MICROSCOPY TODAY

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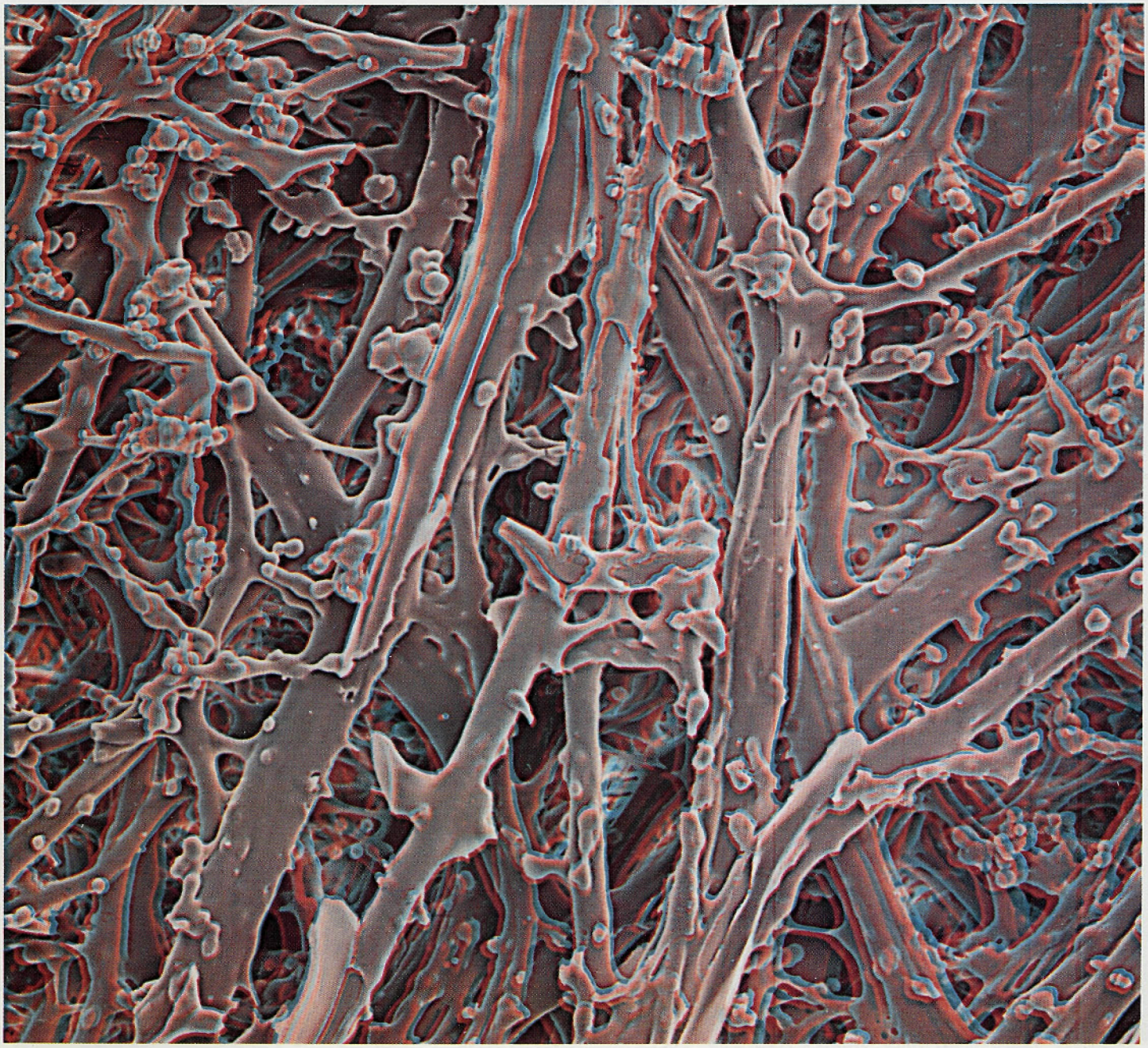
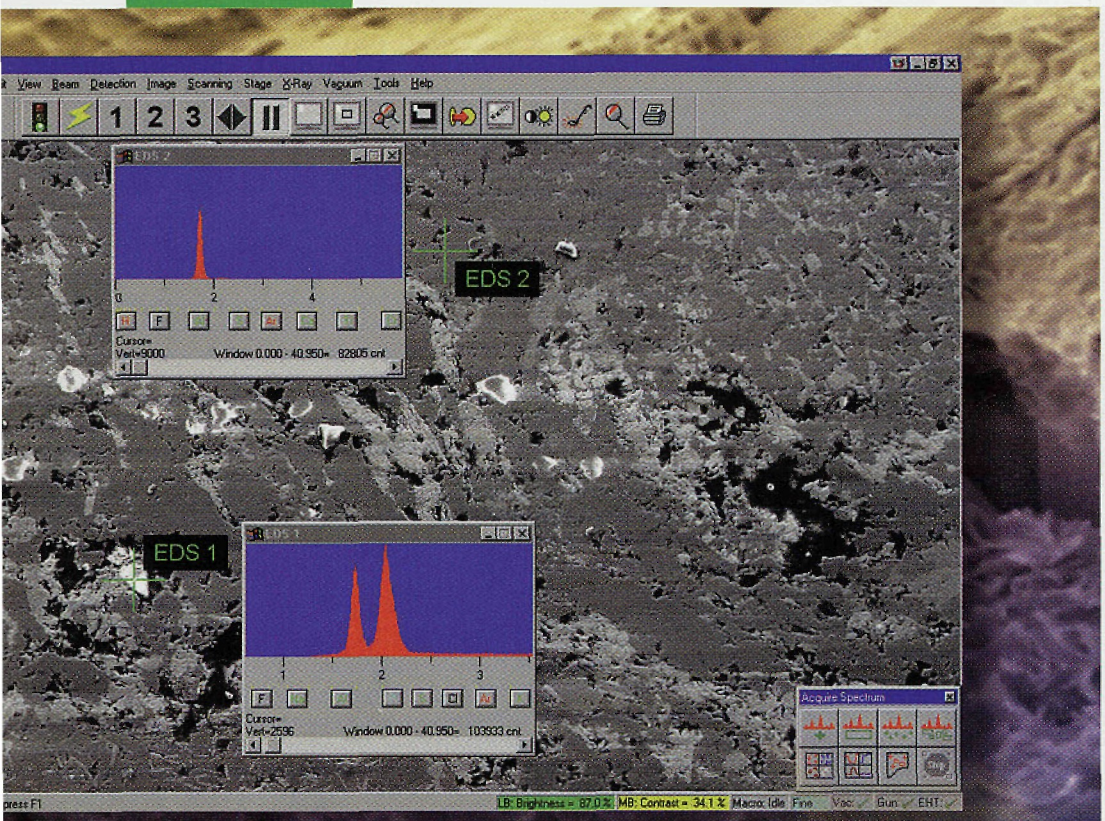


Imaging to Analysis in just two clicks...



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Circle Reader Inquiry #2

Long-Lasting Aqueous Mountants for Light Microscopy

John A. Kiernan,
The University of Western Ontario

The requirements for such a medium are that it won't deposit crystals when it evaporates and that it won't be incapable of evaporating. The second condition probably excludes anything containing glycerol. The first is more difficult. A polymer such as gum, polyvinylpyrollidone or gelatin might fill the bill, especially in the presence of a sugar that can form a syrup that's almost solid. Fructose (levulose) has this reputation, and is a good aqueous mountant. Very cheap, but for some reason somewhat too acidic for basic dyes, but can be buffered. It's not 100% predictable. I've had fructose crystallize under coverslips, but you can quickly remove all the mess with water and then try again.

An old and certain way to make a solid and perhaps permanent aqueous mount is to use a smaller-than-usual coverslip and as little aqueous mountant as possible. Allow a few days for water evaporation at the edges of the coverslip, otherwise the refractive index will be too low and unstained parts of the section will be visible. Sometimes that is wanted, in which case just mount in a minimal drop of glycerol under the small coverslip. It's important to keep the top of the coverslip completely free of dust, condensed moisture and anything else insoluble in xylene. When the aqueous mount with its little coverslip looks OK, give it a swish in clean xylene to remove any grease and apply a larger coverslip in the usual way, as if the preparation were a very thick (200 µm) section. The aqueous mount is now sealed into a resinous medium.

The problem with this is that coverslip thickness is at least 340 µm. This reduces the resolution of a microscope objective without an adjustable "coverslip collar," and if the section itself is a thick one, its lower levels may be beyond the working distance of anything cheaper than a $20,000 water-immersion objective. This won't be a problem unless you're counting mitochondria or peroxisomes, but the unduly thick double coverslip can still be annoying, because it necessitates much fiddling with the fine focus and substage aperture. These annoyances can be reduced.

A refinement of the above technique is especially valuable for cryostat sections collected onto coverslips rather than slides. Use a small or a large coverslip to collect the section. After staining, make the aqueous mount on a larger or smaller coverslip. Wait for evaporation, etc., as explained above, and then do the resinous mounting, with the larger coverslip uppermost and the smaller one applied to the slide. Now there is only the thickness of one coverslip (and an unknown but small depth of one mounting medium) between the specimen and the front lens of the objective.

For dehydrogenase histochemistry (seeing mitochondria in unfixed cells, usually in tissue whose general microanatomy suffers quite a lot to get this result), collection of sections on coverslips is a must. For lipid staining with Sudan dyes, it's often OK to collect the sections on slides and have double thickness coverslipping, because high resolution may not be needed.

Never use any product without finding out and learning about its composition. The results of research are questionable if any part of the methodology is not fully understood by the investigator. This applies even to a mounting medium. How certain can you be that it faithfully preserves the stain? And for how long? And how do you know it's that good? ■

Front Page Image

Outer Surface of Egg Shell

Landscape Format – Original Mag.: 1,500X – Final Mag.: 3,100X – (10 µm = 31 mm)

The image was taken on a JEOL JSM-840 SEM at 5 kV. Digital files were captured at a resolution of 1280 x 960 with a 160 second dwell time. The image was manipulated as indicated in the article "Making Anaglyph Images from SEM Images Using Adobe PhotoShop" by Debby Sherman on page 8 of this issue, and was printed on a Codonics 1660 dye-sublimation printer. Stereo pairs were separated by a 7° tilt.

★ A STEREO IMAGE – VIEW WITH RGB GLASSES ★