



# Using capacitance measurements in EWOD devices to identify fluid composition and control droplet mixing

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## ABSTRACT

The use of capacitance measurements to identify the composition of droplets and monitor mixing in electrowetting on dielectric devices is examined here. Measurements were repeatable at each addressable location, with standard deviations on the order of 0.1 pF and a two-point calibration allowed repeatable differentiation of water–methanol solutions as the capacitance was linear with concentration. Capacitance at addressable locations was monitored throughout the mixing of water–methanol solutions. It was shown analytically and experimentally that the dimensionless capacitance is approximately equal to the dimensionless dielectric constant for practical EWOD applications. The number of cycles required for complete mixing remained constant for periods of actuation between 400 and 1000 ms and applied voltages between 90 and 110 V<sub>RMS</sub>. Although minimizing actuation period and maximizing droplet velocity decreases mixing time, these parameters have little effect on the number of cycles necessary to achieve mixing in EWOD devices. This shows mixing efficiency in EWOD devices is better described by the number of cycles, not the time, required for full mixing.

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## 1. Introduction

Over the past two decades, droplet-based microfluidic devices have been introduced as tools to increase throughput and reduce operating costs of biological protocols (e.g. [1–4]). Device platforms have been introduced to manipulate droplets by chemical [1], thermal [1], acoustic [2], and electrical [3] means. Electrowetting on dielectric (EWOD) is one of the most promising droplet-based microfluidic platforms. These devices apply asymmetric electric fields to manipulate droplets with diameters on the order of 1–2 mm that are confined between parallel plates separated by 50–150  $\mu\text{m}$  ( $\sim 40$ –500 nL) [3–5,7]. Droplets manipulated in EWOD devices have two observable phenomena: (1) asymmetric deformation of the droplet interface and (2) motion of the confined droplet. Some investigations argue that the change in the interface shape drives droplet motion (e.g. [4–6]), while others suggest that the motion is purely an electrical effect (e.g. [7,8]). Despite disagreement on the physical mechanism, these devices have demonstrated the ability to create, move, split, and mix droplets of fluid. They also have low power consumption, high reversibility, and wide applicability to different fluids [3–8]. A comprehensive review of these devices can be found in [9]. One of the proposed advantages of microfluidic systems, and EWOD devices in particular, is the ability

to increase throughput by automating protocols and running them in parallel. Monitoring EWOD devices is often performed manually, but this task must be automated for practical applications.

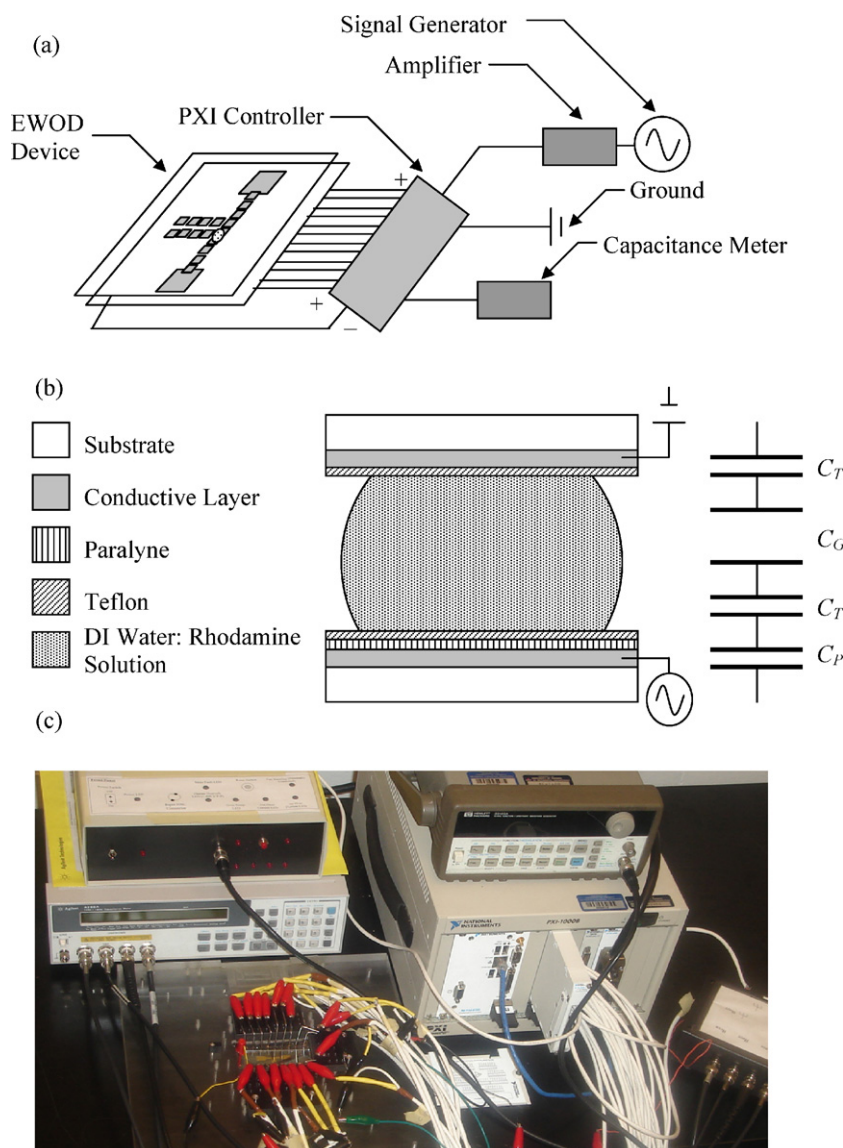
Most systems for automated droplet control in EWOD devices depend on either optical observation [10,11], or capacitance measurements [12–15]. A system that could produce complex droplet paths in an EWOD device by actuating multiple electrodes at variable voltages was simulated in [10]. The proposed design used an optical control system to monitor droplet motion. The presence of a droplet at strategic locations can also be determined using the integrated thin-film photodetector presented in [11]. If the dielectric constant of the droplet differs from that of the surrounding medium, capacitance measurements can be used to determine the void fraction at each addressable location in an EWOD device [12–15]. Since each addressable location in these devices is essentially a parallel plate capacitor, no further fabrication would be required.

One of the earliest investigations using capacitive detection of droplets in EWOD devices was [16] which showed that capacitance measurements could detect droplets. This method has been used successfully to determine optimal usage of electrodes on EWOD devices [12,13] and to create droplets of uniform size [14,15]. This study will examine whether capacitance measurements in EWOD devices can identify fluid composition and monitor droplet mixing.

Mixing fluids is a common problem in most microfluidic devices. On the microscale, Reynolds numbers are very low and viscous mixing dominates. Mixing in EWOD devices is commonly achieved

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**Fig. 1.** Sketch (a) of the experimental facility used in this investigation, (b) the confined droplet device with an electrical diagram of the capacitance, and (c) a photograph of the facility used in this investigation.

by moving droplets along a prescribed path for a specified number of cycles (i.e. [17–19]). With visualization, the mixing time required on EWOD devices was found to be dependent on the actuation frequency, the device geometry, and the path used to mix the droplets [17–19]. Mixing in droplets on the microscale is generally attributed to the folding of the fluid within the droplet as it moves along a specified path (i.e. [19]). Observations of droplet mixing in a pressure driven micromixer can be found in [20]. It has been proposed that fluid motion in EWOD devices can be enhanced and controlled by following more complex paths (i.e. [17,18]) or imposing a time dependant rigid-body rotation [21]. It would be advantageous to be able to monitor droplet mixing without intrusive techniques or optical access to the device.

The objective of this investigation is to use capacitance measurements in EWOD devices to identify fluids of differing composition and to monitor droplet mixing without requiring optical access to the chip, modification of the droplet, or additional fabrication.

## 2. Experimental facility

The experimental facility used in this investigation consisted of the EWOD device and the electrical control system (Fig. 1a).

The EWOD device was made up of two silica glass slides. The upper and lower plates were approximately 25 mm × 75 mm and 75 mm × 75 mm, respectively. Both plates were cleaned using an organic solvent, Piranha, before fabrication. The lower plate was coated with a conductive layer made up of chromium and gold. This conductive layer was patterned into an array of 2 mm × 2 mm electrodes separated by a gap of approximately 60 μm. The bottom plate was also coated with a dielectric layer of Parylene. A Teflon layer was then added to increase the hydrophobicity of the surface. The slides used for the upper plate were purchased with a pre-existing ITO coating. A Teflon layer was also added to this plate. The thickness of the chromium, gold, ITO, Parylene, and the Teflon layers were approximately 10 nm, 100 nm, 150 nm, 1.5 μm, and 50 nm respectively. The Parylene and Teflon layers above the electrical bond pads were manually scratched away to provide good electrical contact.

The spacing between the two substrates is achieved using one piece of double sided 3M Scotch tape with a nominal thickness of approximately 95 μm [22]. The confined droplets in this investigation are solutions of DI water and methanol at various concentrations (Fig. 1b). Droplets of known volumetric concentration were deposited on the bottom substrate using a pipette

before adding the upper plate. The volume of fluid in droplet detection and mixing experiments was 1300 and 650 nL, respectively. The uncertainty in the pipette measurements was 1%. The mixing experiments used droplets of deionized water and a 75% methanol solution. Pure methanol was not used in these experiments as it evaporated during mixing.

Selective application of the electric field in the EWOD device was achieved with a control system consisting of a National Instruments PXI system, an Agilent 33120A signal generator, a custom amplifier, and an Agilent 4288A capacitance meter. The electrical signal was controlled by the PXI system: a PXI controller (PXI 8195), a matrix-switching device (PXI 2529) capable of controlling four inputs with up to 32 outputs, and a PXI GPIB controller. The inputs were the AC source and the capacitance meter. The output channels were connected to bond pads for each addressable location on the EWOD device using a custom built fixture (Fig. 1c). The electrodes were connected to the high voltage source for 350 ms, and the capacitance meter for 50–650 ms. The sequencing of these connections was programmed using Labview Real Time 8.2. The AC source voltage in this investigation was generated using an Agilent signal generator and a custom built amplifier. The actuation frequency in this investigation was held fixed at 10 kHz and the actuation voltage was between 90 and 110 V<sub>RMS</sub>. Control of the capacitance meter was achieved using the GPIB controller in the PXI system described above. The test voltage for the capacitance meter was 1 V<sub>RMS</sub> at a frequency of 1 MHz. The accuracy of this device is given as 0.08% for these parameters. For the magnitude of capacitance examined here, this led to a resolution of approximately 0.20 pF.

### 3. Results and discussion

If each addressable position in the EWOD device is modeled as a number of parallel plate capacitors in series (Fig. 1b), then the capacitance in each measurement volume is given by

$$C_{Fi} = \frac{\epsilon_0 \epsilon_T \epsilon_P \epsilon_{Fi} A}{\epsilon_{Fi}(2\epsilon_P t_T + \epsilon_T t_P) + \epsilon_T \epsilon_P t_G}, \quad (1)$$

where  $\epsilon_0$  is the permittivity of free space,  $\epsilon$  is a dielectric constant,  $A$  is the area of the electrode,  $t$  is the material thickness, and the subscripts  $T$ ,  $P$ ,  $G$  and  $Fi$  denote the Teflon layer, the Paralyne layer, the gap between the substrates, and the fluid in the measurement volume respectively. In order to understand how capacitance measurements in EWOD devices change with droplet composition, it is useful to derive the difference between two capacitance measurements. Using (1), it can be shown that the difference between the capacitance and a reference capacitance is

$$C_{F1} - C_{F2} = \frac{\epsilon_0 A}{t_G} \beta (\epsilon_{F1} - \epsilon_{F2}), \quad (2)$$

where

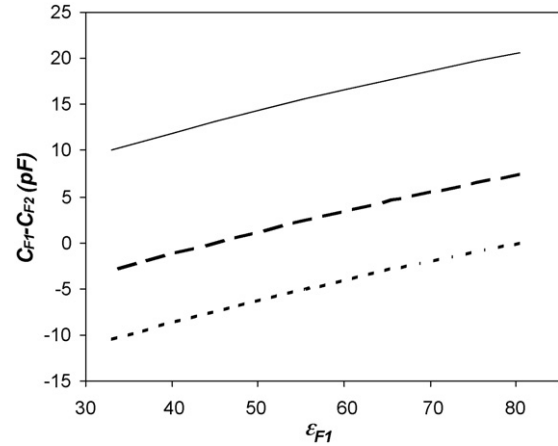
$$\beta = \frac{1}{(4\epsilon_{F1}\epsilon_{F2}/\epsilon_T^2)(t_T^2/t_G^2) + (4\epsilon_{F1}\epsilon_{F2}/\epsilon_P\epsilon_T)(t_T t_P/t_G^2) + (\epsilon_{F1}\epsilon_{F2}/\epsilon_P^2)(t_P^2/t_G^2) + (2(\epsilon_{F1} + \epsilon_{F2})/\epsilon_T)(t_T/t_G) + ((\epsilon_{F1} + \epsilon_{F2})/\epsilon_P)(t_P/t_G) + 1} \quad (3)$$

and the subscripts  $F1$  and  $F2$  denote the fluid being examined and a reference fluid, respectively. As the fluid within the measurement volume ( $F1$ ) changes,  $\epsilon_{F1}$  changes as well. Since  $\epsilon_{F1}$  exists in  $\beta$ , it introduces non-linearity into (2). An order of magnitude analysis can be used to understand the extent of this non-linearity. In most cases,  $\epsilon_{F1}$  and  $\epsilon_{F2}$  will be an order of magnitude larger than  $\epsilon_P$  and  $\epsilon_T$  (Table 1). In the devices tested here  $t_G$  was four orders of magnitude greater than  $t_T$  and two orders of magnitude greater than  $t_P$  (Table 1). The highest order term in the denominator of  $\beta$  is 1. If terms in the denominator of  $\beta$  smaller than  $10^{-2}$  are neglected, then:

$$\beta \approx \frac{1}{(\epsilon_{F1}\epsilon_{F2}/\epsilon_P^2)(t_P^2/t_G^2) + ((\epsilon_{F1} + \epsilon_{F2})/\epsilon_P)(t_P/t_G) + 1}. \quad (4)$$

**Table 1**  
Material Properties.

Parameter (unit)	Value
Dielectric constants:	
Air	1.0
Methanol	33.0
75% methanol	44.9
Water	80.4
Material thickness:	
Teflon (nm)	10
Parylene ( $\mu$ m)	1.5
Gap ( $\mu$ m)	100

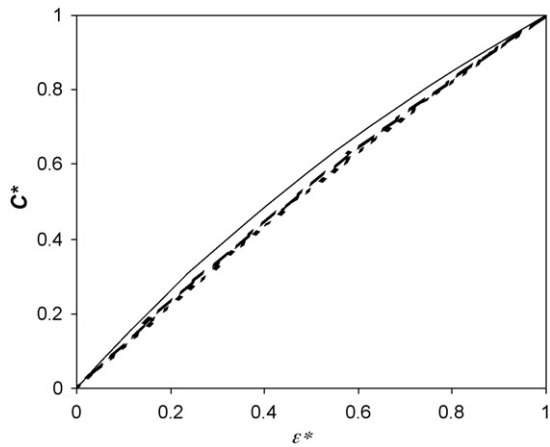


**Fig. 2.** Predicted difference in capacitance with a reference capacitance of air (solid line), 75% methanol (dashed line) and water (dotted line).

The predicted difference in capacitance is shown in Fig. 2 for reference capacitances of air, 75% methanol, and water. In all cases, the increase in the difference between the capacitance and the reference capacitance with  $\epsilon_{F1}$  is approximately linear. The slopes of a linear fit in all cases agree to within 0.4%. This shows that the difference in capacitance is approximately linear in the range of interest in EWOD devices. It is important to realize that the value of  $\beta$  affects the sensitivity of  $C_{F1} - C_{F2}$ . For example, as  $t_P \rightarrow 0$  then  $\beta \rightarrow 1$  and  $C_{F1} - C_{F2} \rightarrow \epsilon_0 A (\epsilon_{F1} - \epsilon_{F2}) / t_G$ . Thus, the EWOD device can be designed to increase the sensitivity of capacitance measurements.

Since the difference in the capacitance is approximately linear with  $(\epsilon_{F1} - \epsilon_{F2})$ , the dimensionless capacitance  $C^* = (C_F - C_{F2}) / (C_{F1} - C_{F2})$  is approximately equal to the dimensionless dielectric constant  $\epsilon^* = (\epsilon_F - \epsilon_{F2}) / (\epsilon_{F1} - \epsilon_{F2})$ . Here, the subscript  $F$ , refers to the fluid in the measurement volume and the subscripts  $F1$ , and  $F2$ , refer to two reference fluids respectively. This

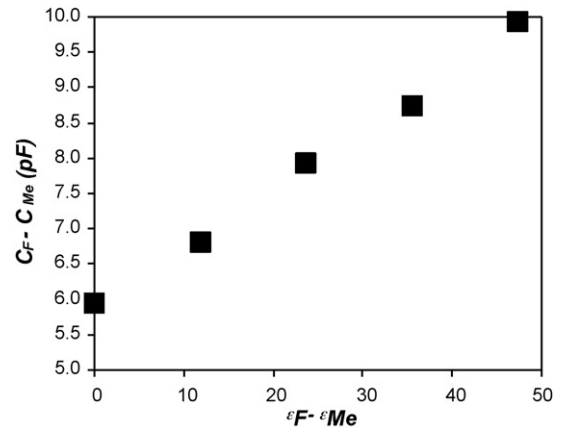
relationship is shown in Fig. 3. For the cases examined here  $F1$  was always water and  $F2$  was air, methanol, or 75% methanol by volume. The difference between  $C^*$  and  $\epsilon^*$  decreases with  $(\epsilon_{F1} - \epsilon_{F2})$  because the non-linearity in (2) decreases with  $(\epsilon_{F1} - \epsilon_{F2})$ . Therefore, the difference in the dielectric constants of the reference fluids should be minimized in order to identify fluids in EWOD devices. As such, the reference fluids should be those with the highest and lowest dielectric constants in an application. When water and methanol are used as the reference fluids, the value of  $C^*$  differs from that of  $\epsilon^*$  by an average of 0.03 and a maximum of 0.05. When water and 75% methanol were used, the average and maximum errors decrease to 0.02 and 0.04 respectively.



**Fig. 3.** Comparison of dimensionless capacitance and dielectric constants when F1 is water and an F2 is air (solid line), methanol (dashed line), and 75% methanol (dotted line).

Multiple capacitance measurements for a water droplet actuated between three electrodes in an EWOD device were taken to confirm repeatability; the distribution ( $G(T)$ ) is shown in Fig. 4. Although the standard deviations in each measurement volume range between 0.05 and 0.08 pF, the standard deviation of the entire population is approximately 0.45 pF. This suggests that although the capacitance measurements are repeatable at every addressable location, measurement calibration is required in order to compare data from different locations.

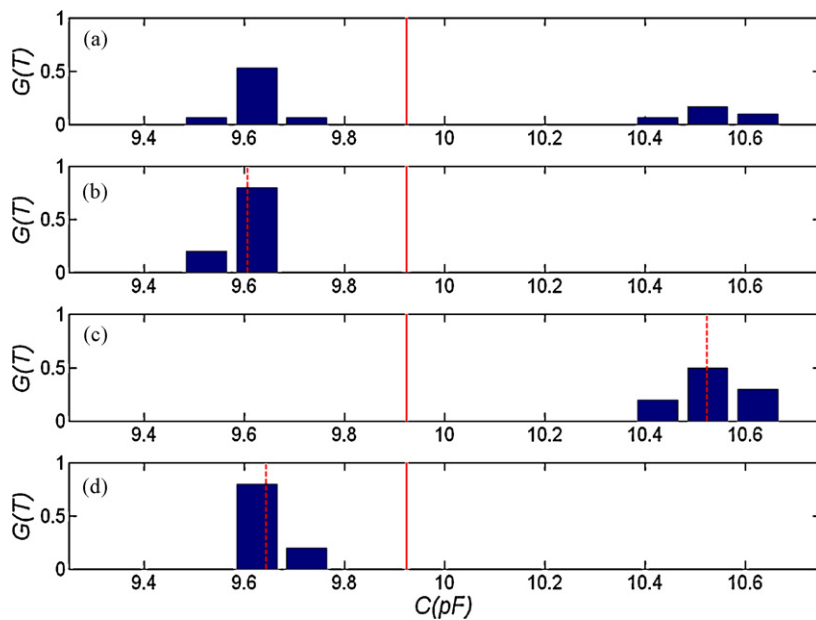
The type of calibration required can be determined by examining the measured capacitance as a function of the dielectric constant (Fig. 5). Here, each point on the curve is an average of 30 measurements distributed over three electrodes. As expected, the capacitance of the mixture increases linearly with  $\epsilon_F$ . The slope of the change in capacitance is approximately 0.08 pF for every unit change in the dielectric constant. This is approximately 0.04 pF per percentage decrease in methanol concentration. Since the experimental resolution is on the order of 0.2 pF, this suggests that it is possible to detect changes in



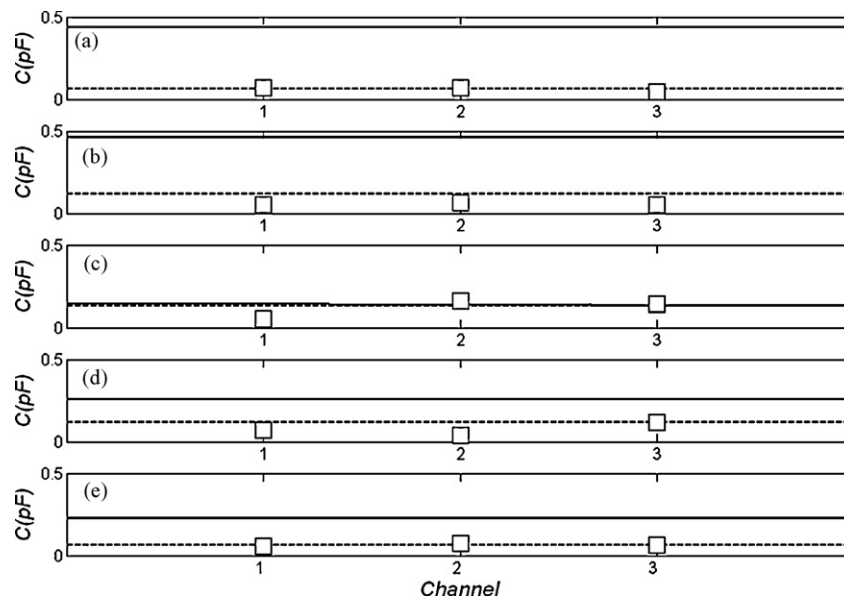
**Fig. 5.** Average  $\Delta C$  as a function of the  $\Delta \epsilon$ .

composition on the order of 5% methanol by volume. Because the change in capacitance is linear with concentration, the relative capacitance at each electrode was found using a two-point calibration. After performing the calibration, the standard deviation of the population of measurements for each solution was below 0.13 pF (Fig. 6). This is closer to the standard deviation on each channel and below the resolution of measurements. This suggests it is possible to identify the composition of solutions on EWOD devices and compare data from any addressable location.

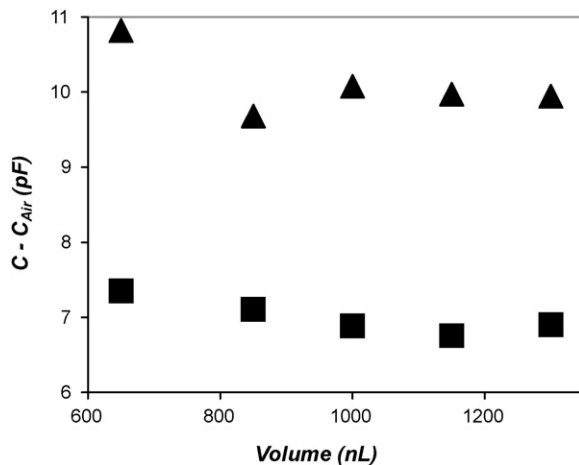
Another possible issue with comparing capacitance measurements of droplets in EWOD devices is the effect that the droplet radius has on the measurements. As droplets move through EWOD devices, they are susceptible to evaporation. This will change their cross-sectional area and could affect the capacitance measurements. The droplet radius was varied between 650 and 1300 nL in this investigation. Water and 75% methanol droplets of various volumes were actuated back and forth over four electrodes a total of 40 times. The average capacitance for each radius was found to agree with the average of the total population to within 5% (Fig. 7). The droplet radius has little effect on the capacitance measurements because the capacitance measurement is a function of the area of



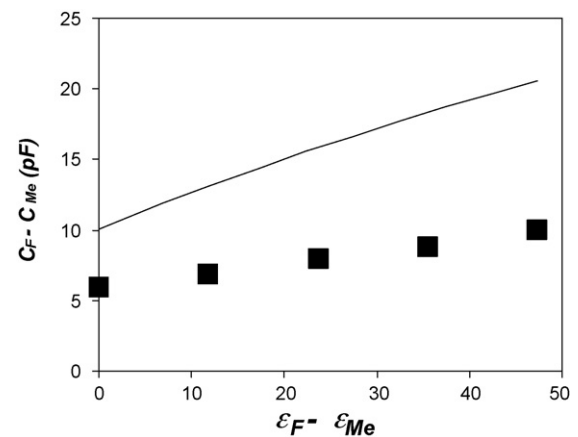
**Fig. 4.** Distribution of capacitance measurements for (a) the population of three addressable locations (b) the first location, (c) the second location and (d) the third location. Also shown are the average of the population (solid line) and the averages of the individual locations (dashed lines).



**Fig. 6.** Comparison of the standard deviation of the measured data on each electrode and the standard deviation of the entire population both before (solid line) and after (dashed line) calibration for solutions with methanol concentration of (a) 0%, (b) 25%, (c) 50%, (d) 75%, and (e) 100%.



**Fig. 7.** Average  $\Delta C$  as a function of droplet volume for water (triangle) and 75% methanol by volume (square).

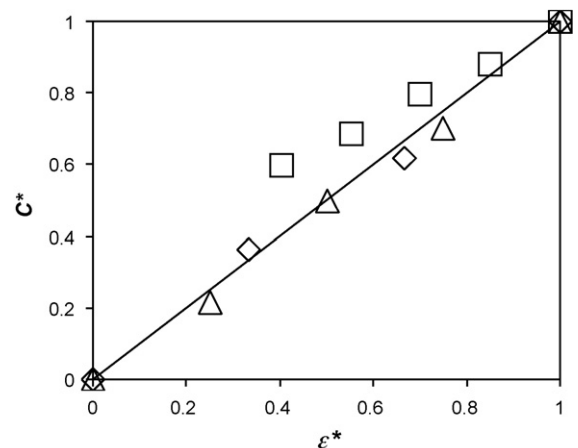


**Fig. 8.** Comparison of the measured (square) and predicted (line)  $\Delta C$  as a function of the  $\Delta\epsilon$ .

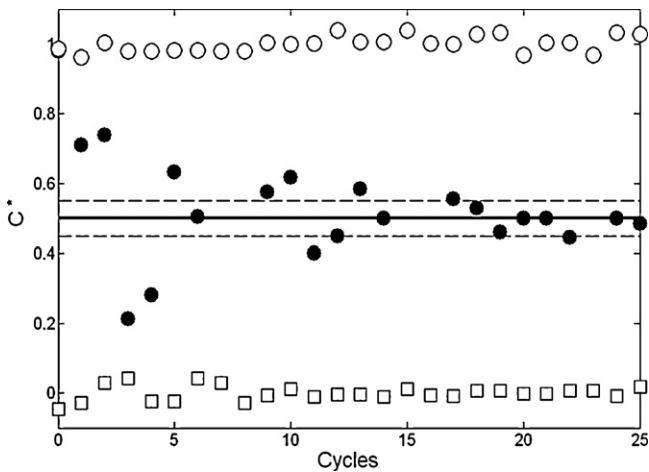
the electrode so long as the droplet fills the entire measurement volume. This has been suggested by [14].

The measured and predicted difference between the droplet's capacitance and that of methanol are presented in Fig. 8. The measured and predicted slopes are 0.08 and 0.22 pF for every unit change in the dielectric constant. This discrepancy shows that the simple series capacitance model presented here does not wholly capture the physics. The disagreement may be the result of parasitic capacitance, or the capacitance caused by the build up of charge at the droplet interface. Although the model presented here does not predict the value of the measured data, it does predict the shape. Since the measured data is still approximately linear,  $C^*$  still provides a valid approximation of  $\epsilon^*$  (Fig. 9). As with the predicted case, the accuracy of approximation increases as  $(\epsilon_{F1} - \epsilon_{F2})$  decreases. From this,  $C^*$  is a reasonable approximation of  $\epsilon^*$  for practical cases, despite the limitations of (2).

The change in dimensionless capacitance  $C^* = (C - C_{Me75}) / (C_W - C_{Me75})$  during the mixing of water and 75% methanol droplets is shown in Fig. 10. Data for the motion of 1300 nL droplets of water and 75% methanol are also provided to show that the value in these control cases does not change due to a change in



**Fig. 9.** Comparison of the measured values of  $C^*$  with water as F1 and F2 as air (square), methanol (triangle) and 75% methanol (diamond). A solid line with a slope of 1 is given to show where  $C^* = \epsilon^*$ .



**Fig. 10.** Dimensionless capacitance measurements from all electrodes during the mixing of water and 75% methanol (closed circle). Control cases of water (open circle), and 75% methanol (open square), and the expected capacitance (solid line) and the experimental resolution (dashed line) are also shown.

droplet volume, or a build up in charge at the interface. Initially, the capacitance measurements in the mixed droplet range between the capacitance of water and the 75% methanol solution. As the droplets are mixed, the capacitance approaches the expected capacitance of a 37.5% methanol solution. Since the capacitance for all electrodes converges in Fig. 9, mixing can be monitored in EWOD devices by measuring the capacitance at a single location along the mixing path.

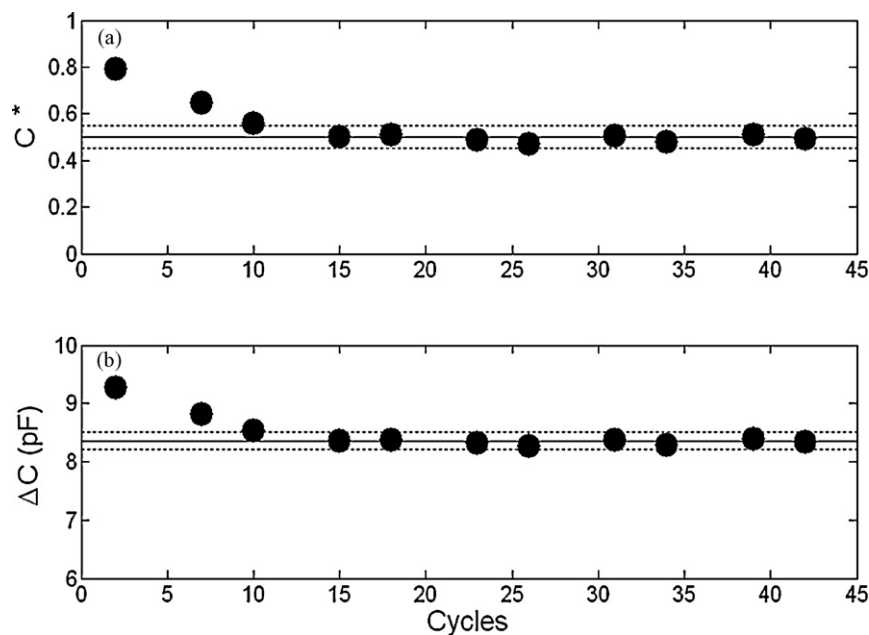
The dimensionless capacitance for a single electrode is shown in Fig. 11a. The capacitance is initially close to that of water and approaches a steady state value over time. If a single electrode is being used, uncalibrated data could monitor droplet mixing. Typical uncalibrated mixing data for a single location is shown in Fig. 11b. The first capacitance measurement from this electrode is approximately 9.3 pF and oscillates about 8.35 pF once mixing is completed. Eliminating the calibration decreases cycle time; however, more

motion cycles are necessary to ensure complete mixing, as the target capacitance is unknown.

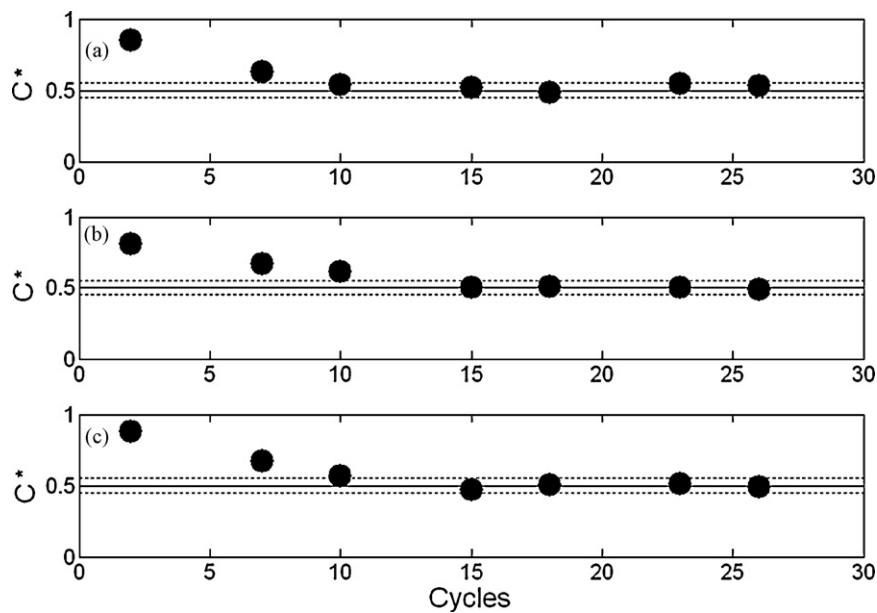
In this investigation, the droplet is considered fully mixed when the capacitance remains at the target capacitance plus or minus the experimental resolution ( $\sim 0.2$  pF). The capacitance measurements in Figs. 10 and 11 come to steady state at approximately 15 cycles, or 6 s. The number of cycles is similar to that reported in [18], and the time required for mixing is similar to that reported by [17] for a device with aspect ratios between 0.33 and 0.5. However, the aspect ratio here is 0.05 and [17] found that mixing time increased with aspect ratio below 0.4. It is unclear if mixing time or number of cycles gives a more appropriate characterization of mixing efficiency in EWOD devices. Mixing time consists of two components (1) the time the droplet is at rest between actuations and (2) the time the droplet takes to travel from one electrode to another. The effect of these parameters will be examined to determine if mixing time or number of cycles gives a better characterization of mixing efficiency in EWOD devices.

Capacitance was used to examine the relative importance of passive diffusion while two droplets were mixed on an EWOD device. This was accomplished by varying the actuation period between 400 and 1000 ms along the EWOD mixer. As the period increases, the droplet sits idle on each electrode for a longer period of time, allowing more time for passive diffusion to take place. Typical results for periods of 400, 600, and 1000 ms are presented in Fig. 12. The capacitance measurements in these three cases are similar, with the droplet achieving steady state at roughly 15 cycles. Viscous mixing inside the droplet dominates over diffusion here since the number of cycles required for mixing remained consistent over the range of periods tested. This is consistent with the literature (i.e. [19,20]) and suggests that EWOD mixers should operate at the minimum period required for repeatable droplet mixing. It also shows that total mixing time is not a suitable parameter to define mixing efficiency in EWOD devices. It is not yet clear if transit time or number of cycles is a more appropriate parameter.

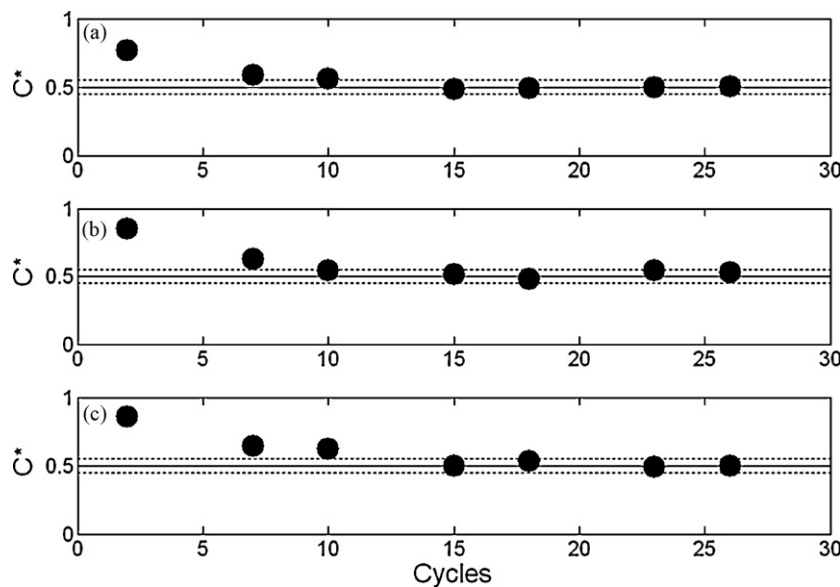
Capacitance measurements were also used to examine the effect of actuation voltage on mixing effectiveness in EWOD devices. The actuation voltage in the device was increased from 90 to 110 V<sub>RMS</sub>. Increasing the voltage increases the droplet velocity provided that



**Fig. 11.** (a) Dimensionless and (b) dimensional capacitance measurements from a single addressable electrode during the mixing of water and 75% methanol. Expected capacitance (solid line) and the experimental resolution (dashed line) are also shown.



**Fig. 12.** Dimensionless capacitance measurements from a single addressable location during the mixing of water and 75% methanol for actuation periods of (a) 400 ms, (b) 600 ms, and (c) 1000 ms. Expected capacitance (solid line) and the experimental resolution (dashed line) are also shown.



**Fig. 13.** Dimensionless capacitance measurements from a single addressable location during the mixing of water and 75% methanol for applied voltages of (a) 90 V<sub>RMS</sub>, (b) 100 V<sub>RMS</sub>, and (c) 110 V<sub>RMS</sub>. Expected capacitance (solid line) and the experimental resolution (dashed line) are also shown.

contact angle has not been saturated [5]. Although droplet velocity was not measured, from inspection the velocity did increase from 90 to 110 V<sub>RMS</sub>. At 90 V<sub>RMS</sub>, the droplet took the majority of the actuation time to move from one electrode to the next, but this time reduced rapidly at higher voltages. Droplets did not repeatedly move the length of the electrode for voltages below 90 V. Contact angle saturation occurs above 130 V with 0.1 M potassium chloride for a dielectric layer thickness of 2  $\mu\text{m}$  [23]. If the onset of contact angle saturation is similar in this investigation, droplet velocity will increase from 90 to 110 V<sub>RMS</sub>. The observed increase in velocity corresponds to a decrease in the droplet transit time. Despite the difference in transit time, the evolution of capacitance with the number of cycles was similar for all voltages examined here, with the droplet achieving steady state at roughly 15 cycles (Fig. 13). Since there is little effect of applied voltage over this range, an increase in the velocity of the droplet does not increase the effective-

ness of mixing in EWOD devices. This is expected for viscosity dominated mixing at the microscale. This also shows that mixing effectiveness in EWOD devices is best described by the number of cycles required, not the transit time for the droplet.

#### 4. Conclusions

Capacitance measurements can determine the composition of a droplet at a specific addressable location and monitor the mixing process on an EWOD device. Dimensionless capacitance for EWOD devices was analytically shown to be approximately equal to the dimensionless dielectric constant. This result was verified experimentally. Since the change in the capacitance was approximately linear, a two-point calibration of the capacitance measurements at each addressable position allowed for comparison of data at every position within the device.

Capacitance measurements are a useful tool to determine the state of droplet mixing in EWOD devices. The capacitance was initially near the values of the first droplet compositions and proceeded to the expected capacitance of the fully mixed solution. Calibrated and uncalibrated capacitance measurements at a single addressable location were sufficient to determine when the solution was fully mixed. Capacitance measurements were taken throughout mixing for actuation periods between 400 and 1000 ms, and voltages between 90 and 110 V<sub>RMS</sub>. It was shown that increasing the idle time between actuations, and the transit time of the droplet during mixing had little effect on the number of cycles required for full mixing. This shows mixing efficiency in EWOD devices is best described by the number of cycles, not the time, required for full mixing.

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