Miniaturized IC Biosensors on a Single Chip for Biological/Biomedical Applications

Abdullah M. Tashtoush
Biomedical systems and Informatics Engineering Department
Yarmouk University
Irbid, Jordan
abdullah.t@yu.edu.jo

***Abstract*— Hybrid biosensors based CMOS Microtechnology and Nanotechnology is high-demand to meet the needs of human's health care and environmental concerns. The sky is limit for R&D biomedical and biological transducers. Heretofore, the semiconductor industry has miniaturized IC biosensors individually, whereas this work will present a single die has set of biosensors implemented within it, which leads to a new generation of multidisciplinary biosensors named as multi-labs-on-a-single chip (MLoC). The invented system will be the most promising and superior transducer in trends of health care human services, as there are a lot of patients in hospitals are contaminated with antibiotic-resistant bacteria, resulting in lavished a lot of money onto the health-care system. Early detection is critical for improved patient care and can help in minimizing the risk of cross contamination between patients. The presented system is introducing different techniques based on CMOS/MEMS technology batch process for early detecting tumors. In addition, the MLoC system will function accurately in biological fields such as detecting pathogen bacterial cell at low concentration levels. The multidisciplinary biosensors miniaturizes four transducers each one is based on a different biosensors approaches; these approaches are based on optical CMOS technology, charge based capacitance measurements (CBCM), electrochemical impedance spectroscopy (EIS) and CMOS microcoils incorporating with interdigitated microelectrode array (IDMA). The fabricated chip has been successfully tested and represented a viable solution for portable, sensitive and rapid detection of pathogens bacterial.**

Keywords-Multidisciplinary, Biosensors, Health Care Applications, MLoC System, IDMA.

I. INTRODUCTION

Each year, a lot of patients in hospitals are infected with antibiotic-resistant bacteria, resulting in non-negligible cost raise to the health-care system. Although most bacteria are harmless to healthy individuals, the symptoms of bacterial infection can be severe for patients with a weakened immune system. Early detection is critical for improved patient care and can help in minimizing the risk of cross contamination between patients.

By far, the main pathogen detection methods use DNA microarray techniques. These methods rely on Petri culture, colony counting and Enzyme-Linked Immunosorbent Assay (ELISA); which also rely on antibodies or nucleic acid polymerase chain reaction (PCR) detection [1]. The reason for this is the high selectivity and reliability of these techniques, which have different strengths and weaknesses. Culture and colony counting is the oldest method and is the one that is generally considered as the reference. It enables the detection of viable cells, but it is labor intensive and takes up to several days to obtain results.

Biosensors are relatively new players in the pathogen detection arena and the use of biological recognition element generally limits their performance. Such recognition elements are mostly antibodies or DNA sequences. While DNA based methods are excellent for their selectivity and long-term stability, they usually are unable to discriminate between viable and non-viable cells. Furthermore, antibody based biosensors are generally very expensive to produce and may suffer from cross binding of other bacteria, which would lead to false results. Most of the commercially available biosensors for bacterial detection are bench type; not suitable for in-field applications, not specific, time consuming; needs hours to days to get the results of the most of the sensors relying on fragile and unstable antibodies as recognition elements.

A high selectivity and specificity, a relatively low production cost, a limited sample preparation time, and the potential for miniaturization are the main advantages of biosensors over conventional analytical methods [2]. Although, the healthcare industry and its high demand is pushing forward the development of biosensors, there have been many suggested applications in food, bio-processing, agriculture, and environment [3].

Miniaturization of biosensors is one of the recent trends aiming towards both increased performance and portability along with low cost for mass production. Developing and producing miniature analytical instruments and devices can achieve a number of advantages; besides rapidly analyzing extremely small amounts of substance, it can also perform on-site analysis and analysis in security areas. Micro/Nano-chip analytical devices that lead to multi/single Lab-On-A-Chip devices are micro-devices that merge microfluidic technology with electrical and/or mechanical functions for analyzing tiny volumes of biological sample [4][5]. Analytical micro-devices can perform a progress through two ways to produce more versatile and smaller instruments. Firstly, developing the entire miniaturized systems in which they are capable and attractive devices for the analysis of pico-, femto-, and atto-molar quantities of biological and

biomedicine samples, and secondly, developing dedicated systems by accelerating the development of chemical sensors, sensor arrays, and microsensors. In addition, miniaturization might be able to produce an instrument combining or hyphenating miniaturized single elements based on different working principles without sacrificing their versatility.

The combination of both the surface-bound antibodies for identification and the surface interaction for detection process is the mechanism of the conventional detection techniques. This technique has limitations because of the inadequate biomolecular binding to the sensing surface. This is because the biomolecular binding is too small to move from the suspension and bind to the functionalized sensing surface. Recently; the specification and the behavior of the biosensors overcame this serious issue due to their high sensitivity, specificity, selectivity and improved accuracy. Therefore, the multi-lab on a single chip technology (MLoC) enhanced the modern detection techniques by including capacitive (CBCM), optical, electrochemical (EIS) and magnetic techniques all on one single chip. CMOS/MEMS technologies, integrated microfluidic channel, sorting, and biomolecular cells identification on a sort of substrates such as glass, polymer or plastic are the basis of modern biosensors (i.e. MLoC, micro total analytical systems (μ-TAS), etc.). Note that miniaturizing of the sensing systems allows faster detection of tiny volumes of biomolecular cells than that of the macroscale analysis techniques, which in sequence facilitates the new generation of biosensors that exhibit rapid biological samples detection, leading it to be fit for point-of-care diagnostics. The performance of the biosensors should be high enough to detect pathogens at low-level concentrations of biomolecular cells, which is enough to cause a disease. Such transducers could play a significant role for monitoring contamination of water supplies with pathogens. Therefore, performing successful detection at low concentration depends on reliability, sensitivity and short timing to obtain results, which become the main characteristics of biosensors. The measurement for this low level of concentration should pass through a protocol starting from the location where the sample picks up. Then the surface goes through modification followed by the treatment that binds it onto the sensing surface until finally the system reads out the results.

The aforementioned approaches so far (the CBCM, Optical and EIS methods) required sample pre-treatment steps and signal amplification strategies, which cause some complications and challenges. Therefore, this work presents a novel microsystem that integrates a fishing system with the interdigitated microelectrodes arrays (IDMA), which may lead to highly functional and versatile biosensor systems, bacteria detection with high sensitivity, and fast-response times based on CMOS microcoil. Magnetic particles are ideal to solve both challenges at once, since the magnetic fields can easily manipulate them.

The novel sensitive chip of multibiosensors integrates state-of-the-art technologies. It allows detection and quantization of biological samples using miniature devices at a lower cost. The device has been fabricated using CMOS technology and has been tested.

II. BIOSENSORS OVERVIEW

Biosensors are analytical devices that combine a biologically sensitive element with a physical or chemical transducer to detect the presence of specific compounds selectively and quantitatively.

In most general cases, it is constructed from a combination of a bioreceptor, the biological component; and a transducer, the detection method. The main function of a biosensor is to transform a biological event into an electrical signal. The conversion is done using measuring techniques like potentiometry, amperometry, thermometry, or photometry, all of which are based on the variation of physical quantities. The method chosen must be simple and of a reasonable size, so that it is cheap and easy to use.

Figure 1 represents the principle of the operation of a biosensor, which is starting from the analyte that can provide all the information needed for its evaluation [5]. This information can be processed and stored for later use. The process of the analysis is starting from the analyte, which is identified by the first connection of a biosensor named “the bioreceptor”. The second stage of a biosensor is the transducer that takes advantage of the biochemical modification of the substrate by the bioreceptor through transforming it into an electrical signal.

A wide range of transducers is available to detect the interaction between the analyte and the biorecognition molecule and convert it into an electronic signal. Electrochemical, optical, thermal, and mass sensitive transduction mechanisms have been used in biosensor development over the past decade [6].

A. Biosensors Techniques

The detection part in the biosensor can be achieved using various techniques, such as optical, capacitive, magnetic, and Electrochemical Impedance Spectroscopy (EIS). The selection of detection technique relies on the nature of the application and the accuracy of the analysis requested. In general, these are the major principles techniques for analyte detection.

Optical sensors make use of the effect of chemistry reaction on optical phenomena, such as Fluorescence spectroscopy, which is a sort of electromagnetic spectroscopy, which analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light of a lower energy. The most popular detection method is laser-induced fluorescence (LIF) for its high sensitivity [7], and absorption spectroscopy, which is a technique in which the power of a beam of light measured before and after interaction with a sample, is compared. Specific absorption techniques tend to be referred to by the wavelength of radiation measured such as ultraviolet, infrared or microwave absorption spectroscopy [8], and chemiluminescence technique [9]. LIF

detection is the most widespread detection method used in the industry. However, its major drawback is the fact that several compounds are not naturally fluorescent, thus further steps are required to change the separation properties of the analytes. Moreover, the high cost and large size of the instrumental set up of the LIF detection are sometimes incompatible with the concept of micro total analytical systems (μ-TAS) [10], especially with the applications when portability and disposability are necessary, such as point-of-care or in-situ analysis.

This is quite the opposite of the electrochemical (EC) technique [11], it is preferably suited to miniaturization, biomedical and biological samples analysis. CMOS technology that is compatible with MEMS devices allows fabrication of biosensor devices such as microelectrodes on a single chip. Consequently, it is leading to a fully integrated system. The principle transducing that is based on the electroanalytical chemistry can achieve more than one method, for instance, potentiometry, voltammetry, and conductometry [12]. Therefore, electrochemical technique is considered and it attracts a large number of scientists and researchers. Biosensors is working along with microfluidic functions that can be readily integrated on microchips; using surface and bulk silicon structure with some materials that are compatible with biological issues, such as PMMA, and PDMS. High-performance detection is strongly requested. Consequently, the final success of a μ-TAS is highly determined by the ability of researchers and engineers to realize detection methods that utilize the advantages of reduced diffusion lengths and confined geometries, while also solving the challenges imposed by such miniaturization [13].

Microfluidic channel fabrication depends on master molds. Fabrication of master molds is the key to the replication technologies. There are three methods that are utilized for master molds fabrication, including micromachining methods [14], electroplating methods [15] and silicon micromachining methods [16]. After master molds have been fabricated, several methods can be applied for the replication step, such as hot embossing, injection molding and casting [17].

B. Biosensors Instrumentation and CMOS Technology

A biosensor is defined as a measuring device that exhibits a characteristic of an electrical nature (charge, voltage or current) when it is subjected to a phenomenon that is not electric. The electrical signal it produces must carry all the necessary information about the process under investigation. The bioreceptor has a particularly selective site that identifies the analyte. Most biosensors make use of existing transducers and the instrumentation already associated with them. The principal modifications are made to the part of the transducer where the biological system is to be situated. Biological systems must be renewed periodically to maintain an optimal activity and the membranes that carry proteins and other reactive substances are much easier to handle if they are removable or disposable components [18].

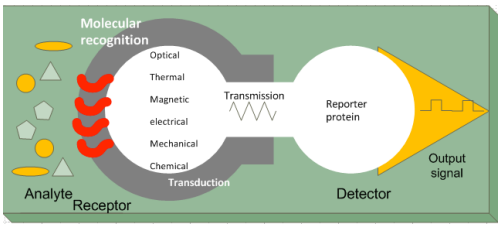

Figure 1: General model for biosensors, differentiating between molecular recognition, transduction and data processing.

The choice of biosensor is often related to the cost of the instrumentation for a given application. Biosensors will be used more extensively in health care in the future, if their total cost, including the instrumentation, is not excessive. In this respect, electrochemical biosensors appear to be well placed, requiring apparatus that is both simple and small. Optical biosensors generally require much larger apparatus with systems of lenses, mirrors, monochromators, and photomultipliers.

All biosensors need a recording system to observe the reproducibility of the signal. This also indicates the nature of the response curve and detects any irregularities. Furthermore, it provides a permanent record of the behavior of the biosensor during both calibration and the determination itself [19].

In the case of biomedical sensors, increasing the information gathering capability per unit volume is often the motivation for integrating electronics in the system. Integration also results in some very promising properties, besides the resulting system is more versatile, it allows drifts to be corrected; fitting and calculation software to be implemented for the measurement based on nonlinear calibration functions; and interferences to be compensated by using sensing arrays. Merging sensing elements with electronic devices for transduction and readout has led to new capabilities, but has also imposed some constraints on the biosensor system; the size of the biomolecular samples [20].

C. Immobilization

In biosensors, functionalization the surface of the bioreceptor is very important step to be occurred in order to integrate the selected biorecognition elements. This is one of the most critical steps in biosensor development because biosensor performance (sensitivity, dynamic range, reproducibility, and response time) depends on how far the original properties of the bioreceptor are kept after its immobilization. Therefore, bioreceptor requires direct or indirect immobilization on transducers to ensure maximal contact and response. Immobilization technique used for the physical or chemical fixation of cells, organelles, enzymes, or other proteins such as monoclonal antibodies, onto a solid support and solid matrix or retained by a membrane, in order to increase their stability and make possible their repeated or continued use. Immobilization has the advantage of stabilizing the protein so that it can be used repetitively. Miniaturizing and developing the sensing surface as a bioreceptor in the biosensors architecture is a challenge and

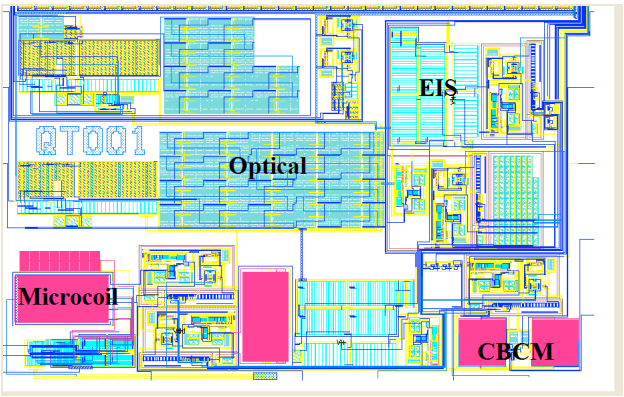

Figure 2: Layout of the entire multibiosensor device on a single chip.

a superior area in enhancing their performance [21]. The sensing properties of a sensor depend on the physical-chemical environment of antibody and antigen-antibody complex, which are in turn determined by antibody immobilization techniques such as adsorption techniques[22], entrapment [23], cross-linkage [24], or magnetic microbeads [25]. Functionalizing the surface of the biosensor; glass or silicon by a chemical material such as epoxysilane, polylysine or aminosilane; facilitates bonding the biological recognition elements such as enzymes [26], antibodies [27] or reporter genes [28]; to the surface; i.e. polymer, glass or silicon of the biosensor.

III. Proposed MLoC System

The capability of the MLoC system complies with employing the advantages of miniaturization that converts the devices from macro scale to micro/nano scale resulting in reduced diffusion lengths and minimized geometries. The miniaturization reduces the cost and time, which helps increase the manufacturing that pushes forward the research and the development in the area of life science and biomedicine applications. The proposed and fabricated single die has all of the biosensors implemented within it using CMOSP35 TSMC technology which leads to a reduction of features that enhances and improves the new generation of multibiosensors, named multi-labs-on-a-single chip (MLoC). Figure 2 shows the layout of the entire proposed MLoC.

A. CMOS capacitance biosensor based on CBCM

The system is designed using TSMC/CMOSP35 technology and it consists of interdigitated capacitor structures (MMCC) and signal detection and processing circuitry. The signal detection and processing is done by a Charge Based Capacitance Measurement (CBCM) circuit was originally proposed as an accurate technique for the characterization of interconnects capacitance in deep submicron CMOS ICs.

The CMOS capacitive based sensors in implementation offer a number of advantages including small size, fast response, and low-cost mass–production. 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 [29]-[34]:

- Enhancing and Improving the sensitivity (improved features) and selectivity of CBCM techniques.
- 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.
- Processing the output signal of the circuit to be readily managing and handling the results.

B. Biosensors Based on VPNP Phototransistor Array

Modern biosensors technologies can provide rapid quantifications of pathogens bacterial cells. Biosensors based-phototransistors are an optical platform capable of highly sensitive and specific measuring of biomolecular interactions in real-time. Biosensors based-phototransistors have been used to detect bacterial pathogens in clinical and food-related samples. Biosensors based-phototransistors can provide sensitive, label-free, and real-time, monitoring of reactions and can quantify the characteristics of biomolecular such as proteins and bacteria, interactions on a surface, including their kinetics, affinity (similarity) and concentration. Biosensors based-phototransistors have been successfully applied to environmental monitoring, biotechnology, medical diagnostics, drug screening, food safety, and homeland security [35]-[41]. The property of the optical biosensor prototype is as following:

- The IC consists of a high-gain phototransistors 32 × 32 array.
- The structure of phototransistor pixel is similar to a bipolar junction transistor and is formed by p-active (emitter)/n-well (base)/p-substrate (collector).
- The phototransistor has one of the highest responsivity (electrical current output/optical power input) of the photodetectors available in the standard CMOS process and particularly in the visible region of the electromagnetic spectrum.
- The vertical phototransistor (VPNP) can produce currents that are several times larger comparing to a comparable sized photodiode.

The optical biosensor IC essentially consists of four blocks; phototransistor array; current to voltage converter; amplifier block; and phase detector. The phototransistor 32 × 32 array of high-sensitivity phototransistors convert the imposed optical signals into electrical current signals and sends the signals to an operational amplifier (op-amp) based circuit that acts as a current-to-voltage converter. The following voltage signals are then amplified by another op-amp based circuit and finally sent to a NXOR based phase detector. The phase detector provides a dc voltage proportional to the phase shift between the detected optical signal and a fixed reference sinusoidal signal obtained from a standard function generator.

The vertical phototransistor is formed by the p-active (emitter)/n-well (base)/p-substrate (collector), and has one of the utmost responsivities of the photodetectors available in standard CMOS process. The inversion emitter improves the overall gain of VPNP for two reasons: first, the

inversion emitter efficiency is inherently higher than shallow diffused emitter and, second, the presence of MOS inversion layer decreases the total base current by eliminating minority carrier surface recombination in the channel area.

C. Biosensor Based on Electrochemical Impedance Spectroscopy (EIS)

EIS is an experimental method of characterizing electrochemical systems. This technique measures the impedance of a system over a range of frequencies, and therefore the frequency response of the system, including the energy storage and dissipation properties, is revealed. Often, data obtained by EIS is expressed graphically in a Bode plot or a Nyquist plot. Impedance is the opposition to the flow of alternating current (*AC*) in a complex system. A passive complex electrical system comprises both energy dissipater (resistor) and energy storage (capacitor) elements. If the system is purely resistive, then the opposition to AC or direct current (DC) is simply resistance. This technique has grown tremendously in stature over the past few years. In recent times, it is being widely employed in a wide variety of biological and life science applications, for instance; microstructure characterization, biomolecular interaction, and particularly pathogen detection.

Often, EIS reveals information about the reaction mechanism of an electrochemical process: different reaction steps will dominate at certain frequencies, and the frequency response shown by EIS can help identify the rate limiting step. Two major limiting factors in mass production and field use of electro-analytical instruments have been considered the size and cost of potentiostats. CMOS potentiostat has performance comparable to bench-top instruments at a fraction of the size, power consumption and cost.

The main function of the integrating CMOS potentiostat is setting the voltage between the working electrode; where the electrochemical reactions of interest take place, and a chemically stable reference electrode. The control amplifier regulates this voltage using feedback to drive a third (counter) electrode. The working electrode current is converted to frequency using signal process unit (AFC). In this paper, we present a fully electronic integrated sensor built in a standard complementary metal-oxide-semiconductor (CMOS) process. To demonstrate the advantages of semiconductor fabrication processes to create a high-performance electrochemical biosensor. The complete system is fabricated within a single chip and built in a standard CMOSP35 process with no post-processing requirements. This integrated CMOS electrochemical sensor capable of performing impedance spectroscopy, potentiometry, voltammetry, and ion-sensitive detection. In this work, we explore the limits of form-factor achievable with electrochemical detection by constructing an integrated sensor directly on an electronically active CMOS substrate. The active CMOS biosensor described here includes off-chip WEs and full potentiostat electronics, including two Op-amplifier for Electrochemical Cell (ECC), current-input analog-to-Frequency converters (AFCs), and data-processing circuitry. A lot of works in this area have reported; integrated potentiostat and conduction-based DNA sensor arrays [42]-[48].

1) Potentiostat

Electrochemical sensing of biomolecules eliminates the need for the bulky and expensive optical instrumentation required in traditional fluorescence-based sensing assays. The block diagram of the microluminometer is shown in Figure 3. It consists of integrated CMOS potentiostat, electrochemical cell and the signal-processing system. The signal-processing system, as shown in Figure 3(c), is a key component of the IC CMOS potentiostat.

It converts the current from the "WE" in to a digital signal, the frequency of which is proportional to the concentration of the contaminant. The integrated potentiostat was composed of a potential controller and a current–frequency converter. The potential controller consisted of two operational amplifiers (TIA) and two load transistors.

The function was to control the potential difference at the interface between the solution droplet and working electrode, where an electrochemical reaction occurred, by reading the input voltage (V_{in}) and adjusting the voltage at the counter electrode (*CE*), so that the potential difference between the voltages at the reference electrode (RE) and working electrode (*WE*) was equal to V_{in}. On the other hand, the current–frequency converter consisted of two operational amplifiers, one-shot, and D flip-flop and a load transistor. The function was to convert the electric current through the working electrode (WE) into a digital signal. The open-loop gain and cutoff frequency of the operational amplifiers were more than 86 dB and roughly 500 kHz, respectively, by setting the control voltage (V_{ctrl}); as shown in Figure 3.

2) Electrochemical cell

Mostly, the electrochemical cell consist three electrodes; counter, reference, and working electrodes, to be fabricated using soft photolithography technology as shown in Figure 4. This cell is also called Microfluidic cell (MFC), and it is developed to have four electrodes instead of three, to handle DNA detecting along with bacteria sensing.

The core of MFC is interdigitated microelectrodes (IDMA) using Au as a good material for immobilization, and the collector (WCE) and generator (WEG) electrodes; as well, where the reference will make of Ag/AgCl and Pt for counter electrode, all has been fabricated on a glass substrate and can be easily made on a polymer material; as well.

D. Biosensor based on CMOS Microcoil embedded with IDMA

A planar microcoil is developing using CMOSP35 to generate magnetic field to attractive the magnetic beads, the property of this device is as follows [49]-[57]:

- CMOS Microcoils 4 × 8 arrays.
- Interdigitated microelectrodes array (IDMA) for impedance measurements.

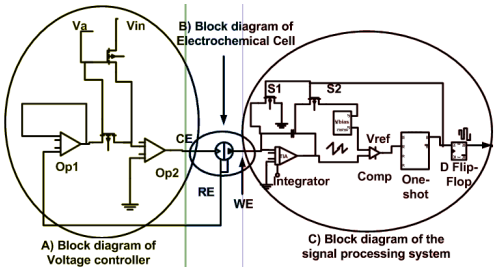

Figure 3: Circuit diagram of EIS.

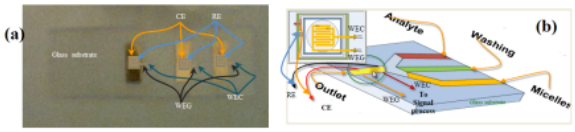

Figure 4: Electrochemical Cell with four electrodes.

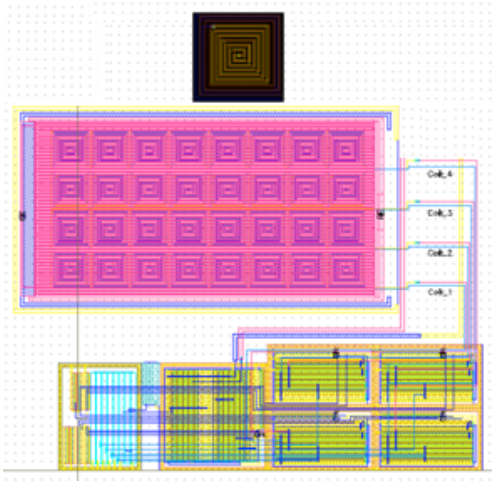

Figure 5: Layout of the magnetic generator along with the control signal and current source circuits.

- Bidirectional Current-source to provide each microcoil individually.
- Signal-process system to measure the output signal.

Figure 5 shows the layout of the magnetic generator along with the control signal and current source circuits, the area of the entire of the circuit is 248.05 µm × 246.725 µm. The magnetic source is building from 32 microcoils in 4 × 8 arrays, each coil look like the inset in Figure 5, which made of three metals; M1, M2, and M3; respectively, connecting with vias, where the input current plug into metal1 (M1) and the output comes out from metal3.

An integrated system; as described here; will have an enormous potential to concentrate and enhance bacterial capture from samples. This integration should improve the detection limit by several orders of magnitude, shorten the analysis and reduce non-specific detection events. Throughout the experimental procedures of setup; a bi-directional microcoil array placed under the biosensor chip; that is interdigitated microelectrodes. The electrode surfaces of the sensor chips will be functionalized with the recognition receptors as specific binding agent. In the proposed project the detection process will be performing through three steps process as follows:

Step 1: Magnetic beads coated with the specific capturing agent and captured bacteria are introduced over the array containing the electrodes coated with the Aptamers; i.e. artificial DNA.

Step 2: **The** magnet will be turned on to attract the magnetic beads, along with the captured analyte; bacteria in our case, on the sensor surface.

As a result, the response of the sensor; impedance; will change due to the added bacteria captured by the fishing system.

Step 3: The magnetic field is then reversed, which causes the unbound magnetic beads to move away from the sensor. Since some magnetic beads are already bound to the sensor, the impedance of the sensor does not reverse to its initial state; meaning Step 1, as opposed to the control sensor. By measuring the difference in sensor response between step 1 and step 3, the measurement perform to be able to determine the amount of magnetic beads, if any, that have bound onto the sensor surface, thus allowing the rapid detection and quantitation of the presence of the specific bacteria.

IV. Results

Several MLOC have been fabricated in order to test them as:

- CMOS capacitance biosensor
- Optical CMOS biosensor.
- Electrochemical impedance spectroscopy biosensor
- CMOS Microcoil embedded with IDMA.

In addition; microfluidic channels have been fabricated for the optical chip and PDMS microchannel credits for MEMS techniques in favor of EIS methods using soft photolithography techniques within cleanroom environmental protocols.

A. CMOS Capacitance Biosensor

The CBCM technique on the MLoC system is tested in appropriate conditions and its performance was validated. The system provided a viable alternative to traditional biological analysis systems, which is mostly time consuming. The system employs an interdigitated capacitor structure in the charge based capacitance measurement technique to detect and process capacitance variations in the presence of targeted biological cells. The system provides a rapid, low power, and miniaturized platform that can be used for mass-production.

Figure 6 shows the variation of the capacitance with respect to a range of frequencies. These results are extracted from the experimental setup.

In the light of the definition of originality, the CBCM techniques fill in the first category where the ideas have been previously published since 1984 but the tools used to create the first biosensor out of four in the MLoC system are CMOSP35, which is one of the most recent technologies. In addition, the reference electrode in this technique is isolated using a special layer made of combinations of CMOSP35 metals, which fill in the third category of originality.

Furthermore, the Signal process unit used to avoid the parasitic capacitance that due to the pins and connectors, which makes the biosensors more reliable and sensitive.

B. Optical CMOS biosensor

E-coli 12 was used to act as a target for a detection process in different concentration and types. The experimental setup involves the integrated CMOS microchip system, an external laser source; 473 nm blue laser; (0.6-25 mW Aquarius Series Blue Laser), and a pinhole was used to eliminate extraneous light from the laser. The laser beam was focused onto the optical chip array using a capillary holder that is adjusted using a translational stage so that the laser beam is passed through the center of the microfluidic channel (MFC). Fluorescence from the MFC was detected with the CMOS microchip that was laid on it. Thereafter, the data was collected using a 2700-Multimeter/Data Acquisition System and National Instruments DAQ516 PCMCIA card installed in a laptop computer. A band pass optical filter (cut-off position: 510 nm, Edmund Industrial Optics) was attached on the CMOS microchip to eliminate the laser scattering.

The bacteria E-coli 12 were prepared in two categories; Gram-positive and Gram-negative. A significant detection voltage was observed when the mixture (bacteria and LB) was pumped through the MFC and the microchip's response was recorded. Figure 7 shows the profile as a result of flowing DI water through MFC, then Gram-positive and negative bacteria in LB media pumping with high concentration (10^9). The profile is a remarkable observation because it shows the behavior of a complex solution alternatively starts with DI water and then pumping bacteria; positive type (BP) lastly pumping Bacteria; negative type; (BN) with high concentration; respectively.

The experiment was repeated after the optical chip was encapsulated using Norland Optical Adhesives. NOA60 are clear, colorless, one part adhesives that contain no solvents. When exposed to ultraviolet light, they gel in seconds and full cure in minutes to give a tough, resilient bond. These adhesives are designed for fast, precision bonding where low strain and optical clarity are required. The experimental result with encapsulation has shown no significant difference from the previous experiment.

Optical-based technique is specifically designed, fabricated, and experimentally validated for realizing a new generation of multi-labs on a single chip (MLoC) system. Attributable to its compact design and multiplex capability, the integrated optical sensor as a part of the MLoC chip worked successfully and provided high-gain and throughput analysis as a tool for the detection of bacteria in medical diagnosis and bacterial pathogen based on its compactness, low cost, multiplex capability, selectivity and sensitive method. The integrated MLoC system as a detector expects to be compatible with conventional microfabricated devices to allow more rapid and high throughput analysis.

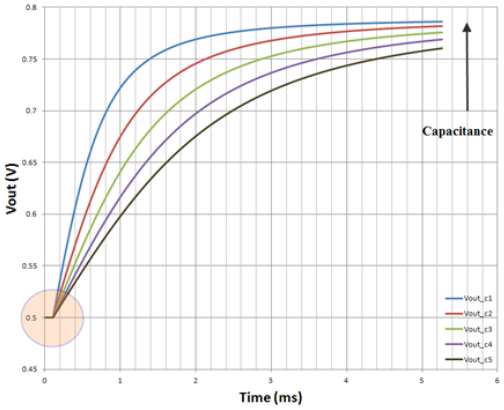

Figure 6: The capacitance measurement using CMOS capacitance biosensor.

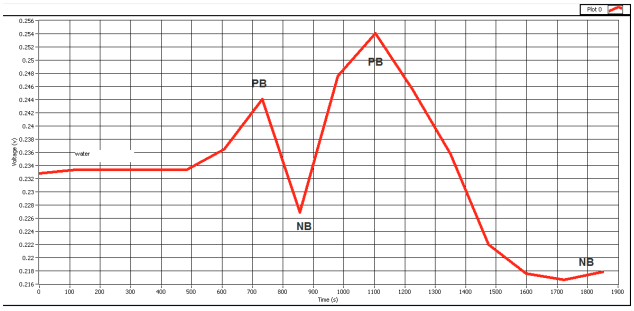

Figure 7: The fluorescence signal for the positive and negative bacteria, with high concentration and DI water after encapsulation.

C. Electrochemical impedance spectroscopy biosensor

Electrochemical-based biosensors are specifically designed, fabricated, and experimentally validated for realizing a new generation of multi-labs-on-a-single-chip (MLoC) system. The synthesis of the electrochemical biosensing with the IDMA biosensor is considered as a part out of four biosensors on the MLoC chip that worked successfully and provided enhancements in the current generation potentiostat system to impart robustness and improve the system performance when compared to the previous generations. Attributable to its compact design, multiplex capability, low cost, selective, and sensitive method, the integrated EIS is considered as a powerful tool for the detection process in medical diagnosis.

This work shows how engineering fields, physics, MEMS, bioelectrochemistry (BEC), and biology are combined at VLSI by replacing the separation between immunosensors and bioreceptors with an integrative approach through utilizing CMOS based biosensing. This work is focused on the interaction between the biosensors and the matching transducer surface nano- architectures with special focus on electronic sensing.

IDMA technology has a potential integrated within MFCI for biomedical and life sciences applications and it is

the main valuable outcome along with the achievement of the miniaturization of biosensors that improves the transducer's sensitivity and selectivity. Presently, EIS incorporated with IDMA based on immunosensors are introduced as solutions for point-of-care diagnosis due to the direct electronic detection that is straightforwardly scalable and integratable into the CMOS technology process.

In the light of the definition of originality, the EIS techniques locate in the first category where the ideas have been previously published. The design in the literature made of a die within 2.25 × 2.25 mm using BiCMOS technology 1.2 µm, and power supply -2V, +7V but the tools for MLoC system are definitely different. In this design, the technology that used is CMOSP35, the area of the overall size of the CMOS potentiostat chip sites is 500 µm by 380.6 µm, and power supply is 3.3V. Furthermore, all of its stages implemented on-chip includes voltage controller and signal-processing unit along with their associating capacitors and resistors.

The experiment was performed using small *AC* potential within a range of frequency from 1 Hz to 100 kHz. The state-of-the-art impedance measurements employ a frequency response analyzer that produces a sequence of alternate current of a range of frequencies. Superimposed with a DC bias current, the current is then applied to the EIS system yielding *AC* current measurements at each selected frequency. Afterwards, the impedance measurements were then analyzed from the excitation function. The results of the process can be recorded and drawn using Nyquist plot, imaginary impedance vs. real impedance, as shown in Figure 8.

The modifications on the electrode surface during the immobilization of antibodies onto the interdigitated microelectrodes array surface caused some variation mainly on the charge-transfer resistance. The changes are due to the antibody protein layer on the electrode surface that established a charge-transfer barrier and the variation increases because of the binding of biological cells on the antibody-immobilized microelectrode surface. The double layer capacitance shows variation for the immobilization of antibodies. In the medium resistance side, the binding biological cells do affect the interface resistance in the microelectrode system by creating resistance along with the medium one causing a little bit of difference from the original value of R_{sol}. The charge-transfer resistance in the semiconductor electrolyte interface can be carried out readily through a Nyquist diagram of the electrochemical impedance spectrum.

The behavior of the system is tested using three different biological samples shown in Figure 9. Generally, the impedance spectrum has two parts a semicircle and a linear line. The semicircle part stands for the charge-transfer process with a diameter equal to the charge-transfer resistance. The linear part stands for diffusion process.

The diameter of the semicircle represents the electron-transfer resistance at the electrode surface. The electrochemical impedance spectroscopy measurements

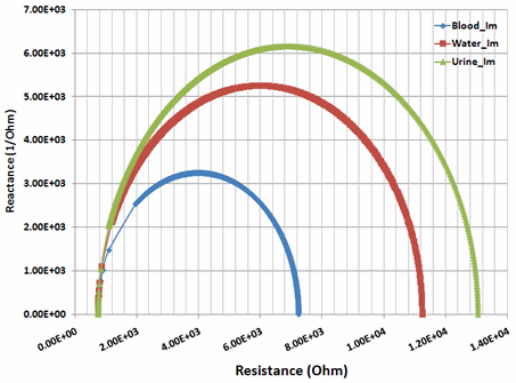

Figure 8: The behavior of EIS after applying three samples.

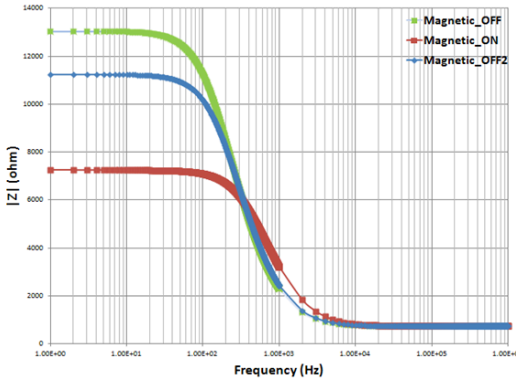

Figure 9: The behavior of the entire process, control and sensing sensor before and after applying magnetic field.

without any amplification shows useful results for the convenience of the IDMA that are used as working electrodes for the detection of bacterial cells bound to its surface. As long as the antibody is spontaneously adsorbed due to the immobilization protocol onto the surface ofmicroelectrodes after coating it by gold, the change in the electrochemical characteristics happened during the binding of the specific antigen. The charge-transfer resistance (R_{ct}) can be figured out from the semicircle in the Nyquist plot that is increased due to the formation of the stable antigen–antibody complex.

D. CMOS Microcoil embedded with IDMA

The experiment ran under small internal *AC* potential to generate the magnetic field in CMOS microcoil arrays, within a range of frequency from 1 Hz to 10 kHz. Afterwards, the results of the process were monitoring and plotting for each as well. The process starts by monitoring the control sensor, which is a bar of electrode immersed in the solution, then after the sensing layer that is immobilizing antibody on the surface of it. Then a combination of the biological cells and the magnetic bead got stable under magnetic field applied B > 0. After removing the magnetic field; B = 0 also the results recorded as well. Each status was plotting using Bode plot, magnitude of impedance vs. frequency, the result of this experiment shown in Figure 9.

In the case of the control sensor, the surface of IDMA not functionalized where the IDMA as a sensing layer is functionalized. Immobilization the antibody onto the surface of the working electrode (*WE*) should take place first follow then immersed "*WE"* into LB media that mixed with biological cells and magnetic bead to be attached.

Consequently, the immunoreaction that took place in between immobilized antibodies and biological cells binding along with the magnetic bead were monitoring and recording for carrying out the difference in charge-transfer resistance R_{ct} and the double layer capacitance; C_{dl}, before and after applying the magnetic field. Figure 9 shows impedance measurements for both biosensors; control and functionalized. The control biosensor, which referred to it on the figure by the green line and signs, it has no modification to the surface of microelectrodes; called bare electrodes, therefore, the impedance shows the maximum due to the low concentration of the biological attached to the surface and the "Magnetic OFF".

The functionalized biosensor where the antibodies immobilized on the surface of microelectrodes, there are two curves, the red line when the "Magnetic ON", and the blue line when the "Magnetic OFF2", the impedance shows the minimum for the case when the "Magnetic ON" and higher for the case when the magnetic released.

However, the latter case still lesser than the control biosensor due the residual of the biological on the surface after releasing the magnetic field. A layer that would reduce the moving and transferring charges in between the electrodes can create because of antibodies immobilization onto the electrode surface that means increasing the R_{ct} resistance. Consequently, this leads mainly to the basic rule that is saying the higher biological cells that bonded to the surface of the electrodes the higher double layer capacitance at low frequency range the minimum impedance measured.

E. Labs on Single Chip

In terms of the transduction techniques used, the four main classes of biosensors that lead to a new generation of MLoC contains capacitive, optical, Electrochemical Impedance Spectroscopy (EIS) and planar microcoils for magnetic field generation techniques.

The modern CMOS technology provides a very high scale device that allows catching the smallest biomedical cells adequately and significantly. Practically and sensibly the new technology is indeed increasing the biosensor sensitivity. In addition; reducing of the biosensor features indeed leads to contract the area and thus the cost and power consumption; accordingly. Implementing MLoC system exhibits eminent capability to achieve high performance of detecting biological samples. The achievement of this objective depends on developing and fabricating a low-cost disposable OFF-chip microfluidic channel interface (MFCI) incorporating it with the interdigitated microelectrode arrays (IDMA) using the soft photolithography technique in the cleanroom environment that will all work along with the MLoC system. At the end, the MLoC system goes through testing and validation. Furthermore, each single biosensor out of four has a new modification in design. For the CBCM technique, a new layer sited on top of the reference capacitor isolates it. This layer consists of two metals that protect it from any external contamination in the course of running the experiment without altering the value of its capacitance. For the optical biosensor, and in the light of the fact that the sensitivity of the optical sensor depends on the exposed area to the light, the surface increases to the VPNP phototransistor 32 x 32 arrays instead of 16 x 16 arrays, also, the chip contains all the required resistors on-chip. For the electrochemical impedance technique (EIS), all of its stages implemented on-chip includes voltage controller and signal-processing unit along with their associating capacitors and resistors. For the CMOS Microcoil biosensors technique, the entire sensor implemented on-chip includes the IDMA sited on the top of the microcoils for magnetic field manipulation. The work includes two types of IDMA; on-chip using CMOS technology for CMOS Microcoil sensor and OFF-chip on MFCI surface using soft photolithograph technique inside the cleanroom. The Labview code has been written to thoroughly control the experiment and provide us with useful information.

V. CONCLUSION

All the aforementioned biosensors worked separately as expected and successfully passed all the validation tests. However, the chip is able to run more than one experiment using the different techniques if a multiplexer is added to the interface of the MLoC system. This could be considered as a future work suggestion.

REFERENCES

[1] U.E. Spichiger-Keller, "Chemical Sensors and Biosensors for Medical and Biological Applications", Wiley VCH, 1998.

[2] J. Irudayaraj and C. Reh, "Nondestructive Testing of Food Quality", Institute of Food Technologists Series, ch11. Wiley-Blackwell, 2008.

[3] V. Rajendran and J. Irudayaraj, "Detection of Glucose, Galactose, and Lactose in Milk with a Microdialysis–Coupled Flow Injection Amperometric Sensor", Journal of Dairy Science, vol. 85, pp. 1357–1361, 2002.

[4] A. Tashtoush, "Novel Multibiosensors and Biomedical Applications", 8th International Conference on Asia Modelling Symposium, 2014, pp. 235-241.

[5] A. Tashtoush, "CMOS Technology for IC Biosensor and Applications, Multi-Labs-On-Single-Chip, (MLoC)", Xlibris Corporation, Bloomington, Indiana, USA, 2013.

[6] G. Ramsay, "Commercial Biosensors: Applications to Clinical, Bioprocess, and Environmental Samples", Wiley-Interscience: New York, 1998.

[7] F. Laugere, R.M. Guijt, J. Bastemeijer, G. van der Steen, A. Berthold, E. Baltussen, P. Sarro, G.W.K. van Dedem, M. Vellekoop, and A. Bossche, "On-chip Contactless Four-Electrode Conductivity Detection for Capillary Electrophoresis Devices", Anal. Chem., vol. 75, pp. 306–312, 2003.

[8] M.A. Ansari, R. Massudi, M. Hejazi, "Experimental and Numerical Study on Simultaneous Effects of Scattering and Absorption on Fluorescence Spectroscopy of a Breast Phantom", Optics & Laser Technology, vol. 41, issue 6, pp. 746-750, 2009.

[9] A.J. Bard, "Electrogenerated Chemiluminescence", ch1 & ch11.

[10] D.R. Reyes, D. Iossifidis, P.A. Auroux, and A. Manz, "Micro Total Analysis Systems. Introduction, Theory, and Technology", Anal. Chem., vol. 74, pp. 2623-2636, 2002.

[11] D.R., Thevenot, K. Toth, and G.S. Wilson, "Electrochemical Biosensors: Recommended Definitions and Classification", Biosens. Bioelectron., vol. 16, pp. 121-131, 2001.

[12] A.J. Bard, M. Stratmann, P.R. Unwin, "Instrumentation and Electroanalytical Chemistry", Encyclopedia of Electrochemistry, vol. 3, Wiley-VCH, New Jersey, 2003.
[13] K. Huikko, R. Kostiainen, T. Kotiaho, "Introduction to Micro-Analytical Systems: Bioanalytical and Pharmaceutical Applications", European Journal of Pharmaceutical Sciences, vol. 20, issue 2, pp. 149-171, 2003.
[14] L. Martynova, L.E. Locascio, M. Gaitan, G.W. Kramer, R.G. Christensen, and W.A. Mac-Crehan, "Fabrication of Plastic Microfluid Channels by Imprinting Methods", Anal. Chem., vol. 69, pp. 4783–4789, 1997.
[15] W. Ehrfeld, and D. Munchmeyer, "Three-Dimensional Microfabrication using Synchrotron Radiation", Nucl. Instrum. Methods Phys. Res. A, vol. 303, pp. 523–532, 1991.
[16] M.J. Madou, Fundamentals of Microfabrication: The Science of Miniaturization, 2002.
[17] Mitchelson, "New High Throughput Technologies for DNA Sequencing and Genomics", Perspectives in Bioanalysis, vol. 2, 2007.
[18] M.L. Yarmush, M. Toner, R. Plonsey, J.D. Bronzino, Biotechnology for Biomedical Engineers (Principles and Applications in Engineering), 2003.
[19] R.F. Taylor, J.S. Schultz, Handbook of Chemical and Biological Sensors, 1996.
[20] D.O. Wipf, R.M. Wightman, "Submicrosecond Measurements with Cyclic Voltammetry", Anal. Chem., vol. 60, no. 22, pp. 2460–2464, 1988.
[21] A. Tashtoush, "Fully IC Label-Free Aptasensor with Interdigitated Microelectrodes in Microfluidic Channel", 8th International Conference on Asia Modelling Symposium, 2014, pp. 124-128.
[22] S.K. Parida, S. Dash, S. Patel, and B.K. Mishra, "Adsorption of Organic Molecules on Silica Surface", Advances in Colloid and Interface Science, vol. 121, pp. 77–110, 2006.
[23] S. Cosnier, "Biosensors Based on Electropolymerized Films: New Trends", Analytical and Bioanalytical Chemistry, vol. 377, pp. 507–520, 2003.
[24] G.T. Hermanson, Bioconjugate Techniques, Academic Press, London, 1996.
[25] J.C. Rife, M.M. Miller, P.E. Sheehan, C.R. Tamanaha, M. Tondra, L.J. Whitman, "Design and Performance of GMR Sensors for the Detection of Magnetic Microbeads in Biosensors", Sensors and Actuators A: Physical, vol. 107, issue 3, pp. 209-218, 2003.
[26] A.L. Smith, Oxford Dictionary of Biochemistry and Molecular Biology, Oxford University Press, 1997.
[27] John Bradley, "Immunoglobulins", Journal of Medical Genetics, vol. 11, pp. 80, 1974.
[28] O.J. Becher, E.C. Holland, "Genetically Engineered Models have Advantages over Xenografts for Preclinical Studies", Cancer Res., vol. 66, no. 7, pp. 3355–3358, 2006.
[29] J.C. Chen, B.W. McGaughy, D. Sylvester, and C. Hu, An On-Chip, Attofarad Interconnect Charge-Based Capacitance Measurement (CBCM) Technique, IEEE, 1996.
[30] J.C. Chen, D. Sylvester, and C. Hu, "An On-Chip, Interconnect Capacitance Characterization Method with Sub-Femto-Farad Resolution", IEEE Transactions on Semiconductor Manufacturing, vol. 11, no. 2, 1998.
[31] R. Bach, B. Davis, R. Laubhan, "Improvements to CBCM (Charge-Based Capacitance Measurement) for Deep Submicron CMOS Technology", Proceedings of the 7th International Symposium on Quality Electronic Design, 2006.
[32] D. Sylvester, J.C. Chen, and C. Hu, "Investigation of Interconnect Capacitance Characterization Using Charge-Based Capacitance Measurement (CBCM) Technique and Three-Dimensional Simulation", IEEE Journal of Solid-State Circuits, vol. 33, no. 3, 1998.
[33] B. Ward, J. Bordelon, S. Prior, B. Tranchina, and J. Liu, "Test Structure and Method for Capacitance Extraction in Mu1ti-Conduc tor Systems", Proc. IEEE 2001 Int. Conference on Microelectronic Test Structures, vol. 14, 2001.
[34] Z. Ning, H.X. Delecourt, L. De Schepper, R. Gillon, "Precise Analogue Characterization of MIM Capacitors Using an Improved Charge-Based Capacitance Measurement (CBCM) Technique", Proceedings of ESSDERC, 2005.
[35] R. Pownall, C. Thangaraj, G. Yuan, P. Nikkel, T.W. Chen, and K.L. Lear, "CMOS Optoelectronic Components for Clock Distribution", Microelectronic Engineering, In Press, 2009.
[36] L. Hannati, P. Pittet, G.N. Lu, and G. Carrillo, "CMOS 32-APS Linear Array for High-sensitivity, Low-resolution Spectrophotometry", Sensors and Actuators B: Chemical, vol. 120, issue 1, pp. 142-149, 2006.
[37] P. Pittet, G.N. Lu, N. Bertoncini, L. Renaud, "CMOS Absorbance Detection System for Capillary Electrophoresis", Materials Science and Engineering: C, vol. 26, issue 2-3, pp. 282-289, 2006.
[38] G. Chen, H. Chen, M. Haurylau, N.A. Nelson, D.H. Albonesi, P.M. Fauchet, E.G. Friedman, "Predictions of CMOS Compatible On-Chip Optical Interconnect", Integration, the VLSI Journal, vol. 40, issue 4, pp. 434-446, 2007.
[39] G. de Graaf and R.F. Wolffenbuttel, "Smart Optical Sensor Systems in CMOS for Measuring Light Intensity and Colour", Sensors and Actuators A: Physical, vol. 67, issue 1-3, pp. 115-119, 1998.
[40] G. de Graaf, R.F. Wolffenbuttel, "Optical CMOS Sensor System for Detection of Light Sources", Sensors and Actuators A: Physical, vol. 110, issue 1-3, pp. 77-81, 2004.
[41] G.S. Sayler, S. Ripp, D. Nivens, and M. Simpson, "Bioluminescent Bioreporter Integrated Circuit; Sensing Analytes and Organism with Living Microoranisms", Journal of Environmental Biotechnology, vol. l, no. 1, pp. 33-39, 2001.
[42] L.J. Yang, Y.B. Li, and G.F. Erf, "Interdigitated Array Microelectrode-based Electrochemical Impedance Immunosensor for Detection of Escherichia Coli O157 : H7", Analytical Chemistry, vol. 76, pp. 1107-1113, 2004.
[43] H. Wang, Y. Chen, A. Hassibi, A. Scherer, A. Hajimiri, "A Frequency-Shift CMOS Magnetic Biosensor Array with Single-Bead Sensitivity and No External Magnet", ISSCC 2009.
[44] L.J. Yang, Y.B. Li, C.L. Griffis, and M.G. Johnson, "Interdigitated Microelectrode (IME) Impedance Sensor for the Detection of Viable Salmonella Typhimurium", Biosensors & Bioelectronics, vol. 19, pp. 1139-1147, 2004.
[45] V. Dharuman, I. Grunwald, E. Nebling, J. Albers, L. Blohm, and R. Hintsche, "Label-free Impedance Detection of Oligonucleotide Hybridisation on Interdigitated Ultramicroelectrodes using Electrochemical Redox Probes", Biosensors & Bioelectronics, vol. 21, pp. 645-654, 2005.
[46] X.H. Li, Y.L. Zhou, T.C. Sutherland, B. Baker, J.S. Lee, and H.B. Kraatz, "Chip-based Microelectrodes for Detection of Single-Nucleotide Mismatch", Analytical Chemistry, vol. 77, pp. 5766-5769, 2005.
[47] A. Hassibi and T.H. Lee, "A Programmable Electrochemical Biosensor Array in 0.18μm Standard CMOS", Digest of IEEE International Solid-State Circuits Conference, 2005, pp. 564-566.
[48] C. Yang, D. Rairigh, and A. Mason, "On-Chip Electrochemical Impedance Spectroscopy for Biosensor Arrays", Proceedings of IEEE Sensors Conference, 2006, pp. 93-96.
[49] S. Eroglu, B. Gimi, B. Roman, G. Friedman, R.L. Magin, "NMR Spiral Surface Microcoils: Design, Fabrication, and Imaging", Concepts in Magnetic Resonance Part B (Magnetic Resonance Engineering), vol. 17B, no. 1, pp. 1–10, 2003.
[50] H. Lee, L. Yong, D. Hamb, and R.M. Westervelta, "Integrated Cell Manipulation System-CMOS/Microfluidic Hybrid", Lab On a Chip, vol. 7, pp. 331–337, 2007.
[51] Q. Ramadan, V.D. Samper, D.P. Puiu, and C. Yu., "Fabrication of Three-Dimensional Magnetic Microdevices with Embedded Microcoils for Magnetic Potential Concentration", Journal of Microelectromechanical Systems, vol. 15, no. 3, 2006.
[52] H. Lee, A.M. Purdon, and R.M. Westervelta, "Manipulation of Biological Cells using a Microelectromagnet Matrix", Applied Physics Letters, vol. 85, no. 6, 2004.
[53] H. Lee, Y. Liu, R.M. Westervelt, and D. Ham, "IC/Microfluidic Hybrid System for Magnetic Manipulation of Biological Cells", IEEE Journal of Solid-State Circuits, vol. 41, no. 6, 2006.

[54] H. Lee, Y. Liu, E. Alsberg, D.E. Ingber, R.M. Westervelt, and D. Ham, "An IC/Microfluidic Hybrid Microsystem for 2D Magnetic Manipulation of Individual Biological Cells", 2005 IEEE International Solid-State Circuits Conference, 2005.
[55] H-M. Hsu, "Investigation on the Layout Parameters of On-chip Inductor", Microelectronics Journal, vol. 37, pp. 800–803, 2006.
[56] U. Lehmann, M. Sergio, S. Pietrocola, C. Niclass, E. Charbon, and M.A.M. Gijs, "A CMOS Microsystem Combining Magnetic Actuation and in-Situ Optical Detection of Microparticles", Transducers, 2007.
[57] M.A.M. Gijs, "Magnetic Particle Handling Microsystems for Miniaturized Analytical Applications", 29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, 2007, pp. 4088–4089.