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Imaging single bacterial cells with electro-optical impedance microscopy

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Abstract

Impedance measurements have been an important tool for biosensor applications, including protein detection, DNA quantification and cell study. We present here an electro-optical impedance microscope (EIM) based on the dependence of surface optical transmission on local surface charge density for single bacteria impedance imaging. We applied a potential modulation to bacteria placed on an indium tin oxide coated slide, and simultaneously recorded a sequence of transmitted microscopy images. By performing fast Fourier transform analysis on the image sequences, we obtained the DC component (signal at the 0 Hz) for cell morphology imaging, and the AC component (signal at the modulation frequency) for the mapping of cell impedance responses with sub-cellular resolution for the first time. Using this method, we have monitored the viability of *E. coli* bacterial cells under two different class of antibiotics treatment with low frequency potential modulation. The results showed that the impedance response is sensitive to the antibiotic that targets at the bacterial cell membrane as the membrane capacitance dominated at low frequency modulation. Heterogeneous responses to the antibiotic treatment were observed at single bacteria level. In addition to the high spatial resolution, EIM is label free and simple, and can be potentially used for the continuous mapping of single bacteria impedance changes under different conditions.

Keywords: single bacteria impedance, cell viability, potential modulation, label-free detection, optical imaging.

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Electrical impedance measures the electrical current response of a sample to an applied potential, which is a powerful label-free biosensing platform for various applications, including tissue, protein and cell studies¹⁻⁴. However, the traditional impedance detection methods are mainly based on the spectroscopic techniques, such as the electrochemical impedance spectroscopy, which measures the averaged impedance over the whole sensor area. At such, it lacks the image capability, making it hard to detect single cell response for the heterogeneous study. The newly developed plasmonic-based impedance microscopy can image the local impedance responses with submicron spatial resolution, and has been used for the study of different cellular processes⁵⁻⁷. However, the plasmonic-based imaging is based on evanescent illumination and the sensitivity exponentially decays as the objects away from the sensing surface, which is not suitable for whole cell impedance measurement. Therefore, a new platform for whole cell impedance mapping is needed.

One critical application of whole cell impedance imaging is the pathogenic bacteria study. Bacterial infection has become an emerging threat to public health, and the situation becomes worse with the rapid development of antibiotic resistance due to the widespread overuse/misuse of the broad-spectrum antibiotics⁸⁻¹⁰. Therefore, it is critical to develop a rapid method for pathogenic bacteria viability test and antibiotic mechanism study to provide better understanding of antibiotic resistance development. Current commercial technologies for rapid pathogenic bacterial detection are mainly genotypic based assays (e.g. multiplex PCR)¹¹⁻¹⁴, which are sensitive but require a series of sample preparation steps and use of primers and enzymes. The response of bacterial cells to the electrical field is an intrinsic feature related with the bacterial cell size and shape, bacteria internal structure, and the electric conductivity and permittivity of different bacterial cell components. Therefore, any differences and changes in the electric components of bacterial cells would have influence on their electrical responses, making the impedance analysis

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3 a good method for the study of pathogenic bacteria response to external stimuli, the determination
4 of bacteria viability, the study of working mechanism of the antibiotic, the identification of
5 different bacteria, and the separation of bacteria from other cells¹⁵⁻¹⁸.
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10 A variety of spectroscopic techniques have been developed for bacteria study, such as the
11 electrochemical impedance spectroscopy¹⁶, dielectrophoresis spectroscopy¹⁹, and electrorotation
12 spectroscopy²⁰. Electrochemical impedance spectroscopy (EIS) is the most used method, which
13 can detect the presence of bacteria with very high sensitivity²¹, and has been used for the detection
14 of foodborne pathogenic bacteria^{16, 22}. Dielectrophoresis spectroscopy is mostly used with the
15 microfluidic device for the separation of different cell component and for the trapping of target
16 bacterial cells²³⁻²⁵. Most of these studies measure averaged signal from a population of bacterial
17 cells, and not provide detailed viability responses (e.g. elongation, permeability change, or pore
18 formation) to the antibiotics, due to the lack of sufficient spatial resolution. Therefore, a capability
19 of imaging individual cells response is needed for studying the cellular viability responses to
20 different antibiotics, considering the cell to cell heterogeneity within a population.
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24 Here we report a label-free method to detect single bacteria impedance by imaging the cell
25 responses to a low frequency potential modulation. By immobilizing *E. coli* O157:H7 on the
26 surface of indium tin oxide (ITO) electrodes, we were able to image the impedance response of
27 each single bacteria with potential modulation. We demonstrated the capability of mapping the
28 bacteria viability via impedance responses with sub-cellular resolution. Using this capability, we
29 have monitored the impedance changes under two different kinds of antibiotics for detailed
30 bacterial viability detection and antibiotic mechanisms study, demonstrating EIM capability for
31 future antibiotic resistance mechanism study.
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Results and discussion

Detection principle

In physics, the impedance measures the current response to an applied electric field, which depends on the resistive and capacitive properties of the sample. Similarly, when the electric field is applied to the bacterial cell, the resistive component, the ionic flow across the conductive bacterial cell wall and cytoplasm, and the capacitive component, the plasma membrane charging, will contribute to the impedance response. As the ion flow and membrane charging induced local charge density change can affect the optical properties of the bacterial components, such as the local refractive index, the electrical impedance can be optically measured with a microscope.

As illustrated in Fig. 1, the optical and EIM images of the immobilized bacterial cells are recorded with an inverted microscope. To image the impedance responses of the bacterial cells, a transparent electrode, indium tin oxide (ITO), was used for both cell immobilization and potential application. The ITO surface was modified with anti-*E. coli* IgG antibody to immobilize the target *E.coli* on the surface. When a potential modulation is applied, the charge density on the cell surface will change with the modulation, which leads to the optical transmittance change and creates a periodic intensity change. By performing fast Fourier transform (FFT), this charge density related intensity changes can be extracted as EIM image. Fig. 1B shows the optical images and the corresponding EIM images. One is the amplitude image and the other is the phase image. Note that the EIM image contrast is proportional to the electrical admittance, which is the inverse of the impedance.

Unlike the conventional EIS, the EIM can resolve local impedance with sub-micro spatial resolution, and monitor the cell-cell heterogeneity to different stimulations. Besides, the EIM is based on the dependence of optical transmittance on surface charge density, which is measured

with simple microscopy, allowing for fast and non-invasive imaging of impedance. The EIM maps the local electrical permittivity and conductivity that reflects local changes in the bacterial cell structure and ionic distribution, providing valuable information for the understanding of bacterial responses to different drug stimulations.

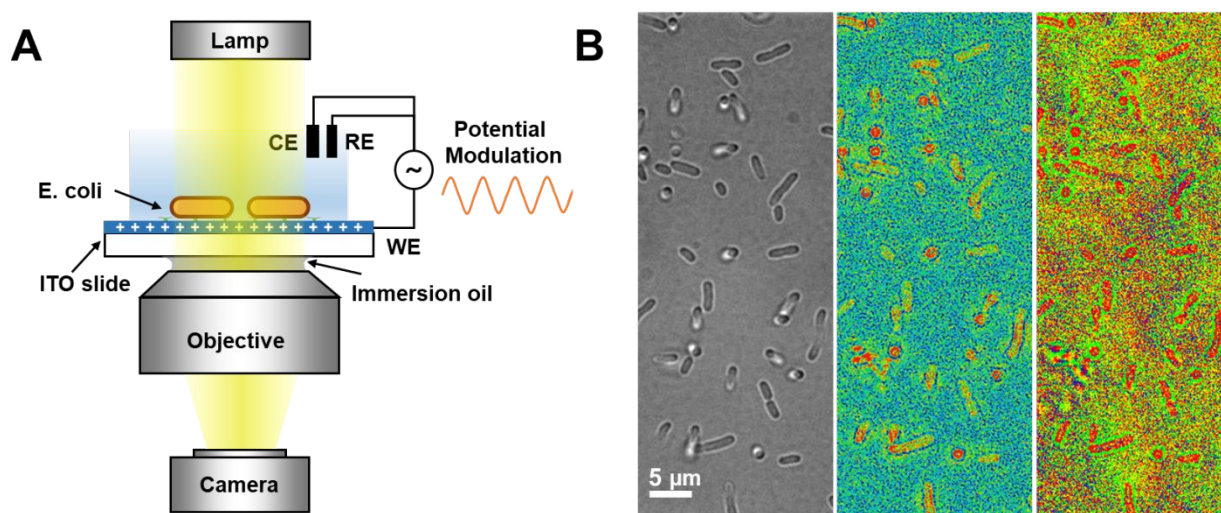


Fig. 1. Detection principle of single bacteria impedance imaging on ITO coated glass surface.

(A) Schematic illumination of the imaging setup based on an inverted microscope with 60x oil immersion objective. The ITO electrode is modified with antibodies for *E. coli* immobilization. An AC modulation potential is applied to the ITO electrode with a standard three-electrode configuration, and the impedance responses of the bacterial cells were created from the potential-induced optical changes. (B) Example of the DC optical (left), AC amplitude (center) and AC phase (right) images of *E. coli* cells.

Single-Cell Impedance Model

Conventional EIS measures the averaged impedance response over many cells on an electrode, which was modeled using a combination of resistors and capacitors. When it comes to single-cell impedance model, a more microscopic one is needed to involve the internal structure of each bacterial cell. Since impedance measures the response of the cells to a small external electrical

field, it can be modeled by local permittivity (ϵ_r) and conductivity (σ) of the bacterial cell structure and the surrounding environment. To be more specific, the impedance model of single bacterial cell can be simplified with a capacitive region, cell membrane, and two resistive regions, including cell wall and cytoplasm, as shown in Fig. 2A. The applied electric field induces ionic flow through the conductive structures and membrane charging that changes the local charge density, which alter the local refractive index and shown as a contrast change in the impedance image.

As shown in Fig. 2B, single bacterial cell is modeled by a sequence of electrical components, including cell wall resistance (R_{wall}), membrane capacitance (C_m), membrane resistance (R_m) and cytoplasm resistance (R_{cyt}). When the potential modulation (V) is applied, the total current can be calculated as

$$I = \frac{V}{R_s + R_{wall} + R_{cyt} + \frac{1}{\frac{1}{R_m} + j\omega C_m}} \quad (1)$$

R_s is the medium resistance, and f is the modulation frequency. Thus, at low frequency modulation, the charge density of the bacterial cell mainly comes from the charge density (Q_c) on cell membrane capacitance, so the membrane resistance (R_m) is not considered here for simplicity. The membrane charge density (Q_c) is calculated as

$$Q_c = \frac{VC_m}{1 + j2\pi f(R_s + R_{wall} + R_{cyt})C_m} \quad (2)$$

The basic principle of our method is that the optical transmittance changes with the charge density on the cell surface, and a linear relationship between charge density and optical intensity is observed with bare ITO surface (Supporting Information S1). Thus, the EIM signal can be interpreted as

$$S = \alpha * Q_c = \frac{\alpha VC_m}{1 + j2\pi f(R_s + R_{wall} + R_{cyt})C_m} \quad (3)$$

in which α is the linear ratio.

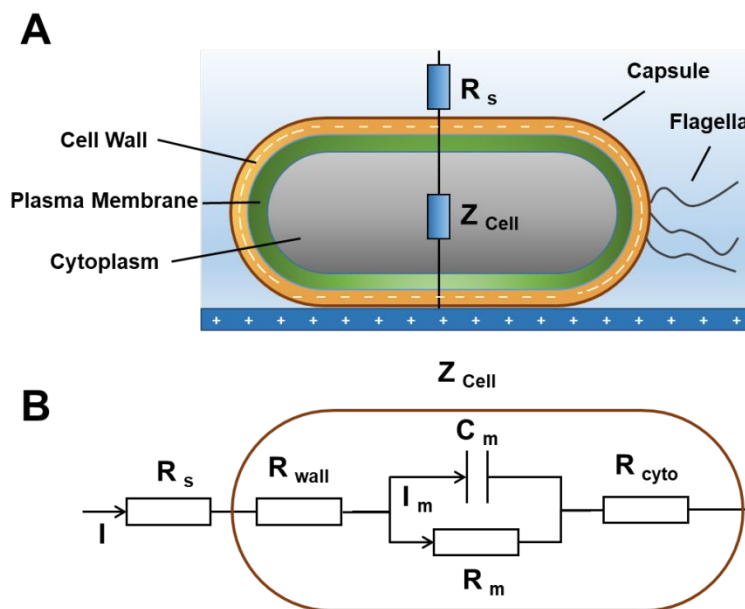


Fig. 2. The single-cell impedance model of *E.coli* cell. A. Schematic diagram of the *E.coli* structure. B. Electric model of different cell components. R_s is the solution resistance and Z_{cell} represents the cell impedance with cell wall resistance (R_{wall}), membrane capacitance (C_m), membrane resistance (R_m) and cytoplasm resistance (R_{cyt}).

Based on this single-cell model, we performed the experiments with potential modulation of different amplitudes and different frequencies to fit the values of the electrical component in our experiment. Fig. 3 shows the AC FFT amplitude of the bacterial cell response under different potential modulation. Fig. 3A presents the signal increases with the modulation amplitude increase from 600 mV to 1000 mV at 10 Hz frequency, and the averaged responses is shown in Fig. 3C. It is reasonable that the signal is linearly increased with the modulation amplitude, from which we can fit the value of Equation 3. Furthermore, to extract the value of each parameter, we measured

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the EIM responses of bacterial cells under modulation with different frequencies, and the result is shown in Fig. 3B. With the modulation amplitude fixed at 1000 mV, we changed the frequency from 5 Hz up to 30 Hz, and a decrease in the impedance signal intensity was observed. By fitting this data with our single-cell model, the average membrane capacitance is derived to be around 0.95 $\mu\text{F}/\text{cm}^2$.

The EIM image maps the local variations in the dielectric and conductive properties, which reflect changes in the bacterial cellular structure and ionic distribution. This information is important for a better understanding of bacterial cellular responses and can be used for the measurement of bacterial viability.

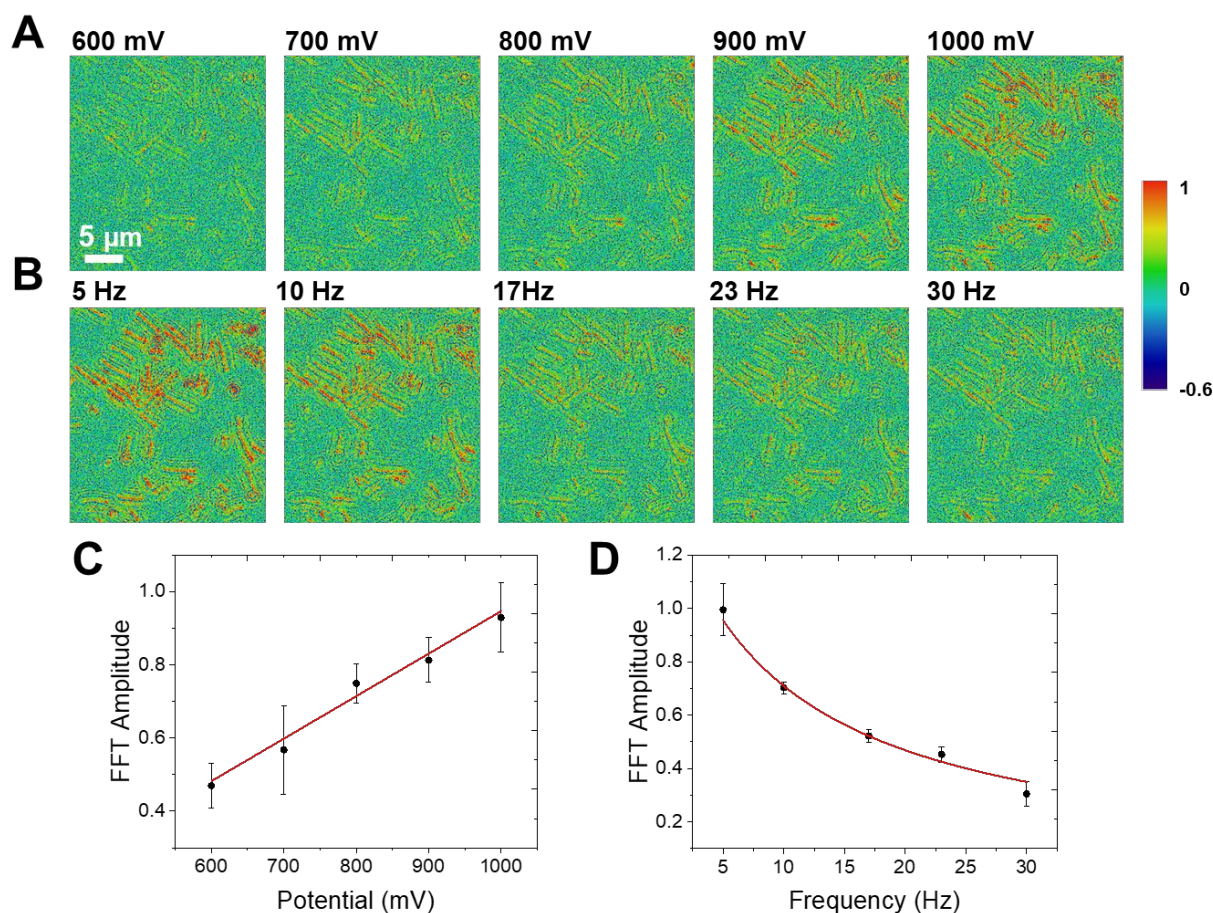


Fig. 3. The EIM images of bacterial cells under different potential modulation. (A) The mapping of bacterial cell impedance responses under AC potential modulation at 10 Hz frequency with an increasing amplitude (600 mV, 700 mV, 800 mV, 900mV and 1000mV). (B) The representative mapping of bacterial cell impedance responses under AC potential modulation at 1000 mV with different frequencies (5 Hz, 10 Hz, 17 Hz, 23 Hz and 30 Hz). (C) A linear relationship is fitted between the EIM signal and potential modulation amplitude. (D) The relationship between the EIM signal and the AC modulation frequency and fitted with the single-cell model. The error bar is the standard deviation of all cells in three repeats.

Bacterial Cell Viability with Antibiotics

To demonstrate the capability of monitoring the bacterial cell viability with EIM detection, the bacteria impedance responses under different antibiotics were measured. Two different types of antibiotics were tested. One is the polymyxin B, which is targeted at the bacterial cell membrane, and the other one is ampicillin that targeted at the bacterial cell wall. Bacterial cell responses were measured with EIM when high concentration dose antibiotic was added. The AC potential modulation used is 500 mV amplitude and 10 Hz frequency. Each modulation was applied for 10 seconds. By performing temporal FFT on the optical images, the impedance amplitude and phase were extracted for impedance comparison. Prior to antibiotic addition, the impedance responses of the bacterial cells were detected as the time zero point. Then, the antibiotic was added to the bacteria sample, and the impedance responses were recorded every 5-10 minutes for 90 minutes to track the cell responses.

Fig. 4 shows the bacterial cell viability responses to 32 $\mu\text{g/ml}$ polymyxin B. The optical images of bacterial cells at five time points of 0 min, 10 min, 30 min, 60 min and 90 min were plotted for viability mapping. The cell morphology from DC response was shown in Fig. 4A, while the AC impedance responses were present in Fig. 4B (FFT amplitude) and 4C (FFT phase). From the optical images, no obvious changes were observed until about 60 min later, while from the AC impedance amplitude images, visible changes can be observed within 10 min in some bacterial cells. Also, the AC impedance amplitude maps the heterogeneity of the bacteria population: some bacteria show changes as early as 10 min, while others show much slower responses. No obvious changes were found in AC impedance phase images. By plotting the averaged impedance amplitude response of all bacterial cells, a clear linear increase was observed (Figure 4D, black line). The variation of different cell responses is shown as the error bar. The averaged impedance

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3 amplitude was normalized by the ITO (no cell area) response for better comparison. In contrast,
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5 the normalized optical contrast of the transmitted images was stable (Figure 4D, red line). The
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7 reason for increased impedance amplitude is because polymyxin B attacks and destabilizes the cell
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9 membrane and changes the membrane permeability²⁶, which increased membrane capacitance, and
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11 lead to the increase of EIM signal.
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15 Furthermore, sub-cellular impedance responses can be resolved by EIM. Gradually increased
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17 number of local hot spots inside of individual cells can be observed in the AC impedance amplitude
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19 images (Fig. 4B), which are likely resulted from internal membrane holes opened by high
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21 concentration polymyxin B²⁷. Furthermore, heterogeneous response to the antibiotics from
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23 individual bacterial cells observed (Fig. 4E). Although most of the bacterial cells show increased
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25 AC impedance amplitude value (Fig. 4E, red lines), two cells (marked by arrows in Fig. 4B, the
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27 90 minutes image) are showing little changes in the amplitude value (Fig. 4E, black and red lines),
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29 indicating these two bacteria may have higher tolerance to polymyxin B. This result shows that
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31 EIM can map individual bacterial cell viability under the treatment of polymyxin B, and could
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33 help to find sub population of drug resistant bacteria within a susceptible strain.
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38 To compare the performance of EIM with the classic impedance spectroscopy, we
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40 simultaneously recorded the total electrical current during the EIM measurement. It is not
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42 surprising that the total current show little change over the 90 min treatment period (Supporting
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44 Information S4), because the current measures the averaged impedance responses of whole sensing
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46 surface, in which the contribution from the low density bacteria is too small to be detected.
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48 Therefore, EIM has the unique advantage for quantification of individual bacterial cell impedance
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50 changes and responses to antibiotic treatment regardless of the cell density.
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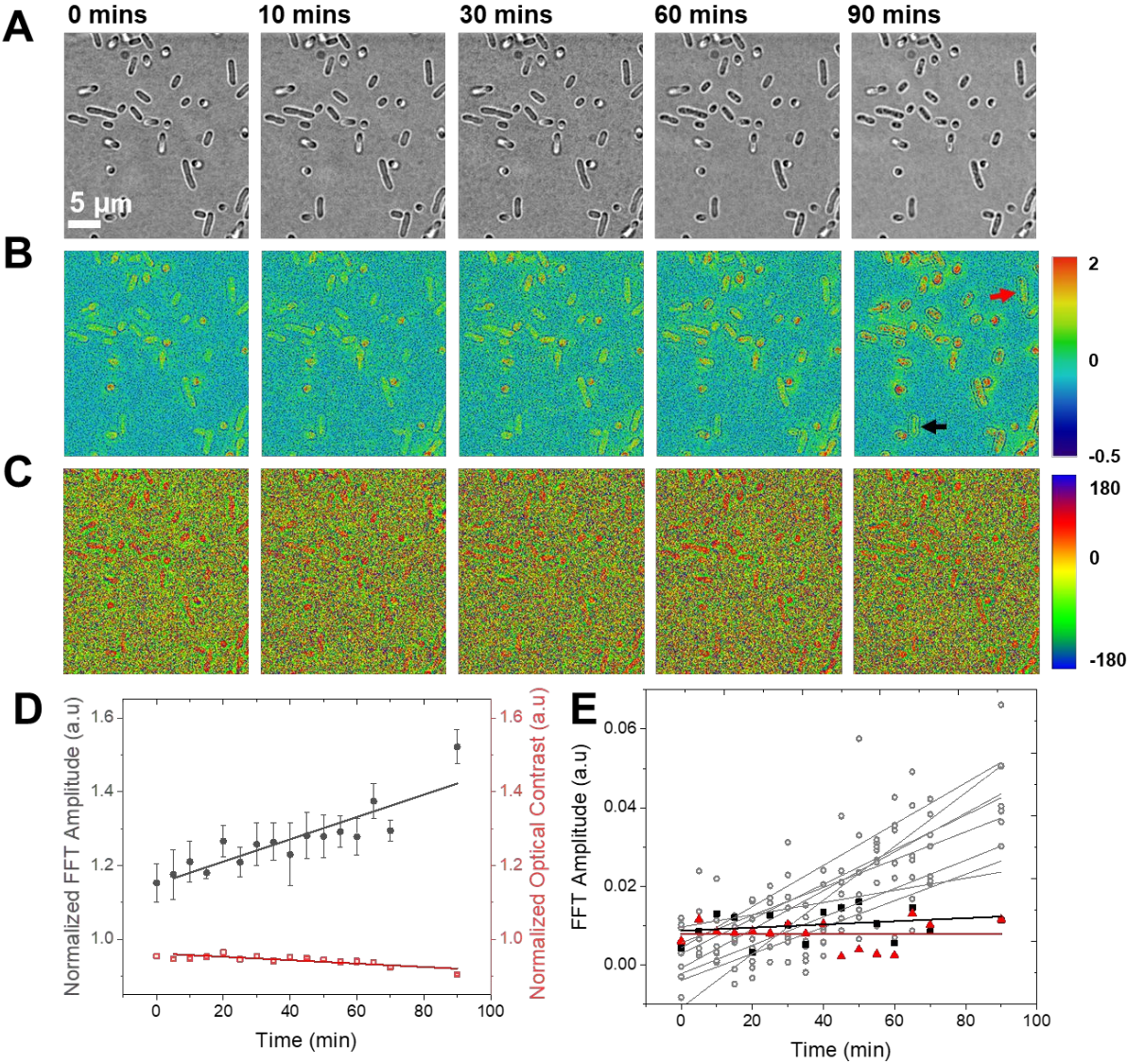


Fig. 4. The bacterial cell response to 32 µg/ml polymyxin B at time points from 0 to 90 min. (A) DC optical images at different time points. (B) AC impedance amplitude image at different time points. Arrows indicating bacterial cells that has little impedance amplitude changes. (C) AC impedance phase images at different time points. (D) The averaged AC impedance amplitude changes over time (black, left axis) and the corresponding optical contrast change of the DC images (red, right axis). Lines are linear fitting to all data points. The error bar is the standard deviation of all cells in three repeats. (E) AC impedance amplitude change of individual bacteria

over time. Dots are AC impedance amplitude for individual bacterial cells in the images at different time points. Lines are linear fitting of data from individual cells. Black and red dots and lines are for the cells marked with arrows in B.

Fig. 5 shows the bacterial cell viability responses to 16 $\mu\text{g/ml}$ ampicillin. The optical and EIM images of bacterial cells at five time points of 0 min, 10 min, 30 min, 60 min and 90 min are presented to visualize the cell viability changes in response to ampicillin. The DC images (Fig. 5A) shows visible morphological changes only at around 60 min and later. Similarly, both AC impedance amplitude (Fig. 5B) and AC impedance phase (Fig. 5C) didn't show obvious change until the cell start to lyse after 60 min. Quantitative analysis also shows no measurable increase was observed in the EIM amplitudes in the first hour (Fig. 5D). This result is expected, because ampicillin inhibits the cell wall synthesis, leading to the bacterial cell lysis, while the EIM signal is mainly from the membrane capacitance at the frequency we measure. The contribution from cell wall resistance to EIM signal is negligible, thus this low frequency EIM is not sensitive to bacterial cell response to ampicillin. Therefore, EIM can help to map the mechanism of action of different antibiotics.

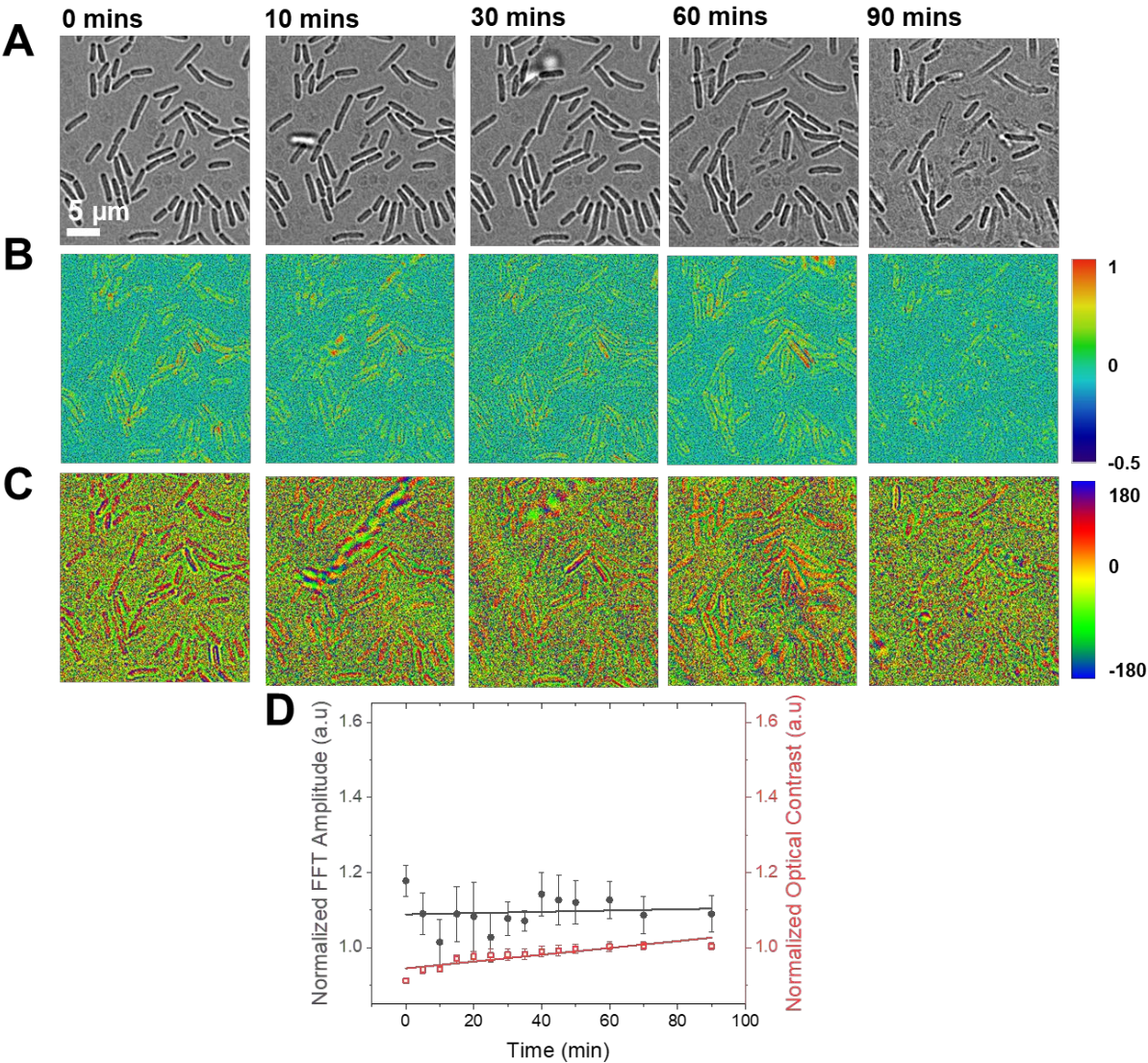


Fig. 5. The bacterial cell response to 16 µg/ml ampicillin at time points from 0 to 90 min. (A) DC optical images. (B) AC impedance amplitude images. (C) AC impedance phase images. (D) Averaged AC impedance amplitude and DC contrast of all bacteria in the image over time. The error bar is the standard deviation of all cells in three repeats.

Conclusions

We have demonstrated a new label-free method for single bacterial cell impedance imaging based on the dependence of optical transmittance with surface charge density. Unlike the

conventional EIS, our method can resolve the local impedance with sub-cellular resolution for single cell viability mapping, which enables the study of the cell to cell heterogeneity in response to the antibiotic treatment. With a single bacterial cell model, our EIM signal is derived to be related to the charge density on cell membrane capacitance in low frequency potential modulation. To demonstrate the capability of our method, the bacterial cell viability under two different types of antibiotics were monitored. Polymyxin B changes cell membrane permeability, which induce cell impedance responses in about 10 min, while ampicillin block cell wall synthesis, which does not induce much change with low frequency EIM signals until cell lyses started. Furthermore, sub-cellular mapping revealed cell structure changes associated with the antibiotic action, providing detailed information about the working mechanism of antibiotics. More importantly, individual bacterial cells having higher tolerance to the antibiotic treatment were identified, which may be used for early detection of the development of resistant strain. By adding broader frequency modulation range and higher frame rate imaging capability, EIM will be able to map the impedance changes of different cell components and monitor the cell viability changes under broader kinds of antibiotics, leading to a new tool for bacteria metabolism and antibiotic mechanism study.

Materials and Methods

Materials

E. coli strain O157:H7 (ATCC 43888) was purchased from American Type Culture Collection (ATCC, Rockville, MD). Goat anti-*E. coli* O157:H7 IgG polyclonal antibody was purchased from Kirkegaard and Perry Laboratory, Inc. (Gaithersburg, MD), and the stock solution (1mg/ml) was made by mixing the antibody with 1 ml 1x PBS and stored at -20 °C. Polymyxin B and ampicillin were purchased from Sigma-Aldrich, dissolved in 1x PBS at stock concentrations of 10 mg/mL

and 2 mg/mL, respectively. 3-Triethoxysilylpropylamine 99% (APTES), N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide 97% (EDC), and sodium acetate (NaOAc) were also purchased from Sigma- Aldrich. All reagents were analytical grade from Sigma-Aldrich, except those stated.

Optical setup

Optical imaging of bacterial cells was performed with an inverted microscope (Olympus IX81) equipped with a high numerical aperture oil immersion objective (CFI Apo TIRF, 60 x, 1.49 NA). The immersion oil used was Cargille Immersion Oil Type A (Cargille Laboratories, #16482). Broad-band light illumination from a 100 W halogen lamp (Olympus, 12V100WHAL-L) was directed from the top of the sample cell (Fig. 1A). Bright field transmitted images were collected by the objective under the sample and recorded as 640x480 pixel 14-bit grey scale image sequences by a CCD camera (Allied Vision Pike F-032) with a frame rate of 106 fps. To prevent the solution evaporation, a coverslip was used to cover the well top and the microscope light was only turned on during measurement when potential modulation applied (10 s for every 5-10 min).

Potential modulation

A Flexi-Perm silicon chamber (a single well cut from a flexPERM® slide, Sarstedt, Inc., #94.6032.039) was placed on top of an ITO coated glass slide (SPI Supplies, 06472-AB) to serve as a bacteria culture well and electrochemical cell, and Lysogeny broth (LB) media was used as electrolyte. To reliably control the potential, a standard three electrode electrochemical measurement configuration was used. The potential on the bacteria was controlled with respect to an Ag/AgCl reference electrode by a potentiostat (Pine Research Instrumentation, ARFDE5) using a platinum coil as counter electrode. Potential modulations with different frequencies and amplitudes were applied to the sample using an external function generator (Agilent, 33220A).

Synchronization between electrochemical measurement and CCD camera was achieved with a data acquisition card (DAQ, USB-6250, National Instruments) to simultaneously record the applied electrical potential and camera trigger signals.

Surface Preparation

The ITO slides were cleaned by sonication for 15 min sequentially in acetone, alcohol and DI water before use. To generate the $-OH$, the ITO surface is activated in the solution of $H_2O:H_2O_2:NH_4OH$ (5:1:1) at 80 °C for 15 min. Then, the ITO slides is immersed to 5% APTES in 95% ethanol for 60 min to functionalize the surface. After the slides were cleaned and dried, a mixture of fresh made 0.4 M EDC and 30 $\mu g/mL$ of antibody in 20 mM sodium acetate (pH 5.5) were added to the slides for 60 min. At last, the slides were cleaned again with water and dried with nitrogen, thus generating an antibody-activated surface for bacteria immobilization.

Bacteria immobilization

A single well Flexi-Perm silicon chamber were mounted on a microscope coverslip, and filled with 0.5 mL LB media and 10 μL of bacterial cells. The filled chamber were placed in a covered Petri dish and incubated for 30 min at 37 °C to let the bacterial cells bind to the surface-attached antibodies. Unattached bacterial cells were washed away with LB medium.

Signal analysis

Fuji ImageJ was used for signal processing. First, the image sequences were normalized with the averaged image result (Z Project) to eliminate the illumination intensity difference. Then, to extract the EIM mapping result of the bacteria, we performed stack temporal Fast Fourier Transform (FFT) using an Fuji ImageJ plugin (developed by Jay Unruh at Stowers Institute for Medical Research in Kansas City, MO) on the image sequence and obtain both the amplitude and phase mapping at the modulation frequency. Furthermore, to compare the FFT amplitude of different time points, we

first tracked all individual bacterial cells over the stack with imageJ particle analysis (Supporting Information S2) and then plotted the optical responses of the bacterial cells at each time point and used it to calculate the corresponding FFT amplitude value at the modulation frequency (Supporting Information S3). For better comparison, the normalized result was calculated by dividing the FFT amplitude of the ITO surface (no cell area). Similarly, the optical contrast value is obtained by first tracking the intensity of bacterial cells, and use it to calculate the corresponding normalized FFT amplitude value at 0 Hz frequency (DC signal).

ASSOCIATED CONTENT

Supporting Information.

Supporting Information Available: Linear relationship between optical responses and charge density with bare ITO; Single cell tracking with imageJ; Comparison of bacterial EIM response with ITO response; Simultaneous electrical current recording;

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Notes

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Reference

1. Ng, S. Y.; Reboud, J.; Wang, K. Y. P.; Tang, K. C.; Zhang, L.; Wong, P.; Moe, K. T.; Shim, W.; Chen, Y., Label-free impedance detection of low levels of circulating endothelial progenitor cells for point-of-care diagnosis. *Biosens Bioelectron* **2010**, *25* (5), 1095-1101.
2. Shamsipur, M.; Gholivand, M. B.; Ehzari, H.; Pashabadi, A.; Arkan, E.; Mansouri, K., Single frequency impedance strategy employed in rapid detection of leukemia cancer cells using an electrospun PES-nanofiber reinforced ternary composite-based cytosensor. *Electrochim Acta* **2018**, *283*, 1498-1506.
3. Nwankire, C. E.; Venkatanarayanan, A.; Glennon, T.; Keyes, T. E.; Forster, R. J.; Ducree, J., Label-free impedance detection of cancer cells from whole blood on an integrated centrifugal microfluidic platform. *Biosens Bioelectron* **2015**, *68*, 382-389.
4. Lei, K. F., Review on Impedance Detection of Cellular Responses in Micro/Nano Environment. *Micromachines-Basel* **2014**, *5* (1), 1-12.
5. Liang, W.; Wang, S.; Festa, F.; Wiktor, P.; Wang, W.; Magee, M.; LaBaer, J.; Tao, N., Measurement of small molecule binding kinetics on a protein microarray by plasmonic-based electrochemical impedance imaging. *Anal Chem* **2014**, *86* (19), 9860-5.
6. Yuan, L.; Tao, N.; Wang, W., Plasmonic Imaging of Electrochemical Impedance. *Annu Rev Anal Chem (Palo Alto Calif)* **2017**, *10* (1), 183-200.
7. Liu, X. W.; Yang, Y.; Wang, W.; Wang, S.; Gao, M.; Wu, J.; Tao, N., Plasmonic-Based Electrochemical Impedance Imaging of Electrical Activities in Single Cells. *Angew Chem Int Ed Engl* **2017**, *56* (30), 8855-8859.
8. Llor, C.; Bjerrum, L., Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. *Ther Adv Drug Saf* **2014**, *5* (6), 229-41.
9. Chattaway, M. A.; Dallman, T.; Okeke, I. N.; Wain, J., Enterohaggative *E. coli* O104 from an outbreak of HUS in Germany 2011, could it happen again? *J Infect Dev Countr* **2011**, *5* (6), 425-U1501.
10. Bouki, C.; Venieri, D.; Diamadopoulos, E., Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review. *Ecotox Environ Safe* **2013**, *91*, 1-9.
11. Lebovitz, E. E.; Burbelo, P. D., Commercial multiplex technologies for the microbiological diagnosis of sepsis. *Mol Diagn Ther* **2013**, *17* (4), 221-31.
12. Sancho-Tello, S.; Bravo, D.; Borrás, R.; Costa, E.; Muñoz-Cobo, B.; Navarro, D., Performance of the LightCycler SeptiFast test Mgrade in detecting microbial pathogens in purulent fluids. *J Clin Microbiol* **2011**, *49* (8), 2988-91.
13. Pasqualini, L.; Mencacci, A.; Leli, C.; Montagna, P.; Cardaccia, A.; Cenci, E.; Montecarlo, I.; Pirro, M.; di Filippo, F.; Cistaro, E.; Schillaci, G.; Bistoni, F.; Mannarino, E., Diagnostic performance of a multiple real-time PCR assay in patients with suspected sepsis hospitalized in an internal medicine ward. *J Clin Microbiol* **2012**, *50* (4), 1285-8.
14. Blaschke, A. J.; Heyrend, C.; Byington, C. L.; Fisher, M. A.; Barker, E.; Garrone, N. F.; Thatcher, S. A.; Pavia, A. T.; Barney, T.; Alger, G. D.; Daly, J. A.; Ririe, K. M.; Ota, I.; Poritz, M. A., Rapid identification of pathogens from positive blood cultures by multiplex polymerase chain reaction using the FilmArray system. *Diagn Microbiol Infect Dis* **2012**, *74* (4), 349-55.
15. Mannoor, M. S.; Zhang, S. Y.; Link, A. J.; McAlpine, M. C., Electrical detection of pathogenic bacteria via immobilized antimicrobial peptides. *P Natl Acad Sci USA* **2010**, *107* (45), 19207-19212.

16. Yang, L.; Bashir, R., Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. *Biotechnol Adv* **2008**, *26* (2), 135-150.
17. Xu, M.; Wang, R. H.; Li, Y. B., Electrochemical biosensors for rapid detection of *Escherichia coli* O157:H7. *Talanta* **2017**, *162*, 511-522.
18. David, F.; Hebeisen, M.; Schade, G.; Franco-Lara, E.; Di Berardino, M., Viability and membrane potential analysis of *Bacillus megaterium* cells by impedance flow cytometry. *Biotechnol Bioeng* **2012**, *109* (2), 483-492.
19. Sanchis, A.; Brown, A. P.; Sancho, M.; Martinez, G.; Sebastian, J. L.; Munoz, S.; Miranda, J. M., Dielectric characterization of bacterial cells using dielectrophoresis. *Bioelectromagnetics* **2007**, *28* (5), 393-401.
20. Holzel, R., Non-invasive determination of bacterial single cell properties by electrorotation. *Bba-Mol Cell Res* **1999**, *1450* (1), 53-60.
21. Braiek, M.; Rokbani, K. B.; Chrouda, A.; Mrabet, B.; Bakhrouf, A.; Maaref, A.; Jaffrezic-Renault, N., An Electrochemical Immunosensor for Detection of *Staphylococcus aureus* Bacteria Based on Immobilization of Antibodies on Self-Assembled Monolayers-Functionalized Gold Electrode. *Biosensors (Basel)* **2012**, *2* (4), 417-26.
22. Wang, Y. X.; Ye, Z. Z.; Ying, Y. B., New Trends in Impedimetric Biosensors for the Detection of Foodborne Pathogenic Bacteria. *Sensors-Basel* **2012**, *12* (3), 3449-3471.
23. Castellarnau, M.; Errachid, A.; Madrid, C.; Juarez, A.; Samitier, J., Dielectrophoresis as a tool to characterize and differentiate isogenic mutants of *Escherichia coli*. *Biophys J* **2006**, *91* (10), 3937-3945.
24. Yang, L., Dielectrophoresis assisted immuno-capture and detection of foodborne pathogenic bacteria in biochips. *Talanta* **2009**, *80* (2), 551-8.
25. Yang, L.; Banada, P. P.; Chatni, M. R.; Seop Lim, K.; Bhunia, A. K.; Ladisch, M.; Bashir, R., A multifunctional micro-fluidic system for dielectrophoretic concentration coupled with immuno-capture of low numbers of *Listeria monocytogenes*. *Lab Chip* **2006**, *6* (7), 896-905.
26. Dixon, R. A.; Chopra, I., Polymyxin B and polymyxin B nonapeptide alter cytoplasmic membrane permeability in *Escherichia coli*. *J Antimicrob Chemother* **1986**, *18* (5), 557-63.
27. Daugelavicius, R.; Bakiene, E.; Bamford, D. H., Stages of polymyxin B interaction with the *Escherichia coli* cell envelope. *Antimicrob Agents Chemother* **2000**, *44* (11), 2969-78.

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