

problems where cell shape analysis is required [3]. Point dropping was encoded into BGSU's software for tracing a contour which can be run by logging onto the anonymous FTP server <elvis.bgsu.edu>

#### References:

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2. Heckman, C.A. and Jamasbi, R.J. (1999). Describing shape dynamics in transformed cells through latent factors. *Experimental Cell Research.* 246, 69-82.
3. Heckman, C.A., Plummer, H.K., and Mukherjee, R. (2000). Enhancement of the transformed shape phenotype by microtubule inhibitors and reversal by an inhibitor combination. *International Journal of Oncology.* 16, 709-723.

## Making Replicas of Surfaces for TEM and SEM

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### Carbon Replicas

Before SEMs were invented and when they were still of relatively poor resolution, one way to see the fine details of a sample surface was to prepare an electron-transparent replica of the sample surface and view it in a TEM. The carbon-coated surface of the sample was shadowed with a heavy metal to make a replica that mimicked the topography of the original surface, in a sample that could be viewed in the TEM.

We have found some value in this old technique; to examine second-phase particles freed from the metal matrix for EDX, diffraction, and morphology studies--while preserving the original position and distribution of the particles, historically called "extraction replication."

To prepare a carbon replica:

1. Polish the specimen to a one micron diamond polish, or equivalent.
2. Lightly etch the surface with the appropriate etch for the metal. When we were using the technique for low-carbon, micro-alloyed steels, we used two percent Nital (2 ml. Nitric acid and 98 ml. methanol) for about 10 seconds to slightly etch the surface. Rinse and dry.
3. Carbon-coat the sample surface with at least 20 nm of carbon in an evaporator.
4. Score the carbon coat with a razor blade into approximately 3 mm squares.
5. Etch the specimen by immersing in a stronger etchant for one to two minutes. For the low-carbon, micro-alloyed steels, we used five percent Nital. You should see the carbon coat start to wrinkle.
6. Submerge the specimen in a dish of distilled water with the carbon-coated side up. The carbon squares should pull free and float to the top of the water. Use a plain copper TEM grid of 75 or 100 mesh to scoop up the carbon squares from below the surface of the water.
7. Blot the grids dry on filter paper, carbon side up.

You should be able to image the replicated surface of the etched material in the TEM, preserved in the carbon film. The second-phase particles that were caught and held by the carbon film should be darker and their position and morphology preserved by the carbon film. They are free of any interference from the matrix metal for EDX and electron diffraction studies.

The best source of recipes for etchants is George F. Vander Voort's book: METALLOGRAPHY Principles and Practice, McGraw-Hill Book Company.

### Cellulose-Acetate Replicas

To sample the morphology of a surface that is too large to go into an SEM, or that is not available for examination or to sample the corrosion product from a surface, a cellulose-acetate replica can be made of the surface and examined in the SEM. Cellulose-acetate (CA) replicating tape and sheet is available from most EM catalogue suppliers. Cut a strip of the tape large enough to cover the area you want to sample, plus a piece at the end for a handle. I usually use about a 10 to 15 mm long strip, about 10 mm wide. Bend the last three mm up at a right angle to make a little handle.

To make a cellulose-acetate replica:

1. Place the strip of CA on a pile of filter papers soaked in acetone and leave it on there for 10 to 20 seconds until the bottom gets sticky. You will have to experiment to see how long to leave it, so the CA gets soft but does not melt away.
2. Put a puddle of acetone on the surface to be replicated. Use the handle on the CA strip to pick it up and put it on the puddle of acetone. Try to exclude any air from under the CA strip.
3. Leave the CA on the sample for at least half an hour, until the acetone is all evaporated and the CA is firm again.

Carefully peel the CA off the sample and trim off the handle. Glue it, replica side up, on an SEM stub. Carbon coat the CA for EDX analysis of any corrosion product that has been stripped off or gold coat it for morphology or fractography. It can also be viewed in variable-pressure mode uncoated. Remember that some features will be reversed, e.g. the "cup-and-comb" features of a ductile fracture will now be a series of bumps. Brittle fracture and fatigue should look the same as the real sample. You can strip multiple replicas from a corroded surface to clean the metal gently and examine the layers of corrosion product.

There are many other specimen preparation protocols using replication techniques that are useful in all fields of electron microscopy. I hope others will be willing to share their knowledge of this art in future Tech Notes.

### Addendum to TJ Collins article on Mounting Medias\*

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A caveat about using fingernail polish cover slip sealing for tissues containing Green Fluorescent Protein (GFP) was given by Chalfie et al, 1994.<sup>1</sup> They reported the chemicals in nail polish interfered with GFP fluorescence, a problem we also experienced in our laboratory. Clontech, a GFP supplier, warns customers about this problem. The chemical in question may be the alcohol that leaches into aqueous mounting media. We dilute permanent acrylic mounting media with its solvent (e.g. xylene or toluene) so the media is thinner than the nail polish, less messy to use, and easier to apply around cover slip edges. Toluene and xylene are not miscible with water and cannot leach into aqueous mounting media although other chemicals may be involved. Diluted media dries rapidly and also prevents 'drying' retraction bubbles from forming under the cover glass with two hard set anti-fade mounting media (Vectashield, Vector Laboratories, CA and Prolong Gold Molecular Probes, OR).

\* Collins TJ, *Mounting Media and Antifade Reagents*, *Microsc. Today*, 14, 1, January 2006.

1. Chalfie M, et al., *Green Fluorescent Protein as a Marker for Gene Expression*, *Science* 263:802-805, 1994.