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V. Sankaran, J.T. Walsh, Jr., D.J. Maitland

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Polarized light propagation in biologic tissue and tissue phantoms

Vanitha Sankaran* [a,b], Joseph T. Walsh, Jr.[a], Duncan J. Maitland[b]

[a]Northwestern University, Evanston, IL 60208
[b]Lawrence Livermore National Laboratory, Livermore, CA 94551

ABSTRACT

Imaging through biologic tissue relies on the discrimination of weakly scattered from multiply scattered photons. The degree of polarization can be used as the discrimination criterion by which to reject multiply scattered photons. Polarized light propagation through biologic tissue is typically studied using tissue phantoms consisting of dilute aqueous suspensions of microspheres. We show that, although such phantoms are designed to match the macroscopic scattering properties of tissue (i.e., the scattering coefficient, μ_s, and scattering anisotropy, g), they do not accurately represent biologic tissue for polarization-sensitive studies. In common tissue phantoms, such as dilute Intralipid and dilute 1-μm-diameter polystyrene microsphere suspensions, we find that linearly polarized light is depolarized more quickly than circularly polarized light. In dense tissue, however, where scatterers are often located in close proximity to one another, circularly polarized light is depolarized similar to or more quickly than linearly polarized light. We also demonstrate that polarized light propagates differently in dilute versus densely packed microsphere suspensions, which may account for the differences seen between polarized light propagation in common dilute tissue phantoms versus dense biologic tissue.

KEYWORDS

Degree of polarization, Stokes polarimetry, multiple scattering, dependent scattering

1. INTRODUCTION

Optical imaging through highly scattering media is a problem that has been studied extensively in a number of fields, including atmospheric (1) and ocean optics (2), and more recently, biomedical optics (3-6). As photons travel through a turbid material, they can be unscattered or weakly scattered and provide information about the sample structure, or they can be highly scattered and contribute to the signal noise. Optical imaging can be enhanced through the discrimination and rejection of multiply scattered from weakly scattered photons. There are many such discrimination methods, including time- and frequency-domain techniques (3,4), trans-illumination laser computed tomography (5), and confocal detection (6). Polarimetry, in which the polarization state of light incident on a sample is compared with the polarization state of light exiting the sample, has recently been under much study as another method of discrimination (7-10). Polarization-based discrimination is based on the premise that ballistic photons retain their initial polarization state whereas diffuse photons do not; thus, multiply scattered photons can be gated out based on their low degree of polarization.

Various studies have been conducted on how polarized light propagates in different tissue phantoms, often using aqueous suspensions of polystyrene microspheres (7-10). Based on the results from these investigations, it has been predicted that circularly polarized light may survive through more scattering events than linearly polarized light in an anisotropically scattering medium such as tissue (7,8). However, we have previously shown that this is not necessarily the case (9). Typically, phantoms are chosen because their absorption and scattering properties match those of tissue; this does not necessarily imply that the polarization properties of the phantom match those of tissue. We have shown that simply matching the gross scattering coefficient of a phantom to a given tissue does not imply that polarized light will propagate similarly in the two samples (9). In this study, we present further evidence that light depolarization by a sample is heavily dependent on the microstructure of the sample, rather than on its macrostructure alone. We begin with a comparison of how linearly and

* Correspondence: Email: sankaran1@llnl.gov; Telephone: (925)424-3101; FAX: (925)424-2778

circularly polarized light propagate in two different tissues, blood, which contains dilute spherical and quasi-spherical scatterers and adipose tissue, which contains dense quasi-spherical scatterers. We then explain these results using tissue phantoms consisting of polystyrene microspheres suspended in water. The effects of scatterer size and density on polarimetric measurements are presented.

2. THEORY

2.1 DEGREE OF POLARIZATION

Degree of polarization is a term used to quantify the fraction of light that has retained its initial polarization after traveling through a turbid material. A degree of polarization of 1 corresponds to completely polarized light whereas a degree of polarization of 0 corresponds to completely unpolarized light. In this study, the propagation of linearly and circularly polarized light through a sample is described using Stokes vector measurements. The Stokes vector, **S**, of light exiting a sample is a 4x1 vector that describes completely the polarization state of the collected light:

$$\bar{S} = \begin{bmatrix} I \\ Q \\ U \\ V \end{bmatrix},$$

where I is the total intensity, Q is the difference between horizontal and vertical linearly polarized components, U is the difference between +45° and -45° linearly polarized components, and V is the difference between right and left circularly polarized components.

Values for the degree of polarization are calculated from each Stokes vector as:

$$p_L(Q,U) = \text{Degree of linear polarization} = \frac{\sqrt{Q^2 + U^2}}{I}, \tag{1a}$$

$$p_C(V) = \text{Degree of circular polarization} = \frac{\sqrt{V^2}}{I}, \text{ and} \tag{1b}$$

$$p(Q,U,V) = \text{Total degree of polarization} = \frac{\sqrt{Q^2 + U^2 + V^2}}{I}. \tag{1c}$$

2.2 RAYLEIGH AND MIE SCATTERERS

In order to compare studies conducted on different samples with different incident wavelengths, it is often useful to speak in terms of Rayleigh and Mie scatterers. Mie scattering applies to particles that are large compared to the incident wavelength whereas the Rayleigh approximation to Mie theory applies to particles that are small compared to the incident wavelength. The relative size parameter, usually notated as α or k_a, can be used to relate the diameter of a scatterer, d, to the incident wavelength, λ (10):

$$\alpha = \frac{\pi d}{\lambda}, \tag{2}$$

Since the scattering of electromagnetic radiation scales linearly to the relative size parameter, α can be used to compare studies with different scatterer sizes and incident wavelengths. For small scatterers ($\alpha \leq \pi/10$), the amplitude of the scattered wavelets is small; thus the infinite series solutions giving the scattered amplitude and phase can be approximated by a sum of only the first few terms in the series. Scattering by these small particles is usually called Rayleigh scattering and is a subset of Mie theory, which is used to describe larger scatterers ($\alpha > \pi/10$).

3. METHODS

3.1 EXPERIMENTAL SETUP

A He–Ne laser (Melles Griot Inc., 05LHP925) emitting a collimated 1.5-mm $1/e^2$ diameter beam of 632.8-nm-radiation was passed through a chopper operating at 2.78 kHz (Stanford Research Systems, SR540), a linear polarizer (LP; Melles Griot Inc., 03FPG009), and into the sample. For circularly polarized light incident on the sample, a quarter-wave plate (QWP; Meadowlark Optics, NQM-100-633) was inserted between the polarizer and the sample. Light emerging from the sample was collected with a 55-mm-focal length, F/2.8 camera lens and passed through subsequent polarizing optics. An iris in the camera lens rejected light scattered beyond a full angle of 22.6°; this ensured that the collected light that was passed through the subsequent polarization optics fell within the acceptance angle of each element. The collected light was first passed through a variable-wave plate (VWP; Meadowlark Optics, LRC-100) oriented at 45° to the horizontal, whose retardance, Δ, was controlled by a voltage input. The light exiting the VWP was passed through a photoelastic modulator (PEM; Hinds Instruments, PEM-90) oriented at 0° followed by a linear polarizer (LP; Melles Griot Inc., 03FPG009) oriented at 45°. The PEM is a wave plate whose retardance, $\delta = \delta_o\cos(\omega t)$, modulated between $\pm\delta_o$ at $\omega = 2\pi(50\text{ kHz})$, thus providing the reference signal for lock-in detection. The collection optics, VWP, PEM and LP constituted the analysis section of the system. All signal components were measured with a ±15 V photoconductive detector (Hinds Instruments, DET-90-002) and a lock-in amplifier (Stanford Research Systems, SR810). To simplify the signal analysis, δ_o was set to 138°; thus, $J_0(\delta_o) = 0$. To isolate U_{sample} and V_{sample}, Δ was set to 0°; U_{sample} was then measured at 2ω and V_{sample} was measured at 1ω. Similarly, when Δ was set to 90°, U_{sample} was measured at 2ω and Q_{sample} was measured at 1ω. I_{sample} was measured at the chopper frequency. The measurement technique used here is similar to that described by Schmitt et al. (7).

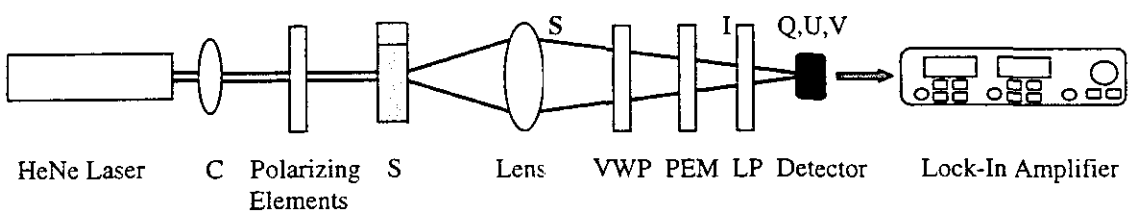

Figure 1. The experimental setup consists of a He–Ne laser, a chopper (C) operating at 2.78 kHz, polarizing elements for the light incident on the sample (S), a variable-wave plate (VWP), a photoelastic modulator (PEM) operating at 50 kHz, a linear polarizer (LP), a photodetector and a lock-in amplifier. The maximum degree of polarization with no sample present was 1.0001.

3.2 SAMPLES

Depolarization by biologic scatterers was studied using porcine blood and porcine adipose tissue. Each tissue was taken from freshly sacrificed 12-month-old slaughterhouse pigs and stored at 5 °C prior to experimentation. The samples were warmed to room temperature 2 h before each experiment and were used within 48 h post mortem. Whole blood was heparinized within 1 h post mortem. Samples of whole blood were placed in a glass cuvette with a pathlength ranging from 0.1 to 5 mm for each experiment. Fat was taken from the abdominal cavity. Sections of fat with an average thickness ranging from 0.15 to 2.9 mm were mounted between glass slides for each experiment.

Depolarization in the tissue phantoms was studied using monodisperse suspensions of 0.102 μm, 0.48 μm, and 0.99 μm-diameter polystyrene microspheres in water (Polysciences Inc., $n_{sphere} = 1.59$, $n_{medium} = 1.33$). Two different experiments were performed with these suspensions. First, for a given size of spheres, a fixed concentration of scatterers was chosen and prepared. Each sample was diluted with distilled water to a concentration ranging from 0.25 to 2.5%, where independent, uncorrelated scattering could be assumed. Samples were placed in cuvettes with pathlengths ranging from 0.1 to 2 cm; the degree of polarization for each sample was measured as a function of cuvette pathlength. Second, for a given size of spheres, a fixed cuvette pathlength was used to make degree of polarization measurements as a function of increasing scatterer concentration. The range of concentrations investigated spanned both dilute, independent scattering and dense, dependent scattering. Concentrations of spheres greater than 2.5% (the initial concentration obtained from the manufacturer) were obtained by centrifuging the sample so that the particles would settle and then decanting the excess water. Spheres with a diameter smaller than 0.48 μm have a prohibitively long settling time and thus could not be studied here. The dense suspensions were sonicated to prevent clumping of the spheres and stirred before each measurement. Static repulsion between the anionically charged spheres should ensure a minimal distance between each sphere and its nearest neighbors; however this could not be verified experimentally. For concentrations of spheres less than 2.5%, the suspension was diluted with distilled water. Samples were placed in a 1-cm-pathlength glass cuvette with flat parallel walls for experimentation.

4. RESULTS & DISCUSSION

4.1 BIOLOGIC TISSUE

Blood was first used to investigate polarized light propagation through the simplest case of dilute, near-spherical scatterers. Degree of polarization results as a function of cuvette pathlength are shown in Figure 2. Blood is a fluid tissue that consists of various cells; one cubic milliliter of blood contains roughly 5,000,000 biconcave disk-like erythrocytes (7 μm in diameter), approximately 7,000 spherical leukocytes (8-18 μm in diameter), and between 150,000 and 300,000 biconvex disk-like platelets (2-4 μm in diameter), floating freely and separated from one another by plasma (11,12). The scatterers in blood predominately fall into the Mie scattering regime for the incident 633-nm radiation used here. For each sample of whole blood investigated, linearly polarized light is depolarized more than circularly polarized light.

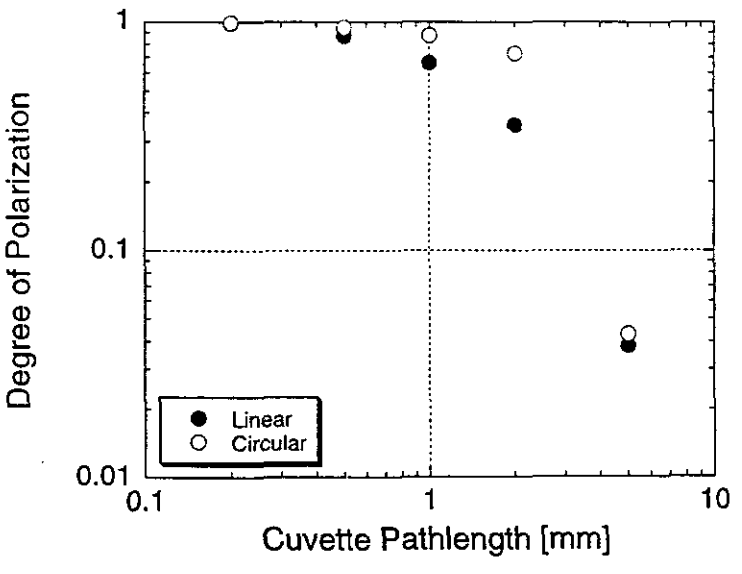

Figure 2. The degree of linear and circular polarization in whole porcine blood as a function of cuvette pathlength.

Adipose tissue was used next to study polarized light scattering by densely packed near-spherical scatterers. Degree of polarization results as a function of tissue thickness are shown in Figure 3. White adipose tissue consists of closely packed unilocular fat cells, each containing a single lipid droplet. The lipid droplet nearly fills the entire cell, causing the cytoplasm and nucleus to become eccentrically placed. Fat cells range in size, with a mature fat cell having the largest diameter of 50-75 μm. The dominant scatterers in fat cells are likely to be much smaller, perhaps the individual lipid droplet (0.5 to 1.5 μm in diameter) or subcellular organelles (11,12). Although the scatterers in adipose tissue span the Rayleigh and Mie regimes, the structures are predominantly Mie-sized. For each tissue sample investigated, circularly polarized light is depolarized more than linearly polarized light, in contrast to the results seen in blood.

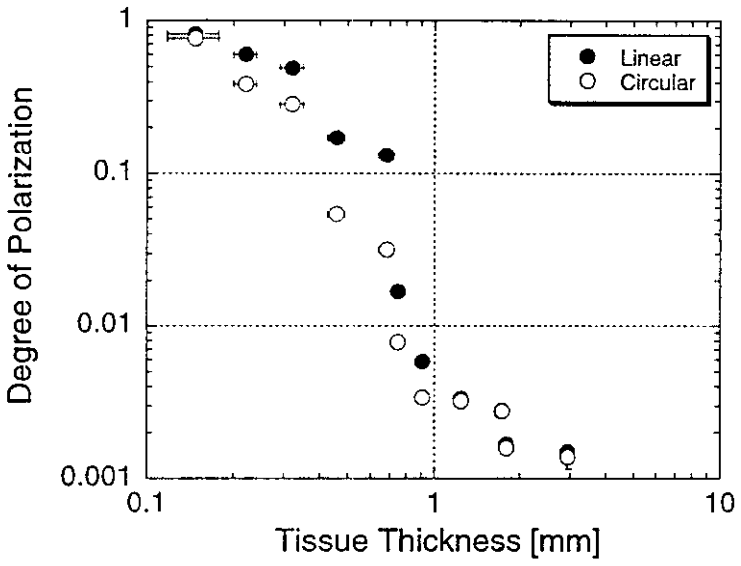

Figure 3. The degree of linear and circular polarization in sections of porcine adipose tissue as a function of tissue thickness.

4.2 TISSUE PHANTOMS: SIZE EFFECT

In trying to describe the differential depolarization behavior seen in the two tissues, phantoms consisting of polystyrene microspheres suspended in water were used. The concentrations of spheres investigated here were dilute enough to assume that the spheres scattered and depolarized light independent of one another. The 0.102-μm-diameter spheres fall into the Rayleigh-Mie transition regime whereas the 0.48 and 0.99-μm-diameter spheres are both Mie-sized. Spheres small enough to fall within the Rayleigh scattering regime were not commercially available. For the 0.102-μm-diameter spheres, linearly polarized light is equally or slightly less depolarized than circularly polarized light. For the larger spheres, the converse is true; circularly polarized light is less depolarized than linearly polarized light. These results are corroborated by other studies in the literature (13,14).

The differential scattering and depolarization of linear versus circular polarization states is an important factor to consider in describing our degree of polarization results. Three different cases can be identified: $p_L > p_C$ (typical of scattering by dilute Rayleigh spheres), $p_L < p_C$ (typical of scattering by dilute Mie spheres), and $p_L \sim p_C$ (typical of scattering in the Rayleigh-Mie transition regime). Blood consists of dilute, large scatterers with size parameters that place them into the Mie regime. The results seen in blood demonstrate a preferential depolarization of linear over circular polarization states, also seen in the results for the dilute, Mie-sized tissue phantoms. Thus, the dilute Mie-sized scatterers found in blood can be well modeled by dilute, Mie-sized polystyrene spheres. Adipose tissue consists of densely packed structures with a distribution of sizes that span the Rayleigh and Mie scattering regimes but are mostly Mie-sized. However, the results seen in the fat tissue exhibit a preferential depolarization of circular over linear polarization states, which is typical of scattering by dilute Rayleigh spheres. The reasons why Rayleigh-like depolarization is seen for scattering by dense Mie-sized scatterers in this tissue are likely a

combined effect of scatterer size and density. Thus, the effect of scatterer density for dilute and dense concentrations of spheres was studied next.

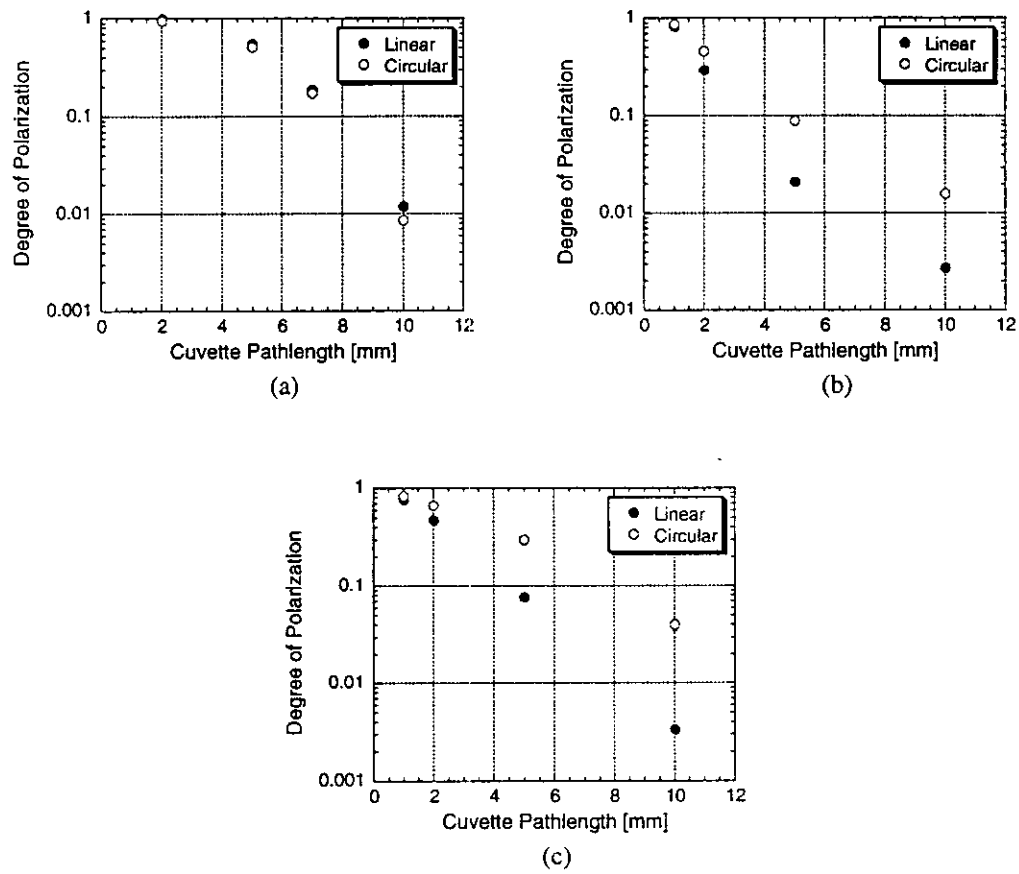

Figure 4. The degree of polarization was measured as a function of cuvette pathlength for aqueous polystyrene microsphere suspensions with a diameter and concentration of (a) 0.102 μm, 1.0%; (b) 0.48 μm, 0.25%; and (c) 0.99 μm, 0.25%.

4.3 TISSUE PHANTOMS: DENSITY EFFECT

The effects of scatterer density was studied using aqueous suspensions of polystyrene microspheres with diameters of 0.48 and 0.99 μm, both of which are Mie-sized spheres. A wide range of sphere concentrations was studied so that depolarization by independently and dependently scattering spheres could be studied. For dilute concentrations of each suspension, as the scatterer concentration increases the degree of linear and circular polarization both decrease. Linearly polarized light is depolarized more quickly than circularly polarized light, a result corroborated both by the results shown in Figure 4 and by reports in the literature (13,14). Above a critical concentration of scatterers, the degree of linear and circular polarization begin to increase with increasing scatterer concentration. The concentration at which there is an inflection in the degree of polarization follows trends seen in previous studies that report changes in unpolarized light attenuation through densely packed microsphere suspensions (15,16). For these dense suspensions, circularly polarized light is depolarized similar to or more quickly than linearly polarized light, comparable to results seen in both the dilute suspensions of smaller (0.102-μm-diamter) spheres and the adipose tissue.

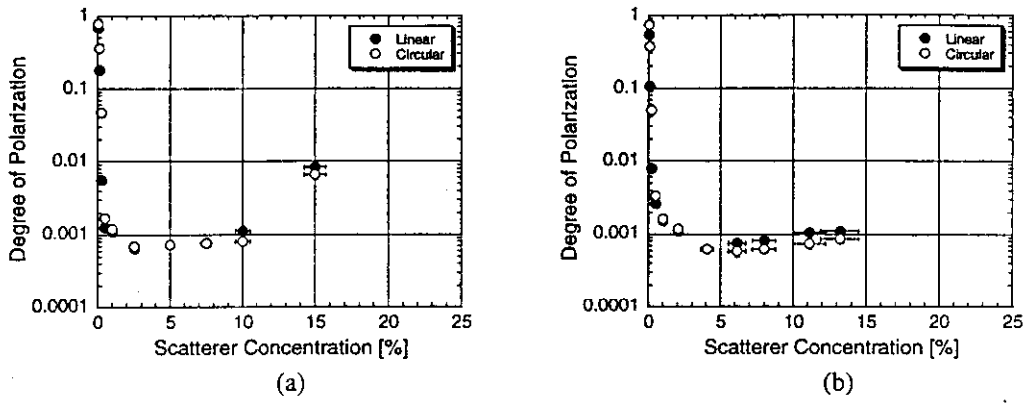

Figure 5. The degree of polarization was measured as a function of scatterer concentration in suspensions with sphere diameters of (a) 0.48 μm and (b) 0.99 μm.

4. CONCLUSIONS

Polarized light propagation has typically been studied in tissue phantoms consisting of microspheres suspended in water. In common phantoms, such as dilute aqueous suspensions of 1-μm-diameter polystyrene spheres and Intralipid, it has been seen that circularly polarized light survives better than linearly polarized light (9,14). However, we found in this study that similar behavior is not always seen in biologic tissue. In porcine blood, which consists of dilute, Mie scatterers, results similar to that seen in common phantoms were found; namely, circular polarization is maintained better than linear polarization. In porcine adipose tissue, however, which consists of densely packed Rayleigh and Mie scatterers, the reverse was true. Linearly polarized light was maintained better than circularly polarized light. The reasons behind why different results were found in the two tissues may be attributed to the differences in the microstructure of the scatterers found in each case.

The effect of scatterer size on polarimetric measurements was first studied using aqueous suspensions of microspheres. Dilute concentrations were used so that independent scattering could be assumed. For small spheres that fell into the Rayleigh-Mie transition region, it was seen that linearly polarized light was maintained better than circularly polarized light. As the sphere size increased (i.e. Mie scattering), the reverse was true; circularly polarized light was maintained better than linearly polarized light. Thus, depending on the tissue to be modeled, a sphere size should be chosen for the phantom to mimic the differential depolarization of linear versus circular polarization states of the tissue under study. However, even though the resulting phantom may mimic the depolarization behavior of the tissue under limited conditions, it is unlikely that the structure of the phantom bears any semblance to the structure of the tissue. For example, even though blood and fat exhibit differential depolarization behavior that is characteristic of Mie and Rayleigh scattering respectively, the physical structures in both tissues are predominantly Mie-sized. Thus, some factor other than sphere size must also be considered if the phantom material is to approach the microstructure of the tissue scatterers. One such factor is scatterer concentration, in a range outside of the independent scattering assumption made here.

Many tissues contain structures that are densely packed and thus cannot be well represented by dilute suspensions of spheres. Thus the next effect that was studied was scatterer concentration for both dilute and dense concentrations of spheres. We found that for dense suspensions of microspheres, polarized light propagation through a dense suspension of Mie spheres is similar to polarized light propagation through a dilute suspension of smaller Rayleigh spheres. Specifically, we observed that for dense concentrations of the 0.48 and 0.99-μm-diameter particles, each of which fall into the Mie scattering regime for dilute concentrations, circularly polarized light was depolarized similar to or more quickly than linearly polarized light. This corresponds to the behavior seen for scattering by dilute suspensions of particles in the Rayleigh-Mie and Rayleigh regions. Therefore adipose tissue, which could be modeled by a suspension of dilute Rayleigh spheres, could perhaps be modeled better using a suspension of dense Mie spheres, which mimics the actual microstructure of the tissue more accurately. In

understanding how polarized light propagates in biologic tissue, and in designing a phantom to mimic a particular tissue, it thus seems likely that both scatterer size and density (in a range outside of independent scattering) should be considered.

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