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A Fast and Switchable Microfluidic Mixer Based on Ultrasound-Induced Vaporization of Perfluorocarbon†

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Mixing two fluids together within a microfluidic device remains, still today, a challenging operation. In order to achieve this goal, a number of effective micromixers have been developed over the years based on the use of either passive or active systems. Typically, passive mixers require no external energy, are more robust, easy to manufacture albeit poorly flexible. Active mixers, on the other hand, rely on external disturbance and are thus more difficult to implement but have proven greater efficacy. Here, we report a particularly effective, remotely-induced and switchable microfluidic mixer, which relies on the concomitant use of ultrasound and a perfluorocarbon (PFC) phase, the latter benefiting from its non-miscibility with most fluids and its low boiling point. More specifically, our approach is based on the localized vaporization of a PFC phase at the focus of a transducer leading to the efficient mixing of two adjacent fluids. The results show that mixing occurs ~100 ms following the delivery of the acoustic pulse, while the laminar flow is re-established on roughly the same time scale. Overall, this method is simple and effective, it does not require tailored channel geometries, it is compatible with both hydrophilic and hydrophobic microfluidic systems, it is applicable to a wide range of Reynolds numbers (10^{-4}<Re<2.10^{0}) and the PFC phase is easily separated from the mixed phase at the end of the run.

Microfluidics has attracted tremendous attention over the past two decades to become a particularly exciting field of research with broad applications in various areas including biology, biotechnology, chemical synthesis and optics.[1] This success is largely due to the fact that microfluidic systems rely on short analysis times, low reagent or biological sample consumption, cheap microfabrication techniques, and most of all, they are generally simple to operate and they benefit from the possibility to integrate different steps of an analysis or a synthesis on a same chip, which is why these systems are often designated as "labs on chips".[2]

Although mixing two liquids together is a key operation in microfluidic devices, it remains challenging due to the laminar character of the flow. Indeed, microfluidic channels have typical dimensions and flow rates that lead to low Reynolds numbers (Re << 10). Under these conditions, as viscous forces dominate over inertia, the mixing is mainly governed by diffusion which translates into slow mixing. This limitation has spurred researchers to develop mixers able to overcome this drawback.[3] These mixers can be classified as active or passive. Hence, passive mixers[4] rely on techniques such as splitting and recombining fluids,[5] multi-lamination or chaotic strategies,[6] such as the herringbone mixer,[7] to increase channel length and tortuosity, fluid contact time and diffusion. They are easy to integrate in a chip as they require no external forces but they generally impose longer mixing length and time. Active mixers,[8] on the other hand, require external stimuli to induce the mixing effect which often leads to complex manufacturing processes, increased fragility and reduced portability. Nonetheless, such systems, which involve magnetic-,[9] thermal-,[10] optical-[11] or acoustic-based[12,13] stimulations, were proven more effective. They benefit from their non-invasive character as well as their remote activation. In this context, Baigl and co-workers[14] recently reported the use of a three-phase system, where the central phase could be affected by an external light stimulus to induce mixing. In line with this remotely-induced mixing approach, we designed a new, extremely effective, switchable and non-geometry dependant ultrasound-triggered mixer based on the localized vaporization of a PFC phase at the focus of a transducer (Figure 1).[15] We report here the results of our endeavour.

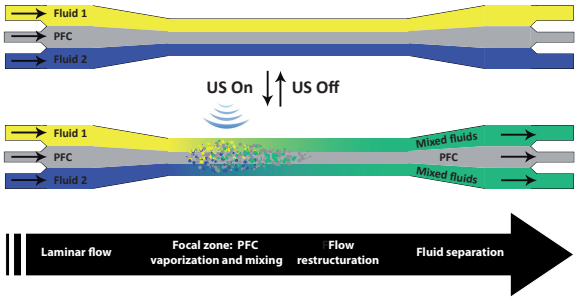

Fig. 1. Ultrasound-induced vaporization of a perfluorocarbon phase for efficient mixing in microfluidic channels.

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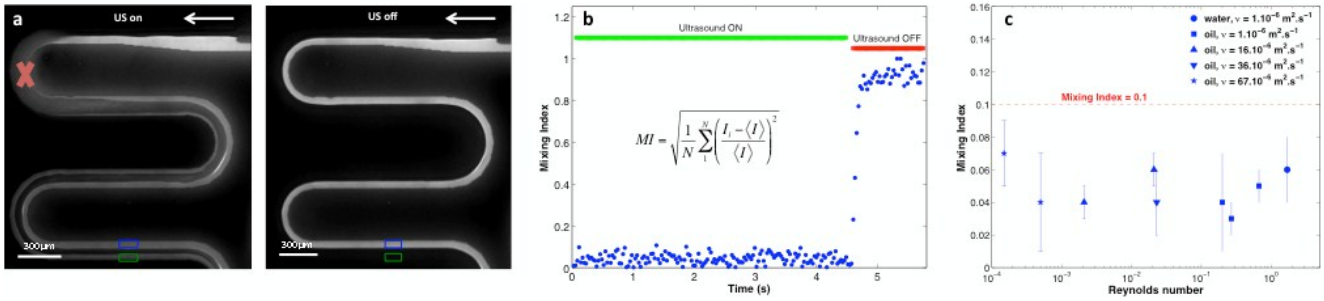

Fig. 2. (a) Representative fluorescence microscopy images corresponding to mixing following **Method A** (with or without ultrasound activation). Ultrasound focus is represented by the red cross. (b) Corresponding mixing index evolution (Q_{tot} = 25 µL/min, v_{oil} = 36.10^{-6} m^2.s^{-1}, Re = 0.023). (c) Mixing index as a function of the Reynolds number.

To allow an efficient ultrasound-triggered mixing within a microfluidic device, we imagined a three-layer co-flow comprised of two external oil or water phases and a central perfluorocarbon (PFC) phase that prevents the mixing to occur in the absence of any ultrasound owing to both its hydro- and lipophobic properties. Indeed, we believed that 1) the PFC layer would be briefly vaporized when submitted to ultrasound solicitation within the focal zone (~600 mm) of the ultrasound transducer, 2) this vaporization phenomenon would induce a brief emulsification of the PFC together with the oil or water external phases leading to mixing, 3) spontaneous demixion of the fluids would occur after crossing the focal zone thus allowing to separate the mixed oil or water phase from the perfluorinated phase and 4) our method would require no specific geometry and allow a switchable mixing of the external phases provided the ultrasound source could be alternatively turned on and off.

Three cases were studied in order to demonstrate the power of our microfluidic mixing method which was initially conceived for both hydrophobic and hydrophilic microfluidic systems. Each case involved a central perfluorocarbon (PFC) phase composed of a 1:1 mixture of perfluoropentane and perfluorohexane running between two external phases. Method A and B involved two external oil phases, one of which was stained with a fluorescent dye (Nile Red, 600 µM) or a suspension of fluorescent beads (10 µm in diameter), respectively, while Method C involved two external water phases, one of which was stained with fluorescein (800 µM).

To quantify the quality of the ultrasound-induced mixing along the channel, two regions (green and red boxes) of equal size were defined at the top and bottom part of the channel and the average fluorescence of these regions was recorded over the sequence of images. As expected, in the absence of ultrasound pulses the two external phases remained well separated by the non-miscible central PFC layer and the average fluorescence of both regions remained unchanged independently of the case studied (Figure 2 and Figure S9 in the Supporting Information). Upon ultrasound excitation, however, we observed a highly emulsified region at the focal zone of the transducer, while downstream, the mixed water or oil phase and the PFC demixed progressively, with the PFC remaining in the middle of the channel due to wetting properties. Most importantly, the fluorescence downstream to the focal zone was equally distributed thus confirming the ultrasound-induced mixing.

To evaluate the efficacy of the mixing, we used a mixing indicator which was calculated using the equation shown in Figure 2,[16] where a mixing index (MI) equal to 1 represents a non-mixing situation while an MI equal to 0 reflects a perfect mixing. Hence, in the absence of ultrasound-excitation, a MI close to 1 was observed independently of the method used, thus showcasing the lack of mixing. In contrast, after ultrasound irradiation, an excellent mixing was observed in both hydrophilic and hydrophobic systems. Indeed, we were able to show that the MI remained unaffected over four decades of Reynolds numbers (10^{-4}<Re<2.10^{0}) with values ranging from 0.03 to 0.07 (Figure 2c). Analysis of the results obtained using the fluorescent beads dispersed in oil also confirmed the excellent mixing of the two fluids as the beads were equally distributed on both sides of the channel downstream to the focal zone (Method B, Figure 3). Once again, the splitting of the beads occurred in a similar fashion for all the Reynolds numbers tested (Figure S10 in the Supporting Information). Most importantly, the efficiency of the mixing compared favourably with all the methods reported so far in the literature.[7b] In particular, to the best of our knowledge, no other micromixer allows to reach a range of Reynolds numbers that covers four orders of magnitude[17] and, more importantly, that embraces both low and moderate values, *i.e.* compatible with viscous and moderately inertial regimes. We believe this will have interesting consequences in chemical engineering, where micromixers are often required to operate on a range of different flow regimes.

As the perfluorinated central phase only vaporizes at the focus of the ultrasound transducer, the mixing event can be activated on demand by simply turning the latter on or off as illustrated in Figure 4 which shows a sequence of mixing and non-mixing situations. Hence, as long as ultrasound were applied to the system, the mixing appeared optimal and stable in time (Figure 4a). As soon as the transducer was switched off, a short transient regime was observed (Figure 4b), while the three incoming laminar flows replaced the mixed fluids in the channel (Figure 4c) to reach a maximal MI corresponding to two perfectly well separated external fluids (Figure 4d). When the ultrasound were switched back on,

(Figure 4e), another short transient regime occurred until a stable mixing regime was attained again (Figure 4g). This transition between two series of mixing and non-mixing events appeared highly reproducible in both efficacy and time; the MI decreasing instantaneously as soon as the transducer was switched on. In addition, both transitions (mixing to non-mixing and *vice versa*) occurred within a second at a flow rate equal to 9.6 µL/min, while these transitions occurred at a significantly higher speed (115 ms and 92 ms) when the flow rates were respectively equal to 18 µL/min and 25 µL/min. This observation tends to show that we are able to adjust the transition time between mixing and non-mixing regimes by simply tuning the injection flow rate.

Another estimation of the mixing time was achieved by measuring the channel length necessary to affect the laminarity of the co-injected fluids. Indeed, when reaching the focal spot of the transducer, fluids enter a transition zone where the trajectories of the beads statistically cover the entire width of the channel downstream to which the flows separate and the beads are equally split. Considering the distance measured to switch from a laminar to a non-laminar situation, we estimated this time around 1.2 ± 0.5 ms the latter being independent of the flow rate (see Supporting Information for details).

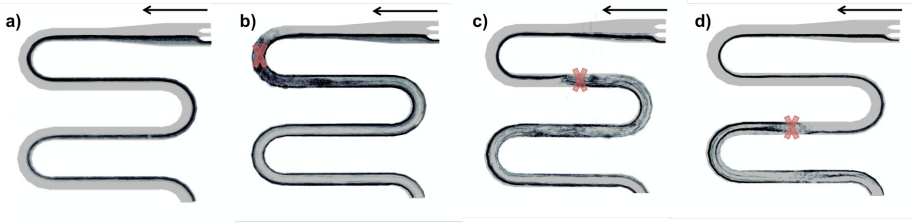

Fig. 3. Mixing of two oil phases according to **Method B** (beads in oil). Ultrasound were focalized in the upper left corner (b), central positions (c and d) or turned off (a). $Q_{pure\ oil} = Q_{oil+beads} = 0.8$ µL/min, $Q_{PFC} = 8$ µL/min.

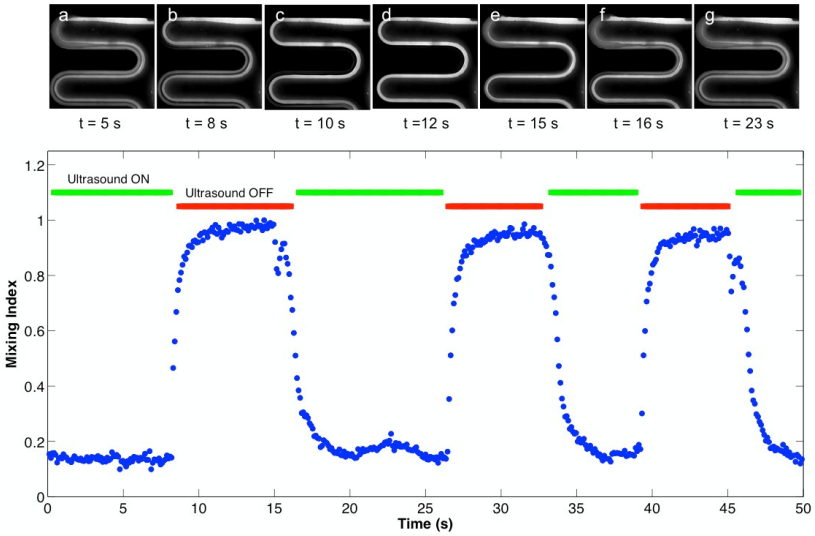

Fig. 4. Reversible mixing of two oil phases (**Method A**, Q_{tot} = 9.6 µL/min). Representative fluorescence microscopy images corresponding to different steps in the sequence of ultrasound excitation of the system. Corresponding mixing index value as a function of time, calculated from the same two regions (green and red).

To showcase the simplicity of the method, we deliberately designed a microfluidic device made of a simple serpentine based on a straight, rectangle-sectioned channel which also allowed to visualize the fluids on a long distance (few mm) while using the same observation window. The aspect-ratio of the channel was chosen low to prevent a droplet regime and thus favour a laminar circulation. This was made possible as in contrast to other mixing methods our mixer appeared to require no specific geometry. In addition, we were also able to show that mixing could be induced at various positions of this same serpentine in a reproducible and effective fashion and with an excellent precision. Indeed, as shown in Figure 3, the trajectories of the beads remained undisturbed in the laminar flow until reaching the focal zone where they split upon local ultrasound stimulation.

Another important feature that was studied concerned the loss of efficacy, which is usually observed with most available mixers, especially passive mixers, when the flow rate is increased. Interestingly, this did not appear to be the case with our mixer. Indeed, we were able to show that the transition between a mixing and a non-mixing situation remained unaffected upon increasing the flow rate with concomitantly no noticeable impact on the efficacy of the mixing (see Figure S10 in the Supporting Information). Thus, the splitting of the beads on both sides of the channel occurred in a similar fashion at flow rates ranging from 9.6 µL/min to 48 µL/min which corresponds to Reynolds numbers ranging from 1 to 2.

While PFC was specifically chosen for its ability to vaporize upon ultrasound-stimulation, it was also chosen for its non-miscible character with most fluids, its high density (density of perfluoropentane and perfluorohexane used here are respectively 1.63 g/mL and 1.67 g/mL at 25 °C), and its wettability in the perspective of incorporating this mixer in a more complex series of operations on a microfluidic chip where the PFC could be separated from the mixed fluids at the end of the run. In order to attain this goal, we designed and built a separation unit that is a replica of the entrance unit except that the size of the channels was modified to allow the central PFC phase to be removed. As the major requirement was that the PFC did not contaminate the collected mixed fluids, the width of the central exit channel was chosen bigger (100 µm) than the ones of the two side exit channels (40 µm). As shown in Figure 5, we were able to demonstrate that the PFC could be easily separated from the mixed fluids.

The use of perfluorinated solvents to induce mixing in microfluidic channels is a new concept. In order to further understand the mechanisms lying behind the emulsification of the PFC, a series of experiments were carried out by changing the ultrasound parameters such as the amplitude of the pulses, the number of cycles within each pulse and the interval between two pulses. The peak-negative acoustic pressure (in MPa) and the total intensity of the focused wave (in Watt/cm^2), which are obtained from these parameters, affect the liquid perfluorocarbon differently. Indeed, mechanical effects such as cavitation are mainly correlated to the peak-negative pressure, while thermal effects such as vaporization are influenced by the total acoustic energy absorbed by the medium (here PFC). As shown in Figure 6, the mixing efficiency

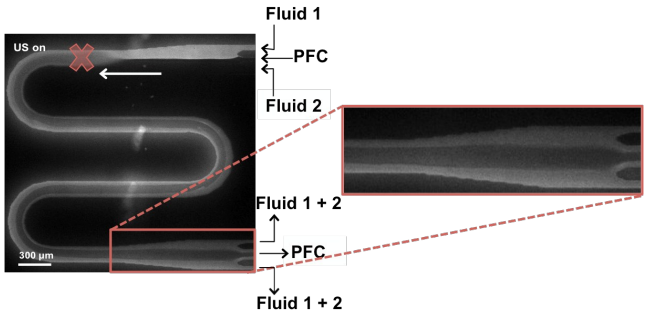

Fig. 5. Microscopy image of perfluorocarbon extraction from the system (**Method A**, Nile Red in oil). The focal zone is represented by the red cross, and flow direction by the white arrow.

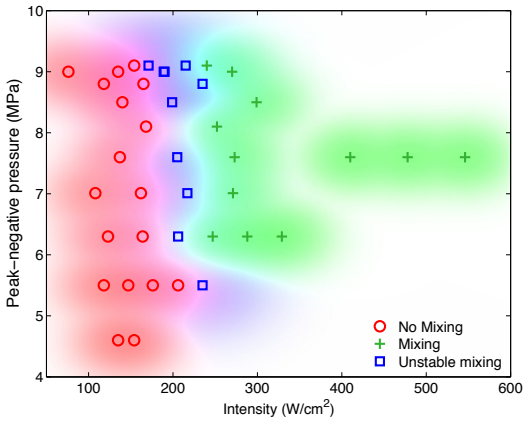

Fig. 6. Evaluation of the mixing efficacy. The interval between the pulses is maintained constant at 100 µs, while the pulse amplitude and the number of cycles were adjusted to obtain respectively an efficient mixing effect (green), an unstable mixing (orange) and no mixing effect (red). These different situations are represented in a phase diagram of the peak-negative pressure (MPa) *vs* the intensity (W/cm^2).

depends mostly on the absorbed acoustical energy and only marginally on the peak-negative pressure, which tends to support a vaporisation-like phenomenon.

In summary, we designed a new and particularly effective active mixer based on the localized vaporization of a perfluorocarbon stream at the focus of an ultrasound transducer. The results show that when the ultrasound are active within the microfluidic channel, the laminar flow is affected in less than 2 ms, while a total switch from a non-mixing to a mixing situation is observed in roughly 100 ms following the delivery of the acoustic pulse. High mixing efficacy was reached, as illustrated by the low mixing index values (MI < 0.2). Overall, this method is simple to implement and highly effective, it does not require tailored channel geometries, it is applicable to a wide range of Reynolds numbers (10^{-4}<Re<2.10^{0}) and it is compatible with both hydrophilic and hydrophobic microfluidic systems. In addition, as a spontaneous separation of the mixed fluids from the PFC occurs downstream to the focal zone, the PFC phase can be easily separated from the mixed phase at the end of the run. We believe that such ultrasound-triggered mixing using PFC-vaporization properties will lead to a new generation of switchable, extremely fast, and highly versatile mixers easily incorporable in microfluidic chips.

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Notes and references

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Abstract. We report here a remotely-induced and switchable control of microfluidic mixing triggered by ultrasound. The method is based on the localized vaporization of a perfluorocarbon (PFC) phase at the focus of a transducer leading to the efficient mixing of two adjacent fluids. Overall, this method is simple and effective over a wide range of Reynolds numbers, it does not require tailored channel geometries, it is compatible with both hydrophilic and hydrophobic microfluidic systems and the PFC phase is easily separated from the mixed phase at the end of the run.