Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information for

Title:

Acoustic radiation forces at liquid interfaces impact the performance of acoustophoresis

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Estimating the acoustic energy density

Samples containing 5-µm polystyrene microbeads were analyzed in the chip to determine the relationship between the ultrasound transducer voltage amplitude (U) and the acoustic energy density inside the microchannel. Microbeads were pre-aligned using the 5 MHz standing wave in the first channel segment (see Figure S1) of the chip and were thereafter focused to the center of the main channel using the 2-MHz standing wave. The location of the beads with respect to the channel side-walls is a function of the acoustic energy density, the acoustic and hydrodynamic properties of the microbead, and the flow velocity profile in the microchannel. For a microbead of known size, density and compressibility the motion path within the separation channel can be calculated numerically. The acoustic energy density in the channel was then estimated by analyzing the position of microbeads when reaching the trifurcation outlet, for a range of ultrasound actuator voltage amplitudes(Augustsson et al., 2012a), taking into account the acoustic radiation force on the particles as well as the ultrasound-induced acoustic streaming (Barnkob et al., 2012). Images of microbeads were acquired for actuator voltages ranging from 0 V_{pp} to 7 V_{pp} and the corresponding acoustic energy was calculated. Figure S shows that the acoustic energy density displays the expected square dependence on actuator voltage (Barnkob, Augustsson, Laurell, & Bruus, 2010).

A 5-µm polystyrene bead has approximately the same acoustophoretic mobility as a white blood cell and it has a lower mobility than most cell-line cells in suspension, so the experiment covers a range of highly relevant acoustic energy densities.

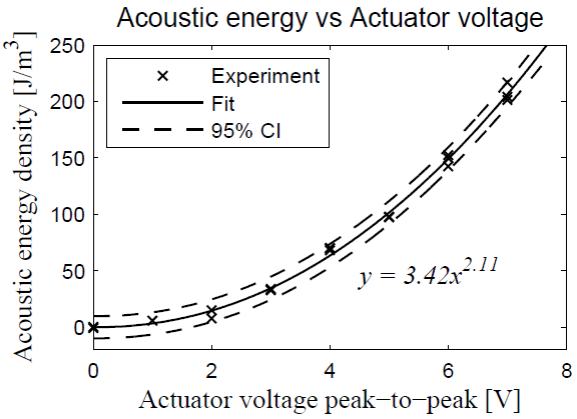

Figure S1: The delivered acoustic energy density in the channel was determined by analyzing the position of particle bands at the trifurcation outlet for increasing transducer voltages and comparing to simulated particle trajectories.

Diffusion vs flow rate

To investigate any molecular diffusion effects at low flow rates that might interfere with the observations of acoustic relocation. Flow rates in the acoustophoresis chip were varied from 12.5 µL/min to 800 µL/min (mean velocities 3.7 mm/s to 237 mm/s) for a solution of NaCl (10 mg/mL, transparent) laminated alongside a solution containing NaCl (9.9 mg/mL) and Evans Blue dye (0.1 mg/mL, blue), **Figure S(a)**. Micrographs of the trifurcation outlet region of the 20 mm long channel were analyzed to find a critical flow rate above which low-flow-rate-effects are negligible. **Figure S(c)** shows that for flow rates below 400 µL/min, which correspond to retention times higher than 0.2 s, the transfer of dye in the central region of the channel increases substantially.

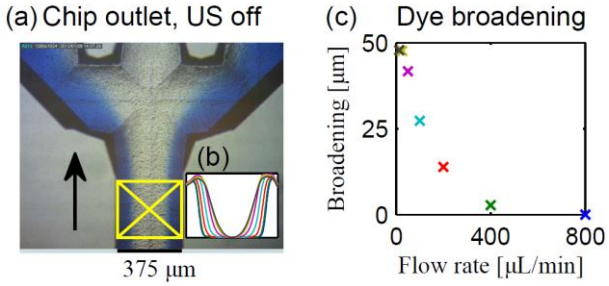

Figure S2: (a) Micrograph of the trifurcation outlet. (b) Evans blue color intensity profile was analyzed within the region of interest (yellow box). (c) The broadening of the dye vs flow rate.

Impact of gravity on liquids of different densities

A COMSOL simulation of the influence of gravity confirms that a 1% density difference between the central and side's liquid is sufficient to perturb the interface between the liquids within the time frame

of one second, Figure S3. This initial perturbation of the interface will impact the rotation pattern of the liquids once the ultrasound is active. If the gravitational perturbation is not present it is likely that the liquids will relocate anyway since configurations of positive mismatch are not stable. Other sources of perturbations that may come into play are interface deformation due to acoustically induced streaming and effects of a non-uniform acoustic field along the vertical axis.

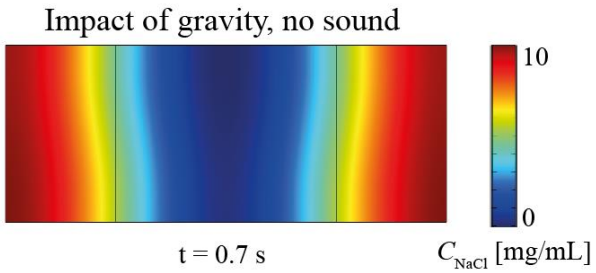

Figure S3: Simulation of channel cross section taking into account diffusion and gravity for NaCl 10 mg/mL laminated on both sides of a stream of water. The time point t = 1 s was chosen to correspond to the flow conditions in the experimental images shown in **Error! Reference source not found.**(a). The thin black vertical lines indicate the positions of the interfaces at t=0.

The origin of the phenomenon

Concentration Central Inlet		**Concentration Side Inlet**		**Relative Mismatch**			**Relocation Energy**
Histopaque (%)	**NaCl (mg/mL)**	**Histopaque (%)**	**NaCl (mg/mL)**	$\tilde{c}$ **(%)**	$\tilde{\rho}$ **(%)**	$\tilde{Z}$ **(%)**	**Eac (J/m3)**
---	2.10	15.0	---	0 ± 0.4	1.0 ± 0.4	1.0 ± 0.8	34.7
15.0	---	---	2.10	0 ± 0.4	-1.0 ± 0.4	-1.0 ± 0.8	ND
---	6.20	43.5	---	0 ± 0.5	3.0 ± 0.5	-3.0 ± 1.0	14.8
43.5	---	---	6.20	0 ± 0.5	-3.0 ± 0.5	3.0 ± 1.0	ND
15.0	---	---	18.0	1.2 ± 0.4	0 ± 0.4	1.2 ± 0.8	34.7
---	18.0	15.0	---	-1.2 ± 0.4	0 ± 0.4	-1.2 ± 0.8	ND
39.3	---	---	47.2	3.0 ± 0.4	0 ± 0.4	3.0 ± 0.8	23.6
---	47.2	39.3	---	-3.0 ± 0.4	0 ± 0.4	-3.0 ± 0.8	ND
---	9.10	15.0	---	-0.5 ± 0.4	0.5 ± 0.4	0 ± 0.8	208
15.0	---	---	9.10	0.5 ± 0.4	-0.5 ± 0.4	0 ± 0.8	ND
---	29.8	50.0	---	-1.7 ± 0.5	1.7 ± 0.5	0 ± 1.0	275
50.0	---	---	29.8	1.7 ± 0.5	-1.7 ± 0.5	0 ± 1.0	ND

Table S1: Observed acoustic relocation energy density for different constellations of Histopaque and NaCl solutions. Error estimates are derived from a 1 % uncertainty in the volumetric measurement when preparing the solutions.