# Polar decomposition of $3 \times 3$ Mueller matrix: a tool for quantitative tissue polarimetry

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Abstract: The polarization properties of any medium are completely described by the sixteen element Mueller matrix that relates the polarization parameters of the light incident on the medium to that emerging from it. Measurement of all the elements of the matrix requires a minimum of sixteen measurements involving both linear and circularly polarized light. However, for many diagnostic applications, it would be useful if the polarization parameters can be quantified with linear polarization measurements alone. In this paper, we present a method based on polar decomposition of Mueller matrix for quantification of the polarization parameters of a scattering medium using the nine element  $(3 \times 3)$  Mueller matrix that requires linear polarization measurements only. The methodology for decomposition of the  $3 \times 3$  Mueller matrix is based on the previously developed decomposition process for sixteen element  $(4 \times 4)$ Mueller matrix but with an assumption that the depolarization of linearly polarized light due to scattering is independent of the orientation angle of the incident linear polarization vector. Studies conducted on various scattering samples demonstrated that this assumption is valid for a turbid medium like biological tissue where the depolarization of linearly polarized light primarily arises due to the randomization of the field vector's direction as a result of multiple scattering. For such medium, polar decomposition of 3 × 3 Mueller matrix can be used to quantify the four independent polarization parameters namely, the linear retardance ( $\delta$ ), the circular retardance  $(\psi)$ , the linear depolarization coefficient  $(\Delta)$  and the linear diattenuation (d) with reasonable accuracy. Since this approach requires measurements using linear polarizers only, it considerably simplifies measurement procedure and might find useful applications in tissue diagnosis using the retrieved polarization parameters.

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#### References

- D. Bicout, C. Brosseau, A.S. Martinez, and J.M. Schmitt, "Depolarization of multiply scattered waves by spherical diffusers: Influence of the size parameter," Phys. Rev. E 49, 1767-1770 (1994).
- A.D. Kim and M. Moscoso, "Influence of the refractive index on the depolarization of multiply scattered waves," Phys. Rev. E 64, 026612, 1-4 (2001).
- 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).
- 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).
- N. Ghosh, H.S. Patel, and P.K. Gupta, "Depolarization of light in tissue phantoms effect of a distribution in the size of scatterers," Opt. Express 11, 2198-2205 (2003).

- N. Ghosh, A. Pradhan, P.K. Gupta, S. Gupta, V. Jaiswal, and R.P. Singh, "Depolarization of light in a multiply scattering medium: effect of refractive index of scatterer," Phys. Rev. E 70, 066607 (2004).
- J.F. de Boer, T.E. Milner, M.J.C. van Gemert, and J.S. Nelson, "Two-dimensional birefringence imaging in biological tissue by polarization-sensitive optical coherence Tomography," Opt. Lett. 22, 934-936 (1997).
- D.J. Maitland and J.T. Walsh Jr., "Quantitative measurement of linear birefringence during heating of native collagen," Lasers Surg. Med. 20, 310-318 (1997).
- 9. M.H. Smith, "Interpreting Mueller matrix images of tissues," Proceedings SPIE 4257, 82 89 (2001).
- G.L. Liu, Y. Li, and B.D. Cameron, "Polarization based optical imaging and processing techniques with application to the cancer diagnostics," Proceedings SPIE 4617, 208 – 220 (2002).
- C.W. Sun, L.S. Lu, C.C. Yang, Y.W. Kiang, and M.J. Su, "Myocardial tissue characterization based on the time-resolved Stokes-Mueller formalism," Opt. Express 10, 1347 – 1353 (2002).
- J. Zhang, S. Guo, W. Jung, J.S. Nelson, and Z. Chen, "Determination of birefringence and absolute optic axis orientation using polarization-sensitive optical coherence tomography with PM fibers," Opt. Express 11, 3262

   3270 (2003).
- O. Kostyuk and R.A. Brown, "Novel Spectroscopic Technique for In Situ Monitoring of Collagen Fibril Alignment in Gels," Biophys. J. 87, 648-655 (2004).
- F. Boulvert, B. Boulbry, G Le Brun, S. Rivet, and J. Cariou, "Analysis of the depolarization properties of irradiated pig skin," J. Opt. A: Pure Appl. Opt. 7, 21 – 28 (2005).
- R.J. McNichols and G.L. Cote, "Optical glucose sensing in biological fluids: an overview," J. Biomed. Opt. 5, 5 - 16 (2000).
- B.D. Cameron and G.L. Cote, "Noninvasive glucose sensing utilizing a digital closed loop polarimetric approach," IEEE Trans. Biomed. Eng. 44, 1221-227 (1997).
- I.A. Vitkin and R.C.N Studinski, "Polarization preservation in diffusive scattering from in-vivo turbid biological media: Effects of tissue optical absorption in the exact backscattering direction," Opt. Commun. 190, 37-43 (2001).
- K.C. Hadley and I.A. Vitkin, "Optical rotation and linear and circular depolarization rates in diffusively scattered light from chiral, racimic and achiral turbid media," J. Biomed. Opt. 7, 291-299 (2002).
- I. Vitkin, R.D. Laszlo, and C.L. Whyman, "Effects of molecular asymmetry of optically active molecules on the polarization properties of multiply scattered light," Opt. Express 10, 222 – 229 (2002).
- X. Wang, G. Yao, and L.V. Yang, "Monte Carlo model and single scattering approx. Of the propagation of polarized light in turbid media containing glucose," Appl. Opt. 41, 792 – 801, (2002).
- D. Cote and I. Vitkin, "Robust concentration determination of optically active molecule in turbid media with validated three dimensional polarization sensitive Monte Carlo calculation," Opt. Express 13, 148–163 (2005). http://www.opticsexpress.org/abstract.cfm?URI=OPEX-13-1-148
- S. Manhas, M. K. Swami, P. Buddhiwant, N. Ghosh, P. K. Gupta, and K. Singh, "Mueller matrix approach for determination of optical rotation in chiral turbid media in backscattering geometry," Opt. Express 14, 190-202 (2006).
- S. Yau Lu and R.A. Chipman, "Interpretation of Mueller matrices based on polar decomposition," J. Opt. Soc. Am. A 13, 1106-1113 (1996).
- 24. R.A. Chipman, "Hand book of optics (polarimetry)," OSA/McGraw-Hill, 22.1-22.35, (1994).
- B.L. Boulesteix, A. De Martino, B. Dre villon, and L. Schwartz, "Mueller polarimetric imaging system with liquid crystal," Appl. Opt. 43, 2824-2832 (2004).
- E. Collett and V. Gazerro, "Polarization measurements in a spectrofluorophotometer," Opt. Commun. 129, 229-236 (1996).
- E. Gar cia-Caurel, A.De Martino, and B.Drevillon, "Spectroscopic Mueller polarimeter based on liquid crystal devices," Thin Solid Films 455-456, 120-123 (2004).
- J. Morio and F.Goudail, "Influence of the order of diattenuator, retarder, and polarizer in polar decomposition of Mueller matrices," Opt. Lett. 29, 2234-2236 (2004).
- M. Wong, M.J. Hendrix, K. Von der Mark, C. Little and R. Stern, "Collagen in the egg shell membranes of the hen," Dev. Biol. 104 (1), 28-36 (1984).
- C.F. Bohren and D.R. Huffman, "Absorption and scattering of light by small particles," Wiley, New York (1983).

# 1. Introduction

Studies on polarization properties of scattered light from a turbid medium like biological tissue have received considerable attention because such measurements can facilitate quantification of useful physiological and morphological parameters of tissue. For example, since the rate of depolarization of incident linearly and circularly polarized light depends on the morphological and optical parameters like the density, size (and its distribution), shape and refractive index of scatterers present in the medium [1-6], this information may be used

for tissue diagnosis. In addition to depolarization property, many constituents of tissue also show intrinsic polarization properties such as retardance (linear retardance arising due to a difference in phase between two orthogonal linear polarization, and circular retardance arising due to a difference in phase between right and left circularly polarized light) and diattenuation (linear diattenuation arising due to the different attenuation coefficients of two orthogonal linear polarization, and circular diattenuation arising due to the different attenuation coefficients of right and left circularly polarized light). For example, collagen is a structural protein present in tissue that has linear retardance property due to its oriented fibrous structure. In various kinds of tissue abnormalities such as actinic keratosis, neurofibroma, psoriasis, erythema and the numerous types of carcinomas (like melanomas, basal cell, squamous cell carcinoma etc.), the structural and functional properties of collagen and other fibrous structures present in tissue show distinct changes, which lead to significant variation in their linear retardance property. Several studies have therefore been conducted to develop techniques for quantification of the magnitude and orientation of linear retardance in tissue and to use this information for diagnostic applications [7-14]. Glucose possesses intrinsic circular retardance property due to its asymmetric (chiral) structure and its presence in tissue leads to rotation of the plane of linear polarization about the axis of propagation (known as optical rotation) [15]. Measurement of circular retardance or optical rotation of scattered light from tissue might thus facilitate non-invasive monitoring of glucose level in human tissue [15-22]. Development of techniques / approaches for the measurement of the different polarization properties of tissue is therefore of considerable current interest and is expected to be useful for a wide variety of diagnostic applications. Since Mueller matrix contains complete information about the polarization properties (retardance, diattenuation and depolarization) of a medium in its different elements, this can be used to quantify these parameters very efficiently. This would however, require suitable theoretical approaches to extract the polarization parameters from the measured Mueller matrix and to interpret these parameters in terms of the physiological and morphological features of tissue. Polar decomposition of Mueller matrix is a robust and efficient approach for interpretation and quantification of these polarization parameters [23]. Indeed, in our previous study, we have successfully used polar decomposition of sixteen element (4 × 4) Mueller matrix for determination of the degree of optical rotation introduced by the presence of chiral substances in a turbid medium using the Mueller matrix measured from the medium [22]. The sixteenelement Mueller matrix that involves both linear and circular polarization measurements [23, 24] provides a complete description of the polarization properties of the medium. However, for practical biomedical applications, it would be useful if the important polarization parameters (e.g., linear and circular retardance) can be quantified with linear polarization measurements alone. This would considerably simplify the measurement procedure and would particularly be suitable for spectroscopic polarimetry where polarization information can be obtained at several wavelengths [25-27]. To accomplish this objective, in this communication, we present a polar decomposition based approach for quantification of the different polarization parameters of a scattering medium using the nine element  $(3 \times 3)$ Mueller matrix measured from the medium. Measurement of the nine elements of Mueller matrix requires just two linear polarizers with nine combinations of input and output state of polarizations.

The paper is organized as follows. Section 2 describes the theoretical foundation of polar decomposition of  $3 \times 3$  Mueller matrix. The experimental methods are discussed in Section 3. The results of studies on various scattering samples and on optical components with known polarization properties, conducted to verify the efficacy of the polar decomposition based approach, are presented in Section 4. Section 5 concludes with discussion on the potential applications of the developed approach and its limitations.

# 2. Theory

### 2.1 Polar decomposition of nine element (3×3) Mueller matrix

Polar decomposition of the Mueller matrix is a tool, which provides informations about the different polarization properties of the sample, namely, diattenuation, retardance and depolarization. The process for Polar decomposition of  $4\times 4$  Mueller matrix into Mueller matrices of a diattenuator  $(M_D)$ , a retarder  $(M_R)$  and a depolarizer  $(M_\Delta)$  has been described in reference [23]. For decomposition of  $3\times 3$  Mueller matrix (M), we follow the similar procedure. As for the  $4\times 4$  matrix, the diattenuation properties (d) of the medium are contained in the first row of the matrix that is used to construct the  $3\times 3$  diattenuation matrix  $(M_D)$ . The inverse of  $M_D$  is multiplied with M to remove the diattenuation and the remaining matrix (M') consists of retardance and depolarization.

$$\mathbf{M}' = \mathbf{M}\mathbf{M}_{\mathbf{D}}^{-1} = \mathbf{M}_{\mathbf{A}}\mathbf{M}_{\mathbf{R}} \tag{1}$$

Where the  $3 \times 3$  depolarization matrix (M<sub> $\Lambda$ </sub>) can be written as

$$\mathbf{M}_{\Delta} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \mathbf{a} & 0 \\ 0 & 0 & \mathbf{b} \end{bmatrix} \tag{2}$$

Here, a and b are linear depolarization coefficients for incident horizontally polarized light and light polarized at 45<sup>o</sup> from the horizontal direction respectively.

The  $3 \times 3$  retardance matrix ( $M_R$ ) consists of both linear and circular retardance and can be written as

$$\mathbf{M}_{R} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos^{2}2\theta + \sin^{2}2\theta\cos\delta & \sin 2\theta\cos 2\theta (1-\cos\delta) \\ 0 & \sin 2\theta\cos 2\theta (1-\cos\delta) & \sin^{2}2\theta + \cos^{2}2\theta\cos\delta \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos 2\psi & \sin 2\psi \\ 0 & -\sin 2\psi & \cos 2\psi \end{bmatrix}$$
(3)

Here,  $\delta$  is linear retardance,  $\theta$  is the orientation angle of the fast axis of the linear retarder and  $\psi$  is optical rotation (circular retardance).

For non-depolarizing samples (a = b = 1), the linear retardance ( $\delta$ ) and optical rotation ( $\psi$ ) can directly be estimated from the matrix M' as

$$\delta = \cos^{-1}[\{(\mathbf{M}_{22}^{\prime} + \mathbf{M}_{33}^{\prime})^{2} + (\mathbf{M}_{23}^{\prime} - \mathbf{M}_{32}^{\prime})^{2}\}^{1/2} - 1]$$
 (4)

$$\Psi = \frac{1}{2} \tan^{-1} \left[ \frac{(\mathbf{M}_{23}^{\prime} - \mathbf{M}_{32}^{\prime})}{(\mathbf{M}_{22}^{\prime} + \mathbf{M}_{33}^{\prime})} \right]$$
 (5)

However, for depolarizing sample, one would need to decouple the depolarization matrix  $M_{\Delta}$  from the matrix M' and find out the retardance matrix  $M_R$ . In order to obtain the values for the depolarization coefficient, we construct a matrix  $M_{DR}$  as

$$\mathbf{M}_{\mathrm{DR}} = \mathbf{M}^{\prime} (\mathbf{M}^{\prime})^{\mathrm{T}} \tag{6}$$

It should be noted here that one of the Eigen values of the matrix  $M_{DR}$  is always unity for a normalized matrix. If no depolarization were present (a=b=1), then the other two Eigen values would be 1 and  $\cos^2\delta$  respectively. In presence of depolarization, the Eigen values would be scaled by the square of the value of the linear depolarization coefficient. If the depolarization of linearly polarized light can be assumed to be independent of the orientation angle of the incident linear polarization vector, that is if  $a=b=\Delta$ , then the remaining two Eigen values of the matrix  $M_{DR}$  would be  $\Delta^2$  and  $\Delta^2\cos^2\delta$  respectively. Hence, the larger of the remaining two Eigen values would be equal to the square of the linear depolarization coefficient ( $\Delta$ ) and thus this Eigen value can be used to find out the value of  $\Delta$ . Once the value of  $\Delta$  is obtained from the Eigen values of  $M_{DR}$ , one can readily construct the depolarization matrix as

$$\mathbf{M}_{\Delta} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \Delta & 0 \\ 0 & 0 & \Delta \end{pmatrix} \tag{7}$$

The inverse of  $M_{\Delta}$  is thereafter multiplied with M' to obtain the retardance matrix  $M_R$ 

$$\mathbf{M}_{\mathbf{R}} = \mathbf{M}_{\Lambda}^{-1} \mathbf{M}^{\prime} \tag{8}$$

Equations (4) and (5) can then be applied on the retardance matrix  $M_R$  to estimate the values for  $\delta$  and  $\psi$ . The orientation angle of the axis of the linear retarder ( $\theta$ ) can also be obtained using the value of  $\delta$  and  $\psi$  in Eq. (3).

It follows from the above development that this approach can be used to estimate four independent polarization parameters namely, the linear retardance  $(\delta)$ , the circular retardance  $(\psi)$ , the linear depolarization coefficient  $(\Delta)$  and the linear diattenuation (d). It is pertinent to note here that there exist important differences in the process for decomposition of  $3 \times 3$ Mueller matrix presented here as compared to the process for decomposition of  $4 \times 4$  matrix developed earlier [22,23]. Unlike the decomposition process of  $4 \times 4$  matrix, a matrix  $M_{DR}$  is constructed here (using the matrix M'), Eigen values of which are utilized to decouple the depolarization matrix  $(M_A)$  and the retardance matrix  $(M_R)$  with the assumption that the depolarization of linearly polarized light is independent of the orientation angle of the incident linear polarization vector. The accuracy of this approach for decomposing the retardance and the depolarization matrices in a depolarizing medium would thus be determined by the validity of this assumption. It should also be noted that the decomposition of Mueller matrix also depends upon the order in which the diattenuator, depolarizer and retarder matrices are multiplied. Based on the order of these matrices, six possible decompositions can be performed. Among these, the group in which the diattenuator matrix comes ahead of the retardance and the depolarization matrix  $[M = M_A M_R M_D]$  always lead to a physically realizable Mueller matrix [28]. The decomposition process discussed here is therefore based on this convention.

#### 3. Experimental methods

A schematic of the nine element Mueller matrix measurement set-up is shown in Fig. 1. The 632.8 nm output from a He-Ne laser (Suresh Indu Lasers, India) was used as excitation source. A linear polarizer (P1) was used to illuminate the sample with desired polarization

states. Another linear polarizer (P2) was used to analyze the polarization states of the light scattered from the sample. The scattered light emerging from the sample was collected with an f/3 lens and after passing through subsequent polarizing optics was imaged onto a CCD detector (ST6, SBIG, USA). While performing measurements in the forward scattering direction, the analyzers and the collection optics were kept at an angle of  $0^{\circ}$  with respect to the direction of the ballistic beam. The collection angle was  $\sim 20^{\circ}$ .

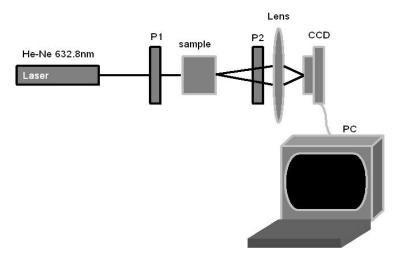


Fig. 1. A schematic of the nine element Mueller matrix measurement set-up

For the measurement of spectral Mueller matrix from the samples, a commercial spectrofluorometer (SPEX, Fluorolog II, USA) was used. The light from a 450W Xenon lamp filtered through the excitation monochromator was used to illuminate the samples. An aperture was kept to limit the spot size to 1 mm. A linear polarizer was kept in front of the sample to generate the three desired input polarization states. The elastic scattering signal was collected at  $\sim 20^{0}$  angle with respect to the exact backscattering direction and were passed through an analyzer before detection. A synchronous scan with zero offset between the excitation and the emission monochromators was used to record the polarized elastic scattering spectra (340 nm - 650 nm) at nine different combinations of the orientation angle of the polarizer and the analyzer. The band-pass for both the excitation and emission monochromator was 1.7 nm and the integration time was kept 0.2 sec. The spectra recorded at different linear polarization channels were corrected for the sensitivity of the instrument to the different linear polarization states (at angles of  $0^{0}$ ,  $45^{0}$  and  $90^{0}$  from the horizontal).

In order to construct the  $3 \times 3$ -intensity measurement matrix  $(M_i)$ , we generated the required three incident polarization states (linear polarization at angles of  $0^0$ ,  $45^0$  and  $90^0$  from the horizontal) and recorded the intensity of the light transmitted (or backscattered) through samples after it passed through the suitably oriented analyzers (linear polarization at angles of  $0^0$ ,  $45^0$  and  $90^0$  from the horizontal). We define polarization state generator (PSG) and polarization state analyzer (PSA) matrix as [24]

$$PSA = \begin{bmatrix} 1 & 1 & 0 \\ 1 & -1 & 0 \\ 1 & 0 & 1 \end{bmatrix} \qquad PSG = \begin{bmatrix} 1 & 1 & 1 \\ 1 & -1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$
(9)

The intensity measurement matrix (M<sub>i</sub>) is related to the sample Mueller matrix (M<sub>s</sub>) as

$$M_i = PSA.M_S.PSG (10)$$

This can also be written as a  $1 \times 9$  vector

$$M_{i \text{ vec}} = W M_{S \text{ vec}}$$
 (11)

Where W is a 9 × 9 matrix given as Kroneker product of PSA with transpose of PSG

$$W = PSA \otimes PSG^{T}$$
 (12)

 $M_{S \, vec}$  is the sample Mueller matrix written in a  $1 \times 9$  vector form.

For an ideal system, W can be written as

$$W = \begin{bmatrix} 1 & 1 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 1 & 0 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & -1 & -1 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & -1 & 0 & -1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & 0 & 1 & 1 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 1 & -1 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 1 \end{bmatrix}$$

$$(13)$$

This kind of construction allows calibration for non-ideal components since one can replace the ideal stokes vector in each column of PSG or each row of PSA by the measured Stoke's vectors that may deviate from ideal value. The errors arising due to the finite extinction ratios of the linear polarizer and the analyzer was incorporated using the generated matrix W corresponding to these polarizers.

The experimental set-up was first used to record Mueller matrix from known optical components such as mirror, diffuser, linear polarizer and quarter wave plates etc. Each of the nine independent measurements required to construct the  $3\times3$  Mueller matrix was repeated three times and the average value was taken to construct the Mueller matrix of the sample. Typical value of error in each element was found to lie between 1-3%. After obtaining satisfactory results from these standard measurements, the set-up was used to record Mueller matrix from the samples investigated in this study. The turbid scattering samples were prepared using aqueous suspension of polystyrene microspheres (Bangs Lab., USA) with mean diameter of either 0.61  $\mu$ m or 2.0  $\mu$ m. The values for scattering coefficient ( $\mu$ s) of the samples were varied by dilution. The samples were kept inside quartz cuvette having path length of either 5 mm or 10 mm while taking measurements. The collagen samples used in

this study were extracted from eggshell membrane. Eggshell membrane is known to contain mainly Type I and Type V collagen. The Type I collagen from the inner shell membrane was extracted by acid-pepsin digestion method [29]. Briefly, the outer membrane was removed by dipping the shell in 1% Triton X and then washing thoroughly with tap water. The shells were then dipped in 1N HCL for 1 hour. The inner membrane consisting of Type I collagen gets separated which was then removed and washed with distilled water. To remove the attached globular proteins, the membranes were dipped in a mixture of 1:1 1N HCL and water containing pepsin powder for 24 hrs in fridge and the Type I collagen was extracted.

### 4. Results and discussion

In order to illustrate the efficacy of the polar decomposition of  $3\times3$  Mueller matrix, we first present results on theoretical calculation of single scattering Mueller matrix of an achiral (having no optical rotatory power,  $\psi=0$ ) spherical scatterer with known size and refractive index. The  $3\times3$  scattering matrix [S ( $\Theta$ ),  $\Theta$  is the scattering angle] for a spherical scatterer with diameter  $d=2.0~\mu m$  at a wavelength  $\lambda=632.8~nm$  was computed using Mie theory [30]. The refractive index of the scatterer (n) and that of the surrounding medium ( $n_{medium}$ ) was taken to be n=1.59 and  $n_{medium}=1.33$  respectively. The computed  $3\times3$  non depolarizing Mueller matrix was decomposed into diattenuation ( $M_D$ ) and retardance ( $M_R$ ) matrices following the procedure described in Section 2 and the values for diattenuation (d), linear retardance ( $\delta$ ) and optical rotation ( $\psi$ ) of scattered light was calculated using Eqs. (4) and (5). The variation of d,  $\delta$  and  $\psi$  as a function of scattering angle  $\Theta$  is shown in Fig. 2.

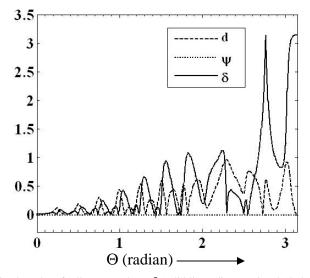


Fig. 2. The values for linear retardance  $\delta$  (solid line), diattenuation d (dashed line) and optical rotation  $\psi$  (dotted line) obtained from polar decomposition of single scattering Mueller matrix ( $\delta$  and  $\psi$  are in radian).

As expected, the value for  $\psi$  is observed to be zero and the values for d and  $\delta$  are seen to vary significantly with increasing value of  $\Theta$ . The observed variation of d and  $\delta$  with  $\Theta$  was previously shown to be originating from the difference in amplitude and phase between the scattered light polarized parallel  $[0.5 \times \{S_{11}(\Theta) + S_{12}(\Theta)\}]$  and perpendicular  $[0.5 \times \{S_{11}(\Theta) - S_{12}(\Theta)\}]$  to the scattering plane [22]. The oscillations observed in the angular variation of d and  $\delta$  are characteristics of monodisperse spherical scatterers and are expected to be washed out in presence of subtle distribution in the size of scatterers [30]. Important to note here that

the values for d,  $\delta$  and  $\psi$  obtained from the decomposition of the  $3 \times 3$  matrix is identical to that obtained using the decomposition of the  $4 \times 4$  matrix (the results were presented in our previous paper, [22]). Single scattering calculations performed on several other achiral ( $\psi = 0$ ) or chiral ( $\psi \neq 0$ ) scatterers having known size and refractive indices confirmed that the decomposition of  $3 \times 3$  Mueller matrix following the approach described in Section 2 yields the same value of d,  $\delta$  and  $\psi$  as for that obtained following the decomposition of  $4 \times 4$  Mueller matrix of scattered light for the same scatterers.

In order to test the experimental validity of the approach, studies were conducted on samples with known polarization and scattering properties. Nine element Mueller matrices were recorded in transmission mode, separately from a linear retarder (linear retardance at 632.8 nm is  $\delta = \pi/2$ ) and from turbid scattering samples prepared using aqueous suspension of polystyrene microspheres with mean diameter of either 0.61  $\mu$ m or 2.0  $\mu$ m. The angle of orientation of the fast axis of the linear retarder was kept at 5° from the horizontal. Mueller matrix was also recorded from the linear retarder with the scattering sample placed in front of it. These experiments were performed using the 632.8 nm line of the He-Ne laser as excitation source. Equation (14) shows the recorded 3  $\times$  3 Mueller matrix (M) for the linear retarder. The value for linear retardance ( $\delta$ ) and the orientation angle of the axis of the linear retarder ( $\theta$ ) were calculated to be  $\delta = 1.56$  and  $\theta = 5.5^{\circ}$  respectively using Eqs. (3) and (4) of Section 2.

$$\mathbf{M} = \begin{bmatrix} 1.000 & 0.007 & 0.051 \\ 0.012 & 0.966 & 0.201 \\ -0.067 & 0.199 & 0.018 \end{bmatrix}$$
 (14)

$$\mathbf{M} = \begin{bmatrix} 1.000 & 0.071 & 0.005 \\ 0.053 & 0.748 & -0.034 \\ -0.008 & -0.001 & 0.738 \end{bmatrix} \tag{15}$$

In Eq. (15), we show the Mueller matrix recorded from a scattering sample prepared using aqueous suspension of 0.61  $\mu$ m diameter polystyrene microspheres ( $\mu_s = 2 \text{ mm}^{-1}$ ). The sample was kept inside a quartz cuvette with path length of 10 mm. The value for the linear depolarization coefficient ( $\Delta$ ) obtained from the decomposition was  $\Delta=0.74$  (where  $\Delta=1$ corresponds to completely polarized light). It is important to note here that the values for the linear depolarization coefficients for incident horizontally polarized light and light polarized at 45° from horizontal direction are comparable for this sample (the value for the elements M<sub>22</sub> and M<sub>33</sub> of the depolarization matrix are nearly equal). Measurements were also conducted on samples with different other concentration of the scatterers. The value for M22 was always found to be nearly equal to the value for M<sub>33</sub> for all the turbid scattering samples  $(u_s = 1-6 \text{ mm}^{-1})$ . These results confirmed that the assumption, the depolarization of linearly polarized light due to scattering is independent of the orientation angle of the incident linear polarization vector, is valid for a turbid medium. This should be expected also because in a turbid medium, depolarization of linearly polarized light takes place mainly because of the randomization of the filed vector's direction by a random sequence of scattering at arbitrary scattering and azimuthal angles. This can be treated to be an incoherent addition of Mueller matrices of isotropic depolarizers and thus the linear depolarization coefficients of the resultant depolarizing Mueller matrix will be the same for the two different incident linear polarization states.

In Table 1, we show the recorded  $3 \times 3$  Mueller matrix (M) from the linear retarder with the scattering sample placed in front of it. The decomposed matrices  $M_\Delta$ ,  $M_R$  and  $M_D$  are also displayed in the table. Following the procedure described in Section 2, the value for linear retardance ( $\delta$ ), orientation of the linear retarder ( $\theta$ ) and linear depolarization ( $\Delta$ ) were estimated to be  $\delta = 1.57$ ,  $\theta = 5.7^{\circ}$  and  $\Delta = 0.75$  respectively. These values are reasonably close to the corresponding values obtained from separate measurements on the linear retarder and the pure depolarizer ( $\delta = 1.56$ ,  $\theta = 5.5^{\circ}$  and  $\Delta = 0.74$ ).

Table 1. The measured  $3 \times 3$  Mueller matrix (M) and the decomposed components for the combination of the linear retarder and the turbid scattering sample.

M						
	<u> </u>	0.061	0.015	]		
	0.045	0.729	0.118			
	-0.023	0.140	0.023	]		
$\mathbf{M}_{\!\Delta}$ $\mathbf{M}_{\!\mathbf{R}}$			$M_D$			
$\begin{bmatrix} 1 & 0 & 0 \end{bmatrix}$	<u> </u>	0	0	1	0.061	0.015
	-0.002	0.969	0.157	0.061	1	0
0 0 0.753	-0.002 -0.043	0.188	0.031	0.015	0	0.998

We also repeated the experiment with the scattering sample kept both in front of and at the back of the linear retarder. In this case, the scattering samples were kept in two 5 mm path length cuvettes with the linear retarder kept at close contact in between the two cuvettes. The angle of orientation of the axis of the linear retarder was kept at  $10^{\rm o}$  in this case. The measured Mueller matrix (M) and the decomposed matrices  $M_{\Delta},~M_R$  and  $M_D$  for this combination of linear retarder and scattering samples are displayed in Table 2. The values  $\delta=1.56,~\theta=10.3^{\rm o}$  and  $\Delta=0.79$  obtained from the polar decomposition approach are again found to be reasonably close to that expected for this combination.

Table 2. The measured  $3 \times 3$  Mueller matrix (M) and the decomposed components for the combination of linear retarder kept in between the two turbid scattering samples.

As noted previously, the accuracy of the present approach for decomposing the retardance and the depolarization matrices is determined by the validity of the assumption that depolarization of linearly polarized light due to scattering is independent of the orientation angle of the incident linear polarization vector. The results presented above show that this assumption is valid in a turbid medium where depolarization of linearly polarized light takes place due to randomization of field vector's direction as a result of multiple scattering. This approach is therefore expected to be applicable in a turbid and multiply scattering medium like biological tissue. Since the 3 × 3 Mueller matrix measurements require just two linear polarizers with nine combinations of input and output state of polarizations, this would allow one to perform spectral polarimetric (quantification of the polarization parameters at several wavelengths) measurements on tissue with relative ease as compared to that for the  $4 \times 4$  Mueller matrix (because of the complexities originating due to the usage of quarter wave retarder at multiple wavelengths). Such measurements could provide additional information and may turn out to be useful for various diagnostic applications. In order to illustrate the accuracy of such spectral polarization measurements, in Fig. 3, we show the wavelength dependence of linear retardance  $[\delta (\lambda)]$  obtained by decomposing the nine element spectral Mueller matrix ( $\lambda = 420 \text{ nm} - 650 \text{ nm}$ ) measured from a linear retarder (linear retardance at 632.8 nm is  $\delta = \pi/2$ ). The data are presented at 5 nm intervals. The corresponding theoretical variation of  $\delta$  with wavelength is shown by squares in Fig. 3. The agreement is seen to be reasonable.

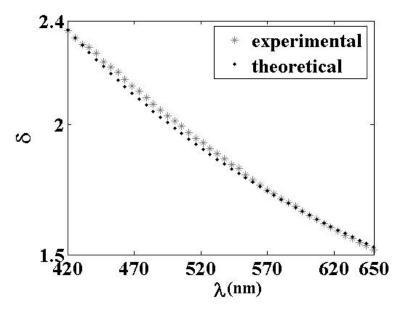


Fig. 3. The wavelength variation of linear retardance  $\delta$  obtained by decomposing the measured nine element spectral Mueller matrix of a linear retarder (symbol asteryx). The theoretical variation of  $\delta$  with wavelength is shown by squares.

Having obtained satisfactory results on estimating the wavelength variation of linear retardance of ideal linear retarder, experiments were performed on Type I collagen extracted from eggshell membrane. On microscopic examination, the collagen showed mesh like oriented fibrous structures. The structural aggregates of collagen (micro-fibrils, fibrils and fibers) are known to produce such oriented fibrous structures in collagen matrix. The spectral Mueller matrix measurements from the collagen samples were performed using the spectrofluorometer. In Fig. 4(a), we show the nine elements of the spectral Mueller matrix recorded from the collagen sample (results are shown for the wavelength range 380 nm - 480 nm). The wavelength variation of linear depolarization  $[\Delta(\lambda)]$ , linear retardance  $[\delta(\lambda)]$  and linear diattenuation  $[d(\lambda)]$  obtained following the polar decomposition of the spectral Mueller matrix is displayed in Figs. 4(b), 4(c) and 4(d) respectively. As can be seen from the figure, the collagen sample shows appreciable values of linear retardance and linear diattenuation. The observed linear retardance and diattenuation is known to originate from the oriented fibrous structure of collagen [8-10, 12, 14]. The collagen contains helix-type molecule binding structures whose anisotropic alignment is the origin of the birefringence property of collagen fibers. Measurements were also repeated on the same sample by changing its physical orientation. This was done by rotating the sample at an angle  $90^{\circ}$  in the plane normal to the direction of propagation of the incident light. The magnitude of  $\Delta(\lambda)$ ,  $\delta(\lambda)$  and  $d(\lambda)$ was not found to change significantly with altered physical orientation of the sample. However, as expected, the orientation angle of the linear retarder  $\theta$ , obtained from the decomposed retardance matrix M<sub>R</sub> was observed to change with altered physical orientation of the sample.

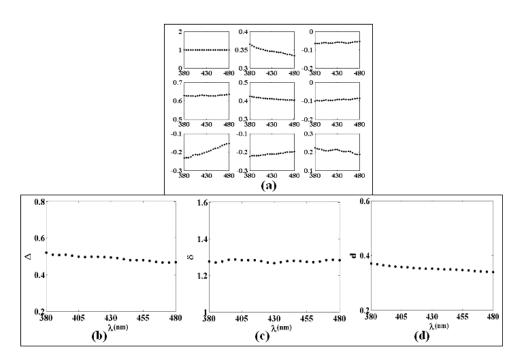


Fig. 4. (a) The nine elements of spectral Mueller matrix recoded from Type I collagen extracted from eggshell membrane. The wavelength variation of (b) linear depolarization  $[\Delta(\lambda)]$ , (c) linear retardance  $[\delta(\lambda)]$  and (d) linear diattenuation  $[d(\lambda)]$  obtained following the polar decomposition of the spectral Mueller matrix.

It should be mentioned here that the estimated value for  $\delta$  (or  $\theta$ ) of the collagen sample cannot be directly related to the microscopic structure or orientation of any individual fibers of the collagen network, rather it can be treated as an average value over the area of the sample probed by the light beam. Though the parameters  $\delta$  (or  $\theta$ ) and d, describes the linear retardance and diattenuation of the sample in an average sense, these would have diagnostic value because in various kinds of tissue abnormalities, the structural and functional properties of collagen and other fibrous structures show distinct changes, which is expected to lead to a change in their net linear retardance (and the orientation angle of the retarder) and diattenuation values. In order to explore this possibility, we have conducted preliminary studies on spectral Mueller matrix measurements from normal and cancerous (squamous cell carcinoma) tissues resected from human oral cavity. Results of measurements on a limited number of tissue samples showed that the values for  $\delta(\lambda)$  and  $d(\lambda)$  are considerably lower in the cancerous tissues as compared to that for the normal tissues. This possibly arises due to the denaturization of collagen (present in the connective tissue layer) in the cancerous tissues, which results in a loss of linear retardance of the tissues. Detailed studies on this aspect and on exploring possibility of using  $\delta$  ( $\lambda$ ) and d ( $\lambda$ ) as diagnostic parameters, is presently underway and the results will be reported after successful completion of the studies.

### 6. Conclusion

To conclude, we have presented a method based on polar decomposition of Mueller matrix for quantification of the different polarization parameters of a scattering medium namely, the linear retardance, the circular retardance, the linear depolarization coefficient and the linear diattenuation using the nine element  $(3 \times 3)$  Mueller matrix measured from the medium. It should however, be mentioned that quantification of the circular depolarization coefficient and the circular diattenuation would necessitate measurement of  $M_{44}$  and  $M_{14}$  elements of the

 $4 \times 4$  Mueller matrix and these therefore cannot be estimated using the decomposition of  $3 \times 3$  Mueller matrix. Further, the decomposition of the  $3 \times 3$  Mueller matrix is based on the assumption that the depolarization of linearly polarized light due to scattering is independent of the orientation angle of the incident linear polarization vector which is valid for a turbid and multiply scattering medium like tissue. The applicability of the approach was tested by carrying out studies on various samples having known scattering and polarization properties. Since the  $3 \times 3$  Mueller matrix requires measurements using linear polarizers only, it considerably simplifies measurement procedure and also allows one to quantify the wavelength dependence of the useful polarization parameters of a turbid medium like biological tissue with relative ease. This approach may thus find useful diagnostic applications.