Monitoring DNA Hybridization with a Simply Manufactured GMR Biosensor

Ting-Jou Ding[1], Jiann-Hwa Lue[1], Ya Lun Tsai[1], Tsung-Hsun Yang[1*], Jenq-Yang Chang[1,2], Wen-Yih Chen[3]

[1] Department of Optics and Photonics, National Central University, Chungli, Taiwan, ROC
[2] Optical Science Center, National Central University, Chungli, Taiwan, ROC
[3] Department of Chemical and Materials Engineering, National Central University, Chungli, Taiwan, ROC
thyang@dop.ncu.edu.tw

Abstract: In this letter, a real-time approach to identify the various DNA molecules hybridized of the integrated micro-fluid with the simplest manufactured guided-mode resonance (GMR) sensor is presented. By monitoring the resonant peak wavelength shift of GMR biosensor, the DNA hybridization experiment can be recognized and clarified. The biosensor exhibited sensitivities about 2.14nm in peak wavelength shift for the detection of the hybridizing of both the capture DNA and the probe DNA. Furthermore, the stability and reliability of nucleic acid hybridization on the GMR sensor are examined by combining the sensor with the fluidic elements. The results reveal that the dynamics of DNA hybridization on the GMR sensor can obviously be monitored in real time.
[Ting-Jou Ding, Jiann-Hwa Lue, Tsung-Hsun Yang, Jenq-Yang Chang, Wen-Yih Chen. **Monitoring DNA Hybridization with a Simply Manufactured GMR Biosensor**. Life Sci J 2012;9(2):1015-1019] (ISSN:1097-8135).
http://www.lifesciencesite.com. 151

Keywords: guided-mode resonance, biosensor, DNA hybridization, simply manufacture, real time monitoring, cost down, reliability, stability

1. Introduction

Label-free DNA biosensors and DNA microarrays tests based on optical technical has become the hottest topics in the field of biological molecule detection in the few years. Detecting the optical signals variety of the complementary (target) DNA strand and the immobilized single-stranded (ss) DNA, can analyze the hybridization effects of specific DNA sequences [1]. The applications of DNA-based diagnostic can be used in various fields of life sciences, such as gene analysis, fast detection of biological warfare agents, monitoring of environmental pollutants, and forensic applications [2].

Conventional biosensors require labeling with external reagents such as enzymes, fluorescent dyes, or both, to enhance the major sensitivity. But, the labeling process would increase additional time and cost demands. Furthermore, the labels on cells will interference the molecular interaction and the cell biology of the target receptor, which leads to false negatives (or positive) [3]. Label-free biosensor can not only avoid the suppression of external reagents (such like enzymes, fluorescent dyes) and time-consuming cause in labeling procedure, but also ensure the life-cell detected completely. Therefore, the free-tag technical has become more and more helpful in the practical applications of medical diagnosis and treatment.

Beside, the miniaturized DNA biosensor and biochip approach has become increasingly important in basic researches into the genetics of disease and also in the more practical applications of medical diagnosis and treatment. Integrated optical devices onto the biosensors such as prism, grating, waveguide, interferometric and ellipsometry possess high sensitivity and small-sized characteristics, which allow them easily, continue monitor and miniaturization [4-10]. Among these optical biosensors, Guided-mode resonant (GMR) biosensor is the most potential one.

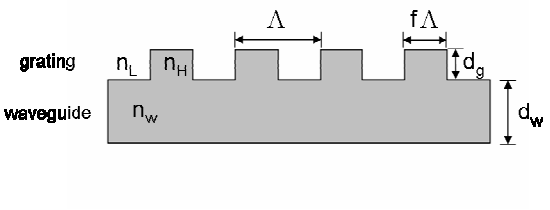
Physically, when one plane wave illuminates a period grating, the diffractive waves may be corresponding to a guided mode of the waveguide under the condition of resonance. Under this condition, the diffractive device would support a sharply reflective and transmittance spectra. With the changing of the refractive index or thickness of a resonant waveguide grating, its resonance wavelength will shift. By using such characteristics to bio- and chemical sensor, real time monitors can attach the biolayer on its surface without any fluorescent tags. This key feature also enables time resolved DNA or protein binding studies to be performed with very high accuracy. Since this technology can provide efficient reflection peaks with narrow linewidths, potentially very high signal to noise ratios can be achieved [11]. GMR sensors have been shown to exhibit high parametric sensitivity, rendering them extremely responsive to small amounts of trace chemicals or biological molecules. In 1998, Z. S. Liu et al reported a new type of narrow-band reflection filter based on guided-mode resonant effects [12-13]. A extremely narrow-

band and low side band reflection filter can be obtained when it is illuminated with TE (or TM) polarization light to a sub-wavelength grating.

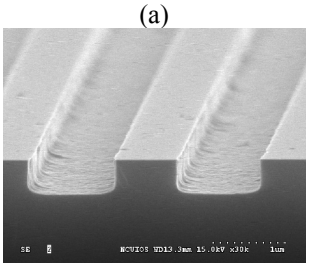
In this letter, a simply manufactured GMR device compound with a micro-fluidics channel biosensor was reported. The GMR optical device is a single dimension grating directly writing on waveguide layer by MEMS technology. By monitoring the reflective light spectrum shift, the DNA hybridization procedure can be detected in real-time. Beside, the experiments also discuss the repeatability of this sensor, by using fresh urea solutions to dehybridized the hybridized DNA. The results reveal that the GMR sensor has good detection sensitivity and good repeatability for DNA interaction dynamics experiments.

2. The Design and Fabrication of Elements

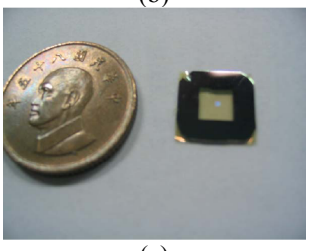
The two-layer GMR biosensor with a refractive index modulated periodic grating and a multiple-guided-mode waveguide layer are proposed to design the ultra-sensitive GMR sensor in this research by the use of simple structures and fabrication methods. The GMR structure under study is shown schematically in figure 1.



(a)



(b)



(c)

Figure 1. (a)The schematic drawing of GMR biosensor and (b) the SEM side view picture of grating and waveguide. (c) The picture of GMR biosensor.

The structure parameters of proposed GMR device are given as below: grating period Λ = 814 nm, grating depth d_g = 100 nm, waveguide depth d_{WG} = 900nm, grating filling factor f= 0.5, both refractive index of grating n_g and waveguide n_{Wg} are 2.05 (Si_3N_4). In consideration of the inducing guided-mode to the structure, the equivalent refraction index of the thin grating layer is design as n_{eff}, [12] which $n_{eff} = [f \times n_H^2 + (1-f) \times n_L^2]^{1/2}$.

There are four processing steps to fabricate the GMR device. First, deposit the Si_3N_4 thin film on both surfaces of the silicon substrate by using the low-pressure chemical vapor deposition (LPCVD). Second, transfer the sub-wavelength grating patterns on the top side of the Si_3N_4 film by using photolithography and e-beam lithography. Third, form square pattern on silicon substrate with photolithography and Inductively-Coupled-Plasma (ICP) dry etching. Finally, remove the silicon substrate beside the waveguide layer by KOH wet-etching.

Figure 1(b) shows the SEM photographs of the side profile of the GMR biosensor. The fabricated parameters of the GMR sensor have a little variation. For instance, the period of grating Λ=811nm, the depth of grating d_g=108.2nm, the thickness of waveguide d_{WG}=891.8nm. In figure 1(c) shows the picture of GMR biosensor.

3. DNA Hybridization Experiment and Results

3.1 Surface modification and DNA hybridization

For DNA hybridization experiment, four preload steps to immobilize DNA on GMR surface are necessary. First, we used plasma enhance chemical vapor deposition (PECVD) to deposit SiO_2 50nm on GMR surface. Second, steep the GMR device in the sulfuric acid and H_2O_2 solution for five minutes, to expose the OH^- group terminals on the SiO_2 surface. Third, immerse the device in the DETA (trimethoxysilylpropyldiethylenetriamine) solution for 30 minutes to a self-assemble monolayer. It provides the connectivity with the amino-groups to be attached to SMPB (4-(p-malemidophenyl) butyrric acid n-hydroxysuiimideester). Final, immerse the GMR sensor in the thiol-DNA solution to let the Capture DNA attached on the GMR biosensor surface. In addition to these immobilizing processes, it is also necessary to hybridize capture DNA complementary to one part of target DNA, and the probe DNA to the other part of target DNA. Figure 2 shows the processes of surface modification and DNA hybridization.

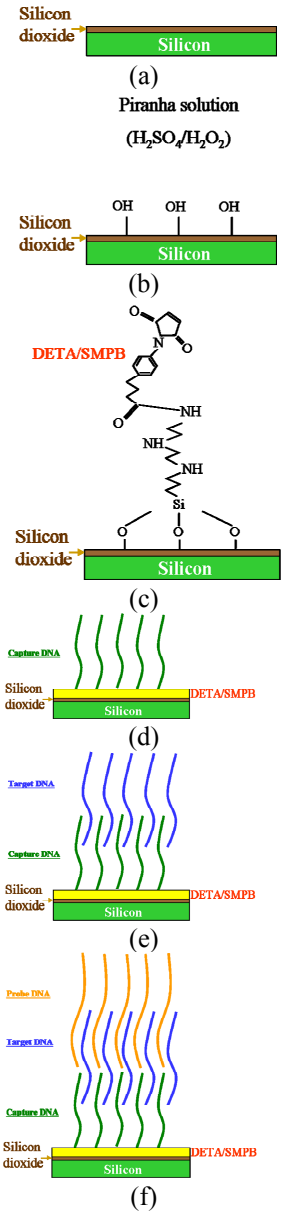


Figure 2. The DNA attaching processes are (a)-(d) and the DNA hybridization processes are (e)-(f).

3.2 Experimental setup

Figure 3 shows the fluid system of GMR biosensor. Figure 3(a) is the schematic diagram of this fluid system. And figure 3(b) is the picture of this system.

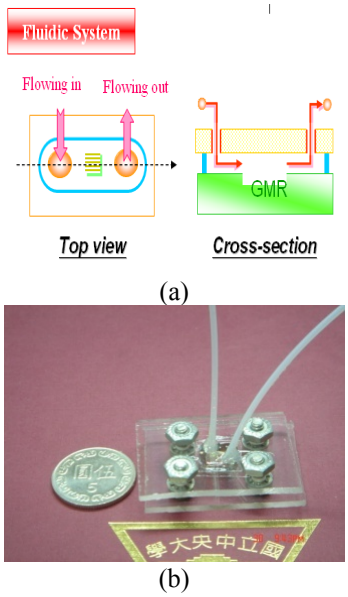
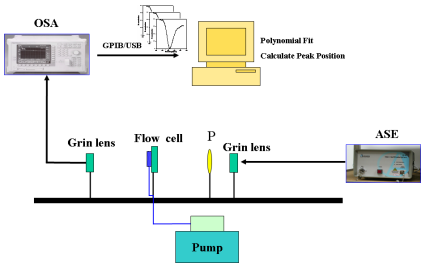
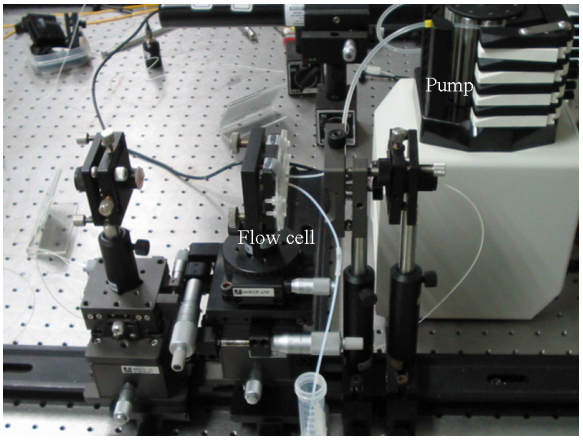


Figure 3. (a) Schematic diagram of GMR biosensor combined with fluid system. (b) The picture of this system.

Figure 4 shows the experiment setup of DNA hybridization. The GMR device is seated in a measurement system including light source, fluidic system, detection and analysis parts which were shown in figure 4(a). Amplified Spontaneous Emission (ASE, Amonics ALS15CL) is used to be a broad band light source. The range of wavelength is between 1525 and 1610 nm. The light emitted continuous waves from ASE light source with conventional communication band wavelengths and coupled into a single-mode fiber. The end of the single-mode fiber with GRIN lens that collimates parallel wave launches to the polarizer and fluidic system normally. The fluidic system includes two parts that are fluidic cell and syringe pump. By the use of the pumping of syringe pump, reaction reagents can flow into and out the fluidic cell through the tubing and react with the specific functional group on the surface of GMR device. Through the fluidic system, the transmission light was collected by GRIN lens and transmitted to Optical Spectrum Analyzer (OSA, Anritzu MS9710B). OSA was applied in this measurement system as a detector. Finally, those data were taken to analyze in personal computers which were connected to OSA with General Purpose Interface Bus (GPIB). Figure 4 (b) shows the picture of experimental setup.



(a)



(b)

Figure 4. The experiment setup of DNA hybridization.

4. Results and Discussion

Figure 5 shows the transmission spectrums of DNA hybridization. When DNA hybridizes, the resonance wavelength will shift to long wavelength apparently. According to Figure 5, molecules attached to the surface and the resonance wavelength shifted. The resonance wavelength of self-assembly monolayer and ligand, DETA/SMPB, is 1535.83nm. When capture DNA attached to SMPB, the resonance wavelength shifted about 1.44nm. Besides, capture DNA and target DNA hybridized target DNA and probe DNA, respectively. The resonance wavelength of hybridizing between capture DNA and probe DNA shifted about 2.14nm. Furthermore, the signal of hybridization is observed easily that the resonance wavelength with DNA hybridization is twenty times the frequency of which without DNA hybridization. We evaluated the total DNA length after hybridization to be 6.9nm, and the minimum resolution of OSA to be 0.07nm. The best resolution of our experiment is 0.23nm in thickness.

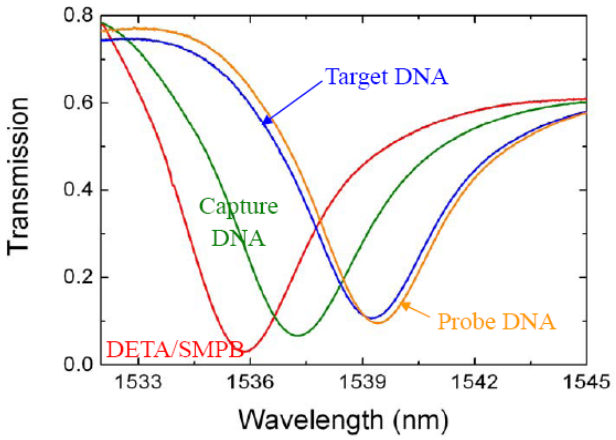
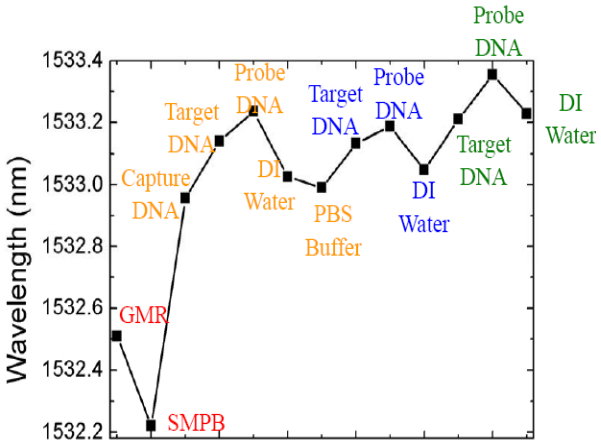
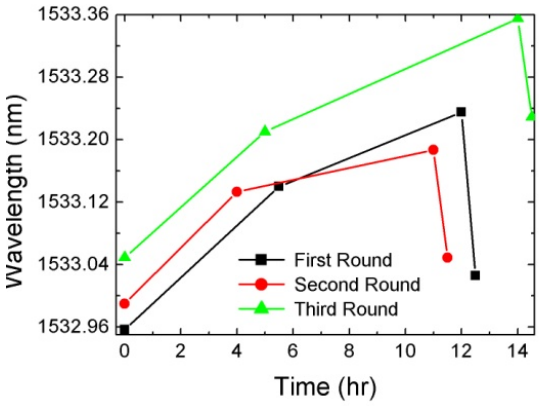


Figure 5. The transmission spectrums of DNA hybridization.

Figure 6 shows the result of DNA hybridization.



(a)



(b)

Figure 6. (a)The transmission spectrum of DNA hybridization and dehybridization. (b) The comparison of three repeat cycles. The result shows that three cycles have similar trends.

In this experiment, we process three cycles of DNA hybridization and dehybridization. The experimental steps show as followed. The GMR biosensor proceeded a sequence of hybridization and dehybridization steps. The hybridization steps were mentioned above and the dehybridization steps which remove target DNA and probe DNA were heated to 80°C for 20 minutes. Then, Put it into PBS buffer solution. With those methods, the reliability and the repeatability of GMR biosensor can be demonstrated.

As illustrated in figure 6(a), the three cycles have similar trends. Figure 6(b) shows the comparison of three cycles. For the repeatability test of the GMR biosensor, DNA can be hybridized and dehybridized successfully.

5. Conclusion

In this paper, we succeeded in applying the wave-guided resonance optical element on GMR biosensor. Differing from conventional sensors, GMR biosensor has many advantages, which include a label-free system, a more minimizing system, a high throughput system, a high sensitivity system, a real-time monitoring system, and, especially, high sensitivity and label-free. The result showed that GMR biosensor has great potential to detect DNA hybridization. We confirmed the feasibility of the detection of SiO_2 multilayer deposition on GMR element. Moreover, the application of DNA hybridization can be successfully applied on GMR biosensor. It provided a technical platform for studies of the bimolecular interaction. Also, the repeatability, DNA hybridization and de-hybridization of GMR biosensor are all demonstrated in this paper. As what's illustrated in figure 3, we combine GMR element with micro-fluid system to make real-time monitoring possible. Moreover, these advantages enables it a powerful biophysical tool to characterize quantitatively how biomolecular will complex form and dissociate apart over time in the future.

Acknowledgments

This wok was partly sponsored by the National Science Council, Taiwan, under the grant numbers of NSC 101-3113-E-008-001 and NSC 101-2911-I-008-501, and also partly sponsored by National Central University, Taiwan, under the grant number of 101G903-2.

Corresponding Author:
Tsung-Hsun Yang, Ph.D.
Department of Optics and photonics, National Central University, Chungli, Taiwan, ROC
No. 300, Jhongda Rd., Jhongli City, Taoyuan County 32001, Taiwan, ROC
E-mail: thyang@dop.ncu.edu.tw

References

1. Sassolas, A., B. D. Leca-Bouvier, L. J. Blum. (2008) DNA biosensors and microarrays. Chemical reviews. 108: 109-139.
2. Cooper, M. A. (2003) Label-free screening of bio-molecular interactions. Analytical and bioanalytical chemistry. 377: 834-842.
3. Cooper, M. A. (2006) Optical biosensors: where next and how soon? Drug discovery today. 11: 1061-1067.
4. Cooper, M. A. (2002) Optical biosensors in drug discovery. Nature Reviews Drug Discovery. 1: 515-528.
5. Fang, Y. (2007) Non-invasive optical biosensor for probing cell signaling. Sensors. 7: 2316-2329.
6. R. Magnusson, S. S. Wang. (1993) Optical guided-mode resonance filter. US patent number 5,216,680.
7. D. Wawro, S. Tibuleac, R. Magnusson, H. Liu. (2000) Optical fiber endface biosensor based on resonances in dielectric waveguide gratings. Biomedical Diagnostic, Guidance, and Surgical-Assist Systems II, Proc. SPIE. 3911: 86-94.
8. A. Cunningham. (1998) Introduction to Bioanalytical Sensors. John Wiley and Sons, New York.
9. Sergiy Patskovsky, Andrei V. Kabashin, Michel Meunier, John H. T. Luong. (2003) Silicon-based surface plasmon resonance sensing with two surface plasmon polariton modes. Applied Optics. 42: 6905-6909.
10. Thurman, S. T., G. M. Morris. (2003) Controlling the spectral response in guided-mode resonance filter design. Applied optics. 42: 3225-3233.
11. S. Tibuleac, D. Wawro, R. Magnusson. (1999) Resonant diffractive structures integrating waveguide gratings on optical fiber end faces. Proc. 1999 IEEE LEOS 12th Annual Meeting. 2: 874-875, San Francisco, CA.
12. S. S. Wang, R. Magnusson. (1993) Theory and applications of guided-mode resonance filters. Applied Optics. 32: 2606-2613.
13. Z. S. Liu, S. Tibuleac, D. Shin, P. P. Young, R. Magnusson. (1998) High-efficiency guided-mode resonance filter. OPTICS LETTERS. 23: 1556-1558.

5/21/2012