I thank Mr. C. J. E. Phillips of the Department of Electrical Engineering, University of Sydney, for his assistance and advice.

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Precise Optical Thickness Measurement of Biological Objects

The measurement of the optical thickness of biological objects, such as cells, in the living state can yield a value for the dry weight of the object[1,2]. Such measurements, however, are difficult to carry out with great precision. A method has previously been described[3] for making very precise measurements of path difference in a reflecting interference microscope, using the principle of phase modulation. This principle can also be applied to the transmission microscope, and an experimental test of this has been made.

The instrument used was of the shearing type, by Vickers Instruments, Ltd. With the quarter-wave plate removed, this gives two superimposed fields of view. One of these is of the illuminated object, the other of the background, and the light forming these fields is polarized in mutually perpendicular directions. The primary image is formed by the objective on the diagonal interface of a split glass cube. This face is aluminized except for a small central rectangle, of such proportions as to seem square when seen at 45°, which 'straddles' the focal plane. Most of the light is thrown out to an eyepiece at the side, in which the image is seen with a small black square superimposed.

Light from the portion of the image falling on the unaluminized portion passes through the split cube and traverses a phase modulator and an analyser, finally entering a photo-multiplier.

The phase modulator is of the type previously described[3], consisting of a slice of a crystal, such as ammonium dihydrogen phosphate, which exhibits the Pockels effect, and provided with electrodes to which an a.c. voltage is applied. The output from the photo-multiplier contains an a.c. component which is detected in a phase-conscious rectifier, the reference voltage for which is derived from that actuating the phase modulator. As is well known, the d.c. output of the rectifier then passes through zero as the path-difference between the two interfering beams passes through an integral number of half wave-lengths.

The path difference can be varied by means of a Babinet-Soleil compensator placed in the beam before it enters the split cube.

The operation is as follows. The object is moved until the black square, as seen in the eyepiece, falls on the clear background outside the object, and the rectifier output set to zero by operating the compensator; the scale of the latter is read. The object is then moved again until a selected part of its image falls on the small black square, and the setting operation repeated. The difference of the scale readings is then proportional to the optical thickness of the object, with a constant of proportionality which can be determined in an obvious way.

There remains an ambiguity of an integral number of half-wave-lengths. This can be resolved by using white light and watching, through the eyepiece, the motions of the central dark fringe.

The size of the unaluminized region in the experiment corresponded to a 4μ square in the object space, using a ×40 objective. Using white light it was possible to make settings repeatable to 1/3,000 of a wave-length, which represented the accuracy to which the compensator scale could be read.

A precision of this order allows for the possibility of measuring the dispersion of optical thickness, thereby providing further information about the object being measured.

I thank Mr. F. H. Smith, of Vickers Instruments, Ltd., for loan of the interference microscope.

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Embedding and Staining Small Nematodes for Electron Microscopy

Investigations of the ultrastructure of nematodes have been restricted to the cuticle[1], testes[2], eggs[3] and muscles[4] of *Ascaris* spp., the intestine of *Ankylostoma caninum*[5], the cuticle of *Strongylus equinus*[6] and the cyst wall of *Heterodera rostochiensis*[7]. The detail of internal structures of small plant-parasitic or free-living nematodes is just beyond the resolving power of the light microscope and workers interested in the examination of these organisms are forced to speculate on the probable physiological activity of areas or organs the detailed structure of which they cannot properly perceive. Despite the obvious need for examination of these organisms under the electron microscope, little progress has been made.

Initial experiments carried out in this laboratory on *Meloidogyne javanica* have shown that the cuticle acts as a barrier to fixatives, dehydrants and embedding media. This coupled with the difficulties involved in cutting and orienting animals which are from 0·5 to 1·0 mm in length is partly why work on the ultrastructure of these organisms has not been extended.

Both ethanol and acetone have proved unsatisfactory dehydrating agents for *Meloidogyne javanica* in conjunction with methacrylate and 'Araldite' as embedding media. However, if the water-soluble aliphatic polyepoxide 'Durcupan *A*' is used[8] as a dehydrating agent after fixation and mechanical perforation of the cuticle, and an 'Epon'[9] mixture is used as an embedding medium, good results are obtained particularly if the sections are stained with potassium permanganate and uranyl acetate.

The method used is as follows: fix at 20° C in 2 per cent osmium tetroxide in 0·1 M phosphate buffer (*p*H 7·0) for 15 min; after 5 min in the fixative cut each nematode or perforate its cuticle; rinse quickly in 0·15 M sodium chloride, dehydrate and embed the nematodes according to the schedule given in Table 1, which is a modification of that described by Stäubli[8].

Finally place in 'Epon' mixture + accelerator in gelatin capsules at 60° C for several days, cut sections with an ultramicrotome and mount these on grids. Stain sections on grids in 1 per cent potassium permanganate : saturated

Table 1

Medium (20° C)	Time (min)
'Durcupan *A*' : water (70 : 30)	30
'Durcupan *A*' : water (90 : 10)	30
'Durcupan *A*' (100%)	45
'Durcupan *A*' : 'Epon' mixture (70 : 30)	30
'Durcupan *A*' : 'Epon' mixture (50 : 50)	30
'Durcupan *A*' : 'Epon' mixture (30 : 70)	Overnight
'Epon' mixture	30
'Epon' mixture + accelerator (*in vacuo*)	30