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Release of *in-situ* fluorometer for Cyanobacteria "*Cyanosens*"

~ Continuous real-time monitoring of Cyanobacteria bloom in flesh water ~

In-situ fluorometer for Cyanobacteria, "*Cyanosens*"

Continuous real-time monitoring of Cyanobacteria biomass.

The bloom of cyanobacteria in flesh water is now regarded a serious problem in Japan. In eutrophic rivers and lakes around the world, this frequency of the blooms is increasing year by year. For water quality management, it is essential to monitor cyanobacteria biomass. The identification and counting of cyanobacteria is traditionally done using microscopy. Using this technique, however, yields only a limited number of samples, because investigators need special skills and the cost of analyzing each sample are high. Therefore, it is difficult to measure the variability of cyanobacteria biomass with high temporal and spatial resolution. In order to monitor their biomass in real-time and continuously, Alec Electronics has developed an *in-situ* fluorometer for cyanobacteria, *Cyanosens*. *Cyanosens* applies Alec Electronics' technological capabilities and their understanding of the optical properties of cyanobacteria to provide and effective tool for the monitoring of cyanobacteria in fresh water.

Cyanobacteria bloom in flesh water.

Cyanobacteria species in fresh water

Phormidium sp. *Anabaena* sp.

We are incubating some species for sensor developments and validations.

1. Introduction

Cyanobacteria appeared around 3.5 billion years ago providing oxygen for Earth's atmosphere. Cyanobacteria are found in almost every conceivable habitat from oceans to fresh water. As well as algae and plant, cyanobacteria take in inorganic carbon and produce organic carbon compounds by photosynthesis. The photosynthesis supports the aquatic food web and the bio-geochemical cycle. Cyanobacteria often bloom in eutrophic fresh water and form blue-green mats on the water surface. The phenomena are called "*aoko*" in Japan. This is one of serious problems in health and water quality around the world. The cyanobacteria blooms in fresh water are almost always formed by *Microcystis* sp. and/or *Anabaena* sp. with a high colony density. In drinking water reservoirs, these blooms cause damage to water purification systems. Moreover, some of the species produce biotoxins, namely cyanotoxin, including microcystin and anatoxin that affect as neurotoxin and hepatotoxin. There have been reports about health problems for humans and animals by cyanotoxin. Moreover, *Phormidium* sp. produce odor compounds (e.g. geosmin etc.). Therefore, for water quality management, it is essential to monitor cyanobacteria biomass in fresh water. Historically, identification and counting using microscopy are the most standard technique for the measurement of cyanobacteria biomass. In order to predict the species-dependent production of biotoxins or odor compounds, microscopy technique is very useful because it can identify species. However, since special skills and professional morphologic knowledge are required to identify them, operators spend much time identifying and counting cyanobacteria, and it is difficult to monitor cyanobacteria biomass over the long term with high resolution. Many investigators, therefore, require an automatic *in-situ* instrument for measuring cyanobacteria biomass. By applying our accumulated technologies for *in-situ* chlorophyll and multi-excitation fluorometer, Alec Electronics has developed the *in-situ* fluorometer for cyanobacteria *Cyanosens*.

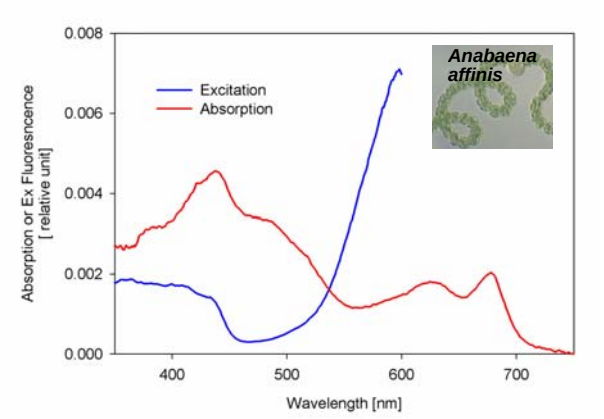

Fig.1 Optical properties of *Anabaena affinis*. *A. affinis* is a major cyanobacteria species in fresh water. Red and blue coloured lines show absorption and excitation spectrum, respectively.

2. Optical properties of cyanobacteria

According to their photosynthetic pigment composition, cyanobacteria have some unique optical properties. Cyanobacteria contain chlorophyll-a (Chla) and phycobiliprotein as photosynthetic pigments. Phycobiliproteins are soluble photosynthetic pigments including phycocyanin (PC), phycoerythlin (PE) and allophycocyanin (APC). In particular, the optical properties and cellular colour mainly depend on the ratio of PC to PE. Figure 1 shows the light absorption and the fluorescent excitation spectra of *Anabaena affinis*. The absorption spectrum is the fraction of incident light absorbed by photosynthetic pigment. On the other hand, the excitation spectrum is the emitted fluorescent intensity as a function of incident light. Figure 1 demonstrates that *(i)* in the blue light region

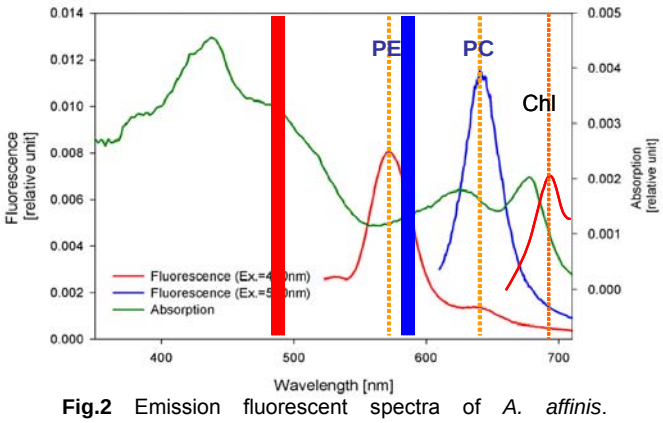

Fig.2 Emission fluorescent spectra of *A. affinis*. Abbreviations, PE, PC and Chl, show phycoerythlin, phycocyanin and Chla fluorescence, respectively.

around 440 nm, cyanobacteria effectively absorb incident light but the emitted fluorescent intensity is lower, *(ii)* on the other hand, although the light absorption efficiency is lower at the green light region more than 550 nm, the fluorescent intensity is the highest in this region. In general, phytoplankton emit fluorescence efficiently in the blue light region. Thus, there is a significant difference of fluorescent excitation property between cyanobacteria and another phytoplankton classes. Figure 2 shows the fluorescent emission property. All phytoplankton emit red fluorescence with the peak around 680 nm, namely Chl fluorescence. Moreover, due to phycobiliprotein including PC and PE, cyanobacteria emit also orange and red fluorescence with the peak around 580 nm and 650 nm. Thus, cyanobacteria have a fluorescent property containing multiple emission bands. These unique optical properties of cyanobacteria are very useful to identify them from phytoplankton community and measure their biomass. *Cyanosens* has been developed based on these optical properties.

3. Features and benefit of *Cyanosens*

Cyanosens is a very compact package with low power consumption (Figure 3). *Cyanosens* can measure three parameters simultaneously: *(i)* cyanobacteria biomass estimated by PC fluorescence, *(ii)* Chla concentration of phytoplankton community estimated by Chl fluorescence, *(iii)* turbidity by aquatic particles measured by backscattering (patent pending). Therefore, *Cyanosens* can provide the information of cyanobacteria biomass, community change and water quality. Moreover, *Cyanosens* has an anti-biofouling wiper to minimize fouling on the optical window. This wiper is very useful for long term monitoring. Thus, *Cyanosens* can be deployed for long periods and be used for early detection of cyanobacteria blooms. *Cyanosens* can also be used for measurements of cyanobacteria community structure and water quality.

Fig.3 *In-situ* fluorometer for cyanobacteria, *Cyanosens*.

4. Sensor performance

Figure 4 (a) shows a relationship of a cyanobacteria culture, *Anabaena affinis*, between the cell density [cells/ml] and the fluorescent intensity. Good linearity was obtained in each fluorescent channel of PC and Chla (r^2=0.99). However, each slope was a quite different between PC and Chl channel. The slope of PC channel was three times as steep as that of Chl. Figure 4 (b) shows a relationship of a diatom culture, *Skeletonema costatum*, between the Chla concentration and the fluorescent intensity. The Chla concentration was

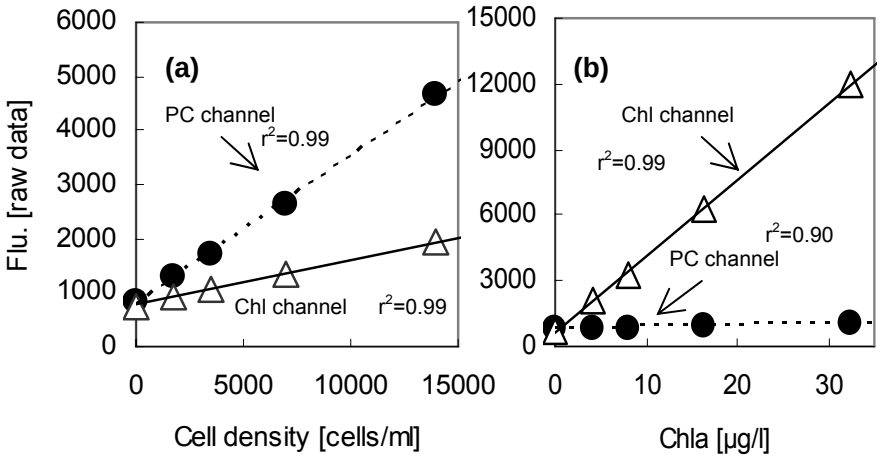

Fig.4 Relationships between (a) PC fluorescent intensity and cells density of cyanobacteria, *A. affinis*., (b) Chla fluorescent intensity and Chla concentration of diatom, *S. costatum*. These tests were done using culture collection.

measured by fluorometric analysis with pigment extraction. When the excitation light for Chla fluorescence exposed to diatom, the intensity of Chl fluorescence increased with a good linearity, depending on the Chla concentration. On the other hand, when the diatom was excited by the excitation light for PC fluorescence, diatom cannot emit any fluorescence. These results indicated PC and Chla channel can measure PC fluorescence from only cyanobacteria and Chl fluorescence from total phytoplankton community, respectively. Thus, *Cyanosens* can simultaneously estimate both biomass of cyanobacteria and phytoplankton community.

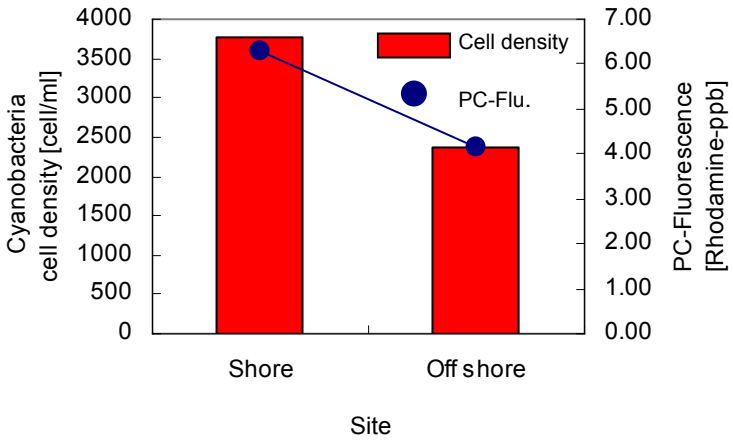

Fig.5 A comparison between cells density of cyanobacteria and PC fluorescent intensity in Lake Biwa, Japan.

Figure 5 shows a comparison between the PC fluorescent intensity (red coloured bars) and the cell density of cyanobacteria (blue coloured dots) measured by *Cyanosens* and microscopy in Lake Biwa, Japan. From microscopy measurement, one of cyanobacteria species, *Microcystis* sp. dominated more than 50% of the phytoplankton community at both sites of shore and off-shore. The variation ratio of PC fluorescence (=1.59) almost corresponded with cells density's one (=1.52). By this field test, it was validated that the PC fluorescent intensity measured by *Cyanosens* was a good index of cyanobacteria's cells density. This demonstrates that *Cyanosens* is capable of monitoring cyanobacteria *in-situ*.

5. Conclusion

Cyanosens is simultaneously able to measure PC, Chl fluorescence and turbidity in real time. So it can extract the spatial and the temporal variability of cyanobacteria biomass, Chla concentration of phytoplankton community, and turbidity with high resolution. In this technical express, we have focused the information of *Cyanosens* on the monitoring for cyanobacteria in fresh water. In the ocean, cyanobacteria is also very important in the bio-geochemical cycle, since marine cyanobacteria with the ability of nitrogen fixation can also grow vigorously in oligotrophic water (e.g. *Trichodesmium* sp.) and fixed nitrogen is likely main input to marine nitrogen cycle. We hope that *Cyanosens* will contribute not only to the monitoring for water quality managements but also to ecological and biogeochemical studies in fresh and marine water.

Notice: The release of *Cyanosens* (data logger and direct-reading cabled type) scheduled soon.

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