Microfluidic Large-Scale Integration: The Evolution of Design Rules for Biological Automation

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Annu. Rev. Biophys. Biomol. Struct. 2007. 36:213–31

First published online as a Review in Advance on February 1, 2007

The *Annual Review of Biophysics and Biomolecular Structure* is online at biophys.annualreviews.org

This article's doi: 10.1146/annurev.biophys.36.040306.132646

1056-8700/07/0609-0213$20.00

Key Words

soft lithography, biological automation, polydimethylsiloxane

Abstract

Microfluidic large-scale integration (mLSI) refers to the development of microfluidic chips with thousands of integrated micromechanical valves and control components. This technology is utilized in many areas of biology and chemistry and is a candidate to replace today's conventional automation paradigm, which consists of fluid-handling robots. We review the basic development of mLSI and then discuss design principles of mLSI to assess the capabilities and limitations of the current state of the art and to facilitate the application of mLSI to areas of biology. Many design and practical issues, including economies of scale, parallelization strategies, multiplexing, and multistep biochemical processing, are discussed. Several microfluidic components used as building blocks to create effective, complex, and highly integrated microfluidic networks are also highlighted.

Contents

INTRODUCTION

Microfluidics: the study and manipulation of fluidic behavior on the nanoliter scale and below

Microfluidics refers to the science and technology of systems that manipulate small amounts of fluids, generally on the nanoliter scale and below. Numerous applications of microfluidics have been developed in chemistry, biology, and other fields (6, 7, 37, 49). Many clever technological inventions have been developed to control fluid behavior in small channels, often taking advantage of the physical properties of the fluid, the channels, and the contents of the fluid (37). In this review, we focus on the development of a particular microfluidic technology that has made enormous strides in the effort to automate biology: microfluidic large-scale integration (mLSI).

mLSI refers to the development of microfluidic chips with hundreds to thousands of integrated micromechanical valves. This technology enables hundreds of assays to be performed in parallel with multiple reagents in an automated manner and has been used in applications such as protein crystallography (12–14), genetic analysis (26), amino acid analysis (36), high-throughput screening (42), bioreactors (10), chemical synthesis (24, 47), and single cell analysis (30). It is a candidate to replace today's conventional biological automation paradigm, which consists of fluid-handling robots.

Although mLSI is not the only microfluidic approach used in these areas, using micromechanical valves gives one the ability to create highly complex, integrated designs without accounting for the detailed properties of the fluids that one is manipulating. Just as the development of digital electronics enabled ever more complex microprocessor designs, mLSI allows one to treat microfluidics as a design problem in which components of various complexity are stitched together in a seamless and transparent fashion.

In electronic LSI, many distinct functional subcomponents such as memory, comparators, counters, multiplexers, and so on are integrated on a single chip to perform application-specific or generic tasks. These higher-level components are assembled from transistors, and in digital electronics the transfer of information in these components is based on the 0 and 1 logic states. Each subcomponent in LSI is well characterized, and a given input produces a reliable and repeatable output. In this way, subcomponents can be treated essentially as black boxes when designing LSI circuits with a limited number of user-defined parameters (i.e., number

of inputs for a multiplexer, step resolution for a counter, threshold voltage for a comparator, etc.). This enables modular design and the use of automated software design tools.

As tasks and experiments to be performed by microfluidics become more and more complex and with hundreds or thousands of components housed on a single chip, digital microfluidics enables a similar strategy. However, until recently, focus has been limited to optimizing individual microfluidic components that are often not easily integrated with each other, and microfluidic systems have been designed using a bottom-up approach. A top-down approach simplifies the design of integrated microfluidic systems on a chip by providing a library of microfluidic components, similar to a cell library in LSI design. This would also allow automated software synthesis tools for both microfluidic architecture-level synthesis (i.e., scheduling and resource binding) and geometry-level synthesis (i.e., routing and component placement) (40). The development of explicit design rules and strategies allowing modular top-down design methodology is an exciting frontier in microfluidic system design.

We review the basic development of mLSI and then discuss design principles of mLSI to assess the capabilities and limitations of the current state of the art and to facilitate the application of mLSI to other areas of biology. Many design and practical issues are discussed, such as economies of scale, parallelization strategies, multiplexing, multistep biochemical processing, and metering strategies. Several microfluidic components that can be used as building blocks to create effective, complex, and highly integrated microfluidic networks are also highlighted. Our goal is to go beyond the details of device fabrication and application already discussed in the published literature and to discuss some of the general design rules that have evolved.

THE MICROMECHANICAL VALVE

The valve is the basic unit of fluid-handling functionality and plays a role analogous to that of the transistor in semiconductor electronics. Early micromechanical valves with moving parts were developed using silicon microelectromechanical systems (MEMS) technology, but were challenging to fabricate and were not incorporated into highly integrated devices because of their complexity (23). They were succeeded by a series of devices that included a silicone rubber membrane integrated onto silicon/glass substrates with chemically etched channel and pressure ports, and were activated either pneumatically or thermopneumatically (3, 31, 34, 35, 45, 51). More recently, the techniques of soft lithography (50) have been used to make monolithic valves from polydimethylsiloxane (PDMS) (43). This advance has led to a number of other valve designs (1, 9, 17), including a seat valve in which the seat is integrated into the PDMS diaphragm by a molded extension, allowing contact with a planar substrate surface (20). Another polymer valve includes a Parylene/PDMS double-layer diaphragm resistant to aggressive chemicals (which otherwise degrade PDMS) (18). Other valves include a single-layer PDMS microchannel device in which valving is achieved by compressing the channel via external pressure at specific points along the microchannel by using Braille pins (10). Weibel et al. (48) have also developed torque-actuated valves in which screws are embedded into the single-layer PDMS device and are manually adjusted to close valves on-chip.

The first mLSI was realized using monolithic membrane valves made with multilayer soft lithography (MSL). Early devices included a microfluidic memory that used 3574 valves integrated on a single chip, as well as a comparator array with 2056 valves (42). These were followed quickly by a number of other devices made with the same fabrication paradigm with diverse applications in biology and chemistry (2, 12–16, 24, 26, 30). If the

Large-scale integration: the ability to integrate hundreds to thousands of functional components on a single device to execute complex tasks

mLSI: microfluidic large-scale integration

High-throughput screening: the ability to screen a sample (or reaction) in a large number of conditions in a highly parallel and efficient manner

MEMS: microelectromechanical systems

PDMS: polydimethylsiloxane

MSL: multilayer soft lithography

definition of mLSI is expanded to include devices with at least 40 valves, then a number of other basic valve technologies have been used to create a large degree of microfluidic integration. These include a digital microfluidic circuit with mixer and storage cells (~40 integrated valves on-chip) (44), a bioreactor with elastomeric valves actuated by a Braille writer (~50 valves) (10), a capillary electrophoresis device for amino acid analysis (~60 valves) (36), and a latching pneumatic valve demultiplexer (~70 valves) (8). A microreactor platform (~100 valves) (18) has also recently been shown. The principles of mLSI do not necessarily depend on the particular type of valve used, and the majority of this review discusses higher-level structures and design strategies that can be used with many different valve types.

Push-Up versus Push-Down Valves Using Soft Lithography

The most densely packed microfluidic valve networks and complex on-chip integrated functionality have been achieved with MSL. The fundamental building block for this platform, the monolithic micromechanical valve shown in **Figure 1*a***, is produced by replica molding from two masters and sealing the layers together (32, 43). Two separate molds are produced: one for the channels and one for the flow channels. A membrane is formed where the control channel and flow channel intersect orthogonally. The flow-channel must have a rounded profile to enable the valve to close completely during actuation. If the control channel layer is bonded on top of the flow-channel layer, push-down valves are formed. The geometry (width, height, and thickness) of the membrane determines the valve actuation pressure, and the valve experiences little hysteresis. Actuation pressures for corresponding valve dimensions and mechanical properties of the valves useful in microfluidic design are discussed in References 21 and 39. Holes can be punched in PDMS to access the control and flow channels independently. The push-down valve allows one to combine liquid control with a DNA array or custom-functionalized surface or device.

Push-up valves are better suited for taller flow channels in applications, including eukaryotic cell manipulation (see **Figure 1*b***). The uniform membrane thickness of this valve enables lower actuation pressures than one finds with push-down valves of similar dimensions. By combining push-up and push-down valves in a single device, as shown in **Figure 1*c***, a high density of valves can be achieved while simplifying channel routing.

For both types of valves, aspect ratios not exceeding 1:10 reduce membrane collapses during fabrication; flow-layer support pillars can be incorporated if channels greater than 1:10 are desired. The porosity of PDMS presents certain design advantages such as sophisticated device priming and the ability to manipulate the environmental equilibrium (11, 46). Monolithic membrane valves compatible with organic solvents can also be made from photocurable perfluoropolyether (33, 19). Further information on PDMS material properties and biocompatibility can be found in References 24a, 25, 28, and 50.

HIGHER-LEVEL COMPONENTS: PUMPS, MIXING, AND METERING

Peristaltic Pump

A linear array of valves actuated in sequence can be used to create a pump. Peristaltic pumping occurs when three membrane valves, shown in **Figure 1*d***, are actuated in the pattern 101, 100, 110, 010, 011, 001, where 0 and 1 represent open and closed valves, respectively. A maximum pumping rate of 2 cm s^{-1} was reported at 100 Hz (14). In this case, the control channels were filled with air, and the valve actuation frequency was limited only by the maximum frequency of the off-chip solenoid control valves. On-chip peristaltic pumping provides the ability to control

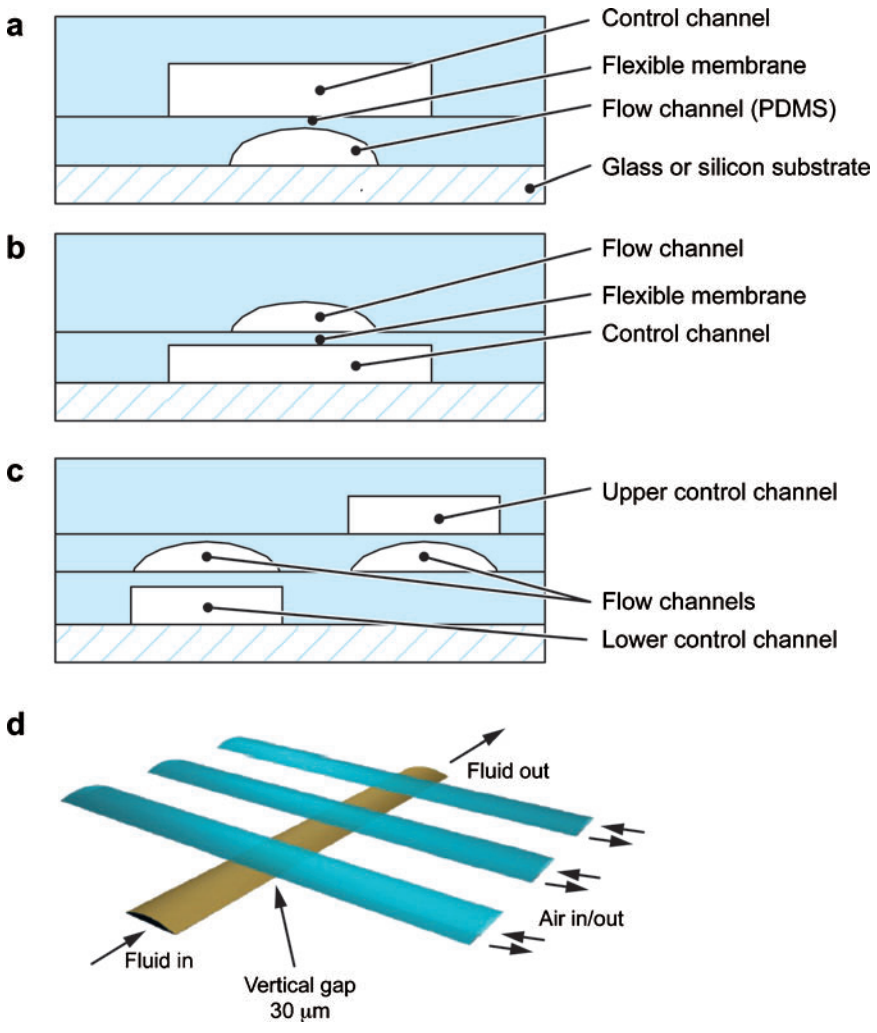

Figure 1

(*a*) A two-layer polydimethylsiloxane (PDMS) push-down microfluidic valve. An elastomeric membrane is formed where the flow channel is positioned orthogonal to the control channel directly above. Fluid flow is out of the page. (*b*) A two-layer PDMS push-up microfluidic valve where a control channel lies orthogonal to and below the flow channel. (*c*) A three-layer device with both push-up and push-down valves. (*d*) Schematic of a linear peristaltic pump using three membrane valves in a series (43).

fluid flows without the need for precisely regulated external pressure sources.

Mixing

One of the fundamental requirements in biological automation is to mix reagents efficiently. A number of clever schemes for continuous-flow mixing have been developed, including chaotic advection in a three-dimensional herringbone-shaped microchannel (38), spiral mixers (4), and the use of hydrodynamic focusing (22). These continuous-flow mixers can often be adapted to batch processing in mLSI by simply adding peristaltic pumps in a closed-loop geometry with the mixers (27). It is also possible to create a simple yet powerful batch mixer by combining a rotary geometry with a peristaltic pump (5) (**Figure 2*a*,*b***). Once reagents are loaded into the device, the loop is sealed and the peristaltic pump is activated. Liquid in the central part of the flow channels travels faster than liquid close to the channel walls. This results in rapid stretching and an increase of the interface between the two reagents and, consequently, a shorter diffusion distance for mixing. Mixing times can be reduced to a matter of seconds compared to several hours for passive diffusion. The rotary pump is a

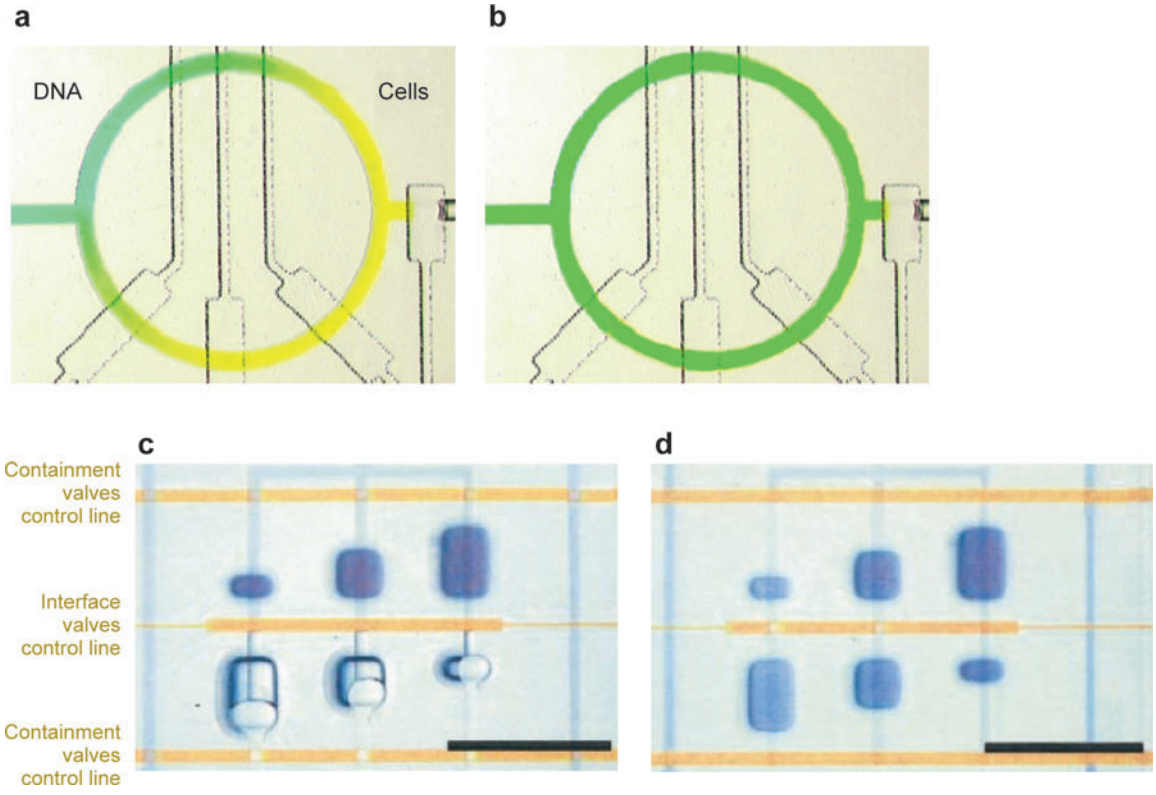

Figure 2

(*a*) Rotary micromixer, where two colored liquids (*aqua*, DNA; *yellow*, cells) are loaded into the mixing loop and completely mixed after actuating the peristaltic pump (*b*) (15). (*c*) Geometric metering via three sets of chambers of different sizes. Yellow channels are membrane-valve control channels. Interface valves are closed, and upper chambers have been filled with colored liquid, while bottom chambers have been filled with clear liquid (11). (*d*) Interface valves are opened and diffusive mixing occurs. (*c*, *d*) Scale bar is 1 mm.

versatile microfluidic component that is useful in a wide variety of biological and chemical assays.

Metering Strategies

Precise metering of small volumes of liquid can be achieved by a number of schemes. Geometric metering involves mixing two reagents, where the volumes are predefined by channel or chamber geometry (13). Chambers of different volumes are separated by an interface valve, as shown in **Figure 2*c*,*d***. First, the interface valve is closed and the chambers are filled with different reagents by dead-end priming. Next, valves at the chamber inlets are closed to define a fixed volume. As the interface valve is opened, mixing occurs by free interface diffusion (13).

Positive displacement cross-injection is an active method of liquid metering and involves serially dispensing an arbitrary number of reagents (11). As seen in **Figure 3*a*–*d***, the reaction junction is first loaded vertically with reagent A. Next, the peristaltic pump is activated for a defined number of cycles, which allows a predictable volume of reagent B to enter the reaction junction. By increasing or decreasing the number of pump actuation cycles, any predefined volume can be metered by calculating the volume of displaced liquid. Finally, the reaction junction is sealed by

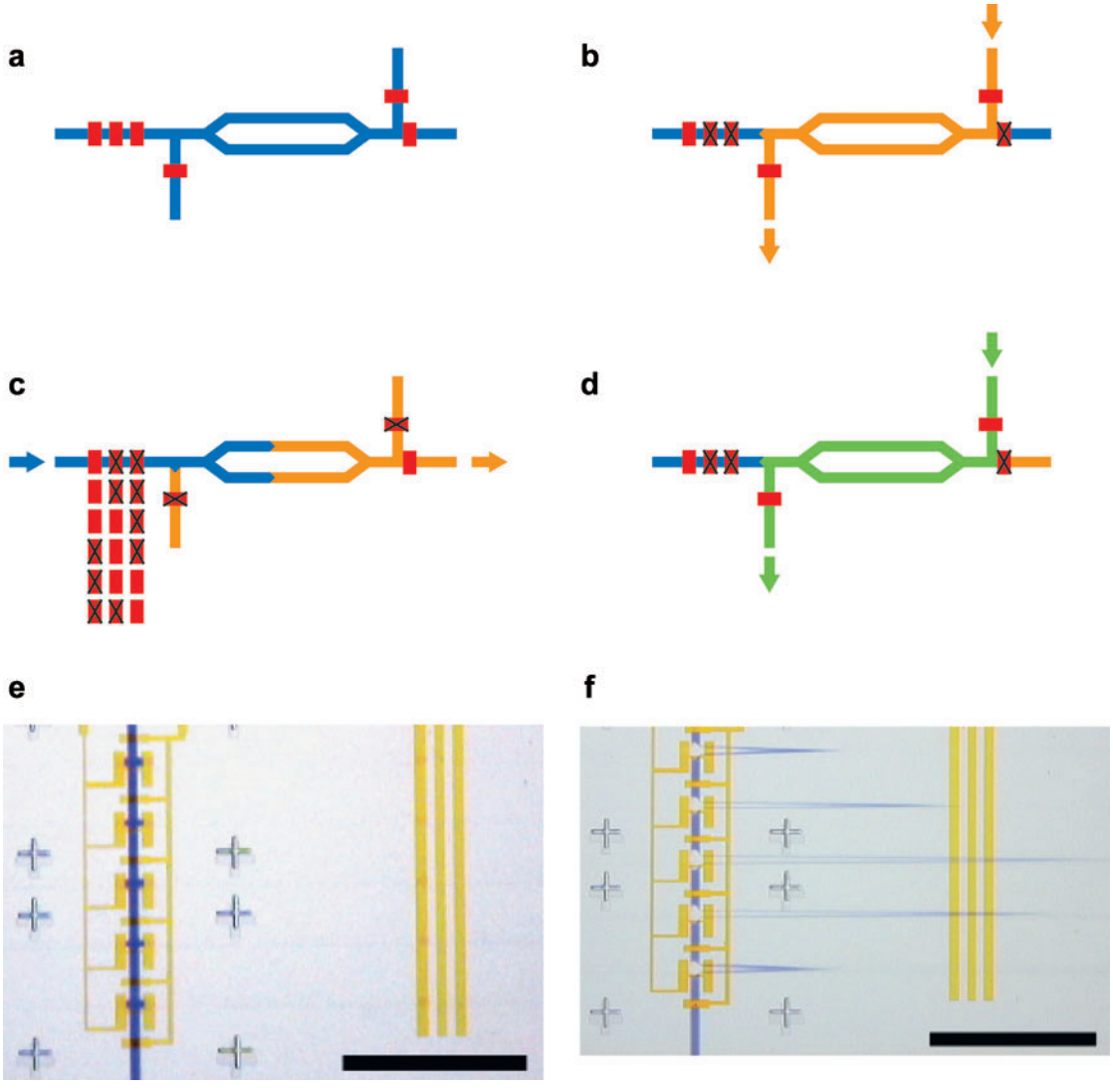

Figure 3

(*a*) Serial positive displacement cross-injection, where valves are shown in red and fluid lines in blue. (*b*) Valves are actuated to allow junction to be loaded with fluid (*orange*). (*c*) A metered amount of blue liquid is injected by activating the peristaltic pump in a predetermined number of sequence cycles. (*d*) Diffusive mixing occurs in the junction (11). (*e*) Parallel active metering, where blue liquid is injected into the vertical column. (*f*) Compartmentalization valves are actuated, and the metered liquid samples are flushed horizontally downstream using a peristaltic pump (11). (*e*,*f*) Scale bar is 2 mm.

inlet and outlet valves, and diffusive mixing occurs. The mixed reagents can then be expelled downstream, and two new reagents can be metered and mixed.

An early version of positive displacement cross-injection was parallelized using the configuration shown in **Figure 3*e*,*f*** (11). The vertical channel is filled with reagent, and the valves compartmentalize the desired volume. The two horizontal valves of each compartment are opened, and each reagent volume is simultaneously pumped downstream in

individual horizontal channels using a peristaltic pump. If the pumping rate is slow enough, the amount of liquid injected is independent of fluidic microchannel resistance and liquid viscosity (11).

HIGHER-LEVEL COMPONENTS: MULTIPLEXED ADDRESSING

Latches: Valves Switching Valves

A microfluidic latch is a powerful tool that enables valves to be actuated and remain actuated without the need for continuous external pressure. Latches are often used to decrease the number of external control ports needed while increasing functional complexity and integration on-chip. **Figure 4*a*** shows such a latch, which uses monolithic membrane valves (41, 42). Pressure is applied through port A to close valve 2. Next, pressure is applied through port B to close valve 1. Then, external pressure can be released from port A, and valve 2 remains closed because of built-up pressure. This pressure can be sustained for >10 min until depressurization occurs via dissipation through pores in PDMS. Hence, control valves are used to actuate other control valves that subsequently restrict fluid flow on-chip.

Grover et al. (8) have also demonstrated a set of latching valves based on a three- or four-valve network. Normally closed seat valves form a vacuum-latching or pressure-latching valve (**Figure 4*b***). In the case of the vacuum-latching valve shown in **Figure 4*c***, the vacuum valve is responsible for holding the latching valve open by sealing a vacuum on-chip, and the pressure valve is responsible for expelling the built-up vacuum upon command. Initially, a pulse of vacuum is applied to the input, and the vacuum valve and latch valve open. Shortly thereafter (120 ms), the vacuum valve closes again because of depressurization of the pressure valve and vacuum valve outputs. Disconnecting the vacuum control input from the chip at this point allows the latch valve to remain open. To close the latch valve, a pulse of pressure is applied to the input, thereby opening the pressure valve and closing the latch valve for up to 10 min, regardless of whether the chip is connected to an external controller.

Nonlatched Multiplexing

Not every memory cell in a semiconductor integrated circuit is connected to a chip pin-out. A variety of schemes are used to reduce the number of pin-outs, including multiplexed addressing. The microfluidic equivalent of a multiplexer is a powerful tool that addresses large numbers of valves with a small number of connections from the chip to the outside world. **Figure 5** shows a schematic of a nonlatched microfluidic multiplexer (42), i.e., pressurizing individual control lines directly actuate valves controlling fluid flow. N vertical flow channels are controlled by $2\log_2 N$ horizontal control channels. Because threshold valve pressure is highly dependent on membrane geometry, valves are formed only where wider sections of the control lines intersect flow lines, and control lines are grouped into sets of complementary (binary) valve pairs, as seen in **Figure 5**. Therefore, pressurizing control lines corresponding to bit 1, 2, 3 (i.e., 1110) allows fluid from channel 1110 = 14 to be chosen.

Although this multiplexer addresses flow channels effectively, cross-contamination between flow channels can occur because of dead volume at the outlet (**Figure 5*b***). A modified multiplexer based on a binary tree design eliminates this problem, as shown in **Figure 6*a*,*b*** (11). Each flow channel incorporates N consecutive bifurcations, allowing 2^N inlet channels to be connected through equivalent fluidic paths. At each bifurcation, valves control the direction of liquid movement. Each flow channel filled with reagent has a corresponding flush channel connected to deionized water. After reagent A is chosen and flows through the outlet of the multiplexer, the corresponding flushing channel is opened. This allows deionized water to remove any reagent A remaining in the

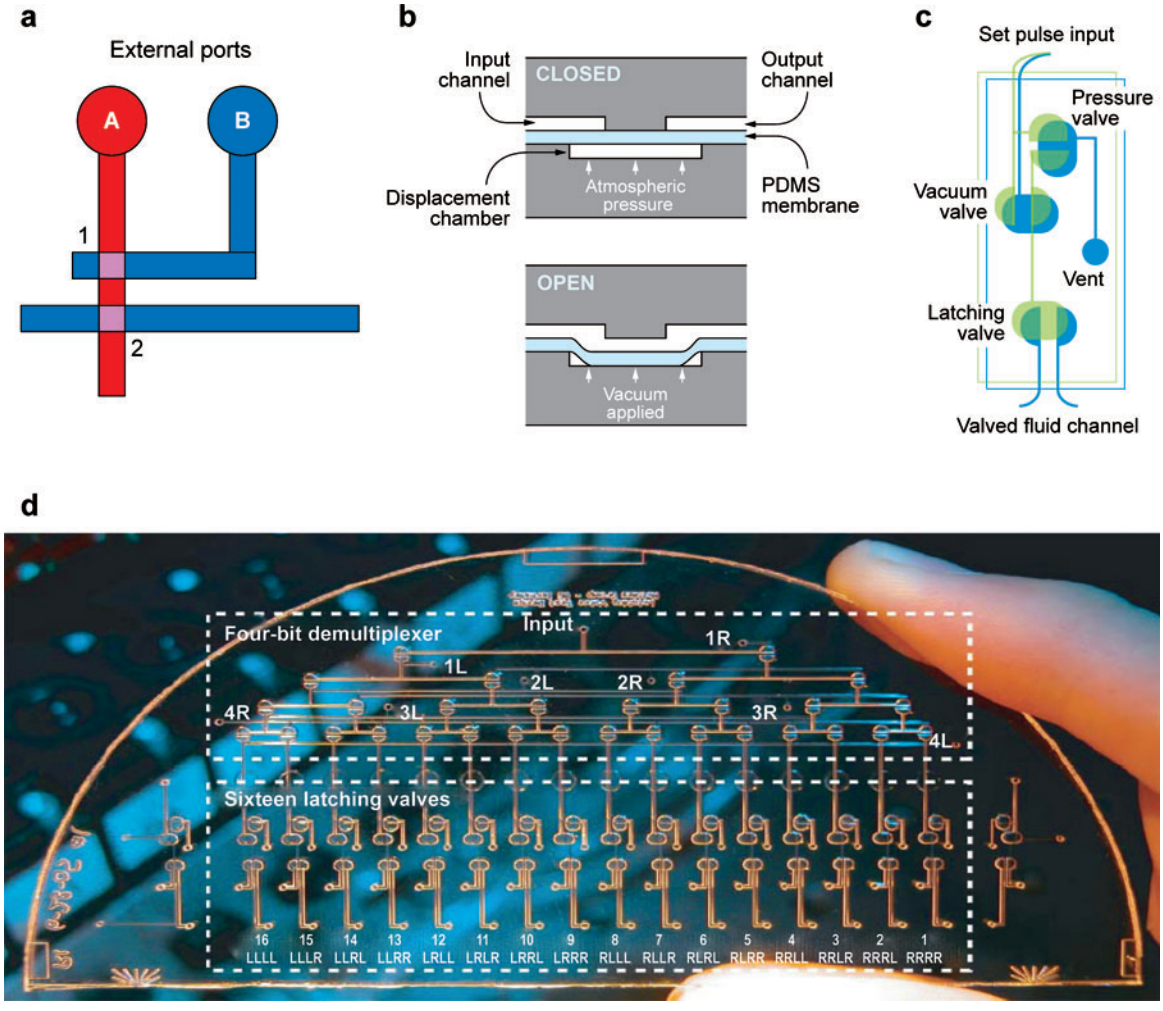

Figure 4

(*a*) A microfluidic latch that uses monolithic membrane valves (*red*, control channels; *blue*, flow channels). Port A is pressurized and closes valve 2. Port B is pressurized and closes valve 1. Pressure supplied to port A can then be disconnected, and valve 2 remains closed. (*b*) A normally closed seat valve. When vacuum is applied to the bottom channel, the diaphragm deflects and fluid can flow freely from inlet to outlet. (*c*) A three-valve network, creating a vacuum-latching valve (8). (*d*) Demultiplexer using vacuum-latched valves, where the input port at the top of the device is supplied by a pressure/vacuum pulse. The upper section of the device houses four rows of valves, where each alternate valve (on each row) is connected to vacuum or pressure off-chip and comprises the demultiplexer. The bottom of the device contains 16 vacuum latch valves activated via the demultiplexer (8).

multiplexer, leaving the multiplexer ready for another reagent to be addressed.

A multiplexer with even higher efficiency than the binary multiplexer described above was recently demonstrated. This device uses a combinatorial scheme in which all possible combinations of addressing valves are used. This strategy allows $N!/(N/2)!^2$ chambers to be addressed using N control lines (for 16 control lines, this corresponds to 12,870 addressable chambers, compared with 256 addressable chambers using the binary method) (18).

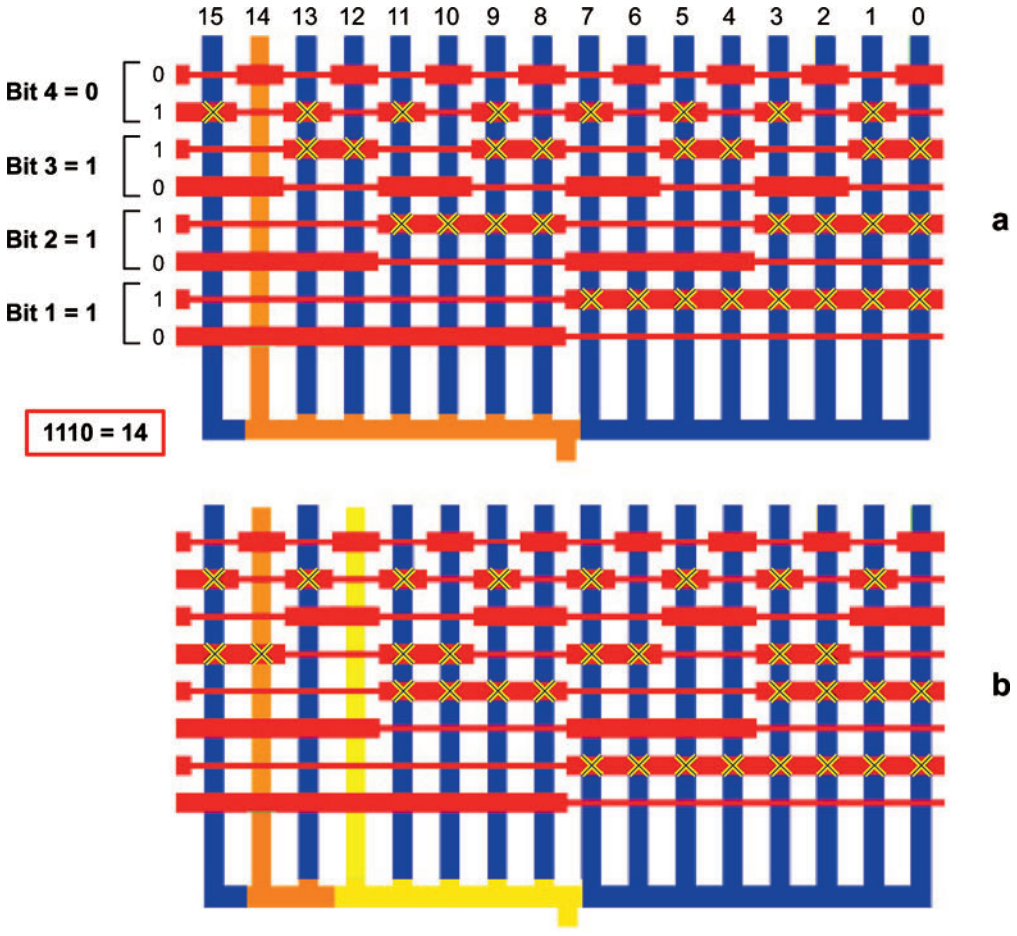

Figure 5

(*a*) Microfluidic multiplexer, where N vertical flow channels can be individually addressed by $2\log_2 N$ horizontal control lines. Valves are created only where a wide control channel (*red*) intersects a flow channel. Narrow channels depict passive crossovers where no valve is created. X represents an actuated valve. (*b*) When each flow line contains different reagents, cross-contamination can occur because of dead volume at the output of the multiplexer. Figure adapted from Reference 11.

For the 16-channel multiplexer in **Figure 6*c***, pressurizing any three of the six control lines results in only one open flow channel. For example, pressurizing control channels 2, 3, and 5 addresses flow channel 7.

Latched Multiplexing

The latching mechanism shown in **Figure 6** was used to realize a microfluidic analogy to a random access memory. This device utilizes two multiplexers based on monolithic membrane valves (row and column multiplexers) and a $25 \times 40 = 1000$ compartment matrix that includes 3574 valves (42). Contents from individual chambers can be recovered using latched multiplexing. Each flow channel is neighbored by two other parallel flow channels. To recover the contents of an individual chamber, the row and column multiplexers control fluid pressure to create a purging flow path via these two parallel flow channels. Each multiplexer can be likened to a memory address register in random access memory.

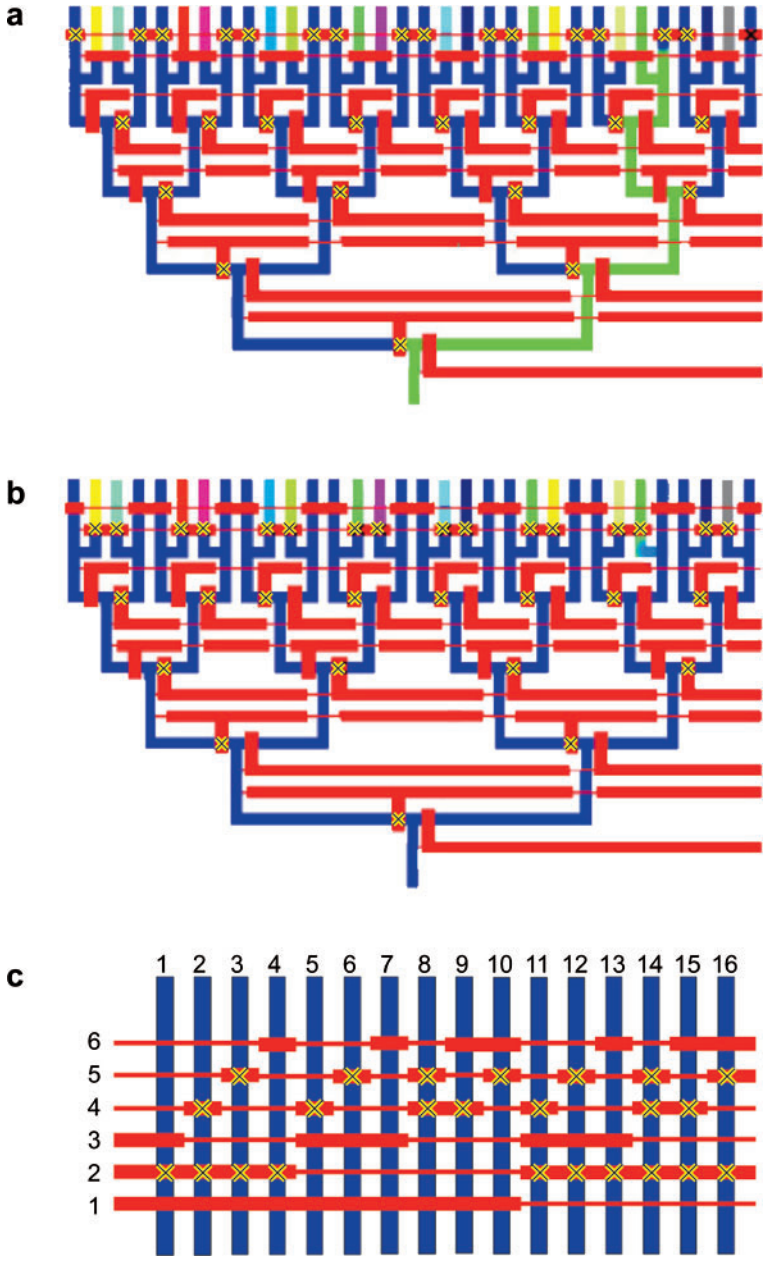

Figure 6

Binary tree format multiplexer that eliminates cross-contamination. (*a*) Green sample is selected. (*b*) Green sample is flushed using adjacent buffer channel (11). (*c*) Combinatorial multiplexer, where N control channels address $N!/(N/2)!^2$ flow channels.

The row multiplexer is responsible for fluid purging and replenishment, and the column multiplexer controls the vertical input/output valves.

A 4-bit (N) binary demultiplexer addressing 16 (2^N) independent vacuum-latching valves and outputting pressure or vacuum pulses has also been demonstrated (8) (**Figure 4*d***). In this case, each row of valves is controlled by a four-connection, two-position solenoid valve and connected in an alternating manner to the valves in each demultiplexer row. Thereby, vacuum or pressure is applied to the even- or odd-numbered valves,

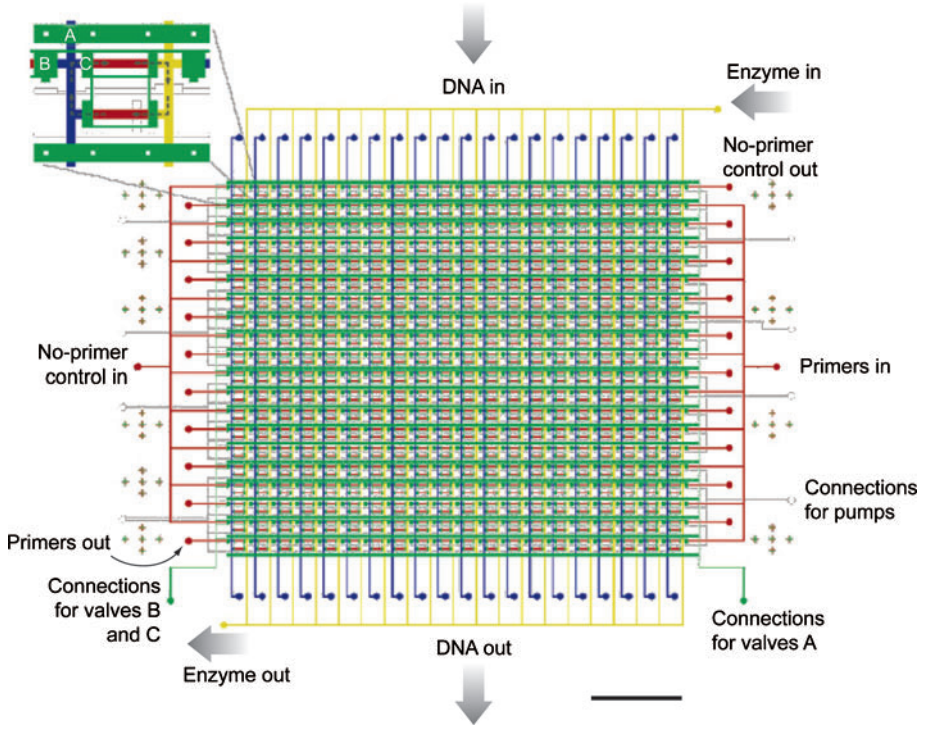

Figure 7

N × N = 400 reaction chamber matrix requires only 41 pipetting steps. Enlargement depicts one reaction chamber: White valves are used as peristaltic pumps and green valves are used for compartmentalizing reagents. Two differently sized green valves are used to compartmentalize reagents at two different pressures during the reagent-loading sequence. This reduces the number of individual control channels needed. Scale bar = 6.4 mm (26).

respectively, and the input signal can be routed to the correct output. This device can be latched every 120 ms, and all valves in the device are in their final chosen state after 2 s.

HIGHER-LEVEL COMPONENTS: COMBINATORIC ECONOMIES OF SCALE

Microfluidic devices offer new economies of scale in biological automation. One example of this is the ability to perform combinatorial experiments with a minimum number of pipetting steps. An N × N (N = 20) matrix of reaction chambers has been demonstrated for 400 PCR reactions, in which only 2N + 1 = 41 pipetting steps were required to set up the experiment (compared with the $3N^2 = 1200$ pipetting steps required when using conventional pipetting into a microtiter plate) (26). This allows 2 μL of reagent to be amortized over as many as 400 chambers. **Figure 7** shows the full microfluidic matrix and an enlargement of one individual reaction chamber (microchannel loop). The chip contains 2860 valves controlled by two independent pressure supply ports. Sets of two differently sized valves (actuated at different threshold pressures) are connected together and used to compartmentalize the reaction chamber when filling with the DNA template, primers, and polymerase. The large valves (270 μm wide) actuate at 110 kPa, and the small valves (96 μm wide) at 260 kPa. Crossover routing (42-μm-wide

tapered channels) also enables a single control line to be used for both sets of valves. This device is composed of three-layers, where the flow layer is sandwiched between upper control lines (push-down valves used for compartmentalizing reagents) and lower control lines (push-up valves used for peristaltic pumping).

A 294-bp segment of human β-actin cDNA fragment was amplified using this chip and the output fluorescence emission monitored (519 nm and 570 nm). Over multiple experiments, 98% of 3200 reaction chambers produced the expected result, and the detection limit for this experiment was 60 template copies/reactors. This chip also enabled a set of all possible forward and reverse primers to be tested on the same DNA template, where the three-component reaction included (*a*) a cDNA template and polymerase, (*b*) forward primers, and (*c*) reverse primers. Other plausible applications for this chip include genetically screening N patients for N mutations, and multistep reactions where intermediate products can be contained in one compartment of the chamber while the remaining compartments are flushed and loaded with reagents needed for a subsequent reaction (26).

HIGHER-LEVEL COMPONENTS: AFFINITY COLUMNS AND THE SIEVE VALVE

An on-chip affinity column enables several sequential processes to be performed and adds yet another dimension of functionality to mLSI. Microbeads, i.e., functionalized polystyrene microspheres, can be trapped upstream of a partially closed valve (16). However, slight variations in pressure can cause large variations in column properties (29). By replacing the variable pressure valve with a sieve valve, a more reproducible column is formed. This sieve valve comprises a rectangular cross-section flow channel, where leakage occurs along the edges of the channel when the valve is closed, while the movement of beads or even cells is blocked. Submicron-sized leakage channels can be created, enabling beads of only a few microns in diameter to be stacked. **Figure 8*a*,*b*** shows an actuated sieve valve and a stacked affinity column upstream of the valve. Another advantage of this sieve valve is that a temporary column or filter can be created and the contents later flushed farther downstream by opening the valve completely (30). These columns can also be used for ion-exchange chromatography (24) and solid-phase synthesis (21a).

Serial Processing

The microfluidic affinity column can be used as a component in a serial process, such as cell isolation, cell lysis, mRNA or DNA purification, and recovery of the product. **Figure 8*c*,*g*** shows a step-by-step schematic of a single bioprocessing unit for such a microfluidic device, which has a loading mode and processing mode of operation (16). Initially, an affinity column of magnetic beads is stacked upstream of a sieve valve. Next, bacterial cell culture is introduced upstream, followed by loading of dilution buffer in a compartment directly downstream. Lysis buffer is introduced to yet another compartment, and the valves are opened and mixed in a rotary pump mixer. Once mixing is complete, the lysate is flushed over the affinity column and the remainder goes to a waste outlet. The nucleic acid bound on the affinity column can be recovered by flushing elution buffer over the column or by opening the sieve valve and recovering the beads.

PARALLELIZATION: INCREASING THROUGHPUT WITHOUT INCREASING CONTROL COMPLEXITY

Effective parallelization is an important component of mLSI. The serial processing scheme described above lends itself to parallelization by using common control lines

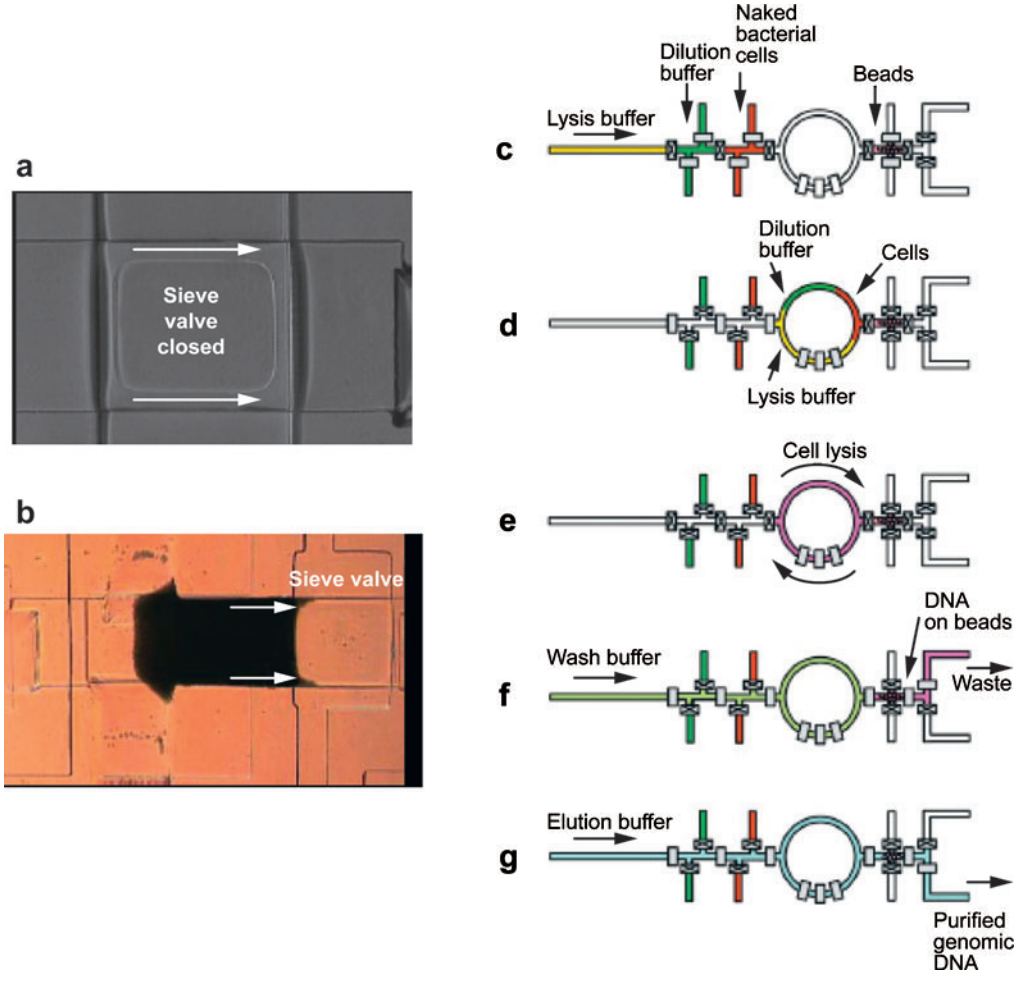

Figure 8

(*a*) Leakage occurs along the edges of a rectangular profiled flow channel when the valve is actuated. (*b*) A bird's-eye view of a stacked affinity column of microspheres upstream of a sieve valve (30). (*c*) A microfluidic device for serial biochemical processing, where a microsphere column is stacked upstream of the sieve valve. Lysis buffer, dilution buffer, and bacterial cell solution are loaded. (*d*) Cell solution is loaded into rotary pump. (*e*) The pump is activated and cells are lysed. (*f*) Wash buffer and lysate are flushed over the columns. (*g*) The sieve valve is opened, and purified genomic DNA on beads is flushed out (16).

to allow loading and processing of multiple reactors simultaneously without increasing the number of control lines needed. Sample volumes can also be geometrically customized in an array of such processing units to allow various dilution factors to be tested. This is a relatively generic processing system that can be used for a variety of biochemical processes (15, 16).

As an example, parallel process lines for cell isolation, cell lysis, mRNA purification, cDNA synthesis, and cDNA purification were integrated on a microfluidic device (30). The sensitivity is sufficient for detecting low and medium copy number transcripts from single NIH3T3 cells. This device uses architecture similar to that of **Figure 8**; however, each reagent sample is flushed directly into

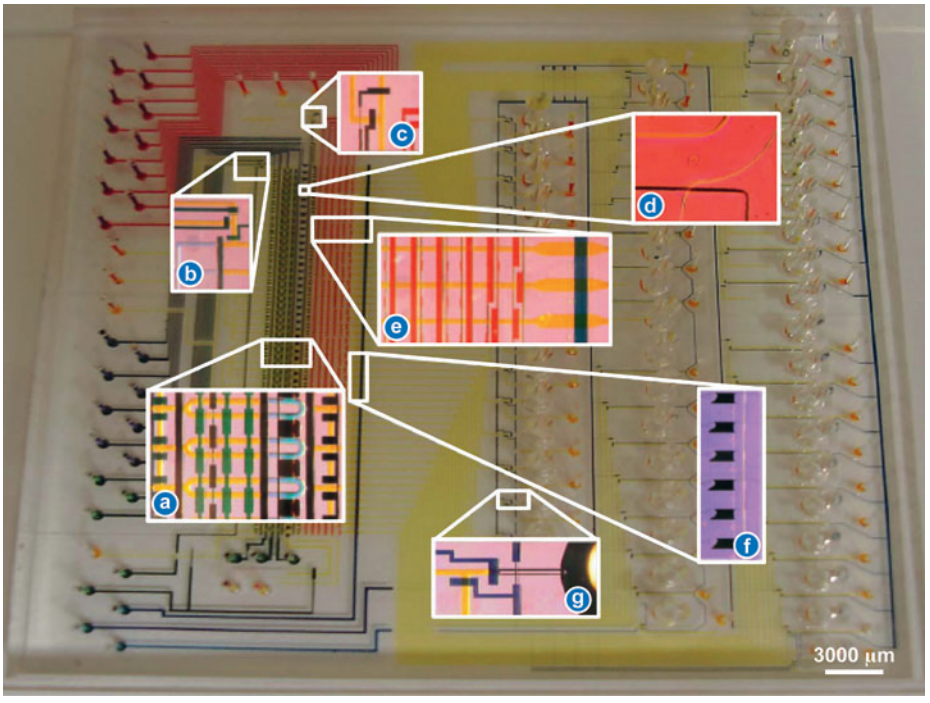

Figure 9

A 50-plex cDNA-synthesis microfluidic device with insets of (*a*) a cell lysis module, (*b*) buffer inputs, (*c*) bead and lysis inputs, (*d*) captured NIH3T3 cell, (*e*) multiplexer and sieve channels, (*f*) stacked bead columns, and (*g*) output, waste, and collection ports (29).

the rotary pump mixer through a sequence of valve actuations, and single cells are trapped in one compartment of the rotary loop. A dilute solution of cells is injected into the rotary loop, and the valves closed when a single cell is seen to enter. The affinity column is stacked with oligo-dT functionalized beads, lysis buffer is loaded into the rotary mixer, and the individual cells and lysis buffer are mixed. Next, the lysate is flushed over the stacked bead column, leaving mRNA bound to the beads. Reverse transcriptase and dNTPs are then flushed over the column, thereby synthesizing cDNA. This concept has been successfully scaled up to a 50-plex device (**Figure 9**). This mRNA isolation and cDNA-synthesis technique is reliable for templates that span four orders of magnitude in copy number and for which the presence of GAPDH and HPRT mRNAs from 0.1 to 1 pg of mRNA can be detected (29).

CONCLUSIONS

From the short list of examples discussed here, one can see that mLSI design components enable a virtually unlimited set of tools for biological automation. This technology is now at a stage at which design can be decoupled from fabrication, which allows users to focus on applications without requiring them to become skilled in the details of fabrication. Going forward, there is a need for computer CAD tools that incorporate mLSI design rules. Soon, designing microfluidic tools for experiments may be as common as designing DNA oligonucleotides.

SUMMARY POINTS

1. mLSI demonstrates excellent economies of scale and is a strong candidate to replace conventional methods of fluidic automation such as pipetting robots.

2. mLSI functional components enable a virtually unlimited set of tools for biological automation, and we are now at a stage where microfluidic system design can be decoupled from fabrication.
3. mLSI based on an MSL platform is excellent for biological and biochemical applications such as screening conditions for protein crystallography, highly parallel and combinatorial PCR, and parallelization of multistep biochemical processes such as cell capture, cell lysis, mRNA purification, and cDNA synthesis for multiple and single cell analysis.
4. There is a need for computer design tools that incorporate mLSI design rules. Soon, designing microfluidic tools for experiments may be as common as designing DNA oligonucleotides.

FUTURE ISSUES

1. mLSI should be made more accessible to researchers in order to apply the technology to an even wider range of biological and biochemical applications. This includes making the MSL mLSI platform readily usable for nonaqueous-based applications such as synthetic chemistry.
2. Researchers should develop an even more mature and specific set of design rules for mLSI.
3. A truly portable microfluidic design and end-user software allowing high-level architecture to be designed without consideration to detailed microfluidic behavior and the specifics of the microfluidic platform to be used should be developed.

ACKNOWLEDGMENTS

This work was supported by NIH 1RO1 HG002644-01A1.

DISCLOSURE STATEMENT

SRQ founded a company that operates in the microfluidics field.

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Volume 35, 2006

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