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The Resolution of Two Particles in a Bright Field by Coated Microscope Objectives

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A theory is presented for determining the diffraction image of two, identical, opaque or transmitting, uniform, individually unresolved particles of finite area located in a uniform, transmitting surround. Numerical calculations are included for the classical Airy type objective, for center-blocked Airy type objectives and for simplified Sonine type objectives which have been coated so as to produce diffraction patterns having central disks of reduced diameter. It is shown that marked improvement in the resolution of two particles can be expected by such coated objectives. The resolving power for two particles increases rapidly with the area of the particles and has unexpectedly steep dependence upon the relative amplitude and phase transmission of particle and surround. The theory, subject to experimental verification, forms a basis for explaining why a microscope as used with transmitted light can resolve considerably better than is indicated by the Airy limit.

I. INTRODUCTION

When the amplitude and phase transmission of the exit pupil of a microscope objective is suitably varied by coating the exit pupil with refracting and absorbing materials, it is possible to reduce the diameter of the Airy disk. Reduction of the diameter of the Airy disk below that of the central maximum in the classical, Airy type, diffraction image is necessarily accompanied by a marked drop in the energy density at the diffraction head and by a corresponding rise in the energy density of the diffraction rings. Therefore, the neighborhood of the geometrical image of two particles will be overlapped by an increasing amount of energy from the diffraction rings resulting from object points in the bright field as the object slide is viewed by objectives which have the same numerical aperture but which produce Airy disks of reduced diameter. The following theory will include the effects of the overlapping, diffracted energy from object points located in the bright field upon the image of two tranmitting or opaque partticles. Each of the particles is assumed to be unresolvable and to have the area A. It is assumed for simplicity that the particles are identical, non-overlapping and have uniform phase and amplitude transmissions. The theory is readily extended to dissimilar, overlapping, unresolved particles. The total energy density in the image plane will be calculated upon the supposition that the source of light is uniform and is large enough to fill the exit pupil of the objective. The objectives may have numerical apertures ranging from low to intermediate values such as 0.7.

The X direction is chosen in the image plane along the line connecting the centers of the geometrical images of the two particles. The origin is located midway between these centers so that the centers are located at $x=\pm Ml$, where M is the magnification of the objective. The total energy density G is a function of both x and y. Consistent with the usual practice, the analysis will be confined to the energy distribution $G(x,0)\equiv G(x)$. The Airy limit of resolution is not a suitable limit for purposes of the following discussion. A more useful and *physical* limit of resolution is defined[1] as the separation $2l$ or $2Ml$ for which

$$\frac{d^2}{dx^2}G(x)=0 \qquad \text{when } x=0. \tag{1}$$

This physical limit is a function of the area and of the amplitude and phase transmission of the particles. It will be shown to decrease with the diameter of the Airy disk when the objective is coated for obtaining the simplest Sonine[2] type diffraction pattern or when the central zone of the objective is made opaque as in the classical method of the ink spot.

II. THE AMPLITUDE AND PHASE EQUATION

The conjugate object and image planes with magnification M are denoted, respectively, by X_0, Y_0 and X, Y. The object plane consists as in Fig. 1 of two, like, uniform particles of area A located at distances $x_0=\pm l$ from the optical axis and of a surround whose amplitude and phase transmission is chosen as reference and hence as the complex number unity. It is supposed that the surround is never opaque. The amplitude and phase transmission of the particles is represented by the complex number

$$f_0=ge^{-i\Delta}, \tag{2}$$

in which g is the ratio of the amplitude transmission of the particles to the amplitude transmission of the surround and in which Δ is the difference between the phase transmissions of the particles and surround considered as positive when the optical path of the particles exceeds that of the surround.

It is supposed that the microscope is adjusted for Köhler illumination and that the condenser diaphragm is adjacent to the first focal plane of the substage condenser. The object slide is then illuminated by substantially plane wave fronts which originate from each point in the condenser diaphragm. The normals to these wave

[1] Ramsay, Cleveland, and Koppius, J. Opt. Soc. Am. 31, 2[illegible] (1941).
[2] Osterberg and Wilkins, J. Opt. Soc. Am. 39, 553 (1949).

fronts have the optical direction cosines p_0, q_0. If the exit pupil is conjugate to the condenser diaphragm, an image of the source of light will be located at the exit pupil. It is supposed that the exit pupil is filled by the source. The amplitude and phase transmissions of the coating applied to the exit pupil are included in the pupil function $P(p, q)$ in which p, q are the optical direction cosines of the axial bundle in the image space. This pupil function includes the phase variation due to spherical aberration and also the amplitude variation on the spherical wave converging upon the axial point $x=y=0$. ϑ_m is the maximum angular aperture of the objective in its image space. The objective is assumed to obey the Abbe sine condition.

The relation between the condenser diaphragm and the plate for supporting the coating material at the exit pupil is similar to the relation between the condenser diaphragm and the diffraction plate in the phase microscope. Only the shape of the opening in the condenser diaphragm and the distribution of the coating materials upon the exit pupil are different. The general equations which appear in the theory of phase microscopy apply therefore to the amplitude and phase distribution $F_0(x, y, p_0, q_0)$ produced over the image plane from the incidence upon the object plane of a single wave front whose normal has the optical direction cosines p_0, q_0. Thus,[3]

$$F_0(x, y, p_0, q_0)=\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f(x_0, y_0, p_0, q_0) \times\exp[2\pi i(p_0x_0+q_0y_0)] \times U(x-Mx_0, y-My_0)dx_0dy_0, \quad (3)$$

in which $f(x_0, y_0, p_0, q_0)$ is the object function which specifies the modification of the amplitude and phase transmission of the object plane due to the presence of the object specimen. The object function is considered to vanish beyond the field of the optical system. $U(x, y)$ is the primary diffraction integral defined by

$$U(x, y)=\int\int P(p, q) \exp[2\pi i(px+qy)]dpdq; \quad (4)$$

$$p^2+q^2\leqq n^2 \sin^2\vartheta_m\equiv n^2\rho_m{}^2=\rho_m{}^2. \quad (5)$$

All distances in Eqs. (3) and (4) are measured in wave-lengths.

As applied to the present problem of two particles in a bright field, the object function is taken as unity for all points x_0, y_0 of the surround and as f_0 over the area of the particles. It is natural to divide the function F_0 of Eq. (3) into two parts F_u and F_d which correspond, respectively, to the undeviated and deviated waves which arise from diffraction at the object plane. Thus,

$$F_0(x, y, p_0, q_0)=F_u(x, y, p_0, q_0)+F_d(x, y, p_0, q_0); \quad (6)$$

$$F_u(x, y, p_0, q_0)\equiv\int\int \exp[2\pi i(p_0x_0+q_0y_0)] \times U(x-Mx_0, y-My_0)dx_0dy_0, \quad (7)$$

in which the domain of integration extends over the entire field of view;

$$F_d(x, y, p_0, q_0)\equiv f\int\int \exp[2\pi i(p_0x_0+q_0y_0)] \times U(x-Mx_0, y-My_0)dx_0dy_0, \quad (8)$$

in which the domain of integration extends over the area of the two particles and in which

$$f\equiv f_0-1=g\exp(-i\Delta)-1. \quad (9)$$

The expression for F_u simplifies greatly whenever it can be assumed that the object field extends over a large number of wave-lengths. This assumption is usually satisfied by a microscope so that with negligible error,

$$F_u(x, y, p_0, q_0)=\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \exp[2\pi i(p_0x_0+q_0y_0)] \times U(x-Mx_0, y-My_0)dx_0dy_0. \quad (10)$$

When U from Eq. (4) is substituted into Eq. (10) and when the integration with respect to dx_0dy_0 is performed, it is found in a direct manner that

$$F_u(x, y, p_0, q_0)=\underset{\substack{x_0=\infty\\ y_0=\infty}}{\text{Limit}}\int\int P(p, q) \times\exp[2\pi i(px+qy)]\frac{\sin 2\pi x_0(p_0-Mp)}{\pi(p_0-Mp)} \times\frac{\sin 2\pi y_0(q_0-Mq)}{\pi(q_0-Mq)}\frac{d(Mp)d(Mq)}{M^2}. \quad (11)$$

When the integrations with respect to $d(Mp)$ and $d(Mq)$ are examined successively in view of the Dirichlet integral[4]

$$\int_{-a}^{a} f(x)\frac{\sin\alpha(x-t)}{\pi(x-t)}dx\rightarrow f(t) \text{ as } \alpha\rightarrow\infty, \quad (12)$$

it is seen that

$$F_u(x, y, p_0, q_0)=\frac{1}{M^2} \times\exp\left[\frac{2\pi i}{M}(p_0x+q_0y)\right]P\left(\frac{p_0}{M}, \frac{q_0}{M}\right). \quad (13)$$

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[3] H. Osterberg, J. Opt. Soc. Am. 38, 686 (1948), Eq. (1).

[4] Churchill, *Fourier Series and Boundary Value Problems,* (McGraw-Hill Book Company, Inc., New York, 1941), p. 69, Eq. (6).

TABLE I. Separations $2L$ in number of Airy units of the two particles at the physical limit of resolution as a function of fk for Airy type objectives.

$-fk$	$2L$
0	0.7767
0.25	0.7610
0.50	0.7339
0.75	0.6780
0.9	0.5903
1.0	0

It has been assumed that each of the two object particles is unresolvable. The centers of the particles are located at $x_0=\pm l$. More specifically, it is assumed that the areas A of the particles are so small that the integral of Eq. (8) may be replaced by the sum

$$F_d(x, y, p_0, q_0)=fA[\exp(2\pi i p_0 l)U(x-Ml, y)+\exp(-2\pi i p_0 l)U(x+Ml, y)]. \quad (14)$$

This simplification of Eq. (8) can be shown to be valid when the effective radius of the particles does not exceed one-half of the Airy limit of resolution.

The amplitude and phase distribution $F_0(x, y, p_0, q_0)$ produced over the image plane by the incidence upon the object plane of a single wave front whose normal has the optical direction cosines p_0, q_0 is therefore given by

$$M^2F_0(x, y, p_0, q_0)=\exp\left[\frac{2\pi i}{M}(p_0x+q_0y)\right]P\left(\frac{p_0}{M}, \frac{q_0}{M}\right)+fM^2A\left[\exp(2\pi i p_0 l)U(x-Ml, y)+\exp(-2\pi i p_0 l)U(x+Ml, y)\right], \quad (15)$$

when the object plane contains two, uniform, unresolved particles of area A in a uniform, transmitting surround and when the particles are located about the points $x_0=\pm l$.

Considerations of the total energy distribution are limited in the following analysis to the line $y=0$. Accordingly,

$$M^2F_0(x, p_0, q_0)\equiv M^2F_0(x, 0, p_0, q_0)=\exp\left(\frac{2\pi i p_0 x}{M}\right)P\left(\frac{p_0}{M}, \frac{q_0}{M}\right)+\frac{fk}{\pi\rho_m^2}\left[\exp(2\pi i p_0 l)U(x-Ml)+\exp(-2\pi i p_0 l)U(x+Ml)\right], \quad (16)$$

in which

$$k\equiv\pi M^2\rho_m^2A=\pi(\text{N.A.})^2A. \quad (17)$$

The product fk represents the essential physical characteristic of the particles with reference to their effect upon the diffraction image. N.A. denotes the numerical aperture of the objective.

III. THE TOTAL ENERGY DENSITY

The total energy density in the image plane is the sum of the partial energy distributions $|F_0(x, y, p_0, q_0)|^2$ for all effective points in the source of light or, equivalently, for all incident wave fronts. Let $G(x, y)$ denote the total energy density. Then it can be shown[5] that

$$G(x, y)=\int\int\frac{S(p_0, q_0)}{n_0^2-p_0^2-q_0^2}\times|F_0(x, y, p_0, q_0)|^2dp_0dq_0, \quad (18)$$

in which the integration extends over the optical direction cosines p_0, q_0 of the family of rays incident upon the object plane and transmitted by the objective. n_0 is the refractive index in the object space.

It will be supposed that the energy density $S(p_0, q_0)$ of the source is uniform such that

$$S(p_0, q_0)=S=1. \quad (19)$$

If the variables of integration are transformed into their polar form in the image space in accordance with the relations,

$$p_0=n_0M\rho\cos\phi;\quad q_0=n_0M\rho\sin\phi;\quad \rho\equiv\sin\vartheta; \quad (20)$$

and if $F_0(x, 0, p_0, q_0)$ from Eq. (16) is substituted into Eq. (18), it is found that

$$G(x, 0)\equiv G(x)=\frac{1}{M^2}\int_0^{\rho_m}\int_0^{2\pi}\Big|\exp(2\pi i\rho x\cos\phi)P(\rho)+\frac{fk}{\pi\rho_m^2}[\exp(2\pi i\rho Ml\cos\phi)\times U(x-Ml)+\exp(-2\pi i\rho Ml\cos\phi)\times U(x+Ml)]\Big|^2\frac{\rho d\phi d\rho}{1-M^2\rho^2}. \quad (21)$$

The choice of limits of integration in Eq. (21) involves the supposition that the exit pupil of the objective is filled by the image of the source. The substitution $P(p_0/M, q_0/M)=P(\rho)$ involves the requirement that the optical system shall have axial symmetry. $\rho_m=\sin\vartheta_m$ where ϑ_m is the maximum angular aperture of the objective with respect to its image space as in Fig. 1.

Equation (21) shows that the essential physical characteristics of the two particles are contained in the product fk, where

$$fk=(ge^{-i\Delta}-1)\pi(\text{N.A.})^2A. \quad (22)$$

[5] H. Osterberg, J. Opt. Soc. Am. 38, 688 (1948), Eq. (19).

The total energy distribution $G(x)$ depends therefore upon the relative amplitude transmission g and the optical path difference Δ of the particles with respect to their surround and upon the unresolved area A of the particles.

The integral of Eq. (21) will be evaluated for the special cases in which fk is real, that is for optical path differences Δ equal to 0 or π. If $\Delta=0$,

$$fk=-(1-g)\pi(\text{N.A.})^2A. \tag{23}$$

If $\Delta=\pi$,

$$fk=-(1+g)\pi(\text{N.A.})^2A. \tag{24}$$

The integral will furthermore be evaluated under the simplifying approximation that the factor $1-M^2\rho^2$ in the denominator may be replaced by unity. Correspondingly, the energy density will be approximated with negligible error for objectives of numerical aperture of 0.25 or less. It is difficult to estimate the errors incurred by this approximation at higher numerical aperture, but it is expected that the degree of approximation will be satisfactory for many purposes when $M\rho_m=\text{N.A.}$ is as large as 0.7.

In summary, the equations for calculating the total energy density become

$$M^2G(x)=\int_0^{\rho_m}\int_0^{2\pi}|\exp(2\pi i\rho x\cos\phi)P(\rho) + \frac{fk}{\pi\rho_m^2}[\exp(2\pi i\rho Ml\cos\phi) \times U(x-Ml)+\exp(-2\pi i\rho Ml\cos\phi) \times U(x+Ml)]|^2\,\rho d\phi d\rho, \tag{25}$$

with

$$U(r)=2\pi\int_0^{\rho_m}P(\rho)J_0(2\pi r\rho)\rho d\rho. \tag{26}$$

Equation (25) can be solved without approximation for the three pupil functions $P(\rho)$ of the following sections. For definiteness, fk is restricted to the special cases of Eqs. (23) and (24).

IV. THE AIRY TYPE OBJECTIVE

The Airy type objective has the simple pupil function

$$P(\rho)=1,\quad 0\leqq\rho\leqq\rho_m; \tag{27}$$

and produces the amplitude, phase distribution

$$U(r)=2\pi\rho_m^2\frac{J_1(Z)}{Z};\quad Z\equiv2\pi r\rho_m. \tag{28}$$

If r_a denotes the distance from the diffraction head to the first zero of $J_1(Z)$,

$$2\pi\rho_m r_a=3.831706\equiv\beta. \tag{28.1}$$

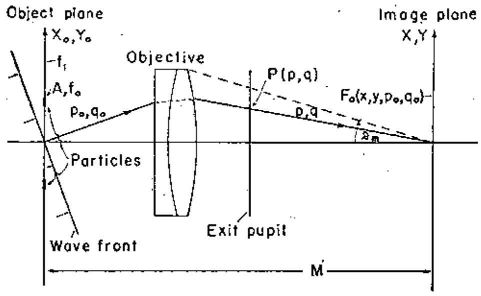

FIG. 1. The optical system and notation.

Let

$$r_a^0\equiv r_a/|M|; \tag{28.2}$$

$$L\equiv l/r_a^0. \tag{28.3}$$

r_a^0 or r_a is the Airy limit of resolution as measured in wave-lengths in the object or image space, respectively. $2L$ is the separation of the two particles as measured in Airy units. Corresponding to $r=x\mp Ml$ in Eqs. (28) it is convenient to set

$$Z_1\equiv2\pi\rho_m(x-Ml)=\beta(x/r_a-L);$$
$$Z_2\equiv2\pi\rho_m(x+Ml)=\beta(x/r_a+L). \tag{29}$$

Both x/r_a and L are therefore measured in Airy units.

When $U(x-Ml)$ and $U(x+Ml)$ are expressed with the aid of Eqs. (28) and (29) and are substituted into Eq. (25) on the supposition that fk is real, it is found that the total energy density $G(x)$ reduces to the

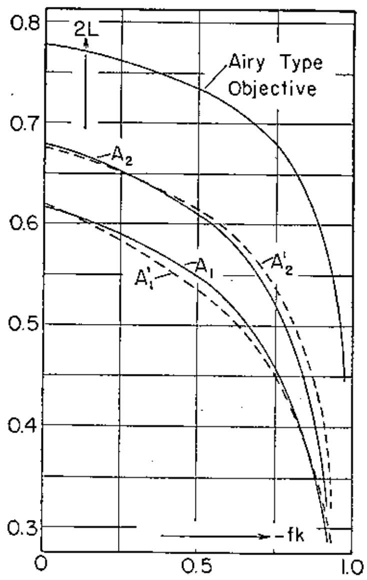

FIG. 2. Separations $2L$ at the physical limit of resolution of two particles. Curves A_1 and A_2 refer to the Sonine type objectives; A_1' and A_2', to the center-blocked Airy type objectives. Subscripts 1 and 2 refer to ratios r_0/r_a of 0.676 and 0.770, respectively.

TABLE II. Separations $2L$ in number of Airy units of the two particles at the physical limit of resolution as a function of fk for two different center-blocked Airy type objectives.

s	r_0/r_a	$-fk$	$2L$
0.6145	0.770	0	0.677
0.6145	0.770	0.25	0.652
0.6145	0.770	0.5	0.615
0.6145	0.770	0.75	0.538
0.6145	0.770	0.9	0.417
0.8551	0.676	0	0.620
0.8551	0.676	0.25	0.580
0.8551	0.676	0.5	0.535
0.8551	0.676	0.75	0.452
0.8551	0.676	0.9	0.355

expression

$$\frac{M^2G(x)}{\pi\rho_m^2}=1+4fk(2+fk)\left[\frac{J_1^2(Z_1)}{Z_1^2}+\frac{J_1^2(Z_2)}{Z_2^2}\right]+16f^2k^2\frac{J_1(2\beta L)}{2\beta L}\frac{J_1(Z_1)}{Z_1}\frac{J_1(Z_2)}{Z_2}. \quad (30)$$

It follows from Eqs. (1) and (30) that the condition for the physical limit of resolution of the two particles by Airy type objectives is equivalent to

$$2(2+fk)J_2^2(\beta L)=\left[2+fk\left(1+\frac{2J_1(2\beta L)}{2\beta L}\right)\right]\times\left[J_1^2(\beta L)+J_2^2(\beta L)-\frac{3J_1(\beta L)}{\beta L}J_2(\beta L)\right]. \quad (31)$$

Separations $2L$ at the physical limit have been calculated from Eq. (31) for several values of fk, and have been listed in Table I and plotted in Fig. 2. Since $2L$ is measured in Airy units, a single calculation includes objectives of any numerical aperture.

V. CENTER-BLOCKED AIRY TYPE OBJECTIVES

Center-blocked Airy type objectives have the pupil function

$$P(\rho)=0,\quad 0\leqq\rho\leqq\rho_1;\qquad P(\rho)=1,\quad \rho_1\leqq\rho\leqq\rho_m. \quad (32)$$

The coating over the central portion of the exit pupil, Fig. 1, is therefore opaque to the axial bundle of rays in the axial cone defined by $p^2+q^2\leqq\sin^2\vartheta_1\equiv\rho_1^2$.

Let

$$s\equiv\rho_1/\rho_m. \quad (33)$$

It follows from Eqs. (26), (29), and (32) that

$$U(x-Ml)/\pi\rho_m^2\equiv u(Z_1)=2[-s^2J_1(sZ_1)/sZ_1+J_1(Z_1)/Z_1];$$
$$U(x+Ml)/\pi\rho_m^2\equiv u(Z_2)=2[-s^2J_1(sZ_2)/sZ_2+J_1(Z_2)/Z_2]. \quad (34)$$

Equation (25) can be integrated after the substitution of $P(\rho)$ and $U(x\mp Ml)$ from Eqs. (32) and (34) to obtain the total energy density $G(x)$ in the form

$$M^2G(x)/\pi\rho_m^2=1-s^2+fk(2+fk)[u^2(Z_1)+u^2(Z_2)]+4f^2k^2\frac{J_1(2\beta L)}{2\beta L}u(Z_1)u(Z_2). \quad (35)$$

The condition for the physical limit of resolution as determined from Eqs. (1) and (35) becomes

$$\left[2+fk+2fk\frac{J_1(2\beta L)}{2\beta L}\right]\left[-s^2\frac{J_1(s\beta L)}{s\beta L}+\frac{J_1(\beta L)}{\beta L}\right]\times\left[-3s^4\frac{J_2(s\beta L)}{(s\beta L)^2}+s^4\frac{J_1(s\beta L)}{s\beta L}+3\frac{J_2(\beta L)}{(\beta L)^2}-\frac{J_1(\beta L)}{\beta L}\right]$$
$$=\left[2fk\frac{J_1(2\beta L)}{2\beta L}-(2+fk)\right]\times\left[-s^3\frac{J_2(s\beta L)}{s\beta L}+\frac{J_2(\beta L)}{\beta L}\right]^2. \quad (36)$$

If s is assigned a value in the range $0<s<1$, $U(r)=0$ at a corresponding distance $r=r_0$ from the diffraction head. This value of r_0 falls in the interval $0.6276<r_0/r_a<1$. As s approaches unity, r_0/r_a approaches 0.6276. This means physically that the radius of the Airy disk cannot be reduced below the fraction 0.6276 of its classical value by means of center-blocked objectives. Separations $2L$ at the physical limit of resolution have been computed from Eq. (36) for several values of fk at two fixed values of s and are listed in Table II.

VI. SONINE TYPE OBJECTIVES

The analysis of the diffraction images and physical limit of resolution for two particles viewed against a bright field will be made for the simple Sonine type of objective whose pupil function $P(\rho)$ obeys the law[2]

$$P(\rho)=1+a_1-a_1\rho^2/\rho_m^2; \quad (37)$$

$$-a_1=\frac{\beta}{2}\frac{r_0}{r_a}J_1(\beta r_0/r_a)/J_2(\beta r_0/r_a). \quad (38)$$

With this choice of pupil function, the radius of the Airy disk is reduced to the fraction r_0/r_a of its value for the Airy type objective.

From Eqs. (26), (29), and (37),

$$U(x-Ml)/\pi\rho_m{}^2 \equiv u(Z_1) = 2J_1(Z_1)/Z_1 + 4a_1J_2(Z_1)/Z_1{}^2;$$
$$U(x+Ml)/\pi\rho_m{}^2 \equiv u(Z_2) = 2J_1(Z_2)/Z_2 + 4a_1J_2(Z_2)/Z_2{}^2. \quad (39)$$

Let $P(\rho)$ and $U(x\pm Ml)$ from Eqs. (37) and (39) be introduced into Eq. (25). The result is

$$M^2G(x)/\pi\rho_m{}^2 = 1 + a_1 + \frac{a_1{}^2}{3} + fk(2+fk)[u^2(Z_1)+u^2(Z_2)] + 4f^2k^2\frac{J_1(2\beta L)}{2\beta L}u(Z_1)u(Z_2). \quad (40)$$

The physical limit of resolution has been calculated from Eqs. (1) and (40) for two values of a_1 from the formulation

$$\psi^2(r)\left[1-\frac{2fk}{2+fk}\frac{J_1(2r)}{2r}\right] = -\phi(r)\alpha(r)\left[1+\frac{2fk}{2+fk}\frac{J_1(2r)}{2r}\right]; \quad (41)$$

$$\phi(r)\equiv rJ_1(r)+2a_1J_2(r);\quad \psi(r)\equiv rJ_2(r)+2a_1J_3(r);$$
$$\alpha(r)\equiv (r+10a_1/r)J_3(r)-(1+2a_1)J_2(r);\quad r\equiv\beta L.$$

The constants a_1 have been chosen so that the corresponding values of r_0/r_a are the same as in Table II. The resulting values of $2L$ as a function of fk are listed in Table III.

VII. COMPARISON OF THE PHYSICAL LIMITS OF RESOLUTION; INTERPRETATION

Separations $2L$ of the two particles at the physical limit of resolution are plotted in Fig. 2 for comparison of the three types of objectives. The two special cases of the Sonine type objective are represented by the curves A_1 and A_2 for which r_0/r_a are, respectively, 0.676 and 0.770. The two special cases of the center-blocked Airy type objective are represented by the curves A_1' and A_2' for which r_0/r_a are also 0.676 and 0.770, respectively. The ratio r_0/r_a may be considered as the actual radius of the Airy disk produced by a "coated" objective measured with the radius of the classical Airy disk at the given numerical aperture chosen as the unit of length. This length is called the Airy unit. The separations $2L$ are measured in Airy units. fk is given either by Eq. (23) or by Eq. (24). The curves of Fig. 2 apply therefore to two, identical, unresolved particles having optical paths which differ from that of their surround by zero or one-half wave-lengths. The area A and the amplitude transmission g of the particles may vary from one set of particles to another.

In the case of the Airy type objective the separation $2L$ is slightly less than 78 percent of the Airy limit when $fk=0$. This is a previously known property[6] which may be used in explaining why microscope objectives can resolve better than is indicated by the Airy limit. It will be noted from Fig. 2 that in the case of the center-blocked and Sonine type objectives the physical limits at $fk=0$ decrease approximately as the ratio r_0/r_a, that is approximately as the ratio of the radius of the contracted Airy disk to the radius of the classical Airy disk. This observation is interpreted to mean that the relative increase in the energy content of the rings in the diffraction image produced by objectives which have been coated so as to reduce the diameter of the Airy disk has but slight effect upon the physical limit of resolution of two infinitesimal particles. Since the curves of Fig. 2 drop at about the same rate with increasing $|fk|$ and hence with increasing area of the particles, this interpretation applies also to finite but unresolved particles.

The drop in $2L$ with increasing numerical value of fk is large. Consequently, microscope objectives may be expected to show markedly higher resolution for particles of increasing $|fk|$. This resolution is improved in so far as may be judged from Fig. 2 by coating the objective so as to reduce the diameter of the Airy disk. Reference to Eqs. (23) and (24) shows that the two particles should be more readily resolved as their area increases. The dependence of the physical limit of resolution upon the transmission of the particles is more complicated but can be ascertained from Eqs. (23) and (24) and the curves of Fig. 2.

The drop in the physical limits of Fig. 2 depends upon the maximum allowable values $|fk|_m$ of $|fk|$. It is important to distinguish three categories for $|fk|_m$. The first category is that which is consistent with the validity of replacing dx_0dy_0 in Eq. (8) by A as in Eq. (14). Let R denote the effective radius of the particles and let R_m be the maximum value of R for which Eq. (14) is a good approximation to Eq. (8). Since R_m may be taken as

TABLE III. Separations $2L$ in number of Airy units of the two particles at the physical limit of resolution as a function of fk for two different Sonine type objectives.

$-a_1$	r_0/r_a	$-fk$	$2L$
1.0872	0.770	0	0.6785
1.0872	0.770	0.25	0.6509
1.0872	0.770	0.5	0.6081
1.0872	0.770	0.75	0.5230
1.0872	0.770	0.9	0.3758
1.3397	0.676	0	0.6175
1.3397	0.676	0.25	0.5867
1.3397	0.676	0.5	0.5470
1.3397	0.676	0.75	0.4583
1.3397	0.676	0.9	0.3320

[6] R. K. Luneberg, *Mathematical Theory of Optics* (Brown University Press, Providence, 1944), pp. 389–90.

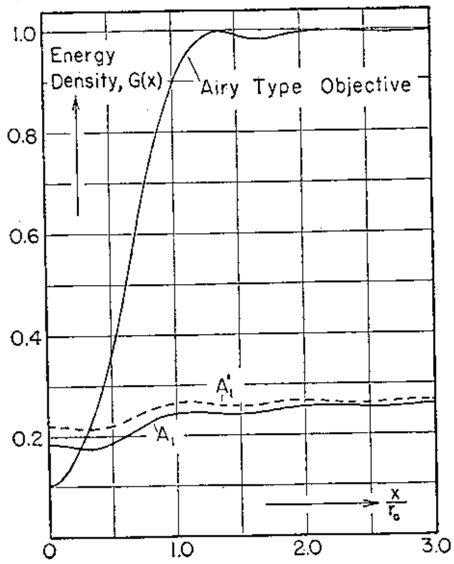

FIG. 3. Diffraction curves for the separation $2L=0.608$ Airy units and for $fk=-0.5$. Curves A_1 and A_1' belong to Sonine type and center-blocked objectives, respectively, whose ratio $r_0/r_a=0.676$.

large as one-half of the Airy limit,

$$R_m=0.61/2\text{N.A.} \tag{42}$$

The maximum allowable area A_m of the particles is correspondingly

$$A_m=\pi R_m^2=0.093\pi/(\text{N.A.})^2. \tag{43}$$

In Eqs. (23) and (24) let g' denote $-(1-g)$ or $-(1+g)$ according as $\Delta=0$ or π. Then, from Eqs. (23), (24), and (43),

$$|fk|_m=0.093\pi^2|g'|$$

or, approximately,

$$|fk|_m=|g'|. \tag{44}$$

Since $|g'|>1$ for particles whose optical path exceeds that of the surround by one-half wave-lengths, values $|fk|_m$ of the first category can readily exceed unity. Equations (14) and (25) contain the restriction that the particles shall not overlap. This restriction reduces $|fk|_m$. Let R_c denote the radii of two like particles which are in contact. Then,

$$R_c=Lr_a/|M|,$$

and

$$A_c=\pi L^2r_a^2/M^2=\pi L^2\beta^2/4\pi^2(\text{N.A.})^2. \tag{45}$$

From Eqs. (23), (24), and (45)

$$|fk|_{nom}=L^2\beta^2|g'|/4,$$

or, approximately,

$$|fk|_{nom}=|g'|(2L)^2, \tag{46}$$

in which the subscripts m, n, and o refer to the maximum allowable value of $|fk|$ in the second category for non-overlapping particles. Since $2L<1$ in Fig. 2, $|fk|_{nom}<|fk|_m$. As an example, the point $2L=0.5$, $-fk=0.9$ on the curve for the Airy type objective can be reached only with particles for which $|g'|=3.6$. Whereas such particles are possible, they are not common in microscopy. It can be expected for reasons beyond the scope of the present paper that the results predicted by Eq. (25) apply with at least fair approximation to the case of overlapping particles until the particles overlap to such an extent that the circumference of one particle passes through the center of the second particle. The extension of the equations to such particles constitutes an extrapolation which will be denoted by the subscript e. It follows that since $R_e=2R_c$,

$$|fk|_{em}=4|fk|_{nom}=4|g'|(2L)^2, \tag{47}$$

where $|fk|_{em}$ denotes the maximum value of $|fk|$ in the third category. The point $2L=0.5$, $-fk=0.9$ on the curve for the Airy type objective can be reached with particles for which $|g'|=0.9$. Particles for which $|g'|=1$ are not uncommon. For example, $|g'|=1$ with opaque particles. The range of fk in Fig. 2 can therefore be realized with observable particles.

It will be noted from Table I that with Airy type objectives the physical limit $2L$ approaches zero as fk approaches -1. Particles for which $fk\rightarrow -1$ as $2L\rightarrow 0$ will have the optical path difference $\Delta=\pi$ and an increasingly great g value. This means that the surround becomes practically opaque. One can expect, accordingly, that transparent particles having an optical path difference of one-half wave-length with respect to an almost opaque surround will belong to the class of most

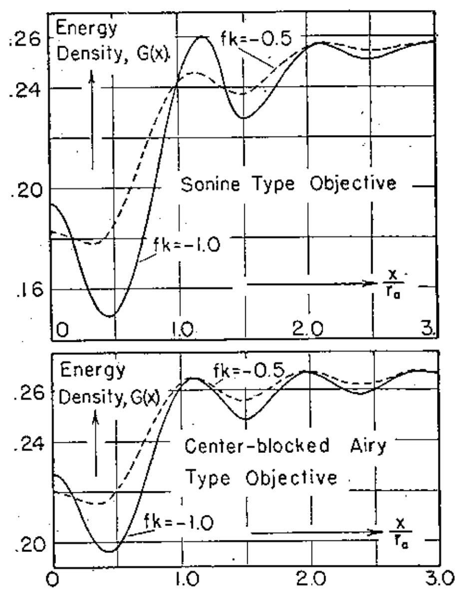

FIG. 4. Comparison of diffraction curves drawn for the two fk values of -0.5 and -1.0. The separation $2L$ is 0.608 and the ratio $r_0/r_a=0.676$ as with the Sonine type and center-blocked objectives of Fig. 3.

readily resolved particles. Small particles of this character are uncommon.

With particles whose optical path difference $\Delta=0$, $fk>0$ when $g>1$. The physical limits for such particles are not included in Fig. 2. The scope of this paper is limited to particles for which $fk<0$. An investigation by Dr. J. Ernest Wilkins, Jr., has shown that the physical limits become higher than 78 percent of the Airy limit when $fk>0$. This phenomenon which occurs with particles whose optical path difference $\Delta=0$ and whose amplitude transmission exceeds that of the surround will be described elsewhere.

At the selected ratios r_0/r_a of Fig. 2, the curves of the physical limits for center-blocked and Sonine type objectives do not differ significantly.

The possible theoretical variation of the physical limit of resolution in the direction of better resolution is impressive. It is emphasized, however, that the physical limit of resolution can only be approached whereas the Airy limit of resolution can be realized.

VIII. COMPARISON OF THE DIFFRACTION IMAGES

The diffraction curves of Figs. 3, 4, 5, and 6 represent the total energy densities computed from Eqs. (30), (35), or (40) with $M^2/\pi\rho_m^2$ set equal to unity. Since the curves are symmetrical about the origin, the abscissas are restricted to the positive values of x/r_a.

The diffraction curves of Fig. 3 are drawn for the case in which $fk=-0.5$ and in which the separation of the two particles is 0.6080 Airy units as measured in the object plane, that is $2l/r_a^0=0.6080$. Reference to Tables I, II, and III will show that at $fk=-0.5$ the separation 0.6080 exceeds the physical limit only for the center-blocked and Sonine type objectives for which $r_0/r_a=0.676$. The curves A_1' and A_1 for these objectives show a noticeable minimum between $x/r_a=0.3$ and $x/r_a=0.4$ Airy units, whereas the curve for the Airy type objective gives no direct indication for the presence of two particles. The curve A_1 for the Sonine type objective is considered to show slightly better contrast than the curve A_1' for the center-blocked objective. The gain in resolution of curves A_1 and A_1' is accompanied by great loss of contrast in the diffraction image with respect to the contrast displayed by the Airy type objective. When fk is decreased from -0.5 to -1.0, the diffraction curves for the separation $2L=0.6080$ show greater contrast and better evidence for the presence of two particles in the manner which is illustrated by Fig. 4 and which is consistent with the trends of Fig. 2.

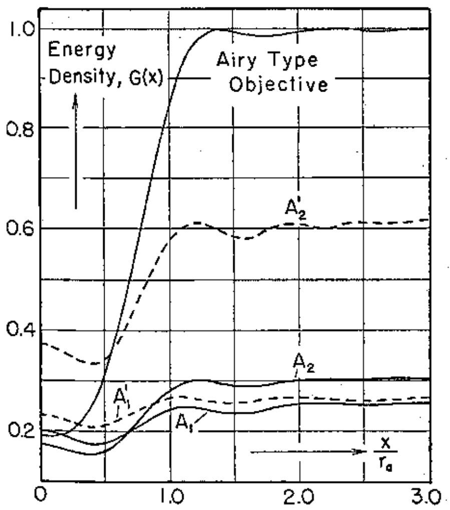

FIG. 5. Diffraction curves for the separation $2L=0.7338$ and $fk=-0.5$. Curves A_1 and A_2 refer to the Sonine type objectives; A_1' and A_2', to the center-blocked Airy type objectives. Subscripts 1 and 2 refer to the ratios r_0/r_a of 0.676 and 0.770, respectively.

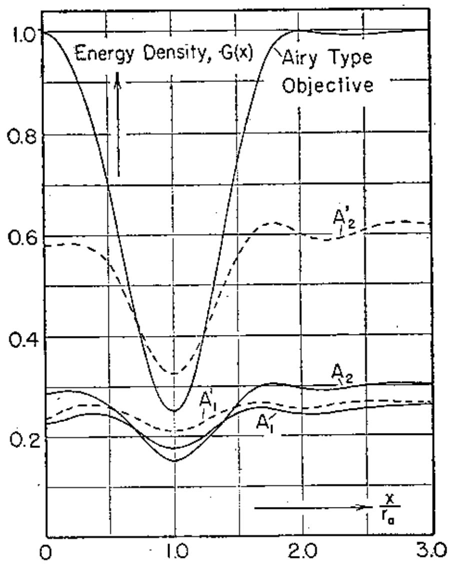

FIG. 6. Diffraction curves for the separation $2L=2$ and $fk=-0.5$. A_1, A_2, A_1', and A_2' have the same meanings as in Fig. 5.

Figure 5 has been drawn for the case in which $fk=-0.5$ and in which the separation $2l/r_a^0$ of the particles is equal to 0.7338, a distance large enough to enable resolution by both sets of Sonine and center-blocked objectives but too small to enable resolution by the Airy type objective. The Sonine and center-blocked objectives show pronounced minima near $x/r_a=0.4$ Airy units, whereas the Airy type objective gives no direct indication for the presence of a second particle. The set of curves A_2 and A_2' for which $r_0/r_a=0.770$ show more contrast than the set of curves A_1 and A_1' for which $r_0/r_a=0.676$. The curve A_2' for the center-blocked objective shows better contrast than the curve A_2 for the Sonine type objective. The best contrast in the diffraction image is shown again by the Airy type objective but this objective fails to resolve the two particles.

The curves of Fig. 6 have been drawn for the case in which $fk=-0.5$ and in which the separation $2l/r_a^0$ of the two particles is equal to 2 Airy units, a distance more than sufficient to enable resolution by all of the objectives. Pronounced minima occur at $x/r_a=1$ in all of the curves. Contrast in the diffraction image is best with the Airy type objective. It is concluded that objectives which have been coated so as to reduce the diameter of the Airy disk can be used with advantage over the Airy type objective only in those cases in which the Airy type objective fails to resolve. A comparison of the curves of Figs. 3–6 indicates also the more general conclusion that in order to resolve two particles with maximum contrast whenever the separations of the particles are too small to enable resolution by the Airy type objective, it is preferable to choose a coated objective whose Airy disk has been reduced no more than is necessary for resolving the two particles.

IX. CONCLUSIONS

(1) The relative increase in the energy content of the rings in the diffraction patterns whose Airy disks have been reduced in radius below the Airy limit has but slight effect upon the physical limit of resolution of a microscope objective for two uniform, identical particles in a transmitting surround.

(2) Objectives which have been coated for reducing the radius of the Airy disk can be expected to show better resolution for two particles than the classical Airy type objective.

(3) With the center-blocked and the particular Sonine types of objectives which have been studied in this report, contrast in the diffraction image decreases steadily as the objective is coated to meet the demand of greater resolution.

(4) Contrast in the diffraction image deteriorates so rapidly with decreasing radii of the Airy disk at fixed numerical aperture that scattering by the optical system is expected to become a major difficulty in realizing the possible gains in resolution.

(5) The separation of the two particles at the physical limit of resolution decreases with unexpected rapidity as the unresolvable area of the particles is increased. This separation has a marked dependence also upon the relative amplitude and phase transmission of the particles with respect to the surround. With properly chosen particles the separation at the physical limit of resolution can drop to 50 percent of the separation at the Airy limit even with the classical Airy type objective.

(6) It is unfortunate that the usual particles of biological research have the least favorable amplitude and phase transmissions for taking advantage of the variation of the physical limit of resolution with the optical properties of the particles. For these particles, whose amplitude and phase transmission is not far different from that of the surround, the physical limit increases to 78 percent of the Airy limit. If the transmission of such particles is made different from that of the surround by staining either the particles or the surround, not only will the particles appear in better contrast but also their separation at the physical limit becomes smaller, that is the particles will be resolved more readily. It is emphasized, however, that the conclusions of this paragraph are not expected to apply without modification to the phase microscope.

(7) If the predicted variation of the physical limit of resolution with the properties of the particles proves to be in substantial agreement with carefully controlled experiments, there will exist a physical basis for explaining why a microscope can resolve considerably better than is indicated by the criterion of the Airy limit.

The authors are indebted to Miss Barbara R. Kloepfer for calculating the physical limits and diffraction patterns of the center-blocked objectives.

Installation of the New England Section of the Optical Society of America

THE New England Section of the Optical Society of America was officially installed at a meeting held in Boston on May 5, 1949. The brief ceremony was conducted by the Secretary for Local Sections, Dr. Stanley S. Ballard, with the assistance of two former presidents of the society, Professor Arthur C. Hardy and Dean George R. Harrison. The speech of the evening was given by Dr. Edwin H. Land of the Polaroid Corporation, who spoke on "Color Translation and Ultraviolet Microscopy." Dr. Land, a Director-at-Large of the Optical Society, is a member of the new section, as are the other three national officers just named.

This new section is the first one formed from a local group organized for the express purpose of petitioning for local section status. This group, the "New England Optical Society," had held three previous meetings, each of which was attended by well over 100 persons. While the charter membership of the new section is drawn largely from the Boston-Cambridge area, it includes representation from western Massachusetts, Connecticut, Rhode Island, New Hampshire, and Vermont.

Officers of the new section are: *President*, DR. DUNCAN E. MACDONALD, Boston University Optical Research Laboratory; *Vice President*, DAVID S. GREY, Polaroid Corporation; *Secretary*, JOHN T. WATSON, Boston University Optical Research Laboratory; *Treasurer*, RUSSELL P. MAHAN, Baird Associates, Inc.; *Councillors*, DR. WALTER S. BAIRD, Baird Associates, Inc., PROFESSOR STANLEY S. BALLARD, Tufts College, DR. ELKAN R. BLOUT, Polaroid Corporation, and PROFESSOR RICHARD C. LORD, Massachusetts Institute of Technology.