



Long period grating based biosensor for the detection of *Escherichia coli* bacteria

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ABSTRACT

In this paper we report a stable, label-free, bacteriophage-based detection of *Escherichia coli* (*E. coli*) using ultra sensitive long-period fiber gratings (LPGs). Bacteriophage T4 was covalently immobilized on optical fiber surface and the *E. coli* binding was investigated using the highly accurate spectral interrogation mechanism. In contrast to the widely used surface plasmon resonance (SPR) based sensors, no moving part or metal deposition is required in our sensor, making the present sensor extremely accurate, very compact and cost effective. We demonstrated that our detection mechanism is capable of reliable detection of *E. coli* concentrations as low as 10^3 cfu/ml with an experimental accuracy greater than 99%.

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1. Introduction

Pathogenic *Escherichia coli* (*E. coli*) is one of the most dangerous agents of food-borne disease. Consumption of contaminated food or water can be deadly, especially for children and the elderly. Although *E. coli* infection is most common in developing countries, many recent outbreaks in Europe and Northern America have been attributed to a strain of *E. coli* which has been identified among the most common causes of diseases related to food safety (WHO, 2008, <http://www.cdc.gov/ecoli/2011/ecolio104/>). Accurate routine testing is crucial for outbreak prevention. Nevertheless, currently available tests require time-consuming amplification of samples. The standard detection process of *E. coli* bacteria takes about 24 h to obtain results from culturing methods. Although more recent detection techniques such as PCR, ELISA and IMS offer a more rapid detection, analysis time of several hours is still required.

For the specific and rapid detection of such pathogens, bio-recognition elements such as antibodies (Rijal et al., 2005; Arora et al., 2011), nucleic acids (DNA/RNA) (Baeumner et al., 2003; Dorst et al., 2010) and bacteriophages (Balasubramanian et al., 2007; Shabani et al., 2008) have widely been used for the specific capturing of the target bacteria. Their binding can be detected by fluorescence labeling methods (Pan et al., 2005; Tombelli et al., 2005) or by label-free methods (Zourob et al., 2005; Nanduri et al.,

2007; Smietana et al., 2011). Each of these recognition elements has its own advantages and disadvantages. For example, recognition based on nucleic acid, though offering high specificity, suffers from the inability to discriminate between viable and non-viable cells (Dorst et al., 2010). For antibody-based recognition elements, the drawbacks are high price, stability and cross-binding to other bacteria which may result in false positives (Balasubramanian et al., 2007). Bacteriophage, on the other hand, offers a superior alternative to other recognition elements in terms of their high specificity, fast binding, easy/low-cost production and stability. Furthermore, the bacteriophage has a unique feature to discriminate between the viable and non-viable cells. In principle the bacteriophage binds to both viable and non-viable bacteria as long as the surface receptor (lipopolysaccharides, teichoic acids, proteins) is intact and recognized by the bacteria. To see whether the bacteria are viable, the experiment can be extended past the latent period (the time from infection to bacterial lysis by the bacteriophage progeny). The signal should change since the intact bacteria are now broken (lysed).

In the fluorescent-label method, the recognition elements are labeled with specific dyes, making this procedure complex, time-consuming and the potential alteration of the properties of the analyte (Balasubramanian et al., 2007). Label-free methods, on the other hand, are based on changes occurring at the sensor surface, on changes in the analyte refractive index (ARI) or on changes in the thickness of the bio-film – all changes that could modify the optical properties of a sensor. This method is not only very fast but also allows real-time monitoring of the biomolecular interactions occurring while the process of detection takes place.

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Several schemes based on the label-free methods, like evanescent wave coupling in grating couplers (Horváth et al., 2003), leaky optical waveguides (Zourob et al., 2005) and SPR (Bae et al., 2004; Oh et al., 2005; Taylor et al., 2005; Homola, 2006) for the detection of *E. coli* have been proposed in recent past. Out of these, the SPR based sensors, both in the angular interrogation regime (Bae et al., 2004; Oh et al., 2005; Taylor et al., 2005) and spectral interrogation regime (Homola, 2006), have received much attention due to their extremely high sensitivity which is necessary for highly accurate bio-measurements. However, drawbacks associated with the SPR based sensors are numerous, such as the incorporation of bulky moving part (the high index prism used in angular interrogation regime) (Bae et al., 2004; Oh et al., 2005), the expensive and elaborated metal deposition requirements to support the surface Plasmon wave, the limitation of operation to the highly lossy visible spectral regime ($\sim 400\text{--}800\text{ nm}$) (Homola, 2006), and the possibility of false positives due to input power fluctuations and/or connector losses associated with the intensity based measurements. To overcome these issues, our group has recently proposed a low-cost long-period fiber grating (LPFG) sensor for detection of *E. coli* bacteria based on physically adsorbed bacteriophages on the optical fiber surface (Smietana et al., 2011). Due to the physical adsorption mechanism and low sensitivity $\sim 700\text{ nm/RIU}$ (as compared to $\sim 1000\text{ nm/RIU}$ achievable using SPR based sensors), the sensor was able to detect only high concentrations of *E. coli*, with no link between bacterial concentration and spectral shift detected.

Here, we present for the first time a compact, ultra high sensitive LPFG ($\sim 2321\text{ nm/RIU}$) based cost-effective, highly accurate, ultra stable, label-free, specific detection of *E. coli* using bacteriophages covalently attached on the optical fiber surface as the recognition moiety. We demonstrated a reliable detection of *E. coli* concentrations as low as 10^3 cfu/ml with an exceptional experimental accuracy ($>99\%$). Using scanning electron microscopy (SEM), we also confirm that these measurements are the result of real bacterial binding to the optical fiber surface and not on the changes in the sample refractive index.

2. Materials and methods

The LPFGs are periodic refractive index variations created within the core region of optical fiber (diameter $\sim 9\ \mu\text{m}$) to redirect part of the optical field from the fiber core region (known as the core mode) to the cladding region (diameter $\sim 125\ \mu\text{m}$) known as the cladding mode of the optical fiber. The specific wavelength at which the field is redirected is known as the resonance wavelength (λ_R) and is expressed as (Kashyap, 2009),

$$\lambda_R = (n_{\text{eff}}^c - n_{\text{eff}}^{\text{cl}}) \Lambda + (\kappa_{c-c} - \kappa_{\text{cl-cl}}) \quad (1)$$

where n_{eff}^c and $n_{\text{eff}}^{\text{cl}}$ are the effective refractive indices of the core and the cladding mode; Λ is the grating period; and κ_{c-c} and $\kappa_{\text{cl-cl}}$ are the self-coupling coefficients of the core mode and the cladding mode, respectively. A small fraction of the cladding mode field, evanescent field, travels outside the optical fiber, interacting with the outer region, changing the $n_{\text{eff}}^{\text{cl}}$ and thus changing the λ_R . The LPFG sensing principle relies on measuring the changes in λ_R , the smaller the period of refractive index variation (Λ) the larger would be cladding mode evanescent field leading to high sensitivity.

2.1. LPFG fabrication

To carry out the experiments we fabricated several highly sensitive LPFGs at our lab in single mode optical fiber SMF-28TM (Corning: NY, 14831 USA). For ease of fabrication the photosensitivity of the fibers was increased by hydrogen loading of the fibers at 150 bar in hydrogen chamber for 15 days. Photosensitive LPFGs

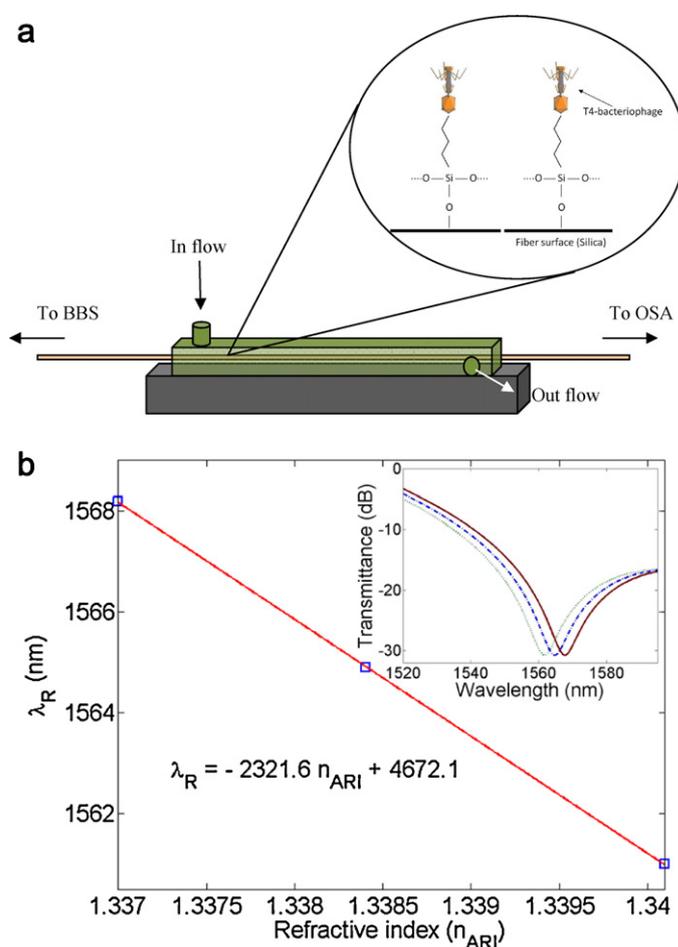


Fig. 1. (a) Schematic of the experimental arrangement; inset shows Si—O—Si covalent binding of the bacteriophages with the optical fiber surface (SiO_2) (b) Variation of the resonance wavelength with ARI; the transmission spectrum is shown in the inset.

were then inscribed into the fiber core using a chromium amplitude mask ($\Lambda = 226.8\ \mu\text{m}$) and a high-power KrF excimer laser (LumonicsTM Lasers: Pulse Master[®]-840) emitting at 248 nm at a pulse repetition rate of 100 Hz, pulse duration of 12 ns and peak pulse energy of 10 mJ. The LPFGs were then annealed at $150\ ^\circ\text{C}$ for 3 h to release the excess hydrogen to stabilize their optical properties. The LPFGs so fabricated have λ_R in the low loss window of telecommunication wavelengths ($\sim 1.56\ \mu\text{m}$) for air as surrounding medium. Since the measurements were to be performed in aqueous solutions the λ_R would shift by $\pm 350\text{ nm}$ shifting away from the low loss regime. To overcome it we slowly etched the cladding of the fiber in 4% HF for $\sim 3\text{ h}$. This shifted the λ_R close to its turning point $\sim 1.58\ \mu\text{m}$, enhancing the sensitivity of the LPFGs (Shu et al., 2002).

2.2. *E. coli* culturing

Frozen stock of *E. coli* B was used to seed Luria-Bertani (LB) media overnight. Bacteria were harvested by centrifugation at $3000 \times g$ for 10 min, followed by washing in phosphate-buffered saline (PBS) buffer. Dilutions of the overnight culture were plated on LB agar to determine the titer, expressed in colony forming units (cfu). Appropriate dilutions of bacteria stock were made in the PBS buffer from the overnight culture stock.

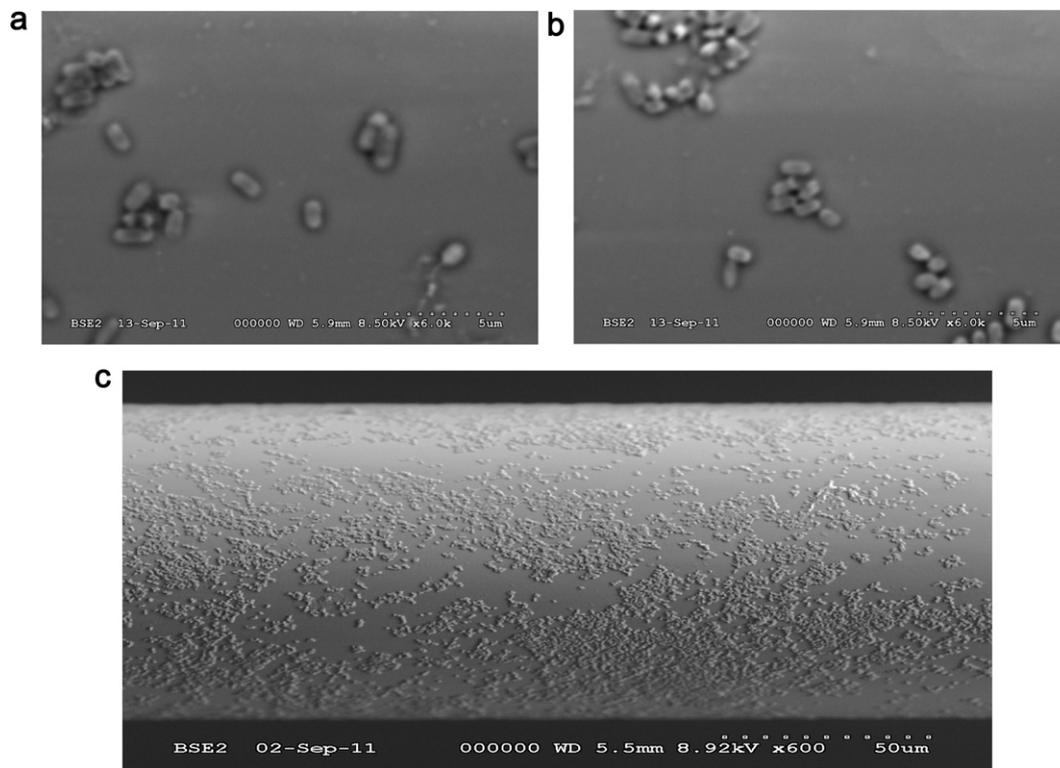


Fig. 2. SEM micrograph of the *E. coli* immobilized on the surface of the LPFG (a) 10^3 cfu/ml of *E. coli*, (b) 10^5 cfu/ml of *E. coli* and (c) 10^9 cfu/ml of *E. coli* concentration in PBS buffer.

2.3. T4 bacteriophage preparation

To produce a stock of T4-bacteriophage solution, 100 μ l of *E. coli* log-phase culture was added to 3 ml of cooled top agar and the mixture was poured onto an LB agar plate until it solidified. Then 100 μ l of phage stock (T4 phage) specific to *E. coli* B was added to the solidified top agar and incubated at 37 °C overnight. A macroplaque was developed on the lawn of bacteria after incubation. The top agar was scrapped off with 3 ml of lambda buffer, the suspension was collected in a 50 ml centrifuge tube, followed by an additional washing of the LB agar plate with another 3 ml of lambda buffer. Three drops of chloroform were then added to the suspension, which was then vortexed and centrifuged at 3500 \times g for 10 min. The phage containing supernatant was then filtered through a 0.22 μ m filter to remove bacterial debris. The phage was freshly prepared for each experiment. The titer of the phage stock was determined by serial dilution of the stock. Phage concentration was expressed in plaque forming units (pfu).

2.4. Experimental set-up

The presence of macrobends along the LPFG region is the primary cause of measurement errors in LPFG based sensors. In order to avoid them we maintained a constant tension along the LPFG throughout the experiments by attaching the fiber near one end of the LPFG and applying a fixed force near the other end of it (see Fig. 1(a)). Light was launched into the fiber using an Agilent-83437A broadband source (BBS) and the transmission spectrum was recorded using an Agilent-86142B optical spectrum analyzer (OSA) with a resolution of 0.02 nm. Prior to the bacteria measurements, the ambient refractive index (ARI) sensitivity of the LPFGs was determined by using analytes of known refractive indices measured by Abbe refractometer (Atago DR-M2) operating at $\lambda = 1.55 \mu$ m. The variation in the λ_R of a typical LPFG in response

to changing ARIs is shown in (Fig. 1(b)), with the transmission spectrum shown in the inset. The slope of the λ_R with the ARI and hence the ARI sensitivity being 2321.6 nm/RIU.

2.5. Bacteriophage immobilization

To covalently immobilize the bacteriophages on the fiber surface we first silanized it by incubating the cleansed LPFG samples in 3-aminopropyltris(trimethylsiloxy)silane (APTES) $\text{H}_2\text{N}-(\text{CH}_2)_3-\text{Si}-(\text{OCH}_3)_3$ (5% in methanol), for 3 h. This resulted in a mono (multi) layer poly silane film covalently bonded to the silica surface of the optical fiber with the amino functional groups on the top of the film (Chandran et al., 2008). The incubated LPFG samples were then rinsed first in methanol and second in deionized water. Next, the amino functional groups exposing on the top of the film were functionalized by an amine-reactive homo-bifunctional cross-linker glutaraldehyde (5% in deionized water), for 30 min. Thus, the amino groups on the top of the LPFG sensor surface were activated to bond with the bacteriophages. The LPFGs were rinsed once again with deionized water to remove the excess glutaraldehyde and then with the PBS buffer to neutralize the surface to pH 7.5. Having activated the surface, the T4 bacteriophages were covalently immobilized through their amine groups on the LPFG surface by immersing the LPFG samples in the bacteriophage solution (10^{10} pfu/ml) for 4 h. The functionalization of the fiber surface by APTES and the eventual covalent binding of the bacteriophages are schematically shown in the inset of Fig. 1(a).

The LPFG sensors were washed copiously with the PBS buffer to rinse off the excess (unbound) phages. This step was followed by incubation of the gratings in bovine serum albumin (BSA) (1 mg/ml) for 30 min to block the non-specific sites on the LPFG surface. The excess BSA was removed by washing the sample LPFGs again in the PBS buffer. At this point, the sensors were all set to bind the bacteria

on the LPFG surface, and we therefore continuously recorded the transmission spectrum right from this stage. After the PBS buffer washed LPFGs, LPFGs were incubated in different concentrations of *E. coli* B bacteria for 20 min, and were finally rinsed again with the PBS buffer. Here, we would like to mention that since bacteriophage is easy and cheap to produce and the long-period fiber gratings is easy to fabricate on widely available telecom-grade standard single mode optical fiber, to avoid contamination and for bio-safety we have used separate LPFGs for different measurements.

3. Results and discussion

Micrographs, of the optical fiber surface, obtained using a Hitachi TM-3000N scanning electron microscope (SEM) at three different concentrations (10^3 cfu/ml, 10^5 cfu/ml and 10^9 cfu/ml) of *E. coli* bacteria bound on the LPFG surface are shown in Fig. 2. To clearly show the extent of *E. coli* coverage on optical fiber surface we have intentionally selected a bigger scale for higher concentration of *E. coli*, 10^9 cfu/ml (Fig. 2(c)). To increase the topographic contrast and avoid the metallization of *E. coli* bacterium we used the back scattering electron (BSE) mode of the SEM. The high-energy electrons originating in the incident electron beam back scatter form of the *E. coli* volume due to the elastic scattering interactions with the *E. coli* bacterium atoms, which are in turn recorded using an asymmetrical, directional BSE detector concentrically placed with the incident electron beam, above the optical fiber. As can be observed from Fig. 2, even for a small concentration of the *E. coli*, 10^3 cfu/ml, the bacteria are well bound on the LPFG surface, with surface coverage increasing as the *E. coli* concentration is increased. Unlike the adsorption-based measurements where the *E. coli* bacteria could easily be wiped away from the fiber surface by subsequent PBS buffer washes, in the present experiments the *E. coli* bacteria remain firmly attached to the fiber surface due to the covalently immobilized bacteriophages on it, making the measurements stable in time. This feature enables us to estimate the amount of bacteria in the solution by monitoring the spectral shifts of the resonance wavelength λ_R as a function of varying *E. coli* concentrations in the PBS buffer. This shift is influenced not only by the changing refractive index of the solution but also by the change in the cladding radius due to the *E. coli* bacteria attached to it: the shifts in resonance wavelength ($\Delta\lambda_R$) with respect to changes in the ARI (Δn_{ARI}) and the cladding radius (Δr_{cl}) can be expressed as:

$$\Delta\lambda_R^m = -A \left(\frac{\partial n_{eff}^{0m}}{\partial n_{ARI}} \Delta n_{ARI} + \frac{\partial n_{eff}^{0m}}{\partial r_{cl}} \Delta r_{cl} \right) \quad (2)$$

where we have disregarded the self-coupling terms as a first-order perturbation (Kashyap, 2009).

The spectral shifts in the λ_R corresponding to the stages of the experiment recorded at the last three steps, namely *pre* PBS buffer wash–Bacteria incubation–*post* PBS buffer wash, are shown in Fig. 3(a). A spectral shift in the λ_R of ~ 2 –4 nm, given the same overlay medium (*pre* and *post* PBS buffer washes) after *E. coli* incubation, is a clear indicator of various concentrations of bacteria binding on the LPFG surface. This has been shown more clearly in Fig. 3(b) where we have plotted the spectral shift of the λ_R between the *pre* buffer wash stage and *post* PBS buffer wash stages against varying *E. coli* concentrations. We observe that the wavelength shift initially increases exponentially with increasing *E. coli* concentrations and then, after 10^6 – 10^7 cfu/ml, it starts saturating as the concentrations of *E. coli* continue to increase in the sample. This can be easily explained by the fact that an increased concentration of *E. coli* in the PBS decreases the refractive index of the solution and at the same time increases the surface coverage of the LPFG surface (see Fig. 2). At high *E. coli* concentrations, however, the sensor surface is almost completely covered with the bacteria and

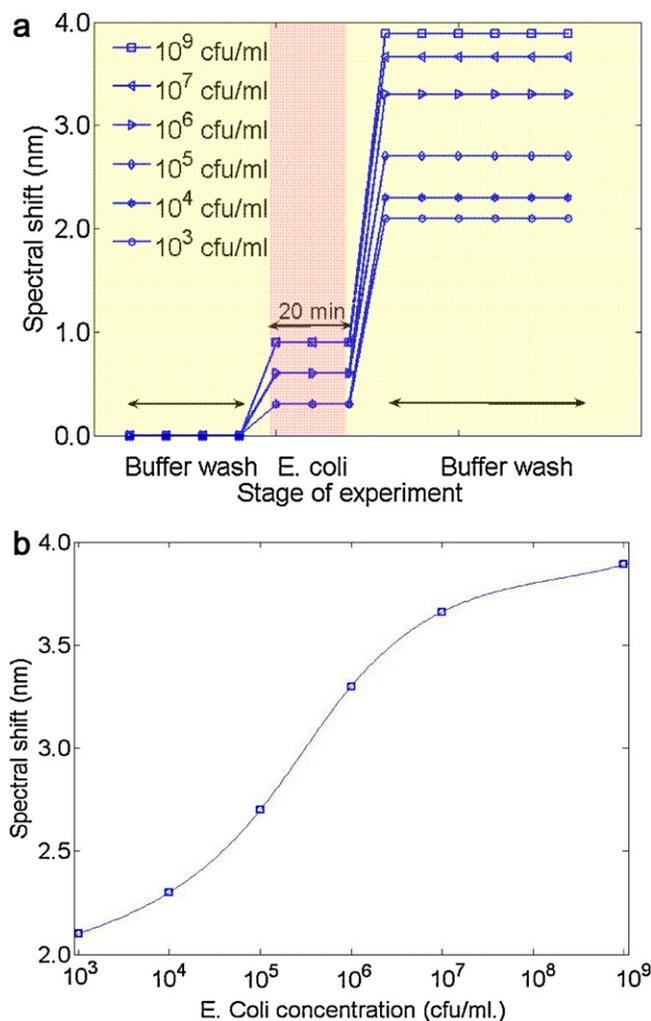


Fig. 3. Variation of (a) the spectral shift of the resonance wavelength prior to and after the *E. coli* incubation on the LPFG, and (b) the spectral shift of the resonance wavelength as a function of the *E. coli* concentration in the PBS buffer.

therefore the resonance-wavelength shift starts saturating. The overall response time of the sensor, after the *E. coli* incubation, is ~ 20 min and the transmission spectrum can be continuously monitored using an optical spectrum analyzer. This sensor, thus, provides an attractive, reliable and very fast way to monitor the growth/decay of *E. coli* culture. The primary cause of low spectral shifts in the λ_R at lower *E. coli* concentrations is the smaller fractional surface coverage area of the LPFG by the *E. coli*. Further, owing to an experimental spectral resolution of 0.02 nm used in our measurements, the experimental accuracy associated with the minimum resonance-wavelength shift observed in our experiments (~ 2.1 nm, corresponding to an *E. coli* concentration of 10^3 cfu/ml) comes out to be $\sim 99.05\%$. We would like to mention here that, although the present sensor is capable of measuring even lower concentrations of *E. coli*, the experimental error associated with such measurements would be greater because of the reduced spectral shifts. For example, using the present LPFGs the experimental accuracy associated with an *E. coli* concentration of 10^2 cfu/ml is 98.95%, which drops down to 98.82% for an *E. coli* concentration of 10 cfu/ml. Nevertheless, since the resonance wavelength is independent of the grating length (Erdogan, 1997; Kashyap, 2009), lower concentrations of *E. coli* (0–100 cfu/ml) could be efficiently measured by increasing the fractional fiber surface coverage by *E. coli*, which could be done by reducing the LPFG length. This, however, will result in a decreased transmission loss at

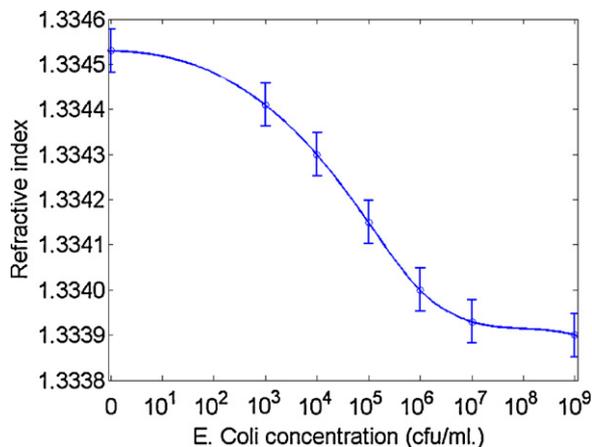


Fig. 4. Variation of the refractive index (measured at $\lambda = 1.55 \mu\text{m}$) of the bio-sample as a function of the *E. coli* concentration in the PBS buffer.

the resonance wavelength (Erdogan, 1997; Kashyap, 2009) making detection challenging. Finally, in order to estimate the influence of *E. coli* bacteria on refractive index variation of the bio-sample we measured the refractive indices of various concentrations of the *E. coli* (in PBS buffer) using an Abbe refractometer (Atago DR-M2) with an accuracy of 0.0001 RIU, shown in Fig. 4. The overall refractive index difference in the PBS solution, as observed from Fig. 4, is $\sim 6 \times 10^{-4}$ RIU for the *E. coli* concentrations varying between 0 cfu/ml and 10^9 cfu/ml. This implies that, based on an exclusive refractive index sensing of the bio-samples, the LPFGs used in our experiments (with a sensitivity of 2321.6 nm/RIU) should yield a net resonance-wavelength shift of ~ 1.3 nm. This shift is much smaller than the ~ 4 nm spectral shifts observed in our experiments: a further confirmation of an efficient *E. coli* binding on the optical fiber surface.

4. Conclusions

In this paper we have presented a fast, reliable, cost-effective, and label-free sensor for detection of *E. coli*. We used a highly accurate spectral interrogation mechanism for real-time monitoring of the bacteria binding on fiber surface by measuring the spectral shift in the resonance-wavelength of the LPFG. Using covalently immobilized T4 bacteriophages on the surfaces of long-period optical fiber gratings, we observed resonance-wavelength shifts ranging

from 2 nm to 4 nm for *E. coli* concentrations varying between 10^3 cfu/ml and 10^9 cfu/ml, with an experimental accuracy larger than 99%. No bacteria dissociate during subsequent washes of the sensor as they do in physical adsorption-based measurement procedures, making the measurements highly stable compared to those of adsorption-based sensors. We showed that the present sensor can efficiently detect a bacteria concentration as low as 10^3 cfu/ml. Even lower concentrations can be detected by increasing the fractional surface coverage of the LPFG by the bacteria through reducing the grating length.

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