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P. M. Davidson

It may readily be shown that specific volumes near the sphere’s surface, calculated from this \( \theta \), will, owing to its defective properties at \( r = a + ct \), be too large by an amount which is \( E(1 + \rho_0 f_0'/2f_0')/2\rho_0 f_0' \) at small values of \( t \), and dies away with increasing time, falling to half its value in a time of order \( a/c \).

If now the value of \( \delta V \) is calculated (in a portion of matter near the surface), averaged over a period of the sphere’s pulsation, it is found that its value when \( t \) is only a small fraction of \( a/c \) is less than the value \( E f_0'/2\rho_0(f_0')^2 \) attained at great \( t \) by two approximate expressions which are: (a) the expression mentioned above, which falls to a half value at a time of order \( a/c \); and (b) two terms, \( \epsilon^2 \Delta - E/\rho_0 f_0' \), where \( \Delta \) has the same sign as \( \sigma B \) and a magnitude equal to the amplitude of oscillation of the specific volume, in this portion of matter, in the established motion. These two terms arise from the terms with factor \( \epsilon^{(r-a-ct)/a} \) in (7), and thus, like the expression (a), decay to a half value at a time of order \( a/c \) (that is, at a time at which the wave has travelled out from the sphere’s surface through a distance of the order of the sphere’s radius).

Reference


The control of amplitude in phase-contrast microscopy

By E. W. Taylor

(Communicated by Sir Thomas Merton, Treas.R.S.—Received 6 November 1946)

[Plates 6 and 7]

Introduction

In the past the biologist has generally resorted to differential staining as a means of rendering visible slight non-homogeneities in his preparations. When such treatment was impracticable, as in the case of living cells, the alternatives were to study the out-of-focus image, to illuminate the specimen with very narrow pencils (with a consequent loss of resolution), or to use dark-ground illumination. Phase contrast offers a means of converting slight changes of refractive index (with the consequent change of wave front) into corresponding changes of amplitude. The method possesses the advantages that the object is accurately focused, that the full aperture of the objective is used and that the eye is particularly sensitive to changes in amplitude.

It also makes possible for the first time the detailed study at full aperture of transparent living tissue in place of the usual stained preparations which may have undergone considerable modification in the course of processing.
The control of amplitude in phase-contrast microscopy

HISTORICAL

The use of a phase plate or 'Z disk' to advance or retard the direct rays going to form an image, with respect to the diffracted rays was first proposed by Professor F. Zernike of Groningen University, and in the original description (Zernike 1934) the method was suggested as a means of increasing the sensitivity of the knife-edge test as applied to the mirrors of reflecting telescopes. In a very practical paper by Burch (1934), the Z test is applied to a decentered ellipsoid and photographs are given of the consequent Zernike fringes.

Thus matters remained for a short time with attention focused on astronomical reflectors, until Professor Zernike turned his attention to the microscope (Zernike 1935) and showed that the principles of phase contrast could be equally well applied, not in this case to the testing of the objective but to the transmutation of a change of phase produced by the object to a change of amplitude in the image.

Professor Zernike's invention was taken up by the firm of Carl Zeiss (German patent, D.R.P. no. 636168, Nov. 1932), and a very practical and well-illustrated contribution on this subject by Kohler & Loos (1941) next appeared in Germany.

A further addition to the literature on the subject was made by Burch & Stock (1942), who not only described the practical advantages of phase contrast in detail but showed how, with a little ingenuity, a standard microscope could be converted to this method of observation.

Reference should also be made to contributions by Richards (1944) and Linfoot (1945), and to papers by Jupnik (1944) and Osterberg (1944) in the U.S.A.

THE KNOWN METHOD

In known systems for phase-contrast microscopy, the image of an illuminated diaphragm placed in or near the front focal plane of the substage condenser is formed in or near the back focal plane of the microscope objective, and in this plane is placed a transparent phase-retarding plate which is such that a relative retardation of approximately $\frac{1}{4}\lambda$ is produced between the phases of the light passing through the image of the clear portion of the condenser diaphragm and that which has been diffracted by the object and passes through the remaining area of the objective aperture.

The condenser diaphragm is preferably in the form of an annulus, for reasons of symmetry, and is mounted co-axially with the condenser. The corresponding phase plate must then consist of a phase-changing annulus coinciding exactly with the image of the condenser annulus formed near the back focal plane of the objective. The phase plate is usually constructed of glass, and its phase-changing properties are obtained either by etching away a portion of its surface or preferably by depositing a suitable thickness of transparent material upon the surface in order to produce the required retardation, the essential feature being an optical path difference of approximately $\frac{1}{4}\lambda$ between the two areas referred to above.
The phase-contrast effects in the image field are produced by interference between light which passes directly through the condenser annulus and the annulus in the phase plate and light diffracted by the object which passes through the remainder of the aperture of the phase plate. Since the latter is often weak, there may be a marked difference in amplitude between the two interfering beams and consequent 'veiling glare'.

Two types of phase-contrast image may be produced. The so-called 'positive' type in which the annulus has a shorter optical path in relation to the rest of the plate and the 'negative' type in which these conditions are reversed. Different types of object or even different parts of the same object may demand differing optical densities of phase annulus, and the purpose of this paper is to describe a convenient means whereby relative amplitude of the direct and diffracted beams may be controlled.

**The new method**

As a means of controlling the amplitude of the direct and indirect beams going to form the image, a polarizer is interposed between the source of light and the object so that the latter is illuminated by plane-polarized light. An annular diaphragm is placed near the front focal plane of the condenser, and the image of this annulus is formed near the back focal plane of the objective in the usual manner. In this plane is a disk of optically active material consisting of an inner annulus of left-handed quartz and a central disk and outer annulus of right-handed quartz (or vice versa), both cut with their axes parallel to the optic axis of the microscope so that the planes of polarization of the direct and diffracted light are rotated in opposite directions through an angle of, say, 45°. The required optical path difference is obtained by means of a normal annular phase plate also mounted approximately in the back focal plane of the objective.

The object which is assumed to be isotropic is viewed in the usual way but with a rotatable analyzer interposed in some position between the phase plate and the eye of the observer. When the object is illuminated by monochromatic light and the vibration direction of the analyzer is parallel to that of the light that has traversed the central disk and outer annulus, the light which has passed through the central annulus is quenched, and at the same time a maximum of diffracted light is transmitted by the remaining portions of the plate. The effects are therefore those associated with dark-ground illumination.

On rotating the analyzer slightly, some light from the annulus is admitted and phase-contrast effects are produced. The light intensity contributed by the annulus to the final image can be varied as the analyzer is rotated through 90° from zero to maximum when the vibration direction of the polarizer is parallel to the direction of vibration of the light passed by the inner annulus, and in this position no diffracted light is transmitted, so that the effects associated with ordinary bright-field illumination slightly modified by the annular aperture are thus observed. If the phase-contrast effects have been positive over this quadrant of rotation of the analyzer,
The control of amplitude in phase-contrast microscopy

they now suddenly reverse and a graduated succession of negative phase-contrast effects is obtained throughout the next quadrant of rotation, ending with the equivalent of dark-ground illumination with the vibration direction of the analyzer again parallel to that of the light which has passed the central disk and the outer annulus.

In short, the phase of the light which has passed through the central annulus and phase plate either leads or lags behind that which has traversed the central disk and outer annulus by one-quarter wave-length, thus causing the phase-contrast effects to alternate from positive to negative as the phase difference changes from $+\frac{1}{4}\lambda$ to $-\frac{1}{4}\lambda$ or vice versa as the analyzer is rotated.

Although this arrangement produces the necessary conditions for phase-contrast illumination strictly for one wave-length only, it is quite suitable for use with the usual source of light employed by microscopists. Filters may be interposed if desired to narrow the band of wave-lengths used.

Various alternative arrangements are possible, but all make use of at least one polarizing element either between the source of light and the object or between the phase-plate and the eye of the observer.

Results

Epithelial cells (plate 6) and an organism, Caryophanus latum (plate 7), were selected as suitable objects, and a series of photographs was prepared by the courtesy of the Director of the National Institute for Medical Research with the direction of vibration of the analyzer set at varying angles as a means of modifying the relationship of amplitude in the focal plane.

The focal length of the objective was 4 mm., the total magnification was about 475 × (plate 6) and 375 × (plate 7). Further details are given in the description of plates on p. 426.

This apparatus was developed in collaboration with Mr B. O. Payne, M.Sc., D.I.C., A.R.C.S., A.Inst.P.

References

Kohler, A. & Loos, W. 1941 Naturwissenschaften, 29, 49.
Description of Plates 6 and 7

Plate 6. Epithelial cells. Objective 4 mm. $\lambda = 5461\,\text{Å}$. $475 \times$

(a) Transmitted light 12%. Phase difference $+\frac{1}{4}\lambda$.
(b) Transmitted light 3%. Phase difference $+\frac{1}{4}\lambda$.
(c) Transmitted light 0%. Dark ground.
(d) Transmitted light 3%. Phase difference $-\frac{1}{4}\lambda$.
(e) Transmitted light 12%. Phase difference $-\frac{1}{4}\lambda$.

Plate 7. Caryophanum latum. Objective 4 mm. $375 \times$

(f) Positive phase contrast. Large amplitude. Phase difference $+\frac{1}{4}\lambda$.
(g) Positive phase contrast. Small amplitude. Phase difference $+\frac{1}{4}\lambda$.
(h) Transmitted light. Narrow cone. Appearance with substage condenser nearly closed.
(i) Negative phase contrast. Large amplitude. Phase difference $-\frac{1}{4}\lambda$.
(j) Negative phase contrast. Small amplitude. Phase difference $-\frac{1}{4}\lambda$.

Cosmic-ray bursts and shower spread under large thicknesses of lead

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(Communicated by H. S. W. Massey, F.R.S.—Received 21 December 1946)

The frequency of occurrence of cosmic-ray bursts under large thicknesses of lead and iron has been measured as a function of the thickness, using a small ionization chamber with and without shielding material round its sides. Experiments have also been carried out with two small chambers side by side, the frequency of occurrence of bursts simultaneously in both chambers being observed under different thicknesses of lead.

While the burst-rate—thickness curves are, on the whole, fairly flat beyond the first maximum, there are indications of a second maximum at large thicknesses in the case of lead. From the experimental results information has also been obtained about the mean and the maximum angular spread of showers, and the increase in shower spread with increasing size of shower.

Introduction

In a previous paper from this laboratory (Mohr & Stafford 1944, hereafter referred to as paper A), the rate of burst production in large thicknesses of lead and iron was measured as a function of the thickness, and a pronounced ‘second maximum’ was found to occur for bursts of from 40 to 70 particles. The chamber used was an upright cylinder with a collecting volume of 221, and ratio of length to diameter of 2.6.

This result was sufficiently unexpected to make it worth while repeating the experiments with a smaller chamber of collecting volume 1.11, and ratio of length
PLATE 6. Epithelial cells. Objective 4 mm. $\lambda = 5461\text{Å}. \quad 475 \times$

(a) Transmitted light 12%. Phase difference $+\frac{1}{4}\lambda$.
(b) Transmitted light 3%. Phase difference $+\frac{1}{4}\lambda$.
(c) Transmitted light 0%. Dark ground.
(d) Transmitted light 3%. Phase difference $-\frac{1}{4}\lambda$.
(e) Transmitted light 12%. Phase difference $-\frac{1}{4}\lambda$. 
Plate 7. Caryophanum latum. Objective 4 mm. 375 x

(f) Positive phase contrast. Large amplitude. Phase difference $\frac{3}{4} \lambda$.
(g) Positive phase contrast. Small amplitude. Phase difference $\frac{1}{4} \lambda$.
(h) Transmitted light. Narrow cone. Appearance with substage condenser nearly closed.
(i) Negative phase contrast. Large amplitude. Phase difference $-\frac{1}{4} \lambda$.
(j) Negative phase contrast. Small amplitude. Phase difference $-\frac{1}{4} \lambda$. 