

The conventional theory of resolving power, as outlined in this section, is particularly appropriate to direct visual observations. With other methods of detection (e.g. photometric) the presence of two objects of such smaller angular separation than indicated by Rayleigh's criterion may often be resolved. In this connexion it is also of interest to compare the relative resolving efficiency of a telescope and a MICRONS Stellar Interferometer (§ 7.3.6). If the presence of two stars is judged by means of the first vanishing of the fringes formed by the interferometer, and if  $d$  is the maximum distance by which its outer mirror may be separated, then according to § 7.3 (34) double stars down to angular separation  $\phi \sim \frac{1}{2} \lambda/d$  may be detected with this instrument. Comparison of this value with (24) shows that, to detect double stars of this separation with a telescope used visually, the diameter  $2a$  of its objective would have to be about 2.44.

8.6.3 Image formation in the microscope

In the elementary theory of resolving power which we have just outlined, light from the two object points was assumed to be incoherent. This assumption is justified when the two objects are self-luminous, e.g. with stars viewed by a telescope. The intensity observed at any point in the image plane is then equal to the sum of the intensities due to each of the object points.

In a microscope the situation is, as a rule, much more complicated. The object is usually non-luminous and must, therefore, be illuminated with the help of an auxiliary system. Owing to diffraction on the aperture of the illuminating system (condenser), each element of the source gives rise to a diffraction pattern in the object plane of the microscope. The diffraction patterns which have centres on points that are sufficiently close to each other partly overlap, and in consequence the light vibrations at neighbouring points of the object plane are in general partially correlated. Some of this light is transmitted through the object with or without a change of phase, whilst the rest is scattered, reflected, or absorbed. In consequence, it is in general impossible to obtain, by means of a single observation, or even by the use of one particular arrangement, a faithful enlarged picture showing all the small-scale structural variations of the object. Various methods of observation have, therefore, been developed, each suitable for the study of certain types of objects, or designed to bring out particular features.

We shall briefly outline the theory of image formation in a microscope, confining our attention first of all to the two extreme cases of completely incoherent and perfectly coherent illumination. Partially coherent illumination will be discussed in § 10.5.2.

(a) Incoherent illumination

We first consider a self-luminous object (e.g. an incandescent filament of an electric bulb). Let  $P$  be the axial point of the object and  $Q$  a neighbouring point in the object plane, at a distance  $Y$  from  $P$ , and let  $P'$  and  $Q'$  be the images of these points (Fig. 8.31). Further let  $\theta$  and  $\theta'$  be the angles which the marginal rays of the axial pencils make with the axis.

If  $a'$  is the radius of the region (assumed to be circular) in which the beam of light converging on  $P'$  intersects the back focal plane  $\mathcal{F}'$  and if  $D'$  is the distance between the back focal plane and the image plane, then, since  $\theta'$  is small,

$$\theta' \sim \frac{a'}{D'} \quad (20)$$

Further, if  $w = \sqrt{p^2 + q^2}$  is the separation of  $Q'$  from  $P'$  measured in "diffraction units" (cf. § 8.3 (35) and § 8.5 (7)), i.e. the sine of the angle which the two points subtend at the centre of the diffracting aperture, then we have to a good approximation, (30)

$$Y' = wD'.$$

Let  $n$  and  $n'$  be the refractive indices,  $\lambda$  and  $\lambda'$  the wavelengths in the object and image spaces, and  $\lambda_0$  the wavelength in vacuum. Then, since according to § 8.5 (16) the first minimum of the diffraction pattern of  $P$  is given by  $w = 0.61\lambda/a'$ , we have, at the limit of resolution

$$Y' = 0.61\lambda \frac{D'}{a'} = 0.61 \frac{\lambda'}{\theta'} = 0.61 \frac{\lambda_0}{n \theta'} \quad (31)$$

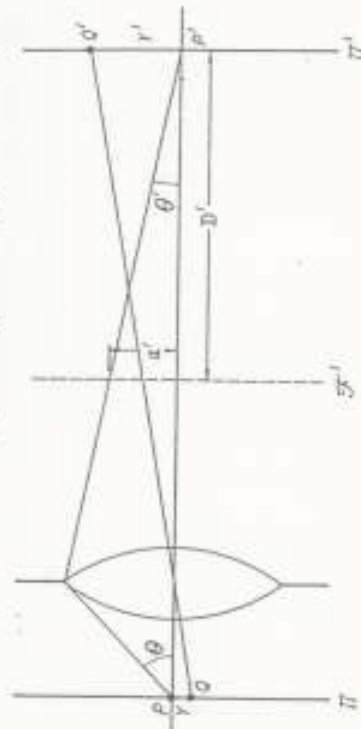


Fig. 8.31. Illustrating the theory of resolving power of the microscope.

A microscope must, of course, be so designed that it gives a sharp image not only of an axial point but also of neighbouring points of the object plane. According to § 4.5.1 the sine condition must therefore be satisfied, i.e.\*

$$n Y \sin \theta = -n' Y' \sin \theta'.$$

Since  $\theta'$  is small we may replace  $\sin \theta'$  by  $\theta'$ . On substituting for  $Y'$  into (31), we finally obtain

$$|Y| \sim 0.61 \frac{\lambda_0}{n \sin \theta} \quad (32)$$

This formula gives the distance between two object points which a microscope can just resolve when the illumination is incoherent and the aperture is circular.

The quantity  $n \sin \theta$  which enters into (32) is the numerical aperture, (cf. § 4.8 (13)) and must be large if a high resolving power is to be achieved. Means for obtaining a large numerical aperture were discussed in § 6.6.

(b) Coherent illumination—Abbe's theory

We now consider the other extreme case, namely when the light emerging from the object may be treated as strictly coherent. This situation is approximately realized when a thin object of relatively simple structure is illuminated by light from a sufficiently small source via a condenser of low aperture (cf. § 10.5.2).

Merc on Abbe

\* The minus sign appears here because  $\theta'$  corresponds to  $-\theta$  of § 4.5.





opaque regions, with period  $d$ . For simplicity the aperture will be assumed to be rectangular with two of its sides parallel to the strips.

According to § 8.6 (3)

$$U(\xi) = C_1' \left( \frac{\sin \frac{k\xi a}{2f}}{\frac{k\xi a}{2f}} \right) \frac{1 - e^{-i\pi a k \xi / 2f}}{1 - e^{-i\pi k \xi d / 2f}}, \quad (40)$$

where for  $U^m$  there has been substituted the expression relating to diffraction on a rectangular aperture and  $C_1'$  is a constant (cf. § 8.5.1). If the rectangular aperture extends in the  $\xi$  direction throughout the range

$$-a \leq \xi \leq a,$$

the disturbances in the image plane is, by (36) and (40), given by ( $C'$  denoting a constant)

$$V(x') = C' \int_{-a}^{+a} \frac{\sin \frac{kx\xi}{2f}}{\frac{kx\xi}{2f}} \frac{1 - e^{-i\pi a k \xi / 2f}}{1 - e^{-i\pi k \xi d / 2f}} e^{-ikx'U'} d\xi. \quad (41)$$

The position of principal maxima of the integrand are given by the roots of the equation  $1 - \exp(-i\pi k \xi d / 2f) = 0$ , i.e. by  $\xi = mf\lambda/d$ , where  $m$  is an integer. Between these principal maxima there are weak secondary maxima. If  $N$  is large, the principal maxima are very sharp and the secondary maxima negligible in comparison. To a good approximation we may then replace the integral by a sum of integrals, each extending from the midpoint  $Q_m$  of the interval between two successive principal maxima to the next midpoint  $Q_{m+1}$ . In each interval we may replace the argument by the central value  $\xi = mf\lambda/d = 2\pi m f / d$ , and obtain for  $V$  the following expressions:

$$V(x') \sim V_0 \sum_{-m < n < m} \frac{\sin \frac{\pi n x'}{d}}{\frac{\pi n x'}{d}} e^{-\frac{\pi n^2 x'^2}{d^2}}. \quad (42)$$

Here

$$\bar{m} = \frac{ad}{\lambda f}, \quad d' = M d = -\frac{D'}{f} d, \quad (43)$$

and  $V_0$  is the integral

$$V_0 = C' \int_{Q_m}^{Q_{m+1}} \frac{1 - e^{-i\pi a k \xi / 2f}}{1 - e^{-i\pi k \xi d / 2f}} d\xi, \quad (44)$$

which, apart from small correction terms in the high orders, is practically independent of  $m$ . The series (42) may be re-written in real form as

$$\frac{V(x')}{V_0} = 1 + 2 \sum_{1 < n < \bar{m}} \frac{\sin \frac{\pi n x'}{d}}{\frac{\pi n x'}{d}} \cos \frac{2\pi n x'^2}{d^2}. \quad (45)$$

Suppose first that the length  $a$  of the aperture is very large. The summation may then formally be extended over the whole infinite range ( $\bar{m} = \infty$ ), and we can easily

verify that the image is then strictly similar to the object. For this purpose we expand the transmission function  $F$  of the grating-like object (see Fig. 8.53),

$$F(x) = F_0 \quad \text{when} \quad 0 < |x| < d/2 \\ = 0 \quad \text{when} \quad d/2 < |x| < d/2 \quad (46)$$

into a FOURIER series

$$F(x) = c_0 + 2 \sum_{m=1}^{\infty} c_m \cos \frac{2\pi m x}{d}. \quad (47)$$

Then

$$c_0 = \frac{F_0}{d}, \quad c_m = F_0 \frac{\sin \frac{\pi m d}{d}}{\pi m} \quad (m = 1, 2, 3, \dots) \quad (48)$$

We see that apart from a constant factor this series is the same as (45).



Fig. 8.53. A grating-like object.

Suppose now that the length  $a$  of the aperture is decreased. If  $a$  is so small that only the zero-order spectrum contributes to the image, i.e. if  $\bar{m} = ad/\lambda f$  is only a fraction of unity, then according to (45)  $V(x') = \text{constant}$ , so that the image plane is uniformly illuminated. (This result is, of course, not strictly true, as we have neglected certain error terms; in reality there is a weak drop in intensity towards the edges.)

If in addition to the zero-order spectrum the two spectra of the first order ( $S_1, S_{-1}$ ) are also admitted by the aperture, i.e. if  $\bar{m} = ad/\lambda f$  is slightly greater than unity, then we see from (45) that

$$\frac{V(x')}{V_0} = 1 + 2 \frac{\sin \frac{\pi x'}{d}}{\frac{\pi x'}{d}} \cos \frac{2\pi x'^2}{d^2}. \quad (49)$$

The image has now the correct periodicity  $x' = d'$ , but a considerably flattened intensity distribution. By increasing the aperture more and more the image is seen to resemble the object more and more closely.

A completely false image is obtained when the lower orders are excluded. If for example all orders except the second are excluded, then

$$\frac{V(x')}{V_0} = 2 \frac{\sin \frac{2\pi x'}{d}}{\frac{2\pi x'}{d}} \cos \frac{4\pi x'^2}{d^2}. \quad (50)$$

so that the image has the period  $x' = d'/2$ ; the "image" shows twice the number of lines that are in fact present in the object.



Finally let us estimate the resolving power. Consider again the situation illustrated in Fig. 8.31, but assume now that the light from  $P$  and  $Q$  is coherent. Then the distribution in the image plane arises essentially from the coherent superposition of the two AXAY diffraction patterns, one centred on  $P'$ , the other on  $Q'$ . The complex amplitude at a point situated between  $P'$  and  $Q'$  at distance  $w_1$  (measured in "diffraction units") from  $P'$  is given by

$$U(w_1) = \left[ \frac{2J_1(kaw_1)}{kaw_1} + \frac{2J_1[ka(w-w_1)]}{ka(w-w_1)} \right] U_0 \quad (51)$$

$w$  being the distance between  $P'$  and  $Q'$  and the other symbols having the same meaning as before. The intensity is, therefore, given by

$$I(w_1) = \left[ \frac{2J_1(kaw_1)}{kaw_1} + \frac{2J_1[ka(w-w_1)]}{ka(w-w_1)} \right]^2 I_0 \quad (52)$$

Now in the case of incoherent illumination,  $P'$  and  $Q'$  were considered as resolved when the principal intensity maximum of the one pattern coincided with the first minimum of the other. The intensity at the midpoint ( $kaw \sim 1.92$ ) between the two maxima is then equal to  $2[2J_1(1.92)/1.92]^2 \sim 0.735$  of the maximum intensity of either, i.e. the combined intensity curve has a dip of about 26.5 per cent between the principal maxima. (This corresponds to the value 19 per cent for a slit aperture—cf. Fig. 7.03.) If we consider a dip of this amount as again substantially determining the limit of resolution, the critical separation  $w = 2w_1$  is obtained from the relation

$$\frac{I(w_1)}{I(0)} = 0.735, \quad (w = 2w_1). \quad (53)$$

The first root of this transcendental equation is  $w_1 \sim 2.57/\lambda a$ , so that the critical separation measured in ordinary units is

$$Y' = 2w_1 D' \sim \frac{2.57}{\pi} \frac{D \lambda'}{a} = \frac{0.82 \lambda_0}{\theta'} = \frac{0.82 \lambda_0}{\pi \theta'}. \quad (54)$$

To relate  $Y'$  to the corresponding separation  $Y$  of the object points we use the sine condition (with the approximation  $\sin \theta' \sim \theta'$ ), and finally obtain for the limit of resolution with coherent illumination the expression

$$|Y| = 0.82 \frac{\lambda_0}{\pi \sin \theta}. \quad (55)$$

Apart from a large numerical factor (which in any case is somewhat arbitrary as it depends on the form of the object and aperture and on the sensitivity of the receptor), we obtain the same expression as in the case of incoherent illumination [eq. (32)]. Thus with light of a given wavelength the resolving power is again substantially determined by the numerical aperture of the objective.

#### (c) Coherent illumination.—Zernike's phase contrast method of observation\*

We have defined a phase object as one which alters the phase but not the amplitude of the incident wave. An object of this type is of non-uniform optical thickness, but

\* For fuller discussion of the phase contrast method see, for example, M. FLEISCHER, *Le contraste de phase en optique et en microscopie* (Paris, *Revue d'Optique*, 1950); and A. H. HEENHUY, H. JURETS, H. OOSTERMAN, and O. W. BUCHANAN, *Phase Microscopy* (New York, J. Wiley & Sons, 1952).

does not absorb any of the incident light. Such objects are frequently encountered in biology, crystallography, and other fields. It is evident from the preceding discussion that with ordinary methods of observation little information about phase objects can be obtained. For the complex amplitude function that specifies the disturbance in the image plane is then similar to the transmission function of the object\* and, as the eye (or any other observing instrument) only distinguishes changes in intensity, one can only draw conclusions about the amplitude changes but not about the phase changes introduced by the object.

To obtain information about phase objects, special methods of observation must be used, for example, the so-called *central dark ground method of observation* where the central order is excluded by a stop, or the *Schlieren method*, where all the spectra on one side of the central order are excluded. The most powerful method, which has the advantage that it produces an intensity distribution which is directly proportional to the phase changes introduced by the object, is due to ZERNIKE and was first described by him in 1935. It is known as the *phase contrast method*.

To explain the principle of the phase contrast method, consider first a transparent object in the form of a one-dimensional phase grating. The transmission function of such an object is by definition (see p. 401) of the form

$$F(x) = e^{i\phi(x)}, \quad (56)$$

where  $\phi(x)$  is a real periodic function, whose period ( $d$  say) is equal to the period of the grating. We assume that the magnitude of  $\phi$  is small compared to unity, so that we may write

$$F(x) \sim 1 + i\phi(x). \quad (57)$$

If we develop  $F$  into a FOURIER series

$$F(x) = \sum_{m=-\infty}^{+\infty} c_m e^{i \frac{2\pi m x}{d}}, \quad (58)$$

then, since  $F$  is of the form (56) and  $\phi$  is real and numerically small compared to unity,

$$c_0 = 1, \quad c_{-m} = -c_m^* \quad (m \neq 0). \quad (59)$$

The intensity of the  $m$ th order spectra is proportional to  $|c_m|^2$ .

In the phase contrast method of observation a thin plate of transparent material called the *phase plate* is placed in the back focal plane  $\mathcal{F}'$  of the objective and by means of it the phase of the central order ( $S_0$  in Fig. 8.32) is retarded or advanced with respect to the diffraction spectra ( $S_{-1}, S_{-2}, S_2, S_{-3}, \dots$ ) by one-quarter of a period. This means that the complex amplitude distribution in the focal plane is altered from a distribution characterized by the coefficients  $c_m$  to a distribution characterized by coefficients  $c'_m$ , where

$$c'_0 = c_0 e^{i\pi/2} = \pm i, \quad c'_m = c_m \quad (m \neq 0). \quad (60)$$

\* Strict similarity would actually be attained only if the objective had an infinite aperture. Because the aperture is always finite, some details of the phase structures can be seen. In some cases the visibility of such "images" is enhanced, at the expense of resolution, by a slight defocusing of the instrument (cf. H. H. HORRIGAN, contribution in M. FLEISCHER, *Le contraste de phase et le contraste par interférences* (Paris, *Revue d'Optique*, 1952), 142).

† F. ZERNIKE, *Z. Tech. Phys.*, 18 (1935), 454; *Phys. Z.*, 38 (1935), 849; *Physica*, 9 (1942), 486, 974.



the positive or negative sign being taken according as the phase of the central order is retarded or advanced. The resulting light distribution in the image plane will now no longer represent the phase grating (67), but rather a fictitious amplitude grating

$$G(x) = \pm i + i\phi(x). \quad (61)$$

Hence the intensity in the image plane will now be proportional to (neglecting  $\phi^2$  in comparison to unity)

$$I(x') = |G(x)|^2 = 1 \pm 2\phi(x), \quad (62)$$

where as before  $x' = Mx$ ,  $M$  being the magnification. This relation shows that with the phase contrast method of observation, phase changes introduced by the object are transformed into changes in intensity, the intensity at any point of the image plane being (apart from an additive constant) directly proportional to the phase change due to the corresponding element of the object.\* When this phase of the central order is retarded with respect to the diffraction spectra (upper sign in (61)), regions of the object which have greater optical thickness will appear brighter than the mean illumination, and advanced regions of a bright phase contrast; when the phase of the central order is advanced, regions of greater spectral thickness will appear darker and one then speaks of a dark phase contrast (Figs. 8.34 and 8.35).

To obtain good resolution, the aperture of the illuminating system is often of annular rather than circular form (cf. § 8.5.2). In this case the annular region of  $\mathcal{F}'$  through which the direct (undiffracted) light passes plays the role of the central order  $S_0$  of Fig. 8.32, and it is this light which must then be retarded or advanced by a quarter period.

The phase-changing plate may be produced by evaporating a thin layer of a suitable dielectric substance on to a glass substrate. If  $n$  is the refractive index of the substrate and  $d$  the thickness of the layer, then for a retardation of a quarter of a period one must have  $d = \lambda/4(n-1)$ . A retardation of the central order by this amount is, of course, equivalent to an advance of the diffracted spectra by three-quarters of a period, and vice versa. It is possible to increase the sensitivity of the method by using slightly absorbing instead of a dielectric coating. We shall return to this point later.

It remains to show that the phase contrast method is not restricted to phase objects of periodic structure. For this purpose we divide the integral (34) into two parts:

$$U(\xi, \eta) = U_0(\xi, \eta) + U_1(\xi, \eta) \quad (63)$$

where

$$\left. \begin{aligned} U_0 &= C_2 \iint_{\mathcal{F}'} e^{-\frac{ik}{z}(x+\nu)} dx dy, \\ U_1 &= C_1 \iint_{\mathcal{F}'} [F(x, y) - 1] e^{-\frac{ik}{z}(x+\nu)} dx dy. \end{aligned} \right\} \quad (64)$$

$U_0$  represents the light distribution that would be obtained in the plane  $\mathcal{F}'$  if no object were present, whilst  $U_1$  represents the effect of diffraction. Now the "direct light"  $U_0$  (corresponding to the central order  $S_0$  of Fig. 8.32), will be concentrated in only a small region  $\mathcal{B}_0$  of the  $\mathcal{F}'$ -plane, around the axial point  $\xi = \eta = 0$ . On the

\* The approximation implicit in the elementary theory of the phase contrast method are discussed by J. FEENEY, *Zentralbl. f. Physik*, 58 (1936), 481; *ibid.*, 58 (1938), 1 and by V. D. KAMIN, *Proc. Phys. Soc.*, B 69 (1956), 1073.

other hand a very small fraction of the diffracted light will, in general, reach this region, most of it being diffracted to other parts of this plane.\*

Suppose that the region  $\mathcal{B}_0$  through which the direct light passes is covered by a phase plate. The effect of the plate may be described by a transmission function

$$A = ae^{i\alpha}. \quad (65)$$

For a plate that only retards or advances the light which is incident upon it,  $\alpha = 1$ ; for a plate that also absorbs light  $\alpha < 1$ . The light emerging from the aperture will be represented by

$$U'(\xi, \eta) = AU_0(\xi, \eta) + U_1(\xi, \eta) \quad (66)$$

so that, according to (36), the distribution of the complex amplitude in the image is given by

$$V(x', y') = V_0(x', y') + V_1(x', y'), \quad (67)$$

where

$$\left. \begin{aligned} V_0 &= AC_2 \iint_{\mathcal{B}_0} U_0(\xi, \eta) e^{-\frac{ik}{z}(x'+y'\nu)} d\xi d\eta, \\ V_1 &= C_1 \iint_{\mathcal{B}_0} U_1(\xi, \eta) e^{-\frac{ik}{z}(x'+y'\nu)} d\xi d\eta \end{aligned} \right\} \quad (68)$$

Now the aperture  $\mathcal{B}$  greatly exceeds in size the region  $\mathcal{B}_0$ , and since  $U_0$  was seen to be practically zero outside  $\mathcal{B}_0$ , no appreciable error is introduced by extending the domain of integration in  $V_0$  over the whole  $\mathcal{F}'$ -plane. Moreover, if  $\mathcal{B}$  is assumed to be so large as to admit all the diffracted rays that carry any appreciable energy, the integral for  $V_1$  may likewise be given infinite limits. Finally, if as before the transmission function  $F(x, y)$  is defined as zero at points of the object plane outside the region covered by the object, the integrals (68) may also be taken with infinite limits. We then obtain, on substituting from (64) into (68), and using the FOUCAULT integral theorem and the relation (38),

$$\left. \begin{aligned} V_0(x', y') &= CA, \\ V_1(x', y') &= C \left[ F\left(\frac{x'}{M}, \frac{y'}{M}\right) - 1 \right] = CF(x, y) - 1 \end{aligned} \right\} \quad (69)$$

From (67) and (69) it follows that the intensity in the image plane is given by

$$I(x', y') = |V(x', y')|^2 = |C|^2 |A + F(x, y) - 1|^2. \quad (70)$$

With a phase object

$$F(x, y) = e^{i\alpha\phi(x)}, \quad (71)$$

and (70) reduces to†

$$I(x', y') = |C|^2 [a^2 + 2(1 - a \cos \alpha - \cos \phi(x, y) + a \cos(x - \phi(x, y)))] \quad (72)$$

\* This point was investigated in detail by J. FEENEY, *Zentralbl. f. Physik*, 58 (1938), 1. See also F. ZERNIKE, *Mon. Not. Roy. Astr. Soc.*, 64 (1934), 382-385, where it is discussed in a somewhat different connexion.

† The special case when  $\alpha = 0$  corresponds to the dark-ground method of observation. According to (72), the intensity distribution is then given by

$$I(x', y') = 2C^2 [1 - \cos \phi(x, y)].$$



Since  $\phi$  was assumed to be small, (72) may be written as

$$I(x', y') = |C|^2 [a^2 + 2a\phi(x, y) \sin \alpha], \quad (73)$$

and, if the phase difference introduced by the plate represents a retardation or advance by a quarter of a period, then  $\alpha = \pm \pi/2$  and (73) reduces to

$$I(x', y') = |C|^2 [a^2 \pm 2a\phi(x, y)]. \quad (74)$$

When the plate does not absorb any of the incident light ( $\alpha = 1$ ) we have again the expression (62). The intensity changes are then directly proportional to the phase variations of the object. With a plate that absorbs a fraction  $a^2$  of the direct light the ratio of the second term to the first term in (73) has the value  $\pm \phi/a$ , so that the contrast of the image is enhanced. For example, by weakening the direct light to one-third of its original value, the sensitivity of the method is increased three times.

## 8.7. FRESNEL DIFFRACTION AT A STRAIGHT EDGE

### 8.7.1 The diffraction integral

Having considered various cases of FRAUNHOFER diffraction, we now turn our attention to the more general case of FRESNEL diffraction.

The basic diffraction integral § 8.3 (28) may be written in the form

$$U(P) = B(C + iS), \quad (1)$$

where

$$B = -A \frac{i}{\lambda} \cos \delta \frac{e^{i(kr + \epsilon)}}{r}, \quad (2)$$

$$\begin{aligned} C &= \iint_{\sigma} \cos (k\ell(\xi, \eta)) d\xi d\eta, \\ S &= \iint_{\sigma} \sin (k\ell(\xi, \eta)) d\xi d\eta, \end{aligned} \quad (3)$$

The intensity  $I(P) = |U(P)|^2$  at the point  $P$  of observation is then given by

$$I(P) = |B|^2 (C^2 + S^2). \quad (4)$$

We must now retain in the expansion § 8.3 (31) for  $\ell(\xi, \eta)$  terms in  $\xi$  and  $\eta$  at least up to the second order.

As before we take the plane of the aperture  $\sigma$  as the  $xy$ -plane. To simplify the calculations we choose as the  $x$  direction the projection of the line  $PP_0$  on to the plane of the aperture (Fig. 8.36). Thus with a source in a prescribed position our reference system will in general be different for different points of observation.

According to § 8.3 (30), we now have  $l = l_0$ ,  $m = m_0$ , so that the linear terms in  $\ell(\xi, \eta)$  disappear. The direction cosines of the rays  $P_0Q$  and  $OP$  are

$$\left. \begin{aligned} l &= l_0 + \sin \delta, \\ m &= m_0 + 0, \\ n &= n_0 + \cos \delta, \end{aligned} \right\} \quad (5)$$

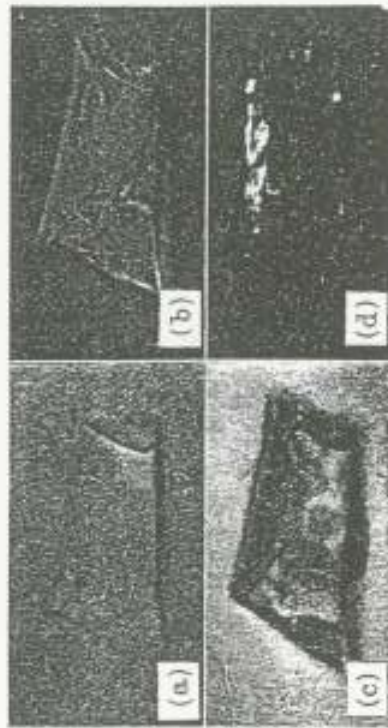


Fig. 8.34. Microscope images of glass fragments ( $n = 1.52$ ) mounted in clartin ( $n = 1.64$ ), 100  $\times$ .

(a), Bright field image; (b) and (c), Phase contrast images; (d), Dark field image. (After A. H. BEHWERT, H. JURST, H. OGYMANNO, and O. W. RICHARD, *Trans. Amer. Microscop. Soc.*, 65 (1946), 126.)

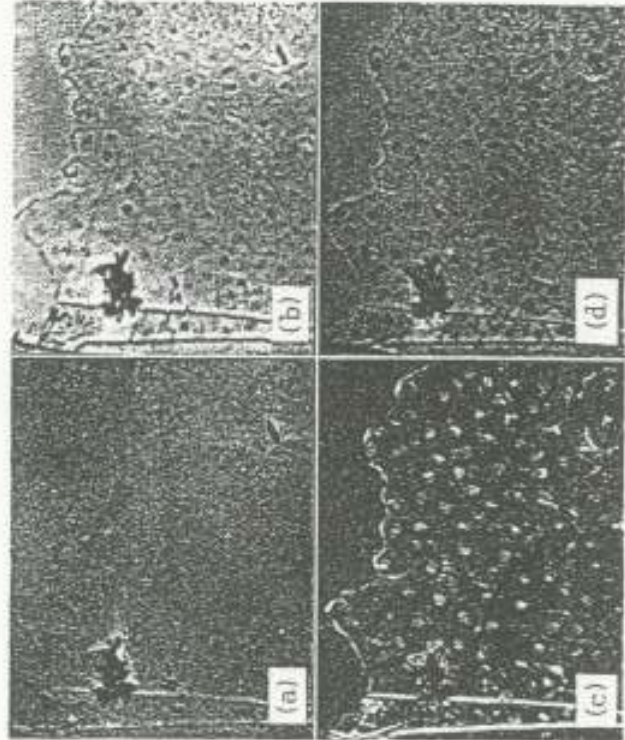


Fig. 8.35. Microscope images of epithelium from frog *Metabac muschiana*, 100  $\times$ .

(a), Bright field image at full aperture, N.A. = 0.26; (b), Bright field image with aperture half filled; (c), Phase-contrast image (bright contrast) at full aperture; (d), Phase-contrast image (dark contrast) at full aperture.

(After A. H. BEHWERT, H. JURST, H. OGYMANNO, and O. W. RICHARD.)