Cryo-Planing of Frozen-Hydrated Samples by Triple Ion Gun Milling (CryoTIGM™)

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Cryo-SEM is particularly efficient at revealing the ultrastructure of biological systems in a near-to-native state and at nanometer resolution. Freeze fracture is conveniently used to prepare interior surfaces of frozen-hydrated samples, but the random nature of fracture does not ensure the passage of the fracture plane through the regions of interest. Cryo-planing by cryo-FIB allows positional control of structural investigation, and smaller structures like single cells can be precisely selected. However, in many situations it would be advantageous to be able to cryo-plane larger areas. To address these issues, we developed the cryo triple ion gun milling (CryoTIGM™) technique. High pressure-frozen samples were trimmed using a custom-built cryo-saw to expose a sample edge, which was brought into contact with a milling mask. Three broad Ar⁺ beams were aimed at the sample edge and removed materials above the mask to create a cross-section in the sample at the level of the mask. Cryo-planed samples were subsequently freeze-etched and coated with Pt to increase contrast. We optimized operating parameters for CryoTIGM™ for a range of samples, including yeast suspensions, mouse liver biopsies, and suspensions of whole sea urchin embryos. Irrespective of the sample type, we find that ion milling with Ar⁺ at an acceleration voltage of 3.0 kV, a current of 1.0 mA/gun, a base temperature of -120°C, and for 2 h results in very smooth cryo-planed area of ~700,000 µm². Analysis of cryo-planed surfaces after freeze-etching and coating indicates that CryoTIGM™ does not induce crystallization of vitreous ice in well-frozen samples. A direct comparison of samples prepared by conventional freeze fracture and CryoTIGM™ revealed that 1) surfaces prepared by CryoTIGM™ are much smoother; 2) cellular and organellar details are observed at comparable high resolutions with good contrast; 3) most importantly, additional ultrastructural features can be revealed.