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An evanescent wave multi-channel immunosensor system for the highly sensitive detection of small analytes in water samples

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ABSTRACT

To simultaneously detect multiple analytes in water samples, we propose an integrated evanescent wave multi-channel immunosensor (EWMI) system. This system employs fluorescence-based detection of the binding of fluorophore-tagged antibodies to the surface of a planar optical waveguide chip. Incident light is coupled into the planar optical waveguide chip via a beveled angle to form eight individual reflection spots for multi-analyte biosensing. Fluorescence emissions are collected by eight multi-mode fibers placed in parallel underneath the chip. A reusable biosensing surface is established via the covalent attachment of the analyte derivative onto the optical waveguide chip. With one spot for 2,4-dichlorophenoxyacetic acid (2,4-D) detection taken as an example, the pre-incubation time, antibody concentration, and incubation time of EWMI system are optimized. 2,4-D, microcystin-LR, bisphenol A, and melamine are simultaneously and specifically detected within an analysis time of about 20 min for each assay cycle. The regeneration of the planar optical waveguide chip allows more than 300 assay cycles.

1. Introduction

Evanescent field-based optical sensors have received considerable attention because they easily achieve miniaturization, and they are significant in remote and multi-analyte sensing [1–4]. Based on the principle of total internal reflection (TIR), the light launched into a waveguide that is placed into a dielectric medium of a low refractive index ($n_{waveguide} > n_{medium}$) reflects all the light within the waveguide when the angle of incident light entering the waveguide, θ, is greater than the critical angle θ_c. When the incident beam is reflected, a portion of the radiation exists in the distal phase as an evanescent wave. This evanescent wave penetrates the distal phase to a distance comparable to the wavelength of light, with an exact penetration depth dependent on the incidence angle and refractive indexes of the two media [5,6].

Since the pioneering report of Hirschfield [5], the evanescent wave resulting from TIR has been widely applied in biochemistry and analytical chemistry. Among the numerous evanescent wave sensor configurations proposed in several decades, two major groups emerge as the most promising. One group is based on the refractive index changes caused by mass adsorption within the evanescent field, such as the traditional surface plasmon resonance technology [7,8] and interferometry [2,9,10]. These methods are associated with attractive features of being in-situ and label free. However, the sensitivities of these methods are inferior [11]. The other group uses evanescent fields to specifically probe sensitized films on the waveguide surface in a process called evanescent wave excitation. Compared with label-free methods, luminescence-based sensors offer the advantages of improved sensitivity independent of the size of the analyte molecule [11–13].

Kronick and Little [14] were the first to use evanescent wave excitation for fluorescence immunoassay. Since then, the applications of the evanescent wave excitation principle for biosensing have been described extensively. Numerous sensor configurations have been proposed, and the two most promising classes of transducers are the planar optical waveguide and optical fiber. Examples of applications of planar optical waveguide are RIANA and AWACSS devices, which were used in the European Union projects [4,15–18]. The configurations of the optical fiber evanescent wave sensors include Analyte 2000, RAPTOR, and EWFI

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systems for environmental water analysis [19–21]. Comparatively, the planar optical waveguide shows several practices, possibilities, and prospects in the development of systems capable of multiple analyte detection in a single sample.

The efficient and accurate identification of the trace concentrations of small organic molecules in drinking water or other media is a key step toward controlling them. Four analytes, 2,4-dichlorophenoxyacetic acid (2,4-D), microcystin-LR (MC-LR), bisphenol A (BPA), and melamine, are selected as targets for the following reasons. 2,4-D belongs to the top 10 pesticides used worldwide [21,22]. In the national standard of China, the limit of 2,4-D in drinking water is 30 μg/L (GB 5749–2005). MC-LR is one of the most common microcystins; it represents a potent tumor promoter that leads to hepatic necrosis and hemorrhage, and it causes apoptosis [21,22]. China introduced guideline values for MC-LR in drinking water in 2002, with a recommended limit of 1 μg/L. BPA is widely used to produce epoxy resins and polycarbonate plastic used in food packaging; however, BPA can mimic the effects of endogenous hormones, estrogens, and androgens by binding to the estrogen receptor and proliferation [23]. In 2008, over 50,000 infants and young children in China suffered from illnesses because of continuous consumption of melamine-contaminated infant milk [24]. Consequently, the Food and Drug Administration (FDA) of the US, the WHO, and the Chinese government set the safety limits of melamine at 2.5 mg/kg for milk and 1.0 mg/kg for infant formula after the melamine accident. Developing an easy and sensitive method for the on-site detection of BPA and melamine is therefore critical for food safety and human public health.

Conventional detection methods for small organic analytes include HPLC, GC/MS and so on. Several immunoassays are also commercially available, and most of them consist of a 96-well plate-based or magnetic particle-based enzyme-linked immunosorbent assay (ELISA) tests [25]. However, these methods depend on heavy manual labor and need relatively large amounts of reagents. Therefore, we propose a novel integrated evanescent wave multi-channel immunosensor (EWMI) system that employs fluorescence-based detection of the binding of fluorophore-tagged antibodies to the surface of a planar optical waveguide chip. In combination with bioaffinity assays, the EWMI system offers benefits, such as a reusable and inexpensive chip, enhanced sensitivity, easy sensor handling and preparation, sample volume reduction, versatility, and low cost per test.

2. Transducer geometry and configuration of EWMI system

2.1. Transducer geometry

The basic structure of a dielectric waveguide consists of a longitudinally extended, high-index optical medium, which is transversely surrounded by low-index media. Using the planar optical waveguide embedded in flat dielectric substrates guides light at a surface on which films are sensitized to specific immobilized chemicals and thus facilitates the real-time interrogation of the optical properties of the films during biochemical reactions [26]. Semiconductor processing, such as photolithography, and ion-exchange procedures have been extensively used for planar waveguide fabrication [4,16–18]. However, the sensor chips need complicated, precise and multi-step fabrication procedures.

We therefore adopt a simple transducer geometry (Fig. 1) as similar as reported by Klotz et al. [15]. It uses a rectangular K9 glass (Jinji Optical Glass Processing Center, Beijing) with a high refractive index as the waveguide core. Only air and the liquid bulk phase surrounding the chip function as the upper and lower cladding. The chip sizes are 60 mm × 15 mm with a depth of 1.5 mm. Incident light is coupled into the waveguide chip via a beveled angle

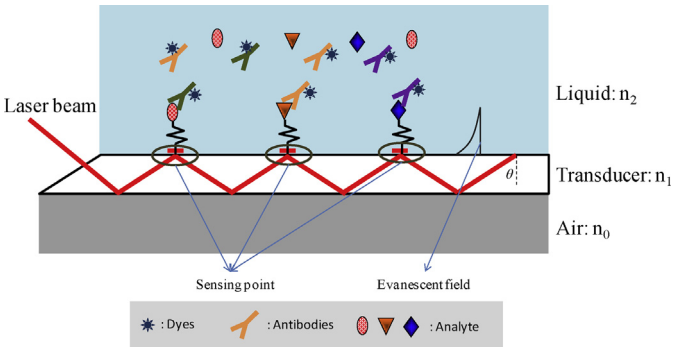

Fig. 1. Principle schematic of the evanescent wave planar optical waveguide chip.

of 45° on one endface, forming eight individual reflection spots for biosensing. The other endface was coated with black paste to absorb the reflection of light. To facilitate the following biomolecular modification and improve the surface finish of chip, a thin layer of SiO_2 film (35 nm) was deposited on the K9 glass chip by magnetron sputtering (PVD coating technology).

The TIR fluorescence principle has been extensively discussed elsewhere [5,6,27], so only a short description is given here. TIR occurs when the angle of incidence exceeds the critical angle θ_c, which is given by

$$\theta_c = \sin^{-1}\left(\frac{n_2}{n_1}\right). \tag{1}$$

In the proposed transducer geometry, the solid phase is K9 glass with $n_1 = 1.5163$, and the liquid phase is water with $n_2 = 1.33$ at an incident light wavelength λ of 635 nm; therefore, θ_c is 61.3°. When TIR occurs, the intensity $I(z)$ of the evanescent wave field present in the liquid phase exponentially decreases with distance from the chip surface into the liquid phase, z:

$$I(z) = I_0 \exp\left(\frac{-z}{d}\right), \tag{2}$$

where I_0 is the evanescent wave intensity at the interface and d is a characteristic distance that describes the penetration depth of the evanescent wave into the liquid phase. The depth can be calculated from λ and the incidence angle of excitation light θ with the use of

$$d = \frac{\lambda}{4\pi}\left(n_1^2 \sin^2\theta - n_2^2\right)^{-1/2}. \tag{3}$$

Therefore, the penetration depth of an evanescent wave is a function of λ and θ. The intensity of I_0 can be calculated with the used of

$$I_0 = I_i |t|^2, \tag{4}$$

where the coefficient $|t|^2$ is given by

$$|t|^2_{\mathrm{TM}} = \frac{4\cos^2\theta}{\left(n_2/n_1\right)^4 \cos^2\theta + \sin^2\theta - \left(n_2/n_1\right)^2}, \tag{5}$$

$$|t|^2_{\mathrm{TE}} = \frac{4\cos^2\theta}{1 - \left(n_2/n_1\right)^2}, \tag{6}$$

where I_i is the intensity of the incident beam, the index "TM" indicates TM polarization, and "TE" indicates TE polarization.

The total fluorescence intensity $F(z)$ at any depth z induced by the evanescent wave is given by

$$F(z) = C(z) I_0 \exp\left(\frac{-z}{d_e}\right), \tag{7}$$

where $C(z)$ is a constant dependent on the characteristics of fluorophore and is simply expressed as its quantum yield (Φ) times the concentration $c(z)$ distributed as a function of z, and d_e is the