High Resolution Imaging on the CM200

Environment

(1) The evening before, set dehumidifier to 20% RH, chiller to 17 °C and air conditioner to 24 °C. This helps to minimise stage drift and ice build-up.
(2) Just before starting your experiment, set the objective lens water flow to 20 l/h. **Do not forget to reset to 30 l/h after finishing the experiment.**

Set-up and Alignment

This is most easily done with amorphous carbon film on the room-temperature holder.

(1) Fill anti-contaminator.
(2) Check HT. If you change the HT, make sure to download the appropriate alignments from the PC.
(3) Select LOW DOSE page.
(4) Select low magnification (e.g. 580 ×) in SEARCH.
(5) Adjust eucentric specimen height.
(6) Select image magnification in EXPOSURE (e.g. 58,000 ×).
(7) Adjust C2 aperture size, spot size and intensity to give a 0.7 or 1.0 s exposure reading when beam just covers film area. 50 μm aperture gives better coherence but fewer electrons.
(8) Align C2 aperture.
(9) Adjust condensor astigmatism.
(10) Switch to diffraction mode, insert and align objective aperture.
(11) Switch to imaging mode and focus image.
(12) Focus beam.
(13) Adjust X and Y beamcoil pivot points. This ensures that no beam tilt occurs when the beam is shifted.
(14) Increase magnification to e.g. 220,000 ×, focus on a piece of dirt and adjust current rotation centre.
(15) Reset illumination conditions for imaging and irradiate specimen for a few seconds.
(16) Select FOCUS S1. Adjust spot size and intensity to cover central circle.
(17) Adjust beam/image shift to 2.5 μm and rotation to 0°.
(18) Repeat 16,17 for S2 but using rotation of 180°.
(19) Select SEARCH. Spot size should be large (e.g. 7) and beam should not cover more than 1 grid square.
(20) Centre (on TV screen) contamination spot/hole generated in 15 using multifunction knobs.
(21) Cycle through modes several times to remove hysteresis effects.
(22) Set exposure to manual 0.7 or 1.0 s.

Imaging

(1) Load and cool specimen. When loading a frozen specimen, rotate stage slowly while monitoring IGP.
(2) Select specimen area in SEARCH mode.
(3) Select S1, increase magnification to e.g. 220,000 × and adjust astigmatism. Check drift rate.
(4) Set magnification to that used for imaging and focus.
(5) Select S2 and set focus to mean of S1 and S2. RSET DEFOCUS.
(6) Choose appropriate defocus e.g. -700 nm for crystal imaging or - 2000 nm for single-particle imaging.
(7) Expose film.
(8) After exposure check that illumination beam is still well centred and intensity is correct in EXPOSURE mode.
(9) Repeat 1 to 8 for next image.

Remember to check rotation centre and beam tilt occasionally. The beam tilt pivot point is sensitive to the objective lens current which will change as you move over the grid.