Depolarization of light in tissue phantoms – effect of a distribution in the size of scatterers

N. Ghosh, H. S. Patel, P. K. Gupta

Biomedical Applications Section,
Center for Advanced Technology, Indore, India –452013.
pkgupta@cat.ernet.in

Abstract: We show that the depolarization behavior of light on propagation through a sample having a mixture of suspension of monodisperse polystyrene microspheres of two different sizes (mean diameter 0.11μm and 1.08 μm) is dominated by the smaller of the two scatterers. In contrast the estimates for the anisotropy parameter (g) for this sample, obtained from goniophotometric measurement, are observed to be closer to the value corresponding to the larger of the two scatterers. These results imply that the depolarization behavior of light in biological tissue (having a distribution of scatterer size) would be different from that of a matched monodisperse scattering sample having the same value of anisotropy parameter (g) and optical thickness ($\tau = \mu_s \times d$, μ_s is scattering coefficient and d being the physical thickness).

OCIS Codes: (170.3660) Light propagation in tissues; (290. 4210) Multiple scattering; (170. 3880) Medical and biological imaging; (170.0170) Medical optics and biotechnology.

References

1. A.D. Kim, M. Moscoso, "Influence of the refractive index on the depolarization of multiply scattered waves," Phys. Rev. E **64,** 026612, 1 –4 (2001).
2. E.E. Gorodnichev, A.I. Kuzolov, D.B. Rozozkin, "Diffusion of circularly polarized light in a disordered medium with large scale inhomogeneities," JETP Lett. **68**, 22 – 28 (1998).
3. D. Bicout, C. Brosseau, A. S. Martinez, and J. M. Schmitt, "Depolarization of multiply scattered wave by spherical diffusers: Influence of the size parameter," Phys. Rev. E **49**, 1767- 1770 (1994).
4. J. M. Schmitt, A. H. Gandjbakhche, and R. F. Bonner, "Use of polarized light to discriminate short path photons in a multiply scattering medium," Appl. Opt. **31**, 6535-6546 (1992).
5. S.L. Jacques, R.J. Roman and K. Lee, "Imaging skin pathology with polarized light," J. Biomed. Opt. **7**, 329 –340 (2002).
6. V. Sankaran, Klaus Schonenberger, Joseph T. Walsh Jr., Duncan J. Maitland, "Polarization discrimination of coherently propagating light in turbid media," Appl. Opt. **38**, 4252- 4261 (1999)
7. V. Sankaran, Matthew J. Everett, Joseph T. Walsh Jr. , Duncan J. Maitland, "Comparison of polarized light propagation in biological tissue and phantoms," Opt. Lett. **24**, 1044-1046 (1999).
8. V. Sankaran, Joseph T. Walsh Jr., Duncan J. Maitland, "Polarized light propagation through tissue phantoms containing densely packed scatterers," Opt. Lett. **25**, 239-241 (2000).
9. V. Sankaran, J. T. Walsh, Jr., and D. J. Maitland, "Comparative study of polarized light propagation in biological tissues," J. Biomed. Opt. **7**, 300 – 306 (2002).
10. W.F. Cheong, S.A. Prahl, A.J. Welch, "A review of the optical properties of biological tissue," IEEE J. Q.E. **26,** 2166 –2185 (1990).
11. N. Ghosh, P.K. Gupta, H.S. Patel, B. Jain and B.N. Singh, "Depolarization of light in tissue phantoms – effect of collection geometry," Opt. Commun. **222**, 93 –100 (2003).
12. C. F. Bohren, and D. R. Huffman, *Absorption and Scattering of Light by Small Particles*, (Wiley, New York, 1983).
13. N. Ghosh, S. K. Mohanty, S. K. Majumder, and P. K. Gupta, "Measurement of optical transport properties of malignant and normal human breast tissues," Appl. Opt. **40**, 176 –184 (2001).
14. E. Collett, "Measurement of the four Stokes polarization parameters with a single circular polarizer," Opt. Commun. **52**, 77- 80 (1984).
15. A. Ishimaru, *Wave propagation and sacattering in random media*, Vol. 1 (Academic NY 1978) Ch. 9, pp 175 –186.

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16. R. Drezek, A. Dunn, R.R. Kortum, "Light scattering from cells: finite difference time domain simulations and goniometric measurements," Appl. Opt. **39**, 3651 – 3661 (1999).
17. R. Marchesini, A. Bertoni, S. Andreola, E. Melloni, and A.E. Sichirollo, "Extinction and absorption coefficients and scattering phase functions of human tissues in-vitro," Appl. Opt. **28**, 2318 –2324 (1989).
18. A. Kienle, F.K. Forster, R. Hibst, "Influence of the phase function on determination of the optical properties of biological tissue by spatially resolved reflectance," Opt. Lett. **26**, 1571 – 1573 (2001).
19. Judith R. Mourant, James P. Frayer, Andreas H. Hielscher, Angela A. Eick, Dan Shen and Tamara M. Jhonson, "Mechanisms of light scattering from biological cells relevant to noninvasive optical-tissue diagnostics," Appl. Opt. **37**, 3586 - 3593 (1998).

1. Introduction

There exists considerable current interest in understanding the depolarization of light on propagation through biological tissue [1-4]. One major motivation for these studies has been the potential of polarization gating for optical imaging of turbid biological objects [5]. Recent studies by Sankaran *et al*. [6 -9] have revealed significant differences in polarized light propagation in biological tissues (porcine adipose, arterial and myocardial tissue) and comparable tissue phantoms (suspension of Intralipid ® - 20% and polystyrene microsphere of 1.072 μm diameter in water). It was observed that for comparable values for anisotropy parameter (g, defined as the average cosine of scattering angle [10]) and the optical thickness (τ) depolarization of both linear and circularly polarized light was considerably more in biological tissue than in tissue phantoms [6]. Another interesting observation was that while for biological tissue linearly polarized light survives through longer propagation distances than circularly polarized light, for tissue phantoms with similar values for anisotropy parameter (g) the opposite result was obtained [7,9]. The observed difference in depolarization between tissue and matched tissue phantoms (comparable τ and g) may arise due to a difference in a large number of parameters like density of scatterers or a distribution in size and shape of the scatterers. Since it is difficult to quantify these parameters in biological tissue, elucidation of the reasons responsible for the observed differences in polarized light propagation through biologic tissue and matched tissue phantoms (comparable g and τ) require careful experiments using well characterized tissue phantoms. Our recent study in this direction has shown significant difference in depolarization of light through two monodisperse polystyrene microspheres suspension both having the same anisotropy parameter (g) and optical thickness (τ) but a different value for scattering coefficient (μ_s, defined as inverse of the scattering mean free path [10]). Depolarization was observed to be significantly more for the sample with higher value of μ_s and lower physical thickness (to ensure constant value for the optical thickness). This has been shown to be due to the fact that when the same collection geometry is used to collect light transmitted from the two matched samples, as is usually the case in most experiments, more multiply scattered photons are collected from the sample with higher μ_s [11]. These results imply that an important reason for the observed higher depolarization in tissues compared with matched phantoms (comparable g and τ) is the fact that the μ_s for the tissue [porcine myocardial tissue ($\mu_s \sim 19$ mm^{-1}, g =0.94) and adipose tissue ($\mu_s \sim 7$ mm^{-1}, g =0.77)] were much higher than that for the corresponding phantoms [suspension of 1.072 μm diameter polystyrene microspheres ($\mu_s \sim 3$ mm^{-1}, g =0.92) and a suspension of intralipid - 20% in water ($\mu_s \sim 0.5$mm^{-1}, g =0.73)] used in the experiments [7].

In this communication we address the second observation of Sankaran *et al*. [7,9] that the relative rate of depolarization of linear and circularly polarized light is different in biological tissues and matched tissue phantoms. Since depolarization of linear and circular polarized light depends on the size of the scatterers [1-3], it is reasonable to expect that a distribution in scatterer size may influence the relative rate of depolarization of linearly and circularly polarized light. With this objective we have investigated the effect of a distribution of scatterer size on the observed depolarization of linearly and circularly polarized light. The

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results obtained show that the depolarization behavior of light on propagation through a sample having a mixture of suspensions of monodisperse polystyrene microspheres of two different sizes (mean diameter of 0.11μm and 1.08 μm) is strikingly similar to that of the monodisperse suspension of the smaller of the two scatterers. In contrast the estimates for the anisotropy parameter (g) for this sample obtained from goniophotometric measurement are observed to be closer to the value corresponding to the larger of the two scatterers. Significance of these results for the observed difference in relative rate of depolarization of linearly and circularly polarized light in tissues and matched tissue phantoms is discussed.

2. Experimental methods

A schematic of the experimental set-up used for steady state polarization measurements is shown in Fig. 1(a). The 632nm output from a He-Ne laser (Suresh Indu Lasers, India) was passed through a polarizer to make it linearly polarized. A quarter wave plate was inserted between the linear polarizer and the sample for generating circularly polarized light when required. An aperture was kept to limit the spot size of the incident laser beam at the sample site to 0.5 mm. The diffused light emerging from the sample was collected with an f/3 lens after passing through subsequent polarizing optics and was imaged onto a CCD detector (ST6, SBIG, USA) with active area of 9 mm × 7 mm. The focal length of the collection lens was 7.5 cm and it was kept at a distance 6 cm away from the distal surface of the sample. The samples were kept in a quartz cuvette with path length of 5mm. An aperture was kept to limit collection angle to ~ 20^o. Scattering samples used in this study were monodisperse aqueous suspensions of polystyrene microspheres (Bangs Lab., USA). The mean diameters of microspheres used were 0.11μm and 1.08μm. We varied the optical thickness (τ) of the samples by changing the μ_s of the samples through dilution. The values for μ_s of the samples were calculated using Mie theory [12,13]. The mixtures of scattering samples were prepared by mixing samples of 0.11μm and 1.08μm spheres suspension in water. For this purpose, first two different scattering samples were prepared using aqueous suspension of 0.11μm and 1.08μm spheres. The concentrations of the spheres were adjusted to have the same value of μ_s (or τ for a fixed path length of 5mm) for the two individual samples. The two scattering samples were then mixed in different volume ratios to prepare samples having mixture of these two different sized scatterers. The degree of polarization of light transmitted through a scattering sample was determined by measuring the four Stokes parameters (I, Q, U, V) [12]. Here, I is the total intensity, Q and U describe the linearly polarized component and V described the circularly polarized component of the collected light. The technique described by Collett [14] was used for the measurement of Stokes parameters. From the measured Stokes parameters, the degree of linear polarization (P_L) and circular polarization (P_C) was worked out as

$$P_L = (Q^2 + U^2)^{1/2} / I \text{ and } P_C = V/I$$

For measurement of spatial distribution of the degree of polarization at the detector, the degree of polarization at individual CCD pixels along the horizontal direction containing the center of the beam were measured using the same method.

A schematic of goniophotometric set-up used for the measurement of scattering phase function from the samples is shown in Fig. 1(b). The 632nm line of a He-Ne laser was used as the excitation source. An optical fiber (core radius 300μm) kept on a rotational stage was used to collect scattered light from different angular positions. The distance between the sample and the collection fiber was fixed at 30 mm, which gives a collection full angle of ~ 1.2^0 at the sample. The signal collected from different angular positions (8^o to 172^o at a step of 4^o) was recorded using a PMT. The laser power incident on tissue was monitored using a beam splitter and Photodiode combination. For goniophotometric measurements the scattering

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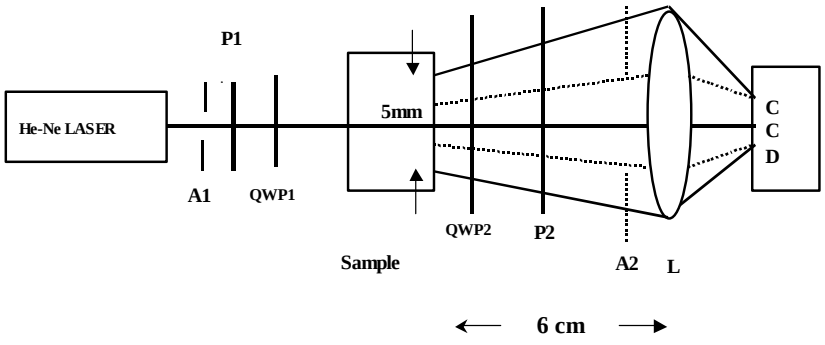
samples were diluted to minimize the multiple scattering effects ($\mu_s \times d << 1$, d being the diameter of the cuvette) and were kept in a cylindrical quartz cuvette.



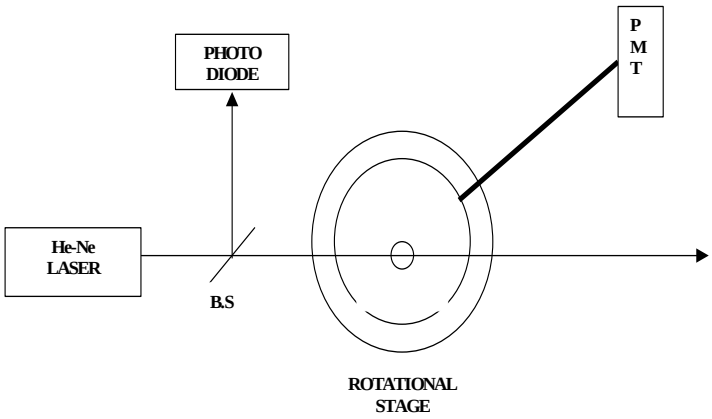


Fig. 1. (a) A schematic of the experimental set –up for steady state polarization measurements. A1 and A2 are apertures, P1 and P2 are linear polarizers, QWP1 and QWP2 are quarter wave plates and L is the lens. (b) A schematic of goniophotometric set-up for the measurement of anisotropy parameter (g).

3. Results and discussion

In Fig. 2(a), we show the measured spatial distribution of degree of linear and circular polarization at the detector for a sample (τ = 8.3) prepared using 0.11 μm diameter spheres. The corresponding results for the sample having the same value of τ but prepared using 1.08 μm diameter spheres are shown in Fig. 2(b). It can be seen from the figures that whereas the spatial profiles of degree of polarization for both linearly and circularly polarized light show distinct peak around the beam center for the sample having smaller g value (g = 0.09 for 0.11μm diameter microsphere suspension), for the sample having larger g value (g = 0.92 for 1.08μm diameter microsphere suspension), the profiles show no distinct peaks.

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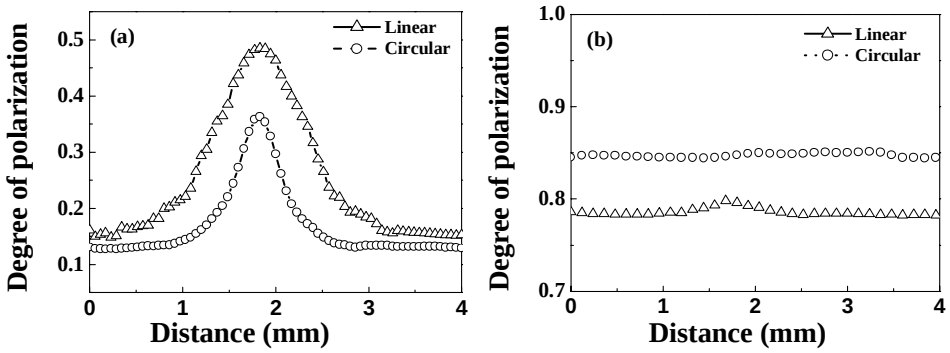

Fig. 2. Measured spatial distribution of the degree of linear polarization (triangles) and circular polarization (circles) at the detector for (a) 0.11 μm diameter polystyrene microspheres suspension in water (g = 0.09, τ = 8.3) and (b) for 1.08μm diameter polystyrene microspheres suspension in water (g =0.92, τ = 8.3).

These results are in agreement with previous reports [4] where it has been shown that for samples with smaller sized scatterers, owing to the isotropic nature of scattering for such medium, polarization is preserved within a narrow cone angle along the direction of propagation of incident light. In contrast for samples prepared using larger sized scatterers, the forward scattering nature of such samples ensures that polarization is preserved even for photons scattered far away from the propagation axis. Further, because in a medium comprised of smaller sized scatterers, large angle scattering of light depolarizes circularly polarized light to a greater extent than linearly polarized light, the spatial profile of degree of polarization for circularly polarized light (FWHM ~ 1 mm) is seen to be sharper than that for linearly polarized light (FWHM ~ 1.45 mm). Measurements were also performed on depolarization of linearly and circularly polarized light as a function of increasing optical thickness of these monodisperse samples.

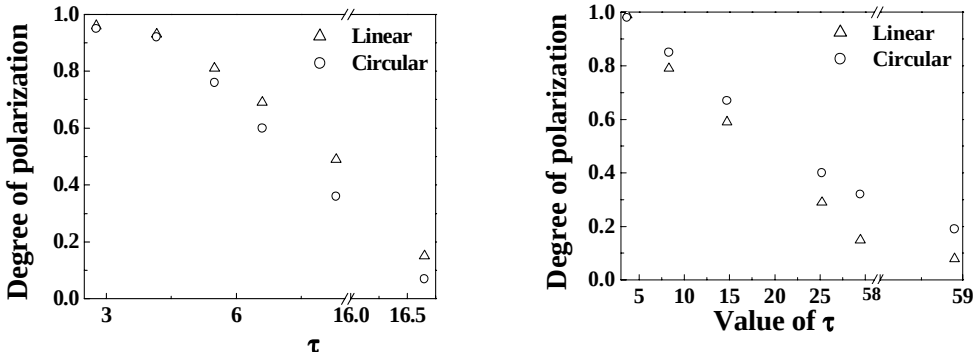

Fig. 3. Measured degree of linear polarization (triangles) and circular polarization (circles) as a function of optical thickness (τ) for (a) 0.11 μm diameter polystyrene microspheres suspension in water and (b) for 1.08μm diameter polystyrene microspheres suspension in water.

The results of these measurements (Fig. 3(a) and Fig. 3(b)) show that, in conformity with previous reports, the degree of circular polarization falls sharper with increasing value of τ than the degree of linear polarization for the samples having smaller diameter scatterers

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(radius of scatterer a < <λ, anisotropy parameter g ≤ 0.3) and the reverse behavior is the case for the samples with larger diameter scatterers (a≳ λ, g ≥ 0.7) [1-3, 11]. Further, it can also be seen that for both linear and circularly polarized light, the state of polarization is maintained for a larger optical thickness (τ) in case of sample with larger diameter scatterers.

In Figs. 4(a) and (b), we show the measured scattering phase function for samples with 0.11 μm and 1.08 μm diameter polystyrene spheres respectively. The phase functions computed using Mie theory are shown by dashed line.

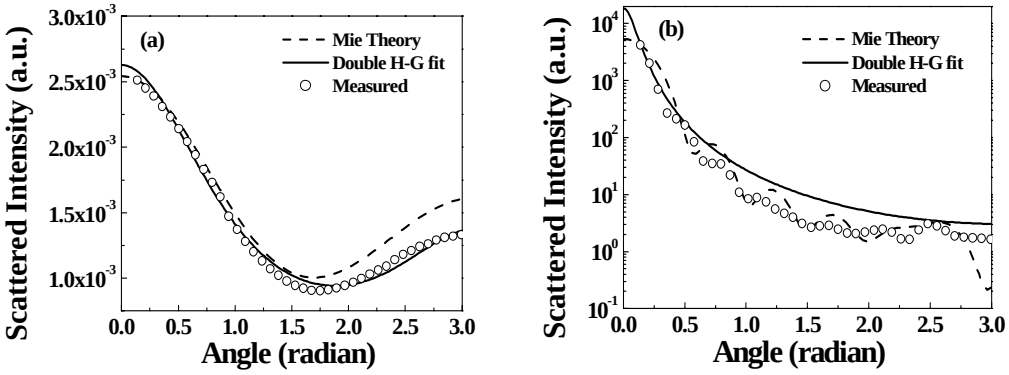

Fig. 4. (a) The measured scattering phase function (open circles), Mie theory computed phase function (dashed line) and the double H-G fit (solid line) to the measured phase function for 0.11 μm diameter polystyrene microspheres suspension in water. Single H-G function did not produce good fit. (b) The measured scattering phase function (open circles), Mie theory computed phase function (dashed line) and the single H-G fit (solid line) to the measured phase function for 1.08 μm diameter polystyrene microspheres suspension in water. The double H-G fit and single H-G fit was indistinguishable.

Henyey – Greenstein (H-G) function has widely been used to fit the measured phase function of a polydisperse medium like tissue [15]. However, it has been observed that a single H-G phase function often underestimates large angle scattering in a polydisperse medium that have significant back scattering contribution [16-18]. A better estimate for g can be obtained from the measured scattering phase function from such medium by fitting it to a phase function composed of two H-G functions one with high positive g value another with a very low negative g value [17,18]. The measured phase functions for both the samples were therefore fitted with a Henyey – Greenstein (H-G) and a double H-G function. For the 0.11μm spheres, a single H-G function could not provide a good fit to the measured phase function because of the presence of strong back scattering lobe from this sample. The estimate for g for this sample using a double H-G fit was 0.12, which is reasonably close to the Mie theory, predicted value of 0.09. For the sample prepared using 1.08 μm spheres, estimate for g from both single H-G as well as the double H-G fit was 0.88. The corresponding estimate from Mie computation was 0.92. It is pertinent to note here that, in Fig. 4(a), double H-G function provides a better fit to the experimentally measured phase function than the phase function calculated by the Mie theory. This we believe is because of a distribution in the size of scatterers. Indeed Mie phase function calculated for a sample having a Gaussian distribution of scatterer size with mean diameter = 0.11 μm and standard deviation σ = 0.011μm was observed to provide an excellent fit to the measured phase function of 0.11 μm spheres. This distribution in size of scatterer is also responsible for the fact that the oscillations observed in Mie computed phase function are washed out in the experimentally measured phase function for 1.08 μm spheres (Fig. 4 (b)).

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In Fig. 5 (a), we show the spatial distribution of the degree of linear and circular polarization at the detector for the sample prepared by using 1:1 and 1:2 volume ratio mixture of 0.11 μm diameter spheres and 1.08 μm diameter spheres suspension both having a value of μ_s= 1.66 mm^{-1}. This ensured that the mixtures of the two samples also had a value of τ = 8.3. It can be seen from the figure that for these mixtures of monodisperse samples, spatial profiles for both the degree of linear and circular polarization show distinct peaks around the beam center. In Fig. 5(b), we show the variation of the degree of polarization as a function of the optical thickness of the samples for the two states of polarization. The degree of polarization was found to be lower for circularly polarized light as compared to linearly polarized light for all values of τ of these samples.

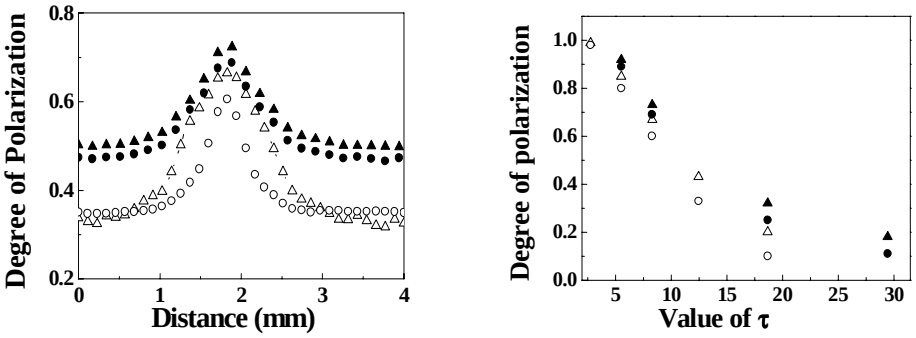

Fig. 5: (a) Measured spatial distribution of the degree of linear polarization (triangles) and circular polarization (circles) at the detector for samples prepared using 1:1 volume ratio (open symbols) and 1:2 volume ratio (solid symbols) mixtures of 0.11 μm diameter spheres and 1.08 μm diameter spheres suspensions (μ_s= 1.66 mm^{-1} and τ = 8.3). (b) Measured degree of linear polarization (triangles) and circular polarization (circles) as a function of optical thickness (τ) for samples prepared using 1:1 volume ratio (open symbols) and 1:2 volume ratio (solid symbols) mixtures of 0.11 μm diameter spheres and 1.08 μm diameter spheres suspensions

The depolarization behavior of Figs. 5(a) and (b) is characteristics of a sample with smaller scatterers ($a << \lambda$, $g \leq 0.3$) (see Fig. 2 (a) and Fig. 3(a)). This observation that smaller scatterers determine the depolarization behavior of light in a mixture of two different sized scatterers may be due to the fact that in a mixture of different sized scatterers, the major contribution of depolarization comes from photons that are scattered at larger angles and that the relative contribution of large angle scattered photons is more from the smaller sized scatterers in the mixture.

In Fig. 6, we show the measured phase function for the sample prepared by 1:1 volume ratio mixture of 0.11 μm diameter spheres and 1.08 μm diameter spheres having the same value of μ_s for individual samples. The estimate for g from a single H-G and double H-G fit were 0.87 and 0.80 respectively. These are much larger than the estimated value of 0.51 using Mie theory. These results are consistent with the earlier reports where significant discrepancy was observed in the estimated values for Mie equivalent scatterer radius of a polydisperse medium using two methods, one based on goniophotometric measurements of phase function and the other using a fit for the measured wavelength variation of μ_s' [=μ_s (1-g)], which was determined from spatially resolved diffuse reflectance measurements at different wavelengths [13, 19].

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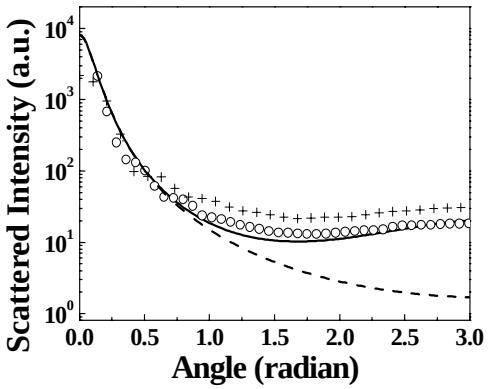

Fig. 6. The measured scattering phase function (open circles), Mie theory computed phase function (line with '+' symbol), single H-G fit (dashed line) and the double H-G fit (solid line) to the measured phase function for samples prepared using 1:1 volume ratio mixture of 0.11 μm diameter spheres and 1.08 μm diameter spheres suspensions.

This discrepancy was shown to arise because of the fact that whereas scatterers with larger size ($a \gtrsim \lambda$) contribute more to the goniophotometric measurements, for diffuse reflectance measurements scatterers with the smaller size ($a << \lambda$) contribute more. Indeed a fit to large angle phase function yielded estimates of Mie equivalent scatterer radius closer to that obtained via diffuse reflectance measurement [13].

It is of interest to relate these results with the observation of Sankaran *et al*. [7] that the relative rate of depolarization of linear and circularly polarized light is different in biological tissues and matched tissue phantoms. Here it is pertinent to note that light scattering from biological tissue arises from scatterers ranging in size from 0.1μm (mitochondria, lisosomes, perixosomes and other sub-cellular structures) to ~10 -20 μm (cell as a whole) [16,19]. Results presented above would therefore suggest that whereas the goniophotometric measurement of g for tissue would be weighted towards the larger sized scatterers the depolarization behavior would be influenced more by the smaller sized scatterers. Therefore, depolarization behavior of biological tissue and a monodisperse tissue phantom having the same g and τ should be expected to be rather different as indeed was observed by Sankaran et al [6,7,9]. These results are further corroborated by the fact that no significant difference was observed in the depolarization behavior of linearly and circularly polarized light in tissue with a narrow distribution of scatterer size (porcine blood) and corresponding matched tissue phantom [9].

4. Conclusion

To conclude we have shown that the depolarization behavior of light on propagation through a sample having a mixture of scatterers of different sizes is dominated by the smaller scatterers (size $a << \lambda$). In contrast, the estimates for the anisotropy parameter (g) for this mixture, from goniophotometric measurement of scattering phase functions, will be closer to the values corresponding to the larger sized scatterers ($a \gtrsim \lambda$). These results have an important implication in that the depolarization behavior of light of biological tissue (having a distribution of scatterer size) would be different from that of a matched monodisperse scattering sample having the same value of anisotropy parameter (g) and optical thickness (τ).

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