

LETTERS TO THE EDITORS

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Interference Microspectroscopy

ONE of the main advantages of interference microscopy over phase-contrast microscopy as normally practised is that certain forms can be used for the quantitative determination of the phase change or optical path difference introduced by the object¹. Not all types of interference microscope, however, are equally suitable for quantitative work, as the phase difference between the interfering beams cannot always be varied continuously. It is therefore useful to have an alternative method of measuring the phase change.

It is well known that, if light from an interferometer system is viewed through a spectrograph, a series of dark bands can be seen crossing the spectrum when a source of continuous radiation is used. When the phase difference between the interfering beams is uniform throughout the field of view these dark bands are straight; but when additional path differences are introduced, as by a refractile object, the bands become displaced and distorted in a manner related to the phase change produced by the object. Thus, if the image of a transparent object such as a cell is projected through an interference microscope on to the entrance slit of a spectrograph, the displacement of the dark bands gives a convenient method of determining the phase change produced at each point of the object the image of which is formed along the slit.

The technique used is similar to that described elsewhere for ultra-violet absorption microspectroscopy². The measurement of the phase change in the object can be applied to the determination of mass as recently described³. An unexpected advantage of the method is that the contrast of the bands seen in the spectrograph is much greater than that of the interference fringes in the field of view. Indeed, this type of method is often used for demonstrating the presence of interference effects which may be suspected but cannot be otherwise detected. This is particularly the case when white light is used and when the path difference between the interfering beams is great. The accompanying photograph illustrates this point. The object was an unstained human oral epithelial cell, mounted in water between a semi-rhodiumized slide and coverslip, as suggested by Merton⁴. A tungsten filament lamp was used as source and the separation between slide and coverslip was rather large. The full illuminating aperture of the condenser was used. In these circumstances, no interference fringes whatsoever could be seen in the field of view and the object, being transparent, was virtually invisible. Fringes were quite easily seen in



Interference microspectrograph of epithelial cell, in visible region, taken under conditions of zero contrast in field of view of microscope

the spectrograph, however, and their contrast could be enhanced photographically without loss of detail.

We thus have the possibility of obtaining precise optical information concerning an object which cannot be seen under the microscope. Moreover, since the full illuminating aperture is used, the resolving power of the microscope itself is high. The contrast, which may be very low or even zero in the microscope, can be largely recovered in the spectrograph. If the apparatus is adjusted to give good fringes and high contrast in the microscope field of view, the contrast in the spectrograph is very much better than that shown. It is not essential to use a tungsten filament lamp, as most high-pressure mercury lamps emit sufficient continuous radiation between the spectral lines to enable the dark bands to be seen. Such lamps are generally preferable for visual work.

If the object contains absorbing material, anomalous dispersion may occur in the region of absorption bands, so that the refractile and absorbing properties may be simultaneously observed. Finally, at those points which introduce zero phase change, the refractive index will be equal to that of the mounting medium at the same wave-length.

I wish to acknowledge the help received as Johnston, Lawrence and Moseley Research Fellow of the Royal Society.

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March 1.

¹ Dyson, J., *Proc. Roy. Soc., A*, **204**, 170 (1950).

² Barer, R., Holiday, E. R., and Jope, E. M., *Biochim. Biophys. Acta*, **6**, 123 (1950). Barer, R., in "Cytology and Cell Physiology" (edit. G. H. Bourne, 2nd edit., Clarendon Press, Oxford, 1951).

³ Barer, R., *Nature*, **169**, 366 (1952).

⁴ Merton, T., *Proc. Roy. Soc., A*, **189**, 309 (1947).

Thermal Measurements on Small Transient Systems

EXPERIMENTS recently carried out in this laboratory have shown that it is practicable to heat the wires of a thermocouple by the passage of an electric current and at the same time to record the temperature of the junction by means of the thermo-E.M.F. A high-frequency current is used in order that the concomitant voltage (order 10 volts) may be separated from the thermo-E.M.F. (order 0.001 volts) by means of inductance-capacitance filters. The apparatus can be used for measuring the thermal constants of poor conductors placed in contact with the couple wires.

It has been found convenient to pass a pulse of current (after which the system returns to its original state) and to make deductions from the E.M.F.-time record obtained during the heating period. If the pulse is long enough for a steady state to be achieved, the highest temperature is inversely proportional to the thermal conductivity; otherwise both conductivity and specific heat have to be considered. Calculations made by Dr. S. Paterson and Miss Jean

Substance with which wire was in contact	Proportion of energy retained by wire after 96 msec.	
	Observed	Calculated
Medicinal paraffin	0.57	0.52
Castor oil	0.48	0.48
Glycerol	0.33	0.38
Water	0.19	0.22