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Cryoplunge™3 and Solarus® 950: a Perfect Duet for High Quality Frozen Hydrated Specimen Preparations for CryoEM

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CryoEM is a powerful tool in the arsenal of structural biologists and soft polymer chemists. It is used to elucidate the high resolution structure of radiation-sensitive specimens such as dispersed biological macromolecular assemblies, 2D crystals, nano-particles and colloids. By embedding the specimen within the thin layer of vitreous ice, the specimen is preserved in essentially its native solution state to near atomic resolution [1, 2].

Two key factors that are critical to obtaining high quality cryoEM data are a properly prepared support substrate and blotting the specimen aliquot to a thin fluid layer prior to freezing. The integrity of this fragile support film is important to the dispersal of the sample on the film, to the uniformity of ice thickness, and to the cleanliness of the resultant ice layer. Blotting the specimen aliquot to a thin film just prior to freezing can be accomplished manually or in an automated fashion; the latter providing more consistently reproducible results. Throughout this procedure, it is important to minimize all sources of contamination that may affect the quality of the preparation [3].

We demonstrate improvements to the experimental protocol by using the Solarus® 950 to prepare the specimen support substrate and the Cryoplunge™3 to prepare virtually contamination free frozen hydrated specimens. The Solarus® 950 uses a hydrogen and oxygen gas mixture providing uniform cleaning of the specimen support, with less sputter damage than other commonly used gas mixtures such as argon or argon/oxygen, resulting in a uniform distribution of the specimen when it is applied to the support film. Cryoplunge™3 is designed to minimize contamination during the plunge freezing process. The blotters are designed to prevent cross contamination. A filter paper loading jig eliminates the need to touch the blotting filter paper with ones hands. The cryogenic workstation is designed to minimize condensation of atmospheric oxygen and water vapor onto the surface of the ethane as well as provide a protective cryo interface for transferring the frozen hydrated specimen grid. A transfer pot allows the frozen hydrated grids to be removed from the workstation while fully submerged in liquid nitrogen. The ethane used to freeze the specimen can be maintained at a temperature just above its melting point, eliminating the need for using metal tools to thaw frozen ethane; another source of contamination.

References

[1] Dubochet, J., et.al. (1988). Cryo-electron microscopy of vitrified specimens. Q Rev Biophys 21, 129-228.
[2] Glaeser, R., et.al. (2007) Electron Crystallography of Biological Macromolecules. Oxford University Press. 150-166.
[3] Steinbrecht, RA., et.al. (1987) Cryotechniques in biological electron microscopy. Berlin: Springer-Verlag. 47-54.

Figure 1: Minimizing contamination during specimen preparation: (a) loading the filter paper disk and protective backer using the loading jig, (b) attaching the filter paper and protective backer onto a blotter, (c) transferring the frozen hydrated grid within the cryogenic atmosphere of the liquid nitrogen workstation, (d, e) frozen hydrated grids within the transfer pot are submerged in liquid nitrogen as they are removed to a storage dewar.

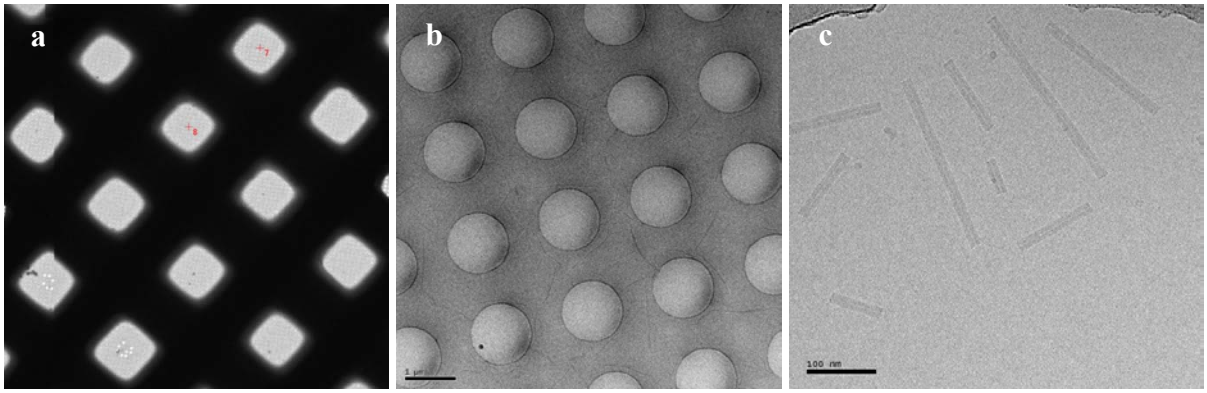

Figure 2: The three images above are an example of the high quality frozen hydrated preparations produced using Solarus® 950 and Cryoplunge™3: (a) Image of an area of several grid squares showing the clean and uniform vitrified ice layer; TEM magnification 140X, ~ 0.01 $e^-/Å^2$. (b) Higher magnification image of a portion of one grid square showing a virtually contamination free vitrified ice layer; 4700X, 0.1 $e^-/Å^2$. (c) Image near edge of one hole showing tobacco mosaic virus particles embedded within the vitrified ice layer; 59KX, 20 $e^-/Å^2$. Specimens were prepared on Quantifoil® R1.2/1.3 macro machined holey carbon grids and plasma cleaned with the Gatan Solarus® 950 for 15 seconds at 50 Watts using the hydrogen/oxygen gas mixture. All images were recorded on an FEI Tecnai F30 TEM with a Gatan 626 70° single tilt liquid nitrogen cryo transfer holder and a Gatan Ultrascan® 4000. Images courtesy of Dr. Chen Xu, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA.