

# A design of spectrophotometric microfluidic chip sensor for analyzing silicate in seawater

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**Abstract.** High quality and continuous in situ silicate data are required to investigate the mechanism of the biogeochemical cycles and the formation of red tide. There is an urgently growing need for autonomous in situ silicate instruments that perform determination on various platforms. However, due to the high reagents and power consumption, as well as high system complexity leading to low reliability and robustness, the performance of the commercially available silicate sensors is not satisfactory. With these problems, here we present a new generation of microfluidic continuous flow analysis silicate sensor with sufficient analytical performance and robustness, for in situ determination of soluble silicate in seawater. The reaction mechanism of this sensor is based on the reaction of silicate with ammonium molybdate to form a yellow silicomolybdate complex and further reduction to silicomolybdenum blue by ascorbic acid. The minimum limit of detection was  $45.1 \text{ nmol L}^{-1}$ , and the linear determination range of the sensor is  $0\text{--}400 \text{ }\mu\text{mol L}^{-1}$ . The recovery rate of the actual water is between 98.1%–104.0%, and the analyzing cycle of the sensor is about 5 minutes. This sensor has the advantages of high accuracy, high integration, low water consumption, and strong anti-interference ability. It has been successfully applied to measuring the silicate in seawater in Jiaozhou Bay.

## 1. Introduction

Silicon is one of the essential nutrients that maintain biological growth in the marine environment. Silicate, a key component in diatoms, radiolarians and foraminifera and other marine organisms, is one of the greatest important sources of silicon in marine ecosystems [1]. Insufficiency of silicate may limit primary production in marine ecosystems; however, excessive silicate concentration may lead to excessive phytoplankton growth, leading to the cause of the occurrence of red tide [2]. So, an accurate and sustainable measurement of marine silicate is of great significance for marine biological resources development and ecological environment protection.

In China, in accordance with the “Specification for Marine Monitoring” (17378.4-2007), there are two methods adopted for arbitration, including a) silicomolybdenum yellow method and b) silicomolybdenum blue method. The former method is rapid but insensitive and susceptible to salinity, it is then not suitable for low silicate concentration determination; in the contrary, the later one, adopted by most of marine monitoring unit, has the advantage of high sensitivity and specificity, even though this method is of time-consuming, tedious, and difficult to achieve the on-line analysis. With



the development of flow analysis technology, various of methods have been applied to measure nutrients in seawater, including flow injection [3-5], sequential injections [6-8], continuous flow [9], and stop flow analysis [8, 10, 11], etc. Among those methods, methods of flow injection, sequential injections and continuous flow are of the most common ones with a quick reaction and relatively simple structure, however with the relatively low reaction efficiency, and those consumptions of reagents or samples are usually large. Another method, stop flow method, has the advantage of excellent reaction efficiency, however the low reaction rate and adverse dispersion effect restricted its application. All these methods, adopting the precise time control and non-equilibrium state analysis which greatly shorten the analysis time, can be used to on-line analyze silicate. It is a break through against traditional conception of which the reaction must be achieved with a physical and chemical equilibrium.

Recently, with the development of Micro and Nano-technology, a “Lab on Chip” technology, an integrated fluid flow analysis system with the advantage of high integration level and speed of mass transfer and heat conduction, has become a new trend of analysis [12, 13]. This new technology contributes to the occurrence of a new generation of in situ marine nutrient sensor, such as nitrate and nitrite microfluidic sensor [14], phosphate sensor [12] and Fe and Mn sensor [15], etc., although these sensors have not yet matured enough to form reliable products. Most of prototypes are not expected to perform multi-step reactions due to deficiency of the reaction efficiency and the increment of the system complexity. In this study, A new microfluidic continuous flow analysis sensor was established and designed for determination of soluble silicate in seawater in situ based on “blue” arbitration method (multi-step reactions). Furthermore, this sensor deployed on research vessel was successfully applied in routine survey in Jiaozhou Bay in China.

## 2. Experimental

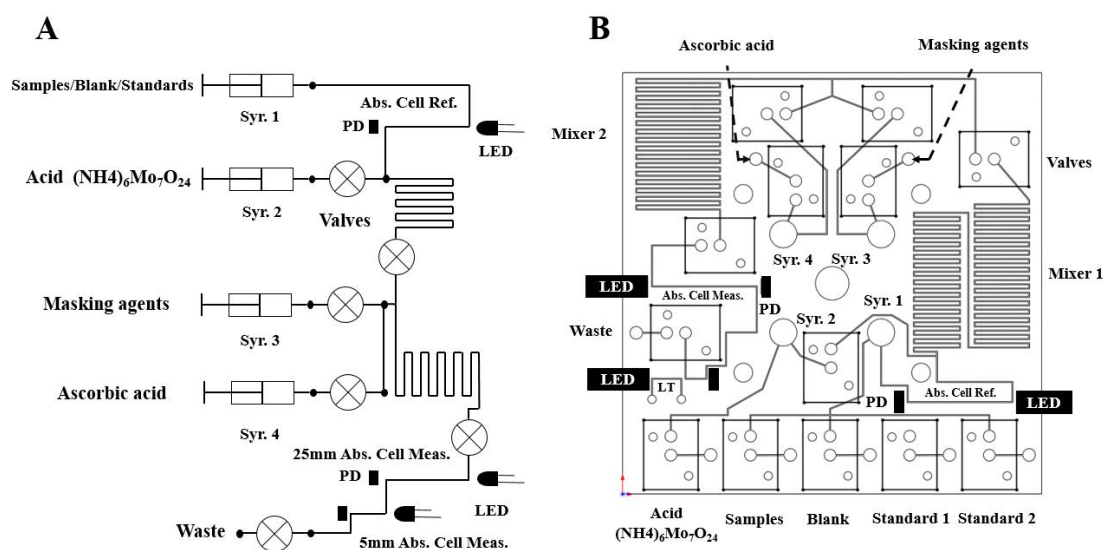
### 2.1. Reagents and chemicals

All the solutions were prepared by Milli-Q ultra-pure deionized (DI) water (Millipore, USA), followed by 30 minutes ultrasonic degassing (Shengyan, SCQ-H300A, China). All the chemical reagents were purchased from Fluka (Sigma-Aldrich, Germany). 100mg L<sup>-1</sup> Silicate standards (NRCCRM, China) were used for calibration. Acidified molybdate reagent of which 11.9 g ammonium molybdate was dissolved in 250 mL water, mixing with 15 mL concentrated sulfuric acid in 150 mL water was used for color developing agent. 26.3 g citric acid dissolved in 250 mL water, followed by adding 0.2 g antimony potassium tartrate used as masking agent to avoid phosphate interference. 10.0 g ascorbic acid dissolved in 200 mL water used as reduction reagent to form silicomolybdenum blue. All above solutions were stored in 4 °C and kept in dark, followed by 1ml chloroform added to prevent biodegradation.

### 2.2. The design of microfluidic chip

The microfluidic chip (figure 1) was produced using 3.0 mm thick tinted polymethyl methacrylate (PMMA) sheets (7C83, Plexiglas, Germany). The PMMA sheets were patterned by the method of micro-milling using Circuit board plotters (LPKF Protomat S61 Micromill). Before micro-milling, sheets were thoroughly cleaned to remove dusts and grease [16]. The purpose using tinted PMMA was for reducing light, which was from ambient sources, as well as those from the LEDs not passing through the analytes, reaching the detector, as reported by Cedric F A Floquet [17]. A chloroform solvent vapor assisted bonding method was used to achieve chip sealing. Previous studies have shown that this procedure could smooth the inner surface of the microchannel and enhance the precision of optical analysis on microchip [14]. Three absorbance cells were designed in microchip, including one reference cell (25 mm length), and two measurement cells with 25 mm as the length for low concentration silicate determination and 5 mm as the length for high concentration silicate determination respectively. Also, three pairs of 810 nm LEDs (Avago Technologies, USA) and photodiodes (TAOS, USA) that directly fixed onto the chip using Norland 68 optical adhesive

(Norland, USA), were used to perform spectrophotometry analysis. For the 5 mm cell, photodiode and LED were spaced apart by 15 mm using a 7mm long “light tube” [14] to transmit light between the LED and the optical absorbance cell, and this design could reject the additional background light when the intense light from LED passing through the short absorbance cell. The light tube was produced by directly milling microchannel on the tinted PMMA sheets and filled with transparent Norland Optical Adhesive 68 with a high refractive index. Two serpentine mixers were deployed with length of 936.5 mm and 684.1 mm respectively to fulfill the reaction of coloration and reduction. All the microchannel were of  $250\ \mu\text{m} \times 250\ \mu\text{m}$ , except for the three optical absorbance cells which were of  $400\ \mu\text{m} \times 400\ \mu\text{m}$ . The total fluidic dead volume was designed as about  $123.7\ \mu\text{L}$ .



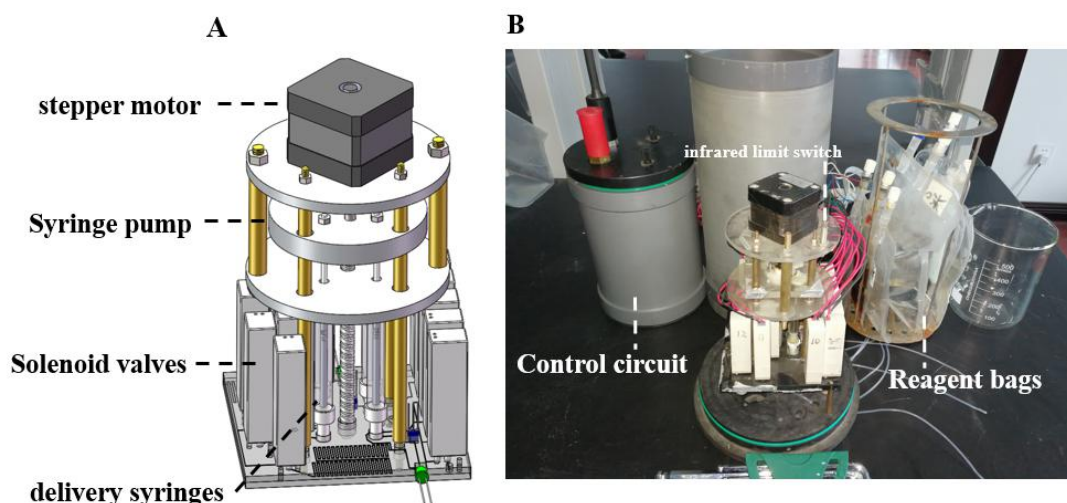
**Figure 1.** Microfluidic design and analysis procedure.

PD: Photodiode, Syr: Syringe, LED: Light-emitting Diode, Abs. Cell Ref: Adsorption Cell Reference, Abs. Cell Meas: Adsorption Cell Measurement, LT: Light Tube.

### 2.3. Description of the sensor

Silicate sensor (figures 2A and 2B) in this study contained four functional modules, including fluid driving system, microchip, control circuit and sensor peripheral. Fluid driving system consisted of syringe pump and 13 microinert solenoid valves (100R10, SMC, Japan) which were directly mounted on the microfluidic chip sealed with inert O ring. The syringe pump was custom-designed with a stepper motor (Dingzhi, China) driving four 2 mL PMMA syringes controlled by two infrared limit switches. The distance between these two switches on the active plate of the pump were pre-calibrated, and this structure formed a syringe pump. The total fluid dead volume (include valves and microchip) of the sensor was about  $383.7\ \mu\text{L}$ . Control circuit consisted of MCU, power module, stepper-motor-based linear actuator, data processing and memory module, etc. MCU used in this sensor was STM32 chip (TI, USA). Power module was considered to perform in two operation mode, which were self-contained and externally powered monitoring. Stepper-motor-based linear actuator adopted DS-5045-003 commercial actuator (Dingzhi, China) to enable and control the stepper motor. A 24 bit A/D converter (ADS1675, Texas Instruments, USA) was used to acquire, convert and amplify data from photodiodes. A 2 GB flash memory card was used to store the signals from the photodiodes and the silicate concentration data. A fault tolerant system was established to guarantee data was not missing or corrupted. The whole micro-analyzer system was enclosed in a watertight PVC tube (figure 2B) with a diameter of 15 cm and 45 cm high.

An external filter unit which consisted of peristaltic pump and  $0.45\ \mu\text{m}$  capsule filter and several reagent bags was equipped as sensor peripherals.



**Figure 2.** Construction model and real product of silicate sensor.

#### 2.4. Analytical process

The analytical procedures involved continuous five steps: (1) sampling of the seawater sample using syringe from environment; (2) injection of the sample and relative reagents in micro-system; (3) the first hybrid reaction to form yellow silicomolybdate; (4) the second hybrid reaction to form blue silicomolybdate, by mixing with masking agent and ascorbic acid; and (5) determination of the absorbance. Before addition of the reagents, the absorbance of the sample, blank, and/or standards were measured in the reference absorbance cell. This step was essential for deduction any background. Both the sample and the reagent flowed through the micro-system at a rate of 80 mL min<sup>-1</sup>, with a ratio of 1:1. The solenoid valve after the mixer were closed when the first mixer was full of the mixture of sample and reagent, and then jerked the syringe for several times to enhance the reaction efficiency. The flow was stopped for 150 s so as to allow color formation in consideration of its low reaction rate for forming yellow silicomolybdate. Blank (artificial seawater) were determined prior to sample in every determination cycle to inspect the baseline, and meanwhile to wash the microchannel to prevent the residue from last determination. The measurement period of the whole determination was about 300 s (including the blank before sampling). Besides, a high (200  $\mu\text{mol L}^{-1}$ ) and low (5  $\mu\text{mol L}^{-1}$ ) concentration standards were carried as inner standard when this sensor was deployed for field test. For ensuring the accuracy and avoid baseline drift, these two standards were periodically determined once every 15 samples.

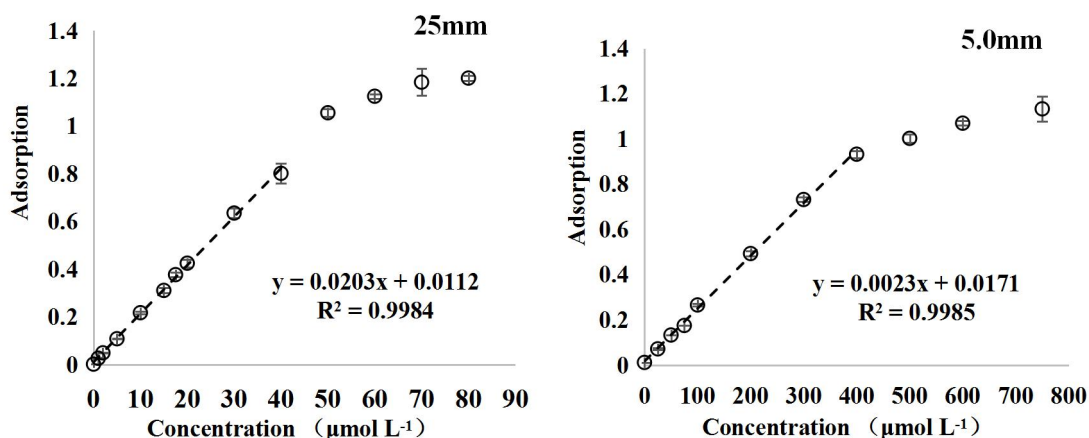
### 3. Results and discussion

#### 3.1. Calibration, precision, and limit of detection

The average concentration of the soluble silicate in seawater is about 36  $\mu\text{mol L}^{-1}$ , with a dramatic variation [1]; in flourish season of diatoms phytoplankton, silicate concentration significantly is reduced to 0.13  $\mu\text{mol L}^{-1}$  [18] due to the uptake of the large amounts of phytoplankton; but in the deep ocean, the concentration of silicate is about 100-200  $\mu\text{mol L}^{-1}$ . Such a large concentration range greatly increases the difficulty to perform analysis of silicate in situ.

In order to pay attention to high and low concentration of the silicate determination, two different optical lengths (25 mm and 5 mm) of absorbance cells were designed on the microfluidic chip. As shown in figures 3A and 3B, the calibration curves for each of the two absorbance cells. For 25 mm absorbance cell, an obvious absorbance change against concentration gradient change is obtained from 0 to 90  $\mu\text{mol L}^{-1}$ , with the best linear measurement range was 0-40  $\mu\text{mol L}^{-1}$  having a regression equation  $A=0.203C_{\text{Si}}+0.0112$  ( $n=7$ ,  $R^2=0.9984$ ); for 5 mm absorbance cell, the concentration gradient change is obtained from 0 to 800  $\mu\text{mol L}^{-1}$ , with the best linear measurement range 0-400  $\mu\text{mol L}^{-1}$

having a regression equation  $A=0.0023C_{Si}+0.0171$  ( $n=7$ ,  $R^2=0.9985$ ). For the quality of the data, the sensor procedure set for measurements below  $30 \mu\text{mol L}^{-1}$ , data from 25 mm absorbance cell is adopted; while above  $30 \mu\text{mol L}^{-1}$ , 5 mm absorbance cell is adopted. The total linear dynamic range is  $0\text{--}400 \mu\text{mol L}^{-1}$ .



**Figure 3.** Calibration of sensor.

A for Calibration of 25mm optical path length, B for Calibration of 5mm optical path length.

In this study, continuous determination of  $7.7 \mu\text{mol L}^{-1}$  and  $132.1 \mu\text{mol L}^{-1}$  samples was made for 11 times, with concentration calibrated using the method of blue silicomolybdate from “Specification for Marine Monitoring” (17378.4-2007). Accordingly, as calculated, the relative standard deviation (RSD%,  $n=11$ ) is 2.1% and 1.4% respectively, indicating that the repeatability and precision are good enough for deployment in seawater; besides, the limit of detection of this sensor, defined as three times the standard deviation of blank sample (artificial seawater without silicate [19]) divided by the number of calibration slope, is  $45.1 \text{ nmol L}^{-1}$  and  $1.6 \mu\text{mol L}^{-1}$  responding to the optical length 25 mm and 5 mm respectively; the limit of quantitation, defined as fifteen times the standard deviation of blank sample (artificial seawater without silicate) divided by the number of calibration slope, is  $225.5 \text{ nmol L}^{-1}$  and  $8.0 \mu\text{mol L}^{-1}$ .

### 3.2. Spike recovery

Recovery experiments were conducted using samples from Qingdao Zhongyuan Wharf ( $120^{\circ}18' \text{ E}$ ,  $36^{\circ}03' \text{ N}$ ) and Zhoushan Banshengdong Wharf ( $122^{\circ}19' \text{ E}$ ,  $29^{\circ}56' \text{ N}$ ). After  $0.45 \mu\text{m}$  filtration, 50% and 100% background concentration of samples were spiked using silicate standard into the samples. As shown in table 1, 98.1%–104.0% recoveries have been obtained by using high and low background concentration samples, indicating the considerable validity and accuracy of this method.

**Table 1.** Determination of seawater sample and recovery tests ( $n=3$ ).

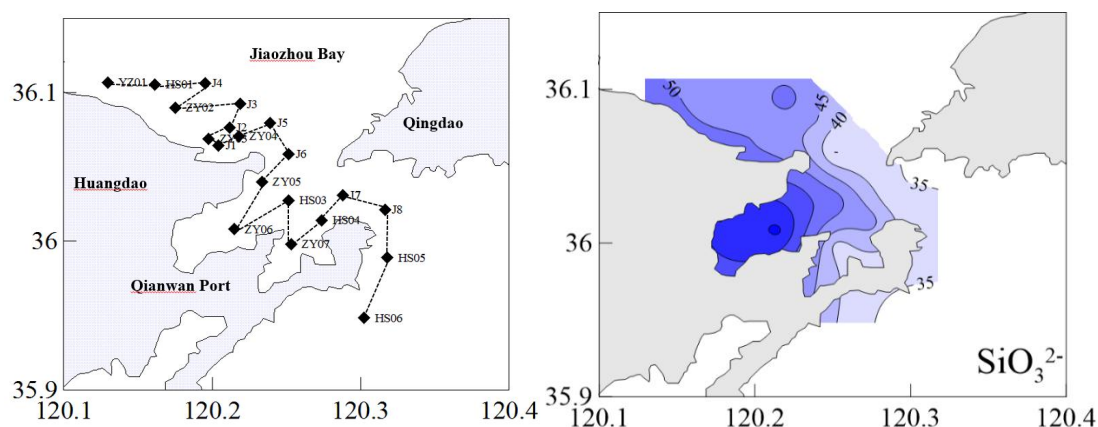
Samples	Salinity	Spikes Conc. ( $\mu\text{mol L}^{-1}$ )	Recovery Conc. ( $\mu\text{mol L}^{-1}$ )	Recovery%
Qingdao Zhongyuan Wharf	30.1	0	$1.7 \pm 0.2$	-
		1.8	$3.6 \pm 0.1$	103.1%
		3.6	$5.2 \pm 0.2$	98.1%
Zhoushan Banshengdong Wharf	25.4	0	$62.3 \pm 0.2$	-
		32.0	$95.3 \pm 0.5$	101.1%
		64.0	$131.3 \pm 0.4$	104.0%



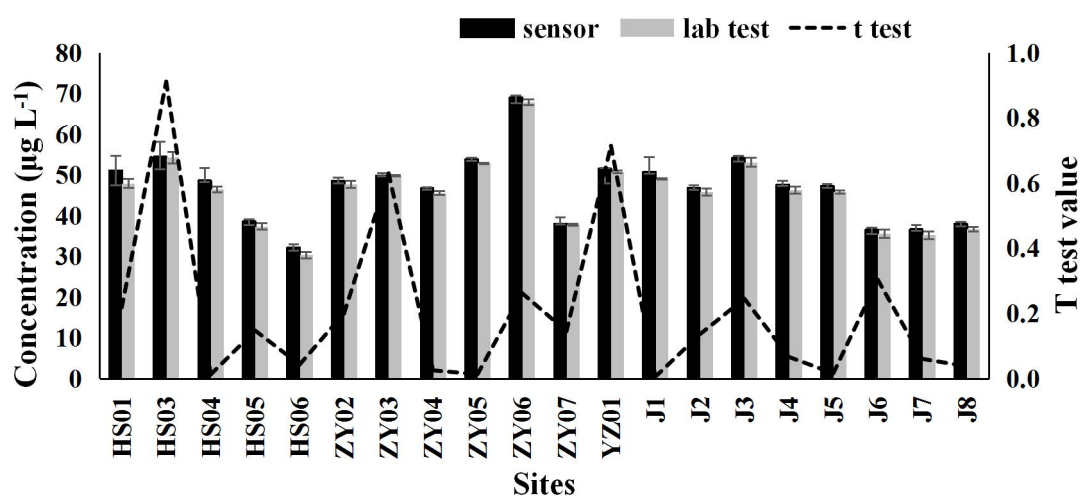
### 3.3. Application for routine monitoring

The microfluidic sensor was also deployed from November 29<sup>th</sup>, 2015 on an oceanographic cruise (about 50 sites) in the southern of Jiaozhou bay Qingdao, and the specific sites and route of this survey showed in figure 4A. The sensor was fixed on a frame of CTD profiler with water bottles, and measured each sample three times to obtain the average and deviation, meanwhile water samples were collected and frozen. Afterwards the samples were taken away to the lab for analyzing as comparisons. Figure 5 shows the comparisons of the data from sensor and lab analysis. As shown by the results that the trend of the data from sensor are consistent with data from lab analysis. Furthermore, significance test (t test) was also conducted to verify the data consistency. In t test,  $\alpha=0.05$ ,  $n=3$ , the critical value of  $t(a, f)$  was obtained as 2.776, indicating that data from sensor is not significantly different from lab analysis, due to all of the t values obtained from this experiment are below the critical value.

In Sea trial, the concentration of silicon converted from the concentration silicate in seawater were 32.2-69.1  $\mu\text{g L}^{-1}$  in the southern of Jiaozhou bay in late November, with 47.1  $\mu\text{g L}^{-1}$  as the average concentration; the maximum value and minimum value occurred in ZY06 site in Qianwan port and HS06 site in Zhucha island respectively. As shown in figure 4B, the silicate contour map indicates that silicate concentration in nearshore is higher than in open sea.



**Figure 4.** Sea trial route and silicate distribution around sea trial area ( $\mu\text{g L}^{-1}$ ).



**Figure 5.** Silicon data from sea trial and comparison with national standard method ( $\mu\text{g L}^{-1}$  Si,  $n=3$ ).

## 4. Conclusion

Based on the reaction of silicate with ammonium molybdate to form a yellow silicomolybdate complex and further reduction to silicomolybdenum blue by ascorbic acid, the new microfluidic continuous flow analysis sensor is designed for determination of soluble silicate in seawater. The measurement period of the whole determination may be about 300s, much less than the time cost (3 h) using lab method described in "Specification for Marine Monitoring" (17378.4-2007). With double light path in situ analysis adopted, this microfluidic sensor has an expanded linear range of 0-400  $\mu\text{mol L}^{-1}$  with 45.1  $\text{nmol L}^{-1}$  as the minimum limit of detection. Offshore experiment with this sensor shows that (1) data from sensor are consistent with lab analysis; (2) this microfluidic may be deployed in situ for continuous analysis, and (3) it has the advantages of high precision and robustness.

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