

Label-free detection of breast cancer cells using a functionalized tilted fiber grating

XIAOYONG CHEN,^{1,4,6} PIN XU,^{2,6} WENWEI LIN,² JIN JIANG,³ HANG QU,² XUEHAO HU,²  JINGHUA SUN,¹  AND YUKUN CUI^{3,5}

¹School of Electrical Engineering and Intelligentization, Dongguan University of Technology, Dongguan 523808, China

²Department of Physics, College of Science, Shantou University, Shantou, Guangdong 515063, China

³Guangdong Provincial Key Laboratory of Breast Cancer Diagnosis and Treatment, Shantou University Medical College Cancer Hospital, Shantou 515041, China

⁴xychen@dgut.edu.cn

⁵yukuncui@yahoo.com

⁶Equal contributors.

Abstract: The detection of circulating tumor cells (CTCs) still faces a huge challenge partially because of low abundance of CTCs (1-10 cells/mL). In this work, a plasmonic titled fiber Bragg grating biosensor is proposed for detection of breast cancer cells. The biosensor is made by an 18° TFBG with a 50 nm-thick gold nanofilm coating over the surface of the fiber, further immobilized with a specific antibody against GPR30, which is a membrane receptor expressed in many breast cancers, serving as bait. In vitro tests have confirmed that the proposed biosensor can detect breast cancer cells in concentration of 5 cells/mL within 20 minutes and has good linearity in the range of 5–1000 cells/mL, which has met the requirement of CTC detection in real conditions. Furthermore, theoretical analysis based on the experimental results shows that the limit of detection can even reach single-cell level. Our proposed biosensor has a simple structure, is easy to manufacture, is of small size, and has a good performance, making it a good choice for real-time, label-free, and milliliter-volume detection of cancer cells in future.

© 2022 Optica Publishing Group under the terms of the [Optica Open Access Publishing Agreement](#)

1. Introduction

Breast cancer is one of the most common cancers in women and the second leading cause of cancer death worldwide, after the lung cancer [1]. The techniques of early diagnosis are gradually developing for assisting the treatment and improving more chances of survival. Nowadays, the main methods for diagnosis of breast cancer are mammography, magnetic resonance imaging (MRI), ultrasound and positron emission tomography (PET-scans). However, such methods require expensive instruments and the diagnostic cost is high. To solve these problems, a noninvasive in-vitro diagnostic method, through detection of circulating tumor cells (CTCs), has been proposed in recent years [2]. Because of the low concentration (1-10 cells/mL in peripheral blood), CTCs detection requires the biosensor with high detection accuracy and low volume sample sensing. Although there have been numerous attempts in previous work, the complete industrialization of medical diagnosis utilizing CTCs detection is still a difficult and huge project [3].

Among all of the available technologies, fiber-optic sensors, especially the tilted fiber Bragg gratings (TFBGs), have been intensively studied for biomedical detection at a low concentration of analytes, owing to multitudinous advantages, such as the small volume, immunity from electromagnetic fields, biocompatibility and high sensitivity [4–7]. The TFBG, which is with periodic refractive index modulation in the doped single-mode optical fiber core, simultaneously possesses advantages of fiber Bragg grating (to measure the temperature) and long-period grating (to measure the surrounding refractive index). The excited cladding modes, observed as a

high-density comb of narrowband spectral resonances with Q-factor of 10^4 in the TFBG spectrum [8], can provide an outstanding sensitivity and a low limit of detection (LOD) for biomedical detection. Furthermore, the core mode in the TFBG spectrum can be used for calibrating the temperature and power fluctuation-induced cross-sensitivities, thus improving the measured accuracy [9]. These properties make TFBG an ideal option fitting with the aforementioned objective.

To further improve the sensor sensitivity, surface plasmon resonance (SPR) technology is applied in the TFBG. The SPR can be observed as an absorption area in the TFBG spectrum, and any perturbations near the metallic surface, such as binding of biomolecules, can be detected using a TFBG-based SPR (TFBG-SPR) sensor. To date, TFBG-SPR sensors have been demonstrated for biomedical and biochemical applications with label-free detection of analytes at very low concentrations, involving but not in all, a cortisol biosensor (sensitivity $0.275 \pm 0.028 \text{ nm/ng.mL}^{-1}$) [10], a cadmium ions sensor (LOD 1 ppb) [11], a glucose detector (LOD 295 pM) [12], a mercury Ions detector (LOD 3.073 pM) [13], an urinary protein biosensor (LOD 1.5 ng/mL) [14], a calmodulin biosensor (LOD 0.44 nM) [15], a thrombin molecule biosensor (LOD 2.5 nM) [16], an AdoHcy biosensor (LOD 1nM) [17], a breast cancer biomarker biosensor (LOD 10^{-12} g/mL) [18], a cytokeratin biosensor (LOD 14 pM) [19], and a hydrogen sensor (LOD 180 ppm) [20]. The aforementioned applications are especially relevant to the detection of cancerous cells whose presence at a low concentration requires a large quantity of samples and labeling detection. Furthermore, the compact size of fiber-optic sensors, especially the TFBG-SPR sensors, enables small-volume detection.

To detect cancerous cells, the biomolecules immobilized on the sensor surface severed as receptors should be specificity to the targets. Based on this principle, many fiber-optic sensors have been proposed, including an Ω -shaped fiber sensor (LOD 12 cells/mL) [21], a multi-resonant optical fiber (LOD 49 cells/mL) [22], a 2D materials-based fiber-optic SPR biosensor (LOD 3×10^{-5} RIU) [23], and a long period fiber grating biosensor (Sensitivity 1700nm/RIU) [24]. Of course, there are also other methods, such as localized SPR (LSPR, LOD 2 cells/mL) [25,26], electrochemistry (LOD 5 cells/mL) [27], fluorescence (LOD 10 cells/mL) [28] and SERS [29], studied for cancerous cells detection, providing performances comparable to or better than the fiber-optic method but at the price of complex system.

Given to the inspiration from the biosensor proposed by Loyez et al. [22] and the great specificity of antibody GPR30 to the breast cancer cell lines BT549 [30], we propose and demonstrate a label-free biosensor for BT549 detection in this work. The sensor was made by a plasmonic TFBG with a surface functionalization of GPR30. To evaluate the performance of the sensor, the LOD and linear response range were studied as well as the selectivity. Our sensor can be fabricated without breaking the structural integrity of the fiber, ensuring the sensing stability and reproducibility. Moreover, the core mode in the TFBG spectrum can be used calibrating the temperature and power fluctuations-induced cross-sensitivities, improving the measured accuracy. The good performances as well as other merits, such as simple structure, easy to manufacture and small size, make our sensor a good option for in-situ, label-free and small-volume detection of cancerous cells.

2. Materials and methods

2.1. Materials

The phosphate buffer saline (PBS) was purchased from Beijing Solarbio Science & Technology Co., Ltd., Beijing, China. The 11-mercapto-undecanoic acid, 1-ethyl(3-dimethylaminopropyl)-carbodiimide (EDC), and N-hydroxy-succinimide (NHS) were purchased from Hefei Bomei Biological Engineering Company, Anhui, China. The Rabbit GPR30 (42 kDa) antibody and fetal bovine serum (FBS) were provided by Guangdong Provincial Key Laboratory of Breast Cancer Diagnosis and Treatment, Shantou University Medical College, Shantou, China. The human

breast cancer cell lines BT549, MCF-7 and SKBR3 were obtained from ATCC (Manassas, VA, USA) and cultured according to the instructions of the manufacturer. These cells were cultured in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10% FCS [30]. The cells were detached by trypsinization and resuspended in culture medium and counted with TC20 automated cell counter (BIO-RAD, Singapore), then diluted in the FBS solution to the concentration as described before experiment. Figure 1(a) shows that our experimenter was working on the cell culture, and Fig. 1(b) is the TC20 automated cell counter used for counting the cancerous cells.

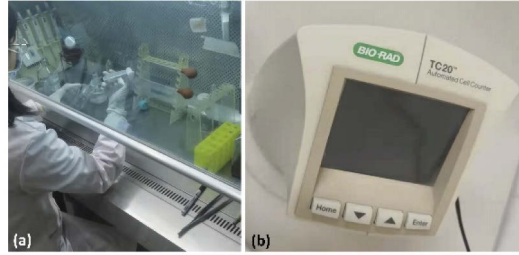


Fig. 1. (a) Experimenter works on the cell culture; (b) TC20 automated cell counter.

2.2. Fabrication of plasmonic TFBG

We used a scanned phase-mask technique [31], as shown in Fig. 2, in a germanium-doped silica fiber (which can also provide a good photosensitivity in the fiber core) without hydrogen process to fabricate the TFBG. Firstly, we fixed the fiber in the position very close to the phase-mask, and then we cylindrically focused a 193 nm pulsed ultraviolet light from an ArF excimer laser (with a power of 3 mJ per pulse and frequency of 200 Hz) through the ± 1 diffraction order phase-mask and onto the fiber. Secondly, we spatially scanned the beam, using an automated micro-displacement platform, through the phase mask and along the fiber, both of which were tilted at an angle θ relative to the direction of the incident beam, and then we successfully inscribed a desired tilt grating into the fiber core within a very short length (< 2 cm). The phase-mask was angled to select the tilt angle of the grating's refractive index planes in the fiber. The resonant wavelength, $\lambda_{clad,i}$, can be written as Eq. (1). Given to our previous study [31], the best spectrum for measurement of refractive index of ~ 1.34 is the TFBG with tilted angles between 15° and 20° . In this work the RI of the solution was ~ 1.34 , therefore we chose a 18° TFBG for measurement.

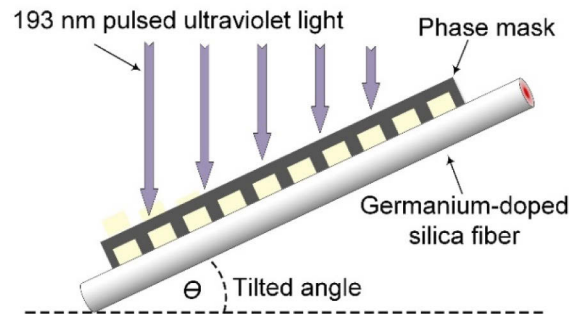


Fig. 2. Schematic diagram of phase-mask technique for TFBG inscription in an optical fiber.

To further improve the sensor sensitivity in measuring perturbations near the fiber surface, a 50 nm-thick gold film was deposited on the fiber surface, through the magnetron sputtering method, to excite surface plasmonic resonance. It should be pointed out here that the time for depositing a gold mirror (> 200 nm) on the TFBG end was 5 minutes, and that for a gold layer around the TFBG was 30 seconds. We usually made the gold mirror first, and during this deposition process a capillary was used to protect the fiber surface from gold deposition. After that we removed the capillary and deposited a 50 nm-thick gold layer over the fiber surface. Figure 3 gives us the morphology and thickness of the gold film over the fiber surface. We can see that the surface is very smooth and the thickness is 48.7 nm, close to the desired thickness of 50 nm. Moreover, a gold mirror was deposited on the downstream of the TFBG to make the sensor work as a reflective probe (as Fig. 4 shows), simplifying the operation during experiment. The surface plasmon wave (SPW) can be written as Eq. (2) [32]:

$$\lambda_{clad,i} = (N_{clad,i}^{eff} + N_{core}^{eff})\Lambda \quad (1)$$

$$\beta_{spw} = \frac{\omega}{c} \sqrt{\frac{\epsilon_s \epsilon_m}{\epsilon_s + \epsilon_m}} \quad (2)$$

where N_{core}^{eff} and $N_{clad,i}^{eff}$ represent the effective refractive indices of the input core mode and excited cladding mode i , respectively. Λ is the grating period. ω is the angular frequency of the light, and c is the light velocity in vacuum. ϵ_m and ϵ_s represent, respectively, the complex relative permittivities of the metal film and the surrounding material. According to Eqs. (1) and (2), the SPW can be excited when phase matching condition is met ($\beta_{spw} = \beta_{clad,i} = \omega \cdot N_{clad,i}^{eff}/c$). Any perturbations, such as molecules bonded on the metallic surface, will change the complex relative permittivity of the surrounding material, ϵ_s , thus leading to the wavelength of the SPW drift accordingly.

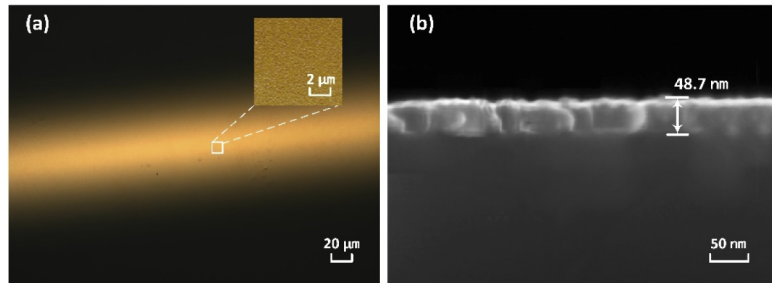


Fig. 3. Surface morphology (a) and thickness (b) of the gold film over the fiber surface.

2.3. Bio-functionalization of the metallic surface

To realize specific detection of breast cancer cells, surface functionalization should be carried out for the plasmonic TFBG. In this work, the GPR30 antibodies were bonded on the metallic surface serving as bio-detectors, as shown in Fig. 4. The surface functionalization includes three steps as follows:

- 1) The plasmonic TFBG was rinsed several times with ethanol and with Milli-Q water for removing the unwanted contaminants on the metallic surface, and then it was immersed in the 11-Mercaptoundecanoic acid solution (1 mM) for 6 hours to generate a self-assembly of a monolayer of mercapto compounds on the metallic surface.

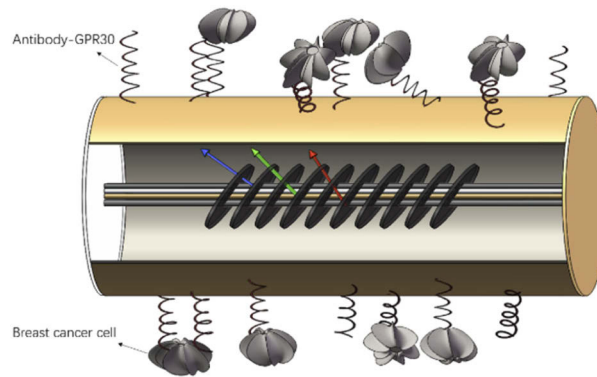


Fig. 4. Schematic diagram of the plasmonic TFBG with functionalized coating.

- 2) The sensor was again rinsed with ethanol and with Milli-Q water for removing the unconnected 11-Mercaptoundecanoic acid, and then it was immersed in a mixed solution which contained 0.5 mL of EDC (0.5 M) and 0.5 mL of NHS (0.2 M) for 30 min to activate the carboxyl on the self-assembly of a monolayer. After that, it was rinsed several times with Milli-Q water to remove the unwanted EDC and NHS.
- 3) The sensor was immersed in the GPR30 antibody solution (20 $\mu\text{g/mL}$, 0.476 μM) for 1.5 hours to bind the GPR30 antibody on the metallic surface. Then, the sensor was immersed in the solution of fetal bovine serum (FBS) for 50 minutes to block the excess carboxyl groups. After that, the biosensor was ready for BT549 breast cancer cells.

3. Experimental system

3.1. Experimental setup

Figure 5 shows the experimental setup, including a broadband source (BBS) with a wavelength range from 1500 to 1620 nm, an in-line fiber polarizer, a 3-paddle fiber polarization controller (PC), an optical spectrum analyzer (OSA) with a resolution of 0.02 nm for monitoring and recording the reflected spectra from the functionalized TFBG, a 1 mL centrifuge tube used as a sample cell for storing the solution of breast cells, and an optical circulator (OC) used for connecting the TFBG to the OSA. The SPW is a transverse magnetic (TM) mode and propagates axially along the metallic surface, that is, only the p-polarized light can meet the matching condition and excite the SPR. In this work, a 3-paddle PC (FPC561, Thorlabs, USA) was used to generate p-polarized light in the experiment. The 3-paddle PC combines a quarter-wave plate, half-wave plate, and quarter-wave plate in series to transform an arbitrary polarization state into any other polarization state. Therefore, we can get the p-polarized light through adjusting each of the three paddles. The inset in Fig. 5 is the practical sample cell together with the fixing device.

Here we should highlight that as all the experiments were done in the ultraclean chamber, which could provide constant temperature and humidity, the temperature-induced cross-sensitivity can be reduced at most. Moreover, the core mode in the TFBG spectrum can be used for calibrating the temperature and power fluctuations induced cross-sensitivities, as the core mode is only sensitive to the temperature but not refractive index. Through referencing the measured results to the initial state of the core mode, we can eliminate temperature and power fluctuations induced cross-sensitivities [33].

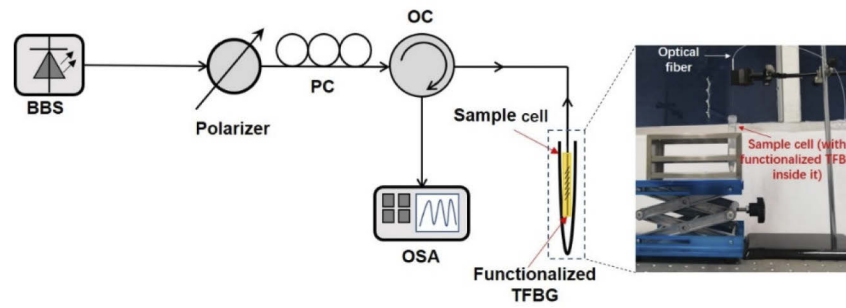


Fig. 5. Block diagram of the experimental setup. The inset on the right side is the practical sample cell together with the fixing device. PC: polarization controller; OC: optical circulator; BBS: broadband source; OSA: optical spectrum analyzer.

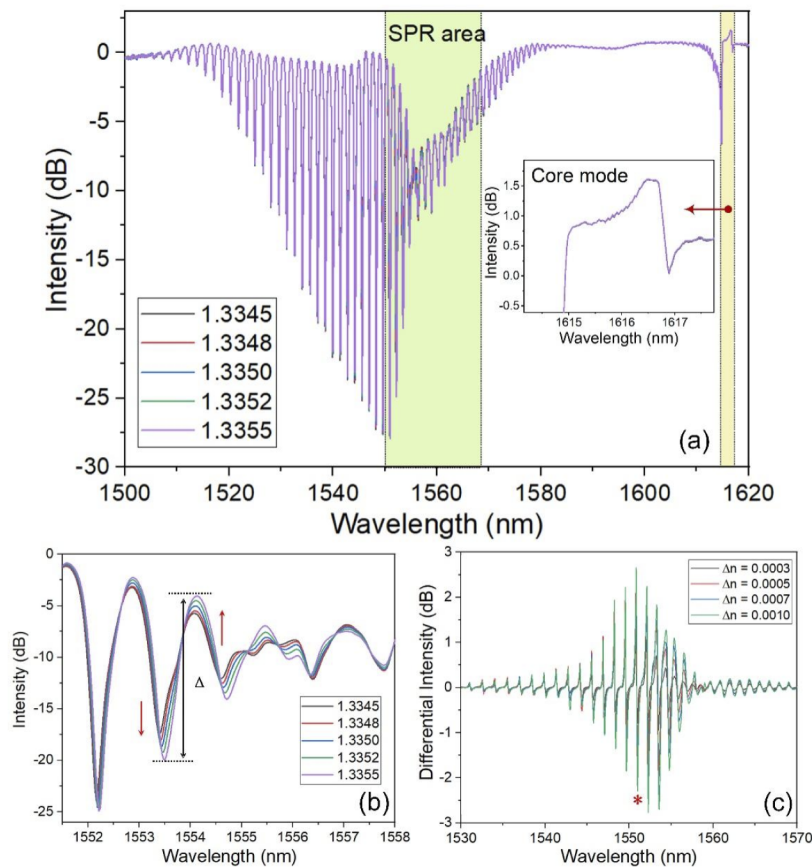


Fig. 6. Working principle of the sensor. (a) Spectral response during measurement. The inset is the zoom-in of the core mode; (b) Zoom-in of the spectrum in the SPR area. (c) Difference method instead of directly reading the spectra used in this work for determining the sensor performance.

3.2. Sensor interrogation

The working principle of our sensor is shown in Fig. 6. The spectral responses were measured with small changes in refractive index. As can be seen, the spectrum in the SPR absorption area, labelled with light green box, varies with the refractive index, and its details can be seen clearly in the zoom-in shown in Fig. 6(b). With the refractive index near the metallic surface increases, the SPR area shift to the long wavelength side. Unlike the conventional plasmonic TFBG biosensor that through measuring a selected cladding mode located in the right side of the SPR center for target biomolecules monitoring [17], here we measure the variation (labelled as “ Δ ” in Fig. 6(b)) between the bottom and the top of the selected cladding mode, which is also called difference method. As the variation trend in the bottom and the top are inverse (as the red arrows in Fig. 6(b) show) during the measuring process, the measuring signal can be efficiently amplified using the difference method compared to the conventional method. Furthermore, we can also eliminate the influences caused by other external factors, such as power fluctuation and fiber-optic devices induced losses, thus improving the measured accuracy. Figure 6(c) shows the spectral responses using the difference method to deal with the data. We can see that, in comparison to the original spectra shown in Fig. 6(a) and Fig. 6(b), the sensing signal is obviously amplified. With the difference method, we can detect breast cancer cells in a low concentration. To simplify the calculation, we select the signal near 1550 nm, labelled with red symbol “*” in Fig. 6(c), for monitoring the detection process.

Here we should point out that if RI variation is larger than 10^{-4} , we can analyze the sensor performance using both wavelength shift and intensity change, as Fig. 6 shows. However, if the RI variation is lower than 10^{-4} , the intensity change is more obvious than the wavelength shift. In this work, when we measured the breast cancer cells with concentration lower than 1000 cells/mL, the RI variation was much lower than 10^{-4} , therefore we chose the intensity variation for measurement. The inset in Fig. 6(a) shows the spectral responses of core mode during the experiment. We can see that it is stable, which means that the experimental system has outstanding stability, and the room temperature also keeps stable during the experiment. The core mode here can be used for calibrating the temperature and power fluctuation induced cross-sensitivities.

4. Results and discussions

4.1. Immobilization of GPR30

In this work, we monitored the process of bio-functionalization of metallic surface through the TFBG spectrum (a selected cladding mode in the SPR absorption area) shown in the optical spectrum analyzer, as detailed in the inset in Fig. 7. The principle can be understood that the intensity of the selected cladding mode decreases with the immobilization of GPR30 on the metallic surface, and the variation trend of intensity will become stable when few of GPR30 can be immobilized on the metallic surface. Hence, through monitoring the intensity variation we can know whether the bio-functionalization is done or not. Figure 7 shows the full process of antibody GPR30 (0.476 μ M) bonded to the metallic surface. It is obvious that the GPR30 was quickly bonded on the surface of the plasmonic TFBG within 20 min, and after that the signal intensity increased slowly and became stable after 50 min, which means that the bio-functionalization of metallic surface was done within 50 min. The inset in Fig. 4 shows the spectral response of the selected cladding mode in real-time. To avoid the influence caused by the excess carboxyl groups on the metallic surface during the detection of breast cancer cells, we immersed the biosensor in the solution of fetal bovine serum (FBS) for 50 minutes to bind the FBS on the excess carboxyl groups, and the results (red circles) are also shown in Fig. 7. The signal intensity gets saturated after 30 min, and the relative variation of intensity is much smaller than that induced by the GPR30. This, on the other hand, means that most of the metallic surface

covers with GPR30. As the FBS cannot interact with the breast cancer cells, the exist of FBS on the biosensor will not affect the detectable results.

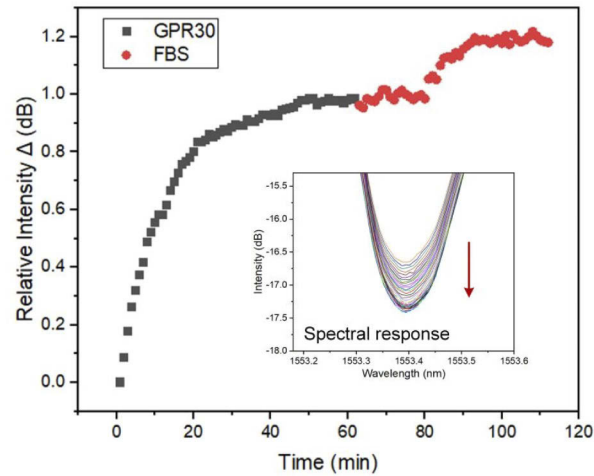


Fig. 7. Intensity variation of the selected cladding mode during the immobilization of GPR30 (black square “■”) on the metallic surface and the FBS (red circle “●”) for encapsulating the excess carboxyl group. The inset shows the spectral response of the selected cladding mode in real-time.

It should be pointed out that the buffer solution for storing the BT549 breast cancer cells in the work is FBS solution, and therefore it is necessary to encapsulate the biosensor with FBS before the detection of breast cancer cells, eliminating the FBS-induced influence. We have also done some experiments to test the performance of the biosensor using BSA instead of FBS to block excess carboxyl groups, and the results were the same. Therefore, to block excess carboxyl groups, either FBS or BSA can be used in this work.

4.2. Detection of breast cancer cells BT549

Before the cancerous cell detection, we used a refractometer (PAL-RI, ATAGO, Japan) to measure the RI values of four different cancerous cell concentrations (5, 10, 100 and 1000 cells/mL), and the measured results are the same, that is 1.3416. This means that it is impossible to distinguish the cancerous cell concentrations based on the RI values. According to the above measurement method, we measured the breast cancer cell BT549 in different concentrations, including 5, 10, 100 and 1000 cells/mL, and the results are shown Fig. 8. We can see, according to Fig. 8(a), that although the variation of intensity caused by the cancerous cell concentration of 5 cells/mL is very small, we can distinguish it from that generated by the FBS solution. Base on the fitting line, the signal becomes stable after 20 min. Figure 8(b) shows a column diagram from a 5-time measurement for both buffer solution and BT549 in concentration of 5 cells/mL. It is obvious that the intensity variation obtained from the solution with BT549 is much larger than that without BT549. Therefore, we can conclude that, in the work, the LOD of our biosensor for breast cancer cell BT549 is 5 cells/mL, and the response time is 20 min. Figure 8(c) presents the measured results including several cancerous cell concentrations. A pronounced variation of intensity, caused by the BT549 immobilized on the GPR30, can be observed when the concentrations of breast cancer cell BT549 are higher than 10 cells/mL. Figure 8(d) shows the linear fit of the measured results in four concentrations. It can be calculated that the linearity is 95.6%, which means that our biosensor has a measured range from 5 to 1000 cells/mL. Figure 9 shows the microscopic views of the biosensor surface before and after the detection. It is clear, as the inset

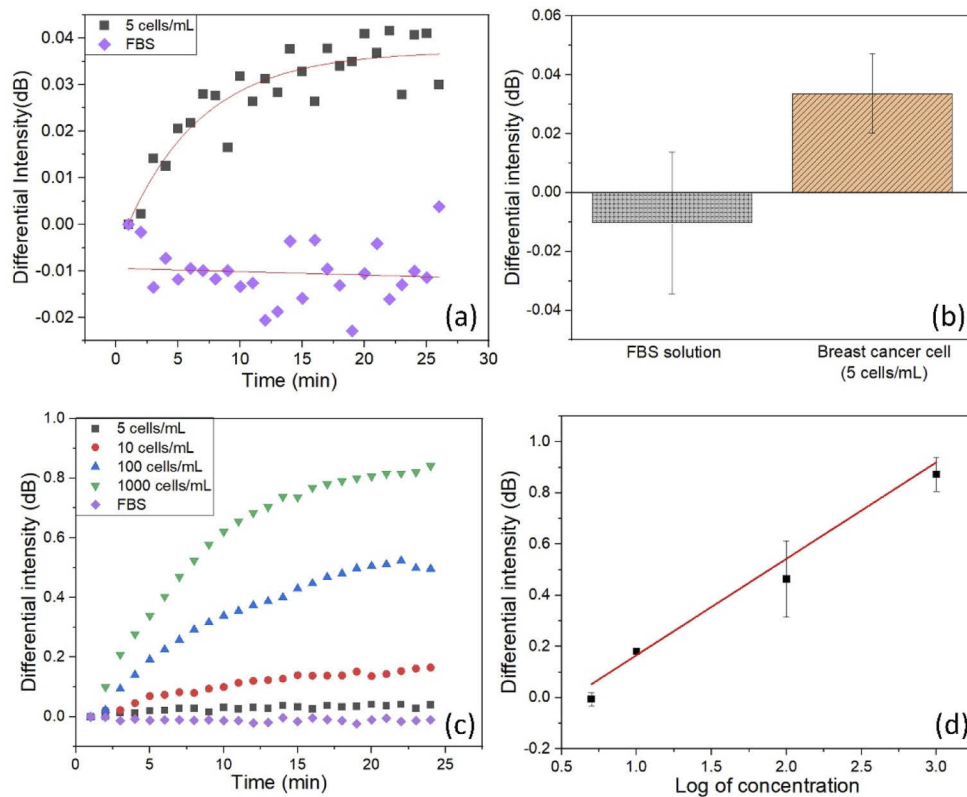


Fig. 8. (a) The detection of cancerous cell BT549 in concentration of 5 cells compared to FBS solution; (b) A 5-time measurement for both FBS solution with and without BT549; (c) Measured results in different concentrations of breast cancer cell BT549; (d) Linear fit ranging from 5 to 1000 cells/mL. The error bar represents the standard deviation from a 5-time measurement.

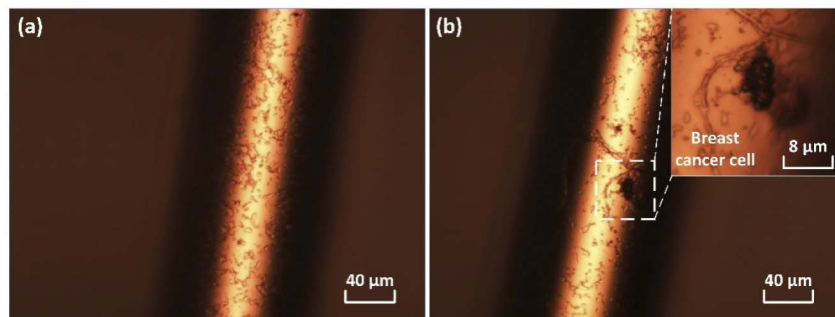


Fig. 9. Microscopic view of the biosensor surface before (a) and after (b) the detection of breast cancer cells. The inset is the zoom-in of a breast cancer cell bonded on the biosensor surface.

in Fig. 9(b) shows, that a breast cancer cell BT549 is attached on the surface of the biosensor after the detection.

It should be pointed out here that Fig. 8(b) and 8(d) are based on a 5-time measurement in the same probe, and we cannot talk about the reproducibility according to these results. However,

our previous work [17] and other reported works [14,22] have shown that the TFBG-SPR sensor possesses reproducibility. The TFBG-SPR sensors fabricated in the same conditions together with the same tilted angle could provide similar spectra and performances in measurements. The functionalization over the metallic surface will determine the specificity and LOD of the TFBG-SPR sensors in biomedical and biochemical measurements.

The theoretical LOD of the functionalized TFBG for breast cancer cell BT549 detection can be estimated by the following equation [34]:

$$\text{LOD} = 3\sigma/S \quad (3)$$

where σ is the standard deviation of the system noise in unit of dB, and S is the sensitivity of the functionalized TFBG in unit of dB/cell (with 1 mL centrifuge tube), which is numerically equal to the slope of the linear fitting in Fig. 8(d). Based on a 5-time measured result shown in Fig. 8(b) and 8(d), it can be calculated the theoretical LOD of our biosensor is 1.3 cells/mL. This means, in ideal conditions, our biosensor for the detection of breast cancer cells could reach almost single-cell level. However, we should point out here that, to reach single-cell detection, a micro-fluidic system is necessary to use for controlling the cell one by one to pass through the biosensor surface, and we will focus on it in our next work.

In order to demonstrate the selectivity, we also use our biosensor to detect two more breast cancer cell lines MCF-7 and SKBR3, and the experimental results shown in Fig. 10 are obtained from 5 independent biosensors. Although the concentrations of both control cells MCF-7 and SKBR3 (10^4 cells/mL) are much higher than the target cell BT549 (10 cells/mL), a significant variation can be observed in the target cell, while the control cells pinpoint a high stability. We can definitely distinguish the target cell from the control cells according to the measured variation of intensity. Therefore, our biosensor shows specificity in measuring breast cancer cell lines BT549. Additionally, the measured results (Fig. 10) from 5 independent biosensors indicate that our biosensor exhibits good reproducibility.

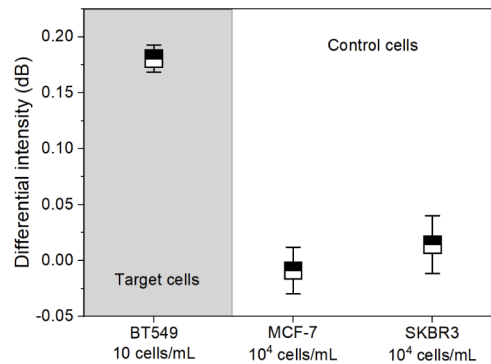


Fig. 10. Measured results of the control cells (MCF-7 and SKBR3, 10^4 cells/mL) in comparison to the target cells BT549 (10 cells/mL).

Table 1 summarizes the performance of our biosensor compared with other methods. As there are many sensors that have been studied for cancerous cell detection, it is impossible to present all of them in comparison to our sensor. Here we show only the sensors that could provide comparable performances as our sensor. According Table 1, we can see that the LOD of our biosensor is similar to the electrochemical method but not as sensitive as the SERS and LSPR methods. However, the SERS method is more complicated and its measured range is not as large as our biosensor. The LSPR method proposed by Ragini Singh et al. [25,26] shows a low LOD and a great linear range, but the detected time is 60 min, much longer than that of our

sensor. Although the etching process and the surface modification with several nanomaterials in the LSPR method will increase the fabricated complexity, the compact size (~ 5 mm) together with the good performances makes it a good potential for practical application. In comparison to the LSPR method and the Ω -shaped fiber, there is nothing broken in the structural integrity of the fiber, making our biosensor more stable. Furthermore, as our biosensor has reached the requirement of CTCs detection (< 10 cells/mL), it can provide a new option for in-vitro cancerous cells detection.

Table 1. Summary of different methods for measurement of cancer cells.

Methods	LOD (cells/mL)	Detected time (min)	Linear Range (cells/mL)	Biorecognition-element	Ref
Ω -shaped fiber	12	37.5	$5-10^4$	Aptamer	[21]
SERS ^a -AuNanostar	1	Not mentioned	$1-100$	Folic Acid	[35]
LSAW ^b	32	27	10^2-10^7	Aptamer	[36]
Electrochemical	5	Not mentioned	$10-10^5$	Antibody	[27]
Fiber-optic with SPR and LSPR	49	5	Not mentioned	Aptamer	[22]
Fluorescence	10	30	10^2-10^7	Aptamer	[28]
LSPR ^c	2	60	10^2-10^6	2-deoxy-D-glucose	[25,26]
TFBG-SPR	5	20	$5-10^3$	Antibody	This work

^aSERS: Surface-enhanced Raman scattering;

^bLSAW: Leaky surface acoustic wave;

^cLocalized Surface Plasmon Resonance

5. Conclusion

We have proposed and experimentally demonstrated a functionalized TFBG for breast cancer cell detection in this work. Experimental results shows that our biosensor can detect breast cancer cell BT549 in concentration as low as 5 cells/mL, and the theoretical LOD can even reach single-cell level. The measured range is from $5-1000$ cells/mL, and the measured time is only ~ 20 minutes. Our biosensor has advantages of simple-design, ease of manufacture, low-cost, and good performances as other methods, which can provide in-situ, label-free and real-time measurement for CTCs in future.

Funding. Basic and Applied Basic Research Foundation of Guangdong Province (2021B1515140029, 2020B1515120041); 2020 LKSF Cross-Disciplinary Research Projects (2020LKSF17B) Key Project of Basic Research and Applied Basic Research in Ordinary Universities of Guangdong Province (2018KZDXM067).

Acknowledgments. The authors thank Penglei Ma and Jiajian Ruan for their guidance on the experiment. Author contributions include X.C.: investigation, writing – review and editing; P.X.: writing - original draft preparation, data processing; X.H.: Methodology; H.Q.: validation, W. L. and J. J.: formal analysis; J.S.: supervision; Y.C.: conception, review, editing and funding support. All authors have read and agreed to the published version of the manuscript.

Disclosures. The authors declare no conflicts of interest.

Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

References

1. E. Garcion, P. Naveilhan, F. Berger, and D. Wion, "Cancer stem cells: beyond Koch's postulates," *Cancer Lett.* **278**(1), 3–8 (2009).
2. H. K. Kordasht and M. Hasanzadeh, "Aptamer based recognition of cancer cells: Recent progress and challenges in bioanalysis," *Talanta* **220**, 121436 (2020).
3. Z. Shen, A. Wu, and X. Chen, "Current detection technologies for circulating tumor cells," *Chem. Soc. Rev.* **46**(8), 2038–2056 (2017).

4. Y.-n. Zhang, W.-g. Xie, J. Wang, and P. Wang, "Study on the effect of carbon nanotube coating on the refractive index sensing sensitivity of fiber modal interferometer," *Opt. Mater.* **75**, 666–672 (2018).
5. X. D. Wang and O. S. Wolfbeis, "Fiber-optic chemical sensors and biosensors (2013–2015)," *Anal. Chem.* **88**(1), 203–227 (2016).
6. J. Albert, S. Lepinay, C. Caucheteur, and M. C. Derosa, "High resolution grating-assisted surface plasmon resonance fiber optic aptasensor," *Methods* **63**(3), 239–254 (2013).
7. D. Chetia, J. M. Gogoi, H. K. Singh, and T. Bezboruah, "Low-cost refractive index sensor with optical fibers attached to a U-shaped glass tube," *IEEE Sens. Lett.* **2**(3), 1–4 (2018).
8. T. Guo, "Fiber grating-assisted surface plasmon resonance for biochemical and electrochemical sensing," *J. Lightwave Technol.* **35**(16), 3323–3333 (2017).
9. T. Guo, F. Liu, B.-O. Guan, and J. Albert, "[INVITED] Tilted fiber grating mechanical and biochemical sensors," *Opt. Laser Technol.* **78**, 19–33 (2016).
10. C. Leitão, S. O. Pereira, N. Alberto, M. Lobry, M. Loyez, F. M. Costa, J. L. Pinto, C. Caucheteur, and C. Marques, "Cortisol in-fiber ultrasensitive plasmonic immunosensing," *IEEE Sensors J.* **21**(3), 3028–3034 (2021).
11. S. Cai, H. Pan, Á. González-Vila, T. Guo, D. C. Gillan, R. Wattiez, and C. Caucheteur, "Selective detection of cadmium ions using plasmonic optical fiber gratings functionalized with bacteria," *Opt. Express* **28**(13), 19740–19749 (2020).
12. F. Wang, M. Lu, H. Yuan, Y. Zhang, W. Ji, C. Sun, and W. Peng, "pM level and large dynamic range glucose detection based on a sandwich type plasmonic fiber sensor," *J. Lightwave Technol.* **39**(12), 3882–3889 (2021).
13. Y. Duan, F. Wang, X. Zhang, Q. Liu, M. Lu, W. Ji, Y. Zhang, Z. Jing, and W. Peng, "TFBG-SPR DNA-biosensor for renewable ultra-trace detection of mercury ions," *J. Lightwave Technol.* **39**(12), 3903–3910 (2021).
14. T. Guo, F. Liu, X. Liang, X. Qiu, Y. Huang, C. Xie, P. Xu, W. Mao, B. O. Guan, and J. Albert, "Highly sensitive detection of urinary protein variations using tilted fiber grating sensors with plasmonic nanocoatings," *Biosens. Bioelectron.* **78**, 221–228 (2016).
15. X. Chen, J. Jiang, N. Zhang, W. Lin, P. Xu, and J. Sun, "Study on a plasmonic tilted fiber grating-based biosensor for calmodulin detection," *Biosensors* **11**(6), 195 (2021).
16. M. Sypabekova, S. Korganbayev, A. González-Vila, C. Caucheteur, M. Shaimerdenova, T. Ayupova, A. Bekmurzayeva, L. Vangelista, and D. Tosi, "Functionalized etched tilted fiber Bragg grating aptasensor for label-free protein detection," *Biosens. Bioelectron.* **146**, 111765 (2019).
17. X. Chen, Y. Nan, X. Ma, H. Liu, W. Liu, L. Shi, and T. Guo, "In-situ detection of small biomolecule interactions using a plasmonic tilted fiber grating sensor," *J. Lightwave Technol.* **37**(11), 2792–2799 (2019).
18. M. Lobry, M. Loyez, E. M. Hassan, K. Chah, M. C. DeRosa, E. Goormaghtigh, R. Wattiez, and C. Caucheteur, "Multimodal plasmonic optical fiber grating aptasensor," *Opt. Express* **28**(5), 7539–7551 (2020).
19. M. Loyez, J. Albert, C. Caucheteur, and R. Wattiez, "Cytokeratins biosensing using tilted fiber gratings," *Biosensors* **8**(3), 74 (2018).
20. S. Cai, L. González-Vila, X. Zhang, T. Guo, and C. Caucheteur, "Palladium-coated plasmonic optical fiber gratings for hydrogen detection," *Opt. Lett.* **44**(18), 4483–4486 (2019).
21. Z. Luo, Y. Xu, L. He, F. He, J. Wu, Z. Huang, Y. Tian, Y. Li, and Y. Duan, "Development of a rapid and ultra-sensitive cytosensor: Ω -shaped fiber optic LSPR integrated with suitable AuNPs coverage," *Sens. Actuators, B* **336**, 129706 (2021).
22. M. Loyez, E. M. Hassan, M. Lobry, F. Liu, C. Caucheteur, R. Wattiez, M. C. DeRosa, W. G. Willmore, and J. Albert, "Rapid detection of circulating breast cancer cells using a multiresonant optical fiber aptasensor with plasmonic amplification," *ACS Sens.* **5**(2), 454–463 (2020).
23. B. Kaur, S. Kumar, and B. K. Kaushik, "2D materials-based fiber optic SPR biosensor for cancer detection at 1550 nm," *IEEE Sensors J.* **21**(21), 23957–23964 (2021).
24. G. Quero, M. Consales, R. Severino, P. Vaiano, A. Boniello, A. Sandomenico, M. Ruvo, A. Borriello, L. Diodato, S. Zuppolini, M. Giordano, I. C. Nettore, C. Mazzarella, A. Colao, P. E. Macchia, F. Santorelli, A. Cutolo, and A. Cusano, "Long period fiber grating nano-optrode for cancer biomarker detection," *Biosens. Bioelectron.* **80**, 590–600 (2016).
25. B. Kaur, S. Kumar, and B. K. Kaushik, "Recent advancements in optical biosensors for cancer detection," *Biosens. Bioelectron.* **197**, 113805 (2022).
26. R. Singh, S. Kumar, F.-Z. Liu, C. Shuang, B. Zhang, R. Jha, and B. K. Kaushik, "Etched multicore fiber sensor using copper oxide and gold nanoparticles decorated graphene oxide structure for cancer cells detection," *Biosens. Bioelectron.* **168**, 112557 (2020).
27. Y. Liu, F. Zhu, W. Dan, Y. Fu, and S. Liu, "Construction of carbon nanotube based nanoarchitectures for selective impedimetric detection of cancer cells in whole blood," *Analyst* **139**(20), 5086–5092 (2014).
28. J. Tan, Z. Lai, L. Zhong, Z. Zhang, R. Zheng, J. Su, Y. Huang, P. Huang, H. Song, N. Yang, S. Zhou, and Y. Zhao, "A graphene oxide-based fluorescent aptasensor for the turn-on detection of CCRF-CEM," *Nanoscale Res. Lett.* **13**(1), 66 (2018).
29. L. Li, M. Liao, Y. Chen, B. Shan, and M. Li, "Surface-enhanced Raman spectroscopy (SERS) nanoprobe for ratiometric detection of cancer cells," *J. Mater. Chem. B* **7**(5), 815–822 (2019).
30. H. Pan, Z. Peng, J. Lin, X. Ren, G. Zhang, and Y. Cui, "Forkhead box C1 boosts triple-negative breast cancer metastasis through activating the transcription of chemokine receptor-4," *Cancer Sci.* **109**(12), 3794–3804 (2018).

31. X. Chen, J. Xu, X. Zhang, T. Guo, and B. Guan, "Wide range refractive index measurement using a multi-angle tilted fiber Bragg grating," *IEEE Photonics Technol. Lett.* **29**(9), 719–722 (2017).
32. X. Dong, H. Zhang, B. Liu, and Y. Miao, "Tilted fiber Bragg gratings: Principle and sensing applications," *Photonic Sens.* **1**(1), 6–30 (2011).
33. V. Marquez-Cruz and J. Albert, "High resolution NIR TFBG-assisted biochemical sensors," *J. Lightwave Technol.* **33**(16), 3363–3373 (2015).
34. H. Zhu, I. M. White, J. D. Suter, M. Zourob, and X. Fan, "Integrated refractive index optical ring resonator detector for capillary electrophoresis," *Anal. Chem.* **79**(3), 930–937 (2007).
35. X. Wu, Y. Xia, Y. Huang, J. Li, H. Ruan, T. Chen, L. Luo, Z. Shen, and A. Wu, "Improved SERS-active nanoparticles with various shapes for CTC detection without enrichment process with supersensitivity and high specificity," *ACS Appl. Mater. Interfaces* **8**(31), 19928–19938 (2016).
36. K. Chang, Y. Pi, W. Lu, F. Wang, F. Pan, F. Li, S. Jia, J. Shi, S. Deng, and M. Chen, "Label-free and high-sensitive detection of human breast cancer cells by aptamer-based leaky surface acoustic wave biosensor array," *Biosens. Bioelectron.* **60**, 318–324 (2014).