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.

1. Go to an empty region on the sample
2. Go to high mag (e.g. 45000x)
3. Insert 150µm C2 aperture
4. Go to spot size 2
5. Check on the FluScreen that beam is centered
6. Digital Micrograph > Camera > Prepare Gain Reference
7. Perform the gain in Linear mode first
8. Adjust Beam Intensity to match the number of count on the camera
9. Proceed to Counting mode
10. Press 'Yes' to aquire the dark frame reference image (take twice 3 min 30)
11. 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%)
12. 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 the microscope user interface.

Prepare the microscope

1. Insert the 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
5. Set the microscope to the imaging conditions you want to use (e.g. mag 45000x, Spot size 6, C2 aperture 30µm)
6. Perform direct alignment in microprobe and nanoprobe as follow:

Microprobe

1. Switch the condenser system to microprobe
2. Open Direct Alignment panel
3. **DO NOT TOUCH GUN TILT/SHIFT**
4. Perform Beam Tilt Pivot point X alignment
5. Perform Beam Tilt Pivot point Y alignment
6. Check C2 aperture centering
7. Perform Rotation Center alignment

Nanoprobe

1. Switch the condenser system to nanoprobe
2. Open Direct Alignment panel
3. Again, **DO NOT TOUCH GUN TILT/SHIFT**
4. Redo Beam Tilt Pivot point X alignment
5. Redo Beam Tilt Pivot point Y alignment
6. Check C2 aperture centering
7. Redo Rotation Center alignment

Eventually, recenter the beam

Objective Astigmatism / Coma-free alignment

This is done in SerialEM. You need to have a sample inserted to do it. Re-use the calibration specimen or a carbon Quantifoil (not a gold grid!). Use the Imaging state you will use for data collection (e.g. mag 45.000kx, spot 6, C2 aperture 30 µm, C2 lens 39.6%)

1. Insert and center Objective Aperture
2. In SerialEM, Focus/Tune > Correct Astigmatism by CTF
3. In SerialEM, Focus/Tune > Coma-free by CTF