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.

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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 of the nerve fibres - a bi 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. (1973), 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 Hancke 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.

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 für 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.)


An Improved Fixation Method for Nerves

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) Suspense the sample by swirling (some people sonicate for several minutes) and use a pipette to transfer a droplet onto a holey, film (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 a resolution with cooled traps over specimen area. A clean vacuum system is needed.

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