Double-resonance long period fiber grating for detection of E. coli in trace concentration by choosing a proper bacteriophage

Y. Chiniforooshan*[a], A. Celebanska [a], M. Janik [a], P. Mikulic [a], F. Haddad[b], J. Perreault[b], W. j. Bock[a]
[a]Photonic Research Centre, University of Quebec in Outaouais, 101 rue Saint-Jean-Bosco, Gatineau (QC); [b]Centre INRS-Institut Armand-Frappier, 531 boulevards des Prairies Laval (QC) Canada

ABSTRACT

There is a critical need of a fast, specific and reliable assay for biological species. To address this need, long period fiber gratings (LPFG) among other fiber optic sensors can be used because of their high sensitivity to changes in surrounding medium. In this work we fabricated and used two over-etched LPFGs. One of them was covered with T4 Phage and the other was covered with MS2 phage that both specifically bind with Escherichia coli (E. coli) bacteria. This bacterium is a major cause of the food contaminations and outbreaks. We showed achieving a highest sensitivity region of the LPFG and the way to fine tune to that region by over-etching the grating. Finally, using the highly sensitive LPFG platform we could detect E. coli at concentrations as low as 100 colony forming units (CFU), by covering the LPFG with an optimized bio-functionalization of the fiber surface with MS2 bacteriophage.

Keywords: Long period fiber grating LPFG, double resonance, biosensor, over etched LPFG, trace detection, T4 bacteriophage, MS2 bacteriophage, E. coli bacteria

1. INTRODUCTION

The contamination of the food and water resources with pathogenic microorganisms is one of the major cause of outbreaks and diseases. Escherichia coli (also known as E. coli) is one of the common reasons of food poisoning. This microorganism is a gram-negative, facultatively anaerobic, rod-shaped bacterium. Most of the E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contaminations. Therefore, early detection capability of this type of bacteria in trace concentration is urgently needed. The biological detection techniques (e.g., PCR and immunological detection) are available today, however, they require highly qualified personnel. Moreover, these techniques are complex, time consuming and costly. Therefore, developing an accurate and fast bio-sensing techniques such as fiber optical bio-sensing for detection of pathogenic strains is needed. Fiber optical biosensors refer to the sensing methods in which the target is selectively captured by a bio-receptor that is attached to the optical fiber. The bio-receptor material, such as antibody, DNA or bacteriophages are used for selective detection. The binding of a target to the host is detectable using an optical process. This process can be simply a fluorescence quenching/turn-on or the changes in the optical absorbance or reflectance that can create the optical signal modulation detectable using evanescent-wave sensors. In more complicated sensors, the optical processes can be the wavelength shift of surface plasmon resonance, reflected resonance (by a tilted FBG) or cladding resonance (coupled by an LPFG).

The LPFGs have recently become widely used for biological analyte detection [1, 2] because of their high sensitivity to the changes in surrounding medium, in addition to their low cost fabrication processes and to their robustness in different environments. The objective of this study is to improve and to optimize our previously developed LPFG based fiber biosensor [3] for effectively detecting of the E. coli bacteria in very low or trace concentration. This goal has been achieved by fabricating a LPFG close to the turning point of the dispersion curve [4], and also by using a more stable and sensitive bio-receptor.

2. METHODS AND EXPERIMENTS

In this work, two LPFGs were used with two different bio-chemical overlays. The final sensor comprised of an LPFG covered by chemical layers that facilitate the attachment of a layer of bacteriophages as the specific bio-receptor for a targeted bacterium. Bacteriophages are natural enemies of the bacteria that infect and replicate within them. Therefore, there are two steps of sensor preparation: LPFG fabrication and bio-chemical surface modification.

* chiy03@uqo.ca; Phone : 1 819 595 3900 ex. 1613 ; http://w3.uqo.ca/labopto/

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2.1 LPFG fabrication

There are two main techniques to inscribe the Long Period Grating in the optical fiber core: the amplitude mask and the point-by-point techniques. In the latter LPFGs are fabricated by the arc induced refractive index changes, by thermal and residual stress relaxation induced by IR radiation or by core micro-machining by Ti:Sapphire femtosecond laser. Although some of the point-by-point methods are simple and cost effective, they are not as repeatable as the amplitude mask technique. Also in some techniques, excitation of the desired higher order cladding modes is questionable (for example using CO_2 laser IR radiation). Therefore, a high-power KrF Excimer laser (Lumonics™ Lasers: Pulse Master ®-840) emitting at 248nm and a chromium amplitude mask ($\Lambda = 215\ \mu m$) have been used for LPFGs fabrication. Then the LPFGs were annealed for 3 hours in 150 °C to stabilize their optical properties. Using this pitch, a double resonance could be excited. Then 10% Hydrofluoric acid (HF 10%) was used to etch the cladding. By reducing the cladding diameter, the double resonances were merged and created a single but broadband resonance. The attenuation of this resonance was reduced by continuing the etching process. Then etching was stopped at an optimized cladding diameter. These LPFGs were over-etched because the higher sensitivity was required for trace detection of the E. coli bacteria. The over-etched attenuation resonance returned to the turning point after bio-overlay attachment to the fiber surface. Therefore, the LPFG reached its highest sensitivity just before attachment of bacteria to the fiber surface.

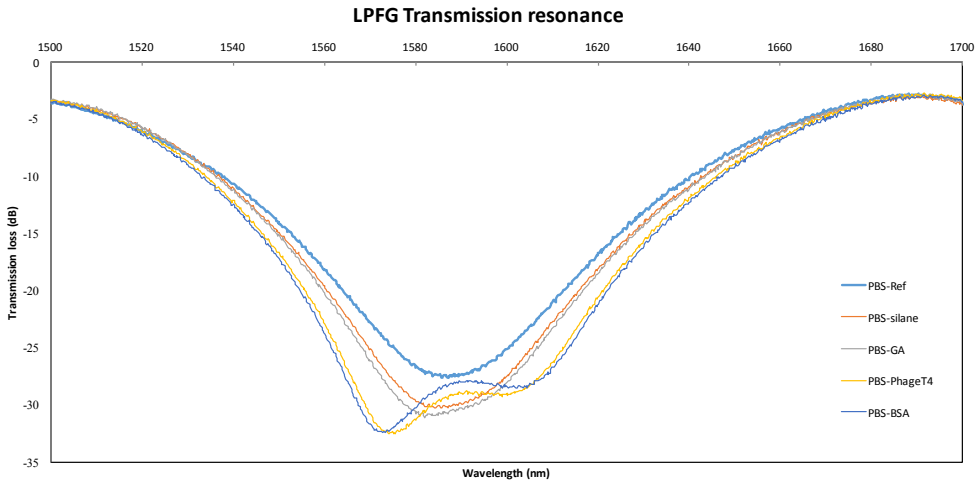

Figure 1. Transmission spectrum of an over-etched LPFG. Attenuation of the single broadband resonance increased by adding chemical layers and the single resonance split into two resonances by adding a layer of bacteriophages.

Figure 1 explains the compensation of the attenuation resonance by attachment of the bio-chemical overlays. As it is shown in figure 1, by adding those layers to the over-etched cladding, the single broadband resonance split to double resonances. Using this method, the sensitivity of the LPFG was boosted to around 7000 nm/RIU in the range of 1.3333-1.3400 RIU.

2.2 Bio-chemical functionalization

The first and crucial step of the LPFG functionalization process includes the pre-cleaning treatment in the mixture of hydrochloric acid and methanol (1:1, v/v) for 30 min, and then in the concentrated sulfuric acid (30 min), in order to remove organic contaminations and to increase the density of superficial hydroxyl groups (Si-OH) [5]. Next, the LPFG was rinsed profusely by Mili-Q water and dried under an argon stream. Cleaning process does not affect the recorded spectrum.

The silanization process was carried out from the (3-Aminopropyl) triethoxysilane (APTES) gas phase according to the modified literature procedure [6]. Deposition from vapor allows for an easy control of the film thickness by the silanization time manipulation. The process was done in a small desiccator filled with argon. Two trays with a 30 µl of APTES and 10 µl of triethylamine (a sol-gel process catalyst) were placed into the chamber and left over 30 minutes. After that, the vials were removed and LPFG was kept in an argon atmosphere for 48 hours for silane layer aging. In the next step, the amine groups were activated by homo-bifunctional cross-linking agent, glutaraldehyde (GLU) through the LPFG incubation in 2.5% GLU solution in phosphate buffer saline pH=7.4 (PBS) for 30 min. Thus, obtained modified-LPFG was very sensitive towards the amine groups present at the bacteriophages surface. For their conjugation, the

sensor was incubated at a bacteriophage solution for 1 hour, followed by its immersion in 2 mg ml-1 bovine serum albumin (BSA) solution in PBS for 30 min in order to block non-specific interaction. This is the last step before the exposure of the sensor to the bacteria solution. We studied two types of bacteriophages, T4 phage and MS2 phage, towards detection of E. coli.

T4 phage has been widely used as a bacteria recognition element in fiber-based sensors [3]. T4 is one of the best-studied phages and has been described in details in case of structure, genome replication, and expression, as well as the morphogenetic pathways. Despite many advantages like great specificity and sensitivity, low cost and speed of production, longer shelf-life, comparing for example to antibodies, as well as high thermal stability [7] there are also several difficulties. Phages, like aforementioned T4, have complex structure what makes them very fragile. It is also very hard to immobilize them on the fiber with a well-defined orientation, what can affect their efficiency and repeatability of detection. In view of that kind of problems we used also another type of phage - MS2. An icosahedral shape and lack of the tail and other surface features make this phage much more stable and robust. Furthermore, the MS2 particle is approximately 27 nm in diameter [8] comparing to approximately 50 X 225 nm T4 phage. Thanks to the shape and the size, the MS2 can uniformly cover the fiber surface with a well-defined orientation. Also, the binding site of bacteria is different. MS2 phage particles contain protein, A which is a receptor capable of binding with bacteria pili. Comparing to T4, MS2 does not have to go to the bacteria cell surface, what facilitates capturing of bacterial host and reducing the time of detection.

2.3 Experiment

The goal in this work was to detect trace concentration of the E. coli bacteria. For this study two over-etched LPFGs (as described in section 2.1) were prepared. Then the LPFGs were characterized by refractive index sensitivity measurements over the range of 1.3333-1.3400 RIU. Each grating was functionalized by the same chemical process as described in section 2.2. Then T4 phages were incubated on LPFG-T4 and MS2 phages were incubated to LPFG-MS2. The E. coli bacteria dilutions were prepared and the bacteria was counted in each dilution. Then each LPFG was tested with different dilutions. In the next section the results of the experiments are discussed.

3. RESULTS AND CONCLUSION

As shown in figure 2 and in figure 3 the measurements were taken when the LPFGs were placed in PBS buffer. Therefore, the surrounding refractive indices are fixed and the changes in the spectrum were related to the attachment of the layers to the LPFGs. Figure 2 shows the resonance wavelengths captured from the LPFG-T4. Several measurements were taken in each step to assure the stability of the signal and the average of resonance wavelengths for each step were calculated. In both LPFGs the double resonances were merged before attachment of bacteriophages and the difference between the two resonances in both LPFGs were 0 in this step.

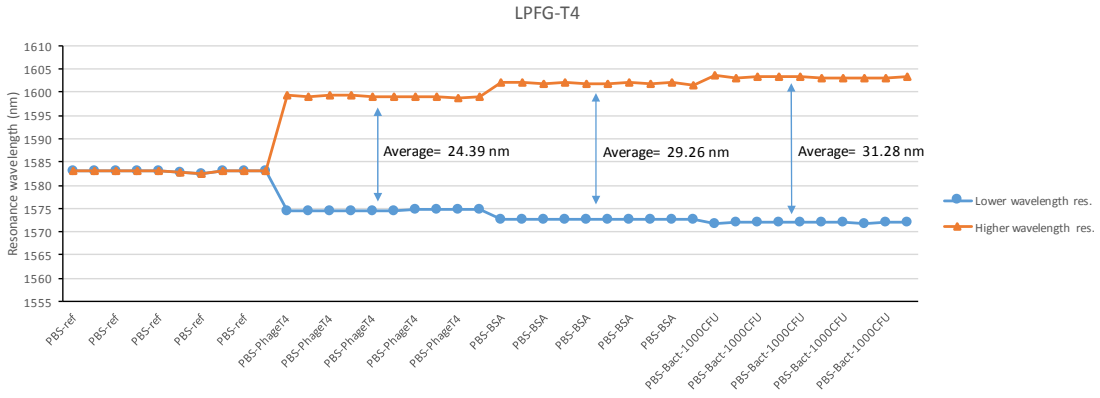

Figure 2. Experimental steps using LPFG-T4 to detect E. coli in minimum concentration of 1000CFU.

The difference between the averages of double resonances after T4 phage attachment is $\Delta\lambda_{res-Ph-T4} = 24.39\,nm$ (LPFG-T4) and after MS2 phage attachment is $\Delta\lambda_{res-MS2} = 29.33\,nm$ (LPFG-MS2) as shown in figure 3 and 4. This results show that the LPFG-MS2 was covered more uniformly with the MS2 phage. This was expected because the MS2 phages are smaller in size and more stable in shape rather than T4 phages. After exposing the LPFGs to the E. coli

bacteria, LPFG-T4 showed $\Delta\lambda_{res-Bact-T4} = 2.0\,nm$ shift because of 1000 CFU of E. coli as shown in figure 3. The lower bacteria concentrations showed no significant shift of the LPFG-T4 spectrum. On the other hand, the LPFG-MS2 showed $\Delta\lambda_{res-Bact-MS2} = 2.7\,nm$ shift for just 100 CFU of E. coli, as shown in figure 4. Therefore, the LPFG-MS2 showed bigger shift for lower concentration of E. coli comparing to the LPFG-T4. Since the physical structures of the gratings were similar, therefore the difference is only because of MS2 phage usage.

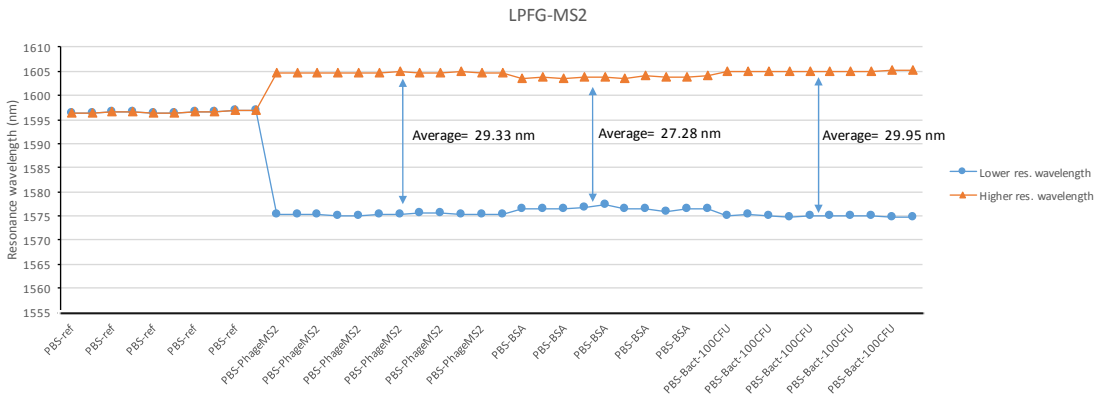

Figure 3. Experimental steps using the LPFG-MS2 to detect E. coli in minimum concentration of 100CFU.

In conclusion, in this study we successfully fabricated two over-etched LPFGs close to their turning point and achieved a very high sensitivity of about 7000 nm/RIU. Then we covered the LPFGs with a stable chemical layer and finally attached a bacteriophage layer to the surface. The whole bio-chemical surface modification and measurement techniques were stable and repeatable during the experiments. Therefore, the comparison of the experiments was possible. Our results showed that using the MS2 phage for detection of E. coli bacteria enhances the limit of detection as expected because of the size and shape of the MS2 phage comparing to the T4 phage. In our experiment we could detect E. coli bacteria in concentration of only 100 CFU while with T4 phage the minimum detectable concentration was 1000 CFU.

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