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Recent advances in microparticle continuous separation

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Abstract: Recent advances in microparticle separation in continuous flow are presented. It is intended for scientists in the field of separation science in biology, chemistry and microsystems engineering. Recent techniques of micron-sized particle separation within microsystems are described with emphasis on five different categories: optical, magnetic, fluidic-only, electrical and minor separation methods. Examples from the growing literature are explained with insights on separation efficiency and microengineering challenges. Current applications of the techniques are discussed.

1 Introduction

Over the last ten years, point-of-use microreactors or point-of-care diagnostic tools have helped to reduce the need for intensive macrochemical plants or long diagnostic procedures. These so-called 'labs on a chip' are built out of several different modules, each of them often achieving similar process functions as a macrochemical plant or a laboratory. Among these modules, the 'separation modules', selectively sort different kinds of particles, often immediately after synthesis or before analysis processes.

Applications of separation techniques at the microscale are broad and versatile. Particle separation is a necessary preparation step in most biological microassays and common in microchemical processing.

In the biomedical field, separation techniques are used for fundamental cell studies where isolation of pure cell types is essential. Separation is a key activity of diagnostic and analysis tools. The field of theranostics, the new driver for bedside diagnostics, and treatment is particularly demanding in separation technology. The management of HIV disease in patients is a typical example in theranostics. Although HIV diagnostic relies on separation of specific human T-lymphocytes from whole blood, its treatment is also adapted in function of the advancement of the disease. Therefore separation is continually needed through the length of the disease [1]. Prenatal diagnosis to determine the outcome of pregnancies and detect conditions that may affect future pregnancies has risen as another big issue among the broad public. Conventional prenatal tests are using invasive techniques, such as amniocentesis or chorionic villus sampling, which can result in abortion or growth abnormalities. Non-invasive prenatal diagnosis can be performed by separating rare foetal cells from maternal blood, consequently avoiding the potential risk of amniocentesis [2–4]. Microseparation techniques are also needed for the detection of cancer cells or the accumulation and counting of various types of cells and bacteria. As a particular example, testicular stem cell transplantation is a potential solution for the recovery of fertility in testicular cancer survivors [5]. However, the effectiveness of the transplantation is based on the number of stem cells transplanted. Testicular stem cells represent a very small fraction of testicular cells collected for this operation (0.03% in the mouse). Enrichment of stem cells is therefore highly desirable. Moreover, there is a risk of contamination by carcinogenic cells in a testicular cell suspension leading to a malignant relapse in the treated patient. Cell sorting is a solution to circumvent the contamination.

Tournaye *et al.* [6] show that the currently available macrotechniques do not allow the total depletion of malignant cells and subsequently illustrates the need for a finer gentle separation of cells.

For agrochemical, cosmetic and pharmaceutical companies, these techniques permit, after a chemical reaction, the separation, at production level, of solid products for post-treatment. In the food industry, potentially harmful bacterial activity is carefully monitored. Separation and enrichment of bacteria is necessary preliminary to analysis [7]. Monitoring of biological weapons is an important activity in the defence sector. In this field, separation is required to detect threatening agents such as Anthrax [8]. All these examples account for the tremendous need of portable, low-cost separation microdevices in a wide range of fields.

The aim of this paper is to provide a critical review of the current technologies available for continuous microparticle separation within microchannels. Because of the growing rate of publications in the field, this review focuses mainly on advances in the last five years. This article describes radical new ways to separate particles, improvement of older methods and novel, cost-effective manufacturing methods. In comparison to recent reviews on particle separation, the emphasis given here is on continuous flow techniques. Although techniques such as electrical, optical or magnetic separations are used in non-continuous flow, the main advantages of continuous-flow devices lie in profitability, minimum residence time under harmful conditions for separated particles, flexibility and integration of downstream functions. Special attention is given to a parallel, simultaneous separation of many subpopulations, providing potential high-throughput and high-recovery processes. This review focuses particularly on the passive separation of particles, not involving external decision making, and does not treat single-cell separation or trapping.

Different separation methods are highlighted and illustrated by relevant examples: (1) optical separation, (2) magnetic separation, (3) hydrodynamic separation, (4) electrical separation and (5) other types of separation.

2 Recent developments

This section describes different kinds of separation techniques within microchannels currently developed as prototypes or already commercially available. A chart summarising the techniques presented in this review is shown in Fig. 1.

2.1 Optical separation

A type of optical separation technique attracting attention is the new field of optical fractionation. Dholakia and coworkers [9] and Grier and coworkers [10] gave proof of the concept of optical fractionation. This method uses the recent advances in manipulation of optical tweezers. Optical tweezers is a manipulation tool developed by Ashkin, where a tightly focused single laser beam is used to trap a single particle [11]. Optical tweezers have been widely used since then to trap single particles in microfluidic systems. Advances in optical manipulation allowed the creation of

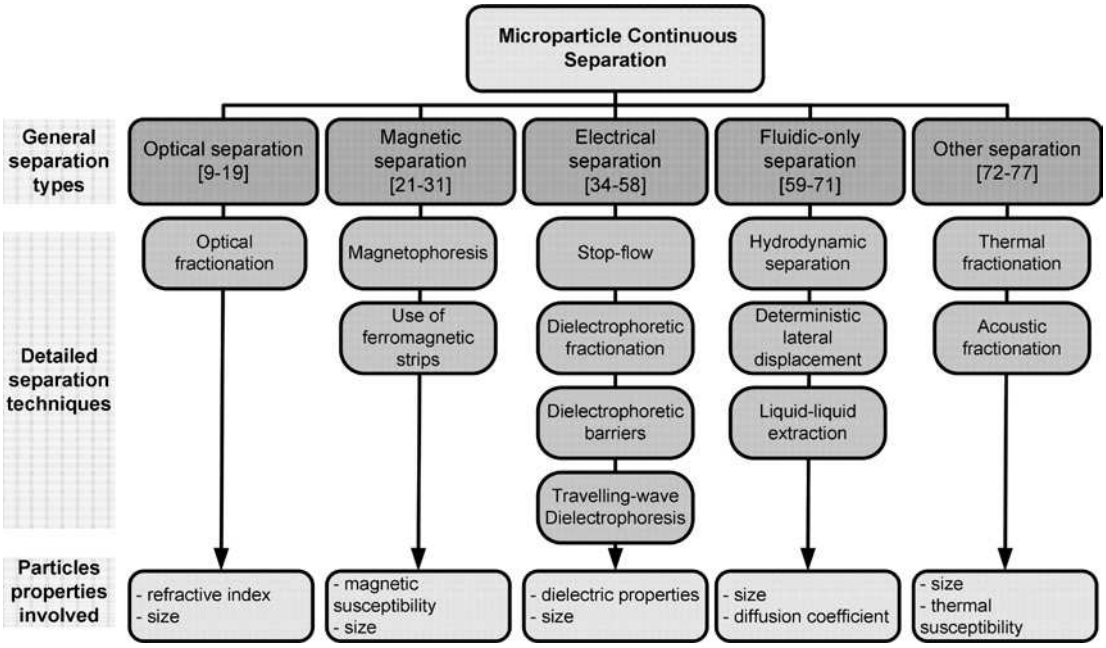

Figure 1 *Chart summarising the different microparticle continuous separation techniques detailed in this review*

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three-dimensional arrays of light traps (optical lattices) using holographic optical tweezers, generalised phase contrast or diffractive optical element and multi-beam interferences for example. In optical fractionation, these methods allow the generation of an optical gradient force called potential energy landscape, which can deflect particles from their natural pathway according to their size or intrinsic properties. Particles experiencing sufficient optical force are kinetically locked in arrays of optical tweezers, whereas other particles flow along the natural stream, as shown in Fig. 2. Dholakia's team reported with this method a sorting efficiency of up to 95% [12]. It was shown in [13,14] that flashing lattices, realised by amplitude modulation of the laser, can help to reduce the congestion of particles during the separation process. Optical fractionation is patented and yet commercially available from BioRyx200 by Arrys Inc [15, 16].

In optical fractionation, the light patterns can easily be tuned making this method very flexible and responsive to new environments. On the other hand, the systems described are able to separate only one species from a mixture. In [17], however, separation of four different colloidal species is demonstrated using an optical lattice created with an acousto-optic deflector (AOD). An AOD is used to spatially control laser light and produce a complex potential energy landscape. In this experiment, a mixture of particles with four different diameters (2.3, 3.0, 5.17 and 6.84 μm), pumped through a microchannel is first focused into a single particle stream by an optical funnel. A following exit ramp with decreasing intensity releases the particles at different heights depending on their size. The smaller particles, which experience the weaker optical trapping force, leave the ramp first. Therefore a continuous parallel separation of four subpopulations is achieved. This method shows a near 100% pure sorting efficiency (all particles retrieved in their own specific group) and a

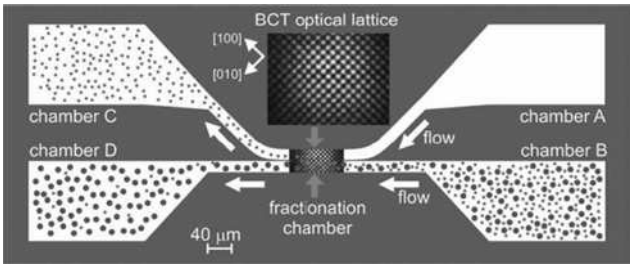

Figure 2 *Optical fractionation concept*

3D optical lattice is introduced in the shared part of the chambers A, B, C and D allowing the separation of species according to their size or optical properties

3D optical lattice is reconfigurable which allows an easy updating of the selection criteria

Scale bar is 40 μm long

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throughput of 40 particles per second. Although this throughput does not reach the throughput of conventional fluorescence-activated cell sorters (FACS), it is claimed that optical fractionation has the potential for higher throughput and might find niche application areas [18]. FACS is a common separation technique where particles are optically interrogated one by one and directed into different outlets depending on the interrogation result. However, FACS requires very expensive apparatus and fluorescence labelling.

Optical separation presents for the most part major advantages in terms of sensitivity, selectivity as well as versatility and permits the sorting of particles in a continuous flow. However, the need for laser sources hinders the easy portability of such systems although the integration of vertical cavity surface emitting lasers arrays offers real opportunity [19]. A real need for miniaturisation of the apparatus is necessary in this field. The scale up of such optical and fluidics microsystems is also a major issue.

2.2 Magnetic separation

Magnetism has many uses in biological and chemical assays such as in drug labelling and targeting, transport, mixing and also separation [20, 21]. In the magnetic separation method, sorted particles have either intrinsic magnetic properties or are labelled with magnetic beads. In the case of an immunological separation, coated antibodies bind specifically with antigens of the targeted cells in an operation called labelling.

The concept of a conventional magnetic separation device is a straightforward and long-established process: a magnet is placed in the vicinity of a column containing the cells to be separated. Magnetically labelled cells are retained in the column, whereas non-labelled cells will be flushed with the buffer allowing the immunological separation of species. The column is removed from the magnet in a second step and flushed to allow the collection of the sorted particles. This kind of separation is termed magnetic-activated cell sorting (MACS). MACS is also a method patented by Miltenyi Biotec but is widely available commercially under other names from manufacturers such as Dexter or StemCell [22, 23]. The efficiency rate of the protocol is often better than 95% [24].

The concept of MACS has been demonstrated at the microscale in order to reduce the volume of the apparatus and add other functions downstream. Benefits and drawbacks arise from the miniaturisation. Miniaturised magnets allow stronger and more precise magnetic fields because of the vicinity of magnets to the microchannels. On the other hand, the fabrication

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of integrated micromagnets requires numerous and expensive manufacturing steps [21, 25]. The use of permanent magnets allows for portable (no electric connection) and autonomous devices; however, if the separation is not fully continuous, the removal of the magnet to flush the channel can cause difficulties. Electromagnets have the advantage to be easily switched on and off; however, their fabrication is more complicated and hampers the energetic autonomy of the system. An example of integrated microMACS was presented by Deng *et al.* [26], in which arrays of posts were manufactured on the bottom of a microchannel to trap magnetic particles. This device showed more efficiency compared with its macroequivalent. However, MACS is a batch process and might slow down further downstream analysis and limit the collection yield.

Some attempts have been made to fabricate continuous flow magnetic separation devices. A technique called 'on-chip free-flow magnetophoresis' was demonstrated by Pamme *et al.* [27, 28]. In this example, a mixture of different magnetic particles and non-magnetic particles is aligned along the wall of a microchannel. A micromagnet placed upon the channel provides a non-homogeneous magnetic field gradient transverse to the laminar flow. Depending on their size and magnetic properties, particles are deflected more or less from their path. The addition of spacers allows the collection of particles in separated outlets as shown in Fig. 3. A continuous-flow, magnetic separation device for the enrichment of foetal cells from maternal blood has been described in a patent by Blankenstein [29]. This device uses the same working principles as the previous example although the cells require to be labelled.

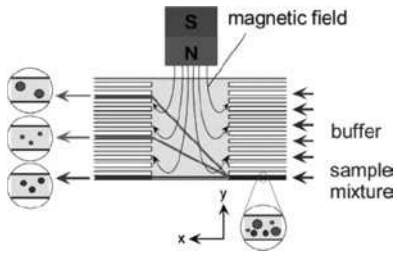

Figure 3 *Magnetic separation principle within a microchannel*

Mixture of different magnetic and non-magnetic particles is injected in a microchannel
Depending on their size and magnetic properties the particles will be more or less deflected from their natural path due to the magnetic field
Non-magnetic particles will not be deflected
Addition of spacers permits the independent recollection of different species [27]
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Another type of continuous magnetic separation was demonstrated by Inglis *et al.* [30]. In this example, some ferromagnetic strips fabricated in a microchannel provide an array-like magnetic field pattern at a given angle to flow direction. Cells selectively tagged with magnetic nanoparticles deflect from the flow path to follow the strips. This technique convincingly demonstrates the separation of white blood cells (WBCs) from human blood as shown in Fig. 4. In another example, Han and Frazier [31] used an external magnetic field to activate a ferromagnetic wire integrated to a microchannel as shown in Fig. 5. Depending on their internal properties, particles will be repelled or attracted by the wire and thus collected at different outlets.

Magnetic separation is very interesting in terms of portability and autonomy. Magnetic fields have never been reported to damage biological particles and therefore allow the gentle sorting of cells. However, the manufacturability of the magnetic-activated separation mechanism can hamper the development of such devices.

2.3 Electrical separation

Originally, most of the first separation techniques for microparticles used electrical forces. Indeed, electric-field-based manipulation is well suited at the microscale because of the ease with which high electric fields can be produced with micron size gaps and voltage of several volts only. Furthermore, in the last ten years, the diversification of micro-electromechanical systems into areas such as chemistry and biology has increased the tendency to combine electrical microsystems within microchannels. There are two main types of electric field-based manipulation, depending on the properties of the particles to be sorted. Electrophoresis, the movement of charged particles in a uniform electric field, is a very well known technique to separate and transport different kinds of charged particles. Detailed reviews

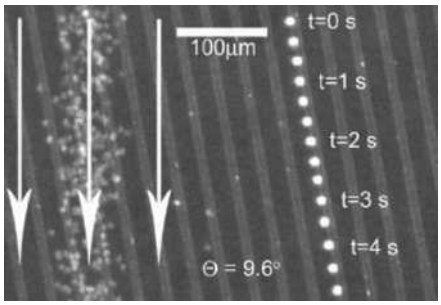

Figure 4 *Separation of red blood cells from white blood cells with magnetic strips*

Superposed images showing the separation of a tagged leucocyte from untagged red blood cells using magnetic stripes
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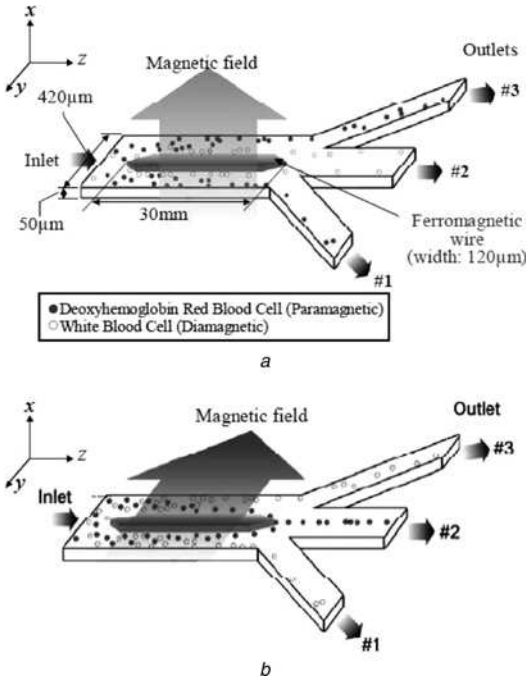

Figure 5 *Separation of red blood cells from white blood cells with a ferromagnetic wire*

Illustration of a magnetophoretic separator
Ferromagnetic wire is incorporated along the length of the microchannel
In the diamagnetic capture mode, an external magnetic field is applied normal to the *x*-axis of the microchannel
In this case the red blood cells will be deflected from the ferromagnetic wire and will flow through the outlets 1 and 3
In the paramagnetic mode, the external magnetic field is applied normal to the *y*-direction of the microchannel, which forces the white blood cells to be deflected at this time from the ferromagnetic wire
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a Diamagnetic capture mode
b Paramagnetic capture mode

on electrophoresis can be found in recent literature [32]. Dielectrophoresis, often confused with electrophoresis, is described by Pohl as 'the translational motion of neutral matter caused by polarisation effects in a non-uniform electric field' [33]. This technique has attracted scientific attention because of its ability to manipulate neutral–but polarisable–particles.

Dielectrophoresis has first been applied to separate cells according to their size or dielectric properties. A number of early articles report the separation or the enrichment of particles using castellated or interdigitated planar electrodes providing an inhomogeneous electric field [34, 35]. These techniques, sometimes referred as stop-flow techniques, often relate to binary separation where a mixture is split into two subpopulations, one usually retained in the channel [36]. Sometimes particles approaching DEP affinities and size are difficult or impossible to separate. To overcome this limitation, Yang *et al.* [7] coupled an antibody recognition and a dielectrophoretic stop-flow technique. In this example, antibodies specific to the targeted bacterial cells are coated above interdigitated DEP arrays, isolated by a thin layer of SiO_2. The mixture of bacterial cells is injected into the channel. When the DEP is actuated, all the cells are concentrated above the electrodes against the flow. During this time, the targeted cells bind themselves with the antibodies. When the DEP is deactivated, the unbound cells flow away, leaving the targeted cells separated in the channel. These techniques report high efficiencies, close to pure recollection of each type of particle and are adopted for high product value and low volume application. Some drawbacks can nevertheless be reported. First, cells are often trapped with positive dielectrophoresis (pDEP) and without careful control of the applied voltage and frequency, might experience very high electric fields, that can damage or destroy them. A guide to the values of frequencies and applied electric fields affecting the cells' viability is given in [37]. In [7], the cells trapped by pDEP stay viable, but an anomalous protein production is reported. Secondly, in general, only one subpopulation can be extracted from a heterogeneous mixture. Finally, even though fluid is continuously drawn through the chip, voltages often have to be turned on and off to collect the separated subpopulations, which can hamper the continuity of the process.

Hydrodynamic forces have been coupled to dielectrophoresis to produce continuous particle separation. This technique uses electrodes to levitate particles to different heights depending on their dielectric properties. The addition of a parabolic flow allows the particles to be dragged away at different velocities. Separation of erythrocytes and latex beads was performed by Rousselet *et al.* [38]. The team of Gascoyne reported separation of different types of leucocytes with a purity after separation up to 98% [39]. This technique uses negative dielectrophoresis to levitate particles above the electrodes and thus protects vulnerable biological particles from high electric fields. However, it has limitations in separation performance. Cells of one type can contaminate the cell population of another type [39].

Other kinds of electrode arrangements have been successfully tested. Choi and Park [40] proposed a trapezoidal planar electrode array providing a specific electric field geometry in a microchannel. Particles focused on one side of the channel are deflected more or less by the electric field depending on their properties. At the end of the microchannel, the addition of spacers helps to collect different particles. Li and Kaler [41] reported an ingenious 'isomotive' electrode arrangement for continuous-flow separation. Isomotive refers to a specific electric field where a

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particle experiences a constant dielectrophoretic force everywhere in the field, a configuration described by Pohl and Pethig [42]. This geometry provides a better separation of species, based only on their dielectric properties.

The use of dielectrophoretic barriers has also been widely and successfully demonstrated. A dielectrophoretic barrier refers to electrodes mounted at the top and bottom in a microchannel. This configuration creates an electrical field barrier which deflects particles from the direction of fluid flow. To separate two different species, the frequency and magnitude of the voltage is set such that one species exhibits dielectrophoretic forces and can be deflected, whereas the other species go through the electrical field barrier without experiencing any force. Fuhr and coworkers [43] were the first team to report such an arrangement in 1998 with serial and parallel non-contact manipulation at high velocity. From 1998, several patents and articles have been published by the same team in collaboration with the German company Evotec Technologies and includes treatment, separating, sorting or confinement of diverse kinds of biological or synthetic particles [44–46]. In a publication by Schnelle, a particle separation is reported using dielectrophoretic forces engendered with curved electrodes and hydrodynamic forces [47]. In [48], a detailed experimental and theoretical study of DEP barriers shows a strong dependency between the channel height and the threshold velocity above which particles may penetrate the barrier. Decreasing the channel height leads to better separation efficiency. In [49], a system named NanoVirDetect is described. This versatile module uses standardised biochips packaging and has individually tunable functions such as focusing, separation, holding of micron or submicron-sized particles and thus can be adaptable to a large number of different particles. Electrodes are made out of nanoporous materials to increase the electrode capacitance. This not only expands the frequency range of dielectrophoretic deflection but also allows the system to be used with higher conductivity medium which is of high interest especially with biological samples often flowing in relatively high conductivity liquids.

Other teams report also the successful implementation of dielectrophoretic sorters, using improved DEP barriers [50–52]. Although the new configurations double the maximum flow speed compared to a classical strip-like electrode, plane electrodes show more temperature rise than the classical geometry, which is a major drawback for sensitive biological or chemical applications.

A focusing device has been fabricated by Morgan *et al.* using a quadrupolar electrode arrangement [53]. In [54], a tripolar electrode arrangement for separation purposes is demonstrated. In this application, two tripolar strip-like electrode arrangements are embedded on each side of the bottom of the channel. Thus, particles experiencing positive DEP are expected to flow near the electrodes, whereas particles with negative DEP affinity are focused along the central axis of the channel. This simple geometry might not be so proper for continuous flow, as particles with positive DEP will probably stick to the electrodes if the flow is too slow, hampering the collection of the species. A high-speed cell-dipping system is proposed in [55] and shown in Fig. 6. A classic pair of strip-like electrodes is mounted at the top and bottom of a 20 μm high microchannel. A large amount of particles is transferred from one reagent to the other in less than 0.5 s at a flow speed of 300 $\mu m\ s^{-1}$. A large diffusion of the dye shown as the second flow stream can be noticed after the first barrier and might be because of some electrokinetic forces or a perturbation in the flow caused by the accumulation of particles on the tip of the barrier. The addition of a spacer between the two streams, as stated in Fuhr's patent, can help to avoid this perturbation [44].

In recent years, new ideas have been developed to enhance performance of DEP separations systems. A different channel geometry for dielectrophoretic focusing of particles is presented by Leu *et al.* [56]. In this device, a mixture of particles is separated in a single particle stream with a combination of DEP

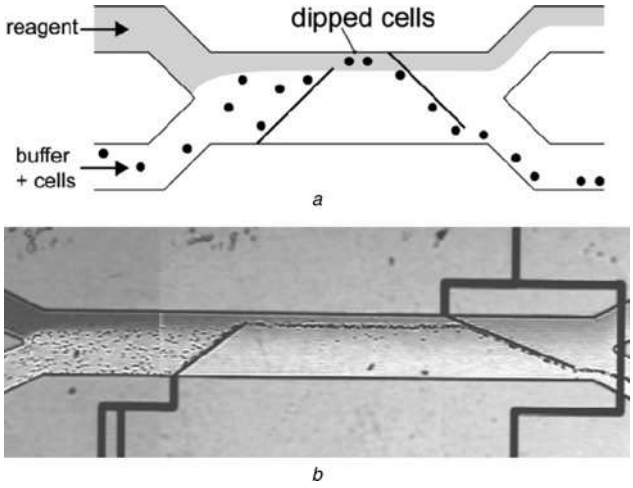

Figure 6 *DEP barriers used for cell dipping*

a Schematic diagram illustrating the concept of cell dipping
Electrodes are mounted at the top and bottom of the microchannel
Population of cells is introduced in one of the inlets
Electrodes, once activated, divert cells from their natural path
Cells are guided to the reagent and from the reagent back to the buffer
b Photograph of the experiment
Transit time in the reagent is 0.3 s [56]
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forces and hydrodynamic forces in a funnel-like channel. Although the result is obviously useful in term of particles analysis, the electrodes need to be embedded on the channel side walls, which is a laborious fabrication step. An interesting insight to improve separation is proposed by the team of Hu [57]. In this approach, labelling of cells with particles that differ in polarisation response enhances the sorting activity. A flow speed of 300 $\mu m\ s^{-1}$ in a 20 μm high channel can be obtained. However, this process requires a supplementary step of labelling. Park *et al.* [58] reported a fan-shaped like electrode geometry creating a unique asymmetric electric field gradient. In this concept, the applied field is continuously varied because of a half-circular type of microchannel and this geometry increases the discrimination power of the device. However, this approach leads to a higher temperature rise.

The use of so-called electrodeless DEP was reported. In this method a homogeneous electric field is created within a microchannel by introducing electrodes at the inlet and outlet. Insulating obstacles are placed within the channel rendering the electric field non-uniform, which leads to the creation of a dielectrophoretic force. Electrodeless DEP prevents electrode fouling and electrode destruction sometimes reported in DEP manipulation. Originally this principle was applied with arrays of insulating posts in a microchannel [59, 60]. In this kind of arrangement, particles sensitive to DEP are trapped between posts, whereas others flow along. This results in a non-continuous system as the voltage has to be turned down to recollect the trapped particle. Using the same concept, Barbulovic-Nad *et al.* realised an original device incorporating an oil droplet at one point of the side wall of a microchannel. Particles approach the base of the droplet in the same fashion and are diverted more or less at the top of the bubble in the highest electric field zone. This allows the parallel collection of different particles [61]. The separation can be tuned by changing the size of the droplet. Renaud and coworkers [62] proposed recently a device embedding large electrodes insulated from the main microchannel by thin, dead-end channels. These effective methods nevertheless require high voltages and create relatively high electric fields, sometimes exceeding the threshold of safe cell manipulation.

Another kind of electrical separation, known as travelling-wave dielectrophoresis (TW-DEP), has been demonstrated. In TW-DEP, particles are transported across arrays of interdigitated electrodes being energised with sinusoidal electric signals [36]. A travelling electric field, produced by phase shifting of the signals, induces a dipole moving in a direction perpendicular to the direction of the electrodes array. As the speed and direction of the motion depends on the particle's dielectric properties, separation of different species can be achieved. Recently, Pethig *et al.* [63] demonstrated the separation of T-cells from monocytes with a superposition of TW-DEP signals. The technique of signal superposition leads to a reduction in the length of the electrode array needed to achieve separation.

To summarise, the use of an electric field for particle separation is a relatively old, established technique. Among the different kinds of microdevices using electric field-based separation in continuous flow, examples include DEP fractionation, DEP barriers and TW-DEP. Many different electrode arrangements related to these reliable and efficient techniques can be found in the literature. Moreover, it has been demonstrated that dielectrophoretic barriers have the potential for greater exploitation. Nevertheless, the use of electrodes introduces an important fabrication step which increases the cost of the microdevice and might hamper mass-manufacturability.

2.4 Fluidic-only separation

To tackle the problem of outer force field requirement in separation devices, researchers proposed recently some techniques which can be termed 'fluidic-only' separation. In these separation methods, particles are sorted exclusively by size, often using only the geometries of microchannels and hydrodynamic forces.

In 2004, the team of Yamada and Seki proposed a pinched-flow separation device [64]. The concept is shown in Fig. 7. A mixture of different-sized particles is injected into a buffer. With the help of another fluid, particles are aligned to a sidewall of a pinched segment, which is subsequently broadened; at this point, hydrodynamic forces act differently on particles, deflecting the small ones away from the big ones. Flow rates of the two inlets and the angle between the two segments are determining factors to sort the different sizes of particles. In 2005, Yamada *et al.* proposed a two-step technique called 'hydrodynamic filtration' to get rid of the need of a second buffer to control the separation; concentration and classification are performed at the same time [65]. A fluid containing particles is injected in the main microchannel having multiple branch points and side channels. Particles do not enter the subchannels when the relative flow rates at the branch point are low. This permits in a first step the withdrawal of the liquid and the concentration of the cells. Increasing stepwise the relative flow rates at each branch point allows the collection of firstly small particles and secondly large particles. The size limit of the sorted particles is determined by a precise distribution of the flow rate at each branch. This technique was proven

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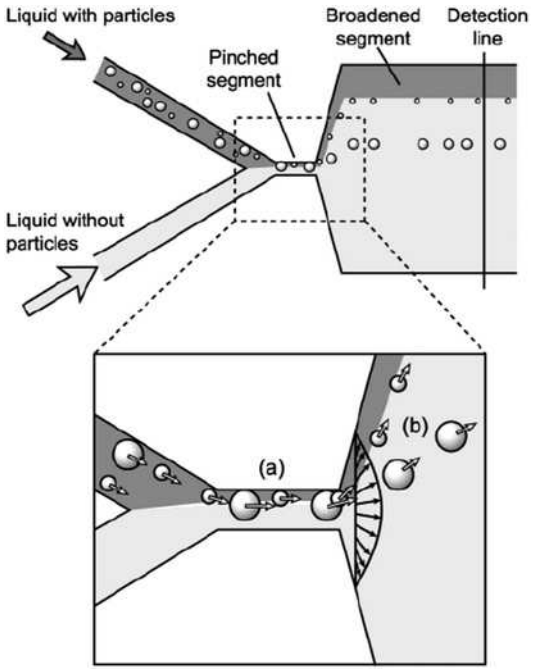

Figure 7 *Separation of different-sized particles in a pinched segment*

Schematic diagram illustrating the principle of pinched-flow fractionation

a In the pinched segment, particles are aligned to one sidewall regardless of their sizes by controlling the flow rates from two inlets

b Particles are separated according to their sizes by the spreading flow profile at the boundary of the pinched and the broadened segments

Reprinted with permission from [64]

by the enrichment of WBCs. Although, RBCs remain present in the fraction of interest, the relative concentration of WBCs in relation to RBCs was increased by 29 times. Fluidic-only separation techniques are particularly relevant in the case of blood plasma separation, where the axial migration of blood cells in microchannels below 300 μm is used for sorting [66–68].

Another technique called deterministic lateral displacement was set up and tested by Huang *et al.* [69]. In this method, microposts are placed in rows within a microchannel. Each row of posts is shifted from the other by a distance which partly sets the critical separating size. The asymmetric bifurcation of laminar flow around obstacles leads particle to choose their path deterministically on the basis of their size. A small particle will have a zigzag displacement path, whereas a large particle will tend to flow straight. After a number of rows, the particles can be collected separately. This method is cost effective and can separate in parallel a large number of different-sized particles with a precision of up to 10 nm. The method was successfully tested for the separation of WBCs and RBCs [70]. However, because of a high risk of clogging because of the numbers of posts employed and the narrow gaps between them, Huang and coworkers [71] presented later an optimised device including additional regions alongside the active sorting arrays. These additional regions collect the larger particles that have been sorted. Although these regions also contain pillars to maintain the same pressure drop across the system, the gaps between the pillars are made larger to avoid clogging. This device removed all the particles larger than 1 μm from whole blood, allowing the collection of pure undiluted plasma. The latest technique is patented in [72]. Recently Li *et al.* labelled CD4+ T helper lymphocytes with 25 μm polystyrene beads in a mixture of WBCs injected in a lateral displacement system [73]. Up to 91% of the specific lymphocytes were therefore separated from the other kind of WBCs. This article demonstrates the possibility of cell subtype sorting with the continuous-flow lateral displacement method.

'Liquid–liquid extraction' is another fluidic-only separation technique widely used in the chemical and biological industries. The concept exploits the preferential affinity or differential diffusion coefficients of solid compounds in a laminar flow of two liquids streams. H-filters developed by Yager *et al.* [74] have used this technique to extract molecular analytes from whole blood. Nam *et al.* [75] proposed a microsystem incorporating this technique. In this device, the injection of cells in a thin stream between two phases allows the cells to partition in the preferred solvent. The sorting efficiency of the microdevice was reported to be 97% which is higher than its macroscale counterpart. However, it is not a versatile technique, as only two types of particles can be sorted at the same time using sometimes very specific solvents. Liquid–liquid extraction is particularly adapted to chemical extraction, such as extraction of hydrocarbons from oil in the petrochemical industry or extraction of organic compounds from extraterrestrial dissolved minerals in space exploration [76, 77].

In conclusion, these fluidic-only separation techniques have the great advantage of not requiring any outer field. Therefore the manufacturing of these devices relies only on microchannel networks. Manufacturing of these channels can be achieved via hot-embossing or microinjection moulding. This holds the potential for cheap and mass-manufacturable devices, which is particularly relevant in the case of point-of-care devices. However fluidic-only separation devices, with the exception of the last example, can separate particles by size only.

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2.5 Other types of separation

A system using an outer thermal field is described in [78]. In this device, a temperature difference is applied across the microchannel. A separation between particles occurs because of the difference of thermal diffusion coefficient. This technique does not have a large separation efficiency and particles are not easily collectable from different outlets.

Acoustic separation can also be found in the literature [79–81]. The last reference demonstrates a separation efficiency near 100% which is comparable with conventional blood cell separation by centrifugation. The three-stage separation device is shown in Fig. 8. Acoustic techniques can be adapted with other techniques to form hybrid systems. Indeed, Wiklund *et al.* [82] presented recently a device combining short-range DEP manipulation with long-range ultrasonic wave (USW) manipulation as shown in Fig. 9. In this device, a DEP force is induced by an electric field between co-planar electrodes at the bottom of the microchannel. A transducer made of a piezoceramic element and a polymethylmethacrylate (PMMA) refractive edge is placed on the glass cover of the chip. The two combined forces permit particle trapping, sorting, concentration and separation with a selectivity up to 90%. This method had been previously described to some extent in a patent [83]. In the latter work, USW was also proposed as a means for transportation of particles. The DEP/USW manipulation looks promising for high-throughput (because of the use of long-range USW forces) and high precision (because of the use of short-range DEP forces) applications.

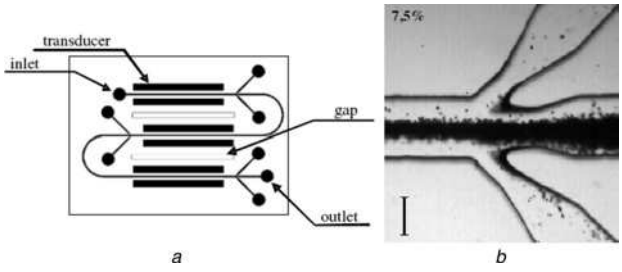

Figure 8 *Sorting using ultrasonic field*

a Schematic of a three-stage microdevice
Particle separation here occurs on each stage of the separator
On each stage, most of the particles in the solution being affected by the ultrasonic standing waves are directed to the side channels of the flow splitter, whereas the diluted solution flows via the central channel and passes to the second stage for further dilution and separation
b Photograph of the experiment in a one-stage microchannel
Particle concentration is 7.5% in volume
Scale bar is 100 μm
Reproduced with permission from reference [78]

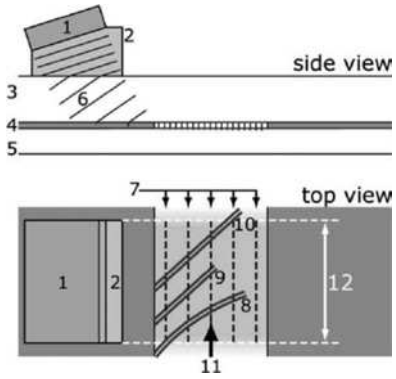

Figure 9 *Sorting using ultrasonic standing wave coupled with dielectrophoresis*

Illustration of the top and side views of the device showing an ultrasonic transducer on a dielectrophoretic chip
Transducer is composed of a piezoceramic plate (1) and a PMMA refractive element (2)
Three-layered chip (3, 4, 5) has three paired dielectrophoretic electrodes (8, 9, 10) on the bottom substrate
Ultrasonic standing wave is perpendicular to the flow (11)
Net force acting on particles is a combination of the ultrasonic wave force, the flow force and the DEP force [81]
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3 Conclusions

Recent advances in continuous microparticle separation have been presented in this paper. The extensive literature present in the field as well as the wide range of applications, illustrates the tremendous interest drawn by continuous microparticle separation. Separation is an important activity in the biological, medical and defence fields, to name but a few. Microparticle separation illustrates well the joined effort of different scientific communities that characterises the route towards integrated lab-on-a-chip devices.

High throughput and high efficiency characterise optical fractionation in continuous flow. Magnetic separation is a gentle separation technique providing potential autonomy for a portable device if used with a permanent magnet. DEP separation is a well-established, robust and reliable technique applicable to a wide range of applications, not only separation but also focusing or dipping. Fluidic-only techniques are gentle, label-free techniques easily mass manufacturable. Other separation techniques such as thermal or acoustic separation can prove useful when coupled with other techniques to form hybrid systems which can manage a wide range of functions.

All techniques presented in this review have the potential for immunological separation, although this is not demonstrated for all. The possibility to label cells with various kind of beads (magnetic, polystyrene) using antibody recognition, opens up the

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future of immunological separation within microchannels. Some of these techniques already have commercial applications, whereas some of the others will undoubtedly find new applications. In general, lab-on-a-chip modules will not always replace conventional laboratories but might find niche markets. In this paper, we have also highlighted new manufacturing trends such as the integration of optical components on a chip and development of cheap, highly mass-manufacturable components.

The future of separation techniques may lie in hybrid techniques combining different methods for better accuracy, efficiency and versatility. Reducing the size of the apparatus surrounding the chip remains by itself a challenge to be overcome for separation applications to be commercially viable.

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