Etched and DNA coated Fiber Bragg Grating based bio-sensor for protein concentration measurement

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In this paper, we propose a novel method for measuring the concentration of the protein (Bovine Serum Albumin- BSA) present in bio-chemical samples. The bio-sensor exploits the inherent characteristics of the Fiber Bragg Grating (FBG) which is coated with a biopolymer-namely, deoxyribonucleic acid (DNA). For increased sensitivity, the FBG was etched with hydrofluoric acid (HF) prior to coating with the DNA. The etched FBGs are sensitive to an external analyte by evanescent field interaction. The sensing mechanism is based on the interaction of the protein with the biopolymer film, which changes the film refractive index and also exerts some stress on the underlying fiber, resulting in a shift in the Bragg wavelength. By analyzing the Bragg wavelength shift, we can calculate the amount of protein present in the sample solutions. A complete experimental analysis, based on the use of an etched and coated FBG for protein concentration measurement, is presented.

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1. Introduction

Proteins form a class of major biomolecules and there is much motivation to detect proteins because they are widely employed to diagnose the presence of diseases. Today, the protein concentration measurement is very important and has significant applications in medical diagnostics, drug discovery, food and biotechnology. Currently analytical techniques like Gas Chromatography-Mass Spectroscopy (GC-MS) and High Performance Liquid Chromatography-Mass Spectroscopy (HPLC-MS) are commonly used. But most of these techniques have the disadvantage that they are expensive, time consuming and require skilled and well-trained technicians to perform the analysis. Therefore an optic fiber based biosensor will be a promising alternative to the classical analytical methods due to its simplicity, relatively low cost, inherent specificity, ability to perform sensing using a small amount of sample and rapid response. In recent years, Fiber Bragg Grating (FBG) sensors are one of the most exciting developments in the field of optical fiber sensors. FBGs make promising candidates as sensors due to their significant sensing advantages, the most important of which is that the information on a measurand is encoded in the reflected or transmitted wavelength from the grating. Thus, the problems associated with the source power fluctuations, bending losses and reflection losses are eliminated.

A standard FBG sensor takes into account the refractive index modulation in the core of an optical fiber that acts to couple the fundamental forward propagating mode to the contra-propagating core mode. The principle of operation of an FBG sensor is based on the shift of the Bragg wavelength when it is under the influence of a measurand. During the two decades, FBGs have been used as optical sensors to measure a wide range of physical parameters including temperature, strain, pressure, loading, etc. [1-3]. Since the light coupling takes place between well-bound core modes that are screened from the influence of the surrounding-medium refractive index (SRI) by the cladding, normal FBGs are intrinsically insensitive to SRI. Therefore normal FBGs cannot be used as chemical sensors or biosensors. To use FBG as an effective refractometric sensor element, the cladding radius around the grating region must be reduced, allowing the effective refractive index of the fiber core to be significantly affected by the refractive index of external medium [4, 5]. As a consequence, shifts are expected in the Bragg wavelength combined with a modulation of the reflected amplitude. A very simple method to reduce the cladding can be the uniform chemical etching of the Bragg grating section of the fiber using hydrofluoric acid. The sensitivity of the sensor depends on the change in the effective index of the core mode, which is related to the change in the refractive index of the biological or chemical sample under test. To date, a number of SRI sensors have been realized using etched FBG structures to measure concentrations of some chemicals or bio samples [6–9].

At present, refractive index sensing based on the etched FBG is an extraordinarily important subject in the bio sensing area which attracts significant research interest. The etched FBGs can detect extremely low concentrations of biochemical target molecules by applying suitable material coatings on their surface. These

coatings selectively react with specific target molecules and result in the refractive index change. The presence of a specific target molecule is detected by analyzing the Bragg wavelength shift of the FBG reflection spectra. Therefore, FBGs are ideal candidates for biomolecular and chemical sensing applications [10,11], if properly complemented with suitable chemically sensitive materials or biorecognition elements. The number of biosensing applications exploiting different designs of FBGs is rapidly growing and they are expected to become a key technology in the next few years [12,13].

In this work, an optic fiber based protein concentration sensor is demonstrated by coating an etched FBG with the DNA bio-polymer material. It is well known that proteins react with high specificity with DNA material. The stress and refractive index (RI) change induced in the biopolymer film during its interaction with proteins depends on the concentration of protein present in test samples. Using a real time monitoring set-up, we recorded the Bragg wavelength changes with changes in protein concentration. By analyzing the Bragg wavelength shift, we calculated the concentration of protein present in a liquid sample.

2. Principle of operation

In a FBG, the guided light is reflected by each interface of different refractive index regions in the core. For a wavelength which satisfies Bragg condition, the scattered light adds up constructively resulting in back reflection with a central wavelength λ_B given by

$$\lambda_B = 2\, n_{eff}\, \Lambda \qquad (1)$$

where Λ is the pitch or periodicity of the grating and n_{eff} is the effective refractive index of the core. The Bragg resonance wavelength of a grating depends on both the effective refractive index of the fiber core and the periodic spacing between the grating planes. Any variation in physical measurands will either change the value of the effective refractive index of the grating period or both of them. Any change in these factors changes the wavelength of the back reflected light. This is the basis of sensing with FBGs. The measurand under monitoring can be assessed by estimating the shift in wavelength of reflected light with and without perturbation.

FBGs have been extensively used as temperature and strain sensors. However, FBGs are intrinsically insensitive to SRI since the light coupling takes place only between well-bound core modes, which are shielded from the influence of the SRI by the fiber cladding. To make the FBG sensitive to changes in the SRI, the cladding radius around the grating region must be reduced. If the fiber cladding layer is partially or fully removed symmetrically along the grating region using chemical etching, the effective refractive index, n_{eff} is significantly affected by the external RI. As a consequence, a shift in the Bragg wavelength combined with a modulation of the reflected amplitude is expected as the SRI changes. The resultant FBG is often termed as an etched, thinned or reduced cladding FBG. Uniformly etched FBGs were first demonstrated by Asseh *et al.* [14]. FBG sensors based on evanescent wave interactions with the external environment are attractive because they transduce the change of index in the surrounding medium to a change of reflected wavelength that can be easily measured. The change in the Bragg wavelength $\Delta\lambda_B$ associated with the chemical etching is given by [15]:

$$\Delta\lambda_B = 2\Lambda\Delta\eta_p(\Delta n) \qquad (2)$$

where Λ is the period of the grating, $\Delta\eta_p$ is the fraction of the total power of the unperturbed mode that exists in the etched region and Δn is the difference between the cladding RI and the surrounding RI. The strong dependence of the effective refractive index on the surrounding RI of the medium, recommends the use of a thinned FBG as a highly sensitive fiber RI sensor. An etched FBG will show maximum Bragg wavelength shift when the SRI is close to the core RI. In a variety of chemical and biological applications, RI sensing is very important since a number of substances can be detected through measurement of the RI. The RI changes with the concentration of bio-chemical solution variation. This, in its turn, energizes the change in core effective index and leads to the Bragg wavelength shift. The shift of Bragg wavelength with the concentration of bio-chemical solution variation could be calculated using the relation mentioned above [16,17].

For bio-sensing applications, the sensitivity and selectivity of etched FBGs can be improved by applying suitable material coatings with high refractive indices along the thinned region. The basis of these FBG bio sensors relies on the use of a suitable measurand-specific material to induce a secondary effect, to which the etched FBG is susceptible. i.e. the interaction of the specific target molecule along with the coating changes the film RI or exerts some strain on the underlying fiber and thus causes a Bragg wavelength shift and a variation in the grating reflectivity. Therefore, concentration measurement of biological samples can be done by analyzing the spectral changes in FBG transmission or reflection spectrum. The basic configuration of the sensor is shown in Fig. 1.

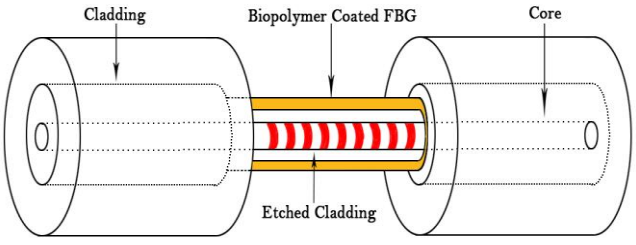

Fig. 1. Structure of the biopolymer coated etched FBG sensor

The bio-sensing device presented in this paper consists of an etched FBG coated by a biopolymer material, namely, DNA. The selective interaction of the protein with DNA results in a change in RI of the material coating. This interaction will also exert some strain on the underlying fiber. As a result, both shift in the Bragg wavelength and reflectivity can be expected based on the concentration of protein in samples. Protein concentration measurement can be done by analyzing the relation between Bragg wavelength shift and amount of protein present in test solutions.

3. Experiments

3.1 Materials & methods

The DNA used for our experiment was commercially available from Sigma Aldrich, USA. The available DNA was a double stranded DNA which is rich in sodium salt. The extracted DNA was in white fibrous form and used without any further purification. It was found that this DNA, from salmon fish was soluble only in water. This property is not amenable for DNA to be dip coated into FBGs, as the resulting coating will be vulnerable to water absorption and will affect the mechanical strength of coated FBGs. Therefore it is necessary to perform certain processing steps [18], so that the DNA will become suitable for fiber coating. DNA is naturally negatively charged due to the phosphate groups in the backbone of the double helical structure. They form electrostatic charged pairs with the sodium cations in aqueous solution. The processing techniques involve the removal of sodium salt by precipitating with a cationic surfactant, hexadecyltrimethylammonium bromide (CTAB). This surfactant replaces the cationic sodium salt with its long alkyl chain containing positively charged Nitrogen by an ion exchange reaction, forming DNA-CTMA (hexadecyltrimethylammonium). The displaced sodium cations then bonds ionically with the bromide anion of CTAB forming NaBr as the byproduct. The CTAB was chosen for our studies because of its long alkyl chain (>16). A shorter chain might induce poor mechanical property and a longer one would damage the double helical structure of DNA by breaking the hydrogen bonds of the base pairs.

The coating material preparation was done as follows. First, the DNA was dissolved in demineralized water at a concentration of 4g/L by allowing it to dissolve for a day. However, magnetic stirring can be done to speed up the process. The DNA solution should be stored at a temperature in the range 20°C to 25°C .CTAB was also prepared by dissolving it in water at a slightly larger concentration (> 4g/L). This ensured that all sodium cations were replaced by the alkyl chain of CTAB. The surfactant solution was added drop by drop to the DNA solution, while stirring was done continuously. The DNA-CTMA lipid complex then begins to precipitate in the solution. The solution was stirred for few hours and the precipitate was removed by filtration in vacuum using a nylon filter with a pore size of 0.45μm. During filtration, additional 3-4 liters of distilled water were added to ensure that any CTAB, that did not bind to DNA, was washed away. The precipitate was then dried in vacuum at 40 °C overnight. The resulting DNA-CTMA lipid complex was not soluble in water; however, it is soluble in organic solvents such as methanol, ethanol, and butanol. So, we have dissolved the lipid complex in butanol and then filtered using a nylon syringe filter of 0.45μm. During this filtration process the temperature was maintained at 60 ^{0}C [19]. The final solution was quite viscous and suitable as the coating material around FBGs. The measured RI of the coating solution was found to be 1.398. The protein powder, Bovine Serum Albumin Fraction V (BSA) was supplied by Sisco Research Laborataries (SRS). The test samples were prepared by dissolving BSA in distilled water in different proportions (50 μg/mL to 300 μg/mL). CTAB was supplied by Ranbaxy fine chemicals Ltd.

3.2. Fabrication of the sensor and experimental set-up

The processes for producing a fiber grating based bio sensor include fiber hydrogen loading, FBG fabrication, fiber cladding etching and film coating. The fabrication of the sensor has been carried out using an FBG with Bragg reflectivity at 1564.28 nm before etching. FBG was written on the middle of stripped section of a photosensitive single-mode fiber by the phase mask technique, using a KrF laser operating at 248 nm. The single-mode fiber used had a cladding diameter of 125 μm and a numerical aperture of 0.14. The core and the cladding refractive indices were 1.4630 and 1.4563, respectively. To enhance the photosensitivity, the fiber was loaded with hydrogen at 100 ^{o}C and at pressure of 1500 psi for 24 hours before the FBG fabrication.

To make the FBGs sensitive to changes in the SRI, the cladding radius around the grating region was reduced by wet chemical etching in a buffered hydrofluoric acid (HF 48 %) solution. For this purpose, HF solution was taken in a special teflon mount, which is non reactive to HF. The experimental set-up for both fabrication process monitoring and for recording the Bragg wavelength shift with variation in protein concentration, is shown in Fig. 2. It comprises of a white light source ([Yokogawa] AQ 4305), a 3dB coupler to collect the reflected spectrum from the sensor head, a teflon mount and an optical spectrum analyzer ([Yokogawa] AQ 6319) for spectral measurements. The teflon mount has an inlet and outlet provision and the dimensions were suitably selected to facilitate the process of etching and sensor operation. To stop the etching process at the desired fiber diameter, the HF solution was removed and to restrict the etching activity the teflon tube was filled with deionized water. After an etching time of 33 minutes, a final blue shift of 0.28 nm in the Bragg wavelength was observed between unperturbed and etched grating with air as external medium as shown in Fig. 3. The etched fiber was cleaned by repeatedly washing with isopropyl alcohol and methnol. The FBG was then dipped in DNA coating

solution for two minutes and withdrawn at a speed slow enough to produce a uniform coating without bead formation. Residual stress after the film deposition was observed which produced a red shift of 0.26nm as shown in Fig. 3. For a precise measurement, the experimental set-up and sample solution temperature were maintained at 25.0 ± 0.5 °C.

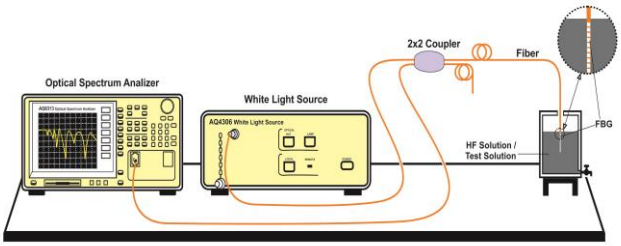

Fig. 2. Experimental setup for FBG etching/ protein concentration measurement

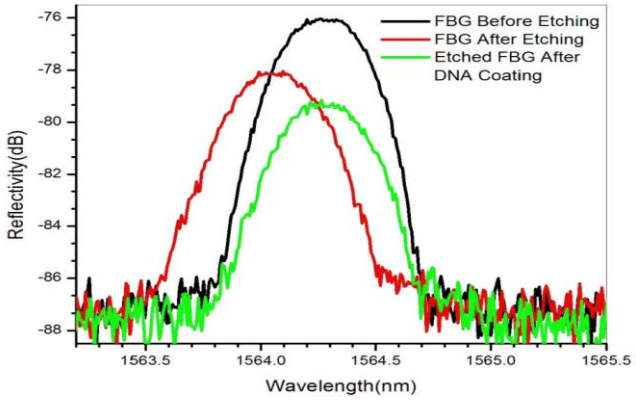

Fig. 3. Bragg wavelength and reflectivity before and after the etching

4. Results and discussion

In our experiment, the Bragg wavelength of the bio polymer coated FBG was monitored, while samples with different protein concentrations were in contact with the sensing region. Since the principle of operation relies on the interaction between the evanescent wave of the fundamental guided mode and the surrounding medium, it is obvious to expect the effective RI variations with change in BSA concentration, and the corresponding Bragg wavelength shift. The concentration of BSA used ranged from 50 µg/mL to 300 µg/mL. Before each measurement, the sensing element was immersed in deionized water to clean any residue left out from the previous measurement. After this cleaning and proper drying, when we exposed the DNA-coated FBG to air, the Bragg wavelength returned to its original wavelength without any deformation in the transmission spectral shape. This demonstrates the reversibility and reusability of the bio-sensor. Sensor responded to concentration changes as soon as new samples were introduced to the teflon cell. But, to get a stabilized output, all readings were taken one minute after the FBG was immersed in the solution.

Reflection spectra of the sensor as a function of protein concentration are shown in Fig. 4. From this figure, it can be seen that the Bragg wavelength of the sensor showed a red shift when it was exposed to a higher protein concentration. The FBG exhibited a total red shift of approximately 0.141 nm when the BSA concentration was gradually changed from 50 µg/mL to 300 µg/mL. Apart from the red shift, there was a reduction in the reflected power with increasing protein concentration. When we expose the bio sensor to protein test samples, it selectively reacts with DNA coating. This causes a modulation of RI at the surface and leads to a change in FBG reflection spectrum. This protein-DNA interaction will also exert some small strain on the underlying fiber. As a combined effect, both red shift in the Bragg wavelength and reduction in reflectivity are observed based on the concentration of protein present in bio samples. Hence, the amount of the Bragg wavelength shift can be directly related to the protein concentration.

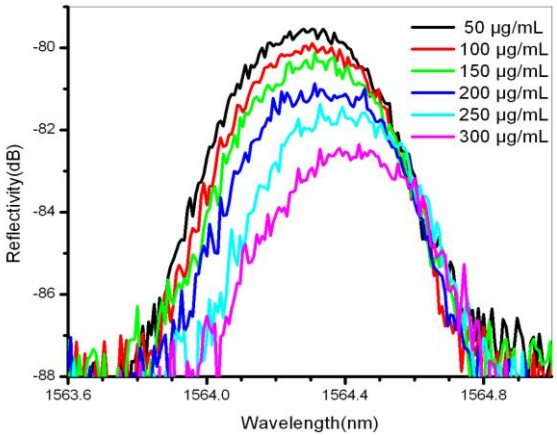

Fig. 4. The spectral response of FBG for different concentrations of protein solutions.

The sensitivity of the coated FBG, when used as a sensor for various weight percentage of protein in distilled water is shown in Fig. 5. It can be seen from the graph that the sensitivity of the sensor increases with increase in protein concentration. The FBG sensor sensitivity was around 0.180 pm/µgmL^{-1} of protein in the lower measurement range and 0.820 pm/µgmL^{-1} of protein in the higher measurement range (150-300 µg/mL).

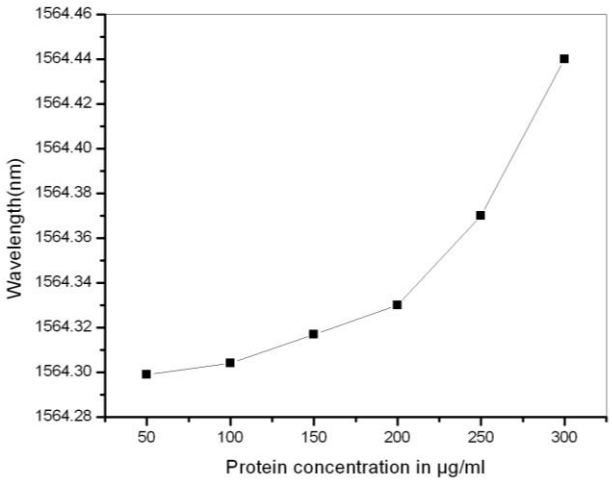

Fig. 5. Bragg wavelengths of the FBG sensors as a function of protein concentrations.

5. Conclusions

We have designed, fabricated and experimentally evaluated a protein concentration sensor based on biopolymer coated FBG refractometer. The bio-sensor can measure the amount of protein by monitoring the Bragg wavelength shift which is induced by the selective interaction of protein with DNA biopolymer coating. The advantages of this type of grating sensor are easy interrogation, it does not involve the use of toxic chemicals, requires a small volume of sample for analysis, and provides the response in real time. This sensor has also shown a very good repeatability. The measurement system may be used to detect biological or chemical changes in the surrounding media. The simplicity and high sensitivity of the sensor make it worthy for medical diagnostics, pharmaceutical, and biomedical sensing applications

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