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 Protocol for Immunostaining of Microtubules and Microfilaments in the Secondary Vascular Tissues of Hardwood Trees for Fluorescence Microscopy

The plant cytoskeleton has been much studied in single cells and primary growth systems. Technical problems have meant that the secondary vascular tissues of trees has been largely ignored. However, the procedure below, combined with the advice on sampling in the previous article "Big is Beautiful" in the November issue (#97-9) of this publication should permit others to explore this fascinating developmental system.

1) Prefix in 100 µM MRS (3-maleimidobenzoic acid *N*-hydroxysuccinimide ester) for *ca.* 1.5 hours

2) Fix in 3.7% freshly-made *p*-formaldehyde in 12.5 mM Piperazine-N,N'-bis[2-ethanesulphonic acid], pH 6.9, (PIPES buffer) for *ca.* 3 hours

3) Trim to final block size, and store, in PIPES buffer overnight *(ca.* 15-20 hours)

4) Dehydrate in ethanol, 30 minutes each at 30, 50, 70 80, 90% and 2 x 30 minutes in 100%. To last 100% step, add equal volume of BMM resin (4 parts of n-butyl methacrylate: 1 part methyl methacrylate, with 5 mM dithiothreitol), leave overnight

5) Replace half volume in vial with pure BMM resin, leave for *ca.* 9 hours

6) Replace all volume with pure BMM resin and leave overnight

7) Replace all volume with pure BMM resin and leave for *ca.*. 5.5 hours

NB: All above steps 1-7 performed at room temperature on rotator in stoppered glass vials

8) Replace all volume with BMM resin & 0.5 % benzoin methyl ether, leave in dark at *ca.* +5° C, rotating, for *ca.* 7 hours

9) Embed in nearly full, stoppered polypropylene capsules, cure overnight with long wavelength ultraviolet light at -20° C

10) Cut sections dry with glass knives at 6-10 µm, transfer to a drop of water on albumen-coated multiwell glass slides

11) Dry down overnight at *ca.* 40° C

12) Process for cytoskeleton staining of a-tubulin (for microtubules) and F-actin (for microfilaments):

a) *ca.* 10 minutes in acetone (with minimal agitation) to remove resin

b) wash 2 x 2 minutes in PBS (NaCl, 8g; KCl, 0.2g; Na_2HPO_4, 1.15g; KH_2PO_4, 0.2g; NaN_3, 0.2g per l; pH 7.3-7.6)

c) block (in solution of 6% BSA; 0.1% fish skin gelatin; 5% goat serum; 0.05M glycine in PBS), for *ca.* 45 minutes

d) primary antibody (20 µl mouse-anti-a-tubulin (Amersham) + 200 µl PBS) or (2 µl mouse-anti-F-actin (ICN) + 200 µl PBS), for *ca.* 2 hours

e) wash 3 x 5 minutes in PBS

f) secondary antibody [20 µl FITC-labeled anti-mouse (Sigma) + 600 µl solution of 1% BSA and 0.1% fish skin gelatin in PBS], for *ca.*1 hour

g) wash 3 x 5 minutes in PBS

h) stain sections in 0.01% toluidine blue till light blue, rinse in PBS

NB: all above steps a-h performed at room temperature: steps c, d and f carried out in sealed plastic box with humid environment

i) mount in Vectashield™ (Vector Laboratories, Peterborough, UK), apply coverslip, store in refrigerator overnight, view next day with appropriate filter combinations.

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Making Silianted Slides for Mounting Sections

We have found the following very useful over the years. I can not take credit, however, as 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

Further information about this method comes from Cohn Henderson. who gives another time schedule, and writes:

"Ultimate" credit for the following belongs to Weetall who published his concept in both *Nature* and *Science* (1969), that enzymes (proteins) could be bound to a silica (glass) surface using AAS.

I expect that there may be earlier sources for this idea.

Rentrope *et al.* did report the use of AAS for insitu hybridization (Histochem J 18:271-276,1986), and Maddox reported on the application of AAS to routine histology (J ClinPathol 40:1256-1257).

I only developed and reported on a very much shortened procedure for silanizing slides (40 sec. as opposed to 2 days; 10 seconds in silane, and three 10 second deionized water rinses) and its application to routine histotechnology. At the time I was unaware of the Maddox reference; it was the referees which directed me to his work.

Henderson, C. 1989. Aminoalkylsilane: an inexpensive, simple preparation for slide adhesion. Journal of Histotechnology 12(2):123-124.

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