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TriPleX™ waveguide-based fluorescence biosensor for multichannel environmental contaminants detection

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ABSTRACT

In order to realize the multi-analyte assays for environmental contaminants, an optical biosensor utilizing laser-induced fluorescence-based detection via the binding of biomolecules to the surface of an integrated TriPleX™ waveguide chip on a glass substrate (fused silica, FS) is described. As far as we know, this is the first demonstration of using the TriPleX™ technology to fabricate the waveguide chip on a FS substrate. The sensor consists of 32 individually addressable sensor patches, which were formed on the chip surface by exploiting 3 Y-junction splitters, creating four equal rows of eight evanescently excited windows in parallel. The basic low-loss SiO_2/Si_3N_4 TriPleX™ waveguide configuration in combination with on-chip spotsize convertors allows for both high fiber-to-chip coupling efficiency and enables at the same time individually optimized high chip surface intensity and low patch-to-patch deviation. Moreover, the complementary metal-oxide-semiconductor compatible fabrication of waveguide chip allows for its mass production at low cost. By taking MC-LR, 2,4-D, atrazine and BPA as the model analytes, the as-proposed waveguide based biosensor was proven sensitive with the detection limits of 0.22 μg/L for MC-LR, 1.18 μg/L for 2, 4-D, 0.2 μg/L for atrazine and 0.06 μg/L for BPA. Recoveries of the biosensor towards simultaneous detection of MC-LR, 2, 4-D, atrazine and BPA in spiked real water samples varied from 84% to 120%, indicating the satisfactory accuracy of the established technology.

1. Introduction

The need for sensitive chemical and biochemical sensors capable of multi-analyte detection in a single sample in the fields of environment, medical care, food, anti-terrorism etc. has become extremely important over the past few decades (Heideman et al., 2012; Lambeck, 1992, 2006; Zhu et al., 2013). Natural toxins, agricultural chemical agents (such as pesticides), industrial chemical agents (such as endocrine disrupting substances) etc. are serious threats for public health (Damen et al., 2014; Shi et al., 2013). The conventional chemical analysis methods for contaminant detection are sensitive and reliable; however, they need complicated and time-consuming pre-concentration procedures, expensive testing cost, and rely on large-scale analytical instruments, which would hinder their applications in some cases, such as in-field and on-line testing (Singh et al., 2012). Therefore many researches focus on multi-analyte sensor development and address the problems inherent in discriminating multiple simultaneous signals without loss in measurement speed, specificity or sensitivity (Ligler et al., 1998).

Integrated optical sensors have proven to be a reliable optical platform for fulfilling these requirements, which combine high sensitivity, miniaturization and possibility of mass-production (Heideman et al., 2012). Most integrated optical sensors are constructed based on the evanescent field detection principle, which show a lot of advantages, such as low background and high sensitivity and selectivity (Colomer-Farrarons et al., 2011; Robbins et al., 2004), acting as a beneficial supplement to traditional chemical analysis techniques. Although various types of integrated optical sensors have been reported in the past (Liu et al., 2017c; Robbins et al., 2004; Wiki et al., 2001), the TriPleX™ waveguide-based optical device receives a lot of attentions because of its remarkable advantages, such as ultra-low optical loss and high configuration flexibility (Duer et al., 2010; Leinse et al., 2012; Prak et al., 2011; Wörhoff et al., 2015). In addition , the TriPleX™ waveguide comprises of alternating CMOS compatible low pressure chemical vapor deposition (LPCVD) layers, which are completely transparent for wavelengths from UV to IR (Heideman et al., 2007). This technology also allows for production of low, moderate and high

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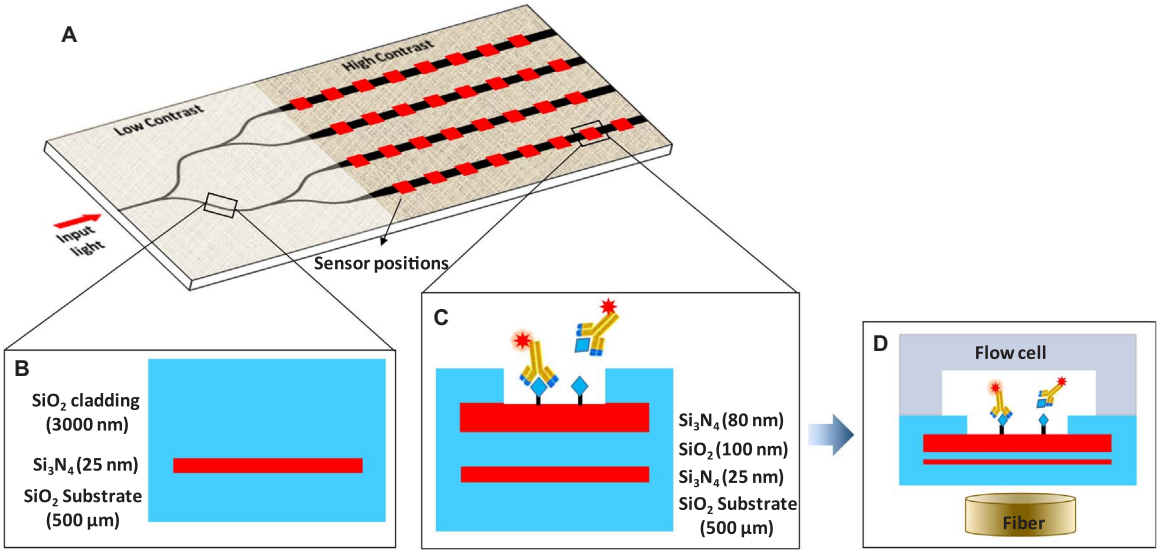

Fig. 1. (A) Schematic diagram of the waveguide chip layout; (B) Cross-section of the optimized single-stripe waveguide structure; (C) Cross-section of the optimized double-stripe waveguide structure and the sensing windows for surface chemistry (D) with the integration of flow cell and polymer fiber for fluorescence collection.

refractive index contrast waveguide geometries (Fédéli et al., 2013). Moreover, the standardized silicon microelectronic technologies guarantee the waveguide devices with the great material homogeneity and the possibility of integrating a complete micro optical system with flexible functions on a single chip. Consequently, different types of TriPleX™ waveguide-based optical sensor have been reported and investigated. Most of them are label free to result in the refractive index sensing, such as microring resonators (MRRs) sensors (Besselink et al., 2016; De Vos et al., 2007; Xu et al., 2008) and Mach-Zehnder interferometer (MZI) sensors (Chalyan et al., 2016; Densmore et al., 2006; Misiakos et al., 2014); however, compared with the fluorescent labeling system, most of such biosensors suffer from limited sensitivity. Even so, to our best knowledge, researches on design of the TriPleX™ waveguide for fluorescent biosensing are still rare.

To meet the technological gap, we developed a 32-analyte integrated optical fluorescence biosensor (IOFB) by designing the TriPleX™ waveguides on a glass (e.g. FS) substrate, to allow for simultaneous detection from the bottom side, and fluid manipulation from the top as shown in Fig. 1. The standard silicon microelectronic technology was exploited for the realization of a multi-channel photonic pattern integrated on one chip, distributing the evanescent excitation light into four-channel branches through the Y-junction splitter. By introducing the indirect competition immunoassay, the waveguide surface was modified with antigen-protein conjugate. Four chemical compounds of great environmental significance, including microcystin-LR (MC-LR), 2, 4-dichlorophenoxyacetic acid (2, 4-D), atrazine and bisphenol-A (BPA), were chosen as a paradigm to validate this system capable of multi-contaminant detection in a single water sample. As far as we know, this is the first demonstration that using the TriPleX™ waveguide technology to fabricate the fluorescent integrated chip on a fused silica substrate for environmental bioassay.

2. Experiment

2.1. Materials

All chemicals and buffers used in this work can be found in Supplementary Information, **Page 2**.

2.2. Waveguide geometry design, fabrication and instrumentation

Considering that most evanescent wave fluorescence biosensors used 520 nm or 635 nm diode laser as the excitation light (Anderson et al., 1996; Han et al., 2016; Huang and Tseng, 2005; Liu et al., 2017a, b), an integrated multi-channel waveguide chip was built using the TriPleX™ technology on a glass (eg FS) substrate, providing the evanescent field with high surface intensity, low loss, good patch-to-patch uniformity both at the wavelength of 520 nm and 635 nm, respectively. The TriPleX™ technology is based on LPCVD processes in which silicon nitride (Si_3N_4) and silicon oxide (SiO_2) are the key materials. These materials have an opposite stress when deposited on a silicon wafer (nitride is tensile and oxide is compressive), therefore stacking them in a multilayer results in a macroscopically low stress layer stack. A variety of geometries can be realized by using LPCVD fabrication process, depending on the different etch depth, layer thickness and waveguide width. Three “standard” TriPleX™ geometries, named as box shape, double–stripe and single–stripe, are extensively studied (Fédéli et al., 2013). Herein, a multilayer-stack of Si_3N_4 and SiO_2 based waveguide chip with 65 mm × 20 mm in the area and 1 mm in depth was proposed to distribute the laser light into 4 branches with 32 separate sensing windows, as shown in Fig. 1**A**.

Figs. 1**B and** 1**C** show the cross-sectional view of two TriPleX™ geometries connected on the chip and windows exposed for the surface chemistry. In the waveguide design, the single–stripe geometry was adopted in the low refractive index contrast area to achieve an optimal coupling efficiency of light from the single-mode fiber to waveguide and to maintain a single-mode transmission area needed for an even split ratio of the power into different waveguide branches. The double–stripe geometry was adopted in the high refractive index contrast area to achieve an optimal intensity of the evanescent field on the surface for better fluorescent excitation. The transition from low contrast (single stripe) to high contrast (double stripe) area was based on an adiabatic change of waveguide thickness from the single waveguide to the double stack waveguide (see Fig. S1). The resulting on-chip spotsize convertors were very low loss, and allowed for individual optimization of the both fiber-to chip coupling section and the sensing windows. Meanwhile, the waveguide width was altered from 1 to 30 µm, using an adiabatic increase in width of the waveguide. The Si_3N_4 layer in the high contract area was exposed to form the sensing window with area of 0.8 mm × 1.5 mm. An extensive simulation was carried out in order to optimize the waveguide chip design (see Section 3.1). The fabrication process was conducted by using the CMOS compatible TriPleX™ waveguide fabrication scheme as depicted in Fig. S1 (Supplementary Information, **Page 3–4).**