Calibration Of Electron Microscopes: How To Do This, How Often, Pit-falls And Problems. M&M 2001 Experts' Session on Core Facility Management

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This article is the third of a series transcribed from the discussion taped during the Core Facility Management session. Bulleted paragraphs indicate comments by individuals attending the session.

D. Sherman: The next topic we are going to deal with has to do with calibration of electron microscopes. This became very important in my situation because we started dealing with people with nanotubes and nanovesicles and with very small things where they needed to have accurate size both in the SEM and the TEM magnification range. My own frustrations dealing with calibration, especially in the in between magnification, spurred me on to ask a couple of experts to give us some insight on what we should be doing with calibration – the problems associated with this and some general information about how to go about doing this in our standard facilities where money is short. We have to have samples that we can use to get the most out of our instruments and that we can put into the hands of students and other users. So with this in mind I asked two individual people to speak based on their expertise, Dr Michael Postek and Dr. John McCaffrey. Unfortunately I was unable to transcribe the taped dialog of Michael Postek's portion on SEM calibration. We hope that Michael will be able to contribute his portion at a later date.

Dr. John McCaffrey will discuss calibration as related to the transmission electron microscope. John has spent most of the past 16 years as the senior microscopist at the Institute for Microstructural Sciences, National Research Council of Canada. His work focused on electron microscopy of electronic and photonic materials. The need for a high quality calibration standard for TEM led to his development of the MAG*I*CAL™ calibration standard. This effort landed him and a colleague in the Guinness Book of Records for inventing 'The Worlds Smallest Ruler'.

Currently John is a scientist with NRCC's Institute for National Measurement Standards, the Canadian equivalent of NIST. He is currently responsible for Medical Radiation Dosimetry calibrations for Canada. I was delighted to be able to entice John to join us this morning to give us an overview of some of the calibration problems and approaches associated with transmission electron microscopes.

J. McCaffrey: Thanks very much and I am pleased to be here. Calibration of the transmission electron microscope (TEM) is a complicated issue. The reason that I had to end up spending a lot of time calibrating TEMs is that I worked in a materials lab where the length scales that were of interest to us were very small. A monstrous feature (to us) would be 10 µm but typically we were dealing with atomic level features. We needed to be able to calibrate our instruments so that our level of accuracy would be of the order of a percent or better over all these ranges of calibration.

I do have a conflict of interest in that I developed the MAG*I*CAL™ calibration sample. John Marc Baribeau and I needed a calibration sample to use in this laboratory. John Marc is a crystal grower. He grew the prototype crystal lattice that eventually became the MAG*I*CAL™ calibration sample. One of the nice things that came from this was that we decided to send it into the Guinness book of records. We now hold the prize for the "world's smallest ruler".

There are three major TEM calibrations. The one that is of interest to most people is the magnification calibration. There is also a calibration that you can do with diffraction patterns, which is a camera constant calibration. Depending on the type of TEM, there is a third type of calibration, mostly of interest to material scientists, where you will have a rotation between the image and the diffraction pattern if you are trying to identify the rotation of a crystallite relative to a substrate. Almost all of this talk on calibration will be on magnification. Giving a 10-minute talk on calibration is virtually impossible so we will just fly through it and pick up the rest of it with questions.

There is a huge range of samples (Figure 1) for calibrating TEMs that apply to various magnification ranges. What's useful here is what we will call the "feature size" on these various samples. For example, something that has a feature size of the order of 5.0 nm will allow you to calibrate features from about 1 nm to about 25 nm. All of these samples on the left side of this figure involve lattice imaging of crystal planes and that is the most accurate measure currently available. Crystal lattice planes are a fundamental constant of nature providing the electron beam does not damage your crystal. So that means it has traceability – it is traceable to fundamental constants of nature.

To put this into perspective, almost every sovereign government has a national institute of measurement standards. Collectively there is the System International of units, the SI units, where internationally it is agreed upon that "this is what a meter is" or "this is what a kilogram is". "Traceability" means that something is certified to be consistent with SI units. I work for the Institute for National Measurement Standards in Canada that is equivalent to NIST in the USA. We calibrate things that are traceable through our institute to the SI units, just as NIST calibrates things that are traceable through NIST to the SI units.

Back to calibrating TEMs! The first thing you want to do with a calibration sample you put in the TEM is make sure that the sample is aligned perpendicular to the electron beam. For example, if you put in a diffraction grating as the TEM sample that has a series of parallel lines and the calibration sample is tilted over, the projection of the lines is going to give you the wrong answer. Your accuracy is going to be way off and you are going to make a mistake. Fortunately, in a lot of cases that problem is solved for you. Most calibration samples are set up so that they are aligned

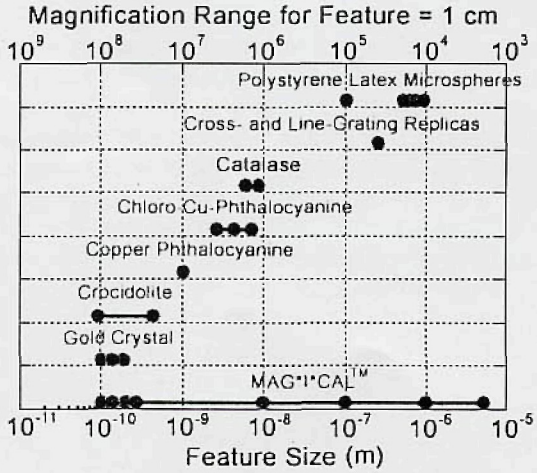

Figure 1

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perpendicular to the beam if you just make sure that your goniometer or sample holders are aligned in the microscope so that the sample is flat.

Another really easy but really effective trick in calibrating TEMs is, (if your microscope allows it), to consistently set the eucentric height. Each time you look at a sample at the eucentric height and in focus it means your objective lens current is the same. That's your equipment reference point for using that microscope. If you remember nothing else from this talk, remember to set your sample at the eucentric height and focus it. Then your objective lens current will be the same within a very small error for every sample observed. You have eliminated 90% of your problems in calibrating right there.

Regarding accuracy of calibration specimens, if the documentation doesn't state an error rate, such as plus or minus 2%, 5% or whatever, you shouldn't be using it. There is no reason for any calibration sample to be used if you don't have a basic idea of what is the error in the sample. If you don't know what the error in the sample is you are going to have a very fuzzy idea of the error in your measurement. I gave some examples here. Again lattice spacings are a constant of nature. Ruled gratings are quite accurate because they can be measured optically too, so you can have an alternate way of measuring them. You have to be a little careful with the microspheres because many are beam sensitive and with an intermediate voltage microscope you can change the dimension of those things. You have many of them on your sample so you have to keep moving to a new area of the sample and get good statistics on things like that. That will eliminate a lot of problems.

When you calibrate the microscope start with the calibration sample at the highest magnification and then start coming down, to eliminate hysteresis effects. Then, when you want to measure something, initially go up to a higher magnification than you want, and then lower the magnification to the level of interest. This will eliminate the same hysteresis errors in you measurement. Your measurements are going to be much more accurate if you do all these things consistently.

A more subtle technique is that you can overfocus and then focus on the sample. Again these are just protocols. If you get in the habit of making measurements always using identical procedures, your accuracy is going to go way up.

Measure off of a negative. If you print it, you have the potential of a few percent error in the transmission from the negative to the print. You are introducing unnecessary error. As a rule of thumb you should make measurements at several different magnification levels and you will see why that is important in just a minute. To convert your measurement on a negative to a magnification factor, measure your feature size on your negative, divide it by the actual feature size that is on your calibration sample sheet, and you come up with a magnification value.

Magnification Calibration, 250 keV, 31 October, 2000

Nominal	Measured	Difference	Nominal	Measured	Difference
650,000	647,000	.995	42,500	44,500	1.047
550,000	508,000	.923	30,600	30,500	.997
420,000	378,000	.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

Figure 2

Figure 2 is an example of magnification calibration off of what I consider is an absolutely fabulous TEM, a Philips EM-430. What is interesting here is in the column where you have a "Nominal" magnification. That's what is listed on the console of your own TEM as your magnification. The "Measured" column is the calibrated value. Most of the Nominal and Measured values are quite close, but there are examples where the difference is over 10%! An example of this is where the value was listed at 420,000x and measured at 370,000x. You can see other examples here where there is a substantial difference between nominal and calibrated values. That's why you calibrate microscopes and that's why you take images at a minimum of a couple of different magnifications. You could calibrate one magnification and be happy with that one. But if you do something wrong (like you don't have the eucentric height set right), then you are going to be off by a lot. If you calibrate at a couple of different magnifications and they are consistent then you know you've probably done a pretty good job. Again it depends on your need for accuracy but the game is to be as accurate as possible.

Now I am going to discuss the Camera Constant Calibration. In TEMs, besides producing images, you can also get electron diffraction patterns which are very powerful tools. To do a camera constant calibration you need a polycrystalline or crystalline sample. In a polycrystalline sample you will take a diffraction pattern at a particular camera length setting and you will end up with an image like in Figure 3. You need to measure across the pattern. The magic number here is a radius value. The problem with polycrystalline samples is that you have to determine where the center of that diffraction pattern is. That is really tough to do. If you can get a single crystalline sample, such as silicon, you can measure across several spacings. In this case you have a more accurate number by measuring 4 spacings and then dividing by 4. The most important problem with doing this kind of calibration is making that measurement accurately. So if you have a single crystal you want to take a short enough exposure so that your spots are sharp. If you have a ring pattern you want to try to find in that ring pattern spots or reflections that are on opposite sides and are fairly small so that you can identify the center of that spot

The Camera Constant Calibration Equation is $\lambda L = dR$. What you are relating here is the accelerating voltage of your beam (λ) that you don't know really all that accurately, and the camera

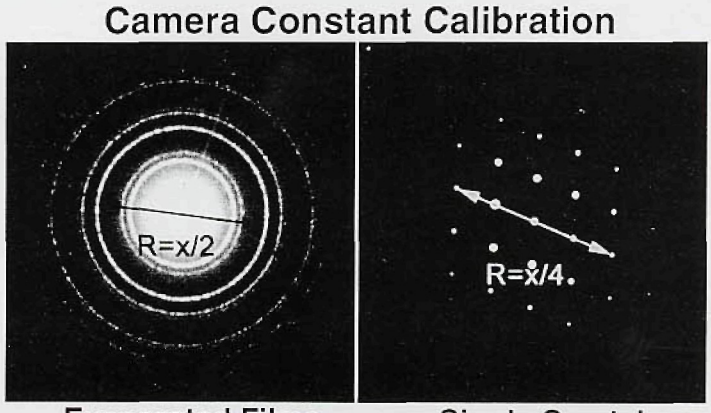

Figure 3

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length (L) that you don't know accurately. However, you are measuring off a crystal where you do know a lattice spacing (D) very accurately; for example, silicon is known to 8 or 10 decimal points, the most highly characterized material on the planet earth because of the semiconductor industry. So you have this fabulous value of what this highly traceable standard is and then it is all up to you as to how accurately you can measure that diffraction ring radius (R, in mm) or the spacing between those spots. Then you come up with a little chart like Figure 4. Let's say if you are using your camera length of 270 you take and make a measure of a radius that is say 6 mm. You know that 6.08 divided by 6 corresponds to a lattice spacing of 1.013 A.

The final calibration that I will discuss is the Image/Diffraction Pattern Rotation calibration. There is a rotation between the image and the diffraction pattern on a lot of microscopes. On newer microscopes they put in an extra lens to eliminate that. What is happening is that as electrons go down a TEM column they are spiraling down in a helix. So your diffraction pattern basically stays still and your image turns as you go up through magnifications. If you see your image changing orientation as you go up through the magnification ranges, you work in materials, and you need to know the orientation of crystallites relative to the rest of the sample, then you need to do this calibration. This is pretty labor intensive. You need to get a sample like these great little molybdenum trioxide crystals (Figure 5) and you take double exposures of the image plus the diffraction pattern. You get a diffraction pattern and you figure out which reflections correspond to which direction and you measure the angle between them. This is what the MAG*I*CAL™ (Figure 5) looks like and you do a similar type thing there. You use the same simple rules: get the eucentric height right, focus, then take the series of double exposures, and you can create your own protocol. The one that I use is angle θ clockwise from the diffraction pattern to image. What you end up with is a horrendous sheet full of data. Let's say you want to work at a magnification of 160,000x and you have your favorite camera length such as the 1200, so you know that the rotation, the angle clockwise from the image to the diffraction pattern, is going to be 51.5°. So then you can relate an image to the crystallographic properties of that particular feature. This is fairly specialized stuff and unless you are heavily into material science and crystals it is probably not going to be relevant. So I will stop there. Thank you very much.

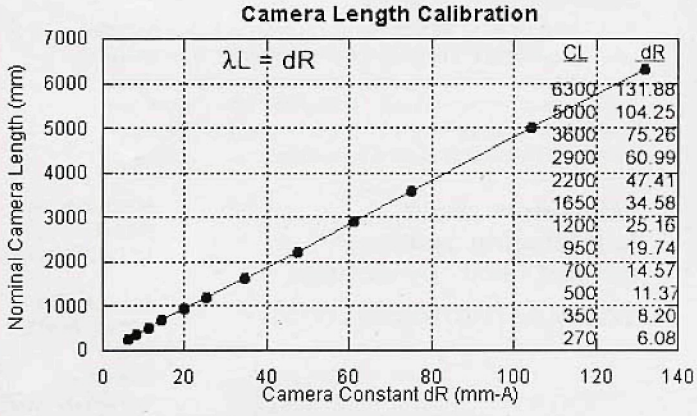

Figure 4

- I ran into some problems in calibration in that I want an inexpensive calibration sample that I can hand to a student. I want to have it around so users can routinely throw it into the microscope and take calibration images at the magnifications that they are using at that particular time. It is even better if it is something that we can incorporate with the sample. In addition we are working in magnification ranges that don't lend themselves necessarily to standards that you can only visualize at very high magnifications like the crystalline lattices. So what are we left with? We are left with things like catalase. But catalase is not a really good sample. Those of you that have spent a lot of time using catalase realize that there is a fair amount of error in the crystals and a lot of it has to do with the way the crystal is lying on the grid, among other things. If you have suggestions of types of samples that can be used in a TEM in a range of 20-60,000x that would be great. The other area in which we are running into problems is below 50,000x. We can use the replica lattice gratings that are readily available through the EM supply houses. However they are not adequate between 50,000x and 100,000x. Once we get way over 100,000x we can get into more of the crystalline-type samples and they are more effective. In SEM we are running into similar type problems. We can use these same types of replica lattice gratings for magnifications of about 1000x and up to about 50,000x but we have a lot of people doing things below 1000x. And they need to have some sort of calibration as well. So it is a problem of what we should use at the very low magnifications in an SEM. Again it needs to be very affordable so that students can routinely use it. So we would appreciate knowing about any suggestions you might have for those very practical types of samples.

J. McCaffrey: Yes. What you described is a common problem. But you came up with a really good solution. If you can have a TEM sample that actually has a calibration reference right on the same sample then you are on your way. Some people have done things like depositing little spheres on grating replicas or taken little spheres and sprinkles them around on their samples so they will have a reference right there. But except in a few very specialized cases most specimens do not lend themselves well to having a reference right in the sample.

With TEM, what I do when I have students or other research-

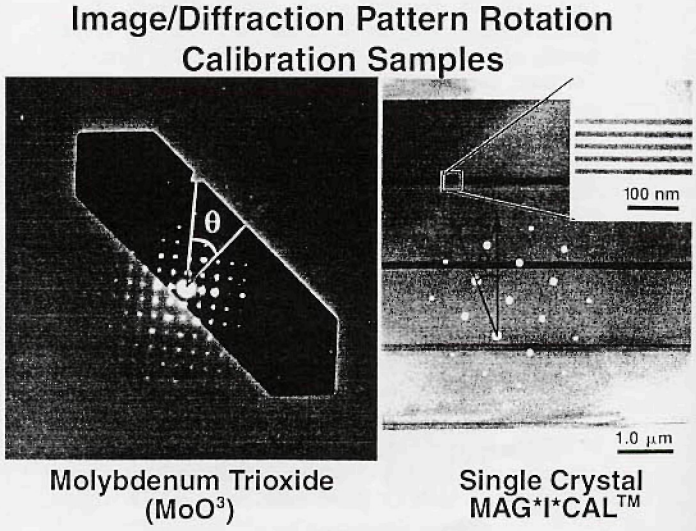

Figure 5

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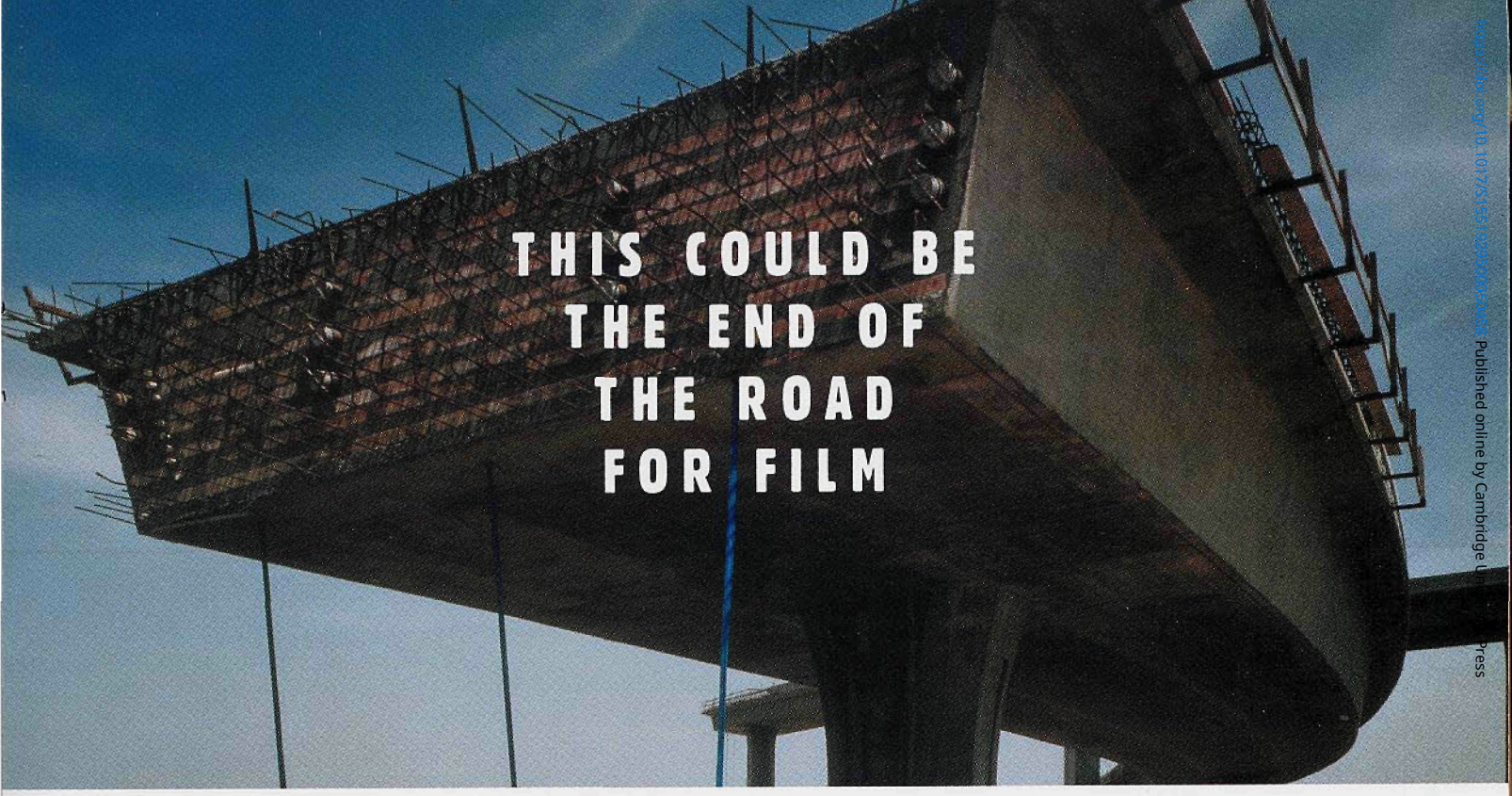

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ers working through those types of problems is teach them good technique in how to do a calibration. Going through the process of doing a calibration tends to make people a lot more careful. They realize that they have to pay attention to these issues. I have them make up a little list of what they have to do: *make the sample perpendicular, *set the eucentric height, *go to a high magnification and then come down, etc. If they are reasonably careful they can routinely hit about 5% accuracy. The people who have really critical things can drop that down to about 2%. But it is a tough problem in a TEM because you cannot just drive over to a new area and look at a calibration sample like you can do in some of the SEMs.

- We have a silicon grating standard that you can buy in a pack of 10. It is 10 µm and for every 10 of those there is a thick line so it works at the lowest magnifications. It is cheap enough with the pack of 10 to give one to the students or have one sitting by each microscope. Of course it is not a traceable standard but works well for most needs. It is available through some of the EM supply houses.

- Obviously if you are working with something that is crucial for measurements you would want to take a calibration picture the same day along side the rest of the samples you are photographing. Some microscopes go into and out of alignment morning and afternoon while other scopes are like rocks and you align them once a year it seems. Do you have any feeling for changes in magnifications over time? If I sit down and do a calibration range from magnification settings can I come in the next week and expect to assume that my electronics are going to be stable enough so I don't have to keep doing these magnification standards on any ongoing basis. Or if I need to do it on an ongoing basis, what might be a ballpark just to keep it in mind...once a month, once a year?

J. McCaffrey: Are you more interested in SEMs or TEMS or both?

- Mostly for the SEM but obviously it would be a consideration for the TEM as well.

J. McCaffrey: As a general rule if you ever have anything major happen to the instrument like you change a lens or a goniometer in a TEM, you absolutely have to do a recalibration. With new instruments it is a good idea to do it monthly and just see how it goes. Electronics will age and cause changes but they are remarkably stable in my experience. I do a TEM magnification calibration generally once a year now and I find only very subtle variations. But we had to have a goniometer changed once that made a difference. We changed a lens once that made a difference. You want to stay right on top of it with major changes but changing a filament or something like that should have no effect on the instrument calibration.

- I think you had mentioned that the ideal situation would be if you could put your calibration standard right on the sample. What would be the degree of shrinkage of, for instance, the polymer sphere in the presence of the beam?

J. McCaffrey: I don't use that so I can't give an example from my own experience. I think even in your catalog it says that the spheres will shrink under exposure to the beam so that is going to be a function of how much energy you are dumping in to it. Presumably the longer the exposure the smaller your sphere but I have never seen anybody measure those. I know that people are aware of beam effects and they avoid going back to the same area. With the spheres the documentation that comes with them say that there is a nominal size range but you need really good statistics because you can have populations of the spheres that are outside that range. You just have to take lots of measurements over an area that has not been exposed to the beam before. But I do not have a number on the effect. I think it is going to be dependent on the exposure on the spheres. ■