doi:10.1017/S1431927610058186
Microsc. Microanal. 16 (Suppl 2), 2010

A 360° Rotatable Cryo-FIB stage for Micromachining Frozen-Hydrated Specimens for Cryo-Electron Tomography

A. Rigort*, F. J. B. Bäuerlein*, T. Laugks*, M. Hayles**, C. Matthisen**, B. Lich**, R. Morrison***, A. Leis****, W. Baumeister* and J. M. Plitzko*

* Max-Planck-Institute of Biochemistry, Department of Molecular Structural Biology, Am Klopferspitz 18, D-82152 Martinsried, Germany
** FEI Company, 5656 GG Eindhoven, The Netherlands
*** Quorum Technologies, 2 Acorn House, The Broyle, Ringmer, East Sussex, BN8 5NN, UK
**** CSIRO Australian Animal Health Laboratory, 5 Portarlington Rd, East Geelong VIC 3219, Australia

Cryo-electron tomography (cryo-ET) is uniquely suited to visualize the supramolecular architecture of thin biological specimens in the frozen-hydrated state. While frozen-hydrated objects, such as organelles [1], prokaryotic cells [2] and peripheral regions or appendages of whole eukaryotic cells [3] can be examined directly by tomographic analysis, the vast majority of the eukaryote cell volume exceeds the maximum accessible specimen thickness, which is 0.5 to 1 µm. This is exacerbated in tomography applications, where the aspect ratio (and thus the apparent specimen thickness) changes considerably during specimen tilting. Cryo-ultramicrotomy is the most common way of dealing with this problem. However, frozen-hydrated sections suffer from compression that cannot be corrected with certainty, and furthermore, yields of sections that satisfy all of the conditions necessary for tomographic imaging are poor. An alternative approach that avoids mechanical deformations is the use of focused ion beam (FIB) instrumentation, where thinning of the frozen-hydrated specimen occurs through the process of sputtering with heavy ions, typically gallium.

In order to visualize macromolecular structures by means of cryo-ET, at a resolution that permits unambiguous identification within the cellular context, it is necessary to prefabricate suitably thin, frozen-hydrated specimens at cryogenic temperatures. In practise this can be realized by micromachining plunge frozen specimens directly on EM grids [4, 5]. However, due to heterogeneity in cell size and morphology, plunge freezing of eukaryotic cells can result in significant variations to local ice thickness. Irregular ice protrusions can obstruct the incident ion beam, especially at very shallow angles (10° or below) and can complicate the accurate ion beam targeting process. It is therefore necessary to approach the specimen from arbitrary directions to obtain extended thin areas at pre-selected sites and minimize adverse effects for tomography, e.g. shadowing due to substantial variations in thickness. By adding a rotational degree of freedom to the cryo-FIB stage, site-specific milling of target regions and selective removal of material that surrounds target structures is possible.

Here we introduce a new 360° rotatable cryo-FIB stage which is adapted to the stage of a Quanta 3D FIB (FEI, Eindhoven, The Netherlands) and a Polarprep 2000T cryo-system (Quorum, East Sussex, UK). The stage permits ion beam milling with respect to sample geometry whilst maintaining the vitreous state of the cells. Together with improved transfer devices [5] and optimized milling strategies, the 360° rotatable cryo-FIB stage facilitates the routine application of cryo-electron tomography to larger cellular structures.

References
[1] Nicastro et al., *J. Struct. Biol.* 129 (2000), 48–56.
[2] Kürner et al., *Science* 307 (2005), 436-8.
[3] Medalia et al., *Science* 298 (2002), 1209-13.
[4] Marko et al., *Nat. Methods* 4 (2006), 215.
[5] Rigort et al., *J. Struct. Biol.* in press.
[6] This research was supported by the European Commission's 7th Framework Programme (grant agreement HEALTH-F4-2008-201648/PROSPECTS) and from the Federal Ministry of Education and Research (BMBF).

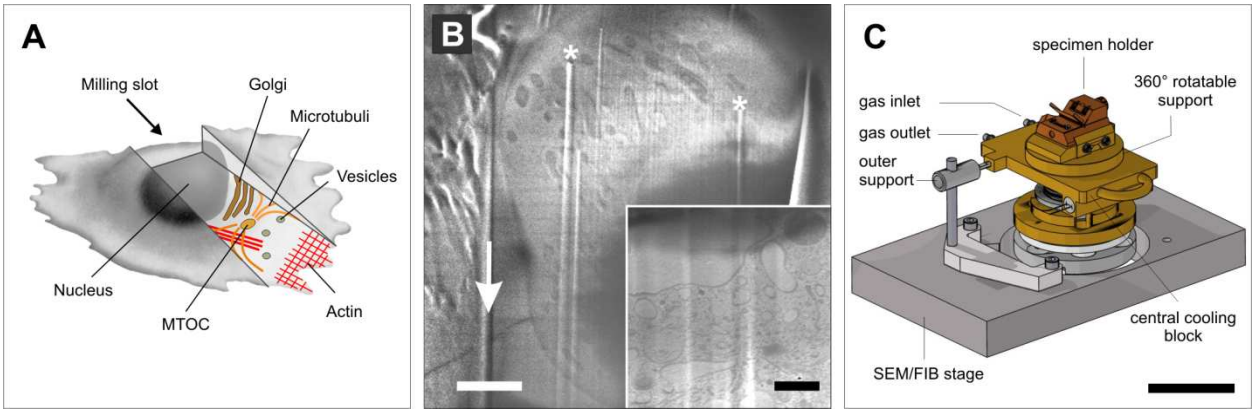

FIG. 1: Potential of cryo-FIB milling for prefabrication of thin, frozen hydrated specimens for cellular cryo-ET. (**A**) Cartoon illustrating the principle of wedge-shaped milling yielding a thinned region for subsequent cryo-ET across a frozen-hydrated eukaryotic cell. (**B**) Cryo-SEM micrograph exhibiting a cut-open view of the cell cytoplasm along a milled area (to the right of the white arrow) within the frozen specimen, revealing the cell membrane. The inset in (**B**) displays vesicular components of the cell's cytoplasm. (**C**) CAD drawing of the 360° rotatable cryo-FIB stage allowing site-specific milling of target regions. Scale bars: (**B**) 2 µm, Inset in (**B**) 500 nm, (**C**) 40 mm.

https://doi.org/10.1017/S1431927610058186 Published online by Cambridge University Press