

# Easy Guide to Calibrating TEM's and STEM's

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This guide provides an introduction to calibrating transmission electron microscopes (TEM's) and scanning transmission electron microscopes (STEM's), as well as (S)TEM's - instruments that can perform in both modes. While not technically correct, all these instruments will be collectively referred to as TEM's for the rest of this Guide. As with most things, there is a wealth of interesting facts and insights to be discovered by exploring this topic further. More detailed explanations can be found in the classic monographs by Edington [1] or in textbooks such as Williams and Carter [2].

Calibrations are the basis for fair trade, development in science and technology, achievement of product quality and demonstration of conformance to international standards. If a company manufactures automobile cylinders in Japan, pistons in Brazil and piston rings in Germany, calibration is a big issue! In the microscopical world, accurate calibrations are critical for all of these reasons, as well as for publication of your work. Errors in calibration may make your work unreproducible, and hence unpublishable.

Because of their tremendous versatility, large magnification range and large variety of camera constants available, many calibrations are required for a TEM, and accurate TEM calibrations take time. On the bright side, once completed, a calibration should remain accurate until a significant failure or change takes place in the microscope column. It's prudent to verify the calibration of a TEM annually as part of your Quality System. If at any time you suspect that something has gone wrong with the column or the electronics, a few quick measurements with a calibration sample will immediately verify or nullify your suspicions!

There are three major calibrations for TEM's. They are the:

- Magnification calibration (images)
- Camera constant calibration (diffraction patterns)
- Image/diffraction pattern rotation calibration (relationship between the images and diffraction patterns)

Instructions for each are given below, illustrated with examples and 'Helpful Hints'.

## Magnification Calibration

The magnification calibration is the most common calibration. It's important to know if the magnification value on the microscope console or on the image is accurate, and if not, how to correct the value. This calibration procedure is simple in concept. A TEM image is taken of a calibration sample with a known (true) feature size. The calibrated (true) magnification is then calculated by divid-

ing the measured feature size on the image by the known feature size. This calibrated magnification value is then used for accurate calculations of feature size whenever this magnification range is used in the future.

### Example:

Suppose that a sample with a feature that is known to be 1.00 micrometer ( $1.00 \times 10^{-6}$  m) in length is imaged, and the nominal (instrument) magnification is given as 10,000X. If the image is measured digitally, or on a film negative, as being 1.10 cm ( $1.10 \times 10^{-2}$  m), compute the calibrated magnification:

$$\begin{aligned} \text{calibrated magnification} &= \text{measured size} / \text{known size} \\ &= 1.10 \times 10^{-2} \text{ m} / 1.00 \times 10^{-6} \\ &= 11,000X \end{aligned}$$

In the future, any time the 10,000X calibration range is used, the operator will know that the calibrated magnification value is 11,000X.

A table should be assembled containing all the nominal and calibrated magnification values. An example is shown in Table 1. You'll notice that the correction is not consistent across the ranges, because each range requires a different set of lens currents, which are set individually by the manufacturer and require some compromises. This table should be dated and posted by the TEM with copies provided to all users so they can correct their measurements. An added bonus is that your fame as a careful microscopist will spread!

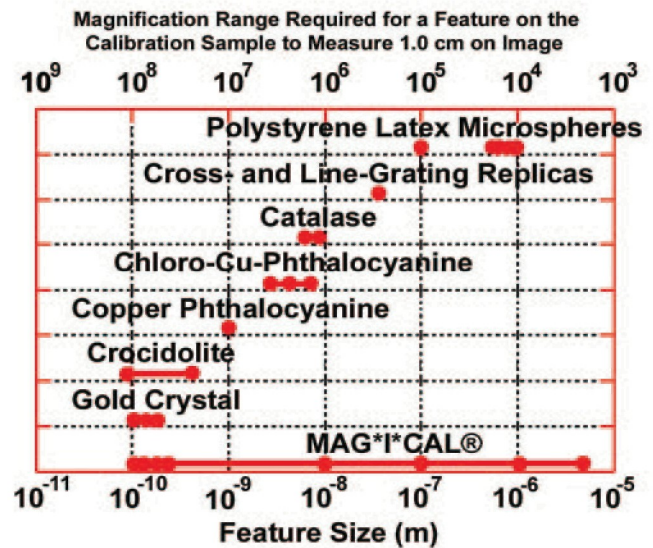


Fig. 1: Some of the TEM calibration samples available for various magnification ranges.

A major asset of TEM's is that over three orders of magnitude in magnification ranges are available. Historically, many magnification calibration samples were required to cover this full range (see Fig. 1). This necessitated 'stitching' all these various sets of calibrations together so that measurements over the complete set of calibration ranges were consistent. If using several calibration samples to cover all the ranges, it is desirable to have two overlapping measurements between each calibration sample. For example, if a catalase sample and a line grating sample were being used to calibrate the middle ranges on a TEM, two overlapping sets of measurements in the ranges between 50,000X and 100,000X would be necessary.

This 'stitching' problem was one of the motivations for the development of the MAG\*I\*CAL® calibration sample. The MAG\*I\*CAL® is made from a single crystal silicon wafer using semiconductor

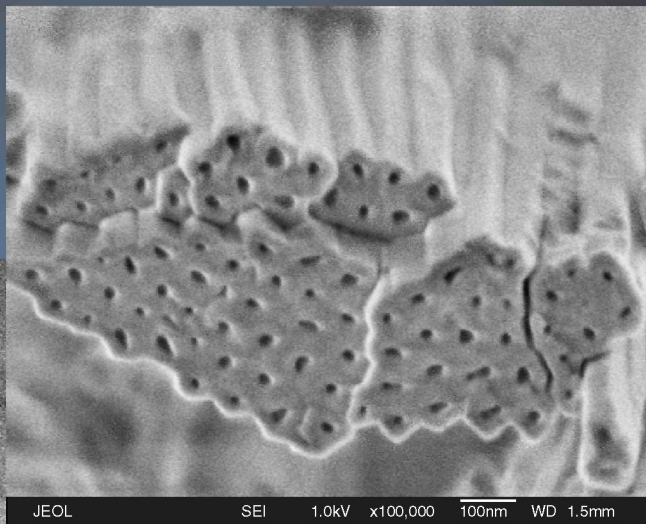
Table 1: Magnification Calibration; 250 keV Philips EM430 TEM, December, 2004.

Nominal	Calibrated	Correction	Nominal	Calibrated	Correction
650,000	647,000	0.995	42,500	44,500	1.047
550,000	508,000	0.923	30,600	30,500	0.997
420,000	378,000	0.900	21,200	21,800	1.028
340,000	347,000	1.021	17,100	17,500	1.023
260,000	267,500	1.023	13,600	14,000	1.029
160,000	161,000	1.006	10,300	10,700	1.039
122,000	124,600	1.021	7400	7750	1.047
88,600	91,200	1.029	5550	5900	1.063
69,000	71,000	1.029	4450	5170	1.162
52,100	54,000	1.036	3900	4200	1.077

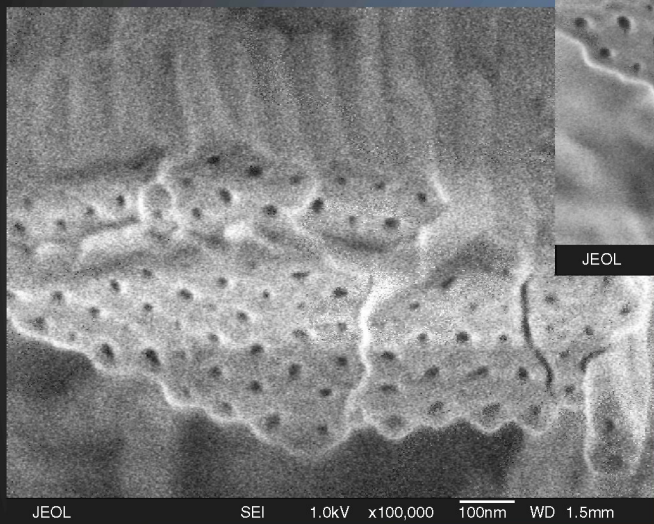
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# Extreme Imaging



Fig. 2: Electron diffraction pattern from single crystal silicon, with the beam parallel to the  $[011]$  zone axis.

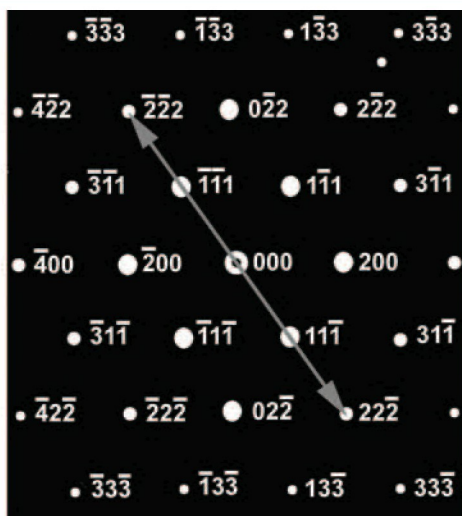


Fig. 3: Indexing of single crystal silicon, from the  $[011]$  beam direction.



Fig. 4: Electron diffraction pattern from an unknown sample, suspected to be aluminum.

technology. The selection of layer thicknesses that make up the calibrated portion of the sample allows it to be used over the entire magnification range of the TEM, ensuring that all calibrations are consistent with each other and saving a substantial amount of time and effort. Since the MAG\**I*\*CAL® is single crystal, it can also be used for the electron diffraction calibrations, described below. Having one calibration sample capable of performing all three of the major TEM calibrations was an additional motivation for its development. Disclaimer: The author of this Guide developed the MAG\**I*\*CAL® calibration sample.

#### Helpful Hints for Magnification Calibration:

- **Sample alignment:** the calibration sample must be perpendicular to the electron beam, or errors will result. A tilted line grating or MAG\**I*\*CAL® sample will result in narrower apparent spacings, and any tilt away from the correct zone axis will make crystal lattice spacings unobservable. Follow the alignment procedure for your calibration sample.
- **Eucentric height:** the calibration sample must be positioned at the eucentric height. This point in the microscope column is fixed and does not change (unless the column or goniometer is mechanically changed). Positioning the sample at this reference point allows the sample to be tilted without changing its height on the optic axis. In addition, objective lens and the intermediate 1 lens (the first lens in the projector system) are then always set to the same value for every sample, so all measurements remain consistent. If there is some difficulty using the eucentric height (top entry STEMs), then standardize on a particular objective lens current, which is another way to provide the same benefits.
- **Hysteresis:** electronic and mechanical devices sometimes overshoot or undershoot when a range is changed, and usually repeat this behavior consistently. When calibrating, start at the highest magnification range and work down to generate consistent hysteresis effects. When acquiring an important image, initially set the TEM to a higher magnification range, and then lower the range to the desired magnification.
- **Overfocus** first, and then focus. This provides fine-tuning of lens hysteresis effects, and will help standardize the lens currents for important measurements.
- **Measure directly** on the film negative if using film. Do not measure from a print. Make several measurements on the nega-

tive and average them, and avoid using the edges of the negative where there can be some distortions from lenses, especially at lower magnifications.

- **Digital images:** there are a wide variety of digital techniques for making measurements. Choose one procedure for all measurements of both calibration samples and test samples, so that all measurements are made in a consistent manner.
- **Verify:** Measure the same feature at 2 or more magnifications and compare the results to avoid errors.
- **Accuracy of the calibration specimen:** - the relative accuracy and reliability of TEM calibrations samples vary. Calibrations using crystal lattice spacings are the most accurate, since measurements are compared with fundamental constants of nature! Ruled gratings are quite accurate but can deteriorate with time and cumulative exposure. Latex spheres can vary significantly in true size, as some types can shrink when placed in an electron beam. Good statistics on spheres (many measurements) are required for accuracy.

#### Camera Constant Calibration

The camera constant calibration is the most common calibration used in electron diffraction. It allows the microscopist to identify the crystal lattice spacings of the material being observed, which helps to identify the material. This identification is accomplished by measuring ring radii of polycrystalline (ring) diffraction patterns, or distances between spots on single crystal (spot) diffraction patterns. This calibration makes use of the Camera Constant equation (derived from Bragg's law with the small angle approximation):

$$\lambda L = d_{hkl} R$$

where:

$\lambda$  = wavelength of the TEM accelerating voltage (nm)

$L$  = camera length (mm)

$d_{hkl}$  = lattice spacing (nm)

$R$  = measured diffraction ring radius from a polycrystalline sample, or measured distance between two adjacent diffraction spots from a single crystal sample (mm)

Note that this definition is set up for nm-mm, but there are many choices for units. For example, a furlong-lightyear is OK, but not recommended! With digitized images, Angstrom-pixel or nm-pixel are good choices.



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The product  $\lambda L$  is called the Camera Constant. While the exact values for ' $\lambda$ ' and ' $L$ ' are difficult to measure accurately, values for both ' $d_{hkl}$ ' and ' $R$ ' are easily acquired by making careful measurements of ' $R$ ' and using a calibration sample with a known value of ' $d_{hkl}$ '. A table should be assembled of Camera Constant values for each combination of TEM accelerating voltage and each nominal camera length ' $L$ ' (the camera length as given on the console). This can be quite time consuming, since we need a complete series of diffraction patterns at all camera lengths for each accelerating voltage. Some microscopists perform all of their diffraction measurements at a favorite accelerating voltage, to cut down on the number of tables needed.

Once this table is complete, the microscopists know that any time they choose a particular camera length ' $L$ ', they can divide that Camera Constant value by the measured diffraction spot spacing or ring radius of their sample, and accurately determine the crystal lattice spacing of that material. This table should be distributed along with the magnification calibration table, as an aid your colleagues and to enhance your now legendary stature! An example will (we hope) make this more understandable.

#### Example:

This example uses a single crystal calibration sample. The calibration can also be performed with a polycrystalline sample. Figure 2 shows a positive image of an electron diffraction pattern taken of single crystal silicon (the MAG\*I\*CAL® sample) with the beam parallel to the [011] crystal zone axis (defined as the [011] beam direction). The diffraction pattern was taken at a nominal camera length of 1650 mm. Figure 3 shows a set of indexed (identified) diffraction spots of silicon calculated for the [011] beam direction, which we can use as a map for identifying the spots on the diffraction pattern. By examining the patterns carefully (notice this is not a 'square' pattern), we can identify the unknown spots in Fig. 2 as lying along one of the {111} systematic rows, as indicated by the double-arrowed line in both images. The double-arrowed line in Fig. 2 indicates a distance ' $x$ ' that is four times the distance between two adjacent {111} spots, so  $R = x/4$ . Our measurement of Fig. 2 gave a value of 44.0 mm, so ' $R$ ' = 11.0 mm. We know that the (111) lattice spacing of silicon is equal to 0.314 nm (available in Handbook of Chemistry and Physics [3], as well as many other references). Therefore, to find the Camera Constant for  $L = 1650$  mm:

$$\begin{aligned} L &= d_{111} R \\ &= 0.314 \text{ nm} \times 11.0 \text{ mm} \\ &= 3.45 \text{ nm-mm} \end{aligned}$$

This value was entered into Table 2, along with the same calculation for the other camera lengths available with this instrument. These values can also be graphed, and the results will be linear if the manufacturers' values for the camera lengths are accurate.

Now that we have our table, we can apply this to real problems. Figure 4 shows a positive image of a polycrystalline diffraction pat-

**Table 2: Camera Constants for a Philips EM430T TEM, operating at 250 kV, December, 2004).**

Camera Length (mm)	$\lambda L$ (nm*mm)	Camera Length (mm)	$\lambda L$ (nm*mm)
270	0.493	1650	3.45
350	0.664	2200	4.63
500	0.986	2900	6.13
700	1.414	3600	7.63
950	1.950	5000	10.63
1200	2.49	6300	13.41

tern acquired from an unknown material at a camera length of 950 mm. The material was suspected to be aluminum. We measure the diameter of each of the first 4 rings counting from the center - the ring marked with the arrows is counted as the third. We then divide these four measurements by 2, since we need the ring radius for the Camera Constant equation. The lattice spacing ' $d$ ' can be determined by dividing the Camera Constant for  $L=950$ , (1.950 nm-mm, from the table) by ' $R$ ', the measured radius of 13.6 mm:

$$\begin{aligned} L &= d_{hkl} R \\ \text{or} \\ d_{hkl} &= L / R \\ &= 1.950 \text{ nm-mm} / 13.6 \text{ mm} \\ &= 0.1434 \text{ nm} \end{aligned}$$

In this way, we produced table 3. The calculated lattice spacing ' $d_{hkl}$ ' from our measurements of is given in column 3. Column 4 lists crystal reflections in aluminum, and column 5 gives the corresponding values of lattice parameters for Al. The match is reasonably good. We concluded that the material was polycrystalline aluminum.

**Table 3: Indexed electron diffraction rings from an unknown sample, compared to polycrystalline aluminum**

	Radius ' $R$ ' (unknown)	Calculated lattice spacing ' $d_{hkl}$ ' (unknown)	Reflection hkl (Al)	Known lattice spacing $d_{hkl}$ (Al)
Ring 1	8.3 mm	0.2349 nm	(111)	0.2338 nm
Ring 2	9.6 mm	0.2031 nm	(200)	0.2025 nm
Ring 3	13.6 mm	0.1434 nm	(220)	0.1432 nm
Ring 4	16.0 mm	0.1219 nm	(311)	0.1221 nm

**Helpful Hints; Camera Constant Calibration:** • **Eucentric height:** the sample must be positioned at the TEM's eucentric height. If that is not possible, then standardize on a particular objective lens current so all images are taken with the sample at the same height in the microscope column.

- **Alignment:** For single crystal calibration samples, ensure that the sample is aligned so that the beam is parallel to the zone axis of the crystal.
- **Hysteresis:** Start at highest camera length and work down to provide consistency and to avoid hysteresis effects. When taking an important diffraction pattern, initially set at a higher camera length, then lower the setting to the desired camera length.
- **(S)TEM:** If your instrument is a true (S)TEM, the Camera Constant calibrations may vary depending on whether you are in STEM or TEM mode. Check this.
- **For diffraction patterns:** the intermediate 1 lens needs to be focused properly on the diffraction pattern. There are several methods to do this and both the standard and unknown must be focused in the same way. In the first way, the condenser is set fully clockwise and then the diffraction spots are focused to the smallest size. In the second way, the objective aperture is inserted into the diffraction pattern and its edge is set to be as sharp as possible. In the third way, a focused convergent beam pattern is formed and the shadow edge of the condenser aperture is focused. Equivalently, if HOLZ lines are present, they are focused. This is the best way because it finds the back focal plane of the objective lens.

#### Image / Diffraction Pattern Rotation Calibration

The image / diffraction pattern rotation calibration is used to identify the orientation of crystalline material relative to other crystals, features or interfaces in your sample. For this calibration, it is

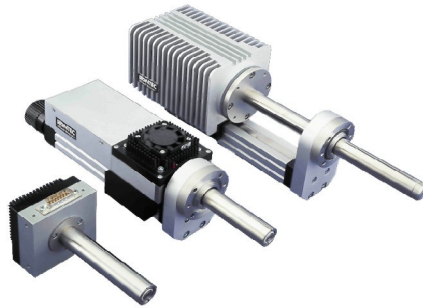
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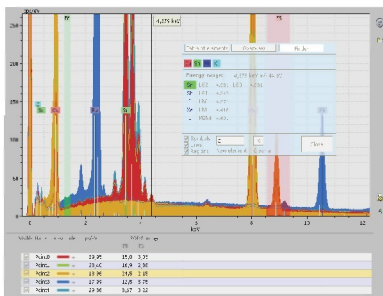


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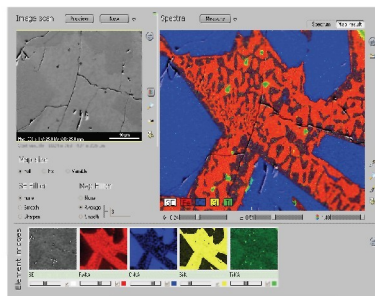
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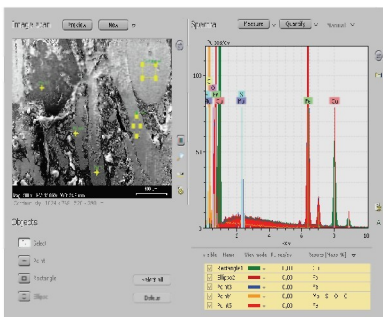
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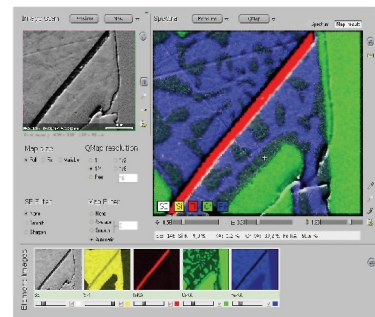
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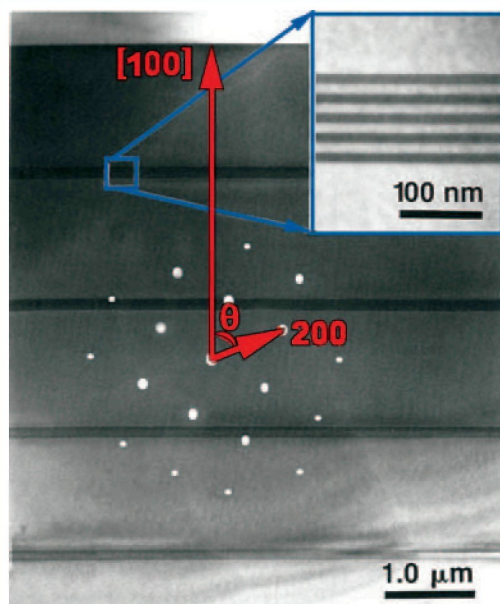


Fig. 5: Double exposure of the image and the electron diffraction pattern from a single crystal silicon region of the MAG\*I\*CAL sample.

necessary to take double exposures of the calibration sample image plus its diffraction pattern, at each magnification range and at each camera length for every accelerating voltage of interest. This is a lot of images! Fig. 5 shows an example of a double exposure of the MAG\*I\*CAL calibration sample. In Fig. 5, the [100] direction is 'up' in the image, but at an angle of about 67° to the (200) reflection (spot) shown on the diffraction pattern. It's important to read the instructions for these calibration samples, because symmetries can cause errors of 180° and/or other angles, depending on the sample. One technique that eliminates this ambiguity can be used with single crystal calibration samples that have distinct features, like the MAG\*I\*CAL or MoO<sub>3</sub> (below). If a convergent beam pattern of the sample is obtained and the condenser is then underfocused (crossover below the sample), a shadow image of the sample will appear in the central (bright field) spot, and the orientation of the sample relative to the diffraction pattern can be recorded.

MoO<sub>3</sub> crystals (which the adventurous can make themselves with ammonium molybdate smoke) can also be used for this calibration, as shown in Fig. 6. The (001) reflection corresponds to the long edge of the crystals [1]. A different relationship may exist for each magnification and camera constant combination, depending on the TEM. There's lots of interesting crystallography here concerning forbidden reflections and the like--see [2] or any other good materials science text.

A table is made of all magnification ranges (for the images) versus all camera constants (for the electron diffraction patterns) to display this data. Choose a standard way of presenting the measurements, such as: "Angle  $\theta$  measured counter-clockwise from diffraction spot to image direction", and include this information with your chart. This will help avoid symmetry errors. See Table 4 for an example. As mentioned, this is a lot of measurements - this table is for only a single accelerating voltage!

**Helpful Hints; Image / diffraction pattern rotation calibration:**

- All of the 'Helpful Hints' for both images and diffraction patterns apply.

Choose the single most useful accelerating voltage and only the most useful magnification ranges and camera lengths, to keep the amount of measurements down to a manageable level. When measuring an unknown sample, make sure to set the instrument to the parameters that are calibrated.

- Choose the single most useful accelerating voltage and only the most useful magnification ranges and camera lengths, to keep the amount of measurements down to a manageable level. When measuring an unknown sample, make sure to set the instrument to the parameters that are calibrated.

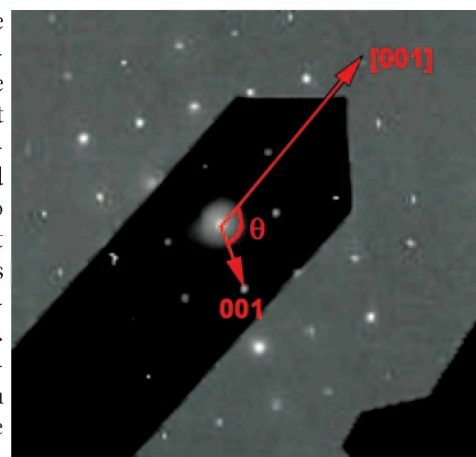


Fig. 6: Double exposure of the image and the electron diffraction pattern from a MoO<sub>3</sub> crystal.

If you have made it this far through the Guide, you should now have a good idea of what's necessary for accurate TEM calibrations. These calibrations take time, but once completed, will make your measurements more accurate and reliable. They will also endear you to all other users of your instrument, so share them with pride! Special thanks to Scott Walck for editing and for adding some of the better lines! This Guide is also available on the website: <http://mag-i-cal.ca> . ■

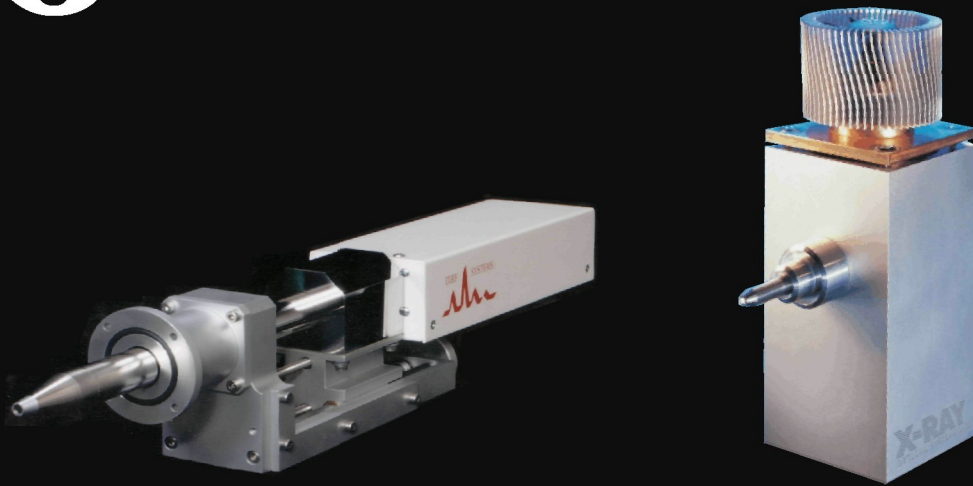
**References**

- [1] J.W. Edington, *Practical Electron Microscopy in Materials Science*, Van Nostrand Reinhold, New York (1976).
- [2] D.B. Williams and C.B. Carter, *Transmission electron Microscopy*, Plenum Press, New York (1996).
- [3] CRC Handbook of Chemistry and Physics, CRC Press, Inc., Boca Raton, Florida 33431 (any year)

**Table 4: Image / diffraction pattern rotation calibration; 250 keV, Philips EM430T**

Angle $\theta$ counterclockwise from diffraction spot to image direction									
Camera Length (mm)	Magnification								
	5550	9400	10300	13600	17100	21200	30600	42500	52100
270	61.0	62.0	67.0	64.5	68.0	63.0	95.5	328.5	330.0
350	49.5	50.5	55.5	53.0	56.5	51.5	84.0	317.0	318.5
500	15.0	16.0	21.0	18.5	22.0	17.0	49.5	282.5	284.0
700	142.0	143.0	148.0	145.5	149.0	144.0	176.5	49.5	51.0
950	139.5	140.5	145.5	143.0	146.5	141.5	174.0	47.0	48.5
1200	135.5	136.5	141.5	135.0	142.5	137.5	170.0	43.0	44.5
1650	130.5	131.5	136.5	134.0	137.5	132.5	165.0	38.0	35.5
2200	123.0	124.0	125.0	126.5	130.0	125.0	157.5	30.5	32.0
2900	115.0	116.0	121.0	118.5	122.0	117.0	149.5	22.5	24.0
3600	105.5	106.5	111.5	107.0	112.5	107.5	140.0	13.0	14.5
5000	86.0	87.0	92.0	89.5	93.0	88.0	120.5	353.5	355.0
6300	64.0	65.0	70.0	67.5	71.0	66.0	98.5	331.5	333.0
Camera Length (mm)	Magnification								
	69000	88600	132000	160000	260000	340000	420000	550000	650000
270	331.5	338.5	339.5	337.0	18.0	41.0	65.0	49.5	85.5
350	320.0	327.0	328.0	325.5	6.5	29.5	53.5	38.0	72.0
500	285.5	292.5	293.5	291.0	332.0	355.0	19.0	3.5	37.5
700	52.5	59.5	60.5	58.0	99.0	122.0	146.0	130.5	164.2
950	50.0	57.0	58.0	55.5	96.5	119.5	143.5	128.0	162.0
1200	46.0	53.0	54.0	51.5	92.5	115.5	139.5	124.0	158.0
1650	41.0	48.0	49.0	46.5	87.5	110.5	134.5	115.0	153.0
2200	33.5	40.5	41.5	39.0	80.0	103.0	127.0	111.5	145.5
2900	25.5	32.5	33.5	31.0	22.0	45.0	113.0	103.5	137.5
3600	16.0	23.0	24.0	21.5	62.5	85.5	109.5	94.0	128.0
5000	356.5	3.5	4.5	2.0	43.0	66.0	90.0	74.5	105.5
6300	334.5	341.5	342.5	340.0	21.0	44.0	68.0	52.5	86.5

# got SEM?



Add MicroX-ray Fluorescence to any Scanning Electron  
Microscope.

Complements:

OXFORD.....

EDAX.....

NORAN.....

PGT.....

