Double frequency technique to measure cell polarizability

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The frequency behavior of cell polarizability is a characteristic of the physiological state of cells, and therefore it can be used for their identification. At present, this frequency behavior is determined by measuring the cell velocity in an alternating inhomogeneous electric field from which the cell polarizability coefficient is then calculated. The measurement error includes the errors of measuring the speed, electric field strength, and the coefficient of proportionality between the speed and the viscous friction force. The proposed double frequency method is free of these errors and provides higher measurement accuracy.

Introduction

The method of dielectrophoresis has been widely used in recent years for cell identification.[1] The possibility of measuring dielectric properties of a cell while studying cell motion in an inhomogeneous alternating electric field was demonstrated in Ref. 2. In this paper we describe single and double frequency techniques to measure cell polarizability, as well as the hardware and software for the determination of electric characteristics of biological particles. The results of measuring erythrocyte polarizability by the double frequency technique are presented.

Theory of single frequency and double frequency dielectrophoresis

A cell when placed in an electric field varying according to a harmonic law acquires induced dipole moment **d**, which is proportional to the electric field strength **E**: $\mathbf{d} = \alpha \varepsilon_0 \mathbf{E}$, where α is the complex cell polarizability coefficient; ε_0 is the dielectric constant of vacuum. If the electric field is inhomogeneous, then the cell experiences the action of the force[3]

$$\mathbf{F} = \frac{1}{2} \mathbf{grad} (\alpha \varepsilon_0 \mathbf{E}, \mathbf{E}) \qquad (1)$$

or, after averaging over time:

$$\langle \mathbf{F} \rangle = \frac{1}{4} \varepsilon_0 \mathrm{Re}[\alpha(\omega)] \mathbf{grad} (E_0^2), \qquad (2)$$

where E_0 is the amplitude of the electric field at the point of the cell location; ω is the frequency of the electric field; $\mathrm{Re}[\alpha(\omega)]$ is the real part of the cell polarizability coefficient. Under the effect of this force, the cell accelerates, until the viscous friction force balances this dielectrophoretic force. Thus, if we measure the cell velocity and calculate the electric field at the point of its location, then we can find the cell polarizability coefficient. The typical dependence of the polarizability coefficient of a living cell on the frequency of the electric field is depicted in Fig. 1.

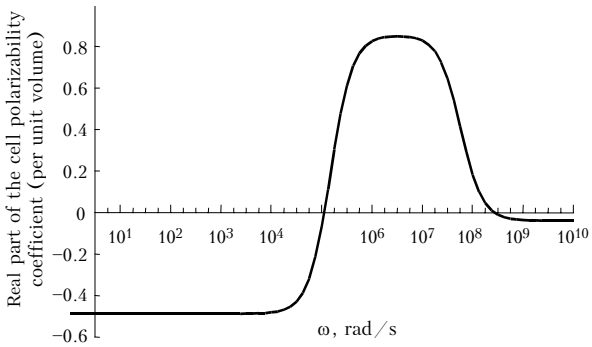

Fig. 1. Typical dependence of real part of cell polarizability coefficient on the frequency of alternating electric field.

The uncertainty in the cell polarizability coefficient is caused both by the measurement error in the cell velocity and by the uncertainties in the parameters used in calculations. These parameters include the electric field strength and the coefficient of proportionality between the cell velocity and the viscous friction force. If the cell is surrounded by other cells spaced by a gap of several cell's radii in size, the electric field is distorted, and the gradient produced by such cells can be comparable with or even larger than the gradient of the electric field produced by electrodes. Cells are near a glass surface, because the areas with the highest electric field strength are just near the electrodes. This changes the viscous friction force. The cell shape should also be taken into account, because some cells are non-spherical.

Analysis of measured dependences for living cells showed that there exists a frequency range (see Fig. 1), in which the real part of the cell polarizability coefficient $\mathrm{Re}[\alpha(\omega)]$ is larger than zero, that is, the cell is pulled in the area with the maximum electric field strength – the so-called positive dielectrophoresis, and the frequency range, in which the real part of the polarizability coefficient is below zero, that is, the cell is pushed out of the area with the high electric field strength – the negative dielectrophoresis. This fact facilitates measurement of the frequency dependence of the cell polarizability coefficient.

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If the voltage, being a sum of two harmonic signals with the amplitudes U_1 and U_2 at the frequencies ω_1 and ω_2, is applied to the electrodes, then the cell experiences the action of the force

$$F_{2d} = K \{\mathrm{Re}[\alpha(\omega_1)]\ U_1^2 + \mathrm{Re}[\alpha(\omega_2)]\ U_2^2\}, \quad (3)$$

where K is the coefficient depending only on the electrode geometry and the cell position. Let $\mathrm{Re}[\alpha(\omega_1)] < 0$ at the frequency ω_1 and $\mathrm{Re}[\alpha(\omega_2)] > 0$ at ω_2. At a proper selection of the amplitudes U_1 and U_2, we may have the situation that the force acting on the cell from the electric field equals zero. This is possible under the following condition:

$$\frac{\mathrm{Re}[\alpha(\omega_1)]}{\mathrm{Re}[\alpha(\omega_2)]} = \frac{-U_2^2}{U_1^2}. \quad (4)$$

Having measured the voltage amplitudes, we can find the ratio of the real parts of the polarizability coefficients. Having fixed the frequency ω_1 and changing the frequency ω_2, we can find the dependence of the cell polarizability coefficient in the frequency region of the positive electrophoresis. The frequency dependence of the cell polarizability coefficient in the frequency region of the negative electrophoresis can be found in a similar way. The dependence obtained is dimensionless. To find the dimensional quantity, we should measure the cell polarizability coefficient at one of the frequencies by use of a single frequency method.

Laboratory setup for measuring cell polarizability

Special laboratory setup has been designed and manufactured for cell observations and measurements of the cell polarizability coefficients under various conditions (Fig. 2).[4–6] It consists of a microscope, video camera, and measurement chamber with electrodes, to which a sum of voltages is applied, the voltages being generated by two computer-controlled G4-158 generators.

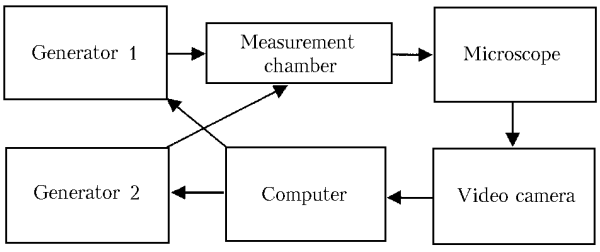

Fig. 2. Laboratory setup for measuring cell polarizability coefficients.

The generators produce alternating voltages with the amplitude $U = 0–4$ V in the frequency range $F = 10^4–10^8$ Hz that are applied to the electrodes of the measurement chamber. The measurement chamber is intended for generation of the inhomogeneous alternating electric field with the preset spatial strength characteristics. It consists of an object glass plate with $0.2 \cdot 10^{-6}$ m thick metal electrodes on one of its side. The gap between the electrodes is $60 \cdot 10^{-6}$ m. The microscope, video camera, and computer allow observation of individual cells and measurement of their radii, as well as the velocity of the translational motion in the measurement chamber. These characteristics are needed to calculate the cell polarizability coefficient by use of a single frequency method. For this purpose, we have developed a program for calculation of the electric field parameters in the measurement chamber. This program was used to estimate distortions of the electric field strength gradient caused by cells in the measurement chamber and to theoretically study trajectories of the polarized cells. Our setup can be used for measurements using both single frequency and double frequency methods.

Video processing program

To measure cell positions and velocities, a Visual C++ 6.0 program was developed. Video frames were entered into a computer with special DC-10 and DV-10 electronic cards for analog and digital frame grabbing. The files with the images stored have the avi extension. The program we have developed divides video into frames. In every frame, the program finds the coordinates of cell centers by determining the maximum correlation of the sampled cell color intensity with the color of a given screen area at a consecutive displacement of the screen part under control. Once the cell coordinates in one frame are determined, the program determines the coordinates of the closest cell in the next frame and thus draws a particle trajectory.

The cell motion depends on the flow rate and the effect of the inhomogeneous alternating electric field. To determine the flow rate, we calculated the mean velocity of all particles before voltage has been applied to the electrodes. The force acting on the cell from the inhomogeneous electric field is calculated from the velocity of cell motion relative to the flow. For this purpose, the flow rate is subtracted from the total cell velocity with the electric field on. For the double frequency method, a feedback is introduced to find, in real time, such amplitude ratio, at which the center of gravity of all cells moves at the speed of the flow rate.

Electric field calculation

The electric field was calculated through determination of the charge distribution over the electrode plates. For this purpose, we solved a system of algebraic equations determining the potential φ_i on the plates from charges q_j at nodes of the computational grid on the electrode:

$$\varphi_i = \sum_{j=1}^{n} \frac{q_j}{4\pi\varepsilon_0 r_{ij}}, \quad (5)$$

where r_{ij} is the separation between the charges q_i and q_j.

The distribution of potential across the interelectrode gap was calculated from the charge

values at the nodes of the computational grid on the electrodes. The electric field strength was calculated from the values of potential at the nodes of the computational grid in the interelectrode gap:

$$E_x = \frac{d\varphi}{dx}\ ,\ E_y = \frac{d\varphi}{dy}\ . \qquad (6)$$

The values of the squared electric field strength at the nodes of the computational grid form a field of the scalar quantity

$$E^2 = E_x^2 + E_y^2. \qquad (7)$$

Calculation of cell polarizability

By making the force (2) acting on the cell from the electric field equal to the viscous friction force acting from the liquid

$$F = 6\pi\eta v r, \qquad (8)$$

where η is the dynamic viscosity coefficient of the liquid, we can find the cell polarizability coefficient

$$\alpha = \frac{24\pi\eta v r}{\varepsilon_0\ \mathrm{grad}(E^2)}\ . \qquad (9)$$

Then the histogram of particles distribution over their polarizability is drawn. Equation (4) is used to draw the frequency dependence of the polarizability coefficient. The voltage step of the generators is 1 dB, and this value corresponds to the 12% error in the cell polarizability coefficient.

Experimental results

Using the setup described above, we have studied the frequency dependence of the polarizability coefficients of human erythrocytes. The polarizability at the frequency of 100 kHz was studied by use of the single frequency method. The measurement results are shown in Fig. 3 in the form of the cell polarizability distribution. The frequency dependence of the erythrocyte polarizability coefficient in the range from 100 kHz to 100 MHz was, in addition, measured by the double frequency method.

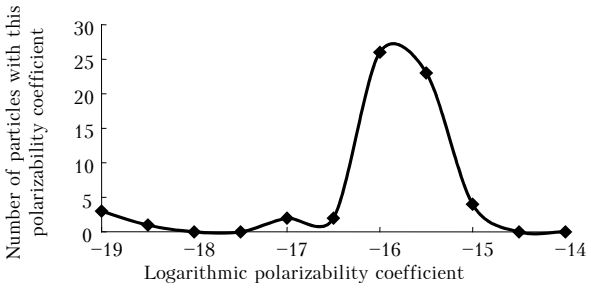

Fig. 3. Erythrocytes distribution over the polarizability coefficient measured using a single-frequency method.

Figure 4 depicts the results of these measurements (12% error) along with the best theoretical dependence calculated by the model, in which the cell is assumed spherical. For this model an analytical solution exists.[4] In addition, Fig. 4 shows the dependence calculated for the model, in which the erythrocyte shape is assumed a biconvex disc. Calculations were carried out by the numerical method described in Ref. 2.

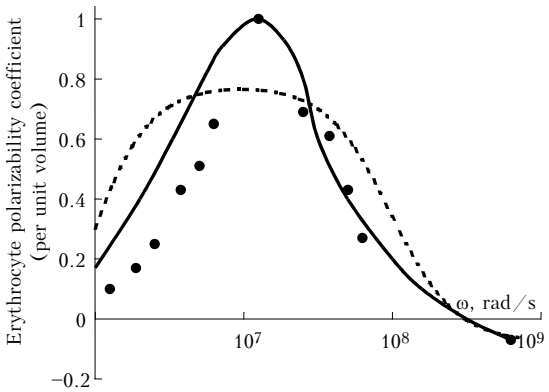

Fig. 4. Frequency dependence of the erythrocyte polarizability coefficient: experimental data (dots), best theoretical dependence calculated for spherical cells (dashed curve), and dependence calculated for the model with erythrocyte cells having the shape of biconvex disc (solid curve).

Conclusion

The hardware and software have been developed for determination of electric characteristics of biological particles having an arbitrary shape in a liquid under the exposure to alternating electric fields in the frequency range from 10 kHz to 100 MHz by both single and double frequency methods. Test experiments on measurement of the erythrocyte polarizability coefficient by the double frequency method have been conducted. The measurement error obtained for the double frequency method turned out to be several times smaller than that for the single-frequency method.

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