MICROSCOPY 101

We appreciate the response to this publication feature - and welcome all contributions. Contributions may be sent to Phil Oshel, our Technical Editor at:

Mr. Phil Oshel (608)833-2885
PO Box 620068 Fax: (608)836-1969
Middleton WI 53562 eMail: oshel@terracom.net

Hydrofluoric Acid Safety

Hydrofluoric Acid is a very dangerous reagent. It is only a weak acid, but it acts like a toxin. So, you can get dangerous amounts on yourself and not know it. The dangers are maiming, amputation, and death. A single drop, if not treated, has caused fingers to be amputated, and a 25 square inch spill can cause death, if not treated properly.

Microfabrication facilities (both academic and industry) use lots of HF, but there are few problems, because they understand what they are doing and have invested in protective gear and the necessary medical supplies. The problems occur in labs who use HF only once or once in awhile. This is where the horror stories typically come from, and I think making mica samples fails into this category.

My advice is: If you are already used to using HF and have all the needed facilities, go ahead with the mica work. It's nothing that you have not done before. If you don't have experience or the equipment for HF work, find a lab on campus who does (a microfabrication facility, for example) and do it there. If there isn't a lab with experience, either don't do the experiment or spend the time and money to do it right. Nobody should die for their research (and with HF, this has happened).

The sci.chem newsgroup has periodic discussions on HF. From those discussions I have a few references that anyone using or planning to use HF should have:

1) Symptoms and treatment of HF injuries. D. Peters and R. Miethchen Journal of Fluorine Chemistry vol.79, pp 161-165 (1996).

2) Recommended medical treatment of HF acid exposure. Allied Signal HF Products Division. Morristown NJ (800) 622-5002.

Ken Westra, MicroFab, University of Alberta

Making Silianted Slides For Mounting Sections

We have found it very useful over the years. I can not take credit, however, the method originally came from G. Farmillo, a technical specialist at Dimension Laboratories, Canada (unpublished).

Silanated Slides
(Aptex: 3-aminopropyltriethoxysilane from Sigma)

1. Wash slides in detergent.
2. Rinse in running tapwater 10-15 minutes.
3. Rinse in distilled water.
4. Rinse in acetone 5 minutes.
5. Coat in 2% (v/v) Aptex in acetone 5 minutes.
6. Rinse in luke-warm running tap water 2 minutes.
7. Rinse in distilled water.
8. Air or oven dry at 40° C in a dust-free area.
9. Wrap, and store at room temperature up to 1 month, or at -20° C for several months. Freshly coated slides are best.

Dr. Sharon Miksys, University of Toronto

Increasing the Intensity of DAB Chromagen in BrdU Immunohistochemistry

I do a lot of BrdU staining and image analysis. While consulting on a cell proliferation study (not here at UNC), there was a processing problem (with the dehydrants) which resulted in decreased, faint and in some cases no visible chromagen (DAB). Also, we had a project here that involved decalcification and we did not get the intensity of staining that I prefer for image analysis. Both of these problems were resolved by the following method which may be helpful (or may not, all things being unequal). I should note that we use the Dako anti-BrdU and the Dako Envision Kit. This is my basic or start off procedure for most of my antibndies without steps 1 to 6 and step 10 is here just a different antibody. The hydrolyzation step and the pepsin step are usually not needed.

BrdU Immunohistochemistry (using the Dako Envision kit)

1) Hydrate slides to double distilled H20 as per standard procedures.
2) Hydrolyze with 4N HCL at 37° C for 20 minutes.
3) Rinse with double distilled H_20 once for 1 minute at room temperature.
4) Transfer slides to double distilled H_20 (kept at 37° C) for 5 minutes.
5) Incubate in pepsin solution (Dako) at 37° C for 15 minutes. All of the remaining steps are performed at room temperature.
6) Rinse twice with double distilled H_20, 1 minute each rinse.
7) Rinse twice with phosphate buffered saline containing 1% Tween 20 (PBSt), 3 minutes each rinse.
8) Place in Blocking Reagent (H_20_2) for 5 minutes.
9) Repeat step 7
10) Place in primary antibody (anti-BrdU) and incubate for 10 minutes; use a 1:200 dilution (20 minute incubation time for preputial gland only).
11) Repeat step 7
12) Place in polymer labeled secondary antibody and incubate for 10 minutes.
13) Rinse well with double distilled H_20.
14) Incubate with working DAB solution (1 drop DAB per 1 ml buffer) for 8 minutes.
15) Rinse well with double distilled H_20.
16) Place in DAB Enhancer solution (Innovex) and incubate for 5 minutes.
17) Rinse well with double distilled H_20.
18) Stain with Aqua Hematoxylin (Innovex) for 35 seconds and rinse with tap water.
19) Place in tap water for 5 minutes.

Dehydrate and coverslip (we use Permount, Fisher) according to standard procedures. The difference between this procedure and our normal one is the pepsin.

Robert Schoonhoven, University of North Carolina\
Linda Meeker, Dow Corning, Midland, MI

Phosphotungstic Acid, Water, and Histologic Stains

If you're preparing Mallory's trichrome stain from scratch, be aware that the phosphotungstic acid (PTA) concentration may be critical. Unless you take pains to ensure reproducibility, you may encounter unpredictable staining results. PTA functions as a dye excluder, so that the operative acid dyes can stain differentially. If not enough PTA is present, the acid dyes will perform as though no PTA is present and NOT stain differentially.

PTA is hygroscopic. It naturally absorbs moisture from the atmosphere. In

the worst case, it appears grossly to be like school paste of the kind provided to me in the dark ages. Bottom line: when you weigh out PTA with much water and add it to a stain formulation, the stain will perform as though no PTA has been added. I've seen the effect in Pap EA formulations, the origins of which are rooted in Mallory's trichrome stain. In such instances, the EA is awful. Light green and eosin stain the same cells so that they are muddy and totally unacceptable, instead of distinctly green and red.

Therefore, dry PTA by removing the screw cap of the bottle and heating the bottle in a warm oven overnight. Prepare a 20% (wt/vol) solution of PTA in 95% alcohol. Thereafter, volumetrically, rather than gravimetrically, dispense the volume required to provide the desired weight of PTA. This approach is convenient. It avoids the hygroscopicity of PTA and facilitates stain preparation. Each 10 mL contains 2 gm PTA. For my EA, for example, use 20 mL per 980 mL of solution to provide the recommended 4 gm per L

P.S.: If anybody knows why Mallory had the insight to use PTA, I'd love to know. Why on earth would anybody choose PTA for this purpose? Mallory must have had a reason.

suspect this same tip would apply equally to phosphomolybdic acid (PMA), which is also a hydrate of variable and uncertain composition, vis-à-vis, water content. I'm not positive, however, as I've not worked with PMA.

Quoting Lillie and Fullmer (p 699): "Although there has been considerable dispute as to the relative efficiency of phosphomolybdic and phosphotungstic acids and as to the proper concentration and exposure time, it seems to make little difference whether one uses one, the other, a combination of both, or neither as long as sections are treated with acid before and during the fiber stain."

References:

Lillie, R.D. and H.M. Fulimer. 1976. *Histopathologic Technic and Practical Histochemistiy*. 1st ed. McGraw-Hill, New York.

Puchtier, H. 1958 No title. J Histochem Cytochem. 6:265.

Gary Gill, Diagnostic Cytology Laboratories, Inc., Indianapolis IN

Tips On Thick Mounting Media And Refractive Index Problems.

Whole mounts of small arthropods, fungi, and other organisms with chitinous cuticles often have problems with contrast. This problem is derived from the similarity of the refractive indices of chitin and your mounting material. I did some work on this issue many years ago and offer the following solutions. These ideas also apply more generally to thick-mount specimens.

1) First, an observation: the refractive index (RI) of chitin is about the same as immersion oil (1.515). As I remember, we tried immersion oil as a mounting material and the chitin all but disappeared.

2) Concerning refractive index changes: you don't have to have much of a refractive index change to increase the contrast. Cargille has a whole slew of mounting oils from which to choose. Water (RI = 1.33) could also be used as a mounting medium. A more permanent mount may be made by making sure that the drop of water does not exceed the edge of the coverslip, then sealing with something like nail polish.

3) Concerning spherical aberration: the microscope's optics expect to see very specific components in the sample prep "sandwich". All the changes which cause spherical aberration (among other things) are derived from Snell's law (see any basic physics book). A higher refractive index will actually accentuate the problem, not solve it. The first thing to do to optimize imaging is to check the barrel of the objectives you are using and make sure that they say "0.17" for coverslip thickness (to order, a number 11/2 is the equivalent). Anything thicker will contribute significantly to spherical aberration. Secondly, when using the higher RI mounting materials, try an objective with a "coverslip correction collar". Adjusting the collar may reduce the spherical aberration problem.

3) Colloids are serious scatterers. The resulting scatter will create glare and haze which obscures information.

For more specifics on coverslips, mounting media, and spherical aberration, may we suggest the book *Optimizing Light Microscopy for Biological and Clinical Laboratories*? Details are available at our website: http://www.MME-Microscopy. com/education

Barbara Foster, Microscopy/Microscopy Education

Indicator For Water In Absolute Alcohol:

Dri-Rite may be used in absolute alcohol to detect the presence of water. This was a suggestion of a former employee of the Texas Children's Hospital, Sybil Horak.

Adding Dri-Rite (blue colored desiccant pellets) to absolute alcohol will turn the alcohol blue. The alcohol should be filtered to remove the solid pellets before use. The absolute alcohol is put on the processor, or wherever absolute alcohol is used, and it. will turn pink when water gets in it. The blue dye will also turn all your tissues blue In the case of small tissue biopsies, the blue color makes all bits and pieces visible. (Pathologists seem to like it because they do not have to stain the gut biopsies with hematoxylin anymore.)

It is important to note that even though the tissues turn blue, the dye will not interfere with any routine, special, or immunohistochemical staining. The blue color also helps with orienting skin biopsies.

However, when we used it we experienced two drawbacks. First, our newer inexperienced histotechs tried to use the blue dye as an indicator of when they had achieved full face of the tissue. They figured once the blue dye was gone, they had full face and could start taking sections. The dye does not penetrate the whole tissue unless the specimen is small. In the case of large tissues this was true, but with small biopsies one could cut away all the tissue without the blue color going away. Perhaps a way to avoid this problem is to add sufficient absolute alcohol so that the blue coloring is a pale color rather than a dark blue.

The second problem we found was pathologists who routinely use hematoxylin or Mercurichrome to mark specific sites as landmarks: those spots were obscured by the blue color. Again perhaps lessening the intensity of the blue coloring would also take care of this.

Using Dri-Rite as a water indicator was met with mixed reviews here, but try it and see for yourself.

Cheryl Rehfeld, Texas Children's Hospital, Houston, TX

A Cheap Holder for Small Reagent Bottles

I have had problems with small quantities of reagents being accidentally tipped over; especially when working with plastics and I wanted to pipette resins from their mixing beaker to a mold. To prevent the problem, I cut an appropriate sized hole in a lid of one of the boxes that microscope slides are sent in. The tube or small beaker can then be placed in the hole and this prevents tipping-over. The holder is free and easy to replace when it gets dirty.

John Tarpley, Amgen Inc, Thousand Oaks, California