Contents

1. STAFF AND EQUIPMENT DIRECTORY 3

2. USER INSTRUCTIONS AND ACCESS PROCEDURES. 4
2.1 INTRODUCTION 4
2.2 SECURITY 4
2.3 RESTRICTED ACCESS 5
2.4 INSTRUMENT BOOKINGS 5
2.5 CANCELLATIONS 5
2.6 OTHER WEBSITE FUNCTIONS 6

3. EMU LABORATORY PROCEDURES 7
3.1 INTRODUCTION 7
3.2 LABORATORY EQUIPMENT 7
3.3 CLEANLINESS IN THE LABORATORY 7
3.4 EQUIPMENT FAILURE 7
3.5 SPECIMEN STORAGE 8
3.6 PHOTOGRAPHIC NEGATIVES 8
3.7 DIGITAL IMAGES 8
3.8 COMPUTERS 8

4. SAFETY IN THE LABORATORY 9
4.1 INTRODUCTION 9
4.2 PERSONAL SAFETY 9
4.3 SMOKING 10
4.4 FIRE IN THE LABORATORY 10
4.5 FLAMMABLE SOLVENTS 10
4.6 HAZARDOUS CHEMICALS 10
4.7 OXIDISING SUBSTANCES 11
4.8 COMPRESSED GASES 11
4.9 LIQUID NITROGEN 11
4.10 TOXIC CHEMICALS 12
4.11 SPILLAGE 14
4.12 WASTE DISPOSAL 15
4.13 SHARPS 15

5. OVERVIEW OF SPECIMEN PREPARATION FOR ELECTRON MICROSCOPY ..16
5.1 SCANNING ELECTRON MICROSCOPY 16
5.2 TRANSMISSION ELECTRON MICROSCOPY 18

6. SPECIMEN PREPARATION EQUIPMENT 21
6.1 INORGANIC SPECIMENS 21
6.2 BIOLOGICAL/ POLYMER SPECIMENS 22
6.3 VARIOUS COATERS 23

7. MICROSCOPES 24
7.1 TRANSMISSION/SCANNING TRANSMISSION ELECTRON MICROSCOPES 24
7.1.1 Hitachi H-7000 TEM/ STEM (1988) 24
7.1.2 Philips CM200 Field Emission TEM/ STEM (1999) 24
7.1.3 VG HB601UX Field Emission TEM/ STEM (Installed at the University of Sydney) 24
7.2 SCANNING ELECTRON MICROSCOPES 24
7.2.1 Cambridge S-360 SEM (1988) 24
7.2.2 JEOL 840 SEM (1986) 25

7.2.3 Hitachi S-900 Field Emission SEM (1991) 25
7.2.4 Hitachi S-4500II Field Emission SEM (1996) 25
7.2.5 FEI Quanta 200 Environmental SEM 25
7.3 ELECTRON PROBE MICRO ANALYSER 25
7.3.1 Cameca SX50 Microprobe (1992) 25
7.4 FOCUSED ION BEAM MILLERS 26
7.4.1 Philips/FEI FIB XP200 FIB (1998) 26
7.4.2 FEI Nova 200 Nanolab 26
7.5 SCANNING PROBE MICROSCOPES 26
7.5.1 Veeco/ Digital Instruments Extended Dimension 3000 SPM (1997) 26
7.5.2 Veeco/ Digital Instruments Extended Multimode SPM (2001) 27
7.6 LIGHT MICROSCOPES 27
7.7 X-RAY MICROSCOPE 27

8. MICROSCOPY RESOURCES 28
8.1 See http://srv.emunit.unsw.edu.au/ and http://srv.emunit.unsw.edu.au/PDF_files/books.pdf 28
8.2 SUPPLIERS 28
8.3 BOOK REFERENCES 28
8.4 MICROSCOPY JOURNALS. 31

UNSW EM Unit Laboratory Handbook, *revised March 2005* (Next revision due in March 2006, unless otherwise required).

1. STAFF AND EQUIPMENT DIRECTORY

Position	*Title*	*Name*	*Phone*	*E-mail*
LA	Mrs	Margaret Budanovic	56392	m.budanovic@unsw.edu.au
TO	Ms	Delphine Chassaing	55581	d.chassaing@unsw.edu.au
TO	Mrs	Sigrid Fraser	56392	s.fraser@unsw.edu.au
TO	Ms	Katie Levick	56390	k.levick@unsw.edu.au
Post-doctoral Research Ass.	Mr.	Damien McGrouther	55581	d.mcgrouther@unsw.edu.au
Director	Prof	Paul Munroe	54435	p.munroe@unsw.edu.au
PO	Ms	Jenny Norman	56391	j.norman@unsw.edu.au
STO	Ms.	Viera Piegerova	56389	viera@unsw.edu.au
PO	Mr.	Barry Searle	56384	b.searle@unsw.edu.au
Deputy Director	Dr	Marion Stevens-Kalceff	55928	marion.stevens-kalceff@unsw.edu.au
PO	Mr	Dong Ming Zheng	56680	dm.zheng@unsw.edu.au

Equipment	*Extn*
Computer Room, Applied Science Building, Room 1002	55581
Cameca SX-50 Probe	56399
Cambridge S360 SEM	56396
Digital Instruments Dimension 3000 SPM	56401
Digital Instruments Multimode SPM	56214
FEI Nova Nanolab 200 - FIB	56395
FEI Quanta 200 Environmental SEM	56401
Hitachi H 7000 Transmission EM	56393
Philips CM200 Transmission EM	56386
JEOL S 840 Scanning EM	56402
Hitachi S4500 Field Emission SEM	56403
Hitachi S 900 Field Emission SEM	56394
Philips / FEI Focused Ion Beam Miller	56387
Skyscan MicroCT – X-Ray Microscope	56396
Laboratory 1 East	56868
Laboratory 2 West	56869

First Aid Officer	Katie Levick	56390	k.levick@unsw.edu.au
Safety Officer	Barry Searle	56384	b.searle@unsw.edu.au

Floor Fire/Emergency Warden	Viera Piegerova	56389	viera@unsw.edu.au
Floor Fire/Emergency Warden	Jenny Norman	56391	j.norman@unsw.edu.au
Enquiries			emu.enquiries@unsw.edu.au

2. USER INSTRUCTIONS AND ACCESS PROCEDURES.

2.1 INTRODUCTION

The Electron Microscope Unit at The University of New South Wales is well equipped with a versatile range of some of the finest microscopes in Australia. The total value of the EMU equipment exceeds 10 million dollars. The EMU policy is to encourage use of the equipment for research and teaching activities and we will work with you to ensure you get the best possible results.

All users are to observe safe and responsible working practices.

2.2 SECURITY

Admission to the laboratory is through validation of your University of New South Wales Staff or Student card. You will be issued with a request to Security to validate your card for entry to the laboratory for the hours 8:00 am to 6:00 pm Monday-Friday. Initially, new users will be closely supervised by one of the EMU staff members who will provide instruction in the operation of the laboratory facilities and safety procedures.

When you have demonstrated you are competent to operate equipment unsupervised you may be granted 24 hour, seven-day access. Access is a privilege granted to you individually once you are trained and judged to be competent and responsible.

The following activities must only be performed from 8AM to 6PM Monday to Friday and must not occur outside the normal business hours.

1. ***Electropolishing of specimens***
2. ***Electro-discharge machining of specimens***
3. ***Biological specimen preparation, including cryo-ultromicrotomy***
4. ***Cryo-SEM sputter coating***
5. ***Sputter coating***
6. ***Use of precision cutting saws***

Users who are using the electron microscopes outside of normal business hours must be aware that out of hours contact numbers for the Unit's Director or Deputy Director are displayed in each microscope room and throughout the Unit. They must also be aware of the University's emergency procedures and that emergency services can be contacted through UNSW Security on 56666.

You must not lend your card to anyone else. You must not give access to any person who does not have valid card. In doing so you become personally responsible for any loss/ damage they cause and/ or any harm they may come to in the laboratory. If you wish to bring an unregistered co-worker into the unit with you, to observe during your session, you must first obtain permission from EMU staff. This person must remain with you at all times while they are in the Unit. It is essential that EMU staff know who is in the Unit for safety reasons.

Similarly, you must not wedge open the security entry door or tamper with the lock. The swipe card system protects EMU's multi-million dollar assets against unauthorised access and misuse. The swipe card system records the identity of the cardholder and the time of its use in any of the EMU doors. So we can readily check who has entered the laboratory in case of any theft or damage. Your ID card may also be used to log on to the microscopes. This records usage and allows EMU staff to check

who was using a particular instrument at any particular time.

All users working in the laboratory must be registered and recorded in the EMU database. Prior to training, new users are required to fill out a form with their name, telephone numbers and University account number and give it to the EMU systems administrator (Dong Ming Zheng). He will also take a digital photo of you for the EMU database. You will also need to provide a login name and password so you can use the Unit's computerised (network) booking and image management system, accessible through any web browser. A folder will be created on the EMU server for you to store your images etc. Your login name will also be your folder name.

2.3 RESTRICTED ACCESS

Your access to the laboratory implies permission to use EMU facilities and operate equipment for which you have been trained and which are specific to your work.
You must NOT use any other facilities until you have received instruction in their use from a member of staff.

Only E.M. Unit staff may access certain parts of the laboratory:

- Any space behind any of the microscopes
- The service corridor alongside the microscope rooms, B003
- The liquid nitrogen store, B023
- The stationery store, B002
- Storage for toxic chemicals
- The toxic chemical refrigerator.

User access to these areas is prohibited. If you believe you need access or get anything from one of these places ask a staff member.

2.4 INSTRUMENT BOOKINGS

The Electron Microscope Bookings system is available on any personal computer in the Unit, on the Campus-Wide Network (CWN) and through the World Wide Web (WWW). ***Telephone bookings are not accepted.***

The Unit Web page is at ***http://srv.emunit.unsw.edu.au.***

Select bookings, enter your login name and password and make a booking.

If any problems are experienced, please advise the systems administrator (Dong Ming Zheng x56680).

The rules for booking instruments endeavour to give the greatest possible access to instruments at times suitable to individual researchers. Bookings may be made four weeks in advance.

We generally prefer you to book for 2-4 hour sessions to give everyone a chance in the day. EMU staff must approve longer sessions in normal working hours. External users who have to travel to the University and those using special techniques (e.g. cryomicroscopy) may be entitled to longer sessions.

2.5 CANCELLATIONS

If you cannot attend a session you have booked, please log on to the booking system, ***cancel your booking and advise the staff as soon as possible*** in order to make the equipment available to other

users.

Users will be charged for microscope session they fail to turn up for and users who consistently fail to turn up for booked time will be excluded from the facility.

2.6 OTHER WEBSITE FUNCTIONS

The EMU website is the gateway for all transactions with the Unit. Newsletters, information about working in the Unit, the equipment, the computer system, staff directory, digital data retrieval, and the booking system may be accessed on the website. Read the introductory material on the website prior to beginning work in the laboratory.

Persons who are either not registered in the EMU database or who have not completed the appropriate EMU risk assessment forms or who have not had any prior consultation and training with EMU staff are not permitted to use these facilities under any circumstances.

3. EMU LABORATORY PROCEDURES

3.1 INTRODUCTION

Initially, you must learn the rules of the laboratory, methods for handling specimens and operation of the equipment. We have a range of in-house operating instructions (see the EMU website), so you will be well informed about operating EMU equipment. You should be familiar with these manuals as part of your instruction. Your probationary period will be shorter if you read the operating instructions and other related material in advance. Manufacturer's manuals for each piece of equipment are also available for use in the laboratory, if required. They may not be removed from the laboratory. Photocopies can be made if required - see EMU Staff.

3.2 LABORATORY EQUIPMENT

Initially, a staff member will introduce you to an instrument. Once you are judged to be able to work safely, you will be encouraged to perform the basic operations on your own. When you have mastered the basic operations you will be introduced to the more advanced operations of the equipment.

You must not try advanced or complex procedures with the equipment unless you have been given approval and you have been trained appropriately. Do not press buttons or initiate operations where you do not know the outcome of such steps.

The Unit provides many small items like pipettes, glassware, forceps, glues etc. for your use. ***Laboratory supplies must never be "borrowed" or removed from the laboratory.***

3.3 CLEANLINESS IN THE LABORATORY

Good working practices are essential to ensure optimum results. It is important to have moderately clean and dust-free working areas and to keep the region in the immediate vicinity of grids in preparation scrupulously clean. Use disposable work surfaces like filter paper circles or Parafilm squares that can be taken fresh when you begin work and discarded once you have finished. Your co-operation is important in helping us to keep the laboratory clean and tidy. Untidy workbenches are a hazard. You must clean up workbenches after you have finished.

When you have finished your work:

- Thoroughly clean any instruments or apparatus you have used and replace them in their drawer or cupboard.
- Replace protective covers where they are supplied.
- Clean up all mess and any spills with paper towelling or appropriate clean up kit.
- Leave the work area clean and tidy for the next person.
- After using electron microscopes, remember to log out.
- Rinse all glassware thoroughly before putting it out to be washed.
- Ensure all flames (spirit lamps, burners) are extinguished – and hotplates and lamps are turned off.

3.4 EQUIPMENT FAILURE

Even the best equipment will give trouble from time to time, and often it will not be your fault. Minimise trouble and delay by ***IMMEDIATELY SEEKING HELP*** when anything seems to be broken, faulty or not working as you expected.

EMU STAFF MUST BE ADVISED OF ANY PROBLEMS IMMEDIATELY

DO NOT TRY TO FIX OR FORCE ANYTHING

These rules not only protect the EMU equipment from damage done by unskilled attempts at repairs; they also protect YOU from hazards associated with the equipment***.***

3.5 SPECIMEN STORAGE

There is limited space for storing specimens in the laboratories. Take your specimens with you. Any specimens or materials permitted to be left in the laboratory must be labelled with your name, the date and a specimen identification***. Any unlabelled material left in the Unit will be thrown out immediately.***

3.6 PHOTOGRAPHIC NEGATIVES

After processing, negatives are stored in B007. If you do not collect them within a week they will be thrown out unless you advise us of any likely absence. We recommend you to scan your negatives into the EMU computer system.

3.7 DIGITAL IMAGES

All the EMU microscopes are fitted with digitisers or CCD cameras that convert an image to a computer file. The digitising is one function of the personal computer beside each microscope. All the computers are networked and each digital image is saved in duplicate on the EMU laboratory network server box from which they can be accessed through the Internet, using a web browser. Several times a month the EMU systems administrator archives all the images.

All the images are accessible from any of the computers in the EMU computer room where you can use software to improve contrast, crop images, label them, make up pages and finally print them out in draft or photo quality form.

If you have lots of your own images a CD-ROM of your own images (up to 600 standard images fit on one CD-ROM) can be burned for you to take away to use on your own computer. You will first have to assemble your images in the directory structure you prefer.

3.8 COMPUTERS

The EMU Electron and Scanning Probe Microscopes and peripherals are operated by specialised computers. Computers with image and word processing software and printers are available for ***registered users*** in the computer room located on Level 10 of the Applied Science Building. If you require access to the computer room in the Applied Science Building please ask an EM Unit staff member for a key. If you require long term access to this facility, EM staff can provide you with a key for a small deposit.

The downloading of unauthorised or inappropriate material and/ or the installation of unlicensed/ illegal/ unauthorised software (including encryption software) on any EMU computer is forbidden. Improper use of EMU computer facilities may result in a review of your access privileges.

4. SAFETY IN THE LABORATORY

4.1 INTRODUCTION

The EMU laboratory is not equipped to handle blood, tissue containing blood, live viruses, and bacteria. None of these types of agents are permitted to be brought into the laboratory.

The laboratory contains various instruments, chemicals and facilities that are hazards if not properly managed. We are committed to having a safe working environment. EMU users breaching the safety rules set out in this Handbook and the UNSW Risk Management Unit will have their access privileges withdrawn.

You must know the EMU safety rules***. You are required to read these rules and to sign a form to stating that you have read and understood them. Tasks which require high levels of risk, for example handling liquid nitrogen, require specific training. We are required to keep a record of that training.***

You must check the safety notice board at the east end of the laboratory for current advisory notices.

If you notice any hazard or have an accident in the Laboratory you must tell the Safety Officer, Barry Searle (x6384) or any other staff member. You must comply with any safety instruction given to you.
http://srv.emunit.unsw.edu.au/EMUSafety2000.htm

All injuries must be reported to the EMU First Aid Officer, Ms Katie Levick (x56390)

UNSW Risk Management Unit also provides information about matters relating to occupational health, safety and environment http://www.riskman.unsw.edu.au/ You should be familiar with relevant sections of this web site.
Further sources of safety information are available at http://www.civeng.unsw.edu.au/safety/start.htm (links & fact sheets) and http://www.proscitech.com.au/msds.htm (Material Safety Data Sheets, see below).

4.2 PERSONAL SAFETY

You must wear protective clothing (***Personal Protective Equipment***, PPE) whenever you are handling chemicals or other hazardous materials. The type of protection required is specified by signage associated with a specific instrument or chemical. You should also be aware of the required PPE for chemicals as specified in the ***Material Safety Data Sheets*** MSDS for the material.

You must wear ***proper shoes*** with enclosed toes in the laboratory. If you wear open toed sandals, thongs or bare feet you will be required to leave.

You must wear a ***laboratory overall/coat*** whenever you are working with chemicals in the preparation areas.

You must wear ***eye protection*** (goggles, or full face mask) when handling corrosive or toxic chemicals or liquid gases. These are provided in the laboratory.

You must wear ***gloves*** when handling corrosive, toxic or sensitising chemicals or liquid gases.
Latex gloves are provided for ordinary work and heavy leather gloves are provided for work with liquid gases and other extremely cold (or hot) materials.

You must not eat or drink in the laboratory. You may eat in the Tea making room or in one of the

offices. Food and drinks can be kept in the bar fridge in the Tea making room. Food and drinks must not be stored in any other refrigerator. Drinks are prohibited near any computer or microscope.

4.3 SMOKING

Smoking in University buildings is forbidden.
Smoking in the laboratory is forbidden.

4.4 FIRE IN THE LABORATORY

If you find a fire:

Call emergency ***56666*** and report

1. What is burning
2. That the fire is in the Electron Microscope Unit, Basement, Red Centre Building.
3. Your name and telephone extension number.

Alert others to the danger.

Assist anyone who is disabled.

Extinguish the fire if you know how to use the nearest extinguisher.

If the fire alarm rings, please leave the building immediately. Please exit via the stairs and assemble at the front of the Red Centre. EM Unit fire wardens, Viera Piegerova and Jenny Norman will advise you how to exit, if required.

4.5 FLAMMABLE SOLVENTS

Solvents such as acetone, ether and propylene oxide are explosive fire hazards. Naked flames (matches, burners, spirit lamps) must be 3 metres distant from any area where solvents are stored or in use as vapour can catch fire and ignite the liquid.

Stock volumes of flammable solvents are stored in a special flammables storage cabinet.
They must not be stored in a refrigerator.
Small volumes are stored at bench level for use at the bench.

All solvents must be disposed of in the SOLVENT DISCARD CONTAINER kept in the waste storage area. Small amounts of solvent can be used on the bench. If you are handling large volumes you must use a fume cupboard.

4.6 HAZARDOUS CHEMICALS

There are 9 classes of chemical hazard. We have no explosives (Class 1) but we have all the other classes of hazard:

Class 2.2	Non-flammable non-toxic gas (argon)
Class 2.3	Toxic gas (chlorine)
Class 3 PGI	Highly flammable liquids boiling point < 35^{O} (diethyl ether)

Class 3 PGII Highly flammable liquids boiling point > 35^{O} (acetone, petrol)
Class 3 PGIII Flammable liquids flash point >23^{o} <60^{o}

Class 4 Flammable solids (cellulose nitrate)

Class 5.1 Oxidising substances (hydrogen peroxide, perchloric acid)
Class 5.2 Organic peroxides (methyl ethyl ketone peroxide, benzoyl peroxide)

Class 6.1 Toxic substances (osmium tetroxide, glutaraldehyde)
Class 6.2 Infectious substances (bacterial cultures, viruses)

Class 7 Radioactive material (our uranium salts are weak emitters)

Class 8 Corrosive substances (hydrochloric acid, sodium hydroxide)

We have collections of ***Material Safety Data Sheets (MSDS)*** for all the materials used in the laboratory. They are also are available through websites such as
http://www.proscitech.com.au/msds.htm

You must read the Material Safety Data Sheets for all the materials ***YOU*** are working with. You must comply with the handling requirements stated in the sheets. You must dispose of the materials as instructed.

The following rules are for your guidance and they are not a substitute for the MSDS information.

4.7 OXIDISING SUBSTANCES

You must wear comprehensive protective clothing: lab coat, goggles, gloves when handling these materials. You must use the fume cupboard with the sash low to protect your upper body.

4.8 COMPRESSED GASES

Only staff members are permitted to move the cylinders and replace empty ones.

Ensure that a suitable pressure regulator is fitted to the nitrogen and argon cylinders. The CO_2 cylinder does not require a pressure regulator.

When about to use a gas, turn on the main valve and check that the reading on the regulator indicates gas in the cylinder.

If the gas flow seems inadequate ask staff to adjust the regulator. Do not attempt to adjust it yourself.

After you have finished close the main valve.

4.9 LIQUID NITROGEN

Liquid nitrogen is associated with two main hazards:

1. Cold burn
2. Evolution of large volumes of suffocating gas

a) Cold burn

Materials cooled by liquid nitrogen will stick to the skin and will tear the flesh if removal is attempted. Some materials also become very brittle at liquid nitrogen temperature. Pipes and vessels containing liquid nitrogen require a protective layer of insulation.

Containers to be used for liquid nitrogen must withstand the rapid changes in temperature and low temperature that will be achieved. In the laboratory we prefer the use of stainless steel Dewars, but steel encased glass Dewars are acceptable. Styrofoam boxes and cups are acceptable as containers for working under liquid nitrogen but they should not be used for transportation

Avoid skin contact with the liquid. Cooled objects should be handled with forceps; fast work is advised as forceps will rapidly cool and stick to your skin.

When transferring liquid nitrogen you must wear insulating gloves and protective face shield.

Pour 1-200 ml into your container and let most of it boil away, to cool your container down before transferring the bulk of the volume. Small splashes on the skin cause little harm. The greatest hazard is having a significant volume pour into a pocket or shoe where it will be held in close contact with the skin and will be unable to leak away.

Ideally, high boots and clothing without pockets should be worn. Thick gloves will protect against casual splashes and will enable hoses to be handled in comfort. In the event of a serious spill on the skin, the area should be immediately flooded with copious cold water.

b) Evolution of gas.

ALWAYS TRANSFER LIQUID NITROGEN IN A WELL-VENTILATED AREA.

One ml of liquid nitrogen produces 637 ml of nitrogen gas. If, whilst transferring liquid nitrogen in an enclosed space, you were to lose consciousness due to low oxygen, you would collapse into a layer of cold nitrogen gas on the floor and death by suffocation would occur.

For this reason you must not work alone in the laboratory (nights or weekends) when you are using large volumes of liquid nitrogen. The nitrogen store is locked after hours to restrict use to staff.

Normal practice is for staff to fill the 10L and 20L dewars from the EMU bulk supply. You may decant liquid nitrogen from the 10 and 20L dewars into 2L dewars as needed.

NEVER TIGHTLY STOPPER A CONTAINER OF LIQUID NITROGEN

Pressure inside the container will quickly rise and a blow out or explosion will result.

4.10 TOXIC CHEMICALS

Many of the chemicals used in electron microscopy are extremely toxic. Their labels advise of specific hazards and action to take in an emergency. Laboratory reagents are only to be used by responsible people with a professional concern for proper procedures.
The advisory labels are small. It is your responsibility to properly inform yourself of possible hazards by reading the MSDS for reagents you propose to use. If you are in any doubt, ask the EMU staff.

TOXIC MATERIALS SPILLS MUST BE CLEANED UP AT ONCE BY E.M. UNIT STAFF.
DO NOT ATTEMPT TO CLEAN UP TOXIC MATERIAL SPILLS YOURSELF.

Seek help or advice if you find yourself in trouble. Some of the more commonly used hazardous

chemicals are listed below, together with safety precautions required when using them.

The essential rules are

- ***to avoid***
  - ***ingestion,***
  - ***skin contact, and***
  - ***inhalation***
- ***Wear a laboratory coat, gloves and goggles.***
- ***Handle toxic and hazardous materials in the fume cupboard with the sash low.***

GLUTARALDEHYDE (AND FORMALDEHYDE)

These are toxic through contact and inhalation. Skin contact can cause allergic dermatitis. These are respiratory irritants and should ALWAYS be handled in a fume cupboard.
Wear LAB COAT, LATEX GLOVES AND GOGGLES when handling the solutions.
Used fixative containing aldehydes should be discarded in a special discard container in the fume cupboard and NEVER down a sink.

Only Staff are to work with concentrated solutions to dilute them to working strength.
Undergraduates must not transport glutaraldehyde solutions outside the laboratory.

SODIUM CACODYLATE

This is a very toxic salt containing ***arsenic*** - a potent ***cumulative poison***. Prolonged exposure to dust and concentrated solutions is harmful. Wipe up all spills. Use concentrated solutions in a fume cupboard, clean up all spills and WEAR GLOVES AT ALL TIMES. Solutions containing cacodylate should be discarded in the Aldehyde special discard container.

OSMIUM TETROXIDE

Solid osmium tetroxide, its solutions AND ITS VAPOUR are extremely toxic. They damage mucous membranes and cause irreversible damage to the cornea of the eyes. Liver damage is caused if the osmium is ingested.

Only EMU staff are permitted to work with osmium tetroxide solid.

Osmium tetroxide in any form must be handled in a fume cupboard with the sash low.

Laboratory coat, safety glasses, and latex gloves MUST be worn when handling osmium tetroxide in any form.

IMMEDIATELY REPORT ANY SPILLS TO A MEMBER OF STAFF.

Dispose of used solutions in the special osmium discard bottle in the fume cupboard.

LEAD AND URANIUM STAINS

These are heavy metal poisons which are cumulatively toxic. Clean up all spills and wash hands after use with soap and warm water. The uranyl acetate we use is depleted and is only slightly radioactive. The greatest risk is through inhalation of the powdered solid. Wear gloves when handling the solid. Avoid raising chemical dust. Clean up all spills. Avoid aerosols of the solution. Store all the uranyl salts in the secure cabinet until wanted for use.

EPOXY RESINS

There are five major hazards associated with resins:

1. ***Carcinogenesis*** - possibly ERL 4206
2. ***Primary irritancy*** - Skin contact dermatitis which can be severe
3. ***Systemic toxicity***
4. ***Environmental pollution***
5. ***Fire hazard*** - most resins are flammable.

The most commonly used epoxy resin used in the laboratory is the low viscosity resin devised by Spurr.

These reactive chemicals may seriously damage your health. You must wear a laboratory coat, goggles and gloves while handling them and you must work with them in a fume hood.

SMOKING, EATING AND DRINKING WHEN USING RESINS IS PARTICULARLY HAZARDOUS

Tie long hair back so it cannot contact the resin and become contaminated. A container for waste resin is provided in the fume cupboard. Paper towels are available for cleaning.

Only disposable containers are to be used for resin. Resin discards and resin-contaminated disposables MUST be polymerised prior to disposal. Dispose all contaminated containers, gloves, and paper towels in the toxic waste garbage bin. Persons handling waste should be informed that incineration might lead to the production of nitric acid, hydrochloric acid, cyanide and CO_2.

In the event of a spill on your skin: WASH WELL WITH SOAP AND COLD WATER. Wash hands well on removal of gloves.

Laboratory contamination

Avoid leaving resin on handles of doors, refrigerators, balances, forceps, telephones etc. Clean off any resin you accidentally transfer while working.

NEVER DISPOSE OF RESIN IN A SINK. Waste must be disposed of in the jar in the fume cupboard. Before consigning to the trash, resin in beakers or pipettes must be polymerised for several days at 60^{o}C.

4.11 SPILLAGE

In fume cupboard, absorb with paper towelling, remove final traces with acetone, place contaminated towelling etc. in a polythene bag for disposal.

For spills occurring

In open laboratory, bench, floor etc

1. Inform a senior member of staff
2. Using respirator, cover spillage with vermiculite, allow time for absorption, remove final traces with acetone-moistened paper towelling and remove all resin-contaminated materials in a sealed polythene bag.

On clothing:

1. Remove at once. (You should be wearing a laboratory coat!)

On skin:

1. Wash with cold water and soap.
2. DO NOT USE ACETONE, WHICH ASSISTS CHEMICALS TO RAPIDLY PENETRATE THE SKIN.

4.12 WASTE DISPOSAL

Waste materials MUST be handled in specific ways as designated by UNSW Risk Management Unit.

http://infonet.unsw.edu.au/poldoc/admax/waste.htm

Waste materials must be disposed of promptly in an appropriately labelled waste container. You must dispose of chemicals and other materials as instructed. No chemical is to be disposed of down the laboratory sinks.

Generally,

1. Resin residues in beakers or pipettes must be polymerised for at least 24 hours at 60°C prior to disposal.
2. Used gloves and plastic pipettes are to be placed in the yellow waste bin.
3. Waste paper is to be placed in the blue recycle bin provided.
4. Broken laboratory glass must be placed in the labelled “Broken Glass” white waste plastic bin.
5. All chemical waste must be placed in the appropriate waste container provided. All waste Containers for removal from the EM Unit are identified using the UNSW Waste Labels.

4.13 SHARPS

“Sharps” is a waste classification term associated with those instruments used to puncture, cut or scrape body parts and includes, hypodermic needles, syringes, lancets scalpel blades, glass Pasteur pipettes, microtome blades, razor blades and other sharp, contaminated sharp metal and glass laboratory waste materials.

All such items MUST be disposed of in the yellow plastic SHARPS BIN.

Needles and razor blade waste for disposal must not be placed into office garbage bags, waste paper bins or any other type of plastic bags.

5. OVERVIEW OF SPECIMEN PREPARATION FOR ELECTRON MICROSCOPY

No two materials or specimens are alike and so the methods used to prepare specimens for examination in the electron microscope will depend not only on the type of specimen that is to be examined, but also the microscope to be used. Every person has different specimens and it is strongly suggested that individual users develop their own methods and protocols for specimen preparation depending on their own specimens and the experiments they wish to perform.

In this section the principal methods of specimen preparation are outlined in rather broad terms. However, this should allow users to understand the decisions they will need to make in specimen preparation.

5.1 SCANNING ELECTRON MICROSCOPY

Specimens for SEM analysis are often described as "bulk" specimens. They are typically 1-3cm in diameter and often require little or no pre-preparation. Different SEMs have different stage sizes that limit the maximum size of the specimen. Typically specimens are mounted on stubs that are 1 inch (2.5cm) in diameter. The description below is generic to specimens of this size.

If specimens are less than 1 inch (2.5cm) in diameter then they can be mounted directly on the stub. This is normally done with double-side carbon tape. The specimen should be strongly adhered to the stub to prevent it from becoming detached during examination. Materials in powdered form can simply be sprinkled on to carbon tape. Different microscopes use different stubs so it is important to ensure that the correct stub is used.

All specimens must be normally a) dry and b) electrically conductive. For metallic specimens this is not normally a problem, however most other specimens are not electrically conductive. Specimens are made electrically conductive by the application of a thin coat of electrically conductive material, usually a metal (Au, Pt, Cr) or carbon. These coatings are usually only a few nanometres thick and do not interfere with the structure of the specimen.

The choice of coating material used depends upon the experiments to be performed. A metallic coating is best when only visual examination of the specimen is required. Heavy metal coatings such as gold ensure a strong electron signal from the specimen and high signal-to-noise images. However, coatings from gold or other metallic elements will interfere with chemical analysis procedures. Therefore if EDS/WDS is to be performed on the specimen a carbon coating is preferable. In general, the carbon coating does not interfere with chemical analysis. For metallic coatings gold is most commonly used and gives high quality images. However, for high resolution imaging (greater than ~ 50,000x) the structure of the gold coating will start to become visible. For specimens to be prepared for high resolution imaging, chromium coating using a specialised high resolution coater is required.

Coatings can be applied by two processes: sputtering and evaporation. Overall, for most applications, the choice of process is not critical. Either carbon or metal can be applied by either method.

Evaporation of gold, for example involves the rapid heating of a short strip of gold wire in a chamber under vacuum. The heating melts the gold which because of its rapid heating (and the vacuum chamber) disperses as a spray of droplets in all directions. This results in a fine coating of gold over both the specimen (and the inside of the vacuum chamber).

Sputtering involves the interaction of a high energy beam, usually of an inert gas such as argon or xenon onto a target, again under vacuum. The atoms sputtered from the target then coating both the

specimen and the inside of the vacuum chamber. For most coaters argon is used, high resolution coaters use xenon (e.g. Xenosput.)

Many 'metallurgical' type specimens are mounted in resin or Bakelite and polished to a high level of smoothness to prepare sections for structural analysis. Even though the specimen may be metallic (and conductive) the resins used are insulators, so the specimen requires coating.

A conductive silver-containing paint, often called "silver dag" is frequently used to adhere specimens to stubs and provide a conductive path from the conducting region of a specimen to, for example, the aluminium stub.

Specimens for microchemical analysis, specifically done by microprobe analysis, must be optically flat. This must normally be polished to a significantly high standard. Metallurgical type specimens are normally mounted in Bakelite or resin for polishing. Geological specimens are usually mounted in glass microscope type slides.

Since SEM's operate under vacuum, specimens must also be dry for SEM analysis. There is a type of SEM, variously known as a variable pressure, controlled pressure or Environmental SEM which operates at low vacuum which allows imaging of hydrated, wet and/or uncoated specimens.

Most "inorganic" specimens are already suitably dry and need no specific treatment. Hard, dry specimens (most inorganic material specimens) or those that are naturally self-supporting need no special treatment. They can be air dried or dried in the vacuum of the coating unit. Insects, for instance usually can be air dried without structural collapse. However, soft specimens, especially those that have a considerable content of water, need special preparation. They may be dried in a variety of ways that can minimise damage.

There are three methods of reducing or bypassing the effect of surface tension when drying specimens.

1. To substitute for the water in the specimen a solvent with lower surface tension such as acetone or ether and then evaporate the substituted solvent in air.

2. To freeze the water in the specimen and sublime the ice under vacuum (freeze-drying). After solvent substitution it can be useful to freeze dry to remove the solvent.

3. To substitute for the water in the specimen a fluid with a suitable critical point and pass the specimens through the critical point.

In air-drying, the damage is caused by surface tension forces which collapse delicate structures as a liquid/gas interface passes through the specimen. Low viscosity solvents (e.g. ether) have lower surface tensions, hence reduce damage somewhat. In freeze drying a solid/gas interface passes through the specimen. In a process called critical point drying there is a phase change from liquid to gas without the formation of a phase interface.

Critical point drying is the most commonly used technique to dry delicate hydrated specimens.

If a liquid and its vapour are sealed into a pressure vessel and heated, the vapour pressure and vapour density will increase as molecules join the vapour phase and the liquid density will decrease. At a particular temperature and pressure (the critical point) the density of the vapour equals the density of the liquid and the boundary between the two phases disappears. At temperatures and pressures above or below the critical point only one phase exists and the surface tension is zero.

A specimen immersed in a liquid passed through its critical point will find itself in the gas phase (i.e. dry) without having passed through a gas/liquid interface. Although collapse of the specimen is

avoided shrinkage of 20-40% can occur.

The critical point of water is 374C and 3270 psi, conditions which will destroy biological tissue. Tissue water must therefore be substituted with a solvent having a less extreme critical point. Carbon dioxide has a suitable critical point of 31C and 1100 psi and is also cheap, non-polluting (note it is a suffocating gas). Critical point drying is performed in a pressure chamber.

5.2 TRANSMISSION ELECTRON MICROSCOPY

Transmission electron microscopy requires specimens that are "electron transparent". The typical thickness of a TEM specimen is 200nm. For some applications much thinner specimens are required. TEM specimens are also, routinely, discs 3mm in diameter.

Therefore the specimen must be reduced from the bulk "as-received" dimensions and in doing so must exhibit a structure which is artefact-free and representative of the bulk material. There are a range of approaches for different specimen types. These notes will only deal in general terms with different approaches.

5.2.1 Powders

Powdered specimens (catalyst and other powders) which are sub-micron in diameter are trivial to prepare. The powder is usually added to ethanol and ultrasonically stirred to create a suspension. A droplet of the suspension is then removed with a pipette and dropped onto a 3mm diameter Cu grid coated with a thin film of carbon. The carbon film is so thin that it is effectively transparent to electrons. The powder particles become suspended on the film and the ethanol evaporates.

Some ceramic materials, which are very brittle, can also be ground up and dispersed as described above. Brittle materials cleave easily during mechanical deformation, through a pestle and mortar, and often flat electron transparent sections can be generated.

5.2.2 Metals and Ceramics

Inorganic materials which are solid in form, for example a piece of aluminium require much more detailed treatment for TEM preparation. Typically, this would involve firstly sectioning a thin 300-500 micron slice from the bulk aluminium. This requires a slow speed diamond saw, which cuts the material slowly to avoid unwanted heating. (The blade is immersed in an oil bath). The saw allows thin sections to be cut with a high degree of precision.

Discs, 3mm in diameter, are then punched from the cut sheet. There are different methods depending on the material. Metals are cut with an "electro-discharge" machine, which uses a copper tube, with a 3mm internal diameter to cut discs. A large current is passed down the tube which is held just above the specimen, all immersed in an oil bath. Sparks (arcing) from the tube to the specimen under a current of several amps cut out a 3mm diameter disc.

Ceramic materials can be cut using an ultrasonic cutter. This uses a steel tube of 3mm internal diameter to cut discs. A slurry containing SiC powder is placed between the specimen and the tube and the tube is vibrated over the surface. The SiC particles embed themselves in the wall of the steel tube and cut into the ceramic.

These 3mm discs are then "profile thinned" so that the centre of the disc is so thin that it is electron transparent (a few 100 nms), but the outside of the disc is not thinned, and remains 200 microns in thickness (it is therefore easy to pick up with tweezers). Thinning occurs until a hole forms in the centre of the disc. If thinning is stopped immediately the hole forms the thin (wedge-shaped) region adjacent to the hole will be suitably thin. Again, different methods are used depending on the

material.

For metals, electropolishing is used. This uses a solution, usually a mixture of an acid and an organic solvent (e.g. 20% nitric acid in methanol) which is sprayed on to the specimen under the action of an electrical current. The specimen becomes the anode in this cell and material is removed. Once a hole is formed a light or laser detects it and stops the polishing process. Electropolishing is most definitely a "black art" and also requires that significant attention be given to safety. However, the process is rapid and takes only a few minutes to make a specimen.

For ceramic materials, ion milling is commonly used. An argon ion beam incident on the specimen at some shallow angle, sputters away atoms from the surface of the material until a hole is formed. Again, once a hole is formed a light or laser detects it and stops the milling. This process is very slow and tedious and it can take many hours to thin a specimen.

Increasingly, "difficult" materials, such as ceramics and semiconductors are thinned using focused ion beam (FIB) milling. This is not dissimilar to the argon ion milling described above, but uses gallium ions in a SEM type environment. This is significantly more precise and faster than through argon ion milling.

5.2.3 Polymers and Biological Materials.

Biological or polymeric materials are normally prepared for TEM examination through a number of steps which involve firstly fixing the specimen, dehydrating the specimen to remove moisture, staining to achieve phase contrast, embedding in a polymer resin and finally sectioning of submicron thick sections to produce material thin enough to be electron transparent. For these materials it is not only necessary to produce thin sections, but ones which are dry. Not all the steps described below are required for polymeric materials.

Normally the specimen is stabilised by fixing and dehydration. This is done in a series of steps. Firstly the specimen must be fixed. Fixatives may be aldehydes or oxidizers. Aldehydes – (e.g. glutaraldehyde) often used in a mix called Karnovsky's fixative. These work by a process in which certain proteins are cross-linked, so forming a gel from the cytoplasmic sol. They have little effect on nucleic acids and lipids, but proteinaceous parts of the cell are well preserved.

Other fixatives are oxidizers - e.g. osmium tetroxide. Osmium reacts at the double bonds in unsaturated lipids, stabilizing them. Osmium deposits are precipitated at the site of reaction and so it also has a staining function. It does not have a significant effect on proteins or nucleic acids. As it is a relatively heavy metal, deposits show up well on the TEM. It is important that excess osmium be washed out e.g. with sodium acetate, or it will oxidize other substrates used in processing, forming precipitates.

Uranium salts such as uranyl acetate are commonly used after osmium fixation. They both fix and stain the specimens.

Dehydration is the next stage in the processing. A series of increasingly concentrated mixtures of some solvent in water is used. For example 25% ethanol in water; 50% ethanol in water; 75% ethanol in water; 95% ethanol in water; 100% ethanol.

Once the specimens are fixed and the moisture removed they are stained. Staining is used to increase the contrast of an organic/biomedical specimen. Often materials which are entirely organic in nature will exhibit very small differences in contrast from one phase to another, which makes discrimination of these phases difficult. Staining involves relatively heavy salts, as described above which differentially attaches to different parts of the structure. Different sections are therefore electron transparent to different degrees and provide some form of image contrast.

Stained specimens are then embedded in some form or resin prior to microtoming. Specimens are usually embedded in resins which cure to a high level of hardness (rather than paraffin wax used in optical microscopy). Common polymers are methyl methacrylates and epoxy resins. These often require curing in an oven to fully set/harden.

Thin electron transparent sections are prepared by a process called microtoming. A knife, made of either glass or diamond, is mounted (in effect) on a micrometer. The entire assembly is viewed through an optical microscope. The knife is then used to shave thin slices from the embedded section. The knife is kept stationary and the specimen is moved over the knife. Glass knives are most commonly used as they are inexpensive and easy to use. Diamond knives are often used for harder specimens (some polymers for example) and are more durable than glass knives. They are, however, relatively expensive. Cut sections are typically about 1 mm^2. The colour of the cut sections is often used to indicate the section thickness. These sections are then attached to copper support grids, 3mm in diameter, for examination in the TEM.

In summary, the above section briefly describes some of the issues important in specimen preparation for electron microscopy. However, as stated above every specimen requires a slightly different approach.

6. SPECIMEN PREPARATION EQUIPMENT

Instruction manuals will be provided prior to training.

6.1 INORGANIC SPECIMENS

6.1.1 Introduction

Materials science/ inorganic specimens are typically metals or minerals or ceramics that require coating, grinding or thinning to prepare them for the electron microscope.

Users are also directed to the references

Transmission Electron Microscopy - A Textbook for Materials Science by David B. Williams and C. Barry Carter (Plenum Publishing 1996, ISBN 0-306-45324-X)
Scanning Electron Microscopy and X-Ray Microanalysis by J.I. Goldstein, Dale Newbury, P. Echlin, D.C. Joy, A.D. Romig Jr., C.E. Lyman, C Fiori & E. Lifshin. (Plenum Publishing 1992, ISBN 0-306-44175-6

The following list describe equipment provided in the E.M. Unit
Prospective users must first be instructed by the appropriate staff (PM, VP).
Please note that suitable protection (lab-coat, latex gloves and eye protection, etc) is required when using this equipment.

6.1.2 Buehler Diamond Saw

for preliminary sectioning of metals and ceramics, most usually for transmission electron microscope examination.

6.1.3 Struers Setacom Precision Saw

for sectioning materials as a preliminary step in specimen preparation.

6.1.4 Gatan Ultrasonic Disc Grinder

for production of 3mm discs prior to thinning.

6.1.5 Precision Dimple Grinder

for the pre-thinning of 3mm discs prior to final thinning.

6.1.6 Concept Electro- Discharge (Edm) Machine

for the preparation of 3mm discs from metallic samples prior to final thinning.

6.1.7 Struers Tenupol-3 Electropolisher

for the final thinning of TEM samples.

6.1.8 Nitric Acid/Methanol Electropolishing Solutions

One of the most commonly used electropolishing solutions is a mixture of nitric acid and methanol. Typical solutions are either 20% nitric acid 80% methanol or 30% nitric acid 70% methanol. Solutions are only to be prepared by Prof Paul Munroe or Ms Viera Piegerova. ***(Extreme care is required as the reaction between these liquids is strongly exothermic)***.

Once the solution has been prepared it should be stored in the appropriate storage cabinet when not in use. Label the storage bottle properly. Include your name and the date. See the Unit's Safety Officer (Barry Searle) for appropriate warning labels. The solution can usually be used several times. When the solution becomes discoloured it must be disposed of safely (see EMU Safety Officer) and request that Prof Paul Munroe prepare a fresh solution.

6.1.9 South Bay Technology Pc2000 Plasma Cleaner

used for cleaning (TEM) specimens

6.2 BIOLOGICAL/ POLYMER SPECIMENS

6.2.1 Introduction

Specimens for electron microscopy must be dry, conductive, or coated with a thin layer of grounded conductive material, prepared with minimal effect on specimen structure. Hard, dry specimens (most inorganic material specimens) or those that are naturally self-supporting need no special treatment. They can be air dried or dried in the vacuum of the coating unit. Insects, for instance usually can be air dried without structural collapse. However, soft specimens, especially those that have a considerable content of water, need special preparation. They may be dried in a variety of ways (air drying, freeze drying and critical point drying)

Detailed instructions for specimen fixing, staining, drying, mounting cryo/ ultramicrotomy are available separately. Users are also directed to the references

Polymer Microscopy Second edition by Linda C. Sawyer & David T. Grubb Chapman & Hall, London 1996. ISBN 0412 60490 6
Scanning Electron Microscopy and X-Ray Microanalysis by J.I. Goldstein, Dale Newbury, P. Echlin, D.C. Joy, A.D. Romig Jr., C.E. Lyman, C Fiori & E. Lifshin. (Plenum Publishing 1992, ISBN 0-306-44175-6

The following list describes facilities provided in the E.M. Unit
Prospective users must first be instructed by the appropriate staff (Sigrid Fraser, Margaret Budanovic).
Please note that suitable protection (lab-coat, latex gloves and eye protection, etc) is required when using this equipment.

6.2.2 Reichert Ultracut E Ultramicrotome

for cutting thin sections of biological or polymer specimens.

6.2.3 Reichert Ultracut E Cryo-Ultramicrotome

for cutting thin frozen sections of soft biological or polymer specimens.

6.2.4 Bromma LKB 7800 Knifemaker

for making glass knives for microtomy. (Diamond knives are also available for special applications. Diamond knives may only be used by staff. See Margaret Budanovic or Sigrid Fraser)

6.2.5 LKB Ultramicrotomy System Pyromitome 11800

6.2.6 Lynx Tissue Processor for Epoxy Embedding

for preparing a number of specimens simultaneously

6.2.7 Baltech Critical Point Drier CPD030

The most common technique used to dry delicate hydrated specimens for SEM investigation.

6.2.8 Sorvall Biofuge primo R

microfuge for the processing/ concentration of cellular material for TEM investigation.

6.3 VARIOUS COATERS

The coaters are used to coat the surface of specimens with a thin layer of selected conductive materials (e.g. Cr, Pt, Au, C etc) prior to microscopy /analysis. Operation manuals are found with the instruments (some are also available from http://srv.emunit.unsw.edu.au). Sputter and evaporative coating options are available.

JEOL vacuum evaporation coaters (JEE-400 and JEE-4B). The JEOL coaters are operated by staff only. Give your samples to Viera Piegerova or Barry Searle for coating ***at least one day before your SEM session.***
Gold, carbon, aluminium, chromium

- ***Polaron E5000 sputter coater.***

Gold (pre-dates magnetron sputtering head and therefore may heat samples during coating)

- ***Balzers carbon fibre carbon coater.***

Carbon

- ***Xenosput high resolution sputter coater.***

Chromium

- ***Emitech K575 dual head turbo chromium sputter coater.***

Chromium, gold

- ***Polaron E6000 sputter coater.***

Gold

- ***EMITECH K-575 sputter coater***

Gold

It is preferable to examine your specimens immediately. If this is not possible, store them (on their stubs) in evacuated vacuum desiccators. The coating does not prevent rehydration. Shrinking and swelling due to hydration changes will produce discontinuities in the conductive film.

7. MICROSCOPES

Operation details are found in the user's manual with the instrument, and are also available from http://srv.emunit.unsw.edu.au

7.1 TRANSMISSION/SCANNING TRANSMISSION ELECTRON MICROSCOPES

7.1.1 Hitachi H-7000 TEM/ STEM (1988)

The H-7000 is mainly used for analysis of thin sections of both biological materials and polymers. STEM produces secondary electron images, transmitted electron images

- Electron beam accelerating voltages from 40-125 kV.
- Capable of 0.5nm Resolution.
- tilting stage, cryo-stage (LN_2)
- Moran Kevex energy dispersive x-ray analyser, MegaView CCD Camera

7.1.2 Philips CM200 Field Emission TEM/ STEM (1999)

The CM200 is used for high resolution imaging and microanalysis

- 200 keV maximum electron beam accelerating voltage
- capable of 0.24 nm resolution
- EDAX EDS detector (chemical analysis from regions as small as 10 nm in diameter)
- Soft Imaging System CCD camera for direct recording of digital images.

Detailed procedures for use of its ancillary equipment (EDAX EDS, SIS CCD camera) are found in their respective manuals. Copies of these manuals are available from Paul Munroe.

7.1.3 VG HB601UX Field Emission TEM/ STEM (Installed at the University of Sydney)

The VG STEM is used for high resolution imaging and microanalysis

- 100kV maximum electron beam accelerating voltage
- bright field imaging (capable of 0.22nm resolution)
- high resolution EDS profiles and mapping
- Parallel Electron Energy Loss Spectroscopy (PEELS)

See Paul Munroe for information about access and training.

7.2 SCANNING ELECTRON MICROSCOPES

7.2.1 Cambridge S-360 SEM (1988)

The S-360 is used for routine scanning electron microscopy and microanalysis.

- secondary electron, backscattered electron (Robinson), specimen current, and EBIC imaging modes are available.
- X-ray elemental analyzer, multi-element mapping, (IXRF http://www.ixrfsystems.com/new.html)
- accessories include a LN cold stage operating down to 77K and a tensile stage operating to 45.5Kg, calibrated readout.
- live image can be recorded on videotape

7.2.2 JEOL 840 SEM (1986)

The JEOL 840 is used for routine microstructural and microanalytical analysis of materials.

- Link AN 10000 X-ray elemental analyzer
- HKL Electron Backscattered Diffraction system

Detailed procedures for use of its ancillary equipment, (Link EDS and HKL EBSD) are found in their respective manuals. Copies of these manuals are available from PRM.

7.2.3 Hitachi S-900 Field Emission SEM (1991)

The S-900 is the highest resolution SEM in the EMU.

- low voltage "in-lens" field emission scanning electron microscope with hot and cold stages
- capable of imaging at very high resolution (0.7 nanometres).
- in-lens configuration limits specimen size to ~5 x 10 x 2.5mm

7.2.4 Hitachi S-4500II Field Emission SEM (1996)

The S-4500 is used for high resolution imaging and analysis.

- high resolution (1.5 nanometres) "below lens", field emission scanning electron microscope with tilting stage.
- below lens configuration means that the specimen size limitation is not as stringent as for Hitachi S-900 SEM.
- Robinson Back-Scatter Electron Detector
- Link ISIS 200 Energy Dispersive X-ray Microanalysis System

Detailed procedures for use of its ancillary equipment, (Oxford Isis EDS, Robinson BSD) are found in their respective manuals. Copies of these manuals are available from Paul Munroe.

7.2.5 FEI Quanta 200 Environmental SEM

The Quanta 200 is an environmental scanning electron microscope which operates under a low vacuum so that specimens may be examined in a hydrated or uncoated state. This overcomes many of the limitations of conventional specimen preparation. It is equipped with an EDAX Energy dispersive spectrometer for elemental chemical analysis and a Renishaw Structural Chemical Analyser (SCA) for identification of chemical compounds.

7.3 ELECTRON PROBE MICRO ANALYSER

7.3.1 Cameca SX50 Microprobe (1992)

The SX50 is used for fully automated quantitative microanalysis using wavelength dispersive spectrometers to analyse the X-ray spectra emitted by electron-irradiated specimens.

- secondary or backscattered electron imaging modes.
- high resolution and high quality x-ray maps.
- detection and non-destructive analysis of almost all elements from Boron (4) and heavier in the periodic table.
- Oxford EDS with Moran Scientific software.
- analysing crystals (Lif, TAP, PET, PCO, PC1, PC2, PC3)
- Instrumental calibration is carried out using certified standard reference materials and suitable data reduction matrix correction procedure SAMx PC-Based software;

Procedures may also be found at the Cameca SX50 web site ***http://cameca.fr/***

7.4 FOCUSED ION BEAM MILLERS

7.4.1 Philips/FEI FIB XP200 FIB (1998)

The FIB is used for precisely controlled milling of materials in preparation for SEM and TEM examination.

- A fine, energetic beam of gallium ions is scanned over the surface of a specimen.
- high beam currents sputter the specimen surface that allows subsurface cross-sections to be prepared.
- At low beam currents, the secondary electrons or secondary ions emitted from the specimen surface, can be detected and used to form high resolution images.

It can also be used for

- electron and ion imaging
- surface modification
    - patterned deposition of conductive or dielectric materials on specimens
    - micro-machining patterns in any material.

Detailed procedures for its use are found in the FIB-200 manual. Copies of the manual are available from Paul Munroe.

7.4.2 FEI Nova 200 Nanolab

The FEI is a dual beam focused ion instrument. It comprises a high resolution ion column with a field emission source electron column. The ion column is conventionally used to prepare cross-sections into specimens and the electron column is used to image them. The Nova is equipped with an EDAX Energy dispersive spectroscopy EPS system for elemental analysis and a TSL Electron Backscattered electron diffraction system for crystallographic analysis. The Nova also includes sophisticated software packages which allow 3-dimensional reconstructions of specimens and automated TEM preparation.

7.5 SCANNING PROBE MICROSCOPES

7.5.1 Veeco/ Digital Instruments Extended Dimension 3000 SPM (1997)

Scanning Probe Microscopy (Atomic Force Microscopy/ Scanning Tunnelling Microscopy) provides non-destructive three dimensional high spatial resolution real space images of surfaces. Images are formed by detecting the local interactions (inter-atomic forces/ tunnelling current, respectively) between the specimen surface and a sharp probe. (STM is possible for conductive specimens only).
The D3000 provides

- high resolution AFM and STM (Nanoscope III controller)
- scanning probe/ fixed specimen configuration. Specimen size is fairly unrestricted.
- extended options include phase, electrical and magnetic imaging, also imaging in a fluid environment is possible.
- Standard probes supplied. (OTESPA, NSC15, OTR8, DNP, DNP-S) See staff

Detailed procedures for use/ options are found in the Dimension 3000 manual.
See also http://www.veeco.com/html/product_bymarket_proddetail.asp?ProductID=178 for applications notes, links etc.

7.5.2 Veeco/ Digital Instruments Extended Multimode SPM (2001)

Scanning Probe Microscopy (SPM) enables non-destructive three dimensional high spatial resolution real space images of surfaces via detection of local interactions between the specimen surface and a sharp probe.
The Multimode provides

- very high resolution AFM and STM. (Nanoscope III controller)
- fixed probe configuration/ specimen is mounted on piezo-scanning tube, hence specimen size is restricted.
- extended options possible include phase, electrical and magnetic imaging, also electrochemical and imaging in a fluid environment.
- Optional anti-vibration configuration (bungee) available for high resolution work.
- Standard probes supplied. (OTESPA, NSC15, OTR8, DNP, DNP-S) See staff

Detailed procedures for use/ options are found in the Multimode manual.
See also http://www.veeco.com/html/product_bymarket_proddetail.asp?ProductID=177 for applications notes, links etc.

7.6 LIGHT MICROSCOPES

Various light microscopes are available in the EMU. For example

- Zeiss W Microscope with phase contrast optics and digital camera attachments.
- Olympus BH-2 Microscope with Universal Illumination system, for the examination of specimens using incident reflected light, polarised light and dispersion staining. The microscope is equipped with a filar micrometer for measurement of particle and fibre size and a DP12 Digital Camera System.

7.7 X-RAY MICROSCOPE

The Skyscan Micro CT enables 3-dimensional reconstruction and analysis of an object to be generated from 2-dimensional x-ray shadow projections.

- X-ray source accelerating voltages from 20-80kV.
- Non-destructive imaging technique requiring no specimen preparation
- Capable of spatial resolutions of 3-5µm
- Best suited to objects within the range 2mm^3 to 15mm^3

See also http://www.skyscan.be for further information.

NOTE:

The ***Microscopy and Imaging Unit*** in the School of Medical Sciences (Faculty of Medicine) has an excellent range of microscopy facilities. This Unit provides access to equipment and services in histology, light microscopy (brightfield, darkfield, DIC, phase contrast, polarisation and fluorescence illumination techniques), laser capture microdissection, laser scanning confocal microscopy, image processing and image analysis. The Unit, found on the first floor of the Wallace Wurth Building, operates on a cost recovery basis. Please contact Maria Sarris on x52469 or m.sarris@unsw.edu.au with any enquiries.

8. MICROSCOPY RESOURCES

8.1 See http://srv.emunit.unsw.edu.au/ and http://srv.emunit.unsw.edu.au/PDF_files/books.pdf for reference materials and links.

8.2 SUPPLIERS

Please ask EMU staff for advice and/ or consult http://srv.emunit.unsw.edu.au/ for links

8.3 BOOK REFERENCES

This is not a complete list.
Please also consult http://srv.emunit.unsw.edu.au/ and
http://srv.emunit.unsw.edu.au/PDF_files/books.pdf for other reference material and links

Scanning Electron Microscopy and X-Ray Microanalysis
A Text for Biologists, Materials Scientists, and Geologists
Joseph L Goldstein A. D Romig, Jr.; Dale E. Newbury; Charles E. Lyman; Charles Fiori; Patrick Echlin, David C. Joy; & Eric Liffshin
ISBN 0-306-44175-6 Second Edition 1992
Plenum Press. New York and London

Advanced Scanning Electron Microscopy and X-Ray Microanalysis,
D. E. Newbury, D. C. Joy, P. Echlin, C. E. Fiori, and J. I. Goldstein,
Plenum Press, New York, 1986.

Maintaining and monitoring the transmission electron microscope.
(Microscopy handbooks; 08)
By S.K. Chapman, ISBN 0-19-856407-4

Polymer Microscopy
Second edition 1996
By Linda C. Sawyer & David T. Grubb ISBN 0412 60490 6
Chapman & Hall, London . Glasgow Weinheim . New York . Tokyo Melbourne Madras

Positive Staining for Electron Microscopy
M. A. Hayat 1975
Van Nostrand Reinhold Company New York/ Cincinnati Toronto/London/ Melbourne
ISBN 0442-25684-1

Stains and cytochemical methods
Hayat. M. A. (1993) ISBN 0-306-44294-9
Plenum Press. New York and London

Principles and Techniques of Electron Microscopy: Biological Applications
M. A. Hayat ISBN: 0521632870; 4th edition (2000)
Cambridge University Press;

Microscopy, Immunohistochemistry, and Antigen Retrieval Methods: For Light and Electron Microscopy
by M. A. Hayat ISBN: 0306467704
Plenum Pub Corp;

Quantitative Electron-Probe Microanalysis
Scott, V.D. & Love, G. & Reed, S.J.B.
(1995), 2nd edition; ISBN 0-13-104050-2;
Ellis Horwood Ltd

Quantitative Electron-Probe Microanalysis,
Scott, Love & Reed,

1995, 2nd Edition, Ellis-Horwood: New York.

Electron Micro-Probe Analysis and Scanning Electron Microscopy in Geology,
SJB Reed, 1996, 2nd Edition ISBN 0-521-41956-5
Cambridge Univ. Press: Cambridge, U.K.

Electron Probe Quantitation
Heinrich, K.F.J. & Newbury, D.E. (1995):.- ISBN 0-306-43824-0;
Plenum Press, New York & London;

Scanning and Transmission Electron Microscopy: An Introduction,
Stanley L. Flegler, John W. Heckman, Jr., Karen L. Klomparens,
Oxford University Press, 1993.

Scanning Probe Microscopy: Theory, Techniques, and Application,
ed. D. A. Bonnell
John Wiley & Sons New York (2000).

Scanning Tunneling Microscopy: Theory, Techniques, and Application,
ed. D. A. Bonnell
VCH Publications New York (1993).

Selected papers on scanning probe microscopes; design and applications
Yves Martin, editor. ISBN 0819418048
Bellingham, Wash. : SPIE Optical Engineering Press, (1995).

Atomic force microscopy for biologists
V J Morris, A R Kirby, A P Gunning.
London : Imperial College Press, (1999)
ISBN 1860941990 :

Cathodoluminescence microscopy of inorganic solids.
B. G. Jacobi, D. B. Holt:
(1990) ISBN: 0306433141
New York: Plenum

Scanning Electron Microscopy: Physics of Image Formation and Microanalysis (2nd Ed)
by Ludwig Reimer, T. Tamir (Editor),
(Springer Series in Optical Sciences, Vol 45) ISBN: 3540639764; 2nd edition (1998)
AL. Schawlow (Editor)
Springer Verlag

Transmission Electron Microscopy: Physics of Image Formation and Microanalysis (4th Ed)
by Ludwig Reimer
(1998) ISBN: 3540625682
Springer Verlag Telos

Transmission electron microscopy: a textbook for materials science
By David B. Williams and C. Barry Carter. 1996
ISBN 0-306-45247-2 (hardbound). ISBN 0-306-45324-X (pbk.)
Plenum Press. New York and London

Working with a Scanning Electron Microscope
By S. K. Chapman ISBN 0 850770 93 9
LODGEMARK PRESS LTD
Bank House, Summerhill, Chislehurst, Kent BR7 5RD, England.

Handbook of biological confocal microscopy
edited by James B. Pawley. 2nd ed.
(1995). ISBN 0306448262

Plenum Press, New York

Confocal microscopy
edited by P. Michaal Conn
(1999). ISBN 0121822087
Academic San Diego London

PRACTICAL METHODS IN ELECTRON MICROSCOPY
Series edited by AUDREY M. GLAUERT

Volume 1
Part I: ***Specimen Preparation in Materials Science***
by P.J. GOODHEW (1973, 2nd printing 1974, viii + 180 pages) (out-of-print)
Part II: ***Electron Diffraction and Optical Diffraction Techniques***
by B.E.P. BEESTON, Robert W. HORNE and Roy MARKHAM (1973, 4th printing 1990, xiv + 250 pages)

Volume 2
Principles and Practice of Electron Microscope Operation
by Alan W. AGAR, Ronald H. ALDERSON and Dawn CHESCOE (1974, 7th printing 1990, xiv + 346 pages)

Volume 3
Part I: ***Fixation, Dehydration and Embedding of Biological Specimens***
by Audrey M. GLAUERT (1975, 7th printing 1987, xii + 208 pages)
Part II: ***Ultramicrotomy***
by Norma REID (1975, 7th printing 1989, x + 138 pages) (out-of-print)

Volume 4
Design of the Electron Microscope Laboratory
by Ronald H. ALDERSON (1975, 2nd printing 1985, xii + 130 pages)

Volume 5
PartI:***Staining Methods for Sectioned Material***
by P.R. LEWIS and D.P. KNIGHT (1977, 6th printing 1990, xvi + 312 pages
Part II: X-ray ***Microanalysis in the Electron Microscope***
by John A. CHANDLER (1977, 4th printing 1987, xvi + 222 pages)

Volume 6
Part I: Autoradiography and Immunocytochemistry
by M.A. WILLIAMS (1977, 6th printing 1990, xx + 218 pages)
Part II: Quantitative Methods in Biology
by M.A. WILLIAMS (1977, 3rd printing 1985, xx + 234 pages)

Volume 7
Image Analysis, Enhancement and Interpretation
by D.L. MISELL (1978, 2nd printing 1990, xvi + 306 pages)

Volume 8
Replica, Shadowing and Freeze-Etching Techniques
by J.H. Martin WILLISON and Arthur J. ROWE (1980, xvi + 302 pages)

Volume 9
Dynamic Experiments in the Electron Microscope
by E.P. BUTLER and KF. HALE (1981, xvi + 458 pages)

Volume 10
Low Temperature Methods in Biological Electron Microscopy
by A.W. ROBARDS and U.B. SLEYTR (1985, xx + 552 pages)

Volume 11
Thin Foil Preparation for Electron Microscopy
by P.J. GOODHEW (1985, xiv + 206 pages)

Volume 12
Electron Diffraction: An Introduction for Biologists
by D.L. MISELL and E.B. BROWN (1987, xvi + 288 pages)

Volume 13
Sectioning and Cryosectioning for Electron Microscopy
by Norma Reid and Julian E. Beesley ***Published by Elsevier*** Amsterdam, New York & London (1991)
ISBN 0444-81190-7

Volume 14:
Cytochemical Staining Methods for Electron Microscopy
by P. R. Lewis and D. P. Knight (1992, 321 pages)

Volume 15
Vacuum Methods in Electron Microscopy
by Wilbur C. Bigelow 1994.

Volume 16
X-ray Microanalysis for Biologists
by Alice Warley 1997

Volume 17
Biological Specimen Preparation for Transmission Electron Microscopy
by Audrey M. Glauert and Peter R. Lewis 1999

Volume 18
Negative Staining Methods for Electron Microscopy
by R.W. Horne and E.A. Munn

8.4 MICROSCOPY JOURNALS.

The following journals are classified as specialist microscopy journals (ISI Journal Citation Reports) and provide useful material, particularly relating to techniques. The majority of microscopy related research is published in the other science, medical, engineering etc journals.

- *HISTOCHEMISTRY AND CELL BIOLOGY*
- *JOURNAL OF ELECTRON MICROSCOPY*
- *JOURNAL OF MICROSCOPY-OXFORD*
- *MICRON*
- *MICROSCOPY AND MICROANALYSIS*
- *MICROSCOPY RESEARCH AND TECHNIQUE*
- *SCANNING*
- *ULTRAMICROSCOPY*
- *ULTRASTRUCTAL PATHOLOGY*