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https://doi.org/10.1017/S1551929500060004 Published online by Cambridge University Press

Acetate Peels

A slick technique for separating thin coatings from their substrate for elemental analysis, along with a bit of history

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It's hard to believe it when you realize you're getting to the point of almost being eligible for "old geezer" status. But along with the title comes the fact that, if you've been paying attention, you've learned some neat stuff along the way. Every once in a while when I'm doing some "routine" task around the lab, I realize that I first did this some 30 years ago! Take making acetate pel replicas, for example. Before scanning electron microscopes came along, peels were the standard technique for looking at surfaces of bulk samples. While the supply houses used to sell "replica-making kits" and may still do so, I found heavy-gauge drawing acetate in sheets from an engineering drawing supply store to be both cheaper and better. That source has probably dried up, what with the advent of CAD-CAM and all that, but the development of energy dispersive x-ray spectroscopy as a routine tool has raised another problem: I believe that 100% of the cellulose acetate sheet in the United States is made with a phosphorous-bearing flame retardant. If you're interested in the phosphorous content of your sample, you've got to make your own acetate sheet.

Aldrich sells a good quality cellulose acetate powder. Simply mix up a small amount with a suitable amount of acetone to make a thin solution. Pour it into a Petri dish and allow the acetone to evaporate out to dryness. A little practice will tell you about proportions and how thick you need to make the sheet so that it will hold up in the process of making the replica. Cut a small square out of your sheet; moisten one side of it with a few drops of acetone, smeared one drop at a time onto the sheet surface with a glass stirring rod, to make a "gooey" spot. (Don't put so much acetone on as to make the entire thickness of the sheet soft.) Then press this spot onto the area you want to replicate, holding it still in place for at least a minute with the fleshy part of your thumb. Allow to dry completely, probably for several hours, then peel off.

I opened suggesting this as a technique for separating coatings from substrates. We routinely do this in the following sequence of steps:

1. First examine the sample of interest in the light microscope.
2. Then examine the sample in the SEM. When you find the area of specific interest, take the sample out of the SEM and figure out a way to mark it with reference marks. We commonly simply scribe an "X" in the surface near the area of interest with a sharp tool of some kind. Then photograph it with the scribe mark in place in the light microscope. (We use a 3-CCD camera and Image Pro software, printing the images in color on a color laser printer.) Now you have a color map to guide you in the SEM, in which you lose what may be important color clues. Take the SEM examination up as high as you need to, to be able to return to exactly the place of interest on the sample. We document everything with many micrographs, using thermal transfer prints made on a Seikosha VP-3500; the prints are virtually free.
3. You may simply be able to peel off the material of interest with a straight-forward acetate peel. Otherwise, if the coating of interest is extremely well bonded to the substrate, you will now have to torture the surface. Immerse the sample in liquid nitrogen, allow it to reach temperature equilibrium, and rapidly bend it back away from the surface of interest. This stretches the top surface beneath the coating and usually frees some of the coating. Quickly heat the sample in a hair dryer to prevent moisture from condensing on the surface. Then make your peel. Carbon-coat the peel and examine it in the SEM. It will contain your "X" scribe, and usually at least some of your coating. You will now be able to return to the surface of your original sample at exactly the same spot from which the coating has been removed and compare compositions of the coating and the spot on which it was adhered.
4. The next step is to take that coating into the TEM. This can be done by washing away the acetate, dropping the coating fragments onto thin carbon substrates on TEM grids. This washing can be done in a specially designed reflux apparatus (condensation washer, available from E.F. Fullam).

This technique has worked with a broad variety of samples. I've often found that attempting to actually produce a controlled cross-section of a material with a thin surface coating is much more trouble than simply fracturing that coating in this way. You have a very good idea of what kind of artifacts you may have introduced by the deformation, as opposed to all the unknowns in any cross sectioning process. ■

NEW RMS MICROSCOPY HANBOOK

Negative Staining and Cryoelectron Microscopy

By J. Robin Harris, Institute of Zoology, University of Mainz

A review by Fred G. Lightfoot, Electron Microscope Consultants

The Royal Microscopical Society has a tradition of providing concise and informative reference handbooks in electron microscopy. With the addition of their latest book they have provided an excellent guide for anyone who may need to better understand the technology and applications of negative staining and cryomicroscopy. This text provides the reader with information guiding him through the most basic preparative procedures and discusses various applications using both conventional and the more esotericaly designed experiments.

Chapter four discusses very candidly the use of negative staining with respect to immunogold labeling and reveals not only various methodologies but also their limitations. I particularly enjoyed the section on 'problems and artifacts of negative staining' in this chapter, because it clearly outlines how one can misinterpret the images that are produced. Also, in chapter five, Dr. Harris has given many examples of negative staining to better understand how one can visualize lipid-membrane structures, viruses and macromolecules with and without computer processing, to mention a few.

In chapter seven he goes into considerable detail outlining the way one may vitrify samples via 'plunge freezing'. Also he discusses the difficulties one may experience in the vitrification of biological samples, which is frequently overlooked in other publications. There is an excellent section (albeit a bit short) on how one can utilize plunge freezing in physiological and time-resolved protocols; a short discussion follows, which touches upon long-term storage of vitrified specimens.

With the excellent image analysis software packages on the market, Dr. Harris discusses the use of this technology in chapter ten. As with any new advancements in the biomedical imaging field, one must be cautious in how to interpret this digitized data. There is no question that negative staining, cryropreparation and cryomicroscopy has become, once again, a method that can be used to better understand and enhance research capabilities in macromolecular biology. I recommend this text to all research scientists who are just starting in the field of cryoelectron microscopy or as a reference to those who need to upgrade their skills in this area.

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