A TECHNIQUE FOR MEASURMENT OF DIELECTRIC PROPERTIES OF CELLS BY SIMULTANEOUS USE OF ELECTROROTATION AND NEGATIVE DIELECTROPHORESIS

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ABSTRACT

We introduce a technique for measurement of dielectric properties of cells using superposed signals, which can simultaneously induce negative dielectrophoretic (nDEP) force and electrorotational (ROT) torque. Cells were trapped and levitated in the center of a 3D octode by the nDEP force and concurrently rotated by the superposed ROT torque. By using the proposed technique, area specific membrane capacitances and cytoplasm conductivities of human leukocyte subpopulations and metastatic human cancer cell lines were analyzed.

KEYWORDS

Dielectric properties of cells, Dielectrophoresis, Electrorotation, Human leukocyte subpopulations, Metastatic human cancer cells

INTRODUCTION

Conventional ROT measurement for analyzing dielectric properties of cells is performed with a polynomial electrode array with a rotating field. This method however suffered from friction, aggregation, and adherence to the electrode in specific frequency ranges. Alternatively, optical tweezers [1] or ac field cage [2] were used for trapping cells during ROT measurement. Nevertheless, these techniques have still limitations due to expensive to use and difficult to simultaneously realize cell trapping and ROT in effective. Therefore, in this paper, we present a ROT measurement technique, which can rotate a single cell while it is trapped and levitated in the center of a 3D octode by simultaneous use of nDEP force and ROT torque.

WORKING PRINCIPLE

When cells, suspended in a 32.6 mS/m low conductivity medium, are injected, they are passing through a microchannel including a 3D octode by capillary force. When a cell reaches the central region of the 3D octode, the nDEP signals with out-of-phase are applied to the 3D octode for generating the nDEP force, as shown in Figure 1. The cell is then trapped and levitated in the center of the 3D octode by the nDEP force. Subsequently, ROT signals with 90° phase difference each other are superimposed to the nDEP signals, thereby generating waveforms superposed by the ROT and nDEP signals. As the superposed signals are applied to the four electrode pairs, the trapped cell starts to rotate. A single-cell ROT spectrum can be efficiently measured because the nDEP force repels other cells from the 3D octode.

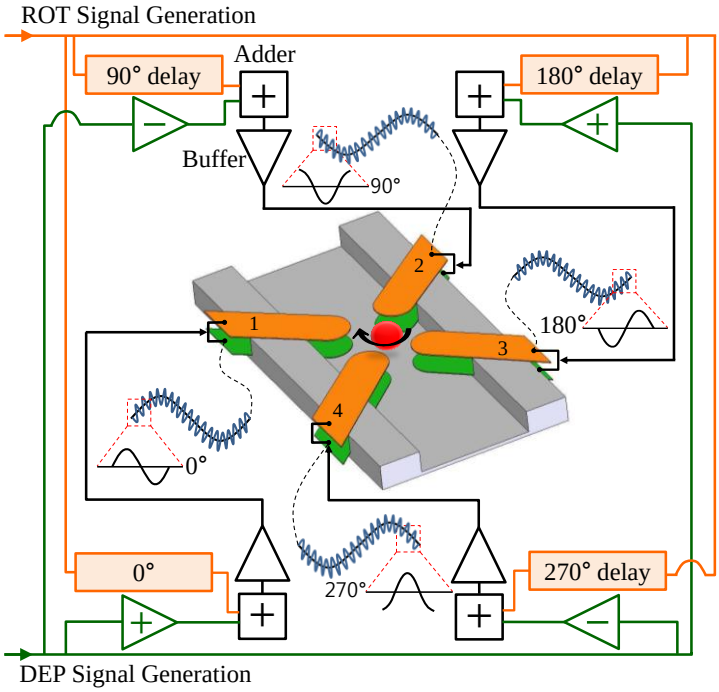

Figure 1. Schematic diagram of the ROT-microchip and the applied superposed signals including the nDEP and ROT signals

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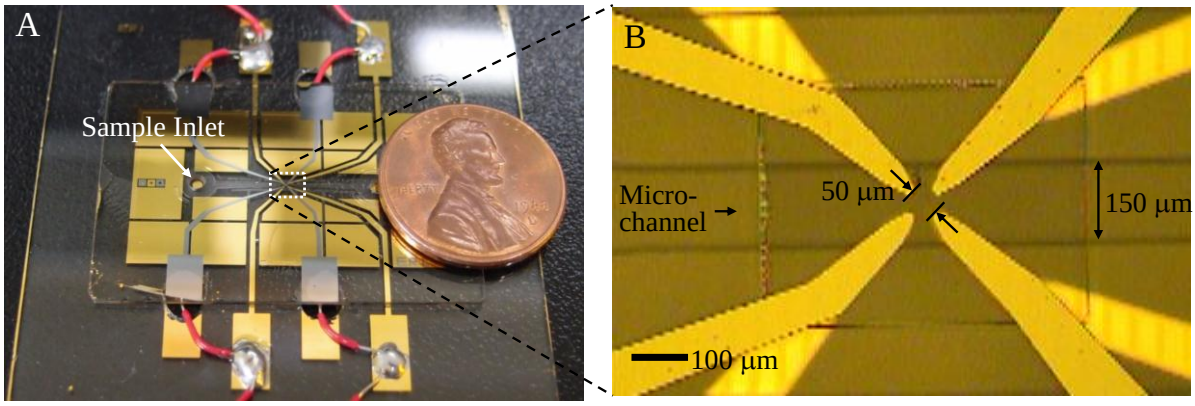

Figure 2. Photographs of (A) the fabricated ROT-microchip and (B) enlarged view of the 3D octode in the microchannel

EXPERIMENT

The ROT-microchip (Figure 2) was fabricated using 0.7-mm thick bottom and top glass substrates and SU8-to-SU8 thermal bonding. For generating the nDEP and ROT signals, we used a two channel function generator and a four channel arbitrary waveform generator. The superposed waveforms were produced by a homemade signal processing circuitry. Using the superposed signals, rotation of a single cell trapped in the center of the 3D octode was measured in a frequency range of 10 kHz to 10 MHz at four points per decade. The rotation was recorded during 30 seconds at each frequency and the rotation rate was calculated.

RESULTS AND DISCUSSION

To observe relation between rotation rate of cell and the nDEP force, rotation rate of a T-lymphocyte was measured for varying peak voltage of the nDEP signal. For the measurement, a T-lymphocyte was trapped in the center of the 3D octode (Figure 3(A)) by a 2 V peak nDEP signal. By the nDEP signal, the T-lymphocyte was levitated at approximately half-height of the microchannel. Figure 3(B) shows the rotation rate of a trapped T-lymphocyte for varying peak voltage of the nDEP signal. As 100 kHz, 0.4 V peak ROT signals were applied to the 3D octode, rotation rates of the T-lymphocyte were measured following the various peak voltages from 0.01 to 5.5 with 20 kHz frequency. For the nDEP signal higher than 1.5-V peak, the rotation rate maintained at 1.4/s. At lower than 1.5-V peak, the rotation rate decreased. It may be caused by a friction between the cell and the substrate, because the cell was not completely levitated. This result also explains that the rotation rate is not influenced by the nDEP force since the cell is levitated.

Using the superposed signals, the ROT spectra of human leukocyte subpopulations (T-lymphocytes, B-lymphocytes, granulocytes, and monocytes) and metastatic human cancer cells (SkBr3 and A549) were measured in a frequency range of 10 kHz to 10 MHz (Figure 4). Dielectric properties of the measured cells (Table 1), such as cytoplasm conductivities and specific membrane capacitances, were extracted by the best fitting between the measured ROT spectra and theoretical results based on the single-shell dielectric cell model. Table 1 shows that

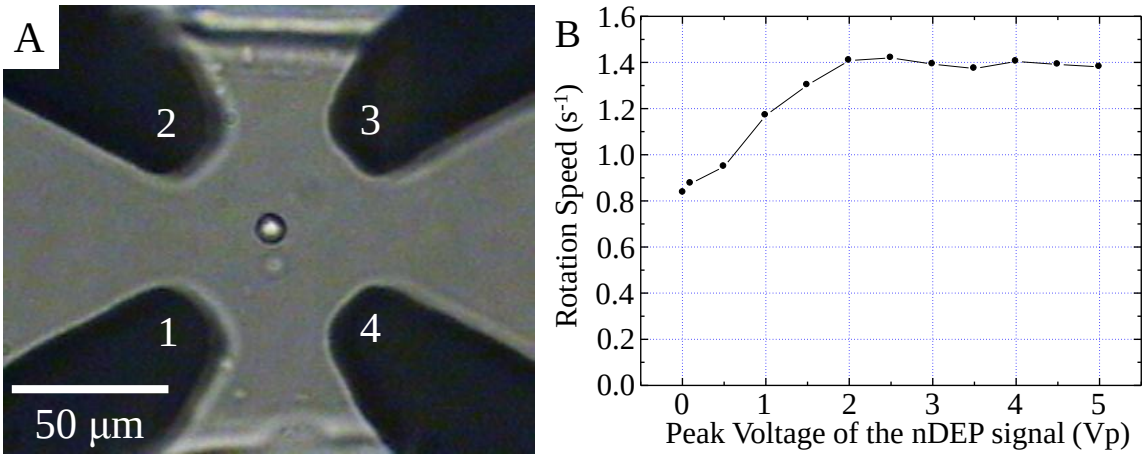

Figure 3. (A) A photograph of a trapped T-lymphocyte in the center of the 3D octode by the quadrupole nDEP force. (B) Measured rotation rate of the trapped T-lymphocyte for varying peak voltage of the nDEP signal.

Table 1. Dielectric properties of human leukocyte subpopulations and cancer cells

Cell type	Number	Radius (μm)	C_{mem} (mF/m^2)	σ_{int} (S/m)	ε_{int}
T-lymphocytes	9	3.6±0.55	7.01±0.91	0.53±0.1	100
B-lymphocytes	8	3.6±0.6	10.33±1.6	0.41±0.1	100
Granulocytes	12	4.3±0.55	9.14±1.06	0.31±0.06	100
Monocytes	6	4.8±0.55	11.77±2.12	0.37±0.15	100
SkBr-3	7	7.34±0.64	14.83±1.74	0.34±0.06	100
A549	12	6.9±1.07	16.95±2.93	0.23±0.05	100

T-lymphocyte has the largest value of the cytoplasm conductivity, σ_{int}, among the leukocytes and granulocyte has the smallest value. Cancer cell lines (SkBr3 and A549) have generally small cytoplasm conductivity comparison with leukocytes. In comparison, T-lymphocyte has the smallest value of specific membrane capacitance, C_{mem}, and monocyte has the largest value. The specific membrane capacitances of the cancer cells exhibited larger than those of human leukocytes, because the cancer cell membrane is usually composed of many folds, ruffles and also pits.

CONCLUSION

This study showed that the electrorotation technology could exactly measure a ROT spectrum of a single cell with high reliability. The biggest advantage of our technique is that it could solve the problem of friction between a cell membrane and the surface of the microchannel. Furthermore, the proposed technique allowed for measuring the ROT spectrum of a cell at the range of high frequency (>100 Hz), which generates positive DEP (pDEP) force on the cell. Although the cell was attracted by the pDEP force, it remained stationary in the center of the 3D octode by the applied nDEP signal. In addition, the rotation rate of a cell according to the frequency of the ROT signal was analyzed based on the single-shell dielectric cell model. The dielectric properties of the normal leukocytes and cancer cell lines could be extracted by the best fitting between the analysis results and the measured ROT spectra. Consequently, the proposed ROT measurement technique using the electrical method only can be used to analyze the accurate dielectric properties of cells, which can be applied for many cellular assays, such as cell identification and classification.

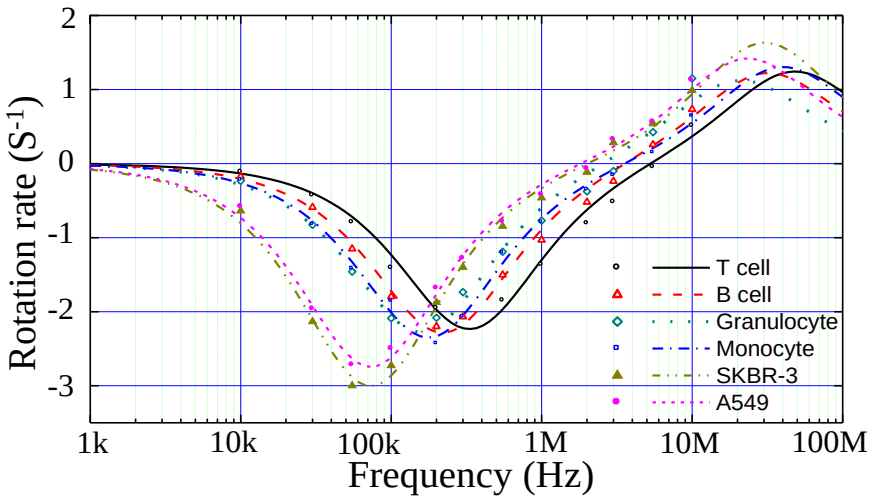

Figure 4. Measured ROT spectra for human leukocytes and metastatic human cancer cell lines suspended in a 32.6 mS/m low conductivity medium. Continuous lines show the best fits of the single-shell dielectric model of the cells.

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