Sensitive DNA biosensor based on a long-period grating formed on the side-polished fiber surface

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Abstract: We demonstrate a sensitive DNA biosensor based on a long period grating (LPG) formed by a photolithograph process on the surface of a side-polished fiber. The biomolecules of the biosensor were immobilized on the silica surface between LPG patterns. The resonance wavelength was red-shifted after the binding of the poly-L-lysine, probe ssDNA and target ssDNA to the sensor surface. The overall wavelength shift after the successful DNA hybridization was 1.82 nm. The proposed LPG-based DNA biosensor is ~2.5 times more sensitive than the previously reported fiber grating-based DNA biosensors.

OCIS codes: (060) Fiber optics and optical communications; (060.2370) fiber optic sensors; (170.0170) Medical optics and biotechnology

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1. Introduction

Biosensors have wide applications, including biomarker detection for medical diagnostics, and pathogen and toxin detection in food and water. However, general biosensors detect analytes using a fluoroimmunoassay. The disadvantage of this method is the requirement of fluorescence labeling of the antigen or target DNA, which requires additional reagents [1]. In addition, the process has a high cost and is complicated and real-time detection is not possible. Therefore, to overcome these disadvantages, many methods have been applied to develop label-free detection biosensors [2,3]. In particular, there has been much interest in fiber optic biosensors using label-free detection because of the potential sensitivity, detection speed and small size, variable and multiple detection of an analyte. Fiber optic biosensors are analytical devices in which the fiber optic device is a transduction element [4].

Many fiber optic biosensors are based on the surface plasmon resonance (SPR) phenomenon [2,4,5]. However, in the case of the SPR biosensors, the SPR property of the biosensor is dependent on the metal, its thickness and biomolecules. Therefore, one should carefully design and fabricate the SPR biosensors. These problems have resulted in a rise in cost. To overcome the disadvantages, biosensors based on a fiber grating have been designed [6-10]. In particular, there has been much interest in fiber optic biosensors with long period grating (LPG) that assists mode coupling [11] at resonance wavelengths that are sensitive to the variation of the external medium of the optical fiber. The advantages of the LPG sensor include simple fabrication and easiness in adjusting the resonant wavelength well within the spectrum of optical source by simply adjusting the grating period. However, because of the low refractive index, small size of biomolecules and high thickness of fiber cladding, the sensitivity of the LPG biosensor is limited. Therefore, several methods such as colloidal gold modified LPG [8], etching fiber cladding [9] and using a high refractive index overlayer [10] have been applied to improve the sensitivity of LPG sensors. However, these methods are disadvantageous because of the complicated and hazardous procedure, and the requirement of additional materials.

We present a new, sensitive DNA biosensor based on a LPG written on the surface of a side-polished fiber. The cladding layer was substantially reduced using the side-polishing method [12] to enhance the interaction between the fundamental fiber core mode and the external medium for high sensitivity. The LPG pattern was formed on the surface of a side-polished fiber using a photolithograph process. The performance of the LPG based DNA sensor was tested using the label free-detection method of selective binding of poly-L-lysine, probe ssDNA and target ssDNA.

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2. Structure and sensitivity of a DNA biosensor based on LPG

2.1 Fabrication of LPG-assisted optical biosensor

LPGs were fabricated on the side-polished fiber sensors using the side-polishing method [12]. Figure 1(a) shows the schematic of the side-polished fiber with an LPG pattern of period Λ. Figure 1(b) shows a microscope image of the surface of the side-polished fiber. In Fig. 1(a), the period Λ is 600 μm. To polish the single mode fiber, we stripped and cleaned the jacket with acetone. The unjacketed fiber was placed in a bent groove in a quartz block and was held by an UV epoxy. The quartz block had a 25 mm length and a 10mm thickness. The quartz block had a bent groove with a radius of curvature R of 250 mm and width of 130 μm. The quartz block was polished until the cladding of the fiber was nearly removed. Photoresist (Az4210) was spread on the polished surface with 2.1 μm of thickness using a spin coater. The designed LPG pattern was formed by UV exposure followed by a development process. The period of the LPG is 600 μm and the length is 25 mm.

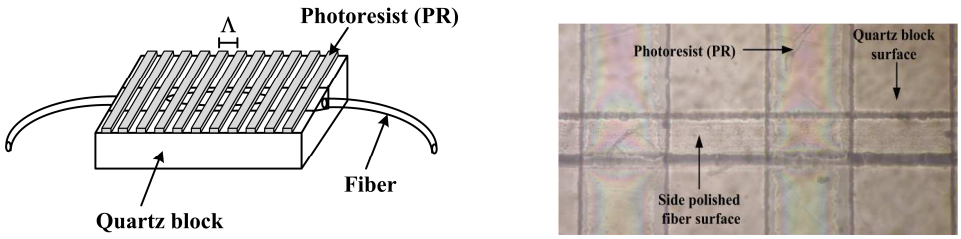

Fig. 1. (a) Schematic of the side-polished fiber with an LPG pattern. (b) Microscope image of the surface of the side-polished fiber with an LPG pattern.

2.2 Sensitivity of LPG-assisted optical biosensor

To analyze the sensitivity of the proposed LPG biosensor, we used refractive index oils of different values. The wavelength shift was observed using a white light source and an optical spectrum analyzer (OSA, Ando AQ6317B, 0.01 nm resolution). Figure 2 shows the normalized transmission spectrum of the LPG fabricated with a period of 600 μm. The transmission spectrum was measured by an OSA. The three different dips in the transmission spectrum indicate three different resonance wavelengths where a fundamental core mode couples to three lossy cladding modes, p, q and r (named for convenience), in the LPG formed on the surface of a side-polished fiber. Note that the resonance wavelengths, corresponding to the r and q cladding modes, vary with the refractive index n of an external medium which are plotted in Fig. 3. As refractive index was increased, the resonant wavelengths shifted to a longer wavelength. When the refractive index changed from 1.333 to 1.454, the shifts of the resonance wavelength for the q mode (Fig. 3(a)) and r mode (Fig. 3(b)) were 84.4 nm and 110 nm towards the longer wavelength, respectively. The corresponding sensitivities were 607.2 nm/RIU and 909.1 nm/RIU for the q and r modes, respectively. The sensitivity of 909.1 nm/RIU was high compared to the values of LPG sensors previously reported [13-15]. In this case, the resolutions of the biosensor were 1.6×10^{-5} and 1.1×10^{-5} RIU for the q and r modes, respectively. This indicates the sensitivity of the LPG biosensor is high when the shift of the resonance wavelength for the r mode is measured rather than that for the q mode. Therefore, in this DNA biosensor, we chose the shift of the resonance wavelength for the r cladding mode for the DNA detection.

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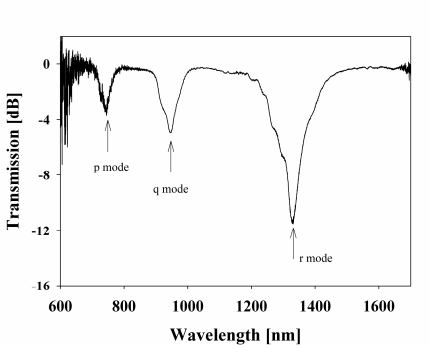
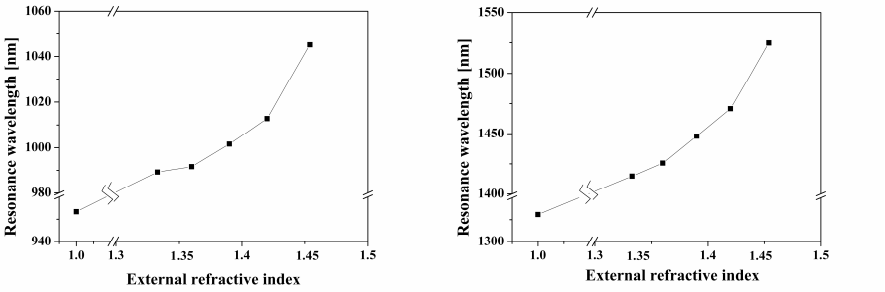


Fig. 2. Transmission spectrum of the LPG in air.



(a) (b)

Fig. 3. Resonance wavelengths with different external refractive indices for (a) the q mode, (b) r mode.

Note that the resonance wavelength determined by the phase matching condition is given by [16] $\lambda = (n_{co} - n_{cl,m})\Lambda$, where λ is the resonance wavelength, Λ is the grating period and n_{co} and $n_{cl,m}$ are the effective indices of the fundamental core mode and the *m*th cladding mode, respectively. Because the effective index of a high order cladding mode is smaller than a low order mode, the mode number of the r mode should be higher than that of the q mode. The biggest dip in Fig. 2 indicates that strong coupling occurred in the LPG between the fundamental core mode and the higher order r cladding mode.

Since the effective index of the cladding mode is dependent on the refractive index of the surrounding medium, the change in the refractive index of the external medium alters the power and resonance wavelength of the LPG. In a typical optical fiber used in a LPG sensor, as the refractive index of the external medium increases, the effective index of the cladding mode increases. Accordingly, the resonance wavelength is blue-shifted. However, in a side-polished fiber, because the cladding of the fiber is removed, the change in the refractive index of the external medium influences the effective index of the fundamental core mode. Namely, as the refractive index of the external medium increases, the effective index of the fundamental core mode increases, as discussed for the refractive index sensor with a thinned fiber Bragg grating [17]. As a result, the resonance wavelength is red-shifted.

3. Label-free DNA detection

Poly-L-lysine (PLL) has an extreme positive charge with NH_3^+ in the side chain and is often used for adsorbing biomolecules with negative charge like DNA (deoxynucleic acid) [18,19]. We used a PLL solution (0.1% W/V in water, the molecular weight =150,000-300,000 g/mol, Sigma), which is commonly used in biology to treat glass slides. The fiber was initially washed with a phosphate-buffered saline solution (NaH2PO4/Na2HPO4 pH 7.4, 150 mM

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NaCl) (PBS) before being modified with poly-L-lysine. The PLL solution was dropped on the surface of a side-polished fiber to form a PLL layer and this state was maintained for 160 minutes at room temperature. The PLL functionalizes the sensor surface with an amino group at the free end, allowing a negatively charged DNA to be immobilized on the sensor surface. Then, the sensor is washed with PBS buffer to remove excess PLL that had not been immobilized on the surface of the side-polished fiber. The 1 μM probe of single-stranded DNA in PBS buffer was then dropped on the surface of the PLL layer and this state was maintained for 130 minutes at room temperature. Then, the above washing process was again performed on the sensor surface. Finally, the 1 μM target single-stranded DNA in PBS buffer was dropped on the surface of a probe ssDNA layer and after 65 minutes the sensor was washed with PBS buffer. About 100 μl of all biomolecules was added and removed using a micro-pipette. Figure 4 shows the molecular structure of PLL, probe ssDNA and target ssDNA. The DNA sequences were (5'-CAG CGA GGT GAA AAC GAC AAA AGG GG-3') for the probe ssDNA and (5'-CCC CTT TTG TCG TTT TCA CCT CGC TG-3') for the target ssDNA.

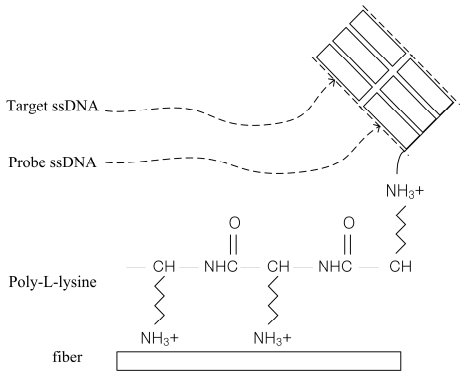

Fig. 4. The molecular structure of Poly-L-lysine, probe ssDNA and target ssDNA immobilized on the surface of a side polished fiber.

The wavelength shift of the LPG sensor was measured for the r mode, which is highly sensitive to external media with n=1.333~1.454. As shown in Fig. 5(a), the resonance wavelength shifted to a longer wavelength as the biomolecular layers formed. When the PBS buffer was dropped on the surface of the LPG sensor, the resonance wavelength shifted to 1411.69 nm. The immobilization of PLL on the sensor shifted the resonance wavelength to 1413.52 nm, indicating that the sensor surface was modified. Then, when the probe ssDNA was immobilized on the PLL layer, the shift of the resonance wavelength was 1416.61 nm. Finally, due to the hybridization with the complementary target ssDNA, the resonance wavelength shifted to 1418.43 nm. The overall wavelength shift induced by the hybridization reaction was 1.82 nm, which is ~2.5 times higher than the previously reported biosensor based on a dual-peak LPG [20] under the same 1 μM target DNA concentration. This shows that our fiber grating-based biosensor is highly sensitive to DNA hybridization in comparison with previously reported DNA fiber grating-based biosensors [7,20]. The resonance wavelengths of the fiber grating-based DNA biosensor after each procedure are plotted in Fig. 5(b). Note that the red-shifts after each procedure are due to the increase in refractive index of the external media by immobilizing the PLL, the probe ssDNA and by hybridizing target ssDNA. To monitor the DNA hybridization after target ssDNA was dropped on the probe ssDNA attached to the sensor surface, the shifts of the resonance wavelength of our LPG-based biosensor were measured in real-time. The wavelength shifts measured against time are plotted in Fig. 6. The

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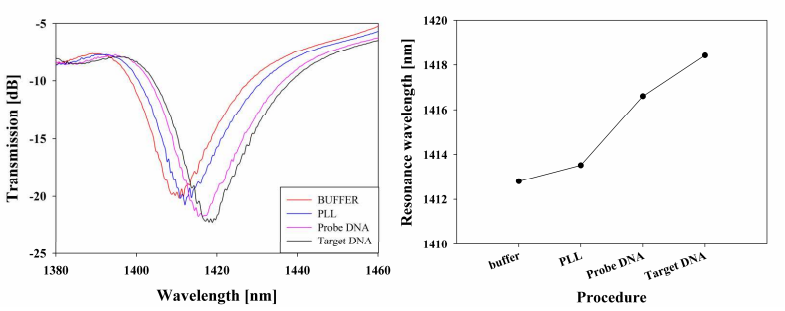
rapid reaction was observed in the first 9 min, indicating a wavelength shift of 0.94 nm, followed by a slow reaction with a wavelength shift of 0.84 nm between 9 min and 65 min.



(a) (b)

Fig. 5. (a) Transmission spectra of an LPG sensor after a sequential procedure of PBS buffer, PLL, probe ssDNA and target ssDNA. (b) The resonance wavelength versus each procedure.

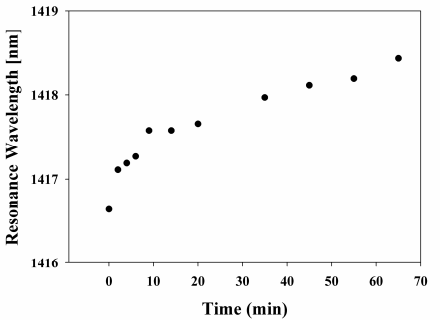


Fig. 6. Resonance wavelength against time during the hybridization of a target ssDNA.

4. Conclusion

We have demonstrated a sensitive biosensor based on LPG written on the surface of the side-polished fiber for the detection of DNA hybridization in real time. The LPG pattern was formed using a photolithograph process. As the refractive index of the surrounding medium of the LPG sensor increased, the resonance wavelength was red-shifted. The LPG sensor was used to detect the hybridization of single strand DNA. The shifts of the resonance wavelength were measured after the binding of the PLL, probe ssDNA and target ssDNA to the surface of the sensor. The overall wavelength shift induced by the DNA hybridization was 1.82 nm, which is ~2.5 times higher than that for the previously reported LPG biosensors. The majority of the wavelength shift occurred in the first 9 min due to the rapid reaction with DNA hybridization, showing a wavelength shift of 0.94 nm. During the experiment, the target ssDNA did not need to be labeled with a fluorescent tag, which is expensive and complicated. Because of its simplicity in fabrication and high sensitivity, the proposed LPG DNA biosensor is promising for the detection of real-time biomolecular interactions.

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