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An array fluorescent biosensor based on planar waveguide for multi-analyte determination in water samples

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ABSTRACT

A planar waveguide-based array immunosensor (PWAI) was described, allowing measurements of up to twenty-four analytes in eight separate channels rapidly, sensitively and simultaneously. In this system, a linear laser light created by a line generator was coupled into a planar optical waveguide via a beveled angle, forming eight individual total internal reflection (TIR) lines. A multi-channel microfluidics cell was employed to isolate the parallel TIR lines physically so as to form eight independent flow channels on the same chip, avoiding the cross-reactivity of antibodies and supporting various bioassay conditions. By employing fluorescent detection with fluorophore-labeled antibodies binding to the surface of the waveguide with the analyte derivative covalently attached, the array immunoassays can realize the multi-analyte biosensing. A model was proposed to guide the design of such a planar waveguide-based evanescent wave biosensor. The proposed system was confirmed with the comparable sensitivity with previously reported waveguide biosensor. In addition to being a multi-channel analytical device for the highly sensitive detection of contaminants, the proposed evanescent wave PWAI can provide the dynamic surface-based biomolecular interaction information regarding the affinity and kinetics with high sensitivity.

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1. Introduction

Optical biosensors have received much attention for decades because of the remarkable advantages such as robustness, immunity to electromagnetic interference, high sensitivity and selectivity, easy integration, and potential portability for in situ and onsite measurements [1–3]. Among them, evanescent field fluorescent biosensors have drawn special attention because of their easy access to miniaturization and for their high sensitivity and selectivity [2,3]. In this type of biosensor, evanescent wave serves as excitation energy to induce fluorophore emission, which can be detected and is proportional to analyte concentration in the sample. Theoretically, sensitivity and selectivity are remarkable natural benefits offered by the combination of evanescence wave and fluorophore labeling. The field intensity of evanescent wave decreases exponentially with distance away from the interface into the lower refractive index material, which selectively excites only the dye molecules adsorbed, attached, or bound to the sensor surface. Evanescent wave essentially confines transducible optical signals within a discrete distance from the sensor surface, minimizing interference or contribution from components in the bulk phase of lower index medium and thus is more sensitive to its analyte [4]. Moreover, the excitation laser beam is away from the biosensing region of waveguide surface via total internal reflection (TIR), thereby easily distinguishing the fluorescent signal from the excitation light and enabling the device with high sensitivity and low detection limit [5–8].

Since the pioneering study of Kronick and Little on the use of evanescent wave excitation for fluorescence immunoassay [9], biosensors based on the principle of evanescent wave-induced fluorescence represent a fairly mature yet still expanding and exciting field of research. The application of planar waveguide transducer to construct evanescent wave optical biosensor shows several practices, possibilities, and prospects in the development of systems capable of multiple analyte detection in a single sample [10]. As a result, many of the landmark studies on evanescent wave fluorescent biosensor based on planar waveguide have been conducted in the past years. Among them, a simple geometry is widely adopted

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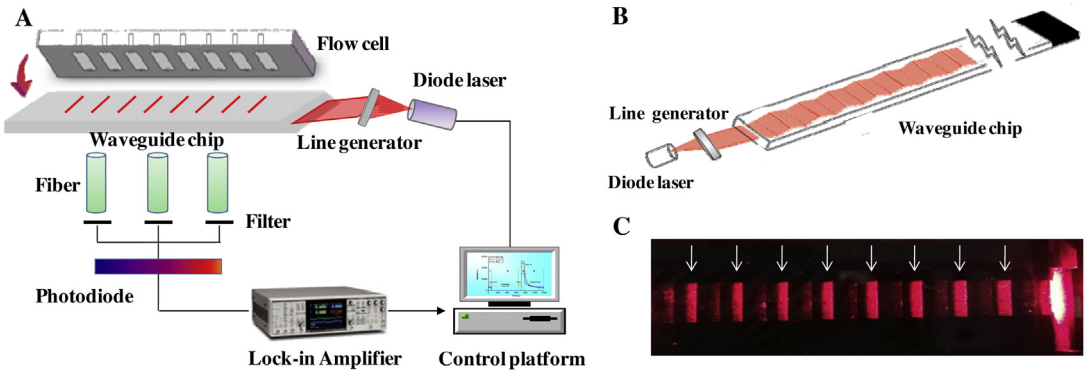

Fig. 1. Schematic diagram of (A) a prototype array fluorescent biosensor PWAI with a removable multi-channel PDMS flow cell; (B) excitation light path inside the planar waveguide transducer; (C) photograph of waveguide chip with eight reflection lines for biosensing indicated by white arrows (Photo by Lanhua Liu).

to form a planar waveguide transducer, the application of such a waveguide to construct RIverANAlyser (RIANA) optical biosensor is a remarkable case [11–14]. In this configuration, a bulk optical glass slide with a polished 45° bevel on one end-face serves as the waveguide core. Air and bulk liquid phases surrounding the waveguide function as the upper and lower cladding, respectively. The incident light is coupled into the waveguide through the beveled angle of the facet. The other end-face of the waveguide is painted with black ink for light absorption. Based on the designed waveguide chip, eight individual total reflection spots have been formed for immunosensing with the established multi-analyte immunoassay approach for the simultaneous detection of microcystin-LR (MC-LR), 2,4-dichlorophenoxyacetic acid (2,4-D), bisphenol A (BPA), and melamine [10]. An upgraded device, planar waveguide-based array immunosensor (PWAI) was designed, based on optical detection principle and immunoassay approach with major improvements in two critical areas. One is the expanded multi-analyte analysis capability allowing for simultaneous measurements of up to twenty-four analytes; and the other is providing detailed kinetics of interaction in an immunoassay, which has never been reported before. Moreover, a novel design approach to the flow cell including integrated microfluidics was proposed to match the waveguide layout. The new device PWAI was characterized concerning relative signal intensity, linear range, detection limit, regeneration and recoveries of real samples. Following the requirements of the drinking water-related legislations around the world, target compounds were selected mainly from the groups of biotoxin such as MC-LR, modern pesticides such as 2,4-D, and endocrine disrupting compounds such as BPA to validate the performance of PWAI.

2. Experiment

2.1. Materials and reagents

2,4-D, MC-LR, BPA, sodium dodecyl sulfate (SDS), bovine serum albumin (BSA), *N*-(4-maleimidobutyryloxy) succinimide (GMBS) and 3-Mercaptopropyl-trimethoxysilane (MTS) were obtained from Sigma-Aldrich and stored at 4 °C before use. The PDMS templates were made from polydimethylsiloxane purchased from Dow Corning Corporation. The fluorescent dye Cy5.5 and *N*-hydroxysuccinimide (NHS) ester were purchased from GE Healthcare Life Sciences. Labeling of the anti-2,4-D-antibody, anti-MC-LR-antibody and anti-BPA-antibody with dye Cy5.5 was performed based on the method as previously described by Mujumdar et al. [15]. The hapten conjugates were synthesized based on the procedure reported by Moorhead et al. [16]. The Cy5.5-labeled antibody and the hapten conjugates were purified and stored at −20 °C in small aliquots for use. All chemicals were analytical grade and were used without further purification. Deionized water was used throughout the experiments. 1 mg/L MC-LR, 100 mg/L 2,4-D and 100 mg/L BPA stock solutions were prepared in methanol solvent and stored at 4 °C before use. Phosphate buffered saline (10 mM PBS with pH 7.4) was prepared using deionized water (18.2 MΩ cm). All the stock solutions were diluted to a series of concentration levels using the 10 mM PBS buffer solution.

2.2. Array fluorescent biosensor system

Fig. 1A shows a schematic of the array fluorescent biosensor PWAI. Briefly, a diode laser of 100 mW at wavelength 635 nm was fitted with a line generator (Huanyuan-Star Laser Ltd., Beijing). It was powered through connecting to the port of a computer and was modulated by a 10 Hz square wave signal, generated by a data acquisition card (DAQ2213, Adlink Technology, China Co., Ltd., Shanghai). The laser beam collimated light was positioned to launch excitation light into the beveled edge of the waveguide chip and propagated along the biosensing area of chip surface via TIR, forming eight individual total internal reflection lines (TIRL). In each TIRL, the evanescent wave field generated on the chip surface interacted with the surface-bound, fluorophore-labeled biomolecules, thereby causing the excitation of fluorescence. The plastic fibers with 1 mm core diameter and the numerical aperture of 0.46 were placed beneath the sensing region to collect the emitted fluorescence through a high pass filter (#28-473, Edmund Optics Inc., USA). The fluorescence signals were then detected by the photo diodes with a lock-in amplifier.

The K9 glass was adopted as the planar waveguide transducer with a reflective index of nm and a size of 65 mm × 25 mm in the area and 1.5 mm in depth. All faces of the K9 glass were polished, and one end-face was beveled to 45° for the light coupling. A removable multi-channel reservoir modules made of PDMS was employed in the system to isolate the parallel TIRLs physically so as to form eight flow channels on the chip surface. The PDMS flow cell containing eight independent linear channels (3 mm in width, 12 mm in length with an interval spacing of 2.7 mm) was clamped onto the surface of a waveguide with a manual chuck. All reagents were delivered by a flow delivery system operated with the peristaltic injection pump. Compared with the array biosensors introduced by USA National Naval Laboratory [17,18], the proposed PWAI can avoid the absorption or scattering of light caused by the attachment of flow cell on the TIRLs even though a reflective cladding on the waveguide surface can partially decrease the loss of excitation intensity [17]. Schematic diagram of the excitation light path of the waveguide chip is shown in Fig. 1B. Fig. 1C shows the photograph of waveguide chip with laser on to form the eight TIRLs for biosensing.

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