A Pulsed Finite-Difference Time-Domain (FDTD) Method for Calculating Light Scattering from Biological Cells Over Broad Wavelength Ranges

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Abstract: We combine the finite-difference time-domain method with pulse response techniques in order to calculate the light scattering properties of biological cells over a range of wavelengths simultaneously. The method we describe can be used to compute the scattering patterns of cells containing multiple heterogeneous organelles, providing greater geometric flexibility than Mie theory solutions. Using a desktop computer, we calculate the scattering patterns for common homogeneous models of biological cells and also for more complex representations of cellular morphology. We find that the geometry chosen significantly impacts scattering properties, emphasizing the need for careful consideration of appropriate theoretical models of cellular scattering and for accurate microscopic determination of optical properties.

OCIS codes: (290.0290) Scattering; (170.6510) Spectroscopy, tissue diagnostics

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1. Introduction

Recent work has suggested that elastic light scattering spectroscopy can provide a valuable, non-invasive means to quantitatively probe tissue morphology. A number of studies have demonstrated that elastic scattering can be used to differentiate normal and diseased tissues in several organ sites including the skin, bladder, and colon [1-5]. More recently, several investigators have reported methods to extract quantitative features related to tissue morphology from reflectance spectra. Mourant *et al.* reported that the size of scatterers could be determined from elastic scattering spectra using Mie theory [6]. This work involved tissue phantoms comprised of polystyrene spheres and Intralipid. Perelman *et al.* described a technique for obtaining nuclear size distributions by Fourier transformation of the scattering spectra [7]. Zonios *et al.* reported a method to extract effective scatterer sizes from colon tissue spectra based on a Mie theory model [5]. In order to isolate the scattering signal due to cell nuclei from diffuse background scattering, which is typically orders of magnitude larger, Backman *et al.* [8] and Sokolov *et al.* [9] proposed techniques based on polarized light scattering spectroscopy. These polarization-based techniques assessed size and refractive indices of cell nuclei using Mie theory models. The use of polarization provided a means to distinguish between single and multiply scattered light and reduced the effects of hemoglobin absorption. All of the studies noted above developed models based on Mie theory which could adequately describe experimental data, offering a potential approach to extracting quantitative information from the scattering spectra.

Although a number of studies have shown promising results using Mie theory models, the ability to match a model with experimental data does not necessarily indicate the model is the best model or even an appropriate one. There is not convincing experimental evidence that justifies the use of Mie theory as an accurate model of cellular and/or nuclear scattering. Mie theory is commonly applied to model scattering from cells largely because it is the only simple analytical model available. In fact, experimental evidence at times suggests a Mie theory model of a biological cell may not be appropriate. For instance, work by Mourant *et* *al.* found that the scatterer sizes in biological cells ranged from 0.4 to 2.0 μm, a size consistent with organelles smaller than a cell's nucleus [10]. Moreover, the same study showed that the nucleus contributed mostly to low angle scattering while smaller organelles contributed to high angle scattering, which may be particularly pertinent to reflectance measurements acquired using fiber-based probes with small source-detector separations. Other studies have also produced results which indicate that Mie theory may not always be a sufficient model. For instance, McGann *et al.* clearly demonstrated that forward scattering of lymphocytes varied inversely with cell volume. This would not be expected from a Mie theory model of scattering and emphasizes that cellular light scattering cannot always be adequately described using the simplest conceivable geometric model [11]. Additional research has described significant changes in backscattering obtained when a coated sphere (cell with membrane) [12] or concentric sphere (nucleated cell) [13] model was used rather than a single sphere model, further emphasizing the importance of a prudent approach to choosing a cell model since the scattering predicted is a strong function of the geometry chosen.

Based on the available experimental evidence, it seems likely that there will be cells which cannot be adequately modeled as a homogeneous sphere. For such cases, a more flexible alternative than Mie theory is required. We provide one possible solution in this paper. The method we describe can be used to calculate light scattering from cells of arbitrarily complicated shape and dielectric structure. Our approach is based on a standard finite-difference time-domain (FDTD) technique [14]. In the past, the primary limitation of the FDTD technique has been the need for an individual FDTD run for each frequency of interest, making the technique impractical for calculations at more than a few frequencies. In this paper, we modify the standard approach incorporating pulse response techniques which provide a means to calculate light scattering properties over a broad frequency range using only a single FDTD run.

Because the computational time requirements of the proposed method are extremely modest, requiring little more time than equivalent Mie theory calculations, the method can be efficiently used to compute light scattering for any desired model of a cell, from models as simple as a single sphere or coated sphere to cell models containing numerous organelles and intricate dielectric structure. The purpose of this paper is two-fold: first, to provide a detailed description of the practical implementation of the method, and second, to use the method to compute the scattering properties of cells modeled using progressively more complex geometries and to demonstrate that the cell model used significantly impacts the scattering properties.

2. Methods

The FDTD algorithm provides a means to numerically solve Maxwell's equations in the time domain. The method is briefly reviewed here. A more detailed explanation is found in [14]. The algorithm begins by discretizing Maxwell's curl equations in space and time, resulting in a set of explicit finite-difference equations. The finite-difference equations are stepped in time, and the electric and magnetic field components at each grid point are alternately updated. To prevent artificial reflections along the edges of the grid, an appropriate boundary condition must be employed. In this paper, the Liao boundary condition was used [15]. For applications requiring a larger dynamic range, the PML boundary condition may be applied to provide at least 80 dB additional suppression [16].

To yield accurate results for a given wavelength, the grid spacing used must be less than wavelength, typically $\lambda/10$ or smaller. At each grid point, the permittivity and conductivity of the medium is specified. The cell is constructed by assigning permittivity values to each cell component. A range of values may be assigned to a particular component if that component is inhomogeneous. Details regarding the application of the FDTD method specifically to biological cells, including refractive index values for specific cellular constituents, may be found in [17-19].