doi:10.1017/S1431927621007509
Microsc. Microanal. 27 (Suppl 1), 2021

Molecular views into cellular functions by in-cell cryo-electron tomography

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Most structural biology focuses on the structure and function of individual proteins and complexes thereof, but falls short of revealing how they come together to give rise to cellular functions. As a consequence, structural and cell biology have traditionally been separate disciplines and employed techniques that were well defined within the realm of either one or the other. Here, cryo-electron tomography (cryo-ET) provides a unique opportunity for obtaining structural information across a wide range of spatial scales - from whole cells to individual macromolecules [1].

We develop and employ advanced sample preparation, imaging and analysis techniques for in-cell cryo-ET. We will discuss our recent open source software developments for automation of cryo-focused ion beam thinning (FIB) guided by three-dimensional correlative cryo-confocal fluorescence microscopy. Preparations of site-specific 'electron-transparent windows' in suitable cellular model systems enable assignment of molecular structures directly from three-dimensional stills and reveal their molecular sociology [2]. We will describe the development of 2D and 3D convolutional neural networks for segmentation of cellular organelles and ultrastructure, and context-dependent localization of individual complexes for systematic interrogation of cellular tomograms. Using the genome-reduced human pathogen *Mycoplasma pneumoniae* as a model system, we further demonstrate the synergistic application of whole-cell crosslinking mass spectrometry and cryo-ET to generate integrative models that reveal transient and low abundance actively transcribing and translating super-complexes [3]. Ground breaking computational advances in data processing and structure determination now open the transforming possibility to image macromolecular complexes in their native cellular context at close to atomic resolution [4]. This technology forms the foundation for a new field in Life Science, often referred to as structural cell biology, which can quantitatively reveal how fine compositional and conformational states of macromolecular complexes are linked to biological functions [5].

References

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[5] JM acknowledges funding from EMBL and a European Research Council Starting Grant (3DCellPhase- 760067).