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DEVELOPMENT OF A MULTI-SENSOR IN SITU FIBER OPTIC FLUOROMETER

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ABSTRACT

Our objective is to develop and evaluate a multi-sensor *in situ* fiber optic fluorometer. The instrument is designed to sample and store in vivo strobe-stimulated fluorescence data at multiple depths and high frequencies (1 Hz). This information may be used for estimating the distribution and abundance of particulate pigment biomass, for supporting models of water column primary production and as a complement to remotely sensed ocean color estimates of pigment biomass. The instrument is unique in that it uses fiber optic technology to increase vertical resolution. While it is theoretically possible to accomplish this task using a large number of commercially available fluorometers, our proposed design would provide a less expensive approach. A laboratory prototype has been built and is being tested. Preliminary results indicate that the instrument meets all the project goals and that low cost, high frequency, high spatial resolution chlorophyll data are obtainable with the current design. Further work is required to develop the seagoing version, and optimize the configuration of the fiber sensors.

SCOPE OF INVESTIGATION

Goals of the Department of Energy's (DOE) Ocean Margins Program (OMP) include quantification of primary production and flux of phytoplankton biomass on the continental shelf and determination of controlling mechanisms. To accomplish these objectives, high resolution time series observations of the distribution and abundance of particulate pigment biomass will be required. Discrete shipboard sampling is inadequate to fully resolve variations in particulate pigment biomass related to changing environmental conditions. Many important physical variables have time scales ranging from minutes to hours. Hence, the coarse temporal resolution provided by discrete sampling techniques may lead to aliasing (e.g. Dickey et al., 1991). Furthermore, shipboard studies have limited duration and may miss episodic (e.g. weather related) as well as seasonal scale events.

The estimation of chlorophyll concentration from in vivo light-stimulated fluorescence has long been recognized as useful for oceanographic applications (e.g. Lorenzen, 1966). The method allows for greatly increased resolution over that possible by discrete measurements of extracted chlorophyll (e.g. Holm-Hansen et al., 1965) or through direct observations of phytoplankton cell concentrations. Furthermore, measurements can be accomplished *in situ*, thus eliminating the need for acquiring and manipulating a water sample. *In situ* fluorometers have long been used to obtain high resolution vertical profiles (e.g. Platt, 1972; Derenbach et al., 1979) and in towed instrument packages for high resolution spatial assessments of chlorophyll concentrations (e.g. Platt, 1972; Aiken, 1977; Herman and Denman, 1977, 1979; Denman and Herman, 1978; Fasham et al., 1985; Strass, 1990). More

recently, they have been deployed on moorings (Whitledge and Wirick, 1983, 1986; Dickey et al., 1986; Falkowski et al., 1988; Walsh et al., 1988; Fukuchi et al., 1988; Walsh et al., 1989) and free-drifting instrumentation (Hitchcock et al., 1989). Thus far, vertical resolution in moored applications of *in situ* fluorometers has been achieved through deployment of multiple individual instruments. Our system would expand the effectiveness of individual strobe and detector packages by the use of multiple optical fibers to allow increased vertical resolution.

The fluorescence yield (R, the ratio of the fluorescence signal to the chlorophyll concentration within the fluorometer detection volume) is variable. Variations in fluorescence yield have been attributed to the following (cf. Falkowski and Kiefer, 1985; Strass, 1990):

- Species composition (Flemer, 1969; Loftus and Seliger, 1975; Slovacek and Hannan, 1977; Yentsch and Yentsch, 1979; Soohoo et al., 1986)
- Light history and photoadaptive state (Steeman-Nielsen and Jorgensen, 1968; Kiefer, 1973a,b; Loftus and Seliger, 1975; Falkowski and Owens, 1980; Perry et al., 1981; Setser et al., 1982; Falkowski, 1984; Falkowski et al., 1986; Neale and Richardson, 1987)
- Nutrient supply (Kiefer, 1973a; Slovacek and Hannan, 1977)
- Physiological state and endogenous rhythms (Loftus and Seliger, 1975; Prezelin and Sweeney, 1977; Cullen and Renger, 1979; Prezelin and Ley, 1980; Harris, 1980)

Besides these factors related to phytoplankton physiology, fluorescence yield may be sensitive to variations of the irradiance within the detection volume, which is subject to changes in particle concentrations and size distributions (Morel and Bricaud, 1981; Baker and Lavelle, 1984; Collins et al., 1985). In addition, fluorescence yield will be affected by the presence of fluorescent substances other than chlorophyll a, such as phaeopigments (e.g. Herbland, 1978; 1988).

In view of the many sources of variation, it is not surprising that fluorescent yield in natural populations has been observed to vary by one order of magnitude (Kiefer, 1973a; Loftus and Seliger, 1975). It is beyond the scope of this proposal to exhaustively characterize all sources of variability. However, such variability must be considered during field applications of the instrument. Previous investigations have shown that reasonable precision in estimates of chlorophyll concentrations from fluorescence yield relationships. Best results were achieved when calibrations were made for a specific area of study (spatial scales of 10-100 km) and adjusted for effects of light-dependent fluorescence quenching (cf. Weber, 1986; Strass, 1990). Examples to support this view include a regression of *in situ* fluorescence versus extracted chlorophyll derived for combined data from various water masses in the Western Mediterranean Sea over a three week period ($r^2 = 0.80$, $N = 456$, D.

Wiesenburg, unpublished). Lohrenz et al. (1993), using a multiple linear regression of fluorescence and depth versus extracted chlorophyll, obtained an r^2 of between 0.72 (N = 76) and 0.90 (N = 117) for data collected from Gulf Stream and Slope water masses over a five week period. Whitledge and Wirick (1986) reported that chlorophyll concentrations estimated by fluorescence agreed within a factor of 2 with extracted chlorophyll measurements for prototype deployments of a moored instrument off Long Island. Since natural variations in chlorophyll concentration encompass several orders of magnitude, this level of precision can provide useful information. Clearly, the interpretation of the fluorescence measurement as an indicator of distributions of particulate pigment biomass necessitates an extensive calibration procedure. Our proposed research will include an evaluation of sensitivity, precision and signal response of a prototype model in conditions where variations in chlorophyll *a* concentration are controlled. Subsequent field tests will take into account the temporal and spatial dependence of the auto-correlation function of fluorescence yield (Strass, 1990).

SIGNIFICANCE OF PROPOSED RESEARCH

The utility of moored fluorometers for providing information about flux of particulate pigment biomass (Falkowski et al., 1988; Walsh et al., 1988; Walsh et al., 1989) and in support of bio-optical models of primary production (e.g. Ryther and Yentsch, 1957; Platt, 1986; Bidigare et al., 1987; Kiefer et al., 1987; Smith et al., 1987; Platt and Sathyendranath, 1988; Keller, 1989; Platt et al., 1989; Sakshaug et al., 1989; Sathyendranath and Platt, 1989; Dickey et al., 1991; Balch et al., 1992). This monitoring capability will allow the identification of periodic high productivity events (e.g. Glover et al., 1988; Lohrenz et al., 1988a; Marra et al., 1990). It has been suggested that inadequate sampling of these episodic events in the open ocean may lead to underestimation of regional primary production (Platt et al., 1989; Dickey et al., 1991). Since primary production in the continental margins is both higher and more variable than in the open ocean (Platt and Subba Rao, 1975; Malone, 1980; Walsh, 1989; Lohrenz et al., 1990), there is a need for high resolution temporal and spatial information about particulate pigment biomass. Another important application of moored vertically resolving fluorometer is that of ground truth applications for satellites, providing both near surface and subsurface information in support of algorithms for estimate of pigment biomass from ocean color. The moored data would also provide data between satellite missions and in periods when satellite measurements are not possible (night and cloudy periods). Finally, the design is sufficiently flexible to be adapted for high resolution spatial and temporal characterization of fluorescent material associated with the benthic boundary (e.g. SEEP I and SEEP II).

PROGRESS TO DATE

The goal of this 2 year project is to develop a multi-sensor fiber optic fluorometer with the following capabilities:

1) autonomous collection of data at a moored location for months long deployments.
2) large number of sensors for increased vertical resolution.
3) high data sampling frequencies (1 Hz).
4) low cost componenets to permit deployment at multiple locations.

In Year 1, the specific objective was to develop a laboratory based single sensor unit that could serve as a prototype detector system for the seagoing version to built in Year 2 and also to serve as a test bed for exploring fiber optic sensor designs.

The laboratory prototype has been built and is being tested. Preliminary results indicate that the instrument meets all the project goals and that low cost, high frequency, high spatial resolution chlorophyll data are obtainable with the current design. Further work is required to develop the seagoing version, and optimize the configuration of the fiber sensors.

Description of Laboratory Prototype Instrument

The laboratory prototype fiber optic fluorometer (FP-1) consists of 3 components: 1) a xenon strobe excitation source, 2) a photomultiplier - phototransistor detection module interfaced to a PC computer, and 3) a fiber optic sensor. The components are all capable of battery operation. The efficient pulsed xenon source is capable of operation over extended time periods. Deployment duration is limited currently by computer power consumption.

Xenon strobe excitation source: The strobe uses a standard "U" shaped xenon tube with integral invertor for high voltage generation. The pulse bandwidth is approximately 20 msec. The strobe firing circuit uses optical isolation to eliminate possible discharge events through the trigger line that is connected to the detection module. Power is supplied by a rechargeable 12 V gel cell with a storage capacity of 3.4 amp h. This will provide power for flashes at the rate of 1 KHz for approximately 34 h. Provision is made for recharge via a standard plug in power module.

The light output of the strobe passes through a set of dichroic filters to a standard SMA connector that couples to the excitation line of the fiber optic sensor. The dichroics block light > 500 nm from entering the excitation line. A second SMA connector is mounted away from the filters in a partially shielded region to provide light to a fiber that illuminates the strobe monitor.

Detection module: The detection module consists of a Hamamatsu HC125-01MOD70 photomultiplier tube (PMT) assembly for measuring fluorescence and a Motorola MFOD100 phototransistor (PT) to monitor the strobe intensity. The detectors are connected to a Computer Boards Inc. PC plug-in board (ADC) capable of data acquisition rates up to 330 KHz. The output of the PMT (0-10 V) is connected directly to channel 0 of the ADC board. The output of the PT is connected to channel 1 of the ADC through a transimpedance amplifier. Power is supplied by +5 V from the ADC. The read fiber from the fiber sensor is couple to the PMT with an SMA bulkhead connector. A narrow bandpass dielectric filter centered at 680 nm is sandwiched between the PMT and the fiber end.

Fiber optic sensor: The fiber sensor consists of two lengths of encapsulated 1 mm diameter acrylic fiber joined at the sensor and terminated with SMA connectors at the detector and excitation ends. At the sensor end flat sections of black encapsulant is removed from each fiber and the two are bound together in parallel such that one fiber illuminates the taget and the other views approximately the same region to within about 1 mm of the fiber surface. The large numerical aperture of the fiber results in a significant overlap between illumination and read volumes.

Operating software: Operating software has been written in modular Borland Turbo C so that modifications can be easily implemented and so that the code can be transported to the data logger in the seagoing system or to other platforms. The PC based software is designed as a development tool to investigate data manipulation strategies and to optimize system performance. The code initializes the ADC board, outputs strobe trigger pulses and provides a series of menus from which to choose the various functions:

OSCILLOSCOPE - real time monitoring of the detector outputs, i.e. voltage vs time.

TAKE DATA - signal averaged data sets collected for a selected number of flashes and stored in either BACKGROUND (no strobe), REFERENCE (strobe intensity monitored by the PT) or SAMPLE (fluorescence detected by the PMT) data registers. Background corrected averaged data are presented graphically for inspection through a cursor and saved as ASCII data files.

SET PARAMETERS - permits selection of ADC sampling rate, number of samples to average, time between samples and ASCII data file names. The sample period can be set from as short as 300 ms (limited by the charging time of the strobe capacitors) to as long as desired.

Two versions of the code have been written. FLUOR.EXE takes 100 ADC data points for each flash event for either the PMT or the PT and alternates between the two every other flash. FLSWICH.EXE alternates between PMT and PT during each flash, thus providing half the time dependant resolution but giving an instantaneous reference reading for the flash.

System Performance

Detection: The system has been tested using water samples collected near Dunedin Harbor, FL and isopropyl alcohol extracts of terrestrial plant material. The water at the sampling site was very clear and is assumed to have the low levels of chlorophyll associated with winter conditions at this locale. Chlorophyll levels in the alcohol extract were estimated from optical absorbance measured in an S1000 spectrometer using standard methods. The fluorescent signal of chlorophyll in alcohol compared to DIW is shown in Figure 1. The DIW signal is due to the EMF pulse of the strobe and can be reduced in the seagoing system by mounting the strobe in a separate housing. Sampling rates of 1 Hz were easily obtainable. Flash to flash variation was very low, indicating that the strobe system is working properly, and the A/D system is capable of catching the peak signal. A serial dilution of this filtered extract in water shows that signal amplitude is directly related to chlorophyll concentration (Figure 2) and that the dynamic range of the system spans from 0.1 to perhaps as high as 200 mg Chl a m^{-3} of water (Figure 3). Further dynamic range is available by decreasing the bias voltage to the PMT, or by using smaller diameter fibers. Fluorescence output in the marine sample was 55 counts for the sample compared to 13 counts for de-ionized water (DIW), indicating a chlorophyll concentration of about 4 mg m^{-3}.

Spatial Resolution: The fiber sensor as configured has a detection volume of about 1 mm^3. Multiple sesors can be easily packed to proved a vertical resolution as fine as 2-3 mm for use near bottom or sea surface boundary layers, or can be spread out along a mooring cable within the limits imposed by fiber attenuation.

Additional Activities in Year 1 and Schedule of Work

Sensor Optimization: Optimization of sensor design with be accomplished by evaluating various design parameters with well-defined fluorescent media (e.g. Rhodamine B, Fluoresbrite 2.24 mm fluorescent beads by Polysciences, Inc., standard pigment solutions). The parameters include: 1) the angle between the excitation and sensor fibers, 2) fiber size, 3) fiber numerical aperture, 4) excitation source, 5) delay time between illumination and fluorescent measurement.

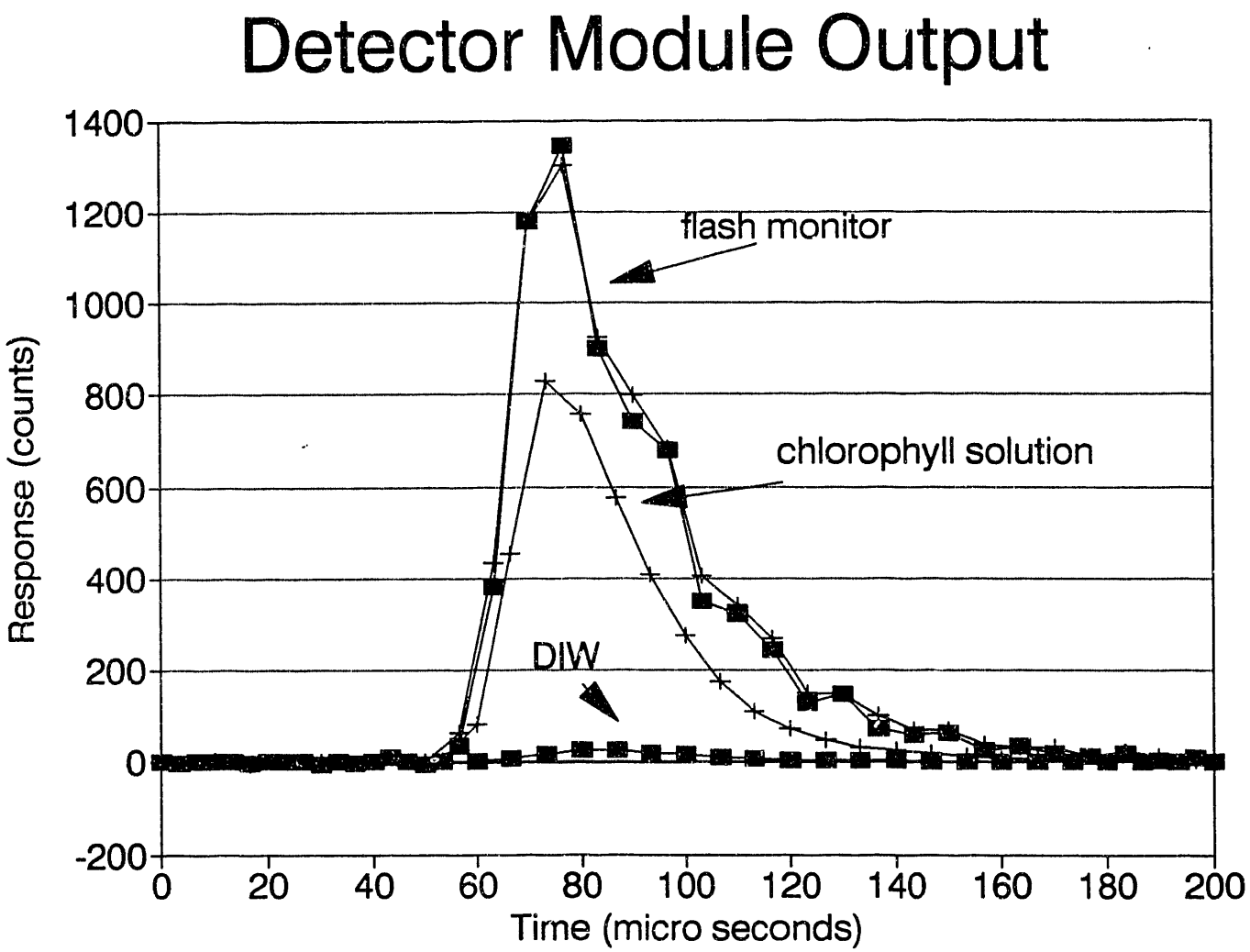
Detector Module Output
1400
1200
1000
800
600
400
200
0
-200
Response (counts)
0
20
40
60
80
100
120
140
160
180
200
Time (micro seconds)
flash monitor
chlorophyll solution
DIW

Figure 1.

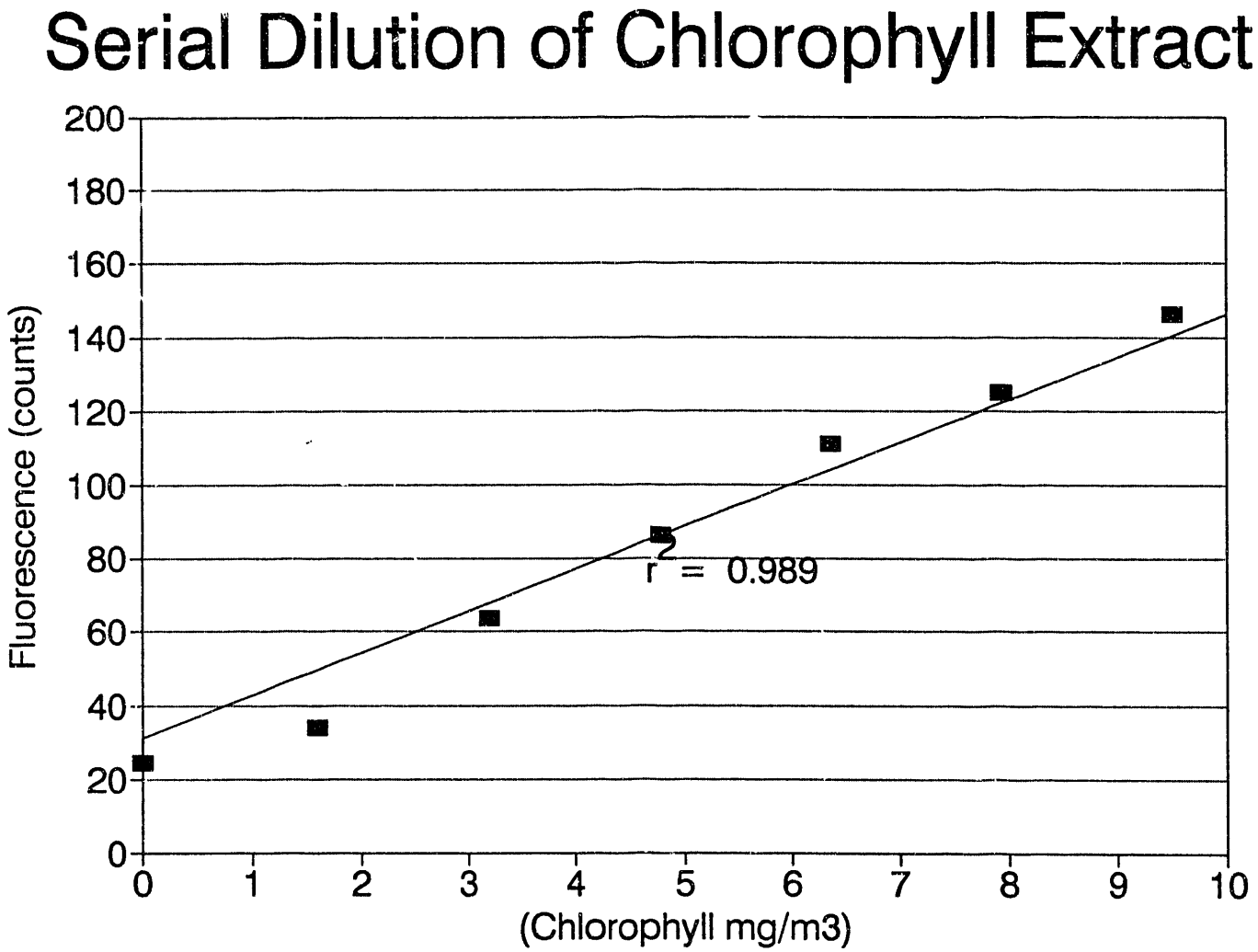
Serial Dilution of Chlorophyll Extract
Fluorescence (counts)
200
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
(Chlorophyll mg/m3)
r² = 0.989

Figure 2.

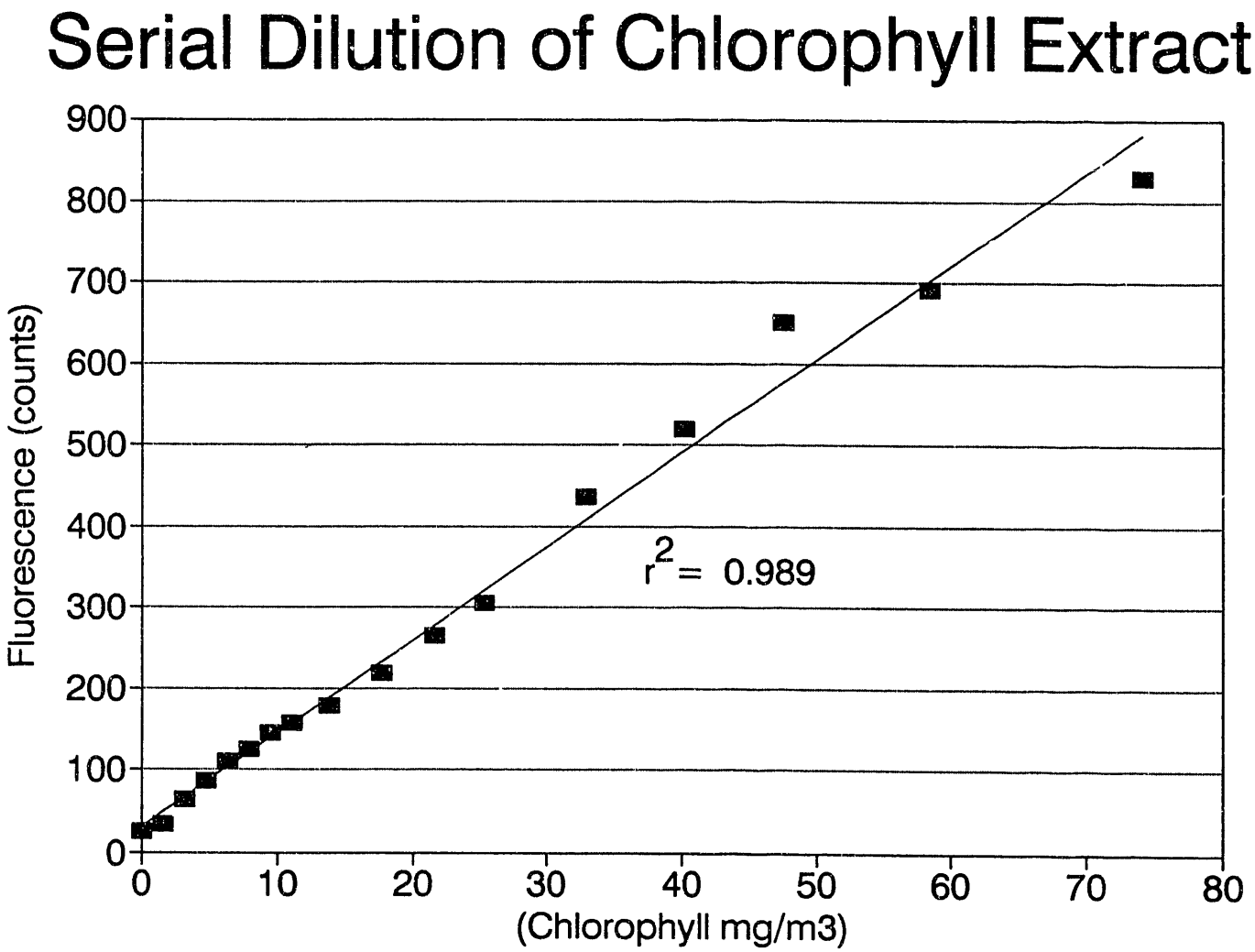
Serial Dilution of Chlorophyll Extract
Fluorescence (counts)
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
70
80
(Chlorophyll mg/m3)
r2= 0.989

Figure 3.

Calibration and evaluation: The FP-1 instrument will be transported to USM for evaluation using defined pigment solutions and representative phytoplankton cultures. Tests will performed in a tank in which chlorophyll concentration can be measured and maintained. For routine analyses, chlorophyll *a* concentration will be determined fluorometrically (Holm-Hansen et al., 1965) by 90% acetone extraction of filtered (GF/F) samples. Pigment composition effects will be evaluated by HPLC determination of photosynthetic pigments (Mantoura and Llewellyn, 1983). Tests will determine sensitivity, dynamic range, precision, instrument drift, linearity, and timing sequence (i.e. the optimal sampling time in response to variable fluorescence response, e.g. Papageorgio, 1975; Falkowski and Kiefer, 1985). In addition, an unanticipated cruise opportunity aboard the R/V *Pelican* in April 1993 will provide preliminary information on the performance of the FP-1 instrument in field conditions. Bio-fouling will be considered as a potential factor influencing the fluorometer signal response. This will be especially critical in field operations. Previous strategies for dealing with fouling problems included both mechanical (Whitledge and Wirick, 1986) and chemical (Whitledge and Wirick, 1983; Dickey et al., 1991) methods. We will initially investigate the use of the anti-fouling clear organo-metallic polymer (OMP-8) used by Dickey et al. (1991). They found this was effective for relatively long term deployments (70 d).

Review and design revision: The sensor and instrument design will be reviewed in light of results of the performance evaluation of the FP-1 instrument and modified as warranted. The Seagoing Instrument (FP-2) will be formulated with respect to the primary objective of providing high quality synoptic fluorescence data from multiple depths, and several secondary criteria: 1) number of sensors that can be deployed, 2) data storage capabilities, 3) power (i.e. potential length of autonomous operation), 4) practicality of deployment and retrieval, 5) cost.

Based on the schedule projected for the first year of this project (see below), we are making good progress.

Year 1

ACTIVITIES OF PRINCIPAL INVESTIGATORS

S. E. Lohrenz (USM)

Dr. Lohrenz is co-principal investigator on the project and primary point of contact for the contract. He has been responsible for administrative operations. He has been directly involved in consultations and decisions regarding design options of the prototype. He also served as the project representative at program planning meetings held at Brookhaven Laboratories. Dr. Lohrenz has had extensive experience in working with marine algal cultures, photosynthetic pigment analyses, and in the measurement of in vivo fluorescence in natural populations. When the prototype instrument is shipped to USM, he will oversee evaluation of sensor performance and calibration procedures in collaboration with Dr. V. L. Asper. Dr. Lohrenz has and will continue to devote 20% of his time on this project.

V. L. Asper (USM)

Dr. Asper is the other co-principal investigator. He has been directly involved in consultations and decisions regarding design options of the prototype. Dr. Asper has had extensive experience in constructing and deploying oceanographic instrumentation in various mooring configurations. When the prototype instrument is shipped to USM, he will oversee evaluation of sensor performance and calibration procedures in collaboration with Dr. Lohrenz. Dr. Asper has spent about 10% of his time on this project. It is anticipated that his efforts will increase to 15% when the prototype is delivered to USM.

Mr. M. J. Morris (contractor, OOI)

Mr. Morris is president of Ocean Optics, Inc., the agency contracted to construct the lab prototype and field instruments. He has had extensive experience in the design of fiber optic based sensors and has been directly involved in consultations and decisions regarding design options of the prototype. He will provide the initial evaluation and approval of the prototype design before it is shipped to USM. Dr. Morris has and will continue to devote 20% of his time on this project.

Dr. R. A. Walters (contractor, OOI)

Dr. R. Walters has been primarily responsible for construction of the prototype instrument and implementation of design and construction options selected by the principal investigators. He is an optical engineer with extensive research and design experience with fiber optical instruments. Dr. Walters has and will continue to devote 20% of his time on this project.

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