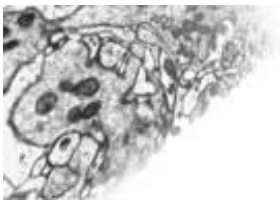


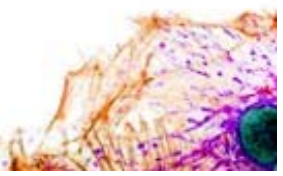
CRG CORE FACILITY TECHNOLOGY SYMPOSIUM

CORRELATIVE LIGHT AND ELECTRON MICROSCOPY

9th July 2013 – 10:00am to 1:00pm
Charles Darwin meeting room (ground floor)

Online registration at www.crg.eu/technology_symposium_130709

CRG Centre de Regulació Genòmica



ABSTRACTS

Lucy COLLINSON

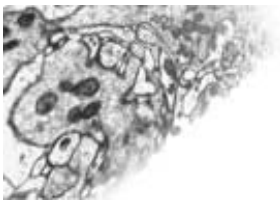
London Research Institute, London UK

"Linking Structure to Function: From Correlated Microscopy to Integrated Microscopy"

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 improving and extending CLEM. We developed 'correlative light and volume EM' to enable visualisation of rare events in tissues and whole model organisms, by combining correlative workflows with new microscopes that can automatically collect large stacks of high resolution images. We applied this technique to locate forming blood vessels undergoing fusion events in a zebrafish model. We developed an in-resin fluorescence (IRF) protocol for mammalian cells, which preserves GFP and mCherry through processing into resin, so that we can directly detect fluorophores and cellular structure in the same ultrathin resin section for CLEM in the next generation of integrated imaging systems. Finally, we used correlative cryo-fluorescence and cryo-soft X-ray microscopy at synchrotron beamlines to image fluorescent proteins within whole mammalian cells, preserved as close to native-state as possible.

Thus, 3D correlative imaging can be applied to a wide variety of samples and biological questions, and is starting to become more accessible to non-specialist laboratories.



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ABSTRACTS

Alberto LUINI

Institute of Protein Biochemistry, Italian National Research Council (CNR), Naples IT

"Recent variants on the correlative microscopy theme"

Correlative Microscopy aims at observing the same cellular object by two different microscopy approaches, and at integrating the information thus obtained to achieve a level of understanding superior to that afforded by each of the two techniques used separately. A most common and fruitful combination of techniques is between light (video) microscopy and electron microscopy (CLEM), which integrates information on the dynamics of the object of interest in living cells with information on the fine structure and the cellular context, or reference space, of the same object. CLEM can thus be considered a super-resolution imaging technique with significant advantages (and disadvantages) over other superresolution approaches. Other combinations are of course possible, each with their distinctive advantages. The assembly and disassembly of multiprotein complexes lies at the core of all cellular functions. We have developed a new form of correlative microscopy that allows the visualization of large multiprotein complexes at the time and place of interest in single cells by combining video microscopy with a novel long-range multiple FRET-based approach.

Ines ROMERO-BREY

University of Heidelberg, Heidelberg DE

"Correlative microscopy: a tool to investigate viral replication"

Correlative light and electron microscopy (CLEM) is a term applied to a number of approaches, all having in common that the imaging and analysis of the same sample employs both methods. On one hand light microscopy (LM) provides a general overview from many cells, helping to identify subpopulations of cells expressing a protein of interest (POI). On the other hand electron microscopy (EM) overcomes the resolution limit of light microscopy, providing a higher resolution image of a particular intracellular event and additional spatial information, the so-called reference space.

By using several correlative fluorescence and transmission electron microscopy (TEM) we have unambiguously shown that hepatitis C virus (HCV) induces the formation of double membrane vesicles (DMVs), surrounding lipid droplets (LDs) and residing in close proximity of endoplasmic reticulum (ER).

Furthermore, CLEM has allowed us to confirm the important contribution of one of HCV non-structural proteins (NS5A) to the formation of DMVs, as well as to have a direct evidence of the drastic effect of an anti-HCV compound on the biogenesis of these HCV-induced mini-organelles, its presumed replication sites.