



After loading the grids, top off LN_2 to "Sample Transfer" level. This is required to cool the Transfer Device (TD) to -196 °C.

Now you are ready to load a sample to TEM.

CLS OSD ⊳ Actions ⊳ Load sample to TEM ⊳ Select the sample to be loaded (any one from the 5 slots). This process takes about 8 minutes and during this time TD evacuates, cools down, and picks up the selected grid. The OSD on CLS shows the progress and prompts the user to detach TD from CLS and attach TD to TEM to complete the sample transfer.

At this point (will show count down time of 1 minute on the CLS OSD)—pull back the black knob outwards ⊳ move the transfer device downwards ⊳ insert the transfer device into the tunnel on TEM. Hold the transfer device for a few seconds to ensure that the airlock is engaged (pay attention to the pump's decrescendo).

Once the sample transfer to the TEM stage is complete another 1 minute countdown will begin. Return TD to CLS—pull the black knob out and move TD upwards to its original position. Once all the way up, the TD will be pumped with dry nitrogen to keep it contamination-free and ready for the next sample.

Important Notes on Tundra TEM Operation

Column Valve: Always close the column valve when the microscope is not in use or in standby mode (this can be easily done using Mico).

TEM Cryocycle: After completing the data acquisition, initiate cryocycle from TEM (OSD ⩥ Actions ⩥ Cryocycle TEM). It is a good practice to perform cryocycle at least once a week. Take the fill-level measuring probe out of TEM Dewar during cryocycle.

CLS Cryocycle: Whenever you use CLS under cryo-conditions, it is imperative to cryocycle CLS after use (**never let LN_2 just dry up and lead to moisture accumulation which will damage CLS**). However, if the CLS was used for room temperature samples, then the cryocycle is not necessary until the message pops up at the OSD or EPU microscope tab.

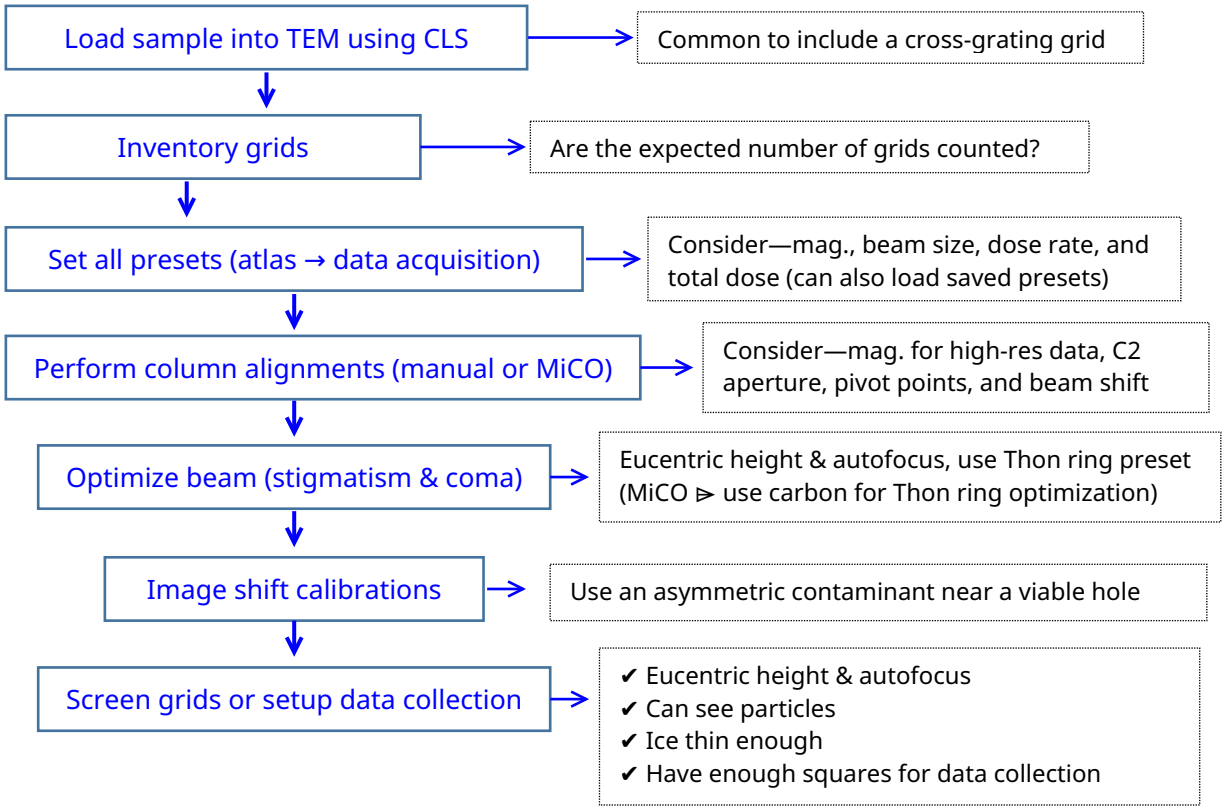
Cleaning TD: Transfer Device (TD) should not require a daily cleaning. DO NOT touch the O-ring area and DO NOT try to clean it with paper tissues. Only the *facility manager* or a Thermo Engineer may clean the TD.

Daily Alignments: Use EPU to perform alignments daily. Make sure there is no grid on the stage and perform alignment either using MiCO or by pressing the recovery button in EPU. Remember to close the column valves after this procedure.

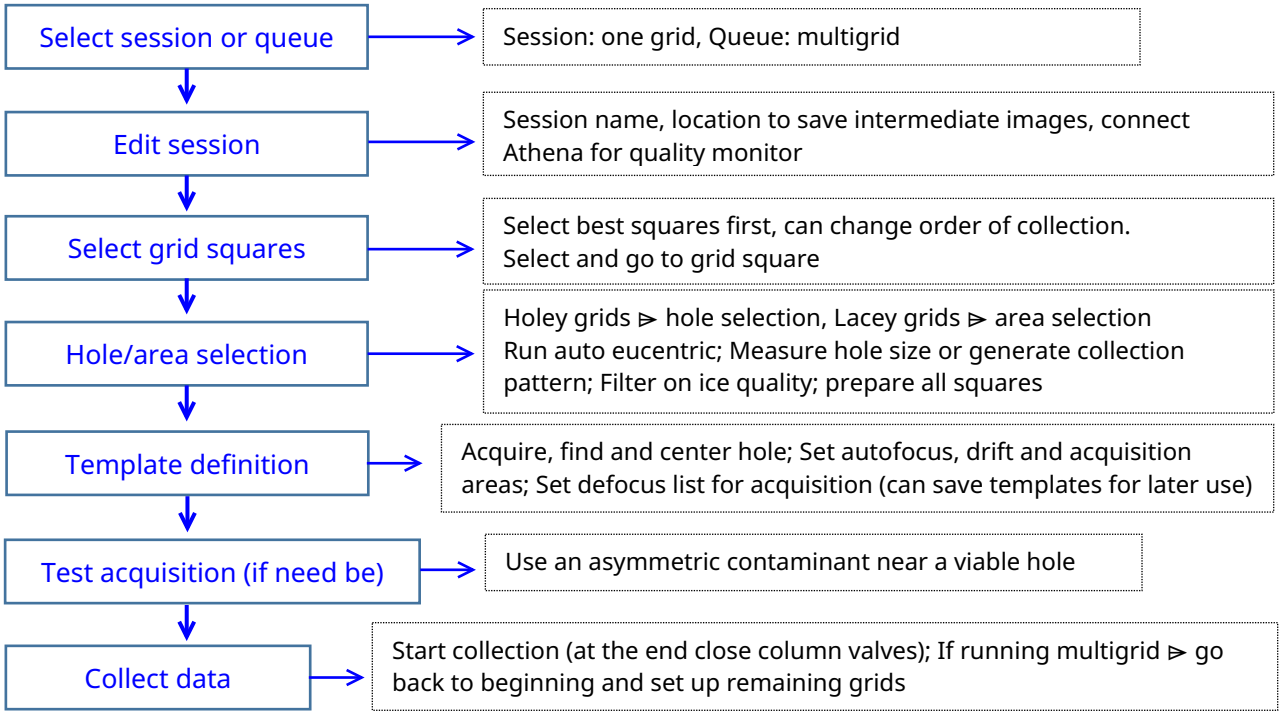
Performing beam optimization: When performing beam optimization it is important to use carbon grids and this procedure should never be performed using Au-foils or any other grids that do not exhibit Thon rings. Also, ensure that you are in the carbon area (not in the grid bar, empty, broken, or hole area) and have proper eucentric height before performing beam optimization.

Data Acquisition: It is highly recommended that automated data acquisition be carried out using EPU.

Tundra Cryo-TEM Operations Cheat Sheet

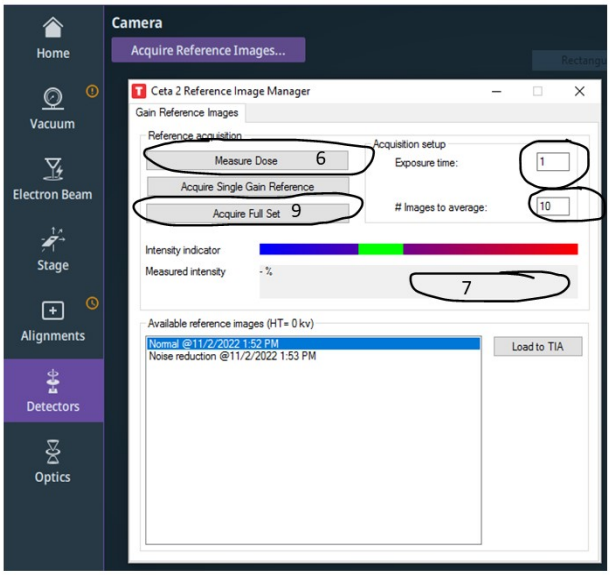


EPU Data Collection Cheat Sheet



Gain Correction in Tundra

1. Perform microscope alignments
2. Go to your data acquisition preset/optics settings
3. Go to an empty area or broken area
4. At the Mico/Apollo window, click ‘Detectors’ tab ⊳ click Acquire Reference Images…
5. This will open a new window: ‘Ceta 2 Reference Image Manager’
6. Click ‘Measure Dose’.
7. This will give the measured intensity (dose). Any dose rate between 10 and 100 electron/pixel/second is good.
8. Calculate exposure time X # Images to average X measured intensity. The resulting value should be around 3000. (e.g. 1.25 (exposure time) × 170 (images to average) × measured intensity (14.13) = 3002.
9. Click ‘Acquire Full Set’.
10.



Step 6= Measure dose

Step 7= Give you measure Intensity

-Exposure time and measure intensity should be similar to the one that is shown in EPU. (Adjust the exposure time is needed

-Change the images to average in such a way that the value of the result should be around 3000.

Step 9=acquire Full set

Objective aperture (μm)	Resolution cutoff (Å)
100	1.7
70	2.43
60	2.84
50	3.4
40	4.26
30	5.67
20	8.51
10	17.02