Determination of the Water Content of Nematode Worms by Interference Microscopy

The ability to estimate cell solids and cell water by interference microscopy depends on 2 things: first, the fact that the specific refractive increment, α (the increase in refractive index/1% increase in concentration) is essentially constant over a wide range of concentration; second, the specific increments for the main cell constituents, protein, amino acids, and lipo-proteins, are very similar. Irrespective of the relative proportions of these substances, therefore, the same value of α can be used, at any concentration, to estimate cell solids and water from refractive index. The value generally used, 0.0018[1,2], is somewhat lower than the increments for the classes of substances listed; this allows to some extent for the lower increments for fats, carbohydrates, and salts. Unless one kind of substance is solely present, the error is considered to be of the order of 5%[3,4].

Estimates are overall assessments, for cells are not uniform in structure and constitution; indeed, the method gains in accuracy from this fact, for the value of α used must be an average figure. There is no reason in principle, therefore, why the method should not be applied to whole organisms, although, as far as I am aware, this has never been done. Greater caution in the interpretation of the results is called for, but the same criteria are applicable; the main difficulties are of a practical nature: whether indeed, it is possible to use the technique with metazoa. In fact, I have found that nematode worms, with their circular cross section and transparent bodies, are very suitable material, and the interference microscope has been used to investigate problems of particular relevance to the ability of certain forms to withstand prolonged desiccation in cryptobiosis (anabiosis)[5,6]. A full account of some of the results will soon be published. In the meantime it was thought that it would be helpful to publish details of the techniques in order to indicate some of the possibilities of the method.

Early attempts, using a $^1/_4$ wave plate and analyser with the Smith-Baker instrument, were of little use. Apart from the difficulty, normally experienced I understand, of accurate matching at extinction, in much of the work optical path differences of 2 or even more wavelengths were encountered. The instrument was therefore used with a fringe field eyepiece usually with $\times$ 40 shearing objective. Living animals were examined in media of different refractive index. Water, and bovine ox plasma fraction V at various concentration in water[4], were commonly employed, sometimes with propylene phenoxetol narcosis[7]. If all superficial water was removed from a specimen, it could be examined in liquid paraffin (mineral oil). This medium, and benzyl benzoate with the high refractive index of 1.568, were also very useful for the examination of dry animals. The latter media could be 'displaced' from the specimens by the addition of water: they were apparently harmless and animals revived even after 1 h immersion in them.

A tungsten light source was used, together with a green filter when monochromatic light was needed: white light was of great help in the identification of fringes when there was considerable retardation by the specimen. With animals which were sometimes moving, sometimes changing in water content, it was found that measurements from photographic prints at standard magnification gave most satisfactory determinations of phase change and specimen thickness. Specimens were therefore photographed, as a routine, with a 35 mm reflex camera. The interference fringes do not possess sharp limits, but their centres are easily pinpointed at the mid line of the animal; on the other hand, estimates of specimen thickness may be difficult and it is therefore important to focus on the animal's margins.

Figure 1 shows a specimen of *Pangrellus redivivus*, mounted in water and photographed through a fringe field eyepiece using monochromatic light ($\lambda = 0.546\ \mu$). The interference fringes are displaced by amounts which vary with the thickness of the animal across its diameter. At the midline, where it is 31 μ thick, the displacement is

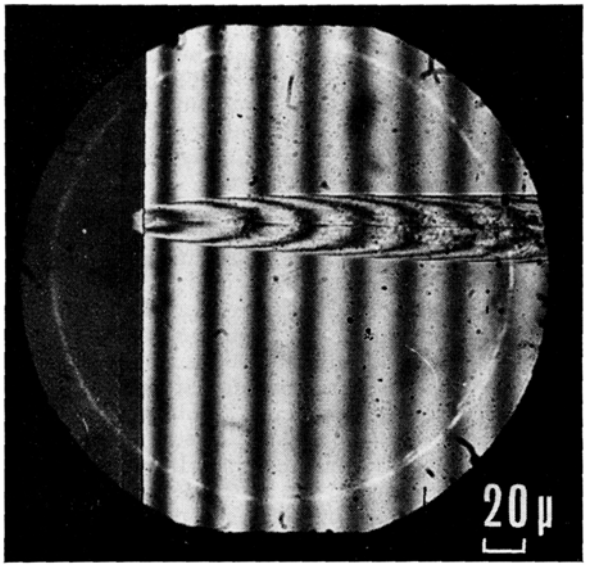


Fig. 1. Larva of *Panagrellus redivivus* in water. Photographed through fringe-field eyepiece of interference microscope.

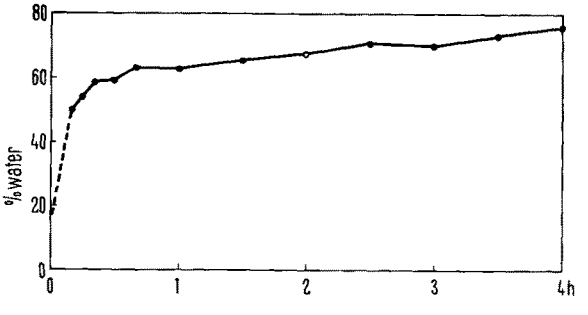


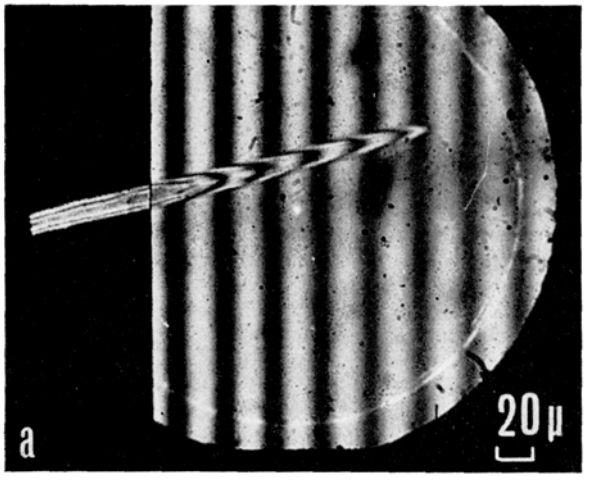
Fig. 2. Water uptake of a single larva of the potato-root eelworm, *Heterodera rostochiensis*. The first determination was made immediately the larva was freed from the egg-shell, 5 min after the dry egg had been immersed in bovine ox plasma. The broken line, indicating the probable water uptake during the first 5 min, is based on measurements of the water content of other dry larvae.

[1] H. G. DAVIES and M. H. F. WILKINS, Nature *169*, 541 (1952).
[2] R. BARER, Nature *172*, 1098 (1953).
[3] H. G. DAVIES, M. F. H. WILKINS, J. CHAYEN and L. F. LA COUR, Q. Jl. microsc. Sci. *95*, 271 (1954).
[4] R. BARER and S. JOSEPH, Q. Jl. microsc. Sci. *95*, 399 (1954).
[5] G. STEINER and F. E. ALBIN, J. Wash. Acad. Sci. *36*, 97 (1946).
[6] M. S. FIELDING, Proc. helminth. Soc. Wash. *18*, 110 (1951).
[7] C. ELLENBY and L. SMITH, Nematologica *10*, 342 (1964).

almost exactly 2 wave-lengths. The refractive index of the specimen at that point is therefore 1.370. If the specific refractive increment of 0.0018 is used, as is usual[3,4], the refractive index would correspond to a value for 'cell solids' of approximately 20%, or a water content of approximately 80%. This is in good agreement with preliminary determinations by more conventional methods[8]. Changes in water content may also be studied continuously on the same specimen (Figure 2)[9]; apart from other considerations, the comparative nature of such observations make assumptions about the value of α of less importance.

The nematode body is not homogeneous across its longitudinal axis, so that a measurement of refractive index at a particular point combines the separate values for cuticle, hypodermis, body fluid, gut, and whatever other organs are in line. Yet, clearly, the regularity of the fringes inside the animal, as shown in Figure 1 is consistent with considerable uniformity in refractive index; the retardation curve of each fringe inside the animal corresponds to the increase in thickness of the specimen to the mid line. An even better example is shown in Figure 3a, for a larva of the vinegar eelworm, *Turbatrix aceti*, which compares favourably in regularity with a glass fibre (Figure 3b). This, however, is not always the case: in certain stages of some species, the intestine, filled with food reserves, is a region where the fringes are difficult to follow. Occasional local differences are clearly shown (note the cuticular lining of the gut in Figure 1) and may be exploited. For example, the water content of the larvae inside the body of the mother in such forms as *Turbatrix* and *Panagrellus* has been estimated and the survival value of the 'viviparous' habit clearly demonstrated: after drying for 10 min at 40% relative humidity, the water content of the female was about 12% while that of the larvae was about 33% (Figure 4).

When an animal is so wide that the secondary image overlaps the primary, measurements may sometimes be made on thinner regions. Measurements on the sharply delimited tail of the marine *Enoplus brevis* indicate a water content of 80%, due allowance being made, of course, for the refractive index of the sea-water in which it is immersed[10].



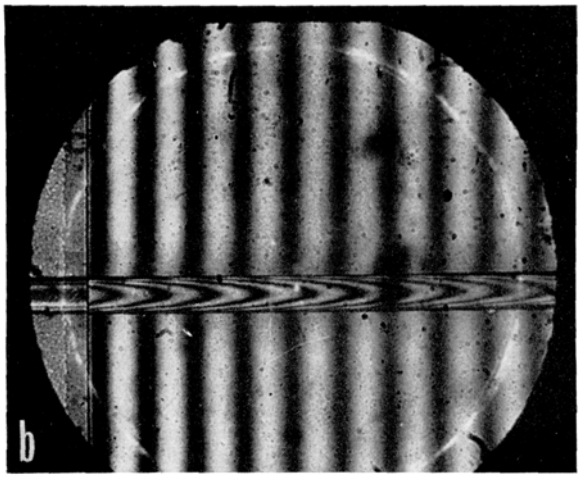


Fig. 3. (a) Larva of vinegar eelworm, *Turbatrix aceti*, in vinegar. (b) Glass fibre in bovine ox plasma.

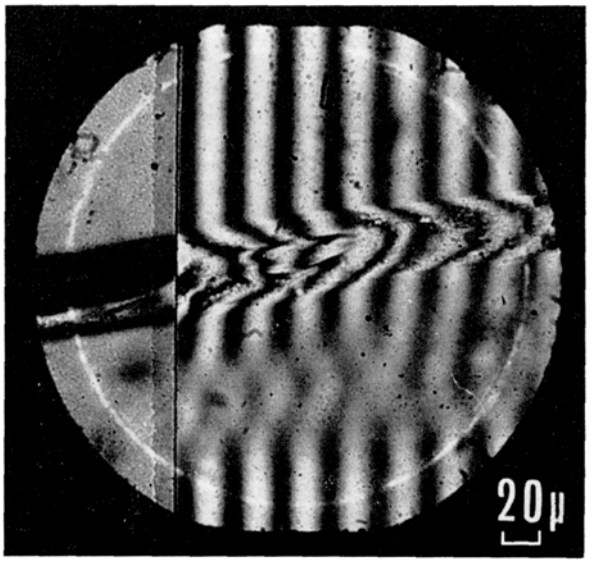


Fig. 4. Dried female of *Turbatrix aceti*, with larva in utero. The higher water content of the larva is indicated by the lower displacement of the fringes where the larvae are present. In liquid paraffin.

Zusammenfassung. Das Interferenz-Mikroskop ist hier zum ersten Male bei Metazoen, nämlich lebenden Nematoden, zur Messung des Wassergehalts benutzt worden. Die Resultate werden beschrieben und die Möglichkeiten weiterer Anwendung diskutiert.

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[8] C. Ellenby and L. Smith, unpublished.
[9] C. Ellenby, Proc. R. Soc. B, in press.
[10] A grant from the Royal Society of London is gratefully acknowledged.