Supplementary Information

An acoustofluidic sputum liquefier

Po-Hsun Huang,[a] Liqiang Ren,[a] Nitesh Nama,[a] Sixing Li,[ab] Peng Li,[a] Xianglan Yao,[c] Rosemarie A. Cuento,[c] Cheng-Hsin Wei,[d] Yuchao Chen,[a] Yuliang Xie,[ae] Ahmad Ahsan Nawaz,[a] Yael G. Alevy,[f] Michael J. Holtzman,[f] J. Philip McCoy,[c] Stewart J. Levine,[c] and Tony Jun Huang*[ae]

[a]Department of Engineering Science and Mechanics, The Pennsylvania State University, PA 16802, USA

[b]Cell and Developmental Biology (CDB) Graduate Program, The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA.

[c]National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892, USA.

[d]Department of Nutritional Sciences, The Pennsylvania State University, PA 16802, USA

[e]Department of Chemical Engineering, The Pennsylvania State University, PA 16802, USA

[f]Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA.

*To whom correspondence should be addressed: junhuang@engr.psu.edu

Mixing performance at different regions in the channel

Using DI water and fluorescein (DI water containing FITC-dextran) as a demonstration of mixing performance, we were able to examine under a flow rate of 30 µL/min, whether such a long channel can result in a complete, uniform mixing of the two solutions solely based on diffusion. As shown in Fig. S1, the mixing performance at different regions, including the beginning, the middle, and the end of the channel, was characterized in terms of fluorescence intensity. It is clear that when the piezoelectric transducer was switched OFF, only an incomplete, partial mixing of the solutions due to diffusion was observed under the flow rate condition. By contrast, once the piezoelectric transducer was switched ON, a complete, uniform mixing of the solutions was achieved throughout the channel, demonstrating the advantage of the active mixing of our acoustofluidic mixer. Given the flow rate condition and the solution (DI water) we used, the Reynold's number was estimated to be roughly 800. Although t is higher than that in a typical microfluidic device, it still falls into the laminar flow regime. For the case of human clinical sputum samples, the precise calculation of Reynold's number is more challenging because the viscosity of sputum varies across a wide range (10^3 – 10^6 times the viscosity of water),[1] depending on the patient status. Besides, no data is provided on the density of the sputum in the literature. Considering the extremely high viscosity of clinical human sputum samples, a longer mixing channel incorporating with active acoustic mixing mechanism is believed to be beneficial for processing human sputum samples.

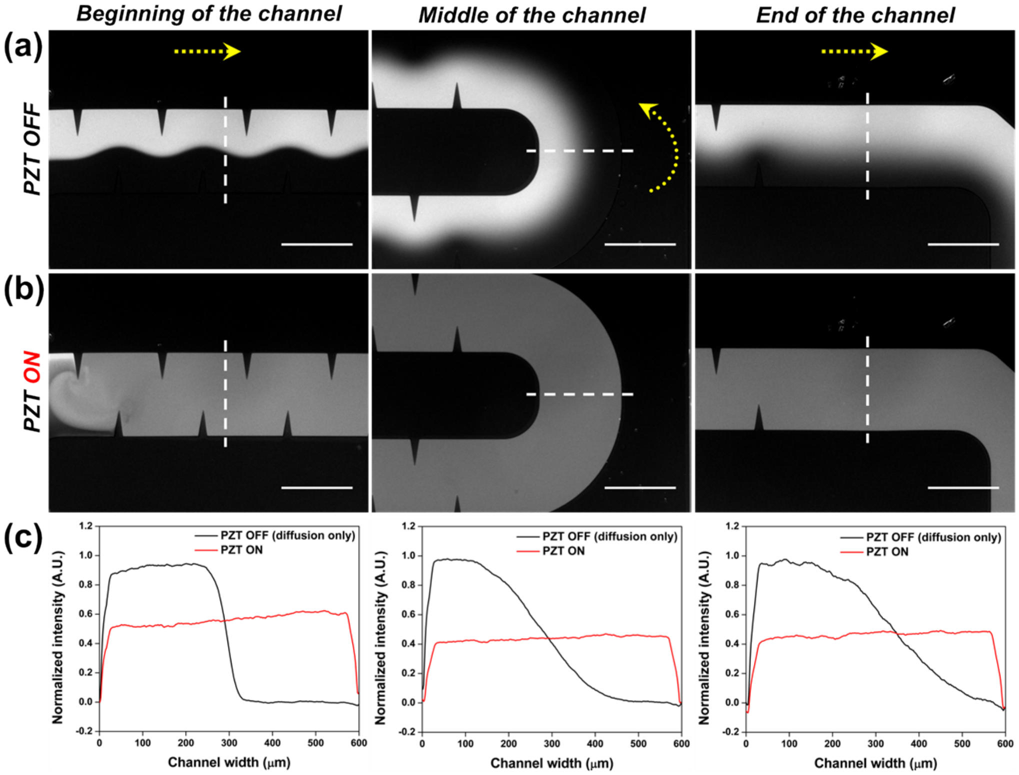

Figure S1. *Characterization of the mixing performance at different regions under a total flow rate of 30 µL/min. (a) When the PZT was switched off: A side-by-side laminar flow pattern was first observed in the beginning of the long channel; partial, incomplete mixing due to diffusion was then observed at the middle and the end of the channel. (b) When the piezoelectric transducer was switched ON: Uniform, complete mixing was observed throughout the channel. (c) Plots of corresponding normalized fluorescent intensity across the width of channel at different regions. The normalized intensity profiles were characterized along the dashed white lines in the figure. Scale bar: 500 µm*

Determination of the working frequency for the piezoelectric transducer

In order to determine the optimized the working frequency for the PZT before conducting liquefaction experiments, we swept the frequency with a 50 Hz increment from 1 kHz to 100 kHz to monitor and characterize the mixing performance at different frequencies. From the experimental results, we found that there were some frequencies at which mixing (incomplete or complete) of the two solutions were observed. Fig. S2 shows the mixing performance at the beginning of the channel when our device was tested with different input frequencies. The results suggest that when tested with a frequency of 5.50 kHz, our device achieved complete mixing with a uniform intensity profile, suggesting that 5.50 kHz may be the optimum working frequency for our sputum liquefier. To be more accurate, we further verify the mixing performance by calculating mixing index for each case. The mixing index was calculated using following equation:[2]

$$M = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\frac{I_i - I_m}{I_m}\right)^2}$$

where I_i is the gray scale value at a given point, I_m is the average gray scale value, and n is the total number of sampled points along the width of the channel. By using this equation, one can know that the mixing index is actually the standard deviation of gray scale values along the width of the channel. A mixing index of 0.5 represents completely unmixed fluids (*i.e.*, distinct laminar flow), while a mixing index of 0.0 stands for completely mixed fluids. In this work, we chose a mixing index below 0.1 as acceptable mixing. The mixing indices were calculated to be 0.433, 0.047, and 0.118 for mixing performance obtained, respectively, using the frequency of 5.75 kHz, 5.50 kHz, and 5.25 kHz. The results, once again, suggest that 5.50 kHz is the optimized working frequency for our acoustofluidic device to uniformly mix two solutions.

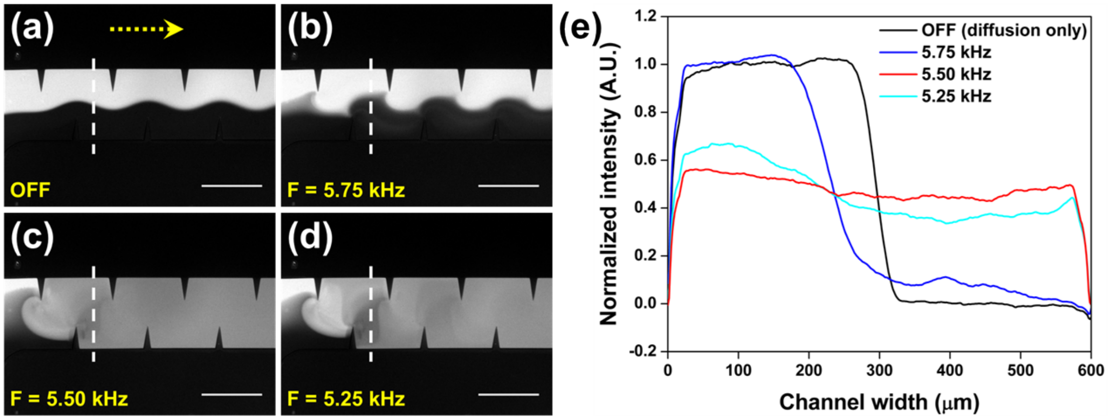

Figure S2. *Characterization of the mixing performance at the beginning of the channel under different driving frequencies of the piezoelectric transducer. (a) A side-by-side laminar flow pattern was observed when the piezoelectric transducer was switched off. (b) 5.75 kHz: Incomplete mixing due to weaker acoustic streaming effect. (c) 5.50 kHz: Uniform mixing was observed. (d) 5.25 kHz: Excellent mixing was achieved, but not as complete as that obtained using 5.50 kHz. (e) Plots of normalized fluorescent intensity across the width of channel. The normalized intensity profiles were characterized along the dashed white lines in the figure. Scale bar: 500 μm*

Reference:

[1] S. K. Kai, Y. Y. Wang, D. Wirtz, and J. Hanes, *Advanced Drug Delivery Reviews*, 2009, **61**, 86-100.

[2] P. Garstecki, M. J. Fuerstman, M. A. Fischbach, S. K. Sia, and G. M. Whitesides, *Lab on a chip*, 2006, **6**, 207-212.