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Angular distribution of fluorescence from phytoplankton

Abstract—The angular distribution of fluorescence for several natural oceanic phytoplankton cultures was measured in the laboratory. Of the six cultures examined, only *Dunaliela tertiolecta* exhibited measurable anisotropy. The anisotropy in this case was ~20%.

In vivo chlorophyll fluorescence is an important phenomenon in biological oceanography. Often used as a method of quantifying the chlorophyll concentration (Yentsch and Menzel 1963), measurement of in situ natural chlorophyll fluorescence has also been proposed as a measure of primary productivity (Kiefer et al. 1989). Natural fluorescence of phytoplankton has been identified as a major contributor to the underwater light field for wavelengths near the chlorophyll fluorescence maximum at 685 nm (Gordon 1979).

Measurements and models of in situ and in vivo chlorophyll fluorescence have assumed that the fluorescent emission from the phytoplankton cells is isotropic. Recently, Gong (1991) applied radiative transfer theory to measurements of natural fluorescence reported by Kiefer et al. (1989). His analysis suggested that the observed variation of the ratio of the upwelling radiance to the downwelling irradiance at 683 nm required that the angular distribution of fluorescent emission be anisotropic. Further analysis (unpubl.) suggests that the dissymmetry factor—the intensity of light fluoresced at 135° with respect to the incident direction of excitation divided by the intensity fluoresced at 45°—would have to be as large as 5 to explain the measurements.

Although fluorescence from dissolved chlorophyll should be isotropic, there are reasons to suspect that when the chlorophyll is encapsulated in a cell the angular distribution of the fluorescence may be anisotropic. For example, a simple mechanism for anisotropy in the fluorescence by phytoplankton is reabsorption of the fluorescent emission within the phytoplankton cell itself. Collins et al. (1985) illustrated in models and measurements that the efficiency factor for the escape of fluorescence emission from the cell can be as low as 66%, thus leading to an efficiency of fluorescence reabsorption of 34%.Combining absorption of the excitation along with reabsorption of the fluorescence, one would expect that the fluorescence emission would be strongest in the backward direction (toward the source of the excitation). On this basis, the anisotropy would be an elevated "backscattering" of the fluorescence and a decrease in the forward direction and would yield a dissymmetry factor >1.

Rigorous electromagnetic theories describing the fluorescent emission by molecules imbedded in otherwise homogeneous spheres (Chew et al. 1976*a*,*b*) have been developed and numerical computations with these theories have shown significant anisotropy in the fluorescent emission, depending on the size and structure (location of the molecule in the sphere) of the fluorescing particles (Kerker et al. 1979). Measurements of small spheres (0.3 μm) containing fluorescent dye have shown anisotropy in the fluorescent emission of a factor of 2 between 20 and 90° (fluorescence being higher at 20°) (Kratohvil et al. 1978, Lee et al. 1978). For a suspension of nonspherical particles, even in random orientation, one would expect anisotropies at least as strong as those for spheres.

Because of the necessity to understand fluorescent emission of phytoplankton for input into radiative transfer models and the possibility that emission may be anisotropic, we have measured the angular distribution of fluorescent emission for several phytoplankton cultures.

The instrument we used to measure the angular distribution of fluorescence is a modified Brice Phoenix light-scattering photometer (Fig.

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1). This instrument was originally designed to measure the light scattered at an angle Θ (the scattering angle) with respect to the direction of the incident beam by a small sample of fluid. Since the fluorescence of phytoplankton occurs near 685 nm, the first modification consisted of replacing the stock photomultiplier tube with an extended red response tube (Burle 4840). To prevent collection of the incident exciting light (436 nm), we placed seven layers of Kodak Wratten filter no. 29 in front of the photomultiplier tube. This filter combination strongly absorbed light at wavelengths <600 nm, while for wavelengths around 690 nm the combination transmitted ~50% of the light. This attenuation of the exciting light was necessary because of the strong forward elastic scattering (no wavelength change) of the phytoplankton cells and the significantly higher sensitivity of the photomultiplier tube in the blue compared to the red. A mercury vapor lamp with a filter excluding all but the 436-nm line was used as the exciting source.

Measurements were taken with both a cylindrical cell (Fig. 1) and a dissymmetry cell (semioctagonal cross-section). With the dissymmetry cell, measurements can be made only at Θ = 45, 90, and 135° while with the cylindrical cell measurements were taken in 5 or 10° increments between Θ = 20 and 135°. With one of the samples, fluorescence was not sufficiently above the system noise, due to low cell number density or low Chl/cell content. In this case measurements were performed with only the dissymmetry cell, which had a scattering volume ~3 times as large as the cylindrical cell and thus 3 times the signal (system noise was independent of signal).

The photometer current was read, after amplification and conversion to a voltage, with a digital voltmeter. An estimate of the error due to system noise was found by recording the range of values observed on the voltmeter during the measurement process. The estimate is shown as error bars on each of the data graphs.

For samples in which rapid settling was observed, the measurement at 20° was repeated after each angular measurement. These 20°

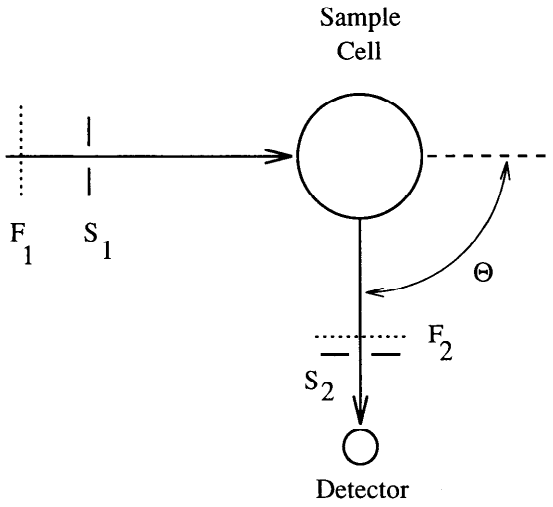

Fig. 1. Schematic of the measurement apparatus. Collimated light from a high-pressure Hg lamp passes through filter F_1, which isolates the line at 436 nm. Slit S_1 defines the beam, which falls on the sample cell. The front surface of the sample cell is actually polished flat to avoid refraction by the glass. The light fluoresced at angle Θ then passes through filter F_2 which absorbs strongly for wavelengths <600 nm. Slit S_2 defines the field of view of the detector (a red-sensitive photomultiplier tube). An additional filter (not shown), which attenuates strongly for wavelengths >500 nm, is sometimes placed between the cell and F_2. This filter absorbs the fluorescence and allows observation of any elastically scattered light transmitted by F_2.

Table 1. Information specific to individual culture types. Growth media types are IMR (Eppley et al. 1967), K (Keller et al. 1987), and f/2 (Guillard and Ryther 1962).

Cell name	Culture No.	Media type	Growth temp (°C)	μg Chl cell^{-1}	Cell diam (μm)	Cell shape
Thalassiosira pseudonana	ccmp 1011	IMR	19	9.87×10^{-7}	4–8	Hockey puck
Nitzschia cf. *constricta*	ccmp 576	IMR	19	2.32×10^{-6}	6	Football
Thoracosphaera heimii	ccmp 1070	K	19	1.61×10^{-6}	10–15	Baseball
Rhizoselenia fragillisima	ccmp 576	IMR	6	3.23×10^{-5}	40	Rectangular forms, long chains
Gonyaulax spinifera	ccmp 409	IMR	19	8.50×10^{-6}	50	Walnut
Dunaliela tertiolecta	ccmp 576	f/2	19	3.79×10^{-6}	6–8	Rugby ball

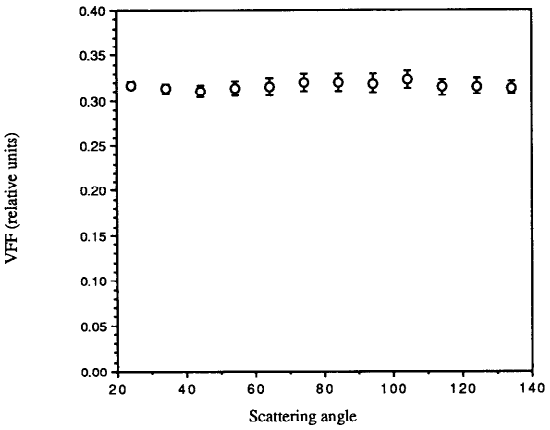

Fig. 2. Volume fluorescence function (VFF) for a dissolved chlorophyll standard, illustrating the isotropic VFF for chlorophyll in solution.

measurements were then used as a normalization to offset changes in cell number density in the sample volume during measurement.

To check and correct for elastic scattering not blocked by the Kodak Wratten filters, we placed a dichroic filter with transmission of 90% in its pass band in front of the receiving optics. This dichroic filter passed light of wavelength <500 nm and blocked light of longer wavelength. With this filter in place, only elastically scattered light was measured. In all of the cultures examined, the elastically scattered light received in the fluorescent signal was negligible for $\Theta > 50°$. This measurement of the elastically scattered light was then used at the smaller angles to correct the fluorescent signal.

The angular fluorescent emission of six cultures was measured: *Thalassiosira pseudonana* (centric diatom), *Nitzschia* cf. *constricta* (pennate diatom), *Thoracosphaera heimii* (calcifying dinoflagellate), *Rhizoselenia fragillisima* (centric diatom), *Gonyaulax spinifera* (dinoflagellate), and *Dunaliela tertiolecta* (chlorophyte without a visible cell wall). Specific growth conditions for each culture are detailed in Table 1. All cultures were grown with a 14 : 10 L/D cycle at ~ 51 μEinst $m^{-2}s^{-1}$. Samples of the culture were counted with an epifluorescence microscope and a Palmer Maloney counting chamber. Chlorophyll values were obtained for each culture by the technique of Yentsch and Menzel (1963). All cultures were obtained from the Provisoli-Guillard Center for Culture of Marine Phytoplankton.

The quantity measured in this experiment is the volume fluorescence function (VFF). The VFF, as defined by Gordon (1979), is the intensity (radiant power per unit solid angle) of fluorescence observed at an angle Θ to the excitation direction (Fig. 1) divided by the product of the incident radiant power (at the excitation wavelength) and the path length through the cell. Because the radiant power of the excitation was not measured (but the source was stable during each set of measurements), we were only able to measure the VFF in relative units; however, such measurements are sufficient to study the angular distribution of the fluorescence.

The first measurements obtained were of a dissolved chlorophyll standard (Fig. 2) obtained from Sigma Chemical Co. As expected, the VFF of the chlorophyll standard was completely isotropic. This measurement was used as a check of our instrumentation and procedures for the culture measurements.

The *R. fragillisima* clone did not grow to sufficient density to allow measurement in the cylindrical cell. For this culture, measurements were obtained at 45, 90, and 135° with the dissymmetry cell. The three measurements indicated that the angular distribution of fluorescence was isotropic, at least to the level of the system noise ($\sim 6\%$, figure not shown).

The cultures of *T. pseudonana, N. constricta, G. spinifera,* and *D. tertiolecta* grew to sufficient density to allow measurement in the cylindrical cell. The VFF for *T. pseudonana* and *G. spinifera* was found to be isotropic to within the experimental noise (Fig. 3). One sample of *N. constricta* showed peculiar structure in the VFF near $\Theta = 60°$ (Fig.4); however, this was not reproduced in a second sample, and we conclude that the VFF of *N. constricta* is also isotropic within the experimental error.

The signal from *T. heimii* was marginally sufficient to allow its observation with the cylindrical cell. A VFF-measurement sequence on it suggested a dissymmetry factor of $\sim 1/2$ might exist; however, when the sample was stirred and a rapid sequence of measurements was made at $\Theta = 45, 135, 45, 135°$, etc., no dissymmetry was observed, indicating that the initial observations were influenced by particle settling.

The only sample that exhibited a reproduc-

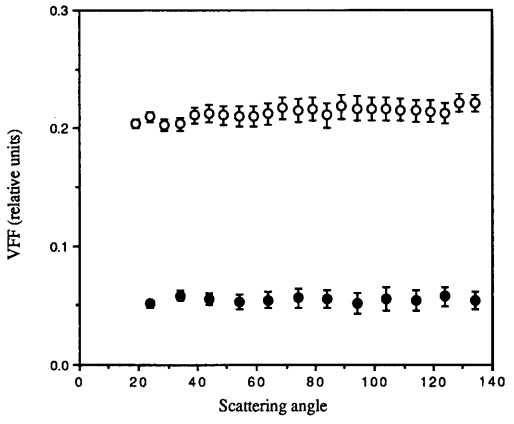

Fig. 3. VFF for cultures of *Thalassiosira pseudonana* (○) and *Gonyaulax spinifera* (●). The cultures exhibited an isotropic VFF within experimental error. Error bars are related to system noise.

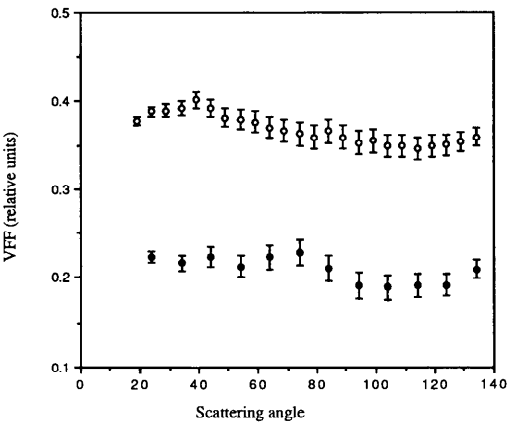

Fig. 5. VFF for *Dunaliela tertiolecta* at two concentrations (0.55×10^5 and 1.1×10^5 cells ml^{-1}). This culture was the only one to exhibit repeatable anisotropy.

ible anisotropic VFF was *D. tertiolecta* (Fig. 5). In this sample, there was an anisotropy of ~15% between the forward and backward direction (40 and 120° scattering angle). This anisotropy was repeatable and independent of cell concentration. Collins et al. (1985) reported this species to have a smaller than typical efficiency for the escape of fluorescence. The intercellular concentration of Chl *a* in our culture was 42×10^{-16} mol Chl *a* $cell^{-1}$ (Table 1, using 893 g mol^{-1} Chl *a*) compared with 34.6×10^{-16} for their shade-adapted cultures.

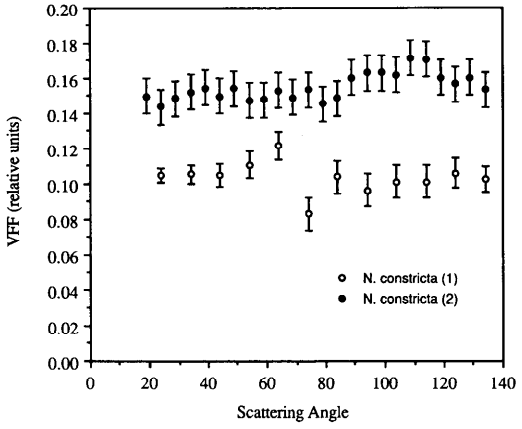

Fig. 4. VFF for two samples of *Nitzschia* cf. *constricta*. The peculiar behavior of the VFF near $\Theta = 60°$ in the first set of measurements is apparently an artifact.

Thus, our culture should have an even stronger reabsorption factor. If reabsorption is a dominating process, this should be an extreme case for anisotropy of the VFF. However, even in this case the anisotropy was low compared to the experimental studies of small particles (Kratohvil et al. 1978). Also, note that the slight enhancement in the forward direction cannot be explained by reabsorption of the fluorescence, as this would cause a depression in the VFF for $\Theta < 90°$.

Although the cultures examined had a diverse range of cell size, volume, and morphology, we have found an isotropic VFF for these phytoplankton cultures in all species examined except of *D. tertiolecta*. In *D. tertiolecta*, a repeatable anisotropy was found, which could cause small errors in both measurements and models requiring, or based on, isotropic fluorescence. In contrast to the strong anisotropy required to explain the observations of Kiefer et al. (1989), the anisotropy reported here was <20%, implying that an isotropic VFF is a reasonable approximation for most applications.

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Erratum

In the article by Pringle et al. (June 1993; V. 38, No. 4), the second sentence in the second paragraph that begins on p. 772 should read "For instance, streams draining some geological areas are low in P, with relatively high N : P ratios (Table 10)."