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, 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.

Figure 1 (left). En face *view of SEM stub in modified shelf bracket, in sputter coater.*
Figure 2 (right). Side view of same.

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

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