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Measurement of the Mueller matrix for phytoplankton[1]

Abstract—Measurement of the light-scattering Mueller matrix of phytoplankton cultures resulted in a characteristic Mueller matrix much like that of seawater.

Voss and Fry (1984) measured the scattering of polarized light by ocean water. We here report measurements of various phytoplankton cultures made to further our understanding of the ocean water measurements.

To quantify changes in polarization due to scattering one must be able to describe the polarization and the transformations that take place. A convenient representation of the polarization of a light beam is the Stokes vector (van de Hulst 1981). The four elements of this vector, labeled I, Q, U, and V, are defined in terms of the electric field as

$$I = E_l^2 + E_r^2, \qquad U = 2E_lE_r\cos\,\delta,$$
$$Q = E_l^2 - E_r^2, \qquad V = 2E_lE_r\sin\,\delta$$

where E_l and E_r, the components of the total electric field, and δ, the relative phase, are defined by

$$E = E_l\cos(kz - wt + e_l)\hat{\mathbf{l}} + E_r\cos(kz - wt + e_r)\hat{\mathbf{r}},$$
$$\delta = e_l - e_r$$

with $\hat{\mathbf{l}}$ a unit vector parallel to the scattering plane and $\hat{\mathbf{r}}$ a unit vector perpendicular to the scattering plane. Qualitatively, I corresponds to the total intensity of the light beam, Q to the degree of linear polarization

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in the $\hat{\mathbf{l}}$ and $\hat{\mathbf{r}}$ directions, U to the degree of linear polarization at 45° to the $\hat{\mathbf{l}}$ and $\hat{\mathbf{r}}$ directions, and V is the degree of circular polarization. The principle of optical equivalence, first derived by Stokes (1853), shows that the Stokes vector is a complete representation of the polarization state of a light beam.

In a suspension with individual particles in random positions, the light scattered from any two of the particles will not have a specific phase relationship, and the scattered light will add incoherently. In this case, the Stokes vector is the sum of the individual vectors. When a light beam is scattered by a particle, or changed by an optical element, the Stokes vector of the light beam undergoes a linear transformation to a new Stokes vector. This transformation can be represented by a 4 × 4 matrix called the Mueller or polarization matrix. Specifically, we have

$$\begin{bmatrix} I' \\ Q' \\ U' \\ V' \end{bmatrix} = \begin{bmatrix} \mathrm{M11} & \mathrm{M12} & \mathrm{M13} & \mathrm{M14} \\ \mathrm{M21} & \mathrm{M22} & \mathrm{M23} & \mathrm{M24} \\ \mathrm{M31} & \mathrm{M32} & \mathrm{M33} & \mathrm{M34} \\ \mathrm{M41} & \mathrm{M42} & \mathrm{M43} & \mathrm{M44} \end{bmatrix} \begin{bmatrix} I \\ Q \\ U \\ V \end{bmatrix}$$

where (I, Q, U, V) is the Stokes vector of the incident light, and (I', Q', U', V') is the Stokes vector of the scattered or transmitted light. The Stokes vector contains all the polarization information that can be measured for a light beam; therefore, the Mueller matrix contains all the polarization information in an elastic scattering process. As with the Stokes vector, the Mueller matrix of a suspension of particles is the sum of the Mueller matrices of the individual particles.

Since light scattering is a nonevasive measuring technique, we can, in principle, get information on orientation, physical structure, and physiological state of phytoplankton as these can all change the Mueller matrix. The measurements presented here were made with an electro-optic light-scattering polarimeter (Thompson 1978; Voss and Fry 1984), which measures the entire Mueller matrix at 1° intervals from 10° to 160° in about 2 min, with an accuracy of about 2–10% depending on the matrix element. All of our measurements were made with an argon ion laser operating at 488 nm.

It is advantageous, when looking at polarization effects in the Mueller matrix, to normalize the matrix to the M11 component

$$\begin{bmatrix} 1 & \mathrm{S12} & \mathrm{S13} & \mathrm{S14} \\ \mathrm{S21} & \mathrm{S22} & \mathrm{S23} & \mathrm{S24} \\ \mathrm{S31} & \mathrm{S32} & \mathrm{S33} & \mathrm{S34} \\ \mathrm{S41} & \mathrm{S42} & \mathrm{S43} & \mathrm{S44} \end{bmatrix}$$

where $\mathrm{S}ij = \mathrm{M}ij/\mathrm{M11}$. In this way, intensity-dependent effects can be isolated from the polarization effects, both to simplify analysis and to highlight the latter. The normalized matrix elements, $\mathrm{S}ij$, can only have values between −1 and +1. All of our measured matrices are normalized electronically in the process of measurement and are presented in normalized form. Errors are quoted in terms of this normalized full-scale value of unity.

Our measurements of ocean water (Voss and Fry 1984) showed the Mueller matrix, at the level of accuracy of our instrument, to be highly symmetric with many zero elements. In trying to understand the zero elements, we undertook a study of phytoplankton cultures. In particular, since the normalized Mueller matrix of a collection of particles is the average of the individual matrices of the particles, more structure may be evident in the matrix for particles of one shape (as in unialgal cultures) than for a collection of many shapes (as in natural ocean water). We chose unialgal cultures of phytoplankton having different shapes, sizes, and composition: *Thalassiosira eccentrica* (a centric diatom, 12–60 microns), *Thalassisira mala* (a centric diatom, 4–9 microns), a coccolithophore *Cycrosphaera* sp. (15 microns), *Porphyridium cruentum* (red algae, 15 microns), and two clones of *Syncehococcus* sp. (a marine cyanobacterium, <1 micron). These were all grown as batch cultures in f/2 media with a 12:12 light/dark cycle and were in log phase growth when measured. The measurements were made in the single scattering regime where the normalized scattering matrix is not de-

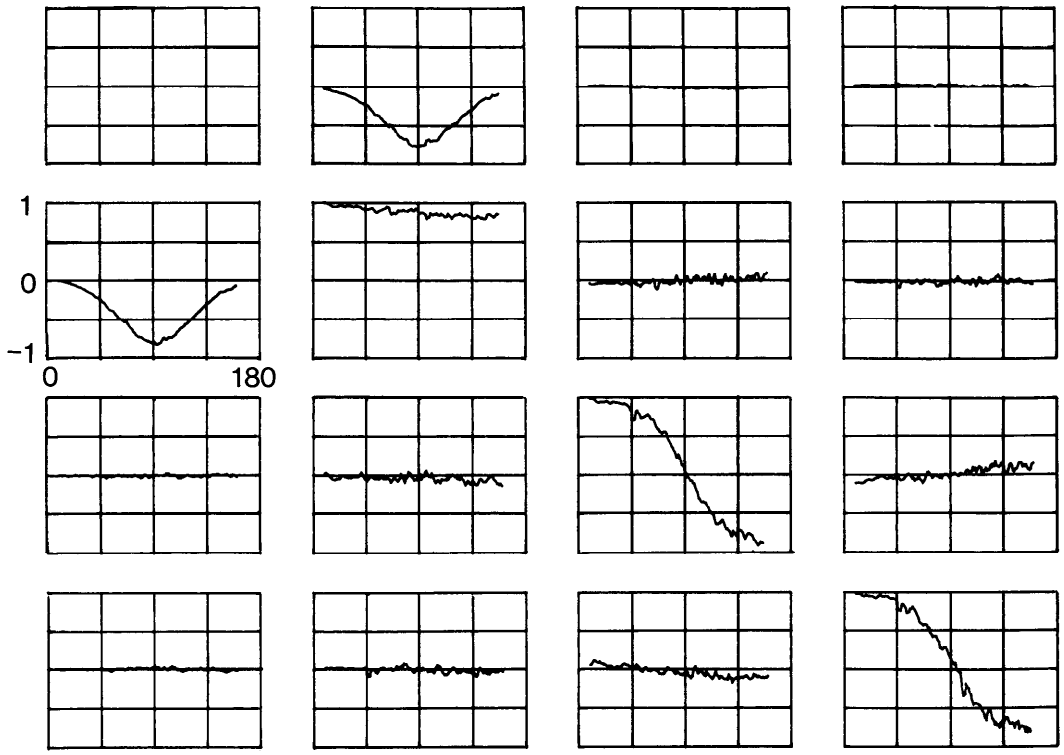

Fig. 1. The Mueller matrix of *Porphyridium cruentum*. The x-axis is the scattering angle in degrees, the y-axis the normalized matrix value.

pendent on particle concentrations. The cultures were added, 10 ml at a time, to 0.5 liter of filtered seawater in the sample cell. The normalized matrix remained constant until very high concentrations (the solutions appeared "milky"), when multiple scattering effects dominated. *Thalassiosira mala* and *P. cruentum* tend to make gelatinous colonies (Hallegraeff 1984; Percival 1978); these were stirred before adding them to the seawater to reduce this effect.

The measured matrices for all cultures were very similar to our measurements of ocean water. Figure 1 shows the matrix for *P. cruentum,* which is similar to that of the other phytoplankton cultures (except *Synechococcus* spp.). The zero off-diagonal elements (other than S12 and S21) in the *P. cruentum* matrix imply that the average orientational and particle asymmetries for a collection of these phytoplankton are smaller than our instrument can measure.

For comparison, we show in Fig. 2 the calculated matrix for particles in the Rayleigh-Gans limit, which holds for particles in which the length-scale times the index of refraction relative to the medium is small compared to the wavelength of light. The matrix clearly has the same general form as that for *P. cruentum.* The most important difference between the culture matrices and that of the Rayleigh-Gans matrix is the S22 element which does not equal 1 at all angles in the culture matrix. The S22 element is 1 in spherical particles and has been shown to vary from 1 in measurements of randomly oriented nonspherical particles (Bottiger et al. 1980). S22 would not be expected to be 1 in the phytoplankton matrices since the phytoplankters were not spherical. Also the S12 and S21 matrix elements do not go all the way to -1.0 at 90° and the S33 and S44 elements do not reach -1.0 in the backward direction; this can be shown to be consistent with the change of the S22 element with angle (Voss 1984).

The only significant difference among the phytoplankton was in the measurements of

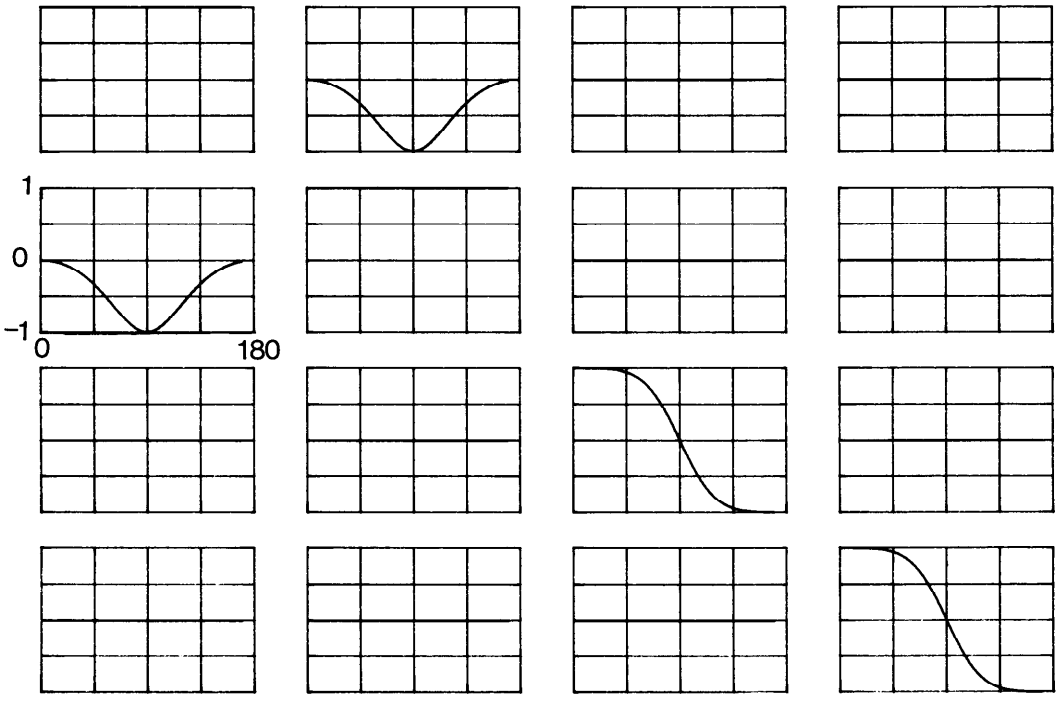

Fig. 2. The calculated matrix for particles in the Rayleigh-Gans limit.

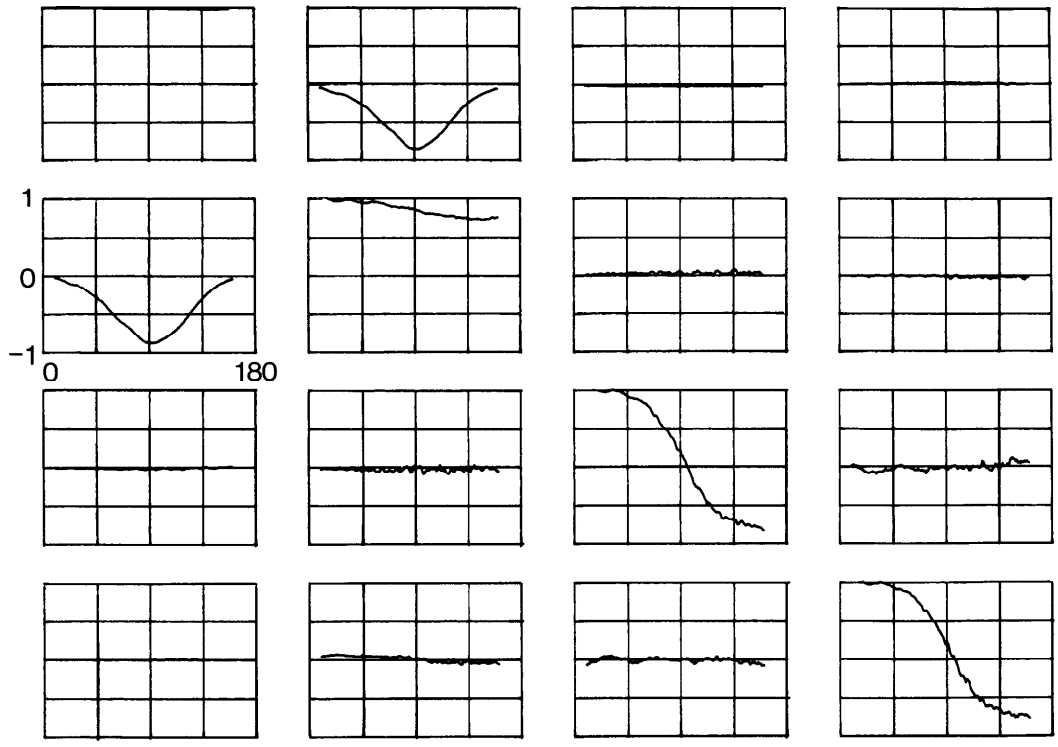

Fig. 3. The measured Mueller matrix for *Synechococcus* sp. clone DC2.

the cyanobacteria, *Synechococcus* sp. clones DC2 and SYN (isolated by R. R. L. Guillard). Although most phytoplankton measurements exhibited S33 and S44 elements which crossed zero at scattering angles between 90° and 95°, the latter exhibited a characteristic feature in which the zero of the S33 and S44 elements occurred at scattering angles of about 100° (Fig. 3). We confirmed this effect by measuring Rayleigh scattering standards before and after each measurement. A possible origin for this difference is the bacterialike cell wall of *Synechococcus* sp. As the index of refraction of the shell of a coated sphere is reduced (from 1.15 to 1.10 with the core index constant at 1.05), the zero point of S33 and S44 shifts by about 4°, from 96° to 100°. We speculate that in *Synechococcus* sp. the shift may be due to a cell wall structure with a lower index of refraction than the cell wall found in eucaryotic phytoplankton, but we are not aware of any measured values of the index of refraction of the *Synechococcus* cell wall.

It is evident from these matrices that at the level of accuracy of our instrument very little physical or physiological information is gained by measuring the whole Mueller matrix. We believe that the reason for the symmetry and zero elements is the small relative index of refraction of the phytoplankton. More information may be gained by more accurate measurements; on the level of one part in 10^4, anisotropies due to chlorophyll dichroism will appear (Voss 1984; Voss and Fry 1984). Finally, measurements of single cells in a flow system could enhance the information content of the matrix by eliminating the smoothing effects that result from averaging over a size distribution.

E. S. Fry
K. J. Voss[2]

Physics Department
Texas A&M University
College Station 77843

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[2] Present address: Visibility Laboratory, Scripps Institution of Oceanography, Univ. Calif., San Diego, La Jolla 92093.