2314
doi:10.1017/S1431927617012235
Microsc. Microanal. 23 (Suppl 1), 2017

Electron Cryotomography of Vitreous Cryosections and Cryo-Focused Ion Beam Milled Lamellae.

Matthew T. Swulius[1,2], Shrawan Mageswaran[1], Grant J. Jensen[1,2]

[1.] California Institute of Technology, Department of Biology, Pasadena, CA. USA
[2.] Howard Hughes Medical Institute, Chevy Chase, MD. USA

Electron cryotomography (ECT) is the highest resolution technique available for the direct imaging of macromolecular structures within a cellular context [1]. While near-atomic resolution crystallographic methods and single particle cryoEM can provide images of individual cellular "puzzle pieces", ECT provides a picture of how these pieces come together inside of the cell. Additionally, there are many complex and dynamic structures that are only assembled within the cell, highlighting the importance of *in vivo* structural biology in the pursuit of a molecular understanding of cellular phenomena. For more than a decade virologists and microbiologists have witnessed the benefits of ECT, due to the small size of intact viruses and most bacteria (< one micron thick), which makes them ideal biological samples for such investigation. Indeed, the structural insight brought by ECT to these areas has been profound, and provides reason to expect that investigation of the vast cellular space remaining unexplored by ECT will provide unforeseeable knowledge about the inner workings of the cell.

Currently there are two techniques for thinning cryo-preserved biological samples that are too thick for investigation by ECT, which includes nearly all eukaryotic and many bacterial and archaeal cells. One method, cryosectioning, uses a cryo-ultramicrotome to cut thin sections of high-pressure frozen material, in an approach analogous to traditional sectioning of plastic-embedded material. The second method, cryo-focused ion beam (cryo-FIB) milling, uses a focused beam of gallium ions to ablate unwanted portions of the sample, leaving behind a thin portion of cryo-preserved material. In the past few years, cryo-FIB milling has received much attention, because it provides a "gentler" approach to cell thinning, but both techniques have their advantages as well as obstacles to be overcome when used in conjunction with ECT.

This report focuses on both the shared and specific obstacles that should be considered when setting out to collect and reconstruct tomographic tilt-series from either cryosections or cryo-FIB milled lamellae, and draws from a comparative overview between both techniques applied to the model organism *Schizosaccharomyces pombe*. The major considerations include 1) determining which method is most appropriate for the question at hand, 2) the challenges of data collection on samples thinned by both methods and 3) the reconstruction methods available for this data once it is in hand.

By comparing tomograms from similar cells thinned by each method, it was clear that cryo-FIB milling maintains detailed molecular and cellular features, as well as larger-scale morphology, by avoiding compression and crevassing artifacts known to occur during cryosectioning (Fig. 1). In particular, membrane continuity was disrupted in cryosections depending on their orientation with respect to the blade edge, and filamentous structures running parallel to the section, such as microtubules, are routinely broken into segments along their length. While studies using cryoFIB milling to address biological questions are relatively few in number [2-4], it is primarily limited by technological advancements needed to increase throughput and ensure successful targeting of specific objects of interest (a task proven possible by fluorescence guided milling [5]). Given such developments, there is good reason to believe that cryo-

FIB milling will continue to prove critical to cell biology as more cellular space is explored in its "frozen hydrated" state.

References:

[1] Oikonomou, C.M. in "Methods in Microbiology", eds. C. Harwood and G. Jensen, (Elsevier, Amsterdam), p. 115.
[2] Engel, B.D., Schaffer, M., Albert, S., et al., PNAS, **112** (2015), p. 11264.
[3] Engel, B.D., Schaffer, M., Cuellar, L.K., et al., eLIFE, **4** (2015), p. 3583.
[4] Hagen, C., Dent, K.C., Zeev-Ben-Mordehai, T., et al., Cell, **163** (2015), p. 1692.
[5] Arnold, J., Mahamid, J., Lucic, V., et al., Biophys J, **110** (2016), p. 860.

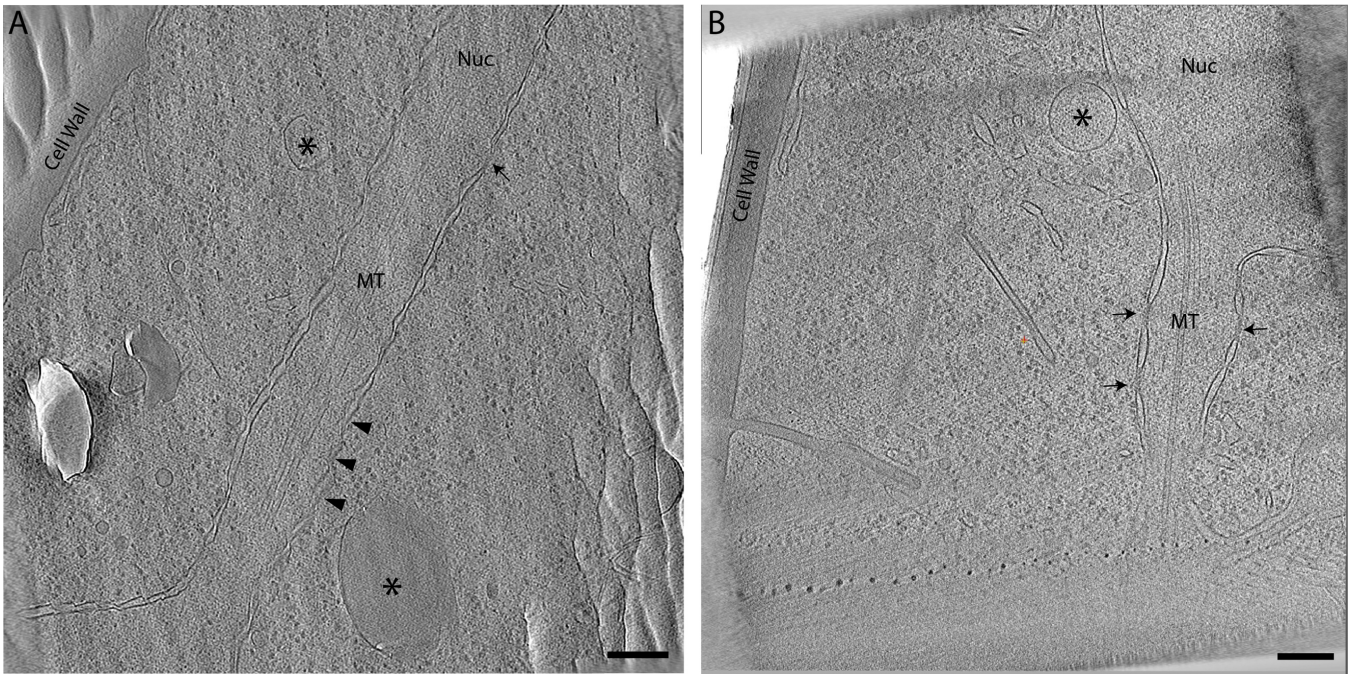

Figure 1 Comparison of tomograms from (A) cryosectioned and (B) cryo-FIB milled *S. pombe* cells. Both tomograms reveal a dividing nucleus (Nuc) with visible nuclear pores (arrows). Arrowheads point out the fractured membrane of the nuclear envelope in (A) and asterisks (*) label membraneous compartments that are elliptical in (A) due to compression and round in (B). Scale bars represent 200 nm.

https://doi.org/10.1017/S1431927617012235 Published online by Cambridge University Press