Practical approach towards the structural analysis of multicellular organisms

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Abstract

Cryo-electron tomography (cryo-ET) reveals the macromolecular and structural organization within cells in a close-to-life state. It is, however, limited to thin specimens, such as peripheral areas of intact eukaryotic cells. Analysis of tissue ultrastructure requires physical sectioning approaches prior to the application of cryo-ET. Here we developed and demonstrated the use of cryo focused ion beam (cryo-FIB) milling for cryo-ET imaging of vitrified, multi-cellular tissues. Combining a modified high-pressure freezing (HPF) procedure, which allows sample vitrification directly on EM grids, with the means for introducing fiducial gold markers on the surface of cryo-FIB milled lamellae, we were able to produce high quality tomograms of frozen *C. elegans* embryos and worms. The method described here offers a general solution for acquiring high-resolution tomograms on thick cells and tissues in different imaging setting. Cryo-ET can now be implemented to resolve macromolecular structures from tissues, thus bridging the gap between developmental and structural biology.