

## STANDARD PREPARATION OF BIOLOGICAL MATERIAL FOR SEM

General Scheme: Fix-rinse-post fix-rinse-dehydrate

NOTE: Have fixative-buffer prepared and in a vial before cutting the material. Have all tools and glassware ready. Material must be placed in the fixative immediately after removal from the plant or animal.

1. Place specimen in buffered glutaraldehyde – 2.5% glut. in 0.1 M phosphate buffer, pH 7.2, 1 1/2 – 2 hours.
2. Rinse tissue 2 times in phosphate buffer, 15 minutes each. The fixative and buffer rinse may be put down the drain with plenty of water. The tissue may be stored for several days in buffer if kept cold (0-4°C) or the process may be continued immediately after the rinse.

NOTE: Steps 3 and 4 optional depending on tissue.

3. Remove rinse buffer and replace with the post-fixative—1% OsO<sub>4</sub> in 0.1 M phosphate buffer, pH 7.2, 2 hours. Always use OsO<sub>4</sub> in the fume hood. The vapors are very harmful to the eyes, nose, and throat. Osmium is very expensive—use only enough to just cover the material.
4. Rinse all traces of unbound OsO<sub>4</sub> out of the tissue with 2 changes of buffer, 15 minutes each. Do not discard OsO<sub>4</sub> down the drain. Place it in the appropriate waste jar within the hood. Keep the jar tightly sealed when not in use.
5. Dehydrate the tissue in a graded series of ethanol or acetone, 15 minutes each step.

40-60-80-95-100-100-100%

Do not contaminate the 100% E+OH bottle with a wet pipet. Keep the lid on the bottle when not being used. During dehydration, take care not to allow the tissue to dry out between transfer of reagents.

6. If there are time constraints, the tissue can be stored at this point (100% E+OH). Seal and label the vials and store in the refrigerator.
7. Dry by critical point drying (see instructions for Critical Point Drying) or by the Hexamethy-disilazane method (see instructions for Hexamethyldisilazane method).

## STEREO MICROGRAPHS IN THE SEM

### A. Taking stereo pairs (Use P/N film if possible)

1. Select a specimen and take one micrograph at a magnification of 300-2000 X. This first micro- will be the left photo and can be taken at 0° or any position (toward the detector) angle. Select some feature near the center and, using a marking pencil, trace over this feature. Rather than marking directly on the CRT, carefully tape a piece of clear plastic over the CRT and mark on this sheet.
2. The second image is recorded after tilting the stage 5-15° towards the detector. It is essential that the magnification is identical on both micrographs, therefore, no further use can be made of the focus controls (objective lens). It is also very desirable to have the same contrast/brightness range in the second (right) image as in the first.
3. Tilt the specimen very slowly, using a rapid scan rate. If it is difficult to keep track of the target feature, reduce the magnification during tilting. An angle of 10° difference between the two exposures is suitable for many specimens. Very small features may require greater tilt than large features.
4. Return the magnification to the original setting and carefully recenter the target feature as necessary. It will be slightly out of focus. Use the Z stage control to focus the image.
5. Reset the contrast and brightness controls to their original values and take the second micrograph. Mark both micrographs as to tilt, orientation, etc.

### B. Mounting and Viewing Stereo Pairs

Place the micrographs under the stereo viewer with the less tilted image on the left and oriented so the tops of both micrographs are toward the left. Move them until a sharp, central, 3-D image appears. Mark corners or other points so that the micrographs can be mounted later in the same position.