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Workshop Proceedings

Applications of *In Situ* Fluorometers in Nearshore Waters

Cape Elizabeth, Maine
February 2-4, 2005

Funded by NOAA's Coastal Services Center through the Alliance for Coastal Technologies (ACT)

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An ACT 2005 Workshop Report

A Workshop of Developers, Deliverers, and Users of Technologies for Monitoring Coastal Environments:

Applications of *In Situ* Fluorometers in Nearshore Waters

Cape Elizabeth, Maine
February 2-4, 2005

Sponsored by the Alliance for Coastal Technologies (ACT) and NOAA's Center for Coastal Ocean Research in the National Ocean Service.

Hosted by ACT Partner organization the Gulf of Maine Ocean Observing System (GoMOOS).

ACT is committed to develop an active partnership of technology developers, deliverers, and users within regional, state, and federal environmental management communities to establish a testbed for demonstrating, evaluating, and verifying innovative technologies in monitoring sensors, platforms, and software for use in coastal habitats.

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ACT WORKSHOP: APPLICATION OF *IN SITU* FLUOROMETERS IN NEARSHORE WATERS

EXECUTIVE SUMMARY

The Alliance for Coastal Technologies (ACT) Workshop "Applications of *in situ* Fluorometers in Nearshore Waters" was held in Cape Elizabeth, Maine, February 2-4, 2005, with sponsorship by the Gulf of Maine Ocean Observing System (GoMOOS), one of the ACT partner organization. The purpose of the workshop was to explore recent trends in fluorometry as it relates to resource management applications in nearshore environments. Participants included representatives from state and federal environmental management agencies as well as research institutions, many of whom are currently using this technology in their research and management applications. Manufacturers and developers of fluorometric measuring systems also attended the meeting.

The Workshop attendees discussed the historical and present uses of fluorometry technology and identified the great potential for its use by coastal managers to fulfill their regulatory and management objectives. Participants also identified some of the challenges associated with the correct use of Fluorometers to estimate biomass and the rate of primary productivity. The Workshop concluded that in order to expand the existing use of fluorometers in both academic and resource management disciplines, several issues concerning data collection, instrument calibration, and data interpretation needed to be addressed. Participants identified twelve recommendations, the top five of which are listed below:

Recommendations

1) Develop a "Guide" that describes the most important aspects of fluorescence measurements. This guide should be written by an expert party, with both research and industry input, and should be distributed by all manufacturers with their instrumentation. The guide should also be made available on the ACT website as well as those of other relevant organizations. The guide should include discussions on the following topics:

   - The benefits of using fluorometers in research and resource management applications;
   - What fluorometers can and cannot provide in terms of measurements;
   - The necessary assumptions required before applying fluorometry;
   - Characterization and calibration of fluorometers;

- Techniques and protocols to overcome some of these limitations (i.e. night-time measurements, ancillary data, implications of instrumentation e.g. changes to LED).

2) Adopt the use of secondary standards so fluorescence measurements can be reported relative to standard solutions in Practical Fluorescence Units (PFU). For example, 110 µg/l $PFU_{fluorescein}$ is the fluorescence that would be measured for a 110 µg/l solution of fluorescein prepared according to standard procedures and measured at a specific temperature. Several fluorophore-specific PFUs should be defined. Changes in instrument response could be tracked using PFUs. Also, measurements of chlorophyll fluorescence in PFUs could be compared between instruments that have the same excitation spectra and emission responses. It follows that PFU readings would be linked to a classification of instruments according to their spectral responses.

3) Develop two-step calibration protocols:

   a) Calibration of instrument response to standard solutions. Calibrate to two or more standard selected fluorophores. These should have different excitation characteristics to allow description of any changes in the fluorometer's excitation spectrum;

   b) Calibration of instrument response to biology. Calibrate to chlorophyll a lab cultures or samples taken during field deployments, and express the results in PFUs relative to the fluorophores. This requires accurate and appropriate determination of a blank (Cullen and Davis, 2003), and should include a measurement of ambient irradiance (e.g., PAR) for characterizing the effects of bright light on fluorescence yield (Cullen and Lewis, 1995).

4) Develop a classification of fluorometers to indicate the degree to which measurements from different instruments might be comparable, and to allow users an efficient means to describe the type of instrument they are using. For example, Level 1 could be defined by excitation (LED, filters) and emission characterizations, and Level 2 could be defined by intensity and duration.

5) Provide a template table of standard specifications for various *in situ* fluorometers. This will be filled in by manufacturers and made available for viewing on the ACT website once the classification scheme is established. This table will need to be updated regularly and should be sent out by manufacturers when shipping new instruments to an end user. A draft version of this table is available in Appendix B to this report. Tables include a column for updating version changes on instruments.

ALLIANCE FOR COASTAL TECHNOLOGIES

There is widespread agreement that an Integrated Ocean Observing System (IOOS) is required to meet a wide range of the Nation's marine product and information service needs. There also is consensus that the successful implementation of the IOOS will require parallel efforts in instrument development and validation and improvements to technology so that promising new technology will be available to make the transition from research/development to operational status when needed. Thus, the Alliance for Coastal Technologies (ACT) was established as a NOAA-funded partnership of research institutions, state and regional resource managers, and private sector companies interested in developing and applying sensor and sensor platform technologies for monitoring and studying coastal systems. ACT has been designed to serve as:

ACT Headquarters is located at the UMCES Chesapeake Biological Laboratory and is staffed by a Director, Chief Scientist, and several support personnel. There are currently seven ACT Partner Institutions around the country with sensor technology expertise, and that represent a broad range of environmental conditions for testing. The ACT Stakeholder Council is comprised of resource managers and industry representatives who ensure that ACT focuses on service-oriented activities. Finally, a larger body of Alliance Members has been created to provide advice to ACT and will be kept abreast of ACT activities.

- An unbiased, third-party testbed for evaluating new and developing coastal sensor and sensor platform technologies,
- A comprehensive data and information clearinghouse on coastal technologies, and
- A forum for capacity building through a series of annual workshops and seminars on specific technologies or topics.

The ACT workshops are designed to aid resource managers, coastal scientists, and private sector companies by identifying and discussing the current status, standardization, potential advancements, and obstacles in the development and use of new sensors and sensor platforms for monitoring, studying, and predicting the state of coastal waters. The workshop goals are to both help build consensus on the steps needed to develop and adopt useful tools while also facilitating the critical communications between the various groups of technology developers, manufacturers, and users.

ACT Workshop Reports are summaries of the discussions that take place between participants during the workshops. The reports also emphasize advantages and limitations of current technologies while making recommendations for both ACT and the broader community on the

steps needed for technology advancement in the particular topic area. Workshop organizers draft the individual reports with input from workshop participants.

ACT is committed to exploring the application of new technologies for monitoring coastal ecosystem and studying environmental stressors that are increasingly prevalent worldwide. For more information, please visit www.act-us.info.

GOALS FOR THIS WORKSHOP

The ACT Workshop on the Applications of in situ Fluorometers in Nearshore Waters was convened on February 2, 2005 in Cape Elizabeth, Maine. Key objectives of the workshop were:

1. To summarize the trends in fluorometry technology as it relates to nearshore applications;

2. To identify uses of *in situ* fluorometers and how they assist coastal managers in fulfilling their regulatory and management objectives;

3. To identify the barriers and challenges with the application of fluorometers in management and research activities; and

4. To recommend a series of actions to overcome these challenges.

ORGANIZATION OF THE WORKSHOP

The one and a half day workshop included both formal presentations and group working sessions. Invited speakers discussed the state of *in situ* fluorometer technologies, including a historical perspective, with respect to what and how they are used in research and management, advantages and disadvantages with the instrumentation and data interpretation, recent developments in the technology and data interpretation, and the future development and application of fluorometers (Table 1).

Table 1. Workshop presentations.

Name and Affiliation	**Title of Presentation**
Dr. John Cullen, Dalhousie University, Halifax, Nova Scotia, Canada	*In Situ* Fluorometers for Coastal Management Applications: Salvation or Curse?
Dr. Andrew Barnard, WET Labs Inc., Philomath, Oregon	The Use of Chlorophyll Fluorometers as an Ecosystem Indicator in Nearshore Environments
Dr. Scott McLean, Satlantic, Halifax, Nova Scotia, Canada	Applications of *In Situ* Fluorometers in Nearshore Waters: An Industry and Research Perspective
Dr. Jan Newton, Applied Physics Laboratory, University of Washington, Seattle, Washington	Management Perspectives on Fluorescence
Dr. Earle Buckley, North Carolina State University and NOAA Coastal Services Center, Charleston, South Carolina	Evaluating the Performance of Available and Emerging Technolo gies

The presentations are available at the GoMOOS website address http://www.gomoos.org/act/fluorometer.html

The workshop format of plenary and breakout sessions insured that expertise, views and concerns from all the sectors were identified and discussed. In the first day's morning breakout sessions, the managers, researchers and industry representatives formed three separate work groups to discuss individual as well as overlapping issues regarding the applications of *in situ* fluorometers in nearshore waters, such as :

- The existing and potential uses of fluorometers;
- Emerging trends and applications;
- The challenges with using fluorometers;
- The limitations of existing technologies;
- Instrument specifications; and
- Ease of use (i.e. calibration, data interpretation).

During the first day's afternoon sessions, the representatives of the various sectors were integrated into three working groups to discuss implementation issues with using fluorometers and interpreting their measurements, and ways for improving and increasing their utility in management as well as research applications. Each group was tasked with making recommendations for improving the current technology for use in monitoring, coastal research and management applications and for overcoming the barriers identified in the morning sessions.

The final day of the meeting was dedicated to the formation of the 12 recommendation for making fluorometers, and the data they provide, more easily utilized and better understood, respectively.

BACKGROUND: INTRODUCTION TO *IN SITU* FLUOROMETERS

Fluorescence is a process of inelastic scattering, whereby a molecule absorbs light energy at a given wavelength, then re-emits some fraction of the energy at a longer wavelength. A fluorescence excitation spectrum for a given substance is the distribution of wavelength-dependent intensity of energy absorbed, whereas an emission spectrum is the distribution of wavelength-dependent intensity of energy emitted. Fluorometers are sensors designed to detect the fluorescent energy emitted by certain molecules of interest, such as chlorophyll a. They work by illuminating a target sample with an excitation beam of light of analyte-specific wavelength, and detecting at oblique angles (ca. 90º) the longer-wavelength fluorescence light emitted by the molecules. When working with pure analyte solutions, the fluorescence value measured is usually proportional to the concentration of the molecules present. However, this relationship can be confounded by the presence of quenching effects in solution or living cells. Chlorophyll fluorescence *in vivo* is a function of light absorbed by all photosynthetic pigments in the targeted sample, whereas in an extract, it is only the light absorbed by chlorophyll molecules. This makes fluorescence of chlorophyll in an extract a poor proxy of chlorophyll fluorescence *in vivo*.

In situ fluorometers are submersible instruments that make measurements to detect chlorophyll a in living algal and cyanobacterial populations occurring in natural aquatic environments. They are particularly useful at providing temporal and spatial estimates of chlorophyll distributions. Since most *in situ* fluorometers use flashing excitation, they are fundamentally different from most common lab instruments. Lab fluorometers can be used for continuous *in vivo* (flow-through measurements), but they are not used for *in situ* measurements. *In situ* fluorometers, on the other hand, can be made to operate like a lab fluorometer in flow-through mode, but cannot be used for discrete sampling mode. The influences of ambient irradiance experienced in the field (outside of the lab) put large constraints on the design of *in situ* fluorometers, further enhancing the difference between the two types of fluorometers. For instance, fluorescence of *in vivo* samples can differ between *in situ* and lab fluorometers during the daytime, because the lab sample will be dark-adapted to some extent, and fluorescence yield will be higher. At night, the differences should be reduced, but the two types of fluorometers will almost certainly have different excitation characteristics, causing dissimilar measurements. As a result, the relationship between the measurements made with lab and *in situ* fluorometers will not exactly be linear in a wide range of conditions. The amount of disagreement is difficult to predict on first principles.

NEED FOR *IN SITU* FLUOROMETERY MEASUREMENTS IN NEASHORE WATERS

Resource managers monitor nearshore coastal environments to assess water quality and to investigate whether anthropogenic influences are causing problems. They are particularly interested in determining ecosystem integrity so they can better manage the natural resources supported by the environment. Managers try to link measurement variables that characterize environmental concerns, and often seek simultaneous measures of multiple water quality parameters. In terms of water quality, key concerns include eutrophication, nutrient status, and the occurrence of harmful algal blooms (HABs). In terms of ecosystem integrity, key concerns are largely related to trophic support (biomass) and primary productivity.

Traditionally, water quality estimates for biomass or trophic status are achieved by analysis of discrete water samples for chlorophyll a concentration. Physical processes inherent in coastal environments (tides, wind, runoff) induce significant temporal and spatial variations in measured parameters, including chlorophyll, and can complicate interpretation of the measurements. Natural phytoplankton populations are also subjected to population dynamics, such as grazing by zooplankton, nutrient and light limitation, sinking, and vertical migration, which further confounds the measurements. Unfortunately, routine (e.g. monthly) discrete sampling, typical of most monitoring programs, does not provide the spatial and temporal measurements necessary to characterize the processes at space/time scales that are necessary for determining unbiased average and peak values, whilst avoiding temporal data aliasing. Furthermore, discrete samples rarely provide the real-time results needed for targeted sampling. As a result, to adequately measure phytoplankton concentrations using discrete methods requires an immense amount of sampling effort. Such efforts create a large number of samples, and the resultant laboratory analytical expenses can be high.

Ecosystem models are becoming more widely available and used by resource management sectors; however, they still require "real-world" data as input, such as *in situ* estimates of biomass, to initialize and ground truth model output. With increased availability and utilization of water quality sensors, such as in situ fluorometers, some of the sampling problems and issues are being addressed. *In situ* sensors provide the ability to sample more thoroughly in space and in time depending on the application. (Figure 1). However, not only do environmental factors independent of chlorophyll induce variability in fluorescence measurements (e.g., changes in fluorescence yield), sensors have inherent performance issues that typically degrade the quality of measurements with time due to instrument drift and biofouling.

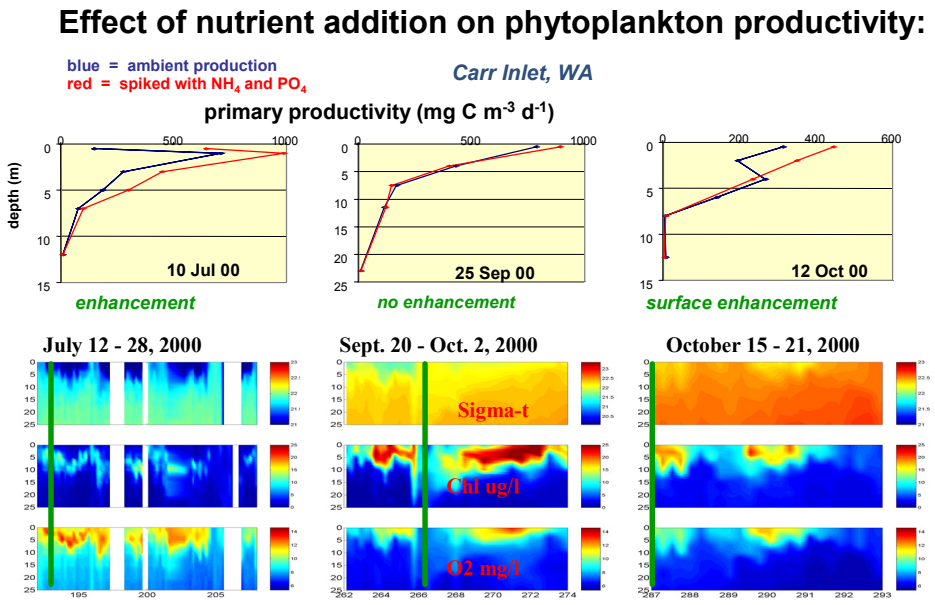

Figure 1. Effect of nutrient addition on phytoplankton productivity based on discrete, shipboard profiles (upper panels) and moored time series of depth profiles (lower panels). The mooring consisted of a profiling buoy equipped with CTD, fluorometer and oxygen sensors (lower panels). The two are shown to point out the differences between discrete station sampling (upper panels) versus a continuous time series of parameters using moored in situ instruments (lower panels). ***(from: Jan Newton, ACT Workshop Presentation, 2005)***

Resource managers are limited by continuously changing mandates while budgets tend to decrease, restricting their ability to improve sampling strategy by increasing routine sampling density or by purchasing more advanced but expensive instrumentation. They also need to maximize the quality of their data in terms of consistent and defendable data sets while minimizing the ambiguity of the measurements and the effort required to gather the data. Though new technologies may reduce operating costs in the long-run, there is the initial cost in personnel time and money required to develop in-house expertise. Furthermore, there is the issue of data consistency when transitioning from a discrete laboratory based protocol to an in situ field protocol.

EMERGING TRENDS

The marine sensor industry is predominantly made up of small companies that fulfill a niche in a given market. These companies focus their efforts on a specialized technology with a limited user group, and often depend on experts working at universities and research institutes for developing and testing new technologies. Development of new sensor technologies is expensive and products are usually introduced to meet new research needs, emphasizing performance, not affordability or ease of use. As researchers using new technologies start to interpret and publish results, useful features become recognized and are enhanced, while less useful features are phased out. As products mature and the data become more accessible and better understood, the community begins to accept the technology, and starts to apply it outside of niche research environments. Ultimately, as the user group grows, sales increase and economies of scale make the technology more affordable to a broader community of users.

Conventional *in situ* fluorometers, the subject of this Workshop, are a good example of a technology in this transition, positioned somewhere between a maturing and accepted technology. There are currently many commercially available fluorometers on the market. The ACT website maintains a database for various technologies and features several fluorometers that are commercially available (www.act-us.info/tech_db.php).

To move a given technology from a highly specialized research tool to an operational tool that can be used by a broader community requires several steps, including:

- Improving the understanding of what the technology can be used for;
- Increasing confidence in the sensor output with verified data comparisons;
- Identifying what the data needs are by the resource management and broader user community;
- Adaptating products that are still highly specialized to meet different user needs;
- Broadening the user group, hence reducing the cost of the technology and making it a more affordable, relatively easy to implement tool for monitoring.

During the past four decades, fluorometers have become an effective tool in monitoring of coastal phytoplankton, as chronicled below:

- 1960's - Flow through fluorometer system utilized (Lorenzen, 1966)
- 1970's - Commercial instruments introduced (flash lamp)
- 1980's - Introduction of PAM fluorometer (Schreiber et al, 1986)
- 1990's - Development of LED fluorometers
- 1990's - Introduction of *in situ* FRR fluorometers (Kolber et al, 1998)
- 1990's - Advancement in antifouling technologies
- 2000's - Progress with physiological fluorometers (Dijkman et al. 1999;
- Gorbunov et al. 2000)

- 2000's - Progress with hyper spectral excitation/emission (Desiderio et al. 1997; Beutler et al. 2002)

Fluorometers are particularly useful for describing relative changes between independent estimates of chlorophyll, and variable fluorescence has advanced our ability to study variations of phytoplankton physiological status and primary production. Emerging trends in fluorometer technology and usage include:

- Development of smaller sensors;
- Application on real-time systems;
- Applications on gliders and AUVs;
- Continued improvement of antifouling systems;
- Easier to use pulse fluorometers;
- Spectral excitation/emission studies;
- Single cell flow-cytometric approaches;
- Light status studies; and,
- Nutrient limitation studies;

Two types of fluorometers are primarily in use today: conventional fluorometers (the focus of this Workshop), and modulated pulse fluorometers.

Conventional fluorometers are used primarily to estimate biomass by estimating the concentration of chlorophyll a using on frequency-intensity flash regime. They are an easy-to-use, inexpensive, and mature technology; however, they do not consider physiology of the plankton being measured. Furthermore, different instruments have different excitation wavelengths, bandwidths and intensities.

Modulated pulse fluorometers change frequency and/or intensity, and are used to better understand physiological characteristics of plankton by providing estimates of photosynthetic parameters that can also be used to calculate primary production (in conjunction with measurements of PAR and dark adaptation). Examples include Fast Rapid Repetition fluorometers (FRR) and (FIRe) fluorometers. The FRR technology provides a rapid pulse sequence for induction and relaxation, and provides much more information on the status of photosynthetic processes than can be obtained from conventional fluorometers. At the moment, these instruments are expensive, require complex data processing and are not as user accessible as conventional fluorometers. Though not specifically the subject of this workshop, selected references that describe these technologies are provided in the bibliography for the interested reader (Schreiber et al., 1986; Kolber et al., 1998; Suggett et al., 2003).

EXISTING AND POTENTIAL USES OF FLUOROMETERS FOR COASTAL MANAGEMENT

The resource management community is trying to better understand the distribution of chlorophyll a, verify remote sensing technologies, and to determine whether there are anthropogenic contributions enhancing blooms in nearshore environments. They would like to be able to use fluorometers to accurately estimate many of the parameters listed below, although it is acknowledged that these may not be achievable with existing technology and/or methods:

- Chlorophyll a;
- Phytoplankton biomass (e.g., as carbon); (NOTE: fluorescence cannot directly estimate this - it can only be modeled, with unimpressive accuracy, from an assumed relationship between chlorophyll and carbon, which like the relationship between fluorescence and chlorophyll, is highly variable);
- Diel and other natural variations in chlorophyll a concentrations;
- Species, including Harmful Algal Blooms (HABs);
- Nutrient status.

Fluorometers offer a useful tool for coastal managers for these types of needs, although at present, they can measure none of these variables accurately without frequent calibration. For one, they are readily available commercially in a variety of price ranges and operational packages. The following is a list of the existing and potential uses of *in situ* fluorometers, which fall broadly into two major categories: 1) monitoring and trend detection, and 2) ground truthing of models and remote sensing data:

1. MONITORING AND TREND DETECTION

- Estimating biomass for quantifying blooms, primary productivity, and mapping spatial patterns of phytoplankton in water masses;
- Providing a proxy for chlorophyll a as an indicator of ecosystem health, and for use in permitting and regulatory activities in regions with nutrient limitation (e.g., develop relationships between carbon to chlorophyll a to help with permit regulations for nutrients);
- Monitoring blooms in anthropogenic stimulated environments, such as harmful algal blooms (HABs);
- Describing the vertical distributions of fluorescence in the watercolumn and identifying the biomass maxima to help determine discrete water sampling positions;
- Long-term monitoring of chlorophyll a as one in a suite of parameters for water quality databases. Data are used to identify natural temporal changes in ecosystem dynamics, and to better understand short-term versus long-term variability (e.g., assessing event responses in a system, such as how storms or other events will influence short and long-term variability in productivity);

- Measuring temporal patterns related to other parameter distributions (e.g., chlorophyll and nutrient concentrations related to bird and fish distributions; inferring nutrient status and response to land use; identifying relationships between chlorophyll a and dissolved oxygen dynamics in estuaries; determining spatial patterns of biomass for mariculture applications);
- Research and development (e.g., biocide development).

2. Ground Truthing of Models and Remote Sensing Data

- Verification and ground truthing of remote sensing and airborne observations;
- Verification and ground truthing of ecosystem model output;
- Providing inputs for temporal and spatial water quality models that link trophic cascades, and nutrient-phytoplankton dynamics.

Challenges with using *In Situ* Fluorometers

The theme for this Workshop was established the opening night during Dr. Cullen's presentation "In Situ Fluorometers for Coastal Management Applications: Salvation or Curse?" Fluorometers have revolutionized the study of the ocean by providing continuous measurements of the variability in phytoplankton and can be deployed on many different platforms. The curse is that they measure fluorescence, not chlorophyll, not cell numbers or particulate organic carbon or nitrogen etc., and thus cannot provide accurate measurements of phytoplankton biomass. Furthermore, fluorescence is very difficult to understand, so simple interpretations of measurements, while tempting, are elusive.

Fluorometers, though seemingly straightforward to use, yield measurements that are complex in nature. Many considerations need to be addressed before they can be applied to give reasonable estimates of chlorophyll, much less phytoplankton biomass or productivity. Measurements produced by fluorometers are affected by physiology (i.e. life stage and types of plankton), external environmental factors (i.e. light, turbidity), excitation characteristics of the sensor, and varying excitation response spectra between phytoplankton species.

To add to the problem, different manufactured fluorometers measure different species of phytoplankton differently, even when calibrated the same way in the lab. This means that fluorometers, though apparently similar in their specifications, will measure different patterns when deployed in the field. These differences may be small, but whether this poses a problem depends on the application of interest. For example, in global warming studies, small differences between fluorometrically inferred biomass can be very important, so replacement of an instrument during a monitoring program could introduce significant bias. The many factors affecting fluorescence (e.g., excitation spectrum and power, excitation frequency and intensity, illuminated volume and shading) all affect fluorescence yield. Standardization of fluorometer design would entail everyone making the same fluorometer, which is unlikely and makes long-term comparisons of fluorescence values and the estimates of chlorophyll they provide problematic. This is due to the fact that a fluorometer does not measure chlorophyll a directly, but

rather chlorophyll fluorescence. The question then becomes, "How should accuracy and precision of a fluorometer be defined?"

Defining What Fluorometers Actually Measure

It is important to remember that fluorescence a measure of *chlorophyll fluorescence*, not *chlorophyll*. There are many technologies available for measuring in situ fluorescence, including the conventional fluorometer, and newer sensors that can sample multiple wavelength excitation and modulated excitation. In terms of the technology, fluorometers use different excitation sources (LEDs or lamps), with different excitation frequencies, duration and intensities. All of these interact differently with physiological and taxonomical influences, since phytoplankton species have different excitation response spectra (Figure 2) and physiological responses to pulses of light. Further complicating the problem is the fact that chlorophyll itself is a highly variable measure of the biomass. Therefore, fluorescence, being an imprecise measure of chlorophyll, is an even more imprecise measure of biomass (Cullen, 1982).

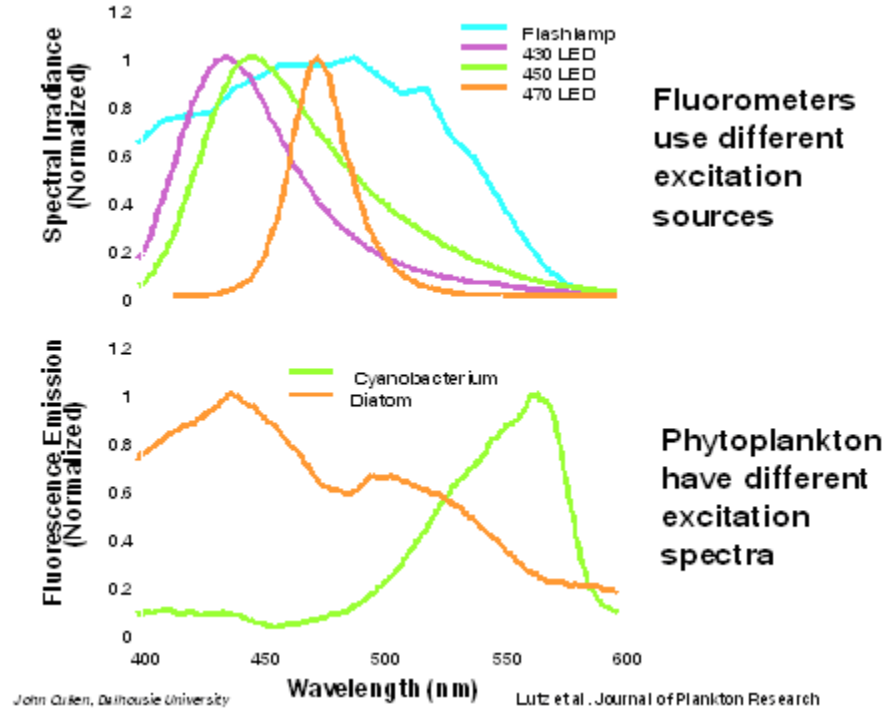

Figure 2. Spectral irradiance vs. wavelength of the fluorometer LED (upper panel, from: John Cullen, 1982); and Fluorescence emission vs. LED wavelength (lower panel, from: Lutz, et al., 2001).

Downwelling solar irradiance has a major influence on fluorescence, causing an apparent inhibition near the surface due to nonphotochemical quenching. This can be corrected for if irradiance is measured at the same time (Cullen and Lewis, 1995). Nutrition and species composition also influence fluorescence yield, increasing the difficulty in relating fluorescence directly to chlorophyll concentration. However, these departures can be a source of useful information, and when measured carefully and with consideration of these influences, fluorescence is still a good general estimation of chlorophyll a, and hence biomass.

Managerial Issues With *in situ* Fluorometers

Resource managers using *in situ* chlorophyll fluorometers are faced with greater complexity than with other types of sensors. Each model responds differently in real-world conditions in the field. The difference is not quantified by manufacturer's specification sheets or by the usual calibration procedures. Even when comprehensive calibration controls sensor performance, physiological variability is so complex that it requires expert interpretation. Few published articles address the complexity of instrument response and physiological variability, and there are currently no standards for data reporting.

Bearing in mind that managers do not want complexity, but rather comparability across time and space scales, the challenges faced by the resource management community with *in situ* fluorometer applications are difficult. Clearly there needs to be a method for robust interpretation and reporting of data as well as community accepted standardized calibration procedures for fluorometers. Particularly for the resource manager, data often need to be defendable in court, and the variation in the data from site to site and temporal variability due to external forces makes this objective difficult to achieve.

More specific questions related to these issues were raised during the workshop discussions:

- How do you defend fluorometrically derived observations?
- Do the data really represent what is occurring in the natural system?
- How are the data quantified to values that give meaningful, consistent and defendable results?
- How can we improve confidence in data that have inherent natural variability?
- What are the variations between manufactured versions of fluorometers?
- What kind of disparity can be tolerated? (Workshop consensus suggests doubling the error is a problem).
- How can the understanding as to what these measurements can be reliably used for be improved across user groups?

Instrument performance considerations were also brought up during the sessions:

- How can we improve confidence in data that contain errors derived during conversions from sensor output to the parameters we are trying to estimate?
- Can the sensor handle temperature stress? Pressure stress? And at what level?
- What is the longevity of the sensor? (weeks to months?)
- What are the electronic drifts?

- What are the fouling issues (i.e. mud, rust, corrosion along with biofouling and growth)?
- How do the antifouling technologies improve longevity? (e.g., wipers work, but require maintenance; flow through can foul with mud; legal antifouling paints)?
- What are the overall operational and maintenance requirements to operate properly?

In order to address these and other points, the community as a whole needs to accept and concentrate on what the technology can do now, aspire to apply this technology correctly, recognize the limitations of the technology, all without losing sight of its value. This will require communication, cooperation, and collaboration between all the sectors involved with research and development and utilization of fluorometers in the broader user community.

WORKSHOP RECOMMENDATIONS AND CONCLUSIONS

The Workshop provided a venue for very enthusiastic discussions on how to improve the existing use of *in situ* fluorometers. To address the challenges and questions raised during the Workshop, a series of recommendations, condensed into 12 points listed below, were prioritized by the participants at the workshop on the final day.

1) Develop a "Guide" that describes the most important aspects of fluorescence measurements. This guide should be written by an expert party, with both research and industry input, and should be distributed by all fluorometer manufacturers with their instrumentation. The guide should also be made available on the ACT website as well as those of other relevant organizations. The guide should include discussions on the following topics:

    - The benefits of using fluorometers in research and resource management applications;
    - What fluorometers can and cannot provide in terms of measurements;
    - The necessary assumptions required before applying fluorometry;
    - Characterization and calibration of fluorometers;
    - Techniques and protocols to overcome some of these limitations (i.e. night-time measurements, ancillary data, implications of instrumentation e.g. changes to LED).

2) Adopt the use of secondary standards so fluorescence measurements can be reported relative to standard solutions in Practical Fluorescence Units (PFU). For example, 110 µg/l $PFU_{fluorescein}$ is the fluorescence that would be measured for a 110 µg/l solution of fluorescein prepared according to standard procedures and measured at a specific temperature. Several fluorophore-specific PFUs should be defined. Changes in instrument response could be tracked using PFUs. Also, measurements of chlorophyll fluorescence in PFUs could be compared between instruments that have the same excitation spectra and emission responses. It follows that PFU readings would be linked to a classification of instruments according to their spectral responses.

3) Develop two-step calibration protocols:

   a) Calibration of instrument response to standard solutions. Calibrate to two or more standard selected fluorophores. These should have different excitation characteristics to allow description of any changes in the fluorometer's excitation spectrum.

   b) Calibration of instrument response to biology. Calibrate to chlorophyll a lab cultures or samples taken during field deployments, and express the results in PFUs relative to the fluorophores. This requires accurate and appropriate determination of a blank (Cullen and Davis, 2003), and should include a measurement of ambient irradiance (e.g., PAR) for characterizing the effects of bright light on fluorescence yield (Cullen and Lewis, 1995).

4) Develop a classification of fluorometers to indicate the degree to which measurements from different instruments might be comparable, and to allow users an efficient means to describe the type of instrument they are using. For example, Level 1 could be defined by excitation (LED, filters) and emission characterizations, and Level 2 could be defined by intensity and duration.

5) Provide a template for a table of standard specifications for various in situ fluorometers. This will be filled in by manufacturers and made available for viewing on the ACT website once the classification scheme is established. This table will need to be updated regularly and should be sent out by manufacturers when shipping new instruments to an end user. A draft version of this table is available in Appendix B to this report. Tables include a column for updating version changes on instruments.

6) Develop training and outreach, because fluorometer usage is complex and confusing, and because standardization is necessary in this era of ocean observing systems (for comparability),.

   Ideas for achieving this are:

   - Convening conference sessions and workshops,
   - Developing graduate courses,
   - NOAA courses,
   - Website training,
   - Have major agencies come together (e.g., USGS, NOAA, EPA) to address standardized fluorometer methods.

7) Continue development of antifouling technology, directly integrated into the instrument.

8) Encourage development and simplification of fluorescence and/or other techniques to assess phytoplankton nutrient status for research management applications (e.g., business development and also addressing the gap between beta and operational management usage).

9) Facilitate communication and collaboration between research and management communities (journals, web portals), and between industry and users (potential user questionnaire between industry and user (on ACT website).

10) Investigate and develop other approaches to measure phytoplankton (biomass, species, productivity) by alternatives. Hold future ACT workshop(s) on "What is the best technology to measure phytoplankton?"

11) Encourage ACT to sponsor a technical panel on fluorescence within IOOS, etc.)

12) Encourage ACT to sponsor technical panel on sensor interoperability within IOOS and GOOS. Ensure industry representatives are engaged.

OUTCOMES

As an outcome of this workshop, the upcoming *in situ* fluorometer verification being conducted by ACT in the spring of 2005 will incorporate many of these recommendations into their activities and will make every effort to provide a tested, standardized protocol for laboratory and in-field evaluations. These protocols and the results of the verification will be made available on the ACT website upon completion (Fall 2005). (Direct outcome of Recommendation 3 and will contribute to Recommendations 1 and 2).

A draft template of the specification table in Recommendation 5 is provided in this report (Appendix B).

ACT will help facilitate training on use of fluorometers via promoting development of the fluorometer guide book, web-based tutorials, and assisting with a course proposed by some of the attending workshop participants, directly as a result of Recommendations 6, 10, and 12.

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aspects of the workshop, facilitated sessions, and prepared the final report. Josie Quintrell (GoMOOS) provided the overall guidance and leadership that made this workshop successful.

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APPENDIX A: BIBLIOGRAPHY

Beutler, M., K. H. Wiltshire, B. Meyer, C. Moldaenke, C. Lüring, M. Meyerhöfer, U.-P. Hansen & H. Dau. 2002. A fluorometric method for the differentiation of algal populations *in vivo and in situ. Photosynth. Res. 72: 39-53.*

Cullen, J. J. and Davis, R. F. (2003). The blank can make a big difference in oceanographic measurements. *Limnol. Oceanogr. Bull.* 12: 29-35.

Cullen, J. J. and Lewis, M. R. (1995). Biological processes and optical measurements near the sea-surface: some issues relevant to remote sensing. *J. Geophys. Res.* 100: 13,255-213,266.

Cullen, J. J. 1982. The deep chlorophyll maximum: comparing vertical profiles of chlorophyll a. *Can. J. Fish. Aquat. Sci.* 39: 791-803.

Desiderio, R. A., C. C. Moore, C. Lantz & T. J. Cowles. 1997. Multiple excitation fluorometer for *in situ* oceanographic applications. *Appl. Opt.* 36: 1289-96.

Dijkman, N., M. Trtílek, D. Kaftan & L. Nedbal. 1999. Measurements of phytoplankton of sub-namomolar chlorophyll concentrations by a modified double-modulation fluorometer. *Photosynthetica* 37: 249-54.

Gorbunov, M. Y., P. G. Falkowski & Z. S. Kolber. 2000. Measurement of photosynthetic parameters in benthic organisms *in situ* using a SCUBA-based fast repetition rate fluorometer. *Limnol. Oceanogr.* 45: 242-45.

Kolber, Z. S., Prásil, O. and Falkowski, P. G. (1998). Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. *Biochemica and Biophysica Acta.* 1367: 88-106. (not commercial until 90's)

Lorenzen, C. J. (1966). A method for the continuous measurement of in vivo chlorophyll concentration. *Deep-Sea Res.* 13: 223-227.

Lutz, V. A., Sathyendranath, S., Head, E. J. H. and Li, W. K. W. (2001). Changes in the *in vivo* absorption and fluorescence excitation spectra with growth irradiance in three species of phytoplankton. *J. Plankton Res.* 23: 555-569.

Schreiber, U., Schliwa, U. and Bilger, W. (1986). Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosyn. Res.* 10: 51-62.

Suggett, D. J., Oxborough, K., Baker, N. R., MacIntyre, H. L., Kana, T. M. and Geider, R. J. (2003). Fast repetition rate and pulse amplitude modulation chlorophyll a fluorescence measurements for assessment of photosynthetic electron transport in marine phytoplankton *Eur. J. Phycol.* 38: 371-384.

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APPENDIX C: DRAFT TABLE OF FLUOROMETER SPECIFICATIONS

Conventional Fluorometer Specifications Level 1

Parameter	Units	Specifications
Excitation Wavelength with tolerance (center wavelength)	(nm)	
Excitation Bandwidth (full width half maximum)	(nm)	
Emission Wavelength with tolerance (center wavelength)	(nm)	
Emission Bandwidth (full width half maximum)	(nm)	

Conventional Fluorometer Specifications Level 2 (number of fluorophores subject to discussion – 2 to 3 were discussed)

Parameter	Units	Specifications
Minimum Detection Limit [1] (Fluorophore $_1$)	(μg/l F_1)	
Minimum Detection Limit [1] (Fluorophore $_N$)	(μg/l F_N)	
Detection Range (Fluorophore $_1$) Detection Range (Fluorophore $_N$)	(μg/l F_1) (μg/l F_N)	
Minimum Detection Limit (Standard Culture A) Minimum Detection Limit (Standard Culture N)	(μg/l chl a) (μg/l chl a)	
Precision[2]	(μg/l F_1)	
Bias[3] Accuracy[4]	(μg/l F_1) (μg/l F_1)	

DRAFT TABLE OF FLUOROMETER SPECIFICATIONS (CONTINUED)

[1] Minimum detection limit (or instrument detection limit defined in Standard Methods) is the concentration of fluorophore solution that produces a signal five times the standard deviation of the instrument signal standard deviation recorded for a blank analysis. The blank for the fluorophore solution should be Nanopure water.

[2] Precision is the equivalent concentration of fluorophore solution that produces a signal equal to the standard deviation (σ) of the instrument signal for a blank analysis. For a normal distribution this represents at 68.26% probability that the error will not exceed $\pm\sigma$. Precision represents the repeatability of the instrument to measure the same sample.

[3] Bias is the consistent deviation of instrument measured values from the true value of a standard solution of known concentration (a standard fluorophore solution) caused by systematic errors.

[4] Accuracy is the combination of bias and precision of an analytical procedure, which reflects the closeness of the value measured by the instrument to the true value of a known standard solution (in this case concentration of a specific fluorophore). The reproducibility of the standard solution itself may limit the accuracy of a measurement and must be considered. Accuracy cannot be assessed using a standard phytoplankton culture as the physiological state of the organisms would be too variable at different measurement locations to be a useable calibration standard. A stable solution of a given fluorophore that can be reproducibly prepared and has a fixed fluorescent yield is appropriate to measure instrument performance.

DRAFT TABLE OF FLUOROMETER SPECIFICATIONS (CONTINUED)

Conventional Fluorometer Characterization

Parameter	Units	Specifications
Source Irradiance [5]	($\mu W/cm^2$)	
Pulse Frequency [5] Pulse Duration [5] Window material [5]	(Hz) (msec)	
Source Beam Width (half angle half power) [5] Pulse Intensity [5] (in water) (calculated value) Sample Volume (in water)	(deg) ($quanta/cm^2/sec$) (cm^3)	
Sample Volume [6]	(OPEN or CLOSED)	
Sampling Method [7] Response Time (one time constant to step change)	(OPEN, FLOW, PUMPED) (msec)	
Linearity [8]	(%FS F_1)	
Temperature Sensitivity (Responsivity)	(%FS F_1/°C)	
Temperature Sensitivity (Offset) Pressure Sensitivity	($\mu g/l$ F_1/°C) (%FS F_1/°C)	

FS = Full Scale

DRAFT TABLE OF FLUOROMETER SPECIFICATIONS (CONTINUED)

[5] Ideally a measure using a calibrated spectrograph in air (can be estimated from a simpler measure such as PAR - make note of measuring device manufacturer/model/units), with pulse duration and pulse width, a calculation of pulse intensity can be made. Measurement should be taken, normal to the source axis and at a distance approximating the center of the sample volume. A correction can be made for the change in illumination geometry in water, knowing the window material and the approximate source beam width (in degrees). Use zero if the beam is collimated.

[6] This describes the measurement volume characteristics. OPEN refers to a sample volume that is exposed to the ambient light field. CLOSED refers to a sample volume that is enclosed (ie dark).

[7] This describes how the sample arrives at the sample volume. OPEN refers to a sample volume in an open sample. PUMPED refers to a sample that is pumped into the sample volume. FLOW refers to a sample that relies on ambient flow to arrive at sample volume. Can be more than one (ie PUMPED or FLOW).

[8] Linearity is expressed as 100 times the maximum deviation from a best fit straight line (of a measured fluorophore standard dilution set vs actual sensor readings) divided by the sensor full scale measurement range.

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