Ultrasonic standing wave manipulation technology integrated into a dielectrophoretic chip

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Several cell-based biological applications in microfluidic systems require simultaneous high-throughput and individual handling of cells or other bioparticles. Available chip-based tools for contactless manipulation are designed for either high-precision handling of individual particles, or high-throughput handling of ensembles of particles. In order to simultaneously perform both, we have combined two manipulation technologies based on ultrasonic standing waves (USWs) and dielectrophoresis (DEP) in a microfluidic chip. The principle is based on the competition between long-range ultrasonic forces, short-range dielectrophoretic forces and viscous drag forces from the fluid flow. The ultrasound is coupled into the microchannel resonator by an external transducer with a refractive element placed on top of the chip, thereby allowing transmission light microscopy to continuously monitor the biological process. The DEP manipulation is generated by an electric field between co-planar microelectrodes placed on the bottom surface of the fluid channel. We demonstrate flexible and gentle elementary manipulation functions by the use of USWs and linear or curved DEP deflector elements that can be used in high-throughput biotechnology applications of individual cells.

Introduction

The miniaturization and automation of cell-based applications in biotechnology is dependent on the development of flexible tools for non-intrusive handling and manipulation of bioparticles (*e.g.*, cells, viruses and functionalized beads) in, *e.g.*, microfluidic chips. Available tools in microsystems have either high spatial accuracy (*e.g.*, dielectrophoresis[1] and laser tweezers[2]) suitable for slow 3D-manipulation of individual particles, or long range (*e.g.*, magnetic fields[3] and ultrasonic standing waves[4]) suitable for high-throughput manipulation of large particle ensembles. Several modern applications in cell biology and biotechnology require well-controlled and gentle handling of individual cells while still maintaining high-throughput in order to achieve sufficient volume. One example of such applications is controlled cell programming by surface-to-surface contact of a cell with a functionalized surface such as a bead, or with another cell.[5,6] Microfluidic systems are suitable multi-purpose platforms for such cell handling as well as for similar applications in biotechnology where long-term and gentle handling of cells are important. In the present paper, we combine short-range dielectrophoretic (DEP) manipulation with long-range ultrasonic standing wave (USW) manipulation for high-throughput handling of individual bioparticles in microfluidic chips.

Dielectrophoresis (DEP) is a powerful tool for handling and characterization of single cells in microfluidic chips.[1] DEP is based on polarization forces on dielectric particles created by inhomogeneous high-frequency electric fields. In a DEP chip, cells can be identified, selected, trapped and characterized one-by-one by the use of DEP and a fluid flow.[7] Different microelectrode geometries are utilized to create different elementary manipulation functions, and a set of such different electrode elements placed successively in the fluid channel creates a miniaturized application-specific "conveyer-belt" device. In addition to manipulative functions, DEP systems can also be used for characterization of cells, *e.g.*, by electrorotation[8] and impedance spectroscopy,[9] and for electrohydrodynamic pumping of the carrier fluid.[10] The breakthrough for DEP in cell-based biological applications was made in the early 90s, when routine production of microstructures and microelectrodes became available.[11,12] Here, electrodes and channels were scaled to similar sizes as the cells ($\sim 10^{-5}$ m), which resulted in high spatial accuracy ("tweezer sharpness") and efficient heat removal. Thus, DEP manipulation is characterized by its localized and short-range force fields, which typically extend a few tens of μm away from the electrodes. Today, a wide range of biological applications has been reported where DEP is used for manipulation of individual particles in microfluidic systems.[7,13,14]

Ultrasonic standing wave (USW) manipulation is a simple and useful method for handling, separation and concentration of large groups of particles or cells.[15] The principle of USW manipulation is based on steady-state acoustic radiation forces, which typically drive particles to the pressure nodes of the standing wave.[16] The technique has long been used in macro-scaled or "mini"-scaled systems with chambers ranging from the mm to the cm scale.[17–19] Examples of biological USW applications in such systems are separation, filtering and agglomeration of suspended particles or cells.[15,20,21] However, in the late 1990s and early 2000s, the first step towards miniaturization was taken by the investigation of sub-mm

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laminar-flow USW chambers for particle manipulation[22–24] and USW separation in microfluidic (sub-100 μm) capillaries.[25] Shortly thereafter, a microfabricated USW silicon–glass chip was developed for particle separation in a suspension,[4,26,27] followed by similar instrumental approaches for manipulation of cells or beads using all-glass,[28] glass–silicon,[29–32] glass–steel,[33,34] or glass–polymer[35,36] as the basis of the microstructure. Furthermore, microscaled USW concentration has also been carried out in microtiter plates for improved sensitivity in bead-based assays.[37] In contrast to DEP, USW manipulation is characterized by its long-range force field, determined by the pressure node spacing in the axial direction ($\sim 10^{-4}$–10^{-3} m). In addition, it is difficult to use ultrasound for localized, three-dimensional single-particle manipulation. The reason is that the lateral force range of a focused USW field can at best be as small as the axial range (*i.e.*, of the order of 10^{-4} m) due to diffraction. Therefore, USW manipulation is mainly considered as a "coarse" tweezer suitable for simultaneous handling of large groups of particles or cells. Finally, we note that USW technology appears to be superior to optical tweezers and DEP for long-term manipulation since cells can be manipulated in microchannels for hours without any detectable damage or stress.[38]

In the present paper, we combine manipulation by short-range dielectrophoretic (DEP) forces with long-range ultrasonic-standing-wave (USW) forces in a microfluidic chip. The system is designed for one-by-one handling of bioparticles, but—in contrast to other individual handling techniques such as DEP and optical tweezers—without compromises in throughput and manipulation time. The combined DEP/USW manipulator is integrated in a transparent glass–silicon–glass chip with the ultrasound coupled obliquely into the fluid channel by an external transducer combined with a refractive element. This allows for the use of high-NA transmission light microscopy for real-time monitoring of unlabelled cells without any contaminating fluorophores or stains. The modular USW/DEP approach also allows for high flexibility, since the traditional single-purpose DEP chip with its static electrode configurations can be used for different kinds of applications when it is combined with external USW transducers. Here, the combined DEP/USW approach is suitable for both high-throughput programming of individual cells as well as for prolonged and gentle manipulation during the programming procedure.

In the context of combining USW and DEP manipulation in microsystems, it is worth noting that acoustic and electric fields have previously been combined for manipulation of particles in suspensions. For example, the competition between the acoustic radiation force and the electrostatic force has been used to separate particles on the basis of their size, charge or stiffness by combining a 500 kHz USW with a DC electric field.[39] Furthermore, a non-resonant 23 kHz acoustic field has been combined with a DC electric field to enhance the performance of filtration processes, *e.g.*, by limitation of the reduction of permeate flux and retardation of membrane fouling.[40] Both these applications are carried out in macro-scaled systems. A miniaturized approach is the combination of high-frequency (8 MHz) USW manipulation and an electro-osmotic flow inside a sub-100 μm capillary for size-selective particle separation.[41] However, neither of these techniques utilizes the combination a short-range field, such as DEP, with the long-range USW manipulation. To our knowledge, the present paper is the first application of a combination of DEP and USW manipulation technologies.

Principles and theory

Dielectrophoretic (DEP) manipulation

A dielectric particle is polarized when it is exposed to an external electric field. The size and direction of the induced dipole depend on the field frequency and the dielectric properties (*i.e.*, the conductivity, σ, and the permittivity, ε). If the AC electric field is inhomogeneous, the frequency-dependent difference in polarizability of the particle and the surrounding liquid induces a force on the particle. If there are no phase gradients in the electric field, the time-averaged force on a dielectric particle of radius r in an electric field E can be expressed in dipole approximation as[7]

$$F_{\mathrm{DEP}} = 2\pi\varepsilon_l r^3 Re(f_{\mathrm{CM}})\nabla E^2_{\mathrm{RMS}} \quad (1)$$

where f_{CM} is the DEP contrast factor (the Clausius–Mossotti factor), which for a homogeneous sphere in an electric field with angular frequency ω is defined as

$$f_{\mathrm{CM}} = \frac{\tilde{\varepsilon}_p - \tilde{\varepsilon}_l}{\tilde{\varepsilon}_p + 2\tilde{\varepsilon}_l}, \qquad \tilde{\varepsilon}_l = \sigma_l + i\omega\varepsilon_l, \qquad \tilde{\varepsilon}_p = \sigma_p + i\omega\varepsilon_p \quad (2)$$

The indices l and p refer to the liquid and the particle, respectively. The sign of the DEP contrast factor, $Re(f_{\mathrm{CM}})$, determines whether the particle is attracted to (positive DEP, $Re(f_{\mathrm{CM}}) > 0$) or repelled from (negative DEP, $Re(f_{\mathrm{CM}}) < 0$) the electrodes. It is only the latter case that is of interest for contactless cell manipulation.

The electric field distribution can rarely be calculated analytically. However, for the simple case of DEP deflectors (two parallel and planar electrodes), the maximum force in the central horizontal plane is[7]

$$F_{\mathrm{deflector}} = \frac{27}{32}\pi^2\varepsilon_l Re(f_{\mathrm{CM}})r^3\frac{U^2}{a^3} \quad (3)$$

where U is the applied voltage over the electrodes and a is the spacing between the electrodes. In our experiments, DEP deflector elements are used for particle and cell manipulation.

Ultrasonic standing wave (USW) manipulation

The steady-state acoustic force in a standing-wave field is a result of a non-linear effect in the time-averaged acoustic radiation pressure around the particle. If only axial forces are considered, the time-averaged acoustic force, F_{USW}, on a spherical particle suspended in a liquid is given by[42]

$$F_{\mathrm{USW}} = \frac{\pi}{2\rho_l c_l^3}\left(f_1 + \frac{3}{2}f_2\right)r^3 p_0^2 v \sin\left(2\pi\frac{z}{\lambda/2}\right) \quad (4)$$

where ρ is the density, c is the sound velocity, r is the particle radius, p_0 is the pressure amplitude, v is the acoustic frequency, z is the axial coordinate, λ is the acoustic wavelength in the

liquid and f_1 and f_2 are dimensionless corrections taking the compressibility of the particle into account, given by

$$f_1 = 1 - \frac{\rho_l c_l^2}{\rho_p c_p^2}, \qquad f_2 = \frac{2(\rho_p - \rho_l)}{2\rho_p + \rho_l} \tag{5}$$

The indices l and p denote the liquid and the particle, respectively. In most practical cases, suspended particles are trapped in the pressure nodal planes of the standing wave. However, if the first expression in brackets in eqn 4 is negative, the particles are instead trapped in the pressure antinodes. This situation is for example obtained if the particles have lower density than the density of the liquid.

If eqn 3 is compared with eqn 4, we see that the DEP force and the USW force have several similarities. Both forces are proportional to the volume of the particle ($\sim r^3$) and to the square of the applied voltage (since the acoustic pressure amplitude, p_0, is proportional to the transducer voltage). In addition, both forces are dependent on contrast factors, determined by the dielectric properties (the DEP case) or the acoustic properties (the USW case) of the particle relative the surrounding liquid. In addition, the applied voltages and frequencies are within the same range for both methods (a few V, a few MHz) for the manipulation of μm-sized particles or cells, which is beneficial for the instrumental arrangement. However, the scales of the field gradients are different, mainly due to diffraction and absorption limitations for focused high-frequency ultrasound.

Field coupling

One advantage by combining DEP and USW is to employ two independent forces that can be applied and controlled without mutual interference. However, it is known that alternating electrical potentials can be generated by ultrasound propagation in conductive suspensions, and *vice versa*.[43] Therefore, it is of interest to investigate to what extent there is any coupling between the electric and ultrasonic fields. The suggested coupling principle has been experimentally verified in electrolytic solutions[44] and in colloidal suspensions.[45] Typically, the generated electric potential is 10^{-5}–10^{-4} V at 10^5 Pa acoustic pressure amplitude. Contrary, the generated acoustic pressure amplitude is ~1–10 Pa for a 10^4 V m^{-1} electric field amplitude at 1 MHz. If these measured values are scaled according to the properties of our system (taking the field amplitudes, frequencies, geometrical dimensions and the force equations (eqns 3 and 4) into consideration), any coupling contribution is less than $10^{-8}\times$ the primary forces ($F_{\text{deflector}}$ and F_{USW} in eqns 3 and 4). Therefore, we conclude that there exists no interference of significance between the DEP and USW fields in the present paper.

Materials and methods

The fabricated chip (GeSim GmbH, Dresden, Germany) was designed to match the electric and fluidic platform of the Cytoman™ system (Evotec Technologies GmbH, Hamburg, Germany). In Fig. 1, an illustration of the cross section of the chip and the transducer is shown. Basically, the transducer is made by a 4 × 7 mm square PZT piezoceramic element (1) glued to a polymethylmethacrylate (PMMA) refractive wedge (2) with an angle of incidence of 17 degrees relative to the chip surface normal. The fundamental resonance frequency of the transducer was 2.12 MHz. The combined DEP/USW chip is a three-layer structure made of a 785 μm thick Pyrex glass plate (3) and a 150 μm thick D263 borosilicate glass plate (5) separated by a 40 μm thick silicon spacer (4) defining the 750 μm wide and 10 mm long microfluidic channel. The glass layers were attached to the silicon spacer by anodic bonding (Pyrex) and gluing (D263). The idea of the oblique coupling of sound into the chip (*cf.* the marked wavefronts (6) in Fig. 1) is to transfer the primarily vertical direction of the incident wave into a primarily horizontal direction inside the fluid channel, by careful matching of the transducer angle and the acoustic properties of the layers (PMMA, borosilicate, silicon) between the transducer and the channel. In addition, this transducer arrangement also allows for high-NA transmission microscopy, since the transducer is not placed directly over the channel. The DEP electrodes were fabricated on the bottom glass surface in the fluid channel, in a ''side-by-side'' co-planar arrangement. The reason for choosing such oriented electrode pairs, instead of the more commonly employed ''face-to-face'' mounted electrode pairs perpendicular to the fluid flow (11), was only a priority of fabrication costs over DEP efficiency. In the experiments, three DEP elements were used, a curved deflector element (8) and two linear deflector elements (9–10) with different lengths. The number of pressure nodes in the USW field (*cf.* dotted lines (7) in Fig. 1) can be chosen by the acoustic frequency (for more details, see the results section). In close vicinity to the points of intersection between the pressure

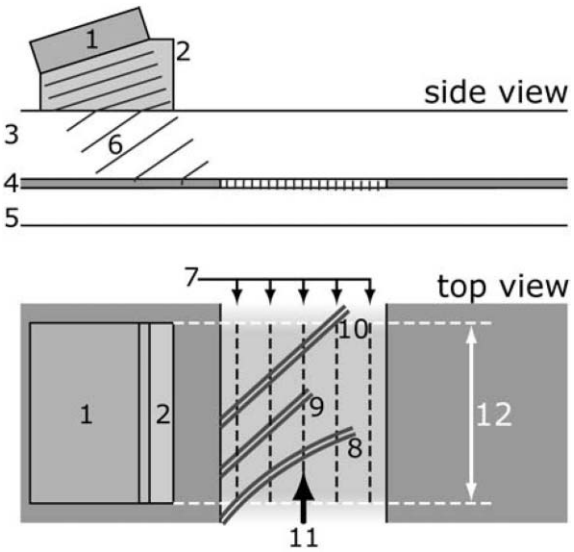

Fig. 1 Illustration of the side and top view of the ultrasonic transducer and the chip. The transducer consists of a piezoceramic plate (1) and a PMMA refractive element (2). The chip consists of a Pyrex (3)–silicon (4)–borosilicate (5) three-layer transparent structure. The wave is coupled into the chip by refraction in each surface (6). At the points of intersection between the USW pressure nodes (7) and the DEP electrodes (8–10), particles are manipulated by the combined USW/DEP/flow forces. The USW is perpendicular to the flow direction (11), and the ultrasonic field is mainly limited to the region matching the width of the transducer (12).

nodes (7) and the electrodes (8–10), particles can be manipulated by the combination of USW forces, DEP forces and viscous drag forces from the fluid flow. Due to the flat channel cross section (width/height ratio = ~20), suspended particles or cells are initially arranged two-dimensionally (since the channel height is of the order of the cell size). Therefore, the manipulation is in principle only performed in the horizontal plane. In addition to the DEP/USW chip, a simple in-house made chip prototype was also fabricated for USW experiments only. This chip is a two-layer structure consisting of 1 mm Pyrex bonded to a silicon wafer with either 40 × 750 μm or 40 × 375 μm rectangular grooves. Both the three-layer combined DEP/USW chip and the two-layer USW chip has ~750 μm drilled holes through the upper glass layer at the inlet/outlet points of the microchannel, and plastic nipples glued to the holes for connection to the tubing.

The chip surroundings consisted of an Olympus IX71 inverted microscope with objectives (5–40×/0.15–0.6NA) and combined epi-fluorescence/bright-field illumination imaging. The transducer was placed on the upper surface of the chip with a small droplet of immersion oil (Type A, Nikon) used as acoustic coupling medium between the transducer and the upper glass layer of the chip. A slight pressure was applied on top of the transducer to minimize the thickness of the immersion oil layer. Teflon tubing and a syringe pump (Model SP2301WZ, WPI) was used to control the laminar flow inside the chip. The flow rate used in all combined DEP/USW experiments was 50 μl min^{-1}, corresponding to a mean flow velocity of 0.5 mm s^{-1}. A Cytoman™ system (Evotec Technologies GmbH, Hamburg, Germany) was used as the DEP generator, and a 15 MHz function generator (Model 33120A, Hewlett-Packard) was used as the USW generator. In all experiments, the employed DEP frequency was 2 MHz and the employed USW frequencies were near the resonances found at 2.12 MHz, 6.6 MHz, 7.5 MHz, 10.8 MHz and 13.4 MHz. The manipulation and handling of cells were modeled by either 10 μm green-fluorescent polystyrene (latex) beads, or 2 μm, 5 μm and 15 μm non-fluorescent polystyrene beads (Polysciences, Warrington, USA). Verification of the beads as a cell model was performed with human histiocytic lymphoma cells (U937).[46] All experiments were carried out with beads or cells suspended in Cytocon™ II-solution (Evotec Technologies GmbH, Hamburg, Germany).

Results

In this section, we report on experimental results when the chip is operated by USW alone, as well as by combined DEP/USW. Operation with DEP only in a similar chip layout has been reported elsewhere.[47]

(1) USW manipulation

When the chip is operated by ultrasound alone, the basic manipulation functions are high-speed alignment (1a), parallel alignment in multiple nodes (1b) one-dimensional aggregation (1c) and fusion of particle lines (1d) (*cf.* Fig. 2–4). The latter experiment (1d) was performed in the three-layer chip, while the others (1a–c) were performed in the two-layer chip.

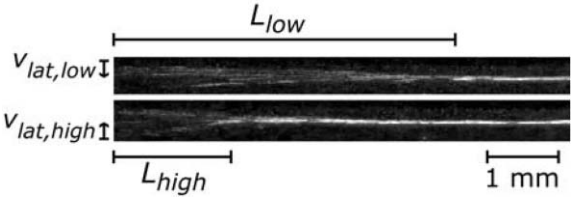

Fig. 2 High-speed USW alignment of 10 μm fluorescent beads at a mean flow velocity of 19 mm s^{-1} and at transducer voltages of 4 V_{RMS} (top picture) and 7 V_{RMS} (bottom picture). The corresponding maximum focusing distances (L) and lateral mean velocity of beads (v_{lat}) are marked for the low and high transducer voltages.

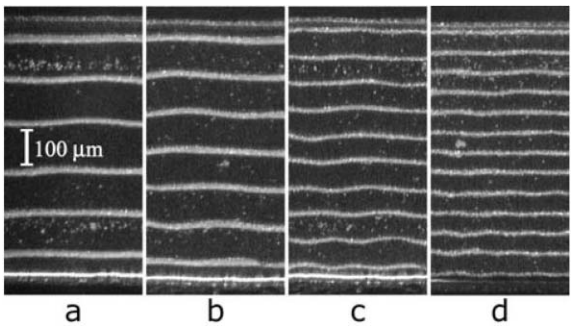

Fig. 3 Parallel USW alignment in multiple nodes, demonstrated with 2 μm latex. 6 nodes at 6.6 MHz (a), 7 nodes at 7.5 MHz (b), 10 nodes at 10.8 MHz and 13 nodes at 13.4 MHz (d). The experiments are performed without a flow.

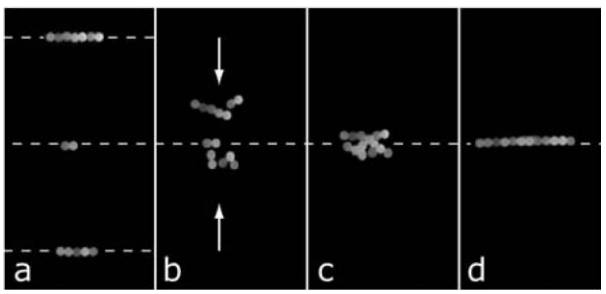

Fig. 4 Fusion of particle subgroups by ultrasonic frequency shift. Fourteen beads (10 μm latex) are trapped as line-aggregates in three subsequent nodes at 6.56 MHz (a). When the frequency is changed to 2.12 MHz, the outer nodes are merged with the center node (b–c), resulting in fusion of the three groups into one (d). Dashed lines indicate nodes.

(1a) High-speed alignment. In order to estimate the maximum ultrasonic forces that can be obtained in our system, we measured the focusing distance, L, of beads at different flow rates and transducer voltages. The principle of the experiment is illustrated in Fig. 2, where fluorescent 10 μm polystyrene beads are pumped externally into the channel from the left. Here, the focusing distance (*cf.* L_{low} and L_{high} in Fig. 2) is defined as the maximum axial distance a bead covers, when it is laterally displaced from the periphery into the center of the channel where the pressure node is. At the acoustic frequency of 2.12 MHz, the beads were aligned within the distance L_{high} = 1.9 mm at a flow rate of 2.0 ml h^{-1} and a transducer voltage

$U_{t,high}$ = 7.0 V_{RMS}. This flow rate corresponds to a mean flow velocity of 19 mm s^{-1} in the 40 × 750 μm^2 (height × width) fluid channel. This, in turn, corresponds to a lateral mean velocity of beads (*cf.* $v_{lat,high}$ in Fig. 2) of 1.9 mm s^{-1} and a maximum alignment time of 100 ms. The same experiment was carried out at 4 V_{RMS} transducer voltage and flow rate of, again, 2.0 ml h^{-1}, resulting in L_{low} = 4.8 mm, $v_{lat,low}$ = 0.7 mm s^{-1} and 250 ms maximum alignment time. If the acoustic force (*cf.* eqn 4) is compared with the viscous drag force from the fluid ($F_v = 6\pi\eta r v$, where η is the fluid viscosity and v is the lateral bead velocity relative the fluid), the acoustic pressure amplitude inside the fluid channel can be estimated from the experimental values for the lateral bead velocities. Here, the lateral mean velocity of 1.9 mm s^{-1} (at 7.0 V_{RMS} transducer voltage) corresponds to an acoustic pressure amplitude $p_0 \approx 0.55$ MPa, and the lateral mean velocity of 0.7 mm s^{-1} (at 4.0 V_{RMS} transducer voltage) corresponds to $p_0 \approx 0.35$ MPa. These pressure amplitudes are equivalent to acoustic forces of the order of 100 pN.

The maximum flow rate that could be used for efficient bead focusing within the 10 mm long fluid channel was approx. 4.0 ml h^{-1}, corresponding to 37 mm s^{-1} mean velocity. At even higher flow rates, the beads moved too fast for manually tracking them by microscopic observation. If the USW alignment performance is compared with DEP alignment by the use of linear deflectors, the USW throughput is two orders of magnitude higher than the typical DEP throughput. The reason is mainly the longer allowed focusing distance in the USW case (several millimetres), while DEP deflection requires a fixed alignment distance defined by the angle and length of the electrodes.

(1b) Parallel alignment in multiple nodes. The ultrasonic resonances of the transducer–chip system are found when the channel width matches a multiple of $\lambda/2$ (where λ is the acoustic wavelength). These were found by investigation of the USW manipulation of 2 μm polystyrene beads in the microchannel during a frequency sweep. In Fig. 3, the higher resonances (beside the fundamental resonance at 2.12 MHz) are shown at the approximate frequencies of 6.6 MHz (a), 7.5 MHz (b), 10.8 MHz (c) and 13.4 MHz (d), respectively. For the combined DEP/USW chip, the resonances around 2.1 MHz, 6.6 MHz and 10.8 MHz could be used for switching between 2, 6 or 10 parallel pressure nodes. The parallel alignment function is particularly suitable for multiplexing, *e.g.*, by guiding different subpopulations of cells or particles in different pressure nodes.

(1c) One-dimensional aggregation. When the chip is operated with ultrasound and without a flow, a function obtained is one-dimensional particle aggregation. This is shown in Fig. 4a, where 10 μm polystyrene beads are aligned and aggregated in three different nodes. Typically, the lateral alignment (perpendicular to the flow) occurs within less than 1 s, while the axial alignment is weaker and has a time scale of ~1 min. Eventually, the beads form one-dimensional stable aggregates where each bead has its well-defined position in the line. This effect has previously been observed by Yasuda,[23] who suggested the use of such line-aggregation for distinction and numbering of cells by keeping track of the position of each cell in the line.

(1d) Fusion of particle lines. The line-aggregation effect (1c) can be combined with the multiple node alignment effect (1b), resulting in fusion of particle subgroups by ultrasonic frequency shift. Fig. 4a–d shows an experiment where three line-aggregates of 10 μm polystyrene beads are trapped in three adjacent pressure nodes, followed by fusion into a single line-aggregate by changing the frequency from 6.56 MHz to 2.12 MHz. Here, fusion only refers to particles merged into the same position, resulting in surface contact between the particles.

(2) Combined DEP/USW manipulation

When USW manipulation is added to a DEP chip, a static DEP deflector can be used for several different purposes. The basic idea is to first align particles by ultrasound, followed by combined manipulation when the particles arrive at the electrode. In this way, dynamic elementary manipulation functions are realized, *e.g.*, high-precision particle trapping, sorting, concentration and separation (2a), particle switching (2b) and fusion of particle subgroups (2c). In the following experiments, the three-layer chip (*i.e.*, the DEP chip) was used.

(2a) Particle trapping, sorting, concentration and separation. In Fig. 5a, four beads (10 μm polystyrene) are trapped in the equilibrium between the USW force, the DEP force and the viscous fluid force. Since each force can be controlled and adjusted separately without any interference from the other forces, trapped beads can be sorted sideways by turning off the ultrasound (Fig. 5b), or straight forward by turning off the electric field (Fig. 5c). The mean flow velocity (0.5 mm s^{-1}) and the angle of the electrode relative to the flow direction (38°) can be used to estimate the force components (47 pN and 61 pN in the axial and lateral directions, respectively). The USW alignment prior to the combined manipulation is advantageous since all incident particles will approach the electrode at predefined lanes along the pressure nodes of the standing wave. It is also possible to perform size-selective separation between 15 μm beads and 10 μm beads. Here, the

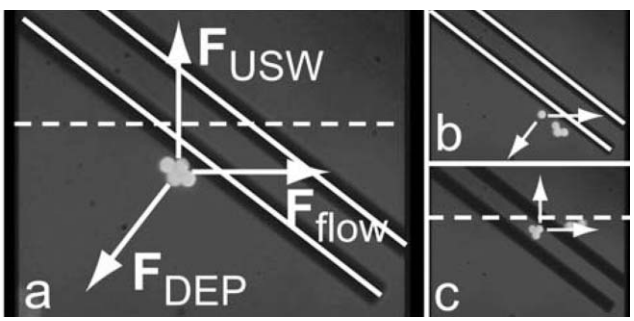

Fig. 5 The basic principle of combined DEP/USW manipulation, showing the USW, DEP and the flow force components on four trapped particles (10 μm latex). Each component can be adjusted separately as shown in (b) and (c), resulting in particle selection following trapping. The dotted line marks the USW pressure node and the solid lines mark active electrodes.

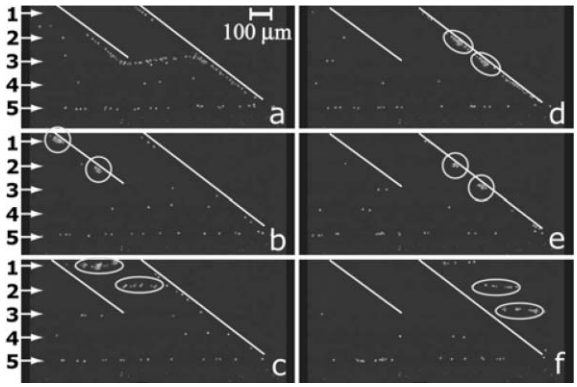

Fig. 6 Particle switching experiments. Merging of USW lanes by DEP (a). Sideways shifting of lanes (b–f). The arrows mark the five USW pressure node and the solid lines mark the electrodes. The ellipses denote the trajectories of bead agglomerates. The distance between the nodes is 110 µm (at 6.6 MHz acoustic frequency), and the time scale is approx. 10 s between each image.

smaller beads pass by the electrodes, while the larger bead is trapped at the electrode. High selectivity (>90%) is obtained if each large (15 µm) bead is trapped and released one-by-one.

(2b) Particle switching. Another possible application of a linear DEP deflector when it is combined with ultrasound is lateral switching of particles by changing "lane" (*i.e.*, pressure node). This is illustrated in Fig. 6, where a short and a long DEP deflector are combined with USW manipulation of 10 µm polystyrene beads. In Fig. 6a, a "slipway" experiment is shown where lanes 1 and 2 are merged with lane 3 at the first deflector, and lane 3 and 4 are merged with lane 5 at the second deflector. Here, the acoustic pressure amplitude is low enough to allow the DEP force to guide the particles to the adjacent lane, but high enough to align the particles into each lane. In Fig. 6b–f, two groups of trapped particles in lanes 1 and 2 (Fig. 6b) are shifted sideways into lane 2 and 3 (Fig. 6e), and finally released (Fig. 6f).

(2c) Fusion of particle subgroups. A manipulation function of interest in, *e.g.*, cell-based assays is the controlled and gentle (surface-to-surface) contact of a cell with a functionalized bead, or with another cell. This kind of application is demonstrated by two different experiments. In the first experiment, USW manipulation is combined with a curved DEP deflector for step-by-step fusion of particle subgroups, see Fig. 7. Here, four minor particle groups (10 µm polystyrene) are held at the deflector in the combined DEP/USW traps. Then, by slowly decreasing the acoustic pressure amplitude the particle group at lane 1 will first be released since the lateral component of the DEP force, defined by the tangent of the deflector, is highest for the first trap at lane 1. Thus, group 1 is combined with group 2 (Fig. 7b), and at even lower acoustic pressure amplitude, group 2 is combined with group 3 (Fig. 7c). Finally, all four particle groups are combined into the fourth trap (Fig. 7d). In an alternative approach, USW manipulation is combined with a linear DEP deflector and USW frequency shift for direct fusion of several particle subgroups. Here, non-fluorescent 5 µm beads are employed to model non-labeled cells. Initially, the homogeneously suspended beads move with

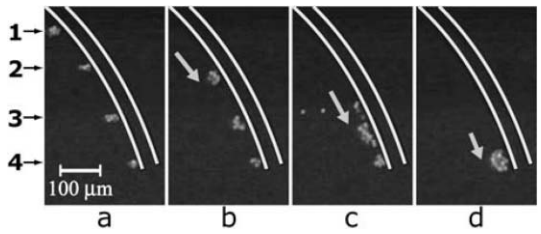

Fig. 7 Fusion of particle subgroups by USWs and a curved DEP deflector. Initially, four subgroups of 10 µm latex are trapped at the electrodes (a). By slowly decreasing the acoustic pressure amplitude, the first subgroup is merged with the second subgroup (b). Then, group 2 is merged with group 3 (c) and finally, all subgroups end up in lane 4 (d). The arrows mark the four USW pressure nodes and the solid lines mark the electrodes. The distance between the nodes is 110 µm (at 6.6 MHz acoustic frequency), and the time scale is approx. 10 s between each image.

the flow with both ultrasound and the electric field turned off (Fig. 8a). When the ultrasound is turned on at 10.8 MHz, the beads align into five lanes (Fig. 8b). In the images, only half of the channel width is visible. Therefore, only five of the ten lanes in total are visible (*cf.* Fig. 3c). After USW alignment, the DEP deflector is turned on, resulting in trapping and accumulation at the five positions in front of the electrodes (Fig. 8c). Then, by changing the frequency from 10.8 MHz to 2.12 MHz, the five nodes are merged into one node (Fig. 8d). The major difference between the two fusion experiments described here (by the use of combined DEP/USW), and the fusion experiment by the use of USW only (1d), is that the combined approach allows for high-precision fusion of single particles.

In order to verify the validity of polystyrene beads as cell models, similar experiments with combined DEP/USW

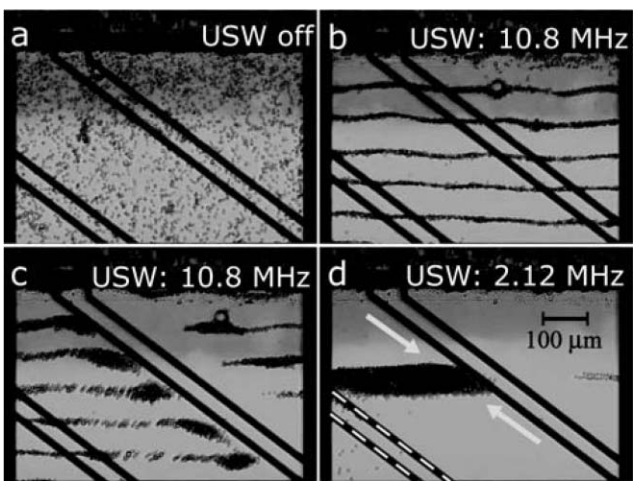

Fig. 8 Fusion of particle subgroups by USW frequency shift and a linear DEP deflector. Initially, beads (5 µm latex) move with the flow in the microchannel (a). When the ultrasound is turned on, they are aligned in 5 lanes (b), followed by trapping and accumulation when the electrode is turned on (c). Then, by changing the ultrasound frequency from 10.8 MHz to 2.12 MHz, the five subgroups are fused into one group (d). The bead concentration is chosen to be relatively high in order to visualize the force fields. The distance between the nodes (in b and c) is 70 µm (at 10.8 MHz acoustic frequency), and the time scale is approx. 10 s between each image.

manipulation were performed with human histiocytic lymphoma cells (U937).[46] The cells could be manipulated with combined DEP/USW, but with lower efficiency. The reason is the reduced efficiency of cell manipulation by the DEP deflector with side-by-side oriented electrodes. However, this is only a technical problem related to the chip fabrication, and could be solved by employing face-to-face mounted electrodes as normally used in DEP chips. Other solutions are to add a conductive surface at the upper glass layer and still use the side-by-side oriented electrodes, or to use a vertical ultrasonic standing wave with a pressure node close to the bottom surface of the microchannel. Finally, it should be mentioned that there was no significant difference in USW manipulation performance between beads and cells. All observed characteristics for USW manipulation with beads were also observed with cells.

Discussion

The most convenient way to handle many particles or cells in a microfluidic channel is to have high visual control and high flexibility of the manipulation tools. In our DEP/USW chip, the channel height has the same scale as a typical cell size. Thus, all particles are easily imaged with a microscope. However, the requirement of the manipulation tools in such a chip is that the force fields must be oriented horizontally within the flat microchannel. While this is customary for a standard DEP chip,[7] it is more difficult to create a horizontal ultrasonic standing wave by external transducers. We have solved this problem by utilizing refraction of the incident ultrasonic wave from an oblique transducer (see Fig. 1). This transducer design has several advantages. Firstly, it is an efficient way to create a directed horizontal standing-wave mode with low energy losses. Furthermore, the glass–silicon–glass chip design allows for any kind of high-NA optical microscopy since the transducer is placed beside the fluid channel. Previous designs of USW chips for horizontal manipulation suggest the use of transverse flexural waves formed either by paired co-planar phase-shifted transducers[29] or by the shear vibration mode of transducers integrated in the upper glass layer,[28] or bulk mode excitation in a direction perpendicular to the intended standing-wave direction in the channel.[30] Neither of these techniques is compatible with high-NA transmission light microscopy. High flexibility is also obtained from the possibility of using differently designed, removable transducers at different positions on top of the chip. Optimally, each of such transducers can produce a tailored standing wave field with defined positions, orientations and spatial extensions (widths) of the pressure nodes. When this technique for coupling USWs into a microchannel is applied to a DEP chip, dynamic elementary functions are realized, unlike the normal static case in a DEP chip with fixed elementary functions for each design of electrodes. Thus, simpler, multi-purpose and more cost-effective DEP chips can be manufactured, with only a few deflector elements instead of the highly complicated application-specific chips available today. Furthermore, it is also possible to combine the DEP/USW manipulation method with an optical tweezer, due to the unhindered optical access from both directions (up/down).

The outlook for using several manipulation tools for cell or particle handling in microchips is promising. For each kind of manipulation function, the most suitable tool available in the ''toolbox'' should be used. For example, while USW is the best tool for large-scale particle alignment, DEP is the best tool for single-particle manipulation. When different tools are combined, both higher flexibility and higher functionality are obtained. For example, the long-range USW can first be used for ''coarse'' manipulation in the whole microchannel, followed by ''fine'' manipulation of individual particles by combined DEP/USW. The use of such coarse and fine tools simultaneously opens new possibilities, *e.g.*, to handle large cell populations with high-throughput and multiplexing, but still in the individual ''one-by-one'' format that makes it possible to study the variance, and not only the average, of a cell group. Finally, it should also be mentioned that the two manipulation methods are technically easy to integrate, since the same electronic driver platform can be used for both the ultrasound transducers and the DEP electrodes.

There are several biotechnology applications that require both high-throughput and individual cell/bioparticle handling. One possible application of a DEP/USW-based cell handling device is to provide a platform for imprinting individual cells with functionalized beads or other cells for controlled surface-contact-induced cell differentiation.[5] The principle is based on copying the natural mechanism of cell–cell communication by the use of artificial immobilization of macromolecules on surfaces, *e.g.* beads, and then to ''program'' the cell by imprinting it with such a bead. Such surface controlled differentiation has been demonstrated on primary cell cultures with immobilized cytokines.[5] The influence of different immobilized extracellular matrix molecules has also been investigated.[48] Thus, an automated miniaturized cell handling device with both DEP and USW manipulation tools is an interesting approach for the realization of a high-throughput instrument for cell programming. An aspect to consider when choosing manipulation tools is time-dependent effects on the cell state and viability. Recent results indicate that ultrasound is preferable for long-term manipulation.[38,49] Thus, DEP can be used initially for short-term high-precision positioning of cells, followed by long-term retention by USW only.

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