MICROSCOPY 101

While pleased with the response to this new newsletter feature, we need your help to make it of value and interesting. So - how about a contribution?

BEEM CAPSULE REMOVAL

I haven't used a razor blade on beem capsules in years. I just used a regular old pair of pliers. I gently squeeze around the capsule and watch it separate from the block, then squeeze the bottom (pyramidal) end until it separates. By separating I mean that you can see that the beem capsule and the epon are not adherent; now there's a layer of air between the two. Then you just carefully squeeze the bottom of the capsule just where the pyramid begins and the block either slides out or pops out depending on how hard you squeeze. If you squeeze too hard or have not completely separated the capsule from the body of the block. You might squeeze right through and rip the polypropylene and then will need a razor blade. The other risk of incomplete separation is damaging your specimen at the tip of the block. If complete separation is accomplished, however, this rarely, if ever, happens.

One other advantage of this method is that, if you're gentle, you can reuse the beem capsules and you don't ruin razor blades - a real money saver!

John G. Aghajanina, Worchester Foundation for Biomedical Research

TEM PHOSPHOR PLATES VS. CCD CAMERA SYSTEMS

The phosphor plates have (potentially) a higher resolution than currently affordable CCD devices. Actually, at the last price (noting that I have not seen the latest plate prices) I saw for the plate system you could probably afford a 2K x 2K CCD camera. The camera is quicker but 2K is just barely good enough if you expect to use the images like you are accustomed to, i.e., like film. A 2K image blown up to 8" is only 250 dpi - not very good. Even an average laser printer will do 600 dpi. The images will be OK if printed with a dye sub printer (300 dpi) but you probably will not be able to enlarge sub areas of the image and get what you would expect from film. Area array CCDs bigger than 2K are still rare.

William Miller

ELECTROPOLISHING

Standard jet electropolishers usually hold the 3 mm disk between two pieces of plastic. It seems to me that the time it takes to get the specimen out of the holder (an operation which occurs at room temperature), or to rinse the acid from the entire holder, could be a problem with extremely sensitive specimens.

One solution is to hold the specimen in a pair of platinum-tipped tweezers (clamped closed). The specimen must be dimpled carefully, or else it will simply dissolve in the acid before it is perforated. By viewing the specimen through a glass beaker with a light bulb positioned behind it, it should be possible to see the hole and quickly cut off the current before the specimen is ruined. I have used this technique in the past to produce TEM specimens in Titanium alloys.

Another issue is the temperature of the electrolyte. Very low temperature is good, because any reaction between the specimen and the electrolyte, which can occur after polishing and before cleaning, will be slowed.

A good reference on electropolishing (with references for further reading) is written by Van der Voort, and I believe has the word "Metallography" in the title.

Wharton Sinkler, University of Pennsylania

CONTROLLING ALGAL GROWTH

Controlling algal growth in closed circuit cooling systems can be a very real problem for most electron microscopes. There are three things that can be done to control it:

1) Algal growth can be greatly retarded by excluding light from all parts of the cooling systems. This involves using opaque tubing, and keeping the water reservoir in the water chiller covered with a light-tight cover at all times.

2) Further control can be achieved by using an algicide. The old standby is a product known as Chloramine-T, which is the sodium salt of N-chloro-p-toluenesulphonamide. This is available from most specialty chemical companies (e.g., Polyscience, Sigma, Aldrich), and is used at the level of about 1 gram per gallon of water in the cooling system (0.25 g/litter).

3) A filter should be installed on the intake to the water line to prevent algae and other solid materials from getting into the cooling lines of the lenses, etc. This filter must be cleaned, and preferably replaced, on a regular basis. (Ref: Vacuum Methods in Electron Microscopy, by W.C. Bigelow, Portland Press, 1994, P. 216)

I have not had any experience with the product called Thermoclean DC; however, we have used the Chloramine-T stuff in our several systems here at the University of Michigan for a number of years with very good success. We use distilled water to avoid scale formation in heated parts of the system (the stuff sold in drugstores is good enough), and add more as necessary from time to time to keep the system up to the necessary operating level. We only replace it when it gets dirty or otherwise contaminated, or when it is lost due to service problems.

Wil Bigelow, University of Michigan

COLLOIDAL GOLD PROBE HALF LIFE

The standard gold probe formulation for electron microscopy, light microscopy, and blotting grades is made up in glycerol. These can be frozen and essentially the shelf life is indefinite. Some have lasted for 5 to 6 years. A non-glycerol formulation is also available and ususally provides a life of 12 - 18 months, but the latter number is about the maximum in my experience.

Don Cox, Goldmark

DE-WAXING AND EPOXY EMBEDDING

Here's what I do when I need to embed paraffin sections on slides. Use a diamond pencil to mark back of slide with area of interest. After removing coverslip with xylene, deparaffinize tissue by soaking in xylene for a couple of hours. Remove excess tissue with razor blade. Rehydrate with EtOH, then wash in NaCacodylate. Postfix with OsO_4, wash and enbloc stain U.A. Dehydrate with EtOH, then P.O. Infiltrate with 1:1 Epon Araldite: P.O. Drain in warm oven on side. Put on 100% Epon Araldite, drain, and repeat. Put 1-2 drops of resin on tissue. Use a flat hardened block that you have previously made in a Beem capsule (add resin to capsule, close lid, and bake standing up on lid). Use the flat end of this block and place it on top of slide, being careful not to introduce air bubbles. Bake. Scrape excess resin from around block, then place on 90 degrees C. hotplate. After a few minutes, pop capsule (with tissue attached) off with pliers. This is not always foolproof, but it usually works for the few samples I do. You can also immerse slide in liquid nitrogen which will break from the capsule. In the past, I've tried inverting a capsule filled with fresh resin over the tissue, but I always ended up with a mess because the resin would leak out. I've also tried putting the slide on top of an overfilled been capsule, then baking, but the slide moves and I end up with air bubbles. My samples are animal tissue, so you may have to modify it for plants.

Debbie Casout, Texas Veterinary Medical Diagnostic Laboratory

A Repeat: Should you find these "notes" of interest and value, we need your contributions. An "approach" to address any issue in microscopy would be appropriate - and appreciated.