1 April 2001

ELSEVIER

Optics Communications 190 (2001) 37–43

OPTICS COMMUNICATIONS

www.elsevier.com/locate/optcom

Polarization preservation in diffusive scattering from in vivo turbid biological media: effects of tissue optical absorption in the exact backscattering direction

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Received 13 November 2000; received in revised form 25 January 2001; accepted 25 January 2001

Abstract

There is considerable recent interest in using polarized light to investigate turbid biological media. Although tissue multiple scattering randomizes incident polarization states, there are circumstances when appreciable degree of polarization can be observed in diffusive scattering. In this study, we use polarization modulation and synchronous detection to examine in the exact backscattering direction the polarization properties of diffusely reflected visible light from hands of human volunteers of varying pigmentation levels. The surviving polarization fraction increases with increasing pigmentation, likely due to preferential loss of highly scattered, long-pathlength photons; this mechanism lowers the average pathlength traversed by the detected light and hence increases the measured polarization preservation. This behavior is contrasted with the overall diffuse reflectance intensity, whose magnitude decreases with increasing absorption. These experiments demonstrate the important influences of medium optical properties on the polarization characteristics of multiply scattered light, which must be further investigated to enable quantitative polarization evaluation of turbid media such as biological tissues.

Keywords: Polarization; Multiple scattering; Biological tissue; Pigmentation levels

When polarized light enters a turbid medium, multiple scattering randomizes the polarization state such that the emerging beam is greatly depolarized. This poses significant challenges when using polarized light to investigate the structure or composition of turbid media such as clouds or biological tissues. The extent to which the depolarization process occurs depends on the number and nature of the scattering events, source–sample–detector geometry, and the polarization properties of the incident beam. Other optical characteristics of the turbid medium, such as the presence of optically active (chiral) molecules, can also affect the depolarization rate [1,2]. For a sufficiently thick turbid medium, of the order of 10–15 transport mean free paths [3,4], the emerging beam is fully depolarized, and thus any potentially useful information encoded in the light's polarization properties is lost. However, in the backscattering direction, partial polarization may

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0030-4018/01/$ - see front matter
PII: S0030-4018(01)01080-X

be retained even in very thick multiply scattering media [5,6]. This effect is particularly strong in the *exact* backscattering direction, when the incoming and the outgoing optical paths in the turbid sample superpose, enabling significant retention of coherence and polarization properties. For example, recent theoretical work indicates that for incident circular polarization, the degree of polarization detected in the backscattering direction should converge asymptotically to ~25% with increasing medium turbidity [3]. Experimentally, polarization properties of back-scattered light have been investigated [7–10]. The unusual coherence properties of light detected in this geometry have been explained in terms of the weak localization concept [7,8,11–13].

Most mammalian tissues are weakly absorbing in the 600–1300 nm wavelength range, allowing appreciable penetration of light; however, the scattering properties of tissues at these wavelengths are significant, meaning that the near-infrared light is subjected to extensive scattering [14]. The polarization information encoded in this diffusely scattered light is potentially useful for optical diagnosis of biological tissues. Applications such as body glucose monitoring [2,15], thermal lesion demarcation [16,17], and microstructural bioimaging [18,19] are currently being explored. However, given the depolarization effect of multiple scattering, polarization studies of optically thick turbid media such as biological tissues may be seriously compromised.

The studies in the exact back-scattering direction are therefore attractive for two reasons – there is the expectation of non-vanishing polarization signals even in extremely thick turbid media, and this configuration is practically convenient, and sometimes unavoidable, for biomedical investigations. It is important to quantify the effect of tissue optics on the polarization properties of back-scattered light. For example, since the degree of polarization surviving in a random media generally decreases as the optical pathlength increases, one would assume that limiting light penetration by increasing tissue absorption would enhance polarization preservation, albeit at the expense of reducing the overall reflectance intensity. In this letter, a novel application of a polarization modulation method is used to investigate this conjecture in human tissue in vivo.

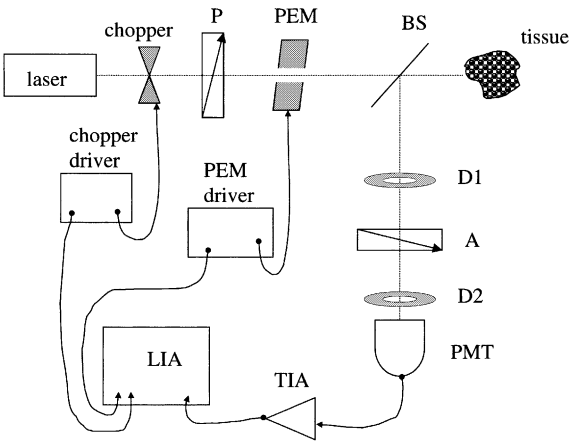

Fig. 1. Schematic of the experimental system for measuring polarization properties of back-scattered diffusely reflected light from a turbid biological sample. The PEM (oriented at 6° off normal incidence with respect to the incident beam direction, to negate the effects of modulated specular interference) oscillates in the plane of the optical table. Polarizer P is at 45° with respect to the plane of the optical table, and the orientation of analyzer A is set to ~20 different angular positions in the 90–270° range. BS: beam splitter; D1 and D2: pinhole diaphragms; PMT: photomultiplier; TIA: transimpedance amplifier; LIA: lock-in amplifier.

The experimental system capable of detecting weak polarization signals in the presence of large diffusive background for light scattered from a random biological medium is illustrated in Fig. 1. Its detailed mode of operation has been described in detail previously [2,10]. Briefly, it consists of a He–Ne laser source, a linear polarizer, and a 50 kHz photoelastic modulator (PEM) to enable time-varying polarization states to impinge on the beam splitter and then onto the turbid sample, and an analyzer/photodetector combination to measure the scattered light intensity and the polarization properties of the scattered light. A transimpedance and lock-in amplifiers enable sensitive synchronous measurement of the resultant photocurrent. A mechanical chopper operating at ~150 Hz is also used; when tuned to its rotation frequency, the lock-in measures the overall light intensity, and when tuned to the PEM's oscillation frequency (and its harmonics), the lock-in signal is sensitive to the polarization

fraction that has survived the sample interactions [18,20–23]. The beam splitter is present to enable detection of light that emerges from the sample centered on the exact backscattering direction [10].

The polarization apparatus was used to measure light back scattered from hands of Caucasian, Asian, and Negroid volunteers of varying pigmentation levels. The differences in skin coloration between the different ethnic groups, and within a single group, are largely due to varying amounts of epidermal melanin, a significant biological absorber [24]. The subjects rested their hands on an optical platform such that the incident beam impinged on the skin in the region between the thumb and the index finger. No additional restraint on the hand was used. No attempt was made to position the skin surface perpendicular to the incident beam, and hence the contribution of specular reflection from the skin surface was minimal. Indeed, what is crucial for retro-reflection detection is that the incident and scattered paths are anti-parallel; with the surface specular effects thus minimized, the detected signal then arises via photons scattered from within the interrogated volume. The spot size was 2 mm in diameter. Collected data consisted of lock-in amplifier readings at the chopper and PEM reference frequencies, and was collected at ~20 different angular positions of the analyzer to enable quantitative data analysis, as described below. The entire measurement procedure took approximately 15 min. Because of the many collected experimental points that decrease the influence of noise in any one measurement, and because of the normalization scheme employed for data analysis that negates the effects of light intensity fluctuations, the results were relatively insensitive to the slight but inevitable subject motion that occurred during the data collection. All measurements were performed at room temperature.

Fig. 2 shows the results from a Caucasian volunteer of light pigmentation and an Asian (Indian) volunteer of moderate pigmentation interrogated with 633 nm He–Ne laser. The vertical axis is the ratio of the lock-in photocurrent at $2f(=100$ kHz), or twice the modulation frequency of the PEM, to the 'dc' signal measured at the chopper frequency of 150 Hz. The horizontal axis

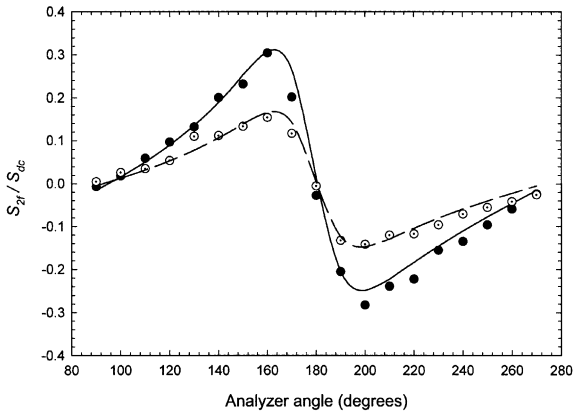

Fig. 2. Measured polarization signals (⊙,●) and theoretical fits (– – –,—) for experiments performed at 633 nm wavelength. The abscissa is the angular orientation of the analyzer, and the ordinate is the detector signal at twice the modulation frequency of the PEM, normalized by the signal at the mechanical chopper frequency. The dotted symbols and line denote results from Caucasian skin of light pigmentation, and the solid symbols and line are from an Asian (Indian) volunteer of medium pigmentation.

is the angular orientation of the analyzer. Negative ratios are due to the lock-in phase change of the 100 kHz signal that occurs with varying analyzer angle. This signal arises from surviving polarized light, whereas both polarized and unpolarized optical fluxes contribute to the 150 Hz photocurrent. The magnitude of the ratio is clearly larger for reflectance from the darker skin subject, indicating higher surviving polarization fraction. Conversely, the overall light intensity represented by the 'dc' signal is about two times lower (see Fig. 4 for further discussion). It thus appears that more light is reflected from the less pigmented Caucasian skin, but its polarization retention is weaker. This is likely due to the increasing polarization loss of the longer pathlength, deeper penetrating photons that have a greater change of scattering back out of tissue and hence being detected in the presence of lightly-melanized epidermis (Caucasian skin) than a heavier-melanized one (Asian skin).

To quantify the surviving polarization fraction using the data from Fig. 2, the optical system was analyzed using Mueller calculus. The Mueller matrices for the various optical components can be found in standard texts [25]. The sample was

modeled as an optical rotator (to account for optically active compounds, e.g. glucose), a retroreflector (to account for back-scattering geometry), and a depolarizer (to account for multiple scattering). [1] The possible effects of linear birefringence of tissue are ignored, but may be included in future developments. [2] As described, the ratio of the signals at the two modulation frequencies becomes

$$\frac{S_{2f}}{S_{\mathrm{dc}}} = \frac{2J_2(\delta)A}{J_0(\delta)A + B + C} \tag{1a}$$

with

$$\begin{aligned} A &= 1.82\beta(0.82\sin\alpha + \sin\alpha\cos 2\theta \\ &\quad + 0.58\cos\alpha\sin 2\theta)B \\ &= 1.82(1 + 0.82\cos 2\theta)C \\ &= -1.82\beta[(0.82 + \cos 2\theta)\cos\alpha \\ &\quad - 0.58\sin 2\theta\sin\alpha] \end{aligned} \tag{1b}$$

where θ is the orientation of the analyzer with respect to the horizontal plane (plane of optical table), α is the sample optical rotation, and $\beta =$ (polarized light intensity)/(total light intensity) is the degree of polarization. J_0 and J_2 are the zeroth and second order Bessel functions, respectively, and their argument is δ, the user-defined retardation setting of the PEM. In the reported measurements, δ was set to 3.469 radians to maximize the $2f$ signal [2]. In deriving Eq. (1), specifically the numerical values appearing in Eq. (1b), a refractive index of 1.45 was used for the glass beam splitter. The unknowns in Eq. (1) are the two sample-specific characteristics α and β, and it is thus possible to perform a non-linear two-parameter fit to the data in Fig. 2, using θ as the independent variable. Although it is also possible to estimate α and β with simpler methods using only two signal ratios [1,2], these approaches are prone to sample variability and measurement artifacts; the fitting method requires more data collection, but is more robust under realistic conditions and less prone to single-measurement mistakes. The fitting of Eq. (1) to the experimental photocurrent ratios was performed using the non-linear regression routine of SigmaPlot 3.0 (Jandel Scientific, San Rafael, CA).

The lines in Fig. 2 show the predictions of Eq. (1) with optimally fitted α and β. For these data sets, the best-fit parameters for the Caucasian volunteer are $\alpha = 2.2 \pm 1.3°$ and $\beta = 17.2 \pm 0.5\%$, and for the Asian volunteer $\alpha = 2.2 \pm 1.3°$ and $\beta = 30.0 \pm 0.4\%$. Thus, darker skin reflection manifests the degree of polarization nearly twice that of skin with lower pigmentation. In this and similar analyzes, we find that the degree of polarization results are determined with excellent precision ($\pm 2\%$) and reproducibility ($\pm 7\%$), and thus can be used to examine real trends in the data. The results for α are less encouraging. We are presently investigating the source of the large uncertainty in the determined optical rotation, and will discuss its behavior in a separate publication.

If the determined degree of polarization is truly indicative of the interrogated volume or the average penetration depth of the detected photons, then the difference in β's seen at 633 nm should be reduced when the same skin types are examined at

[1] It is possible to propose Mueller matrices that differ from the suggested depolarization matrix (non-zero elements at $M_{11} = 1$, $M_{22} = M_{33} = M_{44} = \beta$). For example, to account for different depolarization rates of linear versus circular polarization states, a matrix with non-zero elements at $M_{11} = 1$, $M_{22} = M_{33} = \beta_{\mathrm{L}}$, $M_{44} = \beta_{\mathrm{C}}$ may be more appropriate (β_{L} = degree of linear polarization, β_{C} = degree of circular polarization, $\beta = (\beta_{\mathrm{L}}^2 + \beta_{\mathrm{C}}^2)^{1/2}$ = total degree of polarization), although this representation is non unique as well. It can also be argued that the current one-β-parameter depolarization description is correct, and that the total degree of polarization obtained from the data fits is further decomposable into β_{L} and β_{C} [1]. For the current study, the suggested form of the Mueller depolarization matrix is valid in that the contribution of M_{44}, whether same or different from M_{22} and M_{33}, does not propagate to the final signal expression of Eq. (1).

[2] Experiments with thermally denatured chicken and beef muscle samples have yielded β values similar to those obtained with fresh ex vivo tissues, suggesting that the inclusion of tissue birefringence effects may be relatively unimportant. This result differs from previous findings [16,17] which showed that polarized light signals are sensitive markers of tissue birefringence loss engendered by thermal denaturation. One difference may be that in the present experiments, the light incident on the sample exhibits all polarization states (linear, elliptical, circular) in a time-varying fashion, so that the possible effects of linear sample birefringence, averaged over these different polarization states, are less pronounced. Thus, ignoring birefringence as is done with the present analysis is unlikely to introduce significant errors; work is ongoing to further clarify this issue.

shorter wavelengths. This is because the relative importance of melanin's absorption is diminished as other visible-light chromophores begin to play a role in the 400–550 nm wavelength range. A major tissue chromophore in the visible spectrum is hemoglobin, both in its oxygenated and deoxygenated forms, and its contribution to tissue absorption generally increases at lower wavelengths. The magnitude of the increase depends on blood volume, hematocrit, and oxygenation state. Unlike absorption, the scattering properties of tissues do not change appreciably with modest changes in wavelength [14,24]. Thus, in repeating the measurements at 544 nm, there is a slight increase in the scattering coefficient, a slight increase in melanin absorption, and a significant increase in hemoglobin absorption. Hence, optical effects due to melanin pigmentation differences in the skin types should be diminished by the presence of the hemoglobin absorber common to both Caucasian and Asian skin.

Fig. 3 displays data from the hands of the same volunteers, but now interrogated with 544 nm wavelength light from another He–Ne laser. The fitted parameters are $\beta = 28.2 \pm 0.5\%$ for the Caucasian, and $\beta = 34.0 \pm 0.4\%$ for the Asian volunteers. The degree of polarization values are thus higher than at 633 nm, with the darker skin still exhibiting higher polarization preservation. Importantly, the *difference* in degree of polarization is indeed lower at this less penetrating wavelength than at 633 nm. All of these observations are consistent with the preferential absorption of the long-pathlength photons as affected by changing the tissue type and/or interrogating wavelength.

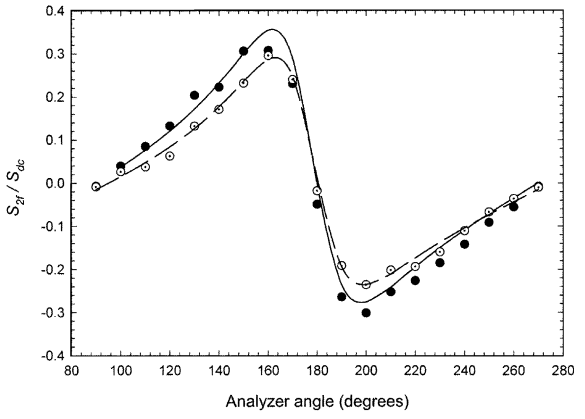

Fig. 3. Measured polarization signals (⊙,●) and theoretical fits (– – –,—) for experiments performed at 544 nm wavelength. See Fig. 2 for further details.

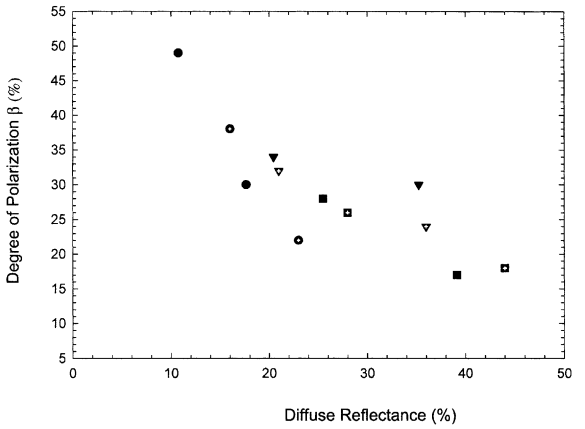

Fig. 4. Degree of polarization versus diffuse reflectance measured at two wavelengths from skin of six volunteers of different pigmentation (12 PEM results and 12 spectrophotometer results). For each pair of like symbols, the left point represents results obtained at 544 nm, and the right point represents results at 633 nm. (■,◘): Caucasian; (▼,▽): Asian; (●,◘): Negroid).

The relationship between the magnitude of the back-scattered light and the amount of retained polarization is explored in Fig. 4. The overall backscattered intensity was measured with a home-made diffuse spectro-reflectometer [26] on the same location on the hand as the PEM studies, and is plotted on the abscissa axis of the figure. (Although the dc signal from the polarization studies is itself a measure of diffuse reflectance, inter-sample comparisons of the dc levels may be inaccurate due to differences in hand positioning, optical alignment, laser power fluctuations, and the like. These sources of noise are fully accounted for in the S_{2f}/S_{dc} normalization procedure described above, so inter-sample comparison of these *ratios* is fully justified. However, for comparing diffuse reflectance intensities, the reflectometer values are more reliable since a reference reflectance is used for each sample spectra.) Data for six subjects at the two wavelengths are summarized – two Caucasians, two Asians, and two Negroid volunteers. For these 12 determined polarization parameters,

12 corresponding diffuse reflectance readings were obtained. As seen in Fig. 4, darker skin types exhibit higher polarization preservation and lower reflected intensity. Thus, an increase in diffuse reflectance generally results in lower polarization fractions. Given the nature of pigmentation diversity and the noise present in any in vivo measurements, the observed inverse relationship is rather strong ($\beta \sim 1$/diffuse reflectance), with a correlation coefficient of $r^2 = 0.78$. An increase in diffuse reflectance magnitude implies reduced absorption, increased light penetration, and larger sampling volume within tissue; these cause a decrease in the degree of polarization by enabling more scattering interactions for the photons that eventually escape the tissue and reach the detector. Thus, the magnitude of β may be indicative of the effective pathlength traversed by the diffusing photons.

In general, the characteristics of light diffusely reflected from a turbid medium depend on its optical properties. In this study, we have applied a simple and sensitive method to extract the degree of polarization of diffusely scattered light, and have examined the effect of tissue absorption on polarization retention at two wavelengths reflected from skin of different human volunteers. These determined degree of polarization values were correlated with diffuse reflectance measurements. Increasing absorption caused a change in both polarization preservation and diffuse reflectance, with an approximate inverse correlation between these two quantities. As the natural skin absorption varied between a light-skinned Caucasian and a heavily-pigmented Negroid, the degree of polarization increased by $\sim$2–3$\times$, whereas the diffuse reflectance lowered by $\sim$3–4$\times$. These changes are more pronounced at a longer wavelength (633 nm) where the difference in melanin pigmentation dominates tissue optical absorption, and decrease toward shorter wavelengths (544 nm) where the variations in melanin levels are masked by contributions of hemoglobin absorption which does not vary significantly between different skin types.

It is encouraging to note the significant polarization preservation easily and reproducibly measurable with the described system in live human subjects. This is because of: (a) the detection geometry which maximizes polarization signals in the exact back-scattering direction, (b) experimental methodology which minimizes the reliance on single data points by enabling a regression fit over $\sim$20 values of the independent variable, and (c) the dual-frequency signal acquisition followed by normalization procedure which eliminates many experimental fluctuations. While this approach yields reliable β values, the noise and reproducibility in the determined optical rotation α is sub-optimal. We are currently working on identifying the source of the error in, and on improving the reliability of, the optical rotation determination.

The dependence of degree of polarization on pigmentation of tissues in particular, and on optical properties in general, must be fully accounted for if the polarization information is to be used for quantitative analysis of biological media. For example, one attractive possibility is to use polarized light to sense the presence, and quantify the concentration of optically active metabolites such as glucose in optically turbid tissues [2,15]. The degree of polarization has been shown to depend on the turbidity of the medium, and also on the amount of optically active species [1,2]. One may conceivably estimate the effective pathlength from the degree of polarization [27] , and then use this in combination with the derived optical rotation value and known glucose specific rotatory power to yield glucose concentration [2]. Much work remains to be done to determine if this approach to glucose sensing is useful, but clearly the variation in the measured polarization properties with tissue parameters that do not depend on glucose per se, such as tissue pigmentation levels and scattering coefficients, must be clearly understood. This study is a part of our continuing effort to establish quantitative applications of polarized light diagnostics in biomedicine.

In conclusion, we have demonstrated that light diffusively scattered in the retro-reflection direction from in vivo human tissues exhibits significant polarization retention that can be reliably measured with a simple polarization modulation/synchronous detection methodology. The deduced degree of polarization increases with increasing tissue absorption. In contrast, the magnitude of

the diffuse reflectance decreases, so that the diffuse reflectance intensity and polarization preservation are inversely correlated. The behavior of polarized light in turbid biological media, and the influence of tissue optics as examined in this study, must be properly quantified to enable reliable biomedical diagnostic applications relying on light polarization.

Acknowledgements

The authors thank Dr. Robert Weersink for providing the diffuse reflectance data. This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada. R.C.N.S. was partially supported by the Harold E. Johns Summer Studentship at the Ontario Cancer Institute.

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