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Tape underlayment rotary-node (TURN) valves for simple on-chip microfluidic flow control

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Abstract

We describe a simple and reliable fabrication method for producing multiple, manually activated microfluidic control valves in polydimethylsiloxane (PDMS) devices. These screwdriver-actuated valves reside directly on the microfluidic chip and can provide both simple on/off operation as well as graded control of fluid flow. The fabrication procedure can be easily implemented in any soft lithography lab and requires only two specialized tools – a hot-glue gun and a machined brass mold. To facilitate use in multi-valve fluidic systems, the mold is designed to produce a linear tape that contains a series of plastic rotary nodes with small stainless steel machine screws that form individual valves which can be easily separated for applications when only single valves are required. The tape and its valves are placed on the surface of a partially cured thin PDMS microchannel device while the PDMS is still on the soft-lithographic master, with the master providing alignment marks for the tape. The tape is permanently affixed to the microchannel device by pouring an over-layer of PDMS, to form a full-thickness device with the tape as an enclosed underlayment. The advantages of these Tape Underlayment Rotary-Node (TURN) valves include parallel fabrication of multiple valves, low risk of damaging a microfluidic device during valve installation, high torque, elimination of stripped threads, the capabilities of TURN hydraulic actuators, and facile customization of TURN molds. We have utilized these valves to control microfluidic flow, to control the onset of molecular diffusion, and to manipulate channel connectivity. Practical applications of TURN valves include control of loading and chemokine release in chemotaxis assay devices, flow in microfluidic bioreactors, and channel connectivity in microfluidic devices intended to study competition and predator / prey relationships among microbes.

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1. Introduction

The simplest microfluidic devices that have been fabricated from PDMS using soft lithography feature uncomplicated microchannel architectures filled with quiescent or flowing liquids controlled either by hydrostatic pressure or external syringe pumps and valves (Beebe *et al.*, 2002; Cho *et al.*, 2003; Duffy *et al.*, 1998; Georgescu *et al.*, 2008; Liu *et al.*, 2008; Walker *et al.*, 2005). The most intricate devices use banks of pneumatically controlled on-chip valves and pumps to control complex bioreactors and microchannel networks (Gomez-Sjoberg *et al.*, 2007; Thorsen *et al.*, 2002; Unger *et al.*, 2000). As microfluidic devices become more widely accepted as practical tools in the scientific laboratory, there is a need for devices of intermediate complexity that perform a particular function well, but at low cost in terms of time, money, and expertise both to fabricate and operate. The full potential of adopting microfluidic technology will not be realized as long as compact, elegant PDMS devices must be controlled by bulky syringe pumps or solenoid valve banks and computerized controllers costing hundreds to thousands times more than the devices themselves. These issues are especially pertinent for applications involving large numbers of devices used for experiments in space-restricted environments, such as cell-culture hoods and incubators, particularly when they involve scientists, technicians, and students who are not microfluidic experts.

Researchers at the Vanderbilt Institute for Integrative Biosystems Research and Education (VIIBRE) are developing a family of low-cost microfluidic devices such as bioreactors (Prokop *et al.*, 2004) and nanophysiometers (Werdich *et al.*, 2004) for investigating the chemotactic and morphogenic behavior of eukaryotic cells (Georgescu *et al.*, 2008; Liu *et al.*, 2008), as well as the ecology and behavior of individual microbes (Wang *et al.*, 2005; Wang *et al.*, 2008). Many of these devices target the long-term observation of a small number of cells subjected to precisely controlled microenvironmental conditions, including chemical gradients of nutrients or signaling molecules, which influence the proliferation, morphology, and developmental fate of individual cells. These applications often require a valve to be opened or closed once or twice during the time course of the experiment, which may be up to a week. Therefore, there is a need for an alternative to pneumatic valves and off-chip controllers.

Numerous valve technologies compatible with microfluidics have been recently developed and described in the literature (Easley *et al.*, 2006; Grover *et al.*, 2003; Gu *et al.*, 2004; Kovacs, 1998; Weibel *et al.*, 2005). However, most previously proposed technologies are bulky or complex to fabricate, require external actuation and power source maintenance, or are not compatible with tall (greater than 20 μm) microfluidic channels. Our goal is to provide a valve that can be easily integrated into microfluidic devices, is simple to fabricate and operate, does not require external power connections, and is capable of reversibly closing a variety of microfluidic channels.

A low-cost valve to compress and block a microfluidic channel was proposed by Weibel *et al.* (Weibel *et al.*, 2005). Their design incorporates a small diameter mechanical screw that is inserted into a blind hole drilled into the PDMS structure directly above a microfluidic channel. When this screw is turned clockwise, the underlying channel is collapsed; subsequent counter-clockwise screw action will allow resilient forces in the PDMS to restore fluid flow in the channel. However, fabrication of the Weibel *et al.* design requires precisely controlled and time-consuming drilling of previously molded PDMS structures to incorporate each individual valve. This approach is not well suited for mass production of microfluidic designs that require a large number of valves, because a single error in alignment or drilling depth can render an entire device useless. Ges *et al.* have advanced this approach by inserting an oversized threaded insert with a screw into a blind hole drilled into

the PDMS to compress the channel located beneath the hole (Ges *et al.*, 2008; Ges and Baudenbacher, 2008). While this eliminates the problem of the screw threads pulling out of the PDMS, it still requires drilling a blind hole to a precise depth into the PDMS, with the concomitant risk of damaging the device. This embedding technique also is not well suited for parallel fabrication of large numbers of valves on a single device.

To overcome these difficulties and to make fabrication and operation more user-friendly, we take a simple and robust approach. Our valves consist of a valve body, which is easily fabricated using a simple injection molding method and a small captive stainless steel 0-80 machine screw (1.5 mm diameter with 3.15 threads/mm). The assembled valves are aligned with the underlying microfluidic network and embedded into the device during a two-step PDMS casting procedure. Valves are fabricated as a strip for ease of alignment and placement, which gives rise to the acronym TURN (Tape Underlayment Rotary-Node) valve. Valves can be placed as strips by matching the underlying microfluidic network to the valve spacing in the mold, or cut out and used individually.

One TURN valve-based microfluidic system that we are developing permits control of multiple crossed gradients of various chemokines and nutrient factors and is designed to be compatible with real-time microscopic examination. Up to four independent gradients may be established simultaneously in a single continuous space in the device called “Hyper-Quad” (Figure 1). Each corner serves as a point source and is connected to a supply reservoir. By loading each supply reservoir with different substances and allowing for diffusion to start at various time points (by opening an inlet valve), cells located within the assay chamber will be exposed to variable crossed gradients that may influence migration rates or morphological transformations. A cross-sectional schematic of a TURN valve and its operation within a microfluidic device are shown in Figure 2.

2. Experimental

2.1 TURN valve fabrication

Valve bodies are pre-fabricated using a simple injection molding method and a custom-machined brass mold (Figure 3). A wide variety of comparable mold designs have been developed for specific applications, with a range of node spacings, flange diameters and thicknesses, and lateral extensions, limited only by the imagination of the user and the several hours required for computer numerically controlled (CNC) machining of the mold. In general, mold fabrication is straightforward. While our molds were fabricated on an industrial-class CNC (RH-20, Milltronics Manufacturing, Waconia, MN), it is also possible to fabricate them on a modest, model-maker’s CNC milling machine (*e.g.*, MicroMill DSLs 3000, MicroProto Systems, Chandler, AZ), or even with a manual milling machine operated by a moderately experienced machinist.

In the molding process, molding screws are first threaded all the way into the mold from the back so that screw threads protrude slightly above its top surface. Then, a light coat of aerosol mold release compound (Smooth-On, Inc.) is applied. Next, thermosetting plastic glue (HS-300, Adhesive Solutions Co. LLC) (FDA approved for food packaging) is delivered by an 80 watt hot-glue gun (GR-24 Pro, Stanley Tools) to injection-mold the valve bodies. Glue is injected into the mold recesses sequentially in one motion, without breaking the glue flow, so as to fill the mold and encapsulate the protruding screws. Molten glue completely conforms to the shape of the mold and the screw threads, providing excellent gripping force in the finished product. After the molten plastic is solidified (typically 10 minutes), the molding screws are partially unscrewed. Excess glue is trimmed with a razor blade to form a smooth surface flush with the top surface of the brass mold. Molding screws

are then completely backed out, and a strip, or tape, of valve bodies is removed from the mold.

Valve fabrication is completed by inserting into the valve bodies a different set of 0-80 screws with polished ends that will act as valve actuators. These are screwed into the valve bodies to be exactly co-planar with the bottom of the valve assembly. Polishing of the screw ends is important to avoid galling of the PDMS surface and eventual rupture of the fluidic channels during valve engagement. Finally, valve tapes, with screws inserted, are rinsed with isopropyl alcohol, dried, and stored under sterile conditions for later assembly into PDMS microfluidic devices.

2.2 Valve incorporation into microfluidic devices

The fabrication of the microfluidic devices used in these experiments is straightforward and is widely described in the literature (Whitesides *et al.*, 2001). Briefly, SU-8 series photoresist (MicroChem) is spin-coated to the desired feature height on the surface of a silicon wafer. A standard contact print photolithographic process is then used to selectively expose photoresist to ultraviolet light. Following development of unexposed photoresist, and hard-baking the polymerized device pattern, a positive relief mold is created. The height of the microfluidic network depends on a particular application and hence the choice of SU-8 resist. We routinely fabricate devices ranging from 1 μm to >300 μm tall.

Next, a 1 mm-thick layer of 10:1 mixture of PDMS and curing agent is poured onto the mold to create the deformable layer of PDMS upon which the TURN valve operates. The whole assembly is then placed in a 70 °C oven (DX 400, Yamato) and allowed to partially cure until the surface is semi-rigid, but still sticky (~25 min). The mold is then removed from the oven and strips of TURN valves are carefully placed on top of the partially cured PDMS in the appropriate locations utilizing tweezers and a low-power stereoscope. The alignment marks built into the photolithographically fabricated master greatly simplify the process of precisely positioning the valves on top of the thin layer of PDMS still rigidly supported by the silicon wafer, as does the rigid linear arrangement of a row of valves in the tape that allows many valves to be installed in a single operation. When all valves are correctly positioned, an additional ~3 mm of PDMS is poured over the entire device to completely encapsulate the valve assembly. After overnight curing, the microfluidic device molded in PDMS and containing the embedded valves is removed from the silicon wafer, access holes are punched for the external fluidic lines, and the whole device is irreversibly bonded to the microscope slide or cover glass by activating the PDMS and the glass surface via a 30 sec low pressure O₂ plasma treatment (PDC-32G, Harrick Co.).

3. Results and discussion

3.1 Controlling multiple flows

These TURN valves are a robust and versatile technology that has enabled us to develop and use a variety of microfluidic flow devices. Figure 4 shows how flow stream composition may be easily and reversibly adjusted by varying the contributions of three input sources using TURN valves. Valves for each of the flow channels were opened and closed multiple times without a failure, and contributions from each of the flows always returned to the original ratios upon complete valve opening. As indicated in Figure 4C, partial closure of the valve reduces total flow through the channel and thus the valve can be used as a simple flow regulator. The actual relationship between flow rate and degrees of valve turns depends on the channel geometry, stiffness of surrounding PDMS (curing agent – polymer ratios) and the thickness of the PDMS layer between the valve and the underlying channel, and it can vary from device to device. However, valve performance is quite reproducible for a single device even after multiple (>10) open-close cycles. Shown in Figure 4E is the change

in flow rate through a single 100 μm wide and 40 μm tall channel as a function of TURN valve closure. The driving pressure was kept constant at 0.5 psi (3.45×10^3 Pa), and the flow was measured using an Upchurch Nanoflow sensor (N-565, Upchurch, Oak Harbor, WA). One turn corresponds to 360° . Each point represents a 30 second average flow rate with standard deviation as error bars. The dotted line shows a sigmoidal fit to the average of 4 closing cycles indicated as (●) on the graph with error bars showing standard deviation of

the flow between the closing cycles. The fitted equation is $y = -7.7 + \frac{5597.3}{1 + e^{\frac{x-1}{0.2}}}$, $R^2 = 0.999$. The inserts depict progression of the channel's ceiling collapse as the valve is being closed. It is clear from the graph that the initial closing (shown as ◆) can be viewed as a "break-in" or conditioning cycle associated with the initial separation of the lower layer of PDMS from the valve body above it. After this initial closing, the compression and restoration forces of PDMS are thereafter quite reproducible resulting in reproducible flows for multiple open/close valve cycles. Even if the valve is backed off half a turn past the "full open" position the flow rate remains constant, indicating that there is no "pull" from the valve screw. In the closed state, the valve can withstand at least 55 psi (3.8×10^5 Pa) of pressure. We have not seen the need to test our devices at higher pressures.

The precision with which we have can position the valve body with respect to the channels beneath it allows us to place a valve over an intersection of multiple channels. As shown in Figure 5 we were able to completely collapse an intersection of four channels and block the flow, thereby isolating each of the three input channels from each other and the output channel.

3.2 Time release of diffusive gradients

In our diffusion studies, TURN valves are indispensable for controlling the loading of chemokine reservoirs and the precise initiation of time-varying concentration gradient fields. The Hyper-Quad device (Figure 1) utilizes sixteen valves: eight are used for loading of four different source reservoirs, four are used to load cells and coat surfaces, and the remaining four are used on suction channels, which independently control the initial onset of diffusion gradient formation from each reservoir and are also used in initial cell loading. In the diffusion chamber of the Hyper-Quad device, experimentally controlled concentration fields are produced by adjusting the time intervals at which the source valves are opened. Figure 6 depicts the evolution of a multi-gradient experiment where individual point sources were activated (valves opened) at different intervals. Independent control of gradient initialization allows experimental flexibility and the ability to compensate for differing chemokine diffusion coefficients, or to provide different sequences of morphogen delivery. Diverse research areas in mammalian cell migration and morphological transformation, as well as topics in microbiological-ecology interactions, would profit from such low-cost, simple devices that are capable of creating stable overlapping gradients of nutrients, chemoattractants, antibiotics, or other molecules of interest within the microfluidic assay chamber.

3.3 Microbial behavior studies

Another interesting application of TURN valves enables experimenters to control connectivity within microfluidic networks. Typical microbial habitats have complex microscale physical, chemical, and biological structure. Channel dimensions and connectivity affect microbial migration and dispersal rates, susceptibility of bacteria to predation, community stability, and net microbial productivity (Anderson and Domsch, 1995; Fenchel, 1969; Ranjard and Richaume, 2001; Wang *et al.*, 2005; Wang *et al.*, 2008).

For example, some of our work has focused on interactions between bacteria and protozoa predators within microfluidic devices (Wang *et al.*, 2005; Wang *et al.*, 2008). Protozoan grazing may enhance net bacterial activities via release, recycling, or limiting nutrients, or through physical disturbance of microbial habitats (Glud and Fenchel, 1999; Hahn and Hofle, 2001; Mattison *et al.*, 2005), leading to enhanced rates of bacterial biodegradation of crude oil, polycyclic aromatic hydrocarbons, and coal tar (Madsen *et al.*, 1991; Rogerson and Berger, 1983; Tso and Taghon, 2006). However, efforts to understand the impact of habitat structure on microbial interactions have been limited by the inability to control the dispersal of microorganisms in microstructured habitats. The combination of microfluidics and TURN valves can provide a powerful yet simple tool that overcomes such problems.

We have demonstrated that TURN valves can provide an effective barrier between bacteria (prey) and protozoa (predator). In Figure 7A we show the total number of protozoa that have crossed the valve aperture. Protozoa *Cyclidium* sp., a hypotrich scuticociliate isolated from estuarine sediment, were first introduced into the input well at a concentration of 0.57×10^6 per liter in artificial sea water (ASW) and were visually observed as they swam up and down the channel and tried to penetrate the closed valve. After a 20 min observation, the valve was opened, and they were counted manually as they passed through. Until the valve was opened, we observed perfect inhibition of the *Cyclidium* sp., which have been previously shown to pass constrictions of $<5 \mu\text{m}$ in microfluidic devices (Wang *et al.*, 2005).

Similar “breakthrough” experiments were performed using *Escherichia coli* (*E. coli*), a motile $1 \mu\text{m}$ rod-shaped gram-negative bacterium. This organism was hybridized with a green fluorescent protein (GFP) plasmid, greatly assisting optical detection even at low magnification. In devices consisting of several habitats in series with microfluidic channels and a TURN valve, bacteria readily dispersed across open TURN valves. The concentration of bacteria used in these experiments was quite high, approximately 10^9 cells/ml suspended in Luna-Bertani broth with $50 \mu\text{g/mL}$ of kanamycin. Within several hours, the bacteria had dispersed throughout the entire open region of the device. We used the crossed device shown in Figure 5A to demonstrate the bacterial impermeability of a closed TURN valve. Bacteria were introduced into one fluidic leg of the closed cross. After a seven-day incubation period the bacteria spread evenly throughout the inoculated channel but were not able to penetrate the closed TURN valve. (Even at high magnification, no fluorescence from GFP bacteria was observed either visually or with long integration times on a cooled CCD camera (Q-Color 5). Figure 7B shows a combination of fluorescent and white light illumination pictures of the closed valve with bacteria aggregating around the collapsed channel ceiling but unable to penetrate the closed valve into the remaining three channels.

3.4 Hydraulic valve

During our experiments with TURN valves, we identified a need to close multiple channels with a single actuator. A single TURN valve can reliably close two parallel unconnected $100 \mu\text{m}$ wide channels that are directly under the center of the TURN valve. However it would be useful to have a more general approach to control multiple independent channels with one single actuator. We have developed a hydraulic technique for controlling multiple shallow channel closures with a single TURN valve. Quake *et al* (Unger *et al.*, 2000) have shown that by applying a pneumatic pressure to the gas- or fluid-filled channels that are located above or below the fluidic network and are separated from it by a thin extensible membrane, it is possible to block the flow in that fluidic network. Even though devices with very complex architecture and functions can be achieved using that approach, such systems require an external gas tank or a compressor and computer-controlled pressure and bleed valves, and often constrain routing of microfluidic and pressure channels through the devices (Gomez-Sjoberg *et al.*, 2007). The external connections can be very useful but require substantial investment in experiment design and infrastructure, and are not well

suited for the use of large numbers of separate devices in an incubator, particularly if the valves need to be opened or closed only once or twice in the lifetime of the device.

We propose a simple system that can close multiple channels via a single manual control and does not require external pressure sources or expensive auxiliary instrumentation. The schematic view of this Self-Contained Single-Control Several-Closure valve (SC³ valve) is shown in Figure 8. It consists of a two-layer device with two microfluidic networks. Located at the bottom are the shallow microfluidic channels that need to be controlled, and above them is an encapsulated hydraulic pressure distribution network for collapse control. Both networks are separated by a thin PDMS membrane and are not in fluidic contact with each other. The hydraulic layer is a closed-loop network, which consists of a large reservoir and channels that distribute the pressure. The pressure distribution channel is positioned in such a way that it overlaps underlying fluidic channels in predetermined locations. A single TURN valve, located over two parallel channels, is used to load and seal the pressure distribution network and its associated reservoir/actuator chamber. The network is in the form of a loop to simplify filling and bubble removal with both ports sealed by a single TURN valve.

In our demonstration device, a second TURN valve is used to control the pressurization of the filled and sealed hydraulic loop and hence the closure of all intersecting fluidic lines. As the TURN valve is actuated and the screw pushes onto the plunger, the fluid is displaced from the collapsing reservoir into the pressure network. Since this is a closed network, the pressure generated by displaced fluid is applied to the membrane, stretches it, and collapses the underlying fluidic channel. Figure 9 shows the closure of a channel using a single SC³ valve. This system is completely autonomous and does not require external connections and power sources.

We have also demonstrated that it is possible to use a low-cost stepping motor and driver (Digi-Key MTSD-V1) and flexible plastic shafts, such as those used in radio-controlled model airplanes, to operate TURN valves automatically or remotely. While this may prove useful for automated loading of large numbers of devices, in this paper we emphasize the minimalist approach of using a small screwdriver to actuate the TURN valve by hand.

4. Conclusion

We have extended the concept of a simple mechanical screw valve system in a manner consistent with ease of soft lithographic microfluidic device manufacture and operation. TURN valves do not require tedious fabrication and use of photocurable polymers, and they can be easily implemented in any laboratory that uses soft lithography. These valves do not require expensive external actuation and control parts and are capable of blocking a variety of microfluidic channels, including those with tall aspect ratios and intersections. They can be used individually or in a long tape or strip, and when employed in passive diffusion devices, can aid in generating adjustable, spatially varying concentration gradients. They provide an extremely tight, cell-impermeable seal and can separate protozoa and bacteria in experimental devices for studying microbial population dynamics. Lastly, the TURN valve concept can be used as efficient and extremely inexpensive actuators in hydraulic systems to close multiple channels simultaneously. TURN valve devices are compact, inexpensive, easy to use, and can be readily moved into different locations or in and out of an incubator without concern for any external pressure connections, pressure leaks, and power sources.

Information regarding the design, construction, and availability of CNC molds for the valve bodies and the corresponding mask layouts is available at <http://www.vanderbilt.edu/viibre/technologies.html>.

Acknowledgments

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Figure 1.
A passive cross-gradient generator (“Hyper-Quad”) with two valve banks for reservoir and cell loading, and four independent reservoir release valves.

Figure 2.

A schematic representation of a typical TURN valve and its operation. Once the screw is rotated clockwise, the walls of the underlying channel are collapsed, thus sealing the channel.

Figure 3.

Fabrication of a TURN valve strip. A) Brass mold with molding screws. B) Hot-glue injection into the mold. C) Valve body with actuation screws flush with the valve seating surface. D) A strip of TURN valves (a tape). E) A cross-sectional view of a TURN valve indicating excellent formation of the threads during the injection molding step. Alternative designs have protrusions on either side of the valve flange for ease of alignment and increased retention by the second layer of PDMS.

Figure 4.

Changes in the flow profile through a three-input (L, C, and R) channel, one-output (lower) channel, hydrodynamic-focusing chip controlled by TURN valve actuation. The center channel is loaded with water while the side channels are loaded with fluorescein solution. Total flow rate was held at 90 $\mu\text{L}/\text{min}$. A) White-light photograph of the device showing the close-up region. B) Fluorescence picture of the channel flow with all valves opened. C) Fluorescence picture of the channel flow with R valve closed and L and C valves opened. D) L and R valves opened and C valve partially closed. E) Change in the flow rate as a function of valve closure for multiple closing cycles for a single 100 μm wide and 40 μm tall microfluidic channel at 0.5 psi of driving pressure. Zero turns correspond to the initial / open state (as shown in Figure 2B) and -0.5 turns correspond to one-half turn outwards from the initial position (shown only for one cycle). Symbols ($\blacklozenge$) indicate changes associated with the initial closure. Dotted line is the sigmoidal fit to the average flow for 4 closing and opening cycles (excluding initial) with the equation and the R^2 as indicated. Inserts are the

fluorescent pictures of the channel filled with fluorescent solution as the valve is being closed.

Figure 5.

Collapse of the microfluidic channel intersection due to TURN valve actuation. A) A photograph of the device. B) An AutoCAD drawing of the photolithographic mask. The dotted circle indicates the location of the TURN valve screw, and the four large arcs guide the placement of the turn valve assembly. C) Fluorescence image of the steady-state microflow with the valve opened. The rightmost input channel is loaded with water while the upper and lower input channels are loaded with fluorescein solution. The output of the device is to the left, as evidenced by the hydrodynamic focusing of the three input channels. D) Fluorescence image of the microflow after valve partial closure (360° turn). E) Fluorescence picture of the completely closed microfluidic intersection (900° turn).

Figure 6.
Time evolution of the concentration profile of four different dyes, with each reservoir being activated at a different time.

Figure 7.

Separation of bacteria and protozoa by a TURN valve. A) The number of protozoa that have crossed the valve aperture as a function of time. B) Fluorescent picture of GFP-modified *E. coli* bacteria with white light background illumination. Note that bacteria were unable to penetrate the closed TURN valve even after 7 days of incubation. 10x objective. The dashed lines indicate the locations of the channels underneath the valve body (dark circle). The insert is the 32x fluorescent view of the bacteria at the edge of the closed valve. The parabolic profile of the GFP *E. coli* reflects the tapering collapse of the channel as it approaches the center of the actuating screw, and shows that the bacteria can penetrate only half-way to the center of the valve.

Figure 8. Schematic view and operation of the Self-Contained Single-Control Several-Closure (SC^3) valve. A) Valve in the open position. B) Valve in the closed position. C) AutoCAD drawing of the microfluidic device topology with an SC^3 valve.

Figure 9.
Fluorescence pictures showing channel closure with SC³ valve.