A digital microfluidic system with integrated electrochemical impedance detection arrays

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Abstract: Electrochemical sensing provides a new way for miniaturization and low cost of equipment. Meanwhile, digital microfluidic (DMF) technology is making lab-on-chip a reality. The two technologies are essential in the point-of-care area. Electrochemical detection on electrodes and DMF-based droplet driving electrode realize their respective functions by means of electrical signals. In terms of working mode, it seems to have certain similarity, and electrochemical sensing seems to be more suitable for integrating digital microfluidic systems. We thus integrated electrochemical sensing on an active matrix (AM) DMF system. As a common transparent conductive glass, indium tin oxide (ITO) has been widely used as the top plate of the digital microfluidic chip. In this work, we used ITO glass with patterned interdigital electrodes as the top plate of the AM-DMF chip. We achieved on-chip droplet volume detection by electrochemical impedance spectroscopy (EIS). We also proposed a novel sample dilution method on the DMF chip. It takes only 30 seconds to dilute solutions into 4 different concentrations. We then analyzed droplet samples of different concentrations with EIS method. And the correlation coefficient of the fitted curve is as high as 0.9964. In conclusion, EIS is a powerful detection technology for fast, high-integration and low-cost detection on DMF system. The integration of EIS and DMF can be used in various biochemical analyses in the future.

1. Introduction
Digital microfluidic (DMF) technology is a key technology in the field of microfluidics, which can precisely control droplets in pico-liter to microliter sizes [1]. With its powerful liquid processing capacity, it can easily realize a series of biological reactions including pretreatment of biological reaction reagents, molecular amplification, immune binding, cell division and so on [2]. However, in order to determine the presence or absence of the disease, and detect the occurrence of biological reactions quantitatively or qualitatively, the chip needs to rely on a series of detection and analysis devices, such as micropore meter based on optical sensor, chemiluminescence instrument, mass spectrometer through mass spectrum analysis, [2,3]. All of the above analysis methods basically need to set up a large detection system outside the chip, which will not meet the urgent needs of human beings for low-cost, miniaturized and portable detection equipment.
Electrochemical detection technology is a conventional means to detect and analyze biological reactions through electrical signals [4]. Among them, electrochemical impedance detection mainly analyzes the biological reaction by analyzing the change of electrical impedance on the sensor electrode [5]. For highly integrated lab-on-a-chip systems, the combination of electrochemical detection technology and digital microfluidic chip is an important solution to realize the integration and miniaturization of sample manipulation, detection and analysis [6,7]. At present, the most common digital microfluidic chip structure is composed of an electrode bottom plate for driving droplets and a glass top plate coated with indium tin oxide (ITO). The orderly control of interlayer droplets is realized by applying preset voltage signal on the bottom electrode. And the top plate serves as the reference ground for the driving signal. We etched an interdigital electrode array on the ITO glass top plate. Therefore, electrochemical impedance spectroscopy (EIS) measurement can be conducted in this microfluidic chip. The three-dimensional schematic diagram of the active matrix (AM) DMF chip is shown in Figure 1(a). In this paper, we firstly built up an equivalent circuit model based on the structure of the chip. EIS measurement result of $\text{K}_3\text{Fe(CN)}_6 / \text{K}_4\text{Fe(CN)}_6$ probe solution and oil fairly verified this model. Then we used EIS impedance for detection of droplet volume. Our next work focused on obtaining droplets of different concentrations through the powerful liquid handling capabilities of the digital microfluidic chip. And through the method of electrochemical impedance sensing, we finally distinguished these on-chip handled droplets of different concentrations within a certain frequency range. The combination of EIS and DMF technology would pave the way for fast, high-integration and low-cost detection, and accomplish various biochemical analyses.

2. Experimental

![Figure 1](image)

Figure 1. (a). Three-dimensional schematic diagram of the AM-DMF chip. The array type interdigital electrodes on the ITO top plate are located directly above the bottom droplet driving electrodes. The electrode contacts of the chip are connected with the external drive module and the detection module. (b). A cross-sectional view of the chip, which is composed of an active digital microfluidic drive chip TFT backplane, an insulating layer, a hydrophobic layer, an ITO top plate with sensing electrodes, and a gap for droplet movement. (c). The design drawing of the 16 sensing electrodes on the ITO top plate of the chip, which are named 1 to 16 in turn, and the electrode pixel design of the TFT back plate. (d). Schematic diagram of impedance measurement system for the ITO top plate electrode.
2.1 DMF chip design and fabrication
Here, the DMF bottom plate is the electrode backplane based on an active matrix technology. The AM digital microfluidic chip integrates thin film transistors (TFT) as active switches into each pixel electrode, thus achieving independent pixel electrode control in a large-scale array [8]. And it realizes droplet manipulation by peripheral driving system. Related research has been reported in previous work. Figure 1 (b) shows the cross section of the chip, including TFT backplane, insulating layer (SiNx), hydrophobic layer (Teflon AF from Chemours), ITO glass top plate with sensing electrodes. 2cSt silicon oil from Dow Corning was used as the medium. Figure 1(c) shows an ITO top plate containing 16 interdigital electrodes with a size of 2mm*2mm. And the TFT backplane is composed of 32*32 AM electrode array with a single pixel size of 1mm*1mm.

2.2 Electrochemical impedance test
All electrochemical measurements were performed on a Gamry Interface 1010E electrochemical workstation. An AC signal with the amplitude of 1Vrms was used. The frequency range was from 10 Hz to 2MHz. Measurements were made using the most common \( \text{K}_3\text{Fe(CN)}_6 /\text{K}_4\text{Fe(CN)}_6 \) probe solution to verify the homogeneity of the sensor electrode.

3. Results and discussion

3.1 Equivalent circuit model analysis of impedance test

![Figure 2](image)

Figure 2. (a). The impedance test equivalent circuit diagram of the sensing electrode on the top plate. (b). Impedance modulus change of EIS Bode plot for the medium oil and the probe solution (c). Nyquist diagram of the medium oil and the probe solution. (d). Phase change of EIS Bode plot for the medium oil and the probe solution.

The impedance measurement in this work is based on a bipolar-electrode arrangement, and the
working principle diagram is shown in Fig. 1(d). Figure 2(a) shows the equivalent circuit diagram of the sensing electrode used for droplet detection on the top plate. It consists of two RC models (naming \( R_4 \) and \( C_4 \)) that simulate the line resistance and line capacitance of the electrode wires, two RC models that simulate the hydrophobic layer on the electrode surface (naming \( R_1, C_1 \) and \( R_2, C_2 \)), and an RC model (naming \( R_s \) and \( C_s \)) that simulates the test solution. Figure 2(b) and (d) show the Bode plots of the medium oil and probe solution measured by the No. 4 sensor electrode. Figure 2(c) shows the Nyquist diagram of measuring medium oil and probe solution by No. 4 sensing electrode. Among them, the green curve represents the data of measuring environmental oil, and the red curve represents the data of measuring the probe solution. The solid line represents the data fitted using the equivalent circuit diagram of Figure 2(a). In Figure 2(b), the impedance decreases as the frequency increases, indicating that the capacitance of the measured object dominates the impedance change [9]. The medium oil and the probe solution can be easily identified by the size of the impedance in the Bode plot. The spectrum line obtained by measuring the silicone oil in Figure 2(c) is a straight line perpendicular to the X-axis, that is, only the impedance of the imaginary part, indicating that the measured medium oil can be regarded as a pure capacitance. The measurement probe solution is in a state where capacitance and resistance coexist. In Figure 2(d), the measured environmental oil phase is constant around 90°, showing pure capacitance characteristics, which is consistent with the conclusion of the Nyquist diagram. The probe solution showed resistance characteristics in the high frequency region.

3.2. Impedance detection of droplet volume

We moved deionized water droplets of different sizes to the bottom of the sensing electrode, and further studied the volume of the droplets on the digital microfluidic chip by means of impedance analysis. We selected three sensing electrodes, naming No. 8 and No. 12, and No. 16, for droplet volume sensing detection. In order to explore the uniformity of these electrodes, firstly, the probe solution with a droplet bottom area of 2mm*2mm was tested through these three sensing electrodes. Figure 3(a) shows the EIS Bode plot measured by these three sensing electrodes, and the error bar represents the standard deviation between the three sensing electrodes. The result shows that these electrodes have a good uniformity. Since the powerline interference frequency is 50Hz, the phase data measured at this frequency has a relatively larger error. We also measured droplets with bottom area of 1mm*1mm, 1mm*2mm, and 2mm*2mm on the three electrodes. As shown in Figure 3(b), there are significant impedance differences among the three droplets of different volumes. Droplets of different volumes occupy different proportions in the impedance test loop area of 2mm*2mm covered with oil. The smaller the volume of deionized water droplets is, the greater the proportion of medium oil will be. It is reported that the dielectric constant of dielectric oil is much smaller than that of deionized water [10]. Therefore, the impedance value of the droplet with smaller bottom area is larger. The error bar represents the standard deviation of the measured droplets under the electrodes at three different positions. It further proves the uniformity of these electrodes. Figure 3(c) shows the relationship between the impedance value and the droplet size at a single frequency of 1KHz. Based on this relationship, we can accurately determine the droplet size on the digital microfluidic chip.

3.3. Sample dilution and impedance detection

Sample dilution is the most common liquid processing behavior in the application of digital microfluidic chips [11]. In our experiment, we demonstrated a method for quantitative dilution of samples on a digital microfluidic chip. We use a droplet with a 2mm*2mm bottom area as the smallest sample unit. As shown in Figure 4(a), we can dilute the 1X PBS sample into four concentrations in only 5 steps. These steps involve (1). Sample injection (2). Droplet tearing (3). Droplet generation (4). Droplet mixing (5). Stir. The sample dilution operation was repeated three times by the digital microfluidic chip.
Figure 3. (a). EIS Bode plots of probe solutions for three different electrodes. (b). The relationship between the impedance amplitude of the three different volume droplets and the frequency. The error bar represents the standard deviation of the droplet measurement at the three different positions of No. 8, No. 12, and No. 16. (c). The relationship between the impedance value and the actual droplet volume at the frequency of 1KHz.

The evenly mixed reagents were finally moved to the sensor electrode for EIS detection. The relationship between phase and frequency obtained under three measurements is shown in Figure 4(b), and the error bar represents the standard deviation between the three measurements. As we can see, in the frequency range of 50Hz-1KHz, the phase angle of deionized water is close to 90°. It means that the capacitance characteristics of the reagent sample dominate in this frequency range. This indicates that the capacitance characteristics of different concentrations of sample reagents may affect the passage of current [12]. Previous research work has reported that the dielectric constant of an ionic solution decreases as the ion concentration increases [13,14]. Therefore, the higher the concentration of the PBS solution is, the greater the dielectric constant and capacitance will be. An increase in capacitance results in a decrease in the capacitive reactance of electrical impedance. However, in this frequency range, the impedance modulus of sample solutions with different concentrations did not change significantly. Therefore, we believe that the change of solution concentration indirectly leads to the change of the impedance spectrum phase at this frequency. In addition, the quantification of the biological response between the electrodes by changes in the electrical impedance phase has been confirmed by researchers [15,16]. We noticed that for the target with different concentrations, the most obvious phase difference is at the frequency around 100 Hz. Figure 4(c) shows a certain linear
relationship between the PBS sample concentration and the phase angle at a frequency of 100 Hz. The correlation coefficient is 0.9964. It can be said that we explain the linear relationship between ion concentration and dielectric constant in the literature from another angle. Therefore, the sensor response at this frequency can be used to quantify the droplet concentration properties.

Figure 4. (a). DMF chip sample dilution, (b). The relationship between the phase and frequency of the four concentration samples, (c). The relationship between the sample concentration and the phase angle at a measurement frequency of 100 Hz.

4. Conclusions
In this paper, we reported a digital microfluidic analysis system based on electrochemical impedance. In this system, the patterned ITO top plate was applied on the DMF chip. The design of the electrode array on the ITO top plate provides the possibility to improve the detection throughput of the system. The AM-DMF chip was fabricated by TFT backplane technology. Sample dilution was completed within 30 seconds. Interdigitated electrodes array combined with electrochemical impedance method realized the detection of the droplet volume and droplet concentration. In the future, EIS method will contribute to the miniaturization and rapid detection of digital microfluidic equipment.

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