ULTRASONIC MICROMIXER FOR MICROFLUIDIC SYSTEMS

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ABSTRACT

This paper describes the design, fabrication and evaluation of an active micromixer. Mixing occurs directly from ultrasonic vibration. The intended use of the device was for integrated micro chemical synthesis systems or for micro total analysis systems. The pattern of inlets, outlet and mixing chamber were formed in glass. The whole flow path was encapsulated by anodic bonding of a Si wafer to the glass. A diaphragm (6 mm x 6 mm x 0.15 mm) was etched on the Si side for oscillation. The ultrasonic vibration originated from a bulk piezoelectric PZT ceramic (5 mm x 4 mm x 0.15 mm), which was excited by a 60 kHz square wave at 50 V (peak-to-peak). Liquids were mixed in a chamber (6 mm x 6 mm x 0.06 mm) with the Si oscillating diaphragm driven by the PZT. A solution of uranine and water were used to evaluate the mixing effectiveness. The entire process was recorded using a fluorescent microscope equipped with digital camera. The laminar flows of uranine solution (5 μl/min) and water (5 μl/min) were mixed effectively when the PZT was excited.

INTRODUCTION

We report an active micromixer using PZT-generated ultrasonic vibration. We believe this prototype should be useful in micro total analysis systems, clinical diagnostics, drug screening and micro synthesis systems.

A microfluidic system can be defined as a system which handles liquids on the order of microliters and picoliters. With regards to the applications of micromachine technologies, portable systems are expected to be realized for on site monitoring and on site synthesis. The benefits of such systems include reduced consumption of samples and reagents, and greater sensitivity by reducing background noise [1]. The ability for parallel analysis is another advantage using microfabrication technologies, implying that the time needed for analytical results can be dramatically shortened. The systems for these applications should integrate elements for acquisition, pretreatment, reaction, separation, posttreatment and detection. The related technology development centers on transportation, mixing and separation of samples. Sophisticated micropumps [2] and micro capillary electrophoresis devices are the benchmarks for sample transportation and separation [3]. The methods for creating the networks of micro channels are well established for substrates of silicon, glass and polymer. Other fundamental components for integrated systems, such as microvalves for flow control and micromixers for reaction, are still under development.

In principle, the mixing of different fluids depends on turbulence and interdiffusion. Magnetic stirrers are the conventional solutions for achieving this in the macroscopic world, but they are difficult to scale down to suit liquids in the micro liter order. Miyake et al. first reported a micromixer [4], using a double layer structure for mixing two liquids. 400 micronozzles (15 μm x 15 μm) were fabricated in the middle separating membrane, so one liquid can be injected into the second stationary liquid through these micronozzles. Similar efforts have been reported recently [5-8], including an array of holes to separate the main flow into partial flows, which are laterally alternated in order to increase the boundary surface between the liquids. Another way to increase the contact area is relatively complex, where repeated structures have been developed to repeat a lamination/splitting process [9-12]. At each cycle, the number of laminates becomes double and the thickness of individual laminate becomes half. This principle is also known to macroscopic mixing of viscous liquids in Kenics mixer [8, 13]. Branebjerg et al. showed the results of the mixing effects in the straight and zigzag channels in both mini- and micro size [14]. Generally, microfluids have very low flow rates (Reynolds numbers << 2000), the flows are laminar, so that all these approaches focus on fabricating structures which enhance the interdiffusion effect in laminar flow. Turbulent mixing is excluded. Such structures can be defined as passive mixers in which no external energy is introduced into the mixing parts. The problems with these passive mixers are that the mixing time is affected greatly by the flow rates and the ratio of liquids flow [15]. The pressure drop through the flow path, especially the micro-array structure, may affect the homogeneity greatly [8]. These devices also face the same problems as those for most micropumps [2]. They are difficult to prime and sensitive to gas bubbles.

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A reliable active micromixer is really necessary for general use applications. Active mixer is defined as the mixer with external energy input to enhance the mixing effect. It should be tolerant towards gas bubble and the mixing effects should be adjustable by changing the level of input energy. Evans et al. reported the design and simulation results of an active micromixer [16]. Its mixing chamber was connected to 2 bubble-actuated pumps through several bubble-actuated valves. The experimental results of this system are expected. We found that turbulence was possible in microfluidics in certain areas. A valveless micropump driven by PZT, which operates at high frequency (in the kHz region), can locally generate turbulence near the areas of the nozzles [17-19]. Using a modified structure, we reported the first active micromixer, which was operated by using turbulence effect to enhance the mixing of liquids [20]. The mixing of liquids started with the work on valveless micropump. However, this working principle makes this type of mixer difficult to be used for general microfluidic applications. The problem is that the flow ratio of solutions for mixing cannot be changed freely. An artificial change of the flow ratio through the application of an external pressure to the fluids may result in malfunction the valveless micropump, making mixing impossible. Therefore, for general use, the mixing unit should be separated from the pumping unit.

Liquid streaming and eddying can be introduced by ultrasonic waves [21]. Zhu et al. reported a micromachined opening with a loosely focused ultrasonic lens. The ultrasonic waves were loosely focused on water-air interface to generate the pressure gradient. Liquid motion was observed when ultrasonic waves were generated at radio frequency [22]. If this principle is effective in a closed chamber, it can be a candidate for an active micromixer. Yasuda et al. reported the mixing effect using standing waves [23]. Instead of a micromachined structure, they used a glass tube modified with PZT as the mixing chamber as well as for other purposes. The mixer driven by a transverse traveling wave was theoretically analyzed [24] and a SAW device was designed [25], but no experimental data is available yet. We have reported the first active micromixer using PZT-generated ultrasonic vibrations [26]. The device worked effectively at 48 kHz. The ratio of solutions for mixing can be set freely by adjusting the pressure applied.

In this paper, we present the quantitative evaluations of active micromixer employing PZT-generated ultrasonic vibrations. The design factors for a mixer for an integrating fluidic system are also discussed.

MATERIALS AND METHODS

The same structure as that employed previously [26] was used in this study. The excitation frequency was increased from 48 kHz to 60 kHz. Conventional photolithographic methods were used in the fabrication of the device. The mixer has two inlets and one outlet. The mixing chamber is in the size of 6 mm x 6 mm and has a depth of 0.06 mm (Fig. 1). The patterns of inlets, outlet and mixing chamber were formed in Pyrex glass #7740 (Iwaki Glass, Japan) by HF isotropic etching (50% HF-69% HNO_3-H_2O in volume rate of 2:1:2) [27]. A pretreatment by thermal annealing [27] was not necessary for the glass wafer we used. A 200 Å thick chromium layer, topped by a 1500 Å thick gold layer, was evaporated as the etching mask. The entire flow path was encapsulated by the anodic bonding of a Si wafer to the glass. Then, an oscillating diaphragm was fabricated on the Si side by KOH etching at 70°C. A 8000 Å thick thermally oxidized SiO_2 layer, topped by a 1000 Å thick LPCVD Si_3N_4 layer, was used as the etching mask. The oscillating diaphragm was designed to cover the mixing chamber area, which had the same length and width as the mixing chamber and a thickness of 0.15 mm. After a Pt/Ti background electrode was sputtered onto the Si side, a piece of bulk piezoelectric PZT ceramic (5 mm x 4 mm x 0.15 mm) was adhered directly onto the oscillating diaphragm using an epoxy resin, XNR 3506 (Nagase-Ciba, Japan) (Fig. 2). The PZT (Type C-82) was purchased from Fuji Ceramics Co. (Fujinomiya, Japan). A function generator (Sony Tektronix AFG320, Japan) connected to a power amplifier (NF Electronic Instruments 4010, Japan) was used to generate the square wave (50 V peak-to-peak in 60 kHz) for the PZT excitation. The mixing performances of the device were measured by mixing water and a fluorescent dye, undiluted uranine (Sodium fluorescein) solution (Yoshida Chem., Japan). External pressure was applied to the liquids using a fluid dispenser (Musashi Engineering Inc.,

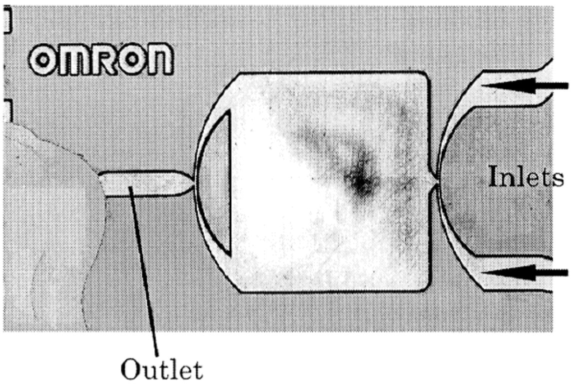

Fig.1 Photograph of our mixing device. Two separated mixers were fabricated on one chip. Each mixer had two inlets and one outlet connected to the mixing chamber (6 mm x 6 mm). The bulk PZT was adhered onto the opposing side.

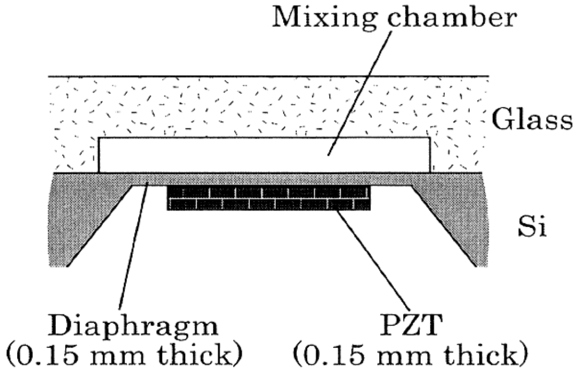

Fig. 2 Schematic drawing of the cross section of the mixing chamber. The glass substrate was etched and anodically bonded to a Si wafer to form the flow channel. Si was etched from the back side to form the oscillating diaphragm and a PZT was adhered onto the oscillating diaphragm.

MS-10DU, Tokyo). The flow rates of both water and uranine solution were 5 μl/min. The flow rates were measured using ϕ0.70 glass micropipettes (Drummond, USA).

A fluorescence microscope (Customer ordered from Olympus, Japan) with a digital CCD camera (FUJIX HC-300Z, Fujifilm, Japan) was used for mixing evaluation. Almost the whole mixing chamber was in the field of CCD camera using the 1.25x objective. The fluorescent filter we used (U-MWIBA, Olympus) has a 460-490 nm filter for excitation and a 515-550 nm filter for absorption. For a quantitative analysis of the mixing ratio, the auto-balance of the CCD camera was disabled. The gain was controlled manually and the exploration time was fixed to 83.3 msec. CCD camera images were saved in uncompressed TIFF format for intensity calculation. The data of fluorescent intensity was converted from TIFF files using the software Scion Image for Windows [28]. For a clear print in black and white, the photos of the fluorescence images were inverted. Water with no fluorescence is shown as white area and the fluorecent uranine is shown as the darker area (Fig. 3).

RESULTS AND DISCUSSION

An active micromixer with a very simple structure has been developed. The entire fabrication process was compatible with conventional microfabrication technologies. It shows great flexibility to be integrated into systems for sample preparation, analysis or synthesis. The principle employing ultrasonic vibrations was shown to be effective for enhancing mixing. It was tolerant towards gas bubbles and no priming process was needed. This is a significant step toward the development of integrated microanalysis and microsynthesis systems.

There is no standard method for evaluating the performance of micromixers. Basically, two kinds of evaluation have been reported: testing by optical absorption and luminescence. Inks [26] and pH indicators [6, 11, 14] are popular choices for observing optical absorption. Optical absorption is a simple characterization method that does not require complex instrumentation, but presents two problems for the testing of the micromixer. Firstly, the optical absorption path is conventionally of the order of a few millimeters, so that the microchannels cannot be tested with good signal-to-noise ratio. Secondly, the Si/Glass structure of the device requires absorption testing to be carried out in the reflective mode. The poor surface quality of an etched structure can affect the quality of the reflected signal [8]. The other evaluating method using luminescence is a better solution. There are no problems from short absorption distance and reflection. However, they need some special instruments. The methods using chemiluminescence (luminol + H_2O_2) [8], bioluminescence (luciferin + luciferase + ATP) [7] and fluorescence (uranine) [4] have been reported. Chemiluminescence is weak, and photomultiplier tubes are generally required for its monitoring. This makes it difficult to observe the distribution of two liquids in the mixing chamber. On the other hand, bioluminescence uses expensive reagents. We think fluorescence is the best solution. The bandpass filters for excitation and absorption greatly reduce the noise level, making this method very sensitive. Previously, we tried to use the fluorescent dye, rhodamine B. This dye was found to stick strongly to glass surfaces, requiring very thorough purging of the mixing chamber for cleaning. Therefore, rhodamine B was unsuitable for real-time monitoring the mixing process. On the other hand, uranine was found to be very bright. It was too bright when mercury lamp illumination was used. Instead, we used normal halogen lamp in our fluorescent microscope and got satisfactory results.

Our device mixed different liquids effectively. The entire mixing process is illustrated in Figure 3. In the standby state, with no ultrasonic vibration, the flow was laminar. Only limited diffusion was observed at the interface of the water and uranine flows (Fig. 3a). Turbulence occurred immediately after the ultrasonic vibration (Fig. 3b). The successive picture was taken 7 sec after Fig. 3b. The mixing spread throughout the entire area of the output (Fig. 3c). When the ultrasonic vibration was terminated, the laminar flows resumed immediately (Fig. 3d). The two liquids mixed rapidly and effectively. The long period of 7 sec for taking the picture shown in Fig. 3c was due to rephrase the CCD camera. The mixing process itself was much faster, about 2 sec from video tape recording [26].

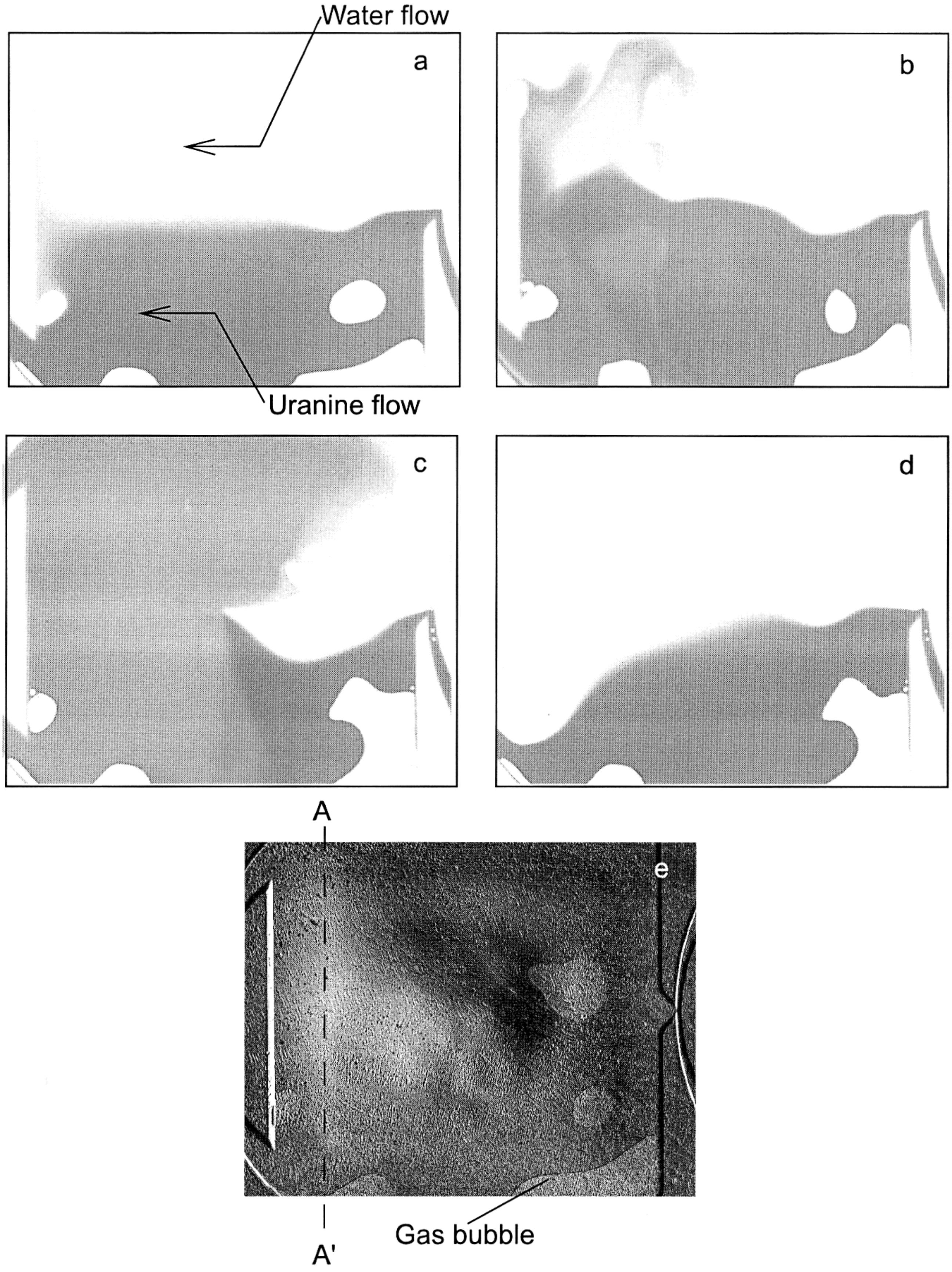

Figure 3. Mixing process (see text). (a) Fluorescent view in the standby state. (b) Ultrasonic ON. (c) 7 sec after ultrasonic irradiation. (d) Ultrasonic OFF. (e) Photograph in bright field.

The mixing ratios for our device were measured near the outlet of the mixing chamber from Fig. 3a and 3c. The intensity measurements were taken along the line AA' (Fig. 3e). The results were shown as Fig. 4. The relative fluorescent intensity here was not linearly proportional to uranine concentration. Our CCD camera was not calibrated [30]. Some gas bubbles existed but did not affect our mixer's performance. The two laminar flows in the standby state became homogenous after ultrasonic irradiation.

We tested the frequency characteristics of our mixer up to 600 kHz (due to the limitation of our power amplifier). Quantitative results were not obtained because no measurable mixing index is available yet. There was no linear relation between excitation frequency and mixing ability. Qualitatively, we observed that the mixing efficiency decreased at ~90 kHz and recovered at ~130 kHz. We chose the excitation frequency as low as possible but without unpleasant noise (around 20 kHz).

We used an oscillating diaphragm in the mixer structure. Theoretically, the mixing effect originated from the ultrasonic irradiation. Thus an oscillating diaphragm is not necessary for acoustic irradiation. In Yasuda's work, fused silica was used to increase the thickness of the mixing chamber's wall [23]. The impedance of ultrasonic irradiation from glass to liquid was matched with the thickness of glass. In our device, the thickness of silicon wafer for making the device was 0.4 mm. The wavelength of 50 kHz acoustic wave in silicon is about 170 mm. So the effect from the change of silicon's thickness could be neglected. The silicon oscillating diaphragm we used was only to prevent the ultrasonic radiation from escaping to the other part of the device and to focus it into the mixing chamber. There is no need to match the ultrasonic impedance at the solid-liquid interface anymore. The actuator can be considered working directly on the liquid. In Yasuda's mixing chamber, the ultrasonic path is naturally isolated by air. But for microfabricated mixing chamber, the isolation of ultrasonic radiation should be carefully accounted for. We thought the oscillating diaphragm structure was the easiest way to achieve ultrasonic isolation.

The mixing chamber can still be optimized. Because of complexities in the physics [21], we cannot give fundamental theory on ultrasonic mixing. Active mixers have disadvantages. Heat generation is unavoidable during the energy input for mixing processes. As indicated by Berg et al. [29], considerable heat can be added to the liquids with ultrasonic irradiation. Our mixer can work continuously. There was no need to interrupt for cooling when water and uranine were used. However, heat will be a problem for temperature-sensitive fluids. The excitation voltage of 50 V was still high. The excitation voltage of 10 or 15 V was preferred for a compact driver unit. We are currently looking for a modified structure for better performance. The experimental results from the focused ultrasonic lens seem promising [22], as focused ultrasounds introduced little heat into liquid. Another approach to reduce the input energy may be to combine with passive mixers' structures to get fast and effective mixing.

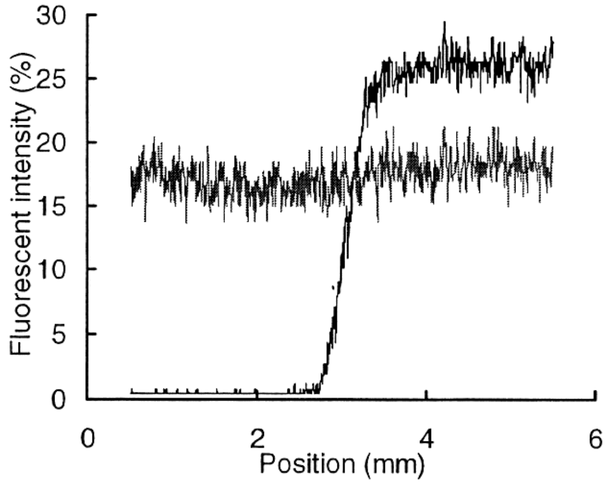

Fig. 4 Mixing effect measured near the outlet at the positions on the line AA' as shown in Fig. 3e before (the "S" line, from Fig. 3a) and after (the line in the middle, from Fig. 3c) ultrasonic emission. Position 0 was defined as the upside of the mixing chamber on the line AA'.

CONCLUSIONS

In MEMS applications, internal moving parts are difficult or impractical to construct, and thus, ultrasonic vibration was proposed. A micromixer using ultrasonic waves, tolerant of gas bubbles and compatible of operating under different flow rates was demonstrated. No significant pressure drop in the mixer was measured. This active micromixer structure exhibits great potential for integration for micro sample preparation, micro total analysis- or microsynthesis systems. This work confirmed that ultrasonic waves are a very powerful tool for microfluidic applications.

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