

Dielectrophoresis Field-Flow Fractionation for Continuous-Flow Separation of Particles and Cells in Microfluidic Devices

Nuttawut Lewpiriyawong and Chun Yang

Abstract The ability of separating particles and cells in continuous flow is always desirable as it allows faster biomedical diagnosis. This chapter presents a review on the development of dielectrophoresis field-flow fractionation (DEP-FFF) technologies for continuous-flow separation of particles and cells in microfluidic devices. The review is mainly focused on the publications between 2005 and 2012. During separation processes, DEP-FFF transports particles and cells with hydrodynamic liquid flow in microchannels and fractionates particles and cells using dielectrophoresis (DEP) force generated perpendicular to the fluid flow direction. In the literature, numerous strategies have been developed to advance the way of generating nonuniform electric field which is required to produce DEP force, and four main strategies are grouped here, including (1) the use of planar electrodes, (2) the use of three-dimensional and sidewall electrodes, (3) the use of insulating topographical structures, and (4) the use of combined planar electrodes and insulating structures. DEP-FFF microfluidic devices can serve as a label-free, non-invasive and most efficient tool for manipulating and separating various biosamples such as particles, cells and DNA based on their polarizabilities in nonuniform electric field.

1 Introduction

Separation of target cells is of broad importance in a large number of applications. In biomedical diagnoses, separation of platelets, white or red blood cells from blood is a common practice in modern medical laboratories and hospitals [1]. To enhance the effectiveness of artificial fertilization, healthy sperm cells must be separated from the unhealthy ones [2]. In environmental monitoring, diagnosis of

N. Lewpiriyawong · C. Yang (✉)
School of Mechanical and Aerospace Engineering, Nanyang Technological University,
Nanyang Avenue 639798, Singapore
e-mail: mcyang@ntu.edu.sg

water quality involves identification, separation and counting of live bacterial cells [3]. For these clinical and industrial applications, centrifugation, chromatography, flow cytometry and fluorescence/magnetic-activated cell sorters (FACS/MACS) are the leading separation and detection techniques [4]. However, these existing macro-scale techniques have the following drawbacks: (1) non-continuous separation process which leads to longer analysis time, (2) requirement of a large volume and modification of samples, and (3) the need for highly trained personnel to operate bulky and costly instruments [4–8].

Lab-on-a-chip (LOC) or micro-total analysis system (μ TAS) is a fast-emerging interdisciplinary technology that integrates laboratory based biomedical and chemical processes onto a single chip with typical size of only millimeters to centimeters [9]. In LOC platforms, microfluidics, consisting of capillary network structures, exploits the characteristics of laminar flow to provide precise control of fluid in nanolitres and to manipulate thousands of cells through either pressure gradient or electrokinetic forces. Thus, microfluidic devices have numerous advantages such as fast analysis, requirement of small amount of sample volume, portability, disposability, etc. These benefits are favorable to the development of future inexpensive clinical and point-of-care diagnostic devices. It is therefore unsurprising that microfluidics has been utilized in manipulation, separation and identification of cells, proteins and DNA, drug delivery, and rapid diagnosis of complex diseases [10].

One of the most important clinical and industrial demands in biomedical diagnosis is the separation of target biosamples in a continuous manner. Field-Flow Fractionation (FFF) was engineered using the principle similar to chromatography with the aim to achieve continuous-flow separation of subpopulations without stationary phase [11]. FFF transports the samples by the means of pressure-driven flow in microchannels and fractionates them using an externally applied field perpendicular to the flow direction. FFF subtechniques are mainly classified by the externally applied field: sedimentation (SdFFF), thermal (ThFFF), flow (FIFFF), magnetic (MgFFF), electrical (EIFFF) and dielectrophoresis (DEP-FFF) [12]. Among these subtechniques, DEP-FFF is the most promising technique that has exploited both microfabrication and microfluidic technologies for continuous-flow separation of various samples such as human leukemia (HL-60) cells [13], breast cancer cells [14, 15], bacteria [16], yeast cells [17] and DNA [18].

DEP is an electrokinetic phenomenon describing the motion of a dielectric particle or cell in nonuniform electric field due to polarization effects [19]. A DEP force is resulted from the nonuniform polarization effects generated by the interaction of induced dipoles with the externally applied AC or DC electric field gradients, thus allowing separation of particles and cells based on their different polarizabilities to their suspending medium [20]. Using this concept, the DEP technique unlike FACS and MACS does not need any pre-treatment to samples such that the sample properties are maintained for further downstream processes. This attractive advantage makes DEP a non-invasive and simple yet effective tool which is uniquely featured for continuous-flow separation of target cells from other interfering particulates in microfluidic devices.

This review article presents advancement of the development of state-of-the-art DEP-FFF technologies for continuous-flow separation of biosamples in micro-fluidic devices. The publications between 2005 and 2012 are the main focus of this review. The article classifies DEP devices into four groups based on the strategies of generating nonuniform electric field for inducing DEP force. These four strategies are: (1) the use of planar (or thin film) electrodes, (2) the use of three-dimensional (3D) and sidewall electrodes, (3) the use of insulating topographical structures, and (4) the use of combined planar electrodes and insulating structures (known as hybrid DEP system). In the review, the theoretical background of DEP is provided and followed by the separation mechanisms and fabrication techniques of the four DEP groups. Finally, the summary section provides possible future research directions for improving DEP technologies in the field of continuous separation of biosamples.

2 Theoretical Background of Dielectrophoresis

DEP refers to the motion of a polarizable particle (or a cell) in a buffer solution under nonuniform electric field due to polarization effects. The effects occur as a result of the interaction between the unevenly distributed induced dipoles in the particle and the applied nonuniform electric field [20]. As shown in Fig. 1a and b, the interaction between the induced net dipole charges and the nonuniform electric field leads to a nonzero columbic net force (F_{DEP}) on the particle [21, 22]. The DEP force can be determined by using the Maxwell stress tensor formulation [23] or the effective dipole moment method [21]. Both methods have been shown to yield an identical DEP force expression. Furthermore, because the effective dipole moment method is much simpler, it has been widely used in the DEP community [24]. The method points out that the effective dipole moment of a neutral particle

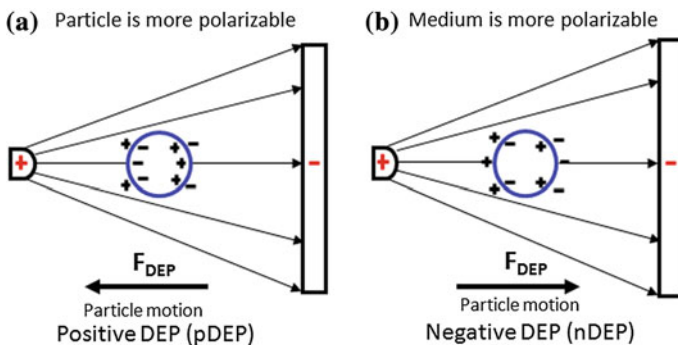


Fig. 1 Illustration of dielectrophoretic (DEP) force. **a** Positive DEP (pDEP) attracting the particle to the high electric field region and **b** Negative DEP (nDEP) repelling the particle away from the high electric field region [22]

polarized by an electric field is defined as the moment of an equivalent free-charge point dipole that is immersed in the same dielectric medium and located at the same point of the particle's center [21]. With the dipole moment method, the time-average DEP force acting on a spherical homogeneous particle of radius a suspended in a dielectric medium with permittivity ε_m is expressed as [21]

$$\langle \vec{F}_{DEP} \rangle = 2\pi\varepsilon_m a^3 \text{Re} \left[f_{CM}(\omega) \right] \nabla \vec{E}_{rms,ac}^2 \quad (1)$$

where ∇ denotes a gradient operation, $\vec{E}_{rms,ac}^2$ is the square of the root-mean-square of local AC electric field and $\text{Re} \left[f_{CM}(\omega) \right]$ is the real part of the Clausius–Mossotti (CM) factor given by

$$f_{CM}(\omega) = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} \quad (2)$$

where ε_p and ε_m are the complex permittivities of the particle and suspending medium, respectively. The complex permittivity is represented by $\varepsilon = \varepsilon' - i\sigma/\omega$ where ε' and σ are respectively the permittivity and electrical conductivity, $i = \sqrt{-1}$, and $\omega = 2\pi f$ is the radian field frequency. Therefore, $f_{CM}(\omega)$ depends on electric field frequency and complex permittivities of the particle and suspending medium. Moreover, $f_{CM}(\omega)$ provides a measure of effective polarization strength, and the value of its real part varies from -0.5 to 1 . When the particle is more polarizable than the suspending medium (i.e., $\text{Re} \left[f_{CM}(\omega) \right] > 0$), positive DEP (pDEP) is generated to attract the particle to the high electric field region as illustrated in Fig. 1a. When the particle is less polarizable than the suspending medium (i.e., $\text{Re} \left[f_{CM}(\omega) \right] < 0$), negative DEP (nDEP) is generated to repel the particle away from the high electric field region as illustrated in Fig. 1b. At high frequency (e.g., $\omega \rightarrow \infty$), the CM factor reduces to $\text{Re} \left[f_{CM}(\omega) \right] = (\varepsilon_p - \varepsilon_m) / (\varepsilon_p + 2\varepsilon_m)$ which is only dependent on the permittivities of the particle and suspending medium. On the other hand, at low frequency (e.g., $\omega \rightarrow 0$), $\text{Re} \left[f_{CM}(\omega) \right] = (\sigma_p - \sigma_m) / (\sigma_p + 2\sigma_m)$ which is only dependent on the electrical conductivities of the particle and suspending medium where the particle conductivity σ_p is the sum of its bulk conductivity ($\sigma_{p,bulk}$) and its surface conductivity ($2Ks/a$). For insulating latex particles ranging from micron to submicron in diameter, $\sigma_{p,bulk}$ is typically negligible compared to the conductivity of suspending medium σ_m , and Ks is the surface conductance ranging from 0.2 to 2.1 nS [25, 26]. In particular, for an applied DC electric field, the time-average DC-DEP force expression is the same as that presented in Eq. 1 as $\vec{E}_{rms} = \vec{E}_{dc}$.

An AC electric field can be biased with a DC electric field. Under an assumption that the AC electric field frequency is low (e.g., ≤ 10 kHz), then $f_{CM,ac} \approx f_{CM,dc}$, and thus the time-average DEP force can be formulated as [27]

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re} [f_{CM}(\omega)] \nabla \left(\vec{E}_{dc}^2(\vec{r}) + \vec{E}_{rms,ac}^2(\vec{r}) \right) \quad (3)$$

If there are spatial changes in the phase (ϕ_x, ϕ_y, ϕ_z) of an AC electric field with their respective magnitudes (E_{x0}, E_{y0}, E_{z0}), the generalized DEP force can be expressed as [28],

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \left\{ \begin{array}{l} \text{Re} [f_{CM}(\omega)] \nabla \vec{E}_{rms,ac}^2 \\ + \text{Im} [f_{CM}(\omega)] \left(E_{x0}^2 \nabla \phi_x + E_{y0}^2 \nabla \phi_y + E_{z0}^2 \nabla \phi_z \right) \end{array} \right\} \quad (4)$$

where $\text{Im} [f_{CM}(\omega)]$ is the imaginary part (or the out-of-phase component) of the CM factor and it indicates whether the induced dipole moment leads ($\text{Im} [f_{CM}(\omega)] > 0$) or lags ($\text{Im} [f_{CM}(\omega)] < 0$) the applied electric field. When the induced dipole moment leads the electric field, the particle moves in the direction opposing to that of the traveling field or vice versa [28].

3 DEP Devices

3.1 Planar (or Thin-Film) Electrodes

With the advancement of microfabrication technologies, planar (or thin-film) electrodes fabricated by using the sputtering and lift-off processes have been extensively integrated in DEP devices [29]. Due to the miniaturized dimensions of microfabricated electrodes, an electric field gradient and the resulting DEP force are significantly enhanced for facilitating manipulations of micro-sized particles and biological cells.

3.1.1 Dielectrophoresis Field-Flow Fractionation (DEP-FFF)

DEP-FFF being one of the FFF subtechniques utilizes DEP force to separate particles and cells in a continuous flow. DEP force is generated due to the presence of nonuniform electric field which is conventionally induced by planar interdigitated electrodes deposited on the chamber floor of a microfluidic device as schematically shown in Fig. 2a. When particles are less polarizable than their suspending medium, they experience repulsive nDEP force that pushes the particles to levitate above the DEP electrodes. On the other hand, particles experiencing pDEP force can be attracted to (or trapped at) the DEP electrodes. Two DEP-FFF operation modes can be classified based on how the particles interact with DEP force. The first operation mode can be used when two different particle groups experience nDEP of difference in magnitude. Figure 2a shows that particles in the DEP-FFF channel can attain different equilibrium heights based on the

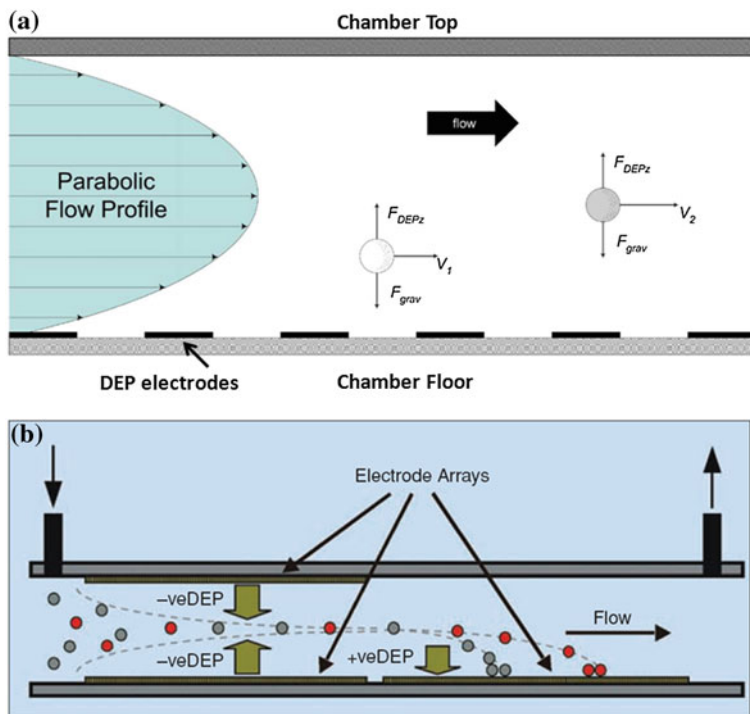


Fig. 2 Separation principle of DEP-FFF. **a** A conventional DEP-FFF device with interdigitated electrodes levitating particles by nDEP force [31]. **b** Combined pre-focusing and fractionation functions using both nDEP and pDEP [35]

balance between nDEP and gravitational forces. The particles at the higher equilibrium height having a higher velocity in the pressure-driven flow will be separated prior to the others [30, 31]. Such DEP-FFF principle has been successfully used for the separation of polystyrene latex beads [32] and human leukemia (HL-60) cells from peripheral blood mononuclear cells [13] as well as for the detection of malaria cells.

The second mode can separate particles undergoing different polarities of DEP forces, namely pDEP and nDEP. The particles experiencing nDEP are levitated above the electrodes while the others undergoing pDEP are attracted to the electrodes. Such the device utilizing the nDEP and pDEP operation mode has been widely exploited for separating various biological samples such as viable from non-viable yeast cells [17, 33], erythrocytes from latex beads [34], different types of white blood cells [35] and cultured human breast cancer M4DA-435 cells from normal blood cells [14, 15]. Continuous DEP cell separation was reported by using a series of short-time flushing; however, the system still needed certain relaxation time for the cells to reach equilibrium after each on-off flushing [36]. In addition to these typical interdigitated electrodes, other electrode designs including

multilayered grid electrode array [37], ring electrodes [38] and combined quadrupole and microwell electrodes [39] were adopted to achieve trapping of microparticles and single cells in DEP-FFF microfluidic devices.

Furthermore, the DEP-FFF technique was advanced by combining pre-focusing and fractionation functions to specifically trap cells into distinct locations [35]. In Fig. 2b, the first section consisting of two interdigitated electrode arrays at the top and bottom of the channel can focus particles into the centre zone of the channel by nDEP. The second section with an electrode array differentially attracts the focused particles by pDEP into distinct bands. Other than particle separation, DEP-FFF devices have also been used for cell lysis and trapping [40], immobilization of probe beads for affinity assay [41], cell genetic detection [42] and analyses of cellular properties such as dielectric, density and deformability [43].

Despite these successes, the separation efficiency of DEP-FFF devices may be compromised due to the following issues. The first issue is related to the overlapping of sample zones which is mainly caused by similar displacement velocity and dispersion of individual subpopulations during separation [11]. However, this issue can be resolved by optimizing the chamber dimensions, applied electric field strength and flow rate. The second issue concerns the exponential decay of electric field strength and thus DEP force at a further distance from the surface of planar electrodes [32, 44]. As a result, the manipulation of cells far away from the electrode surfaces becomes ineffective.

3.1.2 Lateral DEP-FFF

Because of the rapid decay of electric field strength from the surface of conventional DEP-FFF devices, researchers have developed DEP-FFF devices to extend the DEP force across the entire channel height. Dürr et al. [45] devised a microfluidic device with interdigitated electrode pairs which are embedded on the bottom and top of the channel while being inclined with the flow direction. Such electrode configuration is also known as DEP deflector. As shown in Fig. 3a, these double-sided electrodes allow electric field to distribute across the channel height, thus mitigating the issue of exponential decay of electric field and resulting in better manipulation of cells. In Fig. 3b, as the electrodes are inclined at an angle relative to the flow direction, the DEP force acting on particles of different sizes induces distinct lateral displacement of each subpopulation, leading to separation into different outlets simultaneously [46]. The first deflector only deflects large particles to the collecting outlet whereas smaller particles are discarded at the waste outlet. The second deflector further screens out the particles larger than the target size. As a result, separation of mitochondria (empty circles) from a human lymphoblastoid cell line (solid circles) was successfully demonstrated. Furthermore, Chen et al. [47, 48] utilized these electrode deflectors as DEP barriers which allow smaller particles to penetrate, but slow down (or trap) larger particles, thus achieving particle separation and accumulation by size simultaneously.

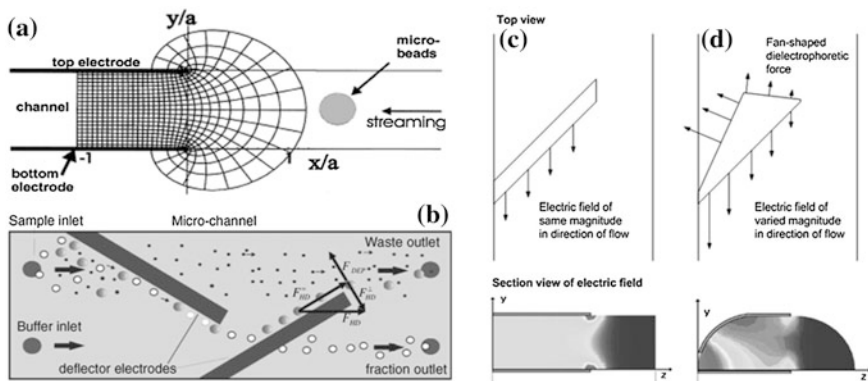


Fig. 3 DEP separation with electrode deflectors. **a** Side view of double-sided electrode deflectors generating electric field across the channel height [45]. **b** Lateral size-dependent separation using two consecutive deflectors [46]. **c** A typical deflector generating electric field of the same magnitude along the flow direction and **d** a 3D fan-shaped electrode DEP system generating electric field of varying magnitude along the flow direction in a half-circular channel [54]

Besides straight DEP electrodes, other electrode designs including trapezoidal, triangular and curved shapes were also widely employed for separation of microparticles and cancer cells [49–51] and focusing of nanoparticles [52]. Ling et al. [53] recently presented a new device with an isosceles triangular ITO electrode array to continuously separate 10 and 15 μm particles with a speed of 1 mm/s. Instead of the lift-off technique, surface micromachining with integrated fluid technology was used to fabricate DEP gating for focusing and separating *Penicillium brevicompactum* (PBC), human T-cells and *E. coli* [16]. However, disregarding the shape of these typical electrode deflectors, electric fields of the same magnitude can only be created along the flow direction as illustrated in Fig. 3c. Park et al. [54] developed a 3D asymmetric fan-shaped electrode system that can produce electric fields of continuously varying magnitudes along the flow direction due to the varying width and depth between deflectors in a half-circular channel (Fig. 3d). Hence, variation in DEP force was produced to increase the sensitivity of lateral sorting of cells. This microfluidic device showed the purities of $81.5 \pm 7.6\%$ for mouse P19 embryonic carcinoma and of $94.1 \pm 4.3\%$ for red blood cells while the typical deflector system could only provide the purities of $79.4 \pm 6.8\%$ for mouse P19 embryonic carcinoma and of $91.3 \pm 3.7\%$ for red blood cells.

It is noted that although the double-sided electrodes permit electric field to distribute along the channel height, the electric field strength is not always uniform along the height and it could vary substantially when there is a need to increase channel height for higher throughput. Therefore, DEP force magnitudes along the channel height are no longer uniform as desired. Interestingly, Liao et al. [55] recently utilized this concept to precisely separate 2, 3, 4 and 6 μm polystyrene particles by size. As illustrated in Fig. 4, because of the presence of the DEP force

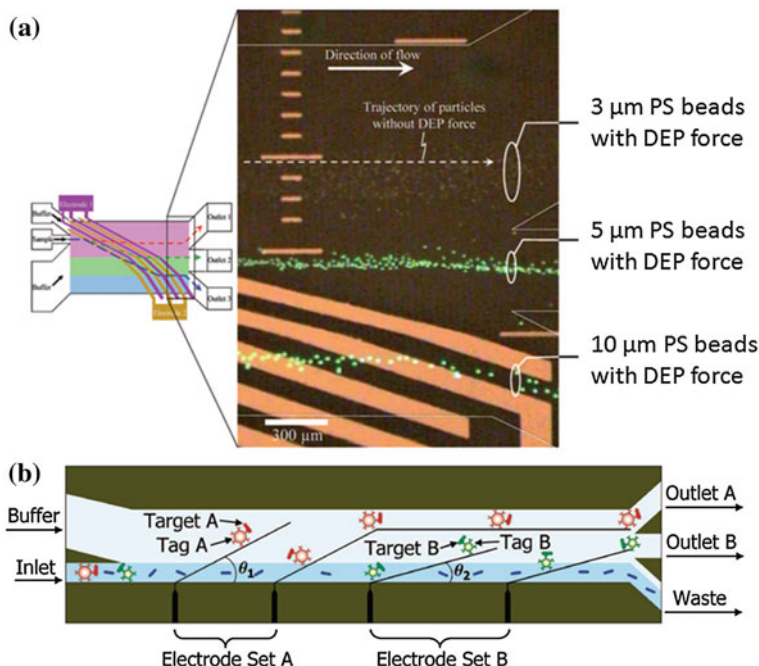


Fig. 5 DEP separation of multiple samples with inclined planar electrodes. **a** Separation of 3, 5 and 10 μm microparticles using piecewise curved electrodes inclined at 9.5° , 14° and 18.4° relative to the flow direction [57]. **b** Separation of multiple bacterial cells with the aid of distinct synthetic DEP tags in multi-target DEP activated cell sorter (MT-DACS) [58]

tags in multi-target DEP activated cell sorter (MT-DACS) as shown in Fig. 5b. The DEP tag A and tag B are 10 μm non-fluorescent and 5 μm fluorescent polystyrene particles coated with anti-bacteria antibodies. Because the tag A is larger than the tag B, the electrode set A inclined with a grater angle ($\theta_1 = 10^\circ$) exerts a larger DEP force on the tag A, thus separating the target A with tag A to the outlet A. Simultaneously, the electrodes set B inclined with a smaller angle ($\theta_2 = 8^\circ$) separate the target B with tag B to the outlet B, while allowing the untagged bacteria to separate at the outlet C. Not only for bacteria, DACS systems were also used to separate microparticles and platelets from dilute blood samples [57, 59–62].

3.1.3 DEP with Modulation in Space or in Time

In previous cases, DEP force is generated with neither modulation in space nor in time because fixed AC electric field is continuously applied to all planar electrodes in the device. This section reviews three DEP techniques which modulate DEP force in space or in time, namely, traveling wave DEP, moving DEP and pulsed

DEP. Using such modulated-DEP techniques, continuous separation of samples can be achieved without the need of fluid flow like conventional DEP-FFF.

Traveling-wave DEP (twDEP)

The working principle of twDEP is based on the application of AC electric field with a typical 90° phase shift to adjacent planar electrodes so as to modulate twDEP force in space as shown in Fig. 6a [63, 64]. According to Eq. (4), the twDEP force is dependent on the DEP force associated with both $\text{Re}[f_{CM}(\omega)]$ for nDEP levitation (or pDEP attraction) and $\text{Im}[f_{CM}(\omega)]$ for translational motion. Depending on the applied field frequency, particles could be transported in the same or opposite direction to that of the traveling wave propagation direction. The translational twDEP velocity of a particle is proportional to the square of the electric field strength and it determines the speed in separation process [65].

Similar to DEP-FFF, there are two twDEP operation modes. The first mode is used when two samples experience different types of DEP force (i.e., pDEP and nDEP) while the second mode is operated when two samples undergo the same type of DEP force (e.g., nDEP). The first mode was demonstrated to fractionate non-viable from viable yeast cells at 35 kHz [63, 65]. The non-viable cells were attracted to the electrodes by pDEP while the viable cells were levitated and eluted by twDEP force. Another important twDEP development using the first mode was the detection of malaria from human blood samples by using the spiral electrode configuration as shown in Fig. 6b [66]. Malaria cells (displayed in green at the

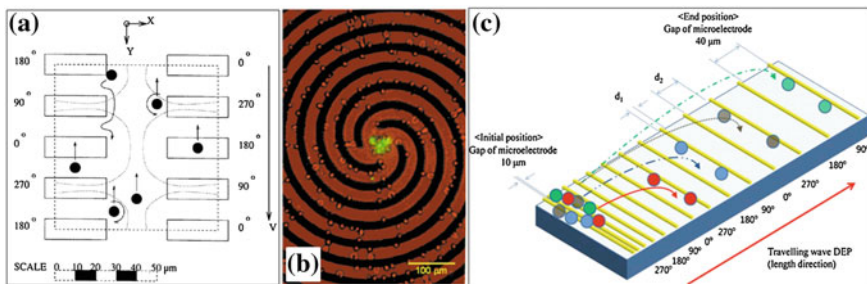


Fig. 6 twDEP electrode configurations. **a** Conventional twDEP electrodes addressed with voltages of 90° phase sequences establishing traveling wave of propagation direction. Depending on the applied field frequency, particles are transported in the same or opposite direction to that of the traveling wave [64]. **b** Spiral twDEP electrodes employed for detecting malaria-infected cells from human blood samples. The infected cells (displayed in green at the centre) are levitated by nDEP and transported towards the electrode centre by twDEP while the normal cells are trapped by pDEP at electrode edges [66]. **c** A very large area ($31 \times 25 \text{ mm}^2$) twDEP electrode array with gradually increasing gaps from 10 to 40 μm produced both nDEP and gradient twDEP forces to balance gravitational force for multiple particle separation by size [67]

centre) exhibited nDEP were levitated and transported towards the centre of the spiral twDEP electrodes while normal cells were trapped by pDEP at electrode edges. This separation platform allows a simple detection of malaria-infected cells in stagnant flow. In the second mode, as both samples undergo the same nDEP force, a difference in the twDEP translational velocity of each sample determines successful separation. Based on this concept, erythrocytes and leukocytes (both undergoing nDEP) were successfully fractionated as erythrocytes move faster than leukocytes in the travelling wave [68]. twDEP-based separation of other samples including latex beads [69] and DNA molecules by size [18] was also demonstrated.

In addition to the above typical twDEP configurations, other twDEP designs have also been reported. As illustrated in Fig. 6c, twDEP electrodes could be positioned with gradually increasing gaps from 10 to 40 μm in a very large area ($31 \times 25 \text{ mm}^2$) [67]. When the gaps are larger, nDEP and gradient twDEP forces, used for balancing gravitational force, become weaker towards the channel end, causing particles to settle at different locations. The device was successfully used to separate 3, 6, 10 and 20 μm particles. Another advanced twDEP technique is the use of twDEP force to balance gravitational force which is produced by inclining the twDEP device at an angle for separation of particles by size [70].

Other than separation in stagnant fluid, twDEP has been implemented for lateral separation of samples in continuous flows. The separation principle is based on two working steps: first, samples are focused to the centre of the separation area, and second, the focused samples are separated laterally by twDEP force. Figure 7a shows a PDMS-based twDEP device with ITO electrodes used for cellular characterization and separation of viable human myelogenous HL-60 cells from the non-viable ones [71]. The device employed hydrodynamic focusing via multiple inlets to the centre. When twDEP with 1 kHz frequency was activated, it was found that the viable cells moved perpendicularly to the flow with a faster speed (4.3 $\mu\text{m/s}$) than the non-viable cells (3 $\mu\text{m/s}$) under a flow rate of 5 ml/min. As a result, the viable cells were collected at the outlet farther away from the centre as compared to the non-viable cells. It was also reported that the use of ITO electrodes is beneficial for efficient tracking and image analysis of cell motion since they are optically transparent and provide much lower background noise as compared to Cr/Au electrodes. Subsequently, a new twDEP device was proposed to implement 3D DEP focusing and 3D twDEP force which can be induced by top and bottom electrode arrays as illustrated in Fig. 7b [72]. The device function was tested to separate red blood cells from *Staphylococcus aureus* bacterial cells with a maximum flow rate of up to 10 $\mu\text{L/min}$ (or linear velocity of $\sim 3 \text{ mm/s}$).

It is worthwhile to note two main limitations of twDEP systems. First, higher electric field strength may speed up the twDEP separation process but it also can impose current-induced Joule heat into the medium and thus can damage the biosamples. Second, with planar electrode structure, twDEP devices still suffer the exponential decay of electric field away from the electrode surfaces, which is similar to the problem encountered in the conventional and lateral DEP-FFF systems.

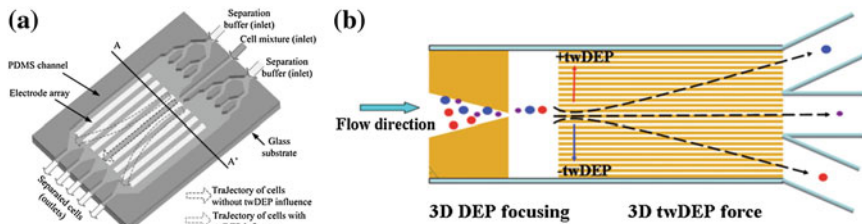
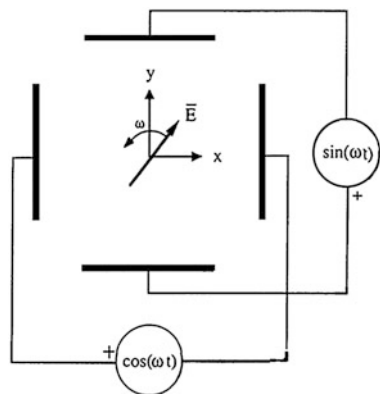


Fig. 7 Focusing-aided twDEP techniques for lateral separation. **a** Hydrodynamic focusing [71] and **b** DEP focusing [72]. twDEP electrodes produce twDEP force perpendicularly to the flow direction causing lateral separation of samples

The twDEP system not only can be used for sample separation but also can be served as a tool for characterizing dielectric properties and frequency-dependent DEP behaviors of particles and cells. After obtaining such information, researches can predict whether cells in a buffer solution experience pDEP or nDEP at a specific frequency, and can be used for performing separation. This twDEP-based characterization tool is named as electrorotation (ROT). Different from the typical twDEP systems, the ROT electrode configuration consists of two pairs of electrodes positioned perpendicularly to each other (Fig. 8). By assigning sine and cosine wave signals to individual pairs, a rotating electric field and thus a rotational torque can be generated on a polarized particle/cell [21]. Since a cosine wave always leads a sine wave by 90° of phase, the resulting electric field rotates anti-clockwise. This rotating wave can be used to levitate the particle/cell above the electrodes by repulsive DEP force and to rotate the particle/cell by the torque in the same or opposite direction to that of the rotating field. The torque exerting on a particle/cell can be expressed as [73, 74]

$$\Gamma(\omega) = -\text{Im}[\vec{p}_{eff}(\omega)] \vec{E}_{ac} \quad (5)$$

Fig. 8 Electrorotation electrode (ROT) configuration consisting of two pairs of perpendicular electrodes assigned with sine and cosine wave signals to induce a rotating electric field and rotational torque on a polarized particle/cell [21]



where $\vec{p}_{eff}(\omega) = 4\pi\epsilon_m a^3 f_{CM}(\omega) \vec{E}_{ac}(\vec{r}, t)$ is the complex effective dipole moment of a dielectric particle/cell. A theoretical study of detailed electrokinetic behaviors of a particle in traveling waves can be found elsewhere [64]. ROT has been widely utilized to characterize dielectric properties (including membrane capacitance, membrane conductance, surface conductance and zeta potential) of latex spheres [26], human leukocyte subpopulations [75], cell membrane [76], and breast cancer cell lines [77]. Details of the apparatus and procedures can be found elsewhere [78]. The current theory usually extracts cell dielectric properties from the measurement of crossover frequencies, indicating the transition frequencies between pDEP and nDEP, or the measurement of peak frequencies, providing the maximum electrorotation rate [79, 80]. However, since the expression of both crossover and peak frequency are nonlinear and cannot be solved analytically, the usual practice is to employ the low frequency (DC) approximations of Schwan [81] which require the measurements to be carried out in a medium with conductivity less than 10 $\mu\text{S}/\text{cm}$. Recently, Lei et al. [80] greatly modified the ROT theory by applying various levels of approximations through scaling analysis, and they gave the full expressions of equivalent cell permittivity and conductivity. Their study showed successful extraction of both membrane and interior properties of cells in suspending media with any conductivity from the peak or crossover frequency measurements. In addition to the DEP characterization of cells, Fuhr et al. [82] demonstrated the separation of plant protoplasts using a ROT system in which cells having distinct membrane capacitances were observed to rotate in different directions and then were separated.

Moving DEP

Moving DEP (mDEP) makes use of the real part of the induced dipole moment in order to simultaneously transport and fractionate particle and cell samples along a microfluidic channel without the need of fluid flow [83]. In operation, either a single electrode or an array of electrodes is sequentially energized at a time, enabling mDEP force to be created or modulated in time. As shown in Fig. 9, the dotted circle represents a cell at the initial time ($t = 0$) prior to energizing the electrode while the patched and solid circles denote the cells after experiencing pDEP and nDEP, respectively. The pDEP cell is attracted to the high electric field region near the energized electrode and the nDEP cell is repelled away from the energized electrode. By energizing subsequent electrodes from $t = T$ to $2T$, the nDEP cell is continuously repelled to the right while retaining the pDEP cell at the first electrode. At this stage, the fractionation of two subpopulations is noticed with a distance of two electrodes apart. At $t = 3T$, the sample transport begins by moving three energized electrodes to the right, causing separated cells to transport with the moving electric field. mDEP was successfully demonstrated to fractionate viable and non-viable yeast cells. Optimization of the device configuration and studies of cell motion under mDEP, drag, buoyancy and gravitational forces were also conducted by Kua et al. [84, 85]. Clearly, the technique reduces the

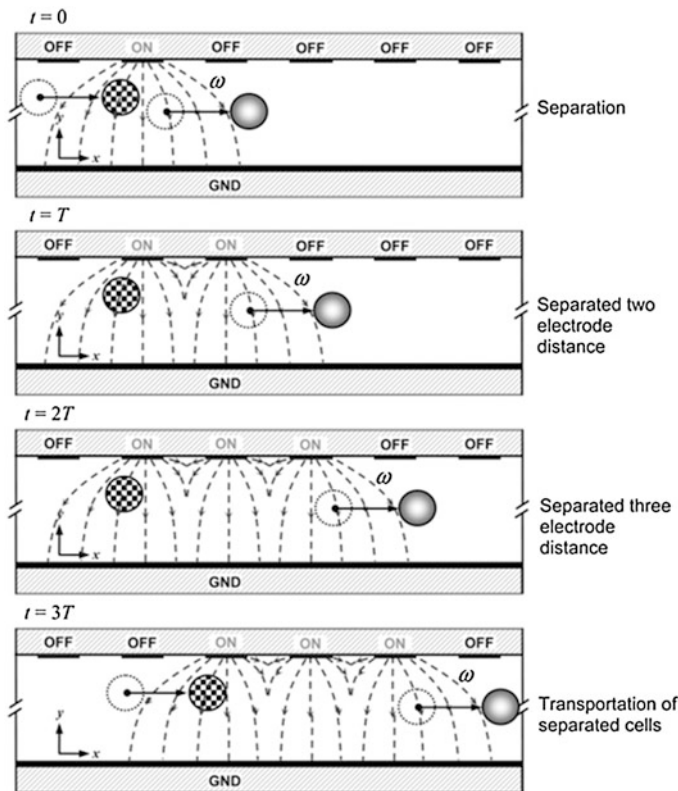


Fig. 9 Moving DEP for fractionation of cells under pDEP (patched circle) and nDEP (solid circle) [83]

complexity of the twDEP system; because the latter requires both the real and imaginary parts of the induced dipole moment. However, mDEP can only fractionate cells in a stationary fluid, and thus suffers low throughput. Furthermore, similar to twDEP, additional improvement in terms of flow-through separation and the decay of electric fields from planar electrode surface is still needed.

Pulsed DEP

Pulsed DEP is the most recent developed technique which employs DEP force with modulation in time for particle separation by size in a DEP-FFF microchannel as depicted in Fig. 10a [86]. In this technique, nDEP force is pulsed in time (or discontinuous) while the frequency of pulsing can be controlled. The suitable pulsing frequency allows the system to reverse the order of separation (That is, eluting the larger particles prior to the smaller ones, and also extracting intermediate-size particles from a heterogeneous sample in a single run).

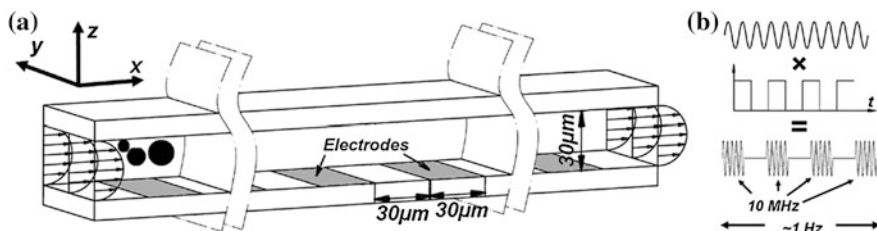


Fig. 10 **a** Schematic of a pulsed-DEP device employing a discontinuous (in time) nDEP force for size-dependent separation of particles [86]. **b** The pulsed-DEP waveform is obtained by time-multiplexing a sine wave with a square wave

The pulsed-DEP waveform is formed by time-multiplexing a sine wave with a square wave as illustrated in Fig. 10b. The analysis of pulsing frequency versus applied voltage provides a recommended operating condition for retaining or releasing any particle size (3, 5 and 10 μm polystyrene beads). The technique was also utilized in combination with a silicon nanotweezer to stretch and trap a single DNA molecule with 50 ms pulses of an 1 MHz AC voltage [87]. The use of pulsed DEP is more advantageous than the conventional AC DEP because when an electric field is pulsing, the electric field-induced flow is lessened, giving rise to the DNA trapping in a more stable environment, and continuous trapping of DNA molecules at the tip of the nanotweezer can be avoided.

3.1.4 Optically-Induced Dielectrophoresis

While many studies focused on DEP techniques with metallic planar electrodes, a new optically-induced DEP technique was developed [88]. As shown in Fig. 11, this technique mainly relies on virtual DEP electrodes made from a photosensitive surface sandwiched by indium tin oxide (ITO)-coated or conductive glass substrates was integrated for the first time. When the photosensitive areas are projected with light, the impedance of projected areas is reduced, thus yielding virtual electrodes for DEP manipulation. The projection of optical images (e.g., circular or parallel electrode patterns) on the photosensitive areas is achieved by combining a light source from a light-emitting diode and a digital micromirror display (DMD) through a 10× objective lens. The photosensitive surface consists of three thin layers: a 50 nm n + hydrogenated amorphous silicon (a-Si:H) layer, an 1 μm undoped a-Si:H layer, and a 20 nm silicon nitride layer. An AC electric field is imposed on both top and bottom ITO-coated electrodes. The intensity of light sufficient to activate virtual electrodes is found to be 100,000 times lower than that used in the laser-based technique such as optical tweezers [89, 90]. Thus, the technique clearly becomes a benevolent tool for cellular applications. This technique is named as optoelectronic tweezers (OET). Because of the flexibility of the OET platform in defining the light-induced electrode patterns, many research groups have proposed a variety of OET systems that can produce dynamic patterns for discriminating particles by size [91], switchable patterns for particle sorting

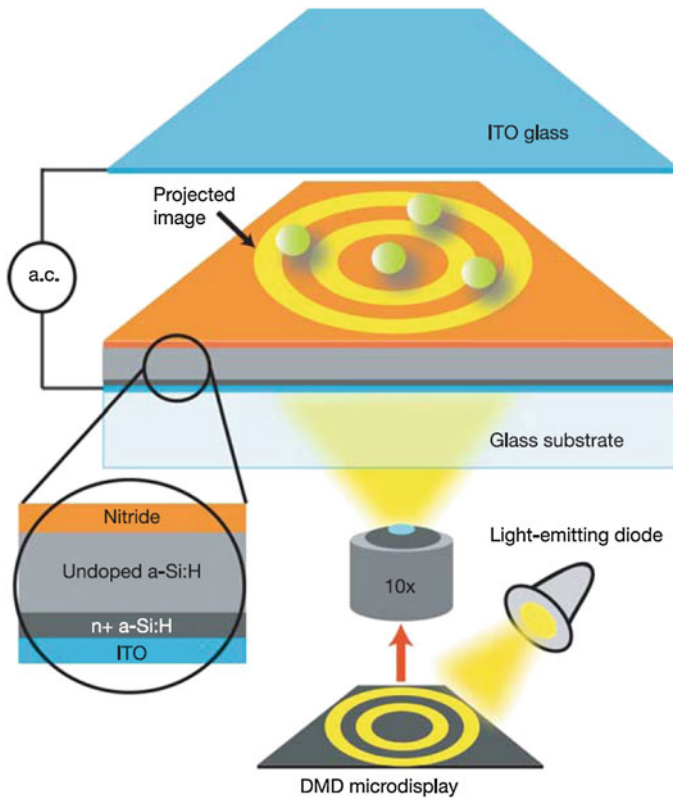


Fig. 11 An optoelectronic tweezer system for DEP manipulation of particles and cells via optically-induced photosensitive electrodes [88]

[92], gray-scale patterns for cell sorting and separation [93, 94], and configurable approaches for cell patterning [95]. Besides lasers as light source, liquid crystal display (LCD) projector [96], len-less direct image [97], LCD integrated with condenser lens [98] were also integrated in the OET systems. In the literature, this OET technology has been continually advanced and employed to manipulate various biosamples such as human B cells (lymphocytes) [88], DNA [99] and CNTs [100], while its applications can be found not only in massively parallel manipulation of cells but also in high-resolution manipulation of single cells [88].

However, like planar electrodes, the OET technique still suffers from an exponential decay of electric fields from the photosensitive surface. Compared to the use of metallic electrodes, the use of ITO-coated glass substrates may also lead to the need of high voltage supply as the light-intensity-dependent impedance of the photosensitive layer is not very sensitive. Furthermore, for fluorescence-based applications, the use of high intensity of light is not always favorable since the differentiation of cells stained by fluorescent dyes may encounter difficulties due to photobleaching.

3.2 Three-Dimensional (3D) and Sidewall Electrodes

To address the issue of the exponential decay of the electric field from the surface of planar electrodes, tremendous emphasis has placed on creating DEP force in a 3D manner. It is desirable that this 3D DEP force can produce strong lateral separation of samples and more importantly can be present uniformly throughout the microchannel height without compromising the throughput. Then cells at any height can experience DEP force of the same magnitude, thereby allowing more efficient separation. To fabricate 3D electrodes, the main fabrication techniques have been developed, such as heavy doping of silicon, pyrolysis of SU-8 photoresist, electroplating of gold (Au) or titanium (Ti), and manual insertion of etched copper (Cu) electrodes, and integration of conducting silver (Ag)-filled PDMS composite electrodes.

Iliescu et al. [101–104] first employed heavily doped 100 μm -thick silicon (Si) as both 3D electrodes and channel walls. The advanced version of Si-based DEP devices with asymmetric doped silicon electrodes is presented in Fig. 12a [105]. During separation, pDEP cells are attracted to the thin electrodes (1 μm -thick amorphous silicon layer) and nDEP cells are levitated and repelled away from the thin electrodes. This technique benefits cell separation by reducing Joule heating and producing a stronger DEP force nearly two times in the vertical direction as compared to the planar direction [105, 106]. In spite of these advantages, it is difficult for users to obtain a desired channel thickness because the channel height depends on the doped Si wafer thickness which is usually much thicker than typical range of microfluidic height. In the meantime, Park et al. presented carbon electrodes for flow-through DEP separation and filtration of cells. With the carbon microelectromechanical system (C-MEMS) technologies, patterned SU-8

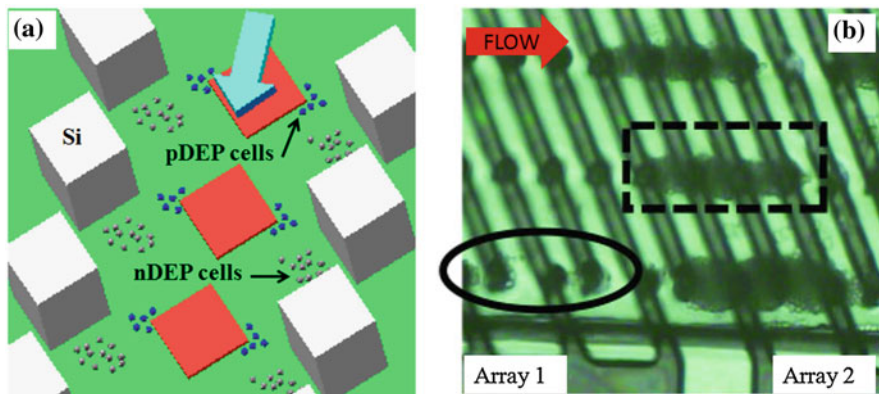


Fig. 12 3D electrodes for continuous particle separation. **a** Heavily doped asymmetric silicon electrodes attracting pDEP cells to the thin electrodes while levitating and repelling nDEP cells away from the thin electrodes [105]. **b** Two arrays of carbon electrodes capturing viable yeast cells at array 1 (ellipse) and non-viable yeast cells at array 2 (rectangle) [108]

photoresist microstructures can be turned to 3D carbon electrodes through pyrolysis usually above 900 °C in an inert environment [107]. As shown in Fig. 12b, dense arrays of high aspect ratio of carbon electrodes as multi-stage filters (electrically independent) were fabricated and demonstrated to fractionate viable from non-viable yeast cells with a flow rate of 10 $\mu\text{L}/\text{min}$ [108]. Numerical analyses of the geometrical effects of carbon electrodes on electric field distributions provided an optimal design [109].

Alternatively, 3D DEP electrodes can be embedded in a sidewall of microchannels to achieve lateral separation of biosamples in continuous flow. Figure 13a depicts a schematic of a DEP device with electroplated Au sidewall electrodes which produce dual-frequency DEP forces causing distinct lateral locations of subpopulations [110]. As this technique controls the channel height by photoresist-coating process, adjustment of the channel height becomes flexible and plausible for high throughput [111]. The device was successfully used to achieve focusing, sorting and separation of beads and biological cells such as mouse neural stem cells and human kidney cells [110–112]. Similarly, sidewall Ti electrodes was also reported for particle manipulation [113]. Using these electroplated sidewall electrodes, the problem of rapid decay of electric fields along the channel height can be improved and the adjustment of channel height is flexible. However, these microfluidic devices are usually made from glass or silicon, and prevention of liquid leakage becomes a problem [111].

With rapid development of soft lithography, polydimethylsiloxane (PDMS) is now widely used as a fabrication material in microfluidic devices, owing to beneficial features: ease of fabrication and bonding, optical transparency, biocompatibility, and low cost [9, 10, 116]. However, it remains difficult to integrate 3D or sidewall metal electrodes for producing AC-DEP in PDMS-based microfluidic devices, because adhesion between metallic electrodes and PDMS is poor [117]. To date, two fabrication techniques have been reported: use of a manual insertion of etched Cu electrodes [114, 118] and fabrication of 3D conducting AgPDMS composite electrodes in a PDMS microchannel [115, 119].

Cetin et al. [118] and Kang et al. [114] reported a manual insertion of etched Cu sheets as sidewall electrodes in PDMS-based devices. The difference of these two studies is that Cetin et al. utilized the sidewall Cu electrodes embedded in a straight channel while Kang et al. [114] introduced a hybrid design using a PDMS hurdle and sidewall Cu electrodes as shown in Fig. 13b. As DEP forces occur only near the corners of the PDMS hurdle, this leads to reduced exposure of electric field exerting on cells and also mitigates negative effects related to Joule heating. Such hybrid-based device was tested to separate microparticles from yeast cells. Even though the device assembly between a glass cover and PDMS channels using oxygen plasma treatment is simple, this device fabrication method may not be effective and convenient because of the following reasons. First, the etching process for patterning Cu electrodes cannot guarantee the exact dimension of electrodes to match with PDMS channels, and thus there exists gaps between the inserted electrodes and PDMS walls. Such gaps can cause fluid leakage and even cell loss during separation (Fig. 13b). Second, similar to the case of heavily doped

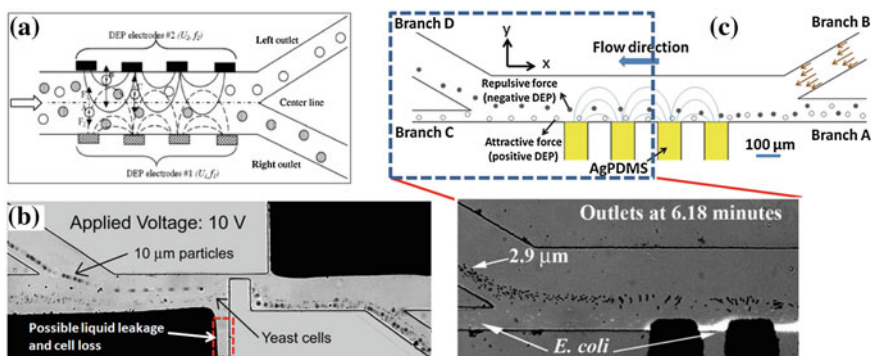


Fig. 13 Sidewall electrodes for continuous-flow separation of cells. **a** Electroplated Au sidewall electrodes with dual-frequency DEP forces resulting in distinct lateral locations of subpopulations [110]. **b** Manually inserted Cu electrodes for separating 10 μm particles by nDEP from yeast cells [114]. **c** Sidewall conducting AgPDMS electrodes for separating *E. coli* from 2.9 μm particles of similar size [115]

silicon, adjustment of channel height is constrained by the availability of Cu sheet thickness. Third, it becomes difficult for batch fabrication when manual insertion of multiple Cu electrodes is necessary to enhance DEP force for higher throughput.

Our group recently developed fabrication technique using the synthesis and the integration of 3D sidewall conducting AgPDMS composite electrodes in PDMS microchannels [115, 119, 120]. As depicted in Fig. 13c, sidewall AgPDMS electrodes are able to attract a tremendous number of *E. coli* and to repel 2.9 μm particles of similar size. The separation efficiency of 97 % was achieved. The devices also demonstrated separation of 5, 10 and 15 μm particles by size [119] and separation of yeast cells from 5 μm particles of similar sizes by polarizability [115]. In addition to the continuous separation, DEP characterization of submicron, microparticles, yeast and bacterial cells were successfully demonstrated [115, 120]. As the AgPDMS composites are synthesized by dispersing 1 μm silver fillers into PDMS gel (85 % w/w), the PDMS cohesion strengthens the bond between the conducting AgPDMS composites and the PDMS channel. Thus, this approach greatly facilitates (1) the integration of the conducting AgPDMS electrodes with PDMS microstructures; and (2) the assembly of the device through bonding between the PDMS channels, with embedded PDMS composites electrodes, and a glass substrate, involving only oxygen plasma treatment. Moreover, since the electrode height is controlled by the photoresist thickness, selection of desirable channel height and integration of multiple sidewall electrodes are flexible and practically possible for batch fabrication. As compared to other 3D or sidewall electrode-based devices, this device with AgPDMS electrodes is deemed the first and important step towards a sole polymer-based microfluidic platform offering multiple functions (e.g., focusing, sorting, separation and characterization of particles and cells).

3.3 Insulator-Based Dielectrophoresis (iDEP)

Instead of utilizing metallic electrodes, Cummings and Singh proposed a new class of DEP devices which consist of an array of electrically insulating posts as shown in Fig. 14a for generating non-uniform electric field [121]. Usually, a DC voltage is imposed via external electrodes placed at the inlet and the outlet. Due to the change of electrical current density around the insulating posts, spatial gradients of electric field can be induced. This insulator-based DEP (or iDEP) offers relatively low costs and is suitable for mass fabrication through the use of injection molding or hot embossing, and suffers less severe electrolysis and fouling problems because of no electrodes fabricated inside channels [122, 123]. In addition, similar to 3D or sidewall electrodes, iDEP can also create 3D DEP forces across the channel height.

The iDEP principle of sample transport is originally based on the DC-driven coupled phenomena, including linear electrophoresis (EP) and electroosmosis (EOF) and nonlinear DEP. EP refers to the motion of an electrically charged sample in a stationary electrolyte solution under an applied electric field while EOF refers to the fluid motion due to the interaction between the applied electric field and electric double layer (EDL) formed next to the charged wall of a microchannel. Generally, EP and EOF occur simultaneously because both the sample and the channel surface are charged. As a result, in iDEP devices, EP and EOF are for transporting samples in a microchannel and DEP force is for separating or trapping the samples.

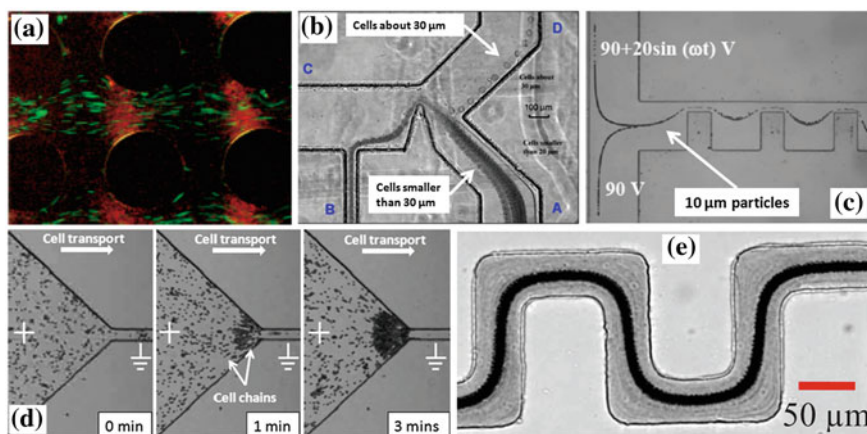


Fig. 14 Use of iDEP for particle and cell manipulations. **a** Glass-etched iDEP chip containing insulating circular posts for DC-based separation of *B. subtilis* (labeled green-Syto[®] 11) and *B. cereus* (labeled red-Syto[®] 17) [121]. **b** DC-induced lateral separation of small and large breast cancer cells by using a triangular PDMS hurdle [124]. Applications of DC-offset AC electric field for: **c** sorting of 10 μm particles using multi-insulating blocks at 2 kHz [125], **d** DEP concentration of yeast cells at 5 kHz [126] and **e** separation of focused yeast cells (black) and unfocused bacterial cells (gray) via DEP focusing in a serpentine channel at 1 kHz [127]

Figure 14a presents the separation of *B. subtilis* (labeled green-Syto[®] 11) and *B. cereus* (labeled red-Syto[®] 17) in a glass-etched iDEP chip containing arrays of insulating circular (quartz) posts [121]. This DC-iDEP principle has been successfully demonstrated for both separating and concentrating submicron particles, DNA, bacteria, protein and blood cells by using various insulator structures such as triangular and sawtooth constrictions and faceted prism microchannels [121, 128–132]. In addition, lateral DC-DEP separation of particles and cells was achieved by using an oil meniscus [133] and PDMS blocks [125, 134]. A serpentine channel was demonstrated for focusing microparticles [135]. Another example is lateral separation of small and large breast cancer cells by using a triangular PDMS hurdle as depicted in Fig. 14b [124]. However, the drawback of the DC-iDEP technique is the requirement of high DC voltages due to the aforementioned coupled linear EP and EOF and nonlinear DEP electrokinetic phenomena [123, 126, 136], and the undesirable consequence leads to Joule heating affecting viability and physiology of the cells [137].

Other than the use of sole DC field, a number of studies have been reported that the use of a DC-offset AC electric field is promising for manipulating particles and cells in iDEP devices. Hawkins et al. [27] employed the DEP force induced by a DC-offset AC electric field to sort and trap particles in a microchannel with 3D constriction along the depth. Their study showed an important finding that with an applied DC-offset AC field, EOF and EP force could be decoupled from DEP force, thus providing independent control of these electrokinetic forces. Lewpiriyawong et al. [125] further discovered that the use of DC-offset AC field is capable of minimizing Joule heating by reducing the total voltage required for particle sorting (as compared to the sole DC field). Moreover, their study showed that the iDEP effect can focus particles in a similar fashion as the well-known hydrodynamic focusing and thus facilitate the particle sorting and separation in a PDMS H-filter with multi-insulating blocks as shown in Fig. 14c. Lewpiriyawong et al. also reported a study using the DC-offset AC electric field to concentrate viable yeast cells in a PDMS channel with a tapered contraction (Fig. 14d) [126]. Their findings showed that the total electric field required to concentrate 15 μm particles could be reduced up to 85.9 % as compared to the sole DC field. Zhu et al. [138] also reported the similar findings showing the reduced voltages for particle focusing in a constriction-integrated PDMS microchannel. Later, Church et al. [127] presented a new technique for size-dependent separation of yeast cells (dark) and bacterial cells (gray) via DEP focusing in a serpentine channel as illustrated in Fig. 14e. Nonetheless, these studies reported similar results that the manipulation of the width of focused particle streams or the enhanced concentration of particles can be achieved by adjusting the ratio of AC-to-DC electric fields.

Although the effect of Joule heating is much less severe in iDEP devices under the applied DC-offset AC electric field, the Joule heating still remains and can be more significant in biological fluids with high conductivity. Hawkins et al. [139] showed that even though the solution conductivity was $>10^{-3}$ S/m, strong electrothermally induced (ET) flow existed near an insulating block with a 25 μm

constriction in a 100 μm microchannel. This ET flow perturbs the local EOF flow field, thus generating fluid circulation (or vorticity). Instead of deteriorating the device performance, their findings showed vorticity-aided separation and trapping of those particles experiencing nDEP. A similar investigation conducted by Sridharan et al. [140] found that counter-rotating fluid circulations can be manipulated to occur at either downstream end alone or both ends of the constriction channel, depending on the applied DC magnitude.

As can be seen from the literature describing from the use of DC electric field to the DC-offset AC electric field, Joule heating causing temperature rise has been a main issue. High temperature not only may kill or lyse the processed samples but also influence the local EOF flow field. Therefore, it is of an importance to consider the effect of Joule heating and the ET flow as crucial factors when designing iDEP devices to deal with biosamples in physiological fluids typically with high conductivity ($\sim 0.1\text{--}1\text{ S/m}$) [141].

3.4 Hybrid DEP

Hybrid DEP combines both planar-electrode-based and insulator-based DEP techniques for continuous separation and trapping of biosamples [142, 143]. The key features of a hybrid DEP system then are (1) the ability to manipulate samples with low voltages like planar DEP systems and (2) straightforward mass fabrication by rapid prototyping like iDEP systems [123]. So far, two types of the hybrid DEP have been proposed: (1) use of liquid electrodes and (2) use of contactless DEP. Figure 15a illustrates a schematic of a liquid electrode DEP system in which the comb-like SU-8 microstructures are fabricated side by side with Ti/Pt planar electrodes [142]. An electric field is produced by planar electrodes (in black) in contact with the flowing fluid and the patterned SU-8 insulator is responsible for creating nonuniform electric field. Using multiple electric field frequencies, the downstream equilibrium locations of subpopulations can be controlled by DEP forces created near insulating structures at both sides of the microchannel. The technique has been successfully used for focusing and continuous separation of particles, live/dead yeast cells, *Babesia bovis*-infected red blood cells and platelets [142, 144–148]. Details of the optimized design of liquid electrodes can be found elsewhere [149]. In spite of the advantages and high performance of the liquid electrode-based devices, the use of SU-8 photoresist as channel walls can be expensive especially for disposable or point-of-care devices. Also, fouling can be an issue if the device is operated with real biosamples (e.g., blood) for a long period of time. The use of inexpensive materials like PDMS or other polymers can be an option to make this technique more promising and reliable for clinical uses.

The second hybrid DEP system is named as contactless DEP (cDEP) because it produces a nonuniform electric field without direct contact between metal electrodes and the sample fluid (Fig. 15b) [143]. This new system clearly can

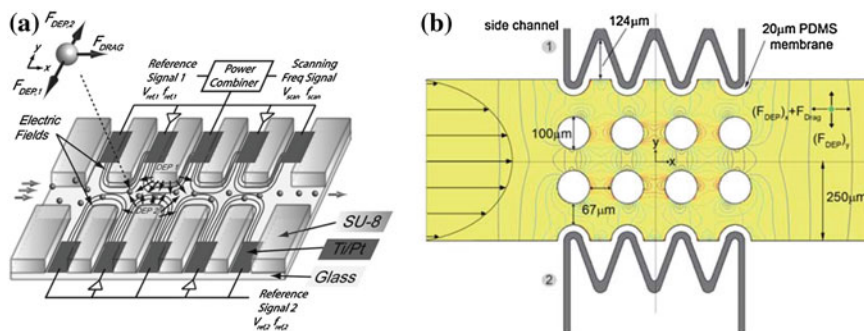


Fig. 15 Two hybrid DEP systems: **a** schematic of a flow-based DEP system with liquid electrodes comprising the comb-like SU-8 microstructures fabricated side by side with Ti/Pt planar electrodes [142]. **b** Contactless DEP device with 100 μm circular insulating posts used as flow-based DEP filtering system where nonuniform electric field is generated from the zigzag microchannel filled with a highly conductive medium separated by a thin PDMS membrane [143]

avoid bubble generation and reduce severe fouling and any possible contamination during separation. To generate electric field in the main channel, an AC voltage is applied via external aluminium electrodes remotely placed at the zigzag microchannel reservoirs. Because these zigzag microchannels contain a highly conductive medium, although separated from the main channel by a thin insulating PDMS layers, a nonuniform electric field and thus the DEP force can be created near the circular posts without directly immersing metal electrodes in the sample fluid. Phosphate buffered saline (PBS) was chosen as the conductive medium filled in the zigzag channels. Such cDEP devices have been demonstrated to successfully separate various biosamples such as live and dead cells [143] and human cancer cells [150]. In addition to these two hybrid DEP designs, researchers also proposed similar hybrid DEP devices by employing other insulating structures such as a micropillar array to discriminate or trap particles by size [151] and inclined rod-like insulators to focus and filter biological cells [152].

4 Concluding Remarks

This chapter presents an overview of the development of DEP-FFF technologies for continuous-flow separation of biosamples in microfluidics. The publications between 2005 and 2012 are the main focus of this report. Based on the strategies in providing nonuniform electric field for DEP force, DEP devices are categorized into four groups: (1) the use of planar (or thin film) electrodes, (2) the use of 3D and sidewall electrodes, (3) the use of insulating topographical structures, and finally (4) the hybrid DEP systems. Despite a plethora of proposed DEP devices and their successes, the efficiency, throughput and selectivity of these reported DEP devices still do not meet the requirements of clinical laboratories and cannot

be competed with the existing commercial biomedical analytical instrument such as flow cytometry and FACS.

The capability of performing continuous separation of biosamples with high efficiency and throughput is highly desirable in DEP microfluidic devices. Currently, most DEP devices can be operated at about 1–10 $\mu\text{L}/\text{min}$ while the working flow rate of clinical and research facilities such as FACS (LSR II cell analyzer, BD Biosciences, San Jose, California) is about 60 $\mu\text{L}/\text{min}$ [72, 153]. In principle, the high throughput of DEP systems can readily be achieved by increasing both the flow rate and the applied voltages. However, this is often not practical because upon increasing voltages, current-induced Joule heating rapidly causes temperature rise in the DEP systems (including both electrode-based and insulator-based systems). As a result, the sample can be potentially killed in high temperature environment (e.g., $>41^\circ\text{C}$) above its physiological temperature even for a short period of time [154]. In addition, bubble generation induced by highly applied voltages can interrupt separation process. One possible solution is to integrate a cooling system near the heat-generating area of electrodes. Recently, Moschallski et al. [46] demonstrated the feasibility of removing heat by attaching a chilled water system to the bottom face of the DEP device. With such cooling system, the device could be operated at 4–10 $^\circ\text{C}$ which can preserve integrity of organelle during the 60 h continuous-flow separation. In addition, a Peltier cooling pad was also used to maintain the desired temperature in a DEP device [155]. Therefore, it is expected to further develop DEP devices with more efficient cooling systems such that higher DEP system throughput can be achieved.

Nanoscale electrodes, typically fabricated via electron beam (e-beam) lithography, may play an important role in future DEP technologies by increasing throughput, efficiency and selectivity in separating rare cells in continuous flow. It has been shown since 1996 that the e-beam lithography allows for the fabrication of electrodes with dimensions from a few micron to 60 nm with ZEP-520 resist [156] or even 20 nm with PMMA resist [157]. In comparison with microscale electrodes, when nanoscale electrodes are energized under similar magnitude of AC voltages, DEP force produced by the nanoscale electrodes can be dramatically increased. Hence, it is anticipated that DEP devices can achieve separation with higher efficiency and also require lesser energy consumption. Moreover, as the electrode area significantly reduces, the effect of Joule heat is also greatly minimized due to the high surface-to-volume ratio [158]. However, in the past five years, DEP devices still relied heavily on microscale electrodes with dimensions of 50–100 μm . Only a limited number of studies were reported to employ microscale electrodes with relatively small dimensions, about 2 μm using e-beam lithography [159] and 10 μm using photolithography [52, 160]. The device functions were manifested to trap 282 nm particles [159], focus 40 nm particles at 2 mm/s [52] and sort 6 μm particles at 10 mm/s [160] under an AC voltage of 15–20 V peak-to-peak. To date, no study has been to utilize such nanoscale electrodes for continuous-flow separation of biological cells. In the near future, the implementation of nanoscale electrodes could be a promising and challenging research direction to realize fast separation and detection of pathogenic rare cells, virus, protein and

DNA with high efficiency, throughput and selectivity. It should be pointed out here that nanoscale insulating posts can also be used to induce highly nonuniform electric field in iDEP devices for enhanced separation efficiency.

Another important ongoing trend is an attempt to increase sensitivity of DEP biosensors for foodborne and waterborne pathogens detection. The presence of pathogens in food and water often results in a deathly impact on many groups of people: the elderly, pregnant women, newborns and even healthy persons [161]. Among pathogenic microorganisms, *L. monocytogenes*, *Listeria*, *Salmonella*, *E. coli* O157:H7 and protozoan (Cryptosporidium) are the leading causes of foodborne and waterborne lethal illness [162–164]. For safe drinking water, the concentration of *E. coli* O157:H7 must be lower than 1 Colony Forming Units (CFU)/ml [165] and around 1–30 CFU/ml for clear clinical symptoms to manifest [166]. Due to such a low concentration, pathogenic detection becomes rather difficult in practical. The plate-culture method is a traditional detection approach (still widely used nowadays) which is tedious, time consuming (a few days), and labour costs (as it requires a well trained personnel to identify bacterial colony morphology to provide the readings) [167]. To date, the detection limit for currently available sensors [e.g., enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR)] are on the order of about 10^1 – 10^6 CFU/ml [168]. Clearly, currently available sensors are still not sensitive enough for detecting those pathogens. However, it has been reported in the DEP community that the utilization of antibodies is promising in improving the selectivity of DEP systems where antibodies can typically be either immobilized on the DEP sensor surface or coated on flowing micro/nanoparticle surface [58, 61, 169]. Yang et al. [170] showed that the DEP-assisted capture efficiency of *Salmonella* cells by immobilized Anti-*Salmonella* antibodies on the DEP chip with planar electrodes was significantly improved from 17.6 to 64 % in a non-continuous separation mode. In a continuous separation mode, Kim et al. [58] achieved separation of multiple bacterial cells with distinct synthetic DEP tags in a DEP device with inclined double-sided electrodes. Their device demonstrated 1,000-fold enrichment of multiple bacterial cells at a throughput of $\sim 4,166$ particles/min in a single pass separation. Similar development was the use of magnetic nanoparticles-antibody conjugates (MNAC) to assist in detecting *E. coli* O157:H7 at concentrations as low as 1.6×10^2 and 1.2×10^3 (numbers of bacteria in 60 nL loaded in a PDMS microchannel). These concentrations are equivalent to 94 and 74 % in capture rates for pure culture and ground beef samples, respectively [164]. Besides planar electrodes, a 25 μm microwire was recently demonstrated for detecting *E. coli* K-12 in baby spinach leaves with the detection limit of 10^3 CFU/ml [165]. Although improved, such detection limit was merely the pathogenic concentration of patients near death [167]. Therefore, the early pathogenic detection is still challenging. Based on the literature, only planar electrodes have been employed in DEP biosensors while other kinds of DEP devices such as 3D/sidewall electrodes, insulating structures or hybrid DEP systems could be used as new biosensing platforms. Last but not least, as mentioned earlier, the cooling system and nanoscale electrodes can be important elements in developing efficient and reliable

biosensors. For more information on DEP fractionation and detection of other rare cells such as blood cells, cancer cells and mammalian cells, the readers may consult an excellent review by Pratt et al. [171].

Acknowledgments The authors would acknowledge the research grant (AcRF RG17/05) from the Ministry of Education of Singapore to CY and the Ph.D. Scholarship from Nanyang Technological University to NL. The authors also are grateful to Dr. Marcos for his comments and critical reading of the manuscript.

References

1. Toner, M., Irimia, D.: Blood on a chip. *Annu. Rev. Biomed. Eng.* **7**, 77–103 (2005)
2. Cho, B.S., et al.: Passively driven integrated microfluidic system for separation of motile sperm. *Anal. Chem.* **75**, 1671–1675 (2003)
3. Greenberg, A.E., Clesceri, L.S., Eaton, A.D.: *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association, American Water Works Association, and Water Environment Federation (2005)
4. Lenshof, A., Laurell, T.: Continuous separation of cells and particles in microfluidic systems. *Chem. Soc. Rev.* **39**, 1203–1217 (2010)
5. Dainiak, M.B., et al.: Cell chromatography: Separation of different microbial cells using IMAC supermacroporous monolithic columns. *Biotechnol. Prog.* **21**, 644–649 (2005)
6. Hulett, H.R., et al.: Cell sorting: automated separation of mammalian cells as a function of intracellular fluorescence. *Science* **166**, 747–749 (1969)
7. Yager, P., et al.: Microfluidic diagnostic technologies for global public health. *Nature* **442**, 412–418 (2006)
8. Bonner, W.A., et al.: Fluorescence activated cell sorting. *Rev. Sci. Instrum.* **43**, 404–409 (1972)
9. El-Ali, J., Sorger, P.K., Jensen, K.F.: Cells on chips. *Nature* **442**, 403–411 (2006)
10. Bilitewski, U., et al.: Biochemical analysis with microfluidic systems. *Anal. Bioanal. Chem.* **377**, 556–569 (2003)
11. Schimpf, M.E., Caldwell, K., Giddings, J.C.: *Field-Flow Fractionation handbook. Principle and Theory*. John Wiley & Sons, Inc., New York (2000)
12. Reschiglian, P., et al.: Field-flow fractionation and biotechnology. *Trends Biotechnol.* **23**(9), 475–483 (2005)
13. Huang, Y., et al.: Introducing dielectrophoresis as a new force field for field-flow fractionation. *Biophys. J.* **73**, 1118–1129 (1997)
14. Yang, J., et al.: Cell separation on microfabricated electrodes using dielectrophoretic/gravitational field-flow fractionation. *Anal. Chem.* **71**, 911–918 (1999)
15. Wang, X.-B., et al.: Cell separation by dielectrophoretic field-flow fractionation. *Acc. Chem. Res.* **72**, 832–839 (2000)
16. Song, H., et al.: Continuous-mode dielectrophoretic gating for highly efficient separation of analytes in surface micromachined microfluidic devices. *J. Micromech. Microeng.* **18**, 125013 (2008)
17. Markx, G.H., Talary, M.S., Pethig, R.: Separation of viable and non-viable yeast using dielectrophoresis. *J. Biotechnol.* **32**, 29–37 (1994)
18. Nedelcu, S., Watson, J.H.P.: Size separation of DNA molecules by pulsed electric field dielectrophoresis. *J. Phys. D Appl. Phys.* **37**, 2197–2204 (2004)
19. Pohl, H.A.: The motion and participation of suspensoids in divergent electric fields. *J. Appl. Phys.* **22**(7), 869–871 (1951)

20. Pohl, H.A.: *Dielectrophoresis: The Behavior of Neutral Matter in Nonuniform Electric Fields*. Cambridge University Press, Cambridge (1978)
21. Jones, T.B.: *Electromechanics of Particles*. Cambridge University Press, Cambridge (1995)
22. Lewpiriyawong, N.: *Continuous Separation and Manipulation of Particles and Cells Using Dielectrophoresis*. Nanyang Technological University, Singapore (2011)
23. Wang, X., Wang, X.-B., Gascoyne, P.R.C.: General expressions for dielectrophoretic force and electrorotational torque derived using the Maxwell stress tensor method. *J. Electrostat.* **39**, 277–295 (1997)
24. Denner, V., Pohl, H.A.: Dielectrophoretic force in electrostatic fields. *J. Electrostat.* **13**, 167–174 (1982)
25. Morgan, H., Green, N.G.: *AC Electrokinetics: Colloids and Nanoparticles*. Research Studies Press, Philadelphia (2002)
26. Arnold, W.M., Schwan, H.P., Zimmermann, U.: Surface conductance and other properties of latex particles measured by electrorotation. *J. Phys. Chem.* **91**, 5093–5098 (1987)
27. Hawkins, B.G., et al.: Continuous-flow particle separation by 3D insulative dielectrophoresis using coherently shaped, dc-biased, ac electric fields. *Anal. Chem.* **79**, 7291–7300 (2007)
28. Wang, X.-B., et al.: A unified theory of dielectrophoresis and travelling wave dielectrophoresis. *J. Phys. D Appl. Phys.* **27**, 1571–1574 (1994)
29. Nguyen, N.T., Wereley, S.: *Fundamentals and Applications of Microfluidics*, 2nd edn. Artech House, Boston (2006)
30. Wang, X.-B., et al.: Separation of polystyrene microbeads using dielectrophoretic/gravitational field-flow-fractionation. *Biophys. J.* **74**, 2689–2701 (1998)
31. Gascoyne, P., Satayavivad, J., Ruchirawat, M.: Microfluidic approaches to malaria detection. *Acta Trop.* **89**, 357–369 (2004)
32. Markx, G.H., Pethig, R., Rousselet, J.: The dielectrophoretic levitation of latex beads with reference to field-flow fractionation. *J. Phys. D Appl. Phys.* **30**, 2470–2477 (1997)
33. Markx, G.H., Rousselet, J., Pethig, R.: DEP-FFF: Field-flow fractionation using non-uniform electric field. *J. Liq. Chromatogr. Relat. Technol.* **20**(16-17), 2857–2872 (1997)
34. Rousselet, J., Markx, G.H., Pethig, R.: Separation of erythrocytes and latex beads by dielectrophoretic levitation and hyperlayer field-flow fractionation. *Colloids Surf., A* **140**, 209–216 (1998)
35. Holmes, D., Green, N.G., Morgan, H.: Microdevices for dielectrophoretic flow-through cell separation. *IEEE Eng. Med. Biol. Mag.* **22**(6), 85–90 (2003)
36. Markx, G.H., Pethig, R.: Dielectrophoretic separation of cells: Continuous separation. *Biotechnol. Bioeng.* **45**, 337–343 (1995)
37. Ino, K., et al.: Manipulation of microparticles for construction of array patterns by negative dielectrophoresis using multilayered array and grid electrodes. *Biotechnol. Bioeng.* **104**(4), 709–718 (2009)
38. Thomas, R.S.W., et al.: Trapping single human osteoblast-like cells from a heterogeneous population using a dielectrophoretic microfluidic device. *Biomicrofluidics* **4**, 022806 (2010)
39. Jang, L.-S., Huang, P.-H., Lan, K.-C.: Single-cell trapping utilizing negative dielectrophoretic quadrupole and microwell electrodes. *Biosens. Bioelectron.* **24**, 3637–3644 (2009)
40. Ramadan, Q., et al.: Simultaneous cell lysis and bead trapping in a continuous flow microfluidic device. *Sens Actuators B* **113**, 944–955 (2006)
41. Auerswald, J., et al.: Fast immobilization of probe beads by dielectrophoresis-controlled adhesion in a versatile microfluidic platform for affinity assay. *Electrophoresis* **26**, 3697–3705 (2005)
42. Lagally, E.T., Lee, S.-H., Soh, H.T.: Integrated microsystem for dielectrophoretic cell concentration and genetic detection. *Lab Chip* **5**, 1053–1058 (2005)
43. Gascoyne, P.R.C.: Dielectrophoretic-field flow fractionation analysis of dielectric, density, and deformability characteristics of cells and particles. *Anal. Chem.* **81**, 8878–8885 (2009)

44. Morgan, H., et al.: The dielectrophoretic and travelling wave forces generated by interdigitated electrode arrays: Analytical solution using Fourier series. *J. Phys. D Appl. Phys.* **34**, 1553–1561 (2001)
45. Durr, M., et al.: Microdevices for manipulation and accumulation of micro- and nanoparticles by dielectrophoresis. *Electrophoresis* **24**, 722–731 (2003)
46. Moschallski, M., et al.: MicroPrep: Chip-based dielectrophoretic purification of mitochondria. *Electrophoresis* **31**, 2655–2663 (2010)
47. Chen, D., Du, H.: A dielectrophoretic barrier-based microsystem for separation of microparticles. *Microfluid. Nanofluid.* **3**, 603–610 (2007)
48. Chen, D.F., Du, H., Li, W.H.: A 3D paired microelectrode array for accumulation and separation of microparticles. *J. Micromech. Microeng.* **16**, 1162–1169 (2006)
49. Choi, S., Park, J.-K.: Microfluidic system for dielectrophoretic separation based on a trapezoidal electrode array. *Lab Chip* **5**, 1161–1167 (2005)
50. Yang, F., et al.: Dielectrophoretic separation of colorectal cancer cells. *Biomicrofluidics* **4**, 013204 (2010)
51. Khoshmanesh, K., et al.: Dielectrophoretic-activated cell sorter based on curved microelectrodes. *Microfluid. Nanofluid.* **9**, 411–426 (2010)
52. Morgan, H., Holmes, D., Green, N.G.: 3D focusing of nanoparticles in microfluidic channels. *IEE Proc.-Nanobiotechnol.* **150**(2), 76–81 (2003)
53. Ling, S.H., Lam, Y.C., Kua, C.H.: Particle streaming and separation using dielectrophoresis through discrete periodic microelectrode array. *Microfluid. Nanofluid.* **11**(5), 579–591 (2011)
54. Park, J., et al.: An efficient cell separation system using 3D-asymmetric microelectrodes. *Lab Chip* **5**, 1264–1270 (2005)
55. Liao, S.-H., Cheng, I.-F., Chang, H.-C.: Precisely sized separation of multiple particles based on the dielectrophoresis gradient in the z-direction. *Microfluid. Nanofluid.* **12**, 201–211 (2011)
56. Yang, F., et al.: Cascade and staggered dielectrophoretic cell sorters. *Electrophoresis* **32**, 2377–2384 (2011)
57. Han, K.-H., Han, S.-I., Frazier, A.B.: Lateral displacement as a function of particle size using a piecewise curved planar interdigitated electrode array. *Lab Chip* **9**, 2958–2964 (2009)
58. Kim, U., et al.: Multitarget dielectrophoresis activated cell sorter. *Anal. Chem.* **80**, 8656–8661 (2008)
59. Doh, I., Cho, Y.-H.: A continuous cell separation chip using hydrodynamic dielectrophoresis (DEP) process. *Sens. Actuators* **121**, 59–65 (2005)
60. Kralj, J.G., et al.: Continuous dielectrophoresis size-based particle sorting. *Anal. Chem.* **78**, 5019–5025 (2006)
61. Kim, U., Soh, H.T.: Simultaneous sorting of multiple bacterial targets using integrated dielectrophoretic–magnetic activated cell sorter. *Lab Chip* **9**, 2313–2318 (2009)
62. Pommer, M.S., et al.: Dielectrophoretic separation of platelets from diluted whole blood in microfluidic channels. *Electrophoresis* **29**, 1213–1218 (2008)
63. Talary, M.S., et al.: Electromanipulation and separation of cells using travelling electric fields. *J. Phys. D Appl. Phys.* **29**, 2198–2203 (1996)
64. Hughes, M.P., Pethig, R., Wang, X.-B.: Dielectrophoretic forces on particles in travelling electric fields. *J. Phys. D Appl. Phys.* **29**, 474–482 (1996)
65. Huang, Y., et al.: Electrokinetic behavior of colloidal particles in travelling electric fields: Studies using yeast cells. *J. Phys. D Appl. Phys.* **26**, 1528–1535 (1993)
66. Gascoyne, P., et al.: Microsample preparation by dielectrophoresis: Isolation of malaria. *Lab Chip* **2**, 70–75 (2002)
67. Choi, E., Kim, B., Park, J.: High-throughput microparticle separation using gradient traveling wave dielectrophoresis. *J. Micromech. Microeng.* **19**, 125014 (2009)
68. Morgan, H., et al.: Large-area travelling-wave dielectrophoresis particle separator. *J. Micromech. Microeng.* **7**, 65–70 (1997)

69. Cui, L., Holmes, D., Morgan, H.: The dielectrophoretic levitation and separation of latex bead in microchips. *Electrophoresis* **22**, 3893–3901 (2001)
70. Kawamoto, H.: Some techniques on electrostatic separation of particle size utilizing electrostatic traveling-wave field. *J. Electrostat.* **66**, 220–228 (2008)
71. Huang, C., et al.: Design and fabrication of an automated microchip-based cell separation device. *Anal. Lett.* **40**, 763–778 (2007)
72. Cheng, I.-F., et al.: A continuous high-throughput bioparticle sorter based on 3D traveling-wave dielectrophoresis. *Lab Chip* **9**, 3193–3201 (2009)
73. Huang, Y., et al.: Differences in the AC electrodynamics of viable and non-viable yeast cell determined through combined dielectrophoresis and electrorotation studies. *Phys. Med. Biol.* **37**(7), 1499–1517 (1992)
74. Zhou, X.-F., et al.: Differentiation of viable and non-viable bacterial biofilms using electrorotation. *Biochim. Biophys. Acta* **1245**, 85–93 (1995)
75. Yang, J., et al.: Dielectric properties of human leukocyte subpopulations determined by electrorotation as a cell separation criterion. *Biophys. J.* **76**, 3307–3314 (1999)
76. Arnold, W.M., Zimmermann, U.: Rotating-field-induced rotation and measurement of the membrane capacitance of single mesophyll cells of *Avena sativa*. *Zeitschrift fuer Naturforschung. Section C. Biosci.* **37**, 908–915 (1982)
77. Cristofanilli, M., et al.: Automated electrorotation to reveal dielectric variations related to HER-2/neu overexpression in MCF-7 sublines. *Clin. Cancer Res.* **8**, 615–619 (2002)
78. Huang, Y., Wang, X., Becker, F.F., Gascoyne, P.R.: Membrane changes associated with the temperature-sensitive P85gag-mos-dependent transformation of rat kidney cells as determined by dielectrophoresis and electrorotation. *Biochim. Biophys. Acta* **1282**(1), 76–84 (1996)
79. Pethig, R.J., Sanger, L.M., Heart, R.H., Corson, E., Smith, E.D., Peter, J.S.: Electrokinetic measurements of membrane capacitance and conductance for pancreatic β -cells. *IEE Proc.-Nanobiotechnol.* **152**(6), 189–193 (2005)
80. Lei, U., Sun, P.-H., Pethig, R.: Refinement of the theory for extracting cell dielectric properties from dielectrophoresis and electrorotation experiments. *Biomicrofluidics* **5**, 044109 (2011)
81. Schwan, H.P.: Electrical properties of tissue and cell suspensions. *Adv. Biol. Med. Phys.* **5**, 147–209 (1957)
82. Fuhr, G., Hagedorn, R., Goring, H.: Separation of different cell types by rotating electric fields. *Plant Cell Physiol.* **26**, 1527–1531 (1985)
83. Kua, C.H., et al.: Dynamic cell fractionation and transportation using moving dielectrophoresis. *Anal. Chem.* **79**, 6975–6987 (2007)
84. Kua, C.H., et al.: Modeling of dielectrophoretic force for moving dielectrophoresis electrodes. *J. Electrostat.* **66**, 514–525 (2008)
85. Kua, C.H., et al.: Cell motion model for moving dielectrophoresis. *Anal. Chem.* **80**, 5454–5461 (2008)
86. Cui, H.-H., et al.: Separation of particles by pulsed dielectrophoresis. *Lab Chip* **9**, 2306–2312 (2009)
87. Kumemura, M., et al.: Single-DNA-molecule trapping with silicon nanotweezers using pulsed dielectrophoresis. *J. Micromech. Microeng.* **21**, 054020 (2011)
88. Chiou, P.Y., Ohta, A.T., Wu, M.C.: Massively parallel manipulation of single cells and microparticles using optical images. *Nature* **436**, 370–372 (2005)
89. Grier, D.G.: A revolution in optical manipulation. *Nature* **424**, 810–816 (2003)
90. Garces-Chavez, V., Dholakia, K., Spalding, G.C.: Extended-area optically induced organization of microparticles on a surface. *Appl. Phys. Lett.* **85**, 031106 (2005)
91. Lin, W.-Y., Lin, Y.-H., Lee, G.-B.: Separation of micro-particles utilizing spatial difference of optically induced dielectrophoretic forces. *Microfluid. Nanofluid.* **8**, 217–229 (2010)
92. Lin, Y.-H., Lee, G.-B.: Optically induced flow cytometry for continuous microparticle counting and sorting. *Biosens. Bioelectron.* **24**, 572–578 (2008)

93. Choi, W., et al.: Programmable manipulation of motile cells in optoelectronic tweezers using a grayscale image. *Appl. Phys. Lett.* **93**(14), 143901 (2008)
94. Cheng, I.-F., et al.: Stepwise gray-scale light-induced electric field gradient for passive and continuous separation of microparticles. *Microfluid. Nanofluid.* **12**, 95–105 (2011)
95. Yang, S.-M., et al.: Dynamic manipulation and patterning of microparticles and cells by using TiOPc-based optoelectronic dielectrophoresis. *Opt. Lett.* **35**(12), 1959–1961 (2010)
96. Lu, Y.S., et al.: Controllability of non-contact cell manipulation by image dielectrophoresis. *Opt. Quantum Electron.* **37**, 1385–1395 (2005)
97. Choi, W., et al.: Lab-on-a-display: A new microparticle manipulation platform using a liquid crystal display (LCD). *Microfluid. Nanofluid.* **3**, 217–225 (2007)
98. Hwang, H., et al.: Interactive manipulation of blood cells using a lens-integrated liquid crystal display based optoelectronic tweezers system. *Electrophoresis* **29**, 1203–1212 (2008)
99. Hoeb, M., et al.: Light-induced dielectrophoretic manipulation of DNA. *Biophys. J.* **93**, 1032–1038 (2007)
100. Lee, M.-W., Lin, Y.-H., Lee, G.-B.: Manipulation and patterning of carbon nanotubes utilizing optically induced dielectrophoretic forces. *Microfluid. Nanofluid.* **8**, 609–617 (2010)
101. Iliescu, C., et al.: Fabrication of a dielectrophoretic chip with 3D silicon electrodes. *J. Micromech. Microeng.* **15**, 494–500 (2005)
102. Yu, L., et al.: Sequential field-flow cell separation method in a dielectrophoretic chip with 3D electrodes. *J. Microelectromech. S.* **16**, 1120–1129 (2007)
103. Iliescu, C., et al.: Bidirectional field-flow particle separation method in a dielectrophoretic chip with 3D electrodes. *Sens. Actuators B* **129**, 491–496 (2008)
104. Iliescu, C., Tresset, G., Xu, G.: Continuous field-flow separation of particle populations in a dielectrophoretic chip with three dimensional electrodes. *Appl. Phys. Lett.* **90**, 234104 (2007)
105. Iliescu, C., Tresset, G., Xu, G.: Dielectrophoretic field-flow method for separating particle populations in a chip with asymmetric electrodes. *Biomicrofluidics* **3**, 044104 (2009)
106. Tay, F.E.H., et al.: Electrical and thermal characterization of a dielectrophoretic chip with 3D electrodes for cells manipulation. *Electrochim. Acta* **52**, 2862–2868 (2007)
107. Park, B.Y., Madou, M.J.: 3D electrode designs for flow-through dielectrophoretic system. *Electrophoresis* **26**, 3745–3757 (2005)
108. Martinez-Duarte, R., Renaud, P., Madou, M.J.: A novel approach to dielectrophoresis using carbon electrodes. *Electrophoresis* **32**, 2385–2392 (2011)
109. Ma, W., et al.: High-throughput dielectrophoretic manipulation of bioparticles within fluids through biocompatible three-dimensional microelectrode array. *Electrophoresis* **32**, 494–505 (2011)
110. Wang, L., et al.: Dual frequency dielectrophoresis with interdigitated sidewall electrodes for microfluidic flow-through separation of beads and cells. *Electrophoresis* **30**, 782–791 (2009)
111. Wang, L., Flanagan, L., Lee, A.P.: Side-wall vertical electrodes for lateral field microfluidic applications. *J. MEMS* **16**(2), 454–461 (2007)
112. Wang, L., et al.: Dielectrophoresis switching with vertical sidewall electrodes for microfluidic flow cytometry. *Lab Chip* **7**, 1114–1120 (2007)
113. Zhang, Y.T., et al.: Titanium-based dielectrophoresis devices for microfluidic applications. *Biomed. Microdevices* **10**, 509–517 (2008)
114. Kang, Y., et al.: Continuous particle separation with localized AC-dielectrophoresis using embedded electrodes and an insulating hurdle. *Electrochim. Acta* **54**, 1715–1720 (2009)
115. Lewpiriyawong, N., et al.: Microfluidic characterization and continuous separation of cells and particles using conducting poly(dimethyl siloxane) electrode induced alternating current-dielectrophoresis. *Anal. Chem.* **83**, 9579–9585 (2011)
116. Xia, Y., Whitesides, G.M.: Soft lithography. *Annu. Rev. Mater. Sci.* **28**, 153–184 (1998)
117. Niu, X., et al.: Characterizing and patterning of PDMS-based conducting composites. *Adv. Mater.* **19**, 2682–2686 (2007)

118. Cetin, B., et al.: Continuous particle separation by size via AC-dielectrophoresis using a lab-on-a-chip device with 3-D electrodes. *Electrophoresis* **30**, 766–772 (2009)
119. Lewpiriyawong, N., Yang, C., Lam, Y.C.: Continuous sorting and separation of microparticles by size using AC dielectrophoresis in a PDMS microfluidic device with 3-D conducting PDMS composite electrodes. *Electrophoresis* **31**, 2622–2631 (2010)
120. Lewpiriyawong, N., Yang, C.: AC-dielectrophoretic characterization and separation of submicron and micron particles using sidewall AgPDMS electrodes. *Biomicrofluidics* **6**, 012807 (2012)
121. Lapizco-Encinas, B.H., et al.: Insulator-based dielectrophoresis for the selective concentration and separation of live bacteria in water. *Electrophoresis* **25**, 1695–1704 (2004)
122. Cummings, E.B., Singh, A.K.: Dielectrophoresis in microchips containing arrays of insulating posts: Theoretical and experiment results. *Anal. Chem.* **75**, 4724–4731 (2003)
123. Shafiee, H., et al.: Contactless dielectrophoresis: a new technique for cell manipulation. *Biomed. Microdevices* **11**, 997–1006 (2009)
124. Kang, Y., et al.: DC-Dielectrophoretic separation of biological cells by size. *Biomed. Microdevices* **10**, 243–249 (2008)
125. Lewpiriyawong, N., Yang, C., Lam, Y.C.: Dielectrophoretic manipulation of particles in a modified microfluidic H filter with multi-insulating blocks. *Biomicrofluidics* **2**, 034105 (2008)
126. Lewpiriyawong, N., Yang, C., Lam, Y.C.: Electrokinetically driven concentration of particles and cells by dielectrophoresis with DC-offset AC electric field. *Microfluid. Nanofluid.* **12**, 723–733 (2011)
127. Church, C., et al.: Electrokinetic focusing and filtration of cells in a serpentine microchannel. *Biomicrofluidics* **3**, 044109 (2009)
128. Chen, D., Du, H.: A microfluidic device for rapid concentration of particles in continuous flow by DC dielectrophoresis. *Microfluid. Nanofluid.* **9**, 281–291 (2010)
129. Chou, C.F., et al.: Electrodeless dielectrophoresis of single- and double-stranded DNA. *Biophys. J.* **83**, 2170–2179 (2002)
130. Lapizco-Encinas, B.H., Ozuna-Chacon, S., Marco, R.-P.: Protein manipulation with insulator-based dielectrophoresis and DC electric fields. *J. Chromatogr. A* **1206**, 45–51 (2008)
131. Jones, P.V., Staton, S.J.R., Hayes, M.A.: Blood cell capture in a sawtooth dielectrophoretic microchannel. *Anal. Bioanal. Chem.* **401**, 2103–2111 (2011)
132. Barrett, L.M., et al.: Dielectrophoretic manipulation of particles and cells using insulating ridges in faceted prism microchannels. *Anal. Chem.* **77**, 6798–6804 (2005)
133. Barbulovic-Nad, I., et al.: DC-dielectrophoretic separation of microparticles using an oil droplet obstacle. *Lab Chip* **6**, 274–279 (2006)
134. Kang, K.H., et al.: Continuous separation of microparticles by size with direct current-dielectrophoresis. *Electrophoresis* **27**, 694–702 (2006)
135. Zhu, J., et al.: DC dielectrophoretic focusing of particles in a serpentine microchannel. *Microfluid. Nanofluid.* **7**(6), 751–756 (2009)
136. Sabounchi, P., et al.: Sample concentration and impedance detection on a microfluidic polymer chip. *Biomed. Microdevices* **10**(5), 661–670 (2008)
137. Rosenthal, A., Voldman, J.: Dielectrophoretic traps for single-particle patterning. *Biophys. J.* **88**, 2193–2205 (2005)
138. Zhu, J., Xuan, X.: Dielectrophoretic focusing of particles in a microchannel constriction using DC-biased AC electric fields. *Electrophoresis* **30**, 2668–2675 (2009)
139. Hawkins, B.G., Kirby, B.J.: Electrothermal flow effects in insulating (electrodeless) dielectrophoresis systems. *Electrophoresis* **31**, 3622–3633 (2011)
140. Sridharan, S., et al.: Joule heating effects on electroosmotic flow in insulator-based dielectrophoresis. *Electrophoresis* **32**, 2274–2281 (2011)
141. Krishnan, R., et al.: Alternating current electrokinetic separation and detection of DNA nanoparticles in high-conductance solutions. *Electrophoresis* **29**, 1765–1774 (2008)

142. Demierre, N., et al.: Focusing and continuous separation of cells in a microfluidic device using lateral dielectrophoresis. *Sens. Actuators B* **132**, 388–396 (2008)
143. Shafiee, H., et al.: Selective isolation of live/dead cells using contactless dielectrophoresis (cDEP). *Lab Chip* **10**, 438–445 (2009)
144. Braschler, T., et al.: Continuous separation of cells by balanced dielectrophoretic forces at multiple frequencies. *Lab Chip* **8**, 280–286 (2008)
145. Tornay, R., et al.: Dielectrophoresis-based particle exchanger for the manipulation and surface functionalization of particles. *Lab Chip* **8**, 267–273 (2008)
146. Valero, A., et al.: A miniaturized continuous dielectrophoretic cell sorter and its applications. *Biomicrofluidics* **4**, 022807 (2010)
147. Nascimento, E.M., et al.: Dielectrophoretic sorting on a microfabricated flow cytometer: Label free separation of *Babesia bovis* infected erythrocytes. *Bioelectrochemistry* **73**, 123–128 (2008)
148. Piacentini, N., et al.: Separation of platelets from other blood cells in continuous-flow by dielectrophoresis field-flow-fractionation. *Biomicrofluidics* **5**, 034122 (2011)
149. Demierre, N., et al.: Characterization and optimization of liquid electrodes for lateral dielectrophoresis. *Lab Chip* **7**, 355–365 (2006)
150. Henslee, E.A., et al.: Selective concentration of human cancer cells using contactless dielectrophoresis. *Electrophoresis* **32**, 2523–2529 (2011)
151. Cui, H.-H., Lim, K.-M.: Pillar array microtraps with negative dielectrophoresis. *Langmuir* **25**, 3336–3339 (2009)
152. Jen, C.-P., Chen, W.-F.: An insulator-based dielectrophoretic microdevice for the simultaneous filtration and focusing of biological cells. *Biomicrofluidics* **5**, 044105 (2011)
153. Pethig, R.: Dielectrophoresis: Status of the theory, technology, and applications. *Biomicrofluidics* **4**, 022811 (2010)
154. Voldman, J.: Electrical forces for microscale cell manipulation. *Annu. Rev. Biomed. Eng.* **8**, 425–454 (2006)
155. Lee, D.-H., et al.: Dielectrophoretic particle–particle interaction under AC electrohydrodynamic flow conditions. *Electrophoresis* **32**, 2298–2306 (2011)
156. Yang, H., et al.: Low-energy electron-beam lithography of ZEP-520 positive resist. In: 1st IEEE International Conference on Nano/Micro Engineered and Molecular Systems (2006)
157. Cumming, D.R.S., et al.: Fabrication of 3 nm wires using 100 keV electron beam lithography and poly(methyl methacrylate) resist. *Appl. Phys. Lett.* **68**(3), 322 (1996)
158. Muller, T., et al.: A 3D microelectrode system for handling and caging single cells and particles. *Biosens. Bioelectron.* **14**, 247–256 (1999)
159. Green, N.G., Morgan, H.: Dielectrophoretic investigations of sub-micrometre latex spheres. *J. Phys. D Appl. Phys.* **30**, 2626–2633 (1997)
160. Holmes, D., et al.: On-chip high-speed sorting of micron-sized particles for high-throughput analysis. *IEE Proc. Nanobiotech.* **152**(4), 129–135 (2005)
161. Koo, O.K., et al.: Targeted capture of pathogenic bacteria using a mammalian cell receptor coupled with dielectrophoresis on a biochip. *Anal. Chem.* **81**, 3094–3101 (2009)
162. Goater, A.D., Burt, J.P.H., Pethig, R.: A combined travelling wave dielectrophoresis and electrorotation device: Applied to the concentration and viability determination of *Cryptosporidium*. *J. Phys. D Appl. Phys.* **30**, 65–69 (1997)
163. Bridlea, H., et al.: Detection of cryptosporidium in miniaturised fluidic devices. *Water Res.* **46**, 1641–1661 (2012)
164. Varshney, M., et al.: A label-free, microfluidics and interdigitated array microelectrode-based impedance biosensor in combination with nanoparticles immunoseparation for detection of *Escherichia coli* O157:H7 in food samples. *Sens. Actuator B* **128**, 99–107 (2007)
165. Kim, S., et al.: A microwire sensor for rapid detection of *Escherichia coli* K-12 in fresh produce. *Innov. Food Sci. Emerg.* **12**, 617–622 (2011)
166. Yagupsky, P., Nolte, F.: Quantitative aspects of septicemia. *Clin. Microbiol. Rev.* **3**, 269–279 (1990)

167. Sengupta, S., Gordon, J.E., Chang, H.-C.: Microfluidic diagnostic systems for the rapid detection and quantification of pathogens. In: Tien, W.-C., Finehout, E. (eds.) *Microfluidics for Biological Applications*, pp. 274–276. Springer, Berlin (2008)
168. Yang, L., Bashir, R.: Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. *Biotechnol. Adv.* **26**, 135–150 (2008)
169. Guan, X., et al.: Rapid detection of pathogens using antibody-coated microbeads with bioluminescence in microfluidic chips. *Biomed. Microdevices* **12**, 683–691 (2010)
170. Yang, L.: Dielectrophoresis assisted immuno-capture and detection of foodborne pathogenic bacteria in biochips. *Talanta* **80**, 551–558 (2009)
171. Pratt, E.D., et al.: Rare cell capture in microfluidic devices. *Chem. Eng. Sci.* **66**, 1508–1522 (2011)

Advances in Transport Phenomena 2011

Wang, L. (Ed.)

2014, IX, 169 p. 76 illus., 25 illus. in color., Hardcover

ISBN: 978-3-319-01792-1