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In-site chlorophyll-a fluorometer based on lock-in amplifier

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ABSTRACT

The content of chlorophyll-a is one of the important indicators of eutrophication in marine ranching. Accurate measurement of chlorophyll-a content is of great significance for the sustainable development of marine ranching. In order to realize in-site detection of chlorophyll-a content in marine ranching, a chlorophyll-a fluorometer based on lock-in amplifier was designed according to the fluorescence characteristics of chlorophyll-a. A high-brightness 460nm LED was used as the excitation source and the chlorophyll-a fluorescence was detected at 680nm by a photodiode through an interference filter. At the same time, a reference light path was used to compensate the change of light source. The response of the sensor was tested using chlorophyll-a solution, the results showed the sensor has good linearity in the dynamic range and the correlation coefficient is 0.996. The noise level was tested using pure water, the test results showed that the sensor has good noise performance and the resolution of the sensor is 0.2μg/L.

Keywords: chlorophyll-a, fluorometer, lock-in amplifier, performance analysis

1. INTRODUCTION

Marine resources are essential natural resources for human development. However, the marine ecological environment has been severely damaged because of overfishing, pollution and coastal infrastructure construction etc. In order to maintain the sustainable development of marine resources, marine ranching has become one of the effective ways to solve this problem[1]. With the development of research and construction in marine ranching, there is an urgent need for instruments and equipment to monitor environmental parameters and biological living conditions in marine ranching. Chlorophyll-a is the most important pigment in phytoplankton. Its concentration represents the content of phytoplankton in seawater[2], which is an important evaluation parameter for the degree of eutrophication[3]. Therefore, in-site monitoring of chlorophyll-a concentration is helpful to timely obtain the ecological environment changing in marine ranching. Methods for measuring chlorophyll-a content in water mainly include spectrophotometry and fluorescence method. Because of the low solubility of chlorophyll-a in water, there is a great error in measuring the absorbance directly. It is often necessary to use ethanol or acetone to grind phytoplankton to extract chlorophyll-a from water. The spectrophotometer is then used to measure the absorbance of the extract at a specific wavelength. Finally, the Arnon formula[4] is used to calculate the concentration of chlorophyll-a in water. Therefore, spectrophotometry is difficult to be applied to the in-site chlorophyll-a concentration measurement. The fluorescence method uses specific wavelength light source to excite chlorophyll-a fluorescence, and indirectly measures the concentration of chlorophyll-a by measuring the intensity of fluorescence. With the advantages of convenience, high resolution, and no pollution, the fluorescence method is widely used in the detection of chlorophyll-a and other organic materials in water.

In this paper, we designed a chlorophyll-a sensor based on fluorescence method and lock-in amplifier. A high-brightness 460nm LED was used to excite chlorophyll-a fluorescence. A high-sensitivity photodiode was used as the fluorescence detector. Lock-in amplifier was used to improve the precision and anti-interference ability of the sensor. The dynamic range of the sensor is 0~200μg/L, and the resolution is 0.2μg/L.

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2. MEASURE PRINCIPLE

2.1 Fluorescence detection principle

Chlorophyll-a has good fluorescence characteristics, its absorption and emission spectrum are shown in figure 1. Absorption peaks of chlorophyll-a occur at 430nm in blue region and 665nm in red region, and the absorbance in blue region is greater than that in red region. Emission peaks of chlorophyll-a occur at 680nm and 750nm in red region, and the emission peak intensity at 680nm is greater than that at 750nm. When designing a chlorophyll-a sensor, in order to acquire the best resolution, we can use 430nm light to excite chlorophyll-a and measure the fluorescence intensity at 680nm. However, when exposed to excitation light (peak at 430nm), chlorophyll-a is unstable. It is quickly converted to several byproducts, such as pheophytin[6].A 460nm light source can be used instead to reduce that problem[7].

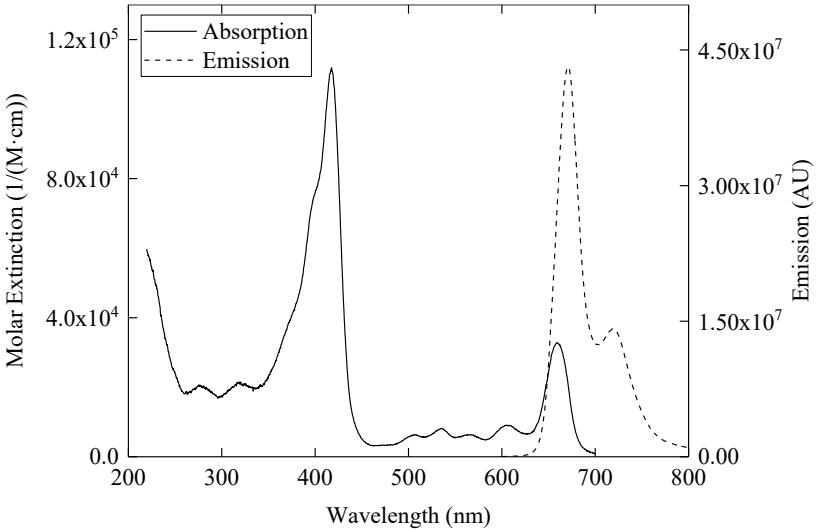

Fig.1 Absorption and emission spectrum of chlorophyll-a

The relationship between fluorescence intensity and chlorophyll-a concentration is based on the Beer-Lamberts law of absorption

$$I_F = \phi I(1-10^{-\varepsilon bc}) \tag{1}$$

Where I is the intensity of incident light, I_F is the intensity of fluorescence, c is the concentration of chlorophyll-a, b is the optical length, ε is the molar absorption coefficient, ϕ is the fluorescence efficiency of chlorophyll-a. When $\varepsilon bc < 0.05$, Eq.1 can be expanded by Taylor formula and the higher order terms can be neglected

$$I_F = 2.3\phi I \varepsilon bc \tag{2}$$

When chlorophyll-a solution is very dilute, fluorescence intensity has a great linear relationship with chlorophyll-a concentration and the fluorescence method has the highest sensitivity.

2.2 Lock-in amplifier principle

Lock-in amplification is a very common method in weak signal detection, which can effectively separate the useful signal from noise[8]. A sinusoidal signal is used as the excitation signal of the system. When measuring the response of the system, a sinusoidal reference signal, which has the same frequency and phase as the response signal, is used to demodulate the response signal of the system. Therefore, lock-in amplifier has a very narrow pass band and a very high quality factor. The structure block diagram of lock-in amplifier is shown in figure 2.

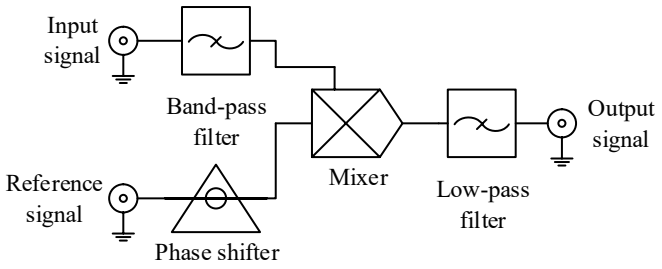

Fig.2 Structure of lock-in amplifier

The input signal and reference signal of the lock-in amplifier have the same frequency. The input signal is the system response signal. The signal first enters a band-pass filter, the center frequency of which is equal to the excitation signal, to initially filter the noise and amplify the signal. The reference signal is transformed into a signal, which has the same phase as the output signal of band-pass filter, by phase shifter. Then the two signals are multiplied by a mixer. Finally, a low-pass filter is used to convert the output signal of mixer to a stable direct current signal. Only the signal with the same frequency and phase as the reference signal can pass through the lock-in amplifier, other noise signals are filtered out. Therefore, the lock-in amplifier has excellent performance in weak signal amplification. In actual use, in order to simplify the circuit system, a square wave is often used instead as an excitation signal.

3. SYSTEM DESIGN

3.1 Optical structure design

The optical structure of the chlorophyll-a sensor is shown in figure 3. The sensor has two optical paths: fluorescence detection path and reference path, and they share the same light source. In order to effectively excite fluorescence, a high-brightness 460nm LED (SEMIB Semiconductor) was used as the light source. The maximum forward current is 150mA. In order to improve the utilization of the light source, a convex lens (Daheng Optics, Φ6, F6) was used to collimate the emitted light of the LED. A 460nm interference filter was then used to improve the monochromaticity of the excitation light. In order to avoid the influence of the excitation light, the fluorescence was detected in the direction perpendicular to the excitation light, and a 680nm interference filter was used to filter the receiving light, only chlorophyll-a fluorescence is allowed to pass through the filter. A high-sensitivity photodiode (Hamamatsu, S2386-5K) was used as the fluorescence detector, and a convex lenses (Daheng Optics, 12.7, F12.7) was used to converge the received light to improve the sensitivity of fluorescence detection. The luminous intensity of LED can be affected by temperature and drive current fluctuations, so a reference path was added in the sensor. Another photodiode was used to detect the light intensity scattered by the light source. The influence of fluctuation can be compensate by the intensity relationship between fluorescence and reference light.

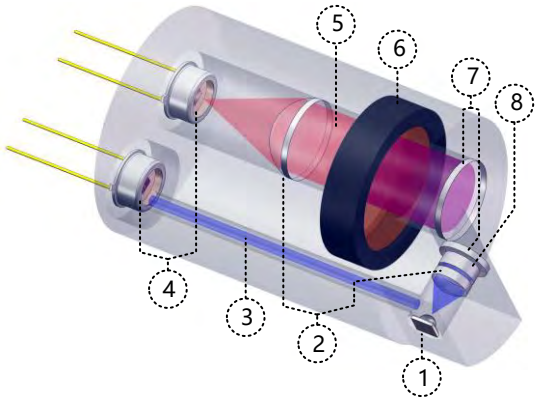

Fig.3 Optical structure of the chlorophyll-a sensor
1. Blue LED 2. Convex lens 3. Reference light path 4. Photodiode 5. Fluorescence detection path
6. Interference filter 7. Quartz glass 8. Interference filter

The light intensity emitted by the LED is I_0 and the fluorescence intensity detected by PD is I_f . The intensity of light used to excite fluorescence is $K_f I_0$, and the intensity of light entering the reference path is $K_r I_0$. Both K_f and K_r are constants and $K_f + K_r < 1$. I_f can be calculated basing on the Eq.2 as follows:

$$I_f = 2.3\phi K_f I_0 \varepsilon bc \tag{3}$$

Suppose I_r is the light intensity received by the photodiode in the reference light path. It can be calculated according to the relation mentioned in the Eq.4.

$$I_r = K_r I_0 \tag{4}$$

The relationship between I_f , I_r and c can be calculated basing on the Eq.3 and Eq.4 as follows:

$$\frac{I_f}{I_r} = \frac{2.3\phi K_f I_0 \varepsilon bc}{K_r I_0} = 2.3\frac{K_f}{K_r}\phi\varepsilon bc \tag{5}$$

Therefore, when the solution is very dilute, the ratio of the two light intensities is proportional to the concentration of chlorophyll-a and it is not affected by light source fluctuations.

3.2 Electronic circuit design

The electronic circuit structure of the chlorophyll-a sensor is shown in figure 4. The main circuits are MCU circuit, LED constant current drive circuit, I/V conversion circuit and lock-in amplifier circuit. STM32F103T8U6 was selected as the MCU chip, its rich peripherals and powerful functions meet the design requirements of the sensor. A constant current drive chip (PowTech, PT4211) was used as the LED driver, it supports PWM modulation and its maximum drive current is 350mA. According to the performance parameters of the chips selected in the sensor, 800Hz square wave was used as the driving signal of the LED. The current signals of the photodiodes in two optical paths are converted to voltage signal through the I/V conversion circuit. The voltage signals enter the lock-in amplifier through an analog switch chip (TI, TS5A3154) and the lock-in amplifier (ADI, AD630) amplifies the output signal of analog switch chip. Then the ADC (TI, ADS1115, 16bits) was used to transform the output signal of lock-in amplifier into digital signal. The communication between the MCU serial port and the host computer was realized through the 485 interface, which meets the requirement of the long communication distance.

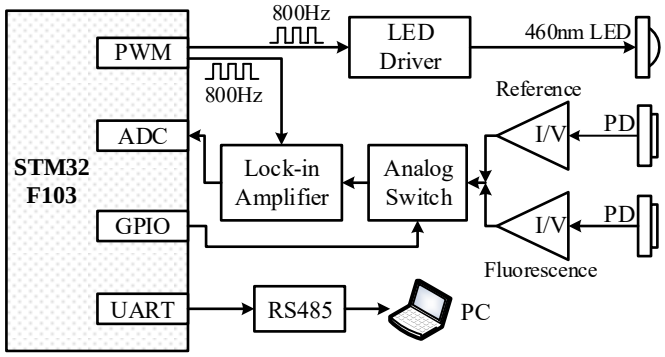

Fig.4 Electronic structure of the chlorophyll-a sensor

D_f and D_r are the analog to digital conversion results in the fluorescence detection path and the reference path respectively. The relation between them can be calculated through the Eq.6.

$$\frac{D_f}{D_r} = 2.3\frac{K_f}{K_r}\phi\varepsilon bc \tag{6}$$

4. EXPERIMENT AND RESULT

4.1 Preparation of chlorophyll-a standard solution

Chlorophyll-a standard solution was extracted from leaves using laboratory grinding method[9]. First, 1g fresh leaves were prepared, wiped with absolute alcohol, cut into pieces and put into a mortar. Then, 1g Calcium carbonate, 1g quartz sand and 5mL ethanol (95%) were added into the mortar and the mixture was ground for 5 minutes with a pestle. The mixture after grinding was placed in a 5mL centrifuge tube and centrifuged at 4000 rpm for 2 minutes. Then the supernatant was poured into a 25mL brown volume bottle and diluted with ethanol (95%) to volume. Because chlorophyll-a is easy to decompose when exposed to light, the standard solution should be used immediately after preparation. To store the standard solution, it should be placed in a refrigerator below 4℃[10] and the store time should not exceed 24 hours.

Then the concentration of standard solution was measured. 3mL standard solution was taken into a 1cm cuvette and the absorbance of the solution at 645nm and 663nm were measured respectively. The chlorophyll-a concentration can be calculated through the Eq.7 (Arnon formula)[4].

$$c = 12.7A_{663} - 2.69A_{645} \tag{7}$$

where A_{645} and A_{663} are the absorbance at 645nm and 663nm respectively. The unit of concentration is mg/L.

4.2 Performance test of the sensor

The dynamic range of the chlorophyll-a sensor is 0~200μg/L. In order to test the response of the sensor at different concentrations, 5 concentrations of chlorophyll-a solution (25μg/L, 50μg/L, 100μg/L, 150μg/L and 200μg/L) were prepared using the standard solution. The test result is shown in figure 5. The circular point in the graph is the response of the sensor, and the dotted line is a straight line fitted through the response.

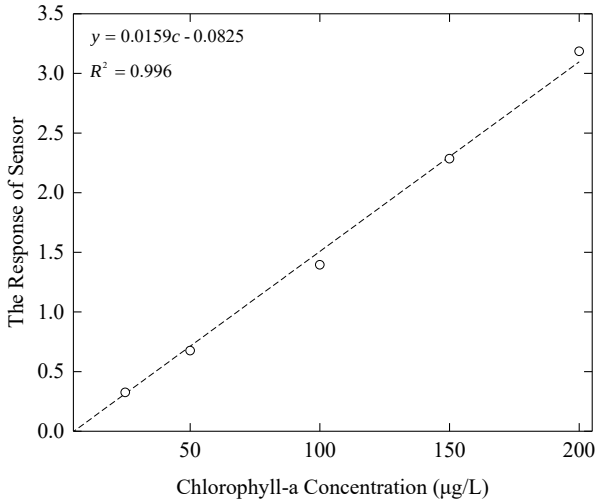

Fig.5 Response curve of the chlorophyll-a sensor

From the figure 5, the relation between the response of the sensor and the concentration of chlorophyll-a can be calculated through the Eq.8

$$y = 0.0159c - 0.0825 \tag{8}$$

where c is the concentration of chlorophyll-a. The linear regression coefficient $R^2 = 0.996$, so the sensor has good linearity in the dynamic range.

Subsequently, the noise level of the sensor was tested using pure water. The same water sample was continuously measured by the sensor for 100 times with 1 minute interval between each measurement. The original ADC sampling data was used

as the output of the sensor. To calculate the noise level, the average of the output was first calculated and then the average was minus by each sampling data. The result is shown in figure 6.

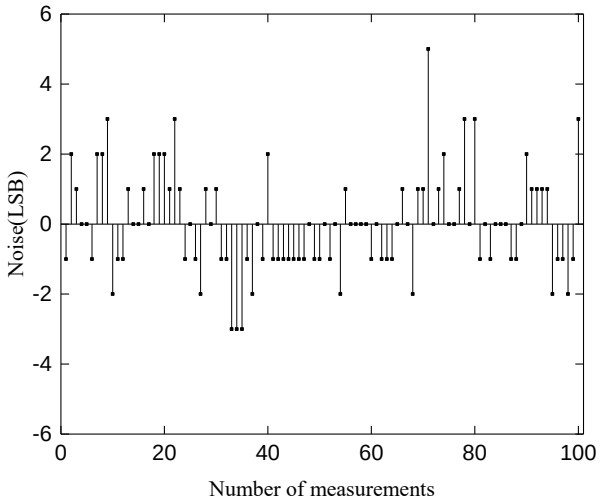

Fig.6 Noise test of the chlorophyll-a sensor

The measurement result shows that the noise level of the sensor is ±5LSB, so the sensor has good stability and reliability. The effective resolution of the ADC is 15bits, and 90% of the ADC dynamic range was used to measure chlorophyll-a concentration. When the signal intensity with 3:1 SNR is used as the fluorescence detection resolution, the resolution of the sensor is 0.2μg/L.

5. DISCUSSION

Fluorescence method can be used to measure the content of chlorophyll-a in water. In this paper, an in-site chlorophyll-a fluorescence sensor was designed based on lock-in amplifier. A high-brightness 460nm LED was used as the light source, which can excite chlorophyll-a fluorescence effectively. A high-sensitivity photodiode was used as the fluorescence detector, the size of the sensor can be reduced while maintaining the accuracy of the measurement. Lock-in amplifier was used to amplify small signals, it can effectively avoid the effects of noise and background light on the measurement results. The performance and noise level of the sensor were tested using chlorophyll-a solution and pure water. The test results shown that the sensor has good linearity and high resolution in the dynamic range and it meets the requirements of actual use.

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