MICROSCOPY 101

With this issue, we start a new feature in this publication: a summary of (hopefully) practical and useful hints related to microscopy. Contributions from our readers will be ***greatly appreciated!***

Hanging Drop Slides

To make a hanging drop slide you will need a depression slide, a square coverslip, some petrolatum, and the liquid suspension of what you wish to view.

Place a small spot of petrolatum on each of the 4 corners of the coverslip. Place a drop of your suspension in the center of the coverslip. Invert a depression slide over the drop, allowing the petrolatum to attach the coverslip to the depression slide. Quickly (but carefully) invert the slide so that the coverslip is oriented "up", and the drop is hanging into the slide depression.

W.L. Steffens, University of Georgia, College of Veterinary Medicine.

Clearing Polaroid Negs

For several years now three SEM labs here at the University of Michigan have not been using sulfite baths for treating Polaroid P/N negatives. Instead, they merely rinse them for an hour or so in warm running water (only a slow flow is needed - just enough to keep the water lukish) and then hang them up by the corner to drain and dry on spring-clip type clothespins that are strung on a piece of rope or wire. This eliminates the cost of the sulfite bath, the problems of disposing of the spent bath liquor, and the ungodly mess that students always produce by splashing the sulfite solution all over the lab. Try it, you might find it satisfactory for your purposes.

Wil Bigelow, University of Michigan

Rapid Processing of Tissue Samples for TEM

For years I have been using a protocol that allows me to section materials the next day. It is modified from a booklet by Millonig.

Fix in buffered GA (3-4 hr.), rinse (45 min.), osmicate (2 hr.), rinse and dehydrate in acetone (3 hr.), infiltrate and embed in Spurr's (about 1 hr.). Instead of agitating each solution for several hours on a rotator to infiltrate, Millonig recommends using a clinical centrifuge at 2,500 rpm for 10-15 min. for each solution. Polymerize overnight at 70° C. You can section the next morning (though I prefer curing for an additional week).

This technique also eliminates the need to make multiple batches of resin or to freeze the unused resin overnight.

Donald L. Lovett, Trenton State College, Dept. of Biology

De-waxing and Epoxy Emmbedding

We routinely dewax thick paraffin sections on microscope slides, and then run them up in resin for TEM, with H & E preps of human biopsies. First, we soak off the coverslip with

xylene. We then rehydrate the section on its glass slide through a series of alcohols (100% = > water) and osmicate the tissue, again on the slide. We then dehydrate the tissue back through 100% alcohol and P.O. and flood the slide with PO/Spurr in 2:1, 1:1, 1:2, and pure Spurr, all while the tissue is still on the glass slide. We then fill a Beem capsule with resin and invert it over the section and oven cure it. The cured (and stuck) capsule and slide is then dropped into a beaker of liquid nitrogen until it is thoroughly chilled. The LN chilled slide/capsule is then transferred quickly to a beaker of hot water. This frees the glass from the block. Remember the tissue is right on the surface of the resin block and the first section off the knife will have tissue in it. So align the block in your microtome carefully.

I assume that one could collect paraffin sections on glass slides, deparaffinize them in the usual way, and proceed as above. If one does not subject the tissue to H&E staining, etc., but osmicate and embed them right after deparaffinizing, your EM results might be better than ours. Tissue that has been embedded in paraffin, and then stained for light microscopy, doesn't have a whole lot of ultrastructure left; although we can often get our diagnosis. Plant tissue may stand up to this better than human tissue.

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