Rapid Microfluidic Mixing

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A preformed T-microchannel imprinted in polycarbonate was postmodified with a pulsed UV excimer laser (KrF, 248 nm) to create a series of slanted wells at the junction. The presence of the wells leads to a high degree of lateral transport within the channel and rapid mixing of two confluent streams undergoing electroosmotic flow. Several mixer designs were fabricated and investigated. All designs were relatively successful at low flow rates (0.06 cm/s, ≥75% mixing), but had varying degrees of success at high flow rates (0.81 cm/s, 45–80% mixing). For example, one design operating at high flow rates was able to split an incoming fluorescent stream into two streams of varying concentrations depending on the number of slanted wells present. The final mixer design was able to overcome stream splitting at high flow rates, and it was shown that the two incoming streams were 80% mixed within 443 μm of the T-junction for a flow rate of 0.81 cm/s. Without the presence of the mixer and at the same high flow rate, a channel length of 2.3 cm would be required to achieve the same extent of mixing when relying upon molecular diffusion entirely, while 6.9 cm would be required for 99% mixing.

The application of microfluidic analytical devices to chemical or biological assays has developed rapidly over the past decade.1–4 Although highly successful, considerable research effort is being focused on overcoming a number of performance limitations, one of which is slow reagent mixing. The difficulty in rapidly mixing reagents results from the fact that the system is often restricted to the laminar flow regime (Reynolds number, Re < 2000) and also because the feature sizes are too small (typically <100 μm) to incorporate conventional mixing mechanisms. Microfluidic devices that require a mixing operation have typically relied upon diffusive mixing by bringing the fluid streams to be mixed together within a single channel.5–11 Therefore, to ensure a completely mixed outlet stream, the mixing channel was simply extended to sufficient lengths. This approach may be acceptable for devices that only utilize low flow rates, but higher flow rates (1 cm/s) or low analyte diffusion coefficients (<10−7 cm2/s) within a system will require excessively long mixing channels.

The small channel sizes and the lack of turbulence in microfluidic systems has led to the development and publication of several “passive” or “static” mixer designs, often operating under pressure-driven flow. For gas-phase mixing, Gobby et al.12 described the mixing characteristics within various T-channel designs and discussed the increase in mixing performance when the two fluids are throttled at the point of confluence. For liquid-phase mixing, Liu et al.13 reported on a 3D serpentine channel that achieved mixing through chaotic advection, a mechanism that becomes more effective as the flow rate increases (Re → 70). The most commonly reported liquid-phase mixing designs that are effective at lower flow rates (Re → 1) utilize multilamina, or flow splitting, techniques.14–18 These designs split the incoming streams into several narrower confluent streams to reduce the mixing equilibration time. The effectiveness of the technique is based on the fact that the equilibration time, t, scales quadratically with the width of the channel, w,

\[ t \sim D^{-1}w^2 \]

where D is the diffusion coefficient of the molecule. For example, if the width of the channel decreases by 2, then the equilibrium time and the channel length is decreased by 4. Once mixing is

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complete, the narrow channels are then brought together in a larger main channel for further transport, processing, or detection.

Although the flow splitting technique is effective, it is still limited by diffusion. To increase the mixing rate beyond diffusion limited, it is necessary to induce off-axis or lateral transport within the channel. This is nontrivial, especially when using electroosmotic flow, which typically has flow rates much lower than pressure-driven flow due to constraints imposed by Joule heating. Only recently has there been a report describing flow splitting for an electroosmotic flow rate of 300 μm/s (Re = 0.0090), but results for higher flow rates were not reported.

In this paper, we will present a novel and easily fabricated static mixer that is able to achieve a high degree of lateral transport and mixing over a broad range of electroosmotic flow rates (0.033 < Re < 0.45). A comparison of the performance of the mixer operating under pressure-driven flow, and at similar flow rates, will also be given.

**EXPERIMENTAL SECTION**

**Reagents and Materials.** Laser-grade Rhodamine B (Acros Organics) was dissolved in 20 mM, pH 9.4 carbonate buffer to a final concentration of 0.11 mM (The accepted SI unit of concentration, mol/L, has been represented by the symbol M in order to conform to the conventions of this journal.) The buffer solution was made using deionized water (Millipore Milli-Q system, Bedford, MA) and was filtered before use with a syringe filter (pore size 0.22 μm).

Microchannels were made using polycarbonate sheet (PC; Lexan, GE Co., M. L. Vernon, IN). Poly(ethylene terephthalate glycol) (PETG; Vivak, DMS Engineering Plastic Products, Sheffield, MA) was used to cover and seal the microchannel substrate. PETG was chosen to seal the microchannels because its glass transition temperature (81 °C) is well below that of PC (150 °C); therefore, thermal sealing can be performed at a temperature that does not cause distortion of the PC microchannel.

**Hot Imprinting Method.** Prior to imprinting, the PC substrate was blown clean with ionized air. Channels were hot imprinted in the substrate material using a silicon stamp with a trapezoidal-shaped raised T-channel. The PC was placed over the silicon stamp, the two items were then placed between two aluminum heating blocks, and the temperature was raised to 155 °C. Next, the assembly was placed in a hydraulic press, and a pressure of 13.8 MPa (2000 psi) was applied for 1.5 h. The imprinted substrate was then removed from the template. Channel dimensions were measured using optical profilometry.

**Laser Ablation Method.** A 248-nm excimer laser system (LMT-4000, Potomac Photonics, Inc., Lanham, MD) was used to ablate microstructures within the preformed, PC microchannel. The excimer laser system, described previously, contained a laser light source, a round aperture (200-μm diameter) for delimiting the size and shape of the beam, a focusing lens (10× compound), a visible light source, a CCD camera to image the ablation process, and a controllable X−Y stage with a vacuum chuck to hold the substrate in place. The X−Y stage was programmed to move linearly at a rate of 1 mm/s, and the mixing wells were ablated at a 45° angle relative to the axis of the main channel. Three laser-ablated-well designs were investigated for this study: (1) a three-well mixer, (2) a four-well mixer, and (3) a four-well mixer followed by a series of six alternating partial wells. During ablation, the average power level per pulse was 2.04 μJ, σ = 0.14 μJ, and was determined by three measurements of 100 pulses with an Energy Max 400 laser energy meter from Molecotron Detector, Inc. (Portland, OR). The pulse frequency was set to 200 Hz, with a constant pulse width of 7 ns. The light exposed a circular area of 1.54 × 10⁻⁶ cm² after focusing.

**Measuring Well Depth and Profile.** The depth of the ablated wells was measured by cutting the substrate with a microtome (Microm HM 335 E, Walldorf, Germany) either perpendicular to the axis of the outlet channel or parallel to the slanted wells. The substrate was cut so that the edge of the substrate was within micrometers of the wells. The wells were then imaged and measured using white light microscopy.

**Microchannel Sealing Procedure.** The preformed microchannels were covered and thermally sealed with a flat piece of PETG (referred to as the “lid”) of similar dimensions to the PC. The lid was placed on top of the channel, and the two pieces were clamped together between microscope glass slides and bonded by heating in a GC oven at 90.0 °C, σ = 0.5 °C for 13 min. For the electroosmotic flow studies, 3-mm-diameter circular holes in the lid provided access to the channels and served as fluid reservoirs. For the pressure-driven flow studies, 0.8-mm-diameter circular holes in the lid, located at the ends of each inlet channel, provided access to insert a section of hollow stainless steel tubing. A 3-mm-diameter hole in the lid and at the end of the outlet channel served as a waste reservoir. For each experiment, the channel arms were fixed to a length of 8 mm.

**Flow Image Acquisition.** Fluorescence imaging of the rhodamine dye was performed using a research fluorescence microscope equipped with a 10× objective, a mercury arc lamp, a rhodamine filter set, and a video camera (COHU, San Diego, CA). Digital images were acquired using Scion Image software and a Scion LG-3 frame grabber (Scion, Inc., Frederick, MD). For each experiment, 40 images were captured at a rate of 60 Hz, averaged, and then recorded.

**Experimental Setup.** To image the mixing under electroosmotic flow, the microchannels were initially filled with the carbonate buffer solution. Then, an equal amount (typically 40 μL) of buffer was placed in one inlet channel reservoir and in the outlet channel reservoir, while the second inlet reservoir was filled with the rhodamine-labeled buffer. Platinum electrodes were then placed in contact with the solution in the reservoirs such that the UV laser energy is dangerous and standard laboratory safety equipment should be used, including protective eyewear.

[20] Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.
[22] Safety considerations: UV laser energy is dangerous and standard laboratory safety equipment should be used, including protective eyewear.
two inlet reservoirs were fixed to ground, and the potential was applied to the outlet channel reservoir, as shown in Figure 1a. Fluorescent images were acquired at several different applied voltages (0 to $-1750 \text{ V}$), beginning with zero applied voltage to verify that there was minimal flow resulting from hydrostatic pressure. The current through the microchannel was determined by measuring the voltage drop across a $100 \text{ k} \Omega$ resistor (typically less than $\frac{1}{1000}$ the resistance of the microchannel) connected to the high-voltage supply in series with the microchannel. For the pressure-driven flow studies, a programmable syringe pump (Harvard Apparatus PHD 2000, Holliston, MA) was connected to the stainless tubing in the inlet reservoirs via Teflon tubing.

**Normalization of the Fluorescence Profiles.** All intensity profiles across the outlet channel were analyzed at distances of 183 or 443 $\mu$m from the T-channel junction. These distances correspond to 5 $\mu$m past the end of the four-well mixer and the four-well mixer with alternating partial wells, respectively. The profiles at these locations were normalized by dividing by the maximum fluorescence signal in the inlet channel. To correct for photobleaching and the temperature dependence of rhodamine dye, the normalized fluorescence profile was multiplied by a constant so that the profile for a perfectly mixed solution would reach a maximum of 0.5. This multiplicative constant was a function of the applied electric field and the distance down the outlet channel. For pressure-driven flow studies, the multiplicative constant extrapolated to zero field was used. Finally, it should be noted that the autofluorescence of the substrate and the lid was not subtracted from the intensity profiles.

**Quantifying Mixing.** To quantify the percentage of mixing, the following formula was used

$$\text{percentage mixed} = 1 - \frac{\sqrt{\frac{1}{N} \sum_{i=1}^{N} (I_i^{0} - I_i^{\text{perf, mix}})^2}}{\sqrt{\frac{1}{N} \sum_{i=1}^{N} (I_i^{0} - I_i^{\text{perf, mix}})^2}} \times 100$$

(1)

where $N$, $I_i^{0}$, and $I_i^{\text{perf, mix}}$ are the total number of pixels, the intensity at pixel $i$, the intensity at pixel $i$ if no mixing or diffusion were to occur, and the intensity of the perfectly mixed solution at pixel $i$, respectively. The intensity profile for $I^0$ across the width of the channel was determined by doubling each pixel intensity of the perfectly mixed intensity profile, $I_i^{\text{perf, mix}}$, for half of the channel width, and then setting the intensity of the opposite half of the channel to zero. The midpoint of the channel was chosen such that the total area under the intensity curve for $I^0$ was equal to the area under $I^{\text{perf, mix}}$. The numerator in eq 1 represents the deviation from perfect mixing and is similar to a formula described by Liu et al.\textsuperscript{13} If the channel cross section were rectangular, rather than trapezoidal, $I_i^{\text{perf, mix}}$ would be replaced by the mean intensity, $I$, and the numerator in eq 1 would then become the standard deviation. The experimental data for a perfectly mixed solution were obtained by placing the same fluorescent buffer, with half

![Figure 1](https://example.com/figure1.png)

**Figure 1.** (a) Configuration of the experimental setup and white light microscopy image of an imprinted T-channel with a series of ablated wells. The dashed line after the ablated wells depicts a distance of 183 $\mu$m down the main channel and is the point where the fluorescence intensity profiles were analyzed. Fluorescence images of electroosmotic flow past the mixer at flow rates of (b) 0.06 and (c) 0.81 cm/s. (d) White light image of a T-channel without the ablated wells and fluorescence images of electroosmotic flow at flow rates of (e) 0.06 and (f) 0.81 cm/s.
of the normal rhodamine concentration, in both inlet channels and then applying the various electric fields under investigation.

**Computational Fluid Dynamics Model.** The computational fluid dynamics package CFD-ACE+ v6.4 (CFD Research Corp., Huntsville, AL) was used to create a 3D geometry of the trapezoidal T-channel with the four slanted wells to determine the electroosmotic flow lines and the concentration profiles of rhodamine within the system. The standard Navier–Stokes equation augmented with an electric body force term for electroosmotic flow was solved to determine the streamlines, and the convective–diffusion equation was solved for the concentration profiles. The fluid properties were set to the physical and thermodynamic properties of water at 298 K, and the incoming rhodamine concentration was set to 0.11 mM with a diffusion coefficient of 2.8 × 10⁻⁶ cm²/s. For the 20 mM carbonate buffer, the Debye thickness on the walls was set to 2.2 cm. To ensure that the outlet boundary conditions did not distort the flow field near the four-well mixer, the outlet boundary was extended to 2 mm past the T-junction.

**RESULTS AND DISCUSSION**

**Effective Electroosmotic Flow Rate in the Main Channel.** Because the electric field in the outlet channel is greater than either inlet channel, Joule heating can become significant at moderate to high applied fields. In a previous study performed by our group in a T-channel at high applied fields, the buffer temperature was shown to increase from 40 to 70 °C from the inlet to the outlet channel. The increase in buffer temperature leads to an increase in the electroosmotic mobility due to the decreased buffer viscosity. The differences in the mobility between the inlet and the outlet channels then lead to the spontaneous formation of a pressure gradient such that pressure-driven flow from each reservoir toward the T-junction occurs to ensure that mass is conserved. This is analogous to the results of electroosmotic flow in a capillary with varying axial z potentials. The effective electroosmotic mobility, \( \mu_{EO,eff} \), in the outlet channel, which incorporates the temperature dependence between the channels and therefore the pressure component, was determined by averaging the mass flux over all of the channels and by using the data for the mobility of carbonate buffer as a function of the applied electric field. The mobility data for an imprinted PC channel were measured by the current monitoring method. From the determined effective electroosmotic mobility and the corresponding effective electric field, \( E_{eff} \), the effective electroosmotic velocity, \( u_{EO,eff} \), in the outlet channel was determined. For an applied voltage of −250 V, the effective parameters were \( E_{EO,eff} \), \( \mu_{EO,eff} \), and \( u_{EO,eff} \) of 3.4 × 10⁻⁶ cm²/V·s, 180 V/cm, and 0.06 cm/s, respectively. For the highest applied voltage investigated, −1750 V, \( E_{EO,eff} \), \( \mu_{EO,eff} \), and \( u_{EO,eff} \) were 7.0 × 10⁻⁶ cm²/V·s, 1160 V/cm, and 0.81 cm/s, respectively.

**First-Generation Designs: Low-Velocity Mixing and High-Velocity Stream Splitting.** Our first microchannel mixer design was a series of laser-ablated wells near the junction of a T-channel as shown in Figure 1a. Since electroosmotic flow is a wall-driven phenomenon, it was anticipated that the fluid would enter and follow the contours of the wells, with the slanted well design used to induce lateral transport across the channel. The channel was 72 μm wide at the top, 28 μm wide at the bottom, and 31 μm deep. The width of an ablated well was 14 μm, the center-to-center spacing between the ablated wells was 35 μm, and the length of the region occupied by the wells from the T-junction was 178 μm. White light microscopy of the well profiles showed nearly vertical side walls and a broad parabolic depth profile. The majority of the well had an average depth of 85 μm below the bottom of the channel. However, due to the finite time required for the laser’s X–Y stage to stop and change directions during fabrication, deep spikes existed at the ends of the wells. The spikes were measured to be 223 μm below the bottom of the channel and occupied less than 20% of the total well volume.

The performance of the slanted-well design was evaluated over a range of effective electroosmotic flow rates from 0.06 to 0.81 cm/s. The captured fluorescence microscopy images for flow rates of 0.06 and 0.81 cm/s are shown in Figure 1b and c, respectively. The corresponding intensity profiles across the channel at a location of 183 μm from the T-junction are shown in Figure 2a and b, respectively. These results are compared with the intensity profiles at the same location for flow without a mixer (Figure 1d–f), a three-slanted-well mixer, and a perfectly mixed solution.
For a low flow rate of 0.06 cm/s, it can be seen from Figure 2a that the slanted well design induced lateral transport and resulted in an outlet stream that nearly approximates the profile of a perfectly mixed solution, with the four-well design performing better than the three-well design. This suggests that the degree of lateral transport is dependent on the number of wells present and that an optimum number of wells for mixing is likely to exist.

For a high flow rate of 0.81 cm/s, Figures 1c and 2b, it was observed that the four-well mixer was able to split the incoming fluorescent stream into two streams of equal fluorescence intensity. Based on this result, it was apparent that at high velocities the fluid enters the well at point 1 in Figure 1a and essentially does not exit the well until it reaches the end, point 2. Also, Figure 2b shows that a reduction in the number of ablated wells results in two exiting fluorescent streams of differing concentrations. Stream splitting may prove useful for performing assays downstream from the wells, and the number of slanted wells can be tailored to provide different dilutions of the incoming analyte. Also, a cascading series of T-channel mixers utilizing stream splitting can be envisioned for performing serial dilutions.

The percentage of mixing for a four-well mixer as a function of the effective electroosmotic flow rate was calculated and is shown in Figure 3 along with experimental results for diffusional mixing if no mixer were present. Results are presented for two locations, 183 and 443 μm past the T-junction. At 183 μm and a flow rate of 0.06 cm/s, it was determined that the four-well mixer achieved 74.7% mixing compared to 32.3% mixing for diffusion. As the flow rate increased to 0.81 cm/s, the four-well mixer achieved 45.8% mixing compared to 5.7% for diffusion.

**CFD Results for the Four-Well Mixer.** Since top-down fluorescence microscopy was used to assess the degree of mixing, the possibility exists that there is incomplete mixing, or lamination, of the fluorescent and nonfluorescent fluids along the depth of the channel that could not be experimentally detected, therefore leading to overstated values in Figure 3. For this reason, the CFD model was used to observe the rhodamine concentration profiles. The CFD model had a constant wall mobility of $3.4 \times 10^{-4}$ cm²/V-s and an electric potential at the outlet boundary such that the electric field downstream from the mixer reached 180 V/cm. These values compare with the experimental parameters for an electroosmotic flow rate of 0.06 cm/s in the mixing channel. The full parabolic shape of the ablated wells was difficult to model; therefore, the well depth was set to 85 μm, the average depth over the majority of the ablated well. We anticipate that the depth and the minimal volume of the deep portions of the well will contribute little to the overall flow field and the degree of mixing.

Figure 4 provides cross-sectional views of the model results for the concentration profiles of Rhodamine B as shown in views a–d as determined from the CFD model. The model results pertain to the experimental scenario of an electroosmotic flow rate of 0.06 cm/s in the mixing channel. Locations c and d are 183 and 443 μm, respectively, from the T-junction.
tion (views c and d). Integration of the concentration profile in Figure 4, view c, over the depth of the channel gives a qualitatively similar profile to the experimental fluorescence measurements as shown in Figure 2a, therefore confirming that the results in Figure 3 are not drastically overstated.

From previous work, we have found that ablated structures have a much higher surface charge than imprinted structures in a variety of polymers. To simplify the model, we set the wall charge across the microchannel and inside the wells to a constant. The fact that the model is in good agreement with the experimental results suggests that the increased surface charge of the ablated wells compared to the surface charge of the imprinted channel contributes little to the mixing mechanism at the low flow rates investigated. It is possible that the differences in the surface charge may become important at higher flow rates; however, computational divergence of the model at higher flow rates has currently prevented a determination of the effect.

Mixing by lateral transport within the wells can be observed with the fluid streamlines shown in Figure 5a,b. The presence of the wells, combined with the wall-driven electroosmotic flow, causes the desired effect of pulling the rhodamine into the wells and transporting it to the other side of the channel as observed in Figures 4 and 5. We have also determined, through the fluid streamlines, that the residence time within any well ranged from a few tenths of a second up to 10 s, with the majority of the fluid residing less than 3 s within a well. The long residence times existed for the fluid molecules deep within the well, and as the flow rate increases it would be expected that these residence times would decrease. We will use the CFD model to optimize the depth and the shape of the wells to reduce the dead volume and to minimize the residence time in the wells.

Second-Generation Design: Mixing at High Velocities. To avoid stream splitting and to achieve complete mixing at high electroosmotic flow rates, a series of six partial slanted wells were ablated after the four-well mixer, as shown in Figure 6a, to redirect...
some of the fluorescent fluid toward the center of the channel and enhance mixing. The overall linear region occupied by the wells was 438 μm. The depth profile of the partial wells was also a broad parabolic shape with the average depth over the majority of the well measured to be 100 μm below the bottom of the imprinted channel. The average depth of the deep spikes at the ends of the partial wells was measured to be 238 μm below the bottom of the channel.

The success of this design under electroosmotic flow can be observed in the fluorescence image in Figure 6b for a flow rate of 0.81 cm/s and also in the intensity profiles shown in Figure 7a. Based on the fluorescence profiles at a location of 443 μm, it was determined that this design achieved 87.2 and 80.5% mixing at flow rates of 0.06 and 0.81 cm/s respectively, as shown in Figure 3. This is a dramatic improvement over 63.8 and 21.8% mixing for a four-well mixer and diffusional mixing, respectively, when evaluated at a location of 443 μm and a flow rate of 0.81 cm/s. Theoretical predictions indicate that a channel length of 2.3 cm would be required to achieve 80.5% mixing if no mixer were present and assuming a flow rate of 0.81 cm/s and a diffusion coefficient for Rhodamine B of 2.8 × 10^{-6} cm²/s.

The mixer design shown in Figure 6a was also analyzed under pressure-driven flow at comparable outlet channel flow rates, u_p. Since pressure-driven flow is not a wall-driven phenomenon, and therefore the fluid would not be forced to enter the wells, the presence of the wells would not be expected to have the same ability to mix the two streams. The intensity profiles at the outlet of the mixer for flow rates of 0.21 and 0.83 cm/s are shown in Figure 7b and are compared to profiles for perfect mixing. The percentage mixing versus the flow rate for pressure-driven flow is also shown in Figure 3. From Figures 3 and 7b, it can be seen that the performance of this mixer design under pressure-driven flow is not as successful as mixing under electroosmotic flow; however, the presence of the wells does induce some lateral flow across the channel.

CONCLUSIONS

We have presented results for the mixing of two confluent streams by a novel and easily fabricated static mixer operating under either electroosmotic or pressure-driven flow. The slanted well design of the mixer was able to induce a high degree of lateral transport across the channel. Since mixing within this design occurs by lateral transport, and is therefore not limited by diffusion, the mixing of streams containing molecules with low diffusion coefficients should also be successful. Finally, due to the multitude of different well and channel configurations that are imaginable (number of wells, well length, well location, well depth, well angle, channel height, etc.), computational fluid dynamics studies are currently being conducted to optimize mixing for either electroosmotic or pressure-driven flow.

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