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Biosensors employing ionic self-assembled multilayers adsorbed on long-period fiber gratings

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ABSTRACT

We demonstrate that ionic self-assembled multilayers (ISAMs) adsorbed on long period gratings (LPGs) function effectively as biosensors using biotin–streptavidin as a demonstration bioconjugate pair. Biotin, streptavidin, and anti-streptavidin were deposited onto the ISAM-coated LPG sequentially, each inducing a measurable resonant wavelength shift of the LPG. Control experiments verified the specificity of the biosensor system. Furthermore, we demonstrate that ISAM coated turnaround point LPGs could serve as highly sensitive biosensors that exhibit a change in transmitted intensity rather than resonant wavelength. Experimental measurements confirm that ISAM-coated LPGs provide an attractive platform for building efficient and high-performance optical sensors.

1. Introduction

Long-period fiber gratings (LPGs) couple light between copropagating modes of an optical fiber. They have been broadly used as spectral shapers [1,2] and mode converters [3] in optical fiber communication systems because of their characteristics such as compactness, low insertion loss, and low back reflection. In addition, single-mode fiber (SMF)-based LPGs have been extensively investigated for use as chemical/biological sensors [4] and other index-modulating fiber devices [5,6] due to their high resolution and high sensitivity to the refractive index of the material surrounding the fiber. Recently, LPGs with nanoscale coatings have attracted many researchers' interest [7–12]. The resonant wavelength shift of an LPG to a thin-film coating of sub-wavelength thickness has been demonstrated experimentally and analyzed theoretically [7–12]. Uniform nanoscale coatings can be achieved by utilizing the Langmuir–Blodgett (LB) technique or ionic self-assembled multilayers (ISAMs) technique. Because LB thin-films have relatively poor mechanical and thermal stability, the ISAM technique is more suitable for practical devices due to its enhanced reliability, tunability and film quality. The ISAM technique [13,14] provides a highly controllable means to build precise, nanometer-thick films on any surface with a minimum charge density, including glass, silicon, metal, etc. Furthermore, a diverse array of materials (e.g., clay platelets, proteins, virus particles, etc.) can be incorporated into ISAM films. Therefore, ISAMs on LPGs provide a robust platform for building efficient sensors.

In this paper we demonstrate that ISAMs adsorbed on LPGs function effectively as biosensors, in which the biotin–streptavidin system serves as the bioconjugate pair. Biotin, streptavidin and anti-streptavidin were deposited onto the ISAM-coated LPG in sequence, each inducing a measurable resonant wavelength shift of the LPG. Additionally, control experiments were conducted to verify the specificity of the biosensor system. Finally, we demonstrate that ISAM-coated LPGs can serve as highly sensitive optical-intensity based biosensors. Experimental measurements confirm that ISAM-coated LPGs provide an attractive platform for building efficient optical sensors, which enhance the flexibility and cost-efficiency of LPG-based devices.

2. Simulations of TAP LPGs

In our biosensor study, a special type of LPG is used that is referred to as a turnaround point (TAP) LPG [3,6,15–18]. In order to clearly illustrate the optical properties of TAP LPG, we simulate the phase-matching curve (PMC) and the spectrum evolution of a

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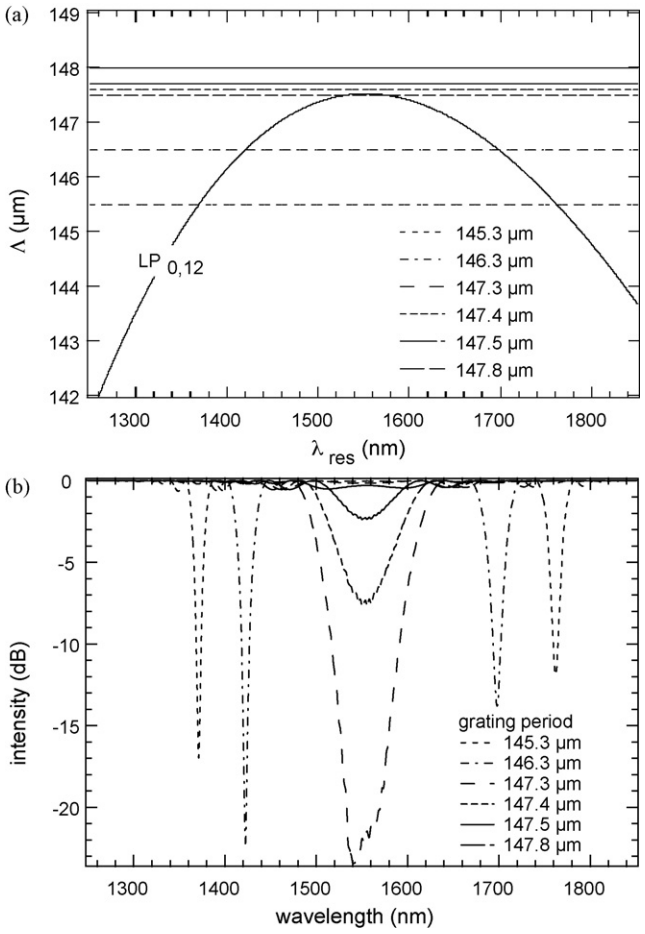

Fig. 1. Simulations of the optical properties of TAP LPG. (a) Solid curve represents the PMC of the mode $LP_{0,12}$ and dashed horizontal lines represent different grating periods (145.3 μm, 146.3 μm, 147.3 μm, 147.4 μm, 147.5 μm and 147.8 μm from bottom to top, respectively); (b) corresponding LPG transmission spectra at different grating periods.

TAP LPG. The fiber parameters chosen are: radius of the fiber core $a = 3.2$ μm, radius of the fiber cladding $b = 59.2$ μm, the surrounding medium is air with the index $n_{air} = 1$, and the core index n_1 and the cladding index n_2 are computed based on Sellmeier's equation. We select coupling from the fundamental mode to a typical high-order mode $LP_{0,12}$ to illustrate the TAP LPG properties. For this mode, the TAP is located at grating period $\Lambda = 147.3$ μm with the resonant wavelength $\lambda_{res} \approx 1550$ nm. We set the length of the LPG as 50 mm and $\overline{\delta n_{core}} = 0.000148$. The details of the simulation method are described in Ref. [9].

The simulated results are shown in Fig. 1. In Fig. 1(a), the solid curve represents the PMC of the mode $LP_{0,12}$ and dashed horizontal lines represent different grating periods (from bottom to top, they are 145.3 μm, 146.3 μm, 147.3 μm, 147.4 μm, 147.5 μm and 147.8 μm, respectively); Fig. 1(b) shows the corresponding LPG transmission spectra at different grating periods. The PMC is a plot of the resonant wavelength at which strong attenuation occurs versus the grating period. We notice that: (i) in Fig. 1(a), the grating period of either 145.3 μm or 146.3 μm intersects the PMC at two separate resonant wavelengths. Correspondingly, the grating spectrum caused by those periods possesses two separate attenuation peaks at those two resonant wavelengths as shown in Fig. 1(b). Each peak has the narrowband characteristic of conventional LPGs. When the period approaches from below the period $\Lambda = 147.3$ μm corresponding to the TAP, the two attenuation peaks move closer to one other. (ii) When the period equals 147.3 μm, the two attenuation peaks merge to become one single broadband strong attenuation peak. The 3-dB and 20-dB bandwidths are much larger than for the conventional LPG (e.g., either of the two separate attenuation peaks at $\Lambda = 145.3$ μm). (iii) When the period increases beyond the period $\Lambda = 147.3$ μm for TAP, the grating period (e.g., 147.4 μm, 147.5 μm and 147.8 μm) does not intersect the PMC at any wavelengths. The broad single attenuation peak thus rapidly decreases in strength. The grating strength (amplitude of the peak) decreases in proportion to the separation between the grating period and the PMC, since the detuning parameter becomes progressively larger. Ultimately, the PMC shifts far enough so that no resonance occurs such as in the LPG spectrum at $\Lambda = 147.8$ μm.

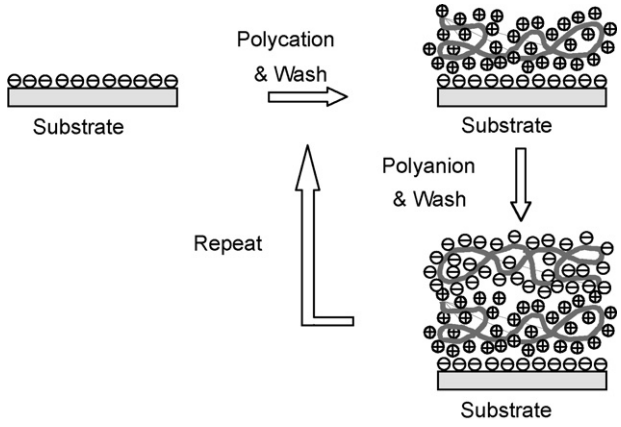

Fig. 2. Schematic of the ISAM film deposition process and illustration of film architecture of the first two layers (one bilayer).

3. Experimental results and discussion

The ISAM deposition process, also known as layer-by-layer (LbL) deposition is schematically shown in Fig. 2. Through alternately immersing a charged substrate into anionic and cationic polyelectrolyte aqueous solutions, a nanoscale multilayer organic thin-film is built due to electrostatic forces. Through use of a polyanion and a polycation solution with appropriate pH values, fine control of the thickness and the refractive index of the ISAM thin-film overlay can be achieved [8]. The combination of one monolayer of polycation and polyanion together is denoted a bilayer. In our experiments, poly(allylamine hydrochloride) (PAH) at pH = 7.5 was used as the polycation, and poly{1-[4-(3-carboxy-4-hydroxyphenylazo)-benzensulfonamido]-1,2-ethanediyl, sodium salt} (PCBS) at pH = 8.0 was used as the polyanion. Under these conditions, one bilayer of PAH/PCBS ISAM films is ~1.5 nm in thickness [8,9]. An LPG serves as the charged substrate. In the grating characterization experiments, an Ando AQ6317 Optical Spectrum Analyzer (OSA) was used. The white-light source we used is the LS-1 Tungsten Halogen Light Source of Ocean Optics Inc., which is a versatile white-light source optimized for the VIS-NIR (360–2500 nm). The fiber optic cleaver we used is Fujikura CT-07. The buffered fiber adapters we used are FC buffered fiber adapters of Fiber Instrument Sales Inc. The halogen light source is coupled into the optical fiber, and the transmitted light is coupled into the optical spectrum analyzer.

For fiber grating sensor applications, it is attractive to be able to completely remove the probe-element coating from the grating without physically modifying the properties of the grating so the grating can be reused multiple times. In the ISAM removal experiments, we compared the LPG spectra taken before depositing the ISAM film with the spectra taken after immersing an ISAM-coated LPG in an acid mixture. The acid mixture consists of 95% sulfuric acid and 5% nitric acid. Removal of ISAM films has previously been

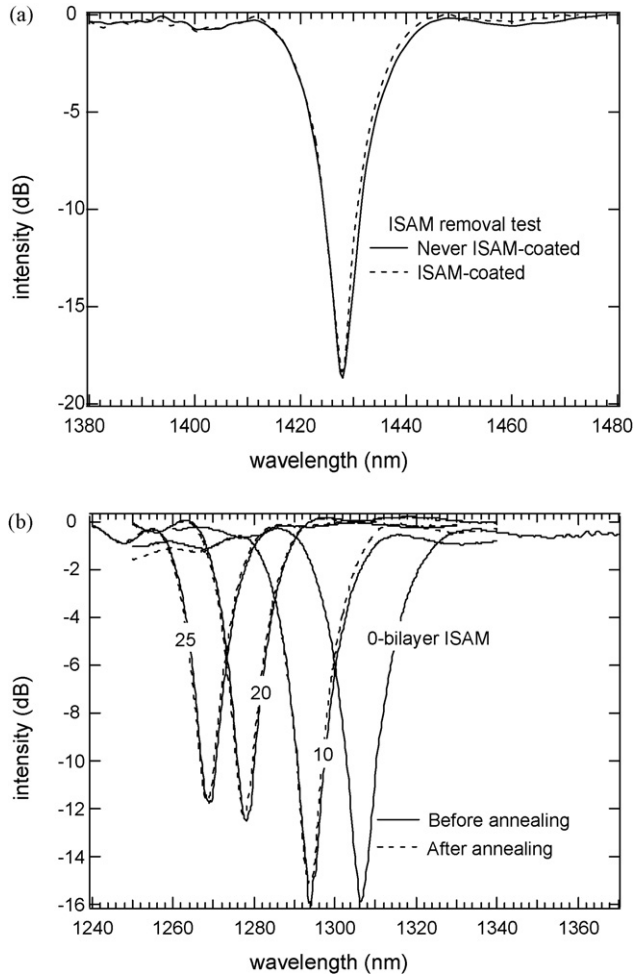

Fig. 3. (a) Comparison of the LPG spectrum taken before depositing an ISAM film on it with the spectrum taken after immersing an ISAM-coated LPG in an acid mixture (95% sulfuric acid and 5% nitric acid). The results were repeated on two separate LPGs with $LP_{0,12}$ coupling. (b) Comparison of the spectrum of a PAH/PCBS ISAM-coated LPG taken before annealing with the spectrum taken after annealing in an oven at 140 °C for 14 h for three different numbers of bilayers. One bilayer of PAH/PCBS ISAM film is ~1.5 nm in thickness.

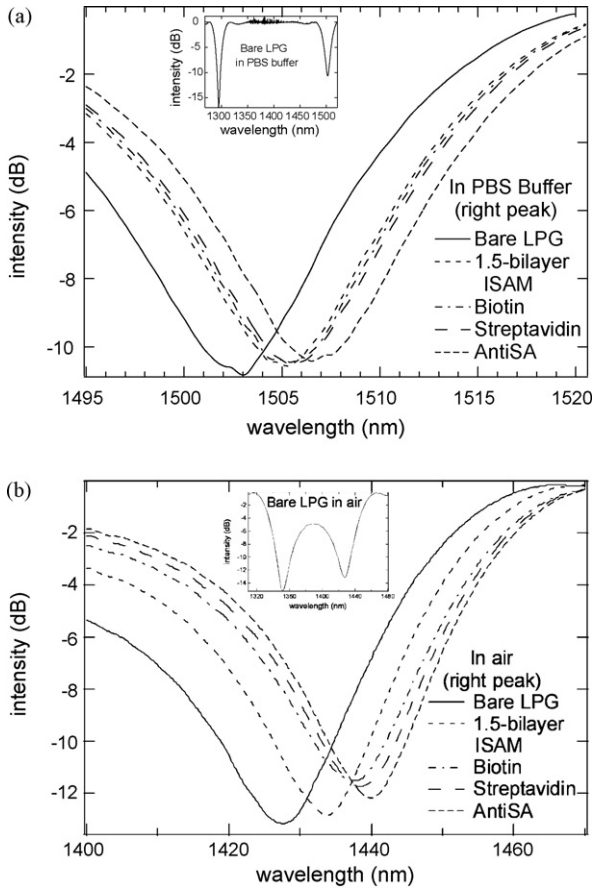

Fig. 4. ISAM-coated LPGs as biosensors. (a) LPG spectrum shift after each immersion step [bare LPG → ISAM film → biotin → streptavidin → anti-streptavidin (AntiSA)], measured in PBS buffer. (b) LPG spectrum shift after each immersion step [bare LPG → ISAM → biotin → streptavidin → anti-streptavidin], measured in air.

demonstrated using piranha solution [19]. First, the acid mixture was put into a beaker and heated up to about 80 °C. Then the ISAM-coated LPG was immersed into the hot acid mixture with only the ISAM-coated section exposed to the acid for approximately 20 min. Fig. 3(a) shows the results for an LPG with the mode $LP_{0,12}$ we tested. The solid and dashed curves represent the grating spectra before coating it with an ISAM film and after coating it with a 25 bilayer PAH/PCBS ISAM film followed by removal in the acid bath, respectively. The LPG spectrum was completely restored with a negligible variation, demonstrating that the ISAM film was completely removed from the LPG. In our experiments, we have utilized this method to reuse the same LPG for ISAM-coating related experiments dozens of times. On one hand it guarantees the repeatability and the comparability of the experimental results, and on the other hand it enhances the flexibility, cost-efficiency, and time-efficiency of the experiments. In addition, we studied the thermal stability of the ISAM film coating. We compared the ISAM-coated LPG spectra taken before and after annealing in an oven at 140 °C for 14 h. We deposited 10-bilayer, 20-bilayer and 25-bilayer PAH/PCBS ISAM films on the same grating and investigated the annealing effect on each of them. The results for annealing the ISAM films on the LPG are shown in Fig. 3(b), in which solid and dashed curves represent the grating spectra before and after annealing, respectively. The grating spectra taken before and after annealing are essentially identical in all three cases, which proves the ISAM film coating on LPGs has excellent thermal stability.

Based on our prior work demonstrating the exceptional sensitivity of LPGs to nanoscale ISAM films [8,9], we have demonstrated the use of an ISAM-coated LPG as a biosensor. In our biosensor model system we used the very strong affinity biotin–streptavidin interaction which is currently one of the most widely used systems for studying a wide variety of biological structures and processes and for clinical diagnostics [15]. Biotin is a water-soluble B-complex vitamin. Streptavidin is a 53 kDa protein with an exceptionally high binding constant with biotin of $10^{15}\ M^{-1}$. The biotin–streptavidin system serves as the bioconjugate pair in this demonstration. The biotin is immobilized on the fiber grating through the biotinylation of PAH, which is the outer layer of the ISAM film deposited on the grating. The buffer solutions used were sodium phosphate buffer and phosphate buffered saline (PBS) solution. The LPG we used was UV-induced on TrueWave RS^{TM} fibers with a grating period of 112 μm and a length of 5 cm. This yielded gratings that couple the fundamental mode to the $LP_{0,14}$ cladding mode, resulting in a TAP LPG [3,6]. As illustrated above, TAP LPGs exhibit either two separate attenuation peaks or a single broadband attenuation peak depending on the properties of the fiber and grating. We first studied a TAP LPG exhibiting two separate attenuation peaks. We deposited a 1.5-bilayer ISAM film (1 bilayer PAH/PCBS followed by 1 monolayer PAH), biotin (1 mg/ml), streptavidin (0.1 mg/ml), and anti-streptavidin (1 mg/ml) onto the LPG in sequence. LPG spectra were taken after each deposition. Fig. 4(a) and (b) shows the right

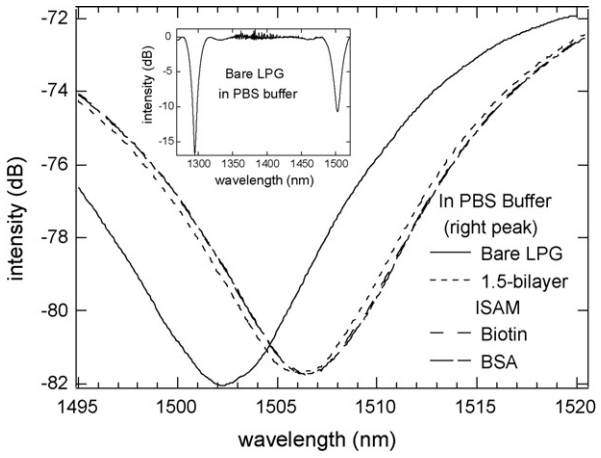

Fig. 5. Control experiment to verify the specificity—LPG spectrum shift after each immersion step [bare LPG → ISAM film → biotin → bovine serum albumin (BSA)], measured in PBS buffer.

(long wavelength) peak of the LPG spectra after exposure to each of these solutions measured when the LPG was immersed in PBS and when the LPG is exposed to the air, respectively. There is a clearly measurable resonant wavelength shift after each deposition step. Each adsorption causes a change of the thickness of the surrounding thin-film and/or a change of its refractive index, which resulted in the resonance shift of the grating. Compared to Fig. 4(a) and (b) shows a larger LPG spectrum shift after each step, which means the sensitivity of the LPG was higher when LPG was operating in air than in the PBS solution. The reason for this is a special feature of the TAP LPG—if the operating range is closer to the TAP (approximately at the midpoint between the two attenuation peaks) of the grating, the sensitivity is larger [3,6].

Additionally, we did a control experiment to verify the specificity of the system which confirmed the observed LPG spectral variations are due to the specific binding of the streptavidin to the biotin [20]. We used the protein bovine serum albumin (BSA) to test the nonspecific binding. After immersing the LPG in the biotin solution, we immersed the grating in the BSA solution (1 mg/ml, pH = 7). As shown in Fig. 5, there was no obvious resonant wavelength shift after immersing the grating in the BSA solution. This suggests that there was no nonspecific binding occurring on the LPG surface.

Finally, in order to demonstrate that ISAM-coated LPGs can serve as optical-intensity based biosensors, we etched the LPG with hydrofluoric (HF) acid such that the split attenuation peaks merged into a single broadband peak and the operating point of the LPG was set near the TAP [6]. The benefits of this optical-intensity based TAP LPG biosensor scheme are as follows: (1) a biosensor system can be fabricated with inexpensive optical components and (2) the sensitivity of the LPG is dramatically increased when the operating range is set near the TAP of the LPG [3,6]. Because the signal consists of a change in intensity at a particular wavelength rather than a shift in the resonant wavelength, inexpensive optical sources (e.g., LEDs) could be used rather than tunable lasers that are often employed in real-time measurement systems and simple photodetectors could be used rather than an expensive optical spectrum analyzer. Fig. 6(a) shows the experimental results of HF etching the LPG to the TAP. As the cladding radius is decreased, the two narrowband attenuation peaks merge into a single broadband peak which then gets weaker as the cladding is further etched, in agreement with the simulations in Fig. 1. While Fig. 1 corresponds to a fixed PMC and varying grating period, the experimental results of Fig. 6(a) correspond to a fixed grating period and a PMC that varies due to the change in the cladding mode propagation constant. The effect on the transmission spectrum is the same in both cases. Fig. 6(b) shows the experimental results of the demonstration of an optical-intensity based biosensor by utilizing the biotin–streptavidin system with the same conditions and procedure described above. Here, each step of additional adsorbed material on the cladding surface brings the LPG closer to the TAP and causes an increase in the attenuation strength. We note that the sensitivity was improved significantly compared to using the conventional narrowband LPG peaks that shift in wavelength, and an optical-intensity based biosensor system could be achieved by tracking the optical-intensity variation at the wavelength of 1386 nm. Because of the ultrasensive characteristic of TAP LPGs, the TAP LPG was characterized in a temperature-controlled room to avoid the temperature effect on the grating. Both the whitelight source and the OSA were warmed up for more than 1 h to minimize their fluctuations before measuring the grating's spectra. The grating's spectrum at each step were measured for three times to ensure the stability of the grating.

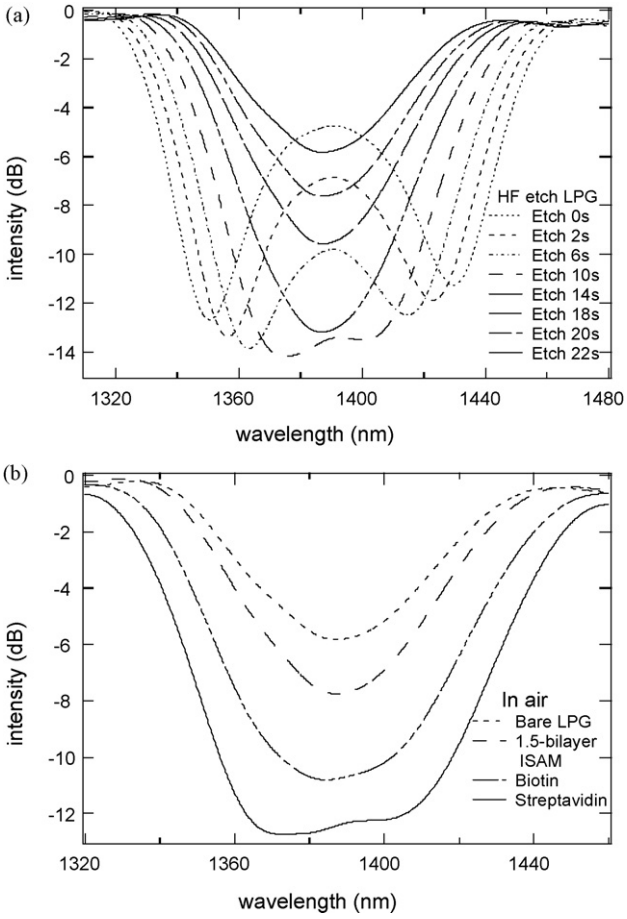

Fig. 6. (a) HF etching of an LPG beyond the turnaround point (TAP) of the LPG. (b) Demonstration of an optical-intensity based biosensor utilizing the biotin–streptavidin system [bare LPG → ISAM → biotin → streptavidin], measured in air.

Furthermore, by taking advantage of the optical-intensity based TAP LPG, we studied the sensitivity versus the concentration of the streptavidin used in the biotin–streptavidin system. The experimental procedure is the same as that in the previous experiments. We obtained 0.0125 mg/ml, 0.025 mg/ml, 0.05 mg/ml, and 0.075 mg/ml streptavidin by diluting 0.1 mg/ml streptavidin. We used the same TAP LPG employed in Fig. 6(b). Fig. 7 shows the experimental results of the sensitivity versus the concentration of the streptavidin (SA). We note that the optical-intensity variation

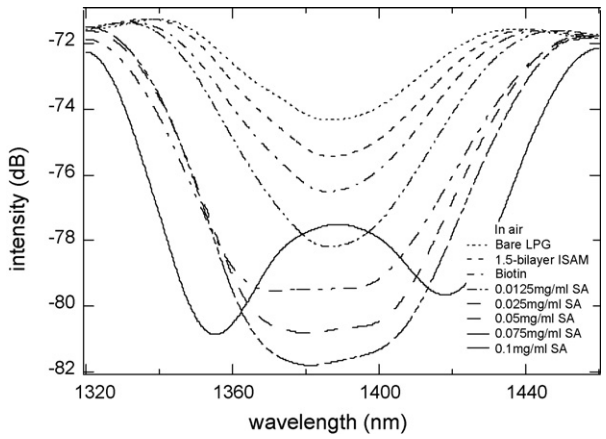

Fig. 7. Transmission spectrum versus the concentration of streptavidin (SA) of an optical-intensity based biosensor using the biotin–streptavidin system [bare LPG → ISAM → biotin → streptavidin], measured in air.

for 0.0125 mg/ml streptavidin is about 1.6 dB. Thus, this biosensor system could detect streptavidin with up to an order of magnitude lower concentration even with standard photodetectors. In addition, Fig. 7 illustrates that the dynamic range for the optical-intensity based sensor operation is from 0 mg/ml to 0.075 mg/ml in this system. Due to the unique characteristic of TAP LPGs, the single broad peak splits into two separate peaks after the concentration of the streptavidin is larger than 0.075 mg/ml. We could etch the TAP LPG further to gain a larger dynamic range as shown in Fig. 6 or use stronger LPGs (e.g., 30 dB TAP LPGs).

4. Summary

In summary, ISAM deposition offers a simple, efficient, and cost-effective method for immobilization of high sensitivity and specificity biorecognition elements onto the optical fiber device. TAP LPGs provide a platform for building highly sensitive biosensors. An optical-intensity based biosensor with sensitivity to concentrations <0.0125 mg/ml streptavidin has been demonstrated with ISAM-coated TAP LPGs. The sensitivity can be further increased by several orders of magnitude beyond that of this initial demonstration through optimizations such as TAP LPGs with stronger attenuation, thicker ISAM films, and improved functionalization of the ISAM film with the probe layer. In a separate publication, we will show that these modifications can lead to sensitivity to <12 ng/ml of streptavidin. The optical-intensity based biosensor also offers a system with low-cost and fast response time. These experimental results have demonstrated that an ISAM-coated LPG provides an attractive platform for building high-performance fiber optic biosensors.

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Biographies

Zhiyong Wang obtained his MS and Ph.D. both from Department of Electrical and Computer Engineering of Virginia Polytechnic Institute and State University in 2000 and 2005, respectively. Currently, he is working with Translational Multimodality Optical Imaging Lab of The Methodist Hospital Research Institute in Houston, TX. His research interests include optical/fiber optic sensors/spectroscopies, biophotonics (e.g., optical imaging, optical microscopy, optical spectroscopy, etc.), fiber/waveguide optics, gratings, plasmonics optics, and self-assembly material.

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Kevin Van Cott completed his B.S. in Chemical Engineering from Purdue University in 1991. He went on to receive his Ph.D. in Chemical Engineering from Virginia Tech University in 1996. At present, he is an associate professor of Chemical and Biomolecular Engineering at the University of Nebraska-Lincoln.

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Siddharth Ramachandran obtained his Ph.D. in Electrical Engineering from the University of Illinois, Urbana, in 1998. He joined Bell Laboratories, Lucent Technologies, as a Member of Technical Staff in November 1998, and subsequently continued with its spin-off, OFS Laboratories. In March 2003, he was promoted to the position of distinguished member of technical staff. Dr. Ramachandran's research focuses on fiber and fiber-grating devices in specialty fibers, with applications to biomedical imaging, lasers, sensors, as well as telecom. He has authored 118 refereed journal and conference publications including 18 invited talks and papers, 2 book-chapters, 30 patent applications, and is the editor of a Springer-Verlag book on "Fiber-based dispersion compensation."

Samir Ghalmi was born in Algiers, Algeria in 1969. He received a B.S. in fluid mechanics from USTHB in Algiers, Algeria in 1991. He earned a B.S. in 1992 and a M.S. in 1993 from the University of Aix-Marseille II, France. In 1998 he received a M.S. in M. Eng. from NJIT, Newark, NJ. From 1999 to 2001 he was an Applications Engineer at Vytran Corporation in Morganville, NJ, where he was developing new processes for fiber splicing and prototyping. Since 2001, he has worked at Bell Labs in Murray Hill, NJ, and then OFS Labs in Somerset, NJ as a member of technical staff in the optical fiber research group. His current interests include high order mode fiber devices, long period gratings and applications in fiber lasers and amplifiers.