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# Self-Pressurized Rapid Freezing (SPRF) using a U-shaped specimen carrier and dual phase freezing.

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Physical fixation of biological specimens is the basic principle underlying a range of specimen preparation methods in electron microscopy aimed at preserving ultrastructure, molecular functionality and recognition, as well as electrolyte distribution. Several methods have been developed involving freezing in the presence of a cryoprotectant, using high heat extraction rates, and under hyperbaric pressure.

High-pressure freezing (HPF) is a technique that allows for a depth of 100-300μm from the specimen surface to be frozen without detectable ice crystallization. At a pressure of ~210 MPa high cooling rates are no longer a prime requirement. HPF has become an accepted technique for the structural preservation of pro- and eukaryotic cells, multicellular organisms and tissues, allowing further processing with cryosubstitution, freeze fracturing and cryoultramicrotomy (CEMOVIS). High-pressure freezing is based on the principle of Le Chatelier and Braun which postulates that if a system at thermodynamic equilibrium experiences a change in one of the physical parameters involved, then the equilibrium will shift in order to minimize that change. In high-pressure freezing this principle explains how an externally applied pressure of ~ 210 MPa prevents water from expanding into low-density ice upon cooling.

Recently we introduced the self-pressurized rapid freezing (SPRF) method based on the same principle of Le Chatelier and Braun (Leunissen & Yi, 2009). SPRF employs plunge freezing of specimens in a sealed capillary tube into a cryogen such as liquid propane or liquid nitrogen. It uses the tendency for water to expand upon cooling thereby generating pressure intrinsically inside the specimen container instead of using an external hydraulic system. The ambient and low-pressure polymorphs of ice as well as supercooled water have a lower density than water at 0°C. Therefore, upon cooling a specimen that is enclosed in a confined space below 0°C pressure will be generated. In practice, pressures comparable to those applied in HPF can be generated in this way. The ultrastructural preservation of unprotected specimens is comparable to that achieved with high-pressure freezing (HPF). Because the pressure is generated inside the specimen holders as a result of the cooling rather than applied from an external source as in HPF, the technique has been baptized Self-Pressurized Rapid Freezing (SPRF).

In straight tubes hexagonal ice is formed on the inner surface of the tube wall, extending towards the centre of the tube. Consequentially, specimens near the tube wall suffer freezing damage. The combined use of a U-shaped capillary specimen carrier (U-tube) and a controlled dual phase plunging procedure allows a spatial separation of the pressure-generating low-density ice formation zone in the U-tube legs from the high-pressure freezing zone in the U-tube arc where the specimen is positioned. This approach, facilitated by a prototype instrument, significantly improves the yield of well preserved specimens.

The tutorial will present the principles of SPRF using both straight tubes and U-tubes.

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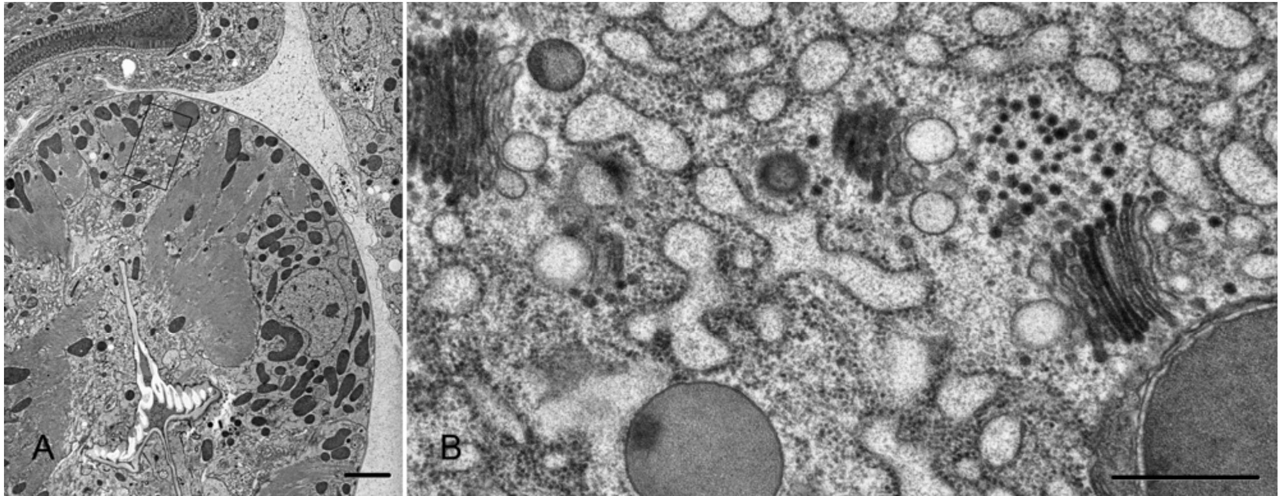


Examples of cryosubstituted C. elegans prepared by SPRF plunging into liquid propane at −180◦C. The specimens were frozen in a pure water environment and no cryoprotectants were added. Panel B is a higher magnification of the boxed-in area in panel A. Scale bars: A = 2 μm, B = 0.5 μm.

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