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High-Resolution Fluorometer for Mapping Microscale Phytoplankton Distributions

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A new high-resolution, in situ profiling fluorometer maps fluorescence distributions with a spatial resolution of 0.5 to 1.5 mm to a depth of 70 m in the open ocean. We report centimeter-scale patterns for phytoplankton distributions associated with gradients exhibiting 10- to 30-fold changes in fluorescence in contrasting marine ecosystems.

Patchiness in phytoplankton distributions is a ubiquitous phenomenon in aquatic ecosystems at spatial scales of millimeters to kilometers (7, 12, 17). At the microscale (<1 m) (10), interactions between biotic (9, 11, 21) and abiotic factors (1, 4, 15) generate spatial variability in the distribution of phytoplankton (5, 7, 14, 19). Understanding the extent and nature of this spatial variability is essential for determining the processes and mechanisms controlling plankton ecology (5) and has direct consequences for population dynamics (1, 21), planktonic ecosystem functioning, and biogeochemical fluxes (3).

How phytoplankton are spatially organized at the microscale remains poorly understood, due largely to technological constraints which have prevented direct measurement of individual distributions, thereby restricting our knowledge of microscale phytoplankton dynamics. Observations of microscale spatial heterogeneity began with measurements of "averaged" ecosystem characteristics determined from water samples collected using discrete sampling devices (14, 16, 19). Recent microprofilers allow continuous high-resolution measurement of in vivo fluorescence but have been limited by resolution or extent (6, 7, 20).

Here we describe laboratory testing, calibration, and field deployment of a laser-based profiling fluorometer, FluoroMAP (fluorescence microscale acquisition profiler; Alec Electronics Co., Kobe, Japan) which measures fluorescence microstructure with millimeter-scale resolution to depths of 70 m. This device can be used for quantification of phytoplankton biomass at spatial scales relevant to individual cells in marine or freshwater.

FluoroMAP. FluoroMAP contains sensors for measurement of pressure and fluorescence (Fig. 1). A blue diode laser (NLPB320; Nichia Chemical Industries. Ltd.) excites chlorophyll fluorescence at 450 nm with a half-bandwidth of 70 nm. Detection is at 640 to 720 nm orthogonal to the excitation and senses a volume of 2 μl.

When FluoroMAP is deployed as a free-fall device, the instrument profiling velocity can be controlled by adjusting the amount of ballast weight at the forward end of the pressure casing. Data obtained at the maximum selectable rate of 512 Hz is transmitted as analog signals via the instrument's deployment cable and is digitized by an A/D converter at a connected laptop computer.

Chlorophyll concentration in cultures. To determine the measurement range and sensitivity of the FluoroMAP fluorescence sensor, a dilution series of the cultured marine alga *Nannochloropsis occulata* was used. With the sensor centrally positioned within well-stirred 6-liter samples, the mean fluorescence was calculated from a 60-s time series of data. Following each measurement set, triplicate 200-ml water samples were collected for determination of the extracted chlorophyll *a* concentration using standard methods (18). Chlorophyll *a* concentration and fluorescence were strongly correlated (r^2 = 0.975; $y = 2591.9x + 152.4$; $P < 0.001$) for concentrations ranging from 0.04 ± 0.01 to 17.78 ± 1.62 μg liter^{-1} (mean ± standard deviation). The data indicated that FluoroMAP accurately measures chlorophyll *a* over the range of concentrations typically encountered in natural aquatic systems.

Cell-to-cell fluorescence variability. Microscale fluorescence measurements may incorporate shifts in the number or changes in per-cell chlorophyll content as a function of the cell size or photosynthetic or physiological state (7). To determine to what extent phytoplankton abundance or photosynthetic or physiological variability influences the measured FluoroMAP fluorescence signal, a laboratory simulated bloom experiment was conducted. Dilute early-stage 15-liter cultures of *Pavlova lutheri* and *Chaetoceros muelleri* supplemented with an initial dose of nutrients (F2 media) were sampled periodically, at the end of consecutive light/dark periods, for 21 days. At each sampling time the FluoroMAP fluorescence signals in well-stirred 6-liter subsamples were determined for 60 s, and the mean fluorescence levels were calculated. After FluoroMAP measurements were obtained, triplicate 1-ml subsamples from each culture were fixed with 2% (final concentration) paraformaldehyde. Flow cytometry was used to determine cell numbers, as well as cell size and per-cell chlorophyll content. Prior to

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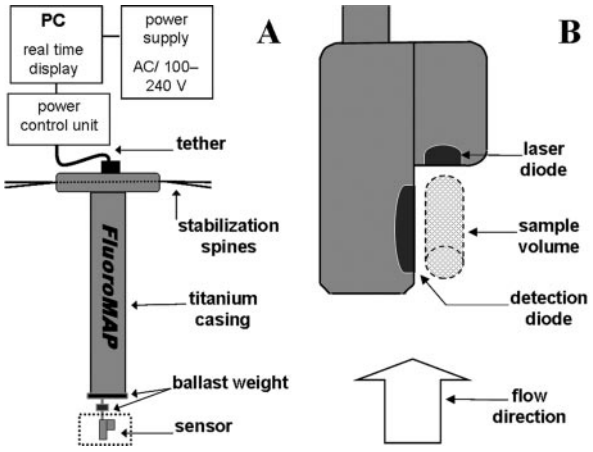

FIG. 1. Schematic diagram of the FluoroMAP fluorometer (A) and close-up of the fluorescence sensor and sample volume (B).

flow cytometric analysis, fluorescent beads (diameter, 1 μm; Molecular Probes, Eugene, Oreg.) were added to samples at a final concentration of approximately 10^5 beads ml^{-1} (8), and all measured cytometry parameters were normalized to the bead concentration and fluorescence. Flow cytometry was conducted using a Becton-Dickinson FACScanto flow cytometer, and populations of phytoplankton were identified and enumerated by using forward-angle light scatter, right-angle light scatter, and red (chlorophyll) fluorescence, using the Win Midi 2.8 flow cytometry analysis software (Joseph Trotter). There was a strong linear correlation between the FluoroMAP fluorescence signals and total cell concentrations for the cultures of *P. lutheri* and *C. muelleri* ($r = 0.961$ and $r = 0.924$, respectively; $P < 0.001$) (Fig. 2). No significant correlations between the FluoroMAP fluorescence signal and cell sizes or per-cell fluorescence were found for either culture. Changes in mean cell size and mean per-cell fluorescence varied by factors of 2.3 and 2.2, respectively, for *C. muelleri* and by factors of 2.1 and 3.0, respectively, for *P. lutheri* during the experimental period. *P. lutheri* exhibited an increase in the ratio of measured FluoroMAP fluorescence signal to cell concentration during the exponential growth stage (Fig. 2A1), indicating that there was physiological acclimation to the growth conditions and enhancement by a diel pattern of increased mean cell size and cell fluorescence ($r = 0.77$, $P < 0.001$) during light cycles prior to cell division during dark cycles. These results demonstrated the ability of the device for direct quantitative measurement of cell concentrations within its dynamic range. The effect of changes in the phytoplankton cellular state on the observed fluorescence signal was found to be small and species specific, which indicates that variability measured using FluoroMAP is directly associated with changes in phytoplankton biomass rather than with shifts in cell size or per-cell chlorophyll content.

Application in contrasting marine systems. FluoroMAP has been successfully deployed in a variety of marine and freshwater habitats. Figures 3 and Fig. 4 show representative profiles selected from 188 casts taken at stations located in (i) the highly productive and well-mixed coastal waters of the eastern English Channel (EEC) (50°40′75″N, 1°31′17″E) during the annual spring *Phaoecystis globosa* bloom and (ii) the oligotrophic oceanic surface waters of the Southern Ocean (SO) (57°37′10″S, 83°40′08″E) on the eastern edge of the Kerguelen Plateau. At each station multiple (≥8) vertical profiles were obtained over a short period (<1 h), and ballast adjustments

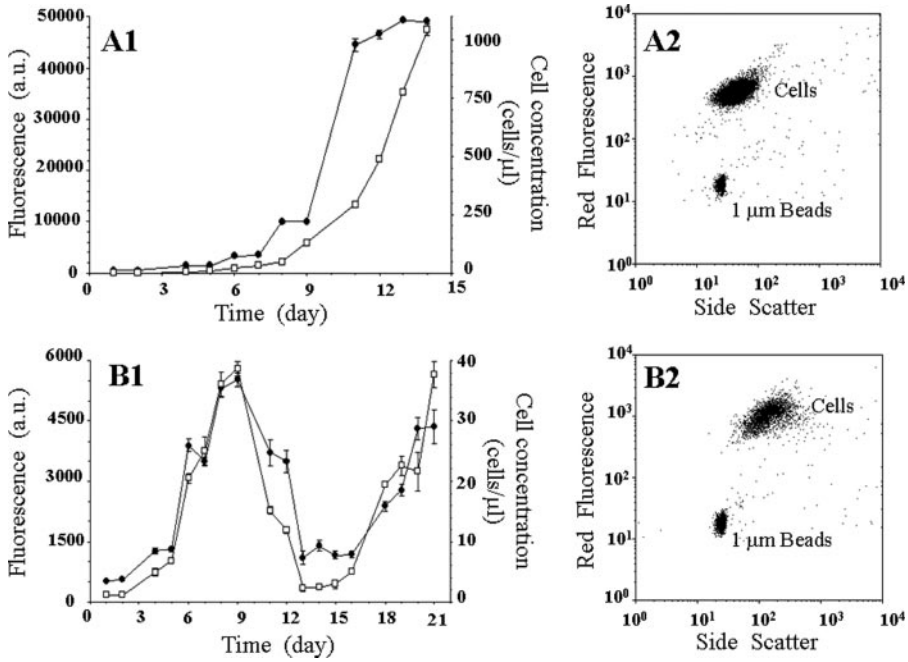

FIG. 2. FluoroMAP fluorescence intensities (●) (mean ± standard deviation; arbitrary units [a.u.]) and cell concentrations (□) (cells/μl) determined by flow cytometry during simulated blooms for cultures of *P. lutheri* (A1) and *C. muelleri* (B1). Representative cytograms show the fluorescence intensity (red fluorescence) and size (side scatter) of populations of *P. lutheri* (A2) and *C. muelleri* (B2).

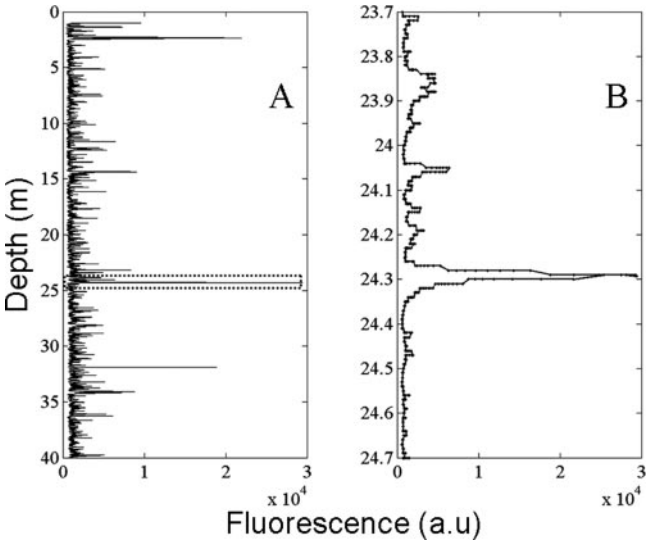

FIG. 3. (A) High-resolution vertical profile of fluorescence intensity (arbitrary units [a.u.]) with depth in the Southern Ocean. (B) Enlarged section of the fluorescence microstructure shown in panel A between 23.7 and 24.7 m, showing a typical peak structure for a 1-m section.

made prior to deployment in response to local hydrodynamic conditions provided spatial resolutions of 0.54 mm and 1.3 mm for EEC and SO profiles, respectively. A comparison of fluorescence time series obtained in the laboratory for a solution of pure water (MilliQ) (290 ± 18 arbitrary units) and for in situ sampling conducted at a fixed depth (17 m) in SO (1,051 ± 1,698 arbitrary units) demonstrated that environmental signals are significantly greater than the background noise (Fig. 5).

Vertical profiles of microscale fluorescence patterns were characterized by multiple point peak structures. Background

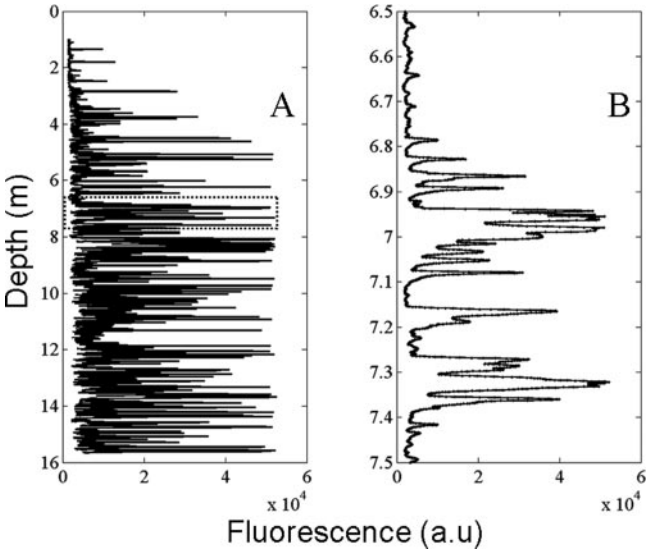

FIG. 4. (A) High-resolution vertical profile of fluorescence intensity (arbitrary units [a.u.]) with depth in the eastern English Channel. (B) Enlarged section of the fluorescence microstructure shown in panel A between 6.5 and 7.5 m, showing a typical peak structure for a 1-m section.

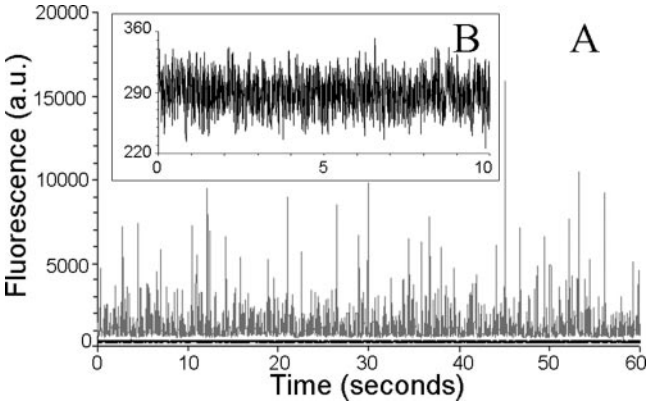

FIG. 5. (A) Comparison of the FluoroMAP fluorescence intensity (arbitrary units [a.u.]) time series determined in situ at a depth of 17 m in Southern Ocean waters (gray) and in the laboratory for a solution of MilliQ water (black). (B) Close-up of the fluorescence intensity of the MilliQ signal in panel A between 20 and 30 s.

concentrations were dominated by intermittent peaks, and the number, intensity, and spacing revealed repeatable patterns for profiles but were significantly different for different systems. For the SO, in contrast to the low background values, the dominant peak structures were typified by single peak morphologies with widths of ca. ≤10 cm (Fig. 3A). The peak structures exhibited sharp gradients over multiple points with up to a 31-fold change in fluorescence over 5.2 cm associated with a peak width of 11.2 cm (Fig. 3B). In the EEC higher background levels linked to the *P. globosa* bloom were associated with an increase in the frequency of dominant peak structures (Fig. 4A). These peaks had distinctive morphological differences compared to those of the SO and were characterized by the occurrence of intrapeak heterogeneity (multiple peaks) within dominant peak structures having widths of ca. ≥10 to 20 cm. Figure 4B shows a representative section of an EEC peak structure, in which there was an 11-fold change in fluorescence over 1.2 cm associated with a peak width of 13.6 cm.

FluoroMAP can provide new insights into phytoplankton ecology through continuous in situ measurement of phytoplankton distributions. Specifically, our observation of repeatable distribution patterns suggests that phytoplankton communities respond in a structured manner to local physical, chemical, and biological conditions. The structures are ecologically significant since the measured gradients revealed a more-than-10-fold change in fluorescence over distances of 1 to 5 cm. These results, obtained by measuring throughout the entire euphotic zone, generalize previous observations of large microscale gradients that were made over only a few tens of centimeters or in unusual marine environments (2, 7, 13, 14, 19). Our overall conclusions are that FluoroMAP shows that biological processes may influence phytoplankton distributions over distances of millimeters to tens of centimeters and that it can quantify the distances over which different species and communities lose their ability to control their positions. This information, in turn, should increase our understanding of plankton reproduction, predator-prey interactions, virus-host

interactions, nutrient uptake, primary production, and the effects of turbulent transport.

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