

Biosensor based on excessively tilted fiber grating in thin-cladding optical fiber for sensitive and selective detection of low glucose concentration

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Abstract: We report a highly sensitive, high Q-factor, label free and selective glucose sensor by using excessively tilted fiber grating (Ex-TFG) inscribed in the thin-cladding optical fiber (TCOF). Glucose oxidase (GOD) was covalently immobilized on optical fiber surface and the effectiveness of GOD immobilization was investigated by the fluorescence microscopy and highly accurate spectral interrogation method. In contrast to the long period grating (LPG) and optical fiber (OF) surface Plasmon resonance (SPR) based glucose sensors, the Ex-TFG configuration has merits of nearly independent cross sensitivity of the environmental temperature, simple fabrication method (no noble metal deposition or cladding etching) and high detection accuracy (or Q-factor). Our experimental results have shown that Ex-TFG in TCOF based sensor has a reliable and fast detection for the glucose concentration as low as 0.1~2.5mg/ml and a high sensitivity of $\sim 1.514\text{nm}\cdot(\text{mg/ml})^{-1}$, which the detection accuracy is $\sim 0.2857\text{nm}^{-1}$ at pH 5.2, and the limit of detection (LOD) is 0.013~0.02mg/ml at the pH range of 5.2~7.4 by using an optical spectrum analyzer with a resolution of 0.02nm.

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1. Introduction

Biosensing technique is a kind of novel microanalysis technology, which has been used to achieve selective, rapid and high reproducible detection at low concentration. So far, it has been applied in the fields of medicine, food safety, environmental monitoring and other bio-related engineering. Due to the compactable sensing platform, remote sensing and in situ measuring characteristic, optical fiber (OF) based biosensors have been attracted more attractions [1]. In principle, OF based biosensors are based on that the interaction between evanescent field of the light propagating through the fiber and the changes of the surrounding molecules or substances of fiber, which leads a changing of mode structure of the optical fiber devices, then the reflection or transmission of the light could be monitored as a function of various parameters.

In general, the standard OF based biosensors didn't show very high refractive index (RI) sensitivity in the aqueous liquid. Many techniques have been applied to increase the RI sensitivity, such as tapered fiber, microfiber, and chemical-etched/side-polished fiber [2–6], in which the penetration of the evanescent field of the guiding light has been enhanced in the surrounding samples. However, the performance of evanescent OF biosensors so much depends on the modified fiber geometry, which will cause several issues associated with careful design and control in the fabrication process thus reducing the reproducibility of the sensor structure, and also will lead to a downgrade in the mechanical strength of the devices. Long period grating (LPG) [7] structure with a period of several hundreds micrometers can couple the fundamental core mode to co-propagating cladding modes, making it intrinsically sensitive to the surrounding medium RI, thus the LPG based biosensors have been widely reported for the detection of bio-related analytes, such as *escherichia coli* bacteria [8], DNA [9] and streptavidin [10]. Because photonic crystal fiber (PCF) possesses the advantages of long optical path, easy light input/output coupling and the accessibility of the channels for

chemical or biological modification, significant research effects have also been focused on the OF biosensors using the PCF directly or inscribed with an LPG structure [11, 12], greatly enhancing the RI sensitivity ($>1000\text{nm/RIU}$ achievable). Although the LPG or LPG-PCF based sensors do not face the issues existed in the tapered fiber or chemical-etched/side-polished fiber sensors, but they will very easily suffer from the thermal or stress cross-talk effect due to the intrinsic properties (highly sensitive to the temperature and stress) of the LPG structure.

Another attractive optical biosensor is based on surface Plasmon resonance (SPR) principle. The conventional SPR sensors using the prism coupling mechanism have shown a very high stability, high sensitivity and low limit of detection (LOD) to the analytes as a consequence of the field enhancement effects of the surface Plasmon polariton (SPP) between the noble metal surface and the external medium [13]. By introducing the SPR technique into the OF to realize the microminiaturization of the conventional SPR sensors, many types like multimode/plastic/PCF OF-SPR based biosensors were developed for the detection of various biochemical analytes, such as explosive vapors [14], TNT [15], apolipoprotein E [16] and prostate-specific antigen [17]. Indeed, an extremely high RI sensitivity (usually $>2000\text{nm/RIU}$) is achievable by these types of OF-SPR sensors, but they are associated with the problem that the polarization state and the distribution of the light among all the possible cladding modes must be controlled and maintain at a high degree of precision, which will require strictly controlled the light launch conditions at the input of the fiber, the absence of fiber motion and short fiber lengths without sharp bends during the experiment [18]. Another disadvantage for these types of OF-SPR sensors, similar to the conventional prism coupling SPR one, is that the 3dB bandwidth of resonance spectrum is very wide (usually $>50\text{ nm}$) at their working wavelength range (600~900nm), leading to a very low Q-factor (<20), seriously affecting measurement accuracy. Broad resonance is also an issue existed in the LPG-structured sensors as their typical 3dB bandwidths are around or larger than 20nm.

In our previous works, we have reported a glucose sensor based on excessively tilted fiber grating (Ex-TFG) inscribed in standard single mode fiber (SMF) [19], but whose sensitivity for glucose detection was low ($\sim 0.298\text{nm} \cdot (\text{mg/ml})^{-1}$). In this work we report here, for the first time a more compact, easily fabricated biosensor based on Ex-TFG structure inscribed in the thin-cladding optical fiber (TCOF) (SM1500, core/cladding diameter: $\sim 4\mu\text{m}/\sim 80\mu\text{m}$) [20], which could be smoothly and easily connected to the standard SMF with extremely low insertion loss, for a more sensitive, much accurate, and selective detection of glucose. Glucose oxidase (GOD) was covalently immobilized on TCOF surface as a specific enzyme for the selective detection of glucose molecules. Since the fiber cladding itself is very thin (only $\sim 80\mu\text{m}$) and smooth, the evanescence field of the cladding mode penetrated into the surrounding medium is greatly enhanced without conduction of chemical etch or side polish to the fiber cladding, thus it can obtain a much higher RI sensitivity ($>1000\text{nm/RIU}$) and meanwhile maintain a good mechanical strength. In addition, in contrast to recently reported LPG based glucose sensor [21], our sensor has higher detection sensitivity for the glucose concentration and a far lower temperature cross-talk effect, and thus it can provide a more stable measurement in the real application. While comparing with the reported OF-SPR based glucose sensor [22], no noble metal deposition or cladding etching is required in our sensor, thus it can greatly simplify the fabrication process. Finally, our glucose sensor shows a much higher Q-factor ($>4 \times 10^2$) than that of LPG or OF-SPR based ones, which will ensure its high detection accuracy.

2. Fabrication and properties of Ex-TFG in TCOF

The Ex-TFGs were inscribed in H_2 -loaded TCOF by using scanning mask technique and doubled frequency Ar^+ laser emitting at 244nm with a power level of $\sim 150\text{mW}$. A custom-designed amplitude mask with a period of $6.6\mu\text{m}$ was tilted at 78° thus producing excessively titled fringes at $\sim 81^\circ$ with grating period of $\sim 32\mu\text{m}$ along the fiber axis in the fiber core, as shown in Fig. 1(a) and (b). Obviously, the Ex-TFGs are similar to LPGs but with much smaller grating period. Thus as a consequence, they couple the light to high order cladding

modes resulting in comb-like resonance spectrum as shown in Fig. 2(a), with much narrower resonance bandwidth ($\sim 2.5\text{nm}$) than that of LPG ($>20\text{nm}$).

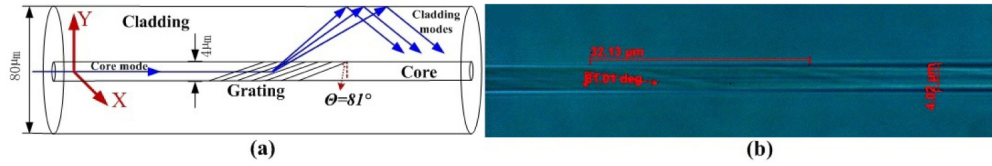


Fig. 1. (a) Structure, (b) Micro image of the core region of the Ex-TFG in TCOF.

The excessively tilted fringes of Ex-TFG in TCOF have induced a significant birefringence to the fiber core, resulting in dual-peak resonances corresponding to the two orthogonal polarization statuses (i.e. TM and TE mode), which can be excited by the polarized light. The zoomed plot in Fig. 2(a) is for a pair of dual-peaks centered in 1555nm to 1585nm region: the blue curve shows the TM mode fully excited when the grating is probed with fast-axis light, whereas the red curve indicates the TE mode fully coupled when probed with slow-axis light; the black curve shows the spectrum when probed with equal-polarized light exhibiting two equally coupled modes of $\sim 3\text{dB}$ strength. According to the analysis [7], the resonance wavelength change of a cladding mode induced by the surrounding RI perturbation can be expressed as

$$\Delta\lambda = -\gamma \frac{u_m^2 \lambda^2 \Lambda n_{sur}}{8\pi^3 r^3 n_{eff-cl,m} (n_{cl}^2 - n_{sur}^2)^{3/2}} \lambda. \quad (1)$$

where, γ is the waveguide dispersion; u_m is the m^{th} root of the zero order Bessel function ($J_0(u) = 0$); r is the cladding radius, n_{sur} is the surrounding RI; n_{cl} is the cladding RI; $n_{eff-cl,m}$ is the effective RI of m^{th} -order cladding mode (TM or TE mode); Λ is the grating period along the fiber axis; λ is the working wavelength of cladding mode. Because the Ex-TFG was produced in thin cladding fiber, the wavelength shift is greatly enhanced as $\Delta\lambda$ is inversely proportional to r^3 . Experimental result showed that the RI sensitivities of the TM and TE mode of this dual-peak could reach $\sim 1526\text{nm/RIU}$ and $\sim 1280\text{nm/RIU}$ at the RI of ~ 1.355 , respectively, as shown in Fig. 2(b), which is ~ 15 times of that ($50\sim 100\text{ nm/RIU}$) of the normal LPG (grating period $\sim 550\mu\text{m}$) and is also comparable to that of the OF-SPR or LPG-PCF based sensors. While the absolute values of temperature sensitivity of TM and TE mode are only $\sim 21.1\text{pm/}^\circ\text{C}$ and $\sim 19.9\text{ pm/}^\circ\text{C}$, respectively, as shown in Fig. 2(b), which are ~ 15 times lower than that ($\sim 360\text{pm/}^\circ\text{C}$) of normal LPG. In addition, experimental results also demonstrated that both the absolute values of temperature and RI sensitivity of the Ex-TFG in TCOF are mode-order related, as they would increase with the increase of the cladding mode order.

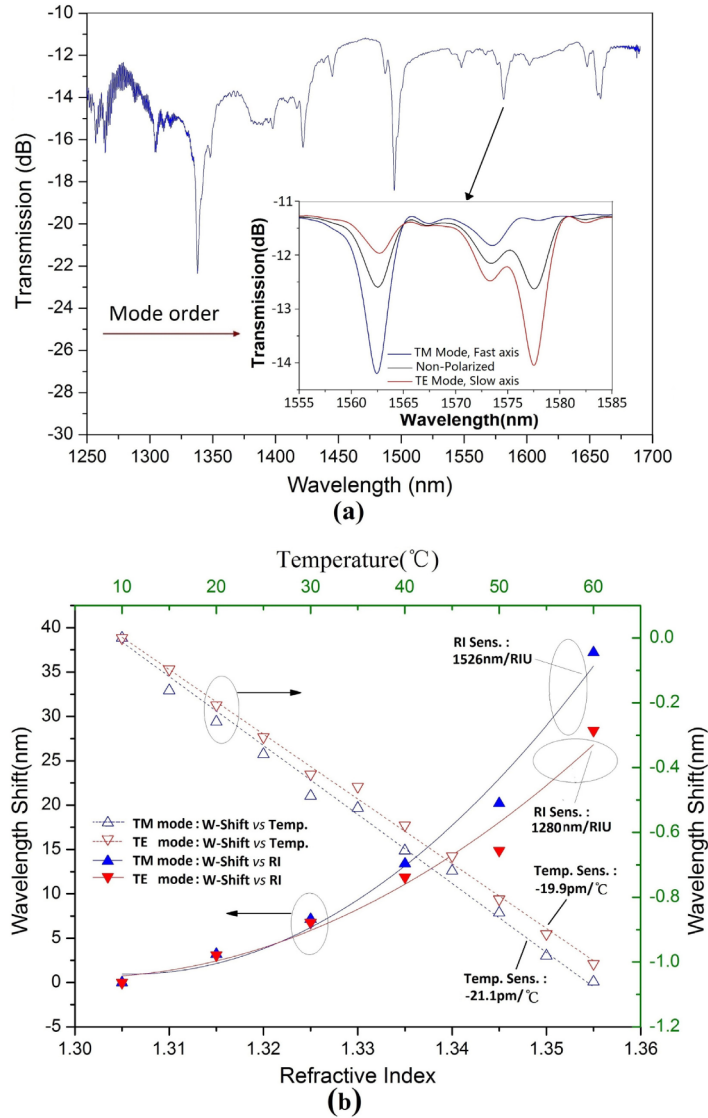


Fig. 2. (a) Transmission spectrum of the Ex-TFG in TCOF. Inset: Zoomed a pair of resonances showing excitation of both TM or TE mode, and equal-polarized light. (b) Experimental results of RI and temperature sensing using a pair of resonances of Ex-TFG in TCOF excited by the light with TM and TE polarization at 1555nm~1585nm in the RI range from 1.305 to 1.355.

3 Surface modification for Ex-TFG in TCOF

3.1 Enzyme immobilization

3-Aminopropyltriethoxysilane (APTES), specific enzyme (i.e., GOD, biological source: fungus *Aspergillus niger*), D-Glucose (>99.5%), acetic acid (AA), sodium acetate (SA), sodium dihydrogen phosphate (SDP), disodium hydrogen phosphate (DHP) were purchased from Sigma-Aldrich Company. The AA and SA were used to prepare buffer solutions with pH 5.2~5.6 while the SDP and DHP were used to prepare buffer solutions with pH 6.0~8.0. All buffer solutions were prepared using de-ionized water from Milli-Q water system. APTES was prepared with concentration of 10%v/v in ethanol solution. The buffer solutions were used to solubilize the enzyme (i.e. GOD) to the concentration of 10mg/ml.

The Ex-TFG in TCOF was initially immersed in HNO_3 solution (5% v/v) for ~2h at ~40°C to remove the contamination and then thoroughly washed by de-ionized water and ethanol. The cleaned TCOF was then immersed in the H_2SO_4 solution (95% v/v in H_2O_2) for ~1h at room temperature to activate the hydroxyl-groups (i.e. '-OH') on the glass surface followed by drying in a convection drying box for ~12h at ~60°C. Then we used the prepared APTES solution to incubate the -OH activated TCOF for ~40 min at room temperature for the silanized process, in which the APTES molecules would assemble to the -OH groups of the glass surface and form free -NH₂ groups on it. Afterwards, the fiber was washed by de-ionized water and ethanol to remove non-covalently bonded silane compounds until the resonance wavelength of the sensor move to a stable point. Finally, the immobilization of GOD molecules on the fiber surface was realized by immersing the silanized TCOF into the prepared GOD solution for 2h incubation, in which the GOD's -COOH groups would bind with the available -NH₂ groups on the surface of the silanized TCOF through covalent interaction. Subsequently, the enzyme-immobilized fiber was washed with buffer solution and de-ionized water again and dried in the air.

3.2 Certification of enzyme immobilization

The confocal microscopy was used to examine every step of the fiber surface modification. Figures 3(a)-3(c) show the micro images of the Ex-TFG in TCOF observed by confocal microscopy (40×) after cleaning, silanization and GOD-immobilization process, respectively. It is clearly shown in Fig. 3(b) that the surface of the grating has been covered by a silane layer. However, there are on obvious differences before and after the GOD immobilized on the sensor surface (see Fig. 3(b) and Fig. 3(c)), here we utilize the intrinsic fluorescent property (excited wavelength at 458nm) of the enzyme to check the effect of the enzyme immobilization. A fluorescent microscope with broad-band light source (containing light with wavelength of 458nm) was used to excite the non-GOD-immobilized and GOD-immobilized TCOFs and the results showed that there were no change in former case but the strong glowing fluorescence can be seen in the later one, as shown in Fig. 3(d), indicating sufficient GOD molecules have been immobilized on the surface of the TCOF.

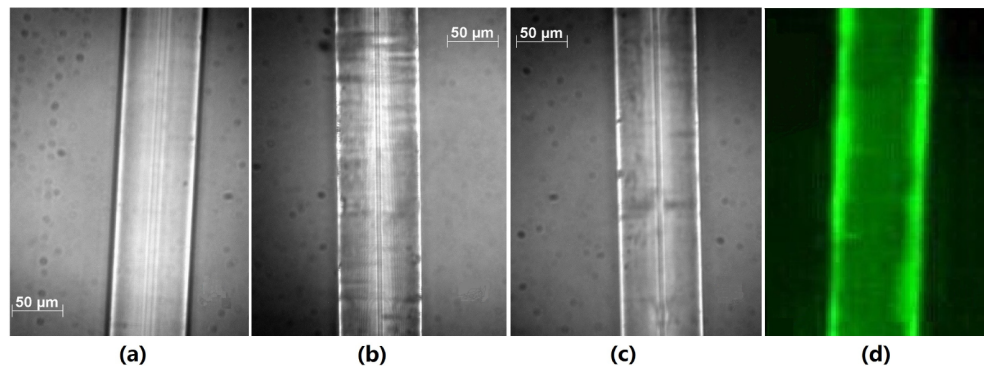


Fig. 3. Micro image of the (a) cleaned TCOF, (b) silanized TCOF, (c) GOD-immobilized TCOF, and (d) fluorescence of the GOD-immobilized TCOF.

In addition, the spectrum evolution of the Ex-TFG in TCOF was monitored in situ throughout the modification process. It should be mentioned here that because the RI of buffer solution used in all the tests was >1.355, which is higher than the cutoff RI of some cladding modes, so we only see the resonances below the cutoff condition. As shown in Fig. 4(a), those cladding modes with resonance wavelengths larger than 1400nm had disappeared when the sensor was immersed in buffer solutions and only two resonances left with the strongest one centered at ~1380nm. The RI sensitivity of the TM mode at ~1380nm was measured for an RI range from 1.345 to 1.370 and it reached ~1168nm/RIU near the RI of ~1.370, as given in Fig. 4(b). The inset of Fig. 4(a) shows the spectra of the TM mode (at ~1380nm) of the bare,

silanized and GOD-immobilized Ex-TFGs as immersed in a blank AA/SA buffer solution (pH 5.2). It can be seen clearly from this inset that the silanized grating has a small but the GOD-immobilized grating has a more significant red-shift compared with the spectrum of the bare grating. This is as expected because the adsorption of additional layer on the fiber surface will change the effective RI (i.e. $n_{eff_cl, m}$, see Eq. (1)) of the cladding mode. Due to the molecular weight (as known to be 156,000) of GOD is much larger than that (only 221.37) of the APTES, thus the GOD layer would impose a relatively large change in resonance wavelength ($\sim 2.386\text{nm}$) compared to that ($\sim 0.534\text{ nm}$) by the silane layer.

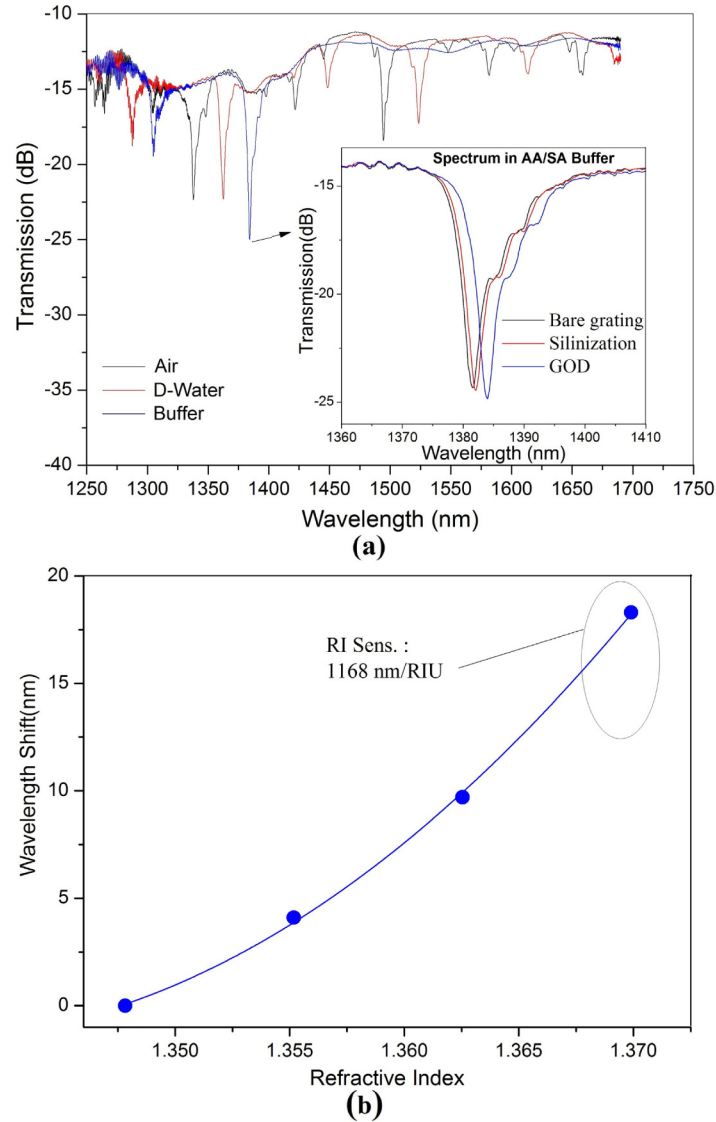


Fig. 4. (a) Transmission spectra of the Ex-TFG in TCOF when exposed in air, immersed in de-ionized water and in blank AA/SA buffer solution (pH 5.2). Inset: spectrum evolution of the TM mode (at $\sim 1380\text{nm}$) of the bare, silanized and GOD-immobilized Ex-TFGs as immersed in a blank AA/SA buffer solution (pH 5.2). (b) Experimental RI sensing properties of the TM mode (at $\sim 1380\text{nm}$) in the RI range from ~ 1.347 to ~ 1.370 .

4 Experiments and discussion

4.1 Experimental setup and measuring principle

The experimental setup to investigate the grating spectral responding to the glucose concentration is shown in Fig. 5, and the inset depicts the chemical link mechanism of GOD on the TCOF surface. Light from a broadband source (BBS, Agilent-83437A, 1200~1700nm) was launched into the fiber and the transmission spectrum was recorded by an optical spectrum analyzer (OSA, AQ-6370B) with a resolution of 0.02nm. A polarizer and a polarization controller (PC) were connected by the SM28 fiber between the BBS and the Ex-TFG in TCOF to adjust and maintain the sensor to work completely in the TM mode (i.e. fast-axis light), since the RI sensitivity of which is larger than that of TE mode (see Fig. 2(b)). The Ex-TFG sensor in TCOF was connected to the SM28 fiber using the fusion splicer and laid inside a sample cell. In every test, the sample cell was sealed to avoid the evaporation of the glucose solution, since which could obviously change the concentration of the glucose solution during the chemical reaction, leading to the spectral of the sensor to red shift. For comparison, a non-modified Ex-TFG in TCOF was also subjected to the evaluation.

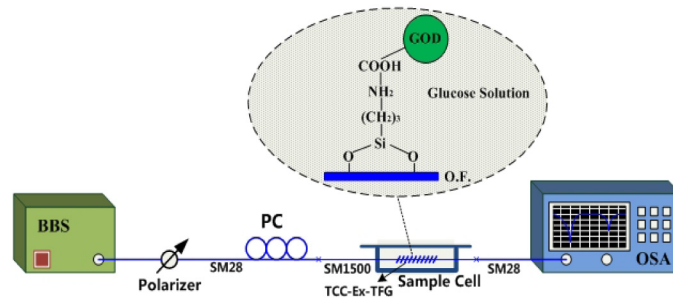


Fig. 5. Experimental setup to detect the glucose concentration by using the GOD-immobilized Ex-TFG in TCOF. Inset: the chemical link mechanism of GOD on the TCOF surface.

The selective detection for glucose is based on the principle that the specific enzyme (i.e. GOD) immobilized on the surface of the sensor will cause the glucose (molecular weight = 180.6) to convert to the gluconic acid (molecular weight = 192.6) during the synthesis process, as shown in Eq. (2), leading to a relatively large RI change even in a dilute (e.g. 0.1~2.5mg/mL) glucose solution, which will in turn, cause a relatively remarkable change in its effective RI of the cladding modes and thus resulting in a detectable shift of the resonance wavelength of the sensor.



In the first set of the glucose detection experiments, the AA/SA buffer solution with pH value of 5.2 was used to configure a set of D-Glucose solutions with concentration of 0.1~5.0mg/ml. Before the test, these D-Glucose solutions were kept for ~24h at room temperature to achieve an equilibrium status (containing two-third β -D-Glucose and one-third α -D-Glucose). Firstly, a blank AA/SA buffer solution (pH 5.2) was introduced in the sample cell, and the observed resonance wavelength of the GOD-immobilized Ex-TFG in TCOF was recorded as the reference. Then the prepared AA/SA buffered solutions (pH 5.2, phase volume = 1mL) of D-Glucose with different concentrations (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mg/ml) were introduced into the sample cell in turn. After each test, the de-ionized water was used to wash the sensor several times to guarantee its resonance wavelength moving back to the reference point.

4.2 Experimental results and discussion

The evolution of the spectrum of the GOD-immobilized Ex-TFG in TCOF varied with the glucose concentration from 0.1 mg/ml to 2.5 mg/ml is shown in Fig. 6(a), indicating that the

spectrum red shift has a good linear relation to the glucose concentration with 0.1~2.5mg/ml. The linear fitting shows that the detecting sensitivity for the glucose concentration (0.1~2.5mg/ml) is $1.514\text{nm}\cdot(\text{mg/ml})^{-1}$ with an R-square 0.96, as shown in Fig. 6(b). The average Q-factor of the sensor, defined by the working wavelength versus FWHM, is calculated to be $\sim 1385\text{nm}/\sim 3\text{nm} \approx 461$ in the same glucose concentration range. In addition, we can see from Fig. 6(b) that a relatively large red shift of resonance wavelength occurred for the first and second test (i.e. the glucose concentration of 0.1 mg/ml and 0.5 mg/ml), however, the red shift of the resonance wavelength had gradually decreased as the glucose concentration changing from 3.0mg/ml to 4.0mg/ml and become nearly unchanged in the concentration range from 4.0mg/ml to 5.0mg/ml. The above results could be explained by that the red shift of the spectrum is related to the change of average RI within the evanescence-field area surrounding the sensor surface, which is closely depended on the number of GOD molecules linked on the fiber surface and the degree of the catalytic reaction (see. Equation (2)) surrounding the fiber surface, so according to Eq. (1) and (2), the more D-Glucose molecules (i.e. the higher the glucose concentration) exist in this evanescence-field area, the greater change of $n_{\text{eff-cl},m}$ will be, then resulting in a greater red shift of the resonance wavelength. Therefore, in the first and second test (i.e. the glucose concentration of 0.1 mg/ml and 0.5 mg/ml), we can see a relatively large red shift due to the sufficient catalytic reaction surrounding the fiber surface. However, as the test number increased, knowing that the activated grating length and fiber diameter is only $\sim 10\text{mm}$ and $\sim 80\text{ }\mu\text{m}$, respectively, thus when the glucose concentration exceeded 2.5mg/ml, there were not enough active enzymes to support a complete oxidation of the glucose molecules, leading to a gradual decrease in the degree of catalytic reaction, thus reducing the red-shift of the resonance. While for the non-modified Ex-TFG in TCOF, it can be seen from Fig. 6 (b) (red triangle) that the variation of resonance wavelength shows no relation to the glucose concentration and the maximum wavelength shift is only $\sim 0.65\text{nm}$ compared with the reference point, manifesting that the red shift of the spectrum for the GOD-immobilized Ex-TFG in TCOF must be induced by the catalytic reaction on the glass surface.

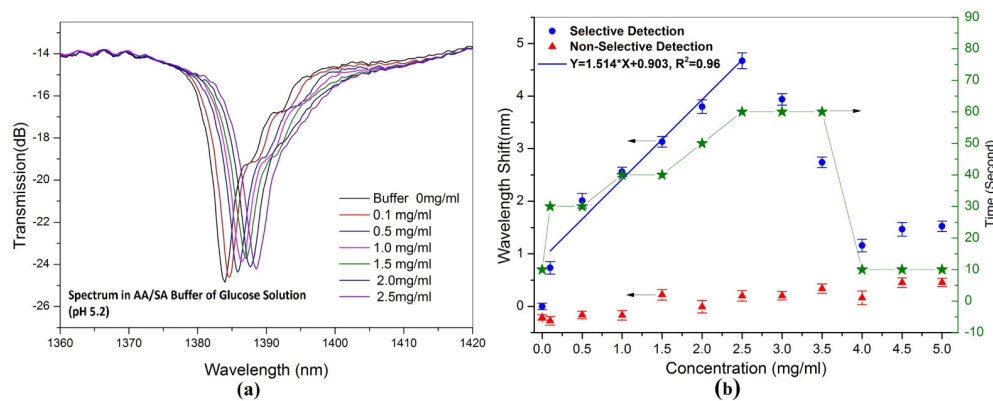


Fig. 6. (a) Spectrum evolution of the Ex-TFG in TCOF with the glucose concentration from 0.1mg/ml to 2.5mg/ml with pH 5.2. (b) Resonance wavelength shift against glucose concentration from 0.1mg/ml to 5.0 mg/ml by using GOD-immobilized sensor (blue circle) and non-modified sensor (red triangle), and the respond time (green star) against glucose concentration by using GOD-immobilized sensor.

The respond time to gain a stable optical spectrum was also investigated in the experiment, as shown in Fig. 6(b) (green star). It can be seen from the figure that the response time for the catalytic reaction increases from $\sim 30\text{s}$ to $\sim 1\text{min}$ as the glucose concentration varying from 0.1 mg/ml to 3.5mg/ml. This is probably due to the fact that the amount and activity of GOD molecules on the fiber surface gradually decreased as the increase in the test number, thus gradually causing a relatively longer period for the oxidation of the D-glucose

molecules surrounding the sensor surface. However, the response time falls back to ~10s again as the test forward to the higher glucose concentrations (4.0~5.0mg/ml), indicating that in this concentration range the catalytic reaction nearly stopped due to the complete consumption of the active enzyme (i.e. GOD) molecules on the fiber surface.

In order to evaluate the repeatability of the sensor, the GOD and silane layers on the TCOF surface were thoroughly removed by using the H₂SO₄ solution (95% v/v in H₂O₂) and HNO₃ solution (20%v/v). Then the above described experiment was conducted again for several times, and the observed average variation in the resonance wavelength shift was only $\sim \pm 0.2\text{nm}$, indicating a good repeatability for the enzyme-modified Ex-TFG in TCOF based glucose sensors.

Other two aspects were also investigated, one was the impact of pH value of the test solution on the sensor response, and another was the detection accuracy varying with pH value. In the experiment, AA/SA and SDP/DHP were used to configure lower pH (5.2~5.6) and higher pH (6.0~8.0) buffer solutions, respectively. Then, the experiments described above were conducted to investigate the performance of the sensor in the pH range of 5.2~8.0. And the results are shown in Fig. 7(a) (blue circles), indicating that the response of the sensor are better in the pH 5.2~7.0 than in pH >7.0 with the best response point at around pH 5.6. This matches the fact that the optimum pH value of the GOD product we used is at ~5.5 and has an activity range of pH 4.0~7.0, therefore the higher pH (>7.0) would reduce its enzyme activity, causing an observably recession (~77% at pH 8.0) in the red shift of the resonance wavelength. However, the sensitivity of the sensor is still as high as $\sim 1.03\text{ nm}\cdot(\text{mg/ml})^{-1}$ at blood pH (~7.4), which is still better than the recently reported LPG based glucose sensor with sensitivity of $\sim 0.806\text{ nm}\cdot(\text{mg/ml})^{-1}$ [21].

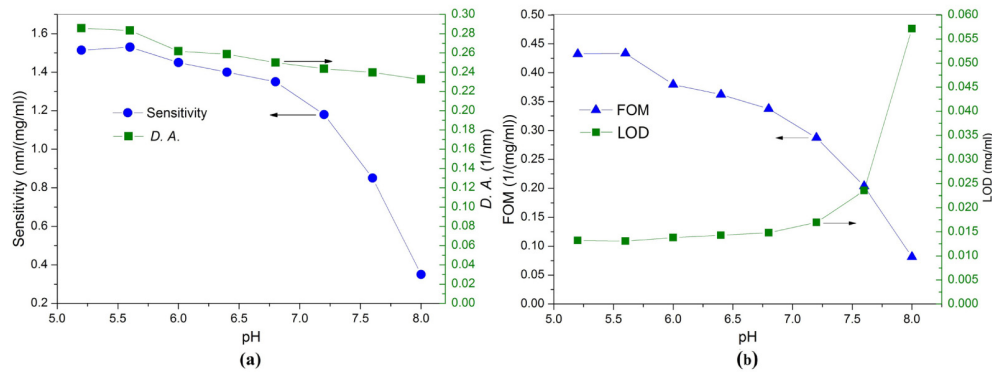


Fig. 7. (a) Variations of the sensitivity (blue circle) and detection accuracy (green rectangle) of the GOD-immobilized Ex-TFG sensor with the pH of the buffer solution of glucose changing from 5.2 to 8.0. (b) Variation of the FOM (blue triangle) and LOD (green rectangle) of the GOD-immobilized Ex-TFG sensor with the pH changing from 5.2 to 8.0 as the glucose concentration range being 0.1~2.5mg/ml.

In this study, the detection accuracy of the sensor is defined as

$$D.A. = 1/\delta\lambda. \quad (3)$$

where, $\delta\lambda$ is the spectral width of the resonance spectrum corresponding to 50% normalized transmitted power (i.e. FWHM), obviously, the narrower the spectral width is, the higher the D.A. of the sensor will be. The detection accuracy was meanwhile determined in this pH range against a constant glucose concentration of 2.5mg/ml, and the result of which is also shown in Fig. 7(a) (green rectangle), manifesting that the D.A. of the sensor decreases from $\sim 0.2857\text{ nm}^{-1}$ to $\sim 0.2325\text{ nm}^{-1}$ as the increase in the pH value (from 5.2 to 8.0) of the test solution. Since the average molecular weight of SDP/DHP is larger than that of the AA/SA, and it is also comparatively larger in the SDP/DHP solution with a relatively high pH value, thus leading to the $\delta\lambda$ of the resonance spectrum to grow wider. However, the variation of $\delta\lambda$

is not large, which increased only from ~3.5nm to ~4.3 nm as the pH changing from 5.2 to 8.0 in the case of glucose concentration being 2.5mg/ml, thus only resulting in ~19% decay (at pH 8.0) in the *D.A.* of the sensor.

In order to depict the combination property of the sensor, FOM (figure of merit) should be used [23]. In our study, FOM is defined as

$$FOM = S/FWHM = S/\delta\lambda = \text{Sensitivity} \cdot D.A.. \quad (4)$$

A higher FOM shows a better sensing property of the sensor. Calculating by using the experimental results in Fig. 7(a), the FOM of the sensor is plot in Fig. 7(b) (blue triangle), indicating that the FOM decreased as the pH increased, and at the blood pH (~7.4), the FOM will reduce by ~40% compared to its' max value (i.e. ~0.43 (mg/ml)⁻¹ at pH 5.6).

The LOD of the sensor for the glucose concentration should be evaluated as well. LOD could be determined by the spectral resolution of OSA versus the detection sensitivity. In the study, the resolution of the OSA is 0.02nm, then according to the sensitivities in Fig. 7(a), the LOD of our sensor varies with pH could be calculated and the results are shown in Fig. 7(b) (green rectangle), manifesting that the LOD is as low as 0.013~0.02mg/ml at the pH 5.2~7.4 as the glucose concentration range being 0.1~2.5mg/ml, which is sufficient for the clinic application.

Knowing that the basic principle of our sensor for the glucose detection is similar to that of the recently reported LPG based [21], OF-SPR based [22] and Ex-TFG in standard SMF based [19] glucose sensors, that is, they have used the specific enzyme (i.e. GOD) to modify the sensor surface for the selective detection of glucose, thus for more detailed comparisons among them, their sensing parameters are summarized in Table 1, showing that the measurement range of glucose concentration are nearly the same for these types of sensors, but the average Q-factor and *D.A.* of our sensor are far better the that of the former two types.

Table 1. Comparison of the Sensing Parameters among LPG based, OF-SPR based, Ex-TFG in standard SMF based and Ex-TFG in TCOF based Glucose Sensors

Parameter	LPG [21]	OF-SPR [22]	Ex-TFG in Standard SMF [19]	Ex-TFG in TCOF
Fiber Material	Silica	multimode plastic clad silica (PCS)	Silica	Silica
Active Length (mm)	20	10	10	10
pH value	5.6	7.4	5.2	5.2
Temperature Sensitivity (pm·°C ⁻¹)	~300	-	~5.30	~20
RI Sensitivity (nm·RIU ⁻¹)	~50~100	>2000	~168	~1168
FWHM (nm)	~20.0	~100.0	~4.5	~3.0
Average Q-factor	~80	~6.5	~352	~461
Measurement Range (mg·ml ⁻¹)	0.1~3.0	0.0~2.6	0.0~3.0	0.1~2.5
Glu Sensitivity (nm·(mg/ml) ⁻¹)	0.806	~13.0	0.298	1.514
<i>D.A.</i> (1/nm)	~0.050	~0.009	~0.2222	~0.2857
R ²	0.92	-	0.99	0.96

As discussed above, the response of the glucose sensor is closely related to the amount of the active GOD molecules immobilized on the fiber surface. Knowing that the fiber cladding diameter and the active length are only ~80μm and ~10mm, respectively, since the former is a fixed parameter, therefore, theoretically speaking, we can fabricate and bio-functionalize a longer Ex-TFG in TCOF to enhance the effective quantity of the GOD molecules on its surface, thus improving its sensitivity and measurement range for glucose detection.

For purpose of evaluating the special detection for the blood glucose and practical utility of the proposed GOD-immobilized Ex-TFG sensor in TCOF, we used it to analyze the glucose content in the clinically analyzed plasma samples provided by local hospital. Firstly, a

plasma sample (phase volume = 1mL) with known glucose concentration of 0.98mg/ml was analyzed to record a reference optical spectrum, and then a set of plasma samples with known glucose concentration from 1.09mg/ml to 2.72mg/ml were analyzed to obtain a calibration curve. Subsequently, the calibrated glucose sensor was thoroughly cleaned by using the H₂SO₄ (95% v/v in H₂O₂) and HNO₃ solution (20%v/v), and then was modified with the GOD again using the same surface modification process. Similarly, the reference optical spectrum was record by analyzing the same plasma sample (glucose concentration of 0.98mg/ml). Finally, several plasma samples (phase volume = 1mL) of diabetic patients were tested by the GOD-immobilized sensor, respectively. The glucoses concentration was calculated from the calibration curve. The results are shown in Table 2 (4th Column), indicating a close agreement with that measured by the biochemical method bone by local hospital (2nd Column).

Table 2. Glucose Concentration of Plasma Samples Analyzed by the GOD-immobilized Ex-TFG in TCOF

Sample	Glucose concentration as hospital test (mg/ml)	Wavelength shift versus the reference optical spectrum (nm)	Estimated glucose concentration calculated by the calibration curves (mg/ml)	Variation to the hospital test (%)
1	1.40	1.42 ± 0.02	1.45	+ 3.57
2	1.75	2.13 ± 0.03	1.81	+ 3.42
3	2.53	2.75 ± 0.02	2.47	-2.37

5. Conclusions

The fabrication and characterization of a glucose sensor based on an Ex-TFG inscribed in TCOF (~4μm/~80μm) have been carried out. The selective detection of glucose is based on the catalytic reaction involving GOD immobilized on the sensor surface through covalent link mechanism. The sensor has clearly demonstrated the advantages of high sensitivity, extremely low temperature cross-talk, high detection accuracy (high Q-factor), easy fabrication and repeatability. By analyzing the glucose content in the plasma samples, feasibility of the proposed GOD-immobilized Ex-TFG inscribed in TCOF for the selective detection of blood glucose was verified, which makes it compatible for practical application. Theoretically, its detection sensitivity for glucose concentration can be further improved by increasing the effective grating length. Combining all these advantages, the proposed Ex-TFG in TCOF could be a good alternative biosensor platform to be applied in various application fields such as disease diagnosis, microbial pathogen detection, life science, food safety control and environmental monitoring.

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