

TEM - TFS Titan Krios G3i: Check-list before starting data acquisition.

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INTRODUCTION

Overview of the direct alignments to be done before starting data acquisition at the transmission electron microscope Titan Krios G3i.

Please find more detailed information about the system here.

Step 1 — Gain Reference and Energy Filter Tuning



- (i) Before data acquisition perform energy filter tuning and acquire a gain reference. Do the same always after microscope and Camera cryocycle (i.e. on Monday Morning) and after a Camera Power Off/Power On cycle.
- Navigate to a void position like a broken grid square, or remove the grid if no voids are available on the grid.
- Prepare the gain references (instructions <u>here</u>).
- Tune the energy filter (instructions <u>here</u>).

Step 2 — Eucentric Height



- (i) The Titan Krios G3i is super stable, only minor alignments are needed before starting data acquisition.
- Do all the alignments with the objective aperture out, and insert it only before starting data acquisition (step 6).
- Move the stage to a thin area of carbon.
- Adjust eucentric height using the EPU autofunction, if you wish, with the "Hole/Eucentric Height" Presets.

Step 3 — Focus



- Make sure you are on carbon, checking the stage location with a Hole/Eucentric Height preview. If you are not, move the stage by right click on a carbon location in the preview image, and selecting "move here".
- Set the data Acquisition Presents to the microscope.
- Bring the Sample to focus condition. Adjust with the focus knob using Thon rings: select into Digital Micrograph: "Process -> Live FFT" and adjust until you don't see any Thon Rings.

Step 4 — Direct Alignments: pivot points and beam shift



(i) Once in focus, proceed with the Direct Alignments.

- The direct alignements are done on the fluoscreen, insert the screen by pressing R1, on the right microscope control panel.
- Activate the pivot point X. Use the **Multifunction X** to you minimize the beam movement.
- Activate the pivot point Y. Use the **Multifunction X** to you minimize the beam movement.
- Center the beam with the beam shift. Use both Multifunction X and Y to center the beam to the K3 position (indicated on the fluoscreen with the green circle)

Step 5 — Direct Alignments: astigmatism and comma free alignments



- (i) You can correct for the astigmatism and comma free alignments with the EPU Autofuction.
- In the AutoFunction tab of EPU, select "Thon Ring" as presets, for the Autostigmate function.
- Start the calibration.
- For the Autocoma, in the same tab select the Autocoma function, and keep the "Thon Ring" presets.
- Start the calibration.
- The coma free alignment is successful when the defocus of both positive and negative beam tilt on the same axis stay the same. The example shows the 2 images from the positive and negative beam tilt along the x axis, presenting the same defocus.

Step 6 — Insert the Objective Aperture



- Insert the objective aperture using the Microscope User Interface (TUI). Click the arrow to display if the objective aperture is inserted. Insert it by clicking on "Objective" button.
- Switch on the diffraction mode on the right microscope control panel.
- Insert the Fluoscreen (R1).
- To observe the objective aperture, click on the image and change the intensity of the live view on the fluoscreen with wheel of the mouse.

Step 7 — Center the objective aperture

Objective Aperture		
Diffraction Spot	Apertures Enable Reset Option Condenser 1 2000 Adjust Condenser 1 Inserted Condenser 2 50 Adjust Condenser 2 Inserted Condenser 3 Manual Condenser 3 Manual Objective 100 Adjust Objective Aligning Selected Area [none] Adjust Selected Area Retracted	s)

- (i) The objective aperture needs to be centered to the diffraction spot.
- Select "Adjust" on the aperture tab, and center the aperture using the Multifunction X and Multifunction Y wheels.

Step 8 — Turbo pump AutoOff



 Make sure that the Turbo pump is set Turbo to Auto Off (otherwise the vibration would affect your data).