We appreciate the response to this publication feature and welcome all contributions. Contributions may be sent to José A. Mascorro, our Technical Editor, at his e-mail address: jmascor@tulane.edu. José may also be reached at the Department of Structural and Cellular Biology, Tulane University Health Sciences Center, 1430 Tulane Ave., New Orleans, LA 70112 and Ph: (504) 584-2747 Fax:

Support Films with Uniform Hole Size

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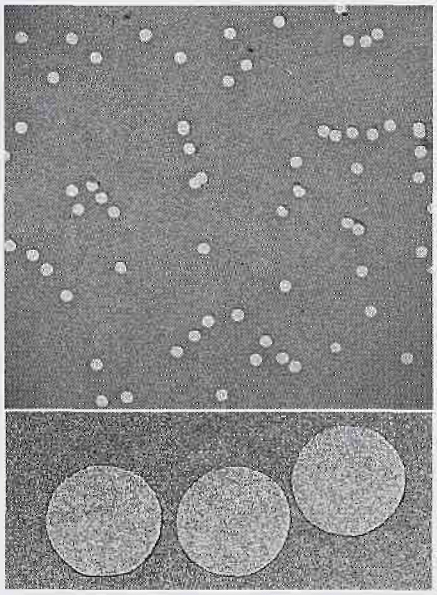
Holey carbon films mounted on standard EM grids are routinely used as specimen supports in electron cryo-microscopy. A droplet of the sample containing, for example, a suspension of virus particles or protein molecules, is applied to the grid, blotted to a thin layer and rapidly frozen. The particles are then imaged where the liquid spans the holes, without interference from an underlying carbon support film. Many recipes for holey films have been presented in the literature, generally employing some method of forming bubbles in a layer of solvent in which a material is dissolved that then forms a discontinuous layer as the solvent evaporates. The methods in use generally produce a wide range of hole sizes and suffer from a large degree of variability. The hole size and distribution often depends on environmental conditions such as humidity. With practice and luck, one can usually adjust the conditions to obtain the desired results. However, a method that would always produce the desired holes would be a frequent advantage.



Here is a method that derives from Martin Muller and Theo Koller at the ETH in Zurich and produces a very nice distribution of holes all of the same size. The holey films are made as replicas of Nucleopore® filters, which have a very smooth surface with uniform, round holes with a diameter that can be chosen for the particular application. A water-soluble parting layer is evaporated onto the filter before evaporating the carbon film, which is then floated off and mounted onto the grids in the normal way. We use Whatman Nucleopore® Track-Etch Membrane filters. We find that with a nominal cutoff of 2.0 µm (cat. No. 110611 for 25 mm diameter filters) the hole size is about 1.5 µm. The filters are taped (using a water-resistant tape) onto a glass microscope slide. The parting layer is sodium metaphosphate, which is available as Victawet from EM suppliers. A bit of the material about the size of a grain of rice is placed in a tungsten basket in an evaporator and heated slowly to evaporate. The Victawet tends to bubble and outgas as it warms, so slow heating is necessary to make sure that it doesn't jump out of the basket. Once the material is all evaporated, carbon is evaporated to the desired thickness. It is a good idea to have both the Victawet and carbon sources placed as close together as possible directly above the filter, in order to avoid forming a film along the inside edge of the holes in the plastic film. The film should then float off onto a water surface as the slide is slowly submerged.

Holey films made in this way may not have as many holes as those made by other methods, but all the holes are the right size. They are free from the plastic residue that may be left from other methods, and the holes have little or no collar around the edge that can adversely affect the ice thickness. These grids are a very effective intermediate between holey films made with the various solvent techniques, which produce random hole sizes, and commercial Quantifoil® grids, which have uniform holes on a regular lattice. ■

Cleaning a Cold Cathode Gauge Tube

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This topic appeared on the MSA list server in December 2002 and January 2003. While cleaning my gauge tube recently, I documented the steps. In this article I describe cleaning a Varian 524-2 gauge tube. There are a number of other cold cathode and penning style gauge tubes on the market, Edwards CP-25 (old penning), CVC GPH-001A (old cold cathode), Pfeiffer IRK 250 (new cold cathode) to name a few. The cleaning steps are very similar. Consult the instructions that came with the gauge tube or visit the vendor's websites.

First, when to clean: I pull the tube to check for cleanliness when the vacuum readings begin to wander erratically up and down scale. When I decided to pull the gauge tube on my evaporator, the readings ranged between $1x10^{-5}$ and $9x10^{-5}$. The meter would not remain steady.

Routine use eventually will result in the need to clean a gauge tube. In evaporators, pumping wet, volatile samples and substances can adversely influence cleanliness. Older systems required that users turn the high vacuum controller on and off. Leaving the controller on when the vacuum is below the recommended level ($1X10^{-2}$ for the Varian 524) for the cold cathode gauge quickly leads to contamination. You can minimize the frequency of required cleaning by having your gauge electronics shut off when the vacuum drops below 10^{-4}. I added a vacuum switch to my old Denton evaporator for that purpose.

Removing the gauge tube: Removing the gauge depends on the type of system it is connected to. Ensure that you have properly vented the vacuum system before attempting to remove the gauge tube. *CAUTION:* the cold cathode gauge is operated by applying a high voltage, up to 4000 VDC, between the anode and cathode (Bigelow). Be sure you have disconnected the control unit before removing the cables to the gauge.

Cleaning: If you utilize a Varian gauge tube, the company makes a convenient kit that includes a few spare parts for disassembling the gauge (Fig. 1). The disassembled gauge is shown in

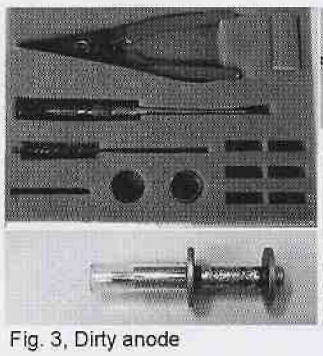

Fig. 1, Varian tool kit

Fig. 2, Disassembled guage tube

Fig. 3, Dirty anode

Fig. 4, Bead blasted clean anode

Fig. 2. When I disassembled the gauge, it was discolored brown and covered in black particles (Fig.3). As mentioned in the list server thread, there are a few ways to clean the anode. An acceptable cleaning method is to use silicon carbide sand paper and water. One contributor suggested an aqueous cleaning protocol. Another common technique is to utilize glass bead blasting at medium pressure (40 psi) for the aluminum anode, and higher pressure (100 psi) for the stainless steel case. Another suggestion from the list suggested using an air eraser for the anode. I use an inexpensive, tabletop bead blaster for this project. When using bead blasting, avoid entraining glass beads in screw threads by using tape and dummy screws to plug holes. When blasting the aluminum anode, only bead blast as long as required to remove the deposits. Afterwards, I use high-pressure air to blow away any remaining beads from the surfaces. The clean bead blasted parts are shown in Fig. 4. Finally, after either cleaning technique you chose, ultrasonically clean the parts in solvent, dry well, and reassemble with gloves as you would any vacuum part. A number of individuals on the MSA list correctly mentioned that the elements will eventually wear to the point of requiring replacement.

Reinstallation: The Varian cold cathode gauge tube utilized in my laboratory installs with a compression fitting. I carefully inspect the O-ring and mating surfaces of the outer gauge tube for dust that could cause a leak. Since the gauge is mounted in a static position, I do not use vacuum grease on the O-ring. Reinstall the cables, reconnect the controller to power, and begin pumping the vacuum system. After installation, I let the system pump at high vacuum for 15 minutes or more before firing the cold cathode gauge to give it time to outgas the solvents used for cleaning. Finally, I make a note of my service in the logbook. ■

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Zigzag Edges in SEM Micrographs

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Multiple specimen holders make it possible to examine several (up to 7) specimens by SEM without the need to vent the microscope, replace the specimen, and evacuate the microscope again. Using such a holder in a Philips XL-30 ESEM frequently showed zigzagged outlines of bacteria obtained at magnifications higher than 10,000x. The zigzag outlines were not associated with charging artifacts as the filter edges were painted with a silver-based cement prior to gold coating. The severity of the zigzag lines varied from day to day. Sometimes they were barely noticeable whereas at other times they developed during a half-day session.

Checking known sources of vibration brought no solution. At that stage, one of us (A.F.Y.) asked subscribers to the listserver of the Microscopy Society of America for advice. Many suggestions emphasized that good images start with a proper setup of the microscope. Ideally, nothing should make any mechanical contact between the inner zone and the outside environment. The wires and tubes should have loose loops between the attachment to the inner and the outer tables. An EDS detector may add to the problem by mechanical or even acoustic means. If hands are clapped loudly while an image is being scanned, decaying vibrations from the clap will be noticeable if the EDS is susceptible. Vibrations may also arise from AC magnetic fields from various sources. If the cause of the vibrations are magnetic fields, the "sawteeth" will be larger at greater working distances. Changing the beam voltage is another useful test; it is easier to do than changing the working distance. Field problems may be tracked down using an AC magnetic field hand meter. Another possible source is a ground loop where the instrument is hooked up to another piece of equipment with a different ground potential. A solution for this is to decide on a single ground potential to use and to hardwire all other equipment to that ground. One should connect the power supply common connections in a way that would provide as low a resistance path as possible to the chosen "common" power connection. In our case, neither heavy equipment being operated in the building nor trucks driven occasionally nearby could be found responsible for the zigzag outlines.

Then, during one session, the ragged edges became so bad (Fig. 1) that the examination had to be terminated. The microscope was vented and the samples were being removed from the multiple specimen holder when it was noted that the holder was somewhat loose. It was re-tightened, the specimens were returned in place and the microscope was started again. The images had a proper appearance and no ragged edges could be found. An examination of the multiple specimen holder (Fig. 2) and its comparison to the single-specimen holder (Fig. 3) indicated that the former would be more susceptible to loosening while the motor-driven stage was moved in order to examine the next specimen. A new stem for the multiple specimen holder was developed (Fig. 4) and the zigzag, ragged edges in the images of bacteria are a defect of the past. ■

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Information about this defect may be viewed in greater detail on the Internet (http://distans.livstek.lth.se:2080/Zigzag.htm)