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Experimental study on light scattering by biological cells with discrete sources method

Jianbin Liu*, Yingxin Zeng, Fang Lin

College of Electronic Engineering South China Agriculture University, Guangzhou, China

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ABSTRACT

In this paper, an experimental apparatus for the analysis of biological cells light scattering in liquid suspensions has been presented. Firstly, an algorithm of the discrete sources method (DSM) is applied to model light scattering from a cell particle. In our model, a monochromatic laser beam scattered by cell particles, which can be inorganic, organic, or biological cells (animal cells and bacteria) and the scattering characteristic is analyzed with morphology and the refractive index of the cell particles. In contrast to traditional volume-based methods which are widely used for light scattering simulation, DSM allows calculation of scattering for all incident angles and polarizations at once. This leads to an essential reduction of the computing time. The DSM algorithm allows using a lower number of elementary sources which results in an increased accuracy of approximation for every harmonic. Secondly, in order to study light scattering in biological cells close to the actual situation, we focus on non-spherical particles in the cell-culture medium. Finally, we demonstrate the light scattering results of bovine kidney cells suspended in the cell-culture medium, and compares with the simulated results.

1. Introduction

Light scattering theory plays an important role in detection and characterization of small particles in various fields of science, for example, remote sensing of aerosols, detection of interstellar dust, quality control in industrial applications and characterization of biological cells. Since, in 1957, Prof. van de Hulst published his well-known book Light Scattering by Small Particles [1]. The study of light scattering by small regular particles has been and still is a subject of great interest in many different scientific disciplines for many years [2–14]. It is well known that most of these applications use theoretical models based on idealized conditions such as perfect spheres, perfect homogeneous particles, symmetric particles, etc. Despite extensive research, knowledge of light scattering of more complex structured particles is still limited. In the past, a variety of different methods has been developed to deal with the scattering problem of, in particular, spherical and non-spherical objects [15–22]. In general, they differ in the approaches used, and consequently, in their capabilities to compute the scattering behavior of various particle classes. Corresponding computer programs are sophisticated, tested, and partly publicly available (see, e.g., [17,18] for a database of numerous programs hosted by the University of Bremen). However, they may lead to slightly different numerical results for a given scattering problem. This can also be the case for various implementations of the same method or even for different versions of the same program. The differences may increase when approaching the limits of the algorithms. On the other hand there are cases where only one single method exists for treating special scattered types so that no comparative calculations with alternative methods are possible for validating the results obtained. In all these cases, it is up to the user to finally judge the accuracy and correctness of the findings. This is, e.g., important in characterization of biological cells. Different scattering models can lead to different results in the data processing and finally to different conclusions.

To study its practical use, we investigated the fulfillment of the reciprocity condition by existing publicly available scattering programs, considering different particles of relatively complex shape. The results of this study are presented here. In Section 2 the numerical simulation of light scattering by different scales cells are given. In Section 3 the experimental apparatus and samples are presented. In Section 4 the numerical results and experimental results are compared and discussed. Some conclusions are given in Section 5.

2. Theory

Originally, the theoretical principles of the DSM were established about 40 years ago independently by Kupradze [23] in the

* Corresponding author. Tel.: +86 13148918189.
E-mail addresses: liujianbin@scau.edu.cn, jbl136@163.com (J. Liu).

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Soviet Union and Yasuura [24] in Japan. The first version of the DSM was published in 1980 by Sveshnicov and Eremin [25]. Since then several research groups have been working on this worldwide. Recently, the method was modified for light scattering by a wide variety of objects, from long fibers [26] to oblate spheroids and concave particles. Also evanescent scattering [27] can be calculated. The DSM is a semi-analytical method. A mathematical statement of the light scattering problem in a frame of DSM consists of Maxwell equations, transmission conditions at the interface and obstacle boundary and infinity conditions to provide the unique solution. An approximate solution is constructed as a linear combination of fields of discrete sources (multipoles or dipoles deposited in a supplementary domain) with certain amplitudes, so that it satisfies all the conditions of a boundary value scattering problem except the boundary conditions on an obstacle surface. The last condition is used to determine the amplitudes of discrete sources using a generalized point matching scheme. Contrary to other methods DSM has the advantage that it allows to estimate a posterior error by calculating the surface residual on the obstacle boundary and efficiently makes use of axial symmetry of the scatterer. In this way computational time is reduced.

A comprehensive overview of the DSM can be found in the book of Eremin et al. [28].

Usually the discrete sources are placed on the axis of symmetry.

Consider scattering in an isotropic homogeneous medium in R^3 of an electromagnetic wave by a local homogeneous penetrable obstacle D_i with a smooth boundary. Let us introduce a cylindrical coordinate system (z, θ, φ) where, z is the axis of symmetry of the particle and θ_i is an incident angle with respect to. Then the mathematical statement of the scattering problem can be formulated in the following form:

$$\nabla \times H_{e,i} = ik\varepsilon_{e,i}E_{e,i},\ \nabla \times E_{e,i} = -ik\mu_{e,i}H_{e,i} \text{ in } D_{e,i} \quad (1)$$

$$\begin{aligned} n_P \times (E_i(P) - E_e(P)) &= n_P \times E^0(P),\ n_P \times (H_i - H_0) \\ &= n_P \times H^0(P) P \in \partial D \end{aligned} \quad (2)$$

and Silver Muller radiation condition for the scattered field at infinity.

Here $\left\{E^0, H^0\right\}$ is an exciting field, n_P is the outward unit normal vector to ∂D, index e belongs to the external domain D_e, $k = \omega/c$, ε, μ are permittivity and permeability, $\mathrm{Im}\varepsilon_e$, $\mu_e \le 0$ (time dependence for the fields is chosen as $\exp\left\{j\omega t\right\}$) and the particle surface is smooth enough $\partial D \Subset C^{(1,\alpha)}$, Then the above boundary-value problem is uniquely solvable [29].

The DSM is based on the conception of an approximate solution. The approximate solution is constructed as a finite linear combination of discrete sources (DS): dipoles and multipoles deposited in a supplementary domain inside the particle with certain amplitudes. Usually as such a domain the axis of symmetry of the particle is used. In case of an oblate particle like erythrocyte, disk or oblate spheroid, it is not always possible to use the axis of symmetry [30]. For this purpose an analytical continuation to a complex plane is constructed. More detailed information can be found in [31]. The deposition of DS in a complex plane allows reducing calculation errors and time of computations.

In case of P-polarized plane wave the exciting field accepts the following form:

$$E^0 = (e_x \cos\theta_0 + e_z \sin\theta_0) \exp\left\{-jk_e(x \sin\theta_0 - z\cos\theta_0)\right\}$$

$$H^0 = -e_y \cos\theta_0 \exp\left\{-jk_e(x \sin\theta_0 - z\cos\theta_0)\right\}$$

where, $k_e = k\sqrt{\varepsilon_e \mu_e}$

To take into account the polarization of the external excitation we use linear combinations of electrical and magnetic multipoles. For this special vector potentials are used. For the P-polarized wave in a cylindrical coordinate system they can be represented as:

$$\begin{aligned} A^{1,e,i}_{mn} &= \{Y^{e,i}_m(\eta, w^{e,i}_n)\cos(m+1)\varphi; -Y^{e,i}_m\left(\eta, w^{e,i}_n\right)\sin(m+1)\varphi; 0\} \\ A^{2,e,i}_{mn} &= \{Y^{e,i}_m(\eta, w^{e,i}_n)\sin(m+1)\varphi; -Y^{e,i}_m\left(\eta, w^{e,i}_n\right)\cos(m+1)\varphi; 0\} \\ A^{3,e,i}_{n} &= \left\{0; 0; Y^{e,i}_0(\eta, w^{e,i}_n)\right\} \end{aligned} \quad (3)$$

Here

$$Y^e_m\left(\eta, w^e_n\right) = \frac{k_e}{i} h^{(2)}_m\left(k_e R_{\eta w^e_n}\right)\left(\frac{\rho}{R_{\eta w^e_n}}\right)^m,$$

$$Y^i_m\left(\eta, w^i_n\right) = j_m\left(k_i R_{\eta w^i_n}\right)\left(\frac{\rho}{R_{\eta w^i_n}}\right)^m$$

$R^2_{\eta w^e_n} = \rho^2 + (z - w^e_n)^2$, $\eta = (\rho, z)$, $h^{(2)}_m$ is a spherical Hankel function and j_m is a spherical Bessel function. Hence, the approximate solution for the P-polarized wave accepts the form:

$$\begin{pmatrix} E^N_{e,i} \\ H^N_{e,i} \end{pmatrix} = \sum_{m=0}^{M}\sum_{n=1}^{N^\xi_m}\left\{p^{e,i}_{mn} D_1 A^{1,e,i}_{mn} + q^{e,i}_{mn}\frac{j}{\varepsilon_\xi} D_2 A^{2,e,i}_{mn}\right\} + \sum_{n=1}^{N^\xi_0} r^{e,i}_n D_1 A^{3,e,i}_n,$$
$$D_1 = \begin{pmatrix} \dfrac{j}{k\varepsilon_{e,i}\mu_{e,i}} \nabla\times\nabla\times \\ -\dfrac{j}{\mu_{e,i}}\nabla\times \end{pmatrix}, \quad D_2 = \begin{pmatrix} \dfrac{j}{\varepsilon_{e,i}}\nabla\times \\ \dfrac{j}{k\varepsilon_{e,i}\mu_{e,i}}\nabla\times\nabla\times \end{pmatrix} \quad (4)$$

The approximation solution for the case of a S-polarized excitation is constructed in a similar way and has the form:

$$\begin{pmatrix} E^N_{e,i} \\ H^N_{e,i} \end{pmatrix} = \sum_{m=0}^{M}\sum_{n=1}^{N^\xi_m}\left\{p^{e,i}_{mn} D_1 A^{1,e,i}_{mn} + q^{e,i}_{mn}\frac{j}{\varepsilon_\xi} D_2 A^{2,e,i}_{mn}\right\} + \sum_{n=1}^{N^\xi_0} r^{e,i}_n D_2 A^{3,e,i}_n, \quad (5)$$

More details can be found in [30].

The constructed approximate solutions Eqs. (4) and (5) satisfy Maxwell equations in Eq. (1) and radiation conditions for the scattered fields at infinity. The unknown vector of amplitudes of DS

$$p_m = \left\{p^{e,i}_{mn}, q^{e,i}_{mn}, r^{e,i}_n\right\}_{n=1}^{N^m_{e,i}}$$

is to be determined from the transmission conditions Eq. (2). As it was mentioned above, DS are situated in a complex plane adjoined to the symmetry axis of the particle. The approximate solutions Eqs. (4) and (5) are finite linear combinations of Fourier harmonics with respect to the φ angle variable. Therefore, after resolving the plane wave excitation into Fourier series with respect to the φ angle, we reduce the two-dimensional approximation problem enforced at the particle surface to a set of one-dimensional problems at the particle generatrix. For solving these problems, the general matching-point technique is applied, more details can be found in [30].

The exactness of the result is provided by stabilization of the scattering diagram and a posterior residual calculation.