

ZOOMING IN ON CELLS AND MOLECULES WITH CORRELATIVE LIGHT AND ELECTRON MICROSCOPY

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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. EM specimens prepared by methods that incorporate chemical fixation and metal staining can provide a wealth of information on the cellular architecture and processes. 3D morphology of cells and tissue can be unravelled in sections of material several hundred nm thick using electron tomography (ET) with transmission EM. 3D imaging of pieces of tissue 100's μm^3 in size can be obtained with serial block face scanning EM (SBF-SEM) to resolve morphological details in the 2-10 nm range. At the molecular level, the fidelity of interpretation is limited because of effects related to the fixation, dehydration and staining. Imaging of cryo-immobilized frozen-hydrated specimens excludes the use of stain and as such the molecular resolution is preserved. The current generation of image detectors and contrast-enhancing phase plates can generate data sets of frozen-hydrated specimens suitable for structural biology studies resolving details in the 0.2 to 2 nm range.

We will present several of our multi-scale light and EM methods in the framework of biomedical applications. To understand the development of blood-filtering structures in kidney organoids, we have used a combination of live cell imaging with 3D , large scale 2D TEM imaging as well as SBF-SEM [1]. In our studies on intracellular virus-induced replication structures, we have used live cell imaging in combination with SBF-SEM to [2, 3]. To enable high precision localization of specifically labelled structures with CLEM, we have developed an approach to perform cryo LM on stained resin-embedded material [4] as well as for super resolution cryo light microscopy on vitrified cells [5]. For our studies focusing on molecular scales using cryo ET on lamella generated with focused ion beam milling, we have significantly improved the reliability of milling. [6]. Results will also be presented that are aimed to a better understanding of the activation steps of our innate immune system on a molecular scale using cryo EM combined cryo ET and sub-tomogram averaging [7, 8].

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