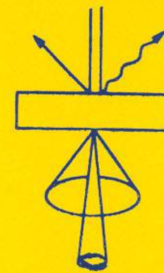
18-2-94

Society of Electron Microscope Technology



THE CRAFT AND ART

of

ELECTRON MICROSCOPY

An afternoon with

STEVE CHAPMAN

2 to 5 p.m. Friday 18 February

Institute of Dental Surgery
Gray's Inn Road
London WC1

Steve Chapman (a Founder member and one-time Chairman of SEMT) has 30 years' experience in the operation, service, design and development of electron microscopes. He runs his own consultancy and training company and conducts courses throughout the world; he is the author of several books. Beginners and experienced electron microscopists alike will benefit from Steve's advice on problems of all types, whether operation-, application- or service-related.

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I hope to be present at the meeting on 18 February 1994

Name

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THE CRAFT AND ART OF ELECTRON MICROSCOPY

Steve Chapman 18.2.94

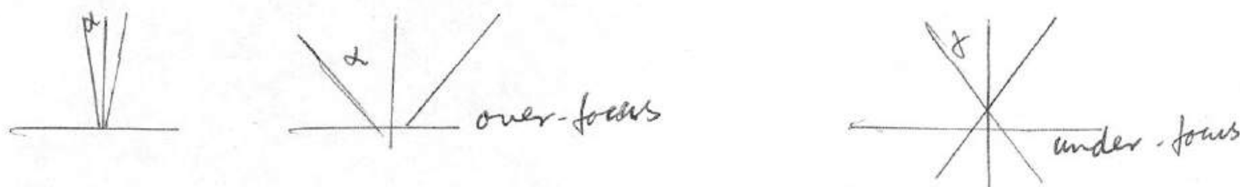
When switching off the microscope, it should be sufficient to wait only about 5 minutes with the water running, for the pump to come off the boil.
But it is better to leave the vacuum system running all the time, in order to get a really good vacuum.
It is better to bleed dry nitrogen into the column rather than air, which cannot be dried much by its rapid passage through silica gel.

Contamination can totally mask resolution, especially in an SEM. It can be reduced by using a liquid nitrogen trap. Contamination is deposited on the lower side of the specimen as well as the upper surface.

After the first 1½ - 3 hours after switching on, the filament supply will be stable and it will not be necessary to over-saturate the filament; the delay is because of the oil-filled tank, with its convection currents etc. Some recent microscopes have a Freon-filled tank instead of the oil-filled tank, and this stabilises within minutes.

The electron gun is designed to work at high kV, and so the filament should last longer at high kV. Increased current emission will give more brightness though shorter life. The SEM does not, of its nature, use the gun flat out; you can drive it as hard as possible, and for imaging saturate it fully. For image analysis it is not necessary to saturate fully. "Only Glauert now uses 60 kV"! A higher kV goes through the specimen faster and therefore causes less specimen damage; Steve often uses 120 kV for negatively-stained material.

The beam should be as near coherent as possible, with small α.



and the condenser should be spread clockwise from crossover to get the optimum configuration. The spot size should be 3-5 um; as you spread the beam, the becomes smaller, giving better coherence.

Astigmatism etc should be corrected on an adjacent area, and then flip to the required area for photography. Even one scan can cause significant contamination.

In SEM, the postion of the specimen relative to the final lens is critical. Rather than make the spot size smaller, take the specimen closer to the lens.

For biological SEM, you don't see the surface of the specimen above 2kV! At 30 kV, the beam goes well into, and even through, biological specimens. Yet some SEMs only offer 25 kV, with no alternatives.

In SEM, the secondary electron image alone is sharper, but does not have shadow or depth; to obtain an image of secondary electron only, tilt the sample away from the detector. Backscattered electrons give contrast, shadow and highlights, because of the elements; to obtain an image using back-scattered electrons without charge, tilt the specimen towards the detector.

In SEM, large spot size is better for back-scatter; small spot size for secondary electrons and less charge. A fast scan rate gives no time for the charge to build up.

Different chamber configurations may suit different people's applications.

Photography:
Set the condenser lens clockwise from crossover to get the optimum for photos; and normally work with slight marks from desaturation, so that you can see when the lens is fully saturated, at high magnification. Set the intensity before focussing for the photo, and if too dim, increase the kV!
The wrong spot size will make the TEM image appear out of focus.

What you see on the screen is not necessarily what you want on the negative. Different people may prefer a different degree of underfocus on the screen, but agree on the final print. Wobbler focus gives true focus; but high resolution structures are at low contrast here. The eye prefers an optimum under-focus; calibrate the number of steps for your own specimens on your own EM. The best spot size is 2.5 um for TEM, which appears about 6 cm on the screen at x 10 K.

For optimum underfocus:
1. focus on a high-contrast feature at double the required photographic magnification; aim for the highest contrast at focus.
2. Stigmate for the highest image contrast - one knob at a time!
3. halve the magnification.
4. set the illumination for photography.
5. focus.
6. take photograph.

In general, you need a slow film, although faster films may be better for high resolution TEM, for 0.3 nm. For cut film, use preferably 4 second exposure - not more than 4 or less than 2 seconds; roll film is different. Kodak 4489 is the best cut film for EM, if developed properly.

When developing cut film, use the dunk and tilt method: 15 seconds in the developer, 3 seconds out and tilt; back for 15 seconds, tilt the other way for 3 seconds, etc. throughout. Wash with water, not stop bath, at the same temperature. In the fixer, dunk and tilt for the first minute of fixation. You want a dense negative!

More electrons give more contrast, not fog. If you are getting fog, check the safelights; these fade, and are not guaranteed beyond 3 - 4 years.

A point source enlarger, with quartz halogen bulb, will give more contrast and more resolution; it will also show all the defects on the negative.

X-ray microanalysis:

put an aperture in the grid holder and send the beam through it; you should get X-rays only from the material of the aperture - this is the hole count test. If you get other X-rays as well, you need the "hard X-ray system" - and extra aperture at the bottom of the upper pole piece.

If you are getting signals of Fe, Ni or Co, these could be from the lens; if Cu, this could be from the specimen holder, grid, SEM stage. Backscattered rays from the specimen can give spurious rays from the lens etc.