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Title: "High Pressure Freezing: Recent Developments and Applications"

High pressure freezing (HPF) is one of the most important specimen preparation advances for biological electron microscopy since the development of glutaraldehyde fixation (Sabatini et al., 1963). HPF provides superior cellular preservation for morphological studies and better antigen retention for immunolabeling work than most conventional, room temperature methods. One powerful advantage of fast freezing is that it is extremely fast compared to conventional chemical fixation methods. Cells are immobilized in milliseconds instead of the seconds to minutes it takes fixatives like glutaraldehyde to diffuse into cells. Fast freezing is also non-selective, i.e., all chemical species are arrested equally whereas chemical fixative cross-linking is selective.

Despite obvious superiority over conventional methods, HPF has not emerged as a routine method for EM specimen preparation. Although the relatively high cost of the machines has had something to do with that, it is also because there have been few applications that absolutely required HPF. Now that is beginning to change as EM studies contribute to the effort to characterize cellular substructure down to the atomic level of detail (Sali et al., 2003).

What are the applications and uses for HPF? First and foremost, it is a technique for preserving high resolution ultrastructural information in biological samples. This makes it ideal, if not essential, for sectioning and tomography of frozen hydrated material (Hsieh et al., 2002; Al-Amoudi et al., 2004) as well as resin-embedded material (McIntosh, 2001; McEwen and Marko, 2001). But it is also emerging as an essential fixation technique for EM studies of the important model organisms such as *Drosophila, C. elegans, E. coli, S. cerevisiae, and Arabidopsis* among others. These are organisms that are difficult to fix well by conventional methods and HPF facilitates accurate phenotype characterization as well as EM immunolocalization studies. In this presentation we will cover these applications of HPF as well as consider the types of high pressure freezing machines, how they work, and how to optimize their use. We will discuss artefacts of HPF and some of the processing problems that are encountered following HPF.

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