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Long-period gratings in photonic crystal fiber as an optofluidic label-free biosensor

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ABSTRACT

Using long-period gratings (LPG) inscribed in photonic crystal fiber (PCF) and coupling this structure with an optically aligned flow cell, we have developed an optofluidic refractive index transduction platform for label-free biosensing. The LPG–PCF scheme possesses extremely high sensitivity to the change in refractive index induced by localized binding event in different solution media. A model immunoassay experiment was carried out inside the air channels of PCF by a series of surface modification steps in sequence that include adsorption of poly(allylamine hydrochloride) monolayer, immobilization of anti-rat bone sialoprotein monoclonal primary antibody, and binding interactions with non-specific goat anti-rabbit IgG (H + L) and specific secondary goat anti-mouse IgG (H + L) antibodies. These adsorption and binding events were monitored in situ using the LPG–PCF by measuring the shift of the core-to-cladding mode coupling resonance wavelength. Steady and significant resonance changes, about 0.75 nm per nanometer-thick adsorbed/bound bio-molecules, have been observed following the sequence of the surface events with monolayer sensitivity, suggesting the promising potential of LPG–PCF for biological sensing and evaluation.

1. Introduction

Photonic crystal fiber (PCF), also termed microstructured or holy fiber, consists of periodically arrayed and axially aligned cladding air channels along the entire fiber length (Knight, 2003; Russell, 2003). The endless flexibility in designing and fabricating PCF of any prescribed cladding microstructure offers a high degree of freedom to tailor the optical properties with dopant-free silica. The open air channels of micrometers in diameter also allow controlled liquid transmission through the PCF waveguide. The light can be manipulated by incorporating fluids in the PCF structure as well (Kerbage et al., 2002; Mach et al., 2002). The synergistic integration of microfluidics with optics thus enables PCF-based fiber optofluidics numerous distinct features compared to its well explored planar counterpart. PCF optofluidics has the advantages of being free-standing (no host substrate), long optical path (limited only by fiber length), high fabrication throughput (via large-scale fiber drawing technique), and easy light input/output coupling and system integration. These features, plus the accessibility of the channels for chemical or biological surface modification, render PCF optofluidics an exciting platform to conduct chem/bio sensing and detection in situ with minimal sampling volume as well as to monitor adsorption or binding events where heat transfer/mass transport is greatly enhanced. Indeed PCF has been increasingly explored for laser interrogation of chemical and biological systems using absorption spectroscopy (Rindorf et al., 2006a), fluorescence spectroscopy (Emiliyanov et al., 2007; Jensen et al., 2004; Konorov et al., 2005), and Raman (including surface-enhanced Raman scattering) spectroscopy (Khaing Oo et al., 2010; Yan et al., 2008).

Long-period grating (LPG) structure with a period of typically hundreds of micrometers is known to couple the fundamental core mode to co-propagating cladding modes in both conventional optical fiber (Vengsarkar et al., 1996) and PCF (Eggleton et al., 1999), resulting in significant attenuation at some resonance wavelengths in the transmission spectrum. The phase-matching condition for LPG is expressed by $\lambda_i = (n^{\mathrm{eff}}_{\mathrm{core}} - n^{\mathrm{eff}}_{\mathrm{clad}(i)})\Lambda$ (Erdogan, 1997), where λ_i is the resonance wavelength of the ith cladding mode coupled by the fundamental core mode, and $n^{\mathrm{eff}}_{\mathrm{core}}$ and $n^{\mathrm{eff}}_{\mathrm{clad}(i)}$ are the effective refractive indices of the fundamental core mode and the ith cladding mode, respectively. The strong dependence of λ_i on $n^{\mathrm{eff}}_{\mathrm{clad}(i)}$ makes LPG inscribed in PCF (LPG–PCF) an refractive index transduction optofluidic platform that is highly sensitive to the changes in $n^{\mathrm{eff}}_{\mathrm{clad}(i)}$ induced by any physical, chemical, or biological perturbations in the cladding structure. The ability to bring a measurant solution inside of the cladding air channels to directly

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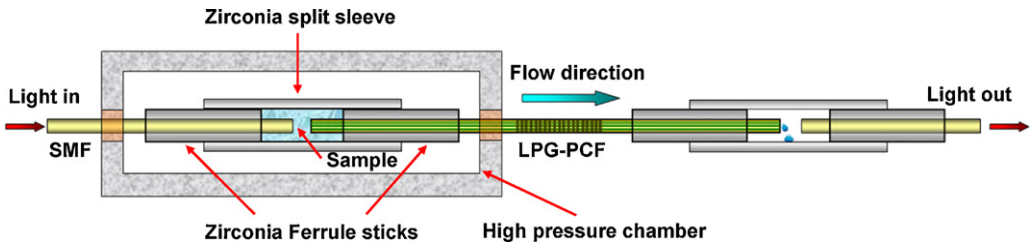

Fig. 1. Schematic of a microfluidic assembly that enables both liquid flow and light transmission measurements through LPG–PCF structure optically coupled with single mode fiber (SMF).

interact with the cladding mode further boosts the refractive index sensitivity of LPG–PCF to bulk solution by 2–3 orders of magnitude (up to $\sim 10^{-7}$ RIU) compared to LPG inscribed in conventional all-solid fiber (He et al., 2008). The LPG–PCF exhibits comparable sensitivity with the PCF sensor achieved by coupling the core mode to a mode in the adjacent fluid-filled waveguide (Wu et al., 2009) yet it is more suited for the refractive index range of aqueous solutions. The LPG–PCF is also comparable with the most-sensitive surface plasmon resonance technique achieved to date (Gopinath, 2010). LPGs have been explored as candidate for evanescent-field sensor to detect the bio-molecules interaction using the all-solid conventional optical fiber, which does not lend it for optofluidics integration. Besides, extra steps were needed to improve the sensitivity, such as forming gratings on side-polished fiber (Jang et al., 2009), etching fiber cladding to dispersion turning points (Chen et al., 2007; Wang et al., 2005) or depositing thicker multilayer coatings on the LPG to excite the transition mode (Pilla et al., 2011). The LPG–PCF platform has excellent prospects of robust sensitivity to surface binding events at the monolayer scale due to the high surface-to-volume ratio and strong evanescent field interaction at the interface of silica and immobilized layers. However, few attempts have been made to effectively couple liquid flow with LPG–PCF so as to achieve a truly integrated optofluidic device (Monat et al., 2007). While the potential of LPG–PCF for biological sensing has been demonstrated in the immobilization of double-stranded DNA on the surface of the cladding air channels (Rindorf et al., 2006b), related studies have been limited to the measurement of single surface event with a sensitivity of $\sim 10^{-4}$ RIU and a broadened resonance.

We report in this paper the first attempt, to the best of our knowledge, to modify the cladding air channels of PCF for biomolecular binding and to use LPG–PCF to monitor each and every step of the multiple modification and binding sequences. Further unique in this study is that we designed and integrated a microfluidic cell that can optically couple light into and out of PCF while enabling continuous liquid flow through the air channels as part of an integral surface modification/binding and in situ measurement scheme. We have demonstrated that the LPG–PCF optofluidics refractive index transduction platform is sensitive to monolayer adsorption/binding event.

2. Materials and methods

2.1. Materials

Poly(allylamine hydrochloride) (PAH), MW~15,000 g/mol, Phosphate Buffered Saline (PBS) 0.01 M, Albumin from human serum (HSA) ≥97% were purchased from Sigma–Aldrich. Anti-rat bone sialoprotein (BSP) (WVID1-9C5) monoclonal primary antibody was obtained from Developmental Studies Hybridoma Bank, Iowa City, IA. Goat anti-rabbit IgG (H + L) (AP307P) was received from Chemicon. FITC-goat anti-mouse IgG (H + L) was obtained from Invitrogen. All chemicals were used as received without further purification.

2.2. Optical and flow-through coupling with PCF optofluidics

The prevailing approach utilized in the experiments with liquid-containing PCF has been to fill the air channels with the solution of interest, followed by separate optical measurements of the solution retained in the PCF via capillary force under static conditions (Rindorf et al., 2006b). This approach suits the purpose of most laboratory research while avoiding the challenge in integrating optically coupled flow cells for continuous transport of solution through the cladding air channels. The ability to inscribe LPG in PCF and to chemically and biologically modify the surface of the air channels under continuous liquid flow while optically accessible for process monitoring is critically important for better process control, enhanced experimental efficiency and consistency, and for system integration. First, it ensures reproducibility in LPG fabrication of solution-filled PCF as solution loss at the open ends of the PCF will otherwise take place under static conditions due to rapid heating and expansion of the solution upon CO_2 laser irradiation, significantly altering the absorption condition in the subsequent laser inscription step. Second, continuous supply of fresh reagent makes surface modification and subsequent rinsing of cladding air channels more efficient. Third, the optical coupling enables in situ measurements of light transmission, hence constantly monitoring the evolution of resonance band during LPG fabrication and the shift of the resonance wavelength as adsorption or binding events take place.

Depicted in Fig. 1 is the schematic of the optically coupled flow cell system constructed for the investigation. It consists of two main parts. One part is in essence a compressed air pressure chamber (~200 psi) that houses the flow entrance end of the PCF and an optically aligned (within ±1 μm) single mode light-source fiber in a zirconia microfluidic reservoir of 50 μl capacity. The other part is the same type of zirconia microfluidic reservoir that houses the exit end of the PCF and a single mode light-collection fiber optically aligned to the same precision as above but under ambient pressure. The pressure difference between the two reservoirs forces continued liquid flow through the PCF air channels during LPG fabrication or surface modification. Liquid addition to or extraction from the reservoirs is done via a syringe. The flow system can be easily disassembled and cleaned to ensure a contamination-free environment.

2.3. LPG–PCF fabrication

The PCF used in this work is ESM-12-2 from NKT Photonics. The cross-section scanning electron microscope (SEM) image of the fiber is shown in Fig. 2. Its microstructure is featured by a hexagonal array of ~3.2 μm cladding air channels with an inter-channel distance of ~8 μm. The ESM-12-2 is endlessly single mode with a large mode area. A CO_2 laser (Synrad, water-cooled 48-1 10W)

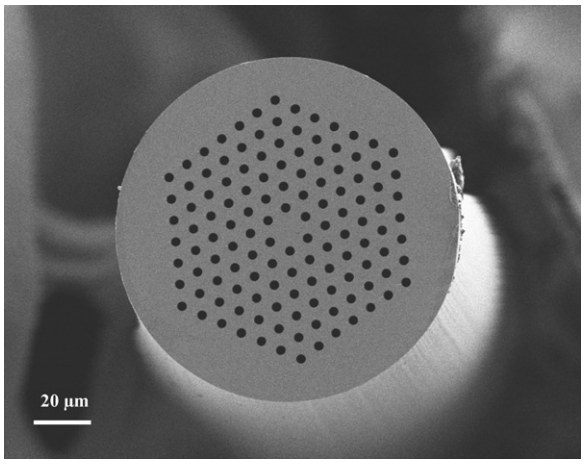

Fig. 2. SEM micrograph of ESM-12-2 PCF. The diameter of the air channels is 3.2 μm. The inter-channel distance is 8 μm.

with closed loop kit to stabilize output power (variation less than ±1%) is employed to inscribe LPG in PCF. The refractive index modulation arises from the so-called opto-elastic effect with its origin stemming from residual stress relaxation in PCF by rapid, localized heating via CO_2 laser irradiation. The excellent power stability of our laser source greatly improves the reproducibility of LPG fabrication. The laser is projected as a 3000 μm × 200 μm spot on PCF with the longer beam profile straddling the fiber in radial direction. The portion of PCF where LPG is inscribed is firmly mounted on a motorized, precision translation stage with 0.2 μm minimum incremental motion. The entire LPG structure is fabricated with laser power and movement of the translation stage controlled by computer interface. An optical spectrum analyzer (OSA, Anritsu MS9710 C) with a Super-K ultra broadband supercontinuum light source is used to measure the transmission spectrum in situ during LPG fabrication. The power and the exposure time of the CO_2 laser are optimized to ensure strongest resonance while avoiding physical deformation of PCF by potentially excessive heating via constant monitoring by a CCD camera for any changes in fiber dimension. A continuous flow of water is maintained in PCF throughout the LPG inscription process.

3. Results and discussion

3.1. The characteristics of LPG–PCF

For comparison, we first fabricated an LPG–PCF in the absence of water in the cladding air channels. The inscription was done at 5 W with 55 ms irradiation for each of the 90 periods with a grating periodicity of 490 μm. Illustrated in Fig. 3(a) are the transmission spectra of the as-fabricated water-free LPG–PCF (red) and the same LPG–PCF subsequently filled with water (blue). (For interpretation of the references to color in this text, the reader is referred to the web version of the article.) The as-fabricated LPG structure exhibits two distinct resonance bands at 1507 and 1553 nm, indicating strong core-to-cladding mode couplings. Subsequent filling with water totally alters the phase matching condition and results in the disappearance of the resonance bands. The broad band over 1400–1500 nm can be attributed to strong water absorption in this range. Shown in Fig. 3(b) are the transmission spectra of the LPG–PCF fabricated under flowing water (blue) and the same LPG–PCF but with subsequent removal of water (red). The inscription parameters are similar as those in the absence of water, except a total of 78 periods in the grating and 80 ms irradiation to compensate for the absorption of CO_2 laser energy by water in the cladding air channels. The water flow rate is about 300 nl/min at 200 psi

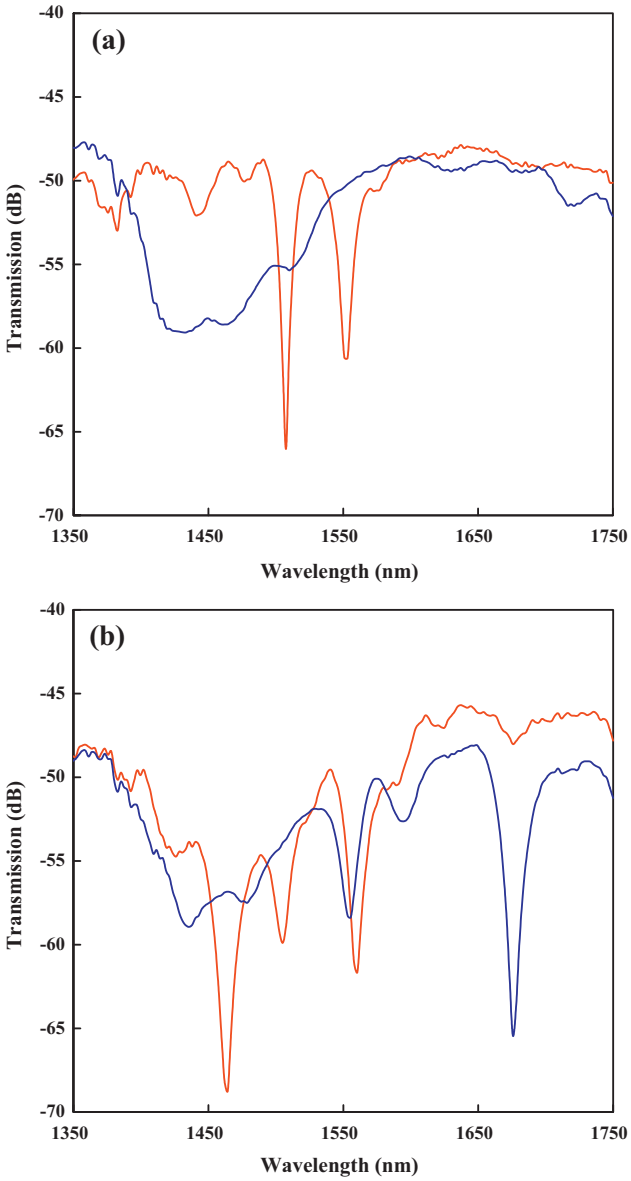

Fig. 3. (a) Transmission spectra of LPG–PCF (a) as-fabricated (red) and filled with water (blue), (b) inscribed in the presence of flowing water (blue) and after water removal from the airchannels (red).

pressure differential between the entrance and the exit of the PCF. In addition to the broad water absorption band over 1400–1500 nm, there exists two main resonance peaks at ~1690 nm and ~1560 nm, evidence of strong core-to-cladding mode coupling. Upon removal of water, mode coupling is retained in the LPG structure but with the resonance wavelengths blue shifted. The lack of resemblance between the water-filled and water-free transmission spectra from the otherwise the same LPG–PCF is a predicted result of the strong dependence of mode couplings on $n^{\text{eff}}{}_{\text{clad}(i)}$. An important point to be made here is that LPG inscription needs to be carried out in a liquid medium, preferably with the index of refraction similar to that of measurant solution in order to preserve the general resonance phase matching condition for sensitive refractive index transduction.

3.2. Biomolecular binding and monitoring by LPG–PCF

Using the LPG–PCF fabricated with flowing water, we demonstrate the measurements of the biomolecular binding events

in situ using an Immunoglobulin G (IgG) antibody and its secondary antibody. In the experiment ~15 cm PCF was used and the length of LPG region is ~4 cm. The effective sampling volume is defined by the total volume of the cladding air channels in the LPG–PCF and is estimated to be ~40 nl in our case. For rapid and sensitive affinity-based biosensing, it is important to control antibody orientation on the refractive index transduction platform for efficient binding. The silica surface of the cladding air channels is inherently negatively charged. It is known that antibody does not interact strongly with negatively charged surfaces (Buijs et al., 1997). In addition, use of low ionic strength polyelectrolyte solutions has proven effective to facilitate controlled adsorption of Immunoglobulin G (IgG) (Chen et al., 2003). The adsorption of antibody to the electrostatic self-assembled polymer monolayer is the simplest way to immobilize biomolecules (Tanvir et al., 2009). The near-zero shear stress and flow rate near the cladding channel surface can minimize the desorption of the adsorbed biomolecules from the channel surface. Hence as the first step, positively charged PAH monolayer is deposited on the air channels via electrostatic interaction. Specifically, PAH is prepared as 0.2 mg/ml solution in Millipore water and adjusted to pH 9. The PAH solution is transmitted through the cladding air channels of the LPG–PCF under 200 psi for 10 min. Unabsorbed PAH is subsequently rinsed by Millipore water for another 20 min. The LPG–PCF is next filled with pH 7.4 PBS to provide baseline for transmission measurements that follow. A 57 μg/ml anti-rat BSP monoclonal primary antibody solution is subsequently introduced into the LPG–PCF for 10 min followed by thorough rinsing with PBS for 20 min. A 0.25 mg/ml HSA solution is further introduced as block protein to prevent non-specific binding on surface not occupied by anti-rat BSP, and followed by 20 min PBS rinsing. 50 μg/ml of non-specific goat anti-rabbit IgG (H + L) solution that is not expected to bind with anti-rat BSP in the PCF channels is then introduced as control, followed by 20 min PBS rinsing. As a final step, a secondary antibody, goat anti-mouse IgG (H + L), is flown through the cladding air channels for specific binding with the primary antibody anti-rat BSP, followed again by 20 min PBS rinsing. The rinsing steps are to remove unadsorbed biomolecules inside the LPG–PCF structure. The rinsing time was determined by tracking the shift of resonance peak until it remains constant. The goat anti-mouse IgG (H + L) antibody is conjugated with fluorescein isothiocyanate (FITC) in the PCF to ascertain the controllability of the surface modification processes in the microscopic air channels over the entire length of the PCF. The confocal fluorescence microscopy measurement of the LPG–PCF after the multiple modification steps shown in Fig. 4 confirms uniform distribution of IgG throughout the cladding air channels.

Optical measurements are carried out in situ and throughout all modification and rinsing procedures. In particular, the resonance band at ~1690 nm (Fig. 3(b) blue) is monitored given its strong mode coupling and sharp resonance feature. Shown in Fig. 5 are the corresponding transmission resonance spectra of the modification and binding events before and after rinsing. The initial resonance peak at 1690 nm undergoes red shift as a consequence of the adsorption of additional layers on the cladding air channels and hence increasingly greater changes in $n^{\text{eff}}{}_{\text{clad}}$ than $n^{\text{eff}}{}_{\text{core}}$. (For interpretation of the references to color in this text, the reader is referred to the web version of the article.) The significant shift at each surface event before and after ringing is a result of the difference in the indices of refraction of the various solutions and the PBS. The adsorption of PAH layer leads to the most striking shift in the resonance wavelength due to the robust nature of the adsorption event and the large molecular weight of the PAH. A resonance wavelength shift of 1.8 nm occurs upon adsorption of the anti-rat BSP on the PAH layer, with an additional 0.2 nm shift as a result of the deposition of the HSA protein block layer. While the introduction of the non-specific goat anti-rabbit IgG solution leads to a

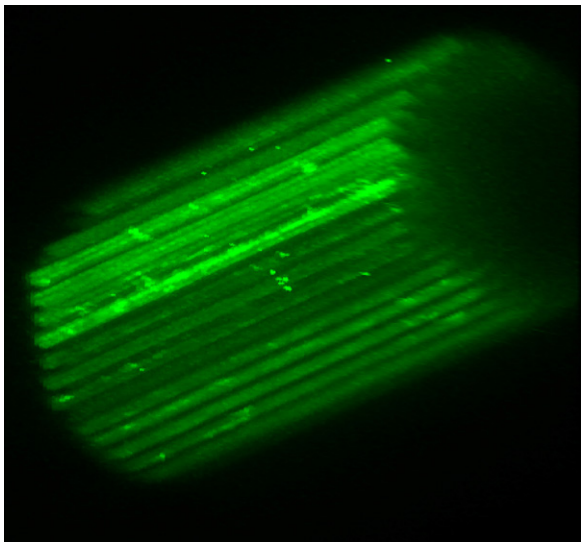

Fig. 4. Confocal fluorescence image of the LPG–PCF containing FITC-goat anti-mouse IgG antibody that is bound to the adsorbed primary antibody goat anti-mouse IgG.

significant shift of 1.3 nm, the measurement after rinsing indicates no detectable binding event, as expected. In fact, the 0.1 nm blue shift may be indicative of possible desorption of anti-rat BSP during PBS rinsing. A major shift of 1.6 nm takes place with the specific binding of goat anti-mouse IgG to anti-rat BSP. Based on our measurements using a phase-modulated ellipsometer, the thicknesses of the anti-rat BSP and goat anti-mouse IgG on silicon substrate are 2.4 nm and 2.1 nm, respectively. Assuming similar thickness values for goat anti-mouse IgG and anti-rat BSP, we estimate resonance peak shift of about 0.75 nm for every nanometer-thick adsorbed/bound bio-molecules.

Summarized in Fig. 6 is the process-dependent resonance wavelength shift of the LPG–PCF, with insets depicting the specific adsorption or binding events. As seen in this figure, the shift over the course of the adsorption and binding follows a steadier trend after rinsing than before rinsing. This distinction can be attributed to the fact that the shift after rinsing is due to directly adsorbed or specifically bound monolayer. On the other hand, the measured

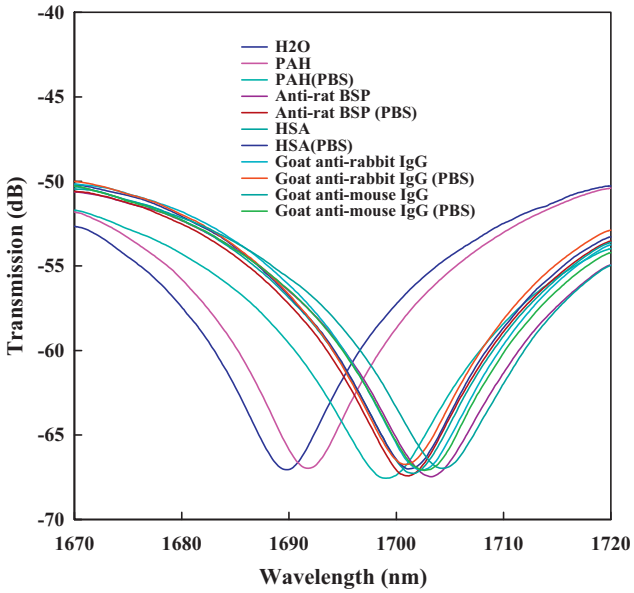

Fig. 5. The shift of resonance wavelength of the LPG–PCF at the various surface events.

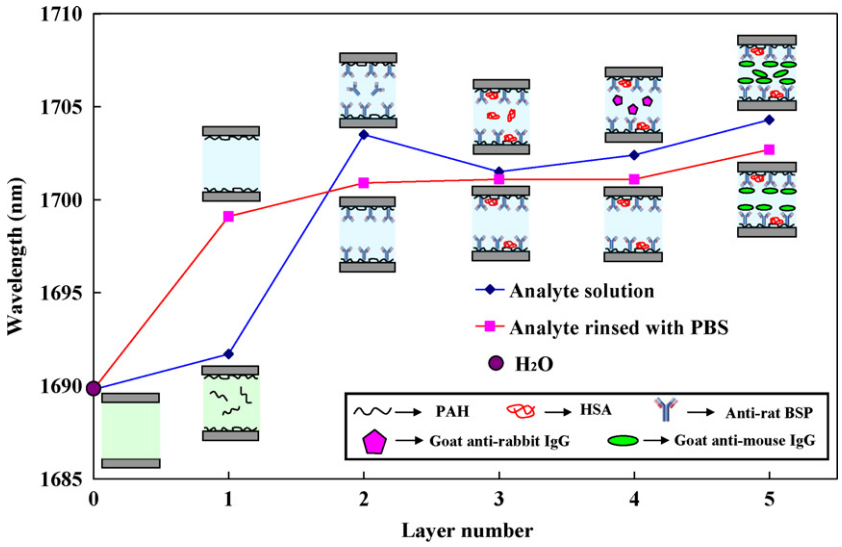

Fig. 6. The shift of resonance wavelength of the LPG–PCF at various surface events before (blue) and after PBS rinsing (red). Insets are depictions of the adsorption/binding events. The protein block is used to minimize the non-specific adsorption of secondary antibody on PAH. The secondary antibody binds specifically to the primary antibody. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

shift before rinsing is a convolution of the contributions from the surface process and the solution which differs from PBS. Rinsing to remove unbound species in the cladding air channels is thus a preferred approach to better elucidate the binding events and for more reliable biological sensing/measurements using the LPG–PCF refractive index transduction platform.

4. Conclusions

Through fabrication of LPG in PCF with continuous water flow and via controlled surface modification of the cladding air channels using polyelectrolyte monolayer for subsequent biomolecular binding interactions, we have shown that LPG–PCF is a powerful optofluidic refractive index transduction platform that exhibits monolayer sensitivity at each and every surface adsorption or binding event. This finding is of great significance in that it represents an important viable alternative to many existing affinity-based biological sensing and measurement approaches while offering many potential advantages such as easy system integration for high throughput, low cost, and massive parallel analysis with low sampling volume. PCF in general and LPG–PCF in specific have the potential to help realize the lab-in-fiber optofluidic concept for basic and applied research that utilizes laser either as an energy source for light-enhanced interactions or as a tool for spectroscopy-based interrogation in geometrically confined systems.

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