

## Laboratory for Advanced Electron and Light Optical Methods

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### Cutting in Samples For TEM Processing and Orientation for Embedding

**I. Purpose:** To cut samples in so that they will be properly fixed and well-preserved for TEM examination.

**II. Materials Needed:**

Paraffin-filled Petri dish  
New carbon-steel razor blades  
Applicator stick with one end shaved down to flat surface  
Specimen vials  
Fixative (typically 4F:1G)  
Pasteur pipet and bulb

**III. Procedure:**

**A. General:** Collect pieces of tissue for EM processing from the surface of previously-fixed samples because **any part of the tissue more than 0.5-1.0 mm from the surface exposed to the fixative will probably have poor fixation quality**. Always make sure that the samples stay wetted during the entire processing procedure. It is also important to make sure that the samples do not wind up stuck to the side of the vials above the liquid level. **If a sample is found adhered to the side of the sample vial, discard it**. If it is the only piece available, run it, but note that it may have dried. **Finally, the vials should contain a volume of fixative 5-10 times that of the size of the tissue.**

**B. Handling of Specific Types of Samples:**

**1. Skeletal Muscle:** Always cut long, thin superficial slices from muscles held in clamps or tied to applicator sticks during primary fixation. Failure to have them held in this fashion will lead to contraction during fixation. Each sample should be flat-embedded, with **2 blocks oriented for longitudinal sections and 2 blocks oriented for transverse sections**. If there is limited sample quantity, make the longitudinal blocks first.

2. **Heart Muscle:** The wall of a heart can be oriented just like a piece of skeletal muscle. If a longitudinal strip of ventricle is processed and embedded so that longitudinal sections can be cut, the image is very similar to that of a longitudinal section of skeletal muscle. It is probably a good idea to orient 2 of the 4 blocks prepared so that they can be cut for transverse sections
3. **Peripheral Nerves:** Arrange them for **transverse sections**.
4. **Samples Where Surfaces are Important (e.g., skin, gut):** Flat-embed the samples such that a surface is either up or down in the mold. When trimming and sectioning, leave some empty plastic beyond the surface of the tissue so that the section will not fold over when exposed to the electron beam.
5. **Samples that are Homogeneous (e.g., liver, kidney cortex):** These samples can be embedded in BEEM capsules, since orientation is not critical. When trimming, trim right down to the edges of the tissue in the block face, since the surface is not critical.
6. **Samples that may have Calcified Areas or Other Hard Substances (e.g., growth plates, fish scales, marrow samples, platelet samples, samples collected from nature that may have dirt associated with them):** Always cut semithin sections of these before attempting to cut ultrathin sections. If there is any evidence of hard materials that could damage a knife edge in the sample, either trim that area away before cutting ultrathin sections or cut sections only with a glass knife. **Skin can sometimes be quite damaging to diamond knives, so investigators requesting the LAELOM do a lot of skin sectioning should provide their own diamond knife.**
7. **Plant Material:** Plant leaves and stems are particularly hard to fix because they are frequently covered with trichomes that trap air bubbles when immersed in fixative. To help wet the plant surface, it is usually best to place the samples in the fixative in a vial and then to apply 10-15 lbs of vacuum from an air line via a stopper with a hole in it to which the vacuum line is attached. As the vacuum is applied, air bubbles will rise from the surface of the plant material. This process can be sped up a bit by rapping the vial on a counter surface sharply while under vacuum and swirling the samples while under vacuum to help dislodge the air bubbles. If the sample does not sink after 5 min, it probably will not sink, so the vacuum can be released at that time.
8. **Samples to be embedded in LR White or other acrylic resins:** Since these samples are typically being prepared for immunolabeling, we usually **do not osmicate them**. The resin is also **not compatible with acetone**, so this step is omitted during dehydration. Finally, **the resin will not polymerize in contact with oxygen**, so the samples are typically put into gelatin capsules filled with LR White resin and the capsules are filled to the top before being capped and polymerized

overnight at 60° C. **Do not use BEEM capsules, because they sometimes dissolve.**