MICROSCOPY 101

*We continue to appreciate contributions to this new publication feature. In addition to items relating to electron microscopy, we hope to publish more material on other microscopy techniques. To do this, and hopefully make the feature of increasing interest and value, we need **your** assistance. Contributions may be sent to Philip Oshel as follows:*
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Curing Resins in Microwave Ovens

There are two approaches in the literature for microwave accelerated curing of resins: 1) Giammara's approach for flat embedding molds and 2) Giberson and Demaree's approach for BEEM capsules. Both methods give excellent results

Start with 50% power for flat embedding molds for 15 minutes (100% power will definitely give you disappointing results). I highly recommend using an appropriately sized water load for your oven during microwave curing in flat embedding molds (otherwise there is simply too much energy in a microwave oven, and specimen damage from overheating will result).

Resins in BEEM capsules are cured by floating them in a water bath and heating by microwave energy to 100° C (maximum!). Curing time is about 75 minutes. See the reference by Giberson and Demaree (1995).

Allow blocks to cool for 15 minutes before removing from the molds.

In addition, microwave curing in an uncalibrated microwave oven is very tricky and usually results in disappointing results (e.g., incomplete curing of blocks). Simple tools (e.g., Agar-saline-Giemsa blocks, neon bulb array) that you can make and a detailed description of how to use them to calibrate your microwave oven are published in *The Microwave Toolbook*. With these tools, you can predict the location within the microwave oven that yields uniform heating and curing of resins.

For safety reasons, the microwave oven must be vented or placed in a flume hood while heating resins.

Some useful references:

Giammara, B., 1993. Microwave embedment for light and electron microscopy using epoxy resins, LR White, and other polymers. Scanning 15:52-87.

Giberson, R.T. and R.S. Demaree, Jr., 1995. "Microwave fixation: understanding the variables to achieve rapid reproducible results." Microsc. Res. Tech. 32:246-254.

Login, G.R. and A.M. Dvorak, 1994. *The Microwave Toolbook. A Practical Guide for Microscopists*. Beth Israel Hospital, Boston. ISBN 0-9642675-0-0. $60.00; sold by distributors of histology and electron microscopy supplies or order by calling (617)667-2034. This book contains a table of curing times for resins tested.

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Cleaning Internal Exoskeletal Structures Of Arthropods For SEM

1) Digest the muscle tissues in either KOH or NaOH (3 pellets in 3 or 4 mL water in watch glass at 70° C). Leaving it in this solution after the tissue has digested will also start to clear the exoskeleton.

2) Partially clear the exoskeleton for examination under transmitted light microscopy by either leaving it in the caustic solution for much longer or by *briefly* boiling the solution. I'm told that lactic acid is also useful for clearing exoskeletons. Light microscopy was useful for detecting overlapping of sternites, relative thickness of sternites, difference between sternite and arthrodial membrane, etc.

3) Clean in ultrasonic bath for extended time (say, 5 minutes) to get all the digested ooze out - obviously with some escape path such as the holes where the head and/or abdomen were.

4) Dry the specimen. We got our best results with Critical Point Drying, though this was because the tingids were a bit flimsy. Your scarabs might be more robust, in which case HMDS (hexamethyldisilizane) might work well, or even just air drying from a volatile solvent (acetone or ethanol?).

5) Dissect the specimen. We use eye surgery scissors, very small blade length which worked on even the smallish specimens. Cut along the dorsal and ventral midlines for two mirror-image samples; in our case, one for SEM and the other for light microscopy.

The order is important, since cutting the specimen first and then digesting the tissue lead to the skeleton just rolling up on drying. But this probably depends entirely on your particular specimen. If given the choice, I'd rather cut first and digest second, making digestion quicker and allowing you to help it along by picking away at the muscle with forceps. The ultrasound cleaning would also be easier. As always in EM, you've just got to find what works with your particular beasts.

The final protocol was worked out by Sue Lindsay, Australian Museum.

Geoff Avern, Australian Museum, Sydney, Australia

I have had excellent results air-drying robust structures such as crustacean mandibles from ethanol. Particularly robust structures can be dried from water if from aquatic arthropods, or just put in a desiccator and dried if from terrestrial arthropods. Curling of drying, dissected specimens can (usually) be prevented by pinning the dissected parts, while moist, in the desired position in a dish or tray filled with wax or silicone rubber. Do not unpin until completely dry. -- Phil Oshel

BSE Imaging And Electron Channel Contrast

It is not uncommon for me to employ electron channelling contrast, usually with stainless steels, nickel superalloys (Inconels), Zirconium alloys, and uranium compounds. The electron channel contrast is a weak signal and is not always easy to produce. Here are some ideas to help get good images:

1. Use back scattered electron (BSE) imaging with large beam currents and high BSE signal gain (contrast). It may be necessary to let any response from areas of higher or lower Z than the matrix of interest go to saturated black and/or white to achieve this.

2. Use a "normal" incident beam (0 degrees tilt)

3. Contrary to some advice I have received, I find that lower beam voltages (10 kV) work better than higher (20-30 kV). I have no proof, but suspect that the lower penetration depth images the surface grains without "confusing and diluting" the image with BSE returns from sub-surface grains with different orientations.

4. *Surface preparation* is "very" important. A very well polished surface, free from surface damage is required or the signal will be obscured. Some materials are easier than others to prepare. On occasion, I have had to send samples