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Automatic separation system for marine meiobenthos based on laser-induced fluorescence technology*

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Abstract: An automatic system for marine meiobenthos separation was developed by using laser-induced fluorescence technology. Rose Bengal was used as organism dye and the spectrums of Rose Bengal were measured. Laser-induced fluorescence system was established to detect marine meiobenthos in sediments. Data obtained from experiments were analyzed by using a mathematical model. The results showed that laser-induced fluorescence technology worked well in the system. The system could select the meiobenthos efficiently and precisely.

Key words: Meiobenthos, Automatic separating system, Optical sensor, Laser-induced fluorescence (LIF), Rose Bengal
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INTRODUCTION

Meiobenthos is defined as marine meiofauna that can pass a screen with 250 μm apertures but cannot pass a screen with 32 μm apertures. It occupies about 80% of the total marine biomass and is of great importance in the marine ecology and the marine mineralogy (Mclntyre, 1969). So that, meiobenthos investigation is a very active research field (Kitazato *et al*., 2003; Gwyther, 2004).

Separating meiobenthos accurately from sediments is crucial in quantificative investigation of meiobenthos, and will influence the reliability and precision of research results. Various separation methods have been developed ever since meiobenthos research began. However most reported methods for separating meiobenthos from sediments are primarily based on the difference of specific mass between meiobenthos and sediment grains, which limit their application (Dillon, 1964; Heip *et al*., 1974; De Jonge and Bouwman, 1977; Nichols, 1979; Schwinghamer, 1981; Jensen, 1982). There is still no formal report on meiobenthos separation system development. At present, picking up meiobenthos manually under microscope is still ongoing in most Chinese and the foreign labs despite of the manual method's large consumption of human power and time.

In order to promote the efficiency and free scientists from the boring manual work, we developed an automatic separation system by applying laser-induced fluorescence (LIF) technology (Li *et al*., 1996), which disregards the properties of the sediments. The system involves the technologies of biology, optics, electronics and mechanics. In this system, LIF is used to identify the organism, photoelectric transducers were used to detect the fluorescence signal, and an electronically controlled robot hand is used to collect the samples (The system is shown in Fig.1).

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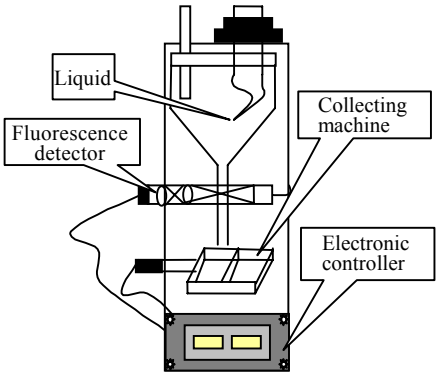

Fig.1 Frame of the separating system for meiobenthos

The LIF detecting technology is the main part of the automatic separation system. In this work, the fluorescence detecting system was established by measuring the spectra of the organism dye, Rose Bengal, to help choose the parameters of the optical parts. A mathematical model of the system signals was set up to analyze the experimental data.

LIF SYSTEM SETTING

Organism dye

We chose Rose Bengal as the organism dye for its good chromaticity and high fluorescence quantum yield (Larkin *et al*., 1999). In order to choose the photoelectric component with appropriate parameters, the fluorescence emission spectrum and absorption spectrum of Rose Bengal dissolved in basic ethanol (96% ethanol:water:2 mol/L NaOH=500:475:257) were measured. There were an obvious absorption peak at 550 nm and an emission peak at 580 nm in the spectra (Fig.2).

System setup

The key problem of the automatic separation system is to determine whether there is organism in the flume based on the weak optical output of the photomultiplier tube (PMT). The laser beam is first filtered, and then continuously focussed on the center of the transparent capillary by the lens. When there is dyed meiobenthos in the mixture liquid flowing through the laser beam, it will be induced to fluorescence with far more intense luminance than that of the background optical noise. When the dispersion laser is blocked by the filter, the PMT detects the fluorescence, and then proportionally generates electronic signals that will be sent to the electronic detector and controller. The compressed meiobenthos cause the fluorescence to project in one direction. Two detecting settings were arranged in different directions to catch more fluorescence signals (The optical layout is shown in Fig.3, Parameters of the optical parts of the setting are shown in Table 1).

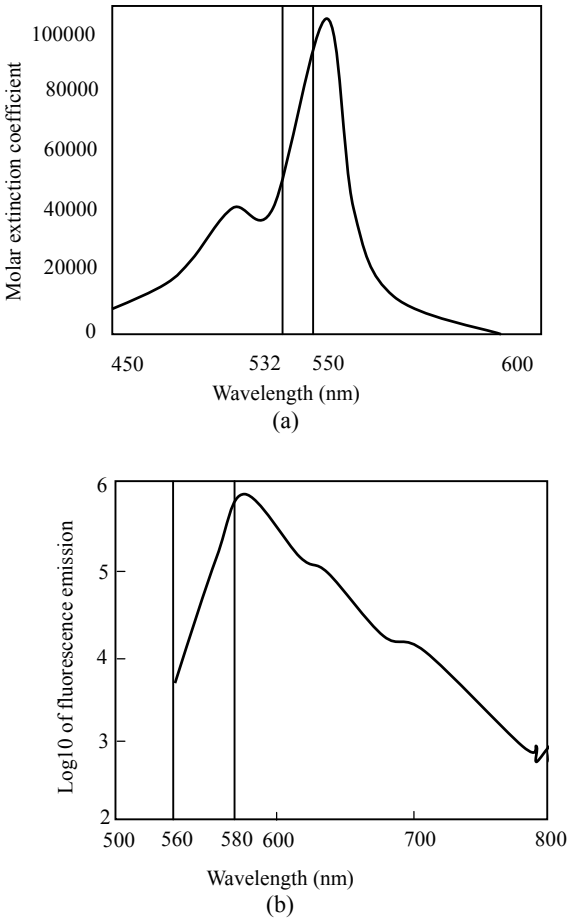

Fig.2 The fluorescence spectra of Rose Bengal (a) Absorption spectrum of Rose Bengal; (b) Emission spectrum of Rose Bengal

MODELING ANALYSIS

The model of the LIF detecting system for the meiobenthos was set up, and the electronic signals of the PMT were analyzed.

The intensity of the fluorescence I is defined by

$$I=a\Phi_0 I_0(1-e^{-\varepsilon lc}) \quad (1)$$

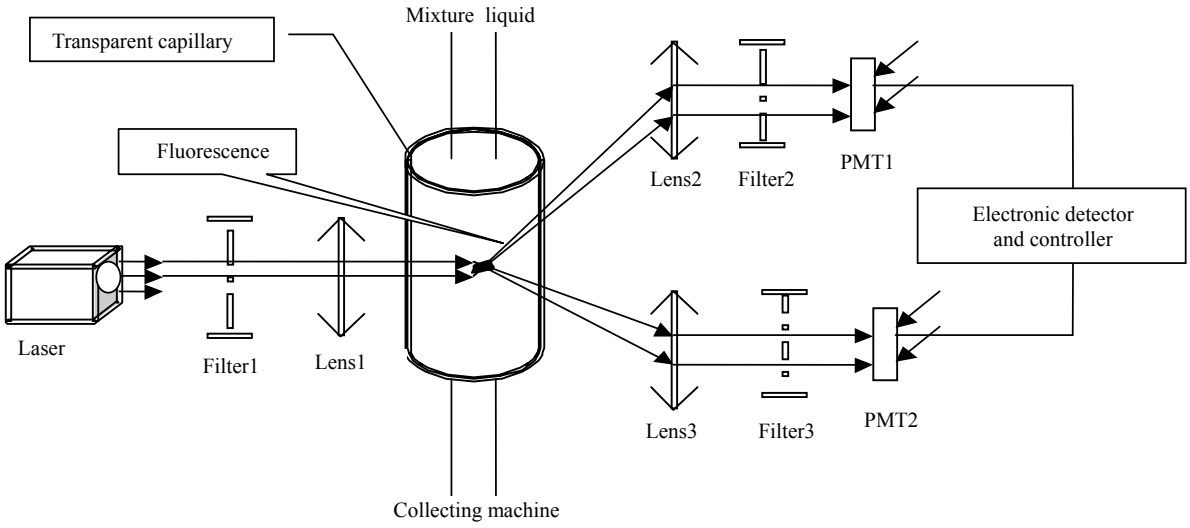

Fig.3 Optical layout of the system

Table 1 Parameters of the optical parts of the setting

Parts	Parameters
Laser	Wavelength λ_0=532 nm; output power W_0=5 mW; output aperture Φ=8 mm.
Filter1	Narrow band-pass filters; center wavelength λ_0=532 nm; bandwidth $\Delta\lambda$=10 nm; transmittance≥50%.
Filter2−3	High-pass filters: λ>560 nm, transmittance≥80%; λ≤560 nm, blocking≈10^{-6}.
PMT1−2	Luminous sensitivity (with 2856K light source) v=2×10^8 V/lm; spectrum response at wavelength of 185 nm~900 nm and the maximum response at 600 nm; dark current of 1 nA; optical window s=7.0×16.0 mm^2; linearity of ±0.5%.

where a is the constant of the instrument, Φ_0 is the fluorescence quantum yield, I_0 is the intensity of the laser, ε is the molar extinction coefficient, l is the light length, and c is the concentration. Because the concentration of the sample was very low, $1-e^{-\varepsilon lc}\approx\varepsilon lc$, and the Eq.(1) could be revised to

$$I=a\varepsilon lc\Phi_0 I_0 \tag{2}$$

With the intensity of the fluorescence I, the luminous flux F is defined by $F=\int_0^{\Omega} I\mathrm{d}\Omega$, where Ω is the solid angle. The fluorescence can be divided into many dot light sources. Considering the position of the PMT and the area of its optical window, the luminous flux F of the fluorescence received by the PMT is given by

$$F=a\varepsilon lc\Phi_0 I_0\int_0^{s}\frac{f_i(r_i,\Delta s_d,\theta)}{r_i^2}\mathrm{d}s \tag{3}$$

where s is the cross section of the meiobenthos. The position of the dot fluorescence is charactered by $f_i(r_i,\Delta s_d,\theta)$, where r_i is the distance between dot fluorescence source and the PMT, Δs_d is the optical area of the PMT, and θ is the angle between the fluorescence and the optical window of the PMT. $\int_0^{s}\frac{f_i(r_i,\Delta s_d,\theta)}{r_i^2}\mathrm{d}s$ is the intensity of all dot fluorescence. Therefore, at luminous sensitivity v of the PMT, the output voltage of the PMT is given by

$$V=Fv=a\varepsilon lc\Phi_0 I_0 v\int_0^{s}\frac{f_i(r_i,\Delta s_d,\theta)}{r_i^2}\mathrm{d}s \tag{4}$$

From the analysis above, we can conclude that the output voltage is mainly influenced by the parameters of $f_i(r_i,\Delta s_d,\theta)$ and the size of the meiobenthos.

RESULTS AND DISCUSSION

The electronic output of the PMT must interface well with the amplifier and be able to drive the collecting machine. The maximal velocity of the flow is 1 m/s, the gain of PMT is adjusted to 4×10^6, the sampling frequency of the A/D transducer is 1024 Hz, data were collected every 2 s. A typical example is shown in Fig.4a. Four peaks in Fig.4a are shown in Fig.4b with expanded scales. The results are discussed in below.

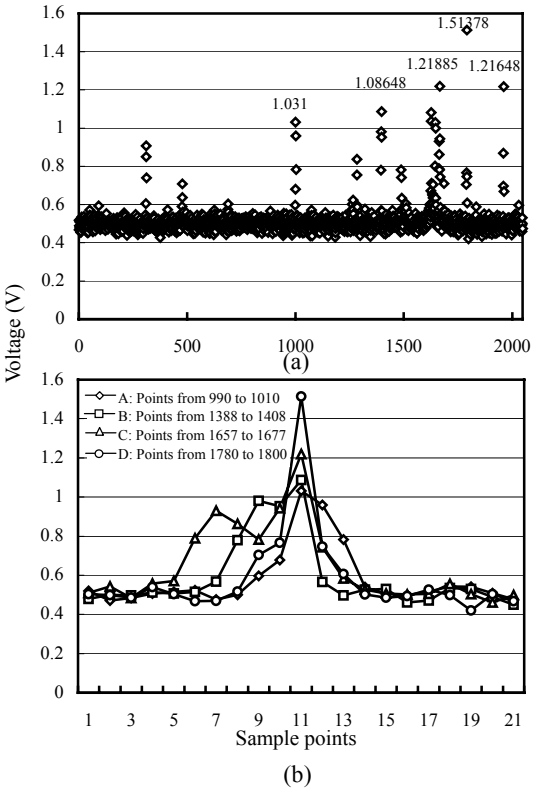

Fig.4 Graphs of the fluorescence data (a) A typical example; (b) Four peaks in (a) with expanded scales

1. As shown in Fig.4a, there are obvious peaks in the background of the steady noise signals. There is a very high voltage output of 1.5137 V, which almost reaches the A/D sampling output limit of 1.6 V. The peaks indicate high fluorescence intensity, which in turn indicate the appearance of dyed organism.

2. The maximum values of the peaks are different. The two results from the modelling analysis can explain this phenomenon. One of the reasons is the different size of the meiobenthos; the other is the angle θ between the fluorescence of the compressed meiobenthos and the optical window of the PMT. That is to say, the fluorescence intensity is not proportional to the size of the organism.

3. Data around every peak first increases and then decreases, which as shown in Fig.4b. The variation indicates that the organism flows with the water through the cross section of the laser beam and the change of the angle θ between the fluorescence of the compressed meiobenthos and the optical window of the PMT.

4. According to Fig.4b, every peak wave occupies about 6 points, indicating that every organism passes through the optical window of the PMT in a similar duration. Therefore, it can be concluded that the appearance of every meiobenthos corresponds to a 6-point wave.

5. The waves are not always so regular and clear as shown in Fig.4b (curves B and C). Actually, there are some strange conjoined waves. After the analysis, we conclude that if the length of the conjoined waves is less than 6 points, there is only one organism whose random motions in the flow cause the aberration of the wave as shown in Fig.4b (curve B), while if the length of two conjoined waves is over 6 points, there must be more than two organisms present proximately as shown in Fig.4b (curve C).

According to the discussions above, experiments are successful. Excellent performance in separating meiobenthos is demonstrated by the obtained precision of above 95%.

CONCLUSION

According to the experiment and the data analysis, the automatic system for separation of marine meiobenthos based on LIF technology can work well. It can select the organisms in the sediments efficiently and precisely. Compared with other conventional detecting methods, LIF technology has the advantages of easy set-up, high sensitivity and resolving ability. Laser induced fluorescence can improve the sensitivity about 2~10 times that of other light sources induced fluorescence. Now, the LIF technology has been developed well, and is widely applied in many fields (Kopp *et al*., 1997; Chuck *et al*., 2002; Michael *et al*., 2004). Because the key part of the automatic separation system is based on LIF detecting technology, a similar technology used in many other biological detecting systems, such as flow cytometry (Lu *et al*., 2004), the success of developing such a set of systems will be of great value in the development of other instruments.

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