

# An Integrated Biosensor for the Detection of Bio-entities Using Magnetotactic Bacteria and CMOS Technology

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**Abstract**—An integrated biosensor for the detection of micron-size biological entities using Magnetotactic Bacteria (MTB) being guided under the control of a few Gauss external magnetic field is briefly described. The proposed biosensor will be implemented onto a silicon substrate compatible with standard CMOS technologies. To validate the proposed concept, a microfluidic device and a microelectronic chip have been fabricated. Pairs of planar microelectrodes are patterned on the bottom of a microchannel and connected with the microelectronic chip. When a microbead pushed by a single MTB passes between a pair of microelectrodes, a variation in impedance is measured by embedded detection circuits. A diagram of the microsystem with emphasis on the sensing principle is first introduced and the technique to implement the microelectrodes is described. Our preliminary experimental results indicate that the fast detection of biological entities, especially bacteria or functional microbeads, can be done with the proposed biosensors.

## I. INTRODUCTION

The detection of pathogenic bacteria in industrial and clinical samples is an important issue in bioengineering.

We report here the use of Magnetotactic Bacteria (MTB) as an actuation method to transport samples between microelectrodes instead of more traditional mechanisms such as dielectrophoresis. The paper also proposes detection circuit with pairs of microelectrodes with the potential of higher sensitivity compared to other existing electrical or optical detection systems. For better performance, all components have been designed to be integrated within a single microsystem without the need for external equipment. The fundamental principle and architecture of such system was first described in [1, 2] and the design of the corresponding microelectronics sub-circuits was reported [3]. Beside portability, short detection time, and improved detection sensitivity, the system provides specificity where targeted pathogenic bacteria can be detected in samples containing other types of bacteria.

## II. OVERVIEW OF SOME PREVIOUS WORKS

Several systems were proposed in the past few years to detect microparticles by measuring their electrical impedance. Although it is beyond the scope of this paper to describe all the system, some main examples are provided. A classical example is the impedance spectroscopy flow

cytometer [4]. This system easily achieves submicron accuracy and as such, can differentiate small polystyrene beads with sizes ranging from 4 $\mu$ m to 6 $\mu$ m using the opacity concept. Moreover, it differentiates ghosts from red blood cells using phase information. A problem with the system is that it uses several external equipments to determine impedance values and thus to differentiate between types of particles. It is a rather complex system, and it is not suitable for in-situ bacterial detection.

Another example is the microelectrode array system used to capture and detect small numbers of bacteria in a millimeter-sized sample [5] by exploiting the high polarizability and dielectrophoretic mobility of single-walled carbon nanotubes (SWNT). A SWNT solution is mixed with the bacteria or nanoparticles in the sample. When applying a high frequency (>100 kHz) AC electrical field via the electrodes, the bacteria are absorbed by the SWNTs via dipole-dipole interaction. Applying the same field on the SWNT-bacteria aggregate by positive AC-dielectrophoresis, the bacteria and the SWNTs tend to form a chain between the electrodes pairs. In other words, they align themselves along the electrodes cathode and anode; thus, forming a conducting line. By measuring the impedance between the pair of electrodes, a detection threshold of 10<sup>4</sup> bacteria/mL is achieved. The problem here is again due to the needs for external equipment.

A somewhat similar microfluidic chip was designed for fast detection of bacteria in diluted samples [6]. Dielectrophoretic force is used to concentrate the bacteria in a chamber (400pL volume) and the detection is then conducted by electrical means. Once more, even if this system provides rapid detection, it still requires external equipment.

An example of a biosensor that does not use electrophoresis was presented lately [7]. In this sensor, specific antibodies are immobilized on the oxide between electrodes in an array like structure. Bacteria are then attached to the antibodies when they pass near them. The chip is washed away from undesired particles and then impedance detection is conducted. The impedance change caused by the bacteria gives information about the concentration of the latter in the sample. The problem with this chip is that a lot of steps are required to perform a single detection.

In this paper, we will present the method used to validate

the concept of the microelectronics circuit mentioned earlier using planar electrodes with the use of MTB. Some preliminary experimental results are also provided.

### III. OVERVIEW OF THE BIOSENSOR

The proposed biosensor as depicted in Fig. 1 is innovative in several ways. To capture pathogenic bacteria into the sensing area, MTB of type MC-1 are used. This type of bacteria responds to the magnetic field through magnetotaxis. Hence, the swimming direction of the MTB can be set by controlling the direction of the magnetic field. MC-1 bacteria swim at an average rate of approximately  $100\mu\text{m/s}$  which makes the sensing time very small for reasonable size chambers [2]. To capture the pathogenic bacteria, MC-1 bacteria are attached to conductive microbeads where some specific antibodies are immobilized. Bacteriophage specific to the targeted pathogenic bacteria are also immobilized on the same bead. When the MC-1 bacteria swim in the sample and encounter a pathogenic bacterium, the bacteriophage attacks the latter and sticks to it. Then, the magnetic field forces the MTB to go toward the sensing area where impedance measurement can begin.

To maximize the detection probability, the sensing area consists of a very dense electrodes array. The electrodes are paired and the detection is done serially. Each pair of electrodes has an XY address and can be accessed independently. There is a detection circuit for each electrodes pair.

There are three possible cases that can occur between the electrodes. First, we can only have the conducting (ionized) medium. This means that nothing has been detected and the

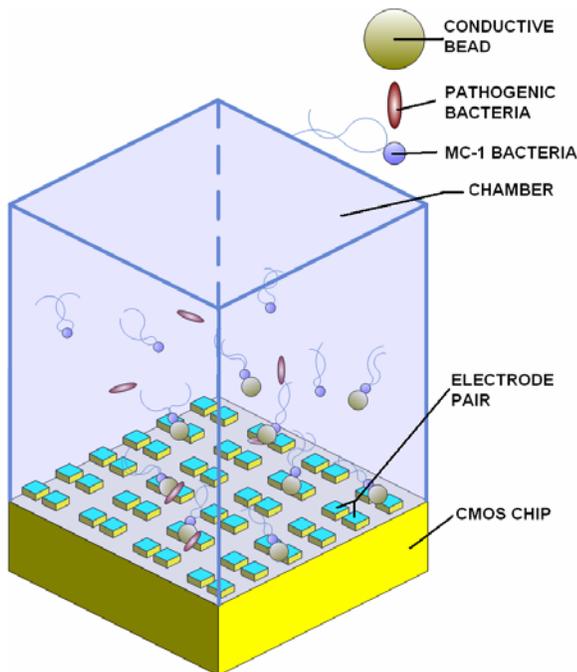


Fig. 1. Proposed biosensor overview  
impedance level would be low. Second, we can have an MC-1 MTB attached to a conductive microbead without a

pathogenic bacterium. In that case, the impedance level is intermediate and is made of the resistance of the microbead and the resistance of the cytoplasm of the MTB. Finally, we may have a MC-1 bacterium pushing a microbead with a pathogenic bacterium attached to it. In such a case, the impedance level is expected to be high and composed of both the resistance of the microbead combined with the cytoplasm of each bacterium.

It is important to note here that if we are seeking such sensitivity in the detection, the voltage across a pair of electrodes must always be greater than the bacterium's membrane breakdown voltage which is approximately 0.2 to 1.5V [8]. In this manner, we can neglect the membranes capacitance and the bacterium becomes conductive or at least we have access to the cytoplasm, which has a relatively high conductivity.

Once this is done, we can compute the concentration of pathogenic bacteria and the proportion of MC-1 bacteria that made it to the sensing area. A standard microcontroller can be used for this step. The same microcontroller can activate and control all the sensing steps.

### IV. MICRO FABRICATED ELECTRODES

To verify the feasibility of detecting a single bacterium, a microfluidic device was designed and fabricated. The main structure of the device was fabricated using the MicraGEM process provided by Micralyne [9]. MicraGEM uses Pyrex glass as its substrate material. Two different depths,  $10\mu\text{m}$  and  $12\mu\text{m}$  can be etched into the Pyrex layer. A monocrystalline silicon layer ( $10\mu\text{m}$ ) is anodically bonded to the Pyrex substrate to form the structural layer. Metal can be deposited on the Pyrex glass and on top of the silicon layer, which allows for the creation of electrodes and/or electronic

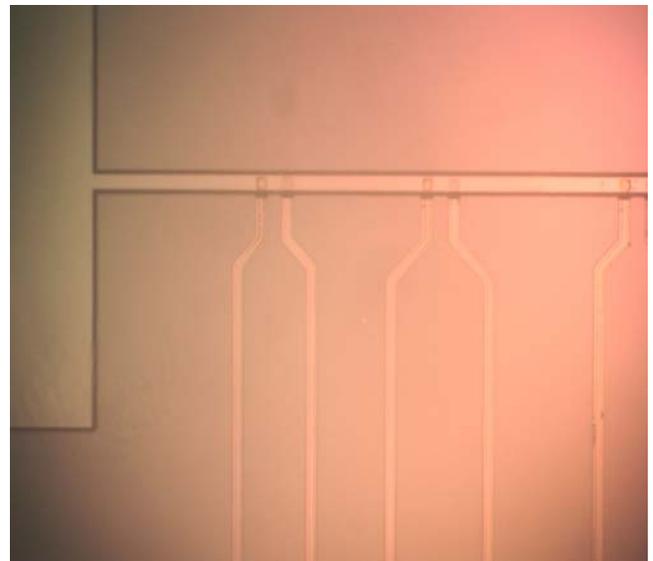


Fig. 2. Photograph of the fabricated microelectrodes

connections. Using this process, we implemented a microchannel ( $52\mu\text{m} \times 12\mu\text{m}$ ,  $W \times H$ ) on the Pyrex substrate. Microelectrodes ( $20\mu\text{m} \times 20\mu\text{m}$ ) for the impedance

measurements are patterned on the bottom of the microchannels. The monocrystalline silicon layer is then used to seal the microchannel and microchamber and to provide inlets for sample injection. The fabricated microdevice is shown in Fig. 2.

### V. IMPEDANCE DETECTION CIRCUIT

As mentioned earlier, for each pair of electrodes, there is a corresponding detection circuit. This circuit is based on the detection of the real component of the impedance. Figure 3 shows a simplified block diagram illustrating the principle of operation of the measurement circuit used.

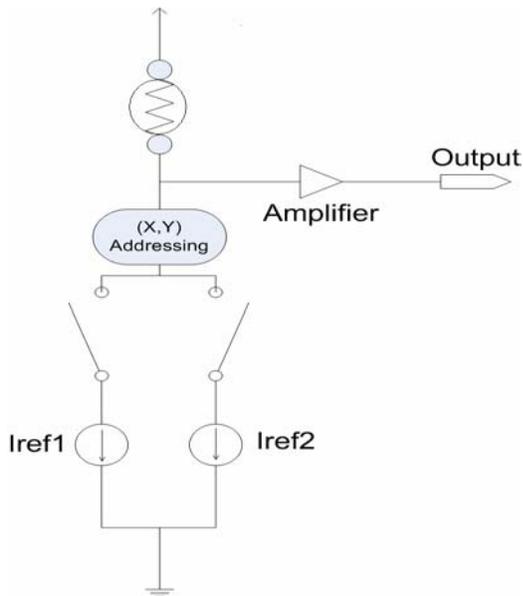


Fig. 3. Simplified circuit diagram of the internal architecture of the impedance measurement microchip.

What the circuit actually does is to determine the level of impedance present between an electrodes pair. To do so, one injects a first reference current from one end of the electrodes pair to the other. The injected current can be adjusted according to some target impedance detection threshold impedance to create a voltage drop between the electrodes. This voltage is then amplified by a digital buffer that has a predefined voltage detection threshold. The digital output reflects whether the detected resistance is higher or lower than the threshold resistance that corresponds to the current injected.

Another reference current corresponding to a second threshold impedance is then injected between the same electrodes pair. When the second current is injected, a new digital output is produced. By using these two digital values, one can determine whether the impedance between the electrodes is low, high, or intermediate.

Since the experimental system is designed to be flexible, the reference currents are not fixed and the user of the

biosensor can calibrate these currents for each specific measurement. Thus, the same circuit can be used to detect a wide range of microparticles and bacteria depending on the technique used to gather them and the selected reference currents. A photograph of the prototype integrated circuit further described in [3] is shown in Fig. 4.

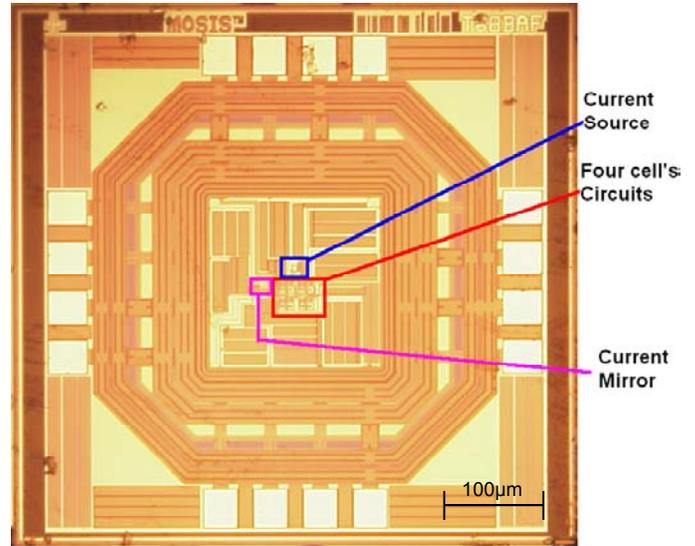


Fig. 4. Picture of the CMOS measurement microchip.

The figure shows the four circuits corresponding to the electronic needed for four pairs of electrodes. In this prototype, electrodes are connected to the circuits using standard pads. In the target microchip, the electrodes will be mounted on the CMOS chip to allow reaching a much higher density. Indeed, a density of more than 1500 cells/mm<sup>2</sup> is achievable. Note that density is limited by the area of the microelectronic circuit.

### VI. EXPERIMENTAL RESULTS

The preliminary results are obtained using two different sets of tests. In the first set of experiments, the objective is to find the suitable impedance thresholds. This is part of the calibration step of the biosensor. Resistors of different values were connected to the electrodes inputs of the CMOS chip (40DIP package). The reference current was then tuned for each resistor to find the one that makes the digital output switch from a digital "0" to a digital "1", or from a digital "1" to a digital "0". This allows us to find the reference currents for which the impedance threshold would correspond to the resistance value of the resistor under test. In the end, we get a characterized CMOS chip ready to be used as a sensor only by choosing the right reference current corresponding to the specific microparticle that one wants to detect. Table I shows some preliminary results for threshold impedance and its corresponding reference current.

Table I. Resistance detection threshold for various injected currents

Threshold Impedance(k $\Omega$ )	Reference Current( $\mu$ A)
36	148
560	35
1500	11

Based on these results, an application requiring low and high threshold impedances of 560 k $\Omega$  and 1.5M $\Omega$ , two reference currents of 35 $\mu$ A and 11 $\mu$ A are needed respectively when a supply voltage of 1.8V is used.

In a second set of tests, the micro-fabricated electrodes and CMOS chip were used together to differentiate between deionized water, magnetotactic bacteria medium at normal temperature and magnetotactic bacteria medium at a higher temperature. We used a microscope to ensure that at each time, the liquid under test was effectively between an electrode pair. We then measured the analog output [3]. The test was done under a reference current of 150 $\mu$ A. We choose this value of electrical current to get a relatively large analog output voltage that can be measured with a standard multimeter.

It is of interest that with deionized water, a voltage close to 0 volt was measured. This is because the impedance is very high in that case, due to the high resistivity of deionized water. For the normal temperature medium, an analog output voltage of 90mV was measured whereas for the hot medium, 190mV was measured. This result is not surprising because the conductivity of our medium (salted water) is much higher than the conductivity of deionized water and it gets even higher for the medium at a higher temperature. This explains why the voltage drop across the electrodes pair is so large.

Note that if one wants to use the biosensor to detect what type of liquid is present between an electrodes pair, it is necessary to compute the average values of impedance corresponding to each type of liquid and then select two relevant reference currents defining the targeted threshold impedance. Based on the values of the digital outputs obtained in response to each current, one can conclude on the nature of the liquid

## VI. CONCLUSION

In this paper we presented a novel biosensor. This biosensor is based on the use of MC-1 magnetotactic bacteria to capture and bring the pathogenic bacteria to the sensing area. The latter consists of an array like structure of microelectrodes that can be selected independently. Each pair of electrodes is connected to a dedicated microelectronic subcircuit. This circuit was fabricated and tested using 20 $\mu$ m  $\times$  20 $\mu$ m electrodes. It was able to differentiate between deionized water, normal temperature

bacterial medium, and hot bacterial medium.

Results are promising. Future works include the fabrication of microelectrodes and microelectronic subcircuit being fabricated on the same CMOS chip using a standard process. The proposed class of biosensor has an excellent potential to provide a low cost solution with faster detection time, increase sensitivity and specificity that works without external test equipments, a feature that no previously reported biosensor concept possesses.

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