

Lab-on-a-chip: microfluidics in drug discovery

Petra S. Dittrich and Andreas Manz

Abstract | Miniaturization can expand the capability of existing bioassays, separation technologies and chemical synthesis techniques. Although a reduction in size to the micrometre scale will usually not change the nature of molecular reactions, laws of scale for surface per volume, molecular diffusion and heat transport enable dramatic increases in throughput. Besides the many microwell-plate- or bead-based methods, microfluidic chips have been widely used to provide small volumes and fluid connections and could eventually outperform conventionally used robotic fluid handling. Moreover, completely novel applications without a macroscopic equivalent have recently been developed. This article reviews current and future applications of microfluidics and highlights the potential of 'lab-on-a-chip' technology for drug discovery.

Electrophoresis

The motion of charged particles in an electrical field towards the opposite electrode.

Photolithography

A fabrication technique to generate small features in micrometre dimensions on microchip substrates such as silicon, glass or polymers.

The search for drugs demands robust and fast methods to find, refine and test a probable drug candidate. The discovery of the elusive molecule with unique qualities out of a nearly unlimited number of possibilities is laborious, time consuming and relies heavily on technological resources that are available for handling small liquid volumes, automation, and high-throughput processing and analysis. An important advance was the miniaturization of entire systems, that is, the introduction of high-density plate formats and nanolitre dispensing systems. The required procedures, however, are extremely diverse and include manifold syntheses and analytical steps *in vivo* and *in vitro*. Performing these processes on a microfluidic chip in a continuous stepwise manner could pave the way for increased speed and automation opportunities.

Microfluidic chips are small platforms comprising channel systems connected to liquid reservoirs by, for example, tubing systems in turn linked to syringes. The size of the channels is in the range of a few micrometres, which greatly facilitates handling of volumes much smaller than a microlitre. Appropriate channel design and integrated tools such as electrodes or a specific surface pattern are now facilitating the incorporation of many operational steps by allowing molecular compounds to pass each unit successively (BOX 1). These individual steps include sampling, sample enrichment (pre-concentration and preconditioning steps, such as filtering), mixing, reaction modules (for example, different heating zones), product separation, isolation and analysis.

Initially, the concept of microfluidics was dedicated to significantly reducing sample consumption and increasing efficiency in separation methods¹. A prominent example is the miniaturization of electrophoresis and related techniques^{2–4}. The high efficiency of electrophoretic separation in channels with small cross-sectional area is achieved through improved heat transfer, and the simplicity of the planar design allows for massive parallelization⁵. Furthermore, the low costs of mass producing microchips and the automation of reaction systems have allowed the adaptation of microfluidic systems for commercial use. Custom-made planar microfluidic chips and entire measuring systems are now commercially sold by companies such as Agilent Technologies, Evotec Technologies, Caliper Life Sciences, Hitachi and Fluidigm Technology⁶.

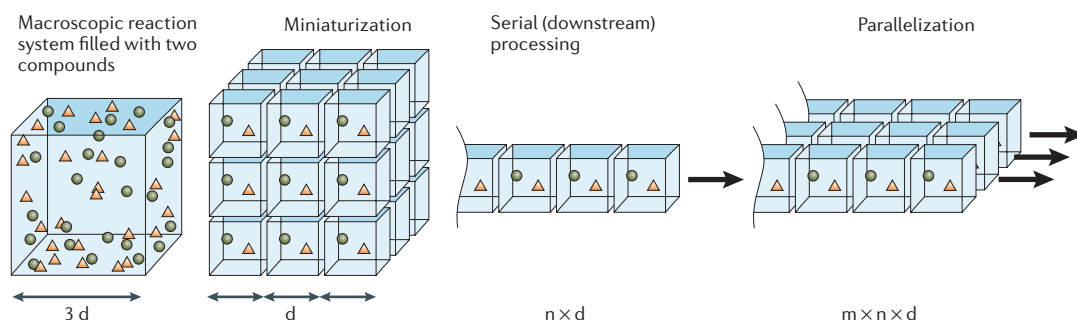
The fabrication of microfluidic chips usually requires cleanroom facilities and specific equipment (see REF. 7; see MEMS and Nanotechnology Clearinghouse, Further information). A great variety of microchip fabrication techniques and materials are available for producing highly sophisticated two- and three-dimensional microstructures with integrated modules. Pumps and valves, mixers, motors and other functional units that assist chemists in the macroscopic lab have been miniaturized^{8,9}. Likewise, sensors and detectors — even optical components — can be integrated on-chip¹⁰. The vast majority of microfluidic devices are, however, simple planar microchips fabricated by photolithography on substrates such as glass, silicon or polymers^{11–14}.

ISAS — Institute for Analytical Sciences,
Bunsen-Kirchhoff-Str. 11,
D-44139 Dortmund,
Germany.
e-mails: dittrich@ansci.de;
a.manz@ansci.de
doi:10.1038/nrd1985

Box 1 | What is gained by miniaturization?

Some simple considerations show what various opportunities can emerge from miniaturization of reaction systems. This is illustrated by means of scaling laws (figure and table) giving the dependence of reaction parameters on the size of the system (for example, reaction volume and diffusion time)^{1,25}. Due to the short distances in microfluidic channels, the transport times of mass and heat are shortened. Fast and controlled heat supply as well as cooling is facilitated due to high surface to volume ratio. Therefore, important running conditions of chemical processes, such as compound concentration and temperature, can be regulated precisely. One key feature of microfluidics is the integration of different functional units for reaction (for example, mixer and heater), separation and detection in a channel network. Therefore, serial processing and analysis can be easily performed in the flowing systems. Furthermore, because space is used sparingly, massive parallelization can be accomplished.

d, length of edge; n and m, numbers of reaction systems serial and parallel, respectively.



Parameter	Macroscopic example	Factor change	Microscopic example
Length of edge	1 mm	d	$1 \mu\text{m}$
Surface	1 mm^2	d^2	$1 \mu\text{m}^2$
Volume	$1 \mu\text{l}$	d^3	1 fl
Number of molecules	10^9	d^3	1
Diffusion time over d ($D = 10^{-6} \text{ cm}^2 \text{ s}^{-1}$)	15 min	d^2	1 ms
Example: in flowing systems			
Linear flow rate	$1 \mu\text{m/s}$	d	1 mm/s
Separation time	$10^5 \text{ s} (>1 \text{ day})^*$	d^2	100 ms
Example: in planar array			
Number of volumes per microwell plate	96	d^2	10^8

*Typically, for example, high-performance liquid chromatography in packed column.

Tightly linked to miniaturization technology is the capacity to image and detect the sample. Reduction of the reaction volume naturally decreases the absolute number of analytes and therefore makes possible the identification and analysis of individual small compounds out of a flowing bulk sample. Therefore, adequate, high-sensitivity detection techniques are indispensable.

Microfluidic systems have been adapted and interfaced to most of the common analytical detection techniques, such as electrochemical methods, mass spectrometry and optical methods including absorption, refractive index variation, surface plasmon resonance, chemoluminescence and fluorescence.

By far the most popular approach for high-sensitivity detection is fluorescence analysis. The high temporal resolution enables real-time measurements. Moreover, fluorophores attached to the analytes can be excited and detected selectively. The excellent sensitivity of fluorescence spectroscopy is further enhanced by reducing the size of the detection volume, which results in high signal-to-background ratios, such as that achieved by confocal setups (laser scanning microscopes) and total internal reflection microscopy. Analysis on the single-molecule-level

in microchips is possible for various applications¹⁵ such as enzymatic assays¹⁶, kinetic studies of protein folding¹⁷ and photo-induced protein conversion¹⁸ and, in particular, for the detection and analysis of DNA^{19–23}.

‘Lab-on-a-chip’ technology is an emerging field, approached by scientists from many disciplines (BOX 2) and exploited for a wide range of applications. The myriad benefits of microfluidic platforms for the study of biological systems, as well as details of fabrication procedures for generating microfluidic chips, have been reviewed in a number of excellent articles^{8–15,24–29}.

The following review highlights current and possible future applications of microfluidics in the process of drug discovery, and focuses on the developments for chemical syntheses, high-throughput screening and cell treatment. FIGURE 1 summarizes the various process steps that can be performed by microfluidic methods, and some of their applications to drug discovery.

Chemical syntheses for compound generation

Although the majority of studies make use of the analytical potential of the microfluidic chips, considerable efforts have also been made to use microsystem technology

Total internal reflection microscopy
An optical method to image fluorescent samples at interfaces, such as in proximity to a glass surface.

Box 2 | Definitions related to 'lab-on-a-chip' technology

'Lab-on-a-chip' technology in the life sciences is a research area that is approached by scientists from different disciplines each having its own 'language' and standards. In the course of time, however, several buzzwords have emerged that are nowadays used in parallel. In the following, we give definitions for the most often mentioned words, being aware that the meanings overlap.

μTAS (micro total analysis system)

An integrated system that performs all steps in an analysis — that is, sampling, sample pre-treatment, sample transport, chemical reactions, analyte separation, product isolation and detection in a microfluidic chip. First defined in 1990, μTAS emphasizes the analytical function of a microfluidic chip.

'Lab-on-a-chip'

Lap-on-a-chip is a term widely used for any kind of research with the goal of miniaturizing chemical and biological processes. It is not a well-defined scientific term. Lab-on-a-chip technologies include microfluidic chips as well as non-fluidic miniaturized systems such as sensors and arrays (the so-called biochips).

Miniaturization

The fact that existing molecular assays, such as chemical binding, chromatographic extractions and electrophoretic separations, can be performed in different volumes without a difference in chemical quality (identical information content) has led to a variety of technologies of fabrications that enable reactions to be scaled-down or 'miniaturized'. Miniaturization is most commonly aimed at particles (beads, packed columns and vesicles), capillaries, gels and fluidic chips.

Microfabrication

Microfabrication describes 'cleanroom' fabrication processes for engineering solid flat substrates. It includes photolithography, direct-write laser (e-beam, X-ray) lithography, wet etching, reactive ion etching, film deposition (plasma, chemical vapour, sputtering) and bonding (eutectic, anodic, thermal, adhesion). It is mostly used for micro-electronic circuit fabrication, but also for MST or MEMS (see below) and 'lab-on-a-chip'.

Microsystem technology (MST) and micro-electromechanical systems (MEMS)

The word MEMS is taken from the electronics industry. MEMS refers to the fabrication of electronic or mechanical components on chips. Similarly, bio-MEMS emphasizes the integration of functional units (particularly electrical manipulation processes) on microchips for biological and biomedical purposes. The term also includes biomedical devices such as chips for minimally invasive surgery and microneedles. In Europe, MST is used to describe MEMS.

Microfluidics

Microfluidics signifies any kind of experimental and theoretical research of liquid streams generated in chips comprising micro-sized channels, including fabrication, handling and practical use of these chips. In a broader sense, streams of gases or fluidized solids/particles in microchips are also included in the definition of microfluidics. For channels with dimensions in the sub-micrometre range, the word 'nanofluidics' is used.

(MST; also known as MEMS, see BOX 2) for preparative applications. In the following section advances in synthetic processes, in particular the production of combinatorial libraries, are described.

Using merging channel geometries (such as T and Y shapes), various reactants can be mixed and inter-molecular reactions induced. The concentrations of reagents can be regulated in space and time, which gives rise to an additional level of control. Moreover, temperature — a key parameter in chemical processes — can be accurately regulated. The improved heat and mass transfer in small-scale reactors justifies positive expectations for preparative applications in microreactors. Indeed, many syntheses have been successfully performed in microreactors. Numerous examples demonstrate the benefits of miniaturizing reactors such as the significantly shortened reaction time — for example, from hours to minutes^{30,31}. In addition, many syntheses

in microreactors yield higher conversion rates and improved selectivity³². A generalization of the results is, however, not admissible and must be drawn for every chemical reaction separately.

In continuous flow microreactors, multi-step reactions can be performed easily. One promising example by Watts *et al.* is the multi-step synthesis of dipeptides giving a substantive yield over a period of 20 minutes³³ instead of a 24-hour procedure in a batch reaction yielding 40–50%. In a follow-up study the group demonstrated the formation of tripeptides³⁴. They also found reduced racemization in a microreactor synthesis compared to a batch reactor³⁵.

Operating under continuous-flow conditions also allows the combination of multiple reaction steps and on-line analysis on a single chip. Shortened reaction times and the direct analytic functioning of a chip could result in faster optimization of reaction parameters (feedback control). Furthermore, this concept of micro synthesis and total analysis system (μSYNTAS) provides a highly effective route for solution-based combinatorial chemistry first demonstrated by Mitchell *et al.*³⁶. In this work, a sub-reaction of the Ugi multicomponent condensation was performed in a distributive micromixing device that was integrated with time-of-flight mass spectrometry. The reaction was carried out between five different secondary amine hydrochlorides (piperidine derivatives) and formaldehyde to produce five different iminium cations. The injection of the piperidine derivatives into a methanol solution of formaldehyde was performed in a sequential manner (serial mode), as well as simultaneously (parallel mode) in a separate procedure. For both modes, the compound library could be derived and fully analysed in real time. The formation of a larger 7 × 3 combinatorial library was also demonstrated. For formation of 21 pyrazole derivatives (Knorr synthesis), seven different dicarbonyl reactants and three different hydrazines were supplied sequentially into a glass microreactor³⁷. No evidence of cross-contamination or carry-over was detected between the two consecutive reactions.

Solution-phase combinatorial synthesis of ($n \times m$) products (from two libraries with the size of n and m compounds, respectively) in a parallel fashion requires the respective number ($n \times m$) of separated reactors (FIG. 2). As a consequence, the complexity of such systems grows rapidly with the number of compounds^{38–40}. Using microchip technology, the need for large space and large quantities of sample can be eliminated, which was shown for a 2 × 2 reaction scheme by Kikutani *et al.*³⁹. They performed combinatorial formation of amides from two different amines and two different acid chlorides. The main challenge of the work, however, was the fabrication of a microfluidic chip that facilitates all possible mixing combinations for any $n \times m$ system (while the number of starting compound reservoirs are $n + m$). It is topologically impossible to disentangle all mixing modules in a system with more than two compounds on a planar chip, which necessitated the fabrication of a three-dimensional channel network.

Ugi multicomponent condensation

An organic reaction between a ketone or aldehyde, an isocyanide, an amine and a carboxylic acid to form a bis-amide. Libraries of low-molecular-mass drug-like compounds can be generated via the Ugi multicomponent condensation.

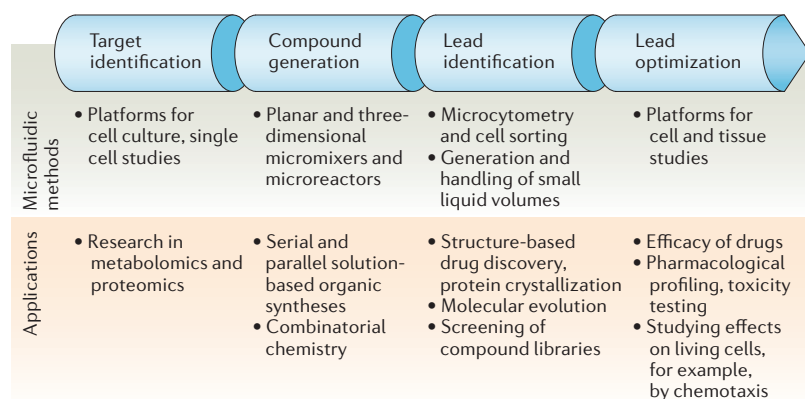


Figure 1 | Microfluidics in drug discovery. The figure depicts microfluidic methods, including respective applications, that are valuable for individual steps in the drug discovery process.

Despite the various examples, the potential of syntheses in microchips has not yet been fully realized. A novel approach demonstrating the potential of microfluidic channels was provided recently⁴¹, in which dry powder with large particle size (>100 μm) was injected into a microchip. By gaseous sheath flow, the powder was fluidized and could be transported through the microchannels. Such devices with reliable particle feeding systems could potentially replace the time-consuming process of weighing powders using a balance.

High-throughput screening and cell sorting

Having produced a library of compounds, the next straightforward step is lead identification. The search for compounds that bind to a particular target molecule, or interfere with, catalyse or modulate a particular reaction, could be performed *in vitro* or *in vivo*. In any case, methods and tools are required that are capable of vetting an extremely large number of compounds in a short time^{42,43}, because the number of possible leads is so vast.

The main efforts to save time and chemical compounds in high-throughput screening applications have resulted in miniaturization of existing technologies — that is, in increasing the well density on microwell plates, thereby decreasing the size of each well. However, currently available robotic systems are limited with regard to the speed of fluid dispensing that is achievable, and the accurate handling of very small liquid volumes that evaporate quickly is another challenge to this approach^{44,45}. Alternatively, screening assays can be performed in a continuous manner using a flowing system that allows the analysis and isolation of compounds on a single chip⁴⁶. The microfluidic channels form a sealed reaction system. After the introduction of compounds, liquid handling is performed exclusively within the channel system, which renders pipetting procedures unnecessary.

Examples for miniaturized continuous-flow separation devices are often based on physical properties of the analytes, such as electrophoretic mobility⁴⁷, refractive index⁴⁸, density⁴⁹ or size^{50,51}. On the other hand, dissolved or suspended particles and cells can be manipulated within the solution, and uncoupled

from their molecular and cellular properties, which makes them amenable to various kinds of secondary control parameters. For example, utilizing fluorescence is widely applicable for screening of libraries (such as phage-display technology^{52,53}) and for viability tests (for example, determination of toxicity), as well as for clinical medicine and basic biological research.

Much effort has been made to develop micro-sized fluorescence-activated cell-sorting devices (μFACS)^{54–63} (microfluidic chips for flow cytometry are also commercially available; for example, Agilent 2100 Bioanalyzer or Cytocon (Evotec Technologies)). The main challenge in such devices is the integration of sorting modules for precisely controlling the direction of the cells when a target cell is detected. One of the first examples of a chip-based sorting device was introduced by Fiedler *et al.* in 1998 using dielectrophoresis (DEP)⁵⁴. Certain electrode configurations were constructed to function either as a funnel, as aligners to break aggregates of cells, as a caging module for cell trapping or as a switching module. Recently, Hu *et al.*⁵⁵ adapted the DEP principle and presented a cell-sorting device that is capable of selecting rare bacterial cells that are attached to a DEP-responsive label.

In 1999, Quake and co-workers developed a microfluidic chip capable of detecting and sorting stained DNA fragments⁵⁶ and fluorescent bacterial cells⁵⁷ with high-throughput rates. The sample was guided by electro-osmotic flow at a T-junction into one of the output channels by proper switching of the electrodes. In following work, a further cell-sorting device was presented in which the transport of cells and sorting modules were solely based on hydrodynamic pumps⁵⁸. Furthermore, a sorting module based on optical forces was reported. In this work, a Y-shaped channel was utilized. Flow to the output channel was asymmetrically biased such that all cells flowed to the waste channel. When a target cell was identified, the optical switch was activated and a focused laser spot deflected the cell to the target output channel⁵⁹.

Although the throughput rates of μFACS devices (maximum several hundred cells per second) are still below commercial FACS machines (with impressive speed of several 10,000 cells per second), they offer the benefits of a continuous operation, including reaction steps preceding and after the sorting process. One example demonstrating this was introduced in 2003⁶⁰. The sorting parameter was the affinity of a fluorescent protein (phycoerythrin) for the bacterial cell membrane performed on the chip prior to the detection of the cells. This chip was adapted to a confocal microscope and was thereby capable of ultra-sensitive detection on a single-molecule scale.

The combination of appropriate biological assays and high-sensitivity detection techniques with such systems will allow identification and isolation of individual cells or molecules⁶⁴. Moreover, the analysis of interactions among molecules that have already been identified as diagnostic or therapeutic targets is facilitated. By attaching target molecules to microspheres (beads), the selection of binding molecules from libraries to peptides/proteins, for example, could be performed⁶⁵.

Dielectrophoresis

The repulsion or attraction of particles in a non-uniform electrical field based on polarization effects.

Electro-osmotic flow

A method to induce flow in a microchannel. An ionic double layer is present at the interface of the (immobile) microchannel (with charged surface) and the mobile fluid (counter-ions are accumulated near the channel surface). Application of an electrical field along the microchannel causes the dissolved ions, together with the bulk fluid, to move to the respective electrode.

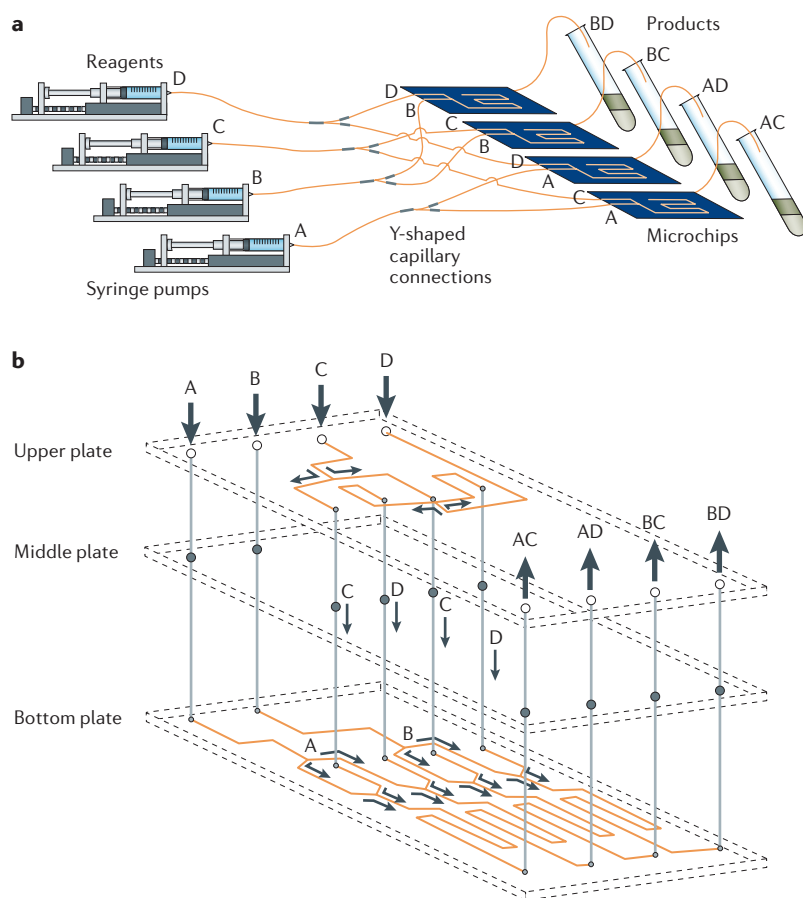


Figure 2 | Combinatorial syntheses in microfluidic chips. **a** | Schematic views of a microreactor system for 2×2 combinatorial syntheses composed of four microchips. **b** | Schematic views of a microreactor system for 2×2 combinatorial syntheses realized in one single three-dimensional microchip³⁹.

Microfluidic generation of small liquid volumes

Screening for products of any intermolecular reaction between probable leads and targets can be facilitated in reaction mixtures that are enclosed in small containers such as liposomes⁶⁶ or emulsion droplets. In microfluidic chips, small (nano- and picolitre) liquid volumes can be generated by the formation of aqueous droplets in a carrier medium, such as a hydrophobic compound or a gas. The ability to precisely control the supply of reagents, to handle small liquid volumes without fast evaporation, and the high-speed formation of droplets with homogeneous diameters of a few μm makes this a valuable tool for screening experiments that rely on high reproducibility such as protein crystallization⁶⁷ and molecular evolution⁶⁸.

By injecting the aqueous phase into the stream of the carrier medium at a T-junction⁶⁹ or by applying focusing techniques⁷⁰ (FIG. 3a,b), small entities with a volume of femtolitre to nanolitre are generated with an exceptional reproducible size that is far beyond the capability of former techniques for emulsification. The droplets form small reaction containers that can be filled with (various) compounds at the very moment of formation. Unlike miniaturized continuous-flow reactors, the

generated droplets form a boundary in three dimensions. The tiny, finite volume enables rapid mixing of reactants and transport along channel networks⁷¹.

Since its first demonstration in microfluidic chips in 2001⁶⁹, the generation of segmented flow has attracted broad interest. Segmented flow has been used among other techniques to carry out chemical and enzymatic reactions, including PCR⁷² and kinetic studies^{73,74}, and in the formation of small particles^{75,76}. It was also used for cell analysis by encapsulating a single cell within one microdroplet in which the cell was later lysed and analysed⁷⁷.

Plug-based systems can probably be adapted for structure-based drug discovery, in which protein crystallization has remained one of the rate-limiting steps in determining three-dimensional macromolecular structures^{78,79}. The small volumes result in a significant reduction in the amount of starting material required, and the environmental conditions can be adjusted very accurately. Ismagilov and co-workers⁸⁰ used aqueous droplets with a volume of ~ 10 nL generated on a microfluidic chip to determine optimum crystallization conditions. Evaluation of the quality of the crystals was subsequently performed by X-ray diffraction⁸¹. In following work, an array of nanolitre plugs of many different reagents was produced that, in a second step, merged with an aqueous stream containing another compound. The usefulness of this approach was demonstrated by functional assays in which a set of enzymes was screened for phosphatase activity. Furthermore, screening of a protein (thaumatin) against multiple crystallizing agents was shown⁸². Preformed plugs were filled with 48 precipitants and merged with a stream of thaumatin, thereby giving rise to 48 crystallization trials.

Aqueous droplets are also a useful tool for molecular evolution⁶⁸, a method that is particularly interesting for the optimization of drug candidates. Aqueous droplets form cell-sized compartments that keep together the genes, the RNAs and proteins that they encode, and the products of their activities. *In vitro* expression of green fluorescent protein (GFP) to directly analyse the contents of individual droplets has been achieved on a microfluidic chip⁸³ (FIG. 3).

Having generated the droplets, different methods of droplet handling and manipulation such as fission, fusion and sorting^{84,85} could be performed. For example, in a sorting module as sketched in FIG. 3a specific droplets can be isolated from the main droplet array.

For all the above applications, a simple planar microfluidic chip is sufficient; the addition of tubings, fittings, syringes and pumps makes the device complete. The simplicity of this set-up is perhaps the reason why there has been extremely fast adaptation of the technology for further applications. Future developments, such as enhancement of droplet formation and integration of different modules, can undoubtedly be expected in the short term.

Continuous droplet formation is one way to create small volumes; the use of micromechanical valves is another. Valves can be independently manipulated and in this respect are advantageous to the previously described droplet-based systems. Appropriate fabrication

Focusing techniques

Narrowing of a fluid stream by applying a sheath flow, for example, in a crossed channel geometry.

Segmented flow

Flow of alternating plugs (droplets) of two immiscible liquids or a liquid and a gas.

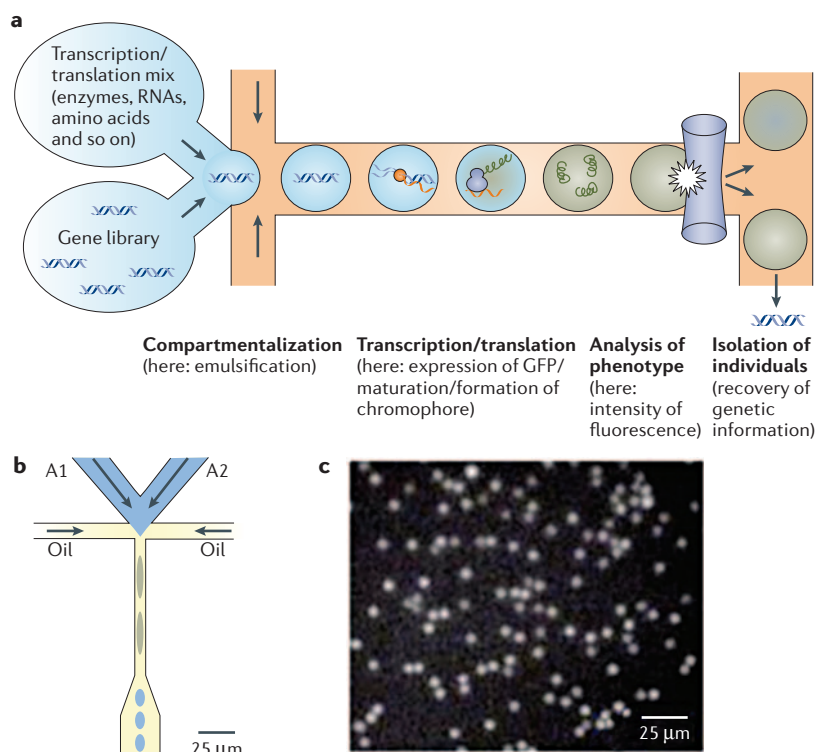


Figure 3 | Screening in microdroplets. **a** | The scheme of the concept of *in vitro* evolution of proteins in a microfluidic channel. **b** | A scheme of the microchip (A1 and A2: aqueous solution containing the translation/transcription mix and a green fluorescent protein (GFP)-encoding plasmid, respectively). During continuous formation of a water-in-oil emulsion, the molecules required for cell-free expression of proteins are mixed with templates from a gene library. *In vitro* expression takes place within the droplets. Analysis could be directly performed and distinct droplets could be isolated by appropriate sorting units within the microfluidic channel. **c** | In the experiments, GFP was used as a model system. After the autocatalytic formation of the chromophoric group, GFP inside the droplets could be determined by fluorescence spectroscopy. Part **c** reproduced, with permission, from REF. 83 © Wiley (2005).

processes, however, are necessary to integrate mechanical valves into the microchannel. The construction of a simple valve is realized by multilayer soft lithography⁸⁶. The chip consists of two layers comprising micro-sized channels. The bottom layer contains the microfluidic channel network, and the other one, situated on top, represents the control layer. By applying pressurized gas in the channels of the control layers, the bottom layer is closed at the intersection of top and bottom channels. Highly complex valve patterns could be constructed to be used, for example, for fluid metering in protein crystallization studies⁸⁷ and high-throughput screening of fluorescence-based single-cell assays⁸⁸. Microchips comprising such mechanical valves have been developed for the commercial market and are currently sold by Fluidigm Technology.

Microfluidic cell treatment

The testing of compounds on living cells is an important part of the drug discovery process, but optimal drug testing requires conditions that are as close to the physiological context as possible. In microfluidics, the

microscale dimensions generated are approaching reaction volumes that are typically found in biological systems. Microfluidic devices therefore make it possible to manipulate single objects of cellular size, and so analysis under controlled yet physiologically relevant environments can be achieved. Moreover, by parallelization of applied methods large numbers of cells can be observed simultaneously—that is, under comparable conditions.

Using microfluidics, cells can be stimulated with spatiotemporal resolution to study, for example, the effect of drug levels on chemotaxis of living cells *in vitro*. It is even possible to deliver reagents to defined domains of the cell surface or cell interior rather than to expose them to the whole cell. Such studies can reveal cell dynamics and phenomena based on (sub)cellular heterogeneity. One simple approach to partial treatment of cells was demonstrated by Takayama *et al.*⁸⁹, in which mammalian cells were allowed to settle onto the floor of a microfluidic channel. Localized perturbation of the cells was achieved by creating parallel streams of different solutions flowing across the cells. Due to a laminar flow, the solutions only mix slowly by diffusion. By this procedure, labelling of subpopulations of mitochondria, as well as disruption of actin filaments in selected regions of the cells, was demonstrated. In such devices, the number of parallel streams that can be generated is, in principle, unlimited. Due to diffusion, however, the boundaries of narrow parallel layers are blurred shortly after generation. On the other hand, diffusion at the boundary allows the formation of gradients. In appropriately designed channel networks gradients with linear, parabolic or periodic shape were generated⁹⁰. The concentration differences could expand over several orders of magnitude and over several different compounds. Applied to cell culture experiments, it allows for rapid acquisition of data for cellular responses depending on the concentration of the compounds the cells are exposed to⁹¹.

The exploitation of multiple laminar streams is not limited to gradients of chemical compounds. Recently, the generation of a temperature step of up to 10 °C was demonstrated by converging two aqueous streams, one of which was heated separately while the other was cooled⁹². In this work, *Drosophila* embryos were positioned at the interface of the two temperature-regulated streams so that the anterior and posterior halves were exposed to different temperatures and therefore developed at different rates.

The formation of networks by laminar streams is an attractive approach for local stimulation of cells, because microfluidic devices comprising a simple two-dimensional network of channels can be fabricated at reasonable cost. The perturbation of the cell happens, however, along the length of the cells, which is to say that the resolution is not three-dimensional.

Another interesting approach for the stimulation of single cells was illustrated by Peterman *et al.*⁹³, using a microchip with small apertures connected to separate microfluidic channels. Driven by electro-osmotic flow, minute quantities of a chemical compound (bradykinin solution) were repeatedly delivered to PC12 cells (a neuronal cell line) adhering to a chip surface, therefore mimicking a biological synapse. The apertures with a

Laminar flow

In the laminar flow regime, no turbulence is observed. As a consequence, two merging fluid streams are flowing in parallel, so that mixing occurs only by diffusion.

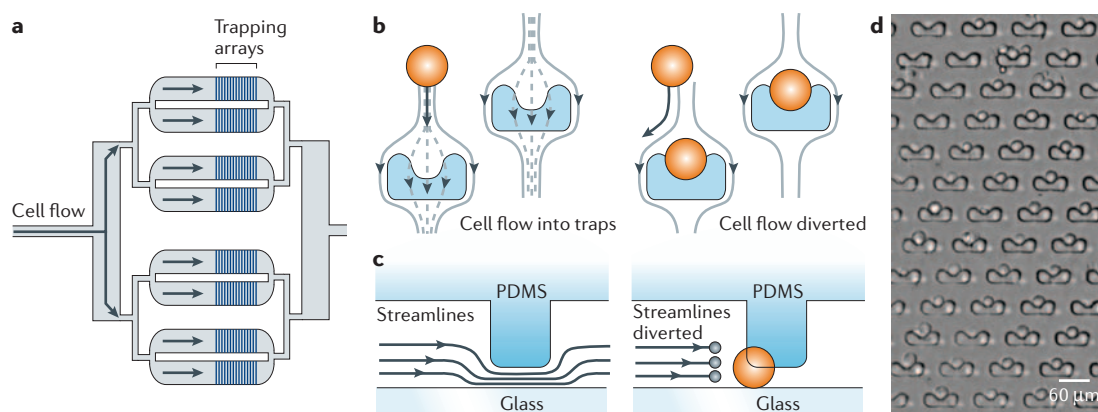


Figure 4 | Trapping cells for large-scale single-cell analysis. **a** | Sketch of the chip. A cell suspension is introduced to the chip and guided to various trapping arrays. Due to the appropriate size of the traps, only one cell can occupy the space, while succeeding cell pass by. The feature, fabricated in PDMS, is designed in such a way that only the cell would be trapped while the buffers continuously flow. Parts **b** and **c** show schemes of the trapping regions, top and side view, respectively. **d** | Trapping of HeLa cells. (Courtesy of L.P. Lee, University of California, Berkeley).

diameter of 5 μm allowed the cells to be investigated on a single-cell level. Decreasing the size of the aperture could further improve the spatial resolution.

Positioning of cells is usually random, and they adhere at any place in the microfluidic channel simply by settling down. Controlled positioning of cells can be facilitated by a pattern on the surface of the channel, resulting in spots of preferential adhesion on the channel's floor. In a novel approach towards array cytometry, cells were held by obstacles incorporated into the channel⁹⁴ while the fluid passed above and at both sides of the cells in the microfluidic channel (FIG. 4). Using obstacles of appropriate shapes and sizes, just one cell per obstacle was trapped, which was applied for studies of enzyme kinetics on a single-cell level.

A growing number of studies are using microfluidic platforms to observe long-term effects and slow cell responses. For this, the microfluidic chip has to be adapted to the needs of living cells. Challenges for cell culturing include the creation and maintenance of required conditions, such as constant temperature and nutrient concentration, and the prevention of metabolite accumulation. Very recently, several approaches have been presented illustrating cell culture experiments of different cell types — bacterial and yeast cells^{95,96}, endothelial⁹⁷, nerve⁹⁸ and muscle cells⁹⁹ — enabling long-term observation of cell growth, differentiation and response. Moreover, there have been promising advances in the design of programmable pumps and valves that could be used to precisely control liquid supply in cell culture experiments¹⁰⁰.

The possible increase of complexity of such devices, which have the potential to replace animal testing, was demonstrated by Shuler and co-workers¹⁰¹. A system designed to mimic cell culture was fabricated on a microchip to allow studies of the effects of chemical exposure on cell metabolism. Mammalian cells were cultured in different compartments of a microchip to represent organs ('lung', 'liver' and 'other'), which were in turn interconnected by microfluidic channels acting as

blood surrogate. A sensor for detecting dissolved oxygen was furthermore integrated into the chip.

The above mentioned cell culture applications of microchips are certainly still at the development stage. But knowledge gained from these examples will help to construct reliable and maybe fully portable cell culture platforms that include automatic regulation of environmental conditions.

Conclusions

The adaptation of microfabrication technology to analytical sciences has provided much scope for new ideas and experimental applications during the past decade. The large body of literature detailing applications of microfluidics beyond proof-of-principle studies demonstrates that the technology is maturing from its infancy. Some challenges still remain, however, before microfluidics platforms can be used to adapt or replace existing assays. Standardization of materials, interfaces and channel geometries will improve the comparability and assessment of experimental procedures and results. Moreover, ease of handling and robustness of systems have to be refined — 'as simple as possible' is certainly a valuable motto in the field of microfluidics.

The development and success of drug discovery is crucially dependent on available technologies. In key areas of drug discovery, such as chemical syntheses, screening of compounds and preclinical testing of drugs in living cells, microfluidic tools can make a useful contribution, and indeed represent an improvement on existing technologies. As discussed, novel reaction, manipulation and analytical steps can be performed with microfluidic systems that are not accessible to other approaches because the key tools for manipulation inevitably have to be of similar dimensions to the analysed sample. Therefore, microfluidics offers innovative technological opportunities for obtaining new information about biological systems.

The power of these methods can only be fully realized if biological assays are developed and adapted effectively to the microscale. A synergistic effort both

Interfaces

The connection between a microchip and its features to a macroscopic system, such as microscopic stages, tubing systems and wiring interconnections.

by developers of conventional biological assays and those working on miniaturization will surely aid the adoption of microfluidics technology by academic

and industrial life scientists in the near future. If this succeeds we can expect exciting technological breakthroughs in the near future.

1. Manz, A., Graber, N. & Widmer, H. M. Miniaturized total chemical analysis systems: a novel concept for chemical sensing. *Sens Actuators B* **1**, 244–248 (1990).
2. Harrison, D. J. *et al.* Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. *Science* **261**, 895–897 (1993).
3. Effenhauser, C. S., Manz, A. & Widmer, H. M. Glass chips for high-speed capillary electrophoresis separations with submicrometer plate heights. *Anal. Chem.* **65**, 2637–2642 (1993).
4. Effenhauser, C. S., Paulus, A., Manz, A. & Widmer, H. M. High-speed separation of antisense oligonucleotides on a micromachined capillary electrophoresis device. *Anal. Chem.* **66**, 2949–2953 (1994).
5. Simpson, P. C. *et al.* High-throughput genetic analysis using microfabricated 96-sample capillary array electrophoresis microplates. *Proc. Natl Acad. Sci. USA* **95**, 2256–2261 (1998).
6. Clayton, J. Go with the microflow. *Nature Meth.* **2**, 621–627 (2005).
The report gives a short supplier guide of companies offering products and services using microfluidics.
7. Madou, M. J. *Fundamentals of Microfabrication: The Science of Miniaturization* 2nd edn (CRC Press, Boca Raton, 2002).
This book gives an introduction to photolithography and describes all common fabrication processes.
8. Nguyen, N.-T. & Wu, Z. Micromixers — a review. *J. Micromech. Microeng.* **14**, R1–R16 (2005).
9. Laser, D. J. & Santiago, J. G. A review of micropumps. *J. Micromech. Microeng.* **14**, R35–R64 (2004).
10. Verpoorte, E. Chip vision — optics for microchips. *Lab. Chip* **3**, 42N–52N (2003).
11. Becker, H. & Gärtner, C. Polymer microfabrication methods for microfluidic analytical applications. *Electrophoresis* **21**, 12–26 (2000).
12. McDonald J. C. *et al.* Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* **21**, 27–40 (2000).
13. Oosterbroek, R. E. & Van den Berg, A. (eds). *Lab-on-a-chip. Miniaturized Systems for (Bio)Chemical Analysis and Synthesis* (Elsevier, Amsterdam, 2003).
14. Xia, Y. & Whitesides, G. M. Soft lithography. *Annu. Rev. Mater. Sci.* **28**, 153–184 (1998).
The review describes the fabrication of microfluidic channels and patterns on surfaces using the polymer PDMS.
15. Dittrich, P. S. & Manz, A. Single-molecule fluorescence detection in microfluidic channels — the Holy Grail in μ TAS? *Anal. Bioanal. Chem.* **382**, 1771–1782 (2005).
Comprehensive reference list of single-molecule detection in microfluidic channels.
16. Rondelez, Y. *et al.* Microfabricated arrays of femtomolar chambers allow single molecule enzymology. *Nature Biotechnol.* **23**, 361–365 (2005).
17. Lipman, E. A., Schuler, B., Bakajin, O. & Eaton, W. A. Single-molecule measurement of protein folding kinetics. *Science* **301**, 1233–1235 (2003).
Forster resonance energy transfer (FRET) is used to determine distance between two chromophores.
18. Dittrich, P. S., Schäfer, S. P., Schwill, P. Characterization of the photoconversion reaction of the fluorescent protein kaede on single molecule level. *Biophys. J.* **89**, 3446–3455 (2005).
19. Foquet, M., Korlach, J., Zipfel, W., Webb, W. W. & Craighead, H. G. DNA fragment sizing by single molecule detection in submicrometer-sized closed fluidic channels. *Anal. Chem.* **74**, 1418–1415 (2002).
20. Tegenfeldt, J. O. *et al.* Micro- and nanofluidics for DNA analysis. *Anal. Bioanal. Chem.* **378**, 1678–1692 (2004).
21. Chan, E. Y. *et al.* DNA Mapping using microfluidic stretching and single-molecule detection of fluorescent site-specific tags. *Genome* **14**, 1137–1146 (2004).
DNA bound with sequence-specific fluorescence tags is stretched in a microfluidic channel. Direct linear analysis (DLA) provides information about spatial location of sequence motifs.
22. Wang, Y. M. *et al.* Single-molecule studies of repressor-DNA interactions show long-range interactions. *Proc. Natl Acad. Sci. USA* **102**, 9796–9801 (2005).
23. Riehn, R. *et al.* Restriction mapping in nanofluidic devices. *Proc. Natl Acad. Sci. USA* **102**, 10012–10016 (2005).
24. Eijkel, J. C. T., DeMello, A. J. & Manz, A. in *Organic Mesoscopic Chemistry* (eds Masuhara, H. & Schryver, F. C.) 185–219 (IUPAC, Blackwell Sciences, Oxford 1999).
25. Reyes, D. R., Iossifidis, D., Auroux, P.-A. & Manz, A. Micro total analysis systems. 1. Introduction, theory, and technology. *Anal. Chem.* **74**, 2623–2636 (2002).
References 25–27 are summaries of recent developments in microfluidics, emphasizing applications, with a two-yearly update.
26. Auroux, P.-A., Iossifidis, D., Reyes, D. R. & Manz, A. Micro total analysis systems. 2. Analytical standard operations and applications. *Anal. Chem.* **74**, 2637–2652 (2002).
27. Vilkner, T., Janásek, D. & Manz, A. Micro total analysis systems. Recent developments. *Anal. Chem.* **76**, 3373–3386 (2004).
28. Squires, T. M. & Quake, S. R. Microfluidics: fluid physics at the nanoliter scale. *Rev. Modern Phys.* **77**, 977–1026 (2005).
Comprehensive and excellent overview of physical phenomena in microfluidics.
29. Stone, H. A., Stroock, A. D. & Adjari, A. Engineering flows in small devices: microfluidics towards a lab-on-a-chip. *Annu. Rev. Fluid Mech.* **36**, 381–411 (2004).
30. Jähnisch, K., Hessel, V., Löwe, H. & Baerns, M. Chemistry in microstructured reactors. *Angew. Chem. Int. Ed.* **43**, 406–446 (2004).
Comprehensive overview of chemical processes in microreactors, grouped into liquid-phase, gas-phase, and gas-liquid reactions.
31. Pennemann, H., Watts, P., Haswell, S. J., Hessel, V. & Löwe, H. Benchmarking of microreactor applications. *Org. Proc. Res. & Dev.* **8**, 422–439 (2004).
Comparison of organic reactions in microreactors and batch reactors.
32. Skelton, V. *et al.* The generation of concentration gradients using electroosmotic flow in micro reactors allowing stereoselective chemical synthesis. *Analyst* **126**, 11–13 (2001).
33. Watts, P., Wiles, C., Haswell, S. J., Pombo-Villar, E. & Styring, P. The synthesis of peptides using micro reactors. *Chem. Commun.* **11**, 990–991 (2001).
34. Watts, P., Wiles, C., Haswell, S. J., Pombo-Villar, E. & Styring, P. Solution phase synthesis of β -peptides using micro reactors. *Tetrahedron* **58**, 5427–5439 (2002).
35. Watts, P., Wiles, C., Haswell, S. J., Pombo-Villar, E. & Styring, P. Investigation of racemisation in peptide synthesis within a micro reactor. *Lab. Chip* **2**, 141–144 (2002).
36. Mitchell, M. C., Spikmans, V., Manz, A. & deMello, A. J. Microchip-based synthesis and total analysis system (μ SYNTAS): chemical microprocessing for generation and analysis of compound libraries. *J. Chem. Soc. Perkin Trans.* **15**, 514–518 (2001).
37. Garcia-Egido, E., Spikmans, V., Wong, S. Y. F. & Warrington, B. H. Synthesis and analysis of combinatorial libraries performed in an automated micro reactor system. *Lab. Chip* **3**, 73–76 (2003).
38. Ismagilov, R. F., Ng, J. M. K., Kenis, P. J. A. & Whitesides, G. M. Microfluidic arrays of fluid-fluid diffusional contacts as detection elements and combinatorial tools. *Anal. Chem.* **73**, 5207–5213 (2001).
39. Kikutani, Y. *et al.* Glass microchip with three-dimensional microchannel network for 2 x 2 parallel synthesis. *Lab. Chip* **2**, 188–192 (2002).
40. Neils, C., Tyree, Z., Finlayson B. & Folch, A. Combinatorial mixing in microfluidic streams. *Lab. Chip* **4**, 343–350 (2004).
41. Vilkner, T., Shihji, A. & Manz, A. Dry powder injection on chip. *Lab. Chip* **5**, 140–145 (2005).
42. Dove, A. Screening for content — the evolution of high throughput. *Nature Biotechnol.* **21**, 859–864 (2003).
43. Bleicher, K. H., Böhm, H. J., Müller, K. & Alanine, A. I. Hit and lead generation: Beyond high-throughput screening. *Nature Rev. Drug Discov.* **2**, 369–378 (2003).
44. Wölcke, J. & Ullman, D. Miniaturized HTS technologies — uHTS. *DDT* **6**, 637–645 (2001).
45. Dunn, D. A. & Feygin, I. Challenges and solutions to ultra-high-throughput screening assay miniaturization: submicroliter fluid handling. *DDT* **5**, S84–S91 (2000).
46. Sundberg, S. A. High-throughput and ultra-high-throughput screening: solution- and cell-based approaches. *Curr. Opin. Biotechnol.* **11**, 47–53 (2000).
47. Raymond, D. E., Manz, A. & Widmer, H. M. Continuous sample pretreatment using a free-flow electrophoresis device integrated onto a silicon chip. *Anal. Chem.* **66**, 2858–2865 (1994).
48. MacDonald, M. P., Spalding, G. C. & Dholakia, K. Microfluidic sorting in an optical lattice. *Nature* **426**, 421–424 (2003).
49. Petersson, F., Nilsson, A., Holm, C., Jönsson, H. & Laurell, T. Continuous separation of lipid particles from erythrocytes by means of laminar flow and acoustic standing wave forces. *Lab. Chip* **5**, 20–22 (2005).
50. Huang, L. R., Cox, E. C., Austin, R. H. & Sturm, J. C. Continuous particle separation through deterministic lateral displacement. *Science* **304**, 987–990 (2004).
51. Takagi, J., Yamada, M., Yasuda, M. & Seki, M. Continuous particle separation in a microchannel having asymmetrically arranged multiple branches. *Lab. Chip* **5**, 778–784 (2005).
52. Visser, A. J. W. G., Kunst, B. H., Keller, H. & Schots, A. Towards sorting of biolibraries using single-molecule fluorescence detection techniques. *Curr. Pharm. Biotechnol.* **5**, 173–179 (2004).
53. Georgiou, G. Analysis of large libraries of protein mutants using flow cytometry. *Adv. Protein Chem.* **55**, 293–315 (2001).
54. Fiedler, S., Shirley, S. G., Schnelle, T. & Fuhr, G. Dielectrophoretic Sorting of Particles and Cells in a Microsystem. *Anal. Chem.* **70**, 1909–1915 (1998).
55. Hu, X. *et al.* Marker-specific sorting of rare cells using dielectrophoresis. *Proc. Natl Acad. Sci. USA* **102**, 15757–15761 (2005).
56. Chou, H. P., Spence, C., Scherer, A. & Quake, S. A microfabricated device for sizing and sorting DNA molecules. *Proc. Natl Acad. Sci. USA* **96**, 11–13 (1999).
57. Fu, A. Y., Spence, C., Scherer, A., Arnold, F. H. & Quake, S. R. A microfabricated fluorescence-activated cell sorter. *Nature Biotechnol.* **17**, 1109–1111 (1999).
58. Fu, A. Y., Chou, H. P., Spence, C., Arnold, F. H. & Quake, S. R. An integrated microfabricated cell sorter. *Anal. Chem.* **74**, 2451–2457 (2002).
59. Wang, M. M. *et al.* Microfluidic sorting of mammalian cells by optical force switching. *Nature Biotechnol.* **23**, 83–87 (2005).
60. Dittrich, P. S. & Schwill, P. An integrated microfluidic system for reaction, high sensitivity detection and sorting of fluorescent cells and particles. *Anal. Chem.* **75**, 5767–5774 (2003).
61. Wolff, A. *et al.* Integrating advanced functionality in a microfabricated high-throughput fluorescent-activated cell sorter. *Lab. Chip* **3**, 22–27 (2003).
62. Johann, R. & Renaud, P. A simple mechanism for reliable particle sorting in a microdevice with combined electroosmotic and pressure-driven flow. *Electrophoresis* **25**, 3720–3729 (2004).
63. Kunst, B. H., Schots, A., Visser, A. J. W. G. Design of a confocal microfluidic particle sorter using fluorescent photon burst detection. *Rev. Sci. Instr.* **75**, 2892–2898 (2004).
64. Yeung, E. S. High-throughput single molecule screening of DNA and proteins. *The Chemical Record* **1**, 123–139 (2001).
65. Nolan, J. P. & Sklar, L. A. The emergence of flow cytometry for sensitive, real-time measurements of molecular interactions. *Nature Biotechnol.* **16**, 633–638 (1998).
66. Chiu, D. T. *et al.* Chemical transformations in individual ultrasmall biomimetic containers. *Science* **283**, 1892–1895 (1999).

67. Kuhn, P., Wilson, K., Patch, M. G. & Stevens, R. C. The genesis of high-throughput structure based drug discovery using protein crystallography. *Curr. Opin. Chem. Biol.* **6**, 704–710 (2002).
68. Tawfik, D. S. & Griffith, A. D. Man-made cell-like compartments for molecular evolution. *Nature Biotechnol.* **16**, 652–656 (1998).
69. Thorsen, T., Roberts, R. W., Arnold, F. H. & Quake, S. R. Dynamic pattern formation in a vesicle-generating microfluidic device. *Phys. Rev. Lett.* **86**, 4163–4166 (2001).
- First demonstration of formation of aqueous microdroplets in hydrophobic layer in microchips.**
70. Anna, S. L., Bontoux, N. & Stone, H. A. Formation of dispersions using 'flow focusing' in microchannels. *Appl. Phys. Lett.* **82**, 364–366 (2003).
71. Song, H., Tice, J. D. & Ismagilov, R. F. A microfluidic system for controlling reaction networks in time. *Angew. Chem.* **115**, 791–796 (2003).
- Mixing of two reactants before droplet formation.**
72. Curcio, M. & Roeraade J. Continuous segmented-flow polymerase chain reaction for high-throughput miniaturized DNA amplification. *Anal. Chem.* **75**, 1–7 (2003).
73. Song, H. & Ismagilov, R. F. Millisecond kinetics on a microfluidic chip using nanoliters of reagents. *J. Am. Chem. Soc.* **125**, 14613–14619 (2003).
74. Roach, L. S., Song H. & Ismagilov R. F. Controlling nonspecific protein adsorption in a plug-based microfluidic system by controlling interfacial chemistry using fluorosurfactants. *Anal. Chem.* **77**, 785–796 (2005).
75. Günther, A., Khan, S. A., Thalmann, M., Trachsel, F. & Jensen, K. F. Transport and reaction in microscale segmented gas-liquid flow. *Lab. Chip* **4**, 278–286 (2004).
76. Shestopalov, I., Tice, J. D. & Ismagilov, R. F. Multi-step synthesis of nanoparticles performed on millisecond time scale in a microfluidic droplet-based system. *Lab. Chip* **4**, 316–321 (2004).
77. He, M. *et al.* Selective encapsulation of single cells and subcellular organelles into picoliter- and femtoliter-volume droplets. *Anal. Chem.* **77**, 1539–1544 (2005).
78. Kunz, I. D. Structure-based strategies for drug design and discovery. *Science* **257**, 1078–1082 (1992).
79. Kuhn, P., Wilson, K., Patch, M. G. & Stevens, R. C. The genesis of high-throughput structure-based drug discovery using protein crystallography. *Curr. Opin. Chem. Biol.* **6**, 704–710 (2002).
80. Zeng, B., Roach, L. S. & Ismagilov, R. F. Screening of protein crystallization conditions on a microfluidic chip using nanoliter-size droplets. *J. Am. Chem. Soc.* **125**, 11170–11171 (2003).
81. Zheng, B., Tice J. D., Roach, S. & Ismagilov, R. F. A droplet-based, composite pdms/glass capillary microfluidic system for evaluating protein crystallization conditions by microbatch and vapor-diffusion methods with on-chip X-ray diffraction. *Angew. Chem.* **116**, 2562–2565 (2004).
82. Zheng, B. & Ismagilov, R. F. A Microfluidic approach for screening submicroliter volumes against multiple reagents by using preformed arrays of nanoliter plugs in a three-phase liquid/liquid/gas flow. *Angew. Chem.* **117**, 2576–2579 (2005).
83. Dittich, P. S., Jahnz, M. & Schwill, P. A new embedded process for compartmentalized cell-free protein expression and on-line detection in microfluidic devices. *ChemBioChem* **6**, 811–814 (2005).
84. Link, D. R., Anna, S. L., Weitz, D. A. & Stone, H. A. Geometrically mediated breakup of drops in microfluidic devices. *Phys. Rev. Lett.* **92**, 054503 (2004).
85. Tan, Y.-C., Fisher, J. S., Lee, A. I., Cristini, V., Lee, A. P. Design of microfluidic channel geometries for the control of droplet volume, chemical concentration, and sorting. *Lab. Chip* **4**, 292–298 (2004).
86. Unger, M. A., Chou, H.-P., Thorsen, T., Scherer, A. & Quake, S. R. Monolithic microfabricated valves and pumps by multilayer soft lithography. *Science* **288**, 113–116 (2000).
87. Hansen, C. L., Skordalakes, E., Berger, J. M. & Quake, S. R. A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion. *Proc. Natl Acad. Sci. USA* **99**, 16531–16536 (2002).
88. Thorsen, T., Maerkl, S. & Quake, S. R. Microfluidic large-scale integration. *Science* **298**, 580–584 (2002).
89. Takayama, S. *et al.* Subcellular positioning of small molecules. *Nature* **411**, 1016 (2001).
90. Dertinger, S. K. W., Chiu, D. T., Jeon, N. L. & Whitesides, G. M. Generation of gradients having complex shapes using microfluidic networks. *Anal. Chem.* **73**, 1240–1246 (2001).
91. Pihl, J. *et al.* Microfluidic gradient-generating device for pharmacological profiling. *Anal. Chem.* **77**, 3897–3903 (2005).
92. Lucchetta, E. M., Lee, J. H., Fu, L. A., Patel, N. H. & Ismagilov, R. F. Dynamics of *Drosophila* embryonic patterning network perturbed in space and time using microfluidics. *Nature* **434**, 1134–1138 (2005).
- Spatial and temporal regulation of embryonic development by exerting a temperature step to *Drosophila* embryos.**
93. Peterman, M. C., Noolandi, J., Blumenkranz, M. S. & Fishman, H. A. Localized chemical release from an artificial synapse chip. *Proc. Natl Acad. Sci. USA* **101**, 9951–9954 (2004).
- Stimulation of individual PC12 cells using a Ca²⁺-sensitive fluorescent dye in a microfluidic chip with several apertures. Chemical substances can be transported to the microapertures and released by electroosmotic flow.**
94. Di Carlo, D., Aghdam, N., Hung, P. J. & Lee, L. P. in *Proceedings of the μTAS 2005 Conference* (eds Jensen, K. F., Han, J., Harrison, D. J., Voldman, J.) 379–381 (TRF, San Diego, 2005).
95. Groisman, A. *et al.* A microfluidic chemostat for experiments with bacterial and yeast cells. *Nature Meth.* **2**, 685–689 (2005).
96. Balagadde, F. K., You, L., Hansen, C. L., Arnold, F. H. & Quake, S. R. Long-term monitoring bacteria undergoing programmed population control in a microchemostat. *Science* **309**, 137–140 (2005).
97. Song, J. W. *et al.* Computer-controlled microcirculatory support system for endothelial cell culture and shearing. *Anal. Chem.* **77**, 3993–3999 (2005).
98. Tayler, A. M. *et al.* A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nature Meth.* **2**, 599–605 (2005).
99. Tourovskaia, A., Figueroa-Masot, X. & Folch, A. Differentiation-on-a-chip: a microfluidic platform for long-term cell culture studies. *Lab. Chip* **5**, 14–19 (2005).
100. Gu, W., Zhu, X., Futai, N., Cho, B. S. & Takayama, S. Computerized microfluidic cell culture using elastomeric channels and Braille displays. *Proc. Natl Acad. Sci. USA* **101**, 15961–15966 (2004).
101. Sin *et al.* The design and fabrication of three-chamber microscale cell culture analog device with integrated dissolved oxygen sensor. *Biotechnol. Prog.* **20**, 338–345 (2004).

Acknowledgements

Financial support by the European Community (CellPROM project, contract No., NMP4-CT-2004-500039), by the Ministerium für Innovation, Wissenschaft, Forschung und Technologie des Landes Nordrhein-Westfalen and by the Bundesministerium für Bildung und Forschung is gratefully acknowledged. We thank all group members and A. J. Garman (AstraZeneca) for helpful discussions and K. Tachikawa for proofreading of the manuscript.

Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

MEMS and Nanotechnology Clearinghouse:

<http://www.memsnet.org>

Access to this interactive links box is free online.