BIO Web of Conferences **129**, 12001 (2024) https://doi.org/10.1051/bioconf/202412912001
EMC 2024

Developing cryo-Volume Electron Microscopy using the JEOL 4700 cryo-FIBSEM to address biological ultrastructural questions

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The Focused Ion Beam Scanning Electron Microscope (FIBSEM) is well suited for site-specific sectioning where the FIB milling exposes a surface that can be imaged either with the FIB or SEM to examine the microstructure within each layer and the interfacial structure between layers. By collecting ultrastructural data from a large volume, to be interrogated in silico; novel understandings of the interconnectivity between many individual cell types can be deduced. These volume Electron Microscopy studies (vEM) overcome two principal limitations of EM: the loss of 3D understanding of ultrastructure and the possibility of "missing" rare ultrastructural events through the limited volume of tissue examined. However, to date, this has only been successfully applied to chemically processed tissue, with their associated artefacts. Non-chemical fixation using low temperature allows cells and tissues to be studied close to their native biological state and has been applied to cryo electron tomography (cryoET) of thin sections of cells and tissues. With cryo lamellae produced from vitrified material by controlled milling using focussed ion beam milling in a dual beam scanning electron microscope at cryogenic temperatures (cryoFIBSEM).

Here we develop cryo vEM (cvEM) to generate larger 3D volumes than possible with cryoET by a sequence of sectioning in a cryo FIBSEM at high resolution and close to the biological native state. By successfully overcoming the high beam sensitivity of vitrified material, lack of heavy metal contrast and significant challenges to the generation of sufficient signal to noise it has been possible to demonstrate the potential of cvEM as a complementary approach to cryo ET capable of generating larger volumes (e.g., an entire cell), and at the same time retaining resolution sufficient to clearly define ultrastructural details at the level of the membrane bilayer (Figure 1). We have incorporated novel scan approaches and evaluated in-painting techniques to manage charge and improve signal to noise (1,2). We also show the workflow can be successfully extended to multicellular volumes by increasing vitrification depth with high pressure freezing, the "waffle" method. By integrating a cryo correlative capability (cryoCLEM) into the workflow, we are able to target regions of interest for cvEM. Data detailing differences between two Euglenoids at the subcellular level has been generated. Clear differences between Eulgena longa and Euglena gracilis Paramylon storage granules and key organelle were observed.

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Future development aim to incorporate lamella lift out with cryo CLEM, cvEM to a predetermined region of interest with cryoET of recovered lamella. A complete and flexible full cryo FIBSEM workflow, the circle would be completed for cryogenic samples for complex biological studies.

Graphic:

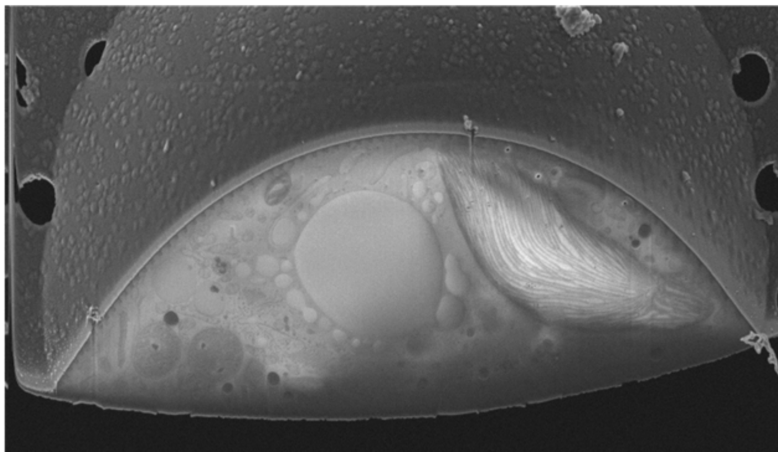

Figure 1. High resolution Image acquired by novel JEOL UHD Scan Interface of *Euglena gracilis* (CCAP 1224/5Z). The intercellular structure is clearly visible with well resolved membrane detail. The image has even contrast and charge is balanced across the entire vitrified sample.

Keywords:

cryo FIBSEM, vEM

Reference:

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