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# Surface plasmon resonance fiber sensor for real-time and label-free monitoring of cellular behavior

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ABSTRACT

This paper reports on the application of an optical fiber biosensor for real-time analysis of cellular behavior. Our findings illustrate that a fiber sensor fabricated from a traditional telecommunication fiber can be integrated into conventional cell culture equipment and used for real-time and label-free monitoring of cellular responses to chemical stimuli. The sensing mechanism used for the measurement of cellular responses is based on the excitation of surface plasmon resonance (SPR) on the surface of the optical fiber. In this proof of concept study, the sensor was utilized to investigate the influence of a number of different stimuli on cells—we tested the effects of trypsin, serum and sodium azide. These stimuli induced detachment of cells from the sensor surface, uptake of serum and inhibition of cellular metabolism, accordingly. The effects of different stimuli were confirmed with alamar blue assay, phase contrast and fluorescence microscopy. The results indicated that the fiber biosensor can be successfully utilized for real-time and label-free monitoring of cellular response in the first 30 min following the introduction of a stimulus. Furthermore, we demonstrated that the optical fiber biosensors can be easily regenerated for repeated use, proving this platform as a versatile and cost-effective sensing tool.



## 1. Introduction

Optical fiber sensors have been extensively employed for detection of a broad range of biological targets such as, nucleic acids (Delport et al., 2012; Ferguson et al., 2000; Yin et al., 2013), antigens (Jang et al., 2009), antibodies (Lepinay et al., 2014; Ruan et al., 2008) and other low and high molecular weight analytes (Amin et al., 2012; Pollet et al., 2011; Silva et al., 2009; Tierney et al., 2009; Wang and Wolfbeis, 2013). Recently there has been an increasing emphasis on the characterization of larger and more complex biological targets such as cells, bacteria (Smietana et al., 2011) and viruses (Bhatta et al., 2010; François et al., 2011). The application of cells as an integral part of the biorecognition scheme is driven by a growing demand for a better understanding of newly discovered cellular signaling pathways, a need for the characterization of drug–cell interactions as well as for a comprehensive evaluation of cellular interactions with their microenvironments (Ghaemmaghami et al., 2012; Khademhosseini et al., 2006).

Optical label-free characterization of cellular behavior can be accomplished by using several approaches. One approach is to apply traditional imaging tools where light propagates in free space and interacts with cells positioned on its path. For instance, imaging using traditional cell phone cameras has been demonstrated as a cost-effective solution that could be integrated into cell culture incubators to monitor cellular responses induced by drugs and other stimuli (Kim et al., 2011, 2012; Zheng et al., 2011).

Another strategy for characterization of cellular responses is to use optical analytical tools where cells would be in a direct contact with the optical elements that confine and guide light. In that case, the detection of cellular reactions is being implemented by utilizing the evanescent field of the light confined by the optical elements. The evanescent field decays away from the surface of an optical element to a distance of a few micrometers, sufficiently deep to penetrate into the cells and probe their optical properties. Changes in the attenuation or phase of the evanescent field penetrating into cells can be used to interpret intra- and extra

*Abbreviations:* SPR, surface plasmon resonance; RI, refractive index; TFBG, tilted fiber Bragg grating; FBS, fetal bovine serum

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cellular changes. In particular, changes in the optical field can be used to monitor cellular adhesion (Lin et al., 2006), detachment (Fang et al., 2006), death (Chabot et al., 2009; Maltais et al., 2012), contraction, a response to osmotic stress (Vala et al., 2013) as well as triggering of diverse intracellular reactions (Fang and Ferrie, 2008; Fang et al., 2006; Yanase et al., 2013) that are very challenging to monitor by other means. So far, the use of analytical tools for cellular characterization has been limited to a few optical platforms such as, the well-established surface plasmon resonance (SPR) (Chabot et al., 2009; Hide et al., 2002; Robelek, 2009; Yanase et al., 2007), resonant waveguide grating EPIC® systems (Fang and Ferrie, 2008; Fang et al., 2006; Scibek et al., 2012) and photonic crystal fibers (Lin et al., 2006). The signal captured by these sensors is real-time, dose-dependent, quantitative, and label-free. A combination of the plasmonic technique with fluorescent labeling can be used to monitor even finer cellular responses such as cellular reorganization and transient contraction as reported by Chabot et al. (2013) if label-free regime is not a requirement.

Although the mentioned optical systems can be used to characterize a wide range of cellular responses, the platforms themselves are bulky and are not designed to be integrated into traditional tissue culture incubators. Handling cells outside incubators during their analysis severely effects cellular viability due to potential contamination and affects cellular metabolism. Additionally, the operation of the platforms mentioned above is complicated and requires highly trained personnel. On the other hand, integration of optical elements such as plain optical waveguides, structured optical surfaces or custom-made fiber would require the development of additional optical infrastructure that would ensure delivery of light to an incubator with sensors wired into a Petri dish and optical signal readout. This would increase the complexity and reduce the practical use of such platform.

In this context, optical sensor platforms that are made using optical fiber offer several competitive advantages such as compactness, remote sensing capabilities, simple integration into cell culture equipment, and potential for *in situ* measurements. When the fiber sensors operate at standard telecommunication wavelengths, as in our case, both the fiber devices and the interrogation equipment become highly cost-effective compared to the more complex and bulkier custom designed optical sensing platforms. Taking into account that the sensitivities reported in the literature for fiber sensors can be similar to those of traditional platforms employing planar optical structures (Sharma and Gupta, 2007), fiber sensors position themselves as a versatile solution that can be used in a range of applications including *in situ* whole cell sensing.

Yanase et al. (2010) showed that a plasmonic fiber biosensor could be used for monitoring cellular response when cells are exposed to a stimulus, i.e. albumin (Yanase et al., 2010). The sensor employed in that study was manufactured using a multimode quartz fiber. The use of multimode fiber, however, limits sensor detection levels and hinders the quality of the detected optical signal. In this study we propose to use a completely different sensor manufactured using a standard telecommunication single mode fiber in order to improve the results. The use of a traditional single mode fiber allows the fiber sensor to be robust and achieve a high level of performance. Moreover, this is the first optical fiber sensor that was fully integrated into conventional cell culture equipment making it possible to conduct the experiments in a controlled environment. This sensor was developed not for "field use" but for *in situ* sensing inside cell culture incubators. Fiber connectorization is very easy to perform with standard low cost instrumentation developed for the telecommunication industry. By conducting all measurements in a conventional incubator, we obtained high cell viability and were able to monitor cellular responses for extended periods of time.

Herein we present the application of a fiber biosensor, a miniature and portable sensor platform, for real-time and label-free monitoring of cellular responses. Particularly, we demonstrate that the plasmonic fiber sensor can be used for characterization of a set of fine cellular responses triggered by selected stimuli. The sensor is manufactured using a single mode fiber and works through the excitation of plasmon waves on its gold-coated surface.

## 2. Experimental

### 2.1. Materials

Fibronectin was obtained from BioLegend (San Diego, CA, USA). Phosphate buffered saline (PBS), Dulbecco′s modified Eagle medium (DMEM), fetal bovine serum (FBS), 10× trypsin and penicillin/streptomycin were purchased from Gibco (Carlsbad, CA, USA). Alamar blue, calcein AM, ethidium homodimer, phalloidin (AlexaFluor 594), and 4′,6-diamidino-2-phenylindole (DAPI) were obtained from Invitrogen Corp. (Carlsbad, CA, USA). Bovine serum albumin (BSA) and sodium azide were supplied by Sigma-Aldrich (St. Louis, MO, USA). Paraformaldehyde was obtained from Electron Microscopy Sciences (Hatfield, PA, USA). 4-well plastic plates were purchased from Fisher Scientific Co LLC (Pittsburg, PA, USA). All reagents were used as received without further purification.

### 2.2. Fabrication of the plasmonic sensors

The sensors used in this work (Fig. 1A) were manufactured using a traditional telecommunication single mode fiber (CORNING SMF28) (Shevchenko et al., 2010b; Shevchenko and Albert, 2007). As reported earlier, the fabrication of sensor elements was completed in a two-step process that involved the fabrication of a resonant grating element followed by a deposition of a gold coating on the surface of the fiber. The gratings were imprinted in the hydrogen-loaded fiber by using UV light passed through a diffractive phase mask, which had a period of 1096 nm. The UV beam was imposed on a fiber with an excimer laser operating at a wavelength of 193 nm. After the inscription process, the gratings were annealed with a heat gun at 400 °C for approximately 1 min and thermally stabilized in the oven at 120 °C for 12 h. The final step was the deposition of a 50 nm thick gold coating on the surface of the fiber. The deposition was implemented using an electron beam evaporation setup (Balzer Evaporator system).

### 2.3. Principle of operation of the plasmonic fiber biosensor

The fiber sensor operates through the excitation of the SPR effect on the surface of the gold-coated fiber (Albert et al., 2013; Caucheteur et al., 2011, 2013; Shevchenko et al., 2010a, 2011; Shevchenko and Albert, 2007). A schematic of the sensor with cells attached to its surface is shown in Fig. 1A. The SPR excitation is achieved by means of a tilted grating imprinted in the core of the fiber. The Tilted Fiber Bragg Grating (TFBG) acts as a resonant element redirecting the light from the guided core to the surface of the fiber. When the wavelength of the light propagating in the fiber is such that a phase matching condition for the excitation of the SPR is satisfied, some of the light escapes from the cladding and is coupled to plasmonic waves on the outer surface of the gold-coated fiber.

The surface plasmon wave is confined at the interface between the gold coating and a dielectric outside of a fiber (or sensed medium) with the evanescent tail of the plasmonic field penetrating deeply in the dielectric. It provides the whole excitation system with an enhanced sensitivity towards refractive index changes that occur at the metal surface. When cells are attached to the surface of the