Fiber-optic immunosensor based on lossy mode resonances induced by indium tin oxide thin-films

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***Abstract*—A novel immunosensor based on lossy mode resonances (LMRs) induced in optical fibers is developed in this contribution. Indium tin oxide (ITO) is sputtered on the optical substrate to generate an LMR in the transmission spectrum. Type G immunoglobulins (IgGs) are then attached to the ITO-coated fiber using (3-glycidyloxypropyl)trimethoxysilane (GPTMS). A phosphate buffer saline solution containing anti-IgGs is used to detect the biological reactions. The presented device is capable of detecting anti-IgG concentrations up to 10 nM. These results will permit the fabrication of biosensors based on a covalent attachment of bioreceptors over an LMR inducing thin-film.**

Keywords—optical fiber; immunosensors; thin-films; lossy mode resonances; indium tin oxide

I. Introduction

Nowadays, developing biosensors is a real challenge, since there is a need for obtaining new devices that give patients a proper early diagnose. In fact, the number of contributions addressing this topic is increasing due to the interest in applying this technology to biomedical engineering [1].

A good example of biosensing applications is to detect the formation of antibody – antigen complexes, as occurs in this contribution. Antibodies have an important role in clinical analytics when performing Enzyme-Linked ImmunoSorbent Assays (ELISAs), since they help diagnose patients suffering from diseases [2]. Type G antibodies or immunoglobulins (IgGs) are biological macromolecules that protect the living beings against bacterial and viral infections. They consist of two parts. The first one, Fc, is similar in every of them and it is what permits to attach the IgGs when designing a biosensor. The second part, Fab, is specifically designed for each target antigen.

Due to the increasing interest of developing biosensors, a wide research is being developed in the last years. In this sense, one of the most focused issues is the development of fiber-optic-based biosensors, which takes in advantage the well-known features that fiber-optics offers to biosensing applications [3]. Several techniques have been developed to solve this issue, including the use of long-period fiber gratings (LPFGs) [4] or the generation of resonances [5]. The final goal is to obtain a detection method based on the wavelength shift of the created resonances, what is more versatile and robust [5].

One of the simplest ways to obtain an optical resonance in the spectrum is to use an optical fiber-based structure that permits to access the evanescent field of the light propagating through the waveguide. Then, the resonant phenomenon can be generated and characterized by just depositing a thin-film of the adequate material onto the fiber. In this sense, this contribution proposes the deposition of an indium tin oxide (ITO) thin-film onto the optical fiber. ITO gives extra properties to the probe: it increases the sensitivity of the device, due to its higher refractive index compared to silica, and it provides a plane surface, what is adequate to covalently adsorb bioreceptors on top of the optical substrate [7].

Moreover, by depositing an ITO thin-film, a specific type of electromagnetic resonance (EMR), lossy mode resonance (LMR), can be generated. Here, it is convenient to remember the three types of EMRs that can be induced by depositing thin-films: long-range exciton-polaritons (LRSEPs), surface plasmon resonances (SPRs) and lossy mode resonances (LMRs) [8]. Currently, there are examples of biosensing applications both in SPRs and LMRs, although this contribution will focus on LMRs, since the idea is to exploit the advantages that make them more suitable when facing a biosensing application. Among them, the fact of reaching sensitivity values near 20,000 nm/RIU [9]. This is interesting from a clinical point of view, since a wide wavelength range where to shift involves to better distinguish between different analyte concentrations.

Thus, the remainder of this work is as follows: in section 2, the optical and chemical materials and the method used to design the LMR-based biosensor is presented. In section 3, the substrate activation and the obtained LMR is characterized and the biodetection process is shown. Finally, some conclusions are extracted on the use of LMRs as biosensing platforms.

II. Experimental Details

A. LMR generation and optical materials

The first step to achieve the biosensor was to generate the LMR. To this purpose, several optical fiber segments from a 200/250 μm (core/cladding diameter) cladding removable multimode optical fiber (CRMMF) were obtained. They were pre-treated as indicated in [7] in order to clean their surfaces and activate them for the next step. Then, a sputtering deposition using an ITO target was performed over the fibers with parameters: Argon partial pressure of 9×10^{-2} mbar and current of 150 mA. Fibers were fixed to a rotator at 5 rps rotational speed and then located under the ITO target. To track the spectrum

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evolution as a function of the deposition time, sputtering times from 15 to 180 seconds were scheduled.

Once the substrates were fabricated, the set-up depicted in Fig. 1 was prepared. Basically, 30 mm-length segments of the ITO-coated fibers were spliced to two CRMMF pigtails. A broadband light source covering a wavelength range from 400 to 1800 nm launched light into one end of the new created waveguide. The other end was connected through a bifurcated optical fiber to both XR1-FLG and a NIR-512 spectrometers (from OceanOptics Inc.), which permitted to monitor a wavelength range from 400 to 1700 nm.

Regarding data processing, every time a transmission spectrum was captured it was referenced to the initial spectrum obtained when the sensor was immersed for the first time in water solution. Additionally, in order to track the LMR shift in wavelength during the whole process, a real-time MATLAB® algorithm based on least squares was programed.

B. Substrate biofunctionalization and chemical materials

Once the first LMR was located at the desired wavelength, the next step was to functionalize the fiber. Thus, the fiber was immersed in 1% (3-glycidyloxypropyl)trimethoxysilane (GPTMS) diluted in toluene for 90 minutes [6]. Then, the goat IgGs adsorption was carried out afterwards by simply immersing the silanized fiber in a PBS solution of IgGs. As plotted in the inset of Fig. 1, GPTMS owns an epoxy terminal on top of the carbohydrate chain, which can be used to directly bind the goat IgGs by immersing the substrates in a 10 mM phosphate buffer saline solution (PBS) at pH 7.4. Thus, 200 µl from the stock IgGs solution in 12 mg/ml concentration were transferred to a 10 ml cuvette and the fiber was then immersed for a maximum of 3 hours. After that, the bioprobe was rinsed in PBS solution to remove the excess of biomolecules that might not have bound.

Finally, the fabricated sensor was immersed in 100 ml of 1 mM PBS adjusted at pH 7.4 during 90 minutes, to make the sensor get used to the environmental conditions. Then, a 2.2 mg/ml stock solution of anti-goat IgGs (anti-IgGs) was added periodically into the detection solution, increasing the anti-IgGs concentration. At the same time, a syringe pumped the whole detection solution to imitate a flowing environment. The LMR minimum was tracked during the whole process.

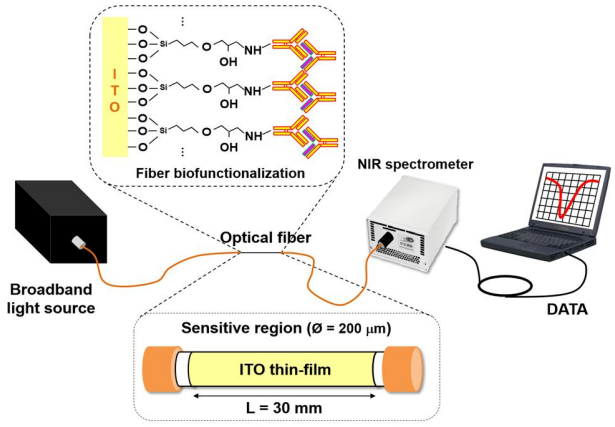

Fig. 1. Schematic set-up designed to monitor the fiber-optic biosensor designed in this contribution. Upper inset shows the fiber biofunctionalization, following the process described in [6].

Although the work presented in this contribution is a label-free detection, the same process was also performed to several non-monitored fibers, in order to corroborate that the anti-IgGs were adequately attached to the thin-film. To this purpose, a solution containing horseradish peroxidase (HRP)-conjugated goat anti-IgGs was used to bind the adsorbed IgGs. This solution also contained phosphate buffer saline (PBS pH 7.4) and 1% of bovine serum albumin (BSA). The presence of BSA is critical, as it prevents the adsorption of IgGs from binding with their non-specific sites. Additionally, 3,3',5,5'-Tetramethylbenzidine (TMB) liquid substrate system was also used to corroborate the formation of IgG – anti-IgG complexes [10].

III. Results and Discussion

A. LMR and ITO thin-film characterization

According to [7,10], several LMRs can be obtained with ITO-coated-based optical fiber sensors. The first LMR is the most sensitive, and it requires depositing less material than to generate the rest of resonances. In this sense, Fig. 2a, shows the first LMR obtained after depositing 30 seconds of ITO. Fig. 2b depicts a SEM image of the ITO thin-film, where its measured thickness is 124 nm and the plane and homogeneous surface as a result of the sputtering process can be checked.

B. Biological attachment and detection

After obtaining the LMR, the next step was to attach some molecules that permitted to bind the bioreceptors to the ITO thin-film covalently. The chemical silanization of GPTMS in toluene has been previously described in the experimental section and in [6]. Thus, the silanized fiber was tested by tracking the LMR minimum after immersing the sensitive area in the PBS solution containing goat IgGs. Here, after diluting the initial IgGs solution in PBS, the IgGs concentration in the cuvette was 232 µg/ml. Since the average molecular weight for a goat IgG is 144 kDal [11], this means 1.61 µM concentration.

As indicated previously, the bioprobe was then cleaned and immersed in 1 mM PBS solution. After that, an increasing concentration of anti-IgGs was achieved by droping a stock solution of 2.2 mg/ml IgGs (15.28 µM) in the PBS solution until the sensor stopped to respond adequately to the biological stimulus. During this time, the LMR wavelength shift was

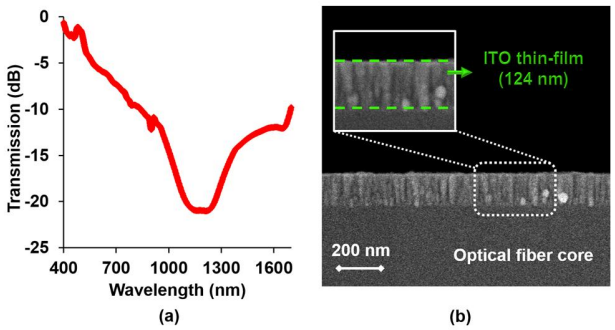

Fig. 2. LMR obtained by sputtering a 30 second-layer of ITO. (b) SEM image of the ITO thin-film, obtaining a thickness of 124 nm.

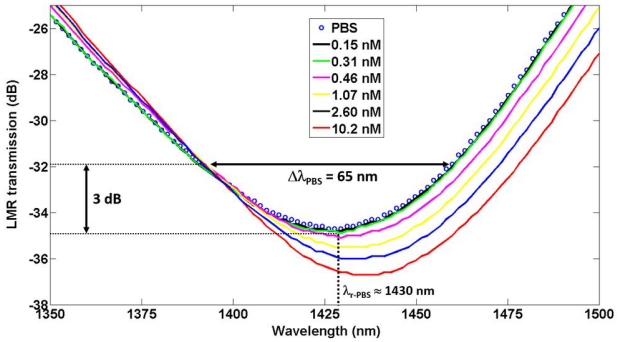

Fig. 3. LMR parameters obtained when addressing the biodetection of the IgG – anti-IgG complexes. It can be observed the red-shift in wavelength as a function of the increasing anti-IgGs concentration.

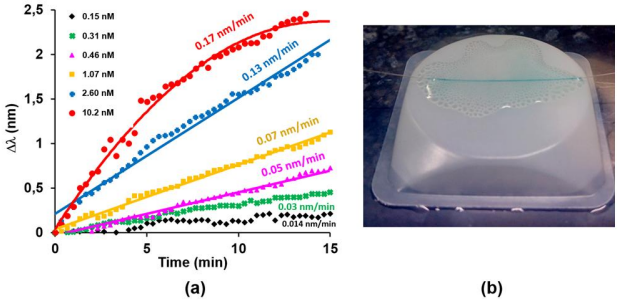

Fig. 4. (a) Evolution of the LMR shift rate as a function of the increasing anti-IgGs concentration. (b) Corroboration of the anti-IgGs presence by means of the TMB-HRP enzyme reaction.

monitored as described in section II.A. Thus, Fig. 3 shows a characterization of the LMR during the anti-IgGs deposition. First, the biosensor was immersed in PBS for an hour to let it stabilize (see blue dotted spectrum). After stabilizing, the LMR was centered at 1430 nm, with a maximum attenuation of 35 dB and a spectral width (3 dB above the minimum) of 65 nm.

Then, as the anti-IgGs concentration increases, the spectrum behavior is monitored, obtaining a wavelength shift from 1430 nm to almost 1440 nm. Fig. 4a shows a change in the shift rate depending on the amount of anti-IgGs as they are accumulated during the detection. For the sake of simplicity, each single slope is extracted and then referenced to its initial wavelength, (the moment after dropping more anti-IgGs solution). This permits to see the differences in the slopes, corresponding to the response of the bioprobe to the increasing IgGs concentration. It is clear that, as more IgGs are in the solution, the slope of the sensor response increases as a function of time.

Finally, some evidence of the anti-IgGs attachment to the bioprobes is shown, based on the TMB-HRP enzymatic reaction mentioned in section II.B. A color change from transparent to blue indicates a correct attachment. The colorimetric reaction was carried out over a small white Teflon® tray. Basically, bioprobes were subjected to 1 ml TMB, carefully dropped over the sensitive area. The color change is clearly visible in the fiber (Fig. 4b), corroborating the presence of anti-IgGs.

IV. Conclusions

This contribution has presented the steps followed to obtain a fiber-optic biosensor based on lossy mode resonances (LMRs) generated with indium tin oxide (ITO) thin-films.

The optimization of the bioprobe has consisted of first depositing a 30 second sputtered-ITO layer onto a cladding removed optical fiber, to generate the LMR and to obtain a plane thin-film where to attach the immunoglobulins (IgGs). Then, a 90 minute-silanization based on (3-glycidyloxypropyl)trimethoxysilane (GPTMS) has been performed in order to covalently adsorb type G immunoglobulins (IgGs). The biosensor detection has been achieved by measuring the LMR shift rate. As a consequence of the increasing anti-IgGs concentration, the wavelength shift speed of the LMR increased from 0.014 nm/min to 0.17 nm/min for a minimum concentration of 0.15 nM to a maximum of 10.2 nM before stabilizing the response.

All things considered, a covalent attachment of bioreceptors has been achieved after an LMR is developed on an optical fiber, which supposes promising results for the design of biosensing applications based on LMRs in the future.

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References

[1] V. Scognamiglio, “Nanotechnology in glucose monitoring: Advances and challenges in the last 10 years”, Biosensors and Bioelectronics Vol. 47, 2013, pp. 12–25.

[2] S.Y. Toh, M. Citartan, S.C.B. Gopinath, T-H. Tang, “Aptamers as a replacement for antibodies in enzyme-linked immunosorbent assay”, Biosensors and Bioelectronics Vol. 64, 2015, pp. 392–403.

[3] X.D. Wang, O.S. Wolfbeis, Fiber-Optic Chemical Sensors and Biosensors (2013-2015), Analytical Chemistry. 88, 2016, pp. 203–227.

[4] F. Baldini, F. Chiavaioli, A. Giannetti, S. Tombelli, C. Trono, "High-performance Label-free Biosensing by Long Period Gratings," in Frontiers in Optics 2015, OSA Technical Digest (online) (Optical Society of America, 2015), paper FTh2E.1. ISBN: 978-1-943580-03-3

[5] L. Wu, H. S. Chu, W. S. Koh, and E. P. Li, “Highly sensitive graphene biosensors based on surface plasmon resonance”, Optics Express Vol. 18, No 14, 2010, pp. 14395-14400.

[6] D. Guoa, M. Zhuoa, X. Zhangb, C. Xua, J. Jianga, F. Gaob, Q. Wana, Q. Li, T. Wanga, “Indium-tin-oxide thin film transistor biosensors for label-free detection of avian influenza virus H5N1”, Analytica Chimica Acta 773, 2013, pp. 83–88.

[7] C.R. Zamarreño, , S. Lopez, M. Hernaez, I. Del Villar, I.R. Matias, F.J. Arregui, “Resonance-based refractometric response of cladding-removed optical fibers with sputtered indium tin oxide coatings”, Sensors and Actuators B: Chemical, Vol. 175, 2012, pp. 106–110.

[8] F. Yang, J.R. Sambles, “Determination of the optical permittivity and thickness of absorbing films using long range modes”, J Mod Opt Vol. 44 (6), 1997, pp. 1155-1163.

[9] F. J. Arregui, I. Del Villar, J. M. Corres, J. Goicoechea, C. R. Zamarreño, C. Elosua, M. Hernaez, P. J. Rivero, A. B. Socorro, A. Urrutia, P. Sanchez, P. Zubiate, D. Lopez, N. De Acha, I. R. Matias, “Fibre-Optic Lossy Mode Resonance Sensors”, 28th Eurosensors Conference 2014, Brescia (Italy), Sep. 7-10 2014, Procedia Engineering ISSN: 1877-7058.

[10] A.B. Socorro, I. Del Villar, J.M. Corres, F.J. Arregui, I.R. Matias, “Spectral width reduction in lossy mode resonance-based sensors by means of tapered optical fibre structures”, Sensors and Actuators B: Chemical Vol. 200, 2014, pp. 53–60.

[11] D. Givol, E. Hurwitz, “Goat Inmunoglubulin G. Peptide chains and terminal residues”, Biochem. J. 115, 1969, 371.