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Dielectrophoresis switching with vertical sidewall electrodes for microfluidic flow cytometry

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Abstract

A novel dielectrophoresis switching with vertical electrodes in the sidewall of microchannels for multiplexed switching of objects has been designed, fabricated and tested. With appropriate electrode design, lateral DEP force can be generated so that one can dynamically position particulates along the width of the channel. A set of interdigitated electrodes in the sidewall of the microchannels is used for the generation of non-uniform electrical fields to generate negative DEP forces that repel beads/cells from the sidewalls. A countering DEP force is generated from another set of electrodes patterned on the opposing sidewall. These lateral negative DEP forces can be adjusted by the voltage and frequency applied. By manipulating the coupled DEP forces, the particles flowing through the microchannel can be positioned at different equilibrium points along the width direction and continue to flow into different outlet channels. Experimental results for switching biological cells and polystyrene microbeads to multiple outlets (up to 5) have been achieved. This novel particle switching technique can be integrated with other particle detection components to enable microfluidic flow cytometry systems.

Introduction

The field of microfluidic lab-on-a-chip aims to develop an integrated platform with multiple functions with applications typically used in chemical or biological analyses.[1,2] Traditional sample preparation steps are usually labor intensive and time consuming, while microfluidic systems provide opportunities for carrying out the entire analysis on a single platform. An ideal sample analysis process should be able to automatically and continuously process the tasks and ensure multiple process steps run in parallel. For biological sample analyses, the object of interest is usually the biological constituents. For chemical analyses, different chemical reagents need to be injected into the microfluidic channel or be sent to different channel branches with precisely controlled volumes. A microfluidic switch is a critical component to regulate the fluids and its constituents in microfluidic networks. Different microfluidic switching techniques have been developed by controlling the flow in microchannels. Active flow control units have been integrated in the channel outlet branches to introduce back pressure to stop the flow in some outlets and direct flow to others. A magnetohydrodynmic (MHD) switch with MHD pumps at the two outlets of a microchannel bifurcating Y junction has been demonstrated for switching flow of electrolyte solutions by

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Lorenz force.[3] Cheng and Liu introduced a time sequence power controlled thermal bubble actuator with hydrophobic patterns in different outlet ports to switch liquid into multiple outlets by capillary force.[4] To utilize the unique laminar flow property of microfluidics, another strategy is to use focused flow to switch the core sample flow by tuning the pressure/flow rate difference of the sheath flows. Focused flow switching utilizes the hydrodynamic properties of the fluid to direct them to desired outlets. This can be accomplished either by pressure driven flow[5,6] or electroosmotic flow.[7–9] When the sheath flow is air, electrowetting assistant patterns can be used to switch the flow.[10] The advantage of focused flow switching is that no moving part is involved and it can be easily expanded to multiple outlets and multiple reagents injection. In most of the biological applications, fluids (like cell media, buffer solutions, *etc*.) are usually used as carrier flow for cells, proteins, DNAs, and other micro organelles to send them to different sample processing steps. The objects of interest in this case can be switched together with the reagent fluid. The flow switching techniques mentioned above can be implemented for this purpose. One typical application of this type of switching is for cell sorting in flow cytometry. Methods of cell sorting demonstrated include MHD switching[11] and a T-switch based on an electrolysis-bubble actuator.[12] Fu *et al*. have demonstrated sorting of cells by electrokinetic pumping,[13] using active pumping and valve control[14] of the flow for a microfluidic fluorescent activated cell sorting device. Instead of switching the flow in the channel, another type of sorting involves switching only the particles without changing the flow pattern. In this method, the objects are pre-focused to the centre of the microchannel. When they arrive at the junction, a force is applied to the particles to deflect them from the flow streamline. This could be achieved by electrostatic force, optical force,[15] or dielectrophoretic force.[16,17]

Dielectrophoresis (DEP) is one of the most widely used techniques for microfluidic manipulation of particles, cells, viruses, DNAs and other objects.[18–20] Microfabricated electrodes enable low power consumption for the generation of DEP forces. DEP trapping has been used to concentrate populations of cells[21] or bacteria[22] from a suspension, while multiple[23] and spiral electrodes have been designed to trap single cells to study the dielectric properties of different cell types by their electrorotation spectra.[24,25] The DEP force together with field-flow fractionation (FFF)[26] has been demonstrated for separation and sorting of cells. Fiedler *et al*.[27] utilized planar electrodes on the bottom and top layer of the microchannel to achieve aligning, caging and switching. Holmes *et al*.[28] put three pairs of electrodes at the junction of channels to achieve two way binary sorting. We have developed a DEP switching technique with vertical electrodes embedded in the microfuidic channel along the sidewalls. With our design, no pre-focusing is required. Objects in the channel can be positioned laterally at different points of the channel by coupled negative DEP forces dictated by the configuration of the electrodes and the signals applied and resulting in them being switched into different channel branches. Up to five-outlet particle switching in a microfluidic device has been demonstrated. This switching technique can be integrated together with other detection and separation methods for flow cytometry applications.

Design and fabrication of 3-D electrodes at the sidewall

Originating from the integrated circuits (IC) industry, most of the microfabrication processes are planar-based techniques. Microelectrodes deposited at the bottom of the microchannel provide non-uniform electrical fields along the height direction, thus forming a vertical DEP force on the chip substrate. As shown in Fig. 1a, when the electrodes are directly deposited at the bottom of the channel, it generates a non-uniform field distribution along the height of the channel. The DEP forces can either attract the particles down to the electrodes or repel them away and lift them up in the channel. Since the electrical field decays exponentially with the distance away from the electrodes, particles far away from the electrodes will be less controlled by the DEP forces. On the other hand, when the electrodes are placed on the

sidewall of the channel as in Fig. 1b, uniform or non-uniform electrical fields can be generated along the width direction of the channel. The corresponding DEP force can then be used to laterally position the particles. Since most of the microfluidic devices are 2-D microchannel networks, this laterally positioning design is more compatible with other on chip analysis steps for continuous manipulation and parallel processing of samples.

One way to overcome the effect that electric field decays from the bottom electrodes is to configure planar electrodes at both the bottom and ceiling of the channels.[27] High aspect ratio electrodes extended along the height of the channel can also generate strong electrical fields covering the whole volume of the channel space to improve the DEP force efficacy with an electrical field gradient along the width of the channel direction. Dead electrical field space can thus be avoided. Different 3-D electrodes for DEP applications have been developed and demonstrated. Iliescu *et al.*[29] demonstrated highly doped 3-D silicon electrodes for DEP manipulation. Voldman *et al.*[30] developed electroplated 3D metal pillars for improving the performance of trapping by DEP. Madou[31] has developed 3-D carbon SU-8 electrodes for DEP extraction of nanofibrous carbon from oil. Yu *et al.*[32] designed microelectrodes on the circumference of an elliptic-like channel for particle focusing. The ability to fabricate 3-D electrodes extends the realm of electrical manipulation in microfluidics, and provides many advantages that can not be achieved by simple planar electrodes. We have developed a process to make vertical electrodes in the sidewall of the microchannel so that the electrodes serve as part of the channel sidewalls and do not interfere with the flow in the channel. Multiple layer SU-8 photolithography and metal deposition and electroplating techniques were used to make the microchannel and electrodes respectively.[33] Fig. 2 shows the SEM pictures of the interdigitated electrodes before the channel layer was coated (Fig. 2a and 2b) and after the channel is coated and aligned with the electrodes (Fig. 2c and 2d).

DEP switching principle and simulation

When interdigitated electrodes are set at the sidewalls of the channel, lateral DEP force can be generated which can attract or repel the particles to and from the channel walls with the electrodes. If another series of electrodes is configured to face the first set of electrodes, this second DEP force can balance the DEP force generated from the first electrode array. Fig. 3a illustrates the force balance of particles between the two sets of sidewall electrode arrays. A signal (U_1,f_1) is applied to the first set of electrode arrays (to the right of the flow direction), generating negative DEP force F_1 on the particles towards the second set of electrodes to the left of the flow direction. A second signal (U_2,f_2) is applied on the left-side electrodes to generate a counter force F_2 to the first set of electrodes on the right. By varying the magnitude and frequencies of the voltages, the forces from these two sets of electrodes can balance each other, and the particles can therefore be positioned at the equilibrium points. As shown in Fig. 3a, when a cell is close to electrode #1, like the one at point B, the DEP force F_1 from electrode #1 is larger than the DEP force F_2 from electrode #2, the cell will be pushed to the left. With the movement away from the right-side electrodes, the DEP force F_1 decreases and F_2 increases until it reaches an equilibrium position where F_1 is equal to F_2 at point C. The line that passes through point C along the channel direction is the equilibrium line for the particles under the signals applied. The two DEP forces can be adjusted by changing the voltage U or the frequency f, so that the equilibrium line can be tuned to any place along the width direction. The fluid can continuously flow into the channel and carry different cells to the desired outlets.

Negative DEP forces are used instead of positive ones, as negative DEP force pushes the objects away from the electrodes, a and positive one attracts them to the sidewalls. When the particles are attracted toward a sidewall by the positive DEP force of the electrode array, this

force becomes stronger and the force from the other electrode arrays decreases so that the particles will be attracted to the sidewall eventually. Even when the two positive DEP forces were equal, the equilibrium position is not a stable point. Any shift of the particle from the equilibrium point will terminate this balance and it becomes attracted to one of the two sides of the channel.

If there is only spatial non-uniformity of the electrical field without a change in the phase of the electrical field, the DEP force can be simplified as:

$$\vec{F}_{\mathrm{dep}}=2\pi\varepsilon_{\mathrm{m}}a^{3}\mathrm{Re}\,[K\,(\omega)]\,\nabla E^{2} \tag{1}$$

Where a is the radius of the particle, ε_{m} is the medium's permittivity, and E is the electrical field. K is the Clausius Mossotti factor.

The electric field distribution and therefore the DEP forces in the channel can be simulated with a commercial FEM program CFD-ACE (ESI group). Both the voltage amplitude and the frequency can be tuned to change the equilibrium points of the particles in the channel. When different voltages at the same frequency are applied to two sets of electrodes, the equilibrium points can be adjusted towards the electrodes with lower voltage. The equilibrium point is determined by:

$$\nabla E_1^2|_y = \nabla E_2^2|_y \tag{2}$$

where E_1 and E_2 are the electrical fields generated by the two sets of electrodes respectively. Fig. 3 shows the simulated results for the gradient of the electric field squared along the y (the width of the channel) direction, which is proportional to coupled DEP forces. Each curve corresponds to different positions along the width direction. When only electrodes on one side are turned on, the equilibrium line is at the opposite side of the channel as in Fig. 3b. When the same voltage is applied to both sets of electrodes as in Fig. 3d, the equilibrium line is centred at $y = 50$ μm place. When different voltages are applied to the electrodes, the equilibrium line will be tuned towards the lower amplitude set of electrodes, as in Fig. 3c.

Another option to tune the equilibrium points is to have different frequencies applied on the electrodes. In this case, the DEP forces from the top and bottom electrode arrays can be tuned by changing the Clausius Mossotti factor K, which can be adjusted by varying the frequency of the signals applied on the electrode arrays. The equilibrium position is given by:

$$\mathrm{Re}[K\,(\omega_1)]\nabla E_1^2|_y = \mathrm{Re}[K\,(\omega_2)]\nabla E_2^2|_y \tag{3}$$

When the voltages are same for both opposing electrode arrays, the electric fields at different places along the y axis are different. With the changing frequencies, the y value that satisfies eqn (3) will be different and will correspond to the equilibrium position of the particles in the channel.

For different types of cells or beads flowing through the channel, the switching can be achieved by tuning the amplitude of the voltages. In this case, even if the objects have different DEP properties, the forces from the left and right electrode arrays applied on each individual type of objects are only determined by the non-uniformity of the electric field, as shown in eqn (2), so that the equilibrium positions are same for different types of

particulates. When different frequencies are applied on the two electrode arrays, the difference in the Clausius Mossotti factors will cause a difference in the equilibrium points for different types of cells/beads. As a result, they will be positioned at different equilibrium points along the width of the channel, which could be used for flow through separation or sorting.[34] This effect is being investigated further in preparation of a future publication.

Flow rate dependence

When the particles are flowing in the channel, they are not only experiencing a DEP force along the width of the channel (*y* axis) direction, but also a DEP force along the *x* direction which is due to the electrical field gradient.

In the centre of the channel, there are some points corresponding to the electrical field minimum which traps the particles when the flow rate is not strong enough to carry them through the channel. The flow rate must be high enough to overcome the DEP force along the *x* axis direction. For a particle with radius *a*, the hydrodynamic force is given by the Stokes' law:

$$\vec{F}_{\mathrm{hd}}=4\pi\eta a\vec{v} \tag{4}$$

where is the relative velocity between the particle and the flow. In order for the flow to generate enough hydrodynamic force to carry the particles, it must overcome the DEP force along the channel *x* direction, therefore the minimum flow rate is given by:

$$v_{\min}=\frac{\varepsilon_{\mathrm{m}}a^2}{2\eta}\mathrm{Re}\,[K]\,\frac{\partial E^2}{\partial x} \tag{5}$$

When the flow rate is less than $v_{\min}$, the beads will be trapped in some virtual cages by the DEP forces. When individual beads coming from the inlet on the left enter the switching region with DEP electrodes, the DEP force along the *x* axis direction hold them from the hydrodynamic force of the flow and trap the beads in a series of virtual traps in the channel. However, when the flow rate is higher than a maximum $v_{\max}$, objects will flow through the DEP zone without enough time for them to be deflected to the equilibrium position. This maximum flow rate can be derived quantitatively by the following approximation.

For the movement in the *y* direction:

$$2\pi\varepsilon_{\mathrm{m}}a^3\mathrm{Re}\,[K]\,\frac{\partial E^2}{\partial y}=4\pi\eta a y' \tag{6}$$

To ensure that all the objects reach the equilibrium positions, the one that is furthest from the equilibrium must be accounted for. The time for objects furthest away from the equilibrium to reach their equilibrium position can be approximately given by:

$$\tau=\frac{w}{y'} \tag{7}$$

where *w* is the width of the channel. The time for objects exposed to the DEP zone is determined by $\tau' = l/v$, *l* is the total length of the switching zone. For effective lateral positioning, $\tau \leqslant \tau'$ so that the flow rate should satisfy:

$$v_{\max} = \frac{2l}{w\eta}\varepsilon_m a^2 \mathrm{Re}[K]\frac{\partial E^2}{\partial y} \quad (8)$$

The ideal flow rate that ensures lateral positioning/switching is $v_{\min} < v < v_{\max}$. When the flow rate is lower than the minimum flow, the DEP force in the flow direction is larger than the hydrodynamic force and the particulates are shunted or trapped by the DEP forces. For flow rates larger than the maximum flow, the particulates pass the DEP electrodes without being deflected enough to reach the equilibrium positions. From the formulation above, the maximum flow rate for lateral positioning is proportional to the ratio of the length of the electrode array to the width of the channel. The larger the number of the electrodes in an array is, the higher the flow rate that can be flowed through the channel to ensure successful switching.

Experimental results

Focusing of microbeads

Polystyrene microbeads (Molecular Probe Inc., OR, USA) were diluted into DI water after sonication. Then the DEP solution was introduced into the channel with a controlled flow rate by a PicoPlus (Harvard Apparatus, MA. USA) syringe pump. The left and right electrode arrays were connected to a dual channel function generator (Tektronix AFG320). With two separate input signals. The trajectory of the particle motion was recorded using a CCD camera (Photron FASTCAM). The microbeads appear to have negative DEP property in the selected medium with different frequencies.

When there was no DEP effect, the beads randomly flow in the channel (Fig. 4a). When 10 V/10 MHz signals were applied to the left and right electrodes, the negative DEP forces from the electrodes push them away from the sidewall. Since the forces from the two sets of electrodes balance each other in the width direction of the channel, the beads are focused in the centre of the channel. Fig. 4b shows the same population of beads as circled in Fig. 4a are focused to the middle of the channel after they pass through the DEP electrode arrays.

Switching of cells

An isotonic medium (8.5% sucrose (w/v), 0.3% dextrose (w/v) dissolved in doubly deionized water, conductivity adjusted to 0.1 mS cm^{-1} with 1640 RPMI using ThermoOrion conductivity/pH meter) is used as the DEP buffer medium for cells. Mouse neural stem cells were isolated from E12.5 cortical regions and cultured as neurospheres, as previously described.[35] Cells were dissociated by trituration and placed in the isotonic media for DEP at physiological pH, with a density of 10^4 cells ml^{-1}. The channel was first flushed with trypsin for about 2 minutes to prevent the cell adhesion to the channel walls. It is found that the mouse neural stem cells experience a negative DEP force at frequencies lower than 100 kHz and a positive DEP force at higher frequencies. When electrode arrays on one side of the channel were activated with 10 V/10 kHz, the negative DEP force will push the cells to the opposite side of the channel and they will flow into the corresponding outlet, as shown in Fig. 4c and 4d. Since cell switching operates in the negative DEP regime, the lower frequency ranges (<50 kHz) result in compromised cell viabilities in our observations. In order to avoid cell death due to the electric fields, longer switching regions must be employed to enable switching at lower voltage amplitudes.

Multiplex switching

Multiple outlet channels were designed and fabricated to verify the switching. Since the K factor for the microbeads is almost constant in the solution with respect to frequency,

switching by voltage amplitude difference applied on the two sets of electrodes was employed. When the beads are flowing through the DEP switching zone in the channel, their trajectory can be adjusted to be away from their original stream line.

Unlike most of the DEP sorting configurations, which require focusing of the objects and can only switch between two outlets, this switching scheme can be extended to channels with multiple outlets ($n > 3$). Normal flow switching requires stopping flow to the outlet branches and energy input is needed to overcome the flow momentum. DEP switching instead redirects the objects from the laminar stream line and aligns them towards different outlets. The same frequency (10 MHz) but different amplitudes of voltage are applied to the left and right electrode arrays to generate differential DEP forces. Since the beads are experiencing negative DEP forces, the force from the electrodes with higher voltage amplitude will be stronger to deflect the beads towards the lower amplitude electrodes. The equilibrium points for the beads can be anywhere along the width of the channel direction, and enables expansion of the switching to more than two outlets. Fig. 5 shows the switching of microbeads in a five–outlet channel design. The beads are switched to five different outlet channels sequentially.

Conclusions

A dielectrophoresis switching device with vertical microelectrodes in the sidewall of microchannels has been designed, fabricated, and tested. This device generates lateral forces along the width direction of the channel which is balanced by a second DEP force from another set of electrodes on the opposing channel wall. Under the effect of coupled DEP forces, objects of interest can be positioned at any equilibrium position in the microchannel. When cells or objects flow through the microchannel, the equilibrium positions can be tuned to have them switched to different outlets. Switching of biological cells and microbeads to up to five outlets has been demonstrated by our current design. Only two electrical signal inputs are required to switch the particles to multiple channels. This type of switching can be the backend of other cell or bead separation techniques, such as field flow fractionation or fluorescently activated cell sorting (FACS) to switch multiple types of objects at different times in a continuous manner. The switching method presented can enable high throughput multiplexed microfluidic flow cytometry in the future.

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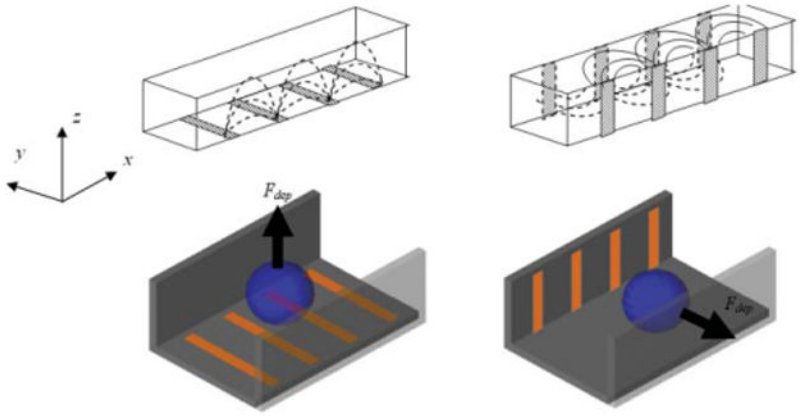

Fig. 1.

(a) Schematic views of planar electrodes at the bottom of the microchannel and the electrical field lines. The DEP force on the particle is along the *z* axis direction. (b) Non uniform electrical field distribution for electrodes at the sidewall of the channel, giving a DEP force along the lateral direction of the channel.

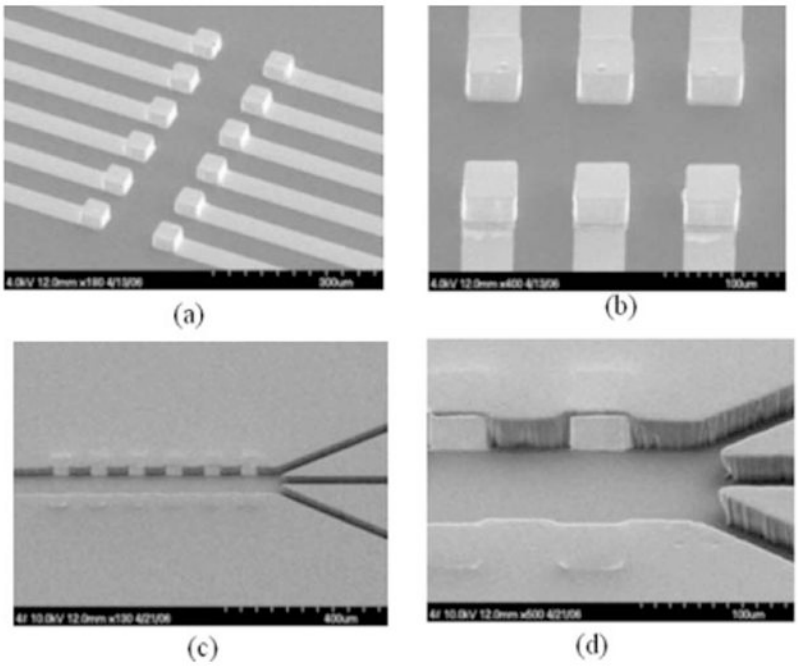

Fig. 2.

(a) SEM pictures of the sidewall vertical interdigitated electrodes before coating the channel layer. (b) Close up view of the electrodes. (c) Final construct of the vertical electrode arrays in the sidewall of the microchannel. (d) Close up view of the electrodes and channel sidewall at the junction.[33]

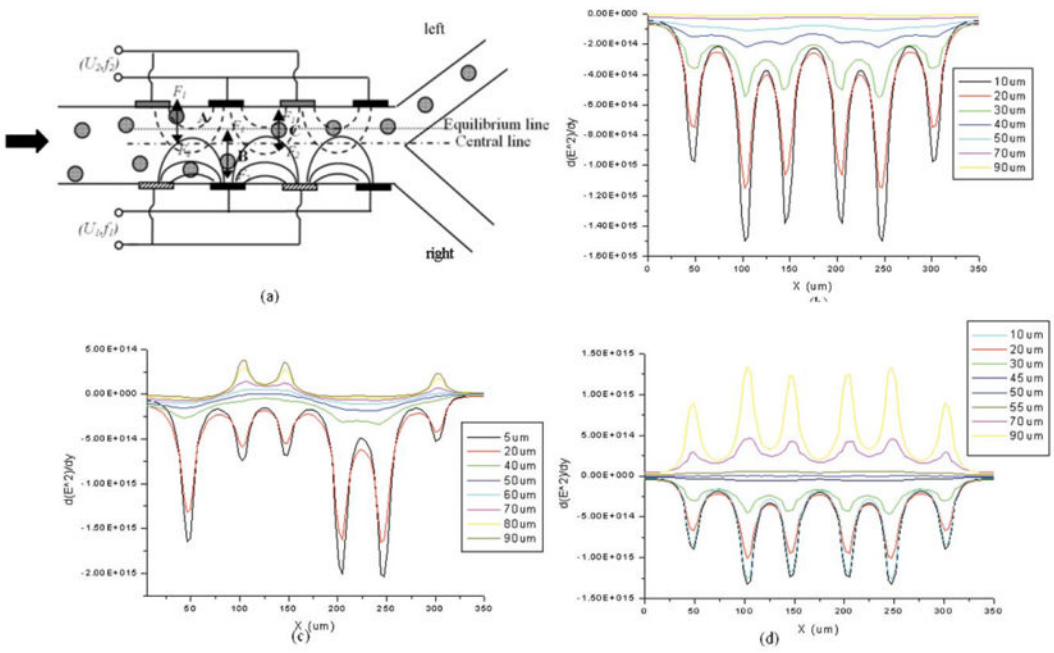

Fig. 3.

(a) Principle of the DEP switching design. Particles are flowing through the channel by the free flow in the DEP switching zone, force balance plots are shown for the particles with negative DEP properties in the switching region. *U*: magnitude of the voltage, *f*: the frequency. (b) 10 V on right electrodes, 0 V on left electrodes, the minimum DEP points are adjacent to the left electrodes, where $y = 100\ \mu m$. (c) Right 10 V, left 3 V, the balancing points are tuned to be near $y = 60\ \mu m$ along the y axis. (d) 10 V voltage is applied on both of the left and right electrodes. The balancing points are the centre of the channel at $y = 50\ \mu m$ place along the channel width. Each of the lines shows the relative DEP forces along the width of the microchannel direction. The lines where $y = 0\ \mu m$ and $y = 100\ \mu m$ correspond to the right and left sidewalls with electrodes, respectively.

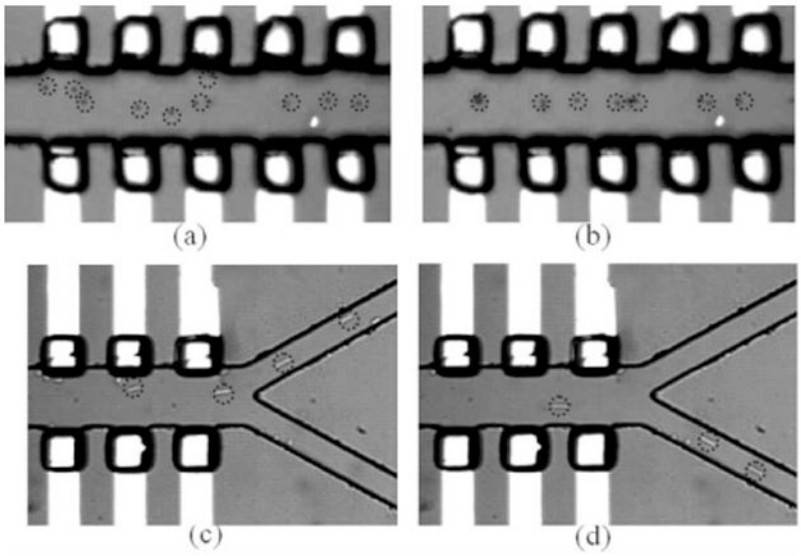

Fig. 4.

(a) Microbeads randomly flow in the channel without DEP effect, (b) negative DEP forces from both the top and bottom electrodes focus the beads in the centre line of the channel. (c) Mouse neural stem cells flowing in the channel are switched to the right channel branch with left DEP electrode arrays on. (d) Cells are switched to the left channel branch with right DEP electrode arrays on. The cells are elongated because of the flow rate is too fast for the camera to capture.

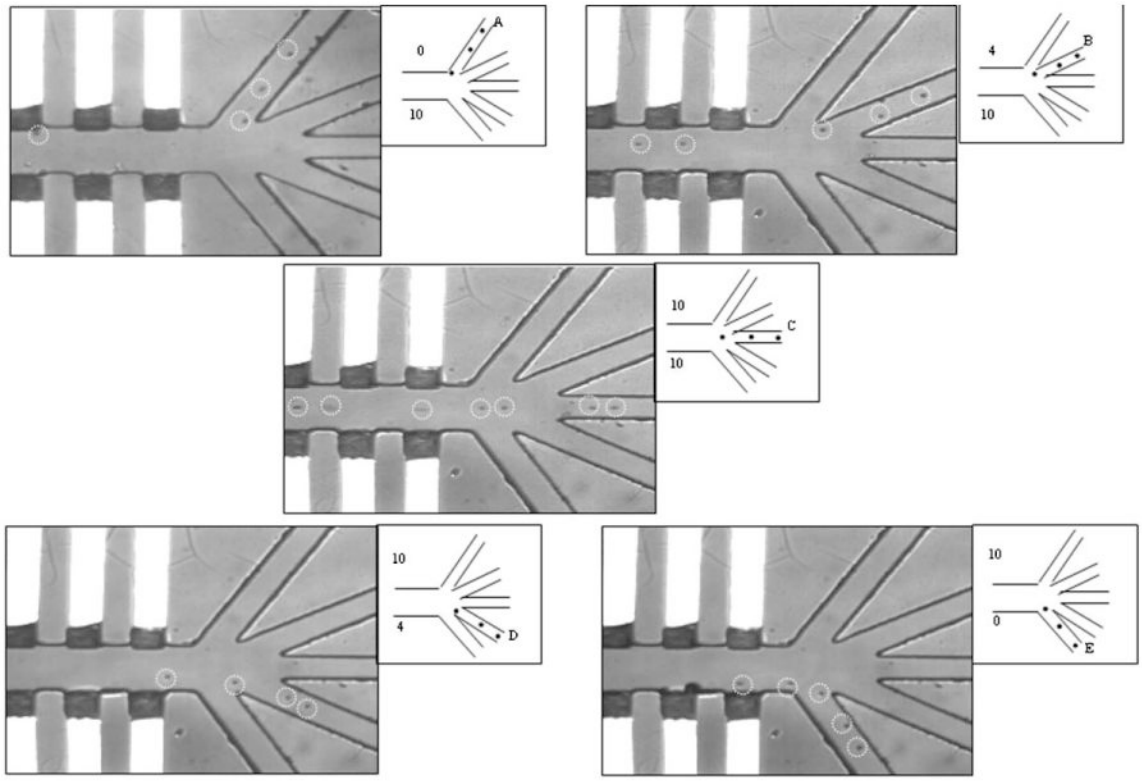

Fig.5.

DEP switching of the microbeads to five different outlets with different voltages applied on the electrode arrays. The frequency applied on all the electrodes is 10 MHz and the voltage applied on the left and right electrodes are A(0,10), B(4,10), C(10,10), D(10,4), E(10,0). The microbeads are circled to clearly show the switching.