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Ultrasonic Mixing in Microfluidic Channels Using Integrated Transducers

Goksen G. Yaralioglu,* Ira O. Wygant, Theodore C. Marentis,† and Butrus T. Khuri-Yakub

Stanford University, Ginzton Laboratory, Stanford, California 94305

This paper presents a microfluidic mixer that uses acoustic stirring created by ultrasonic waves. The ultrasound is introduced into the channel by integrated piezoelectric transducers. The transducers are made of a zinc oxide thin film, which is deposited on the bottom surface of a quartz substrate. The poly(dimethylsiloxane) channel is aligned to the transducers on the top surface of the substrate. The transducers are designed for operation around 450 MHz. The main mechanism of the mixing is the acoustic stirring of the fluid perpendicular to the flow direction. The radiation pressure that is generated by the transducer causes the stirring inside the microfluidic channel. The performance of the mixer is characterized by mixing phenolphthalein solution and sodium hydroxide dissolved in ethyl alcohol. Flow rates on the order of 1−100 μL/min are used. The transducers are driven by 1.2 V_{rms} sinusoidal voltages at 450 MHz.

The miniaturization of fluidic systems has given rise to many exciting applications in biotechnology and biochemistry.[1−4] Micromachining allows the fabrication of laboratories on chips where fluids are manipulated, transported, and tested in volumes ranging from picoliters to microliters. In addition to being portable, the advantages of the miniaturization include reduced analysis time, requirement of minute amounts of chemical reagents, and potential for parallel analysis for increased throughput.

However, a problem arises due to the small channel dimensions, which usually range from a couple of micrometers to 500 μm. In such channels, the flow is laminar in nature. Reynolds number, which is the ratio of the inertial forces to the viscous forces, can be used to characterize the flow. It is given by[5]

$$Re = \rho v D_h/\mu \qquad (1)$$

where ρ is the fluid density, v is the flow velocity, and μ is the viscosity. D_h represents the hydraulic diameter and, for rectangular channels, is given by

$$D_h = 2ab/(a + b) \qquad (2)$$

where a and b are the cross sectional dimensions of the channel. The Reynolds numbers involved in the field of microfluidics are usually below 100.[6] This prevents turbulent flow. Lack of turbulent flow limits the effective mixing of the fluids under investigation. On the other hand, for most of the fluidic devices, the performance of the device is determined by its ability to mix different agents in a small channel or chamber. Fast chemical analysis requires well-mixed chemicals in microfluidic channels.

In small channels where flow is laminar in nature, the mixing is dictated by diffusion. Approximate average time for a small portion of a fluid to diffuse a distance L can be estimated by[5]

$$T_D = L^2/D \qquad (3)$$

where D is the diffusion coefficient of the liquid. The above equation can be used to predict the order of time scale of mass diffusion. As it suggests, one can dramatically reduce the mixing time by reducing the diffusion length required for mixing or increasing the contact area between two different liquids while keeping the volume constant. This observation leads to simple passive mixer configurations where the diffusion length is shortened by the multilayer lamination of the two different fluids in the same channel. In one approach for obtaining multilayer lamination, two fluids are fed into the channel from a set of alternating small channels.[7] In another approach, the laminar flow of the two fluids is split into half horizontally; these two parts are then combined side by side in another channel. This geometry is repeated until the desired number of lamination layers is obtained in the channel.[8−10] Another way of increasing the contact area is to inject one liquid into the other through nozzles.[11] This injection creates microplumes of the injected fluid in the host fluid and,

* Corresponding author. E-mail: goksenin@stanford.edu.
† Current address: Harvard Medical School, Boston, Ma 02115-5750.

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thus, decreases the diffusion length drastically. An important difference between these methods is that, in the latter, mixing takes place in a chamber rather than a channel. In addition to lamination mixers, there is also another type of mixer that uses chaotic advection.[12] These mixers make use of the fact that chaotic fluid trajectories may occur in zigzag-shaped channels, even at small Reynolds numbers.[13] However, to get enough chaotic motion for efficient mixing, complicated channels in 3D should be built.[14,15] Another chaotic advection mixer, which works well for Reynolds numbers greater than 0 but less than 100, was recently proposed by Stroock et al.[16] The proposed mixer uses bas-relief structures on the floor of the channel. These structures create chaotic mixing inside the fluid. However, the length of the channel should be on the order of centimeters. In summary, passive mixers do not necessitate any external source but they require either fabrication of highly complicated structures or relatively long channels.

In addition to the passive mixers discussed above, active mixers are also used in microfluidic channels. The operation principle of most active mixers depends on introduction of chaos into the flow by means of an external source, rather than the complexity of the channel structures. For example, an electrokinetical chaotic mixer uses ac electric field-induced dielectrophoretic (DEP) forces.[17] DEP forces fold and stretch the interface of the two fluids and introduce chaotic flow. However, this method requires that electrodes be placed under the channel. The electrodes should be isolated from the liquid by an insulating layer. Another electrokinetic mixer is proposed by Oddy[18] et al. In this design, electrokinetic instability is introduced in a chamber by means of high ac electrical field strengths (10−100 V/mm) applied through platinum electrodes. The disadvantage of this mixer design is that low-frequency ac voltages on the order of kilovolts are required for efficient mixing. In an alternative method, a thermal bubble-actuated pump is used to enhance mixing by introducing a wavy interface between the fluids.[19] Another type of active mixer mimics large-scale magnetic bar mixers. Lu et al. developed a microfluidic channel with magnetic microstirrers inside the channel.[20] However, this device should be operated in a rotating magnetic field that is usually provided by a macro-scale magnetic stirrer hot plate.

As a fluid actuation method, ultrasound is also widely used for mixing in channels and in small chambers. Liu et al. demonstrated mixing in a chamber using acoustically induced microstreaming.[21] In their design, a piezoelectric disk is used to excite the air bubbles trapped in the top layer of the chamber at 5 kHz. The reported mixing time is 6 s for 40 V peak-to-peak excitation. In a similar configuration, Yang et al., employed a higher frequency of around 60 kHz and 50 V peak to peak.[22] The downside of the latter configuration is that the temperature increases inside the chamber by 16 °C. In addition to these longitudinal resonance devices, Tsao et al. demonstrated mixing using Lamb wave devices where the Lamb waves are excited on the thin plate that forms one side of the channel.[23] The waves are generated by means of interdigital transducers integrated with the thin plate. The operation frequency of this device is 5 MHz. On the radio frequency side, Rife et al. reported a microfluidic device at 50 MHz.[24] This device uses acoustic streaming for pumping and mixing.

In this paper, we present a simple microfluidic channel with integrated piezoelectric transducers. The operation frequency is in the vicinity of 450 MHz. The transducers generate acoustic streaming inside the channel, enhancing the mixing.

EXPERIMENTAL SECTION

Device Fabrication. The microchannels used in our experiments are made from poly(dimethylsiloxane) (PDMS) by casting silicone rubber on a silicon mold. First, the channel geometry was patterned on a thick positive photoresist (13 μm) spin coated on a silicon wafer. Then the silicon wafer was etched with a deep reactive ion etcher (STS Deep RIE) until the desired channel height had been reached. The surface of the silicon wafer was deactivated using a silanization process.[25] This process prevents the silicone rubber from sticking to the wafer and eases peeling of the rubber from the mold. The degassed PDMS (Sylgard 184 silicone elastomer, Dow Corning) was later cast onto the mold on a level table. The PDMS was cured at 65 °C for 4 h.

The ultrasonic transducers were fabricated on a quartz wafer. As a first step, a 3000-Å-thick gold layer was deposited and patterned on the quartz wafer. A thin film of (8 μm) zinc oxide was deposited on top of the gold electrodes using a magnetron sputtering technique.[26] A shadow mask was used to cover only the desired parts of the wafer. Then, the top electrode (3000-Å e-beam evaporated gold) was deposited and patterned by liftoff. Later, holes at the ends of the channels were mechanically drilled into the quartz wafer to enable inlets and outlets.

The final microfluidic device was obtained by aligning the fabricated transducer and the channel under a microscope. To enhance the adhesion between the PDMS and the quartz, the surface of the PDMS was activated for 30 s in oxygen plasma (DRIE 100A plasma etcher, Drytek) before sticking PDMS onto the quartz wafer. The plasma power and the pressure were set to 100 W and 200 mTorr, respectively. In the final device, the channel height was 50 μm and the channel width was 300 μm. The cross section of the fabricated device is shown in Figure 1a. The device was glued on a piece of printed circuit board, which has soldered high-frequency connectors. The electrical connection was achieved

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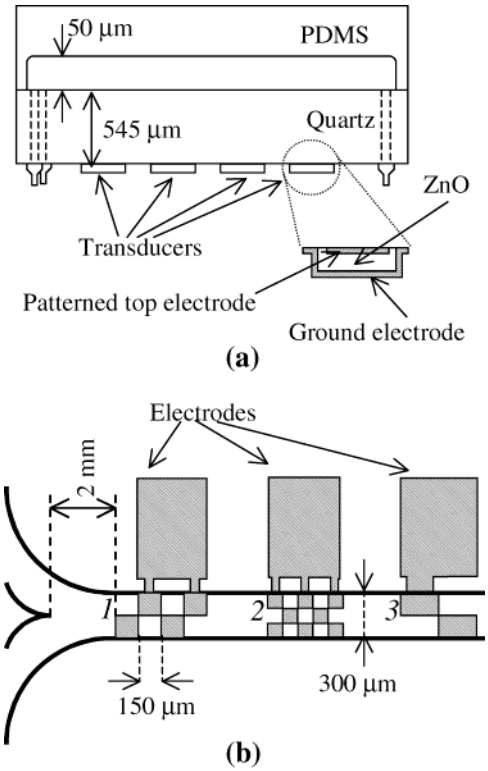

Figure 1. (a) Cross section of the fluidic channel. The piezoelectric material (ZnO) is sandwiched between two electrodes. (b) The electrode pattern of the first three transducers. The ground electrode lies along the channel and it is not shown in the figure for clarity. The edge of the first transducer is 2 mm away from the junction of the two input channels.

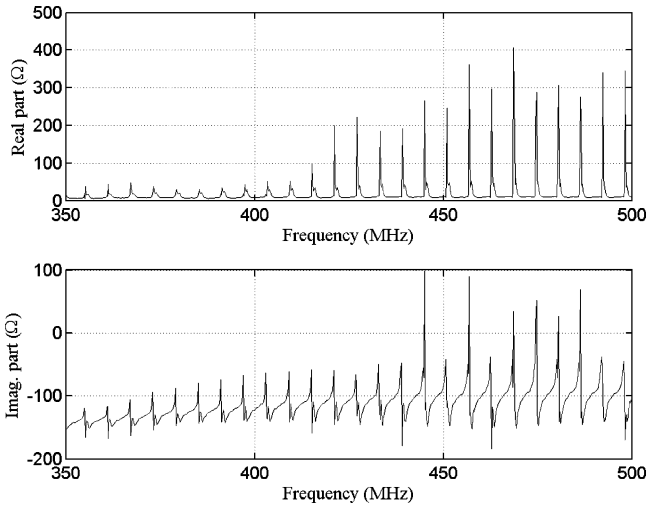

Figure 2. Electrical input impedance of the first transducer.

by gluing a piece of 2-mil gold wire between the connectors and the transducer electrodes using silver ink. The channel was composed of two converging input channels to one main channel as shown in Figure 1b. The transducers were distributed evenly along the main channel on the other side of the quartz wafer. To explore the effect of the electrode pattern, we fabricated transducers with various electrode patterns, as shown in Figure 1b.

Figure 2 shows the electrical input impedance of the first transducer depicted in Figure 1b, when the channel was empty. The impedance is measured using a network analyzer (HP8751A, Hewlett-Packard, Inc.). The longitudinal resonances of the 545-μm-thick quartz wafer reveal themselves as strong peaks, as shown in the figure. The sound velocity is 6040 m/s in quartz, and the corresponding separation between the peaks in the figure is 5.5 MHz. For maximum power transfer into the liquid, the transducer should be operated at one of these longitudinal resonances. The results of a pulse-echo experiment for a similar transducer indicated that the transducer has a 3-dB bandwidth of 25% at 450 MHz.[27]

Mixing Experiments. In microchannels, there are different methods for the evaluation of mixing, such as monitoring the color change with pH, measuring the intensity of fluorescent beads, or observing the color distribution of dyed beads in the channel. In our experiments, we used a pH indicator (phenolphthalein) and a base (NaOH). The solutions were prepared as described by Liu et al.[14] The colorless solutions generated a pink color when they were mixed. By observing the intensity of the color, one can estimate the mixed amounts of the two solutions. In the experiments, phenolphthalein and NaOH solutions were introduced into the main channel from the two inlet channels shown in Figure 1b and the color was monitored over the region following the transducer.

The experimental setup was composed of a syringe pump, a microscope, and a CCD camera. The fluids were supplied to the mixing device using Tygon tubing (3350 Silicone, inner diameter $^1/_{32}$ in.). One end of the tubing was attached to the small hollow stainless steel pieces that were aligned and glued over the mechanically drilled holes in the device. The other end was attached to a syringe pump (PHD 2000, Harvard Apparatus, Inc.) that was capable of driving two syringes (Gastight, No. 1001, Hamilton Co.) simultaneously. The flow rate was set to various values between 2 and 30 μL/min per channel. Therefore, the total flow in the main channel was twice the set value on the pump. The transducers were driven by a high-frequency signal generator (Fluke 6060, Fluke Co.). We observed mixing by using a CCD camera (2200 series, Cohu Inc., San Diego, CA) attached to a stereomicroscope (SMZ-2T, Nikon Inc.). The channel was illuminated from the topside by a halogen lamp at an oblique angle. During the experiments, we kept the illumination constant and turned off the autogain feature of the camera.

RESULT AND DISCUSSION

We produced 320 × 240 pixel color images of the channel using the CCD camera. Phenolphthalein and NaOH solutions flowed through the channel as shown in Figure 3a. The laminar flow was observed in the main channel before the transducer. The diffused chemicals generated a pink line at the interface of the two fluids. The yellow color of the solution before the transducer is due to the gold electrode covering the bottom surface of the substrate where the transducers are fabricated. Figure 3b shows the zoomed image in the vicinity of the transducer. For this particular image, the flow rate was 10 μL/min in the channel. The granular appearance of the image is due to the reflection from the rough surface of the gold. The rapid mixing of the chemicals was observed as shown in Figure 3b just following the transducer. The image in Figure 3b was chosen from the video stream, and it was the first frame after the transducer was turned on.

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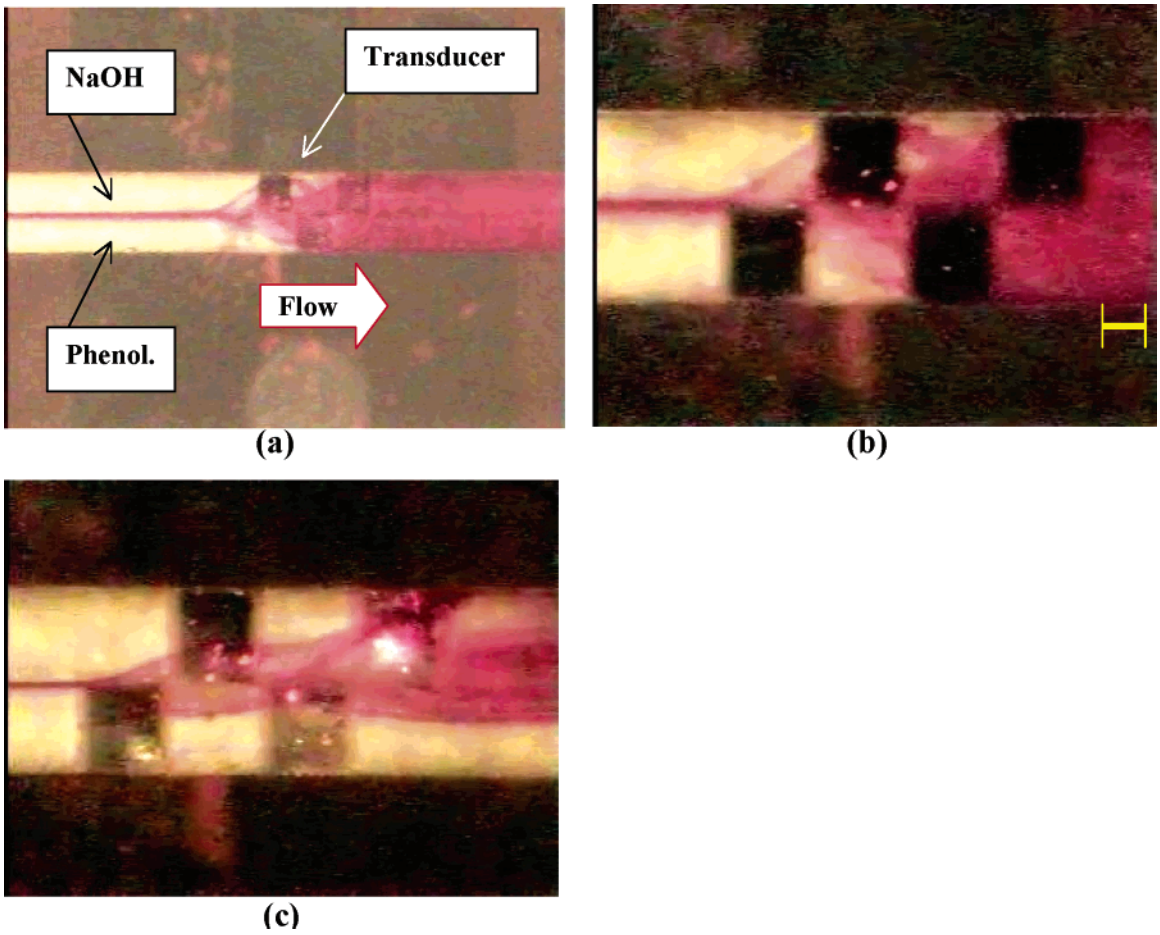

Figure 3. (a) Laminar flow before the transducer and mixing after the transducer. The flow rate is 10 μL/min in the main channel. The transducer is driven by 30 mW. The image is 1.5 mm by 2 mm. (b) Zoomed view of the transducer. The image is 650 μm by 860 μm. The flow rate is 10 μL/min. (c) The flow rate is 60 μL/min. On all the images, lighting conditions were kept constant but the brightness levels were autobalanced to improve the appearance of the images.

Before acquiring the images, the drive frequency was fine-tuned until the acoustic streaming over the transducer was maximized. At this frequency, which is 445.88 MHz, maximum power was coupled into the liquid. Uniform mixing across the channel was observed at an available power level of 15 dBm (30 mW, 1.2 V_{rms}). However, the electrical input impedance of the transducer was not matched to the source impedance (50 ohms) at the operation frequency. The power delivered to the transducer was therefore less than 15 dBm. The measured reflection coefficient of the transducer at the frequency of operation is 0.57, and the corresponding delivered power is 13 dBm (20 mW). Figure 3c shows the mixing at 15 dBm when the flow rate is 60 μL/min. As the flow rate increases, color uniformity across the channel degrades.

Figure 4 shows the intensity of the red color across the channel for various flow rates. Due to the nonuniform reflection from the gold surface, the measured intensity varies, even when the channel is filled with an opaque liquid. To reduce this variation, the red level of the image across the channel was averaged over the region as indicated by the yellow bar shown in Figure 3b. The depicted area is composed of 30 vertical lines. The corresponding width of the region is 82 μm and it is 100 μm away from the edge of the transducer. For flow rates below 10 μL/min, we obtained very uniform intensity distribution across the channel as shown in Figure 4. The associated Reynolds numbers, *Re*, are 0.34 and 0.86

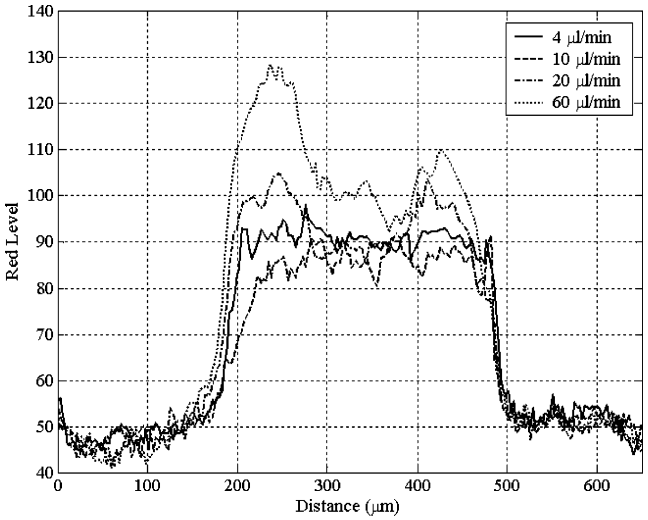

Figure 4. Intensity of the red color for different flow rates. The intensity is averaged over the region, as indicated by yellow bar shown in Figure 3b.

for flow rates of 4 and 10 μL/min, respectively. To calibrate the measurements, we premixed the two solutions in a large beaker and flowed them through the main channel without changing the lighting condition. In this case, the obtained red level was 90. As the flow rate increased beyond 50 μL/min, the uniformity started

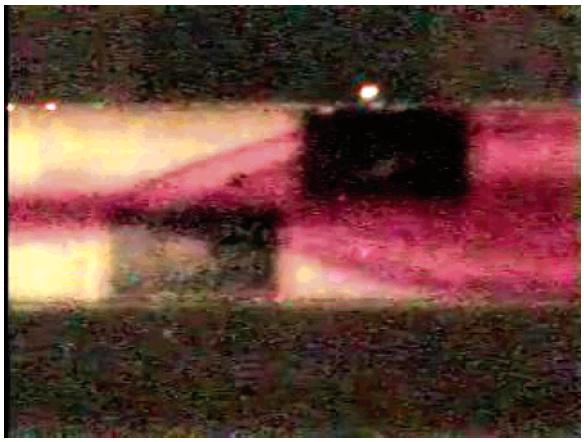

Figure 5. Mixing using the third transducer. The flow is from left-hand side to right-hand side. Flow rate is 10 μL/min in the channel.

to degrade and the red level close to the bottom of the image ($\sim$200 μm) increased to 130, which is the red level of the unmixed yellowish solution.

We also tested the other electrode configurations shown in Figure 1b. For example, Figure 5 shows the image obtained using the third transducer. However, the other electrode configurations did not perform as well as the first one in terms of color uniformity across the channel.

The primary mechanism of mixing in the channel is acoustic streaming. When the transducers are excited by sinusoidal signals, they generate a time harmonic acoustic field inside the fluid that propagates perpendicular to the flow direction. The propagating acoustic wave creates a second-order dc pressure, which is known as the Langevin acoustic radiation pressure,[28] and it is given by

$$\Omega = p_i^2/2\rho c^2 \quad (4)$$

where p_i is amplitude of the time harmonic acoustic field and c is the sound wave propagation velocity inside the liquid. The amplitude of the acoustic field on the transducer can be calculated by

$$p_i^2 = 2\rho c P_E/A \quad (5)$$

where P_E is the coupled electrical power and A is the area of the transducer. Due to the acoustic absorption inside the liquid, the radiation pressure drops as the wave moves away from the transducer, creating a pressure gradient across the channel. As a result, acoustic streaming is generated, which produces stirring and effective mixing. In water, the acoustic waves attenuate 0.22 dB/(mf^2), where f is the frequency (in MHz). Therefore, for our channel, the acoustic attenuation at 450 MHz is 2.23 dB for waterlike liquids. Using eq 5, one can estimate the harmonic pressure over the transducer as 816 kPa (assuming 20 mW electrical power input to the transducer). At the top of the channel (50 μm away from the bottom), this harmonic pressure drops down to 612 kPa, due to the absorption in the liquid. The corresponding radiation pressures are 148 and 83 Pa. This results in a pressure gradient of 65 Pa over 50 μm. If we assume a 150 μm by 150 μm rectangular channel of 50 μm height over each segment of the transducer, it is possible to calculate the vertical fluid velocity. The fluid resistance for rectangular channels is calculated by Foster et al.[29] For our geometry, the resistance is 3.37×10^9 N s/m.[5] Therefore, the above-mentioned pressure drop results in a fluid velocity of 85.7 cm/s. This calculation assumes the transducer is radiating into a half-space and the standing wave pattern does not affect the pressure distribution over the transducer. Moreover, it is also assumed that the fluid resistance of a water block is calculated as if a rigid channel encapsulates it. The above calculation is a very rough approximation but can be used to estimate the order of magnitude of the vertical flow inside the channel.

The main mechanism of the mixing is the acoustic streaming of the liquid above the transducer perpendicular to the flow inside the channel. This streaming pushes the fluid elements toward the top of the channel as well as sideways. Therefore, patterning the electrodes in checker configuration, as shown in Figure 1, introduces the different parts of the laminar flow into each other. The most successful electrode configuration was found to be the first configuration shown in Figure 1 where alternating sections of the transducer covers half of the channel.

It is also useful to calculate the diffusive mixing time. Using eq 1, assuming the characteristic length of 150 μm and waterlike solutions flowing in the channel (the diffusion coefficient for water is 10^{-9} m^2/s), the mixing time is estimated to be 22.5 s. We observed mixing in a much shorter time.

Problems commonly associated with using ultrasound for mixing include the formation of bubbles and unintended heating of the fluid. We did not observe any bubble formation inside our microfluidic channel. Previously, we reported the use of ultrasound for heating and temperature measurement.[27] Our experiments showed that, for a similar channel geometry, water was heated 4 °C at 20 dBm when there is no flow. Therefore, at 15 dBm (3 times less power) and at a constant flow inside the channel, we expect the heating to be less than 1 °C.

CONCLUSION

We have presented a novel active mixer, which uses acoustic streaming generated by integrated ultrasonic transducers. The acoustic streaming is perpendicular to the flow direction and results in mixing of the fluids flowing inside the channel. The device presented is easy to build and does not require fabrication of any complex structures. In addition, it does not use any moving parts. The mixing occurs at the same rate as the ultrasound propagation from the bottom of the channel to the top of the channel, on the order of microseconds. We also observed that, at higher flow levels (tens of microliters per minute), one has to increase the power level to get a uniform mixing across the channel.

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