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Anomalous diffraction by arbitrarily oriented ellipsoids: applications in ektacytometry

Geert. J. Streekstra, Alfons G. Hoekstra, and Robert M. Heethaar

Anomalous diffraction by an arbitrarily oriented ellipsoid with three different axes is derived. From the resulting expression the relationship between the shape of the ellipsoid and the intensity pattern is immediately evident: The axial ratio of the elliptical iso-intensity curve equals the axial ratio of the elliptical projected area of the ellipsoid. A comparison of anomalous diffraction with calculations performed with the T -matrix method reveals that the anomalous diffraction approximation is highly accurate for single ellipsoidal red blood cells. Application of the expression for anomalous diffraction by ellipsoids to a population of red blood cells shows that, even in a red-cell suspension as examined in an ektacytometer, the axial ratio of the iso-intensity curves is equal to the mean axial ratio of the red blood cells in the population. In ektacytometry this relationship between cell shape and intensity pattern is commonly assumed to hold true without reference to the light-scattering properties of the cells. The results presented here show that this assumption is valid, and we offer a profound theoretical basis for it by considering in detail the light scattering by the red blood cells.

Key words: Anomalous diffraction, T -matrix method, red blood cell, oriented ellipsoid, ektacytometer, iso-intensity curve, deformation, red-cell population.

1. Introduction

A technique for quantifying the deformability of red blood cells is ektacytometry.¹ In an ektacytometer a diluted suspension of red blood cells is sheared in a Couette flow between two coaxial transparent cylinders (Fig. 1). As a result of the forces exerted by the flow, the red cells take on ellipsoidal shapes with the longest axis oriented at an angle ψ relative to the streamlines of the flow.² He-Ne laser light is sent through the sheared suspension and scattered by the ellipsoidal red blood cells. The resulting intensity pattern is projected on a screen, scanned by a video camera, and analyzed by an image-analyzing system.^{3,4}

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In the intensity pattern, points of equal intensity build up elliptical curves, the iso-intensity curves. The axial ratio of the iso-intensity curves reflect the mean deformation of the red-cell population.

Although in ektacytometry the measuring system is well described and tested,^{1,3-5} little attention is paid to the light scattering by the ellipsoidal red blood cells. Since the aim of the technique is to obtain the mean deformation of a cell population, insight into the relationship between the cell deformation and intensity pattern on the screen is of major importance.

For the calculation of the intensity pattern caused by a cell population, a correct description of the light scattering by a single ellipsoidal cell is required. In a previously published paper on this subject⁶ the validity of the anomalous diffraction theory of van de Hulst⁷ was shown for spheres with the size and refractive index of a red blood cell. This theory is accurate for spheres with size parameter $\alpha \gg 1$ and with relative refractive index m in the range in which $|m - 1| \ll 1$. Both conditions apply to the ellipsoidal red blood cells as well, and therefore, as is shown in this paper, anomalous diffraction correctly describes the light scattering by ellipsoidal cells that are oriented with an arbitrary angle ψ relative to the flow.

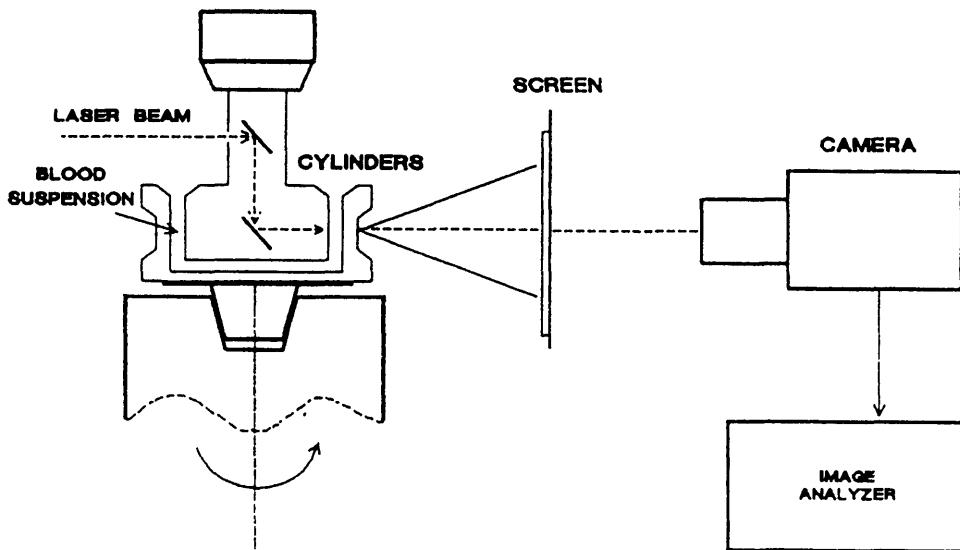


Fig. 1. Measuring configuration of the ektacytometer.

The validity of anomalous diffraction for the oriented ellipsoids is demonstrated by comparison with the *T*-matrix method.^{8,9}

Finally, to gain insight into the relationship between the deformation of the cells in a sheared suspension and the measured intensity pattern, the angular scattering of a realistic red-cell population as present in an ektacytometer is calculated by use of the anomalous diffraction theory.

2. Angular Scattering by an Arbitrarily Oriented Ellipsoid

The anomalous diffraction of an ellipsoid with its semiaxis oriented along the coordinate axes of a coordinate system $[x, y, z]$ is treated in an earlier paper on this subject.⁶ The resulting equations that describe the anomalous diffraction for these ellipsoids are repeated here, and the theory is extended for the case of an arbitrarily oriented ellipsoid. Subsequently the anomalous diffraction by an ellipsoidal red blood cell is discussed.

Consider a plane wave with intensity I_0 traveling in the positive z direction of a Cartesian coordinate system $[x, y, z]$. Let the projected area of an ellipsoidal particle with the $a > b > c$ axes be perpendicular to the z axis and situated at the origin of the system. The two longest axes a and b of the ellipsoid coincide with the axes of the elliptical projected area. The shortest axis of the ellipsoid c is oriented along the z axis parallel to the direction of the incident light. In the anomalous diffraction approximation the intensity I_A of the pattern in a point $\mathbf{P}(x, y, z)$ was found to be⁶

$$I_A = I_0(1/k^2r^2)|S(\nu)|^2, \quad (1)$$

with

$$S(\nu) = \alpha^2 \int_0^{\pi/2} [1 - \exp(-i\phi_{\max} \sin \tau)] J_0(\alpha\nu \cos \tau) \times \sin \tau \cos \tau d\tau,$$

$$r = (x^2 + y^2 + z^2)^{1/2},$$

$$\nu = \frac{1}{r} [(x^2/q) + qy^2]^{1/2},$$

$$\alpha = k(ab)^{1/2} = (2\pi n_{\text{med}}/\lambda_0)(ab)^{1/2},$$

$$q = a/b,$$

$$\phi_{\max} = 2kc|m - 1|.$$

In Eq. (1) $J_0(u)$ is the zeroth-order Bessel function of u , λ_0 is the wavelength of the light in vacuum, and n_{med} is the refractive index of the medium surrounding the ellipsoidal particle.

An important implication of Eq. (1) is that a constant value of ν represents a collection of points in space with equal intensity. From the definition of the parameter ν we can conclude that these points build up elliptical iso-intensity curves with axial ratio q of the elliptical projected area. The elliptical iso-intensity curves are rotated 90 deg compared with the orientation of the ellipsoid: With the ellipsoid directed along the x axis the elliptical iso-intensity curve is oriented along the y axis.

In Appendix A the extension to the case of an arbitrarily oriented ellipsoid is derived. The resulting equations show that the projected area remains elliptical. The difference from the case in which the semiaxes are oriented along the coordinate axes is that the elliptical projected area is oriented with an

angle $\delta + \Phi$ relative to the x axis (for the definitions of δ and Φ , see Appendix A and Fig. 7). In the anomalous diffraction approximation the angular scattering by an arbitrarily oriented ellipsoid with semiaxes a, b , and c turns out to be equal to the scattering by an equivalent ellipsoid with semiaxes a', b' , and c' oriented along the $x'', y'',$ and z axes, respectively. Consequently, we can calculate the scattered intensity using Eq. (1) with $x, y, z, a, b,$ and c replaced by $x'', y'', z, a', b',$ and c' . An important implication of the latter conclusion is that, even in the case in which the ellipsoid is arbitrarily oriented, the iso-intensity curves remain elliptical. Furthermore the axial ratio of these iso-intensity curves are equal to the axial ratio of the projected area, and the iso-intensity curves are rotated by an angle $\delta + \Phi$ in the x - y plane.

A. Anomalous Diffraction of an Oriented Ellipsoidal Red Blood Cell

In an ektacytometer the red blood cells are oriented at angles of 0–20 deg relative to the applied Couette flow.^{2,10,11} With the flow parallel to the x axis the semiaxes a and c of the ellipsoid are in the x - z plane and oriented with an angle ψ relative to the x and z axes, respectively (Fig. 2). Because of the stable orientation of the ellipsoidal red blood cells in the flow, the direction of the b axis is along the y axis.^{2,10}

One consequence of the orientation of the ellipsoid is that the projected area deviates from the elliptical area when $\psi = 0$. As shown in Appendix A the shape of the projected area is still elliptical. The only difference with the orientation angle $\psi = 0$ is that the longest axis of the elliptical projected area a is re-

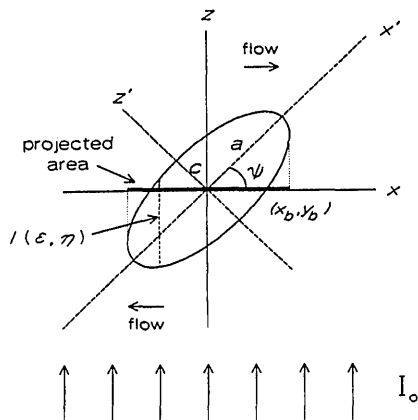


Fig. 2. Ellipsoidal particle with semiaxes $a, b,$ and c illuminated by a plane wave of intensity I_0 . The longest axis a is oriented with an angle ψ relative to the flow. The b axis is oriented along the y axis of the coordinate system, perpendicular to the x and z axes. Traversing the ellipsoid, the light is phase shifted with magnitude $k|m - 1|l(\epsilon, \eta)$ compared with the light traveling along the particle. The border of the projected area is defined by the coordinates (x_b, y_b) .

duced to a' by a factor of f where

$$a' = fa, \\ f = \left[\cos^2 \psi + \left(\frac{c}{a} \right)^2 \sin^2 \psi \right]^{1/2}. \quad (2)$$

The semiaxes of the elliptical projected area are along the x and y axes in these circumstances.

A second difference with the previous situation [Eq. (1)] is the magnitude of the phase shift of the light traversing the particle. Not only the maximal phase shift ϕ_{\max} is changed but also the local phase shift $\phi(\epsilon, \eta)$ at any point $(\epsilon, \eta, 0)$ on the projected area. In the anomalous diffraction theory, $\phi(\epsilon, \eta)$ is given by^{7,12,13}

$$\phi = k|m - 1|l(\epsilon, \eta), \quad (3)$$

where $l(\epsilon, \eta)$ is the distance a ray of light travels when traversing the particle at position (ϵ, η) on the projected area. Within the projected area of an oriented ellipsoidal red blood cell, $l(\epsilon, \eta)$ can be shown to be (see Appendix A)

$$l(\epsilon, \eta) = 2c' \left[1 - \left(\frac{\epsilon}{a'} \right)^2 - \left(\frac{\eta}{b} \right)^2 \right]^{1/2}, \quad (4)$$

where $c' = c/f$.

Summarizing the expressions in this section and using the results in a former paper,⁶ we can state that in the anomalous diffraction approximation the angular scattering by an oriented ellipsoidal red blood cell with semiaxes $a, b,$ and c is equal to the scattering by an ellipsoid with a zero orientation angle with semiaxes $a', b,$ and c' . Consequently the only modification in the calculation of the scattered intensity I_A [Eq. (1)] is that c is replaced by c' and a by a' . With the latter conclusion in mind the relationship between the shape of the iso-intensity curves in the intensity pattern and the shape and orientation of the ellipsoid is immediately apparent. The axial ratio of the projected area $q' = a'/b$ is equal to the axial ratio of the elliptical iso-intensity curves in the intensity pattern.

B. T-Matrix Method

The validity of the anomalous diffraction by oriented ellipsoids was checked with the T -matrix method. The T -matrix calculations were performed with the computer programs supplied by Barber and Hill.⁹ Since the desired size parameters and volumes of the ellipsoids are relatively large, double precision was required and the arrays used in the programs were extended in the way recommended by the authors. Additionally the convergence tolerance in the computer program, T1.for, was set to 5% for angles as large as 80 deg in the calculation of the largest particle (size parameter, $\alpha = 19$; axial ratio, $c/a = 0.6$).

3. Angular Scattering by a Population of Ellipsoidal Red Blood Cells

In a blood sample a distribution of geometrical and mechanical properties of the cells exists, which determines their shapes in flow. In general at a certain shear rate the size of the semiaxes of each ellipsoidal red blood cell is a function of its volume V , surface area A , membrane mechanical properties, viscosity of the cell interior, and the viscosity of the medium surrounding the cells.^{10,14}

To be able to calculate the light scattering of a realistic cell population as present in an ektacytometer, one considers the cells to be maximally deformed and oriented along the streamlines of the flow ($\psi = 0$). When maximally deformed the cells are prolate ellipsoids and the sizes of the semiaxes are independent of the mechanical properties of the cells.² For this particular situation the calculation of the distribution of the semiaxes is straightforward. Since in physiological circumstances A is proportional to V ,¹⁵ the size of the semiaxes can be obtained from V only. With q being the axial ratio a/b of the spheroid, the set of equations necessary for the calculation of the semiaxes at a certain volume V is given by

$$A = 2\pi \left(\frac{3V}{4\pi q} \right)^{2/3} \left[1 + \frac{q^2}{(q^2 - 1)^{1/2}} \arctan(q^2 - 1)^{1/2} \right],$$

$$V = \frac{4}{3} \pi \frac{a^3}{q^2},$$

$$A = \kappa V, \quad (5)$$

where κ is the empirically derived proportional constant relating A and V .

Besides the influence of V on the shape of the cells, the relative refractive index m is also a function of V . The value of m is a second-order polynomial function of the hemoglobin concentration Hb in the cells.⁶ The hemoglobin content of a red blood cell is essentially constant, independent of the volume of the cell.¹⁶ As a result the Hb concentration of a cell with a certain volume V can be calculated from

$$Hb(V) = Hb_{\text{mean}} \left(\frac{V}{V_{\text{mean}}} \right), \quad (6)$$

where V_{mean} is the mean volume of the cell population and Hb_{mean} is the corresponding hemoglobin concentration. When Eq. (6) is applied, m turns out to be a function of V :

$$m(V) = g_p \left[Hb_{\text{mean}} \left(\frac{V}{V_{\text{mean}}} \right) \right], \quad (7)$$

where g_p is the second-order polynomial equation relating Hb and m .⁶

Application of Eqs. (5)–(7) means that the properties that determine the scattering by a spheroidal red blood cell (i.e., m and the semiaxes of the spheroid) are a function of V only. Assuming that the volume

of the red blood cells is distributed normally,¹⁵ we can obtain the mean of the scattered intensities of the cells within the population $I_{\text{mean}}(\theta)$ from

$$I_{\text{mean}}(\theta) = \int_{V_{\text{cells}}} wt(V) I(V, \theta) dV, \quad (8)$$

where

$$wt(V) = \frac{1}{\sigma_d(2\pi)^{1/2}} \exp \left[-\frac{1}{2} \left(\frac{V - V_{\text{mean}}}{\sigma_d} \right)^2 \right],$$

$I(V, \theta)$ is the scattered intensity of a single spheroid with volume V , and σ_d is the standard deviation of the volume distribution.

4. Results

Before application of anomalous diffraction to a red-cell population it is necessary to investigate the accuracy of the anomalous diffraction for ellipsoids. For this purpose anomalous diffraction was compared with T -matrix calculations. In the calculations no normalizing factors were used. Figure 3 shows the results of anomalous diffraction and T -matrix calculations for oblate and prolate spheroids with the relative refractive index of a red blood cell [$m = 1.05$ (Ref. 6) and $\psi = 0$]. The results show good agreement between anomalous diffraction and T -matrix calculations and a negligible effect on the polarization of the incident light on the intensity pattern to a scattering angle as large as ~ 15 deg.

In the case of an oblate ellipsoid oriented at an angle $\psi = 45^\circ$ (Fig. 4) the agreement is still good but somewhat less pronounced. Especially for the smallest ellipsoid [Fig. 4(a)] a significant difference between anomalous diffraction and T -matrix results is observed. Although in both cases the anomalous diffraction curve that is related to the b axis (θ in the y - z plane) agrees well with T -matrix calculations, the curves related to the a axis (θ in the x - z plane) have a first minimum that is shifted compared with the T -matrix calculations. This shift is less pronounced for the largest particle [Fig. 4(b)], confirming the expectation that anomalous diffraction becomes more valid for larger particles.

A phenomenon that is not incorporated in the anomalous diffraction approximation is a possible asymmetry in the intensity pattern because of the orientation of the particle. Figure 4(a) clearly demonstrates that for both positive and negative θ , compared with the symmetrical anomalous diffraction curve, the first minimum is shifted toward the left in the T -matrix calculations.

Note that even for the largest ellipsoid the volume of the particle is only approximately one third of the volume of the red blood cell. Consequently the anomalous diffraction describes even better the light scattering by single elliptical red blood cells.

With the results of the scattering by a single ellipsoid in mind, it is legitimate to use anomalous diffraction for the calculation of the light scattering of

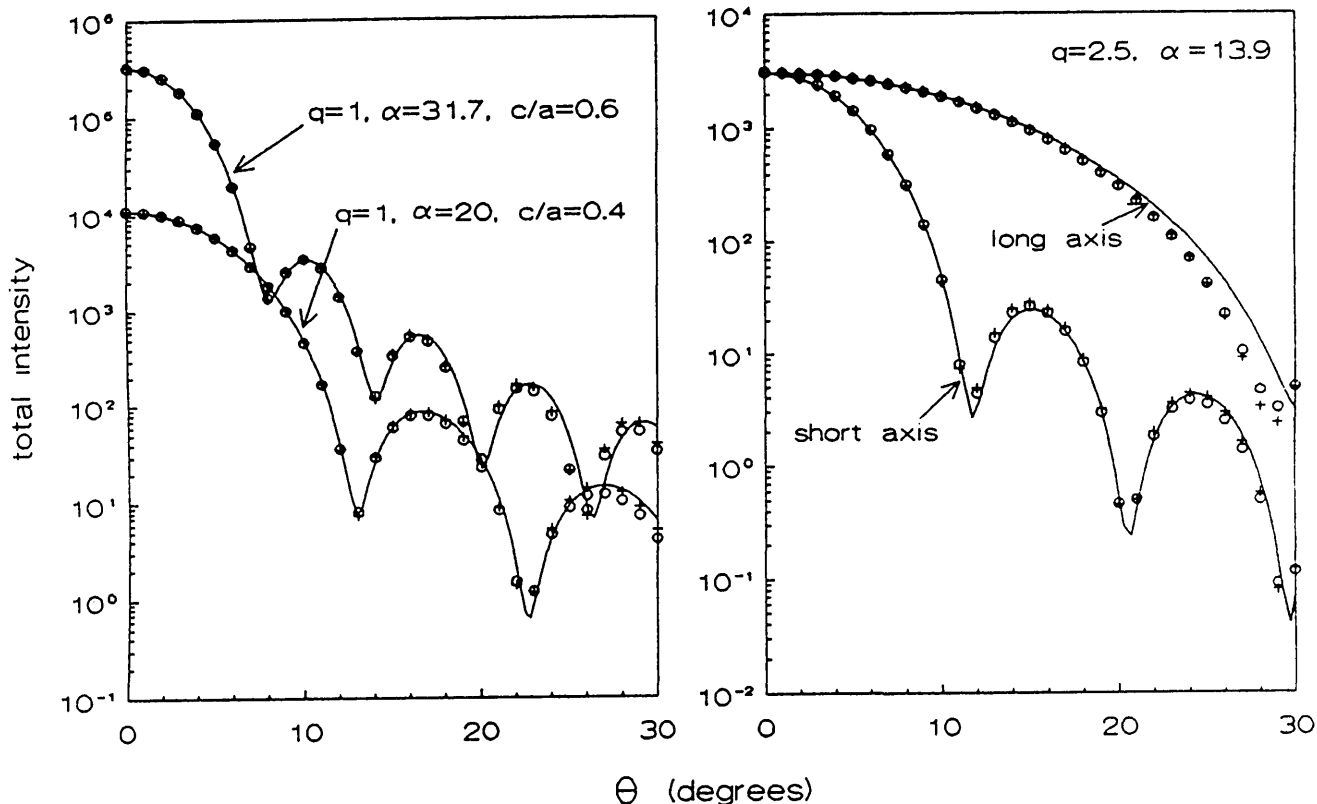


Fig. 3. Anomalous diffraction (curves) and T -matrix calculations (markers) of oblate spheroids oriented with the symmetry axis parallel to the incident light (left) and a prolate spheroid (right) oriented along the x axis of the Cartesian coordinate system (\circ , parallel polarized incident light; $+$, perpendicular polarized incident light). Right, the long axis and the short axis denote the angular scattering with θ in the y - z and x - z planes, respectively.

a red-cell population. In Fig. 5 the angular scattering of both a single cell with volume $V_{\text{mean}}(I_s)$ and the mean intensity of a cell population as described in Section 3 (I_{mean}) is plotted in one graph. The mean volume and the standard deviation of the population have the physiological values of 95 and 15 μm^3 , respectively.^{2,15} The constant κ relating V and A is 1.42 μm^{-1} . In the graph the scattering angle θ is in the x - z and y - z plane for the short and long axes of the pattern, respectively.

It is striking in Fig. 5 that, along the short axis of the pattern, the difference between I_s and I_{mean} is much larger than the corresponding difference along the long axis. Whereas along the short axis the deviation of I_{mean} from I_s is highly significant even within the first minimum in I_s , along the long axis I_s and I_{mean} are nearly indistinguishable. The latter observation can be understood by looking in somewhat more detail at the influence of the volume distribution on the semiaxes a and $b = c$. When we apply Eqs. (5) to the cell population, the longest semiaxis a varies between 3.69 and 11.94 μm within $V_{\text{mean}} - 3\sigma_d < V < V_{\text{mean}} + 3\sigma_d$. For the short axis b the corresponding variation is relatively much smaller (i.e., between 1.67 and 1.80 μm). Since in the anomalous diffraction approximation the short axis of the intensity pattern is related mainly to the long axis of the ellipsoidal cells, it is understandable that along

this shortest axis the differences between I_s and I_{mean} are the greatest.

5. Discussion

In the literature results obtained by ektacytometry are commonly interpreted without reference to the light-scattering properties of the red blood cells.^{1,3-5,17-19} The axial ratio of the iso-intensity curves in the intensity pattern is considered to reflect the deformation of the red-cell population.²⁰ Although it is generally acknowledged that scattered light can act as a source of information about the shape of asymmetrical particles, the exact relationship between the observed intensity pattern and particle morphology is far from trivial. In a single particle system, we can illustrate this relationship by considering the scattering of a spheroid with a certain axial ratio q oriented perpendicular to the incident light. In the limit where the volume of the spheroid is small compared with the wavelength, the resulting iso-intensity curves are circular and no shape information is obtained from the intensity pattern. For a spheroid with a large-size parameter and a relative refractive index near 1; however, the shape information is obtained directly from the iso-intensity curves in the intensity pattern. In intermediate cases some of the shape information is lost. This phenomenon of lost shape information is reflected in Fig. 3 (right),

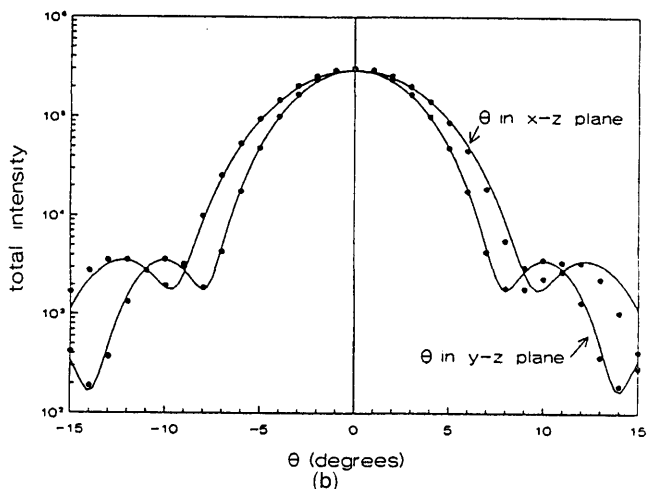
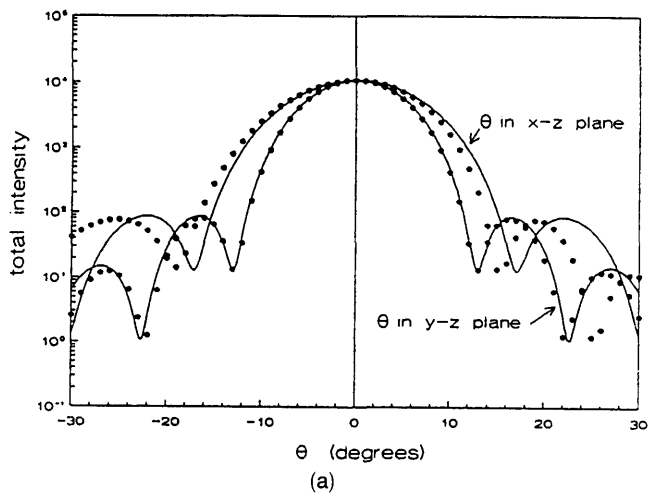


Fig. 4. Anomalous diffraction (curves) and T -matrix calculations (dots) for oblate spheroids ($q = 1$) with orientation angle $\psi = 45^\circ$ and parallel polarized incident light (top, $\alpha = 20$, $c/a = 0.4$; bottom, $\alpha = 31.7$, $c/a = 0.6$).

where in the T -matrix calculations the first minimum of the longest axis of the intensity pattern is shifted toward a smaller angle compared with anomalous diffraction.

The results in Section 4 show that within a scattering angle of ~ 15 deg the anomalous diffraction approximation for oriented ellipsoids is highly accurate in the calculation of the light scattering by ellipsoidal particles with the size and relative refractive index of a red blood cell. Although the T -matrix method would at first sight be the best theory for the calculation of the light scattering of ellipsoidal particles, limitations in this method make it less useful for red blood cells. First, the T -matrix method is in the implementation used here⁹ limited in aspect ratio and size of the ellipsoidal particle. Only particles with volumes less than approximately one third of the volume of a red blood cell can be handled with this method. A second objective against the present implementation of the T -matrix method is that it is not possible to calculate the light scattering by an ellipsoid with three independent axes as present in

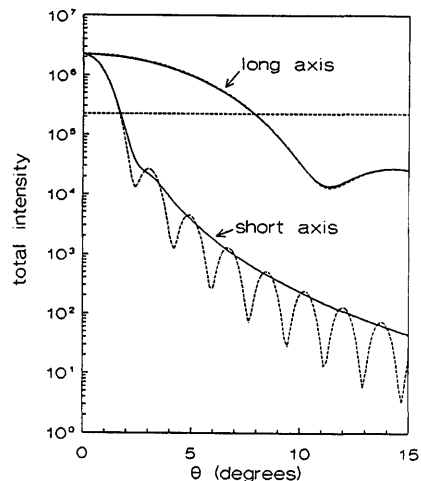


Fig. 5. Angular scattering of a cell population (solid curve, $V_{\text{mean}} = 95 \mu\text{m}^3$; standard deviation, $15 \mu\text{m}^3$) and a single red blood cell (dashed curve, $V = 95 \mu\text{m}^3$) calculated by use of anomalous diffraction. The long axis and the short axis denote the angular scattering with θ in the y - z and x - z plane, respectively. The horizontal dashed line is drawn at intensity $I(\theta) = I(0)/10$.

ektacytometry. These limitations on the size and shape of the ellipsoid are not present in the anomalous diffraction approximation.

Another important advantage of anomalous diffraction compared with the T -matrix method is the small computer time necessary to calculate an intensity pattern. The intensity at any point can be calculated by the one-dimensional integral of Eq. (1). However, most important is that, using the anomalous diffraction approximation, we gained direct physical insight into the relationship between the intensity pattern and the shape of the cells. Even for a population with a considerable spread in the volume of the ellipsoidal cells it is possible to relate the shape of the mean cell of the population to the intensity pattern. Figure 5 shows that when the iso-intensity curves are scanned at a level above 10% of the intensity at a zero scattering angle (dashed horizontal line), the aspect ratio of the iso-intensity curves belonging to the mean cell in the population equals the corresponding aspect ratio in the intensity pattern produced by the whole cell population.

At moderate and low shear rates the orientation angle ψ deviates from zero.¹⁰ For an ellipsoidal red blood cell, $\psi \leq 20$ deg. With $(a/c) \leq 0.29$,² the axial ratio q' that is measured by the ektacytometer is maximally 5% underestimated compared with the axial ratio q of the cell [Eq. (2)]. Only at the lowest shear rates will this underestimation be large compared with the experimental error of $\sim 2\%$ in q' .

With the latter conclusion in mind, even for cases of moderate shear rate (where $\psi \neq 0$), the anomalous diffraction can be used to obtain the sizes of the axes of the mean cell in the population. The axes of the ellipsoids are quantities that are necessary in obtaining the mechanical parameters of the cell membrane and therefore of importance in the rheology of red blood cells.^{10,14}

6. Conclusions

In the anomalous diffraction approximation the angular scattering of an arbitrarily oriented ellipsoid is developed. A comparison with the T -matrix method reveals that the approximation describes the light scattering of ellipsoidal particles with size and the relative refractive index of a red blood cell correctly within scattering angles of ~ 15 deg.

The axial ratio of the elliptical projected area of the ellipsoidal red blood cell is equal to the axial ratio of the isointensity curves in the observed pattern. This conclusion holds true for both a single cell and an *in vitro* cell population.

In its range of validity the anomalous diffraction theory as presented here is a powerful tool in obtaining the shape and size information of ellipsoidal particles from the observed intensity pattern.

Appendix A

Consider a particle situated at the origin of a Cartesian coordinate system $[x, y, z]$. The particle is illuminated by a plane wave traveling in the z direction with intensity I_0 and wave vector \mathbf{k} .

In the anomalous diffraction approximation the scattered intensity I_A at any point (x, y, z) in space is given by^{6,7}

$$I_A = I_0 \frac{S(\beta, \gamma)}{k^2 r^2}, \quad (\text{A1})$$

where

$$S(\beta, \gamma) = \frac{k^2}{2\pi} \iint_{A_{sc}} \{1 - \exp[-i\phi(\epsilon, \eta)]\} \\ \times \exp[ik(\epsilon\beta + \eta\gamma)] d\epsilon d\eta, \\ r = (x^2 + y^2 + z^2)^{1/2}, \\ \beta = x/r, \quad \gamma = y/r.$$

A_{sc} is the area obtained by the projection of the particle onto the x - y plane, and $\phi(\epsilon, \eta)$ is the phase shift of the light traversing the particle at position $(\epsilon, \eta, 0)$ on this projected area. The phase shift $\phi(\epsilon, \eta)$ is a function of both the relative refractive index of the particle and the distance $l(\epsilon, \eta)$ a ray travels when traversing the particle at $(\epsilon, \eta, 0)$:

$$\phi(\epsilon, \eta) = k|m - 1|l(\epsilon, \eta). \quad (\text{A2})$$

For a particle of any configuration the problem that remains is finding the shape of the projected area A_{sc} and an expression for $l(\epsilon, \eta)$.

Let the particle be an ellipsoid with semiaxes a , b , and c oriented along the coordinate axes of a particle frame $[x', y', z']$. The orientation of this particle frame relative to the laboratory frame is defined by two rotation angles ψ and ξ (Fig. 6). Starting with the laboratory frame, we obtain the particle frame by a rotation ψ of the x axis and the z axis in the x - z plane

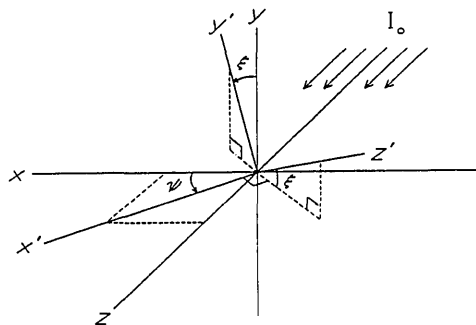


Fig. 6. Orientation of the particle frame $[x', y', z']$ relative to the laboratory frame $[x, y, z]$. The orientation of the particle is defined by a rotation ψ in the x - z plane followed by a rotation ξ around the x' axis.

followed by a rotation ξ of the y and the z axes around the x' axis. The coordinates in the particle frame $[x', y', z']$ are related to the laboratory frame $[x, y, z]$ by

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \begin{pmatrix} \cos \psi & 0 & \sin \psi \\ \sin \psi \sin \xi & \cos \xi & -\cos \psi \sin \xi \\ -\sin \psi \cos \xi & \sin \xi & \cos \psi \cos \xi \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix}. \quad (\text{A3})$$

Since in the particle frame the semiaxes a , b , and c are oriented along the coordinate axes x' , y' , and z' , respectively, the ellipsoid that borders the particle satisfies the equation

$$(x'/a)^2 + (y'/b)^2 + (z'/c)^2 = 1. \quad (\text{A4})$$

Substitution of Eq. (A3) into Eq. (A4) gives the second-order polynomial equation

$$Az^2 + Bz + C = 0, \quad (\text{A5})$$

where

$$A = \left[\frac{\sin^2 \psi}{a^2} + \cos^2 \psi \left(\frac{\sin^2 \xi}{b^2} + \frac{\cos^2 \xi}{c^2} \right) \right],$$

$$B = b_1 x + b_2 y,$$

$$b_1 = 2 \cos \psi \sin \psi \left[\frac{1}{a^2} - \left(\frac{\sin^2 \xi}{b^2} + \frac{\cos^2 \xi}{c^2} \right) \right],$$

$$b_2 = 2 \cos \psi \cos \xi \sin \xi \left(\frac{1}{c^2} - \frac{1}{b^2} \right),$$

$$C = c_1 x^2 + c_2 y^2 + c_3 xy - 1,$$

$$c_1 = \left[\frac{\cos^2 \psi}{a^2} + \sin^2 \psi \left(\frac{\sin^2 \xi}{b^2} + \frac{\cos^2 \xi}{c^2} \right) \right],$$

$$c_2 = \left(\frac{\cos^2 \xi}{b^2} + \frac{\sin^2 \xi}{c^2} \right),$$

$$c_3 = -2 \sin \psi \cos \xi \sin \xi \left(\frac{1}{c^2} - \frac{1}{b^2} \right).$$

The border of the projected area of the ellipsoid is built up by the coordinates (x_b, y_b) for which Eq. (A5) yields only one root; hence

$$B^2 - 4AC = 0. \quad (\text{A6})$$

By substitution of x_b and y_b in the expression for A , B , and C the equation that defines the border of the projected area is found to be

$$Dx_b^2 + Ey_b^2 + Fx_b y_b = 1, \quad (\text{A7})$$

where

$$D = \left(c_1 - \frac{b_1^2}{4A} \right), \quad E = \left(c_2 - \frac{b_2^2}{4A} \right), \quad F = \left(c_3 - \frac{b_1 b_2}{2A} \right).$$

The projected area that is defined by Eq. (A7) is elliptical. The semiaxes a' and b' of this ellipse are oriented with an angle δ relative to the x and y axes, respectively. For a' , b' , and δ it is found that

$$\begin{aligned} \delta &= \frac{1}{2} \arctan \left(\frac{F}{D - E} \right), \\ a' &= \left(\frac{1 - \tan^2 \delta}{D - E \tan^2 \delta} \right)^{1/2}, \\ b' &= \left(\frac{1 - \tan^2 \delta}{E - D \tan^2 \delta} \right)^{1/2}. \end{aligned} \quad (\text{A8})$$

Inside the borders of the projected area there are always two roots of Eq. (A5). The expression for $l(\epsilon, \eta)$ is found by subtraction of the two roots:

$$l = \frac{(B^2 - 4AC)^{1/2}}{A} = \frac{2}{\sqrt{A}} (1 - Dx^2 - Ey^2 - Fxy)^{1/2}. \quad (\text{A9})$$

With the coordinates ϵ and η in the directions of the a' and b' axes for $l(\epsilon, \eta)$ we can finally write

$$l(\epsilon, \eta) = 2c' \left[1 - \left(\frac{\epsilon}{a'} \right)^2 - \left(\frac{\eta}{b'} \right)^2 \right]^{1/2}, \quad (\text{A10})$$

where $c' = 1/\sqrt{A}$.

A complete arbitrary orientation of the ellipsoid in the laboratory frame can be achieved by an additional rotation Φ of the projected area in the x - y plane (Fig. 7). This rotation angle Φ should be added to the angle δ that is introduced by the rotation ξ of the b axis [Eqs. (A7) and (A8)].

The interpretation of the expressions for the borders of the projected area [Eqs. (A7) and (A8)] and for the phase shift [Eqs. (A2) and (A10)] is straightforward: In the anomalous diffraction approximation the light scattering of an ellipsoid with three independent axes a , b , and c and orientation angles ψ , ξ , and Φ is equal to the light scattering by an ellipsoid with semiaxes a' , b' , and c' . The semiaxes a' , b' , and c' are oriented along the x'' , y'' , and z axes, respectively

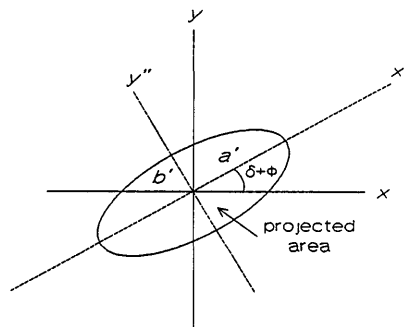


Fig. 7. Projected area of an ellipsoidal particle with semiaxes a , b , and c and orientation angles ψ , ξ , and Φ . The projected area is an ellipse with semiaxes a' and b' along the x'' and y'' axes. The x'' and y'' axes are rotated by an angle $\delta + \Phi$ relative to the laboratory frame.

(Fig. 7). An important implication of the latter conclusion is that the scattered intensity can be calculated with the original formula [Eq. (1)] for an ellipsoid with its semiaxes along the coordinate axes. Furthermore it can be shown that the volume of the original ellipsoid is equal to the volume of the ellipsoid with semiaxes a' , b' , and c' .

From the theory that is developed in this Appendix it is apparent that we can obtain the scattered intensity resulting from the presence of an arbitrarily oriented ellipsoid by carrying out the following steps:

(1) Calculation of the corresponding point (x'', y'', z) in the $[x'', y'', z]$ frame, taking into account the rotation of the projected area:

$$\begin{pmatrix} x'' \\ y'' \end{pmatrix} = \begin{bmatrix} \cos(\delta + \Phi) & \sin(\delta + \Phi) \\ -\sin(\delta + \Phi) & \cos(\delta + \Phi) \end{bmatrix} \begin{pmatrix} x \\ y \end{pmatrix}. \quad (\text{A11})$$

(2) Calculation of the scattered intensity with Eq. (1), in which x , y , a , b , and c are replaced by x'' , y'' , a' , b' , and c' , respectively.

If the b axis of the ellipsoid remains oriented along the y axis ($\Phi = 0$ and $\delta = 0$), the semiaxes of the elliptical projected area a' and b' are oriented along the x and y axes. In this case the resulting expressions for a' and c' as calculated from Eqs. (A8) and (A10) are

$$\begin{aligned} a' &= fa, \\ c' &= c/f, \\ f &= \left[\cos^2 \psi + \left(\frac{c}{a} \right)^2 \sin^2 \psi \right]^{1/2}. \end{aligned} \quad (\text{A12})$$

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