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Placing Molecules in a Cellular Context Using Light, Eelectron and X-Ray Microscopy

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Fluorescence microscopy is a powerful tool for localising proteins within biological samples. However, information is limited to the distribution of the tagged protein, telling us little about the ultrastructure of the surrounding cells and tissues, which may be intimately involved in the biological process under study. Electron microscopy overcomes the resolution limitation inherent in light microscopy and can reveal the ultrastructure of cells and tissues. However, protein localisation tends to be complex and is often dependent on the availability of 'EM-friendly' antibodies. Correlative light and electron microscopy (CLEM) combines the benefits of fluorescence and electron imaging, revealing protein localisation against the backdrop of cellular architecture. In this talk, I will introduce several ways in which we are developing 3D CLEM for application to biological samples.

To image rare events in cells, tissues and whole model organisms, we developed Correlative Light and Volume EM (CLVEM), which combines correlative workflows with microscopes that automatically collect large stacks of high resolution images. The Serial Block Face Scanning Electron Microscope (SBF SEM) consists of a miniaturised ultramicrotome mounted inside the SEM chamber, which cuts resin-embedded samples using a diamond knife prior to imaging the exposed surface with the electron beam. In this way, thousands of images can be automatically collected through the volume of the sample [1]. We used Correlative Light and SBF SEM to analyse the reformation of the nuclear envelope during mitosis in lipid-depleted mammalian cells, using the GFP-C1 construct to detect diacylglycerol in cell membranes [2]. Fast automated data acquisition allowed us to move towards high throughput quantitative CLEM at a rate of one whole cell per day.

During this development work, it became clear that several technical challenges associated with CLEM are exaggerated when working in 3D. Firstly, the accuracy of the overlay between light and electron images (which is critical to successful localisation of molecules to cellular structures) becomes more difficult as sample size increases. Secondly, with data acquisition becoming more automated, the bottleneck in the workflow becomes the data analysis step. Thirdly, artifacts associated with chemical fixation, heavy metal staining, dehydration and resin embedding are exacerbated when imaging in three dimensions, and this can critically affect the outcome of high resolution imaging experiments in some biological systems. We are addressing these challenges as follows…

To increase protein localisation precision, we developed an 'In-Resin Fluorescence' (IRF) protocol that preserves the activity of fluorescent proteins in resin-embedded cells and tissues [3]. Once cut into ultrathin sections, out-of-plane fluorescence is removed resulting in 'super-resolution' light microscopy in the axial direction, which increases the accuracy of the LM-EM overlays. Localisation precision is further increased by imaging the IRF sections *in vacuo* in the next generation of commercial integrated light and electron microscopes (ILEM, Fig.1): the SECOM ILSEM (DELMIC) and the iCorr^{TM} ILTEM (FEI). We are seeking to further improve accuracy by developing integrated super resolution light and electron microscopes.

To facilitate and expedite data analysis, we collaborate with computer vision scientists to design algorithms to automatically detect and select subcellular organelles in EM images, including the nuclear envelope, the endoplasmic reticulum and vesicles. This work is continuing with the ambitious aim of automating the process of recognition and 3D model generation for all cell organelles.

Finally, I will show how we are moving into the world of the native state cell, using synchrotron-hosted soft X-ray microscopes to image whole vitrified cells, without the need for chemical fixatives, stains or sectioning [4,5,6]. Our aim is to capture the most volatile cellular structures and elucidate their function. I will use the example of autophagy, a critical cellular process in cell health and disease, to illustrate the potential of this correlative cryo-fluorescence and cryo-soft X-ray workflow to answer otherwise intractable biological questions [7].

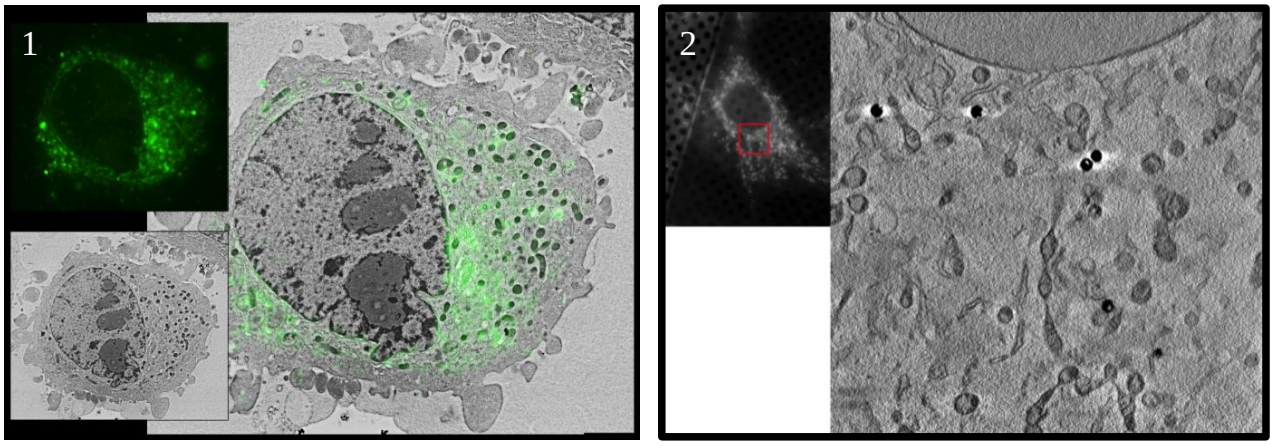

Figure Legends. Figure 1 shows the localisation of diacylglycerol (top) in mammalian cell membranes (bottom) using GFP-C1 as a probe, imaged in an integrated light and scanning electron microscope [3]. Figure 2 shows a whole vitrified HeLa cell, imaged using a correlative cryo-fluorescence (left) and cryo-soft X-ray tomography (right) workflow [4,5,6].

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