Real-time detection of DNA interactions with long-period fiber-grating-based biosensor

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Using an optical biosensor based on a dual-peak long-period fiber grating, we have demonstrated the detection of interactions between biomolecules in real time. Silanization of the grating surface was successfully realized for the covalent immobilization of probe DNA, which was subsequently hybridized with the complementary target DNA sequence. It is interesting to note that the DNA biosensor was reusable after being stripped off the hybridized target DNA from the grating surface, demonstrating a function of multiple usability. © 2007 Optical Society of America

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During the past decade, optical biosensors capable of detecting biomolecular interactions have become valuable tools for use in medical diagnosis and life sciences as well as environmental monitoring and other applications. The high-efficiency immobilization technique has been developed to enable the functionalization of silica glass support [1]. Several optical biosensors have been presented that use DNA microchips, a microcavity, an optical ring resonator, a planar waveguide, and optical fiber gratings [2–8]. However, some of these demonstrated optical biosensors have limitations for real-time hybridization studies and monitoring hybridization kinetics. Here we implement an optical biosensor based on a dual-peak long-period fiber grating (LPFG) for detecting biomolecular interactions at a silica–liquid interface with the advantages of high sensitivity, real-time monitoring, and reusability.

To achieve high-sensitivity detection of the designed biomolecular interaction, the most sensitive LPFG structures should be used. It has been reported that the coupling condition of LPFGs with relatively short periods are close to the dispersion turning points, resulting in conjugate dual-peak cladding modes that are extremely sensitive to external perturbations [9,10]. Several dual-peak LPFGs with a relatively small period (~160 μm) were UV inscribed in H_2-loaded SMF-28 fibers employing the point-by-point method and a frequency-doubled Ar laser. All the gratings were annealed at 80 °C for 48 h to stabilize their optical properties. Figure 1 shows the spectral evolution of a 30 mm long LPFG with a period of 161 μm during the UV inscription process. It is clear that with increasing UV exposure the conjugate dual peaks were increasing in strength and moving close to each other as the coupling condition approached the dispersion turning point.

The ability of LPFGs to couple light from the fiber core mode to cladding modes allows optically detecting the change in refractive index at the grating surface. This thus provides an optical detection method to monitor biochemical and biomolecular interactions. Figure 2 shows the basic scheme of the functionalization of a LPFG as a DNA-array biosensor. Silanization is a process for modification of the glass surface to adsorb biomolecules. The LPFG surface is first silanized, followed by the activation of cross linkers to facilitate the immobilization of probe DNA and then to be used to monitor *in situ* the hybridization of targeted DNA. The DNA hybridization process modifies the refractive index of the LPFG surface, thus resulting in its spectral shift. By demodulating the spectral shift, the designed DNA hybridization can be monitored with high sensitivity.

All the biochemical experiments were performed in a fume cupboard. To minimize the bend cross sensitivity, the dual-peak LPFG with a 161 μm period was placed straight in a V-groove container on a Teflon plate, and all the chemicals and solvents were added and withdrawn from the container by carefully pipetting.

Prior to silanization, the LPFG was cleaned by immersion in 5 M hydrochloric acid (HCl) for 30 min at room temperature, followed by rinsing in deionized

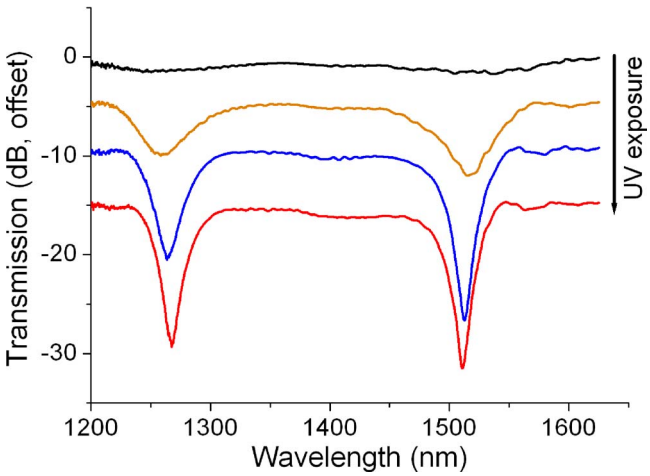

Fig. 1. (Color online) Spectral evolution of a dual-peak LPFG with a period of 161 μm under increased (arrow direction) UV exposure.

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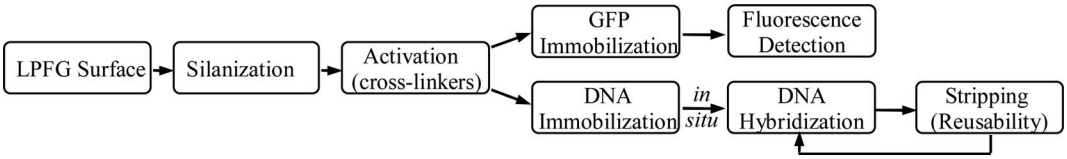

Fig. 2. Basic scheme of the functionalization of LPFG fiber surface for the generation of biosensors.

(DI) water three times and drying in the air. Silanization of the LPFG surface was performed by immersing the cleaned grating sample in fresh 10% 3-aminopropyl-triethoxysilane (APTS) (Sigma-Aldrich Company Ltd.) for 30 min, also at room temperature.

To immobilize biomolecules covalently to the glass surface, a chemical bond has to be formed between a functional group of biomolecules and the amino group of the linker [2]. As it is well known in bioconjugate chemistry, dimethyl suberimidate [DMS, the molecular structure shown in Fig. 3(b)] is water soluble and membrane permeable and is one of the best cross-linking agents to convert the amino groups into reactive imidoester cross-linkers [11]. The imidoester functional group is one of the most specific acylating groups available for the modification of primary amines and has minimal cross reactivity toward other nucleophilic groups in proteins [12]. For activation of the fiber surface, the silanized LPFGs were immersed in 25 mM DMS in phosphate-buffered saline (PBS) solution for 35 min at room temperature. The activated sensors were rinsed by DI water three times, dried, and stored in a vacuum desiccator for immobilization of either protein or probe DNA.

To provide a simple method to determine whether biomolecules are immobilized on the fiber glass surface, green fluorescent protein (GFP), which is an intrinsically fluorescent protein that has been used extensively as a tool in biology to enable imaging [13], was employed to detect the attachment of protein onto the fiber surface. The DMS-activated LPFG fiber, as described above, was incubated in 1 mg/ml GFP in PBS for 16 h at room temperature. For GFP fluorescence detection, the GFP-deposited fiber was observed under a Zeiss Axioskop microscope with a UV light source by using appropriate filters (Filter Set 9, with excitation wavelength between 450 and 490 nm, and the emission filter LP515, which allows wavelengths above 515 nm to be viewed). The image was captured and is shown in Fig. 3(a), exhibiting successful protein immobilization. For comparison, an untreated fiber was also observed under the microscope; no fluorescence was observed (not shown).

The immobilization of probe DNA process was carried out by incubation of the activated LPFG in 1 μM probe DNA (as shown in Table 1) in PBS for 16 h at room temperature. The LPFG was measured at the beginning and end of the immobilization process, and Fig. 4(a) shows the measured spectra of the red peak of its dual peaks (the peak at the longer wavelength side). Note that an offset wavelength of ~70 nm from the original position (Fig. 1) of the red peak was due to the thermal annealing and the immersion in PBS solution. By defining the resonance wavelength using the centroid calculation method, we noticed that there was a blueshift in wavelength of 254 pm after 16 h of deposition, indicating that the grating surface has been modified. We also noticed that the transmission loss of this peak has increased by ~1.5 dB. This could be caused by some roughness of the immobilized grating surface.

Hybridization was executed with the target DNA listed in Table 1. After cleaning with DI water, the grating sensor was rinsed in 6×SSPE (0.9 M NaCl, 0.06 M NaH_2PO_4, and 0.006 M EDTA), then immersed in fresh 1 μM target DNA in 6×SSPE buffer for 60 min at room temperature. The grating wavelength was monitored *in situ* throughout the hybridization process, and the hybridization-induced wavelength shifts against time are plotted in Fig. 4(b), showing a nonlinear characteristic. We may regard that there are two stages associated with the hybridization reaction process in 60 min. The rapid reaction occurred in the first 3 min, showing a wavelength shift rate of 86 pm, followed by a much slower reaction process with a rate of 9 pm/min from 3 to 60 min. The overall wavelength shift induced by the hybridization reaction in 60 min is ~715 pm. In comparison with a previously reported biosensor based on a core-etched fiber Bragg grating [8], our dual-peak LPFG biosensor has not just achieved a three times higher reaction rate but also maintained the fiber robustness.

For realizing a practical optical biosensor, reusability is an important and must-have function. To this end, we have assessed the reusability of our LPFG sensor. The above DNA-hybridized LPFG sensor was washed three times in a freshly prepared stripping buffer of 5 mM Na_2HPO_4 and 0.1% (w/v) sodium dodecyl sulfate (SDS) at 95°C for 30 s [2], then

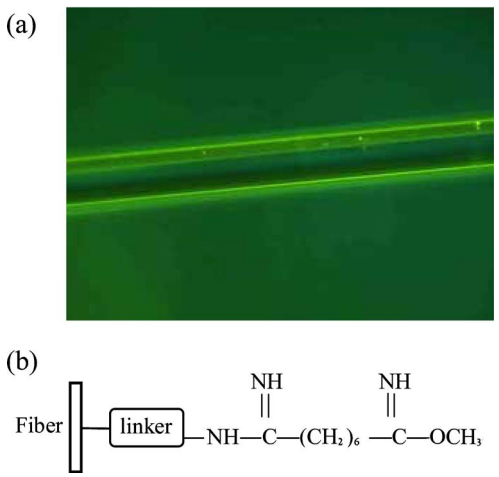

Fig. 3. (Color online) (a) Fluorescence on the fiber surface indicates (b) successful DMS activation.

Table 1. Sequences and Modifications of the Probe and Target Oligonucleotides

Oligonucleotide	5′ End Modification	Sequence	3′ End Modification
Probe	none	GCA CAG TCA GTC GCC	NH_2
Target	none	GGC GAC TGA CTG TGC	none

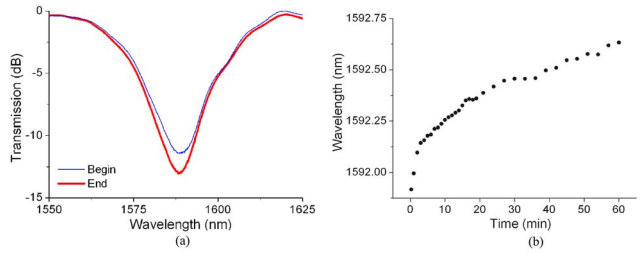

Fig. 4. (Color online) (a) LPFG's spectra monitored at the beginning and end of probe DNA immobilization process. (b) Wavelength evolution of grating sensor against time during hybridization of target DNA.

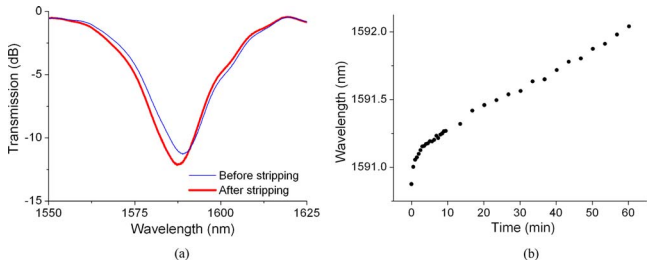

Fig. 5. (Color online) (a) Spectra of the LPFG before and after the stripping procedure. (b) Wavelength shift against time during the rehybridization process.

rinsed with DI water and dried for the rehybridization. The grating spectra, as shown in Fig. 5(a), were obtained before and after the stripping procedure. A blueshift of 1257 pm was observed after the stripping, indicating that the hybridized target DNA was being released back into solution by denaturation. After stripping, the sensor was rehybridized by immersion in 2 μM target DNA (a doubled concentration of DNA was used to enable detecting a larger spectral change) in 6×SSPE buffer for 60 min at room temperature. A 1165 pm wavelength increase was measured over 60 min [Fig. 5(b)], showing a wavelength shift rate of 119 pm/min in the first 3 min followed by 16 pm/min to 60 min. Although the enhanced sensitivity showing in this reuse hybridization is only responding to the higher concentration of target DNA, it clearly demonstrates the success of the sensor reusability.

In conclusion, a novel optical biosensor based on a dual-peak LPFG has been successfully demonstrated and used for detection of the DNA interactions in real time with high sensitivity. Our experiment clearly shows that the covalent linkage between the probe DNA and silanized LPFG surface has not been affected by the repeated heating and cooling stripping procedure. The effective noncovalent probe–target DNA bond, along with its resistance to breakdown, makes the LPFG an ideal reusable biosensor for biomolecular interaction monitoring. It may be possible to further enhance the biosensing sensitivity by employing lightly etched LPFG structures as we demonstrated before [10]. It will also be interesting to look at the advantage of this biosensor in further biomolecular interaction, for example, in developing a probe to discriminate between single nucleotide polymorphisms [14].

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