A Simple and Direct Biomolecule Detection Based on a Microwave Resonator

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ABSTRACT

This paper presents a simple and direct biomolecule detection scheme based on a coplanar waveguide (CPW)-to-slotline ring resonator whose resonant frequency is 3.4 GHz. Its sensitivity enhancing technique and characterization is also reported. When a biomolecule is attached on the resonator, it directly detects the biomolecule through its resonant frequency change without any additional transduction procedures. The microwave biosensor is composed of a substrate and a metal resonator (nickel and gold). For higher sensitivity, the structure was modified by adopting an insulation layer between a nickel and a gold layer. Two types of the microwave biosensor were characterized through biotin-streptavidin reaction. Measurement results showed that resonant frequency decreased 65 MHz and 10 MHz for the original type, and 79MHz and 18 MHz for the modified type when biotin and streptavidin is attached, respectively.

Keywords: biomolecule detection, coplanar waveguide, slotline ring resonator, sensitivity enhancing technique

1 INTRODUCTION

Due to well-established micromachining technologies, there are various kinds of a biosensor based on surface resonance plasmon (SPR) [1], fluorescence [2], electromechanical transduction [3, 4] and a nano-material [5]. Even though these biosensors are successful and useful, they often need bulky measurement equipments, complex fabrication steps, time consuming pre/post processes, or off-site verification. For example, a fluorescence-based biosensor needs preprocessing such as fluorescence labeling and off-site verification steps through fluorescence imaging. This makes biological analysis system complex and expensive.

One of the candidates for simpler and easier biomolecule detection is a micro-calorimeter [6, 7], which detects thermal energy released or absorbed during biological reactions. It does not need immobilization, which makes biological transduction process simple and easy. However, it needs relatively complex and time consuming fabrication steps such as bulk micromachining. Another new emerging candidate for biosensing applications is a microwave passive because its structure is simple and it does not need post process and off-site verification. Its feasibility for bio/chemical sensing application was reported [8].

In this study, it is presented a structurally simple and direct biomolecule detection method. For structural simplicity, it is utilized a microwave passive, slotline ring resonator with coplanar waveguide (CPW) feeding line, whose resonant frequency is 3.4 GHz (From now on, it is named an 'original resonator'). The original resonator is composed of a substrate and just one metal layer. At the same time, the original ring resonator was modified for higher sensitivity (This is named a 'modified resonator'). Sensitivity enhancement of the modified resonator was achieved by additionally utilizing a region on which most of electromagnetic field is concentrated.

After realization of the two types of the resonant biosensor, they were characterized through biotin and fluorescence-molecule-labeled streptavidin. Detail description for design, fabrication, experimental protocol and results is presented in following sections.

2 MATERIALS AND METHODS

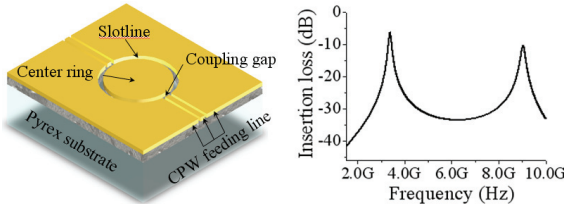
2.1 Design

For developing a simple biomolecule sensing scheme, a microwave resonator, CPW-to-slotline ring resonator, was designed. Characteristics of the microwave resonator whose resonant frequency is 3.375 GHz was examined by using a commercial electromagnetic (EM) simulator, IE3D (Bay Technology Group. Inc., USA). Overall dimension of the resonator was 17 by 9 mm in its length and width. Table 1 lists design parameters, Fig. 1 shows schematic of the original resonator and its simulation result. The original resonant biosensor is composed of just two layers, a Pyrex (BoroFlat33) substrate and a metal layer (nickel and gold).

As shown in Fig. 2, most of EM field is concentrated on the slotline. However, the original resonant biosensor does not use the slotline region as sensing area because there is no gold layer, which provides biotins with binding sites. For higher sensitivity, it is necessary to utilize a region on which EM field is high, and to increase an effective sensing area. In this sense, a dielectric layer was introduced for electrical insulation and for forming a gold layer on the slotlilne as well as center ring and outer ring region. Figure 3 is schematic and cross-sectional view of the modified resonant biosensor.

Table 1. Design parameters of the resonant biosensor

Parameter	Dimension
Overall dimension (length x width)	17 x 9 mm
Diameter of the sensing region	6.2 mm
Width of the slot	100 μm
Width of the feeding signal lines	80 μm



(a) Schematic (b) EM simulation result

Figure 1: Schematic and EM simulation result of the original resonator

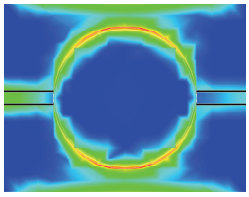


Figure 2: Electromagnetic field distribution on the CPW-to-slotline ring resonator

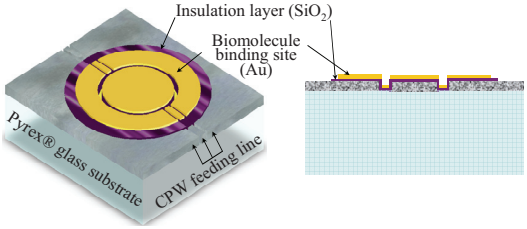
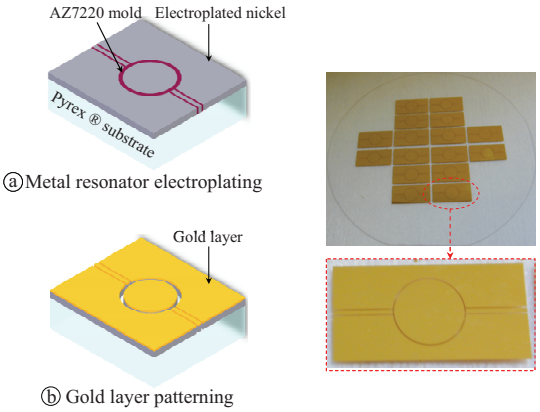


Figure 3: Schematic and cross-sectional view of the modified resonant biosensor

2.2 Fabrication

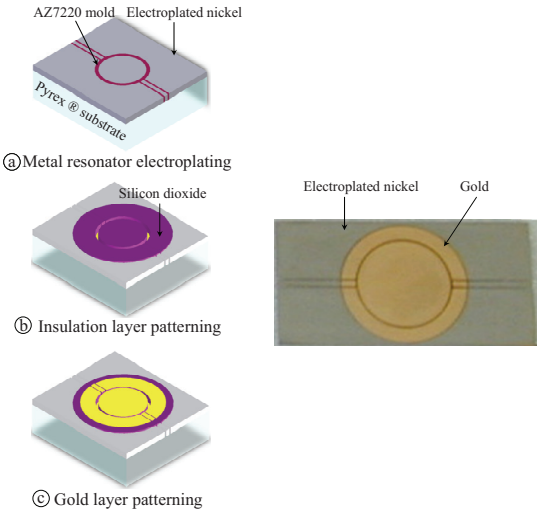
Fabrication of the original resonator can be summarized to define metal resonator and gold. It starts from seed layer deposition (3000 Å-thick copper on 500 Å-thick titanium) followed by 2 μm-thick nickel electroplating. Then 2000Å-thick gold layer was defined to provide biomolecules with binding sites, and then each die was separated. Figure 4 shows simplified fabrication sequence and optical photograph of a fabricated original resonant biosensor.

Fabrication of the modified resonator can be summarized to define metal resonator, an insulation layer, and gold. It starts from seed layer deposition (same thickness with the original resonator) followed by 2.5 μm-thick nickel electroplating. Then a dielectric layer, 2000 Å-thick silicon dioxide, was deposited and patterned with HF solution. A 500 Å gold layer was defined on a 200 Å-thick chrome layer. Finally, each die were separated. Figure 5 is fabrication steps and its result.



(a) Fabrication sequence (b) Optical photograph

Figure 4: Simplified fabrication process and optical photograph of the original resonant biosensor



(a) Fabrication sequence (b) Optical photograph

Figure 5: Simplified fabrication process and optical photograph of the original resonant biosensor

2.3 Biomolecule Preparation

Characteristics of the two resonant biosensors were examined by using biotin and streptavidin whose specific reactivity has known to be very high. In this study, biotin-HPDP (hexyl-3'-(2'-pyridyldithio) propionamide, Pierce Biotech. Inc., USA) was used to provide a ligand to streptavidin (Sigma-Aldrich, Inc., USA). Pyridyldithiol reacts with a thiol group (-SH) to form stable disulfide bond. To verify whether biotin and streptavidin is attached on the slotline region of the modified resonant biosensor, streptavidin-FITC (fluorescein isothiocyanate) was used. Concentration of the biotin and streptavidin was 0.1 *mg/ml*. Phosphate buffered saline (PBS) solution of pH 7.2 was used for washing.

3 MEASUREMENT AND RESULT

At first, the original resonant biosensor was characterized and then it was verified how much sensitivity of the modified biosensor is enhanced.

Characterization of the original resonant biosensor starts from measuring its first harmonic resonant frequency which was 3.410 GHz. Then 5 μl biotin was immobilized on the gold surface with 5 μl dithiothreitol (DTT). Unbound biotins to the gold surface were washed away by 200 μl phosphate buffered saline (PBS). Resonant frequency after biotin immobilization was measured after all PBS solution was dried out, which was 3.345 GHz. For checking reaction status between biotin and streptavidin, FITC labeled streptavidin was used. 10 μl fluorescence-labeled streptavidin was hybridized for 1 hour, and PBS washing was carried out. Finally, resonant frequency (3.335 GHz) was recorded after PBS dried out for 20 minutes. Figure 6 is experimental setup and Fig. 7 is a fluorescence image which indicates that biotin and streptavidin were well bound to the gold surface. Figure 8 shows resonant frequency shift as biotin and streptavidin was bound to the gold surface. Sensitivity was 260 MHz/mg for biotin and 20 MHz/mg for streptavidin, respectively.

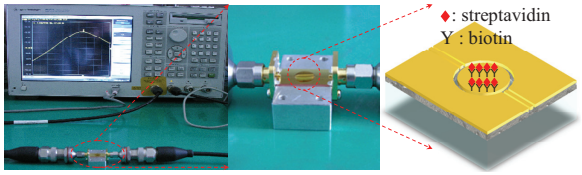


Figure 6: Experimental setup for characterization of the biosensors

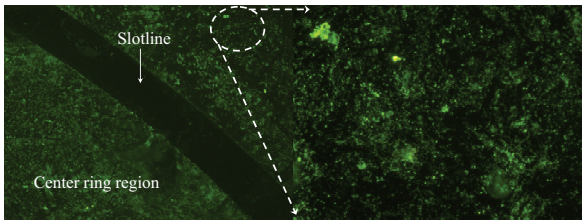
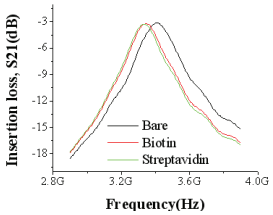
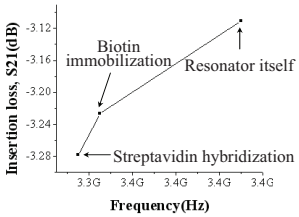


Figure 7: Fluorescence image that shows biotin and streptavidin is attached on the surface of the original resonator except slotline region



(a) Frequency spectrum



(b) Insertion loss change

Figure 8: Frequency spectrum and its change as biotin and streptavidin is attached on the original resonator surface (Resonant frequency and insertion loss value decreases as biotin and streptavidin attached on the resonator surface)

Exactly same experimental procedures were carried out to check characteristics of the modified resonant biosensor. According to the fluorescence images, it was verified that FITC labeled streptavidin is hybridized on slotline as well as center ring and outer ring region (Fig. 9). First harmonic resonant frequency of the modified resonant biosensor was 3.362 GHz, and it shifted to 3.283 GHz and 3.265 GHz as biotin and streptavidin is attached, respectively. Figure 10 shows resonant frequency spectrum and shift of the modified resonant biosensor for biotin and streptavidin. Sensitivity was calculated to 316 MHz/mg for biotin and 36 MHz/mg for streptavidin, which is 18% and 40% increased result compared to the sensitivity of the original type for each.

Table 2 summarizes measurement results. According to the results, their resonant frequency decreases as biotin and streptavidin is attached on the surface of the resonators. This phenomenon can be explained by following reasons. When biomolecules binds to surface of the resonators, dielectric constant of the resonators is changed. This leads change on effective dielectric constant of the resonator, which results in resonant frequency shift. This can be expected according to a theory [9] and is supported by a previous study [10].

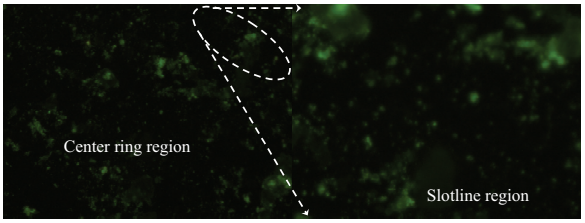
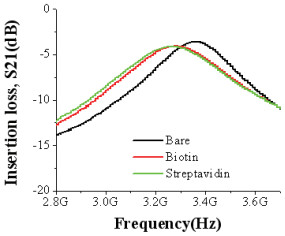
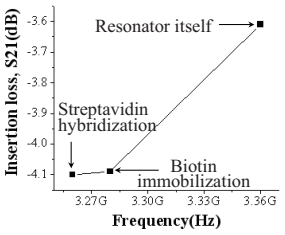


Figure 9: Fluorescence image that shows biotin and streptavidin is attached on all gold surface of the modified resonator including slotline region



(a) Frequency spectrum



(b) Insertion loss change

Figure 10: Frequency spectrum and its change for the modified resonant biosensor

Table 2. Measured sensitivity for two resonant biosensors (f_r: resonant frequency, Δf: resonant frequency change)

		Resonator		Biotin		Streptavidin
Original resonant biosensor	f_r	3.410GHz		3.345GHz		3.335GHz
	Δf		65 MHz		10 MHz	
	Sensitivity	Biotin: 260 MHz/mg, streptavidin: 20 MHz/mg				
Modified resonant biosensor	f_r	3.362GHz		3.283GHz		3.265GHz
	Δf		79 MHz		18 MHz	
	Sensitivity	Biotin: 316 MHz/mg, streptavidin: 36 MHz/mg				

4 CONCLUSION

For simple and direct biomolecule detection, a microwave passive, CPW-to-slotline ring resonator whose resonant frequency is 3.4 GHz was presented, and its application feasibility to biosensing field was reported. Conventional biosensors often need bulky optical instrument, time consuming pre/post processing steps or off-site verification.

The proposed resonant biosensors have simple structure, easy to fabricate, do not need time consuming pre/post process and off-site verification process. The original resonator was fabricated with just one metal layer, and was characterized. Measurement results showed that its sensitivity is 260 MHz/mg for biotin and 20 MHz/mg for streptavidin in terms of resonant frequency change, respectively.

For sensitivity enhancement, the original resonator was modified by increasing sensible area and by utilizing a region on which electromagnetic field intensity is concentrated. For prohibiting electrical shot, a nickel resonator and a gold layer was separated by dielectrics, silicon dioxide in this case. Sensitivity was calculated to 316 MHz/mg for biotin and 36 MHz/mg for streptavidin, which is 18% and 40% enhanced result compared to the sensitivity of the original type for each.

Bioanalysis systems have been being developed with two branches. One is extremely accurate solution. The other is very cheap one (e.g. disposable) even though its sensitivity is sacrificed to an extent. In this study, target application is latter one. Due to simplicity of the proposed resonant biosensor, it is expected that the proposed microwave resonant biosensor can a candidate for exploring cost competitive and disposable bioanalysis system.

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