Basic Microscope Alignment

The microscope should be roughly aligned when you arrive.

There is no need to do a complete alignment every session, if the microscope behaves strangely, ask for help!

You should perform only two or three steps:

- Camera gain reference
- Direct alignment
- Objective Astigmatism / Coma-free alignment (OPTIONAL for screening!)

Gain reference of the K2 camera

This is done in Digital Micrograph (DM) on the K2 server.

- Go to an empty region on the sample
- Go to high mag (e.g. 45000x)
- Insert 150µm C2 aperture
- Go to spot size 2
- Check on the FluScreen that beam is centered
- Digital Micrograph > Camera > Prepare Gain Reference
- Perform the gain in Linear mode first
- Adjust Beam Intensity to match the number of count on the camera
- Proceed to Counting mode
- Press 'Yes' to aquire the dark frame reference image (take twice 3 min 30)
- At the end of the dark ref update, you will be prompt to lower the dose: insert 30µm C2 aperture, go to spot size 6, adjust C2 lens to 39.6%)
- Lift the Fluscreen and aquire Counting Gain Reference Image (you DON'T need to match the number given by DM)

Direct alignment

This is done in microscope interface.

1. Insert calibration specimen (position 1 in the autoloader)
2. Find an unbroken square
3. Bring sample to eucentric high
4. Press Eucentric Focus on the right control panel

Open the Direct alignment tab

DO NOT TOUCH GUN TILT/SHIFT

Objective Astigmatism / Coma-free alignment

This is done in SerialEM.