Strategies for Preventing Detachment of Sections from Glass Slides

Continued from page 22.

Recommended Reading

The following books include detailed discussions of methods for promoting the adhesion of sections to slides.

Culling, C. F A., Allison, R. T. and Barr, W. T. 1985. Cellular Pathology Technique. 4th ed. Butterworths, London.

Gabe, M. 1976. Histological Techniques (English ed., transl. E. Blackith & A. Kavoor). Masson, Paris.

Kiernan, J. A. 1999. Histological and Histochemical Methods: Theory and Practice. 3rd ed. Butterworth-Heinemann, Oxford.

Lillie, R. D. and Fullmer, H. M. 1976. Histopathologic Technic and Practical Histochemistry. 4th ed. McGraw-Hill, New York.

Table 3: Enclosing Slides in a Nitrocellulose Film

General Considerations: This method is applicable to any kind of sections mounted on slides. Paraffin sections must be dewaxed and placed in 100% alcohol (ethanol, methanol or isopropanol). Frozen or cryostat sections must be dehydrated.

What you need:	What to do:
1. Slides with mounted sections, in 100% alcohol. 2. Ether-alcohol: Diethyl ether (anaesthetic ether is suitable): 500 mL Ethanol (100%): 500 mL 3. 0.5% Nitrocellulose This contains 2.5 g of nitrocellulose in 500 mL of ether-alcohol. It may be made from a solid form of nitrocellulose (such as parlodion) or by dilution of a more concentrated solution. Commercial LVN (low viscosity nitrocelluose) is sold as a 20% solution. The 0.5% solution can be kept for a few years, but see safety note below. 4. 70% alcohol. The alcohol concentration is not critical. Add 30 volumes of water to 70 volumes of 100% or 95% ethanol. **Safety Note:** Nitrocellullose solutions are highly inflammable and great care should be taken	1. Take the slides to absolute alcohol, in a straining rack. 2. Immerse in the nitrocellulose solution for about 30 seconds, with occasional agitation to ensure that the edges of the slides as well as their surfaces are contacted by the liquid. 3. Lift out the slide rack, shake off excess liquid and wait until they have partly dried by evaporation. This end point is indicated by a change in the luster of the glass surfaces. 4. Immerse the rack of slides in 70% ethanol for about 2 minutes, to harden the nitrocellulose, than take to water and carry out the staining procedure. 5. Optional: The nitrocellullose film, which is itself stained in some techniques, may be removed. Immerse the slides in ether-alcohol, 2 minutes, after dehydration and before clearing in xylene.

Ruthenium Tetroxide: A Complementary Fixative and Stain to Osmium Tetroxide

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Ruthenium tetroxide (RuO_4), which is a stronger oxidizing agent than osmium tetroxide (OsO_4), reacts well with some of the more polar lipids that fail to show a reaction with OsO_4[1]. It has been demonstrated that the use of RuO_4 can overcome the failure of OsO_4 to visualize epidermal intercellular lamellae[2,3]. RuO_4 reacts strongly with both saturated and unsaturated lipid molecules, as well as with proteins, glycogen, and monosaccharides. RuO_4-fixed membranes appear thicker than those fixed with OsO_4 do[4].

RuO_4 penetrates tissue very slowly and sometimes uneven, patchy preservation may occur[5]. The use of vibratome sections is recommended to optimize penetration of the RuO_4 fixative[6].

There are some artifacts of fixation that limit RuO_4 as a postfixative. For example, the distinct pattern of keratin bundles I have observed within epithelial cornified cells of OsO_4-postfixed tissue often have a disrupted, chewed-up appearance with RuO_4-postfixed tissue. Thus when RuO_4 is used as a postfixative with experimental and pathological tissue, I recommend using a complementary postfixative of 1% OsO_4 or 1% OsO_4 with 1.5% potassium ferrocyanate to fully confirm any interpretations of abnormality. RuO_4 is also a useful stain for polymers and their blends[7].

A mixture of formaldehyde-glutaraldehyde-ruthenium tetroxide has been used as a fixative[8]. A more typical procedure that I have used routinely with success consists of:

1) Prefix with 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7 for a minimum of 1 hour at room temperature.
2) Follow with 3 buffer rinses.
3) Postfix with 0.2% RuO_4 in the same buffer at pH 7 for 1 hour at room temperature.
4) Specimens should be rinsed in 3 changes of distilled water before dehydrating in ethanol or acetone.

Care should be taken in handling RuO_4. It is a strong oxidizing agent and reacts violently with filter paper and alcohol. It should be protected from UV light and stored in a refrigerator. A separate waste container should be used for disposal of RuO_4, which should not be allowed to come in contact with alcohol, ether, benzene, pyridine or other organic compounds. Always handle in a hood. In case of a spillage, use a sodium bisulfite solution to decompose the RuO_4 and flush with plenty of water.

RuO_4 comes in a 0.5% aqueous solution that can be obtained from Electron Microscopy Sciences, Fort Washington, PA (www.emsdiasum.com) or Polysciences Inc., Warrington, PA (www.polysciences.com). A 0.67% aqueous solution can be prepared from a kit supplied by SPI Supplies, West Chester, PA (www.2 spi.com). ■

1) Gaylarde, P. and I. Sarkany. 1968. Science, 161:1157.
2) Eichelberger, H.H., *et al.* 1994. Proc. Microscopy and Microanalysis 270.
3) Swartzendruber, D.C., *et al.* 1995. J. Inves. Dermatol. 194:417.
4) Peltarri, A. and H.J. Helminen, *et al.* 1979. Histochem. J. 11:599.
5) Madison, K.C., *et al.* 1987. J. Inves. Dermatol. 88: 714.
6) van der Meulen J., *et al* 1996. J. Microsc. 184:67.
7) Trent, J.S. 1984. Macromolecules 17:2930.

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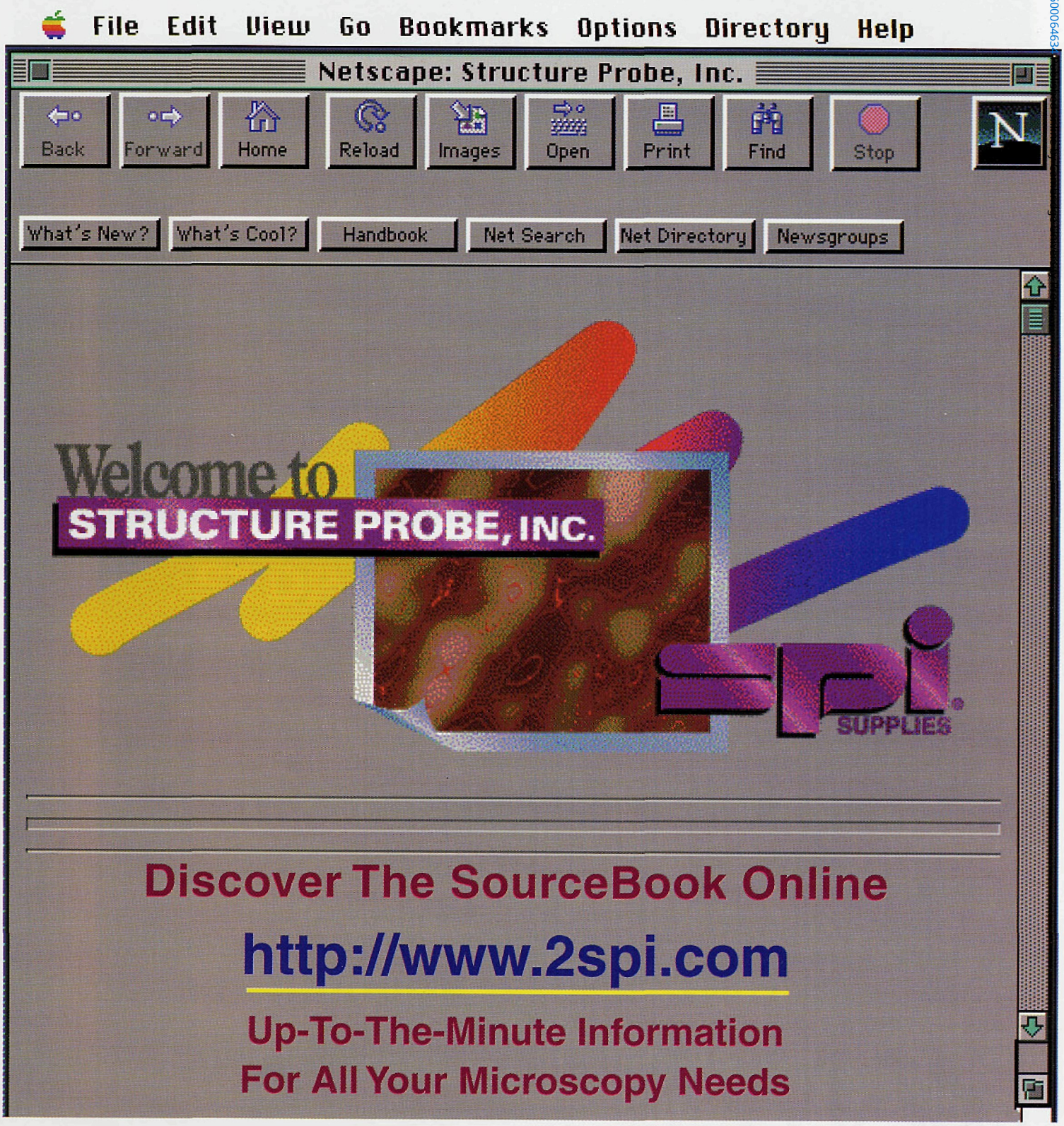

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