In Situ Multi-Excitation Chlorophyll Fluorometer for Phytoplankton Measurements

Technologies and Applications Beyond Conventional Fluorometers

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***Abstract*—In this paper, we present the technology and evaluate the performance of a newly developed in situ fluorometer, *Multi-Exciter*, which can measure the excitation spectra of chlorophyll fluorescence both automatically and continuously. The *Multi-Exciter* has nine wavelength excitation LEDs to measure the excitation spectra. The fluorometer measures the discrete excitation spectra of phytoplankton. Then, the phytoplankton species composition can be estimated by solving the optimization problem. The fluorometer has a good linearity that is less than 0.1μg-Chla/l, and reduces errors (<1.0%FS) in turbid water caused by reflectance induced by the excitation lights. These performances show that the fluorometer enables accurate measurements of phytoplankton excitation spectra with a wide range suitable for use in the field. The classification performance of the device is also evaluated in a laboratory setting.**

I. INTRODUCTION

Measuring biomass and compositions of phytoplankton is necessary to monitor water quality and the health of the ecosystem. Chlorophyll concentrations have been used as an index of the biomass. In the 1960s, in vivo fluorometry for the estimation of chlorophyll-a concentrations was introduced to oceanographic communities [1]. This methodology has been valuable in advancing an understanding of the spatial and temporal distribution of phytoplankton biomass. However, the present in vivo fluorometry has been insufficient to obtain information about the compositions of phytoplankton. This is one reason why a manual methodology with microscopy has been widely used. The analysis of phytoplankton compositions has become increasingly important with the explosion of harmful algal blooms (HAB) during the past several decades. HAB is produced by specific phytoplankton species and the spatial and temporal magnitudes vary rapidly. Therefore, to monitor phytoplankton compositions, oceanographic communities require autonomous technologies to identify phytoplankton species and types.

Phytoplankton groups have different pigment compositions. Therefore, the group can be identified by its composition [2]. However, pigment compositions in water cannot be quantified directly. Optical properties of phytoplankton change because of the pigment composition. Therefore, the compositions can be estimated from the optical properties. By measuring optical properties of phytoplankton, phytoplankton groups are indirectly identified.

Because the optical properties significantly depend on the pigment composition, absorption and excitation spectra of phytoplankton exist. Measuring absorption spectra of phytoplankton in the field is quite difficult because the absorbance is very low and interfered by other properties (i.e. absorption and scattering of CDOM: Colored Dissolved Organic Matter and suspended particles). Compared with absorption spectra, excitation spectra can be easily measured. All phytoplankton, including Cyanobacteria, have a fluorescent emission around 680 nm. This emission characteristic in aquatic environments is unique. Excitation spectra of phytoplankton can be obtained with high S/N ratios. Therefore, excitation spectra measurements are suitable to extract optical properties of phytoplankton. Previous scientific papers suggest that we can classify phytoplankton groups using the excitation spectra [3, 4].

We developed an in situ multi-excitation fluorometer, *Multi-Exciter*, which measures the excitation spectra of phytoplankton. The *Multi-Exciter* measures nine wavelength excitation spectra. The *Multi-Exciter* both quantifies the total phytoplankton biomass (chlorophyll-a) and estimates the phytoplankton group compositions using the observed excitation spectra. To enhance its utility, the fluorometer was developed to have high sensitivity and reduce the noise-effect of reflectance from suspended particles in water. Through mathematical processing, phytoplankton groups are identified and quantified.

II. OPTICAL DESIGN AND TECHNOLOGY

The *Multi-Exciter* has nine wavelength LEDs to illuminate a water sample. The center wavelengths of the nine LEDs are 375, 400, 420, 435, 470, 505, 525, 570, and 590 nm, respectively. Figure 1 shows the spectral characteristics of the LEDs. The excitation wavelengths of the LEDs are selected by considering the maximum absorption of the photosynthetic pigments, which appears at a shorter wavelength than 600 nm. The *Multi-Exciter* detects fluorescent signals from 630 nm to about 1000 nm, because phytoplankton commonly emits a distinguishable red fluorescence near 680 nm. The detector of the *Multi-Exciter* is a Si-photodiode. The optical filters are used for the excitation LED and the photodiode detector to intercept the stray light generated through light scattering. Figure 2

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shows a schematic of the optical filter characteristics used in the *Multi-Exciter*.

The *Multi-Exciter* has temperature, depth, and turbidity sensors. In addition, this instrument has a mechanical wiper to prevent bio-fouling on the optical window. Consequently, the instrument provides stable and accurate optical data during the deployment period. Figure 3 shows the exterior appearance of the *Multi-Exciter*.

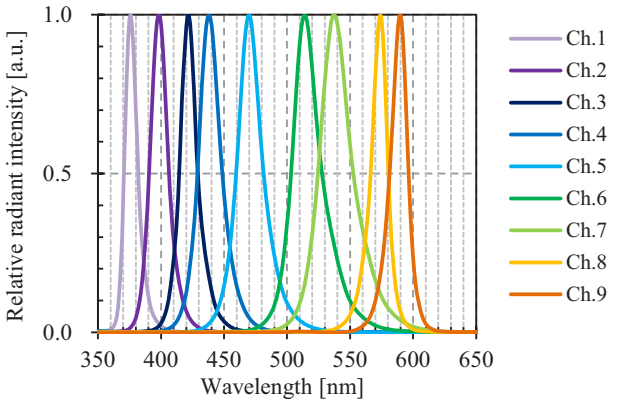


Figure 1. Spectral distributions of the nine LEDs.

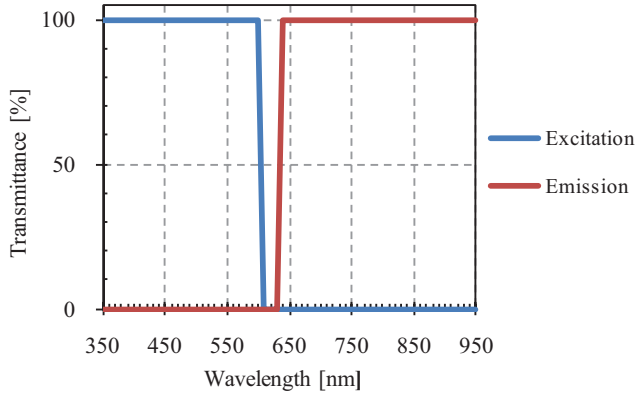


Figure 2. Characteristics of the optical filters.

III. PROCESSING OF PHYTOPLANKTON GROUPS

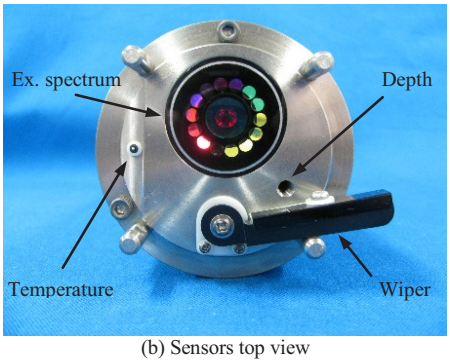
A. Modeling of the excitation spectra

The *Multi-Exciter* measures the nine-wavelength excitation fluorescence spectrum, $\mathbf{y}$, in the water sample. The spectrum is described as the following vector form:

$$\mathbf{y} = [y_1, y_2, \cdots, y_9]^t \in \mathbf{R}^9$$

where t is the transpose of a vector, and $\mathbf{R}^9$ is the set of nine-dimensional real-valued vectors.

(a) Multi-Exciter overview



(b) Sensors top view

Figure 3. Appearance of the *Multi-Exciter*.

It is assumed that there are m groups of phytoplankton in the water sample. Each group has a different spectral characteristic. Here, we defined the specific spectrum as the spectrum normalized by the corresponding chlorophyll-a concentration. The specific spectra of m groups are expressed as follows:

$$\mathbf{s}_i = [s_{i1}, s_{i2}, \cdots, s_{i9}]^t \in \mathbf{R}^9 \quad (i = 1, 2, \cdots, m)$$

The excitation spectrum in the water sample, $\mathbf{y}$, is reconstructed using the specific spectrum and the corresponding chlorophyll-a concentrations:

$$\begin{aligned}\mathbf{y} &= c_1\mathbf{s}_1 + c_2\mathbf{s}_2 + \cdots + c_m\mathbf{s}_m \\ &= \mathbf{Sc}\end{aligned}$$

where c_i indicates the chlorophyll-a concentration of each phytoplankton species in the water sample. The chlorophyll-a concentrations, $\mathbf{c}$, and the excitation spectra of the phytoplankton groups, $\mathbf{S}$, are described as:

$$\mathbf{c} = [c_1, c_2, \cdots, c_m]^t \in \mathbf{R}^m, \quad \mathbf{S} = [\mathbf{s}_1, \mathbf{s}_2, \cdots, \mathbf{s}_m] \in \mathbf{R}^{9\times m},$$

where $\mathbf{R}^{9\times m}$ indicates the set of $9 \times m$ matrices.

B. *Algorithms*

The estimated chlorophyll-a concentrations of each group in the sample, $\hat{\mathbf{c}}$, are described using the following vector:

$$\hat{\mathbf{c}} = [\hat{c}_1, \hat{c}_2, \cdots, \hat{c}_m]^t \in \mathbf{R}^m .$$

$\hat{\mathbf{c}}$ is calculated using a constrained least-squares method, which minimizes the norm of the residual between the observed spectrum (using the fluorometer $\mathbf{y}$) and the reconstructed spectrum composed of the specific spectrum and the corresponding estimated chlorophyll-a, $\mathbf{S}\hat{\mathbf{c}}$. This problem is formulated as the following optimization problem:

$$\min_{\mathbf{c}} \quad \| \mathbf{y} - \mathbf{S}\hat{\mathbf{c}} \|^2$$
$$\text{s.t.} \quad \hat{c}_i \geq 0 \quad (i = 1, 2, \cdots, m)$$

where $\| \cdot \|$ is the Euclidean norm of the vector. The above constraint is based on a priori information that the chlorophyll concentrations are nonnegative.

IV. PERFORMANCE

A. *Responses to Chl-a*

Figure 4 explains the relationship between the fluorometer outputs and the chlorophyll-a concentrations in the Diatom alga *Cheatoceros* sp. determined by a fluorometric method with the extracted samples. This result suggests the fluorometer outputs of the nine-channel device have good linearity to less than 0.1 µg-Chla/l.

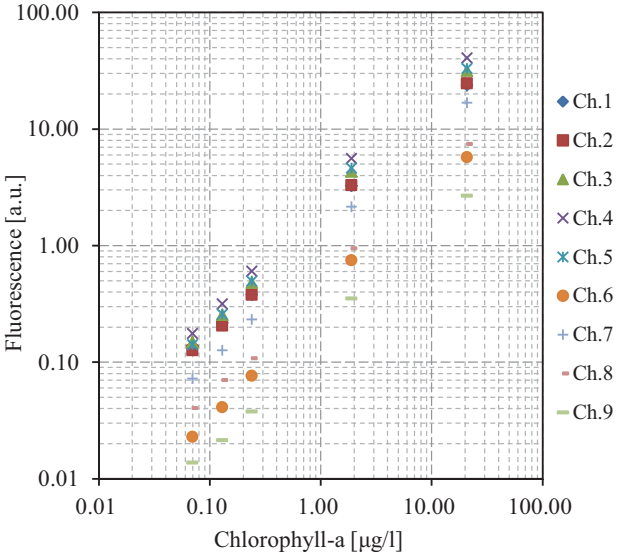


Figure 4. Responses to Chl-a concentration.

B. *Responses to suspended solids*

In turbid water, the excitation light is reflected by suspended matter (e.g. minerals and detritus). The instrument overestimates the fluorescent intensities if the reflectance lights come through the detector. To quantify the effect of the reflectance on measurement, we measured the responses against a wide range of Kaoline solutions in a bucket. After putting Kaoline powder into 2 L of distilled water, the fluorescence sensor outputs were measured. At that time, the turbidity was observed using an IR-backscattering turbidity sensor. The reflectance effects were within 0.6% FS at 109 FTU [Figure 5].

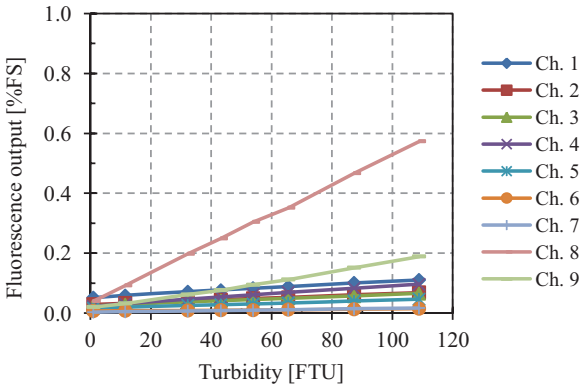


Figure 5. Responses to suspended solids.

C. *Specific spectra of phytoplankton*

Figure 6 shows the specific spectra of three pigment-type phytoplankton (Diatom: *Cheatoceros* sp., Green algae: *Nanochloropsis* sp., Cyanobacteria: *Microcystis* sp.) observed using the fluorometer. All spectra are normalized using the fluorescent intensity at 430 nm or 570 nm. The fluorometer observed different fluorescent properties depending on the pigment compositions among the three types. *Microcystis* sp. indicates a quite different optical characteristic with a high excitation ratio (570 nm:430 nm) because only Cyanobacteria have phicobili-protein pigments with a maximum absorption around 570 nm.

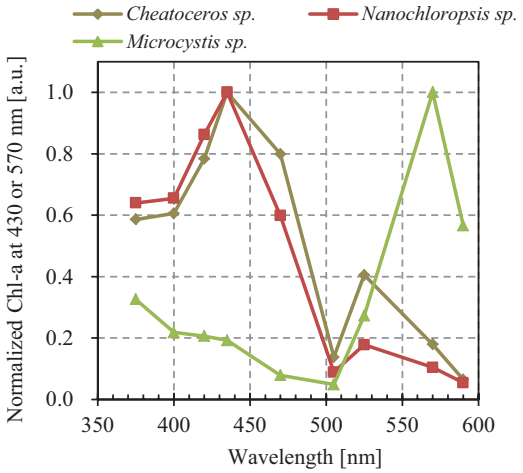


Figure 6. Specific spectra of phytoplankton.

D. Evaluation of the classification

The classification performance described in the above algorithm was evaluated in mixed culture samples (Diatom: *Cheatoceros* sp., Green algae: *Nanochloropsis* sp., Cyanobacteria: *Microcystis* sp.). To process the phytoplankton groups, the specific spectra (see section III-B) were pre-prepared. By changing the mixing ratios, the test was carried out in seven different samples. Figure 7 shows the relationships between the estimated and the actual chlorophyll-a concentrations. The Cyanobacteria and Green algae species match both values with errors of less than 6%. In the Diatom species, the estimated values explain the variation but are overestimated compared with the actual concentrations. The maximum error was approximately 45%. The estimated errors were caused by the mismatch of the fluorescent intensities per chlorophyll-a concentrations between the specific spectra of the actual samples and the prepared samples.

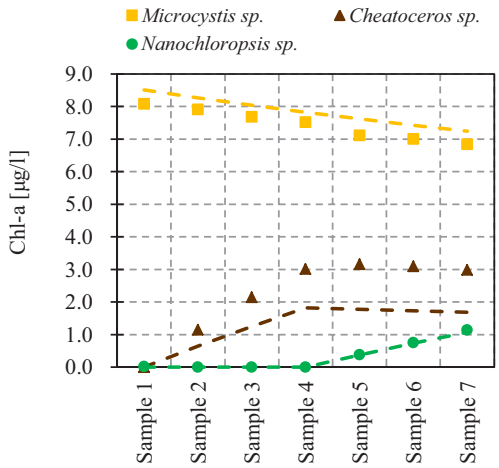


Figure 7. Estimates of the concentration. Colored dots indicate the estimated chlorophyll-a concentrations, and dashed lines represent the actual chlorophyll-a concentrations.

V. CONCLUSION

The multi-excitation fluorometer, *Multi-Exciter*, provides accurate excitation spectra of phytoplankton with high sensitivity for chlorophyll-a concentrations and low sensitivity for the reflectance from suspended particles. Using a mathematical process, phytoplankton groups can be estimated. To achieve accurate classifications, the processing algorithm should be slightly modified. New methods to identify phytoplankton groups using specific fluorescent ratios should be considered in the future. Distinguishing between Diatom and some Dinoflagellate species may be possible because of their different fluorescent properties around 470 nm [5]. The *Multi-Exciter* has good potential for phytoplankton research beyond that of conventional fluorometers.

ACKNOWLEDGMENTS

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