Instrumentation for Cell Capacitance Measurements

Switching Sinusoidal Excitations for Studying Cell Membrane Transport

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***Abstract*— This project aims at the design of new instrumentation techniques for studying the electrical properties of the cell as a means of better understanding the cellular transport processes. The approach is to integrate the concept of a lock-in amplifier on a single-electrode platform. Switching sinusoidal excitations can be used to assess small but measurable changes of cell capacitance, which can be used to make inferences about cell surface area changes during the transport processes. In this study, a cell membrane model circuit has been simulated in software. In addition, a proof-of-concept instrumentation has also been implemented with an embedded system. The results have shown the feasibility of obtaining accurate real-time measurements of the cell capacitance by time-multiplexing the sinusoidal excitation and the voltage measurement via a single patch-clamp electrode.**

***Keywords*— cell capacitance; instrumentation; signal processing; cell membrane transport; simulation; embedded system**

I. Introduction

For years scientists have wanted to gain a deeper understanding of the cell's chemical behaviors. More specifically, how often do cells endocytize or exocytize substances? Is it possible to map these processes when they occur? Based off of the finding that cellular capacitance is directly proportional to cell surface area, we have a way to observe these processes. When cell membrane transport occurs, the surface area across the cell increases, which in turn increases the capacitance of the membrane [1]. This can be exploited by the fact that capacitance can be calculated from the phase shift between two signals [2]. So, calculating the phase delay between the input and the output signals in a circuit simulating the electrical properties of a cell membrane can give insight into the cell capacitance. The primary hardware used for these measurements is a sensitive patch clamp amplifier, a source for the stimulus signal, and a phase sensitive detector. The purpose of this study is to determine the effectiveness of a new and innovative method for the patch clamp. The stimulus signal is modeled as a pulse wave amplitude modulated by a sinusoidal wave envelope, which signifies current injection and simultaneous capacitance measurements in the circuit.

II. Methods

In order to effectively test the capacitive measurement technique, a software and hardware simulation of the signals was created. The input and output measurements and trends after using the signal to drive the circuit modeling a cell membrane were observed.

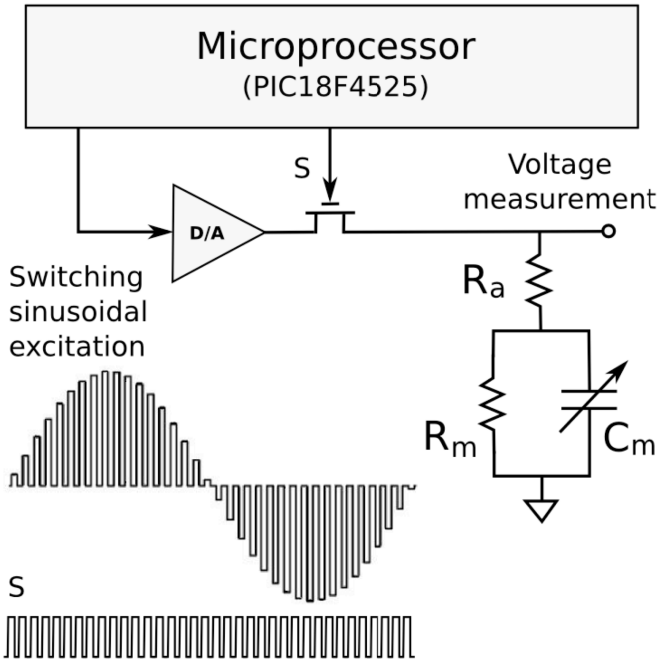

Fig. 1. Schematic diagram of the hardware simulation system for cell capacitance measurements. The cell membrane is modeled by a 3-element RC circuit. The microprocessor generates a switching sinusoidal excitation, which is time-multiplexed with the voltage measurement via a MOSFET switch controlled by the S signal.

A. Software Simulation

The software simulation of the circuit model was done using Simulink (Mathworks, Natick, MA). A pulse wave amplitude-modulated by a sine wave represents the switching sinusoidal excitation shown in the hardware block diagram in Fig. 1. A switching signal (S) is used to turn on the MOSFET switch during the duty cycle of the sinusoidal excitation. The MOSFET is turned off when the pulse wave returned to zero to prevent momentary discharging of the capacitor (C_m). In this simulation, the values for the 3-element cell membrane model were chosen as follows: access resistance R_a = 500 KΩ, rans-membrane resistance R_m = 5 MΩ, and membrane capacitance C_m = 10 pF. The actual parameter values for live cells vary over a broad range depending the types of cells. A report suggests that a typical cell has Ra = 20 MΩ, Rm = 1000 MΩ, and Cm = 5pF [3].

B. Hardware Implementation

Based on the software simulation a proof-of-concept instrumentation system was built. The hardware system was developed with a microprocessor (PIC18F4525, Microchip,

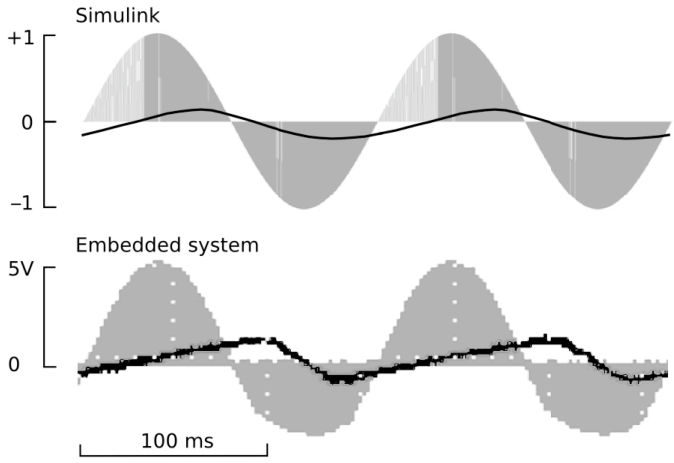

Fig. 2. The switch sinusoidal excitation (grey) and the measured voltage waveform (black) from the software simulation (top) and the embedded instrumentation system (bottom).

Chandler, AZ). As shown in Fig. 1, the embedded system generates the sinusoidally modulated pulse wave and the switching signal (S) to drive the MOSFET switch. The only difference between the software simulation and the hardware implementation was the resistor and capacitor values in the circuit. Some extremely high resistor values or low capacitor values could not be obtained in hardware simulation due to supply constraints. Component values were scaled to commonly available values. Although these components were changed, the time constant for both circuits was kept uniform. In addition, the clock of the PIC18F452 microprocessor was slower than the desirable frequency in the software simulation. Thus, a slower pulse wave frequency was used on the hardware platform.

III. Results

As shown in Fig. 2, the switching sinusoidal excitation was able to induce a sinusoidal response with a phase shift in the voltage measurement. This was observed in both the software simulation and the hardware implementation. The phase shift was comparable: about 40° in the software simulation and about 50° in the hardware implementation. In addition, both of the input and output signals match the simulation model, despite a slight distortion of the input signal in the hardware implementation due to the excessive current drawn by the MOSFET.

In the software simulation (Fig. 2 top), the signals more closely match the cell membrane capacitance models, due to the ideal component models in software. However, because the hardware and the software display the same characteristics and a phase shift roughly equal to the expected phase shift (45°), these results are consistent.

IV. Conclusion

The overall goal of this research was to assess the capacitance in a cell membrane model based a phase shift in the induced voltage in response to a sinusoidal excitation. The significance of this project lies in the combination of the lock-in amplifier concept [4] and the patch clamp technique with a single electrode [5]. The lock-in amplifiers are useful for measuring small signals under noise conditions. The sinusoidal modulation and demodulation process can significantly improve the single-to-noise ratio by rejecting noise outside the very narrow frequency band of the signals. However, a lock-in amplifier usually requires two ports: one for input and one for output. Thus, the traditional lock-in amplifier instrumentation does not lend itself to a patch clamp setting where a single electrode is used to access the cell. This project provides a solution to the application of the lock-in technique to the single-electrode setting. By using a switching sinusoidal excitation, it is possible to time-multiplex current injection and voltage measurement on a single electrode while introducing a sinusoidal modulation. The feasibility of the approach has been demonstrated in this study with the observation of a phase-shifted sinusoidal induced voltage of the same frequency.

Another advantage of the approach lies in the use of a single sinusoidal excitation. Previously reported methods for measuring cell capacitance often require multi-frequency excitations [6] or non-sinusoidal excitations [7]. Furthermore, with a fast digital signal processor it is possible to deliver a cycle of the switching sinusoidal waveform within a relatively short time interval (on the order of 0.1 ms). Thus, this technique will provide a high temporal resolution, which is essential for real-time monitoring of exocytosis and endocytosis activities occurring on the order of 1 ms.

For future work, estimation algorithms will be developed to measure the cell capacitance based on the magnitude and phase of the induced voltage in response to the switching sinusoidal excitation. Additional simulation work will be carried out to assess the accuracy of measuring small cell capacitance changes on the order of 1 fF due to vesicle transports across the cell membrane.

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