Electronic Supplementary Material (ESI) for Lab on a Chip.


# A High-sensitivity Lab-on-a-chip Analyzer for Online Monitoring of Nitrite and Nitrate in Seawater Based on Liquid Waveguide Capillary Cells

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# Supplementary Information

## Contents

**Section 1: Chip photo**

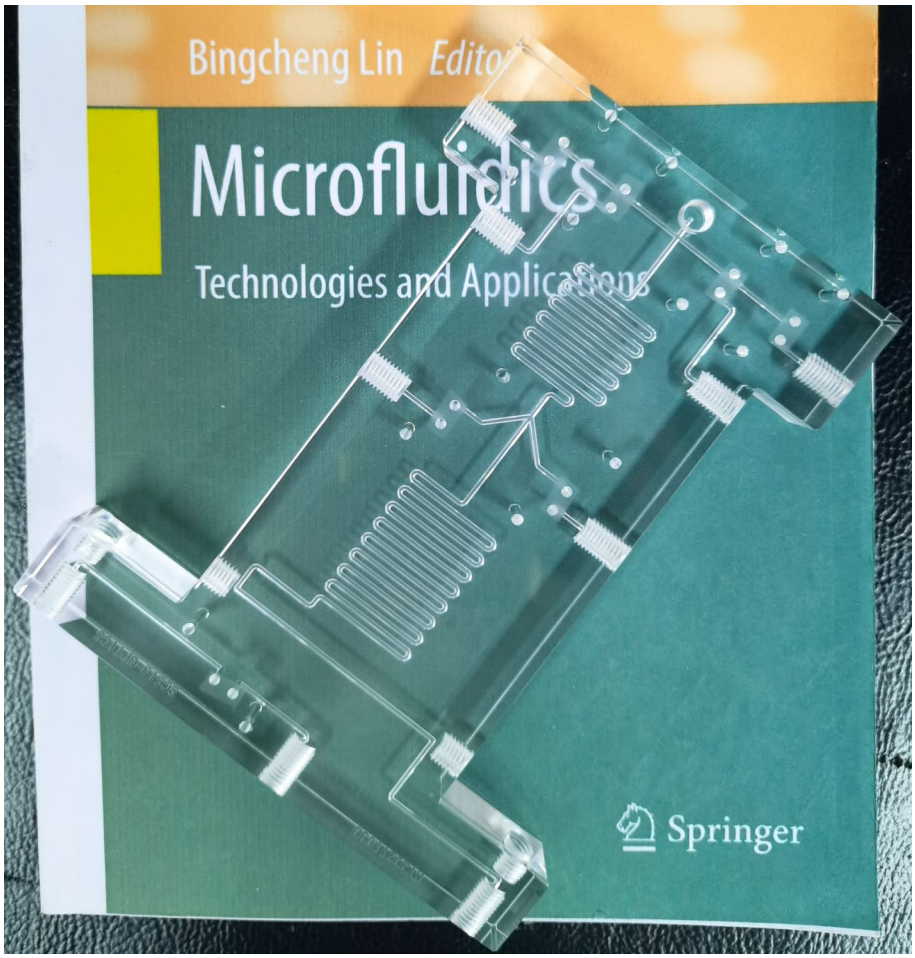


**Figure S1.** Chip photo.

**Section 2: Reagents preparation**

All reagents are analytical pure grade and prepared in ultrapure water (resistivity ≥ 18.2 MΩ•$cm^{-1}$) and were purchased from Guangzhou Chemical Reagent Factory.

| Solution | Preparation |
|---|---|
| **Sulfanilamide solution** | Dissolve 1 g sulfanilamide ($NH_2SO_2C_6H_4NH_2$) in 70 mL hydrochloric acid solution [V(HCI, ρ = 1.19 g•$cm^{-3}$) : V(ultrapure water) = 1 : 6] and dilute with ultrapure water to 100mL. |
| **N-nethylenediamine dihydrochloride (N.E.D.) solution:** | Dissolve 0.1 g NED ($C_{10}H_7NHCH_2NH_2$•2HCI) in 100 mL ultrapure water. |
| **Ammonium chloride ($NH_4Cl$) solution (buffer solution)** | Dissolve 10 g ammonium chloride ($NH_4Cl$) in 1000 mL ultrapure water and adjust the pH to 8.5 with about 1.5 mL of ammonia ($NH_3$•$H_2O$, ρ = 0.90 g•$mL^{-1}$). |
| **Hydrochloric acid (HCl) solution** | Measure 83.5 mL of hydrochloric acid (HCI, ρ = 1.19 g•$cm^{-3}$) and dilute to 500 mL with ultrapure water. |
| **Copper sulfate ($CuSO_4$) solution** | Dissolve 10 g copper sulfate ($CuSO_4$•$5H_2O$) in 1000 mL ultrapure water. |

## Section 3: Cadmium reduction column

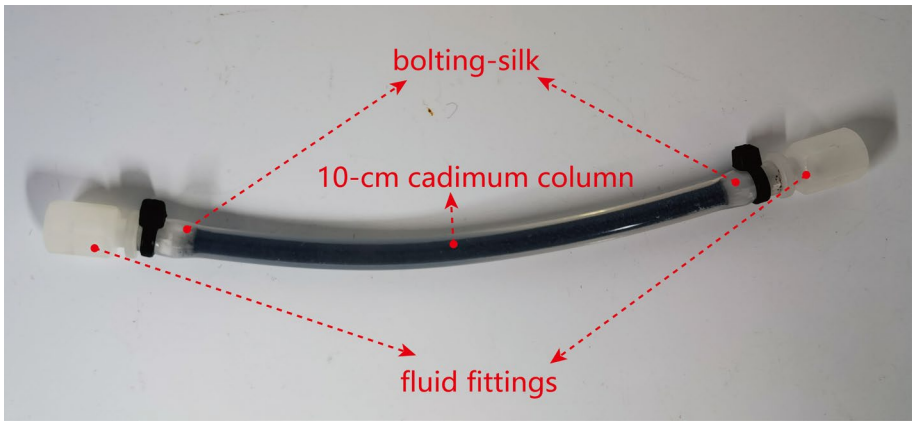


**Figure S2.** Photo of the cadmium reduction column.

### 3.1 Description of the reduction column

Both ends of the reduction column were connected with a 400-mesh (38 μm) Nylon bolting-silk and a fluid fitting to prevent granules from blocking the channel and to facilitate the connection. The reduction column was activated before detection.

### 3.2 Procedures and results of the reduction analysis

The ammonium chloride solution was used as the buffer solution. Reduction procedures for nitrate to nitrite in this study are based on the previous work [1] and described in the steps 19~22 of Table S1 (Section 4). 2 μM nitrate and nitrite standard solutions at salinity 35 were used to calculate the reduction ratio with the following equation:

$$Reduction = \frac{A(NO_3^-)}{A(NO_2^-)}$$

where $NO_3^-$ and $NO_2^-$ are the absorbance of nitrate and nitrite standard solutions, respectively. Figure S4 illustrates the variation of the reduction ratio of the reduction column with increasing detection times. The reduction ratio dropped to 95% after 46 detections and then reached 100% after reactivation.

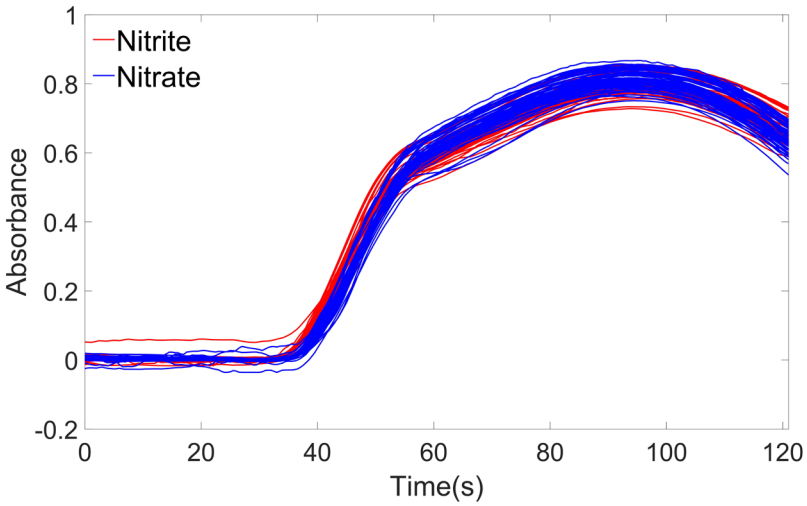


**Figure S3.** Absorbance of nitrite and nitrate standard solutions over time during the measurement step in the reduction experiments.

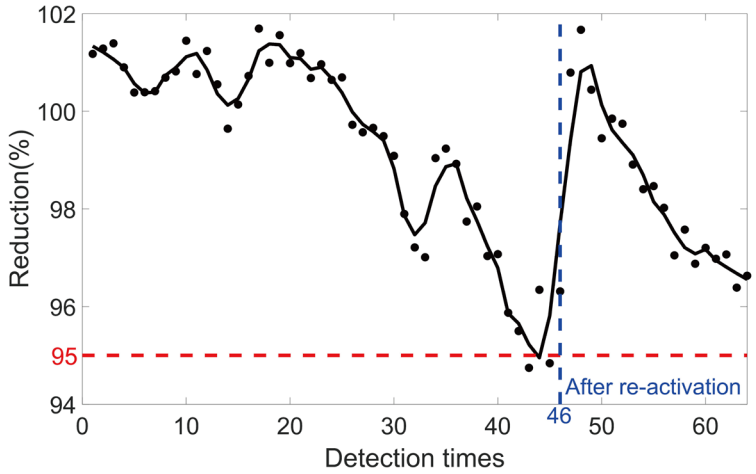


**Figure S4.** Variation in reduction ratio with increasing detection times. The blue dashed line at the position of 46 represents re-activation.

**Section 4: Analytical procedures**

**Table S1**. Analytical procedures of NOx with two standards (Based on Figure 1A).

| Step | Detection Object | Action | Pumps | Valves Open | Description | Loop |
|---|---|---|---|---|---|---|
| 1 | Standard 1 (STD1) | Flush | **P1:** Withdraw | V3 | Withdraw STD1. | Repeat 3 times |
| 2 | | | **P1:** Inject | V7 | Flush the flow channel with STD1. | |
| 3 | | Spectrum acquisition | / | / | The detection system adjusts the integration time (assumed as ***IT0***) of the spectrometer to keep the light intensity at 543 nm at a high level and then collects the spectrum at 543 nm **as the initial value: $I_0$**. | / |
| 4 | | Injection | **P1:** Withdraw | V5 | Withdraw sulfanilamide into the holding channel (H.C.) in a specified proportion. | / |
| 5 | | | **P1:** Withdraw | V6 | Withdraw N.E.D. into the H.C. in a specified proportion. | / |
| 6 | | | **P1:** Withdraw | V3 | P1 withdraws the STD1 to the maximum. | / |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | | Measurement | **P1:** Inject | V7 | Solutions in the H.C. are propelled by P1 through the reaction channel (R.C.), internal and external absorption cells, and the spectrum at 543 nm is simultaneously collected **(defined as $I_t$)** by the spectrometer with the integration time of ***IT0***. | / |
| 8 | \ | Clean | **P1:** Withdraw | V1 | Withdraw ultrapure water. | Repeat 3 times |
| 9 | | | **P1:** Inject | V7 | Flush the flow channel with ultrapure water. | |
| 10 | Standard 2 (STD2) | Flush | **P1:** Withdraw | V4 | Withdraw STD2. | Repeat 3 times |
| 11 | | | **P1:** Inject | V7 | Flush the flow channel with STD2. | |
| 12 | | Spectrum acquisition | / | / | Same as step 3. | / |
| 13 | | Injection | **P1:** Withdraw | V5 | Same as step 4. | / |
| 14 | | | **P1:** Withdraw | V6 | Same as step 5. | / |
| 15 | | | **P1:** Withdraw | V4 | P1 withdraws the STD2 to the maximum. | / |
| 16 | | Measurement | **P1:** Inject | V7 | Same as step 7. | / |
| 17 | / | Clean | **P1:** Withdraw | V1 | Same as step 8. | Repeat 3 times |
| 18 | | | **P1:** Inject | V7 | Same as step 9. | |
| 19 | Sample | Flush | **P2:** Withdraw | PV1 | P2 withdraws $NH_4Cl$ solution. | Repeat 3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 20 | | | **P2:** Inject | V2, V7 | P2 injects $NH_4Cl$ through the reduction column. | times |
| 21 | | | **P2:** Withdraw | / | P2 withdraws sample. | Repeat 3 times |
| 22 | | | **P2:** Inject | V2, V7 | P2 injects sample through the reduction column. | |
| 23 | | Spectrum acquisition | / | / | Same as step 3. | / |
| 24 | | Injection | **P1:** Withdraw | V5 | Same as step 4. | / |
| 25 | | | **P1:** Withdraw | V6 | Same as step 5. | / |
| 26 | | | **P2:** Withdraw | / | P2 withdraws sample to the maximum. | / |
| 27 | | Measurement | **P2:** Inject | V2, V7 | Solutions in the H.C. are propelled by P2 through the reaction channel (R.C.), internal and external absorption cells, and the spectrum at 543 nm was simultaneously collected. | / |
| 28 | | P1 return | **P1** | V7 | P1 returns to the initial position. | |
| 29 | / | Clean | **P1:** Withdraw | V1 | Same as step 8. | Repeat 3 times |
| 30 | | | **P1:** Inject | V7 | Same as step 9. | |

**Table S2**. Analytical procedures of nitrite (Based on Figure 3).

| Step | Detection Object | Action | Pumps | Valves Open | Description | Loop |
|---|---|---|---|---|---|---|
| 1 | Standard | Flush | **P1:** Withdraw | V3 | Withdraw STD. | Repeat 3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 | (STD) | | **P1:** Inject | V7 | Flush the flow channel with STD. | times |
| 3 | | Spectrum acquisition | / | / | Collect the spectrum at 543 nm as the initial valve. | / |
| 4 | | Injection | **P1:** Withdraw | V5 | Withdraw sulfanilamide into the holding channel (H.C.) in a specified proportion. | / |
| 5 | | | **P1:** Withdraw | V6 | Withdraw N.E.D. into the H.C. in a specified proportion. | / |
| 6 | | | **P1:** Withdraw | V3 | P1 withdraws the STD to the maximum. | / |
| 7 | | Measurement | **P1:** Inject | V7 | Solutions in the H.C. are propelled by P1 through the reaction channel (R.C.) and **interior absorption cells (5 cm)**, and the spectrum at 543 nm was simultaneously collected. | / |
| 8 | \ | Clean | **P1:** Withdraw | V1 | Withdraw ultrapure water. | Repeat 3 times |
| 9 | | | **P1:** Inject | V7 | Flush the flow channel with ultrapure water. | |

**Table S3**. Description of reactivation procedures of the cadmium column.

| Step | Action | Description | Loop |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 | HCl solution flush | P2 withdraws the HCl solution and injects it through the reduction column. | Repeat 3 times |
| 2 | $CuSO_4$ solution flush | P2 withdraws the $CuSO_4$ solution and injects it through the reduction column. | Repeat 3 times |
| 3 | $NH_4Cl$ solution flush | P2 withdraws the $NH_4Cl$ solution and injects it through the reduction column. | Repeat 3 times |
| 4 | Sample flush | P2 withdraws the Sample and injects it through the reduction column. | Repeat 3 times |

**Section 5: Optimization of SIA by orthogonal experiments (internal 5-cm absorption cell)**

### 5.1 Mixing method:

Moving the injected zones (sample/blank/standard and reagents) in the R.C. back and forth could achieve efficient mixing in SIA. As shown in Figure S5, the mixing method in the microfluidic system of this study are as follows: (i) the sulfanilamide (Reagent 1) and N.E.D. (Reagent 2) are sequentially injected into the H.C. in a certain proportion (Figure S5A), (ii) the zones in the H.C. are propelled forward to the R.C. (Figure S5B), and (iii) the mixing zones in the R.C. are moved back and forward for specific times by the syringe pump and then reside in the R.C. for a period of time for efficient mixing.

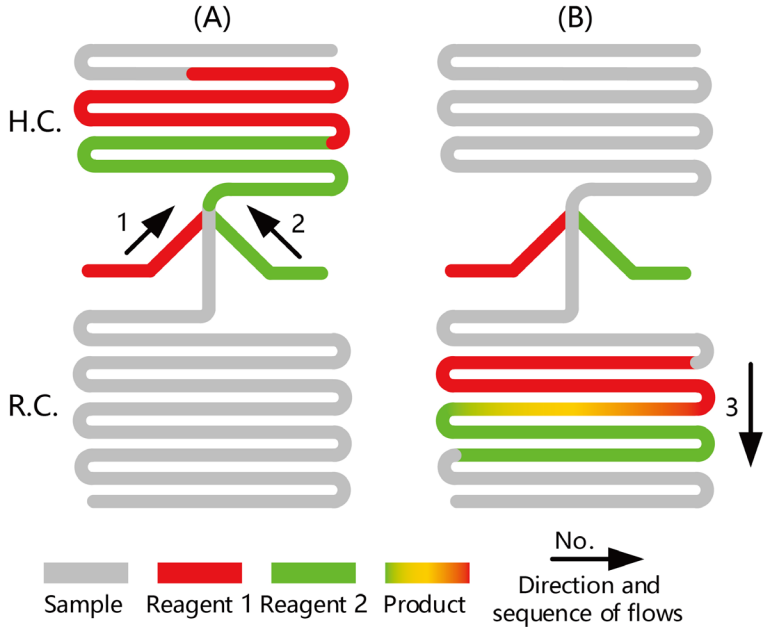


**Figure S5.** Schematic diagram of SIA regime in the micromixer of this study.

### 5.2 Description of the three key parameters:

- **Ratio**: the ratio of each reagent injected into the H.C to the volume of the syringe (volume of the H.C. is 20% of the syringe volume);
- **Reciprocation**: times of the reciprocating motion of the zones in the R.C.;

- **Residence time**: the residence time of the product after reciprocation.

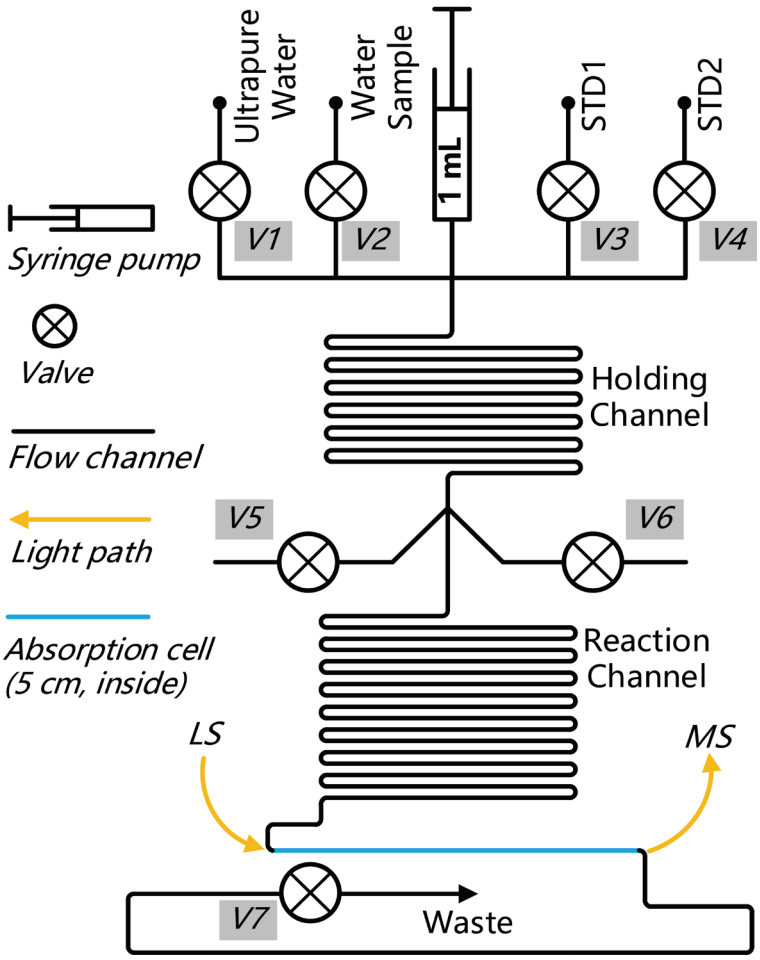


**Figure S6.** Fluidic pathway and control diagram of the microfluidic system for orthogonal experiments.

### 5.3 System setting:

In the orthogonal experiment, the ratio of each reagent was set as 1, 2, 4, 6 and 8 %, reciprocation was 0, 1, 2, 3, 4 and 5, and the residence time was 0, 30, 60, 120, 180 s. A 4 μM nitrite standard solution prepared with ultrapure water was used as the sample and the absorbance was measured by the internal 5-cm absorption cell. The speed of flow in the channel and absorption cell was stabilized at $10^{-3}$ m•s$^{-1}$ (P1 in Figure 1A takes 133.5 s for a full stroke, flow rate is 450 μL•min$^{-1}$) throughout the measurement (step 7, Table S2, Section 4), resulting in a stable and constant *Re* of 10.

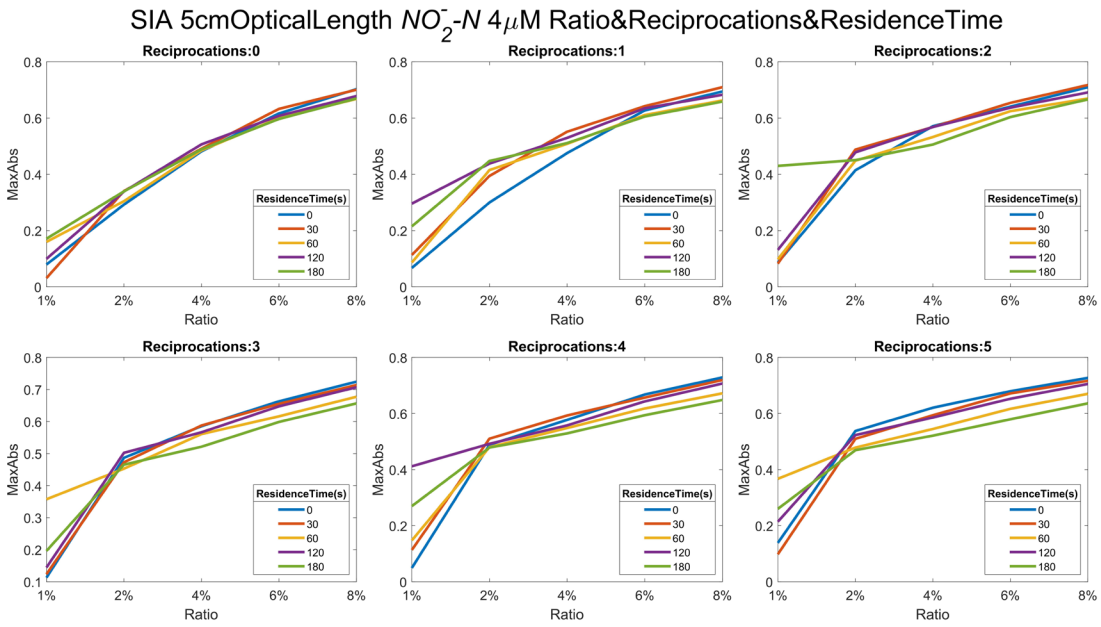


**Figure S7.** Changes of MaxAbs with ratios at different reciprocation and residence times.

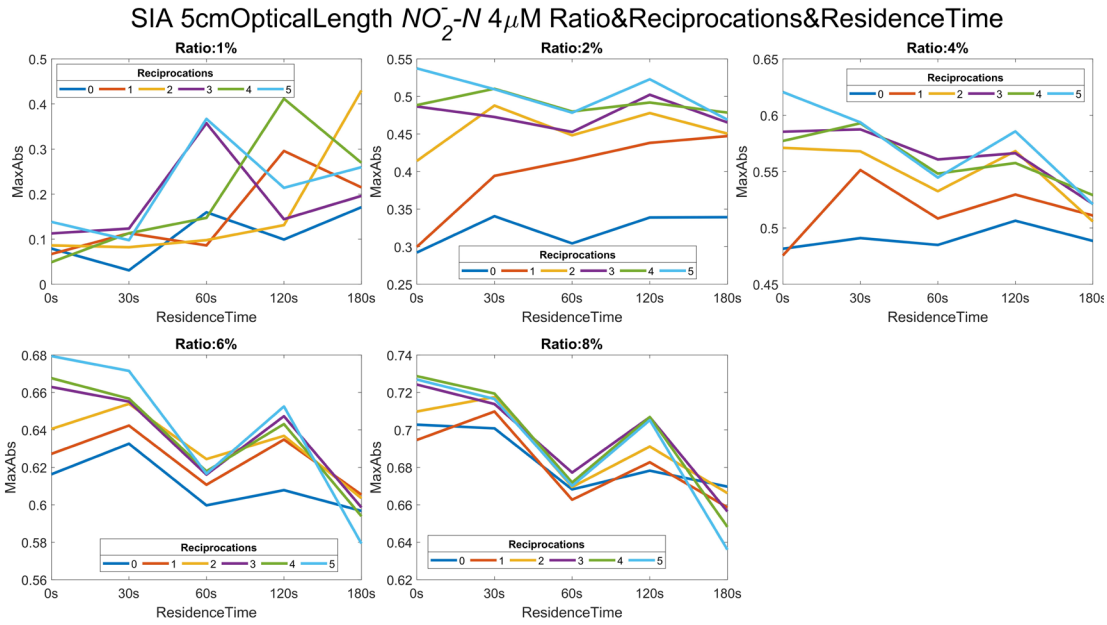


**Figure S8.** Changes of MaxAbs with residence times at different ratios and reciprocation.

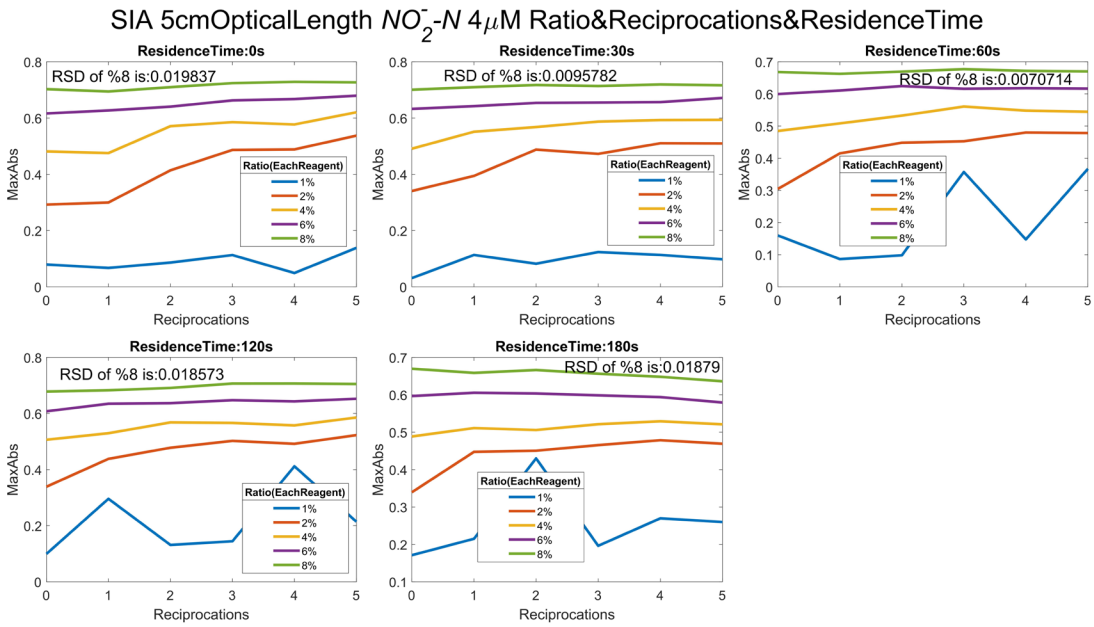


**Figure S9.** Changes of MaxAbs with reciprocation at different residence times and ratios.

**Section 6: Effect of salinity**

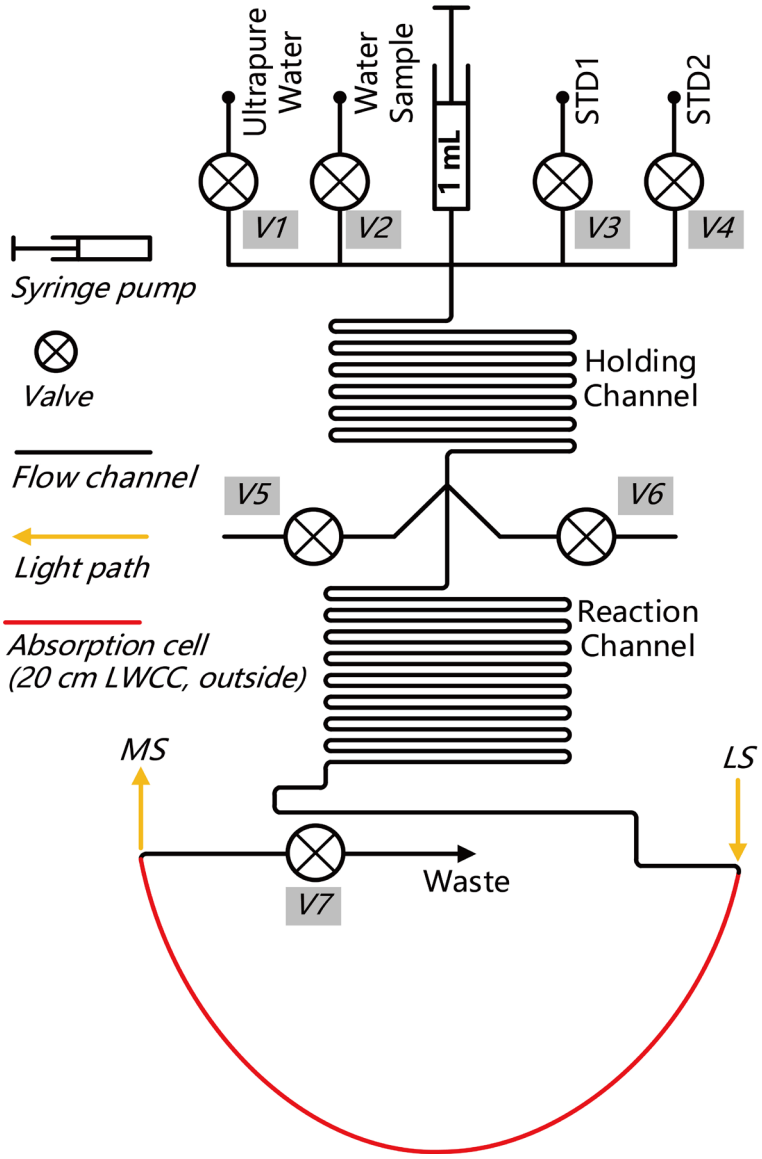


**Figure S10.** Fluidic pathway and control diagram of nitrite system with external 20-cm LWCC.

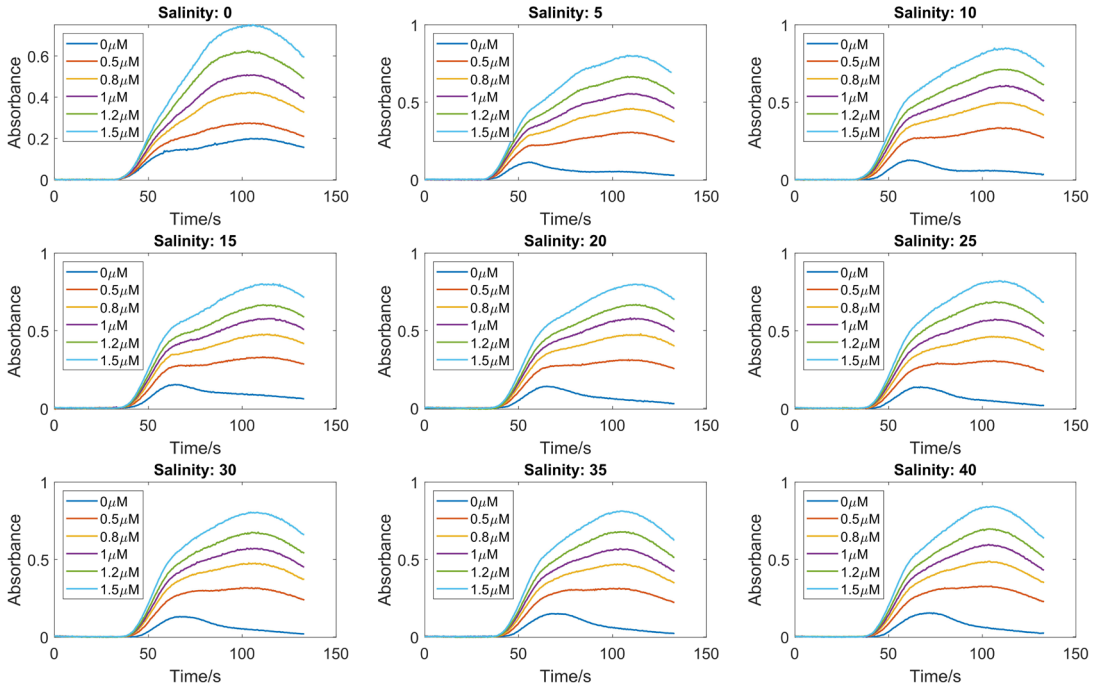


**Figure S11.** Absorbance of nitrite standard solutions with different salinities over time during the measurement step.

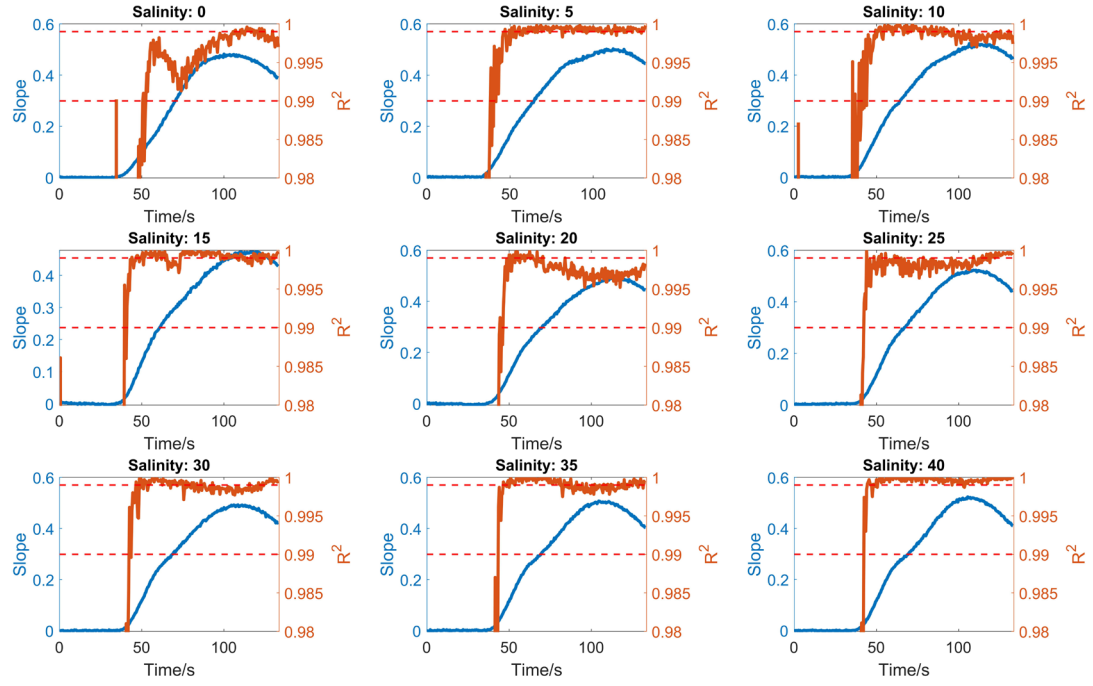


**Figure S12.** Curve fitting (CF) results of nitrite standard solutions with different salinities during the measurement step.

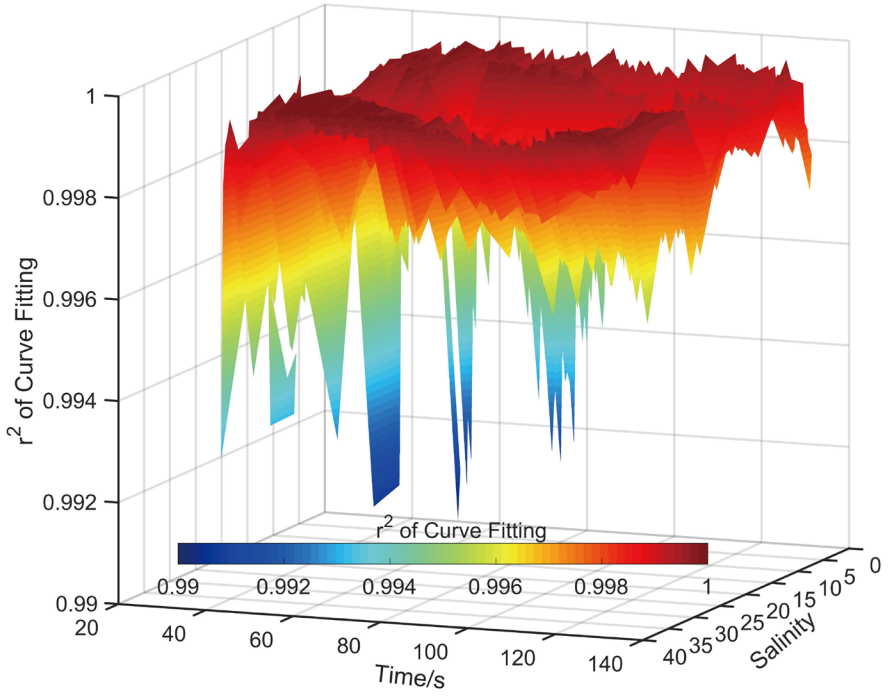


**Figure S13.** R square ($r^2$) of the standard curves for different salinities with an external 20-cm LWCC absorption cell.

**Section 7: Carryover effect**

The carryover effect of the 20-cm LWCC detection system was analyzed. Carryover of this system is determined by the times of flush with the ultrapure water after each detection (see 'Clean' action in the Table S1 and Table S2, Section 4). The method for detecting carryover and the calculation of carryover coefficient ($kco$) were in accordance with the method and equations in the previously published article [2].

A blank solution and a 2 μM nitrite standard solution were used as the low and high concentration samples, respectively, and both with a salinity of 35. Absorbance over time during the measurement step with flush times of 0, 1, 2, 3, 4 and 5 are plotted in Figure S14B. $kco$ measurements ($kco(Detection)$) and corresponding $kco$ to the LOD ($kco(LOD)$) for different flush times are shown in Figure S14A. The $kco(Detection)$ starts to be less than the $kco(LOD)$ after 2 flushes and stabilizes at 0.006 after 3 flushes. 3 flushes were programmed in the 'Clean' step after each detection to prevent measurements from the effect of carryover. The time consumption of 3 flushes is 6 min, total duration of 'Flush' and 'Clean' in Table S2 is 12 min.

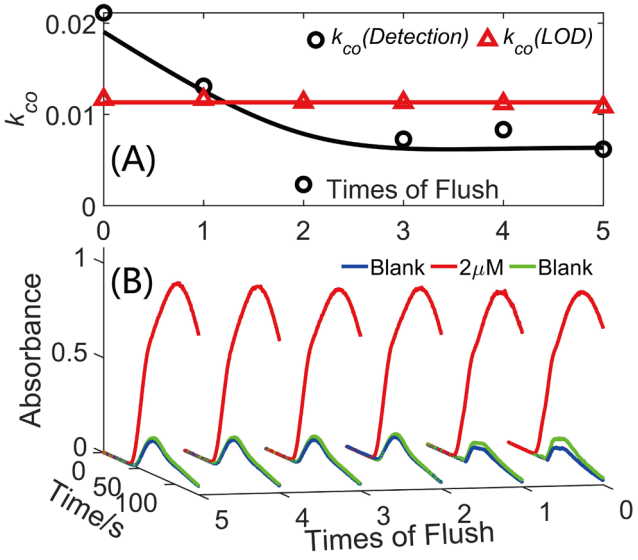


**Figure S14.** (A) Carryover effect of different flush times. (B) Variations of absorbance of different flush times with detection time under flow detection.

## Section 8: LOD calculation

The LOD is calculated by the following equation:

$$\mathrm{LOD} = \frac{3S_0}{k}$$

Where $S_0$ is the standard deviation of the blank samples for multiple consecutive determinations, and $k$ is the slope of the standard curve.

Nitrite standard solutions were used in LOD experiments, with unit of μM.

With the internal 5-cm absorption cell, the $S_0$ (n = 15) is 0.0027 (Figure S15) and $k$ is 0.0865. According to the above equation, the LOD is about 0.0936 μM.

With the external 20-cm absorption cell, the $S_0$ (n = 15) is 0.0025 (Figure S16) and $k$ is 0.5013. According to the above equation, the LOD is about 0.0150 μM.

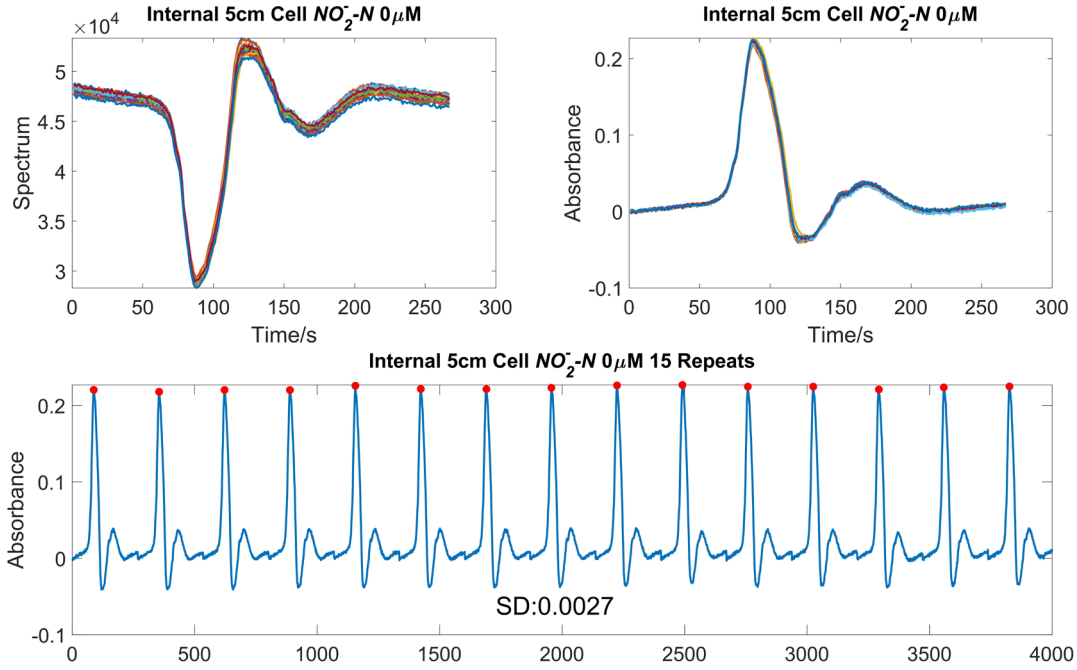


**Figure S15.** Results of 15 consecutive measurements of blank samples using the internal 5-cm absorption cell.

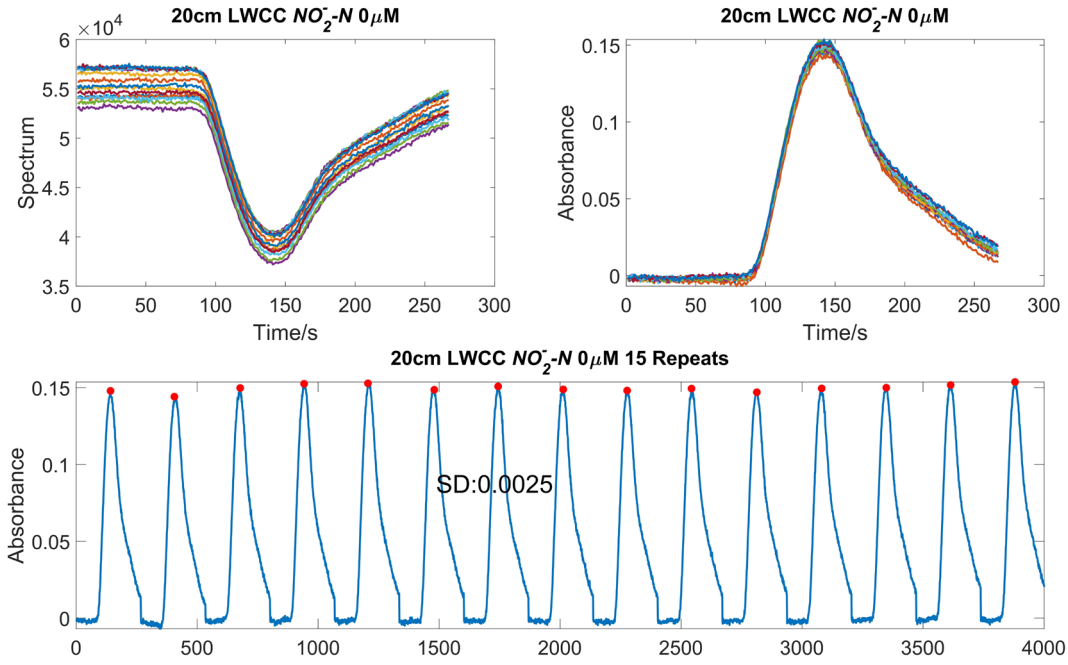


**Figure S16.** Results of 15 consecutive measurements of blank samples using the external 20-cm absorption cell.

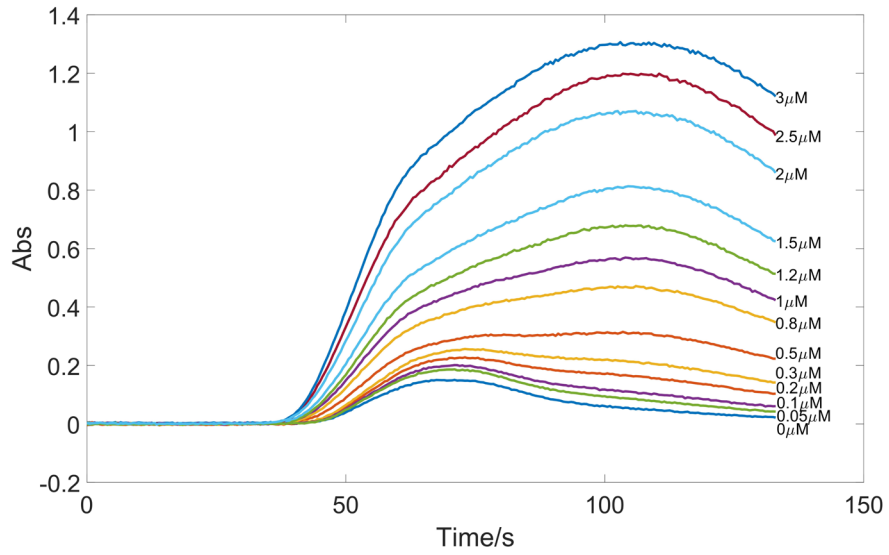


**Figure S17.** Absorbance over time of nitrite standard solutions with a concentration range from 0 ~3 μM and a salinity of 35 with the 20-cm LWCC detection system.

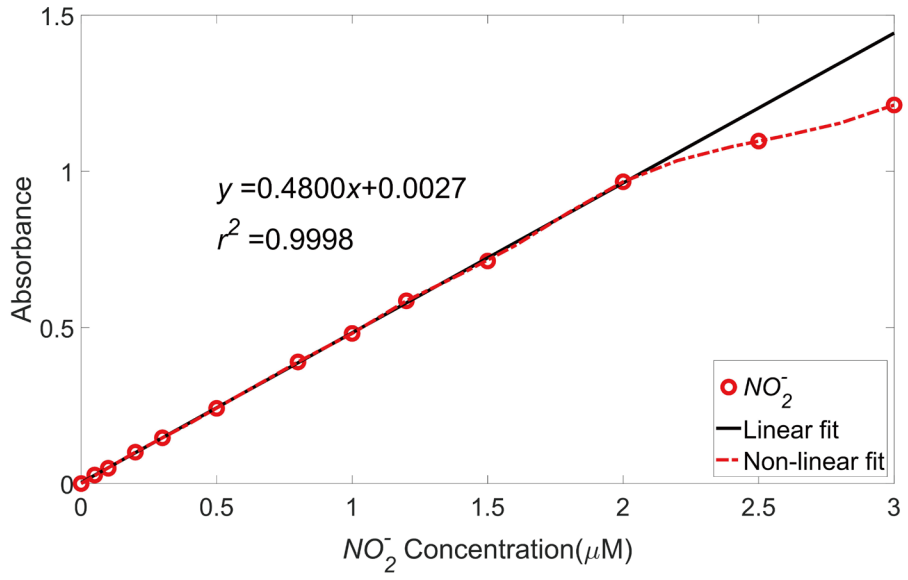


**Figure S18.** Linear regression for the external 20-cm LWCC absorption cell for nitrite standards.

## Section 9: Long-term trial in laboratory

### 9.1 Experimental details:

During the trial, a 0.5 and 1.5 μM nitrite standard solution prepared with ultrapure water were used as the low ($STD_L$) and high ($STD_H$) concentration calibration solutions, respectively. The samples were nitrite solutions with concentrations of 1 and 1.5 μM prepared from artificial seawater with salinities of 0, 5, 10, 15, 20 and 35. Analytical procedures were based on the Table S2 and Figure S7. The detection sequence was ultrapure water (blank), $STD_L$, $STD_H$ and sample solution (corresponding to *V1*, *V3*, *V4* and *V2* in Figure S7, respectively). Each detection requires 15 min for flush (6 min), analysis (3 min) and clean (6 min), and one complete automatic detection for blank, $STD_L$, $STD_H$ and sample took 1 h in this trial.

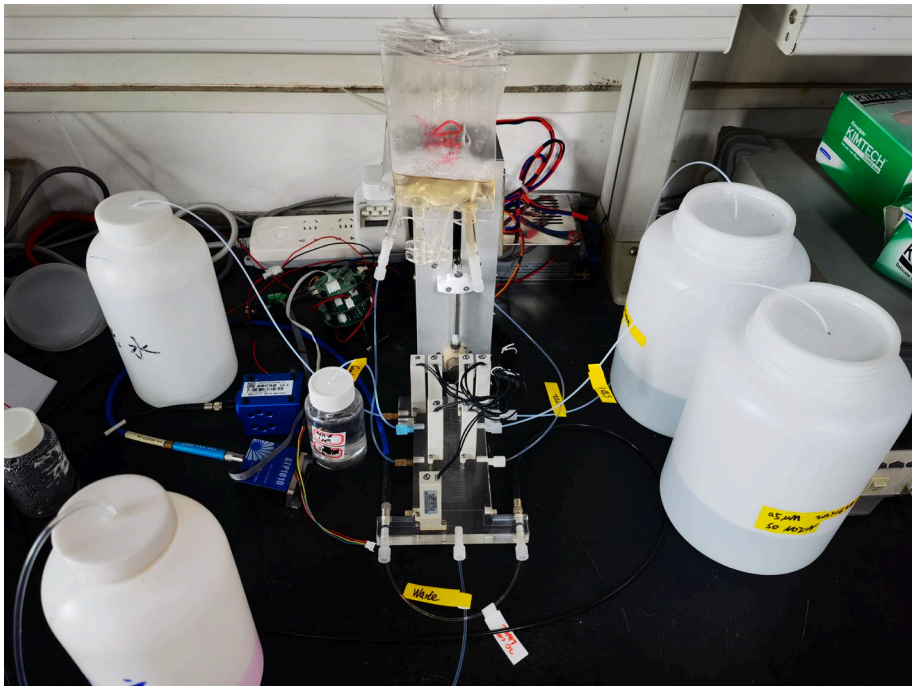


**Figure S19.** Photo of the system during the long-term monitoring trial in laboratory.

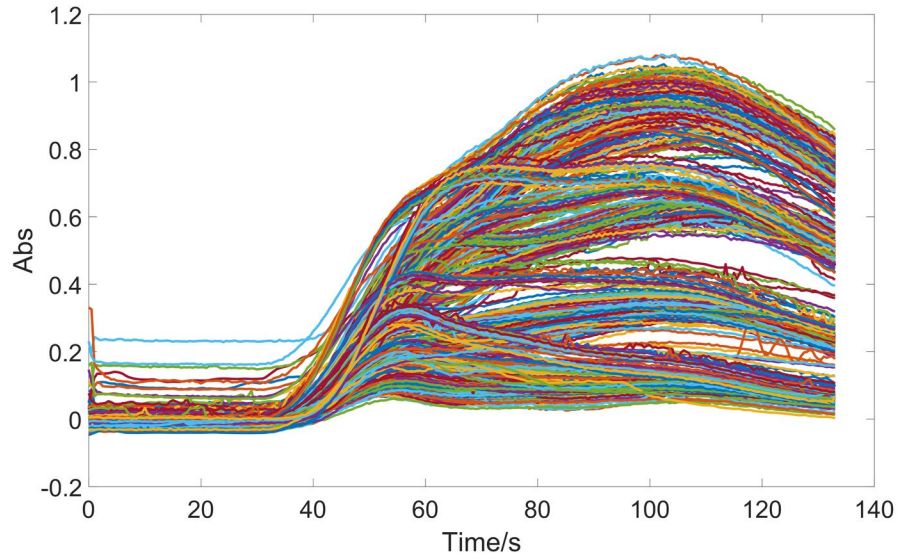


**Figure S20** Absorbance over time during the measurement step for all detections during the long-term monitoring trial in laboratory.

**Table S4.** Definitions of calibration methods for C1 ~ C5

| Method Number | Calibration Curve of Each Detection |
|---|---|
| C1* | Corrected CF by the Intercept offset of $STD_L$ |
| C2 | CF result of Blank and $STD_L$ |
| C3 | CF result of Blank and $STD_H$ |
| C4 | CF result of $STD_L$ and $STD_H$ |
| C5 | CF result of Blank, $STD_L$ and $STD_H$ |

***:** C1 calibrates the CF measured in the laboratory by one standard solution and then the sample concentration was calculated from the corrected CF and the absorbance of the sample, as applied in the previously published article [2].

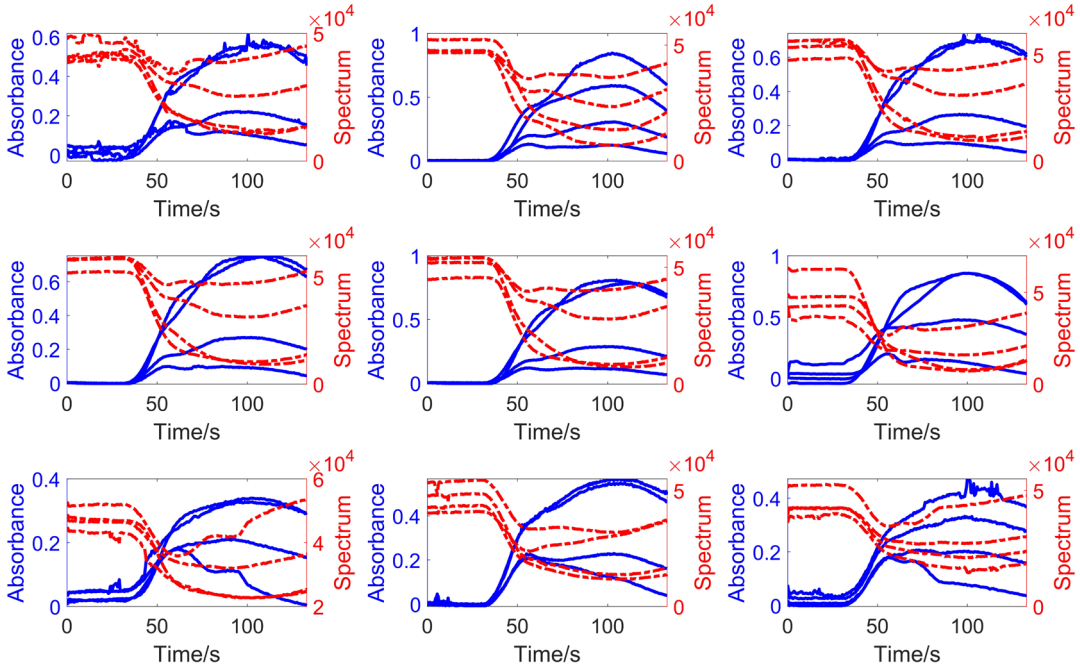


**Figure S21.** Absorbance and spectrum over time during the measurement step for the detections in the red box in Figure 3A.

## Section 10: Stations of “Xisha Scientific Research Cruise”

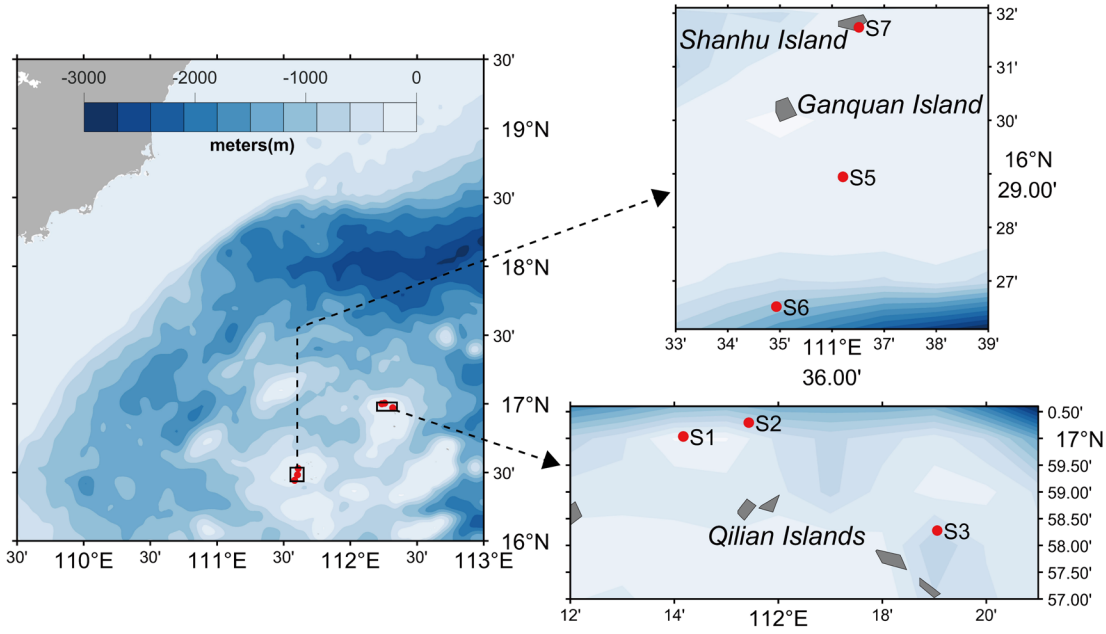


**Figure S22.** Stations map of the “Xisha Scientific Research Cruise”.

## Section 11: Fieldwork

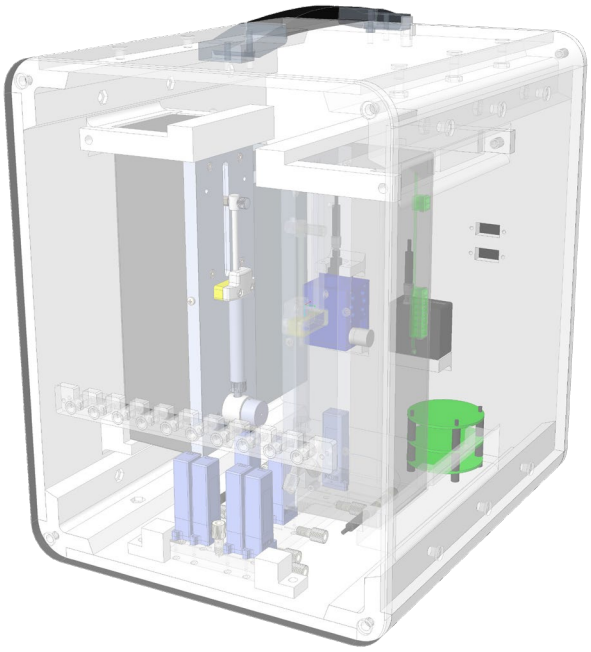

**Figure S23.** 3D model of the portable analyzer prototype for online determination of nitrite and nitrate in seawater.

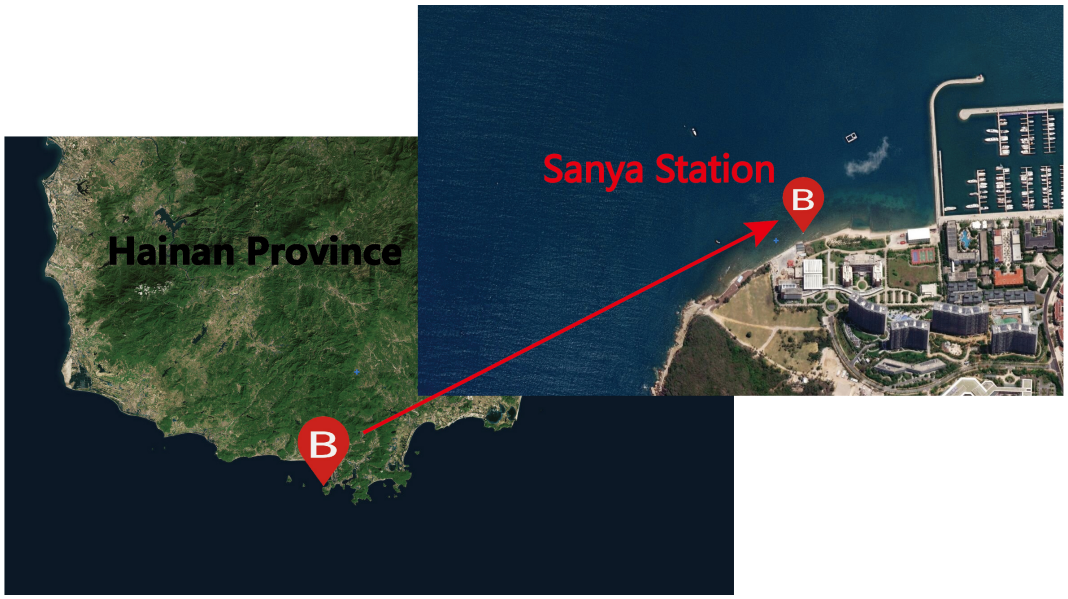


**Figure S24.** Location of Sanya Station.

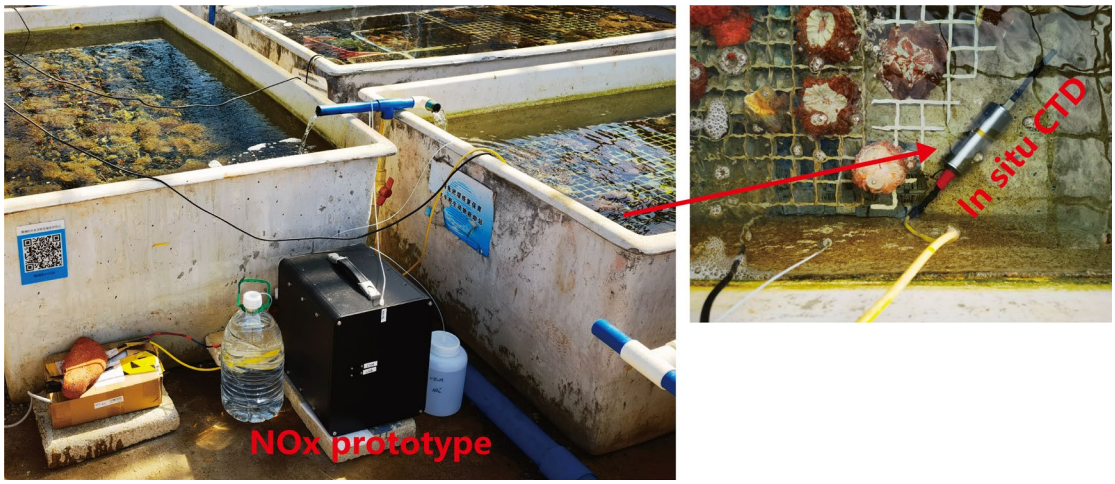


**Figure S25** Photo of the analyzer and self-contained CTD deployed at Sanya station.

### 11.1 CTD failure description

The hermetic shell of the self-designed CTD was made of 316L stainless steel, while the external magnetic switch was made of corrosion-resistant aluminum alloy. Since the galvanic corrosion between the switch and shell during the in situ deployment in seawater resulted in rot of the switch and subsequent corrosion of the interior of the CTD by seawater, salinity and temperature data are only available for the first week of the deployment and the date range is November 23th to November 30th, 2023.

**Section 12: References**

(1) Gui, J. The quick in situ measurement of nutrient in seawater and its applications. M.Sc. thesis, University of Chinese Academy of Sciences, 2014.

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