Microfluidic Impedance Flow Cytometer for Stem Cell Discrimination

Instruments: HF2IS, HF2TA
Options: HF2IS-MF

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Introduction

Adult pluripotent stem cells have been presented as an alternative in the stem cells research to replace the utilization of embryonic stem cells which pose ethical dilemma. The possibility of harvesting adult pluripotent stem cells for medical purposes resulted in a growing interest in stem cell research. In particular, researchers need to identify differentiation state of stem cells in a fast and reliable way. The process in which a stem cell turns to a particular mature cell with specific germ layer type is called differentiation. Stem cells can differentiate into three types of germ layers: ectoderm, mesoderm or endoderm, which make up different tissues and organs of the body.

To monitor the differentiation state of stem cells, biochemical assays (e.g. RT-PCR, qPCR, and microarrays) have been developed [1]. However, these types of bio-assays are time-consuming, labour intensive, and require bulky instrumentations. Another issue is that these methods are often invasive i.e. cells are altered or even damaged. Non-invasive method like microscopic observation is also commonly used to monitor phenotypic changes of differentiated stem cells. However, not only is microscopic observation time and labour intensive, it is also not ideal for quantitative analysis. On the other hand, fluorescence activated cell sorting, or FACS, can facilitate quantitative investigation of stem cell differentiations [2]. Nevertheless, FACS fluorescent biomarkers or antibodies are applied to cell surfaces are considered invasive and can also be complex and costly to set up.

Microfluidic electrical impedance-based measurement is a label-free, non-invasive technique to detect and discriminate cells [3]. The biological cells can be modeled as a network of RC circuits. The parameters such as cell size, cell membrane, cytoplasm, and nucleus etc. can all be represented by a combination of resistors and capacitors. In turn, the cell will have a frequency response dependency reflecting the cell structure (4). This is illustrated in Figure 1. In general, as frequency goes higher, the AC signal penetrates more into the cell, which allows a more detailed look at the inner structure. This technique is often known as microfluidic impedance flow cytometry.

Figure 1. Frequency response of a single cell
Impedance flow cytometry can be applied to detect the morphological and electrophysiological changes in dielectric properties (i.e., membrane capacitance and cytoplasmic conductivity) associated with stem cell differentiation. In this application note, an example is described for microfluidic channel/microfluidic chamber impedance measurement with integrated electrodes for identifying differentiation state of mouse embryonic carcinoma cell line, P19. The microfluidic impedance flow cytometer setup designed by CFD Research Corporation\(^1\) will be presented. A description of a microfluidic impedance flow cytometer setup as well as the measurement data will be presented. The analysis method based on support vector machine (SVM) on the cell differentiation proposed by CFD Research Corporation will also be introduced and discussed in the results section.

Impedance Flow Cytometer Setup and Design

Overall Setup

Figure 2 shows an impedance flow cytometer setup for identifying differentiation state of P19 cells. The syringe pump pumps the stem cell solution into the microfluidic device where the change of impedance due to the passage of cells is measured. For this experiment, an AC excitation signal is generated by the Signal Output port of the HF2IS Impedance Spectroscope to excite the integrated electrodes in the microfluidic device. In addition to the electrical impedance measurement, a digital camera is also used for simultaneous imaging and cross-correlation with the collected impedance data. This optical imaging is essential for developing a cell classification scheme SVM to be discussed later.

Microfluidic device design

The microfluidic device itself is composed of one horizontal flow channel with two arm channels as shown in Figure 3. The channels were fabricated in PDMS layer bonded onto the glass substrate with plasma bonding. Co-planar gold electrodes were deposited directly onto the glass substrate prior to bonding which are positioned on top of both the arm channels and the main channel for impedance measurement purposes. The width of the main channel and the arm channels are 500 \(\mu\)m and 320 \(\mu\)m, respectively, with the channel depth of 27 \(\mu\)m.

Figure 2. Microfluidic impedance flow cytometer setup for identifying differentiate state of stem cells

Figure 3. Microfluidic impedance flow cytometer design

The paths between the main channel and the arm channels consist of micropores (40 \(\mu\)m x 40 \(\mu\)m). An AC excitation voltage from the sine wave generator of the HF2IS Impedance Spectroscope excites the main channel electrode. The resulting AC currents can be measured at both arm channels’ electrodes. Whenever a particle passes through micropore 1 or 2 and under the arm electrodes, the value of the measured AC current \(I_1\) and \(I_2\) changes. It was shown that the probability of simultaneous passage of cells through the two micropores is about 0.01% which is even more insignificant in sufficiently diluted solutions [1]. The HF2TA Current Amplifier provides a transimpedance gain which also converts the current into voltage before the measurement signal enters the HF2IS Impedance Spectroscope, whose differential input subtracts the two measured signals from two electrodes. The differential signal will be constant if no particles pass through either micropores. Otherwise, the signal will either be positive or negative depending on which micropore a particle passes through. The PC attached to the HF2IS Impedance Spectroscope through USB can then be used either to observe the measurement in real time, or to save data for further post processing.

The advantages of using such microfluidic flow cytometer for cell detection are as follows. The dual-micropore design doubles the throughput of the detection compared to a single-channel device. In addition, the micropores serve as

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\(^1\) [www.cfdrc.com](http://www.cfdrc.com)
reference to each other which helps to reduce common mode noise. Furthermore, each micropore represents the dominant element of the overall impedance of the arm channel. This, combined with the distinctive impedance of a cell compared to the medium solution, generates a large variation of impedance as a cell passes through the micropores. This in turn gives a larger signal-to-noise ratio in the impedance measurement.

As usual, the measured signal contains information on the biophysical properties such as size, double-layer capacitance, membrane capacitance and cytoplasm resistance, all of which are essential parameters for detecting changes in morphology and membrane structure of the stem cells in various differentiation states.

Sample preparation

Separated and mixed samples containing polystyrene beads and P19 cells were prepared with a concentration of about $10^5$ ml each. The total volume of the mixed sample was 2 µl. In particular, differentiation was induced in the P19 cells towards a neuronal lineage [1]. In short, the preparation produces both differentiated and undifferentiated P19 cells which were used to test the microfluidic impedance flow cytometer.

The beads were used as training data for a signal processing algorithm based on support vector machine (SVM). SVM is a classification engine which can be "trained" with known input and output data to make the classification of cells, in this case, more accurate.

Experimental Results and Analysis

Experimental conditions

To test whether the flow cytometer functions well in detecting impedance changes, measurements were made with separate beads and undifferentiated P19 cells, and a mixture of both at an AC frequency of 50 kHz. Both the impedance measurement and the microscope images were taken to ensure better accuracy. Figure 4 shows the results from both the microscope as well as the amplitude spikes related to the impedance changes caused by particles and cells flowing through the micropores. The positive and negative spikes are the result of differential micropores measurement as described previously. It can be seen in (c) that the measured amplitude for P19 cells is in average smaller than that of the beads (i.e. on average 0.54 V compared to 1.24 V). The histogram in (d) shows the amplitude spike measured for each particle and cell detected. This was only possible due to microscope images since it would be difficult to distinguish the type below certain amplitude measured. In order to achieve reliable detection in the flow cytometer without optical aides in normal operation, accurate particle classification boundary condition parameters must be defined. This is where the SVM comes into play.

Particle classification with SVM

SVM can be used to improve the classification accuracy of cells in the impedance detection when amplitude detection is no longer sufficient. The principle of SVM is to train a model with the known inputs and the known outputs i.e. +1 for cells detected and -1 for beads detected. These so-called training and validation data would then be used to optimize the SVM classification algorithm [1]. To evaluate accuracy, a final test would be carried out with known inputs and unknown outputs where the trained SVM is used to predict the output i.e. whether a cell or a bead is detected.

The SVM machine developed for this case has two main interrogation parameters:

- Real and imaginary part of the measured spikes
- Frequencies response at 50 kHz, 250 kHz, 500 kHz and 1 MHz

![Figure 4](image-url)

Figure 4. (a) beads only (b) undifferentiated P19 cells only (c) mixed sample (d) histogram of mixed sample based on measured amplitude change
The four frequencies must be applied simultaneously to the main channel electrode in order for the measurement to be effective. A frequency sweep would be too slow. The simultaneous measurement is provided by the multi-frequency function of the HF2IS Impedance Spectroscope. The multi-frequency allows a simultaneous measurement at up to 8 different frequencies. Figure 5 shows a comparison of detection between training data and SVM post-processed data at different frequencies. The real part of the spike amplitude is plotted vs. the imaginary part.

Several conclusions can be drawn from this comparison. First of all, the distinction of undifferentiated P19 cells and beads is much clearer with the SVM engine as shown in the plots on the right side. The distribution of cells and beads detected match very well to the prepared test sample. Furthermore, the spread of real part of the amplitude remains small across both types of particles across all four frequencies while the spread of the imaginary part increases with frequency. This is due to the fact that at higher frequencies, the capacitive components of the particles start to dominate in the overall amplitude measured.

Opacity as an interrogation parameter

Figure 6 is a plot showing detected signals at 50 kHz of differentiated and undifferentiated P19 cells using the impedance flow cytometer. One can quickly notice that the two types of cells are literally undistinguishable without further classification. The main reason is that at 50 kHz, the resistive element (i.e. real part) of both types of cells are fairly similar, and the capacitive element (i.e. imaginary part) is more or less negligible at this signal frequency range. It is therefore better to measure at high frequencies where the difference in cell membrane capacitance between two types of cells can be distinguished more visibly. In this case, a measure called opacity was introduced as an interrogation parameter in the SVM. Opacity is defined as the ratio of the measured amplitude at high and low frequency [4]. It serves to normalize the raw data since opacity is almost independent of the cell size as well as helps to minimize the measurement effect caused by the position of the cells relative to the electrodes. In short, opacity gives a good representation of the dielectric properties of the cells detected.

With opacity as an additional parameter, SVM was applied to classify detected undifferentiated and differentiated P19 cells. Figure 7 shows the opacity of a mixed sample calculated from the simultaneous measurement at 1 MHz and 50 kHz. The black curve represents the classification boundary from the trained SVM. One can clearly see an improvement of the discrimination compared to Figure 6. Measurements performed at 250 kHz and 500 kHz did not provide a clear opacity. In consequence, a high enough frequency i.e. 1 MHz must be applied in order to detect the cell dielectric differences in two types of P19 cells.
Conclusions

In this application note, a microfluidic impedance flow cytometer designed by CFD Research Corporation was presented for the purpose of discriminating differentiation state of mouse embryonic stem cells. With the multi-frequency capability of the HF2IS Impedance Spectroscope, several AC frequencies can be generated and measured simultaneously to detect cell impedance changes. A support vector machine (SVM) algorithm was then developed to improve the accuracy of classification in detecting beads, undifferentiated P19 cells, and differentiated P19 cells. In the case of discriminating beads and P19 cells, the accuracy was improved to 95%. In the case of discriminating undifferentiated and differentiated P19 cells, the detection accuracy is 87%. The high frequency measurement capability and the multi-frequency function of the HF2IS Impedance Spectroscope are unique features that render the implementation of the SVM possible.

User Benefits

The HF2IS Impedance Spectroscope and the HF2TA Current Amplifier from Zurich Instruments allow users to reliably analyze both the static and dynamic (i.e., time varying) impedance of biological cells by simultaneously measuring the voltage and the current. The users also benefit from the multi-frequency measurement which gathering information on different structure of the cells at the same time. In addition, HF2IS Impedance Spectroscope provides:

- 2 integrated signal generators in the frequency range from quasi-DC to 50 MHz
- Very low filter time constant and high sampling rate to capture fast dynamic events
- Fast parametric sweeper for obtaining sample amplitude and phase response as a function of frequency, bias voltage or signal amplitude

The all-digital platform with PC based control also allows users to observe and record data in real time. The post-processing can be combined with instrumentation control through the use of application programming interfaces (i.e., LabVIEW, MATLAB, Python, C) provided free of charge by Zurich Instruments.

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References

[1] H. Song et al., Lab on Chip, 2013, 13, 2300