



Mie Tyndal scattering of spheroidal RBC and WBC: T-matrix calculations

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Abstract

The electromagnetic problem has been solved for randomly oriented nonspherical and inhomogeneous structures with complex refractive indices $1.33018 - 2.09 \times 10^{-8}i$ (immersed in water) due to the T -matrix method. The light scattering properties of spheroidal erythrocytes/leukocytes for each condition were obtained using so-called Mie theory. In this study, the spheroidal erythrocyte is assumed to have a radius of 2:78 mm; giving it a volume equivalent to the average actual erythrocyte, and is illuminated by a plane wave of $1 \frac{1}{4}$ 873 nm: The T -matrix elements are obtained by numerical integration in each condition. Comparisons for erythrocytes/leukocytes with oxygenated hemoglobin and immersed in water, and its results for large, randomly oriented spheroids, show that the applicability range of the scattering process depends on the imaginary part of the refractive index and is changed for different elements of the scattering matrix.

Keywords: Mie-Tyndall theory, Light scattering, T -matrix method, Spheroidal erythrocyte, Leukocyte

1. Introduction

It is known that light scattering from biological cells is dependent on the morphological and biochemical structure of the cell. Increasing the present understanding of the interaction of light with tissue at a cellular level will promote the development of optical diagnostic techniques potentially capable of rapid, noninvasive assessment of cell biology. The most important biological particles such as blood cells can be effectively detected by means of light scattering, which provides a noninvasive, express, fully automated analysis. Nevertheless, the polymorphism and complexity of blood cells pose specific approaches in simulation, measurement, and

interpretation of light scattering data. First of all, analysis of a blood cell assumes the presence of water as a surrounded medium that leads to a negligibly small difference between refractive indices of cell and medium. This fact supposes the usage of well-known approximations in the simulation of light scattering from blood cells. On the other hand, the complexity of blood cells in shapes and internal structure assumes the use of delicate effects in light scattering such as depolarization, optical activity, etc. In general, the approximations do not provide a simulation of these light scattering effects. Consequently, in order to characterize a blood cell from light scattering, exact

light scattering methods should be applied for light scattering simulation.

Recently, correct compensation for light scattering improves the accuracy of the absorption measurement, but mainly because scattering properties themselves provide interesting information on the morphological properties of the blood cells (Kashima et al., 1995). However, the macroscopic scattering parameters do not yield detailed morphological information on the blood cell. To be able to extract microscopic information, such as cell size, shape, and alignment, one needs a detailed and explicit model. Such methods are available based on the solutions of Maxwell's equations. A blood cell has a size of the order of 10 times larger than the wavelength in the optical region. For that reason, researchers have applied the Lorentz–Mie theory to evaluate the influence of the size of randomly oriented blood cells on optical scattering properties by performing Mie calculations for spheres with different blood cell equivalent size and comparing the theoretically obtained scattering properties with those measured. The successful results of that study indicate that size rather than shape affects the light scattering from a suspension with randomly oriented cells (Steinke et al., 1998; Nilson et al., 1998).

The aim of the research described in this paper is to develop an increased understanding of how light interacts with erythrocyte on a cellular level using the T -matrix method to predict cellular scattering patterns. We investigate the light scattering properties of an ensemble of randomly oriented, identical spheroidal particles by the T -matrix method and how scattering is influenced by changes in cellular size and refractive index, and volume fraction, and by changes in the refractive index of the medium surrounding a cell. The normalized scattering intensity, elements of the scattering matrix, and the phase functions are computed by integrating the solution for a simple arbitrary oriented spheroid over all the orientations in 3-D space.

2. Basic Concepts and The T-Matrix Formalism

Erythrocytes are extremely poor conductors of electricity compared with the dilution, which cause a measurable change in electrical resistance. When a narrow beam of infrared light is made incident upon blood, light passes through the crowded blood cells in a zig-zag manner, is reflected, refracted at the membrane, and absorbed in the hemoglobin solution

in the cells. In particular, infrared light was chosen that light transmission through a turbid solution will increase with increasing wavelength by a factor λ^4 ; and was considered suitable rather than any other visible light in many studies (Tomita et al., 1983). Light scattering by spheroidal particles is specified by the following five physical quantities (Asano et al., 1980): (1) particle size relative to the wavelength of incident wave, (2) eccentricity of particle, (3) complex refractive index m relative to that of the surrounding medium, (4) orientation of particle to the incident wave, and (5) the observation direction. We consider a single particle illuminated by a beam with irradiance I_i . The total power scattered by this particle is W_s and it is proportional to the incident irradiance. This proportionality can be transformed into an equality by means of a factor C_s :

$$W_s = C_s I_i \quad (1)$$

where C_s has acquired the name scattering cross section and it must have dimensions of area. At sufficiently large distances, r , from a scattering center of bounded extent, the scattered field, \mathbf{E}_s , decreases inversely with distance and is transverse:

$$\mathbf{E}_s \approx \frac{e^{ik(r-z)}}{ikr} \mathbf{X} \mathbf{E} \quad (kr \gg 1) \quad (2)$$

where k is the wave number of the incident plane harmonic wave $\mathbf{E}_i = \mathbf{e}_x E (E = E_0 e^{ikz})$ propagating along the z -axis. The vector scattering amplitude is written as \mathbf{X} :

The scattering cross section is also obtained from the vector scattering amplitude by integration over all directions:

$$C_s = \int_{4\pi} \frac{|\mathbf{x}|^2}{k^2} d\Omega \quad (3)$$

Polarization changes upon reflection are described by decomposing electric fields into components parallel and perpendicular to the directions of the incident and scattered waves, for describing scattering by particles. With this decomposition the relation between fields can be written (Bohren et al., 1983; Barber et al., 1990; Van de Hulst., 1981; Bayvel et al., 1981):

$$\begin{bmatrix} \mathbf{E}_{\parallel s} \\ \mathbf{E}_{\perp s} \end{bmatrix} = \frac{e^{ikr}}{-ikr} \begin{bmatrix} S_2 & S_3 \\ S_4 & S_1 \end{bmatrix} \begin{bmatrix} \mathbf{E}_{\parallel i} \\ \mathbf{E}_{\perp i} \end{bmatrix} \quad (4)$$

where \parallel and \perp denote parallel and perpendicular, respectively. The elements of the amplitude scattering matrix are complex-valued functions of the scattering directions.

The T -matrix method is based on the surface integral representation of the electric field and on expansions of the surface fields in sets of vector functions that are complete on the unit sphere. The incident, reflected, and internal fields are expanded in vector wave functions. From the boundary conditions it is possible to derive a relation between the expansion coefficients of the scattered and the incident fields. The coefficients are related by a matrix referred to as the T -matrix.

It subsequently was applied to scattering problems under the name extended boundary condition method, EBCM (Varadan et al., 1998; Barber et al., 1975). Linearity of the field equations and boundary conditions implies that the coefficients in the spherical harmonic expansions of the field scattered by any particle are linearly related to those of the incident field. The linear transformation connecting these two sets of coefficients is called the T -matrix. This method is based on the direct numerical solution of the time-dependent Maxwell's equations and the popularity of this method is due to its ability to model a large variety of light propagation phenomena and material effects such as scattering, diffraction, reflection, absorption, gain, and polarization effects. It also successfully models material anisotropy, dispersion, and nonlinearities without any pre-assumptions and approximations for the nature of the optical field and its behavior. This method is well suited for the design and analysis of sub-micron devices with very fine structural details.

The T -matrix solution makes the method particularly suitable for the investigation of scattering by randomly oriented particles. After the T -matrix has been calculated, the solution for any particle orientation relative to the incident beam is obtained by multiplying a new set of incident field coefficients by the previously calculated T -matrix to obtain a new set of scattered field coefficients. Averaging over different orientations until the scattered field converges produces the final result. By utilizing integral representations of these fields, a relation between the expansion coefficient for the incident and the scattered fields is obtained.

The incident field in surrounding medium is regular at the origin and is thus expanded in regular waves:

$$\mathbf{E}_i(\mathbf{kr}) = E_0 \sum_{\nu=1}^{\infty} D_{\nu} [a_{\nu} \mathbf{M}_{\nu}^1(\mathbf{kr}) + b_{\nu} \mathbf{N}_{\nu}^1(\mathbf{kr})] \quad (5)$$

where E_0 is the amplitude of the incident field, D_n is a normalization constant, and a_{ν} and b_{ν} are the expansion coefficients. \mathbf{M} and \mathbf{N} are vector spherical harmonic functions which include Bessel, Hankel, and trigonometric functions.

The internal field within the object is expanded in the same regular vector waves:

$$\mathbf{E}_{int}(\mathbf{mkr}) = E_0 \sum_{\mu=1}^{\infty} [c_{\mu} \mathbf{M}_{\mu}^1(\mathbf{mkr}) + d_{\mu} \mathbf{N}_{\mu}^1(\mathbf{mkr})] \quad (6)$$

where c_{μ} and d_{μ} are the expansion coefficients of the internal field. The scattered field is expanded in outgoing spherical waves:

$$\mathbf{E}_S(\mathbf{kr}) = E_0 \sum_{\nu=1}^{\infty} D_{\nu} [f_{\nu} \mathbf{M}_{\nu}^3(\mathbf{kr}) + g_{\nu} \mathbf{N}_{\nu}^3(\mathbf{kr})] \quad (7)$$

where f_{ν} and g_{ν} are the expansion coefficients characterizing the scattered field.

Thus, the scattered expansion coefficients are obtained by multiplying the known expansion coefficients of the incident field by the T -matrix:

$$\begin{bmatrix} f_{\nu} \\ g_{\nu} \end{bmatrix} = -[\mathbf{T}] \begin{bmatrix} a_{\nu} \\ b_{\nu} \end{bmatrix} \quad (8)$$

The elements of the T -matrix are composed of computationally tolerable surface integrals. Since the information about the scattering behavior of a particle is incorporated in the $[\mathbf{T}]$ matrix, $[\mathbf{B}][\mathbf{A}]^{-1}$; the computational procedure is to calculate the $[\mathbf{T}]$ matrix for a particular particle and then store it for later use in other programs. The incident and scattered wave directions and polarizations are defined in the laboratory frame. The scattering calculations are performed in the particle frame. However, the direction and polarization of the incident and scattered fields have been defined in the laboratory frame. The T -matrix was applied to scattering from blood cells in the shape of oblate spheroids. In order to get an acceptable accuracy, it was necessary to use quadruple precision, i.e., real numbers with 32 digits precision. It was not possible to obtain accurate results for blood cells with realistic shape. It seems that the T -matrix method has to be pushed to its limits in order to give accurate results for blood cells.

3. Modeling and Numerical Implementations

In the presented study, we modeled Mie scattering by spheroidal erythrocyte/leukocyte structure, which has

$m = 1.40175 - 2.93 \times 10^{-5}i$ and $m = 1.33018 - 2.09 \times 10^{-8}i$ as complex refractive index for oxygenated hemoglobin and immersed in water, respectively. $a = 2.78 \mu\text{m}$ was used as radius of the sphere and $\lambda = 873 \text{ nm}$ as wavelength of the incident light (Fig. 1).

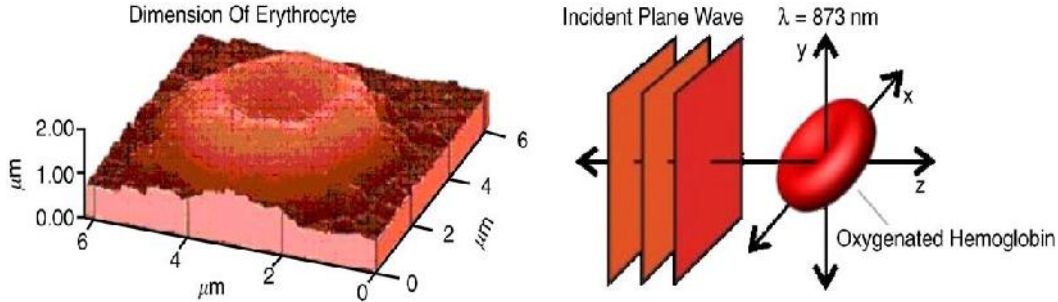


Figure 1. Dimension of erythrocyte and configuration for light scattering by oxygenated hemoglobin.

Numerical calculations of the internal or the scattered intensity for a spheroidal erythrocyte using the T -matrix approach consist of three steps: (1) Evaluate the surface integrals. These integrals contain combinations of Bessel, Hankel, associated Legendre, and trigonometric functions, all of which must be numerical. (2) Solve for the internal or scattered electric field expansion coefficients. (3) Sum expressions containing the expansion coefficients over a finite number of terms to obtain the electric field, intensity, or cross-section quantity of interest.

Firstly, the T -matrix was established for future scattering calculations. Converged results were

obtained for larger size parameters (20) and larger axial ratios (~2) in arbitrary units by implementing the T -matrix method using double-precision variables. Convergence was tested for series of functions.

For these two conditions, we calculated the normalized scattered intensities vs. scattering angles (Fig. 2), the differential scattering cross section vs. scattering angles (Fig. 3) and the differential scattering cross section vs. scattering angles (Fig. 4), for both transverse magnetic (TM) and transverse electric (TE) polarizations. As a function of inscribed spheroid, the internal intensities are calculated along the z-axis for spheroidal erythrocytes in perpendicular polarization (Fig. 5).

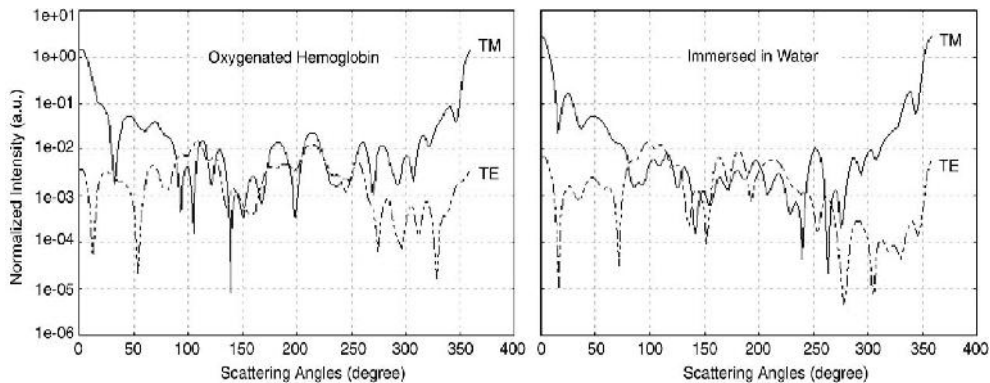


Figure 2. Normalized intensity vs. scattering angles for oxygenated hemoglobin and immersed in water for two polarizations.

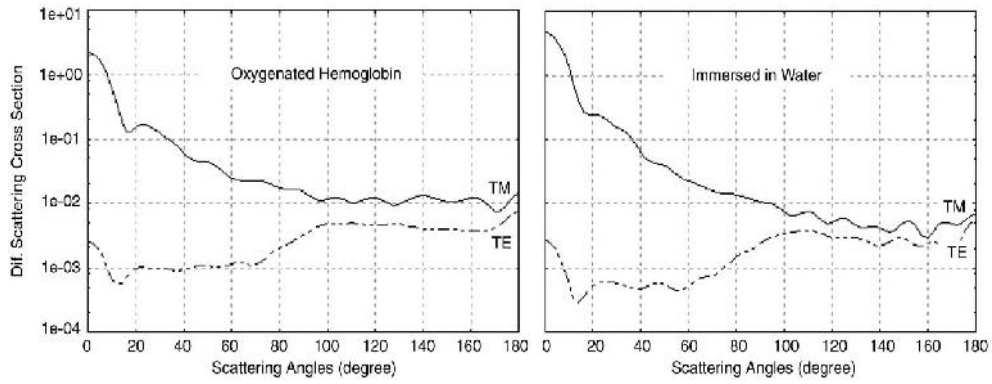


Figure 3. Differential scattering cross section vs. scattering angles for randomly oriented oxygenated hemoglobin and immersed in water for two polarizations in three-dimensions.

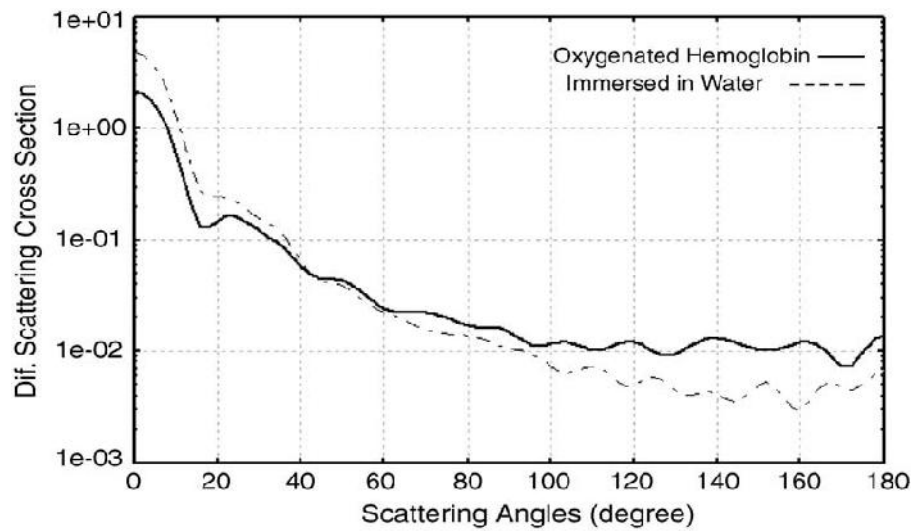


Figure 4. Differential scattering cross section vs. scattering angles for randomly oriented oxygenated hemoglobin and immersed in water in three-dimensions.

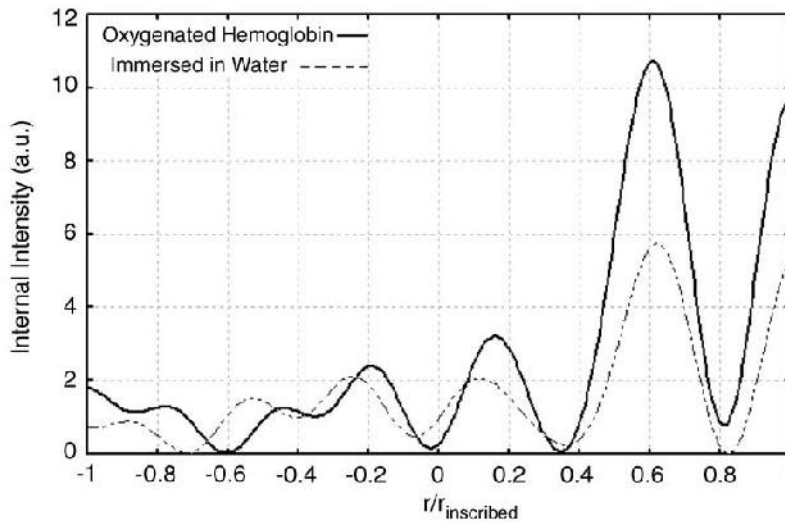


Figure 5. Internal intensity along the z-axis as a function of inscribed spheroid.

Calculated results for one of these test cases are given in Fig. 6. The calculated quantities are P_{11} ; $P_L = -P_{12}/P_{11}$; and $P_X = 1 - P_{22}/P_{11}$; for a spheroid erythrocyte with a refraction index of $1.40175 - 2.93 \times 10^{-5}i$; where, P_{11} is the phase function, P_L is the degree of linear

polarization of the scattered light for un-polarized incident wave, and P_X is the depolarization ratio, the ratio of depolarized light to total scattered light.

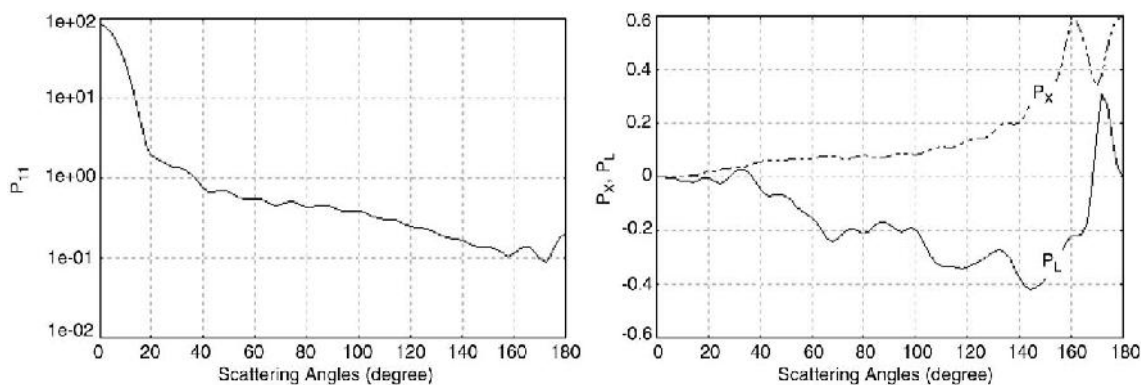


Figure 6. Phase functions and their comparison for oxygenated hemoglobin with a refraction index of $1.40175 - 2.93 \times 10^{-5} i$.

4. Discussion

In this paper, we illustrated the use of scattering theory to biological cells and the development in the context of optical studies on whole blood, essentially a suspension of biconcave ellipsoid red blood cells, but similar problems exist for other cells. The T-matrix method gives a good insight into light scattering by erythrocytes, in particular the relation between the scattering intensity and the refractive index of the pattern is immediately evident in this approximation. These findings will be useful for the interpretation of the disease with refractive index and shape of erythrocytes. The results for WBC very close to the RBC calculations and therefore we did not discuss it in detail here.

Our calculations confirmed the applicability of T-matrix method to the scattering by erythrocytes/leukocytes despite the deviation of the cell from isotropic particle to anisotropic particle.

Our approximation gives quite good results for near small-angle scattering, but does not adequately describe the scattering over the entire angle range. The results showed that the imaginary part of the refractive index of cells plays a significant role in light scattering from cells, and the volume fraction of erythrocytes with the different refractive index affects both the total amount of scattered light and the angular distribution of scattered light. Our results also demonstrate the need for more accurate measurements of the refraction index of cells and cell components for more accurate

determination of the scattering patterns. These computations parallel and are very close to experimental results of spectroscopic measurements for erythrocytes (Hammer et al., 1998; Yaroslavsky et al., 1997; Sloot et al., 1988; Streekstra et al., 1993; Steinke et al., 1988; Lisovskaya et al., 1988; Lisovskaya et al., 1999; Ataulakhanov et al., 2002). The numerical calculation and experimental results indicate that scattering properties are strongly influenced by biochemical and morphological structure.

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DOI: 10.22192/ijarbs.2017.04.04.013	

How to cite this article:

Farkhanda F.Rzayeva, Arif M.Efendiyev, Nargiz Isayeva, Amirullah M.Mamedov. (2017). Mie Tyndal scattering of spheroidal RBC and WBC: T-matrix calculations. *Int. J. Adv. Res. Biol. Sci.* 4(4): 94-100.
 DOI: <http://dx.doi.org/10.22192/ijarbs.2017.04.04.013>