Monitoring Microfluidic Interfacial Flows Using Impedance Spectroscopy

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Abstract

Microfluidic platforms capable of complex on-chip processing and liquid handling enable a wide variety of sensing, cellular, and material-related applications across a spectrum of disciplines in engineering and biology. However, there is a general lack of available microscale sensors capable of non-optically monitoring and quantifying on-chip fluid motion. Hence, many microfluidic systems are confined to the laboratory because their use requires optical microscopy. Here, we present a method for dynamically tracking laminar interfacial flows in microfluidic channels non-optically using impedance spectroscopy. Using a microfluidic T-channel, we generate a liquid interface by co-flowing two different electrolyte streams side-by-side. The interface is driven through an array of "displacement" electrodes where it is electrokinetically deflected across the microchannel. The interfacial flow is monitored downstream using an array of interdigitated "impedance" electrodes which dynamically measure the electrochemical impedance near the surface of the flow channel. We demonstrate that the impedance spectrum is sensitively influenced by the position of the deflected interface. While laminar fluid interfaces are ubiquitous to microfluidic flows and used extensively in rheology and biomolecular detection, it is currently difficult to measure their position non-optically. The sensing method presented here enables the interfacial position to be dynamically determined without a microscope and provides a new tool for lowering the barriers to operating microfluidic devices outside the confines of a traditional laboratory.

Keywords: Electrical Impedance Spectroscopy, Microfluidics, Liquid Interface, Fluidic Dielectrophoresis

Introduction

Microfluidics offers an attractive platform for performing miniaturized chemical and biomolecular analysis. Particularly useful is the ability to embed multiple laboratory steps, including preparation and chemical detection, into a microfluidic chip for automated sample processing and analysis. An important engineering design challenge in microfluidics is to develop new *in situ* analytical tools that monitor these operations within the confines of the microchannel network. Fluorescent-microscopy, for example, has been used to perform noninvasive quantification of liquid properties and flow velocity in microchannels [1]. Other techniques such as *in situ* Raman spectroscopy [2], imaging FTIR [3] and cyclic voltammetry [4] have been used to capture microscale chemical images of microfluidic channel surfaces and fluidic flow patterns. Another strategy relies on employing electrochemical impedance spectroscopy (EIS) to monitor electrical changes on microfluidic surfaces [5] and cells [6] in contact with electrolyte. Because EIS can be performed with micro-electrodes integrated on-chip, it has potential to be a powerful tool for rapid non-optical monitoring of microfluidic processes.

EIS is used for monitoring complex surface processes in microfluidic space without the use of fluorescent labels and fixatives. By detecting variations in impedance as a function of field frequency, EIS, for example, can be used to non-optically detect proteins [7,8] and DNA [9,10] through monitoring protein binding and DNA hybridization on electrode surfaces, where these interactions influence current flow and the corresponding impedance spectrum [17,18]. EIS can also be used to quantify cell growth [11,12]. Cells, for example can be grown on the surface of patterned EIS micro-electrodes [13]. The electrode impedance has been shown to be influenced by the cell type and cell state [14-16].

Because EIS can be easily integrated into microfluidic flow channels, its utility could be extended to non-optically monitoring fluidic behavior for monitoring on-chip mixing [19] and routing operations [20] where multiple fluid streams are routed, mixed, and pumped across a network of microchannels. In this work, we demonstrate how to

use EIS for non-optically monitoring fluid motion produced by an electrokinetic flow. One feature of low Reynolds number (Re) flows is that multiple liquid streams can flow side-by-side without convectively mixing. The result is that well-controlled microfluidic laminar interfaces can be created between both miscible and immiscible liquids. Interfacial flows are ubiquitous in low Re systems and play important roles in microfluidic applications in rheology [21], chemical detection [22], molecular mass sensors [23], immunoassays [24], DNA hybridization [25], and kinetic analysis [26,27].

Recently, we reported a new electrokinetic method for manipulating these liquid interfaces [28]. The technique was demonstrated using two fluids with different electrical properties driven side-by-side in a microfluidic channel. When an alternating current (AC) electric field was applied perpendicular to the direction of flow, the laminar interface polarized and the fluid electrohydrodynamically displaced across the flow channel. The motion of the fluid interface was shown to be an effective electrokinetic method for assessing fluid electrical conductivity and dielectric constant [29], and for detecting biomolecular binding [30]. In order to perform these fluidic measurements, however, the liquid interface required constant optical monitoring with confocal microscopy. While, effective, a non-optical approach could offer a smaller, less costly and potentially automated means to perform interfacial measurements.

In this work, we present a non-optical EIS technique for dynamically tracking the position of a miscible microfluidic liquid interface under electrokinetic flow. Our method utilizes two different electrode arrays: an upstream parallel point electrode array to polarize and induce electrokinetic flow at a liquid interface and a downstream series of interdigitated electrodes to dynamically measure the EIS on the microchannel surface. We show that the magnitude of the impedance ($|Z|$) is sensitive to the position of the liquid interface and can be used to track interfacial motion in response to electrokinetically-induced fluid flow. We propose that variations of this system may be useful for non-optically monitoring the dynamics of microfluidic interfaces in electrokinetics, rheology, biomolecular detection, and microfluidic mixing applications.

We begin by describing electrokinetic flow and interfacial displacement (sec 2.1). We then describe the experimental strategy for producing these flows (sec 2.2-2.3) and the experimental setup used to dynamically measure impedance (2.4). Finally, sec. 3 presents impedance results obtained for a range of electric field frequencies and interfacial positions.

2. Experimental

2.1 Electrokinetic Flow

AC electric fields have long been exploited to manipulate the fluidic contents of microfluidic systems. When an electric field is applied across a charged surface or within a non-neutral liquid domain, an electrical stress is produced which can drive fluid flow. In liquids, these electrokinetic flows occur when a field is applied tangential to a charged surface (e.g electro-osmosis) or across regions of fluid with spatial gradients in electrical properties (e.g electrothermal flow). Recently, a new type of electrokinetic flow was discovered in the vicinity of laminar liquid interfaces formed between two miscible aqueous electrolytes [28]. The phenomenon is known as fluidic dielectrophoresis (fDEP), which describes the ability to deflect a laminar liquid interface across a microchannel using an external AC electric field [29]. The interface is created using a microfluidic T-channel device where two fluids are forced to flow side-by-side. Each liquid has a different electrical conductivity (σ) and dielectric constant (ϵ) such that a large electrical mismatch exists at their interface. fDEP motion is created using a perpendicular electric field produced from an array of parallel point electrodes integrated on the surface of the microfluidic channel (Fig 1a). For a liquid interface subjected to a time varying monochromatic electric field, the displacement is directly proportional to the real part of the interface polarizability factor, $K(\omega)$, which is function of field frequency (ω), electrical conductivity and permittivity [29]:

$$Re[K(\omega)] = \frac{(\epsilon_2 - \epsilon_1)\tau^2\omega^2}{(\epsilon_2 + \epsilon_1)(\tau^2\omega^2 + 1)} + \frac{(\sigma_2 - \sigma_1)\tau^2\omega^2}{(\sigma_2 + \sigma_1)(\tau^2\omega^2 + 1)}, \quad (1)$$

where $\tau = \left(\frac{\varepsilon_2 + \varepsilon_1}{\sigma_2 + \sigma_1} \right)$ is the characteristic Maxwell-Wagner charge relaxation timescale at the interface between the two liquids. As depicted in Eq. (1), because the interfacial polarization (e.g. charging) is driven by both conductive and dielectric charging, displacement is a function of the electrical properties of each fluid stream and the AC electric field frequency. At frequencies on the order of 100 kHz, for example, the magnitude of interface displacement is governed solely by differences in the electrical conductivity between the two co-flowing fluids, which we define here as the interfacial conductivity $(\sigma_2 - \sigma_1)$. At high frequency (typically > 10 MHz), however, the displacement is driven by the interfacial permittivity $(\varepsilon_2 - \varepsilon_1)$. Finally, at intermediate frequency the interface behavior is sensitive to both electrical and dielectric differences. Because of the low and high frequency attributes of fDEP, if one fluid phase has a greater conductivity $(\sigma_1 > \sigma_2)$ and the adjacent fluid has a greater dielectric constant $(\varepsilon_2 > \varepsilon_1)$, the direction of the interface displacement will reverse at a critical frequency and there will be a characteristic cross-over frequency (COF) where no interface displacement is observed. This occurs when the interface's polarizability factor is zero, which can be expressed as [29]:

$$\omega_{COF} = \frac{1}{2\pi} \left[\frac{(\sigma_1 - \sigma_2)(\sigma_1 + \sigma_2)}{(\varepsilon_2 - \varepsilon_1)(\varepsilon_2 + \varepsilon_1)} \right]^{1/2}. \quad (2)$$

At low frequency below the COF, the high conductive fluid displaces across the flow channel (Fig 1b). At the COF the net charge on the interface is zero and no displacement is observed (Fig 1c). Finally, above the COF the interface displaces in the opposite direction (Fig 1d) and the low conductive high dielectric fluid displaces across the channel. We next discuss the experimental setup for measuring the displacement direction and magnitude non-optically using EIS.

2.2 Design and Fabrication of Microfluidic Device with Embedded Electrokinetic and Impedance Electrodes

The experimental design requires a laminar interface and two different types of

electrode arrays to displace and subsequently detect the interface position. We used a microfluidic “T-channel” device to create the liquid interface. Two fluid streams were supplied to the microfluidic device using a low-cost constant pressure source flow system [31]. The microfluidic device was fabricated using standard soft photolithography and microfabrication techniques. Microchannel electrodes were fabricated using wet chemical etching. Glass cover slips (50x30 mm, no.1, Fisher Scientific) were coated with 2 nm of chromium and 50 nm of gold using electron beam evaporation. The cover slips were patterned with photoresist (Shipley 1813) and exposed metal was etched using gold and chromium etchant. The resulting electrode pattern was then aligned and bonded to a soft lithographically fabricated T-channel device. To fabricate this device, the “T-channel” pattern was lithographically fabricated to a silica wafer using SU-8 3050 photoresist (Microchem Corp.). A 10:1 mixture of polydimethylsiloxane (PDMS) elastomer and curing agent was poured atop the wafer and baked at 85 °C for 30 minutes. The PDMS was gently peeled off the wafer and cut out of the mold. Fluid ports were punched with a 0.75 mm diameter biopsy punch (Ted Pella, Inc.). The electrode patterned coverslip was then exposed to oxygen plasma (Jelight, Model 42A), the PDMS microchannel was exposed using a handheld tesla coil (Electro-Technic Products Inc. Model BD-20) and the two substrates were immediately aligned and sealed under an inverted microscope. The assembled device consisted of a main flow channel 150 μm in width and 65 μm in height with an upstream displacement (parallel-point) and a downstream impedance (45°-interdigitated) electrode array (Fig 1). To perform an experiment, the fluid interface was subjected to an electric field using the parallel-point electrode array and forced to displace by fDEP at different field frequencies (Fig 1b-d). As fluid exited the first displacement array, the interfacial stress ceased. Because the inertial influence on the flow is minimal ($\text{Re} < 1$), the fluid interface remained fixed in a displaced position immediately after exiting the fDEP array. We then determined the deflection position by measuring the magnitude of the impedance using a second array of interdigitated electrodes.

2.3 Chemicals and Reagents

The liquid interface was composed of two fluids, each with a different electrical conductivity (σ) and dielectric constant (ϵ). When forced to flow side-by-side at low Reynolds number these two fluids formed an interface with a large electrical mismatch between them. Each stream was injected at a constant flow rate (10 $\mu\text{L}/\text{min}$) into the device using a low-cost flow controller equipped with an externally pressurized fluid-filled cryogenic vial [31]. We labelled each fluid with a different Alexa Fluor fluorescent dye to accurately image the interface position using confocal microscopy. Shown in Fig 1, the electrical interface was formed by flowing a left-most (green) 1x PBS solution ($\sigma_1 = 0.29 \text{ mS}/\text{cm}$; $\epsilon_1 = 78$) with 10 ng/mL of Alexa Fluor 488 (Invitrogen). The right-most (red) high dielectric stream ($\sigma_2 = 19 \mu\text{S}/\text{cm}$; $\epsilon_2 = 110$) was comprised of 0.8M 6-aminohexanoic acid (Sigma-Aldrich) (AHA) labeled with 10 ng/mL of Alexa Fluor 594 (Invitrogen). AHA is a water-soluble zwitterion used for increasing the dielectric constant of aqueous solution [28]. Prior to fluorescent labeling, the AHA solution was polished with 1 g/mL Dowex MR-3 (Sigma) ion exchange resin to remove trace salts and reduce solution conductivity. The COF of this electrolyte system was measured using previously published methods [29] and found to be 6.2 MHz.

2.4 Impedance Measurement

We utilized the upstream parallel-point array to drive fDEP flow across the channel and a second downstream 45°-interdigitated array as an impedance sensor. The parallel-point electrodes were axially separated by 20 μm and symmetrically bridged the width of the microchannel. We used electrodes with sharp points to focus the electric field to the tip of the electrodes and to provide increased contact with the PDMS and glass substrate along the main flow channel walls. A function generator (Rigol DG4102) was connected to the fDEP electrodes and delivered an AC electric field to displace the interface across the channel. The downstream impedance electrodes were interdigitated and positioned at a 45° angle relative to the flow direction to maximize the sensitivity of the array to changes in interfacial position. An impedance spectrometer (Sciospec ISX-5) was connected to the impedance electrode array and used to measure the magnitude of the impedance as a function of interface position. For

all impedance measurements, a sine-modulated AC potential of 50 mV was applied to the electrode array and the magnitude and phase angle of impedance were measured over an excitation frequency range between 100 kHz to 10 MHz.

3. Results and discussion

3.1 Imaging Interfacial Position During Electrokinetic Displacement

Fig 1b-d depicts a top-view of the interfacial motion of the interface over the length of the fDEP electrode array when a 10-volt peak-to-peak (V_{pp}) potential was applied across the displacement electrodes at three different field frequencies (1 MHz, 6.2 MHz, and 20 MHz). When the electric field frequency was 1 MHz, the conductive PBS (green) stream displaced across the interface (Fig 1b). When the COF (6.2 MHz) was applied, both conductive and dielectric forces were equal, and the interface remained fixed as it passed through the electrode array (Fig 1c). Finally, at a frequency above the COF (20 MHz) the deflection reversed direction and the high dielectric stream (red) displaced across the microchannel (Fig 1d).

3.2 Characterization of Electrokinetic Interfacial Flow Using Impedance Spectroscopy

Because the interfacial-driven motion of the fluid ceases upon exiting the displacement electrode array, the interface's displaced position can be accurately determined using the downstream impedance electrode array (Fig 2). The top-view micrographs shown in Fig 2a-c illustrate that when the interface was subjected to either a low or a high frequency electric field, a greater degree of either high conductive-low dielectric PBS or low conductive-high dielectric AHA covered the sensing electrodes at low and high field frequency, respectively. To observe the 3D structure of the interfacial flow field, 2D confocal micrographs were captured above the impedance electrode array for three different electric field frequencies: 1, 6.2 and 20 MHz (Fig 2). Since the displacement electrodes are thin co-planar films (~52 nm) and confined to the microchannel surface, the electrical stress responsible for driving flow was localized

near the surface of the microchannel. In order to satisfy mass conservation, this local electrokinetic flow was countered by a pressure driven back flow at the top of the channel which produced a “tilted” interface, as shown in the 2D confocal micrographs in Fig 2. Because the impedance electrode array is also confined to the microchannel surface, the impedance measurements were only sensitive to the electrical properties of the fluid domain very near the surface where the electric field was capable of penetrating into the liquid domain. Therefore, differences in interfacial position produced by fDEP will create changes in both the local conductivity and dielectric constant of the fluid near the impedance sensor.

In order to determine the optimum impedance conditions for measuring interfacial position, we first determined how upstream fDEP displacement influences downstream impedance over a range of impedance excitation frequencies. An electrical interface was created by co-flowing solutions of PBS and AHA, and then deflected at frequencies below (1 MHz) and above (20 MHz) the COF, and when no field was applied (e.g. the position at the COF). For each interface position, we performed an impedance frequency sweep from 100 kHz to 5 MHz to determine the magnitude of impedance for different interfacial positions (Fig 3). Below the interfacial COF at 1 MHz, the fDEP electrodes polarized and forced the high conductive (green) stream to cover a larger area on the impedance electrode array. Conversely, when we applied a high frequency above the COF, the high dielectric stream displaced across the interface and the impedance sensor was exposed to fluid with lower electrical conductivity. The impedance data was consistent with the electrical changes that the interface position produces in the vicinity of the impedance electrodes. When high conductive-low dielectric PBS covered a greater portion of the impedance electrode array, the impedance decreased, while the opposite was seen when the low conductive-high dielectric AHA stream displaced across the impedance electrode array. Because the interface position is not influenced by the electric field at the COF, the magnitude of the impedance at the COF is identical the case when no displacement field was applied since the interface does not displace at the COF. Shown in Fig 3, the interface deflection produced the greatest change in the magnitude of the impedance ($|Z|$) at an

impedance frequency of ~500 kHz. Based on these experiments, we measured $|Z|$ at a frequency of 500 kHz for all subsequent experiments in this work.

3.3 Determining Interface Position Using Impedance Spectroscopy

With the impedance excitation frequency fixed at 500 kHz, we next measured $|Z|$ as a function interface position for three different applied voltages (5 V_{pp}, 10 V_{pp} and 15 V_{pp}) delivered across the interface using the upstream displacement electrode array. For each applied voltage the fDEP frequency was continuously swept from 1 - 20 MHz, and then back to 1 MHz while simultaneously measuring $|Z|$ at the downstream impedance array. Shown in Fig 4, when the interface is centered at the COF, $|Z|$ was found to be 32.5 k Ω for all three voltages applied. However, when an fDEP frequency below the COF was applied, the high conductive PBS stream covered a larger portion of the impedance sensor surface and the impedance decreased from 25 k Ω to 15 k Ω at an applied voltage of 5 V_{pp}. When the fDEP frequency increased above the COF, the high dielectric fluid covered a greater portion of the sensor surface and $|Z|$ increased to 45 k Ω . The impedance was also influenced when the electrical interface was subjected to larger displacement voltages. This increase was particularly noticeable at high frequencies (> COF) where more low conductive, high dielectric AHA buffer covered a greater area of the impedance sensor. In order to visualize the interface, we captured 3D confocal micrographs of the interface when a 20 MHz AC electric field was applied at voltages of 5, 10 and 15 V_{pp}. Shown in the micrographs in Fig 4, the AHA stream (red) covers a greater electrode area with increasing applied voltage. While there is a large change in impedance with increasing voltage at high frequencies, the three $|Z|$ datasets are not as strongly influenced at low frequencies (< COF). This is because changes in the position of the high conductivity buffer on the impedance electrodes do not influence the impedance to the same magnitude as the AHA buffer. Lastly, when the interface COF was applied to the interface, the interface did not displace in the electric field, and therefore the impedance did not change with applied voltage.

Next, we determined if it was possible to distinguish the COF between two fluid

interfaces with different electrical conductivity mismatches. In Fig 5, we show impedance results for two different interfacial conductivities – one where $\Delta\sigma = \sigma_2 - \sigma_1 = 0.27$ and a second interface where $\Delta\sigma = 0.61$ mS/cm. Shown in Fig 5, we observed a difference in measured impedance for the two different interfaces, where the impedance measurements for a given interface position increased with interfacial conductivity. For an alternative way to analyze these results, we normalized the impedance of each dataset by the initial impedance value when no electric field was applied using a time-averaged baseline impedance reading that was taken prior to applying an electric field at the displacement electrodes. Shown in Fig 5b, we see that both fluidic systems begin and end at the same dimensionless impedance, $|Z|^*$, but their COF's are different and increase with interfacial conductivity, as is consistent with previous fDEP experiments [29].

4. Conclusions

In this paper, we presented a novel method for detecting an electrokinetically displaced liquid interface using impedance spectroscopy. Using an AC electric field, we forced a laminar fluid interface to deflect across a microchannel using fDEP and then measured the position of the deflected interface using a downstream impedance electrode array. The interface was composed of two co-flowing fluids - one stream had a greater electrical conductivity and the other had a greater dielectric constant. When the interface was exposed to a low frequency AC electric field, high conductive fluid stream displaced across the channel and covered a larger surface area of the impedance sensor, which reduced the magnitude of the impedance. As the frequency was increased to the COF, the interfacial position moved to its original position which reduced the amount of conductive fluid over the impedance array. At higher electric field frequencies above this COF, the interfacial deflection reversed direction and the high dielectric, low conductive stream covered a larger surface of the impedance sensor and produced an increase in impedance. This method was able to electrically detect interfacial displacement and measure the interface COF non-optically at a resolution consistent with experiments performed with confocal microscopy. We believe this

method provides a new sensing technique for non-optically imaging microfluidic flow fields. While successful at detecting motion induced by electrokinetic flows, we observed that hydraulic-induced changes in interfacial position through increased flow rate also influenced the impedance measurements. Future work will focus on developing an equivalent circuit model to account for this influence and to accurately predict the limit of detection for which interface motion can be non-optically measured.

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Figure Captions

Figure 1. Confocal micrograph with two embedded electrode arrays. (a) Two co-flowing fluids with varying electrical properties in are driven into a microfluidic t-channel device. Each inlet channel (75 μm in width) merges with a main 150 μm wide main channel to create a sharp liquid interface. One stream has a larger conductivity (green), while the adjacent stream (red) has a large permittivity. The main channel has two embedded electrode arrays - displacement electrodes actuate the liquid electrical interface and sensing electrodes measures the local impedance. (b) A 1 MHz ac electric field is applied to displacement electrodes, displacing the high conductive stream (green) into the low conductive stream (red) A dotted white line shows the original interface position when no electric field is applied. (c) At a COF of 6.2 MHz the interface does not deflect in the field. (d) At 20 MHz the high dielectric stream (red) displaces into the low dielectric stream (green).

Figure 2. Top-down confocal micrographs of the interface position taken above the impedance sensing electrodes for three different electric field frequencies applied at the upstream displacement electrodes. A magnified 3D confocal z-stack is depicted below each top-down micrograph. For each image pair, a dotted white line highlights the interface position when no field is applied. (a) At a field frequency of 1 MHz the high conductive stream (green) is driven across the channel surface. The corresponding 3D confocal stack shows that an increase in conductive green fluid covering the surface of the sensing electrodes. (b) The COF, 6.2 MHz, is applied and the liquid interface remains fixed. (c) The direction of displacement reverses at 20 MHz and the high dielectric stream displaces into the low dielectric stream. The 3D image shows the direction reversed, leading to a decrease in conductive stream covering the impedance electrodes.

Figure 3. The magnitude of the impedance $|z|$ versus applied excitation frequency for different fDEP interface positions. Three frequency sweeps were performed in order to determine the optimal frequency for the impedance spectrometer. When 1 MHz is applied to the displacement electrodes, the high conductive-low dielectric (green) stream covers more impedance sensor electrode area and the impedance magnitude decreases. When a 20 MHz electric field is applied the high dielectric-low conductive (red) stream occupies a larger sensing electrode area and the impedance increases. The magnitude of impedance remains the same when the field is off and the COF is applied.

Figure 4. Magnitude of the impedance for different fDEP frequencies at an impedance excitation frequency of 500 kHz. The interface was displaced at 5, 10 and 15 V_{pp} . As voltage increased, the displacement magnitude increased. This is illustrated in the 3D confocal images taken at a fDEP frequency of 20 MHz. As depicted, as the voltage increased the low conductive (red) stream covered a greater electrode surface area. This is quantitatively shown - the magnitude of impedance increases with increasing voltage.

Figure 5. Impedance taken at an excitation frequency of 500 kHz versus applied fDEP frequency for two different interfacial conductivity differences. (a) The magnitude of impedance for varying applied frequencies, 1 – 20 MHz. The interface with a lesser interfacial conductivity has a larger measured impedance. (b) The magnitude of impedance for both systems is rendered dimensionless to compare the two datasets. The impedance measurement is able to non-optically determine that the COF increases with increasing interfacial conductivity.

Figure 1

Figure 2

(a) 1 MHz

(b) 6.2 MHz

(c) 20 MHz

Figure 3

Figure 4

Figure 5