An analytical formula for determining the electrical impedance between a single adherent cell and sensor substrate

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Received August 28, 2022; revised October 2, 2022; accepted October 6, 2022; published online October 31, 2022

1. Introduction

In recent years, a number of investigations have revealed that an understanding of living cell behaviors is particularly important for new drug development and early cancer detection. Therefore, living cell analyses have applications to drug discovery and disease diagnosis. The numerous methods for living cell analyses have been proposed in previous works. Raman spectroscopy, quantitative phase microscopy and flow cytometry are some of the optical measurements. However, these optical methods require the cells to be labeled for staining and the measurement systems are large. On the other hand, quartz crystal microbalance and surface acoustic wave sensors do not require cell labeling. These methods are non-invasive, and the measurement systems can be much smaller than the optical systems. However, they are not suitable to detect the changes in cell morphology or cell-to-cell junction quality. Electrochemical measurements have long been used to quantitatively analyze morphological changes of cells; these measurements have some advantages such as non-invasion, real-time, small measurement system, and no-labeling. There are three major electrochemical measurement methods. First, the potentiometry has the capability of measuring potential changes caused by oxidation-reduction reaction. The glucose sensor and pH sensor, which are used in cell analysis, employ this measurement method. In addition, this measurement method enables to measure changes in the action potential which neuron and muscle cells cause. Second, the amperometry is the way of measuring the electroactivity of oxidation-reduction reaction on the interface as electrical current. Some studies reported that the amperometry sensor can detect molecules released from cells. Third, the impedance measurement is a non-invasive way of detecting the changes in ionic current paths around cells, conductivity of cytoplasm, and cell membrane permittivity. Previous studies showed that the impedance measurement has the capability to detect morphological change, evaluation of toxicity, cell junction, cell adhesion, cell proliferation, apoptosis, and necrosis. In particular, electrochemical impedance spectroscopy (EIS), which is the impedance measurement by sweeping frequency, provides various information such as morphological and electrical cell changes simultaneously. Hence EIS is optimal for living cell analyses. In previous works, various electrode structures were proposed for electrochemical living cell analyses in the impedance measurement. Planar interdigitated electrode arrays are fabricated at low cost, and the theory and simulation methods are well-established. Parallel-facing electrodes are used in the flow cytometry for analyzing single cells, and this electrode structure has the capability of screening single cells with high throughput. Microelectrode arrays can identify where a target cell exists and map it at moderate resolution. In addition, the use of microelectrode arrays enables the whole measurement system downsizing through the application of complementary metal–oxide–semiconductor (CMOS) technology. As with parallel-facing electrodes, microelectrodes achieve high throughput screening by using CMOS technology. Besides, their sensitivity is in general higher than that of conventional electrode structures. Thus, microelectrodes are attracting considerable attention. As mentioned above, EIS measurement using microelectrodes has been expected to play important roles in living cell analyses. In EIS measurements, equivalent circuit model (ECM) is employed for quantitative analyses of various cell changes from the impedance change. An appropriate analytical formula for ECM is essential to obtain the parameters such as conductivity, permittivity, and cell-size by fitting experimental results. The analytical formula also provides insights in the most optimized design of the sensor geometry. In general, cell-to-cell interactions should affect original characteristics of cell populations and tissues. Thus, single-cell analysis, taking into account the heterogeneous behaviors of a single-cell will be appropriate. There are some studies using microelectrodes with their size, ranging from 1.0 to 8.0 μm sizes used for cell impedance mapping and some others reported theoretical analysis of single-cell behavior when using such electrodes with computer simulation. However, there is no report on the appropriate analytical formula for cell-substrate impedance with such small electrodes. As noted above, in general, appropriate
formulae are essential for quantitative analyses and the several parameters which describe properly the cell characteristics in living cell analyses. Giaever et al. proposed a formula for describing the impedance between an adherent cell and an electrode.\(^4\)\(^4\) Nevertheless, it was assumed that the electrode was large; and this formula is inapplicable when the electrode is smaller than the cell.

In this work, we derived an analytical formula for cell-substrate impedance when the electrode is smaller than the cell. The accuracy of the formula was verified through two numerical simulations. We swept the electrode radius in the first simulation and the height between cell and substrate in the second simulation. From the first simulation, we observed a negligible error between the theoretical value from analytic formula and numerical simulation result. The second simulation showed that our formula well-described the cell-substrate impedance when the cell adheres to the substrate. Thus, our formula can describe cell-substrate impedance when the electrode is smaller than the cell. It will be useful for the experimentalists in identifying cell behaviors and designing optimized sensor structures.

2. Theory

2.1. Giaever’s theory

Giaever et al. proposed a formula for describing the impedance between an adherent cell and electrode as shown in Fig. 1, where \(z\) and \(r\) are the height and radius, respectively. Note that this formula was derived under the assumption that the electrode was sufficiently larger than the cell, and that there are many cells on the electrode. Assuming that the potential drop in the cell-substrate gap on the vertical \(z\)-direction can be neglected, the electric potential in the cell can be given as the solution to the following differential equation\(^4\)

\[
\frac{d^2V(r)}{dr^2} + \frac{1}{r} \frac{dV(r)}{dr} - \gamma^2 V(r) + \frac{1}{h \sigma_{sol}} \left( \frac{V_n}{Z_n} + \frac{V_m}{Z_m} \right) = 0.
\]

(1)

The working electrode (WE) has a constant electric potential \(V_n\) [V], and the potential above the cell is set to be \(V_m\) [V]. The potential at the edge of the cell is given as the multiplication of current through this boundary and the specific resistance \(R_s\) \([\Omega \cdot m^2]\) which represents the resistance between cells per unit area. The \(\sigma_{sol}\) \([S \cdot m^{-1}]\) indicates the conductivity of the solution, and \(h\) [m] is the height of the cell-substrate gap. The \(Z_n\) \([\Omega \cdot m^2]\) is the specific impedance due to the double-electrode on the electrode, as described by the following equation

\[
Z_n = \frac{1}{j \omega \varepsilon_{sol} \lambda_0}.
\]

(2)

where \(j\) and \(\omega\) \([s^{-1}]\) are the imaginary unit and angular frequency, respectively, and \(\varepsilon_{sol}\) \([F \cdot m^{-2}]\) is the electrical double-layer capacitance per unit area, given by

\[
\varepsilon_{sol} = \frac{\varepsilon_0 \varepsilon_r}{\lambda_0}.
\]

(3)

Here, \(\varepsilon_0\) \([F \cdot m^{-1}]\), \(\varepsilon_r\), and \(\lambda_0\) [m] are the permittivity of the vacuum, relative permittivity of the medium solution, and the Debye length, respectively. The \(Z_m\) \([\Omega \cdot m^2]\) is the specific impedance caused by a capacitance \(Z_m\) \([F \cdot m^{-2}]\) through the cell, which consists mainly of two series capacitances due to the upper cell membrane \(C_{mem,upper}\) \([F \cdot m^{-2}]\) and bottom membrane \(C_{mem,bottom}\) \([F \cdot m^{-2}]\), given by

\[
C_m = \left( C_{mem,upper} + C_{mem,bottom} \right)^{-1} \approx \frac{C_{mem}}{2},
\]

(4)

where \(C_{mem}\) \([F \cdot m^{-2}]\) is the capacitance of a cell membrane per unit area, given by

\[
C_{mem} = \frac{\varepsilon_0 \varepsilon_{mem}}{d_{mem}}.
\]

(5)

Here, \(\varepsilon_{mem}\) and \(d_{mem}\) [m] are the relative permittivity and thickness of the cell membrane, respectively. Using Eq. (4), the specific impedance through the cell is written as

\[
Z_m = \frac{1}{j \omega C_m}.
\]

(6)

Finally, the parameter \(\gamma\) \([m^{-1}]\) in Eq. (1) is defined as

\[
\gamma = \sqrt{\frac{1}{h \sigma_{sol}} \left( \frac{1}{Z_n} + \frac{1}{Z_m} \right)}.
\]

(7)

Assuming that electric potential drop occurs mainly under the cell, \(V_m\) can be set to zero. Calculating the electric current from the obtained potential \(V(r)\), the following equation for the impedance \(Z_{Giaever}\) \([\Omega \cdot m^2]\) will be obtained:

\[
Z_{Giaever}^{-1} = \frac{1}{Z_m} \left( \frac{Z_n}{Z_n+Z_s} + \frac{Z_m}{Z_m+Z_s} \cdot \frac{k_i}{2} \cdot \frac{R_s}{h \sigma_{sol}} \right) + \frac{1}{Z_n+Z_s}.
\]

(8)

where the \(I_i(x)\) is a modified Bessel function of the first kind of order \(n\) with non-dimensional variable \(x\), and \(R_s\) [m] indicates the radius of the cell (the corresponding equation in Ref. 44 has been corrected in Ref. 45). If only a single-cell is on the electrode, \(R_s\) becomes zero because it is associated with intercellular resistance between many cells.\(^4\)

2.2. Formula describing cell-substrate impedance when using a microelectrode

Figure 2 shows schematics of a situation where an electrode smaller than the cell is used for single-cell analysis. In

\[\text{Fig. 1. (Color online) Schematic image of the Giaever's model. WE is the working electrode, and } t_{c} \text{ is the cell radius.}\]
Fig. 2. (Color online) (a) Schematic image of single-cell analysis using a microelectrode smaller than the cell. A indicates the area between the cell and the electrode, and B indicates the area under the cell except in region A. (b) Relationship between the potential and the current over an infinitesimal distance \( dr \) of region A in (a). (c) Relationship between the potential and the current over an infinitesimal distance \( dr \) of region B in (a).

Fig. 2(a), the cell-substrate gap is divided into two regions A and B, and the formula is derived from the relationship between the potential and current over an infinitesimal distance in each region. The potential at the WE, electrolyte above the cell, and electrolyte near the cell-edge are given by \( V_e \), \( V_c \), and \( V_s \), respectively. Figures 2(b) and 2(c) represent the relationships between potentials and currents in regions A and B in Fig. 2(a), respectively. For simplicity, we assume that the potential in cell-substrate gap depends only on \( r \), and neglects the potential drop along the \( z \)-axis, as has been done by Giaever et al. as well. This is acceptable because the distance between the cell and substrate is sufficiently small. In region A in Fig. 2(a), the relationship between the potential and the current is expressed by Giaever’s model as follows\(^{44}\):

\[
-dV_A = I_A \frac{1}{2 \pi r \sigma_{na}} dr,
\]

\[
V_c - V_A = \frac{Z_m}{2 \pi r dr} dI_e,
\]

\[
V_A - V_e = \frac{Z_m}{2 \pi r dr} dI_e,
\]

\[
dI_A = dI_e - dI_c,
\]

where \( V_A(r) \) [V] and \( I_A(r) \) [A] are the potential and the current at the radial position \( r \) as shown in Fig. 2(b). The \( I_e(r) \) [A] and \( I_c(r) \) [A] are the total currents flowing out of the WE and through the cell, respectively. From Eqs. (9)–(12), we obtain the differential equation for \( V_A(r) \)

\[
\frac{dV_A}{dr} + \frac{1}{r} \frac{dV_A}{dr} = \frac{1}{\eta_{na}} \left( \frac{1}{Z_n} + \frac{1}{Z_m} \right) V_A
\]

\[
+ \frac{1}{\eta_{na}} \left( \frac{V_e}{Z_n} + \frac{V_c}{Z_m} \right) = 0.
\]

The general solution to Eq. (13) is given by

\[
V_A(r) = C_A I_0(\gamma_A r) + D_A K_0(\gamma_A r) + \frac{1}{Z_n + Z_m} (Z_m V_e + Z_n V_c),
\]

where \( K_n(x) \) is the modified Bessel function of the second kind of order \( n \), with a non-dimensional variable \( x \). The \( \gamma_A \) [m\(^{-1}\)] is identical to \( \gamma \) in Eq. (7), and the \( C_A \) and \( D_A \) are the constants of integration determined from the boundary condition. As \( K_0(\gamma_A r) \) tends to infinity as \( r \) tends to zero, \( D_A \) must be set to zero, giving

\[
V_A(r) = C_A I_0(\gamma_A r) + \frac{1}{Z_n + Z_m} (Z_m V_e + Z_n V_c).
\]

In region B in Fig. 2(a), the bottom of the region is not the WE, but the non-conducting substrate as shown in Fig. 2(c), i.e. \( dV_e = 0 \). Therefore, the differential equation and its solution for the potential in region B can be obtained by setting the \( Z_n \) to infinity in the Eq. (14). The potential then becomes

\[
V_B(r) = C_B I_0(\gamma_B r) + D_B K_0(\gamma_B r) + V_c,
\]

where \( V_B(r) \) [V] indicates the potential at the point of the \( r \) coordinate in region B. Here, \( C_B \) and \( D_B \) are the constants of integration, and the \( \gamma_B \) [m\(^{-1}\)] is defined as

\[
\gamma_B = \frac{1}{h \sigma_{na} Z_m}.
\]

We set three boundary conditions to determine the coefficients which are \( C_A, C_B \), and \( D_B \). First, we defined \( r_e \) [m] and \( r_c \) [m] as the WE radius and cell radius, respectively. The potential and current must be continuous at the boundary between the regions A and B \( (r = r_c) \) in Fig. 2(a), i.e. \( V_A(r_c) = V_B(r_c) \) and \( I_A(r_c) = I_B(r_c) \). Hence, we obtain the following equations
Next, the potential at the edge of the cell \((r = r_e)\) is set to be \(V_e\), i.e. \(V_b(r_e) = V_e\). Therefore, we obtain the following equation
\[
C_A \frac{d}{dr} \left[ \frac{V_e}{r} \right] - \frac{V_e}{r_{i0}} = \frac{Z_m(V_e - V_c)}{Z_n + Z_m} = 0. \tag{18}
\]
\[
C_A \frac{d}{dr} \left[ \frac{V_e}{r} \right] - \frac{V_e}{r_{i0}} = \frac{Z_m(V_e - V_c)}{Z_n + Z_m} = 0. \tag{19}
\]

We now introduce a coefficient matrix to solve simultaneous equations Eqs. (18)–(20) for \(C_A\), \(C_B\), and \(D_B\)
\[
A \left[ \begin{array}{c} C_A \\ C_B \\ D_B \end{array} \right] = \left[ \begin{array}{c} (1 - k^2)(V_e - V_c) \\ 0 \\ V_a - V_c \end{array} \right], \tag{21}
\]
where \(A\) is the following coefficient matrix
\[
A = \left[ \begin{array}{ccc} I_0(\gamma_A r_e) & -I_0(\gamma_B r_e) & -K_0(\gamma_B r_e) \\ I_1(\gamma_A r_e) & -K_1(\gamma_B r_e) & kK_0(\gamma_B r_e) \\ 0 & I_0(\gamma_B r_e) & K_0(\gamma_B r_e) \end{array} \right]. \tag{22}
\]

and \(K\) is defined as
\[
k = \gamma_B \frac{Z_n}{Z_a} = \frac{C_m}{C_{di0} + C_m}. \tag{23}
\]

Note that \(k\) does not depend on frequency and is constant within the range of \(0 < k < 1\). The inverse of \(A\) is given by
\[
A^{-1} = \left[ \frac{A_1 \ A_2 \ A_3}{A_1 \ A_2 \ A_3} \right] = \left[ \begin{array}{ccc} \Delta \ A_1 & \ A_2 & \ A_3 \\ \ A_1 & \ A_2 & \ A_3 \\ \ A_1 & \ A_2 & \ A_3 \end{array} \right]. \tag{24}
\]

Thus, the cell-substrate impedance for a unit area is described by the following equation
\[
Z_e = \frac{V_e - V_c}{I_e}, \tag{38}
\]
where \(Z_e\) \([\Omega]\) is the impedance between the cell and substrate. The \(I_e\) is derived via integration of \(dI_e\) in Eq. (10) with respect to \(r\) as follows
\[
I_e = \int_0^r \frac{2\pi r}{Z_n} \int_0^r r(V_e - V_A(r)) dr. \tag{39}
\]

and \(C_A\) is given by Eq. (35) with \(V_e = V_c\).}

2.3. Comparison with Giaever’s formula

The derived formula Eq. (40) must be the same as Giaever’s formula Eq. (8) with \(R_0 = 0\) (corresponding to a single-cell situation) when \(r_c = r_e\). Taking the limit of \(C_A\) as \(r_c \to r_e\)
\[
\lim_{r_c \to r_e} C_A = \frac{1}{I_0(\gamma_A r_e)}(V_e - V_c)(1 - k^2)
\]
\[
= \frac{1}{I_0(\gamma_B r_e)} \frac{Z_m}{Z_n + Z_m}(V_e - V_c). \tag{41}
\]

Thus, \(Z_e\) for a unit area equals to that given by Giaever’s formula in the limit \(r_c \to r_e\), as expected.

3. Simulation

We performed two numerical simulations to verify the accuracy of our formula by using the model shown in Fig. 3. In this simulation model, actual thickness of the cell-substrate gap (denoted as \(h\) in Fig. 3) is considered as a realistic representation of actual situation. As the potential drop along the \(z\)-axis is neglected in our proposed formula in Sect. 2, comparison with the numerical simulation might reveal the validity and limitations of our formula, which will be discussed later. The parameters used in the simulation are listed in Table. I. In this simulation, the total cell capacitance \(C_m\) in Eq. (4) is set to be that of a cell membrane capacitance \(C_{mem0}\). This does not cause any loss of generality in the
was applied to the WE without DC bias.

stepped by 5 V, and 1m1 and m 2.

Double-layer capacitance per unit area46)

| Parameter                          | Unit       | Value  |
|-----------------------------------|------------|--------|
| Double-layer capacitance per unit area46) | F m⁻³      | 0.89   |
| Solution relative permittivity47)  |            | 78     |
| Solution conductivity49)          | S m⁻¹      | 1.5    |
| Cell membrane thickness46)        | nm         | 5.0    |
| Cell membrane relative permittivity46) |           | 5.0    |
| Cell membrane conductivity48)     | S m⁻¹      | 1.0 x 10⁻⁹ |

The following results and discussions, and in fact it can be a reasonable approximation that the electrical double-layer capacitance is regarded as sufficiently larger than that of the bottom capacitance, which is sometimes the case when the whole-cell has a hemispherical shape as shown in Fig. 2. The simulation was performed in axisymmetric 2D to take advantage of the geometrical cylindrical symmetry of the geometry. First, time-dependent simulations were performed while a sinusoidal electric potential of amplitude 5.0 mV was applied to the WE without DC bias. Without any loss of generality, the Vc and Vs were set to zero potential. The frequency was swept from 10² to 10⁷ Hz in logarithmic steps. Second, we calculated impedances from the ratios of the complex representations of the input voltages to those of the simulated currents. In addition, the frequency characteristics of the impedance magnitudes and phases were calculated via the discrete Fourier transform and are shown in a Bode plot. In the following two simulations, r f was set to 50 µm and an insulation boundary condition was imposed on the substrate. In the first simulation, we swept r f from 1.0 to 4.0 µm stepped by 1.0 µm with h fixed at 100 nm. In the second simulation, we swept h from 100 to 500 nm stepped by 50 nm with r f fixed at 1.0 µm. The numerical simulations were performed using COMSOL Multiphysics 5.6, where the differential forms of Maxwell’s equations are solved in this software.

4. Results and discussion

Figure 4 shows a Bode plot of the simulated impedance fixed at h = 100 nm (dots) and the theoretical impedance calculated using Z c (solid lines). In Fig. 4, the formula describes well the simulated impedance over a wide range of frequencies, regardless of the value of r f.

Figure 4 shows that the electrical double-layer capacitance at the interface between the electrode and solution is dominant from 10² to 10³ Hz because the phase difference starts with -90°. Assuming that the non-dimensional variable x satisfies x ≪ 1, the modified Bessel functions of the first and the second order are approximated by the following equations

\[ I_0(x) \simeq \frac{1}{\sqrt{2\pi x}} \exp(x), \]

\[ K_0(x) \simeq -\ln\frac{x}{2} - C_e, \]

where \( C_e \) is the Euler’s constant. At a sufficiently low frequency, the \( \gamma_A r_f \ll 1 \) and \( \gamma_B r_f \ll 1, \Delta, C_A, \) and \( Z_c^{-1} \) can thus be approximated using following equations:

\[ \Delta \simeq -k \frac{1}{\gamma_B r_f}, \]

\[ C_A \simeq -r_f(1 - k^2)(V_c - V_s)\gamma_B K_1(\gamma_B r_f) \]

\[ = -(V_c - V_s)(1 - k^2) \]

\[ = -(V_c - V_s)\frac{Z_m}{Z_m + Z_n}, \]

\[ Z_c^{-1} \simeq \frac{2\pi r_f Z_m I_1(\gamma_A r_f)}{Z_n(Z_n + Z_m)\gamma_A} + \pi r_f^2 \frac{1}{Z_n + Z_m} \]

\[ = \pi r_f^2 \left( \frac{Z_m}{Z_n + Z_m} \right) \frac{I_1(\gamma_A r_f)}{Z_n + Z_n} + 1 \]

Finally, the impedance of the electrical double-layer capacitance is dominant at sufficiently low frequency, as seen in Fig. 4.

Above 10⁶ Hz, the phase difference becomes closer to -90° in Fig. 4, thus this frequency range is the region where the capacitance is becoming dominant. This capacitance consists of the electrical double-layer capacitance at the metal/solution interface and the capacitance of the cell membrane. In general, the impedance of the cell membrane (Z m) is much larger than that of the electrical double-layer capacitance (Z c). Therefore, Z m is dominant at a high frequency. Assuming that the non-dimensional variable x satisfies x ≫ 1, the modified Bessel functions of the first and second orders can be approximated by the following equations

\[ I_0(x) \simeq I_1(x) \simeq \frac{1}{\sqrt{2\pi x}} \exp(x), \]

\[ K_0(x) \simeq K_1(x) \simeq \frac{\pi}{\sqrt{2x}} \exp(-x). \]

From these approximations, at a sufficiently high frequency at which \( \gamma_A r_f \gg 1 \) and \( \gamma_B r_f \gg 1, \Delta, C_A, \) and \( Z_c^{-1} \) can be approximated using following equations:

![Fig. 3.](Image) The simulation model in cylindrical coordinate.
Hence, the sum of $Z_n$ and $Z_m$ is dominant at sufficiently high frequency.

In Fig. 4, at around $10^5$ Hz, the phase difference approaches $-20^\circ$. Thus, the distributed impedance which consists of the solution resistance and $Z_n$ is dominant. Moreover, the slope of $|Z|$ approaches 0 as $r_e$ decreases at about $10^5$ Hz. As the resistance is less dependent on the frequency, this is attributed to an increase in resistance. At around $10^5$ Hz, the electric current through $Z_m$ is negligible because it is sufficiently large. Therefore, region B consists of only solution resistance. As a consequence, the extension of region B with a decrease in $r_e$ increases the solution resistance.

In Fig. 4, errors between the simulated and theoretical impedances are not evident, because Fig. 4 is a log-log graph. Therefore, we evaluated the error rates defined by

$$\text{Error rate [\%]} = \left( \frac{|Z_{\text{Simulation}}| - |Z_{\text{Theory}}|}{|Z_{\text{Theory}}|} \right) \times 100. \quad (53)$$

Figure 5 shows the frequency characteristics of the error rates calculated using Eq. (53). In Fig. 5, all error rates are less than 1.0 % below $10^6$ Hz, regardless of the value $r_e$. Thus, the formula describes well the cell-substrate impedance. However, at around $10^6$ Hz, the error rate increases. This is attributed to the potential drop on the $z$-axis which the derived formula does not consider.

To examine the potential drop across the $z$-axis, the vertical electric field at the middle of the cell-substrate gap was monitored during the simulation because it is approximately proportional to the potential drop across the gap. As explained in Sect. 3, the time-dependent simulations are performed while a sinusoidal electric potential is applied to WE. During the simulation, the vertical electric field in the
cell-substrate gap changes both in time and radial coordinate \( r \) in a complex manner. Therefore, we took the maximum values at each coordinate \( r \) during the simulation period and plotted as a function of \( r \), which is equivalent to an “envelope function” of time-dependent vertical electric field. Such maximum vertical electric field curves are shown in Fig. 6 for (a) \( r_e = 1.0 \mu m \) and (b) \( r_e = 4.0 \mu m \) at two different frequencies. As can be seen, the maximum vertical electric field is larger at high frequency, which indicates that the potential drop on the \( z \)-axis at high frequency is larger than that at low frequency. Such potential drop might be the main source of the increasing error at high frequency range in Fig. 5.

Figure 7 represents the error rates between the impedances simulated at a fixed \( r_e = 1.0 \mu m \) and the theoretical impedance of Eq. (40). In Fig. 7, the error rates increase as \( h \) increases. As with the first simulation, this is attributed to the potential drop on the \( z \)-axis which the formula does not take into account. The results in Fig. 5 shows that the error rates between theoretical and simulated impedances are not more than 2.0 \%, thus the formula can describe appropriately cell-substrate impedance regardless of the WE radius. The results in Fig. 7 shows that the error caused by the potential drop on the \( z \)-axis increases as \( h \) increases, thus the formula is appropriate when \( h \) is sufficiently small. As the actual cell-to-substrate distance is from 50 to 100 nm when a cell adheres to an electrode, the error caused by the potential drop on the \( z \)-axis might be negligible in actual application of our formula.

It should be noted that the model used in this study is based on axisymmetric geometry, which is valid only when the microelectrode is exactly at the center of the cell. Of course, this assumption is not always valid in actual experimental setup. However, we confirmed that the resulting impedance is not largely changed if the electrode position change is within approximately 1.0 micrometer. Therefore, in experimental setups, cells can be cultured on a dense microelectrode array and one electrode near the center of the cell under test can be selected to be used in impedance measurement as proposed in a previous report.

![Fig. 5.](image1.png) (Color online) Error rate between the simulated impedance and theoretical impedances calculated by Eq. (40), as shown in Fig. 4.

![Fig. 6.](image2.png) (Color online) Maximum vertical electric field at the middle of cell-substrate gap with respect to the radial coordinate \( r \) for (a) \( r_e = 1.0 \mu m \) and (b) \( r_e = 4.0 \mu m \). Legends indicate frequencies.

![Fig. 7.](image3.png) (Color online) Error rate between the simulated and theoretical impedances calculated by Eq. (40) at a fixed \( r_e = 1.0 \mu m \). Legends indicate \( h \).
in-depth study on the impact of electrode position relative to the cell, as well as modeling of impedance under such realistic situation may be done in future.

5. Conclusion
We proposed a formula for describing cell-substrate impedance when the electrode is smaller than a single-cell. We performed two simulations to verify the accuracy of the formula. The first simulation revealed that our formula can appropriately describe cell-substrate impedance regardless of the electrode radius. The second simulation showed that when cell-substrate gap is large, the potential drop on the z-axis cannot be neglected, and our formula becomes inapplicable. However, for realistic cell-substrate gap, error rates are as small as 2.0%. Our formula would enable quantitative analysis of cell parameters when the electrode is smaller than the cell.

Acknowledgments
This work was supported by JSPS KAKENHI under Grant Number 19K04539.

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