doi:10.1017/S1431927616012162
Microsc. Microanal. 22 (Suppl 5), 2016

Zooming in on Cells and Macromolecules with Correlative Light-Electron Electron Microscopy.

A. J. Koster

Molecular Cell Biology, Leiden University Medical Centre, Leiden, The Netherlands.

In correlative light and electron microscopy (CLEM) imaging modalities are combined to study cellular processes. Fluorescence light microscopy (FM) enables the imaging of dynamic events in relatively large fields of view exploiting a wide range of available fluorescent markers, while electron microscopy (EM) can reveal structural macromolecular arrangements in their cellular context in relatively narrow fields of view at nm-scale resolution [1].

EM specimens prepared by conventional methods can provide a wealth of information on the cellular architecture and processes. 3D morphology of cell systems and tissue can be shown in sections of material several hundred nm thick using electron tomography with transmission EM (TEM). 3D imaging of material 100's nm^3 in size can be obtained with serial block face scanning electron microscopy [2]. At the molecular level, the fidelity of interpretation of conventionally prepared specimens is limited because of effects related to the chemical fixation and staining. Imaging of cryo-immobilized frozen-hydrated specimens excludes the use of stain and the molecular resolution is preserved. Images of frozen hydrated specimens have an inherent low contrast and low signal to noise ratio because of their electron dose sensitivity and the lack of heavy atoms. Recent technological improvements of image detectors and contrast-enhancing phase plates for TEM have improved the contrast cryo EM datasets resulting in an increase of structural biology applications [3].

For applications of EM, FM can be instrumental for the selection of areas of interest. An overview of CLEM developments will be given and labelling methods [4] will be illustrated on biological systems, such as on fluorescently labelled bacteria [5], virus-induced replication structures, and blood-filtering structures in tissue [7]. In addition, applications of cryo-EM on molecular structures will be shown obtained at the Netherlands Center of Electron Nanoscopy (NeCEN) [8, 9].

References:

[1] H Patwardhan *et al*, Nat Struct Mol Biol **21** (2014), p. 841.
[2] MJ Mourik *et a*l, J Microscopy **259** (2015), p. 97.
[3] RM Glaeser, Nat Methods **13** (2016), p. 28.
[4] DM Van Elsland *et al*, Chem Sci **7** (2015), p. 752.
[5] K Celler *et al*, Nat Comm **7** (2016), p. 1.
[6] HM van der Schaar *et al*, mSphere **1** (2016), p. 104.
[7] MJ Mourik *et al*, Blood **125** (2015), p. 3509.
[8] CA Diebolder *et al*. Science **343** (2014), p. 1260.
[9] TH Sharp *et a*l, Cell Rep **15** (2015), p. 1.
[10] RI Koning *et al*, (2016) in press.

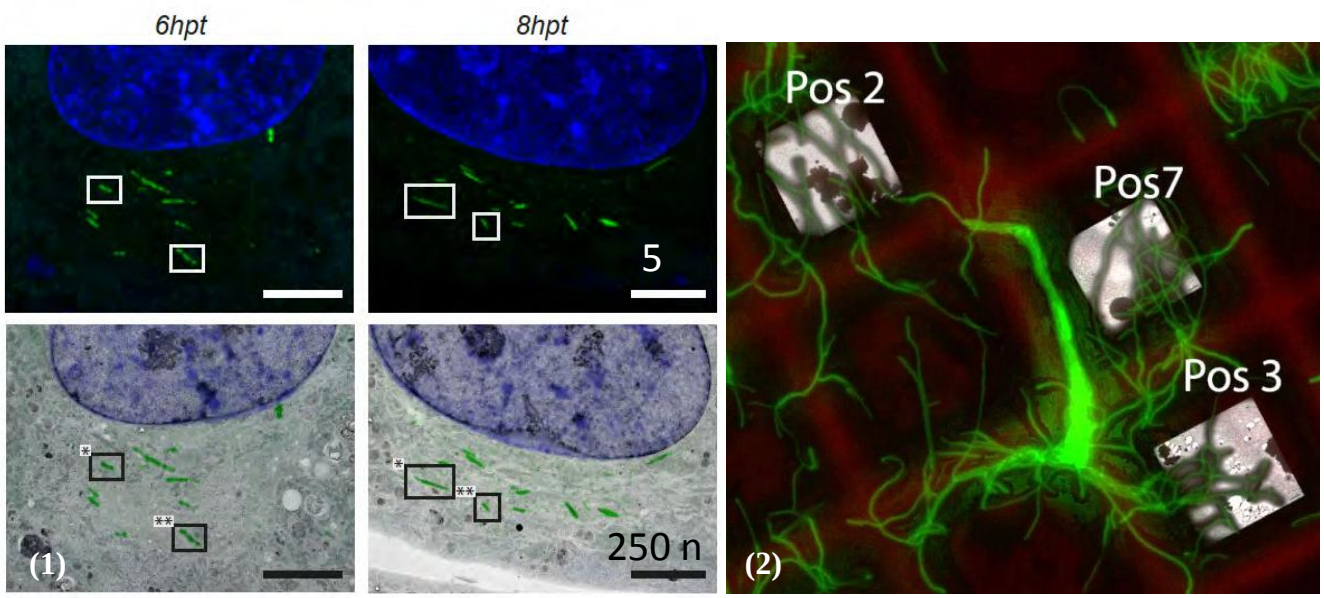

Figure 1. CLEM on stained biological structues material. Biogenesis of Weibel Palade Bodies (WPB). Fluorescence images of HUVECs showing forming WPBs 6 and 8 hours post-transfection (hpt) (green). Nuclei stained with DraQ5 (blue). Described in [7].

Figure 2. CLEM on frozen-hydrated structures to investigate membrane remodelling in Streptomyces bacteria [5].

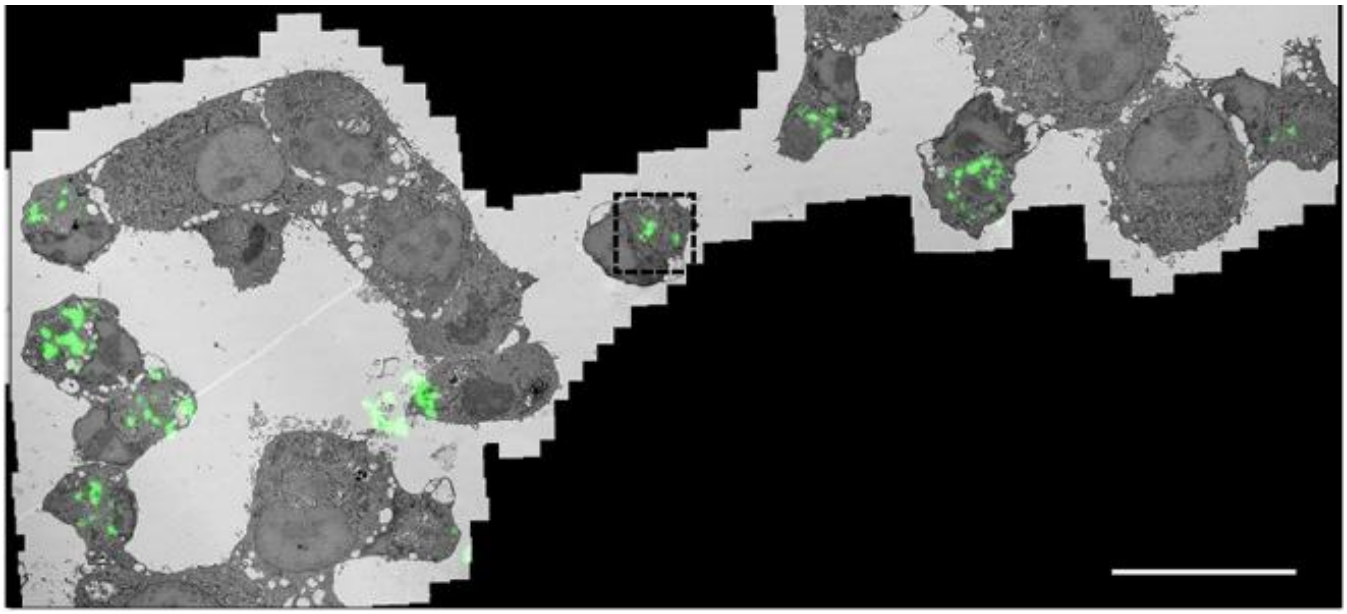

Figure 3. 3A(S11aa2) localizes to regions of interest that have tubule and double-membrane morphologies. BGM GFP(S1-10) cells were infected with CVB3-3A(S11aa2), stained with MitoTracker deep red FM, and monitored by live-cell imaging. Z-stacks of cells emitting GFP fluorescence were taken, and cells were processed for EM. CLEM overlays were made using MitoTracker deep red FM as an orientation guide, and 3A-GFP signal was aligned to the corresponding EM images. Described in [6].

https://doi.org/10.1017/S1431927616012162 Published online by Cambridge University Press