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On Measuring Section Thickness

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Section thickness can be measured by placing beads of some kind on the top and bottom surfaces of the section. This is then a simple parallax problem.

Imagine the specimen in cross section. If there are two particles, one vertically above the other they are separated by the film thickness T. Tilt the film through an angle A and in plan view the particles will separate by a distance D. This can also be extended to account for two particles not vertically above each other but I'll stick to the easy case for the explanation.

Take two negatives one at zero tilt and one at tilt of A and measure the separation D. The thickness can be calculated by T=D/sinA.

The direction of the tilt axis must be known for the measurements and it is easy to see that the larger the tilt angle and the more accurately the separation is measured, the more accurate the measurement will be. Tilt at both positive and negative angles to get a more accurate result.

It is important to think about what how the surfaces will be marked, as the markers must be distinguishable at two different tilt angles. Crystals may go in and out of contrast, making them difficult to follow. If spheres are used, they will probably be measured to the sphere edge. This will be on the circumference which is sitting above the surface by the radius of the sphere. If there are two spheres (one on the top surface of the section and one on the bottom), then both radii must be subtracted to get the correct section thickness (or one diameter if the spheres are the same size). It can be very difficult to see where the sphere touches the surface when observed in plan view, but the edge is easy to see.

Of course the alternative is to re-embed and cross section to measure the thickness directly. ■

Reference to this method may be found in:

Hawes, C.R. 1981. Application of high voltage electron microscopy to botanical ultrastructure. Micron, Vol. 12 No. 3. pp 227-257.

This works references the original stereo measurement work:

Nankivell, J.F. 1963. The theory of electron stereo microscopy. Optik, Vol. 20. pp 171-198.