

A Simple Method for Orientating Small Objects for Sectioning, with Special Regard to Nematodes

BY

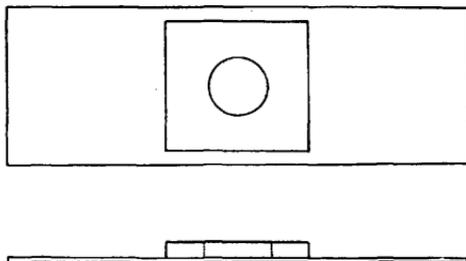
C. OVERGAARD

(From the Institute of General Zoology, University of Copenhagen)

With one Text-figure

FOR solving certain anatomical problems in free-living nematodes serial sections are necessary. Various methods of orientation proved unsatisfactory for these small objects (in this case 700μ in length and 30μ in width). The following method was worked out and found excellent:

In a drop of water the nematode is heat-paralysed, stretched at $50-60^{\circ}\text{C}$., and pipetted into Bouin's mixture (Graupner). After 24 hours it is transferred to 30 per cent. alcohol (4 hours), 50 per cent. alcohol (3 hours), a mixture of 50 per cent. alcohol and aniline (1:3) (1 hour), pure aniline (1 hour), bergamot oil (3 hours), paraffin wax I (3 hours), paraffin wax II (1 hour), in which it is embedded.



TEXT-FIG. 1

All the manipulations except the last one are performed in the usual way and practically no shrinkage takes place.

The difficulties in orientation preceding the embedding are overcome in the following way:

A square glass plate 2 mm. thick and with side of 22 mm., provided with a central circular hole of diameter 15 mm., is fixed by means of Canada balsam to a microscope-slide (see Text-fig. 1). When the Canada balsam has dried the hole is filled with the paraffin wax II, into which the object is transferred from paraffin wax I for orientation and embedding. By means of a heated needle the object is placed 1 mm. above the bottom in the paraffin

wax, which as far as possible is kept in a liquid state by cautious heating over a small flame. These manipulations are performed under a binocular microscope at about 40-fold magnification. Under these circumstances it is easy to orientate the object in such a way that the intended plane of section is exactly parallel with the bottom. When the object has been placed in position, the slide is immediately plunged into water of 10–12° C. (If the water has a higher temperature, the needles of solid paraffin will have the same order of size as the object, and a very loose and unsatisfactory embedding results.)

When the paraffin wax has become solid a block with sides about 5 mm. long is cut out enclosing the object.

A block of wood with a block of paraffin wax fused on it is placed in the microtome and sections are cut until a convenient area of its surface is plane.

By means of a forceps the object block with the plane lower surface downwards is held 5 mm. above the block in the microtome. A very hot lancet is placed in the interspace between the two blocks (without touching either of them). When the radiant heat has melted a fine film of paraffin wax on the two surfaces, the blocks are rapidly fused to each other. After cooling, the block-holder of the microtome is lowered 2 mm. by means of the screw and sectioning may take place. If the radiant heat has been applied accurately, the plane of section will be quite parallel to the plane lower surface of the block containing the object.

Thus an exact orientation (at magnification $\times 40$) is obtained and maintained during sectioning in a purely mechanical way without the drawbacks of a subjective estimation, which is practically impossible in the case of these small objects even if they have been stained beforehand.

By employing this method it has been possible to obtain exactly longitudinal sections in a large number of these small objects, which cannot be seen with the naked eye.

Exactly the same technique has been satisfactorily employed for the sectioning of *Protura* and *Mallophaga*—without the use of diaphanol.