Obtaining shape information of marine microorganisms using polarized-light scattering

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***Abstract*—Polarized-light scattering methods have shown promising potential in characterizing the structural properties of complex biological tissues. Similar methods can also obtain the shape and intracellular information of aqueous suspended microorganisms. In this paper, we measured the Mueller matrix of algae and an alga phantom which consists of cylindrical and spherical scatterers. We also used a Monte Carlo (MC) model containing spheres and randomly oriented infinitely long cylinders to reconstruct the experimental results of cylindrical microorganisms, such as *Synedra* sp. and the phantom. Based on the analysis of the phantom's polarization properties, the relationship between the depolarization ratio and the ratio of scattering coefficient of sphere versus cylinders (RSCSC) is given, which proves the potential of polarized-light scattering methods to obtain shape information of the microorganisms.**

Keywords—Marine microorganism, shape, polarized-light, scattering

I. Introduction

Marine microorganisms, which are responsible for approximately half of the Earth's primary production, play important roles in global issues, such as climate changes and nutrient cycling [1-2]. Many techniques have been developed to detect marine microorganisms *in situ* for environmental monitoring or scientific research [3-4].

Along with the rapidly emerging new photonics technologies, a significant number of research institutions and commercial entities are engaged in research, development and manufacturing of optical instrumentations for oceanographic research and observation [5]. Optical scattering methods have been used in the studies of marine microorganisms and promised the ability for *in situ* detection [6].

One way to measure the marine microorganisms is to find out the quantitative relationship between the scattered light and the physical properties of the scattering particles suspended in water [7]. *In situ* measurements of inherent optical properties (IOPs) of aquatic particles show promise in studies of particle dynamics. Most models of IOPs of marine particles assume that particles are spheres, yet most of the particles that contribute significantly to the IOPs are non-spherical. Many researchers suggest that particle shape affects significantly the IOPs of particles [8]. However, some researches argue that, since the phytoplankton particles are randomly oriented, their shape should not have a major influence on the polarization characteristics of the scattered radiation [9]. Recent reports show that, for the randomly-oriented rod-shaped particles, infinitely long cylinders can be used as a scattering model when the aspect ratio (AR= length/diameter) is bigger than 3 and the diameter is close to the wavelength of the scattered light [10].

Scatterer's shape has important effects on the polarization of the scattered light. We have found that the shape of the scatterers and their alignments will introduce anisotropy into tissues and affect their polarization properties. We have developed techniques to quantitatively characterize such properties [11-13]. We also developed a Monte Carlo (MC) simulation program [14] and a sphere-cylinder scattering model to study polarized-light scattering in complex anisotropic media such as biological tissues [15-17].

In this paper, we approximate the microorganisms as a mixture of microspheres and randomly oriented infinitely long cylinders suspended in water. The ratio of scattering coefficients of these spheres versus cylinders (RSCSC) is related to the shape of the microorganisms. Mueller matrix M is believed to contain all the information of polarization properties of the sample [18]. We measure the angular distribution of the scattered polarized light and use Mueller matrix M to analyze the different polarization scattering behaviors of the two types of scatterers.

$$M \equiv \begin{bmatrix} m11 & m12 & m13 & m14 \\ m21 & m22 & m23 & m24 \\ m31 & m32 & m33 & m34 \\ m41 & m42 & m43 & m44 \end{bmatrix} \tag{1}$$

By comparing the experiments of algae and their MC simulations, we find that the different polarized-light scattering between these algae mainly attributes to their different shape. Analysis based on the experiment and MC simulation of glass fibers (used as phantom) tells that polarized-light scattering methods can obtain the shape information of the scatterers.

II. Methods

Fig.1 shows the schematics of the experimental setup which is able to measures the angular distribution of Mueller matrix elements of the samples. 532nm light from a 40mW solid-state

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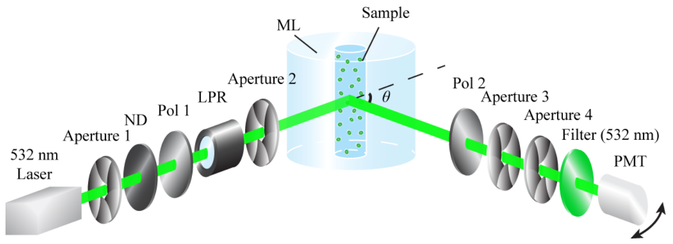

Fig. 1. Schematics of the experimental setup. Pol 1, Polarizer; ND, neutral density filter; LPR, liquid crystal polarization rotator; ML, matching liquid; Pol 2, analyzer; Filter(532 nm), 532 nm narrow band spectral filter; PMT, photomultiplier tube.

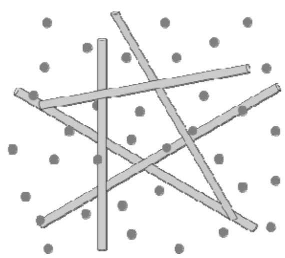

Fig. 2. Schematics of the sphere-cylinder model used in this paper.

laser is attenuated by the neutral density filter (ND) and then passes through a linear polarizer (Pol 1) and a liquid crystal polarization rotator (LPR, Meadowlark Optics) before it illuminates the sample. Linear polarization of the incident light is controlled by varying the voltage applied on the LPR. Scattered photons from the sample pass through the rotating analyzer (Pol 2), two apertures (Aperture 3 and 4) and a 532nm narrow band spectral filter, then are detected by a photomultiplier tube (PMT, Hamamatsu Photonics). Water is used as the refractive index matching liquid (ML) around the sample container.

A Monte Carlo program [14] and a sphere-cylinder scattering model (SCSM) [13-16] are used to simulate the angular behaviors of Mueller matrix of the sample. Validity of the program and the scattering model has been tested in previous studies [13-17]. In the present Monte Carlo simulations, the sphere-cylinder scattering model consists of spheres and randomly oriented infinitely long cylinders suspended in water, shown as in Fig.2, which describes the polarized-light scattering of the aqueous suspended non-spherical scatterers.

III. Results

A. Polystyrene Spheres

In present paper, considered elements include m11, m12, m22, and m33. The setup measures these elements' angular distributions (from 28-120degree) of the aqueous suspended polystyrene spheres. The scatters' average diameter is 2.0μm and refractive index 1.59. Fig. 3 shows the experimental, MC simulated, and Mie calculated results. Mie calculated results

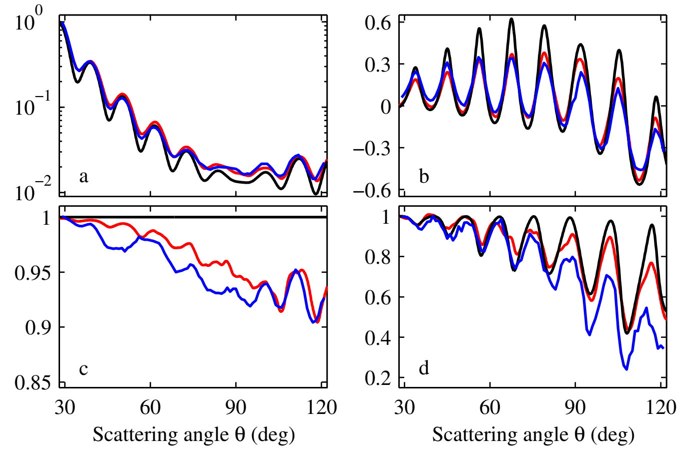

Fig. 3. Experimental (blue), MC simulated (red) and Mie calculated (black) results of the angular distribution of Mueller matrix elements of the aqueous suspended polystyrene spheres. a: m11. b: m12/m11. c: m22/m11. d: m33/m11. "deg" means degree.

give the Mueller matrix of the single sphere based on Mie theory [18]. One can find that the positions of the extrema (peaks and valleys) match well. Best agreement between MC simulated, Mie calculated and measured results was found for spheres with a diameter of 2.08μm. With scattering coefficient of $0.2cm^{-1}$, MC simulation evens out the maxima and minima of the simulated results, such as m11, m12/m11, m33/m11, and obtains the declined curve of m22/m11. There are some small mismatches between the simulated and measured m22/m11 and m33/m11 (especially at large angles) duo to measurement errors and systematic errors. However, the agreement between MC simulated, Mie calculated and measured results in Fig. 3 is seen to be good for these Mueller matrix elements.

B. Algal Samples

The Mueller matrices of both *Synedra* sp. and *Chlorella vulgaris* aqueous suspended samples are measured respectively. Axenic culture of *S.* sp. (FACHB 1296) and *C. vulgaris* (FACHB 31) were obtained from Culture Collection of the Freshwater Algae of the Institute Hydrobiology (FACHB Collection, Wuhan, China) and cultured at 25 °C in D1medium and BG-11 medium, respectively.

Within exponential growth phase, the average diameter of *S.* sp. is about 7.0μm and the length is about 150μm. Fig.4 (left)

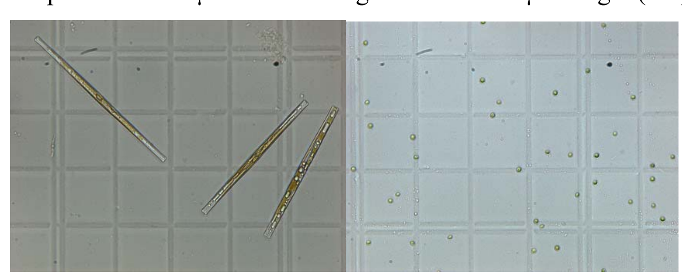

Fig. 4. Microscopic images of the *S.* sp. (left) and *C. vulgaris* (right). The length of the side of the minimal square of the blood cell counting chamber is 50.0μm.

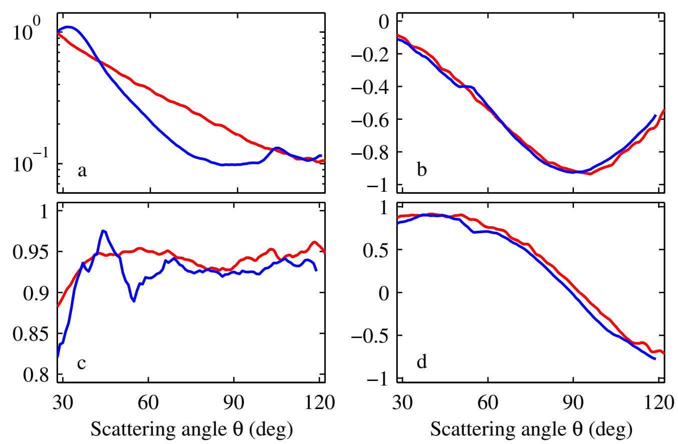

Fig. 5. Measured Mueller matrix elements of *S.* sp. (blue lines) and simulated results (red lines). a: m11. b: m12/m11. c: m22/m11 d: m33/m11. "deg" means degree.

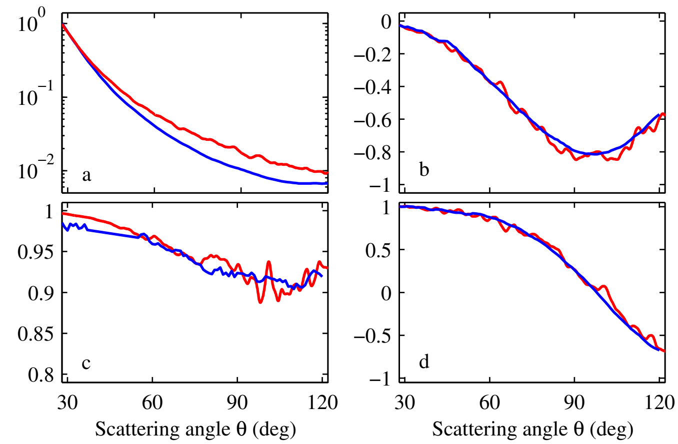

Fig. 6. Measured Mueller matrix elements of *C. vulgaris* (blue lines) and simulated results (red lines). a: m11. b: m12/m11. c: m22/m11. d: m33/m11. "deg" means degree.

gives the microscopic images of the individual alga which shows its cylindrical shape and internal structure. The experimental results are shown in Fig.5 (blue lines), one can find that there are increasing courses both for m22/m11 (values from 0.82-0.95) and m33/m11 (values from 0.80-0.90) at the interval of 28-40degree. Different from *S.* sp., shown as Fig.4, the shape of *C. vulgaris* is mostly spherical. The average diameter is about 3.0µm. The experimental results are shown in Fig.6 (blue lines). From Fig. 6, there are declination in the interval of 28-40degree, which is totally different both for m22/m11 and m33/m11 from those of *S.* sp., shown as Fig.5.

MC simulations are shown in Fig.5 and 6 (red lines) too. According to the microscopic image Fig.4, in the model for *S.* sp., the randomly oriented infinitely long cylinders with the diameter 7.0µm are chosen for the main outer shape, and the spheres with 0.2µm diameter is used for the contribution of the internal structures. The refractive indices of scatterers are 1.40, and the imaginary parts are not considered for their weak absorption at 532nm. The refractive index of the surround water is 1.33. And *C. vulgaris* is modeled by two kinds of spheres, 3.0µm and 0.2µm diameters respectively; and the refractive index settings are the same as *S.* sp.. From Fig.5, m11 is not reconstructed well, partially due to the systematic error of the setup, especially when the concentration of sample of *S.* sp. is very low.

As shown inFig.5 and 6, the experimental and simulated results are fitted well, especially for the m22/m11, m12/m11 and m33/m11, which indicated that the MC model could equivalently reconstruct the polarized-light scattering properties of these two kinds of algae. However, differences are also reconstructed, which means the differences root in the shape of two algae, that is *S.* sp. is cylindrical but *C. vulgaris* is spherical.

IV. Analysis and Discussion

To simplify the analysis of the effects of shape on the polarized-light scattering, pieces of glass fiber are used as the phantom of the cylindrical algae. From the scanning electronic microscope (SEM) image, shown as Fig.7, the glass fiber is about 10.0µm in diameter; and it's l about 1mm in length. In addition to this, more information can be found, such as some microstructures on the surface of the fibers, which implicates that the phantom contains not only cylinders but also small spheres.

Experimental results are shown in Fig.8 as blue lines and the MC simulated results are shown as red lines. The equivalent model consists of cylinders and spheres of 10.0µm diameter, and the main features are reconstructed, especially for m11, m22/m11 and m33/m11 around 50-90degree, although differences between both are obvious at other angles. More careful examinations indicate that adding more other scatterers, such as smaller spheres, will make the simulated m33/m11 and m22/m11 curves close to the experimental results. However, in this paper, we try to pay attention to the contribution of cylinders to the polarized-light scattering, and omit the detailed noise.

Gordon has considered the dependence of IOPs on aspect ratio, and told that when the aspect ratio of the scatterers is bigger than 3, the extinction efficiency and the backscattering probability are close to those of an infinitely long cylinder [10]. We have tested in the simulations different sample components, such as only cylinder, only spheres and their mixture, and find that 1) if the sample consists of only cylinders or only spheres, one cannot reconstruct the experimental curves; 2) in the mixture case, the cylinders dominate the main line style, such

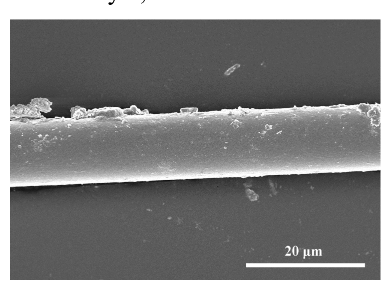

Fig. 7. Scanning electronic microscope (SEM) image of the glass fiber.

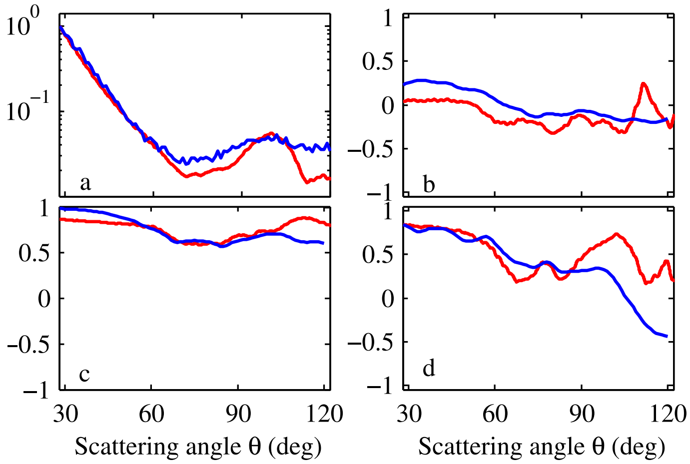

Fig. 8. Measured Mueller matrix elements of glass fiber (blue lines) and simulated results (red lines). a: m11. b: m12/m11. c: m22/m11. d: m33/m11. "deg" means degree.

as the angles of peaks and valleys, and the spheres affect the values.

More MC simulation have been done and collected in Fig.9a in which the scattering coefficient of spheres is changing but that of the cylinders is fixed. In Fig.8, blue lines are experimental curve. The values of the simulated m22/m11 rise as the scattering coefficient of spheres increases. Depolarization ratio D (= 1 – m22/m11) is a measure of the deviation from pure spherical scatterers [18]. Bronk et al. have shown that D is sensitive to the length changes, and D is lower with smaller length, and they confine their conclusion in the condition that the sample is capped cylinder and AR is small [19].

From the view of our model, smaller length means higher RSCSC. To quantitatively study the effect, values around 76degree (near the minimum of the experimental curve) are recorded and plotted in Fig.9b and fitted with double-exponential function (solid line). The monotonous curves shown Fig.9b tells that D decreases as RSCSC. That is D will be lower as the cylinders become less, or in other words, as the spheres become more. This is consistent with that of Brook, despite of the confinement, which indicated that RSCSC is a parameter similar with AR to describe the shape information of the sample. In addition, the monotonously declined curve in Fig.9b tells that the shape information of the sample can be obtained from the measured m22/m11 or D.

V. Conclusion

Shape information is important property of the marine microorganism. This paper presents a polarized-light scattering model and a phantom of microorganism. The model is composed by the spheres and randomly oriented infinitely long cylinders. The phantom contains pieces of glass fibers of rough surface. Monte Carlo simulation based on the model can reconstruct the main features of the experimental data of *Synedra* sp. and the phantom. Further, simulation studies reveal

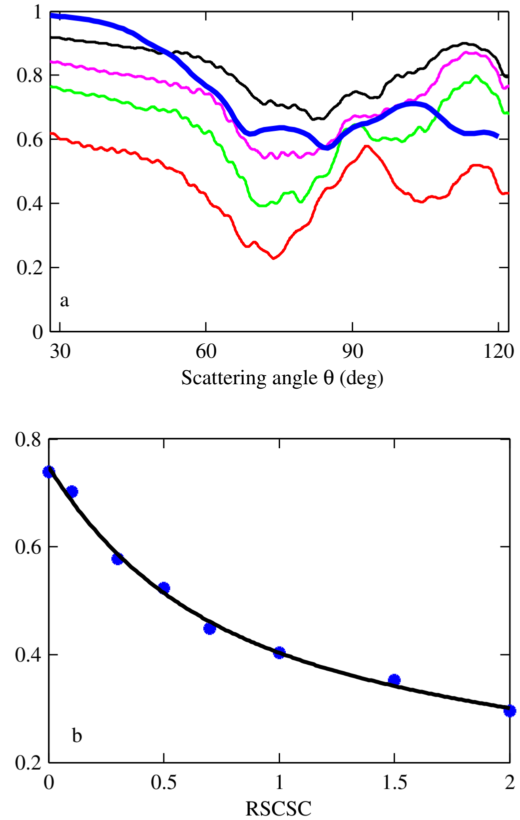

Fig. 9. m22/m11 curves versus the changing scattering coefficient of the spheres (a), 0 (red), 0.03 cm^{-1} (green), 0.07 cm^{-1} (magenta) and 0.2 cm^{-1} (black), but that of cylinders is fixed at 0.1cm^{-1}. The corresponding values (points in b) at 76 degree, and the fitted lines (solid in b). "deg" means degree.

that the values of depolarization ratio at certain angles decrease as the ratio of scattering coefficient of sphere versus cylinders, which indicates the potential of polarized-light scattering methods to obtain shape information of the microorganisms.

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