CORRELATIVE LIGHT AND ELECTRON MICROSCOPY (CLEM) — ULTRASTRUCTURE LIGHTS UP —

P. Verkade[1], B. Giepmans[2], W. Voorhout[3], S. Kinder[4] & F. Braet[5]
[1]Wolfson Bioimaging Facility, University of Bristol, Bristol, UK; Department of Cell Biology, University of Groningen, University Medical Center Groningen, The Netherlands; [3]FEI Eindhoven, The Netherlands; [4]Carl Zeiss, Australia; [5]Anatomy & Histology – School of Medical Sciences / ACMM, University of Sydney, Australia
E-mail: p.verkade@bristol.ac.uk; b.n.g.giepmans@umcg.nl; wim.voorhout@fei.com; simon.kinder@zeiss.com; filip.braet@sydney.edu.au

KEY WORDS: Combined imaging, probes, cryo, sample preparation, labelling strategies, modelling.

The combined use of light microscopy (LM) and electron microscopy (EM) has become increasingly important to our understanding of the structure and function of tissues and cells at the molecular level [1]. Such a combination of two or more different microscopy techniques, preferably with different spatial- and temporal-resolution limits, is referred to as 'correlative light and electron microscopy (CLEM)' [2]. This tutorial will comprise of presentations from leading international and application experts in the rapidly evolving field of CLEM.

14:00 - 14:45 CLEM - Techniques: Paul Verkade will provide an overview of the various techniques and strategies that are currently available in the CLEM field. There are many ways to perform a CLEM experiment and a variety of microscope modalities (e.g. transmission or scanning on the EM side) can be combined, either on separate instruments or as completely integrated solutions. In general a CLEM experiment can be divided in 3 parts; probes, processing, and analysis. Each of these 3 sections will be discussed in this tutorial. Paul Verkade will focus on the processing part. He will discuss relative simple strategies using gridded coverslips [3] but also highlight technically more challenging technologies involving cryo fixation and further preparation [4]. In the last part he will touch on the analysis part, where the LM image has to be matched with the EM data, an often overlooked part of the whole experiment.

14:45 - 15:15 FEI CLEM Solutions: FEI has recently introduced a suit of correlative solutions to overcome the experimental and practical hurdles and make CLEM more efficient: CorrSight, a dedicated high-end fluorescence microscopy system offering CLEM-specific functionality and automation of important workflow steps including the following: -Live cell imaging of dynamic events in intact cells or organisms with environmental control, -Direct chemical fixation with automated microfluidics and -Vitrified sample imaging with automated liquid nitrogen supply; MAPS, a software tool bridging modalities by correlating any light microscopy image with SEM and SDB ultra-structural information; and lastly, iCorr, a light microscope module integrated into the Tecnai family of TEMs. This integrated approach gives users a faster and more accurate approach to CLEM. The iCorr designed to greatly speed up and automate CLEM experiments, resulting in LM image stitches covering a large area of the sample, and automated LM-TEM image overlays at defined positions of interest.

15:30 - 16:15 CLEM - Probes: Ben Giepmans will first focus on recent developed labeling strategies for molecules that allow CLEM [5]. These include particles (gold, quantum dots) to highlight endogenous proteins, but also genetically-encoded probes, as well as traditionally

used stains for light microscopy that aid in EM-analysis of samples. Probes that can only be detected in a single modality, and require image overlay, as well as combinatorial probes that can be visualized bot at LM and EM will be discussed. In addition, published [6] and new approaches for large scale EM to visualize macromolecules and organelles in the context of organized cell systems and tissues will be covered (www.nanotomy.nl). Matching the areas of acquisition in CLEM and EM will not only increase understanding of the molecules in the context, but also is a straight forward manner to combine the LM and EM image data. Covering a wide variety of probes and approaches for image overlay will help to enable (new) users to implement CLEM to better understand how molecules (mal)function in biology.

16:15 - 16:45 Zeiss Shuttle & Find for Life Sciences - Bridging the Micro and Nano Worlds: ZEISS has released Shuttle & Find the first easy-to-use, highly productive workflow from a LM to an SEM and vice versa, allowing researchers to bring together the micro and nano worlds. The solution works with upright, inverted and stereo LMs, including confocal and super resolution systems and all SEM and FESEM microscopes from ZEISS. Due to the open nature of the system researchers can use a variety of sample preparation techniques, to optimise the imaging of biological processes and structures. The system design allows multiple regions of interest to be imaged, stored and recalled on the SEM. With the acquired images from the LM and SEM, Shuttle & Find allows researchers to simply correlate and overlay images obtained with your LM and SEM after image acquisition. This is especially useful for cell biology, neurobiology, studies of host-parasite interaction and analysis of symbiotic relations. With simple operation and semi-automated calibration procedures the system greatly increases researchers time efficiency and decreases the turnaround time needed to acquire, process and output CLEM experiments.

References

[1] Su Y, et al. Multi-dimensional correlative imaging of subcellular events: combining the strengths of light and electron microscopy. Biophys Rev 2010:2;121-135.
[2] Jahn KA, et al. Correlative microscopy: providing new understanding in the biomedical and plant sciences. Micron 2012:43;565-582.
[3] Van Weering J, et al. Studying membrane traffic in high resolution. Methods in Cell Biology 2010, Volume 96: Electron Microscopy of Model Systems. Pp. 619-648.
[4] Brown E, et al. Capturing endocytic segregation events with HPF-CLEM. Methods in Cell Biology 2012, Volume 111: Correlative Light and Electron Microscopy, 175-201.
[5] Giepmans BN. Bridging fluorescence microscopy and electron microscopy. Histochem Cell Biol 2008:2;211; Sjollema KA, et al. Correlated light microscopy and electron microscopy. Methods Cell Biol 2012:111;157.
[6] Ravelli RB, et al. (2013) Destruction of tissue, cells and organelles in type 1 diabetic rats presented at macromolecular resolution. Sci Rep. 3:1804.