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Portable and automated fluorescence microarray biosensing platform for on-site parallel detection and early-warning of multiple pollutants

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ABSTRACT

The portable and automated fluorescence microarray biosensing platform (FMB) that employed a compact hybrid optical structure, microfluidics, and microarray biosensors was constructed for on-site parallel detection of multiple analytes. In the FMB, a hybrid optical structure that composed of a 1 × 4 single mode fiber optic coupler, four fiber optic switches, a single-multi mode fiber optic bundle coupler was at the first time developed for the transmission of the excitation light and the collection and transmission of multi-channel fluorescence signals. Through the control of fiber optic switches, the parallel fluorescence assay of four channels could be achieved using only one excitation light and one photodiode detector on the basis of the time-resolved effect. This optical design not only greatly increased the efficiency of light transmission and fluorescence collection and detection sensitivity of the FMB, but also allows the miniaturization and portability of the whole system because of few optical separation elements used and no requirement of rigorous optical alignment. Taking Microcystin-LR (MC-LR), 2,4-D, atrazine (ATZ), and bisphenol A (BPA) for example, the application potential of the FMB to rapidly and parallelly detect four typical pollutants in real water with high sensitivity and specificity was demonstrated. The limits of detection of MC-LR, 2,4-D, ATZ, and BPA were 0.04 μg/L, 0.09 μg/L, 0.02 μg/L, and 0.03 μg/L, respectively. The FMB could also achieve early-warning of pollutants thanks to its ability of rapidity, high-frequency, and multiple-analyte detection. The FMB has significant implications as a multiplexable, portable, rapid, and quantitative detection platform for pollution accidents and water quality management to satisfy the increasing demands of alerting and protecting civilians.

1. Introduction

Nowadays, monitoring requirements of environmental pollutants are ever-increasing because of their widespread distribution and frequent pollution accidents [1,2]. The great interest has been prompted in developing fast, portable, field deployable, and highly robust monitoring techniques, which may provide key information for early-warning and pollution control and treatment [2–4]. The quantification of pollutants has currently been limited to traditional analytical methods, such as gas chromatography and mass spectrometry. Although highly accurate and sensitive, these methods are generally associated with shortcomings of time-consuming pretreatment process, professional operations, sophisticated and costly instrumentation, thus failing to achieve the real-time, on-site, high-frequency monitoring of pollutants [3,5,6]. In remote areas or low-resource areas, samples have to be collected and sent back to laboratories owing to a lack of rapid and effective monitoring instruments. These inconvenient and low-efficient routine procedures are laborious, time-consuming, and expensive. Moreover, these delays may result in a failure to make prompt decisions for dealing with pollution accidents. As a result, location-based pollutant monitoring and data collection, especially for parallel detection of multiple pollutants, are still a great challenge for water management departments, and it is essential to develop field-deployable, rapid, low-power, cost-effective, and miniaturized analytical devices [5,7].

Optical biosensors are garnering significant interests because of their potential to provide sensitive and specific, real-time, high-frequency, and high-throughput detection of pollutants in complex matrices with minimal sample preparation [3,6,8,9]. Great advance in

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developing multiplexed optical biosensors platform for quantitative detection of multiple pollutants has been made through integrating evanescent wave fluorescence biosensors with microfluidic technology [9–11]. The evanescent wave, generated on the optical waveguide surface when the excitation light transmits via total internal reflection (TIR), induces the fluorophore emission [9,12]. Because of exponential decay of the field intensity of evanescent wave with distance (typical penetration depth < 100 nm), only the fluorescence-tagged biorecognition molecules bound to the biosensor surface can be excited. Therefore, the evanescent wave naturally confines transmissible fluorescence signals within a minimal distance from the biosensor surface, which can minimize the interference and contribution from the bulk solution, thus greatly improving the sensitivity and signal-to-noise ratio (SNR) [9,10]. The fiber optic and planar waveguide chip were the most commonly used transducers to develop the evanescent wave microarray biosensors. The remarkable cases were the RIANA and RAPTOR [9,10,13,14], in which employed a special planar waveguide chip or commercial fiber-optical microarray to achieve simultaneous detection of multiple pollutants. However, the optical detection systems of these instruments are expensive, complex, and inflexible, which requires sophisticated optical design and coordination of various optical elements (e.g. light sources, detectors, and lens) [3,10]. These make them difficult to miniaturize, and the reconfiguration of the optical detection systems is time-consuming and very expensive if the directions of any optical elements are inaccurate [13–15]. Therefore, developing novel fluorescence microarray biosensors with simple optical structure, that are portable, capable of multiplexed analysis, sensitive, and easy to use, still remains a great challenge [16–18].

Herein, a novel device, fluorescence microarray biosensing platform (FMB), was designed to meet the field-detection requirements of trace multiple pollutants based on evanescent wave fluorescence immunoassay principle, which allowed parallel measurements of up to four analytes. In this platform, a compact hybrid optical structure, employing a 1 × 4 single mode fiber coupler (SFC), four 1 × 1 fiber optic switches, and a single-multi mode fiber bundle coupler (SMFBC), is employed for transmission of excitation lights and collection and transmission of four channels fluorescent signals. Through the control of fiber optic switches, the fluorescence signals of four channels can parallelly be detected using only one excitation light and one photodiode detector based on the time-resolved effect. Taking four model pollutants (e.g. Microcystin-LR(MC-LR), 2,4-D, atrazine (ATZ), and bisphenol A (BPA)) for example, the ability of the FMB for highly sensitive detection of multiple pollutants was verified. MC-LR, a family of hepatotoxic cyclic heptapeptides, originates from the freshwater cyanobacteria [19,20]. The MCs exposure from surface and drinking water may result in animal-poisoning and human health diseases, even death. 2,4-D and ATZ are widely used pesticides that have been routinely detected in surface and drinking water [21,22]. BPA, a monomer for polycarbonate plastics, is a xenoestrogenic endocrine-disrupting chemical and has been widely monitored in the water and food [23,24]. Using the FMB, the rapid and parallel detection of four analytes with high sensitivity and specificity were achieved in one detection cycle without pre-treatment. This more comprehensive analysis generates a detailed analysis of the pollution state of water quality. The FMB may achieve automated data processing and generation of alarm signals when the concentration of pollutant exceeds the pre-set threshold value. The result data and the alarm signals are directly sent to the remoted control center or water quality management department through a wireless communication module.

2. Experimental section

2.1. Materials and chemicals

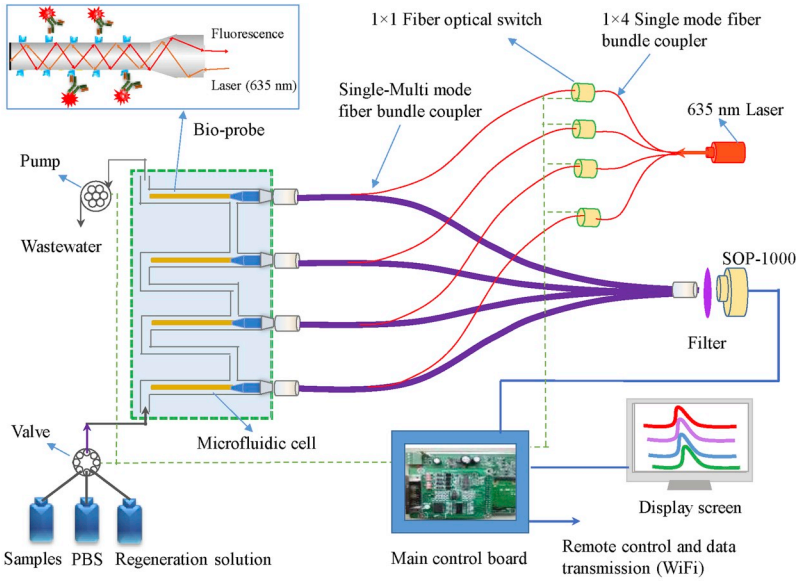
Atrazine (ATZ), Microcystin-LR (MC-LR), Bisphenol A (BPA), 2,4-D, (3-aminopropyl)-trimethoxysilane (APTES), bovine serum albumin (BSA), glutaraldehyde (GA), and sodium dodecyl sulfate (SDS) were supplied by Sigma-Aldrich Co. (Shanghai, China). H_2O_2, H_2SO_4, and all other reagents, unless specified, were purchased from Beijing Chemical Agents (Beijing, China). The SDS solution (0.5%, pH = 1.9) was applied for the regeneration of the biosensors.

Monoclonal anti-2,4-D, anti-ATZ, anti-MC-LR, and anti-BPA antibodies were from Reliance Co. (Beijing, China). The hapten-carrier proteins, such as BPA-OVA, 2,4-D-OVA, ATZ-BSA, and MC-LR-OVA, were developed by our research group. All antibodies were labeled by Cy5.5. The stock solutions (100.0 μg/mL) of 2,4-D, ATZ, BPA, and MC-LR were prepared using the methanol, respectively, and stored at 4 °C. The standard solution was prepared from its stock solution through serial dilutions in phosphate buffer solution (PBS, 0.01 M, pH = 7.4).

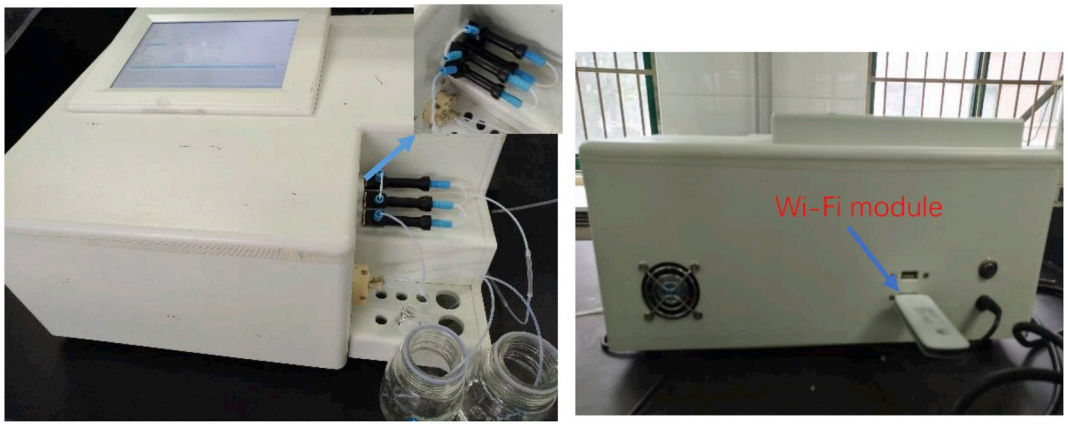
2.2. Design of portable fluorescence microarray biosensing platform (FMB)

The portable fluorescence microarray biosensing platform (FMB) is constructed for simple, sensitive, and parallel analysis of multiple targets, and its design scheme is shown in Fig. 1. The FMB consists of optical module, fluidics, data processing and control unit, and wireless communication module. In the optical module, a 635 nm miniature laser diode (LD, 10 mW, Huayuanstar Photoelectricity Co. China) is used as the excitation light source. The Cy5.5 labeled antibodies can be effectively excited by 635 nm excitation light and the corresponding maximum emission appears at 695 nm. A compact hybrid optical structure, including a SFC, four 1 × 1 fiber optic switches, a SMFBC, and four fiber optic biosensors, is used for the transmission of the excitation light resources and the collection of multi-channel fluorescent signals. This optical design not only greatly improves the efficiency of light transmission and fluorescence collection and the detection sensitivity and signal-noise-ratio (SNR) of the FMB, but also allows the miniaturization and portability of the whole system because of few optical separation elements used and no requirement of rigorous optical alignment. The LD connects with SFC, which has couple ratio of 25% and less than 0.15 dB insertion loss for each single mode optic fiber. The excited light sequentially enters four multi-mode quartz optical fibers (φ 600 μm, NA = 0.22) of SMFBC through its four single-mode optic fibers under controlment of fiber optic switch. The proximal end of each multi-mode optic fiber of SMFBC is linked to the proximal end of one fiber optic biosensor. The far end of four multi-mode optic fibers of SMFBC are combined as a fluorescence signal output port, from which the fluorescence signals of four channels can be sequentially detected by one photodiode detector (SOP-1000, Reliance Co. China). For achieving the parallel assay of the fluorescent signals of four channels, four fiber optic switches sequentially turn on (1-2-3-4-1 … …) and the excitation light enters one of four fiber optic biosensors in one time. The switching interval of fiber switches was set as 1s. The fluorescence molecules near to the biosensor surface are correspondingly excited by evanescent wave, and partial fluorescence couples back into the biosensor. The fluorescent signals of four channels are parallelly detected using one SOP-1000 on the basis of the time-resolved effect thanks to the use of fiber optic switches. Fiber optic switch is the key element in modern optic communication. We pioneer to apply it for constructing the fluorescence array biosensor platform due to its excellent light control capability. To remove cross-talk and reject scattered excited lights, a bandpass filter (Edmundoptics, US) is placed at the front of the detector SOP-1000.

The fluidics are composed of a pump, a valve, and four tandem microfluidic cells (φ 800 μm, 35 mm length) with one inlet port and one outlet port. The scheme of excitation light and fluorescence path of a fiber optic biosensor is demonstrated in inset of Fig. 1A. The photograph of microfluidic cells is shown in inset of Fig. 1B, respectively. Four fiber optic biosensors are respectively embedded in four microfluidic cells, respectively. A black peristaltic pump (Baoding Lange Co., China) is used to introduce various fluids, and the rate of flow was 30 μL/min when its speed was set as 10 r/min. The Six-position valve



(A)



(B) (C)

Fig. 1. Fabrication of FMB system. (A) Scheme of the FMB. The FMB applies an innovative incorporation of a compact optical structure with fiber optic switches, thus resulting in a powerful assay tool to parallelly detect four analytes on the basis of the time-resolved effect. (B) The forward photo of the FMB system, inset: four tandem microfluidic cells. (C) The back photo of the FMB system with a Wi-Fi communication module.

(VICI Valco, Interchim, USA) with one outlet port and six inlet ports is applied to control the selection of different fluids, such as buffer, samples, and regeneration solution. Both pump and valve are procedurally controlled through the user software.

To improve the storage and computing capacity of the FMB, a minicomputer is used. The analysis procedure (e.g. baseline, sample detection, and regeneration) of pollutants can be programmed in the user surface. The sample assay can be automatically performed according to a set procedure. The FMB can also perform data processing and communication. A wireless communication between the FMB and the host server is achieved using the Wi-Fi module (Huawei, China). Another key requirement of portable devices for in-field detection is maintaining low power-consumption when a continuous and prolonged monitoring is necessary, which provides useful and timely pollutant information to the end users. The FMB needs a power supply of only 12 V and power consumption of only 5 W, which can be provided by a portable power module that guarantees a duration of 6 h in experimental condition modality.

2.3. Functionalization of biosensor

The functionalization of biosensors was performed through immobilizing hapten-carrier proteins onto their surface according to a previous method with minor revision [15,16]. Briefly, the quartz optical fiber with the core diameter of 600 μm (NA = 0.22) with a 5.5 cm length is used to prepare biosensor. A 3.0 cm of the coating layer is removed to regarded as sensing section. The combined tapered fiber-optic biosensor is initially prepared by the HF acid based tube etching

method. After etching, the effective length and diameter of sensing section is about 3.0 cm and 225 μm, and the tapered part is about 0.3 cm. Then, the biosensors are treated by piranha reagent (H_2SO_4/H_2O_2, 3:1) for 1 h, and are washed. The biosensors are immersed in the APTES (2% in toluene) for 120 min, rinsed distilled deionized water, and dried in N_2. These biosensors are placed in heterobifunctional cross-linker solution (4% GA) for 60 min. Rinsing with PBS, the biosensors are functionalized using various hapten-carrier protein conjugates (1.0 mg/mL), such as BPA-OVA, ATZ-BSA, 2,4-D-OVA, and MC-LR-OVA for 100 min, respectively. The biosensors are immersed into BSA solution (2.0 mg/mL) for 120 min to block its nonspecific adsorption sites.

3. Results and discussion

3.1. Fabrication and characterization of optical module in the FMB

The fabrication of the FMB is a result of multidisciplinary cross and integration, which involves optics, biosensor, circuit design, computer, signal and data processing, and other technologies. One key technology of the FMB is its optical module. To simplify the structure of portable FMB, improve its efficiency of the optical transmission, and achieve the parallel detection of four targets, a compact optical system with minimum optical elements was constructed and response for the transmission of excitation light and collection and transmission of fluorescence of four channels. As previous described, the excitation light is divided into four quarter using a 1 × 4 SMFBC, and each single-mode fiber optic of SMFBC links with a 1 × 1 fiber optical switch. Therefore, four 1 × 1 fiber optical switches, consisted of one optical subsystem with its electrical driver interface, controls the excitation light to sequentially enter each fiber optic biosensor in four cells on the basis of time-resolved effect. This design is essential to achieve the parallel assay of multi-targets. The input port of one fiber optic switch connects with one single-mode optic fiber of the SMFBC and its output port connects with the single-mode optic fiber of the SMFBC. The optic fiber switches control the excitation light to enter one single-mode optic fiber of the SMFBC. The proximal end of each multi-mode optic fiber of the SMFBC links with one fiber optic biosensor placed in the microfluidic cell. When the excitation light enters one fiber optic biosensor, the evanescent wave excites the fluorescence labeled antibodies bound onto the biosensor surface. Partial fluorescence couples back to fiber optic biosensor and transmits in their corresponding multi-mode optic fiber of SMFBC and is detected using the SOP-1000. Taking use of the time-resolved effect, the fluorescence signals of four channels can be parallelly detected by the SOP-1000.

The feasibility of FMB to parallelly detect fluorescence signals of four channels was investigated. Four fiber optic sensors without modification were embedded into four microfluidic cells, and PBS and free Cy5.5 solution was sequentially introduced over four cells. When PBS was added, the four baselines were obtained because part of the excitation light was backed into the detector, which contributed to the Fresnel reflection and back reflection. The relative standard deviation (RSD) of all baseline signals in different Channels was less than 4.8% (Fig. 2A–E). When 1×10^{-7} mol/L Cy5.5 was introduced into four tandem cells and one fiber optic switch worked, the fluorescence signal of only one channel could be detected (Fig. 2A–D). However, when all fiber optic switches worked, four fluorescence signal traces were detected (Fig. 2E). As shown in Fig. 2E, the fluorescence signals of four channels could be efficiently discriminated using the switch interval of 1 s. These results indicated that the fluorescence signals of four channels could be parallelly detected without notable cross-talk effect based on the time-resolved effect through controlling fiber optic switches. Although it was difficult to quarter the beam split of SFC and to enable all fiber connectors and fiber optic sensors be consistent, the RSD of fluorescence signal peak values in four channels was less than 10%, indicating that the detected fluorescence signal of different detection channels was uniform. Furthermore, the RSD of the fluorescence signals of each channel in three tests was less than 6.5%, demonstrating that the FMB was very stable. It should be noted that a Si-based photodiode detector SOP-1000 was employed for fluorescence detection in the FMB. Previous study demonstrated that the resolution of SOP-1000 was 50 fW and was very suitable for low fluorescence detection. Moreover, it only needed power supply of 5 V, could tolerate electromagnetic field interference, and its price was less than 1/6 price of the PMT assembly (> 600 USD/ea) [17,18].

3.2. Parallel immunoassay principle of multi-pollutants using FMB

We next integrated FMB with optic fiber biosensors modified by various hapten-carriers to test the feasibility of FMB to parallel immunodetection of multiple pollutants based on indirect competitive immunoassay mechanism (Fig. 3A). The working principles of the FMB were as follows. Four biosensors modified by various hapten-carrier proteins, including MC-LR-OVA, 2,4-D-OVA, ATZ-BSA, and BPA-OVA, were placed into channel 1, 2, 3, and 4 in FMB, respectively. To achieve simultaneous detection of four pollutants, four fluorescence labeled antibodies, including anti-MC-LR, anti-2,4-D, anti-ATZ, and anti-BPA antibody, were mixed with four pollutants of various concentrations. During pre-incubation, the portion of fluorescence labeled antibodies specifically bound to their targets and the occupied binding sites of these antibodies were proportional to the concentration of pollutants in the sample. Then, the mixture was introduced into the microfluidic cells, and different fluorescence labeled antibodies with remaining binding sites bound with their respective hapten-carrier proteins (e.g. BPA-OVA, 2,4-D-OVA, ATZ-BSA, and MC-LR-OVA) modified onto the biosensors. The fluorescence labeled antibodies bound onto the biosensor surface were excited by the evanescent field, and part of fluorescence coupled back into the biosensor. The fluorescence signals of four channels were sequentially detected by the SOP-1000 based on the time-resolved effect through controlling fiber optic switches. In the absence of targets, the most antibodies bound with the biosensors, and the highest fluorescence signals were detected. The lower fluorescence signals were detected with increasing the concentration of targets because the more binding sites of antibodies were occupied by targets in solution and did not bind with hapten-carrier proteins immobilized onto the biosensors. After a detection cycle, the biosensors could be completely regenerated by SDS solution. The whole process, including baseline, sample, reaction, and regeneration, was less than 10 min.

To validate the performance of the FMB, we initially conducted the cross-reactivity analysis of various fluorescence-labeled antibodies, which was essential for the specificity and reliability of immunoassays. When one fluorescence labeled antibody (1.5 μg/mL) was added into the cells, high fluorescence signal was detected in only one channel containing its corresponding hapten-carrier proteins modified biosensor detected and few fluorescence signals were detected in other three channels (Fig. 3B–E). When the mixture of four fluorescence labeled antibodies was pumped over the cells, four fluorescence signal traces were obtained in four channels (Fig. 3F). Correspondingly, the fluorescence intensities of different channels were similar with those of one fluorescence-labeled antibody used. These results indicated that four antibodies exhibited high specificity for their respective antigens, and the cross-reactivity responses of different antibodies were few. That was to say, different fluorescence labeled antibodies specifically bound with their respective hapten-carrier proteins modified on the biosensors' surface. Moreover, few free fluorescence labeled antibodies in bulk solution was excited by evanescent wave because of its limited penetrated depth (< 100 nm). These results demonstrated that the FMB was qualified for surface-based immunodetection of multiple pollutants.

3.3. Parallel detection of multiple pollutants using the FMB

Quantification analysis of four pollutants was carried out using the

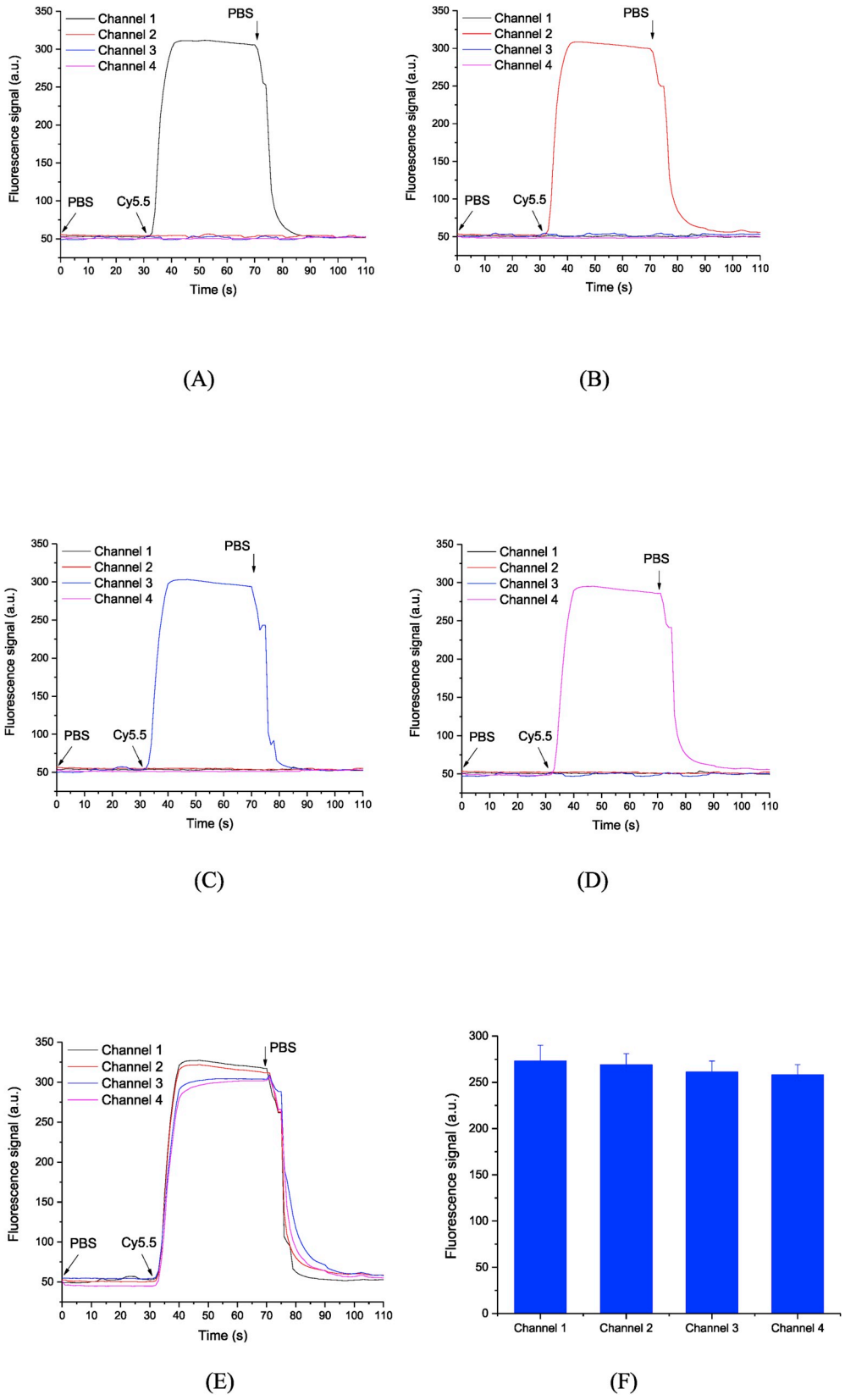
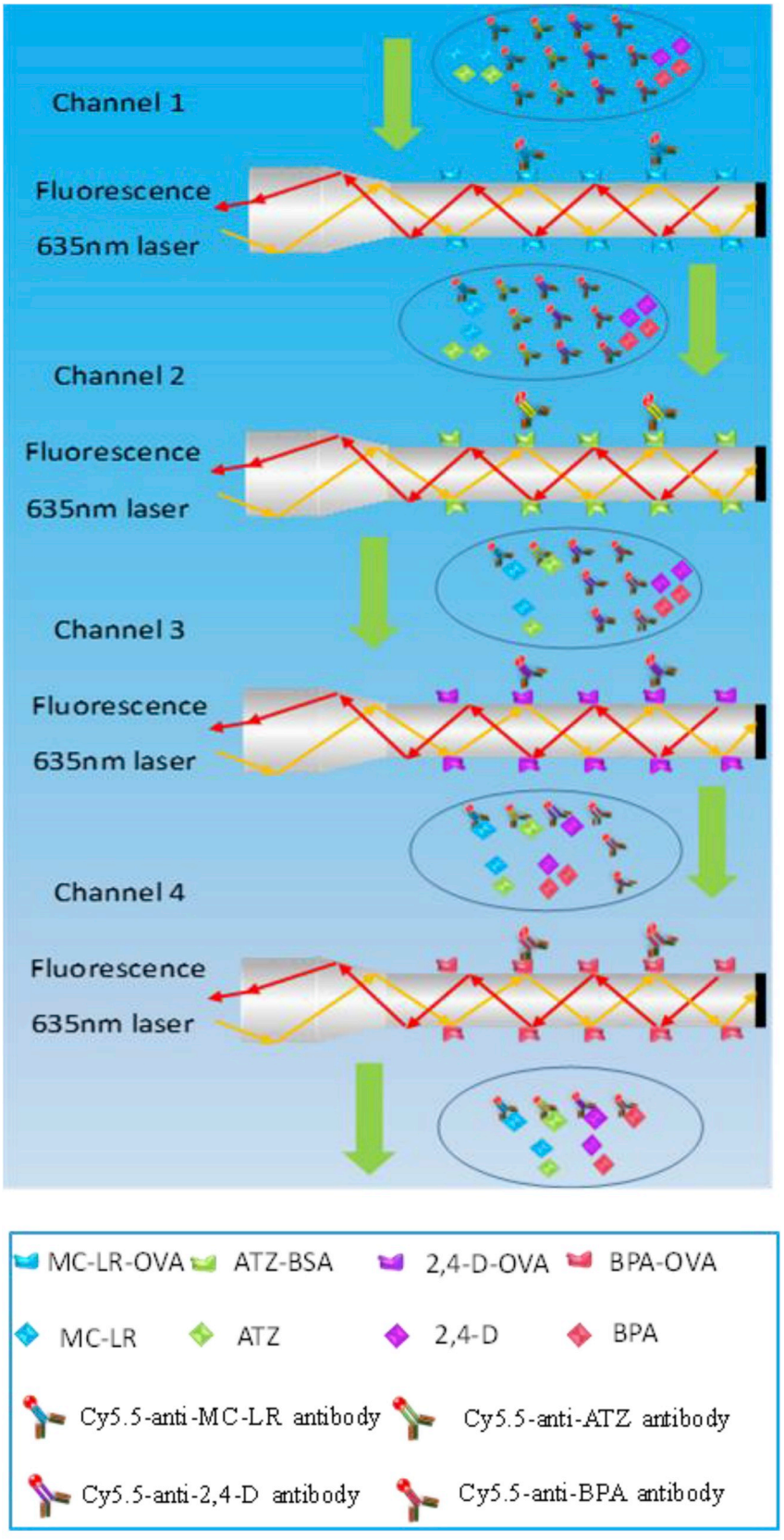


Fig. 2. Characterization of optical module in the FMB. (A–D) The fluorescence signal traces of four Channels when one fiber optic switch from Channel 1 to Channel 4 turned on, respectively, and 1×10^{-7} mol/L Cy5.5 was introduced into four tandem cells. (E) The fluorescence signal traces of four Channels when all fiber optic switches turned on and 1×10^{-7} mol/L Cy5.5 was introduced into four tandem cells. (F) The net fluorescence intensities of four channels in three tests (the concentration of Cy5.5 was 1×10^{-7} mol/L), calculated as subtracting the baseline value from the peak fluorescence intensity. Each experimental point is the average of three measurements and error bars are also shown.



(A)

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Fig. 3. The feasibility of parallel immunoassay of multiple pollutants using FMB. The biosensors modified by MC-LR-OVA, 2,4-D-OVA, ATZ-BSA, and BPA-OVA, were embedded into channel 1, 2, 3, and 4, respectively. (A) Scheme of parallel immunoassay of four pollutants using FMB. (B–D) The cross-reactivity analysis, and the typical fluorescence signal traces when one of four fluorescence labeled anti-MC-LR, ATZ, 2,4-D, and BPA antibody was pumped into four tandem cells. The concentration of all antibodies was 1.5 μg/mL. (F) The typical fluorescence signal traces when the mixture of four antibodies was introduced into the four tandem cells. The concentration of four antibodies used was 1.5 μg/mL. (G) The fluorescence intensities of four channels in the FMB when one fluorescence labeled antibody or the mixture of four fluorescence labeled antibodies were introduced into four tandem cells. Each experimental point is the average of three measurements and error bars are also shown.

FMB based on indirect competitive immunoassay principle. Four hapten-carrier-protein modified biosensors were embedded into four tandem microfluidic cells to regarded as biorecognition element as well as transducer, respectively. 80 μL sample containing four pollutants of various concentrations and 80 μL four fluorescence-labeled antibodies (1.0 μg/mL) was initially mixed for 4 min. The mixture was then introduced into four cells for 4 min reaction. The fluorescence signals of four channels were parallelly detected, and typical signal traces were demonstrated in Fig. 4A–D. The higher concentration of four pollutants in the sample resulted in more fluorescence labeled antibodies to bind with free pollutants in solution and to prevent the antibodies from binding to the biosensor surface. Therefore, the increasing concentrations of four pollutants resulted in the proportional decrease of the fluorescence intensity in their corresponding channels. To obtain dose-response relationships of targets, the net fluorescence intensity F was calculated through subtracting baseline value as follows.

$$F = \text{peak fluorescence intensity} - \text{baseline fluorescence intensity} \quad (1)$$

The dose-response curves of four pollutants were normalized with expressing the fluorescent intensities of each sample as a ratio to the fluorescent intensity of blank sample without pollutants. The normalized value is calculated as following.

$$I = F_s/F_b \quad (2)$$

where F_b is the net fluorescent intensity of the blank sample, and F_s is the net fluorescent intensity of the samples. The dose-response curves of four pollutants are plotted against the concentration logarithm of four pollutants using a four-parameters logistic equation (Supporting information), respectively. In Fig. 4E, all of these error bars are the RSD of the data points ($n = 3$) that are less than 5.3%, demonstrating a good stability of the FMB to detect multiple pollutants.

For a novel analyzer, the LOD and the dynamic range are two key parameters to embody its performance. From Fig. 4, the LODs were 0.04 μg/L for MC-LR, 0.09 μg/L for 2,4-D, 0.02 μg/L for ATZ, and 0.03 μg/L for BPA, which were evaluated using three times of the standard deviation of the blank sample [25,26], and all of them met the requirements to detect these pollutants in the drinking water standard of China (GB 5749–2005). The dynamic detection ranges are 0.09 μg/L ~109.6 μg/L for MC-LR, 0.18 μg/L ~99.8 μg/L for 2,4-D, 0.04 μg/L ~110.5 μg/L for ATZ, and 0.05 μg/L ~110.1 μg/L for BPA, which were calculated considering the target concentration range between 10% and 90% of the dynamic single range [27]. The performance of the FMB was highly comparable with those of other biosensors (Table S1) [13,21,28–33], validating its high sensitivity for the parallel detection of multiple pollutants. The FMB, compared with other array biosensors [9,10], has a simpler and more compact optical structure, which allows the FMB to be more portable, miniaturized, and practicable, which are essential for on-site rapid detection of multiple pollutants. In additional, the use of reusable biosensors also enhances detection accuracy and practical applicability and reduce the detection cost [26].

3.4. Practical application of the FMB

Achieving highly sensitive and rapid on-site detection of pollutants without any pre-treatment is essential for early-warning of pollution accident and making prompt treatment decisions.[3] The attractive performance characteristics of the FMB hold great promise for rapid on-site detection and early-warning of pollution threats to take a rapid countermeasure action. To investigate application potential of the FMB to measure multiple pollutants in real water samples, the FMB was brought to the Yellowstone section and Yichang section in the Yangtze river by a vehicle (Fig. S1, inset: left) and a boat (Fig. S1, inset: right), respectively. The on-site detection of four pollutants in the Yangtze river, the biggest river in China, was performed. Because the concentrations of four pollutants in real samples were lower than the LOD of the FMB and HPLC, the recovery experiments of the spiked samples were performed to evaluate the feasibility of FMB in detecting real water samples. Four pollutants of three concentrations were parallelly spiked into two water samples. The spiked water samples were mixed with four fluorescence labeled antibodies and detected as described method above. The recoveries of the spiked water samples ranged from 80.0% to 120.0% (Table 1). Satisfactory variations were also showed with RSDs of less than 8.0%. These demonstrated the satisfactory accuracy of the FMB and a negligible interference from the real water samples. Furthermore, the detection antibodies prepared with antibody diluent solution exhibited sufficient stability in room temperature, and retained higher than 96% and 93% of their original responses after 10 days and 20 days, respectively (Fig. S2). The fiber optic biosensors could retain more than 100 times assay with few significant activity loss (< 5% decrease) (Fig. S3). These are important for the practical application of the FMB for pollutant detection.

In our software, the limit values of various pollutants are held depending on pollutant type and sampling site location. The detection value of various pollutants obtained by the FMB are real-time recorded and a comparison of these values with their respective limit values is performed, respectively. An observed increase in the detected pollutants will raise the alarm on a possible contamination event. The detection results and automatic alert messages will be real-time transmitted wirelessly to server station via the wi-fi communication. In the future, it is possible to use multiple portable biosensors to construct a large detection system based on a wireless network. With different biosensors in each device, the whole system will exhibit more robust and powerful performances, and discriminate more types of pollutants. Such on-site detection and rapid warning are benefit for timely decontamination and are greatly expected to protect our civilians and environment.

4. Conclusion

In summary, real-time monitoring is essential to understand multiple water quality parameters of pollution water and take remedial action as and when the need arises. Here, the FMB was constructed for the real-time parallel monitoring of multiple analytes and now field-ready for rapid and portable deployment. The integration of the compact optical structure, microfluidics, and array biosensors allowed the miniaturization of the FMB systems. The optical structure of the FMB greatly improved light transmission and fluorescent collection efficiency and its detection sensitivity. The ability of the FMB to inexpensively, quantitatively, and rapidly detect multiple pollutants with a high sensitivity and without the need of pre-treatment was exhibited, which represents a significant improvement over current pollutant screening technologies (e.g. test strips, ELISA). Moreover, the FMB capable of determining multiple analytes allows a reduction in time and in reagent and sample consumption. Other characteristics are that one photodiode detector is used to parallelly detect fluorescent signals of multiple channels on the basis of the time-resolved effect, the use of

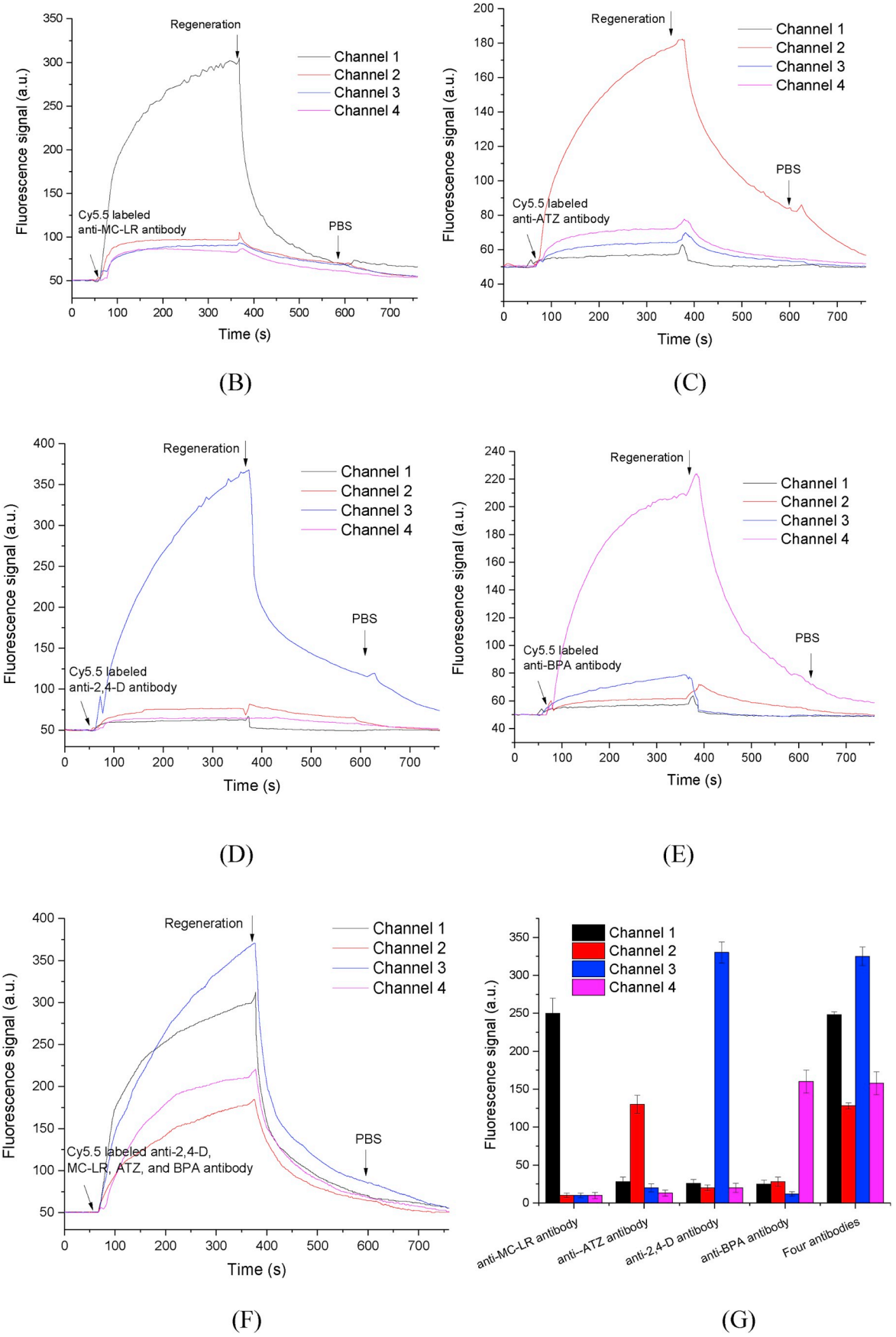

Fig. 3. (*continued*)

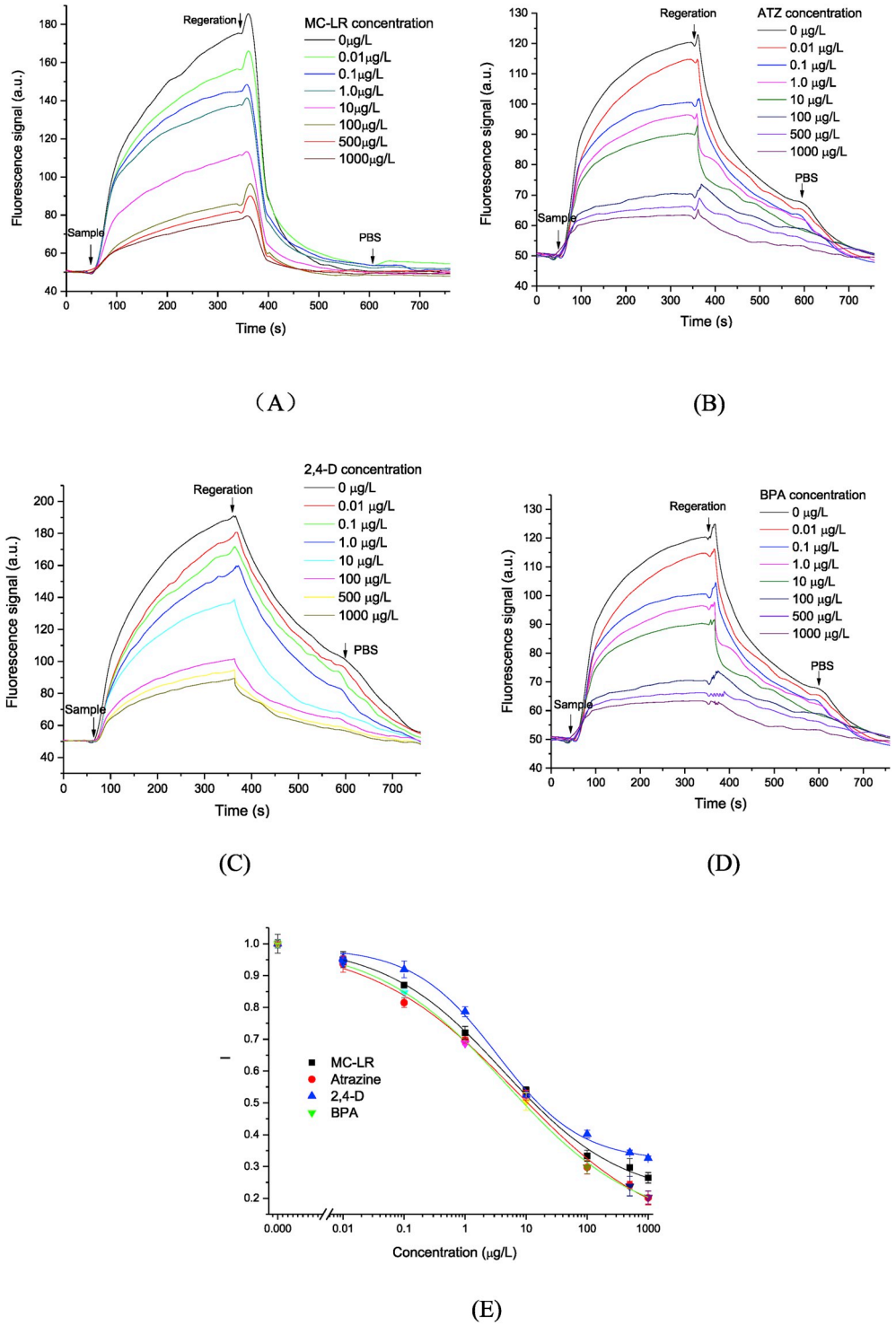

Fig. 4. Parallel detection of four pollutants using the FMB. (A) Typical fluorescent signal traces of MC-LR detection (1.0 μg/mL anti-MC-LR antibody); (B) Typical fluorescence signal traces of ATZ detection (1.0 μg/mL anti-ATZ antibody); (C) Typical fluorescence signal traces of 2,4-D detection (1.0 μg/mL anti-2,4-D antibody); (D) Typical fluorescence signal curves of BPA detection (1.0 μg/mL anti-BPA antibody); (E) Dose-response curves of four pollutants. Each experimental point is the average of three measurements and error bars are also shown.

reusable biosensors enhances detection accuracy and practical applicability, automated data processing and generation of alarm signals can provide rapid early-warning information. All of these were very important in environmental pollutant accidents, especially in remote or resource-limited areas. In a word, the FMB has significant implications as a rapid accessible quantitative assay tool for accidents and routine water quality detection to satisfy the increasing demands of alerting and protecting civilians against possible direct exposure to pollution threats. Although our focus was on monitoring of pollutants in water, the proposed technology could be also used for the parallel detection of multiple analytes in food and in medical diagnosis through using fiber optic biosensors modified by other hapten-carrier proteins and their corresponding fluorescence labeled antibodies.

Table 1
Recovery of the proposed methods towards MC-LR, 2,4-D, ATZ, and BPA in real water samples originated from Yichang section and Yellowstone section in the Yangtze river, respectively.

Samples	pollutants	Spiked (μg/L)	Detected (μg/L)	RSD (%)	Recovery (%)
The water samples from Yichang section	MC-LR	0.5	0.55	2.49	110
		1.0	1.08	1.04	108
		2.0	1.96	5.01	98
	ATZ	2.0	2.24	5.25	112
		5.0	4.86	3.43	93
		10.0	10.9	1.26	109
	2,4-D	1.0	0.9	2.97	90
		10.0	10.7	7.86	107
		20.0	21.6	3.17	108
	BPA	1.0	0.91	5.04	91
		5.0	5.63	1.49	113
		10.0	8.4	3.89	84
The water samples from Yellowsone section	MC-LR	0.5	0.6	5.72	120
		1.0	0.98	7.47	98
		2.0	1.72	2.38	86
	ATZ	2.0	2.36	7.33	118
		5.0	5.79	4.22	116
		10.0	8.0	5.71	80
	2,4-D	1.0	1.10	6.03	110
		10.0	8.4	4.83	84
		20.0	20. 8	3.48	104
	BPA	1.0	1.16	4.89	116
		5.0	5.24	3.44	105
		10.0	8.61	4.86	86

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.120650.

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