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A highly sensitive and label free biosensing platform for wireless sensor node system

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ABSTRACT

In this paper, we propose a radio-frequency (RF) biosensor platform based on oscillation frequency deviation at 2.4 GHz. Its feasibility is experimentally demonstrated with the well-known biomolecular binding systems such as biotin–streptavidin and deoxyribonucleic acid (DNA) hybridization. For a basic principle of our biosensing system, the impedance of a resonator with the biomolecular immobilization is at first varied so that the corresponding change results in frequency change of an oscillator. Especially, to enhance the sensitivity of the proposed system, a surface acoustic wave (SAW) filter having a high-Q factor (~2000) is utilized. From the resulting component, even a small change of oscillation frequency can be transformed into a large output amplitude variation. According to the experimental results, it is found that our system shows the low detectable limit (~1 ng/ml) and fast response time (~real-time) for different target biomolecules, i.e. streptavidin and complementary DNA (cDNA). As a result, we find that our device is an effective biosensing system that can be used for a label-free and real-time measurement of the biomolecular binding events.

1. Introduction

During the last decade, radio-frequency (RF) systems for bio/medical applications received a lot of attention due to their suitability for non-invasive, non-contact, and non-destructive detection capability as well as their low cost and low power consumption characteristics (Buchli et al., 1989; Shen et al., 1997; Zhang et al., 2001). In recent years, the RF biosensor platforms have been expansively investigated for the diverse healthcare applications associated with wireless communication system including radio-frequency identification (RFID), sensor node and so on (Kim et al., 2006; Farahi et al., 2007; Sun et al., 2009; Malhotra and Chaubey, 2003). However, suitable biosensing devices and circuits have not been investigated at microwave frequencies. For this reason, various RF biosensing schemes have been introduced for sensing of the antigen-antibody reaction as well as of cells. For examples, the biosensing devices utilizing interdigital capacitors (IDCs), single-walled carbon nanotubes (SWCNTs) and metamaterial elements have been studied for the detection of label-free biomolecular interactions (Lee and Yook, 2008; Lee et al., 2010a, 2010b; Kallemudi and Gurbuz, 2011). In addition, the micro-electromechanical system (MEMS)-based RF biosensors for living cell detection have been also studied (Dalmay et al., 2010a, 2010b; Grenier et al., 2010). These approaches reveal clear possibilities in RF biomolecular sensing, but they still bear key disadvantages, such as need of expensive equipment, complex measurement system, which is a RF probe system associated with vector network analyzer (VNA), and sophisticated micro-fabrication process. In case of metamaterial inclusions for biosensing, to enhance sensitivity of the biosensing scheme, a resonator of high-Q performance has been essentially required.

In the present work, an RF active sensing system based on oscillation frequency deviation (OFD) at 2.4 GHz (Kim et al., 2012) is applied for detection of well-known and important biomolecular binding systems, such as biotin–streptavidin and DNA hybridization. These coupling systems were employed as following reasons: the biotin–streptavidin coupling system can be utilized to study a wide variety of biological structures and processes. Actually, it has proven to be particularly useful in the detection and localization of antigens, glycoconjugates, and nucleic acids by employing biotinylated antibodies, lectins, or nucleic acid probes. Meanwhile, DNA hybridization is a molecular biology technique that can measure the degree of genetic similarity between pools of DNA sequences. In addition it is usually used as molecular diagnostic agents.

2. Materials and methods

2.1. Principle and configuration of system

Fig. 1(a) shows the operating principle of the proposed RF biosensing system. The system consists of an oscillator, a SAW filter, and a power detector. The biosensing mechanism of our system is as

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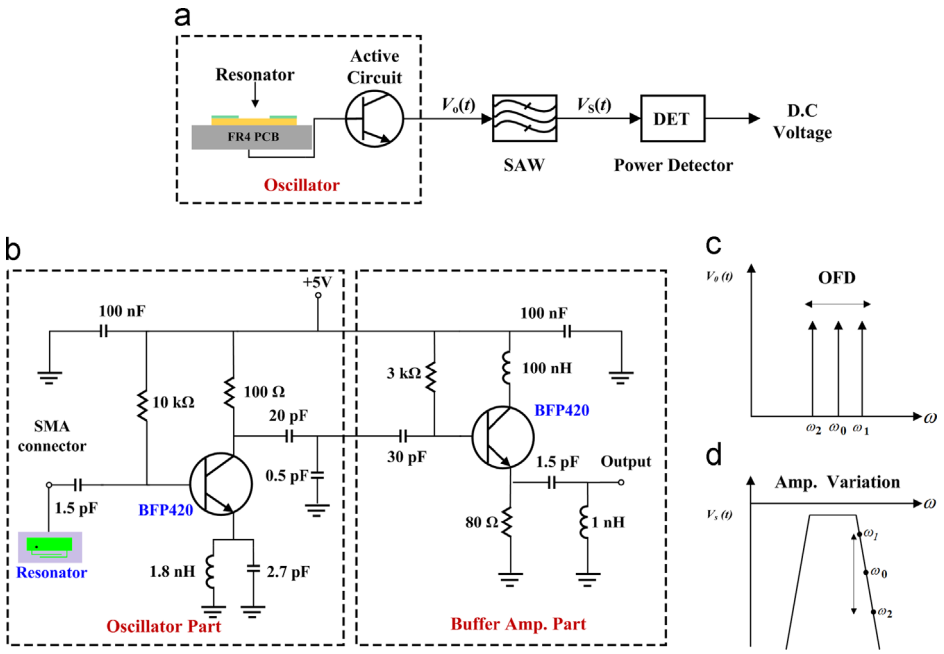

Fig. 1. Schematic diagram of proposed RF biosensing system. (a) Operating principle of the biosensing system. (b) Schematic of oscillator and buffer amplifier. Output spectra of the oscillator (c) and of the SAW filter (d).

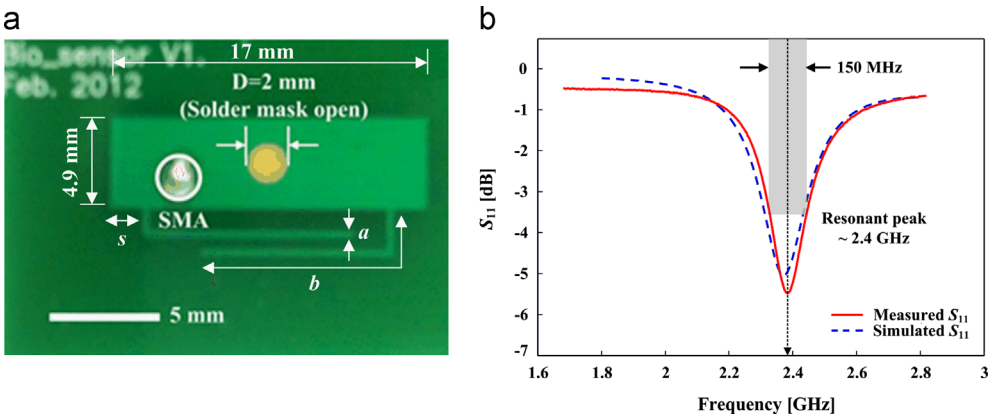

Fig. 2. Fabricated resonator for biosensing system and its resonant characteristic. (a) The fabricated resonator coated with masking layer (green) except sensing part. The length and width of the rectangular patch are 17 mm and 4.9 mm, respectively. The dimensions of the two folded arms are a=0.3 mm and b=12.2 mm, and these are symmetrically located at the corners of the rectangle at an offset of s=0.3 mm. (b) Simulated and measured result of the resonator. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

follows: first, the oscillation frequency is changed by the impedance variation of the planar resonator due to the biomolecular immobilization. Here, the immobilization has an effect on the surface characteristic of the resonator, thus changing the resonator impedance component, such as resistive (R), inductive (L), and capacitive (C). Moreover, the resonator plays important roles in sensing as well as feedback component in our system. Since the target biomolecules are much small in size and weight-mass, the frequency deviation due to the immobilization is extremely small. For above reason, with conventional circuit topologies the detection capability is not sensitive enough for medical applications. To overcome this difficulty, a surface acoustic wave (SAW) filter is used at the oscillator output to increase the sensitivity of the system. Assuming that the impedance variation and resulting frequency deviation are in the locking range of the oscillator, the amplitude of the SAW filter output can be maximized in the skirt frequency region. As a result, even though the frequency deviation is small, it can be transformed into a large output amplitude variation by the SAW filter. In the last stage, an RF power detector is adapted in the output of the SAW filter to transform the frequency deviation of the oscillator to the variation of DC voltage, which can be easily digitized for digital signal processing. With this approach, the oscillation frequency deviation can be eventually used for biosensing as illustrated in Fig. 1(a). In particular, Fig. 1(b) shows in detail the schematic diagram of the 2.4 GHz amplifier and the cascade type resonator combined with an emitter follower-type buffer amplifier (Takaoka and Ura, 1980; Regis et al., 1998). Here, the buffer amplifier of the emitter follower-type is used to prevent a strong mutual interaction between the oscillator and the filter because the oscillation condition can be changed by the rapid impedance variation at the skirt frequency range of the SAW filter. Fig. 1(c) and (d) represents the output spectra of the oscillator and the SAW filter, respectively.

2.2. Simulation and fabrication of sample

Fig. 2(a) shows the fabricated resonator associated with the oscillator in our system. This is fabricated on a dielectric substrate

(ε_r=4.4 and tan δ=0.01) and the metallic surface of the top (resonator) and bottom (ground plane) electrodes is coated with gold. The overall size of the sensing part, including the ground plane is about 30 mm by 20 mm. The top surface is coated with masking layer, and circular sensing area having a diameter of 2 mm is not coated with masking layer for detection of the biomolecular interaction. To predict the resonant characteristics of the resonator, simulations have been performed with a 3D full-wave electromagnetic solver. The resonance occurs in the vicinity of 2.4 GHz with 3-dB bandwidth of 150 MHz; thus, the loaded Q value is about 15. From the obtained results, the simulated results are in good agreement with the measured result, as shown in Fig. 2(b). Conventionally, a high-Q resonator is used in oscillator design to improve the frequency stability and phase noise characteristic (Everard, 2001). However a low-Q resonator is preferable in our system due to the fact that the frequency instability is utilized for sensing of biomolecular (Kim et al., 2011). Note that the suitable impedance control around a bipolar transistor can be achieved with an oscillation condition at the resonant frequency (Rhea, 1995; Giannini and Leuzzi, 2004).

2.3. Experimental set-up and performance of system

Fig. 3(a) shows the schematic of biosensing platform associated with measurement system including power supply, digital multimeter, and spectrum analyzer. In this work, the applied voltage and current for biosensing system are 6 V and 75 mA, respectively. The spectrum analyzer is used to confirm the oscillation frequency deviation, and the output voltage of the sensing system, which is DC voltage, is measured by a digital multimeter. Fig. 3(b) shows the frequency response of the used SAW filter (SA2441AM from SAWNICS). The designed oscillator operates at 2.52 GHz which is in the skirt frequency range of the SAW filter, and the average rate of change in the magnitude variation is 1 dB/MHz in the skirt region. The output amplitude variation is then detected by an RF power detector (AD8312 RF detector from Analog Devices Inc.). Fig. 3(c) shows the measured result of the power detector and its detection range is from −45 to 0 dBm. The expected DC voltage variation of the RF power detector output is about 120 mV_{dc} under the oscillation condition. As a result, it is found that our system can be used for a biosensing platform with simple and convenient measurement equipment.

2.4. Immobilization of biomolecule

In order to verify the feasibility of the biosensing device, the biotin–streptavidin and DNA hybridization are used. For the functionalization of the sample, we have exploited the fluorescent image with and without antibody (Lee et al., 2012). From the previous study, the biological binding process for immobilization on gold surface is performed as shown in Fig. 4. Here, a thiol (–SH)-linked biotin and ssDNA are used because the anchor group is responsible for the chemical link with the gold surface. In addition gold and sulfur create a solid bond that gives stability to the linked biotin and ssDNA. Thus, it has been widely used as a biological receptor of many gold-based biosensors (Seifert et al., 2010; Haeussling et al., 1991; Pradier et al., 2002). In this work, the biological process is as follows: in case of biotin–streptavidin binding, first, all samples are simultaneously immobilized with 25 μg/ml concentration of biotinylated thiols in deionized (DI) water for about 1 h. Next, to deactivate and block the excess reactive groups remaining on the surface, the devices are treated with bovine serum albumin (BSA) of 25 μg/ml concentration for about 1 h. Finally, biotin has been coupled with different streptavidin concentrations, i.e. 100, 10, and 1 ng/ml in DI water about 1 h. Similarly, the biological process of DNA hybridization has progressed with the same method as the biotin–streptavidin system. All samples are also immobilized with 25 μg/ml concentration of thiol-linked ssDNA (5′-CTA gAA TTC TgC CAC TTT ATA CAT TCC-3′) in phosphate buffer solution (PBS) for about an hour. After treating with BSA, the ssDNA was coupled with three different target DNA (5′-ggA ATg TAT AAA gTg gCA gAA TTC TAg-3′)

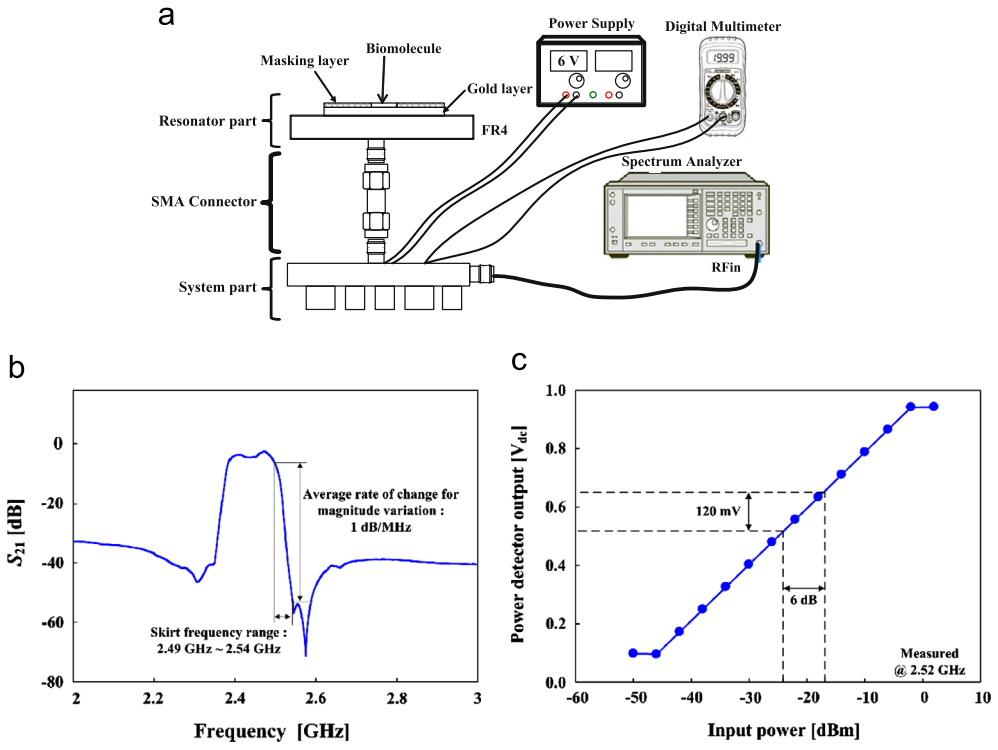

Fig. 3. Schematic of biosensing platform and its component characteristics. (a) The biosensing platform associated with measurement system. (b) Frequency response of the SAW filter. (c) Voltage response of power detector at 2.52 GHz.

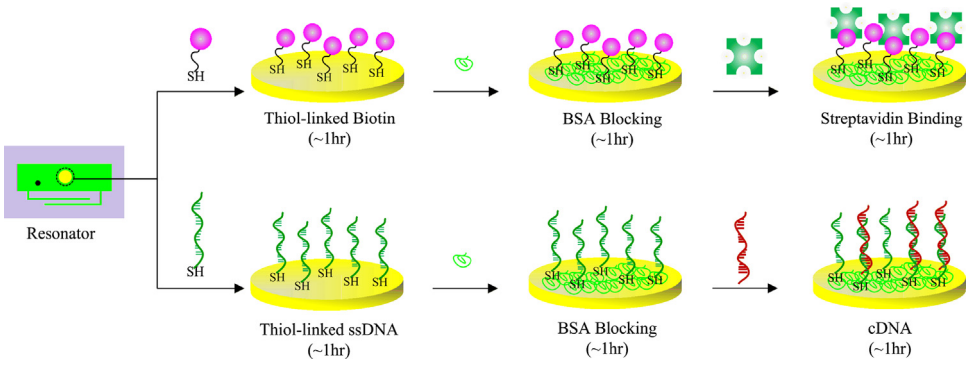

Fig. 4. Schematic representation of biotin–streptavidin and DNA hybridization.

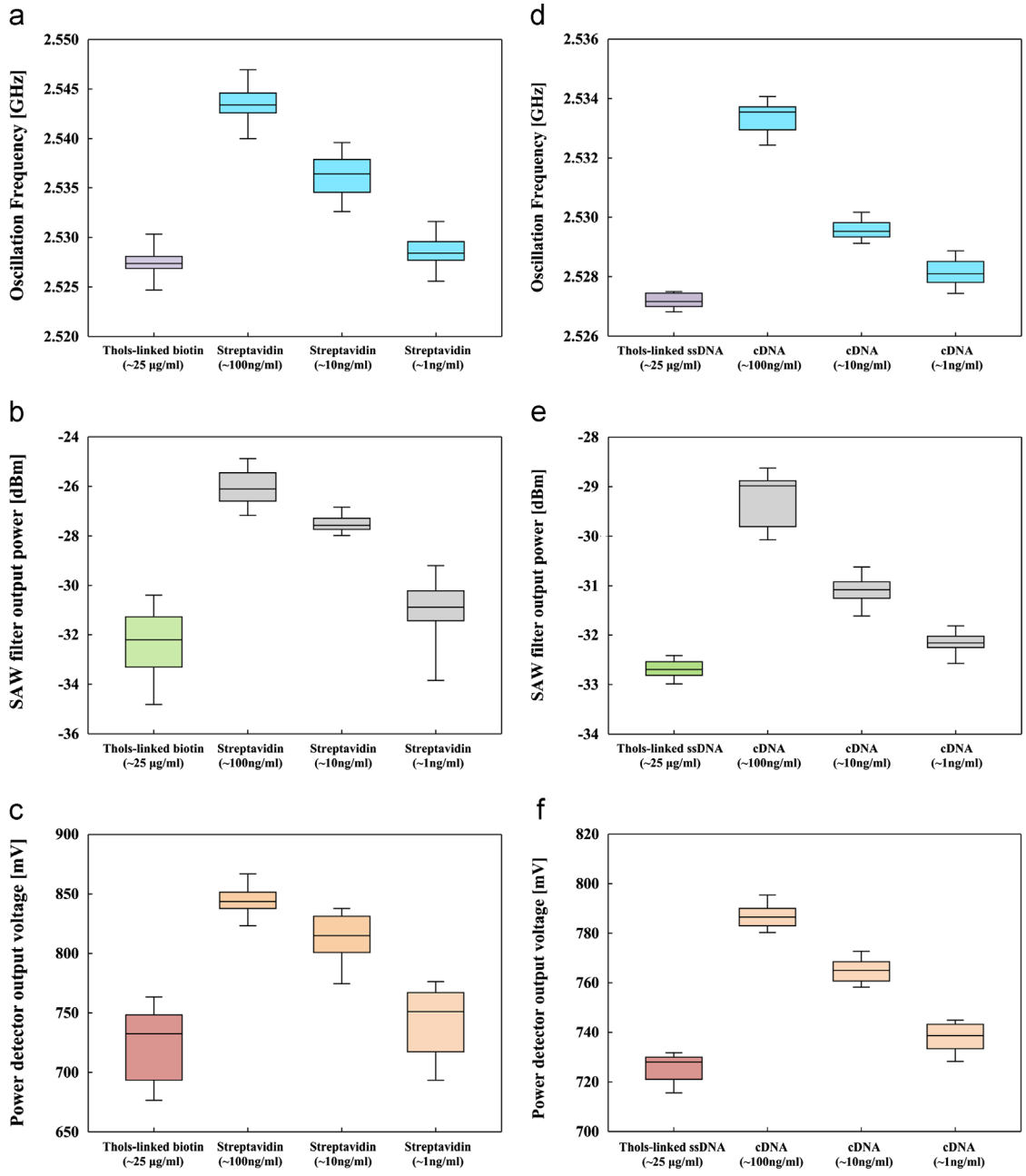

Fig. 5. Frequency, amplitude, and voltage as the biomolecular immobilization. (a) Oscillation frequency deviation, (b) output power, and (c) power detector of the biotin–streptavidin binding system. (d) Oscillation frequency deviation, (e) output power, and (f) power detector of the DNA hybridization system.

Table 1
Summary of experimental results.

Configurations		Biotinylated thiols (~25 μg/ml)	Streptavidin[a]			Thiols-linked ssDNA (~25 μg/ml)	cDNA[a]		
			(100)	(10)	(1)		(100)	(10)	(1)
Oscillation frequency deviation [MHz]	Max	10.4	27.1	19.6	11.7	7.5	14.1	10.1	8.9
	Avg	7.5	23.5	16.5	8.6	7.2	13.4	9.6	8.1
	Min	4.5	19.8	12.6	5.4	6.8	12.4	9.1	7.4
SAW output power variation [dBm]	Max	9.5	15.0	12.4	10.7	7.4	11.2	9.2	8.0
	Avg	7.5	13.8	12.2	8.6	7.1	10.6	8.7	7.6
	Min	4.9	12.6	11.8	5.7	6.9	9.7	8.4	7.3
Power detector voltage variation [mV]	Max	184	288	258	197	152	216	193	165
	Avg	144	264	235	165	146	207	185	158
	Min	96	242	193	111	135	201	178	148

[a] Unit of streptavidin and cDNA concentration is ng/ml.

concentrations, i.e., 100, 10, and 1 ng/ml in PBS for about 1 h. Finally, after washing with pure DI water or PBS, the samples are measured with the proposed measurement system.

3. Results and discussion

3.1. Sensing parameters of the system

Before starting the biological process, we have tested the effect of frequency deviation of DI solution on the resonator. Three samples are used and each sample is measured five times for oscillation frequency deviation. After drying process, the measured samples show slight differences compared to bare samples. However, it is found that the DI solution causes a minimal effect on OFD. The other samples (15) have been also prepared for biological process, and each sample is measured five times for the variations of oscillation frequency, SAW filter output power, and the power detector output voltage for each biomolecule.

3.2. Detection of target biomolecules

Fig. 5(a)–(c) shows the boxplot of the measured results of the afore-mentioned sensing parameters for thiols-linked biotin and streptavidin with different concentration levels of streptavidin and cDNA. Each sensing parameters of biotinylated thiols exhibit smaller variation compared to the streptavidin with different concentration levels, i.e. 100, 10, and 1 ng/ml. This is a reasonable result because biotin (~647 Da) (NanoScience Inc.) is of lower weight-mass than streptavidin (~55 kDa) (Jackson ImmunoResearch Laboratories Inc.). Meanwhile, Fig. 5(d)–(f) also shows the boxplot of the measured results of sensing arameters for thiols-linked ssDNA and cDNA with different concentrations. Similarly, the thiols-linked ssDNA shows smaller variation to the sensing parameters of cDNA with different concentration levels. In our work, ssDNA (~8.1 kDa) and cDNA (~8.4 kDa) (Bio Basic Canada Inc.) for DNA hybridization have been used. Note that the sensing parameters of ssDNA show smaller variation compared to the cDNA with different concentrations. In particular, even though the difference in weight-mass between the ssDNA and cDNA is not large, our biosensing system clearly shows the discrimination for the DNA hybridization. From the obtained results, it is found that the sensing parameters exhibit approximately linear shifts with three different streptavidin as well as cDNA concentration levels. Thus, it is clearly demonstrated that our system associated with oscillator is able to detect ng/ml concentration level and the estimated limit of detection (LOD) is about 1 ng/ml. The average variations of each sensing parameter for four different configurations, i.e. biotinylated, biotin–streptavidin, ssDNA and cDNA, are summarized in Table 1. According to Table 1, the biotinylated thiols and thiols-linked ssDNA with the same concentration (~25 μg/ml) show similar variation to three sensing parameters. This is due to the use of the same thiols substrate to the two antibodies, i.e. ssDNA and biotin. In case of target biomolecules, i.e. streptavidin and cDNA, streptavidin shows the larger variation compared to cDNA because of weight-mass difference between two biomolecules.

3.3. Validation and limitation of the system

Our system shows the performance of label-free detection and rapid response as a sensing platform. After drying a immobilized sample, it is possible to immediately recognize the biomolecular binding events by the real-time method. The detectable limit of our system is considerablely improved compared to the conventional RF active biosensing system, which is composed of a voltage controlled oscillator (VCO) and an interdigital capacitors (IDCs), because the frequency of the system is shifted about 2 MHz (from 1.9438 GHz to 1.94175 GHz) in the presence of 800 ng/ml cardiac-reactive protein (CRP) antigen (Heves et al., 2009). However, in order to directly detect a target biomolecule in the current system, the immobilized sample should be dried because the effect of solution is greatly influenced by the resonant frequency.

4. Conclusions

We have proposed an RF biomolecular sensing system based on OFD and experimentally demonstrated for label-free biomolecular detection at 2.4 GHz. The biosensing system shows the rapid and sensitive frequency response for two different biomolecules, i.e., streptavidin and cDNA. In addition we find that the frequency shift of our system can reflect the difference of weight-mass of these target biomolecules. Furthermore, it is a cost-efficient, simple and direct biosensing platform with the resonator-based RF active system that does not require a complex and sophisticated equipment. However, for recognizing the biomolecular binding event, the immobilized resonator should be dried. Thus, in order to overcome the limitation of our system, it is necessary to additionally improve a RF system that can detect the target biomolecules as well as cells in diverse solutions. If the limitation is resolved, we expect that our system becomes a very powerful candidate for wireless biosensing node in biomedical as well as environmental field in the future.

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