

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 wavelength 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.

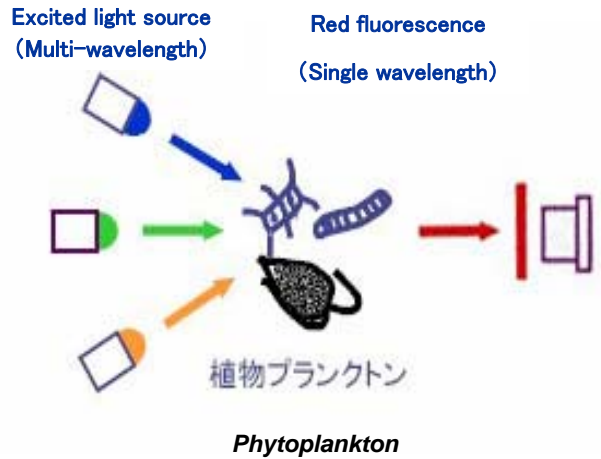


Fig. 1. Conceptual drawing on multi-wavelength excitation fluorescence photometer

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

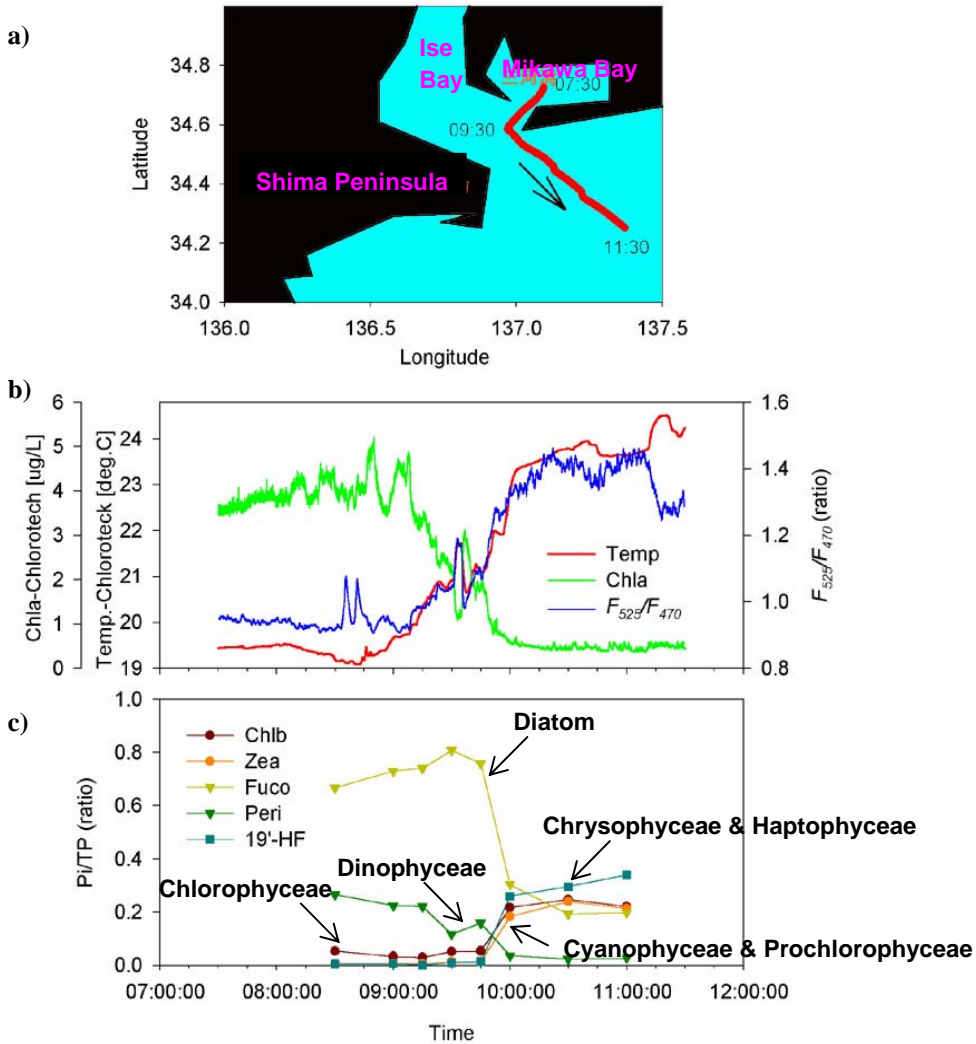
Table 1. Specifications on in-vivo multi-wavelength excitation fluorescence photometer.

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 F_{525}/F_{470} 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 F_{525}/F_{470} 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 F_{525}/F_{470} 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 F_{525}/F_{470} 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.

Self-Cleaning Moorings

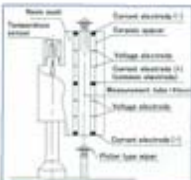
Wiper Technology Proves Effective in Tests in Japan's Coastal Waters

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The development of instrumentation in the ocean for the past half a century has been a challenge, especially in coastal areas where fisheries and aquaculture activities are concentrated. Some other disciplines, marine geophysics and oceanography, also require accurate, long-term measurements of the ocean's physical and chemical properties. This is typically done by anchoring chemical sensors or pumps on the instrument surface around the ocean.

A simple, yet effective, alternative is to mechanically clean the sensing surface at regular intervals. Japan-based ALEC Electronics, manufacturer of oceanographic instrumentation, has developed a wiper-type self-cleaning mooring system for the last 20 years. The system is employed in a variety of oceanographic surveys, ranging from the collection of water samples to continuous monitoring of water quality.

This article presents the results from a long-term deployment of a newly developed mooring system called the Compact CTW (CTW for short). The sensor has a piston-wiper mechanism that periodically sweeps the measurement cavity of the conductivity sensor. Over a 10-day test in coastal waters off Japan, the wiper effectively kept the measurement cavity free of biological growth, resulting in accurate conductivity measurements and a long service period.



What is the Problem?
 Marine bio-fouling can affect any artificial surface that is immersed in seawater. The fouling results from the attachment and subsequent growth of low marine life forms, such as bacteria or algae. These microbial micro-organisms provide the necessary environment for larger organisms, such as bivalves or seaweeds, to grow. The initial development of the mooring facilities requires the presence of organisms, oxygen and light. However, bio-fouling occurs primarily in shallow coastal waters where sunlight and nutrients are abundant and wave action causes organisms to be swept onto the instrument.

In particular, water column water continues the development of micro-organisms.

A variety of methods are available to prevent bio-fouling. Typically, coatings containing silver or antibiotics are applied to the instrument surface. Traditionally, these coatings would contain toxic materials, such as lead or copper. Modern compounds contain the metal ion cadmium. While these coatings can be easily applied to instrument mooring parts, it is often impossible to use them on the sensors directly without interfering with their operating principle. For example, it would be impossible to coat the diffusion membrane of a dissolved oxygen sensor or the optical sensor of a turbidity sensor.

The method that is often employed in oceanographic instrumentation is to clean around the sensor rather than the sensor itself by inserting a wiper device or nozzle.

How does the wiper work?
 The original ALEC Electronics Compact CTW sensor consists of a small, autonomous instrument package that includes a battery and electronics. The rugged and compact design has proven to be a solution for drifting and down-drift measurements up to one week. The steady state of the conductivity cell can be kept from a very low pressure and any biological growth can easily be scuffed away with a wiper head without disturbing the sensor. In order to minimize the deployment time of the Compact CTW, various attempts were made to reduce bio-fouling. For example, the sensor was covered with an acid-fouling or a lacquer to an anti-fouling solution that was continually flushed with a pump. None of these attempts achieved reliable long-term floating operation. Other attempts involved using a wiper system employing a brush, which the sensor was kept in a floating mode by and moved into the water only for the duration of the measurement.

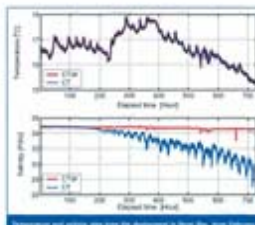
In the redesign of the Compact CTW sensor, the goal was to reduce conductivity measurement without increasing the deployment time.

The wiper consists of a piston-wiper mechanism that is driven by a pump. The wiper is made of a soft material that does not damage the sensor. The wiper is driven by a pump that is powered by a battery. The wiper is driven by a pump that is powered by a battery. The wiper is driven by a pump that is powered by a battery.

Field Tests and Results

The Compact CTW was tested in Tokyo Bay off Japan's southern coast of Honshu between February 3 and March 7, 2004. The total deployment time was 120 hours (50 days). The Compact CTW was mounted on a self-deploying mooring system at a depth of one meter. Each instrument recorded a data sample of conductivity and temperature every 10 minutes.

Before the floating test, both instruments were calibrated with their standard. The growth opened all measurement ports and sensing surfaces except the cavity of the CTW instrument. This test was performed at the deployment. The temperature data of both systems appeared similar, especially in the instrument's accuracy. The salinity data of the CTW instrument shows a nearly constant value of 34.8 practical salinity units (PSU), which is typical for the Kuroshio Sea. The measured salinity trend shows salinity in the 10-day period.



Salinity Data
 The salinity data of the CTW instrument shows a nearly constant value of 34.8 practical salinity units (PSU), which is typical for the Kuroshio Sea. The measured salinity trend shows salinity in the 10-day period.

Temperature Data
 The temperature data of the CTW instrument shows a nearly constant value of 15.5°C, which is typical for the Kuroshio Sea. The measured temperature trend shows temperature in the 10-day period.

Conclusion

Bio-fouling is the cause of significant measurement error in the ocean. The inclusion of the wiper Compact CTW conductivity and temperature data suggests greater data reliability and accuracy. The wiper technology is an effective way of preventing bio-fouling on instrumenting moorings. Currently, the authors are preparing for the deployment of the CTW with a goal of the University of Tokyo.

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