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Cite as: Rev. Sci. Instrum. 90, 035004 (2019); https://doi.org/10.1063/1.5074134
Submitted: 22 October 2018. Accepted: 08 March 2019. Published Online: 28 March 2019

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Label-free biosensing using a microring resonator integrated with poly-(dimethylsiloxane) microfluidic channels

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ABSTRACT
Microring resonators have shown promising potential for highly sensitive, label-free, real-time detection of biomolecules. Accurate quantitative detection of target molecules through use of photonic integrated circuits has been demonstrated for environmental monitoring and medical diagnostics. Here, we described the design, fabrication, and characterization of a highly sensitive, label-free microring optical resonator integrated with poly-(dimethylsiloxane) microfluidic channels, which consumes only 30 µl of sample solution. The resonance wavelength shifts resulting from the change in the effective refraction index can be measured in situ, and thus the binding events on the resonator surface, including antibody immobilization, blocking of the resonator surface, and the specific binding of antibody and antigen, can be recorded throughout the entire experimental process in real time. We measured the binding events for the detection of human immunoglobulin G. The system had a detection limit of 0.5 µg/ml, a value substantially (14 times) lower than that of a previously reported microring resonator. To verify the usefulness and adaptability of this technique, human epidermal growth factor receptor 2 was used for the detection. The microring optical resonator was able to monitor reactions between biological molecules in real time and thus can be used in quantitative detection and biological sensing with little sample consumption.

I. INTRODUCTION
Biochemical sensors are powerful detection and analysis tools with diverse applications in biomedical research, environmental monitoring, and health care. According to the different output physical signals, biochemical sensors can be grouped into functional types such as optical biosensors (including microring biosensors), electrochemical biosensors, and mechanical biosensors (microcantilever sensors). Among these sensors, the silicon microring resonator, a highly sensitive, label-free, real-time, rapid response, and efficient optical sensor, has become one of the most promising photonic integration platforms. Its promise can be mainly attributed to the combination of a high contrast in refractive index (RI) and the availability of complementary metal oxide semiconductor (CMOS) fabrication technology, as well as the substantial experimental cost reduction associated with the use of electronics fabrication facilities. Silicon microring resonators can be integrated with microfluidic technology, thus providing a relatively stable liquid environment. Using advanced fluid handling techniques for photonic devices at or below the micrometer scale has the potential to provide compact, effective sensors for lab-on-a-chip tools.

Microring resonators take advantage of the highly sensitive evanescent field generated near silicon-on-insulator (SOI) photonic waveguides, owing to a contrast in RI between core silicon and silicon dioxide. The evanescent field is a type of electromagnetic wave produced at the boundary of two different media. Its amplitude decays exponentially with increasing vertical dimension.
to the boundary. Biorecognition molecules, such as antibodies and aptamers, are immobilized on the resonator surface. When a solution containing target analytes, such as specific antigens, flows over the resonator surface, the binding of antibodies to antigens causes the effective RI to change, thus shifting the resonance wavelength. The effective RI is related to the RI of the microring and the microring’s surroundings. Resonance occurs when the change in the optical path length of the resonator is exactly a whole number. Microring resonators can sense the effective RI changes induced by analyte binding on the surface. By examining the shift in resonance wavelength, the binding of biomolecules to target-specific capture agents can be monitored. The resonance wavelength is defined as

\[ \lambda = 2\pi R n_{eff}/m, \]

where \( m \) is an integer (\( m = 1, 2, 3 \ldots \)), \( \lambda \) is the wavelength of light, \( R \) is the radius of the microring, and \( n_{eff} \) is the effective RI.

Human immunoglobulin G (IgG), the most abundant immunoglobulin in serum, plays an important role in the immune response. Many analytical methods are currently available to detect IgG, including enzyme-linked immunosorbent assay (ELISA), and protein chip and fluorescein labeling methods. Fluorescein labeling and ELISA are sensitive and selective methods for detection. However, either target molecules or biorecognition molecules must be fluorescently tagged, a process that is often difficult, typically requiring extensive sample pretreatment or lengthy procedures, and may increase experimental cost. In contrast, microring resonators, allow for label-free detection of molecules in their natural forms on the chip surface without the added complexity of the forms on labels or enzymatic tags. Microring resonators have the notable advantages of being label-free, being able to detect multiple analytes in real time to provide \( in situ \) monitoring, and having lower analytical costs. Thus, this method is relatively easy and inexpensive to perform.

Here, we demonstrate the design, fabrication, and characterization of a highly sensitive, label-free microring optical resonator, integrated with poly-(dimethylsiloxane) (PDMS) microfluidic channels, which consumes only 30 \( \mu l \) of the sample solution. Using different concentrations of ethanol solutions with known refractive indices, we determined the RI detection sensitivity and the detection limit (DL). We demonstrated the label-free, quantitative detection of IgG by using a microring resonator and measured the resonance wavelength shift resulting from the effective RI changes caused by the specific binding between antibodies and antigens. Furthermore, the binding events on the resonator surface, including antibody immobilization, blocking of the resonator surface, and specific binding of antibody and antigen, were recorded during the entire experiment in real time. To demonstrate the usefulness and adaptability of the presented technique, we also demonstrated the label-free detection of human epidermal growth factor receptor 2 (Her2).

II. MATERIAL AND METHODS

A. Materials and apparatus

IgG and goat anti-human IgG were purchased from Sango Biotech (Shanghai) Co., Ltd. Human Her2 protein and anti-Her2 monoclonal antibody were purchased from Sino Biological, Inc. Bovine serum albumin (BSA), 3-aminopropyltriethoxysilane (APTES), glutaraldehyde (GAD) solution [25% (w/v) in water], and phosphate-buffered saline (PBS; 0.1M phosphate buffer containing 0.9% sodium chloride, pH 7.5) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were of analytical reagent grade and were used as received.

SOI resonator chips and microfluidic channels (30 \( \mu l \)), both made in house, were used. A temperature control system was purchased from Shimax (MAC50, JP), and 24-well polystyrene microplates were purchased from Costar (Corning, NY, USA).

B. Resonator design, fabrication, and characterization

Figure 1 shows an SEM micrograph of the home-built SOI microring resonator with a radius of 20 \( \mu m \) used in the experiment. The circular section is the microring waveguide, and the structure beside the ring is the straight waveguide. In this work, microring resonators were fabricated on a SiO\(_2\) substrate (RI: 1.44) with a Si core layer (RI: 3.45). Owing to the large RI difference between Si and SiO\(_2\), most optical fields were restricted in the higher index Si waveguide. In order to achieve the high sensitivity, the most key design rule is to improve the overlap between the optical mode and the analyte. Among the single microring resonator, one of the key parameter is the thickness of the silicon waveguide; with thinner waveguide, more optical modes will overlap with the analyte. If we use thinner waveguide, we can get higher sensitivity. Based on sensitivity and micro-nano processing technology, we have chosen a microring of 20-\( \mu m \) radius, a silicon waveguide of 220 nm \( \times \) 450 nm, and a gap of 160 nm between the straight waveguide and the ring.

The microring resonators were patterned by using standard optical photolithography and formed by using reactive ion etching (RIE). Our chips were fabricated at the Interuniversity Microelectronics Centre (IMEC), where 150 nm line or gap can be processed. The fabrication was started by spin-coating approximately
280–320 nm of photoresist on an SOI wafer, then drying for 90 s at 90 °C (microfabrication process sequence in Fig. S1). After conventional photolithography, the pattern of the photoresist was transferred to the SOI wafer through inductively-coupled plasma RIE. The photoresist was then removed, thus allowing the analyte to contact the sensing waveguide surface. Finally, the resonator chip was cleaned in a solution (1:1 H2SO4/30% H2O2) for 10 min to remove the protective photoresist coating and any residual organic contaminants introduced from fabrication and then washed with copious amounts of deionized water.

C. Fabrication of the microfluidic channel system

In the process of detection, steady control of sample solutions flowing through the chip surface is crucial. Microfluidic channels, one of the most commonly used methods for the detection of target molecules with little sample consumption, not only ensure the activity of target and biorecognition molecules but also make the modification of the chip more efficient.

Owing to the high sensitivity of the microring, the output spectrum is easily affected by external factors, such as vibration, temperature, and dust. Many materials are available with a broad range of optical, mechanical, and chemical properties. Polymers have been mostly used as substrate materials for microfluidic devices in recent years.27,28 We designed the system of microfluidic channels integrated with a microring resonator, which prevented salt coalescence on the chip surface, owing to liquid volatilization in an open environment. The microfluidic channels consumed only 30 µl of sample solution, thus potentially allowing for substantially lower experiment costs.

We used two acrylic polymers for easy processing, poly-(methylmethacrylate) (PMMA) and poly-(dimethylsiloxane) (PDMS) to fabricate the microfluidic channels. PDMS is a type of macromolecule organic silicon compound, which has good biocompatibility and is widely used in biological microelectromechanical systems, as a gap filling agent or lubricant, and in contact lenses.29,30

First, we placed the microring chip in the PMMA base. Then, the precise PDMS microfluidic channels were aligned, and another PMMA base was deposited to produce a seal. Simultaneously, inlet and outlet holes above the base were made to form solution channels. As shown in Fig. 1, the base encapsulated the surface of the chip, thus not only protecting the chip but also forming the solution channels.

D. Measurement setup

The resonator consisted of two parts: the integrated optical waveguide structure and the microfluidic channel system. Solutions were controlled by a peristaltic pump, which supplied a continuous flow of solution through the microfluidic channels and output pipes, then to the waste solution bottle. We use the broadband light source and optical spectrum analyzer to monitor the wavelength shift of the microring resonator sensor. Light from the broad band light source was coupled into the waveguide of the resonator through polarization-maintaining lens fibers. After a series of coupling oscillations, the output light was collected by a spectrometer through the output optical fiber. For the optical measurement system, a three-dimensional adjustable high precision optical test platform was used to accurately adjust the input and output optical fibers and chip. In addition, a high precision temperature controller was applied to adjust the experiment temperature. The control of the entire measurement setup was automated by a computer (measurement setup in Fig. S2). The polarization used is TM mode.

E. Preparation of the microring

The surface of the sensor was modified with APTES and glutaraldehyde before being integrated with the microfluidic channels. The chip was first cleaned in piranha dip (3:1 H2SO4/30% H2O2) for approximately 10 min (with stirring) to remove the protective photoresist coating and any residual organic contaminants introduced from fabrication, and then it was washed with copious amounts of deionized water and dried under nitrogen gas. To promote antibody adhesion to the silicon surface, the chip surface was immersed in a solution of 2.5% APTES (1:100 deionized water/ethanol) for 2 h at 4 °C, then rinsed with ethanol and deionized water, and dried under nitrogen gas. The resonator chip was incubated with 5% glutaraldehyde (GAD) in deionized water for 1 h and rinsed with deionized water three times. Next, we integrated the chip with microfluidic channels as described in Sec. II C. The temperature control of the microfluidic channels was 27 ± 0.01 °C, and the room temperature was controlled to 25 ± 0.1 °C. The functionalization procedures of the chip were performed in the microfluidic channels. PBS containing 100 µg/ml of anti-IgG monoclonal antibody was flowed through the microfluidic channels and over the resonator surface at a rate of 4.2 ml/h for 10 min, and then the chip was incubated for 1.5 h to allow functionalization. A rinse with PBS was performed to remove any loosely bound antibody. The microfluidic channel was then filled with 5% solution (w/v) of BSA in PBS for 40 min for blocking. After these procedures, the functionalized microring was washed with PBS and was then ready to use, as shown in Fig. S3.

III. RESULTS AND DISCUSSION

A. Stability of resonance wavelength shift

The stability of the signal when no biomolecular interaction takes place on the chip surface is crucial for a low detection limit. To determine whether the microfluidic channels system was able to measure wavelength shifts with high signal stability, we monitored the real time resonance wavelength shift in an open liquid environment and microfluidic channels by using the same chip [Fig. 2(a)]. In an open liquid environment, the chip is placed on a glass slide or in a petri dish, and then 2 ml of liquid is added to cover the chip. In an open liquid environment, the resonance wavelength shifted 110 pm in the short wavelength direction after 10 min in the buffer solution [black line in Fig. 2(a)]. After the experiment, there was abundant salt on the chip surface. One reason for this deviation may have been salt precipitation during the process of water loss, thus changing the effective RI constantly and causing a continued resonance wavelength shift. In the microfluidic channel system, the continuous liquid flow and small volatile area provided the chip with a stable buffer solution environment, and thus the resonance wavelength was in the steady state [red line in Fig. 2(a)]. These findings indicated the stability of the microfluidic channels system, suggesting that the microfluidic channels system was essential for the accuracy of detection.
Temperature is another major contributor to unwanted changes in effective RI in biochemical detection. We placed the chip in ethanol with or without a temperature control device. The resonance wavelength changed with changes in room temperature and shifted 30 pm in the long wavelength direction after 30 min without a temperature control device [Fig. 2(b)]. The wavelength was essentially in a stable state at a constant temperature of 25°C. These results suggested that temperature stability was also very important for the accuracy of detection.

B. Refractive index sensing

To demonstrate the microring sensing ability and to characterize the RI sensitivity of the resonator, the resonator was calibrated by using different concentrations (0, 2, 4, 6, and 8M) of ethanol. Its resonant wavelength was measured when the ethanol solution was flowed across the microring surface. The results showed a linear shift in the resonant wavelength with increasing ethanol concentration (Fig. S4). Higher ethanol concentrations with a larger RI caused a larger shift in the resonant wavelength. From the slope of the curve, we obtained an RI sensitivity of 140.15 nm/RIU unit (RIU). There are many kinds of the silicon ring resonators and we summarize them in Table I. As we known, the most key performance is the sensitivity. In order to achieve the high sensitivity, the most key design rule is to improve the overlap between the optical mode and the analyte. The strip waveguide based on TE or TM is robust and reliable for the fabrication and can achieve the 135 nm/RIU in a previous reported study. In this paper, the sensitivity is improved by the using of the TM mode. As we know, the TM mode has more overlaps with the analyte than the TE mode, and so maybe that is why we can achieve the higher sensitivity than the convention single microring sensor based on TE mode. Slot waveguide and sub wavelength grating is also a very competitive solution and can push the sensitivity to almost twice/three as compared to the strip waveguide. But they suffer from the problem that the minimum linewidth of slot or subwavelength grating is challenge for the fabrication process. They need high resolution Lithography technology. It will introduce extra cost in the fabrication. The detection limit (DL) was defined as

\[
\text{DL} = \frac{\sigma}{\text{RI sensitivity}},
\]

where \(\sigma\) is the system noise which can be obtained from the system filled with a blank solution. The DL reports the smallest RI

| TABLE I. Comparison of the sensitivity of different microring resonator. |
|-----------------|-------------------|----------------|
| MRR based on strip waveguide working at TE | 70 | 32 |
| MRR based on ultra-thin strip waveguide working at TE | 100 | 33 |
| MRR based on strip waveguide working at TM | 135 | 34 |
| MRR based on the slot waveguide | 298 | 35 |
| MRR based on the sub wavelength grating | 490 | 36 |
| MRR based on strip waveguide working at TM | 140 | This work |

FIG. 2. Real time wavelength shifts of the microring caused by environmental factors. (a) Comparison between wavelength shifts obtained in PBS with or without microfluidic channels. (b) Comparison between wavelength shifts obtained in ethanol with or without a temperature control device.
change that can be measured. The resolution of the spectrometer used in the experiment was 4 pm. From Fig. 2(b), we can get the system noise which is 8 pm. Thus, we determined the DL to be $5.708 \times 10^{-5}$ RIU.

C. Real-time detection of human IgG

The resonance wavelength shifts resulting from effective RI changes can be measured in situ, and thus, the binding events on the resonator surface, including antibody immobilization, blocking of the resonator surface, and specific binding of antibody and antigen, can be recorded during an entire experiment in real time (Fig. 3). We recorded the resonance wavelength every 30 s. The resonance wavelength remained essentially unchanged in PBS and shifted 408 pm after 1.5 h in the anti-IgG solution and then reached the steady state. The spectra did not significantly change after a rinse with PBS, thus suggesting that the antibody had been steadily immobilized on the microring surface. The BSA coating buffer caused a net shift of 132 pm after 40 min. After the baseline resonance wavelength was stabilized in the PBS solution again, the human IgG solution was flowed over the surface. After the chip was rinsed with PBS, 5 µg/ml IgG solution was circulated in the microfluidic channels. For both concentrations of IgG, the effective RI changed when the antigen-antibody complex formed on the chip surface. The shift signals for human IgG solutions were 44 and 90 pm for 1 and 5 µg/ml IgG solution, respectively. The curve did not show an obvious shift during a continual rinse with PBS, thus indicating that the resonance wavelength shift was caused by an RI change on the resonator surface due to the specific binding of antigen rather than to chemical molecules in the liquid.

To further determine whether the measured wavelength shift was induced by the specific binding on the microring surface, the microring was flowed with the BSA solution at a high concentration (5 mg/ml) after 5 µg/ml IgG (Fig. 4). Although the BSA concentration was much higher than the antigen concentration, the measured net shift (10 pm) was much less than that of the microring with specific binding on the surface (90 pm) and slightly greater than that of the microring in PBS (4 pm), as shown in Figs. 3 and 4. The results again suggested that the measured wavelength shift of the microring was generated by the specific binding interaction between the anti-IgG antibody and IgG on the microring surface.

The density of biorecognition molecules on the chip surface may affect the wavelength shift; thus, we compared the wavelength shifts on different chip surfaces for antibody immobilization and blocking of the resonator surface under the same modified conditions. The wavelength shifts were measured when the chip surface was successively covered with anti-IgG monoclonal antibody and...
BSA solutions. As seen in Fig. S5, the wavelength shift in antibody immobilization was approximately twice that in BSA blocking. One reason for this deviation might be that anti-IgG flowed over the chip earlier than BSA, thus affording the anti-IgG monoclonal antibody more opportunities to occupy positions on the chip surface. Another reason may be that BSA has a smaller molecular weight than anti-IgG. The wavelength shifts on different chip surfaces in the same modified condition were nearly identical. These results thus established a foundation for quantitative detection.

D. Quantitative detection of human IgG

To explore the quantitative detection capability of microrings, solutions of human IgG with various concentrations were circulated in the microfluidic channels after the baseline resonance wavelength was stabilized in PBS. The resonance wavelength shifts were monitored in situ for approximately 60 min for each concentration. The real time monitoring of the resonance wavelength in response to various concentrations of human IgG is shown in Fig. 5. Different new sensors were used in each experiment. The measured values showed jumps attributed to the resolution of the spectrometer used in the experiment (4 pm). The shift signals were 12, 52, 64, and 88 pm for 0.5, 2.5, 5, and 10 µg/ml human IgG solutions, respectively. These results indicated that the specific binding between the anti-IgG antibody and IgG appeared to cause the resonance wavelength shift. In addition, with increasing of antigen concentration, the shift increased. The results demonstrated that the microring based on the evanescent field was sensitive enough to detect IgG (7.23–16.85 mg/ml in human serum) and to quantify the IgG. The developed system had a detection limit of 0.5 µg/ml, 14 times lower than the detection limit (7.1 µg/ml) of a previously reported microring resonator for IgG detection.

To verify that this technique was applicable to other analytes, a microring resonator for human epidermal growth factor receptor 2 (Her2), an important breast cancer biomarker, was developed through the same procedure described above for IgG. Overexpression of Her2 protein occurs in approximately 25% of human breast cancers and leads to a particularly aggressive disease. The shift signals were 96 and 125 pm for 2.5 and 5 µg/ml human Her2 solutions, respectively (Fig. 6). We verified that the use of our developed system was able to sensitively detect Her2 at a concentration as low as 2.5 µg/ml, a value comparable to the detection limit (5.0 µg/ml) of a previously reported microring resonator for Her2 detection. The high sensitivity of the sensing system for both IgG and Her2 may be mainly attributed to microfluidic channels, which provide a relatively stable liquid environment, and also to optical waveguide structure of the sensor. As we know, the TM mode have more overlap with the analytes than the TE mode, so maybe that is why we can achieve higher sensitivity than the convention single microring resonator based on TE mode. For the single microring resonator, one of the key parameter is the thickness of the silicon waveguide so that the thinner the waveguide, the more optical mode will overlap with the analytes. We get a higher sensitivity based on the 220 nm × 450 nm waveguide. On the basis of these findings, our developed system composed of a microring optical resonator and PDMS microfluidic channels can be used for target molecule detection, providing the advantage of high sensitivity, label-free, quantitative, rapid, and real time detection.

IV. CONCLUSION

We demonstrated the design, fabrication, and characterization of a highly sensitive and label-free microring optical resonator, integrated with PDMS microfluidic channels, which avoided salt coalescence on the chip surface resulting from volatilization in an open environment, and required only 30 µl of sample solution. The microfluidic channels significantly decreased the experimental cost. The RI detection sensitivity was determined to be 140.15 nm/RIU, and the RI detection limit was determined to be...
5.708 × 10^{-5} \text{RIU}. We demonstrated label-free, real-time, quantitative detection of IgG by measuring the resonance wavelength shift resulting from effective RI changes caused by the specific binding between antibodies and antigens. The detection limit of IgG was as low as 0.5 µg/ml, a value 14 times lower than that detected by a previously reported microring resonator. To verify the usefulness and adaptability of this technique, Her2 was used for the detection. The advantages of the low sample volume required, the convenience, and the capability of real-time, label-free, quantitative detection suggest that the microring optical resonator may hold promise for testing in a point-of-care environment. With these capabilities, microring resonators may open up new perspectives in drug discovery, clinical medicine, healthcare, and environmental monitoring.

**SUPPLEMENTARY MATERIAL**

See supplementary material for a detailed microfabrication process sequence of microring resonator, the entire measurement setup of the sensing system, and the schematic diagram of antibody immobilization. Furthermore, the supplementary material contains wavelength shifts caused ethanol solutions and corresponding variance in the shift curve when the chip was coated with antibody and blocked with the BSA solution.

**ACKNOWLEDGMENTS**

This work was supported by the National Natural Science Foundation of China (Nos. 11872355 and 11627803), the Fundamental Research Funds for the Central Universities (No. WK2480000002), and the International S&T Cooperation Program of China (ISTCP) (No. 2013DFE13090).

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