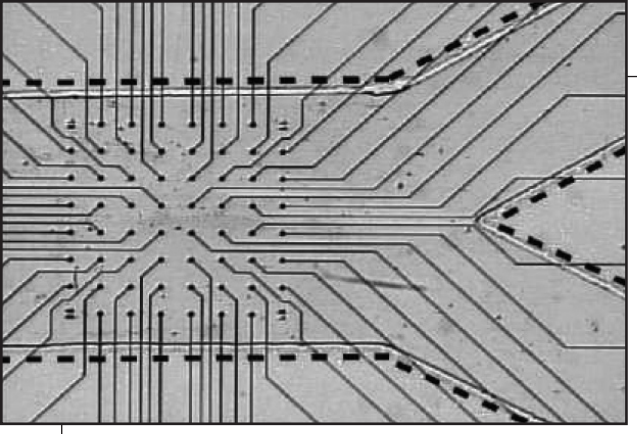


"Whether by aiding the poor or accelerating development of therapies, microanalysis systems seem poised to change quality of life across the globe."

Microfluidics:

New Channels for Biological Research

By Michelle Siao

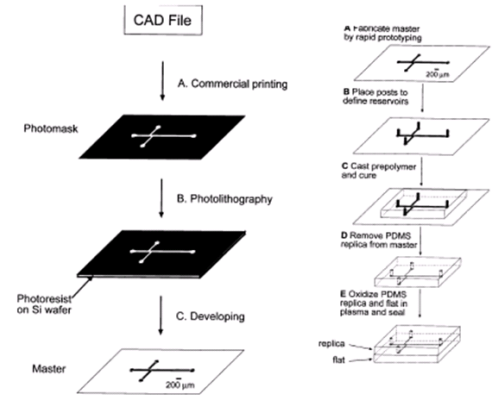
The new chip that is advancing scientific research can be less than a square inch in area. Etched upon its surface are any number and configuration of pathways just 10 to 100 millionths of a meter wide. But it's not a computer chip.

An innovation in a field that arose only in the 1990s, the "lab on a chip" is a microfluidics device whose tiny channels and compartments are providing new approaches to cellular and molecular biology. Whether it features pockets for biochemical analysis or single-lane alleys of cell growth, this microanalysis system is allowing researchers to address basic questions with clarity, speed, and cost-efficiency. Though such devices have not yet been integrated into our homes and offices, they may be on their way. And, already, the answers they have provided are putting healthcare advances on the horizon for both the developed and developing worlds.

How Cells Grow With the Laminar Flow

Microfluidics is the study or technological implementation of small amounts of liquids, perhaps only 10^{-9} to 10^{-18} liters, that flow through channels 10 to 100 μm wide (Table 1) (1). The first series of analytical devices were based on designs straight out of microelectronics, so perhaps it is not surprising that they were made of silicon and glass; however, these surfaces are expensive, fragile, and impermeable to gases like oxygen necessary for many biological studies (1).

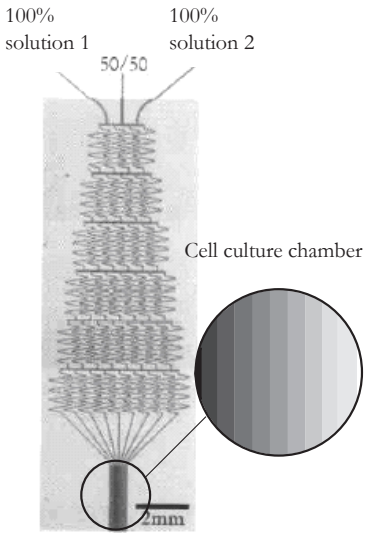
To avoid these problems, researchers began to implement elastomers—rubbery substances with crosslinking molecules—particularly one called poly(dimethylsiloxane) (PDMS). Because this material is soft, cheap, breathable, and low in toxicity, it is perfect for developing high throughput systems of analysis, including those involving cell cultures. PDMS devices can not only be produced and copied quickly, but can also be custom-made to almost any shape through a combination of photolithography and replica molding (Box 1) (2). Accessories like pumps and valves may be added at leisure. Unlike silicon, PDMS is transparent and can thus undergo microscopy or optical scanning. Still, some experiments call for rigid channel walls or chemicals that would dissolve PDMS, so silicon and glass microsystems, as well as plastic ones, are still useful platforms (3). Like their applications, the design and mechanisms of chips are varied, demonstrating the creativity of their makers and the adaptability of the technology.



credit: figures based on McDonald *et al*, 2000

Box 1. PDMS: poly(dimethylsiloxane) designed, molded, and sealed

Making a microfluidics device can be performed through a combination of photolithography and replica molding. Lithography refers to a means of patterning; in the case of photolithography, this patterning is created by light. First, the desired design is created through a computer program, and then printed out on a high-resolution transparency. The printed image can then be placed above a silicon wafer, and block areas from light exposure. When the wafer, layered with a photosensitive chemical, is developed, the blocked areas are washed away, while the light exposed areas are raised. This is the "master" print against which PDMS can be shaped.



credit: Whitesides and Stroock, 2001

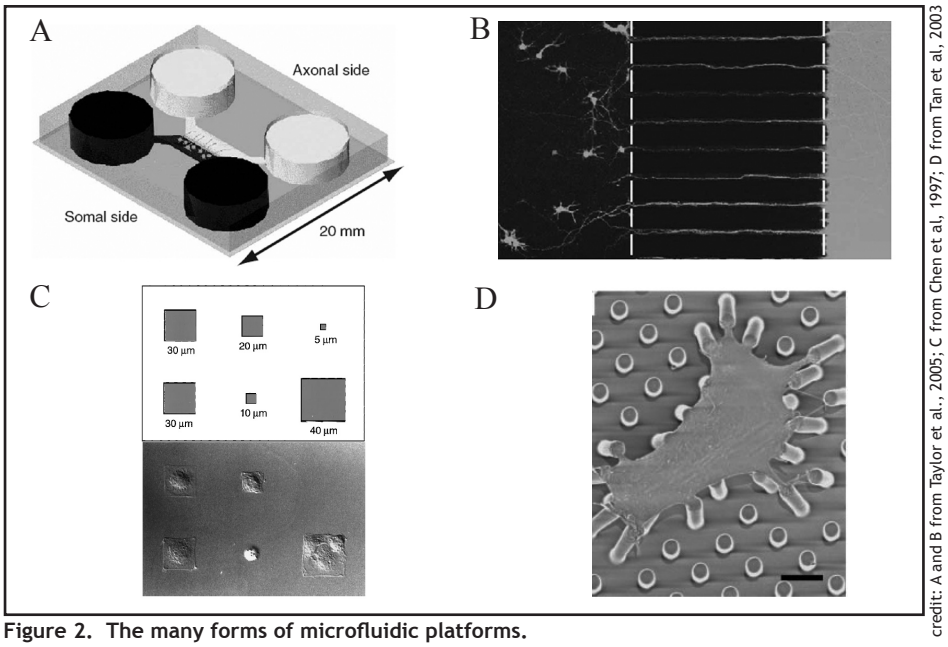
Figure 1. Creating a concentration gradient. Exhibiting the property of laminar flow, minute streams run side by side such that molecules slowly diffuse from one stream to another. Thus, from a few solutions, microchips can generate several streams with varying intermediate concentrations.

Microfluidic devices take advantage of the fact that liquids behave differently when in small volumes than they do in large quantities. In the latter scenario, mixing fluids swirl and eddy. But when minute streams meet, they flow side by side, such that mixing of solutions involves only diffusion of molecules between the streams. This property, called laminar flow, allows microchannels to establish concentration gradients in which the amount of a dissolved chemical progressively increases or decreases (Figure 1) (4). Whether this chemical is a hormone or a drug, exposing cells to these gradients can shed light on how cells send and receive signals, or on how a substance might help or sicken a cell. One recent study, for example, has used microchannels to demonstrate how neural stem cell cultures react to different concentrations of a growth-promoting factor, or protein (5). As the concentration increased, so did cell proliferation, while differentiation into a specific cell type decreased. Other studies have observed cancerous activity in gradients, such as the movement of leukemia cells towards the chemical CXCL8, or of breast cancer cells towards a protein called epidermal growth factor (6,7). More detailed understanding of biochemical interactions, as revealed by these and similar tests, will support the development of medical treatments.

Another advantage of the microchip is its ability to direct growth of cell parts into chemically isolated environments. One novel platform featured two chambers connected by a series of channels with high fluidic resistance (Figure 2a and 2b).8 The maintenance of more volume in one chamber provided a force opposing molecular diffusion between the solutions in each compartment. Just 3 μm in width, the channels were only large enough to allow outgrowth of axons, the long thin branch that extends from neurons to send outgoing signals. Only when an axon grew from one chamber and reached the dye in the other did the rest of the cell fluoresce. The separation of microenvironments allowed the group to cut the axons in the second chamber, then observe gene expression changes, regeneration, and the effect of soluble factors to aid regeneration. Thus, future experiments could study not just uptake of dye, but also uptake of genes or drugs for therapeutic purposes such as treatment of spinal cord injury.

Seeking patterns of growth on patterned surfaces

Because PDMS is so easily manipulated, its form is not restricted to channels. Growth-permissive islands can be patterned to almost any shape, from circles, squares, and teardrops, to smiley faces and block letters (Figure 2c) (9). All fancy aside, the mechanical interactions between a cell and its environment affect its internal organization, development, and life-or-death decisions. Thus, observations of single cells or small populations of them are essential to understanding how they function and how we might later manipulate them. Two types of cell adhering substances have been used in recent studies. The first is extracellular matrix (ECM), the body's network of proteins and other molecules that supports cell growth. The second, fibronectin, is a glycopro-



credit: A and B from Taylor et al., 2005; C from Chen et al., 1997; D from Tan et al, 2003

Figure 2. The many forms of microfluidic platforms. Microfluidics devices can be designed to take on a variety of shapes depending on the needs of a given cell culture. (A) shows the design of a chip that demonstrates the separation of neural cell bodies from axons, branches that send outgoing signals to other cells. (B) Entire cells fluoresce when axons extend through microchannels from the cell body chamber to the compartment containing dye from entering the alternate chamber. In (C), square growth islands have been coated with fibronectin, to which cells adhere and take on different appearances depending on island size. (D) demonstrates the use of fibronectin-treated PDMS posts as a culture platform. The force applied by a growing muscle cell can be quantified according to inflection of a given post.

Table 1. Scaling down: a key to the notation for small units

prefix	abbreviation	value
milli-	m	one thousandth (10^{-3})
micro-	u	one millionth (10^{-6})
nano-	n	one billionth (10^{-9})

tein, or a sugar-protein hybrid molecule, that binds to cell membranes and also ECM. By using custom-cut PDMS as a "stamp" and these substances as the "ink," scientists can pattern glass coverslips to restrict cell growth to imprinted areas. This technology has shed light on how the ECM controls the cytoskeleton, the framework that supports cell shape and internal trafficking of molecules, and how communication between the two determines the orientation of cell division (10). PDMS can also be used for cell culture instead of glass. One microsystem with an array of fibronectin-treated PDMS posts measured the forces exerted by a muscle cell that bent the columns as it grew (Figure 2D) (11).

This technology has already begun to expand to development of three-dimensional PDMS scaffolds (12,13). Because cells in the body indeed interact with neighbors beyond a horizontal plane, 3D models and integrated networks may more accurately relate chemical communication and mechanical stress among cells, and hopefully lead to the engineering of tissues.

Packaging the Improved Lab

Cell culture is just one of many functions that microchips can serve. Used in concert with myriad accessories, ranging from valves to temperature controls and electrodes, chips can be customized to execute entire lab methods. For example, the steps of a polymerase chain reaction (PCR) and electrophoresis can be performed in a series of compartments to test for genetic markers (Figure 3) (14). Detected and quantified in chips, target molecules can be relayed to and further examined in linked devices such as mass spectrometers, which can identify and characterize proteins (15). The ability to control small volumes greatly eases isolation of individual cells for lysis and analysis of constituent enzymes or messenger RNA, the nucleic acid strands necessary for expressing DNA sequences on which their codes are based (16,17). Additionally, single-cell capture can be advantageously integrated with patch-clamp techniques, which monitor the voltage changes in individual neurons (18). In general, miniaturizing these and other methods will promote efficiency and reproducibility of assays that may elucidate molecular and cellular mechanisms.

Such technological potential is the inspiration behind research that is directing chips towards use for the home and for civilian safety. In the future, chips developed to quantify cholesterol levels or detect influenza viral strains might be sold as home-testing devices (19,20). Based on the interaction of an enzyme with fluorescently labeled DNA, one biosensor could be marketed as a lead detector (21). Another chip can capture a forensic sample's epithelial, or membranous lining, cells in a microchannel, through which only sperm would flow unbound to a collection chamber; this simple separation could greatly increase the effectiveness and turnover rate of subsequent DNA analysis for sexual assault cases (22).

The concept of using microfluidic devices for security purposes is not new. In fact, it was funding from the US Defense Advanced Research Projects Agency that catalyzed development of the young technology through the 1990s (1). In recent years, relevance to homeland security has not been ignored. One prototype is capable of detecting micromolar concentrations of several nerve agents in less than four minutes (23). Chips could also play a critical role in detecting the presence of, not just lead, but weaponized pathogens. In general, however, use of microdevices by the military would fulfill the need for various highly portable medical tests where time and resources are limited.

Yet the US government, the Gates Foundation, other international donors, and certainly many scientists recognize that these powerful but easy-to-use devices could have an even wider impact in developing countries. In these areas, where rampant infectious disease is a common cause of death and clean water is often unavailable or rare, microchips

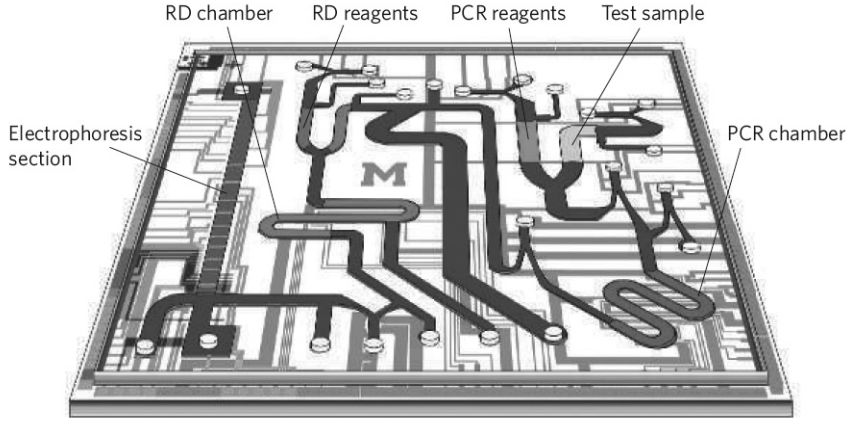


Figure 3. Lab on a chip: A genetic test from start to finish.
Shown above is a schematic diagram of a chip that performs a polymerase chain reaction (PCR) and electrophoresis within minutes. A test sample exposed to PCR reagents and is reacted to amplify, or make multiple copies of, the few original DNA or RNA fragments present. From there, the sample undergoes restriction enzyme digestion (RD) that cuts up fragments further. When subject to an electric current, the fragments travel through molecules in the electrophoresis channel. Short nucleic acid strands less hindered by channel molecules can move farther down, whereas long bulky strands remain farther up. The relative position of bands indicates the presence or absence of a target gene in the sample. This test could be used domestically to speed up lab procedures, or in developing countries to diagnose dangerous infections.

would greatly improve effectiveness of patient treatment and attempts to control the spread of maladies. Simple strip tests for several pathogens such as gonorrhea and syphilis are proving useful in this regard; however, strips are not available for enough diseases, and in the case of P. falciparum strain malaria tests, previous infection or exposure to benign malarial molecules can sometimes cause ambiguous results (3). Genetic kits are available for tuberculosis and HIV. These tests, however, may require substantial training of technicians or refrigeration, which is impractical if electricity is unavailable or inconstant (3).

Customizing chips could make up for these deficiencies and more. In addition to performing currently unavailable tests, portable chip readers with disposable sample inserts could be automated to perform complicated tasks while giving a clear result, easily read by technicians with minimal training (3). While some standard cultures and protein assays can take days to verify infectious diarrhea, micro scale tests could be completed within minutes, allowing patients to be diagnosed and appropriately treated during a single visit (24,3). Chips would need to contain non-perishable reagents within a hardy disposable, so improvements beyond brittle glass and silicon systems for PCR genetic analysis will be necessary. Cost of production has yet to be reduced to pennies for successful mass distribution. But developments in diagnostic platforms look promising so far. Severe acute respiratory syndrome coronavirus (SARS-CoV) tests on microchips are faster and have higher positive rates when compared to conventional methods (25). Artificial human papilloma virus 16 sequences, which are involved in the development of cervical cancer, can be detected at low micromolar concentrations (26). And the spectrum of other detectable pathogens encompasses influenza strains, HIV-1, dengue, tetanus, syphilis, and hepatitis C (20,27-30). These chips would not be cures in themselves, but would enhance delivery of available treatments and help healthcare workers monitor the spread.

The biological applications of microfluidics only scratch the surface of the technology's potential. Other avenues of research are using chips to study or enhance chemical reactions, create new optical systems, and solve mathematical problems (1,31,33). How soon and how successfully these devices will be commercialized and widely used by the general public remains to be seen. Nevertheless, whether by aiding the poor or accelerating development of therapies, microanalysis systems seem poised to change quality of life across the globe, and it will be interesting to watch how they develop in academic laboratories and beyond. H

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References:

1. Whitesides GM. The origins and the future of microfluidics. *Nature* 442: 368-73. 2006.
2. McDonald JC, Duffy DC, Anderson JR, Chiu DT, Wu H, Schueller OJ, Whitesides GM. Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* 21(1): 27-40. 2000.
3. Yager P, Edwards Y, Fu E, Helton K, Nelson K, Tam MR, Weigel BH. Microfluidic diagnostic technologies for global public health. *Nature* 442(7101): 412-8. 2006.
4. Whitesides GM and Stroock AD. Flexible methods for microfluidics. *Phys Today* 54(6): 42. 2001.
5. Chung BG, Flanagan, Rhee SW, Schwartz PH, Lee AP, Monuki ES, Jeon NL. Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. *Lab Chip* 5: 401-6. 2005.
6. Walker GM, Sai J, Richmond A, Stremler M, Chung CY, Wikswo JP. Effects of flow and diffusion on chemotaxis studies in a microfabricated gradient generator. *Lab Chip* 5(6): 611-8. 2005.
7. Wang SJ, Saadi W, Lin F, Nguyen CMC, Jeon NL. Differential effects of EGF gradient profiles on MDA-MB-231 breast cancer cell chemotaxis. *Exp Cell Res* 300: 180-89. 2004.
8. Taylor AM, Blurton-Jones M, Rhee SW, Cribbs DH, Cotman CW, Jeon NL. A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nat Methods* 2(8): 599-605. 2005.
9. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. *Science* 276(5317): 1425-8. 1997.
10. Thery M, Racine V, Pepin A, Piel M, Chen Y, Sibarita JB, Bornens M. The extracellular matrix guides the orientation of the cell division axis. *Nat Cell Biol* 7(10): 947-53. 2005.
11. Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc Natl Acad Sci USA* 100(4): 1484-9. 2003.
12. Powers MJ, Domansky K, Kaazempur-Mofrad MR, Kalezi A, Capitani A, Upadhyaya A, Kurzawski P, Wack KE, Stolz DB, Kamm R, Griffith LG. A microfabricated array bioreactor for perfused 3D liver culture. *Biotechnol Bioeng* 78: 257-69. 2002.
13. Leclerc E, David B, Griscom L, Lepioufle B, Fujii T, Layrolle P, Legallaisa C. Study of osteoblastic cells in a microfluidic environment. *Biomaterials* 27: 586-95. 2006.
14. El-Ali J, Sorger PK, Jensen KF. Cells on chips. *Nature* 442: 403-11. 2006.
15. Lion N, Rohner TC, Dayon L, Arnaud IL, Damoc E, Youhnovski N, Roussel C, Josserand J, Jensen H, Rossier JS, Przybylski M, Girault HH. *Electrophoresis* 24(21): 3533-62. 2003.
16. He MY, Edgar JS, Jeffries GDM, Lorenz RM, Shelby J, Chiu DT. Selective encapsulation of single cells and subcellular organelles in to picoliter and femtoliter-volume droplets. *Anal Chem* 77(6): 1539-44. 2005.
17. Hong JW, Studer V, Hang G, Anderson WF, Quake SR. A nanoliter-scale nucleic acid processor with parallel architecture. *Nat Biotech* 22(4): 435-9. 2004.
18. Seo J, Ionescu-Zanetti C, Diamond J, Lal R, Lee LP. Integrated multiple patch-clamp array chip via lateral cell trapping junctions. *Appl Phys* Lett 84: 1973-5. 2004.
19. Kim L, Vahey MD, Lee HY, Voldman J. Microfluidic arrays for logarithmically perfused embryonic stem cell culture. *Lab Chip* 6: 394-406. 2006.
20. Pal R, Yang M, Lin R, Johnson BN, Srivastava N, Razzacki SZ, Chomistek KJ, Heldsinger DC, Haque RM, Ugaz VM, Thwar PK, Chen Z, Alfano K, Yim MB, Krishnan M, Fuller AO, Larson RG, Burke DT, Burns MA. An integrated microfluidic device for influenza and other genetic analyses. *Lab Chip* 5(10): 1024-32. 2005.
21. Chang IH, Tulock JJ, Liu J, Kim WS, Cannon DM JR, Lu Y, Bohn PW, Sweedler JV, Cropek DM. Miniaturized lead sensor based on lead-specific DNAzyme in a nanocapillary interconnected microfluidic device. *Environ Sci Technol* 39(10): 3756-61. 2005.
22. Horsman KM, Barker SL, Ferrance JP, Forrest KA< Koen KA, Landers JP. Separation of sperm and epithelial cells in a microfabricated device: potential applications to forensic analysis of sexual assault evidence. *Anal Chem* 77(3): 742-9. 2005.
23. Wang J, Zima J, Lawrence NS, Chatrathi mP, Mulchandani A, Collins GE. Microchip capillary electrophoresis with electrochemical detection of thiol-containing degradation products of V-type nerve agents. *Anal Chem* 76(16): 4721-6. 2004.
24. Guerrant RL, Van Gilder T, Steiner TS, Thielman NM, Slutsker L, Tauxe RV, Hennessy T, Griffin PM, DuPont H, Sack RB, Tarr P, Neill M, Nachamkin I, Reller LB, Osterholm MT, Bennish ML, Pickering LK, Infectious Diseases Society of America. *Clin Infect Dis* 32(3): 331-51. 2001.
25. Zhou X, Liu D, Zhong R, Dai Z, Wu D, Wang H, Du Y, Xia Z, Zhang L, Mei X, Lin B. Determination of SARS-coronavirus by a microfluidic chip system. *Electrophoresis* 25(17): 3032-9. 2004.
26. Gulliksen A, Solli LA, Drese KS, Sorensen O, Karlsen F, Rogne H, Hovig E, Sirevag R. Parallel nanoliter detection of cancer markers using polymer microchips. *Lab Chip* 5(4): 416-20. 2005.
27. Sia SK, Linder V, Parviz BA, Siegel A, Whitesides GM. An integrated approach to a portable and low-cost immunoassay for resource-poor settings. *Angew Chem Int Ed Engl*. 43(4): 498-502. 2004.
28. Zaytseva NV, Montagna RA, Baeumner AJ. Microfluidic biosensor for the serotype-specific detection of dengue virus RNA. *Anal Chem* 77(23): 7520-7. 2005.
29. Herr AE, Throckmonrton DJ, Davenprot AA, Singh AK. On-chip native gel-electrophoresis-based immunoassays for tetanus antibody and toxin. *Anal Chem* 77(2): 585-90. 2005.
30. Wang CH and Lee GB. Automatic bio-sampling chips integrated with micro-pumps and micro-valves for disease detection. *Biosens Bioelectron* 21(3): 418-25. 2005.
31. Vezenov DV, Mayers BT, Conroy RS, Whitesides GM, Snee PT, Chan Y, Nocera DG, Bawendi MG. *J Am Chem Soc* 127(25): 8952-3. 2005.
32. Chiu DT, Pezzoli E, Wu H, Stroock AD, Whitesides GM. Using three-dimensional microfluidic networds for solving computationally hard problems. *Proc Natl Acad Sci USA* 98(6): 2961-6. 2001.