Processing Tissues for EM Analysis

1. Initial Consultation

   Because EM projects can be extensive and expensive, the EM Core Facility invites all researchers to meet with core staff before a project is started to discuss the scope of the project, how to procure and fix tissues, time line, budgetary concerns, and any other general issues which might arise. The initial consultation, lasting 1 hr or less, is free of charge. After discussing your project, any time spent by core staff in designing experiments or researching information is charged a consulting fee of $35 per hr.

2. Tissue Procurement and Initial Fixation

   Perfusion fixation

   One good way to preserve tissue ultrastructure for EM analysis to perfuse with fixative, remove the tissue, cut into many small pieces, and place into fresh fixative. The fixative usually includes glutaraldehyde, formaldehyde, and an appropriate buffer such as sodium cacodylate. The specific percentage of chemicals can be based on articles you want to model your project after or can be done using routine formulations for EM such as Karnovsky's fixative. For any perfusion study in animals, you must obtain proper IACUC approval and you must perfuse oxygenated neutral pH buffer prior to the fixative. Each solution must be perfused under low pressure using a peristaltic pump so that the results are uniform. For more detailed help with perfusion methods, please ask core staff.

   Immersion fixation

   For projects where perfusion is not an option, tissues should be excised quickly and immersed in fixative. If you have a number of things to do with each tissue, priority must be given to the EM study. If tissues sit awhile without fixation the tissue structure degenerates, and this is particularly evident if you want to do electron microscopy.

   After the tissue is excised, it should be rinsed in PBS or Hank's balanced salt solution at pH 7.4 (to wash off blood), blotted on filter paper (to remove excess buffer) and placed into a Petri dish containing fixative (enough to keep the tissue covered-the fixative smells bad and so this step is best done in the fume hood wearing proper lab safety clothing and glasses). The large excised tissue should be cut into many smaller pieces with a sharp scalpel blade. Cutting the tissue into small pieces is VERY important for initial fixation. Each tissue has its own characteristics, so core staff will advise about how to cut tissues so that the orientation is correct. After the tissue is cut as required, they are transferred to a labeled glass scintillation vial (or Eppendorf tube) containing fixative. It is VERY important that you label the vials clearly with minimal information; we will transfer the information for each tissue vial to our core log-book and re-assign a core accession number. Samples can be stored overnight at 4 degrees C or brought directly to core staff. Please communicate with core staff before tissue drop-off to ensure someone is in the room to accept your samples.

3. Tissue Processing

This step takes the longest to do, about 4 days. In the process, tissues are washed and postfixed, dehydrated, and infiltrated with resin. The tissues are then placed in molds with liquid resin that is cured (hardened) for 48 hours. Because this process takes 4 days to do, core staff requests that tissues to be submitted at the beginning of each week (Mon or Tues) for processing that week. If not submitted by Tuesday, tissues will remain in fixative or buffer until the next week. It is possible to handle 12 vials for processing at one time. Thus, you should take advantage of this limitation by holding experiments until there are 12 vials to run at once or taking samples from multiple organs that might be useful to the project.

Processing 12 vials (limit) of tissue will cost about $700 in time and labor (BIDMC price).

4. Sectioning

   After curing the resin, thick sections (0.5 μm) will be cut and stained with Toluidine Blue prior to thin sectioning for EM analysis. Core staff does this to a) ensure that the structures you want to examine are in that particular piece of tissue and that the orientation is appropriate, and 2) to ensure proper tissue fixation. If one block is unsuitable, then another block from that group will be thick sectioned. Cutting thick sections takes about 0.5 hr (30 min) per sample in time and labor. Core staff will evaluate all tissues in this manner unless specifically instructed not to do so. We will return all thick sections to you; the thick sections can be used for photodocumentation and can be published instead of paraffin sections.

   Thin sectioning thickness is 70-90 nm and these sections are placed onto 3 mm diameter nickel grids. The grids are formvar and carbon coated by core staff to stabilize the sections in the electron beam. Prior to analysis, 2 or 4 grids are contrast stained by hand with Uranyl Acetate and Lead Citrate.

5. Imaging

   All users have the choice to have core staff analyze the experiment for them (service work) or to work independently on the EM to analyze the experiment themselves. Service work is best agreed-upon prior to beginning (see #1, initial consultation) in terms of how much time is spent and the scope of the project. Training is required for all independent use of the electron microscope and users are expected to abide by the usage guidelines established by core personnel.

6. Communicating and Documenting Results

   After the images are collected there is an option to reconvene and discuss the findings of the Service Work. Any time spent in analyzing data/results, writing Service Reports, trouble-shooting problems, meeting to discuss results, etc… will be charged a Consultation Fee of $35 per hr. Images can also be taken by core staff and sent to you for interpretation. Images are collected in digital format and require that you provide a flash drive to transfer. The digital images can also be printed on 8x10 sheets of photo paper and given to you as a “hard copy”. Please discuss with core staff so that the communication of results meets your needs.