MICROSCOPY 101

We appreciate the response to this publication feature and welcome all contributions. Contributions may be sent to our Technical Editor Phil Oshel, oshel1pe@cmich.edu

Getting Epoxy Semi-Thin Sections to Stick to Glass Slides.

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Semi-thin sections don't always want to stick to glass slides, but subbing slides is usually not needed. For sections about 2 µm thick and no larger than 4 mm on a slide, this simple method works well for me:

- 1) Clean 1x3 inch glass slides with an ethanol rinse, then air dry at room temperature or blow down with a hair dryer.
- 2) Collect sections on a drop or two of distilled water on the slide, transferred there from the microtome with a clean fine tipped artists brush. Collect about 8-12 sections per drop.
- 3) Warm the slide beneath the water drop from below using an alcohol lamp, fairly hot, but not to boil, of course. After drying by heating the sections stick quite well.
- 4) Stain, usually with 0.2 μm filtered toluidine blue, again heating but gently this time, for about a minute, until stain "develops" the section.
- 5) Rinse that stain off with distilled water from a squirt bottle, even directing the spray right onto the sections to get rid of any precipitate. Dry again gently with flame.

The heating is the trick. There should not be any need for subbing or otherwise treating slides other than cleaning them.

SEM Stub Holders for Sputter Coating at 90° Tilt

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It can be difficult to get an even layer of metal coating when sputter coating a sample with steep sides, a rounded lower surface, or one that doesn't make complete contact with the surface of the specimen stub. It can be particularly difficult to get sputtered metal in under the sample, and so an insulating sample (like most polymers and biological specimens) may have little or no electrical path to ground. This results in charging, and increased chances of specimen heating and beam damage in the SEM.

These stub holders were made to fix that problem. A couple of metal shelf brackets, which slide into pegs and support the shelf,



Figure 1 (left). En face view of SEM stub in modified shelf bracket, in sputter coater.

Figure 2 (right). Side view of same.

were modified. The brackets are small 90° angles with a hole in one face and a peg on the other face. To modify them, first the 90° angle of the bracket is bent slightly past 90°. When a stub is slid into the hole on the one face, the angle keeps the stub from falling out. Next, the bracket's peg is ground down until the peg of the bracket is the correct size for the stub-hole of the coater stage.

To use, the stub holder is inserted into the sputter-coater, and a stub is slid into the hole of the holder (Figs. 1 & 2). The stub can then be sputter-coated for a short amount of time, and then the stub rotated on the holder. The stub can be sputter-coated again, and rotated, and so on until all sides of the sample have been coated.

Digital Cameras and the TEM

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Resolution and pixel number is a recurring question in microscopy, especially in regards to appreciating or visualizing the image's resolution once an image is captured. For instance, what is the effect of changing a one megapixel camera for a three megapixel camera? It is important to consider nanometers (or micrometers) per pixel, which will determine the ultimate resolution available. Of course Nyquist will tell you that you can't push things to the single-pixel

dimensions—a couple of pixels is more likely the limit. It is also important to remember that raw pixel count alone is meaningless. The image formation process must be considered. The camera needs to be matched to the phosphor for optimum cost and performance. Excess pixels in the camera beyond the resolution of the phosphor will just waste money. Insufficient pixels will forego potential resolution. With regard to TEM camera systems, I would like to think that systems are fairly well matched by the designers, at least now that the costs of CCDs are coming down.

On the computer screen, imaging software can display the images, or portions thereof, at one pixel of image per one pixel of screen. Many screens are setup so that pixels are not terribly obvious to the eye from normal viewing distance. Therefore, it will be difficult to notice one pixel more or less without zooming in on the image. The software will have full access to the image data and can make measurements down to the pixel level.

The printed image also raises visualization issues. Multiple dots are required to render a single pixel, at least for those printers (laser and many inkjets) where a dot is either there or not. A pattern of dots is needed together to represent shades. Therefore, the printed pixels per inch is practically an order of magnitude less than the dots per inch. Then there are the "truth in labeling" issues. What is the printer genuinely capable of? Once again, the resolution of the eye comes into play, which is quoted at about 500 pixels (250 pixel pairs) per inch at 20 inches, but I don't think I would be appreciating one pixel more or less at that printed resolution. I have a hard time seeing jagginess in real-world, 1024-pixel-wide-images printed at 5 inches. Zooming is necessary for me to see individual pixels clearly.

So it's time to get back to the original question about three megapixel cameras versus one megapixel cameras. My opinion is that you will only marginally appreciate the greater number of pixels on the screen or in a printed image. If the software maps images to the screen pixel by pixel, the image collected at a given magnification will be bigger on the screen and each pixel will represent a smaller dimension. The same software will probably print the image the same size, and the question will be whether your eye will be able to appreciate the finer detail. It will offer larger prints (or more zoom) before pixel jagginess appears to the same degree. For image analysis, the pixels will be finer at a given magnification (field of view). Therefore, one should be able to perform measurements on smaller features than before.

Silicon Cross-Section Sample Preparation (Cleaving)

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I have found the best way to cleave [001]-oriented silicon wafers is rather different compared to GaAs or InP. The problem is that Si prefers to cleave on (111) planes rather than (110) and so one gets an angled face with the cleave, which is usually rather uneven and often doesn't run straight. This is even worse when cleaving close to an existing edge, which attracts the crack front as it propagates. Good for making low-angle cleaved specimens, but a problem otherwise. It is possible to make Si cleave on (110) by cleaving the wafer without any support.

So, to cleave Si on (110): make a single scribe mark on the top surface with a good sharp diamond a couple of millimeters long at the edge of the wafer along the [110] direction. Then, hold the wafer just between forefinger and thumb in both hands, with the top wafer surface under the fingers and the scribe mark between the fingertips. Put a thumbnail under the scribe mark and then bend the wafer down, pulling apart slightly at the same time. If it cleaves well, it will do so very quickly with an audible 'ping'. It's a good idea to do this over a large clean surface in case either part is dropped.

This is not hard to do on a whole wafer (although trying not to drop an eight inch wafer is fun), but as the pieces get smaller, it gets more difficult. It should be possible to get an 8 mm wide strip, but a 5 mm strip might be difficult or impossible. A wafer could be back-thinned, but of course this isn't straightforward for something more than one inch in diameter, and any scratches on the back might make the cleave deviate from its path. Like a lot of these things, it's a lot easier to demonstrate than describe in text, and it takes a little practice to get the hang of it. I would try it on a few spare wafers first. A ten millimeter wide strip could be cleaved and then ground down to a couple of millimeters before mounting for SEM.

The {110} planes lie perpendicular and parallel to the major flat or notch on a Si wafer, and of course there's no point trying to cleave a wafer along any other direction but the <110>.

