doi:10.1017/S1431927616001768
Microsc. Microanal. 22 (Suppl 3), 2016

Fabrication of Frozen-Hydrated Sections by Focused Ion Beam(FIB) Method

Jianguo Zhang[1], Gang Ji[1], Xiaojun Huang[1], Shuoguo Li[1], Fei Sun[1], Wei Xu[1]

[1.] The Center for Biological Imaging (CBI), Institute of Biophysics, Chinese Academy of Science, 15 Datun Road, Beijing, China

Cryo-electron tomography is an important research method to study the cell ultrastructure in a near native state, especially in the structural biology and cell biological fields[1]. There are a few cellular specimens that bacterial and eukaryotic cells can be examined directly by cryo-TEM [2, 3], but eukaryotic and tissue are difficult to realize for the specimen thickness. The primary method to thin the frozen-hydrated cells and tissues is cryo-ultramicrotomy [4-6]. This method prepares the specimen by high pressure frozen, and then cutting the specimen by the cryo-ultramicrotomy, but there are some unavoidable problems and produced some artifacts.The specimen preparation is the limitation for the tomography technical development.
Here, we continue the development of the FIB method milling cryo-hydrated single cell specimen.First, we design three-quarters of the molybdenum grid with lacey support film that can keep the grid direction of FIB milling and data collection. Second, this grid is hard enough for the transfer process and has the less limitation of data collection TEM.

Experiments were performed using an FEI Helios NanoLab 600i Dual Beam SEM (FEI Corp., OR), with a field emission electron source and in-lens electron detectors, and employing ion columns with gallium ion sources. Low temperature experiments were carried out using a Quorum PP3000T cryo-transfer system (Quorum Technologies, East Sussex, UK). During the specimens preparation and transfer processes, all the experiments were performed under -180 ℃. For the processing of frozen-hydrated specimens on the grid, A new shutter was designed to accept the common size grid as show in Fig.1. A special designed molybdenum grid, with 1/4 grid missing, was used for the FIB milling and Cryo-EM imaging processes. The gap of the grid can ensure the uniform direction for FIB milling and tomographic tilt series in TEM. The cover in the shutter is kept close all the time, except FIB milling and grid transfer. The cover open and close processes were operated by the freeze-fracture knife in the preparation chamber in the PP3000T.

We study the small volume cell specimen of less than 10 μm by plunge-frozen method, the milled region imaged by SEM (Fig.2A). There are 3-5 milled regions per grid that is in the SEM image. The enlarge view of the milled region can recognize the cryo-hydrated section in SEM view (Fig. 2B) and the thickness of section could be measured approximately in the FIB view direction in the Fig. 2C. In the Fig. 2D and E the low dose projection image of the E. coli cell indicated the high quality vitreous state with less damage after FIB milling.
Another small size cell experiment is ascaris suum sperm cell milled by FIB, the low dose projection image of the cryo-hydrated section is show in the Fig. 2F. The section area is over 20×8 μm, in this region, and there is sufficient thin lamella for the common general accelerated voltage electronic penetration for the TEM investigation, and this large view is enough to explore internal structures in the biological specimen. Fig. 2G show extensive a part of cytoplasm structure of an ascaris suum sperm cell, while the clearly recognize the reflector body, vesicle and membrane organelle, et al. Fig. 2H is the enlarge view of reflector body structure.

For the development of cryo-ET method, the manly limitation is the preparation of the suitable thin specimen. Cryo-FIB method is a hopeful method to reduce the mechanical artifacts to preparing high quality cryo-hydrated section for tomographic study.

References:

[1] A. Rigort, E. Villa, F.J.B. Bäuerlein, B.D. Engel, J.M. Plitzko, 111 (2012) 259-281.
[2] M. Cyrklaff, A. Linaroudis, M. Boicu, P. Chlanda, W. Baumeister, PLoS One 2 (2007) e420.
[3] U.E. Maurer, B. Sodeik, K. Grunewald, Proc Natl Acad Sci U S A 105 (2008) 10559–10564.
[4] A.W. McDowall, J.-J. Chang, R. Freeman, J. Lepault, C.A. Walter, J. Dubochet, Journal of Microscopy 131 (1983) 1-9.
[5] A. Al-Amoudi, J.-J. Chang, .et.al., EMBO J. 23 (2004) 3583-3588.
[6] C.-E. Hsieh, A. Leith, C.A. Mannella, J. Frank, M. Marko, 153 (2006) 1-13.

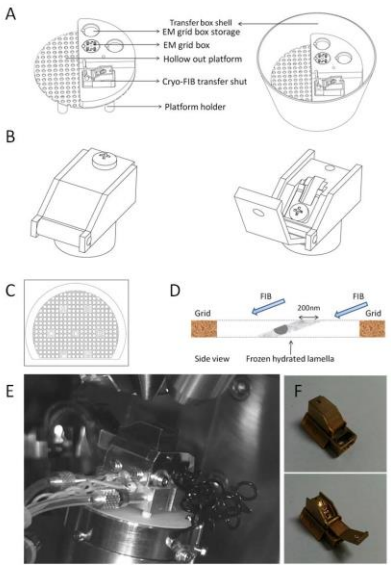

Fig. 1 Cryo-FIB transfer station and cryo-FIB shuttle.(A) Perspective drawing of the cryo-FIB transfer station with the cryo-FIB shuttle.(B) The cryo-FIB shuttle loaded with a specimen grid, with the specimen protection shutter open (left) or closed (right).The shutter cover (arrow) is at 30 relative to the base.A special designed molybdenum grid, with 1/4 grid missing. Schematics of Parallel FIB milling for producing vitrified cell lamella. An overview of the cryo-FIB shuttle mounted on an FEI Helios Nanolab 600i DualBeam FIB/SEM system with a Quorum PP3000T cryo-stage.

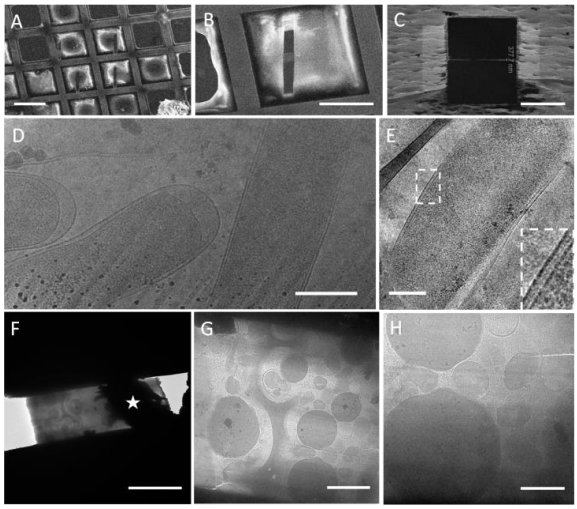

Fig. 2 Cryo-FIB milling of frozen-hydrated E. coli and Ascaris Suum sperm cell. (A) Cryo-scanning electron micrograph of vitrified E. coli cells on an EM grid after FIB milling. (B) Magnified view of the milled region. (C) Vertical view of the milled region from FIB direction. (D and E)Low-dose cryo-EM projection images from FIB-mill E. coli cells. (F) Cryo-lamella imaged at low magnification of Ascaris Suum sperm cell. (G) Medium magnification of (F) lamella. (H) High magnification of the (G) [Scale bars, (A)100μm, (B) 50 μm, (C) 10μm,(D) 500nm, (E) 300nm, (F)10μm, (G)2μm,(H)1μm.]

https://doi.org/10.1017/S1431927616001768 Published online by Cambridge University Press