Scientific Technical Development Co. Ltd.

ISO 9001:2015 CERTIFIED

MAG*I*CAL®

Calibration Reference Standard for TEM

Instruction Book

Please read thoroughly before use!

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TECHNOORG LINDA

MAG*I*CAL® Reference Standard for TEM

FRAGILE !

The MAG*I*CAL® Calibration Reference Standard that you are receiving now looks like Figure A, below. If not handled carefully, it will look like Figure B. This Standard is only about 60 µm thick (about the diameter of a human hair) even at the thicker outer edge. It is held safely in place in a strong titanium grid, but if you touch the central silicon portion with your tweezers, the standard can shatter. Please be careful! Vacuum tweezers on the Standard edge are recommended. If not available, use very fine regular tweezers VERY carefully and gently.

For proper handling and to avoid damaging your MAG*I*CAL calibration sample please visit our website at www.technoorg.hu to watch the tutorial video.

Fig. A: MAG*I*CAL® Standard as shipped. Diameter of central perforation is ~ 0.1 mm

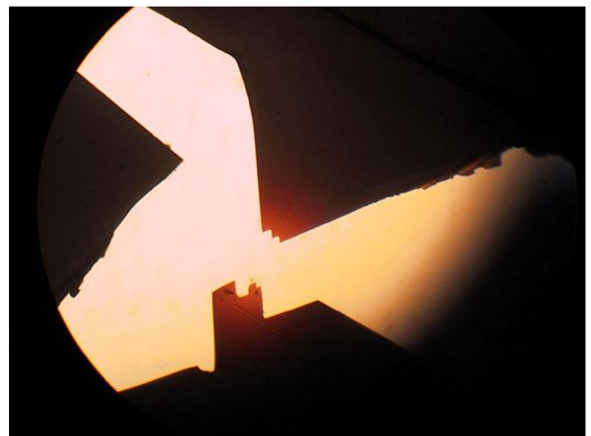

Fig. B: This Standard was damaged by lack of care while handling with tweezers.

Photographs courtesy of South Bay Technology, Inc.

MAG*I*CAL® Reference Standard for TEM

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The MAG*I*CAL® Calibration Reference Standard for transmission electron microscopy (TEM) is the Gold Standard for TEM calibration, and acknowledged in the Guinness Book of Records as the “World’s Smallest Ruler”.

The MAG*I*CAL® was developed in a world-class Solid State Physics laboratory during the mid-1990’s to perform the three major calibrations for transmission electron microscopy:

- the image magnification calibrations,
- the camera constant calibrations (indexing diffraction patterns),
- the image/diffraction pattern rotation calibrations.

Please read the instructions carefully to enjoy the full capabilities of the MAG*I*CAL®.

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MAG*I*CAL® Reference Standard for TEM

Reference Standard Serial No.: Date:

Certificate of Calibration

The MAG*I*CAL® calibration reference standard is a cross-sectional TEM sample of a silicon-based semiconductor multilayer. It consists of five sets of five ~10 nm thick $Si_{0.81}Ge_{0.19}$ alloy layers, alternating with ~10 nm thick pure silicon layers. The device-quality epitaxial layers were grown by Molecular Beam Epitaxy (MBE) as strained layers on a single crystal silicon <001> substrate. All calibrated values incorporate the strain affects.

The four sets of alternating layers (superlattices) provide dark and light contrast in the TEM, and were directly calibrated by high resolution transmission electron microscopy (HREM) in reference to the {111} lattice spacing of silicon, as measured on the single crystal silicon substrate. This spacing is known to be 0.313 560 11 (8) nm [1]. The uncertainty in measurement across each of the full superlattices is less than one atomic layer at both the top and bottom interfaces: Δt (superlattice) < 0.5%.

The variation in layer thicknesses across the wafer material used for the current series of MAG*I*CAL® calibration reference standards was measured by double crystal x-ray diffraction (DCXRD) mapping as < 1%. The estimated uncertainty in all sets of calibrated values (other than the individual thin SiGe and Si layers) assumes a normal distribution, and is the combination of all uncertainties added in quadrature. The **overall uncertainty** on the calibrated values listed on the 'Layer Thickness Values' sheet is:

$$\Delta t < 2\ \%$$

Multiplying the overall uncertainty by a coverage factor of k=2 gives a confidence level of approximately 95%. An user can fine-tune the calibrated values and decrease the uncertainty of their individual calibration reference standard by verifying the calibrated values with reference to the Si {111} lattice spacing in the substrate.

Replace the MAG*I*CAL® Calibration Standard three years after the date on this Certificate of Calibration, or earlier if contaminated. One year limited warranty on defects in manufacture. Replacement or refund provided by the distributor.

[1] CRC Handbook of Chemistry and Physics, CRC Press, Inc., Boca Raton, Florida 33431

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TECHNOORG LINDA

MAG*I*CAL® Reference Standard for TEM

Certificate of Traceability

The MAG*I*CAL® calibration reference standard is manufactured from a single-crystal silicon wafer, and so incorporates a fundamental constant of nature into the standard itself - the crystal lattice constant of silicon. All calibration markings on the standard are directly referenced to this natural constant. The calibrated values can be verified by the user by observing the crystal lattice image of the silicon substrate and validating the calibrated values.

National Metrology Institutes (NMI's) such as NIST in the USA, NPL in Great Britain, etc., currently do not certify any measurements less than approximately 0.1 micrometers. NMI's certify measurements as traceable to fundamental constants of nature, but they do not certify natural constants. The MAG*I*CAL® calibration reference standard does not require NMI certification since the calibration is directly traceable to a natural constant available on the standard itself (the crystal lattice spacing of silicon) and all calibrated values can be verified by the user. The crystalline lattice spacing is an intrinsic property of a material. For pure silicon, the spacing has been well characterized and documented by the scientific community. It is known to better than 8 decimal places (0.313 560 136 (8) nm [1].). The current, best-known value can be obtained from a recent CRC Handbook of Chemistry and Physics [1], and many other references.

Documents that request a reference to traceability for the MAG*I*CAL® reference standard should be filled in with "*natural constant*" or "*directly traceable to a constant of nature [1]*".

[1] CRC Handbook of Chemistry and Physics, CRC Press, Inc., Boca Raton, Florida 33431

Aligning the Reference Standard . . .

The MAG*I*CAL® calibration reference standard is made from a single crystal of silicon, and therefore has many very useful attributes as a TEM standard. To take advantage of these attributes, mount the standard in the TEM sample holder with the epoxy line of the standard parallel to the stem of the TEM sample holder (see Fig. 2, next page). Translate the standard to bring into view the calibration marks close to the thin edge near the perforation in the center of the sample (arrows, Fig. 2), and focus. Put the TEM into diffraction mode, choose the smallest camera length, and adjust the intensity control (condenser control) back and forth to find the proper value of over-focus that forms the striking pattern called a Kikuchi pattern, similar to Fig. 1 below, but perhaps rotated. These Kikuchi bands are formed by elastic Bragg scattering of previously inelastically scattered electrons.

The intensity control can be adjusted so that either a diffraction spot pattern or a Kikuchi pattern can be observed. The standard should be tilted to move the Kikuchi pattern so that the central “intersection” in this diagram (where the largest number of bands intersect) is centered on the brightest (zero order) diffraction spot in the electron diffraction pattern (adjust the intensity control back and forth). It may be necessary to translate the standard to slightly thicker or thinner regions to get a clear Kikuchi pattern.

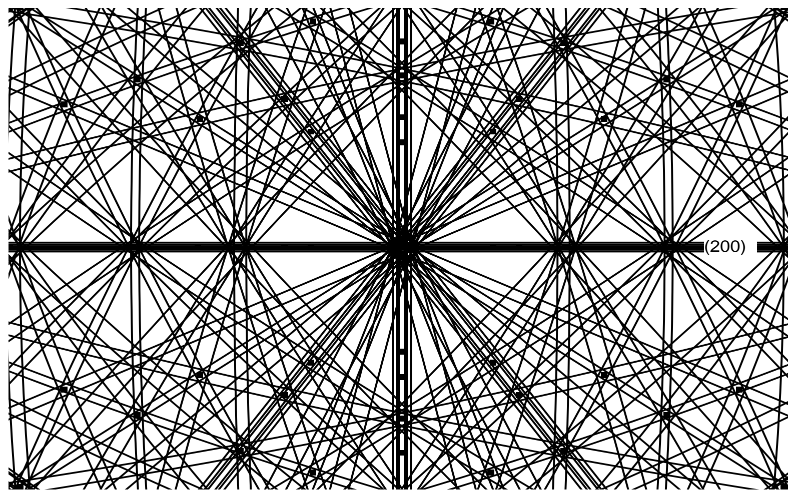


Fig. 1: Kikuchi pattern of single crystal silicon viewed down the <011> zone axis. The broad horizontal band in the center of this figure is between the {200} Kikuchi lines.

MAG*I*CAL® Reference Standard for TEM

Image Magnification Calibration

The MAG*I*CAL® calibration reference standard for transmission electron microscopy (TEM) consists of an ion-milled cross-section of a silicon wafer on which a series of calibration marks were deposited. The spacings between these calibration marks are very accurately known, and are included on the accompanying sheet entitled “LAYER THICKNESS VALUES”. There are four regions on the standard that contain the calibration marks. These four areas are marked by arrows on both the drawing and the photograph in Fig. 2, two on either side of the thin epoxy line which joins two pieces of the silicon wafer which are mounted face-to-face. The central perforation in the standard is small, as shown in the drawing (approximately 0.1 mm in diameter - see the color image). Note that the epoxy line is very thin.

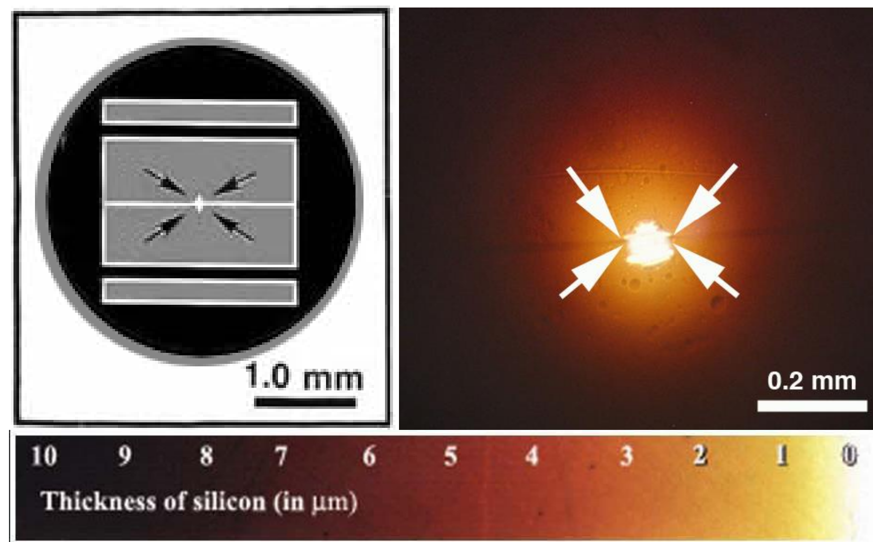


Fig. 2: Diagram, photograph and color/thickness scale of the MAG*I*CAL® reference standard. The arrows on the drawing and photograph indicate the four regions on the standard where the calibration marks may be found.

The magnification calibration is the most common calibration, since it is 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 reference standard with a known (true) feature size. The calibrated (true) magnification is then calculated by dividing 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 (see example below).

STEP 1: Mount the MAG*I*CAL® in the TEM sample holder so that the MAG*I*CAL® epoxy line is parallel to the stem of the TEM sample holder.

STEP 2: Find one of the regions of interest (marked by arrows in Fig. 2) by translating the standard in the microscope, and focus on the layered structure (Fig. 3).

STEP 3: The sample height should be adjusted so that the MAG*I*CAL® is at the eucentric position (see TEM manual). On microscopes with no sample height adjustment, the objective lens current must be monitored and kept constant at each magnification value.

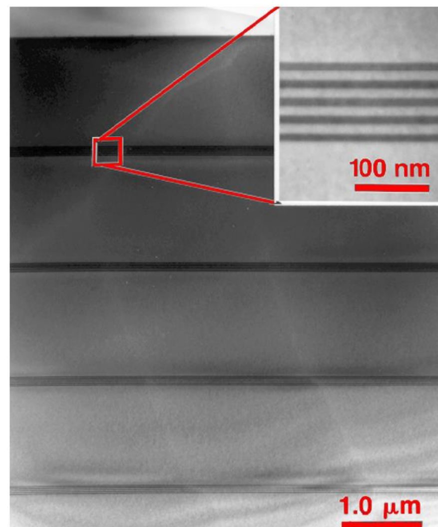


Fig. 3: TEM micrograph of the MAG*I*CAL®.
Inset shows a higher magnification image of one of the layered structures. Four identical structures are found at each of the four locations shown by arrows in Fig. 2.

STEP 4: Switching to diffraction mode, tilt the MAG*I*CAL® so that the electron diffraction pattern or the Kikuchi pattern is exactly centered. The earlier page, "Aligning the Reference Standard) gives more detail on how to do this. The TEM electron beam should be exactly perpendicular to the MAG*I*CAL® reference standard (the TEM beam being parallel to the crystal zone axis of the silicon crystal material which forms the MAG*I*CAL®). If using the Kikuchi pattern, the image should look similar to Fig. 4.

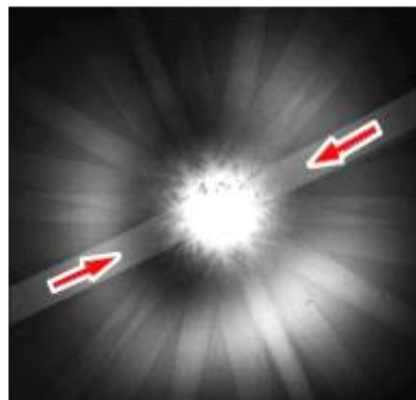


Fig. 4: Kikuchi pattern of the [011] crystal zone axis of Si.
The arrows show the location of the broad band between the {200} Kikuchi lines. When the electron diffraction pattern is centered anywhere along this band, the electron beam will be parallel to the layered structure, and result in accurate layer thickness values.

STEP 5: Focus the microscope on the region shown in Fig. 3 at the highest magnification range available. A series of micrographs or digital images at all magnification ranges should be taken starting at the highest magnification range and working down to the lowest. The measured length on the micrograph or digital image can then be compared to the calibrated values on the attached sheet labeled "Layer Thickness Values".

STEP 6: The most accurate calibrations at the highest magnification ranges (> 400,000) can be accomplished by forming a lattice image of the Si material below the layered structure (Fig. 5). Measure the *perpendicular* distance across a large number of (111) lattice fringes, then divide this length by the number of fringes. This gives the measurement on the micrograph or digital image that corresponds to the (111) lattice spacing of Si (0.31356011 nm.).

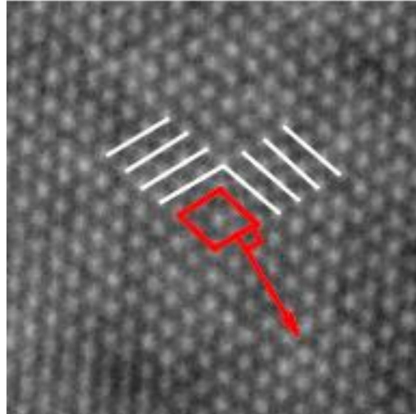


Fig. 5: Lattice image of Si viewed down the [011] zone axis, recognizable by the central diamond shape formed by the rows and columns of Si atoms. The lines marked parallel to the upper two sides of the diamond shape indicate (111) planes, with an interplanar spacing of 0.3135428 nm. Note that measurements must be made perpendicular to the (111) planes, since the planes viewed from this zone do not meet at right angles.

STEP 7: Make up a table of calibration values similar to Table 1 below, recording the Nominal Magnification (the magnification given on the TEM) with the Calibrated Magnification (the actual magnification determined by comparing the measured feature size from your micrograph or digital image, and the actual feature size, as taken from the attached sheet labeled "Layer Thickness Values").

Table 1: Magnification Calibration; 250 keV

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	7,400	7,750	1.047
88,600	91,200	1.029	5,550	5,900	1.063
69,000	71,000	1.029	4,450	5,170	1.162
52,100	54,000	1.036	3,900	4,200	1.077

Example:

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

calibrated magnification = measured size / known size
= $1.10 * 10^{-2}$ m / $1.00 * 10^{-6}$
= 11,000X

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

Helpful Hints for Magnification Calibration:

- **Sample alignment:** the calibration reference standard must be perpendicular to the electron beam, or errors will result. A tilted MAG*I*CAL® reference standard will result in narrower light contrast material spacings, and broader dark contrast material spacings. Any tilt away from the correct zone axis will make crystal lattice spacings unobservable. Follow the procedure given in 'Aligning the Reference Standard'.
- **Eucentric height:** the reference standard must be positioned at the eucentric height. This point in the microscope column is fixed and does not change. Positioning the standard at this reference point allows the standard to be tilted without changing its height on the optic axis, so it stays in focus. In addition, the 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 lower ranges, generating 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 negative and average them, and avoid using the edges of the negative where there can be 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 reference standards 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.

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 distances between spots on a single crystal (spot) diffraction pattern, or ring diameters on a polycrystalline diffraction pattern. 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:

λ = wavelength of the TEM accelerating voltage (nm)
L = camera length (mm)
d_{hkl} = lattice spacing (nm)
R = measured distance between two adjacent diffraction spots on a single crystal diffraction pattern or half the diameter on a polycrystalline diffraction pattern (mm)

Note that this definition is set up for nm-mm, but there are many choices for units. With digitized images, Angstrom-pixel or nm-pixel are good options.

The product 'λL' is called the Camera Constant. While the exact values for 'λ' 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 reference standard with a known value of 'd_{hkl}'. A table similar to Table 2 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 of their sample, and accurately determine the crystal lattice spacing of that material. An example may make this more understandable.

Table 2: Camera Constants for a TEM, operating at 250 kV.

Camera Length (mm)	λL (nm*mm)	Camera Length (mm)	λL (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
1,200	2.490	6300	13.41

Example:

Fig. 6 shows a positive image of an electron diffraction pattern taken of single crystal silicon (the MAG*I*CAL® reference standard) 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. Fig. 7 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 spots in Fig. 6 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. 6 indicates a distance 'x' that is four times the distance between two adjacent {111} spots, so R=x/4. Our measurement of Fig. 6 gave a value of 44.0 mm, so 'R'=11.0 mm. We know that the (111) lattice spacing of silicon is nominally equal to 0.314 nm (available in Handbook of Chemistry and Physics [1], as well as many other references). Therefore, to find the Camera Constant for 'L'=1650 mm:

$$\begin{aligned} \lambda L &= d_{111}\, R \\ &= 0.314 \text{ nm} * 11.0 \text{ mm} \\ &= 3.45 \text{ nm} * \text{mm} \end{aligned}$$

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.

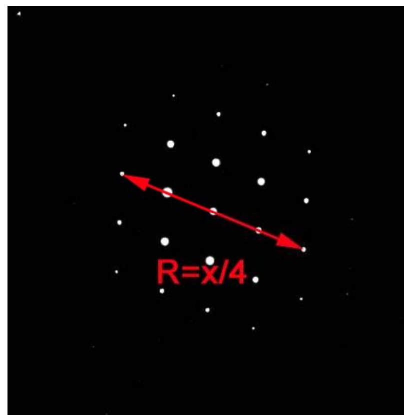


Fig. 6: Electron diffraction pattern from single crystal silicon, with the beam parallel to the [011] zone axis.

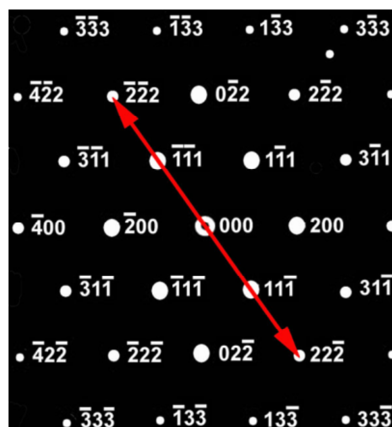


Fig. 7: Indexing of single crystal silicon diffraction pattern, with the beam parallel to the [011] zone axis.

Helpful Hints for Camera Constant Calibration:

- **Eucentric height:** the standard 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 the MAG*I*CAL® and single crystal TEM 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, and 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 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.

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 necessary to take double exposures of the reference standard image plus the reference standard diffraction pattern, at each magnification range and at each camera length for every accelerating voltage of interest. This is a lot of images!

Fig. 8 shows an example of a double exposure of the MAG*I*CAL® reference standard (plus an inset image showing the detail of one of the layered structures). In Fig. 8, 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 (see Figs. 6 and 7). To measure the rotation calibration values, draw a line from the zero order diffraction spot up through the (200) spot. Also draw a line from the zero order spot in a direction perpendicular to the layered structure. The angle θ between these two lines is the rotation calibration value for this magnification/camera length condition, and can now be used to identify crystal direction in unknown samples, when the images and diffraction patterns of the unknown material are taken at the same magnification/camera length condition.

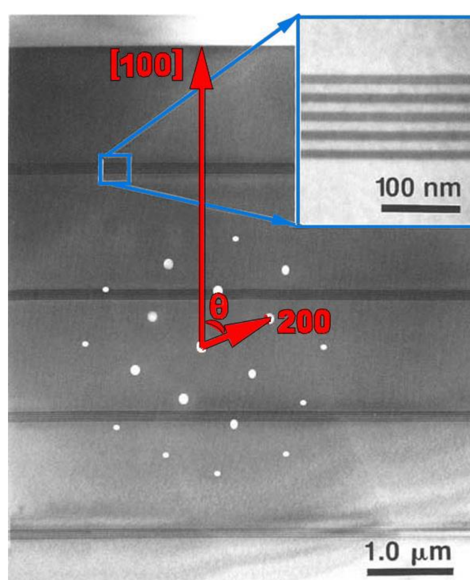


Fig. 8: Double exposure of the image and the electron diffraction pattern from a single crystal silicon region of the MAG*I*CAL® reference standard.

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 θ measured counter-clockwise from diffraction spot to image direction",* and include this information with your chart. This will help avoid symmetry errors. See Table 3 for an example. As mentioned, this is a lot of measurements - this table is for only a single accelerating voltage!

Table 3: Image / diffraction pattern rotation calibration – 250 keV.

Angle θ counterclockwise from diffraction spot to image direction									
MAGNIFICATION >	**5550**	**9400**	**10300**	**13600**	**17100**	**21200**	**30600**	**42500**	**52100**
CAMERA LENGTH									
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
MAGNIFICATION >	**69000**	**88600**	**132000**	**160000**	**260000**	**340000**	**420000**	**550000**	**650000**
CAMERA LENGTH									
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

Symmetries can cause errors of 180° and/or other angles – see Fig. 7. Two techniques that eliminate this ambiguity can be used with the MAG*I*CAL® reference standard. First, to confirm the identity of the (200) diffraction spot, slowly translate the standard 'up' through the layered structure and past the top surface while viewing the diffraction pattern. As the standard is translated past the top surface into empty space, the diffraction pattern will fade and finally disappear first from the direction corresponding to the (200) diffraction spot. Second, if a convergent beam pattern of the standard is obtained and the condenser is then underfocused (crossover below the standard), a shadow image of the standard will appear in the central (bright field) spot, and the orientation of the standard relative to the diffraction pattern can be recorded.

Helpful Hints for Image / diffraction pattern rotation calibration:

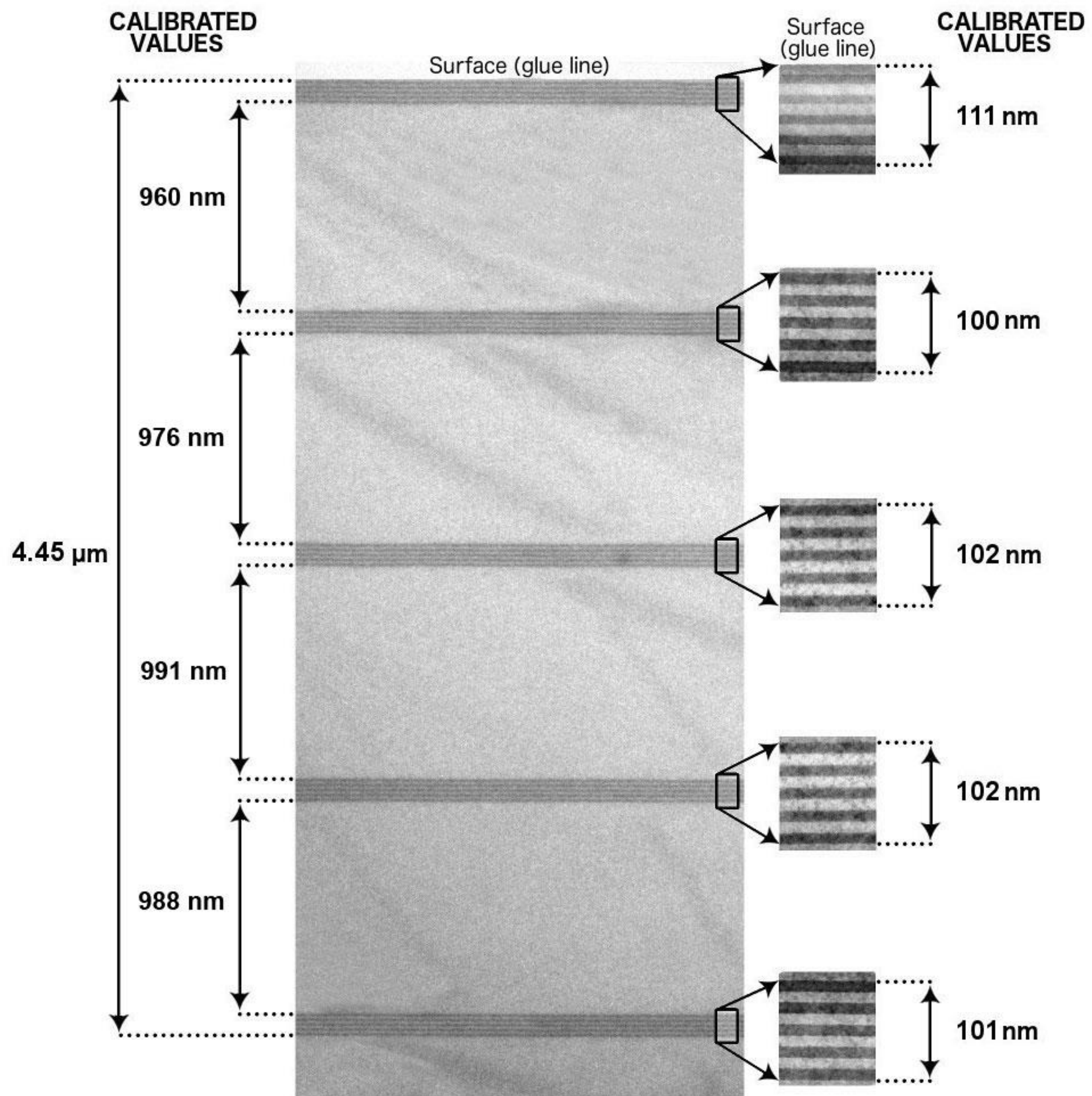
- All of the 'Helpful Hints' for both images and diffraction patterns apply.
- Choose the single most useful accelerating voltage and only the most useful magnification ranges and camera lengths, to keep the amount of measurements down to a manageable level. When measuring an unknown sample, make sure to set the instrument to the same parameters that were used for the calibration.

MAG*I*CAL® Reference Standard for TEM

Layer Thickness Values

Rev. 5

Calibrated values = ± 2%



To improve accuracy when making measurements with CALIBRATED VALUES, always measure across the largest distance visible on MAG*I*CAL® Calibration Standard. For example, at high magnification, always measure across a complete superlattice (the five darker bands and four lighter bands in bright field image). At lower magnifications, measure across one or several of the 0.xx µm layers, remembering to add in the thickness of any intervening superlattices.

For accurate measurements of structures of 100 nm or less, the TEM must be calibrated against the (111) lattice spacing of Si available on the MAG*I*CAL calibration standard.