Design of a 24-Channel Impedance Measurement System for Living Cell Impedance Spectroscopy

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Abstract—This work presents a 24-channel impedance measurement system for living cell detection and monitoring. This system is designed based on the successful result from the proof-of-concept effort of the single-channel system published in [1]. The 24-channel system is implemented by duplicating the single-channel system and running in parallel to enable simultaneous multi-channels recording. The measured impedance results can be used to estimate the number of specific living cells in a collected bio-sample. The 24-channel system consists of a PC and a customized hardware system, designed for impedance measurement. The data communication structure design is organized based on the comprehensive OSI layers concept for completeness. Testing results show that the 24-channel system is able to detect the changes in impedance spectrum for the endothelial progenitor cells (EPC).

I. INTRODUCTION

Living cell impedance spectroscopy has emerged as a major biological and medical research field for early detection of diseases and studying the effects of drugs on living cells for the pharmaceutical industry. Cell impedance spectroscopy provides detailed insights into the physiological and biochemical functions of living biological cells. This enables the construction of functional cell models based upon physiological and pathological information. Research has been done in studying the effects of drugs on the behaviors of living cell in real-time by measuring the extent of the adhesion of a cell population onto micro-electrode array and this was done by monitoring the impedance changes from individually controlled micro-electrodes [2].

In the living cell analysis experiments, the magnitude and phase of the cell impedance are measured at a range of voltages from a few mV to 1V and frequencies from 1Hz to 10MHz depending on the electrical characteristics of the cell. The requirement of measuring a wide range of voltages and frequencies imposes challenges on hardware design of the impedance measurement system. A proof-of-concept effort had been carried out and showed that the proposed modified auto-balancing bridge method was working properly [1].

In [2], an array of micro-electrode was used to estimate the cell population in a sample and this required a multi-channel recording system for fast and efficient measurement. In this project, a 24-channel impedance measurement system is designed to work in conjunction with a 24 micro-electrode array build inside a bio-testing fixture. This full system setup will be used for further research on the distribution of cell population.

II. 24-CHANNEL IMPEDANCE MEASUREMENT SYSTEM DESIGN

A. Proof-of-concept

A proof-of-concept effort has been carried out and published in [1] to test the effectiveness of the proposed measurement method. The basic principle of the measurement method is shown in Fig. 1(a). The cell impedance $Z_c$ can be calculated using the voltage at “A” terminal divided by the current flowing across $R$ based on equation (1). Fig 1(b) shows a single-channel system for impedance measurement. The measured result is consistent with the conceptual value.

$$Z_c = \frac{V_{oc}}{I_R}$$

![Figure 1](image-url) (a) Principle of the impedance measurement method (b) Block diagram of the single-channel impedance measurement system

B. System Architecture

The system architecture of the design is shown in Fig. 2. There are 5 building blocks for the whole 24-channel system, i.e. a PC installed with LabVIEW and NI PCI-6551 HSDIO card, a master FPGA card, a backplane board, a DAC daughter card and 12 ADC daughter cards. The PC serves to
provide a graphical user interface for the operations of the system through the HSDIO card and LabVIEW program. A master FPGA card is designed to interface with the HSDIO card and control the operations of the rest of the daughter cards based on the user commands. A backplane board is designed to provide the connections among all the daughter cards that plugged into the backplane board. The DAC daughter card consists of circuit functions to generate the sinusoidal excitation signals over a wide range of frequencies and amplitudes for measurement. The ADC daughter card consists of 2 channels converting the current flowing through the bio-electrodes into voltage and further digitizes it for digital processing. Each daughter card is also equipped with an FPGA for operation controlled and data processing.

The lower layer of the data communication structure of the 24-channels system is designed based on the concept of the OSI 7 layers structure. To implement the functions of the whole system, the operations are broken down into smaller building blocks and mapped into each of the 7 layers as shown in Fig. 3. Basically the data communication structure of the system can be categorized into two portions, i.e. upper layer and lower layer.

The upper layer provides a graphical user interface for the user to control the operation of the system and display the 24-channel measurement results accordingly. The basic operations such as to start and stop a measurement, to control the characteristics of the excitation signal, to compute the impedance based on data collected and displayed the results onto logarithmic scale graphs for each channel can be mapped to the top three layers of the OSI structure. A LabVIEW program is written to translate a user’s inputs into digital commands and send to the system through the NI PCI-6551 HSDIO card.

The lower layer of the data communication structure represents the activities between the master FPGA card and 13 other daughter cards. For physical layer, the communications among the FPGAs on all cards are connected through a shared bus on the backplane board. In the data-link layer, FPGA on each card is programmed with a simple master-slave bus protocol so that the data can be sent from one card to another on the shared bus and bus congestion will not occur. For network layer, each card is assigned a unique address through the switch settings so that it can be identified on the system bus. In the transport layer, upon receiving the command from the upper layer, the system is controlled by a finite state machine implemented inside the master FPGA card. The FSM will activate the respective control lines and send the corresponding data through data-link layer.

D. Hardware Design

In this project, four different types of PCBs are designed, i.e. master FPGA card, DAC daughter card, ADC daughter card and the backplane board. The layouts of all boards are shown in Fig 4. For data communication between all the 14 boards, one 7-bits wide address bus and one 16-bit wide bi-directional data bus are adopted for the system design.

For the master FPGA card design, an ACEX FPGA is used to decode the command from the HSDIO card, assert the corresponding control lines and carry out write or read operations for designated daughter card. The master FPGA will also need to read the measured data from each ADC card and send back to the HSDIO card for further processing. The backplane board consists of the address bus, bi-directional data bus and control lines. Termination resistors are used for both the address and data bus to improve the rise and fall time of the signals on the buses. A master clock is also generated on the backplane board to be used for bus synchronization across all the daughter cards.

The DAC daughter card generates the excitation signal for impedance measurement. A CYCLONE III FPGA on-board receives the commands through the address and data buses from backplane board and controls the DAC circuit to generate the excitation signal accordingly. The ADC daughter card consists of two ADC channels circuit. A CYCLONE III FPGA is also designed on-board to receive the commands from backplane board through the address and data buses to control the ADC circuit accordingly. A digital FIR low pass filter is also implemented to improve the signal-to-noise ratio of each channel before passing the final measured data to LabVIEW for further processing.
E. LabVIEW

The LabVIEW program provides a graphical user interface between the user and the system as shown in Fig. 5(a). The user can activate the channels to be measured and select the amplitude and frequency for excitation signal generation. A frequency sweeping function from 100Hz to 1MHz is programmed for automatic measurement and the corresponding measured impedance data from each channel are plotted onto respective logarithmic graphs as shown in Fig 5(b).

III. RESULTS AND DISCUSSION

The 24-channel system is tested with a bio-fixture that contains a sample of endothelial progenitor cells (EPC) with CD34 marker. The existence of this EPC cells had been used to measure angiogenesis, which reportedly predicts tumor recurrence. The preparation methods of the cell sample are described in [3]. The test setup for the whole system is shown in Fig. 6. The bio-fixture contains an array of 24 gold-plated electrodes so that the cells from a sample can adhere onto the surface of the electrodes. The excitation signal is applied to the reference electrode which is common to the rest of 23 working electrodes as shown in Fig. 7. The currents flowing through the 23 working electrodes are measured simultaneously by the 24-channels system when the excitation signal is applied to the reference electrode. The impedances appearing on all working electrodes are then plotted on the respective logarithmic graphs.

A control experiment is conducted in order to detect the changes of impedance in the presence of cells using the system. The experiment is first carried out by measuring the impedance of a sample of a standard phosphate buffered saline (PBS). The device is then loaded with cells in PBS and the impedance is measured again. The number of bio-cells used is enough to cover the areas of the electrodes as a monolayer of cells. The excitation signals with peak-to-peak...
amplitude between 27mV to 55mV yield the optimum signal-to-noise ratio with this experiment setup. For a typical impedance spectrum $|Z(f)|$, ten data points per frequency decade are taken to be equidistant on the linear scale. The time taken to acquire one data set by sweeping the frequency from 100Hz to 1MHz is about 1 minute.

The impedance spectroscopy graph for PBS solution without cell for channel P1-3 is shown in Fig. 8(a). The experiment is repeated by injecting the bio-cells into the PBS solution and the result is shown in Fig. 8(b). The impedance change graph is plotted in Fig. 8(c). Increase in the impedance is clearly observed at frequencies around 100kHz and beyond after the cells are loaded. Fig. 9 shows the experimental results for all 24-channel. Variation in impedance is observed for electrodes with different sizes. Four electrodes on the bio-fixture are broken and appeared to be high impedance during the measurement.

Through the testing, the 24-channel system detected the changes in impedance with cells attached onto the micro-electrodes array. Further analysis will be conducted to study the 24-channel impedance changes graphs for a sample and to determine the number of specific cells contained in the sample. Simultaneous multi-channel recordings mechanism of the system has greatly reduced the cycle time needed to conduct the experiment.

IV. CONCLUSIONS

The working principle and systematic approach to design a 24-channel impedance measurement system for cellular impedance spectroscopy are presented. The full system was tested with the living cultures of EPC and the impedance measured across the frequency range is consistent with the experimental results shown in [1] and [2]. This prototype design has been proven to work with the 24 micro-electrodes array for impedance spectroscopy of living cell.

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REFERENCES