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Targetting automatic determination of species composition in phytoplankton !

Successful development of a multi-wavelength excitation fluorescence photometer

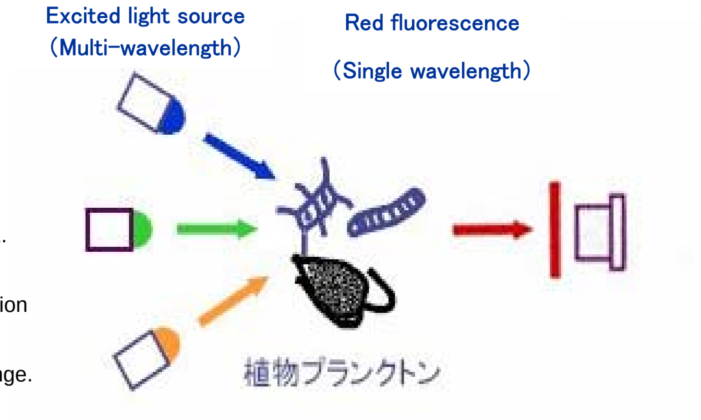
Alec Electronics Co., Ltd. is collaborating with the Research Institute for Cell Engineering(RICE), located in Amagasaki City, Japan to develop an instrument that can automatically identify the species composition of phytoplankton populations. The prototype of a multi-wavelength excitation fluorescence photometer has been completed. This issue reviews the unit and the result of a recently completed in-situ test.

Introduction

The measurement of living chlorophyll fluorescence (hereafter, in-vivo) is popular as it provides an indication of the biomass of a phytoplankton population. The popularity of this measurement has resulted in the demand of in-situ fluorescence photometers such as the Alec Electronics Clorotec. Although measuring Chlorophyll-a (hereafter, Chl-a) serves as an index of the biomass of a phytoplankton population, it does not identify the specific species composition. Phytoplankton is the foundation of the marine ecosystem and greatly affects the workings of our environment. It is well known that a certain species of phytoplankton may cause Red Tide, which can seriously damage human life. In order to avoid these incidents, it is very important to continuously monitor the species composition. Presently, in order to estimate the species composition in phytoplankton it is common to observe sea water samples with a microscope or to extract and quantify the pigment composition with expensive analyzers. These methods are not only time consuming and labor intensive, but also they require superior technical skill of the researcher and moreover, there are limited sample numbers. Accordingly, oceanographic and marine researchers have asked for a quick and inexpensive automatic measuring instrument to monitor the species composition of phytoplankton. In response to our clients' requests we have embarked on the development of an in-vivo multi-wavelength excitation fluorescence photometer.

Fundamental principle

Phytoplankton photosynthesize by absorbing optical energy. In turn, they emit a few percent of the optical energy absorbed as red fluorescence. The Alec Electronics in-vivo multi-wavelength excitation fluorescence photometer works by irradiating each different excited wave-length of light in the phytoplankton, then it measures the intensity of emitted red fluorescence for each excited wavelength. Please refer to Fig 1. The intensity of emitted red fluorescence theoretically depends on the absorption spectrum characteristic of the phytoplankton [change of absorption efficiency per respective color (wavelength)]. It has been theorized that the phytoplankton's absorption efficiency decreases from blue to green, to orange. I.e. when blue, green and orange excited lights are irradiated at the same Intensity, the intensity of red fluorescence emitted is at a maximum for the color blue then decreases with green and orange respectively. This optical absorption characteristic is determined by the pigment composition of the phytoplankton. Accordingly, by measuring the excitation fluorescence characteristic [change of the fluorescence intensity per each color (wavelength)] the species composition of the phytoplankton can be predicted. Thus, the in-vivo multi-wavelength excitation fluorescence photometer assists in determining the species composition of the phytoplankton population.



Phytoplankton

Fig. 1. *Conceptual drawing on multi-wavelength excitation fluorescence photometer*

Outline

The in-vivo multi-wavelength excitation fluorescence photometer developed is shown in Fig. 2. As the unit is extremely light and compact, it is easily handled and operated. It has a total length of 156mm, a diameter of 69mm and a weight of 250g in water. The red central circle shown is the sensor that detects the fluorescence. A red optical filter is provided on the protective window to protect against interference from spurious excited rays and natural light. The light emitting diodes (LED) which are the source of excited light are located around the fluorescence detection window. The central wave-length of each LED is 375, 400, 470, 505, 525, 568, 590 & 612nm. Table 1 shows the specifications of the in-vivo multi-wavelength excitation fluorescence photometer.

Fig. 2. *In-vivo multi-wavelength excitation fluorescence photometer*

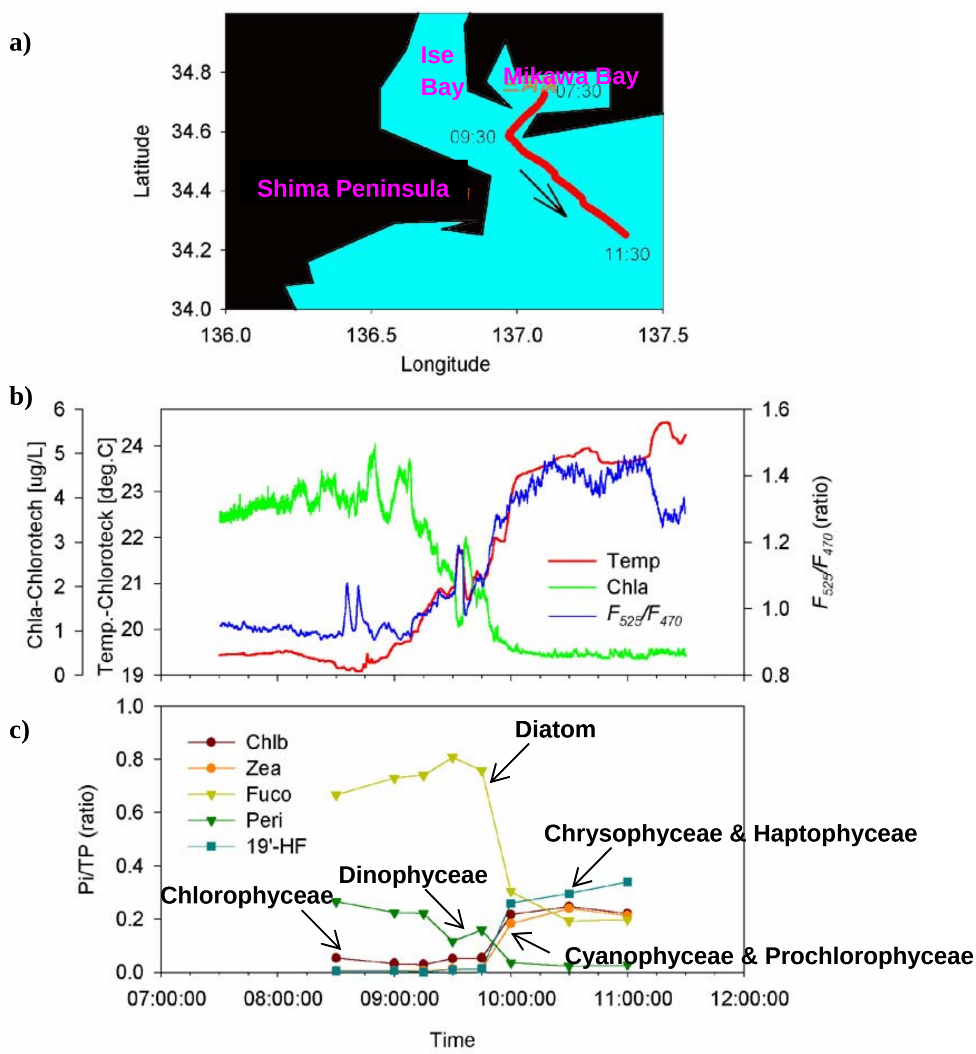
Table 1. *Specifications on in-vivo multi-wavelength excitation fluorescence photometer.*

Parameters	Fluorescence intensity
Sensor type	Back-scattering
Receiving wave-length[nm]	680
Excitation wave-length[nm]	375、400、470、505、525、568、590、612
Communication	RS-232C
A／D Conversion	16-bit digital conversion
Power supply	5VDC
Power consumption	70mA（only for sensor sonde）
Weight	760g in air, 250g in water（Excluding cables）
Depth rating	200m equivalent

Field experiment results

Alec Electronics and the Research Institute for Cell Engineering (RICE) conducted a field experiment (Project #BO-04-26) from the Tokai region up to the Kumano Nada waters with R/V Bousei Maru of the Tokai University. We made simultaneous observations with our in-vivo multi-wavelength excitation fluorescence photometer and our Clorotec (Chlorophyll-a instrument). The two units were installed so that sea water from 3m depth was pumped over the sensors and fluorescence was continuously measured. The results of the experiment from Mikawa Bay to just off Shima Peninsula (Fig.3(a)) is presented below.

Fig.3(b) illustrates the fluorescence strength ratio F525/F470 in the excited wave-lengths of 525nm & 470nm measured by the multi-wavelength excitation fluorescence photometer and the space variation of temperature & Chlorophyll-a measured by the Clorotec. At just off the Shima Peninsula, after passing through Mikawa and Ise Bays which are strongly affected by coastal water, the water temperature rose and the concentration of Chlorophyll-a fell sharply. Accordingly, the F525/F470 ratio varied sharply in and out of the bays (it went up to 1.3 or more off the Shima Peninsula). Compared with the variation of the pigment composition shown on Fig 3(c), the F525/F470 ratio was lower at the Mikawa and Ise Bays due to the wide distribution of diatoms & flagellates there. It also turns out that the composition ratio of a group of small phytoplankton (chrysophyceae, haptophyceae, cyanophyceae, etc.) went up by 1.2 or more in the same area. Therefore, it was verified that the F525/F470 ratio is a good index to indicate the composition ratio of two groups (diatoms and flagellatae & small phytoplankton) in the phytoplankton.



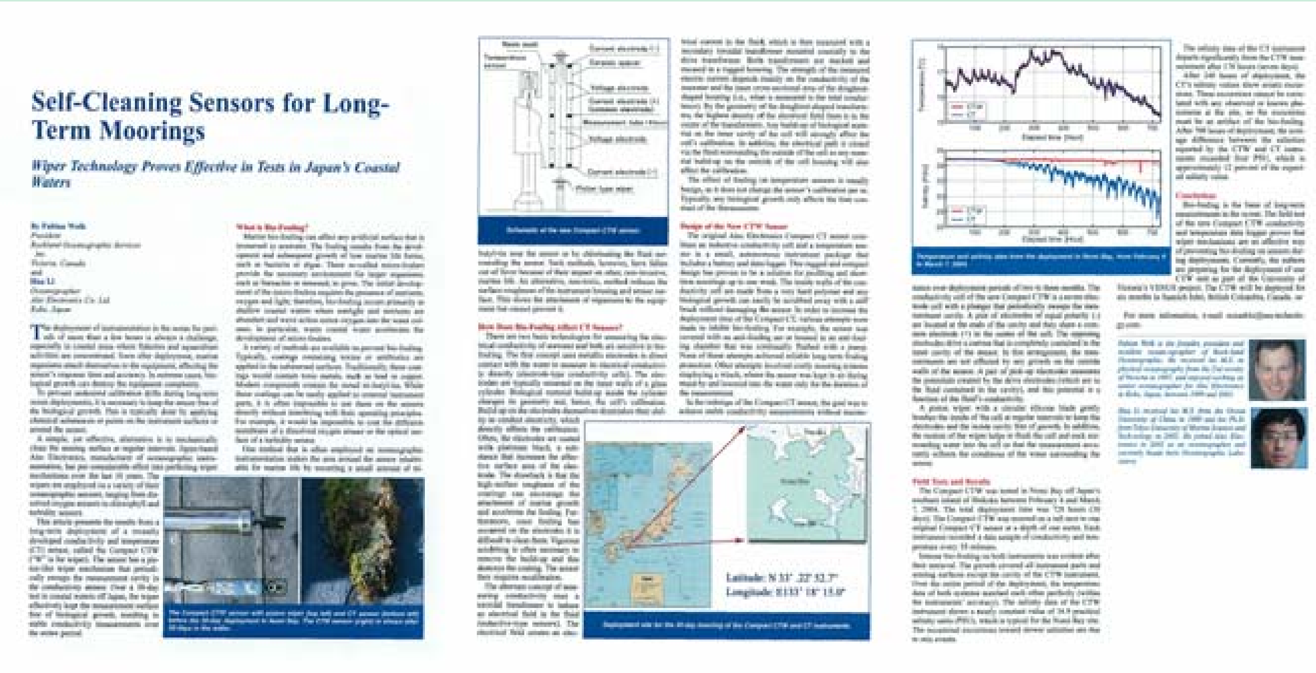
a)Navigation route of R/V Bousei Maru b) Result done by Clorotec (Chl-a concentration & water temperature) & in-vivo multi-wavelength Excitation fluorescence photometer（Fluorescence intensity ratio of 525nm & 470nm）c) Index ratio of the pigment composition of phytoplankton

Future outlook

This field experiment confirmed that the in-vivo multi-wavelength excitation fluorescence photometer is an effective tool to quickly determine the species composition in a phytoplankton population. However, the raw data measured by the in-vivo multi-wavelength excitation fluorescence photometer gives the fluorescence intensity but does not directly predict the species composition. Therefore, in order to determine the species composition with the in-vivo multi-wavelength excitation fluorescence photometer, an analysis algorithm has to be developed. The Research Institute for Cell Engineering (RICE) is accumulating fundamental data for the development of the algorithm. It has investigated the relationship between excited fluorescence characteristics and pigment composition by cultivating more than ten kinds of phytoplankton. The algorithm will be developed based on the data observed in conjunction with the instrument's characteristics. We will continue our efforts to develop the in-vivo multi-wavelength excitation fluorescence photometer so that the species composition of phytoplankton can be easily obtained without requiring complicated data processing by our clients.

COMPACT-CTW introduced world-wide

The English manuscript of the newly-developed piston-wiper type water temperature/conductivity sensor introduced in the ALEC TECHNICAL EXPRESS 3rd. issue (September, 2004) has been published in the February 2005 issue of SEA TECHNOLOGY. We have received over a thousand inquiries from all over the world including Canada & U.S. It is our pleasure to have developed a marine oceanographic instrument that has received such overwhelming interest.



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