Chamber for Critical-Point Drying of Nematodes and Other Biological Specimens

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Processing nematodes for scanning electron microscopy requires transfer of specimens through a series of fixatives and dehydration fluids (1, 2). Preferably, all steps of fixation, dehydration, and drying are performed in the same vessel to avoid repeated handling of specimens. De Grisse (2) described small chambers made of nylon mesh and glass tubing for this purpose, and Marchant (3) constructed a similar device from small polypropylene vials and Millipore[R] filters. In our experience, the joints between chamber walls and screens in the first design (2) were not always tight enough.

Rostgaard and Christensen (4) stated stringent requirements for such a chamber and made one from brass and aluminum stock. Its construction, however, involved extensive precision machine work. This paper describes a simpler, larger variant of the same basic design which is fabricated by modification of commonly available hardware. Threaded components allow a tight fit between screens and chamber walls, and minimize the chances of losing small specimens.

The basic material used to construct this unit was a standard brass compression union for 9.5-mm (3/8 inch) flexible copper tubing (Coronet Part Manufacturing Co., 883 Elton St., Brooklyn, N.Y. 11208). Half of the threaded portions on each end of the middle piece of the fitting were cut off. The thickness of both clamping nuts was also reduced (by half) by cutting a portion from the threaded end. On the threadless end of one clamping nut, additional material was removed so that only a short collar was left. A copper washer of 12.7-mm diam was placed between each nut and the middle piece. The various inner diameters of all parts were uniformly reamed to 10mm. A nylon screen with 18-μm openings (Tobler, Ernst and Traber, Inc., 420 Saw Mill River Rd., Elmsford, N.Y. 10523) was attached to the copper washers with cyanoacrylate adhesive (Eastman 910). All parts were cleaned with metal polish and then washed in ethanol in an ultrasonic cleaner. For dimensions of the finished chamber, see Fig. 1.

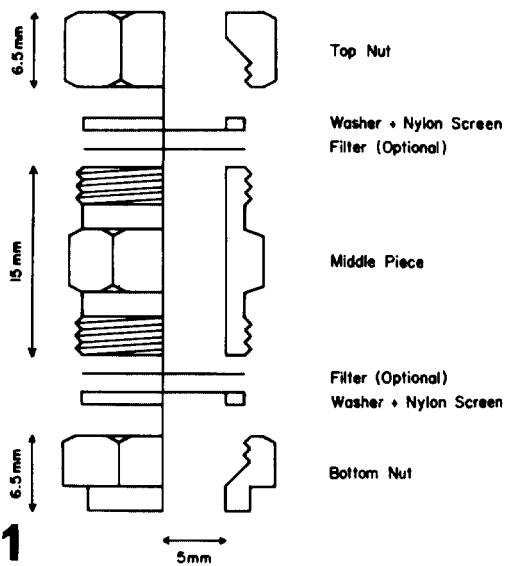

FIG. 1. Diagram of side view and longitudinal section of all parts of the universal chamber.

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In the chamber described, nematodes are transferred through a series of solutions contained in small petri dishes and then into the critical-point drying apparatus (1). Since osmium tetroxide reacts with brass, post-fixation and the first washing step thereafter must be performed in glass containers only. After critical-point drying, the nematodes are picked off the bottom screen and positioned on a piece of double-coated adhesive tape (e.g. Scotch® Tape No. 410) on an SEM-specimen carrier.

For small tissues or specimens, Nucleopore® membrane filters (Nucleopore Corp., 7035 Commerce Circle, Pleasanton, Ca. 94566), with a pore size slightly smaller than the specimens, are used in addition to the nylon screens. Available pore sizes range from 0.03 to 8μm. When filters are used, the chamber can be put on a sidearm flask connected to a vacuum pump to draw liquids through the chamber faster. For this purpose, the arrangment is as in Fig. 2, or also temporarily without top nut, screen and filter, to facilitate addition of liquids with a pipette. After critical-point drying, the specimens often can be transferred, together with the bottom filter, to an SEM specimen carrier. The filter is attached to the carrier with conductive silver paint. If an appropriate filter pore size is chosen, this chamber can be used universally for processing biological specimens of a wide range of sizes.

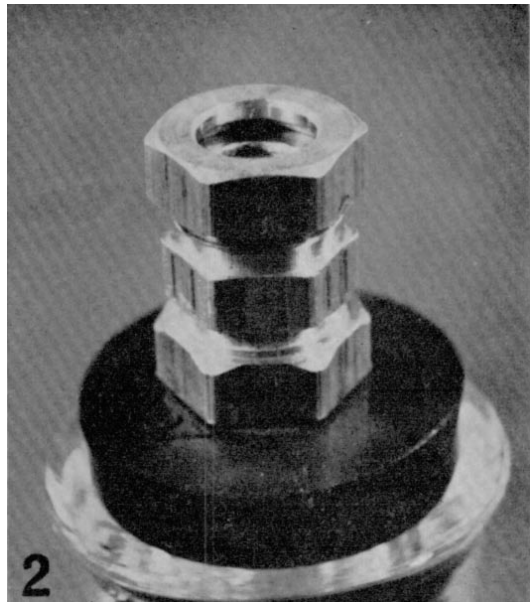

FIG. 2. Chamber connected to a vacuum flask.

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