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`
PO Box 620068
Middleton WI 53562
(608)833-2885

(608)833-2885
eMail: oshel@terracom.net

PLease note NEW address

An Alternative to Toluidin Blue for Staining of EPON-Sections

My favorite method is:

1) Put a small drop of concentrated sulfuric acid on the mounted epon section.
2) Count to 10 slowly, then rinse it off under the tap.
3) Put a drop of any basic dye on the section. Toluidin blue is as good as any, but neutral red, safranin, basic Fuchsin, etc. are OK too. I like toluidine blue best. It doesn't need to be an alkaline solution; any pH is OK.
4) Wait for about a minute, rinse under the tap and thoroughly air-dry
5) Optionally treat with 100% alcohol (this removes a little of the stain: not usually necessary), followed by xylene and a resinous mounting medium.

Some people prefer to mount in epon, which takes longer but gives better transparency. Others don't mount at all, but examine directly in immersion oil, as for a blood smear.

The sulfuric acid pretreatment (a traditional histochemical trick, but destructive to non-plastic sections) changes hydroxyl groups and some amines to half-sulfate esters or sulphoamino groups, both of which ionize as strong acids. Consequently, a basic dye at any pH will bind electrostatically to pretty well everything in the tissue, and you get metachromatic effects too: purples and reds with blue dyes, orange with red dye

A reference for this application of sulphation may be found in *Histological and Histochemical Methods: Theory and Practice*, Chapter 10, page 143, of the 2nd ed. (Kiernan, 1990, Pergamon Press, Oxford) but this has been out of print for about a year. It will also be in Chapter 10 in the 3rd ed. (Butterworth-Heinemann, Oxford, in press, due out early 1999).

John A. Kiernan, The University of Western Ontario

A Tip on Scanning TEM Negatives with Flatbed Scanners

Many 10 and 12-bit per channel flatbed scanners offer a transparency adapter for scanning film negatives. However, scanning TEM negatives in the negative transparency mode - the obvious thing to do - often yields horrible results that might make you think the scanner is no good. The problem arises because the negative transparency scanning mode is intended for low-contrast photographic negative film, not the high-contrast film used in TEM. Scanner driver programs typically contain built-in contrast compensation (a kind of gamma correction) for photographic negatives. Scanning a TEM negative with this compensation applied causes severe posterization, *i.e.*, loss of gray levels, in the 8-bit output image. The posterization effect might not be obvious in grayscale histograms taken over the entire scanned image, but will appear as a stairstep effect in linescan profiles taken across high-contrast image features.

To avoid posterization in scanning negatives, try the positive transparency scanning mode. The image contrast can be inverted with settings in the scanner control program, or during later manipulations with Photoshop. The scanner software may still apply built-in contrast compensation, but it won't be as severe as that in the negative transparency mode. Unfortunately, most scanner drivers provide no (or limited) user control over the built-in film contrast compensation. Microtek's ScanWizard program, for one, contains an option to force linear gamma in the positive transparency mode but not in the negative transparency mode. I sometimes find the forced linear gamma mode useful for very high-contrast micrographs.

This tip might help, but it won't teach how to produce optimum scans. For that, I highly recommend the book *Real World Photoshop 4* by David Blatner and Bruce Fraser, Peachpit Press, 1997.

Larry Thomas, Washington State University

How To Always Have Fresh H_2O_2 Solutions on Hand

Hydrogen peroxide solutions eventually deteriorate in storage, shelf life being shorter the higher the percentage of H_2O_2. This can happen even more quickly if there is accidental contamination of the stock solution (*e.g.*, by metal). I have had this happen twice, and think the cause was using a syringe and needle to withdraw a small volume of 30% H_2O_2 for dilution. A week later, the 30% H_2O_2 was 100% H_2)!

Urea hydrogen peroxide is a stable, solid alternative. It is in the Sigma catalog: #U1753 (powder) and U8879 (tablets, each containing 1.75 mg of H_2O_2). It is a clathrate, with urea molecules making a cage round the H_2O_2.

Weigh it out as if it were 35% H_2O_2, and make a stock solution (1% wt: vol in water is convenient) for further dilution. Use 1/35 g = 28.6 mg for 100 mL of 1% H_2O.

This stock 1% solution can be replaced every day or two, so it's always fresh.

John A. Kiernan, The University of Western Ontario, London, Canada

A Recipe For Eosin Plus Phloxine For Hematoxylin-Eosin Staining

Eosin has been the traditional counterstain for hematoxylin when staining tissue for light microscopy. A synthetic dye, eosin has an affinity for acidophilic components in cells[1]. There are many different formulae for eosin depending on the intensity of staining of tissue components desired. Eosin Y gives a pink-yellow coloration: eosin B, pink-blue: and picric acid* can be added to intensify connective tissue staining. Eosin Y is the dye most commonly used in formulations today.

Eosin is pH dependent. At pH 4.5 - 5, eosin stains erythrocytes and keratin red/orange; collagen, pink; and smooth muscle, light pink. With the addition of phloxine B, collagen stains bright pink-rose in contrast to the pink smooth muscle.

This laboratory has used both commercially prepared and laboratory prepared eosin solutions. The directions for the laboratory-prepared solution[3] are as follows:

Eosin Y (C.I. 45380)	1.0gm
Phioxine B (C.I. 45410)	0.1 gm

Distilled water	110 mL
Alcohol, 95%	780 mL
Glacial acetic acid	4 mL

Combine and stir until dyes are dissolved. Immediately ready for use.

Store at room temperature. Shelf life at least 2 years. Filter before use.

Staining time: 15 seconds to 2 minutes.

Note: To prevent dye crystals from precipitating from the solution, rinse slides in 70% alcohol prior to eosin stain. If the precipitate appears on the slides, rinse slides in absolute alcohol with a level of alcohol higher than that of the eosin stain. This can be accomplished with both manual or automatic staining.

*Picro-Eosin[3]:

Eosin Y	1.6 gm
80% alcohol	720 mL
Saturated picric acid	80 mL

Dissolve eosin in alcohol: add picric acid solution. Mix well. Filter

References:

1. Preece, A. 1972. *A Manual for Histologic Technicians.* Little, Brown and Co., Boston.
2. Feldman, A. Anatech Ltd., Battle Creek, MI., personal communication. 3. Prophet, E. 1992. *Laboratory Methods in Histotechnology* Armed Forces Institute of Pathology, American Registry of Pathology, Washington. D.C.

Cheryl Crowder, Louisiana State University

Freeze-Drying Fungal Hyphae

I've done some preliminary work using Leica's freeze-drying unit on fungal material. These were prepared to maintain chemical integrity. I've freeze-dried Bergamot *(Monard didyma)* leaves with a powdery mildew fungal infection and yeast *(Saccaromyces cerevisae)*. The leaves came out fine but the fungal hyphae were collapsed. The yeast came out very nicely. I ran both fixed (Karnovsky's) and unfixed pieces. All were cryo prepared by propane plunge freezing. All were examined at 2.0 kV uncoated. Overall I was pleased with the results, I haven't had a chance to go over all of the samples but the chemical fixation seemed to be better for maintaining the fungus. I would run both unfixed and fixed as I believe the mechanical agitation of adding a liquid solution may have washed away some fungal material. Aldehyde and/or Osmium vapor fixation would likely be less disruptive, and preliminary results look promising.

Freeze-drier time and temperature schedule was:

48 hours @ -80° C
36 hours @ -60° C
24 hours @ -40° C
12 hours @ -30° C
12 hours @ -15° C
24 hours @ -50° C
6 hours @ +l0° C
30 hours @ +20° C

Vacuum was maintained with a cryosorption pump.

Images are on my web page. The unfixed yeast pics are there already. Follow the links from Research Projects to Sample prep for SEM...
http://www.personal.psu.edu/ejb11

Edward J. Basgall, The Pennsylvania State University

A Simple And Cheap Holder For Staining Several TEM Grids At A Time

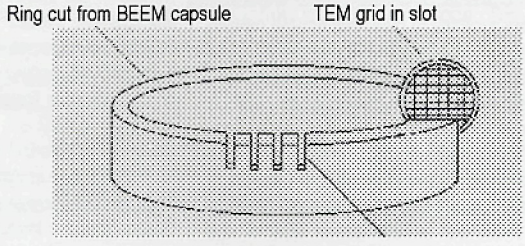
Take ring-shaped slices of BEEM capsules 4-5 mm high, cut evenly spaced perpendicular slits along one side of the ring, leaving room on the other side for pinching the ring which opens the slits.

Then place the grids into the slits, grabbing just the rim of the grid. The grids are held in place when the rings are released.

It takes a bit of practice to get the slits spaced right and to learn how to get several grids into the slits without losing the first ones you put in.

Stain using 10 mL beakers, submersing the grid rings in the stain, and rinse by bobbing the rings up and down in water, holding the rings with tweezers. Excess water can be blotted from between the wet grids with points of filter paper.

This takes more stain, but I think the results are cleaner. Sometimes I produce a "lucky" ring that will hold 6 giids for me!



Note: slot and grid placement shown is for drawing clarity only

Julie Gross, University of Connecticut Health Center