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Fiber optofluidic biosensor for the label-free detection of DNA hybridization and methylation based on an in-line tunable mode coupler

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ABSTRACT

An optical fiber optofluidic biosensor for the detection of DNA hybridization and methylation has been proposed and experimentally demonstrated. An in-line fiber Michelson interferometer was formed in the photonic crystal fiber. A micrhole in the collapsed region, which combined the tunable mode coupler and optofluidic channel, was fabricated by using femtosecond laser micromachining. The mode field diameter of the guided light is changed with the refractive index in the optofluidic channel, which results in the tunable coupling ratio. Label-free detections of the DNA hybridization and methylation have been experimentally demonstrated. The probe single stranded DNA (ssDNA) was bound with the surface of the optofluidic channel through the Poly-L-lysine layer, and the hybridization between a short 22-mer probe ssDNA and a complementary target ssDNA was carried out and detected by interrogating the fringe visibility of the reflection spectrum. Then, the DNA methylation was also detected through the binding between the methylated DNA and the 5-methylcytosine (5-mC) monoclonal antibody. The experiments results demonstrate that the limit of detection of 5 nM is achieved, establishing the tunable mode coupler as a sensitive and versatile biosensor. The sensitive optical fiber optofluidic biosensor possesses high specificity and low temperature cross-sensitivity.

1. Introduction

The development of nucleic acids diagnostics has became a powerful tool in the diagnosis of diseases, genetic disorders and pathogen infection. Compared with conventional fluorescence-labeled oligonucleotides with dyes, label-free methods utilize the immobilized single-stranded DNA (ssDNA) to hybridize a complementary sequence in order to form a double-stranded structure, which promise to offer sensitivity, selectivity, and low cost for the detection of DNA hybridization (Sun et al., 2014; del Río et al., 2015). Besides, DNA methylation has been proven to be related to almost all types of cancers, which is a new generation of cancer biomarkers (Szyf, 2004). Myriad technologies for the analysis of DNA methylation mainly composed of a modification step (e.g., bisulfate conversion (Wang et al., 2013)) and an analysis step (e.g., polymerase chain reaction [PCR] (Fraga et al., 2002), fluorescence methods (Wang et al., 2009), light scattering techniques (Zhao et al., 2011), and so on). They not only took several hours to modify and analyze the DNA methylation, but also required a complex device with many steps (Lee et al., 2014). Therefore, there is considerable interest in the development of a flexible and low cost fiber optofluidic biosensor for the label-free detection of DNA hybridization and methylation.

In recent years, several fiber optic biosensors for the detection of the DNA hybridization have been developed, which can be approximately classified into three types: gratings, interferometers, and surface plasmon resonance (SPR). With regard to fiber gratings, such as fiber Bragg gratings (FBGs) (Chryssis et al., 2005), tilted fiber Bragg gratings (TFBGs) (Sun et al., 2014), and long-period fiber gratings (LPFG) (Jang et al., 2009; Chen et al., 2007), the DNA hybridization changes the effective refractive index (RI) of the fiber grating, which results in a shift of the resonant wavelength for the fiber grating. However, the sensitivity of the grating based biosensor is limited because of the small size of biomolecules and high thickness of fiber cladding. Although several methods have been applied to improve the sensitivity of grating based biosensor, such as colloidal gold modified LPFG (Tang et al., 2006), reflective microfiber grating biosensor (Sun et al., 2014), or

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side-polished LPFG (Jang et al., 2009), the complicated procedure and additional materials also increase the cost of the biosensor. With regard to interferometers, the optical path difference (OPD) of the fiber interferometer is sensitive to the RI change of the DNA hybridization. Various fiber interferometers have been researched in order to split and recombine different optical modes (core mode and cladding modes), such as the thin-core based disposable fiber-optic biosensor (Yin et al., 2013), nonadiabatic tapered optical fiber sensor (Zibaii et al., 2011), and microfiber-assisted Mach-Zehnder interferometer (Song et al., 2016). Although the optical fiber interferometers for the detection of DNA hybridization have many attractive characteristics, such as high sensitivity, linear response, and small size, it suffers from serious temperature cross-sensitivity, which also changes the OPD of the fiber interferometer. With regard to SPR, surface bound electromagnetic waves are formed at the interface between a metal and a dielectric (Zagorodko et al., 2014). SPR is one of the most suitable methods for the real-time detection and monitoring of binding events in biological system due to its high sensitivity, light weight, and long-distance signal transmission for remote operation (Pollet et al., 2009). However, the SPR property of the biosensor is highly dependent on the metal, which need carefully design and fabricate the SPR biosensors.

In this paper, we propose a tunable mode coupler optofluidic biosensor for the detection of DNA hybridization and methylation. A microhole in the collapsed region was fabricated by using the femtosecond laser micromachining in an in-line photonic crystal fiber (PCF) Michelson interferometer. The mode field diameter of the guided light is changed with the RI in the optofluidic channel, which results in the tunable coupling ratio. Label-free detections of the DNA hybridization and methylation have been carried out and detected by interrogating the fringe visibility of the reflection spectrum. The proposed tunable mode coupler fiber optic biosensor is desirable to develop new strategies for label-free, real-time, and sensitive detection of DNA hybridization and methylation.

2. Tunable mode coupler in the PCF interferometic biosensor

The operation principle of a conventional PCF Michelson interferometic biosensor is shown in Fig. 1(a) (Milenko et al., 2012). In the PCF, a section of the collapsed region was fabricated due to the strong electric arc discharge (Xiao et al., 2007), and the free end face of the PCF was filmed with a gold film. At the collapsed region, part of the core mode couples to cladding modes due to the mode-field mismatch. The fundamental core mode and cladding modes are propagated along the PCF with different effective RI, and reflected back at the filmed end. Then the cladding modes recouple back into the core and interfere with the fundamental core mode at the collapsed region. Due to the different effective RI between the core and cladding modes, a PCF Michelson interferometer was formed (Wong et al., 2011). The effective RI of the cladding mode is changed by the surround RI induced by the DNA hybridization or methylation, making the variety of the OPD of the PCF Michelson interferometer. Compared with this PCF interferometic biosensor, the proposed tunable mode coupler biosensor is different significantly.

In the proposed biosensor, an optofluidic channel was created in the middle of the collapsed region by using a femtosecond laser micromachining, as shown in Fig. 1(b). Due to the optofluidic channel, the entire collapsed region can be divided into three sections. When the guided light propagates from the core of the PCF to the end of the section i (collapsed region), the mode field diameter (MFD) of the propagation light is MFD_1. Then the guided light propagates in the section ii (microfluidic channel), and the MFD of the propagation light at the end of the optofluidic channel (MFD_2) can be expressed as (Villatoro et al., 2007)

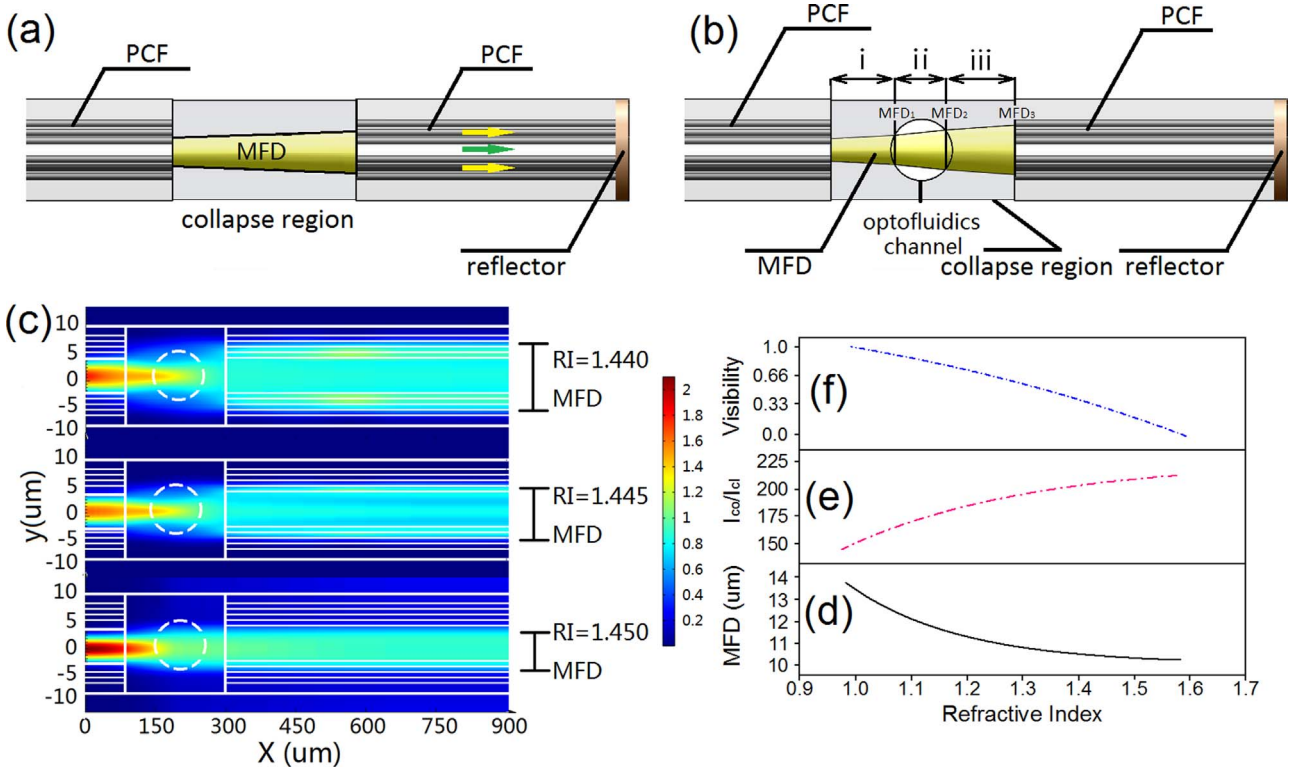

Fig. 1. The schematic diagram of (a) the PCF interferometic biosensor, (b) the PCF optofluidic biosensor. (c) The simulation of modes coupling with different refractive index. The relationship between the refractive index and the (b) MFD (c) coupling ratio and (d) visibility.