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 and redo the Correct astigmatism by CTF step once.

Configuration of the multihole / multishot acquisition

Do this configuration just before starting the acquisition on one of the squares selected for the whole acquisition after correction of the image shift in x280 and LD mode imaging states.

- On the square map, mark the positions of the 4 holes at the corners of a 9 holes square.
- Go to the center hole with **Go to marker** and properly center on the hole with **Stage shift**.
- Press View in LD mode (ensure that defocus is below -20)
- Open Menu: *Navigation > Montaging & grids > Set multi*
- Change the parameters of the multishot for calibration
- Click on the central hole for positioning
- Adjust the number of shots per hole (in general 3)
- Adjust the distance (72 in general for R 2/2 grids)
- Menu: *Navigation > Montaging & grids > Find hole in regul* to configure
- Select regular pattern 3 x 3
- Calibrate after ensuring proper centering on the center hole
  - Move by Image shift to the first corner
  - Center properly in the hole using zoom if necessary

    - Save the position by clicking on **Save image shift**
    - Proceed to the next corner and repeat

- Save the settings in *Menu* > *Settings* > *Save*

Beam tuning

- Ensure that LD modes View and Record are perfectly aligned
- Calibrate the proper centering in Record/View/Focus/Trial modes

- Precisely center Record beam with Direct Alignments/Beam shift
- Check **Continuous update**
- Press View and recenter if necessary with set add beam shift
- Then cycle several times between the modes until the beam stop moving
    - Press **Record** and correct Direct Alignments/Beam shift
    - Press **Focus** and set add beam shift
    - Press **Trial** and set add beam shift
    - Repeat

- Uncheck **Continuous update**

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