Detection of bacteria using bacteriophages as recognition elements immobilized on long-period fiber gratings

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Abstract: The paper presents for the first time a study of long-period gratings (LPGs) applied for label-free detection of specific bacteria using physically adsorbed bacteriophages. For the purposes of the experiment a number of highly sensitive LPGs working at the turning point of phase matching curve was fabricated in SMF28 fiber using UV exposure. We show that the device allows for real-time monitoring of phenomena taking place on the sensor's surface, including phage-bacteria interactions. For the applied conditions a resonance wavelength shift of ~1.3 nm induced by bacteria binding was observed.

OCIS codes: (280.4788) Optical sensing and sensors; (060.2370) Fiber optics sensors; (050.2770) Gratings; (170.1610) Clinical applications; (170.4580) Optical diagnostics for medicine; (280.1415) Biological sensing and sensors.

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1. Introduction

The rapid and specific detection of pathogenic bacteria is extremely important for human and animal health safety and diagnostics. The main target areas where rapid detection is required include the food industry, bodies of water (such as lakes, pools, and water reservoirs), hospitals and bioterrorism-endangered sites. During the last decade a number of incidents were reported in North America and Europe in which thousands of people suffered and were hospitalized as a result of bacteria-related infections [1–3]. Bacteria detection is in even higher demand in developing countries, where microbial diseases are responsible for major health problems and high death rates. In most cases, the cause is contamination of food or water supplies by certain strains of *Escherichia coli* (*E. coli*) bacteria.

Conventional microbiological methods for determining the cell counts of bacteria employ selective culture, biochemical, and serological characterization [2]. More recently introduced methods include liquid and gas chromatography combined with mass spectrometry [4]. Despite the high selectivity and sensitivity offered by these methods, a number of drawbacks limit their universal application, notably the length of time required to yield a result, which typically runs from days to weeks. These detection methods are designed specifically for laboratory use and cannot be expected to serve in the field. Moreover, the supporting systems

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are expensive, and require complicated sample preprocessing as well as specialized operator training.

Major research efforts are currently focused on the development of efficient and cost-effective sensor devices for detection of pathogenic bacteria. A field-ready biosensor can be conceived of as a fast device that is potentially inexpensive, easy to use, portable and highly sensitive [2]. In general, biosensors are composed of a biological recognition element acting as a receptor, and a transducer, which converts the biological activity into a measurable optical, electrical or mass-based signal. The recognition element can be derived from enzymes, whole cells (bacteria, fungi, yeast, animal or plant cells) and other affinity-based elements (antibodies, nucleic acids, molecular imprinted polymers or phages), which specifically bind to individual targets or groups of related targets [4]. In the case of bacteria detection, the sensing process is most often based on the interaction between bacteria and biological recognition elements such as antibodies and nucleic acids (DNA/RNA) [3, 4]. However, the use of nucleic acid-based elements suffers from the lack of discrimination between viable and nonviable cells, while for antibody-based recognition elements, the drawbacks are high price, susceptibility to hostile environmental stresses and cross-binding to other bacteria, which may result in false positives [5].

A specific recent research thrust has been investigation of the use of bacteriophages (phages) as recognition elements. The phages identify their host by specific receptor molecules on the outside of the bacterial cell, infect the host and use it as a factory for their own replication [4]. The advantage of phages is their sensitivity and high specificity to bacteria. Moreover, these small viruses are harmless to humans and animals, are much less expensive and faster to produce than antibodies, and have a far longer shelf life. Phage-based recognition receptors also show very high thermal stability. It has been shown that phages retain detectable binding ability for more than 6 weeks at 63°C, and for 3 days at 76°C [6]. Furthermore, they can be immobilized onto the sensing device in much the same way as antibodies or nucleic acid. In contrast to other novel recognition elements, phages can also be immobilized by simple dip coating, where a physical adsorption mechanism is employed [5–7].

From the point of view of instrumentation, methods using fluorescent labels and label-free methods have both been applied for phage-based bacteria optical detection [4]. When the fluorescence response is to be measured, the phages must be labeled with specific dyes. This procedure is complex and time-consuming, and thus complicates the sensor preparation [5]. Label-free methods on the other hand offer simplicity in sample preparation. They are based on change in a mass bound to a sensor surface, which induces variations in its optical properties, i.e., refractive index (RI) and in the thickness of the bio-film. This approach allows for real-time measurements of the analyte concentration and kinetics, as well as the thermodynamic binding parameters [8]. A number of optical sensors have been developed for label-free detection of bacteria based on surface plasma resonance (SPR) or resonant mirrors (RM) effects [1, 5, 9]. Both these effects offer very high sensitivity to variations in properties of the external medium.

In this paper we focus on the specific and label-free detection of *E. coli* using T4 phages [4, 7] as recognition elements immobilized onto the surface of long-period gratings (LPGs). LPGs are a periodic modulation of the refractive index along the length of an optical fiber [10]. Under special phase-matching conditions, the grating will couple the fundamental core mode to discrete cladding modes that are rapidly attenuated due to absorption and scattering. The coupling from the guided mode to the cladding modes is wavelength-dependent, so one can obtain a spectrally selective loss. A number of sensors based on LPGs have been proposed for temperature, strain, hydrostatic pressure, bending and RI sensing [11, 12]. Due to its high RI sensitivity, an LPG platform can perform real-time monitoring of interactions between bio-molecules at the surface of the grating. LPGs have already been applied in the field of label-free biosensing, e.g., for monitoring of DNA hybridization [13, 14], protein

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adsorption [15, 16] and antibody-antigen reactions [17, 18]. The advantages of the LPG sensor include simple fabrication using several available methods and easy adjustment of the resonant wavelength [19, 20]. It must be noted that a number of investigated liquids, including the buffer and bacteria solutions used in some experiments, may contain a certain amount of salt, which would accelerate corrosion of metal-containing devices [21]. For this reason, the LPG surface must be corrosion-resistant as well as biocompatible. Due to its simplicity, specifically the absence of fragile or corrosive metal overlays, the device is even reusable in some cases [13, 17]. To the best of our knowledge, the work reported in this paper is the first attempt at real-time monitoring of the presence of specific bacteria using LPG-based measurements.

2. Experimental details

The LPGs used in our experiments were written by UV-exposure of hydrogen-loaded SMF-28 fibers, using a Pulse Master 840 high-power KrF excimer laser (λ=248 nm) from Light Machinery and a chromium amplitude mask (period Λ=169.7 μm). Five-centimeter lengths of the fiber were exposed for 5 to 10 minutes to various energy levels in the range from 290 to 360 mJ. To release the hydrogen and thus stabilize the optical properties of the LPGs, all the gratings were annealed at 150°C for 3 hours after exposure.

The spectral response of the LPG structures (λ = 1160 to 1660 nm) was monitored using an Agilent 86142B optical spectrum analyzer and an Agilent 83437A broadband light source. To minimize the bend cross-sensitivity, the gratings were mounted diagonally in a U-shape holder. Then the grating was placed in several V-groove containers filled with specific liquids. In order to determine the RI sensitivity of the sample LPGs, several mixtures of glycerin and water were prepared and their RIs (n_D) were determined using a VEE GEE PDX-95 refractometer working with an accuracy of +/– 10^{-4} RI unit (RIU). Since temperature variations have an influence on the RI of the liquids, as well as playing an important role in phage physical adsorption [22], the procedure was started when all the liquids, especially those normally stored in the refrigerator, reached room temperature.

The experimental procedure employing bio-liquids was as follows. LPG samples were first cleaned with methanol, then washed several times with deionized water, rinsed in phosphate-buffered saline (PBS) and immersed for 4 hours in a T4 bacteriophage solution (10^{10} pfu/mL in PBS solution, where pfu stands for plaque forming unit; pH 7.5). To remove the unbound cells the LPGs were rinsed several times with PBS after immersion. Next, to block non-specific sites on the surfaces of the sensor, the samples were immersed in bovine serum albumin (BSA) solution (1 mg/mL) for 30 minutes [4]. Then the samples were again rinsed several times with PBS and introduced to *E. coli* K12 bacteria solution (10^8 cfu/mL in PBS, where cfu stands for colony forming unit) for 20 minutes. Finally the samples were again rinsed several times with PBS in order to remove unbound bacteria. Reference experiments were performed by skipping the T4 phage immobilization step in the procedure described above. The main steps of the experiment are schematically shown in Fig. 1.

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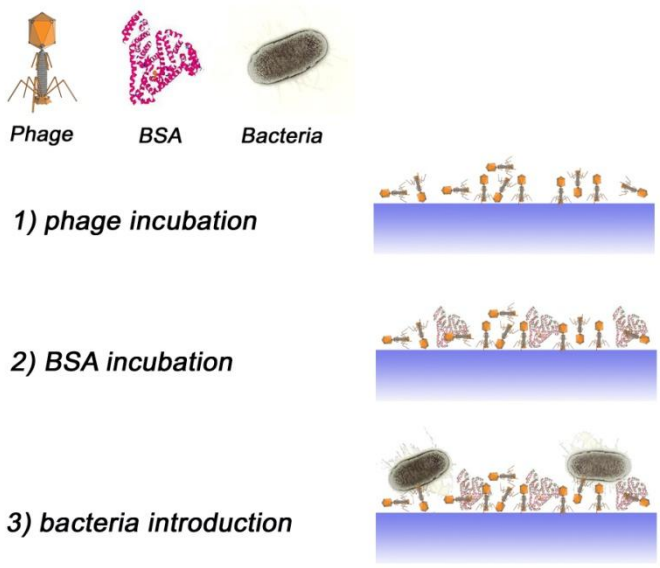

Fig. 1. Schematic showing the main steps of the experiment (Phage, BSA and Bacteria are not to scale). Between these steps the LPG samples were rinsed with PBS.

3. Results and discussion

The unique feature of LPGs that sets them apart from other fiber gratings is their high sensitivity to changes in the external RI n_{ext} [23]. However, when only very small variations are expected in the properties of the surface bio-film, the sensitivity of the LPGs must be maximized. This sensitivity is typically defined as a shift of the resonance wavelength λ_{res}^{m} induced by a measurand [11]. Equation (1) describes a wavelength-dependent coupling from the guided core mode (LP_{01}) to the mth cladding mode (LP_{0m}),

$$\lambda_{res}^{m} = \left(n_{eff}^{01} - n_{eff}^{0m}\right)\Lambda \tag{1}$$

where n_{eff}^{01} is the effective refractive index of the propagating core mode, n_{eff}^{0m} is the effective refractive index of the mth cladding mode and Λ is the period of the LPG. In the case of external RI sensing, a resonance shift can be analytically described by Eq. (2), where u_m is the mth root of the zeroth-order Bessel function of the first kind, and r_{cl} and n_{cl} are the radius and refractive index of the fiber cladding, respectively [11].

$$\frac{d\lambda_{res}^{m}}{dn_{ext}} = -\lambda_{res}^{m} \cdot \frac{\frac{d\lambda_{res}^{m}}{d\Lambda}}{n_{eff}^{01} - n_{eff}^{0m}} \cdot \frac{{u_m}^2 {\lambda_{res}^{m}}^3 n_{ext}}{8\pi {r_{cl}}^3 n_{cl}(n_{eff}^{01} - n_{eff}^{0m})({n_{cl}}^2 - {n_{ext}}^2)^{3/2}} \tag{2}$$

It was found that when Λ is short enough, due to the existence of a turning point in the phase matching curves, it is possible to couple energy into the same cladding mode at two discrete wavelengths, resulting in the appearance of dual resonant peaks [24]. This phenomenon can be obtained in the SMF28 fiber for the LP_{011} mode in the investigated spectral range when the period is Λ ~170 μm. At the turning point, where $\left|\frac{d\lambda_{res}^{m}}{d\Lambda}\right| \to \infty$, the gratings are extremely sensitive to external perturbations and that is why these conditions

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have already been employed in the field of biosensing [13, 16]. With the increase in n_{ext} , the peaks increase their spectral distance. The sensitivity is highest when the turning point is reached, so that the spectral distance between the resonances is minimized, yet the peaks must have enough spectral separation to precisely define the resonance wavelengths [11, 13, 16, 20]. In case of this experiment well defined resonance wavelength and simultaneously high sensitivity were achieved when the spectral distance was about 150 nm. It must be emphasized here that the spectral separation of the peaks is strongly dependent on even tiny variations in fiber properties, in the grating period and in exposure parameters [25]. In our experiment, we observed that only for a certain set of applied fabrication parameters, namely exposure time and energy (6 min./360 mJ or 10 min./290 mJ), was it possible to achieve clearly visible dual-peak resonances. Devices fabricated using these parameters show very high RI sensitivity and high visibility of the resonances only when the gratings are immersed in an aqueous solution, i.e., n_D~1.333 (Fig. 2 inset). It must be noted that we did not apply here any resonant wavelength tuning by means of surface etching [20]. The etching would result in surface damage and would have an influence to phages incubation. When the grating is in air, only single and broad resonance can be seen in the investigated spectral range. Measurements of the RI sensitivity of these LPGs performed using various water/glycerine mixtures show that the sensitivity is constant in the investigated RI range (Fig. 2). For the red-shifting resonance, which is slightly more sensitive than the blue-shifting one [11, 13, 25], sensitivity typically reaches 570 nm/RIU. As for the PBS buffer, it can be seen that it also respects this relation. It should be noted that in this part of the experiment, the applied liquids are treated as an infinite medium and the response is exclusively dependent on their RI.

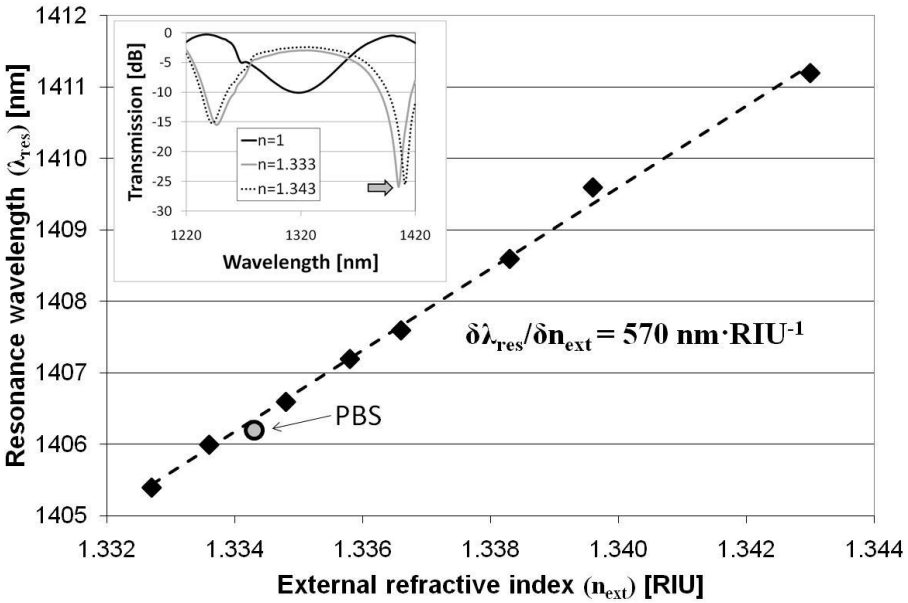

Fig. 2. Resonance wavelength shift induced by small changes in n_{ext}. Results are shown for water/glycerin solutions as well as PBS. The inset shows transmission spectra for selected n_{ext}. Traced resonance is marked by arrow.

The results of the experiments with bio-liquids are compared in Fig. 3 for samples with and without a phage adlayer. The variation in the state of the LPG surface, i.e., modification in the RI of the bio-overlay, as well as its thickness in this case, results in a clear spectral shift of the resonances. During the phage incubation stage, the phages are sticky and physically adsorbed well to the surface of the LPG, a process that is marked by a red shift of the

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resonance with incubation time. An increase in sensor response with time during phage incubation has been observed by other authors using different instrumentation techniques. They attributed the effect to an increase in the density of the phage-based overlay [5, 26]. The observed effect may have been slightly amplified in our experiment by some evaporation of the solution during the 4-hour incubation. Such evaporation results in an increase of the liquid density and the concentration of phages and thus an increase in the RI of the bio-overlay.

In the following stage of the experiment, a decrease of the resonance wavelength is observed. This effect can be explained by the removal of the loosely bound cells from the fiber surface when the LPG is rinsed with PBS [5, 26]. The adsorbed cells create a thin adlayer with an external RI equal to that of the PBS. When PBS responses before and after the introduction of phages are compared, a shift of ~3.7 nm induced by the adlayer can be seen. The RI of this kind of adlayer (protein) is typically assumed to be from 1.56 [5] to 1.57 [26]. The height of T4 phages is estimated to be ~200 nm [22, 27], however their orientation on the surface is random, and the surface coverage is usually low [9]. This distribution was shown to result in average surface coverage of about 2 nm, when incubation took 2-3 hours and a gold surface was used [5]. A higher thickness/density of the adlayer was obtained by Nanduri et al. [9] when flow cell was used. Surprisingly, it was found that differences in the surface coverage of phages had no significant effect on the sensitivity of bacteria detection [5].

In the next step, the sample LPGs were immersed in BSA-containing solution so that the protein would block non-specific sites on the surfaces of the sensor. We performed a number of control experiments where we introduced bacteria to unmodified LPG surfaces and after BSA blocking agents. We did use green fluorescent protein expressed *E. Coli* for testing the surface treated and untreated with BSA using fluorescence microscope. We confirmed that the BSA coated surface showed significant reduction in non-specific adsorption.

Like the earlier stage where phages were adsorbed, this step in the experiment resulted in further red shifts of the resonance of ~4.7 nm and ~1.5 nm for the phage adlayer sample and the sample with no adlayer, respectively. The difference in the size of the shifts is mainly due to differences in the sensitivity of the two samples. It is known that a high-RI nano-overlay modifies the RI sensitivity of an LPG [15, 16, 28]. Here, the phage-based adlayer in essence forms an overlay and thus increases the RI sensitivity of this sample. This accounts for the fact that the shift induced by adsorption of BSA for the sample with phages on the surface is more than 3 times as large as for the other sample. In neither case does rinsing with PBS decrease the shift. This observation possibly indicates that during the 30-minute experiment, most of the BSA from the liquid adsorbed to the fiber or walls of the container, so that the liquid had the same RI as the PBS.

The final step of the experimental procedure involves the introduction of bacteria followed by multiple rinses in PBS. For both types of sample (with and without phages), the presence of bacteria induced a further red shift of the resonances. This red shift takes place during the first several minutes of the final experimental stage and is followed by slight decrease with time and then stabilization of the response in time. The shift for the phage-coated sample is higher than for the sample with no phages. This effect can again be explained by the higher RI sensitivity of samples with high-RI phage-based nanocoating. With the PBS rinses following the introduction of bacteria, two effects are evident for both types of samples: at first an increase of the response and then its slow decrease and stabilization with consecutive rinsing cycles.

The initial red-shift noticed at the start of PBS rinsing is difficult to explain. The phage-bacterium interaction investigated by other authors was shown to cause a time-related structural change in the surface-bound bacteria, possibly due to collapse of the cell [29]. The effect observed in [29] was correlated to an increase in mass density, which in turns increases RI. However, in our experiment the effect can also be seen for samples where no phages were present, so its origins must not be due simply to the increase in mass density.

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As far as the decrease and stabilization of the response with rinsing cycles is concerned, it can be seen that for samples with a phage adlayer, the response stabilizes after only 5 cycles, while about 15 cycles are required for samples with no phages. The difference suggests that unbounded bacteria are removed more easily from the surface of LPGs with a phage adlayer. The decrease is most probably related to the release of some still loosely bonded phages together with some bacteria. The relatively fast stabilization of the response for the sample with phages and the red-shift of ~1.3 nm after the introduction of bacteria proves the phages-bacteria binding effect. The value of the shift achieved, within the range from 1 to 2 nm, is similar to values obtained by other authors who applied LPGs for biosensing [13, 14, 15].

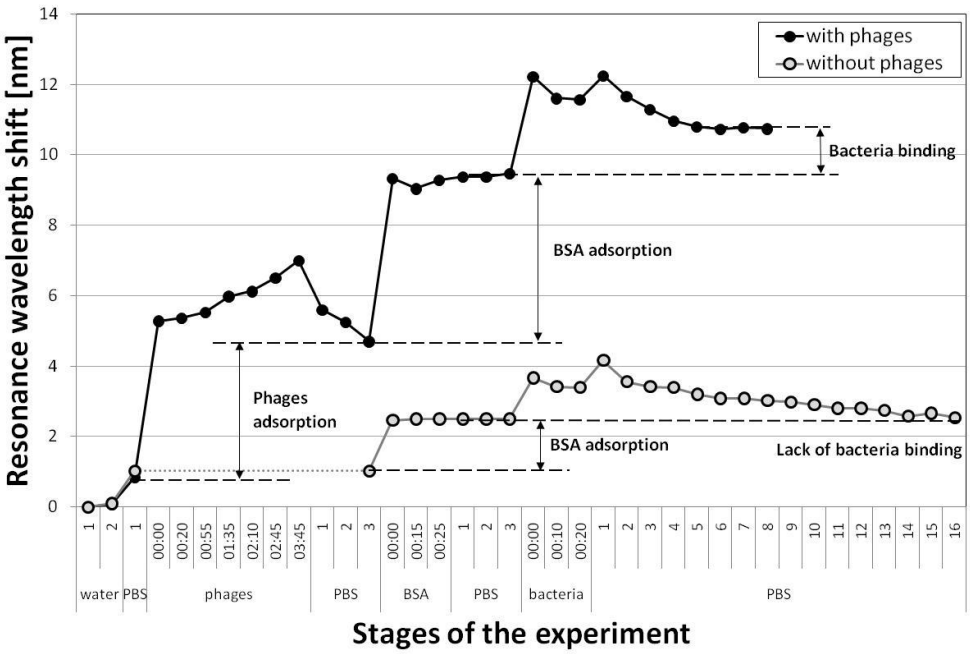

Fig. 3. Resonance wavelength shifts, referred to initial value for water surrounding the samples, at various stages of the bacteria experiment compared for sample with and without phages adsorbed to the surface of LPG. In the case of phages, BSA and bacteria, time of the procedure was given, where for PBS consecutive rinsing cycles are marked.

4. Conclusions

This study shows for the first time that a low-cost LPG-based transducer can be successfully used to monitor bacteriophage-bacteria interactions. When immobilized to the LPG surface, a T4 phage can act as a recognition element for detection of *E. coli* K12 cells. The approach demonstrates a rapid, direct, and label-free means of detecting specific bacteria using phages. The observed resonance wavelength shift of ~1.3 nm induced by bacteria binding is within the range of results obtained by other authors investigating LPGs as biosensors. Moreover, the results obtained here suggest that further improvement of the device's sensitivity could be realized by application of a thin high-RI overlay on the surface of the LPG.

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