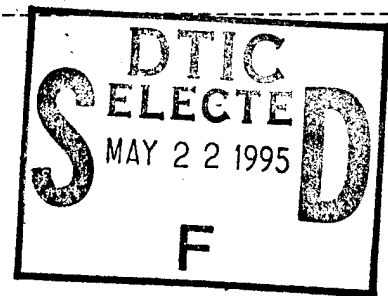


Final Report
ONR Grant N00014-89-1391-A0060



This grant action was to support research in optical oceanography. Specifically two efforts were supported: 1) an experimental study of the optics of individual marine particles and; 2) the editing of a state of the art text on ocean optics. Under the support of the subject grant the first of these activities was completed fully and successfully, and the second action was begun, with subsequent support under a separate but related grant providing the funds for completion.

The study of the optics of individual particles was aimed at assessing the role of particle shape in defining the volume scattering function. This study involved a combination of experimental tests with the EPICS Flow Cytometer at Bigelow Laboratory for Ocean Sciences, as well as some fundamental theoretical analysis of the results. The results were quite promising in demonstrating that particle asphericity (i.e. "non-roundness") could be determined with a parameter involving the forward-scattering-normalized cross-polarized 90-degree scattering values. This result was consistent with the theoretical predictions of polarized light scattering by spheres and non-spherical particles. Of course these results were highly simplified, by virtue of the use of idealized particles.

Two issues for further study developed from these results: 1) what is the theoretical explanation for the forward-scattering normalization? and ; 2) how can these results be expanded to define gradations of asphericity of naturally occurring particles? The results also suggested that this cross-polarization technique could be useful for discriminating living particles from the inherently spherical globules of oil dispersions characteristic of a polluted water sample. Future research will focus on these efforts.

The full detail of the experimental results for the work covered under the subject grant is contained in the peer reviewed article which was published as a result of this work. That publication is:

Spinrad, R.W. and J. Brown. 1993. Effects of asphericity on single-particle polarized light scattering. *Applied Optics*. 32: 6151-6161.

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Effects of asphericity on single-particle polarized light scattering

Richard W. Spinrad and Jeffrey Brown

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Polarized light scattering from individual particles has been analyzed to determine the effects of particle shape. Flow cytometric techniques were used on samples of spherical microspheres and naturally occurring marine algae. An analog of the depolarization ratio was obtained by using crossed polarizers in the source and detector of the flow cytometer. Results suggest that differences between the polarized light scattering of spheres and aspherical particles are not discernible unless the scattered intensities are normalized to the forward scattering, which is roughly equivalent to particulate cross section. This research indicates that polarized light scattering, when normalized to particle size, may provide an indication of the extent of asphericity of hydrosols.

Introduction

For marine particulate matter, the factors affecting light scattering are the size, shape, and refractive index distributions of the suspended material. The significance of size and refractive index have long been recognized. Until recently, the influence of particulate shape has been ignored or underestimated, largely because of the difficulties of controlled experimentation on individual particles and the complications of appropriate theoretical treatment. Recent studies, however, have shown that particulate shape may be a significant parameter in the assessment of unpolarized light scattering and attenuation.¹⁻² Theory suggests that this will be especially true in the case of polarized light.

Research in the area of algal utilization of visible radiation also suggests that marine phytoplankton may be able to exploit the polarization of underwater light fields.³⁻⁸ Theoretical treatments of radiative transfer in the oceanic environment have also shown that the traditional scalar assessments (i.e., in which polarization effects are generally ignored) may be unrealistic; polarization of sunlight may occur at levels as high as 20% in the underwater light field.⁹ If sunlight is polarized to any extent in the underwater light field and if particle sphericity is a controlling

factor in the scattering of polarized light, then it is essential to understand the nature of scattering of polarized light by marine particles.

A fundamental question regarding applications of marine particulate light scattering, therefore, is the capability for discrimination of particle shape through the use of polarized light nephelometry. In earlier studies it was demonstrated that for bulk samples of marine organisms, the polarized light-scattering characteristics were significant.¹⁰⁻¹² One conclusion drawn by these researchers was that "... measurements of single cells in a flow system could enhance the information content... by eliminating the smoothing effects that result from averaging over a size distribution."¹² More specifically, the issue is whether currently existing capabilities for *in vitro* assessment of individual particles can be used to demonstrate in a statistically significant manner that aspherical particles polarize or depolarize incident radiation at a level that is significantly different from that of spherical particles.

Studies with single individual particles have been undertaken to demonstrate the capability of specific cells to polarize or depolarize incident radiation,⁵⁻⁸ although questions still remain about the statistical validity of these results and the applicability of these results to large populations of living organisms. That is, how representative of a whole population are the results obtained from single isolated cells?

To answer these questions requires a statistically large data set of the polarized light-scattering characteristics of a broad size range of individual spherical and aspherical particles. The goals of this research were to demonstrate that on a statistical basis the

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effects of particle shape could be qualitatively identified and that a technique could be developed to optically discriminate both rapidly and repeatedly, aspherical particles from spherical particles. An additional goal was to provide the information needed to characterize the quantitative effects of sphericity on polarization of scattered light by marine organisms.

Flow cytometry is a technique that has been used successfully for a wide variety of studies of the optical properties of marine particles. In studies with controlled light algal populations, researchers have used the capabilities of flow cytometers to measure light scattering by many individual particles in order to study the growth-related optical variability of phytoplankton,¹³ the natural range in the refractive index of phytoplankton,¹⁴⁻¹⁵ and the structural nature of phytoplankton.¹⁶ A body of methodologies has been developed for sophisticated analyses of the light-scattering properties of individual marine particles, using flow cytometric techniques. Many other laboratory techniques have also been developed for highly controlled, high-resolution analysis of single isolated marine particles.^{17,18} These techniques have provided much of the insight for studies with flow cytometry, in which the large number of particles that are analyzed offers excellent statistics to study some of the more subtle optical processes.

Theory

For the purposes of modeling (using the vector form of the radiative transfer equation), one must have some sense of even the qualitative nature of the polarized light scattering by aspherical particles. Some analytical models have been developed as approximation techniques for estimating polarized light scattering by aspherical particles.¹⁹

Polarized light scattering is best assessed through the direct measurement of the Mueller matrix²⁰ and the angular dependence of each of its components.^{21,22}

$$\begin{bmatrix} 1 & S_{12} & S_{13} & S_{14} \\ S_{21} & S_{22} & S_{23} & S_{24} \\ S_{31} & S_{32} & S_{33} & S_{34} \\ S_{41} & S_{42} & S_{43} & S_{44} \end{bmatrix}, \quad (1)$$

where the elements S_{ij} (having dimensionless values between -1 and 1) define the intensity-normalized (i.e., divided by S_{11}) polarization effects of the scattering medium (in this case, the individual particles) for a particular angle of scattering. The matrix product of the Mueller matrix for the medium (i.e., particles in this experiment) and the Stokes vector that defines the polarization of the input beam is the resultant beam with all of its polarization defined:

$$\begin{bmatrix} I_t \\ Q_t \\ U_t \\ V_t \end{bmatrix} = \begin{bmatrix} 1 & S_{12} & S_{13} & S_{14} \\ S_{21} & S_{22} & S_{23} & S_{24} \\ S_{31} & S_{32} & S_{33} & S_{34} \\ S_{41} & S_{42} & S_{43} & S_{44} \end{bmatrix} \times \begin{bmatrix} I_i \\ Q_i \\ U_i \\ V_i \end{bmatrix}, \quad (2)$$

where the subscripts i and t refer to incident and transmitted, respectively, and the elements of the Stokes vector correspond qualitatively to the intensity (I), the degree of orthogonal linear polarization (Q), the degree of 45° linear polarization (U), and the degree of circular polarization (V).

Hunt and co-workers⁵⁻⁸ performed detailed measurements of the angular variability of the components of the Mueller matrix for isolated individual marine particles. Their results suggest that aspherical particles may show a propensity to circularly polarize unpolarized light, and to depolarize circularly polarized light.

Bohren and Huffman²² described the utility of the Mueller matrix elements and their measurement to characterize the propensities for polarization by any particle. In effect, the cross polarization can be defined as

$$\frac{1}{2}(S_{11} - S_{22}). \quad (3)$$

When this term is normalized by the S_{11} term, which is the unpolarized scattered intensity, one gets a form of the depolarization ratio, which is typically written as

$$\rho = 1 - \frac{S_{22}}{S_{11}}. \quad (4)$$

One indicator of asphericity is the degree to which the depolarization ratio diverges from zero.²² The S_{22} element is 1.0 for perfect spheres, and for large collections of nonspherical particles it has been shown that the S_{22} element is significantly different from 1.0.²³ In addition, Bohren and Huffman²² showed that with crossed polarizers (i.e., linear polarizers with their planes of polarization oriented orthogonally, and one placed at the source, the other at the detector in a light-scattering system), one can effectively measure the following combination of scattering elements:

$$\frac{1}{4}(S_{11} + S_{12} - S_{21} - S_{22}). \quad (5)$$

Voss and co-workers¹⁰⁻¹² discovered that for natural marine samples as well as for laboratory cultures of marine phytoplankton (in which particle concentrations were low enough to satisfy the single-scattering approximation), the Mueller matrix was characterized by zero or near-zero values for all off-diagonal elements except S_{12} and S_{21} , and that the values of S_{12} and S_{21} were virtually identical [for spheres or infinite cylinders with $S_{12} = S_{21}$ and $S_{22} = 1$, Eqs. (4) and (5) are equal to zero]. In that case, expression (5) becomes

$$\frac{1}{4}(S_{11} - S_{22}), \quad (6)$$

suggesting that, for marine phytoplankton the use of crossed polarizers can yield the depolarization ratio [Eq. (4)] to a good approximation.

Table 1. Algal Cultures

Clone Identification	Species	Type	D (μm) ^a	Shape
A	<i>Chlorella</i> sp.	Chlorophyte	3.06	Ovoid
DUN	<i>Dunaliella tertiolecta</i>	Chlorophyte	7.17	Ovoid
8613 C (f/2)	<i>Emiliana huxleyi</i> (naked)	Coccolithophore	4.38	Encrusted sphere
8613 C (f/50)	<i>Emiliana huxleyi</i> (plated)	Coccolithophore	4.38	Plated sphere
13-1	<i>Thalassiosira oceanica</i>	Diatom	4.65	Cylinder
3H (slow growing)	<i>Thalassiosira pseudonana</i>	Diatom	4.30	Cylinder
3H (fast growing)	<i>Thalassiosira pseudonana</i>	Diatom	3.90	Cylinder
EXUV	<i>Prorocentrum minimum</i>	Dinoflagellate	14.1	Irregular pear
AMPHI	<i>Amphidinium carterae</i>	Dinoflagellate	9.46	Irregular pear
OMEGA 48-23	Unidentified	Prasinophyte	2.89	Ovoid
ID2	<i>Cryptomonas</i> sp.	Cryptophyte	5.34	Ovoid
3C	<i>Rhodomonas salina</i>	Cryptophyte	6.54	Ovoid

^aMean equivalent spherical diameter.

It is also important to note that the polarized scattered light is also going to be affected by both the size and refractive index of the particles. The study of Fry and Voss,¹² however, demonstrated that the effect of refractive index change is primarily one of shifting the angular scattering characteristics for individual Mueller matrix elements S_{33} and S_{44} . The size of the particle will be manifested in the magnitude of the S_{11} term; the normalization by S_{11} effectively removes the changes in scattering caused exclusively by size.

Experimental Design and Methods

In order to assess the significance of particle shape on depolarization qualitatively, one must remove the effects of size and refractive index. Copolarized and cross-polarized light-scattering measurements for polydisperse particles can be normalized to some simultaneous measure of particle size. It will be shown that in the current study, forward-angle light scattering can provide an unambiguous measure of particle cross section independent of particle type (e.g., synthetic spheres or marine algae). It is also reasonably well established that polarization changes are minimal in the region of near-forward scattering.¹¹ The variability induced by particulate refractive index can be circumvented by performing separate studies on groups of particles having nearly identical refractive indices. The conclusions of the study can then be drawn by comparing the statistics taken from within each group.

Algal Cultures

Cultures used in these experiments were selected from the Provasoli-Guillard Culture Collection of Marine Phytoplankton at Bigelow Laboratory for Ocean Sciences. Table 1 gives a description of these monoclonal algal cultures. Choices of cultures were made to give a variety of particle shapes and sizes. Two cultures of clone 3H were grown under two different growth (nutrient) conditions in turbidistats, yielding a fast- and slow-growing version of the same species (of presumably different cell morphologies).

Microspheres

Measurements of algae were to be compared with spherical latex calibration microbeads. A description of the microspherical beads appears in Table 2. Bead lots were carefully selected to ensure uniform spherical shape and size, and a range of sizes similar to the range of sizes of the algae was chosen for the purpose of comparison. Simultaneous measurements of the algal cultures and beads were made by using two different instruments: a flow cytometer to measure individual particle scattering and a particle size analyzer.

Flow Cytometric Measurements

The flow cytometer used was the Coulter EPICS V Flow Cytometer and Cell Sorter (Coulter Electronics) of the MacIsaac Individual Particle Analysis Facility at Bigelow Laboratory. This instrument is equipped with a linearly polarized wavelength tunable 50-W argon-ion laser as a light source. Sample particles are injected into a laminar flow that intersects the laser beam. A full description of this instrument can be found in Yentsch *et al.*²⁴ For this experiment the flow cytometer was configured for simultaneous measurements of chlorophyll fluorescence, 90° light scatter, and forward-angle light scatter. The flow cytometer was equipped with linear polarizers and

Table 2. Microspheres

Manufacturer	Type	Diameter (μm)
Flow Cytometry Standards Corp.	Nonfluorescent	2.0
Coulter Electronics	Nonfluorescent	5.0
Flow Cytometry Standards Corp.	Nonfluorescent	7.5
Flow Cytometry Standards Corp.	Nonfluorescent	11.8
Seragen Diagnostics, Inc.	Nonfluorescent	15.0
Flow Cytometry Standards Corp.	Nonfluorescent	18.0
Polysciences, Inc.	Fluorescent	3.2
Flow Cytometry Standards Corp.	Fluorescent	7.4
Coulter Electronics	Fluorescent	9.2
Coulter Electronics	Fluorescent	20.2

appropriate spectral bandpass filters, mounted in front of each of the two detectors (the forward-scatter photodiode and the 90° angle-scatter photomultiplier tube). The polarizing filters could be rotated and removed or relocated easily between experimental runs.

Flow cytometric data were collected in real time and appeared on a monitor in the form of frequency histograms with number of particles along the ordinate and relative signal intensity of an optical parameter (fluorescence, forward scatter, or 90° scatter) along the abscissa, which is divided into 256 channels. All parameters could be collected simultaneously and in linear or logarithmic mode. Adjustments could be made to individual parameter gain settings and laser power output to control signal strength and proper scaling along the horizontal axis. Settings have been shown to be consistently linear, so that in comparing results of different gain or laser power, simple multiplication factors normalize values to the same relative scale.

All beads and algae were run with the laser tuned to a wavelength of 488 nm. This wavelength causes excitation of the pigment of the fluorescent beads and the chlorophyll in the algae. The fluorescent emission is received by a separate photomultiplier from the 90° scattered light, and the signal is processed as a separate parameter that is useful in gating or preselecting only the particles in a sample that do fluoresce (i.e., living chlorophyll-containing algal cells or fluorescent pigmented beads).

Before experimental runs the instrument was calibrated with spherical fluorescent beads of a known uniform size. By gating on the parameter of fluorescence and collecting the parameter of forward-angle light scatter, which is a function of size, that parameter can be effectively calibrated by running a number of different size beads and locating the mean channel value (the mean of the frequency histogram) for each bead size along the axis representing the intensity of the forward-angle scatter signal. This calibration procedure was also used as a check of instrument stability, simply by periodically rerunning the beads and correcting for any changes in channel value caused by instrument drift.

For each fluorescent bead and algal culture in Tables 1 and 2, the experiment was first run with the forward-angle light scatter unpolarized. One run was done with the 90° scatter horizontally polarized or cross polarized (because the laser beam itself is always vertically polarized); a second run was made with the 90° scatter vertically or copolarized; and a third run was made without the polarizing filter on the 90° scatter. The same sample was then run three times with the forward-angle light scatter horizontally polarized, alternating between horizontal, vertical, and no polarization of the 90° light scatter. Finally, three similar runs were performed with the forward-angle light scatter vertically polarized. We repeated this procedure for each particle,

yielding nine separate runs for each before going to the next particle selection.

In most cases and for each polarizing filter combination, data were acquired by gating on the fluorescence parameter and collecting the parameters of forward-angle light scatter, 90° light scatter, and log-scale fluorescence. For the nonfluorescent beads the gating parameter was forward-angle light scatter. The laser power and all parameter gain settings were recorded as well during each sample run. Typically, 10,000 beads or cells were collected per run. For each of the frequency histograms generated, the mean channel number was recorded and later corrected for both the laser power and gain used for each parameter collected.

Particle Size Analysis

A Coulter Counter ZM (Coulter Electronics) was used for particle concentration measurements and for particle sizing. The instrument was equipped with a 50- μm orifice and a C-1000 Channelyzer and computerized data-acquisition package to aid in accurate statistical sizing and concentration determination. The instrument was precalibrated for sizing with

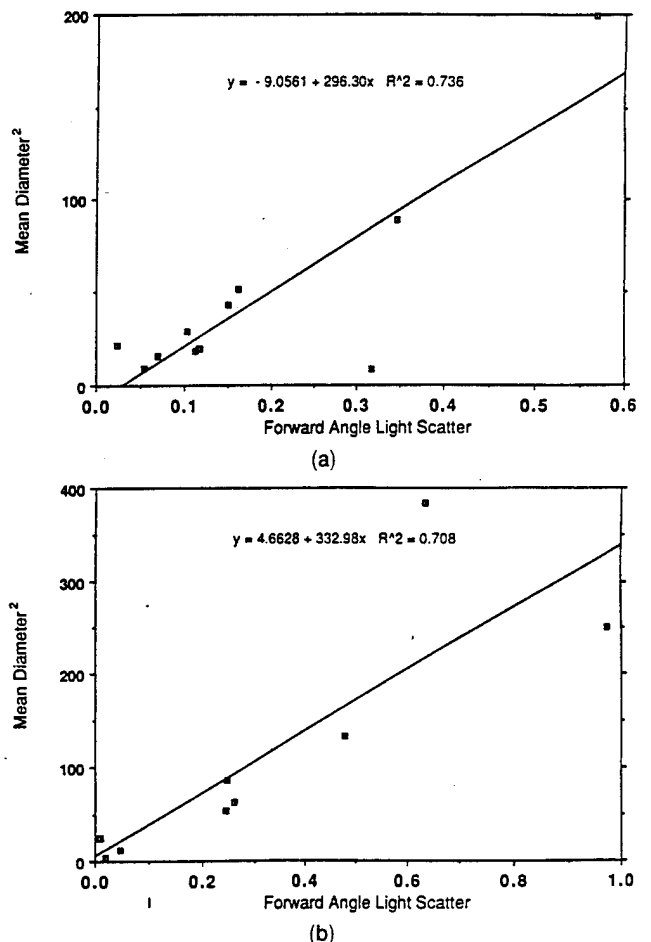


Fig. 1. Mean values of the forward-angle light scattering as measured on the flow cytometer for varying cross sections (given as the square of the equivalent spherical diameter) of (a) algae and (b) microspheres.

some of the same beads used in the experiments. These, again, were of a known uniform size and spherical shape. All the beads and algae were then sampled for size (equivalent spherical diameter and volume), which is calculated internally from the impedance volume as each particle passes through the electrically charged orifice. The algae samples were from the original culture flasks rather than from the diluted samples from the transmissometer tank.²⁵ The upper and lower limits of the size distributions were recorded as references for subsequent counting of the diluted samples taken from the transmissometer tank. For each concentration, 500 μL was sampled and all samples were counted in triplicate. Concentrations were expressed in number of particles per milliliter.

Results and Discussion

Forward-angle light scattering has previously been demonstrated to be a good independent indicator of particle size.²⁶ In fact in this study the same conclusion held. Figure 1 shows that, regardless of particle type, the forward-angle light scattering was well correlated with particle cross section. The correla-

tion coefficient and the relative slope of the regression were nearly invariant for particle type (see Fig. 1 for values of r^2 , for algae and beads, the relative slopes were 296 and 333, respectively). As a result, forward-angle light scattering was used as a parameter for normalization to particle size. All future references to normalization imply a correction of a given parameter to the specific value of the forward-angle light scattering for a specific particle.

Figure 2 shows the comparison of results for the measurement of unpolarized 90° light scattering versus cross-polarized 90° light scattering. The figure shows that for the algae and the beads there was reasonably good correlation between the cross-polarized light scattering at 90° , $\beta_h(90)$, and the unpolarized light scattering at 90° , $\beta_u(90)$. The slopes of the regressions were nearly identical also (0.95 for the algae and 1.08 for the beads). Differences in the intercept, or offset between the beads and the algae are attributable to gain changes in the detector and are not significant, because gain was held constant for all algae or all beads but may have changed between the two particle types.

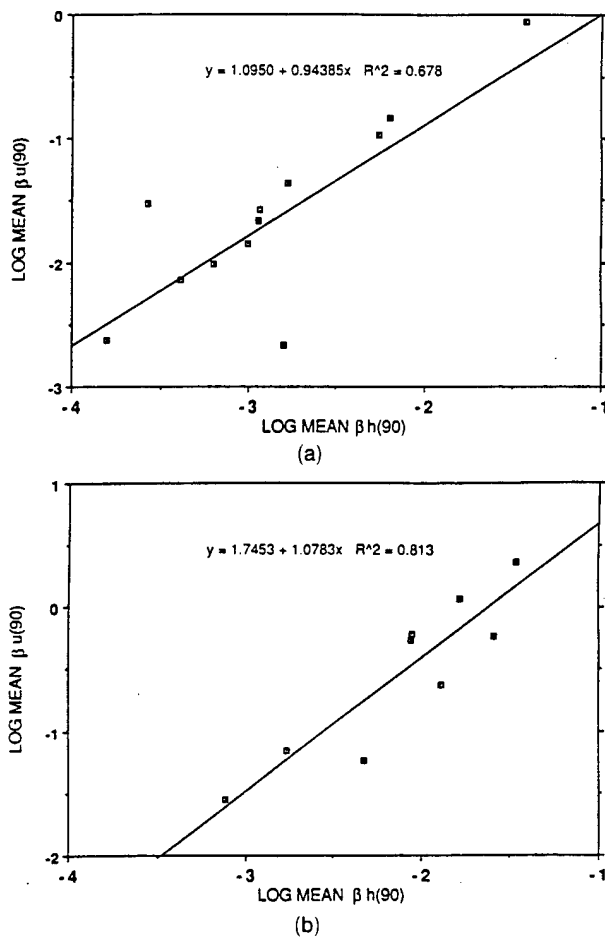


Fig. 2. Log of the mean value of the unpolarized light scattering measured at 90° , $\beta_u(90)$, versus the log of the mean value of the cross-polarized light scattering measured at 90° , $\beta_h(90)$, for (a) algae and (b) microspheres.

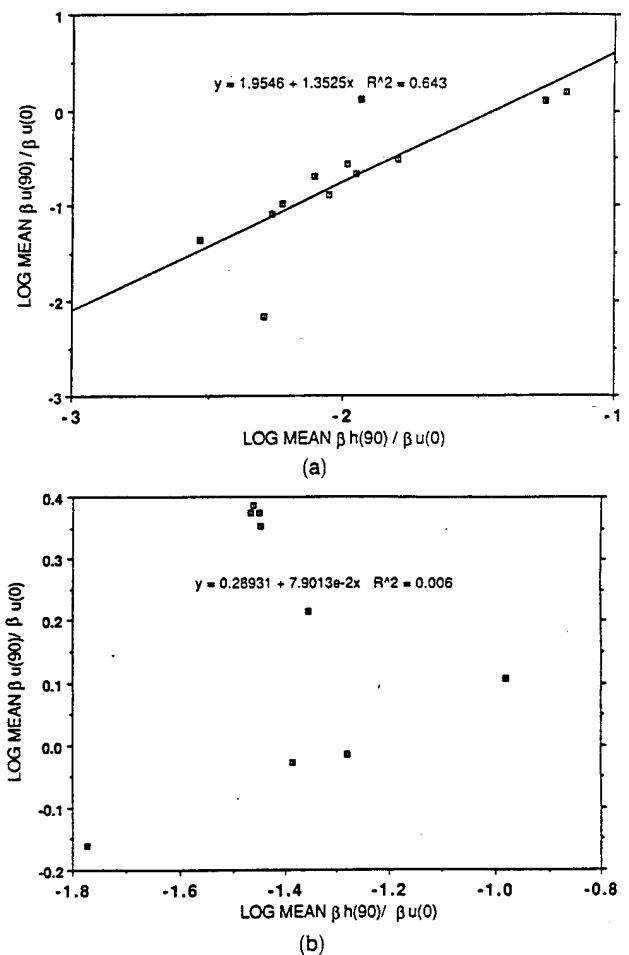


Fig. 3. Same measurements as shown in Fig. 2 but normalized to the unpolarized forward scattering, $\beta_u(0)$, for (a) algae and (b) microspheres. Normalization is mean $\beta_u(90)$ divided by mean $\beta_u(0)$.

However, when normalized to forward-angle light scattering (i.e., size) the correlation of the cross- to unpolarized light scattering (as shown in Fig. 2) showed a large difference between the aspherical algae and the spherical beads (Fig. 3). As predicted by the theory of depolarization by aspherical particles, the algae had a much higher correlation coefficient than the beads (0.643, virtually unchanged for the algae, and 0.006 for the beads).

Similar results were obtained in comparing the cross-polarized and copolarized light scattering at 90° . Figures 4 and 5 show, respectively, the unnormalized and the normalized plots of cross-polarized light scattering to copolarized light scattering at 90° . Again, although the algae and beads had similarly high correlation coefficients before normalization (0.70 and 0.85, respectively), once size effects were removed the algae still had a much higher correlation coefficient [Fig. 5(a), 0.46] compared with the normalized beads. The spherical beads, when corrected for size effects, were found to have almost no correlation between cross-polarized light scattering and copolarized light scattering [Fig. 5(b), $r^2 = 0.070$].

Table 3 shows the results of the regression analyses

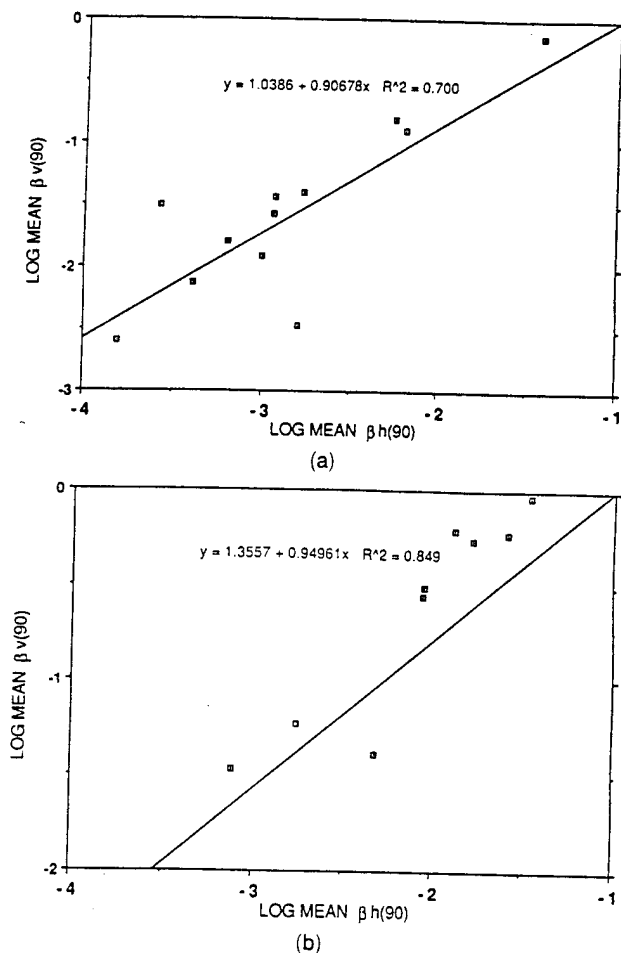


Fig. 4. Log of the mean value of the copolarized light scattering measured at 90° , $\beta_v(90)$, versus the log of the mean value of the cross-polarized light scattering measured at 90° , $\beta_h(90)$, for (a) algae and (b) microspheres.

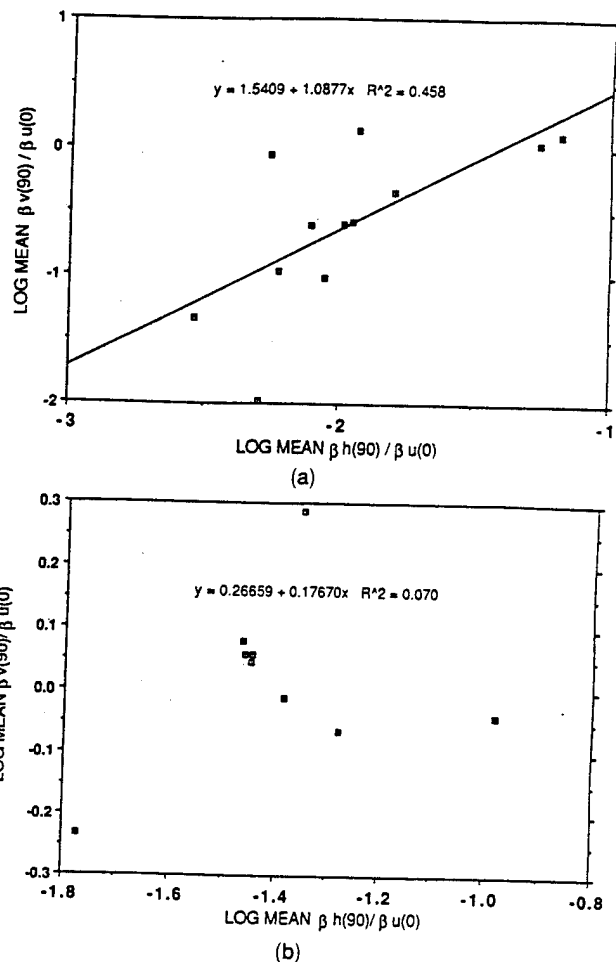


Fig. 5. Same measurements as shown in Fig. 4 but normalized to the unpolarized forward scattering, $\beta_u(0)$, for (a) algae and (b) microspheres. Same normalization method as in Fig. 3.

for the light-scattering studies. There could still be some influence of particle size on the results shown in Figs. 3–5, because the range of particle sizes for all of the algae (equivalent spherical diameters of 2.89–14.11 μm , with a mean of 5.8 μm) was different from that of all of the beads (2–20.2 μm , with a mean of 9.9 μm). To test the hypothesis further that the polarized light scattering was most strongly influenced by particle shape, rather than size, we subjected a subset of the particles identified in Tables 1–3 to additional

Table 3. Statistics of Correlations

Regressed Terms ^a	r^2	
	Algae	Beads
Log $\beta_h(90)$ versus log $\beta_u(90)$	0.68	0.81
Normalized log $\beta_h(90)$ versus log $\beta_u(90)$	0.64	0.01
Log $\beta_h(90)$ versus log $\beta_v(90)$	0.70	0.85
Normalized log $\beta_h(90)$ versus log $\beta_v(90)$	0.46	0.07
FALS versus D^2	0.74	0.71

^a $\beta_h(90)$ is cross-polarized scattering at 90° , $\beta_u(90)$ is unpolarized scattering at 90° , $\beta_v(90)$ is copolarized scattering at 90° , FALS is unpolarized forward-angle light scattering, and D^2 is the squared particle diameter.

Table 4. Particles Used for Subset Analysis

Particles	Equivalent Spherical Diameter (μm)
Algae (<i>Chlorella</i> sp.)	3.06
Beads (PSI) ^a	3.20
Algae (<i>Cryptomonas</i> sp.)	5.34
Beads (Coulter) ^b	5.00
Algae (<i>Rhodomonas salina</i>)	6.54
Beads (FCSC) ^c	7.40
Algae (<i>Dunaliella tertiolecta</i>)	7.17
Beads (FCSC) ^c	7.50
Algae (<i>Amphidinium carterae</i>)	9.46
Beads (Coulter) ^b	9.20

^aPolysciences, Inc.

^bCoulter Electronics.

^cFlow Cytometry Standards Corp.

Table 5. Statistics of Correlations of Particle Subset

Regressed Terms ^a	r^2	
	Algae	Beads
Log $\beta_h(90)$ versus log $\beta_u(90)$	0.98	0.54
Normalized log $\beta_h(90)$ versus log $\beta_u(90)$	0.93	0.04
Log $\beta_h(90)$ versus log $\beta_v(90)$	0.99	0.70
Normalized log $\beta_h(90)$ versus log $\beta_v(90)$	0.97	0.15

^aThe definition of terms is the same as that of Table 3.

statistical analysis. The subset of particles consisted of five matched pairs of algae and beads, each pair being of nearly equivalent size (i.e., equivalent spherical diameter). The results of a regression analysis (identical to the technique used for the full populations) for this subset are shown in Tables 4 and 5 along with an identification of the particles used.

The results shown in Tables 4 and 5 were the same as those for the full population. Unpolarized and copolarized scattered light intensities were reasonably well correlated to cross-polarized scattered light intensities for both the algae ($r^2 = 0.98$ and 0.99 , respectively) and the beads (0.54 and 0.70 , respectively). Again, however, when the polarized light scattering was normalized to the unpolarized forward scattering the statistics for the algae were virtually unchanged ($r^2 = 0.93$ and 0.97 , respectively), but the correlations for the beads degraded dramatically (r^2 of 0.04 and 0.15). These results confirm that for algae and beads of the same size, after normalization to the forward scattering, there are no significant correlations between copolarized, unpolarized, or cross-polarized light scattering at 90° for spherical particles, but there are highly significant correlations for aspherical particles.

Conclusions

In this study methods were developed to determine analogs of several elements of the Mueller matrix qualitatively for a variety of particles. The results show that light scattering by individual particles can be measured in a manner by which spherical and aspherical particles can be discriminated in a statisti-

cally significant manner. More significantly, this result is in complete agreement with the relative changes predicted by a rigorous treatment of the theory of polarized light scattering.

The method of size normalization used in this experiment is one that would inherently yield no changes if the effects of polarization were defined in a linear sense. That is, comparing Figs. 2 and 3, for example, one should expect that by dividing each variable [$\beta_h(90)$ and $\beta_u(90)$] by the same term, $\beta_u(0)$, there should be no effect on the values that are derived, because

$$\frac{\beta_h(90)}{\beta_u(0)} = \frac{\beta_h(90)}{\beta_u(90)} \cdot \frac{\beta_u(90)}{\beta_u(0)} \quad (7)$$

In fact, this is the effect that was observed for the algae, suggesting that the unpolarized light scattering in the forward direction is responding in a fashion that is linearly proportional to the cross-polarized and unpolarized light scattering at 90° . This, however, is clearly not the case for the spheres. For different size spheres, the relationship between the unpolarized forward scattering and the cross- or unpolarized 90° scattering is clearly nonlinear. In the simplest reduction of this concept these results suggest that for two different size spheres, an increase in forward (unpolarized) scattering will not be reflected in equal increases of both unpolarized and cross-polarized scattering at 90° ; more significantly, this disparate change in cross-polarized and unpolarized 90° scattering relative to unpolarized forward scattering is not constant for spherical particles.

Correlation coefficients for regressions of cross-polarized scattering to copolarized or unpolarized scattering are unaffected by size normalization for aspherical algae, and are reduced by approximately an order of magnitude for spherical beads. Cross-polarized light scattering works well to separate aspherical particles from spherical particles qualitatively, only if size effects are removed. Given the very significant differences in the measurements made in this experiment, it is clear that there is real potential for the development of techniques for discriminating shapes of particles in oceanic waters. Such a capability would serve a wide range of research needs, because the shape of a marine particle is often a clue to its nature and origin. Future research in this area should focus on developing even more refined capabilities for discriminating many subclasses of nonspherical particles (e.g., pennate versus centric diatoms).

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