Setup an automated data collection at Glacios

Beam microprobe and nanoprobe calibrations

Prepare the Cross Grating grid for calibration

- 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 Toogle 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

- Introduce the Objective aperture
- Redo the Correct astigmatism by CTF step once