Spatial pattern characterization of linear polarization-sensitive backscattering Mueller matrix elements of human serum albumin sphere suspension

Ping Sun · Xianping Cao · Hanxu Sun ·
Mengmeng Sun · Min He

Received: 29 October 2012 / Accepted: 18 February 2013 / Published online: 4 April 2013
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Abstract Human serum albumin (HSA) nanometer or micron particles represent promising drug-carrier systems. The azimuthal and radial variations of a linear polarization-sensitive backscattering Mueller matrix were experimentally studied in two cases: the scattering particle was smaller or larger in size to the probing wavelength of 780 nm. The results show that the twofold and fourfold structures are characteristic of small particle size suspension, whereas the eightfold structure is characteristic of large particle size suspension. Moreover, for both particle size suspensions, the element patterns have strong radial dependence when the suspension concentration and the incident power of laser change. In addition, for both particle size suspensions, the rotational symmetry of each element is lost in the case of oblique incidence but the multifold structure is maintained. Some suggestions for applications of Mueller matrix imaging in biomedical optics are provided.

Keywords Backscattering · Linear polarization · Mueller matrix · Turbid media · Human serum albumin

1 Introduction

Several non-invasive medical diagnostic techniques for pathological and cancer tissues are associated with the nature of light–tissue interactions [1]. Polarized light is important in

P. Sun (✉) · X. Cao
Department of Physics, Beijing Area Major Laboratory of Applied Optics, Beijing Normal University,
Beijing 100875, China
e-mail: pingsun@bnu.edu.cn

H. Sun
School of Automation, Beijing University of Posts and Telecommunication, Beijing, 100876, China

M. Sun · M. He
College of Science, Leiden/Amsterdam Center for Drug Research,
Leiden University, Leiden, Netherlands

biomedical applications such as investigating the asymmetric (chiral) nature of biological molecules [2–4], determining blood glucose concentrations [5–7], and differentiating normal and precancerous cells in superficial tissue layers [8, 9]. The polarization state and intensity of light change as it propagates through a biological tissue. The Mueller matrix contains information of light scattering in a turbid medium and thus shows great promise for the efficient use of polarization parameters [1]. The Mueller matrix is a four-by-four matrix generally denoted as $M = [m_{ij}]$ ($i = 1-4$; $j = 1-4$) [10, 11]. The first three-by-three submatrix (m_{ij} , $i = 1-3$; $j = 1-3$) is only related to linearly polarized incident light and scattering light at different polarized directions, whereas the last column (m_{ij} , $i = 1-4$; $j = 4$) is related to circularly polarized incident light with right helicity and linearly polarized scattering light. The last row (m_{ij} , $i = 4$; $j = 1-4$) is reversed. Investigations into the effect of a controlled turbid medium of microscopic objects on every polarization state showed that the spatial pattern of the Mueller matrix was influenced by the mean scattering particle size [12, 13], concentration [13], shape [14], structure [15], and optical properties of the turbid medium [16]. For example, Dillet et al. [12] measured the average particle size and concentration of emulsions by comparing experimental polarization patterns with Monte Carlo simulation. Cameron et al. [16] presented a method of Mueller matrix imaging that estimates the scattering coefficient contribution as a function of particle size in complex mixtures of polystyrene spheres (PST). Theoretical and experimental studies, so far, have shown that the first submatrix changes significantly with parameters of the turbid medium [7, 8, 11–20].

Nanometer and micron particles made of human serum albumin (HSA) represent a promising strategy for targeted drug delivery to tumor cells by enhancing the drug's bioavailability and distribution and reducing the body's response towards drug resistance [21, 22]. HSA particles have an important potential application for drug-carrier systems not only because they are relatively easy to prepare and their size distribution can be easily monitored but also because they are biodegradable and non-antigenic for the human body [23]. HSA sphere suspension, indeed, is a biologically turbid medium compared with traditional tissue-simulating phantoms like fat emulsion and PST sphere solution. Therefore, the study of the interaction between light and HSA sphere suspension is more meaningful than tissue-simulating phantoms for the application of Mueller matrix imaging in biomedical optics. This paper aims to investigate the azimuthal and radial variations of patterns of linear polarization-sensitive Mueller matrix elements, i.e., the first submatrix from HSA sphere suspensions. Through studying the dependence on the size of the scatterer, suspension concentration, incident angle of light, and laser illuminating power, we strive to provide some useful suggestions for applications of Mueller matrix imaging in biomedical optics.

2 Materials and methods

2.1 Preparation of HSA sphere suspensions

The HSA particles were prepared by a desolvation process [23]. A 25-ml HSA solution with a certain concentration in a beaker was transformed into particles by the continuous addition (1 ml/min) of a desolvating agent, i.e., ethanol. The solution was constantly stirred (900 r/min) at a certain temperature until the HSA suspension changed from a clear solution to a white turbid liquid. Glutaraldehyde solution was added to achieve particle

Table 1 Preparation parameters of different particle sizes

Particle size (nm)	Concentration (%)	Temperature (°C)	Ethanol (ml)
94	2	2	40
190	4	15	40
733	4	20	40
1350	4	30	40
4240	10	25	25

cross-linking for 24 h after protein desolvation. The resulting particles were purified by vacuum distillation. Three cycles of centrifugation with subsequent redispersion of the sphere doubled the original volumes in water. Table 1 shows the detailed parameters of various particle sizes. The HSA sphere suspensions were used in experiments within a week of preparation.

The sphere particle sizes of five HSA suspensions, which were measured by the Laser Particle Size Analyzer (Horiba LB-550), followed logarithmic Gaussian distribution with average particle sizes of 94, 190, 733, 1,350, and 4,240 nm. The analysis of sphere morphology showed uniform spheres under a scanning electron microscope (SEM: Hitachi S-4800). Figure 1 shows an SEM image of the 1,350 nm-sized sphere particle. Similar results were obtained for the other particle sizes.

2.2 Imaging method

Figure 2 shows a schematic diagram of the experimental setup. A semiconductor laser (Beijing LASER OptoMechatronic) with a wavelength of 780 nm was used as the light source. A skewed-illumination geometry was implemented to avoid glare. A polarizer P_1 was placed in front of the laser and the emergent light was linearly polarized in the horizontal direction. Because the light from the semiconductor laser was partially polarized, a half-wave plate (HW) was placed behind P_1 to change the linear polarization direction from horizontal to another direction that simultaneously kept the output intensity unchanged. The image of the backscattered light distribution on the sample surface was captured by a black-and-white charge-coupled device camera (CCD, DH-SV1420FM, China Daheng Group Inc.) mounted with a lens (M0814-MP, Computar). The CCD chip

Fig. 1 SEM image of the 1,350 nm sized particles

Fig. 2 Schematic diagram of the setup for Mueller matrix imaging, P_1 , P_2 : polarizers; HW : half-wave plate; *cell*: suspension container

had 1392×1040 pixels with a pixel size of $4.6 \mu\text{m} \times 4.6 \mu\text{m}$. The CCD camera had a dynamic range of 12 bits. The linearly polarized incident light was located at a specific angle with respect to the receiving direction. The incident beam was located on the same plane where the scattered light was received. The intensity of the images in the linearly polarized light from the scattered light was captured when a polarizer P_2 was placed in front of the CCD. A quartz sample cell ($10 \text{ cm} \times 10 \text{ cm} \times 2 \text{ cm}$) was filled with 180 ml HSA sphere suspension. The three facets and the bottom of the sample cell were blackened except for the frontal surface to avoid stray light.

The linear polarization-sensitive Mueller matrix elements were calculated from nine recorded polarization reflectance images, which were different combinations of incident and detection polarization states. The nine combinations were HH/HV/HP, PH/PV/PP, and VH/VV/VP. Each measurement was represented by two letters, where the first letter denotes the incident polarization state and the second letter denotes the detection polarization state. The x -axis was chosen as the reference axis and H, V, and P were selected as the linearly polarized light that have polarization directions aligned with the x -axis, y -axis, and at 45° toward the x -axis, respectively. For example, the HP element represents the image acquired with incident polarization H and detection polarization P. The time of measurement of the nine original images took about 2'. The linear polarization-sensitive Mueller matrix elements are calculated from the original images as follows [10, 11]:

$$\begin{aligned}
 M_{LS} &= \begin{pmatrix} m_{11} & m_{12} & m_{13} \\ m_{21} & m_{22} & m_{23} \\ m_{31} & m_{32} & m_{33} \end{pmatrix} \\
 &= \frac{1}{2} \begin{pmatrix} HH+HV+VH+VV & HH+HV-VH-VV & 2(PH+PV)-m_{11} \\ HH-HV+VH-VV & HH+VV-HV-VH & 2(PH-PV)-m_{21} \\ 2(HP+VP)-m_{11} & 2(HP-VP)-m_{12} & 4PP-2PH-2PV-m_{31} \end{pmatrix} \quad (1)
 \end{aligned}$$

3 Experimental results

In this study, all images were normalized to the maximum intensity of the respective m_{11} element and the images were drawn in the form of a contour plot.

3.1 Influence of particle size

The linearly polarized incident light was located at a 30° angle with respect to the receiving direction, i.e., the normal direction on the frontal surface of the sample cell. The power of incident light on the sample was adjusted to 0.8 mW. The suspension concentration was 0.3% by weight and the average particle sizes were 94, 190, 733, 1,350, and 4,240 nm. Figure 3 shows the relationships between the matrix M_{LS} patterns and the particle sizes. The

Fig. 3 Matrix M_{LS} patterns of the HSA sphere suspension of 0.3% at a 30° incident angle with different particle sizes: **a** 94, **b** 190, **c** 733, **d** 1,350 and **e** 4,240 nm. Each image displays a $2.8 \text{ cm} \times 2.8 \text{ cm}$ area of the surface

M_{LS} patterns of all elements except m_{11} have a strong azimuthal dependence. The twofold and fourfold symmetric patterns are typical in elements for small scatterers (small in size with respect to the wavelength), i.e., in the Rayleigh regime. While for large scatterers (large in size to the wavelength), i.e., in the Mie regime, the eightfold structure is typical for the elements of the two rows from the bottom in matrix M_{LS} . It is worth noting that the fold number of the element patterns begin to increase for the 733 nm sphere suspension whose scatterer is similar in size to the wavelength.

To quantify the influence of sphere size, we used a one-dimensional (1D) curve to show each element intensity as a function of the azimuthal angle ϕ . From the 1D curve we obtained quantitative information including pattern contour, intensity sign, and value. For example, Fig. 4 shows the intensity of element m_{22} for different particle sizes as a function of the azimuthal angle ϕ . The center of the plots is the light entry point and the radius is 6 mm. The azimuthal angle ϕ ranges from 0° to 360° . Several important features of the plots shown in Fig. 4 can be predicted from the azimuthal dependence. The intersection points of the 1D curve and the red straight line ($I = 0$) correspond to the black parts of the element pattern images. So, the number of intersection points is equal to the number of pattern fold. In Fig. 4 a–e, there are 2, 2, 7, 8, and 8 intersection points for 94, 190, 733, 1,350, and 4,240 nm sphere suspensions, respectively. In addition, one can more accurately determine the sign and value of the intensity from the 1D curve.

3.2 Influence of concentration

We chose two different particle size sphere suspensions. One was a 94 nm sphere suspension and the other was a 4,240 nm sphere suspension. The linearly polarized incident light was

Fig. 4 One-dimensional curve of element m_{22} intensity I for different particle size suspensions as a function of the azimuthal angle ϕ

maintained at a 30° angle, whereas the incident light power on the sample was adjusted at 4 mW for 94 nm and 0.8 mW for the 4,240 nm sphere suspensions, respectively. The suspension concentrations were chosen as 0.3%, 0.5%, and 1%. Figure 5 shows the relationships between the M_{LS} patterns and the suspension concentrations. For the 94 nm sphere suspension, the azimuthal dependence of the patterns for all elements except m_{21} did not change as the concentration increased. The element m_{21} changed from a twofold to a fourfold structure. For the 4,240 nm sphere suspension, however, both the azimuthal and radial dependence of the element patterns, including the second and third row, varied. The azimuthal dependence significantly changed simultaneously with the shrinking of radial dependence.

3.3 Influence of the incident power of the laser

We chose 94 and 1,350 nm sphere suspensions. We varied the suspension concentration and the incident power of the laser. Figure 6 shows some experimental results of the relationships between the matrix M_{LS} patterns and the incident power of the laser. In the

Fig. 5 Matrix M_{LS} patterns 94 nm (top) and 4,240 nm (bottom) sphere suspensions with different concentrations: **a** 0.3%, **b** 0.5%, and **c** 1%. Each image displays a 3.5 cm $\times$ 3.5 cm (94 nm) or 2.5 cm $\times$ 2.5 cm (4,240 nm) area of the surface, respectively

Fig. 6 Matrix M_{LS} patterns 94 nm and 1,350 nm sphere suspensions with different incident laser power: **a** 94 nm, 0.4 mW, **b** 94 nm, 0.78 mW, **c** 94 nm, 4 mW, **d** 1,350 nm, 0.21 mW, **e** 1,350 nm, 0.87 mW, and **f** 1,350 nm, 5 mW. Each image displays a 3.3 cm $\times$ 3.3 cm (94 nm) or 2.5 cm $\times$ 2.5 cm (1,350 nm) area of the surface, respectively

experiments, the linearly polarized incident light was located at 30° . The 94 nm sphere suspension had a 0.9% concentration and the 1,350 nm sphere suspension had a 0.3% concentration. The incident power of the laser on the samples was 0.4, 0.78, and 4 mW for the 94 nm sphere suspension and 0.21, 0.87, and 5 mW for the 1,350 nm sphere suspension. In Fig. 6, for the 94 nm sphere suspension, the azimuthal dependence of all elements except for m_{21} remained almost the same as the laser power changed. Only the element m_{21} changed from a twofold to a fourfold structure. For both particle size suspensions, the M_{LS} pattern has strong radial dependence.

3.4 Influence of the incident laser angle

Again using 94 nm and 1,350 nm sphere suspensions, the incident power on the sample was adjusted to 0.65 mW. The concentration of the 1,350 nm sphere suspension was 0.3% and the linearly polarized incident light was at 30° , 55° and 78° angles. The corresponding backscattering angles to the incident angles 30° , 55° , and 78° were 150° , 125° , and 102° , respectively. Figure 7 shows the relationships between the matrix M_{LS} patterns and the incident angles. The element patterns did not vary with the backscattering angles for the two types of suspension. However, the rotational symmetry of each element was destroyed in the case of oblique incidence compared with the case of normal incidence [13].

4 Analysis

Figure 3 shows that the sphere suspensions with different particle sizes display different azimuthal patterns of the backscattering Mueller matrix. This study has revealed spin

Fig. 7 Matrix M_{LS} patterns of the 94 and 1,350 nm sphere suspensions with different incident angles: **a** and **d** 30° , **b** and **e** 55° , **c** and **f** 78° . Each image displays a $3.3 \text{ cm} \times 3.3 \text{ cm}$ and $2.0 \text{ cm} \times 2.0 \text{ cm}$ area of the surface for the 94 (*top*) and 1,350 nm (*bottom*) sphere suspensions, respectively

angular momentum (SAM) and orbital angular momentum (OAM) interactions of light propagating in a turbid medium [24–26]. The symmetric azimuthal patterns in the various elements reflect the different interaction types taking place during the scattering process. In fact, the twofold, fourfold, or eight-fold azimuthal pattern critically depends on the nature of interaction and on the geometric evolution of the phase introduced by Berry [27]. We qualitatively provide detailed reasons for the different azimuthal patterns with the theory of optical phase vortex described by Schwartz and Dogariu [24]. They constructed a geometrical phase called *spin space*, where the unit vector represented the direction of the spin. Left and right circular waves, traveling in the same direction, were represented by unit vectors pointing in opposite directions. Changes in both the propagating direction and helicity were represented in this spin space with the added benefit that the sign of the geometrical phase emerged naturally. Light that traveled along a planar trajectory and emerged with a flipped helicity acquired no geometrical phase. This happened because the trajectory in the spin space returned to its initial direction without leaving the plane. If some contribution emerged circularly polarized with the same helicity as the incident beam (an even number of helicity flips), then it acquired a phase vortex. If, on the other hand, it followed an odd number of helicity flips the wave did not acquire a phase vortex. This is a manifestation of total angular momentum (TAM) conservation. The SAM associated with helicity and the OAM associated with phase vortices. The SAM and OAM were redistributed in a scattering process in order to satisfy TAM conservation.

According to the rule of TAM conservation, Schwartz and Dogariu [24] wrote the outgoing field by introducing a helicity-maintaining parameter $\alpha(r)$ (where $0.5 \leq \alpha(r) \leq 1$) and obtained some theoretical simulations. If $\alpha(r) = 1$, the scattering process is completely helicity preserving. If $0.5 < \alpha(r) < 1$, the scattering process is the result of multiple events in which the light is scattered at very small angles, i.e., the scattering light became a paraxial polarized wave. Thus the helicity is maintained with a very high probability, which is appropriate for Mie scatterers. In this case, an eight-fold structure appeared in the elements m_{22} , m_{23} , m_{32} , and m_{33} . If $\alpha(r) = 0.5$, the helicity mixed thoroughly, which is appropriate for Rayleigh scatterers. In this case, two-fold symmetric patterns appeared in the elements m_{12} , m_{13} , m_{21} , and m_{31} and a fourfold structure appeared in the elements m_{22} and m_{33} by theoretical simulation. In Fig. 3, the twofold and fourfold azimuthal patterns dominate in the elements for 94 and 190 nm sphere suspensions and the eightfold for 730, 1,350, and 4,240 nm sphere suspensions. These results are in accordance with the simulations given by Schwartz and Dogariu [24].

In Section 3.2, the corresponding scattering coefficients to the concentrations of 0.3%, 0.5%, and 1% for the 94 nm and 4,240 nm sphere suspensions, which are calculated with the Mie theory [28], are 0.6, 1.0, 2.0 cm^{-1} and 24.9, 41.5, 83.0 cm^{-1} , respectively. Thus, the transport mean free paths (MFP) are 5~17 cm and 0.1~0.4 cm for the 94 nm and 4,240 nm sphere suspensions, respectively. The wavelength of the illuminating light is obviously far smaller than these values, so the dominant scattering events are due to the low-order scattering in the present experiments. Here, we explain the experimental results shown in Fig. 5 with two relationships between the depolarization factor or contrast and the number of scattering events undergone by light before exiting from weakly scattering media [29, 30]. Figure 8 shows two curves that illustrate these relationships. When the suspension becomes denser, the number of scattering events increases and thus the diffused backscattering light increases. Multiple scattering gives rise to a much broader spatial distribution. However, the depolarization factor is growing as well and the contrast diminishes. Towards the strongly diffusive regime where light paths become more and more tortuous, the deterministic behavior is overwhelmed by the disorder and the contrast tends to become zero. For

Fig. 8 Relationships between the depolarization factor or contrast and the number of scattering events

the 94 nm sphere suspension, the radial dependence of the patterns extended as the concentration increased. In the experiment, when the suspension concentration was more than 2%, the patterns disappeared. For the 4,240 nm sphere suspension, the contrast went down and the depolarization factor rose up with increase of concentration. When the suspension concentration was smaller than 0.3% in the experiment, a broader spatial distribution was observed. Hence, a qualitative agreement is obtained between the theory and the experiment. The azimuthal patterns of element m_{21} changed because the helicity-preserving and helicity-nonpreserving components changed when the concentration varied.

The case of laser power was similar to the condition that the suspension concentration changed when other parameters were fixed. In fact, the number of photons propagating in the suspension increases when the laser power increases. As a result, the probability of a scattering event is likely to happen. A broader spatial distribution was observed in Fig. 6. The azimuthal patterns of element m_{21} also changed because the helicity changed. The experimental results show that too weak or too strong laser powers result in unclear patterns of some elements like m_{31} and m_{32} . Thus, a suitable power value exists for a clear Mueller matrix image. At this time, images have maximum contrast.

To explain Fig. 7, we calculated numerically the 2D intensity pattern map of M_{LS} with the single-scattering approximation Mie theory [31]. In calculation, the refractive index of the sphere was 1.41 [32], the refractive index of the water was 1.33, and the HSA density was 1.18 g/cm³ [33]. The numerically determined matrix elements show good agreement with experimental data. Taking the element m_{22} of the 1,350 nm sphere suspension as an example, Fig. 9 shows its pattern with a different incident angle of the laser. The rotational symmetry was destroyed in the case of oblique incidence and the larger the incident

Fig. 9 The theoretical m_{22} patterns of 1,350 nm sphere suspension with the single-scattering approximation Mie theory at different incident angles of 0°, 30°, 55° and 75°

Fig. 10 Images of a narrow laser beam with normal (a) and oblique (b) incidences illuminating Intralipid solution

angle was, the greater the rotational symmetry was destroyed. The Monte Carlo simulation results of the first nine elements of the Mueller matrix for 2,000 nm PST suspension also demonstrated that the rotational symmetry diminished with oblique incidence [18].

In our previous studies [34, 35] we found that when a narrow laser beam with normal incidence illuminated semi-infinite turbid media (Intralipid solution), the reflectance diffusion center overlapped the light entry point as shown in Fig. 10a. Hence, the scattering light is isotropic and the azimuthal pattern of the element has rotational symmetry like

Fig. 11 Nine original recorded polarization reflectance images of 1,350 nm sphere suspension of 0.35% with an incident angle of 30°

Hielscher's results [13]. However, for obliquely incident light, the diffusion center related to the light entry point had a horizontal shift Δ away from the light entry point, which was in accordance with the steady-state diffusion theory with two sources [36]. Figure 10b shows the case of oblique incidence. Consequently, the scattering light is anisotropic and the azimuthal pattern of the element losses its rotational symmetry as shown in Fig. 7.

In fact, the original nine recorded polarization reflectance images can give a phenomenological explanation. For example, Fig. 11 shows the original images of 1,350 nm sphere suspension of 0.35% with an incident angle of 30° , where the HV image is called a cross-image. It can be seen that the rotational symmetry is broken but the fold number is maintained. Dogariu [29] had reported that the incidence angle of the illuminating laser beam seemed not to influence substantially the features of the cross-pattern, except for an asymmetry of the pattern being more visible at low-volume fractions. In the present work, similar results were obtained.

5 Conclusions and remarks

The dependence on the size and concentration of the scatterers of the backscattering azimuthal pattern of the Mueller matrix elements is present in HSA. This inspires us to think that the Mueller matrix approach may be a promising tool in biomedical optics. Here, we take HAS particles surface-modified with transferrin as an example. The transferrin receptor (TFR) is the ubiquitous cell surface glycoprotein related to cell proliferation and is overexpressed in cancers like liver, breast, pancreatic, and lung compared to normal tissues because of the higher iron demand of malignant cells for fast growth and division. Hence, transferrin-mediated drug delivery systems have been developed and studied for tumor-specific targeting in vitro and in vivo [37, 38]. HSA particles, as an efficient drug delivery system, have the potential to be used to deliver the maximum amount of drugs (such as Noscapine for breast cancer, Rifampicin for lung cancer, and Erlotinib for liver cancer, etc.) to target sites at a rate and concentration that permits optimal therapeutic efficacy, while reducing any undesirable side effects to a minimum. Particles between 3 and 7 μm will be taken up by the liver and spleen, particles above 7 μm will be entrapped by the lung, and particles on a nanometer scale will be circulated in blood vessels [39, 40]. When the drug is absorbed by the tumor cell, the HSA particles degrade simultaneously. As a result, the concentration and the size of HSA spheres reduce compared to the origin. If we use the Mueller matrix imaging technology to detect some types of tissues, we possibly judge the drug therapeutic efficacy and diagnose the cancer stage.

In the case of oblique incidence, the rotational symmetry of each element was broken, although the fold number was unchanged. It is better to use a normal or small (not larger than 30°) angle in order to analyze the pattern variations accurately in practice. Laser power is also a factor in biomedical optics, since injuries can result if the power value exceeds a safe threshold. Accordingly, Mueller matrix images will have a low SNR and contrast if the power value is too small. Therefore, it is necessary to select an appropriate power value in the application of Mueller matrix imaging.

The factors of measurement errors in the experiment should be assessed. The main sources of measurement uncertainty originate from two factors. The first is the accuracy of the optical component including the polarizer and half-wave plate. In the experiment, the minimum resolution angle of the optical components is 0.5° . The second is the high-frequency speckle noise originating from CCD. It is reduced with a median filter.

In conclusion, the azimuthal and radial variations around the incident point of light were observed by viewing diffuse-backscattered Mueller matrix images with linearly polarized light. The experiments were implemented on two cases: the scattering particle is smaller or larger in size than the wavelength of the probing beam. The results show that twofold and fourfold structures are characteristic for small particle size suspension, whereas the eightfold structure is characteristic for large particle size suspension. Moreover, for both particle size suspensions, the element patterns have strong radial dependence when the suspension concentration and the incident power of laser change. In addition, for both particle size suspensions, the rotational symmetry of each element is lost in the case of oblique incidence but the multifold structure is maintained. The explanation for the azimuthally symmetric patterns in Mueller matrix elements with an optical vortex is helpful to understand the physical origin of the changes via the scattering properties of the turbid medium.

Acknowledgements This work was supported by the Natural Science Foundation of Beijing (Grant No. 4102031).

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