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Electron microscopy: imaging structures at the nanoscale for cell and structural biology. Applications to Vascular Diseases, Infections, and Immunology

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Electron microscopy: imaging structures at the nanoscale for cell and structural biology. Applications to Vascular Diseases, Infections and Immunology.

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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 that incorporate chemical fixation and metal staining steps can provide a wealth of information on the cellular architecture and processes. 3D morphology of cell systems and tissue can be unravelled in sections of material several hundred nm thick using electron tomography with transmission EM (TEM). 3D imaging of material 100's μm^3 in size can be obtained with serial block face scanning electron microscopy[2].

At the molecular level however, the fidelity of interpretation of conventionally prepared specimens is limited because of effects related to the fixation and staining. Imaging of cryo-immobilized frozen-hydrated specimens excludes the use of stain and as such 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 and signal-to-noise ratio of cryo EM datasets resulting in an increase of structural biology applications[3].

For applications of EM, FM can be instrumental as a tool for selection of areas of interest. An overview of CLEM developments will be given and workflows of different types of CLEM and labelling methods[4], will be illustrated by results obtained on a variety of biological systems, such as on fluorescently labelled bacteria[5], virus-induced replication structures[6] and blood-filtering structures in tissue[7]. In addition, applications of cryo-EM on molecular structures will be illustrated by results obtained at the Netherlands Center of Electron Nanoscopy (NeCEN)[8, 9, 10].

References

1. Patwardhan et al. (2014). A 3D cellular context for the macromolecular world *Nat Struct Mol Biol* 21(10):841-5.
2. Mourik et al. (2015). Towards the imaging of Weibel-Palade body biogenesis by Serial Block Face – SEM *J Microscopy* 259(2):97-104.
3. Glaeser (2016). How good can cryo-EM become? *Nat Methods* 13:28-32
4. Van Elsland et al. (2015). Detection of bioorthogonal groups by correlative light and electron microscopy allows imaging of degraded bacteria in phagocytes. *Chem Sci* 7:752-758.
5. Celler et al. (2016). Cross-membranes orchestrate compartmentalization and morphogenesis in Streptomyces. *Nat Comm*. 2016 Jun 13;7:ncomms11836. doi: 10.1038/ncomms11836.
6. Van der Schaar et al. (2016). Illuminating the Sites of Enterovirus Replication in Living Cells by Using a Split-GFP-Tagged Viral Protein *mSphere* Jul 8;1(4). doi: 10.1128/mSphere.00104-16.
7. Mourik et al. (2015). Content delivery to newly forming Weibel-Palade bodies is facilitated by multiple connections with the Golgi apparatus *Blood* 125(22):3509-16.
8. Diebolder et al. (2014). Complement is activated by IgG hexamers assembled at the cell surface *Science* 343(6176):1260-3.
9. Sharp et al. (2015). Heterogeneous MAC Initiator and Pore Structures in a Lipid Bilayer by Phase-Plate Cryo-electron Tomography. *Cell Rep* 15(1):1-8.
10. Koning et al. (2016). Asymmetric cryo-EM reconstruction of phage MS2 reveals genome structure in situ. *Nat Comm*. Aug 26;7:12524. doi: 10.1038/ncomms12524.