Development of a flow cell system to quantify analyte-receptor binding kinetics utilizing guided-mode resonance sensors

D. Wawro, S. Zimmerman, Y. Ding, and R. Magnusson
Resonant Sensors Incorporated, 202 E. Border Street, Mailbox 201, Arlington, Texas 76010, USA, wawro@resonantsensors.com

E. Moss, and J. Michalik
Automation & Robotics Research Institute at the University of Texas at Arlington, 7300 Jack Newell Blvd. S., Fort Worth, TX 76118, USA, emoss@uta.edu

ABSTRACT

A novel flow cell system integrated with a guided-mode resonance (GMR) sensor element is presented. The real-time association/disassociation binding interactions of the bacteria *S. aureus* with an immobilized specific IgG antibody layer have been measured. A microfluidic flow cell made from poly(dimethylsiloxane) (PDMS) through a molding process is bonded to the polymer GMR sensor element to create a sealed chamber. The results obtained show that the microfluidic flow cell sensor system can be used to monitor the entire association/dissociation binding cycle in real time, which is not possible with the microwell-based static system. For key applications such as environmental monitoring, a flow cell geometry can be advantageous for real-time screening of biotoxins.

Keywords: flow cell system, guided-mode resonance, biosensor

1 INTRODUCTION

Under the correct conditions, thin-film structures containing waveguide layers and periodic elements exhibit the guided-mode resonance (GMR) effect [1]. These resonant leaky-mode structures, tunable on change of refractive index and/or thickness, have clear applications for real-time monitoring of biochemical interactions. When these sensors are illuminated with a broadband light source, a specific wavelength of light is reflected and/or transmitted at a particular angle. Binding interaction of an immobilized receptor with an analyte (Figure 1) can be monitored, without use of chemical tags, by following the corresponding resonance wavelength shift with a spectrometer as denoted in Figure 2. Since the resonance layer is polarization sensitive, separate resonance peaks occur for incident TE and TM polarization states. This property provides cross-referenced data points that can be used to increase detection accuracy. The sensor element can be prepared with standard surface chemistries to covalently attach a selective detection layer (such as antibodies or aptamers). The sensor is multifunctional as only the sensitizing surface layer needs to be chemically altered to detect different species. This new sensor technology is broadly applicable to medical diagnostics, drug development, industrial process control, and environmental monitoring.

In 1992, Magnusson et al. [1] suggested application of the guided-mode resonance effect for sensor applications and disclosed GMR filters that were tunable on variation in resonance structure parameters [2]. Wawro et al. suggested new GMR biosensor embodiments as well as new possibilities of applications of these when integrated with optical fibers [3]. Later, Cunningham et al. also discussed the use of these resonant elements as biosensors [4].

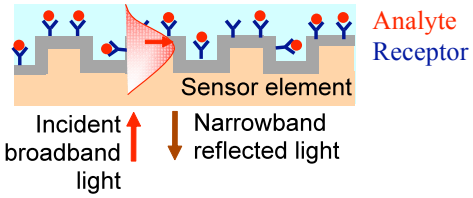

Figure 1. Schematic of a guided-mode resonance sensor.

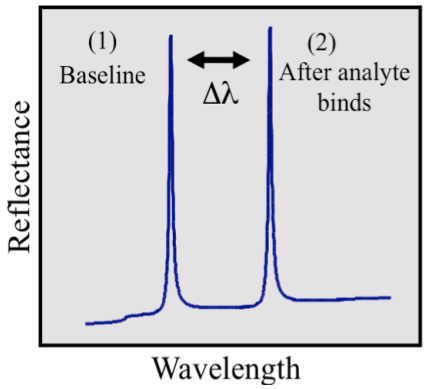

Figure 2. As binding events occur at the sensor surface, resonance-peak shifts can be tracked in real time.

In this paper, a flow cell system integrated with a GMR sensor element is presented. The association/disassociation binding cycle is monitored for the protein-cell interactions of the antibody IgG and the microbial *S. aureus*. Engineered systems of this type may prove effective in characterizing many key biomolecular and cell binding reactions under conditions of dynamic liquid flow. Additionally, for applications such as environmental monitoring, a flow cell geometry can be advantageous for real-time screening of biotoxins. This development is the chief aim of this research.

2 FABRICATION

The GMR biosensor devices are designed using rigorous coupled wave analysis (RCWA) [5]. A single-layer waveguide-grating design is used in this work. The submicron periodic structures are fabricated in polymer materials by molding methods. The grating structure is subsequently coated with a thin layer of a high refractive index film (such as TiO_2 or HfO_2) to create the final sensor device. The sensor is designed to have a dual-resonance (one for each polarization state) and operate in the near-IR region.

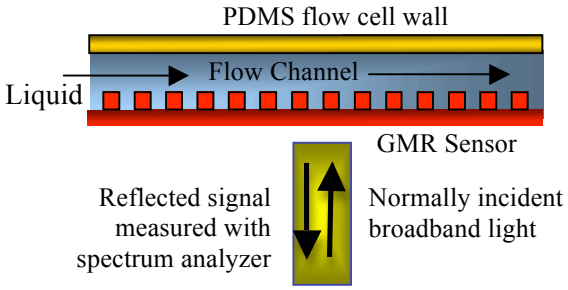

Figure 3. A microfluidic flow cell chamber made from PDMS through a molding process is bonded to the sensor element. Inlet and outlet tubing is inserted into both ends of the flow cell and sealed.

The single-channel flow cell structures are made in PDMS (Sylgard® 170, Dow Corning). PDMS is a common material to use for microfluidic applications due to its ease of processing, the ability to achieve a range of feature sizes, and its mechanical and optical properties [6]. The flow cell mold master is made out of acrylonitrile butadiene styrene (ABS) plastic that is printed using a 3D printer. A 3D design for the mold (inverse of the channel layout) is created in a CAD software program and then sent to the 3D printer for processing. A complex 3D structure is printed using both the ABS plastic and a support material that is later dissolved away in a warm detergent bath. The microfluidic cell is fabricated by pouring uncured PDMS silicone elastomer into the plastic mold. After a short thermal treatment, the PDMS solidifies and easily releases from the mold. The bonding surface of the chamber is then coated with a thin layer of uncured PDMS, which is next brought into contact with the sensor element substrate and allowed to cure (Figure 3). Inlet and outlet tubing is then inserted into both ends of the flow cell and sealed. In this work, a single micro channel with an external pump is used for sample delivery to the sensor element (Figures 3 and 4). However, this method can be directly expanded to a highly parallel system with integrated valves and channels that allows for multiple types of biological assays to be conducted simultaneously using the label-free GMR sensor method described.

A prototype flow cell system integrated with a guided-mode resonance sensor element has been constructed and tested using the spectroscopic Vides™ bioassay reader system previously developed at Resonant Sensors Incorporated (RSI) (Figure 4). White light is incident on the sensor element at normal incidence, and the reflected signal is measured using an optical spectrum analyzer. Changes in the resonance peak location during a binding event are tracked as a function of time. The flow cell cross-section is approximately 10 mm x 2mm, with a flow velocity of approximately 1.7 mm/sec. The beam diameter is approximately 2 mm, and is scanned across the bottom of the sensor plate to measure the response in several locations using an integrated dual-axis translation stage. In this work, two sensor spots are monitored during the experiment. This approach can be readily extended to a high-density sensor chip that is pre-sensitized to detect a large variety of analytes in a single sample.

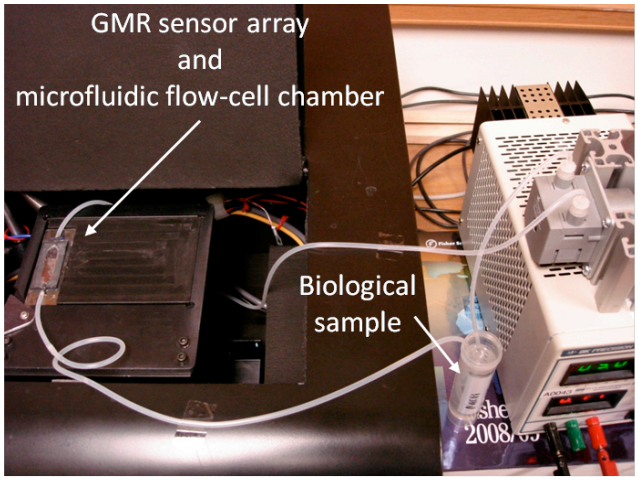

Figure 4. GMR sensor array system with microfluidic flow cell chamber. The biological sample is continuously pumped through the flow cell to monitor the association/dissociation binding cycle.

3 SENSOR VALIDATION

Initial chemical tests are performed to validate operation. The results are shown in Figure 5. An ionic

NSTI-Nanotech 2009, www.nsti.org, ISBN 978-1-4398-1783-4 Vol. 2, 2009

self-assembled polymer bi-layer is deposited on the sensor surface by adding consecutive solutions of the cationic polymer (step A) poly(allylamine hydrochloride) (PAH) and the anionic polymer (step B) polystyrene sulfonate (PSS). This resultant polystyrene surface provides an interface to easily adsorb proteins or antibodies. A buffered saline rinse (step C) is used to remove unbound material between binding cycles. A 1% bovine serum albumin (BSA) solution is then introduced (step D) and the binding kinetics monitored. The BSA quickly adsorbs to the polystyrene surface. Dissociation of the BSA from the sensor surface (between steps E and F) occurs as a neat buffer solution is flowed over the sensor element. Finally, a NaOH solution (step F) is added to the flow cell to regenerate the sensor. As shown in Figure 5, after regeneration the resonance peak shift returns to the starting location (at t=0), indicating the BSA and ionic polymer layers are completely removed. Thus, this flow cell sensor system can be effectively used to monitor the entire association/dissociation binding cycle.

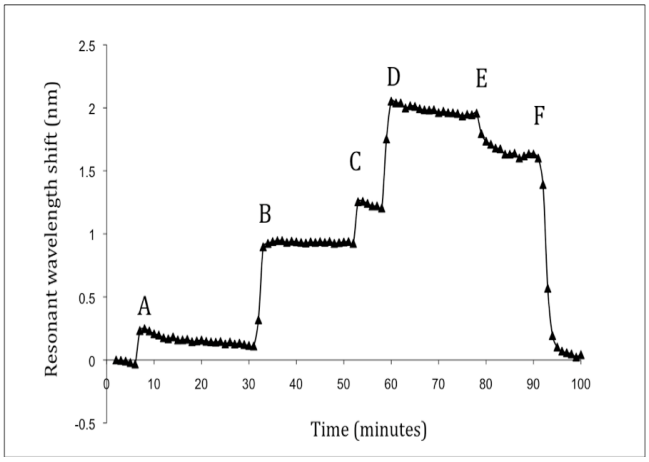

Figure 5. GMR sensor response in buffer at t=0 followed by (A) cationic polymer PAH attaching a monolayer to the sensor surface, (B) anionic polymer PSS binding, (C) buffer rinsing, (D) addition of 1% bovine serum albumin, (E) buffer rinsing, and (F) rinsing in a NaOH solution to remove all bound materials thereby completely regenerating the surface as shown.

4 MICROBIAL TESTS

For microbial tests, *S. aureus* is selected as the analyte of interest due to recent interest in food screening, medical diagnostics, and homeland security. A GMR sensor is bonded to a microfluidic flow cell chamber, as described above. To impart selectivity, an antibody layer that specifically binds to *S. aureus* is immobilized on the sensor surface. First, an ionic self-assembled monolayer is deposited on the sensor surface, as described in the previous section. Antibodies are immobilized on the device surface by adsorption to impart selectivity. Anti- *S. aureus* (Mouse IgG) is flowed into the fluidic chamber and incubated overnight at 4°C. The plate is washed by continuously flowing 10 ml phosphate buffered saline (PBS)/Tween through the fluidic chamber in preparation for blocking. The plate is blocked for one hour using a standard immunoassay diluent (1% BSA in PBS). The plate is then washed again by continuously flowing 10ml PBS/Tween through the fluidic chamber, and is ready for use.

For detection of live bacteria, a neat standard stock solution is prepared from *S. aureus* (ATCC #35556) cultured in our laboratory. The cell count is verified using a hemocytometer. A dilution in PBS is prepared to obtain a concentration of 6.25 x 10^5 cells/ml. An experiment to detect the presence of *S. aureus* in a PBS solution is performed using the sensor reader depicted in Figure 2, Changes in the resonance peak location for both TE polarization and TM polarization are tracked in real-time, as shown in Figure 6. In this design, the TE and TM resonance peaks have similar sensitivities, providing dual data points for each detection event. After a buffer baseline is measured, the *S. aureus* solution is continuously flowed through the microfluidic flow cell chamber for 70 minutes. Binding of the bacteria to the antibody is clearly seen in the binding curve (association) in Figure 6. The input solution is then changed to neat PBS buffer, and the dissociation of the staph bacteria from the immobilized antibody is measured for 30-minutes. Lastly, a NaOH solution is introduced to regenerate the sensor surface. In this step, removal of the bacteria, antibodies, and ionic polymer layers from the sensor are measured. All reactions occur at room temperature.

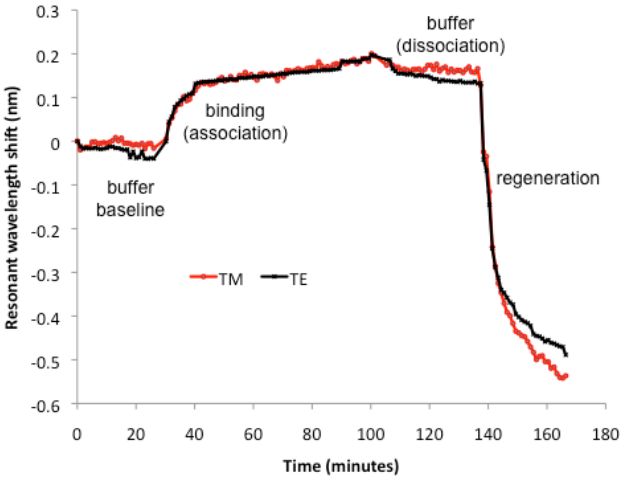

Figure 6. Real-time sensor response measured as the microbial *S. aureus* binds and dissociates to the immobilized anti-*S. aureus* selective layer.

5 CONCLUSIONS

The results show that the microfluidic flow cell system can be used to monitor the entire association/dissociation-binding cycle, which is not feasible with the microwell-based static system. Current research is in progress aiming to expand the microfluidic flow cell chamber into a highly parallel and automated system to allow for multiple types of biological assays to be conducted simultaneously using the label-free GMR sensor method described.

REFERENCES

[1] R. Magnusson and S. S. Wang, “New principle for optical filters,” Appl. Phys. Lett. 61(9), 1022-1024 (1992).

[2] R. Magnusson and S. S. Wang, "Optical guided-mode resonance filter," US patent number 5,216,680, June 1, 1993.

[3] D. Wawro, S. Tibuleac, R. Magnusson, and H. Liu, “Optical fiber endface biosensor based on resonances in dielectric waveguide gratings,” Biomedical Diagnostic, Guidance, and Surgical-Assist Systems II, Proc. SPIE, 3911, 86-94 (2000).

[4] B. Cunningham, P. Li, B. Lin, and J. Pepper, “Colorimetric resonant reflection as a direct biochemical assay technique,” Sens. Actuators B 81, 316-328 (2002).

[5] T. K. Gaylord and M. G. Moharam, “Analysis and applications of optical diffraction by gratings,” Proc. IEEE 73(5), 894-937 (1985).

[6] S. R. Quake and A. Scherer "From Micro- to Nanofabrication with Soft Materials." Science 290(5496): 1536-1540 (2000).