

Setup an automated data collection at Glacios

Beam microprobe and nanoprobe calibrations

- Introduce the Cross Grating Grid in the column.
- Go to x34 Imaging status.
- Select 2 positions: a carbon square and an empty square.
- Introduce the Condenser 2 of 30 um
- Go to x280 Imaging status.
- In DigitalMicrograph click View and place the mouse arrow on a feature.
- Use Alpha Toggle 20° in Stage² window (TEM) to rock the grid.
- Correct with **Z axis knobs** on the console.
- Repeat a second time.
- Go to x45k Imaging status
- In Low Dose Control window (SEM) click on Go to: Record.
- Push the **Eucentric Focus** knob on the console.
- Inset the screen (FC).
- Push the **Wobbler** knob on the console.
- Adjust the Z height on the screen with the **Z axis knobs** on the console.

Microprobe

Focus the beam and center it (with the trackball).

- Go to microprobe (TEM)
- Push the **Eucentric Focus** knob on the console.
- In the **Direct Alignments** window:

1. Adjust the [Beam tilt pp X](#)
 - Click on **Beam tilt pp X**
 - Adjust with **Multifunction X/Y** knobs
 - Click **Done** at the bottom of the window.
2. Adjust the [Beam tilt pp Y](#)
 - Click on **Beam tilt pp Y**.
 - Adjust with **Multifunction X/Y** knobs.
 - Click **Done** at the bottom of the window.
3. Adjust the [Beam shift](#)
 - Click on **Beam shift**.
 - Adjust with **Multifunction X/Y** knobs.
 - Click **Done** at the bottom of the window.
4. Adjust the [Rotation center](#)

- Click on **Rotation center**.
- Adjust with **Multifunction X/Y** knobs.
- Click **Done** at the bottom of the window.

Nanoprobe

Focus the beam and center it (with the trackball).

- Go to nanoprobe (TEM)
- Push the **Eucentric Focus** knob on the console.
- Check the Condenser 2 centering and adjust if necessary.
- In the **Direct Alignments** window:
 1. Adjust the [Beam tilt pp X](#)
 - Click on **Beam tilt pp X**
 - Adjust with **Multifunction X/Y** knobs
 - Click **Done** at the bottom of the window
 2. Adjust the [Beam tilt pp Y](#)
 - Click on **Beam tilt pp Y**
 - Adjust with **Multifunction X/Y** knobs.
 - Click **Done** at the bottom of the window
 3. Adjust the [Beam shift](#)
 - Click on **Beam shift**
 - Adjust with **Multifunction X/Y** knobs
 - Click **Done** at the bottom of the window
 4. Adjust the [Rotation center](#)
 - Click on **Rotation center**
 - Adjust with **Multifunction X/Y** knobs
 - Click **Done** at the bottom of the window

[After all calibrations save the settings](#)

- Menu: *Settings* > *Save*
- In **Low Dose Control** window check **Continuous update**
- Click on Update it
- Uncheck **Continuous update**
- Menu: *Settings* > *Save*

Correct astigmatism

- Verify that the **Turbo Auto Off** Vacuum option is checked (TEM)

- Go to x45k Imaging status
- In the Camera and script window (SEM) open the **Setup**
- In Record Tab, set 2 sec exposure, processing unnormalized, align frames, save frames and the objective diaphragm out
- If the signal is too weak, increase the time, concentrate the beam or change the carbon area
- **++Correct astigmatism by CTF** * Menu: Calibration > Focus & Tuning > Correct astigmatism by CTF * Open FFT window with Shift+F * Repeat several times until the corrected parameters have converged * **++++Coma free align by CTF** * Menu: Calibration > Focus & Tuning > Coma free align by CTF * Open FFT window with Shift+F * **++Coma vs image shift**
 - Menu: Calibration > Focus & Tuning > Coma vs image shift
 - Open FFT window with Shift+F
 - If the signal is too weak, increase the time, set binning, concentrate the beam or change the carbon area
 - parameters for R 1.2/1.3 grids: 5 μm
 - parameters for R 2/2 grids: 6 μm

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