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A microscopy technique based on bio-impedance sensors

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Abstract

It is proposed a microscopy for cell culture applications based on impedance sensors. The imagined signals are measured with the Electrical Cell-Substrate Spectroscopy (ECIS) technique, by identifying the cell area. The proposed microscopy allows real-time monitoring inside the incubator, reducing the contamination risk by human manipulation. It requires specific circuits for impedance measurements, a two-dimensional sensor array (pixels), and employing electrical models to decode efficiently the measured signals. Analogue Hardware Description Language (AHDL) circuits for cell-microelectrode enables the use of geometrical and technological data into the system design flow. A study case with 8x8 sensor array is reported, illustrating the evolution and power of the proposed image acquisition.

Keywords: Electrical Bio-impedance; Biometric circuits; Sensory systems; Microelectrode; ECIS. Cell culture.

1. Introduction

Imaging techniques based on optical signals have been used to display experiments done at cell culture labs to monitor the final result of assays. Cell cultures must be taken out from the incubator and placed on the microscopy plate to be observed, changing the temperature (37º) and CO_2 conditions, with possible contamination risk. This process is repeated each observation time. Some approaches to fully integrated labs or Lab-on-Chips (LoC) are focused on optical emitting sources to monitor cells. They require a light emitter [1, 2, 4] to detect biomarkers. Other techniques are based on capacitive sensing [5, 6].

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Many biological parameters and processes can be sensed and monitored using its impedance as marker [7-11], with the advantage of being a non-invasive and relatively cheap technique. Cell growths, activity, changes in cell composition and shape or in cell location are some examples [12-15]. Electrical Cell-substrate Impedance Spectroscopy (ECIS) [13, 14], based on two-electrode setups, allows the measure of cell-culture impedance and to define biological parameters (material, internal activity, motility and size) of cells and its relationship with the environment [17]. The main drawback of ECIS technique is the need of managing models to decode the electrical performance of the full system composed by the electrodes, medium and cells. Several works have been developed in this field. In [14], impedance is deduced from electric field equation solution at the cell-electrode interface, giving a three parameter based model. h, the cell-electrode distance, R_b, barrier resistance and r_{cell}, cell radius. In [15, 16], finite element simulation (FEM) are executed for solving electrical field considering the whole structure. This method gives one parameter model (R_{gap}) for describing the gap or cell-electrode interface resistance. In both, the derived model considers the confluent phase [14] or a fixed area covered by cells [15]. The latest was extended in [16] to several cell sizes, allowing to define the cell-electrode covered area as the main model parameter.

This work considers an alternative imaging technique based on impedance measurements. It is proposed to culture the cells on top of the sensors, and to continuously acquire data of cells state inside the incubator. Non optical signals are needed, real-time monitoring can be performed, and contamination risks are reduced. To this end, impedance sensor sensitivity curves based on the cell size and density are presented and applied to cell location and cell culture imaging. The system in Fig. 1 employs a two dimensional electrode array as sensors [19] together with CMOS circuits for impedance measurements [20]. Microelectronic circuits must be designed to work with constraints imposed by the electrode sensors. The whole system in Fig. 1 can be fully-integrated in CMOS technologies [19]. Electrical models reported for the electrode-cell interface description [14-16] are the key for matching electrical simulations to real systems performance and hence decoding correctly the experimental results. This kind of system can be used for cell culture real-time monitoring with ECIS. The process to extract useful cell-microelectrode models is described at section 2, with simulations of a simplified system for cell size detection, that uses AHDL format for electrode simulation. Section 3 illustrates a real-time cell culture monitoring case. Conclusions are highlighted at section 4.

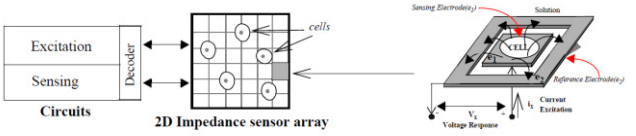

Fig. 1. Simplified system set-up: circuits and 2D electrode sensor array. Each sensor is has e1 and e2 electrodes.

2. The electrode - cell model

The electrode impedance in ionic liquids has been rather extensively investigated [12]. The main components describing the electrical performance of an electrode metal inside a solution are four: the double layer capacitance, C_1; the transfer resistance, R_{ct}, caused by the electron transfer flowing through the electrified interface; the Warburg impedance, Z_W, due to limited mass diffusion from the electrode surface to the solution. The electron transfer resistance R_{ct} is in series with the mass diffusion limited impedance Z_W. Finally, as the current spreads out to the bulk solution, the electrode has a solution conductivity determined by series resistance: the spreading resistance R_S. These parameters depend on technology, medium and geometry. Figure 1 illustrates a two-electrode impedance sensor useful for ECIS technique: e_1 is the sensing electrode and e_2 the reference one. Electrodes can be manufactured in CMOS process with metal layers [15] or using post-processing steps [19]. The model in Fig. 2 considers that e_1 sensing surface could be total or partially filled by cells. For the two-electrode sensor in Fig. 1, e_1 is the sensing area A, and $Z(\omega)$ the impedance by unit area of the empty electrode. When e_1 is partially covered by cells in a surface A_c, $Z(\omega)/(A-A_c)$ is the electrode impedance of the non-covered area, and $Z(\omega)/A_c$ is the impedance of the covered area. R_{gap} models the current flowing laterally through the electrode-cell interface. The parameter ff, called *fill factor*, is zero for $A_c = 0$ (e_1 empty), and one for $A_c = A$ (e_1 full). It is defined $Z_c(ff = 0) = Z_{nc}$. The relative changes at impedance magnitude,

$$r = \left| Z_{nc} - Z_{nc} \right| / Z_{nc} \qquad (1)$$

gives an easy interpretation of curve sensitivity. The graphics of *r* versus frequency are plotted in Fig. 3, for a cell-to-electrode coverage *ff* from 0.1 to 0.9 in steps of 0.1, using R_{gap} = 90 kΩ. The electrodes size is 32 x 32 μm^2 [9, 10, 11]. It can be identified the frequency range where the sensitivity is high at 100 kHz. For a given frequency, each value of the normalized impedance *r* can be linked with its *ff*, being possible the cell detection and estimation of the covered area A_c. The *r*-curve sensitivity is optimal for frequency values around 100 kHz, as predicts the FEM simulations [15, 16]. The R_{gap}=90 kΩ was selected with the maximum value of *r* curve and *ff*=0.69, for a cell size of 30 μm diameter. Impedance sensor curves at Fig. 3 were obtained using SpectreHDL mixed-mode simulator, with Analog Hardware Description Language (AHDL) for circuits in Fig. 2.

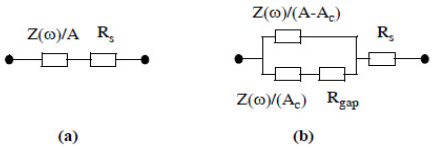

Fig. 2. Proposed model for an electrode-solution-cell model with area *A*, uncovered with cells (a) and covered and area A_c (b).

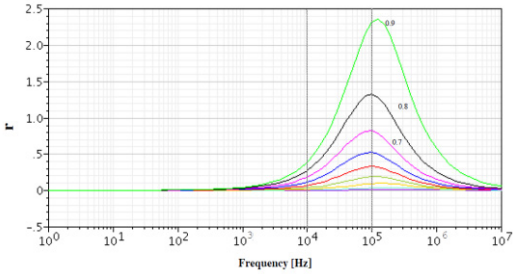

Fig. 3. Normalized impedance *r* vs frequency. Curves correspond to a fill factor in the range of 0.1 (near empty) to 0.9 (near full).

3. Cell-culture imaging results

To proof the proposed idea, it was chosen a study case with 8x8 two-electrode array. The input to be analysed is a low density MCF-7 epithelial breast cancer cell culture in Fig. 4a. The objective is to employ the area parameterized electrode-cell model and the proposed circuits [20] to detect their location. The pixel size is 50x50μm^2, similar to cell dimensions. Figure 4a shows the grid and its overlap with the image. Circuits associated to each e_1 electrode in the array were initialized with their corresponding fill factor (*ff*). Matrix in Fig. 4b is obtained for this example. Full system electrical simulations were done at 10 kHz obtaining the value of the voltage response magnitude V_m for all pixels (Fig. 5). When measuring each pixel, V_m value becomes constant and then is acquired. In Fig. 5 displays the waveforms obtained for the amplifier output voltage, voltage V_m, and excitation current i_x for all pixels. These values are used to calculate their normalized impedances *r*. Fig. 6 represents the 8x8 *ff*-maps, in which each pixel has a grey level depending of its *ff* value obtained (white is empty and black full). Fig. 6 shows the *ff*-map for the input in Fig. 4a. Using the parameterized curves shown in Fig. 3, at 10 kHz frequency, the *ff* parameter was calculated for each electrode from V_m simulated values in Fig. 5. The results are represented in Fig. 6 for 10 and also 100 kHz. As Fig. 3 predicts, better matching is found at 100 kHz because normalized impedance is more sensitive than at 10 kHz. In both cases, errors obtained in *ff* values are below the 1%, so matching with input is excellent.

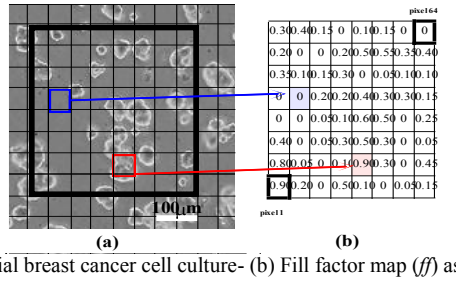

Fig. 4. (a) 8x8 pixel selection in ephitelial breast cancer cell culture- (b) Fill factor map (*ff*) associated.

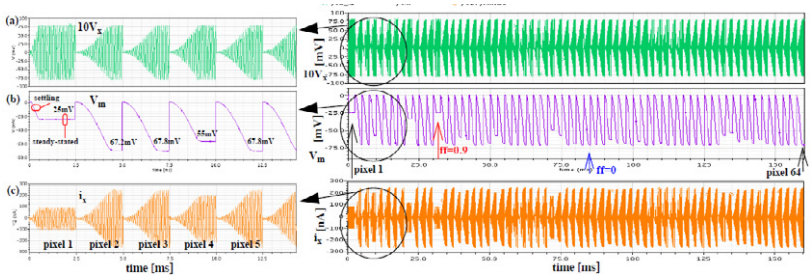

Fig. 5. Simulated waveforms for (a) $\alpha_{ia}V_x = 10V_x$, (b) V_m and (c) i_x signals for the 64 electrodes at 10 kHz.

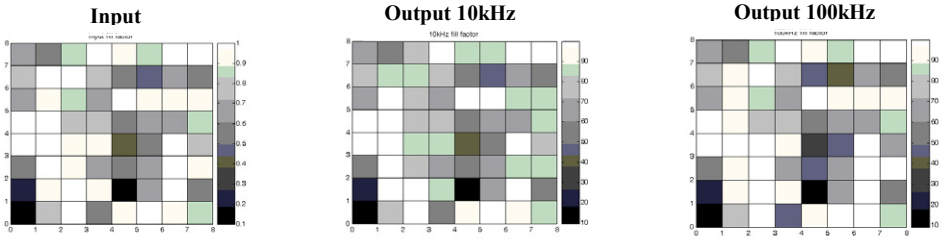

Fig. 6: Fill factor maps obtained for (a) input image. (b) Simulations at 10 kHz. (c) Simulations at 100 kHz.

4. Conclusions

The application of a two-wire set-up enables the proposed system for impedance sensing of biological samples to be useful for 2D imaging. An electrical model based on the overlapping area is employed in both system simulation and image reconstruction for electrode-cell characterization, allowing the incorporation of the electrode design process on the full system specifications. Electrical simulations reproduce the ECIS technique, giving promising results in cell location and imaging, and making possible the use of our system for other real-time applications such as cell index monitoring and cell tracking.

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