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Short Note

A New Device for Preparing Complementary Replicas[1]

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Summary The construction and method of use of a new device for preparing complementary replicas are described. The device is of a simple construction and provides highly satisfactory and reproducible results.

Introduction

Since the introduction of the method by Steere (6) and Moor *et al* (4), freeze-fracturing has been extensively employed for examining the details of cell and membrane organizations. It is now generally believed that the process of freeze-fracturing reveals internal hydrophobic planes of the membrane (1, 2), and exposes two membrane fracture faces, PF and EF-faces according to the convention by Branton *et al* (3). The conventional freeze-fracture preparation allows visualization of only one half of the fracture faces, but examination of the complementary faces revealed by the complementary replica method (7) is necessary for a correct understanding of the freeze-fractured ultrastructures.

The present short technical note describes a new device for preparing complementary replicas which can be installed in a JEE-AFE freeze-etching apparatus which is currently used in our laboratory. The device described here is of a simple construction and provides highly satisfactory and reproducible results.

Method

Device Figure 1 shows a unit constructed for preparing the complementary replicas. Figure 1-a shows a block-holder for fixing a fracture-block to a specimen stage of the freeze-etching apparatus (JEE-AFE). Figure 1-b shows a fracture-block in closed state and Fig. 1-e shows the same block in open state. The upper and lower block are joined with a hinge. Holes for holding specimens are bored through the upper block to the lower block. There are three holes and three specimens can be fractured at the same time. Figure 1-c shows a pair of specimen holders consisting of two

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fine tubes with holes in semi-circular form. Figure 1-d shows the cap for securing the specimen holder to the fracture-block. Except for a part of the block-holder which is made of teflon (* in Fig. 1-a), all parts of the unit are made from brass.

Procedure [1] A pair of fine tubes of the specimen holders is joined as shown in Fig. 1-c, and the holes of the tubes are loaded with specimens. They are frozen by the appropriate cooling rate (generally very rapidly), depending upon the experimental purpose, and stored in a liquid nitrogen bath. [2] The fracture-block is cooled down to $-196°C$ by immersing it in liquid nitrogen. The specimen holders are inserted into the holes of the closed block and covered with caps in liquid nitrogen. [3] The loaded block (b in Fig. 2) is immediately transferred onto the block-holder (h in Fig. 2) which was previously installed on the specimen stage (s in Fig. 2) of the freeze-etching apparatus and a copper-constantan thermocouple (t in Fig. 2) is fixed to the lower block for measuring the temperature of the block itself. [4] After instalment of the fracture-block to the freeze-etching apparatus, the chamber is evacuated to approximately 1×10^{-5} torr. During evacuation, the liquid nitrogen tank (l in Fig. 2) is cooled for preventing the contamination of the specimens. The temperature of the fracture-block is gradually raised at a rate of approximately 10°C/min. The size of both the upper and lower block is designed in such a way as to attain the same rewarming rate. [5] When the temperature of the fracture-block reached $-100°C$, the upper block is forced open by the knife-arm (a in Fig. 2) to an open state, and as a result the specimens are fractured. [6] The fracture surfaces are replicated by platinum-carbon immediately after fracturing or after etching for a few minutes. Then, the replicas are prepared followed by routine method (5). Since the form of replicas is semi-circular, it is quite easy to match the complementary portions.

Figures 3 and 4 show some results obtained using this device and the specimens show good complementation. It is expected that this device will be applicable for preparing the complementary replicas and will be give a correct understanding of the freeze-fractured ultrastructures.

Acknowledgments

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Literature Cited

1) Branton, D. 1966 Fracture faces of frozen membranes. *Proc. Nat. Acad. Sci. U.S.A.*, 55: 1048–1056.
2) Branton, D. 1969 Membrane structure. *Ann. Rev. Plant Physiol.*, 20: 209–238.
3) Branton, D., Bullivant, S., Karnovsky, M. J., Moor, H., Mühlethaler, K., Northcote, D. H., Packer, L., Satir, B., Satir, P., Speth, V., Staehlin, L. A., Steere, R. L. and Weinstein, R. S. 1975 Freeze-etching nomenclature. *Science*, 190: 54–56.
4) Moor, H., Mühlethaler, K., Waldner, H. and Frey-Wyssling, A. 1961 A new freezing ultramicrotome. *J. Biophys. Biochem. Cytol.*, 10: 1–13.
5) Shimada, K. 1977 Effects of cryoprotective additives on intracellular ice formation and survival in very rapidly cooled HeLa cells. *Contr. Inst. Low Temp. Sci.* B 19: 49–69.
6) Steere, R. L. 1957 Electron microscopy of structural detail in frozen biological specimens. *J. Biophys. Biochem. Cytol.*, 3: 45–60.
7) Wehrli, E., Mühlethaler, K. and Moor, H. 1970 *Exp. Cell Res.*, 59: 336–339.

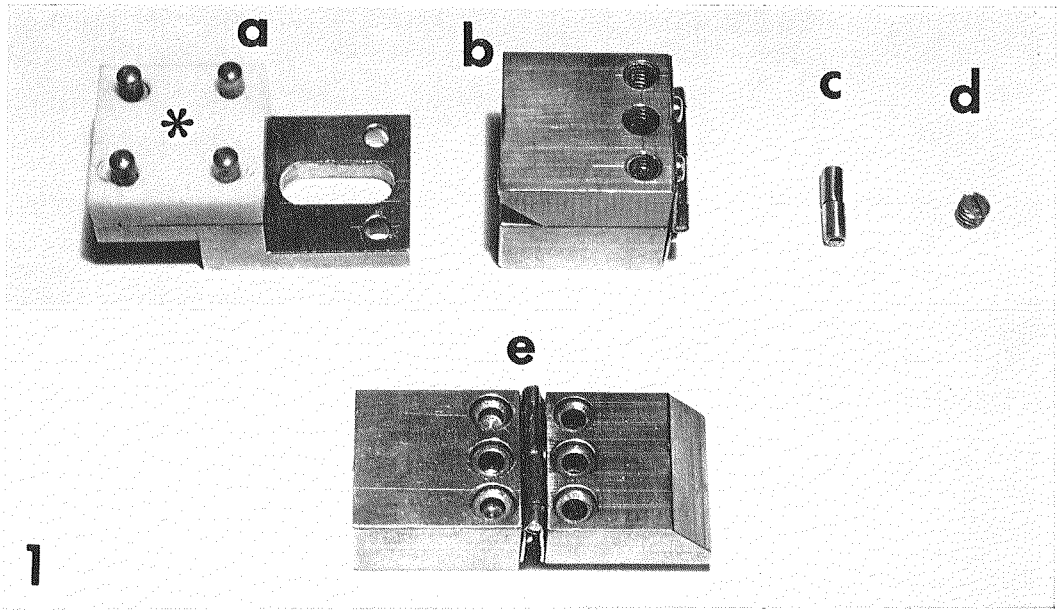
Explanation of Figures

Fig. 1. A unit for preparing complementary replicas. a, a block-holder; b, a fracture-block in closed state; c, a pair of specimen holders; d, a cap; e, a fracture-block in open state. ×1.

Fig. 2. A photograph of the fracture-block (b) and block-holder (h) installed in the freeze-etching apparatus. (t) thermocouple, (a) arm of knife, (l) liquid nitrogen tank, (s) specimen stage.

Fig. 3. Electron micrographs of two complementary replicas of a red blood cell, suspended in isotonic saline solution, frozen by direct immersion in liquid nitrogen, fractured at −100°C and etched to −94°C and immediately replicated. a, PF-face; b, EF-face. ×53,000.

Fig. 4. Electron micrographs of two complementary replicas of a yeast cell, suspended in distilled water, frozen by direct immersion into Freon 22 kept at approximately −160°C, fractured at −100°C, and immediately replicated. ×32,000.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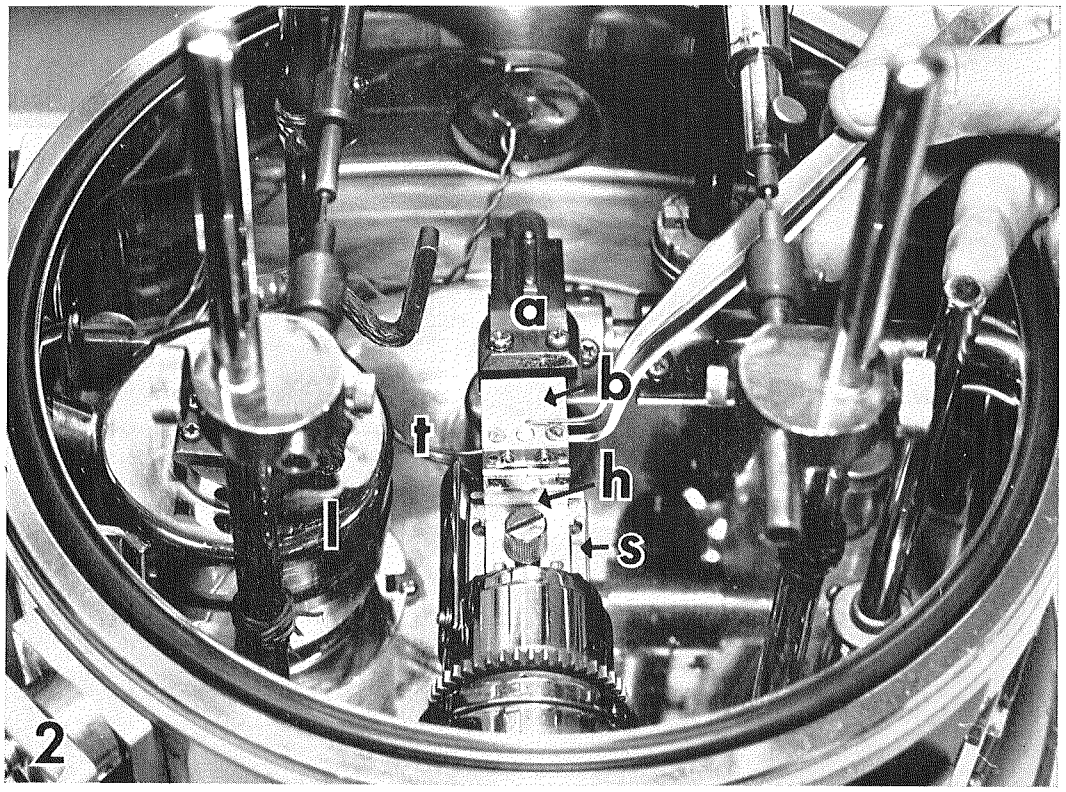
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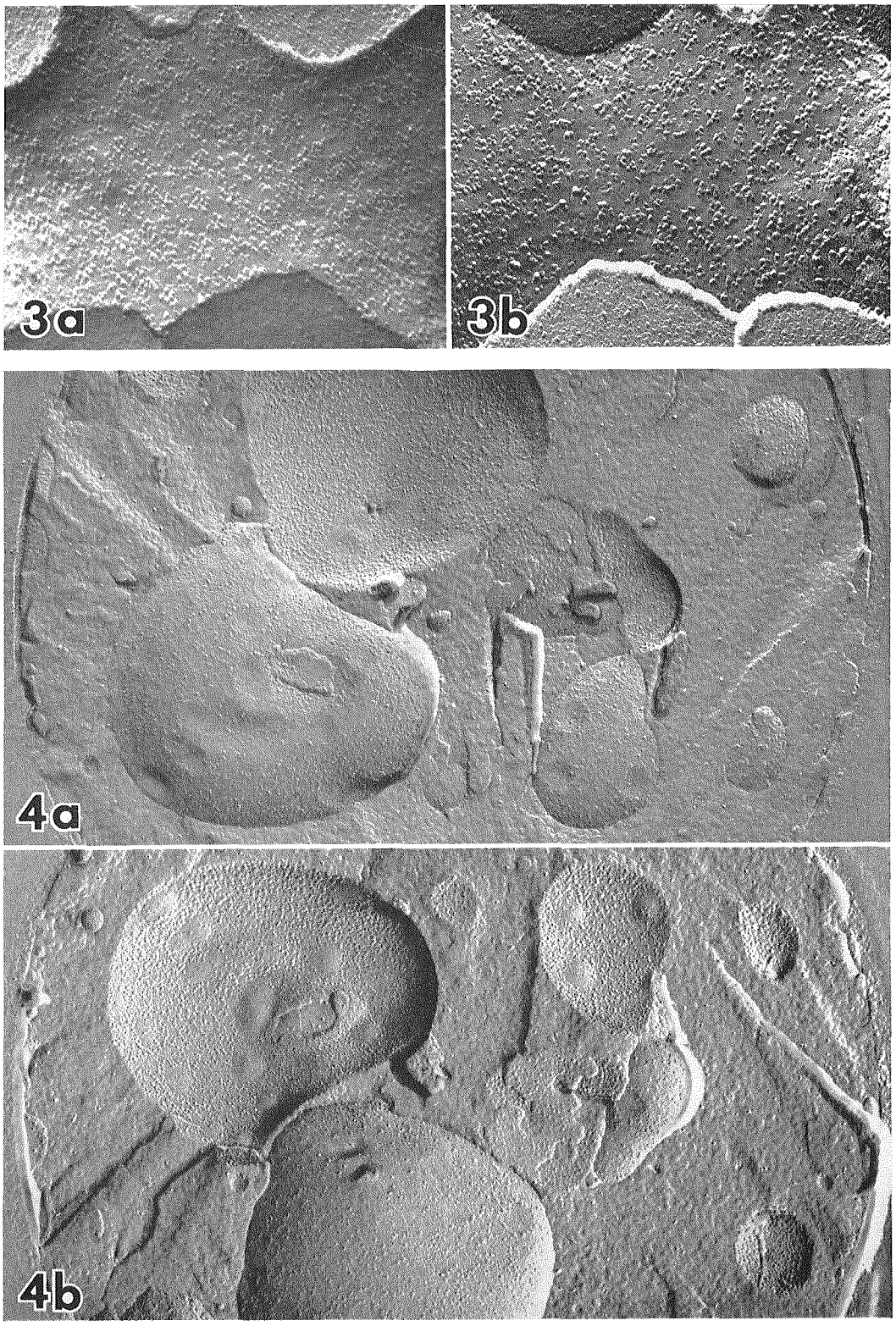
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