IMPROVED BACTERIAL AND VIRAL RECOVERIES FROM COMPLEX SAMPLES USING ELECTROPHORETICALLY ASSISTED ACOUSTIC FOCUSING


Lawrence Livermore National Laboratory, USA

ABSTRACT

We describe an electrophoretically assisted acoustic focusing technique to rapidly extract and enrich viral and bacterial loads from ‘complex samples,’ applied in this case to human nasopharyngeal samples as well as simplified surrogates. The acoustic forces capture and remove large particles (> 2 µm) such as host cells, debris, dust, and pollen from the sample. We simultaneously apply an electric field transverse to the flow direction to transport small (≤ 2µm), negatively-charged analytes into a separate purified recovery fluid using a modified H-filter configuration.

KEYWORDS: Sample preparation, acoustic focusing, electrophoresis, filtration

INTRODUCTION

Automated front-end sample preparation technologies can significantly enhance the sensitivity and reliability of biodetection assays [1]. We are developing advanced sample preparation technologies for biowarfare detection and medical point-of-care diagnostics using microfluidic systems with continuous sample processing capabilities. Our device uses acoustic forces to capture and remove particles larger than 2 µm while transporting smaller, negatively-charged analytes into a separate purified recovery fluid using an electric field transverse to the flow direction.

Hunter and O’Brien combined transverse electrophoresis and acoustic focusing to measure the surface charge on large particles [2], but to our knowledge, our work is the first demonstration combining these two techniques in a continuous flow device. Marina et al. demonstrated superimposed dielectrophoresis (DEP) and acoustic focusing for enhanced separations [3], but these devices have limited throughput due to the rapid decay of DEP forces away from the electrodes. Previous work demonstrated acoustic focusing of microbeads [4] and biological species [5] in various geometries.

THEORY

Both acoustic standing waves and electric fields exert forces over the entire fluid volume in microchannels, thus allowing channels with larger dimensions (> 100 µm) and high throughputs (10-100 µL/min) necessary to process real-world volumes (1 mL). We utilize an acoustic standing wave with a single node in the center of the channel to generate acoustic forces that focus the particles to this plane (Figure 1a). The electric field, applied transverse to the flow field, transports negatively charged particles such as viruses through the acoustic standing wave (Figure 1b).
EXPERIMENTAL

We experimentally characterized our device by determining the biological size cut-off where acoustic radiation forces no longer transport biological particles. Figure 2 shows images of E. Coli (~1 µm) and yeast (~4-5 µm) flowing in a microchannel (200 µm deep, 500 µm wide) at a flow rate of 10 µL/min. The E. Coli does not focus in the acoustic field while the yeast focuses at the channel centerline. This result suggests the acoustic size cut-off for biological particles in our device is between 2 and 3 µm.

We demonstrated transverse electrophoretic transport of a wide variety of negatively-charged species, including fluorophores, beads, viruses, E. Coli, and yeast. Figure 3 shows the electromigration of a fluorescently labeled RNA virus (MS2) across the channel with continuous flow from left to right.

RESULTS AND DISCUSSION

To demonstrate the capabilities of our device, we simultaneously separated yeast cells and 40 nm polystyrene microbeads (virus-like particles). These results, shown in
Figure 4, are an example of how this device can be used to remove large particles (> 2 µm) such as host cells, debris, dust, and pollen from a sample while capturing the smaller charged particles such as viruses.

![Figure 4. (a) Yeast cells and a mixture of yeast cells and virus-like particles are injected into the top and bottom streams, respectively. (b) Acoustic field turned on (yellow arrows), and only yeast cells focus at the center of the microchannel. The virus-like particles are ‘acoustically invisible’ and remain in the bottom-half of the channel. (c) Electric field turned on across the microchannel (orange arrow), and the small virus-like particles electromigrate through the acoustic capture region.](image)

CONCLUSIONS

We demonstrated the effectiveness of our electrophoretically assisted acoustic focusing device by separating virus-like particles (40 nm fluorescent beads, selected to aid in visualization) from a high background concentration of yeast contaminants (see Figure 4). Our device allows for the efficient recovery of virus into a pre-selected purified buffer while large background contaminants are acoustically captured and removed. We also tested the device using clinical nasopharyngeal samples, both washes and lavages, and demonstrated removal of unknown particulates (>2 µm size) from the sample. Our future research direction includes spiking known amounts of bacteria and viruses into clinical samples and performing quantitative off-chip analysis (real-time PCR and flow cytometry).

ACKNOWLEDGEMENTS

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-CONF-404844

REFERENCES


