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:

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A Hint For Reducing Background For Immuno-TEM

Non-specific background staining is occasionally a problem with some antibodies and labeling systems. If normal washing fails to reduce the background you might want to try using a high salt buffer for washing. Phosphate buffered saline, or any other recipe with NaCl in it, is probably around 150 mM (0.9%). Try boosting the NaCl to 5X normal. This would make it 750 mM, or 4.5% by weight.

If high salt buffer is used, remember to incubate the grids in a couple of changes of regular strength (150 mM) saline before going to the next step, to get the salt back into the range of physiologic strength.

An example would be to wash the grids on five drops of high salt buffer for about 2 minutes each, followed by two drops of normal salt buffer for about 2 minutes each. Use this routine after incubating on the primary antibody or after any incubation you suspect may be contributing to background (secondary antibody, colloidal gold, etc.).

I have not found a good explanation for why this works, although it probably changes the conformation of proteins, alters their overall charge, and makes them less likely to bind to the surface of the sectioned material.

High salt concentrations are often used in biochemistry to precipitate proteins out of solution and to wash chromatography columns. Something similar is probably happening on the surface of the section, i. e., only those antibody molecules which have bound specifically to antigenic sites are able to stay on the section in the presence of high salt concentrations.

Robert (Bob) Chiovetti. E. Licht Company

An Improved Fixation Method for Nerves

The following fixation method is better than vascular perfusion for nerves from laboratory animals, and it might be adapted for biopsies.

1) Anaesthetize the animal and expose the part of the nerve to be fixed. While looking at the nerve through a dissecting microscope, manipulate the limb (or other part of the body) to stretch the nerve until its transverse striations (bands of Fontana) just disappear. (The bands of Fontana are due to undulations (waves) of the nerve fibres - a bit of microanatomy that was cleverly deduced before 1700, long before the fibres themselves had been properly seen.)

2) Having straightened the nerve's fibres in this way, drip the fixative (a buffered glutaraldehyde and formaldehyde mixture) onto the exposed nerve.

3) Apply a pellet of cotton wool soaked in the fixative, and wait for 30 to 60 minutes.

4) Remove the desired specimen and immerse it in fixative for a further 6 to 12 hours. Wash in buffer, post-osmicate, embed, section, stain (if desired), *etc.*

The superiority of this method over simple immersion or perfusion was demonstrated by Morris *et. al.* (1972), in one of four classical papers on the ultrastructure of the earliest stages of axonal regeneration. For the story of the bands of Fontana (a conspicuous but rarely discussed feature of nerve anatomy), see Hanicek 1986 (and also papers in J. Anat. by Beam & others, 1970s). My second graduate student, Bruce Stelmack, was able to obtain useful measurements of external (with myelin) and external (axon) nerve fiber diameters in 4 µm paraffin sections of facial and sciatic nerves of rats that had been fixed by Morris *et. al.*, methods.

1. Hanicek, 1986. Anatomy & Embryology 174:407-411.

2. Morris, J H., A.R. Hudson, and C. Weddell. 1972. A study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy, IV. Changes in fascicular microtopography, perineurium and endoneurial fibroblasts. Zeitschrift fur Zellforschung 124:165-203. (This is THE classic in its field. Weddell was the boss. His work in the early 1940s with J. Z. Young and W. Holmes provided the scientific foundation of modern peripheral nerve repair surgery.)

Stelmack, B. M. and J. A. Kiernan. 1977. Effects of triiodothyronine on the normal and regenerating facial nerve of the rat. Acta Neuropathologica 40:151-155.

John A. Kiernan, The University of Western Ontario

A Tip For Primary Antibody Elution:

I have performed the eradication of the initial primary antibody and its detection chemistry in the past and used a microwave pepsin/HCl treatment after the first chromagen was developed. The procedure is detailed in Surgery, 1995. 118:957-66.

In this application we stained the same cells with two different cytoplasmic monoclonal antibodies (sequential immunohistochemical [IHC] staining). We had only one section to work with since the specimen was very limited. The first antibody was stained and photographed, the chromagen was then removed (AEC), elution treatment was performed and the next antibody was stained. We ran a control on another specimen and omitted the application of the second primary antibody, but applied all other detection reagents, and developed for the chromagen. These controls were consistently negative, showing that the signal-generating complex had been removed by the elution or other unknown step.

To do it again, I would consider multicolor immunofluorescence.

A problem that I had with using Vector Red and AEC or Vector Red and DAB in simultaneous IHC staining was that the HRP chromagen covered up the Red – even under fluorescent excitation of the Vector Red. This is why we eventually tried a sequential format. If one is staining a nuclear and a cytoplasmic target most simultaneous systems work great, but when one is labeling two abundant cytoplasmic targets, problems can develop with chromagen overlapping. This is where I believe multicolor immunofluorescence would be great, because one could perform various wavelength excitations, or benefit by simultaneous blended colors.

Timothy Plummer, Mayo Clinic, Rochester, Minnesota

We Examine Carbon Nanotubes As Follows:

1) Add the specimen to a small volume of either acetone or methanol and "trial and error" will be needed to determine the exact dilution, so try different dilutions.

2) Suspend the sample by swirling (some people sonicate for several minutes) and use a pipette to transfer a droplet onto a holey, filmed (Butvar or Formvar) and carbon coated grid (I suggest purchasing from commercial source)..

3) Allow to air dry and examine in TEM looking for tubes suspended over the holes (best resolution)

The TEM should be set up for hi resolution with cooled traps over specimen area. A clean vacuum system is needed!

John J. Bozzola, Southern Illinois University