A Miniature Fluorometer for Oceanographic Applications

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ABSTRACT

A fluorometer intended for ocean measurements of dye patches achieves small size, low power consumption, and high sensitivity. Its performance is reported using fluorescein sodium as the fluorescent agent, although the principle is applicable to any dye that fluoresces at a wavelength sufficiently different from the excitation wavelength. An implementation in the Naval Research Laboratory's advanced microstructure profiler is described in detail, and results from static calibration and dynamic response tests are given.

1. Introduction

Fluorometers have long been used in oceanography, especially for determining chlorophyll concentration (D'Sa et al. 1997; Cowles et al. 1989). Until recently, these have depended on an incandescent or stroboscopic broadband light source (e.g., Hitchcock et al. 1989). However, since light from only a narrow portion of that band is suited to excite the fluorescent material, this approach requires relatively large amounts of input power to obtain the narrow band required for fluorescent excitation. This inefficiency requires a large instrument volume dedicated to power supplies and controllers. Unwanted energy outside the excitation band must then be absorbed in the filters and dissipated as heat. Moreover, heat released from the incandescent source or strobe itself must be dissipated to the environment to avoid unacceptably high temperatures in the instrument.

A new generation of instruments that avoid many of these size and power problems is now emerging. For example, the WETStar model, manufactured by WET Labs, intended for measurement of ocean chlorophyll, is already available commercially. It offers a straight-through flow tube, to which a pump can be attached to minimize response time. The optical path is transverse to the flow tube. A pair of light-emitting diodes fitted with blue filters provide light for excitation, while fluoresced light passes through a red filter before falling on the photodiode receptor. Response is boosted by use of a mirror and lense.

The design introduced here is optimized for measuring concentrations of fluorescein dye, which is used in studies of ocean mixing because of its detectability at very low concentrations. Our application has been to augment the suite of measurements on the Naval Research Laboratory's Advanced Microstructure Profiler (AMP), a variant of an instrument originally developed by M. Gregg at the University of Washington's Applied Physics Laboratory. This free-fall device reports data back through a slack fiber-optic cable that also serves as a recovery tether. The AMP is battery powered and normally makes a large number of profiles in succession without being retrieved on board. This usage dictates the basic fluorometer design requirements: small size to permit rapid response, minimum hydrodynamic flow disruption, and physical integration among the other sensors; low power to minimize battery drain and avoid heat contamination of nearby sensors; flushing by normal motion of the package through the water, thus avoiding use of a pump; and high sensitivity to detect dye patches for as long as possible while they diffuse.

2. Design principles

In Fig. 1a, a 3.3-kHz clock is used to switch a light-emitting diode (Fig. 1b) on and off at the clock fre-

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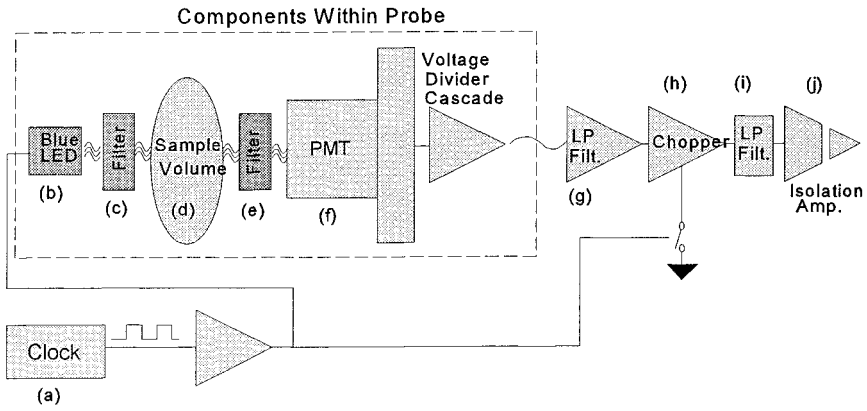

FIG. 1. Schematic of overall fluorometer design.

quency. The light-emitting diode is chosen to have a narrow bandwidth of emitted light that will bracket the excitation band of the fluorescent molecule. Light from the diode passes through a blue optical filter (Fig. 1c) and illuminates a volume of water containing the dye (Fig. 1d). In constructing the prototype instrument, we chose fluorescein sodium dye as the fluorescent material. The light-emitting diode and blue filter were chosen to emit light around a wavelength of 490 nm. The dye is excited at this wavelength and fluoresces at a wavelength near 575 nm. Emitted light passes through a green optical filter (Fig. 1e) and into a miniature photomultiplier tube (PMT) (Fig. 1f). The exciting LED (Fig. 1b), the optical filters (Figs. 1c,e), the sample volume (Fig. 1d), and the PMT (Fig. 1f) are mounted in an inline configuration. Filter (Fig. 1c) is chosen so that most of the light at the exciting wavelength passes though. Filter (Fig. 1e) is chosen so that nearly all light from filter (Fig. 1c) is blocked, while most of the light at the emitting wavelength is passed. Output from the PMT enters an initial electronic bandpass filter (Fig. 1g) to reduce very high-frequency noise and to center the signal between the LED on and off conditions. The signal is then fed through an electronic synchronous chopper (an inverting rectifier—see appendix), synchronized to the clock frequency, so that the only signal passed though the chopper is that synchronized with light from the LED. The signal is then low-pass filtered (Fig. 1i) and becomes only a function of the fluorescence. Chopping and centering of the chopped output reduces the effects of stray ambient light and of unwanted fluorescence caused by stray light that may have leaked into the control volume. An isolation amplifier (Fig. 1j) is employed to couple the output signal to other data collection and processing circuitry without affecting signal and power grounds of the ancillary circuits.

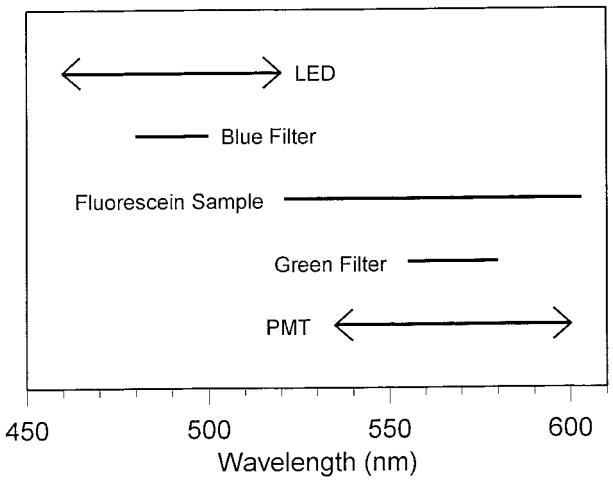

FIG. 2. Bands of emission, transmission, or response for components given in Fig. 1. Bands for the LED and PMT are greater than shown.

3. Implementation

Optical characteristics of this inline arrangement are given in Fig. 2. The blue optical filter is a Satlantic TO5-Cust/490 with pass band from 480 to 500 nm; the green is a Satlantic TO8-Cust/555 with pass band from 555 to 575 nm. These were chosen to be mutually blocking and to match the characteristics of fluorescein sodium ($C_{20}H_{10}O_5Na_2$, 10% aqueous; Sigma Chemical Company). The PMT is a Hamamatsu R5600U TO-8.

An exterior view is given in Fig. 3. In operation, water approaches the probe from the top, enters through the 11 entrance ports, passes through the interior sample volume, and leaves via the exit ports. Diameter of the probe is 32 mm and overall length is 151 mm. The 11 entrance and exit ports are 3.9 mm in diameter. An internal wire passageway, marked at each end by exterior plugged holes, carries power to the LED. An exploded view (Fig. 4) shows the arrangement of interior components.

4. Sensitivity and static calibration

Testing was done by immersion in a well-stirred bath of fluorescein diluted with distilled water. Output of the

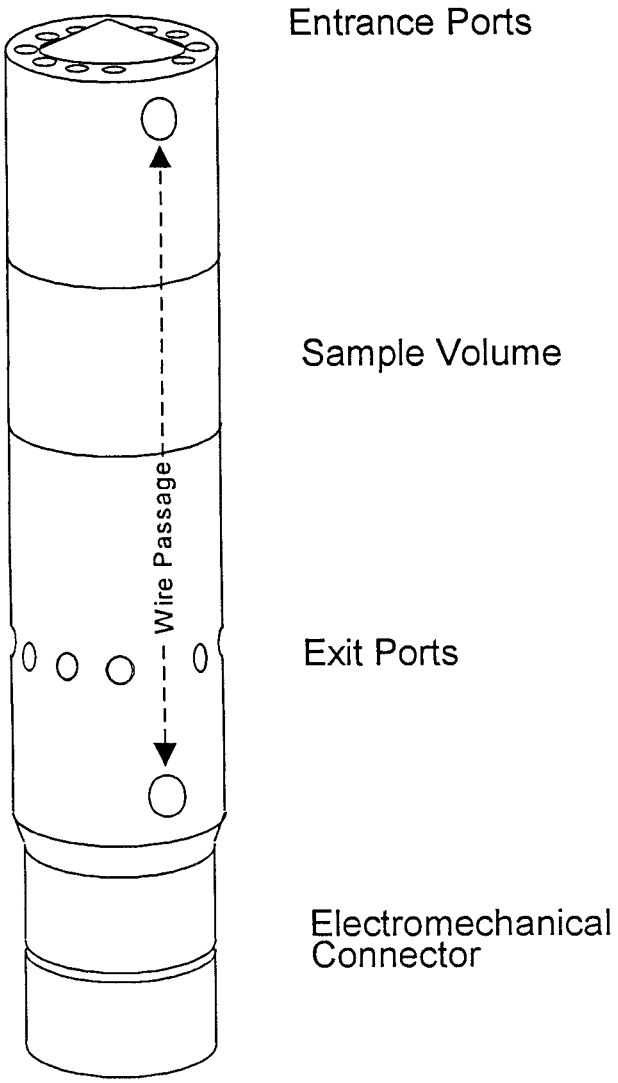

FIG. 3. External view of AMP fluorometer.

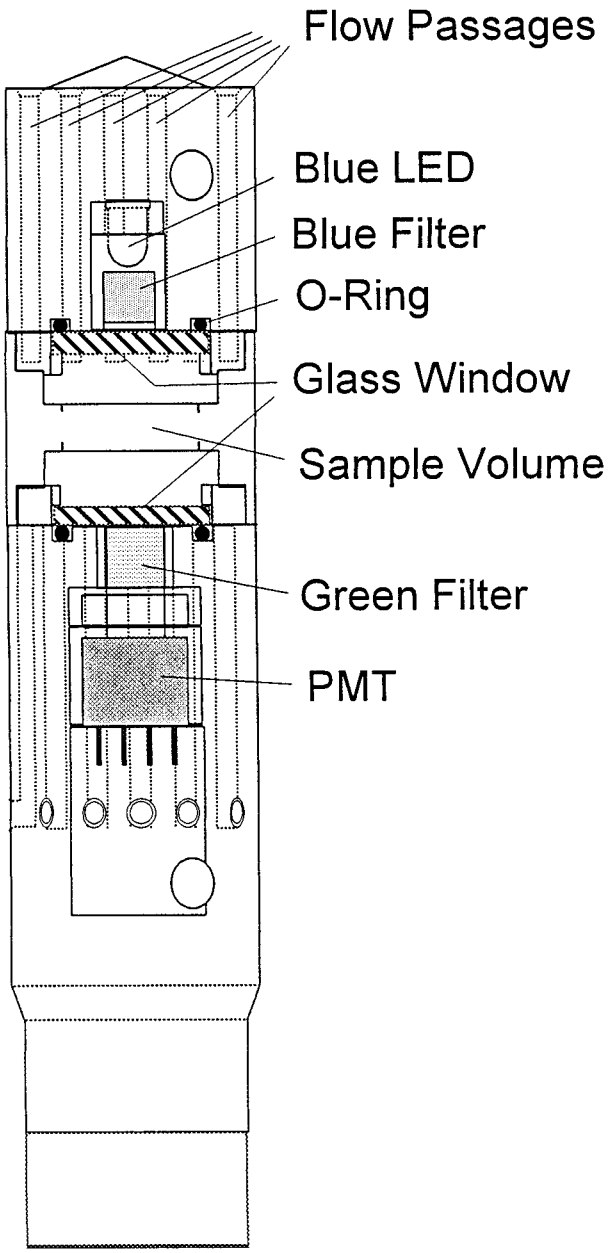

FIG. 4. Internal layout of major components.

unit, expressed as counts from the A/D converter output, is given as a function of dye concentration in Fig. 5. Over the broad central range of concentrations, from approximately 10 to 2000 parts per trillion (ppt), output is roughly linear on a log–log scale. Departure from linearity at high concentrations can be attributed to light attenuation by dye over the optical path; concentrations greater than about 50 000 ppt effectively blocked light transmission. At low concentrations, the signal was masked by electronic noise from the circuit board for concentrations below about 30 ppt. But by viewing output from the PMT with an oscilloscope, the lower limit of detectability could be extended to about 3 ppt. However, as we note in the discussion, these very low concentrations are unlikely to be seen in the ocean because of naturally occurring contaminants.

5. Dynamic response

Transient response of the probe has been evaluated under simulated operating conditions, addressing especially flushing of the sample chamber. A plastic container some 2.5 m high and 1.0 m in diameter was fitted midway up with a viewing window and dye injection port. The lower part was filled with saltwater of salinity about 30 and the rest with freshwater so that a sharp density interface was formed at the level of the injection port. A layer of dilute fluorescein dye was introduced at this interface and allowed to spread across it, forming a layer a few centimeters thick. AMP was then allowed

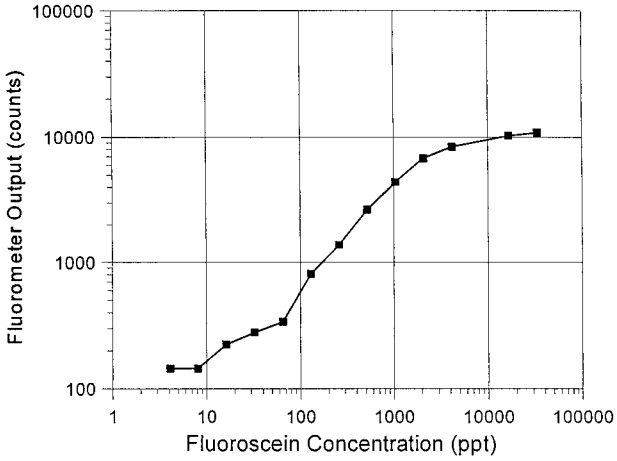

FIG. 5. Static calibration in a well-mixed bath with various dye concentrations.

to make a controlled fall in such a way that the fluorometer passed through the dye layer at 1 m s^{-1}, approximating the speed used for ocean measurements.

Results of this test are given in Fig. 6. Response of the microconductivity sensor (left panel) indicates the salinity interface sharpness; response of the fluorometer is given in the right panel. A delay equivalent to 10 cm of instrument travel is evident in fluorometer response relative to that of conductivity. Of this, 4 cm is attributed to vertical separation between the conductivity sample point and the fluorometer sample volume, and the remaining 6 cm to retardation of flow through the fluorometer passageways. The probe flushes after about 20 cm, and this is the limiting factor for spatial resolution.

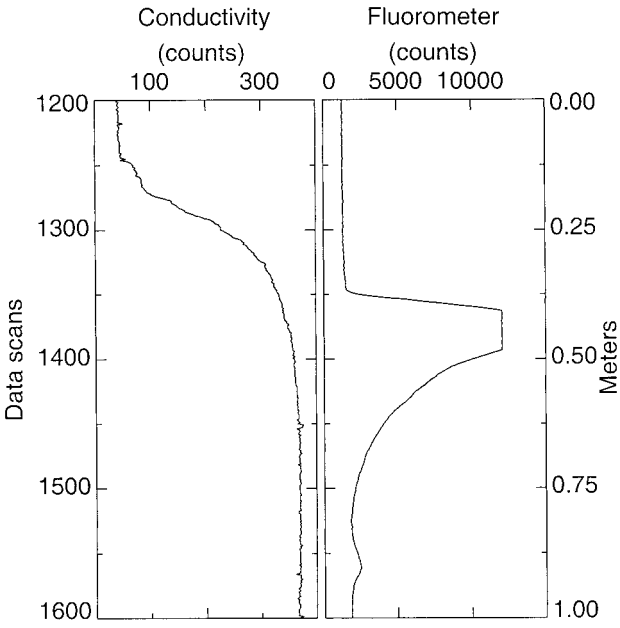

FIG. 6. Typical transient response measured during passage through a dye layer.

6. Discussion

The fluorometer we have described has a potential measurement range of fluorescein sodium concentrations between 30 and 50 000 ppt. Small diameter, 32 mm, is achieved by use of a light emitting diode, miniature PMT, and high-quality optical filters in an inline configuration. Overall length is 151 mm. Spatial resolution of approximately 20 cm is obtained without use of a pump in an external flow of 1 m s^{-1}, which is provided by the descent rate of our free-fall instrument. Power consumption is kept to about 1 W, which occurs primarily in the LED and power supply for the PMT. Low heating of the instrument follows from low overall power consumption, and heat dissipated in the sensed volume is kept low by the narrow bandwidth of excitation light. These properties make the device well suited to measurement of ocean dye patches from a free-fall instrument where size and power consumption are limiting.

High common mode rejection of undesired fluorescence arise from synchronous operation of the diode light source and chopper circuit and from centering the output signal between levels corresponding to diode on and off states. The technique minimizes the effect of any stray light that might enter the sample chamber. We have in fact found the output to be independent of background light level during field operations—it is the same near the surface as at depth. But this may be due in part to effective exclusion of outside light by the probe geometry. By the same argument, we expect little effect from bioluminescent bacteria. *Vibrio fischeri,* for example, might otherwise register as fluorescein since it emits light with a spectral peak near 550 nm (Mobley 1994), overlapping the band of our green filter.

However, synchronous chopping does not discriminate against natural fluorescence. Most troublesome in natural waters is yellow matter, which fluoresces over a broad spectral band (Mobley 1994) and occurs in a wide range of concentrations. Raman scattering is not expected to play a significant role. Light that has passed through the blue filter and been Raman scattered will fall primarily between 570 and 600 nm, and so will be largely blocked by the green filter with its pass band of 545–565 nm.

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APPENDIX

Chopper Circuit

The chopper or inverting rectifier operates in the following manner (Fig. A1). When the diode is on, the control signal from the clock is on and switch S1 is open. Therefore, the potential at the positive input *P* to the operational amplifier *A* is equal to *e*-in. This forces

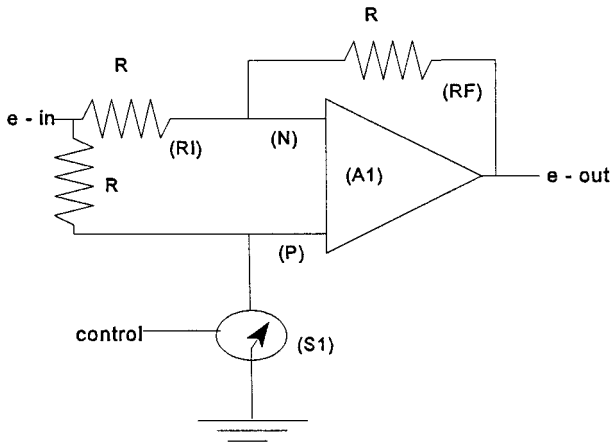

FIG. A1. Schematic of synchronous chopper circuit.

the potential at the negative input *N* to be *e*-in. Thus no current flows through feedback resistor (RF) and the potential at the output is *e*-in. When the diode is off, the control signal closes switch S1, forcing potentials at *P* and *N* to ground. As RF is equal to the input resistor (RI), the current through RI must be equal in magnitude and opposite in sign to that through RF, thus forcing *e*-out to be $-(e\text{-in})$. A lowpass filter (Fig. 1) centers the zero point of the photomultiplier output. Thus (for a constant fluorescence) the input signal changes from $+E$ to $-E$ and the coherent demodulator rectifies the voltage to a uniform $+E$ except for a small transient during switching. This transient is smoothed by the final lowpass circuit.

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