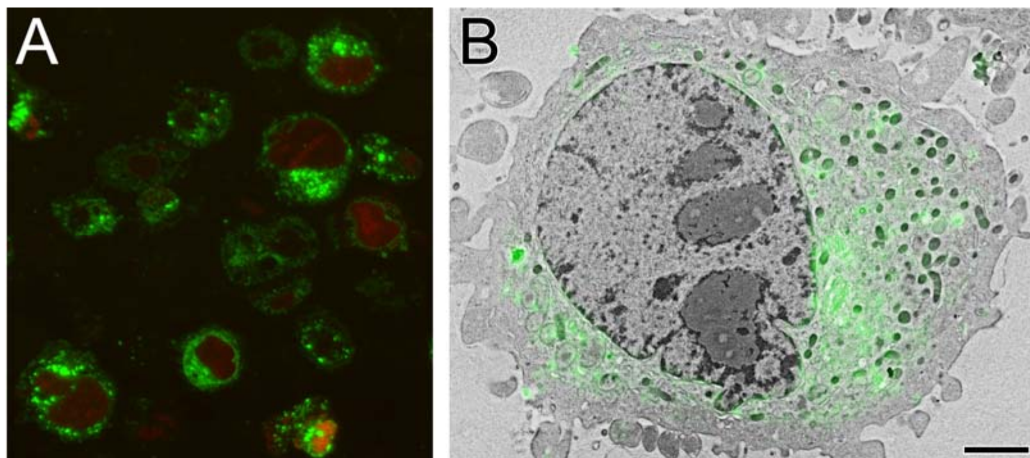
Sample preparation for integrated light and electron microscopy

Christopher Peddie
Electron Microscopy STP, The Francis Crick Institute, London

Fluorescence microscopy is a fundamental tool in cell biology and provides powerful functional protein localisation data. For a complete understanding of cellular function however, the provision of structural context is crucial. Electron microscopy can provide this ultrastructural information, where the inherent resolution limit of light is no longer a problem. Indeed, protein localisation can itself be studied directly by electron microscopy using a variety of established immunolabelling techniques, each of which has its own merits; but, these methods are ultimately restricted by structural compromises and the availability of suitable antisera.

Correlative light and electron microscopy techniques allow direct translation of functional protein localisation data onto a detailed backdrop of cellular architecture. However, the disconnection between imaging modalities and requirement for complex intermediate specimen preparation steps present significant limitations. With the recent development of integrated light and electron microscopes, a revolution in correlative techniques is underway, and these instruments have the potential to increase the speed and precision of correlative experiments, whilst simultaneously widening accessibility.

Despite this, routine procedures for preserving fluorophores in specimens compatible with the vacuum of the electron microscope were until recently falling behind the rapidly moving technological front line. We have developed a protocol to preserve cloned fluorophores in cells and tissues embedded in resin with sufficient contrast for electron imaging, and have shown that several fluorophores are stable and active in resin-embedded cells in the vacuum of integrated light and electron microscopes. Here, I will outline our sample preparation method, and briefly discuss how super-resolution light microscopy and 3D electron microscopy will be integrated into future imaging workflows.



A: Light microscopy of GFP-C1 and mCherry-H2B in a 70 nm HM20 section using a widefield epifluorescence light microscope. B: Electron micrograph with overlaid GFP-C1 signal acquired from a 200 nm HM20 section using the SECOM integrated light and scanning electron microscope. The fluorescent signal corresponds to the nuclear envelope and nucleoplasmic reticulum, and other membranous structures within the cytoplasm including Golgi stacks and endoplasmic reticulum. Scale bar – 2 µm.