New Instruments and Methods of Measurement

ELECTRON INTERFERENCE AND PHASE MICROSCOPY

V. I. MILYUTIN

Usp. Fiz. Nauk **74**, 553-566 (July, 1961)

1. INTRODUCTION

FOLLOWING the performance of experiments on the interference of electron waves,[1] the practical utilization of this phenomenon began to assume importance and the first electron interferometers were constructed. In some instances electron interference was subsequent to the splitting of coherent electron beams by thin monocrystalline layers,[2] while in other instances the beams were split by an external electric field.[3] Electron interference seemed to be especially promising for the purposes of electron microscopy. Electron interference was actually observed for the first time during electron microscope investigations. Fresnel interference fringes at the boundaries of underfocused and overfocused electron microscope images have been studied thoroughly. There have also been investigations of numerous interference effects arising when deformed monocrystalline layers are observed in an electron microscope. The interference of two electron beams, which is analogous to Fresnel interference in light optics, has also been observed when a special electron biprism is inserted in an electron microscope. In this case, of course, the electron microscope no longer functions as a microscope, but rather as a precision electron-beam instrument. The biprism is a deflecting electric field between a thin conductive filament and two plane parallel plates. The electron source is a very narrow pencil of electrons formed by double crossover in a stabilized electron gun. The electron beam is split while traversing the deflecting field of the biprism; the biprism filament can be either positive or negative. When the two beams are subsequently superimposed an interference picture is formed similar to that resulting when light passes through a glass biprism. It has been shown experimentally that coherence of the interfering beams is ensured by the smallness of the source, as well as by the monochromaticity and stability of the emitted electron beam.

It must be mentioned that these requirements for the beam, as well as the small angle between interfering beams that is required for the observation of interference patterns, make experiments on electron interference extremely complicated in practice. However, the highly developed technology of electron microscopy makes it possible to perform these experiments and to use the effects for practical purposes.

One of the first applications was the determination of internal potentials in different solids from the phase changes of coherent electron beams traversing the solids.[4] Phase shifts considerably greater than those of light beams were observed.[5] Large phase shifts of electron waves traversing thin objects, which naturally interest specialists in electron microscopy, permit the successful utilization of electron interference for a more intensive study of the microstructure and composition of objects, and also improve image contrast in an electron microscope.

In the present article we shall describe briefly the first attempts to combine the electron-microscope image of an object with the interference of electrons traversing the object, i.e., an electron interference microscope, as well as experiments on phase contrast in electron microscopes.

2. AN ELECTRON INTERFERENCE MICROSCOPE

The idea of combining an image with the interference of the waves traversing the object is not new. In light optics the appropriate instruments were constructed a long time ago. Linnik's microinterferometer[6] was constructed for the investigation of reflecting objects. V. A. Savin[7] devised a similar instrument for the study of transparent bodies. In the Linnik microinterferometer (Fig. 1) rays reflected from an object S_1 and forming its image in the focal plane S' of the microscope eyepiece will interfere in this same plane with a portion of the beam that is reflected from the perfect optical surface S_2. The plate P splits the light into coherent rays 1 and 2. In this way an interference pattern is superimposed on the image in the focal plane of the eyepiece; interference fringes are deformed in accordance with the microprofile of the object. This instrument is used to investigate the quality of surface treatments.

An instrument in which a microscope image and the deformed interference pattern of an object are observed simultaneously is customarily called an interference microscope.

The construction of an interference microscope for electron waves became possible after an electron biprism had been devised and the fundamental characteristics of electron interference had been determined. The construction of an electron interference microscope was first attempted in the laboratory of G. Möllenstedt,[8-10] where one form of an electron biprism had previously been developed. The biprism is placed in the path of the electron beam within an

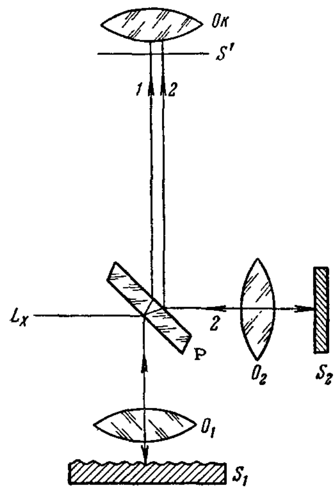


FIG. 1. Arrangement of the Linnik microinterferometer.

electron microscope, between the object and the objective lens, and induces the coherent splitting of the beam that is required for interference (Fig. 2a).

The biprism can also be positioned differently,—between the objective and the image plane, for example (Fig. 2b). Highly increased magnification results in the latter case.

A coherent beam traverses two adjacent regions of the object plane; in Fig. 2 these regions are represented by a thick line and thin line, respectively. The biprism splits the electron beam into two parts (Fig. 2a), which are converged by the objective and interfere in the crosshatched region. Interference fringes parallel to the biprism filament are formed in the image plane. The traversal of the object does not affect coherence but does induce a phase shift that displaces the interference fringes in the image. The image plane therefore contains simultaneously an image and an interference pattern modified by the object that supplies additional information regarding the microstructure of the latter. Even when the degree of image contrast is inadequate to reveal details the modified interference pattern can supply phase information about such details.

An electron interference microscope must satisfy more stringent requirements than a conventional electron microscope. The electron gun must produce a more intense beam. An additional cylindrical lens assists in forming a narrow pencil of electrons (the source for the biprism), usually in the shape of a slit. The projection system consists either of axisymmetric electron microscope projection lenses or magnetic quadrupole lenses that enhance the final image intensity.

We present a few photographs illustrating the operation and possibilities of an electron interference microscope. Figure 3 shows interference patterns obtained in the absence of an object, with two different voltages applied to the biprism filament. Figure 4 shows the edge of a carbon film and an interference pattern in which the bands have been shifted clearly by the film. This indicates that the phase of the electron waves has been changed while traversing the film. Figure 5 shows three MoO_3 crystals in the interfer-

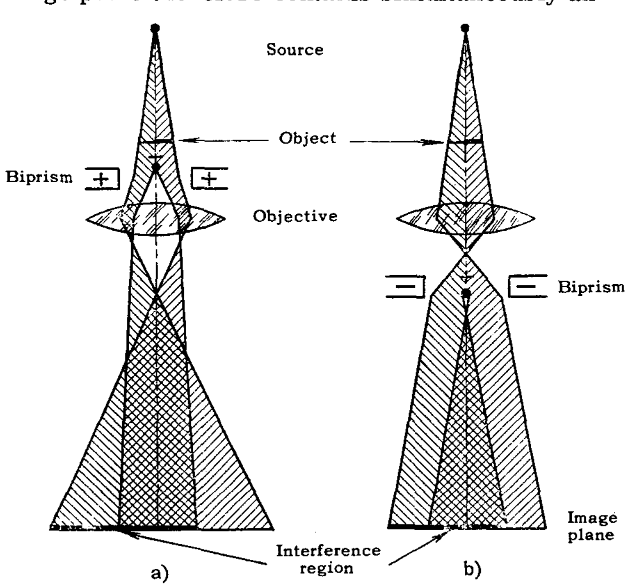


FIG. 2. Two possible arrangements of an electron interference microscope.

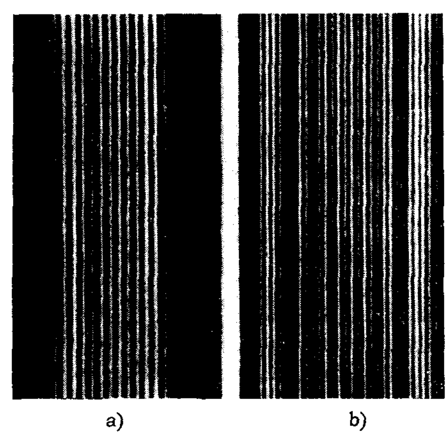


FIG. 3. Interference bands in the absence of an object. × 12 500. Voltage on biprism filament: a) – 7 v; b) – 9.5 v.

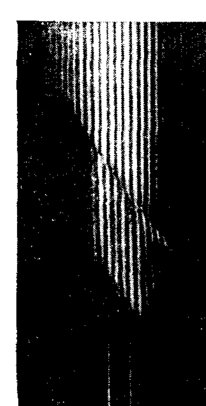


FIG. 4. Interference image of carbon film edge. × 8000.

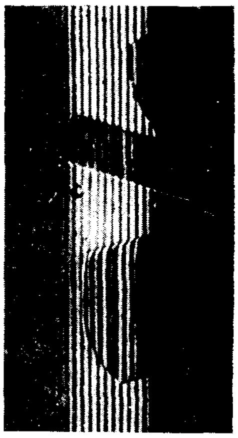
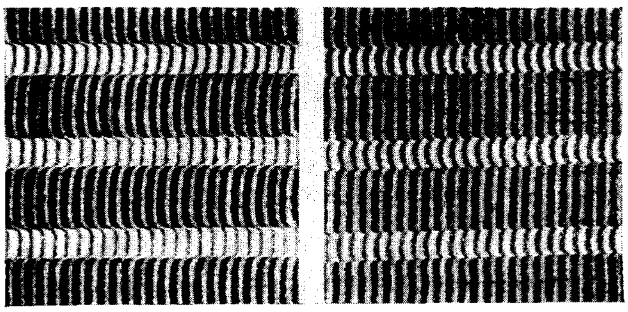


FIG. 5. Interference image of MoO_3 crystals. × 7600.



a) b)

FIG. 6. Influence of contact potential difference on electron interference pattern. a) Silver layer (in form of stripes) upon copper layer; b) Copper stripes upon silver layer.

ence field. Different crystal thicknesses produce different shifts of the interference fringes.

Figure 6 illustrates clearly the influence of contact potential difference on the interference pattern produced when electrons traverse two thin layers of different metals in contact. Interference bands are shifted to the right (Fig. 6a) by areas of a copper layer that has been coated with silver. Similar copper coating of a silver layer shifts bands to the left (Fig. 6b). The contact potential difference between these two metals is easily determined from the magnitude of the shift.

These and similar photographs obtained with an electron interference microscope can be used to investigate the microrelief of an object, and the thicknesses and densities of individual parts. Additional phase information results from the different internal potentials of the parts. It is known that electron scattering and internal potentials do not depend uniquely on the atomic weight. Thus if two different metallic layers are deposited in such a way as to yield identical electron scattering, they cannot be distinguished in a conventional image, but the shifts of the interference bands will not be identical. Figure 7 represents two shadings of a crystal produced by oblique deposition of gold and aluminum in different directions. Interference fringes are shifted differently in the two regions, with the greater shift for aluminum.

FIG. 7. Interference pattern of a crystal shaded with aluminum and gold in different directions. × 10 000.

Reference 11 describes an interesting attempt to obtain an interference image of thin ferromagnetic films. Conditions for the coherent splitting of an electron beam are here created in the object itself (the ferromagnetic film). Splitting occurs at the boundaries of the spontaneous magnetization regions (domains). When an electron beam traverses two adjacent regions magnetized in opposite directions (produced by special treatment of the layers in a variable magnetic field), the Lorentz force will deflect electrons in opposite directions (Fig. 8). Regions of convergence (K) and divergence (D) will then be formed near the domain walls. When the coherence conditions for deflected beams are satisfied, electron interference in the convergence region can be observed on a screen. The interference pattern is somewhat distorted by electrons traversing transition regions between domains. It appears, however, that the intensity of these electrons in the viewing plane is uniformly distributed in the convergence region and comprises only an insignificant fraction of the average intensity of the interference fringes. The intensity of the interference pattern in the final image plane for a given electron velocity depends on the magnetic deflection, width of the transition region, and number of fringes.

Figure 9 shows one possible electron-optical arrangement for obtaining a magnified shadow image of a ferromagnetic foil with a superimposed interference pattern. In Fig. 10 a bright band representing the convergence region and a dark band representing the di-

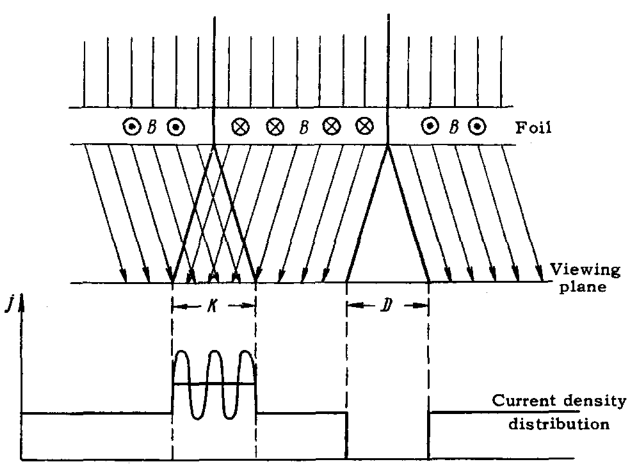


FIG. 8. Deflection of electrons passing through ferromagnetic foil.

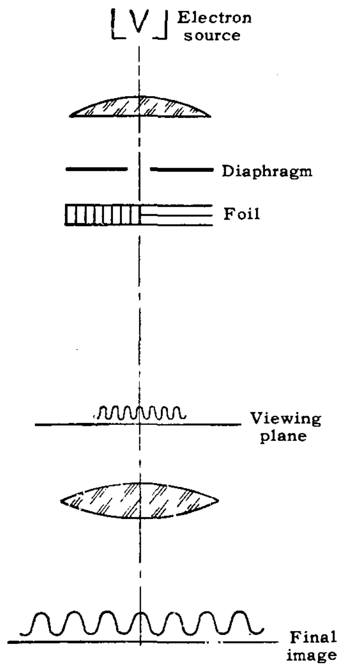


FIG. 9. Arrangement for obtaining interference image of ferromagnetic foil.

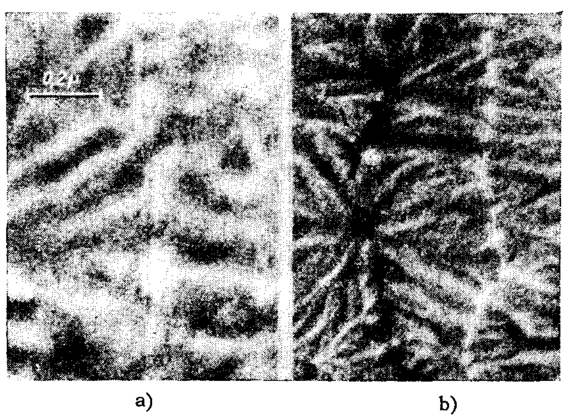


FIG. 10. a) Shadow image of ferromagnetic film; b) the same, enlarged.

vergence region are visible in addition to the microstructure of the ferromagnetic foil. An interference pattern is visible within the bright band, especially when the image is enlarged (Fig. 10b). This interference pattern can be used to study the magnetic properties of domains. For example, in agreement with theory,[12] the phase of electron waves traversing domains is influenced directly by the magnetic field of the latter.

3. PHASE CONTRAST OF ELECTRON-MICROSCOPE IMAGE

Image contrast in an electron microscope depends generally on the absorption, scattering, and phase shifts of electron waves traversing the object. However, both theory and experiment show the practical absence of electron absorption in a majority of objects, while the principal role in image contrast is played by phase shifts.

Different methods for improving image contrast are based on Abbe's general wave theory,[13] according to which the mechanism of image formation for trans-illuminated objects is as follows. A beam of parallel coherent rays passing through an object undergoes diffraction scattering at all points of the object's microstructure. The objective forms, in its focal plane, a diffraction image of the illumination source (the primary diffraction image), consisting of a central and noncentral diffraction maxima (Fig. 11). The interference of waves proceeding from the primary diffraction image produces a secondary diffraction image, which is the microscope image of the object. The intensity distribution in the image plane determines image contrast. Abbe's theory shows that the character of the final image is completely determined by the characteristics of the primary diffraction image within the aperture of the objective. The image is completely similar to the object only when all maxima resulting from the structure of the object impinge on the objective. Moreover, image-object similarity and image contrast can be affected by artificial modification of the primary image.

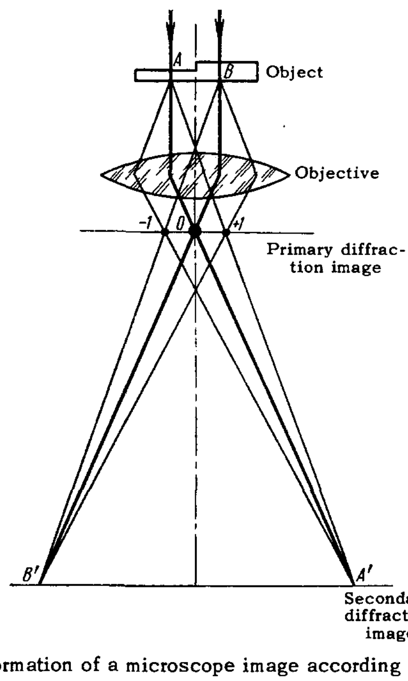


FIG. 11. Formation of a microscope image according to Abbe.

If, for example, one screens the central maximum (in central dark-field illumination) or the central maximum and the noncentral maxima on one side (in *oblique dark-field illumination*), image contrast is enhanced in some instances.

The most promising procedure for electron microscopy lies in modification of the primary image for the purpose of changing the phases of waves proceeding from the central and noncentral maxima. This alters the interference conditions and, consequently, changes the intensity distribution in the final image plane, with enhanced contrast as the ultimate result. The method of artificial phase shifts in the central or noncentral maxima for the purpose of enhancing image contrast is called phase contrast, and was first suggested by Zernike for light microscopy.[14]

We shall now study the essential nature of this phase contrast method using as an example a transparent object consisting of a small article with the refractive index n_1 imbedded in a homogeneous film with the refractive index n (Fig. 12). Let the object be illuminated by parallel coherent rays. The amplitudes of the rays passing through the film and small article will be identical, but the phases will differ by

$$\delta = 2\pi \frac{d(n-n_1)}{\lambda}, \tag{1}$$

where d is the thickness of the film, and λ is the wavelength of the illumination. The equations of vibrations at points of the film and small article will be, respectively,

$$y_1 = a\cos\omega t \text{ and } y_2 = a\cos(\omega t + \delta). \tag{2}$$

Figure 12 shows vector diagrams for both vibrations. y_2 can be represented by two components, one of which is in phase with y_1, while the phase of the other differs from y_1 by $\delta/2 + \pi/2$. The equation for y_2 can be replaced identically by

$$y_2 = a\cos\omega t + 2a\sin\frac{\delta}{2}\cos\left(\omega t + \frac{\delta}{2} + \frac{\pi}{2}\right).$$

This system of two waves (y_1 and y_2) emerging from the film and the small article can be replaced by a different system of two waves, one of which comes from all points of the object including the small article,

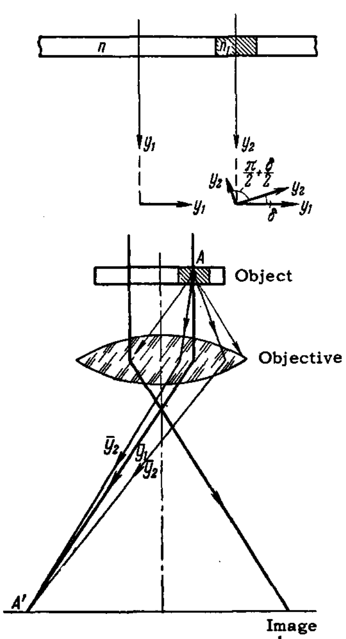


FIG. 12. Formation of an image by phase contrast.

while the other wave comes only from the latter. The equations of these vibrations are

$$\bar{y}_1 = a\cos\omega t,\ \bar{y}_2 = 2a\sin\frac{\delta}{2}\cdot\cos\left(\omega t + \frac{\delta}{2} + \frac{\pi}{2}\right). \tag{3}$$

The $\bar{y}_1$ rays pass through the object without scattering, are collected at the focus of the objective, and form the principal maximum (Fig. 12). The $\bar{y}_2$ rays are diffracted while passing through the small article and form spectra in the focal plane of the objective that differ in phase by the angle $\delta/2 + \pi/2$ from the principal maximum. The transmitted $\bar{y}_1$ rays provide uniformly bright illumination (background) in the image plane. Only slight illumination is produced by the diffracted $\bar{y}_2$ rays (with the amplitude $2a\sin\delta/2$), since δ is relatively small as a rule. The image intensity of the small article (of the point A, for example) depends on the interference of the $\bar{y}_1$ and $\bar{y}_2$ rays, which differ in phase by $\delta/2 + \pi/2$.

If a phase plate is positioned at the location of the principal maximum in the rear focal plane of the objective, in order to change the phase of transmitted rays by the angle Δ (neglecting the small number of $\bar{y}_2$ rays passing through the principal maximum), the vibrations in the image plane (at point A', Fig. 12) will be

$$\bar{y}_1' = a\cos(\omega t - \Delta), \qquad \bar{y}_2' = 2a\sin\frac{\delta}{2}\cos\left(\omega t + \frac{\delta}{2} + \frac{\pi}{2}\right). \tag{4}$$

As a result of interference the amplitude of the resultant vibration in the image of the small article will be

$$\bar{a}^2 = a_1^2 + a_2^2 + 2a_1a_2\cos\varphi, \tag{5}$$

where

$$a_1 = a, \quad a_2 = 2a\sin\frac{\delta}{2}, \quad \varphi = \frac{\delta}{2} + \frac{\pi}{2} + \Delta.$$

We can therefore write

$$\bar{a}^2 = a^2 + 4a^2\sin^2\frac{\delta}{2} + 4a^2\sin\frac{\delta}{2}\cdot\cos\left(\frac{\delta}{2} + \frac{\pi}{2} + \Delta\right). \tag{6}$$

or

$$\bar{a}^2 = a^2\left[1 + 4\sin\frac{\delta}{2}\left(\sin\frac{\delta}{2} - \sin\left(\frac{\delta}{2} + \Delta\right)\right)\right].$$

The brightness contrast between the image and the background is given by

$$k = \frac{a^2 - \bar{a}^2}{a^2}$$

(where $\bar{a}$ is the amplitude of the vibrations determining image brightness for the small article, and a is the amplitude determining background brightness), or

$$k = 4\sin\frac{\delta}{2}\left[\sin\left(\frac{\delta}{2} + \Delta\right) - \sin\frac{\delta}{2}\right]. \tag{7}$$

It is evident that $k = 0$ when $\Delta = 0$.

The largest value of k results when $\Delta = \pi/2 - \delta/2 \approx \pi/2$:

$$k_{\max} = 4\sin\frac{\delta}{2}\left(1 - \sin\frac{\delta}{2}\right) \approx 4\sin\frac{\delta}{2}. \tag{8}$$

The case $\Delta = \pi/2$ produces positive contrast, i.e., the small article is darker than the background (Fig. 13a). $\Delta = -\pi/2$ (or $\frac{3}{2}\pi$) produces negative contrast ($k_{max} \approx -4 \sin \delta/2$), in which the small article is brighter than the background (Fig. 13b).

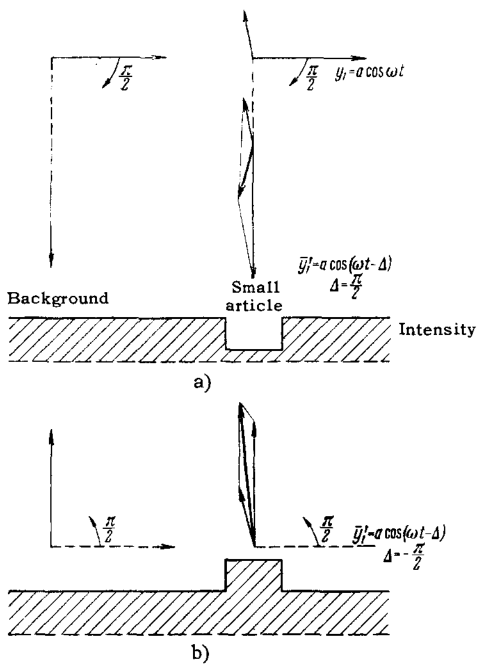


FIG. 13. Production of (a) positive and (b) negative phase contrast.

We have been considering the simplest case. A phase grating is customarily used as a typical object for theoretical investigation and experimental checks; in this case image formation is more complex but the phase contrast method remains essentially unchanged.

In an electron microscope the possibility of producing phase contrast is associated, on the one hand, with the possibility of obtaining coherent electron beams for illuminating the object, and, on the other hand, with the possibility of preparing a suitable phase plate to modify the phase difference between the rays from the principal and noncentral maxima.

With regard to the first of these factors, experiments on electron interference have shown that it is entirely possible to produce coherent electron beams. It is also possible to prepare a phase plate. As already mentioned, when electrons pass through matter a considerable phase shift results; for example, a collodion film 180 A thick induces a phase shift of the order $\pi/2$ in the case of 60-kv electrons. It must be kept in mind, of course, that because of the small wavelength of electrons the diffraction maxima in the primary image are very close and can even overlap partially. In order to screen out only the central maximum or only the noncentral maxima, the phase plate must be very small.

The first attempts to prepare suitable phase plates and to obtain phase contrast of the image in an electron microscope were made in Japan by Kanaya et al.[15] The same workers had previously carried out a theoretical study of phase contrast for the simplest kind of object in the form of a uniform circular disk.* They calculated the intensity distribution of electron waves in the image plane, resulting from the interference of waves diffracted by the object, when the phase plate lies in the path of these waves. When the phase plate blocks diffracted waves (i.e., screens noncentral maxima, as in Fig. 14a) positive (or dark) contrast results, i.e., a dark disk against a bright background. When a phase plate in the form of a small circular disk blocks transmitted rays (i.e., screens the principal maximum, as in Fig. 14b), negative (bright) contrast results, i.e., a bright disk against a dark background.

The vector diagrams below the figures explain the effects of the phase plates clearly. In the first case (a central aperture) the amplitude is reduced compared with the background. In the second case (a disk at the center) the amplitude is enhanced compared with the background. In the absence of a phase plate, amplitude gradations in the image plane are insignificant.

The degree of phase contrast for the center of an object (the so-called macrocontrast) for both cases was calculated from the intensity distribution in the image plane:

$$k = \frac{I_1 - I_2}{I_2} \approx \frac{(1-p)^2 \Delta\delta^2 - p^2 \Delta\delta^2}{(1-p)^2 \Delta\delta^2}, \tag{9}$$

where I_1 is the intensity of the object, I_2 is the intensity of the background, $\Delta\delta$ is the phase shift induced when electrons pass through the object, and $p = (R_s/R_a)^2$ (Fig. 14). Electron microscope experiments performed with a phase plate containing apertures indicated the possibility of constructing a phase electron microscope. It is much more difficult to use a phase plate in the form of a disk, since the electron beam is focused in the rear focal plane of the objective at the point where the phase plate must be located. The beam intensity in this region can be ten times greater than at the object, resulting in contamination and charging up, or burning through, of the plate.

With a specially prepared phase plate in the form of a collodion or carbon film containing 5 — 50 micron apertures, Kanaya et al.[15] were the first to obtain a phase contrast image. Figure 15 shows two photographs of molybdenum oxide on Formvar, without and with a phase plate. The latter case shows more clearly the fine structure of the interference bands for the crystal.

Figures 16 and 17 are photographs of a biological object, for which phase contrast can be especially

*The theory of phase contrast in electron microscopes is also discussed by V. Glazer in his book Osnovy elektronnoi optiki (Fundamentals of Electron Optics), Gostekhizdat, Moscow, 1[illegible]

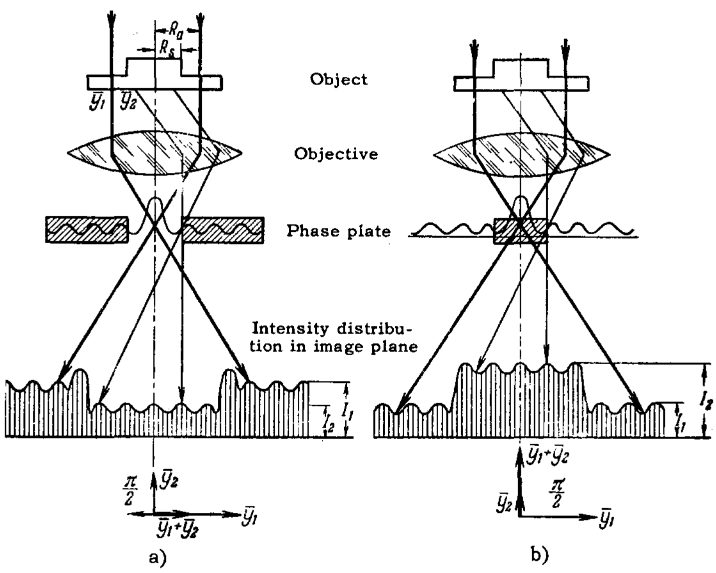


FIG. 14. Different types of phase plates. a) Plate with aperture; b) plate in form of disk.

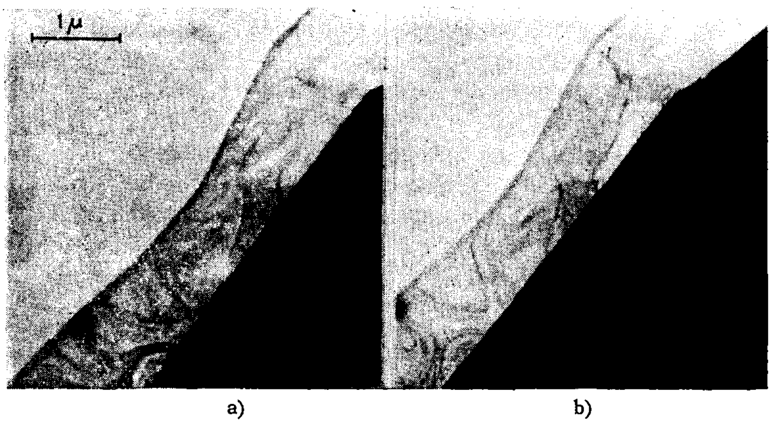


FIG. 15. Image of molybdenum oxide on Formvar. a) Without phase plate; b) with phase plate.

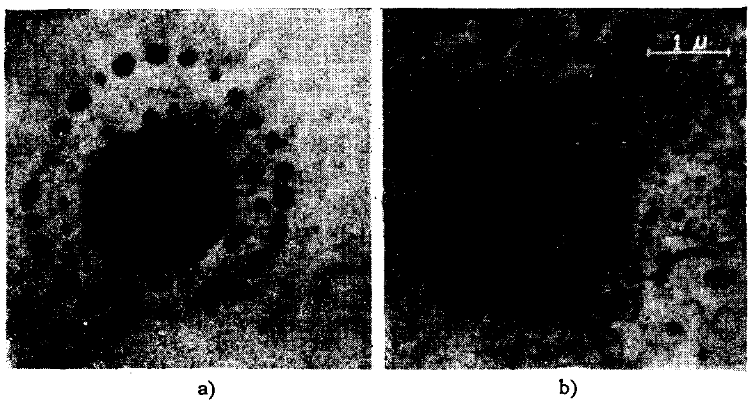


FIG. 16. Image of section through cell serum from the parotid gland of a dog. a) Without phase plate; b) with phase plate.

useful. Images of section through cell serum from the parotid gland of a dog were obtained (a) without and (b) with a phase plate. It is evident that the microstructure of an object is revealed more clearly by means of phase contrast.

Electron phase contrast has also been investigated recently by French workers,[16] who used a 100-kv magnetic electron microscope. The phase plate was prepared by depositing a 150-A mixture of boron and carbon on a collodion film with a 0.3-micron slit. The layer thickness required for a $\pi/2$ phase shift was monitored by means of the interference fringe shift in an electron interference microscope. The phase plate was not placed in the focal plane of the objective, but was positioned between the intermediate lens and the projector at the point where the second diffraction image of the source is formed. In this arrangement the separation between the diffraction maxima is

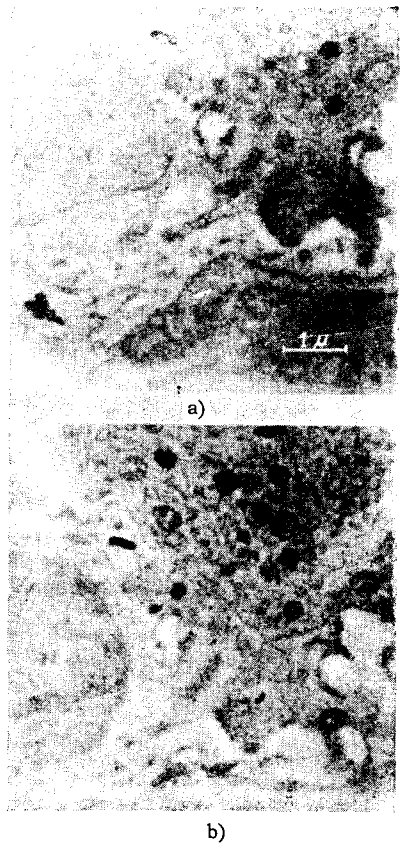


FIG. 17. Image of the same object as in Fig. 16. a) Without phase plate; b) with phase plate.

greater and the electron beam density is smaller, making it easier to overcome the difficulties mentioned above. It is difficult, of course, to adjust the phase plate aperture with respect to the source image.

Figure 18 illustrates the phase contrast obtained with this arrangement.

[1] V. I. Milyutin, Usp. Fiz. Nauk **65**, 665 (1958).
[2] Marton, Simpson, and Suddeth, Phys. Rev. **90**, 490 (1953); Rev. Sci. Instr. **25**, 1099 (1954).
[3] G. Möllenstedt and H. Düker, Z. Physik **145**, 377 (1956).
[4] G. Möllenstedt and M. Keller, Z. Physik **148**, 34 (1957).
[5] E. G. Ramberg, J. Appl. Phys. **20**, 441 (1949).
[6] V. P. Linnik, Doklady Akad. Nauk SSSR, No. 1 (1933).
[7] V. A. Savin, Doklady Akad. Nauk **59**, 177 (1949).
[8] G. Möllenstedt and R. Buhl, Physik. Blätter, **13**, 357 (1957).
[9] R. Buhl, Z. Physik **155**, 395 (1959).
[10] G. Möllenstedt, European Regional Conference on Electron Microscopy, Delft, August 29, 1960.
[11] Boersch, Hamisch, Wohlleben, and Grohmann, Z. Physik **159**, 397 (1960).
[12] Y. Aharonov and D. Bohm, Phys. Rev. **115**, 485 (1959).
[13] E. Abbe, Schultzes Archiv f. mikr. Anat. **9**, 413 (1873).
[14] F. Zernike, Z. tech. Physik **16**, 454 (1935); Physica **9**, 686, 974 (1942).
[15] Kanaya, Kawakatsu, and Ishikawa, Bull. Electrotech. Lab. **21**, No. 11 (1957).
[16] Faget, Ferre, and Fert, Compt. rend. **251**, 526 (1960).

Translated by I. Emin

FIG. 18. Image of collodion film. a) Without phase plate; b) with phase plate.