Supporting Information

A single inlet two-stage acoustophoresis chip enabling tumor cell enrichment from white blood cells

Maria Antfolk, Christian Antfolk, Hans Lilja, Thomas Laurell, and Per Augustsson**

S1. Chip photograph

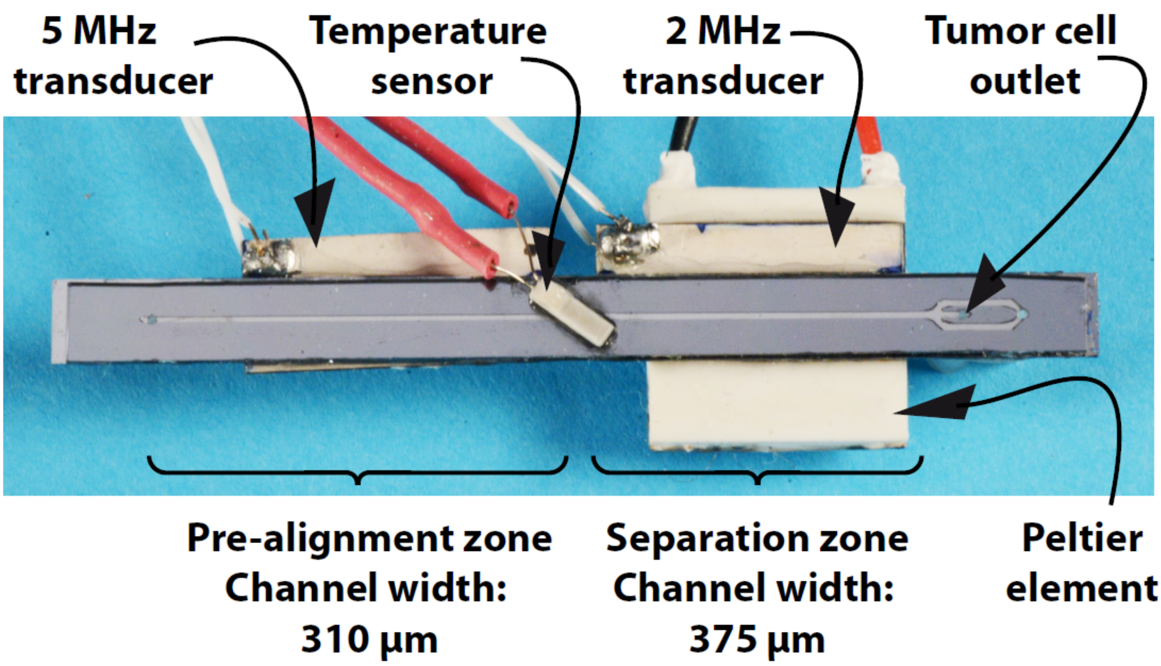

Figure S1. Photograph of the microfluidic chip. A temperature sensor (Pt-1000) is glued on top of the chip. Two piezoelectric transducers for pre-alignment (left) and separation (right) are glued to the backside of the chip. The chip and transducers are glued to a Peltier element positioned underneath the separation transducer for temperature control. Liquid in- and outlets are connected from the backside of the chip through holes in an aluminum base plate.

S2. Comparison between random distribution, hydrodynamic pre-positioning, and single inlet acoustic pre-alignment.

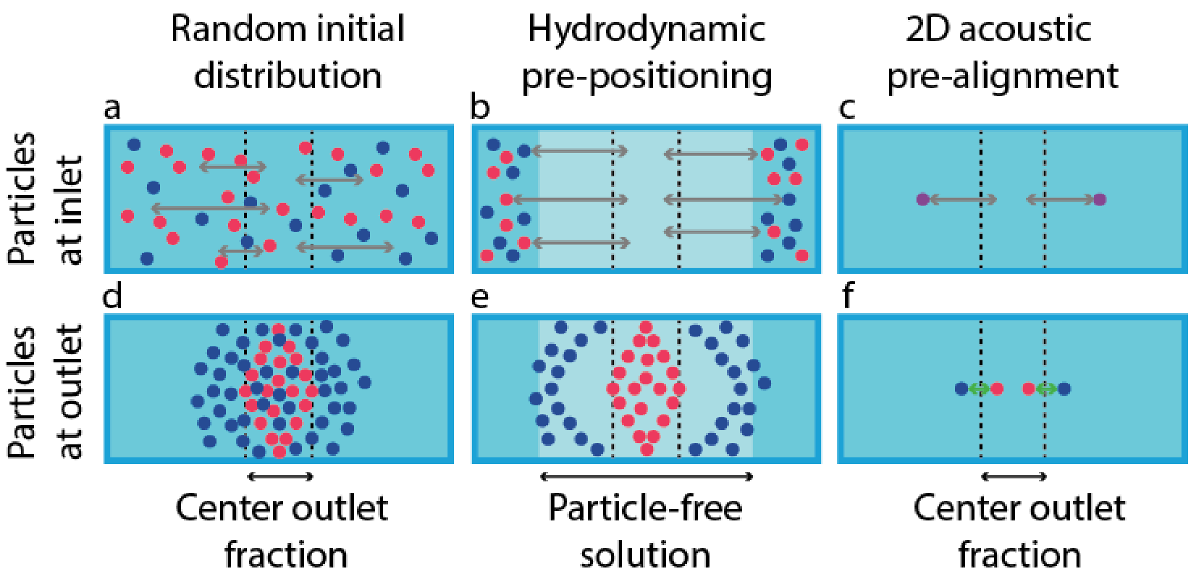

Figure S2. Cross-sections of the microchannel comparing three modalities of pre-alignment prior to acoustic sorting. Grey arrows indicate the sideways displacement and green arrows the particle-particle distance between the 5-µm (blue) and 7-µm (red) particles after separation. Particle-free solution is shown in lighter blue and overlapping particle sizes are shown in purple. (a-c) Particle distributions at the inlet of the separation channel after pre-alignment and (d-f) particle distributions at the end of the separation channel. Dashed lines indicate the center fraction of the total flow that will exit through the center outlet. The particles will be separated when one of the populations crosses these lines while the other one does not. The focusing patterns are highly influenced by the microchannel flow velocity distribution which causes particles flowing near the top and bottom of the channel to have longer retention times in the acoustic field than particles flowing at mid-height. It is evident that methods (a) and (b) cannot achieve deterministic separation of particles.

S3. The impact of pre-alignment in height

The separation efficiency is dependent on the degree of particle pre-alignment. The degree of alignment along the width of the channel can be easily determined visually, while the alignment in height is harder to determine unless more advanced microscopy is employed . Assuming perfect height and sideways pre-alignment for a single particle passing through the channel, the particle would be situated at $z = ½\ h = 75$ µm, where h is the channel height, and along $y = ¼\ w$ or $¾\ w$ where w is the channel width. This position corresponds to the fastest moving fluid regime as dependent on the Hagen-Poiseuille flow profile. Any particle flowing above or below this point will travel in a slower flow regime and will therefore have a longer retention time in the channel, allowing a longer time for focusing and thus the opportunity to travel further towards the channel center. A simulation was carried out to determine the required degree of height focus of the 5- and 7-µm particles to be correctly sorted (Figure S3). In the simulations the 7-µm particle has been kept at a channel height of 75 µm as this is the position where the particle has the shortest amount of retention time in the channel to move towards the center. Any 7-µm particles deviating from this position in height would have the possibility to move further towards the channel center and would thus overlap less with the 5-µm particles. Instead, the 5-µm particle height position (focusing height) has been varied to determine the relative position of this particle compared to the 7-µm particle (particle distance) when it arrives at the interface to the center outlet flow stream. A negative particle distance means that the 5-µm particle has already passed the 7-µm particle as it reaches the center fraction. From Figure S2 it can be seen that the 5-µm particle will cross the path of the 7-µm particle when it is focused to a height of about 22 µm, meaning that the sideways displacement will be identical. The height of 22 µm might indicate that the particles can be allowed to deviate from a perfect height focus to some extent. This would be true in a perfect fluidic system where no fluctuations in the flow existed and the particles thus only had to be separated by an infinitesimal amount to be separable. It would also assume particle populations without any size deviation. In reality, however, this is not attainable and thus the particle-particle distance between the two particle populations after separation needs to be approximately 10-15 µm (data not shown) to be separable into different outlets, depending on the stability of the fluidic system and the size deviation of the two populations. Since the experiments described in this paper clearly showed that 5-µm and 7-µm particles could be separated, that indicates that the pre-alignment in both height and width mode was sufficient.

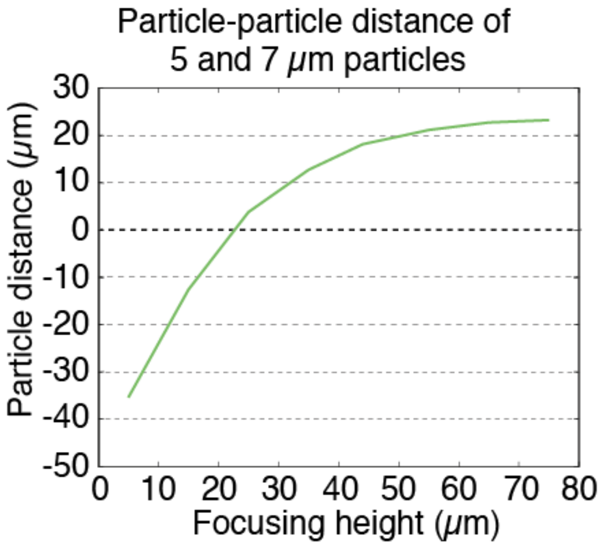

Figure S3. Distance between particles after separation for 5- and 7-μm particles at the point where the 7-μm particle reaches the interface of the center outlet flow stream, as a function of the position in height of the 5-μm particle. A negative number indicates that the 5-μm particle has outrun the 7-μm particle before it reaches the central stream. The flow rate of the central outlet is here 25% of the total flow rate.

S4. Sorting optimization for increased width of sorting channel

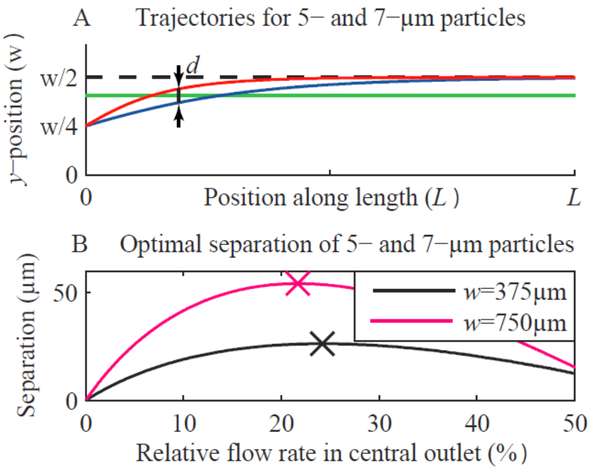

Figure S4. (A) Simulated trajectories of 5-μm (blue) and 7-μm (red) polystyrene microparticles starting from an initial position of ideal pre-alignment in width and height. The black vertical line indicates the point of maximal separation, the green line indicates the interface between the side and central outlet flow streams and the dashed black line indicates the channel center. (B) Plot showing the maximum achievable particle-particle distance versus the relative center outlet flow rate when sorting pre-aligned 5- and 7-μm particles in chips with widths of 375 μm and 750 μm.