Supporting Information for

Isolation of a Low Number of Sperm Cells from Female DNA in a Glass-PDMS-Glass Microchip Via Bead-Assisted Acoustic Differential Extraction

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Abstract: Here we describe our determination of trapping efficiency over a range of cell and particle concentrations. Experiments are described that show the effect of the secondary radiation force, and what happens when a minimum number of trapped particles is not achieved.

Cell concentration-dependent trapping efficiency

The Laurell group established the limitations associated with attempting to trap a small number of particles in a diluted sample, showing that single-particle capture is possible under certain conditions[37]. When considering that sperm cells may be outnumbered several hundred-to-one in a typical sexual assault sample, a high capture efficiency is paramount for our acoustic device. Trapping efficiency with this described GPG system was explored with both fluorescently-tagged polystyrene 6 μm beads, as well as the analyte of focus here, sperm cells. As shown in **Figure S1**, the trapping efficiency, defined as the number of retained particles / number of total infused particles × 100%, increased as the particle concentration was increased. Both fluorescent beads and sperm cells could be trapped with an efficiency exceeding 80% at concentrations greater than ~100 cells/particles per microliter. However, below this concentration, the trapping efficiency dropped precipitously, reaching ~20% at the lowest tested concentration of ~10 cells/particles per microliter. It is interesting that trapping of sperm cells at this concentration appears to be more effective than with 6 μm polystyrene beads, and may suggest that either the surface properties of sperm cells or their density/compressibility somehow allow for more effective aggregation in the trap zone.

The sharp decrease of trapping efficiency in dilute samples (below 100 cells/particles per μL) can be attributed to the difficulties in aggregate formation. Hammarström et al. showed that shifting from the acoustic streaming to a trapping regime was dependent on both the bead size and the number of particles and, interestingly, this could be honed for ~500 nm sized beads.[27]. The formation of a particle aggregate mainly requires two sequential stages: (1) upon entering the acoustic field particles are slowed down by the primary radiation force (PRF) and recruited to the nodal plane of the USW; (2) when particles move together and the inter-particle distance small enough, the secondary radiation force (SRF) begins to dominate, pulling particles into a compact aggregate that resists hydrodynamic drag[29]. Since the SRF is inversely proportional to d to the 4^{th} power, (d is the inter-particle distance)[29], the SRF diminishes dramatically as the inter-particle distance increases; hence, particles separated by even a miniscule distance are not subject to adequate SRF to maintain aggregation.

To better demonstrate this, we tracked the movement of single cells at very dilute cell concentrations (~1 particle/μL) during acoustic trapping. The result of tracking the movement of two particles during a failed trapping process is given in **Figure S2A**, where it is clear that, when two particles enter the acoustic field (x-position = 600) there is a rapid drop-off is y-position within the trapping site, leading to a decrease in the inter-particle distance (**Figure S2B**). However, if the inter-particle distance isn't reduced to the point where the SRF can act effectively, the drag force dominates and particles begin to separate again. It is for this reason that the sperm cell trapping efficiency of dilute semen samples with ~1cell/μL is only 18±3 % (n=3). The generation of an effective SRF that induces aggregate formation is highly dependent on the inter-particle distance decreasing rapidly as the particles transit the acoustic field. One approach for facilitating this is to increase the acoustic power to generate a stronger PRF to induce trapping, however, one must be cognizant of issues resulting from heat generated by the PZT, which may adversely affect trapping and/or cell integrity.

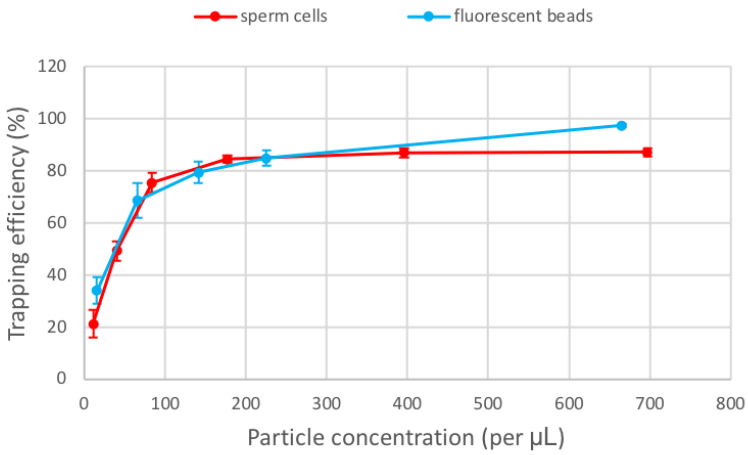

Figure S1. Dependence of trapping efficiency on particle concentration (n=3, error bars represent standard deviation). Samples were infused at a flow rate of 30 µL/min and Vpp=12. Below 100 particles per microliter, significant drop-off in efficiency is observed.

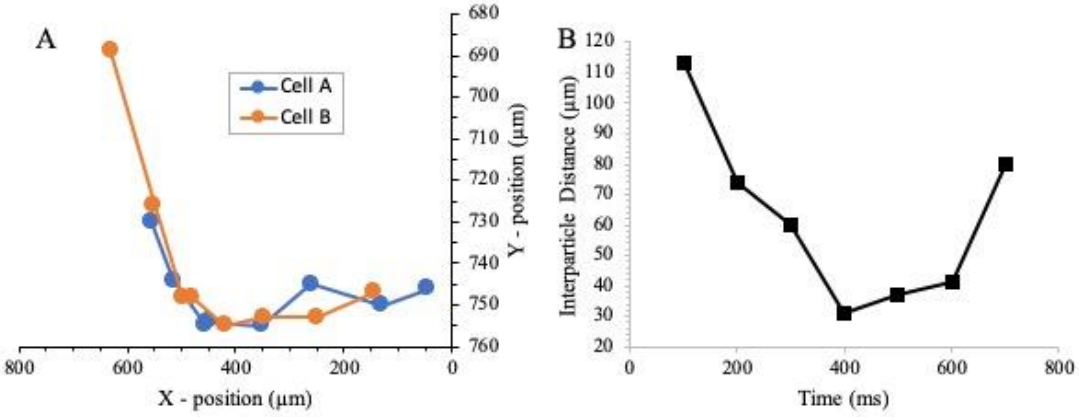

Figure S2. Trajectories and inter-particle distance of two cells during a failed acoustic trapping process. (A) 2-dimensional positional data for two cells, reported as distance from the bottom of the channel (y-position) and distance from the edge of the channel (x-position). Data points were collected at 0.1s intervals, so moving left to right in the figure goes from t = 0 to t = 800 ms. (B) Change of inter-particle distance between cells during the 800 ms trapping event.