Lab on a Chip

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PAPER

Semi-autonomous liquid handling *via* on-chip pneumatic digital logic†‡

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This report presents a liquid-handling chip capable of executing metering, mixing, incubation, and wash procedures largely under the control of on-board pneumatic circuitry. The only required inputs are four static selection lines to choose between the four machine states, and one additional line for power. State selection is simple: constant application of vacuum to an input causes the device to execute one of its four liquid handling operations. Programmed control of 31 valves, including fast coordinated cycling for peristaltic pumping, is accomplished by pneumatic digital logic circuits built out of microfluidic valves and channels rather than electronics, eliminating the need for the off-chip control machinery that is typically required for integrated microfluidics.

Introduction

Microfluidic large-scale integration[1] enables complex on-chip fluid control, including systems for precise metering, dilution, and reaction of multiple reagents.[2–4] However, such devices typically rely upon unwieldy off-chip pneumatic and electronic components that interface to the chip through a maze of tubing. While this control method has proven highly effective for many laboratory functions, it is less ideal for applications such as point-of-care diagnostics, where simplicity and portability are key. The implementation of digital logic circuits out of microfluidic valves and channels could potentially enable fully self-contained systems that are controlled by onboard circuitry, thus eliminating the need for off-chip controls.[5]

A number of microfluidic digital logic components have been reported, from fundamental logic gates to combinatorial and sequential circuits, driven by either liquid or gas pressure.[6–9] We utilize a gate architecture pioneered by the Mathies group that is built around a normally off elastomeric membrane valve (Fig. 1), which can be treated analogously to a NMOS transistor in the construction of digital circuits.[6] Elsewhere, we report the design and implementation of self-oscillating circuits and the use of such circuits to control peristaltic pumping, which requires rapid, highly coordinated actuation of multiple valves.[10]

In this work, we integrate multiple peristaltic pumps in a ring mixer architecture[3] and add a combinatorial logic block for selective activation of the required pumps and valves to execute four functions: **meter**, where two liquids are loaded into the device at a specific ratio; **mix**, where the liquids are mixed together using an on-chip rotary pump;[11] **incubate**, where the liquid mixture is left stationary (*e.g.* for timed reactions); and **wash**, where the mixing chamber is flushed with a buffer solution. Each of the four states is switched on by the activation of a single input line. A network of Boolean logic gates then route control signals to the appropriate components in order to accomplish the selected function. Thus, an operator can select between a set of integrated microfluidic operations without the need for computer control.

Materials and methods

Devices were fabricated as previously described.[6] Briefly, channels and valves were patterned in glass wafers (Telic Co., Valencia, CA, USA) by photolithography and HF etching. Access holes for off-chip connections were drilled through the glass with diamond-tipped grinding bits (McMaster-Carr, Robbinsville, NJ, USA). Via holes were punched through 250 μm thick PDMS membranes (Rogers Corp., Carol Stream, IL, USA). Finally, two complementary glass wafers and a matching PDMS membrane were aligned and assembled, with the membrane in the middle of the stack (Fig. 1a). Adhesion forces between the PDMS and glass wafers were sufficient to hold the device together.

Resistor values were determined *via* circuit modelling using PSPICE/OrCAD (Cadence Design Systems Inc., San Jose, CA, USA). Pneumatic resistances were calculated based on channel dimensions[12] to form a lumped-element resistor network that was modelled in PSPICE as an equivalent electrical circuit, where the ratios of the electrical resistances corresponded to the ratios of the pneumatic resistances in the microfluidic circuit. Valves were approximated as either perfect conductors or infinite resistors, depending on their opened or closed state. Resistor values were then adjusted in order to achieve the desired steady-state transfer function behaviour in the PSPICE circuit model. Electrical voltage swings between the supply and ground voltage levels corresponded well to pneumatic pressure swings between

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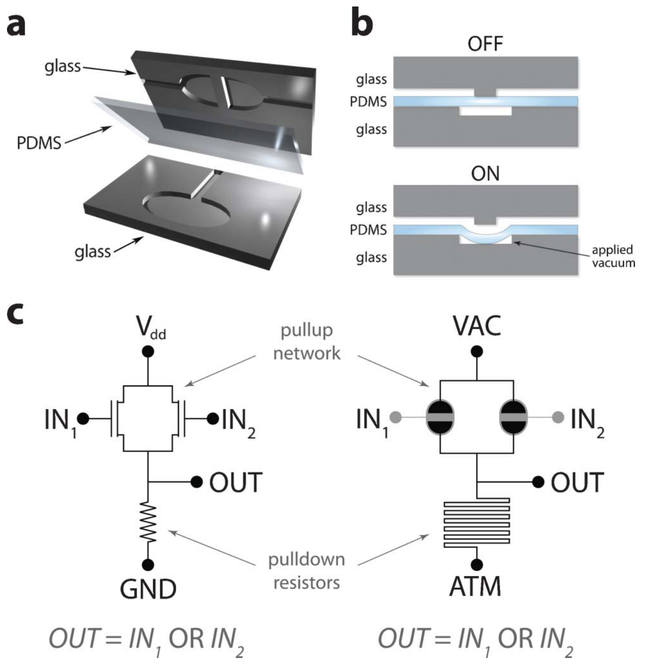

Fig. 1 Normally closed elastomeric membrane valves serve as the fundamental building block of microfluidic digital logic. (a) Exploded view of a three-layer valve. (b) Cross-sectional diagram of a valve. Vacuum applied to one side of the PDMS layer causes the membrane to deform, connecting the two channels on the opposite side. (c) Microfluidic networks of pneumatic valves and channels behave analogously to electronic digital circuits. Shown here is an electronic OR gate and its pneumatic counterpart.

the supply and ground pressure levels, thus the behaviour of the pneumatic circuit could be predicted effectively by the behaviour of the modelled electrical circuit.

Design and results

A general scheme for metering and mixing with a rotary mixer has previously been reported by Urbanski *et al.*[3] Metering is accomplished geometrically by loading specific fractions of the ring, and then mixing is accomplished by circulating the liquids around the ring (Fig. 2). Circulatory flow requires peristaltic pumping, which can be accomplished by placing three valves in series and actuating the valves in a ripple pattern.[13] During the different phases of ring mixer operation, specific valves must be opened, closed, or cycled in order to pump and route liquids appropriately. This typically requires an off-chip connection to each valve, in order to apply pneumatic pressure under computer control.

In our system, the control signals for driving peristaltic pumping are generated on chip through the use of an oscillator circuit.[10] Three inverter gates are connected in a loop, creating an inherently unstable circuit that oscillates at a frequency that can be defined by adjusting circuit parameters such as resistance and capacitance. Pumps can be switched on and off by controlling the supply of power to each oscillator.

Binary logic is represented in our implementation by differences in pneumatic pressure, with vacuum representing binary 1 and atmospheric pressure representing binary 0.

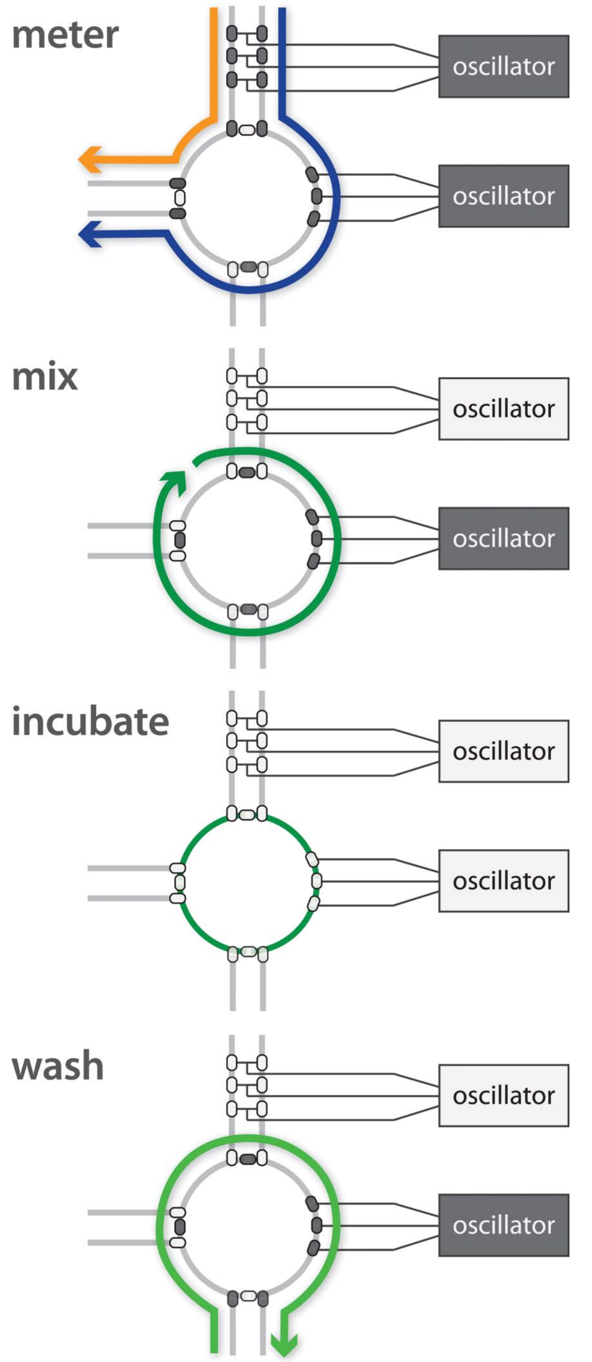

Fig. 2 Schematic diagram of the four liquid-handling operations. Arrows represent the direction of liquid flow during each state. Specific valves must be opened during each state in order to route the liquids properly. Open valves and active pumps (driven by on-chip oscillators) are shown in dark grey, while closed valves and inactive pumps are white. The metering step illustrated here measures out two liquids at a 1 : 3 ratio.

Elastomeric valves (Fig. 1) are analogous to NMOS transistors in that they are normally closed and require the application of vacuum (binary 1) to the gate in order to open. Power to the chip is supplied as a single vacuum line, which can be driven by a mechanical or manual pump, or even the pull of a syringe.[10] Ground connections are simply holes to connect to atmospheric

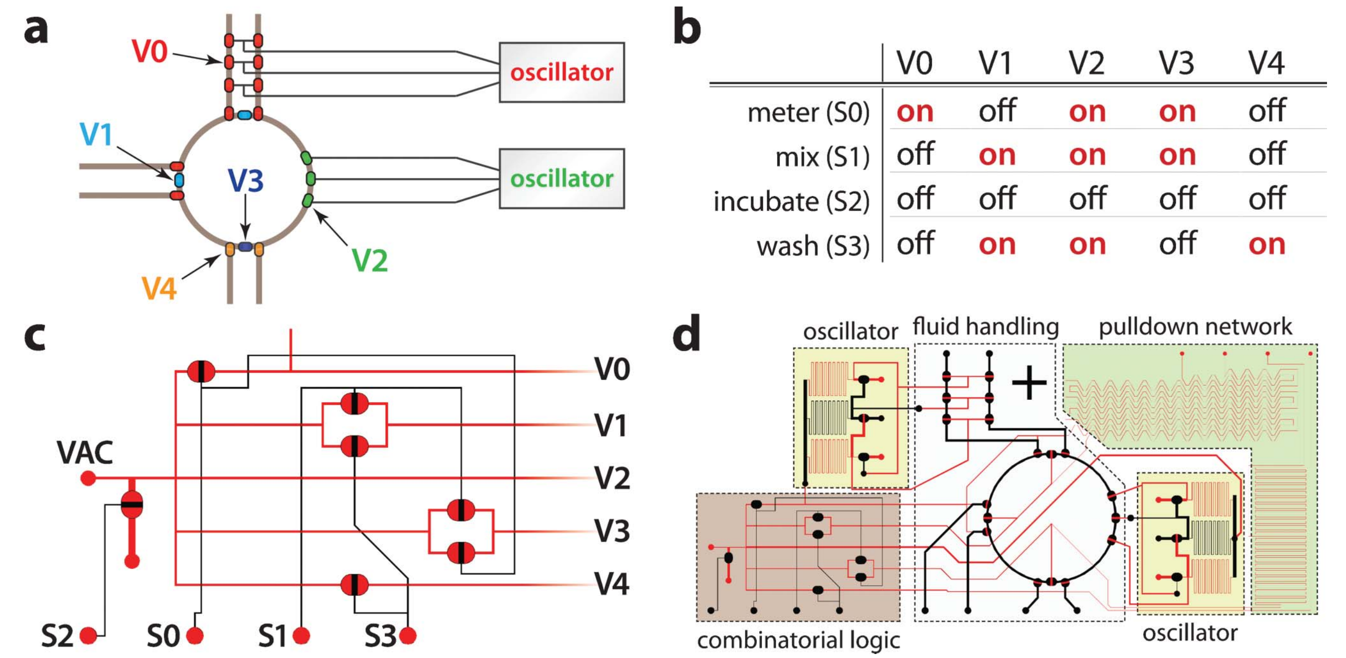

Fig. 3 Design of combinatorial Boolean logic circuitry. (a) Valves and oscillators are organized into groups that should be actuated simultaneously. (b) Table showing the required activation of valve groups (V_0–V_4) during each liquid handling state (S_0–S_3). (c) Implementation of above table in microfluidic digital logic gates. Vacuum supply is to the left. (d) Layout of complete device. Liquid handling components, including the ring mixer, are seen in the center while control elements are to the left and right. On-chip oscillators are used to drive peristaltic pumps for routing and mixing fluids.

pressure. Resistors are simply long channels. Together, these elements provide the ability to mimic designs from the NMOS-logic circuit family, which provides a wide library of existing designs from electronics.

To achieve semi-autonomous liquid handling, the activation of a single input line must subsequently activate a specific set of pumps and valves to accomplish a particular operation. The routing of these multiple signals is accomplished by a combinatorial Boolean logic block, which was designed as illustrated in Fig. 3. For ease of notation, the four liquid handling states (meter, mix, incubate, and wash) are denoted as S_0 through S_3. All on-chip valves and oscillators involved with fluidic control were grouped based on which states they were active in, as seen in Fig. 3a. For example, all valves in group V_0 should open and close together, depending on the operation being executed. The table in Fig. 3b summarizes the activation of valve groups required to accomplish each liquid handling operation. With this table, simple Boolean equations can be created that show when a given valve group should be active:

$$V_0 = S_0$$

$$V_1 = S_1 + S_3$$

$$V_2 = S_0 + S_1 + S_3$$

$$V_3 = S_0 + S_1$$

$$V_4 = S_3$$

For example, all valves in group V_1 will be open during state 1 (mixing) and state 3 (flushing). In Boolean logic, this is equivalent to an OR gate: in state 1 OR state 3, valves in group V_1 should be activated. These Boolean equations can then be translated into the actual mask design for the combinatorial logic block, as seen in Fig. 3c. Fig. 3d shows the layout of the entire device, illustrating the combination of control logic blocks and fluid handling blocks together on the same chip.

Operation of the completed device is shown in Fig. 4. A video is also included in the online supplement.† Static activation of single control lines was effective in triggering the coordinated activation of multiple pumps and valves to accomplish liquid handling operations. Blue and yellow food coloring were mixed using the device, in order to illustrate its various functions. Full mixing required about 50 s, as judged by observing color uniformity, which is comparable to previous reports.[2] Reagents could be sucked into a dry unprimed chip by using just the on-chip peristaltic pumps. Alternatively, initial priming of all the fluid lines with water was helpful for eliminating bubbles.

Discussion and conclusions

We have illustrated that Boolean logic can be implemented in microfluidics and utilized to coordinate the actuation of numerous integrated valves to accomplish fundamental liquid handling functions. Four static inputs, plus an additional static vacuum line for power, were sufficient to control a network of 31 microfluidic valves. While operations such as on-chip peristaltic pumping typically require computer control, our system requires simple static on-off inputs, which can be accomplished, for example, by manual twist valves.

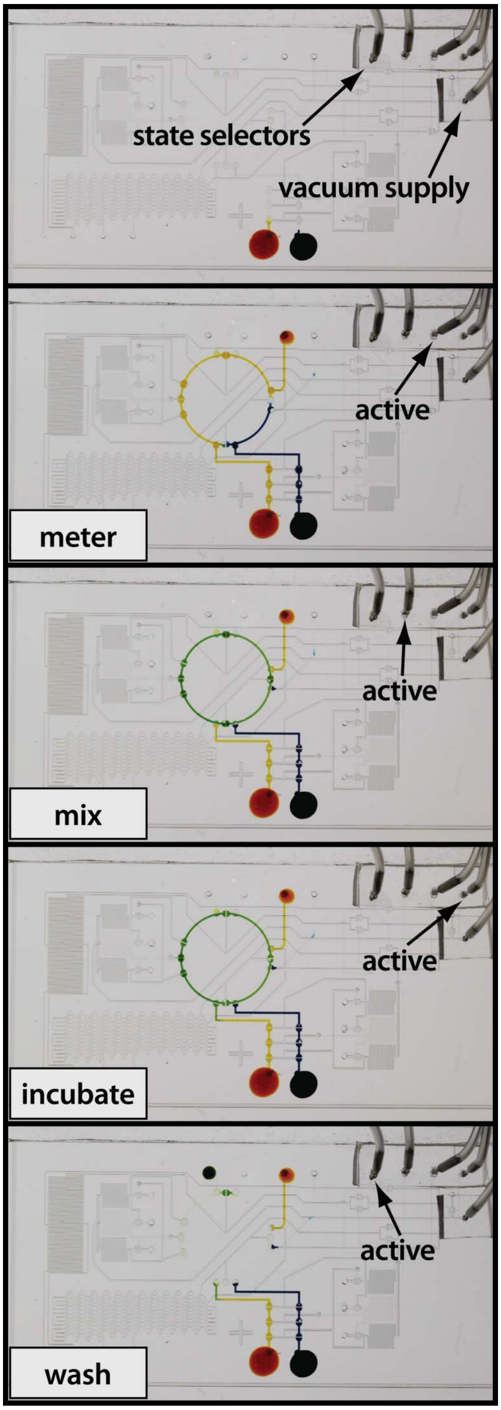

Fig. 4 Captured video frames illustrate device operation. A single connection to the right supplies constant vacuum for power. Each liquid handling state is selected by activating one of the four input lines. All fluid pumping and routing is achieved by on-chip circuitry.

A previous report by Rhee *et al.*[9] similarly employed pneumatic digital logic to control liquid handling, although their lack of peristaltic pumping precluded operations such as circulatory flow. Significantly, their system was controlled externally through a serial input, that is, a series of pneumatic pulses, encoded in time, travelling along a single pneumatic line. In contrast, our system is controlled by four static parallel inputs. This design decision makes our system amenable to control by on-chip circuitry such as finite state machines (FSM). The FSM is a classic microcontroller architecture that can step through a series of operations according to a set of programmed rules. At each particular program step, or state, the FSM calculates its next state based on its current state and any available inputs. An FSM could be used to trigger liquid handling control lines according to a timed, programmed sequence. In the future, the combination of an FSM, a clock reference, and a liquid handling circuit could potentially realize self-contained fully autonomous liquid processing chips. These single-chip systems could offer significant advantages in terms of size, ease of use, and manufacturing cost (since they are fully batch fabricated), compared to current systems that are composed of both microfluidic and electronic components, plus a pneumatic system to interface between the two.

Limited chip real estate may pose a significant challenge to realizing a complete system-on-a-chip. Indeed, in the device reported here, the control logic already occupies more than half of the chip. While microfluidic circuits may not be able to replicate the same Moore's Law scaling achieved by microelectronics over the past four decades, significant reductions in pneumatic gate size should be possible. Moderate improvements in gate density ($\sim$100 gates per chip) may be sufficient to enable enough programmable functionality to implement a microcontroller that can execute, for example, a broad diagnostic panel. The limits of microfluidic digital logic circuits have only begun to be explored.

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