Common Screening with EPU Standard Operating Procedure

version 1.2

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1. Purpose

1.1. Screening cryo-EM grids with EPU software (ThermoFisher Scientific)

2. Definitions:

2.1. EPU is an automated data collection software from ThermoFisher Scientific (TFS).
2.2. TEMUI is the user interface of the microscope
2.3. TEM Image Analysis (TIA) must be running to ensure EPU is communicating with TEMUI
2.4. Imaging Presets in EPU:

2.4.1 Atlas
a. Create a grid map with stitching image tiles at low dose
b. Optic and camera settings are relatively fixed for each microscope

2.4.2 Grid Square
a. Depends on mesh size of the grid (can see one grid square)
b. Illumination covers the entire grid at a small dose rate.
c. Linear mode or counting mode (camera setting) with binning x2.

2.4.3 Hole/Eccentric Height
a. Field of view covers 3~ 4 holes.
b. Illumination covers ~ 15um at a small dose rate.
c. Linear mode or counting mode (camera setting) with binning x2.

2.4.4 Data Acquisition
a. Beam diameter meets a parallel illumination and fringe free conditions.
b. Dose rate is within camera sensitivity range.
c. Beam edge doesn't reach adjacent targets.
d. Exposure time is not too long to achieve the required dose (~ 50 e/A^2, depends on sample).
e. Electron counting mode with dose fractionations.

2.4.5 Auto Focus
a. Same as Data Acquisition with shorter exposure and 2x binning.

2.4.6 Thon Ring
a. Same as Data Acquisition with 2 s exposure and 2x binning.

2.4.7 Drift Measurement
a. Same as Data Acquisition except with shorter exposure and binning.

2.4.8 Zero Loss
a. Same as Data Acquisition except with shorter exposure and binning.

3 Supplies & Equipment

❑ Microscope

❑ Software: TEMUI, TIA, EPU

4 Procedure:

4.4 <u>Microscope and Software Checks</u>

4.4.1 Microscope's states are within specifications such as vacuums, HT, emission and temperatures.
4.4.2 Stop Data Acquisition of the previous session on **EPU** and close column valves.
4.4.3 Cassette with grids have been loaded (see Loading SOP)

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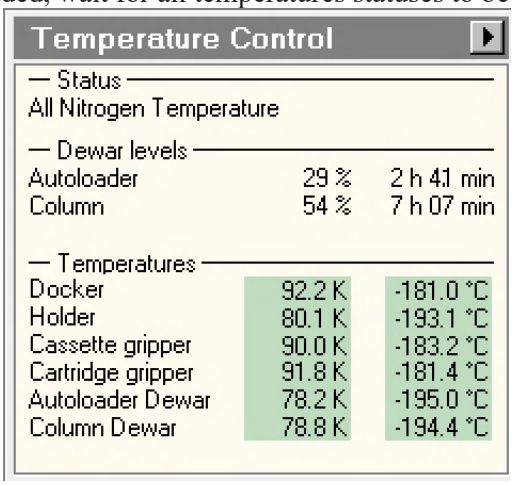
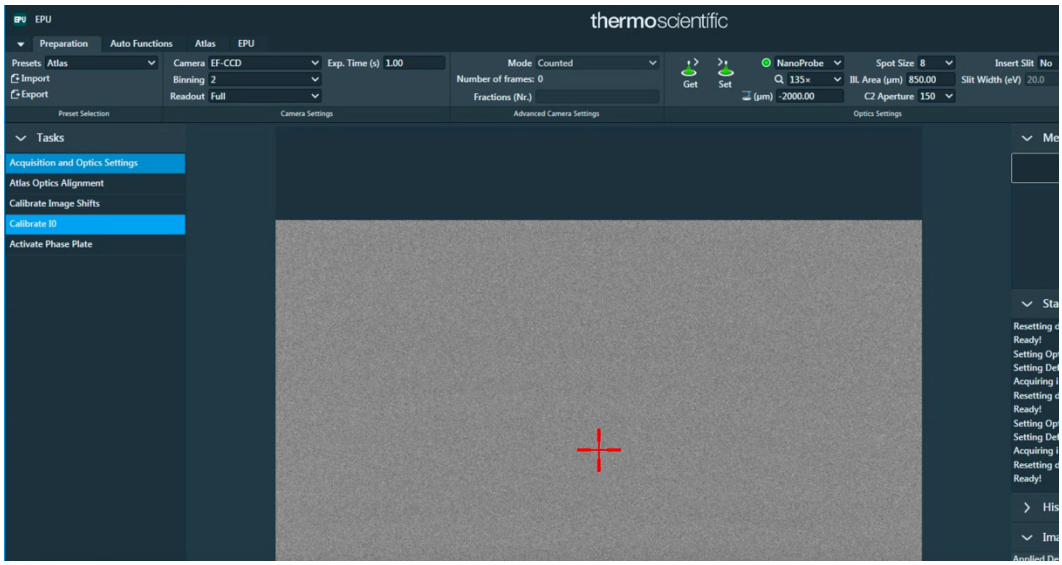
4.5 Making Grid Atlases with EPU

4.5.1 After grids are loaded, wait for all temperatures statuses to be green AND <-160C



4.5.2 Select **Inventory** on the **Autoloader** panel and wait till the loaded grids have been detected correctly in the cassette

4.5.3 Open **EPU** software, click **Preview** to take an image using **Atlas** preset (Column valve should be open)



4.5.3.1 **IF** there is a beam shift, insert FluScreen. In **TEMUI Tune** tab, go to subsection **Direct Alignments** then select **Beam shift**. **DO NOT TOUCH GUN TILT AND GUN SHIFT.** Proceed to 4.2.4 if no beam shift adjustment is needed

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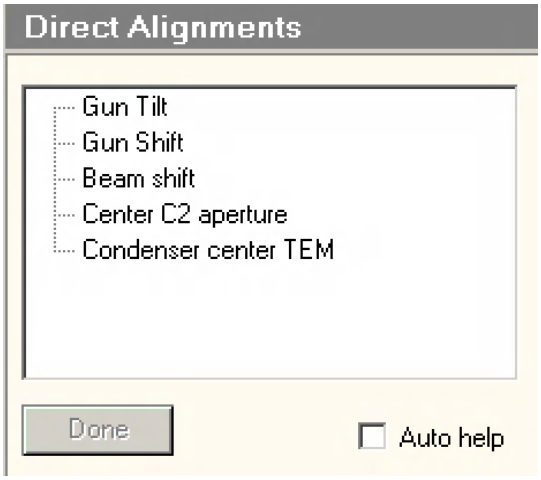
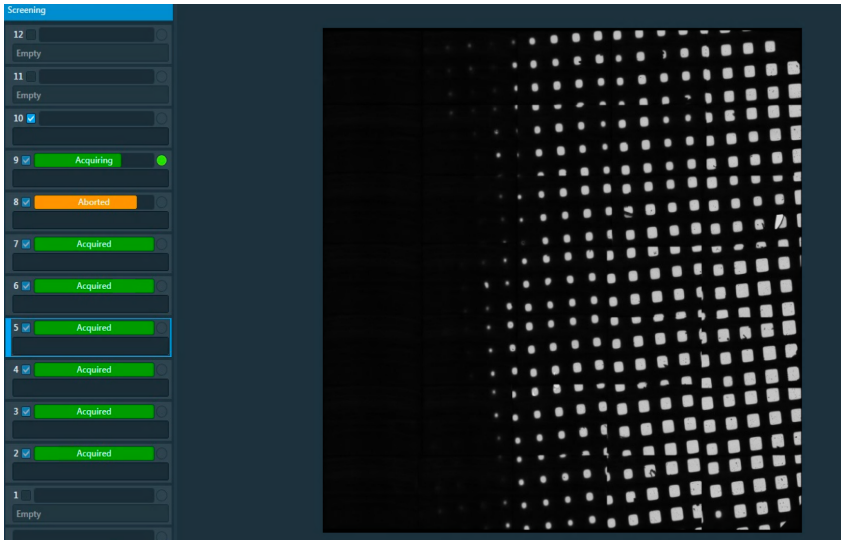


4.5.3.2 On the hand panel, use **MF-X** and **MF-Y** knobs to center beam over the green circle.
4.5.3.3 Click **Done** after adjustment. Press **R1** to lift the screen
4.5.3.4 Take another **Preview** to ensure the beam is centered

4.5.4 Select the **Atlas** tab in **EPU** > **Session Setup** task
4.5.5 Select **New Session**, and enter a Name for the session: Name should be yearmonthday-projectnumber-atlas, for example 20210414-CT21-atlas
Image format = MRC, Output folder = X, click **Apply**
4.5.6 In the **Screening** section, check the cartridges with grids and select **Start** to begin. It'll take 10-15 minutes to map each grid

4.6 Screening Grid Squares

4.6.1 After Atlasing, evaluate ice thickness and grid quality. A good grid should have at least three quarters of thin area



4.6.2 Load a grid of interest by clicking on the slot number and selecting **Load from sample**
4.6.3 When loading is done, pick a square (usually a thinner square). Right click on the square, then select **Move stage here**
4.6.4 Go back to **Preparation** tab, select **GridSquare** preset and take a **Preview**. Adjust the magnification so that you only see one square without much cutoff
4.6.5 Go to **AutoFunctions**, select **Hole/EucentricHeight** preset and run **Auto-eucentric by beam**

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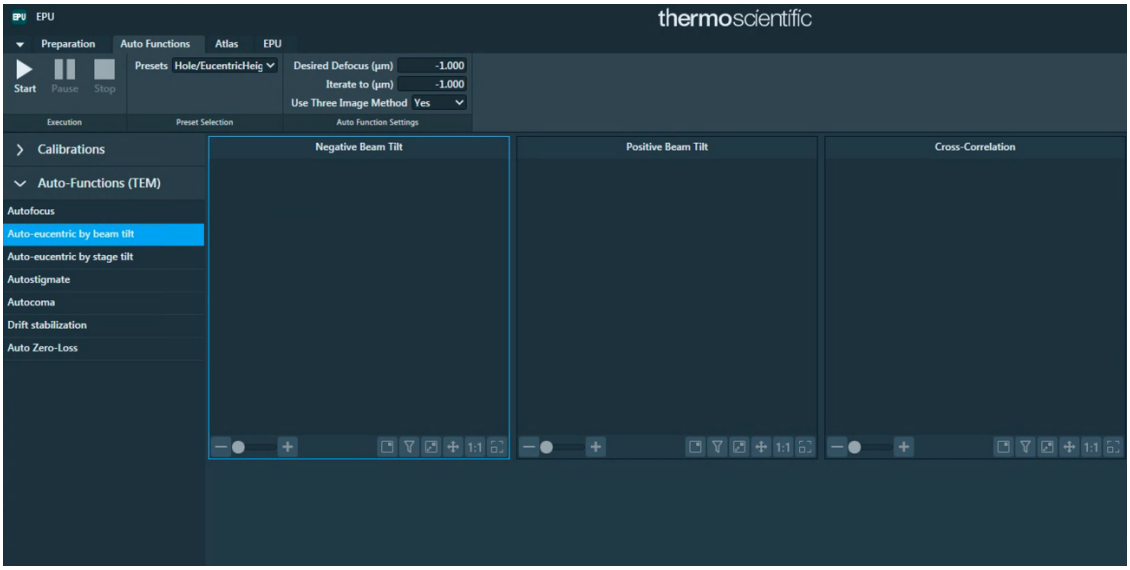
tilt to find the eucentric height

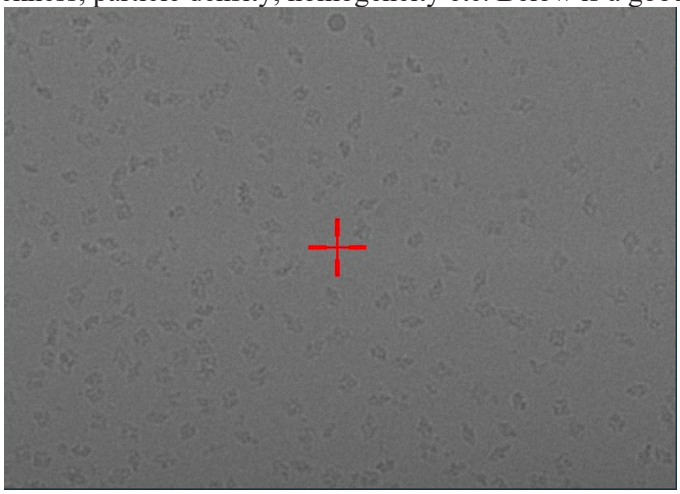


4.6.6 Go back to **Preparation** tab and take a preview at **Hole/EucentricHeight** preset

4.6.7 Move stage over a hole and capture at **DataAcquisition** preset (**DataAcquisition** preset allows you to set up optics for data collection); Make sure the pixel size is adjusted to your sample

4.6.8 **Autofocus (optional)**: this is performed before capturing an image at **DataAquisition** preset. Move stage on carbon at **Hole/EucentricHeight**, and run **Autofocus** task with **Autofocus** preset under **AutoFunctions** tab

4.6.9 Evaluate ice thickness, particle density, homogeneity etc. Below is a good image:



4.6.10 Screen at least one hole on the same square and repeat 4.6.3-4.6.9 to screen other squares on the same grid. Load the next grid to screen if needed

4.6.10 Image Shift Calibration

4.6.10.1 After screening and all presets are determined, Image Shift Calibration is required for each session

4.6.10.2 On the atlas map, find a feature, such as a piece of ice contaminant on a square. Right click and select **Move stage here** to move the stage onto the square

4.6.10.3 Set Presets as **GridSquare**, and **Preview**

4.6.10.4 Move stage close to the feature and run **Auto-eucentric by beam tilt** with **Hole/EucentricHeight** preset under **Autofunctions**

4.6.10.5 **Preview** at **Hole/EucentricHeight** and make sure you can find the feature and center it in

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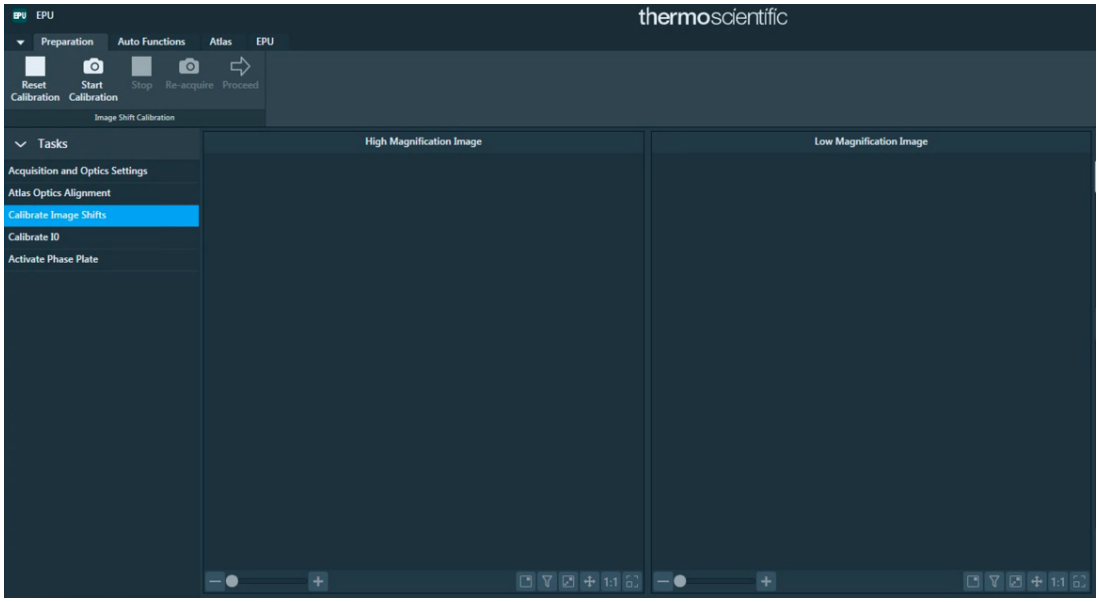
the image. If the feature isn't visible, you may need to insert the FluScreen and move the stage to find the feature

4.6.10.6 **Preview** at **DataAcquisition** preset and make sure the feature is visible. If not, you may need to insert the FluScreen and move the stage to find the feature

4.6.10.7 Select **Preparation** tab > **Calibrate Image Shifts** task, and click **Start Calibration**, and follow the instructions. Use **Proceed** to continue and double click on the feature to center it



5 Chemicals: N/A

6 Waste Disposal: N/A

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