

SINGLE CELL DIELECTRIC SPECTROSCOPY IN A MICRO-CHANNEL

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ABSTRACT

We propose the idea of single cell dielectric spectroscopy for future clinical applications such as cell therapy and regenerative medicine. The structure of the microfluidic device is carefully designed to suppress the influence of electrode polarization on the dielectric spectra of single cells. Beads of uniform size distribution is used to determine the detection limit. As feasibility tests for biological samples, the dielectric spectra of single cells are measured for two kinds of cell lines and compared to those measured for the suspensions of the same cell lines.

KEYWORDS: Dielectric spectrum, Blood cell, Electric property, Cytometer, Micro-channel, Equivalent circuit

INTRODUCTION

Dielectric spectroscopy of living cells has been widely used to assess the electric properties of the cell constituents such as the membrane and cytoplasm from the dielectric spectrum of a cell suspension on the basis of interfacial polarization theory. Here we call this classical technique as “macroscopic” dielectric spectroscopy, because generally more than several millions of cells in the suspension are required for the measurement. Macroscopic measurements of lymphocytes [1] show that each kind of lymphocytes has its specific relaxation spectrum in the MHz region and provide the electric parameters of the corresponding lymphocyte, although they inherently represent the average values of all the cells in the suspension. On the other hand, single cell dielectric spectroscopy we propose here, which rapidly measures and analyzes dielectric spectra of single cells passing one by one through a micro-channel as shown in Figure 1, allows discriminating different cells without staining them. Moreover, in this system, target cells detected on the basis of spectral difference are specifically collected in the cell sorting area of the same device, though details are not discussed in this paper. We believe this system is helpful for future clinical applications such as cell therapy and regenerative medicine. Our system is different from other flow-cytometric systems based on electric detection principles previously proposed [2] or realized [3] in that it accurately measures complex capacitances over frequencies around the relaxation frequency while effects of electrode polarization are suppressed, and the number of frequencies for simultaneous measurement is larger so as to obtain the dielectric spectra of single cells.

Electrode polarization, which originates from an electric double layer at the interface between an electrode and an aqueous solution of electrolytes, often covers up the relevant cell dielectric spectrum. Therefore, in a macroscopic dielectric spectroscopy, physical and numerical compensation techniques have been proposed and applied. In single cell measurement, capacitance increment and conductance decrement due to a cell between a pair of electrodes is so small in contrast to the macroscopic case that the effect of compensation is critical. In this paper, we focus on our solution to the compensation of electrode polarization in the measurement of single cell dielectric spectra, the implementation, and the results.

THEORY

Dielectric spectroscopy deals with the relative complex permittivity of a cell obtained from a measurement of capacitance and conductance by considering the system including a cell suspension as a simple parallel circuit of a capacitor and conduc-

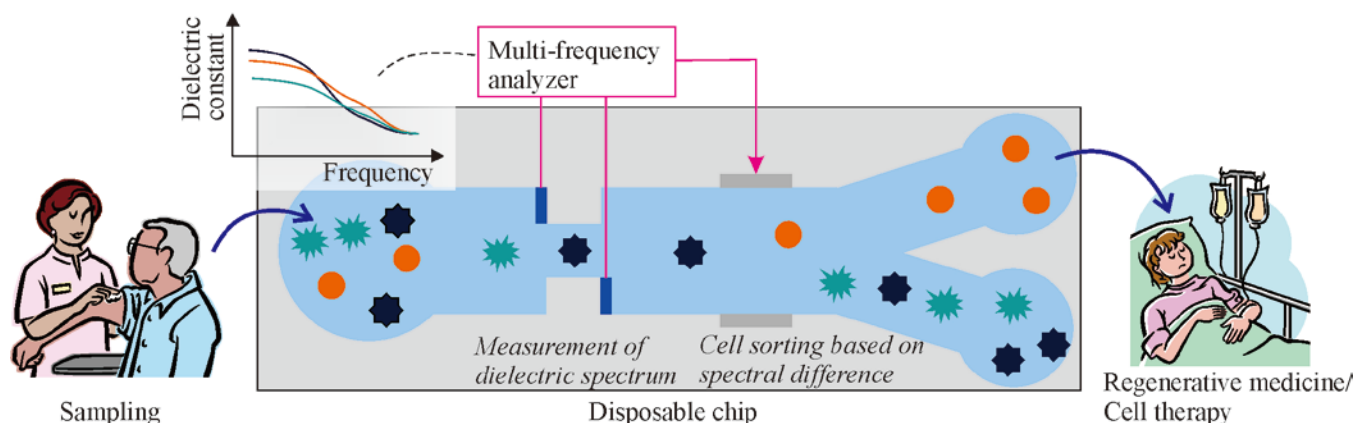


Figure 1: Concept of single cell dielectric spectroscopy with a sorter.

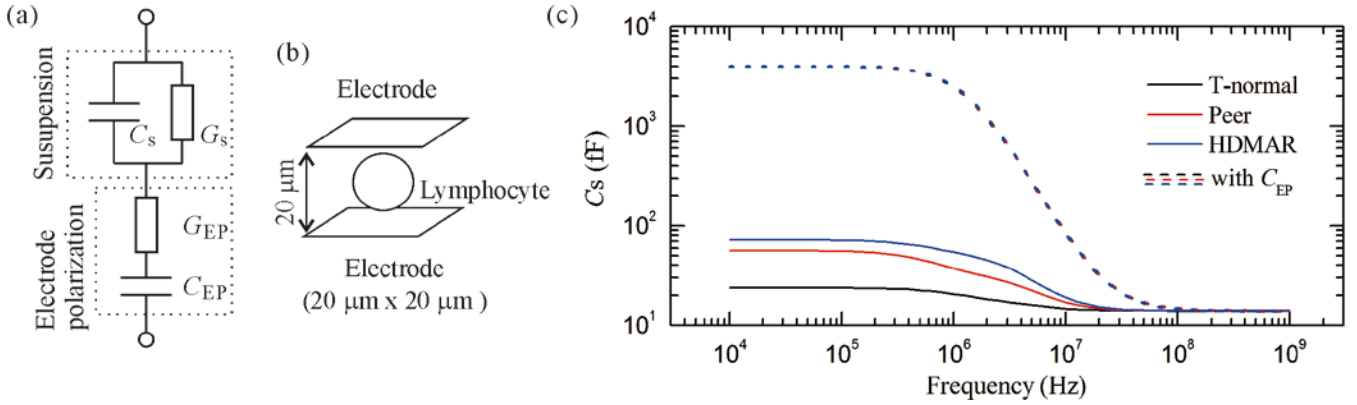


Figure 2: (a) Equivalent circuit of a cell suspension, (b) simple measurement cell for a single cell, and (c) analytical capacitance spectra of single T-lymphocytes at a center of measurement cell with and without considering electrode polarization.

tor (Figure 2a). Without the existence of electrode polarization, the ideal single cell dielectric spectra of three kinds of T-lymphocytes in PBS measured with a $20 \times 20\ \mu\text{m}^2$ electrode pair (Figure 2b) were calculated analytically from the parameters in Ref.[1] and shown in Figure 2c. However, the analytical dielectric spectrum considering the capacitance of electrode polarization C_{EP} (the value of $10\ \text{mF/m}^2$ was taken from literatures as a consensus value for metal electrodes) indicates that the electrode polarization covers up the real dielectric spectrum and almost no difference among the three can be observed. In this case, subtraction of the spectrum of PBS for compensation will result in poor signal to noise ratio experimentally.

The characteristic frequency of electrode polarization, f_{EP} , is estimated by $G_s/2\pi C_{EP}$. In the case of the system in Figure 2b the f_{EP} is about 1.3 MHz. The equation tells us that increasing the electrode surface area and decreasing the net conductance leads to the decrease of f_{EP} and reveal the real spectrum of a single cell. Inspired by such an insight, here we propose the fluidic structure to suppress the influence of electrode polarization in Figure 3a with the cross section of the measurement cell and the potential distribution. Cells continuously flow one by one from one channel to the other, crossing the small center hole whose diameter is almost the same as the cells. Even though the a.c. voltage is applied between two electrodes much larger than the hole, the potential drop mainly occurs through the hole and the dielectric spectrum of a single cell is measured only when the cell passes through the hole. The dielectric spectra of the single T-lymphocytes at the center of the hole were simulated by 3D finite element method using COMSOL (COMSOL AB) and shown in Figure 3b, where the spectrum of the medium (PBS) is subtracted to clearly understand the lymphocytes' spectra. Although in the region below 200kHz the influence of electrode polarization can be seen, the intrinsic spectrum shape of each T-lymphocyte can be observed in the higher frequency range due to the low f_{EP} (~ 1.0 kHz). Even if the electrode polarization still exists, the spectrum is clearly recognized.

EXPERIMENTAL

The parasitic capacitance originating from electrode polarization does not affect measurement of complex capacitance purely attributable to the cell in a medium. As a consequence of engineering system design for the analyzer and flow instruments, we decided the optimal structural parameters as in Figure 3a and implemented the design into a micro-channel that is made of layered polymer films and has embedded electrodes. An originally developed high-speed analyzer that enables simultaneous measurement of capacitance and conductance over 16 frequency points (maximum) in the range from 0.1 to 20 MHz (variable) with sampling rate of 2.5×10^3 per second was used for the experiments. While capacitance and conductance

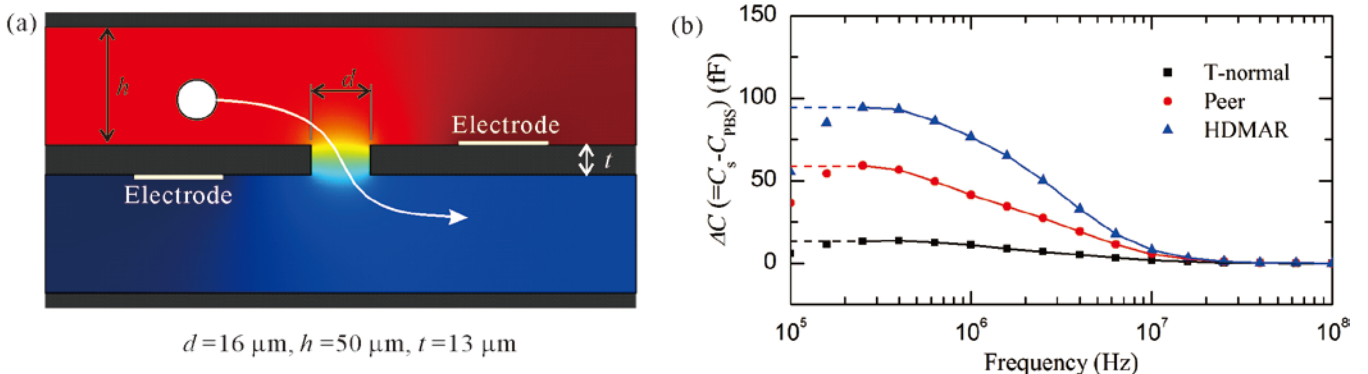


Figure 3: (a) Cross section of the measurement cell with electric potential distribution, and (b) analytical capacitance spectra of single T-lymphocytes at the center of the hole with and without considering electrode polarization.

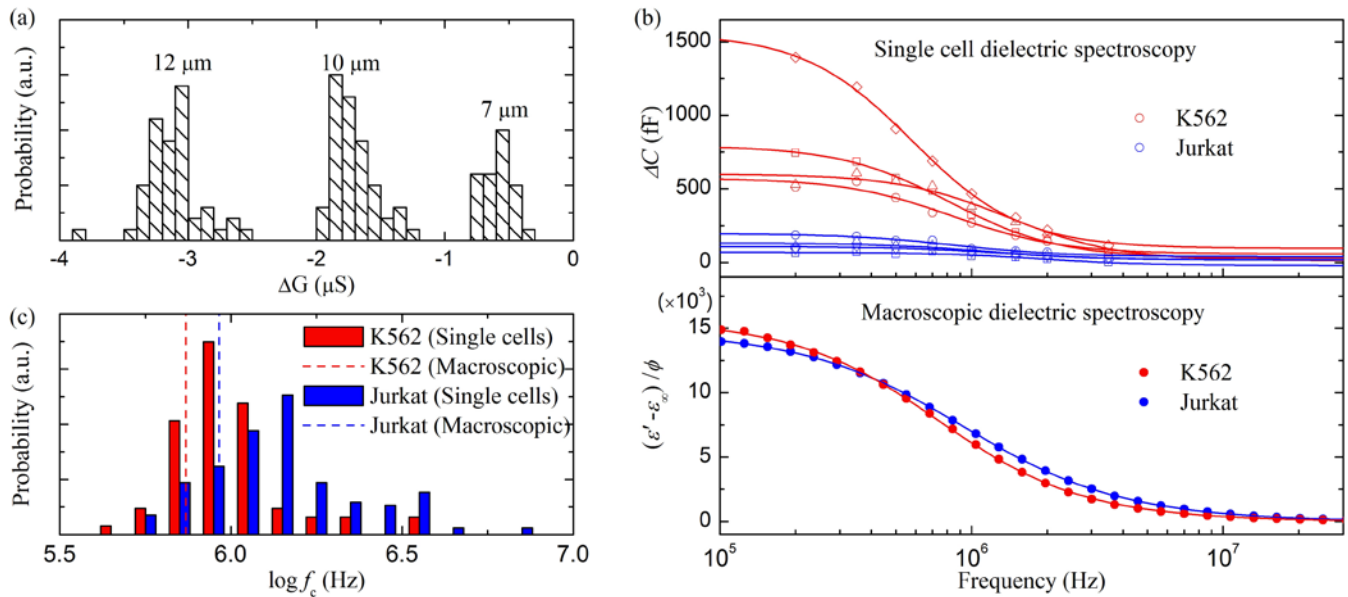


Figure 4: (a) Detected conductance difference of 7, 10, and 12 μm polystyrene beads, (b) real part of dielectric spectra of K562 and Jurkat cells taken by single cell dielectric spectroscopy (the upper panel) and “macroscopic” method (the lower panel), and (c) relaxation frequency distributions of Jurkat and K562.

at each frequency were continuously measured, the peak values in time-series data are detected with the originally developed software and subtracted by the baseline values to suppress the influence of the drift caused by subtle temperature change and the residual capacitance of electrode polarization in order to evaluate the dielectric spectrum of each single cell.

RESULTS AND DISCUSSION

Figure 4a shows that the distributions of conductance differences with respect to the baseline conductance are clearly separated for three kinds of polystyrene beads with different diameters.

A simple biological experiment was performed for two kinds of cell lines, K562 and Jurkat. In Figure 4b, the dielectric spectra from single cells and macroscopic ones are compared. The macroscopic spectrum for each cell line is broader in width than the corresponding single cell spectra. Even though the broadness has been usually explained by the existence of a high-frequency relaxation due to the nucleolus, the cell size distribution should be taken into account, either. On the other hand, the spectrum for a single cell fits well with the theoretical spectrum from Debye’s formula, and we can discriminate two kinds of cell lines from the dielectric spectra. The parameters that characterize the spectrum such as a relaxation frequency are obtained by fitting the spectrum to the Debye’s formula, as shown in Figure 4c that indicates the cell lines have different distributions.

CONCLUSION

Single cell dielectric spectroscopy was proposed with the structure of the device for accurate measurement of complex capacitances over frequencies around the relaxation frequency while effects of electrode polarization are suppressed. The results show that the single cell spectrum can be measured and the cell lines of K562 and Jurkat have different distributions of the dielectric parameters.

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