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Improved anticontaminator for cryo-electron microscopy with a Philips EM 400

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SUMMARY

Electron microscopy of frozen-hydrated specimens is frequently impaired by atmospheric humidity, introduced together with the cold specimen and subsequently condensing on it. This effect can be prevented in the Philips 400 electron microscope operating with the cold stage PW6591 by an improved anticontaminator described in this note. The retractable anticontaminator is formed by two adjustable liquid-nitrogen cooled blades placed just above and below the specimen. It does not interfere with any other feature of the microscope.

INTRODUCTION

Frozen-hydrated samples must be observed in the electron microscope at a temperature where water sublimation is negligible. They thus form an excellent trap for any water molecules in the surrounding atmosphere. Under the normal working conditions of a modern electron microscope, the partial pressure of water in the specimen chamber is so low that the layer formed on the specimen by ice condensation is negligible. The situation is different after the transfer of a cold specimen into the microscope. This is particularly so when atmospheric humidity is allowed to condense on the cryo-specimen holder in regions which are not actively cooled and therefore degas once in the high vacuum of the specimen chamber. The result is an increased partial pressure of water in the specimen chamber during the few minutes following transfer. Generally, the standard microscope anticontamination system cannot adequately condense all this humidity at once. Some of the water molecules condense on the cold specimen. This ice layer which subsequently builds up, is vitreous when the temperature is below that of devitrification (around 135 K) and cubic above this temperature. The layer thickness depends on the transfer conditions, but in the absence of special protection it makes the observation of biological specimens difficult if not impossible. This is particularly the case when working with a simple cryo-specimen holder such as PW6591 for the Philips (Eindhoven, The Netherlands) 400 electron microscope.

The condensation of a disturbing water layer can be avoided in several ways. The principle of the new Philips cryo-transfer system PW6361/00 and of the Gatan (Pittsburgh, U.S.A.) system for Jeol or Philips microscopes, relies on reducing contact between the cold specimen

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holder and atmospheric humidity during transfer and on improved shielding of the cold specimen, until the high vacuum is restored in the specimen chamber. We found that the cryopumping effect of an improved anticontaminator also provides a satisfactory solution for the work with the simple cryo-specimen holder PW6591 in the Philips 400 electron microscope. The system has been used successfully for several thousand cryotransfers in our laboratory during the last 4 years (Dubochet *et al.*, 1982, 1983; Adrian *et al.*, 1984). We will give here a description of this improved anticontaminator.

DESIGN

A general drawing of the anticontamination system is given in Fig. 1 and precise details for use with an EM 400 equipped with $\pm 60°$ goniometer pole pieces (type PW 6003) can be seen in the detailed workshop plans which are available on request. The system must be modified in order to fit other microscopes of the Philips 400 series (e.g. the twin lens).

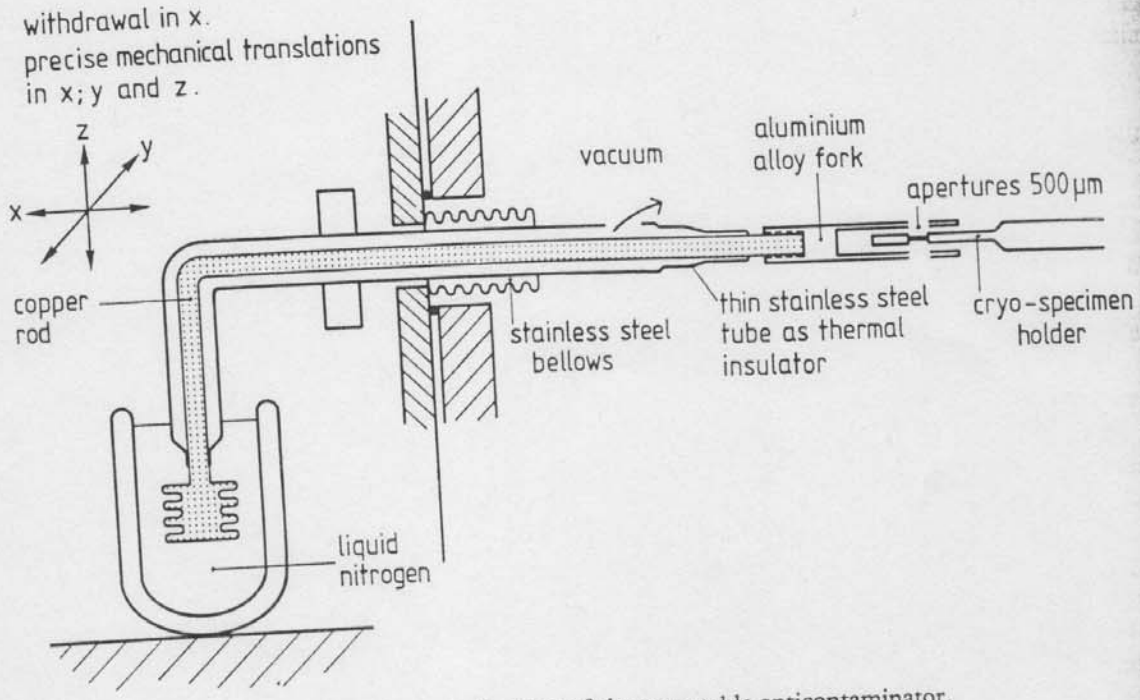

Fig. 1. Schematic drawing of the retractable anticontaminator.

The retractable anticontaminator is mounted on one of the unused ports of the microscope and can replace the standard anticontaminator. It consists of two aluminium blades positioned just above and below the specimen in which two small, well-aligned holes are drilled to allow the beam access to the specimen. A 700 or 500 μm diaphragm is pressed into each blade for astigmatism-free operation. The blades themselves are cooled by conduction through a vacuum-isolated copper rod from an external liquid nitrogen dewar. Vacuum sealing of the moving parts is made with welded stainless steel bellows. The welding of the bellows is the only part of the construction which requires special equipment. The blades can be positioned with three perpendicular fine adjustment screws.

PERFORMANCE

When retracted, the anticontamination system does not interfere with any normal function of the microscope. When introduced, the field of view is limited to the dimension of the hole in the blades (e.g. 500 μm). Specimen movement is unimpaired but the permissible tilt-angle is

limited to *c.* 23°. Damage may result if the specimen holder or the anticontaminator is introduced with the specimen tilted by more than 23°. A full tilt series of a frozen specimen can, of course, be made with the blades retracted. The steady state temperature of the anticontaminator is 95 K and it cools from room temperature to 100 K within 20 min. The response to cooling with liquid nitrogen is shown in Fig. 2.

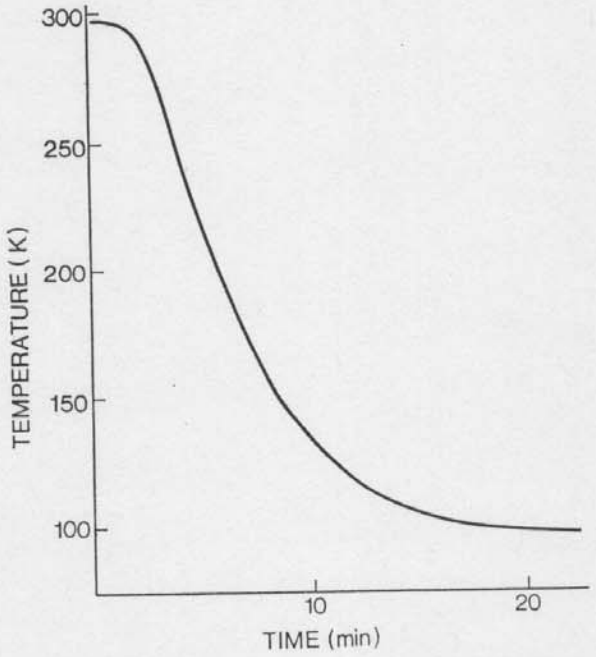

Fig. 2. Plot of temperature versus time during cooling of the anticontaminator.

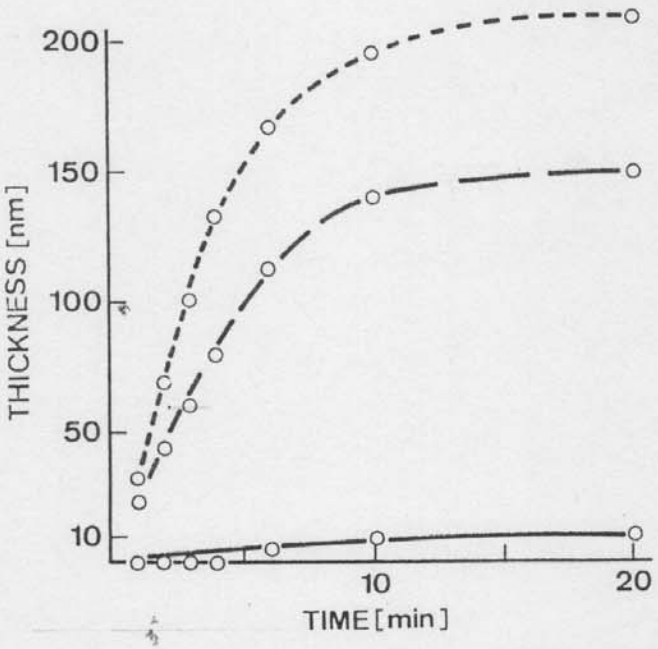

Fig. 3. Typical building up of a condensed vitreous ice layer on a carbon film, transferred from liquid nitrogen into the electron microscope and kept at the lowest possible temperature. The time origin is the moment of insertion of the specimens in the specimen chamber. Temperature: about 100 K. ———, with blade anticontaminator inserted; - - - - -, with blade anticontaminator retracted; — —, with standard anticontaminator.

Typical measurements (using the method described in Dubochet *et al.*, 1983) of the ice layer thickness which builds up on a thin carbon film transferred from liquid nitrogen into the microscope and then maintained at the lowest possible temperature are shown in Fig. 3. Slight variations in transfer conditions (atmospheric humidity; time to establish the preliminary vacuum) can easily change these values by a factor of 2.

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