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Determining the Micron Marker Distance or Magnification of a Microscopic Image

Paul Beauregard
Chemist and Electron Microscopist, Greensburg, PA
beaurega@westol.com

Here's how I always calculated and converted between micron marker distances and magnifications on negatives and prints.

General Procedure and Set of Rules:

1. Take the magnification and convert it to k×. Let's say you get 50 k×.
2. That number in millimeters is the length of ONE micron at that magnification of 50 k×.
3. So in this example, 50 mm is one micrometer (or 5 mm is 0.1 µm on higher mag prints). You could try to draw a calibration bar 50 mm long and label it as 1 µm, but the print labeling area might not be wide enough for that. What do you do if the distance for one micron is 750 mm? In either case, one can use one-tenth of that millimeter distance and label it with a 0.1µm marker bar.
4. Always determine the one micron distance first and then convert up or down from there. This procedure can be used in the reverse order stated and is the more common procedure.

This mag rule works for TEM, SEM, OM, et al. It is easy to remember and it usually does not take a calculator to figure out the mag and micron markers distance changes. Just stick with 1 and 0.1 micron markers. Then all you need to have is a millimeter ruler.

Here's a few examples of how to use this procedure or set of rules. Let's say you see an image in Microscopy Today that shows an internal calibration bar in the image and it says 0.1 µm. So what is the magnification in MT? Did the author know how much his image would be reduced or enlarged? No. Say the 0.1 µm bar is 6 millimeters long. Convert that to a one micron distance and you get 60 millimeters for one micrometer. The mag of the image in MT is 60 k× regardless of what the labeling under the image says or the article says.

Here's an optical micrograph calculation example. Your print was determined by a stage micrometer calibration to be 37 ×. That is 0.037 k× for one micron. Therefore, one micron is 0.037 mm but you can't draw that length on your photo micrograph. So use 3.7 mm and label the 3.7 mm calibration bar as representing 100 microns.

To calibrate an optical microscope magnification, you need a calibration standard called a stage micrometer, which is a high-quality ruler with typical lengths from 0.1 mm to 20 mm and may have divisions as small as 2 micrometers. Beautifully made NIST traceable step and measurement standards can be purchased from VLSI (vlsistandards.com) for all types of measurements. We used their step standard for quality checking on Mirau interferometry calibrations and setups. The VLSI standards are not cheap but they are certified traceable and ISO9001 certified.

Geller Microanalytical Laboratory Inc. (gellermicro.com) also sells their MRS-5 standard that is a similar traceable calibration standard and Geller is an ISO-9000 accredited lab. Mag*I*Cal is a calibration standard for TEM that consists of four sets of five nominally 10 nm thick $Si_{0.81}Ge_{0.19}$ alloy layers, alternating with nominally 13 nm thick pure silicon layers. The Si {111} lattice planes (0.03136 nm) layers are imaged in the TEM as are the band thicknesses to establish the calibration. The same procedure can be used to calibrate ALL of the magnifications possible on any microscope: use one of the traceable standards mentioned above to calibrate as many of the magnifications as possible by making images that are rich in larger and smaller features so you can calibrate these additional features. Then make images at any other magnifications that the instrument is capable of where the standard's feature size in too small or large, but include one or more of the additional features that you indirectly calibrated in the previous step. Use these additional feature images to calibrate the magnification of those images where the standard is inappropriate. For example, a high magnification TEM image of a semiconductor device cross section may contain the Si lattice, which can be used to calibrate that magnification. However, that same image may contain integrated circuit layers with thicknesses' ranging from 10's to a hundred nm or so. These layer thicknesses' can be measured on the image containing the Si lattice and then lower magnification images can be taken to indirectly calibrate these lower magnifications, and so on. Make a table of the instrument magnification readout vs. the actual magnification at that magnification step vs. the percent error at that magnification. It is not uncommon to see errors from 1% to 5% or more. Many microscopists still use inexpensive common carbon grating replica (cheaper) for TEM calibration at lower magnifications. For image analysis, you need to photograph a standard, calibrate as above, and "pull" it into your CRT with your software. Then you can calibrate your software directly against your known distance standard. Do both the x and y axis directions.

This mag rule is very helpful and easy to use "on the fly". I gave a presentation with a 35 mm slide of a TEM micrograph displayed onto a large projector screen. Some guy asked, "What is the actual magnification on the screen?" I picked up a meter stick, measured the distance for the 0.1 µm calibration bar as 150 mm, multiplied by ten to get the one micron distance, multiplied by 1000, and I said, "Close to 1.5 million ×." ■

A Very Simple Method for Quickly Making Large Numbers of Measurements on Micrographs

Ron Anderson
Microscopy Today, Largo, FL
microscopytoday@tampabay.rr.com

It has been in the back of my mind to write this up for MT since I retired from a certain large computer company. Inasmuch as Paul's article above is a perfect lead-in, there is no time like the present. Our lab supported a semiconductor integrated circuit and a ceramic substrate manufacturing facility. We were continually required to measure circuit line widths on plan-view specimens and layer thicknesses on cross-section specimens for both semiconductor and

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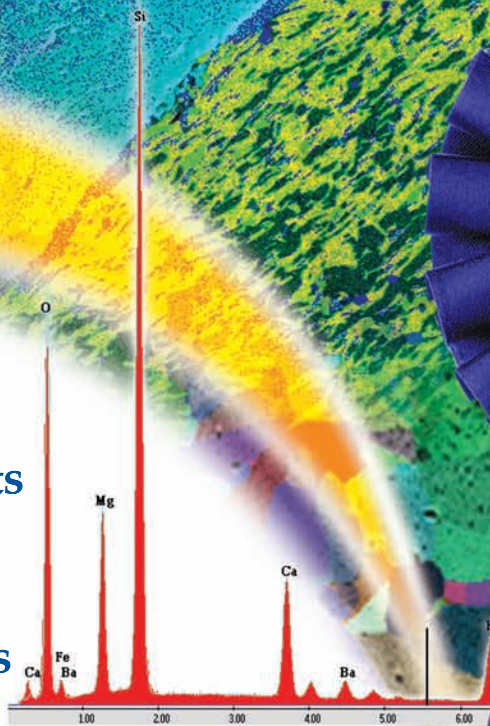

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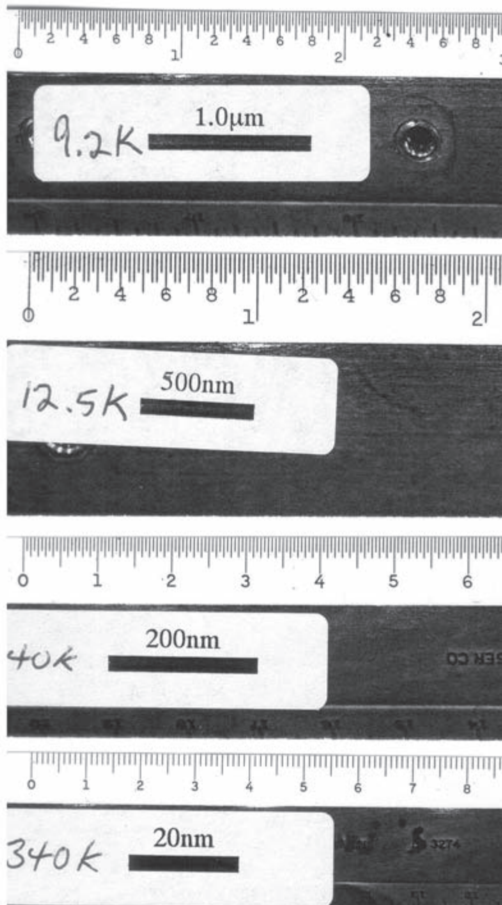
ceramic substrate specimens and we were often asked to determine thin film grain size and ceramic raw material particle size data. A large number of measurements were required for each specimen to guarantee statistically sound data. We had image analysis software available that we used whenever we could, but often found that measuring things on a system using grey-level image analysis as input simply did not work. This is especially true for thin film grain size determination when using diffraction contrast TEM images for input. If there was a large degree of preferred orientation in the subject grains, the grey level differences from adjacent, similarly oriented grains were not detected and the image analysis software incorrectly measured and reported a single large grain. Trying to measure grain size data when the subject was a copper thin film with twin structures was impossible. Determining particle size data from a micrograph where there was particle overlap was similarly problematical. In ALL of the above cases, the analyst, looking at the micrographs, could easily distinguish what was and was not a grain boundary and the identity of overlapped grains by eye. I suspect similar difficulties exist in the analysis of biological specimens.

A simple solution was adopted: use a ruler and measure the objects manually! Before you throw the magazine against the wall, read a little further. You can make the measurements on the monitor screen if you wish but it is easier to make a print. Using the procedure Paul recommended, calibrate the final print with regard to magnification. It is important to standardize the whole process so that the entire operation from recording the image to putting an enlarged paper print on the desk is calibrated with regard to magnification. After this protocol is done once, it should be checked now and then for any drift.



Starting with the knowledge of how long a calibrated micron marker should be for every print magnification to be used, make a custom ruler for prints at that magnification as follows:

1. Obtain two actual rulers: a centimeter ruler marked in millimeters and an "inch" ruler divided in decimal parts of an inch (not fractions of an inch).

2. See which of these rulers comes closest to measuring the calibrated micron mark on the print and determine by what percentage the ruler is off. For example, if one ruler measures a 100 nm micron mark at 115 units then the ruler is "off" by 15%.

3. Take the ruler to a copy machine that can enlarge and reduce the resulting copy by integer percentage points and set the copier enlargement factor so that the resulting copy of the ruler image measures 100 nm at "1" on the ruler's scale. Cut out the enlarged/reduced image of the ruler and place a label on it that corresponds to the print micron marker and displays the instrument's magnification. This is useful so that you will know that "1" on the ruler's scale = 100 nm. Use a pair of dividers on the figures provided to convince yourself that the rulers measure microns or nm as per the associated scale. Note that which ruler is used to make the enlargement/reduction in the copy machine makes no difference. The ruler used to collect data is a custom ruler that came out of a copier and measurers neither cm nor inches. It is a straight thing with decimal markings on it! Those decimal markings are like a long micron bar with a scale on it that you can move around. It is simply a way to directly read a value of distance on the print with a calibrated scale. It is similar to the Ernest F. Fullam ruler used to read the distances on either a #1000 or #1002 carbon grating replica image.

In use when a large number of measurements need to be taken on a series of micrographs, the quickest approach is to use two people: one person with the ruler and a marker pen and the second person sitting at a lab computer running spreadsheet software. Person 1 applies the ruler and calls out a dimension *in the correct units* and marks the grain, or particle, or line width, location with the marker pen to avoid repeating that measurement. Two people can perform about 50 or so measurements per minute and when done, the spreadsheet can report all of the measurement statistics with a couple of mouse clicks.

We realize that there are digitizing tablets to perform the same function I'm describing on prints and software that allows one to draw lines on objects on a computer monitor whose lengths can be calibrated and statistics presented that overcome the problems with making automated measurements using gray scale level discrimination as input. All of these systems cost money to implement compared to the basically free tools and protocols suggested in this note. Further, when you compare this method with any other image analysis system and you include all the set-up time for the computer system per job, the rulers are usually faster for getting the job done and you don't have to worry about the data. ■

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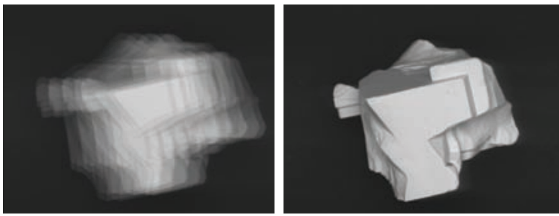

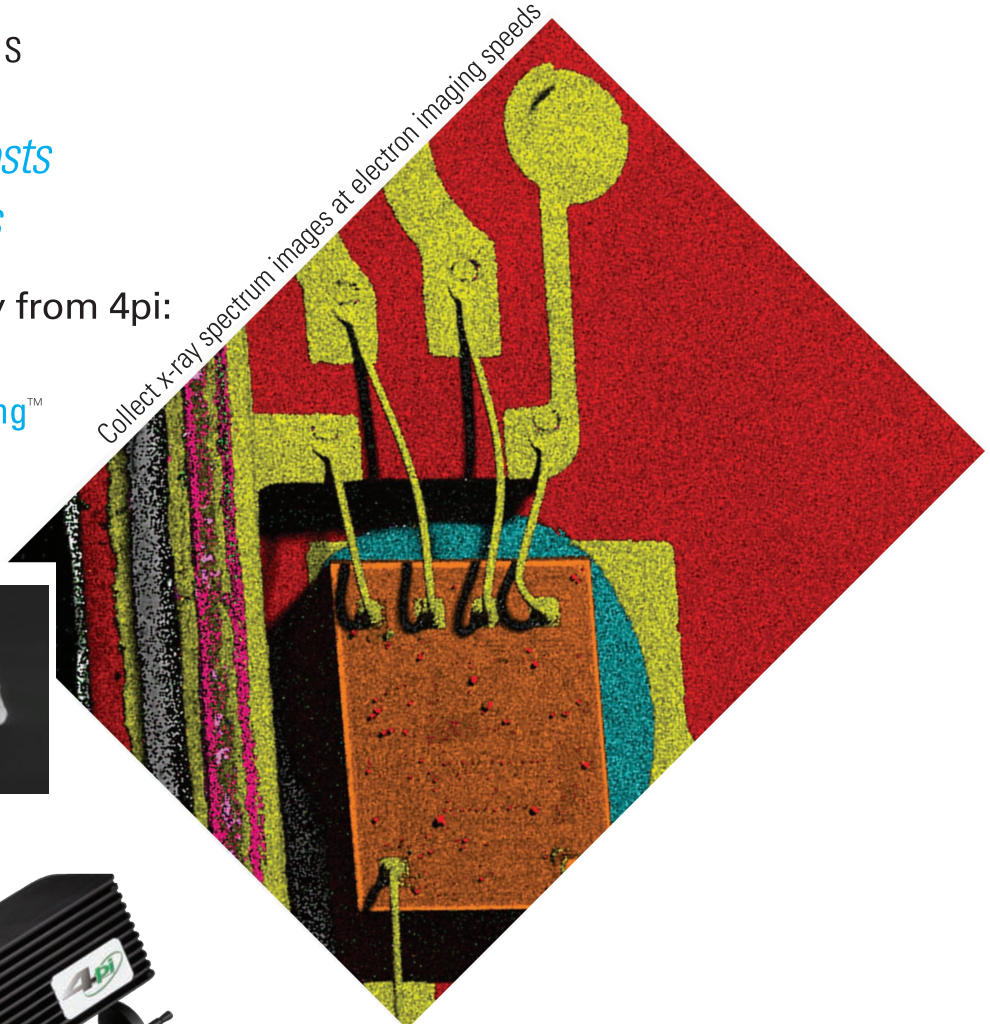

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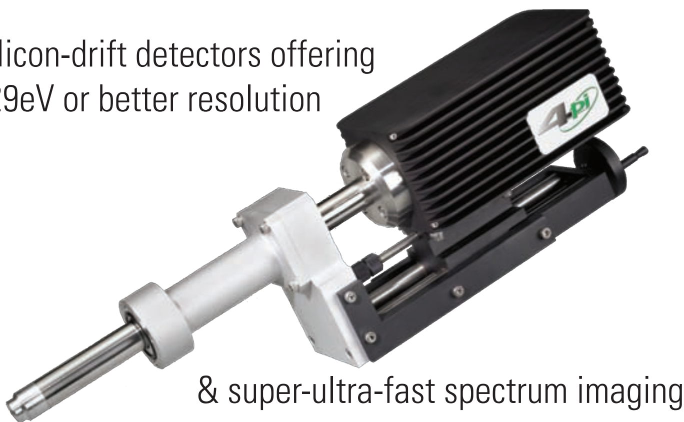

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