Sorting by distance: hydrodynamic control of droplets traffic

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Abstract LoCs are usually realized through monolithic devices. Samples are processed by passing them through a preset sequence of elements. This is achieved by exploiting fixed microfluidic channels. To increase reusability of LoCs, their effectiveness and flexibility, it is crucial to be able to control and direct droplets’ motion. Accordingly, in this paper we propose and assess a passive sorting device in which the path followed by a droplet can be controlled through a series of junctions simply modulating the distance between droplets in microfluidic channel.

Keywords Droplet · Passive sorting · Traffic control

1 Introduction

The use of droplets as confined microreactors in microfluidic devices has growing application in chemical and biological experiments, since droplets offer many advantages such as very low consumption of reagents and samples, fast mixing, and avoid cross-contamination. (Teh et al 2008; Yang et al 2010).

Several operations can be performed on droplets from their generation, such as splitting, merging, sorting, mixing, storing, etc (Teh et al 2008). Each of these unit operations corresponds to a distinct processing step of a biochemical protocol. In general, a droplet-based lab-on-a-chip is a network of microchannels integrating a serial combination of unit operations to suite the requirements of a specific biochemical experiments (Kintses et al 2010).

The current challenge of droplet-based microfluidics, is the improvement of LoC technology by the development of a programmable versatile platform integrating multiple processing protocols in parallel, to achieve high-throughput and multiplexing for screening applications and chemical synthesis. Infact,
most of the existing microfluidic devices are single-purpose or perform only limited sets of fixed processing operations.

Moreover, today’s programmable droplet microfluidic chips typically rely on active control of droplets movements based on valves or on microelectronic technologies integrated inside the microfluidic device (e.g. electrowetting on dielectric, EWOD). Realization of such systems require a complex multilayer microfabrication process; also, the EWOD devices are not suitable for some biological settings due to problems of biocompatibility of electrical signals on some cells or biomolecules (Link et al 2006; Yuh et al 2008; Yang et al 2010; Huang et al 2011).

To implement the advocated vision of a versatile, simple, and cheap droplet-based LoC, it is crucial to be able to control and direct droplets’ motion. As a result many methods have been developed to act precisely on individual droplets guiding them along a desired path; these mainly rely on sorting devices and microfluidic logic gates. Droplets sorting in general, is coupled with detection methods to extract specific sub-populations of droplets; based on their chemical content or size the droplets are guided toward one arm of a bifurcation. Sorting, can be performed actively or passively. Active methods use valves (Abate et al 2010), DEP (Agresti et al 2010; Ahn et al 2006), electric forces (Link et al 2006; Ahn et al 2011), thermocapillary effect (Baroud et al 2007a,b) and surface acoustic waves (SAW) (Franke et al 2009) to manipulate droplets for selective sorting. These manipulation methods provide a precise control of droplets flow but suffer for many drawbacks, such as need for external electronic and optic control, and need for a complex multilayer fabrication. Conversely, passive methods not require in-chip or off-chip electronics and complex and costly production process, since they exploit only pure hydrodynamic or surface forces to sort the droplets by their size (Tan et al 2008, 2004; Xu et al 2012), viscosity (Hatch et al 2013), and resistance (Cartas-Ayala et al 2012). However, guiding the droplets using passive methods is a complex problem that requires understanding of droplet motion dynamics through networks of microchannels. The complexity lies in the fact that the presence of droplets in a microchannel affects the pressure field and modifies the flow field in the microchannel network in a time-dependent way (Fuerstman et al 2007b; Sessoms et al 2009, 2010; Cybulski and Garstecki 2010). However, these effects have been exploited not only to design sorting passive devices, but also to implement microfluidic logic gates that control the droplet motion steering hydrodynamically the flow of bubbles/droplets into a network of microchannels by means of other properly timed bubbles/droplets (Prakash and Gershenfeld 2007; Cheow et al 2007); so they require perfect synchronization that may be hard to be achieved without a droplet-on-demand generator (Gu et al 2011).

Here, we propose a new passive methodology to guide droplets: this is a sorter capable to selectively direct a droplet along a desired path based only on the programmable control of inter-droplet distance. Unlike the above mentioned sorter, it is independent of the properties of the droplet such as size or chemical content, requires a simple one-step fabrication process and can be
cascaded with potential application in parallel or controlled protocols. Indeed, our sorter can be integrated to create a passive platform in which each droplet can be directed toward a different intended destination for further process, e.g. toward a different set of serial unit operations or different detection method. For example, it can be useful to create a multi-purpose chip that can be used for different types of experiments allowing to select the serial set of unit operations to involve or to execute parallel analyses and syntheses that require different reaction or incubation time, such as measurements of enzymatic kinetics (Han et al 2009) and determination of clotting time (Song et al 2006). Usually, parallel processing in passive microfluidic droplet-based devices is achieved by splitting droplets (Link et al 2004; Liu et al 2009) at a junction or equally distributing the droplets on two paths (Cristobal et al 2006); however these devices lack of programmability to select a predetermined path for droplets.

The inter-distance between droplets has already been used as mean to encode information (Fuerstman et al 2007a; De Leo et al 2012a,b) and to design a hydrodynamic filter able to direct a train of droplets into the shortest arm of a bifurcation (Engl et al 2005). In (Fuerstman et al 2007a) information is encoded in the interval between droplets which flow through a cascade of two asymmetric loops exhibiting with reverse topology so as to allow reconstruction of the information at the destination.

Based on the above relevant results, we believe that a new type of passive sorting device can be introduced, in which droplets are hydrodynamically directed by exploiting only information encoded into the distance between droplets flowing in the microfluidic channels. The resulting microfluidic devices will allow to combine LoC specific functionalities in more powerful and competitive systems for both analyses and syntheses.

2 Theoretical model

The approach we propose is inspired by communication solutions fully consolidated in data transport. There, in order to exchange information between entities, data structures referred to as packets are defined. Each packet consists of an header and a payload: the header encodes signaling information such as the address of the receiving entity; the payload contains the information to be manipulated by the receiving entity.

In this paper we suppose that the samples which should be chemically treated or analyzed are included in a payload droplet, that is a droplet containing the sample which is the object of the biochemical process to be performed by a set of unit operations. This is feasible because the production of droplets of alternating composition has been already demonstrated (Zheng et al 2004).

In order to appropriately direct the payload droplet towards the desired set of unit operations, our sorting scheme exploits another droplet which we call the header droplet; this is used for encoding the destination address, i.e. the information about the intended destination of the payload droplet. In particular, in our sorter the distance between the header and the payload
droplets determines the selection rule of the second droplet at a bifurcation; so, by using a data transport terminology, we can say that the destination address of the payload droplet is encoded in the distance $D$ between the header droplet and the payload droplet.

In other words our sorting functionality will exploit $D$ in the same way a switching device in a data network uses the address information for guiding the packet towards the correct network user. For this reason the microfluidic sorter we are going to discuss acts as a “microfluidic switch” able to guide any (payload) droplet towards the appropriate destination pipe of the microfluidic system. The sorting device here proposed consists of the T-junction circuit represented in Figure 1. The inlet is connected to a pipe which bifurcates in $B$ in two opposite pipes denoted as pipe 1 and pipe 2. The points at the end of pipe 1 and pipe 2, denoted as $A$ and $C$, respectively, are connected by a channel characterized by very low hydrodynamic resistance, which we call bypass channel. Then, pipes 1 and 2 continue into pipes 3 and 4, respectively. The bypass channel connecting $A$ and $C$ guarantees that the pressure at the above two points is equal. Accordingly, the pressure difference between points $B$ and $A$, which we denote as $\Delta P_{i}^{(BA)}$, is equal to the pressure difference between points $B$ and $C$, which we denote as $\Delta P_{i}^{(BC)}$. This occurrence makes the droplet behavior in the sorting device represented in Figure 1 dependent on the geometric characteristics of pipe 1 and pipe 2 only (Cristobal et al 2006).

In order to demonstrate this and to derive the design parameters which allow the sorter to decode the destination address and therefore to guide the payload droplet into one of the two pipes, we study the microfluidic circuit representing our sorting device shown in Fig. 2(a). This circuit can be solved by considering the equivalent electric circuit in Fig.2(b) and exploiting the analogy between the the Ohm’s law ($\Delta V = RI$) and the Hagen-Poiseuille’s law $\Delta P = R_{hyd}Q$, with the pressure difference $\Delta P$ replaced by the voltage difference $\Delta V$, and the flow rate $Q$ replaced by the electric current $I$. In both Figures 2(a) and 2(b), note that $R_{i}$ represent the equivalent resistance of the pipe $i$.

A droplet arriving at a bifurcation always enters the pipe with higher flow rate (Fuerstman et al 2007a; Cybulski and Garstecki 2010). Therefore, to appropriately direct a droplet, initially we want to evaluate how to set the T-junction characteristics in such a way that $Q_{2} > Q_{1}$ (or analogously $I_{2} > I_{1}$) when the header droplet arrives at the bifurcation point.

By applying the Kirchhoff laws we have

$$\begin{align*}
I_1 &= I_1 + I_2 \\
I_1 - I_{R_1} - I_{R_{by}} &= 0 \\
I_2 + I_{R_{by}} - I_{R_2} &= 0 \\
I_1 R_1 - I_2 R_2 + R_{by} I_{R_{by}} &= 0 \\
V_{out,1} + V_{R_3} + R_1 I_1 - R_2 I_2 - V_{R_4} - V_{out,2} &= 0
\end{align*}$$

(1)
By moving to the hydrodynamic domain, the solution of the system of equations in (1) gives:

\[
\frac{Q_1 - Q_2}{Q} = \frac{1}{R_1 + R_2 + R_{by}} \left[ \frac{(R_2 - R_1)(R_3 + R_4)}{(R_3 + R_4) + R_{by}(R_1 + R_2)} + R_{by} \left( \frac{(R_2 - R_1) + (R_4 - R_3)}{(R_3 + R_4) + R_{by}(R_1 + R_2)} + \frac{2}{Q}(P_{out,2} - P_{out,1}) \right) \right]
\]

Fig. 1 Distance-based switching.

Usually, we can assume \( P_{out,2} \approx P_{out,1} \) since in the end all fluids will be collected in a sink at the atmospheric pressure. Furthermore, we realize the bypass channel with a very large width (when compared to other pipes in the circuit) so that \( R_{by} \ll \min\{R_3, R_4\} \).

If the above conditions are satisfied, eq. (2) can be rewritten as

\[
\frac{Q_1 - Q_2}{Q} \simeq \frac{R_2 - R_1}{R_1 + R_2 + R_{by}} + \frac{R_{by}}{R_1 + R_2 + R_{by}} \frac{R_4 - R_3}{R_4 + R_3}
\]

(3)
Finally taking into account that usually $R_3$ and $R_4$ are very large when compared to the other hydrodynamic resistances involved in the circuit, since they are resistances of microchannels which should implement unit operations (e.g., mixing, merge, etc.), we can further approximate eq. (3) as follows:

\[
\frac{Q_1 - Q_2}{Q} \approx \frac{R_2 - R_1}{R_1 + R_2 + R_{bg}} \tag{4}
\]

Exploiting the condition in eq. (4) the sign of $Q_1 - Q_2$ will depend on the sign of $R_2 - R_1$. In (Cristobal et al. 2006) this result was used to allow an alternate distribution of droplets among two pipes. In this work, instead, we exploit this result to direct droplets along the desired path by the introduction of a resistance imbalance between $R_1$ and $R_2$. In fact, if $R_2 < R_1$ and thus $Q_2 > Q_1$, then the header droplet enters pipe 2 when arrives at the bifurcation point $B$, as desired.

In order to impose the relationship $R_2 < R_1$, we exploit the expression of the hydrodynamic resistance, $R$, of a rectangular channel with length $L$, height $h$, and width $w$, traversed by a monophase flow, at low Reynolds number, that is (Bruus 2007)

\[
R = \frac{\Delta p}{Q} \approx \frac{12\mu L}{h^3 w (1 - 0.63h/w)} = \frac{L}{w} \quad \text{with } h < w \tag{5}
\]

where $\mu$ denotes the fluid viscosity and $\alpha$ is defined as follows:

\[
\alpha = \frac{12\mu}{h^3(1 - 0.63h/w)} \tag{6}
\]

Accordingly, in order to have $R_2 < R_1$, that is the header droplet enters pipe 2 at the bifurcation point $B$, from eq. (5) the length of pipe 2 must be smaller than the length of pipe 1:

\[
L_2 < L_1 \tag{7}
\]
In order to appropriately control the direction taken by the payload droplet when it arrives to the bifurcation point $B$ we must impose that the presence of the header droplet in pipe 2 inverts the sign of the difference between the hydrodynamic resistances of pipes 2 and 1, respectively.

This is feasible because the presence of a droplet in pipe 2 increases the hydrodynamic resistance of such pipe. We denote the increased resistance as $R'_{2}$. In the literature, the increase in microchannel resistance due to the presence of a droplet or bubble has been widely discussed since it is a complex problem that involves many features, such as the ratio between the viscosities of the continuous and the dispersed phases, the droplets/bubbles velocity, their size, channel geometry, the capillary number $Ca$, etc (Bretherton 1961; Hodges et al 2004; Sessoms et al 2009; Vanapalli et al 2009; Glawdel et al 2011). Although it is difficult to accurately predict the resistance added by a droplet in a pipe, several studies confirm that the hydrodynamic resistance $R'$ of a micro-channel with a rectangular section, containing $n$ droplets can be approximated as (Wong et al 1995; Jousse et al 2006; Baroud et al 2010)

$$R' = R(\eta_r \phi + 1 - \phi) + n \left[ 3.15 f(\eta_r) \frac{\sigma}{h} Ca^{2/3} \frac{1}{v \cdot w \cdot h} \right]$$  \hspace{1cm} (8)

where

- $R$ is the hydrodynamic resistance in case no droplets are currently in the micro-channel;
- $L_C$ and $L_D$ represent the lengths of the channel and the droplet, respectively;
- $\phi = n \cdot L_D / L_C$ is the fraction of the micro-channel occupied by the droplets;
- $f(\eta_r)$ is a function of the viscosity ratio varying between 1 and about 1.5 when $\eta_r$ spans from 0 to $+\infty$ (Hodges et al 2004);
- $v$ is the velocity of the droplets.
- $\sigma$ is the surface tension coefficient.

In the following we will find approximated expressions for the hydrodynamic resistance in two cases which are typical of several application scenarios:

- Case 1: when $\eta_r > 1$, as for example in the case of oil droplets dispersed in water;
- Case 2: when $\eta_r << 1$, as for example in the case of nitrogen bubbles dispersed in water.

\textbf{2.0.1 Case 1}

$\eta_r > 1$: in this case the resistance of a pipe containing droplets can be approximated by (Jousse et al 2005, 2006):

$$R' = R(\eta_r \phi + 1 - \phi) = \zeta_{LC} \mu_{\nu}(\eta_r \phi + 1 - \phi)/w_C$$  \hspace{1cm} (9)
where \( \zeta = \frac{\alpha}{\mu_c} \) and \( \alpha \) is the expression defined in eq. (6). Accordingly, the conditions we are looking for, that is \( R_2 < R_1 \) and \( R'_2 > R_1 \) can be equivalently rewritten as

\[
\begin{aligned}
&\quad L_2 < L_1 \\
&\quad \zeta[\mu_c L_2 + L_D(\mu_d - \mu_c)] > \zeta \mu_c L_1
\end{aligned}
\] (10)

So it follows that the sorting device design must satisfy the following condition:

\[
0 < L_1 - L_2 < L_D \left( \frac{\mu_d}{\mu_c} - 1 \right)
\] (11)

2.0.2 Case 2

\( \eta_r << 1 \): in this case the term \( \eta_r \phi \) in the eq. (8) can be neglected, whereas \( f(\eta_r) \) can be approximated as \( f(\eta_r) \approx 1 \). Accordingly, if we define \( R_D \) as in (Jousse et al 2006; Parthiban and Khan 2012)

\[
R_D = 3.15 \frac{\sigma}{h} C a^{2/3} \frac{1}{U \cdot w \cdot h}
\] (12)

where \( U \) is the droplet velocity, then the hydrodynamic resistance can be approximated as follows:

\[
R' \approx R(1 - \phi) + nR_D
\] (13)

If there is only one droplet in the channel, that is, \( n = 1 \), then eq. (13) can be rewritten as

\[
R' \approx \alpha \left[ L_C - L_D + \frac{3.15 \cdot (1 - 0.63h/w)}{12} C a^{-1/3}h \right] / w_C
\] (14)

In the following we set \( h/w = 1/2 \). Therefore, in the above equation it should be \( L_D < 0.18C a^{-1/3}h \) so that \( R' > R = \alpha L_c \); otherwise the channel resistance would decrease in spite of the presence of a droplet in the pipe as observed experimentally (Parthiban and Khan 2012).

Finally, by imposing that the two pipes 1 and 2 have the same \( h \) and \( w \) and only differ for their length, we must guarantee that the following relationships hold

\[
\begin{aligned}
&\quad L_2 < L_1 \\
&\quad \alpha(L_2 - L_D + 0.18C a^{-1/3}h) > \alpha L_1
\end{aligned}
\] (15)

So it follows that the device design must satisfy the following relationship:

\[
0 < L_1 - L_2 < 0.18C a^{-1/3}h - L_D
\] (16)

Moreover, other guidelines should be considered while designing the sorter device. As a first consideration, droplets break up at the bifurcation point \( B \) must be prevented (Link et al 2004). Also, observe that the distance between consecutive droplets changes as they traverse different cascaded sorters. Such changes should be considered while designing the device. Suppose that the distance between two droplets entering the \( i \)-th MNI is \( D_{HP}^{(i)} \) and suppose
that the second droplet will follow the first one in pipe 2. Furthermore, let us call $v$ the velocity of the droplets in the pipe leading to the bifurcation point $B$ and $v_2$ the velocity of the droplets in pipe 2. The distance between the two droplets when they leave the $i$-th MNI (which we denote as $D^{(i+1)}_{HP}$) will be equal to

$$D^{(i+1)}_{HP} = v_2 \cdot \frac{D^{(i)}_{HP}}{v} \quad (17)$$

The velocities $v$ and $v_2$ are proportional to the flow rate in the corresponding pipes; specifically, the following relationship holds:

$$Q_2 \approx \frac{L_1}{L_1 + L_2} \cdot Q \quad (18)$$

If we use eq. (18) in eq. (17), we obtain

$$D^{(i+1)}_{HP} \approx D^{(i)}_{HP} \cdot \frac{L_1}{L_1 + L_2} \quad (19)$$

Accordingly, if we set the distance between the header and payload droplets to $D_{HP}$, at the entrance of the $i$-th sorter such distance will be

$$D^{(i)}_{HP} \approx D^{(1)}_{HP} \cdot \prod_{j=1}^{i-1} \frac{L_1^{(j)}}{L_1^{(j)} + L_2^{(j)}} \quad (20)$$

Then, if we want to drive droplets toward the $i$-th outlet we set:

$$D^{(i)}_{HP} < L_2^{(i)} \quad (21)$$

### 3 Methods and materials

#### 3.1 Computational fluid dynamic analysis

To assess the device functioning computational fluid dynamic (CFD) analysis was carried out using OpenFOAM software which implement the volume of fluid (VOF) method (OpenFOAM 2012).

In order to show how and if the designed sorting device can correctly work also when cascaded, we performed simulations considering two devices connected as depicted in Figure 3. As we already outlined, the bypass channel is a crucial design element in the circuit. Note that it must be large enough to guarantee very low hydrodynamic resistance while it must be designed in such a way that droplets do not enter it. In Figure 4 we show a zoomed view of the
portion of the bypass channel attached to the rest of the circuit, that is point \( A \) in Figure 1. In Table 1 we give the values of the parameters characterizing the geometry used in Figure 3.

We performed two sets of simulations respectively for \( \eta_r > 1 \) and \( \eta_r \ll 1 \). For the sake of conciseness, we only detail the case of oil droplets dispersed in water (\( \eta_r > 1 \)) and report briefly our observations on the case of nitrogen bubbles dispersed in water (\( \eta_r \ll 1 \)). Accordingly, we have set the viscosities of the dispersed and continuous phases to \( \mu_c = 1 \text{ mPa} \cdot \text{s} \) and \( \mu_d = 10 \text{ mPa} \cdot \text{s} \), and densities to \( \rho_c = 1000 \text{ Kg/m}^3 \) and \( \rho_d = 930 \text{ Kg/m}^3 \) with the interfacial tension between the two phases \( \sigma = 0.0365 \text{ N/m} \). We have imposed a no-slip
boundary condition at the channel walls and a static contact angle of 140. The parameters characterizing the geometry given in Figure 3 must satisfy eq. (11), where $L_D = 150 \mu m$.

By using the values given in Table 1 we verify that

$$0 < L_1^{(i)} - L_2^{(i)} = L_1^{(i+1)} - L_2^{(i+1)} = 600 \cdot 10^{-6} < L_D \left( \frac{\mu_d}{\mu_c} - 1 \right) = 1.35 \cdot 10^{-3}$$

3.2 Device fabrication and experimental setup

We have realized a prototype of the device described so far which is shown in Figure 5. Microfluidic devices are fabricated in poly(dimethylsiloxane) (PDMS) polymer according to the fast prototyping technique described in (Zanoli et al 2012). Briefly, PDMS channels bearing circular reservoirs at the ends of each channel were created by replication from masters in polyvinyl chloride. Replicas were formed from a 1:10 mixture of PDMS curing agent and prepolymer (Sylgard 184, Dow Corning, USA). The mixture was degassed under vacuum, poured onto the master in order to create a layer with a thickness of about 34 mm, and then left polymerizing for 48 h at room temperature on a plain surface. The PDMS mold bearing the negative pattern of the masters was peeled off from the master surface and repeatedly washed with ethanol, ultra-pure water, and dried before use. PEEK tubes (UpChurch Scientific) were inserted in the circular. The irreversible adhesion of PDMS molds on microscope cover glasses was obtained after 30 s air plasma etching of cleaned surfaces. Processsing of surfaces with air plasma was carried out by using a Femto Diener Electronics plasma cleaner system using a 40-kHz generator. After the air plasma etching, treated surfaces were quickly placed in contact with each other and the new device placed at 60 C for 30 min. The device includes a T-shaped droplet generator (see Figure 5(c)) in which the stream of dispersed phase that flows from the vertical channel, is sheared by the continuous phase that flows from the horizontal channel. The prototype transverse dimensions ($w = 400/\mu m$ and $h = 80/\mu m$) are higher than the simulated device because of fabrication constraints.

3.3 Experimental setup

The fluids we have used are a fluorinated oil, FC-3283 ($\mu_c = 1.3$ mPa·s, $\rho_c = 1820$ kg/m$^3$) and an aqueous flow ($\mu_d = 1$ mPa·s, $\rho_d = 1000$ kg/m$^3$) added with a food dye. A surfactant (PFO) is added to the continuous phase to lower the interfacial tension facilitating droplet formation and stabilization of the emulsion.

These two phases were chosen to test our model in an intermediate case between a low $\eta_r$ (Case 2, discussed above) and a high $\eta_r$ (Case 1, discussed above) cases in which we have found an approximation for $R_D$. In this way we can demonstrate that our device works properly also in this case in which
4 Results and discussion

4.1 Simulation results

We have considered two cases. In the first case, the payload droplet must be delivered to $out_i$, whereas in the second case the payload droplet must be delivered to $out_{i+1}$.

Accordingly, in the first case the distance between the droplets entering the $i$-th sorter must be such that when the payload droplet arrives at the bifurcation point the header droplet is still in pipe 2. To this end, such distance $D^{(i)}_{HP}$ must satisfy the following relationship

$$D^{(i)}_{HP} < \frac{L_1^{(i)} + L_2^{(i)}}{L_1^{(i)}} \cdot L_2^{(i)} - \delta_{Margin} = 800 \ \mu m$$  \hspace{1cm} (22)

where $\delta_{Margin}$ is a confidence margin utilized to absorb fluctuations in the actual value of the distance between the header and the payload droplets which could cause errors in the delivery of the payload droplet. The value of $\delta_{Margin}$ must be set by considering that the distance between droplets changes as they move forward in the microfluidic channels as clarified by eq. (??). In our simulations we have chosen $\delta_{Margin} = 100 \ \mu m$ and $D^{(i)}_{HP} = 600 \ \mu m$ which satisfy the relationship in eq. (22).

We show two snapshots of the simulation output representing:
In Figure 6(a) we observe that the distance $D_{HP}^{(i)}$ satisfies the condition in eq. (22), whereas in Figure 6(b) we observe that the payload droplet enters the pipe leading to $out_i$. In this case the header droplet will travel toward the $out_{i+2}$.

In the second case in order for the payload droplet to go towards $out_{i+1}$, we must guarantee that:

- the distance between the header droplet and the payload droplet before they arrive at the bifurcation point $B$ of the $i$-th sorter, $D_{HP}^{(i)}$, must be larger than $((L_2^{(i)} + L_1^{(i)})/L_1^{(i)}) \cdot L_2^{(i)} + \delta_{\text{Margin}}$;
Fig. 7 Simulation results for the case in which the payload droplet is addressed to the (i + 1)-th outlet.

- the distance between the header and the payload droplets before they arrive at the bifurcation point \( B \) of the (i + 1)-th sorter, \( D_{HP}^{(i+1)} \), must be smaller than \([L_1^{(i+1)} + L_2^{(i+1)}]/L_1^{(i+1)} \cdot L_2^{(i+1)} - \delta_{Margin}\).

By recalling that \( D_{HP}^{(i+1)} \approx D_{HP}^{(i)} \cdot L_1^{(i)}/(L_1^{(i)} + L_2^{(i)}) \) we obtain

\[
\frac{L_1^{(i)} + L_2^{(i)}}{L_1^{(i)}} - L_2^{(i)} + \delta_{Margin} < D_{HP}^{(i)} < \frac{L_1^{(i)} + L_2^{(i)}}{L_1^{(i)}} \cdot \frac{L_1^{(i+1)} + L_2^{(i+1)}}{L_1^{(i+1)}} \cdot L_2^{(i+1)} - \delta_{Margin}
\]  

(23)

Therefore we have set \( D_{HP}^{(i)} = 1.1 \) mm, which satisfies the condition in eq. (23), and we represent the corresponding simulation results in Figure 7(b). More specifically in the above figure we show three snapshots of the simulation output representing:

- the situation immediately before the header droplet arrives at the bifurcation point \( B \) of the \( i \)-th sorter (see Figure 7(a));
- the situation immediately after the payload droplet leaves the bifurcation point \( B \) of the \( i \)-th sorter (see Figure 7(b));
- the situation immediately after the payload droplet leaves the bifurcation point \( B \) of the (\( i + 1 \))-sorter (see Figure 7(c)).

To further verify the device functionality we performed another set of simulations using nitrogen bubbles dispersed in water. We observed that in this
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Fig. 8 Simulation results of streamline pattern in the bypass channel at different droplet positions. Provare ad allargare la figura per fare vedere dove si trova la goccia.

case the device works correctly also if the condition (16) is not satisfied but the header bubble clogs up the bypass connection when the payload bubble enters the bifurcation. The obstruction indeed causes the same inversion of the flow in the bypass channel as when the droplet travelling in pipe 2 increases the resistance of this channel. The streamline patterns are shown in Fig. 8: when a droplet travels along channel 2 the flow in the bypass channel is from 1 to 2, while it is inverted if a droplet travels along channel 1.

4.2 Experimental testing

Experimentally, we tested the validity of results from theoretical model and simulations. We obtained two regimes depending on inter-droplet distance: an alternating regime in which two consecutive droplets have followed an opposite path and a filtering regime in which both are sorted in the pipe 2. In Figures 9 we show two snapshots representing:

- the situation before the header droplet arrives at the bifurcation point B (Figure 9(a)). Note that the distance between the header droplet and the
payload droplet is such that the header droplet is still inside pipe 2 when the payload droplet arrives at the bifurcation
– the situation after the payload droplet leaves the bifurcation point $B$ (Figure 9(b)). Note that the payload droplet has entered pipe 1, as expected.

In Figures 10 we show two snapshots representing:
– the situation before the header droplet arrives to the bifurcation point $B$ (Figure 10(a)). Note that the distance between the header droplet and the payload droplet is such that the header droplet has already left pipe 2 when the payload droplet arrives at the bifurcation point $B$.
– the situation after the payload droplet leaves the bifurcation point $B$ (Figure 10(b)). Note that the payload droplet has entered pipe 2, as expected.

However, we have observed that, also for fixed values of $Q_c$ and $Q_d$, the inter-droplets distance is not constant, but varies within a range of values as
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Described in Fig. 12; this may be avoided adding a fluidic resistor as is explained in (Fuerstman et al. 2007a). So, the sorting device in addition to operate correctly depending on the inter-droplet distance, should be robust against these variations. First, we consider the case in which the payload droplets have to be directed to out2 and the header droplet to out1. In order to evaluate the correct behaviour of the sorting device and verify the validity of the eq. (19)-(21) we have performed measurements of the inter-droplet distance at which the droplets arrived at the bifurcation and distance between each droplet at the bifurcation point and the previous one that flows into branch 2 for $Q_d = 0.6 \mu\text{L/min}$ and $Q_c = 6 \mu\text{L/min}$. As depicted in Fig. 12, despite of the oscillations of inter-distance, the inequality (21) is respected so we expected that the device operation would be robust against these variations. To verify that the Fig. 11 shows a probability density describing the number of correct path choices for the different range values of inter-droplet distance. The accuracy decrease from 75% to 25% as the inter-droplet distance increase. For distances $D < 1\text{mm}$ (not shown) the reliability of the device is low because are more frequent coalescence and consequent break-up events at the junction. In Fig. 12, the distance values over 7.5 mm are obtained for $Q_d = 0.2 \mu\text{L/min}$ and $Q_c = 8 \mu\text{L/min}$, the right sorting events in this case are droplets flowing on the same path, toward the out2. The accuracy increase from 50% to 87% as the inter-droplet distance increase. We attribute the mistaken sorting events to the imperfections in the microchannel at the junctions between the two branches and the bypass channel, caused during the first step of fabrication process, i.e. the creation of the mold pattern by a cutting plotter. These imperfections randomly slow down a droplet, this deceleration affects the inter-droplet distance and lead to an incorrect path choice by the following droplet. Moreover, the T-configuration of the channel increase the time taken by a droplet to choose for one or the other pipe further reducing the inter-droplet distance. So, taking into account these problems, the measured results for the sorter accuracy were reasonably good.
5 Conclusions

In this paper we have introduced a new passive sorting scheme to guide droplets in microfluidic devices in order to both increase their flexibility and decrease their cost. We have addressed the design rules for both the architecture and functionalities to be implemented. In particular the sorting function has been proposed, analyzed, and assessed through simulations. Results of our test have confirmed the feasibility of this new technology.
References


Baroud CN, Gallaire F, Dangla R (2010) Dynamics of microfluidic droplets. Lab Chip 10


Song H, Li HW, Munson MS, Van Ha TG, Ismagilov RF (2006) On-chip
titration of an anticoagulant argatroban and determination of the clotting
time within whole blood or plasma using a plug-based microfluidic system.
Analytical Chemistry 78(14):4839–4849
guidance for control of droplet volume, chemical concentration,
and sorting. Lab Chip 4:292–298
Tan YC, Ho YL, Lee AP (2008) Microfluidic sorting of droplets by size. Mi-
crofluidics and Nanofluidics 4(4):343–348
Vanapalli SA, Banpurkar AG, van den Ende D, Duits MHG, Mugele F (2009)
Hydrodynamic resistance of single confined moving drops in rectangular
microchannels. Lab Chip 9
nal capillaries. part 2. drag, fluid pressure and fluid flow. Journal of Fluid
Mechanics 292
parallel trains of droplets using a railroad-like channel network and guiding
tracks. Lab Chip 12:3936–3942
systems. TrAC Trends in Analytical Chemistry 29(2)
algorithm for the synthesis of digital microfluidic biochips. Computer-Aided
Design of Integrated Circuits and Systems, IEEE Transactions on 27(11)
Marchelli R, Spoto G (2012) Peptide nucleic acid molecular beacons for the
detection of pcr amplicons in droplet-based microfluidic devices. Analytical
and Bioanalytical Chemistry
composition in microfluidic channels and applications to indexing of con-
centrations in droplet-based assays. Analytical Chemistry 76(17):4977–4982