Laboratory for Advanced Electron and Light Optical Methods
College of Veterinary Medicine
1060 William Moore Drive
North Carolina State University
Raleigh, NC 7
Tel: 919-513-6202
Fax: 919-513-6464
Email: Michael_Dykstra@ncsu.edu

Computing Micrometer Bar Sizes

Many journals request that internal micrometer bar references be added to electron micrographs to be published. The bars should be designed to be between 1 and 2 cm long. For example, if the final print magnification is 57,800 a line 57.8 mm long would represent 1 μm (dividing the final print magnification by 1000 will always give the line length, in mm, that would represent 1 μm). A 57.8 mm line would be too long and a 5.78 mm line (representing 0.1 μm) would be too short, so it would be appropriate to make a line 11.6 mm and state in the figure legend that it represents 0.2 μm.

Another question that arises concerns determining the real size of an object in a photograph of known magnification. Divide the size of the object of interest in the print (measured in mm) by the print magnification to determine the true size of the object in μm. For example, if an object measures 30 mm in diameter in a photograph with a final magnification of 60,000, the true diameter of the object is 0.5 μm.

Calibrating the TEM and SEM

1. TEM

To calibrate the TEM, several commercially available size references may be purchased. Diffraction grating replicas with an array of parallel lines (2160 lines/mm or 54,864 lines/in) or crossed lines (2160 lines/mm in both directions) can be photographed and the distance between the lines measured (center to center) on the negative. The size-determination procedure in appendix A will then yield the actual magnified distance between the lines, which can be compared with that shown by the electron microscope magnification readout.

A suspension of catalase crystals spread on a Formvar-coated grid (or on sections, for an internal reference) and negatively stained will provide a lattice plane spacing of 8.75 nm and 6.85 nm, which can be photographed and measured as above.

Graphitized carbon may be purchased on grids to measure line-to-line spacing of 0.34 nm, which is the guaranteed resolution of most standard biological transmission electron microscopes sold today. Oriented gold crystals may be purchased on prepared grids that have plane spacing of 0.204 nm, 0.143 nm and 0.102 nm for even more stringent resolution checking.

2. SEM

Polystyrene spheres may be purchased from various electron microscopy supply houses in standard diameters from 0.085 □m to 1.091 □m. They can be attached to poly-L-lysine-coated coverslips, air-dried, sputter-coated and examined with the SEM. Photographing them at a specific magnification and then comparing the diameter calculated from measuring the photograph to the actual diameter specified by the manufacturer will establish the accuracy of the magnification readout of the SEM.