CHAPTER

c0018 New Sensors for Ocean Observing: The Optical Phytoplankton Discriminator

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CHAPTER OUTLINE

s0010 1. INTRODUCTION

p0010 Phytoplankton are integral to complex natural processes such as the carbon cycling, food web dynamics, coastal hypoxia events, and harmful algal blooms (HABs). Identification and quantification of phytoplankton are listed as high-priority measurements needed to address six of the seven societal goals identified in the Integrated Ocean Observing System (IOOS) Summit[1] and were listed as core variables for observatory systems.[2,3] Similarly, chromophoric dissolved organic matter (CDOM) is the primary constituent that is absorbing light in the ocean and often exceeds even the light absorbed by phytoplankton.[4] As a result, CDOM dominates ocean color, plays a critical role in photobiology and photochemistry, photoproduction of CO_2,[5] as well as controlling the absorption of light energy and subsequent impacts on heat flux[6] and other ocean–climate interactions. The IOOS Summit[1] included CDOM among its 26 high-priority variables required to address three of its seven societal goals. The Optical Phytoplankton Discriminator

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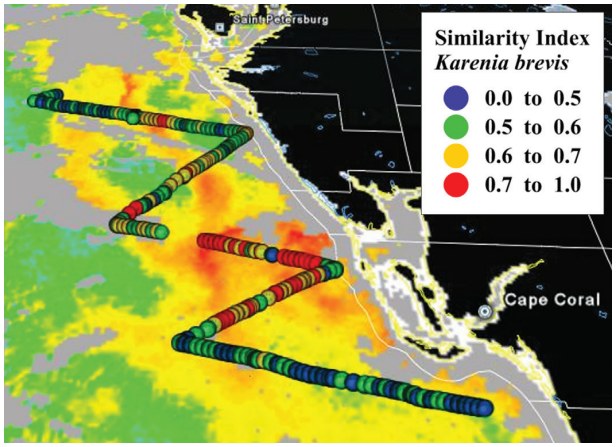
organic matter (FDOM) as a proxy for CDOM. Fluorescence-based CDOM instruments, however, measure only a small subset of DOM molecules that have the aromaticity and conjugation to fluoresce. The FDOM:CDOM relationship varies with the source of CDOM, its lability, and its light exposure history. Significantly, FDOM cannot provide spectral slope information.

p0040 Unlike FDOM determinations, spectrophotometric CDOM absorption measurements directly quantify the desired light absorption properties in both coastal and oceanic waters, provided adequate sensitivity and accuracy are obtained. Spectral slope data can be readily derived from full spectrum absorption. Conventional path lengths of both laboratory spectrophotometers and field absorption instrumentation are typically limited to 10 or 25 cm, respectively, which limits some open ocean applications. Corrections for salinity, temperature, and scattering[31] are also applied for the most exacting work. The most sensitive commercially available CDOM absorption instrument and the OPD utilize liquid-core waveguide (LCW) technology, in which the difference in refractive index between sample and waveguide wall results in a highly efficient internal reflection, permitting an illumination of the core to be transmitted through a coiled waveguide and resulting in path lengths of 200 cm or more. Operational issues common to all LCW instruments include fragility of silica capillary, bubble artifacts, condensation, and clogging of small lumen apertures. Additionally, because light transmission through the LCW is a function of the sample refractive index in addition to the sample absorption,[32] data collection requires careful accounting for temperature and salinity differences between references and samples. The commercial LCW instrument, though limited to manual benchtop operation, has advanced the sensitivity of CDOM analysis and resulting knowledge.[4,32,33] The OPD provides similar absorption measurement sensitivity with the additional feature of unattended, in situ, automated operation.

s0015 2. HISTORY OF THE OPD

p0045 In a set of laboratory experiments, Millie et al.[34] utilized in vivo absorbance spectra to discriminate different light acclimation states of *K. brevis* cultures grown under differing light levels. Results from those experiments on a single species provided evidence that there might be utility in the use of absorbance spectra to discriminate multiple taxonomic groups of phytoplankton. Subsequently, taxonomic groups were discriminated in theoretical mixes of absorbance spectra collected from multiple monospecific cultures.[35] A stepwise discriminant analyses was used to differentiate mean-normalized absorbance spectra for laboratory cultures of *K. brevis* from absorbance spectra of a diatom, a prasinophyte, and peridinin-containing dinoflagellates. Wavelengths delineated by the stepwise techniques were associated with the accessory carotenoids. Unfortunately, the comparative absorption by the carotenoids in the green, yellow, and orange wavelengths was much less than the absorption by chlorophyll in the blue and red wavelengths, limiting the sensitivity of that approach. Furthermore, the absorbance attributable to class-specific groupings of accessory



f0015 **FIGURE 2**

The near surface *Karenia* sp. similarity indexes (SI) determined by a shipboard OPD on November 8, 2005. Background image is MODIS remote sensing fluorescence line height.

Remote sensing image courtesy of USF-IMARS.

s0020 3. METHODOLOGY

p0060 Photopigments of plants and algae are light-harvesting molecules that function to channel light energy into the photochemical pathway for photosynthesis or to shunt excess light energy away from the photochemical pathway when there is a risk of damage from too much light energy.[45] There are approximately 45 known plant pigments found in marine microalgae, each with a slightly different molecular structure.[46] These differences in molecular structure yield differences in the shapes of light absorption spectra for each pigment. The absorption spectrum of an individual plant pigment can be modeled as the sum of a set of Gaussian curves centered at wavelengths of maximum absorption by the light-absorbing chemical structures. The absorption spectrum of any phytoplankton cell is the sum of the absorption spectra of all the pigments making up the cells pigment complement modified by factors such as cell size and the concentration of pigments within the cell (pigment-packaging effects).

p0065 The OPD method is a computational means of highlighting the absorption characteristics of plant photopigments, removing or minimizing the absorption and scattering characteristics of nonpigmented components of the bulk sample, and then fitting a set of known taxonomic class photopigment signatures to the highlighted photopigment absorption characteristics. To accomplish this, the bulk water particle absorbance spectrum is subjected to derivative analysis, and then that derivative spectrum is compared to the derivative spectrum of the known target taxa yielding a similarity index (SI).

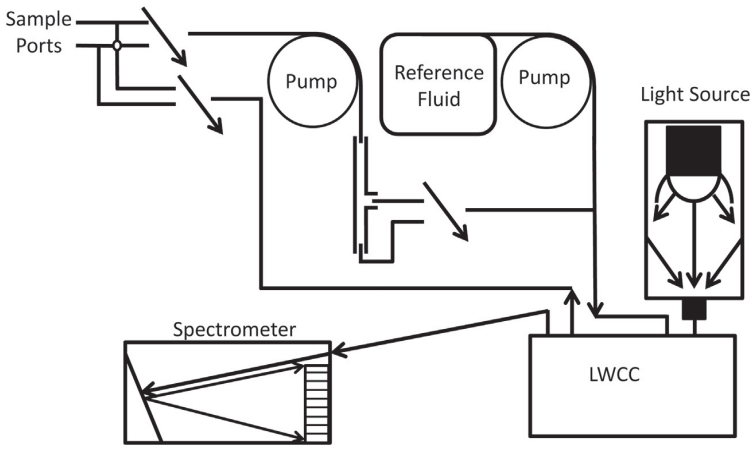
p0090 Absorption by CDOM is determined using the standard approach where the natural logarithm of the ratio of the light transmission through the CDOM containing water sample to the light transmission through "pure" water is scaled by the optical path length of the water-containing cell. CDOM absorption is an exponential function of wavelength and can be expressed as follows:

$$a_{CDOM}(\lambda) = a_{CDOM}(\lambda_S) * e^{-S*(\lambda-\lambda_s)} \quad (1)$$

where $a_{CDOM}(\lambda_S)$ is the absorption value at a "standard" wavelength (λ_S), typically 400 or 440 nm), and S is the exponential slope of the CDOM absorption spectrum at λ_S. By accepting the standard form of the CDOM absorption spectrum (Eqn (1)), it is possible to completely describe a CDOM absorption spectrum by reporting just $a_{CDOM}(\lambda_S)$ and S. To determine those two parameters for any CDOM absorption spectrum, first, the spectral absorption values are transformed by the natural log (ln), and then a least squares linear regression is fit to the transformed absorption values over the wavelength range from 380 to 500 nm. The best fit linear coefficients, intercept and slope, then represent $\log_e(a_{CDOM}(\lambda_S))$ and S, respectively. During every sample cycle, the CDOM absorption is calculated.

s0025 4. SYSTEMS LEVEL INTEGRATION

p0095 The OPD is a system of fluidic, optical, and computational systems that obtains a water sample, illuminates the sample with a calibrated light source, and measures the transmission spectrum through the water sample (Figure 3). There are two



f0020 **FIGURE 3**
Schematic of major OPD components including the fluidic pathways.

and a whole water transmission spectrum ($I(\lambda)_w$) is collected. For this sample, a dark spectrum is not collected because it follows immediately after the previous filtered (CDOM) sample. Calculation of SI and CDOM absorption spectra are completed, and results are stored and transmitted as specified by the user. If the OPD is ~~set up~~ configured to continuously cycle, only the portion of cycle described ~~that comes after~~ proceeding the CDOM reference is repeated. The CDOM reference cycle is repeated on an adjustable schedule, but usually every 8 to 10 cycles to account for the development of fouling in the LWCC, changes in the light source spectrum, and drift in the spectrometer. Additionally, if there will be a delay before the next cycle, a small volume of CDOM reference water is pumped into the LWCC to displace fouling organisms and compounds and to inhibit growth.

p0110 Computational, electronic, fluidic, and optical systems are controlled by a low-power Persistor Instruments, Inc. CF2 microcontroller. This processor handles all data management, processing, and communications capabilities of the OPD. Communications with host systems, which can include external communications systems (LP units) or independent control and communications systems (HP units on AUVs), are handled by RS232 serial standard protocol.

p0115 The Optical Phytoplankton Detector provides a relatively low-cost way to monitor phytoplankton community structure and CDOM. On a systems level, an LP OPD capable of operating unattended for approximately one month has a purchase price of under $30,000. Operating costs are on the order of $1000 per deployment, including costs of operating marine vessels to deploy and recover units. A single trained operator is capable of maintaining approximately four instruments. An HP OPD for deployment on an AUV has a purchase cost on the order of $40,000, in addition to the cost of the AUV, and it can run with minimal user intervention on the order of two weeks. In comparison, shipboard survey work costs on the order of $10,000−20,000 per day for vessel operations and personnel, plus the cost of scientific staff on board, and sample processing costs once grab samples are returned to shore. For the purpose of regional HAB monitoring, the OPD is capable of identifying domains of interest for shipboard surveys without the cost associated with large-scale surveys.

s0030 5. APPLICATIONS

p0120 The OPD is a modular instrument, in that components can be adapted for different water types with varying optical transmission properties and phytoplankton concentrations. In offshore environments, namely oligotrophic waters, planktonic and CDOM concentrations can be extremely low, requiring an increase in instrument sensitivity. This can be achieved by increasing the sample volume and path length, for planktonic and CDOM detection, respectively, to result in a measurable change in absorbance. Similarly, in extremely turbid waters, absorbance by CDOM in the water can extinguish the characteristic transmission spectra of planktonic cells contained within the sample volume, necessitating a shortened waveguide.

instrument for continuous position information in time and space. Variants of the CDOM mapper have been designed that incorporate updated sensor components to prevent obsolesce, to measure conductivity and temperature for refractive index modeling and correction, and to prevent and remove bubbles in the sampling pathway. The most recent variant of the CDOM mapper has increased ease of operation to make it possible to integrate into the SeaKeeper Discovery Yachts Program, increasing available deployments to include both scientific research vessels and private vessels of opportunity.

s0035 6. VALIDATION AND RESULTS

p0135 Because of the remote, unattended nature of OPD deployments, it was often not feasible to directly verify the results telemetered back to the laboratory. Data from several projects that employed the OPD (Table History1) were used in the validation of the method. To accurately conduct a validation exercise, it was necessary to assure that the OPD and the comparison method utilized the same water sample very closely in very spatially ~~close to the~~ same time. Phytoplankton communities, especially at bloom concentrations, can be ~~spatially very~~ patchy. Photo-acclimation of photopigments can change pigment compliments and the resulting absorbance signature on minute time scales. Fortunately, there have been studies, both laboratory and field, that conducted simultaneous sampling and processing. Additionally, comparison methods are subject to their own inaccuracies, making it necessary to place caveats on validation results. For instance, the use of optical microscope enumeration of phytoplankton taxonomic classes has been the de facto standard method for many years. Although the optical microscope is a powerful tool when used by skilled taxonomists, it is very time consuming and problematic when identifying very small cells. Because *Karenia* sp. cells are large and very distinctly shaped, optical microscope enumeration provides very accurate data for comparison to the OPD estimations of *Karenia* sp. However, the diatom class, for instance, includes a wide range of species with varying sizes and shapes. Some are large and uniquely shaped, making practical the use of microscopic enumeration for numerous samples. Conversely, very small-sized diatom species with nondescript shapes (at optical microscope resolution) are difficult to enumerate accurately by optical microscopes in large numbers of samples. The upshot of these issues in optical microscope enumeration is that there were no complete taxonomic enumerations of community structure to use in validation of the OPD community structure estimates. There are molecular techniques for identification of taxonomic groups, but few simultaneously provide comprehensive coverage of all the possible groups. Chemotaxonomic classification of class-level taxonomy, utilizing high-performance liquid chromatography (HPLC), is a widely accepted approach to dealing with relatively large numbers of samples and for including the full complement of taxonomic classes in natural water samples.

s0040 7. FUTURE DEVELOPMENT/PLANS

p0165 The OPD has been developed for over a decade and has been implemented across a broad geographic range including North America, from the Eastern Pacific to the Western Atlantic, around the Gulf of Mexico, and in the Great Lakes. An OPD has made its way across the Mediterranean, around the Arctic, and there have been deployments in Mexico. With this experience, there are several developmental pathways that will enhance future development. Improvements fall into the domains of expanding species identification capabilities, enhancing CDOM measurement, and expanding the user base.

p0170 To identify plankton species and allocate species to a community structure using the OPD, characteristic fourth derivative spectra must be maintained on file. These spectra are obtained by running isolated plankton samples through the OPD. Typically, plankton are collected during bloom conditions, or they are isolated and grown in culture to detectable levels. A rigorous effort to generate absorption spectra from cultured samples must be conducted and verified by testing plankton from both culture and wild blooms. Additional validation of OPD results against known samples of mixed cultures and against ambient samples determined through more complete molecular and microscopic methods is desirable. The present library of species files would benefit from including phytoplankton from different geographic regions, and a database of libraries would enable researchers and operators of monitoring stations to access and share species files.

p0175 The OPD has demonstrated success in responding to naturally varying levels of CDOM in the natural sampling environment. For OPD to provide CDOM measurements as absorption coefficients (m^{-1}) for research purposes, remote sensing validation, and generating hybrid in situ remote sensing products, a thorough validation and calibration of CDOM measurement must be demonstrated. Simultaneous analyses of CDOM via OPD and benchtop spectroscopy would be performed on estuarine samples that cover a range of both CDOM and salinity values, as well as a matrix of constructed, fixed CDOM-varying salinity samples. The resulting dataset would allow an algorithm to correct measured absorbance with actual CDOM absorption coefficients by accounting for variations in refractive index between sample and reference salinity. The successful algorithm to compensate CDOM absorbance for changing salinity would motivate that integration of a conductivity cell within the OPD, similar to that designed as part of CDOM mapper, as well as the integration of this algorithm into the automated sampling sequence.

p0180 To integrate additional sensors, such as a conductivity cell and thermistor into the OPD, as well as to maintain compatibility with the upcoming generations of ocean sampling platforms, it will eventually be desirable to convert the OPD hardware from the Persistor CF2 processor to an ARM Linux architecture. This would enable a more flexible computing infrastructure and additional computing power to calculate plankton community structure and chlorophyll *a* biomass contributions in real time. It has already been determined that the Slocum Glider will make a similar transition from the Persistor series of processors to the more flexible Linux architecture.

p0185 In the Gulf of Mexico, the Gulf of Mexico Coastal Ocean Observation System (GCOOS) currently supports a number of OPD installations in the eastern Gulf. In addition, GCOOS is currently seeking to expand observing systems including, among other systems, an extended harmful algal bloom monitoring system, of which the OPD could be an integral component. The Sarasota Operations of the Coastal Ocean Observation Laboratories (SO-COOL) model of combining fixed and mobile HAB monitoring instruments with real-time data telemetry and distribution to end users can easily be extend to accommodate additional sampling sites. An effective model would be an instrument exchange program that would support sites around the Gulf, maintaining continuous OPD operations with minimal instrument/site downtime and minimal replication of technical expertise.

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