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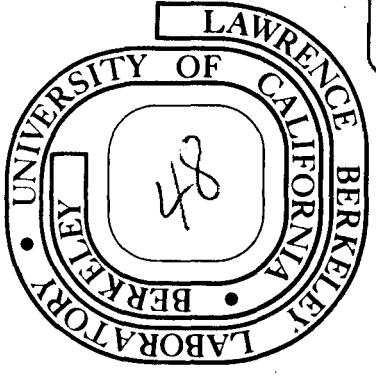

DISCLAIMER

A MODIFIED AIRLOCK DOOR FOR THE INTRODUCTION OF FROZEN SPECIMENS INTO THE JEM 100B ELECTRON MICROSCOPE

by

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ABSTRACT

An airlock door modification for the JEM 100B electron microscope is described. This modification permits prefrozen specimens to be introduced into the microscope without frost forming on the specimen or the specimen warming excessively. The imaging of hydrated biological material using frozen specimens is demonstrated with crystalline catalase as a test specimen.

INTRODUCTION

One method for maintaining specimen hydration in the electron microscope is to rapidly freeze the specimen and observe it at as low a temperature as is possible (1, 2). One of the advantages of this technique is the relatively minor instrument modifications that are necessary. These are limited to a liquid nitrogen cooled stage and suitable methodology for the introduction of a prefrozen specimen into the vacuum of the electron microscope. The cold stage manufactured by JEOL for the JEM 100B electron microscope can achieve a lattice resolution of 3.4Å at room temperature and can therefore be used for all but the highest resolution work. Its resolution at liquid nitrogen temperature is somewhat less, but that is still not a limitation at this time for the imaging of hydrated biological specimens.

For the introduction of frozen specimens into the electron microscope several techniques have been proposed. Heide and Grund (2) have proposed a "diving bell" type of apparatus. The frozen specimen is first mounted in the specimen holder under liquid nitrogen. The specimen holder is then placed in a hollow "diving bell" which is filled with gaseous nitrogen, also at liquid nitrogen temperature. The diving bell is then closed off with a plug, which has a narrow channel winding around the base to allow for evacuation but which does not allow water molecules to diffuse into the chamber. The closed diving bell can then be placed in the airlock port and the airlock evacuated.

The basic principles of a much simpler cold sink and frost protector can be incorporated in a device for introducing frozen specimens into the JEM 100B electron microscope. This is similar to the device previously

described for the Hitachi HU-11 (3), although the specimen exchange mechanism in the case of the JEM 100B is more complicated. It is only necessary to modify the airlock door so that it can accept the specimen holder when it is mounted in the frost protector. Specimen exchange can then be accomplished in the conventional manner.

DESCRIPTION OF THE AIRLOCK DOOR

A drawing of the airlock door modification for the JEM 100B is shown in Fig. 1. The airlock door consists of a body of nickel plated brass and a rotating stainless steel drum. The drum has two positions for mounting the frost protector and specimen holder and one position for receiving a frozen specimen from the microscope column. Stainless steel was chosen for the rotating drum because it is more durable than plastic and has a low thermal conductivity for a metal. Its thermal conductivity is greater than the plastics available, but this has been found at the present time to serve as a real advantage. It is most undesirable to place a specimen holder on the cold stage if the specimen holder is colder than the stage. Because the specimen holder has a temperature of -196° C when it is first removed from a liquid nitrogen bath, and the cold stage maintains a temperature of only -130° C, the specimen holder must be warmed by about 70° C before it can be safely placed on the stage. This is accomplished by keeping the specimen holder in the airlock for about 30 seconds. If a colder stage were available, this time could be shortened, or plastic might be substituted for the stainless steel.

The stainless steel drum is rotated by means of an aluminum handle. To indicate the position of the specimen holder in the drum when the airlock door is closed, a spring loaded detent stop is built into the back

of the rotating drum. This indicating system is similar to that provided in the standard airlock door for the JEM 100B. Rotation of the drum is necessary for the specimen exchange device to engage the specimen holder.

A cold specimen receiver made of Teflon is built into the rotating drum for the purpose of accepting a cold specimen from the cold stage after observation has been completed. A wire spring in the back of the receiver prevents the specimen from falling out when the airlock door is opened. A cold specimen receiver of plastic or some other flexible material is necessary because the cold specimen holder could expand during warming and become locked into a metallic receiver.

The cold sink and frost protector is made of copper and has an inside taper which matches that of the specimen holder. At the back of the frost protector, on the outside, is a stainless steel ring with a circular groove cut in it. This groove engages another detent stop built into the back of the rotating drum. This detent stop prevents the cold sink and frost protector from being drawn into the specimen chamber of the microscope along with the specimen holder.

RESULTS

Frozen specimens sandwiched between thin hydrophilic support films have been prepared by a technique described previously (3, 4). Once prepared they are frozen directly in liquid nitrogen and placed into the precooled specimen holder, also under liquid nitrogen. The specimen holder is then placed in the cold sink-frost protector and transferred to the airlock door using a specially constructed forceps. The airlock door is immediately shut and the airlock evacuated. The length of time for this transfer is less than two seconds from the time the specimen holder

and frost protector are removed from the liquid nitrogen until the airlock begins evacuating. The airlock is normally evacuated within ten seconds.

High resolution electron diffraction patterns have been obtained from frozen, unstained catalase crystals (1) thus demonstrating excellent preservation of structure in the frozen state. Electron diffraction has also been used to measure the critical exposure for fading of the electron diffraction pattern from frozen catalase (4). It was found that there is a ten fold improvement in the critical exposure of frozen, hydrated catalase relative to that of hydrated catalase at room temperature. This improvement in critical exposure has helped to overcome the disadvantage resulting from the lower contrast in unstained specimens and has enabled lattice images of hydrated catalase to be obtained.

In Fig. 2a is shown an image of a catalase crystal embedded in ice. The image was recorded using the minimal exposure technique of Williams and Fisher (5). The complete absence of frost particles attests to the efficiency of the technique described here for introducing frozen specimens into the electron microscope. Bend contours from the surrounding ice are visible. It is interesting to point out that these bend contours do not extend entirely across the crystal. This is an indication that the surrounding ice film itself does not extend entirely across the catalase crystal. This probably occurs by a process in which the capillary action effect causes the sandwiching windows to come in contact with the upper and lower surfaces of the protein crystal. In this way the thinnest possible hydrated specimen thickness can be obtained. In addition we also point out that the presence of the surrounding ice, as evidenced by the bend contours, demonstrates that the frozen specimens are indeed

hydrated and not lyophilized.

A greater enlargement of the micrograph, as shown in Fig. 2b, permits a lattice image of the crystal to be visualized. The optical diffraction pattern of the image (inset) shows a weak spot in the second order of the 68Å unit cell dimension indicating a resolution of 34Å is present.

Radiation sensitivity and lower contrast make high resolution imaging more difficult for hydrated specimens. The radiation damage limitation can be avoided if experiments are limited to electron diffraction. This alternative sacrifices the most important feature of the electron microscope which is its imaging capabilities. If these imaging capabilities are to be retained, then techniques for recording statistically noisy images in conjunction with spatial averaging (6) must be applied.

REFERENCES

1. K. A. Taylor and R. M. Glaeser, Science 186, 1036 (1974)

2. H. G. Heide and S. Grund, J. Ultrastruct. Res. 48, 259 (1974)

3. K. A. Taylor and R. M. Glaeser, Rev. Sci. Instrum. 44, 1546 (1973)

4. K. A. Taylor and R. M. Glaeser, in preparation

5. R. C. Williams and H. W. Fisher, J. Mol. Biol. 52, 121 (1970)

6. R. M. Glaeser, I. Kuo and T. F. Budinger, 29th Ann. Proc. Electron Microscopy Soc. Amer., 1971, p466.

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FIGURE CAPTIONS

Figure 1.

Drawing of the modified airlock door for the introduction of frozen specimens into the JEM 100B electron microscope. The door consists of a nickel plated brass body with a rotating stainless steel drum. There are two positions in the drum for receiving the combination specimen holder-frost protector, and the third position for the cold specimen receiver alone is made of Teflon. The frost protector is made of copper and has a stainless steel ring in the back. The inside of the frost protector is machined to conform to the taper of the specimen holder.

Figure 2.

Electron micrograph of a catalase crystal embedded in ice. a. A relatively low magnification image showing bend contours in the surrounding ice and the complete absence of frost particles on the specimen. b. An enlargement showing the lattice of the protein crystal. An optical diffraction pattern from this area is shown in the inset.

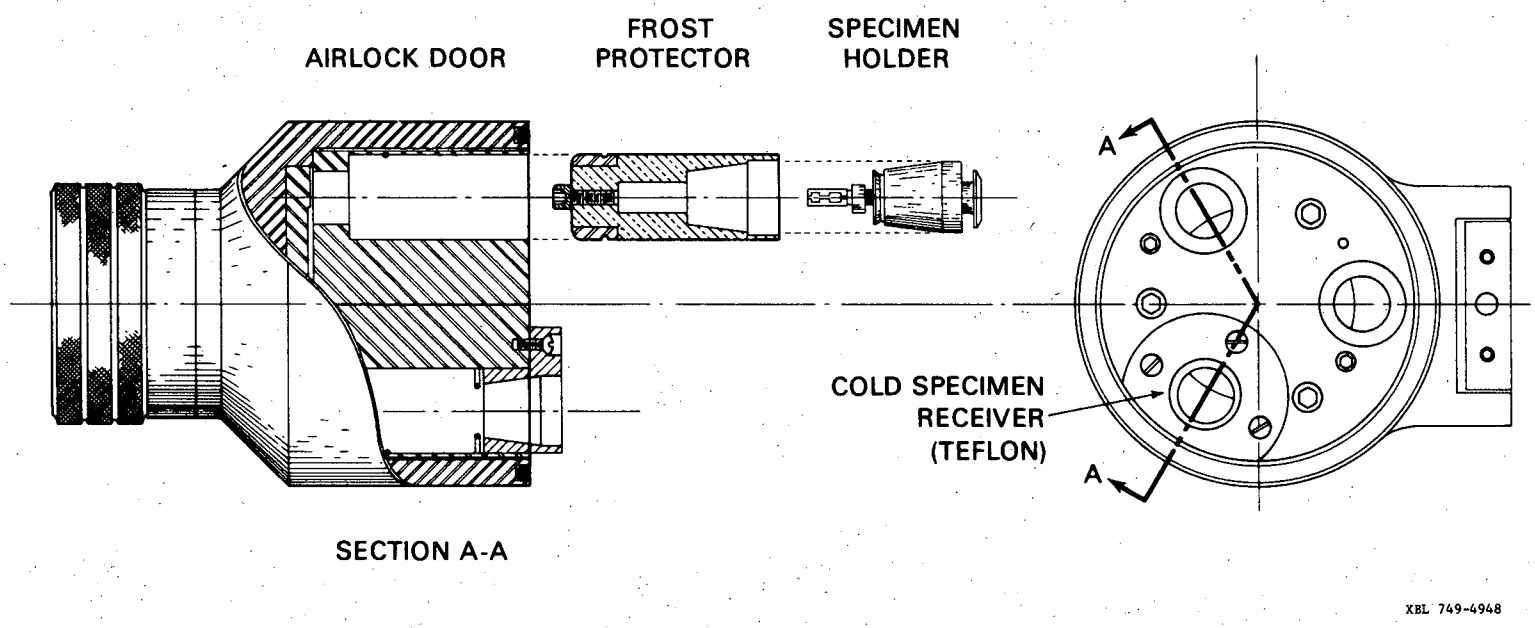
AIRLOCK DOOR
FROST PROTECTOR
SPECIMEN HOLDER
A
A
COLD SPECIMEN RECEIVER (TEFLON)
SECTION A-A
XBL 749-4948

Fig. 1

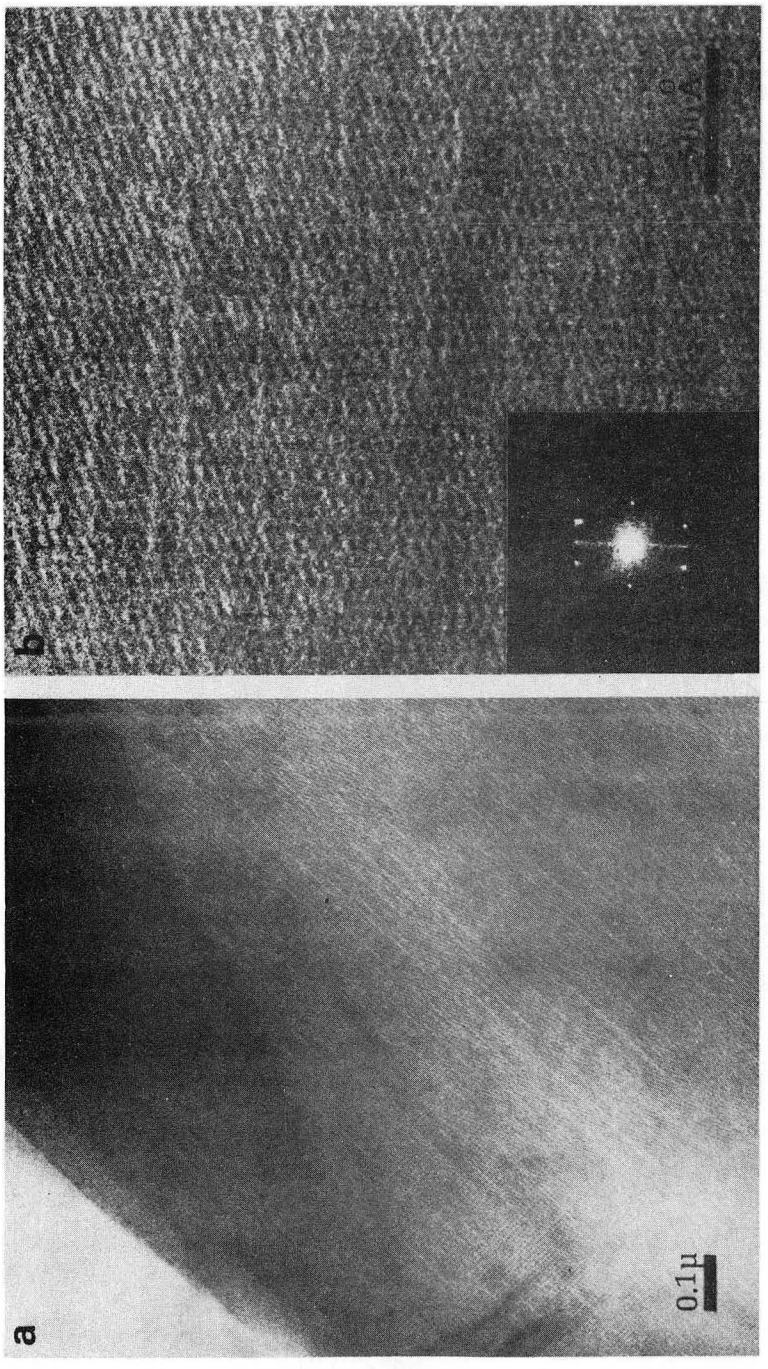

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Fig. 2

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