

Chapter 7 Freeze-Substitution and Freeze-Drying

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1 Introduction

Freeze-substitution (FS) and freeze-drying (FD) are dehydration techniques by which the water is gently removed from a frozen specimen. Both techniques can serve as a link between cryofixation and conventional thin sectioning at room temperature (Fig. 1). They are, therefore, hybrid techniques combining the advantages of the low temperature and the room temperature specimen preparation. With respect to the danger of artefacts, these procedures are much more obscure than “pure” cryotechniques, such as freeze-etching or cryosectioning. Both, FS and FD, are known from light microscopy and have been used in electron microscopy since its early days (for refs. of the older literature see Bullivant 1970; Rebhun 1972; Robards and Sleytr 1985), but only during the last dozen years a breakthrough can be noticed, which is mainly due to improved cryofixation. As for any other cryotechnique in biological electron microscopy, for successful FS and FD the main prerequisite is also good cryofixation with as little freezing damage as possible (see Bachmann and Mayer, Chap. 1; Sitte et al., Chap. 4; Dubochet et al., Chap. 5; Moor, Chap. 8; this Vol.).

Freeze-substitution dissolves the ice in a frozen specimen by an organic solvent, which usually also contains chemical fixatives. It has to be carried out at a temperature low enough to avoid secondary ice-crystal growth. After completion of FS, the temperature can be raised without this risk, since water is now absent. Except for low temperature embedding, infiltration with the embedding resin is done at room temperature followed by heat polymerization. The result is a plastic-embedded specimen which can be sectioned and stained in the routine way.

Freeze-drying eliminates the frozen water by sublimation in a vacuum chamber. Again, the temperature must be sufficiently low to avoid secondary freezing damage. When the specimen is dry, it may be warmed to room temperature for plastic embedding and ultramicrotomy as above.

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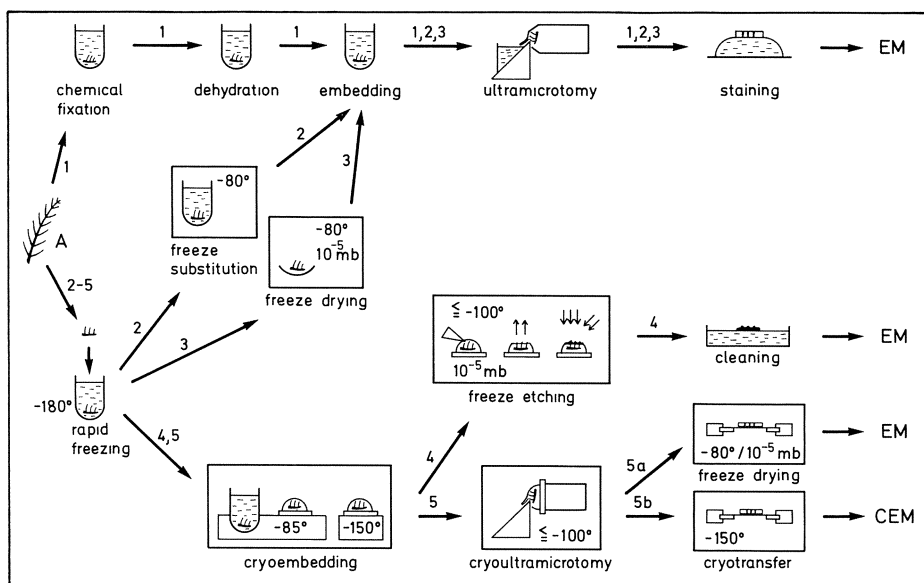


Fig. 1. Flow chart comparing the principal steps of conventional specimen preparation (1) and cryofixation with its various follow-up procedures (2–5). Rapid freezing is demonstrated by immersion of a small insect antenna (A) into liquid propane (for other techniques see Sitte et al., Chap. 4, this Vol.). Freeze-substitution (2) and freeze-drying (3) link cryofixation with embedding and sectioning at room temperature. Cryoembedding (cryoglueing) with butyl benzene and heptane renders small specimens amenable to freeze-etching (4) and cryoultramicrotomy (5) respectively. An electron microscope with a cold stage (CEM) is essential for the study of frozen-hydrated cryosections (5b). Temperatures other than room temperature are given in centigrades, pressure in millibar (Steinbrecht 1984)

Plastic infiltration and thin sectioning are, however, not the only techniques to combine with FS or FD. If the dry specimens are thin enough, they may be directly viewed in the transmission electron microscope after metal shadowing (see Gross, Chap. 10, this Vol.). Freeze-dried bulk specimens can be observed in the scanning electron microscope after metal coating (Sect. 3.4; see also Marshall, Chap. 13, this Vol.). Freeze-drying of ultrathin cryosections is important for microanalysis to increase contrast and stability (see Zierold, Chap. 6, this Vol.).

This chapter provides an overview of the theoretical and experimental data on the methodology of FS and FD, it then describes the most generally used procedures, and closes with a critical evaluation of the present status with selected examples of applications.

2 Methodology: Theoretical and Experimental Data

2.1 Water in the Cell and Its Removal

Both, FS and FD, are essentially dehydration processes. Our current problems in understanding these processes are mainly due to our incomplete knowledge of