A Submersible in-Situ Highly Sensitive Chlorophyll Fluorescence Detection System

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Abstract. Chlorophyll concentration in water is an important indicator of water quality monitoring. In this paper, the method of fluorescence analysis for the detection of chlorophyll in water is introduced in detail, and a design scheme of in-situ chlorophyll fluorescence detection instrument is proposed. Effectively improve the efficiency and accuracy of chlorophyll fluorescence detection in water by optimizing optical path, fluorescence signal modulation, filtering and other anti-interference methods; Adopt a low-power design scheme to extend the instrument's in-situ continuous detection time. The test results show that the linear response correlation coefficient R of the self-designed water chlorophyll detection instrument is above 0.999 with an accuracy of 0.02μg/L. The in-situ measurement time can reach 3 months, reaching the level of similar high-end instruments abroad.

1. Introduction
An important indicator of water quality monitoring is the concentration of phytoplankton in the water, and the concentration of phytoplankton is the antenna pigment contained in it - chlorophyll \( \alpha \). Chlorophyll \( \alpha \) is the main source of phytoplankton fluorescence in vivo, and the fluorometry is generally used for quantitative detection [1]. After sampling from the water and testing in the laboratory, the results generally lag behind the real situation of the ecosystem. The in-situ measurement has more practical meaning. Due to the complexity of natural water environment, low chlorophyll concentration and long duration of in-situ monitoring, there are higher requirements for anti-interference, accuracy and power consumption of measuring instruments [2]. Underwater in-situ testing instruments provide scientific researchers with simpler and more efficient means of detection, with important scientific and social value, but the current market is dominated by imports. This paper designs and implements a high-precision in-situ underwater chlorophyll fluorescence detection instrument, which has practical meaning for improving the water quality monitoring standard in China and changing the situation that the market is monopolized by imported products.

2. Detection method
Chlorophyll \( \alpha \) is a kind of fluorescent compound. Irradiating water with a certain wavelength of excitation light, chlorophyll \( \alpha \) in water is excited to produce fluorescence, and the intensity of fluorescence is proportional to the concentration. By detecting the intensity of the excited fluorescence, the concentration of chlorophyll \( \alpha \) in the water can be converted. Widely applied imported high-end
products, such as SCF SVF (Seapoint, USA) and Cyclops (Turner Designs, USA) underwater fluorescent probes, their measurement principles are based on fluorescence analysis [3, 4]. The in-situ chlorophyll sensor designed by the thesis mainly includes three parts: optical, electronic and shell packing.

2.1. Optical Module
Light path is the key to system design. Thus, selecting an appropriate excitation source, photodetector, and filter according to the chlorophyll fluorescence spectral characteristics; In the optical path design, try to avoid interference light other than fluorescence entering the detector to cover the useful signal.

The optical module of the fluorescence detection system is shown in Fig.1. According to the emission and excitation spectrum [5] of chlorophyll α and the detection principle, two blue-violet light-emitting diodes (LED) with the wavelength of 430 nm are selected for excitation light sources, arranged at 180°. A monochromator (the passband is 430 nm ± 15 nm) is placed in front of the LED to remove the variegated light generated by the light source. When LED irradiates water, chlorophyll α in water is stimulated to emit fluorescence at 680 nm [6]. Two measures were taken in the design to avoid excitation light entering the detection window: One is to design the detection window at an angle of 90° to the light source, and the other is to install a monochromator (passband 685 nm ± 15 nm) in front of the detection window. The optical window is made of artificial corundum. In order to improve the efficiency of fluorescence detection, an optical lens is also used in front of the monochromator to focus the incident fluorescence so that it can reach the photosensitive region of the photosensor to the utmost extent.

![Figure 1. Optical design.](image)

2.2. Electronic Module
The structure of the electronic module is shown in Fig.2. The main functions of the electronic module are: Amplifying and filtering the weak fluorescent signal; modulating the excitation light and...
synchronous demodulation of the fluorescent signal; Sampling analog signals; data processing (digital filtering, storage, reading) and communication.

2.3. Signal Detection, Amplification and Filtering
A silicon photodiode S1336-44BK (Hamamatsu, Japan) was used as a fluorescence detector, which has a relatively high response (0.38 A/W) to fluorescence at 680 nm and low dark current (60pA). Its sensitivity, power consumption, response speed and temperature characteristics can meet the requirements of in-situ measurements. The photodiode has no internal gain and the output signal is very weak. Because the preamplifier will affect the sensitivity and signal-to-noise ratio of fluorescence detection, the noise requirement, gain and bandwidth are fully considered in the design. Under normal circumstances, the background light (such as sunlight, lamplight) has a low frequency, close to DC, but the fluorescent signal is a modulated signal with a certain frequency. In order to avoid strong background light to saturate the amplifier output, use a bandpass filter to block DC and suppress high frequency noise generated by the I/V conversion circuit. In this way, only the fluorescence signal with modulation frequency is allowed to pass through, excluding the interference of ambient light and improving the dynamic range of fluorescence detection. After two-stage amplification, fluorescent signal will be demodulated.

2.4. Low Power Microprocessor
Microprocessor (MCU) is the core of testing instrument, which executes control, data processing and communication instructions. In in-situ offline status, the probe is placed under water for continuous operation and powered by a lithium battery. During this period, the MCU cannot be completely shut down and is always in a monitoring state. Therefore, the contribution of the MCU's own power consumption to the total power consumption of the system must be considered. Considering the ultra-low power mode, event-driven capability, on-chip peripherals and operational efficiency, the 16-bit mixed-signal microprocessor F1611 in MSP430 series can meet the requirements of the design.

2.5. Excitation Light driver, Modulation and Signal Demodulation
In order to avoid the influence of temperature drift and resistance accuracy on the driving current for the light source, MAX1916 was used to drive the excitation light source. MAX1916 is a constant-current LED driver integrated circuit with low power, and can drive three light sources simultaneously. The maximum driving current of each light source is 60mA, and the matched-degree can reach 0.3%.

The modulation signal for LEDs is generated by the timer of MCU, which is output to the EN pin of MAX1916 after being driven. The demodulation circuit of the fluorescence signal consists of an analog switch and a differential amplifier. Synchronous demodulation has high frequency selectivity and can effectively obtain amplified and filtered fluorescent signals.

2.6. Sampling, Communication, and Data Storage
Choose a 24-bit Δ-Σ type AD for analog-to-digital conversion of this signal, which has flexible power configuration mode, high dynamic range, low power consumption (standby mode 4mW, dormancy mode about 500μW) can fully meet the needs well.

The test data can be transmitted synchronously in on-line detection. During in-situ detection, the probe is disconnected from the power supply and data cable and test data needs to be temporarily stored. After the detection is completed, the cable is connected and the data is uploaded to the host computer. Flash storage is used in the instrument, and the capacity is determined by the data structure and the required continuous monitoring time. According to test standards, RS-232 serial communication interface is used to improve the communication distance and anti-interference ability between the probe and the host computer.
3. Power Consumption Testing
Table 1 shows the results of the power consumption test of the main modules. It can be seen that the current consumed by the LED driver is much larger than other modules. The detection frequency will affect the proportion of each module in the power consumption of the whole system. If the detection is frequent, the light source contributes the most to the overall power consumption, and the battery life is determined by the excitation light module. However, in the case where the detection interval is long, the LEDs illuminate for no more than 2 seconds in a single detection, and the average power consumption is much lower. In this case, the primary factor affecting the battery life is the power consumption of the standby mode and the peripheral circuit. The longest duration of in-situ detection can be calculated from the data in Table 1, the testing frequency, as well as the battery capacity. When the detection interval is set to 10 minutes, the lithium battery with a capacity of 2000mAh can support the in-situ monitoring for no less than 3 months.

Table 1. Power consumption testing.

| Module     | LE D | Storage (working) | MCU (Standby) | AD | Signal Processing | Peripheral Circuit(Standby) |
|------------|------|-------------------|---------------|----|-------------------|-----------------------------|
| Voltage/V  | 5    | 3.3               | 3.3           | 3.3| 3.3               | 5                           |
| Current/mA | 64.8 | 2.5               | 2.43          | 0.08| 16.9              | 8.94                        |

4. Tests and results
A self-developed in-situ chlorophyll fluorescence detector HEM and imported Seapoint products are shown in Fig.3. The linearity of two kinds of fluorimeters was tested in the laboratory. Each time 500μL of a standard chlorophyll solution having a concentration of 3mg/L was added to 1L of water and stirred well, and then the chlorophyll concentration was measured by two devices. The results of HME and Seapoint chlorophyll analyzer are given respectively in the two curves of Fig.4. It can be seen that both curves have good linearity. The linearity of the HME is very close to that of SCF, both above 0.999. The detection accuracy of HEM is 0.02μg/L, which reaches the level of similar high-end instruments abroad.

![Figure 3. HEM and Seapoint](image1)

![Figure 4. Linear response characteristics](image2)
In-situ comparison tests were performed on HME and Seapoint SCF chlorophyll α fluorometers in the offshore waters of XiangShan Port, NingBo. The two probes are fixed on the experimental platform with a water depth of 25 meters, and the test is performed every 30 minutes. The curve of chlorophyll concentration detected by the two probes is shown in Fig.5. The linear correlation coefficient of the two sets of data is 0.921.

5. Conclusion
This paper implements an in-situ chlorophyll sensor based on fluorescence analysis method. The test results show that the detection accuracy and linearity reach the level of similar high-end imported products, which is conducive to breaking the monopoly of imported products in the domestic market and has a good industrial prospect.

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