Sample preparation for Transmission electron microscopy (TEM)

TEM is a microscopy technique whereby a beam of electrons is transmitted through an ultrathin specimen, interacting with the specimen as it passes through it. An image is formed from the electrons transmitted through the specimen, magnified and focused by an objective lens and appears on an imaging screen, a fluorescent screen in most TEMs, plus a monitor, or on a layer of imaging plate, or to be detected by a sensor such as a CCD camera.

Biological materials contain large quantities of water. To be able to view it in the electron microscopy, the first stage in preparing is the fixation, one of the most important and most critical stages. We need to fixed it in a way that the ultrastructure of the cells or tissues remain as close to the living material as possible. The sample is then dehydrated through an acetone or ethanol series, passed through a “transition solvent” such as propylene oxide and then infiltrated and embedded in a liquid resin such as epoxy and LR White resin. After embedding the resin block is then thin sectioned by a process known as ultramicrotomy, sections of 50 - 70 nm thickness are collected on metal mesh 'grids' and stained with electron dense stains before observation in the TEM. Sectioning the sample allows us to look at cross-sections through samples to view internal (ultra)structure. Many modifications to the basic protocol can be applied to achieve many different goals, immunogold labeling for example; the in situ localization of specific tissue constituents using antibody and colloidal gold marker systems.

Every sample is different. Please consult with the EM Staff before starting a project.

Support film on TEM grids

Formvar film is useful for the support of ultrathin sections on the finer mesh grids. Using of support film are ideal for those applications requiring large viewing areas without grid bar interference. These films must be strong, clean and remain attached to the specimen grids during specimen preparation.

A Formvar film covered with a "light" layer of carbon will help to stabilize the film when the film is exposed to the electron beam.

Sectioning with ultramicrotome

Materials for TEM must be specially prepared to thicknesses which allow electrons to transmit through the sample, much like light is transmitted through materials in conventional optical microscopy. Because the wavelength of electrons is much smaller than that of light, the optimal resolution attainable for TEM images is many orders of magnitude better than that from a light microscope.

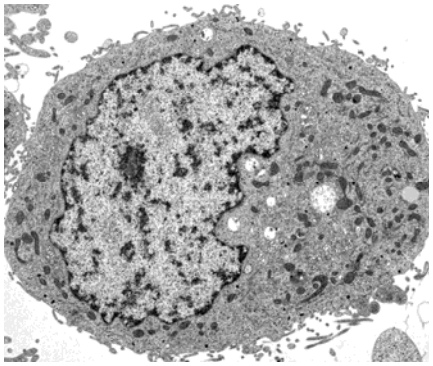
The block is cut into semithin sections (1 µm) with a glass knife, using an ultramicrotome. The sections are then stained with Toluidine Blue for LM for orientation, and for selecting of a small area for ultrathin sectioning. Ultrathin sections are made at 50-70 nm using a diamond knife and placed/collected on a grid of metal.

Positive staining

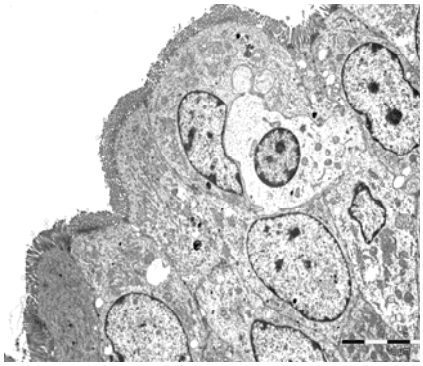
Details in light microscope samples can be enhanced by stains that absorb light; similarly TEM samples of biological tissues can utilize high atomic number stains to enhance contrast. The stain absorbs electrons or scatters part of the electron beam which otherwise is projected onto the imaging system. Uses heavy metals such as lead and uranium to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects).

Heavy metal salts attach to various organelles or macromolecules within the sections to increase their electron density and they appear dark against a lighter background. Uranyl ions react strongly with phosphate and amino groups so that nucleic acids and certain proteins are highly stained. Lead ions bind to negatively charged components and osmium-reacted areas (membranes).

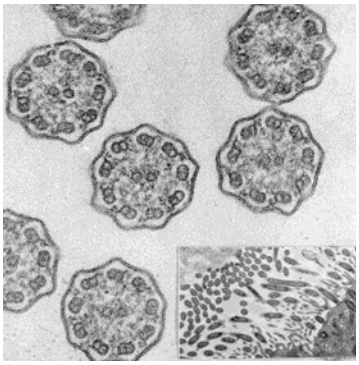
Grids are stained with heavy metals, such as uranyl acetate and lead citrate. The grids, with the specimen side down, remain in 4% uranyl acetate for 25 minutes and are then rinsed in a series of four beakers of pure water. After rinsing, the grids are then stained with 1% lead citrate for 5 minutes, rinsed again in pure water, and stored in a grid box.



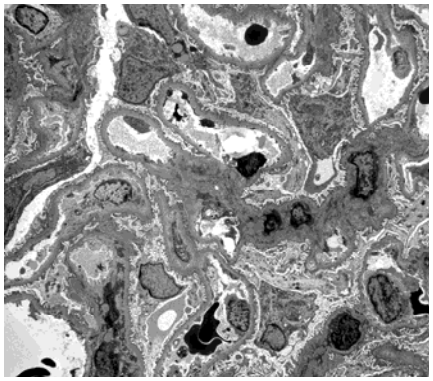
PC-3 cell, a prostate cancer cell line.



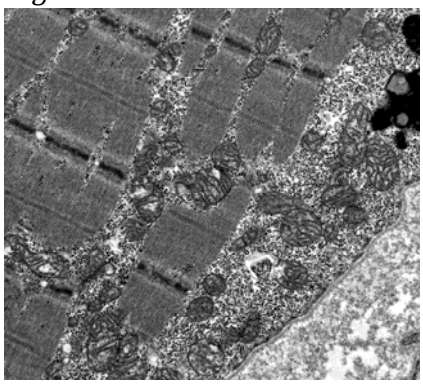
Columnar cells from small intestine, showing cilia and organelles.



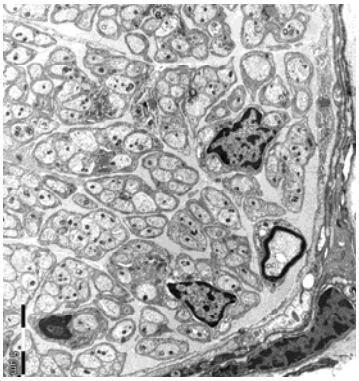
Cilia, transverse section.



Glomerulus in kidney.



Skeletal muscle.



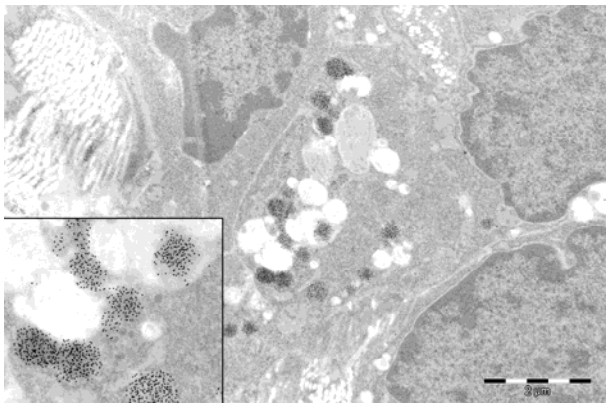
Nervus vagus, rat

Immunogold labelling

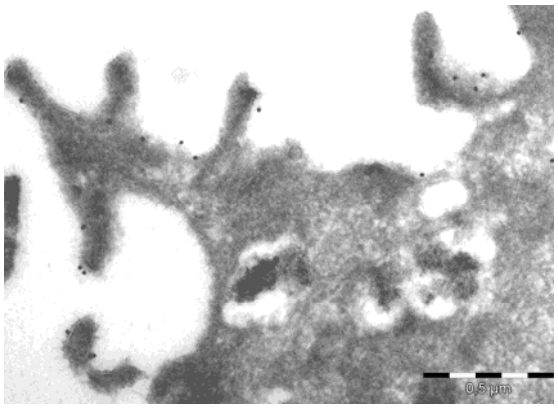
This technique uses antibodies to detect the intracellular location of structures of particular proteins by electron microscopy. Ultrathin sections are labelled with antibodies against the required antigen and then labelled with gold particles. Gold particles of different diameters enable two or more proteins to be studied.

EMLab can offer post-embedding immunogold labelling of samples in resin (Epoxy, LR White and Lowicryl) and on frozen hydrated ultrathin sections (Tokuyasu-method).

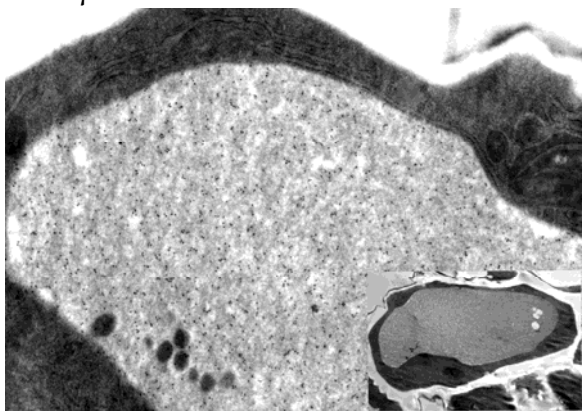
The investigator must supply the primary and secondary antibodies. The investigator should do immunolabelling at the fluorescent light microscopy level before attempting it at the EM level.



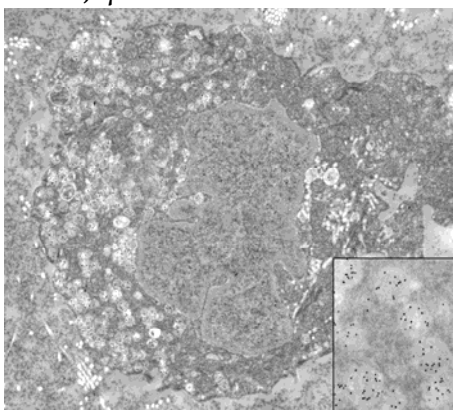
Stomac cancer tissue, conventionally fixed and embedded in epoxy resin. Immun-olabelled with anti-CgA, enhanced gold labelling.
Bar: 2 µm.



Visualizing of cell-surface located epidermal growth factor (EGF) receptors in cultured A431 cells (epidermoid carcinoma) using Tokuyasu-method.
Bar: 0,5µm.



Immunogold labelling of myrosin cell in Arabidopsis thaliana with antibody against TGG1. The plant was freeze substituted and embedded in lowicryl HM20 resin.



Stomac cancer tissue, poorly differentiated, conventionally fixed and embedded in epoxy resin. Immunolabelled with anti-CgA.

Cryo techniques/Low temperature

Cryo-ultramicrotomy is the ultrathin sectioning of unfixed/fixed, cryo-protected and/or rapidly frozen samples at very low temperatures. The Leica EM FC6 cryochamber is designed for low temperature sectioning of samples at temperatures from -15 to -160°C.

Freeze substitution is a process where the water molecules within the samples are exchanged with a solvent (usually methanol or acetone), then, the solvent with a resin (Lowicryl, LR White or Epoxy resins). This method, working at temperatures below 0ºC, reduces the loss of components from the sample and minimizes the denaturization of the proteins. In the end, the sample is fully infiltrated with pure resin. Polymerization of the resin is performed outside (Epoxy resin) or inside the machine when using Lowicryl resin. This latter resin is polymerized under a UV lamp, starting at -45ºC, then gradually moving up to room temperature (Lowicryl HM20). At the end of the process hard plastic blocks are generated ready to be cut by ultramicrotomy. The Leica EM AFS is capable of freeze substitution, progressive lowering of temperature techniques, and low temperature embedding and polymerization of resins using UV light instead of heat for improved preservation of ultrastructure and antigenicity.

TEM services include:

- Conventional specimen fixation, dehydration and embedding.
- Specimen sectioning:
    - Semithin sectioning (1 µm) with Toluidine blue stain
    - Ultrathin sectioning (50 – 70 nm) of resin embedded material (Epoxy, LR White, Lowicryl etc)
    - Cryo-ultrathin sectioning for Tokuyasu-method (sucrose-infiltrated)
    - Cryo-ultrathin sectioning for X-ray microanalysis (unfixed)

- Freeze substitution followed by resin embedding (Epoxy, LR White, Lowicryl etc)
- Immuno labelling of sections (resin and frozen)
- Positive staining
- Preparation of samples for X-ray microanalysis
- Coating of grids (formvar)
- Image processing (software iTEM and TIA)