

Controlled microfluidic switching in arbitrary time-sequences with low drag†

Cite this: *Lab Chip*, 2013, 13, 2389

Cassandra S. Niman,^{*a} Jason P. Beech,^a Jonas O. Tegenfeldt,^a Paul M. G. Curmi,^{bc} Derek N. Woolfson,^{de} Nancy R. Forde^f and Heiner Linke^{*a}

The ability to test the response of cells and proteins to a changing biochemical environment is of interest for studies of fundamental cell physiology and molecular interactions. In a common experimental scheme the cells or molecules of interest are attached to a surface and the composition of the surrounding fluid is changed. It is desirable to be able to switch several different biochemical reagents in any arbitrary order, and to keep the flow velocity low enough so that the cells and molecules remain attached and can be expected to retain their function. Here we develop a device with these capabilities, using U-shaped access channels. We use total-internal reflection fluorescence microscopy to characterize the time-dependent change in concentration during switching of solutions near the device surface. Well-defined fluid interfaces are formed in the immediate vicinity of the surface ensuring distinct switching events. We show that the experimental data agrees well with Taylor–Aris theory in its range of validity. In addition, we find that well-defined interfaces are achieved also in the immediate vicinity of the surface, where analytic approaches and numerical models become inaccurate. Assisted by finite-element modelling, the details of our device were designed for use with a specific artificial protein motor, but the key results are general and can be applied to a wide range of biochemical studies in which switching is important.

Received 11th February 2013,

Accepted 4th April 2013

DOI: 10.1039/c3lc50194a

www.rsc.org/loc

Introduction

In many single-molecule and single-cell studies it is of interest to use microfluidic systems to explore a molecule's or cell's response to changes in the surrounding chemical environment. For instance, microfluidic switching devices have been used to study ion translocation in ion channels in response to quick changes in ion concentration,^{1–3} and neuron cell response to transmitters and drugs,^{4,5} and they have enabled performance measurements of single motor proteins in response to controlled changes in chemical environment.⁶

A limitation of existing techniques is the inability to switch three or more fluids in an arbitrary time sequence while

keeping drag forces on surface-adhered molecules low. This ability is needed, for example, to power chemically artificial molecular motors, which can be designed to produce mechanical work from cyclical changes of the chemical potential in their environment.

To illustrate the usefulness of more advanced microfluidic devices for single-molecule studies, we describe briefly the function and microfluidic requirements of the artificial protein motor Tumbleweed (TW).⁷ It comprises a central hub linking three different repressor proteins (“feet”) that bind and unbind to specific sequences in a DNA track in the presence of three different ligands in solution. To power the motor, one needs to cyclically change the concentration of the three ligands, indicated by solutions I, II, and III in Fig. 1.⁸ The desired flow pattern is characterized by two time scales: t_d , the dwell time that one fluid is in the region of the motor and t_s , the time it takes to switch between fluids. The requirements on the fluidic system can be summarized as follows: the system must have the ability (i) to cyclically change three or more fluids in arbitrary order (the order I–II–III or III–II–I controls the direction of motor motion) and (ii) to achieve many cycles in sequence (one for each stepping event). Furthermore, (iii) fluid interfaces must be well defined ($t_s \ll t_d$), and (iv) low fluid velocity must be maintained to minimize mechanical disturbances to the functioning of the TW motor.

Finally, because the DNA track will be immobilized on a surface, the above conditions need to be realized (v) in the

^aNanometer Structure Consortium (nmC@LU) and Division of Solid State Physics, Lund University, Box 118, 221 00 Lund, Sweden.

E-mail: cassandra.niman@ftf.lth.se; heiner.linke@ftf.lth.se; Tel: +46 46 222 4495
Tel: +46 46 222 4245

^bSchool of Physics, University of New South Wales, Sydney, New South Wales 2052, Australia

^cCentre of Applied Medical Research, St. Vincent's Hospital, Darlinghurst, New South Wales 2010, Australia

^dSchool of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

^eSchool of Biochemistry, University of Bristol, Medical Sciences Building, University Walk, Bristol BS8 1TD, UK

^fDepartment of Physics, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3lc50194a

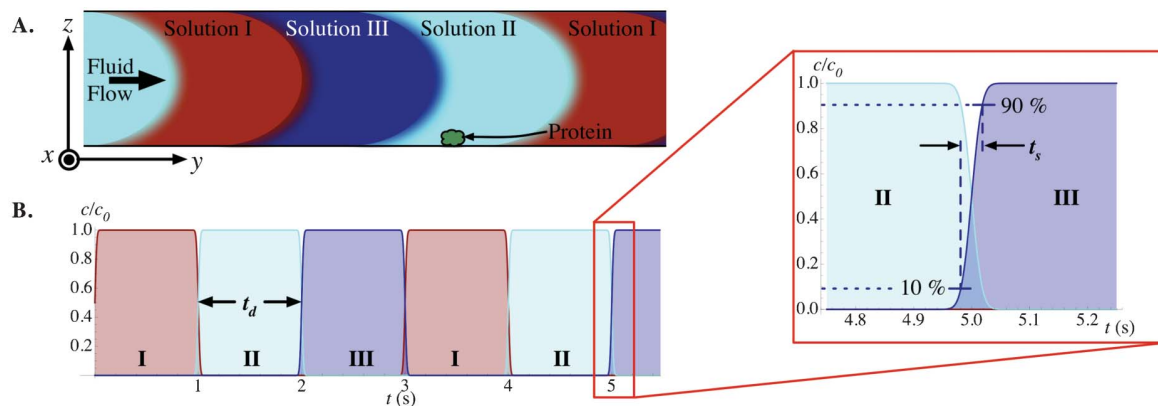


Fig. 1 Illustrative schematics of the desired properties of a fluid switching device. A, The desired fluid flow in a microfluidic channel, with a surface adhered protein. B, Resultant changes in each fluid's relative concentration at the location of the protein. The time one fluid spends in the region of the protein is defined as t_d and the time to switch from 90% to 10% of one fluid concentration is defined as the switching time, t_s .

immediate vicinity of a device surface, that is, the possible existence of a boundary layer must be taken into account. Whereas this set of conditions may appear specific to TW, the general ability to achieve well-defined fluid switching surrounding surface-adhered particles with low flow in arbitrary time sequence will be of interest to a wide range of studies. For example, like TW, natural molecular motors produce mechanical work by cycling through a series of conformational states linked to ligand-binding states. The ability to control the presence of ligands (typically ATP and its hydrolysis products) in arbitrary time sequence would enable experiments to further illuminate the roles of these conformational changes.

Existing techniques for changing the chemical environment around surface-adhered particles fulfil some, but not all of the above requirements (i)–(v). The uncaging of caged particles upstream from the region of interest⁴ offers a small t_s ,⁹ but not the ability to be switched easily many subsequent times. The lateral manipulation of fluid streams around a surface-adhered particle by changing the input pressures on different inlets^{3,10,11} is simple, but does not allow arbitrary time sequences (for example, a sequence I–II–III–I–II–... is not possible with only three inlets using this technique). The use of patch clamps or optical tweezers to move a particle between parallel fluid streams^{1,2,6} does not allow one to create arbitrary fluid patterns responsively during an experiment. These last two limitations can be addressed using valves to switch between fluids, but off-chip valves,⁵ or on-chip valves with large footprints,¹² can have problems with diffusive mixing in inlets connected to closed valves. On-chip valves and other design techniques aim at solving this problem, but add much complexity to device fabrication.¹³

Here we introduce a device design that fulfils the above requirements (i)–(v) by directing the desired fluid to the region of interest and directing unwanted fluid streams to collection outlets on the chip. By changing relative applied pressures we can switch between fluids in any order, while maintaining a low flow speed to avoid excessive drag or shearing. Keeping the

fluid moving to waste outlets allows for a sharp fluid interface close to the inlet to the main channel.

In the following we describe the device design in more detail, including the use of finite-element modelling that provides a qualitative picture of the role of convection and diffusion, and helps optimize the design. Then, we present experimental data on the solution-switching performance, obtained using fluorescent solutions. Importantly, in addition to monitoring the bulk-level performance using epifluorescence microscopy, we use total internal reflection fluorescence microscopy (TIRFM) in order to determine the switching time between fluids in the immediate vicinity of the surface, at the location of surface-adhered particles. The experimental data agree well with theory in its range of validity.^{9,14,15} In addition, we find experimentally well-defined fluid interfaces are achieved in the immediate vicinity of the surface, where analytic approaches and numerical models become inaccurate. Finally, we discuss the benefits and possible drawbacks of this device for use with TW or in other single-molecule or single-cell studies.

Experimental methods

Device design

The overall device layout is shown in Fig. 2. To enable switching between three different solutions in any arbitrary, controlled time sequence, the design includes U-shaped access channels with inlets each containing a different fluid connected directly to waste outlets. The fluid streams coming from each inlet are then controlled using different relative pressures on each inlet/waste-outlet pair. When a fluid is not needed in the region of interest it is pushed to a waste outlet. To direct a fluid to the main channel the pressure on that inlet is increased relative to the others. In Fig. 2E a close-up of the interface between the two laminar flow streams near one inlet is shown. The arrows indicate the fluid velocity direction and relative magnitude. By maintaining flow in this area, one

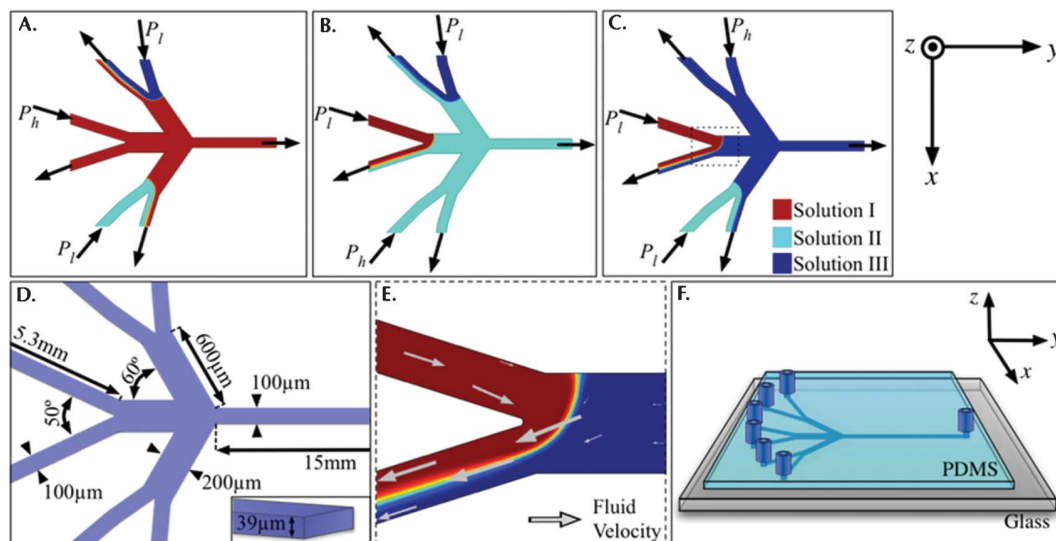


Fig. 2 Device design for switching between three fluids in arbitrary time sequence. Each inlet is connected to a reservoir containing one of the three fluids. For each inlet, there is one outlet to a waste reservoir. This allows for the disposal of unwanted fluids until the time a given fluid is needed. A, B and C are simulated images of the three switching configurations, I, II and III respectively, in the steady state (see simulations section for more details). The fluid that is desired in the main channel is given the highest pressure, P_h , while the other two are kept at P_l , a pressure intermediate between P_h and the pressure in the waste outlets. The arrows indicate the flow direction for inlets and outlets. The colours indicate three different fluids. D, Dimensions of the devices used in experiments and simulations; the entire channel is 39 μm high. E, Close-up of the laminar flow stream interface from the region marked in C. The grey arrows show the proportional velocity magnitude and direction. F, Overview of device design in PDMS bonded to glass with silicone reservoirs (not drawn to scale; the overall device size is about 2.5 cm $\times$ 2 cm $\times$ 0.5 cm).

keeps the boundary between the fluids sharp until the switch to a higher pressure on this inlet occurs.

Device fabrication

The PDMS microfluidic device was fabricated using a master mould consisting of SU8 2050 (MicroChem, MA, USA) spin-coated onto a silicon wafer and patterned using UV lithography in a contact mask aligner (Karl Süss, Munich, Germany). The master was given an anti-sticking treatment in tridecafluoro-(1,1,2,2)-tetrahydrooctyl-trichlorosilane (F13-TCS) (Sigma Aldrich, St. Louis, MO, USA) to facilitate demoulding. The PDMS polymer base and curing agent (Sylgard 184, DowCorning, Midland, MI, USA), 10 : 1, were mixed, degassed, and poured onto the master mould, then cured at 80 $^{\circ}\text{C}$ for 1 h. After peeling the PDMS off the master mould, holes were poked through each inlet and outlet. The resulting channel is 39 μm high and has the dimensions shown in Fig. 2D. The PDMS was bonded to a no.1 cover slip (Menzel-Gläser, Braunschweig, Germany) after both were exposed to oxygen plasma for 30 s (Plasma Preen II-862, Plasmatic Systems, Inc, North Brunswick, NJ, USA). The thin cover slip is necessary to image the inner surface of the device with low working distance TIRF objectives. Prior to bonding, the cover slip was rinsed in detergent (2% Helmanex (VWR, PA, USA)) once, rinsed in MilliQ water to remove dust, dried in N_2 and finally baked at 80 $^{\circ}\text{C}$ for 1 h, to dry any water left after cleaning, before bonding. Lastly silicone tubes, 3 $\times$ 5 mm (VWR, PA, USA), cut 10 mm long, were sonicated in detergent (2% Helmanex), then twice in MilliQ water to remove dust and dried with nitrogen, before being glued around the punched holes on top of the PDMS device using a silicone adhesive

(ElastosilA07, RTV-1 silicone rubber, Wacker Silicones, Munich, Germany), allowing for fluidic connections to external tubing. An overview of the final device is shown in Fig. 2 F.

Experimental setup/procedures

Pressure-driven flow and control of differential pressures to inlets and waste outlets was achieved using a Fluigent microfluidic control system (MFCS-FLEX-3C system, pressure range 0–69 mbar (Fluigent, Paris, France)). Three channels were connected to a FLUIWELL-4C system with 2 ml vials connected to the device ensuring that no air was in the system. These vials act as a reservoir for additional solution so we could run the device for more than 4 h. A script in the software provided with the Fluigent system was used to change the pressures at each inlet after a specified dwell time, t_d , between P_h and P_l as is illustrated in Fig. 2 A, B and C. Two sets of measurements are presented here, for P_h and P_l set at 2000 Pa and 200 Pa, and 7000 Pa and 2500 Pa above atmospheric pressure respectively. In all cases, the waste outlets were at atmospheric pressure. We determined (below) that these two pressure pairs resulted in average flow speeds of $v = 3.3 \text{ mm s}^{-1}$ and $v = 8.3 \text{ mm s}^{-1}$, respectively. This corresponds to flow rates in the main channel of $0.7 \mu\text{l min}^{-1}$ and $1.9 \mu\text{l min}^{-1}$, respectively. The simulations and the discussion concern the lower pressure settings.

To be able to visualize fluid flow, we used three solutions with different concentrations of fluorescein (Life Technologies Corporation, Paisley, UK): solutions I and II contained 0.45 M

(high fluorescence) and 0.15 M fluorescein (intermediate fluorescence), respectively, in MilliQ water.

The solutions were filtered using 0.2 µm sterile Supor® membrane syringe filters (VWR, PA, USA). Solution III was MilliQ water (no fluorescence). The fluorescein solutions in the device were imaged using an inverted Nikon Eclipse TE2000-U microscope using either epifluorescence or TIRFM with a modified Nikon laser TIRFM system (Nikon Corporation, Tokyo, Japan) and an Andor iXon-EM+ camera, model DU-897D (Andor Technology, Belfast, Northern Ireland). The 488 nm laser line from a Spectra-Physics Stabilite 2018-RM laser (Spectra-Physics, Santa Clara, CA, USA) was used for TIRFM.

Simulations

The system was modelled using finite-element simulations in COMSOL Multiphysics (COMSOL AB, Stockholm, Sweden). Two models were considered qualitatively. Model I considered the entire channel geometry in two dimensions with the shallow channel approximation, which accounts for the top and bottom channel walls with a body force term added to the Navier–Stokes equation (eqn (1)). Model II focused on the device's main channel where all experimental measurements were taken (see Fig. 2), represented by a two-dimensional rectangle of size 15 mm × 39 µm, with the aim of determining the cross sectional profile of the fluid interface near the inlet and further down the channel. For models I and II we used a stationary solution for the “laminar flow”, and a second time-dependent solution using the velocity field from the first solution as the initial conditions. The time-dependent solution was solved with both “laminar flow” as well as “transport of diluted species.” Here we assume the diluted solute does not influence the viscosity or density of the fluid.

The “laminar flow” approach used the Navier–Stokes equation for an incompressible fluid,

$$\rho \frac{\partial \bar{v}}{\partial t} + \rho(\bar{v} \cdot \nabla) \bar{v} = \nabla \cdot [-\bar{p} + \mu(\nabla \bar{v} + (\nabla \bar{v})^T)] - 12 \frac{\mu \bar{v}}{d_z^2} + \bar{F} \quad (1)$$

$$\rho \nabla \cdot \bar{v} = 0 \quad (2)$$

where ρ is the density, μ the dynamic viscosity and $\bar{v}$ the velocity of the fluid, $\bar{p}$ is the pressure relative to the outlets, d_z is the shallow channel depth, and $\bar{F}$ is all other body forces, such as gravity ($\bar{F} = 0$ in our simulations). In the first step of the solution, we set $\partial \bar{v} / \partial t = 0$ in eqn (1) (time-independent solution).

To model the diffusion of fluorescein (transport of diluted species) we used the convection–diffusion equation

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) + \bar{v} \cdot \nabla c = R \quad (3)$$

and the continuity equation for the flux in and out of the channel

$$\bar{N} = -D \nabla c + \bar{v} c \quad (4)$$

where c is the concentration of the solute, R is the source or drain of the species, D is the diffusion coefficient and N is the total flux.

The material in the channel was set to have the viscosity and density of water. The sidewalls were defined by no-slip boundaries with no flux. Inlets were given the appropriate pressure relative to the outlets, set at 0 Pa. Inlets were also set to have fluid entering with the fluorescein concentrations used in the experiment, and $D = 4.9 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ (fluorescein).¹⁰

In model I each inlet was set to have the same concentration of solute as was used experimentally, and the pressures were set to change using step functions, similar to the pressure changes caused by the Fluigent system in the experimental setup.

In model II the channel was set to initially contain a solution with a concentration of 1 mM solute. For this model the pressure to the inlet remained constant while the concentration of the fluid entering was set to a step function to simulate pure water entering the main channel.

The pressure used, 466 Pa relative to the outlet, was chosen to yield the average fluid velocity in the channel of 3.3 mm s^{-1} . The results of this simulation at $t = 0.1 \text{ s}$ and $t = 3.4 \text{ s}$ after the fluid enters the inlet on the left are shown in Fig. 3. We can see that there is the expected parabolic profile in the main channel near the inlet at $\sim 0.5 \text{ mm}$ from the beginning of the main channel (indicated as a red dot in Fig. 4) as well as spreading of the interface due to diffusion of the solute over time when the interface has reached 12 mm.

Results and discussion

To demonstrate the basic functionality of our device, Fig. 4 A–C shows epifluorescence images of the device in its three states of operation, with the solution in the main channel being switched in the sequence I–II–III (see supplementary movie†). The observed laminar flow patterns shown are qualitatively very similar to the results from our simulations (Fig. 2 A–C) confirming consistency between experiment and simulation. Fig. 4 E shows data (in this case obtained by TIRFM) demonstrating that the order of cycling solutions can be reversed, to III–II–I, establishing this central performance objective.

We were also interested in the sharpness of the interface between two solutions upon switching, and specifically in the quality of switching very near a surface. In this context, there is a possible trade-off: on the one hand, maintaining a high flow speed is desirable to minimize inter-diffusion between fluids, needed to fulfil requirement (iii) as defined in the Introduction; on the other hand, the flow speed should not be too high⁵ to avoid excessive drag or shear stress as per requirement (iv), and to allow mixing by diffusion near the boundary, and thus interrupt the possible formation of a static layer near the boundary that could result from the no-slip boundary condition valid at a low Reynolds number. Therefore, we performed TIRFM measurements of the time evolution of the fluorescein concentration within about 100

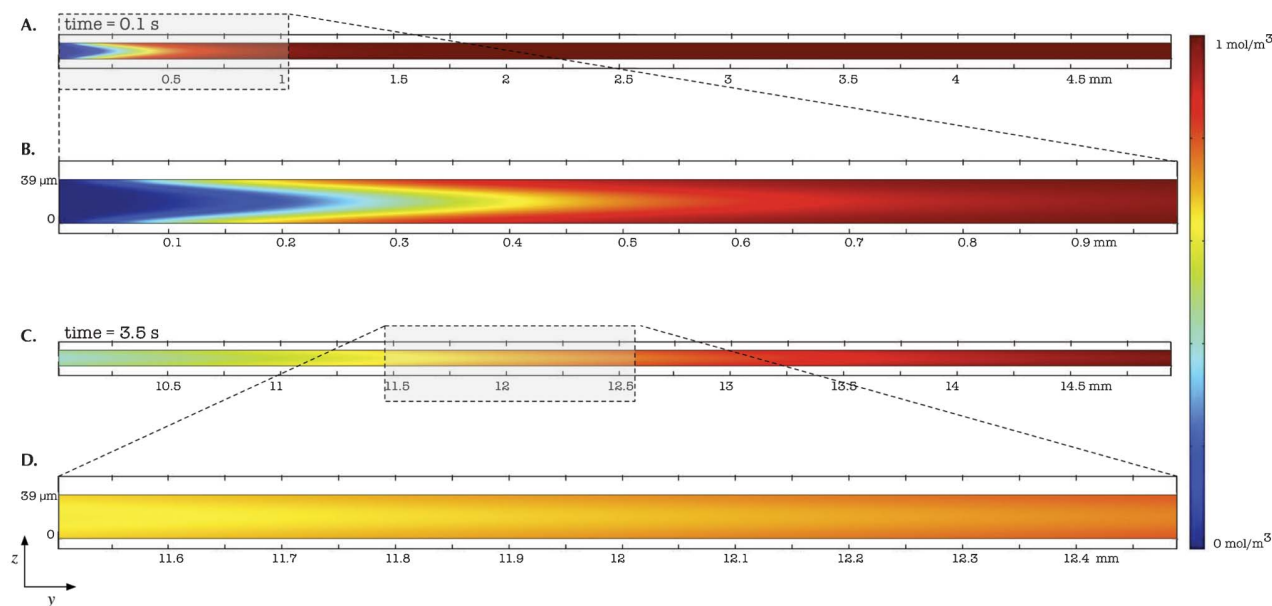


Fig. 3 A close-up of a COMSOL simulation for a rectangular channel 15 mm by 39 μm with a pressure drop of 466 Pa across $y = 0$ mm and $y = 15$ mm, initially full of water containing 1 mM solute. At $t = 0$ s water without a solute is introduced to the boundary held at 466 Pa. Top and bottom boundaries are set as “no-slip” boundaries. A, The range $0 \text{ mm} < y < 5 \text{ mm}$ at $t = 0.1 \text{ s}$. B, Close-up at the fluid interface, for the range $0 \text{ mm} < y < 1 \text{ mm}$ which shows the result of the parabolic velocity profile acting on the fluid, as well as the diffusion of the solute across the boundary between fluids. C, The range $10 \text{ mm} < y < 15 \text{ mm}$ at $t = 3.5 \text{ s}$, which shows the result of the Taylor diffusion spreading the fluid interface. D, Close-up of the range $11.5 \text{ mm} < y < 12.5 \text{ mm}$ for comparison with B. In our study we are most interested in the change from one fluid to the next within a thin layer at the wall ($0 \text{ nm} < z < 100 \text{ nm}$).

nm of the boundary as a result of switching between solutions I, II, and III.

The TIRFM data shown in Fig. 4D were collected in the region of the channel shown as a red dot in Fig. 4A when switching with $t_d = 10$ s. From this data we see that well-defined fluid switching is achieved within 100 nm of the boundary. Fig. 5 shows how diffusion and the parabolic flow profile gradually smears out the interfaces as we move along the channel, and further away from the inlet.

To characterize the quality of switching between fluids, we define the fall time t_s as the time to go from 90% intensity to 10% intensity (see inset to Fig. 4D), and graph the measured t_s as a function of the position y along the channel (see Fig. 6). We focus our discussion on the fall time, but note that the rise times behave the same within experimental error.

The measured values can be compared to theory as follows. The Taylor-Aris model^{14,15} takes into account diffusion and convection leading to a higher effective diffusion coefficient for the diffusion in the y direction (along the channel). The theory is valid when the time for diffusion in the z direction within the channel is much shorter than the travelling time of the interface, ensuring complete vertical mixing by diffusion, and thus preventing the formation of a boundary layer. More precisely this theory is valid when

$$\frac{y}{v_0} > \frac{a^2}{288D} \quad (5)$$

where y is the distance from the entrance of the interface, v_0 is

the average velocity of the fluid, $2/3$ the maximum velocity in the centre of the channel, and a is the height of the channel.

We define

$$y_0 = \frac{a^2 v_0}{288D} \quad (6)$$

such that the Taylor-Aris theory is valid at distances $y \gg y_0$ from the inlet of the main channel. In our case $y_0 = 32 \mu\text{m}$.

Using the effective diffusion coefficient for convection and diffusion

$$k = D + \frac{\kappa(av)^2}{D} \quad (7)$$

with $\kappa = 1/210$ for a rectangular channel,⁹ one obtains the following expression for the concentration as a function of the time, $c(t)$, after a fluid enters a rectangular channel:

$$c(t) = c_0 \frac{1}{2} \left(1 + \text{Erf} \left[\frac{y - vt}{2\sqrt{tk}} \right] \right) \quad (8)$$

where c_0 is the fluid concentration.

In Fig. 5 we show, as triangles, circles and squares, an example set of the TIRFM data for three different y values, 1 mm, 6 mm, and 12 mm. Also shown as solid and dashed lines are calculated concentrations $c(t)$ (eqn (8)) for the same y -values as a function of time.

The average flow speed (averaged over the channel cross section), $v = 3.3 \text{ mm s}^{-1}$, for this pressure setting was determined by fitting $c(t)$ to the experimental data for $y = 12$

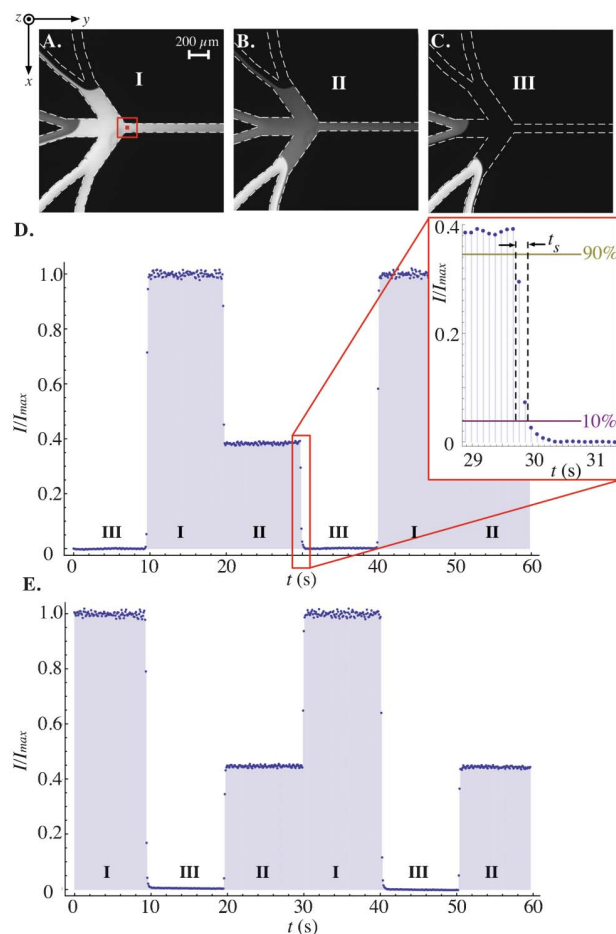


Fig. 4 A–C, Epifluorescence images taken of the device at different stages in the switching process (dashed lines outline the channel). The fluid flow in the main channel is from left to right. States I, II and III correspond to the different pressure states as in Fig. 2A, B, C (see supplementary movie). TIRFM was used to acquire images of the intensity measured in the region of interest as indicated by the red dot in A in the centre of the red square. D, An example of TIRFM data taken when $t_d = 10$ s for the dwell times of states III–I–II–III–I–II. The inset shows how t_s is found for each boundary between fluids. E, Result of changing the order of switching between the fluids.

mm where the Taylor–Aris theory is known to be valid; the same v was then used for the $c(t)$ curves for $y = 1$ mm and $y = 6$ mm, in good agreement with the experimental data. Using the same procedure we determined an average flow speed of 8.3 mm s^{-1} for the higher-pressure settings (see Methods).

Fig. 6 shows, as lines, values of t_s extracted from theoretical graphs of $c(t)$ as shown in Fig. 5, as a function of y for different v . In the range $y < 100y_0$, where the condition $y \gg y_0$ is not fulfilled the curves for t_s are shown as dashed lines. Experimental data from TIRFM experiments for the two pressure settings are also shown. Each experimental data point represents the mean of four or more consecutive t_s values extracted from one data set, and the error bars represent the standard deviation of those values. This was done to reduce the uncertainty introduced by the finite frame rate, which limits the resolution of the raw data in Fig. 5.

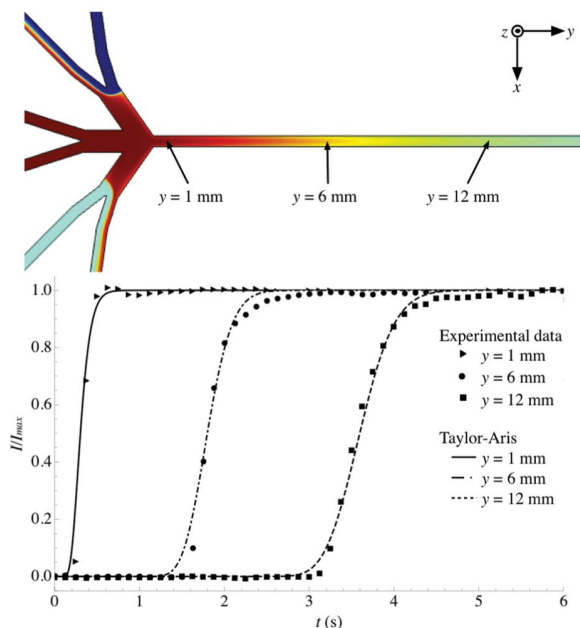


Fig. 5 The top panel shows the device design and the image from a COMSOL simulation where the red fluid was recently introduced in the channel. Below is shown the data taken with TIRFM at $y = 1$ mm, 6 mm, and 12 mm with triangles, circles and squares respectively. The solid and dashed lines show the predicted results assuming Taylor–Aris diffusional spreading with an average fluid velocity 3.3 mm s^{-1} at each y value.

Comparing experimental and theoretical data, we found that for $y \gg y_0$ the measured t_s are in good agreement with the corresponding theoretical curves. Crucially, the experimental data extend into the range where the condition $y \gg y_0$ is not satisfied, where no theoretical estimate of t_s near a boundary is available, and where one may be concerned that effective solution switching near a boundary may not be possible. We found that the experimental t_s are generally smaller than predicted by extrapolating the Taylor–Aris theory into this regime, demonstrating a good quality of switching even very near the channel surface.

In the region where Taylor–Aris theory is valid a constant concentration along the z -direction is expected. Where Taylor–Aris theory is not valid, one expects the switching times near the boundary to be slower than at the channel centre, so that the measured rates are expected to be a lower limit of the switching times.

The data shown is for a particular case where the solute studied is fluorescein. If one were to consider a different solute it is not immediately obvious how switching time would be affected by changing the diffusion coefficient, D . Using eqn (7) and (8) to extract switching times numerically we find that t_s is decreased for larger D , as a consequence of the improved diffusional mixing (see ESI for more details).

We now consider the force exerted on a surface-bound particle by the flow used here. Using a velocity profile of the fluid with average velocities of 3.3 mm s^{-1} and 8.3 mm s^{-1} in a rectangular channel¹⁶ we found fluid velocities of about $40 \text{ } \mu\text{m s}^{-1}$.

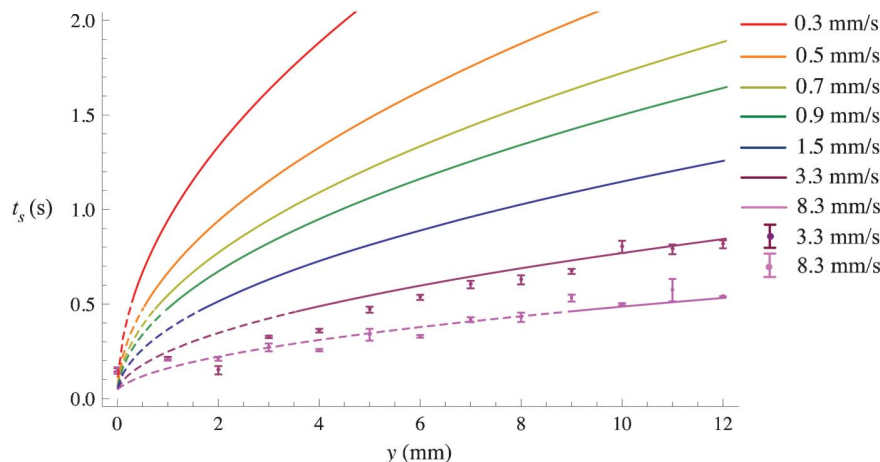


Fig. 6 The switching time, t_s as a function of position y along the channel ($y = 0$ as defined by the red dot in Fig. 4). The curves show the predicted results for switching times t_s from the Taylor–Aris model for different average fluid velocities, as indicated. The solid lines indicate where the theory is within its range of validity, $y \gg y_0$ (see eqn (5) and (6)). The dashed lines show the region where $y < 100 y_0$. Also shown are the switching times measured with TIRFM at multiple positions along the channel for two pressure settings, corresponding to $v = 3.3 \text{ mm s}^{-1}$ and $v = 8.3 \text{ mm s}^{-1}$ as indicated.

s^{-1} and $100 \mu\text{m s}^{-1}$, respectively, at a distance of 100 nm from the channel surface. For our experiments with TW, we intend to use a quantum dot (QD) attached to the TW *via* a biotin-streptavidin linker.¹⁷ In that case, most of the drag force due to fluid velocity will be on the QD. Using Stokes' law for a QD with radius 10 nm ,¹⁸ a flow speed of $100 \mu\text{m s}^{-1}$ corresponds to a drag force of about 0.02 pN . This is much less than the load force of $F_{\text{load}} \approx 0.5 - 1 \text{ pN}$ against which TW is expected to be able to step (based on a step size of $\Delta x = 11 \text{ nm}$ and the condition that $F_{\text{load}}\Delta x$ must be less than a few kT , where $kT \approx 4 \text{ pN nm}$).¹⁹ Also in more general biochemical contexts, a drag force of 0.01 pN can be considered very small: applied over the typical size scale Δx of several nm for a protein, a force of the order of $F \approx 1 \text{ pN}$ is needed to perform work $F\Delta x$ comparable to the magnitude of natural, random fluctuations in kinetic energy of scale kT . This establishes that our device can be used for rather disturbance-free experiments on surface-adhered particles on the order of 10 nm in size.

We achieved experimental switching speeds of $t_s = 0.2 \text{ s}$ near the entrance to the main channel and close to the surface (Fig. 6). The lower limit of time between switches, t_d , is determined by the device geometry and our pressure control system (Fluigent): because the fluid needs to move beyond the three sections of channel branching and into the main channel before the next fluid switch occurs, our device cannot reliably switch between fluids in less than one second at these low pressures. To improve performance (to reduce switching times) one could increase the overall pressure on the device, thus increasing the fluid velocity in the branching channels and reducing the time between switches. Similarly, switching times could also be decreased by decreasing the length of these branches, or by increasing the device depth to decrease the fluid resistance in these branches.

To use the device over extended time periods it is worth noting that about 0.5 ml h^{-1} of fluid passes through the device

(to all four waste reservoirs) under the conditions we have used.

A similar design using waste outlets connected to each inlet was recently used in a micropipette device.²⁰ Here, we used an on-chip integration of this approach, allowing, in principle, the up-scaling to an arbitrary number of fluids with little time added to device fabrication.

Conclusions

We have demonstrated a microfluidic device that can be used to change the chemical environment in the immediate vicinity of a microchannel surface in arbitrary and changeable time order, with sub-second switching between solutions, and at low fluid velocity. This device fulfils all of the specific requirements (see Introduction) for use with a chemically powered molecular motor such as TW, but is equally useful for a wide range of other experiments where real-time control of the chemical environment is useful. We demonstrated the device for three different solutions, but in principle, the same design can be used for use with more than three different fluids. A key result is the experimental demonstration that fluid switching is achieved very close ($<100 \text{ nm}$) to the channel surface. This is possible due to diffusion perpendicular to the channel walls, such that the chemical composition near the channel walls is the same as in the bulk of the channel, in spite of the non-slip boundary condition that prevents fluid flow at the surface. Our experimental data show that this fluid switching near the surface works in practice also outside the range of validity of the Taylor–Aris theory.

Acknowledgements

We acknowledge financial support from the Human Frontier Science Program (RGP0031/2007), nmC@LU, and the Knut and Alice Wallenberg Foundation. We thank Maria Hansson and Masoomah Ghasemi for their preliminary efforts towards this work as summer students. Also, we thank Henrik Persson, Martina Balaz and Neville Gilhooly for valuable discussions.

Notes and references

- 1 F. Sachs, Practical limits on the maximal speed of solution exchange for patch clamp experiments, *Biophys. J.*, 1999, **77**(2), 682–690.
- 2 J. Sinclair, *et al.*, Stabilization of high-resistance seals in patch-clamp recordings by laminar flow, *Anal. Chem.*, 2003, **75**(23), 6718–22.
- 3 G. Ohlsson, *et al.*, Solute transport on the sub 100 ms scale across the lipid bilayer membrane of individual proteoliposomes, *Lab Chip*, 2012, **12**(22), 4635–4643.
- 4 D. L. Pettit, *et al.*, Chemical two-photon uncaging: a novel approach to mapping glutamate receptors, *Neuron*, 1997, **19**(3), 465–71.
- 5 P. B. Allen, *et al.*, Pressure-driven laminar flow switching for rapid exchange of solution environment around surface adhered biological particles, *Lab Chip*, 2010, **10**(6), 727–733.
- 6 P. R. Bianco, *et al.*, Processive translocation and DNA unwinding by individual RecBCD enzyme molecules, *Nature*, 2001, **409**(6818), 374–8.
- 7 E. H. Bromley, *et al.*, The Tumbleweed: towards a synthetic protein motor, *HFSP J.*, 2009, **3**(3), 204–12.
- 8 N. J. Kuwada, G. A. Blab and H. Linke, A classical master equation approach to modeling an artificial protein motor, *Chem. Phys.*, 2010, **375**(2–3), 479–485.
- 9 A. J. Bae, C. Beta and E. Bodenschatz, Rapid switching of chemical signals in microfluidic devices, *Lab Chip*, 2009, **9**(21), 3059–3065.
- 10 P. Chen, *et al.*, Hydrodynamic gating valve for microfluidic fluorescence-activated cell sorting, *Anal. Chim. Acta*, 2010, **663**(1), 1–6.
- 11 B. Kuczenski, P. R. LeDuc and W. C. Messner, Pressure-driven spatiotemporal control of the laminar flow interface in a microfluidic network, *Lab Chip*, 2007, **7**(5), 647–649.
- 12 S. E. Hulme, S. S. Shevkoplyas and G. M. Whitesides, Incorporation of prefabricated screw, pneumatic, and solenoid valves into microfluidic devices, *Lab Chip*, 2009, **9**(1), 79–86.
- 13 M. A. Unger, *et al.*, Monolithic microfabricated valves and pumps by multilayer soft lithography, *Science*, 2000, **288**(5463), 113–116.
- 14 G. Taylor, Dispersion of soluble matter in solvent flowing slowly through a tube, *Proc. R. Soc. London, Ser. A*, 1953, **219**(1137), 186–203.
- 15 R. Aris, On the dispersion of a solute in a fluid flowing through a tube, *Proc. R. Soc. London, Ser. A*, 1956, **235**(1200), 67–77.
- 16 H. Bruus, Theoretical microfluidics, *Oxford Master Series in Condensed Matter Physics*, 2008, New York, Oxford University Press.
- 17 A. R. Dunn, *et al.*, Single Qdot-labeled glycosylase molecules use a wedge amino acid to probe for lesions while scanning along DNA, *Nucleic Acids Res.*, 2011, **39**(17), 7487–7498.
- 18 Invitrogen. *Qdot Steptavidin Conjugate*, 2007 2007-September-12 [cited 2012 June]; Product Page]. Available from: <http://probes.invitrogen.com/media/pis/mp19000.pdf>.
- 19 N. J. Kuwada, *et al.*, Tuning the performance of an artificial protein motor, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2011, **84**(3).
- 20 A. Ainla, *et al.*, A multifunctional pipette, *Lab Chip*, 2012, **12**(7), 1255–6.