# CARBON FILMS AND SPECIMEN STABILITY

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Plates 16 and 17

In recent years a subject which has received little attention is the question of motion of the specimen relative to the objective lens of the electron microscope. In ordinary electron microscopy, where resolutions better than 30 A are not required, one can tolerate drift of the object (during a 5 second exposure of the plate) of perhaps 1 to 3 A per second, while for high resolution microscopy this figure may drop as low as 0.2 to 0.6 A per second. When we consider that the distance between adjacent atoms of many substances is on the order of 1 A, it is clear that our stability requirements are rather stringent. For purposes of analysis we can find two general sources of specimen drift: motion of the specimen holder arising in the stage mechanism and motion of the specimen relative to the grid. We will consider these in order.

Inherent in the design of any useable stage mechanism is its ability to move the specimen over distances hundreds of thousands of times greater than the size of objects one desires to resolve. Any part of this mechanism subject to mechanical relaxation or to sensitivity to vibration may cause undesired motion of the specimen. Proper adjustment of the stage mechanism will minimize the mechanical problems mentioned above, but may not be permanent. There remains, in addition, the long mechanical pathway between the specimen and the objective which is subject to thermal effects during warmup, drafts around the microscope, etc. Although the stage may be perfectly stable in one position, this is no guarantee that it is stable in another position. For this reason it is difficult to be certain that the mechanism is operating properly. Operating practice leading to consistently good results from the microscope requires certainty on this point. In addition, if other sources of image drift, such as drift in the power supplies, are present as well, one is in doubt as to where to look for the trouble. These points, in my opinion, are strong arguments for eliminating insofar as possible all sources of stage drift. The device to be described provides an arrangement whereby the chances for motion of the specimen holder are very substantially reduced. While this device has been primarily designed for the RCA types EMU-1 and -2 microscopes, it probably can be adapted to certain other instruments as well. Essentially, the specimen holder is arranged so that it rests on top of the objective pole-piece and is in contact with the stage only when it is being moved. Text-fig. 1 shows how this is achieved.

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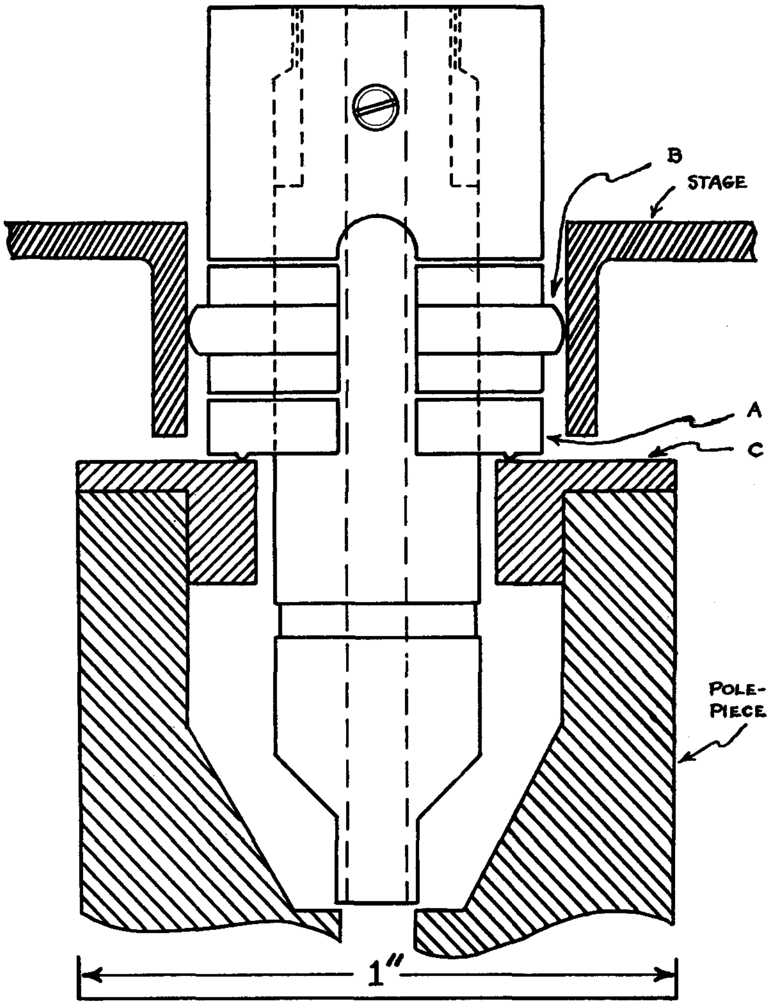
A new top is made for the holder and is provided with a skirt *A*, resting on a platform *C*, inserted into the top of the pole-piece. A raised ring *B*, encircles the barrel of the holder and fits within the bore of the specimen stage. The ring is machined so that it clears the stage by about ¼ mil. In use, the specimen is moved in the usual fashion and, when the specimen is properly located, the stage is backed off so that it is no longer in contact with the specimen



TEXT-FIG. 1. Modified specimen holder for RCA type EMU-1 and -2 microscopes. The holder rests on a split ring *C*, inserted into the top of the objective pole-piece. The elevated ring *B*, is machined to clear the hole in the stage by about ¼ mil. Thus after proper positioning of the specimen the stage may be backed off leaving the specimen holder resting free on top of the pole-piece.

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holder. The holder then rests free on the pole-piece and is thus unaffected by possible mechanical or thermal disorders of the stage mechanism. In order to prevent rocking of the holder, the skirt is upset at three points on its circumference thus providing three-point support. A wide slot at the back of the holder clears the tongue which is present in the old style stage. The barrel also has two horizontal slots so that the bearing ring *B*, may be expanded to compensate for wear.

The inconvenience resulting from play between the holder and stage is more than compensated for by positive security from drift. In any case, one soon becomes accustomed to it and can devote his attention to eliminating other sources of image motion.

A second source of object motion may arise between the object and the supporting grid. Specimens, particularly tissue sections, mounted on formvar or collodion films, tend to exhibit a slow drift which can be correlated with the position and intensity of the beam. Many areas of such sections often have an apparently inexhaustible capacity to move in some direction. In addition to this, it is possible, by injudicious application of the beam to tear the substrate thus usually ruining an entire grid opening for electron microscopy. Carbon films as substrates for tissue sections essentially eliminate these difficulties.

Since a number of workers appear to have had difficulty in preparing satisfactory carbon films, I propose to spend some time discussing the methods we use. The techniques described here have previously appeared in less detail in reference 1. The apparatus used is shown in Fig. 1.

Graphite spectroscopic electrodes of high purity are used.[1] They are sharpened to fine points in a pencil sharpener. One electrode is faced off on No. 600 carborundum paper to a diameter of about 1 mm., and the other to a diameter of ¼ to ½ mm. One rod is clamped rigidly and the other slides freely in a closely fitting guide tube. Contact between the rods can be maintained with a spring, but it is preferable to use a weighted bar which operates reproducibly and does not deteriorate with use. The force between the rods should only be great enough to ensure contact during the evaporation. The grids, having previously been coated with a formvar film, are placed for evaporation at a distance of about 10 cm. from the rods. After the carbon is deposited the formvar is removed in ethylene dichloride.

Evaporation commences when sparking from the carbons is first noticed, films being formed at this temperature. It was soon found, however, that films were not strong unless formed at higher temperatures. The evaporator used in this work is provided with a variable autotransformer to control the current through the carbon rods; this control is advanced rapidly until sparking is noted and is then advanced somewhat further to deposit the film. If too high a temperature is used there is danger of rupturing the formvar films. Formvar is used as the substrate rather than collodion because of its greater heat resistance thus providing a much greater percentage of intact squares than when collodion films are used.

For dissolving away the formvar the carbon-coated grids are inserted edgewise into a container of ethylene dichloride and deposited on a wire mesh

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[1] National Carbon Co. No. L3806; 3/16 inch by 12 inches

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screen. Care must be taken not to disturb them at this time lest the carbon films become dislodged. After a time the wire screen bearing the grids is lifted carefully from the solvent and as much solvent as possible is blotted off in order to avoid contaminating the grids with traces of dirt and solute which may be in the solvent.

A well formed carbon film will be very shiny and show no interference colors. If excessively high evaporation temperatures are used, wrinkling of the heated formvar substrate will result in a wrinkled carbon film which appears dull to the naked eye. The pencil sharpener and carborundum paper used to prepare the tips of the carbons should not be used for other purposes because of possible contamination of the carbons. Under the conditions described, the total evaporation time is between 1 and 5 seconds. The degree of vacuum does not appear to be critical, although 0.1 $\mu$ Hg is satisfactory.

I cannot stress too strongly the excellence of carbon films for routine work with tissue sections when resolutions in the neighborhood of 20 to 50 A are expected. Areas do not have to be examined for drift since there is none (*cf.* Fig. 2). The section does not have to be prepared by careful exposure to the beam since it is usually impossible to tear the carbon film by exposure to normal crossover intensities. The use of this substrate in the Cytology Laboratory at The Rockefeller Institute over the period of 1 year has made loss of micrographs through specimen drift a rarity.

Two important limitations of carbon films exist and should be mentioned. Carbon is brittle and does not stretch in contrast to formvar or collodion. This means that when deposited on slit grids or grids with large openings the chances of breakage of the carbon film by deformation of the grid in handling are greatly enhanced.

A second point to notice with carbon films arises owing to the relatively high density of the carbon. Measurements were kindly made by Dr. Rothen of The Rockefeller Institute of the thickness of carbon films and of formvar films suitable for section substrates. The values obtained ranged between 100 and 150 A, the carbon films having about the same thickness as formvar films. Now, the density of amorphous carbon is reported to be 1.8 while of graphite, 2.25. The density of formvar is 1.5 to 1.6. Thus, the electron optical thickness of the carbon film is 20 to 30 per cent greater than that of a formvar film of the same physical thickness. In addition to this, we remember that some of the formvar may be removed by the electron beam so that its final thickness will be less than that measured. Lest I overemphasize this question, Fig. 4 is presented to show a section part of which is unsupported, part with one layer of carbon and part with two.

A way in which one can utilize the high stability of the carbon and at the same time have high transparency of the substrate is to use carbon films containing holes. A number of years ago Jaffe (3), described a method for forming

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holes in collodion films by condensing moisture on the film before the solvent evaporated. Collodion films are cast on glass slides, the holes being formed by moisture condensed from the breath as the solvent evaporates. Depending on the amount of moisture so condensed, large or small holes may be formed. Carbon is then deposited on grids bearing this collodion which subsequently is removed in solvent (2). Fig. 5 shows at low power the appearance of a film with large holes. Sections are self-supporting over such holes although substantially less deformation of the section results if an extremely thin film of formvar is put on first.

One further application of carbon films involves the preparation of films with small holes, ¼ to 2 μ in diameter, as shown in Fig. 3. These make ideal test specimens for determining excellence of compensation, image stability, etc. They are much superior to carbon black since the holes are nearly round, lie in one plane, and are stable. The contrast is sufficiently high so that compensation good to perhaps 30 to 50 A resolution may be carried out visually at a magnification of 20,000.

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## EXPLANATION OF PLATES

### PLATE 16

FIG. 1. View showing the arrangement of the evaporator for the preparation of carbon films. The rod on the right is firmly fixed, while the rod on the left slides freely in a closely fitting tube. Contact between the rods is maintained by the bent strip of metal, pivoted on one end and resting against the end of the sliding electrode. A flexible wire (not shown) is connected between the metal strip and an appropriate base-plate electrode to provide electrical continuity with the sliding graphite rod.

FIG. 2. View of a mitochondrion bisected by the edge of the torn carbon film. The tear, which extended across the entire grid opening, was present before mounting the section. This micrograph is included to show the absence of drift of carbon substrates even under these extreme conditions. × 50,000.

FIG. 3. Showing the use of small holes in carbon films as test objects for compensating for pole-piece asymmetries. The micrographs are taken from below focus (bottom) through focus to overfocus (top). × 100,000.

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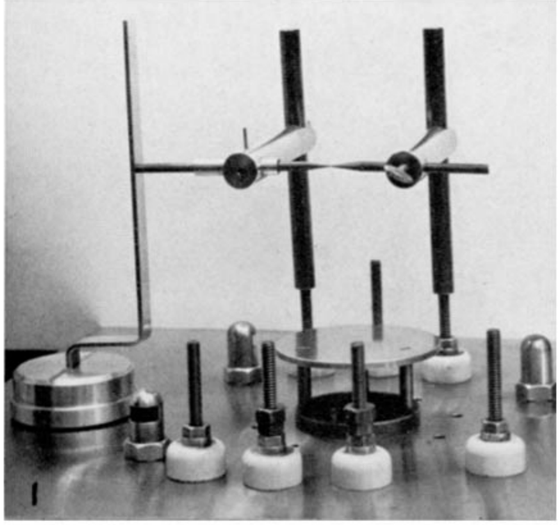

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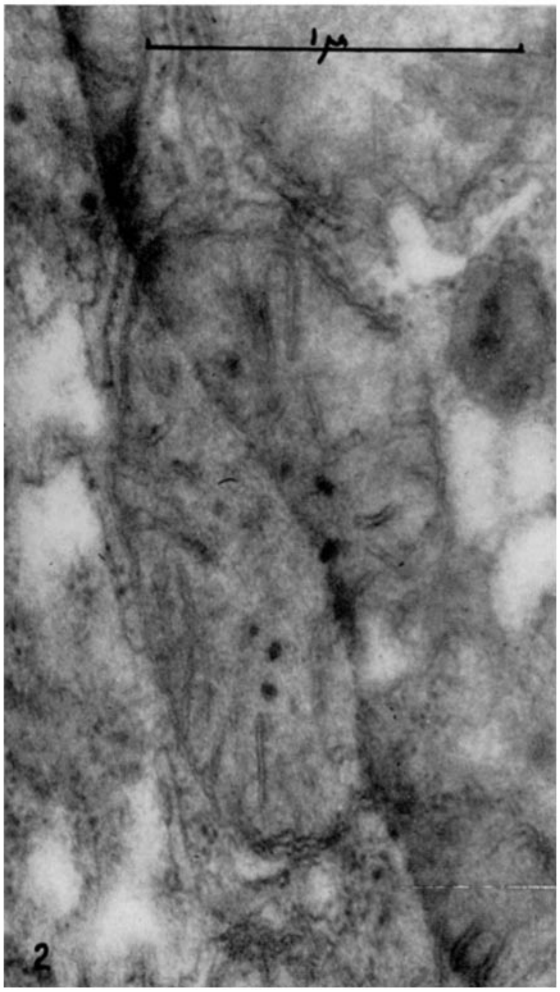

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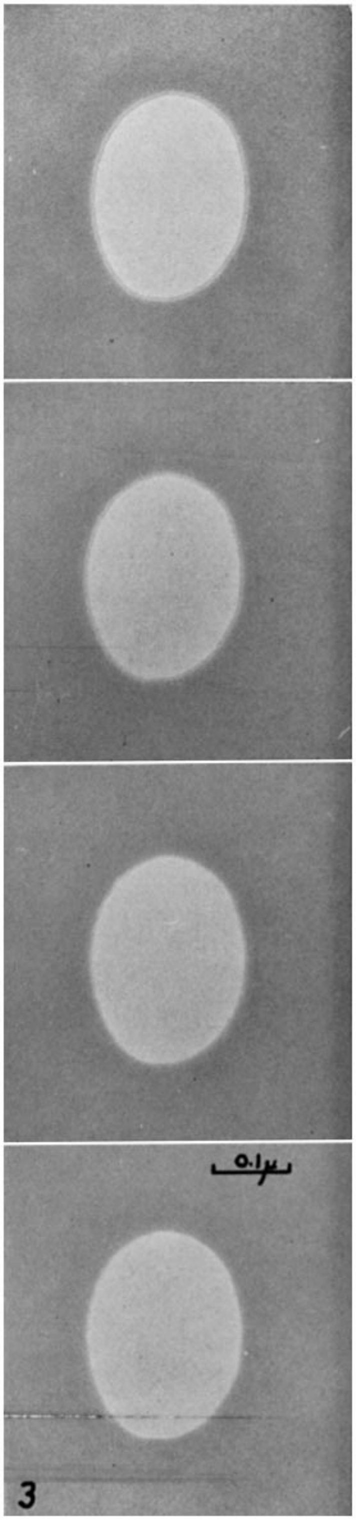

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(Watson: Carbon films and specimen stability)

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PLATE 17

FIG. 4. Showing the transparency of a carbon film. One portion of the section is unsupported where the carbon film is folded away. In the center of the micrograph is a wedge-shaped region having two layers of carbon, while the final area of the section is supported by a single layer of carbon. × 15,000.

FIG. 5. Carbon films can be prepared with large holes through which sections may be viewed without substrate. There appears to be some increase in contrast and resolution. × 16,000.

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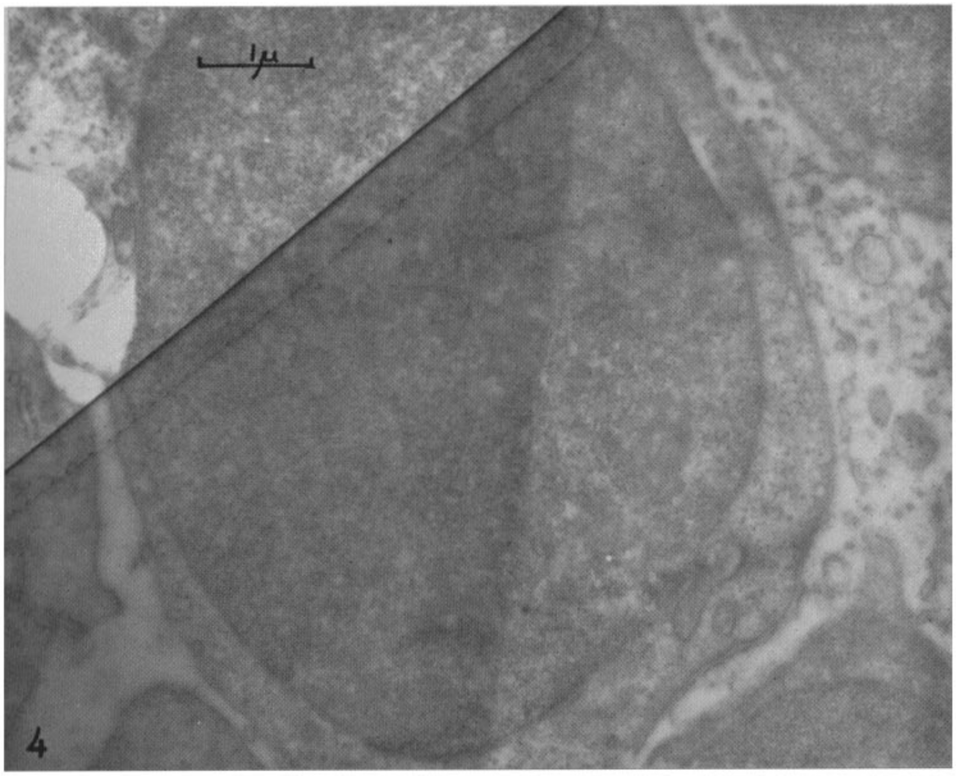

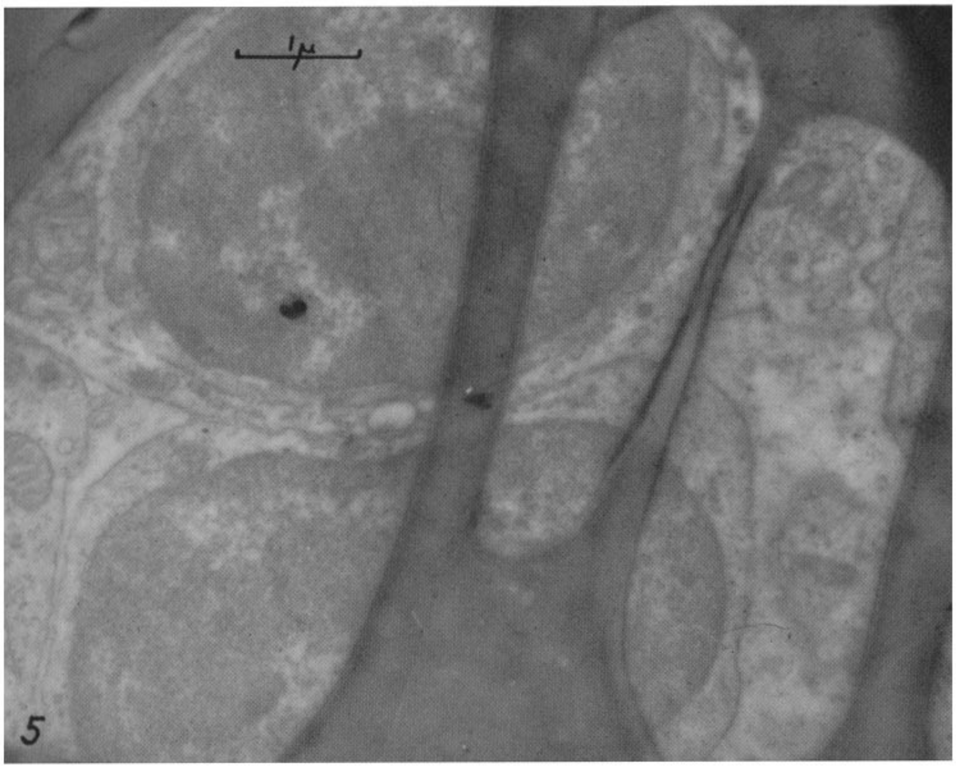

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