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Morphological characterization of cells in concentrated suspensions using multispectral diffuse optical tomography

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ABSTRACT

Based on a non-spherical model of particle scattering, we investigate the capabilities and limitations of a T-matrix based inverse algorithm to morphologically characterize cells in concentrated suspensions. Here the cells are modeled as randomly orientated spheroidal particles with homogenous dielectric properties and suspended in turbid media. The inverse algorithm retrieves the geometrical parameters and the concentration of cells simultaneously by inverting the reduced scattering coefficient spectra obtained from multispectral diffuse optical tomography (MS-DOT). Both round and spheroidal cells are tested and the role of multiple and higher order scattering of particles on the performance of the algorithm is evaluated using different concentrations of cells.

1. Introduction

Morphological characterization of cells plays an important role in clinical diagnosis [1–6]. For example, tumorous cells are crowded and enlarged compared to normal cells [1]. Microscopy is routinely used to examine the cellular morphology, but it is not applicable to *in vivo* tissue. It has been shown that multispectral diffuse optical tomography (MS-DOT) is a promising modality for imaging of cellular morphology since it is non-invasive and can be used to characterize *in vivo* cells located deep in tissue [7]. The basic idea of MS-DOT is to illuminate the tissue by near-infrared light and collect the scattered light along the tissue boundary. By analyzing the spectra of scattered light, one can retrieve information regarding the morphology and composition of cells.

It is known that MS-DOT or other optical based particle sizing methods are dealing with an ill-posed inverse problem that requires some *a priori* information about the cells in order to obtain stable and unique solutions [7,8]. For example, the spherical model of the Mie scattering theory is widely used so far. However, cells generally have complex irregular shapes; thus the effect of non-sphericity cannot be adequately addressed by the Mie based techniques [9]. This necessitates a non-spherical model of particle scattering for accurate cellular sizing. The T-matrix or extended boundary conditions method is one of the most powerful and widely used tools for rigorous computation of electromagnetic scattering by single or an aggregate of non-spherical particles [10,11]. In the T-matrix approach, the incident and scattered waves are expanded in terms of incoming and outgoing vector spherical wave functions. The relationship between the coefficient of the incident and scattered waves is established through a transmission matrix (i.e., the T-matrix). The main advantage of T-matrix representation is that it is independent of the incident/scattered wave directions and that it depends only on the shape, size, orientation and refractive index of the particles [10].

The application of the T-matrix method to assess the morphology of spheroidal particles has been reported in several publications [12–14]. However, these studies did not demonstrate the ability to retrieve the concentration of particles or the methods used were not applicable for characterization of cell aggregates in deep tissue. The purpose of our current work is to demonstrate and evaluate our MS-DOT-T-matrix based inverse algorithm for characterization of cells in concentrated suspensions. In our method, the scattering spectra of cell suspensions provided by MS-DOT are used to retrieve the statistical parameters of cells including mean size, volume fraction and aspect ratio through the T-matrix based algorithm. Both round and spheroidal cells embedded in turbid media are studied. This work is the first demonstrating the application of MS-DOT for morphological characterization of cells suspended in a culture medium, by analyzing diffusely scattered light collected along the phantom boundary.

2. Methodology

2.1. Theoretical summary and the inverse algorithm

The first step in our technique is to shine laser beams into a mixture containing the cells to be characterized. The light sources

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are placed sufficiently far from the suspensions. The presence of cells influences light scattering and propagation within the suspensions. Afterwards, photon densities along the medium boundary are recorded by some detectors for further analysis. We suppose that cells have spheroidal shapes and they are dispersed in suspensions with random orientations.

The photon propagation in suspensions satisfies the following photon diffusion equation, which can be rigorously solved by the finite element method [7]:

$$\nabla\cdot(D(r)\Phi(r,\omega))-[\mu_a(r)-i\omega/c]\Phi(r,\omega)=-S(r,\omega) \quad (1)$$

where $\Phi(r,\omega)$ is the photon density, ω is the angular frequency, $D(r)=1/3(\mu_a(r)+\mu_s'(r))$ is the diffusion coefficient, $\mu_a(r)$ is the absorption coefficient, $\mu_s'(r)$ is the reduced scattering coefficient, c is the speed of light in the medium, $S(r,\omega)$ is the source term, and an $\exp(-i\omega t)$ time variation is assumed. Once the reduced scattering spectra are recovered using the MS-DOT algorithm, as elaborated in Ref. [7], they have to be further processed to recover the morphological parameters of the cells.

At each computational node, the measured reduced scattering coefficient is related to the particle morphology parameters through the following expression:

$$\mu_s'(\lambda)=\phi\int_{r=r_{\min}}^{r=r_{\max}}\int_{\varepsilon=\varepsilon_{\min}}^{\varepsilon=\varepsilon_{\max}}\frac{e^{-(1/2)\left(\frac{(r-r_m)^2}{\delta_m^2}+\frac{(\varepsilon-\varepsilon_m)^2}{\delta_\varepsilon^2}\right)}}{2\pi\delta_r\delta_\varepsilon}\left(1-\frac{\alpha_1^1(r,\varepsilon)}{3}\right)\langle C_{sca}(r,\varepsilon)\rangle\,dr\,d\varepsilon \quad (2)$$

The reduced scattering coefficient, at a wavelength λ, is denoted by the symbol $\mu_s'(\lambda)$. $r\in[r_{\min}r_{\max}]$ is the equal-volume radius of the scattering particle. $\varepsilon\in[\varepsilon_{\min}\varepsilon_{\max}]$ is the aspect ratio, which is the fraction of the equatorial radius to the polar radius of the spheroidal particle. We have utilized a bivariate normal distribution function to describe the random variations in the cell size and aspect ratio. The mean values of the particle radius and aspect ratio are represented by the symbols r_m and ε_m, respectively. The concentration of cells is represented by the symbol ϕ , which represents the number of cells per cubic millimeter. The following relationship holds between the cell volume fraction and its concentration:

$$VF\,(\%)=0.1\phi\ (\text{in million per cubic millimeter})\times\frac{4\pi}{3}r_m(\text{in }\mu m^3) \quad (3)$$

δ_r and δ_ε are the standard deviations of the cell size and aspect ratio, respectively. In this work we assumed that the standard deviations are 10% of their corresponding mean values. $\langle C_{sca}(r,\varepsilon)\rangle$ denotes the averaged scattering cross section which can be calculated using the T-matrix method. $\alpha_1^1(r,\varepsilon)$ is a coefficient obtained by expanding the phase function [15]. The values of $\langle C_{sca}(r,\varepsilon)\rangle$ and $\alpha_1^1(r,\varepsilon)$ are *a priori* calculated and stored in a T-matrix database.

In order to recover the cell size ($2r_m$), aspect ratio (ε_m) and the concentration (ϕ), we first need to find the best estimates for these parameters, in a range of their possible values, by minimizing the error function $\chi^2=\sum_{\lambda_j}(\mu_s'(\lambda_j)^o-\mu_s'(\lambda_j)^c)^2$, where $\mu_s'(\lambda_j)^o$ is the observed reduced scattering coefficient and $\mu_s'(\lambda_j)^c$ is the calculated one from Eq. (2) and $\lambda_j\ j=1,2\ \ldots$ are the measurement wavelengths.

The updates for unknown parameters are determined by solving

$$(J^TJ+\gamma I)\Delta\zeta=J^T\Delta\chi \quad (4)$$

where γ is an appropriately chosen regularization parameter, I is the identity matrix, the vector $\Delta\chi=[\mu_s'(\lambda_1)^o-\mu_s'(\lambda_1)^c,\ldots]^T$ represents the error between the observed and calculated reduced scattering coefficients and $\Delta\zeta=[\Delta\phi\quad\Delta\delta_r\quad\Delta r_m\quad\Delta\delta_\varepsilon\quad\Delta\varepsilon_m]^T$ is the vector which updates the parameters of interest. We can obtain explicit expressions for the elements of the Jacobian matrix J by direct differentiation of Eq. (2) with respect to the model parameters.

2.2. Experimental setup and data collection

Briefly, light from one of the five continue-wave laser modules was transmitted to an optical switch, which sequentially passes it to 16 pre-selected points at the surface of the phantom for 2D imaging experiments. 16×16 measured data was then input into our MS-DOT reconstruction algorithm to generate a 2D cross-sectional image of the phantom [7]. Measurement data at five wavelengths of 733, 775, 808, 840, and 915 nm were used for the image reconstructions.

For cellular sizing, depending on the cell type and its refractive index, an *a priori* stored T-matrix database with different combinations of cell size and aspect ratio was used to calculate the reduced scattering coefficient [16] and recover the unknown morphological parameters using the inverse algorithm [15]. A separate T-matrix database was used for characterization of the scattering Intralipid particles in the background [17,18]. Cells to be characterized were suspended in a glass tube which was embedded off-center in the cylindrical background phantom consisting of 1% Intralipid and India Ink (Fig. 1).

3. Results and discussion

3.1. Chinese Hamster Ovary (CHO) cells

CHO cells are one of the most commonly used cell cultures in biological and medical researches. CHO cells have epithelial morphology in culture and grow attached to the surface of a flask. While attached to the surface, CHO cells have polygonal and elongated shapes as shown in Fig. 2a. The size of CHO cells is about 14–15 μm with refractive index of ~1.38 [19–21]. Optical scattering from CHO cells cannot be accurately measured while they are attached to the plastic surface. Hence, we utilized a serum free medium to suspend CHO cells following the procedure described in Ref. [22]. While in suspension, the irregular-shaped CHO, being anchorage-dependent cells, change their morphology

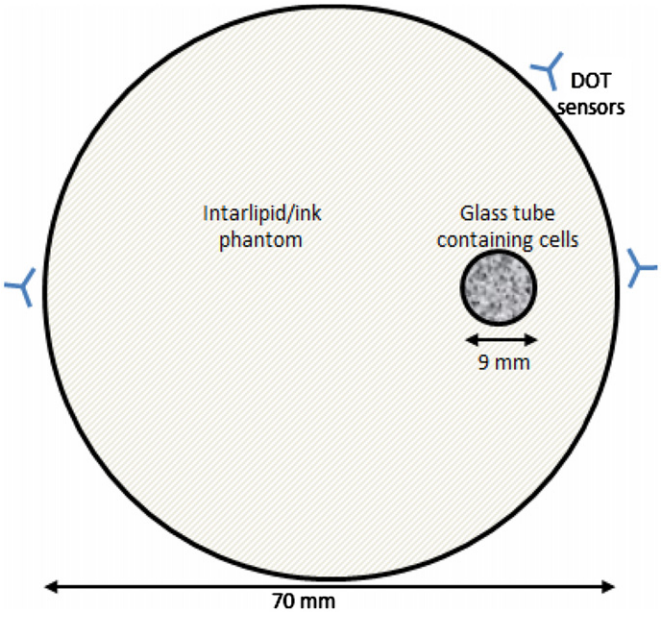

Fig. 1. Diagram of the experimental setup for MS-DOT measurements.