MAPPING OF LARGE AREAS WITH THE ELECTRON MICROSCOPE

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One of the limitations of conventional techniques in electron microscopy comes from the bars of the grids which periodically interrupt the field. This was pointed out by Gasser (1), for example, who attempted to get total fiber counts in cross-sections of cat nerves. Techniques have been developed to mount serial sections for electron microscopy (2), but it is often desirable to have uninterrupted fields for the examination of sections with appreciable dimensions in both axes. A more recent report gives a method for the preparation of unsupported thick films for the examination of large areas with the electron microscope (3). A similar method has been applied successfully in the investigation of total numbers of nerve fibers found in the cross-sections of various nerves, including submicroscopic ones.

Grids for unsupported films were prepared by cutting out about 40 grid squares from standard (2 mm.) grids with a sharp razor blade and flattening the cut edges of the copper against glass (see insert in Fig. 1). Formvar films were prepared on glass slides and floated upon a water bath in the usual manner. A number of Formvar solutions were tried, but it was found that the most successful films were prepared from two layers of 0.1 per cent solution. The special grids were placed upon the floating film and picked up together by moving a wire mesh down upon them and through the water. The films were then covered with a 100 to 150 A thick carbon layer. It was possible to prepare a reasonably large number of grids successfully in this manner. The sections were cut with a diamond knife on a Porter-Blum ultramicrotome and picked up squarely over the unsupported portion of the film. The sections were examined and photographed in a Siemens Elmiskop I electron microscope at magnifications of less than 1000 using the intermediate projector lens connected to a rheostat for continuous magnification. The magnification was selected in a manner such that the image of the 50 x objective aperture was just outside of the field of view. Focusing was achieved by removing the objective aperture and bringing the objective lens control to the point where the minimum contrast in the image was seen, and then replacing the aperture before taking photographs. Calibration of the magnification was accomplished by taking an area in the image at a higher fixed magnification and comparing the corresponding distances in the same region in the low magnification map. The accelerating voltage was 60 kv. and the condenser II lens was completely defocused. Nerves and nerve tracts up to 0.4 mm. in diameter were mapped by this technique. These included the circumesophageal connective, the nerves in the large chelated leg, and the ventral nerve cord in the crayfish, the circumesophageal connective in the roach, and the optic nerve of the mouse. Allowing for proper overlap of the fields, from 12 to 36 plates were required for each map. Fig. 1 shows a montage of the circumesophageal connective of the crayfish. The negatives were developed for 15 minutes in Finex-L developer and, following routine processing, were printed at a ¥ 3 enlargement. The prints were fitted together by matching each print with its neighbor, taping them loosely together with cellophane tape, and then cutting through the median line of overlap with a razor blade. After removing the cut-off overlap areas, the adjacent prints were applied together on the reverse side with cellophane tape. Because the magnification at the edge of a negative is slightly higher than at the center, it was not possible to achieve a perfect

1 Ansco, Binghamton, New York.
fit of adjacent prints in all cases. The finished montage was mounted on white board under glass and then rephotographed and reduced.

The significance of the results is of physiological interest and will be reported elsewhere. It can be said, however, that in the case of the map of the crayfish circumesophageal connective, it was possible to make an accurate count of practically all of the fibers as seen in cross-section, including many less than 0.3 \( \mu \) in diameter. A representative fine fiber bundle is shown in the insert. The classically reported counts (4) are now revised upwards by over 60 per cent. It is our experience that the method of preparing large montages as described above is so simple that we are considering using it routinely.

**SUMMARY**

Carbon-stabilized formvar films are strong enough to support entire sections over large areas of a copper grid from which some of the grid squares have been cut out. This technique has been utilized to produce electron micrograph montages of the following nerves: the circumesophageal connective, the chela nerve, and the ventral cord of the crayfish, the circumesophageal connective of the roach, and the optic nerve of the mouse. Direct counts of the nerve fibers in the entire cross-sectional area of these nerves, by including the submicroscopic ones, provides for the first time an accurate method for determining total numbers of nerve fibers.

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**REFERENCES**


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**FIGURE 1**

Reproduction of a montage reduced to approximately one-fifth size of 33 electron micrographs of the crayfish circumesophageal connective. Scale line is equal to 50 \( \mu \). Magnification, \( \times \) 500. Insert, placed in the axoplasmic area of a giant fiber, shows a fine fiber bundle approximately 2 \( \mu \) in width.

Insert in the upper left hand corner represents the modified grids used for unsupported films.