NANO 703/703L
Lab: TEM Magnification Calibration
Due: Following Lab Session

BACKGROUND

Before we begin to acquire images on the transmission electron microscope (TEM), we should calibrate the magnification. In particular, we should develop a simple approach to determine the scale of an image based on the magnification that is indicated on the microscope. The magnification M gives the ratio of a lateral image dimension H to the corresponding dimension h of the object:

$$M \equiv H/h$$

Rotation or inversion of the image with respect to the object is ignored in this discussion.

The TEM itself has been calibrated such that the indicated value corresponds to M on the fluorescent viewing screen. For example, at $M = 10^5$ (written $10^5\times$, or $100\,\mathrm{K}\times$), an image feature of length $H = 1\ \mathrm{cm}$ on the screen corresponds to an object feature of length $h = 0.1\ \mu\mathrm{m}$ (using $h = H/M$). We will assume that the existing microscope calibration provided by the manufacturer is accurate.

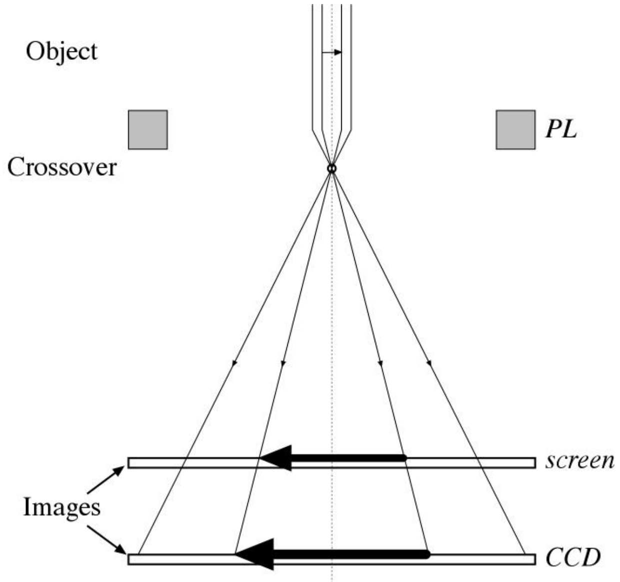

Fig.: Simplified ray diagram showing the relative size of an image on the CCD and viewing screen. *PL* is a projector lens.

A retractable, digital camera (CCD) is mounted above or below the TEM viewing screen; the camera must be inserted to obtain a digital image. The CCD image is actually formed on a phosphor screen. The optical image is directed to the CCD chip with a fiber-optic bundle or prism. The screen determines the effective area of the CCD. The optical image generated on the phosphor screen is coupled to the CCD chip by a lens. The image produced on the phosphor is scaled to produce the image of different size on the CCD chip.

The illumination in the TEM is very nearly parallel to the optic axis, which, leads to very large depth-of-focus (the distance range over which the image remains in focus.) In fact, *any* projection image of a thin object generated from a point source of illumination will have infinite depth-of-focus (see Fig.). Because of this large depth-of-focus, the image focus is the nearly the same in every post-specimen plane. Thus, the image focus on the CCD is essentially identical to that in the plane of the viewing screen.

Magnification in the TEM occurs in stages; each lens produces a magnified image, of which the image of a preceding lens is the object. The final lens in the system is a projector lens, which has a roughly fixed focal length under typical imaging conditions, magnifying the image from the preceding lens by a factor of roughly 20 to $50\times$. The focal length of the projector lens under these conditions is on a scale of about 1 cm, so the focal point is relatively stationary with respect to both the viewing screen and the CCD, which are positioned approximately 30-40 cm below the lens. The magnification on a particular image plane is proportional to the distance from crossover. This allows us to find a simple relationship between the magnification on the CCD and that on the viewing screen.

Magnification Factor

The size H_1 of an image feature on the CCD scales linearly with the size of H_0 of the same feature on the viewing screen, where the proportionality constant is the magnificiation factor F :

$$H_1 = F \cdot H_0$$

Thus, the magnification on the CCD (M_1) is proportional to that on the screen (M_0) by the same factor, i.e.:

$$M_1 = F \cdot M_0$$

Calibration Constant

An object feature with actual size h will produce an image on the CCD with size H_1:

$$H_1 = M_1 \cdot h = n \cdot P$$

where P is the effective pixel size of the CCD, (available from the manufacturer's literature), and n is the size of the image feature in pix.

The calibrated pixel size is then:

$$\frac{h}{n} = \frac{P}{F \cdot M_0} = \frac{C}{M_0}$$

where C is the CCD calibration constant, which is independent of magnification.

A plot of n/h vs. M has slope $1/C$:

$$\frac{n}{h} = \frac{M_0}{C} \qquad (1)$$

Special units can be chosen to help remember the use of C :

$$[C] = \mathrm{nm} \cdot (\mathrm{K}\times)/\mathrm{pix}$$

For example, we often seek the actual size h of an unknown feature with image size n (in pix) at indicated magnification M_0 :

$$h = n \cdot C / M_0$$

Projected Dimensions

In practice, it is useful to note the dimensions of the CCD projected onto the viewing screen. For a CCD with $n_x \times n_y$ square pixels, the dimensions are $(n_x \cdot P) \times (n_y \cdot P)$. The projected dimensions can then be found:

$$L_x \times L_y = (C \cdot n_x) \times (C \cdot n_y) \qquad (2)$$

In this lab, we will test the validity of (5) by measuring n/h at several values of M_0. We will then determine the following:

1) the calibration constant C ;

2) the magnification factor F ;

3) the CCD dimensions projected onto the plane of the viewing screen

GOAL

Determine the magnification calibration constants for the CCD camera on the TEM.

PROCEDURE

Experiment

The calibration sample will be a thin replica of an optical grating, coated with a low density of latex spheres. The grating can be used as an absolute calibration standard; documentation of the grating period is provided by the manufacturer. The absolute calibration at lower magnification will be used to extend the calibration to higher magnification.

Create a table of measurements, as shown below:

M_0 $(K\times)$	$h(\mathrm{nm})$	$n(\mathrm{pix})$	$n/h(\mathrm{pix/nm})$
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Populate the table with values at several magnifications covering a broad range.

Calibration at low magnification is easily accomplished by measuring the span of at least one full grating period. At higher magnifications, a single grating square may extend beyond the field-of-view of the CCD. Use one of the following methods when you reach this range:

i) Create an image montage spanning several grating periods.

ii) Extend the calibration by calibrating an image at the highest range for which calibration data has been obtained. Then measure the size h of a relatively small, but well-defined, feature at the highest calibrated magnification. Increase the magnification to the target value and measure the size n of the same feature in pix. Save both images with the same trial-number index. Record n/h as a single point in the table.

iii) Use lattice fringes of known spacing from a standard specimen (e.g., gold).

Analysis

1) Determination of C:

a) Plot n/h vs. M on either a linear-linear or a log-log graph using plotting software. Label the axes.

b) Fit n/h vs. M to a linear function intercepting the origin (i.e., $y = a \cdot x$). Record the slope $a = 1/C$.

c) Evaluate C from the inverse of the slope. Express C with units of $\mathrm{nm} \cdot (\mathrm{K}\times)/\mathrm{pix}$.

2) Determination of F:

a) Obtain the physical pixel size P (in $\mu\mathrm{m}/\mathrm{pix}$) for the CCD camera from the manufacturer's literature. Record the value.

b) Determine the magnification factor F for the CCD ($F = P/C$). Compare the value of F to unity.

3) Determination of projected CCD dimensions:

a) Obtain the size $n_x \times n_y$ (in $\mathrm{pix} \times \mathrm{pix}$) of the CCD from the manufacturer's literature. Record the size.

b) Determine the projected CCD dimensions $L_x \times L_y$ using (2).

4) Record the values of C, F, and the projected CCD dimensions, as well as P and $n_x \times n_y$.

REPORT

Your report should describe the procedure for measuring the calibration constant for the CCD camera. Include the data in both table and graph form, representative images, and results of the analysis.