Training-qualification scheme for EM usage.
Before starting an EM-based project the planed work must be discussed and, after agreement, a brief (a couple of lines) project outline submitted to Bio-Imaging.

A] Specimen preparation protocols for resin sections, cryo-sectioning/ immunoEM and negative staining.

(i) Basics of fixation, dehydration and embedding for resin sections.
Use of the microtome for cutting resin sections (MT2 qualification).
a] trimming blocks
b] making glass knives (use of LKB knife maker – KM1 qualification) including attaching and sealing tape boat
c] aligning knife to block face
d] cutting and recovering sections onto grids
e] cleaning and care of instrument (care of diamond knives)
f] staining grids with uranyl acetate and lead citrate
g] formvar-coating grids (optional)

Once the user is confident/competent then they will be qualified to book and use the microtome (MT2 and KM1 qualifications) for themselves during normal working hours (9.00 – 5.00, Monday to Friday).

(ii) Cryo-sectioning (MT1 qualification), immunolabelling.
a] fixation protocols, gelatin embedding, and cryo-protecting and freezing samples (LN_2 handling.)
b] making support films and coating grids - use of vacuum coating unit (VCU1) for carbon coating and/or glow discharging grids.
c] making glass knives / care and handling of diamond cryo-knives
d] LN_2 handling and setting-up the cryomicrotome (MT1 qualification)
e] cutting and retrieving cryosections
f] immunolabelling cryosections, staining and embedding sections
g] cleaning and care of instrument.

(iii) Negative staining.
a] coating grids – formvar-carbon; carbon films
b] use of vacuum coating unit (VCU1 qualification).

Outline protocols and standard operating procedures for all of the above will be posted on the Bio-Imaging Technical Microsite.

B] Basic use of the Leo912 TEM (TEM1 qualification).
An introduction to the EM will be given covering the basic features of the instrument, principals of beam-specimen interactions, image formation and specimen contrast (apertures), methods of recording images. Before a person will be qualified to book the TEM they will have to be familiar and confident with the following procedures.

1] Inserting and removing specimen holder from microscope; loading grids into rod.
2] Switching on filament-HV and obtaining image.
3] Use of magnification and illumination switches.
4] Focusing.
5] Basic alignment – Z height; centring specimen; objective wobbler; specimen tilting.
6] LowMag imaging – for searching grid; choice and alignment of apertures.
7] Knowing when basic alignment is not correct.
8] Use of CCD camera to record images, saving images (archiving to each individuals/labs storage space on Servalan or Dunn4) etc.

A short summary of procedures 1-6 will be given to each user to help them become familiar with the operation of the microscope. Once these procedures have been mastered and the user is confident then they will be qualified to book and use the microscope (TEM1 qualification) for themselves during normal working hours (9.00 – 5.00, Monday to Friday).

Basic use of the Tecnai 12 TEM (TEM2 qualification).
The FEI Tecnai 12 TEM is controlled through a computer interface and two control panels and users will again have to be familiar and confident with the following procedures. While the actual operating procedures are somewhat different from the LEO 912 the principals behind the procedures are the same.

1] Logging on to the microscope computer and opening appropriate software.
2] Inserting and removing specimen holder from microscope; loading grids into rod.
3] Operating 'column valves' function, switching on HT and filament and obtaining image.
4] The lay-out of the two control panels; use of magnification and illumination controls.
5] Focusing.
6] Basic alignment – Z height; objective wobbler; specimen tilting.
7] LowMag imaging – for searching grid; choice and alignment of apertures.
8] Use of CCD camera to record images, saving images etc.

C] Advanced use of the Leo912 TEM
(Required for 'out-of-hours' working which will require swipe-card access).

1] Setting-up CCD camera and in particular obtaining reference images; use of RTFFT for focusing; image analysis package.
2] Review of basic alignment procedures with reference to specimen tilting [also critical for optimising images on CCD camera].
3] Changing the filament; checking gun-beam alignment (centring filament/gun tilt etc) and aligning the microscope.
4] 'Calibrate all' and stigmators.
5] Start-up and shut-down of microscope.

Advanced use of the Tecnai 12 TEM
(This will be required for 'out-of-hours' working that will require swipe-card access).

1] Start-up and shut-down of microscope.
2] Changing the filament; checking gun-beam alignment (centring filament/gun tilt etc) and aligning the microscope.
3] Changing apertures.

D] SEM
Fixation
Critical point dryer (CPD1)
Sputter coater (SCU1)

Use of SEM.

Outline protocols – standard operating procedures for all of the above are posted on the Bio-Imaging Technical Microsite.