

FS-8500 Provides Superior Freeze Substitution Preparation

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Through the years, the process of freeze substitution has progressed through the introduction of better reagents, protocols, and especially new equipment. In keeping with this progress, RMC has introduced its latest automated freeze substitution instrument.

Freeze substitution allows the processing of bacteria, cells, organelles, and tissue samples from a cryo-stabilized state. Generally, the result of this process is a tissue embedded in plastic at room temperature that is easily cut on an ultramicrotome. This procedure allows better ultrastructural preservation, antigenicity and temporal information, while still providing a familiar representation of cell and organelle structure. The sample is quickly stabilized, frozen in less than a second. Standard room temperature fixation takes from several minutes to several hours. Masking of short term processes like muscle cell contraction and firing of nematocysts, as well as substantial reformation of the cell can take place, creating an artifact ridden image. Freeze substitution is free from most of these effects.

The specimen is frozen by any number of methods, such as high pressure freezing, which will minimize ice crystal formation. The solid sample is immersed in a solvent, often combined with a fixative. Dry acetone or methanol typically are the preferred solvents. Freeze substitution is usually done at temperatures around -90°C [1]. Substitution times, temperatures, and ramp times have an effect on sample quality. There are a vast number of substitution time-temperature regimens in the literature, extending from as short as 90 minutes to beyond 5 days [2]. The choice of embedding media is another factor to be considered, depending on the anticipated final use of the block. All of these parameters offer the researcher a puzzling number of options, both which to use as well as what equipment might be available to accomplish the chosen protocol.

In the past, many labs used dry ice, a Styrofoam mailing container, a metal block of a particular mass, drilled with multiple holes for the processing vials [3]. In the more advanced laboratories, a temperature logging thermometer was used to record the sample conditions during the substitution procedure. This resulted in well described procedures, producing good results, working well in one lab, but difficult to reproduce at other facilities [1]. The use of ultra low temperature freezers has been tried, but proved difficult to control and work in. The contamination of this equipment was also a problem, given the toxicities of the fixative and the plastic monomers used for low temperature embedding. The automated freeze substitution instruments introduced by several companies successfully eliminated the variables associated the equipment mentioned above. In addition, these instruments reduced labor, increased safety, and generally made freeze substitution a viable process, applied to a wide variety of samples.

The new FS-8500 features sample agitation, which reduces the time necessary to completely substitute

even the larger high pressure frozen samples. The LN₂ cooling system is new and uses an active pumping system for the first time. LN₂ is delivered to the cooling area only as needed. This greatly reduces the LN₂ consumption and allows long duration substitution runs, up to 7 days without refilling, regardless of the temperature settings of the substitution chamber. Longer substitution runs can be accommodated by an upgraded filling system which does not cause temperature variation in the samples during the filling procedure. Progressive Lowering of Temperature (PLT) procedures can be done with this unit without the excessive LN₂ use previously experienced. The addition of a specimen temperature sensor allows monitoring the actual sample conditions throughout the run. The FS-8500 is controlled by a laptop computer which allows great flexibility and ease in creating protocols and also allows accurate logging of the actual conditions of a procedure including both chamber and sample temperatures. Alarms can be set to notify a user of an impending solution change. Included with the FS-8500 is a stereo microscope work station, augmented with high intensity LED's, to ease sample manipulation. Interchangeable sample disks accommodate a variety of readily available sample containers for individual preferences and protocols. All these features make the FS-8500 a user friendly instrument able to process multiple specimens in a rapid, reproducible, and documented manner.

References:

- [1] K McDonald, D Struder, and P Walther, *Microscopy & Microanalysis* (2007), pp. 442.
- [2] K McDonald, and R Webber, *Journal of Microscopy* **243** (2011), p. 227.
- [3] T Giddings, *Journal of Microscopy* **212** (2003), pp. 53-61.

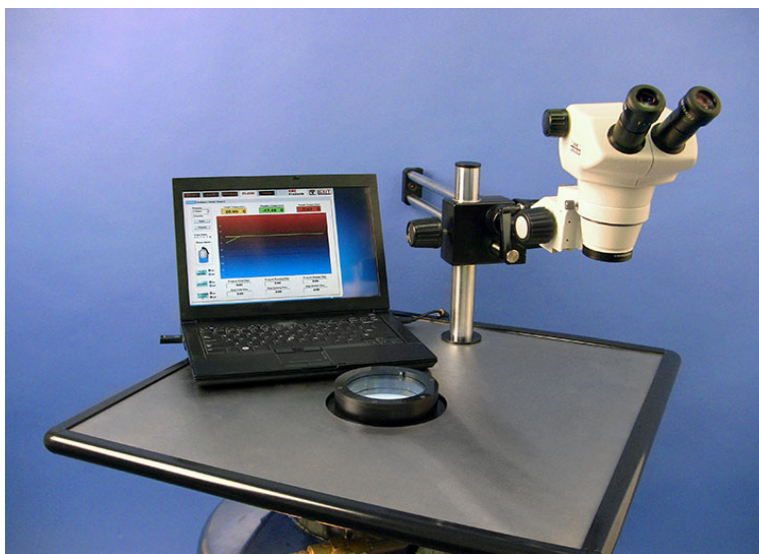


Fig. 1 New FS-8500 Freeze Substitution system showing work station and laptop controller.