On a method of increasing contrast in microscopy

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[Plate 7]

When an object is viewed under the microscope, its visibility depends on its opacity, its colour and the extent to which it differs in its refractive index from the surrounding medium. There are a number of methods by which the contrast of a transparent object, whose refractive index differs slightly from the surrounding medium, can be enhanced. These include dark-ground illumination and, in the special case of bi-refringent materials, the use of polarized light. The most recent and successful addition to these methods is the phase-contrast technique which owes its inception to Zernike (1934) and has since been the subject of investigation by a number of other workers.

It has occurred to me that a powerful method of increasing contrast in the case of transparent substances, in particular certain specimens of biological interest, would be provided by placing the object under investigation between two half-silvered or half-metallized surfaces which would thus constitute an interferometer. Our knowledge of the interference phenomena which occur under such conditions has been greatly enlarged in a recent series of papers by Tolansky (1943a, b, 1944a, b, 1945, 1946).

For the present purpose it may be noted that a large number of effective reflexions between the plates, giving rise to very narrow fringes, is not essential. The first experiments were carried out by half-silvering two plates of glass (which were not optically flat) so that the amount of light reflected from a surface was about 40 to 50%. A small amount of pyroxyline was dissolved in amyl acetate and a fine spray was allowed to fall on one of the silvered surfaces by the use of an atomizer. When the amyl acetate had evaporated, the surface of the plate was covered with a large number of thin flat disks of pyroxyline. A drop of water was put on the plate and the second plate was firmly pressed down, the water which was driven out being removed with a piece of filter paper. The plates were put on the stage of a microscope and examined with a low-power objective, the source of light being a mercury arc lamp. The two yellow rays were absorbed by a thick sheet of didymium glass, the visible rays being in effect limited to the green line at \(\lambda 5461\) A and the violet line at \(\lambda 4359\) A. If the two plates had been optically flat and parallel to one another the field would (in the absence of the pyroxyline disks) have been uniform in colour, but as they were not perfectly flat the field was crossed by a number of broad bands which formed a kind of contour map showing the distance between the plates. With glass of
reasonably good quality these bands were for the most part broad in comparison with the objects under investigation. At many parts of the field the colour alternates from green to violet, whilst at some points there was an approximate coincidence between the maxima and somewhat neutral bands with intervening dark spaces were seen. The pyroxyline disks presented a remarkable appearance, being in general conspicuously different in colour or intensity to the particular part of the background against which they were seen. A disk might be seen as a green circle on a violet background or vice versa, and the thickening which occurred at the edges of the disks was manifest in many cases from the appearance of a narrow circle of different intensity or colour. These pyroxyline disks, when examined in the same way on unsilvered glass, were difficult objects to see. At this stage of the investigation the use of silvered surfaces was abandoned. Whilst these half-silvered surfaces are of the highest efficiency from an optical point of view they were so easily scratched or otherwise damaged that it was found preferable to use half-platinized surfaces in which the platinum had been "burnt" into the glass and which were not impaired by any ordinary usage. The half-platinized surfaces were prepared by a slight modification of the method described by Rheinberg (1920). The plates were flowed over with a solution consisting of chloroplatinic acid 2·1 g., collodion 2 g., ethyl alcohol 77 c.c., acetone 33 c.c., which was compounded by dissolving the chloroplatinic acid in the alcohol and the collodion in the acetone and then mixing the two solutions. When the plate had been allowed to dry in a warm place it was put on a flat plate of stainless steel about $\frac{1}{2}$ in. thick, a foot long and 4 in. in width. One end of the plate was heated by means of a bunsen burner to a temperature estimated at 500 to 600° C and the plate gradually pushed from the cold end to the hot end until the collodion had burned off and the platinum was left as a mirror on the glass. After cooling, the plate was flowed over with a similar solution in which about 0·2% of bismuth chloride with a few drops of hydrochloric acid was substituted for the chloroplatinic acid. On burning off the collodion a slight bloom was left on the platinum, but this bloom was removed by waving the bunsen flame over the surface until it disappeared. With a little practice the thinnest microscope cover-glasses (about 0·17 mm. in thickness) can be successfully coated without distortion and the surfaces are remarkably durable and permanent.

Certain difficulties arise when objectives of short focus and large N.A. are used. Since the rays pass through the plates in a wide cone, and since the difference of path of the successively reflected beams is $2d\mu \cos \theta$, where $d$ is the distance between the plates, $\mu$ the refractive index of the medium and $\theta$ the angle which the rays make with the normal, it follows that the colours are diluted to a degree depending on the angle of the cone subtended and the distance between the plates. Moreover, since the interferometer constitutes an angular filter it follows that the objective cannot be uniformly illuminated, with the consequent loss of some resolving power. There are several methods which might be adopted to mitigate this difficulty, and it is proposed to make further experiments in this direction; possibly by the use of an annular stop or a zone plate immediately below the substage condenser.
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I have found, however, that the following rather inelegant and unorthodox but very simple method gives excellent results. A piece of ground glass is placed on the stage of the microscope with the ground surface uppermost, or alternatively the underside of the platinized slide is ground. The image of a mercury lamp after traversing the didymium glass filter and after reflexion from the mirror of the microscope is brought to a focus on the ground-glass surface by means of a lens of 1½ in. focus working at f/3·1. This results in the objective being illuminated in a manner determined by the polar curve of the ground glass, the greatest intensity being along the optical axis. This method has been used with objectives down to 4 mm. focus, and highly coloured fringes with good definition have been obtained.

As a test object I have, following Burch & Stock (1942) used wet epithelial cells from the dorsum of the tongue. The cells are easily detached from the surface of the tongue, and a drop of the saliva containing them (with as few bubbles as possible) is laid on the platinized slide. The cover-glass is laid down on the drop and gently squeezed down under a piece of filter paper which removes the liquid which exudes from the sides. Many of these cells show very poor contrast when viewed in the usual manner by transmitted light, and indeed some of them can only be found with difficulty, but when seen between the half-platinized plates they are even more striking than the pyroxyline disks; the structure can be seen in great detail, and variations in thickness are clearly shown as changes in colour or intensity. I have not yet had the opportunity to try these methods with a 2 mm. oil-immersion objective, but there seems to be no reason to suppose that good results will not be obtained.

For practical purposes the method seems to be limited to specimens not exceeding a few microns in thickness. For greater thicknesses the loss either of contrast or of resolving power seems prohibitive. For the highest degree of contrast it would be necessary to illuminate the specimen with parallel or nearly parallel light. This would reduce the resolving power to a degree which would seriously limit the application of the method. The distribution of intensity over the objective resulting from the use of the ground glass provides a useful compromise between loss of contrast and loss of resolving power: but it is essential that the distance between the semi-reflecting surfaces should be small.

There is another method which may be useful occasionally for the study of very small particles in the case in which they can be examined in a dry state.

When a film of cellulose nitrate or cellulose acetate is laid on a plate of glass with a film of water between the cellulose film and the glass, the water will evaporate through the film, which is left in optical contact with the glass. Owing to the contraction of the film on drying it is necessary to paint over the junction of the film and the bare glass with a solution of gelatine which prevents the film from curling up and detaching itself from the glass on drying.

It is possible to prepare films of silvered or platinized cellulose material by silvering or platining a sheet of glass (in the case of platining the treatment with bismuth must be omitted) and by pouring over the surface, for example, a 2½ % solution of cellulose nitrate in amyl acetate. When the solvent has evaporated the
film can be detached from the glass and usually carries the silver or platinum with it. In a similar way metallized films of cellulose acetate can be prepared by the use of a 15\% solution of cellulose acetate in acetone which is spread over the silvered or platinized glass with a steel straight-edge with copper wires of 30 to 40 s.w.g. as distance pieces along the edges of the glass.

If a piece of half-metallized film prepared in this way is laid on a half-metallized sheet of glass with a film of water between the surfaces and the water is allowed to evaporate, the two metallic surfaces are left in optical contact. If the water between the film and the glass contains any solid particles the cellulose film is raised by these particles on drying, and on examining the plate through a microscope with a mercury arc as a source of illumination, each particle is seen to be surrounded by a concentric series of interference fringes. By counting the number of rings and estimating the fraction of a ring, the thickness of the particle can at once be determined, \( N \) rings denoting a thickness \( \frac{1}{2} N \lambda \).

In the same way a fragment of a fibre will be flanked by a series of linear fringes which follow the contour of the fibre and which provide a measure of its thickness.

The fringes which are produced in this way differ conspicuously from the Newton fringes which are seen when a half-silvered convex surface is laid on a half-silvered plate. Owing to the manner in which the cellulosic film is distorted by the particle, the fringes are closest together at the centre of the system and get wider and wider apart until zero difference of path is reached at a distance from the centre at which the two metallic films pass into metallic contact.

It will be seen, however, that the exact law governing the separation of the rings is irrelevant in so far as the determination of the thickness of the particle is concerned, since it is only their number and an estimate of a fraction of a ring which is required. If the mercury line \( \lambda = 4358 \) A is used the first maximum would denote a thickness of 0-00022 mm., whilst a particle which raised the film to a height such that the first minimum fell on the centre of the particle would have a thickness of half this value or 0-00011 mm. The method provides a simple means of measuring very small particles and fibres with a considerable degree of precision.

There is, however, a further consideration which may prove to be of greater importance. A perceptible darkening can be observed at a separation which is much smaller than the separation corresponding to the first minimum, and it seems possible that with a sufficiently powerful source of illumination and films of high reflecting power particles as small as \( 10^{-8} \) mm. might be detected.

It is hoped that these methods may find some useful application in the biological field.

Note added December 1946. Since this paper has gone to the printers my attention has been called by Dr C. R. Burch, F.R.S., to the work of A. M. Frederikse (Acta Brev. Neerl. 111, 8/9 (finit 10, x. 1933)) in which the interferometer has been applied to the study of biological specimens. Frederikse's paper covers a part of the work described in this communication and I am glad to be able to call attention to his work.
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REFERENCES

Tolansky, S. 1943b Phil. Mag. 34, 555.
Tolansky, S. 1944b Phil. Mag. 35, 120.

DESCRIPTION OF PLATE 7

Figure 1. Epithelial cells from the tongue with groups of bacteria between half-platinized surfaces photographed in the light of the two mercury yellow lines. These cells are colourless and transparent (imperfect focusing).

Figure 2. The same cells photographed in mercury green light (bacteria nearly invisible).

Figure 3. The same cells photographed in mercury violet light (× 450).

Figure 4. Two particles of rouge, showing ten and one complete rings respectively, between a platinized pellicle of cellulose acetate and a platinized plate.

The spectra of flames containing oxides of sulphur

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(Communicated by Sir Alfred Egerton, F.R.S.—Received 30 May 1946)

[Plates 8 and 9]

The spectra and characteristics of hydrocarbon and related flames containing small amounts of SO₂ and SO₃ have been studied; observations have been made with a view to elucidating the mechanism of carbon formation in flames.

Band systems due to Sₓ, SO, CS and SH occur; this is the first record of the SH band in emission. The mechanism of the formation of these radicals is discussed. The outer cones of flames containing SO₂ show strong ultra-violet continuous emission, and this is provisionally attributed to direct association between SO₂ and atomic oxygen to give SO₃. Failure to obtain luminous reaction between SO₂ and atomic oxygen in a discharge tube at room temperature indicates that this reaction requires an activation energy. The formation of SO₃ cannot be used as a quantitative test for atomic oxygen in flames.

SO₃ increases carbon deposition in hydrocarbon flames and it is considered that it can induce chain reactions leading to the polymerization and decomposition of hydrocarbons. These chain reactions are maintained by free radicals which may be produced either by direct reaction of sulphur trioxide with hydrocarbons or indirectly through the formation of peroxides. SO₃ and H₂S, which reduce carbon deposition, either inhibit the chain processes or remove carbon as soon as it is formed.
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