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Lab-on-chip microfluidic impedance measurement for laminar flow ratio sensing and differential conductivity difference detection

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We present a microfluidic impedance device for achieving both the flow ratio sensing and the conductivity difference detection between sample stream and reference buffer. By using a flow focusing configuration, with the core flow having a higher conductivity sample than the sheath flow streams, the conductance of the device varies linearly with the flow ratio, with $R^2 > 0.999$. On the other hand, by using deionized (DI)-water sheath flow as a reference, we can detect the difference in conductivity between the buffer of core flow and sheath DI-water with a high detection sensitivity of up to 1 nM of sodium chloride solution. Our study provides a promising approach for on-chip flow mixing characterization and bacteria detection. *Published by AIP Publishing.*
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The recent developments of microfluidics and dielectrophoresis (DEP) systems^{1–4} have enabled a myriad of applications in flow sensing,⁵ micro-mixing,⁶ and biomedical operations such as impedance flow cytometry,⁷ bacteria and virus detection,^{8–11} cell sorting,^{12–14} and cell enumeration.^{15,16} The miniaturization of these microfluidics devices offers the benefits of requiring a less sample volume, faster analysis, precise fluid handling, and reduced biological and chemical wastes.^{17,18} DEP induces lateral movement of cells or bacteria through the electropolarization effects under a non-uniform electric field,^{19,20} which allows the focusing and immobilization of cells and bacteria in a microfluidic chamber for detection and classification. On the other hand, impedance spectroscopy allows for the continuous monitoring of the electrical properties such as the absolute impedance, phase, conductance, and capacitance of the sample in a microfluidic network.²¹ Nonetheless, despite these advances in DEP impedance based microfluidics, few works have been done on the impedance analysis involving the flow focusing configuration.

In this work, we report a DEP impedance microfluidics system for realizing both the flow ratio sensing and the differential conductivity difference measurement capability. We measure the conductance of the flow focusing microfluidic system, with the core flow having a higher conductivity sample than the sheath flow streams to detect the changes in the flow ratio. The applications of such a flow ratio sensing system include on-chip continuous monitoring of fluid mixing and characterization of the diffusion length by placing multiple sets of interdigitated microelectrodes at several downstream locations. On the other hand, the ability to detect the differential conductivity difference between sample stream and reference buffer would ultimately provide a convenient way for tracking cell proliferation and allow for pathogen detection for marine applications such as bacteria detection for ballast water compliance tests.

The schematic diagram of the microfluidics impedance measurement setup is shown in Fig. 1(a). The device comprises a flow-focusing microchannel and an interdigitated gold microelectrode array. The microchannel has two inlets—core flow and sheath (side) flow. The microelectrodes were connected to an impedance analyzer (MFLI lock-in amplifier, Zurich instrument) with a built-in AC voltage source for the impedance measurements and characterization. The microelectrodes, shown in the inset of Fig. 1(a), were fabricated using standard cleanroom photolithography techniques with positive photoresist (AZ9260, Microchemicals GmbH) and magnetron sputtering with 10 nm of chromium, Cr, as the seeding layer followed by the deposition of 200 nm of gold onto a 4 in. Borofloat 33 glass wafer (supplementary material, Fig. 1).

The interdigitated microelectrodes have 50 pairs of fingers. The finger length of the microelectrodes is 500 μm , while both the finger width and gap are 25 μm . There are two large square contact pads, with both the width and length being 5 mm, for establishing electrical connection with the impedance analyzer via spring-loaded test probes (PA3FS, Coda pins) (supplementary material, Fig. 2). Subsequently, a layer of approximately 100 μm SU-8 photoresist (Microchemicals GmbH) was aligned and spin-coated on top of the gold electrode and glass wafer. After developing with a developer, the SU-8 microchannel has a width of 600 μm and a length of 10 mm (supplementary material, Fig. 3). A layer of 10 mm thick polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning Inc.) was cured and punched with holes for the fluidic connectors to cover the opening of the SU-8 microchannel. The PDMS layer was uniformly pressed against the SU-8 layer and glass wafer through a customized computer-numerical-control (CNC) machined aluminum fixture with four M4 wing nuts to provide a good sealing for the microchannel, and a high flow rate of up to 100 $\mu\text{l min}^{-1}$ could be achieved without fluid leak.

The impedance of the microfluidics device varies with the choice of bulk fluids and AC voltage and frequency. Figures 1(b) and 1(c) show the variation in the absolute impedance and phase angle of the device in response to the

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FIG. 1. (a) Schematic diagram of the microfluidics platform for the impedance measurement. The interdigitated microelectrodes are made up of 50 pairs of fingers with a length of $500\ \mu\text{m}$, a width of $25\ \mu\text{m}$, and a gap of $25\ \mu\text{m}$. (b) and (c) Frequency response for the device's absolute impedance and phase angle of the device in response to the AC frequency from 100 Hz to 5 MHz for DI-water at total flow rates of $1\ \mu\text{L min}^{-1}$ and $10\ \mu\text{L min}^{-1}$. The standard deviations are $< 1.50\ \text{k}\Omega$ and $< 0.717^\circ$ based on five repeated measurements. (d) Equivalent circuit for the impedance analysis. C_{de} is the dielectric capacitance, C_{dl} is the double layer capacitance, and R_{sol} is the buffer resistance. (e) Equivalent parallel circuit representation of the impedance measurement device with core and sheath fluids flowing in the flow focusing configuration.

AC frequency from 100 Hz to 5 MHz for deionized (DI)-water at flow rates of $1\ \mu\text{L min}^{-1}$ and $10\ \mu\text{L min}^{-1}$. [Supplementary material](#), Fig. 4 shows the resistance and capacitance frequency response. The resistance of the device is larger for a higher flow rate at AC frequency less than 200 kHz. The resistances of gold electrodes and connection wires are of the order of about $1\ \Omega$, and both of them are negligible compared to the resistance of DI-water and low concentration of sodium chloride (NaCl) solution in the microchannel. The impedance of DI-water at $10\ \mu\text{L min}^{-1}$ varies from $471.5\ \text{k}\Omega$ down to $903.5\ \Omega$ when AC frequency sweeps from 100 Hz to 5 MHz [Fig. 1(b)]. When a pair of electrodes is in contact with the electrolyte, two thin layers of opposite charges will form close to those electrode surfaces, which generates a double layer capacitance.²² We adopt the widely used equivalent circuit shown in Fig. 1(d) to analyze the impedance response.^{15,22,23} These double layer capacitances (C_{dl}) are assumed to be in series with the bulk fluid resistance R_{sol} , forming a C_{dl} - R_{sol} - C_{dl} branch. When an AC voltage source is applied on the electrode, a dielectric capacitance will be generated due to the polarization of the bulk fluid.²⁴ This dielectric capacitance, C_{de} , of the solution is assumed to be parallel to the C_{dl} - R_{sol} - C_{dl} branch.¹⁵ Since the branches of the interdigitated electrode array are perpendicular to the flow direction of both the fluids, the equivalent impedances of these two bulk fluids (Z_{core} and Z_{sheath}) are connected in parallel [Fig. 1(e)].

At low AC frequency, the dielectric capacitance branch is inactive and acts as an open circuit.²³ The measured phase angle is therefore a competition between the resistance of the electrolyte and the electric double layer (EDL) capacitance.

The device impedance is mainly contributed by C_{dl} and R_{sol} in the frequency range of 100 Hz to 10 kHz, with the former dominating below 100 Hz.²³ In a high frequency range, the dielectric capacitance dominates the impedance response, resulting in the phase angle approaching -90° [Fig. 1(c)]. For the applied AC frequency of less than 10 kHz, the impedance of the device can be expressed as

$$\mathbf{Z} = R_{sol} + \frac{1}{j\pi f C_{dl}} = |\mathbf{Z}|e^{j\theta}, \quad (1)$$

where $|\mathbf{Z}|$ and θ are the magnitude and the phase angle of the impedance, respectively.

In order to realize the functionality as a flow ratio sensor, we characterized the relationship between the conductance and capacitance with the flow ratio, λ , using a high-conductivity diluted seawater buffer ($57 \pm 2\ \mu\text{S/cm}$ or $99 \pm 2\ \mu\text{S/cm}$) as core flow and DI-water ($0.05\ \mu\text{S/cm}$) as side flow [Figs. 2(a) and 2(b)]. The total flow rate was set to $1\ \mu\text{L min}^{-1}$ with an AC voltage output of $0.3\ V_{pp}$ at 1 kHz. We limit the voltage output of the lock-in-amplifier to less than $1\ V_{pp}$ and with frequency increased to 1 kHz for seawater experiments as the maximum allowable current for the lock-in-amplifier is 10 mA. The syringes were carefully washed 10 times before each experiment. By increasing and decreasing λ in a step function, we observed that the conductance increases and decreases correspondingly for both the diluted seawater samples. If the sheath flow has a higher conductivity than the core flow, the trend for the conductance and capacitance variation with the flow ratio would be reversed ([supplementary material](#), Fig. 5). Figures 2(c) and 2(d) shows

FIG. 2. (a) and (b) Conductance and capacitance plot for the flow ratio, $\lambda = 1/4, 1/2$, and $3/4$ with diluted seawater samples ($57 \pm 2 \mu\text{S/cm}$ and $99 \pm 2 \mu\text{S/cm}$) as core fluid and DI-water ($0.05 \mu\text{S/cm}$) as side flow. (c) and (d) The average conductance and capacitance values scale linearly with the flow ratio, with $R^2 > 0.999$. (e) and (f) Investigation of the detection limit of the impedance measurement for conductance and capacitance of the device with core flow of DI-water, 1 nM, and 1 μM NaCl and side flow of DI-water.

the average conductance and capacitance value at each of the flow-ratio step. Indeed, the conductance scales linearly with λ with $R^2 > 0.999$. For the core-sheath focusing flow, the total impedance of the parallel impedance circuit [Fig. 1(e)] can be expressed as

$$\frac{1}{Z_{\text{total}}} = \frac{\lambda}{Z_{\text{core}}} + \frac{1-\lambda}{Z_{\text{sheath}}}, \quad (2)$$

where Z_{core} and Z_{sheath} are the measured impedances with core and sheath flow, respectively. Since the conductance of sea water (which is the reciprocal of real part of Z_{core}) is much higher than that of DI-water, it is fair to assume that the $C_{\text{dl}} - R_{\text{sheath}} - C_{\text{dl}}$ branch due to DI-water is negligible. Therefore, from Eqs. (1) and (2), the corresponding total conductance and capacitance can be simplified as

$$G_{\text{total}} = \lambda \frac{1}{R_{\text{sol,core}}}, \quad (3)$$

$$C_{\text{total}} = \frac{\lambda}{2} C_{\text{dl,core}}. \quad (4)$$

Hence, the measured conductance (the reciprocal of R_{total}) and capacitance vary linearly with the flow ratio λ [Figs. 2(c) and 2(d)].

On the other hand, we assessed the detection sensitivity and limit of the microfluidic impedance sensor by repeating the experiment with both DI-water and highly diluted samples of NaCl solutions up to 1 μM and 1 nM concentrations. Figures 2(e) and 2(f) show the normalized conductance and capacitance measurement at various λ steps at 200 Hz, an

AC voltage of 1 V_{pp} , and a total flow rate of $10 \mu\text{l min}^{-1}$. The normalized conductance values are obtained by dividing the conductance with the average conductance value for $\lambda = 1/4$ at the beginning of the experiment from $t = 0$ to 300 s. Likewise, the capacitance values are normalized in the corresponding manner. Unexpectedly, when DI-water is used as both the core and sheath buffers, the average normalized conductance increased by a small margin of 2.3% at $\lambda = 3/4$. A similar trend was observed even though we repeated the experiment three times. Moreover, we observed an increase of approximately 4% and 10% in the normalized conductance at $\lambda = 3/4$ for 1 nM and 1 μM NaCl, respectively. As evident in [supplementary material](#), Fig. 6(a), there is a statistically significant difference in the normalized conductance values between 1 nM NaCl-DI-water and DI-water-DI-water configurations, with the t -test p -value $\ll 0.05$. The average values of the normalized conductance are 1.023 ± 0.002 and 1.040 ± 0.004 , respectively. Similarly, the capacitance distributions at $\lambda = 3/4$ also exhibit a statistical significance difference with the t -test p -value $\ll 0.05$ [[supplementary material](#), Fig. 6(b)]. This shows that our device is capable of performing high sensitivity differential conductivity measurements with a resolution up to 1 nM NaCl concentration with reference to DI-water.

For the case of DI-water and low concentration of NaCl solution in the steady state, it is reasonable to assume identical phase angles of Z_{core} and Z_{sheath} . Thus, the total resistance and capacitance can be simplified as

$$\text{Re}(Z_{\text{total}}) = \frac{R_{\text{sheath}}}{\lambda(R_{\text{sheath}}/R_{\text{core}} - 1) + 1}, \quad (5)$$

$$\text{Im}(\mathbf{Z}_{\text{total}}) = \frac{1}{\pi f C_{\text{sheath}}} \frac{1}{\lambda(C_{\text{core}}/C_{\text{sheath}} - 1) + 1}. \quad (6)$$

Further simplifying Eqs. (5) and (6) leads to the conductance, G_{total} , and capacitance, C_{total} , of the equivalent circuit, which are given by

$$G_{\text{total}} = \frac{\lambda(R_{\text{sheath}}/R_{\text{core}} - 1) + 1}{R_{\text{sheath}}}, \quad (7)$$

$$C_{\text{total}} = \frac{1}{2} C_{\text{sheath}} [\lambda(C_{\text{core}}/C_{\text{sheath}} - 1) + 1]. \quad (8)$$

Therefore, both the conductance and capacitance are linearly proportional to the flow ratio [Figs. 2(e) and 2(f)]. While the conductance and capacitance increase linearly as we increased λ from 1/4 to 1/2 and 3/4, we observed hysteresis effects where the conductance and capacitance values do not return to their original level when we reduced the flow ratio. This could be attributed to the electrode-polarization effect.¹ Furthermore, the Peclet number for the experiments is approximately 239.3, which indicates that the diffusion effect between the core and sheath flow is negligible (supplementary material, Calculation 1).

Another interesting phenomenon we observed is that the time required to reach the steady state impedance value for the DI-water–DI-water case is much longer than that for the high conductivity diluted seawater solution. The same trend was found to be true for the low concentration NaCl solution as well. We speculate that this is due to the difference in the electric double layer (EDL) properties of the two fluids flowing through the microchannel.^{25–28} The EDL capacitance could be affected by the size and distribution of cations and anions in the EDL. For electrolytes with low conductivity, neither the electrolyte resistance nor the EDL capacitance dominates the total impedance of the circuit since the pure DI-water impedance phase angle is approximately 16° at low AC frequency [Fig. 1(c)]. The EDL profile could be altered immediately after we change the flow ratio, which takes a few minutes to reach the new steady state because of slow fluid velocity close to the electrode surfaces. It is possible to conduct the experiment at higher AC frequency to mitigate the EDL effect on the impedance measurement and achieve the steady state in a shorter time.

However, for applications of bacteria detection and bio-affinity sensing such as protein binding, a low AC frequency of less than 1 MHz is typically used for higher detection sensitivity.^{1,2,23} Zou *et al.* demonstrated that a relative impedance change before and after mouse anti-rabbit IgG protein binding could only be detected for frequency less than 1 kHz.²³ In the low frequency range, the deposition of bacteria and binding of protein would create a new charged capacitance layer in series with C_{dl} , thus lowering the C_{dl} and increasing the total impedance.^{23,29} From the differential conductivity difference measurement experiment, we see a statistical difference between DI-water and 1 nM NaCl solution with normalized conductances of 1.023 and 1.04 at $\lambda = 3/4$. Since the conductivity of DI-water is $0.05 \mu\text{S cm}^{-1}$, the conductivity of the 1 nM NaCl solution can be evaluated from Eq. (7) and is estimated to be $0.0511 \mu\text{S cm}^{-1}$, and thus, the detection resolution of the impedance measurement

device is approximately 1.1 nS cm^{-1} . For frequencies from 10 to 100 kHz, the effective particle conductivities for 5×10^7 cells ml^{-1} of *Bacillus subtilis*, *Micrococcus luteus*, and *Escherichia coli* are $935 \mu\text{S cm}^{-1}$, $1557 \mu\text{S cm}^{-1}$, and $412 \mu\text{S cm}^{-1}$, respectively.³⁰ Hence, for the case of *E. coli* bacteria, the limit of detection is estimated to be in the order of 133 cell ml^{-1} . This is consistent with the detection limit of 300 CFU ml^{-1} reported by Kim *et al.* and 100 CFU ml^{-1} reported by Suehiro *et al.*^{8,31}

In conclusion, we have presented a microfluidic impedance measurement scheme for achieving both the flow ratio sensing and differential conductivity difference detection. We have characterized and proven the linear relationship between the measured conductance and the flow ratio between the core and sheath buffers. The ability to detect the flow ratio in real-time, without the need for fluorescence imaging or image processing, would ultimately enable future applications in microfluidic mixing. On the other hand, we have also demonstrated differential conductivity measurements for highly diluted sodium chloride buffers with a detection sensitivity of up to 1 nM concentration, which is of pivotal importance for high sensitivity detection of cancer cells and bacteria detection for lab-on-chip applications.

See supplementary material for more details of the device fabrication, chip integration, channel configuration, frequency response, statistics for limit of detection, and Peclet number calculation.

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