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# Separation of Yeast Cells from MS2 Viruses Using Acoustic Radiation Force

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**Separation of Yeast Cells from MS2 Viruses Using Acoustic Radiation Force**

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We report a rapid and robust separation of *Saccharomyces cerevisiae* and MS2 bacteriophage using acoustic focusing in a microfluidic device. A piezoelectric transducer (PZT) generates acoustic standing waves in the microchannel. These standing waves induce acoustic radiation force fields that direct microparticles towards the nodes (i.e., pressure *minima*) or the anti-nodes (i.e., pressure *maxima*) of the standing waves depending on the relative compressidensity between the particle and the suspending liquid.[1] For particles larger than 2 μm, the transverse velocities generated by these force fields enable continuous, high throughput separation.

Extensive work in the last decade [2-4] has demonstrated acoustic focusing for manipulating microparticles or biological samples in microfluidic devices. This prior work has primarily focused on experimental realization of acoustic focusing without modeling or with limited one-dimensional modeling estimates. We recently developed a finite element modeling tool to predict the two-dimensional acoustic radiation force field perpendicular to the flow direction in microfluidic devices.[1] Here we compare results from this model with experimental parametric studies including variations of the PZT driving frequencies and voltages as well as various particle sizes and compressidensities. These experimental parametric studies also provide insight into the development of an adjustable 'virtual' pore-size filter as well as optimal operating conditions for various microparticle sizes.

Figure 1 shows a typical experimental acoustic focusing result for microparticles (diameter = 2.0 μm) in a 500 μm wide by 200 μm deep microchannel. In this case, the PZT driving frequency and voltage are, respectively, 1.459 MHz and 6.6 V. The microparticles tightly focus (full width half maximum (FWHM) ~30 μm) less than 30 s after the initiation of the acoustic field.

We simulated the same geometry and operating conditions for comparison. The surface plot in Figure 2 illustrates the two-dimensional pressure field orthogonal to the flow direction (*x*-direction) from the simulation. The superimposed vector plot shows the acoustic radiation force in this plane. The dark regions and the light regions respectively represent the nodes and anti-nodes of the acoustic pressure field. The corresponding force field predicts acoustic focusing at the center of the microchannel, which is confirmed by the experimental results shown in Figure 1.

We demonstrated the separation of *Saccharomyces cerevisiae* (typical cell size of 4-6 μm depending on the cell growth stage, measured using a Coulter counter) and MS2 bacteriophage (typical diameter ~30 nm [5]) using acoustic focusing (Figure 3). A mixture of *S. cerevisiae* and MS2 labeled with Ribogreen was prepared and injected into one inlet of the microchip (i.e., half of the microchannel was filled with the sample). We varied driving voltages from 1.96 to 4.76 V, while fixing the driving frequency at 1.459 MHz and flow rate at 20 μl/min. The acoustic radiation force did not affect the MS2 viruses, and their concentration profile remained unchanged. Increased driving voltages enhanced the acoustic focusing of the yeast cells thereby achieving good separation. We are able to achieve yields of > 80% and sample purities of > 90% in this continuous-flow sample preparation device.

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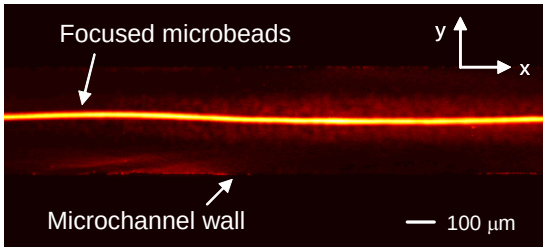


***Figure 1.*** *A representative acoustic focusing of micro particles (d = 2.0 μm). The driving frequency and voltage were 1.459 MHz and 6.60 V, respectively. Flow rate was 20 μl/min, and a 4x objective (N.A. = 0.1) was used.*

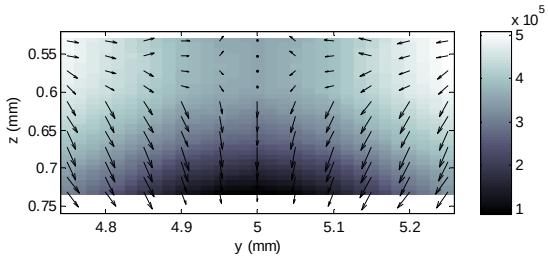


***Figure 2.*** *Two-dimensional (cross-section of the microchannel) numerical estimate of the acoustic pressure field and the acoustic radiation force field. The dark regions and the light regions respectively represent nodes (low acoustic pressure) and anti-nodes (high acoustic pressure) in the acoustic pressure field. The driving frequency was set to 1.481 MHz. The acoustic radiation force field (vector plot) predicts a strong acoustic focusing at the nodes (the center of the microchannel).*

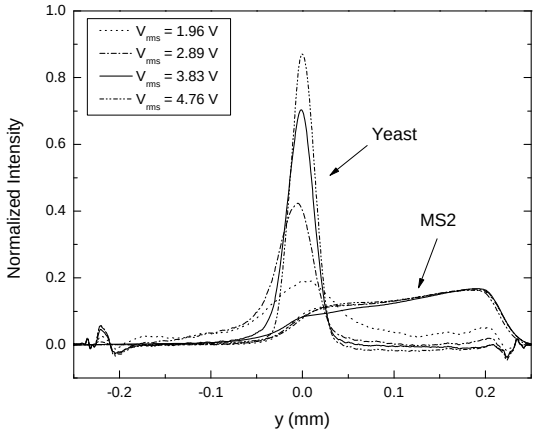


***Figure 3.*** *Separations of a mixture of yeast cells and MS2 viruses. Concentration profiles (normalized intensity) across the microchannel width direction (y) are shown for the driving voltages from 1.96 to 4.76 volts. The driving frequency and flow rate were 1.459 MHz and 20 μl/min, respectively. Samples were initially introduced only at the right hand side of the microchannel.*

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