Fiber-optic sensor for bacteria detection based on intensity-modulated SPR by monochromatic excitation

A.S. Arcas[1,*], F.S. Dutra[1,2], R.C.S.B. Allil[1,3], M.M. Werneck[1,4]

[1]Federal University of Rio de Janeiro, Nanotechnology Program/COPPE, RJ, Brazil
[2]PETROBRAS Research and Development Center, RJ, Brazil
[3]Institute of Chemical, Biological, Radiologial and Nuclear Defense, Brazilian Army Technological Center, Rio de Janeiro, Brazil
[4]Federal University of Rio de Janeiro, Electrical Engineering Program/COPPE, RJ, Brazil
*Corresponding author: ariadny@lif.coppe.ufrj.br

ABSTRACT

This study describes the development of a low cost and ease-to-fabricate sensor for bacteria detection based on intensity-modulated surface plasmon resonance (SPR) by monochromatic excitation in a plastic fiber optic (POF). U-shaped probes with 8-mm bend diameter made of 1 mm poly(methyl methacrylate) POFs were fabricated for SPR sensing. Gold thin films with 30, 50, 70 and 100 nm thickness were deposited on the U-shaped region by RF magnetron sputtering. Anti-*E. coli* antibodies were immobilizated on the surface of gold deposited over the fiber. Tests with *E. coli* demonstrated a detection limit of 10^4 UFC/mL.

Keywords: Bacteria sensor, *E. coli* sensor, Surface Plasmon Resonance, SPR, Plastic optical fiber, POF.

1 INTRODUCTION

Escherichia coli (*E. coli*) O157:H7 is a human pathogen of animal origin that can cause serious illness if ingested. The most common routes of *E. coli* contamination are drinking water, vegetables, undercooked meat, unpasteurized milk and by bathing in polluted rivers and seas [1] [2]. Its importance as a public health was observed in the United States outbreak occurred in 1982 on which 8,598 cases of people had been infected in the Oregon and Michigan cities [3]. Besides the public health problem, an *E. coli* outbreak may result in financial losses to the industry and agriculture, as occurred in Germany in 2011, which caused $ 1.3 million in losses to farmers and industries [4]. Therefore, food quality is an issue of global importance that must be ensured by constant monitoring. In order to predict and prevent further outbreaks, fast and reliable detection methods are crucial.

Yet, conventional detection methods such as culture-based are far from being rapid and reliable. An alternative method to culturing is the polymerase chain reaction (PCR). The analysis time of a PCR-based biosensor is shorter (~5 to 24 h) [5], however, PCR cannot differentiate between live and dead cells. In addition, a certain time for samples preparation are required prior to analysis [6].

Biological detection through refractive index (RI) measurements can be performed in several ways by the use of optical fiber sensors. Surface Plasmon Resonance (SPR) is one of these methods and is used widely as a detection principle in different fields. SPR is very attractive because it can attain resolutions as high as 10^{-6} RIU [7].

This study focuses on the developing a biosensor based on intensity-modulated SPR, excited by monochromatic light. The sensor is made of a U-shaped POF sensor for having greater sensitivity than the straight ones. The sensor is coated with a gold thin film immobilized with anti-*E. coli* antibodies for specific bacteria detection. Preferred use of gold instead of silver or copper is due to its high chemical stability.

U-shaped geometry does not require removing fiber cladding for using intensity-modulated SPR, which is difficult in practice. In straight fibers, the evanescent wave does not reach the gold film because of cladding thickness, however, in a bent fiber, higher-order propagation modes go through to the cladding producing evanescent field in the gold and medium.

To the best of our knowledge, this is the first biosensor for *E. coli* detection that uses this technique. This scheme reduces the cost because it requires no optical spectrum analyzer (OSA) to measure the output spectrum of the transmitted light which goes through the U-shaped probe. With a simple and easy-to-fabricate detection system, this scheme has a larger possibility of ending up in a commercial and large-scale production.

2 MATERIALS AND METHODS

2.1 U-shaped Probes Fabrication

Multi-mode Mitsubishi Rayon Eska GH 4001 POF with 1-mm diameter made of poly(methyl methacrylate) (PMMA) core and fluorinated polymer cladding with 10 μm in thickness was used. The RI in the visible range of interest is about 1.49 for PMMA and 1.41 for fluorinated polymer.

The POF was cut into several 10-cm-long sections and both end surfaces were cleaved and polished with polishing paper for a better light coupling. Then, the 10-cm-long sections were bent around a mold and heated at about 70 °C for 15 s to produce U-shaped probes with 25 mm length and 8 mm waist diameter. This task was carried out with the aid of a custom-made device shown in the Fig. 1.

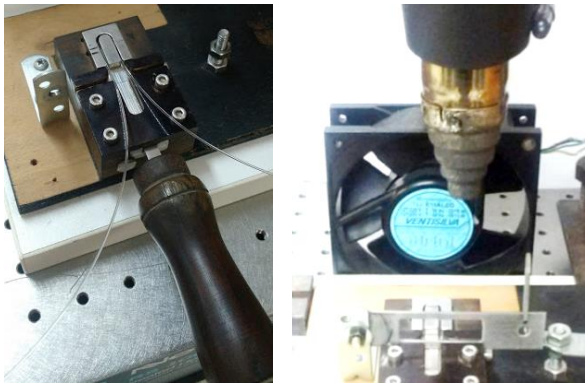

Figure 1: Custom-made device for molding U-Shaped probes (*left*). Hot-air gun and cooler used for heating and cooling the probes (*right*).

2.2 Gold Thin Film Deposition

A sputtering system (Aja International, USA) with RF magnetron was used for gold depositon over the U-shaped region. Prior to gold coating, the probes were cleaned in 100 % isopropyl alcohol for 2 minutes (not exceeding this time in order to avoid fiber cracking), and then washed in ultrapure water and dried with ultrapure nitrogen.

The gold sputtering process was done with argon flow rate of 12 sccm (standard cubic centimeters per minute) at a pressure of 4×10^{-3} mbar. A 40 W RF power was applied to ionize the gas (plasma). The U-shaped probes were placed on the substrate base of the sputtering located 10 cm above from the gold target and tilted 45° from vertical, as shown in Fig. 2. The substrate base was rotated at 20 rpm during the deposition process in order to evenly expose the probes to the same deposition rate.

The gold deposition rate in these conditions is about 3.5 nm/min. Probes were exposed to the sputtering process for 8, 14, 20 and 28 minutes to produce probes coated with 30, 50, 70 and 100 nanometer thickness of gold.

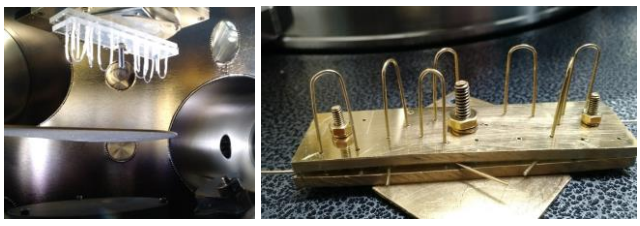

Figure 2: Bare U-shaped probes installed inside the sputtering chamber 10 cm above from the gold target and tilted 45° from vertical (*left*). Au coated U-shaped probes after sputtering (*right*).

2.3 Sucrose and Bacterial Solutions

Aqueous solutions with RI varying from 1.333 to 1.375 were prepared by dissolving sucrose in ultrapure water. Solutions refractive indexes were measured by Abbe refractometer (Quimis, Brazil, Model Q767BD).

E. coli O55 was obtained from Oswaldo Cruz Institute (FIOCRUZ) in Rio de Janeiro, Brazil. Bacterial cultures were prepared on a plate containing tryptic soy agar (TSA), incubated at 37 °C for 24 hours. The bacterial suspensions for sensor testing were prepared in a saline water solution at 0.85 %. Bacteria concentrations were obtained by dilution and confirmed by two methods: McFarland turbidity standards and absorption spectroscopy (Pro-Tools UV-1800 spectrometer). The absorbance of *E. coli* concentration of 10^8 CFU/mL (colony forming unit) was beetwen 0.08 and 0.1 at a wavelength of 625 nm [8].

2.4 Immobilization

Polyclonal anti-*E. coli* antibody from rabbit (Bio-Rad Labs, Brazil) was diluted to the concentration of 5×10^{-4} mg/mL in PBS. After gold coating, the probes were immersed into ethanol solution containing cysteamine 4 mM at 25°C for 2 hours. Then they were rinsed with 100% ethanol and treated with a 40 mM NHS/100 mM EDAC solution in PBS buffer for 1 hour at room temperature. They were further rinsed with PBS buffer and ultrapure water. The sensors prepared with cysteine, NHS and EDAC were immersed in antibody solution with PBS for 1 hour at 20°C. Unbound antibody molecules were removed by washing with PBS and subsequent immersion in BSA solution for 30 minutes.

2.5 Optical and Electronic Setup

Fig. 3 shows a block diagram of the optical and electronic setup used for the measurements of surrounding RI changes.

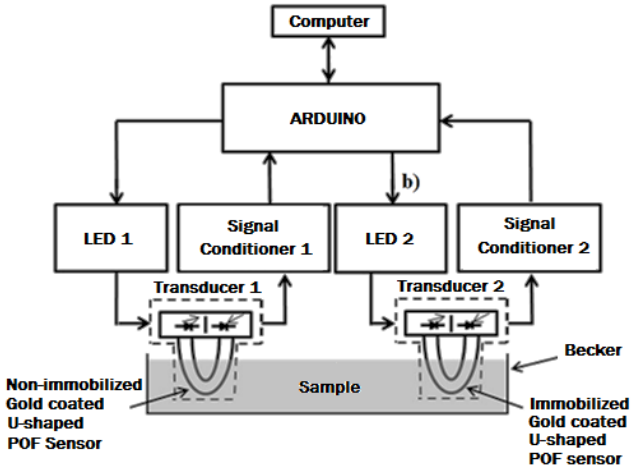

Figure 3: Block diagram of the optical setup [9].

The configuration consisted of two sets of 880 nm LED powered by a current source controlled by an Arduino Microcontroller and connected to the end of the bent fiber. The light is received by the photodiode located on the opposite fiber end. The light intensity is modulated by SPR absorption in function of the variation of RI of the medium. For wavelengths around 880 nm, the photodiode achieves maximum gain.

The optical and electronic set-up allows the use of two U-shaped probes at the same time. This is useful for checking immobilization effect referencing during tests.

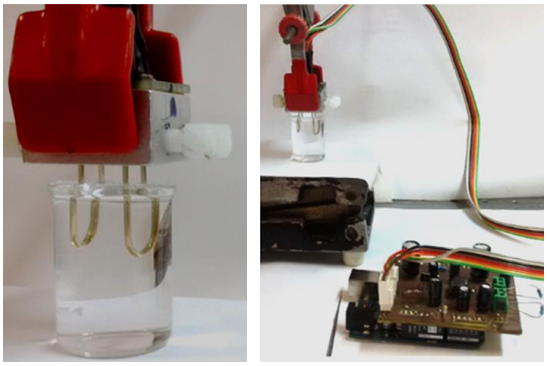

Figure 4: Immobilized and reference U-shaped probes immersed into bacteria solutions (*left*). Optical and electronic set-up photo (*right*).

3 RESULTS AND DISCUSSION

3.1 Refractive Index Sensitivity

At first, the sensors were tested with sucrose solutions under different RI into the operation range for bacteria detection in order to verify their linearity and sensitivity. Six sensors were fabricated and tested for each gold thickness.

For the 30-nm and 50-nm gold coated probes, the sensors showed a non-linear behavior and very low sensitivity to the RI variation. Therefore, theses sensors are definitely not useful for sensing.

The 70-nm and 100-nm gold coated probes were tested in sucrose solutions with RI from 1,33 to 1,38. The results are shown in Fig. 5 and Fig. 6.

Each point in the plot is the average value of twenty measures. The sensor output voltage has been set to be 3 V for the water refractive index. In this operating range, both sensors present linear response as a function of the RI.

Estimated linear regression equation and its coefficient of determination (denoted R^2) for each sensor are shown in Fig. 5 and Fig. 6. The average sensitivities of 70-nm and 100-nm gold coated probes are respectively 6.01 V/RIU and 8.25 V/RIU. The highest standard deviation of the measurements is the order of 10^{-3} RIU for both gold thickness.

Notice that the output signal increases with increasing RI. This behavior for the fiber optic sensor by SPR is in accordance with results from [10] and [11]. A simple explanation for this phenomenon is that the light incident on the metal-dielectric surface will be more or less absorbed by the plasmon ressonance effect generated by the incident beams with angle greater than the critical one. The curvature of the U-shaped probes allows high order propagation modes to go to the cladding and senses the external medium.

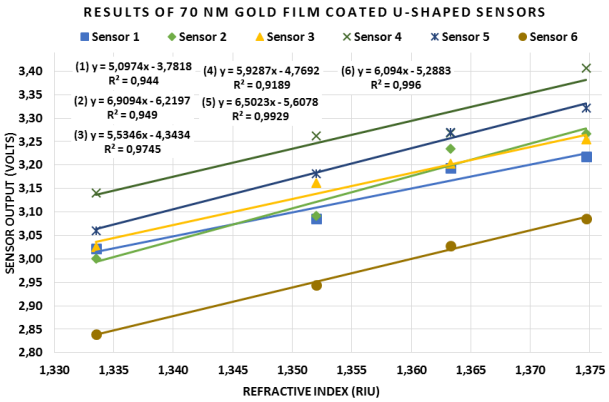

Figure 5: Results of 70-nm gold coated sensors in refractive index of sucrose solutions measurements.

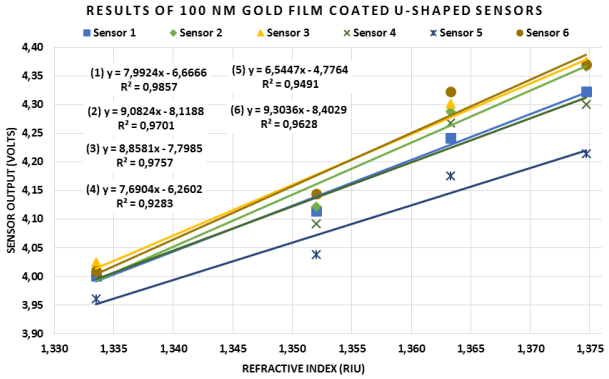

Figure 6: Results of 100-nm gold coated sensors in refractive index of sucrose solutions measurements.

3.2 Experiments with Bacteria

In this section, we present the results obtained with immobilizated 70 nm gold coated U-Shaped sensor against *E. coli*. This gold thickness was chosen instead of 100 nm just for the purpose of saving the gold target.

Fig. 7 shows the results of 70-nm gold coated sensor in an *E. coli* concentration of 10^8 CFU/mL. The immobilized sensor showed an increase in output voltage with time due to the increase in the surrounding RI caused by immunocapture effect as the antibody layer keeps capturing the bacteria present in the water. The output of reference sensor remained constant which confirms the properly operation of the immobilized sensor for *E. coli* detection.

However, at lower concentrations, the sensor was not able to measure the small changes in the surranding RI. It was supposed that the optical and electronic set-up did not present enough sensitivity at this extreme of the range.

In order to verify the functionality of the sensor, tests were carried out with a spectrometer (Model HR-400, Ocean Optics) and a white light source. The wavelength of interest is 845 nm (as close as possible to 880 nm, the peak of Si photodiode sensitivity). It was not possible to test at 880 nm because this wavelelngth is outside the spectrometer range.

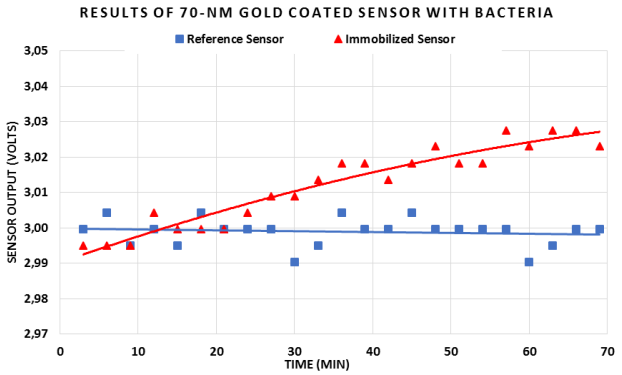

Figure 7: Results of the 70-nm gold coated U-Shaped sensor under a bacteria concentration of 10^8 CFU/mL.

In this optical configuration, the 70-nm gold coated U-shaped sensor was able to measure an *E. coli* concentration of 10^4 CFU/mL. Fig. 8 shows the results for operation time of 70 min.

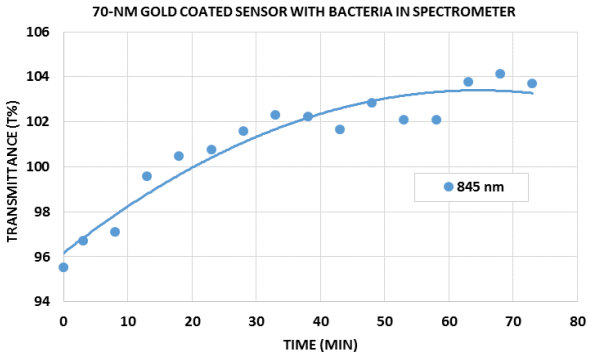

Figure 8: Results of the 70-nm gold coated U-Shaped sensor under a bacteria concentration of 10^4 CFU/mL obtained with the spectrometer for 845 nm.

4 CONCLUSION

The purpose of this work was to develop a low cost, portable and ease-to-fabricate biosensor for the detection of *E. coli* bacteria by intensity-modulated SPR excitated by monochromatic light. Plastic optical fiber was chosen because it is more resilient to damage and easier to terminate, polish, and fold. U-shaped geometry allows to use intensity-modulated SPR without the need to uncladding the fiber. Layers of gold thin film were deposited on the surface of the cladded U-shaped plastic optical fiber by sputtering method. Gold is chemically more stable than other metals and has efficient and well-known immobilization protocols.

The 30-nm and 50-nm gold coated sensors showed a non-linear behavior and very low sensitivity to the surrounding RI variation and thus they are not useful for sensing. Nonetheless, 70-nm and 100-nm gold coated sensors showed properly linear responses for the range from 1,33 to 1,38 RIU. Experimets with *E. coli* showed that the sensor is able to detect concentration of 10^8 CFU/mL in the optical and electronic set-up used and 10^4 CFU/mL in the spectophotometer.

In order to improve the limit of detection, the following solutions shall be adopted: to fabricate a highest sensitivity optical and electronic set-up, to investigate more efficient protocols for bacterial adhesion, and to choose a led wavelength with higher response in SPR

For the first time this technique of detection through gold-plated U-shaped sensors by intensity modulation is used to measure concentrations of bacteria, and the results demonstrated that the proposed biosensor can be an efficient, low cost and portable tool for routine analysis of potability and balneability of water and food quality. This technique can be applied to other types of bacteria by simply immobilizing the antibody of the bacteria to be detected.

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