Time Series Measurements of Chlorophyll Fluorescence in the Oceanic Bottom Boundary Layer With a Multisensor Fiber-Optic Fluorometer

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An in situ multisensor fiber-optic fluorometer (MFF) has been developed to acquire long-term chlorophyll fluorescence measurements in the oceanic bottom boundary layer to characterize the finescale pigment structure at vertical spatial scales comparable to physical measurements. The eight fluorescence sensors of the MFF are composed of dual optical fibers of varying lengths (1.5–8 m), with the fiber ends oriented at 30° to each other and enclosed by a small light baffle. Strobe excitation blue light is passed through one of each pair of optical fibers and stimulated chlorophyll fluorescence is carried back to a photomultiplier. Two sets of four fluorescence sensors assigned to high- and low-sensitivity photomultiplier detectors enable chlorophyll \( a \) measurements in two ranges, 0–50 mg m\(^{-3}\) and 0–200 mg m\(^{-3}\), respectively. Aspects of the design of the fiber-optic sensor are described that were intended to optimize detection of fluorescence signals and minimize interference by ambient light. The fiber-optic sensor outputs were stable with minimal instrument drift during long-term field operations, and measurements were not affected by turbidity and ambient light. A vertical array of fiber-optic fluorescence sensors supported on a tripod has been deployed at coastal sites for up to seven weeks and chlorophyll fluorescence was obtained with sufficiently high vertical spatial and temporal resolution.
The multisensor fiber-optic fluorometer 
a. The general description of the MFF system

The MFF system used four excitation xenon strobe lamps (model LS-128A; EG&G Electro-optics) and two photomultiplier detectors (Hamamatsu HC-125), thereby providing a capability for independent fluorescence measurements at eight locations using variable length dual fiber-optic sensors (Fig. 1). Excitation light from each strobe was conveyed through two excitation optical fibers to two sensors. The two excitation optical fibers were epoxied into a single SMA fiber-optic connector and then inserted into an SMA mating adapter on the strobe housing. An additional fiber inserted into the strobe housing conducted the strobe light to a photodetector. Detection fibers from the sensors conveyed fluorescence emission light to the two photomultipliers provided with a housing to hold a pair of red filters and mating adapters for the detection fibers. The basic optical configuration of the excitation and detection system of a single fluorescence sensor of the MFF is shown in Fig. 2. The broadband excitation light is focused through a lens and a blue interference filter (Omega Optical 455DF) with maximum transmission at 455 nm and bandwidth of 70 nm at FWHM (full width at one-half of maximum) into a 600-μm core diameter optical fiber (3M FP-600-UHT). The blue excitation light is conducted by the fiber to the sensor sampling volume. The detection system consists of a detection fiber that conducts the fluorescence emission light from the sampling volume through a combination of a red interference filter (Rolyn Optics) with a peak transmission at 676 nm and a 665-nm high-pass filter (Rolyn Optics RG665) into a photomultiplier.

The electronics of the MFF is controlled by a Tattletale microcomputer (Onset Computers model 7) interfaced with a 500-kHz analog-to-digital converter (Ocean Optics, ADC500) and a 120-Mbyte hard disk for data storage. The software was written in C programming language that allowed control of instrument operations. These included the sampling interval and signal averaging of the strobe stimulated fluorescence, background signal subtraction (i.e., detector signal in absence of strobe excitation), data storage, switching to standby low-power mode between samplings, and communication with a personal computer. A 12-V battery pack consisting of alkaline batteries supplied power to the system. The four xenon strobe lamps in the MFF are triggered in sequence by the microcomputer and typically have a total flash duration of about 100 μs and a FWHM duration of only about 8.2 μs. As the peak fluorescence intensity corresponds to the peak in strobe intensity, the signals from the photomultiplier and the reference photodetector were digitized by a 12-bit analog-to-digital converter at a high sampling frequency of 500 kHz for 200 μs (100 samples per strobe flash) and the signal stored on hard disk. A typical fluorescence value obtained during a laboratory experiment or field operation consisted of an averaged response from 10 strobe flashes that was recorded along with the time stamp and the sensor number. An additional strobe flash was used for monitoring strobe intensity by the photodetector. A serial port on the MFF enabled programming and computer control for setting up of the MFF operating parameters and downloading of stored data files for further processing.

The pressure case was constructed of T-6061 aluminum and was 1 m long and 0.2 m in diameter. A double O-ring end cap was fitted with 16 optical penetrators (Ocean Optics, Inc.) or feedthroughs to couple the internal–external optics of the fluorometer. The pressure rated optical feedthroughs had SMA adapters at either end to couple to SMA connectors and a short
length internal 600-μm fiber that allowed light to be coupled from fibers mated on either end of the feed-through. One set of eight optical penetrators was used to couple excitation blue light from the four strobes to the sensors, and another set of eight penetrators coupled the fluorescence emission light from the sensors to the two photomultipliers located inside the pressure casing. Index matching liquid (Norland Products, Inc.) was used to enhance the efficiency of coupling of signals between the fibers and the optical feedthroughs. A four-pin underwater connector permitted serial communication between an external computer and the Tattletale microcomputer, thus enabling instrument control without opening and closing the pressure case. The sensor fiber lengths varied from 1.5 to 8 m, allowing remote fluorescence measurements to be made at varying distances from the instrument.

b. The dual-fiber sensor

Key considerations for the fiber-optic sensor design were the geometrical configuration of the fibers, fiber type, and a light baffle to minimize ambient light interference while simultaneously providing minimum impedance to water flow. Selection of the optical fibers was based on cost, performance, and ease of use in the ocean environment. The light-detection efficiency of an optical fiber depends on the numerical aperture and the fiber core diameter: the active sampling volume increases with larger values of both the parameters (Plaza et al. 1986). However, large core optical fibers are susceptible to breakage and difficult to handle during field operations in the oceanic environment. A trade-off is therefore involved in the selection of the core size for the optical fibers. The optical sensor for the fluorometer is made up of a pair of polished ends of the excitation and emission fibers that are oriented at an angle to each other. The active sampling volume is a cone defined by the numerical aperture, the fiber core diameter, and the sensor geometry. The overlap between the excitation and collection cones is the region of sampling volume from which fluorescence is measured.

Experiments were conducted to select fiber type and the best geometrical configuration for the fiber-optic fluorescence sensor, with the parameters of interest being the interferable angle, the distance between the bare polished fiber ends, and the optical fiber-core diameter. The fluorescence response for different configurations was evaluated in a solution of coproporphyrin (Aldrich Chemical, Inc.), a substance with fluorescence properties similar to chlorophyll. The use of this solution enabled fluorescence response of the optical fibers to be evaluated without the variability due to chemical degradation of chlorophyll. Fluorescence intensity was maximal for a 30° interferable angle for the 600- and 1000-μm-diameter silica-core optical fibers (Fig. 3). In comparison, experiments with the 200- and 400-μm silica-core fibers gave lower fluorescence response than the 600-μm silica fiber, while measurements with plastic-core fibers indicated high levels of fiber background emission (data not shown). Though the 1000-μm-diameter fiber gave slightly higher fluorescence signal (Fig. 3), the 600-μm core fiber was selected because of flexibility and low minimum bend radius. The fiber sensor ends were separated by a minimal distance of 0.1 mm or less. The 30° interferable angle between the excitation and detection fibers, beside giving a maximal fluorescence response, also provided easy handling of the fiber-optic sensors during field operations of the MFF. The silica-clad silica core fibers had a kevlar-reinforced polyvinyl chloride (PVC) jacket. At the sensor end, the PVC jacket and kevlar were removed to optimize the alignments between the excitation and detection fibers.

c. The light baffle

In situ chlorophyll fluorescence measurements can be affected by interference from ambient red light, especially during daytime operations (Hitchcock et al. 1989). Initial field trials of the fiber-optic fluorometer confirmed the need for a light baffle to prevent ambient light from affecting chlorophyll fluorescence measurements (data not shown). Though the fluorescence emission signal due to chlorophyll in the sampling volume could be separated from the ambient background signal by background subtraction or other synchronous detection techniques, the net effect of high background signal due to ambient light containing photons in the red wave band was a reduction in the fluorescence dynamic range measured by the instrument. This problem was addressed by construction and testing of a series of sensors with different light baffle designs (D’Sa et al. 1994). The selected design of the light baffle (Fig. 4) provided for minimal ambient light interference and only moderate impedance to water flow. The baffle was constructed out of PVC and composed of three cylindrical blocks of 9 cm in diameter and 2.5 cm thick. The fiber holder block had two holes oriented at a 30° angle. Excitation and emission fibers with polished ends were secured in the holes with epoxy. A center block was an
inverted V-shaped 2.5-cm-thick hollow cylinder. The bottom block had two sharply inclined holes to allow settling particles and fluid to exit the baffle. The blocks were held together by bolts that were inserted in three holes drilled at a 120° separation angle at the center of the V-grooves. The 1.25-cm spacers were placed on the bolts and between the blocks to separate them. The two inverted V-shaped 360° openings around the baffle allowed water to flow through the baffle and the dual-fiber sampling volume. A flume experiment using a dye (thymidine blue) indicated that at an approximate flow speed of 15 cm s⁻¹, the dye had a residence time of 1 s, while at 25 cm s⁻¹ flow speed, an approximate residence time of 0.5 s was recorded. These high flow rates were only used to determine the water residence time within the sample volume and revealed the minimum impedance provided by the baffle to the water flowing through the sampling volume. Analysis of sensor flow characteristics and saturation excitation irradiance indicated an increased phytoplankton fluorescence yield within the dual-fiber-optic sensor sampling volume (D’Sa 1996). A laboratory experiment was conducted to test the effect of the baffle walls on the fluorescence response of the dual fiber sensor by enclosing the fiber sensor in volumes—678, 381, 172 (baffle volume), and 22 cm³—and monitoring its fluorescence response in a diluted phytoplankton culture solution. A steady fluorescence signal of 581 counts was measured by the fluorescence sensor for the different baffle enclosures and indicated that the baffle, if necessary, could be further reduced in size and made smaller than other miniature fluorometers.

3. Performance evaluation of the multisensor fiber-optic fluorometer

a. Stability and drift analysis

In applications involving long-term unattended instrument operation, such as the bottom boundary layer deployments of the multisensor fiber-optic fluorometer, measurement stability is an important concern. The fluorescence measured by the different sensors should reflect the chlorophyll concentration in the medium as determined by the calibration coefficients (assuming constant fluorescence yield). However, due to various factors such as environmental stress on sensors and fibers, fiber degradation, biofouling, and strobe and electronic drift, the instrument response may vary. To monitor instrument stability, a number of laboratory calibrations were conducted on the eight-sensor fiber-optic fluorometer using stirred laboratory-grown pure phytoplankton cultures and by referencing the fluorescence output of the baffled fiber-optic sensors to the fluorescence of a “standard” Turner Designs fluorometer 10-005R, which was previously calibrated in acetone-extracted chlorophyll. The long-term fluorescence response of the dual fiber-optic sensors (Fig. 5) were
monitored prior to and following a 7-week field deployment in the United States east coast by comparing the fluorescence response of the MFF sensors in laboratory-grown phytoplankton culture with the fluorescence response of the same culture on the Turner Designs fluorometer and indicated no degradation in sensor performance. Sensor s7 shows higher fluorescence response after the deployment and could have been due to adjustments made to the sensor and better light coupling between the optical feedthrough and the sensor fibers. Performance degradation in some sensors occurred mainly due to biofouling. To minimize biofouling effects, the antifouling agent TBT (tributyl tin) was applied to the inside of the baffle and around the fiber-optic sensors. The antifouling agent was not applied to the fiber faces as it would have affected the optical properties of the sensor.

Sensors have different responsivities and calibration slopes (Fig. 6) due to the characteristics of each sensor and the different sensitivities of the detectors. Pure phytoplankton cultures were used in the calibration of the MFF sensors using the dilution method. The chlorophyll concentration of the culture was determined fluorometrically (Parsons et al. 1984) using a Turner Designs fluorometer. There exists a high degree of correlation between the chlorophyll concentrations and in vivo fluorescence ($r^2 \geq 0.9$ for most sensors). The measurement range of chlorophyll concentration for the sensors assigned to the high- and low-sensitivity photomultipliers are 0–50 mg m$^{-3}$ and 0–200 mg m$^{-3}$, with detection limits of 0.1 mg m$^{-3}$ and 0.5 mg m$^{-3}$, respectively. The lower fluorescence detection limits (equals $2\sqrt{2}$ times the standard deviation of the mean of the baseline signal) in fluorescence counts for the eight sensors of the MFF are shown in Table 1 and were obtained from seven measurements taken in artificial seawater during various calibration runs.

We evaluated the possibility that the fluorescence signal was affected by drift in fluorometer components such as the strobe and electronics. The strobe intensity and photomultiplier background signal were recorded during a 7-week deployment of the MFF in the bottom boundary layer on the United States east coast and are shown in Fig. 7. Analysis of peak strobe signal intensities and their background revealed stable operation of all four strobes. The excitation light intensities for the four strobes were about the same, but the relative strengths measured by the photodetector varied due to the positioning of the optical fibers carrying the strobe light in relation to the photodetector and is the reason for the different average peak intensities recorded (Fig. 7a). Monitoring of the strobe intensity allowed for the scaling of the fluorescence signal in case of drift in the xenon strobe lamp during long-term deployments. Analysis of the time series photomultiplier background signal (i.e., the photomultiplier signal in absence of the strobe excitation light) during the deployment also indicates minimal effects of detector and electronic drift in the signal (Fig. 7b). Background signal for the two photomultipliers during the whole deployment period varied by less than 10 counts out of 4096 and includes intersampling variations (at 1-h interval) and drift (Table 2). These measurements also indicate that the baffle was very efficient at minimizing any contributions of diel ambient light variations to the fluorescence signal. Changes in instrument temperature (due to the MFF being exposed to different water masses during the 7-week field deployment) also did not appear to cause

### Table 1. Lower fluorescence detection limit for the eight fiber-optic sensors used in the MFF. Values in fluorescence counts for the mean, standard deviation, and detection limit are shown.

<table>
<thead>
<tr>
<th>Fluorescence counts</th>
<th>Sensor 1</th>
<th>Sensor 2</th>
<th>Sensor 3</th>
<th>Sensor 4</th>
<th>Sensor 5</th>
<th>Sensor 6</th>
<th>Sensor 7</th>
<th>Sensor 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>51.0</td>
<td>29.85</td>
<td>25.85</td>
<td>30.14</td>
<td>41.71</td>
<td>93.85</td>
<td>40.0</td>
<td>44.85</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>38.18</td>
<td>17.45</td>
<td>11.11</td>
<td>24.97</td>
<td>24.4</td>
<td>45.05</td>
<td>38.09</td>
<td>35.41</td>
</tr>
<tr>
<td>Detection limit</td>
<td>107.9</td>
<td>49.3</td>
<td>31.3</td>
<td>70.6</td>
<td>69.0</td>
<td>107.7</td>
<td>127.4</td>
<td>100.1</td>
</tr>
</tbody>
</table>
any significant drift in electronics. Table 2 summarizes the overall performance of the four strobes and the two PMTs used in the fluorometer. The standard deviations of the peak intensities of the four strobes are quite small in relation to the mean peak signal.

b. Scattering and turbidity analysis

The problem of optical backscatter arises because of two main factors: 1) the blue filter on the excitation optics and the red filter on the detection optics do not perfectly isolate photons outside their cutoff wave bands and the possibility of strobe excitation light leakage into the detection system exists for the dual-fiber-optic sensor and 2) high turbidity in the water column due to high concentration of particulate matter may affect fluorescence measurements, as well as enhance nonfluorescence light backscattering. Experiments were conducted to evaluate the magnitude of extraneous signals that were not related to chlorophyll fluorescence and approaches to minimize their contribution.

The optical filter backscatter experiment was conducted to identify and minimize the coupling or leakage of the excitation light from the excitation to the detection system of the fluorometer. Transmission characteristics of broadband blue filters analyzed on a spectrophotometer showed the presence of a small bandpass in the red that could affect fluorescence measurements due to the leakage of red light from the excitation to the detection system. The fiber-optic fluorometer was operated with the excitation and detection fibers facing each other in a 180° opposing orientation, and the filter combination with the best optical isolation between the excitation and detection system of the fluorometer was determined from this experiment. Different filter configurations were evaluated for the detection and the excitation system. A blue interference filter on the excitation optics and a combination 665-nm high-pass filter and a red interference filter were selected for the detection optics. The high-pass filter was located between the interference filter and the detector, with the interference filter having its reflecting side facing the sensor fibers.

To evaluate the effect of different concentrations of nonfluorescing particulate matter (PM), the response of the fiber-optic sensors in waters of different diatomaceous earth concentrations was determined. Results of the experiment show that high turbidity levels do not affect the fluorescence signal of the MFF. The fluorescence output of a low- and high-sensitivity fiber-optic sensor (Fig. 8a) in waters containing zero chlorophyll concentrations indicate no consistent trend with increasing PM concentrations of up to 1500 mg L$^{-1}$. The fluorescence values obtained in this experiment may not correspond to those of the calibration (Fig. 6) since the MFF performance was optimized before the field deployment. The transmittance of the same water samples containing PM was measured on a 1-cm pathlength spectrophotometer (Fig. 8b) and confirms that backscattering of excitation light does not contribute significantly to observed signals. Effects of increasing PM concentrations added to the medium containing phytoplankton indicates no effect on the fluorescence signal (Fig. 8c).

4. Measurement of time series chlorophyll distribution in the bottom boundary layer

The MFF was attached to a 5-m-tall tripod along with benthic acoustic stress sensors (BASS) (Williams et al.)
Fig. 8. (a) Response of the fiber-optic sensors (numbers 2 and 6) of the MSFF to different concentrations of particulate matter (PM) measured using diatomaceous earth. (b) Percentage transmission of the same samples containing diatomaceous earth measured using a 1-cm-pathlength spectrophotometer. (c) Response of the low- and high-sensitivity fiber-optic fluorescence sensors (number 1 and 5) to different concentrations of PM added to a medium containing phytoplankton culture with a concentration of 118 mg m$^{-3}$ of chlorophyll $a$.

1987). The remote fiber-optic sensors of the multisensor fluorometer were oriented facing the seabed and placed at different heights above the bottom along one leg of the tripod at depths corresponding to the BASS sensors. The MFF was programmed to acquire chlorophyll fluorescence data from the remote fiber sensors at 40-min intervals. Each fluorescence value was composed of an averaged response from 10 strobe flashes for each sensor and was stored on the hard disk along with ancillary data. Time series estimates of near-bottom pigment concentrations (Fig. 9) were obtained at a 20-m site near Duck, North Carolina, at four discrete depths within 5 m from bottom [at 4.44, 2.55, 1.2, and 0.24 m above bottom (mab)] from 23 July (yearday 204) to 22 August 1994 (yearday 234). Pigment estimates were obtained from a predeployment calibration conducted with laboratory-grown pure phytoplankton culture (green algae—Nannochloris) based on the assumption of a constant fluorescence yield. Chlorophyll measurements at the four depths shown correspond to the high dynamic range MFF sensors that did not saturate at the high pigment concentrations encountered in the bottom boundary layer. Time series of estimated pigment concentrations were quite variable throughout the dataset and show a number of episodes with high pigment concentrations ($>100$ mg m$^{-3}$) that remained for periods of time varying from 0.5 days to approximately 2 days during the first half of the deployment period (day 204 to day 218). These were recorded at all four levels in the bottom boundary layer. For the duration of deployment two distinct distribution patterns were discernible. One was a high activity period at all levels in the bottom boundary layer prior to day 218, followed by a lower activity at depths closer to the seafloor and some high pigment levels at the uppermost levels. Following day 218 and the occurrence of a frontal storm, a large signal was recorded by the upper two sensors that persisted for a number of days but was not detected by the lower two sensors. In a subsequent paper (in preparation), we examine the relationship of observed variability to the physics and meteorology of the site.

5. Summary and conclusions

The multisensor fiber-optic fluorometer provides a unique capability to obtain fluorescence time series at different depths simultaneously. Thus far, vertical resolution in moored applications of in vivo fluorometers has been achieved through deployment of multiple instruments. The two photomultipliers and four strobes in the MFF allows for up to eight sensors on one instrument. Addition of xenon strobe lamps or a photomultiplier in the MFF would accomodate more fluorescence sensors. The fiber-optic system expands the effectiveness of individual strobe and detector packages by use of multiple optical fibers to allow increased vertical resolution, long-term unattended operations, and the design of small-sized fluorescence sensors allowing flexibility to place the fiber-optic sensors in close proximity to one
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REFERENCES