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Highly sensitive detection of molecular interactions with plasmonic optical fiber grating sensors ☆

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ABSTRACT

Surface Plasmon resonance (SPR) optical fiber biosensors constitute a miniaturized counterpart to the bulky prism configuration and offer remote operation in very small volumes of analyte. They are a cost-effective and relatively straightforward technique to yield in situ (or even possibly in vivo) molecular detection. The biosensor configuration reported in this work uses nanometric-scale gold-coated tilted fiber Bragg gratings (TFBGs) interrogated by light polarized radially to the optical fiber outer surface, so as to maximize the optical coupling with the SPR. These gratings were recently associated to aptamers to assess their label-free biorecognition capability in buffer and serum solutions. In this work, using the well-acknowledged biotin–streptavidin pair as a benchmark, we go forward in the demonstration of their unique sensitivity. In addition to the monitoring of the self-assembled monolayer (SAM) in real time, we report an unprecedented limit of detection (LOD) as low as 2 pM. Finally, an immunosensing experiment is realized with human transferrin (dissociation constant $K_d \sim 10^{-8}$ M^{-1}). It allows to assess both the reversibility and the robustness of the SPR-TFBG biosensors and to confirm their high sensitivity.

1. Introduction

Surface Plasmon resonance (SPR)—the excitation by light of surface Plasmon waves propagating at the interface between a noble metal (most often gold or silver) and a dielectric finds many applications in (bio)chemical sensing (Homola, 2006; Andersson et al., 2010; Dostálek et al., 2007). Thanks to their micrometric scale dimensions, optical fiber SPR sensors enable practical applications not possible with the bulky Kretschmann prism configuration, such as remote operation in very small volumes of the order of microliters (Slavik et al., 2002; Jeong et al., 2013). To excite SPR in optical fiber-based plasmonic sensors, light initially confined in the fiber core has to be locally outcoupled and brought into contact with the surrounding medium. Practically, this can be achieved either mechanically by polishing (or etching) the cladding so as to expose the evanescent wave to the surrounding medium or by using radiative fiber gratings (refractive index modulations imprinted in the fiber core thanks to its intrinsic photosensitivity) such as long period gratings (LPGs) or tilted fiber Bragg gratings (TFBGs). They both couple the core mode to cladding modes that propagate towards the cladding-surrounding medium interface and are therefore sensitive to surrounding refractive index (SRI) changes (Verma et al., 2008; Schuster et al., 2012; Shevchenko et al., 2010; Baldini et al., 2012). In this work, we make use of TFBGs that inherently possess all the advantages of optical fibers. In comparison to other optical fiber SPR configurations, they gather the additional benefits of keeping the fiber integrity while yielding intrinsically temperature-insensitive measurements (Albert et al., 2013), which is particularly worthwhile to obtain highly sensitive SRI measurements.

A standard fiber Bragg grating (FBG) consists of a refractive index modulation in the core of an optical fiber that is imprinted perpendicular to the propagation axis. An FBG acts as a selective mirror in wavelength, reflecting some wavelengths around a characteristic one, so-called Bragg wavelength. The latter is sensitive to temperature and mechanical strain but, as it results from light coupled in the fiber core, it is not influenced by SRI changes. A TFBG corresponds to a core refractive index modulation angled by a few degrees with respect to the perpendicular to the propagation axis. This induces two kinds of couplings: the self-backward coupling of the core mode at the Bragg wavelength and numerous couplings between the core mode and backward-going cladding modes at resonance wavelengths below the Bragg wavelength. Contrary to the Bragg resonance, the cladding

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mode resonances are modified by SRI changes (Laffont and Ferdinand, 2001; Caucheteur and Mégret, 2005; Chan et al., 2007), depending on the SRI value with respect to their own effective refractive index value.

TFBGs yield SPR generation when a nanometric gold coating is deposited all around the fiber section, which further increases their sensitivity to SRI changes (Caucheteur et al., 2011; Voisin et al., 2011). It reaches more than 500 nm/refractive index unit (RIU) in the SRI range between 1.32 and 1.42, yielding an intrinsic resolution better than 2×10^{-6} RIU when using a measurement device accurate to 1 pm (as provided by interrogation techniques based on a tunable laser source). This high resolution combined to a quality factor of 10^5 and signal-to-noise ratio greater than 50 dB opens the path to biodetection (Shevchenko et al., 2011). Another important feature of these sensors is their self-immunity from temperature fluctuations as the Bragg wavelength provides an absolute reference. Measurements are therefore normalized with respect to the Bragg wavelength shift to get this intrinsic compensation.

In practice, the SPR generation can only occur when the light launched into the TFBG is polarized radially to the fiber surface. A remarkable property of TFBGs is that they excite spectrally distinct cladding mode families with fields radially polarized (so-called P-polarized modes) and azimuthally polarized (S-polarized modes) at the cladding boundary (Caucheteur et al., 2011). P-polarized modes couple to the SPR and are therefore strongly attenuated with respect to neighboring cladding mode resonances, which is not the case for S-polarized modes.

It was recently demonstrated that gold-coated TFBGs functionalized with aptamers can be used for label-free biosensing (Shevchenko et al., 2011). Protein detection in buffer and serum solutions was achieved in micromolar concentrations. In this work, we use the well-acknowledged antibody–antigen affinity mechanism to further assess the unique protein detection and quantification capabilities of these SPR-TFBGs. We are aware to go one step backward in terms of biomaterials technology with respect to the use of aptamers but this is useful to evaluate the performances of the proposed biosensors in terms of both sensitivity and limit of detection (LOD) and to compare them with other optical fiber biosensors. We report two kinds of experiments, with the biotin–streptavidin pair and with human transferrin. The biotin–streptavidin couple has been used as a reference in a wide variety of new device studies and in a lot of bioanalytical applications (Scott Phillips and Cheng, 2007; Takahashi et al., 2012). This great interest is largely due to the exceptionally high affinity (dissociation constant $K_d = 10^{-15}\,M^{-1}$) and the subsequent stability of this non-covalent interaction. The most straightforward method to immobilize biotin on a gold surface is a self-assembled monolayer (SAM) composed of alkanethiols functionalized with biotin (Seifert et al., 2010). This is what we have exploited in this work.

As a follow up to (Shevchenko et al., 2011; Caucheteur et al., 2013), we exploit here the differential behavior of cladding mode resonances in the vicinity of the SPR mode to monitor the SAM formation and to finely measure streptavidin concentrations. For this, functionalized SPR-TFBG biosensors were immersed in phosphate buffered saline (PBS) with streptavidin molecules at very low concentrations ranging from 10^{-11} to 5×10^{-4} g/ml. We report the highest spectral variations ever obtained with gold-coated TFBGs biosensors, allowing to reach a limit of detection equal to 2 pM.

Finally, an immunosensing experiment is reported with human transferrins. They are iron-binding blood plasma proteins that control the level of free iron. They are also associated with the immune system and reflect the presence of pathologies such as iron deficiency anemia. It is thus clinically relevant to identify and quantify such proteins. In this work, they were chosen for their K_d value equal to $10^{-8}\,M^{-1}$ to demonstrate that our immunosensor can operate well in real biochemical conditions. Transferrins also allow to demonstrate the reversibility of the binding, the regeneration of the biosensor and to further assess its robustness. To do so, an SPR-TFBG with antibody transferrin immobilized on gold was put into contact with different human transferrin solutions (concentrations ranging between 10^{-7} g/ml and 10^{-3} g/ml). The regeneration is obtained with glycine and NaCl.

2. Materials and methods

2.1. Materials

2.1.1. Tests with the biotin–streptavidin couple

Biotinylated alkyl thiol (mercaptoundecyl-hexaethyleneoxy biotin amide) was purchased from Nanoscience Instrument (Belgium). Ethanol in HPLC grade was purchased from Sigma-Aldrich (Belgium) and used without further purification as solvent for thiols incubation. Alexa Fluor-488-streptavidin was purchased from Invitrogen (Belgium). Its molecular weight is equal to 55,090 g/mol. Phosphate buffer saline (PBS) solution from PAA laboratories (Austria) was used as solvent for streptavidin anchoring.

2.1.2. Tests with human transferrin

6-Mercaptohexanoic acid (90%) purchased from Sigma-Aldrich (Belgium), Human Holo-Transferrin from R&D systems (UK), Antibody anti-Transferrin from AbD Serotec (Germany), N-hydroxysuccinimide (NHS) from Sigma-Aldrich (Belgium), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) from Sigma-Aldrich (Belgium), glycine at pH=2 from Sigma-Aldrich (Belgium) were used as received. HBS-EP 1X purchased from GE Healthcare (Belgium) was diluted 10 times with Milli-Q water before use.

2.2. Fabrication of the SPR-TFBGs

1 cm-long TFBGs were manufactured into hydrogen-loaded telecommunication grade single-mode optical fiber using a 1090 nm period uniform phase mask and a frequency-doubled argon-ion laser emitting at 244 nm. The mask was tilted in the plane perpendicular to the optical fiber axis, as illustrated in Albert et al. (2013). TFBGs were obtained by a single scan of the laser beam (width: 0.6 mm; averaged power: 60 mW) along the phase mask at the speed of 20 μm/s. An external tilt angle of 6° was chosen with respect to the perpendicular to the optical fiber axis to ensure strong coupling to cladding modes characterized by an effective refractive index close to water (1.33). Right after the inscription process, the gratings were annealing at 100 °C during 24 h to remove the residual hydrogen and to stabilize their physical properties.

A 50 nm gold coating (99.99% purity target) was then deposited on the TFBGs using a standard sputtering process. The vacuum was obtained in the chamber starting from ambient air. Two consecutives depositions were made in the same conditions, with the optical fibers rotated by 180° between the two processes to ensure that 100% of the surface is covered by gold.

2.3. Development of the SPR-TFBG biosensors

2.3.1. Biotinylation of the SPR-TFBGs outer surface

The gold-coated TFBGs were first thoroughly rinsed with ethanol and cleaned under ozone atmosphere to remove unwanted contaminants. To obtain the SAM at the outer surface of the sensors, they were then immersed in a solution of biotinylated thiols (mercaptoundecyl-hexaethyleneoxy biotin amide) dispersed in ethanol. Thiols incubations were done during 16 h at room temperature in a 1 mm thick

capillary tube sealed at both ends to prevent solvent evaporation. At the end of the incubation, the functionalized gold-coated TFBGs were removed from the tube and again rinsed with ethanol, prior to their use for streptavidin quantification.

2.3.2. Grafting of the transferrin antibody on the SPR-TFBGs outer surface

Binding of transferrin antibody on the gold coating was processed in several steps. SPR-TFBGs were first cleaned with ethanol and ozone just before immersion in a solution of mercaptohexanoic acid (2 mM) for 12 h to allow the formation of a SAM of thiols on the gold coating (step **1**). Before binding the antibody, the SPR-TFBGs outer surface was activated by immersion in a mix of 200 μl NHS (75.0 mg/ml) and 200 μl of EDC (11.5 mg/ml) for 15 min (step **2**). The antibody was suspended in acetate buffer (10 mM; pH=5) at a concentration of 30 μg/ml and put into contact with the activated SPR-TFBG for 30 min (step **3**) (Aubailly et al., 2011).

2.4. Methods

2.4.1. Experimental set-up

SPR-TFBG transmitted amplitude spectra were recorded by means of an optical vector analyzer (OVA) from LUNA Technologies. The OVA is a stand-alone equipment that combines (among other components) a tunable laser and a powermeter. It is commonly used to measure the complex response of an optical device both in amplitude and in phase. Here, it is essentially used for its high measurement resolution (1.25 pm) and fast scanning rate (~1 s to cover the full SPR-TFBG spectrum). It works as follows: light emitted by a tunable laser is launched into the device under test (functionalized gold-coated TFBG placed in a microfluidic system) through an external linear polarizer. It is used to control and orient the input state of polarization so as to excite P-polarized light. The fiber output is then directly connected to the OVA input (powermeter entrance) so as to measure the TFBG transmitted spectrum corresponding to the P-polarized light. It is worth mentioning that care was taken to avoid polarization instabilities (use of short fiber lengths, strong curvatures avoided and ambient temperature kept constant to within 1 °C). For immersion, in order to use volumes of solutions of the order of microliters, SPR-TFBGs were placed in a capillary tube of 500 μm of inner diameter and solutions were injected using an in-house developed microfluidic system. Transmitted spectrum measurements were taken in static solutions, roughly 2 min after injection in the capillary tubes. Solutions were pushed away using an equivalent volume of PBS.

Fig. 1 displays the P-polarized mode transmitted amplitude spectrum of an SPR-TFBG immersed in salted water (refractive index close to 1.38). It shows comb-like cladding mode resonances whose minimum amplitudes are aligned to a Gaussian envelope, except for some cladding mode resonances around 1550 nm that couple to the SPR and are consequently strongly attenuated. The Bragg wavelength appears at the right end side, centered on 1602 nm. In the following, the Bragg wavelength (sensitive only to temperature changes and not to changes of the surrounding refractive index medium) is used to decorrelate the surrounding temperature influence on the sensor response through the monitoring of its shift. This is an intrinsic feature of our device, which is very interesting in practice as a change of 0.1 °C induces a surrounding refractive index change of 10^{-5}, thus comparable to the resolution of our sensing platform.

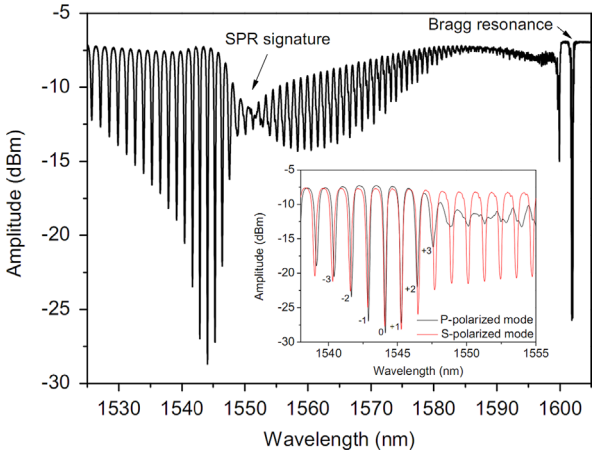

Fig. 1. Amplitude transmitted spectrum of an SPR-TFBG immersed in salted water (refractive index close to 1.38). In inset, close-up around the SPR signature for the two orthogonal polarization modes.

2.4.2. Demodulation of SPR-TFBGs biosensors

To retrieve the information about molecules concentration in the transmitted amplitude of SPR-TFBGs, we make use of a couple of resonances, according to Caucheteur et al. (2013). The inset of Fig. 1 displays a close-up around the SPR signature for the P-polarized and S-polarized modes. Modes are labeled consistently according to Caucheteur et al. (2013). As demonstrated in Shevchenko et al. (2011), the SPR mode is the most sensitive to refractive index changes at the sensor outer surface. However, for slight SRI shifts such as those generated by biomolecules attachment, the SPR mode is too attenuated and too broadened to yield precise measurements. Fortunately, the transmitted amplitude spectrum of the SPR-TFBG presents other worthwhile mode resonances, most particularly the 0 and +2 modes. It was indeed demonstrated that the wavelength shift of the 0 mode and the amplitude variation of the +2 mode are the most relevant grating features to quantify slight SRI changes. In this work, we will also exploit these resonances to detect and quantify macromolecular interactions.

3. Results and discussion

3.1. In situ monitoring of the SAM formation of biotin

During 12 h, transmitted amplitude spectra of the SPR-TFBG immersed in thiols solution were recorded using light polarized radially to the fiber outer surface (P-polarized mode). Fig. 2 shows the local evolution of the amplitude and the wavelength of relevant cladding mode resonances as a function of time. The increase of the wavelength and of the peak-to-peak amplitude indicates a red shift of the SPR. During the first hours, both parameters present an exponential variation, revealing that the refractive index changes at the sensor outer surface, this one affecting the evanescent wave coupled by the sensor. After about 9 h, the wavelength shift of the 0 mode is saturated. The amplitude of +2 mode continues to slightly evolve up to about 15 h, time after which the SAM formation is complete. These results reflect the full potential of the SPR-TFBGs biosensors: when the wavelength shift of the 0 mode becomes limited due to slight SRI changes, the amplitude change of the +2 mode can be used with more accuracy. The results also confirm that the future SPR-TFBG biosensor can be used in situ as a diagnostic tool during the incubation to ensure that it has been properly realized.

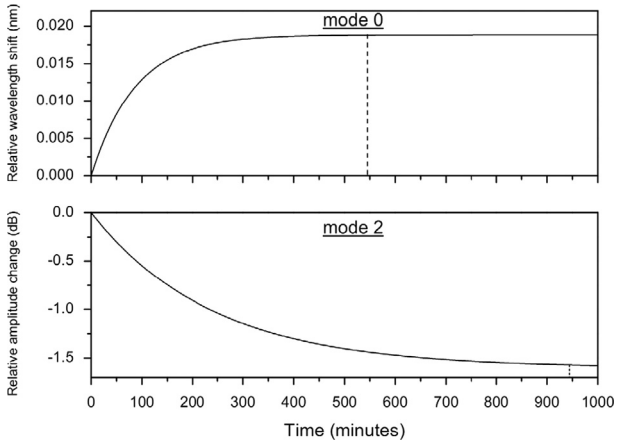

Fig. 2. Wavelength shift of the 0 mode and relative amplitude change of the +2 mode and as a function of the SAM formation of biotinylated thiols.

3.2. Quantitative detection of macromolecular interactions based on the streptavidin/biotin affinity

Biotynilated SPR-TFBGs were immersed in different streptavidin solutions with concentrations ranging between 10^{-11} and 5×10^{-4} g/ml. Their refractive index was measured with a refractometer Reichert AR 200 at 589 nm. Every solution is characterized by a refractive index that matches the one of PBS, with an uncertainty of 10^{-4} corresponding to the resolution of the refractometer. This control ensures that the obtained data can be attributed to the binding of streptavidin molecules and not to undesired refractive index variations. Streptavidin molecules used in this work possess a fluorescent tag and are characterized by a molecular weight equal to 55,090 g/mol. The fluorescent tag was used to verify the correct adhesion of streptavidin molecules through microscopy. As done previously for SRI changes, the precise quantification of the streptavidin concentration requires the local monitoring of selected cladding mode resonances. It is worth to mention that the variation of the SPR signature caused by macromolecular interactions is evaluated relative to measurement done in PBS, which is thus taken as a reference.

Fig. 3 depicts the evolution of the wavelength and the amplitude of the two relevant cladding mode resonances relative to their value in the reference spectrum as a function of the streptavidin concentration. The data presented in Fig. 3 have been obtained on 5 different sensors prepared and tested in the same experimental conditions. They reveal that the lowest streptavidin concentration (LOD) that our sensor is able to detect is of the order of 10^{-10} g/ml, corresponding to 2 pM. For the sake of comparison, LOD levels for some recent optical fiber configurations are listed below:

- LOD$\sim 10^{-6}$ g/ml for nanoscale coated long period fiber gratings, which are radiating gratings particularly sensitive to the surrounding medium (Wang et al., 2009);
- LOD better than 10^{-4} g/ml for long period gratings working in transition mode (Pilla et al., 2009);
- LOD$\sim 10^{-6}$ g/ml for photonic crystal fiber-based interferometers present an LOD of the order of 10^{-6} g/ml (Hu et al., 2012);
- LOD = 6 pM for optical fiber tips coated with gold nanodot arrays realized from the electron beam lithography (Lin et al., 2010).

As a consequence, an LOD of 10^{-10} g/ml makes our biosensor configuration particularly competitive with respect to other advanced optical fiber probes and to commercially available solutions based on the bulky Kretschmann prism.

As commonly done in the field, the LOD was simply estimated from the actual protein concentration in the sample volume used for the experiments. In doing so, the LOD could be slightly overestimated as it normally refers to the concentration in the active biosensing surface area (smaller than the total volume used taking into account the penetration depth of the light outcoupled from the optical fiber and the diffusion rate of biomolecules towards the sensor surface creating a depletion zone, according to Sheehan and Whitman (2005).

It is worth mentioning that the measured wavelengths are corrected with respect to the slight Bragg wavelength shift (2 pm maximum) attributed to temperature changes. The wavelength shift of the 0 mode reaches 84 ± 1.25 pm at 10^{-4} g/ml. Amplitude variations of the +2 mode reaches 1.15 ± 0.05 dB and allow the detection of streptavidin up to 2×10^{-4} g/ml, value for which saturation is reached. Therefore, it comes out that, at this concentration, all the functionalized surface is covered by streptavidin molecules.

During our experiments, the complete modification of the SPR signature took only 2 min to occur after immersion. This brief response time is attributed to the very strong affinity between biotin and streptavidin, on one hand, and to the fact that the sensor offers a localized sensitive area (only 4 mm^2 for a 1 cm long TFBG), on the other hand.

3.3. In situ monitoring of the transferrin antibody SPR-TFBG functionalization

The evolution of the SPR-TFBG amplitude spectrum is monitored in real time during the steps (**1**–**3**) required to sensitize the biosensor, as described in Section 2.3.2. Fig. 4(a) presents the SPR signature at the beginning and at the end of the immersion in the solution of mercaptohexanoic acid (step **1**). The variation of the amplitude of the cladding mode resonances reflects the global wavelength SPR shift caused by the SAM formation. Again, we have tracked the amplitude of the +2 mode (arrow on Fig. 4(a)). The amplitude evolution of this cladding mode is shown in Fig. 4(b). We observe that the peak-to-peak amplitude of the cladding mode resonance increases during about 9 h and then, the amplitude stays stable.

Then, the SAM anchored on the gold surface of the SPR-TFBG is activated (step **2**) with equal volumes of NHS and EDC for 20 min. Finally, the modified SPR-TFBG is immersed in a solution of antibodies (step **3**), suspended in 10 mM acetate buffer at pH 5.0 at a concentration of 30 μg/ml. The anchoring of the transferrin antibody on the SAM is monitored during the process by tracking the amplitude of the cladding mode resonance indicated by an arrow in Fig. 4(c). This figure shows the transmitted amplitude spectrum of the SPR-TFBG with and without antibody. To obtain the reference measurement, the SPR-TFBG with SAM is immersed in acetate buffer that has the same refractive index than the antibodies solution. The amplitude variation of the resonance shown in Fig.4(d) indicates a red shift of the SPR and allows concluding that the sensor has to be immersed during 23 min to achieve the complete binding of antibodies.

3.4. Detection, regeneration and repeatability of the SPR-TFBG immunosensor

Here, regeneration cycles were operated: the transferrin antibody SPR-TFBG was brought into contact with a human transferrin solution. Then, the antigen was eliminated with a pulse of glycine (pH = 2) for 1 min. Fig. 5 (a) shows a regeneration cycle. This figure compares the amplitude spectrum of the sensor in buffer (reference), in antigen solution (10^{-3} g/ml) and in buffer after the elimination of antigens. We observe that the antibody–antigen

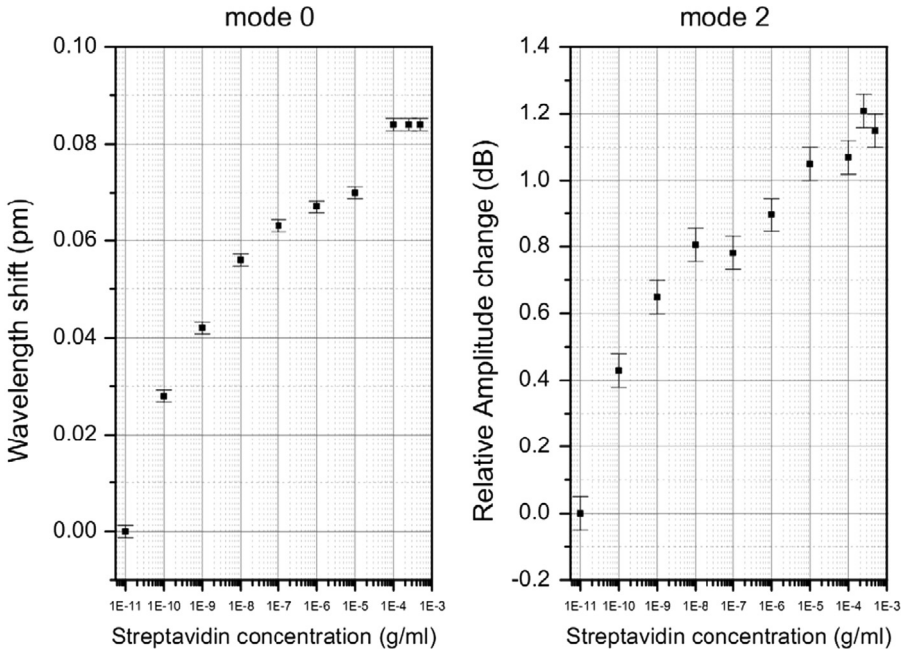

Fig. 3. Evolution of the peak wavelength (left: 0 mode) and the peak amplitude (right: +2 mode) as a function of the streptavidin concentration.

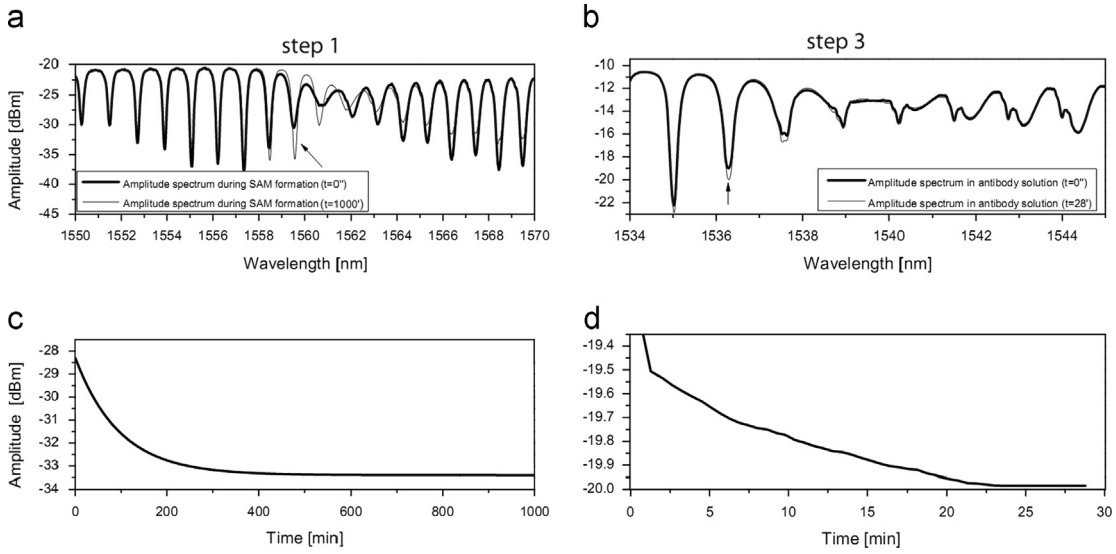

Fig. 4. (a) Comparison of the amplitude spectrum with and without the SAM at the metallic surface, (b) evolution of +2 during SAM formation in the solution of mercaptohexanoic acid, (c) comparison of the amplitude spectrum with and without the antibodies, and (d) evolution of +2 mode during the binding of antibodies.

interactions are detected by a red shift of the SPR of about 2 nm. We also appreciate the proper elimination of antigens as the amplitude spectrum after the immersion in acidic solution matches perfectly the reference amplitude spectrum. We repeated three regeneration cycles with the same antigen solution (10^{-3} g/ml) to prove the good repeatability of the sensor. Fig. 5(b) shows a bar graph with the mean amplitudes of +2 mode indicated by an arrow in Fig. 5 (a) when the biosensor is in antigen solution and in buffer after regeneration. The first bar indicates the reference amplitude of the +2 mode, which varies within a range of 0.05 dB. The second bar gives the mean amplitude for three measurements in antigen solution (10^{-3} g/ml). We obtain a change of about 4.5 dB in comparison with the reference value and a standard deviation of 0.18 dB. Finally, the last bar indicates the mean amplitude after regeneration. We observe a difference of 0.04 dB with the reference value. This difference is lower than the resolution of the measuring device. These measurements confirm the reversibility of the SPR-TFBG and yield repeatability equal to 0.18 dB. As a consequence, the sensor can be calibrated for antigen quantification. Fig. 6 depicts the +2 mode resonance for different values of antigen concentration ranging between 10^{-7} and 10^{-3} g/ml. Taking into the change with respect to the buffer and the error bars, the limit of detection ranges here between 10^{-7} and 10^{-6} g/ml. As our main purpose was again to quantify the LOD of the device, we did not investigate the full range of sensitivity. The tested values confirm that our biosensor behaves well in the range of transferrin concentrations to be measured in case of anemia. The selectivity of the biosensor configuration is inherent to the antibody/antigen couple used in this work. It has been tested with bovine serum albumin (BSA—5 μM). The

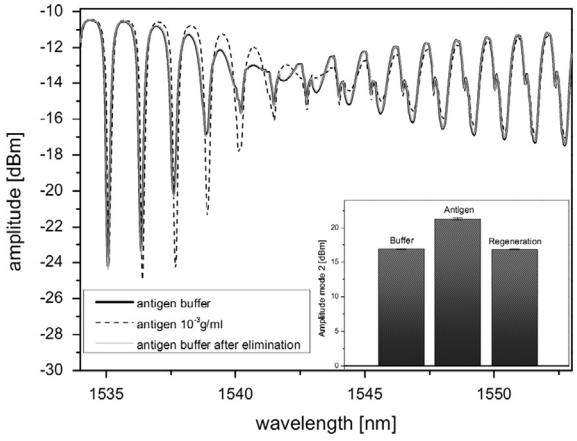

Fig. 5. Comparison of amplitude spectra for a regeneration cycle ($n=1.335$), (inset) bar graph showing the mean amplitude of +2 mode for different steps of the regeneration cycle.

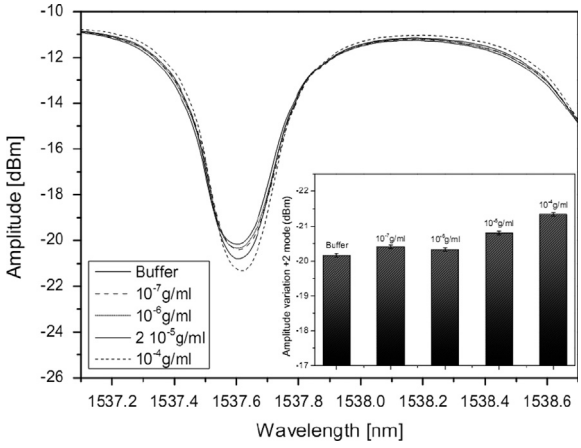

Fig. 6. Amplitude spectrum evolution of the +2 mode for different values of antigen concentrations, (inset) bar graph showing the amplitude variation of the +2 mode for different concentrations (± 0.05 dB).

amplitude change observed with respect to the PBS solution (taken as a reference) falls within the measurement error and is therefore not presented in Fig. 6.

4. Conclusion

The optical fiber biosensor configuration reported in this work consisted gold-coated TFBGs interrogated by light polarized radially to the optical fiber outer surface, so as to maximize the optical coupling with the SPR. Neighboring cladding mode resonances exhibit a differential sensitivity in response to slight SRI changes better than 10^{-5} RIU. This peculiar behavior has been exploited to assess the protein detection and quantification capabilities of these SPR-TFBGs. For this, a SAM of biotin has been formed on the sensor outer surface to quantify streptavidin molecules diluted in PBS. We have demonstrated that a single SPR-TFBG is capable not only to monitor the SAM formation in situ but also to accurately quantify very low streptavidin molecules concentrations down to 2 pM. The SPR-TFBG is a biosensor able to detect antibody–antigen interactions. We based the measurement on transferrin and anti-transferrin affinity. Regeneration of the biosensor was achieved and allowed the detection of several samples. Moreover, this sensor presents a good repeatability. Therefore, the SPR-TFBG can be calibrated for quantification of antigen concentrations. This biosensor configuration combines the advantages of both SPR sensors and optical fiber sensors. It is prototyped to allow in situ measurement, remote interrogation in very small volumes and real-time monitoring.

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