# Microscale Bioanalytical Systems

**Deirdre R. Meldrum and Mark R. Holl**

Microfluidic phenomena are present in all of nature's exquisitely designed biological systems (*1*). A complex oxygen transport network consisting of plasma, red blood cells ~6 to 8 μm in diameter, and capillaries ~8 μm in diameter carries sustenance to cells throughout the body, including the eyes through which you are reading this article. Without microfluidic channels and diffusion transport systems, life as we know it would not be possible (*1*).

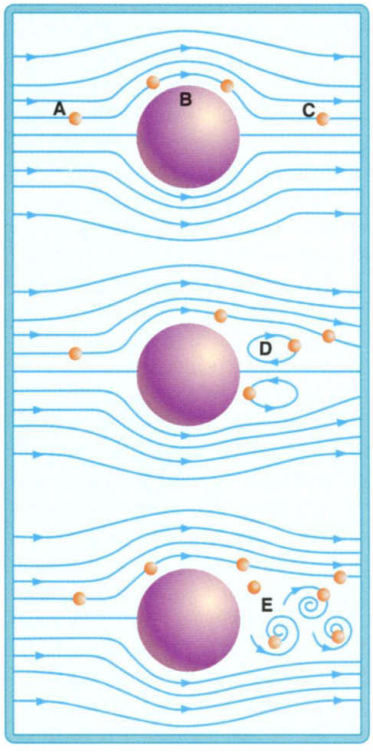
Most microfluidic systems draw their advantages from low thermal mass and efficient mass transport, as well as from large channel surface area–to–channel volume ratios. Manmade microfluidic systems have been made possible by microfabrication methods developed for the electronics industry. Microfluidic systems are of intense interest because of their characteristic controlled mixing that expedites chemical reactions, reduced consumption of expensive materials, high sensitivity, and efficient product yields. Here, we aim to share a summary of the applications and design of microfluidic devices. Welcome to an expansive world of the very small.

Microfluidic systems are composed of fluid channels and chambers with critical dimensions of tens to hundreds of micrometers (for reference, a relatively fine human hair is about 80 μm in diameter). Integrated systems of microfluidic channels combined with pumps, valves, and detectors are known as Micro Total Analysis Systems (μTAS) or "lab-on-a-chip" systems (*2*, *3*). Modern microfluidic devices are generally hybrids of various materials and processes integrated with electro-optical components for detection and, importantly, manipulation of molecules of interest (*2*).

Rapid and efficient mixing of components is critical in the control of chemical reactions. In nature, the limit of diffusion has determined, in part, how single-cell organisms evolved and how they behave (*4*). Microfluidic channels provide specialized environments for controlling mixing. Only laminar flow exists in confined spaces such as microchannels, because fluid viscosity, not inertia, dominates fluid behavior. Therefore, fluid flow in these systems is not turbulent or random. In macrofluidic systems, a much larger fluid mass is in motion and inertial forces can dominate and lead to turbulent conditions (see figure, this page). In laminar flow, two nonreacting fluids that merge will flow smoothly next to each other as diffusion brings the two solutions into a well-mixed equilibrium. This is achieved by layering the fluid or minimizing the channel dimensions (see figure, next page). The equilibration time is proportional to the square of the diffusion distance, in this case the microchannel dimension in the direction of the concentration gradient.

The small dimensions of microfluidic devices cause the ratio of surface area–to–volume to be large. Capture of specific molecules and particles is achieved by exploiting the binding affinity of these substances to specialty coatings of the wall surfaces. Captured molecules and particles can be released all at once (as a bolus) by a change in conditions local to the capture site (e.g., heating or reagent change) when desired.

Microfluidic devices are used with aqueous and nonaqueous solvent mixtures that carry cells or dilute chemical reactants or indicators. Air bubbles are quite a problem in microfluidics because they change the device response dynamics, alter fluid flow patterns, and may lodge where a measurement needs to be made. Air bubble entrapment occurs most often when solvents do not wet the channel surface. To reduce the incidence of bubbles, devices are usually filled with the use of special protocols that include a pretreatment of the channel surface to facilitate solvent interaction.




**Fluid flow around a cylinder. (A to C)** At very low flow rates around an obstacle (purple), viscous forces dominate and the path of the fluid is completely reversible. A particle (orange) can retrace its path on a fluid streamline in the reverse direction. **(D)** At higher flow velocities, the effects of the inertia of the fluid are observed by the creation of stable recirculating patterns. **(E)** At yet-higher flow velocities, turbulent eddies are generated.

Typical uses of microfluidic systems include sample preparation for mass spectrometric analysis, fluid and particle routing, detection and control of chemical reactions, mixing of solutions, and separations. These operations are used in a number of different techniques, such as process analysis, environmental monitoring, clinical diagnostics, drug discovery, cell culture, cell manipulations, protein analysis, polymerase chain reaction (PCR), DNA sizing, and sequencing (*3*). In electrospray ionization mass spectrometers, microfluidic devices provide small sample volumes at low flow rates and in high-density microarray formats for high-throughput analysis.

One of the key tools in DNA analysis is PCR, in which a desired section of DNA is exponentially amplified by cyclically heating and cooling the reaction mixture. Applications of microfluidics-based PCR systems include sensitive monitoring of the presence and/or expression of a specific gene of a patient, tracking the effectiveness of a drug, or detecting the presence of tiny amounts of contaminating DNA (*5*). These microfluidic systems use electrophoresis in microchannels, similar to capillary electrophoresis, to perform high-quality, high-speed separation of DNA fragments, enabling rapid, parallel genotyping applications (*6*). In many cases, the speed of operation is measured in seconds, a fraction of the time required for macroscale capillary-based electrophoresis systems. The quality of separation in such microfluidic systems is also often better due to their ability to inject

D. R. Meldrum and M. R. Holl are in the Genomation Laboratory, Department of Electrical Engineering, University of Washington, Seattle, WA 98195–2500, USA. E-mail: deedee@ee.washington.edu (D.M.); holl@u.washington.edu (M.R.H.)

picoliter-sized samples in a focused spot at the entrance of a microfluidic channel. Electrophoresis systems have been developed for applications as diverse as sequencing the genomes of organisms, protein characterization, and forensic analysis (*7–13*).

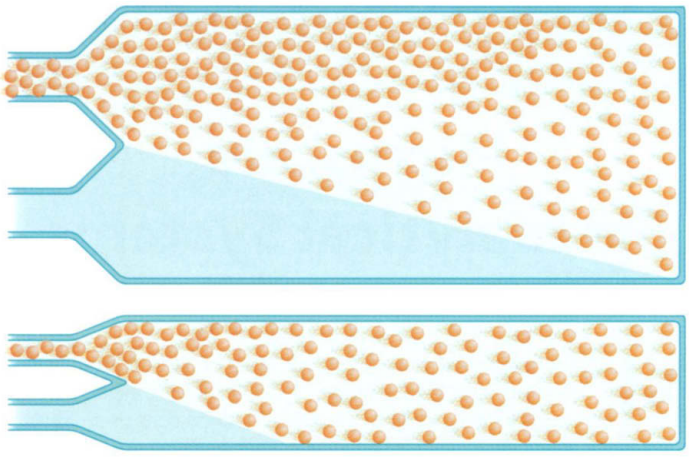
Dielectrophoresis provides another means of using electric fields for manipulating particles such as DNA, viruses, proteins, and cells. Electrodes are used to generate a nonuniform alternating current (AC) in a channel with particles and fluid. The nonuniform field induces an electrical polarization on uncharged particles to produce a dielectrophoretic force that can be used to collect them. This is analogous to clothes becoming electrostatically charged in a clothes dryer (see www.dielectrophoresis.org for more information). Dielectrophoresis has been demonstrated in a microfluidic device as an effective method of discriminating between infected and uninfected blood cells at concentrations of 1 infected cell in more than $10^5$ normal cells (*14*).

Electrofusion of two cell types to generate a third cell using battery-operated power supplies and microelectrodes has been demonstrated (*15*). When combined with chip-based flow cytometry to sort and isolate fused cells into chambers for culturing, the survivability of the fused cells can be determined. Cells have also been sorted using a microfabricated fluorescence-activated cell sorter (μFACS). Such a device is disposable, eliminating possible contamination and cleaning; it has more sensitive optical detection; and it is easier to set up (*16*). Integrating the output of a μFACS directly with microfluidic PCR chips, DNA sequencing chips, and systems for analyzing single protein molecules will enable quick, direct, automated analysis of a cell's contents.

Fluorescence is the most commonly used detection method in microfluidics. Fluorescence assays in these devices often use a large external laser to excite fluorescence and a microscope lens and photomultiplier tube (PMT) to collect and detect the signal. Promising detection methods more readily miniaturized for microfluidics applications include blue light-emitting diodes (LEDs) instead of a laser and silicon photodiodes instead of PMTs (*11*). Electrochemical detection methods that convert chemical signals directly to voltage in microelectrodes can be used to detect analytes and DNA fragments as well as amino acids, carbohydrates, and organic acids (*17*).

One of the challenges in microfluidic devices is the interface between the macroscopic and the microscopic worlds. Caliper Technologies Corp. (Mountain View, CA) has developed one solution with their "sipper" technology: A glass capillary is physically integrated in a perpendicular orientation to the plane of the chip and can be used to sip samples from wells in a standard microplate. The chip accesses samples sequentially from a microplate with the capillary sipper, mixes the samples with cells flowing through a microchannel, labels the cells with dyes, and reads fluorescent signals. Advantages of this approach over standard macro-scale methods is high sensitivity, using only 150 cells per sample; a wide dynamic range; low reagent consumption of 10 nl per sample; and high throughput of 500 samples per hour (*18*).

Cutting-edge research in microfluidics has pushed the science and technology to systems that are three orders of magnitude smaller, resulting in chambers with critical dimensions of tens to hundreds of nanometers. Nanofluidic systems are so small that these channels approach molecular dimensions and the well-characterized rules of microfluidics may no longer apply (*19*). Nanofluidics have been applied to DNA sizing (*20*) and have been demonstrated for the direct reading of the nucleotide sequence of a single-stranded DNA (ssDNA) (*21–24*). In this method, the ssDNA is pulled through a nanopore slightly larger than the diameter of the ssDNA molecule. Changes in the electrical current that flows through the pore are correlated with the movement of the different bases (A, G, C, or T) of the DNA molecule as they pass through the opening. Agilent Technologies (Palo Alto, CA) and Harvard University's Daniel Branton are codeveloping a nanopore sequencer on the basis of this idea that DNA can be shuttled through a $10^{-9}$ m hole at a rate of a million bases per second. With this method, it would take less than 2 hours to sequence an entire genome (*25*).



**Two fluid streams, same average flow velocity.** In both channels, the particles (orange) are assumed to have identical diffusion coefficients. The lower channel has a fluid layer thickness one-half of that shown in the upper channel. Because the rate of diffusion is identical in both channels, equilibrium mixing occurs in the lower channel ~4 times faster.

Many see a parallel between microcomputing and microfluidics. In the early days of the computer, mainframes were accessible only to a small population, whereas nearly every home has a computer now. With advances in microfluidics, healthcare testing currently performed in central laboratories may soon move to point-of-care testing by individuals using their own microfluidic devices (*26*). A general consensus within the microfluidics community is that 2003 will be a breakthrough year for commercialized microfluidic systems. Just as microelectronics have completely changed the world we have realized for ourselves, micro- and nanofluidic systems of our creative design promise yet another transformation that will be felt by all though too tiny to be seen.

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