Autonomous Microfluidic Capillary System

David J. Juncker,*†,‡ Heinz Schmid,† Ute Drechsler,† Heiko Wolf,¶ Marc Wolf,‖ Bruno Michel,¶ Nico de Rooij,¶ and Emmanuel Delamarche*,‡

Zurich Research Laboratory, IBM Research, 8803 Rüschlikon, Switzerland, Institut de Microtechnique, Université de Neuchâtel, 2000 Neuchâtel, Switzerland, and Departement Forschung, Universitätsskliniken Basel, 4031 Basel, Switzerland

The transport of minute amounts of liquids using microfluidic systems has opened avenues for higher throughput and parallelization of miniaturized bio/chemical processes combined with a great economy of reagents. In this report, we present a microfluidic capillary system (CS) that autonomously transports aliquots of different liquids in sequence: liquids pipetted into the service port of the CS flow unidirectionally through the various sections of the CS, which comprises a 15-pL reaction chamber, into the capillary pump. A CS can thus be operated by simply delivering the different samples to its service port. The liquid transport concept presented here is advantageous because the pumping and valving functions are integrated into the device by means of capillary phenomena, and it therefore does not require any external power supply or control device. Thus, arrays of CSs can easily be formed by cloning a functional CS. Alternatively, the flow of liquids in CSs can also be interactively tuned if desired by (i) forcing the evaporating of liquid out of the capillary pumps and (ii) by contacting a secondary, removable capillary pump to the embedded ones. We illustrate the possibilities of CSs by conducting a surface immunoassay for a cardiac marker, within 25 min, on an area of 100 × 100 μm², using 16 sequential filling steps.

Here, we present a concept for a microfluidic system that integrates all of these attributes. All actions necessary to supply and flush a reaction chamber with multiple solutions—including pumping, valving, and synchronization—are effected by the capillary pressure, which is “coded” into the design of the flow path.² Such a microfluidic capillary system (CS) is autonomous. Its liquid transport mechanism is reminiscent of the one used by trees.⁵–⁷ The most distal elements are the main contributors to the flow, and there is no need for moving parts.

FUNDAMENTAL CONCEPTS

The spontaneous motion of a tiny drop of liquid deposited on a structured surface is governed by capillary phenomena resulting from the interplay between the surface tension of the liquid and the chemistry and geometry of the solid interfaces and leads to the overall minimization of the free energies between the solid, liquid, and vapor interfaces.⁸ These capillary phenomena can be exploited to fill microstructures spontaneously⁹–¹¹ and to valve,¹²¹³ guide,¹⁵¹⁶ and actively displace¹⁷–²¹ liquids along surfaces.

M miniaturization of biological and chemical processes requires transporting and mixing liquids at the microscopic scale; this is the realm of microfluidics. Here, the motion of liquids is dominated by phenomena other than at the macroscopic scale, and it therefore necessitates devising microfluidic systems with novel and tailored liquid-handling capabilities. Many efforts are directed toward the integration of such capabilities into a truly miniaturized system, but so far microfluidic systems still require external macroscopic actuators, cumbersome fluidic connections, and electromechanical interfaces, which limit their scaling down and portability.¹⁻³ An ideal microfluidic system should be straightforward to fabricate, self-contained, and simple to use, yet flexible, robust, free of dead volumes, and easily duplicated to form arrays.

* To whom correspondence should be addressed. E-mail: (D.J.) jun@zurich.ibm.com; (E.D.) emd@zurich.ibm.com.
† IBM Research.
‡ Universität de Neuchâtel.
¶ Universitätsskliniken Basel.


The capillary pressure \( P_c \) of a liquid–air meniscus in a rectangular microchannel is

\[
P_c = -\gamma \left( \frac{\cos \alpha_b + \cos \alpha_c}{d} + \frac{\cos \alpha_l + \cos \alpha_r}{w} \right)
\]

where \( \gamma \) is the surface tension of the liquid, \( \alpha_{b,l,r} \) are the contact angles of the liquid on the bottom, top, left, and right wall, respectively, and \( d \) and \( w \) are the depth and width of the channel, respectively. Each wettable (\( \alpha < 90^\circ \)) wall of the CS contributes to generating a negative pressure in front of the liquid and to drawing the liquid into the channel. Washburn\(^{23} \) derived the hydrodynamic solution of capillary filling for conduits of constant cross section. If the cross section varies, however, smaller cross sections will produce a correspondingly higher differential capillary pressure. Thus, from eq 1, it is possible to calculate precisely how to vary the cross section of a channel to drain a finite volume of liquid from one region to another and, further, to predict the flow rate between these regions. For example, by adopting an arborescent structure for the flow path,\(^{24} \) large quantities of liquid can be displaced unidirectionally to new regions of ever-increasing capillary pressure. Here, we designed the geometry and wettability of the channel network in such a way that the liquid is transported along a “programmed” and functional succession of “flow” and “stop” actions, which are synchronized by (i) the delivery of aliquots of solutions into the service port and by (ii) their subsequent drainage out of the service port.

**EXPERIMENTAL SECTION**

The microfluidic CSs were fabricated in double-side-polished Si wafers (Siltronix, Geneva, Switzerland) using photolithography and a deep reactive ion etcher (STS ICP, Surface Technology Systems plc, Newport, U.K.) in a four-step procedure. Top structures and the upper half of vias were patterned on one side, the reaction chamber and the lower half of vias on the other. The photoresist and oxide masks corresponding to the structures and vias were transferred into the Si sequentially.\(^{16} \) Ten nanometers of Ti (adhesion layer) and 50 nm of Au were sputtered (LA440S, VonArdenne Anlagetechnik GmbH, Dresden, Germany) to render their surfaces hydrophilic and derivatized with a thiolated poly(ethylene glycol) (Rapp Polymere, Heidelberg, Germany) to render these surfaces nonwettable. The other parts of the CS were microcontact-printed on both sides with a flat poly(dimethylsiloxane) (VonArdenne Anlagetechnik GmbH, Dresden, Germany) to both sides of the structured Si wafer. Networks of CSs were diced and microcontact-printed on both sides with a flat poly(dimethylsiloxane) (PDMS; Sylgard 184, Dow Corning, Midland, MI) stamp, which was inked with an ethanolic solution of eicosanethiol to render these surfaces nonwettable. The other parts of the CS were derivatized with a thiolated poly(ethylene glycol) (Rapp Polymere, Heidelberg, Germany) to render their surfaces hydrophilic and protein-repellent.

The elastomeric substrates for the deposition of proteins were \( \sim 1 \text{-mm-thick pieces of PDMS} \) cured against the bottom of a Petri dish. This PDMS was formulated using Sylgard 184, low-molecular-weight cross-linkers, and a methylhydroxiloxane homopolymer to increase the hardness of the material. The elastomeric substrates were separated from the CS after the patterning processes and rinsed with deionized water prior to being dried under a stream of N\(_2\).

Deionized water was produced with a Simplicity 185 system (Millipore). Chemicals were from Fluka and proteins from Sigma, unless otherwise indicated. Human C-reactive protein (CRP), the monoclonal anti-CRP antibodies used for capture and recognition (labeled with fluorescein isothiocyanate (FITC) green, \( \lambda_{FE} = 520 \) nm), were purchased from Hytest (Turku, Finland). Solutions of BSA were dissolved in phosphate buffer saline (PBS; BupH, Pierce, Rockford, IL) at 1\( \text{mg/mL} \) and passed through 0.22-\( \mu \text{m} \) filters (Millipore). The solutions of proteins used in adsorption experiments were prepared in PBS, whereas the analyte and recognition proteins were prepared in a 1% solution of bovine serum albumin (BSA) in PBS. The rabbit antibodies labeled with tetramethylrhodamine isothiocyanate (TRITC, red, \( \lambda_{FE} = 570 \) nm) were prepared as a 500 \( \mu \text{g/mL} \) solution.

The secondary capillary pump was formed by two hydrophilic cover slides held together—but with a 90-\( \mu \text{m gap} \)—by double-sided Scotch tape (3M). They were contacted by their edges with the embedded capillary pumps to extract liquid as desired. A stream of N\(_2\) was directed through four Cu tubes (each \( \sim 0.5 \text{ mm} \) in diameter), located a few millimeters above the embedded capillary pumps to force evaporation, and regulated using a pressure valve.

Video sequences were shot with a digital camera (Coolpix 990, Nikon) affixed to an ocular (Figure 2 and video 1) and an inverted microscope (Eclipse TE 300, Nikon; Figure 4 and video 2). The fluorescent images were obtained using a microscope (Labophot-2, Nikon) equipped with optical filters, captured by a cooled, low-noise CCD camera (ST-8, SBIG, Santa Barbara, CA), and analyzed using a homemade software programmed in Labview (National Instruments).

**RESULTS AND DISCUSSION**

Figure 1 is a sketch of an autonomous microfluidic CS and illustrates how to use it. The user needs only to deliver the required quantity of each solution in the desired sequence to a service port, from where the liquid flows through the open and closed sections of the microchannel into the capillary pump (CP), while never emptying the closed section (which includes the reaction chamber) of the CS. Figure 2 shows how two (out of four) arrayed and nearly identical CSs (etched into a Si wafer) are filled with nanoliter volumes of solutions. Video 1\(^25\) shows in real time how each of these four CSs is addressed at least twice and how one CS is filled and flushed four times in sequence. The cross section of the CSs shown in Figure 2 and in video 1 is schematically represented in Figure 3a and b, and its dimensions are described in Table 1. The pressure curves for a wetting and a dewetting liquid–air interface (solid and dashed line in Figure 3c) were calculated for deionized water under the assumption that the liquid surface tension (\( \gamma = 70 \text{ mN m}^{-1} \)) and the wettability of the surfaces of the CS are invariant. The slight difference between these pressures originates from the hysteresis between the advancing and receding contact angles.\(^{26} \) The capillary pressure that generates the motion of the liquid is the difference in the pressure of the wetting meniscus in the CP and that of the

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(25) Videos 1 and 2 are available as supporting material CS1.avi and CS2.avi, respectively, on http://www.zurich.ibm.com/st/microcontact/video.
dewetting meniscus in the filling port or access channel (Figure 3c). As indicated, the CP in this device produces sufficient capillary pressure to drain both the service port and its appended access channel. The constriction (inlet via) just before the reaction chamber was designed to be the smallest structure of the CS and has the highest capillary pressure. It plays the role of a valve, which pins the interfacial meniscus of the dewetting liquid and protects the reaction chamber from adventitious drying. We therefore call it a capillary retention valve (CRV). The functionalities defined by the structure of the CS used throughout this report thus include (i) pumping the solution from the service port to the reaction chamber, (ii) draining the service port, (iii) stopping the flow when the service port is empty to keep the reaction chamber wet, and (iv) enabling multiple cycles. This series of actions is effective to transport sequences of liquids and is performed autonomously by the CS.

The dynamic description of the liquid motion necessitates an exact value of the flow resistance of the rectangular channels, which can be calculated with a Fourier series. We derived a first-degree approximation, which converges to the exact solution when the flow is steady and laminar. The flow resistance is nearly invariant once the liquid has passed the reaction chamber, enabling the transport of multiple samples of different solutions under similar flow conditions.
Table 1. Hydraulic Parameters of the Microfluidic Capillary System

<table>
<thead>
<tr>
<th>sectiona</th>
<th>service port</th>
<th>access channel</th>
<th>inlet via (μm)</th>
<th>reaction chamber (μm)</th>
<th>outlet via (μm)</th>
<th>capillary pump²</th>
</tr>
</thead>
<tbody>
<tr>
<td>length</td>
<td>1.5 mm</td>
<td>2 mm</td>
<td>150</td>
<td>500</td>
<td>150</td>
<td>6 mm</td>
</tr>
<tr>
<td>width</td>
<td>1.5 mm</td>
<td>140 μm</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>42 μm</td>
</tr>
<tr>
<td>depth</td>
<td>150 μm</td>
<td>150 μm</td>
<td>30</td>
<td>50</td>
<td>30</td>
<td>250 μm</td>
</tr>
</tbody>
</table>

a Advancing and receding contact angles of deionized water: on the walls of the channels, 45° and 33°; on the elastomeric substrate, 123° and 100°. The inlet via functions as capillary retention valve. These parameters have been varied as follows: l, 50–5000 μm; w, 50–100 μm; d, 4–50 μm. From 1 to 15 channels run in parallel, depending on the position, with a total volume of 800 nL.

The time periods needed for the successive exchanges of one solution in the pipet with the next one (during which no movement of liquid is observable in the reaction chamber) were cut out of the sequence shown in video 2, but the exact total time is reported in Figure 4. The successive filling and exchange of each solution in the four reaction chambers, however, is shown in real time. The subsequent filling and exchange of each solution in the four reaction chambers, however, is shown in real time. All reaction chambers remain permanently wetted because the CRV protects the liquid from draining them and, importantly, also prevents the entrapment of air in the conduits.

The integrated microfluidic CSs fabricated with different dimensions (see Table 1) all exhibited the same basic function-

\[ D = \frac{1}{12\eta} \left( \frac{a^2}{b} \right) \left( \frac{R_h + A}{6b} \right) \Delta P = \frac{1}{\eta} \frac{\Delta P}{R_{FR}} \]  

where \( a \) and \( b \) are either the width or the height to satisfy the condition \( b \geq a \), \( R_h \) is the hydraulic radius defined as \( R_h = 2A/P \), with \( A \) and \( P \) being the cross section and perimeter of the conduit, respectively, \( L \) is the filled length of the conduit, \( \Delta P \) is the difference of pressure between the two liquid fronts, and \( \eta \) is the viscosity of the liquid. For simplification, the geometric features can be written as the flow rate resistance \( R_{FR} \) of a channel section, which sums up to the total value: \( R_{FR} = 8.5 \times 10^{15} \text{ m}^{-3} \) for the CS represented in Figure 3. The normalized \( R_{FR} \) is shown in Figure 3d and reveals that \( R_{FR} \) accumulates mainly in the via and the reaction chamber of the microfluidic system and, importantly, that the contribution of the CP is negligible because of its arborescent architecture. As the flow resistance is nearly constant in the CP, this pump will draw aliquots successively added to the service port at a constant rate.

This microfluidic capillary system can be readily cloned to form ensembles because each entity contains the entire functionality, rendering parallelization trivial. We fabricated four-CS arrays and verified the flow properties experimentally by sequentially flushing the reaction chambers of these arrays with colored liquids, as shown in Figure 4 and in real time in video 2.25 In this example, all liquids supplied after the first aliquot had a flow rate close to the calculated value of 220 nL s⁻¹ and an average speed of \( v_{av} = 55 \text{ mm s}^{-1} \) in the 500-μm-long and 50-μm-deep reaction chamber. The time periods needed for the successive exchanges of one solution in the pipet with the next one (during which no movement of liquid is observable in the reaction chambers) were cut out of the sequence shown in video 2, but the exact total time is reported in Figure 4. The successive filling and exchange of each solution in the four reaction chambers, however, is shown in real time. Note that the water dispensed after the blue solution contains 5 μm polystyrene beads that reveal the direction and the speed of the flow of liquid in the reaction chamber. All reaction chambers remain permanently wetted because the CRV protects the liquid from draining them and, importantly, also prevents the entrapment of air in the conduits.

<Figure 4. Images (extracted from a video) illustrating the autonomous transport of liquids in four separate reaction chambers. The chambers (500 × 80 × 50 μm³) were sealed with a layer of PDMS through which the video was recorded. These chambers are filled sequentially with 150-nL aliquots of water (a) and of water containing colorants (b, c); flushed, and filled with water again within a few seconds (d, e). This result is also available as video 2.25>

Notably but accordingly generated different flow rates. The design of the CP was unchanged and therefore produced an almost identical pressure for all variants. The different dimensions of the reaction chambers, however, alter the \( R_{FR} \) according to eq 2. As mentioned above, the main contribution to \( R_{FR} \) precisely originates in the reaction chamber for this CS, and therefore, the size of the reaction chamber can be tailored to control the flow rate. When only the dimensions of the reaction chamber are varied, only the \( R_{FR} \) of this section of the flow path needs to be recalculated. The total \( R_{FR} \) of the original CS with a reaction chamber of 500 μm × 80 μm × 50 μm (length × width × depth) can thus easily be compared with CSs having different reaction chambers. For instance, a CS with a reaction chamber of 500 μm × 80 μm × 10 μm would have a total \( R_{FR} \) that is ~11 times larger and a flow rate of ~11 nL s⁻¹ for water, which, for a delivered volume of 200 nL of aqueous solution, will result in a continuous flow lasting for ~18 s. The length (and width) of the reaction chamber can also be modified to tune \( R_{FR} \). For example, a CS with a reaction chamber of 100 μm × 100 μm × 10 μm (for the calculation of \( R_{FR} \), an effective length of 80 μm was taken to account for the reduced flow path length due to the inlet and the outlet vias at the extremities of the reaction chamber) has a total \( R_{FR} \) that is approximately twice that of the original CS. Experiments corroborated all the above calculations. Very long flow times might be desirable for certain bio/chemical processes and can in principle be achieved using an appropriate design. However, in such a case, controlling the evaporation of the reagents from the service port becomes increasingly important. The flow rate quickly reduces as the depth of the channels becomes shallower, and

eventually it becomes impossible to transport even small volumes (e.g., 100 nL) within a reasonable time. Therefore, it is preferable to adjust the total \( R_{\text{FR}} \) of the CS by means of the pressure generated in the CP for each application.

The CSs presented here are three-dimensional but can also be made planar, in which case the contribution of the reaction chamber to the total \( R_{\text{FR}} \) may be less significant. We prefer to use three-dimensional CSs because they can easily be filled from the backside, while a reaction can occur in the chamber on the front (processing) side.\(^{(29)}\) Reactants dispensed into the service port of a CS will not flow when the connected CP is entirely filled. The size of the CP has to be increased at the expense of the overall compactness of the CS to pump more solutions and larger volumes of liquids. A small CP can be sufficient, however, if (i) liquid is actively evaporated out of it using an air stream,\(^{(29)}\) a heat source, or a sorption agent\(^{(30)}\) or if (ii) a secondary CP can be reversibly connected to the embedded one when desired. Either of these methods creates the opportunity to control interactively the flow rate inside a single CS, or in arrays of CSs, in which case the flow of liquid in the various CSs can be synchronized. A large air stream, or a series of small ones, can be directed onto all individual CPs and force the evaporation. Such active evaporation permits flushing volumes in excess of the CP capacity without affecting the pressure “coded” into the different sections of the CS. In the other case, it is possible to prefill many service ports and trigger additional flow from them to their respective reaction chambers by contacting all embedded CPs with a single, large secondary CP. The key for controlling the flow of liquids here is the synchronization of the capillary pressure of the secondary CP with the pressures generated by the arrayed CSs. The CSs and the removable capillary pumps can be designed to generate a capillary pressure coordinated with respect to each other, so that only the desired, and predetermined, movement of liquid is generated. In particular, the capillary pressure of each CRV needs to be higher than that of the secondary CP. If this is the case, the functionality of the newly formed device can be identical to the one contained in each original CS.

We found these concepts robust and suited to transport solutions such as water, water with surfactants, buffers, electrolytes, ethanol, and solutions containing biomolecules or beads. We were able to deliver between 0.8 and 20 nL s\(^{-1}\) (using passive and forced evaporation, respectively) of solution to a 6-\( \mu \)m-deep reaction chamber, which, using dispensed aliquots of 300 nL, is sufficient to ensure a continuous supply to the reaction chamber for at least 6 min, because there are no dead volumes. Slow flow rates can thus be tuned to achieve a homogeneous concentration of the reactants by diffusion in the reaction chamber, for example.

We used CSs for patterning small areas on PDMS surfaces (Figure 5). PDMS was used because this elastomer induces the deposition of proteins from solution\(^{(22,28)}\) and can seal the reaction chamber of a CS.\(^{(16)}\) The amount of labeled protein adsorbed directly from solution to the 50 \( \times \) 50 \( \mu \)m\(^2\) area of the PDMS substrate covered by the reaction chamber (15 \( \mu \)L in volume) was followed using fluorescence microscopy (Figure 5c). The measured fluorescence reflects the deposition conditions, of course, and has a root-mean-square (rms) intensity variation of only 2.5\% on the central 80\% of the surface. The quality of this patterned area of proteins compares favorably with the typical inhomogeneities of protein-microarraying techniques,\(^{(1)}\) in which drying effects, uncontrolled surface wetting phenomena, and the absence of a rinse step impair the quality of the patterns of proteins. The utility and potential of CSs for standard biological assays is illustrated with a solid-phase sandwich immunoassay (Figure 6).\(^{(31)}\) Monoclonal antibodies against CRP were adsorbed on PDMS, and after a blocking step using BSA, they were used to capture CRPs from a sample according to the procedure described in Figure 6a. CRP is an indicator of general inflammatory response and of special interest for the prognosis and diagnosis of coronary diseases,\(^{(32)}\) e.g., myocardial infarction. The CRPs captured on the surface were then detected using fluorescently tagged anti-CRP antibodies: Figure 6b shows the two (out of the four) zones of the substrate in which the immunoassay and an adjacent negative


Figure 5. Deposition of fluorescently tagged antibodies from a solution drawn through a reaction chamber onto an elastomeric substrate. (a) The 15-\( \mu \)L reaction chamber is visible by looking through the transparent substrate using an optical microscope. (b) Multiple aliquots (~200 nL each) of pretreatment solutions, sample (IgG–TRITC), rinsing solutions (PBS), and blocking solutions (BSA) were flushed through the reaction chamber of the CS. (c) This fluorescence micrograph of the enclosing substrate, but after separation from the CS, reveals a highly homogeneous deposited pattern of antibodies that mirrors the outline of the chamber. The rms variation of the fluorescent intensity within the dashed rectangle is only 2.5\%. Keys for such a homogeneous pattern are the controlled flow conditions in the reaction chamber and the rinsing steps, which can, for instance, prevent the sample from drying on the spot.
control were performed. All the solutions for this experiment, i.e., 4 \times 16 \times 200 \text{ nL} aliquots (4 arrayed CSs \times 16 sequential filling steps) were distributed manually in the microfluidic CSs, and the entire assay was performed in less than 25 min. Such a large number of solutions could be filled into these CSs because forced evaporation and a secondary CP were used to interactively tune the flow and extract liquid in parallel from all embedded CPs. The flow rate was optimized for each step; it was high during the rinsing steps and low when the sample containing the CRP was loaded into the service port of the CS. A lower flow rate of the solution containing the analyte provided reaction times of up to 5 min despite the minute volumes and thereby increased the sensitivity of the assay. The preliminary results obtained here serve as proof of concept, and the performance of CSs can most certainly be improved by further optimizing their design and the experimental procedures.

CONCLUSION
The microfluidic CS introduced here combines effectiveness and convenience of use because it is autonomous. Such a CS is self-sufficient and arrayable per se, and multiple systems can be interconnected to form networks. The liquid transport can, if desired, be tuned using a tailored secondary CP, air convection, or both, which is not more difficult to do for multiple CSs than for a single one. Because the CP is at the end of the flow path and also acts as the waste collector, a simple conduit is sufficient to connect the unique service port to a reaction chamber, no adverse mixing of samples occurs before they react, and the system is free of coupling elements. In principle, the CP could be tailored to generate much higher capillary pressures by making it porous or filling it with a porous material or with a gel and thus exploit them to propel the liquids through a large number of functional elements such as sampling zones, valves, mixers, and reaction chambers. The concepts presented here may contribute to the building of microfluidic systems useful for portable diagnostics, combinatorial assays, and applications requiring high throughput. Further fundamental improvements can be foreseen via the combination of autonomous CSs with selective valving (which can distribute liquids according to their composition) and could lead to “smart” CSs. At last, the similarities and differences our CSs bear to models found in Nature—e.g., to tall trees that have the perplexing ability to transport liquid from their roots to their leaves 100 m above ground—also reveal that there should still be ample room for perfection of microfluidic CSs.

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