Immunoassays using tilted fiber Bragg gratings: an overview

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**ABSTRACT**

Biosensors are expected to provide fast, sensitive, and robust detection at low cost. Despite all these constraints which weigh on the development of emerging technologies and conception of new prototypes, the major challenge is still to carry out measurements in complex matrices and hard-to-reach environments. Optical fibers are perfectly suited to fulfill these requirements. In this paper, we investigate the use of tilted fiber Bragg gratings (TFBGs) photo-inscribed in the core of telecommunication-grade optical fibers as biosensors. Thanks to their high refractive index sensitivity, they are able to track molecular interactions happening on their surface. We present different strategies to use them for label-free immunoassays. Bare, gold-sputtered, gold electroless-plated (ELP), and hybrid configurations were functionalized with antibodies, aiming at the detection of cancer biomarkers. We discuss the relative performances of these four configurations and show that each leads to singular key features, driving their own selection as a function of the target application. The optrodes were tested in laboratory settings but also in gelled phantoms and in human resected lung tissues to study the surface plasmon excitation inside complex media, and to discriminate the nature of the tissue through biomarkers detection.

**Keywords:** Immunoassay, Optical fiber grating, Biomarkers, Biosensing, Plasmonics.

**INTRODUCTION**

Optical fiber sensors have known cutting-edge technological improvements in the recent years. Many architectures have already proven their skills in biosensing, especially unclad fibers, tapered fibers, long period fiber gratings (LPFGs), fiber Bragg gratings (FBGs), and also bend-etched or U-bent fibers, among others.

These optical fiber architectures are often associated with thin metal coatings to generate surface plasmon waves and subsequently increase their sensitivity to the surrounding refractive index. When bioreceptors such as antibodies are grafted on top of this metal film, molecular sensing at low concentration is possible, and only requires small volumes.

There are numerous types of bioreceptors that can be implemented on the optical fiber surface to specifically detect molecules of different nature (proteins, DNA, cells, etc.). Among these possibilities, the use of antibodies remains a gold standard to detect proteins on many platforms (ELISA, point-of-care diagnostic tests, etc.) and are called immunoassays.

In this paper, we have worked on tilted fiber Bragg gratings (TFBGs) permanently inscribed in the 8 µm core of standard single-mode optical fiber (SMF-28). TFBGs are short-period fiber gratings provoking a backward coupling of light from the core to the cladding, where it is guided into tens of resonances. This yields in a comb-like spectrum with discrete and narrowband (FWHM < 200 pm) resonances. Using this technology, we overview four different TFBGs configurations for immunosensing purpose: bare-TFBGs, gold-sputtered TFBGs, gold electroless-plated TFBGs and a hybrid coating combining sputter-coater and electroless plating. In this work, the target is the cytokeratin 17 protein, identified as a lung cancer biomarker. Its overexpression in tumorous tissue is targeted for its discrimination from healthy tissue.

1. **MATERIALS AND METHODS**

Bare-TFBGs were first cleaned by immersion inside a piranha solution made of H₂SO₄/H₂O₂ (4:1) during 15 minutes. They were then silanized through (3-aminopropyl)trimethoxysilane (APTMS) 1% in methanol for 20 min. at room temperature.
temperature. These silanized fibers were then immersed into 20 µg/mL anti-cytokeratin 17 antibodies for two hours at room temperature. They were then rinsed in PBS (Figure 1a).

Gold-sputtered TFBGs were prepared after a cleaning using piranha solution. They were placed inside the vacuum chamber of a sputter-coater under argon, at 10^{-4} mbars. After the plasma deposition of 50 nm of gold, the fibers were thermal annealed at 200°C during 1h30 to increase the gold adhesion on the silica surface. The gold film was sputtered horizontally in two steps with a rotation of the samples between two depositions. A self-assembled monolayer (SAM) was then performed using S_{2}PEG_{6}COOH at 2 mM in ethanol during 16h at RT. Then, optical fibers were immersed in NHS (N-hydroxysuccinimide 0.1M)/EDC (N-(3-Dimethylaminopropyl)-N’-Ethylcarbodiimide Hydrochloride 0.5M) in pure water for 20 min. After that, the fibers were immersed in 20 µg/mL anti-CK17 antibodies during 1h30 (Figure 1b).

The electroless deposition method required a first cleaning in piranha solution, followed by a silanization in APTMS 1% as aforementioned. They were then rinsed in methanol and dried in the oven during 15 min at 80°C. After that, the sensors were immersed in a commercial solution of 10 nm diameter gold nanoparticles for 1h at RT. They were then placed in the plating solution made of 0.4 mM NH_{2}OH.H_{2}SO_{4} and 3 mM HAuCl_{4}.3H_{2}O for the gold coating. Once plated, the fibers were bio-functionalized as for the gold-sputtered strategy (Figure 1c).

Finally, the Hybrid-TFBGs were gold-coated with an extremely thin gold sputtered-layer (4 nm) further expanded thanks to electroless plating. After the right gold deposition determined by the live monitoring of the signal during the plating process, the fibers were bio-functionalized as for the other two gold-coated strategies (Figure 1d).

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![Diagram](https://ebooks.spiedigitallibrary.org/conference-proceedings-of-spie)
2. RESULTS AND DISCUSSION

The use of bare-TFBGs leads to a spectral acquisition without need for polarization control, as these gratings are weakly sensitive to polarization effects. The maximum sensitivity to SRI changes is obtained for the so-called cut-off mode, i.e. the cladding mode resonance for which the effective refractive index is just above the one of the surrounding medium. Tracking the effective wavelength/power shift of this mode reveals the intrinsic properties of TFBGs (Figure 2a).

The immobilization of a gold sputtered-film (~ 50 nm) on the TFBG surface allows the generation of surface plasmon waves (SPR), while the polarization needs to be finely controlled (p-polarization). This configuration presents a singular feature consisting of cladding modes attenuation, revealing the SPR excitation. Sensitive modes are located at this point, and the neighbor modes are usually tracked for biosensing. A scanning electron microscopy analysis (SEM) was also performed to screen the surface of the gold sputtered film (Figure 2b).

The third approach is a more flexible process relying on the gold concentration used, and plating time. The electroless process (ELP) bring the possibility to track the deposition of the metal in real time and monitor its effect on the TFBG spectral content. It is therefore easier to optimize the plating to achieve the highest sensitivity. However, a classical interrogation method is not the optimum read-out technique to ensure this refinement. The selection of the correct state of polarization during the deposition is challenging. For this reason, the use of polarization dependent loss measurement (PDL) was adopted (Figure 2c).

Finally, a hybrid configuration (gold sputtered layer of 4 nm followed by ELP) leads to the live monitoring of the TFBG signal during the ELP while showing similar SPR attenuation as the one obtained with fully sputtered configurations17.

Figure 2. (a) Bare-TFBG amplitude spectrum monitored in PBS with the sensing part located at the cut-off area. (b) P-polarized spectrum of a sputter-coated TFBG with the most sensitive modes located at lower neighbor wavelengths of the SPR attenuation. A SEM analysis of the surface was performed. (c1) Transmitted amplitude spectrum before and after the ELP process. (c2) The same plating leads to a pronounced change in the PDF spectrum, also showing an attenuation. (c3) SEM image of the ELP deposition, which shows the presence of different sizes of gold particles and growing gold islands along the fiber surface.
The four afore-mentioned configurations were implemented for CK17-protein detection. Our experimental results were obtained by immersing the probes successively in growing CK17 concentrations from $10^{-12}$ g/mL to $10^{-6}$ g/mL (Figure 3). The relative amplitude shifts were computed after 5 minutes in each condition, with reference to PBS buffer. Error bars correspond to the standard deviation computed for three experiments performed in the same experimental conditions, with different probes. As expected, the bare-TFBG configuration shows the smaller shifts while the sputter-coated probes benefit from larger plasmonic amplifications. ELP and hybrid platforms show intermediate sensitivities, but the hybrid configuration shows significant shifts after only $10^{-10}$ g/mL. All these experiments were compensated for potential temperature variations thanks to the reference Bragg mode (core mode coupling) present at the right end of all spectra, that is not sensitive to SRI changes.

![Figure 3. Cytokeratin-17 detection in PBS. (a) Detection with bare-TFBGs. (b) Detection with sputter-coated TFBGs. (c) Detection with ELP-TFBGs. (d) Detection with the hybrid gold deposited TFBGs. Mean ± sd, based on three experiments/condition.](image)

After these in vitro experiments, our immunosensors were also tested in resected human tissues after insertion inside adapted packaging, allowing their insertion inside soft matter. The catheters were specifically designed to allow the interactions with the surrounding medium but with sufficient protection for the sensing area. Optical fibers were first inserted inside healthy tissues and then inserted in tumorous resected samples from the same patient. The first insertion in the healthy part plays the role of the reference for the following tumoral monitoring.

The CK17 protein was present in all the analyzed tumors but with a higher expression rate in the neuroendocrine case analyzed, which also lead to a higher sensing response by our biosensor. Negative controls with probes without antibodies grafted on their surfaces were also performed, leading to a lower response under the 0.5 dB defined as threshold (Figure 4).
Other cancer types such as adenocarcinomas or squamous cell carcinomas were also tested and showed a significant shift while inserted in the tumor part. The influence of the surface blocking is important, as the presence of cells and blood inside the lungs can increase the noise level and lead to non-specific signal variations. The immobilization of the device is also needed for the measurements as the polarization state has to be defined for the SPR excitation. The only moving part of the setup is the tumor, which is placed on top of an electric automated lift, moving at constant speed. The data were acquired during 5 minutes to allow sufficient protein interactions with the fiber surface.

![Figure 4](image-url)

Figure 4. (a) Picture of the optical fiber probe inserted inside a packaging, which is immersed in the lung tumoral tissue. (b) Sensor responses (ratio tumoral vs. healthy) in different cancer cases analyzed, after 5 minutes.

Immunohistochemistry was also performed on the same analyzed tissues to assess the overexpression of CK17 in tumoral parts in comparison with their related healthy parts. Histological sections cut from the analyzed samples were therefore stained using 3,3′-diaminobenzidine (DAB) to specifically color the CK17 proteins in brown. Their expression rate was calculated through mean percentage of CK17-expressed areas, achieved by an automated image processing software.

![Figure 5](image-url)

Figure 5. (a) Picture of the optical fiber probe inserted inside a packaging, which is immersed in the lung tumoral tissue. (b) Sensor responses (ratio tumoral vs. healthy) in different cancer cases analyzed, after 5 minutes. (c)
3. CONCLUSION

Optical fiber gratins are increasingly studied for biosensing. There remains rooms for improvements to improve the reproducibility and reliability of these probes, especially when they are associated with thin metal coatings. In this road towards the production of robust immunosensors, we have studied four different TFBGs configurations, combining gold coating methods with biofunctionalization strategies. We have presented an overview of their characteristics from their manufacturing to their use as biosensors.

The bare-TFBG strategy shows the lowest sensitivity due to the absence of SPR enhancement but depicts interesting assets, especially because it can be used straightforwardly without taking care of the polarization effects.

The gold sputtered TFBGs lead to the highest sensitivity for the detection of CK17 proteins (LOD close to 1 pg/mL) and is adequate for complex matrices analyses, as it was implemented inside a catheter to target lung cancer biomarkers in situ.

The gold electroless plating is a rapid method to generate a gold interface and allows a live monitoring in solution during the deposition, which is not possible inside the vacuum chamber of a sputter-coater. It also presents high adhesion on the silica surface thanks to the silanization of the glass. The detection of our targets began at 1 ng/mL when the read-out is based on PDL analysis.

The final configuration tested is called “hybrid” and was made of a thin sputtered film followed by the electroless plating technique. This final strategy is a good compromise to monitor the deposition while reaching high sensitivity comparable to fully sputter-coated gratings.

All these optical fiber-based immunosensing platforms find their place in a wide variety of applications, driven by their own specification.

REFERENCES


