ARAŞTIRMA MAKALESİ / Research Article

Nano-Amplification Strategy Using Charge-Based Capacitance Measurement for Pathogenic Bacteria Detection

Patojenik Bakteri Algılama İçin Sarj Tabanlı Kapasitans Ölçümü Kullanarak Nano-Amplifikasyon Stratejisi

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Abstract

Objective: This work will present a nano-amplification strategy for microfabricating Multi-Lab-On-Single-Chip (MLoC) system to enhance the level of detection and also reduced the number of probe pads for clinical and health care applications, such as pathogenic disease.

Material and Method: The Charge-Based Capacitance Measurement (CBCM) technique used Complementary Metal-Oxide-Semiconductor (CMOS) with 350 nm technology, the implementation material is doped silicon. The system employs an interdigitated capacitor structure, charge based capacitance measurement circuitry to detect and process the variation of the capacitance, which is a function of the permittivity in the presence of targeted bacteria that is applied to the sensing capacitor but not to the reference one.

Findings and Results: The system appeared good sensitivity for low level concentration of bacterial comparing to the literature results. The circuit was measuring the capacitance changes around 100 aF. The noise at the output of the circuit is around 78.7μV.

Conclusion: CMOS biosensor-based charge-based capacitance measurement system has been fabricated, testing and experimentally validated for bacterial pathogens cell detection. Three important requirements of such biosensor systems including functionalized sensing capacitance, passivating the reference capacitance and interface circuit were discussed. The system provides a rapid, low power, and miniaturized platform that can be mass-produced.

Keywords: Biosensors, CBCM, Escherichia coli, health applications, MLoC system, permittivity

Özet

Amaç: Bu çalışma, patojenik hastalık gibi klinik ve sağlık uygulamalarında tespit seviyesini arttırmak ve prob pedlerinin sayısını azaltmak için Multi-Lab-On-Single-Chip (MLoC) sistemi mikro imalatı için bir nano-amplifikasyon stratejisi sunmaktadır.

Materyal ve Metod: Uygulama malzemesi katkılı silikon, Şarj bazlı kapasitans ölçüm (CBCM) tekniği, 350 nm teknolojisi ile Tamamlayıcı Metal Oksit-Yarıiletken (CMOS) tekniği kullandı. Algılayıcı kapasitöre uygulanan, ancak referans olana uygulanmayan hedeflenen bakterilerin mevcudiyetinde oluşan geçirgenliğin sonucu olan, sistem kapasitansın varyasyonunu tespit etmek ve işlemek için interdijite kapasitör yapısı, yüke bağlı kapasitans ölçüm devresi kullanıldı.

Bulgular: Sistem, literatür sonuçlarına göre düşük seviyede bakteriyel konsantrasyon için iyi bir duyarlılık ortaya koymuştur. Devre, 100 aF civarında kapasitans değişikliklerini ölçüyordu. Devrenin çıkışındaki gürültü 78.7μV civarındadır.

Tartışma: CMOS biyosensör bazlı şarj bazlı kapasitans ölçüm sistemi, bakteriyel patojenlerin hücre tespiti için test edilmiş ve deneysel olarak doğrulanmıştır. Fonksiyonel algılama kapasitansı, referans kapasitansı ve ara-yüz devresinin pasifleştirilmesi de dahil olmak üzere bu tür biyosensör sistemlerinin üç önemli gerekliliği tartışılmıştır. Sistem seri olarak üretilebilen hızlı, düşük güç ve minyatür bir platform sağlar.

Anahtar Kelimeler: Biyosensörler, CBCM, Escherichia coli, sağlık uygulamaları, MLoC sistemi, dielektrik sabiti

Introduction

The biomedical transducers development is widespread as one of the superior industrial trends; thanks to CMOS technology that pushes forward this development and credits to Microelectromechanical systems (MEMS) technology that has also made most of the research in the domain of health detection and measurements achievable. MLoC system is one of these accomplishments credited to CMOS Technology. In this paper, CMOS-Biosensors based capacitive biosensors for biomolecular detection is proposed. The integrated biosensor is employing bacteriophage or phage organisms as recognition elements to detect deadly bacteria such as E-Coli and Salmonella at low level. The system works based on monitoring the changes in capacitance signals caused when the target bacteria are attached to the sensing interface. The system is designed using TSMC/CMOSP35 technology and it consists of Metal-Metal Comb-Capacitors (MMCC) and signal detection and processing circuitry. A Charge Based Capacitance Measurement (CBCM) circuit that was originally proposed as an accurate technique for the characterization of interconnects capacitance in deep submicron CMOS ICs[1] does the signal detection and processing. The phage organisms are immobilized on the surface of the capacitor and together they form the sensing interface. The CMOS capacitive based sensors in implementation offer a number of advantages including small size, fast response, and low-cost mass production. Presently, much of the bacterial analysis is done in clinical laboratories, which is time consuming and requires extensive professional expertise. In addition, the majority of the available commercial devices are bulky; not suitable for field applications, and growth-based, which means that the presence of bacteria has to reach a high threshold level in order to get a reading from the apparatus. The growth-based technique is time consuming as it takes from hours to days to get the results. The proposed implementation is a real-time bacterial sensor micro-system that holds a great promise for versatile applicability in food safety, national security, and clinical diagnostics. This sensor system design falls back on the use of specific bacteriophage, which are viruses that recognize specific receptors on the bacterium surface with extreme selectivity and sensitivity. The phages bind to the surface of the bacterium and inject genetic material. Capacitive sensors offer many advantages besides their capability in straightforwardly sensing electrode motion; they can also detect conductive, or the dielectric properties of a biomaterial site onto the sensing capacitor. For that reason; the capacitance approach measurements technology is introduced as a powerful technique in biosensor applications due to its tremendous capability, stability and low-noise signal in sensing process.[3,4]

Materials and Methods

CMOS technology has become the dominant technology for designing and implementing multi-biosensors on a single chip, named Multi-Lab-on-single-Chip (MLoC) [5]. MLoC includes CMOS-based capacitive biosensors along with phototransistors, Electrochemical, and Magnetic transducers all on a single chip. The aim of MLoC integrated system is reducing costs, size and time. MLoC is a multidisciplinary system, which is usually composed of several separate approaches of biomedical fields of expertise. In this paper, CMOS-based capacitive biosensors design and implementation are fully unfolded to act as a biomolecular detection transducer. This transducer employs bacteriophage or phage organisms as recognition elements to detect at low-level of deadly bacteria such as Escherichia coli, and Salmonella.

Capacitance Biosensing Circuit

Real-time, point of care bacterial detection is of great value to medical industries alike. Biosensor

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microsystems experience considerable technological growth and are attracting significant interest in terms of research and development. Following several recent food recalls and instances of food poisoning in people and pets, there is a growing need to monitor bacterial organisms such as *E-Coli* and *Salmonella* in agricultural products and prepared/packaged food. An enabling methodology to face the above challenge is to develop a miniaturized, simple, and field-usable sensor system for real-time bacteria monitoring. Generally, biosensors couple a physical transducer with a specific recognition element. Bacterial sensors typically use antibodies attached to the sensor's surface to bind to specific antigens present on the bacterial cell wall. While antibodies offer some degree of selectivity, they are expensive to produce and suffer from environmental instability. Bacteriophages are a class of viruses that bind to a specific bacterium in order to reproduce themselves. The specificity of this recognition is unique, inasmuch as certain phages are able to differentiate between individual strains of the same species. The unique specificity of this recognition offers a promising platform for the development of bacterial sensors. To that end, methods enabling the efficient attachment of phages onto sensor surfaces need to be developed.

CBCM Biosensor System Design

Immunosensors act on the principle that the untouchable response of certain biological species; such as pathogen bacterial, to contaminants will produce antibodies, which in turn can be measured. To reduce the cost and time required for the accurate clinical analysis, the biosensor-based capacitance immunosensors was developed. This immunosensor for on-site screening and monitoring of contaminants determines the level of contamination by measuring the variation of capacitance on the contaminated capacitor; called sensing capacitor, with respect to clean capacitor; called a reference capacitor. The CMOS-based capacitance biosensor works when contaminant biomolecules bound to specific antibodies on the sensing electrode. When a biomolecular sample is inserted into the microfluidic channel embedded with Interdigitated Microelectrodes Array (IDMA) as sensing electrode, the sensing field passes through the biomaterial.[6] The presence of the biomolecular material alters the dielectric properties yielding to alter the capacitance accordingly. The capacitance will change in relationship to the thickness or density of the biomaterial.[7] The capacitance is determined by the compound dielectric constant of the bulk analyte mixture. For that reason, the capacitance change can be either positive or negative depending on whether the analyte has a higher or lower dielectric constant k; leading to show significant variation with biomaterial properties or with frequency. Afterward, the measurements are recorded as difference signals between sensing capacitor and a reference capacitor.

Interdigitated Microelectrodes Array (IDMA)

IDMA are widely used in developing biosensors for monitoring the catalyzed reaction of enzymes, the biomolecular recognition events; of specific proteins, nucleic acids, whole cells, antibodies or antibody-related substances, growth of bacterial cells or the presence of bacterial cells in the aqueous medium. IDMA have been integrated with CBCM technique in order to miniaturize the conventional electrodes, enhance the sensitivity and use the flexibility of electrode fabrication to suit the conventional electrochemical cell format or microfluidic devices for variety of applications. Some researchers elaborated on different IDMA geometries their fabrication materials and design parameters, and types of detection techniques.[8,9] IDMA were fabricated on glass wafers and investigated to obtain optimal oxidation and reduction reactions. Therefore, IDMA employs to increase the sensor capacitance performance in a tiny biomolecular volume. A custom CMOS capacitance

sensor for cell proximity detection has been designed using the topology shown in Fig. 1. CBCM based capacitive sensor circuit has been reported.[10,11] In general, the behavior of the CBCM technique can be characterized based on the current of each arm of the CBCM circuit that is amplified by a current mirror, integrated and converted to a voltage level using a simple capacitor (Cint). A differential amplifier (DA) finally subtracts the resulting voltage outputs of both sensor circuits. We note that the only important limiting factor on the accuracy of this sensor circuit is due to the input signals of the DA. The biosensor employs a differential capacitor architecture using the sensing and reference capacitors to achieve improved resolution and signal-to-noise ratio. The sensing and reference capacitors are identical interdigitated structures with a metal layer placed over the structures for immobilizing the phage organisms and protecting against hydration.

This work is focusing on IDMA with CBCM technique as biosensors for their applications in pathogens detection. The biosensor system is designed using CMOSP35 process available through the Canadian Microelectronics Corporation. The interdigitated capacitors are implemented using the metal layer available in CMOS process. Another metal layer is placed over the capacitor structure. Passivation (dielectric) layer protects the CMOS circuit and the reference capacitor where only the sensing capacitor exposed to the sample by opening the dielectric layer over it. In addition, the unexposed region of the CMOS system is covered with epoxy after the chip is fabricated as protection for use in aqueous samples. The majority of impedimetric sensors, as previously reported in the literature,[12-14] have two main techniques for pathogens detection these are whether Faradaic impedance of the bioreceptor immobilized on the electrode surface or non-Faradaic impedance detection of biomolecule interactions. The latter is less reported in literature and mainly consists in attaching the capture probe in between the two electrodes.

Since the distance between electrodes is directly related to the sensitivity of the transducer, CMOS capacitive biosensors with IDMA configurations are introduced in biological applications in order to take advantage of IDMA that have a narrow gap between the electrodes. The electric field generated in one of the IDMA is distributed in the area where the target interacts with the bioreceptor, creating a change of the generated electric field, which is detected on the second IDMA.

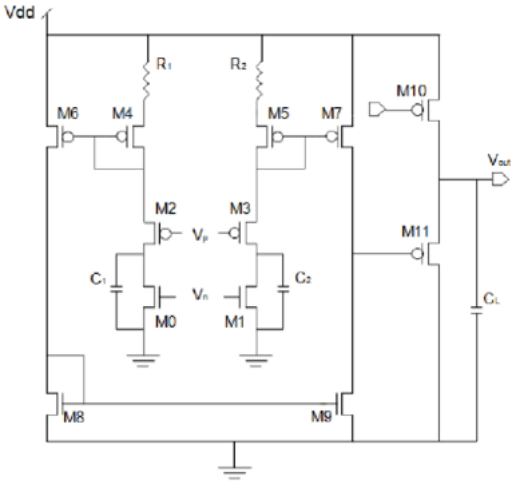

Figure 1. CBCM circuit topology.

The ionic media is a drawback in this kind of sensors, because of the pre-dominant electrical spreading resistance of the solution. A schematic diagram of the interdigitated capacitor; named as Metal-Metal Comb-Capacitors (MMCC) is shown in Fig. 2. The capacitors are fabricated exclusively with layers and processing steps available in the standard CMOS process sequence. Electrode E1 (Blue) is made from the first metal layer while electrode E2 (orange) is a stack of the first and the second metal layers[15,16] While many of the processes used for MEMS fabrication are not compatible with the CMOS IC process, depositing a sensor material onto a previously fabricated CMOS circuit can create a very useful category of sensors. In this work, we propose a CMOS capacitance biosensor composed of immunosensors bioreporters; genetically engineered pathogens bacterial cells, deposited onto IDMA that is fabricated through CMOS technology process provided by CMC. The bioreporter used for this work immobilizing on the surface of the sensor capacitance made of IDMA. The immunosensor is detected by measuring the variation of the capacitance as function of the concentration of bacteria.[17]

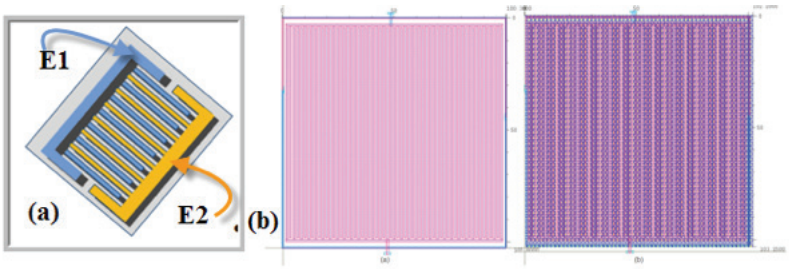

Figure 2. IDMA: (a) A schematic diagram (b) Layout for sensing and reference capacitors.

The computed electrode configuration was chosen to enhance the sensitivity of the biosensor by maximizing the sensing area in the region with strong electric field due to small gap between the fingers of the capacitor.[10] Fig. 2a shows a layout of the IDMAs capacitor. It consists of 30 electrode pairs and occupies an area of 100.3 µm x 102.8 µm, where the IDMA s sensor area is similar to reference electrode except in one aspect that's an isolated layer is laid on the latter electrode to protect it from any contamination from the ambient. The width and spacing of single electrodes are 1 µm and 0.6 µm; respectively, as shown in Fig. 2b, where the height of the microelectrode is defined by CMOSP35 technology and the extracted capacitance value is 500.005fF. A capacitive biosensor is designed by utilizing a couple of capacitors each composed of IDMA. The reference capacitor is covered and protected entirely while the sensing capacitor is modified and works separately and chemically by sensitive polymer. The presence of the polymer on the top of the sensing capacitor aims to absorb the biorecognition elements to readily binding the pathogen bacterial cell. Therefore; any variation on the surface will directly change the capacitance of the electrode and thus electrically detected and recorded.

The Block diagram of the CBCM integrated with Interdigitated Microelectrode array and signal processing system and the layout of the entire CMOS capacitance biosensor is shown in Fig. 3. Using CMOS technology to make the IDMA, the capacitive biosensor shows signs of unprecedented robustness and more sensitivity owing to large area of the sensing surface and small electrode gap (0.6 µm) besides other advantages of IDMA that is aforementioned in this literature.[18,19] Such technology allows going further in detecting tiny biomolecular sample in health applications. The mechanism of this technology lies on characterizing the immunoreactions that occur on the sensing surface by capacitive parameters.

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The CMOS capacitance approach offers more advantages compared to other techniques. Besides high sensitivity owing to the presence of IDMA, exhibits real-time detection property and is less expensive compared to optical techniques.[19] Usually, IDMA has been used to study the electric properties of thin layers and membranes due to short electric field diffusion depth generated by IDMA. In addition, the short field diffusion depth has also established to be valuable either to observe local alterations in the electric parameters of bulk solutions that take place close to the sensing surface or to detect the existence of particular dielectric objects on the sensing surface.

In our case, IDMA is used as a sensing surface that functions by specific antibodies against a target pathogen bacterial cell. During the capacitance detection of pathogen bacterial, the dielectric characteristics of cells can be cut down to the single-shell model. The bound of pathogen cells on the sensing surface is predictable to disturb the electric field the flow of electric currents either Faradiac or non-Faradiac current leading to impedance increase. For that reason, binding the insulating pathogens on the sensing surface by biomolecular recognition of antibodies yields to boost the resistance of the bulk solution and decrease the capacitance of the solution in between the micro-bands.

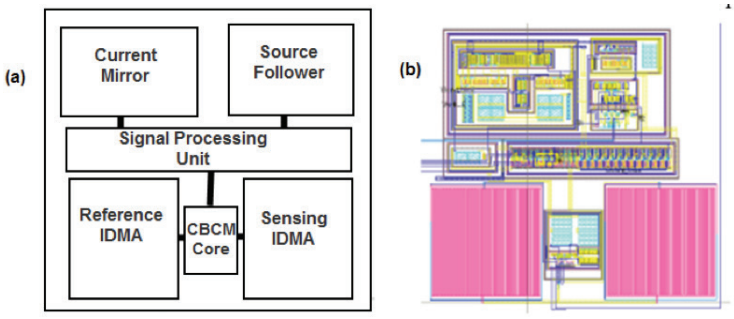

Figure 3. CBCM: (a) Block diagram, (b) Layout.

The diffusion of the electric field close to the sensing surface depends on the size pathogens bacterial cell. In addition, the more binding cell to the sensing surface by biomolecular recognition of antibodies the superior signal of the transducer can be detectable.[20]

Biosensor System Design and Architecture

Capacitive immunosensors are based on altering electrical conductivity at a constant voltage, caused by immunoreactions that specifically generates or consumes ions. Fernandez-Sanchez et al. (2004) developed a disposable, non-competitive capacitive immunosensor for PSA.[21]

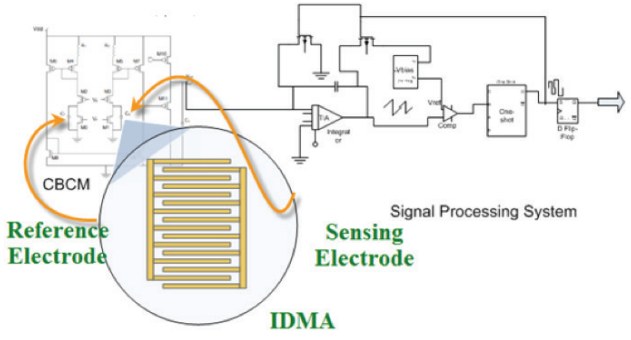

Figure 4. CBCM Schematic Biosensor circuit.

This work presents femto-molar detection pathogens bacterial sensor based on CMOS capacitance. Fig. 4 illustrates the entire capacitive biosensors detecting system. The binding of pathogen bacterial cells and immobilized antibodies on functionalized sensing surface produce negative charges, which form an electrode-electrolyte capacitance interface.[22] By measuring, the capacitance as biosensor response, the rapid detection and quantification of the presence of the specific bacteria is achieved.

Label-free pathogens bacterial biosensing approach along with interdigitated microelectrode arrays under external applied electrical field is no more limited to observing the existence of the live pathogens due to the variations in characteristics of the electrochemical architecture of the actuators but also it has the capability to detect pathogens in its two situations either live or dead cells. Fig. 4 illustrates the biosensor functionalization of the sensing surface with the immunoglobulins (i.e. antibodies) in the CMOS biosensor based CBCM detection approach that provides specificity for the target pathogen bacterial.

The live pathogen bacterial bound to the immunoglobulins on the electrode disturbs the surface-restricted electric field thus the capacitance between the electrodes decreases due to changes in the permittivity of the media, which can be detected as the positive signal for the detection. By contrast, dead bacterial cells are not voluminous enough to induce noticeable changes in the electric field lines distribution.[20]

The biosensing surface built with IDMAs is used as the transducer-sensing surface that will functionalize by specific antibodies against a target pathogens bacterial strain. IDMAs have many advantages besides their short electric field penetration depth that are capable to analyze the electric properties of thin layers and membranes. They are significant enough in electrolyte medium to observe narrow changes in the electric parameters of the electrodes surface.[23] In addition, IDMA can be employed to detect the presence of particular dielectric objects on the surface of electrodes.[24] After the sensing surface represented by interdigitated microelectrodes is exposed to applied electrical field, capacitive detection is performed to analyze and study the behavior of the " and the permittivity of the solution is measured and recorded accordingly as long as they are dunking in the fluid system.[12] Electrolyte medium alike the metallic conductor can be acting upon Ohm's law:

$$R_{sol} = \frac{E}{I} \qquad (1)$$

Where Rsol is the resistance of the body of solution in ohms (Ohm), E is the potential difference (V) and I is the current (A). By definition, the conductance G is the inverse of the resistance R of a consistent body of uniform cross section. In the electrolyte solution, the conductance G is given by:

$$G = \frac{1}{R} = \frac{A}{l}k \qquad (2)$$

Where k is the specific conductance with units -1.m-1. Since it is difficult to define the geometrical parameters A and l, any fluidic cell has to be standardized with a solution can be determined as follows:

$$K_{cell} = \frac{l}{A} = kR \qquad (3)$$

Resistance

$$R = \frac{\rho\, l}{A} = \frac{l}{\sigma\, A} = \frac{K_{cell}}{\sigma} = \rho K_{cell} \qquad (4)$$

Conductance:

$$G = \frac{1}{R} = \frac{\sigma A}{l} = \frac{A}{\rho\, l} = \frac{\sigma}{K_{cell}} = \frac{1}{\rho\, K_{cell}} \qquad (5)$$

is the cell constant (m-1) can be found out by measuring the resistance Rsol of a cell filled ,the

specific conductance k of any solution can be determined based on practical tentative resistances values using any of the equations mentioned above. By definition, specific conductivity is the inverse of the specific resistance of an electrolyte measured between two electrodes 1 cm^2 in area and 1 cm spaced out. The higher the concentration of ionic ingredient, the higher the conductivity will be present in the system where ", of the electrolyte is significantly a function of temperature. The double layer capacitance in the electrolyte behaves alike two parallel plates' theory. Thus, the capacitance in the electrolyte electrode interface can be given as follows:

$$C = \frac{\epsilon A}{d} = \frac{\epsilon}{K_{cell}} = \frac{\varepsilon_0 \epsilon_r}{K_{cell}} \quad (6)$$

Where, 0 is the electric constant equal to 0 ≈ 8.854 pF m−1 and the permittivity r is the relative dielectric of the material between the plates. In the light of what was is proportional to the measured values of the resistance Rsol and inversely proportional to the capacitance C [Source: PAC, 1974, 37, 499 (Electrochemical nomenclature) on page 511]. Indeed, the constant cell is given by:

$$\mathcal{K}_{cell} = \frac{l}{A} = kR = \frac{\epsilon}{C} = \frac{R}{\rho} \quad (7)$$

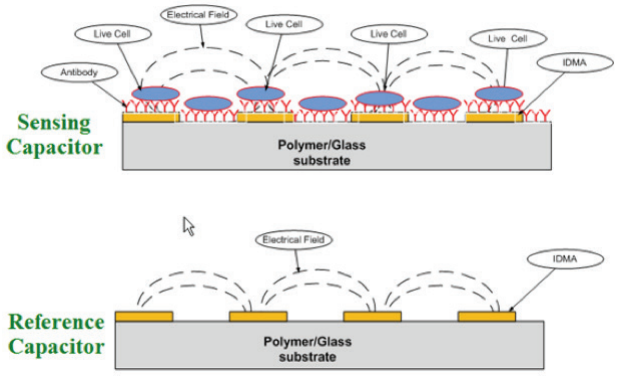

Figure 5. Surface sensing binding with pathogens.

For capacitive detection of pathogens bacterial, a suitable frequency should be selected under a specific electric field applied in presence of pathogen bacterial binding to the surface of the electrodes via the biomolecular recognition of antibodies. Therefore, the entire system will be perturbed, and the variation will be detectable and measurable. In addition, the geometries of the electrodes and interface gap between electrolyte and electrode are playing a significant role in the cell constant. As soon as the surface of the sensing transducer is modified by filling it with biomolecular sample as a new dielectric in between the interdigitated electrodes leading to variation in the capacitance C:

$$C = \frac{A\,\epsilon_r \varepsilon_0}{d} \quad (8)$$

Biosensor based capacitance measurements with IDMA rely on the proximity gap between fingers to generate strong electrical field between the fingers; yielding a transducer with high performance. The mechanism of this technique depends on the generated electrical field, where it starts from one side and ended on the other finger of IDMA as shown in Fig. 5. The electrical field is spreading in the gap where the target cell acts together with the bioreceptor. Antibodies for label process create a disturbance of the generated electric field. The latest criterion has tiny volume due to the membrane potential of cells breaks down and the ions in the cell are driven out.

Dead cell alike live cell still has the capability to be binding to the antibodies as well, but their variation in capacitance properties of the interface is unlikely. Consequently, the dead cell presence in the medium is weak so their influence on the capacitance is less significant than the live cell.

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Therefore, the capacitance plotting of the two categories will distinguish between the behaviors of each criterion under the same conditions. Electric field is much smaller so that they are distinguished from the live cells. Differentiate the pathogen status live or dead is achieved thanks to IDMA configuration. By contrast, capacitance approach is not susceptible to these issues since the transducer can differentiate the volume of the insulating cell via the perturbation of the surface-confined electric field measured at a proper frequency range. The interdigitated transducers have a finger width of 1 µm and gap between fingers of 0.6 µm. In this geometry, 80% of electric field lines and currents are confined within a distance equal to half of the pitch.[25] This layout makes the detection of micrometer-sized dielectric objects such as live bacterial cells most sensitive.

The target bacteria get attached to the immobilized phages on the sensing capacitor which changes the capacitance of the comb capacitor by interrupting the electrical field between interdigitated fingers as shown in Fig. 5. The phages are not immobilized on the reference capacitor and hence it does not experience any capacitance changes with the changes in bacterial concentrations. The CMOS Charge Based Capacitance Measurement (CBCM) circuit measures the difference of the capacitance between the sensing and reference capacitors and provides a voltage output.[1,3] CMOS capacitive biosensor can then be used on different applications such as Multi-labs-on-a chip (MLOC) and micro technology system.

Sensitivity

Sensitivity plays an important role in any design; particularly in biosensors applications. Sensitivity points to the quantity of the output signal; current or voltage alterations, due to an alteration in the gap between the target and the sensing surface. The sensitivity can be readily obtained graphically by plotting the output voltage versus the gap size where the slope of the line is the sensitivity. The sensitivity of the biosensor is proportional to the dielectric constant of the biomaterial.[6]

CMOS Charge Based Capacitance Measurement (CBCM) Circuit

Charge based capacitance measurement (CBCM) method was originally proposed as an accurate technique for the characterization of interconnects capacitance in deep submicron CMOS ICs.1 Fig. 7 shows the principle of operation in which two signal pulses Vp and Vn are applied to two pairs of nMOS and pMOS transistors in order to frequently charge and discharge the sensing CS and reference capacitor CR. The Vp and Vn signals of Fig. 7 consists of two non-overlapping signals. The purpose of these non-overlapping waveforms is to ensure that only one of the two transistors in the basic test structure is conducting current at any given time.[10]

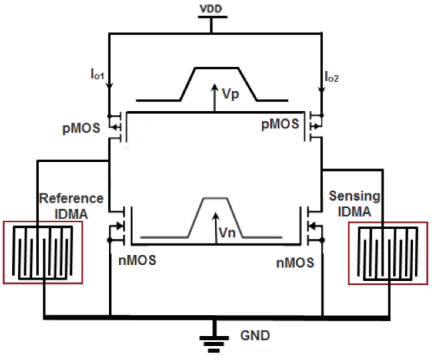

Figure 7. Schematic representation of CBCM.

Thus, short-circuit current from Vdd to ground is eliminated. When the PMOS transistor turns on, it will draw charge from Vdd to charge up the target interconnect capacitance. The charging DC currents ID1 and ID2 can be obtained from the following equations

$$I_{D1} = V_{DD} \cdot f \cdot C_1 \quad (9)$$

$$I_{D2} = V_{DD} \cdot f \cdot C_2 \quad (10)$$

Where Vdd and f are the power supply voltage and the frequency of clock pulses (Vp and Vn) respectively. Based on this method, the subtraction of charging/discharging currents ID1 and ID2 measured through high precision DC ammeters is proportional to C = C1−C2 which is obtained from equation[26]:

$$\Delta C = C_1 - C_2 = \frac{I_{D1} - I_{D2}}{f \cdot V_{DD}} \quad (11)$$

Fig. 1 is the schematic of the CBCM circuit. M0-M3 is the CBCM core that can transfer C between the two capacitors C1 and C2 into I between two currents ID1 and ID2. M4- M9 is the current mirror used to read out and amplify (AI) I. The gain of the current mirror stage is mainly decided by the aspect ratios of M8 and M9. Where M10 and M11 form the source follower (Av) used to drive the load capacitor that is used as a low pass filter. Resistors R1 and R2 are used to balance the offset caused by the fabrication mismatch and the offset caused during the epoxy encapsulation and packaging. The relationship between the current ID and capacitance C in both current arms shown in Fig. 1 is estimated by[2]:

(12)

CMOS Capacitive Biosensors and Nano-Amplification Strategy

In terms of the transduction techniques used, the three main classes of biosensors are optical, electrochemical and piezoelectric. Out of the three, optical methods appear to be the most sensitive, with surface plasmon resonance and waveguide-based devices being the technological spearhead. As for Electrochemical biosensors, they are cheaper than optical ones. They can be amperometric or impedimetric, depending on whether they monitor a current as a function of potential or the resulting sensor impedance as a function of frequency. The advantage of impedimetric methods is that, unlike amperometric, they do not need of enzymatic labels in order to detect. On the other hand, the level of pathogens in a contaminated sample is often below the detection limits.[27] The piezoelectric immunosensor is thought to be one of the most sensitive analytical instruments developed to date, being capable of detecting antigens in the pico-gram range. A piezoelectric sensor that could reliably detect the mycobacterium antigen in biological fluids would be of enormous use. For instance, detection of the antigen in saliva could constitute a noninvasive method of screening high-risk populations. Almost all current methods of diagnosing tuberculosis (TB) have drawbacks. They tend to be either nonspecific or too time-consuming. In most cases of pulmonary and extra-pulmonary TB, diagnosis depends upon culturing the mycobacterium organism, a process requiring 4-8 weeks.[28] To overcome this problem, sample pre-treatment steps and signal amplification strategies are usually required. The recent biosensor employs a differential capacitor architecture using the sensing and reference capacitors to achieve improved resolution and signal-to-noise ratio. The sensing and reference capacitors are identical interdigitated structures with a metal layer placed over the structures required for immobilizing the phage organisms and to protect it against hydration. The target bacteria get attached to the immobilized phages on the sensing capacitor which changes the capacitance of the comb capacitor by interrupting the electrical field between inter-digitized fingers. The phages are not immobilized on the reference capacitor and hence it does not experience any capacitance changes with the changes in bacterial concentrati-

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ons. The CMOS Charge Based Capacitance Measurement (CBCM) circuit measures the difference of the capacitance between the sensing and reference capacitors and provides a voltage output.[11]

Results and Discussion

In this section, simulation and experimental results of sensing capacitance, interface circuit, and interdigitated MMCC capacitance are presented and discussed. Measurement setup shows the interface capacitance that determines the frequency of the electrodes charging and discharging transients. A comparator compares the interdigitated microelectrode potential with a reference voltage Vref producing a digital signal at its output whose frequency is inversely proportional to capacitance. The integrator accumulates the current signal until its output voltage reaches the threshold of comparator. Then, one-shot resets the integrator and triggers the D-Flip Flop. The output frequency of D-Flip-Flop is the digital representation of the variation of the sensing capacitor.[1] To minimize error and make the sensors simpler, the output can be read as a voltage without any signal processing.

Simulation

The transient output voltage of the interface circuit has been simulated by Spectra S and Cadence for different values of input sensing capacitances (Cs1). Results are reported in Fig. 9 and the linear relationship between output voltage and input sensing capacitances is highlighted in Fig. 10 where the reference capacitance Cs2 is 500fF. As shown in these figures, this design results in 0.38 mV/fF sensitivity:

$$S = \frac{\Delta V}{\Delta C} = 0.38 \frac{mV}{fF} \quad (13)$$

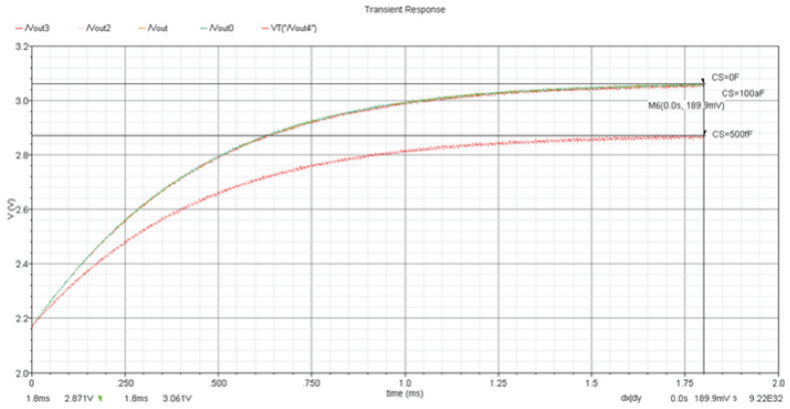

Figure 9 Simulation results showing the voltage output of the CBCM.

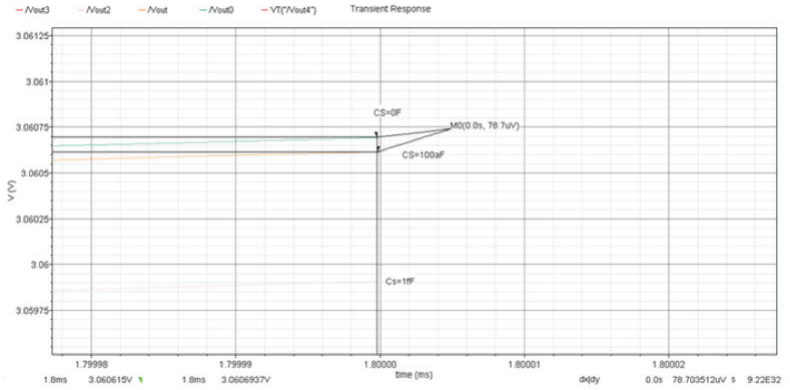

Figure 10 The resolution of the circuit in measuring the capacitance.

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In the CBCM circuit, if there is an unexpected offset in the capacitor C, the value of resistor R can be varied to cancel the offset. Normally, the value of 1/fC is much larger than R. Hence varying the value of the resistance will not have a significant effect on the sensitivity of the circuit. The SPICE simulation result of the circuit is shown in Fig. 9. The output voltage changes from 2.871 V to 3.061 V, as a response to the variation in capacitance from the initial 500 fF to 0 F. We notice from the output of the circuit Fig. 10 that the noise is around 78.7μV, which means the resolution of the circuit in measuring the capacitance changes is around 100 aF. When similar devices are used on both sides of the CBCM structure, the parasitic capacitances associated by M1-M3 are removed through the capacitance subtraction. The changes in capacitance signals are translated into current signals and are amplified and read out by subsequent processing.

Discussion

Fig. 11 shows the variation of the capacitance with respect to the frequency was carried out from the experimental setup. Fig. 12 illustrates the capacitance measurements within low frequency. In CMOS technology, the dynamic power is so far considered. The power consumption can be figured out for capacitance load CL = 1 μF, Vdd = 3.3 V, fc =200 Hz using the following equation:

$$P_D = \frac{1}{2} C_L f_C V dd^2 \quad (14)$$

The power dissipation is then around 1.09 mW.

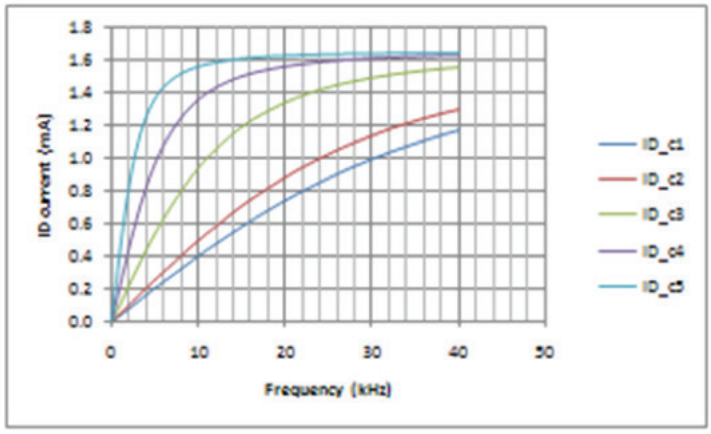

Figure 11 The capacitance measurement.

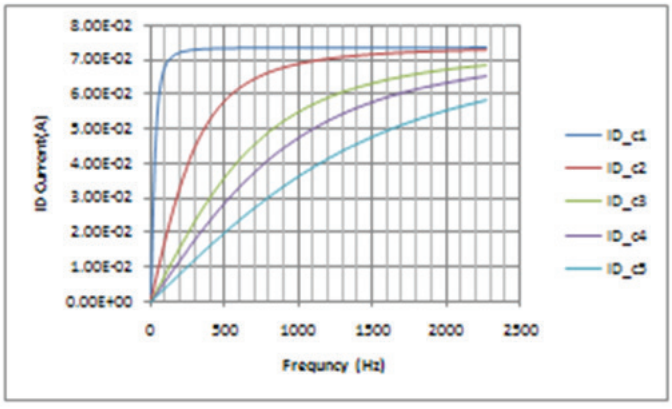

Figure 12 The capacitance measurement at low frequency range.

Conclusions

The system is designed using CMOSP35/TSMC technology and it consists of Metal-Metal Comb-Capacitors (MMCC) and signal detection and processing circuitry. The CMOS capacitive based biosensor offers a number of advantages including small size, fast response, and low-cost mass production. MLOC system capacitive based is specifically designed, fabricated, and experimentally validated for bacterial pathogens cell detection. Nonetheless, the achieved specifications are well suited for other biosensor applications such as DNA hybridization. Three important requirements of such biosensor systems including functionalized sensing capacitance, passivating the reference capacitance and interface circuit were discussed and the important applications have been introduced. The system provides a rapid, low power, and miniaturized platform that can be mass-produced.

Notes for the Editor

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