Specimen Preparation

There are preparation facilities for electron microscope specimens on both sides of campus.

There are many ways of preparing specimens for electron microscopy. The EMS staff are available to help investigators determine the best way of getting the information they need from their specimens. The following is intended only as a general guideline.

Scanning Electron Microscopy Specimens

The size of the SEM specimen depends on which instrument you need for your analysis. The Hitachi S-3000N is able to accept specimens up to 150mm in diameter (although parts of the specimen cannot be accessed) and 20mm high. The JEOL JSM-6320F can accept specimens up to 30mm in diameter and 10mm high. In general, however, the amount of material should be kept as small as possible, especially if it is non-conducting.

- Conducting specimens can be imaged in both SEMs without any further specimen preparation.

- Dry Non-conducting specimens can be looked at in the S-3000N in low vacuum mode or at low accelerating voltages, however they may need coating with a conducting film in order to reduce charging at high vacuum. The EMS is able to coat with either carbon, gold/palladium, platinum/palladium or, for high resolution imaging, chromium.

- Wet Life Science specimens will normally require some processing (similar to 1-3 in TEM life science section below) to remove water before they can be introduced into the microscope vacuum system and may need coating with a conductive film to enhance contrast and reduce charging. Some hydrated specimens vcan be looked at directly in low vacuum, however, dehydration will take place.

Cressington208HR.jpg
Cressington Sputter Coater used to coat non conducting specimens with Pt/Pd or Cr

SEM_samplestub.jpg
SEM powder sample stuck to SEM stub using a carbon sticky dot

Specimens are attached to sample stubs using double sided carbon sticky dots or conductive tape.

Transmission Electron Microsopy Specimens

All EMS TEMs take standard size specimens (3mm in diameter). The maximum thickness of the specimen that can be seen through depends on the density of the material, the accelerating voltage of the microscope and the resolution of the analysis to be carried out - typically less than 0.1µm. Total thickness of the specimen should not be greater than 0.5mm. The EMS has extensive specimen preparation facilities to convert bulk material into transmission thin samples of the correct diameter.

Specimens can be either self supporting (i.e. the whole specimen consists of one material) or supported on a grid or a slotted 3mm washer.

Al_TEM_spec.jpg
3mm Self supporting Al TEM specimen, thinned to perforation

C_coated_Cu_grid.jpg
3mm C coated Cu grid onto which small particles can be deposited from an alcohol dispersion

The first stage of specimen preparation usually involves cutting the larger bulk specimens into a smaller, easier to handle sized specimen that can be processed into a transmission thin specimen for TEM. This applies to both life and materials science specimens where the sample should be no thicker than 1mm to minimize either fixation time (life science) or polishing time (materials science).

Life Science

All life science specimens need to be processed to remove the water from the specimen and replace it with a plastic resin without introducing artifacts. Typically, for TEM, this is a multistage process involving:

1. **Primary Fixation** - to halt degradation of tissue (eg 4% glutaraldehyde in phospate buffer for at least 4 hours). Rate of penetration is slow and sample must be less than 1mm thick in one dimension to acheive good fixation.
2. **Washing** - in phosphate buffer to remove glutaraldehyde
3. **Secondary Fixation** - to stabilize cell components (eg 1% Osmium Tetroxide in phosphate buffer)
4. **Dehydration** - to replace water with ethanol (increasing ethanol concentrations from 50% to 100% in 5 steps)
5. **Infiltration** - to replace ethanol with a transitional solvent (eg Propylene oxide) then replace the transitional solvent in the specimen with resin (33% resin in propylene oxide increasing in 4 steps to 100% resin. Typical resins used include LR White and LX112.
6. **Embedding** - to enclose the resin impregnated specimen in resin block.
7. **Curing** - leave the blocks for a period of time (24hours at 60oC) to set.
8. **Thick sectioning** - Use the microtome to cut thick sections which are then stained in toluiodine blue for optical examination to confirm area of interest is present

Microtome.jpg
Leica UCT ultramicrotome

cutting-sm.jpg
Schematic of the diamond knife cutting the sample showing possible artifacts - compression and cracking

9. **Ultramicrotomy** - to cut transmission thin slices from the resin block containing the specimen and deposit the slices onto a support grid.

10. **Staining** - to increase the contrast of the images obtained by staining parts of the structure with a heavy metal stain (eg uranyl acetate - a nuclear stain or lead citrate - a cytoplasmic stain)

Materials Science

For material science specimens preparation will depend on the type of sample.

Brittle or powder materials science specimens can often be crushed, dispersed in alcohol using an ultrasonic bath, and a drop of liquid placed onto a grid supporting an amorphous thin film. Choice of film material and grid material will depend on the experiment. Typically amorphous carbon films are used on copper grids, however we also have silicon oxide films and nickel and molybdenum grids. For high temperature applications (>500C) molybdenum grids should be used.

Self supporting material science specimens typically are cut using a diamond saw, polished down to 100-200 micrometer using silicon carbide paper on a polishing wheel, then a 3mm disc is cut from the material using a disc punch, ultrasonic cutter or slurry cutter. The central region of the disc can be pre-thinned using a dimpler to a few tens of micrometers before final thinning, to perforation, is carried out by Ion Beam Thinning or Electropolishing.

310 Disk Punch.jpg
South Bay Model 310 3mm Disc Punch - for metals and alloys

380 sonic cutter.jpg
South Bay Model 380 3mm Ultrasonic cutter - for semiconductor materials

360 slurry cutter.jpg
South Bay Model 360 3mm Slurry Cutter - for general purpose cutting

For cross sectional specimens the procedure is more involved:

1. Decide on the orientation of the TEM specimen you require.
2. Stack and bond the substrates together using a thin layer of a permanent glue (e.g M-Bond). Clamp or weigh down the stack and allow 24 hours for the glue to set.
3. Mount the stack onto a glass slide using wax
4. Carefully slice the specimen using a diamond wheel saw

650 Diamond Saw.jpg
Specimen on glass slide is lowered onto a slowly rotating diamond wheel which cuts the specimen. Position of the specimen, and slice thickness, can be accurately set using a micrometer

Grinder_bottom.jpg
Specimen mounted onto a specimen stub that fits into the Disc Grinder

Grinder_on_wheel.jpg
Disc grinder on a silicon carbide covered wheel. The amount of material removed can be set using the micrometer which moves the specimen and stub relative to the bottom face

5. Prethin the specimen down to 100-200 microns using Silicon carbide paper and diamond lapping film on the polishing wheels. The specimen is wax bonded to a Disc Grinder or Tripod Polisher mount which controls the geometry of the slice during polishing.
6. Bond the specimen onto a 3mm aperture grid using permanent glue (e.g M-Bond). Leave for 24 hours to set.
7. Use the dimpler to thin the center of the specimen further (optional)

515 Dimpler.jpg
South Bay Model 515 Dimpler

515 dimpler head.jpg
Dimpler head showing specimen on rotating mount and rotating polishing wheel retracted from specimen

8. Mount the specimen into the ion mill specimen holder and, at 15 degrees, thin the specimen in the ion mill to perforation. For cross sectional specimens it is usual to rock the specimen rather than full rotation during thinning. Reduce the milling angle to 4-8 degrees and mill for an additional 1-2 hours to increase the thin area.

1010 specimen holder.jpg
Specimen is clamped between two plates before being loaded into the ion mill

1010 Ion Mill.jpg
Fischione EAF-1010 Ion Mill

1010 Ion Mill inside sm.jpg
Specimen and holder inside ion mill

For more information, using the equipment in EMS-E, see our General Guide to Cross-Sectional TEM Specimen Preparation [Cross-Sectional_Preparation.pdf].

For more specific information about specimen preparation please get in touch with EMS staff.

Equipment

We have the following equipment available in the MSB laboratory:

- Sputter coaters (Cressington 208HR (Cr & Pt/Pd) and Polaron E5100 (Pt/Pd & Au/Pt)) for depositing conductive metal films on non-conductive SEM specimens.
- Glass Knife Maker (LKB 7801B) to make glass knifes for the ultramicrotomes.

- Ultramicrotomes (Leica Ultracut UCT and Reichart Ultracut E) to slice material at <100nm thickness using either diamond or knives.
- Leica Automatic Freeze Substitution (AFS) to dehydrate then chemically fix samples at low temperatures in preparation for various treatments including embedding in resins
- Reichart Metal Mirror Cryofixation System (MM80E) and Universal Cryofixation System (KF80) to rapidly freeze the surfaces of specimens.

In the SES (Science and Engineering South) laboratory:

- Diamond saw (South Bay Technology 650) for slicing bulk material
- Ultrasonic Cutter, Disc Cutter and Rotary Slurry Cutter (South Bay Technology 380, 310 and 360) for preparing 3mm discs.
- Polishing Wheels (South Bay Technology 900 and 910T) for mechanical thinning using silicon carbide paper or diamond lapping films.
- Tripod Polishers (South Bay Technology 590W) and Disc grinders (Gatan 623) used in conjunction with polishing wheels for final mechanical polishing down to ~100 microns.
- Automated Polishing System (Allied High Tech Multiprep)
- Dimpler/ Polisher (South Bay Technology 515) to selectively remove material from center of the specimen.
- Twin Jet Electropolisher (Fischione 110) to electrochemically thin specimens to perforation.
- Ion Mills (Fischione 1010 and M1050) which use argon ion beams to remove material to perforation.
- Nanomill (Fischione M1040) for focused low energy argon ion beam cleaning of specimens
- Ultramicrotome (Leica UCT) to slice material at <100nm thickness using a diamond knife.
- Plasma Cleaner (South Bay Technology PC200) to remove hydro-carbon contamination at low powers or remove material from the specimen at high power.