NATURE|Vol 437|29 September 2005|doi:10.1038/nature04163

Controlled microfluidic interfaces

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The microfabrication technologies of the semiconductor industry have made it possible to integrate increasingly complex electronic and mechanical functions, providing us with ever smaller, cheaper and smarter sensors and devices. These technologies have also spawned microfluidics systems for containing and controlling fluid at the micrometre scale, where the increasing importance of viscosity and surface tension profoundly affects fluid behaviour. It is this confluence of available microscale engineering and scale-dependence of fluid behaviour that has revolutionized our ability to precisely control fluid/fluid interfaces for use in fields ranging from materials processing and analytical chemistry to biology and medicine.

The effects of gravity and inertia dominate our experiences of the physical world. But as systems are reduced in size, phenomena such as diffusion, surface tension and viscosity become ever more important; at the micrometre scale they can dominate and result in a world that operates very differently from the macroscopic world we perceive and live in[1]. Purcell provided a fascinating peek into such a world populated by microorganisms[2], showing that *Escherichia coli*, with a size of about 2 μm, moves more slowly than diffusing nutrients and waste. This means that rather than actively search for its food, *E. coli* can forage just as efficiently by simply waiting for food to diffuse past. There are plenty of other processes where nature uses the micrometre scale to its advantage. For instance, gas exchange occurs with relatively slow rates within our lungs through diffusion, but nevertheless is efficient overall because it can take place over a large total surface area of about 80 m^2 provided by large numbers of very small air spaces (the alveoli). Similarly, muscle contraction is triggered by calcium ion diffusion, yet large muscles are often activated very rapidly. In this case, the muscle fibres are highly ordered and consist of micrometre-sized repeating structural units; this design keeps diffusion distances short so that the ions can rapidly reach their target destination.

Unlike nature, we are only just beginning to harness microscale phenomena for practical use. This contrasts with our understanding of the behaviour of particles and fluids at the microscale, which has a long history that can be traced back to capillary experiments by Hagen and Poiseuille in the middle of the nineteenth century[3,4]. Navier and Stokes provided important contributions to fluid dynamic theory in the beginning of the nineteenth century[5,6], and Taylor extended the field with his studies of diffusion under laminar flow in the 1950s[7]. But practical, creative use of this knowledge has had to await the availability of technologies for building microscale systems in a controlled and repeatable manner. That started to happen in the early 1980s, which saw the emergence of micro-electromechanical systems[8] (MEMS). MEMS aimed to integrate electronics and mechanical elements such as sensors and actuators on a common substrate, by adapting the advanced fabrication capabilities of the microelectronics industry. The same fabrication technologies were subsequently also used to create devices for containing and controlling fluid at the micrometre scale[9,10], giving rise to the field of microfluidics. Much of the original motivation for microfluidics arose out of developments in biology that call for the ability to manipulate fluids on the cellular length scale, and by the desire to provide cheap and efficient diagnostic tools that require only small sample volumes[11]. Microfluidic systems have now been improved to the state where they are commercially available for biomolecular separations (Caliper Life Sciences) and emerging as promising tools for high-throughput discovery and screening studies in chemistry and materials science[12,13]. But beyond the manipulation of liquids as such, microfluidic systems also let us exploit the scale-dependence of interface properties to develop a wide range of other applications, as we aim to illustrate here.

A striking demonstration of the potential for exquisite control of liquid interfaces at the micrometre scale appeared in 1992: a drop of water moves autonomously uphill when placed on a smooth surface that is treated so as to have a gradient in hydrophobicity[14]. Since then, many successful approaches have been developed for adjusting substrate sur-

Box 1 | The importance of scale

Reynolds number

The Reynolds number relates the ratio of inertial to viscous forces. Viscosity, the internal friction of a fluid, produces a resistance to shear and a tendency for the fluid to move in parallel layers known as laminar flow; and inertia, the tendency of a body in motion to retain its initial motion, counters laminar flow and can ultimately result in turbulent flow. Quantitatively, the Reynolds number is calculated as $Re = av/\nu$, where v is the velocity scale of the fluid, a is a characteristic distance of the system (in the case of flow through a pipe, a would be the pipe diameter), and ν is the kinematic viscosity of the fluid.

Peclet number

The Peclet number, *Pe*, provides an indication of the relative importance of diffusion and convection, diffusion being the random thermal motion of molecules within their surrounding environment and convection the transport as a result of bulk motion of a fluid[23]. The Peclet number is defined as $Pe = U_a H/D$, where U_a is the average velocity of the flow, H is a characteristic length of the system perpendicular to the direction of the flow and D is the diffusion coefficient of the particle or molecule of interest.

Capillary number

The ratio between viscous and capillary forces is given by the capillary number. Capillarity is the rise or depression of a liquid in a small passage, such as a thin tube. Water inside a glass capillary tube will have a concave meniscus that is in equilibrium because of a pressure difference across the interface. Such a pressure difference exists whenever a liquid surface is curved (as in the case of liquid drops or soap bubbles[27]), with the higher pressure found on the inner side of the curve. The capillary number is given by $Ca = U\mu/\gamma$, where U is the velocity of the flow, μ is the viscosity of the fluid, and γ is the surface tension.

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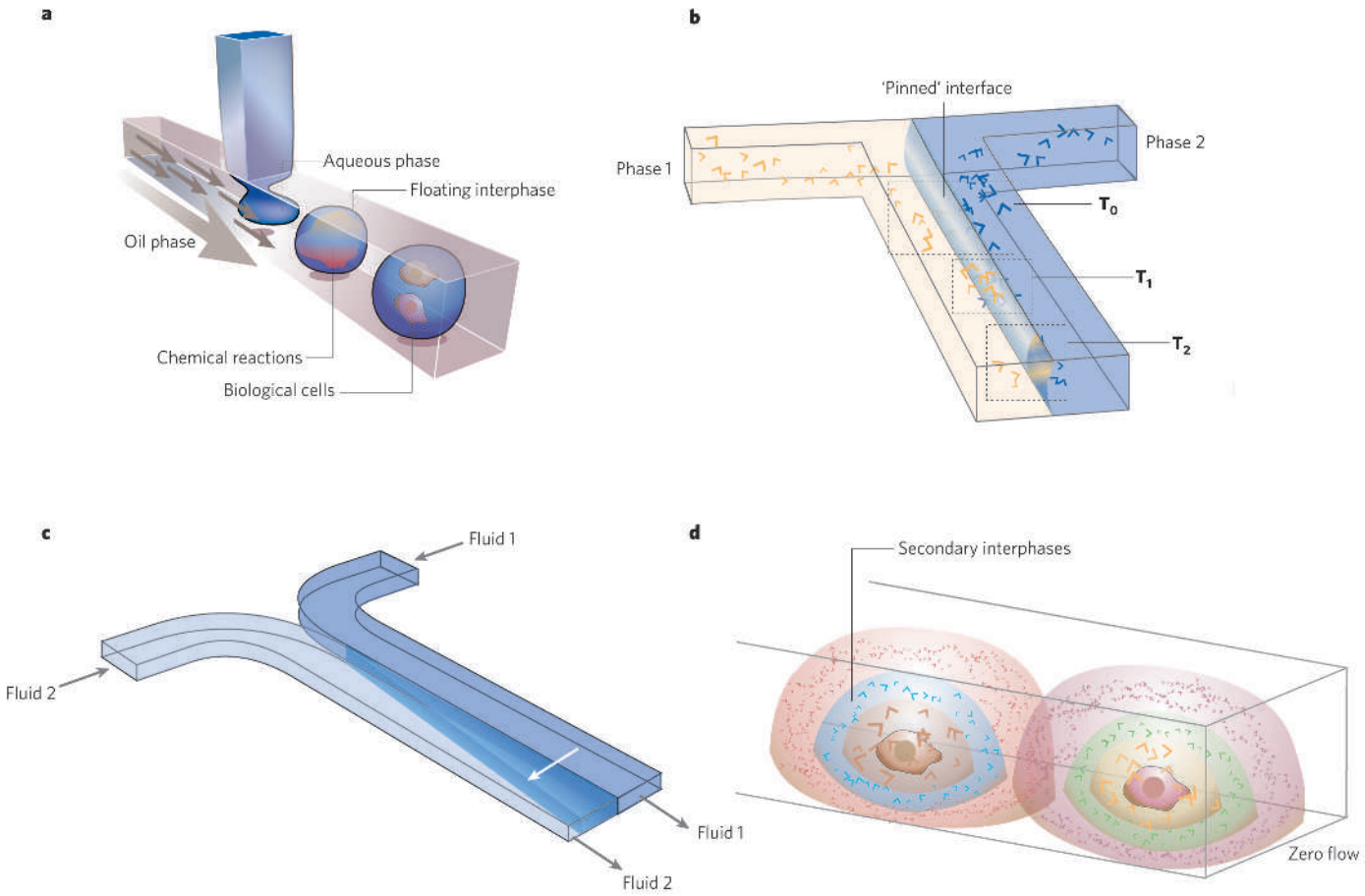

Figure 1 | Microfluidic interfaces provide unique functionality. a, Floating interfaces between immiscible fluids can be used to produce droplets of precise shape and varying content. These can act as microscale containers with permeable walls for performing and analysing reactions, creating custom magnetic or protein-coated vesicles, or transporting cargo. **b**, Pinned interfaces between immiscible liquids are created by selective surface patterning of a microchannel. They can be used to create vertical interfaces between liquid and air that, defying gravity, can hold liquids without breaking, or to stabilize the interface between immiscible fluids allowing interfacial chemistry to form real walls. **c**, Moving interfaces between miscible liquids are created under laminar flow conditions between two streams of fluid flowing together because they do not mix except by diffusion, creating a diffusive interface with predictable geometry. **d**, Secondary interfaces arise in microscale channels because transport is mainly due to diffusion (convection-free environments). Diffusion can create complex but predictable patterns (interfaces) of solutes based on the diffusivity of the solutes.

face properties as a means of manipulating liquid drops, and sophisticated methods capable of controlling surface properties both temporally and spatially are now at our disposal[15–18]. One such method — known as electrowetting — uses electrical control of contact angle to manipulate liquid droplets in real time[19]. This control capability can be used in digital microfluidics, the processing of discrete fluid packets that is of interest for the development of clinical diagnostic assays[20,21]. But it is the ability of microfluidics to harness interfaces that is continuing to open new avenues of inquiry and application, and is the focus of this review.

In our discussions, we will go beyond the classical view of an 'interface' as the thin boundary layer that separates two distinct phases of matter (each of which may be a solid, a liquid or a gas) and that has properties distinct from those of the bulk material on either side. In addition to such classical interfaces, we also consider *de facto* interfaces such as the diffusive layers that appear if miscible fluids are brought into contact or a solute source is placed in a fluid[22]. A common theme is the precise control that microfluidics offers over the interface, permitting many applications that were not previously possible.

Fluid at the microscale

An obvious effect of shrinking a system to the micrometre scale is the huge increase in surface area relative to volume, often by several orders of magnitude. For a fluid, the effect allows for more efficient mass and heat transfer in microsystems: relatively more interface is available for transfer to occur, and less total mass or energy needs to be transferred to reach the final state. Therefore both the creation and the homogenization of solute or temperature gradients are faster as system size is reduced.

Fluid behaviour in reduced dimensions will also be increasingly influenced by viscosity rather than inertia. In the case of microfluidic systems with simple geometries, this results in laminar flow. (Such behaviour occurs if the Reynolds number *Re*, which gives the ratio of viscous to inertial forces, is small; see Box 1). In laminar flow, diffusion can be effective for moving and mixing solutes on micrometre length scales. The relative importance of diffusion and convective bulk flow for transporting solute and solvent molecules is given by the Peclet number *Pe* (see Box 1), and can be readily adjusted through the choice of flow velocity and the dimensions of the system used.

The large ratio of surface area to volume typical for microfluidic systems ensures that surface tension can profoundly influence fluid behaviour. If surface tension varies along a surface or interface as a result of thermal or concentration gradients, for example, so-called Marangoni flows[23,24] can arise and effectively homogenize the thermal or concentration gradients; the convective flows may even be used to

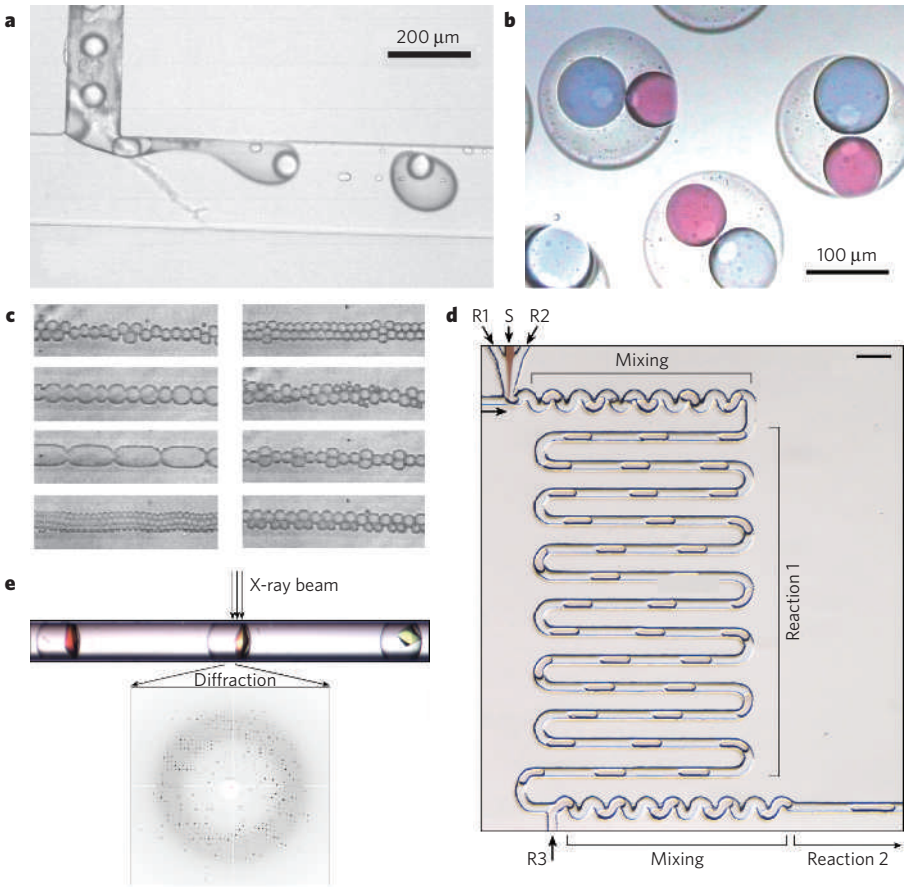

Figure 2 | Droplets as containers. Microfluidic technology allows droplets to be generated that can be used as containers in which different kind of reactions can be carried out in a controlled way. **a**, **b**, A T-junction is used for generating monodisperse double emulsions, with the single emulsion produced by another T-junction upstream with opposite wettability properties (hydrophobic/hydrophilic). Potential applications include encapsulation of therapeutic agents for targeted drug delivery and extractions across the thin layer separating the internal droplets. Reprinted with permission from ref. 34. Copyright (2003) American Chemical Society. **c**, The competition between capillary and viscous forces in a T-junction is used to generate three-dimensional patterns ('necklaces' and zigzag, for example). The goal is to study how systems operating far from equilibrium can produce regular patterns. Reprinted with permission from ref. 33. Copyright (2001) American Physical Society. **d**, A microfluidic platform for performing a two-step reaction in which droplets are used as containers. Aqueous reagents R1 and R2 are merged in a T-junction to form a droplet immersed in oil. Mixing is aided by chaotic advection generated by shear stresses on the interface of the droplets, as they flow through a microchannel with alternating curves. After mixing the droplets flow through a longer channel to allow the reaction to proceed. At the end of the channel, another T-junction permits the injection of a third reagent R3, and the process can be repeated as desired. Reproduced with permission from ref. 44. Copyright (2004) Royal Society of Chemistry. **e**, Protein crystals are formed inside droplets in a glass capillary. The diffraction pattern of the crystal can be obtained directly without removing the droplets from the capillary. Reprinted with permission from ref. 45. Copyright (2003) Wiley-VCH.

move droplets and pump fluid[25,26]. But the most significant surface tension phenomenon is probably capillarity — the rise or depression of a liquid in a small passage, driven by capillary forces which according to the Young–Laplace equation[27] become more significant relative to other forces such as gravity as a system's size is reduced[28]. Surface properties can be selected to influence the competition between viscous forces and capillary forces. This makes it possible to adjust the competition (which is quantified by the capillary number *Ca*; see Box 1) to control the generation, break-up and coalescence of droplets.

Comprehensive and quantitative reviews of fluid behaviour at the microscale are provided elsewhere[29,30], but the brief summary above already shows that it can differ markedly from that seen on the macroscale.

Our aim here is to illustrate that microfluidic systems offer a wealth of ways to exploit this unique behaviour — to create and control interfaces and make use of their interface properties. We will focus on interfaces between fluids (liquids and gas). In the case of immiscible fluids we consider droplets of one liquid dispersed in another, where the 'floating interface' between the two phases can act as a semipermeable container wall (Fig. 1a). Immiscible fluids flowing next to each other near a solid surface can also be separated by stable 'pinned interfaces', which are maintained by the action of capillary forces and may act as membranes (Fig. 1b). If the fluids are miscible, there is clearly no defined interface: as the fluids are brought into contact they will mix over time, ultimately yielding a homogeneous fluid. But under laminar flow conditions, the boundary between two miscible fluids moving next to each other and mixing only through diffusion can be regarded as a dynamic or 'moving interface' that can be manipulated and put to practical use (Fig. 1c). Similarly, the diffusive layer forming around a stationary solute source, referred to here as a 'secondary interface', can play a functional role (Fig. 1d).

Immiscible fluids

Emulsions — droplets of one liquid dispersed in another — have attracted scientific interest ever since Rayleigh in 1879 studied the break up of fluid jets projected on another fluid[31], with the factors controlling the formation and stability of such droplets established[32] by Taylor in 1934. These and other early investigations all used bulk mixtures of immiscible phases to produce large quantities of droplets having a wide range of sizes. Moreover, the practical use of emulsions has long been based on their bulk properties. But with the advent of microfluidics, we can now easily manipulate individual droplets and

precisely control their properties. It has, quite simply, transformed the field.

Dispersion and floating interfaces

A simple microfluidic device for producing and manipulating droplets is the 'T-junction' (Fig. 2a): the T-shaped channel geometry forces two flows of immiscible liquids to merge in such a way that one liquid forms droplets dispersed in the other[33]. The droplet-forming phase can be selected by adjusting the hydrophobicity of the device walls at the junction and the relative flow rates of the liquids[34]. The use of T-junctions in series with alternating surface wettabilities produces monodisperse double emulsions that are useful for encapsulation applications or extractions across the thin layer separating the internal droplets and the continuous phase[34,35] (Fig. 2b). When reversing the flow direction, T-junctions with differently sized exit channels will passively sort droplets according to size[36] or break large droplets into smaller ones with controlled sizes[37]. Despite its simple design, the T-junction provides precise control over droplet formation (Fig. 2c), making it i deally suited for commercial uses that require parallel, high-throughput predictable droplet creation.

Dispersed droplets may also be created using a microfluidic extension of Rayleigh's approach, with two streams of one liquid flanking a stream of a second immiscible liquid and the combined two-phase flow then forced through a small orifice. The pressure and viscous forces exerted by the outer fluid ultimately force the inner fluid to break into droplets, either in or just downstream of the orifice. The fabrication of a planar microchannel system uses simple soft lithography, making it straightforward to adjust not only flow rates but also the geometry of the microchannel design to ensure selective generation of droplets over a range of different sizes and at different rates[38,39]. The method is easily adjusted to produce droplets of various compositions (see also Fig. 1a), as demonstrated by the successful synthesis of monodisperse microparticles[40] and nanoparticles[41] from solutions that allow the droplets to be solidified *in situ* after their formation (by, for example, photopolymerization).

In addition to allowing controlled production of droplets, microfluidic devices also provide an opportunity for precisely manipulating generated droplets. Owing to this combination of capabilities, individual dispersed droplets may serve as floating containers or reaction vessels that can be loaded with different reagents for kinetic measurements[42]: once a reaction medium has been added and mixed in, the spatial position of the droplet moving continuously along a known path within a microchannel will correlate with reaction time. That is, a given position in the channel will correspond to the same kinetic state, so signals can be collected from several successive droplets and integrated to improve the signal-to-noise ratio, making it possible to monitor even relatively fast reactions with millisecond time resolution or better[43]. The system is readily extended to studying controlled multi-step reactions by adding new reagents at selected downstream locations[44] (Fig. 2d). A variation of the method allows efficient screening for optimum protein crystallization conditions by using aqueous droplets in a linear array. Droplets containing different protein solutions alternate with droplets containing salt solutions of different concentrations (see Fig. 2e). Once the array is formed, the difference in osmotic pressure between the alternating static droplets drives the diffusion of water through the oil and thus creates a wide range of different crystallization conditions[45] while requiring only small quantities of often precious protein material.

Dispersed droplets offer the potential to manipulate or analyse small fluid volumes and thus allow experiments that require only small quantities of reagents (which may be very costly). But the droplet size is so small that solutes will quickly diffuse from the centre to the interface. Depending on composition and affinity, this might result in solutes selectively diffusing out of the droplet or adhering to the interface. If adhesion occurs, the large surface area relative to volume can prove problematic, particularly if the droplet size is decreased so much that adhesion greatly depletes the solute in the droplet interior. In the case of proteins, adhesion to the droplet interface is often associated with a conformational change, which may become permanent[46]. This tendency to stick to the interface can be prevented by using appropriate surfactants[47]. The effect has also been used to advantage for the fabrication of mechanically stable hollow protein spheres[48] that might serve as biocompatible 'smart' containers for drug delivery.

At the time of writing, new manipulation methods continue to emerge. For example, microdroplets may be levitated in gas or vacuum using magnetic or acoustic forces[49], and asymmetric laser heating of the liquid/liquid interface between an aqueous droplet and its surrounding immiscible fluid can induce Marangoni flows to move the droplet[50]. In yet another approach, dispersed droplets are exposed to amphiphilic magnetic nanoparticles that accumulate and align at the droplet interface; the resultant nanoparticle 'coat' then chaperones the liquid droplet in response to an applied external magnetic field[51].

Patterned surfaces and pinned interfaces

A mixture of water and oil in a macroscopic vessel will separate into two phases, with gravitation and the density difference between the fluids ensuring that a horizontal interface separates the oil at the top from the water at the bottom. In micrometre-sized systems, capillary forces can overcome gravitation and be used to create precisely controlled vertical interfaces, or 'virtual walls', between water and air[52]. This requires sufficiently strong capillary forces to 'pin' the water/air interface in position and counteract the action of gravity, which drives water to 'fall' and spread out horizontally. To achieve this, the internal surface of a microchannel is patterned to create hydrophilic and hydrophobic paths. Water molecules will adhere to the hydrophilic channel surface, with surface tension preventing the liquid from invading hydrophobic regions. Aqueous solutions introduced into the patterned microchannel will thus be confined to the hydrophilic regions (see also Fig. 1b), provided the pressure difference across the water/air interface does not exceed a critical value determined by the Young–Laplace equation. The virtual wall between the streams provides a large gas/liquid interface for efficient removal of dissolved gas species such as oxygen from the water stream under continuous operation[53]. The large surface area provided by virtual walls, and the relatively small volume of the streams to be treated, ensure efficient transport between liquid and gas phases. In this respect, microfluidic devices mimic the alveoli in our lungs, whose large surface area to volume ratio similarly allows rapid exchange of O_2 and CO_2 between air and blood. These systems are not limited to removing dissolved gas from liquid; they could also be used to passively adjust the pH of a buffer solution by exposing it to CO_2 across a virtual wall. Or imagine triggering a chemical reaction within a microfluidic device by introducing a gas-phase species through a virtual wall, or using chemical reactions to generate a gas to be used elsewhere. If airborne analytes are captured into the liquid phase, the system might be used for continuous sensing applications.

It is straightforward to extend the basic idea underlying virtual walls to immiscible liquids flowing side by side (or even on top of each other) in a microchannel. Because the interface between such liquids tends to be unstable owing to differences in liquid properties, patterning the interior microchannel surface to create regions with different wettabilities allows capillary forces to stabilize both vertical[54] and horizontal[55] liquid/liquid interfaces. Such 'pinned interfaces' allow for rapid and precise control over the contact time between the two phases, which are typically an organic liquid and an aqueous solution. Moreover, pinned interfaces are produced within seconds, whereas it can take tens of minutes[56] to establish a stable liquid/liquid interface in a macroscopic system through the action of gravity. These features make microfluidic pinned interfaces attractive for applications such as the study of drug partitioning behaviour[56] and enzymatic reactions[57], solvent extraction of metal ions[58,59], and the execution of multiphase reactions[13] and phase-transfer reactions[60].

Pinned interfaces can also be harnessed more directly. For instance, a stable pinned interface between appropriately chosen aqueous and

organic liquids can serve as the site for an interfacial reaction; if an interfacial polymerization is conducted, the pinned interface is transformed into a real membrane[54] (Fig. 1b) that is readily functionalized (for instance by immobilizing an enzyme on one of its sides[61]). This approach has allowed the formation of a membrane incorporating a peptide crosslinker (N. O. L. Viernes and J. S. Moore, personal communication), so that exposure to a peptide-cleaving enzyme solution leads to a breakdown of the membrane-forming polymer; the resultant fluid leakage then serves as a visual indicator for the presence of the enzyme (Fig. 3). Instead of serving as indicators or sensors, interfacial reactions can also be used to create materials. Particularly when using photopolymerization and suitable surfactant molecules, a wide range of interfaces (including the menisci formed at the solid/liquid/air interface) can be transformed into stable microstructures with unusual shapes, such as microneedles with smooth curved sidewalls[62].

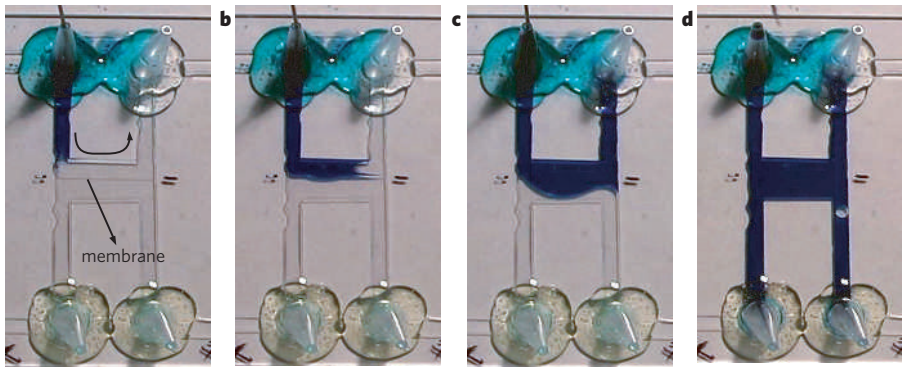

Figure 3 | Pinned interfaces. The use of 'pinned' aqueous/organic liquids creates a stable interface where chemistry can occur, for example to create polymer walls. Making use of enzymatic cleavage of peptides, one can create a biosensing or bio-dissolving wall. Here the wall is created through the interfacial reaction of adipoyl chloride and a lysine-terminated peptide creating a thin wall with a known peptide sequence as a crosslink. When exposed to a solution containing appropriate enzymes the peptides are cleaved, the wall becomes more porous and the dyed enzyme-containing solution leaks through the membrane. (Eventually there may be complete dissolution of the wall; not shown.) **a**–**d**, Sequential images of membrane breakdown. **a**, **b**, A dyed buffer solution containing chymotrypsin is flowed by capillary action into the top half of the channel. **c**, The solution permeating through the membrane indicates that enzyme cleavage has caused a change in the membrane porosity. **d**, The readout channel is completely filled. Such walls should find use as biosensors with simple visual readouts (as shown) or as intelligent valves that can make process decisions based on changes in the local environment, thereby gating flow to appropriate paths. Courtesy of J. S. Moore, University of Illinois at Urbana-Champaign.

Miscible fluids

The interface between immiscible fluids is easily recognized as the common boundary separating the phases. But if fluids are completely mixed, there is clearly no interface. Still, two miscible fluids, usually liquids, brought into contact will have a boundary between them that disappears as the fluids start mixing. This boundary region can act as a *de facto* dynamic interface that changes with time[22] (Fig. 1c) and some of its properties may resemble those of the interface between immiscible fluids[63,64]. Moreover, if two or more miscible liquids move next to each other under laminar flow conditions, then their diffusive interface can be controlled and used[65].

Laminar flow and moving interfaces

Laminar flow ensures that mixing between streams in contact with each other occurs only through diffusion. If conditions exist such that the Peclet number is high, mixing will be negligible (see Fig. 1c). At the interface between streams of miscible liquids the contact time is so short that the interface is kinetically stable and remains sharply defined. At lower flow velocity, the liquids are in contact for longer and mix through diffusion: a diffusive interface forms between the fluids, flows and broadens downstream, as the contact time increases.

Laminar flow and diffusion were first put to practical use by Giddings, who used the interface between aqueous streams flowing through microchannels as a 'virtual membrane' for protein fractionation[66–68]. The success of this approach demonstrated that membrane-like performance can be obtained without the potential fouling problems associated with real membranes, and that the effective membrane thickness — the width of the diffusive layer — can be adjusted by simple changes in flow rate. This work, which largely pre-dates what we now regard as microfluidics, used readily available components to create channels of micrometre dimensions in height and millimetre dimensions in width and length. Such simple microchannels suffice for maintaining laminar flow because it is the smallest dimension that largely determines the ratio of inertial to viscous forces (the Reynolds number is a function of channel geometry via hydraulic diameter). That is, in three-dimensional space the scaling of one dimension to the micrometre scale is often sufficient to harness the forces that are dominant at that scale.

Still, the ease with which laminar flow can be realized in modern microfluidic devices allows for particularly effective and precise control over multiple streams of miscible liquids and exploitation of the interfaces between them. In 1997, microfluidics as we know it today was used to tap into the potential of diffusion and laminar flow[69,70], in the shape of the 'H-filter'. This device merges two separate fluid streams in a central channel and then separates them again into individual channels; the flow regime throughout is laminar. One of the fluids carries particles or solutes of different sizes (the sample stream), while the other is particle-free (the extraction stream). The moment the fluids are in contact, particles start diffusing from the sample to the extraction stream. Diffusivity depends inversely on solute size according to the Stokes–Einstein equation[23], and provided the contact time between the streams is adjusted appropriately, only the smaller solute(s) will enter the extraction stream. Downstream of the central channel, the fluid is split and the extracted solute collected. To achieve fluid splitting without any gross mixing[69], the two streams need to move with equal velocity and steady flow — conditions that can be challenging to realize experimentally. Moreover, the H-filter requires continually flowing liquids so that the performance of the diffusive interface can be controlled; solutes are therefore extracted at the expense of being diluted.

Like the H filter, the 'T-sensor'[71] merges two fluid streams into a common channel to create a controlled diffusive interface. One stream typically contains an analyte, the other a tracer compound such as a fluorescent dye or dye-labelled antibody that can interact with the analyte and provide a signal for optical detection. By monitoring the broadening of the interface during the early stages of diffusive mixing, it is then possible to determine diffusion coefficients (from which analyte size can be extracted), analyte concentrations, reaction kinetics and binding affinities[72]. An attractive and useful feature of the T-sensor is that the reagents start to interdiffuse and react the moment the two fluid streams are in contact, so the time available for diffusion and reaction correlates with the distance the fluid travels subsequently. An outside observer will therefore 'see' the course of the reaction or diffusion as a still image, and reaction kinetics and diffusion distances can be measured as a function of distance rather than time. This allows the optical signal to be integrated over time to improve sensitivity, making the T-sensor a robust device that is straightforward to implement (in contrast to the H-filter, where the need for stream splitting constitutes a serious complication). At the time of writing, this basic system has been developed for use in molecular mass sensors[73], chemical assays[74], membraneless microfluidic fuel cells[75,76], and immunoassays[72].

The laminar nature of fluid flow through microchannels permits many streams containing not only different substances but also different concentrations of the same substance to flow side by side. As a result, concentration gradients with complex profiles can be generated by feeding a small number of fluid streams with initial concentrations of diffusible substances into a pyramidal microfluidic network[77,78]. As the streams travel down the network, they are repeatedly split, and some combined with neighbouring streams and allowed to mix by diffusion within a channel before being split and combined again. At the end of the network, many individual fluid streams containing solutions with different concentrations combine in a broad channel that will have a complex concentration profile perpendicular to its flow direction. In contrast to the concentration gradients produced with conventional methods, the profile in this microfluidic system is stable and can be maintained over long periods — characteristics that make the system attractive for studying processes that require gradients, such as chemotaxis[79,80] and intracellular protein trafficking[81]. In contrast to concentration, the local temperature within microfluidic channels can be externally imposed and permanently maintained[82–85]. This makes it possible to superimpose temperature and concentration gradients in one microfluidic system for high-throughput two-variable experiments.

Laminar flow of multiple liquid streams through microchannels can also serve as a microfabrication tool that is applicable to a broad range of materials, including metals, polymers, inorganic crystals and ceramics[86,87] (see also Fig. 4a). The chemical composition of the liquids is chosen such that material is deposited onto or etched away from the inner microchannel walls, with the reactions that create the desired structures occurring either between the streams and the contacted channel surface, or at the interface between the streams. For instance, using an etching solution sheathed by an inert liquid results in a narrow trench, the width of which can be adjusted by controlling the relative flow rates of the fluids used. If neighbouring streams carry components that become reactive upon mixing, then etching or material deposition occurs only at locations where the microchannel surface is exposed to the diffusive interface between the streams. Again, adjustment of relative flow rates provides control over the width and location of the structures fabricated.

Laminar flow at high Peclet number, where almost no mixing occurs, is an effective and widely applicable approach for 'sheathing' one fluid with another. It has been used to contain monomers, which are then photopolymerized 'on the fly' as they exit from the microfluidic device[88]; the method thus allows continuous generation of micrometre-thick fibres and tubes that retain the geometry of the original fluid (Fig. 4b). The process can be used to 'freeze' the diffusive interface, providing insight into directed polymer growth (Fig. 4c). Sheathing is also useful for suppressing non-specific adsorption of analyte to microchannel walls[89] (a problem that becomes more significant as microfluidic devices are shrunk further).

Zero-flow and secondary interfaces

Owing to their small size, microfluidic systems have flow patterns that tend to be dominated by viscous forces; this allows precise control and use of laminar flows. But the dominance of viscous forces makes microfluidic systems also ideally suited for realizing purely diffusive ($Pe = 0$), convection-free environments that are almost impossible to achieve in macroscopic systems (see also Fig. 1d). Only under such conditions will solute released from a source diffuse in all directions with equal probability, its concentration decreasing with increasing distance from the source. The extent of this diffusive layer, which we term a 'secondary interface', depends on the rate of solute release (or production) at the source and on the solute's diffusivity. If microchannel walls are close, solute will accumulate in a predictable way.

A microfluidic chip for screening of protein crystallization conditions[12] takes advantage of such purely diffusive solute transport: compartments are filled with protein solution and precipitant solution, which on opening of a connecting valve mix through diffusion only. Such free interface diffusion is known to make it easier for high-quality crystals to nucleate and grow, but has so far rarely been implemented because of the considerable difficulty of achieving diffusive mixing in large-scale systems.

Flow-free microfluidic systems also offer intriguing opportunities for the study of processes such as cell division and migration, intercellular communication and the emergence of cell polarity during development (where molecular gradients are known to play an important role). For instance, cell proliferation studies on a number of different cell types have revealed that the proliferation characteristics are markedly different when using microchannels instead of traditional mass culture systems[90,91]. To understand this difference in behaviour, consider the rather different environments experienced by the cells: in the constrained medium within a microchannel, signalling molecules

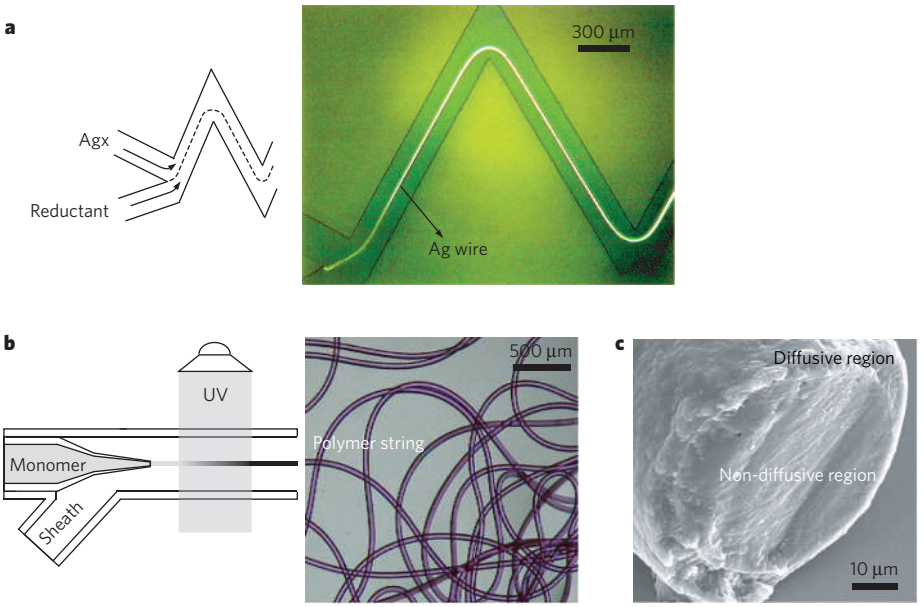

Figure 4 | Interfacial reactions. Control of the time of contact between two streams in laminar flow is important in these microfabrication processes. **a**, Two solutions containing the components of an electroless silver-plating solution flow together in a PDMS microchannel, producing a deposited continuous silver wire at their interface. Image on the left reprinted with permission from ref. 86. Copyright (1999) American Association for the Advancement of Science. Image on the right courtesy of P. J. A. Kenis, University of Illinois at Urbana-Champaign. **b**, Rapid photopolymerization of flowing laminar streams ('on-the-fly' polymerization) allows the continual creation of microscale strings. The smoothness of the interface can be controlled by altering the components in each stream. The addition of multiple sheath flows allows the creation of tubes with controlled size and content[88]. Reproduced by permission of the Royal Society of Chemistry. **c**, Radially directed polymer growth is seen when two miscible fluids are used, one containing a photoinitiator and one without the photoinitiator. The diffusive interface is 'frozen' by photopolymerization allowing high-resolution imaging of the diffusion region between the flows. Courtesy of S. Lee, Dankook University.

produced by a given cell (autocrine signals) or surrounding cells (paracrine signals) can accumulate, whereas such signalling molecules will be diluted or even removed by the convective flows that inevitably arise in mass culture systems or flowing microfluidic systems (see also Fig. 1d). Culturing in microchannels in the absence of flow, where transport is purely by diffusion and the size of the system prevents extensive dilution, appears to increase the sensitivity of proliferating cells to the effects of soluble factors[91]. Similar effects may explain why the efficiency of embryo development improves in microchannels under no-flow conditions[92]. So microfluidics should open new opportunities for studying cell signalling, where convection-free culture conditions allow signalling molecules secreted by a cell to form diffusive layers and influence the secreting and surrounding cells. Of course, cells in 'real' living systems are unlikely to experience environments of either laminar flow or no flow at all; still, the ease of creating a range of microenvironmental conditions should allow complementary investigations to characterize and understand cellular processes more fully.

Broadening the range

As we have seen, microfluidics provides us with a powerful way of exploring and exploiting fluid behaviour at a scale where diffusion, viscous drag and surface tension can dominate. Of the applications that are now emerging, we are particularly excited about the unique opportunities for exploring cellular processes. But as the breadth of material and methods presented in this review illustrates, microfluidics can influence a vast range of fields and topics. In fact, whenever we need to use or analyse a fluid, microfluidics could add a new dimension to the task. Cellular autocrine/paracrine signalling mechanisms in development and pathological conditions can now be explored in new ways. Questions of interfacial instabilities and their role in complex systems may become more tractable[33]. Basic polymerization dynamics and interfacial reactions can be more carefully examined and turned to better advantage[87]. Basic cellular transport mechanisms might be studied using engineered controlled interfaces to validate proposed models, such as the selective phase model for transport through nuclear pore complexes by hydrophobic exclusion[93]. It is likely that the future will see a maturing in the way microfluidics are applied: moving beyond the demonstration stage, microfluidics will become an integral tool for formulating and answering these and many other fundamental cross-disciplinary questions. ■

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Acknowledgments We thank J. Moorthy and D. Kim for help in preparing the manuscript.

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