



Design & construction of a tapered optical fiber sensor for the detection of streptavidin protein

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Abstract

This paper presents the fabrication process and operation of a bio-sensor based on tapered optical fiber by using optical spectrum shift in the presence of PBS buffer and dissolved streptavidin. The taper-fiber sensors are 18 and 24 μm in diameter and are fabricated by use of an oxy-butane torch. The optical spectrum is obtained using a broadband light source and an optical spectrum analyzer (OSA) in a 40 nm wavelength range. The existence of streptavidin on the tapered fiber surface is confirmed. The experiments on PBS buffer have shown that, the output spectrum demonstrates a red-shift by adding the streptavidin. The maximum sensitivity obtained is 29000 nm/RIU.

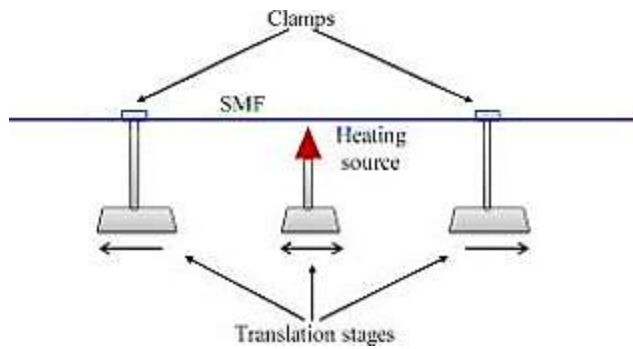
Keywords: tapered fiber, biosensor, streptavidin, biological buffer

1. Introduction

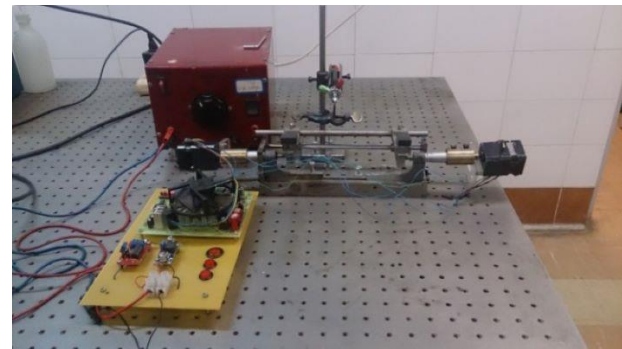
Today, the use of biosensors has become widespread. A biosensor is an instrument that detects tissue, cell, or any chemical element, using specific biochemical reactions by isolated enzymes, usually electrically, optically or thermally [1]. The human senses of smell and taste, which detect different odors and tastes, or the immune system, which detects millions of different types of molecules, are examples of natural biosensors. Optical biosensors have attracted great attention due to their various applications in industrial services and medical, healthcare diagnostic, biomolecular interaction detection, environmental monitoring, and so on [2]. Optical fiber sensors based on different sensing mechanisms, such as interferometers [3], evanescent-field fiber sensors [4], and surface plasmon resonance (SPR) sensors [5-7] could be used for biological detection owing to their desirable advantages such as small and flexible shape, low sample consumption, good specificity, low-cost fabrication, and rapid and sensitive detection in real time [8,9]. An interesting type of optical biosensor is the tapered fiber-optic sensor, whose detection is based on the overlap of the evanescent electromagnetic waves of the fiber with the environment whose refractive index is

measured. When the total internal reflection occurs in the optical fiber, at the core-clad interface, the electromagnetic wave tunnels into the clad, which is called the evanescent field. Although this field disappears quickly in the fiber cladding, removing a few micrometers from the fiber cladding, can increase the penetration depth (the distance from which the field strength is reduced to 1/e of its peak value) [10,11]. Due to the tiny diameter of the tapered optical fiber, the amount of penetration depth and the intensity of the evanescent wave field can be considerably high [12]. For this purpose, the optical fiber is tapered so that the evanescent wave of the core can be used outside the fiber. This makes the optical fiber very sensitive to changes in the refractive index of the environment. When the light is passed through the fiber, the refractive index of the environment outside the fiber affects how light is propagated inside the fiber. By analyzing the output light intensity, the characteristics (type and amount) of the peripheral substance can be obtained [13,14].

In the work done by Mena et al., a tapered fiber optic biosensor (TFOBS) fabricated by an optically controlled HF chemical etching is demonstrated. This device is a



(a)



(b)

Figure 1. (a) Schematic representation of tapered optical fiber construction system and (b) Laboratory equipment for tapered optical fiber fabrication using air-gas flame.

label-free sensor for detecting glucose concentration. A single-mode TFOBS controls the reaction rate by changing the HF concentration and monitoring the optical power variation at the fiber output. This method used two samples of tapered fiber optics with different diameters of 25 and 35 μm . In this experiment, changing the glucose concentration between 0 and 50 mg/ml in the wavelength range of 1536 to 1555 nm, shows that in a constant wavelength, the power decreases as the glucose concentration increases. In addition, it is observed that the sensitivity increases significantly with decreasing taper diameter and approaching the core. However, measuring glucose concentration at these fiber diameters requires great care due to its fragility and high sensitivity. Nevertheless, due to the use of relatively low-cost materials for the sensor fabrication, the fabrication process of glucose concentration sensors is made affordable and optimized. The sensitivity of the tapered fiber optic with a diameter of 35 μm is equal to $4.89 \times 10^{-4} (\text{mg/ml})^{-1}$ in the detection range of 0.481 mg/ml [15].

In another experiment by Urrutia et al., protein sensors based on tapered optical fiber were examined and analyzed using sputtering and layer by layer methods. In the layer-by-layer method, Au-based coatings consisting of poly cation poly (allylamine hydrochloride) and negatively charged SiO_2 nanoparticles followed by charged Au nanoparticles are deposited onto a nonadiabatic tapered optical fiber. In the sputtering method, tapered optical fibers are coated with a gold thin film using the DC-sputter deposition process (Quorum Emitech K575×Sputter Coater, from Quorum Technologies) with a partial pressure of argon of 8×10^{-2} mbar and current of 70 mA. The sensors in both methods are covered with gold nanostructures at the conical region. Biotin was attached covalently to gold molecules immobilized on an optoelectronic silicon transducer. Then, the sensors are tested in streptavidin solution with a concentration range of 2.5 nM to 1.33 μM , and their responses are analyzed. Both sensors show a logarithmic response (streptavidin concentration versus light signal intensity). Their observations show that for sensors of both methods, the transmission intensity decreases by increasing the concentration of streptavidin solution in the wavelength range of 500-650 nm. The limits of detection for the sputtering method and layer-

by-layer sensor respectively, were obtained at 8.06×10^{-10} and 2.71×10^{-10} M [16].

In this paper, we have used a biosensor based on tapered optical fiber for detecting streptavidin and the junction formation between biotin and streptavidin is used for this purpose. Two tapered optical fibers are used for measurements, and biotin is immobilized on their surface.

In section 2, the fabrication of the tapered optical fiber, and in section 3, the steps of biotin immobilization and preparation of the biosensor surface are fully described. Section 4 describes the observation of changes in the optical fiber spectrum and the detection of streptavidin. In section 5, the recorded output spectrum is described using an optical spectrum analyzer, and finally, in section 6, the paper is concluded.

2. Construction of tapered optical fiber

There are several ways for the fabrication of the tapered optical fiber. These methods are divided into two general categories: the heat and pull method and the chemical method. The most important advantages of the heating and pulling method are the smoothness of the surface of the tapered fiber and its simplicity and fastness. Due to these advantages, we used this method.

A part of the optical fiber is heated to the melting point. Tension to one or both ends of the fiber, makes its softened part tapered. The heat source can be a flame [17], a CO_2 laser [18], or an electric spark [19]. We used an air-gas combination flame in this experiment (Figure 1(a)). The optical fiber is held by magnetic clamps. An optical fiber stretching device has been used, with two stepper motors pulling the fiber from both sides at a speed of approximately 1 mm/s. Figure 1(b) shows the equipment used.

3. Immobilization of streptavidin on the sensor surface

3.1. Immobilization and its methods

To create a suitable biosensor, the biological component must be properly connected to the transducer, which is called the immobilization process. There are various methods for immobilization, including physical immobilization, ionic immobilization, physical trapping, chemical immobilization, cross-linking, etc. In some of



Figure 2. NHS-Biotin was prepared in the laboratory.

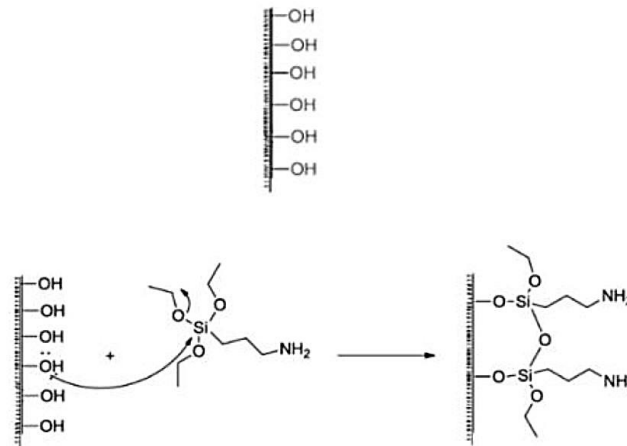


Figure 3. Hydroxylation and amination of the silica surface.

these methods, a diagnostic agent on auxiliary materials such as membranes, polymers, nanocomposites, etc., are immobilized. Even in some cases, the immobilized agent is placed in the volume of a matrix material to increase selectivity. In this case, analytes compatible with the holes in the matrix will be detected. We have used the chemical immobilization method, which will briefly be discussed.

3. 1. 1. Chemical immobilization

Chemical immobilization involves the formation of a covalent bond between an auxiliary solid surface of an optical biosensor with a detecting element. In this method, the surface of the auxiliary substance is modified by chemical reaction to form the groups -OH, -NH₂, -COOH, etc [1]. This method is one of the oldest immobilization methods and is mainly used today.

3. 2. Biotin-streptavidin bond

Streptavidin is a protein produced by the bacterium streptomyces that has a high tendency to bind to biotin and is used as a protein in diagnostic medical applications. Due to this high tendency, streptavidin is used as an intermediate molecule to bind and paste biotinylated molecules to each other. The interaction between streptavidin and biotin is known as the strongest non-covalent bond in nature. The streptavidin molecule has four binding sites capable of binding to biotin. Streptavidin is also used as a probe to detect of biotinylated molecules in biomolecule analysis methods.

That's why we used the biotin-streptavidin pair in this experiment.

3. 3. NHS-biotin preparation

In this experiment, we have to put biotin on the surface, and because biotin can not bind directly to the fiber surface, the NHS ester must be used. The NHS-Biotin combination synthesis is explained first: 0.45 g of N-hydroxysuccinimide is mixed with 0.53 g of biotin, and 0.75 g of DCC solution is added to the mixture. Then, the mixture is heated until a white precipitate forms in the solution. The white precipitate is smoothed, and ether is added to it. The resulting precipitate is called NHS-biotin (Figure 2).

3. 4. Hydroxylation and amination of the silica surface

The surface of the optical fibers is not ready to react yet. By washing the surface with Piranha solution, the OH group is activated on the surface and ready to react. By adding APTES to the optical fibers, the NH₂ groups are created on the surface and the surface is activated. This process is called functionalization (figure 3).

3. 5. Adding biotin to the surface with the help of NHS

We dissolve NHS-Biotin in DMSO solvent and pour it several times on the surface of the optical fibers to bind the biotin to the surface of the optical fibers with the help of NHS after the required time (2-3 hours) has passed.

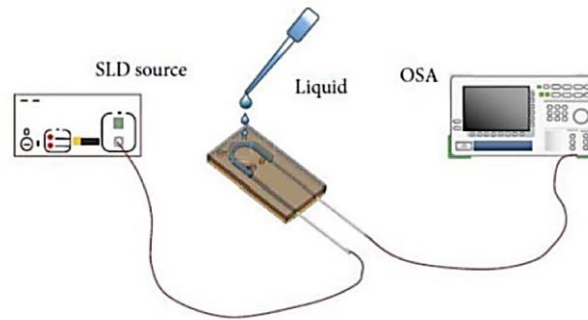


Figure 4. Test configuration of output spectrum changes by buffer injection into the container: data acquisition using an OSA.

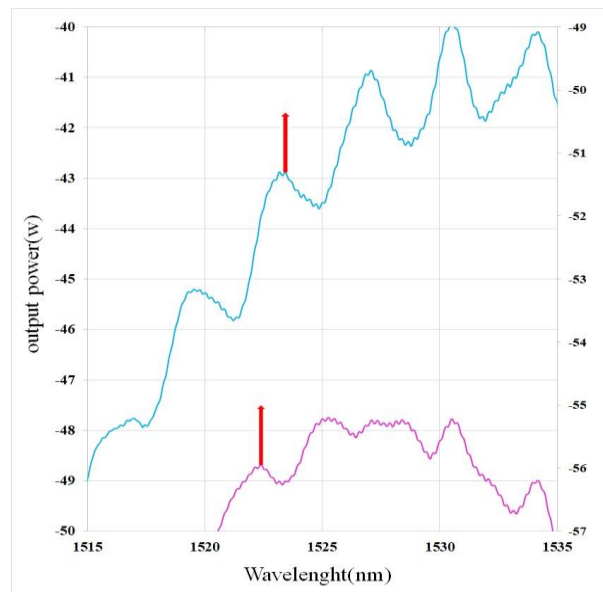


Figure 5. Output spectrum of tapered fiber (24 μm) in the presence of air and PBS buffer.

4. Setup for observing changes in fiber optic sensor and detecting the presence of streptavidin

To bind streptavidin to the surface of optical fibers, a suitable buffer must be prepared in which streptavidin is solved and injected into the cell. For this purpose, PBS buffer with a refractive index of 1.3341 and pH=7.4 is used.

The refractive index of the environment around the optical fiber is equal to 1.003 (air) and changes to 1.3341 with the injection of PBS buffer. A broadband light source (super luminescence diode) in the wavelength range of 1520-1560 nm and an optical spectrum analyzer (OSA) are used to observe the effect of the refractive index change around the tapered optical fiber (Figure 4).

The light enters the tapered optical fiber by ordinary optical fiber. It is sent from the other side to the OSA to observe and record changes in the spectrum due to the presence of streptavidin in the PBS buffer, which changes the refractive index around the sensor.

5. Output spectrum recorded using an OSA

Figure 5 shows the spectrum of tapered fiber in the presence of air and a PBS buffer. The length of the tapered optical fiber is 1 cm, and its waist diameter is 24 μm .

As can be seen, the wavelength of one of the peaks in

the presence of the air is 1522.5 nm, and the wavelength of the same peak shifts to 1523.3 nm, when we inject the PBS buffer into the sample cell. This means that the output spectrum is displaced by 0.8 nm in the presence of PBS buffer relative to air.

Figure 6 shows the spectrum of the second tapered fiber. The length of this tapered optical fiber was 1 cm and its waist diameter was 18 μm .

In this case, the wavelength of one of the peaks in the presence of the air is 1534.15 nm, and shifts to 1536.0 nm, when the PBS is injected. Therefore, the wavelength of the peak shifts by 1.85 nm.

We now examine the effect of adding streptavidin in the optical spectrum. Figure 7 shows the output spectrum of the first tapered optical fiber. It is observed that the peak wavelength of 1526.9 nm changes to 1525.8 nm when PBS buffer with streptavidin is injected into the cell.

We add the second buffer to compare the resulting spectrum with the first buffer. The arrow on the spectrum indicates the wavelength of a specific peak which should be traced. As can be seen, the arrow indicates different wavelengths for that peak before and after the second buffer injection, which indicates that the streptavidin is immobilized on the surface of the tapered fiber. This is the goal of this work.

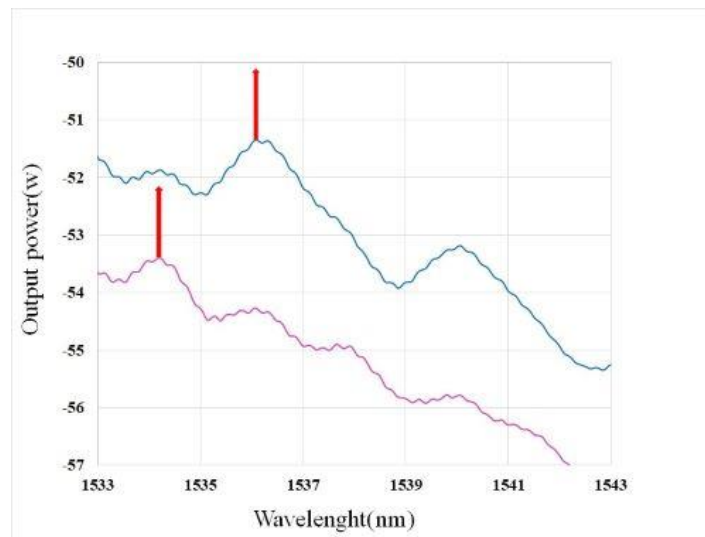


Figure 6. Tapered fiber optic output spectrum (18 μm) in the vicinity of air and PBS buffer.

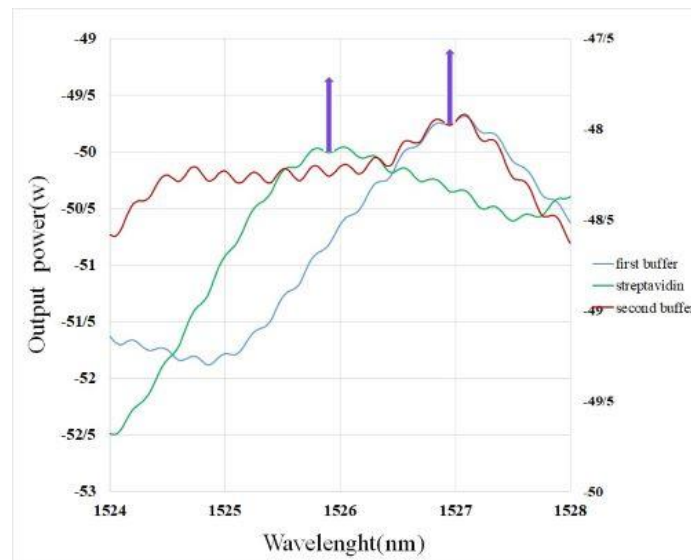


Figure 7. First tapered fiber output spectrum (24 μm) in the presence of PBS buffer and streptavidin.

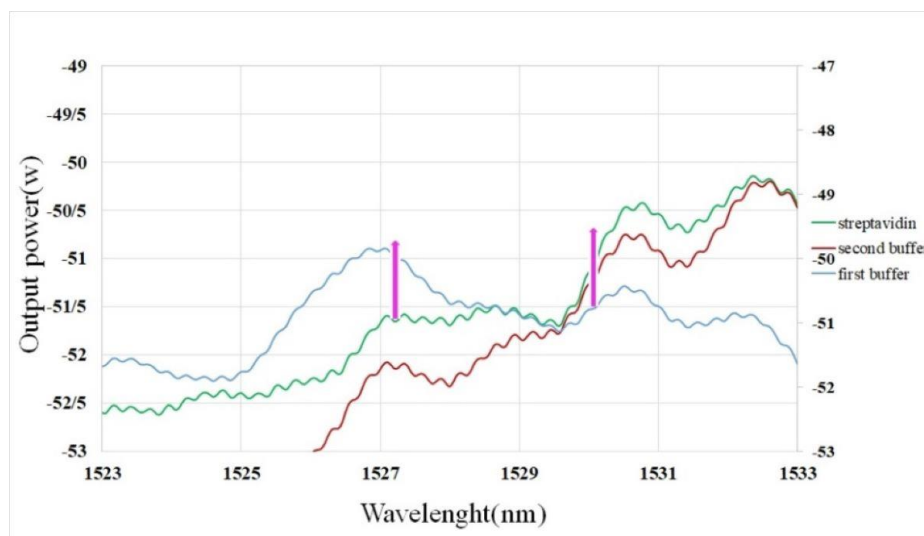


Figure 8. Second tapered fiber output spectrum (18 μm) in the presence of PBS buffer and streptavidin.

The same experiment was done with the second fiber. Figure 8 shows the wavelength of the tapered fiber (18 μm) for the second measurement. When PBS buffer is

injected, the wavelength is 1530.05 nm. By injecting streptavidin buffer, the wavelength changes to 1527.15 nm.

Table 1. Wavelengths of the output spectrum recorded by the analyzer (OSA)

| Test Number | Sensitivity(nm/RIU) |
|-------------------|---------------------|
| The first sensor | 11000 |
| The second sensor | 29000 |

At each step in which the PBS buffer is injected with streptavidin into the sample cell, the wavelength spectrum shifts relative to the previous state in which the tapered fiber was surrounded by PBS buffer. A summary of the measurement results is given in table 1. Comparing the results of the two-sample cells, it can be seen that the tapered optical fiber with a diameter 18 μm used in this experiment has the better result, because the sensitivity begins to increase considerably when decreasing to the core diameter [15].

As can be seen, the maximum displacement of the sample cell of 18 μm was 2.9 nm, and the highest sensitivity was 29000 nm/RIU. After re-injecting the PBS buffer into the sample cell, the spectrum of the samples no longer returns to its original state and the spectrum is more like the PBS buffer with streptavidin. This result indicates that streptavidin is not removed from the optical fibers; that is, the capture of streptavidin

on the surface of the sensor is confirmed.

6. Conclusion

This experiment aimed to show that the streptavidin in the buffer was bonded to biotin, which was immobilized on the surface of the optical fibers. This caused the spectrum of PBS buffers before and after the addition of streptavidin to be unequal. In all measurements, the output spectrum of the samples (tapered optical fibers) shifted to the left of the graph after streptavidin injection. The results show that the fabricated sensor could detect streptavidin in the buffer surrounding the surface of tapered optical fibers.

Founding

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