Mounting and Storage Specimens

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Grids

Specimen support grids are now almost universally 3.05 mm in diameter, except for a few high resolution stages and some very old instruments. They are available in a vast range of materials and designs. One catalogue lists 86 types in a total of 10 materials. The reason for this proliferation is to enable one to control the following:

(a) the amount of support the specimen needs (unsupported areas range from 20 μm to 1 mm in extent);

(b) the material of the grid, so that it neither interferes with X-ray analysis nor reacts with the specimen;

(c) the labeling of specific regions of a specimen (many grids have identification marks for relocation of interesting fields).

The cheapest most widely used supports are copper grids at a spacing of 100 bars in^{-1}. Most grids have a shiny side and a dull side. Opinions differ as to the best side on which to mount the specimen but if a consistent practice is adopted it is always known which way up in the microscope the specimen was mounted.

Disc Specimens

The advantage of preparing a 3 mm disc specimen is that it will need no support in the microscope. However, there is an exception to this rule. Many brittle or porous materials are liable to break up on handling and such materials can be supported by sticking them to a copper grid with a single large hole in the centre. This provides a ductile support to be handled by the tweezers without obscuring any of the thinned areas (with luck!).

Thinned Foils

All foils except 3 mm disc specimens will need support in the microscope. In order to maximize the area available for viewing a grid with the widest spacing consistent with its supporting the specimen should be chosen. This could well be a 50 mesh grid. If a 400 or 700 mesh grid has to be used more than three-quarters of the specimen will not be visible. However, it should be remembered when examining a non-conducting or thermally sensitive specimen that the grid also acts as an electrical and thermal conductor and there would then be a reason for using a fine spacing.

Attaching the foil to the grid may be a problem. Some foils seem to stick remarkably well with no assistance. However, many foils will need to be secured lightly on the grid. Although folding grids, in which the foil can be trapped between two meshes, are available these are not recommended for "materials" specimens since the specimen is nearly always damaged as the grid is folded. It is better in almost every way to use a sharpened toothpick to apply a very small drop of lacquer (e.g. Lacomit) to one or two corners of the specimen and to attach it to the grid just at these points. It is often easier to do this using a bench magnifier since the drop of lacquer should be of submillimetric dimensions.

Support Films

Any specimen which cannot be supported by a grid alone requires a thin electron-transparent support film to be mounted on the grid first. Powders and fine particulate specimens are the most common examples. Many types of support films are in use but it is only necessary to describe three: the Formvar (polyvinyl format) film which is suitable for fairly undemanding work, the carbon film when greater stability is required, and the perforated carbon film for high resolution work where the presence of any support films is to be avoided.

A Formvar film is very easily and quickly made. One drop of a solution of 0.5 per cent Formvar in chloroform is placed on a glass slide. A glass rod is used to wipe this drop uniformly over the whole slide. The resultant film will float off onto the surface of water as the slide is slid obliquely in Figure 1 (a). The easiest way to transfer the film onto 3 mm grids is to put several grids on a folded piece of metal gauze, submerge this in the dish and then bring the whole ensemble up beneath the floating film (Figure 1b) The continuous film will break around the edge of each grid as it is then lifted from the gauze, leaving each grid coated with Formvar. The stability of such films to the electron beam can be improved by coating them with a very thin layer of carbon. About 3 nm (scarcely visible on the porcelain dish) is adequate.

A pure carbon film is more stable than a coated Formvar film. This can be made by depositing carbon onto either freshly cleaved mica or a glass slide wiped with diluted Teepol and then wiped dry. In both cases the film, which can be of any desired thickness, should lift off very easily as the slide is immersed into water (Figure 1a). The same technique as described in the previous paragraph can be used to transfer the film onto 3 mm grids. If the film is very thick it may need to be broken from around each grid with a needle.

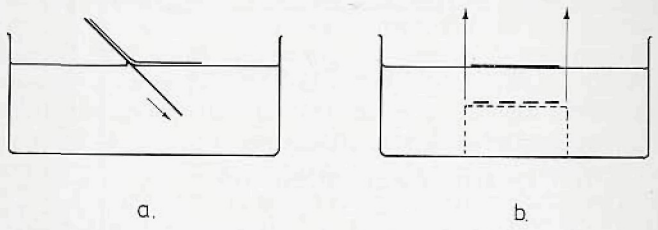

Figure 1:: (a) Lifting off a replica or carbon film. The substrate is slid gently into a water bath. The carbon will remain on the surface. If this does not work first time it can be repeated and the leading edge of the film can be teased with a needle. (b) Picking up a carbon film on grids. The 3 mm grids are arranged on a mesh beneath the floating film. The mesh is lifted out of the water bath through the film, which will adhere to the grids. Each grid can subsequently be lifted off the mesh, when the film will break around the grid leaving a continuous film over the whole grid area.

The third type of support film is a perforated carbon film which can be used to support fine particles, some of which are likely to overhang a hole, or to carry a second carbon film which is too thin to be self-supporting. The manufacturing process combines both the previous techniques: to a 2 per cent solution of Formvar in chloroform is added 1-2 per cent of glycerin (the greater is the amount of glycerin, the larger is the number and size of holes). This mixture is agitated in an ultrasonic bath until the glycerin is well dispersed (emulsified) and then a glass slide is coated with one drop, as previously described for Formvar films. The glycerin is now dissolved out in methanol by immersing the whole slide in methanol for at least an hour. The slide is then allowed to dry and is carbon coated as described above for plain carbon films. The Formvar is then dissolved off in chloroform, either by immersing the whole slide in chloroform or by mounting the film on a grid which is then washed with chloroform as illustrated in Figure 2. There are many ways of making perforated carbon film, and a commonly used alternative procedure is to mount the Formvar films on grids before dissolving out the glycerin, carbon coating, and removing the Formvar.

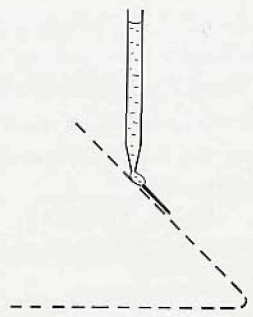

Figure 2. Washing a grid or replica. The grid is placed on a piece of wire mesh folded so as to hold it at about 45°. A fine pipette is used to drop solvent gently above the grid, over which it will then wash.

Once mounted on a grid all the above films should be fairly stable. The coated grid can be immersed in further baths of liquid, for example to pick up a second film. Fine particulate material is generally mounted on a support film by dispersing it ultrasonically in an appropriate liquid (the choice depending on the wetting behavior), placing a single drop of the specimen on a coated grid, and allowing it to dry. The key point is to ensure that the dilution of the suspension is correct; if it is too concentrated the particles will be piled on top of each other,

and if it is too dilute the particles will be difficult to find on the support film. The correct dilution can only be established by trial and error.

Specimen Storage

A Petri dish is adequate for temporary storage, say between preparing the specimen and deciding whether it is worth keeping. A piece of filter paper in the bottom can be marked into areas with a pencil and several specimens can be stored. It is possible to buy an insert for 50 and 90 mm dishes which is divided into numbered squares separated by raised ridges designed to minimize the risk of grids being mixed up.

Some sort of grid box is needed for permanent storage. The problems to be avoided are electrostatic charging of the grid, specimen, or box and mechanical damage to the specimen while it is inserted or removed from the holder. Several designs of plastic box with slits for 50 or 100 grids are available commercially. Grids can also be stored individually in gelatine capsules (diameter 5 mm) since only the edges of the grid or disc then touch the capsule and the risk of damage is minimized. Individual capsules can be stuck to a piece of card for labeling and storage. Some workers believe that the gelatine capsules can contribute to specimen-borne contamination in the microscope but the present author has seen no evidence of this.

The golden rules concerning specimen storage are: LABEL IT and DO NOT LEAVE SPECIMENS IN THE MICROSCOPE ROOM. (They will disappear). ■

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Zepto and Yocto - Continued from page 6

this direction as early as 40 years ago. He referred to "a staggering small world that is below." There is this powerful intellectual challenge to move where there is "plenty of room at the bottom." But there is an additional powerful force, perhaps not always recognized. On our overcrowded planet we are in danger of running out of energy, out of accessible raw materials, out of space. Short of throttling our activities, we must resort to smaller and smaller scale.

So, while the next generation may well forget Groucho, Harpo, and Zeppo, they will have to live with zepto and yocto. ■

This article is one of a series of Dr. Gabor B. Levy's essays on science and society containing thought-provoking editorials previously published in International Scientific 'Communications' journals. The book expresses the author's point of view on such subjects as Our Society, Our Economy, Ethics, Lawyers and the Law, Health and Medicine, Statistics, Science. Pseudoscience, Metrology, and New Directions. Size 8½" x 5½". Price only $10.99 plus $3.50 for shipping and handling. Order now by check or money order, as supplies are limited. International Scientific Communications, Inc., 30 Controls Drive, Shelton, CT 06484.