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A fiber-based fluorometric system for *in situ* algal classification

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ABSTRACT

We develop a fast fiber-based *in situ* fluorescence system for phytoplankton taxonomy. The fluorescence is excited by high power LEDs with different wavelengths through a customized fiber bundle to the place of interest. Sinusoidal-amplitude-modulation (SAM) techniques are employed. The detection time is shorten to 0.1 s because the fluorescence is excited and demodulated by different wavelengths simultaneously. The fluorescence signal is demodulated by a digital lock-in amplifier. The device is tested in Shenzhen Bay, South China Sea.

1. Introduction

Degraded water quality from nutrient pollution coupled with the global climate change promote the development and persistence of algal blooms which has been a major environmental issue around the globe [1–3]. Knowledge of algal species composition and their population dynamics is essential for understanding the development of algal blooms. The traditional group-specific assessments of microalgae include microscopy [4], flow cytometry [5], and high performance liquid chromatography (HPLC) [6]. These methods are often labor-intense and time-consuming. Moreover, these sample analysis approaches require samples being collected before obtaining the results, which limits the use of these methods for monitoring or supervision tasks. Most importantly, these traditional methods cannot provide real-time and *in situ* measurements due to the time lag between sample collection and sample analysis. It is difficult to obtain fine spatial and temporal dynamics of microalgae because samples are often integrative by nature and enumeration of samples are time-consuming [7,8].

Chlorophyll fluorescence analysis offers an alternative approach and potentially allows *in situ* estimate of algal concentration. In 1963, Yentsch et al. proposed using the fluorescence emission of photosystem II (PS II) measured around 685 nm as a measure of chlorophyll (Chl) content of algae [9]. In the 1980s, Ulrich Schreiber first presented a pulse-amplitude modulation (PAM) Chl fluorometric method [10,11]. In the PAM configuration [12], four different light sources illuminate algae sample sequentially to get the fluorescence from algae pigments. The fluorescence signal is processed by a lock-in amplifier after a 685 nm filter. In this way, Chl concentration is determined accurately by the precise measurements of fluorescence signals. A typical product of fluorescence meter, Phyto-PAM [13] can provide a resolution of 0.1 ug/L measuring Chl a. However, sampling processing time is almost 1 min, which limits its use for real-time applications. Meanwhile a sample extraction procedure before the real measurement is also required, so the actual state of the sampled algae may vary from its original state. Beutler et al. designed a submersible probe combining light source, detector, analog-to-digital convertor (AD), Micro Controller Unit (MCU) to one module and obtained vertical distribution of algae [14]. The resolution and detection time remain the same level as the PAM method. Recently, we have developed a sinusoidal-amplitude-modulation (SAM) method for Chl a measurement [15]. The SAM method enhances the use ratio of excitation light energy in the demodulation process, thus improving the detection limit of Chl a concentration.

In this paper, we present a fast fiber-based *in situ* fluorometric system for group-specific assessments of microalgae. A customized fiber bundle is used to guide the excitation light energy to the place of interest. A submersible probe without electronic components is designed to improve the efficiency of gathering

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fluorescence. *In situ* experiment is carried out to verify the measurements in Shenzhen Bay, South China Sea.

2. Materials and methods

2.1. Measurement principles

Fluorescence emission around 685 nm is widely accepted as a measure of Chl concentration in microalgae in aquatic systems [9,16]. The photosynthetic algal pigments absorb a part of the luminous light energy. The absorbed energy is re-emitted in the form of fluorescence by the chlorophyll-a of the photosystem (PS II) antenna system, which is made up of a Chl a-containing core and species-dependent peripheral antenna. The species-dependent peripheral antenna influence the excitation spectrum of the fluorescence. Different algae species has different peripheral antennae in the PSIIs, so the spectral distribution in the fluorescence excitation spectra can be used as fingerprints [14,17] for species-determination.

The measurement of a water sample yields a vector:

$$M = (F_1, F_2, F_3) \tag{1}$$

With F_1, F_2, F_3 being the fluorescence intensity excited by different wavelengths. The subscript 1, 2, 3 represents blue, green, amber LEDs respectively. By analysis the measurement of a certain individual specie of algae we can get a base vector:

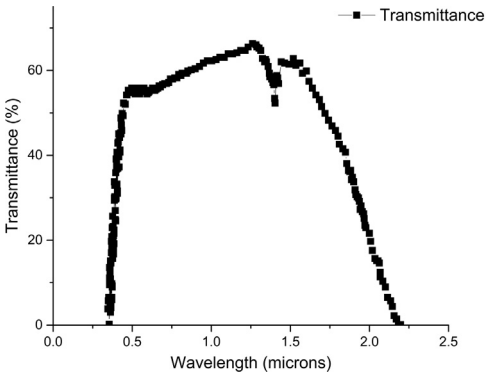
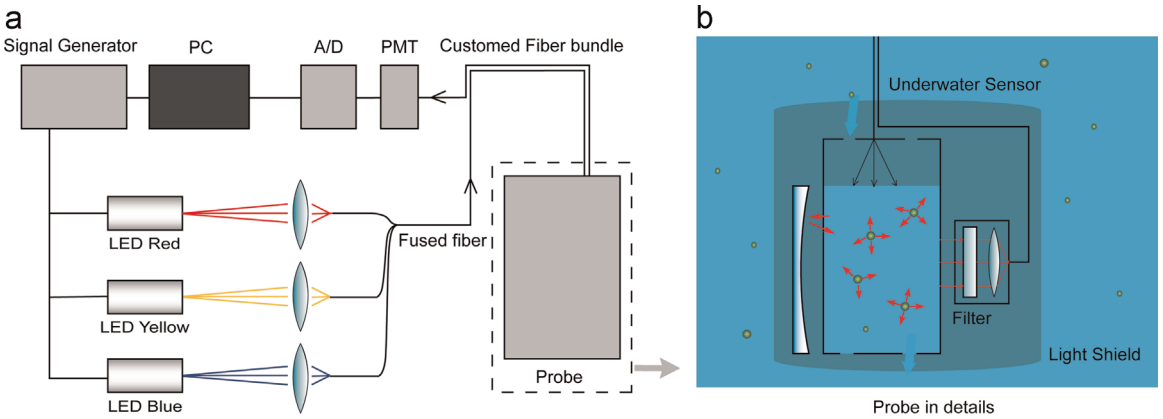


Fig. 2. Transmission spectra of the customized fiber used in this *in-situ* Chl fluorometer.

$$N_K = (f_{k1}, f_{k2}, f_{k3}) \tag{2}$$

With f_{k1}, f_{k2}, f_{k3} being the fluorescence efficiency of kth species in terms of fluorescence intensity per unit Chl a concentration. The concentration of each group, a_k can be obtained by solving the linear regression problem for:

$$M = \sum a_k \times n_k \tag{3}$$



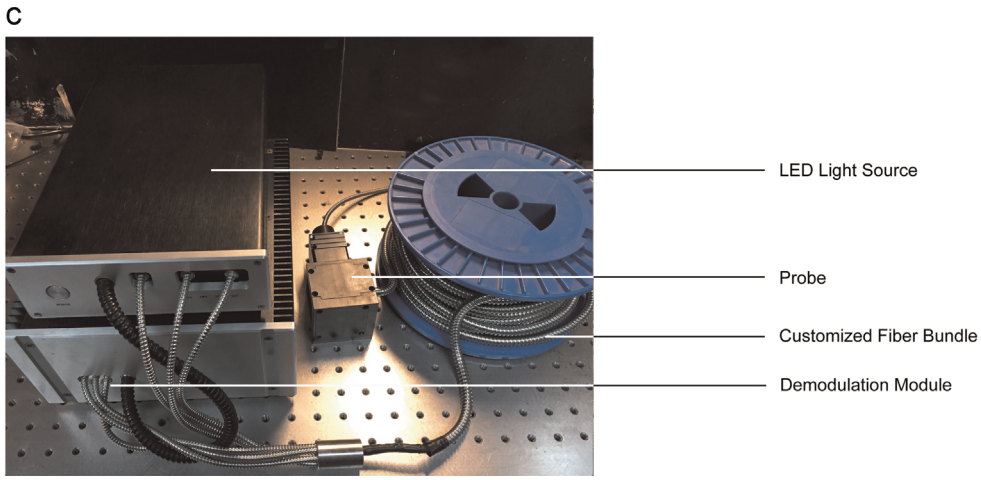


Fig. 1. (a) System schematic of a fiber-based *in-situ* Chl fluorometer: signal generator: sinusoidal signal generator; PC: personal computer; A/D: 16 bit analog to digital convertor, convention rate 2 MHz; PMT: photomultiplier; Probe: submersible probe. (b) Details of the submersible probe. (c) Photograph of the fluorometric system: LED light source contains sinusoidal signal generator, LEDs, LED driver and power supply; demodulation module contains A/D convertor and PMT.