System Design of an Integrated Measurement Electronic Subsystem for Bacteria Detection Using an Electrode Array and MC-1 Magnetotactic Bacteria

Jaouad El Fouladi, Walder André, Yvon Savaria *Senior Member IEEE*, and Sylvain Martel, *Member, IEEE, EE and CE Departments, École Polytechnique de Montréal, Canada*

Abstract

This paper presents a novel technique for bacteria detection. The proposed system uses MC-1 magnetotactic bacteria and measures impedance to detect the presence of pathogenic bacteria. An electrode array is connected to respective cells that are fully integrated for impedance detection. The simulated performance shows that the circuit that was designed is robust. It can detect impedances ranging from about 3KΩ to at least 100MΩ. The only limits that we have for detecting large impedances are the operation frequencies and leakage currents. The circuit is thus very robust and can adapt to a wide range of uncertainty.

I. Introduction

IMPEDANCE is an electrical parameter fundamental to characterize conductive materials─especially in electrical circuits. One application is the measurement of the impedance for biological materials. Previous works in the field of bioengineering have demonstrated the electrical conductivity of these materials, and have derived equivalent electrical circuit models [1]. As a result, the measurement of the impedance constitutes an important avenue to characterize materials properties in the field of medicine, biomedical and bioengineering [2]. Recently, a chip for tissue impedances measurement has been reported [2], and a preliminary biosensor design based on magnetotactic bacteria has been proposed [6].

Although there are many publications related to impedance measurement, very few are relevant with respect to the design of an integrated microelectronic chip for in-situ impedance measurement applicable to biosensors based on magnetotactic bacteria. In fact, impedance measurement of biological cells was mainly reported using external impedance analyzers [4].

This paper presents the design of a novel integrated microelectronic circuit for in-situ impedance measurement of biological cells, such as bacteria. This circuit is designed to allow detecting the presence of specific bacteria in a biological sample. The principle of operation of the proposed system [5] is such that a large number (possibly hundreds) of electrode pairs are needed in order to increase the detection probability. There are many degrees of freedom in the design of an effective electrode array for bacteria detection and the exploration of these degrees of freedom is conducted as part of a concurrent on-going research. The characteristics of these electrodes are expected to have a strong influence on the level of impedance that corresponds to detection of a bacterium. Moreover, physical constraints such as achieving an exact alignment of target cells between the electrode pairs can also affect the measurements. Based on those considerations, the proposed design, presented in a conceptual form in figure 1, must be robust and flexible, allowing the measurement of impedance that vary over a wide range. As shown in figure 1, each cell comprises an address decode module, which allows the selection of a chosen pair of electrodes using an X-Y wire grid, and two current sources allowing the determination of three ranges of impedances (low, medium and high). As will be shown in this paper, it enables adjusting the measurements over a wide range of impedance variation. This paper is divided in five sections. In section II, an overview of some relevant previous works is done, while in section III and IV the circuit for impedance measurement, and the corresponding simulation results are presented. Finally our key results are summarized in section V.

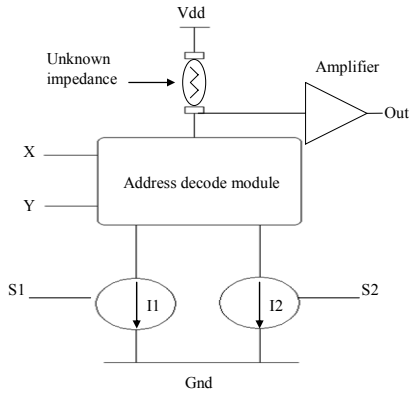

Fig. 1. Block diagram of the internal architecture of the impedance measurement microchip.

II. Overview of Previous Works

Several systems have been developed to characterize biological materials using conventional microelectronic circuits, microfluidic devices or microelectromechanical

systems (MEMS). An example, is the system that integrates electronic circuits for in-situ tissue impedance measurements [3]. It uses the four-point method to measure the unknown impedance of an electrode. In our application, we assume a direct contact between the bacteria and the electrodes; thus, neglecting the electrode-electrolyte interface impedance. Moreover, according to the form factor of the micro bead used as bacterial carrier, electrodes used in four-point measurements are not suitable.

Other designs have been developed to capture and to detect small number of bacteria in a fluid sample [4]. They are based on external impedance analyzers to measure the impedance between some electrodes pairs.

Another example is the microfluidic biochip used for impedance spectroscopy [5], which measures concentration variation in biological solutions. Since a change in the electrolyte concentration is due to the bacterial metabolic activity; a change in impedance indicates the presence of living bacteria in a sample.

In previous work from some of the authors, a biosensor system based on MC-1 magnetotactic bacteria [6] was recently proposed. It combines a microelectronic device used to scan an array of electrodes for impedance measurement, and a microfluidic circuit to shelter the magnetotactic bacteria that are used to capture and to align the pathogenic bacteria across pairs of electrodes prior to impedance measurements. The circuit presented in the rest of this paper has been designed to fit the preliminary design of the biosensor based on magnetotactic bacteria. As this system uses magnetotactic bacteria to capture other pathogenic bacteria [6] it is faster and more efficient than previous ones. Indeed, the average speed of an MC-1 is 200 μm/s. In contrast, the detection of bacteria based on impedance spectroscopy can take up to a week.

III. Proposed circuit for Bacterial impedance Measurement.

As shown in Fig. 1, the principle of operation of our system is based on detecting impedance levels across electrode pairs laid out in an array, in order to indicate the presence or absence of bacteria to be detected. The system developed by our team is the first of its kind. This leads to a great deal of uncertainty with respect to its specifications. This uncertainty results in a lack of solid data on the impedances of the bacteria to be detected, and the beads on which the bacteria are attached, as well as the impedance of the electrodes. Moreover, research on these characteristics is done at the same time as the development of the microelectronic part. Consequently, at the time of writing this paper, we must design a circuit that can adapt to a wide range of situations

Preliminary system design led us to assume that there are three distinguishable impedance levels that need to be detected. The first one is when there is nothing between a selected pair of electrodes. In this case, the impedance is the highest and it nominally tends towards infinity (leakage through the fluid supporting the bacteria). The second case is when only the conductive bead carried by the magnetotactic bacteria is located between the selected pair of electrodes. As a result electrodes are shorted and the impedance is the lowest (at least the contact impedance of electrodes to bead considering imperfect alignment). Finally the last case is when a bacterium is found by a magneto tactic bacterium and brought back between a pair of electrodes. In that last case, the expected value of impedance is intermediate. As we mentioned before, at the time of completing the design, the exact thresholds between these levels are not known, and the circuit to detect these impedance levels was developed to operate properly in spite of wide uncertainty ranges.

Let us now consider means of detecting these three impedance levels. The proposed approach is to inject successively two reference currents between two electrodes, generating a voltage at one end of the electrode pair. By setting a threshold voltage, the generated analog voltage is converted into a digital signal. The detected analog voltage varies according to equation 1:

$$V_{out} = V_{dd} - R_u \times I_{ref} \qquad (1)$$

where, R_u is the unknown impedance, I_{ref} is the reference current used to measure the impedance, V_{out} is the output voltage and V_{dd} is the power supply voltage.

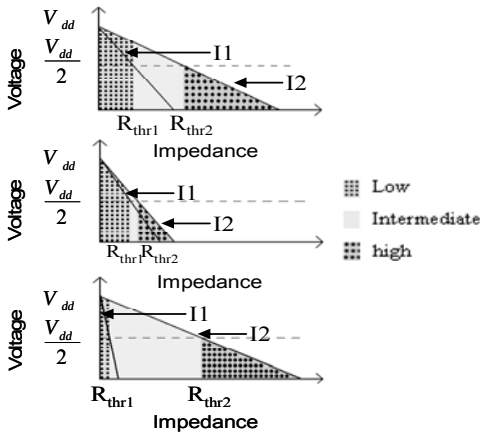

Fig. 2. Graphical representation of the discrimination concept used to distinguish between levels of impedance.

Figure 2 presents various output curves that could be observed for some reference currents and impedance levels. It should be noted that the current determines the slope of these curves. The figure also shows that by quantizing the analog voltage according to some threshold voltage, it is possible to detect the desired levels of impedances. In this example we chose a voltage threshold of $V_{dd}/2$ that defines resistance thresholds R_{thr1} and R_{thr2} separating regions of interest.

The figure also shows that it is possible to distinguish between high and low impedances even if the ratio between these impedances varies by large factors. Indeed, the proposed circuit allows adjusting detection thresholds

independently over wide ranges. This is done by injecting suitable reference currents with external sources. As these two reference currents can be adjusted independently, the detected impedance threshold can be adjusted as desired.

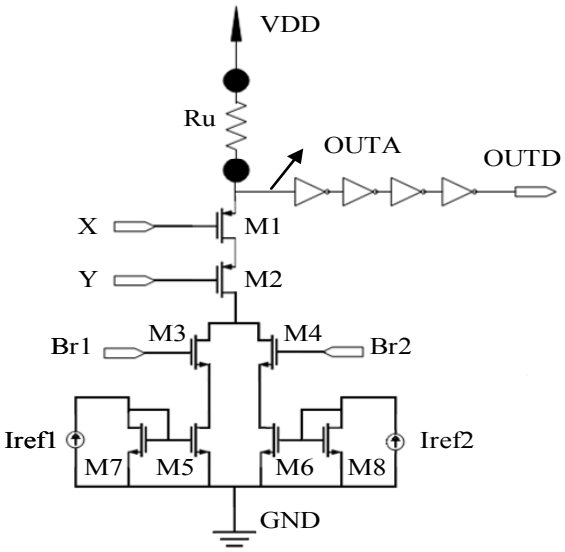

Fig. 3.Schematic of a cell.

A transistor level schematic diagram of the proposed circuit is shown in figure 3. In this circuit, transistors M1 and M2 act like simple switches and are used to select the active cell using and X-Y control grid. Recall that our system is based on a micro electrodes array, and individual cells in that two dimensional array can be selected with X-Y digital control signals. Transistors M3 and M4 are used to select the active branch that sets the current to be injected into the pair of electrodes. Note that to distinguish three impedance levels, two different reference currents must be injected in succession. The resulting analog voltages (OUTA) can be converted to digital signals (OUTD) with a chain of 4 inverters.

To activate the two detection thresholds, it is thus necessary to activate successively M3 and M4. The currents that come from signal external to the array are injected in each cell by current mirrors formed by M5–M7 and M6–M8 respectively. Note that transistors M7 and M8 are not part of the cells that compose the array. They are shared by all cells and their gate voltages are made available to all cells in the array that need to produce a copy of each reference current. This is illustrated in figure 4 that sketches the complete circuit for an array of 4 cells.

IV. Simulation Results.

In preparation for laying out this circuit, detailed circuit simulations were conducted to validate it. Figure 5 presents the results of a resistance sweep for a resistor located between a pair of electrodes. The simulated interval is from 5kΩ to 45kΩ and the reference current was set to 500µA.

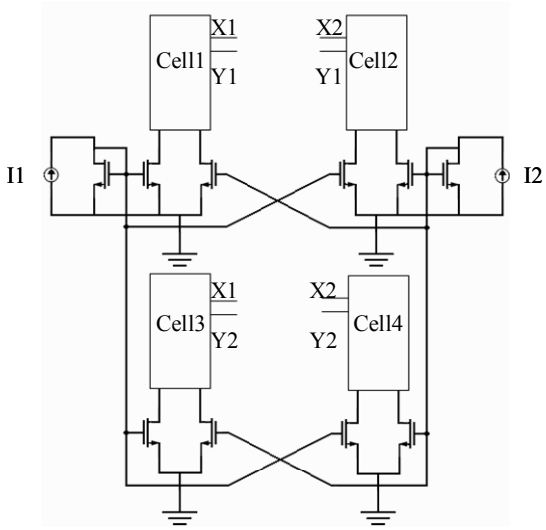

Fig. 4. Four cell array block diagram.

Curve A shows how the analog output voltage of the cell changes with impedance. This circuit simulation result generally confirms the expected behavior shown in figure 2. However, the voltage on OUTA does not reach zero, but it rather saturates around 0.5V. This is due to the fact that the current mirror used is not ideal when the voltage is low and the transistors (M5 or M6) enter their triode region.

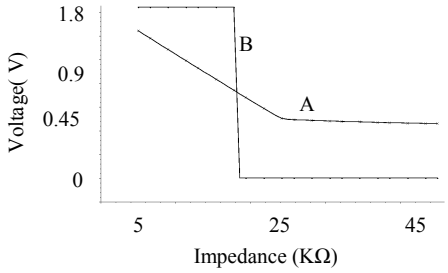

Fig. 5.Impedance sweep simulation result.

Curve B shows the digital output at node OUTD. In this simulation, the inverters were designed to have a threshold of 0.9V which corresponds to $V_{dd}/2$. The reported simulation result confirms that when OUTA crosses that threshold, OUTD switches between a logical "1" and a logical "0". That corresponds to an impedance threshold of approximately 20kΩ. The following analytic expression gives a good approximation of this threshold impedance

$$R_{thr} = \frac{I_{ref}}{V_{thr}} \frac{W_{5,6}}{L_{5,6}} \times \frac{L_{7,8}}{W_{7.8}} \qquad (2)$$

Where R_{thr} is the impedance threshold, I_{ref} is the reference current used, V_{thr} is the voltage threshold used to define the digital decision values and finally $W_{5,6}$,$L_{5,6}$,$W_{7,8}$ and $L_{7,8}$ are the channel width and length of transistors M5, M6, M7 and M8 respectively.

Table I shows the result for various tests made with different reference currents to determine the threshold

impedance that the circuit can reach. Above 4 mA, the circuit saturates, but it can work with currents lower than the 50 nA reported in Table I.

Table I. Resistance detection threshold for various injected currents with *W5*/*L5* = 4/2µm and *W7*/*L7* = 40/2µm (TSMC 180 nm CMOS technology)

Injected Current(µA)	Resistance Threshold(KΩ)
0.05	180000
1	8970
10	905
500	18.4
1000	9.2
4000	2.9

Table I shows that we can set a threshold as low as 3kΩ. The system is mainly limited by the input impedance of the current mirrors which becomes significant for small impedances. For the highest threshold, we determined that the intrinsic noise introduced by the impedance to be measured, which can be evaluated with Eq. 3, can be neglected

$$V_{noise} = \sqrt{\frac{2kT}{\pi C_{electrode}}} \qquad (3)$$

In Eq.3 *k* is the Boltzman constant, *T* is the temperature and $C_{electrode}$ is the capacitance of the electrode. For instance, if we have a capacitance in the range of 1 pF and T=300K.we obtain a noise voltage amplitude of 52µV. The latter is very small with respect to the threshold voltage of $V_{dd}/2$. If we neglect leakages in the liquid supporting the bacteria, our analysis indicates that the maximum threshold impedance the proposed circuit can reach is limited mainly by the frequency of operation and is giving by Eq. 4

$$R_{\max} = \frac{1}{2\pi f C_{electrode}} \qquad (4)$$

Where *f* is the frequency of operation, $R_{\max}$ is the maximum threshold impedance achievable and $C_{\text{electrode}}$ is the capacitance of the electrodes. We can also express the relation between the minimum reference current that can be used and the frequency as:

$$I_{\min} = fV_{dd}\pi C \qquad (5)$$

Where *f* is the frequency of operation, $C_{\text{electrode}}$ is the capacitance of the electrode, $I_{\min}$ is the minimum current reachable and V_{dd} is the power supply voltage. For example, if we wish to have a frequency of operation of at least 1000Hz, an $I_{\min}$ as low as 5.65nA could be used, which corresponds to a maximum threshold impedance of 159MΩ. Thus the circuit can work properly with bacteria characterized by a wide range of impedances as long as there is a sufficient relative difference between the impedances that characterize the situations of interest.

Finally Figure 6 shows a typical result that one can obtain with our system. This simulation was made with reference currents of 20µA and 6.6uA. It is a transient simulation of 1 µs duration. The impedance to be detected was 600KΩ. The result is a sequence of two digital symbols: 10. This means that the impedance between the electrodes is at an intermediate level. This is the expected response as the selected bias currents define low and high threshold impedances of 500KΩ and 1MΩ.

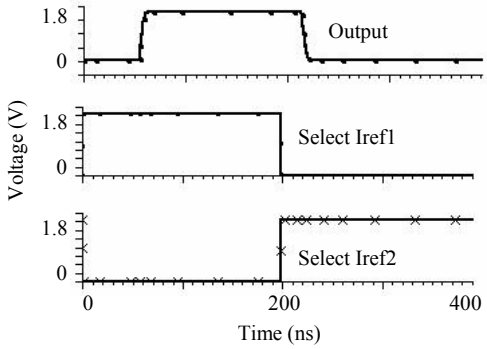

Fig.6. A typical simulation result

V. CONCLUSION.

In this paper, we presented a novel system for bacteria detection based on the use of MC-1 magnetotactic bacteria and impedance measurement of target bacteria. We presented the detection method used as well as the proposed circuit. The latter comprises an array of electrodes and can function with impedances varying over a wide range. It differs from existing systems by the fact that it is completely integrated and does not require external measurement equipment. The technique used in this system was never used before, thus data on the impedances of the target bacteria is unavailable. The electronic subsystem proposed here was designed to be very robust. For instance it can be adjusted to detect impedance thresholds ranging from few kΩ to more than 100 MΩ..

REFERENCES

[1] M.R. Stephen, and C.A. Evangelyn, "Design and fabrication of a microimpedance biosensor for bacterial detection", IEEE sensor journal vol.4, No.4, 2004, pp. 434-440..

[2] A. Rerkratn, K. Chitsakul, and M. Sangworasil, "4-channel bio-impedance measurement system for medical applications", Procedings, 10th International Annual Conference, ISIC-2004, pp. 411-414.

[3] A. Yufera, G. Leger, E.O. Rodriguez-Villegas, J.M. Munoz, A. Rueda, A. Ivorra, R. Gomez, N. Noguera, and J Agillo, "An integrated circuit for tissue impedance measure", 2nd Annual International Conference IEEE-EMBS 2002, pp. 88-93.

[4] Z. Ronghui, W. Ping, and C. Hsueh-Chia, "Bacteria capture, concentration and detection by alternating current dielectrophoresis and self-assembly of dispersed single-wall carbon nanotubes" Electrophoresis 2006, 27, pp. 1376–1385.

[5] R. Gomez, R. Bashir, A. Sarikay, M.R. Ladisch, J. Sturgis, J.P. Robinson, T. Geng, A.K. Bhunia, H.L. Apple, and S. Wereley, "Microfluidic biochip for impedance spectroscopy of biological species", Biomedical Microdevices, V3, n3, 2001, pp. 201-209.

[6] Z. Lu, O.D. Truong, W. Andre, and S. Martel, "Preliminary design of a biosensor based MC-1 magnetotactic bacteria", Biosensor 2006, Toronto Canada.