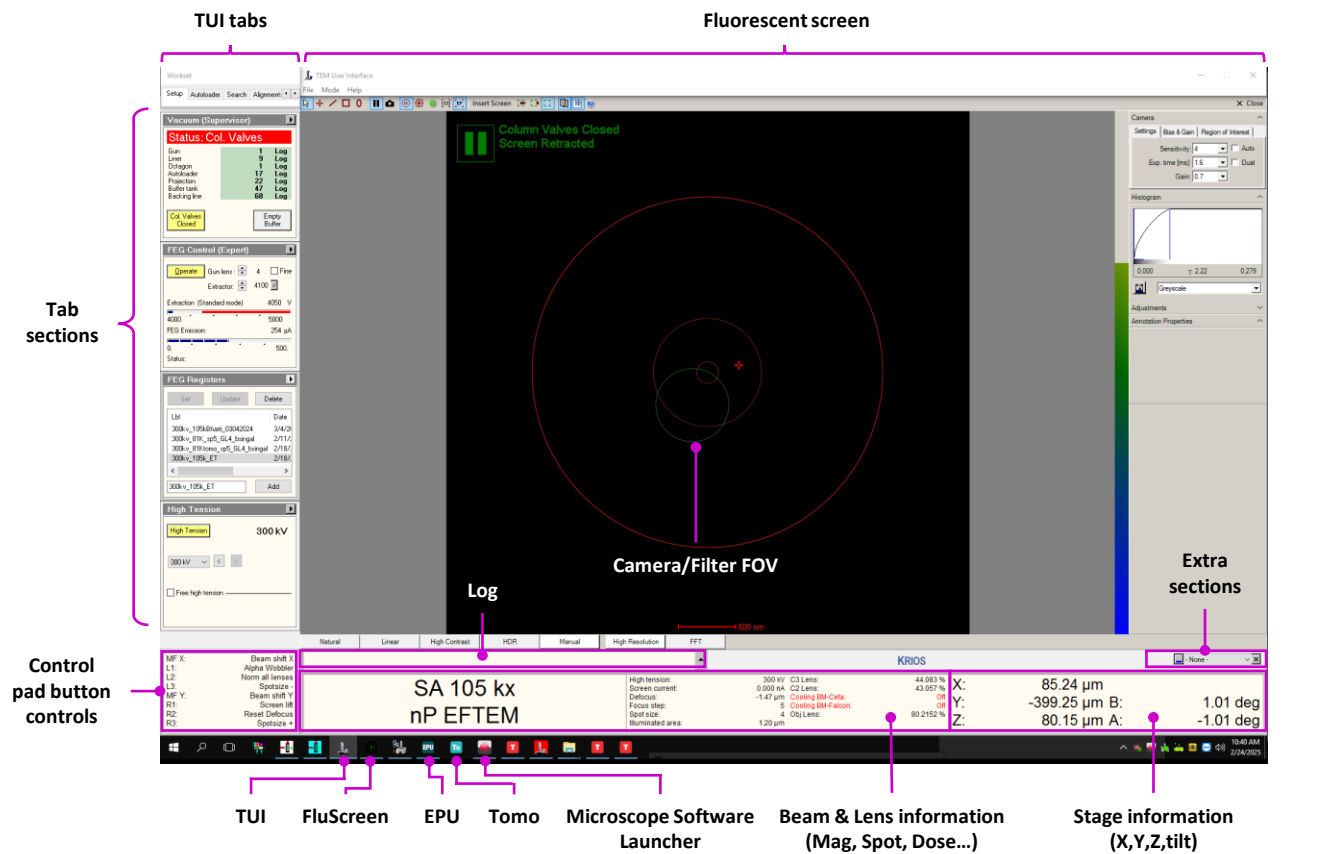


TEM1 Basic Protocol

Pictographic Version
TFS Titan Krios G2 + Bioquantum + Gatan K3

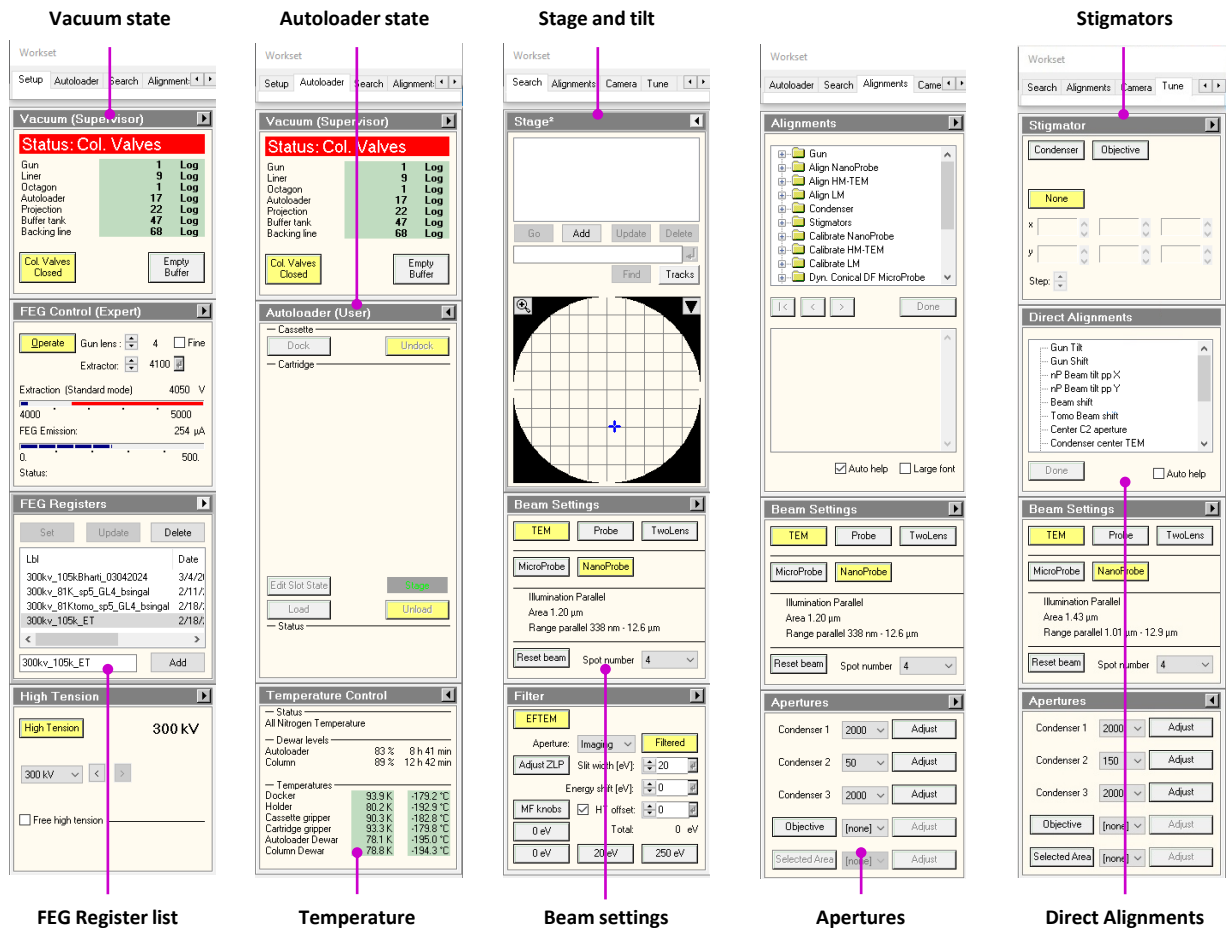
David Fernandez-Martinez, PhD
Stanford CryoEM Center (CEMC)
2026

The TEM1 User Interface (TUI)



TEM1 TUI Tabs and sections, in order

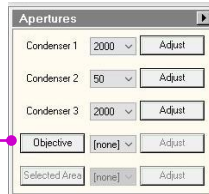
Digital Micrograph (Third Screen) -----●



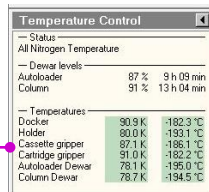
Initial Microscope Setup

1 Preliminary checks

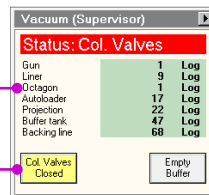
Objective aperture should be out



Temperatures should be around -180C



Optimal vacuum values

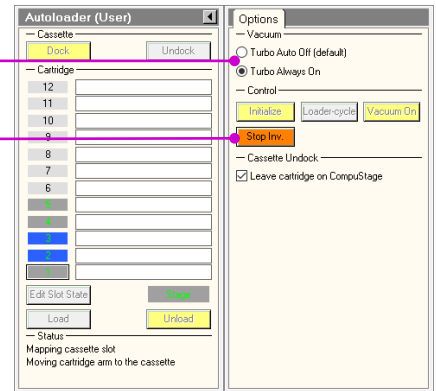


Column valves closed

2 Loading grids

Turn Turbo ON for Inventory and OFF after

Inventory will recognize loaded grids



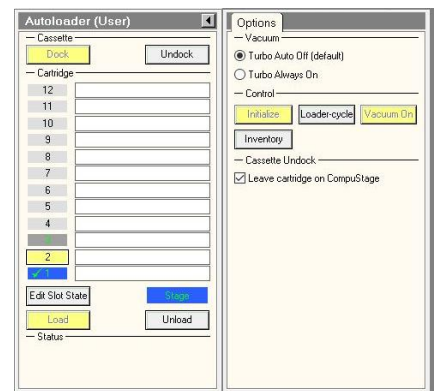
Grids should be recognized after Inventory

Blue: Slot with Grid

Gray: Slot without grid

Yellow: Grid on column

Load a grid (Preferably X-grating or carbon) to start setting up your session



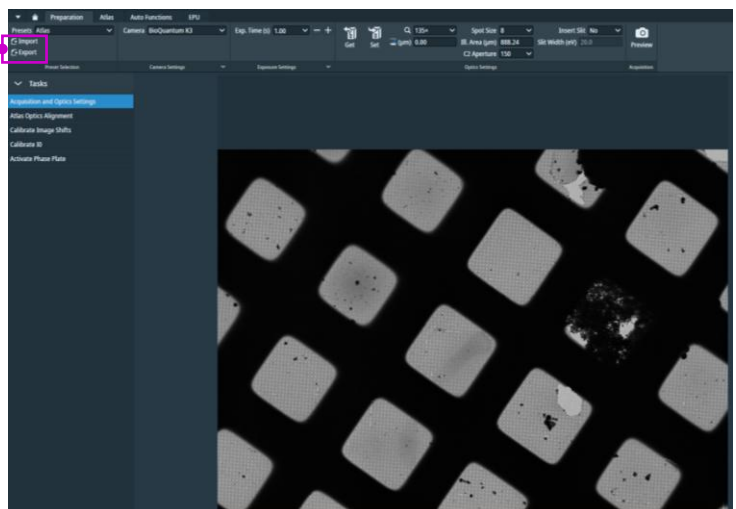
Initial EPU setup

1 Establish the magnification presets for your specific grid

- Import presets from Preset folder before moving forward

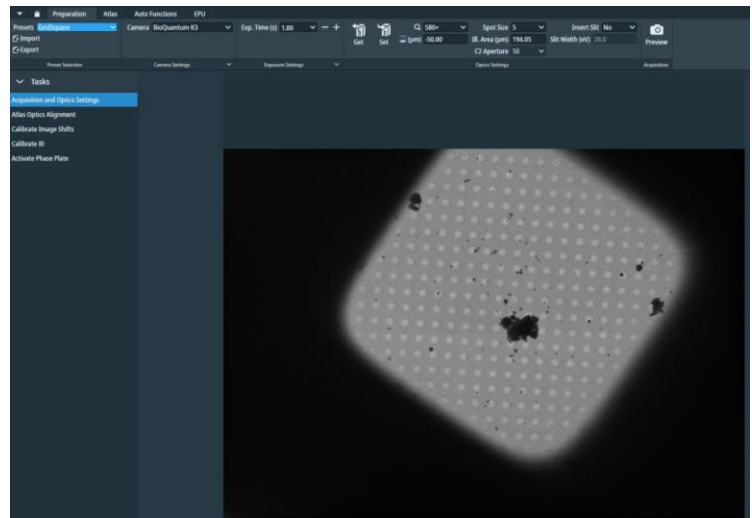
Atlas preset

- Lowest magnification
- Make sure beam fully covers camera
- ~16 squares on FOV



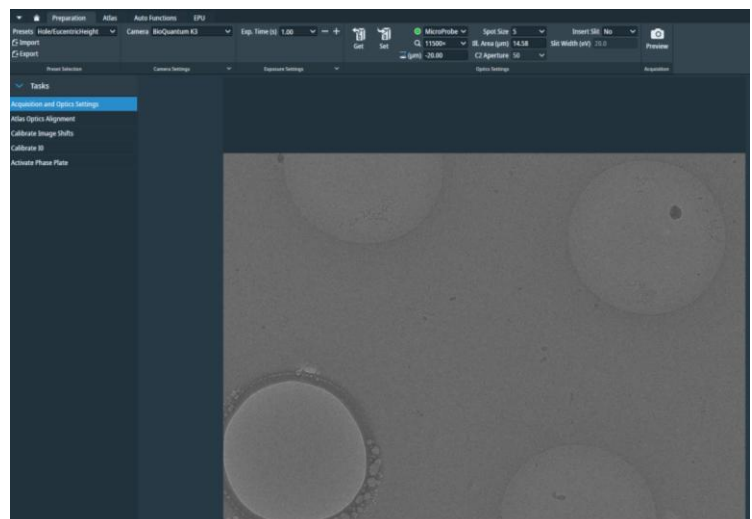
Gridsquare preset

- LM preset
- Auto-eucentric before taking image
- Make sure beam fully covers camera
- One full square on FOV when centered
- Sufficient contrast to clearly identify foil holes



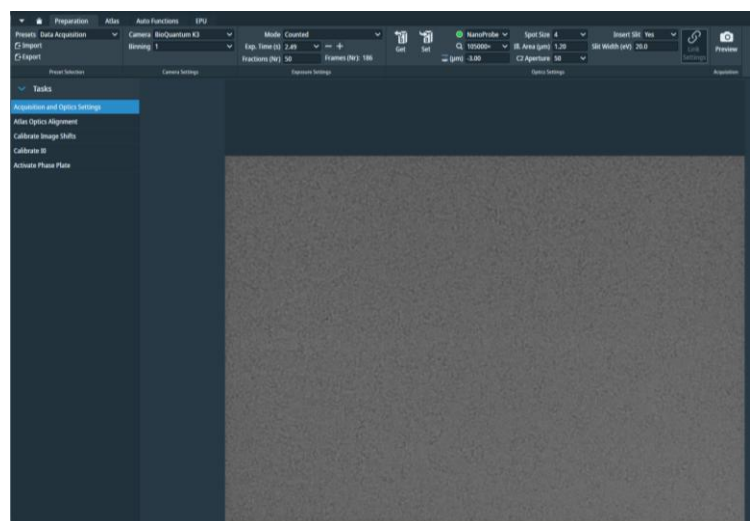
Hole/Eucentric preset

- Hole Centering, Auto-eucentric Height and Template Definition preset
- Contrast should allow to clearly see holes, use spot size and defocus
- Ideally 1 hole in FOV, with parts of surrounding 8 holes

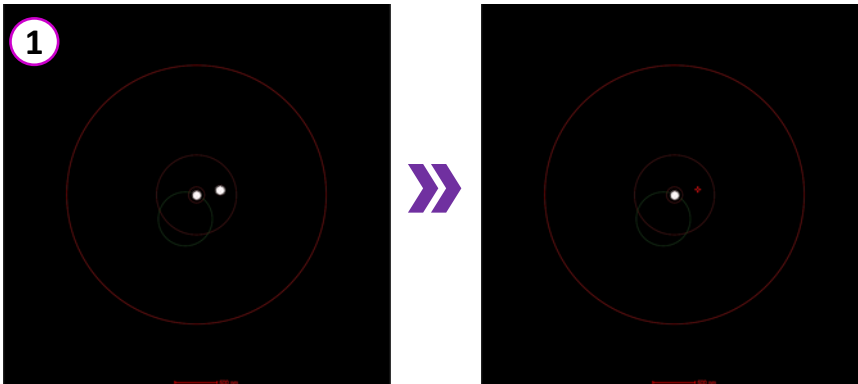


Data acquisition preset

- Magnification used for collection and focus
- Sample dependent, make sure you see sufficient particles per FOV
- Dose rate of 40-60 e should allow for sufficient contrast
- Adjust Spot Size and Intensity to place dose in green section of dose rate bar
- Link remaining presets to this one

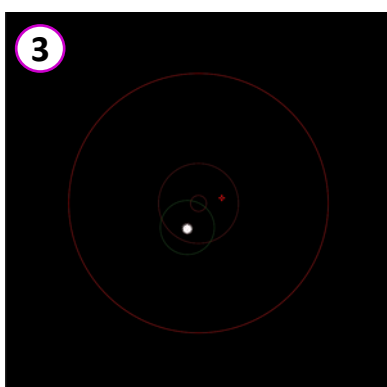
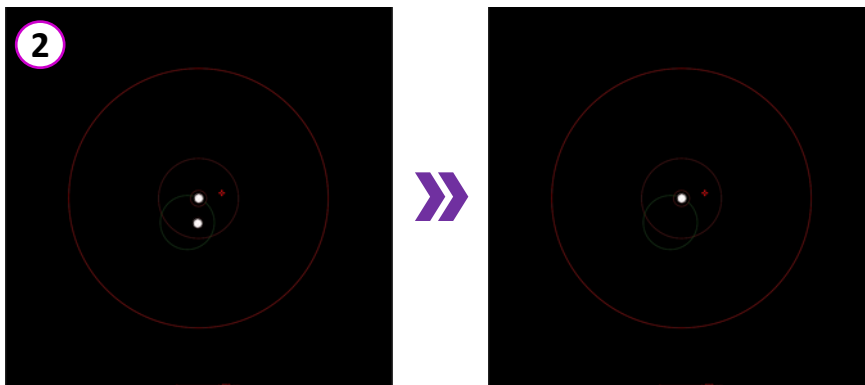


- Press “Eucentric Focus” on Control Pad, center the beam and cover camera with beam beforehand
- Use fluorescent screen with screen down (Press “Insert Screen” or R1 in control pad)



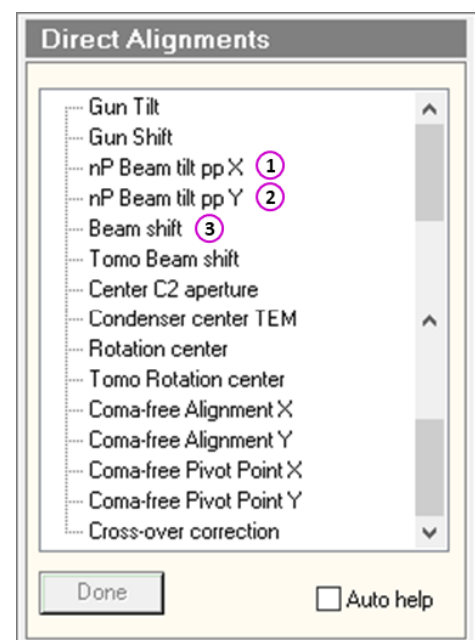
nP Pivot points X/Y

- Smallest beam size
- Correct with Multifunction X
- Make beam 1 spot



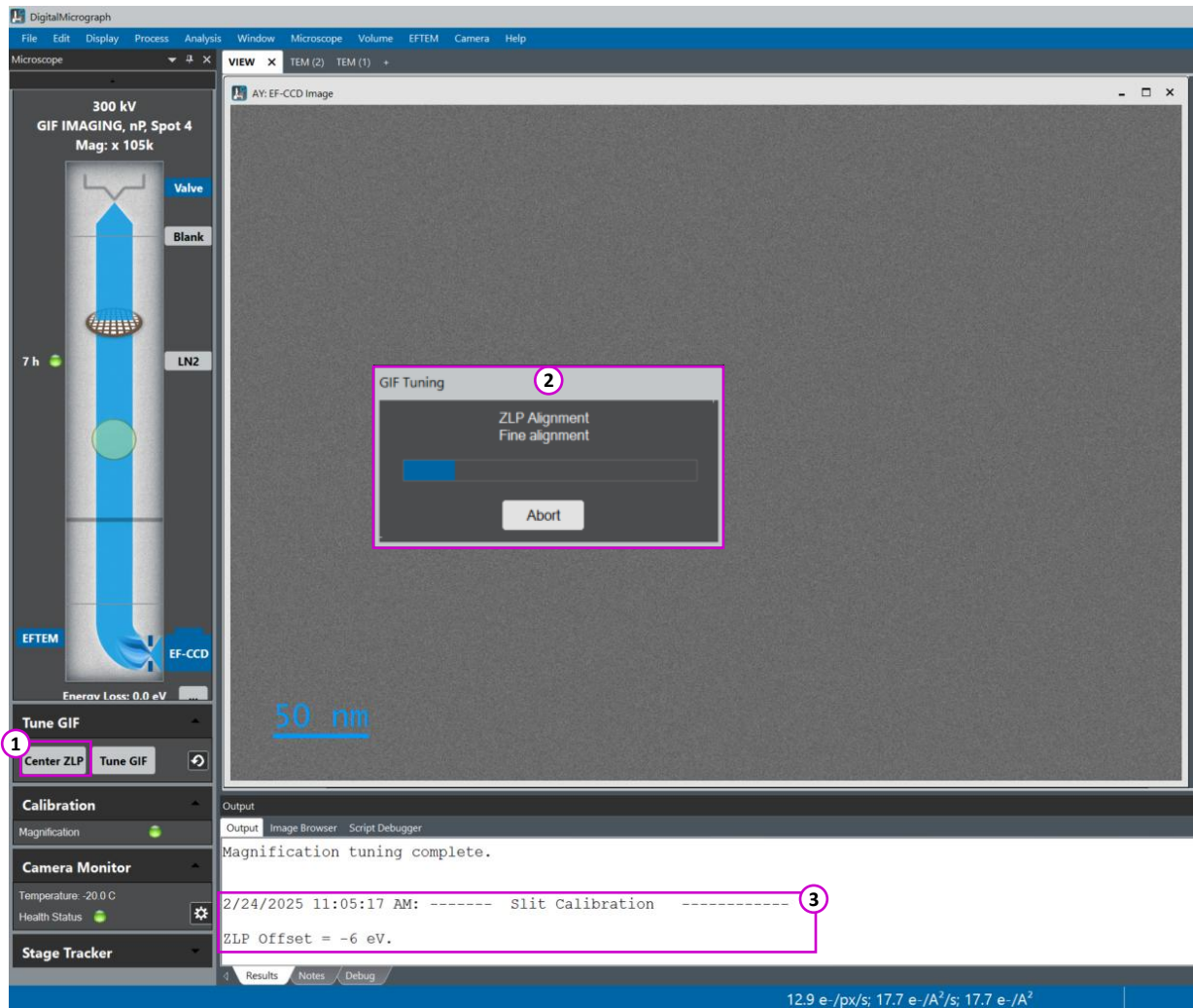
Beam Shift

- Small spot
- Center on filter (green)
- Do after each step



3 Zero-loss peak centering

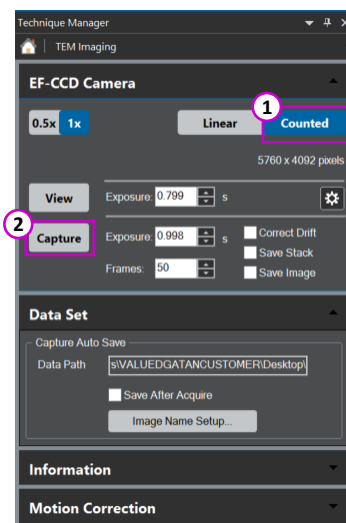
- While still in the empty square, go to the **Digital Micrograph software** (rightmost screen) to recenter the Bioquantum energy filter
- If it does not work or filter needs calibration, lower spot size and recenter beam.



4 Adjust Dose in Data Acquisition Mode

Measure dose

- In Data Acquisition preset, make sure you are on an empty area
- Ensure beam is centered on camera using Fluorescent Screen
- Change beam diameter or spot size until dose rate is $\sim 15 \text{ e-/pix/s}$
- The dose rate ($\text{\AA}$) is magnification-dependent

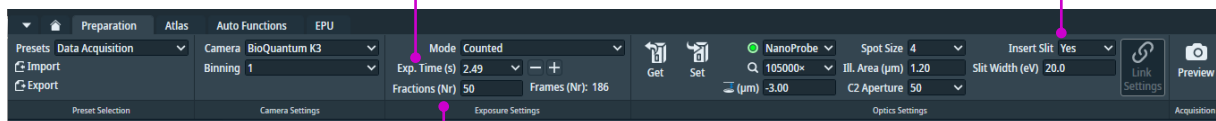


Dose rate (pixel)	Dose rate ($\text{\AA}$)	Total Dose
12.9 e-/px/s; 17.7 e-/Å²/s; 17.7 e-/Å²		

Calculate and Annotate Dose

Consider the dose you want to use ($\sim 40\text{-}60\text{ e-}/\text{\AA}^2$)
Calculate exposure time by dividing total dose by dose rate ($\text{\AA}$)

Bioquantum may need a wider slit

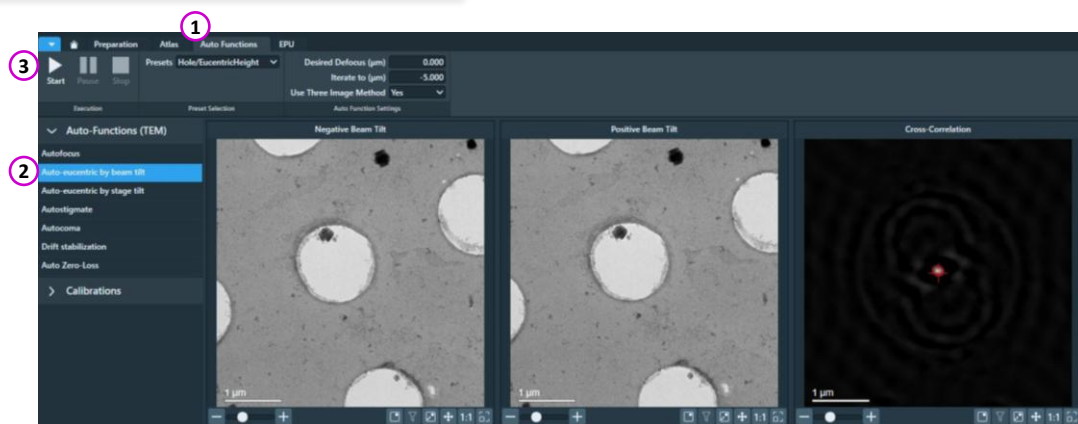


It is recommended to input a number of fractions so that $1\text{ e-}/\text{fraction}$

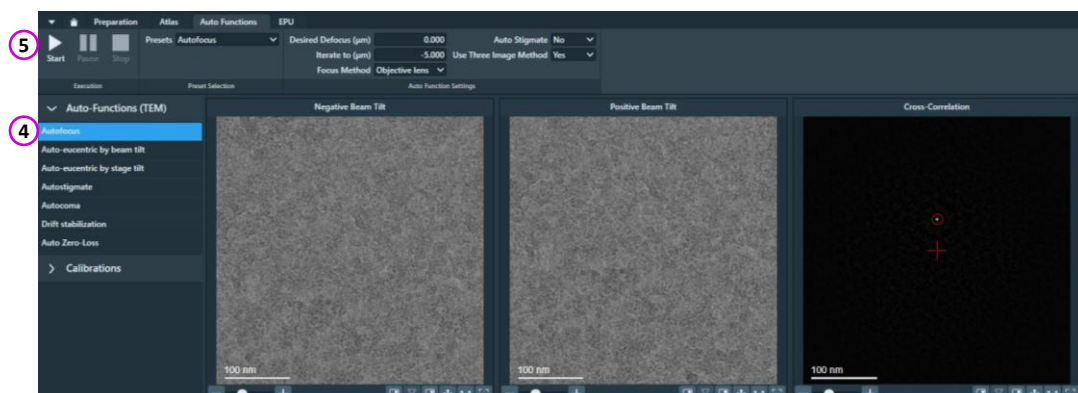
5 Set Optics Settings For Image Shift Calibration

- Navigate to a square with a feature that can be identified at all Presets, then run Auto-eucentric and Autofocus jobs

Auto-Eucentric Height



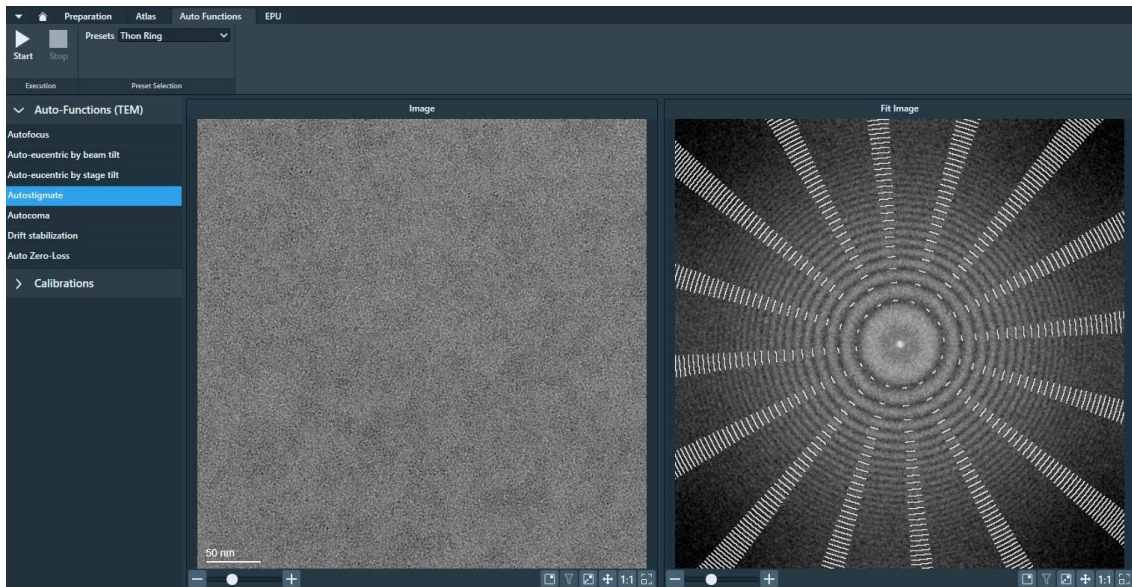
Autofocus



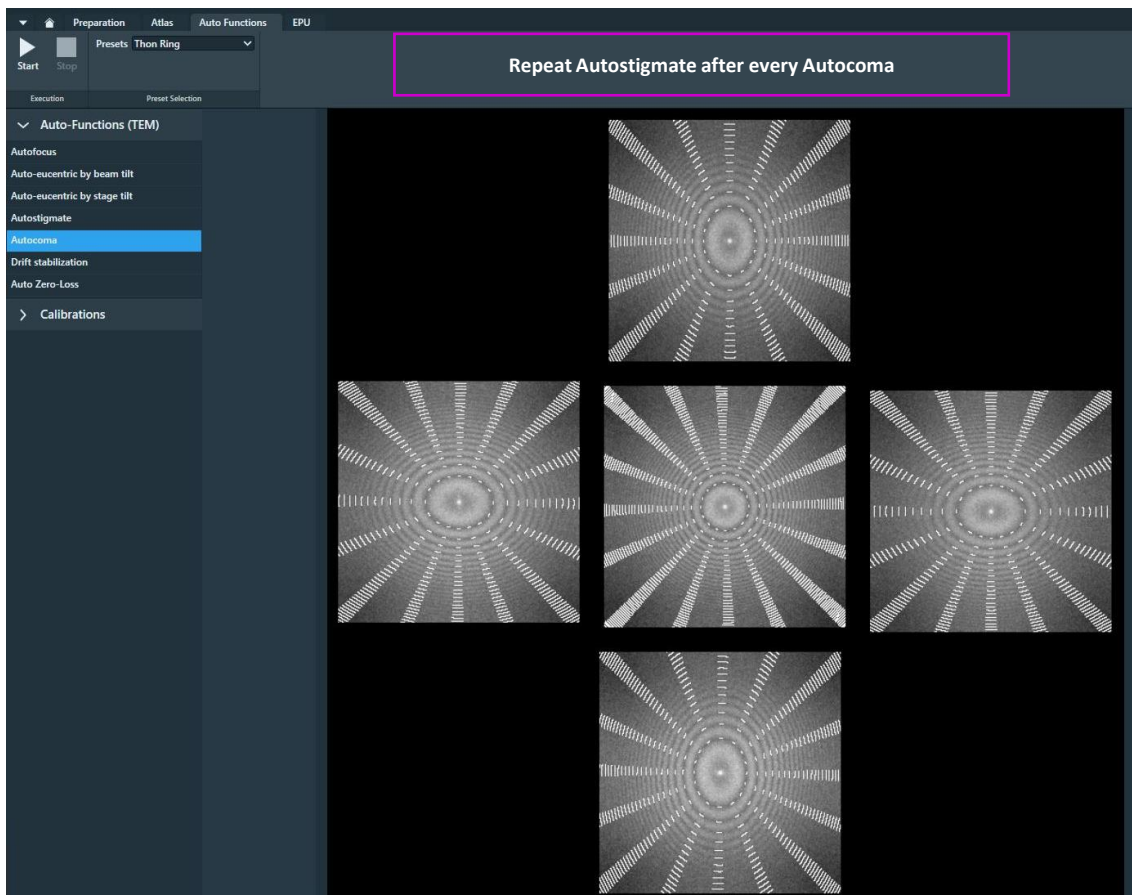
6 CTF correction: Objective Stigmatism and Coma-Free Alignment

- Corrections must be performed on carbon after autofocus
- Ask staff if you need help

Objective Stigmatism



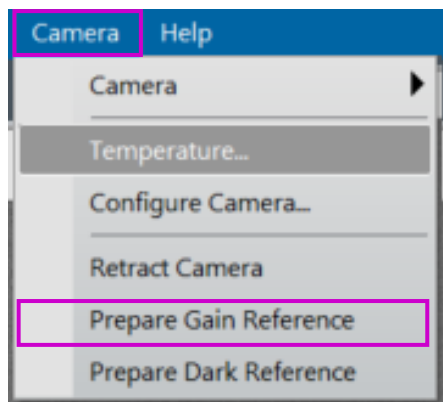
Coma-Free Alignment/Coma Correction



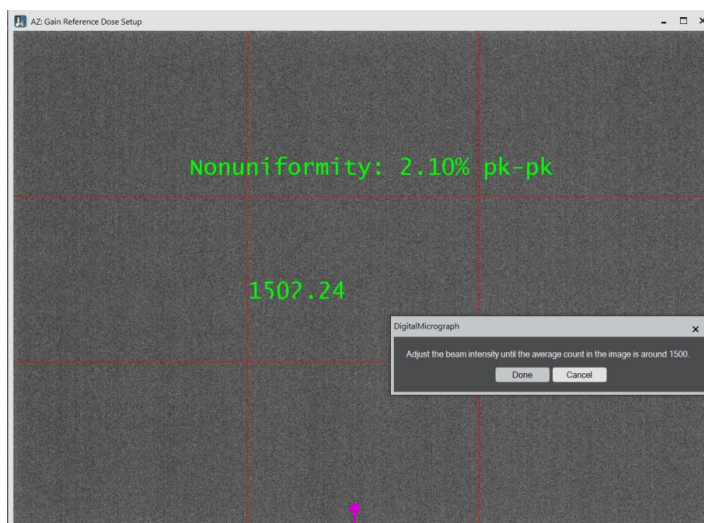
7 Gain Reference Acquisition

- Ensure that the beam is on empty square and at Data Acquisition magnification
- Follow all the directions on-screen after initializing Gain Reference

1 Select Gain Reference

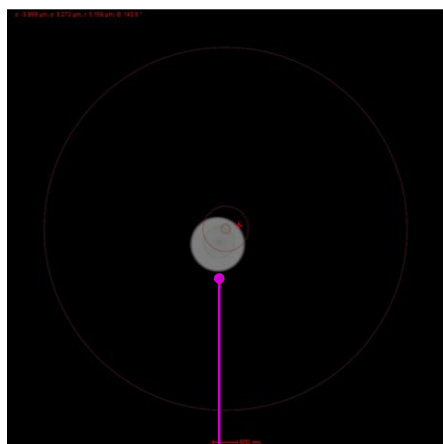


3 Record Gain reference in Linear Mode



Try C2 150 and low spot sizes to reach 1500

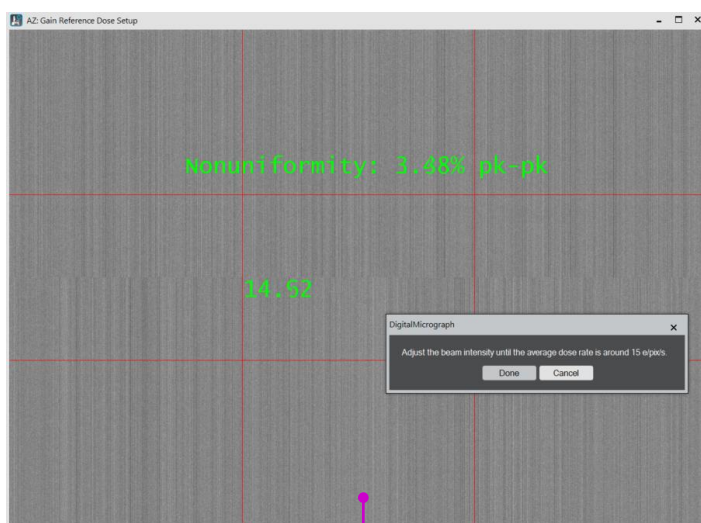
2 Confirm beam is centered



Center beam on energy filter (green circle)

4 Record Gain reference in Counting Mode

DO NOT START UNTIL YOU CHANGE TO DATA ACQUISITION MAGNIFICATION

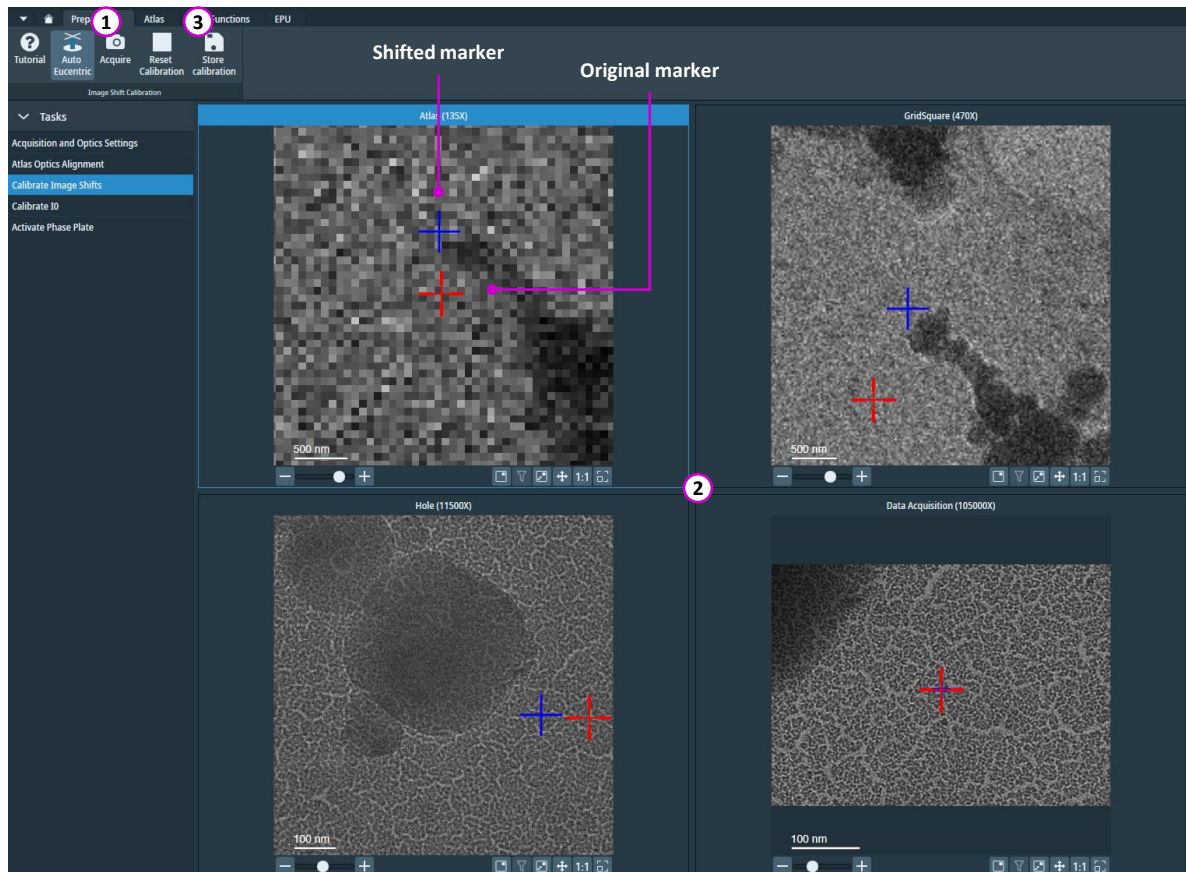


Try C2 50 and medium spot sizes to reach 15
(close to Data Acquisition settings)

Gain Reference File Path: /sdf/group/cryoem/exp/YYYYMM/YYYYMMDD-CSXX_TEM1/*collectionID*/raw/GainRefs/*.mrc

8 Image Shift Calibration

- Find a recognizable feature at all magnifications and center it at Data Acquisition magnification
- Double-click on same feature in every magnification



Atlas Acquisition

The screenshot shows the NVIDIA DLI Session Management interface. The 'New Session' button in the top-left navigation bar is highlighted with a red box. The 'Session Setup' tab is selected, displaying the following configuration:

- Name:** YYYYMMDD_GroupANDORUser_Sample_Atlas
- Image format:** MRC (selected), TIFF
- Output folder:** Z:\YYYYMMDD_Group\ANDORUser_Sample
- ☐ Set as default storage folder
- Apply** button

YYYYMMDD_CSXX_userInitials_sample

The screenshot shows the Atlas software interface. At the top, there are tabs for Preparation, Atlas, Auto Functions, and EPU. Below these are buttons for Start, Skip, Stop, Reset Subsequence, and a dropdown for Start Position (set to Close to center). A Number of Tiles input field is set to 12. To the right are buttons for Close Cal. Values, Load Sample, and a subsequence icon. Below the main toolbar are tabs for Acquisition, Atlas Settings, Options, and AutoSuborder. On the left, a Tasks panel shows a list of grids (12 to 2) with checkboxes. Grid 4 is selected. At the bottom, there are buttons for Single Atlas and Acquire. Annotations with pink lines point to the Number of Tiles field, the Start button, the Acquisition tab, the selected grid (4), and the loaded grid (2).

Number of tiles to stitch starting in an outward spiral. Blank = All the grid

When all grids are selected, press Start

Selected grid for Atlas acquisition

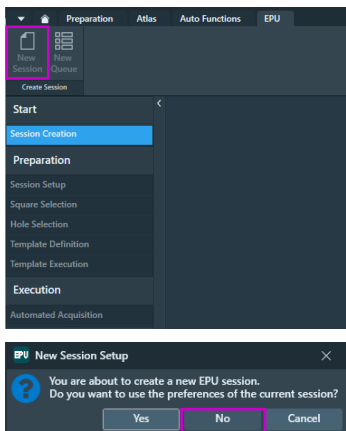
Loaded grid

Loaded grid

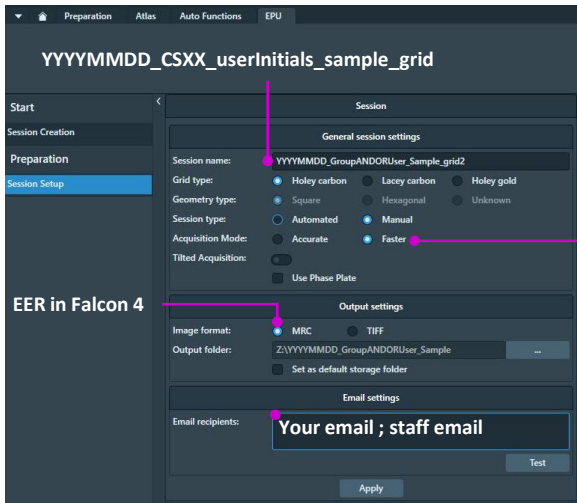
Ice-quality classification of gridsquares

Setting an EPU session

1 Create a session

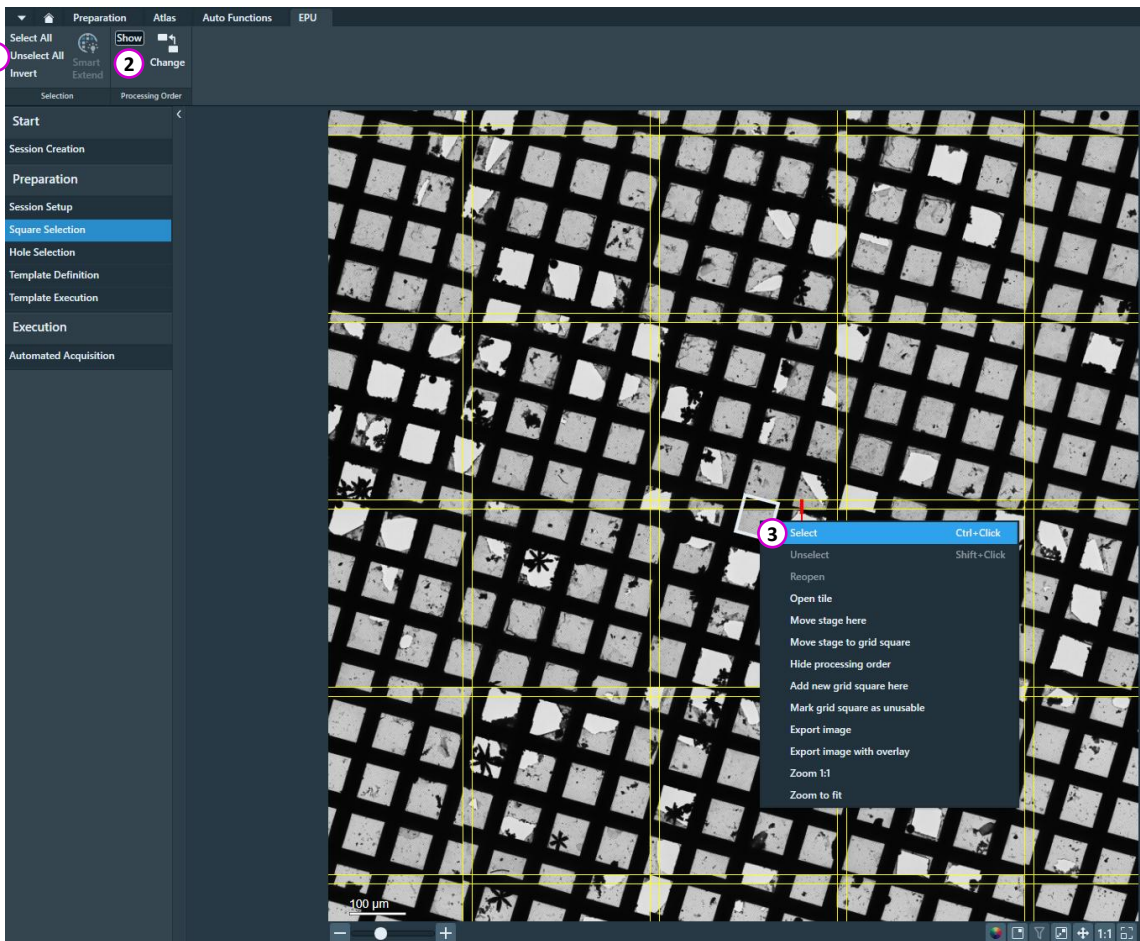


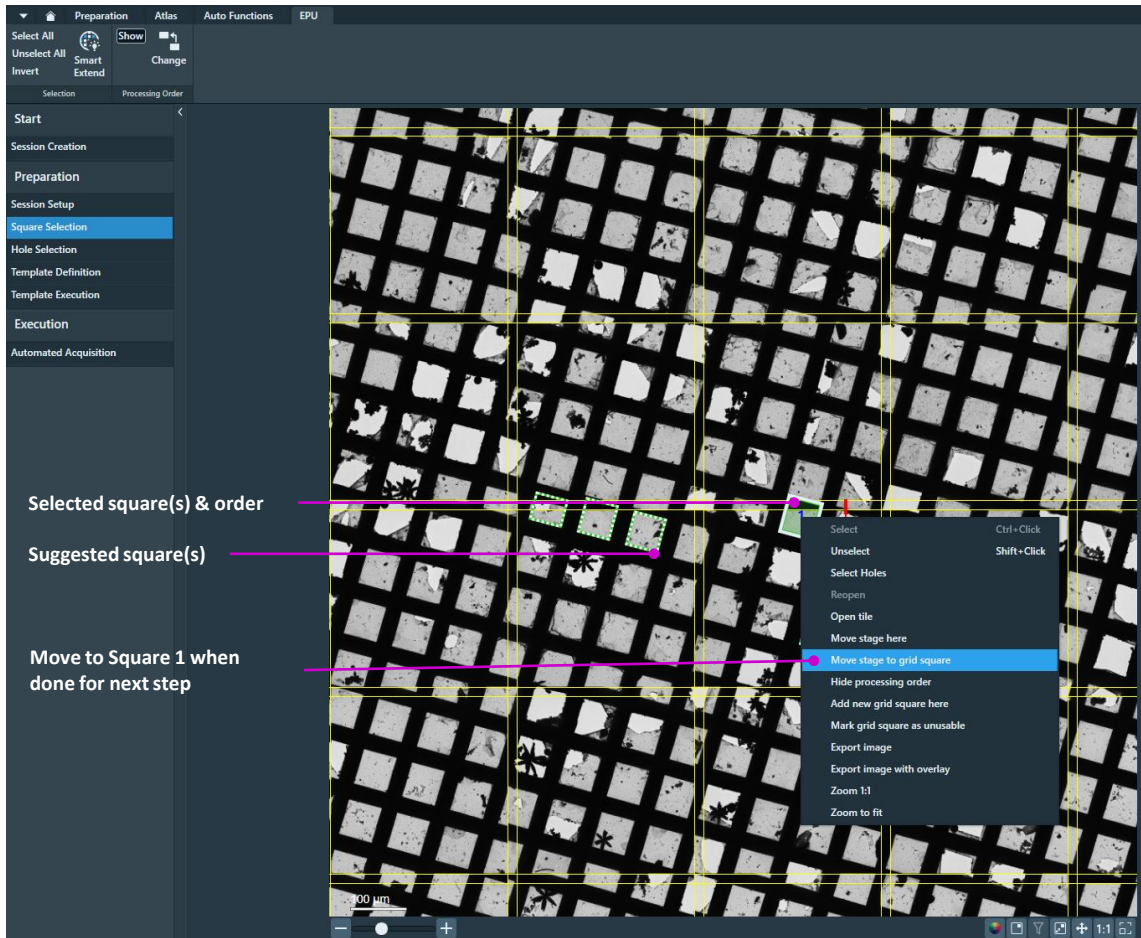
2 Determine session settings



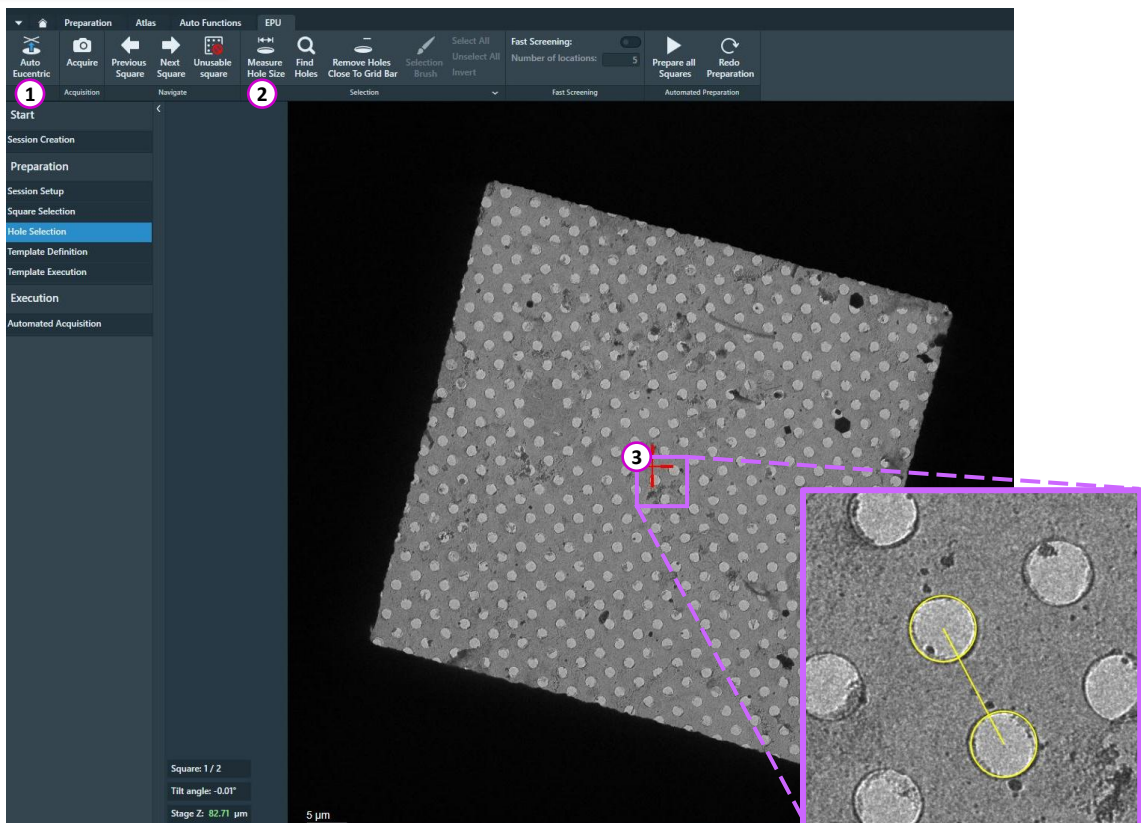
AFIS mode (300-500 exposure/hr)
Requires good alignments

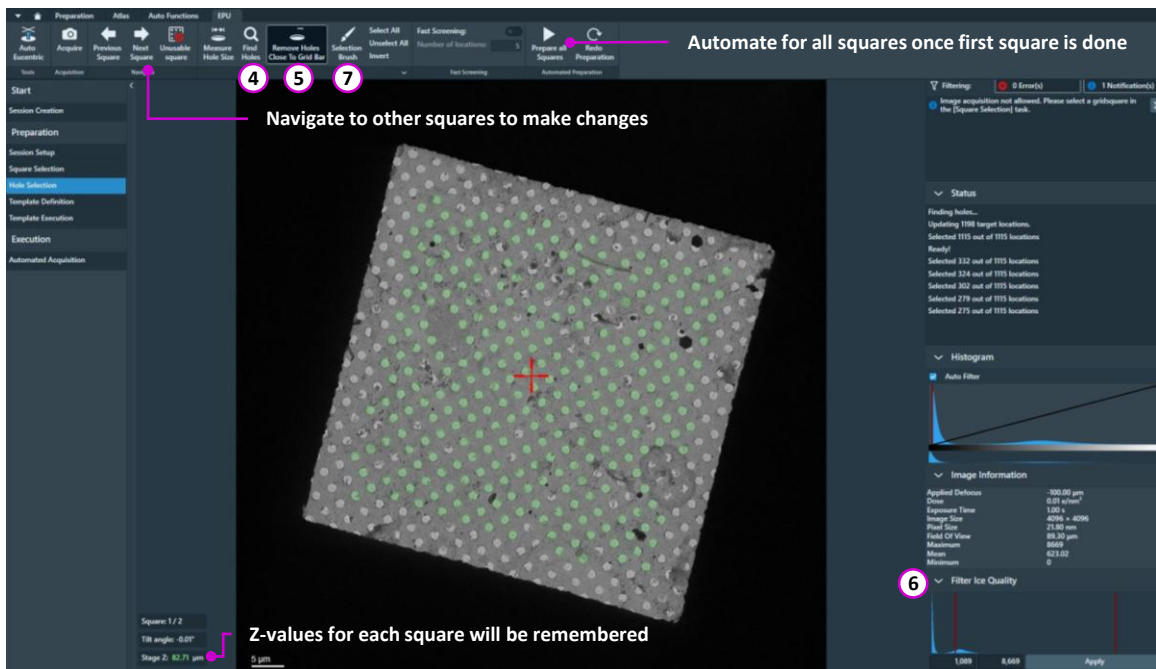
3 Square selection



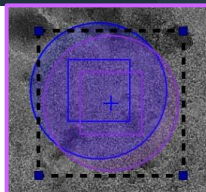
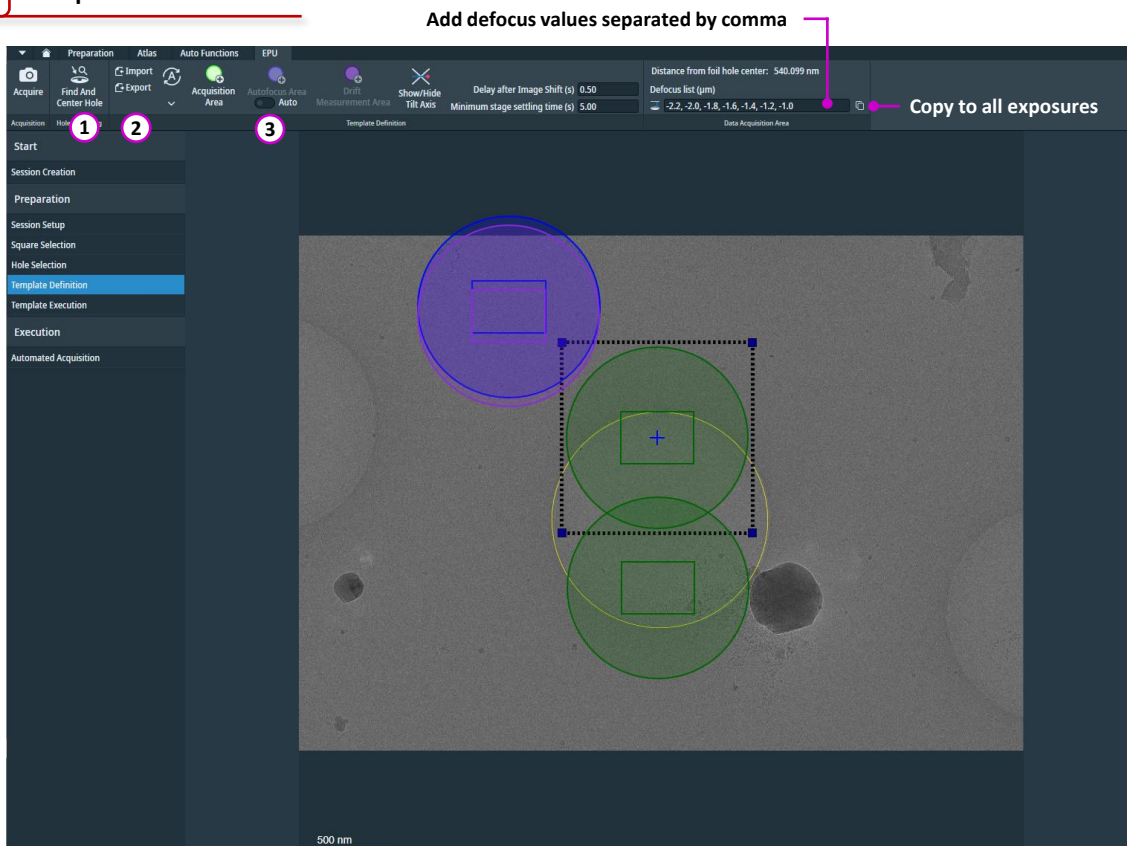


4 Hole selection

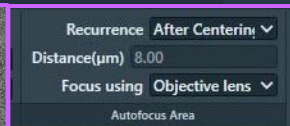
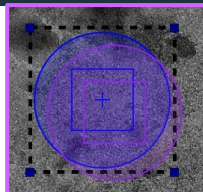




5 Template Definition

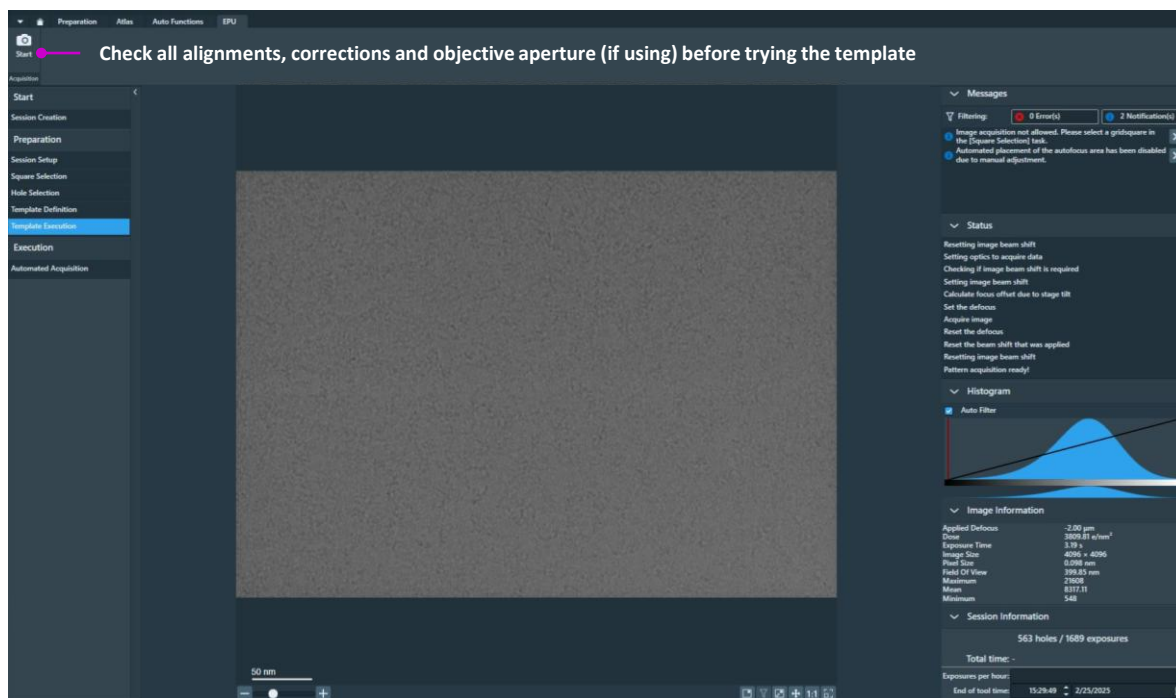


- If stable beam/stage, use "Once Per Gridsquare"



- Recurrence can also be set up to "After Distance"

6 Template Execution



7 Automated Acquisition Setup

