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Sorting drops and cells with acoustics: acoustic microfluidic fluorescence-activated cell sorter
Sorting drops and cells with acoustics: acoustic microfluidic fluorescence-activated cell sorter†

Lothar Schmid,a David A. Weitzb and Thomas Franke*abc

We describe a versatile microfluidic fluorescence-activated cell sorter that uses acoustic actuation to sort cells or drops at ultra-high rates. Our acoustic sorter combines the advantages of traditional fluorescence-activated cell (FACS) and droplet sorting (FADS) and is applicable for a multitude of objects. We sort aqueous droplets, at rates as high as several kHz, into two or even more outlet channels. We can also sort cells directly from the medium without prior encapsulation into drops; we demonstrate this by sorting fluorescently labeled mouse melanoma cells in a single phase fluid. Our acoustic microfluidic FACS is compatible with standard cell sorting cytometers, yet, at the same time, enables a rich variety of more sophisticated applications.

Introduction

Fluorescence-activated cell sorting (FACS) is a widely used technology that is a critical tool in molecular biology, genetics and immunology.1,2 It is often used to select subpopulations of cells from a large number of cells in drug screening and diagnostics and has developed into a commercially available standard technique. It also has great potential for emerging biotechnologies such as systems biology, rare cell detection or stem cell isolation.

FACS uses laser interrogation based on fluorescence or scattering to identify single cells followed by the encapsulation of cells in drops that form an aerosol and that are sorted at very high rates through application of an electric field.

Many operational and technical difficulties associated with cell sorting arise in auxiliary operations. Cells growing in culture must be sterile to maintain their viability. Practical procedures to ensure the quality of the sorted sample are both time-consuming and costly; for example, RNase-free processing entails sterilization and cleaning of the complete tubing and filter system of the FACS machine, requiring considerable time. Moreover, because of the high acquisition cost of commercial FACS machines, cell sorting service is generally offered by central facilities.

Microfluidics technologies enable affordable, desk-top-scale platforms suitable for individual lab use. The sample chambers are disposable, eliminating the need for complex cleaning and sterilization, and consume very low volumes of reactants. In addition, efforts to provide a biosafety environment to handle potentially biohazardous materials are greatly reduced because the sample is always self-contained and aerosol production is eliminated; thus, operation in a hood or biosafety lab is typically not required.

However, realizing a microfluidic fluorescence actuated cell sorter still remains challenging because microfluidic actuation and deflection mechanisms of cells are slow and inflexible. Many devices exploit dielectrophoretic forces that depend on the volume and dielectric contrast of the sorted objects and the continuous medium.3 Yet, to osmotically balance the cytosol, cells are suspended in cell buffer media that possess a low dielectric contrast as the cells and which cannot be replaced with a high-contrast agent. Similarly, magnetic actuated sorting suffers from dependence on magnetic susceptibility contrast and is also volume dependent. Recently, acoustic radiation forces in a gradient of a standing acoustic wave field have been used to deflect cells,4–10 but in this approach the forces depend on both compressibility contrast and cell size. All of these actuation mechanisms critically depend on the volume of the objects and small objects are inherently more difficult to sort. This also makes it difficult to sort cell populations that have a wide distribution of sizes.

In this way a low and size-dependent deflection force also results in slow sorting rates. Methods for enhancing sorting forces are limited. To overcome this drawback, other techniques have been employed: immunopolystyrene beads are used to selectively bind to target cells, both to label cells and to enhance sorting by increasing their effective dielectric or paramagnetic contrast.11,12 Alternatively, cells can be encapsulated into fluid containers or drops that provide sufficient contrast with the continuous fluid.13–15 Yet, both methods require the

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*a Lehrstuhl für Experimentalphysik I, Soft Matter Group, Universität Augsburg, Universitätsstr. 1, 86159 Augsburg, Germany. E-mail: Thomas.Franke@glasgow.ac.uk

b Department of Physics and School of Engineering and Applied Science, Harvard University, 29 Oxford Street, Cambridge, Massachusetts 02139, USA

c Chair of Biomedical Engineering, School of Engineering, College of Science and Engineering, University of Glasgow, Rankine Building, Oakfield Avenue, G12 8LT, UK

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addition of supplemental additives, either beads or immiscible fluid, to achieve sorting. Another alternative is the use of fluid-switching techniques which do not depend on volume or contrast.\textsuperscript{16–19} Instead, they employ fluid valves that direct the fluid flow into a collection channel. However, these techniques suffer from a low response time, limiting switching rates to a few hundreds per second.

In this paper, we overcome the limitations of the commonly used microfluidic sorting techniques using actuation by using travelling surface acoustic waves. These have been used previously for mixing and moving droplets on a substrate,\textsuperscript{20} lysing cells,\textsuperscript{21} and mixing in microfluidic channels\textsuperscript{22} as well as particle concentration\textsuperscript{23} and fluid nebulization,\textsuperscript{24} employing the acoustic streaming effect. Travelling waves have also been used to generate an acoustic radiation force on particles, which is size dependent and allows for size separation and filtering. While the acoustic radiation force depends on particle volume and acoustic impedance contrast,\textsuperscript{7} acoustic streaming actuates particles independent of their properties\textsuperscript{25–27} and is therefore particularly useful for small particles.

We introduce an acoustic sorting technique that is able to sort cells and other objects at high rates and independent of their size or contrast, which is important for bacteria and virus sorting. In our microfluidic device we select and deflect cells and objects directly from the bulk solution without enhancement with responsive labels or other additives which introduce sample contamination. Cells that are interrogated using laser-based detection are sorted by acoustic actuation using a surface-acoustic wave device into two, or even more, employing the acoustic streaming effect. Travelling waves have also been used to generate an acoustic radiation force on particles, which is size dependent and allows for size separation and filtering. While the acoustic radiation force depends on particle volume and acoustic impedance contrast,\textsuperscript{7} acoustic streaming actuates particles independent of their properties\textsuperscript{25–27} and is therefore particularly useful for small particles.

Experimental results and discussion

We sort using acoustic actuation and deflection based on the level of an interrogation signal. Our acoustofluidic hybrid device for sorting combines conventional soft lithography with surface acoustic wave technology (SAW) to drive microflows. To generate a SAW on the chip we use interdigitated transducers (IDT). We vapor deposit those comb-like electrodes on a transparent piezoelectric substrate made of lithium niobate and generate a mechanical acoustic wave by applying a high-frequency electric signal to the IDT. SAWs excited in this way typically propagate at very low attenuation along the substrate surface. However, if we deposit a fluid such as water on top of the substrate the surface wave gets strongly attenuated and the acoustic energy couples into the fluid.\textsuperscript{28} We exploit this effect to deflect and sort the sample in microfluidic channels as shown in Fig. 1 (see also the ESI† Movie 1).

We fabricate the microfluidic device using a polydimethylsiloxane (PDMS) molding process and attach it to a thin PDMS layer of a few micrometers thick to form a sealed and closed microchannel device. The PDMS film features a small post that is carefully aligned below the sorting region of the microchannel. To accomplish the hybrid device the sealed PDMS mold is then adjusted on top of the piezo-substrate with the sorting region close to the IDT to enhance optimal coupling of the acoustic energy into the fluid as shown in Fig. 2. This enables us to couple the acoustic wave into the microfluidic channel at a precise location.\textsuperscript{25} Alternative methods have employed a single-layer device attached directly to the substrate; however, these methods lead to a more complex flow profile with several counter-rotating vortices\textsuperscript{22,26} and generally provide less control of the acoustic path. The design of the microfluidic channels contains a sample inlet and a sheath flow for hydrodynamic focusing of the sample before sorting into two or more outlets. By controlling the sheath flow rate, sorting errors are prevented because drop separation can be optimized to ensure that only one drop appears at once in the sorting region. Furthermore, using slightly asymmetric flows, we calibrate the flow
conditions to align the sample trajectory and guide it into the waste channel in the absence of acoustic actuation. Careful alignment of the trajectory is also important to make sure that the sample flow passes through the center of the optical interrogation spot that detects the trigger signal for sorting. To optionally control the spacing between objects and to meet the specific requirements of the sample, we added another side channel further upstream to dilute the concentration of the sample. This is particularly useful to prevent the fluid shear-stress-induced coalescence of densely packed drops when sorting at extreme speeds. To enhance the robustness of the sorter and maintain the balanced flow conditions, we introduce shunts between the two outlet channels to decouple the acoustic sorting region from any pressure disturbances further downstream in the microfluidic device that affect the hydrodynamic outlet resistance. Such pressure fluctuations do arise when there are variable numbers of drops in the collect and waste channels or simply when connecting tubing for sample collection.

We trigger the sorting on the level of a fluorescence signal of the sample in the interrogation area of a focused laser spot. The fluorescence signal is detected with a photomultiplier tube and analyzed in real time. If the intensity of the fluorescence signal exceeds a preset threshold we trigger a high-frequency generator to send a pulse to the IDT to excite a SAW on the hybrid chip to diverge the sample.

We demonstrate fluorescence-activated droplet sorting using picoliter-volume aqueous drops dispersed in a continuous fluorocarbon oil phase that is stabilized with a biocompatible surfactant. We produce fluorescent and non-fluorescent drops in a separate PDMS drop-maker device and mix both populations. To enable optical discrimination and control of sorting in a transmitted light microscope we added the stain bromophenol blue to the non-fluorescent drops to blacken them as shown in Fig. 1. The monodisperse mixture of drops is reinjected into the high-speed acoustic sorter at a high volume ratio. The drops then flow into a constriction to force them into a single file line. This facilitates timing and prevents coincidence of drop occurrence in the interrogation or sorting region that potentially causes false sorts. Dense packing of drops allows for the precise control of their flow rate by carefully tuning the sheath flow via the side channels. We select drops based on a preset fluorescence intensity threshold and acoustically direct them into the select channel.

Using this acoustofluidic hybrid device, we are able to sort drops at rates of several kHz.

The sorting speed depends on the duration required for the interaction between the drop and the acoustic field and the intensity of the acoustic wave power. Therefore, the maximum sorting rate of the sorter is limited by the minimum duration of the sorting pulse $t_{\text{pulse}}$. If the pulse is shorter, the deflection will not be sufficient to move the droplet to the collect channel. Furthermore, the droplet is only deflected during the time it is traveling through the actuation region above the post $t_{\text{act}}$. For optimal performance $t_{\text{act}}$ is adjusted to $t_{\text{pulse}}$ by carefully tuning the sheath flow rate. To sort consecutive droplets independently, only one droplet can be in the actuation region at once. Therefore the droplet separation time must be adjusted to $t_{\text{sep}} = t_{\text{pulse}}$. For non-aligned particles like cells in bulk fluid, the number of particles in the actuation region is Poisson distributed, which necessitates a trade-off between sorting rate and sorting yield and purity. However, when sorting droplets in a single file the edge-to-edge alignment in our droplet sorter eliminates this problem, allowing for adjustment of $t_{\text{sep}}$ with a very low standard deviation. In our experiments, a minimum of $t_{\text{sep}} = 330 \mu s$ with perfect sorting can be achieved, corresponding to a sorting rate of 3000 cells per second at a moderate SAW power of 15 dBm (see also section sorter performance).

This acoustic sorting principle in PDMS channels is not restricted to sorting into only one collection channel but is easily extended to sort drops into multiple outlet channels to separate a more complex population of drops. To demonstrate this versatility and flexibility of the device we sort drops into three categories. Here, we use two populations of fluorescence-labeled droplets with different fluorescence intensities in addition to a third population of non-fluorescent drops. The design of the channel was customized by adding a third outlet as well as a second PDMS post as shown in Fig. 1 (a-c). Non-fluorescent droplets are not actuated and drift into the central waste outlet. Fluorescent droplets are sorted either into the upper or into the lower outlet channel depending on their fluorescence intensity. We use tapered IDTs that allow us to selectively couple the acoustic wave either through the right (upper) or through the left (lower) post, thereby driving the droplets either up or down, respectively.

The applicability of our acoustic device is not only limited to guiding and sorting droplets but also enables direct manipulation of cells suspended in cell buffer media without...
prior encapsulation and formation of drops. This procedure is similar to the fluid handling of commercial FACS machines that use only a single fluid.

We demonstrate acoustic cell sorting for B16F10 mouse melanoma cells. These cells are highly invasive and metastatic and are used in cancer research to screen drugs that reduce tumor growth\textsuperscript{31} and inhibit metastasis.\textsuperscript{32,33} To enable identification with our detector before sorting, the cells were treated with Calcein-AM, a fluorescence dye that is sensitive to cell metabolic activity and plasma membrane integrity.\textsuperscript{34,35} This preparation yields a cell population with varying fluorescence intensity. To regulate the sorter we set a fluorescence intensity threshold. For sorting, cells suspended in phosphate-buffered saline (PBS) were injected into the sample inlet and aligned by a co-flowing sheath flow containing the same PBS solution. The sample flow is forced to form an extended fluid jet that aligns the cells as shown in Fig. 3.

In contrast to droplet sorting, a constant cell rate cannot be achieved since cells cannot be densely packed. They tend to form clusters caused by mutual adhesion and conglutination. In exactly the same way as in sorting with commercial FACS machines, the number of cells in the sorting region is therefore Poisson distributed. However, the mean rate can be regulated by adjusting cell concentration, sample flow rate and sheath flow rate.

**Theory of acoustic streaming**

The dissipation of a sound wave in a medium leads to an acoustic force that generates acoustic streaming of fluids in the same direction as the propagation. An acoustic wave with intensity $I_\text{ac}$\textsuperscript{1−βx} (with initial intensity $I_\text{ac}$ and attenuation coefficient $\beta$) that propagates in the \textit{x}-direction at velocity $c$ carries a momentum flux $I_\text{ac}c^{-1}e^{-\beta x}$ and permanently loses momentum over the attenuation length $\beta^{-1}$. Therefore, momentum conservation implies an exchange of momentum from the attenuated wave to the fluid. This gives rise to a volume force acting on the fluid\textsuperscript{36}

$$F = \beta c^{-1}I_\text{ac}e^{-\beta x}$$

that can be considered as an external bulk force in the equation of motion for the fluid, with $\beta^{-1} = 975 \ \mu m$ for water.\textsuperscript{37}

Commonly, in microfluidics the equation of motion reduces to the non-inertial Stokes equation. However, acoustics is one of the few effects in microfluidics where inertial terms have to be explicitly taken into account. The actuation mechanism of acoustic streaming is evoked by an exchange of momentum that is inherently an inertial effect. Moreover, in our sorting device acoustic streaming induces a flow velocity component perpendicular to the syringe-driven flow direction in the microchannel. For discontinuous changes of flow direction, inertia effects become increasingly important, as can be observed at sharp corners in a channel. Therefore, to describe the fluid flow we have to consider the full Navier–Stokes equation in our simulations. (For a detailed discussion and comparison of inertia and non-inertia flows see ESI† discussion 1: inertial effects and Fig. S1.)

**Simulations and experiments**

To quantitatively model the acoustic flow field and acoustic streaming we simulate the effect of the surface acoustic wave (SAW) on the flow field in the microchannel using COMSOL Multiphysics (version 4.2a) and consider the full Navier–Stokes equation. We model a stationary laminar flow of an incompressible fluid and account for the acoustic streaming using a volume force calculated from the intensity profile of the SAW on the chip surface. The propagation direction of the wave (and therefore the direction of the force) is along the \textit{yz}-plane tilted by a Rayleigh angle of 21.8° with respect to the \textit{z} axis.

For comparison, the flow field has also been measured experimentally using particle image velocimetry (PIV). As shown in Fig. 4d, e, our simulations reproduce the key features of the experimental velocity field quite well. Both experimentally observed vortices in Fig. 4e are obtained from the simulations and agree quantitatively with the measured size and position. The distances between the vortex centers compare well, and the value and direction of velocity are also
reproduced: the highest velocities are close to the central flow, while the lower velocities appear in the backflow regions, as expected. We qualitatively understand the resultant flow field by considering that the volume force acts only on the region of the post in the middle of the sorting region, creating a pair of vortices, with a flow mainly in the $y$-direction above the post and a backflow in the $-y$-direction to the left and right of the post to comply with the equation of continuity for the incompressible fluid volume.

**Sorting of cells and jet deflection**

To directly apply the results of our acoustofluidic simulations to a realistic sorting scenario we study the acoustic streaming effect on the fluid jet of our cell sorting experiment that is used to align cells in the sorting region as shown in Fig. 4f, g. When the SAW is switched off, the fluid from the sample inlet and the embedded cells travel along a straight trajectory towards the outlet along a jet. This jet is visualized in the microscope by using slightly different solvents for sample and sheath fluid that causes small differences in optical densities. When the SAW is activated, the initial Poiseuille flow is modified by an additional acoustic streaming force. As a result the jet and the embedded cells are deflected. This experimental setup has been simulated by adding pressurized inlets and outlets to the SAW simulations. In the simulations we follow the streamlines of tracer particles which are initially positioned in the center of the channel (parallel to the $x$-axis) at different $z$-heights. In Fig. 4 we show the initial position (f) and the position directly after a pulse of 1 ms duration (g) superimposed on the experimental data. There is a good match of both, with the general shape of the jet as

Fig. 4 (a) Top view of the simulation. The color plot shows the actuating volume force acting above the post, which is dampened in the $y$-direction. The colored line shows the vertical section along the $yz$-plane that is plotted below. The rectangle shows the region of the comparison plot d and e. (b) Top view of the simulation showing the $xy$-velocity along a mid-plane section of the channel. A strong central stream bifurcates into two backflow arms, generating a double vortex. (c) Side view of the simulation (section along the $yz$-plane) showing in-plane velocity. Due to geometric constraints, the flow is mainly in the $y$-direction even though the force is predominantly in the $z$-direction (at a Rayleigh angle of 21.8°). (d, e) A comparison of simulations (d) and PIV measurements (e) of the flow field in the sorting region is in good agreement with the distance of the two vortices as well as a high velocity in the middle (above the post) and a lower velocity in the backflow region. Velocities are normalized. (f, g) Overlay of the experimental image of acoustic deflection of the sample jet with simulation results. (f) Initial position of the tracer particles before SAW actuation. The particles are aligned along the sample liquid, which, in the absence of acoustic actuation, is confined by the sheath flow to a thin jet along the center of the channel, finally flowing into the waste (bottom) outlet. (g) A 1 ms SAW pulse deflects the jet, driving it upwards, so that it will later flow into the collect (upper) channel. The points denote the simulated deflection of fluid particles which are initially positioned along the undeflected jet at 3 different $z$-heights: (red) $10 \mu m$ above the channel bottom; (green) center; and (blue) $10 \mu m$ below channel top. The simulations reproduce the deflection as well as the broadening of the jet due to different deflection velocities along the $z$-axis. The scalebar denotes $50 \mu m$. 

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well as the small broadening of the jet width due to different vortex velocities along the z-axis.

Materials and methods

Preparation of cells and droplets. For droplet sorting, monodisperse droplets of aqueous solution suspended in an apolar bulk phase were generated using drop makers as described elsewhere. As aqueous solutions we used 20 mM bromophenol blue for the non-fluorescent black droplets and 1 mM and 0.3 mM fluorescein solution for the fluorescent droplets. The osmolarity of the solutions was matched by adding sucrose to avert osmotic volume exchange between closely packed droplets. For the apolar bulk phase we used fluorocarbonated oil (3M™ Novec™ Engineered Fluid HFE-7500). To stabilize the surface of the droplets, we added 1% w/v of a PFPE-PEG block copolymer, which is a biocompatible surfactant. The different droplet populations were produced separately and mixed prior to sorting.

For cell sorting we used B16F10 mouse melanoma cells. To enable fluorescence detection, the cells were treated with a fluorescent dye that is sensitive to cell metabolic activity (Calcein-AM, Sigma-Aldrich). This preparation yields a cell population with varying fluorescence intensity. The cells were suspended in isosmotic phosphate-buffered saline (PBS) solution. The density of the liquid was matched to the cells bound and the device can be pulled off the silicon mold.

The PDMS device is then placed on top of the SAW chip, with the PDMS post positioned in front of the IDT along the path of the SAW.

Sorting device and operation. Optical detection is performed with a 473 nm 150 mW DPSS laser focused on the channel. The fluorescence signal of the samples was detected with a photomultiplier tube (Photosensor Module H9858-20, Hamamatsu Photonics, Germany). The signal is analyzed in real time with a custom LabView routine (LabView 2009, National Instruments, Germany) embedded in a field programmable gate array (NI PCI-7833R Virtex-II 3M Gate R, National Instruments, Germany). If the intensity of the fluorescence signal exceeds a preset threshold, a delayed TTL-level pulse is generated and fed into a high-frequency generator (Rohde & Schwarz SMP02) to pulse-modulate its output. The resulting high-frequency pulse is amplified (ZHL-2, Mini-Circuits) and fed to the interdigitated transducer, where it excites a surface acoustic wave (SAW) on the piezo-chip to diverge the sample.

For the triple sorter, additional frequency modulation and two different thresholds are used. If the signal exceeds the lower threshold, a pulse is generated as above. If the signal exceeds the upper threshold, an additional frequency modulation signal is generated and fed to the HF generator. The two frequencies are adjusted to ensure that the SAW generated by the unmodulated signal couples to the left post, and the SAW generated by the modulated signal couples to the right post, resulting in deflection to the top or the bottom outlet, respectively.

Sorter operation was recorded with a microscope (Olympus CKX41) and a high-speed camera (Photron Fastcam 1024 PCI).

Fluidic device setup. The fluidics is driven by syringe pumps (PHD 2000 Infusion, Harvard Apparatus). Typical sample flows are 25 μl h⁻¹. The sheath flow rates are adjusted to ensure that, in the absence of actuation, the particles to be sorted flow into the default channel, but near the edge of the junction, so that they can be easily deflected into the collect channel. Sheath flows are typically in the range of 1000–2000 μl h⁻¹.

Flow field measurements and simulations. For measuring the flow field, a suspension of polystyrene microbeads with a diameter of 2 μm was used (Polybead® Microspheres 2.00 μm, Polysciences Europe). The beads were diluted 1:100 v/v with a mixture of 15% v/v OptiPrep and 85% v/v PBS, with
the density of the liquid matched to the bead density to eliminate buoyancy.

The movement of the beads was recorded with a high-speed camera (see above) and analysed with particle image velocimetry.‡

The simulations were done with COMSOL Multiphysics (version 4.2a). A stationary laminar flow of an incompressible fluid was modeled using the full Navier-Stokes equation.

The acoustically generated volume force is calculated from the intensity profile of the SAW on the chip surface. We approximate the profile by assuming unidirectional SAW propagation along the y-direction and interference of the waves excited by single IDT electrode fingers. This results in a typical diffraction profile \( I_{\text{SAW}}(x) \propto \sin^2 x/b \) and a half-width of the central maximum \( b = 125 \mu m \). The absolute intensity and the ratio of the SAW diffracted into the PDMS post can be estimated from delay line measurements. Since impedance matching between PDMS and water is very good, practically all of the wave’s energy is transmitted into the liquid. Thus, the intensity of the acoustic wave on top of the post is:

\[
I_w(x,y) = I_0 \exp\left(-\frac{(y-y_0)}{a_{\text{SAW}}}\right) \sin^2\left(x/b\right)
\]

with the SAW attenuation length \( a_{\text{SAW}} = 213 \mu m \) and \( I_0 = 0.15 \text{ W m}^{-2} \) for an electric signal of 15 dBm and the position of the post edge \( y_0 \). Attenuation in water can be neglected because the attenuation length is much higher than the channel height. The propagation direction of the wave (and therefore the direction of the force) is along the \( yz \)-plane tilted by a Rayleigh angle of 21.8° with respect to the \( z \) axis.

**Conclusions**

We have introduced a versatile SAW-actuated microfluidic sorter (SAWACS) with sorting rates of 3000 s\(^{-1}\) at a moderate acoustic power of 15 dBm. In our acoustic method we gently direct cells into the collect channel. We apply only low shear forces because we switch fluid volumes instead of applying forces directly to the target cell. The amplitude of the electric voltage that we use to excite the acoustic wave on the piezoelectric substrate is lower than 1.8 V at the highest power we used. For such moderate acoustic amplitudes we have demonstrated good cell viability.‡ Because we switch the flow, the method does not require additional responsive particles to enhance sorting. Sorting does not need magnetic or dielectric labeling and can operate in the same way under different physiological conditions as well as on different targets. Unlike electric methods, it is independent of the electrolytic buffer used to suspend the cells. The streaming velocity and sorting speed could be further optimized by using a higher SAW frequency, thus decreasing the attenuation length.\(^{43-45}\)

The sorting rates achieved by our traveling wave device are considerably higher than those achieved by standing wave based devices so far. While these devices can achieve high throughput when separating particles in bulk by size, they report longer switching times\(^6\) and sorting rates of 222 droplets s\(^{-1}\). As the wavelengths in standing wave devices have to be comparable to channel width, optimization by increasing SAW frequency is not possible. Furthermore, the PDMS channel has to be aligned very precisely to the substrate.

When operating the device as a cell sorter, the handling of cells is similar to commercially available FACS machines because it uses single phase flow. Cells that enter from a sample reservoir move through a tube into the interrogation area and are sorted directly from the continuous cell buffer medium into multiple outlet channels based on the level of the fluorescence signal. In this mode our sorter has the same advantage as conventional FACS sorting that does not require additives.

However, our microfluidic sorter has a number of improved capabilities compared to FACS. While FACS requires the dispersion of samples into drops that produce the potential biohazardous aerosol, drop formation is not required for our sorter and the device is fully self-contained. This greatly simplifies the safe handling of biohazardous materials. According to the International Society for Analytical Cytology (ISAC), aerosol production is the most critical and serious issue for biosafety concern.\(^{46}\) The acoustic platform we suggest avoids this drawback by sorting directly from the continuous single-phase buffer solution. This not only reduces biosafety concerns but also greatly reduces the cost of maintaining and containment of the cell sorting system. Because the chip is disposable it makes elaborate cleaning procedures of the system unnecessary. Furthermore, the absence of drop formation dramatically simplifies optical detection and makes interrogation of the sample more reliable.

The sorter that we have demonstrated is not only limited to cell sorting but can also sort different types of objects independent of their size or physical properties. To show this versatility we have sorted drops of an emulsion. Drops are useful for cell encapsulation and can be loaded with a biological or synthetic cargo. They enhance studies of protein expression of cells and are useful in many ways, for example, for assays and as drug delivery containers. Using drops enables qualitatively new studies in many fields including directed evolution and drug screening. Based on their fluorescence level we were able to demonstrate fast droplet sorting into two and multiple channel outlets at high rates of up to 3000 s\(^{-1}\) without any sorting errors.

This rate limit is mainly caused by the occurrence of droplet coalescence above a certain shear stress in the alignment channel. Since this shear stress is proportional to droplet rate, it sets an upper limit to the sorting rate.

However, novel alignment methods like acoustic focusing,\(^5\) which can be easily integrated into the device, do not rely on geometric constraints for alignment and therefore substantially reduce shear stress. In combination with increasing droplet
stability achieved through optimization of droplet surfactants, this coalescence limit can be overcome.

The sorter is also easily integrated into available fluidic platforms and technology. Since our sorting channel device is optically transparent, it can be combined with existing optical flow cytometric detection methods. Applicable SAW-based alignment methods using standing acoustic waves to align cells\(^{6,47}\) can be simply integrated into our device. By increasing acoustic power, improving droplet alignment and parallelization, our sorter can also be optimized to further increase sorting rate and efficiency even more. In addition, new methods to precisely control the volume of drops with acoustics are straightforward to implement.\(^{48}\) Furthermore, since our sorter works by fluid switching, it is not only limited to droplets or cells but can also sort any detectable object independent of size. Recent advances on detection in microfluidic systems enable a high-throughput analysis of nanoparticles.\(^{49}\) In combination with our device, this enables sorting of particularly small objects such as nanoparticles, viruses and bacteria, and even proteins or DNA.

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