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Applications of microfluidics in chemical biology

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This review discusses the application of microfluidics in chemical biology. It aims to introduce the reader to microfluidics, describe characteristics of microfluidic systems that are useful in studying chemical biology, and summarize recent progress at the interface of these two fields. The review concludes with an assessment of future directions and opportunities of microfluidics in chemical biology.

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Introduction

'Microfluidics' refers to the science and technology of manipulating fluids in networks of channels with dimensions of ~5–500 μm; its history has been the subject of a recent review by Whitesides [1••]. Microfluidic systems transport volumes of fluid that vary from microliters (10^{-6} l) to femtoliters (10^{-15} l) in channels that are usually embossed in the surface of a polymer, but are occasionally fabricated in glass (Figure 1). Soft lithography is a set of techniques used frequently to produce microfluidic systems, and is based on embossing channels in a thin slab of a polymer; poly(dimethylsiloxane) (PDMS) is the material most commonly used in academic laboratories [2,3]. The techniques of soft lithography provide an inexpensive and convenient alternative to conventional methods of microfabrication based on laser- or e-beam writing and photolithography in glass or silicon. The use of soft lithography also makes possible the fabrication of prototype microfluidic systems in short periods of time (typically less than one day), and the generation of multiple copies of a device in several hours.

PDMS has several attractive properties for the fabrication of microfluidic channels for use with aqueous systems of biochemicals and cells: it is soft, flexible, biocompatible, electrically insulating, unreactive, transparent to visible and ultraviolet light, permeable to gases, and only moderately permeable to water. Its surface can be oxidized easily to present primarily Si-(OH) groups; this reaction makes PDMS hydrophilic, and, more importantly, allows PDMS to be sealed to other polymers, and to glass, typically without an adhesive. Many of these characteristics make PDMS particularly useful for studying biological systems, such as cells and small organisms [4]. The primary disadvantages of PDMS as a material for microfluidics are that it absorbs a range of organic solvents and compounds (particularly alkyl and aryl amines), and that its surface properties can be difficult to control [5]. Rolland *et al.* have demonstrated recently that cross-linked perfluoropolyether elastomers have many physical properties in common with PDMS but do not absorb many of the organic solvents that PDMS does; sealing layers of this polymer to itself or other materials is more difficult than sealing PDMS using the oxidative route [6].

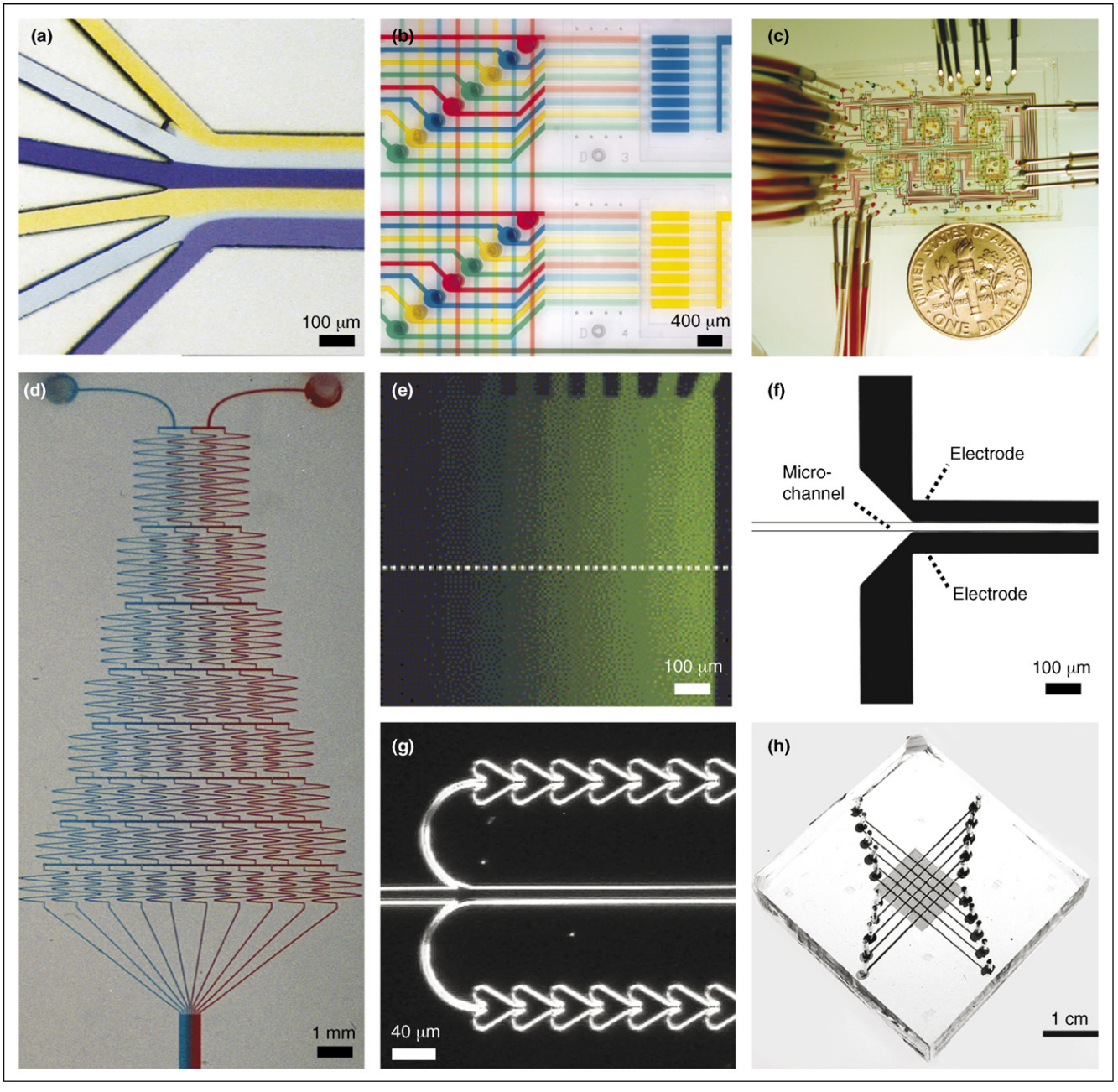
Microfluidics has several features that have attracted users in biology, chemistry, engineering and medicine. It requires only small volumes of samples and reagents, produces little waste, offers short reaction and analysis times, is relatively cheap, and has reduced dimensions compared with other analytical devices. In addition, microfluidics offers structures with length scales that are comparable to the intrinsic dimensions of prokaryotic and eukaryotic cells, collections of cells, organelles, and the length scale of diffusion of oxygen and carbon dioxide in tissues (every cell, *in vivo*, is no more than ~100 μm from a capillary). These characteristics make microfluidics particularly useful in studying biology and biomedicine [7].

The number of applications of microfluidics in biology, analytical biochemistry, and chemistry has grown as a range of new components and techniques have been developed and implemented for introducing, mixing, pumping, and storing fluids in microfluidic channels. Despite rapid progress in this area, there remain several unsolved problems: preparing and introducing samples; interfacing microfluidic channels with the human hand; working with a range of sample volumes (e.g. Vacutainers, a drop of blood, a biopsy sample, a single cell); and portability.

Characteristics of microfluidics with relevance to chemical biology

Microfluidics is an *enabling* technology. It makes possible the exploration of biological systems — from molecules, through cells, to small, multicellular organisms such as

Figure 1



Images of **(a,b)** microfluidic channels, **(f,g)** microfluidic components and **(c,d,e,h)** microfluidic devices. (a) Laminar streams of solutions of dye (in water) flowing in a microfluidic channel. The fluid is flowing from the six channels on the left into the central channel on the right where flow is laminar. Reproduced from [43] with permission. Copyright 2005, ACS. (b) An image of a section of the Topaz Dynamic Array for high-throughput protein crystallization. The integrated fluidic circuit contains microfluidic chambers, channels, and pumps. Image courtesy of Fluidigm Inc. (http://www.fluidigm.com). (c) A microfluidic chemostat in which an intricate system of plumbing is used to grow and study bacteria. Reproduced from [9•] with permission. Copyright 2005, AAAS. (d) A microdiluter system in which two fluids are repeatedly split at a series of nodes, combined with neighboring streams, and mixed. At the end of the network of channels, the streams of fluid carrying different concentrations of green and red dye are combined and produce a gradient. Reproduced from [19] with permission. Copyright 2001, AAS. (e) A linear gradient of fluorescein produced using the microdiluter shown in (d). Reproduced from [19] with permission. Copyright 2001, AAS. (f) An image of metal wires fabricated 10 μm away from a microfluidic channel (40 μm diameter). The metal structures have a variety of uses — for example, as electromagnets for producing electromagnetic fields in adjacent microchannels. Image courtesy of A Siegel (unpublished data). (g) Microfluidic channels for directing the movement of cells of swimming bacteria. Image courtesy of W DiLuzio (unpublished data). (h) A combinatorial tool based on two layers of PDMS microfluidic channels bonded together at right angles to each other, and separated by a thin membrane. Reproduced from [22] with permission. Copyright 2001, ACS.

Caenorhabditis elegans — in ways that are not possible using larger systems. Below we provided a summary of the characteristics of microfluidics that are particularly useful in probing biological systems using small molecules.

Miniaturization

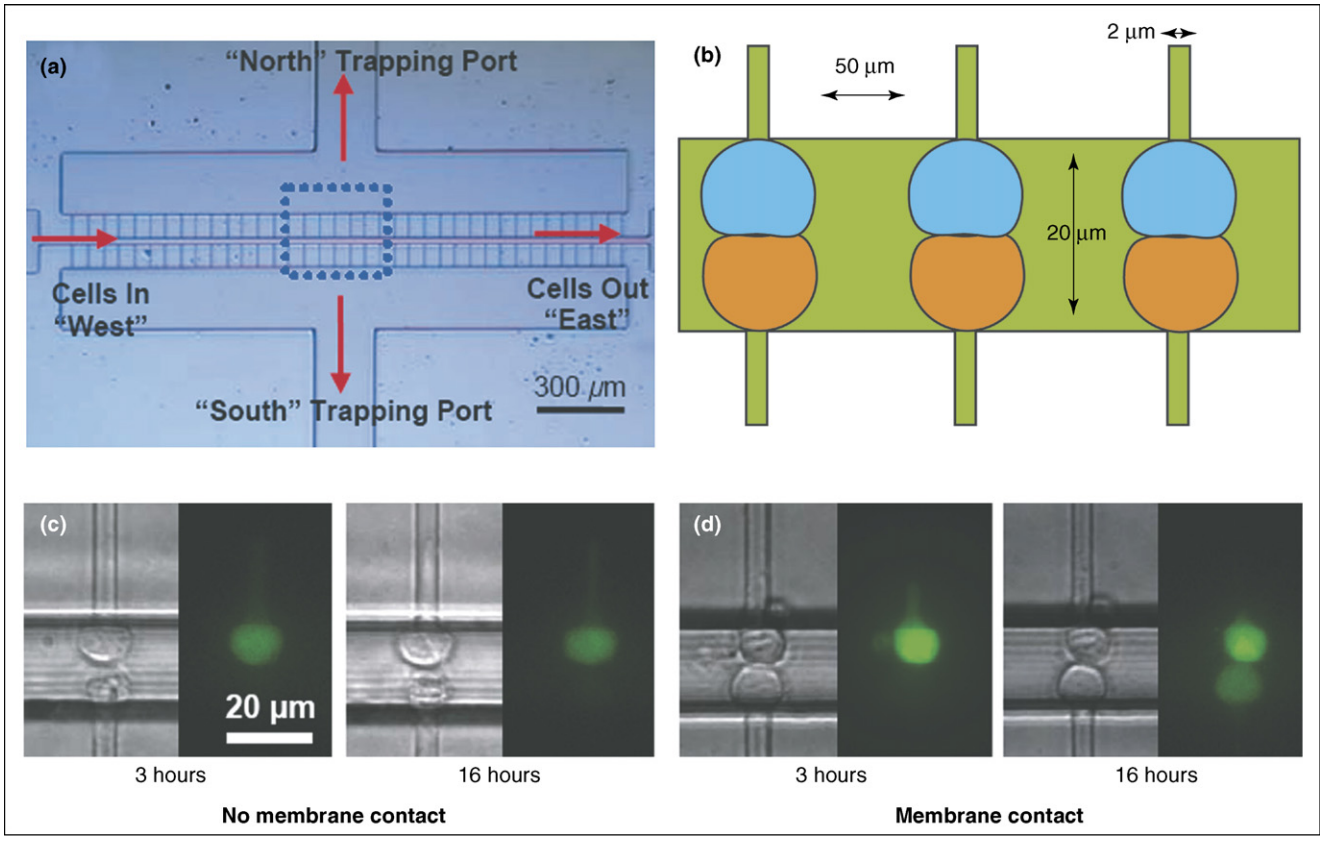
Microfluidic systems allow small numbers of cells, or even single cells, to be isolated, manipulated, and examined [8•,9•,10]. The microchannels in microfluidic systems provide a mechanism for introducing nutrients and reagents, exchanging media, and removing waste from cells or small samples of tissue. Single cells can be isolated physically in microfluidic systems while keeping them in fluidic contact; this controlled contact allows them to exchange small molecules, growth factors and other proteins, and thus allows studies of signaling and toxicology on small scales [11•,12•] (Figure 2).

Small volume, large surface area

As the physical dimensions of channels decrease, their surface-to-volume ratio increases, and the rates of heat and mass transfer increase due to steeper gradients in temperature and concentration. Large surface areas can, in some cases, be beneficial (e.g. in gas exchange), or detrimental (e.g. in the non-specific adsorption of proteins from solution onto the walls of the container). Several techniques minimize the non-specific adsorption of proteins and other biomolecules on the walls of microfluidic channels. For example, a wide range of studies have identified oligomers of ethylene oxide (poly(ethylene glycol); PEG) as coatings that reduce the adsorption of proteins and cells on surfaces [13,14]. Huang *et al.* describe another approach for preventing the non-specific adsorption of proteins to PDMS channels by coating the walls with n-dodecyl-β-D-maltoside [15].

An important characteristic of working with small fluidic systems is that the volumes of samples, reagents and cells that are needed are quantitatively small. Minimizing waste can be particularly important when working with biologically hazardous material. Lee *et al.* described a particularly relevant example: the multistep synthesis of an ^{18}F-labeled probe in a microfluidic system for PET scanning [16•].

Figure 2



A microfluidic system for engineering cell–cell contact. **(a)** An image of a microfluidic system for bringing pairs of mammalian cells in membrane–membrane contact. The dashed line highlights the area of the device illustrated in **(b)**. (b) A cartoon of the region of the device in which cells were trapped. The blue and orange spheres represent cells. **(c)** The top cell was loaded with an intracellular fluorescent dye (calcein AM); the bottom cell contained no dye. In the absence of membrane–membrane contact between the cells, dye was not transferred from the top cell to the bottom cell. **(d)** When the two cells were brought into membrane–membrane contact, the transfer of dye occurred. Reproduced from [12•] with permission.

Scaling out

Microfluidics facilitates a characteristic that we refer to as 'scaling out': that is, thousands of identical microfluidic structures can be produced in an area that is several square centimeters; these structures make it possible to carry out many experiments in parallel. A relevant example are microfluidic chips produced by Thorsen *et al.* that have an area of 5 cm^2 and contain a network of 256 microchambers (each with a volume of ~750 pl) that are addressed individually using integrated pneumatic valves [17]. We believe that scaling out is of increasing importance in biology as the field moves toward quantitative data because it allows many parallel experiments to be performed on cells or organisms under identical conditions, and thus provides data that will allow a meaningful estimate of the reproducibility and reliability of biological measurements.

Automation

The integration of techniques for introducing samples, pumping, storing, mixing and metering out fluids in microfluidic systems is beginning to lead to the miniaturization of laboratory instruments. Automation is used in many integrated microfluidic devices, particularly in systems in which pneumatic valves pump fluids, colloids and cells into addressable chambers where reactions or analyses are carried out [17]. Automated techniques for distributing reagents in parallel microfluidic channels compete with expensive liquid handling robots that dispense fluids in 96-, 384- or 1536-well plates.

Laminar flow

The Reynolds number (Re) is a dimensionless quantity that describes the behavior of fluids, and is defined by $Re = \rho v d/\mu$, where ρ is the density of fluid (g/cm^3), v is the velocity of the fluid (cm/s), d is the hydraulic diameter (cm) of the channel, and μ is the viscosity of the fluid (g/cm·s). Below a Reynolds number of ~2000, flow is laminar; above this value the flow is turbulent. In channels with a surface-to-volume ratio characteristic of microfluidic systems, the flow of fluid is dominated by viscous dynamics and is laminar. Adjacent streams of miscible fluids flowing through microchannels flow side-by-side, with mixing only by diffusion at the interface of the streams. Several groups have exploited this characteristic to produce stable gradients of small molecules, proteins and growth factors [18•,19,20,21••]. These gradients are typically perpendicular to the flow of fluid, can be produced on surfaces or in solution, and have temporal and spatial stability.

Applications of microfluidics in chemical biology

Microfluidic systems are beginning to appear in a sufficiently wide range of applications that it is not practical to survey them completely in a limited space; a recent set of articles in a *Nature* Insight section on microfluidics provides a good, recent overview over this area [1••]. Here, we have selected several recent papers that we think will be of interest to the community of chemical biologists, and organized them into categories; we summarize them below.

Arrays

Microfluidic systems consisting of crossed sets of microchannels provide a way of studying the interaction of large numbers of molecules with proteins or cells in a combinatorial layout. Ismagilov *et al.* described a combinatorial tool based on two layers of PDMS microfluidic channels bonded together at right angles to each other, and separated by a thin membrane (Figure 1h) [22]. The membrane permitted chemical contact between the two layers of channels and kept small particles from crossing the two streams. This system was used in assays for pathogenic microorganisms, but, more generally, it offers the opportunity to conduct a wide range of different types of assays (for example, antibody-based assays for anti-gp120 for AIDS) requiring the formation of precipitates. In principle, this kind of device should make it possible to examine several samples of serum for the presence of proteins.

Gradients

The manipulation of laminar streams of fluids (Figure 1a) in microfluidic channels makes it possible to create gradients of almost arbitrary complexity of small molecules, growth factors and other proteins in solution and on surfaces. Methods that use a common attachment scheme based on biotin and avidin are particularly well developed [18•]. Microfluidics enables the formation of gradients that cannot be generated using other techniques, including very steep gradients of concentration that extend over several orders of magnitude, gradients with complicated profiles, and gradients of gradients [18•,19,20,21••,23,24] (Figure 1e).

Several groups have studied the behavior and differentiation of cells in response to chemical signals in channels. One of the earliest examples of interactions between cells and chemical gradients was by Jeon *et al.*, who studied the chemotaxis of human neutrophils on a gradient of interleukin-8 in a microfluidic channel [25]. Other groups have studied the migration and behavior of neutrophils on gradients of proteins [20,26]. Pihl and co-workers used a microfluidic system to profile molecules for pharmacological activity [24]. The authors created an integrated device in which gradients of drugs were produced and their activity against voltage-gated K^+ ion channels was measured (at different concentrations) by patch clamping individual CHO cells exposed to different regions of the gradient. Chung *et al.* created gradients of growth factors to study the growth and differentiation of human neural stem cells [27]. Gunawan and co-workers studied the migration and polarity of rat intestinal cells on gradients of extracellular matrix proteins in microfluidic channels [28].

Laminar flow can also be used to create gradients in temperature. Luccetta *et al.* created a thermal gradient in a microfluidic channel whose dimensions were designed to study the effect of temperature on the development of an embryo of *Drosophila melanogaster* by exposing different parts of the same embryo to fluids at two different temperatures [29•].

Microdiluters

Microfluidic diluters ('microdilutors') are systems in which solutions or liquid reagents are carried through a series of controlled dilutions, and then used in assays (Figure 1d) [19,30]. These dilutors perform some of the functions of 96-well plate assays, but use smaller quantities of reagents, and are less labor-intensive. Microdiluters that perform multiple cycles of dilution are the microfluidic version of a multiwell plate. Microdiluters are just beginning to be used in biochemistry: for example, Jiang has used a microdiluter to carry out a miniaturized, parallel, serially diluted immunoassay for analyzing multiple antigens [31].

Gel structures

One useful property of soft lithography is that it can be used to form microstructures in gels, or can be used in conjunction with gels. Microfluidic systems fabricated in agar, agarose and calcium alginate form biocompatible structures that can be infused with small molecules, nutrients or proteins. Cabodi *et al.* fabricated microfluidic systems in alginate, and studied mass transfer in the channels [32•]. Khademhosseini *et al.* captured fibroblasts in 'corrals' molded in poly(ethylene glycol) within microfluidic channels for high-throughput screening [33]. Gel structures can serve as containers in which cells can be grown in the presence of small molecules that diffuse through the gel structure. Takeuchi and co-workers used slabs of agarose with embossed microfluidic chambers for growing cells of *Escherichia coli* in the presence of small molecules that alter the phenotype of cells [11•]. Campbell *et al.* have studied reaction-diffusion systems involving cleverly designed gels fabricated using soft lithography; although these methods have not been used in biochemical systems, their potential in these systems is obvious [34].

Droplets

Several different microfluidic systems have been described for producing droplets of liquid (typically 10–500 microns in radius) dispersed in a second liquid. Droplets produced in microchannels can, in appropriately designed droplet-generators, be almost monodisperse, and have a volume that may be as small as several picoliters; such droplets have been used in a range of applications. For example, they serve as liquid bioreactors for encapsulated cells [35•]. Plugs of fluids with nanoliter volumes form the basis of systems for screening conditions for biochemical reactions and the crystallization of proteins. Chen and Ismagilov have reviewed this area recently [36].

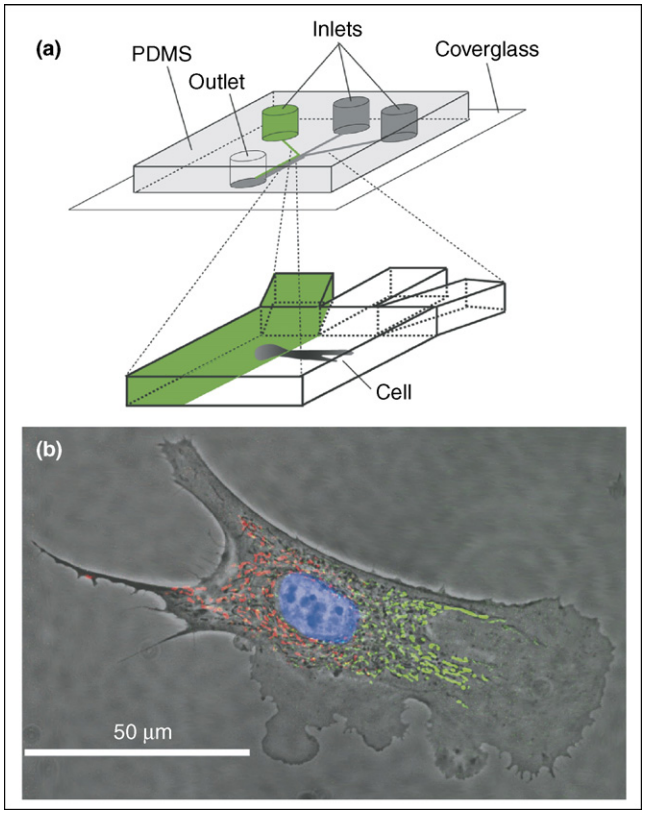
Painting cells

Microchannels provide a convenient mechanism for treating cells — or portions of cells — with soluble reagents carried in laminar flows with interfaces smaller than the dimensions of a cell. Takayama *et al.* exposed selected regions of mammalian cells to fluids containing fluorescent dyes (that is, 'painted' them) by positioning cells at the interface of two different streams of fluids flowing at low Reynolds number [37] (Figure 3). The authors used this technique to: i) study the movement of populations of mitochondria in cells; ii) disrupt actin filaments in cells; iii) detach selected regions of cells from surfaces; and iv) create gradients of small molecules in single cells [38].

Studies of single cells

As we mentioned briefly above, single cells can be isolated and manipulated in microfluidic systems. This characteristic has been used to study the biochemistry

Figure 3



A microfluidic system for positioning small molecules with subcellular resolution. **(a)** A cartoon depicting the treatment of selected regions of mammalian cells with reagents using laminar streams of fluid. **(b)** An image of a bovine capillary endothelial cell treated with Mitotracker Red CM-H_2XRos (left side of cell) and Mitotracker Green FM stain (right side of cell); the nucleus was stained with the DNA-binding dye, Hoechst 33342. Reproduced with permission from [37].

and biophysics of single cells. Wu *et al.* constructed microfluidic compartments with picoliter volumes for carrying out chemical cytometry on single Jurkat T cells [39]. Cai and co-workers measured the expression of β-galactosidase in single cells of *E. coli* in a microfluidic system with single molecule sensitivity [40••].

Devices

Much of the work in polymeric microfluidic systems has focused on the design and fabrication of components, and their combination into devices. There is now a substantial level of competence in the choice of materials and in the design of pumps, valves, filters, and the myriad of other components that must be available to fabricate complex systems. New work has used co-fabrication (the fabrication of *all* of the microsystems needed for a device at one time) to make an on-chip light source, tunable dye laser, magnetic separators, heaters, and other sophisticated components. Related methods have been used to fabricate varieties of cell- and sperm-sorters, and micro-variants of continuous stirred-tank reactors for microbiology [8•,9•,41]. The rapid advance in the physical science and technology of microfluidics is the product of a growing community of researchers, of which some of the names not so far mentioned or cited are Klavs Jensen, Seth Freden, Chris Chen, David Weitz, Piotr Garstecki, Howard Stone, John Wikswo, Paul Yager and others. It is now time to begin to apply these systems and devices to real problems in biochemistry; this work is just beginning.

Conclusion and future directions

Microfluidics brings the advantages of small volumes of fluids, reagents, and waste, and small numbers of cells, to chemical studies on biological systems. The techniques of soft lithography are within the reach of biologists and biochemists, and make it possible to prototype microfluidic structures rapidly. Public foundries — facilities designed to fabricate microfluidic devices, typically using soft lithography — have been established at several universities (Harvard, California Institute of Technology, and University of Washington) to eliminate most of the technically difficult steps in fabricating microfluidic systems and to extend the technology to the chemical and biological communities.

Microfluidics is an enabling technology that will make possible new applications in biological sciences; we believe it will play an important role in the development of cell-based assays, single-cell assays, phenotypic screens, gene-expression profiling, patient-specific assays, high-throughput and combinatorial methods, and techniques and devices appropriate in cost and performance for bioanalytical detection in developing economies, in home health care, and by first responders.

The future of microfluidics offers a range of interesting and new opportunities. The most important is the application of existing technology to problems in biochemistry and biomedicine. Beyond that, however, there are several new opportunities in learning: i) to interface microfluidic systems with microelectronic devices; ii) to carry out new kinds of experiments involving very large numbers of experiments run in parallel to measure the reproducibility of biochemical (or more importantly, cell biological) experiments [42•]; iii) to carry out specialized types of biochemical synthesis (e.g. synthesis of ^{18}F-labeled compounds for PET [16•]; and iv) to incorporate optical and electromagnetic technologies in chip-based devices in an integrated way for manipulating samples and for their analysis.

Microfluidics has much to offer the field of chemical biology. Chemical biology, in return, has an opportunity to stimulate research in microfluidics by asking questions that require the development of new capabilities and materials. The techniques and tools that arise from this successful merger will broaden our understanding of biological interactions and may have applications in other areas of science.

Acknowledgements

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