Detection of Heterogeneous Cells in Cell Spheroids by Applying High-Frequency Second-Order Sensitivity Matrix Electrical Impedance Tomography (HSSM-EIT)

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ABSTRACT The high-frequency second-order sensitivity matrix electrical impedance tomography (HSSM-EIT) method has been proposed to detect heterogeneous cells in cell spheroids by coupling the high-frequency and second-order sensitivity matrix electrical impedance tomography (EIT). The sensitivity matrix with the first and second-order terms of Taylor’s formula (Jacobian and Hessian) is applied to the image reconstruction of cell spheroids with the high-frequency injected current at 1 MHz, at which the impedance reflects intracellular contents to visualize the cytoplasm conductivity distribution of cell spheroids. The cell spheroids with five composition percentages of the wild type (WT) and green fluorescent protein type (GFPT) of MRC-5 human lung fibroblast cell line are 100/0%, 75/25%, 50/50%, 25/75%, and 0/100%, and were cultured to mimic heterogeneous cells. As a result, the cell spheroid images reconstructed by HSSM-EIT clearly visualize the heterogeneity stage rather than the images reconstructed by general first-order sensitivity matrix EIT; moreover, the cytoplasm conductivity of the cell spheroid is decreased with the increase of GFPT percentage. In order to confirm the cytoplasm conductivity reconstructed by HSSM-EIT, an equivalent circuit model containing a cell spheroid and extracellular fluid is employed to calculate the cytoplasm conductivity $\sigma_{cyto}$ from the measurement of electrochemical impedance spectroscopy. The result shows that $\sigma_{cyto}$ is also decreased with the increase of GFPT percentage, which shows the same trend as the cytoplasm conductivity reconstructed by HSSM-EIT.

INDEX TERMS Cytoplasm conductivity, electrical impedance tomography (EIT), heterogeneous cell detection, high-frequency, second-order sensitivity.

I. INTRODUCTION

THE DETECTION of heterogeneous cells in cell spheroids is important in tissue engineering [1] and regenerative medicine [2]. Heterogeneous cells appear in tissues as a result of disease, abnormal differentiation, and drug stimulation, which are often accompanied by changes in the internal protein expression [3]. Proteins attract free ions in unlike charge, resulting in changes in the amount of free charge that affects intracellular conductivity, which provides a new solution for heterogeneous cell detection in cell spheroids [4].

In order to detect heterogeneous cells in cell spheroids, the fluorescent method is generally employed [5], [6]. The HT29 (colon cancer cell) spheroids were detected by measuring the...
expression of LGR5 (a marker of colon cancer cells) using immunofluorescent staining [7]. The colon cancer spheroids were detected by measuring the expression level of markers CD44 and MUC2 using immunofluorescent staining [8]. Although the fluorescent method accurately detects heterogeneous cells, it relies on the small molecule labeling technique which requires loading a certain amount of labeling molecules in cells. Cells loaded with labeling molecules are no longer suitable for culturing tissues and organs due to molecular contamination.

In order to address the shortcoming, electrical impedance spectroscopy (EIS) is widely applied for the detection of heterogeneous cells in cell spheroids as a noninvasive method [9], [10]. A novel method for spheroid detection based on two unique impedance characteristics at 60 kHz and 1 MHz measured by a planar sensor was proposed, which showed impedance successfully reflected the effects of anti-cancer drugs on spheroids and the increase of breast cancer (MCF-7) spheroid biomass [11]. A capillary system with electrodes to detect spheroids by impedance measurement was proposed, which showed changes in morphology, apoptosis, and necrosis were reflected by EIS [12]. EIS detects changes in the complex impedance of cell spheroids but lacks information to determine whether the changes in complex impedance are caused by position, diameter, shape, or composition. Therefore, an approach that measures multiple dimensions and provides more information is needed.

Recently, the electrical impedance tomography (EIT) method, a noninvasive method for constructing spatial distribution, has rapidly evolved into a broadly employed method for detecting heterogeneous cells in cell spheroids [13], [14]. A single miniature EIT sensor was applied to measure the 3-D cell culture viability in the 3-D printed scaffolds at the β-dispersion frequencies of the cell membrane, which are 10 and 100 kHz [15]. An imaging method of extracellular ion concentration distribution considering the ion transport characteristic was proposed to detect the cell spheroid type by combining the Cole-Cole model and the low-frequency EIT method [16], which were successful in detecting the viability and type of cell spheroid. However, these methods have some shortcomings which are: 1) due to the capacitive property of the cell membrane, the low-frequency injected current only passes through the extracellular region. The EIT method at a low frequency only detects the cell membrane and extracellular fluid and does not reflect the intracellular composition [17] and 2) the EIT method using the Jacobian matrix as the sensitivity matrix is unsuitable for reconstructing complex fields. A complex field consisting of the extracellular solution and cell spheroid due to the cell structure is required to be reconstructed at high-frequency measurement. The adoption of the Jacobian matrix simplifies the measured impedance and reconstructed conductivity to a linear relationship, which results in the conductivity distribution of complex fields cannot being accurately reconstructed [18]. In order to overcome the above-mentioned shortcomings, two approaches are applied: 1) applying the high-frequency injected current around 1 MHz that passes through the cell membrane to measure the intracellular field [19] and 2) applying the second-order sensitivity matrix EIT (SSM-EIT) to reconstruct the complex field for addressing the characterization of cell spheroid composition [18].

The objectives of this study are: 1) to apply the high-frequency SSM-EIT (HSSM-EIT) method to the detection of heterogeneous cells in cell spheroids; 2) to visualize the heterogeneity stage and measure the cytoplasm conductivity of cell spheroid by analyzing the reconstructed images by the HSSM-EIT method; and 3) to confirm the cytoplasm conductivity reconstructed by HSSM-EIT by calculating the cytoplasm conductivity from EIS measurement data using an equivalent circuit model.

II. HIGH-FREQUENCY SECOND-ORDER SENSITIVITY MATRIX EIT

The HSSM-EIT method is proposed by coupling the high-frequency (hf-EIT) and SSM-EIT to achieve the detection of heterogeneous cells in cell spheroids. The SSM-EIT refers to the study by Ding et al., which improved the reconstruction accuracy of complex fields [18].

A. SECOND-ORDER SENSITIVITY MATRIX EIT

Fig. 1(a) shows the relationship sketch between boundary voltage $U$ and conductivity distribution $\sigma$ by simulation. $\Delta \sigma_{\text{sim}}$ is the variation of conductivity, $\Delta U$ is the resulting boundary voltage response of $\Delta \sigma_{\text{sim}}$. $\Delta \sigma_{1\text{order}}$ is the reconstructed conductivity by the first-order sensitivity matrix, and $\Delta \sigma_{2\text{order}}$ is the reconstructed conductivity by the second-order sensitivity matrix. Compared with $\Delta \sigma_{1\text{order}}$ based on a linear relationship of $U$ and $\sigma$, $\Delta \sigma_{2\text{order}}$ reconstructs the nonlinear relationship between $U$ and $\sigma$ to a certain extent, where $\Delta \sigma_{2\text{order}}$ is closer to $\Delta \sigma_{\text{sim}}$.

The nonlinear relationship between boundary voltage $U$ and conductivity distribution $\sigma$ is expanded by Taylor’s formula as

$$U(\sigma) = U(\sigma_0) + \frac{\partial U(\sigma)}{\partial \sigma} \bigg|_{\sigma=\sigma_0} (\sigma - \sigma_0) + \frac{1}{2} \frac{\partial^2 U(\sigma)}{\partial \sigma^2} \bigg|_{\sigma=\sigma_0} (\sigma - \sigma_0)^2 + o((\sigma - \sigma_0)^2)$$

(1)

where $U \in \mathbb{R}^{m \times 1}$ is the measurement boundary voltage, $\sigma \in \mathbb{R}^{n \times 1}$ is the reconstructed target of conductivity distribution, and $\sigma_0$ is the reference conductivity distribution. In (1), the first-order term is the Jacobi matrix, and the second-order term is the Hessian matrix, where the Hessian matrix is described as

$$H(\sigma) = \frac{\partial^2 U}{\partial \sigma_i \partial \sigma_j} \bigg|_{\sigma=\sigma_0}$$

(2)

where $i$ and $j$ are the index numbers of matrix $H$, respectively. As the general approach, the first-order term (Jacobian...
Figure 1. HSSM-EIT. (a) Second-order sensitivity matrix. (b) \( \beta \)-dispersion and high-frequency EIT.

The second-order term (Hessian matrix) is adopted as the sensitivity matrix to simplify (1) into a linear form as follows:

\[
\Delta U = J \Delta \sigma \quad (3)
\]

where \( \Delta \sigma \) is the difference between the reference and target conductivity, \( J \) is the Jacobian matrix, and \( \Delta U \) is the boundary voltage reaction of \( \Delta \sigma \). The EIT algorithm is simplified by (3), which ignores important information about the second-order term. By adding the second-order term (Hessian matrix) as shown in (2) to the sensitivity matrix, (1) is modified to

\[
\Delta U_k = J_k \Delta \sigma + \frac{1}{2} \Delta \sigma^T H_k \Delta \sigma, \quad k = 1, 2, \ldots, m \quad (4)
\]

where \( J_k \) is the \( k \)th row of \( J \). \( H_k \) is described as

\[
H_{k-ij} = \frac{\partial^2 U_k}{\partial \sigma_i \partial \sigma_j}, \quad k = 1, 2, \ldots, m, \quad i = 1, 2, \ldots, n, \quad j = 1, 2, \ldots, n \quad (5)
\]

where \( k \) is the number of boundary measurements; \( i \) and \( j \) are the row and column of matrix \( H \), respectively. To shorten the computational time, a diagonal matrix termed \( D_k \) constructed by the diagonal elements in \( H_k \) is used instead of \( H_k \). Equation (4) is expressed as

\[
\Delta U_k = J_k \Delta \sigma + \frac{1}{2} \Delta \sigma^T D_k \Delta \sigma = T_k \Delta \sigma, \quad k = 1, 2, \ldots, m \quad (6)
\]

where \( T_k \) is the new sensitivity matrix containing the first-order and second-order terms.

Transforming (6) according to the regularization algorithm, \( \Delta \sigma \) is described as

\[
\Delta \sigma = \left( T^T T + \lambda R \right)^{-1} T^T U \quad (7)
\]

where \( \lambda \) is the hyperparameter, and \( R \) is the regularization matrix by the Tikhonov prior [20]. \( \Delta \sigma \) is reconstructed by iteration as

\[
\Delta \sigma^{i+1} = \Delta \sigma^i + \left( T^T T + \lambda R \right)^{-1} T^T \left( T \Delta \sigma^i - U \right) \quad (8)
\]

where \( i \) is the number of iteration times. Specific calculation processes and details are published by Ding et al. [18].

B. HIGH-FREQUENCY EIT

In the EIT measurement, a high-frequency current of 1 MHz is injected to measure the boundary voltage which reconstructs the spheroid conductivity. Fig. 1(b-1) shows the

- Capacitive property of cell membrane
  - Low-frequency EIT
  - High-frequency EIT

- Objective: Extracellular conductivity
- Objective: Intracellular conductivity

- Frequency range of \( \alpha \)-dispersion
- Frequency range of \( \beta \)-dispersion
- Frequency range of \( \gamma \)-dispersion

- Frequency of cell membrane interface polarization
- Frequency for intracellular part

- Frequency range of \( \alpha \)-dispersion
- Frequency range of \( \beta \)-dispersion
- Frequency range of \( \gamma \)-dispersion

- EDL of electrode
- Extracellular fluid
- Cellular organization
- Macromolecular
- Small molecules
representation of \( \alpha \), \( \beta \), and \( \gamma \) dispersions, where \( f_\alpha \), \( f_\beta \), and \( f_\gamma \) are the frequency of \( \alpha \)-dispersion, \( \beta \)-dispersion, and \( \gamma \)-dispersion; and \( \Delta f_\alpha \), \( \Delta f_\beta \), and \( \Delta f_\gamma \) are the frequency range of \( \alpha \)-dispersion, \( \beta \)-dispersion, and \( \gamma \)-dispersion [21]. Polarization reasons in the complex permittivity of biological cells are generally considered to be caused by \( \alpha \)-dispersion due to electrode polarization, \( \beta \)-dispersion due to the interfacial polarization of cell membrane, and \( \gamma \)-dispersion due to the reorientation of small molecules [17]. Thus, the measurement targets from low to high frequencies are electric double layer (EDL) of the electrodes [22], ion diffusion in extracellular fluid, cellular organization, macromolecular rotation, and small molecule polarization, respectively [17]. In previous studies, the injected current’s frequency was set as 10 kHz to detect the outer surface of the cell spheroid but was slightly inadequate for detecting the intracellular field [23], [24]. Fig. 1(b-2) shows the capacitive property of the cell membrane, where the low-frequency current passes through the liquid and the high-frequency current passes through the liquid and cell [25]. In order to detect the internal composition of the cell spheroid, a high-frequency current capable of passing through the cell membranes is employed.

III. MATERIALS AND METHODS

A. CELL-SPHEROID CULTURE

Two types of MRC-5 Human Lung Fibroblast Cell Line (MRC-5 SV1TG1, RIKEN Cell Bank, Tsukuba, Japan) of wild type (WT) and green fluorescent protein type (GFPT) were measured in this study, where WT is the normal cell and GFPT mimics the heterogeneous cell. Fig. 2(a) shows the schematics of two types. Compared to WT, GFPT expressed green fluorescent proteins in the cytoplasm [26]. As mentioned before, heterogeneous cells appear accompanied by changes in the internal protein expression due to disease, abnormal differentiation, and drug stimulation [4]. Therefore, GFPT containing green fluorescent proteins mimics the heterogeneity of the cell spheroid.

WT and GFPT were cultured in a 96-well microplate (Microplate Round Bottom 96 Well with Lid—IWAKI, AGC, Japan) to produce the cell spheroid. The spheroids with a diameter of approximately 400 \( \mu \)m were produced by adding a total of approximately 6400 cells and 200-\( \mu \)l Dulbecco’s modified eagle medium [(DMEM (1X), Gibco, Japan)] in each well of the microplate, where DMEM is a basal medium widely used to support the growth of a wide variety of mammalian cells. The five composition percentages of WT and GFPT for culturing the cell spheroid were 100/0%, 75/25%, 50/50%, 25/75%, and 0/100%, which simulate the heterogeneity of cell spheroids. After approximately 24 h of culture, the cell spheroids were available for experiments. Fig. 2(b) shows the cell spheroids under five compositions.

B. EXPERIMENTAL SETUP

Fig. 3 shows the experimental setup composed of an EIT sensor with eight electrodes arranged in a circular array of 2-mm diameter, a multiplexer for switching injection and measurement electrode combinations, an impedance analyzer (IM3570, HIOKI, Japan) with a frequency range of 4 Hz–5 MHz for measuring the complex impedance, a triaxial micromanipulator (MM-3, NARISHGE, Japan) for fine-tuning the position of the microtube, a microscope (Main body: VH-5500, Lens: VH-Z20R, KEYENCE, Japan), a PFA microtube (1-4423-02, ASONE, Japan) with a size...
of 0.2 × 0.4 mm for fixing the cell spheroid, and a PC for controlling the entire measurement system and recording the data. The EIT sensor was developed by printing eight electrodes and eight electric lines on the circuit board, and specific design parameters, materials, and other details are shown in our previous study [16].

C. EXPERIMENTAL METHOD AND CONDITIONS

The cell spheroid was removed from the microplate containing DMEM and then placed on the end of the microtube under microscope with a micropipette. Next, the cell spheroid was moved and fixed in the EIT sensor covered with sucrose solution by adjusting the micromanipulator. After that, the measurement was started, and data was recorded by PC. Table 1 shows the experimental conditions. The cell spheroid was composed of two types of MRC-5 cells: 1) WT and 2) GFPT. The composition percentage of WT and GFPT in five compositions of the cell spheroid was 100%–0%, 75%–25%, 50%–50%, 25%–75%, and 0%–100%. The measurement frequencies were 0.05, 0.1, 0.5, 1, and 2 MHz, where high-frequency data was for EIT imaging, and multifrequency data was for fitting the equivalent circuit. The injected current was 0.01 mA, and the total measurement time was 60 s. The EIT measurement was performed by the adjacent method, whose electrode pair number was 28 [27]. With the measurement at five frequency points and 28 electrode pairs, one image was obtained every 4 s. Since the EIT images were saved as 256 × 256 pixel and the EIT sensor size was 2 mm, the spatial resolution was 7.8125 μm/px.

D. IMAGE RECONSTRUCTION

In the inverse problem, the conductivity \( g \) was calculated by resistance \( R_s \) measured at 1 MHz as

\[
g^{k+1} = g^k + (T^T T + \lambda R)^{-1} T^T \Delta R_s \tag{9}
\]

where \( \lambda \) is the hyperparameter, and \( R \) is the regularization matrix by Tikhonov regularization [20]. \( k \) is the number of iteration times in the Gauss–Newton iterative method supported by the EIDORS software (ver. 3.10) to solve (9) by iteration [28]. \( \Delta R_s \) is normalized resistance as

\[
\Delta R_s = \frac{R_{si} - R_{rs}}{R_{s\text{max}}} \tag{10}
\]

of 0.2 × 0.4 mm for fixing the cell spheroid, and a PC for controlling the entire measurement system and recording the data. The EIT sensor was developed by printing eight electrodes and eight electric lines on the circuit board, and specific design parameters, materials, and other details are shown in our previous study [16].

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\[
\Delta R_s = \frac{R_{si} - R_{rs}}{R_{s\text{max}}} \tag{10}
\]
of compositions 1–3 changed significantly, and compositions 3–5 changed slightly.

Fig. 5 shows the image reconstruction of five compositions at 1 MHz. Fig. 5(a) shows the image reconstructed by HSSM-EIT. The region with significantly higher conductivity than the background was the cell spheroid. The conductivity of the cell spheroid region was decreased with the increasing GFPT percentage. Fig. 5(b) shows the image reconstructed by the general first-order sensitivity matrix EIT (FSM-EIT). The images showed artifacts close to the electrodes, and the conductivity of cell spheroids was not prominent enough compared to the artifacts. Comparing Fig. 5(a) and (b), the adoption of HSSM-EIT improves the image reconstruction accuracy in cell spheroid detection. Fig. 5(c) shows the measurement of cytoplasm conductivity.

$$\sigma_{sp}$$ was the total conductivity of cell spheroid in the red region, and $$\sigma_{all}$$ was the total conductivity of the sensor area in the yellow region. $$\sigma_{sp}$$ and $$\sigma_{all}$$ were measured by ImageJ software which is widely used in image processing [30]. Fig. 5(d) shows the evaluation of HSSM-EIT by calculating the conductivity ratio $$\sigma_R$$, where $$\sigma_R = \sigma_{sp}/\sigma_{all}$$. The larger the value of $$\sigma_R$$, the less the interference, that is, the more accurate the reconstruction result of the target of interest. $$\sigma_R$$ (HSSM-EIT) is higher than $$\sigma_R$$ (FSM-EIT) in all spheroid compositions, which shows the advancement of the HSSM-EIT method. Fig. 5(e-1) shows the Feret diameter of cell spheroids measured by ImageJ software, where the Feret diameter is widely used to evaluate the cell diameter [31]. Fig. 5(e-2) shows the Feret diameter $$d$$ of cell spheroids measured from the images by FSM-EIT, microscope, and HSSM-EIT. Compared with $$d$$ (FSM-EIT), $$d$$ (HSSM-EIT) was closer to $$d$$ (Microscope), which also indicates the advancement of the HSSM-EIT method.

Fig. 5(f) showed the average conductivity of cell spheroid $$\tilde{\sigma}_{sp}$$ measured by ImageJ software, where $$\tilde{\sigma}_{sp}$$ was the average conductivity in the red region reconstructed by HSSM-EIT. The results indicated that the cytoplasm conductivity was decreased with the increasing GFPT percentage.
The cytoplasm conductivity of compositions 1–3 was changed obviously, and that of compositions 3–5 was changed slightly.

V. DISCUSSION

A. APPLICABILITY OF HSSM-EIT IN THE DETECTION OF HETEROGENEOUS CELLS IN CELL SPHEROIDS

MRC-5 cells are widely used in cell detection and microfluidic research [32], [33]. In this study, two types of MRC-5 cells are measured, where WT stands for normal cells, and GFPT mimics heterogeneous cells. The cell spheroids with different compositions mimic the heterogeneity stage. HSSM-EIT successfully detects heterogeneous cells in cell spheroids by measuring the cytoplasm conductivity, which demonstrates applicability in detecting cells with changes in conductivity due to changes in composition, such as detecting the tumor cell content in tissue.

B. DECREASE OF CYTOPLASM CONDUCTIVITY OF CELL SPHEROID WITH INCREASING GFPT PERCENTAGE

In this study, the decrease in cytoplasm conductivity of the cell spheroid with the increasing GFPT percentage is due to the presence of green fluorescent protein in the cytoplasm of GFPT compared to WT.

1) Since the resistance of a single GFPT is greater than that of a single WT [34], the resistance of cell spheroids is increased and the conductivity is decreased with the increase of GFPT percentage.

2) Since the green fluorescent protein in the cytoplasm of MRC-5 is negatively charged, the most abundant potassium ions are bound around the green fluorescent protein (Intracellular: Na\(^+\) 4 mM, K\(^+\) 135 mM, Ca\(^2+\) \(80 \times 10^{-6}\) mM, H\(^+\) \(10^{-4.1}\) mM [35]). The permittivity is increased due to the increase in bound charge. The conductivity is decreased due to the decrease in free charge [26].

C. HIGH FREQUENCY CURRENT IN CELL SPHEROID DETECTION

The high-frequency current of 1 MHz was applied to measure the cytoplasm conductivity. Since the \(\beta\)-dispersion frequency is sensitive to the cell membrane, measuring the intracellular part requires the current above the dispersion frequency to pass through the cell membrane [36]. Fig. 6 shows the spectral data of composition 2 measured by electrodes 1–5. Fig. 6 (a) shows the relationship between phase \(\theta\) and frequency \(f\), where the \(x\)-axis was \(f\), and the \(y\)-axis was \(\theta\). The curve indicated the presence of a peak at 0.5 MHz, which is considered the \(\beta\)-dispersion of the experimental sample due to interfacial polarization of the cell membrane. Therefore, in this experimental condition, the current at 1 MHz was applied to detect the intracellular conductivity. Fig. 6(b) shows the curve of resistance \(R_s\), where the \(x\)-axis was \(f\), and the \(y\)-axis was \(R_s\). The curve indicated that \(R_s\) tended to stabilize over 0.5 MHz, which indicated that the current had crossed the cell membrane and the intracellular fraction had been fully involved in the impedance contribution.

D. CONFIRMATION OF HSSM-EIT

By HSSM-EIT, we knew the cytoplasm conductivity was decreased with the increasing GFPT percentage. To confirm the cytoplasm conductivity reconstructed by HSSM-EIT, the EIS measurement data by electrode pair 1–5 was also used to calculate the cytoplasm conductivity using an equivalent circuit model.

Fig. 7(a) shows the equivalent circuit model containing cell spheroid and extracellular fluid, where \(C_M\) was the cell membrane capacitance, \(R_{cyto}\) was the cytoplasm resistance, and \(R_s\) was the extracellular fluid resistance. The model, whose cytoplasm and cell membrane were series connection and the cell spheroid and extracellular fluid are parallel connection, had been widely applied to cell spheroid and cell suspensions [37]. \(R_{cyto}\) was calculated by fitting the equivalent circuit model with the measured complex impedance by ZView software, where the complex impedance \(Z^*\) was described by

\[
Z^* = \frac{1}{\frac{1}{\omega C_M + R_{cyto}} + \frac{1}{R_s}} = \frac{(\omega C_M R_{cyto} - 2j)R_{cyto}}{\omega C_M R_{cyto} + \omega C_M R_s - 2j}.
\]

(11)

After calculating \(R_{cyto}\), cytoplasm conductivity \(\sigma_{cyto}\) was calculated as follows:

\[
\sigma_{cyto} = \frac{G_d}{S}
\]

(12)
Fig. 7(b) shows the fitting results of composition 2, where the black dots were fitting data, and the orange dots were experimental data. $R^2$ was the determination coefficient, which was 0.96. Fig. 7(c) shows calculated $R_{cyto}$ and $\sigma_{cyto}$. $R_{cyto}$ was increased with the increasing GFPT percentage. Conversely, $\sigma_{cyto}$ was decreased with the increasing GFPT percentage, which showed the same trend of cytoplasm conductivity reconstructed by HSSM-EIT.

In this study, the EIS and HSSM-EIT methods both successfully detected a decrease in cytoplasm conductivity with the increasing GFPT percentage. However, in contrast to EIS, HSSM-EIT also detects the size, shape, and location of cell spheroids, as shown in Fig. 5, which is an advantage that EIS does not have.

VI. CONCLUSION
This study proposed an HSSM-EIT method to detect heterogeneous cells in cell spheroids. The key findings of this study are as follows.

1) The HSSM-EIT method was proposed by coupling the high-frequency and SSM-EIT to detect heterogeneous cells.
2) The heterogeneity stage of cell spheroids was successfully visualized by the HSSM-EIT method. The cytoplasm conductivity of the cell spheroid was decreased with the increasing GFPT percentage.
3) The equivalent circuit model confirmed the cytoplasm reconstructed by the HSSM-EIT method by calculating the cytoplasm conductivity $\sigma_{cyto}$ which showed the same trend of cytoplasm conductivity reconstructed by HSSM-EIT.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Songshi Li: Funding acquisition, Methodology, Total planning, Experiment, Validation, Manuscript writing, and editing. Daisuke Kawashima: Funding acquisition, Supervision, Manuscript review, and Project administration. Zengfeng Gao: Algorithm support, program support. Masahiro Takei: Funding acquisition, Supervision, Manuscript review, and Project administration.

DECLARATION OF COMPETING INTEREST
There are no conflicts to declare.

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