Chipping in to microfluidics

Devices that allow nanolitre volumes of liquid to be manipulated on "labs-on-chips" are revolutionizing the way biological research is carried out, describe Carl Hansen, Kaston Leung and Payam Mousavi

Imagine stepping off the edge of a swimming pool, only to find that your foot deflects the surface of the water without breaking it, as if held by some impenetrable skin. As you walk forward, the water continues to support you; but if you take a running leap and bring your full weight down on the surface, then it snaps open to envelop you without a splash. Rather than plunging to the bottom of the pool, however, you stop abruptly as your kinetic energy is instantaneously dissipated in the fluid. You flail your arms in an attempt to return to the pool’s edge but make no progress, merely bouncing back and forth with each stroke.

While such an experience would come as some surprise to a human used to experiencing life on the macroscopic scale, this is precisely how fluids behave when confined to micrometre-wide channels. On the micro-scale, surface tension and viscosity dominate fluid dynamics, as our imaginary swimmer would discover (see box on page 27). These phenomena cause the chaotic turbulence that characterizes macroscopic flow to disappear and be replaced by "laminar flow" in which fluid flows in parallel layers with little or no mixing between them. Physicists, chemists and biologists are seeking to exploit the novel physical properties of this microfluidic regime for applications ranging from materials synthesis to drug discovery.

Handling liquids is a big part of nearly all experimental chemistry and biology, and it is usually carried out with traditional hand-held micropipettes or robotic liquid-handling systems. The advantage of microfluidic technology is that it allows far smaller, sub-nanolitre, volumes of precious reagents to be manipulated precisely. This economy of scale means researchers can carry out exhaustive experiments that would otherwise be prohibitively expensive. Microfluidic technology also allows experiments to be miniaturized and automated on compact "lab-on-a-chip" systems, thereby freeing scientists from repetitive work while making experiments more accurate and reproducible. Less than 20 years old, this idea is transforming biological and medical research in the same way that the miniaturization of electronics has benefited computation, and it is set for wider applications in clinical and environmental sensing.

But microfluidic research means much more than just miniaturizing chemical and biological experiments. The unique physical properties of the microenvironment, including laminar flow, allow experiments that are difficult or impossible in macroscopic systems, ranging from the analysis of single cells to highly efficient mixing for protein crystallization. And recent work that incorporates droplets and bubbles into microfluidic systems is even opening up a new world of chemical computation in which digital bits of information and chemical payloads are carried in the same package.

Labs on chips

Microfluidics dates back only to the early 1990s, when the fabrication techniques used in the booming micro-electronics industry were adapted to produce the first lab-on-a-chip systems. Tiny quantities of biological or chemical reagents are shuttled around networks of microscopic channels in postage-stamp-sized devices by external hydraulic pressure, electric fields or microfabricated pumps. Early microfluidic systems were primarily based on the hard materials used in microelectronics, such as glass and silicon. Such systems proved successful in allowing electric fields to separate complex mixtures of molecules in solution based on their differing mobilities, which is important for sequencing the human genome, for example.

However, the photolithography and etching techniques adapted from the microelectronics industry are expensive and inflexible. In particular, glass and silicon can not be easily fashioned into sealable valves. Without valves to control the flow, a microfluidic circuit is essentially hard-wired and the flow through any branch of the circuit depends on the pressure or electric potential applied to all its outlets. But if valves are present, they can be opened or closed using an external control, allowing researchers to rewire the network during an experiment.

In an attempt to incorporate valves into microfluidics, in 2000 Marc Unger of Stephen Quake's group at the California Institute of Technology turned to silicone rubber - a material that is about 100,000 times softer than silicon or glass. Unger extended techniques developed by George Whitesides at Harvard University in the US to fabricate channels and valves by bonding together multiple layers of rubber, each moulded from a silicon master made using conventional pho-
tollithography. He formed valves from a perpendicular crossing of two channels in adjacent layers separated by a thin rubber membrane. Applying pressure to the fluid in one channel causes it to expand and thus constrict the flow in the other channel, by squeezing a rubber group had moved from early demonstrations containing just a few working valves per square centimetre to a large-scale system with thousands of valves.

Just as transistors are the basic element in electronics, microvalves can be combined to create more advanced devices such as pumps and micromixers, which can then be linked together to form complex fluidic networks. Recently Emil Kartalov of the University of Southern California has extended the rubber-moulding technique to construct 3D circuits by connecting the channels in different layers to each other.

The laminar flow of fluids on the micro-scale offers distinct advantages when designing microfluidic circuits. For low Reynolds numbers, the Navier–Stokes equations of flow become linear, in contrast to the chaotic turbulence of macroscopic fluids. The equations are then easily solved analytically or numerically and the solutions scale with the input. Doubling the pressure doubles the velocity, for example. However, this also means that there is no mechanism for amplification or feedback, which are vital properties of an electronic circuit. By incorporating feedback into fluidic networks we can create more powerful devices that behave differently based on the fluidic inputs to the system, without having to control the networks externally using valves. Introducing nonlinear effects to laminar flow is difficult, but exciting progress has been made recently by constructing networks in which two fluid phases, such as oil and water, flow together.
We can generate droplets with a volume of $10^{-15}$ litres that let us study enzymes produced not from whole cells but from single DNA molecules.
reagents to mix quickly is vital.

In macroscopic fluids, mixing is accelerated by turbulence. But in the laminar regime, two fluids simply flow alongside each other, and mixing only occurs when single molecules diffuse across the lines of flow. However, ultra-fast mixing can still be achieved in microfluidic flows using “chaotic advection” – similar to the kneading of dough. This was accomplished by Rustem Ismagilov’s group at the University of Chicago by forcing a droplet through alternating winding turns and straight sections in a microchannel. As the droplet travels through a straight section, viscous drag at the channel walls creates two symmetric circulating flows that have the effect of stretching and folding the fluid layers. When the droplet flows through one of the winding sections, it moves at different velocities relative to the inside and outside walls of the curve, thereby causing it to reorient with the line separating the two reagents perpendicular to the direction of flow. With successive cycles of stretching, folding and reorientation, the fluid layers get thinner, which allows the reagents to mix rapidly through the diffusion of molecules between the layers.

Micro-scale fluids do not experience turbulence, so the reagents within a droplet must be mixed using a different process called chaotic advection – similar to the kneading of dough. This was accomplished by Rustem Ismagilov’s group at the University of Chicago by forcing a droplet through alternating winding turns and straight sections in a microchannel. As the droplet travels through a straight section, viscous drag at the channel walls creates two symmetric circulating flows that have the effect of stretching and folding the fluid layers. When the droplet flows through one of the winding sections, it moves at different velocities relative to the inside and outside walls of the curve, thereby causing it to reorient with the line separating the two reagents perpendicular to the direction of flow. With successive cycles of stretching, folding and reorientation, the fluid layers get thinner, which allows the reagents to mix rapidly through the diffusion of molecules between the layers.

On the other hand, viscous and surface forces scale only linearly with the length: the viscous force is approximated by the product of shear stress and surface area \( F_{\text{visc}} \sim \mu v d / d^2 \); and the surface force by the product of surface tension, \( \sigma \), and length \( F_{\text{surf}} \sim \sigma d / d^2 \).

Taking the ratio of the relevant forces generates non-dimensional numbers that describe the relative importance of these effects. For instance, the Weber number \( W_e = \rho v^2 d / \sigma \) describes the relative importance of inertial and surface forces, and tells us, for example, how big and how fast an insect can be before it will break through the surface of a pond. Perhaps the most celebrated of these numbers is the Reynolds number \( Re = \rho dv / \mu \), which describes the relative importance of inertial and viscous forces. Microfluidics involves low flow at low Reynolds numbers, typically much less than 1.

The characteristic behaviour of flow at high Reynolds numbers is turbulence: tiny perturbations in the initial conditions result in vastly different subsequent behaviour and the flow profiles are random and fractal. For example, the Reynolds number describing the flow around a professional cyclist is approximately \( 7 \times 10^6 \).

In contrast, the Reynolds number describing the swimming of an \( E. \text{coli} \) bacterium is approximately \( 3 \times 10^3 \). Inertial effects are completely suppressed and the flow is said to be laminar. In this highly viscous regime, water behaves more like molasses and different fluids flow alongside each other instead of mixing. The advantage of laminar flow when designing microfluidic systems is that the fluid-flow equations become linear and do not depend explicitly on time. This makes it much easier to calculate how fluids will flow in different channel geometries.

**Bubble logic**

Microfluidic systems can act much more than just miniature test tubes by using the nonlinearities of two-phase microfluidic systems to combine chemistry with computation. Thorsen’s investigations of droplet formation showed that if the fraction of sample to carrier volume is sufficiently low, then the carrier fluid quickly removes each drop, resulting in the periodic generation of highly uniform microdroplets. However, as the sample fraction is increased, nascent droplets emerge before their neighbours have left the vicinity of the injector. Long-range coupling between the droplets can then lead to fantastically complex and dynamic patterns including regular arrays of droplets, helical structures, well-ordered “crystals” and chaotic aperiodic trains of drops. The richness of these systems can be exploited to allow microfluidic devices to be used to process information.

In pursuit of this goal, Manu Prakash and Neil Gershenfeld at MIT have recently built simple microfluidic
Manu Prakash and Neil Gershenfeld at the Massachusetts Institute of Technology have created a microfluidic system that performs logical operations using bubbles of nitrogen as bits. Shown here is the microfluidic equivalent of an electronic flip-flop (a bit of memory that switches from one state to another when triggered). It works by storing and dislodging nitrogen bubbles from a structure comprising two connected elliptical lobes, one representing "0" and the other "1" (a). A bubble tends to minimize its surface energy by adopting a shape with the smallest surface area, so it will remain indefinitely in one of the elliptical lobes until perturbed. The state of the flip-flop is changed by sending a "toggle" bubble into the inlet (b), which is diverted to the unoccupied lobe by the resistance presented by the stored bubble (c). It then dislodges the stored bubble via the flow through the channel connecting the two lobes (d, e), leaving the flip-flop in the opposite state to before the bubble's arrival (f).

Logic gates that use closely spaced bubbles of nitrogen as "bits". Each bubble bit that passes through the fluidic network changes the effective impedance of the surrounding channels, thus determining the trajectory of subsequent bits. Prakash developed a four-terminal device that simultaneously implements AND and OR operations on the incoming streams of bubbles; and a bistable "flip-flop" device that allows bubbles to be stored in and recovered from a digital memory (figure 3). Since bubbles are both the inputs and the outputs of each gate, one device can drive another to build up complex logical operations. For example, Prakash and Gershenfeld combined three AND gates to build a ring oscillator.

Although it seems unlikely that bubble-based microchips will replace their silicon counterparts in computers anytime soon, droplets do have the unique ability to carry both digital information and a chemical payload in the same package. This opens up the possibility of performing a "chemical computation" in a microfluidic logic circuit. For instance, one could design a fluidic network for automated combinatorial chemistry that would take a selection of basic chemicals as inputs and generate a library of chemical compounds in isolated droplets as the output. A major challenge in developing such systems is to extend logical operations from well-behaved nitrogen bubbles to real biological samples where the properties of the fluid, including the surface tension and viscosity, are less controlled.

An even more far-out example of microfluidic computation could use the reversibility of laminar flow to encrypt and decrypt information. Turbulent flow is fundamentally irreversible – you can never "unstir" milk from coffee – but laminar flow can be made to precisely retrace its steps by reversing the pressure driving it. Earlier this year, George Whitesides and Michael Fuerstman at Harvard University investigated this reversibility by forcing a series of droplets to flow through a symmetrically branching channel.

At a low flow rate the droplets pass through one at a time and choose a path randomly, but as the rate increases the presence of one droplet in a branch inhibits the next, causing the droplets to follow alternating paths. At higher rates still, the network shifts to a quasi-periodic behaviour with multiple frequencies, and finally becomes chaotic and unpredictable. Nevertheless, by reversing the flow, Whitesides and Fuerstman were able to exactly recover the initial sequence in which droplets arrived at the branch. Thus a signal could be represented by choosing the initial time intervals between droplets, which is drastically altered (encrypted) by passing droplets through the bifurcation, but can be recovered (decrypted) by switching the pressure.

**Complete control**

The two-phase microfluidic devices described so far allow droplets to be formed, stored, and used in logical operations. However, these systems are somewhat limited in that they are "passive" – there is no external control other than the input of fluids. Furthermore, although nonlinear effects create feedback between the droplets themselves, this feedback is not connected to the chemical reactions going on inside the droplets. More direct external control is needed to develop microfluidic systems that can be programmed to carry out different chemical reactions and to respond to experimental results.

In 2006 Weitz and colleagues at Harvard incorporated electric fields into a microfluidic system to achieve more control over the creation, recombination, splitting and sorting of droplets than is possible with purely flow-based systems. In their system, metal electrodes patterned onto a piece of glass are used to apply an electrical field that charges up a droplet as it is formed. Increasing the electric field exerts a force on the emerging droplet, causing it to break off before it has the chance to grow. In this way, droplet sizes can be tuned over three orders of magnitude, and since the droplets retain an electric charge, they can be further manipulated downstream.

A major advantage of this system is that it can fuse two droplets containing different reagents in order to carry out multi-step chemical reactions. It is usually difficult to fuse droplets because surfactants are deliberately added to stop them from breaking up; and droplets must be made to cross the prevailing flow in order to arrive at the same location. But by using two injectors with electrodes of opposite polarity, pairs of droplets with equal and opposite charge can be generated at the same time. Electrostatic attraction between the droplets then forces them to cross the flow and co-
alesce into a neutral mixed droplet. Electric fields can also be used to split and re-charge droplets so that multiple fusing and splitting operations can occur in a network; and to sort charged droplets by forcing them to choose a particular path rather than a random one at a branch. As this sorting mechanism requires no moving parts, it can attain very high switching frequencies.

An alternative way to control microfluidic circuits is to build microvalves into a two-phase system, thus combining the advantages of both technologies. In such a system, droplets are formed by actively pumping fluids through valves, rather than using a flow-based system like Thorsten’s T-junction. This has the advantage that the formation of droplets is not affected by the varying physical properties of the carriers and reagents, and does not require flow rates to be precisely tuned. Using this method, the current authors and colleagues have recently developed a fully programmable microfluidic system that provides arbitrary control over droplet chemistry, timing, spacing and size (figure 4). Our device also allows chemical feedback: real-time image analysis allows us to determine what is inside a droplet, and the droplet can then be directed to an appropriate part of the chip using valves.

We recently used our device to automatically find optimal chemical conditions for proteins to form crystals—an important step in macromolecular structure determination by X-ray crystallography. We first systematically determined the solubility of the protein in a wide range of chemical conditions, and then used this information to automatically identify the optimal concentrations of protein and solvent to maximize the chance of crystal growth for a set of model proteins. The programmability of two-phase systems with integrated valves means they can be easily adapted to other applications, from optimizing cell-culture conditions for protein production to screening chemical libraries for the discovery of new bio-active compounds. One challenge will be to make the devices smaller for use outside the lab: while the fluidic chips themselves are tiny, the solenoids that are used to switch the valves, the tubing that supplies the fluids and the image analysis tools are all bulky.

Beyond the lab
Two-phase fluid flows bring a rich array of new physical phenomena and many practical advantages to microfluidic research. Fundamental studies of two-phase fluid physics at the micro-scale will undoubtedly lead to better control over lab-on-a-chip applications. Two-phase microfluidic systems are providing a systematic brute-force approach to experiments in biology and chemistry laboratories that would otherwise be impractical, and allowing fundamentally new types of measurement through rapid mixing and compartmentalizing of single molecules or cells.

As the field of microfluidics advances, even more exotic applications are emerging. For example, Claus Dieter Ohl and colleagues at the University of Twente in the Netherlands have recently used the sudden bursting of bubbles, known as cavitation, to create turbulence in microfluidic channels, thereby accessing a wealth of properties not available in laminar flow. They generated a bubble by depositing energy using a pulsed laser beam and found that jets and vortices form as the bubble collapses. The jets could potentially be used for microfluidic pumping and the vortices for mixing on microsecond timescales.

As microfluidic technologies become more reliable and portable they will find widespread uses outside the biology lab, including point-of-care clinical diagnostics, industrial-scale materials engineering and environmental monitoring. Although significant steps have been taken towards realizing this potential, there are many outstanding challenges, such as developing reliable storage of reagents, gaining better control over the adsorption of molecules on to droplet surfaces, developing more scalable systems for droplet fission and fusion, and creating new actuation and sensing schemes for portable devices. Such advances will undoubtedly require insights from disparate disciplines including physics, micro-scale engineering, chemical synthesis, molecular and cell biology, and medicine. Indeed, perhaps the biggest challenge facing microfluidics research will be training scientists to communicate and work effectively across these disciplines.

More about: Microfluidics
B T C Lau et al. 2007 A complete microfluidic screening platform for rational protein crystallization J. Am. Chemical Soc. 129 454–455
M Prakash and N Gershenfeld 2007 Microfluidic bubble logic Science 315 832–835
H Song et al. 2003 A microfluidic system for controlling reaction networks in time Angewandte Chemie (International Edition) 42 768–772
T M Squires and S R Quake 2005 Microfluidics: Fluid physics at the nanoliter scale Rev. Mod. Phys. 77 977–1026