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Two-layer multiplexed peristaltic pumps for high-density integrated microfluidics

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A B S T R A C T

The integration and operation of a large number of components is needed to enable ever more complex and integrated chemical and biological processes on a single microfluidic chip. The capabilities of these chips are often limited by the maximum number of pumps and valves that can be controlled on a single chip, a limitation typically set by the number of pneumatic interconnects available from ancillary hardware. Here, we report a multiplexing approach that greatly reduces the number of external pneumatic connections needed for the operation of a large number of peristaltic pumps. The utility of the approach is demonstrated with a complex microfluidic network capable of generating and routing liquid droplets in a two-phase flow. We also report a set of design rules for the design and operation of multiplexed peristaltic pumps, based on a study of the effect of the number of valves per pump and the valve-to-valve distance on the performance of peristaltic pumps. The multiplexing approach reported here may find application in a wide range of microfluidic chips for chemical and biological applications, especially those that require the integration of many different operations on a single chip and those that need to perform similar operations massively in parallel, in sub-nanoliter volumes.

1. Introduction

The advent of VLSI (Very Large Scale Integration) microfluidics has enabled multi-step and high-throughput applications with massively parallel operations to be performed on a single chip [1–4]. Key to the development of VLSI microfluidics was the capability to integrate a large number of microfluidic components, such as valves, pumps, chemical reactors and analytical chambers, on a single chip with high density. Of all these microfluidic components, valves and pumps have received the most attention, because these active components are responsible for routing the fluids in almost any complex network of microchannels.

Different approaches to integrate the valves and pumps in microfluidic networks, which primarily differ in the actuation principle of these active components, have been reported in the literature. For example, researchers have integrated pumps based on electrochemical [5], electrostatic [6], and pneumatic [7] actuation and valves based on magnetic [8], piezoelectric [9] and pneumatic [10] actuation. Several of these technologies for integration have been summarized in a recent review [11]. However, most of these technologies are *not* amenable to integration into complex high-density microfluidic chips, due to the intensive nature of the required fabrication procedures, actuation crosstalk between adjacent components, and /or incompatibility of the actuation principle with the chemical or biological process the chip is supporting. A key exception is the pneumatically actuated valves and pumps, which possess a small footprint, fast response time, can be easily integrated, and do not suffer from the limitations mentioned above. Typically, the actuation of pneumatic microfluidic valves and pumps is based on the actuation of a thin membrane by pressurized air in a control layer that is positioned over a network of microchannels embedded in a fluid layer [7]. Implementation of pneumatic pumps and valves has enabled multi-step and high-throughput applications in which massively parallel operations can be performed on a single chip. Examples include the synthesis of radiolabeled imaging probes [2], Sanger sequencing of DNA [1], integrated genetic assays [3] and high-throughput sorting for drug screening [4].

Generally, a highly dense microfluidic chip with several valves and pumps requires many pneumatic connections (tubing) to an external pneumatic controller. Constraints with respect to the size and cost of the ancillary control hardware place a limit to the number of pneumatic valves and pumps that can be integrated on a single chip. Hence, an approach to actuate many valves and pumps with a minimal number of external pneumatic connections is crucial to the further development of highly dense microfluidic chips.

To address this chip interfacing issue, microfluidic multiplexers have been reported for pneumatic, as well as non-pneumatic valves and pumps, analogous to the multiplexers in electronic cir-

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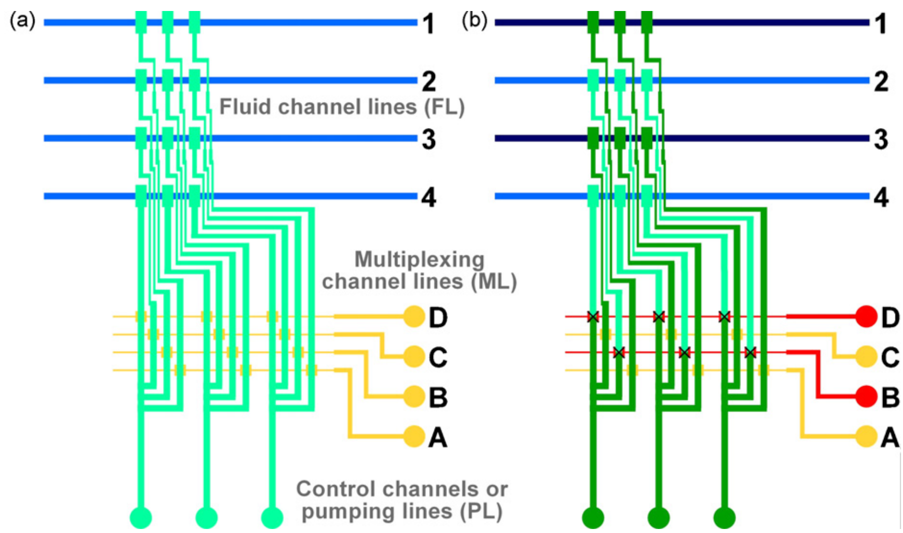

Fig. 1. Microfluidic chip demonstrating the implementation of multiplexed peristaltic pumps. The four fluid channel lines (FL, blue) are each controlled by their own peristaltic pump connected to control channels or pumping lines (PL, green), which in turn are each controlled by their own multiplexing channel lines (ML, yellow). The circles represent external pneumatic connections for the corresponding pumping and multiplexing lines. (a) The chip at rest, with no multiplexing lines actuated. Actuation of the pumping lines would result in fluid flow in all four fluid lines. (b) The chip with two multiplexing lines (ML-B and ML-D) actuated, as indicated in red, which prevents the pumps of FL-2 and FL-4 from being actuated. The pumping lines now can only actuate two of the four pumps (as indicated with dark green), allowing for fluid to be pumped only in FL-1 and FL-3 (dark blue). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)

cuits. Liu et al. [12] used three buffer streams with different pH values to control several pH-responsive hydrogel valves, while Vyawahare et al. [13] used thin wires made of shape memory alloys to control multiple valves and peristaltic pumps with a single wire. However, these *non-pneumatically* actuated valves and pumps are not as versatile as their pneumatic-equivalents in terms of compatibility with on-chip processes and ease of integration into high-density microfluidic networks. One of the earliest microfluidic multiplexers for *pneumatic* valves was reported by Thorsen et al. [14], who developed a binary multiplexer to address $2^{n/2}$ valves with n external connections (control lines). This binary microfluidic multiplexer has since been used in several applications where a high-density microfluidic chip was required, such as gene expression [15], protein crystallization [16] and combinatorial chemistry [17]. In order to further improve the binary multiplexer, Lee and Cho [18] developed ternary and quaternary multiplexers for pneumatic valves by using more than one threshold value for the valve actuation pressure, as opposed to a single threshold pressure used in a binary multiplexer. This ternary and quaternary multiplexing scheme allowed control of $3^{n/2}$ and $4^{n/2}$ valves, respectively, using only n control lines. The first example of multiplexing of the valve lines or the control lines, instead of the fluid channels, was reported by Kawai et al. [19]. Their approach allowed the number of external pneumatic connections required to actuate $2^{n/2}$ valves to be reduced from n (as in the case of traditional binary multiplexers) to $2\log_2 n + 2$.

Although several examples of microfluidic multiplexers for pneumatic valves have been described in the literature, no reports of similar multiplexers for pneumatic pumps exist. Peristaltic pumps, which comprise of a series of pneumatic valves (3 or more valves each), are most commonly used in microfluidics [7]. Although these peristaltic pumps transport fluids at lower flow rates (approximately 1 nL/s) compared to the traditional syringe pumps (greater than 10 nL/s), the peristaltic pumps offer much finer, local control over the flow, both in terms of the fluid response to the on-off switching of the pump and the precise regulation of the flow rate. Additionally, the peristaltic pumps can be integrated on-chip, thus reducing the external ancillaries (no syringe pumps) and the overall system size. Since each peristaltic pump comprises of 3 or more pneumatic valves, the on-chip integration of these pumps rapidly increases the number of control lines needed. Unfortunately, the on-chip peristaltic pumps require specific sequences of valve operation that are unattainable by the various approaches for multiplexing of valves reported previously.

In this paper, we report an approach for multiplexing pneumatic valves that for the first time allows for a large number of peristaltic pumps to be controlled with a limited number of external pneumatic connections. Whereas some of the previously reported approaches for the multiplexing of microfluidic valves require three-layer assemblies [19], the microfluidic chips with multiplexed peristaltic pumps reported here can be assembled from only two layers, thus greatly simplifying chip fabrication and assembly. We demonstrate the utility of these multiplexed peristaltic pumps in a microfluidic chip that integrates droplet-generation and routing capabilities. We also specify a set of design rules for integration of multiplexed peristaltic pumps in microfluidic chips.

2. Design of multiplexed peristaltic pumps

2.1. Design of multiplexed peristaltic pumps

A microfluidic peristaltic pump typically consists of a series of three or more pneumatic valves placed in parallel over a single fluid channel [7]. Individual pneumatic valves consist of a thin membrane, which separates a layer that contains the fluid channel and a layer that contains the control channel (see Fig. S1 in supplementary information). The application of pneumatic pressure in the control channel deflects the membrane into the fluid channel, which stops the flow. Sequential actuation of the pneumatic valves in a peristaltic pump results in pumping action and unidirectional motion of fluid in the fluid channel (see Fig. S2 in the supplementary information for a typical sequence). A single peristaltic pump requires individual pneumatic connections to an external pneumatic controller for each of its valves. Hence, the number of pumps that can be integrated on a single chip will be limited by the number of pneumatic connections available from the ancillary equipment.

To reduce these scaling limitations, we propose a novel approach for the multiplexing of pneumatic valves that enables the integration of multiplexed peristaltic pumps as shown in Fig. 1(a). The chip consists of four microfluidic fluid channel lines (FL, blue, 1–4 in the figure) each being driven by its own 3-valve peristaltic pump that is operated by control channels or pumping lines (PL, green). Note that all four sets of the pumping valves are connected to the same pneumatic inlet, i.e. the first valve for each pump is connected to one inlet, and so on for the second and third valves (or for any additional valves that may be present in a given pump). A set of four multiplexing channel lines (ML, yellow, A–D in the figure) is routed across all the pumping lines. Each multiplexing line has multiplexing valves that can selectively close off the three pumping lines associated with the peristaltic pump of a given fluid line. At the same time, the multiplexing line will not affect the pumping lines associated with the pumps of the other fluid lines, because the sections of the multiplexing line that intersect the unrelated pumping lines are designed to be as thin as possible. Consequently, the surface area of the membrane between the fluid channel and the control channel is small, so the membrane will not deflect completely at the pressures normally used to actuate the valves. For example, opening or closing multiplexing line A (ML-A) will open or close only the pumping lines for fluid line 1 (FL-1), and so on.

Fig. 1(a) shows the chip at rest, with none of the multiplexing lines actuated. Actuation of the pumping lines would result in fluid flow in all four fluid lines. Fig. 1(b) shows the multiplexed operation of the peristaltic pumps. In this configuration, ML-B and ML-D are actuated or closed, which implies that the peristaltic pumping lines for FL-2 and FL-4 are also closed, and hence the peristaltic pumps cannot pump fluid in those channels. However, ML-A and ML-C are open, so when the pumping lines are actuated with a typical pumping sequence, the valves for FL-1 and FL-3 will pump fluid in those channels. The multiplexing scheme shown in Fig. 1 reduces the number of required external pneumatic connections from 12, in case of a non-multiplexed configuration, to 7, in case of the multiplexing approach introduced here. Later, we will elaborate on the general scaling rules of this multiplexing approach.

The multiplexing configuration shown in Fig. 1 is achieved in an actual microfluidic chip by the assembly of two layers. The upper layer contains the control channels or the pumping channels that operate push-down valves (those that form the peristaltic pumps), and the lower layer contains the fluid channels, as well as the multiplexing channels that operate push-up valves to select which pumps are to be actuated (Fig. 2). For clarity of illustration, only a single valve of a pumping channel (i.e. part of each peristaltic pump) and a single valve of a multiplexing channel are shown. This two-layer fabrication approach could also be used for the much simpler fabrication and assembly of the previously reported chips based on three-layer fabrication [19].

A key design feature of the multiplexing approach introduced here is the use of push-up valves in the multiplexing channels to selectively close different sets of pumping channels, and the use of push-down valves that form the peristaltic pumps associated with the different fluid channels. Not only does this approach reduce the number of layers required to create the multiplexing configuration from three to two, it also reduces the actuation pressure needed in the multiplexing lines. Simultaneous actuation of the pumping lines and the multiplexing lines is required for the multiplexed operation of the peristaltic pumps. Therefore, the actuation pressure in the multiplexing lines (p_{ML}) has to be larger than that in the pumping lines (p_{PL}), so that p_{ML} is high enough to close the multiplexing valves while acting against p_{PL}. For the geometries and dimensions used here, a pressure difference of approximately 20 psi is required to close the push-down valves of the peristaltic pumps. Thus, a pressure of 40 psi would be needed to actuate the multiplexing valves to overcome the pressure in the pumping lines. However, push-up valves require lower actuation pressures compared to push-down valves due to the differences in the geometry of the valve and the channel (see supplementary information) [20]. Here, the multiplexing valves are designed to be

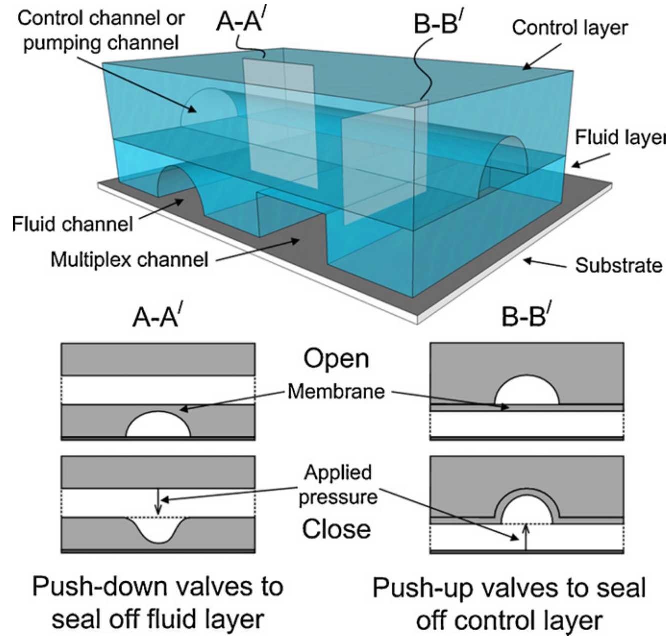

Fig. 2. Schematic illustration of the integration of push-down and push-up valves into a single two-layer chip. The lower fluid layer contains the fluid channels, as well as the multiplexing channels that operate push-up valves to select which pumps are to be actuated. The upper layer contains the control channels (pumping channels) that operate the push-down valves of the pumps. This approach enables the multiplexing approach introduced here, which involves selective actuation of different peristaltic pumps.

push-up valves, so a p_{ML} of only 30 psi is needed to close the valves completely.

2.2. Key aspects of the multiplexing approach for peristaltic pumps

The multiplexing approach introduced here allows for a significant reduction in the number of external pneumatic connections necessary to operate a given configuration of peristaltic pneumatic pumps. In a traditional non-multiplexed configuration of peristaltic pumps (NMP), the number of connections needed, N_{NMP}, would be

$$N_{NMP} = N_{pumps} \times N_{valves}, \tag{1}$$

where N_{pumps} is the number of peristaltic pumps in the chip and N_{valves} is the number of valves used per pump. In contrast, the multiplexing approach introduced here (MP) reduces the number of external connections, N_{MP}, to

$$N_{MP} = N_{pumps} + N_{valves}. \tag{2}$$

The difference between N_{MP} and N_{NMP} will become more significant when the number of pumps and the valves per pump in a microfluidic chip increases. Our proof-of-principle chip consists of 4 pumps with 3 valves per pump, so 12 pneumatic connections would have been needed for a non-multiplexed configuration, whereas only 7 connections are needed in the multiplexed design shown in Fig. 1. Upon increasing the complexity of the chip to 10 pumps comprising of 4 valves each, N_{NMP} would be 40, whereas N_{MP} would be only 14 for the multiplexing approach introduced here.

Apart from requiring fewer external pneumatic connections, the multiplexing approach introduced here offers two additional advantages. Firstly, the multiplexing channels are integrated in the same layer as the fluid channels, thereby reducing the number of layers from three to two. This reduction in number of layers greatly facilitates the assembly of high-density microfluidic chips containing multiplexed pumps and valves. Secondly, due to the fact that the corresponding valves of multiple peristaltic pumps are connected to the same pneumatic inlet, a single actuation sequence of the pumping lines results in simultaneous operation of all the connected pumps. Furthermore, in our multiplexed design, individual pumps can rapidly be switched on/off by actuation of only a single multiplexing line. In contrast, a non-multiplexed configuration would require each valve of each pump to be actuated individually. As a result, the operation of multiple pumps using our multiplexing approach is significantly simplified from a software (programming) and hardware (time required for execution of a single command) point of view, compare to the non-multiplexed configuration. This simplification becomes particularly important for the operation of chips with a large number of pumps and valves.

Multiplexing of peristaltic pumps can also be implemented using the previously reported binary multiplexing approach for individual valves [14]. However, the multiplexing approach introduced here can address valves of multiple peristaltic pumps simultaneously (parallel operation), whereas the conventional binary multiplexing approach can address each valve of each pump sequentially (serial operation). The latter is much more operationally intensive, which becomes an issue especially in cases where many pumps need to be actuated in short periods of time. The advantage gained by the exponential decrease of external connections for the conventional (serial) multiplexing approach compared to the additive decrease of external connections for the (parallel) multiplexing approach introduced here, is offset by reduced throughput in large microfluidic networks, where simultaneous operation of or switching between multiple peristaltic pumps is required.

One drawback to the multiplexing approach introduced here is that sets of multiplexed pumps can only be operated in a 'single mode' at a time. The interconnected pumps are all actuated using the same sequence, so fluid flow will be at an identical rate and in the same direction. However, this drawback can be overcome by performing the desired actuations sequentially (e.g. different pump cycle rates) while switching between different multiplexing lines, so fluid flow in different fluid lines can be controlled independently, with the only sacrifice being a reduced throughput (e.g. serial versus parallel operation of multiple pumps).

3. Fabrication of the multiplexed peristaltic pumps

The fabrication procedure for the multiplexed valve design (Fig. 2) is schematically illustrated in Fig. 3. The fabrication process for the multiplexed valve design followed the typical procedure for multilayer soft lithography for PDMS-based microfluidics [7], except that here, different channel functionalities were integrated in the same layer using different photoresists.

First, the 35-μm thick multiplexing channels were patterned on a silicon wafer using negative photoresist (SU-8 50) via standard lithographic techniques. The negative resist yields channels with vertical sidewalls and rectangular cross-sections, as is necessary for complete closure of the push-up valves [20]. Next, 12-μm thick fluid channels were patterned on the same wafer using positive photoresist (SJR-5740). The positive photoresist was heated to just above its glass transition temperature to reflow the resist, resulting in a rounded semi-circular cross-section for the fluid channels (Fig. 3(a)). This semi-circular profile is needed to ensure that the push-down valves completely seal off the fluid channel [7].

On a second silicon wafer, the 35-μm thick control or pumping channels of the control layer were patterned using a different, more viscous positive photoresist (50XT) than that used for the fluid channels. To create the rounded profile, the positive resist was reflowed by heating to just above its glass transition temperature. Conventionally in multilayer PDMS chips, the control channels are

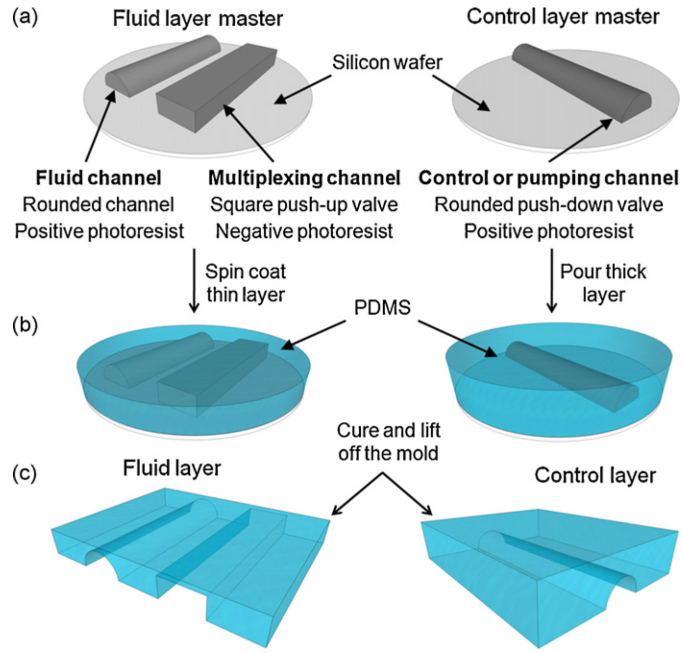

Fig. 3. Fabrication procedure for the two-layer multiplexed valve structure shown in Fig. 2. (a) Positive relief masters (photoresist on silicon) for the two layers. The fluid layer consists of the rectangular multiplexing channels patterned using negative photoresist, and the rounded fluid channels patterned using reflowed positive photoresist. The control layer consists of the control or pumping channels patterned using thick reflowed positive photoresist. (b) Replica molding of the masters: a thin layer of PDMS was spun on the master for the fluid layer, while a thick layer of PDMS was poured on the master for the control layer. (c) After curing the PDMS, the thick PDMS control layer was lifted off its master and aligned and bonded to the PDMS pattern of the underlying fluid master layer. Subsequently, this combined mold was bonded and sealed against a glass substrate to form the structure shown in Fig. 2.

patterned using negative photoresist. However, positive photoresist was used to pattern the control channels here to obtain rounded channels after reflow, because the multiplexing valves need to be able to close of these channels (pumping channels) through push-up valve architectures.

After photoresist patterning and reflow, a silane monolayer was evaporated onto the masters to prevent the adhesion of PDMS to the silicon substrates. Next, a thin layer of PDMS was spun on the master for the fluid layer, while a thick layer of PDMS was poured on the master for the control layer. After curing the PDMS, the thick PDMS control layer was lifted off its master, aligned to the thin PDMS pattern of the underlying fluid master layer, and bonded using standard procedures for multilayer soft lithography [7]. Finally, the two-layer PDMS assembly was bonded to a glass substrate, after activation of the surfaces using plasma, to form the structure shown in Fig. 2. Further details of the fabrication procedure are provided in the supplementary information.

4. Application of the multiplexed peristaltic pumps in microfluidic chips

4.1. Characterization of the peristaltic pumps

First, we characterized the individual peristaltic pumps with respect to valve geometry and valve spacing to arrive at an optimized configuration that will result in maximum fluid displacement rate (flow rate). For this purpose, we created a microfluidic chip for the manipulation of two-phase flows. Specifically, we used peristaltic pumps to merge an aqueous and an oil stream at a T-junction to generate a two-phase flow comprised of water droplets in an oil stream. Further details of the droplet-generation are pro-

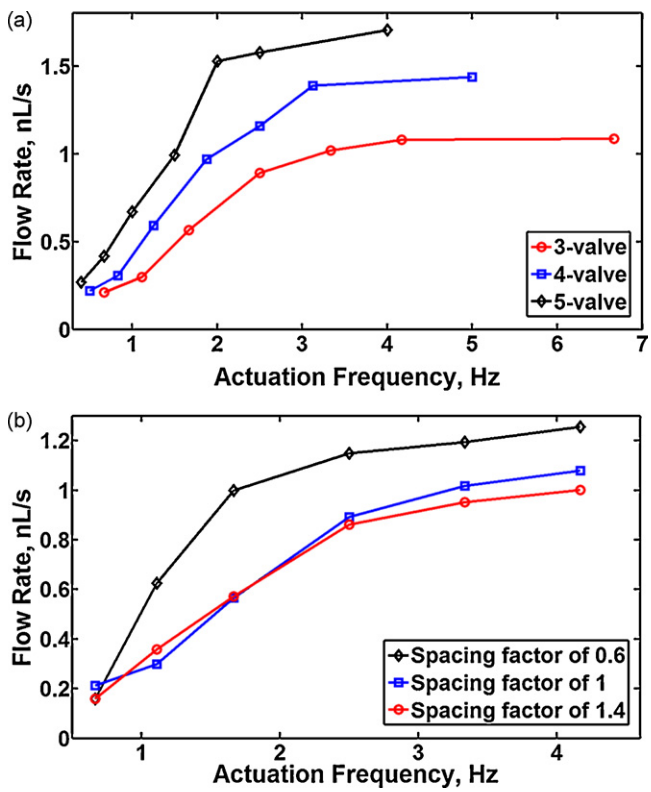

Fig. 4. Characterization of peristaltic pumps: flow rate as a function of (a) the number of valves per peristaltic pump and (b) the spacing between individual valves, with the number of valves fixed at three.

vided in the supplementary information. We first studied the effect of the number of valves (varied from 3 to 5) in a peristaltic pump on the flow rate in 125 μm wide and 12 μm tall fluid channels. The valves of the peristaltic pumps were 150 μm wide, 35 μm tall, and spaced apart by 150 μm. The flow rate was measured optically under a microscope by tracking the position of a droplet over time.

Fig. 4(a) shows the plot of flow rate versus actuation frequency, the rate at which the individual valves in the peristaltic pumps are opened and closed (as opposed to the rate for an entire pumping cycle), for peristaltic pumps comprised of 3, 4, or 5 valves. A maximum flow rate of 1.7 nL/s was observed for the 5-valve pump at an actuation frequency of 4 Hz. For all three pumps, the flow rates increased as the actuation frequency increased, until the flow rates began to saturate at approximately 5 Hz. This saturation behavior is due to the fact that the dynamic deflection of the valve membrane can no longer follow the actuation frequency, which results in a transfer of lesser volume of fluid for a partially closed valve. This saturation behavior is in agreement with the predictions of previously reported analytical models for these types of microfluidic configuration [21–23]. We also observed no measurable flow rate at frequencies higher than 10 Hz, which is again a typical characteristic of the frequency response of peristaltic pumps [7,21–23]. The flow rate increases with increasing number of valves, due to the fact that the volume of fluid advanced per cycle also increases [22]. Although higher number of valves result in higher flow rates, the additional valves results in an increase in the size of the chip and also the number of required external connections. Hence, the final number of valves in a single pump will depend on the requirements and space constraints of the end-application.

Next, we studied the effect of valve spacing on the flow rate, with the number of valves per pump fixed at three. The fluid channels were 125 μm wide and 12 μm tall, while the pumping valves were 150 μm wide and 35 μm tall. The spacing between the valves was varied between 90, 150, and 210 μm, to result in ratios of valve spacing to valve width of 0.6, 1, and 1.4, respectively. We define this ratio as the spacing factor. Fig. 4(b) shows a graph of the flow rate as a function of actuation frequency for the three pumps with different spacing factors. The same trend as in Fig. 4(a) is observed, in that the flow rate increases with increasing valve frequency, saturates at approximately 5 Hz, and drops to zero for frequencies on the order of 10 Hz.

In addition, the results shown in Fig. 4(b) indicate that the flow rates are higher when the gap between the valves in a pump is less than the size of the valves themselves. The peristaltic pumping mechanism relies on the fact that the entire downstream fluid line will be advanced by a volume of liquid equal to the volume directly underneath a valve, when that valve is deflected downward. However, these pumps are fabricated out of PDMS, which is a soft elastomeric material and can be easily deformed. Therefore, when a valve is actuated, the volume of the liquid displaced not only moves the fluid column but deforms the PDMS channel walls as well. Liquid in the channel far away from the actuated valve will be unaffected, because some of the displaced volume will be used to deform the PDMS, before it slowly relaxes back to its original state. The farther the valves are spaced apart within a peristaltic pump, the less of the actual liquid volume displacement is transferred to the next valve, resulting in lower flow rates.

4.2. *Implementation of multiplexed peristaltic pumps in a microfluidic chip*

After characterizing the operation of individual peristaltic pumps, we used the 2-layer multiplexing approach described in Section 2 (Fig. 1) to create a microfluidic chip with multiplexed peristaltic pumps shown in Fig. 5. This chip consists of two fluid lines (FL-1 and FL-2), each being driven by its own multiplexed 3-valve peristaltic pump (blue), which in turn are each controlled by separate multiplexing lines (ML-A and ML-B). The fluid lines and the multiplexing lines are molded in the thinner lower PDMS layer, while the pumping lines are molded in the thicker upper layer, following the 2-layer approach described in Section 3 (Fig. 3). The center column of images in Fig. 5 shows the entire chip for three different stages of the valve actuation – parts (a), (b), and (c) – while the magnified images on the left and right show close-up views of the pumping valves and multiplexing valves, respectively. Each individual control line, either a multiplexing or a pumping line, was actuated using a single dedicated solenoid valve connected to a single external pressure source. A dedicated solenoid valve for each control line allows for independent actuation of the control lines, as is required for the operation of peristaltic pumps and for choosing an arbitrary set of peristaltic pumps using the multiplexing scheme discussed here.

In Fig. 5(a), none of the valves are actuated and all fluid lines are open. In Fig. 5(b), ML-B has been actuated by applying a pressure of 30 psi, thereby closing its three valves which restrict flow in the underlying pumping lines associated with FL-2. In Fig. 5(c), the three pumping lines have been actuated by applying a pressure of 20 psi, while the valves on ML-B are still closed, so only the pumping valves of FL-1 are actuated (closed), while the valves of the peristaltic pump on FL-2 remain unaffected (open). Note that the 10 psi net difference in the actuation pressures for the pumping and multiplexing lines is sufficient to completely close the push-up multiplexing valves, as discussed in Section 2.2. A video showing the multiplexed operation of this chip, fluid being pumped in FL-2, but not FL-1 because ML-B has been actuated, is provided as supplementary material. *The selective actuation of one set of peristaltic pumping valves of a set of two, of which all valves are connected to common pneumatic inlets, demonstrates one of the key aspects of the multiplexing design introduced here*. In this example of 2 multi-

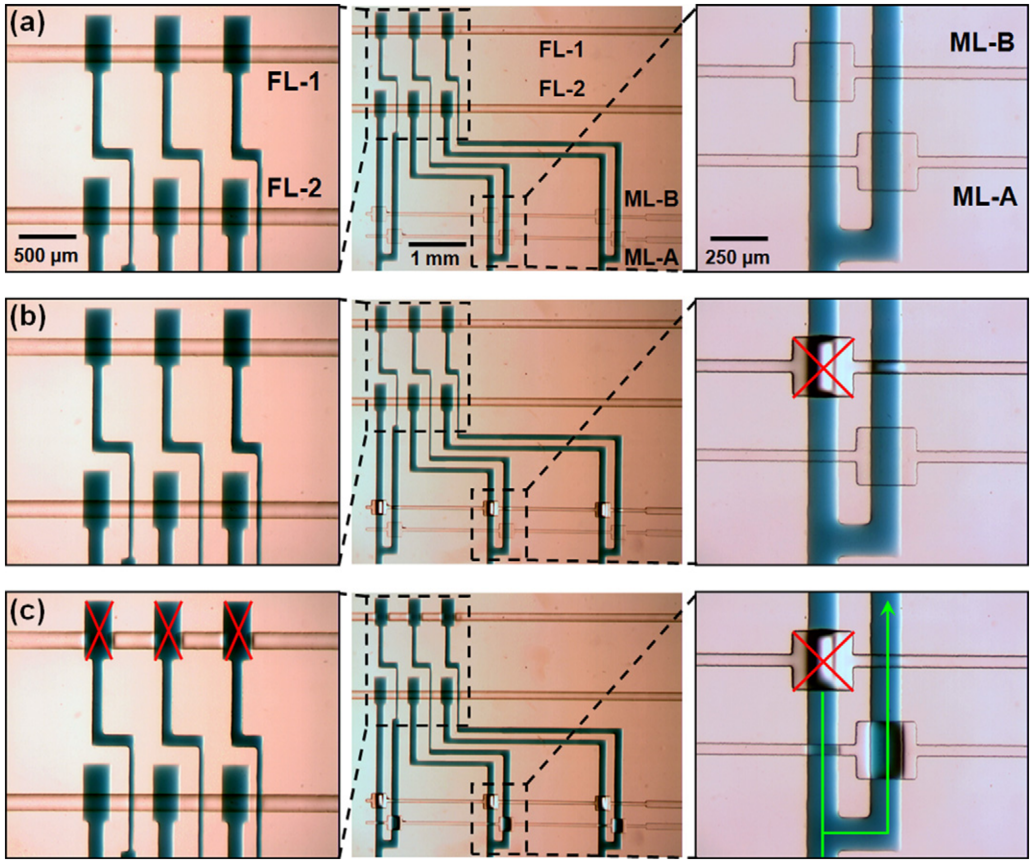

Fig. 5. Microfluidic chip to demonstrate the operation of multiplexed peristaltic pumps. The fluid lines (FL-1 and FL-2) and the multiplexing lines (ML-A and ML-B), rendered colorless in figure, were molded in the lower PDMS layer. The pumping lines (blue) were molded in the thicker upper layer. (a) All the valves and all the lines are open. (b) ML-B is actuated (represented by a red cross through one of its valves), thereby closing off the pumping lines beneath it. (c) All the pumping lines are actuated, allowing the valves of the pump for FL-1 to also be actuated, while ML-B remains closed, so the valves of the pump in FL-2 remain unaffected. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)

plexed pumps, the number of external connections is only reduced by 1 (5 instead of 6), but, as explained in Section 2, the difference between the required number of external connections for a multiplexed and a non-multiplexed chip rapidly increases with an increasing number of peristaltic pumps and valves within a single chip.

4.3. A microfluidic chip with integrated multiplexed peristaltic pumps for routing of droplets

Next, we demonstrated the application of multiplexed peristaltic pumps in a microfluidic chip designed to generate and route droplets. In recent years, droplet-based microfluidics have received increased attention in the fields of biological and chemical synthesis and analysis, due to the advantages of efficient mixing, reduced dispersion of reagents, and the ability to perform parallel experiments. These advantages and the many applications of droplet-based microfluidics have been discussed in detail in recent review articles [24–27]. The microfluidic chip (Fig. 6) for routing droplets consists of two droplet-generating T-junctions, capable of introducing droplets of two different liquids to a main inlet channel that leads into a 3×3 node channel array. The 3×3 array is connected to 11 routing channels, where each routing channel is actuated by its own 4-valve peristaltic pump. The pumps are used to advance the droplets along the vertical and horizontal channels within the array. The dimensions of the pumps are the same as the peristaltic pumps discussed in Section 4.1, with a spacing factor of 1. These 11 pumps are arranged into 3 groups of multiplexed pumps, thereby reducing the number of external connections necessary for those pumps from 44 to 23. The peristaltic pumps used for droplet-generation via the T-junctions were not multiplexed, because droplet-generation does require that the pumps drive the water and oil streams at different rates simultaneously, which is less straightforward using our multiplexing approach, as discussed in Section 2. However, in systems for routing droplets in larger arrays, the number of pumps needed for droplet-generation will be small compared to the number needed for routing droplets. Consequently, the loss in efficiency caused by using non-multiplexed pumps for droplet-generation will not be significant.

Fig. 6 shows a schematic illustration of the design and the fabrication procedure for the microfluidic chip. The chip was fabricated using the two-layer fabrication procedure discussed in Section 3, but with slight differences, which are discussed in the supplementary information. The resulting chip is shown in Fig. 7, with all channel/lines filled with a differently dyed solution to aid in visualization: fluid channels are red, multiplexing channels blue, droplet-generating pumps yellow, and the multiplexed pumps purple.

To demonstrate the capabilities of the multiplexed peristaltic pumps in an integrated microfluidic system, the chip shown in Fig. 7 was used to generate discrete liquid droplets, and then route these droplets through the 3×3 channel array. Optical micrographs of a single droplet's path at different instants of time are shown in Fig. 8. A water droplet (dyed orange) was generated in a carrier oil stream (FC 40) using the standard approach for droplet-generation via a T-junction in a two-phase flow [28]. Details of the generation of droplets using a T-junction are provided in the

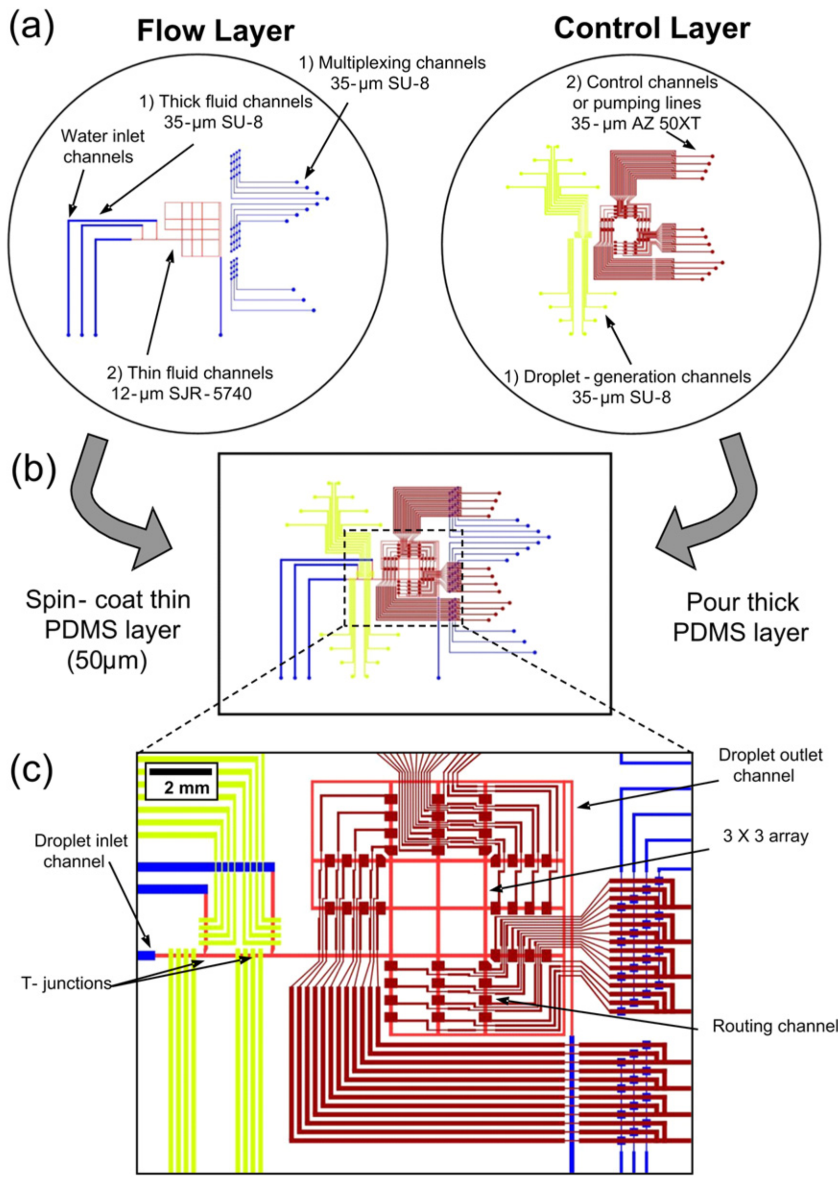

Fig. 6. Channel layout and fabrication procedure for the microfluidic chip designed to generate and route droplets. (a) The fluid layer master consists of the multiplexing channels (blue) and the inlet fluid channels (also blue) patterned first using 35-μm SU-8 (negative photoresist), followed by the fluid channel array (red) patterned next using 12-μm SJR-5740 (positive photoresist). The control layer master consists of the peristaltic pumping channels and valves for the generation of droplets (yellow) patterned first using 35-μm SU-8, and the multiplexed peristaltic pumping channels (maroon) patterned next using 35-μm AZ 50XT (positive photoresist). (b) Schematic illustration of the completed chip fabricated by assembly and alignment of a spin-coated thin layer of PDMS replicating the fluid layer master (~50 μm) and a thicker, replica-molded PDMS layer of the control layer. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)

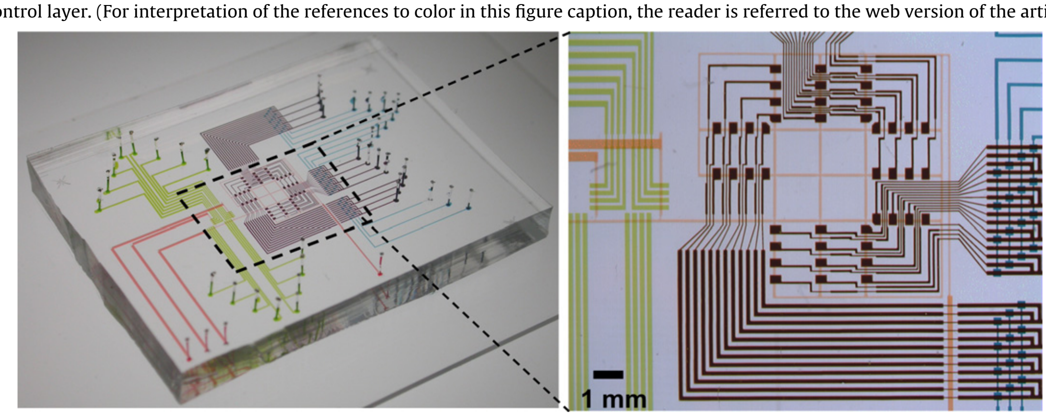

Fig. 7. Optical micrograph of the PDMS-based microfluidic chip to generate and route droplets. The red fluid channels and the blue multiplexing channels with push-up valves were molded in the thinner lower PDMS layer. The multiplexed peristaltic pumping channels with push-down valves (purple) and the droplet-generation pumping channels with push-down valves (yellow) were molded in the thicker upper PDMS layer. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)

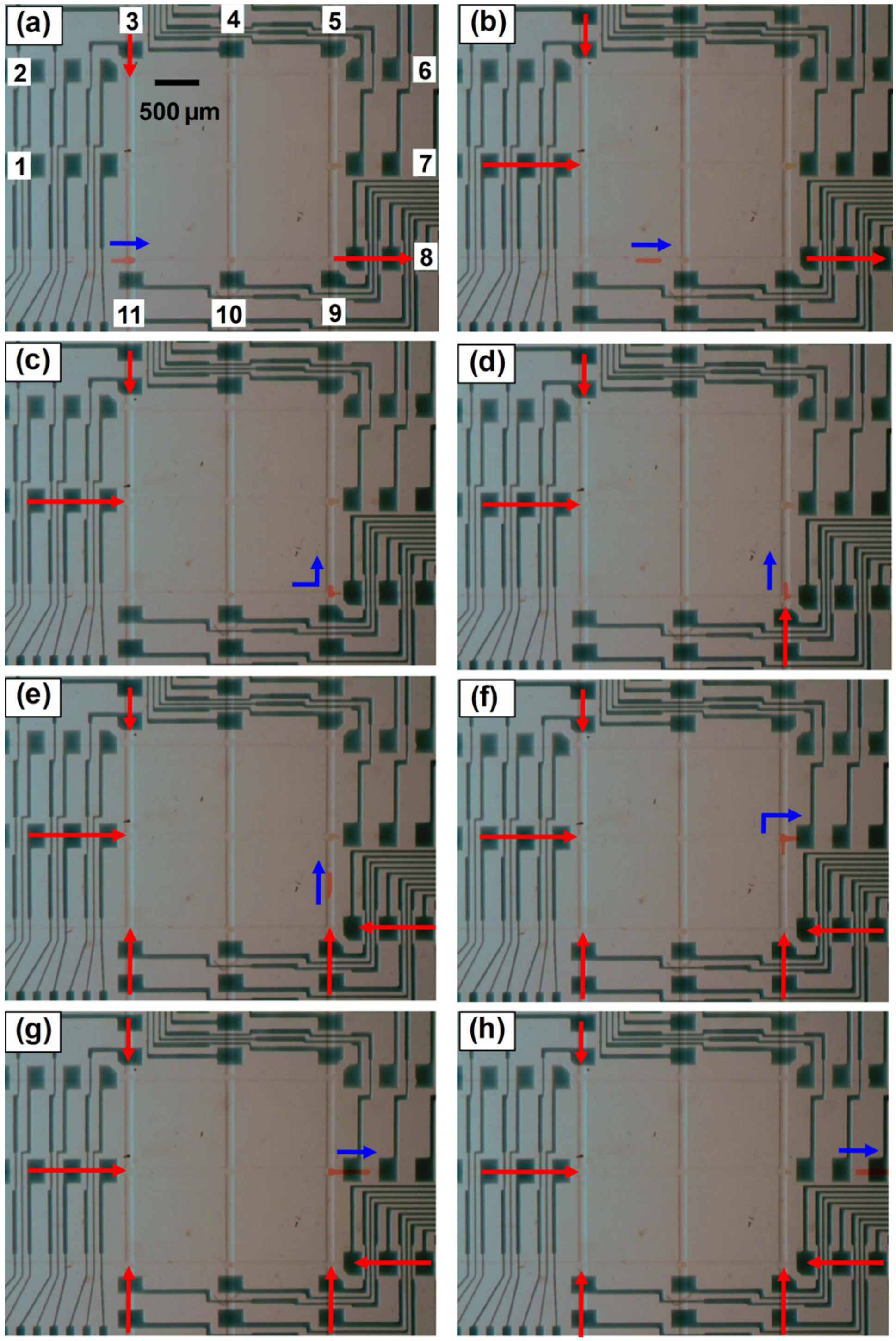

Fig. 8. Optical micrographs of a water droplet (orange) being manipulated through the droplet-routing chip. Red arrows indicate that a given pump is pumping in the direction of the arrow. Blue arrows indicate the position and direction of the droplet. The orange color of the water droplet has been enhanced in the figure for clarity. (a) The water droplet is introduced into the 3 × 3 array using the upstream oil pump and Pumps 3 and 8. (b) The droplet continues through the array using the concerted action of Pumps 1, 3 and 8. (c) The droplet reached the bottom right corner and Pump 8 was switched off. (d) Pump 9 was switched on to drive the droplet upward, along the right side of the array. (e) When the droplet was past the corner, Pump 8 was switched back on, now pumping in the opposite direction, along with Pump 11. (f–h) The droplet reached the next intersection and was driven out of the array (toward the right) by the concerted actuation of Pumps 1, 3, 8, 9 and 11. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)

supplementary information. The water droplet is advanced from the droplet-generation section to the 3 × 3 node array by driving the upstream oil pump in the droplet-generation section (which can be seen in Fig. 7), along with the inward and outward pumping of multiplexed Pumps 3 and 8, respectively, which can be seen in Fig. 8(a).

In Fig. 8(b), the droplet has entered the 3 × 3 array, and Pump 1 was switched on to help drive the droplet towards the right. In Fig. 8(c), the droplet has reached the lower right node, and must be directed upward, instead of being allowed to continue along its current straight path. At this point, Pump 8 (the pump nearest to the droplet) was switched off. In Fig. 8(d), the droplet was advanced around the corner and up the right side of the array by switching on Pump 9. Once the droplet cleared the intersection, Pump 8 was switched back on, and pumped inward, along with Pump 11 (Fig. 8(e)). When the droplet reached the next intersection in Fig. 8(f), the flow generated from Pump 1 forced the droplet to again change directions. Fig. 8(g) and (h) shows the droplet being pushed towards the outlet channel under Pump 7, and then finally out of the array. At this point another droplet could be introduced into the array and similarly routed to other locations on the grid. Fig. 8 clearly demonstrates the utility of the multiplexed peristaltic pumps to perform complex operations on a single integrated chip. A video of the droplet-routing experiment shown in Fig. 8 is provided as supplementary material.

5. Design rules for integration of multiplexed peristaltic pumps

Key design rules for integration and operation of multiplexed peristaltic pumps in high-density microfluidic chips emerged from the experiments described above are as follows:

1. The multiplexing design for peristaltic pumps shown in Fig. 1 will reduce the number of required external pneumatic connections from $N_{pumps} \times N_{valves}$ for a non-multiplexed configuration (N_{NMP}) to $N_{pumps} + N_{valves}$ for the multiplexed approach (N_{MP}) introduced here. N_{pumps} and N_{valves} denote, respectively, the number of peristaltic pumps and valves used per pump in the microfluidic chip. By comparison of N_{NMP} and N_{MP}, one can decide whether or not to implement a multiplexing approach at the expense of a less intuitive channel network design and associated fabrication.
2. The sections of the pumping lines that connect the valves of the peristaltic pumps to the pneumatic inlet (green lines in Fig. 1) need to be as thin as possible because they cross other fluid lines between actuation valves, thereby setting a lower limit to the valve-to-valve spacing. As discussed in Section 4.1, the maximum achievable flow rate decreases upon increasing the distance between adjacent valves of a peristaltic pump. Hence, these thin sections of the pumping lines should be made as narrow as possible, within the limits of the fabrication procedure.
3. Push-up valves should be used for the multiplexing lines and push-down valves for the pumping lines, and not vice-versa, to allow for the multiplexed chip to be fabricated using only two layers. Furthermore, as already specified in Section 2, the lower required pressure for actuation of push-up valves reduces the required pressure for actuation of the multiplexing lines from >40 to 30 psi.
4. Typically, the use of peristaltic pumps comprised of 4 valves is preferable over 3-valve pumps in heavily multiplexed microfluidic networks. Pumping lines have to cross in-between adjacent valves of multiplexed peristaltic pumps. The reduced performance due to the increased valve-to-valve spacing can be compensated by increasing the number of valves per pump, as discussed in Section 4.1. Note that the number of multiplexed pumps per set is limited, because for each additional pump, an additional pumping line has to cross between the valves of the other peristaltic pumps within the same multiplexed set.

6. Conclusions

In this paper, we introduced a novel multiplexing approach for integration and operation of pneumatic peristaltic pumps in microfluidic chips. The multiplexing approach greatly reduces the number of external pneumatic connections required for the operation of sets of peristaltic pumps, which is important for the development of ever more complex VLSI microfluidic chips. We demonstrated the utility of multiplexed peristaltic pumps by generating and routing droplets in a 3×3 microfluidic array. The implementation of both push-up valves and push-down valves within the same chip enabled integration of two of the three necessary channel networks, the multiplexing lines and the fluid lines, in a single layer. As a result, these multiplexed microfluidic chips can be obtained by alignment and assembly of just two microfluidic molds. This simplified soft-lithographic fabrication procedure facilitates integration of multiple sets of multiplexed pumps into complex microfluidic chips, because the fabrication procedures minimizes alignment issues associated with the assembly of chips comprised of 3 or more layers.

Many parameters can have a profound effect on the performance of individual peristaltic pumps, as well as on multiplexed sets of these pumps. We characterized the performance (i.e. achievable flow rates) of individual peristaltic pumps as a function of the number of valves and valve-to-valve spacing. We expressed the results of this study in a set of design rules for the integration and operation of multiplexed peristaltic pumps in high-density microfluidic chips. The multiplexing approach reported here may find application in a wide range of microfluidic chips for chemical and biological applications, especially those that require the integration of many different operations on a single chip and those that need to perform similar operations massively in parallel in sub-nanoliter volumes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.snb.2010.07.012.

References

[1] R.G. Blazej, P. Kumaresan, R.A. Mathies, Microfabricated bioprocessor for integrated nanoliter-scale Sanger DNA sequencing, Proceedings of the National Academy of Sciences 103 (2006) 7240–7245.
[2] C.-C. Lee, G. Sui, A. Elizarov, C.J. Shu, Y.-S. Shin, A.N. Dooley, J. Huang, A. Daridon, P. Wyatt, D. Stout, H.C. Kolb, O.N. Witte, N. Satyamurthy, J.R. Heath, M.E. Phelps, S.R. Quake, H.-R. Tseng, Multistep synthesis of a radiolabeled imaging probe using integrated microfluidics, Science 310 (2005) 1793–1796.
[3] R. Pal, M. Yang, R. Lin, B.N. Johnson, N. Srivastava, S.Z. Razzacki, K.J. Chomistek, D.C. Heldsinger, R.M. Haque, V.M. Ugaz, P.K. Thwar, Z. Chen, K. Alfano, M.B. Yim, M. Krishnan, A.O. Fuller, R.G. Larson, D.T. Burke, M.A. Burns, An integrated microfluidic device for influenza and other genetic analyses, Lab on a Chip 5 (2005) 1024–1032.
[4] C.B. Rohde, F. Zeng, R. Gonzalez-Rubio, M. Angel, M.F. Yanik, Microfluidic system for on-chip high-throughput whole-animal sorting and screening at subcellular resolution, Proceedings of the National Academy of Sciences 104 (2007) 13891–13895.
[5] H. Suzuki, R. Yoneyama, Integrated microfluidic system with electrochemically actuated on-chip pumps and valves, Sensors and Actuators B: Chemical 96 (2003) 38–45.
[6] R. Zengerle, J. Ulrich, S. Kluge, M. Richter, A. Richter, A bidirectional silicon micropump, Sensors and Actuators A: Physical 50 (1995) 81–86.
[7] M.A. Unger, H.P. Chou, T. Thorsen, A. Scherer, S.R. Quake, Monolithic microfabricated valves and pumps by multilayer soft lithography, Science 288 (2000) 113–116.
[8] K.W. Oh, R. Rong, C.H. Ahn, Miniaturization of pinch-type valves and pumps for practical micro total analysis system integration, Journal of Micromechanics and Microengineering 15 (2005) 2449–2455.

[9] G. Waibel, J. Kohnle, R. Cernosa, M. Storz, M. Schmitt, H. Ernst, H. Sandmaier, R. Zengerle, T. Strobelt, Highly integrated autonomous microdosage system, Sensors and Actuators A: Physical 103 (2003) 225–230.
[10] W.H. Grover, R.H.C. Ivester, E.C. Jensen, R.A. Mathies, Development and multiplexed control of latching pneumatic valves using microfluidic logical structures, Lab on a Chip 6 (2006) 623–631.
[11] K.W. Oh, C.H. Ahn, A review of microvalves, Journal of Micromechanics and Microengineering 16 (2006) R13–R39.
[12] C. Liu, J.Y. Park, Y. Xu, S. Lee, Arrayed pH-responsive microvalves controlled by multiphase laminar flow, Journal of Micromechanics and Microengineering 17 (2007) 1985–1991.
[13] S. Vyawahare, S. Sitaula, S. Martin, D. Adalian, A. Scherer, Electronic control of elastomeric microfluidic circuits with shape memory actuators, Lab on a Chip 8 (2008) 1530–1535.
[14] T. Thorsen, S.J. Maerkl, S.R. Quake, Microfluidic large-scale integration, Science 298 (2002) 580–584.
[15] J.F. Zhong, Y. Chen, J.S. Marcus, A. Scherer, S.R. Quake, C.R. Taylor, L.P. Weiner, A microfluidic processor for gene expression profiling of single human embryonic stem cells, Lab on a Chip 8 (2008) 68–74.
[16] C.L. Hansen, M.O.A. Sommer, S.R. Quake, Systematic investigation of protein phase behavior with a microfluidic formulator, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 14431–14436.
[17] G.A. Cooksey, C.G. Sip, A. Folch, A multi-purpose microfluidic perfusion system with combinatorial choice of inputs, mixtures, gradient patterns, and flow rates, Lab on a Chip 9 (2009) 417–426.
[18] D.W Lee, Y.-H. Cho, High-radix microfluidic multiplexer with pressure valves of different thresholds, Lab on a Chip 9 (2009) 1681–1686.
[19] K. Kawai, Y. Shibata, M. Kanai, S. Shoji, Multiplexed pneumatic valve control system for large scale integrated microfluidic circuit (LSIMC), in: Twelfth International Conference on Miniaturized Systems for Chemistry and Life Sciences, San Diego, California, USA, 2008, pp. 683–685.
[20] V. Studer, G. Hang, A. Pandolfi, M. Ortiz, W.F. Anderson, S.R. Quake, Scaling properties of a low-actuation pressure microfluidic valve, Journal of Applied Physics 95 (2004) 393–398.
[21] F. Goldschmidtboing, A. Doll, M. Heinrichs, P. Woias, H.J. Schrag, U.T. Hopt, A generic analytical model for micro-diaphragm pumps with active valves, Journal of Micromechanics and Microengineering 15 (2005) 673–683.
[22] J. Goulpeau, D. Trouchet, A. Ajdari, P. Tabeling, Experimental study and modeling of polydimethylsiloxane peristaltic micropumps, Journal of Applied Physics 98 (2005) 044914–44919.
[23] J. Xie, J. Shih, Q. Lin, B. Yang, Y.-C. Tai, Surface micromachined electrostatically actuated micro peristaltic pump, Lab on a Chip 4 (2004) 495–501.
[24] G.F. Christopher, S.L. Anna, Microfluidic methods for generating continuous droplet streams, Journal of Physics D: Applied Physics 40 (2007) R319–R336.
[25] A. Huebner, S. Sharma, M. Srisa-Art, F. Hollfelder, J.B. Edel, A.J. deMello, Microdroplets: a sea of applications? Lab on a Chip 8 (2008) 1244–1254.
[26] H. Song, D.L. Chen, R.F. Ismagilov, Reactions in droplets in microfluidic channels, Angewandte Chemie International Edition 45 (2006) 7336–7356.
[27] S.-Y. Teh, R. Lin, L.-H. Hung, A.P. Lee, Droplet microfluidics, Lab on a Chip 8 (2008) 198–220.
[28] T. Thorsen, R.W. Roberts, F.H. Arnold, S.R. Quake, Dynamic pattern formation in a vesicle-generating microfluidic device, Physical Review Letters 86 (2001) 4163.

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