The Third International Congress on
Optical Particle Sizing
August 23-26, 1993 Yokohama, Japan

(18)

Anomalous diffraction as a tool for the characterization of red blood cells in Ektacytometry

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

*Dept. of Medical and Physiological Physics, University of Utrecht,
University Hospital/H02.101,
P.O. Box 85500, 3508 GA Utrecht, The Netherlands.

**Dept. of Computer Systems, University of Amsterdam,
Faculty of Mathematics and Computer Science,
Kruislaan 403, 1098 SJ Amsterdam, The Netherlands.

Abstract

Ektacytometry is a technique to measure the deformation of red blood cells in flow. In an ektacytometer, the red cell deformation is obtained from the light scattered by ellipsoidally deformed red blood cells. Although the aim of the technique is to obtain the cell deformation from the isointensity curves in the observed intensity pattern, a correct theory that offers insight in the relation between cell shape and intensity pattern is absent. By comparison with T-matrix calculations, the Anomalous diffraction approximation (AD) turns out to be a correct theory and a useful tool for the description of the light scattering by ellipsoidal red blood cells. Using the AD approximation, it appears that the effect of the imaginary part of the relative refractive index of a red blood cell is negligible. In a red cell population with equal amounts of deformable and undeformable cells, the application of AD reveals that the axial ratio of the isointensity curves in the pattern is dependent on the intensity level of the curves.

1 Introduction

Ektacytometry is an optical technique that is applied to quantify the deformability of human red blood cells[1-3]. In an ektacytometer, the deformation of the cells is obtained from the scattered laser light that is sent through a sheared suspension of red blood cells. Figure 1. shows the setup of the system. A diluted suspension of red blood cells is sheared in a Couette flow between two coaxial transparent cylinders. The hydrodynamic forces cause the red blood cells to deform into ellipsoids that are aligned with a small angle relative to the flow direction [4].

The He-Ne laser beam, that is sent through the suspension by means of two mirrors, is scattered by the red cells and the resulting intensity pattern is projected on a screen. In the intensity pattern, points of equal intensity build up elliptical curves: the isointensity curves. The axial ratio of the isointensity curves is considered to reflect the mean deformation of the red cell population.

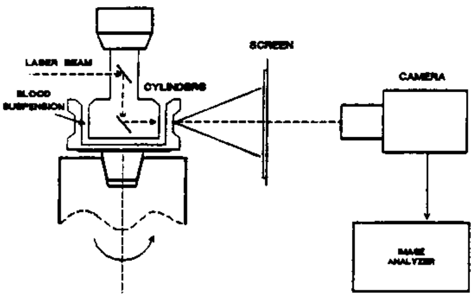

Fig. 1. Setup of the ektacytometer

In literature concerning ektacytometry, the technique is commonly used without reference to the relation between the shape of the cells and the intensity pattern on the screen. In a paper of Zahalak and Sutera [5], Fraunhofer diffraction by a prolate ellipsoid with zero orientation angle is derived and is claimed to be the most appropriate theory to the intensity patterns produced in an Ektacytometer.
However, comparison with Mie theory revealed that Fraunhofer diffraction is not the correct theory for spheres with size and refractive index of a red blood

cell [6]. The anomalous diffraction introduced by van de Hulst [7] turned out to be a more appropriate theory.
For the interpretation of the intensity pattern caused by a cell population as present in an ektacytometer, a correct theory that offers insight in the relation between cell shape and the light scattered by these ellipsoidal cells is required. In this paper the applicability of the anomalous diffraction (AD) approximation as a tool for the characterization of the ellipsoidal red blood cells is investigated. The validity of the AD approximation for ellipsoids is tested by comparison with calculations performed by the T-matrix method [8,9].
A second objective is to investigate the influence of the imaginary part of the relative refractive index on the intensity pattern. Finally, the influence of a fraction of less deformable cells within the cell population, as present in some hemolytic anemias [11,12], is studied.

2 Anomalous diffraction by a suspension of ellipsoidal particles.

In the anomalous diffraction theory both the light that is traveling along and traversing the particle is taken into account in the calculation of the diffraction integral [6,7]. Since the refractive index inside the particle differs from the refractive index outside the particle, the light traversing the particle is phase shifted compared to the light traveling along the particle. An important assumption in the AD theory is that there is negligible deflection and reflection of the light at the particle-medium interface. This assumption and the fact that the diffraction integral is used for the calculation of the intensity pattern implicates that the theory is valid for particles with large size parameter α ($\alpha >> 1$) and relative refractive index m near 1. Consider a single ellipsoidal particle with semi axes $a > b > c$, situated in the origin of a Cartesian coordinate system [x,y,z] and oriented with the longest axis in the x-direction (Fig. 2.).

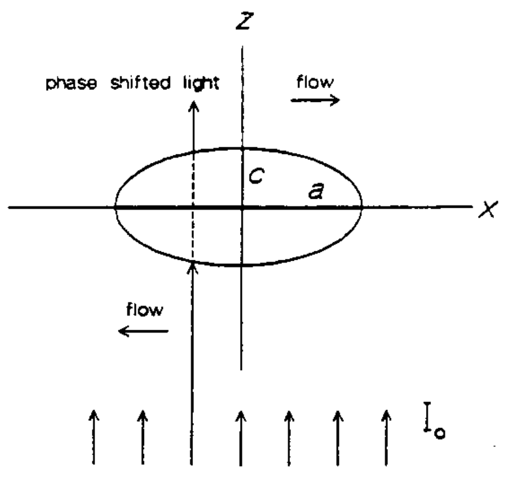

Fig. 2. Ellipsoidal particle illuminated by a laser beam.

The particle is illuminated by a plane wave traveling in the z-direction.
In the anomalous diffraction approximation the intensity I at a point (x,y,z) far from the particle is given by [6]:

$$I = I_o (1/k^2 r^2) \, |S(v)|^2 \, , \qquad (1)$$

with

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

$$\alpha = k\sqrt{ab},$$

$$\phi_{max} = 2kc(m-1) ,$$

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

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

In Eq. (1) I_o denotes the intensity of the incident wave, $J(u)$ is the first-order Bessel function of u, k is the magnitude of the wave vector of the light in the medium surrounding the particle and q is the axial ratio a/b of the ellipsoidal particle.
From the equation it is clear that all the points in space with a fixed value of v build up curves of equal intensity. On a screen perpendicular to the direction of the incident light these isointensity curves are ellipses with an axial ratio q equal to the axial ratio of the ellipsoidal particle.

3 Results

The accuracy of the anomalous diffraction approximation for ellipsoids was investigated by comparision with the T-matrix method. The T-matrix calculations were performed using the computer programs supplied by Barber and Hill [9]. For our relatively large size parameter and axial ratio, the computer programs were modified by using double precision variables and by extension of the arrays in the way recommended by the authors.
Figure 3. shows the comparison of the T-matrix method and anomalous diffraction for a spheroidal particle with relative refractive index of a red blood cell ($m = 1.05$). The results of the anomalous diffraction compare well with the T-matrix calculations up to a scattering angle of 15 degrees. A comparable agreement is observed if an imaginary component up to a value of 0.1 is added to m (data not shown).

cell [6]. The anomalous diffraction introduced by van de Hulst [7] turned out to be a more appropriate theory.
For the interpretation of the intensity pattern caused by a cell population as present in an ektacytometer, a correct theory that offers insight in the relation between cell shape and the light scattered by these ellipsoidal cells is required. In this paper the applicability of the anomalous diffraction (AD) approximation as a tool for the characterization of the ellipsoidal red blood cells is investigated. The validity of the AD approximation for ellipsoids is tested by comparison with calculations performed by the T-matrix method [8,9].
A second objective is to investigate the influence of the imaginary part of the relative refractive index on the intensity pattern. Finally, the influence of a fraction of less deformable cells within the cell population, as present in some hemolytic anemias [11,12], is studied.

2 Anomalous diffraction by a suspension of ellipsoidal particles.

In the anomalous diffraction theory both the light that is traveling along and traversing the particle is taken into account in the calculation of the diffraction integral [6,7]. Since the refractive index inside the particle differs from the refractive index outside the particle, the light traversing the particle is phase shifted compared to the light traveling along the particle. An important assumption in the AD theory is that there is negligible deflection and reflection of the light at the particle-medium interface. This assumption and the fact that the diffraction integral is used for the calculation of the intensity pattern implicates that the theory is valid for particles with large size parameter α ($\alpha >> 1$) and relative refractive index m near 1. Consider a single ellipsoidal particle with semi axes $a > b > c$, situated in the origin of a Cartesian coordinate system $[x,y,z]$ and oriented with the longest axis in the x-direction (Fig. 2.).

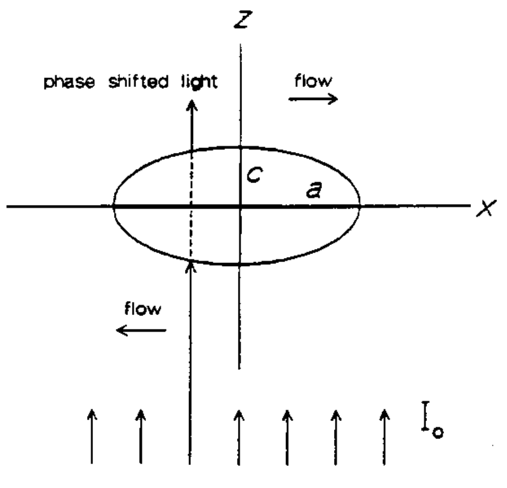

Fig. 2. Ellipsoidal particle illuminated by a laser beam.

The particle is illuminated by a plane wave traveling in the z-direction.
In the anomalous diffraction approximation the intensity I at a point (x,y,z) far from the particle is given by [6]:

$$I = I_o(1/k^2r^2)\,|S(v)|^2 \,, \qquad (1)$$

with

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

$$\alpha = k\sqrt{ab},$$

$$\phi_{max} = 2kc(m-1),$$

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

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

In Eq. (1) I_o denotes the intensity of the incident wave, $J(u)$ is the first-order Bessel function of u, k is the magnitude of the wave vector of the light in the medium surrounding the particle and q is the axial ratio a/b of the ellipsoidal particle.
From the equation it is clear that all the points in space with a fixed value of v build up curves of equal intensity. On a screen perpendicular to the direction of the incident light these isointensity curves are ellipses with an axial ratio q equal to the axial ratio of the ellipsoidal particle.

3 Results

The accuracy of the anomalous diffraction approximation for ellipsoids was investigated by comparision with the T-matrix method. The T-matrix calculations were performed using the computer programs supplied by Barber and Hill [9]. For our relatively large size parameter and axial ratio, the computer programs were modified by using double precision variables and by extension of the arrays in the way recommended by the authors.
Figure 3. shows the comparison of the T-matrix method and anomalous diffraction for a spheroidal particle with relative refractive index of a red blood cell ($m = 1.05$). The results of the anomalous diffraction compare well with the T-matrix calculations up to a scattering angle of 15 degrees. A comparable agreement is observed if an imaginary component up to a value of 0.1 is added to m (data not shown).

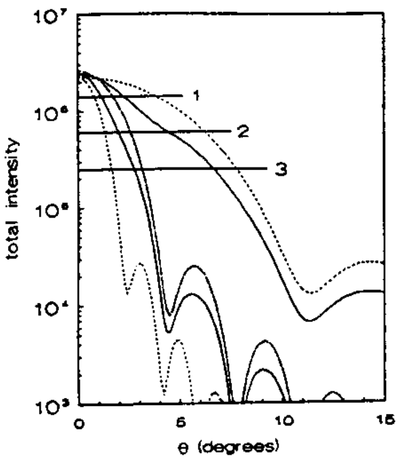

Fig. 5. Intensity pattern of a cell population with equal amounts of deformable cells (q=4.7, α=48.5, V=95 fl) and undeformable cells (q=1, α=56.9, V=95 fl). The dashed curves represent the intensity pattern of the single deformable (- - -) and the undeformable (— —) cells. The solid curve (——) is the intensity pattern of the cell population. The horizontal lines denoted by 1, 2 and 3 indicate the intensity levels I(0)/2, I(0)/4 and I(0)/10 respectively.

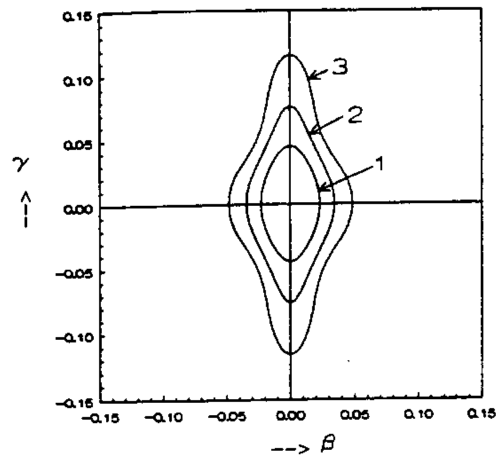

Fig. 6. Isointensity curves 1, 2 and 3 at intensity levels I(0)/2, I(0)/4 and I(0)/10. The axial ratio q_p are 1.9, 2.2 and 2.4 respectively. The coordinates β and γ are normalized distances on the screen i.e. $\beta = x/r$, $\gamma = y/r$.

In this approximation, the intensity pattern is build up by interference of the Fraunhofer light and the phase shifted light that just traversed the particle. From the results presented in Fig. 3. can be concluded that within the scattering angles where the intensity is scanned in an ektacytometer, anomalous diffraction is a valid theory for ellipsoidal red blood cells.

In the anomalous diffraction approximation, the relation between cell shape and intensity pattern is still straightforward. Like in Fraunhofer diffraction the axial ratio of the isointensity curves in the pattern is equal to the axial ratio of the cell. Even in a population of uniformly deformed red blood cells with a distribution in the size parameter α, the latter conclusion holds true.

The situation is different if the cell population consists of a mixture of deformable and undeformable cells. In hemolytic anemias, like sickle cell disease, a fraction of the cells is undeformable due to the stiffening of the red cell membrane. In measurements with blood cells of patients with sickle cell disease, the intensity patterns that are qualitatively the same as shown in Fig. 6. are observed [11,12]. In contrast with a population of uniformly deformed red blood cells, in this case the relation between cell shape and intensity pattern is not straightforward. The isointensity curves are not elliptical any more (Fig. 6.) and the axial ratio of the curves is depends on the intensity level of the curves. Decreasing the intensity level corresponds to an increase of the axial ratio. The observed axial ratio q_p is not equal to the mean axial ratio q_{pop} of the population (in Fig. 6., q_{pop} = 2.85).

These results point out that it is not possible to define one unique axial ratio q_p from the pattern in case the cell population is a mixture of deformable and undeformable cells. Only if the levels of the isointensity curves are equal, results of different blood samples can be compared. On the other hand, since the shape of the isointensity curves is dependent on the level, it should be possible to obtain the amounts of deformable and undeformable cells and the axial ratio of the deformable cells from one single intensity pattern by detection of the isointensity curves at different intensity levels.

5 Conclusions

By comparison with T-matrix calculation the anomalous diffraction approximation appears to be a valid theory for the description of the light scattering by ellipsoidal red blood cells in ektacytometry. The imaginary part of the refractive index of the cell interior has negligible effect on the intensity pattern.

In a red cell population of uniformly deformed red blood cells, the anomalous diffraction approximation reveals that the axial ratio of the cells is equal to axial ratio of the isointensity curves in the pattern. In case the cell population consists of both deformable and undeformable cells the axial ratio is dependent on the levels of the isointensity curves. The measurement of these cell populations with an ektacytometer cannot be interpreted correctly without reference to this intensity level.

From the calculations shown in this paper can be concluded that the anomalous diffraction approximation is a useful tool in the quantification of the deformation of red blood cells in an ektacytometer offering insight in the mechanisms by which the cells scatter the light.

This work was supported by the Dutch Ministry of Economic Affairs, Sondag Optische Instrumenten B.V., and The Netherlands Organization for Scientific Research, grant NWO 810-410-041.

6 References

[1] *M. Bessis and N. Mohandas:* A diffractometric method for the measurement of cellular deformability. Blood Cells *1* (1975) 307-313.

[2] *N. Mohandas, M. R. Clark, M. S. Jacobs, and S. B. Shohet:* Analysis of factors regulating erythrocyte deformability. J. Clin. Invest *66* (1980) 563-573.

[3] *M. R. Hardeman, P. Goedhart, and D. Breederveld:* Laser diffraction ellipsometry of erythrocytes under controlled shear stress using a rotational viscosimeter. Clin. Chim. Acta *165* (1987) 227-234.

[4] *S. R. Keller and R. Skalak:* Motion of a tank-treading particle in a shear flow. J. Fluid. Mech. *120* (1982) 27-47.

[5] *G. I. Zahalak and S. Sutera:* Fraunhofer diffraction pattern of an oriented monodisperse system of prolate ellipsoids. J. Colloid Interface Science *82* (1981) 423-429.

[6] *G. J. Streekstra, A. G. Hoekstra, E. J. Nijhof, and R. M. Heethaar:* Light scattering by red blood cells in ektacytometry: Fraunhofer versus anomalous diffraction. Appl. Opt. *32(13)* (1993) 2266-2272.

[7] *H. C. van de Hulst:* Light scattering by small particles. Wiley, New York 1957.

[8] *P. Barber and C. Yeh:* Scattering of electromagnetic waves by arbitrarily shaped particles. Appl. Opt. *14* (1975) 2864-2872.

[9] *P. W. Barber and S. C. Hill:* Scattering by axisymmetric particles: T-matrix method, in: Light Scattering by Particles: Computational methods. World Scientific, New Jersey 1990, pp. 79-185.

[10] *L. Reynolds, C. Johnson, and A. Ishimaru:* Diffuse reflectance from a finite blood medium: applications to the modeling of fiber optic catheters. Appl. Opt. *15* (1976) 2059-2067.

[11] *C. Allard, N. Mohandas, and M. Bessis:* Red cell deformability changes in hemolytic anemias estimated by diffractometric methods (ektacytometry). Blood Cells *3* (1977) 209-221.

[12] *M. Bessis and N. Mohandas:* Laser diffraction patterns of sickle cells in fluid shear fields. Blood Cells *3* (1977) 229-239.