Simple, robust storage of drops and fluids in a microfluidic device†

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We describe a single microfluidic device and two methods for the passive storage of aqueous drops in a continuous stream of oil without any external control but hydrodynamic flow. Advantages of this device are that it is simple to manufacture, robust under operation, and drops never come into contact with each other, making it unnecessary to stabilize drops against coalescence. In one method the device can be used to store drops that are created upstream from the storage zone. In the second method the same device can be used to simultaneously create and store drops from a single large continuous fluid stream without resorting to the usual flow focusing or T-junction drop generation processes. Additionally, this device stores all the fluid introduced, including the first amount, with zero waste. Transport of drops in this device depends, however, on whether or not the aqueous drops wet the device walls. Analysis of drop transport in these two cases is presented. Finally, a method for extraction of the drops from the device is also presented, which works best when drops do not wet the walls of the chip.

Introduction

In microfluidics, drops are considered as "micro-reactors", as each drop can be the site of an independent experiment.[1–3] Distinct processing steps include formulation, drop creation and mixing of the contents of the drop. This paper will focus on related subsequent steps: on-chip drop storage and extraction, which are useful for the class of devices where drop processing occurs on-chip.

Drop formulation and drop creation, which are conceptually distinct steps, are often intimately related. In this paper drops will always be an aqueous phase while an immiscible oil will be the continuous phase. Popular techniques for continuous drop generation are "flow focusing"[4] and the "T-junction"[5] where the flow of water solution is cut by a flow of oil. These two methods allow drop production at a frequency as high as tens of kilo-Hertz, but have several shortcomings. One is that formulation is limited to gradual composition variations in successive drops.[3] A second shortcoming is that there is a start-up time before the flows are steady enough to produce drops of the desired size and frequency and thus it is inevitable that some solution is wasted. Mixing the drop's contents is achieved by chaotic advection when the drop passes around a corner.[3] An alternate approach to generate drops employs microfluidic valves and pumps to meter precise amounts of reagents into a mixing chamber[6] and then create a drop of the mixed solution using a T-junction where the flow is regulated by valves.[7] This method has the advantage that drops of arbitrary composition can be created in any sequence. Disadvantages are that valve based mixing and drop creation is slow, yielding about a drop per minute and that the manufacture and operation of valve based devices is difficult.

No matter how drops are created, drop storage becomes a crucial issue for experiments that require following each specific drop over time. For single phase flows one sample storage method includes a digital "chemical memory" whereby multiplexed valves can address individual storage chambers. This technique is relatively complex and difficult to use because it involves multi-layer PDMS devices and a large array of multiplexed valves.[8] The valve based storage methods have been extended to drops,[7] but still require a relatively complex supporting infrastructure to manufacture and operate these devices.

Recently, we implemented two methods to use surface tension forces generated by the shape of the microfluidic channels to guide drops into storage wells.[9] These methods required surfactant to prevent the contents of different drops from mixing together. Our goal here is to address drop storage with the twin objectives of minimizing external intervention from the user and avoiding drop-to-drop contact, thereby eliminating the need of a surfactant to stabilize the drops. Drops interact with channel junctions in complex ways. Consider the case where channel hydrodynamic resistance is the dominant factor influencing the choice of which channel a drop enters. Because the drop itself increases the channel resistance the presence of a drop in a channel can influence whether subsequent drops enter a different channel. Designing the hydrodynamic resistance of a channel network is a passive way to control droplet operations in microfluidic devices. Numerous devices have been developed to accomplish disparate tasks such as drop sorting, splitting, formulation and synchronization.[10–14]

Here we describe a single microfluidic device and two passive methods that can (1) store drops created in a previous step upstream, which we call "create then store", and (2) simultaneously create and store drops, which we call "store and create". This device is based on capillary valves[15] and changes of hydrodynamic resistances induced by the presence of drops introduced into the channel network. Furthermore, this

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microfluidic design also has the advantage to allow drop extraction from the storage wells by simply reversing the direction of flow in the device.

Experimental

Materials

We generated droplets using two different techniques and chip designs; "flow focusing"[4] and "peristaltic pumps".[7] In both cases, devices have been made in polydimethylsiloxane (PDMS, Sylgard 184 (Dow Corning)) using standard soft lithography fabrication methods[2,9,16] and detailed fabrication instructions are included in the supplementary section of our previous work.[9] The PDMS device is sealed on a glass slide coated with a thin layer of PDMS such that all walls of the channel are PDMS. Each chip has a "bypass" storage region, illustrated in Fig. 1, consisting of channels of height $h = 110$ μm. The width of the main channel and storage wells, w_w, is 150 μm, the width of the bypass, w_b, is 75 μm, the width of the restriction, w_R, is 15 μm and the length of the restriction, L_R, is 150 μm. An AutoCad file of all the devices used in these experiments is included in the electronic supplementary information.†

The continuous phase is a fluorinated oil, FC-3283 (3M), a perfluorinated tertiary amine with the formula $N(C_3F_7)_3$. It is used either as is or with 2% in weight of a proprietary surfactant R24 (provided by Raindance Technologies, Inc.). R24 is based on DuPont's Krytox, a perfluoropolyether, which has been carboxylated. The surfactant R24 is used to prevent drop coalescence and wetting of water droplets on PDMS. The dispersed phase is either pure water, or water to which food coloring has been added (1% by volume of Hanneford Foods Blue) for flow visualization.

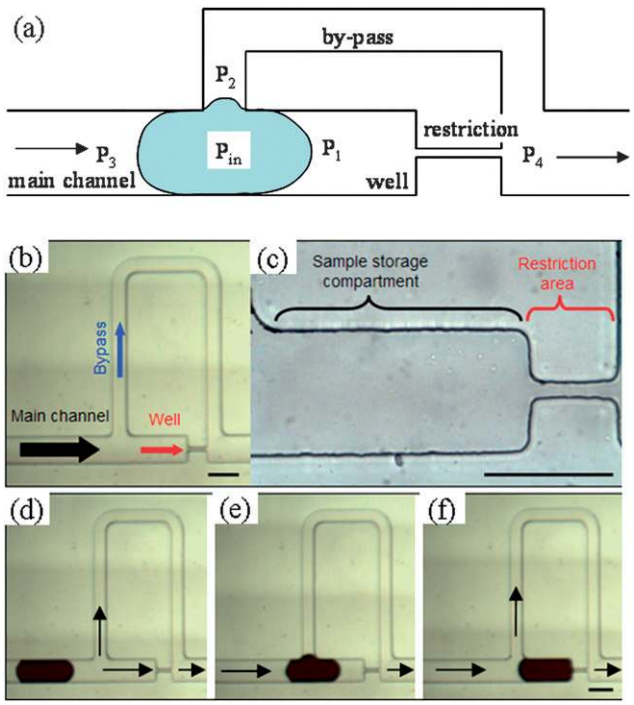

Fig. 1 Bypass storage well. (a) Plan view of one storage unit. The main channel bifurcates into the "well", where the drop is stored, and the "bypass" channel. Channel widths: main and well are 150 μm, bypass 75 μm and restriction 15 μm. The channel height is 110 μm. **(b)** Photograph of one storage unit. **(c)** Details of the storage region consisting of the wide well and narrow restriction used to prevent the drops from leaving. **(d,e,f)** A sequence of images showing a drop docking in a well. A movie of the "create, then store" process is in the ESI (Movie S1).† Scale bar 150μm.

Contact angle

The pendant drop method and Axisymmetric Drop Shape Analysis (ADSA) have been used to measure the interfacial tension of water/oil (FC-3283) and water/oil/surfactant (FC-3283 / 2% R24 surfactant).[17–20] The presence of surfactant decreases the interfacial tension: $\gamma_{FC\text{-}3283} = 40$ mN/m and $\gamma_{FC\text{-}3283/2\%R24} = 12$ mN/m. The manufacturer reports the viscosity of FC-3283 as $\mu = 1.3 \times 10^{-3}$[kg m^{-1} s^{-1}] and the density of FC-3283 as 1820 [kg/m^3].

Jamin pressure

The measurement of the pressure drop across a stationary drop in a channel, known as the Jamin effect[21–23] has been made using a PDMS microfluidic device with a single channel (Fig. 2). The two ends of the oil channel were connected to two bottles of oil (the continuous phase) and the bottles were mounted on a vertical rail. Their height difference was set to control the differential pressure across the single drop in the channel. In order to place a single drop of water in a channel we manufactured a "drop on demand" device[7] to create drops using on-chip valves as indicated in Fig. (2).

Results and discussion

Bypass storage

High density microwell plates are an important class of devices. Their virtues are small volume, isolation of the contents of one well from another, and the precise location of the drops in space which renders such devices amenable to automated detection. We recently developed a platform named the "Phase Chip" in which nanolitre drops stored on a PDMS chip have their water content reversibly varied in order to measure and control phase transitions.[9] Simplified, but precise and robust methods for creating, storing and extracting droplets in this type of device are the topic of this paper.

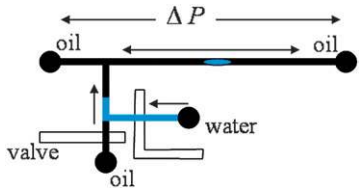

Fig. 2 Drop on demand. Schematic of a "drop on demand" device used to measure the Jamin effect. A single drop is produced in the T-junction by operating PDMS valves. Then the valves are closed and a differential pressure is applied between the oil inlet and outlet. The drop velocity is measured as a function of pressure. An AutoCAD file of the actual device is included in the ESI (S7.dwg).†

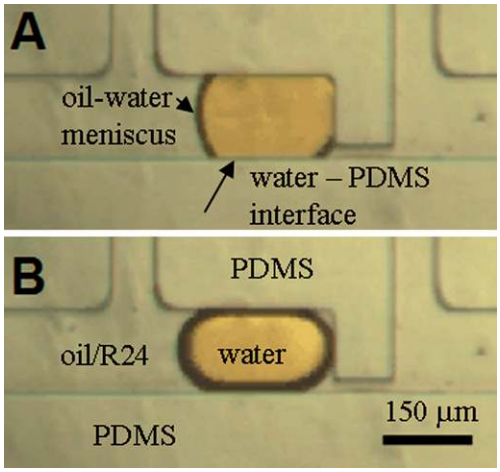

Fig. 3 Wetting and non-wetting drops. (A) Wetting: A drop of water is stored in a well. The rest of the device is filled with the fluorinated oil FC-3283. The drop wets the PDMS walls as evidenced by the lack of optical contrast between the drop and PDMS channel. **(B)** Non-wetting: Photograph of the same drop as in (A) taken 10 min after the addition of surfactant R24 (2%) to the oil. The contact angle is increased and the optical contrast is greater compared to (A). A movie is included in the ESI (Movie S2).†

During the storage process, we observed two different behaviors depending on whether it is the aqueous drop or the fluorinated oil that wets the PDMS channels. In Fig. 3, there is a difference in the optical contrast in photographs of drops that wet the channels and those which are separated from the channel by a thin layer of oil. Non-wetting aqueous drops were produced by using the fluorinated oil, FC-3283, for the continuous phase to which was added the surfactant R24 at 2% concentration. This surfactant also has the benefit that it prevents the drops from coalescing.

Create, then store droplets

We have developed a new way to store drops of aqueous solution driven by a continuous flow of oil. This method, which we call "create, then store" is passive in the sense that in operation only a steady flow of fluid through the device is required. The droplet, created upstream from its storage location, is guided by a combination of hydrodynamic flow and capillary valves into a storage well. Fig. 1 shows a picture of a single storage element consisting of a main channel that enters a junction which has two channels on the exit. One channel, labeled the "well", begins as a continuation of the main channel and then narrows by a factor of ten at a restriction. There is a second channel labeled the "bypass" oriented at 90 degrees to the main channel and of half the width that serves as an alternative flow path when the well is occupied. At the junction between the well and the bypass the flow coming from the main channel splits into two parts. For a single phase fluid the splitting of the flow between the two channels depends solely on their respective hydrodynamic resistances. However, when a drop enters a junction between two channels, a combination of capillary forces and the relative hydrodynamic resistance of the channels determine whether or not a drop will go through one channel or another, or even break into two drops.[10,24] A movie (S1) illustrating the storage process is included in the ESI.†

Flow partition

The fractions of the total flow from the main channel that enters the bypass and well channels were measured by co-flowing a stream of water with dye next to a clear water stream, as shown in Fig. (4). In the case shown in Fig. (4), all of the dye flows into the bypass so the following relationship holds: $m_{\mathrm{dye}} = b_{\mathrm{dye}}$ where m_{dye} and b_{dye} are the dye currents [vol/sec] in the main and bypass channels, respectively. The dye currents are a fraction of the total current in each channel; $m_{\mathrm{dye}} = f_m m$ and $b_{\mathrm{dye}} = f_b b$ with m and b the total currents in the main and bypass channels, respectively, and f_m and f_b the fraction of dye in the main and bypass channels, respectively. Conservation of flow states that $m = b + w$, with w the total flow in the well. Therefore, the fraction of the total flow that flows down the bypass channel is $b/m = f_m/f_b$. The fraction of dye in each channel was measured using the Plot Profile function of Image J (http://rsbweb.nih.gov/ij/) directly from the photograph in Fig. (4) ($f_m = 0.48, f_b = 0.70$), from which we determine that 70% of the flow goes down the bypass and the remaining 30% of the flow enters the well.

When we first designed this device we naively expected the drop to flow down the path of least resistance and predicted that the drop would take the bypass path as 70% of the flow passes through that route. Therefore, we were surprised when drops entered empty wells rather than the bypass channel.

Capillary valve for non-wetting drops

In Fig. (1c–e), a drop of water, dyed black, in a continuous stream of oil is moving from the left to the right in the main channel and enters the junction. The drop must deform in order to enter the narrower bypass channel. Therefore the pressure across the drop must exceed the Laplace pressure if the drop is to enter the narrower channel and thus the narrow opening of the bypass channel acts as a capillary valve.[15] If the drop followed

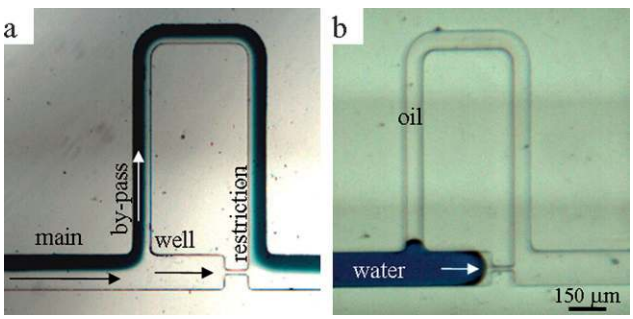

Fig. 4 Hydrodynamic resistance of channel network. (a) Clear and dyed water are introduced as co-flowing streams in the main channel and bifurcate at the junction of the bypass and well channels. Dyed water occupies 48% of the main channel and 70% of the bypass channel implying that 70% of the total flow is carried by the bypass channel and 30% flows through the well. **(b)** The device is filled with transparent oil and then a plug of colored water is flowed into the device. Although the resistance of the bypass is much less than the well, none of the water enters the bypass until the water completely fills the well, demonstrating that the bypass functions as a capillary valve.

the path of least hydrodynamic resistance it would flow down the bypass, which carries 70% of the total flow, as illustrated in Fig. (4). However, we observe that the drop does not enter the bypass until a critical drop velocity of ~1 mm/sec is exceeded. Fig. 1a is a drawing of the drop in the junction and indicates locations in the device of the pressures described in the equations below. The sum of the pressure drops (P [N/m^2]) from the inside of the drop (P_{in}) to where the bypass rejoins the main channel (P_4) must be equal thus

$$(P_{\text{in}} - P_2) + (P_2 - P_4) = (P_{in} - P_1) + (P_1 - P_4) \quad (1)$$

For the case where the aqueous drop does not contact the channel, but instead the continuous oil phase wets the channel, the Laplace pressure accounts for the pressure drop between the inside and outside of the drops. We assume the drop is non-wetting on the channel walls and thus the radii of curvature are set by the channel dimensions.

$$P_{\text{in}} - P_1 = 2\gamma(1/h + 1/w_w) \text{ and } P_{\text{in}} - P_2 = 2\gamma(1/h + 1/w_b) \quad (2)$$

where h[m] is the height of the channel and is the same everywhere, while w_w[m] and w_b[m] are the widths of the channel in the well and bypass region, respectively, and γ[N m^{-1}] is the surface tension. The pressure drops $P_1 - P_4$ and $P_2 - P_4$ arise from the flow of oil through the restriction and bypass, respectively. Now consider the case where the drop is moving slightly below the critical velocity, v_c[m/s], when the drop covers the opening to the bypass channel, but does not enter the bypass channel. In this case there is no flow in the bypass channel and thus

$$P_2 = P_4 \quad (3)$$

However, the drop moves forward into the well and in doing so forces oil to flow through the restriction leading to a pressure drop

$$P_1 - P_4 = Q_{\text{c}}R \quad (4)$$

with Q_{c}[m^3 s^{-1}] the critical flow through the restriction and R[kg m^{-4} s^{-1}] the resistance of the restriction given approximately by

$$R = \frac{12\mu\, L_R}{h\, w_R^3} \quad (5)$$

With L_R, w_R, and h the length, width and height of the restriction, respectively and with μ[kg m^{-1} s^{-1}] the viscosity of the oil. Rearranging these expressions leads to an expression for Q_{c}:

$$2\gamma(1/w_b - 1/w_w)/R = Q_{\text{c}} \quad (6)$$

Experimentally we measure the flow Q_{c} by measuring the velocity of a drop, v_c, as it advances into the well as illustrated in Fig. 1e. All the flow through the well also goes through the restriction so we have $Q_{\text{c}} = v_c A = v_c\, h\, w_w$with A the cross-section of the well. Putting all this together yields:

$$\frac{\gamma\left(\frac{1}{w_b} - \frac{1}{w_w}\right) w_R^3}{6\mu\, L_R\, w_w} = v_c \quad (7)$$

If the velocity, v, of the drop in the well is less than v_c then the pressure difference $P_1 - P_2 < 2\gamma(1/w_b - 1/w_w) = 160$ Pa is too small for the bypass to function and the bypass acts as a closed capillary valve. In contrast, if $v > v_c$ then the drop enters both the well and bypass channels and splits in two. Substituting the material parameters into eqn (7), the formula for the critical velocity yields $v_c = 1.5$ mm/s, consistent with observation.

After entering a well the drop continues to flow downstream until it encounters the restriction at the bottom of the well, which prevents the drop from moving further. The minimum pressure needed to drive a drop through the restriction is the Laplace pressure given by $\Delta P_{\text{res}} = 2\gamma(1/w_R - 1/w_w) = 1400$ Pa.

Typically an external pressure of between 10^3 and 10^4 Pa is used to drive drops at velocities of ~1 mm/s in the channels because the external pressure needed to achieve this velocity is a function of the length and radius of the tubing connecting the chip, as well as the geometry of the channels on the chip.

Sequential loading

After a first drop is trapped in a well, consider what happens when a following drop enters the junction (Fig. 5a, ESI Movie S1).† Since the first drop plugs the restriction all the flow is directed down the bypass channel. Thus when a second drop enters the junction it is driven to the entrance of the bypass channel. If the pressure difference across the drop is not sufficient to overcome the Laplace pressure needed to drive the drop into the bypass channel, or in other words, if the capillary valve is "closed", then flow of oil through the device will stop as both the bypass and well channels are blocked, in which case the external pressure difference across the device of 10^3–10^4 Pa would be applied across the drop. In practice, as the drop begins to occlude the bypass channel the resistance to flow increases and therefore the pressure difference across the drop also increases. Once the pressure exceeds the Laplace pressure for a drop with one part in the bypass and one part in the main channel (which is 160 Pa for our case) the drop enters the bypass and flows downstream; *i.e.* the capillary valve opens. Since the restriction channel is five times narrower than the bypass channel the bypass opens first and the pressure across the drop stored in the well is insufficient to drive the drop through the restriction. The movie S1† shows this process. A device consisting of 500 storage units linked in series on a 4 cm × 4 cm area is illustrated in Fig. 5b. The drop length is 1100 μm and the drop volume is 25 nl. The variation in drop size is 10%.

The drop storage does not depend on spacing between drops, which makes our method robust. This is in contrast to another storage method in which the presence of a drop in the bypass channel is necessary in order to store the next drop in a well.[25]

Effect of wetting on storage: pinning pressure, or the Jamin effect

In the absence of the surfactant R24 we observed that water droplets that are longer than the width of the channel are in contact with the PDMS (Fig. 3). Apparently the oil drains from between the water drop and PDMS channel walls, leaving the water in direct contact with the PDMS. In these circumstances the drop is pinned to the walls and to move the drop at all requires application of a finite pressure across the drop to overcome the pinning. The pressure needed to dislodge a drop was typically observed to be a few hundred Pascal, which is of the

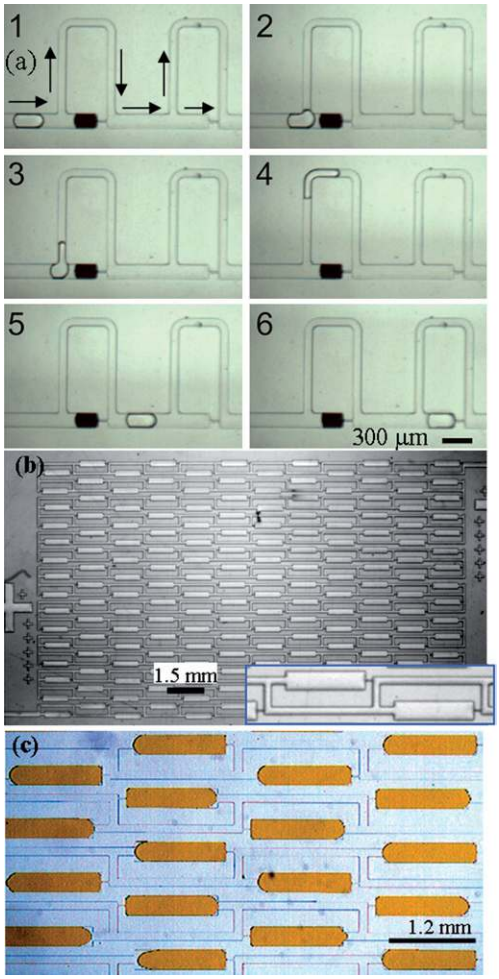

Fig. 5 Sequential loading of wells. (a) (1) After the first well is filled (see Fig. 1c-e and Movie S1)† with a dyed drop of water a second clear drop is introduced. The dyed drop blocks flow through the restriction. (2,3) The clear drop enters the bypass channel. (4) The drop moves down the bypass and (5,6) enters the second well in a manner identical to how the first drop entered the first well shown in Fig. 1c-e. **(b)** High density storage chip (empty) of a symmetrical design with 90 wells/cm^2. Each well is 200 μm wide and the volume is 25 nl. The AutoCAD file for this device is in the supplementary material (S7.dwg).† **(c)** Wells filled with dyed water; channels filled with oil.

order of the pressure needed to drive a drop from the wide main channel into the narrow bypass channel; therefore drop pinning is large enough to disturb the storage process. The phenomenon of a pinning pressure for drops that wet the walls of channels was first described in 1860 by Jamin[21,22] who observed that if a pressure P is applied at the entrance of a capillary filled with water and bubbles of gas the fluid will flow only if the pressure difference P applied across the drop is higher than a pressure ΔP_{Jamin}. The physics of the Jamin effect is contact angle hysteresis; the advancing and receding contact angles of a drop moving in a channel are different if the drop wets the channel, as illustrated in Fig. (6). This generates a Laplace pressure, ΔP_{Jamin}, across the drop, which must be exceeded for the drop to move;

$$\Delta P_{\text{Jamin}} = n2\gamma(\cos\alpha_r - \cos\alpha_a)/r = n2\gamma(1/R_r - 1/R_a) \qquad (8)$$

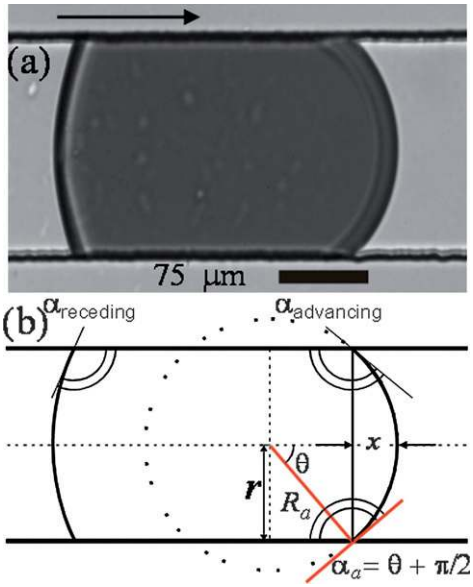

Fig. 6 Contact angle hysteresis. (a) Image of a stationary drop that was moved last in the direction indicated by the arrow. **(b)** The advancing and receding angles are defined. For each meniscus the radius of curvature (R_a, R_r) is determined as indicated for the advancing meniscus using the formula $R_a = (x^2 + r^2)/2x$. The advancing angle is determined from $\sin\theta = r/R_a$ where r is half the width of the channel and x is the distance between the apex of the drop and the line connecting the points where the drop contacts the channel. For this drop $\Delta P_{\text{Jamin}} = 350$ Pa.

with n the number of drops in the capillary, γ the oil/water surface tension, $\alpha_{r,a}$ the receding and advancing contact angles, respectively, r the capillary radius, and $R_{r,a}$ the radii of the receding and advancing menisci, respectively. The magnitude of the Jamin pressure, ΔP_{Jamin}, is proportional to the number of drops and inversely proportional to the radius of the capillary. The physical origins of contact angle hysteresis are varied. It can arise from chemical heterogeneity, surface roughness, or because the receding edge of the drop is exposed to a surface that has recently been in contact with water in contrast to the advancing edge, which moves on a surface that was last exposed to oil.[23]

Our particular case of a water drop in a channel of square cross section is slightly different than the cylindrical capillary studied by Jamin, but we chose to analyze it the same way as Jamin by equating the diameter of the capillary in the expression for ΔP_{Jamin} with the width of the square channel in our devices. To measure the pinning pressure we built a microfluidic device consisting of a single channel and designed such that we could control the number and size of drops in the channel such as shown in Fig. 6. We incrementally varied the differential pressure across a drop on a single channel and measured the velocity of the drop. Fig. 7 shows the drop velocity as a function of the differential pressure across the two ends of the channel. When using surfactant to prevent wetting, the speed of the drop is directly proportional to the applied pressure. There is no pinning pressure and the advancing and receding contact angles of the drop are equal. But when there is no surfactant and the drop wets the PDMS channel walls, a finite pressure must be applied before the drop begins to move (ΔP_{mov}). The data presented in Fig. 6 and 7 are for water droplets in FC3283 fluorinated oil in

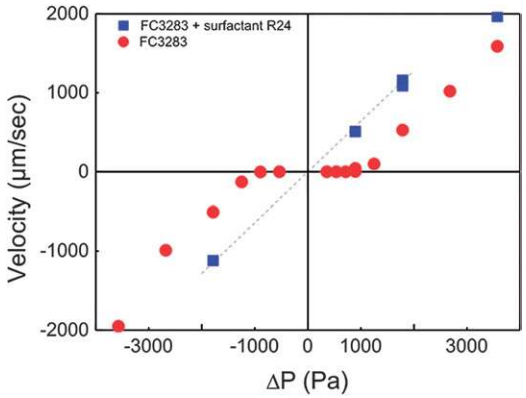

Fig. 7 Drop velocity vs. pressure. Two experiments are performed on single drops in a square channel (Fig. 6). In the first experiment a drop with surfactant (squares) moves with a velocity proportional to the applied pressure. In the second experiment a drop without surfactant (circles) did not move until a critical pressure $\Delta P_{mov} = 350$ Pa was exceeded. The drop moved at a few microns per second until the pressure exceeded 1000 Pa. Above this pressure the change in velocity with change in pressure was the same for drops with and without surfactant. A movie illustrating the Jamin effect is in the ESI (Movie S3).†

untreated PDMS channels. For this particular trial the experimental minimal pressure required to move a drop was $\Delta P_{mov} = 350$ Pa. For the same drop at rest, the calculated Jamin pressure was $\Delta P_{Jamin} = 322 \pm 85$ Pa, which was calculated using the measured advancing and receding angles, channel width, and oil/water surface tension. The drop moved very slowly, but steadily, when the external pressure was varied between 350 Pa and 1000 Pa, as shown in Fig. (7). At higher pressures the slopes of the velocity *vs.* pressure curves for the drop with and without surfactant become equal, indicating that the drop becomes completely free above 1000 Pa. We observed a variation of a factor of two in the Jamin pressure for different chips and for measurements repeated the next day. This is not surprising given that the surface properties of PDMS are known to change with processing conditions and with time.

A robust experimental observation consistent with the theory of the Jamin effect was that ΔP_{mov} was proportional to the number of drops in the channel, which we verified for one, two and three drops. Another robust observation was that the pressure needed to dislodge a drop, ΔP_{mov}, was independent of the length of the drop, also as predicted for the Jamin effect. Movies S3a, S3b, and S3c illustrating the Jamin effect are included in the ESI.†

Drop transport using constant pressure

For the class of microfluidic devices based on valves, it is natural to drive drops using constant pressure because the valves are pressure operated. However, for drops that wet the channel walls we often observed undesirable behavior, such as a drop stopping when the receding end of the drop appeared as if it was barely covering the entrance to the bypass as shown in Fig. (8a). If the drop actually did completely block both the bypass and well channels then the full external pressure would be across the drop because there would be no flow at all in the entire device. The external pressure (typically greater than 10^4 Pa) exceeds by far

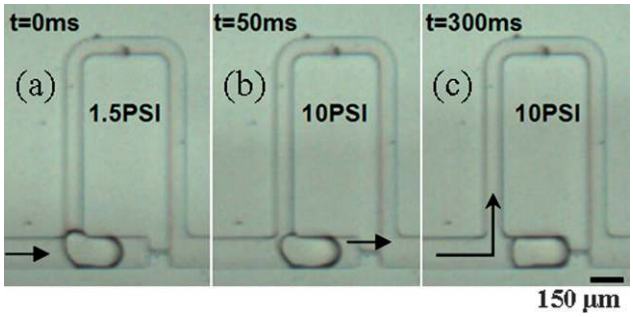

Fig. 8 Failure to enter well. (a) Drop is stopped at the junction between the bypass and well (see Fig. 1). Oil must leak past the drop otherwise the 1.5 psi external pressure would move the drop. **(b,c)** Increasing the pressure to 10 psi drives the drop into the storage well. A movie of these images is in the ESI (Movie S4).†

the pressure needed to drive the drop through the junction into either the well or bypass channels. The explanation for the drop stopping is that the drop actually does not completely close the entrance to the bypass channel, but there is a small leakage path. In this case there is a pressure difference across the drop caused by the oil flowing through the bypass channel, but it must be less than the pressure necessary to dislodge the pinned drop.

The simplest way to get the drop moving is to increase the external pressure to the device. The limitation to this solution is that if the pressure is increased too much then the drop will rupture at the junction between the main channel, bypass and well. The second solution is to use surfactant to create a lubricating layer between the drop and PDMS wall and thereby eliminate contact angle hysteresis. The third solution is to treat the channel surface to prevent the drop from coming into contact with the channel and thereby eliminate pinning which leads to the difference in the advancing and receding contact angle. We have observed that drops which are stabilized against coalescence with the R24 surfactant are sometimes pinned to bare PDMS walls. In this case, when the first drop is stuck at the well/bypass intersection, the second drop will push the first into the well before the second one passes through the bypass and so on for the successive drops. If the emulsions are stabilized the drops don't coalesce on contact and operation of the device is robust and simple.

Often, however, for one or another reason, it is not possible to add surfactant to stabilize drops against coalescence or eliminate pinning of the drops to the channels. In this case we adopted a two step strategy for drop storage. In the first step, after its creation, the drop is driven through the device at a low pressure to prevent any drop breakage. When the drop is stopped at the entrance of the trap due to pinning, the oil pressure is then increased for a short time to overcome the pinning and push the drop into the well shown in Fig. 8 and in movie S4.† To generate and store drops, we used a valve based design, shown in Fig. 9 to generate droplets[7] but included two inlet lines of oil that were valve controlled but maintained at different constant pressures, the first line is at "low" pressure to drive the drop until the drop reaches the well/bypass junction, and the second oil line at "high" pressure to nudge the drop into the well. In a fashion similar to previous work[7] our device was operated in an open-loop mode using chip based valves and pumps controlled through a computer in a specific sequence to first create a drop, drive the

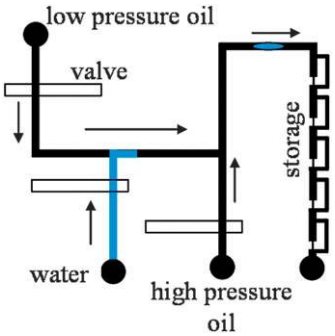

Fig. 9 Create, then store chip. Schematic of a chip to create drops with valves and store them in bypass wells as illustrated in Fig. 8 and in ESI movie S4.† The low pressure was used to create drops at the water-oil T-junction. The high pressure was used to overcome pinning forces and load drops in the storage wells. The AutoCAD file for the actual design is included in the ESI (S7.dwg).†

drop through the device by actuating the low pressure oil line for few seconds to be sure that the drop reached the entrance of the well and then switching to the high pressure line for the storage. In this manner drops could be reliably generated and stored sequentially.

We note that this last case, of storing drops that are not stabilized against coalescence could only be accomplished by developing a complex valve driven device and operating the chip with a precise and finely tuned sequence of timed events. This is not a recipe for an inexpensive, robust device. Further, we note that if the drops are pinned then it is not possible to store drops that are generated in a continuous fashion. In other words, one cannot store drops in this device if the drops are generated by flow focusing, as it is necessary to wait for the first drop to be stored before creating the second.

Store and create droplets simultaneously with zero waste

In contrast to the previous storage method ("create, then store"), in which drops are stored in locations downstream from where they are created, we have developed a simple and robust method to simultaneously store drops in the same location where they are created. This method, which we call "store and create", allows us to create hundreds of drops on a chip with zero loss. The "store and create" method, illustrated in Fig. 10 uses the exact same bypass and well design described previously (see Fig. 1). Simply stated, instead of storing a sequence of pre-formed drops into a series of wells, we flow a long plug of aqueous sample containing no surfactant whose volume is many times that of an individual well into a device completely pre-filled with oil (no surfactant is required). The aqueous plug is then followed by oil. The sequence of steps to create and store drops is illustrated in Fig. 10 and in Movie S5.† To recapitulate, first the entire chip is primed with oil. When the aqueous plug enters a storage region it displaces the oil and fills the well with aqueous phase. Once the well is filled the plug flows through the bypass channel. The aqueous plug is stopped by the oil-filled restriction located at the down-stream end of the well, which acts as a capillary valve identically as described previously for drops. The priming of the device with oil is not strictly necessary as an air-water interface at

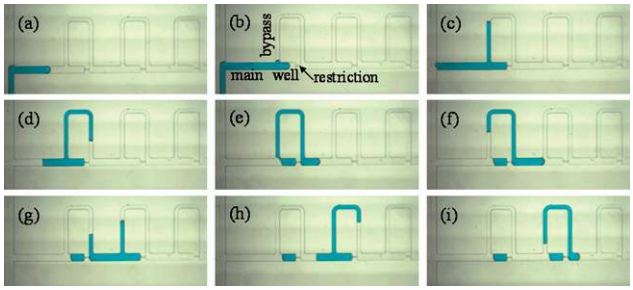

Fig. 10 Store and create drops. (a) Initially the entire bypass storage device (see Fig. 1) is primed with clear oil. Dyed water is flowed into the device. **(b)** Water enters the well, but not the bypass. **(c,d)** Once the well is filled the bypass capillary valve opens. **(e, f)** The oil, which is pushing the water, cuts the water stream in two creating a drop in the first storage well. **(g, h)** The water fills the second well and opens the second bypass capillary valve. **(i)** The oil cuts the water stream in the second well creating a drop in the second storage well. A movie of this "store and create" process is in the supplementary material (Movie S5).†

the restriction would also function as a capillary valve. But because PDMS is permeable to air, after some short amount of time the air in the restriction would diffuse into the PDMS and vanish. Without an air-water interface the restriction would no longer function as capillary valve. Therefore we use fluorinated oil as it has a very low solubility in PDMS. After all the water has entered the device, oil, which follows the aqueous plug also flows through the bypass and thereby isolates the aqueous phase in the wells. This process is automatically repeated until the aqueous plug is entirely consumed (or all the wells have been filled). The number of wells filled is the ratio of the volume of the aqueous plug to the volume of a single well.

We emphasize the simplicity of operating this device, which occurs in two steps. First the entire device is dead-end filled with oil. This is simply done with a hand held syringe. Next the aqueous sample, usually between 100 nl and a 1000 nl volume is aspirated into a narrow tubing (typically 300–700 μm ID) that has been pre-filled with oil. Then the aqueous plug and oil is injected into the device in one sequence. This can also be done with a hand held syringe. The fact that a 95% fill rate is achieved with a hand held syringe is a testament to the robustness of this method.

Drop extraction

To extract the droplets from the storage area we merely reverse the flow of oil through the device. The restriction acts as a one-way capillary valve; a high pressure is needed to push a drop from the well through the restriction, but only a low pressure is needed to have the drop flow in the opposite direction out of the well into the main channel. It is desirable to extract the drops in the same order they were stored. But often, especially when the drops contain biological materials such as proteins, the drops will wet and adhere to the PDMS walls. In this case when we reverse the flow of oil the drops leave the wells in a random order as each drop is pinned to the PDMS to a different degree. To facilitate extraction in general we introduce a surfactant into the oil just before extraction. In the example illustrated in Fig. 3 the oil is FC3283 and the surfactant is R24 at 2% volume fraction. It

took approximately 10 min for the drops to detach after the surfactant was flushed through the device, as shown in Movie S2.† The optical contrast between the drop and PDMS wall is increased when the drop detaches from the wall (Fig. 3). Detached drops have no pinning pressure to overcome in order to move. In this case, reversing the flow of oil on the device leads to all the droplets leaving the wells at the same time, as shown in Movie S6.† This has the consequence to preserve the order of samples; when the flow is reversed the last drop stored is extracted first and the first drop stored is extracted last. This method provides a solution to extract stored samples and keep track of each sample. Additionally, this extraction method can be used regardless of the method to store and create the drops. However, depending on how much protein is stored in the drop, how long the drop has been stored in the device, and how much the drop has shrunk during storage we often observe the drop wetting the PDMS even in the presence of surfactant. To make this extraction method more robust, further experiments are necessary.

Conclusion

We developed a simple microfluidic design to store drops on-chip. Two methods were described. In the first, "create then store", stabilized drops of water in oil were stored in a PDMS chip. Although we stored water drops in oil, this method will also work for any object carried by a single phase flow, such as cells or beads. The most attractive feature of this system is that the storage process is achieved passively. Samples are directed to wells by hydrodynamic flow and capillary forces with minimal external intervention from the user. The second method which we call "store and create" is used to simultaneously store and create hundreds of droplets with zero loss and requires no surfactant. This method has a high success rate (over 95% of wells are filled) and is so robust that the device can be operated by a hand held syringe. The extraction process is also simple and operates by introducing surfactant and then reversing the flow in the device.

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