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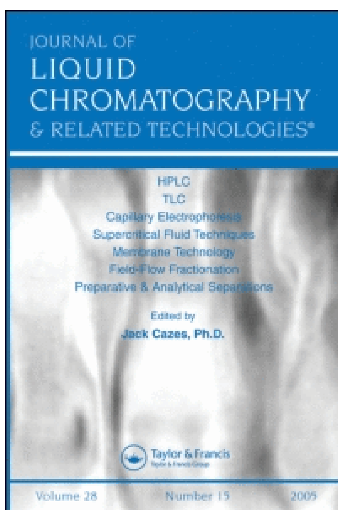

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Torsten Müller[a]; Thomas Schnelle; Gabriele Gradl; Stephen G. Shirley; Günter Fuhr[a]
[a] Institut für Biologie, Berlin, Germany

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MICRODEVICE FOR CELL AND PARTICLE SEPARATION USING DIELECTROPHORETIC FIELD-FLOW FRACTIONATION

Torsten Müller,[1,]* Thomas Schnelle,[2] Gabriele Gradl,[2]
Stephen G. Shirley,[2] Günter Fuhr[1]

[1] Humboldt-Universität zu Berlin
Institut für Biologie
Invalidenstr. 42
D-10115 Berlin, Germany

[2] EVOTEC BioSystems AG
Schnackenburgallee 114
D-22525 Hamburg, Germany

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ABSTRACT

We describe the use of a micro system for dielectrophoretic field flow fractionation (DFFF) of particles and cells. Micro-objects are separated on the basis of differences in size and/or passive dielectric properties, respectively. Bow-like strip electrode pairs have been proven to be the most effective separation systems.

INTRODUCTION

Modern methods in molecular biology, drug screening, diagnostics, and biotechnology frequently make use of single cells, inorganic or polymer particles. An operation playing a central role in such processes is the separation of individuals.

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Particles suspended in liquids can be separated by using centrifugal forces and hydrodynamic flow,[1] ultrasonic forces,[2] magnetic forces,[3,4] laser tweezers,[5] and electric field mediated forces.[6] However, due to the low difference in magnetic susceptibility of cells and water magnetic fields are only useful for such applications involving ferromagnetic particles which are bound to the cells. Centrifugation and hydrodynamic flow work well for separation of large numbers of particles. However, sorting rare events or handling of single cells requires individual objects to be moved reversibly or held at defined positions.[1] Ultrasonic standing waves used in the megahertz range can concentrate cells and particles at submillimeter distances but individual handling of single cells is not possible. Cells can only be held against a flow of 8 µm/s.[2,7]

Currently, laser tweezers[8-11] and high frequency electric field tools[12] are being studied for single cell manipulation. A difference in the refractive index of particle and liquid allows laser trapping of single objects in the focus region.[13] The limited ability to multiplex the focus region makes automated sorting of a larger number of particles by light trapping inaccessible.[14]

Dielectrophoresis (DEP) utilises differences in electric polarizability between particles and the surrounding liquid. When a dielectric particle is exposed to an external electric field it polarises, with the size and direction of the induced dipole depending on field frequency and dielectric properties. If the polarizability of particles is lower than that of the surrounding medium, they are repelled from regions of high field strength towards the field minima (negative, nDEP). If the polarizability of particles is higher they are attracted to regions of high field strength (positive, pDEP).[6] The advantage of negative DEP in particle manipulation is that the objects are forced to regions of low field strength, minimising the electric loading which may damage cells. Additionally, using a special electrode configuration, individual cells suspended in solution could be guided over any distances desired and caged contact-free in octode arrangements against streaming of about 50 µm/s.[15,16]

Recently, the great potential of dielectrophoresis for separation of blood cells from bacteria,[17] tumor cells from blood cells,[18,19] live and dead yeast cells,[20,21] and an enrichment of CD34+ blood stem cells[22,23] have been described. The use of negative and positive dielectrophoresis alone or in combination with a fluid flow to separate micro objects was described by Becker et al. (1995)[18] as DEP migration and DEP retention, respectively. The combination of DEP forces with flow for particle separation has been reported.[24-26] A further development was dielectrophoretic/gravitational field-flow fractionation (DEP/g-FFF) to separate polystyrene micro beads of different size and/or dielectric properties[27] or cultured human breast cancer cells from normal blood cells from each other.[28] These applications use planar microdevices driven with low frequencies of about 5-50 kHz, large electrode array of about 350 parallel gold electrodes, and low conductive medium.

In this paper, we describe micro-object separation based on differences in size or dielectric properties using nDEP in combination with hydrodynamic flow in three dimensional micro electrode structures. Thus, each cell fraction retrieved is subjected to gentle conditions.

The functional elements are two electrodes processed top and bottom of a micro channel. The dielectrophoretic and hydrodynamic forces acting in this system have been described elsewhere.[29] We show how several different electrode designs can be used in particle and cell separation tasks in low and highly conductive solutions.

The reduced electrode surface area and high frequency electric field minimize the electric loading which may damage cells.

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EXPERIMENTAL

Manufacturing of micro-electrode structures has been described elsewhere.[15,16] Briefly, electrodes (Ti/Pt or Au of 170 nm and 500 nm thickness, respectively) were produced lithographically on glass substrates or silicon. 3-D micro channel systems were built up by using spacers. Typical channel height is about 40 µm and width of 800 µm (Figure 1).

The microdevice consists of two electrode pairs to align the bioparticles, and from a funnel and a curved electrode pair, three different elements. The beginning of curved electrode pair (8 µm width) and the end of the upper aligner pair (25 µm width) form a dielectrophoretic nozzle of 40 µm width. After the bow electrodes are five additional spacers of 40 µm in width forming sub channels. Three copies of the separation unit are processed on the chip.

The electrodes were connected to a frequency generator (Hewlett Packard HP 8116 A, USA) producing ac square waves in a frequency range between 1 kHz and 20 MHz and voltages between 2 and 14 V_{rms} (typically 4 V_{rms}). The generators were connected to the liquid-filled micro-chamber with a high frequency high density connector (40 pins) via 15 cm long cables. A laboratory-made distribution box (driven by Reed relays) allows control of the voltage for some electrode pairs. The output signals at the electrode chamber were permanently monitored with a digitizing oscilloscope (TDS 210, Tektronix USA).

Suspensions were introduced into the microsystem channels using a syringe pump (SP 260p, WPI, USA). The continuous flow in the channel was controlled within the range of 2-100 µL/h. Particle behaviour was monitored via a microscope-camera system (Metallux 3, Leica, Germany, JVC, TK-1280E, Germany) and recorded on video tape. Close to the electrodes, particles

experiencing negative DEP were lifted into the central plane.[29] In order to determine the actual velocity at a given point of the profile we analysed the half images of the video tape using the jog shuttle mode of the recorder.

Particles, Cells, and Media

Standard Dow Latex particles (Serva, Heidelberg, Germany) of 6.4 µm, 10 mm, 15 µm, and 20 µm were diluted in water by means of ultrasonic treatment in a Sonorex system (Bandelin, Berlin, Germany). The standard deviations of these particle diameters give an overall size range of 3µm to 33 µm. Mixtures of PBS (phosphate buffered saline solution; Seromed/Biochrom, Berlin, Germany) and 0.3 M inositol solution were used.

The human T-hybridoma cell line (*Jurkat*) was grown in RPMI 1640 medium (GIBCO Life Technologies, Karlsruhe, Germany) containing 10% fetal calf serum (Seromed/Biochrom, Berlin, Germany), penicillin and streptomycin at 100 IU / mL each (Seromed/Biochrom, Berlin, Germany) and washed twice in PBS prior to the experiment. Suspension conductivity for cell experiments was adjusted between 0.3 S/m and 1.5 S/m by diluting with PBS or 0.3 M inositol solution.

Whole blood was drawn from a healthy donor by needle punction and diluted into PBS/inositol solution.

The conductivities of all media were measured with a conventional two-electrode conductometer (WTW, LF 325 Weilheim, Germany). The conductivity of the bioparticle suspensions were between 0.3 S/m and 1.5 S/m. For particle separation conductivities down to 1.7 mS/m were used.

Theoretical

The time averaged dielectric force acting on a spherical dielectric particle of radius R in an ac electric field (E), it is given by:

$$\left\langle \vec{F}_d \right\rangle = 2\pi\varepsilon_1 R^3 \left[\mathrm{Re}\left(f_{CM}\right) * \vec{\nabla} E_{rms}^2 \right] \qquad (1)$$

where the Clausius -Mossotti-factor f_{CM} for a homogenous sphere has been found to be

$$f_{CM} = \frac{\tilde{\sigma}_p - \tilde{\sigma}_1}{\tilde{\sigma}_p + 2\tilde{\sigma}_1}$$

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with

$$\tilde{\sigma}_{l,p} = \sigma_{l,p} + i\omega\varepsilon_{l,p}$$

where σ and ε describe conductivity and absolute permitivity of particle (index p) and liquid (index l). For more complicated particles, ε_p and σ_p in eq. (1) have to be replaced by effective frequency dependent values (see for an overview).[30] Negative DEP ($Re[f_{cm}] <0$) can be assured at low and high field frequencies if the conductivity and permitivity values of the liquid exceed those of the particle.

A particle showing negative DEP experiences a repelling force near a face to face mounted strip electrode pair. The repelling dielectrophoretic force F_{DEP} can be written as

$$F_{DEP} = 2\,\pi\varepsilon_l\,(R/a)^3\,Re[f_{CM}]U^2_{rms}\,f(w/a) \qquad (2)$$

with applied voltage U, electrode distance a and electrode width w. For very broad electrodes ($w \rightarrow \infty$) the geometric function f was found to approach $27\pi/64$.[15] For arbitrary ratios of width and spacing numerical calculations are necessary.

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RESULTS

Figure 1B shows the variation of dielectrophoretic force with the ratio of electrode width to spacing. The full line corresponds to a finite difference approach verified experimentally using latex beads as probe and evaluating the equilibrium between Stokes drag in a streaming and repelling DEP force.[29] The two points represent values of bow and aligner electrodes in our chip. To avoid loading of biological objects the width/height ration should be less than 1.

Straight electrode pairs can be used for dielectric particle alignment and filtering in a stream (see Figure 1A).

For bow electrodes, the repelling DEP force component in the flow direction depends on the distance from the channel wall, enabling particle separation. The dielectrophoretic force varies with particle volume (for dielectrically identical particles) while the Stokes force scale varies with particle radius. Therefore, particles of different sizes leave the bow at different places.

Large particles escape further from the channel boundary (Figure 1A). Note, that at distances greater than one channel height from the side walls, the fluid velocity profile hardly changes with position.

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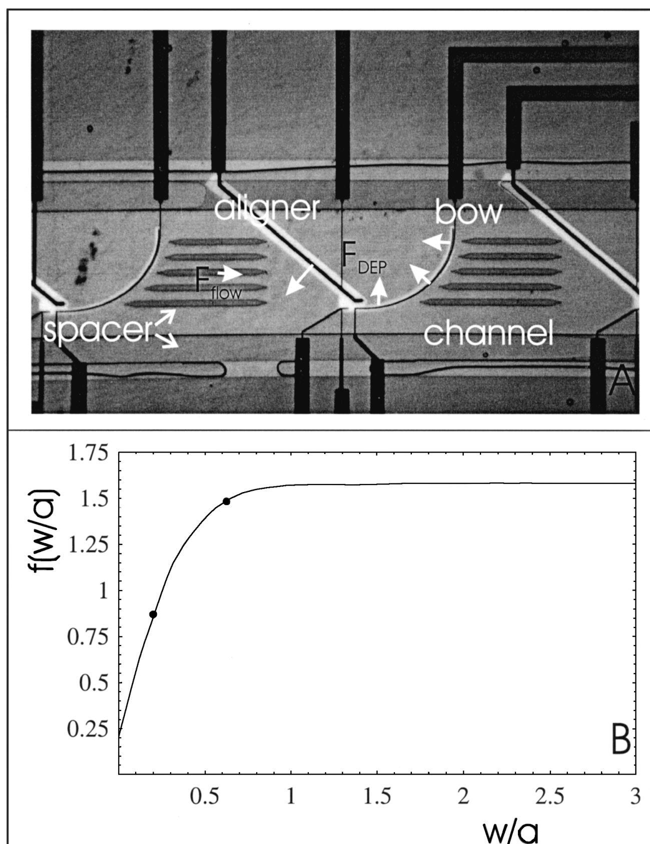

Figure 1. Experimental set-up. Central part of the micro structure with channel of 800 µm width. The light region around the electrode structures correspond to openings in the passivation layer. Alignment and particle separation based on balance of Stokes drag forces in the flow (F_{flow}) and dielectric forces (F_{DEP}). Geometric scaling factor f of dielectric force between face-to-face mounted strip electrodes (see eq. 2). The curve was obtained numerically using a finite difference approach and taking into account the liquid-glass interfaces (for more details see ref. 29). w and a represent electrode width and spacing respectively. The points refer to aligner and bow electrode pairs in Figure 1A.

Table 1 summarizes the conditions for efficient separation of latex beads. The best separation was realized at flow rates of about 800 µm/s for a voltage of U_{rms}= 3.75 V. All four particle fractions could be separated within a distance of about 100 µm.

Table 1

Deflection of Latex Beads by the Bow Electrodes at Different Streaming Velocities*

Bead Size (μm)	Velocity (μm/s)	Location (μm)
20	300 - 1000	0
	1000 - 1300	0 - 90
15	300 - 833	0
	833 - 1000	0 -90
	1000 - 1300	90 - 200
10	300 - 500	0
	650 - 750	90 - 130
	800 - 1000	240 - 270
	1000 - 1300	250 - 350
6.4	300 - 400	0
	400 - 550	80 - 130
	550 - 800	150 - 240
	830 - 1000	340 - 360
	1000 - 1300	540 - 550

* In the channel shown in Figure 1A, the positions were latex beads pass the bow electrodes were determined. The electrodes were driven with 3.75 V_{rms} at 5 MHz. The fluid velocity corresponds to actual value in the profile, distances are given as vertical component measured from the tip of the bow electrode. The channel width was 800 μm and the bow electrode extends from 125 μm to 655 μm.

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Depending on the particle size and streaming profile, the particle stream follows the curvature of the electrodes (3.75 V at 5 MHz) until hydrodynamic force exceeds the dielectrophoretic. Smaller particles pass the curved electrodes earlier. The subdivision of the channel supports the spatial separation of the particle fractions (Figure 1A). A decrease of the flow rate down to 400 μm/s resulted in repelling of all particles without a separation, whereas higher flow rates of 1300 mm/s reduced the distances between the escape positions / separation output (see Figure 1A).

According to eq. 2, it is also possible to separate particles of equal size but with different dielectric properties. We were able to separate a mixture of *Jurkat* cells (10-15 mm in diameter) from equally sized latex beads. Due to their

Table 2

Separation Efficiency of Lymphocytes from Red Blood Cells at Different Flow Rates Using Straight Strip Electrodes*

Pumping Values (μL/h)	Streaming Velocity (μm/s)	Voltage H(Vms)	Frequency (kHz)	Filtration of Red Blood Cells	Filtration of Jurkat Cells
			PBS/Inositol, 0.32 S/m		
4	62	3	630	No	Yes
4	62	4	630	Yes	Yes
4	62	4	1260	No	Yes
8	123	4	630 - 1260	No	No
8	123	5	630 - 1260	No	Yes
10	154	5	630 -1260	No	No
10	154	6	630 -1260	No	Yes
			PBS, 1.5 S/m		
10	154	4	630 - 10000	No	No
10	154	5	630 - 10000	No	Yes
15	240	5	1260 - 10000	No	No
15	240	6	1260 - 10000	No	Yes

* The experimental conditions are identical described in Figure 2. Filtration efficiencies of more than 98% were termed with "yes," otherwise, "no."

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higher effective conductivity and permitivity, cells in physiological media experience a lower dielectrophoretic force per volume than latex beads.[16] Therefore, we used straight electrodes with larger relation of width to height (Figure 1b) instead of the thinner bow electrodes. Successful separations were performed in 0.5 S/m PBS/inositol solution at a flow rate of 75 μm/s and electrode drive of $3V_{rms}$ at 5 MHz. In addition, we were able to separate *Jurkat* cells from red blood cells due to the differences in size (10-15 μm and 5-7 μm in diameter, respectively) and dielectric properties[32] of both species (Table 2).

Frequencies of 630 kHz to 1.26 MHz were effective for separation in 0.32 S/m PBS/inositol solutions. In PBS (1.5 S/m) the frequency could be turned up to 10 MHz without influence on the separation result. In both cases the *Jurkat* cells were deflected by nDEP whereas the erythrocytes passed the electrodes (Figure 2). Table 2 summarizes the experimental conditions under which a separation of erythrocytes and Jurkat cells could be achieved.

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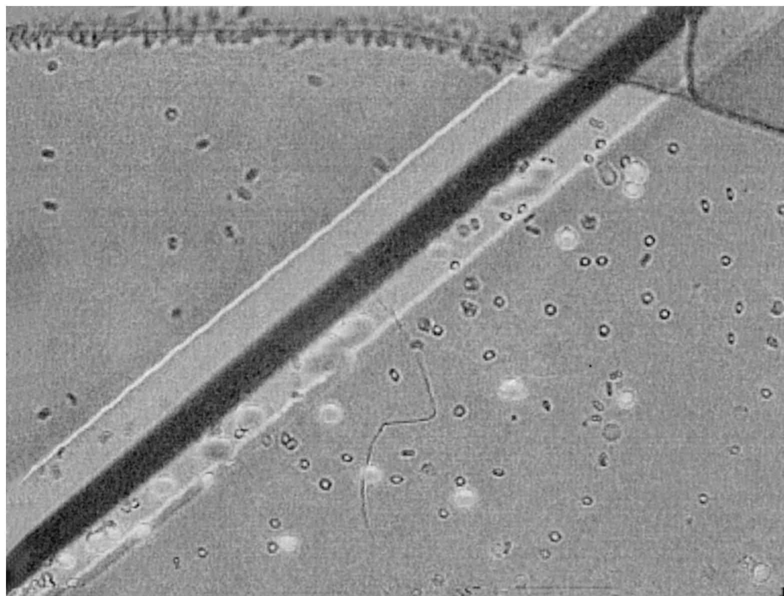

Figure 2. Separation of lymphocyte from whole blood components using straight strip electrode pairs. A mixture of *Jurkat* cells ($2.5*10^6$ /mL) and whole blood ($1.8*10^7$ red blood cells/mL) in PBS/inositol solution were introduced into the micro structure. For better differentiation the *Jurkat* cells were labelled using 0.01% fluorescein diacetate in separation buffer. The flow rates were adjusted to between 2 and 15 µL/h. The electrodes were driven using frequencies between 670 kHz and 10 MHz and amplitudes of 3-6 V_{rms}. The separation efficiency is given in Table 2.

DISCUSSION

We show that dielectric field-flow fractionation on-a-chip can be achieved using a simple electrode geometry. Separation of bioparticles can be done either according to their size and/or to dielectric properties. Both is of great impact for biotechnological, medical, and chemical applications. In contrast to other dielectric based separation methods[24-28] using the fluid profile, these microelectrode devices allow continuous spatial sorting of particles. Since the frequency and amplitude of the electrode drive can be easily controlled, the devices are easily tunable. The integration of several fluidic outputs could complete this microdevice. Separation could also be based on the balance of dielectrophoretic and inertial or other volume forces (instead of Stokes forces in a flow).

Microstructure based biotechnology is a fascinating and rapidly developing field of research which will extend into the next century. Right now, there is progress in integrating these microtools into complete hybrid systems - so called "lab-on-a-chip."[32-35] Dielectrophoretic microtools have great potential for life science applications since they allow manipulation of single cells and particles in suspension .

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