

not be extinguished. A study of the fringe coincidences as described by eq. (6) leads to an estimate of the angular distance between the edges of the star. Michelson obtained the following values in seconds of arc:

Betelgeuse	0.047''
Antares	0.040''
Arcturus	0.022''

Since the distances of these stars from the solar system (their parallaxes) are known from other measurements, their linear diameters can be calculated. These diameters turn out to be of the order of 10^8 km, which is about one hundred times the diameter of our sun and about equal to the diameter of the earth's orbit.

43. The Microscope

Heimholtz¹ treated the resolution of the microscope in the same way as that of the telescope. For an object let us take two luminous points a distance d apart and located in the lower focal plane F_1 of the objective; see fig. 89.

The objective produces an image of these points at infinity because the spherical waves emitted by the two points leave the objective as plane waves forming two bundles of parallel rays, the directions of which differ by an angle α . If the medium on both sides of the objective is air, then this angle is the same as the angle between the two central rays from the objects to the optical center of the objective. The value of this angle is d/f , where f is the lower focal length of the objective. If a medium of greater index of refraction $n > 1$ occupies the space between the object and the objective (immersion in oil), then for small angles of incidence the law of refraction gives

$$\alpha = n d/f.$$

We assume that the objective is, from the point of view of geometrical optics, a perfectly corrected system of lenses. The rim of the objective and all other diaphragms are projected as geometrical images (real or virtual) by the succeeding lenses. The smallest of these images is called the "exit pupil".

¹Die theoretische Grenze für die Leistungsfähigkeit der Mikroskope, Ann. d. Physik, 1874. Fraunhofer stated much earlier (Bayrische Akademie June 14, 1823) that the limit of effectiveness of a microscope depends on diffraction.

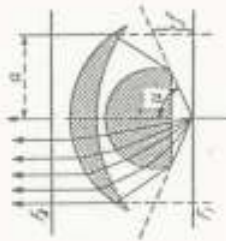


Fig. 89.

Paths of rays in a microscope. F_1 and F_2 are the front and rear focal planes.

of the lens system and it limits the size of our ray bundles.¹ In general the exit pupil is the virtual image of the rim of the front lens.² Let the radius of the exit pupil be a .

$$(2) \quad A = \frac{n \sin u}{\lambda}$$

is called the "numerical aperture"; from the elementary geometrical optics of lens systems (sine condition, Sec. 48) it follows that $a = f \sin u$, where $2u$ is the angle of opening of the object ray cone. Hence, the definition (2) of A becomes

$$(2 a) \quad A = n \sin u.$$

Each of our two ray bundles produces at infinity the Fraunhofer diffraction image of the exit pupil as described by eq. (36.9). Hence the microscope reproduces each of our luminous points in the form of a diffraction pattern (central field plus diffraction rings). In order to render these patterns observable at a finite distance, the eyepiece contains a converging lens on whose focal plane the diffraction patterns are reproduced. This image is observed through the eyepiece which acts as a magnifying glass.

This projection of the image upon a finite plane is of no concern to the theoretical investigation of resolution. Instead, the original diffraction pattern at infinity can be treated directly. As in the case of the telescope, eq. (42.1), we are then led to the result $n d/f = 0.61 \lambda/a$, which, according to the definition (2) of A , we can also write in the form

$$(3) \quad d = 0.61 \frac{\lambda}{A}.$$

Two luminous points are resolved only if the distance between them is greater than that given by (3).

Recalling the meaning (2 a) of A , we note that while the resolving power of the telescope depends on the size of the objective, the resolving power of the microscope depends on the angle u subtended by the objective at the specimen.

→ A. ABBE'S THEORY OF THE MICROSCOPE ←

With Heimholtz's theory the question of the resolving power of the microscope is essentially settled. What remains to be explained is the remarkable effect which the manner of illumination (bright or dark field) has on the resolution of different tissue structures even though it does not influence the resolution of two-point objects. This is where Abbe's theory is of importance. Abbe regarded the object as a diffraction grating (amplitude

or phase grating). Because of the thinness of the specimens observed and the low depth of focus of powerful objectives, these gratings can be considered the plane and their extent as to depth can be neglected. If we illuminate the object with coherent light parallel to the axis of the microscope, then plane waves emerge from the object in the directions of the grating spectra of various orders. Those orders which are emitted inside the angle of opening u are collected in the form of a Fraunhofer diffraction pattern on the upper focal plane F_2 of the objective which is located above the objective and very close to it. This pattern is easily observed with the eye piece removed. But the rays continue, and at infinity or on the focal plane of the collector lens they combine into a more or less faithful image of the object grating.

If the spectra on the upper focal plane of the objective are stopped down even further than this is done by the exit pupil, or if an objective of smaller aperture is used, the image becomes less distinct. If with oblique incidence, for instance, at least two spectra are present, then one only sees a sinusoidal structure without other details. If only one spectrum is admitted, the image disappears in a uniformly illuminated surface. It is also possible to simulate a structure that does not exist. If, for instance, the first order spectrum is stopped but the second order spectrum is allowed to pass, a grating with twice the actual number of lines appears. For the correct grating period to be just visible, at least both first order spectra must appear at the edge of the exit pupil of the objective; this means that the first order spectra may emerge from the objective at angles of at most $\pm u$. From the formula for the grating spectrum (32.2) we obtain for air and perpendicular incidence¹

$$(4) \quad \sin u \geq \alpha = \frac{\lambda}{d}.$$

If, on the other hand, the object and the first lens of the objective are embedded in a medium of index of refraction n (immersion), then λ must be replaced by the smaller wavelength $\lambda' = \lambda/n$; the grating spectra then crowd closer together, which explains the significance of immersion from the point of view of Abbe's theory. The condition (4) becomes now

$$(4 a) \quad \sin u \geq \alpha = \frac{\lambda'}{d} = \frac{\lambda}{n d}.$$

By (2 a) this means

$$(5) \quad A = n \sin u \geq \frac{\lambda}{d}.$$

We can now explain the advantage of *oblique* illumination. Let us assume, for instance, that the zeroth order spectrum falls on one edge of the exit pupil and that the first order spectrum falls on the opposite edge; this situation still suffices to show the existence of a structure and to reproduce its correct grating constant but no other details. The angle between the two spectra can now be twice as large as before or the spacing of grating lines can be half that of before. In place of eqs. (4 a) and (5) we obtain

$$(6) \quad 2 \sin u = \alpha - \alpha_0 = \frac{\lambda'}{d} = \frac{\lambda}{n d},$$

$$(6 a) \quad A = n \sin u = \frac{1}{2} \frac{\lambda}{d}, \quad \text{hence} \quad d = 0.5 \frac{\lambda}{A},$$

which involves only the small improvement in the ratio 0.5:0.61 over (3). If, instead of the spectra of zeroth and first order, the first and second order spectra or spectra of two still higher orders are used, then one speaks of "dark field illumination". The direct light (zeroth order spectrum) does not enter the objective and with no object in place the field of vision remains dark. For $n \sim 1.6$, $\sin u \sim 1$, $A \sim 1.6 n$ numerical estimate yields according to eq. (6 a)

$$d \sim \frac{\lambda}{3}.$$

Smaller spacings can be resolved only by the use of shorter wavelengths; an ultraviolet microscope equipped with quartz and fluorite lenses which is effective down to $\lambda = 0.2 \mu$ or an electron microscope. In the case of the latter the use of hard cathode rays makes the resolving power theoretically almost infinite.

These considerations are valid not only for the one-dimensional gratings which we have had in mind so far, but also for arbitrary plane structures which, according to the Fourier theorem for two dimensions, can always be considered as a superposition of cross gratings. The structure of the object and the structure of the diffraction image in the objective focal plane are "reciprocally" related to one another; one is the "Fourier-transform" of the other; see Vol. VI, eq. (4.13). This "reciprocity" means that the diffraction image of the second of the above structures again yields the structure of the original object. Also in the case of two-dimensional structures any loss of diffraction spectra resulting from stopping down reduces the similarity between the final image and the structure of the object.

¹ α and α_0 continue to stand for direction cosines; see footnote 1, p. 304.

B. SIGNIFICANCE OF PHASE GRATINGS IN MICROSCOPY

As an example, we shall consider the "laminary profile" of fig. 70. Like any pure phase grating, it is completely invisible if the image is perfect, for neither the retina nor the photographic plate can perceive phase differences. We would like to be able to see this grating as a set of bright and dark fringes, that is, as an *amplitude grating*. To accomplish this it suffices, according to the concluding remarks in Sec. 36 D, to shift the phase of the zeroth order spectrum by $\pi/2$ with respect to the phases of the spectra of higher orders. F. Zernicke¹ produced this phase shift in the following way: a glass plate is placed in the focal plane of the objective. A thin layer of transparent material is attached to the center of this plate where for axis-parallel illumination the spectrum of zeroth order appears. While the spectra of higher orders are unaffected, the phase of the zeroth order spectrum is changed by an amount which depends on the thickness of the thin layer and its index of refraction relative to the surrounding medium. In order for the phase change to be $\pi/2$, the path difference must be $\lambda/4$ and the thickness

$$d = \frac{\lambda}{4}(n - 1).$$

This is a real "quarter wave plate"; the layer is less than 1μ thick in contrast to the "quarter wave plate" of crystal optics, see Sec. 36 B, where the thickness was determined by the small difference $n_2 - n_1$ between the indices of refraction in the two principal directions of oscillation rather than by the much larger difference $n - 1$ between the indices of refraction of the plate and its surroundings.

This *phase contrast method* of Zernicke is perhaps the most sensitive way of making very weak phase structures visible. Previously microscopists had been obliged to use more or less oblique illumination. The resulting loss of several spectra caused blurred images of the object. The Zernicke method, on the other hand, fully utilizes the tissue structure and makes it visible to the eye in the same way as an ideal staining method would do.

C. LUMINOUS AND ILLUMINATED OBJECTS

Because of the great successes of Abbe's theory, it was thought for a long time that Helmholtz's theory applied only to *luminous sources*, while in the treatment of *illuminated objects* only Abbe's theory was believed to hold.²

¹Z. f. techn. Physik, Vol. 11, 1935.

²Such as in the review article by O. Lummer and F. Reiche: Die Lehre von der Bildentstehung im Mikroskop von Ernst Abbe, 1910.

However, Laue¹ proved by means of a simple hypothetical experiment that the image of any small luminous object, for instance a glowing Wollaston wire, is perfectly complementary to the image of the same object when illuminated by an external source. If the wire is in a cavity of constant temperature, then according to the laws of radiation it is entirely invisible. The radiation emitted by the wire and that originating from the walls of the cavity and absorbed and re-emitted by the wire combine to produce the same radiation density as that of the background. The same holds for observations with a microscope: the images resulting from self-luminous objects and those resulting from homogeneous illumination of equal brightness of the same objects must structurally complement one another entirely. The resolution of neighboring objects must be the same in both cases.

To be sure, the illumination is not homogeneous in the case of a microscope. With the usual arrangement of a lower illuminating mirror, the illumination is merely as uniform as possible within the aperture. However, the illumination from above is missing, but this cannot make much difference as long as the object is not (or only slightly) reflecting. We may think of this illumination from above as added or we may just omit it. The rays which do not enter the aperture are equally insignificant. Therefore Laue was justified in applying the law of black-body radiation to the microscope.

The superiority of Abbe's point of view becomes apparent only with oblique illumination of the type used in dark field observations. For, Helmholtz's theory assumes that all points of the object radiate uniformly in all directions, and this is generally not true for the elements of a tissue structure.

44. On Young's Interpretation of Diffraction

Even before Fresnel, Thomas Young³ attempted to find a wave-theoretical explanation for the diffraction phenomena which had been discovered by Grimaldi. Young assumed that the incident light undergoes "a kind of reflection" at the edges of the diffraction opening and he explained the diffraction fringes in terms of the principle of interference which he had discovered as caused by the interaction between these edge rays and the incident light rays. In this way he achieved a qualitative understanding of the diffraction pattern of the slit in particular. However, Fresnel in his prize

¹M. von Laue, Zur Theorie der optischen Abbildung, Ann. d. Phys. Lpz. 49, 1914.

³Phil. Trans. Roy. Soc. London 20, 1802.