A PLANAR, INTEGRATED TOTAL INTERNAL REFLECTION SENSOR FOR BIOFOULING DETECTION

K. H. Nam^{1,2*}, W. Choi³, J. Yeo⁴, S. H. Ko⁴ and Liwei Lin¹

¹Berkeley Sensor and Actuator Center, University of California, Berkeley, CA USA

² Research Center of MEMS Space Telescope, Ewha Womans University, Korea

³LG Innotek, Inc., Korea

⁴Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology, Korea

ABSTRACT

A planar, integrated total internal reflection (TIR) sensor for the characterizations of biofouling has been demonstrated based on angular interrogation of Fraunhofer diffraction. This sensor is made of a two-mask process to have optical prism and built-in waveguides of $4 \times 0.25 \mu\text{m}^2$ in cross section area. The core and cladding layer of the waveguide are made of silicon nitride and silicon dioxide, respectively and a 780nm in wavelength light source is used in the experiments. Water, ethanol, acetone and glycerol, have all been tested to illustrate the basic refractive index sensing principle of the prototype sensor. Biofouling measurements show that after been immersed into milk as the testing liquid, the surface refractive index of a prototype TIR sensor shifted continuously to as much as 0.0089 for a 9-hour test. As such, this technique could be useful to various biofouling control and monitoring applications, including water desalination, medical, marine and electronic device industries.

INTRODUCTION

Biofouling is the result of unwanted accumulation of biological substances on surfaces which are constantly exposed in aqueous environments. These include surfaces of pipeline systems, sinks, marine equipments, and even blood vessels in human body. Substances deposited and adhered on these surfaces can form thin films composed of bacteria, algae, fungi, and inorganic matters. Leaving unattended, these biofilms can result in reduced efficiency of aquatic equipment and increased efforts in system maintenance. For example, thick biofilms are hard to remove by using common antimicrobial agents and could dramatically increase the equipment maintenance cost [1]. Today, the mechanisms and control of the biofouling process is still far from full understanding and one fundamental and practical issue is to monitor the growth of biofilms.

The current state-of-art biofouling sensors are bulky and expensive and they utilize different techniques to characterize biofouling such as fiber optics [2], light intensity [3], and bioluminescence [4]. Most of these aforementioned sensors are only operational under laboratory conditions. On the other hand, the sensing technique of total internal reflection principle such as the angular interrogation type (AIT) sensors [5] as illustrated in Figure 1(a), has been proven to be sensitive but has not been applied to the area of biofouling detection probably limited by its large size and the requirement of a rotating light source. Here we propose to use a built-in waveguide with a prism-coupler to replace the moving part of a conventional AIT sensor as shown in Figure 1(b) to take the advantage of Fraunhofer diffraction occurring at the end of the waveguide. As a result, this work presents three distinctive accomplishments for biofouling sensors: (1) microscale devices by batch manufacturing; (2) built-in waveguide coupled with prism; (3) demonstration of total internal reflection as the basic sensing principle. Because the proposed architecture is based on batch microfabrication processes for low-cost manufacturing, we believe that the proposed biofouling sensor could be applicable to various applications, including maintenance and control for water desalination and marine equipments.

Figure 1: (a) Conventional AIT sensor (with rotating light source), (b) sensor for this work (with built-in waveguide).

WORKING PRINCIPLE

Figure 2 illustrates the sensing principle of the proposed biofouling sensor which consists of a light source, an integrated waveguide-prism structure and a detector. The waveguide is designed to deliver light from the open-end of the waveguide to the prism-coupler and the reflected light can come out through the side-facet of the prism. The surface of the prism is exposed to the aqueous environment where biofouling occurs. Diffraction occurs at the input edge (interface of the waveguide and prism) to spread light into various directions such that the output signals at the detector side can accomplish measurements of various angles without the need to dynamically change the light emitting angles. By observing the movement of the positions of the critical points from total internal reflection due to the formation of biofouling, the shift of refractive index of medium being sensed due to biofouling can be calculated. This design enables (1) the reduction of sensor size without moving parts, (2) the elimination of scanning time, and (3) planar integration of the whole system. Furthermore, small-size sensors further enable possible multi-point local detections for better sensing accuracy.

Figure 2: Sensing mechanism. Laser light source passes through the waveguide and diffraction occurs at the waveguide/prism interface. The reflection of laser light due to biofouling is detected at the output and the position of total reflection is recorded.

THEORETICAL ANALYSIS

The output signals captured by the detector can record the position of the critical point of reflected light as shown in Figure 2, and refractive index of the media is calculated by the derivation based on Fresnel equation as expressed in Equation 1. Figure 3 illustrates the light passing paths. In this illustration, L represents the distance of total light travel from the input edge of the prism to the CCD, and D is the distance of light travel after the reflection point. Δd is the shift of critical point on CCD detector. The basic optical Snell's law states:

$$\begin{aligned} \theta_{\text{critical point}} &= \sin^{-1}(n_2 / n_1), \quad \Delta\theta = \Delta d / L \\ \Rightarrow n_{\text{biofouling}} &= n_{\text{nit}} \sin(\theta_{\text{crit_i}} + \Delta\theta) \end{aligned} \quad (1)$$

where $\theta_{\text{crit_i}}$ and $\Delta\theta$ are the critical angle at initial stage (without biofouling) and the total angle shift of the critical point due to biofouling during the experiment, respectively. Both $n_{\text{biofouling}}$ and n_{nit} represents the refractive indices of biofouling surfaces and silicon nitride, respectively.

Figure 3: Schematic diagram of light paths where L , D , $\Delta\theta$, and Δd are total distance of light travel, distance between reflection facet of prism and CCD, total angle shift of the critical point due to biofouling, and shift of critical point on CCD, respectively.

θ_{prism} is a design parameter for the prism and it is selected such that the initial critical point will position appropriately on CCD for easy observation. For this study, θ_{prism} was set as 41° , which is close to the incident light angle at total internal reflection from silicon nitride to water without biofouling at 39.29° .

DESIGN AND FABRICATION

A simple two-mask fabrication process is used as illustrated in Figure 4 to make the built-in waveguide with integrated prism. A $2.5\mu\text{m}$ -thick silicon dioxide (bottom cladding) layer and $0.25\mu\text{m}$ -thick silicon nitride (core layer) were deposited on a silicon substrate (Fig. 4a). The body of the prism-coupler and waveguide were defined using the first mask. (Fig. 4b) Additional $2.5\mu\text{m}$ -thick of oxide (top cladding) was deposited on top of the structure (Fig. 4c), and the final structure was defined using the second mask by RIE (reactive ion etching) to have smooth sidewall surfaces and good aspect ratio (Fig. 4d). Finally, the end of the waveguide is opened by wafer dicing and a polishing process is conducted to reduce the insertion loss of light.

Silicon nitride was used as a core layer due to its higher refractive index than that of silicon oxide. For the core layer, standard stoichiometric silicon nitride was deposited using LPCVD, and its refractive index was measured as 2.10. The cross sectional area of the waveguide is determined by optical simulation as

$4 \times 0.25\mu\text{m}^2$. Here, we set the thickness of the core layer by using the maximum thickness for a single mode of light under the designed geometry of rectangular waveguide in this study. The cladding layer needs to be thick enough to prevent excessive propagation leakage of light into the silicon substrate. Optical simulations have been preformed and $2.5\mu\text{m}$ -thick oxide was chosen.

Figure 4: Microfabrication procedure: (a) silicon oxide and silicon nitride are deposited as bottom cladding and core layer of the waveguide, respectively; (b) prism-coupler body and waveguide are defined using first mask; (c) silicon oxide is deposited as a top cladding layer; (d) final structure is defined using the second mask with smooth side-wall surfaces to reduce optical noises.

Figure 5a shows a fabricated device where the cross section of the waveguide has a designed dimension of $4 \times 0.25\mu\text{m}^2$ and the smallest prism has 1, 0.86 and 1.33mm in length for the exit, input and exposed edges (as illustrated in Fig. 3), respectively. The designed prism angle for the prism is 41° . Two larger sensors have also been fabricated with the same prism angle but larger edges (5 and 10 mm for the input edge, respectively) to characterize the size effects. Diffraction occurs at the end of the waveguide as shown in Figure 5b, as the result of Fraunhofer diffraction. Figure 5c shows reflected light at the interface between prism and aqueous medium.

Figure 5: (a) Microscale sensors on a chip, (b) diffraction pattern from waveguide, and (c) reflection pattern.

A Polydimethylsiloxane (PDMS) channel was constructed and placed on the sensor chip as shown in Figure 6 as the interface for liquid media and the sensor in experiments. The size of the channel was determined in accordance with the viscosity of liquid media being measured to prevent leakage. For the prototype experiments, milk was used as the media and microchannel has 200 or $300\mu\text{m}$ in height. The height of the channel reduces as biofilms grow thicker during experiments and it is important to have enough initial height cushions to prevent the blocking of liquid supply. Furthermore, liquid media was provided with a flow rate at $0.1\sim 0.5\text{mL/hr}$ to minimize the accumulation of unwanted floating substances. Table 1 summarizes the important parameters during experiments.

Figure 6: PDMS microchannel on a sensor chip; (a) optical microphoto; (b) cross-sectional view of the PDMS channel.

Table 1: Summary of Total Internal Reflection Sensor for Biofouling

Component	Dimension
Cladding thickness	2.5 μm
Core thickness	0.25 μm
Width of waveguide	4 μm
Input edge (3 sensors)	1, 5, and 10 mm
PDMS channel height	200 and 300 μm

EXPERIMENTAL RESULTS AND DISCUSSIONS

Calibration

Prototype sensors have been designed to have a critical point without biofouling at about one-eighth of peak-to-peak distance of Fraunhofer diffraction from the 0th peak. More than 50% of light intensity concentrated in the region of first one-fourth peak-to-peak Fraunhofer diffraction and that region has been selected as the main observation area. Once the sensor was tested with water, ethanol was tested and results were compared as a mean of calibration.

Refractive Index

Direct refractive index measurements were conducted for several liquids, including water, ethanol, acetone and glycerol, to illustrate the basic sensing principle as a refractive index sensor. The measured refractive index values were in good agreement with values in literature as illustrated in Figure 7. *Reference Line* in the figure is a line which shows how far each measurement are off from the numbers in literatures. The error range of the measurements for four media with different refractive indices was less than ± 0.002 Refractive Index Unit (RIU).

Biofouling Sensing

Biofouling changes the optical properties of biofilms. When the thickness of biofilms increases, their refractive index increases accordingly [6]. The state-of-art biofouling sensors based on optical detection have not been examining the issue of changes in refractive index of biofilms and this could be a source of measurement errors [7, 8]. Here, surface refractive index between the prism-coupler and media is measured and characterized with respect to time to monitor biofouling.

Figure 8a shows the typical output signals captured by CCD and Figure 8b is the intensity profile for the milk tests after 2, 4 and 8 hours into experiments, respectively. It is observed that the critical point gradually moved rightwards and the corresponding refractive indices variations can be calculated to quantify biofouling characteristics. The captured signals are the convolution of Fresnel reflection and Fraunhofer diffraction, and Figure 8b shows this convolution on the curve of the 0th order of the diffraction pattern. For the convolution, diffraction pattern does not move, while critical point shifts along the diffraction pattern in accordance with

the change of refractive index of medium being measured. This output signals are in good agreement with theoretical derivation:

Figure 7: Experiment results for media of known refractive indices, including water, ethanol, acetone, and glycerol, with comparisons to literature values.

$$I(\theta) = I_d(\theta_d) \times R(\theta) \quad (2)$$

$$= I_0 \left[\sin c \left(\frac{\pi d}{\lambda} \right) \right]^2 \cdot \frac{n_1 \cos(\theta) - n_2 \sqrt{1 - \left(\frac{n_1}{n_2} \sin(\theta) \right)^2}}{n_1 \cos(\theta) + n_2 \sqrt{1 - \left(\frac{n_1}{n_2} \sin(\theta) \right)^2}}$$

where I_d is the intensity distribution of end-waveguide diffraction, and R is the reflectance at the facet of prism-coupler. θ_d is a design parameter for sensor fabrication, and it determines the position of 0th peak of Fraunhofer diffraction. Fraunhofer diffraction is a far-field optical phenomenon; therefore, the minimum size of the geometric dimension of the sensor is hundreds micrometer and the distance of total light traveled is not less than several tens of micrometers.

Output signals were analyzed by using image software, Image-pro Plus (Media Cybernetics, USA), and Figure 8c shows the numerical results which clearly show the detection of critical points. In this figure, the critical point is the point after which intensity of output signal rapidly drops. Flat region comes from light patterns induced by Fraunhofer diffraction, and after the critical point, the output signal drops because of decrease of reflectance.

Figure 9 shows three independent sensor outputs in terms of pixel positions (beginning and end of output profiles) with respect to time during the 9-hour experiment. The downward movements of TIR critical points (top symbols) are results of biofouling and the corresponding refractive index change is as much as 0.0089. Variations between each individual measurement could be mainly due to the random process of biofouling accumulation as well as fabrication variations of different sensors [9]. From the output signals, the position of critical point is extracted as a point of rapidly dropping its intensity using the image software. The bottom curve in the figure represents the measurement points (bottom symbols) of *Reference Point* (most left point of the output signal in the inset), and it barely changed during whole experiment. Since intensity of output signal before the critical point is in the region of total internal reflection, the reference point, as well as all other points in that region, does not shift and change. Therefore, measurement of the unchanging reference point verifies that the shifts of critical point came from the change of refractive index on the sensing surface.

Figure 8: (a) Output light signal captured by CCD; (b) intensity profile clear showing the critical points for each patterns; (c) Analysis of output signals using image software.

Figure 9: Microscale sensing experiment result for observation of biofouling formation. Average refractive index change for biofouling formation of milk was 0.0089 for 9 hours of experiment.

For sensing experiments, polarized laser source was directly coupled with the opening of the built-in waveguide of the sensor as shown in Figure 10. The wavelength of light source being used was 780nm because biofilms can be extracellular polymeric substances (EPS), sediments and bacteria combinations. They have different optical properties but usually have little absorbance at this and higher wavelength. Furthermore, reflectance is high in the region when the wavelength of light is greater than 700 nm, and this means that the output signal of the sensor is strong in this range.

Figure 10: Experimental setup for biofouling sensing experiment. The wavelength of light source is 780 nm at which the absorption of biofouling is minimized (for maximum reflection.)

CONCLUSION

We have demonstrated the feasibility of utilizing a planar, integrated total internal reflection sensor for characterizing the biofouling process. This work details the working principles of sensing mechanism, design, fabrication and characterizations of the biofouling sensors, including image analysis and experimental verifications. The TIR sensor for this work utilizes a simple optical design with a built-in waveguide as a TIR sensor. Furthermore, the fabrication process is simple by using only two masks for the entire fabrication process. Several liquids have been tested as base-line characterizations for the sensor, and measured refractive index were in good agreement with the values given by manufacturers (literature) with the error range of less than ± 0.002 Refractive Index Unit. During the biofouling experiments, the sensor measured the change of surface refractive index of a testing liquid (milk) and a shift as much as 0.0089 during a 9-hour test has been measured as the result of biofouling.

REFERENCES

- [1] J.W. Costerton, P.S. Steward, and E.P. Greenberg, "Bacterial Biofilms: A Common Cause of Persistent Infections", *Science*, Vol. 284, pp. 1318-22 (1999).
- [2] D.F. Merchant, P.J. Scully, and N.F. Schmitt, "Chemical tapering of polymer optical fibre", *Sensors and Actuators A: Physical*, Vol. 76, pp. 365-71 (1999).
- [3] J. Klahre and H-C. Flemming, "Monitoring of biofouling in papermill process waters", *Water Research*, Vol. 34, pp. 3657-65 (2000).
- [4] P. Angell, A.A. Arrage, M.W. Mittelman, and D.C. White, "On line, non-destructive biomass determination of bacterial biofilms by fluorometry", *Journal of Microbiological Methods*, Vol. 18, pp. 317-27 (1993).
- [5] J. Homola, S.S. Yee, and G. Gauglitz, "Surface plasmon resonance sensors: review", *Sensors and Actuators B: Chemical*, Vol. 54, pp. 3-15 (1999).
- [6] M.Leitz, A. Tamachkarow, H. Franke and K.T.V. Grattan, "Monitoring of biofilm growth using ATR-leaky mode spectroscopy", *Journal of Physics D: Applied Physics*, Vol. 35, pp. 55-60 (2002).
- [7] M.G. Trulear and W.G. Characklis, "Dynamics of biofilm processes", *Water Pollution Control Federation*, Vol. 54, pp. 1288-1301 (1982).
- [8] R. Bakke, R. Kommedal and S. Kalvenes, "Quantification of biofilm accumulation by an optical approach", *Journal of Microbiological Methods*, Vol. 44, pp. 13-26 (2001).
- [9] J. Wimpenny, "Ecological determinants of biofilm formation", *Biofouling*, Vol. 10, pp. 43-63 (1996).

CONTACT

*K. H. Nam, tel: +1-510-642-8983; koonam@me.berkeley.edu