LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

LLNL-CONF-402587

Improved Bacterial and Viral Recoveries from 'Complex' Samples using Electrophoretically Assisted Acoustic Focusing

K. Ness, K. Rose, B. Jung, K. Fisher, R. P. Mariella, Jr.

March 28, 2008

Micro-TAS
San Diego, CA, United States
October 14, 2008 through October 16, 2008

Disclaimer

Topic No. 1.5 Separation Methods
Oral or Poster
Paper ID No: 0527

Improved Bacterial and Viral Recoveries from 'Complex' Samples using Electrophoretically Assisted Acoustic Focusing

Kevin Ness, Klint Rose, Byoungsok Jung, Karl Fisher, and Raymond P. Mariella Jr.
Lawrence Livermore National Laboratory (LLNL), Livermore, CA 94551, USA
Phone: (925) 423-1856, Fax: (925) 423-2373, Contact Email: ness5@llnl.gov

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. Here we report 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 [Micronics US Patent 5,716,852].

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. Both acoustic standing waves and electric fields exert significant 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).

Previous work demonstrated acoustic focusing of microbeads [4] and biological species [5] in various geometries. We experimentally characterized our device by determining the biological size-cutoff where acoustic radiation pressure forces no longer transport biological particles. Figure 1 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-cutoff for biological particles in our device lies between 2 and 3 μm.

Transverse electrophoresis has been explored extensively in electric field flow fractionation [6] and isoelectric focusing devices [7]. We demonstrated transverse electrophoretic transport of a wide variety of negatively-charged species, including fluorophores, beads, viruses, *E.Coli*, and yeast. Figure 2 shows the electromigration of a fluorescently labeled RNA virus (MS2) from the lower half of the channel to the upper half region with continuous flow.

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 3). Our device allows for the efficient recovery of virus into a pre-selected purified buffer while 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).

Word Count: 499

This work were performed under the auspices of the U.S. Department of Energy
by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-CONF-402587

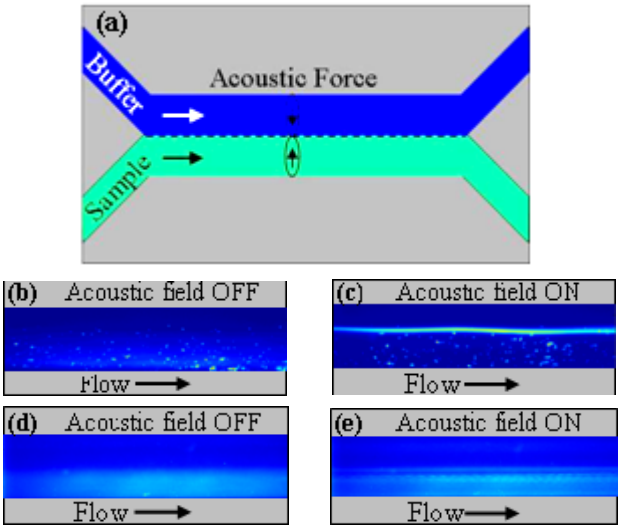

Figure 1. Acoustic focusing of yeast and E.Coli in our microfluidic device (top view) (a) Sample and buffer streams converge in the main channel where the acoustic forces are applied. Yeast (b) and E.Coli (d) flowing with the field off. (c) Yeast (~4-5 μm) is focused to the channel centerline while the (e) E.Coli (~1 μm) is not affected by the acoustic field. The bright spots in the lower half of the channel in images (b) and (c) are yeast cells adsorbed to the glass walls.

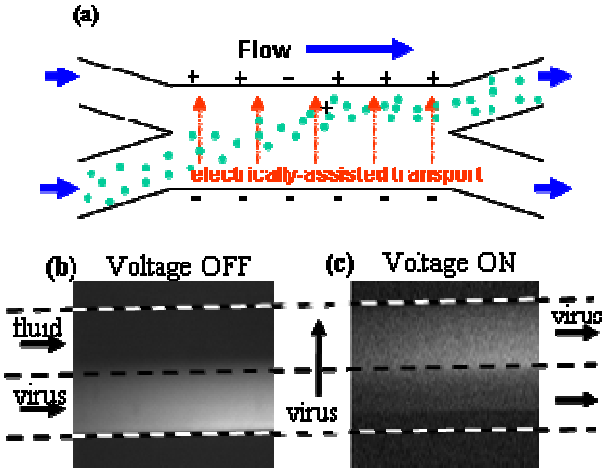

Figure 2. Electrophoretic transport of fluorescently-labeled MS2 virus. (a) The cross-flow electrophoresis device continuously processes sample with fluid flow from left-to-right and electrophoretic transport from bottom-to-top. Fluorescently-labeled MS2 virus before (b) and after (c) the electric field is applied at a downstream location. (b) Virus flowing in the sample stream when the applied voltage is off. The clear liquid side of the channel is dark, indicating a lack of virus. (c) Transport of virus from the lower half of the channel to the upper half when an appropriate voltage is turned on. All images are top view.

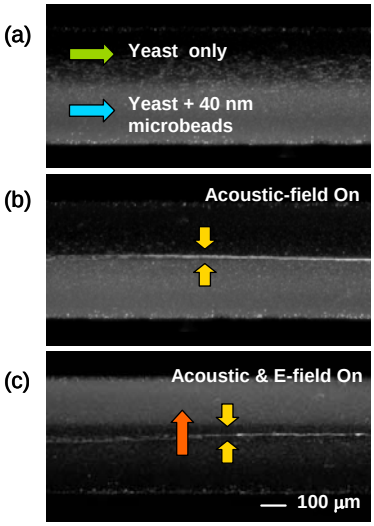

Figure 3. Multi-field separation of yeast cells and 40 nm polystyrene microbeads (virus-like particles). (a) Yeast cells and a mixture of yeast cells and virus-like particles are injected into the top and bottom streams, respectively. (b) Acoustic field is turned on (yellow arrows), and only yeast cells are focused at center of the microchannel. The virus-like particles are 'acoustically invisible' and remain in the bottom-half of the channel. (c) Electric field is also turned on across the microchannel (orange arrow), and the small virus-like particles electromigrate through the acoustic capture node to the top region. All images are top view.

REFERENCES:

1. Johnson, S.R., D.H. Martin, C. Cammarata, and S.A. Morse, *Alterations in Sample Preparation Increase Sensitivity of PCR Assay for Diagnosis of Chancroid.* Journal of Clinical Microbiology. **33**(4): p. 1036-1038.
2. Hunter, R.J. and R.W. O'Brien, *Electroacoustic Characterization of Colloids with Unusual Particle Properties.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 1997. **126**: p. 123-128.
3. Marina, O., M.D. Ward, and G. Kaduchak. *Combination Ultrasonic- Dielectrophoretic Particle Traps for Particle Trapping and Sample Purification in a Microfluidic Channel*. in *Nanotech*. 2006. Boston.
4. Fisher, K. and R. Miles, *Modeling Acoustic Radiation Forces in MicroFluidic Chambers.* Journal of the Acoustical Society of America, 2008. **123**: p. 1100-1104.
5. Petersson, F., A. Nilsson, C. Holm, H. Jönsson, and T. Laurell, *Separation of Lipids from Blood Utilizing Ultrasonic Standing Waves in Microfluidic Channels.* The Analyst, 2004. **129**: p. 938-943.
6. Schimpf, M.E., K. Caldwell, and J.C. Giddings, *Field Flow Fractionation Handbook.* 2000, New York: Wiley-Interscience.
7. Macounova, K., C.R. Cabrera, and P. Yager, *Concentration and Separation of Proteins in Microfluidic Channels on the Basis of Transverse IEF.* Analytical Chemistry, 2001. **73**: p. 1627-1633.

This work were performed under the auspices of the U.S. Department of Energy
by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-CONF-402587