MEASUREMENTS OF BIOLUMINESCENCE IN DEEP SEA

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Abstract

We have designed and built a photon counting system which measures low intensities of bioluminescence in deep sea. The system comprises a CCD-TV camera, two-dimensional image intensifier and video cassette recorder. Using this system we measured the vertical profile of bioluminescence *in situ* at the Suruga Trough and Nankai Trough to a depth of 3600 m and analyzed cultivated them.

Key words: Bioluminescence in deep sea, Photon counting system, Cultivated and in situ measurement.

1 Introduction

Bioluminescence *in situ* has been observed and measured both in the epipelagic zone of the ocean and at greater depths using a variety of submarine photometers designed for use with surface ships[1]–[9] and manned submersibles[10]–[13]. Laboratory observations on single types or even on individuals[14]–[20] have identified characteristics such as flash intensity and duration, circadian rhythm, and sensitivity to various stimuli. These numerous investigations, in the laboratory as well as in the ocean[21]–[23], have included studies of the distribution of potentially luminous organisms, direct measurements of the kinetics, intensity and spectral properties of the bioluminescent response, considerations of the biochemistry, behavioral ecology, and regulation of light emission by individual groups of marine organisms.

2 Materials and methods

Sampling site and apparatus:

The sampling sites were located in the Suruga Trough (the point, S-5, Longitude 138° 34.0'; Latitude 34° 28.0' N) about 27 km offshore at a depth of 2750 m and the Nankai Trough (the point, S-6, Longitude 138° 30.0' E; Latitude 34° 07.6' N) about 60 km offshore at a depth of 3600 m. Water samples were collected with 70-liter Niskin bottles

mounted on ten points from a depth of 10 m to 2750 at the S-5 point and twelve points from a depth 10 m to 3600 m at the S-6 point.

The measurement of stimulated bioluminescence was performed on board. The water samples of one liter were flown through a dark box of the photon counting system which comprises a CCD-TV camera, two-dimensional image intensifier and video cassette recorder as shown in Fig.1a. The water samples of one liter were flown for three minutes and was filtered using a membrane filter of 0.45 μm for collecting bacterium. The microorganism and solids stick on the filter surface. The filter provides a source of bioluminescence stimulation and the focus is on the surface of the membrane filter. These images enter directly to an two-dimensional image intensifier, the data of a two-dimensional light intensity distribution are recorded by the CCD-TV camera and the video cassette. The video cassette stores a huge amount of data which can be retrieved as necessary for analysis in laboratory for luminescence intensity, wavelength, and characteristics and shape of the luminescent pulse.. The video cassette is processed by the image processor and the personal computer; in this way the two-dimensional image is converted to an absolute number of photons and images at 1/30 s intervals are also available. One example of a sample image on flowing through the system is shown in Fig.2. The strong flash continued for several seconds as Fig.2 and vanished.

The microorganisms stuck on the membrane filter were put on an culture medium [1] at a short time after recording on a video cassette and cultured; growth at 3°C for several days. The focus was on the surface of the cultured membrane filter and the luminescent image was recorded as same way as *in situ* as shown in Fig.1b. Strong irradiations of cultured membrane filters are visible in a dark room and many colonies (diameter; about 1 mm) of bacterium grew. Some of colonies irradiated as shown in Fig.3.

3 Results and Discussion

The spot of strong irradiation as Fig.2 or 3 was counted with a depth of a Niskin bottle using the video cassette, image processor and personal computer. Fig.4 plots the intensity of spots per one liter corresponding with Fig.2 (flown through). The intensity of spots in Fig.4 decreases with the function expressed by $300 \cdot \exp[-0.03X(m)]$ till the depth of 200 m and fluctuates at deep sea.

Fig.5 plots the luminescent intensity out of the spot in Fig.4. The luminescent intensity decreases with the function expressed by $1.1 \times 10^4 \cdot \exp[-0.03X(m)]$ till the depth of 200 m as same as Fig.4, but the intensity at the place S-5 or S-6 is constant deeper than 200 m.

Fig.6 plots the number of luminescent colonies corresponding with Fig.3. The number of colonies or luminescent colonies are few at the near surface of the S-6 sea and bottom of the S-5 and S-6 sea. The number of colonies were many at near surface of the sea, but the multiplication of luminescent bacterium was suppressed after the results of competition.

The photon counting system which measures low intensities of bioluminescence in deep sea is effective to measure the vertical profile of bioluminescence.

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[1] the component of the caluture medium; sea water filered, 1 ℓ; peptone, 2 g; yeast extract, 0.5g; agar, 15 g;

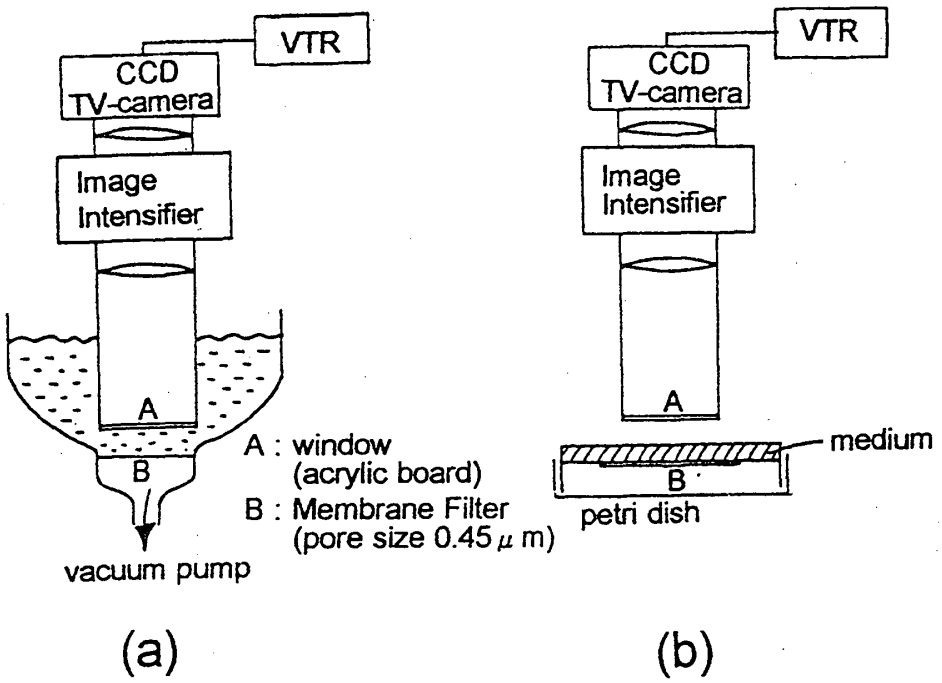

Figure 1: Photon counting system of measuring bioluminescence. (a); with flowing through the sea water, (b); at the culture medium.

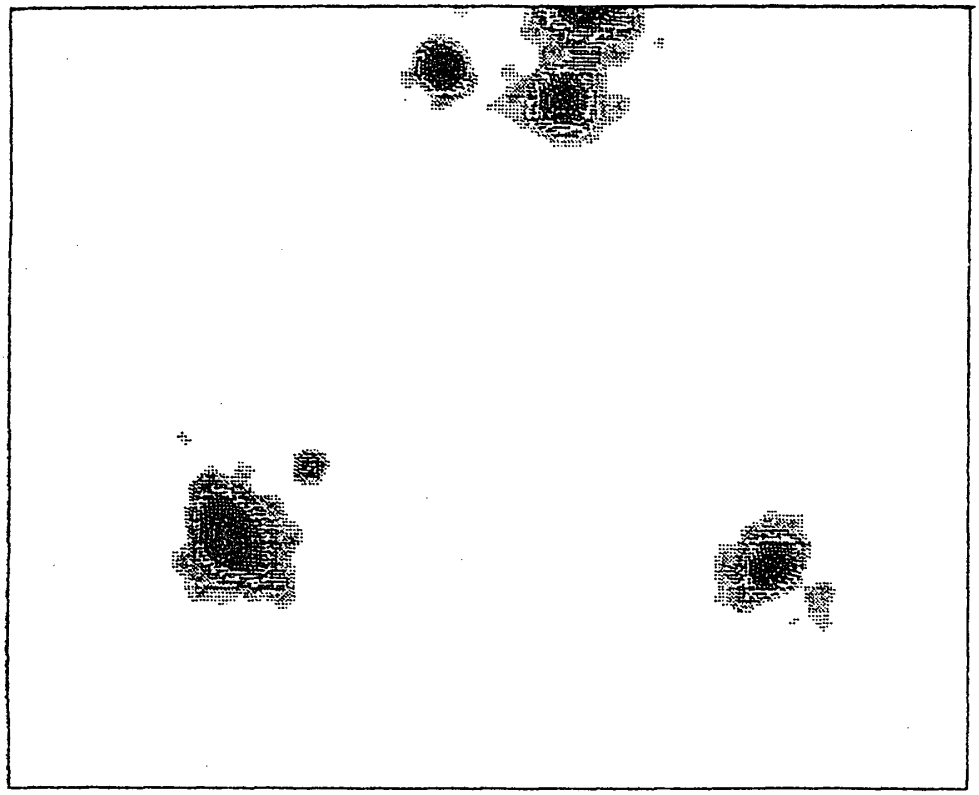

Figure 2: One example of a sample image on flowing through the system.

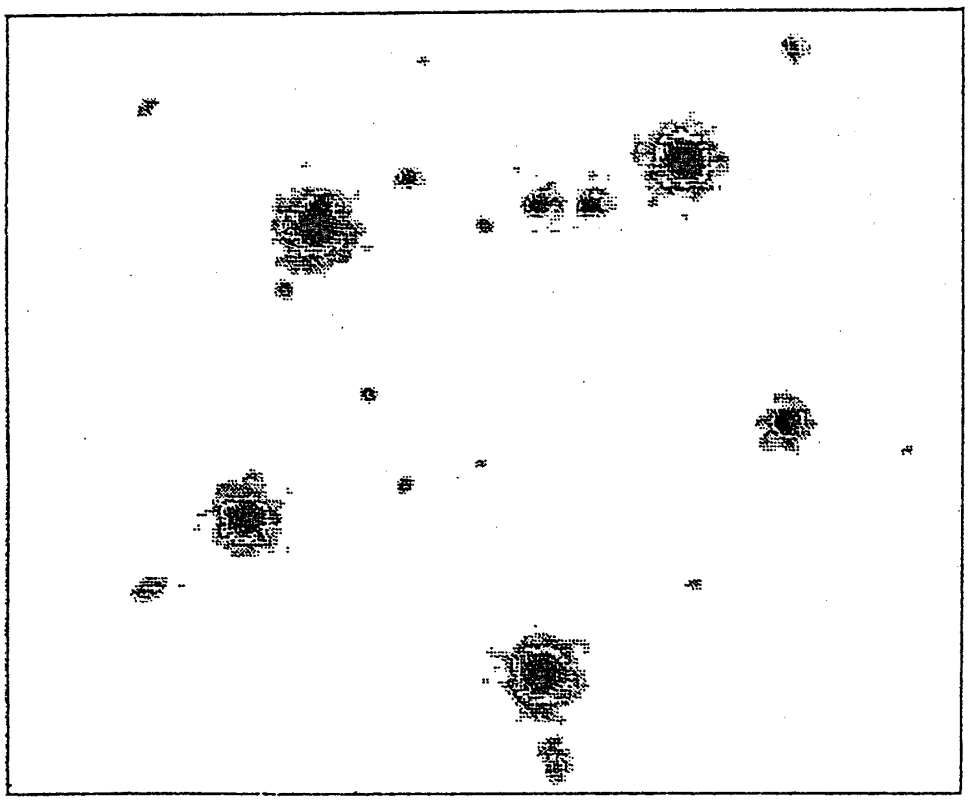

Figure 3: One example of the luminescent image on the surface of the cultured membrane filter.

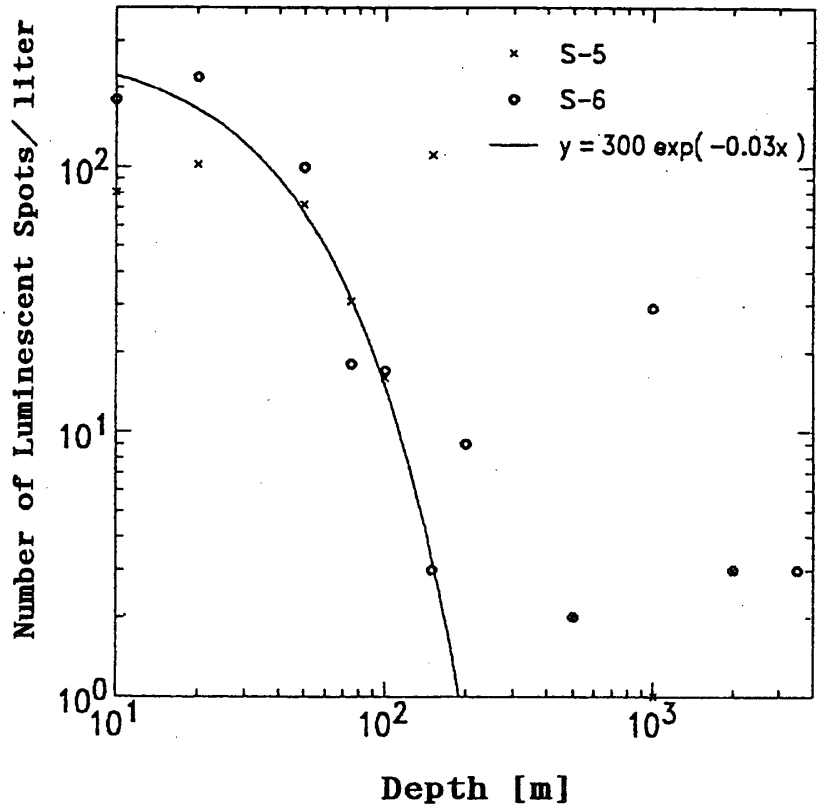

Figure 4: The intensity of spots per one liter corresponding with Fig.2 (flown through).

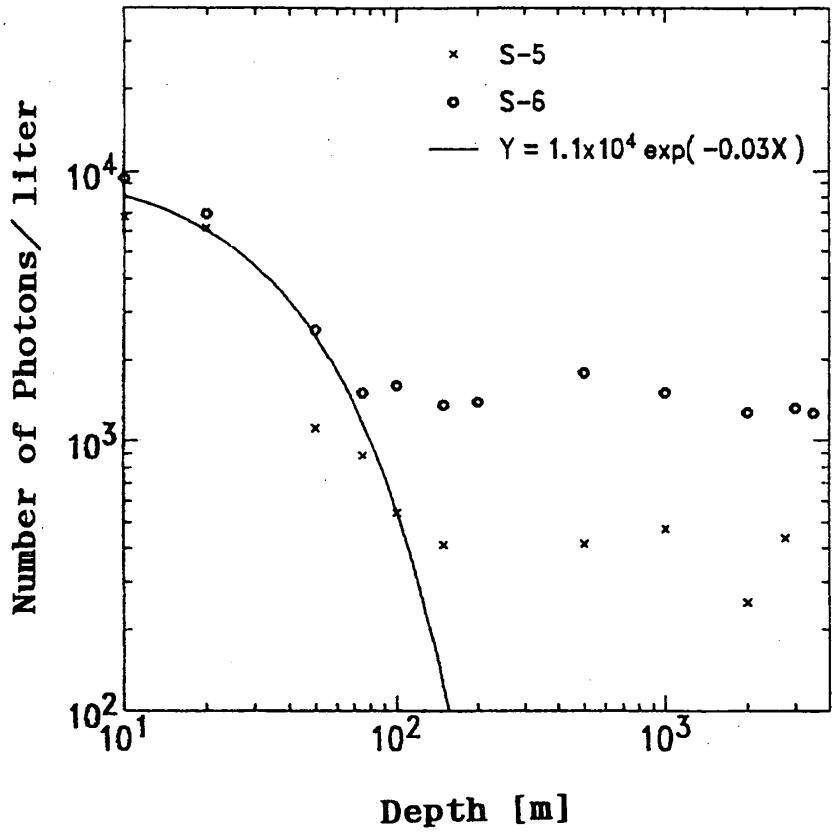

Figure 5: The luminescent intensity out of the spot in Fig.4.

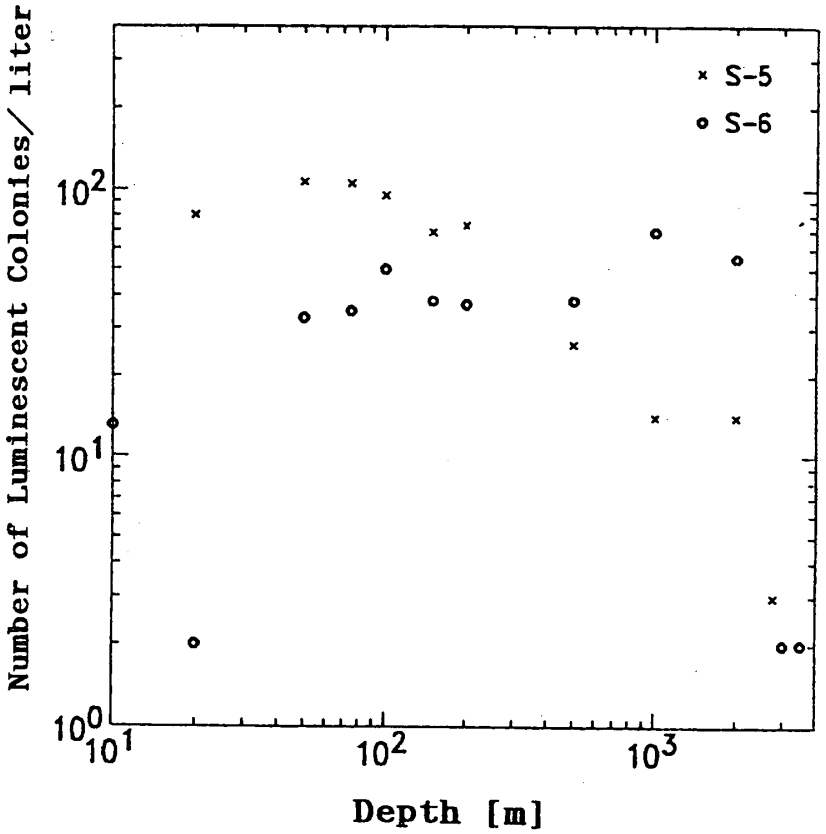

Figure 6: The number of luminescent colonies corresponding with Fig.3.

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