A New Lab-on-Chip Transmitter for the Detection of Proteins Using RNA Aptamers

Ferhat Tasdemir[#1], Samet Zihir[#2], Emre Ozeren[#], Javed H. Niazi[#], Anjum Qureshi[#], S. Saravan Kallempudi[#], Mehmet Kaynak*, Rene Scholz*, Yasar Gurbuz[#3]

[#] *Sabanci University, Faculty of Engineering and Natural Sciences, Tuzla, 34956, Istanbul, Turkey*

**IHP Microelectronics, Im Technologiepark 25 15236 Frankfurt (Oder), Germany*

[1]ferhat@sabanciuniv.edu

[2]sametzihir@sabanciuniv.edu

[3]yasar@sabanciuniv.edu

Abstract— **A new RNA aptamer based affinity biosensor for C-Reactive Protein (CRP), a risk marker for cardiovascular disease was developed using interdigitated capacitor (IDC), integrated in Voltage Controlled Oscillator (VCO) and output signal is amplified using Single Stage Power Amplifier (PA) for transmitting signal to receiver at Industrial, Scientific and Medical (ISM) band. The Lab-on-Chip transmitter design includes IDC, VCO and PA. The design was implemented in IHP 0.25µm SiGe BiCMOS process; post-CMOS process was utilized to increase the sensitivity of biosensor. The CRP was incubated between or on interdigitated electrodes and the changes in capacitance of IDC occurred. In blank measurements, the oscillation frequency was 2.464GHz whereas after RNA aptamers were immobilized on open aluminum areas of IDC and followed by binding reaction processed with 500pg/ml CRP solution, the capacitance shifted to 2.428GHz. Phase noise is changed from -114.3dBc/Hz to -116.5dBc/Hz.**

I. Introduction

Biosensors which have fast, direct and label-free responses make them attractive for biological applications. There are different kinds of detection mechanisms used for biosensors. Some detection mechanisms require quantification of proteins whereas in Interdigitated Electrode-based capacitors (IDC), labeling is not a requirement which also minimizes the cost. In conventional IDC based biosensors, the biosensor is built on glass and IDC is made up of gold because the immobilization of RNA aptamers on gold is easy and better [1]. In these kinds of biosensors, readout circuit will be problematic if complex IC circuit is needed to read and process the signal. Therefore IC compatible biosensors are more efficient for Lab-on-Chip (LoC) applications.

For these kinds of applications, unlicensed ISM band which covers 2.4-2.5 GHz is used. RF integrated circuits like Voltage Controlled Oscillator (VCO) and Power Amplifier (PA) can be implemented using various technologies. Compared to other technologies, SiGe BiCMOS has advantages. SiGe BiCMOS technology has better performance than standard Si CMOS technology for this frequency ranges. Because of combining cost and integration advantages of Si, SiGe BiCMOS leads other high-performance technologies such as GaAs HBT, SiGe HBT. [2] Therefore for this biosensor design, IHP SiGe BiCMOS technology was selected.

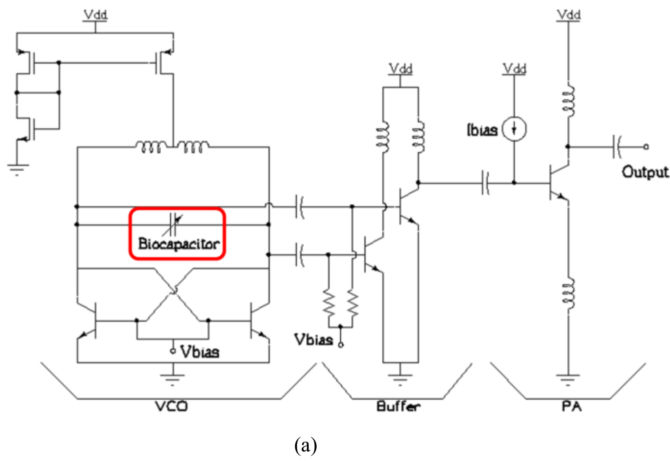
In this work, we aimed to develop label-free, new affinity biosensor using RNA-aptamer functionalized IDC-VCO for detection of C- Reactive Protein (CRP), a biomarker of cardiovascular disease. The biological signal is converted and transmitted using VCO and PA blocks at ISM band. The chip was designed with IHP 0.25 µm SiGe BiCMOS process which is advantageous to obtain high frequency and power signals compared to standard CMOS processes. In second section the circuit design will be explained, each block will be examined in detail, in third section the measurement flow and measurement results will be demonstrated.

II. Circuit Design

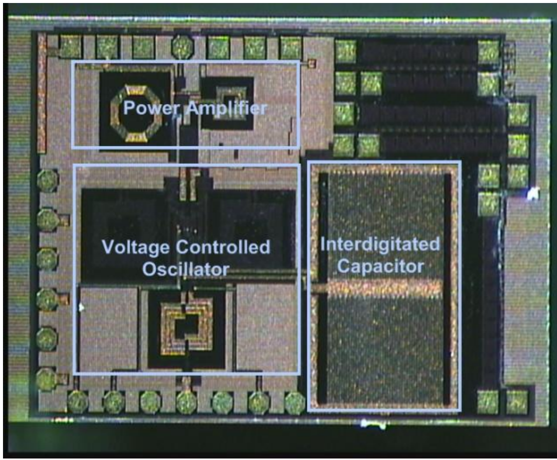
The proposed biosensor design consists of three important blocks, IDC, VCO and Single Stage PA. The working principle of the design is based on converting biological signal to electrical signal. Initially the biological signal will be converted to capacitance with IDC block. This capacitance will be converted to frequency at ISM band with VCO. Finally, PA amplifies the signal that VCO generates. Each block is illustrated in Fig. 1 and will be explained in detail. The schematic and fabricated/optical picture of the transmitter-based biosensor for Lab-on-Chip Applications presented in Fig 1 (a) and Fig. 1 (b), respectively. The interdigitated electrode-based capacitor used in the transmitter for converting biomolecules (proteins) into electrical signal is presented in Fig. 1 (a) as bio-capacitor and as interdigitated electrodes in Fig. 1 (b).

A. Interdigitated Electrode-Based Capacitors (IDC)

IDC consists of parallel electrodes to increase the parallel plate capacitance between two different potentials. Top Metal 2 (Metal 5) of IHP 0.25µm process was used for IDC capacitor design. As indicated in Focused Ion Beam (FIB) Microscopy image of IDC in Fig. 2, the gap between each electrode and width of each electrode was chosen to be 5µm.



(a)



(b)

Fig.1 (a) Schematic illustration, (b) Optical Image of fabricated Chip

Main role of IDC in design is converting biological signal to electrical signal. As it is shown in Fig. 1, the capacitor is used as the varactor for VCO. The main reason why it is modeled as varactor is, its capacitance changes when a dielectric material is placed between/on electrodes. The capacitance of IDC is related to the permittivity of the sample that is put resides on/between the electrodes.

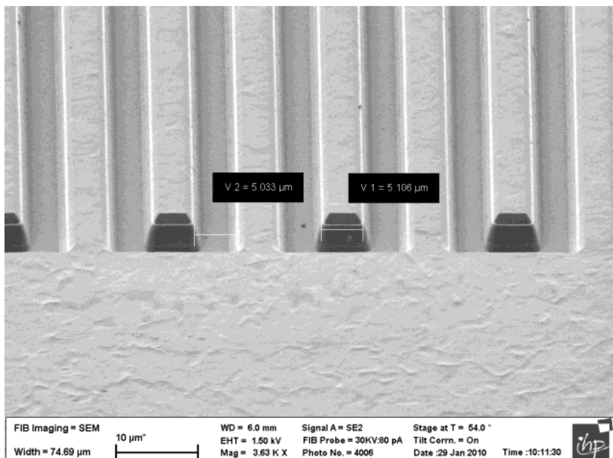
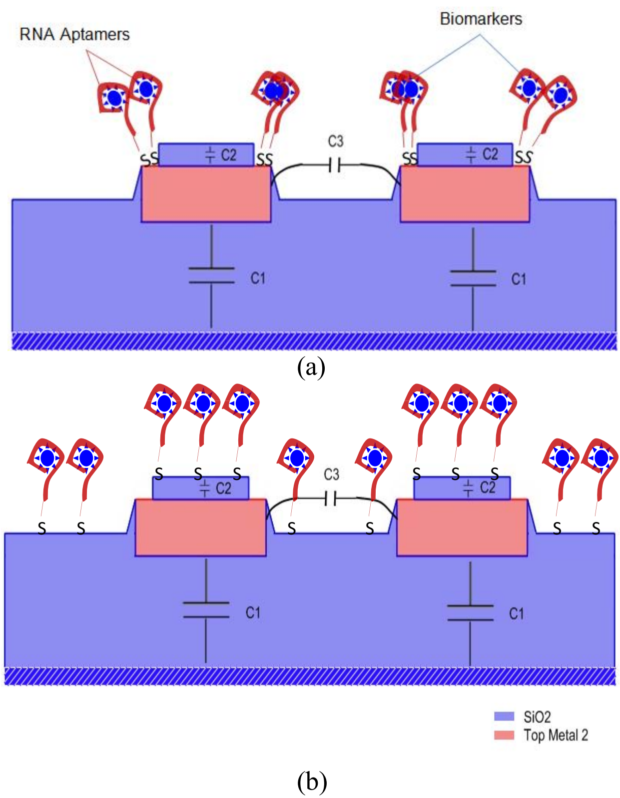


Fig. 2 FIB image showing IDC Structure



(a)

(b)

Fig. 3 Modeling IDC when RNA aptamers are bind to (a) Aluminum (b) SiO_2

The capacitance modeling of IDC is as shown in Fig. 3. Each electrode have three capacitor components; C1, C2, C3. C1 is capacitance between electrode and ground which is constant and cannot be changed externally. Its value is based on standard process according to manufacturer. C2's value is determined with fringing capacitance. As a result of having so thick Top Metal2 layer, the effect of fringing capacitance is not enormous. Furthermore, this value can be manipulated externally but change will not be high. C3 results from the parallel plate capacitance between electrodes. Even though there will be a offset part of C3, which is the capacitance of SiO_2 at sides of electrodes, the most efficient way to change the capacitance of IDC is to change C3.

RNA-aptamers were immobilized on aluminum (Top Metal2 material) and measured the change in IDC brought on by the binding effect. As shown clearly in Fig. 3(a), immobilization on aluminum showed considerable effect on C2 because the immobilization occurred on top of the electrode. In Fig. 3(b), the expected amount of aptamers that are immobilized can be increased because the possible RNA aptamer immobilization area on SiO_2 will be much higher compared to RNA-aptamers immobilized only on aluminum. Immobilization on SiO_2 requires more processing steps, affecting the sensitivity of biosensor. Therefore the RNA molecules are immobilized on aluminum instead of SiO_2.

B. *Voltage Controlled Oscillator (VCO)*

For VCO design –Gm topology was used as it can be seen in Fig. 1. Main aim of this design was to optimize phase noise.

To achieve low phase noise, current source is made by RF-PMOS transistors, which are known as their low phase noise behavior. In addition to this improvement, coupling capacitor which increases the phase noise, was not used at –Gm block for better phase noise behavior [3].

Because of base voltages are not isolated from collector voltages signal amplitude is limited but signal amplitude at oscillator stage is not an important specification because this signal is amplified at later stages. Buffer stage is needed for matching between Power amplifier and Oscillator while adding power gain to the signal. Finally PA stage is used to achieve high output power level.

C. Power Amplifier (PA)

In this design Single Stage PA was used to improve the output signal by compressing the harmonics further and increasing the output power level. While increasing output power level, the working frequency range was tried to be kept wide.

As presented in Fig. 1(a), to obtain maximum power transfer, output impedance of oscillator with its buffer must be conjugate match to input impedance of Power Amplifier.

III. Measurement Set-up and Results

For measurement of the LoC Transmitter chip, the produced chips were packaged and bonded. Except from probe connections and IDE capacitor, the chip was isolated with non-conductive epoxy. Hence bond-pads and other areas of the chip are protected from biochemical processes.

For biosensor measurements, a modified 44-mer single-stranded RNA aptamer that specifically binds to C-reactive protein (CRP) [4] was used as a biorecognition element for the detection of CRP. First, the RNA aptamer was custom synthesized through thiol-modification with C_6 linker at 5' end (Bioresearch Tech. Inc.). The modified RNA aptamer had the following sequence 5′-HS-(C_6)-GCCUGUAAGGUGGUCGGUGUGGCGAGUGUGUUAGGAGAGAUUGC-3′, which used for immobilization on the capacitors of the VCO. CRP was purchased from Sigma (USA) bearing a Cat. No. C4063.

After the chemicals were gotten ready, the capacitor surface was cleaned and the immobilization process was performed under sterile conditions. Self-assembled monolayer (SAM) formation on the capacitor surface was performed by incubating with 2 µl of 10 µM thiol-modified RNA aptamer in sterile distilled water for 2 h [5]. After the SAM formation, the capacitor electrodes of the VCO was gently washed twice with 2 µl sterile distilled water and dried with a stream of N_2 gas.

After immobilizing RNA aptamers, the binding reactions were carried out by incubating 1 µl of CRP in phosphate buffered saline (PBS) under sterile conditions with 500pg/ml concentration on IDC region of VCO. First the CRP was allowed to incubate on the electrode surface immobilized with modified RNA aptamer for 15 min. The unbound CRP was removed by washing twice with sterile PBS followed by washing with sterile distilled water. The moisture content was removed by gentle drying with a stream of N_2 gas and the frequency change was recorded.

In this design, the RNA-aptamer immobilization was performed on aluminum because it requires less processing steps that require only two steps. On the other hand, the immobilization on SiO_2 requires four processing steps [6,7]. Increasing number of processes may result in increased

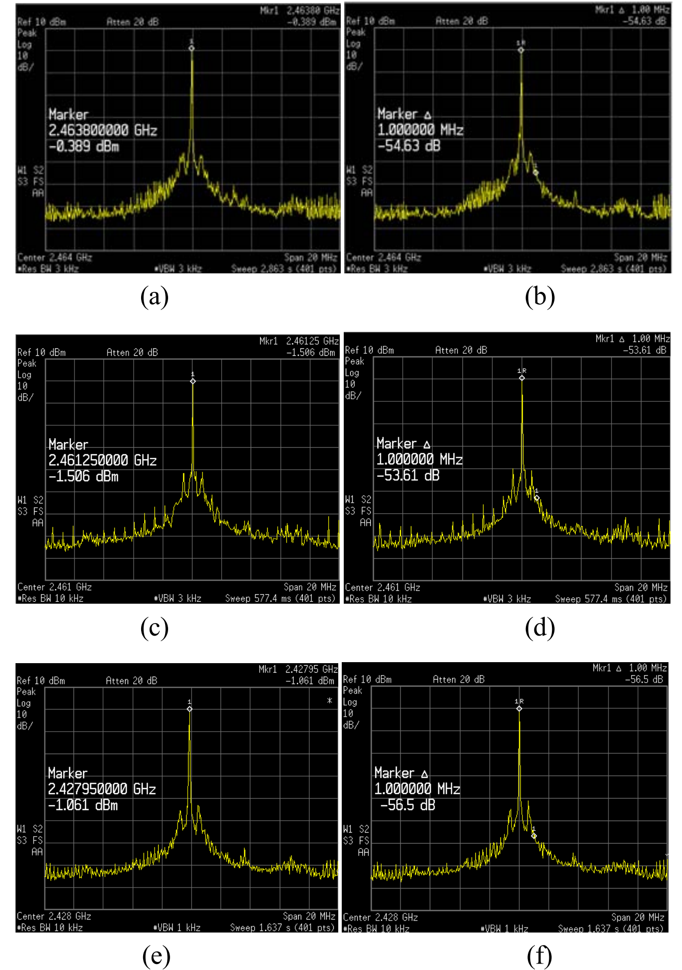


Fig. 4 (a) Frequency Spectrum (b) Phase Noise of Blank Chip; (c) Frequency Spectrum (d) Phase Noise after immobilization of RNA aptamer on ICD; (e) Frequency Spectrum (f) Phase Noise after binding protein with using 500pg/ml CRP solution

detrimental effects on IDC, since salt particles deposit even if the cleaning is thorough. Therefore, there is a trade-off between immobilization on aluminum and SiO_2. We made initially immobilization on aluminum and immobilization of RNA aptamers on SiO2 will be applied as further improvement of this project.

Measurements were taken for blank chip, after immobilization process and after binding reactions were performed. To observe the stability of biosensor, results were taken three times for each measurement step. The output signal was stable and each triplicate result for same sample gave consistent results.

The frequency and phase noise measurement results obtained with 20MHz span are given in Fig. 4. Frequency

measurements, obtained three times for each step, have 50 kHz error margin. Frequency value for blank chip was measured as 2.4648GHz and phase noise at 1MHz offset is -114.3dBc/Hz. After immobilization of RNA aptamer process finished, the oscillation frequency shifted to 2.4613GHz and phase noise was -113.61dBc/Hz. This small amount of change is acceptable because the effect of RNA aptamers to capacitance change was expected to be small. After the last step (binding reaction), new frequency value shifted to 2.4280GHz and the phase noise was decreased to -116.5dBc/Hz. The frequency value decreased, because the permittivity of dielectric between and on the electrodes was increased. This results in decrease in frequency because of inverse relationship between capacitance of biosensor and output frequency of VCO which can be clearly shown in the following equation.

$$f = \frac{1}{2\pi\sqrt{LC}} \quad (1)$$

By using Eqn. 1, which relates to capacitance and frequency, the capacitance values were calculated as well. The inductance value is 1.6nH, after using the measurement results; the capacitance value for blank chip is 2.603pF. After SAM, the capacitance value has shifted to 2.608pF, which means that there is a 5fF change referenced to blank measurements. The capacitance value was 2.680pF for 500pg/ml CRP solution which supports that there is an effect of binding CRP to immobilized aptamer on capacitance value of IDC. The capacitance change is 77fF compared to blank chip measurements.

Furthermore, measured output signal levels of transmitter with and without protein bound are close to each other. As Fig. 4(a) and 4(c) shows, the output signals for blank chip and protein bound chip are -0.369dBm and -1.061dBm. The VCO signal is amplified enough to be transmitted. The noise signals are suppressed by PA to obtain reliable measurement results.

TABLE I
COMPARISON OF MEASUREMENT RESULTS

Measurement Condition	Osc. Freq. (GHz)	Pout (dBm)	Phase Noise (dBc/Hz)	Cap. (pF)
Blank Chip	2.4648	-0.369	-114.3	2.603
After SAM	2.4613	-1.506	-113.61	2.608
After 500pg/ml CRP Solution	2.4280	-1.061	-116.5	2.680

IV. CONCLUSIONS

A new RNA aptamer immobilized on IDC based affinity biosensor for detection of CRP, a risk marker for cardiovascular diseases integrated with VCO and PA for LoC applications at ISM band has been presented. The design was fabricated with using 0.25µm IHP SiGe BiCMOS process with post-CMOS process to thin the passivation layer on top of IDC to increase the sensitivity. The oscillation frequency for blank chip is 2.4648GHz that corresponds to 2.603pF capacitance value of IDC and phase noise is -114.3dBc/Hz at 1 MHz offset. The RNA aptamer was immobilized on aluminum and the binding protein process was performed with using 500pg/ml CRP target solution. After binding process, the frequency decreased to 2.4280GHz and the capacitance changed by 77 fF compared to blank measurements with no CRP.

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REFERENCES

[1] O. Gul, E. Heves, M. Kaynak, H. Basaga and Y. Gurbuz, “Label-free, capacitive immunosensor for protein detection”, in *IEEE Sensors 2006*, pp. 600-603, 22-25 Oct. 2006

[2] O. Esame, I. Tekin and Y. Gurbuz, “A 4.5-5.8 GHz Differential LC VCO using 0.35 µm SiGe BiCMOS Technology”, in *European Microwave Integrated Circuits Conference 2006,* pp. 417-420, 10-13 Set. 2006

[3] S. Bahramirad, J.G. Atallah and S. Albrect, “A low Phase Noise VCO for MultiBand Wireless Transceivers”, International Conference on Design&Technology of Integrated Systems in Nanoscale Era, 2-5 Sept. 2007, pp. 148 - 153.

[4] A. Bini, S. Centi, S. Tombelli, M. Minunni and M. Mascini, "Development of an optical rna-based aptasensor for c-reactive protein", *Analytical and Bioanalytical Chemistry*, vol.390, no.4, pp. 1077-1086 2008.

[5] M. Yang, H. Yau and H. Chan, "Adsorption kinetics and ligand-binding properties of thiol-modified double-stranded DNA on a gold surface", Langmuir, vol.14, no.21, pp. 6121-6129 1998.

[6] J.C. Love, L.A. Estroff, J.K. Kriebell, R.G. Nuzzo and G.M. Whitesides, “Self-Assembled Monolayers of Thiolates on Metals as a Form of Nanotechnology”, American Chemical Society, 2005, vol.105, no.4, pp. 1103-1170 2005

[7] L.A. Chrisey, G.U Lee and C.E. O’Ferrall, “Covalent attachment of synthetic DNA to self-assembled monolayer films”, Nucleic Acid Research, 1996, vol.24, no.15, pp.3031-3039 1996