

A Quick Note on Freeze-Fixing Tissue in Liquid Nitrogen

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There are two possibilities for plunge freezing in LN₂: one is "normal" liquid nitrogen -- as it comes from the dewar, the other is slush nitrogen.

If using liquid nitrogen without slush, then something like isopentane, ethane, or propane (ordinary cooking propane will do) must be used. The caveat is that using these liquid gases is a serious fire and explosion hazard, especially since liquid oxygen forms at liquid nitrogen temperatures, and dissolves into the liquid hydrocarbon. These gases can be used safely (I have done so) but they take care and understanding of what's happening, and a safe place to dispose of the cryogen. Keep in mind that the isopentane, etc., is enriched in dissolved oxygen and as it is heavier than air, it likes to move along floors, etc. Also, liquid isopentane, ethane and so on are reasonably effective explosives.

A simpler and much safer way is to use slush nitrogen. Slush nitrogen is produced by placing beaker (or whatever) full of liquid nitrogen in a vacuum chamber and then pulling a one atmosphere vacuum with a good, meaning high-capacity, rotary vacuum pump or the like. Pump on the liquid nitrogen for 10 or 30 minutes until it starts to form a slush. This slush can be used for plunge freezing without isopentane or other agents.

Plain liquid nitrogen can't be used for plunge freezing because of the Leidenfrost effect. What happens when water is dropped on a very hot skillet: Instead of immediately boiling,

the water drop survives for a while, skittering around on the skillet. The heat of the skillet flash-evaporates a layer of water vapor, which then insulates the drop and keeps it from boiling. The same thing happens when plunging tissue into liquid nitrogen. The relatively hot tissue flash-evaporates some liquid nitrogen, creating an insulating layer of nitrogen gas around the specimen. This both slows down the rate of freezing and creates a longer temperature gradient over which heat must leave the specimen. This gives more time for ice crystals to form and grow, creating more ice damage. Isopentane, ethane, etc., do not exhibit this effect.

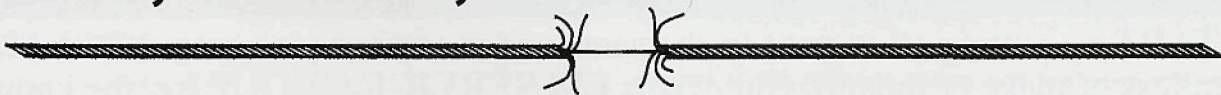
It is possible to minimize or avoid the Leidenfrost effect with liquid nitrogen by rapidly moving the specimen in the liquid nitrogen until the temperature is equilibrated. This requires enough room to move the specimen, and presents the possibility of spilling or splashing the liquid nitrogen.

Then there are the slam-freezing methods, where the tissue is quick frozen by slamming it (literally) onto a polished metal block held at liquid nitrogen temperature. This is rapid and avoids the Leidenfrost effect, but there is the obvious potential for tissue damage. The slamming is done with some force, and is not a gentle act, but it can produce good results.

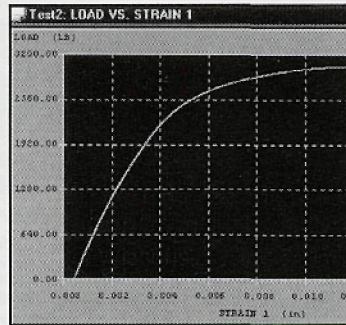
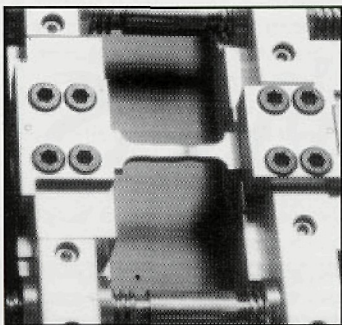
Having said all that, plunging into liquid nitrogen would give better freezing than just sticking a specimen to a piece of metal and setting it in a cryostat. Holding the metal at liquid nitrogen temperatures before sticking on the tissue also avoids the Leidenfrost effect, but the freezing rate is not good, and well frozen tissue is only found very close to the metal surface.

It must be added that freezing-rate requirements for light microscopy are much less stringent than they are for electron microscopy, and freezing methods that are unacceptable for EM

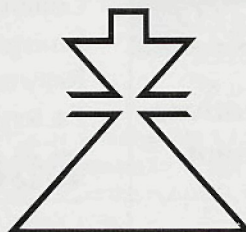
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work fine for LM. Even here, though, there is an issue: intracellular ice crystal formation or dehydration* may change the location of macromolecules within a cell or its subcellular compartments. So correct localization of molecules may require the most stringent freezing conditions (as for EM), even though the study is being done at the light microscopic level, and the morphology looks fine and there is no visible damage. ■

*Much of the morphological change in freeze-damaged tissues is not due so much to ice-crystals as to dehydration. As water freezes, it excludes other molecules and atoms, and "pulls in" water molecules from other areas. The loss of these water molecules dehydrates the area from which they migrated, so causing morphological changes.)

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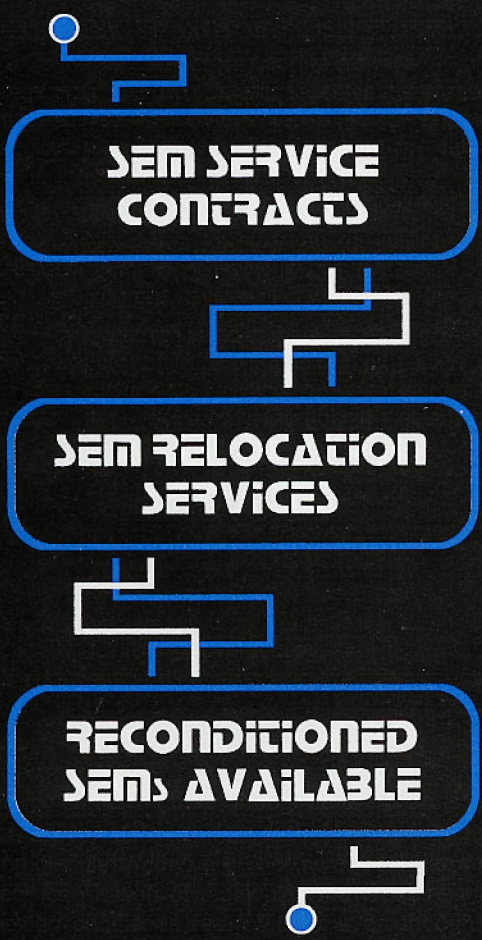
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