NANOFLUIDICS

A fork in the nano-road

Symmetry is usually prized in nature, but the deliberate skewing of symmetry in nanofluidic devices can lead to elegant new ways of sorting biomolecules.

Robert Austin
is at the Department of Physics, Princeton University, Princeton, New Jersey 08544, USA.
e-mail: austin@princeton.edu

Separating the components of biological samples is of critical importance in many areas of research and technology. This is not necessarily a straightforward task, however, as samples typically consist of a rich mixture of objects with different sizes, shapes and other properties such as charge. Although there are many ways to sort complex mixtures, those based on size discrimination are perhaps the most simple. Because the mobility of various objects in a given sample will depend on their size, the obvious approach is to make a matrix consisting of some average size pore, inject a slug of the sample and observe the dispersion as it passes through the medium. From gels to capillary electrophoresis, this is the fundamental technology that drives separation techniques.

However, there are several problems with this basic technique. A fundamental one is that the pores clog if the objects are too big and the flow rate then becomes a function of the fraction of open pores. Another problem is that the separation is a batch process — a slug is injected and then dispersed and analysed. It is often desirable to collect larger amounts of separated material for further analysis, but batch techniques are not well suited to such applications because they make inefficient use of time. They are also difficult to optimize interactively as each sample must be run and analysed before a parameter can be changed. In a continuous process, such as spraying water on a fire from a hose, the parameters can be smoothly changed in a continuous way to quickly optimize the process.

Whereas the holes in a conventional sieving gel are the same in all directions (isotropic), nanofabrication can be used to make a sieving medium that is not symmetric (anisotropic) — a physicist would say that the material has 'skewed' symmetry. In practical terms, it means that objects can move more easily in one direction through an array (for example, west-to-east) rather than another (for example, north-to-south). If designed properly, as objects move through such an asymmetric medium they can be deflected to one side, and the magnitude of the deflection can be some function of a property of the object — such as its size. In this way, a complex mixture is fanned out along a direction orthogonal to the mean direction of the flow and the fractionated components can be continuously collected, possibly with very high resolving power.

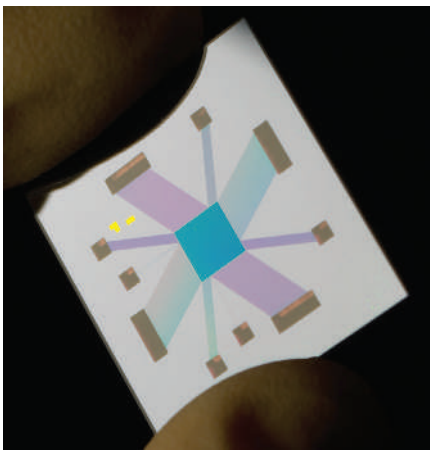

Figure 1 A microfabricated nanofluidic device in which an anisotropic nanofilter array (centre) is connected to injection and collection channels for analysing samples.

On page 121 of this issue, Jongyoon Han and co-workers[1] from the Massachusetts Institute of Technology report a wonderful example of the actual implementation of this idea. They etch a silicon wafer to produce a two-dimensional periodic array consisting of two sets of orthogonal channels, one set being much deeper (300 nm) than the other (55 nm). Driven through this filter (Fig. 1) by two perpendicular electric fields, the molecules travel along the deep channels, occasionally hopping to an adjacent channel through a connecting narrow passage (Fig. 2). A convenient analogy for this process can be found in one of the sayings of Yogi Berra — baseball great and latter-day philosopher — who, when giving directions to his house, would say, "when you get to a fork in the road, take it."

Imagine a straight road — tilted to one side by the land it crosses — connected to narrower roads that fork off to either side. As you travel down the main road the tilt of the land forces you to move at an angle, pushing you towards the side roads along one edge. Going straight down the main road won't get you to your destination, but going down the narrow roads is slow. At each fork that you come to you have to make the decision to continue straight on — but not towards your goal — or take the fork and travel towards your goal, but slowly. How well you move on the narrow roads will influence your choices. In the nanofilter array, the final destination of a molecule will vary depending on how many turns it takes from the main road and so different molecules can be separated accordingly.

The operating principles of Han's filter builds on work of Harold Craighead's group in Cornell on entropic trapping[2], which is the origin of our slow road analogy. In this earlier research, it was observed that when floppy biological polymers (such as DNA) moved from open to confined spaces the confining structure made the floppiness less likely: we say it squeezed out the disorder, or the entropy. Nature doesn't like to lose disorder (entropy) and so these nanoslits gave rise to an entropic force that altered the mobility of the polymer as a function of its length in a predictable way. However, this variation in mobility — which is a function of molecular size — was only used for separations performed in a batch process. The approach used by Han and co-workers incorporates a skewed symmetry[3,4] to the entropic trap, providing options for moving straight through an array or sideways through narrow spaces.

In order for this scheme to work, the forces pushing the objects down the straight road and the side roads have to be absolutely uniform[5] over a large area, otherwise the objects will follow a curved, rather than straight, path. By ensuring that the forces driving the particles do not change with their position in the array, the total velocity of the objects is a function of the relative mobility along two orthogonal axes. Because it is easier to move along a deep channel than a

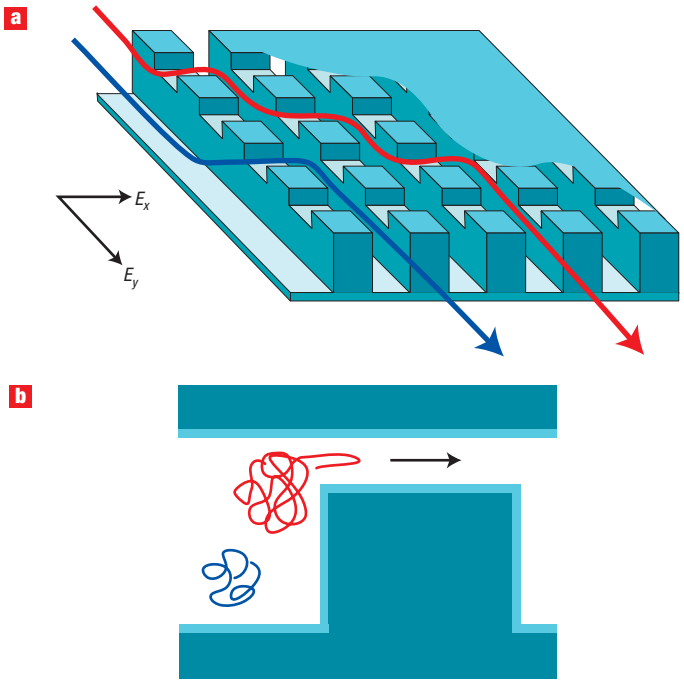

Figure 2 The periodic nanofilter array. **a**, As molecules are driven through the array by the orthogonal electric fields (E_x and E_y) they take different paths depending on how easily they can pass from one deep channel to the next through the narrow channels. In this example, the two paths shown correspond with molecules that have, in relative terms, high (red arrow) and low (blue arrow) probabilities of passing through the narrow gaps. **b**, A cross-section view showing how molecules move from one deep channel to the next through a narrow passage. In this example of an entropic trap, the longer molecule (red) has a greater rate of passage through the constriction than the shorter one (blue) because it has a greater probability of deforming its shape to fit through the gap.

narrower one, the molecules move at angles to the applied field and these deflections are a function of molecular size. This is a key concept: since the molecules fan out as they move, it is possible to run this device in a steady-state mode!

To continue our analogy on how to get to Yogi Berra's house, the side roads can be in various forms of bad shape. They can simply be narrow, and force floppy molecules (like DNA) to be confined. This is the entropic trap. They can be sticky, like a muddy road. This is electrostatic sieving, and charges on the surface of the array walls can be used to sort molecules based on charge. They can be full of potholes that you have to swerve around and this is what we call Ogston sieving, where molecules can be sorted based on their size. Depending on the molecule that one wishes to sort, the side roads can be made to behave in different ways. Because of the rich variety of side roads that can be constructed using nanofabrication techniques, the process described by Han and colleagues can be used to separate a vastly wider range of materials than previous techniques, including proteins.

There is significant potential for further development of this work. In particular, clogging could be reduced dramatically by modifying Han's array to make the size of the asymmetric structures a function of their position on the nanopatterned surface. For example, you could begin with a very coarse asymmetric structure and as you proceed down the flow direction it would only be the smaller objects that are subsequently deflected into ever finer-patterned surfaces. This is a powerful idea.

Although the physics of this concept will be a challenge to develop for all of the objects studied, it is clear that the ideas of broken symmetry, homogenous two-dimensional flow patterns, and generalized entropic forces have been combined to develop a qualitatively new way to separate biological objects over a huge size range in a steady-state manner by fanning them out in space and in time.

References

1. Fu, J. *et al. Nature Nanotech.* **2,** 121–128 (2007).
2. Han, J. & Craighead, H. G. *Science* **288,** 1026–1029 (2000).
3. Duke, T. A. J. & Austin, R. H. *Phys. Rev. Lett.* **80,** 1552–1555 (1998).
4. Huang, L. R. *et al. Nature Biotechnol.* **20,** 1048–1051 (2002).
5. Huang, L. R. *et al. Tech. Dig. Int. Elect. Dev. Mtg.* 363–366 (2001).

NANOLITHOGRAPHY

Going round in circles

With their unique mechanical and electronic properties, carbon nanotubes are appealing building blocks for the construction of nanoscale devices. Now, in an Olympic feat of nanoengineering, Chad Mirkin, George Schatz and co-workers from Northwestern University in the USA have demonstrated how carbon nanotubes can be bent into circular structures, as shown in this image, using a patterned surface as a template (*Nano Lett.* doi: 10.1021/nl062258e; 2007). Bending nanotubes is of particular interest because it allows their unusual curvature-dependent properties to be studied.

Dip-pen nanolithography was used to coat a gold surface with a monolayer comprising circular islands of hydrophilic molecules surrounded by a sea of hydrophobic ones. When the nanotubes were placed on this surface, they assembled along the boundaries between the hydrophobic and hydrophilic regions, forming circles with diameters as small as 100 nm (although the rings in this image are several micrometres across). The formation process is controlled by two opposing forces — the strain energy involved in bending a nanotube and the van der Waals forces between the nanotube and the molecules on the surface. Although nanotube rings have been made previously, this approach can be used to pattern circles of defined diameters on a surface.

Stuart Cantrill