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A simple integrating-sphere fluorometer for monitoring the growth of benthic microalgae*

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Abstract : A new type of fluorometer was developed to monitor biomass of cultured benthic microalgae which adhere firmly to culture vessel. The sample chamber of the fluorometer consists of an integrating sphere which diffuses both excitation and *in vivo* fluorescence, and thus the homogenization adhering cells is not required for the fluorescence monitoring. Significant correlation was found between the relative *in vivo* fluorescence intensity and the chlorophyll *a* concentration for a benthic diatom, *Navicula* sp. (r=0.987) and dinoflagellates, *Prorocentrum lima* (r=0.974) and *Prorocentrum* sp. (r=0.966). This fluorometer is reliable for biomass monitoring of benthic microalgae.

1. Introduction

The ratio of chlorophyll *a* fluorescence intensity of intact plant cells (referred to as *in vivo* fluorescence) to the unit amount of chlorophyll *a* varies among algal species and with the physiological state of the cells (ISHIMARU *et al.*, 1985). However, this ratio is constant during the logarithmic growth phase of a clonal culture, because the physiological state of the alga is constant. Thus, the direct measurement of *in vivo* fluorescence can be used to provied a rapid estimate of phytoplankton biomass in culture tubes (BRAND *et al.*,1981; WATRAS *et al.*, 1982). *In vivo* fluorescence also has been used to estimate biomass of benthic microalgae which can be suspended homogeneously by agitation (BOMBER *et al.*, 1988). However, agitation is thought to retard the growth of some species (DURAND , 1987). Additionally it can be difficult to dislodge cells of some taxa, especially adhering diatoms, from the wall of culture tubes.

We designed an integrating-sphere fluorometer (abbreviated as ISF hereinafter) that can be used to estimate the growth of benthic microalgae without agitating the culture. A culture tube is placed in the integrating sphere that diffuses both excitation light and *in vivo* fluorescence.

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2. Materials and Methods

2–1. Instrument

Figure 1 shows the diagram of the instrument. A high pressure mercury lamp (SL-HG-1; Toshiba Co.) was used with a starter (SLS-5; Irie Manufacturing Co.) as a source of excitation light and placed horizontally. A motor cooling fan (MB6Z-B; Oriental Motor Co.) was attached to the end of the lamp cover in order to dissipate heat of the lamp. The light from the mercury lamp was passed through a color glass filter (V44; Toshiba Co.) which allowed the transmission of 436 nm light for chlorophyll *a* excitation. The excitation light passing through the filter was introduced two the integrating sphere via an acrylic light guide (opening 21 × 10mm, height 15mm). The integrating sphere was assembled from two hemispheres made from acrylic resin (inner diameter 150mm). The inside of the sphere was painted with non-reflective white (Acrylic Spray; Nippon Paint Co.) in order to diffuse both excitation light and *in vivo* fluorescence. A window for the excitation light was situated at the bottom of the integrating sphere for effective irradiation, while a hole for the insertion of the culture tube was made at the top. The bottom of the culture tube where microalgae adhered abundantly was positioned near the center of the sphere. A photomultiplier (R-636; Hamamatsu Photonics

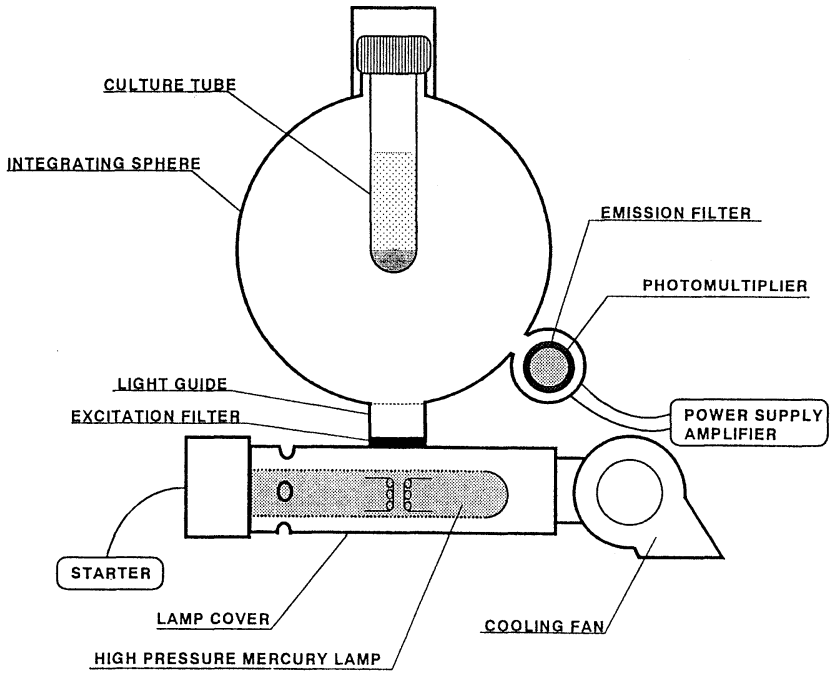

Fig. 1. Schematic diagram of the integrating-sphere fluorometer (ISF).

Co.) was used to detect *in vivo* fluorescence. This detector was wrapped with an acetate filter (SC66; Fuji Photo Film Co.) to eliminate light with wavelenghs shorter than chlorophyll *a* fluorescence. The position of the photomultiplier was adjusted to receive minimum effect of excitation light. The signal from the photomultiplier was measured using an amplifier which also contains a high voltage source for the photomultiplier (AT-100AM, Shimadzu Manufacturing Co.). Every junction was sealed with black PVC tape (Scotch Brand Tape; 3M) or black silicone sealant (Bath cork; Cemedine Co.) to prevent light leakage. The exterior was painted with non-reflective black.

2-2. Performance test

Various species of benthic microalgae, including *Navicula* sp. (a diatom), *Prorocentrum lima* and *Prorocentrum* sp. (dinoflagellates) were used to determine relationships between relative *in vivo* fluorescence intensity obtained by ISF and biomass. Patterns of their adhesion differed among the three species: *Navicula* sp. adhered uniformly around bottom of the culture tube, *Prorocentrum lima* formed numerous lumps consisting of dozens of cells that adhered around the bottom of the glass tube, while *Prorocentrum* sp. growth pattern was intermediate to those of the other two species. Each microalga was inoculated into culture tubes (25 mm ϕ,150mm L, screw capped; Pyrex) containing 25 ml of modified T1 medium (OGATA *et al.*, 1987; without nitrilotriacetic acid, but with ammonium chloride and selenous acid). These cultures were maintained for several days at 26.5 $\pm$0.5℃ under an illumination of ca. 1.0×10^{16} quanta • $cm^{-2}sec^{-1}$with a 14:10 hr light-dark cycle using cool-white fluorescent lamps (FL40SS; Toshiba Co.). The culture tubes were repositioned randomly once a day to minimize the effects of environmental (mostly light) variation.

Qauantity of chlorophyll *a* was chosen as an index of the biomass because increase of chlorophyll *a* corresponds directly to increase of its biomass during the logarithmic growth phase (HANSMANN , 1977).

At each measurement, the signal reading of ISF was set initially to zero by inserting a test

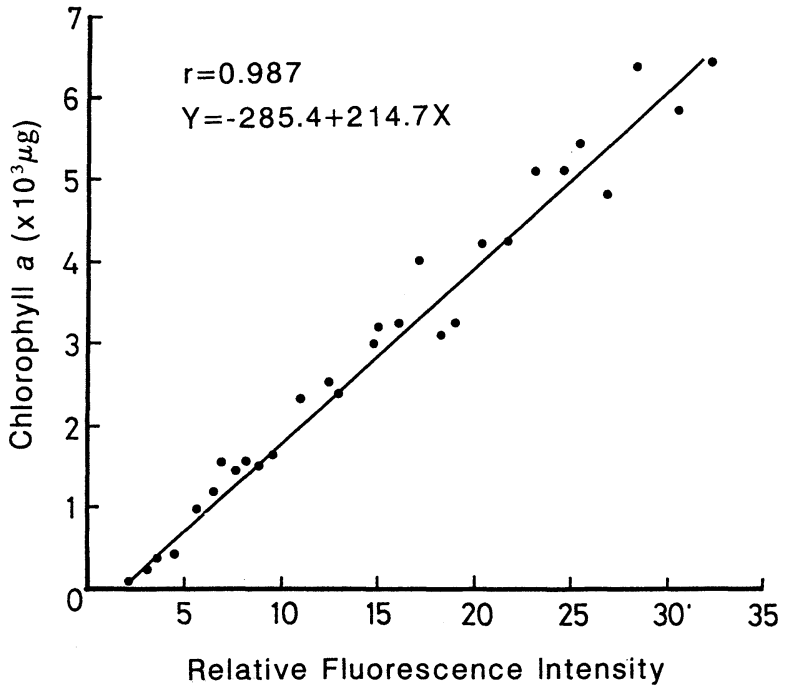

Fig. 2. Relationship between chlorophyll *a* concentrations and relative *in vivo* fluorescence intensity for *Navicula* sp.

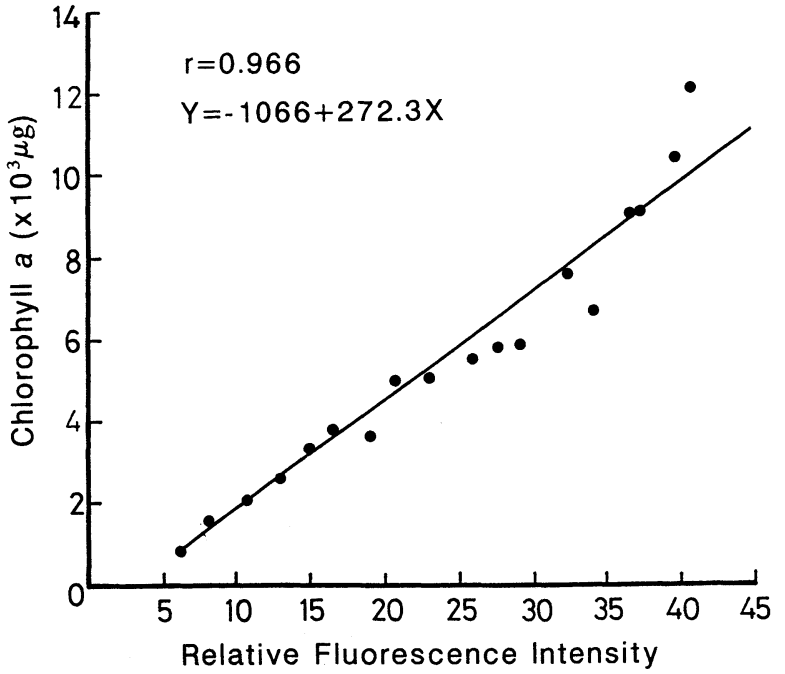

Fig. 3. Relationship between chlorophyll *a* concentrations and relative *in vivo* fluorescence intensity for *Prorocentrum lima*.

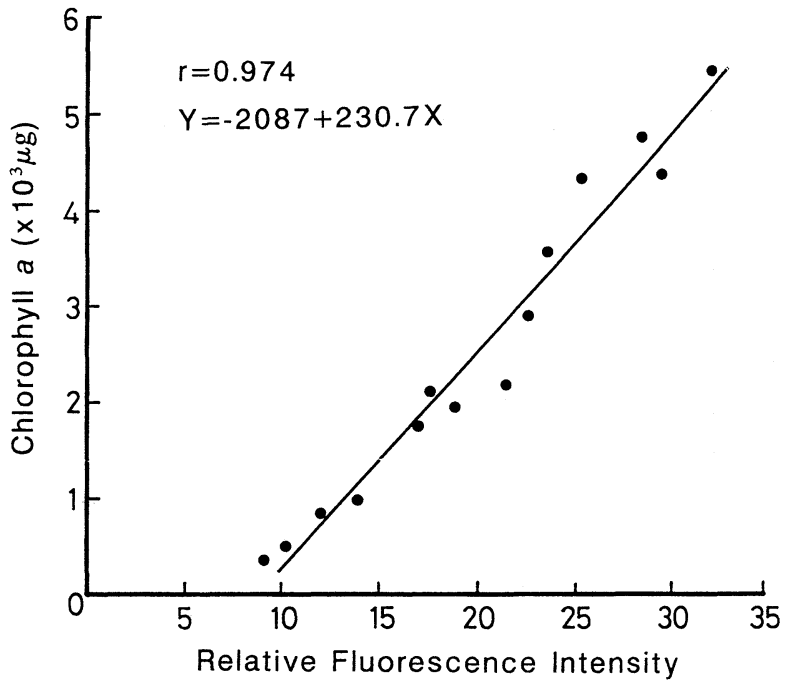

Fig. 4. Relationship between chlorophyll *a* concentrations and relative *in vivo* fluorescence intensity for *Prorocentrum* sp.

tube (same as the culture tube) containing filtered (0.22 μm) and sterilized seawater. Signal reading standardization was also done by inserting a standard culture tube containing fluorescent acrylic resin.

Culture tubes were selected arbitrarily and measured for relative *in vivo* fluorescence intensity by ISF. Culture medium in each tube was then filtered with glass fiber filter (GF/C; Whatman) to collect cells in suspension. The filter was put back into the tube, and 10 ml of dimethylformamide was added to the tube to extract chlorophyll *a* (SUZUKI and ISHIMARU, 1990) from cells both on the filter and attached to the tube wall. Concentrations of chlorophyll *a* were then determined by the fluorescence method (STRICKLAND and PARSONS, 1972) using a fluorometer (Type 10R; Turner Designs Co.).

Relationships of liner plots between relative fluorescence intensity obtained by ISF and its chlorophyll *a* concentration were determined using the least squares method.

Triplicate cultures of each species were maintained in condition described above and measured for *in vivo* fluorescence by ISF every two days to determine the growth.

3. Results and Discussion

Linear plots of the relative fluorescence intensity obtained from the ISF and the chlorophyll *a* concetration are shown in Fig. 2, 3 and 4. Typical growths of the three benthic microalgae monitored by the ISF were also shown in Figs. 5-a, b and c.

Coefficients of correlation between the relative fluorescence intensity and the chlorophyll *a* concentrations were 0.987, 0.974 and 0.966 for *Navicula* sp., *Prorocentrum lima* and *Prorocentrum* sp., respectively. Furthermore, the correlations were high between the relative fluorescence value of 2 and 33 for *Navicula* sp., 10 and 32 for *Prorocentrum lima*, 6 and 38 for *Prorocentrum* sp. At the time of growth rate measurement, each intercept corresponded with the logarithmic growth phase of each species (Figs. 5-a, b and c), thus the increase of the rate of the relative fluorescence intensity was a valid measure of the rate of chlorophyll *a* increase, which, in turn, corresponded to biomass increase for determination of growth rate.

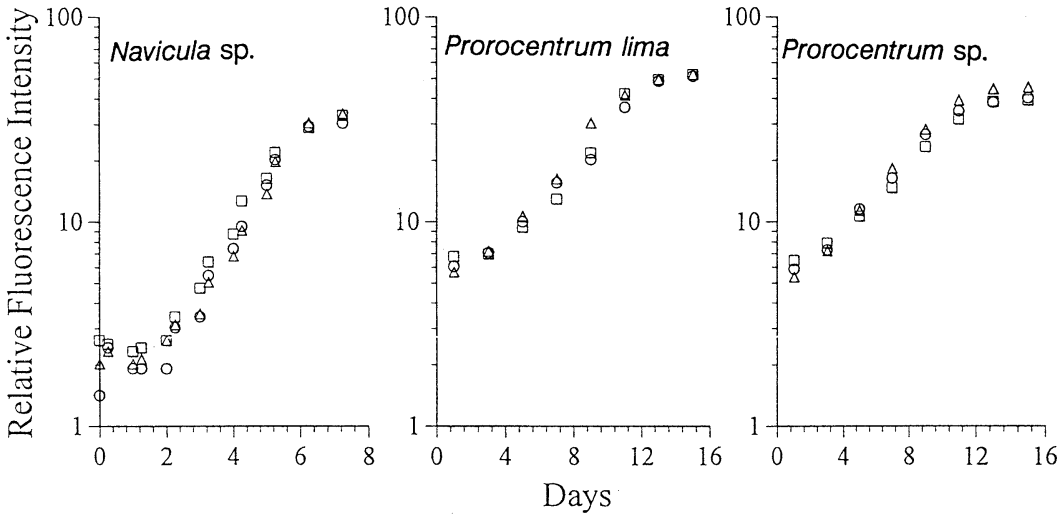

Fig. 5. Typical growths of *Navicula* sp. *Prorocentrum lima* and *Prorocentrum* sp. in

All of the tests exmained showed reliable performance of the ISF when determining the growth rate of benthic microalga. In particular, the strong relationship between the concentration of chlorophyll *a* and the relative fluorescence value of the ISF was evident for a wide range in biomass for *Navicula* sp. This resulted because the benthic diatoms had a tendency to adhere uniformly to the culture vessel, not making lumps of cells which could be a cause of self-absorption of *in vivo* fluorescence.

Meanwhile, correlation values between the relative fluorescence intensity and the concentration of chlorophyll *a* for other cultures showed that differences in microalgal adhesion patterns might yield less accurate estimates of biomass because the diffusion of both excitation light and *in vivo* fluorescence inside the integrating sphere was less homogeneous. Furthermore, some of the fluorescent light coming directly from the culture tube and received by the photomultiplier may yield excessive values. A method should be devised to eliminate the effects of direct light but enabling the photomultiplier to receive diffused light without any interruption.

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付着性微細藻の生長測定のための積分球蛍光々度計

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要旨：培養器の壁面に強く付着する微細藻類の生長を *in vivo* 蛍光のモニターによって測定するために，新型の蛍光々度計（Integrating-Sphere Flurometer, ISF）を開発した。この蛍光々度計のサンプル室は積分球からなり，励起光及び *in vivo* 蛍光をサンプル室内で積分する。このため，培養容器を強く振とうすることなく付着性微細藻類の生長をモニターすることが可能となった。性能を試験するために付着珪藻の *Navicula* sp.，付着性渦鞭毛藻の *Prorocentrum lima* と *Prorocentrum* sp. を培養し，相対 *in vivo* 蛍光値とバイオマスとしてのクロロフィル*a* 量との相関を求めた。その結果，何れの種についても，相対 *in vivo* 蛍光値とクロロフィル *a* 量との間には高い相関関係（*Navicula* sp. では r＝0.987, *Prorocentrum lima* では r＝0.974, *Prorocentrum* sp. では r＝0.966）が認められ，本蛍光々度計が培養容器中の付着性微細藻のバイオマスを正しくモニターできることを確認した。