ENHANCED PATHOGEN DETECTION AND CELL CONCENTRATION USING ACOUSTOPHORETIC DEPOSITION ON SURFACES

Martyn Hill^{1,*}, Filip Plazonic¹, Chloe Rose¹, Andrew Walls², Xunli Zhang¹, Dario Carugo¹, and Peter Glynne-Jones¹

¹Engineering Sciences, Engineering and the Environment, University of Southampton, UK ²Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, UK

* Email: m.hill@soton.ac.uk; Tel.: +44-23-8059-3075

There is a pressing need for simple, rapid and effective ways of concentrating and capturing bacteria and bacterial spores for detection and identification [1]. Acoustofluidic approaches can act on a large number of cells simultaneously over a relatively wide area, allowing cells to be imaged, concentrated, or captured in a flow-through system. This paper discusses the use of planar ultrasonic resonator systems for high throughput 2D cytometry, for cell concentration and finally for cell capture on functionalized surfaces. Recent results demonstrate the capture of bacterial spores on antibody functionalized surfaces and it is shown how the same technology can be used to capture cells from more complex fluids such as whole blood.

Acoustophoretic forces tend to move cells to regions of low acoustic pressure and within planar resonator systems [2] it is generally simplest to generate forces towards the centre of a flow-through chamber as shown in Fig. 1 (a) (a half wave resonance). Such a half wave mode can be used for particle concentration in low aspect ratio channels [3] but in the wide channels of these resonators the fluid behaviour makes the extraction of a high concentration challenging [4]. Such a half-wave mode can successfully be used to move particles into the focal plane of an imaging system [5]. This approach not only places particles or cells at the imaging focus, but has the additional significant advantage that within a highly laminar flow, all the particles in this optical focal plane will travel through the imaging field at identical velocities, allowing particle tracking during imaging without motion blur. This has allowed 2D imaging of beads and cells at very high throughputs – up to 200,000 beads per second [5].

A better approach to concentrating bacteria is to force them to a surface rather than to the centre of a channel. Early acoustic designs for moving particles to a surface were very sensitive to precise layer thicknesses [6], but by using just a thin layer to form the reflector of the device so that the reflection is effectively from the low acoustic impedance air boundary, produces a more robust field [7]. Such an approach for concentrating bacteria, as shown in Fig. 1 (b), can be used to increase the concentration of *E. coli* by a factor of 60 and at a flow rate of 20 ml/hr [4].

This flow-through concentration requires a further identification stage but a more efficient approach is to detect bacteria of interest within the flow-through chamber itself. This can be achieved using the approach shown schematically in Fig. 2 in which the machined stainless steel device shown in Fig. 3 incorporates a "thin reflector" that comprises a cover-slip with antibody functionalization for bacterial capture. Fig. 4 shows an image of a slide with spots functionalized to capture *Bacillus globigii* (BG) spores using a spore concentration of 10⁵ per ml and a flow rate of 10 ml/hr. The same approach has also been used successfully to isolate Basophils from diluted whole blood.

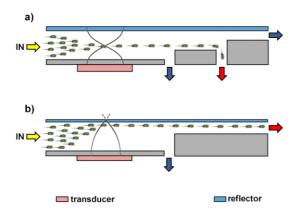


Fig. 1 Representation of (a) half-wave and (b) thin reflector mode of operation of a planar resonator used for bacterial concentration [4].

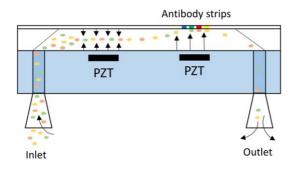


Fig. 2 Schematic of in-device bacterial capture using antibody strips

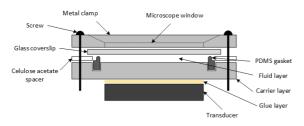


Fig. 3 Further details of construction of bacterial capture device.

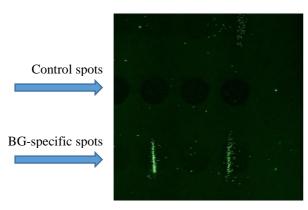


Fig. 4 Image of BG spore capture showing spores captured on functionalized spots in the lower part of the image

REFERENCES:

- [1] C. Paez-Aviles, E. Juanola-Feliu, J. Punter-Villagrasa, B. Del Moral Zamora, A. Homs-Corbera, J. Colomer-Farrarons, *et al.*, "Combined Dielectrophoresis and Impedance Systems for Bacteria Analysis in Microfluidic On-Chip Platforms," *Sensors* (*Basel*), vol. 16, Sep 16 2016.
- [2] P. Glynne-Jones, R. J. Boltryk, and M. Hill, "Acoustofluidics 9: Modelling and applications of planar resonant devices for acoustic particle manipulation," *Lab on a Chip*, vol. 12, pp. 1417-26, 2012.
- [3] M. Evander, A. Lenshof, T. Laurell, and J. Nilsson, "Acoustophoresis in wet-etched glass chips," *Analytical Chemistry*, vol. 80, pp. 5178-5185, Jul 2008.
- [4] D. Carugo, T. Octon, W. Messaoudi, A. Fisher, M. Carboni, N. R. Harris, *et al.*, "A thin-reflector microfluidic resonator for continuous-flow concentration of microorganisms: a new approach to water quality analysis using acoustofluidics," *Lab on a Chip*, vol. 14, pp. 3830-3842, 2014.
- [5] R. Zmijan, U. S. Jonnalagadda, D. Carugo, Y. Kochi, E. Lemm, G. Packham, *et al.*, "High throughput imaging cytometer with acoustic focusing," *RSC Advances*, vol. 5, pp. 83206 83216, 2015.
- [6] S. P. Martin, R. J. Townsend, L. A. Kuznetsova, K. A. J. Borthwick, M. Hill, M. B. McDonnell, *et al.*, "Spore and microparticle capture on an immunosensor surface in an ultrasound standing wave system," *Bio-sensors and Bioelectronics*, vol. 21, pp. 758-767, 2005.
- [7] P. Glynne-Jones, R. J. Boltryk, M. Hill, N. R. Harris, and P. Baclet, "Robust acoustic particle manipulation: A thin-reflector design for moving particles to a surface," *The Journal of the Acoustical Society of America*, vol. 126, pp. EL75-EL79, 2009.