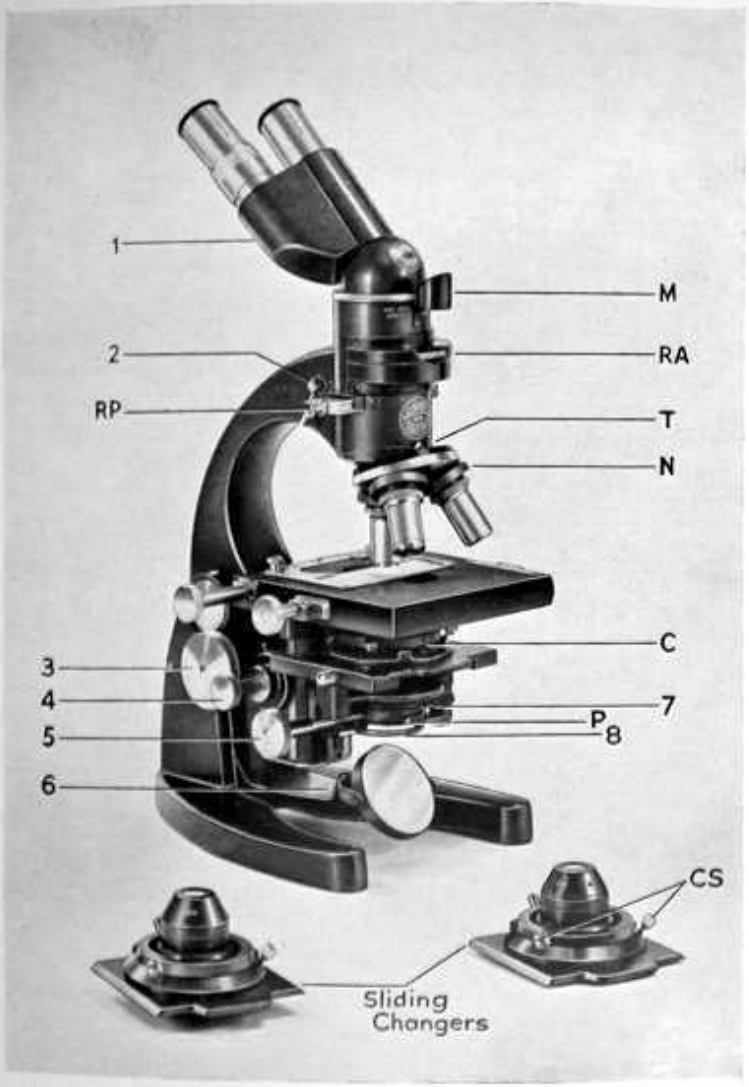


PLATE 1

1: Binocular eyepiece. 2: Body-tube lock. 3: Coarse adjustment. 4: Fine adjustment. 5: Substage focus. 6: Mirror bracket. 7: Substage iris. 8: Filter ring.

RP: Quarter-wave plate. M: Scale magnifier. RA: Rotating analyser. T: Tubelength corrector (on left). N: Nosepiece. C. Condenser. P. Polariser. CS: Condenser-adjusting screws.

# THE BAKER INTERFERENCE MICROSCOPE

SECOND EDITION

C. BAKER of HOLBORN LTD
Metron Works, Purley Way, Croydon, Surrey
and
244 High Holborn, London, W.C.1
1955

FIRST EDITION 1954
SECOND EDITION 1955

**ERRATA**

Page 3, line 12 : read "...the iris diaphragm (7) "
Page 17, line 35 : read "...mixture of different phase-relationships "
Page 22, line 23 : read " ..simultaneously or alternately "
Page 23, line 17 : read " striae " for " strike "
Page 28, line 34 : read "...not exceed $\lambda/2N$
Plate 8, captions : read " *Upper : Right hand side,*"
" *Lower :* Area immediately to the *left*..."

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CONTENTS

## ACKNOWLEDGMENTS

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For the photomicrographs on Plates 3 and 4 we are indebted to Dr. Ambrose, and for Plate 5 (D) to Dr. J. M. Mitchison and Professor M. M. Swann and the *Quarterly Journal of Microscopical Science*. We are grateful for facilities kindly afforded us for photomicrography by Dr. Ambrose at the Chester Beatty Research Institute and by Professor Causey and Mr. Edwards, Department of Anatomy, Royal College of Surgeons of England.

C. BAKER OF HOLBORN LIMITED.

1 September, 1955.


*The Baker Interference Microscope is the subject of British Patent 639014 and U.S. Patent 2601175. As improvements in design are introduced from time to time, illustrations used in this book may not correspond in every detail to an instrument as delivered.*



# PART ONE

## Introduction

INTERFERENCE microscopy is a method for examining phase changing specimens which has been known and discussed for a considerable time, but a general appreciation of its practical value has arisen only of recent years, largely as a result of experience with the now well-established phase contrast technique of Zernike.

Phase contrast and interference microscopy both provide intensity contrasted images of phase changing specimens by means of optical interference phenomena, but the important characteristic of the latter method is that the mutually interfering beams which produce the contrast are generated by an interferometer system incorporated into the microscope itself, thus avoiding dependence upon diffraction by the object structure.

Two advantages follow immediately from this independence. First, that contrast can be obtained for features causing phase changes which are too gradual to diffract an adequate proportion of the light outside the phase step of a phase contrast microscope, thus enabling phase gradients as well as abrupt changes to be perceived. This results in an image which brings out the general morphology of the specimen better than phase contrast does, and which bears a resemblance to that obtained by staining methods. Secondly, the direct illumination need no longer be restricted to a narrow portion of the aperture, thus avoiding the well-known artifacts that result from severe stopping-down, such as the confusing images of the substage stop formed by features which are partly out of focus.

The foregoing are of undoubted advantage from the observational standpoint, but of *even greater importance* are the facilities that the instrument affords for measuring the precise amount of phase change produced. It is possible to see phase changes of less than 1/120 wavelength, and under good conditions changes of 1/300 wavelength have been observed.

The interference microscope thus permits both observation and measurement of the various phase changes produced by transparent objects and this alone is often extremely valuable; but this value is greatly enhanced if the phase changes produced by objects can be converted into, for example, biochemical information about the total protein content of a living cell, or its water content. How this particular information can be obtained from phase measurements has been recently disclosed by workers in this field. It would be impossible to attempt a review of this valuable work in the confines of this publication, but formulae are given in Part Two for converting the measured phase relationships into refractive indices, and in addition the reader is referred to the papers listed in the Bibliography.

*Description of the Instrument*

The optical design of the Baker interference microscope was developed by Mr. F. H. Smith in the firm's laboratories; while in mechanical design it closely follows the Baker Series 4 instruments, with important modifications to meet the specialised requirements of an interference microscope (Plate 1). The Series 4 microscopes are described in a separate publication, but it may be convenient to recapitulate their chief characteristics for reference here.

Coarse and fine focussing movements operate on the stage, while the body-tube remains stationary, although its position in relation to the stage of the instrument can be changed by releasing the lever on the right hand side of the limb and sliding the tube in its dovetails. The lever operates a cam and locking pad which hold the tube firmly. The body should be firmly supported by the left hand whilst this adjustment is being made. This arrangement of fixed body and focussing stage has distinct advantages, particularly in photomicrography. Focussing controls are in a convenient, low position, enabling the hands to be rested on the bench; whilst the milled heads for coarse and fine adjustment are placed in close proximity, so that both can be reached by a single movement of the hand.

A Swing-out polariser. The rotation of this element controls the intensity relationship between the double-refracted beams, permitting the out-of-focus image to be extinguished for normal transmitted-light conditions.

B Double-refracting plano-concave lens.

C The double-refracted rays entering the Abbe condenser.

D Double-refracting plate cemented to the front lens of the condenser rendering it bi-focal.

E Double-refracting plate rendering the objective bi-focal.

F The re-combined double-refracted rays.

G Quarter-wave plate.

H The re-combined rays circularly polarised in opposite directions by the quarter-wave plate.

J Rotatable analyser, with swing-out section, calibrated in degrees.

K The phase relationship between the circularly polarised rays is adjusted by the analyser.

L Final image exhibiting interference between the in-focus image of the object superimposed upon the out-of-focus image.

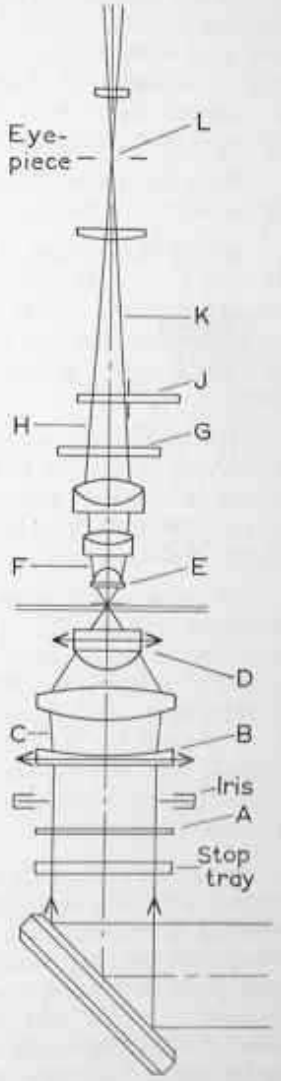



PLATE 2

General diagram of the layout of the Baker Double-refracting Interference Microscope.

In the case of the interference microscope, each objective requires its own special condensing system. To facilitate the easy and rapid change of condensers the well-known sliding type of changer after Akehurst is provided, in which each unit is mounted on its own dovetailed plate, which slides into its corresponding fitting on the substage.

Plate One shows the general appearance of the interference microscope and the positions of the different parts.

Below the condenser (C) is situated the polarising plate (P) with a lever to control its orientation, and mounted on a hinged bracket to enable it to be swung out of the axis. Above the polariser is the iris diaphragm (D) and below it the tray for colour filters, as found in conventional substage arrangements.

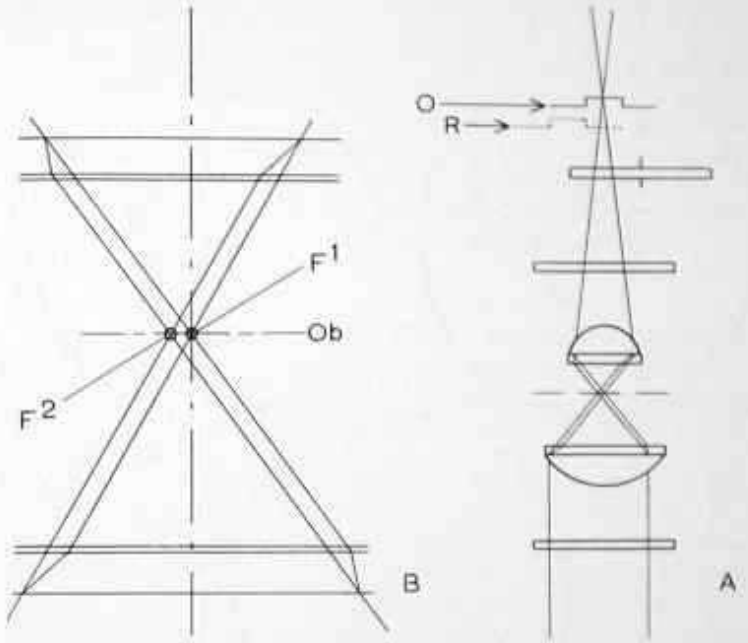
The condensers are pre-centred and placed in their correct orientation in relation to their changer slides, and *no attempt should be made to unscrew any part of them.* The pairs of knurled screws (SC) on the upper surfaces of the plates control the tilt of the systems, as explained below (page 22).

The objectives are mounted on the usual type of rotating nosepiece changer (N). Unlike normal objectives, *these must never be removed* from their nosepieces and are, in fact, pinned in position to prevent this accident from happening. In order that other objectives may be used on the stand, provision is made for the entire nosepiece with its objectives to be removed from the body-tube on dovetail slides and replaced by other nosepieces containing alternative interference objectives or normal objectives.

Situated in the body-tube above the nosepiece is the tube-length correcting lens (T) for the binocular tubes. This is mounted in a swing-aside fitting controlled by a lever; on the left side of the body and above it is the quarter-wave retardation plate (RP) on a slide marked with the 'in' and 'out' positions. Above the quarter-wave plate is the rotating analyser (RA) graduated in degrees and with its 'out' position marked by a red line on the graduation circle.

Interchangeable monocular and binocular eyepiece fittings screw into the upper end of the body-tube; when the former fitment is in use the tubelength compensating lens *must be*






TEXT-FIGURE 1

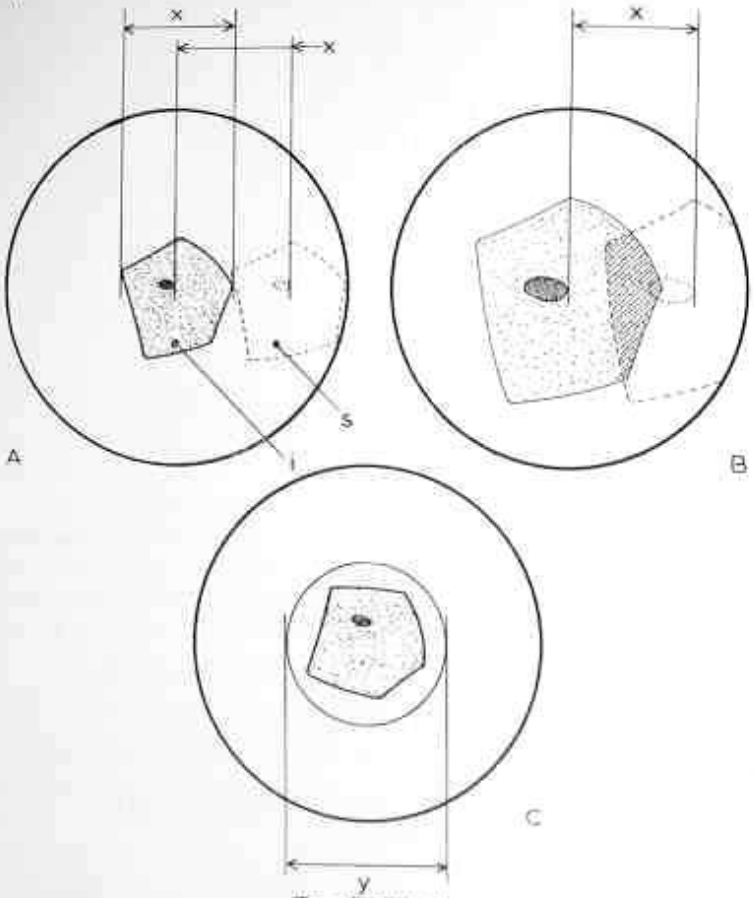
Diagrammatic representations of the paths of rays through a shearing system (not to scale).

A: The basic parts of the instrument, see also Plates 1 and 2.
O: object wave with focus profile.
R: reference wave with sheared profile.

B: Enlargement of the space between the condenser and the objective.
F¹: Object focus.
F²: Reference focus
Ob: Object plane.

*swung aside.*

The mirror bracket plugs into the tail of the limb by a three-pin fitting and is thus readily removable if it is desired to use the microscope in a horizontal position or on a Projectolux Base. For photomicrography the Projectolux camera unit is available. Full particulars of the Projectolux and camera unit can be furnished on request.




TEXT-FIGURE 2

Diagrams to illustrate the appearances seen with the shearing and the double-focus systems.

A: Shearing system showing complete separation of the true image (i) and the sheared one (s).
$x$ = separation between optic axis and centre of reference area = maximum size of cell which can be viewed without overlap.

B: Shearing system showing some overlap between the true image (i) and the sheared one (s).
$x$ = separation between optic axis and centre of reference area. Shaded portion represents that area of the true image overlapped by the sheared image and therefore invalid for mensurement.

C: Double-focus system:
$y$ = diameter of the reference area available for making measurements on the cytoplasm of the cell shown, the size of which must be less than the circle of diameter $y$.

*Note*: The numerical values of $x$ and $y$ for the different objectives are given on page 6. (Diagrams not drawn to scale).



### *Choice of Objective System*

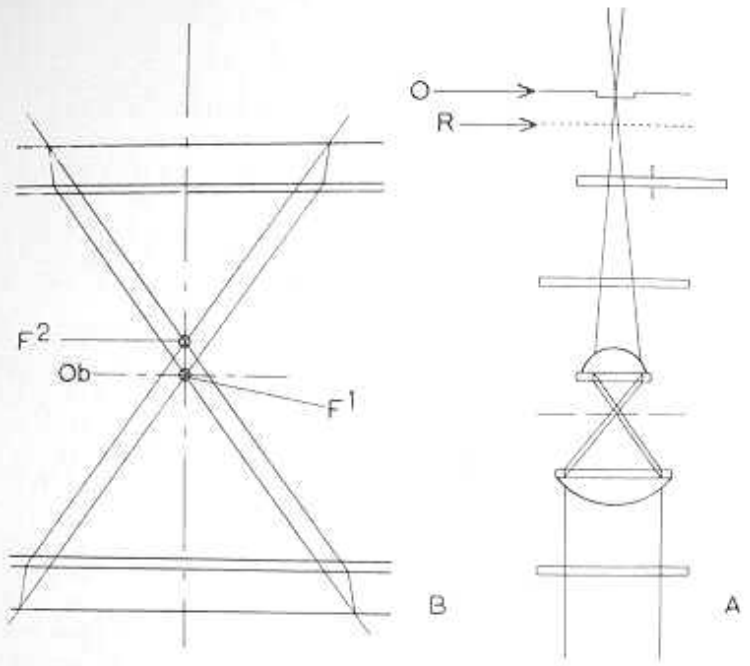
The *Shearing System* objectives are designed for making sensitive measurements upon separated features, e.g., single cells; but they are not so well suited for continuous specimens such as sections: for these the *Double Focus* systems are more suitable, but measurements with these will not be quite so accurate as with the former. The following notes explain the essential optical characteristics of these two alternatives.

### *The Optical Systems*

*(a) Shearing System.* Text figure 1 illustrates this form of the instrument. The same portion of the luminous source is imaged by the condenser in two laterally separated areas in the region of the object, while the subsequent effect of the special form of objective brings the images in these two separated areas into coincidence in the image plane, which consequently contains a correctly focussed image of the specimen superimposed upon a mutually coherent laterally displaced reference area. These interfere in a manner which is controlled by the double-refracting phase-shifting system, which also permits the interference contrast to be varied between positive, central darkfield and negative contrast.

The *Reference Area* with which any given feature of the object is interferometrically compared lies in a laterally displaced region of the field, isolated from the object feature, see Text figure 2. Thus, the shearing system permits a complete spatial segregation between the object feature and the reference area, when the former is below a size dependent upon the character of the objective in use.

In the case of the ×10 shearing objective, the centre of the reference area is separated from the optic axis by a distance of approximately 330 microns; for the ×40 system, this distance is about 160 microns; and for the water immersion × 100 system, the corresponding distance is about 27 microns. These dimensions signify that for the three systems, objects of up to 330$\mu$, 160$\mu$, and 27$\mu$ respectively in diameter can be viewed *without any overlap* between the object and its reference area. Complete freedom from overlap, however, is not as a rule necessary, and measurements can usually be effected in *any area free from overlap*. Text figure 2 (A, B).




TEXT-FIGURE 3

Diagrammatic representations of the paths of rays through a double-focus system (not to scale).

A: The basic parts of the instrument, see also Plates 1 and 2.
O: object wave with focus profile.
R: background reference wave.
B: Enlargement of the space between the condenser and the objective.
$F^1$: Object focus.
$F^2$: Reference focus.
Ob: Object plane.

(*b*) *Double-Focus System.* Text figure 3 illustrates the optical layout of the microscope for this system, in which the optical properties of the matched condenser and objective pairs impart double-focus effects.

The double-focus systems differ from those already described in that the reference area for any given feature of the object, instead of being located in a laterally displaced region,



surrounds the feature, Text figure 2 (C).

The resulting images interfere in a manner which again is controlled by the double-refracting phase-shifting system.

Instructions for setting up and using the Interference microscope are given in Part 3, but the following section, explaining some basic theoretical principles, may be of assistance when using the instrument for more precise quantitative work.

## PLATE 3

Chromosomes in living cells of *Locusta*.

Taken with × 40 shearing objective.

*Upper*: Analyser of interference microscope set for positive contrast.

*Lower*: Analyser set for negative contrast.

(i) Cell at *lower* left centre.

Resting stage showing fine chromosome threads.

Note difference in contrast of nuclear sap (low refractive index).

(ii) Cells at *lower* right centre and extreme right.

Leptotene stage with fully developed single chromosome threads.

(iii) *Upper* centre.

Pachytene stage. Homologous chromosomes have paired at corresponding points and the transverse bands along the length of the thread can be seen. Massive X-chromosome at top of nucleus.

(*Courtesy of* Dr. E. J. Ambrose,
Chester Beatty Research Institute, London.)

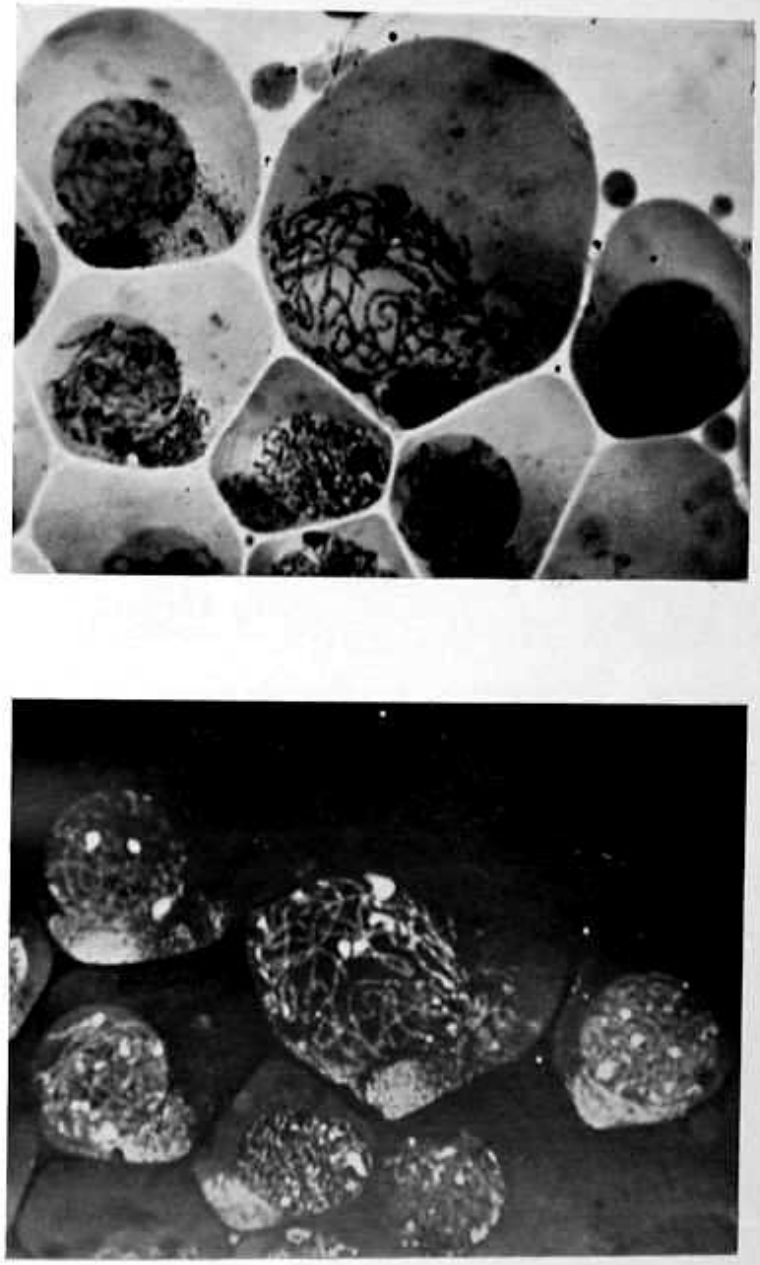


PLATE 3

## Plate 4

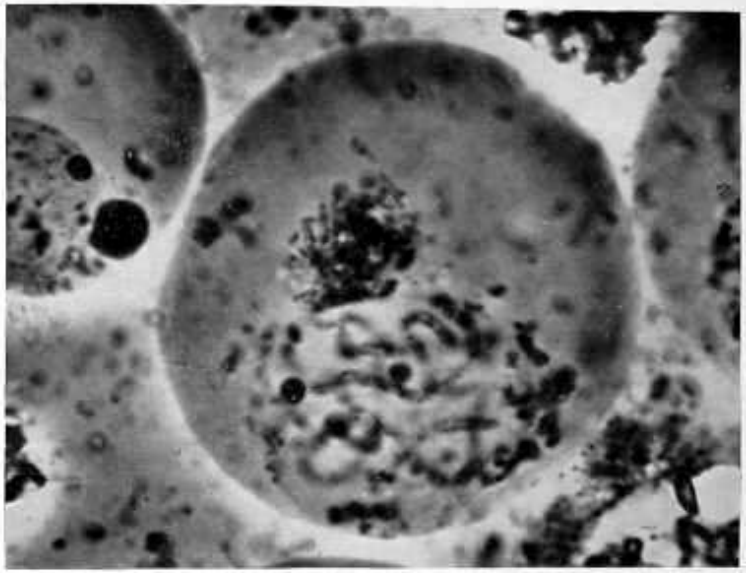
Chromosomes in living cells of *Locusta*.
Taken with × 100 double focus objective

*Upper*: Positive contrast.

*Lower*: Negative contrast.

Central cell is at pachytene stage. Nucleus in lower part of cell. The two homologous strands of the paired chromosomes are clearly resolved. They can be seen to be associated at corresponding chromomeres. In one pair, which runs horizontally across the nucleus, the separate strands appear to be relationally coiled.

(*Courtesy of* Dr. E. J. Ambrose,
Chester Beatty Research Institute, London)



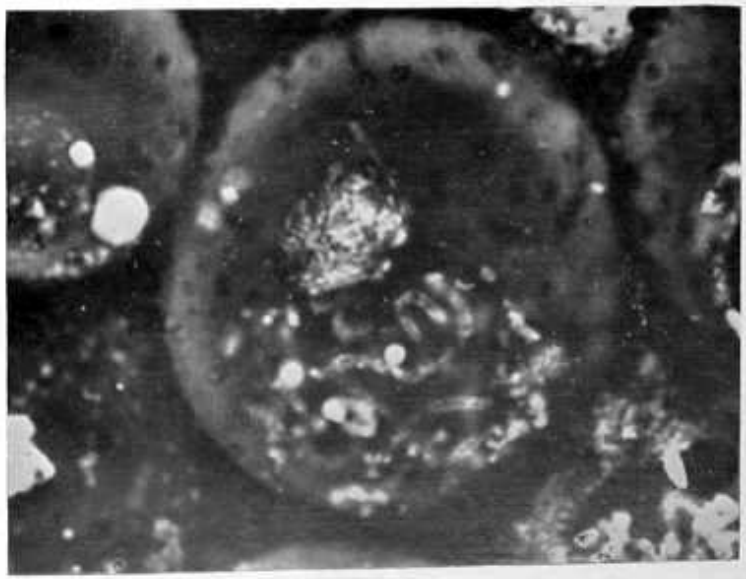


Plate 4

# PART TWO

## Theoretical Treatment

Phase changes exhibited by objects made visible by interference microscopy can be measured, and from the quantitative knowledge of these phase changes, much useful information can be derived (see References).

Interference phenomena can be used to determine phase relationships between mutually interfering beams of light and it will be seen later how these can be converted into useful information concerning microscopic objects. The essential step in this process is to cause the object to modify the phase relationship between the beams, so that the measured phase relationships can be directly related to the optical path-length-changing properties (optical thickness) of the object. The interference microscope achieves this selective action on the part of the object by obtaining the mutually interfering wave trains in the combined beams from different portions of the object area, whereby a wave train proceeding from an object feature becomes combined with a wave train from another portion of the object area, conveniently referred to as the *comparison area* or *reference area*. Consequently, the final image is a compound one comprising two superimposed mutually different views of the object area which are interferometrically compared. This image-doubling is achieved by double-refraction due to anisotropic crystalline material in the objective system.

Pairs of wave trains can mutually interfere only if they originate from precisely the same portion of the light source, which means that the two superposed different images of the object area must be illuminated by identical images of the light source in perfect point-to-point registration upon each other. In other words, the image-doubling power of the objective must be exactly compensated out for the light source by introducing a precisely complementary form of image-doubling between the source and the object space, i.e., in the condenser system. This is carried out by the incorporation of double-refracting crystalline elements in this system as well





as in the objective. The essential optical systems are diagrammatically shown in Plate 2 and Text figures 1 & 3.

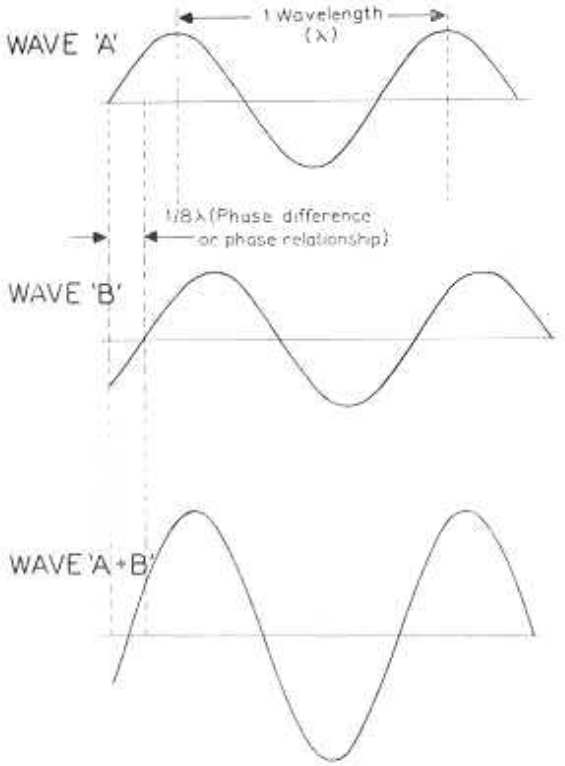
Owing to the well-known wave-like behaviour of light, the intensity which results from the combination of two beams originating from precisely the same portion of a luminous source depends not only upon the individual intensities of the beams, but also upon the distance between a crest of one wave train and a corresponding crest of the other. This principle is diagrammatically illustrated in Text figure 4 (a-d), which shows how two entirely different wave *amplitudes* (A + B and A$^1$ + B$^1$) can arise from the super-position of pairs of identical wave trains, only differing by the distance between successive crests in the two pairs (45° or $\frac{1}{8}$ wavelength in A + B; 135° or $\frac{3}{8}$ wavelength in A$^1$ + B$^1$). This crest-to-crest distance is conventionally known as the *phase relationship* or *phase difference*.

### *Vector Diagrams*

Although the wave diagrams of Text figure 4 clearly illustrate the dependence of the resulting intensity upon the phase relationship between the combined beams, a more convenient and exact means of representation, which is customarily employed, is the vector diagram. Two such diagrams, illustrating the two conditions in Text figure 4 (a, c) are shown in Text figure 4 (b, d). Each vector is a straight line whose length is proportional to the amplitude (crest-to-trough displacement distance) of one wave train and whose direction represents the position of a crest of that train for one moment of time. Since this moment is arbitrary, all that is really relevant is the directional relationship (conventionally known as *phase angle*) between the two vectors representing the two wave trains.

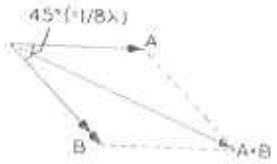
It will be seen that the vector diagram achieves considerable geometrical simplification and this facilitates the derivation of a simple formula establishing the resultant amplitude obtained by the superposition of two identical wave trains. For the special case, where the two trains have one and the same amplitude, 'A,' this formula is

$$R.A. = 2A \cos\frac{\theta}{2}$$




TEXT-FIGURE 4a

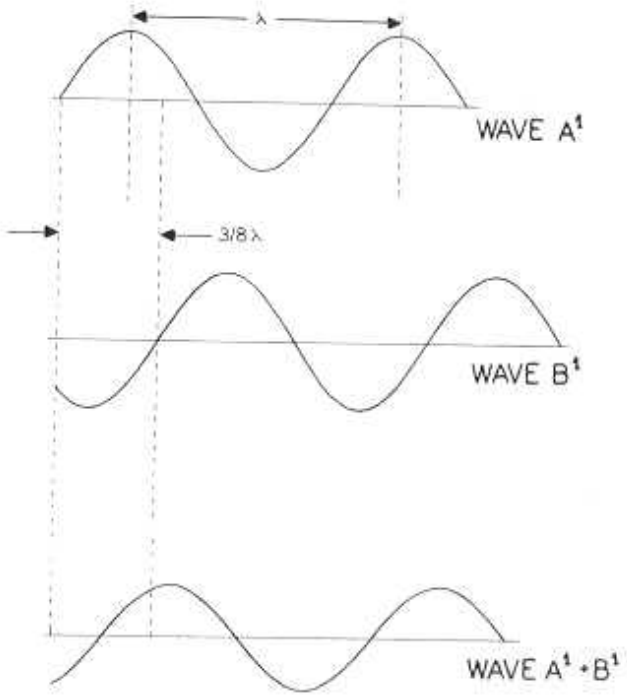
Wave diagram illustrating the combination of two wave trains with a phase difference of $\frac{1}{8}\lambda$.




TEXT-FIGURE 4b

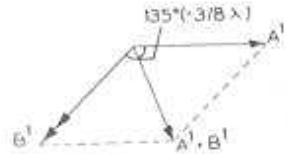
Vector diagram illustrating the above condition.





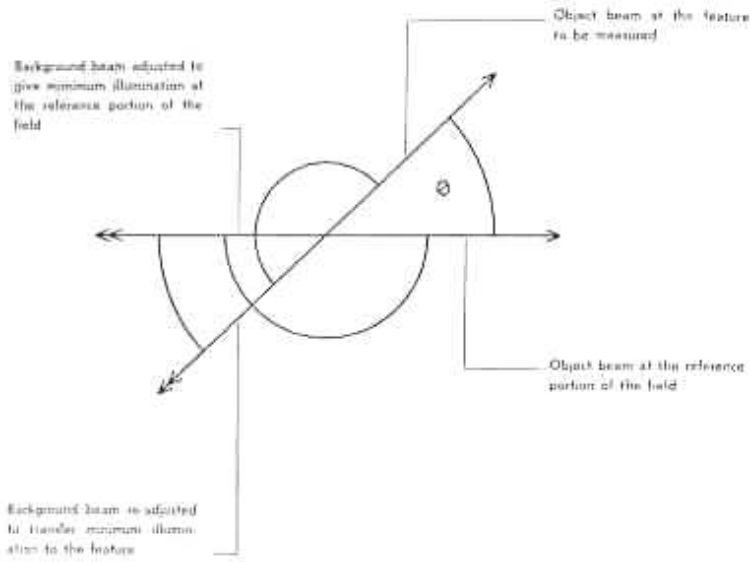

TEXT-FIGURE 4c

Wave diagram similar to 4a except that the phase difference is increased to $\frac{3}{8}\lambda$.




TEXT-FIGURE 4d

Vector diagram illustrating the above condition.




TEXT-FIGURE 5

Vector diagram illustrating the transference method of phase measurement.

where R.A. is the resultant amplitude and $\theta$ is the phase angle between the trains. In general it is only relative amplitude which is of interest, so that 'A' can be omitted from the equation. In practical double-beam interferometry this special case of amplitude equality obtains to a sufficiently close approximation.

*Minimum Illumination Condition*

A case of particular importance is where the phase angle $\theta$ is 180 degrees, for then $\theta/2$ becomes 90 degrees, for which the corresponding cosine value is zero. The amplitude for this special condition is, therefore, also zero, so that the illumination is at a minimum.

This condition of minimum illumination can be used to establish a known phase relationship between two wave trains, to serve as a datum against which other phase relationships can be measured. How this can be done is vectorially illustrated in Text figure 5. A phase-changing system for varying the phase relationship by any known amount is adjusted to



give minimum illumination for the portion of the visual field to which it has been decided to quantitively relate another portion where the phase relationship is different. The 180 degree phase relationship for the reference portion of the field having been thus established, the phase changing system is again adjusted, this time to establish the same minimum illumination condition for the other portion of the field. The amount by which the phase relationship has to be changed to move the minimum illumination condition from one portion to the other is clearly the difference in phase relationship between these two portions.

*Direction of Measurement*

From the same figure, however, it can be seen that there is an ambiguity as to whether the phase angle is to be measured in a clockwise or anti-clockwise direction. The correct direction for the angular phase measurement corresponds with the actual direction of the phase modification being measured. That is to say, that a phase advance must be measured in the phase advance direction and a phase retardation in the corresponding retardation direction. In practice, the direction of the phase modification is usually known, or can be ascertained in a manner to be explained later (Part 4).

*Design of the Present Instrument*

There are various well-known methods for manually changing the phase relationship by known amounts, but many of these are excessively coarse for the very small phase changes which are frequently encountered, for example, with cytological material. However, with interferometer systems of the double-refracting type, such as that employed in the Baker interference microscope, the circumstance that the two interfering wave trains are polarised in mutually perpendicular planes enables one to employ a birefringent compensator to effect the phase change. Various forms of these are familiar to users of normal polarising microscopes. The chosen compensator is very simple and has the additional merit that the measuring principle involved is absolute, so that the uncertainties of calibration are avoided.

The mutually perpendicular transverse vibration directions of the two polarised wave trains are converted into corresponding circular vibrations by transmission through a

PLATE 5

A, B, C: Fresh epithelial cell, taken with ×40 shearing objective.

D: Sea Urchin egg, taken with ×10 double-focus objective.

(A) Analyser set to darken background (223 deg.).

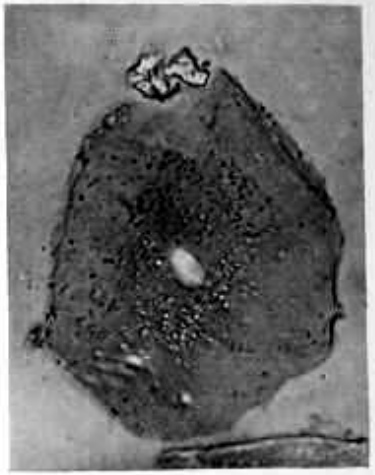
(B) Analyser set to darken cytoplasm (185 deg.).

(C) Analyser set to darken nuclear region (121 deg.).

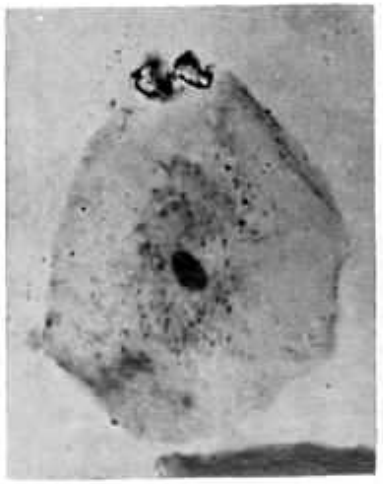
The analyser was being rotated in an anti-clockwise direction, indicating progressively increasing retardation.

(D) Sea Urchin egg, with fertilisation membrane, mounted without pressure. This prepatation exhibits 6 fringes, denoting a phase difference of 6λ (*Courtesy of* Dr. J. M. Mitchison.)

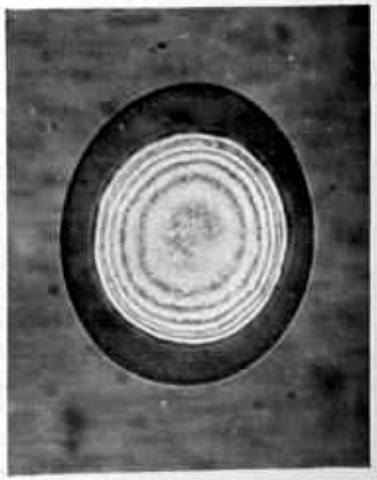
A



B

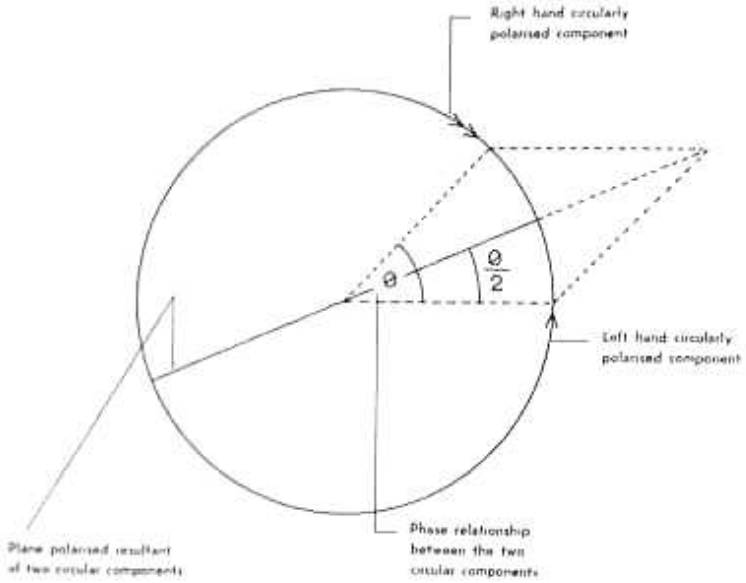


C



D

PLATE 5

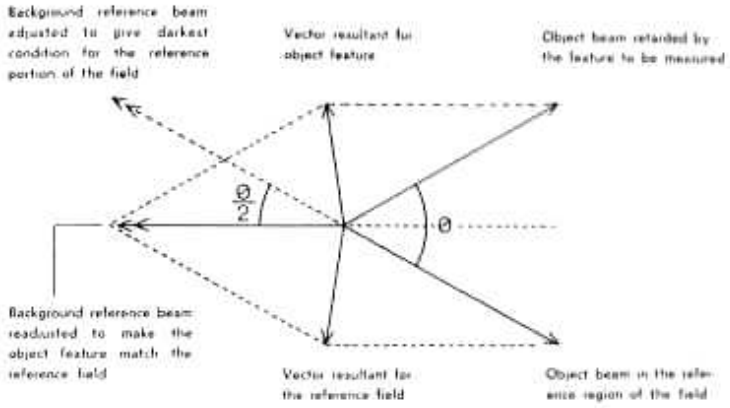



TEXT-FIGURE 6

Showing the plane polarised resultant of two circularly polarised components.

birefringent quarter-wave retardation plate whose vibrations are inclined at 45 degrees to those of the incident wave trains. Because the plane polarised vibrations of the incident wave trains are mutually perpendicular, the circular vibrations into which they are converted by the quarter-wave plate, occur in opposite directions, the one clockwise and the other anti-clockwise. The advantage thereby gained is that the resultant of the two combined circular vibrations of equal amplitude occurring in opposite directions is always a plane polarised vibration, inclined in precisely the same direction as that of the vector-resultant of the vector diagram for the phase relationship between the two same trains. How this happens is diagrammatically explained in Text figure 6, from which it can be seen that the inclination of the plane polarised resultant of the two oppositely circular-polarised wave trains is directly proportional to $\theta/2$, where $\theta$ is the angular phase relationship between the wave trains. The angular inclinations of the plane






TEXT-FIGURE 7

Vector diagram illustrating the matching method for measuring very small features.

polarised resultants of the various phase relationships can be read off directly from a goniometer analyser set to minimise in turn the intensity of each resultant required (Plate 5 A, B, C). The foregoing explanation is summarised in the Appendix figure 1.

*Matching Method*

Phase measurements obtained by transferring the minimum illumination condition from the reference portion of the field to the position being measured by means of the goniometer analyser, are extremely useful but tend to become uncertain when the features being measured are too small for the minimum illumination condition to be precisely determined by the eye. For such cases a useful procedure is to adjust the goniometer analyser to obtain equality of illumination between the reference field and the minute feature. This is a highly sensitive condition which tends to render the feature invisible against its reference background. The vector diagram for this method is shown in Text figure 7. It will be seen that the corresponding phase adjustment is precisely half that required to transfer the datum minimum illumination condition to the feature and that the corresponding goniometer analyser rotation is consequently only $\theta/4$ where $\theta$ is the required angular

phase relationship. This method is applicable to isolated granules, particles and filaments.

*Half-shade Eyepiece*

This is an additional piece of apparatus which greatly enhances the accuracy with which phase measurements can be made. The eyepiece field contains a small area where there is a uniform instrumentally produced phase change surrounded by a sharply defined boundary. As a result the two portions of the field generally assume different intensities which, however, become equal for a very sharply defined position of the analyser. The feature to be measured is made to straddle the boundary between the two portions of the field, the condition of equality being obtained for the reference area and then for the feature. See also page 31 and Text figure 9; also Plate 8.

*Integral Wavelengths*

The foregoing account of phase measurement has assumed that the phase relationships are less than one wavelength, that is to say less than 360 degrees. The measurement of phase angles in excess of 360 degrees is slightly complicated by the circumstance that the vector diagrams for phase relationships which differ by integral multiples of 360 degrees are identical. In other words, the above methods are not directly applicable to the determination of integral numbers of wavelengths. Although phase modifications exceeding 360 degrees are rarely encountered in biological work, it may be necessary to know the modified techniques for dealing with them.

Since the phase modification introduced by an object feature is merely the result of a displacement of the associated wave train along its direction of propagation, the magnitude of the phase modification is virtually inversely proportional to the wavelength of the wave train, a given linear displacement (path difference) containing more short-wave crests than long-wave ones. Consequently, illumination by white light, or light including a wide range of wavelengths, gives rise to a mixture of different wavelengths. Only for the special case where there is no path difference between the pairs of wave



trains will there be one and the same phase relationship for all the different wavelengths simultaneously. For a polarising system, therefore, this is the one unique condition for which a neutral coloured minimum illumination condition can be obtained. This neutral minimum, conveniently referred to as 'black,' therefore serves as a reference datum for the determination of path differences for any number of wavelengths. All that is required is a device which will directly modify the actual linear, path-length relationship.

In its simplest form such a device consists of a wedge made from birefringent material such as crystalline quartz, cut parallel to the optical axis. Sliding such a wedge across the optical aperture provides a variable thickness of birefringent material and therefore serves to vary the path difference between the wave trains, provided that its own vibration directions are approximately parallel to those of the wave trains.

*Fringe Eyepiece*

Perhaps the most convenient arrangement employing such a wedge is the fringe eyepiece (see p. 33). The wedge with its scale is pushed through a slot in the eyepiece mount to positions which give, say, first the black datum for the reference portion of the field and secondly for the feature being measured. The wedge calibrations may be in terms of linear path difference instead of angular phase, since this simplifies the calculation of the refractive indices of features whose thicknesses are known. It is not, of course, essential to select the black interference colour as the datum, any other of the sequence of interference colours may be chosen, but black is probably the most easily recognised. Wedges can also be used in monochromatic light to produce a system of fringes for estimating phase changes by the well-known method of fringe deformation. (Plate 9).

*Refractive Index Calculations*

The refractive index, $\mu$, can be readily found if the thickness '$t$' of the object is known ('$t$' can be directly measured

for objects which can be assumed to be spherical or cylindrical by measuring their diameter):

$$\mu = \frac{\theta\,\lambda}{360t} + n$$

where $\theta$ is the change in phase relationship (in degrees) produced by the object, $\lambda$ is the effective illuminating wavelength expressed in the same units as 't,' and 'n' is the refractive index of the reference portion of the object space, usually the fluid in which the material is immersed. If the actual linear path difference (p.d) is directly known, for example, by employing a p.d.-calibrated wedge in heterochromatic light, then this formula simplifies to

$$\mu = \frac{\text{p.d.}}{t} + n$$

If 't' is not known but it is possible to change the medium in which the material is immersed, then the refractive index can be found from the following equation, where 'A' is the phase difference produced by the object immersed in the medium whose refractive index is '$n_1$,' and 'B' is the corresponding phase difference for the changed medium of refractive index '$n_2$.'

$$\mu = A\frac{(n_2 - n_1)}{A - B} + n_1$$

A helpful feature of this equation is that the phase differences 'A' and 'B' need not be expressed in any particular form, so all that is necessary is merely to use the actual reading differences taken direct from the phase control of the microscope.

*Volume and Dry-Mass Calculations*

Refractive index values are often very useful in many fields of research, but it is only recently that this has become true for cytology. The important discovery that the increase in refractive index resulting from a one per cent. increase in concentration of the solid substances contained in cells is 0·0018, to within 10 per cent. for all such substances, has made it possible to calculate the concentration when the refractive



index is known. Thus, if zero concentration is taken as equivalent to a refractive index of 1·334, the concentration of cytological substances is

$$C = \frac{\mu - 1{\cdot}334}{{\cdot}0018}$$

where C is the concentration in terms of percentage and $\mu$ is the refractive index as measured by one of the above methods.

If one is interested only in the combined amount of cytological substances, it is not necessary to know the refractive index because the equivalent thickness of the combined dry substances is proportional to path difference. Consequently, the equivalent volume of dry, concentrated substances in solution is proportional to the product of the cell's mean path difference (m.p.d.) and its area. Since the increase in refractive index due to a one per cent. increase in concentration is 0·0018 to a close approximation, the solid substances contained in the cell, and considered in isolation from the water component, are optically equivalent to a substance having a refractive index equal to 1·334 + 0·180 = 1·514, provided that the surrounding medium is water. So the average equivalent thickness of concentrated substances $(t)_m$ is

$$t_m = \frac{\text{m.p.d.}}{1{\cdot}514 - n}$$

where m.pd. (mean path difference) $= m\theta\lambda/360$, and n = refractive index of the surrounding water, usually about 1·334. Consequently, the total equivalent volume of dry substances is

$$V = A_e\left(\frac{\text{m.p.d.}}{1{\cdot}514 - n}\right)$$

where $A_e$ is the area of the cell in the same units squared as m.p.d. and V in the same units cubed.

The dry volume may be converted into dry mass for an assumed value of specific weight. For the majority of cases a value of 1·25 grammes per ml. is a close enough approximation. More detailed information is available in the appropriate references at the end of this manual.

# PART THREE

## Instructions for Setting-up the Instrument

### *Notes on Illumination*

The usual light sources are perfectly satisfactory on account of the high light transmission of the optical system. Excellent results can be obtained with the customary Köhler illumination. For precise phase measurement a mercury green filter is advisable and a neutral for obtaining interference colours in white light (see pages 26 & 29). For preliminary setting-up, however, the filament structure of the lamp in the back focal plane of the objective which results from Köhler illumination[1] should be destroyed by introducing a diffusing screen between the lamp and the substage condenser, since this structure mars the visibility of the interference figure in the back focal plane of the objective.

When available, the ideal source is probably a mercury vapour lamp, with its entire visible region used for obtaining interference colours and a mercury green filter for precise phase measurements.

The substage iris diaphragm should be used in a manner similar to that which is customary in normal bright field microscopy, i.e., it should be opened as wide as is compatible with adequate contrast. This applies with particular force to the double-focus type of system (page 23). The shearing interference systems (page 22) do not suffer in contrast from severe stopping down. When using the ×100 double-focus water immersion objective, the substage iris should be completely opened. Only *distilled* water should be used as the immersion medium for both types of ×100 objective. Neither immersion oils nor organic solvents should be allowed to come into contact with the fronts of any of the objectives supplied with the interference microscope.

[1] See "Manipulation and Maintenance of C. Baker's Microscopes"—p. 8.





### *Adjustment of the Instrument*

(Refer to Plate 1 for the positions of the components and controls named in the following paragraphs).

1. Insert and lock into the substage slides the condenser unit (C) appropriate to the objective being used and rack up to stage level.

2. Rotate the lever of the substage polariser (P) to the 'off' position to eliminate the second interference beam and thus produce normal bright field conditions.

3. *Focus on a preparation* and select a reasonably clear area. Adjust the condenser focus if necessary.

4. Rotate the substage polariser lever to the 'on' position.

5. With the quarter-wave plate (RP) 'in' (i.e., with '$\lambda/4$ in' visible on the slide) and the goniometer analyser (RA) in the body on the operating portion of its scale (the 'out' position is marked by a red line interrupting the scale), remove the eyepiece and inspect the back focal plane of the objective by the use of the telescope provided. In diffused white light this should exhibit an interference pattern, which can be traversed across the aperture by turning the goniometer analyser. By this process a fringe can be brought into the centre of the aperture and subsequently broadened out to fill it as uniformly as possible by adjustment of the two condenser levelling screws (SC) either simultaneously or alternatively.

Typical appearances in the back focal plane of a ×40 objective are reproduced on Plate 6. It will be found of assistance to compare these illustrations with the figures actually seen, until one becomes quite familiar with the working of the instrument.

### *Shearing Systems*

When white light is used, rotation of one substage screw only, or rotation of both in opposite directions, will cause a series of interference fringes to move across the objective aperture, thus permitting selection of the required order of

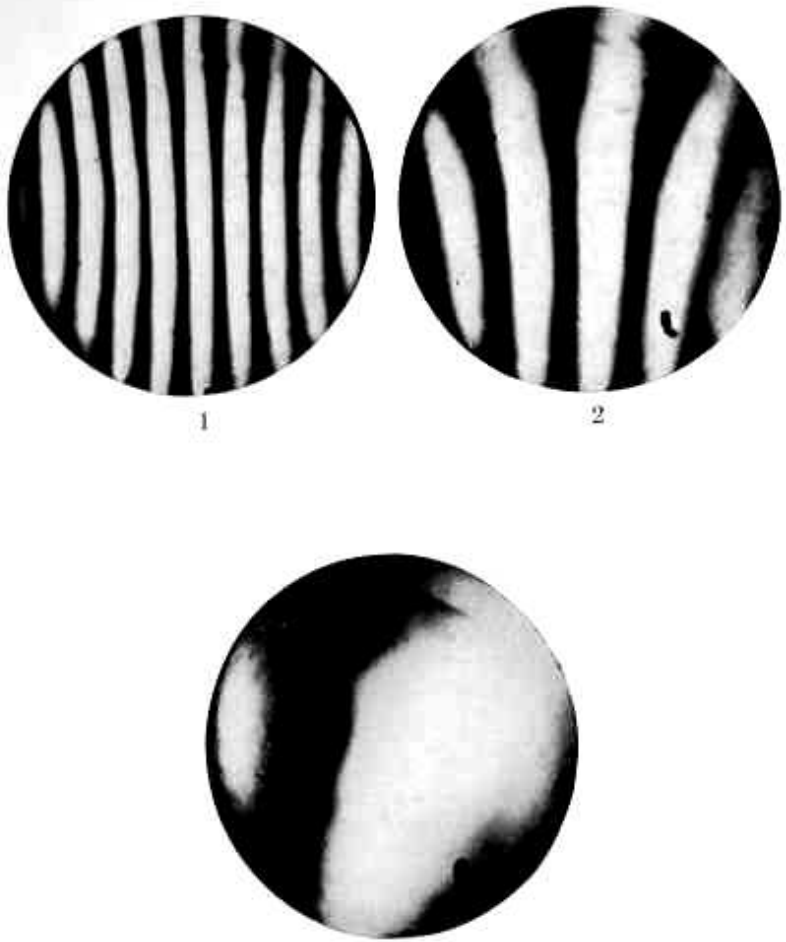


PLATE 6

Illustrating the progressive broadening of the fringes seen in the rear focal plane of a double-focus objective when the levelling screws are appropriately adjusted.

1: System completely out of adjustment.

2: System still out of adjustment, but fringes broader.

3. One fringe commencing to fill the aperture. Ideal adjustment is achieved when one fringe has completely filled the aperture.



interference. When this order, or fringe, has been brought to the centre of the objective aperture, both screws should be simultaneously operated in the *same* direction to spread this selected fringe over the major portion of the aperture. Whether the rotation of the two screws should be clockwise or anti-clockwise to bring this about is at once apparent because the incorrect direction will make the fringe narrower instead of broader. It should be noted that for each shearing system there is only one fringe which can be spread over the major portion of the aperture. Peripheral portions of the aperture which may be slightly beyond the spread of the chosen fringe can be masked out by adjustment of the substage iris diaphragm and this method can be employed when it is desired to use orders other than the optimum one.

While making these adjustments slight irregularities can sometimes be seen in the objective aperture and these are usually due to strike in one or other of the crystal optics. Care is always taken, however, to ensure that such irregularities are too small to impair the performance of the instrument.

If the present copy of this manual has been issued with an instrument, a supplementary leaf will have been inserted in the front stating the optimum order fringe to be used for each of the shearing objectives; and the analyser settings to be used for each of the adjustments.

### *Double-Focus System*

When the double-focus system is in use a series of fringes in the aperture of the objective indicates that the microscope is out of adjustment, as above; but in this instance one does not have to select the order in which the instrument is to work. The goniometer is rotated until a fringe lies across the centre of the aperture, then one of the substage screws is employed to broaden this fringe, and the other to check any resultant rotation of the pattern.

### *Need to Check Adjustment*

Each time the object or the object-slide is changed, the adjustment of the microscope must be checked, whether the

shearing or the double-focus system is being used.

When the most uniform illumination in the objective aperture has been obtained, the instrument is correctly adjusted and it remains only to remove the viewing telescope and to replace the eyepiece and examine the specimen. This should exhibit strong interference contrast, which can be varied by rotation of the goniometer analyser (see plates 3, 4 and 5).

# PART FOUR

## Phase Measurements

The most direct method of measuring the phase differences introduced by various features of the specimen is to rotate the goniometer analyser to a position which causes a clear reference portion of the field adjacent to the selected feature to assume a clearly recognisable hue or density, and then again to rotate the goniometer analyser to a second position which causes the selected feature to assume the same appearance as was previously obtained for the reference portion of the field.

Twice the angle through which the goniometer is rotated from the first to the second position is the fractional part of a wavelength, expressed in degrees, by which the selected feature has changed the phase relative to the adjacent reference area (see Text figure 5 and page 13 for theoretical explanation).

The correct direction in which to rotate the goniometer analyser is determined by the sense of the phase changing property of the features being measured. The phase changing system of the instrument is so designed that a phase retarding feature (one having a refractive index higher than the surrounding medium) requires an anti-clockwise rotation for all the objectives except the ×100 double focus system. For a phase advancing feature the corresponding rotations are, of course, reversed.

If the sense of the phase change to be measured is not already known from the nature of the preparation, this can be found by, for example, the Becke line test[1].

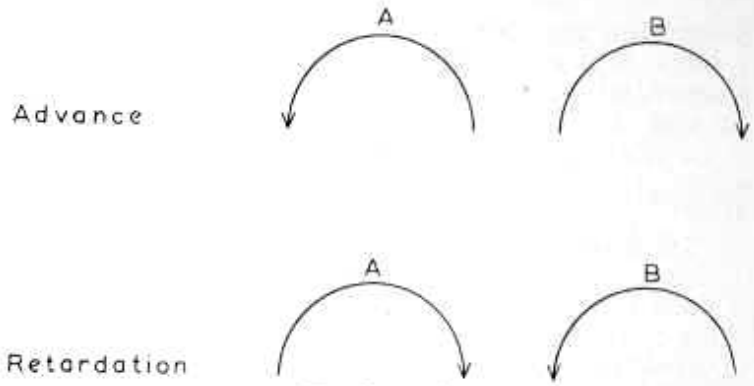
[1] When light impinges upon the interface between two media whose refractive indices differ by a substantial amount, the light is reflected and refracted towards the one having the higher refractive index and results in the appearance of a fine white boundary line. It is usually necessary to reduce the cone of illumination to see this effect clearly. As is well known the line moves *inside* the object upon *increasing* the distance between the preparation and the objective (lowering the stage of this particular instrument) when the object retards the light, and vice versa. A good deal of biological material is too geometrically amorphous to produce a definite 'line,' but the object either brightens or darkens relative to its surround, and these appearances correspond to the line moving 'in' and the line moving 'out.'

The Half-shade Eyepiece

The Fringe-field Eyepiece

PLATE 7






TEXT-FIGURE 8

Chart showing the directions in which the gonimeter analyser has to be rotated to secure advance or retardation of the reference beam with different objectives.

A: with the *double-focus* ×100 water immersion objective.

B: with all other types and powers of objective.

Before attempting to apply the Becke test the second beam should be suppressed by rotating the polariser situated below the condenser to the 'off' position.

When the phase change is too small to provide adequate Becke line conditions, then it can be assumed that it is less than 180 degrees, in which case the correct rotation direction is the one which causes the feature to change towards the original reference appearance as soon as rotation starts.

The recommended reference appearence is the darkest condition obtainable in monochromatic light, preferably mercury green. It is, however, possible to use such a readily recognisable colour as purple, obtained by the use of unfiltered light.

The phase changes due to features which are too small for their densities to be readily recognised can be measured by the matching method instead (see Text figure 7 and page 15), in which case the goniometer analyser is rotated from the reference position only far enough for the small feature to match its surround. The required phase change is then *four times* the angle through which the analyser has been rotated. This method is often useful for such small isolated features as

bacteria and fine filaments. It is often useful also for such features as granular inclusions in cells: the cytoplasm surrounding the inclusion then being used as the selected reference portion of the field.

*Practical Examples*

1. The Becke test indicated a feature showing a *retardation* (brightening of the feature upon increasing the distance between the objective and the slide), therefore a rotation of the analyser in an anti-clockwise direction using a ×40 objective was required to darken the object. Twice the angle between the reading for darkness of the surround and darkness of the feature gave the angular measure of the amount by which the feature was *retarding* the light relative to the surrounding material.

2. The Becke test was negative, indicating a phase change of less than 180 degrees. Rotation of the goniometer analyser in a clockwise direction with the ×100 objective from the 'dark surround' position darkened the object, thus again indicating a *retardation*, and twice the angular difference between the readings indicated the amount of retardation, as before.

*Phase Changes Greater than One Wavelength*

(a) *Gradual Changes.* The above methods of measurement suffices provided it is known that the phase changes being measured are less than one wavelength. With living biological material this is usually the case, and even in the rare cases when it is not, the maximum phase change is practically always approached by a gradient of phase change which starts at less than one wavelength, resulting in the formation of a fringe system upon the object (see Plate 5, figure D). The whole number of wavelengths associated with the maximum change is then merely the number of dark fringes between the thin edge of the gradient and the region being measured. Any reversal of the sense of the gradient can be detected by rotation of the goniometer analyser, when the fringe or fringes in the reversed region will move in a direction opposed to the motion



of the fringes in the other region of the gradient.

(b) *Abrupt Changes.* There is, however, a special case to which the fringe-counting method for determining integral numbers of wavelengths does not apply. This is when the boundary of the phase change is substantially vertical, resulting in an abrupt transition of phase change. There are no fringes to be counted and a more elaborate technique is therefore required.

*Fringe Eyepiece*

The best method for determining integral numbers of wavelengths is to employ a fringe eyepiece (see p. 33) in place of the standard one; and to adjust the quartz wedge to transfer the blackest appearance of the reference portion of the field to the region being measured, white (unfiltered) light being used. The number of fringes which traverse the region while this adjustment is being made corresponds to the required number of integral wavelengths. When using this ocular, both the goniometer analyser and the quarter-wave slide should be in their respective 'out' positions.

A method which has the advantage of not calling for instrumental elaboration is to obtain the integral number of wavelengths from two phase readings instead of one, the first being made with light of one mean wavelength and second with light of a different mean wavelength. It is, of course, essential that the two readings are obtained from two separate settings for the reference portion of the field, corresponding to the two wavelengths.

A formula for this method is as follows: —

$$\text{Phase change} = (A_1 - A_2)\frac{\lambda_2}{\lambda_2 - \lambda_1}$$

where '$A_1$' is the phase difference with light of shorter wavelength $\lambda_1$, and '$A_2$' is the corresponding phase difference with light of longer wavelength $\lambda_2$. It is advisable to ignore the fractional components of the result, since this has already been accurately obtained in the form of $A_1$. The difference between the two wavelengths should not exceed $\lambda_2/N$, where N is the number of wavelengths being measured. This raises

no practical difficulties, because abrupt changes exceeding five wavelengths are extremely improbable, especially with biological material.

Good quality commercial filters will usually be found adequate, even in white light, and the mean visual wavelengths are usually obtainable from the manufacturers. It is recommended that one of the two filters should be the mercury green one previously mentioned (see page 21). The second filter could then be blue. If a mercury vapour source is used, then the green filter will provide the single green line, the second filter can be one designed to transmit only the blue line.

# APPENDIX

*Practical Notes*

1. The ×100 objectives are corrected for *water* immersion and must *never* be oil immersed, or cleaned with organic solvents.
2. It is advisable to ensure that No. 1 cover glasses of a *measured* thickness of 0.18 mm are employed for all preparations. This applies equally to both dry and water immersion systems.
3. More than usual cleanliness in respect of condenser and objective surfaces, slips and covers, is necessary with the interference microscope.
4. The objectives are permanently fixed in the nosepiece and no attempt should be made to remove them.
5. The front component of the ×100 objective is nearly flush with the mount, so that particular care should be taken not to press it on the slide. *This objective is fragile.*
6. If a fringe eyepiece is employed both the quarter-wave plate and the analyser must be placed in their respective 'out' positions.
7. When using Köhler illumination it is particularly important to see that the filament image is centred on the aperture of the substage condenser; this can be checked either by direct inspection of the condenser iris diaphragm or by examination of the back focal plane of the objective.
8. When obtaining the interference figures in the back focal plane of the objective for setting-up purposes, insert a ground screen in front of the lamp to destroy the Köhler image of the filament.
9. If an attempt to obtain a setting of the goniometer analyser is frustrated simply by the 'out' position of the scale appearing in the window, this condition is immediately rectified by turning to the diametrically opposite side of the scale.

10. Make sure the sliding changer of the substage is pushed completely 'home' when changing condensers, and is properly locked in position.

*Interference Colours*

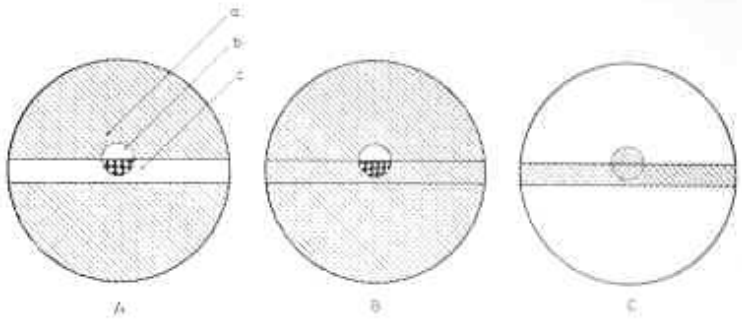
In general, the intensity variations associated with the phase variations produced by an interferometer system when illuminated by monochromatic light are transformed into colour variations when the light contains a mixture of widely differing wavelengths. This phenomenon results directly from the circumstance that any given linear optical path difference between the interfering beams corresponds to phase differences which are inversely proportional to wavelength. For example, if one considers an interferometric field across which there is a uniform gradient of increasing optical path difference it is apparent that the corresponding gradients of phase difference are inversely proportional to wavelength. Consequently the field will be crossed by fringes whose spacing is proportional to wavelength, so that a mixture of wavelengths results in a corresponding mixture of fringe spacings. For instance, red fringes will be more widely spaced than will be blue ones, resulting in a cyclic variation of the red-blue intensity relationship. This variation in intensity relationship applies, of course, to all the colours present in the illuminating beam so that there is a corresponding progressive variation in the colours seen in the interferometric field.

The colours associated with given optical path differences for white-light illumination when the analyser is set to make the zero path difference appear black, are set forth in the table (p. 35). Orders higher than 3 are not included because their hue saturation is insufficient for the estimation of path differences.

*Half-Shade Eyepiece*

The accuracy of visual phase difference measurement with a normal interference microscope is limited by the precision with which a convenient condition, such as that of minimum brightness, can be recognised. Not only is this particular






TEXT-FIGURE 9

Diagrams to illustrate the appearances seen when using the Half-shade Eyepiece.

A: Non-balance condition.

B: Balance for the reference area (*Reading no.* 1).

C: Balance for the object (*Reading no.* 2).

*a*: Reference area.

*b*: Object.

*c*: Half-shade strip intersecting the field.

condition often marred by flare and slight errors of adjustment, but it can be very difficult to recognise when the object to which it has been transferred by instrumental phase adjustment is very small in relation to the field of view, owing to the disturbing effect of glare from the rest of the field.

The half-shade eyepiece has been introduced to greatly increase the accuracy of phase measurement by exploiting the well-established fact that the eye is far more precise in its estimation of equality of illumination between two adjoining areas in the field, than it is in recognising a selected level of illumination at any given region. The Baker half-shade eyepiece carries a 45° inclined viewing prism having a totally reflective face which receives the primary image of the object formed by the microscope objective. A central horizontal strip of the reflecting face is coated with an aluminium film. Owing to the different character of metallic reflection from total reflection at a dielectric/air interface, the two perpendicularly plane polarised beams of the double-refracting interference microscope system suffer a relative phase shift at

PLATE 8

Typical appearance using the half-shade eyepiece.

Taken with ×40 shearing objective, mercury green line.

*Upper*: *Left hand* side, analyser setting giving a close match between half-shade strip and background (177 deg.).

*Lower*: Area immediately to the *right* of the very prominent particle on the boundary shews matching condition (41 deg.).

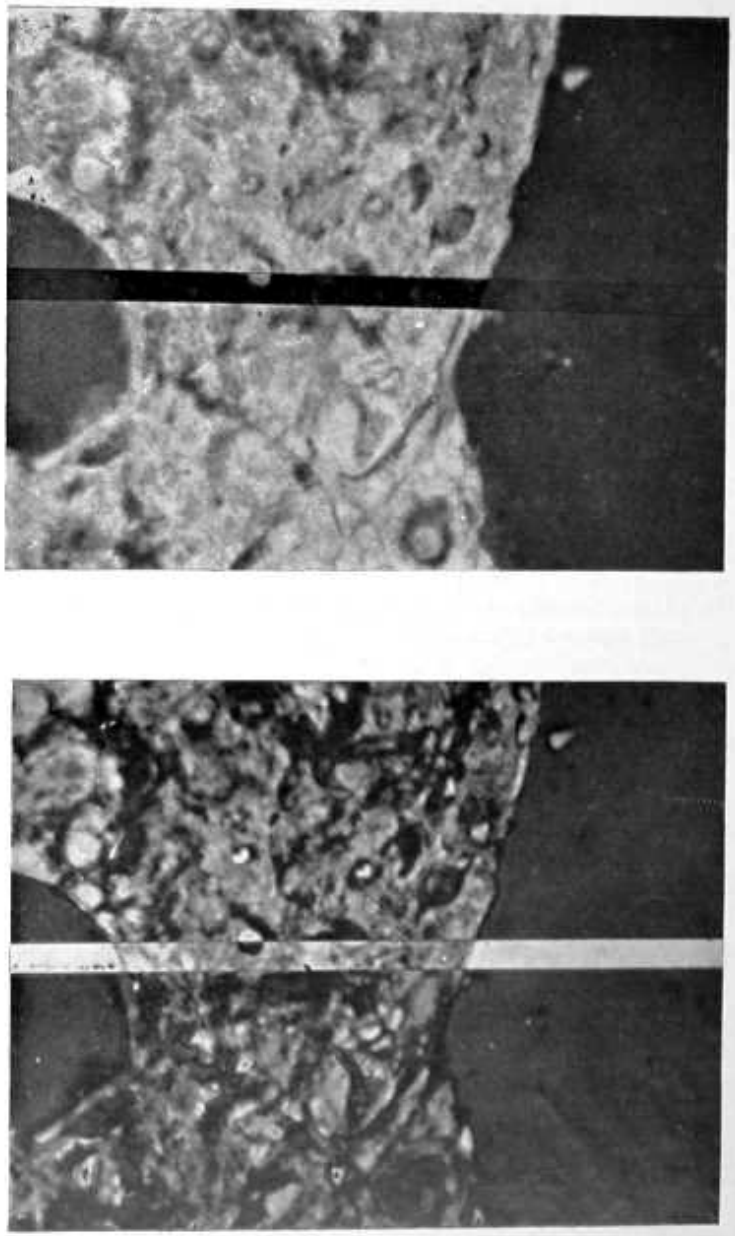


PLATE 8

this metallized strip. In other words, the strip is exactly equivalent to an artificial object producing a constant and uniform phase change with extremely clean-cut edges and consequently changes its relative luminosity when the phase changing system is varied. There are two such phase settings for which the strip matches the surrounding field. One of these two positions is extremely sensitive, and is clearly recognised because it yields a markedly *lower over-all brightness* than does the other, insensitive condition. The normal measuring procedure consists in adjusting the phase system to yield the sensitive balance condition for the reference portion of the field and then to make a further adjustment to transfer this same sensitive balance condition to the feature whose phase shift is being measured, the latter having been previously brought into a position where it is straddled by at least one edge of the strip. In general, the accuracy is more than four times greater than with the normal, direct method.

The prismatic, locally metallized face, together with the primary image of the object, are viewed through a low-power auxiliary microscope provided with a screw-in form of focussing adjustment. This arrangement permits the metallized strip to be focussed with the necessary accuracy, and also allows the use of alternative eyepieces.

The metallized prism is carried in a horizontal slide, which also carries an adjacent prism without a metallized strip, and which can be pushed into the optical system when it is desired to return to normal viewing.

The half-shade eyepiece unit screws on to the microscope body in place of the normal monocular tube or binocular head and is locked in place when it is squared-on to the microscope limb. The unit contains its own phase-changing system, so it is necessary to ensure that the analyser and quarter-wave slide in the body are both in their respective 'out' positions.

*Fringe-Field Eyepiece*

Since the introduction of the Baker interference microscope, experience has shown that special circumstances may



exist for which the uniform field provided by this instrument is less useful than would be a field crossed by a system of straight interference fringes. For the uniform field condition, phase changes in the specimen can be measured by adjustments of the analyser, but when it is preferred to make rapid visual estimates, one has to rely upon the information provided by the hues of the interference colours seen in white light. This method depends upon an uncommonly precise aquaintance with interference colours and is not usually favoured.

If, however, relevant optical conditions can be modified in such a way as to produce a field which is crossed by straight interference fringes, visual estimates of phase changes in the object can be made by observing the extent to which the object deforms the straightness of these fringes.

The straight fringes are produced by introducing an optical path difference between the object beam and reference beam which linearly increases across the field. In the absence of localised phase changing features, this uniform gradient of optical path-difference produces, in monochromatic light, a system of evenly spaced interference fringes so that the corresponding distribution of intensity can be used to determine the phase-change produced by some localised feature. This method is useful when photographic densitometric apparatus is available.

The apparatus for producing the fringes consists of a Ramsden eyepiece with focussing eye-lens at the focal plane of which is a quartz wedge supported in a metal carrier which is inserted into a slot provided with a rotation movement through 90°. Between the two eyepiece lenses is an analyser plate in a slide to allow the analyser to be placed in or out of the optical system. The eyepiece unit is pushed into the top of the monocular tube and then secured by the knurled screws after having been orientated with the analyser slide in a diagonal position. The fringes can then be placed either in a vertical or horizontal position by revolving the carrier in the slot into a position which is respectively horizontal or vertical. A shortened monocular tube is provided to compensate for the additional 'tubelength' occupied by the slot below the eyepiece. Both the quarter wave-plate compensator and revolving analyser in the body of the microscope must, of course,

PLATE 9

Typical appearances using the fringe eyepiece.

Taken with ×40 shearing objective, mercury green line.

*Upper*: Shews displacement of the fringes by the siliceous girdle of a diatom.

*Lower*: Fixed, unstained section of cat piturity to demonstrate a specimen in the fringe field. Mounted in liquid paraffin (refractive index 1·47). Optical thickness, 0.28 $\lambda$; $\lambda = 0.546\ \mu$.

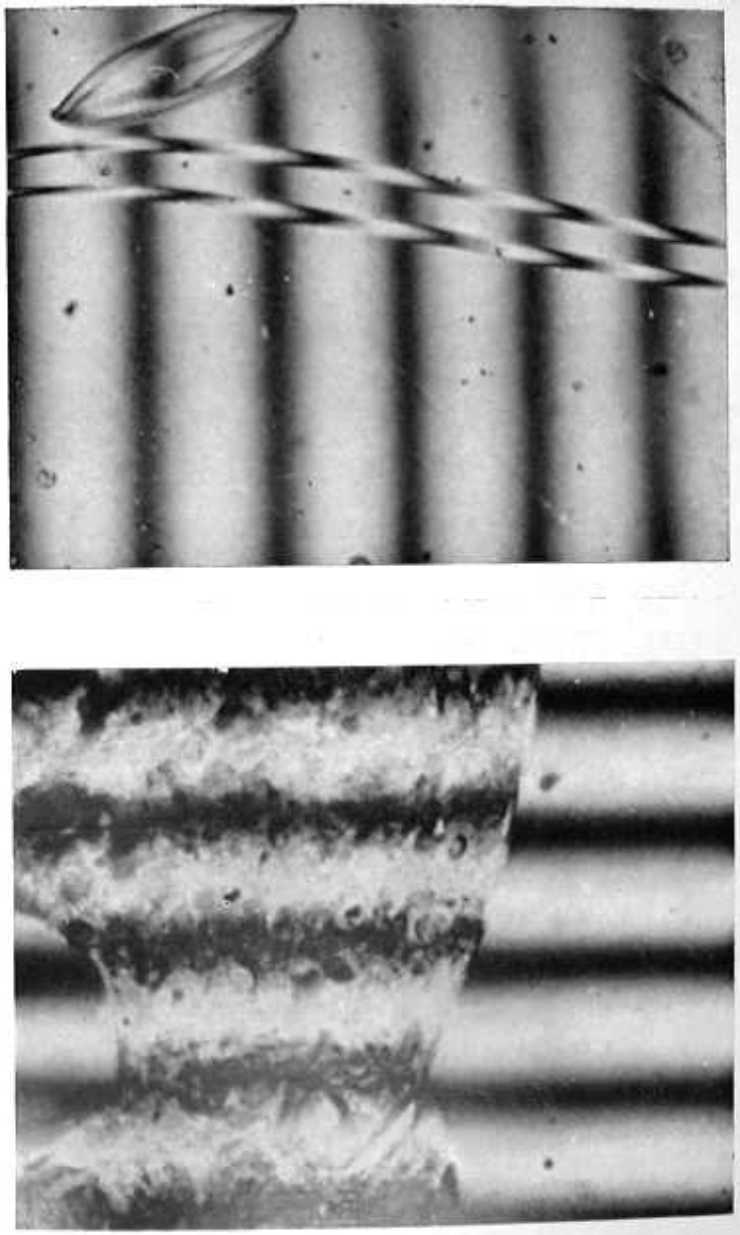


PLATE 9

be in their respective ' out ' positions.

To permit viewing of the back of the objective, the whole eyepiece is easily removed by loosening the securing screws

If it is desired to change over to normal uniform field conditions without exchanging the eyepiece for a standard one, it is only necessary to push the eyepiece analyser to its ' out ' position and move both the quarter wave compensator and analyser in the body to their respective ' in ' positions.

Normal quartz wedge compensators can also be provided in metal carriers for this eyepiece in place of the steeper wedges required for the fringe field condition. Such compensators are also available with a calibrated scale for which use the eyepiece is provided with a cross-lined graticule. In this form, the eyepiece can, of course, be used with a normal polarising microscope for the estimation and measurement of birefringent retardations.

## NEWTON'S COLOUR SCALE

**(Modified from Quincke)**

| *No.* | *Retardation* $\lambda = 589\ m\mu$ | *Order* | *Interference colours between crossed Nicols* |
|---|---|---|---|
| 1 | 0 | 0 | Black |
| 2 | 40 | | Iron-grey |
| 3 | 97 | | Lavender-grey |
| 4 | 158 | $\frac{1}{4}$ | Greyish blue |
| 5 | 218 | | Clearer grey |
| 6 | 234 | | Greenish white |
| 7 | 259 | | Almost pure white |
| 8 | 267 | | Yellowish white |
| 9 | 275 | | Pale straw-yellow |
| 10 | 281 | | Straw-yellow |
| 11 | 306 | $\frac{1}{2}$ | Light yellow |
| 12 | 332 | | Bright yellow |
| 13 | 430 | | Brownish yellow |
| 14 | 505 | $\frac{3}{4}$ | Reddish orange |
| 15 | 536 | | Red |
| 16 | 551 | | Deep red |
| 17 | 565 | | Purple |



| *No.* | *Retardation* $\lambda = 589\ m\mu$ | *Order* | *Interference colours between crossed Nicols* |
|---|---|---|---|
| 18 | 575 | | Violet |
| 19 | 589 | 1 | Indigo |
| 20 | 664 | | Sky-blue |
| 21 | 728 | | Greenish-blue |
| 22 | 747 | | Green |
| 23 | 826 | | Lighter green |
| 24 | 843 | | Yellowish green |
| 25 | 866 | | Greenish yellow |
| 26 | 910 | $1\frac{1}{2}$ | Pure yellow |
| 27 | 948 | | Orange |
| 28 | 998 | | Bright orange-red |
| 29 | 1101 | | Dark violet-red |
| 30 | 1128 | | Light bluish-violet |
| 31 | 1151 | 2 | Indigo |
| 32 | 1258 | | Greenish blue |
| 33 | 1334 | | Sea-green |
| 34 | 1376 | | Brilliant green |
| 35 | 1426 | $2\frac{1}{2}$ | Greenish yellow |
| 36 | 1495 | | Flesh-colour |
| 37 | 1534 | | Carmine |
| 38 | 1621 | | Dull purple |
| 39 | 1652 | | Violet-grey |
| 40 | 1682 | | Greyish-blue |
| 41 | 1711 | | Dull sea-green |
| 42 | 1744 | 3 | Bluish-green |
| 43 | 1811 | | Light green |
| 44 | 1927 | | Light greenish grey |
| 45 | 2007 | | Whitish grey |
| 46 | 2048 | | Flesh-red |

## APPENDIX FIGURE 1

Diagrammatic summary of the basic phase measuring system.

7: At $A_1$ the analyser is set to darken surrounding portion of field, and at $A_2$ to darken the object element. Twice the angular difference between the settings ($\theta$) is the angular phase difference required.

6: R.S. is the vibration direction resulting from combination of the circular vibrations shown at (4), and R.O. is the corresponding direction for the combined vibrations at the image of the phase retarding element in the preparation.

5: Both waves reach the image plane, but only the object wave (O.W.) reproduces the profile modification due to the phase retarding object element, background wave (B.W.) having been defocussed by the double refracting objective. The two phase relations which result are shown by the vectors.

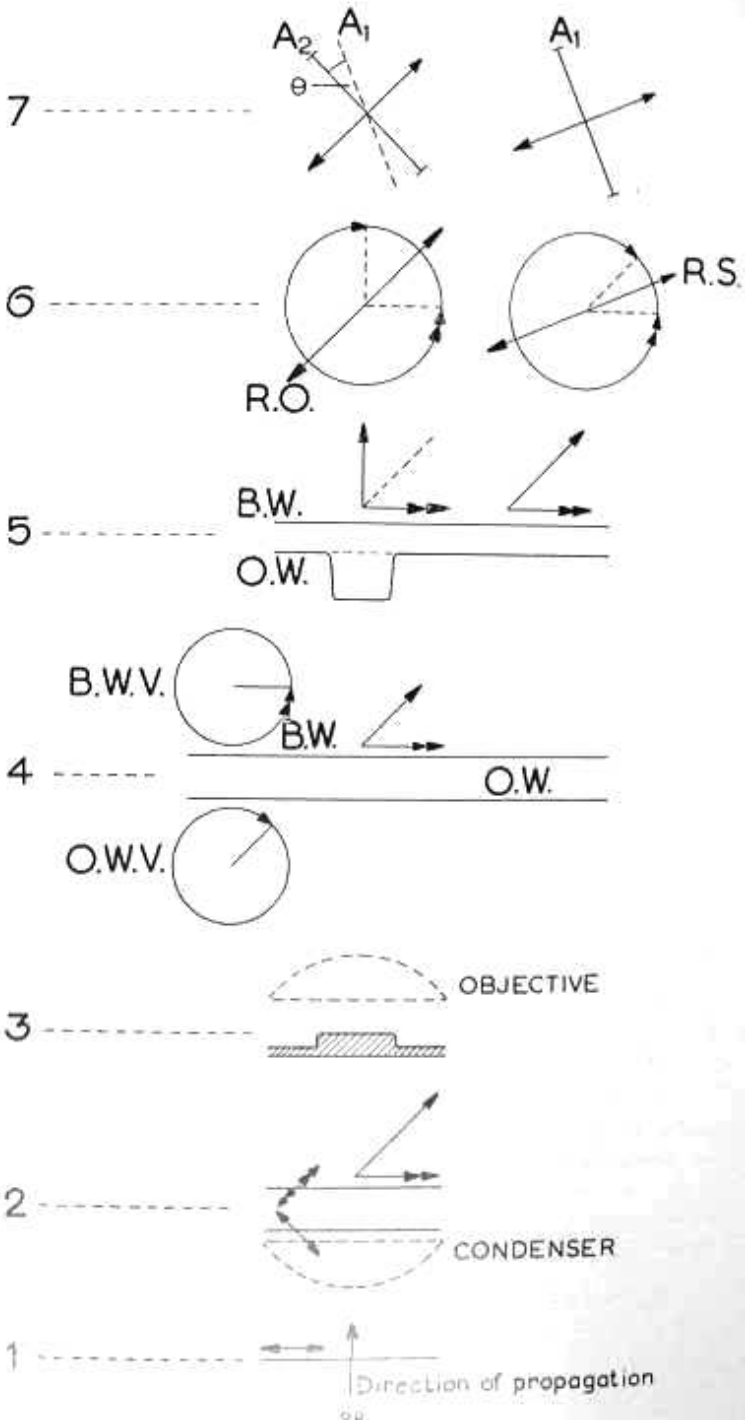
4: Both waves leaving the quarter-wave plate circularly polarised in opposite directions as shown by circle diagrams. The positions of the rotating arrows reproduce phase relationship shown by vector diagram.

3: Represents phase retarding element in the preparation.

2: Two waves derived from a single wave by the double-refracting plate. The resulting vibration directions shown by double arrows. Phase relationship between waves indicated by vector diagram.

1: Single wave front, double arrow indicating plane polarisation after leaving polariser.





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