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Application of a laser fluorometer for discriminating phytoplankton species

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ABSTRACT

A portable laser-induced fluorescence system for discriminating phytoplankton species has been developed. It consists of a high pulsed repetition frequency (10-kHz) microchip laser at 405 nm, a reflective fluorescent probe and a broadband micro spectrometer. The measured fluorescent spectra were overlapped by various fluorescent components, and were then decomposed by a bi-Gaussian mixture model. A spectral shape description index was designed to characterize fluorescent spectral shapes for descriminating the phytoplankton species cultured in our laboratory. Using clustering analysis, the samples of eight phytoplankton species belonging to two divisions of Bacillariophyta and Dinophyta were divided into six categories: 1) *Chaetoceros debilis*, *Thalassiosira rotula*; 2) *Prorocentrum donghaiense*, *Prorocentrum dentatum*; 3) *Gymnodinium simplex*; 4) *Alexandrium tamarense*; 5) *Karenia mikimotoi*; and 6) *Akashiwo sanguinea*. The phytoplankton species belonging to Bacillariophyta were well separated from those belonging to Dinophyta. In addition, the phytoplankton species belonging to Dinophyta were successfully distinguished from each other at genus level. The portable system is expected to be used both in vivo and in the field.

1. Introduction

Phytoplankton are microscopic drifting algae that fix carbon dioxide by photosynthesis and are the base of food webs in most aquatic ecosystems [1]. They are the main source of primary production in both marine and freshwater habitats [2]. The composition of phytoplankton communities can be highly variable in space and time [3]. Characterization of phytoplankton community composition, therefore, requires frequent, high-resolution sampling in both space and time [4]. Bacillariophyceae and Dinophyceae, are major bloom-forming algae in the Chinese seas. To be able to differentiate different taxa within Bacillariophyceae and Dinophyceae is of great importance in marine environment [5]. A variety of approaches has been used to discriminate and determine phytoplankton species, such as labor-intensive microscopic methods [6–8], high performance liquid chromatography [9,10], fluorometric approach [11–13], and an approach based on absorption spectra [14–16]. These currently available methods for determinating micro algal population distribution in waters typically lack the ability of being used in situ and are low in temporal resolution; and they are often costly in terms of man-hours [17]. Among these methods, fluorometric methods have become more and more commonly used because they can work both in vivo and in situ [18–20]. The laser-induced fluorescence (LIF) technique is a well-known fluorometric technique for rapid water environment monitoring, which is based on the measurements of laser-induced water emission spectrum, to obtain qualitative and quantitative information about the in-situ fluorescent constituents of living phytoplankton cell and organic matter [21–23]. Compared to traditional fluorometric techniques (e.g., stimulated by light emitting diodes, or xenon lamps), the spectrally narrow laser emission provides improved selectivity and efficiency of excitation; it also reduces the spectral overlap between the water Raman scattering and fluorescence bands of aquatic constituents [24].

Fluorometric taxonomic method is mainly based on algal feature that various taxa possess different types of antenna and accessory pigments, which can be detected accurately by a fluorometric equipment [25,26]. Excitation of different pigments by light of varying wavelengths, therefore, results in characteristic fluorescence excitation spectra for the major algal groups [27,28]. Nowadays, a number of spectral characteristic analysis methods is utilized in processing fluorescence signals, such as wavelet analysis [29,30], linear discriminant analysis [4,31], band ratios [32,33] and principal component analysis [34]. Using these methods, the fluorescence spectra are often recorded as a multi-dimensional matrix of intensity and spectral

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parameters. Various organic compounds are manifested in such a matrix as specific structural singularities representing the fluorescent components [35].

Nowdays, laboratory benchtop scanning fluorometers, especially the excitation emission matrices (EEMs), which are capable of measuring tunable excitation and emission spectra, are often used for differentiating algal populations [36–38]. Such instruments, however, are too bulky for routine use in the field, and the measurement scans takes a long time [39]. More recently, in situ fluorometer which are capable of measuring automated characterization of plankton communities was developed [40–42]. This type of instrument has excellent resolution but requires high power [4]. In addition, it often uses only one or a few channels to measure one specific parameter and does not provide full and detailed spectral information about other fluorescent constituents for more comprehensive characterization of aquatic environment [21]. In this paper, we present a laser fluorometric taxonomic method for differentiating algal populations to meet the measurement requirements in field. A light-weight LIF system for fluorescence measurements in aquatic environments was developed. A bi-Gaussian mixture model was utilized to decompose the overlapped fluorescence emission spectra into various fluorescent components, and a spectral shape description index was designed to characterize different fluorescent spectral shapes for discriminating eight phytoplankton species cultured in our laboratory. The LIF system consists of a high pulse repetition frequency microchip laser, a reflective fluorescent probe and a broadband hyperspectral micro spectrometer. Its crucial advantages include: higher signal-to-noise ratio improved by high pulse repetition frequency laser [43], and adequate and detailed spectral information about fluorescent constituents provided by a broadband hyperspectral micro spectrometer, especially useful in complex aquatic environments. Currently, the fluorescence band ratio approach is widely used in many commercial instruments, but it has limited ability to discriminate between more complex mixtures; this approach is sensitive to changes in background fluorescence, and is easily biased due to acclimative changes in the representative organisms' excitation spectra [13,44]. In contrast to the band ratio approach, the bi-Gaussian mixture method using detailed spectral shape information can separate groups that are more similarly pigmented. The reliability of the laser fluorometric taxonomic approach was tested by several experiments in the laboratory.

2. Material and methods

2.1. Phytoplankton culture

Eight species belonging to seven genera of two divisions were obtained from the State Key Laboratory of Satellite Ocean Environment Dynamics in the Second Institute of Oceanography of China. These species, most recorded as algae bloom species in the Chinese seas [45,46], are listed in Table 1. Each strain of the eight species was grown in f/2 medium with a 500 mL conical flask by light incubator (Leading Tec Inc.) [47]. And a parallel study was conducted. The culturing temperature was 20.5 °C, the salinity was about 35, and the irradiances were 85, 110, and 150 Wm^{-2}. The light:dark cycle was 12:12 h. The culture period was 18 days for these phytoplankton species.

Table 1
Phytoplankton species used in the study.

Species	Abbreviation	Genus	Division
Chaetoceros debilis	*Cd*	*Chaetoceros*	*Bacillariophyta*
Thalassiosira rotula	*Tr*	*Thalassiosira*	*Bacillariophyta*
Prorocentrum donghaiense	*Pd*	*Prorocentrum*	*Dinophyta*
Prorocentrum dentatum	*Pt*	*Prorocentrum*	*Dinophyta*
Akashiwo sanguinea	*As*	*Akashiwo*	*Dinophyta*
Gymnodinium simplex	*Gs*	*Gymnodinium*	*Dinophyta*
Karenia mikimotoi	*Km*	*Karenia*	*Dinophyta*
Alexandrium tamarense	*At*	*Alexandrium*	*Dinophyta*

2.2. LIF equipment

A portable laser fluorometer was used to record the complete visible LIF emission spectra of algal cultures. A photograph of the LIF equipment is shown in Fig. 1 and the schematic is shown in Fig. 2. A micro violet laser with 405 nm wavelength, a fiber-optic fluorescent probe module with a dichroic beamsplitter, a sample holder module, and a broadband micro CCD spectrometer are integrated to form the system. The main characteristics of the LIF system are summarized in Table 2. Detailed description of the system can be found in [48]. The weight of the whole system is about 1.7 kg, and is small in size. Therefore, it is easy for transport and can be used aboard a small boat. The water Raman signal is often used for fluorescence normalization that accounts for the highly variable optical properties of natural waters [49–52].

2.3. Data pre-processing

All the fluorescence spectra were pre-processed using MATLAB® (The Mathworks, Natick, MA). Each of the raw spectra was changed

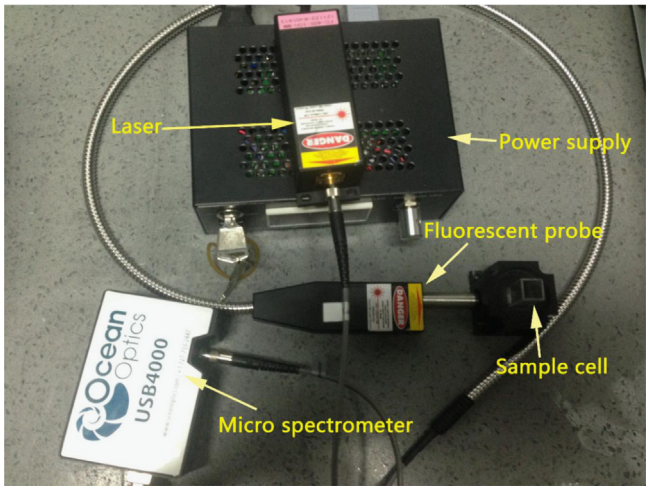

Fig. 1. Photograph of the optical system.

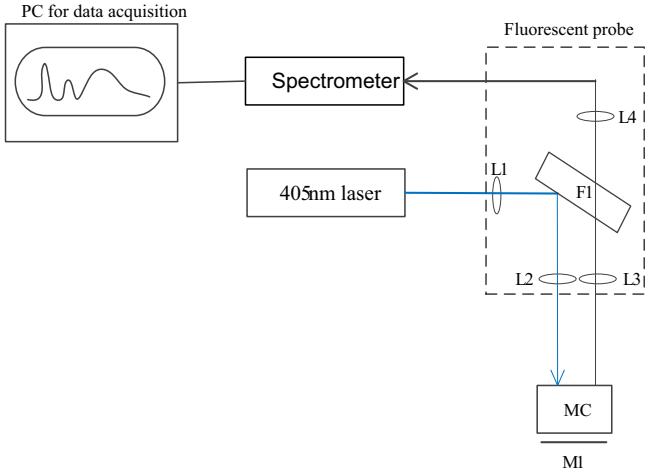

Fig. 2. System chart of the LIF system. MC – measurement cell; F1 – dichroic beamsplitter; L1, L2 and L4 – focusing lenses; L3– collecting lenses; M1– reflecting mirror.