We appreciate the response to this publication feature and welcome all contributions. Contributions may be sent to José A. Mascorro, our Technical Editor, at his e-mail address: jmascor@tulane.edu. José may also be reached at the Department of Structural and Cellular Biology, Tulane University Health Sciences Center, 1430 Tulane Ave., New Orleans, LA 70112 and Ph: (504) 584-2747 (504) 584-1687

Microwave Processing of *Drosophila* Tissues for Electron Microscopy

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Insect tissue is often difficult to prepare for electron microscopy because of the impenetrable barrier surrounding the body tissues. *Drosophila* salivary glands have been used for numerous studies because of the large size of the cells and their large polytene chromosomes. Early TEM studies of salivary glands (1) used a protocol that took several days. We were able to achieve excellent preservation and good ultrastructure in *Drosophila* salivary glands and imaginal discs from Stage L3 larvae using microwave processing in a protocol requiring less than 2 hours.

We used a Pelco Laboratory microwave (model #3451) equipped with a Cold Spot, Steadytemp chiller/recirculator run at 15° C, and vacuum chamber (Ted Pella, Mountain Lakes, CA) (2). The heads and attached salivary glands were removed from the animals and placed in PBS. The tissue was transferred to Pelco prep-eze specimen holders (#36157) for ease of handling. Our goal was to use the microwave effect, not the heating effect, to prepare the tissue (3). For conventional TEM the glands and discs were prepared as follows:

1. Tissues were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, at power level 1(100 W) for 1 minute on-1 minute off-1 minute on. The power level was then changed to power level 4(450W) and the tissue was placed under vacuum (using house vacuum) in the chamber and pulsed for 30 seconds on-30 seconds off-30 seconds on 3 times. They were then removed from the microwave and allowed to sit for 15 minutes on the bench at room temperature.

2. Following two brief rinses in 0.1 M cacodylate buffer containing 0.3M sucrose on the bench, the tissue was post-fixed in 2% osmium tetroxide containing 0.8% potassium ferricyanide for 20 seconds on-20 seconds off-20 seconds on at power level 1 (100 Watts) twice with cold spot at 15° C. The tissue was removed from the microwave and allowed to sit for 15 minutes at room temperature.

3. After rinsing with distilled water, the tissue was dehydrated in an alcohol series: 50%, 70%, 95%, 100 (times 3) at power level 3 (350 watts) for 30 seconds each step.

4. The tissue was infiltrated in 1 part 100% alcohol:1 part embed 812 resin (Electron Microscopy Sciences, Fort Washington, PA) for 8 minutes at power level 3 (350 watts). They were changed into 2 parts resin:1 part 100% alcohol and microwaved for 10 minutes at power level 3. The tissue was placed into 100% resin and microwaved for 8 minutes at power level 3 (350 watts).

5. The discs and salivary glands were removed from the mouthparts and oriented in a flat embedding mold. The resin was hardened overnight in a 60° oven.

6. Sections 1µm to 1.5 µm were cut using a histoknife (Diatome) and dried flat on slides on a hotplate. Thin sections 50-70 nm were cut using a Diatome diamond knife and post stained with 5% aqueous uranyl acetate and lead citrate. Specimens were examined in a Phillips 410 Electron Microscope at 60 KV accelerating voltage.

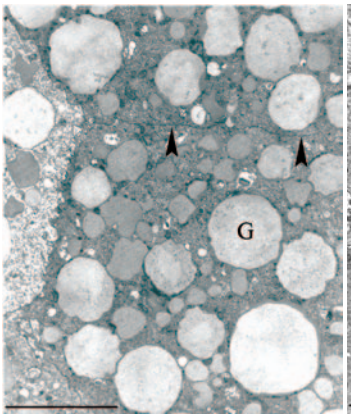

Figure 1. Drosophila salivary gland showing granules(G) and septate junction (arrowheads) between the epithelial cells. Scale bar 5µm

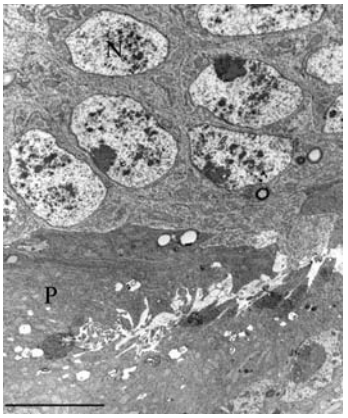

Figure 2. Drosophila wing imaginal disc showing the nuclei (N) of the epithelial cells and the peripodial cell layer (P). Scale bar 5 µm

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Mechanical Polishing Methods for Metal Samples for EBSD

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Electron backscatter diffraction is a powerful technique that has gained momentum in materials science research over the last ten years. Recent advances in electron microscope technology and automation has made EBSD a more viable and routine analytical tool [1].

Sample preparation is a key component in the use of EBSD due to the nature of the backscattered signal. Pattern formation is from the top 10-50 nm of the sample surface, and therefore, mechanical damage remaining from sample preparation will result in a poor quality signal [2]. Several methods of sample preparation have been investigated that vary in abrasive material used, preparation time, and material system. Some approaches have used a combinatorial approach of silicon carbide papers, aluminum oxide papers, and aluminum oxide suspension with a polishing cloth to prepare tantalum [3]. This protocol has yielded acceptable results, but proved to be extremely time consuming. Other methods for preparing metals, such as steels, have utilized electro-polishing with a perchloric acid electrolyte solution [4]. Electropolishing is an excellent method for preparing samples for EBSD, but can be difficult to accomplish for a wide range of materials due to the large number of acid solutions required. Recently, work has been done using focused ion beam thinning to produce samples suitable for EBSD, but problems can arise due to amorphous damage and

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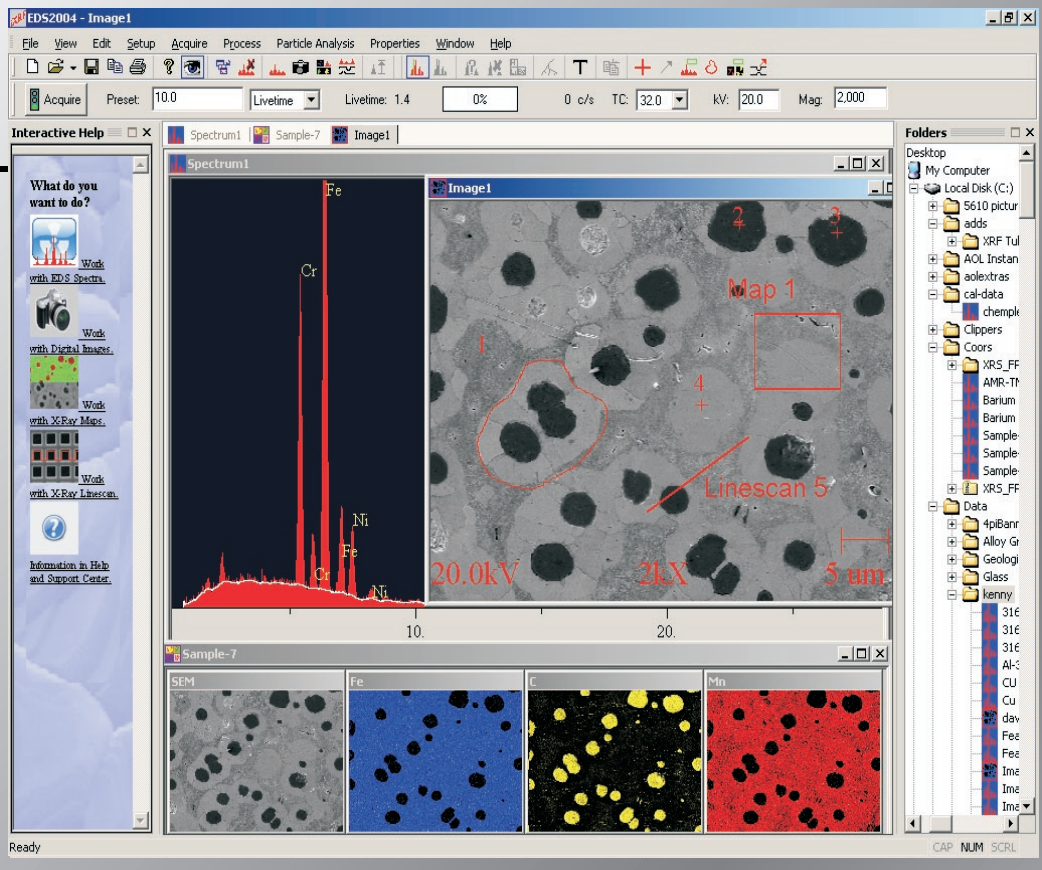

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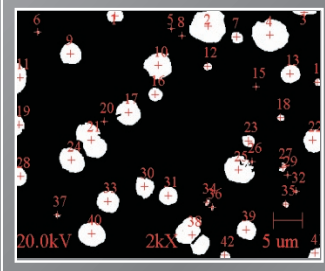

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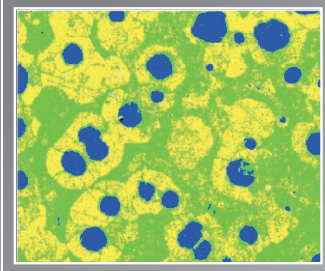

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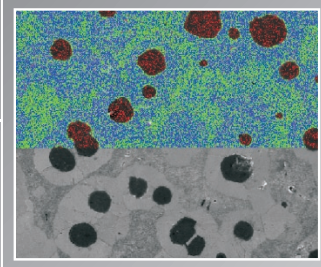
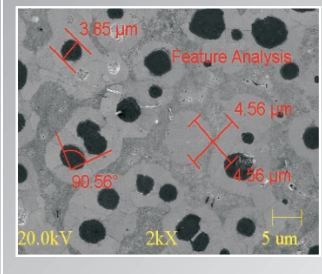


Image Processing



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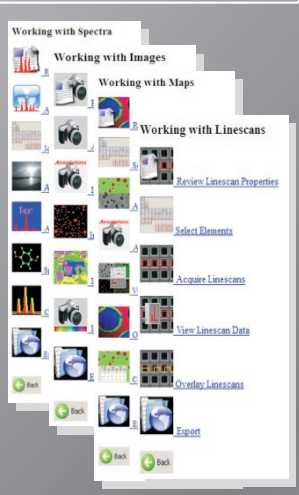

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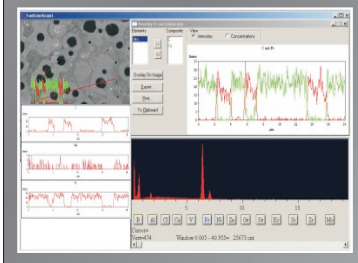

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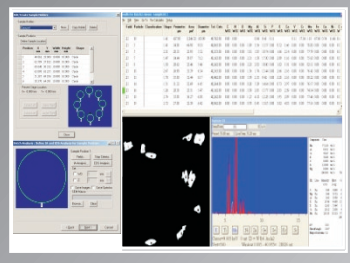

Spectral Matching

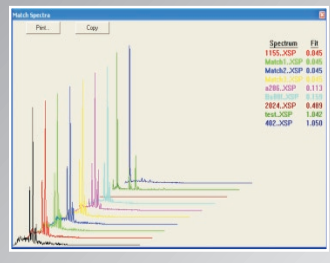

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FIG.1. Polishing setup used for the preparation process.

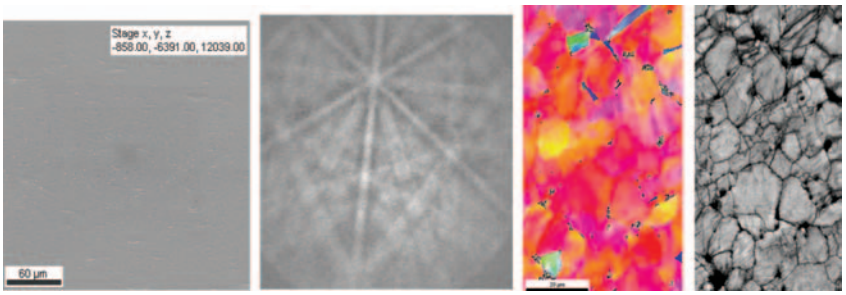

FIG. 2. A) Surface image of the brass sample taken using SEM, B) EBSD pattern acquired from the sample; C) Image quality map of the polished sample demonstrating a good, well polished sample.

strain induced from ion beam bombardment [5].

A simple approach of mechanical polishing utilizing standard metallographic processes has been used on a wide variety of materials to produce EBSD quality samples. The technique uses a semi-automatic polishing machine combined with a precision lapping fixture to control the polishing process. Thickness control of each step is critical in the ability to eliminate mechanical deformation of the sample and to ensure that a high quality EBSD pattern will be obtained. Metal samples of unknown compositions of brass, stainless steel, and copper were prepared using a Model 920 Lapping and Polishing System combined with a Model 150 Lapping and Polishing fixture. A series of silicon carbide abrasive papers from 240, 400, 600, 800, 1000, and 1200 grits were used for preparation, each taking 5-10 minutes per step. Final polishing was done using 1µm diamond suspension on Satin cloth followed by 0.05µm colloidal silica polishing on a MultiTex™ polishing cloth for 5-10 minutes. The equipment setup is shown in Figure 1, with the results given in Figure 2. The image quality map shows a high quality surface finish that is deformation free, and the EBSD pattern is easily acquired using these preparation methods.

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High Pressure Freezing User Group Meeting Launched at M & M 2004, More to Come

The nation's first High-Pressure Freezing (HPF) User Group Meeting, hosted by by BAL-TEC RMC, a joint marketing alliance comprised of BAL-TEC AG, Boeckeler RMC Products, and TechnoTrade International, Inc., was successfully launched during M & M 2004 on Sunday, August 1, in Savannah, Ga. About 40 attendees came from all over the U.S., ranging in disciplines from plant pathology, biology, and neuroscience, to chemistry and pharmaceuticals. All have either used or plan to use high-pressure freezing as a method to freeze specimens intended for examination in the electron microscope (EM).

High-pressure freezing is especially used for freezing large tissue specimens without requiring structure-altering freeze protective additives. It is ideally suited for use in conjunction with subsequent freeze substitution, followed by low temperature embedding and polymerization for sectioning in a conventional ultramicrotome for morphological preservation and immunogold labeling. HPF is also well suited for subsequent replication by freeze fracturing in a freeze etching system for transmission electron microscopy (TEM), subsequent cryosectioning for cryoTEM and cryo scanning electron microscopy (SEM) investigations.

Summary of Presentations

Presenters for the meeting included Robert Apkarian of Emory University in Georgia, who showed how HPF is used in imaging self-assembled organic hydrogels by high-resolution cryoSEM. Allison Vendemeene of Arizona State University, a post-doc in Robbie Roberson's lab, showed how superior morphologic preservation of yeast for TEM tomography could be accomplished with HPF. Her images detailed the improved morphology of samples difficult to preserve with traditional chemical fixatives.

Maryann Martone of the University of California, San Diego, gave a popular presentation on how to use an XML-based database called NeuroSys to catalog and manage large data sets of HPF information.

Roger Wepf of Beiersdorf, AG, Germany, gave two presentations. His first was on closing the gap between histology and ultrastructure research by using 3D Correlative Microscopy with HPF. He explained the advantage to imaging one and the same sample by using HPF followed by a modified freeze substitution process. This allows investigation of the identical sample in the light microscope (histology), the confocal microscope (3D morphology) and the TEM (ultrastructure). The aim of this approach is to have 3D datasets from the identical structure at different resolution and content levels, a so-called multimodal image dataset for further structural investigations.

Wepf's second presentation discussed HPF for high-resolution cryo-SEM. Here, he showed the advantage of the different freezing techniques and compared the various structural data one can obtain by cryo-SEM.

David Hall of the Albert Einstein College of Medicine in New York, presented the advantages of low-temperature fixation for *C. elegans* by using HPF and freeze substitution techniques. HPF captures fast membrane events that are lost during slow chemical fixation. He also detailed freeze substitution protocols to preserve antigenic activity for immunogold labeling.

The Next Meeting

Plans are in the works to incorporate a regular HPF User Group meeting at future M & M meetings. If all goes as planned, the next one will be held on Sunday, July 31, 2005 in Hawaii. HPF users or potential users who wish to be on the invitation list for the user group should contact Dave Roberts of Boeckeler Instruments, Inc., at (800) 552-2262 or e-mail him: dave@boeckeler.com.