# MAGNIFICATION CALIBRATION PROCEDURE

**SPI# 02901-AB**
**SPI# 02902-AB**

## MAGNIFICATION CALIBRATION

The nominal magnification reading in an electron microscope may be no more accurate than 5%, even when the instrument is new. For any accurate work, the instrument needs calibrating with a known standard of length.

Whenever an instrument is calibrated, the lens currents must be cycled (by a "standardization button", if it is fitted), or by cycling the lens currents up to maximum and then down to the operating value. In this way, the effects of hysteresis are standardized. The value of the lens currents for each magnification should be recorded, so that the conditions can be reproduced.

While working at low magnifications, care should be taken to measure the grating spacings only in the central area of the plate. The image distortion is always at its worst in the low magnification range, and is most noticeable near the edges of the plate.

**02901-AB** 2,160 lines/mm Diffraction Grating Replica
This specimen is a replica of a 2,160 lines/mm parallel line diffraction grating. When imaging the specimen, it should be kept in mind that the line spacing is 0.463um and the pattern will not be visible until the imaging system is set to resolve that level of detail – around x2,500. At this magnification, the lines of the pattern will be just over 1mm apart.

To calculate the electron microscope magnification using the pattern of the diffraction grating replica:

Take the measurement, in mm, between as large a number of lines of the replica pattern as possible. Apply the following formula:

**Magnification = A x 2160/B**

**A** is the distance, in mm, between the first and last line measured.
**B** is the number of spaces between the first and last line measured.

**02902-AB** 2160 lines/mm Cross Ruled Grating
The grating is ruled in two directions at right angles. It permits both a magnification check and also gives an easy visual impression of any image distortion, through distortion of the ruled pattern.

To calibrate the electron microscope using the diffraction grating replicas SPI#'s 02901-AB and 02902-AB of 2,160 lines per mm = 0.463 um per line:

1. Make a series of electron micrographs of a grating at the different magnification settings of the microscope (meter setting or dial taps).

2. Using the negatives, measure the width of as many lines together (bar and space) as possible with a millimeter rule placed perpendicular to the lines.

3. Divide this measurement by the number of lines measured to get the average line width.

4. Multiply this number by 1,000 to convert from millimeters to microns and divide the resulting number by the line spacing of the grating:

   (0.463 um for 2,160 lines per mm)

   The result is the actual image magnification for the corresponding microscope Magnification setting. Curves may be drawn plotting microscope magnification Settings against the actual magnifications, as determined above, to determine the Intermediate image magnifications of the microscope.

At the higher magnifications where no more than two lines can be micrographed, a sharply defined piece of dirt or other structure on the grating may be calibrated at a lower magnification. This known dimension may then be used to determine the higher magnification by dividing the space measured at the higher magnifications by the actual space of the dirt.

After calibration with a grating replica, reproducibility of a given magnification from specimen to specimen is within approximately +/-10%.

For most electron microscopes, recalibration should be carried out at least once a year and certainly immediately after any electrical or mechanical work on an instrument.

**Care of Grating Replica Specimen**

When not in use the replica should be kept in the vial. The replica surface may be damaged if touched. Never try to clean it. Care must be taken to avoid bending the grid as distortion may cause the replica film to fracture. When viewing in the TEM begin at low magnification with a low illumination level. Increase the illumination a little beyond comfortable viewing level then reduce it. This helps to stabilize the specimen. Before moving the specimen to view another grid square, reduce the illumination and magnification to starting levels again.