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DOWN THE RESOLUTION ROAD: FREEZE-FRACTURE REVISITED?

Stephen W. Carmichael,[1] Mayo Clinic

It must have seemed rather fantastic back in the fifties, when Russell Steere[2] froze chunks of plant viruses (tobacco mosaic virus, tobacco ringspot virus, and squash mosaic virus) in drops of water, planed them freehand with a scalpel blade, made a replica of the surface, and examined the replica in a transmission electron microscope. But that was the birth of freeze-fracture and freeze-etch methodology that yielded enormous amounts of information about the morphology of membranes. Now Yamamoto and Tashiro[3] have sectioned mammalian tissue and examined the surface with the atomic force microscope. The surprising thing is that they were able to visualize considerable morphology! Could we be going down the same road?

Yamamoto and Tashiro perfused rats with a mixed aldehyde and embedded small pieces of kidney and liver in LR White resin. Ultra-thin sections were mounted in collodion-carbon-coated nickel grids with the newly-sectioned surface facing "up." They were examined unstained with an Olympus atomic force microscope in the contact mode. They consistently observed depressions that corresponded to plasma membranes, basal laminae, mitochondria, and chromatin blocks in the nucleus. The depressions ranged from 6 to 30 nm. The surface of the cytoplasm showed strong relief, 3 to 4 nm on average. Protrusions could be seen on the complimentary surface. Other specimens embedded in Epon had relatively smooth surfaces. The authors pointed out that whereas Epon covalently links to tissue proteins, the links are apparently much weaker in resins such as LR White. The LR White appears to act in a manner similar to ice in a freeze-fractured specimen.

The atomic force micrographs that accompany the article look hauntingly like freeze-etched images, except that the scale bar is in nanometers, rather that micrometers. While the morphology is intriguing, Yamamoto and Tashiro point out that the real value of their technique is that antigenic epitopes may be revealed at the surface of such ultra-thin sections, and subsequently visualized with appropriate immunolabelling.

It will be interesting to see where this new methodology takes us. For now it looks like we're headed down the same road we were half a century ago when freeze-fracture was introduced as a new way to prepare specimens for the transmission electron microscope. The obvious difference is that we are now looking with new instruments that give us orders of magnitude increases in resolving power. Yamamoto and Tashiro have shown us another way to use this power! ■

1. The author gratefully acknowledges Akitsugu Yamamoto, Department of 1st Physiology, Kansai Medical University, for reviewing this article.

2. Steere, R.L., Electron microscopy of structural detail in frozen biological specimens, *J. Biophys. Biochem. Cytol.* **3**:45-60, 1957.
3. Yamamoto, A. and Y. Tashiro, Visualization by an atomic force microscope of the surface of ultra-thin sections of rat kidney and liver cells embedded in LR White, *J. Histochem. Cytochem.* **42**:1463-1470, 1994.

Front Page Image
Orientation Imaging Micrograph Depicting Grain Orientations of Beryllium Weld Area

Welding causes significant microstructural changes in both the weld area and the adjacent heat affected zone (HAZ). The image shown is that of the microstructure of a weldment of Beryllium as seen by orientation imaging microscopy and is based upon 40,000 individual crystallite lattice orientation measurements using the backscatter Kikuchi diffraction (BKD) method in the SEM. Be, a hcp crystal structure material, offers potentially significant challenges to the BKD technique in that the atomic number is so low. Nevertheless, images of reliable quality were obtained as evidenced by the structure shown. The colors on the image are computer generated and high light the grain boundary morphology. Thin black lines indicate a change in orientation between crystallites of 5 to 15 degrees, while thick black lines are changes in orientation of more than 15 degrees between neighboring measurements. The elongated grains show the structure of the weldment with the smaller equiaxed grains being representative of the heat affected zone. Interrogation of the data indicates that the crystallographic texture of the material is very weak, especially in the HAZ. However, analysis of individual lattice orientations reveals that a number of texture components which exist in the weld area are not present in the neighboring structure. It is significant that such orientations arise during the solidification procedure subsequent to a welding pass even though they do not exist in the original structure. In addition, a number of orientations which are present in the original material are not generated in the weld area. The effects of welding on the local texture of a material can be thoroughly interrogated using orientation imaging microscopy.

The Orientation Imaging Micrograph on the front cover and the preceding analysis have been furnished by TexSEM Laboratories, Inc. (TSL), headquartered in Provo, Utah. TSI offers technologically advanced products and services to quantify and analyze the microstructures of crystalline materials. TSL pioneered the development of Orientation Imaging Microscopy™ (OIM™), combining scanning electron microscopy, high resolution video technology, and leading edge software to automate the rapid capture, analysis, and imaging of crystallographic data obtained via electron backscattered patterns (EBSP).

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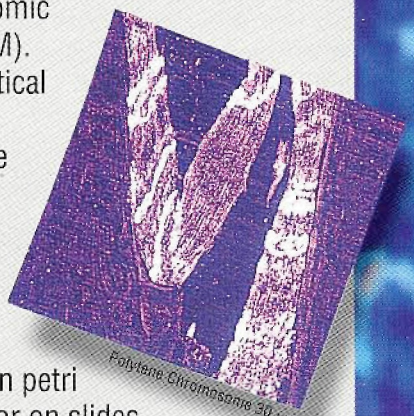

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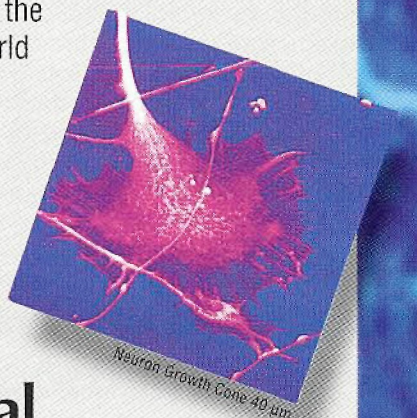

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AFM images courtesy of the Iowa State University (Dr. E. Henderson and Dr. P. Haydon).

Protein DNA Complex 1.2 µm

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