Spatial concentration distribution analysis of cells in electrode-multilayered microchannel by dielectric property measurement

Jiafeng Yao,^{1,a)} Tatsuya Kodera,¹ Hiromichi Obara,² Michiko Sugawara,¹ and Masahiro Takei¹

¹*Department of Mechanical Engineering, Chiba University, Chiba 263-0022, Japan*

²*Department of Mechanical Engineering, Tokyo Metropolitan University, Tokyo 192-0397, Japan*

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The spatial concentration distribution of cells in a microchannel is measured by combining the dielectric properties of cells with the specific structure of the electrode-multilayered microchannel. The dielectric properties of cells obtained with the impedance spectroscopy method includes the cell permittivity and dielectric relaxation, which corresponds to the cell concentration and structure. The electrode-multilayered microchannel is constructed by 5 cross-sections, and each cross-section contains 5 electrode-layers embedded with 16 micro electrodes. In the experiment, the dielectric properties of cell suspensions with different volume concentrations are measured with different electrode-combinations corresponding to different electric field distributions. The dielectric relaxations of different cell concentrations are compared and discussed with the Maxwell-Wagner dispersion theory, and the relaxation frequencies are analysed by a cell polarization model established based on the Hanai cell model. Moreover, a significant linear relationship with AC frequency dependency between relative permittivity and cell concentration was found, which provides a promising way to on-line estimate cell concentration in microchannel. Finally, cell distribution in 1 cross-section of the microchannel (X and Y directions) was measured with different electrode-combinations using the dielectric properties of cell suspensions, and cell concentration distribution along the microchannel (Z direction) was visualized at flowing state. The present cell spatial sensing study provides a new approach for 3 dimensional non-invasive online cell sensing for biological industry. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4929824>]

I. INTRODUCTION

Micro-scale cell measurement and manipulation is a key process for establishing multicellular systems with their medical applications by biological and medical researchers collaborating with mechanical engineering experts. Therefore, the construction of three-dimensional (3D) cellular systems (e.g., Bio Assembler¹) became a hot topic to understand the multifaceted properties of cells and focus on those properties, develop the world's fastest measurement and separation techniques with the application of microfluidic systems.²

In this situation, one of the popular methods is flow cytometry,^{3,4} which is a common technology that simultaneously measures and then analyses multiple physical characteristics of single cells, as they flow in a fluid stream through a beam of light. The properties measured include the particle's relative size, relative granularity or internal complexity, and relative fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell scatters incident laser light and emits fluorescence.⁵ However,

^{a)}E-mail: jiaf.yao@chiba-u.jp

the flow cytometry method has a low cell sensing speed and the extra fluorescer is apt to influence the cell functions.

An alternating method without extra fluorescer is called impedance spectroscopy, which has been deeply and extensively applied to cell sensing by Asami and his team.^{6–10} They detect the cell characteristics (e.g., concentration, cell size, and survival state) by the measuring the dielectric properties of cells, which is regarded as dielectric material due to its highly non-conducting membrane. Their research results prove that impedance spectroscopy is an effective, non-invasive, and on-line cell sensing and manipulation method.

Electrical Impedance Spectroscopy (EIS) as a measuring technique detects the dielectric properties of materials, which can reveal the spectra of objects caused by the external electric field's interaction with the dipole moment of objects.¹¹ It is also regarded as an experimental approach that describes electrochemical systems. This method gauges system impedance over a series of frequencies. Thus, frequency response involving dissipation properties and energy storage is disclosed. Most of the time, data gathered through electrochemical impedance spectroscopy is graphically conveyed in Nyquist plots or Bode plots.¹²

On the other hand, biological cells show frequency-dependent dielectric dispersions includes ion dispersion (termed α dispersion) at lower frequency, interfacial polarization (termed β -dispersion or the Maxwell-Wagner dispersion) related to the plasma membrane at medium frequency and additional dispersions (termed γ -dispersion) at higher frequency.^{13,14}

A common cell can be simply divided as three elements based on the Hanai Cell Model:¹⁵ wall, membrane, and cytoplasm. These three elements contribute to different frequency-dependent dielectric dispersions. The dispersion in the cell wall has been a relatively neglected area in dielectric research, despite being of great importance in understanding the dielectric properties of micro-organisms.¹⁶ For the cell membrane, Asami and his team^{17,18} demonstrated that the β -dispersion is mainly observed caused by the interfaces between the membrane and the outer solutions. The membrane surrounding the cell has a lipid bilayer structure, which is extremely thin of 4–10 nm in thickness, and has incorporated into it large amounts of proteins. As a result, the value of the membrane's relative permittivity has been given values ranging from typically 2–10.¹⁹ Transport of ions through the membrane is highly regulated by trans-membrane channel proteins, and under normal circumstances, the membrane can be regarded as highly nonconducting.^{16,20,21}

The α dispersion caused by the ion diffusion in the electrical double layer around the cell wall or membrane is also commonly neglected since this dispersions happens at very low frequency, and strongly influenced by electrode dispersion.

From the previous discussion on the cell elements, cell membrane can be regarded as the key factor to realize the cell sensing by impedance spectroscopy. Therefore, at a specified frequency range of 100 kHz–10 MHz where membrane shows β -dispersion, excellent linear relationships, in which correlation coefficients were more than 0.99, were obtained between values of cell permittivities and concentrations of various cells (e.g., *Escherichia coli*, *Aspergillus niger*, human leukemia, MDBK) It was also found that the linearities were maintained practically under cultivating conditions in which temperature, pH, and salt and substrate concentrations were changing during cultivation. In addition, the cell survival state was also detected by the different dielectric properties of membranes between living and dead cells.^{22–25} These results prove that the dielectric properties of cell membranes provide a useful information for cell sensing by Impedance Spectroscopy method.²⁶

Measurement of dielectric properties of cells has been used in many applications, such as single-cell printing,²⁷ cell discrimination,²⁸ and cell trapping.^{29,30} However, the cells in the applications are difficult to be measured as a spatial distribution, mainly due to the simple structure of the micro-device. To visualize the inner distribution of cells in a micro device, the non-invasive on-line spatial cell sensing technique is necessary in the current situation.

The present study proposed a non-invasive on-line measuring method for spatial concentration distribution of cells (i.e., 1 cross-section at X and Y directions together with along the microchannel at Z direction) by combining the dielectric properties of cell suspensions with the specific structure of an electrode-multilayered microchannel. First, the dielectric properties of

cell suspensions with different concentrations were measured by impedance analyzer using different electrode-combinations corresponding to different electric field distributions; then, the dielectric properties are analysed and discussed with the Maxwell-Wagner dispersion theory and a cell polarization model established based on the Hanai cell model; finally, by using the dielectric properties of the cell suspensions and the specific structure of the electrode-multilayered microchannel, the non-invasive online measurement of spatial concentration distribution of cells in the microchannel at a flowing state is conducted.

II. EXPERIMENT

A. Experimental setup

The experimental setup is shown in Fig. 1, which is composed of two syringe pumps (A and B), an electrode-multilayered microchannel, a Faraday cage, an impedance analyzer, and a PC. Syringe pumps A and B were used to control the flow velocity and connected to inlets A and B of the microchannel. The microchannel was shielded by the Faraday cage to reduce the amount of noise by external electromagnetic. The impedance analyzer (HIOKI, IM3570) measured the impedance of the objects by applying the AC excitation signal with a range of frequencies. The PC was used to process and analyse the impedance spectra.

The microchannel is fabricated by MEMS processes, and the details of the fabrication method and process are described by Choi *et al.*³¹ The detailed structure of the electrode-multilayered microchannel is shown in Fig. 1(a). Three inlets and three outlets are fabricated to meet the requirement of multiphase flow study, for example, in cell manipulation study, one inlet injects liquid, one injects normal cell, and the last one injects abnormal cells. In the present study, two inlets are employed to inject liquid and cells and the last one is blocked as a standby inlet. As shown in Fig. 1(b), the cross-section of the main flow-channel is $D = 800\ \mu\text{m}$, and the length is $Z = 20\ \text{mm}$. The microchannel is made up of quartz glass ensuring good transparency and offering high heat resistance as well as less chemical reactivity. Eight layers are stacked and each layer (layer distance $d = 80\ \mu\text{m}$) embed 5 electrodes (cross-section distance $Z_0 = 4.5\ \text{mm}$, electrode thickness $k = 10\ \mu\text{m}$) made from platinum along the microchannel by photolithography technique. As shown in Figs. 1(c) and 1(d), the inner shape of the cross-section is diamond. Therefore, different electrode-combinations in 1 cross-section can realize 2 dimensional sensing (X and Y directions), while, 5 cross-sections are used to realize the 3rd dimensional sensing (Z direction).

B. Experimental conditions

Dried yeast cells (*S. cerevisiae*, Euroscarf) in living state with different concentrations in suspensions were employed as samples in the present study.

The preparation procedure of the required suspensions is described. First, powder of yeast cells were mixed with about 3 ml of purified water at room temperature (about $25\ ^\circ\text{C}$) in a tube; then, the tube was immersed in warm water of $37 \pm 1\ ^\circ\text{C}$ for 30 min. Next, a small amount of the cell suspensions in the tube were transferred into a capillary as four-fifths of the capillary capacity to measure the concentration after a centrifugal treatment. Finally, the required volume of purified water was calculated and added into the tube to mix the suspension with volume concentrations of 10%, 20%, 30%, and 40%. For each concentration, a volume of about 1 ml was prepared for experiment, since the maximum volume of the syringe is 1 ml.

C. Experimental method

To measure the dielectric properties of cell suspension in Microchannel with different electrode-combinations, the electric field distribution in 1 cross-section is necessary to be clarified. COMSOL Multiphysics software is taken as the simulation tool for calculating the electric field distribution.

The mathematical formulation for the applied AC electric field can be understood from the governing equations as follows:

FIG. 1. (a) Experimental setup for measurement of dielectric characteristics of cells. (b) Schematic of the electrode-multilayered microchannel. (c) 1 cross section in the microchannel. (d) Microscope image of 1 cross section in the microchannel.

$$\mathbf{E} = -\nabla V, \quad (1)$$

where $\mathbf{E}$ is electric field and V is applied potential. The theoretical expectation of AC electric field is getting influenced by geometry and permittivity.

Electric field distributions with different electrode-combinations are shown in Fig. 2. More electrode pairs generate a larger electrode field, which is compared among electrode

FIG. 2. Electric field distribution of electrode-combinations E1–E6, AC voltage $V_{pp}=5$ V, applied frequency $f=1$ MHz, relative permittivity of water $\epsilon'_{\text{water}}=78.3$.

combination E1–E3. Furthermore, the electric field generated by each electrode-combination only accounts for part area of the cross-section, especially for E1, E4, E5, and E6; therefore, the cells distribution in the cross-section can be approximately measured by different electrode-combinations of E1, E4, E5, and E6.

Therefore, electrode-combinations in 1 cross-section of the microchannel as shown in Table I were selected. The dielectric properties of the cell suspensions were measured at static and flowing state by using different electrode-combinations.

Before experiment, the microchannel was washed three times by injecting purified water into three inlets with a syringe, respectively. The syringe needle is disposable to prevent contamination.

Static measurement was conducted to study the dielectric properties of cells. In the static measurement, the cell suspensions were injected into the microchannel by syringe pumps A and

TABLE I. Electrode-combinations in 1 cross-section of the microchannel.

Measuring state	Electrode combinations (E1-E6)		
Static (measurement in 1 cross-section)
	E1 (2-pair at bottom)	E2 (3-pair at bottom)	E3 (4-pair at bottom)

Flowing (measurement in 5 cross-section)	E4 (2-pair at top)	E5 (2-pair at left)	E6 (2-pair at right)

B simultaneously, and then the impedance data were obtained by impedance analyzer. Based on the electric field distribution, three patterns of electrode-combinations were used to compare the dielectric properties of living and dead cells with different suspension concentrations. The measuring procedure is as follows: first, find out the optimum electrode-combination, which was realized by using different pairs of electrodes for each concentration; then, clarify the relationship between relative permittivity and cell concentration, which was accomplished by using the optimum electrode pair which corresponds to the largest relative permittivities to test the different cell concentrations.

Flowing measurement was conducted to study the spatial concentration distribution of the cells in microchannel using the dielectric properties of cells. In the flowing measurement, syringe pumps A and B were connected to the two inlets of the microchannel. The cell suspensions with concentration of 10% were used. The mean flowing velocity pushed by the syringe pump in the microchannel is $v_{\text{mean}} = 0.87$ mm/s. The AC frequency of 1 MHz was used and the measurement was repeated as 300 steps. In the experiments, the 3rd cross-section was used with different electrode-combinations to measure the cell distribution in the microchannel in X and Y directions at 1 cross-section; then, five cross-sections were used with the optimum electrode-combination found in the 3rd cross-section (Z_3) to measure the flowing characteristics of cells for Z direction along the microchannel.

A series of complex impedance Z and phase angle θ were measured by impedance analyser at frequency range from 60 kHz to 4 MHz and AC current of 0.01 mA in a step-wise manner.

The complex admittance Y ($Y = 1/Z$) is expressed by the real and imaginary parts as

$$Y = G + j\omega C_c, \quad (2)$$

where G is conductance, j is imaginary unit, ω is angular frequency, and C_c is the capacitance of cell suspension, which is calculated by Eq. (3),³² the dimensions are depicted in Fig. 3

$$C = \frac{\varepsilon_0 \varepsilon' w}{\theta} \ln \left(1 + \frac{a}{b} \right), \quad (3)$$

where a is the length of the electrode along the microchannel, b is the radius of the plate point nearest to the electrode intersection, w is the width of the electrode, ε' is relative permittivity, and ε_0 is vacuum permittivity. Since different electrode-combination corresponds to different inner electrode distance, for convenience, we define the cell constant (C_0) as

$$C_0 = \frac{\varepsilon_0 w}{\theta} \ln \left(1 + \frac{a}{b} \right). \quad (4)$$

For the occasion of 2 pairs of electrode, the total cell constant equals to the sum of cell constant of each pair of electrodes.

$$C_0 = \frac{\varepsilon_0 w}{\theta} \ln \left(1 + \frac{a}{b} \right)$$

FIG. 3. Method to calculate cell constant of 1 pair of electrode in a cross-section of microchannel.

Then, the conductance can be expressed by the cell constant and conductivity as

$$G = \sigma C_0 / \varepsilon_0. \quad (5)$$

On the other hand, the capacitance of the cell suspension including stray capacitance (C_r) can be expressed as

$$C_c = \varepsilon' C_0 + C_r. \quad (6)$$

In order to obtain the stray capacitance and remove it, the relative permittivity of air ($\varepsilon'_{\text{air}} = 1$) and water ($\varepsilon'_{\text{water}} = 78.3$) are substituted into Eq. (6). Then C_c and C_r are calculated. Finally, the relative permittivity (ε') and dielectric loss (ε'') are calculated by following equations:

$$\varepsilon' = \frac{C_c - C_r}{C_c}, \quad (7)$$

$$\varepsilon'' = \frac{G(\omega) - G(\omega = 0)}{\omega C_0}. \quad (8)$$

Since in micro-scale devices, the influence of ion polarization induced by electrical double layer on the value of permittivity is much higher than that of millimetre-scale devices, the relative permittivity and dielectric loss in Eqs. (7) and (8) were normalized as follows:

$$\varepsilon'^* = \frac{\varepsilon'(\omega)}{\varepsilon'_{\text{water}}(\omega = 1 \text{ MHz})}, \quad (9)$$

$$\varepsilon''^* = \frac{\varepsilon''(\omega)}{\varepsilon''_{\text{water}}(\omega = 1 \text{ MHz})}. \quad (10)$$

Based on these theoretical equations, the results are presented as follows.

III. RESULTS AND DISCUSSION

A. Dielectric properties of cells in static measurement

Fig. 4 compares the relative permittivities of the cell suspensions with different electrode pairs at bottom of the 3rd cross-section in microchannel. As an example, 10%-E1 means living

FIG. 4. Dielectric spectra of cell suspensions with different electrode-combinations (E1–E3) of the 3rd cross-section in microchannel.

cell suspensions with concentration of 10% by using electrode-combination E1 of cross-section in microchannel.

For each electrode-combination, the relative permittivity (ϵ'^*) decreases linearly as the frequency (f) increases until $f = 60$ kHz, which is caused by electrode polarization. Similar phenomenon was also found in our previous study.³³ Schwan³⁴ reported that electrode polarization causing a serious problem influencing the measurement result is due to the electrical double layer between metal electrodes and an electrolyte solution at low frequencies. In order to avoid the influence of electrode polarization, the dielectric spectra at over $f = 60$ kHz are mainly studied in the experiments.

At the frequency range from $f = 60$ kHz to $f = 4$ MHz, cell suspensions show quite broad polarizations. These are mainly caused by interfacial polarizations (also called Maxwell-Wagner polarization) at the interfaces among the different materials of which a cell is composed.¹⁶ The interfacial polarizations generate large differences of relative permittivities of cell suspensions with different concentrations.

For each cell concentration, the electrode-combination of 2-pair at bottom (E1) corresponds to the largest relative permittivities. That is because higher concentration of biological cells is in the area of bottom of the microchannel due to deposition, which leads to a higher dielectric relaxation.

Fig. 5 presents the linear relationships with AC frequency dependence between cell concentration and relative permittivity using 2-pair of electrodes (E1) at 3 different frequencies: $f_1 = 190$ kHz, $f_2 = 1$ MHz, and $f_3 = 2.8$ MHz. Excellent linearities are obtained in all cases, and the slope of line decreases as the frequency increases. Similarly, by using electromagnetic induction method, Asami *et al.*¹⁰ obtained the linear relationship between cell concentrations of immobilized yeast in the gels and the values of electric capacitance at 1 MHz with correlation coefficient of 0.998.

However, this linear relationship obtained by dielectric measurement method is a promising way to estimate on-line cell concentration in Sec. III B in Microchannel. The linear equations are concluded in Table II.

Fig. 6(a) shows the dielectric loss at the frequency range from 100 kHz to 3 MHz. The dielectric loss ϵ'' increases as the cell concentration increases for each curve. Because the intra-cellular components with high conductance increase as cell concentration increases as shown in Eq. (8).

For the characteristics of dielectric loss ϵ''^* , several peak values appear between frequencies of 100 kHz and 3 MHz for each cell concentration. The frequency corresponding to the peak

FIG. 5. Linear relationship with AC frequency dependence between the relative permittivity and the concentration of cell suspensions with 2-pair of electrodes (E1) at $f_1 = 190$ kHz, $f_2 = 1$ MHz, and $f_3 = 2.8$ MHz.

TABLE II. Linear equations with AC frequency dependency for cell concentration calculation in Fig. 5.

Applied frequency	Linear equation	Coefficient of determination R^2
$f_1 = 190 \text{ kHz}$	$\Phi_1 = (e^{f^*} - 0.78)/7.80$	0.9927
$f_2 = 1 \text{ MHz}$	$\Phi_2 = (e^{f^*} - 0.78)/1.54$	0.9866
$f_3 = 2.8 \text{ MHz}$	$\Phi_3 = (e^{f^*} - 0.78)/0.52$	0.9036

value is relaxation frequency. Therefore, 4 relaxation frequencies ($f_{\text{relax}1}$, $f_{\text{relax}2}$, $f_{\text{relax}3}$, and $f_{\text{relax}4}$) are found from 100 kHz to 3 MHz.

This phenomenon could be explained by a cell polarization model shown in Fig. 6(b), which is established based on the Hanai cell model,¹⁵ explains the dielectric spectra of cells and provides a useful information to reveal the cell structure and concentration. When a biological cell is suspended in a solution, several dielectric dispersions are formed due to the complex structure of the cell.

The Electrical Double Layer (EDL) is the result of the variation of electric potential near surface of the cell and has a significant influence on the behavior of cells and other surfaces in contact with solutions, which contributes to α dispersion at low frequency ($f_{\text{relax}1} = 210 \text{ kHz}$).

Furthermore, β dispersion (interfacial polarization or the Maxwell-Wagner dispersion³⁵) is raised from the different component of a cell (i.e., wall, membrane, and cytoplasm). At the interface between different components in contact, the polarization takes place in the nearby atoms caused by electromagnetic interaction between them. Assuming the two components were made from "ordinary" matter where the nucleus is composed of neutrons and protons surround by the electrons, the electrons on the surface facing each other will repel and cause a polarization on that surface. Therefore, three components of cells, those are cytoplasm, membrane, and cell wall, which lead to three interfaces, correspond to 3 relaxation frequencies ($f_{\text{relax}2} = 1.8 \text{ MHz}$, $f_{\text{relax}3} = 2.6 \text{ MHz}$, and $f_{\text{relax}4} = 3.1 \text{ MHz}$). Especially, the highest relaxation frequency $f_{\text{relax}4} = 3.1 \text{ MHz}$ is due to the polarization between the suspension and the cell cytoplasm, when the cell membrane capacitance is effectively short-circuited.³⁶ The relaxation frequency is also used to identify the cell size and survival status by employing α dispersion around cells and β dispersion in membrane.²²

As a summary, the dielectric properties of biological cell suspension provide a possibility to perform the non-invasive measurement of cell distribution in a microchannel.

FIG. 6. (a). Relaxation frequency of different cell concentrations. (b) Cell polarization model in solution, the dielectric dispersions include α dispersion caused by electrical double layer (EDL) around cells at $f_{\text{relax}1} = 210 \text{ kHz}$, β dispersions (the Maxwell-Wagner dispersion) caused by the interfacial polarization among cell wall, membrane, and cytoplasm at $f_{\text{relax}2} = 1.8 \text{ MHz}$, $f_{\text{relax}3} = 2.5 \text{ MHz}$, and $f_{\text{relax}4} = 3.1 \text{ MHz}$.

B. Cell distributions in flowing measurement

By using the dielectric properties of biological cells in the electrode-multilayered micro-channel in Sec. III A, non-invasive measuring results of spatial cell distribution in flowing measurement are presented and discussed.

Since each electrode-combination generates an electric field in part area of the cross-section as shown in Fig. 3, the cell distribution can be measured by different electrode-combinations. Fig. 7(a) shows the measuring result of cell distribution in the 3rd cross-section (Z_3) when they are flowing through the microchannel with an initial concentration of 10% at a mean velocity of $v_{\text{mean}} = 0.87 \text{ mm/s}$. Four kinds of electrode-combinations are employed to measure the cell distributions at three different positions in the 3rd cross-section (Z_3) of the microchannel. The relative permittivities obtained from the electrode-combination at top are staying lowest, which means the fewest cells are suspending at the top area of the microchannel. The relative permittivities obtained from the electrode-combination at left and right side also almost remain constant over time, a little higher than that of the top area. The difference of the relative permittivities between these 2 kinds of symmetrical electrode-combinations is mainly due to the uneven distribution of cells in the microchannel, of course, measuring error could not be excluded. In addition, the relative permittivities measured from the electrode-combination at bottom increase over time and tend to be a constant value from $t = 25 \text{ s}$. As a consequence, the cell sedimentation in the 3rd cross-section (Z_3) of the microchannel is found from the different permittivities obtained from different electrode-combinations.

For the reason of cell sedimentation, Bryan *et al.*³⁷ measured the density of yeast cells and obtained that $\rho_{\text{yeast}} \approx 1200 \text{ kg/m}^3$, which is higher than that of purified water as a solution $\rho_{\text{solution}} = 1000 \text{ kg/m}^3$ at room temperature. The gravitational force F_g is given by³⁸

$$F_g = V_{\text{yeast}}(\rho_{\text{yeast}} - \rho_{\text{solution}})g, \quad (11)$$

where V_{yeast} (m^3/s) is the volume of yeast cell and g (m/s^2) is the acceleration due to gravity.

The magnitude of the velocity of cells u_g (m/s) in a gravitational field is expressed by³⁸

$$u_g = \frac{V_{\text{yeast}}|\rho_{\text{yeast}} - \rho_{\text{solution}}|g}{\gamma} = \frac{2r^2|\rho_{\text{yeast}} - \rho_{\text{solution}}|g}{9\mu}, \quad (12)$$

where γ (kg/s) is the friction factor of the cell in the fluid. For a spherical cell, $\gamma = 6\pi\eta r$, where r (m) is the cell radius and μ [kg/(s m)] is the dynamic viscosity of solution. For the present condition, the viscosity of water at 30°C is $\mu_{\text{water}} = 0.798 \times 10^{-3} \text{ kg/(s m)}$, then the

FIG. 7. (a) Cross-sectional cell concentration distribution when cells are flowing through the 3rd cross-section of (Z_3) of the microchannel with different electrode-combinations E1, E4, E5, and E6 (initial cell concentration $\Phi = 10\%$, mean velocity in microchannel $v_{\text{mean}} = 0.87 \text{ mm/s}$, $f = 1 \text{ MHz}$, cell density $\rho_{\text{yeast}} \approx 1200 \text{ kg/m}^3$, solution density $\rho_{\text{solution}} = 1000 \text{ kg/m}^3$). (b) Cell concentration distribution along Microchannel from Z_1 to Z_5 .

sedimentation velocity of yeast cell in the microchannel was calculated as $u_g \approx 16 \mu\text{m/s}$. In order to suspend cells in a solution, the same density of the solution with that of cells is necessary.

Furthermore, the quantitative results of the cell distribution is presented in Fig. 7(b) by using the second linear equation ($f_2 = 1 \text{ MHz}$) in Table II. Cell concentration distribution is realized by using 5 cross-sections of the microchannel. The cell suspension with an initial concentration of $\Phi = 10\%$ flows through cross-sections Z_1 – Z_5 in sequence at a mean velocity of $v_{\text{mean}} = 0.87 \text{ mm/s}$. At the first cross-section (Z_1), the cell concentration increases gradually over time, from 0% to 14%. At the same time, take $t = 35 \text{ s}$ as an example, the cell concentration decreases along the microchannel, which visualizes the cell accumulation from Z_1 to Z_5 due to sedimentation.

In consequence, measurement of cell concentration distribution along Microchannel is realized by using 5 cross-sections of the electrode-multilayered microchannel.

IV. CONCLUSIONS

Spatial concentration distribution of cells in a microchannel was realized by combining the dielectric properties of cell suspensions with the specific structure of the electrode-multilayered microchannel. The present study is concluded as follows:

- (1) Dielectric properties of cell suspensions were clarified. The electrode-combination of 2-pair electrodes corresponds to a higher relative permittivity at MHz frequency comparing with other combinations, which were explained by the Maxwell-Wagner dispersion theory. Then, 4 relaxation frequencies ($f_{\text{relax}1} = 210 \text{ kHz}$, $f_{\text{relax}2} = 1.8 \text{ MHz}$, $f_{\text{relax}3} = 2.6 \text{ MHz}$, and $f_{\text{relax}4} = 3.1 \text{ MHz}$) of cell suspension are found from 100 kHz to 4 MHz, which was analyzed by a cell polarization model established based on the Hanai cell model. Moreover, a significant linear relationship with AC frequency dependency (e.g., $R^2 = 0.9866$ at $f = 1 \text{ MHz}$) between relative permittivity and cell concentration was established, which provides a promising way to estimate on-line cell concentration in Microchannel.
- (2) Cell sedimentation was found by employing 1 cross-section (X and Y directions) of the microchannel with different electrode-combinations, and the sedimentation approaches to be constant at $t = 35 \text{ s}$. Therefore, cell distribution in 1 cross-section of Microchannel can be measured with present method.
- (3) Cell concentration was calculated with the linear relationship between relative permittivity and cell concentration. The cell concentration decreases along the microchannel, which visualizes the cell accumulation from Z_1 to Z_5 due to sedimentation. Therefore, cell concentration distribution along Microchannel was visualized at flowing state.

The present study provides a non-invasive on-line spatial cell sensing technique for biological industry. Future work will investigate the difference of dielectric properties between living and dead cells in a suspended state, in order to realize on-line 3D cell sensing and separation between living and dead cells in microchannel.

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