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Analysis on Micro-mixing Enhancement through a Constriction under Time Periodic Electroosmotic Flow

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Abstract

Numerical analyses of the recently proposed micro mixer based on periodic electroosmotic flow through a constriction were conducted. The validity and accuracy of the numerical model developed were verified experimentally. The model allows the prediction of operating parameters such as alternating current (AC) amplitude and frequency for optimum mixing. For accurate simulations of flow behavior such that optimum operating conditions could be determined, the electromigration effect of charged fluorescent dye added to the fluid stream must be considered. This effect, which is generally ignored, can be incorporated in the convection diffusion equation. Important factors that govern the mixing efficiency of the mixer, such as the contact area between the two fluids and the amplitude of fluid interface oscillation were quantified based on experimental and numerical results. Detailed error analyses were performed to investigate the sensitivity of simulation results to the variability of constriction width, diffusion coefficient, and fluorescein ion mobility. This also provides an understanding on the fabrication requirement for efficient mixing.

Keywords: Micro-mixing, electroosmotic flow, numerical simulation, fluorescent dye, electromigration

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1 Introduction

In recent years, lab-on-a-chip (LOC) devices have found numerous applications in chemical analysis and biomedical diagnosis. Micromixer is an important element in a LOC device where rapid mixing of samples and reagents are required. However, the Reynold numbers for flows in these micro-sized devices are typically very small due to low flow rate and small channel dimensions. As such, micro-mixing through inertial/viscous turbulence is typically difficult. Therefore, various micromixers have been developed to enhance the mixing efficiency of flow streams in microfluidic channels.

All micromixers can be generally divided into two categories, namely a passive mixer and an active mixer (Chang and Yang 2007). For a passive micromixer, no additional energy is supplied to enhance the mixing efficiency. Passive micromixers normally incorporate various geometrical features such as grooves on the channel floor (Stroock et al. 2002) or obstacles (Jeon and Shin 2009) along the channel to promote mixing between the fluid streams. Other passive approaches to enhance mixing efficiency include splitting and recombining flow streams (Hardt et al. 2006), introducing surface charge heterogeneity on microchannel wall (Erickson and Li 2002) and exploiting viscoelastic instability (Gan et al. 2007; Lam et al. 2009).

In contrast, external energy is supplied to an active micromixer for mixing enhancement. Sinusoidal pressure can be applied to enhance mixing efficiency in a T-mixer (Glasgow and Aubry 2003; Sun and Sie 2010). AC electromagnetic field has been employed to enhance mixing of ferrofluids (Wen et al. 2009). Applying high frequency alternating current (AC) voltage at symmetrical (Ng et al. 2009) or asymmetrical (Sasaki et al. 2006) planar electrode promotes local flow circulation above the electrodes, thus improving mixing efficiency.

A novel micromixer which enhances mixing efficiency through a constriction under time periodic electroosmotic flow (see Fig 1) was introduced by us recently (Lim et al. 2010). The micromixer can be considered as an active mixer because it requires the addition of sinusoidal voltages to the direct current (DC) driving voltage. The micromixer can be fabricated easily because it does not

involve any three dimensional geometries or embedded metallic structure. It can be easily integrated into various systems which are driven by time periodic electroosmotic flow or pressure driven flow. Mixing efficiencies obtained with the proposed micromixer at various AC frequencies and amplitudes were reported.

However, we had previously investigated the mixing mechanism theoretically, with a simple, idealized model. Upon further investigation, though the simple model gives an insight on the understanding of the mixing mechanism and the role of the governing parameters on mixing efficiency, it is of insufficient accuracy to provide good quantitative agreement to experimental observations such as the amplitude of fluid interface oscillation and the AC frequency for optimum mixing efficiency. This is due to the various necessary approximations and assumptions for a simple model which can be analyzed theoretically. As such, it could not be utilized for an accurate prediction of the mixing efficiency, nor for the optimization of the micromixer.

We report here in-depth numerical analyses which could provide an accurate representation of the flow and mixing conditions of the proposed micromixer. The validity and accuracy of the numerical model developed were also verified experimentally. The model allows accurate predictions and thus the determination of the operating conditions for optimum mixing. This provides future users the option to optimize the mixer without expensive experimental trials. In the course of developing the accurate numerical model, important aspects for mixing enhancement such as the fluid interface amplitude fluctuation and the mixing contact area were computed and obtained from numerical simulation. The fluid interface oscillation amplitude was measured experimentally and compared with numerical simulations. The effect of electromigration of the fluorescent dye added to the stream on the flow behavior, which is generally ignored and hardly been discussed, is elucidated. Indeed, it is discovered that for accurate simulations, this effect must be considered to avoid significant error in the predictions. Detailed error analyses were performed to investigate the sensitivity of variation in constriction width, diffusion coefficient and mobility on the mixing efficiency. This analysis also provides an indication on the fabrication requirements for key geometrical parameters.

2 Experimental Details

The micromixer investigated consists of a T-shaped microchannel with dimensions shown in Fig 1. The length of microchannel from the inlet to the T-junction is 2.5mm and the length from the T-junction to the outlet is 7.5mm. A constriction of 40 μ m wide and 100 μ m long was introduced right at the T-junction. The depth of the channel is 50 μ m. The microchannel was fabricated with PDMS through soft lithography technique with negative photoresist SU-8 patterned on a silicon wafer. The PDMS microchannel was then bonded to a glass slide with oxygen plasma to form a closed channel. Details on fabrication were described in our previous paper (Lim et al. 2010).

The buffer solution employed in the experiment is 1mM sodium bicarbonate (NaHCO_3) solution. For visualization of the flow pattern and mixing efficiency evaluation, fluorescent dye (disodium fluorescein) was added to one of the fluid stream. The micromixer was observed under an inverted microscope (Nikon Eclipse TE2000-S) and the dye was excited under mercury lamp illumination. The concentration of fluorescent dye was controlled such that the fluorescent solution has the same conductivity as the buffer solution. This was to eliminate any conductivity gradient between the two streams, thus preventing electrokinetic instability (Lin et al. 2004b). Images were captured with a charge-coupled device (CCD) camera (HiSense MkII) at 5 frames per second (fps). The resolution of the CCD camera is 1344 x 1024 pixels with 12 bits gray scale. From the captured images, mixing efficiency can be quantified based on the change of fluorescent dye intensity when the two streams mixed. Important parameters for the analysis of mixing mechanism such as the amplitude of fluid interface oscillation and contact area between the two fluid streams can also be obtained from the images.

Two out-of-phase AC voltages with similar DC bias were applied at the two inlets to induce time-periodic electroosmotic flow. The DC voltage applied V_{DC} was 270V and the AC amplitudes were set to $1/3V_{DC}$, $2/3V_{DC}$, V_{DC} and $4/3V_{DC}$. The AC frequencies, f applied are 2.1Hz, 4.1Hz, 6.1Hz, 8.1Hz, 12.1Hz and 16.1Hz. Due to the periodic nature of the flow pattern, by having the ratio of the applied AC frequencies to the capturing frequency to be non-integer numbers,

mixing images at different phases of the whole cycle can be captured under a constant frame rate (5 frames per second). With mixing efficiency evaluated and averaged with these images, a good representation for the average mixing efficiency over the entire cycle can be obtained. The voltages were generated by a function generator (AFG3022 Tektronix) and amplified through a voltage amplifier. An oscilloscope (Hameg Instrument Combiscope) was employed to monitor the voltage applied.

It has been found in our previous investigation that mixing efficiency can be increased significantly as compared to the normal T-shaped channel with the addition of a simple constriction at the T-junction. The oscillation of fluid interface induced by the out-of-phase AC voltages creates alternate plugs of fluid in the constriction. At the exit of the constriction, the formation of thin crescent shaped fluid layer enhances the mixing efficiency due to the increase in contact (mixing) surface area between the two solutions. The amplitude of fluid interface oscillation and the contact surface of the two fluids are important parameters that govern the mixing efficiency. Therefore, images in the vicinity of the T-junction were captured and analyzed to quantify these two parameters.

3 Numerical Simulations

Our previous theoretical model, although elucidated well the mixing mechanism, lacks sufficient accuracy for a good representation of the flow field, and thus not suitable for the determination of the operation condition for optimum mixing. Thus, a numerical model was developed. Numerical simulations to simulate the flow in the micromixer were performed with finite element method (FEM) software: Comsol Multiphysics. The simulation domain is shown in Fig 2. In the simulation, only one fifth of the length of the microchannel was simulated to save computation time without sacrificing simulation accuracy. The simulation domain was meshed with 17394 triangular elements. The electrostatic potential, ion concentration, velocity, and applied potential were discretized with second order elements while the pressure was discretized with linear elements. Convergence test was performed with higher number of elements for steady-state solutions and the numerical error was found to be negligible with the mesh employed. Details on numerical simulation are described in Section 3.1 and 3.2.

3.1 Governing Equations

Electric field is applied across the two inlets and outlet to generate electroosmotic flow. The governing equation to describe the electric potential distribution in a neutral medium is given by the Laplace equation:

$$\nabla \cdot \nabla \phi = 0 \quad (1)$$

where ϕ = electric potential.

Charge accumulation at the surface of the microchannel produces an electrical double layer (EDL), which has a non-zero charge density. The applied electric field exerts force on this thin charged layer and induces fluid movement. After obtaining the electric potential distribution, the flow field can be solved with the continuity equation and the Navier-Stoke equation for an incompressible Newtonian fluid:

$$\nabla \cdot \mathbf{u} = 0 \quad (2)$$

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho(\mathbf{u} \cdot \nabla) \mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u} + F_e \quad (3)$$

where $\mathbf{u}$ = fluid velocity, ρ = fluid density, p = pressure, μ = fluid dynamic viscosity and F_e = external force exerted by the electric field on the fluid. Three models are typically employed to couple the electric field applied to the fluid movement, namely: slip velocity model, Poisson-Boltzmann (PB) model and Nernst-Planck (NP) model. PB and NP model requires solving additional equations which describe the positive and negative ion distributions in the solution. This requires very fine mesh at the boundary of the wall (in the vicinity of the EDL layer) to resolve the steep changes of wall static potential and ion distribution.

A simpler model, the slip velocity model ignores the velocity variation near the wall and assumes that the flow is induced by a moving wall at a slip velocity, given by:

$$\mathbf{u}_{\text{slip}} = \frac{\varepsilon_0 \varepsilon_r \zeta}{\mu} \nabla \phi \quad (4)$$

where ε_0 = permittivity of free space, ε_r = relative permittivity, ζ = zeta potential and E = applied electric field. In the slip velocity model, the external force F_e is set to 0 since there is no net charge in the bulk of the solution. Despite its simplicity, the slip velocity model can describe well the bulk fluid flow under time periodic electroosmotic with good accuracy (Marcos et al. 2004; Yan et al. 2009). Therefore, the slip velocity model is adopted in this numerical analysis.

Various fluorescent dyes are employed for mixing visualization and evaluation. Fluorescent dyes can be categorized as ionic dyes and non-ionic dyes. Non-ionic dyes or neutral dyes includes BODIPY 493/503 (Kim et al. 2009) and fluorescein (Shin et al. 2007). An unfavorable characteristic of a neutral dye is its poor solubility in aqueous buffer solutions. On the contrary, ionic dyes dissolve easily in aqueous solutions and are commonly employed since most studies on micromixers involve aqueous buffer solutions. Ionic dyes can further be categorized into positive dyes and negative dyes. Positive dyes such as Rhodamine B (Lin et al. 2004a; Chen et al. 2008) and Rhodamine 123 (Ng et al. 2009) dissolve in water and produce fluorescent species which are positively charged. Similarly, negative dyes such fluorescein disodium (Gan et al. 2007; Lim et al. 2010) and BODIPY 492/515 disulfonate (Ng et al. 2009) dissolve in water and produce fluorescent species which are negatively charged. It is noted that the dissolution of these dyes will not cause any electrical non-neutrality. For example, the dissolution of disodium fluorescein molecules in water produces 2 positive sodium ions (with +1 ion charge number) and 1 fluorescein ion (with -2 ion charge number), and electrical neutrality is maintained.

Due to the high solubility in aqueous buffer solution, ionic dyes are widely employed in various studies of micromixer. However, in most of the numerical simulations of flows in micromixers, the dye distribution is often described by the convection-diffusion equation:

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) = -\mathbf{u} \cdot \nabla c \quad (5)$$

where c = concentration of dye and D = diffusion coefficient of dye. The dye is often assumed to be convected by the flow (Yan et al. 2009; Lin et al. 2004a; Fu et al. 2005) and the implicit assumption is that it is unaffected by electromigration due to the applied electric field. This is only true if the dissolution of the dye will

not result in charged species. However, for the fluorescent dye, the fluorescein ion (with -2 ion charge number) is charged and not electrically neutral. For charged ion species, the Nernst-Planck equation should be employed:

$$\frac{\partial c_i}{\partial t} + \nabla \cdot [-D \nabla c_i - u_i c_i \nabla \phi] = -\mathbf{u} \cdot \nabla c_i \quad (6)$$

where c_i = concentration of ionic dye and u_i = ionic mobility. The third term on the left side of Eq. 6, which is often neglected, describes the electromigration effect on the charged dye concentration distribution. Eq. 6 reduces to Eq. 5 if electromigration is neglected. The effect of electromigration of charged dye in flow simulation of micromixers will be considered explicitly in this study as it was discovered that its omission will lead to significant error in predictions.

The electromigration of fluorescein ion consists of two components: migration due to the applied electric field across the channel to drive the flow and due to electric potential from the charged channel wall. Only the electromigration due to the applied electric field was included in the model. The electromigration component due to the charged wall has been neglected because it diminishes after a small distance away (in the order of tenths of nanometer) from the wall due to the shielding effect of electrical double layer. The error induced by this assumption will be discussed in Section 4.5.

3.2 Boundary Conditions

To solve the governing equation and to compare the simulated results with experiments, suitable boundary conditions have to be applied. Two out-of-phase sinusoidal AC voltages with a similar DC bias were applied at the two inlets in the experiments. The DC voltage, V_{DC} applied was 270V and the AC voltages, V_{AC} applied are $1/3 V_{DC}$, $2/3 V_{DC}$, V_{DC} and $4/3 V_{DC}$. Although the length of the T-junction was reduced by 5 times in the simulation, the applied voltage cannot be assumed to be $V_{DC}/5$ in the simulation. This is because the presence of constriction affects the potential distribution across the channel. The constriction length is unchanged despite the whole channel length is reduced by five times.

To establish the appropriate voltage boundary condition, a simulation for the electric potential distribution in the T-mixer with dimensions the same as the physical dimensions was performed based on the Laplace equation (Eq. 1). The voltages at locations A, B and C (see Fig 2) which corresponded to the voltages at the upper inlet, lower inlet and outlet in the simulations were determined from this simulation. The voltage at C was set to zero and the voltages at A and B were determined with reference to C. The DC voltage and AC amplitude at the inlets obtained with this simulation for various experimental conditions are listed in Table 1. The channel walls were assumed to be electrically insulated. Other boundary conditions are summarized in Table 2.

For flow field simulation, slip velocity boundary condition is adopted here. According to the Smoluchowski equation (Eq. 4), zeta potential is required to compute the slip velocity. Zeta potential for the buffer solution, 1mM sodium bicarbonate (NaHCO_3) solution, was measured with current monitoring method. The experimental details for current monitoring for the determination of zeta potential are as described in the literature (Huang et al. 1988). The zeta potential of the PDMS/glass microchannel in the buffer solution was found to be -59.8mV (with standard deviation of 2.2mV) based on five experimental runs. Since one of the fluid streams was mixed with fluorescent dye, the zeta potential for the 1mM NaHCO_3 solution with the dye was also measured. The zeta potential for 1mM NaHCO_3 with 0.06mM of disodium fluorescein dye was measured to be -60.9mV (with standard deviation of 4.0mV). To further verify the effect of ionic dye on flow rate and zeta potential, the same buffer solution with another commonly used fluorescent dye, Rhodamine B with concentration of 0.03mM was also measured and the zeta potential was found to be -57.5mV (with standard deviation of 2.9mV). With and without the addition of the dyes, the differences in zeta potentials are well within one standard deviation. Thus, the addition of a small amount of fluorescent dyes such as disodium fluorescein and Rhodamine B to the buffer solution does not modify the zeta potential significantly. Therefore, a constant zeta potential was assumed throughout the whole channel. The experimental results also show that the addition of fluorescent dye does not induce any significant additional body force on the fluid as expected. In various studies on micro-mixing under electroosmotic flow (Lin et al. 2004a; Yan et al.

2009; Fu et al. 2005), similar assumption was made. Since no external pressure was applied, pressure at the inlets and outlet were set to zero.

The normalized dye concentrations at the upper and lower inlets were set to 1 and 0 respectively for the ease of mixing efficiency evaluation. The boundary conditions corresponded to the dyed streams from the upper inlet and the undyed streams at the lower inlet in the experiments. Various constants employed in the simulations are listed in Table 3. Diffusion constant of fluorescein in water at 21.5°C as calculated from Wilke-Chang correlation is $4.9 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ (Rani et al. 2005). The estimation of the mobility of fluorescein ion is detailed in Section 4.1

3.3 Dimensionless Parameters

To obtain the dimensionless parameters governing the flow, Eq. 1, 2, 3 and 6 are non-dimensionalized by introducing the following variable:

$$\phi^* = \frac{\phi}{\phi_{DC}}, \mathbf{u}^* = \frac{\mathbf{u}}{U}, t^* = \frac{Ut}{W}, \mathbf{x}^* = \frac{\mathbf{x}}{W}, p^* = \frac{p}{\rho U^2}, c_i^* = \frac{c_i}{c_{i0}} \quad (7)$$

where ϕ_{DC} = DC potential applied, reference velocity $U = -\frac{\varepsilon_0 \varepsilon_r \zeta}{\mu} E_{DC}$, with E_{DC} = DC electric field in the downstream channel and W = width of downstream channel. E_{DC} was constant in the series of experiments conducted since the voltages applied at the two inlets had the same AC amplitude and were out-of-phase by 180°.

The governing equations can be rewritten in dimensionless form:

$$\nabla \cdot \nabla \phi^* = 0 \quad (8)$$

$$\nabla \cdot \mathbf{u}^* = 0 \quad (9)$$

$$\frac{\partial \mathbf{u}^*}{\partial t^*} + (\mathbf{u}^* \cdot \nabla) \mathbf{u}^* = -\nabla p^* + \frac{1}{Re} \nabla^2 \mathbf{u}^* \quad (10)$$

$$\frac{\partial c_i^*}{\partial t^*} + \mathbf{u}^* \cdot \nabla c_i^* = \frac{1}{Pe} \nabla^2 c_i^* + \alpha \nabla \cdot (c_i^* \nabla \phi^*) \quad (11)$$

where Reynold number $Re = \frac{\rho UW}{\mu}$, Péclet number $Pe = \frac{UW}{D}$ and $\alpha = \frac{u_i \phi_0}{WU}$.

For this series of simulations and experiments, $Re = 0.276$, $Pe = 501$ and $\alpha = 6.67$. Here α is a dimensionless parameter that depicts the ratio between electro-migrative transport and convective transport. If α is much smaller than 1 (migration is small compared to convection), the electromigrative term can be neglected and Eq. 11 reduces to the normal convection-diffusion equation. However, it is clear that electromigrative term cannot be neglected here because α is much larger than 1.

Another important dimensionless parameter can be identified by non-dimensionalizing the applied potential boundary condition. The following dimensionless variables are introduced:

$$\phi^* = \frac{\phi}{\phi_{DC}}, \phi_{DC}^* = \frac{\phi_{DC}}{\phi_{DC}}, \phi_{AC}^* = \frac{\phi_{AC}}{\phi_{DC}} \quad (12)$$

where all applied electric potentials are normalized with the applied DC potential ϕ_{DC} . The dimensionless applied voltage then becomes:

$$\phi^* = 1 + \phi_{AC}^* \sin\left(\frac{\omega W}{U} t^*\right) \quad (13)$$

where Strouhal number $St = \frac{\omega W}{U}$ with $\omega = 2\pi f$. Here, the Strouhal numbers for AC frequencies of 2.1Hz, 4.1Hz, 6.1Hz, 8.1Hz, 12.1Hz and 16.1Hz respectively are 2.15, 4.20, 6.25, 8.29, 12.39 and 16.49.

4 Results and Discussions

The experimental and numerical results will be presented in this section.

4.1 Ionic Mobility of Fluorescent Dye

The ionic mobility of the fluorescent dye is required to simulate the ion concentration distribution with the Nernst-Planck equation (Eq 6). The mobility of fluorescein ion is known to vary with various factors such as temperature, pH, ionic strength and buffer composition (Kim et al. 2006; Whang and Yeung 1992; Persat et al. 2009). Although mathematical models (Jaroš et al. 2002; Bahga et al.

2010) are available to predict the mobility of ion, there are quantitative discrepancies between the mobility value measured experimentally with the value predicted by the models (Bahga et al. 2010). An experimentally measured mobility value of fluorescein ion under the experimental conditions of this study (buffer solution of 1mM NaHCO₃ with pH = 7.2) is not available in the literature.

Incidentally, the configuration of our micromixer is similar to the conventional apparatus for measuring ion mobility with capillary electrophoresis (CE) (Wang et al. 2007; Bahga et al. 2010; Pumera et al. 2002; Whiting et al. 2008). Conventional CE system generally involves the injection of fluid plugs into a separation channel where the ions are separated based on their mobilities and the migration times of the ions can be determined. In our micromixer, the fluorescent property of fluorescein ion allows us to observe the movement of the fluid plugs directly. Based on this concept, a simple method was adopted here to estimate the mobility value of fluorescent ion species.

Images for the experimental condition $f=2.1\text{Hz}$ and $V_{AC}=4/3V_{DC}$ at the exit of the constriction were captured. The ionic mobility could be determined by inspecting the velocity of the moving crescent-shaped pattern. The images were post-processed with MATLAB and the intensity profiles along the centreline of the channel for two consecutive images were obtained. According to Nyquist criterion, the capturing frequency has to be more than two times the characteristic frequencies of the flow pattern to avoid aliasing. In this case, the Nyquist criterion was fulfilled because the capturing frequency was 5Hz while the frequency of the flow pattern inspected was 2.1Hz. By inspecting the shift of peaks for the two consecutive intensity profiles, the velocity of the moving crescent pattern could be obtained (see Fig 3). This shift distance divided by the time interval gave the velocity of the moving crescent, v_c .

The velocity of the moving crescent is the result of two transport effects: fluid electroosmotic flow and electromigration of fluorescent dye. After deducting the contribution from electroosmotic velocity v_{eo} , the velocity component due to electromigration, v_m can be determined:

$$v_m = v_c - v_{eo} \quad (14)$$

The electromigration velocity v_m is related to ionic mobility u_i by

$$v_m = -u_i \nabla \phi \quad (15)$$

Substituting Eq. 4 and Eq. 15 into Eq. 14, and rearranging, an expression for ionic mobility is obtained:

$$u_i = -\frac{1}{\nabla \phi} \left(v_c - \frac{\varepsilon_0 \varepsilon_r \zeta}{\mu} \nabla \phi \right) \quad (16)$$

The negative of gradient for electric potential ϕ is the electric field. The electric field is expected to be non-uniform at the vicinity of the constriction due to the abrupt change in geometry. Numerical simulation shows that the electric field is uniform after approximately 200 μm downstream from the constriction. Therefore, only intensity profiles more than 200 μm downstream from the constriction were analyzed because the electric field was uniform and could be easily determined from numerical simulation.

Zeta potential ζ for the solution can be determined with current monitoring method (see Section 3.2). Therefore the electroosmotic flow velocity v_{eo} can be calculated with the Smoluchowski slip velocity equation (Eq. 4). Since the electroosmotic flow profile is plug-like, the flow velocity is identical regardless of the location across the width or depth of the channel. With Eq. 16, the average mobility of fluorescein ion was determined to be $-2.53 \times 10^{-8} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$ (with standard deviation of $0.25 \times 10^{-8} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$) based on twelve pairs of images.

The mathematically predicted fluorescein mobility at pH 7.15 for 1mM buffer concentration (Persat et al. 2009; Bahga et al. 2010) is $-3 \times 10^{-8} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$. However, the mobility has not been measured experimentally at this concentration. The mobility value measured by our proposed method at 1mM buffer concentration (pH=7.2) was found to be 16% lower than the value predicted by the mathematical model. Bahga *et al* (2010) measured the mobility of fluorescein ion at pH 7.15 with capillary electrophoresis method and also found that the measured values were 5% to 20% lower than the theoretical values at various concentrations of buffer solution (HEPES-sodium hydroxide). This

suggests that our measurement is within the reasonable range of experimentally measured mobility value.

Although this proposed technique has the potential for the determination of ion mobility, it will require more rigorous benchmarking. This will be investigated in more details in our future work. For the present investigation, error analyses are presented in Section 4.5 to investigate the sensitivity of our numerical results to various parameters, including the uncertainty of the determined mobility value.

4.2 Amplitude of Fluid Interface Oscillation

Snapshots of numerical results for mixing conditions at various AC frequencies are shown in Fig 4. At the T-junction, the fluid interface oscillated due to the applied AC voltages which were out of phase by 180 degree. The fluid interface oscillation is an important process of the mixing mechanism and closely related to the mixing efficiency of the mixer. If the amplitude of oscillation is larger than the constriction width, alternate plugs of fluid are formed in the constriction. In addition, the discrepancy induced by the exclusion of the electromigration effect of fluorescent dye can be easily observed quantitatively from the prediction of the amplitude of fluid interface oscillation.

The amplitude of the fluid interface oscillation was measured experimentally by analyzing the fluorescent images captured. The image intensity profile along the centreline along the vertical channel was obtained and normalized with the maximum and minimum intensities (see Fig 5). The fluid interface location is the point at which the normalized intensity is 0.5. Three experimental runs were performed for various frequencies at a constant AC amplitude $V_{AC} = 4/3 V_{DC}$. 50 images were captured for each run. A MATLAB program was written to process the images to obtain the time-dependant interface location. Curve fitting on the data was performed with the program to obtain the amplitude of fluid interface oscillation. The amplitude of oscillation was obtained from numerical results in a similar manner.

Experimental results obtained are compared with the numerical simulation results (see Fig 6). The numerical simulation which included the electromigration term in the Nernst-Planck equation produced oscillation amplitudes agreeing very well with the experimentally obtained results. However, if the electromigration term was neglected, the amplitudes of oscillation obtained by numerical simulation were approximately double that of the experimental amplitudes. This illustrates that electromigration effect is indeed an important consideration, and that it must be included in the simulation of dye concentration distribution for obtaining reliable predictions. Failure to account for the electromigration effect in simulation had resulted in significant simulation inaccuracy.

The amplitudes of fluid interface oscillation for various AC amplitudes and frequencies were obtained from numerical simulation and normalized with the half width of the constriction (see Fig 7). The normalized oscillation amplitude A^* is an important variable that governs the mixing efficiency of the micromixer. It is concluded that A^* decreases with increasing frequency and decreasing AC amplitude.

4.3 Mixing Efficiency

The experimental mixing efficiencies of the micromixer were evaluated based on the captured fluorescent images. The concentration c_i at a point for a given image is represented by the pixel intensity value I_i , with the limiting values of concentration c_{max} and c_{min} corresponding to I_{max} and I_{min} respectively. By normalizing the pixel intensity $\bar{I}_i$ as $(I_i - I_{min}) / (I_{max} - I_{min})$, the mixing efficiency, η_{eff} is defined as,

$$\eta_{eff} = \left[1 - \frac{\sqrt{\frac{1}{N} \sum_{i=1}^N (\bar{I}_i - \bar{I}_\infty)^2}}{\sqrt{\frac{1}{N} \sum_{i=1}^N (\bar{I}_{0i} - \bar{I}_\infty)^2}} \right] \times 100\% \quad (17)$$

where N is the total number of points examined in the cross-stream direction, $\bar{I}_i$ is the normalized intensity at each point, $\bar{I}_\infty$ is the normalized intensity in the complete mixing state (value 0.5) and $\bar{I}_{0i}$ is the normalized intensity at each point where no mixing occurs. $\bar{I}_{0i}$ is equal to 0 for the stream without fluorescent dye or

1 for the other stream with fluorescent dye, with the number of pixels proportional to the flow rate of the streams. For our experimental condition of both streams having equal flow rate, the expression $(\bar{I}_{0i} - \bar{I}_{\infty})^2$ takes the value of $(\pm 0.5)^2$. The mixing efficiencies of the micromixer at various parameters were evaluated at 1200 μ m downstream of the T-junction over the width of the channel. The instantaneous mixing efficiencies for flows with AC electric field were time variant. Therefore, mixing efficiencies were averaged over the whole cycle.

The mixing efficiencies based on numerical simulations were obtained similarly. The concentration profile of fluorescent dye along the cross-stream direction was output from COMSOL and mixing efficiency at the downstream location was calculated based on Eq. 17 with a program written in MATLAB. The numerical results were compared with our previous published experimental results (Lim et al. 2010).

From Fig 8, it can be observed that the mixing efficiencies predicted by simulation are in good agreement with experimental results. Some discrepancies between experimental and numerical mixing efficiencies are noted at high frequency especially for high AC amplitude. This could be due to the variability of the constriction width due to micro-fabrication error and the uncertainty of the value of diffusion constant for the fluorescent dyes adopted from the literature, which could be slightly different from the actual value. However, this does not affect the overall trend of the results and analysis on mixing mechanism. Detailed error analyses are contained in Section 4.5 to address these discrepancies.

Mixing efficiency with just DC voltage applied was only 38%. Mixing efficiency increased as two out-of-phase AC voltages were applied in addition to the DC voltage. Maximum mixing efficiencies as high as 86% were achieved in the frequency range of 4Hz to 6Hz. Increasing the frequency beyond 8Hz gradually reduced the mixing efficiency. High AC amplitude produced higher mixing efficiencies for all frequencies.

4.4 Contact Area for Diffusive Mixing

The improvement of mixing efficiency is attributed to the increase of contact area between the two fluids due to the formation of crescent pattern after the constriction due to the sudden expansion of the channel. The increase of contact area was quantified based on numerical results. Since the numerical simulation was performed in a two dimensional space, the contact area could be represented by contact length instead. Numerical simulation is advantageous in this analysis because the results can be output at any particular time of interest to show the images at different phases of the cycle. Analysis of experimental results on this aspect was limited by the capturing speed (frame rate) of the CCD camera.

The contact lengths within the area confined by the area shown in Fig 9 were obtained at different phases of the cycle. To obtain a clear outline of the contact length, post-processing of the numerical results was performed. A threshold of normalized concentration $c_i=0.5$ was set to reveal the contact line of the two fluids. The contact lines were measured with ImageJ software and normalized with the width of the channel. Fig 10 shows the contact length obtained at various AC frequencies and amplitudes.

If only a DC voltage is applied, mixing only occurs through diffusion at the straight interface at the middle of the two streams. Applying two out-of-phase AC voltages leads to the formation of contact interfaces which span across the entire width of the channel due to the formation of crescent-shaped pattern after the constriction. This simple mechanism effectively increases the contact length by 3.5 to 4.5 fold at the frequency range of 4Hz to 8Hz. Higher contact length allows more mixing through diffusion across the interface. If the frequency is below 2Hz or beyond 16Hz, the normalized contact lengths decrease to 2 only (see Fig10a). From Fig 10b, the contact length is increased with higher AC amplitude. This is because higher AC amplitude results in large crescent or wavy patterns that span across the whole width of the channel, thus creating larger contact area.

4.5 Error Analyses

Detailed error analyses were performed to investigate the discrepancy between numerical and experimental results, especially at high AC amplitudes and frequencies. In particular, the sensitivities of the simulation results to the uncertainties in the width of constriction, diffusion coefficient and fluorescein mobility were examined. The error induced by omitting electromigration due to the channel wall electric potential will also be discussed.

The average width of the constriction was measured to be 40 μm , with a standard deviation of 3 μm based on 14 microchannels. However, the actual width of the constriction varies from 31 μm to 45 μm due to variations induced during micro-fabrication. To investigate the uncertainty induced by assuming an average constriction width of 40 μm in our simulation, the simulation for the case of $V_{AC}=4/3V_{DC}$ was repeated with minimum and maximum constriction widths of 31 μm and 45 μm . It can be observed that the mixing efficiencies at high frequency are more sensitive to the variability of constriction width as compared to low frequency (see Fig11a). If the constriction width was 31 μm instead of the average 40 μm , the mixing efficiency at $f=16\text{Hz}$ increases by 9.1% whereas the mixing efficiency at $f=4\text{Hz}$ only increases by 4.7%. The mixing efficiencies results are sensitive to the variation of constriction width, especially at $f=12\text{Hz}$ and $f=16\text{Hz}$ because the fluid interface oscillation amplitudes are very small. The ratio between the oscillation amplitude and the constriction width is an important parameter that governs the mixing efficiency. Small deviations of constriction width from the average value (+5 μm to -9 μm) induce relatively large error in the simulated mixing efficiency because the oscillation amplitude at 12Hz and 16Hz are only 27 μm and 17 μm respectively.

Diffusion coefficient of fluorescein ion has been measured by a few researches (Petrásek and Schwillé 2008; Culbertson et al. 2002; Zero et al. 1983; Du et al. 2006). The reported value of diffusion coefficient varies from to $4.25 \times 10^{-9} \text{m}^2 \text{s}^{-1}$ to $6.3 \times 10^{-9} \text{m}^2 \text{s}^{-1}$. The value of diffusion coefficient as calculated with Wilke-Chang correlation is $4.9 \times 10^{-9} \text{m}^2 \text{s}^{-1}$ (Rani et al. 2005) which is the value adopted in our simulation in Section 4.2 to 4.4. The simulations were repeated with the two extreme values of diffusion coefficients reported in the

literature (see Fig 11b). If the higher value of diffusion coefficient ($6.3 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$) was adopted in the simulation, the mixing efficiencies for $f=0\text{Hz}$ and $f=16\text{Hz}$ increase by 6.6% and 5.2% respectively while the mixing efficiency for $f=6\text{Hz}$ only increases by 1.7%. The mixing efficiency is sensitive to diffusion coefficient at low (0Hz to 2Hz) and high frequency (12Hz to 16Hz) while the mixing efficiency is not affected significantly at the intermediate frequency range (4Hz to 8Hz). This is due to the fact that for intermediate frequency range, the solutions are almost completely mixed at the downstream cross section where the mixing evaluation is performed (as indicated by the high mixing efficiency for $f=4\text{Hz}$ to $f=8\text{Hz}$). As such, the concentration gradient has been reduced significantly and the diffusion process becomes less dominant. This translates to the mixing efficiency being less sensitive to the uncertainty in diffusion coefficient. In contrast, for the low and high frequencies, the mixing efficiencies are low and significant concentration gradient still persists across the channel. Therefore, the diffusion process remains significantly important in contributing to mixing efficiencies. Thus, mixing efficiencies are sensitive to the uncertainty in diffusion coefficient at low and high frequencies.

The measured values of fluorescein mobility in the literature can vary significantly from $-0.3 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ to $-3.7 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ (Kim et al. 2006; Whiting et al. 2008; Whang and Yeung 1992; Cheng et al. 1990) at room temperature depending on pH, concentration and type of buffer solution. However, since the experimental conditions of the measured mobility value in the literature are different from our experiment, these values were not adopted in the simulation. Instead, simulations were repeated with the theoretically predicted mobility of $-3 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ (Bahga et al. 2010) for comparison with the simulation results with our measured mobility ($-2.53 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ in 1mM NaHCO_3 buffer solution at $\text{pH} = 7.2$) in Section 4.1. The mixing efficiencies at low frequencies (0Hz and 2Hz) are very sensitive to the uncertainty of the mobility value. However, the mixing efficiencies at higher frequencies (4Hz and above) do not vary significantly with the variability of mobility value (see Fig 11c). Therefore, the discrepancies observed at high frequencies simulation are not likely to be caused by the uncertainty in mobility measurement.

To model the electromigration of fluorescein ion due to wall potential, Poisson-Boltzmann equation has been introduced to describe the electric potential, ψ of the charged wall.

$$\psi = \frac{2c_0 F}{\varepsilon_r \varepsilon_0} \sinh\left(\frac{e \psi}{k_b T}\right) \quad (18)$$

where c_0 = bulk concentration of ion, F =Faraday constant, e =unit electron charge, k_b = Boltzmann constant, T = absolute temperature. The wall potential is added to the electromigration term in the Nernst-Planck equation through the principle of superposition of electric potential.

$$\frac{\partial c_i}{\partial t} + \nabla \cdot [-D \nabla c_i - u_i c_i \nabla(\phi + \psi)] = -\mathbf{u} \cdot \nabla c_i \quad (19)$$

To capture the steep changes of electric potential and ion concentration near the wall in the electrical double layer, the mesh near the wall was refined. The simulations of $f=4\text{Hz}$ and $f=16\text{Hz}$ with $V_{DC}=4/3V_{AC}$ have been repeated with consideration of the electromigration due to wall potential. The ion concentrations at 1.2mm downstream obtained from simulations with and without wall potential are compared in Fig 12. The curves for both cases overlapped each other across the width of the channel in the bulk region. The only difference is that the simulations with wall potential show the ion concentration rises sharply near the edges of the channel. However, since the electrical double layer region is in the order of nanometers, the effect of wall potential on the overall mixing efficiency which is evaluated across the entire width of the channel is negligible. Therefore, the omission of electromigration due to wall potential in the simulations reported in Sections 4.2 to 4.4 has resulted in increase computation efficiency, but without sacrificing accuracy.

5. Conclusion

Numerical and experimental investigations have been performed on the mixing mechanism of a micromixer based on periodic electroosmotic flow through a constriction. Dimensionless analysis and numerical simulation reveal that electromigration of charged fluorescent dye species is an important phenomenon which cannot be neglected as it can affect the flow behavior significantly. Failure

to consider electromigration in the simulation could result in significant discrepancies (as large as 50%) between experimentally observed and numerically predicted fluid interface oscillation amplitudes.

Important factors that govern the mixing efficiency of the micromixer such as fluid interface oscillation amplitude and contact length between two fluid streams were quantified based on experimental and numerical results. The proposed micromixer can effectively increase the contact area between the two fluids through the expansion of alternate fluid plugs from a constriction. Optimum Strouhal number which maximizes the contact area ranges from approximately 4.2 to 8.3. Larger AC amplitude as compared to the DC driving voltage was also found to produce higher contact area. The increase of contact area between the two fluids facilitates mixing through diffusion as they are convected downstream. Based on the numerical model, applied AC amplitude and frequency can be optimized to obtain the desired mixing efficiency.

Error analyses have been performed to investigate the sensitivity of the mixing efficiency to the uncertainties in constriction width, diffusion coefficient and fluorescein ion mobility. Mixing efficiency is found to be more sensitive to the uncertainty of constriction width at high frequency. Uncertainty of diffusion coefficient affects the mixing efficiency more significantly at low and high frequencies, as compared to the intermediate frequencies (4Hz to 8Hz). The uncertainty of fluorescein ion mobility affects the mixing efficiency significantly at low frequency only.

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Table 1 Voltage boundary condition for numerical simulation

Experimental conditions	DC bias in simulation, ϕ_{DC} (V)	AC amplitude in simulation, ϕ_{AC} (V)
$V_{AC}=1/3V_{DC}$	65.31	17.95
$V_{AC}=2/3V_{DC}$	65.31	35.91
$V_{AC}=V_{DC}$	65.31	53.87
$V_{AC}=4/3V_{DC}$	65.31	71.82

Table 2 Summary of boundary conditions ($\mathbf{n}$ = unit vector normal to boundary)

Variable	Condition	Boundary
Applied potential, ϕ	$\phi = \phi_{DC} + \phi_{AC} \sin(2\pi ft + \pi)$	Upper inlet
	$\phi = \phi_{DC} + \phi_{AC} \sin(2\pi ft)$	Lower inlet
	$\phi = 0$	Outlet
	$\mathbf{n} \cdot \nabla \phi = 0$	Channel wall
Velocity vector, $\mathbf{u}$ and pressure, p	$p = 0$	Inlets, outlet
	$\mathbf{u} = \frac{\varepsilon_0 \varepsilon_r \zeta}{\mu} \nabla \phi$	Channel Wall
Dye concentration, c_i	$c_i = 1$	Upper inlet
	$c_i = 0$	Lower inlet, outlet
	$\mathbf{n} \cdot (-D \nabla c_i - u_i c_i \nabla \phi + \mathbf{u} c_i) = 0$	Channel Wall

Table 3 Symbols and values of constants in numerical simulation

Constant	Symbol (unit)	Value
Diffusion coefficient of fluorescein ion	$D/\text{m}^2\text{s}^{-1}$	4.9×10^{-10}
Unit electron charge	e/C	1.602×10^{-19}
Faraday constant	F / Cmol^{-1}	96485
Boltzmann constant	$k_b/\text{m}^2\text{kg s}^{-2}\text{K}^{-1}$	1.381×10^{-23}
Mobility of fluorescein ion	$u_i/\text{m}^2\text{V}^{-1}\text{s}^{-1}$	-2.53×10^{-8}
Temperature	T/K	298
Relative permittivity	ϵ_r	80
Permittivity of free space	$\epsilon_0 / \text{Fm}^{-1}$	8.85×10^{-12}
Density of water	ρ / kgm^{-3}	1000
Zeta potential	ζ/V	-0.0598
Viscosity of water	$\mu/\text{kgm}^{-1}\text{s}^{-1}$	8.90×10^{-4}