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.

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.

<table>
<thead>
<tr>
<th>What you need:</th>
<th>What to do:</th>
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<tr>
<td>1. Slides with mounted sections, in 100% alcohol.</td>
<td>1. Take the slides to absolute alcohol, in a straining rack.</td>
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<tr>
<td>2. Ether-alcohol: Diethyl ether (anaesthetic ether is suitable). 500 mL.</td>
<td>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.</td>
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<td>3. 0.5% Nitrocellulose This contains 2.5 g of nitrocellulose in 500 mL of ether-alcohol.</td>
<td>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.</td>
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<tr>
<td>4. 70% alcohol.</td>
<td>4. Immerse the rack of slides in 70% alcohol, 2 minutes, after dehydration and clearing in xylene.</td>
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<td>The alcohol concentration is not critical. Add 30 volumes of water to 70 volumes of 100% or 95% ethanol.</td>
<td>5. Optional: The nitrocellulose 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.</td>
</tr>
</tbody>
</table>

Safety Note: Nitrocellulose solutions are highly inflammable and great care should be taken.

Ruthenium Tetroxide: A Complementary Fixative and Stain to Osmium Tetroxide
Henry Eichelberger, Binghamton University – SUNY

Ruthenium tetroxide (RuO₄), which is a stronger oxidizing agent than osmium tetroxide (OsO₄), reacts well with some of the more polar lipids that fail to show a reaction with OsO₄. It has been demonstrated that the use of RuO₄ can overcome the failure of OsO₄ to visualize epidermal intercellular lamellae. RuO₄ reacts strongly with both saturated and unsaturated lipid molecules, as well as with proteins, glycoprotein, and monosaccharides. RuO₄-fixed membranes appear thicker than those fixed with OsO₄.

RuO₄ penetrates tissue very slowly and sometimes unevenly. Patchy preservation may occur. The use of vibratome sections is recommended to optimize penetration of the RuO₄ fixative.

There are some artifacts of fixation that limit RuO₄ as a postfixative. For example, the distinct pattern of keratin bundles that have observed within epithelial cornified cells of OsO₄-postfixed tissue often have a disrupted, chewed-up appearance with RuO₄-postfixed tissue. Thus when RuO₄ is used as a post-fixative with experimental and pathological tissue, I recommend using a complementary postfixative of 1% OsO₄ or 1% OsO₄ with 1.5% potassium ferrocyanate to fully confirm any interpretations of abnormality. RuO₄ is also a useful stain for polymers and their blends.

A mixture of formaldehyde-glutaraldehyde-ruthenium tetroxide has been used as a fixative. 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% RuO₄ 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 RuO₄. It is a strong oxidizing 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 RuO₄ 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 RuO₄ and flush with plenty of water.

RuO₄ comes in a 0.5% aqueous solution that can be obtained from Electron Microscopy Sciences, Fort Washington, PA (www.emsdiasmum.com) or Polyscience 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.2spi.com).
