MICROSCOPY 101

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Polymerizing Methacrylate Resins With Minimal Shrinkage

Methacrylate resins have had a bad rap for shrinking and bubbling during polymerization. However, with care, it is possible to polymerize samples in methacrylate (at least certain kinds) with minimal shrinking. This was, to my knowledge, first demonstrated by Carlemalm *et al.*, 1982 in their development of a mixture of methacrylates that could be polymerized at low temperature. Under the trade name of "Lowicryl", these mixtures went on to fame and glory in the immunocytochemistry field. Key advances they made to eliminate the shrinking and bubbling included the use of UV-driven polymerization, catalyzed by benzoin ethyl ether or benzoin methyl ether, and control of the light intensity to avoid too fast polymerization.

In my own work, I have been using a mixture of butyl- and methyl-methacrylate, similar in composition to one of the Lowicryl resins mentioned above, but with no cross-linker. We have measured the size of a sample after fixation and again after embedding and found no detectable change in size. The samples measured are roots maize and *Arabidopsis thaliana.* While we cannot rule out shrinkage altogether, our measurements suggest that it is at least less than a few percent.

The general protocol we use can be found in Baskin *et al.*, 1992 and Baskin *et al.*, 1996. The resin comprises 80% butylmethacrylate, 20% methyl methacrylate, 0.5% benzoin ethyl ether, and IO mM DTT

Particularly relevant for minimizing bubbling are the following details: We have a UV box with a 15W UV bulb (long wavelength UV, 365 nm max.) on the bottom. About 10 cm distant, we have a sheet of clear acrylic plastic. We put our samples in flat-bottomed BEEM style capsules. These sit nicely on the acrylic. We place a strip of household aluminum foil between the capsules and the plastic, and we place a tent of foil a few centimeters above the capsules. The box is also lined with shiny metal. In this way the capsules receive diffuse light, mostly from the sides. We polymerize at 4°C for 4 to 6 hours.

There is sometimes a kind of tunnel or "tornado" of air in the upper middle part of the polymerized plastic. Although this might indicate that the resin does contract with polymerization, as mentioned above, our measurements of the size of our samples shows that the samples themselves are not appreciably compressed.

Baskin, T.I., C.H. Busby, L.C. Fowke, M. Sammut, and F, Gubler. 1992. Improvements in immunostaining samples embedded in methacrylate: Localization of microtubules and other antigens throughout developing organs in plants of diverse taxa. Planta 187: 405 - 413.

Baskin, T.I., D.D. Miller, J.W. Vos, J.E. Wilson, and P.K. Hepler. 1996. Cryofixing single cells and multicellular specimens enhances structure and immunocytochemistry for light microscopy. Journal of Microscopy 182:149 - 161.

Carlemaim, E., R.M. Garavito, and W. Villiger. 1982. Resin development for electron microscopy and an analysis of embedding at low temperature. Journal of Microscopy 126,123 - 143.

Tobias I. Baskin, University of Missouri

Hints To Correct For Skirt Effects From Plural Scattering When Doing EDS In A Low-Vacuum SEM

It may not always be possible to obtain single scattering conditions, for instance, because of specimen geometry or because the pressure should be high enough to maintain liquid water in the specimen chamber. Under plural scattering conditions you can use a micromanipulator (which is an optional accessory for the ESEM) to insert a fine needle of the kind used for field ion microscopy or scanning tunneling microscopy in the beam path. The needle should not contain elements that are present in the sample. Take one spectrum with the tip of the needle covering the point of interest. This spectrum will contain characteristic peaks from the needle plus the skirt spectrum. Take another spectrum with the needle slightly retracted from the point of interest. This spectrum will contain the spectrum from the point of interest, the characteristic peaks trom the needle plus the skirt spectrum. Remove the peaks stemming from the needle from both spectra and subtract the first spectrum trom the second spectrum. This will to a good approximation yield the spectrum from the point of interest.

A variation of this method is useful for line scans: Cover the sample with a metal foil (containing only elements not present in the sample) along the line where you wish to scan. Now make two line scans, one on the foil very close to the edge, the other on the part of the sample that is not covered with foil but very close to the edge of the foil. Since the skirt is broad, the skirt spectrum will be almost the same for neighbouring points on the toil and just outside the foil. A skirt-corrected line scan can therefore be obtained for each point In the line scan by subtracting the count rate for a given element measured in the point on the foil trom the count rate measured in the corresponding point outside the roil. Since the skirt is most intense around the beam target, you will not completely get rid of the skirt effects. Our experimental results show, however, that if the distance between the corresponding points is d, then skirt effects from farther away than around 2d are removed.

J.B. Bilde-Soerensen, Risoe National Laboratory, Denmark

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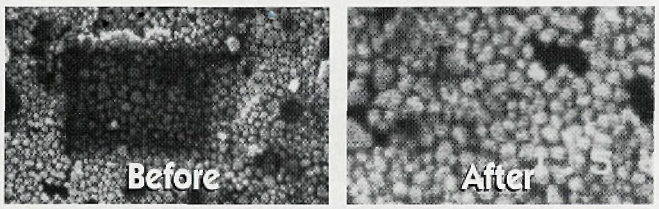

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