Title:

MONTE CARLO INVESTIGATIONS OF ELASTIC SCATTERING SPECTROSCOPY
APPLIED TO LATEX SPHERES USED AS TISSUE PHANTOMS

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Monte Carlo investigations of elastic scattering spectroscopy applied to latex spheres used as tissue phantoms.

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ABSTRACT

An optical-fiber-coupled, elastic-scatter spectrometer has proven effective in discriminating between malignant and non-malignant tissue in the human bladder and gastrointestinal tract. The system injects broadband light into the tissue with an optical fiber and spectrally analyzes the returning light collected by an adjacent fiber. The collected photons have experienced multiple scattering events and therefore arrive at the analysis fiber after traveling varied paths. The diameter of the source fiber is comparable to its separation from the collection fiber. The diffusion model is inappropriate for this geometry; therefore, Monte Carlo simulations are used. In addition, the size of the scattering sites in tissue are expected to be of the same order as the excitation wavelengths, and Mie theory is expected to provide the best description of the scattering and extinction. We will present and compare the results of simulations and measurements of the elastic scatter signal for suspensions of latex spheres in hemoglobin solutions of varying concentrations.

1. INTRODUCTION

We have built an optical biopsy system (OBS) capable of discriminating among tissue types and conditions. This system is based on spectral analysis of broad-band light after scattering through the tissue. The light is delivered by an optical fiber and collected with a second adjacent fiber with both fibers in contact with the tissue surface (see Figure 1). Photons entering the tissue can be elastically scattered, inelastically scattered, or absorbed. In tissue, the scattering-cross-section (μ_s) can be up to 100 or more times greater than the absorption-cross-section (μ_a) for visible and infrared wavelengths. It is therefore expected that the amplitude vs. wavelength distribution of photons emerging from the tissue depends upon the details of cellular and sub-cellular structure of the tissue, as well as the tissue biochemical composition. Since changes in cellular architecture are often associated with tissue pathologies, such as malignancy, spectral analysis of light transported through the tissue is a potential diagnostic tool for malignancy and other tissue abnormalities. This system has proven successful in a preliminary *in vivo* study of patients known to have bladder tissue abnormalities¹ and in another preliminary *in vivo* study of gastrointestinal cancer.²

Most analytical models of the interaction of light with tissue employ the radiative transport equation or approximations to it such as the P1 approximation (diffusion theory) and the P3 approximation. Alternatively, Monte-Carlo simulations have been used. In the P1 and P3 approximations only the first few terms of a spherical harmonic expansion of the light fluence are used. Therefore, these approximations are inaccurate for light distributions that are far from isotropic, which is the case for the OBS geometry. The P3 approximation, however, is a significant improvement over the P1 approximation and is in closer agreement with Monte-Carlo simulations.³ Monte-Carlo simulations are used in this study

not only to overcome this geometric limitation, but also to examine the details of the scattering events.

Figure 1. The Optical Biopsy System analyzes light interacting with a small volume of tissue. Scatter and absorption are the most significant interactions.

Cellular components of tissue that are expected to dominate the elastic scattering process have dimensions comparable with or larger than the wavelengths of the spectral band used to probe the tissue ($0.3\text{--}1.0\ \mu$). Therefore, the scattering characteristics are best described by Mie theory or multiple-scattering extensions of Mie theory. Although the particle size-to-wavelength ratio in tissue is such that Mie theory is necessary, only a few investigators have compared measured values with those predicted by Mie theory. Measurements of the angular light-scattering distribution for a dilute solution of particles agreed with Mie theory predictions for both red blood cells and hollow latex spheres.^{4,5} Experimentally measured values of μ_s for a solution of polystyrene spheres were within 20% of the values determined from Mie theory calculations from 400–700 nm.⁶ Das et al. also found good agreement between some features predicted by Mie theory and transmission experiments through latex sphere suspensions.⁷ In acknowledgment of the highly forward scattering seen in tissue, predictions of optical parameters are frequently made using the Henyey-Greenstein phase function⁸. Liu has discussed the deficiencies of that phase function and has developed a two-parameter phase function which matches the Mie distribution much better in the forward direction.⁹ Liu's expression, however, does not differ significantly from the Henyey-Greenstein expression at large angles and also fails to account for the highly directional structure at large angles in the Mie scatter distribution. Zaccanti et al. report using phase functions evaluated with Mie theory in semianalytic Monte Carlo simulation of photon transport through tissue.¹⁰ In this article we report experimental measurements of elastic scatter from suspensions of polystyrene microspheres in aqueous solutions of hemoglobin, and initial efforts to model the measured elastic-scatter spectra. We used Mie theory to determine the angular and amplitude scattering properties of the spheres, and Monte Carlo computation to describe the resultant photon transport.

2. SCATTER MEASUREMENTS

We prepared 20-ml aqueous suspensions of latex microspheres (Duke Scientific Corporation) according to Table 1, below, such that $\mu_s = 100 \text{ cm}^{-1}$ which is comparable to tissue values ($\sim 100\text{-}200 \text{ cm}^{-1}$) in the visible and near infrared. The particles have a density of 1.05 g-cm^{-3} and a refractive index of 1.59 at 589 nm.

Sphere dia. (cm)	Uniformity	Conc. (%/wt)
0.96×10^{-4}	1.3 %	0.25
3.7×10^{-4}	4.5 %	1.0

Table 1. These are the starting stocks to which the hemoglobin was added.

Hemoglobin was added to these aqueous suspensions after the scatter spectra are measured. The final hemoglobin concentration was $25 \text{ } \mu\text{M}$ ($\mu_a \approx 2.5 \text{ cm}^{-1}$ @ 420 nm and $\approx 0.3 \text{ cm}^{-1}$ @ 580 nm). The oxygenation was not controlled, so we directly measured the extinction coefficient of a hemoglobin solution prior to mixing with the sphere suspensions.

The elastic-scatter spectrometer for these experiments consists of a broad-band source (tungsten filament lamp), an optical fiber probe, and a dual CCD-array spectrometer for analyzing collected light. Light was delivered by a $400\text{-}\mu$ -diameter fiber collected by two $200\text{-}\mu$ -diameter optical fibers located at 500 or $1250 \pm 100 \text{ } \mu$ with respect to the source fiber (center to center). Photons entering the specimen interact by multiple scattering off the suspended spheres and absorption by the hemoglobin. A small fraction of the photons enter the spectrometer fibers after multiple-scattering and absorption events, and are analyzed in the spectrometer. The spectral response of the spectrometer, lamp, and fibers are eliminated from the processed spectra by dividing by the signal obtained from a (95-100 %) diffuse reflector as in the following equation:

$$I(\lambda) = \frac{I_{\text{sig.}} - I_{\text{offset sig.}}}{I_{\text{ref.}} - I_{\text{offset ref.}}}$$

$I_{\text{sig.}}$ is the spectrometer signal of interest, $I_{\text{offset sig.}}$ is the spectrometer offset when measuring the signal, $I_{\text{offset ref.}}$ is the offset when measuring the reference, and $I_{\text{ref.}}$ is the signal from the diffuse reflector. The offsets arise from the CCD dark current and dc offsets from readout electronics. Figure 2 shows the spectra from the $3.7 \text{ } \mu$ suspensions. The spectrometer signal amplitude is arbitrary, so the two pairs of signals are normalized to agree at 650 nm to avoid confusion.

Figure 2. These spectra will be compared with Monte Carlo + Mie theory.

3. COMPUTATIONAL METHODS

The details of light scattering in tissue, described by Mie theory, may be important when describing transport in the vicinity of the light source. The model system for comparing the computational model with experiment was chosen to have values for μ_s , μ_a , and g comparable to those seen in tissue. For the Mie calculations, we used the computational algorithms published by Bohren and Huffman¹¹ and the Monte-Carlo computations draw heavily from the code distributed by Wang and Jacques¹².

3.1 Mie Scatter

The Mie scalar results are expressed as several infinite series of Bessel functions, and their evaluation is based on computationally-demanding numerical methods such as those described by Bohren et al.¹¹ The angular scatter is described by similar expansions including the spherical harmonics and can also be found in Reference 10. Figure 3 shows a sample Mie theory scatter calculation of Q_s , Q_b , and g for polystyrene microspheres with refractive index 1.59 in water. The results are displayed relative to a dimensionless ratio of sphere radius to wavelength, and a single calculation provides results for any chosen range of radius and wavelength. The quantity Q_b is the scattering efficiency in the backwards direction (180°) and Q_s is the integrated scatter coefficient.

Figure 3. This set of results represents the scattering efficiencies for polystyrene microspheres.

The extinction coefficient (Q_x) is identical to Q_s in the absence of absorption. With the assumption that the single-particle interactions are not history dependent, Q_s , Q_x , and g can be related to the transport coefficients μ_a , μ_s , and μ_s' by the following relations, where A_s and N_s are the sphere cross-sectional area and number density respectively.

$$\mu_s = N_s A_s Q_s, \quad \mu_a = N_s A_s (Q_x - Q_s), \quad \text{and} \quad \mu_s' = N_s A_s Q_s (1-g)$$

These quantities provide calculated transport coefficients for the Monte-Carlo computation, but we still need to compute the angular scattering distribution ($P(\theta)$).

Angular scatter distributions (phase functions) used for modeling light transport in tissue have typically used a one-parameter phase function expression proposed by Henyey-Greenstein³ for convenience. Figure 4 compares the Henyey-Greenstein phase function with Mie theory at smaller angles for $\lambda = 0.3$ and 0.5μ . The g 's calculated from Mie theory at the appropriate wavelength are the parameter values required for the Henyey-Greenstein phase function. Liu's phase function would match the Mie results well at angles smaller than the first minimum for proper choice of the two free parameters, but cannot account for local minima seen in the Mie theory scatter distributions for some values of r/λ (see figure 5.).

Figure 4. Mie and Henyey-Greenstein phase functions are quite different at the angles with strongest scatter probabilities even though $g = \langle \cos\theta \rangle$ is identical for both.

Figure 5. The Mie and Henyey-Greenstein phase functions are radically different at high angles. This behavior is significant when collection geometry heavily weighs backscattered light. The Liu phase functions is not significantly different from the Henyey-Greenstein phase function at large angles.

Scatter distributions calculated from Mie theory are used for the phase function needed in the Monte Carlo simulation of light transport in the microsphere suspensions.

3.2 Monte Carlo Transport

Our Monte Carlo computational method for light transport in tissue is a modification of the Monte Carlo computer code distributed by Lihong Wang and Steven Jacques¹¹. These authors also provide an in-depth discussion of the requirements for Monte Carlo simulations of light transport in tissue. Three distinct functions are required. One is a function that maps the step size between interactions onto a uniform distribution of random numbers. This produces a random set of travel steps. Another function maps the angular scatter probability onto a second uniform distribution of random numbers. This is where we incorporate the phase function from the Mie theory calculations. The final function maps the rotation around the initial trajectory. It simply scales the uniform random distribution onto 2π axially because of the cylindrical symmetry of each scattering event. The overall computational strategy is to choose a particle size and density, a hemoglobin concentration, and a wavelength range and increment corresponding to the experiment we wish to simulate. Then for each wavelength, we compute μ_s and the angular scatter distribution from Mie theory, look up μ_a from a table based on our measurements of the hemoglobin absorption, and run a Monte Carlo simulation with these parameters as input.

The step size (s_i) for any iteration in the propagation is given by $s_i = -\ln(\xi)/(\mu_s + \mu_a)$. The angle of scatter is, however, much less straightforward. Wang et al.¹² use the Henyey-

Greenstein phase function which has a simple algebraic representation. The Mie theory angular distributions come from numerical computations of series expansions and do not lead to an algebraic mapping. We can, however compute a table of cumulative probabilities. The cumulative probabilities then provide a one-to-one mapping between a uniform distribution of random numbers and the scatter angles, which preserves the scatter distribution computed from Mie theory. Specifically, we have a table for which the values are the angles and the cumulative probabilities for scattering into those angles. A random value between 0 and 1 inclusive is chosen from the set of uniformly distributed values. This random value is equal to its cumulative probability distribution and is the ordinate for interpolation of the scatter angle. The major shortcoming of this method is that for parameters appropriate to visible and near infrared light in tissue, the cumulative distribution is unevenly distributed and can lead to interpolation errors.

4.0 MODEL EVALUATION

The scatter spectra have an arbitrary amplitude scale factor, so we are looking at only the shape of the spectra, and we have scaled the results for convenient display. Figure 6 shows calculations for the 3.7μ dia. spheres. The simulation results corresponding to the fiber at separations of 500 and 1250 μ are labeled near and far respectively. The Monte Carlo results for the near fiber are seen to be less influenced by the hemoglobin absorption in the range 450 to 650 μ . Below 450 μ , however, the hemoglobin absorption so dominates the process, that there is little difference between the near and far detector locations other than signal-to-noise ratio. Figure 6 also shows the mean number of interactions ($\langle n \rangle$) experienced by the particles before entering the near detector and μ_s , computed from single-particle Mie theory. The periods and phase of the ripple in the simulations match that in μ_s and show that the ripple propagates through the simulations.

Figure 6. This figure shows a typical Monte Carlo run. The Mean Interactions trace is associated with the near Monte Carlo signal.

The higher-frequency oscillations on all curves in fig. 6 are the ripple structure from single-particle Mie theory. Reference to fig. 3 shows that the local minima in the ripple on μ_s correspond to the peaks in the backscatter (scatter at 180°). The curve labeled $\langle n \rangle$, is obtained by counting the number of interactions a photon experienced prior to entering the near fiber. It varies between 10 and 30 events even though the mean for all photons entering the suspension is several hundred interactions before being absorbed or leaving the tissue.

Monte Carlo simulations were run for the 0.96 and 3.7 μ dia. suspensions described in section 2.0. Figure 7 compares the simulations with experiment for the 3.7- μ sphere suspension.

Figure 7. The amplitudes and baselines for both detectors are adjusted for clarity of display. The oscillations observed in the simulations are present in the signals at much smaller amplitude.

Monte Carlo simulations using a phase function generated from single-particle Mie theory describe some features well, but show some discrepancies. The much larger amplitude of the calculated ripple is the most obvious. This is most likely the result of using a uniform particle size for simulations as opposed to the nearly 5 percent spread in actual experimental particle size. Since the period of the ripple depends on the particle size, a mixture of particle sizes will reduce the ripple. The other notable discrepancy is an overestimate of the calculated signal strength at wavelengths greater than 0.6 μ , which is notably greater for the more distant fiber. Since the hemoglobin absorption is lowest at wavelengths greater than 0.6 μ , it is possible that the model is overestimating the role of hemoglobin at shorter wavelengths.

The results for the $0.96\ \mu$ spheres are shown in Figure 8. The measured spectrum for the far fiber did not agree with simulations. At this time the observed spectra are not understood and we intend to repeat the experiment.

Figure 8. The simulations for the $0.96\ \mu$ dia. spheres show the same over estimate of the signal at wavelengths greater than $0.6\ \mu$ seen for the $3.7\ \mu$ sphere Monte Carlo results.

At the near fiber, the Monte Carlo results for the $0.96\text{-}\mu$ dia. sphere suspension are consistent with observation, with the exception of the over-estimate at wavelengths greater than $0.6\ \mu$. The ripple structure seen in the spectra for the $3.7\text{-}\mu$ dia. sphere suspension is not observed for the $0.96\text{-}\mu$ dia. sphere suspension. For the smaller particle size it is of lower amplitude and longer period and is consequently not as noticeable. More important, though, is that both the simulation and experiment for the $0.96\text{-}\mu$ -dia. spheres show a dip in the spectrum near the hemoglobin α and β lines. This dip is either not observed or reduced in both simulation and experiment for the $3.7\text{-}\mu$ -dia. spheres despite the fact that the hemoglobin concentration was the same for both solutions.

5.0 CONCLUSIONS

The use of a phase function calculated from Mie theory instead of an analytical expression such as the Henyey-Greenstein phase function in Monte Carlo simulations has several advantages including: 1. The ability to model photon transport over a broad range of wavelengths without any free parameters. 2. The ability to follow particle trajectories in greater detail than the use of a phase function characterized only by having the same average $\cos(\theta)$ of the scattering angle, but an erroneous distribution of scatter angles. The reduction in the dip observed in the spectra at the hemoglobin α and β absorption bands in both experiment and simulation for the $3.7\text{-}\mu$ -dia. sphere suspension as compared to those for the $0.96\text{-}\mu$ dia. sphere suspension with the same μ_a illustrates the importance of the angular scatter distribution in determining photon transport through tissues in geometries such as that employed by the OBS.

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