

On-chip processing of particles and cells via multilaminar flow streams

Mark D. Tarn · Maria J. Lopez-Martinez · Nicole Pamme

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Abstract The processing of particles, cells, and droplets for reactions, analyses, labeling, and coating is an important aspect of many microfluidic workflows. However, performing multi-step processes is typically a laborious and time-consuming endeavor. By exploiting the laminar nature of flow within microchannels, such procedures can benefit in terms of both speed and simplicity. This can be achieved either by manipulating the flow streams around the objects of interest, particularly for the localized perfusion of cells, or by manipulating the objects themselves within the streams via a range of forces. Here, we review the variety of methods that have been employed for performing such “multilaminar flow” procedures on particles, cells, and droplets.

Keywords Continuous flow · Microfluidics · Microparticles · Cells · Droplets · Trapping · Deflection · Multilaminar flow

Introduction

The ability to control the movement of particles and cells, or the fluidic environment around them, is an important factor in many microfluidic applications. Such temporal and spatial control can be of particular value for cell studies [1, 2], with the ability to expose cells to stimuli and monitor the effects. Similarly, microfluidic techniques allow the precise manipulation of microparticles, which are extensively employed as solid supports for performing separations and immunoassays because of their small size, large surface-to-volume ratio, and

variety of (bio)chemical surface functionalities [3–5]. Particles and cells are commonly handled in two ways: either by being trapped within a channel and consecutively exposed to new reagents, or by continuously deflecting these species from a stream of sample into a stream of washing solution as part of a separation. In both cases, it is possible to take advantage of the laminar flow that is present within microfluidic channels, for reducing procedural time and labor in the former example (trapping), and for moving beyond simple lateral separations in the latter example (continuous flow). By generating multiple parallel laminar flow streams that contain reagents and washing solutions, powerful analytical techniques can be developed for the study of particle- and cell-based processes, such as immunoassays, DNA analysis, and cell studies, whether they are in a trapped format or are being continuously deflected through a microchannel.

Laminar flow

While flow inside a large diameter pipe is often turbulent, with chaotic flow streams being present, the flow within microchannels is laminar, meaning that fluid streams flow alongside each other. Laminar flow occurs when the viscous forces dominate the inertial forces, and whether flow is turbulent or laminar can be determined by the Reynolds number, Re (dimensionless), of a system, as shown in Eq. 1 [6]. When Re is <2000 , the flow is laminar, and when Re is >2000 , it is turbulent. Reynolds numbers are typically <1 in microfluidic channels, and so flow is almost always laminar:

$$Re = \frac{\rho r_h v}{\eta} \quad (1)$$

where ρ is the density (kg m^{-3}), η is the dynamic viscosity ($\text{kg m}^{-1} \text{s}^{-1}$), r_h is the hydraulic diameter of a channel (m), and

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M. D. Tarn · M. J. Lopez-Martinez · N. Pamme (✉)
Department of Chemistry, The University of Hull, Cottingham Road,
Hull HU6 7RX, UK
e-mail: n.pamme@hull.ac.uk

v is the average fluid velocity (m s^{-1}). The hydraulic diameter, r_h , of a noncircular channel can be calculated using Eq. 2, where A is the cross-sectional area of the channel (m^2) and P is the wetted perimeter of the channel (m).

$$r_h = \frac{4A}{P} \quad (2)$$

An important consequence of this laminar flow regime is that when multiple liquids are pumped into a microfluidic channel they flow side-by-side in a highly stable manner. Mixing between adjacent streams occurs only by diffusion, unless features have been included that induce other types of mixing [7, 8]. The distance that a particle (whether it is a molecule, a nano- or microparticle, a cell, or a droplet) will migrate by diffusion in a given time can be determined by the Einstein–Smoluchowski equation (Eq. 3):

$$x = \sqrt{2 D t} \quad (3)$$

where x is distance (m), D is the diffusion coefficient of the particle ($\text{m}^2 \text{s}^{-1}$), and t is time (s). The time that a particle is able to diffuse will depend on the length of the channel and the flow rate of the liquids within it. The diffusion coefficient of a particle can be calculated using the Stokes–Einstein equation, as given in Eq. 4:

$$D = \frac{k_B T}{6 \pi \eta r} \quad (4)$$

where k_B is the Boltzmann constant ($1.38062 \times 10^{-23} \text{ J K}^{-1}$), T is temperature (K), and r is the radius of the molecule/particle/cell (m). Typical diffusion coefficients of small molecules in liquids are on the order of $D \approx 10^{-9} \text{ m}^2 \text{ s}^{-1}$, though larger species have lower values of D and thus move more slowly through a fluid. The $k_B T$ component is an estimate of the translational kinetic energy of a particle, whereas the lower expression of the equation refers to Stokes' law, which describes the viscous drag force, \mathbf{F}_{vis} (Newtons), on a particle as it moves through a fluid at a velocity of \mathbf{u} (m s^{-1}):

$$\mathbf{F}_{\text{vis}} = 6 \pi \eta r \mathbf{u} \quad (5)$$

Utilizing the equations above enables the tailoring of microfluidic systems for performing different types of processes, all because of the predictable nature of the laminar flow and diffusion. With this in mind, the interface of two or more co-flowing laminar streams becomes important [9]. Early microfluidic developments included that of the H-filter [10], a separation system in which two streams, a sample and a buffer stream, are brought together in a short channel before being split again. This allows small molecules, which diffuse faster, to migrate into the buffer stream while the larger, slower molecules remain in the sample stream.

The introduction of chemical reagents into co-flowing streams enables the synthesis of organic or inorganic products as the components of each stream diffuse into each other and react, with the capability of introducing further reagents downstream for multi-step synthesis [11, 12]. By flowing a sample stream and a reagent stream together, antigens in the sample can rapidly diffuse into the reagent stream, in which they are labeled with a fluorescent antibody, allowing immunoassays to be performed in a T-sensor configuration [13–15]. In addition to chemical products and antibody–antigen complexes, it is also possible to perform microfabrication at the laminar flow interface, including the generation of silver or polymer wires, selective etching of materials, and crystal formation [16, 17]. It is also possible to micropattern the channel topography by employing laminar flow streams of etching and non-etching solutions [18], or by chemical treatment of the channel surfaces [19]. The patterning of cells, culture media, and proteins can also be achieved using laminar streams [20–22].

Importantly, the generation of laminar flow streams yields stable and controllable environments in which to manipulate cells and particles, either by controlling the medium around such species or by manoeuvring the species through these environments. Typical, conventional examples of this will be given in the following section.

Particle and cell handling

When it comes to performing particle and cell-based processes in microfluidic devices, there are two general methods by which this can be achieved [23]. One technique is to trap the particle within the channel via a number of techniques [24], before consecutively pumping reagents and washing solutions over the trapped species, although this remains a laborious and time-consuming endeavor. A different method involves continuous flow processing, which utilizes laminar flow for performing continuous particle and cell separations in a similar fashion to the H-filter [25–29]. Here, the particles or cells are introduced into the chamber as part of a sample stream, before being deflected laterally into an adjacent washing stream and thus achieving separation. In recent years, however, both trapping and continuous protocols have been incorporated into microfluidic devices that feature multiple reagent and washing streams, allowing localized, simultaneous or multi-step reactions and assays to be performed in a simple, rapid, and elegant manner.

In this article, we will review the combination of multiple laminar flow streams with the application of particles, cells and droplets, in which these objects are either trapped within microchannels or manipulated whilst in continuous flow. This review is intended to go beyond the norms of conventional trapping and continuous flow-based procedures, which for trapped species usually involves their consecutive exposure

to sequentially injected solutions, and for continuous flow processes normally involves the off-chip mixing of particles with a sample to achieve binding to a target analyte, before performing on-chip deflection of the particles from the sample stream into a wash stream. We will first give a general overview of the different types of processing techniques that can be achieved using multilaminar flow, before providing details of the variety of ways in which particles and cells can be manipulated within these flow streams.

Multilaminar flow processing

There are many methods of handling particles, cells, and droplets for performing (bio)chemical processes using multiple laminar flow streams, as illustrated in Fig. 1. These “multilaminar flow” procedures can largely be split into two main categories: *trapping*-based processes and *continuous flow* processes.

Processing of trapped particles and cells

When a cell is trapped across the width of a microfluidic channel, typically via adherence of the cell to the channel wall or by using microfabricated posts, it is possible to achieve the **simultaneous exposure** of different parts of it to various stimuli by having laminar flow stream provide multiple reagents, as shown in Fig. 1a. This allows the influence of different reagents on the cell to be studied concurrently, or to then observe the migration of different species within the cell once they have been labeled by the simultaneous reagent streams. Alternatively, a reagent can be present in only a single, focused flow stream while the remaining streams contain buffer solution, allowing the **localized exposure** of this one reagent to a precise region of the cell. Such a technique is particularly well suited to perfusion studies as it allows far greater spatial control of reagents compared to conventional techniques.

Instead of being trapped such that it crosses the entire width of the channel, it is possible to trap a particle or cell in a much smaller region within the device. Once this is achieved, the relative flow rates of the multilaminar flow streams can be adjusted to allow “**stream switching**” (or “**flow switching**”), in order to expose the trapped species to alternating reagents and washing solutions, as illustrated in Fig. 1b. This is caused by increasing the flow rate of only one reagent, forcing it to generate a wider stream within the channel such that it encompasses the trapped species, before returning the flow rate to its initial setting to equalize the streams again. This can be repeated with the other washing and reagent streams as desired, and allows much faster switching of the chemical environment compared with batch methods in which the solutions are injected one after the other.

A variation of the stream switching method involves the use of forces to “**shuttle**” the trapped species itself back-and-forth across stable multilaminar streams, as demonstrated in Fig. 1c. This effectively achieves the same outcome as in stream switching, except that in this case the laminar streams remain unchanged. Such a technique lends itself particularly well to optical tweezers, although methods such as the patch clamp have also been applied whereby the object is held in place while the microfluidic device itself is moved on a translation stage. It is also useful when further processing is required as the particle/cell can be released from its trap, which is somewhat more difficult when the object is adhered to the chamber surface or trapped by posts.

Continuous flow processing of particles and cells through laminar streams

Instead of trapping particles and cells and exposing them to reagents and washing solutions, as in traditional batch reaction/assay methods and the stream switching and shuttling multilaminar flow techniques, it is also possible to continuously introduce particles/cells into a multilaminar flow chamber and manipulate their trajectory as they travel through the channel. When particles/cells continuously enter a chamber containing alternating reagent and washing streams, they can be deflected laterally across it, passing from one side of the chamber to the other (Fig. 1d). As they migrate across the chamber they pass through each stream sequentially, allowing consecutive reaction and washing procedures to take place on the particle surface in a single step. This technique is essentially a “**one-shot**” process, with the number of reactions depending on the number of reagent streams, but is highly suited to procedures that have a small, set number of steps, such as immunoassays.

An alternative to the one-shot method is to employ a “**zig-zag**” strategy, in which the particles are deflected back-and-forth across the reagent streams while in continuous flow (Fig. 1e), allowing multiple reactions with only a small number of streams. This method is particularly suited to procedures that require repeated reactions with only a small number of different reagents, such as in the deposition of layers onto particles. However, when only one reaction is required per reagent stream, such as in immunoassays, it is possible to simply “dip” the particle into the reagent and deflect it back into the washing stream using the zig-zag motion.

Rather than deflecting particles across a wide chamber, they can be allowed to migrate through a single main channel that has multiple side channels acting as inlets and outlets in order to introduce short sections of reagents and washing solutions into the main channel (Fig. 1f). This “**sequential exchanger**” thus relies on methods to keep the particles within the main channel instead of being lost to one of the side-outlets, which can be achieved by the design of the channels or by the application of forces. This has the advantage of requiring only that the particle

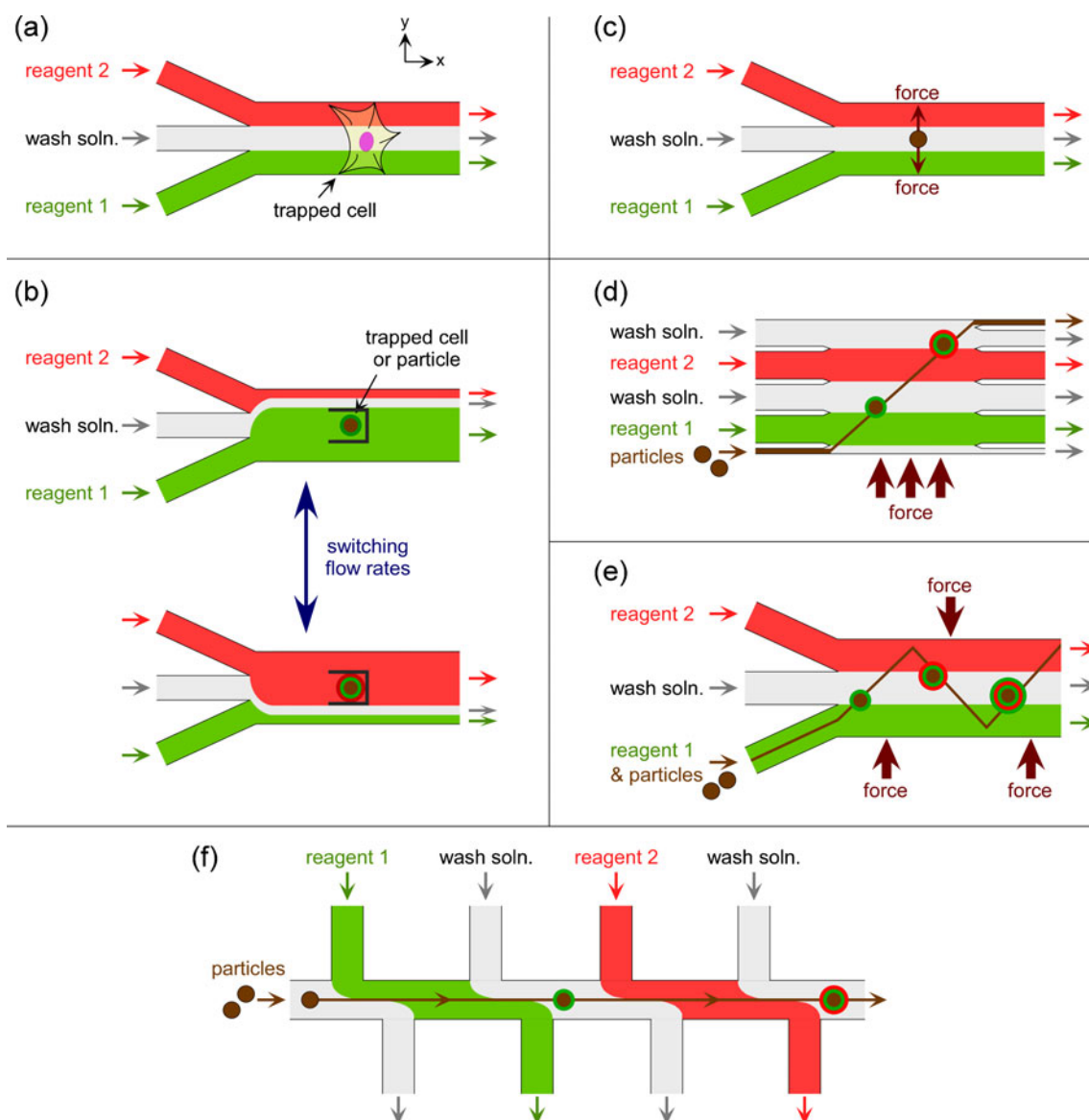


Fig. 1 Common types of multilaminar flow systems for performing cell or particle-based processes. **(a)** Simultaneous and/or localized exposure of a cell to different stimuli while it is trapped across the width of a microchannel. **(b)** “Stream switching,” whereby particles are captured within a trap and exposed to different reagents by varying the relative flow rates of the solutions. **(c)** Application of trapping forces to “shuttle”

a particle back-and-forth across several reagent streams. **(d)** Continuous deflection of particles across multilaminar flow streams in a “one-shot” operation. **(e)** Continuous “zig-zag” deflection of particles back-and-forth across multilaminar streams. **(f)** Continuous motion of particles through sequentially exchanged reagent and washing solutions via arrays of channels branching into and out of the main channel

or cell be kept in a relatively straight line, rather than trying to deflect it over larger distances, but care must be taken to ensure that the objects are not lost into the outlet channels.

As will be demonstrated throughout this review, each of these different types of multilaminar flow processes, both trapping- and continuous flow-based, can be achieved via a number of techniques that include the use of channel designs, flow regimes, and the application of various forces. Prior to that, however, it is worth mentioning the importance of determining the extent of diffusion within such multilaminar flow systems.

Measuring diffusion in multilaminar flow systems

When performing multilaminar flow processes in which two reagent streams are separated by a washing stream, it is important to ensure that reagent molecules are unable to diffuse across this separating stream and react with the other reagent. Therefore, it is useful to study the extent of diffusion in the multiflow system. If the radii of the molecules being used are known, it is possible to calculate the theoretical diffusion using Eqs. 3 and 4. There are also several ways in

which this can be achieved experimentally, such as by the introduction of different colored inks or food dyes into the laminar stream, with the color change at the interface being examined, while pH indicators and reagents of known pH values can also be employed. However, when colored solutions are being used, it can be difficult to distinguish the color change from the background. Therefore, it is advantageous to instead use reagents that are colorless (or have only a faint color), but which form a colored or fluorescent product when mixed in order to better visualize and quantify the diffusion of the streams. Several reagents have been employed in this way within the literature and a selection is given here, though many more reagents would also be applicable.

The Fluo-3 and Fluo-4 range of molecules, commercially available from Invitrogen (Life Technologies, Inc., Carlsbad, CA, USA), are non-fluorescent compounds that bind strongly with calcium (II) ions to form fluorescent complexes. Thus, by introducing alternating laminar reagent streams of Fluo-3 and calcium (II) chloride (CaCl_2) into a microfluidic device, the cone of diffusion can be visualized by the fluorescence signal across the channel width (Fig. 2a) [30]. A similar but more cost-effective approach involves the use of acidified eosin, which exhibits a low fluorescence signal at pH values <3 , but yields a strong fluorescence signal at higher pH values. This effect can be implemented on-chip by flowing adjacent streams of acidified eosin and sodium hydroxide into the channel, and measuring the resultant fluorescence [31]. A simple yet effective method is to utilize the complexation of iron (III) ions with thiocyanate ions. Here, streams of colorless iron (III) sulphate and potassium thiocyanate solutions can be pumped into the channel side-by-side, with the iron thiocyanate product generated at the interface being a dark red color that can be easily visualized against the colorless background (Fig. 2b) [32]. Furthermore, the use of Fe^{3+} and SCN^- ions, because they are very small species with high diffusion coefficients, gives an indication of the largest amount of diffusion that could be expected in a multilaminar flow system for a given channel design and flow rates.

Having given an overview of (1) laminar flow, (2) types of multilaminar flow processing, and (3) methods of determining the diffusion in a multilaminar system, we will now describe the various ways in which particles and cells can be employed within such platforms in terms of their handling and manipulation, in both trapping and continuous flow-based formats. The following sections will describe: (1) the adhesion/binding of particles and cells to the channel surface, (2) the ways in which channels can be designed to manipulate particles and cells, by the use of pillars and also by utilising interesting flow effects, (3) the variety of force fields that can be applied to particles and cells to either trap them in place or to continuously deflect them through a microfluidic device, and (4) the use of biomolecular motors for the controlled transport of “cargo.”

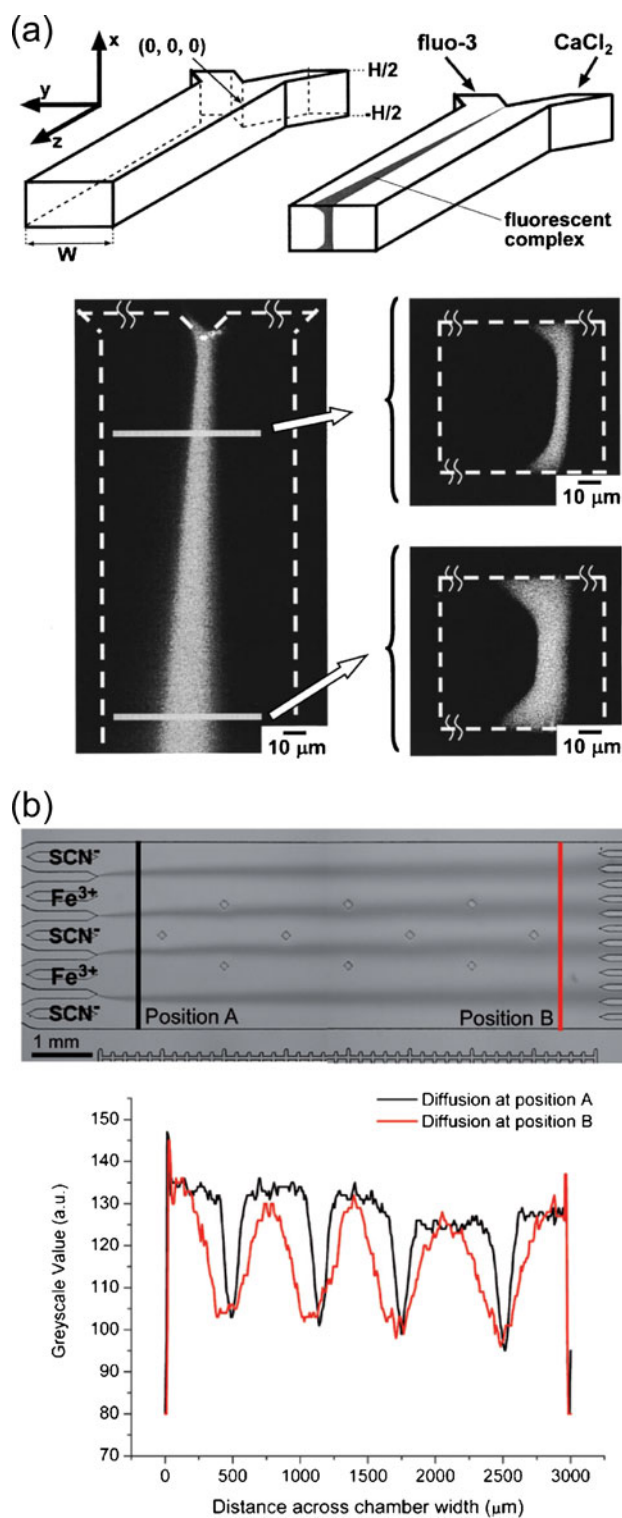


Fig. 2 Methods of experimentally determining the extent of diffusion between laminar flow streams. **(a)** Co-flowing streams of Fluo-3 and Ca^{2+} ions produce a fluorescent compound (Reprinted with permission from [30]. Copyright 2000, American Institute of Physics). **(b)** Laminar streams containing colorless solutions of Fe^{3+} and SCN^- ions generate a dark red complex at their interface (Reproduced from [32] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b904724g>)

Adherence of cells onto surfaces for trapping-based processes

Multilaminar flow streams can be applied to the delivery of membrane-permeable molecules to selected subcellular domains within living cells that have been trapped across a microchannel, in such a way as to be exposed simultaneously to several laminar flow streams. This can allow different sections of the cell to be exposed to different stimuli, or can enable the localized exposure of a single reagent to a certain location. Such a technique enables excellent spatial discrimination and visualization of the processes occurring inside the cells (see Fig. 1a), and was first developed by the group of Whitesides as a method they called PARTCELL (*partial treatment of cells using laminar flows*) [33], which involved the adherence of a cell to the channel surface. Takayama *et al.* [33] utilized this technique to deliver solutions containing fluorescent tags to opposite poles of a bovine capillary endothelial (BCE) cell, selectively labeling the mitochondria in these distinct regions and allowing their movement to be studied (Fig. 3a). The disruption of cytoskeletal actin filaments in spatially defined regions of cells was also examined via treatment with lactrunculin A in one of the laminar streams. This work was later expanded upon [34], both theoretically and experimentally, while the localized detachment of portions of individual BCE cells from the channel substrate was also performed via treatment with trypsin-EDTA, in order to observe the cell's reorganization and reattachment to the channel surface. Selected treatment of the cell surface was achieved using laminar streams of fluorescently labeled colloidal particles and proteins (wheat germ agglutinin, WGA), the latter enabling observation of cell-surface glycoprotein endocytosis. PARTCELL was also utilized by Sawano *et al.* [35] for studying the lateral propagation of epidermal growth factor (EGF) signals after localized delivery of fluorescently-labeled EGF to the surface of live COS cells, which had been adhered to the glass channel surface via poly-L-Lysine.

Lucchetta *et al.* [36] developed a laminar flow system similar to PARTCELL for the study of the *Drosophila melanogaster* fruitfly embryo, and how changes in temperature affect the biochemical network (Fig. 3b). A single embryo was adhered to double-sided tape, and a two-part moulded poly(dimethylsiloxane) (PDMS) chip was assembled around it such that the cell was situated in the center of the main channel. The anterior and posterior halves of the embryo were exposed to two laminar flow streams of differing temperatures in order to rapidly supply and remove heat, resulting in the embryo developing at different rates within the two halves. In later work, the flow and temperature transfer within the microfluidic system were characterized more fully [37], and new findings yielded a greater understanding of the mechanisms and pathways in cell cycle regulation [38].

Nie *et al.* [39] developed a multilaminar flow device for the generation of "wound edges" and the study of the subsequent "wound-healing" via cell migration in a device fabricated from PDMS on a collagen-coated Petri dish. Fibroblast cells were grown to confluence within the device, and a localized stream of trypsin-EDTA was introduced to selectively detach all but a single lane of cells, forming a clean wound edge without damaging the cells on the leading edge (Fig. 3c). Cell medium was pumped through the device and the migration of the cells from the wound edge into the fresh medium was monitored. van der Meer *et al.* [40] took this wound-healing assay further by applying the method to endothelial cells in order to evaluate its viability compared to conventional techniques.

Villa-Diaz *et al.* [41] were able to culture single human embryonic stem cell (hESC) colonies in a device fabricated from PDMS channels on a cell culture plate. Laminar flow reagent streams were employed in two instances: one in which selected portions of the colony were subjected to fluorescent staining of the cell nuclei, and the other in which part of the colony was dissociated with trypsin-EDTA solution and the collected cells analysed. Li *et al.* [42] studied the partial transfection of cells using laminar flow regimes. COS-7 cells were adhered to a poly-L-Lysine treated glass channel surface and grown to near-confluence. Two laminar flow streams containing different transfection mixtures were pumped over the cells for several hours, and the cells were then incubated in fresh medium for later determination of the transfection efficiency via fluorescence microscopy.

Liu *et al.* [43] developed a microdevice in which cells were adhered within a cell culture chamber. In a second microfluidic chip layer, solutions were allowed to flow above the chamber, reducing the shear stress on the cells while enabling spatially selective reagent delivery to them via the laminar flow reagent streams passing above. Lee *et al.* [44] studied the damage of selected portions of neuronal networks and its subsequent regeneration as a means of interrogating nerve injury (Fig. 3d). After obtaining neurons from *Aplysia californica* and culturing them in PDMS channels sealed to a tissue culture dish, a laminar stream of detergent was introduced that transected the exposed neurites within a localized area, allowing subsequent observation of their regeneration in different culture media.

The above examples have generally focused on the application of laminar flow for localized reagent delivery by introducing a reagent stream that was directed and focused by streams of buffer solution, with the relative flow rates largely unchanged. However, by trapping a cell or particle in a microchannel, it is possible to "switch" the reagent and washing streams by altering their relative flow rates (see Fig. 1b), in order to consecutively expose the object to a reagent and then immediately wash it. Lee *et al.* [45] employed this technique for the single molecule analysis of F₁-adenosinetriphosphatase

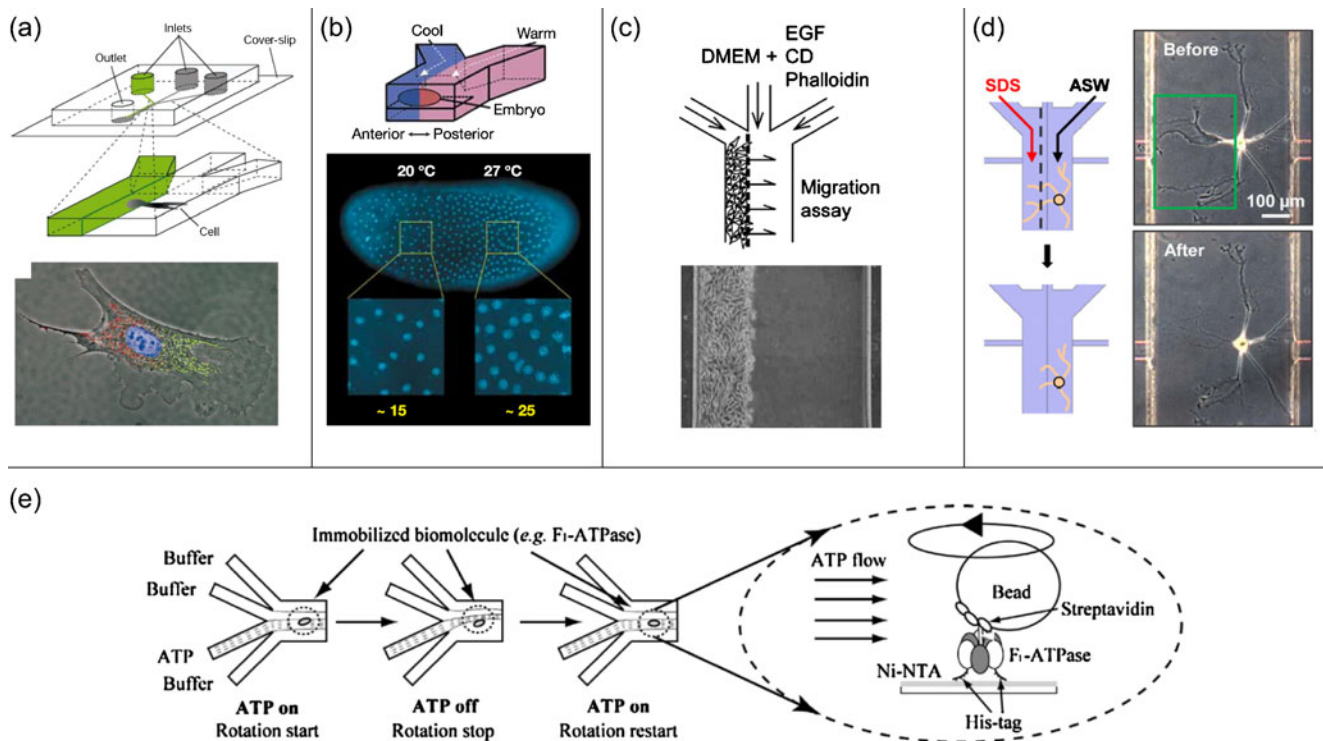


Fig. 3 Adherence of biomolecules to a channel surface. **(a)** The PARTCELL technique applied to the study of mitochondrial movement and changes in cytoskeletal structure by localized exposure of stimulants (Reprinted by permission from Macmillan Publishers Ltd: Nature [33], copyright 2001). **(b)** Study of a *Drosophila melanogaster* fruitfly embryo by exposing each half to hot or cold conditions (Reprinted by permission from Macmillan Publishers Ltd: Nature [36], copyright 2005). **(c)** Selective positioning of fibroblast cells via laminar flow patterning for

migration assays (Reprinted from [39], Copyright 2007, with permission from Elsevier). **(d)** Localized damage of neuronal networks for the study of neuroregeneration [44]. (© IOP Publishing. Reproduced by permission of IOP Publishing. All rights reserved. <http://dx.doi.org/10.1088/1741-2560/10/3/036020>). **(e)** Analysis of an adhered molecule, with stream switching used to expose the molecule to a stimulus (Reprinted from [45], Copyright 2006, with permission from Elsevier)

(F_1 -ATPase), which was anchored to a Ni^{2+} -nitrilotriacetic acid (NTA) coated microchannel surface via a His₁₀ tag present on the F_1 -ATPase (Fig. 3e). Rotation of the F_1 -ATPase was induced when it was exposed to a stream of adenosine triphosphate (ATP) via stream switching, and the rotation was stopped when the stream was switched such that the F_1 -ATPase was in buffer solution. Hersen et al. [46] used a similar technique to monitor signal processing by the hyper osmolar glycerol (HOG) MAP kinase pathway in yeast cells. The cells were adhered to the channel surface before being exposed to an oscillating osmotic shock by switching between streams of varying reagent concentration.

Patch clamping for trapping-based procedures

The patch clamp technique involves the formation of a high-resistance electrical seal between the tip of a glass micropipette and a “patch” of cell membrane that contains only one or a few ion channels of the cell, allowing the registration of ion channel activity. Of importance to this review is the fact that a patch clamped cell is effectively trapped in place while

experiments are performed. Sinclair et al. [47] employed the patch clamp technique to the trapping of single cells for multiflow studies. The trapped cell was held in place in an “open” microfluidic device, with multilaminar flow streams exiting the actual channels and retaining their laminar nature in an open space (Fig. 4a). The microdevice was then moved via a translation stage in order to expose the stationary cell to different stimuli within the laminar streams, in a similar but opposite manner to the “shuttle” method (see Fig. 1c). By introducing different ion channel ligands into the laminar streams, their effect on the ion channels of the trapped cell could be determined as the cell was sequentially exposed to each reagent. In addition, the laminar flow was actually found to increase the resistance of the clamp, generating superior sealing properties that enabled longer recording times and decreased noise levels [48]. The concentration “landscape” and the diffusion zone between the laminar flow streams were characterized by scanning nano-electrode amperometry [49]. The system was later employed for the exposure of cells to varying concentrations of receptor agonists, for the study of the formation of short-term memory circuits by GABA_A receptors [50], the controlled assembly of GABA_A receptor

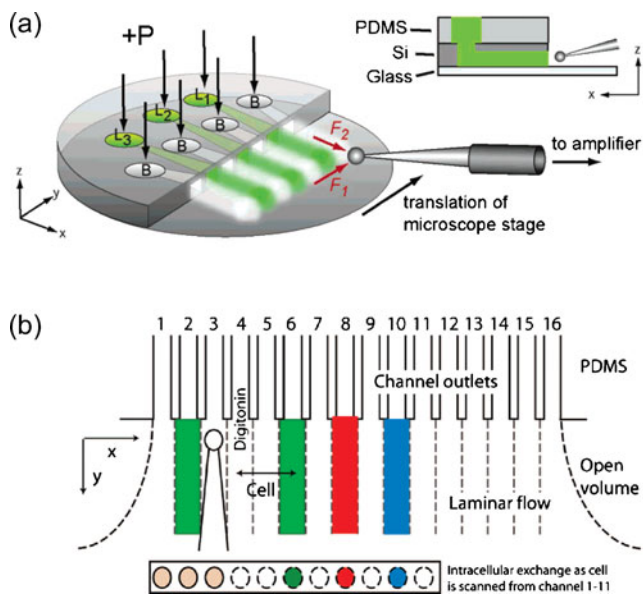


Fig. 4 (a) Patch clamping of cells for exposure to multilaminar flow streams. The cell was held in place while the “open” microfluidic device was moved via a translation stage (Reprinted with permission from [47]. Copyright 2002 American Chemical Society). (b) Poration of patch-clamped cells and for study of the uptake of chemicals into the permeated cells (Reprinted with permission from [53]. Copyright 2009 American Chemical Society)

populations [51], and dose–response curves for GABA_A with varying temperature [52]. In later work, the poration of patch clamped cells was investigated by moving the cell first into a stream of digitonin to create the pores, and then into reagent streams to observe the uptake of chemicals into the permeated cells (Fig. 4b) [53].

Physical barriers and objects for trapping and continuous flow processing

Trapping via microposts and microgrooves

As an alternative to the adherence of biomolecules to channel surfaces for multilaminar flow processes, it is also possible to trap such species using physical objects incorporated into the design of the microfluidic devices. Blake et al. [54] developed a three-layer PDMS microfluidic device in which a brain slice was held in place via micropost arrays situated above and below it (Fig. 5a). This allowed perfusate to reach the upper section of tissue as well as the underside, while a narrow stream of Na⁺-free solution was focused onto the midline of the brain slice and the respiratory motor output monitored via suction electrodes. Meier et al. [55] employed two posts for the trapping of a root of a live *Arabidopsis thaliana* plant across a microchannel. Using multilaminar flow streams, a portion of the root was treated with stimuli, resulting in the spatially-controlled growth of new root hairs (Fig. 5b). Taylor

et al. [56] examined synaptic responses by preparing two populations of neurons within different chambers, and having their dendrites grow into microgrooves that linked the two chambers (Fig. 5c). The synapses trapped within the microgrooves were then perfused with stimuli within a narrow region, and the subsequent effects observed.

Continuous flow processing via deterministic lateral displacement

Deterministic lateral displacement (DLD) is a technique that was originally developed for the separation of particles and cells in continuous flow [57]. DLD utilizes an array of microposts within a microfluidic chamber, with each row of posts being slightly off-set from the previous one. When a sample is pumped through the device, small particles are able to weave around the posts in a relatively straight line with the direction of flow. Larger particles that are above a critical size are unable to follow the flow streams upon encountering the posts and are instead “bumped” laterally, resulting in size-based separation. Morton et al. [58] took the principle of DLD further and applied it to particle and cell-based multilaminar flow processes. The deflection of 3 μm red particles across a stream of 0.5 μm green particles that were following the laminar flow regime was demonstrated as a proof-of-principle (Fig. 6a), along with the deflection of 3 μm particles through a stream of dye. The technique was then applied to the labeling of blood cell platelets by deflecting them through a stream of labeling dye and into a washing stream. A more sophisticated process involved passing *E. coli* cells first through a tracer stream of green fluorescent protein (GFP) in order to label the cells, and then into a stream of lysing agent. Here, the cell was lysed and its contents (including the GFP), which were now below the critical size threshold, followed the laminar flow streams instead of being deflected any further. By staining the chromosomes of the *E. coli* cells, their trajectory could be tracked following the cell lysis step, with the chromosomes found to continue being deflected laterally afterwards (Fig. 6a).

Continuous flow processing via microfluidic pinball

Kantak et al. [59] utilized pillars as part of a “pinball”-inspired microfluidic device for depositing alternating layers of polyelectrolytes onto droplets. The chip consisted of three laminar streams within a main channel, with two different polyelectrolytes in the outer streams and wash solution in the central stream (Fig. 6b). The microchannel featured a “zig-zag” (see Fig. 1e) arrangement of pillars throughout its length. Droplets were generated within the first polyelectrolyte stream and then followed the path of the pillar arrangement, traversing back-and-forth between the polyelectrolyte and washing streams as

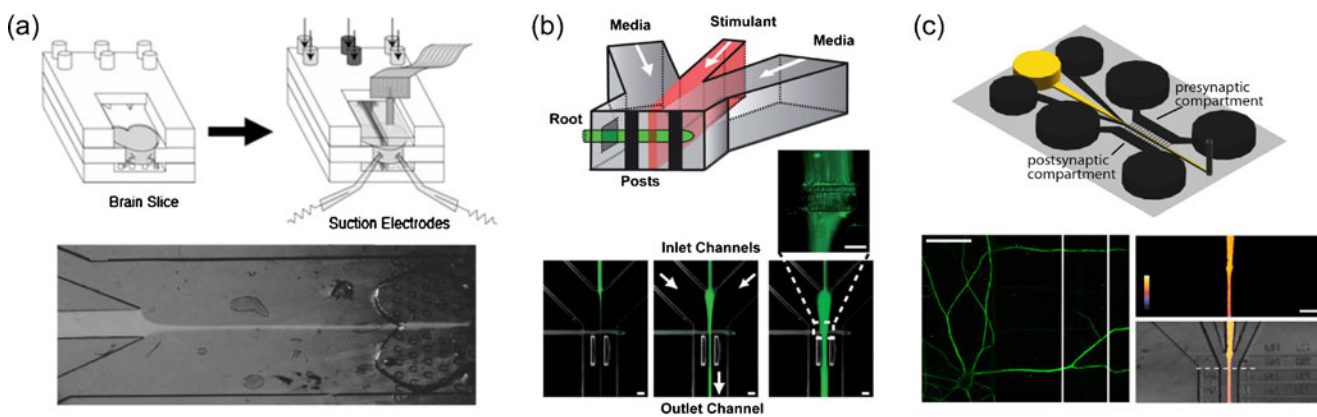


Fig. 5 Trapping via microposts and microgrooves. (a) Perfusion of a focused stream over a brain slice held in place via microposts, allowing perfusate to reach both the upper surface and the underside of the tissue (Reproduced from [54] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b704754a>). (b) Chemical stimulation and

analysis of *Arabidopsis thaliana* root that was held in place with post (Reproduced from [55] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/c004629a>). (c) Localized perfusion of stimuli to neuronal dendrites trapped in microgrooves (Reprinted from [56] Copyright 2010, with permission from Elsevier)

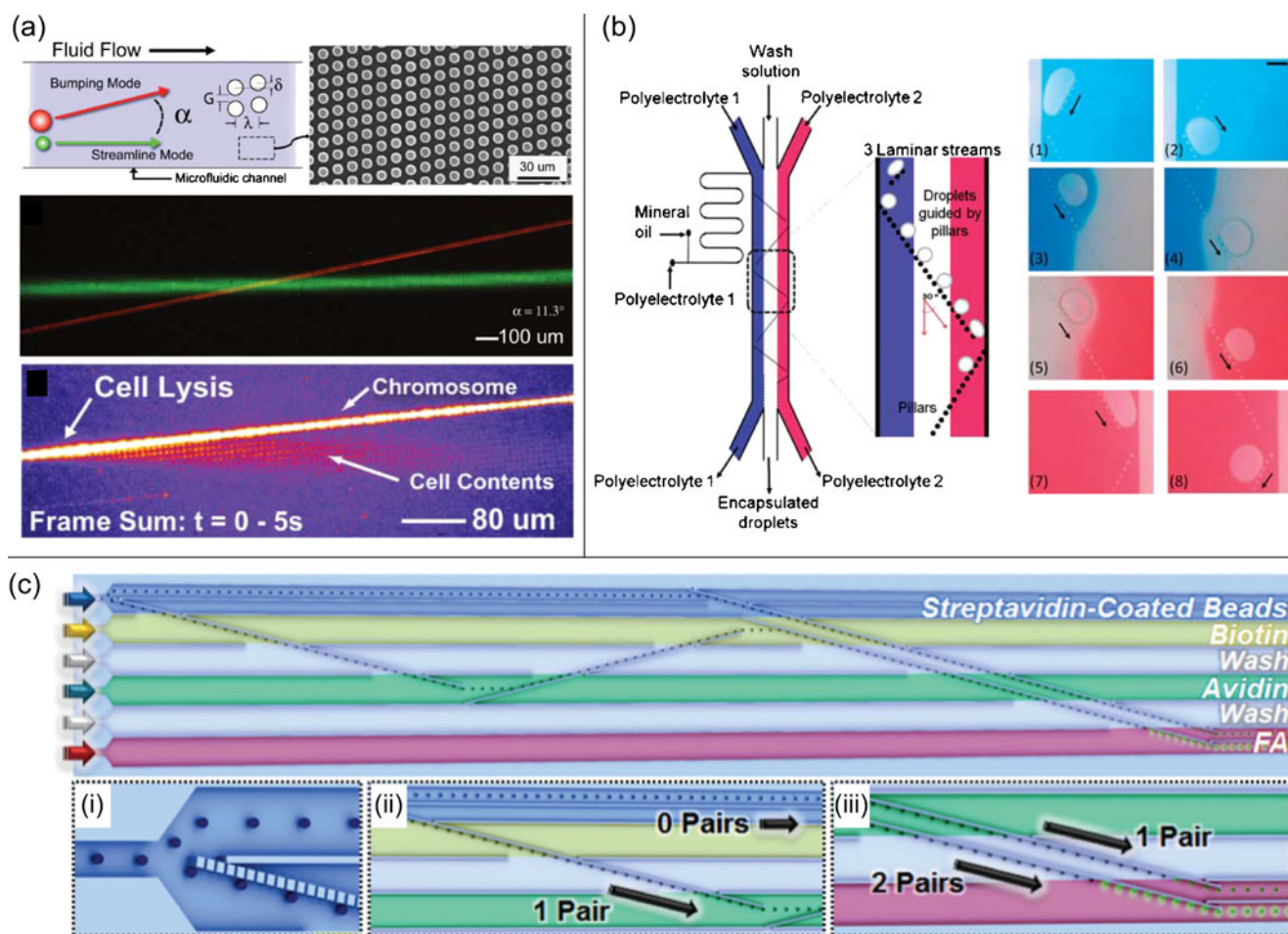


Fig. 6 The application of pillars and posts for the continuous deflection of particles, cells and droplets through multilaminar flow streams. (a) Deterministic lateral displacement (DLD) for cell lysis and labeling in a one-shot scenario (Reproduced from [58] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b805614e>). (b) Microfluidic pinball, featuring a zig-zag pillar arrangement for layer-by-

layer deposition of polyelectrolytes onto droplets (Reproduced from [59] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/c0lc00381f>). (c) Microfluidic array railing (μ PAR) for the deflection of beads into and out of laminar flow streams in both one-shot and zig-zag and motions (Reproduced from [60] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/c2lc40610a>)

they flowed through the channel. Six polyelectrolyte layers were generated in less than 3 min using this technique.

Continuous flow processing via micropost array railing

A similar principle to the pinball method was exploited by Sochol et al. [60] in a setup they named “micropost array railing” (μ PAR), illustrated in Fig. 6c. Multiple alternating reagent and washing streams were generated within a microfluidic chamber, with the pillar arrangement allowing multiple paths through the streams in a one-shot operation (see Fig. 1d) and zig-zag pattern (see Fig. 1e) that could be utilized simultaneously for multiplexed processing. Streptavidin-coated beads were introduced into the device and deflected via the posts through alternating streams of biotin and avidin, before finally labeling them with biotinylated fluorescent aptamer. With the combination of one-shot and zig-zag post arrays, multiple bead populations were prepared that featured between zero and four pairs of avidin–biotin spacer units between the bead surface and the fluorescent aptamer. The system’s viability for biological applications was also tested by deflecting bovine aortic endothelial cells (BAECs) through multiple dye solutions.

Continuous flow processing via microtrains and microfluidic rails

A very different type of railing system, developed by the group of Kwon, does not use posts to “bump” particles and cells in the desired direction but, instead, involves the fabrication of “microtrains” that follow a monorail “track” through the microfluidic channel [61, 62]. The device was designed such that a groove (the rail) is incorporated along the center of the roof of the channel. The microtrains were fabricated in situ using optofluidic maskless lithography [63], derived from continuous flow lithography [64], in which a photocurable liquid within the microchannel is exposed to UV light through a photomask or spatial light modulator, generating a free-flowing polymeric microstructure within the microchannels. When the microtrain is fabricated, a “fin” is formed within the groove of the channel roof, restricting the movement of the microtrain such that it is only capable of following the groove (Fig. 7a).

Chung et al. [61] developed a series of devices in which multilaminar flow streams were employed for the self-assembly of structures formed from microtrains. Using a technique with some similarities to sequential exchange methods (see Fig. 1f), the heterogeneous assembly of microlatches was performed along a main channel into which side channels intersected (Fig. 7b). Microtrains fabricated from different polymers in each channel were delivered to the main channel in such a way that they connected to each other along a main assembly rail, akin to carriages being

added to a train. Multilaminar flow streams were also utilized for the fabrication of a two-dimensional heterogeneous pattern (Fig. 7c). Five laminar flow streams composed of two polymers were generated in a microfluidic chamber from top-to-bottom, and square microtrains were polymerized inside each stream. Following polymerization, the microtrains were moved out of the laminar streams on rails that were orientated perpendicular to the laminar polymer streams, and formed an array at the end of the rails. This method was applied to the generation of an array of fluorescently labeled HeLa and HEK293 cells. Railing was also applied to the zig-zag method (see Fig. 1e) of continuous flow processing via the use of a sinusoidal rail that repeatedly moved the microtrain back-and-forth across three laminar flow streams [62]. The rate of penetration of the microtrains between each interface was theorized to be faster than 100 times per min.

Channel design and controlled flow rates for continuous flow processing

Pinched flow

Pinched flow fractionation is a technique that was originally developed for the continuous flow separation of particles and cells [65]. Here, a stream containing particles and a stream containing buffer solution are forced into a narrow channel together, and both the particles and the solution in which they are suspended are “pinched” against the sidewall of the channel by the buffer stream. The narrow channel then opens up into a wide chamber, and the particles enter the chamber in a position dependent on their radius, since the distance between the center of the particle and the wall against which it is pinched determines the laminar streamline that it will follow. Chiang and West [66] adapted this pinched flow method for the continuous flow processing of HeLa cells by replacing the buffer solution with one containing a ligand, namely epidermal growth factor (EGF), for stimulation of the cells (Fig. 8). A second pinched flow section allowed the cells to be moved into a quenching solution. With this, the quenched flow analysis of EGF receptor auto-phosphorylation transitions was achieved, with the switching of cells from one environment to another taking place within milliseconds.

Hydrodynamic filtration

Hydrodynamic filtration is another technique originally developed as a continuous flow separation method [67]. The device consists of a main flow channel with a series of branching channels. As a particle suspension flows through the main channel, some of the liquid is directed out of it via the side branches. In continuous flow separations this removal of liquid results in particles being drawn to the sidewalls, and

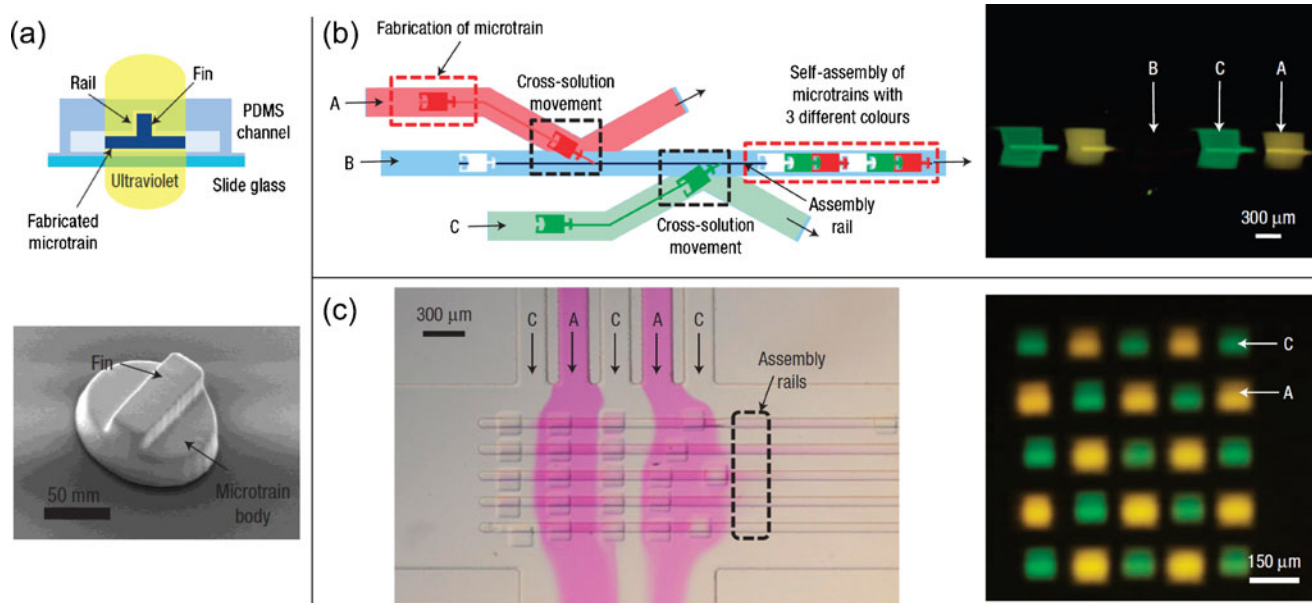


Fig. 7 Guiding “microtrain” particles via microfluidic rails. **(a)** Fabrication of the microtrains by exposure of monomer solution to UV light in a channel featuring a microgroove. **(b)** Cross-solution movement of particles to form multi-colored trains. **(c)** Array formation of microparticles,

with fabrication occurring within a region of five laminar flow streams, after which the particles were moved out of the laminar streams via the railing system (Reprinted by permission from Macmillan Publishers Ltd: Nature Materials [61], copyright 2008)

different sizes of particles exit the device through different branch channels depending on the flow resistance of the channels. Importantly, this filtration effect relies on the fact that when the ratio between the volumetric flow rate of the side channel to the main channel is low enough, particles or cells larger than a critical size will remain in the main channel, even when they are flowing along the sidewall and when the side branch is wider than the particle or cell.

This phenomenon was later utilized for the continuous flow treatment of cells by Yamada et al. [68] in the form of a sequential exchanger (see Fig. 1f), with streams of reagents and washing solutions entering the main channel via side channels, and then being removed via a series of outlet “drains.” The cytotoxic effect of the surfactant Triton X-100,

commonly used to solubilize the cell membrane, on HeLa cells was studied by introducing the surfactant from a side channel while the cells traversed the main channel. The cells passed through the surfactant and into fresh buffer further down the main channel, and the effect of exposure time of the cells was investigated between 17 and 210 ms. The digestion of the cell membrane and isolation of the nuclei of adherent (HeLa) and non-adherent cells (JM) was also examined using different surfactants at various concentrations (Fig. 9a) [69]. The digested cell membranes and the cytoplasmic contents were removed from the device via the side channels, thereby allowing collection of the nucleus from the main channel.

Yang et al. [70, 71] developed a similar device that was designed for performing particle-based immunoassays towards the monitoring of inflammatory responses during cardiac surgery. Streptavidin-coated microbeads were introduced into the main channel and passed through sequentially exchanged reagent solution and buffer solution. The reagent solution consisted of fluorescently labeled biotin that became bound to the particles, while the buffer solution washed any unbound materials from the particle surfaces, and fluorescence detection of the particles was performed downstream.

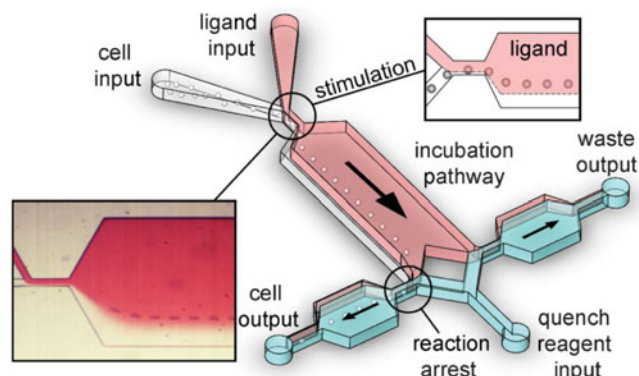


Fig. 8 Continuous pinched flow deflection of cells through consecutive ligand and washing streams (Reproduced from [66] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/c3lc41297k>)

Dielectrophoresis for trapping and continuous flow processing

While the particle and cell handling methods described above have relied on effects intrinsic to the design of the microfluidic

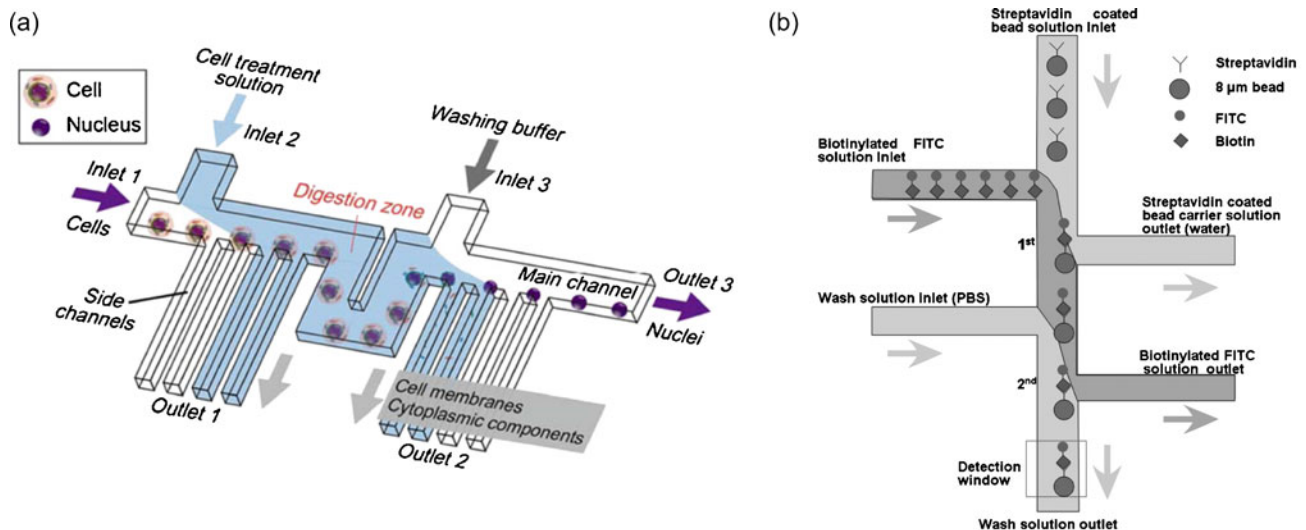


Fig. 9 Hydrodynamic filtration for the sequential exchange of reagents and washing solutions. **(a)** Digestion of cell membranes in treatment solution for isolation of the nucleus (With kind permission from Springer Science and Business Media: [69], Figure number 1a). **(b)** Streptavidin-

biotin binding on particles towards the application of hydrodynamic filtration for continuous flow immunoassays (Reprinted with permission from [70])

device or the adherence of biomolecules to the surface, it is also possible to apply external forces in order to manipulate particles and cells. Dielectrophoresis (DEP) is one such force that has been used extensively for a number of applications in microfluidics [72, 73] and involves the motion of uncharged but polarisable species in an inhomogeneous electric field. When subjected to an electric field, dipoles are induced in the material, polarizing it in the direction of the applied field. In a homogeneous electric field, the electrostatic forces on each end of the dipole would cancel each other out and there would be no net movement of the particle or cell. When subjected to an inhomogeneous field, however, the forces on each side of the dipole are different, and the net force results in movement of the particle. The dielectrophoretic force, \mathbf{F}_{DEP} (Newtons), on a dielectric particle can be calculated from Eq. 6 [72]:

$$\mathbf{F}_{\text{DEP}} = 2 \pi \varepsilon_m r^3 \text{Re}(f_{\text{CM}}) \nabla E_{\text{rms}}^2 \quad (6)$$

where ε_m is the absolute permittivity of a medium (F m^{-1}), $\varepsilon_m = \varepsilon_r \varepsilon_0$ (with ε_r being the relative permittivity of a medium, and ε_0 being the permittivity of free space, $8.854 \times 10^{-12} \text{ F m}^{-1}$), r is the radius of the particle or cell (m), ∇E_{rms} is the gradient of the root mean square (amplitude) of the electric field (where E_{rms} has units of V m^{-1}), and $\text{Re}(f_{\text{CM}})$ is the real component of the Clausius–Mossotti factor, given by Eq. 7:

$$f_{\text{CM}} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad (7)$$

where ε_p^* and ε_m^* are the complex permittivity of the particle and the surrounding medium, respectively. When

$\varepsilon_p^* > \varepsilon_m^*$, then f_{CM} is greater than zero and the particles will migrate towards the region of highest electric field because of a positive dielectrophoretic force (pDEP). When $\varepsilon_p^* < \varepsilon_m^*$, then f_{CM} is less than zero and the particles will move away from the region of strongest electric field because of a negative dielectrophoretic force (nDEP).

Seger et al. [74] demonstrated the application of dielectrophoretic forces for multilaminar flow systems using microfluidic devices with integrated electrodes. A stream switching device (see Fig. 1b) was fabricated with a cell trap composed of electrodes positioned in the center of a chamber (Fig. 10a). Human blood cells were captured within the electrode trap, and were then consecutively exposed to a dye stream and a buffer stream by changing their relative flow rates. A continuous flow zig-zag system (see Fig. 1e) was also developed in which electrodes were orientated diagonally across the chamber with respect to the fluid direction. As cells followed the electrodes they dipped into and out of a laminar flow stream containing a dye solution (Fig. 10b). Tornay et al. [75] later presented an updated version of this cell dipping platform for the functionalization of particles, referred to as a particle exchanger. Here, electrode arrays were patterned along the particle inlet side of the device rather than across the chamber, and these applied a nDEP force to deflect avidin-coated particles into a fluorescently labeled biotin reagent stream (Fig. 10c). By cascading one particle exchanger into another, the particles were then deflected by nDEP out of the reagent stream and into a washing buffer stream in the second particle exchanger. Later optimization of the device [76] involved the widening of the particle exchange chamber in order to introduce further laminar reagent streams, deflect particles

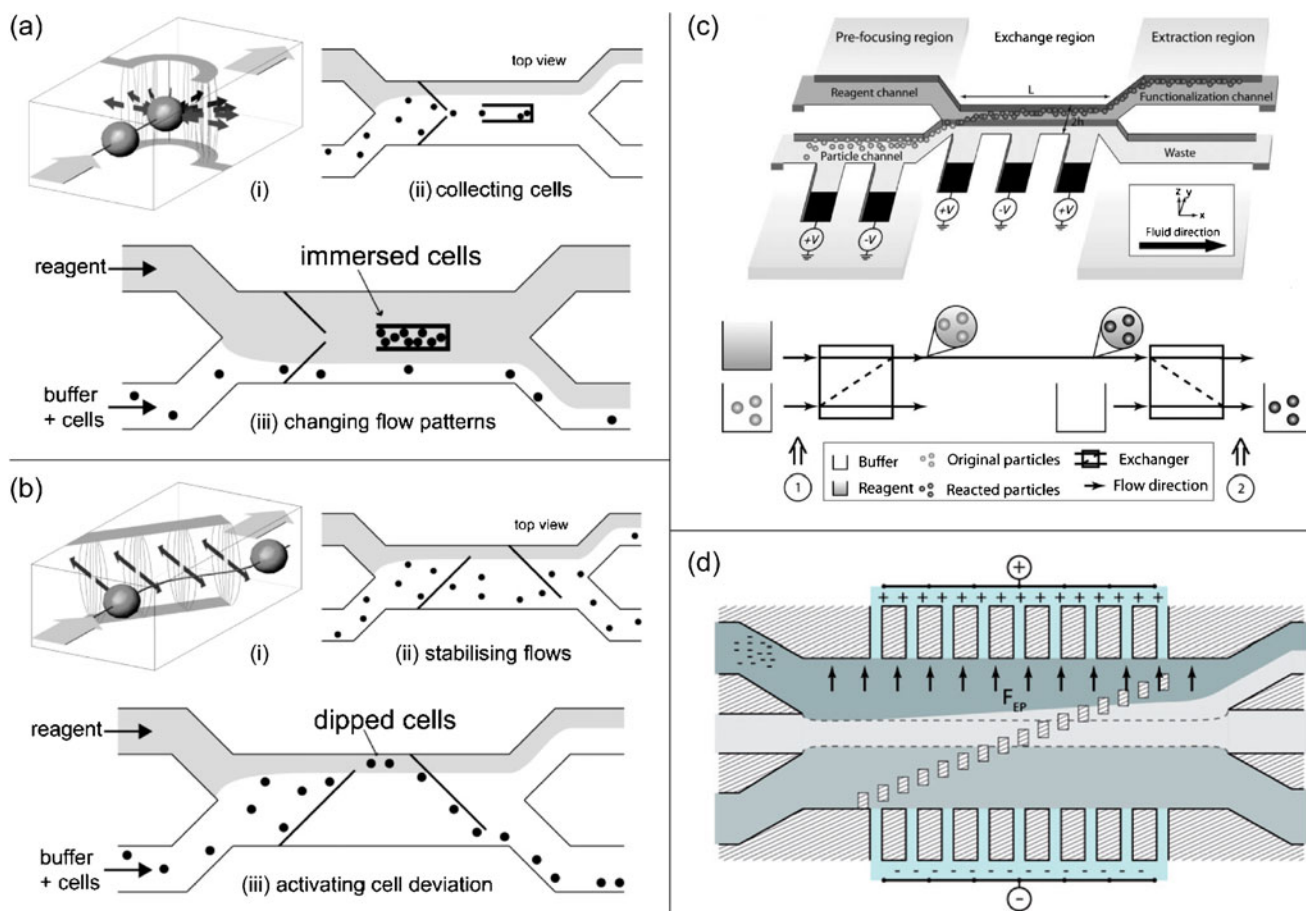


Fig. 10 The use of dielectrophoretic forces in multilaminar flow systems. (a) Capture of particles or cells in an electrode trap for their exposure to solutions via stream switching. (b) Electrodes applied for the continuous zig-zagged deflection of particles or cells into and out of a reagent stream ((a)-(b) Reproduced from [74] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b311210a>). (c) A “particle exchanger” device, featuring two exchange chambers for performing

continuous reaction and washing of particles (Reproduced from [75] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b713776a>). (d) Wide chamber with electrodes placed across it for deflecting particles through three laminar streams (Reproduced from [76] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b816043k>)

over longer distances, and to eliminate problems with diffusive mixing of the reagent and washing streams (Fig. 10d). Here, a shifted array of electrodes was fabricated across a wide chamber, with the effect that as the particles entered the device they were “bumped” from one electrode to another, similar to a DLD device except that electrodes were used rather than pillars. This allowed the deflection of particles across the entire width of the wide chamber. Furthermore, the final laminar flow stream contained a negatively charged reagent that was drawn towards the upper set of electrodes by electrophoretic forces, ensuring that no mixing could occur across the central wash stream.

Magnetism for continuous flow processing

Magnetism is one of the most commonly known forces and one of the easiest to apply. As such, it has found significant

use in many microfluidic applications [77–79]. In most instances, this involves the manipulation of magnetic particles or magnetically labeled cells via an applied magnetic field, which can be provided by permanent rare-earth magnets or by electromagnets. The magnetic force, F_{mag} (Newtons), acting on a particle or cell is given by Eq. 8:

$$F_{mag} = \frac{(\chi_p - \chi_m)V_m(\mathbf{B} \cdot \nabla)\mathbf{B}}{\mu_0} \tag{8}$$

where χ_p is the magnetic susceptibility of the particle (dimensionless), χ_m is the magnetic susceptibility of the medium (dimensionless), V_m is the volume of material in the particle that is affected by the magnetic field (m^3), \mathbf{B} is the magnetic flux density (T), $\nabla\mathbf{B}$ is the gradient of the magnetic flux density ($T\ m^{-1}$), and μ_0 is the permeability of free space ($4\pi \times 10^{-7}\ H\ m^{-1}$). The difference between the magnetic susceptibilities of the particle and medium ($\chi_p - \chi_m$) is known as

the effective magnetic susceptibility ($\Delta\chi$) of the particle. If $\Delta\chi$ is positive then the particle will move towards the region of highest magnetic field. If $\Delta\chi$ is negative, then the particle will be repelled away from the region of highest magnetic field. When using magnetic particles or magnetically labeled cells, χ_p is much larger than χ_m , yielding a highly positive $\Delta\chi$ value and magnetic attraction of the particle or cell towards the magnet. It should also be noted that Eq. 8 only holds true provided the magnetization of the particle reaches saturation, while Shevkopyas et al. [80] have developed an equation for \mathbf{F}_{mag} when the particle is not saturated.

Examples of the use of magnetic forces in multilaminar flow devices typically involve the use of one-shot (see Fig. 1d) and zig-zag (see Fig. 1e) methods, often being derived from the continuous separation technique of on-chip free-flow magnetophoresis [81]. This involves pumping magnetic particles into a chamber alongside a series of laminar flow streams containing buffer solution. By applying a magnetic field across the chamber the magnetic particles are deflected perpendicular to the direction of flow, being drawn into the buffer streams and thus separated from the original sample stream. This format was adapted by Peyman et al. [82] for the continuous processing of magnetic particles in a one-shot format by introducing alternating laminar streams of reagent and washing solutions into the chamber (Fig. 11a). As the particles were deflected across the chamber by a permanent magnet they crossed each stream consecutively, allowing multiple reactions and washing steps to be condensed into a single, rapid process. A proof-of-principle demonstration of the system involved the deflection of streptavidin coated magnetic particles through a single stream of fluorescently labeled biotin, allowing binding of the biotin such that the particles became fluorescent [32, 82]. The platform was later extended to immunoassays, first involving the deflection of streptavidin coated particles through a single stream of fluorescently labeled, biotinylated mouse IgG [31], and later through two reagent streams of biotinylated IgG and then fluorescently labeled secondary IgG antibody [32]. The work has since been demonstrated by Tam et al. [83] for a C-reactive protein (CRP) sandwich immunoassay by deflecting primary CRP-antibody coated particles through streams of CRP and then fluorescently labeled secondary antibody. The format has also been extended to one-step DNA hybridization by deflecting single-stranded DNA (ssDNA) coated magnetic particles through a stream of fluorescently labeled complementary ssDNA [31, 84]. Vojtišek et al. [84] then applied the system to a two-step DNA hybridization in which ssDNA coated particles were deflected first through a laminar stream of complementary ssDNA to allow binding, before the now double-stranded DNA was passed through a stream of PicoGreen intercalator to fluorescently tag it. Tam et al. [85] have also demonstrated the fluorescent labeling of magnetically coated yeast cells, so-called “cyborg cells” [86], by

deflecting them through a single reagent stream containing fluorescently tagged, positively charged polyelectrolyte solution.

Baier et al. [87] presented models and simulations for this one-shot style of approach. They investigated cell separations in which magnetic particles would be deflected through a stream of cells, thus capturing target cells and removing them from the sample stream and into a buffer stream. The models took into account a range of forces and effects, including the magnetic forces involved, binding kinetics, the binding of multiple beads per cell, sedimentation, shear and stirring effects, and particle trajectories and velocities.

Kim et al. [88, 89] developed a zig-zag (see Fig. 1e) multilaminar flow system for the continuous separation of target cells from a sample stream, but notably went beyond the practiced technique of introducing a sample already containing particles into a chip and then deflecting those particles into a buffer stream [25]. Here, functionalized magnetic particles were deflected by a magnet into a laminar stream containing a mixture of T-cells and erythrocytes, whereupon the T-cells became bound to the particles. Further downstream, a second magnet deflected the particles back into their original stream, thus achieving a separation. An improved design was later demonstrated [90] in which a curved channel was employed, yielding a three times higher recovery rate of the T-cells compared to the original straight channel.

Sasso et al. [91] also utilized a zig-zag style multilaminar device (Fig. 11b), in this case for immunoassays, which built upon the previous work of the group involving hydrodynamic filtration [70, 71]. A proof-of-principle one-step reaction was demonstrated in which streptavidin coated particles were deflected into and out of a stream of fluorescently labeled biotin. A two-step sandwich immunoassay was then performed with the deflection of antibody coated particles into and out of a stream of C3a antigen, then into and out of a stream of fluorescently labeled secondary antibody. An updated design involved the use of a three-layered device in which each step of the immunoassay took place in a different layer [92]. Here, incubation times were increased by introducing a spiral incubation channel during each reaction step, allowing the concurrent detection of IL-6 and TNF- α . A variant of this spiral-based device was employed for the continuous monitoring of C3a antigen during simulated cardiopulmonary bypass (CPB) surgery with donor blood [93]. Blood was continuously sampled from the CPB circuit and filtered, and the extracted plasma pumped into the immunoassay device where the C3a was captured via the magnetic particles. Only this first step of the immunoassay was achieved on-chip, but the system nonetheless showed potential for the continuous monitoring of blood during surgery.

Ganguly et al. [94] developed a one-shot multilaminar flow device in which streptavidin coated magnetic particles were deflected via a permanent magnet through a reagent stream of

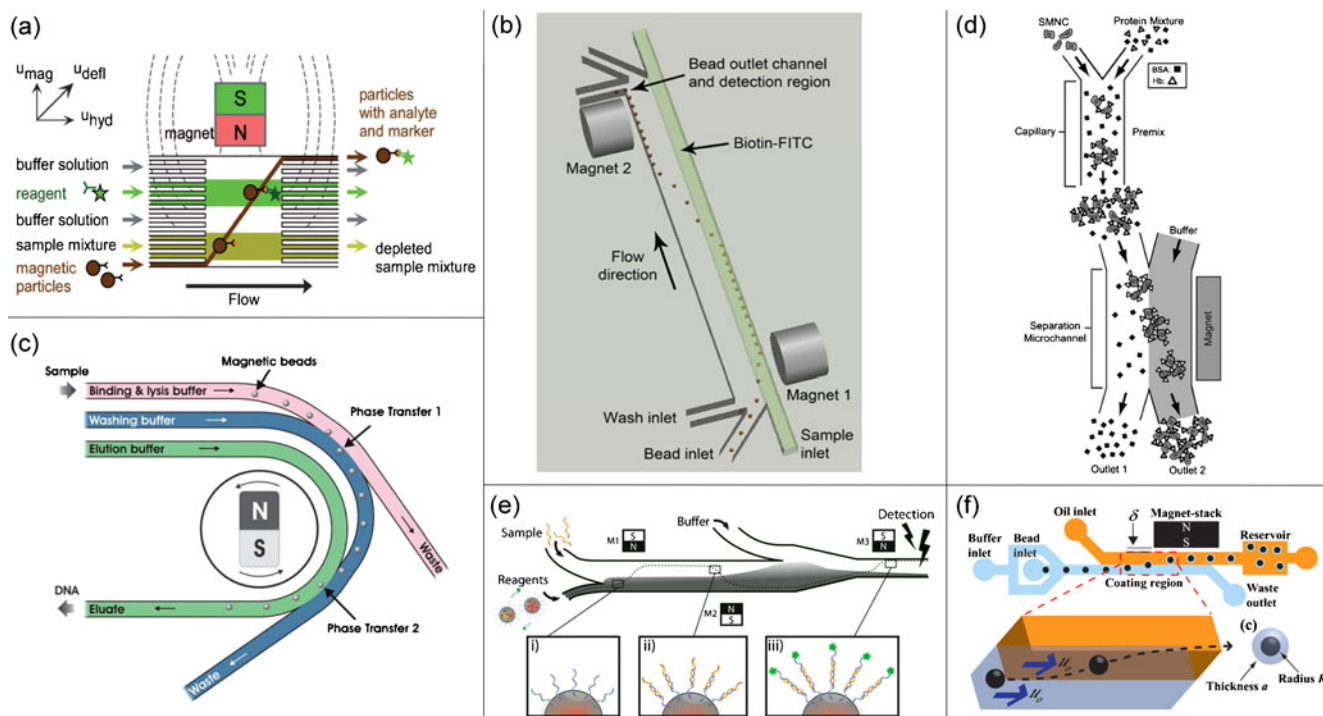


Fig. 11 Continuous flow processing utilizing magnetic forces. **(a)** A one-shot device for performing two-step sandwich immunoassays (Reproduced from [32] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b904724g>). **(b)** A zig-zag platform for performing immunoassays (With kind permission from Springer Science and Business Media: [91], Figure number 1a). **(c)** Phase-transfer magnetophoresis, employing a rotating magnet for the collection, washing, and elution of DNA (Reproduced from [96] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/>

[c0lc00129e](http://dx.doi.org/10.1039/c0lc00129e)). **(d)** Two-stage capture and separation of proteins (With kind permission from Springer Science and Business Media: [99], Figure number 1). **(e)** Zig-zag device for the multiplexed genetic detection of sexually transmitted infections using barcoded magnetic particles (Reprinted with permission from [100]. Copyright 2013 American Chemical Society). **(f)** Coating of magnetic particles by pulling them through the interface between two immiscible liquid phases (Reprinted with permission from [101]. Copyright 2011 American Institute of Physics)

biotinylated, fluorescently labeled oligonucleotides and into a final washing stream. Here, the particles were allowed to aggregate at the sidewall of the channel and their fluorescence intensity was measured. Numerical models of the platform were also prepared towards optimization of the system in terms of analyte concentration, particle characteristics, flow velocity and fluid viscosity [95].

Karle et al. [96] utilized a form of one-shot processing as part of a “phase-transfer magnetophoresis” system for the continuous extraction of DNA from *E. coli* (Fig. 11c). *E. coli* was lysed off-chip and its DNA was bound to magnetic particles that were pumped into the microfluidic device, which consisted of interconnected curved channels. The particles were deflected by a rotating magnet into a channel containing wash buffer before being deflected downstream into a channel containing elution buffer to release the DNA from the particle surfaces for off-chip analysis. Interestingly, the magnetic particles in this system did not pass through the chip individually, but instead formed short chains of particles that moved in the opposite direction to the rotating magnetic field [97].

Zhou et al. [98] developed a platform containing a “target acquisition by repetitive traversal” (TART) incubator and a

separation chamber towards its application for separations and immunoassays. The incubator consisted of a serpentine channel in which particles were deflected back-and-forth into a sample stream to ensure binding of target molecules, before passing into the chamber where the particles were separated from the sample stream via free-flow magnetophoresis. Lee et al. [99] developed a similar device in which nanoparticle agglomerates were mixed with a protein sample of hemoglobin and bovine serum albumin (BSA) in a capillary before passing into a chamber for separation (Fig. 11d). The particles bound to the hemoglobin, allowing their separation from the mixture.

Gao et al. [100] developed a zig-zag immunoassay platform for the multiplexed genetic detection of HIV, hepatitis B, and syphilis (Fig. 11e). Fluorescent magnetic microparticles that were “barcoded” with quantum dots (QDs) were fabricated, and capture probes for each infectious disease were conjugated to a different type of barcoded particle. These particles were first deflected into a sample stream to allow the capture of detection probes for each of the infectious pathogens before being deflected back into the original stream where fluorescently labeled reporter probes became bound to the captured

genetic targets. The particles were finally deflected into a stream of fresh buffer for washing and fluorescence detection. The fluorescence signal from the reporter probes indicated the presence and concentration of infectious disease, while the fluorescence of the QDs provided the identity of the disease.

Tsai et al. [101] demonstrated a system in which magnetic particles were deflected from a stream of aqueous solution into a stream of an immiscible oil, resulting in the coating of the particles with a thin layer of water (Fig. 11f). The thickness of the water layer was controlled the flow rates of the solutions, whereas the number of particles aggregated in a single coating was determined by the magnetic field strength and the interfacial tension between the two solutions. The platform was later employed for the measurement of ultralow interfacial tension by studying the behavior of the particles as they approached the liquid–liquid interface [102].

A relatively recent development in the manipulation of magnetic particles through multiple solutions is that of “immiscible filtration assisted by surface tension” (IFAST), pioneered by the group of Beebe [103]. This technique involves introducing reagents and washing solutions into a series of chambers, through which appropriately functionalized magnetic particles are sequentially pulled via a moving magnet, similar to one-shot continuous methodology, for extractions and immunoassays. However, since the IFAST technique utilizes chambers of stationary liquids rather than continuously flowing multilaminar streams, and due to the field itself having grown substantial enough to warrant its own review article, the available literature will not be reviewed any further here.

Diamagnetic repulsion

While the attraction of magnetic objects to a magnetic field is intuitive and well-known, the phenomena of diamagnetic repulsion is much less known and applied. Referring to Eq. 8, when the difference in magnetic susceptibility ($\Delta\chi$) between the particle (χ_p) and the medium (χ_m) is positive, the particle will be attracted towards the region of highest magnetic field, but when $\Delta\chi$ is negative the particle is actually repelled from the region of highest field. This is most easily achieved when the particle is diamagnetic ($\chi_p < 0$) and the surrounding medium is paramagnetic ($\chi_m > 0$), the latter of which can be accomplished by preparing solutions containing paramagnetic ions (e.g., Mn^{2+} , Gd^{3+}) or ferrofluid. This is particularly useful since all materials are actually diamagnetic, although if the material exhibits any other type of magnetism (e.g., ferromagnetism, paramagnetism) then the diamagnetic behavior will be masked.

Diamagnetic repulsion has been presented as being applicable to the continuous separation of particles and cells via on-chip free-flow diamagnetophoresis (also known as negative magnetophoresis) [104–106]. This technique was adapted by Tam et al. [83] for performing continuous multilaminar flow

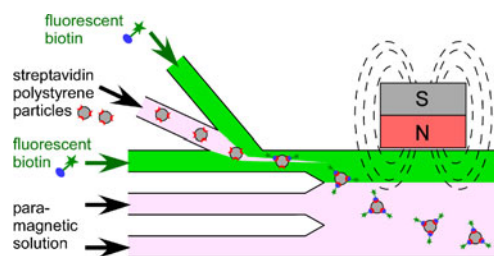


Fig. 12 Diamagnetic repulsion for the continuous deflection of streptavidin coated polystyrene particles into and out of a fluorescently labeled biotin stream, with all solutions containing paramagnetic ions. (Redrawn from [83])

processing via diamagnetic repulsion (Fig. 12). Streptavidin-coated polystyrene particles in a paramagnetic solution of manganese (II) chloride were introduced into the flow focusing section of a microfluidic device between two streams of fluorescently labeled biotin in MnCl_2 . Further downstream, the particles and biotin streams entered a chamber alongside a stream of MnCl_2 washing solution. A permanent magnet was used to repel the particles through the biotin reagent stream and into the washing solution, allowing fluorescence detection of the treated particles.

Acoustic forces for continuous flow processing

The generation of ultrasonic standing waves creates stationary pressure gradients that exert a force on particles or cells suspended in a media. This acoustic force will cause such species to move either to a pressure node or to an anti-node, depending on the physical properties of the particles. The acoustic force, F_{ac} (Newtons), exerted on a particle or cell is directed along the axis of the incident wave, and is given by Eq. 9 [27, 107]:

$$F_{ac} = \frac{\pi p_0^2 V_p \beta_m}{2 \lambda} \phi(\beta, \rho) \sin(2kx) \quad (9)$$

where p_0 is the pressure amplitude (Pa), V_p is volume of the particle (m^3), β_m is the medium compressibility (Pa^{-1}), λ is the acoustic wavelength (m), x is the distance from a pressure node (m), and k is the wave number, which is defined by $2\pi/\lambda$; ϕ is the acoustic contrast factor, which is given by Eq. 10:

$$\phi(\beta, \rho) = \frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} \frac{\beta_p}{\beta_m} \quad (10)$$

where ρ_p is the density of the particle (kg m^{-3}), ρ_m is the density of the medium (kg m^{-3}), and β_p is the particle compressibility (Pa^{-1}). When ϕ is less than zero (as for many cells and particles) the species will migrate towards the pressure node, whereas if ϕ is greater than zero (as for gas bubbles and lipids) then the particles will move to the anti-node. The

theory and application of acoustic forces in microfluidics has been reviewed by Laurell *et al.* [107], while a series of Focus articles about acoustofluidics has recently been presented in *Lab on a Chip* [108].

Acoustic forces have been exploited for multilaminar flow applications in the form of sequential exchangers (see Fig. 1f). Augustsson *et al.* [109] constructed a silicon-glass device that featured a main channel with several side channels acting as inlets and outlets for the introduction and removal of new solutions (Fig. 13). An ultrasonic transducer generated a standing wave across the device, creating a pressure node along the center of the main channel. As particles or cells moved through the main channel, they would be pushed towards the outlets upon the introduction of a new exchange solution into the device, but the acoustic forces would draw them back to the pressure node in the center of the channel. Thus, polystyrene particles and red blood cells could be transferred from their initial solution to a wash buffer solution with high efficiency and minimal cross-over of the starting solution. This acoustophoresis-based sequential exchanger was later adapted by Augustsson *et al.* [110–112] for the analysis of proteins and peptides that were loosely bound to ion-exchange microbeads or sperm cells. The bead or cell samples were introduced into the main channel and sequentially drawn through solutions containing different pH values, with different populations of surface-bound peptides being eluted in the various pH buffers. Once eluted, the peptides could be collected from the side channel outlets, purified by solid-phase extraction, and analysed by MALDI-MS.

Optical tweezers for trapping and manipulation of particles and cells

Optical forces can be applied for the trapping and movement of particles and cells in the form of optical tweezers. When a laser beam is directed at a particle, it exerts a “radiation pressure” upon it, while a gradient force drives particles with

a higher refractive index than the surrounding medium towards the focal point of the laser, the region of highest optical density [113]. By manipulating these phenomena, it is possible to trap a particle or cell in the focused light of a laser beam, after which the trapped species can be manoeuvred by moving the position of the focal point. In general, the optical force, F_{opt} (Newtons), on a particle can be expressed by Eq. 11 [114]:

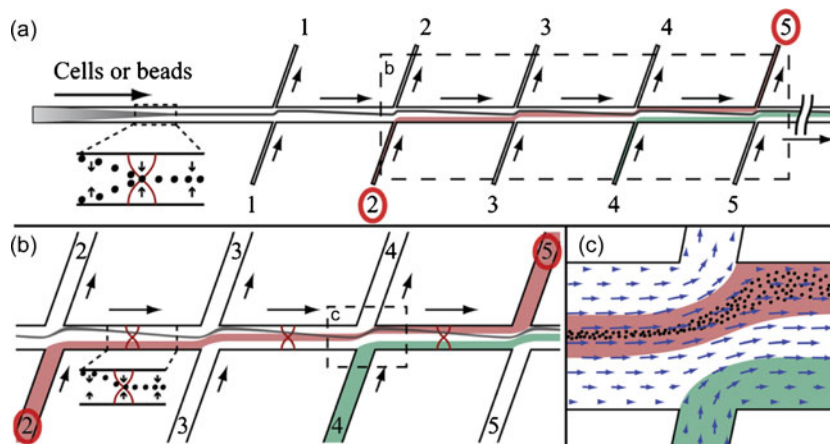
$$F_{\text{opt}} = \frac{n_1 P}{c} Q \quad (11)$$

where n_1 is the refractive index of the surrounding medium, P is the laser power incident on the particle, c is the speed of light ($2.998 \times 10^8 \text{ m s}^{-1}$), and Q is the trapping efficiency factor that depends on the size, shape, material, and position of the particle with respect to the spatial profile of the beam. Because of the fine control offered by optical tweezers, they have found many applications in microfluidic devices, particularly for the manipulation of cells [113–115].

Optical tweezers have been employed as a component of several multilaminar flow platforms. Eriksson *et al.* [116] demonstrated the use of optical tweezers to “shuttle” (see Fig. 1c) yeast cells back-and-forth across two laminar flow streams, one containing growth medium and one containing sodium chloride, for the study of osmotic shock (Fig. 14a). The size of the trapped cell was monitored to determine the effect of single and multiple osmotic shocks on the cell volume. The exposure of trapped yeast cells to reagent streams for 30 min was also monitored in order to test the ability to perform studies over a longer time scales. In an extension of this work, a spatial light modulator was used to trap 25 yeast cells in an array and shuttle them between two environments [117]. In separate experiments, signalling pathways within the cells were monitored during exposure to osmotic shock, and after movement from glucose-rich to glucose-free media.

Boer *et al.* [118] investigated the use of optical tweezers as part of a stream switching (see Fig. 1b) multilaminar flow

Fig. 13 Sequential exchange of reagent and washing solutions in a main channel, with acoustic forces maintaining the position of the particles within the center of the channel (Reproduced from [111] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/c2lc40200a>)



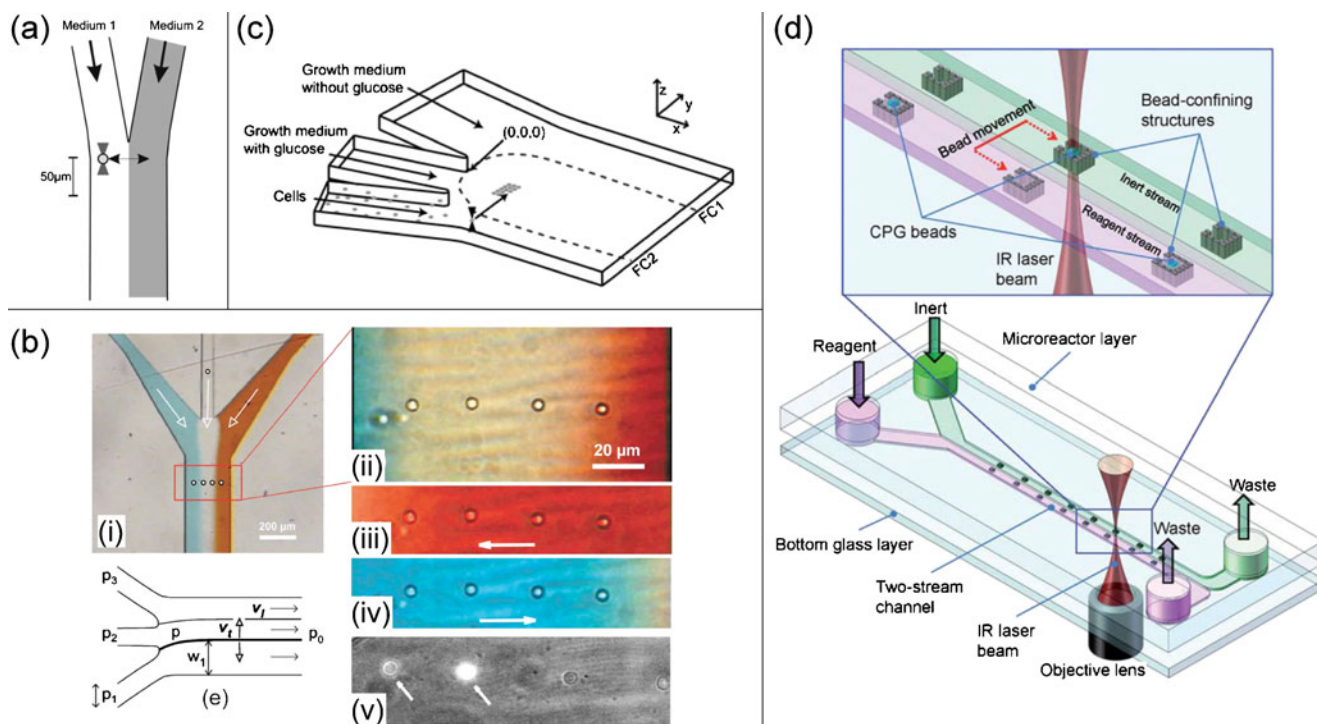


Fig. 14 The application of optical tweezers to multilaminar flow systems. (a) The shuttling of yeast cells between a reagent stream and a buffer stream (Reproduced from [116] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b613650h>). (b) The simultaneous capture of four particles, which are subsequently exposed to different reagents via stream switching (Reprinted with permission from [118]. Copyright 2007 American Institute of Physics). (c) The capture and arrangement of cells for exposure to glucose-free and

glucose-rich solutions via stream switching (Reproduced from [119] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b913587a>). (d) Transport of particles between traps within two co-flowing streams, allowing the synthesis of DNA by replacing the solution in the reagent stream while the particles are held in the washing stream (Reproduced from [120] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/c0lc00577k>)

system. The application of four lasers allowed four polystyrene particles to be trapped simultaneously across the width of a channel, and the relative flow rates of three laminar flow streams of dye were varied to expose the particles to each one (Fig. 14b). Eriksson et al. [119] also employed optical tweezers as part of a stream switching system, in combination with the adherence of cells to the channel surface for studying the effect of changes in glucose availability on yeast cells (Fig. 14c). Cells were captured from their inlet stream via optical tweezers and positioned on the channel surface in the chamber, onto which they were adhered. An array of cells was formed, after which the relative flow rates of the streams were altered to expose the cells to either glucose-rich or glucose-free environments. Studies were also performed to determine the effect of the laser on cell reproduction, with findings indicating that no negative effects were induced by the laser.

Wang et al. [120] developed a device that combined the manipulative capabilities of optical tweezers with traps formed from physical barriers for bead-based DNA oligonucleotide synthesis (Fig. 14d). The platform consisted of a main channel through which two laminar flow streams were generated, one inert stream and one reagent stream. Within each stream were a series of physical traps fabricated from micropillars, and

optical tweezers were used to move controlled pore glass (CPG) beads between them. By consecutively replacing the reagent stream with solutions containing different nucleotides,

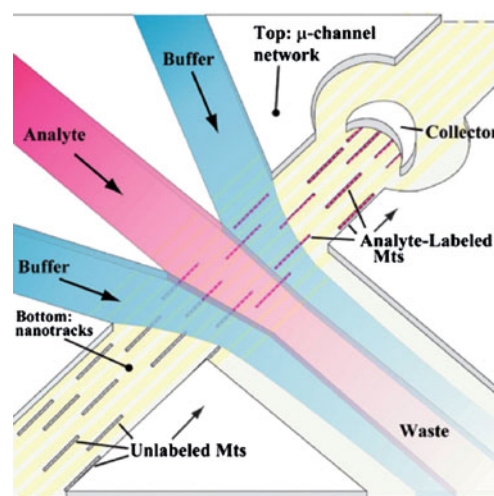


Fig. 15 The application of biomolecular motors in which microtubules (Mts) are transported by ATP-fueled kinesin for the capture of analyte from a laminar flow stream (Reproduced from [122] with permission of The Royal Society of Chemistry. <http://dx.doi.org/10.1039/b900753a>)

Table 1 Comparison of various parameters between the different particle and cell handling techniques employed in multilaminar flow platforms

Mechanism	Trapping / continuous	Sample	Application	Force	Flow rate	Residence time	Advantages	Disadvantages	Ref.
Adherence	Trapping (LE, SS)	Cells Embryos Single molecule	Localized stimulation Induced rotation	N/A	60 mm/s	0 – 2.5 h	No external forces required	Often requires surface treatment	[33]
Patch clamp	Trapping (OS)	Cells	Ion channel activity Cell poration	N/A	3 mm/s	0 – 120 s	Precise positioning	Not a universal method	[53]
Microposts / Microgrooves	Trapping (LE)	Plant root Neuron dendrites	Localized stimulation	N/A	50 mm/s	0 – 150 min	No external forces; Precise positioning	Risk of blockages	[36]
Deterministic Lateral Displacement	Continuous (OS)	Particles Cells	Labeling Cell lysis	N/A	50 – 500 $\mu\text{m/s}$	< 8 s	No external forces	Relatively complex chip design	[58]
Pinball	Continuous (ZZ)	Droplets	Polyelectrolyte coating	N/A	150 $\mu\text{L/h}$	< 15 s	No external forces	Not a flexible system	[59]
Micropost array railing	Continuous (OS, ZZ)	Microparticles Cells	Binding assays Cell deflection	N/A	0.6 $\mu\text{L/min}$	1 min	No external forces	Not a flexible system	[60]
Microtrain railing	Both (LE, OS, ZZ)	Microtrains Cells (in trains)	Cross-solution movement Array formation Zig-zag deflection	N/A	Not given	Not given	No external forces	Relatively complex setup	[61]
Pinched flow	Continuous (SE)	Cells	Quenched flow analysis	N/A	100 – 400 mm/s	0 – 2 s	No external forces	Requires careful consideration of chip design	[66]
Hydrodynamic filtration	Continuous (SE)	Cells Microparticles	Cell membrane digestion Binding assays	N/A	15 – 60 $\mu\text{L/min}$	17 ms – 3.64 s	No external forces	Relatively complex chip design	[68, 69]
Dielectrophoresis	Both (SS, OS, ZZ)	Cells Particles	Cell immersion/dipping Binding assays	28 – 55 pN	0.25 – 4.2 mm/s	3 s	Force controlled by electric field; Intrinsic	Complex fabrication; Fouling of electrodes	[74–76]
Magnetism	Continuous (OS, ZZ)	Magnetic particles	Immunoassays DNA hybridization Extractions	1.3 – 16 pN	0.06 – 200 $\mu\text{L/min}$	2 s – 25 min	Contactless; Simple setup	Requires magnetic properties or label	[32, 92, 100]
Diamagnetism	Continuous (OS)	Microparticles	Particle coating Binding assays	1 pN	1 $\mu\text{L/h}$	~ 40 s	Contactless; Intrinsic	Weak force; Magnetic medium needed	[83]
Acoustics	Continuous (SE)	Microparticles Cells	Buffer exchange Peptide analysis	Not given (typically 100 – 400 pN)	60 – 120 $\mu\text{L/min}$	0.35 s	Contactless; Intrinsic	Relatively complex setup	[109, 110]
Optical tweezers	Both (SS, Sh)	Cells Microparticles	Exposure to stimuli Oligonucleotide synthesis	2 – 425 pN	0.7 – 200 $\mu\text{L/min}$	0.125 s – 30 min	Contactless; Precise control	Relatively complex setup	[116, 118–120]
Biomolecular motors	Continuous (OS)	Microtubules	Binding assays	N/A	100 mm/s	~ 100 s	Precise control of motion	Requires nanotrack	[122]

It should be noted that the values and information are based on those applied to specific procedures, and by no means represent the limitations of each system. LE = localized exposure; SS = stream switching; Sh = shuttling; OS = one-shot; ZZ = zig-zag; SE = sequential exchange

the beads could be transferred into and out of the nucleotide solution as required until an oligonucleotide sequence of 25-nt had been synthesised on their surfaces.

Biomolecular motors for continuous flow processing

Biomolecular motors are a form of “synthetic nanomachine” capable of the capture, transport, and release of cargo [121]. These motors consist of microtubule protein filaments that are transported by surface-immobilized kinesin motor proteins in the presence of ATP fuel, moving 8 nm per hydrolysis of ATP. The constriction of microtubules within nanotracks fabricated on the surface of a microfluidic channel enables directional control over the movement of the microtubules, which can be functionalized in order to transport cargo from one location to another.

These biomolecular motors were applied by Kim et al. [122] to a one-shot operation (see Fig. 1d) for the labeling of microtubules with analyte. Multilaminar streams were generated in a microfluidic channel, consisting of washing buffer solutions and a central reagent solution of fluorescently labeled streptavidin (Fig. 15). Nanotracks, featuring immobilized kinesin, were imprinted on the lower surface of the microfluidic channel, oriented perpendicular to the main channel. When biotinylated microtubules were introduced into the nanotracks and in the presence of ATP, they migrated along the tracks, thus passing through the streams of streptavidin and washing buffer. Finally, the now streptavidin-labeled microtubules were collected in a horseshoe structure at the end of the nanotracks and their fluorescence intensities were measured.

Conclusions

Laminar flow is an intrinsic component of microfluidic systems and can be exploited in a variety of ways in order to treat particles and cells for studies, reactions, and immunoassays. By careful manipulation of the flow, it is possible to localize the exposure of stimuli on specific areas of trapped cells or to repeatedly expose trapped particles or cells to different reagents by changing the relative flow rates of the solutions. The continuous deflection of particles and cells through multiple reagent and washing streams yields large decreases in processing times, with multi-step procedures reduced into a single, rapid process. Such deflection can be achieved using microfabricated structures such as pillars and grooves within the channels, or by the application of a number of different forces for controlled manoeuvring of the particles or cells. These numerous particle and cell handling methodologies have been summarized and compared in Table 1. Of course, each method has its own advantages and disadvantages, and some of the major points in both of these cases are given for each method in the table.

Multilaminar flow processing using particle and cells provides many advantages in terms of time and fine control over both the fluid flow and the manipulation of the objects themselves. With such a variety of particle-handling methods available, ultimately the choice of which one a researcher uses will depend on several factors including cost, complexity, availability, the accuracy and precision of the fluid or particle control, and the flexibility of the system. There are, of course, important issues that would benefit from further development in order to both improve the systems and to broaden their applicability. For example, improvements in reliability and robustness would be welcome in all cases, while greater throughput in the continuous flow methods would be highly beneficial. Many of the assay-based formats would profit from interfacing technologies that would allow a sample to be injected into one of the laminar flow streams in order to reduce setup times and the potential for human error, or enable continuous sampling for longer term studies. Integrated detection would also be a welcome addition to many of the assay formats, particularly those with a view to portability. In addition, although many different particle handling techniques have been employed for multilaminar flow systems, it can be envisaged that several other techniques could also be applied with relative ease, including optoelectrofluidic forces [123], thermophoresis [124], diffusiphoresis [125], inertial forces [126] that have included manipulation based on cell/particle shape and deformability [127], surface acoustic waves (SAW) [128], optoacoustic tweezers [129], and laser-induced (optothermally generated) bubbles [129, 130]. This would potentially allow a wider range of applications to be explored, while offering further options to the operator in terms of having more tools available with a different set of advantages and disadvantages.

With the ability to utilize the inherent fluid properties present in microchannels to form multiple adjacent reagent and washing streams, and with the sheer range of tools available for the manipulation of both the fluid flows and the objects within them, it is clear that multilaminar flow systems are, and will remain, an important part of many microfluidic platforms for a vast range of (bio)chemical processes.

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