Correlated LM/EM - Embedding and sectioning of microinjected cells

- Cells are seeded onto CELLocate coverslips (Eppendorf) and microinjected with a fluorescent marker and protein/DNA of interest.

- After required incubations cells are fixed in 4% paraformaldehyde in PBS and the location of the injected cells on the locater grid is recorded using by fluorescence and phase/DIC imaging using water immersible objectives and a CCD camera. Up to this point the cells can be used for either immunofluorescence or EM

- For EM the cells are then post fixed with 1% glutaraldehyde in PBS followed by 1% OsO4 (with or without 1.5% potassium ferrocyanide) in 0.1M cacodylate buffer, dehydrated in graded ethanols and embedded in epoxy resin. To infiltrate/embed the coverslips overfill plastic tubes with resin and invert the cover slip cell side down onto the top of the tubes avoiding trapped bubbles. Before polymerisation invert the tube and cover slip to allow any bubbles to rise from the cover slip.

- Dip the cover slip briefly into liquid nitrogen and split off from the resin leaving the cells and an impression of the grid on the block. The location of the cells can be found by comparing the phase contrast and fluorescence CCD images with the grid impression prior to trimming and sectioning.

- The region of injected cells is serially sectioned and sections collected on plastic/carbon coated slot grids contrasted with uranyl acetate and lead citrate.

- Cells are located at the EM by comparing the position of cell profiles in the section with the injected cells on the CCD images. Note that the arrangement of the cells on the block face will be a mirror image of that in the CCD image. So for comparison it may be helpful to invert the CCD image before examining the sections at the EM. The cells are then located by comparing the pattern of sectioned cell profiles with the phase contrast/fluorescence images.