Passive Droplet Control in Microfluidic Networks: A Survey and New Perspectives on their Practical Realization

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Abstract

Two-phase flow microfluidics is a promising platform for realizing Lab-on-a-Chip (LoC) devices, that perform different laboratory functions on a single chip. This is accomplished by processing droplets, containing biological/chemical samples, by different elements each performing specific operations. Usually, the sequence of elements in which droplets are processed is fixed, which limits the flexibility, effectiveness and reusability of such LoC devices. Recently, microfluidic networks for two-phase flow microfluidics have been introduced with the aim of realizing programmable and flexible LoC devices. In particular, the goal is to dynamically and passively assign the droplets' path through a microfluidc network, which enables to reuse the LoC devices for different laboratory functions. This paper presents the state-of-the-art and discusses various aspects on the practical realization. First, we provide a survey on microfluidic networking, including passive switching, network topologies and validation methods. Second, we propose a simple Droplet-on-Demand (DoD) system, which allows generating droplets at prescribed times and with prescribed volumes – a crucial accomplishment in order to exploit the potential of microfluidic networks for practical purposes. We verify its functionality through experimental results. Third, we describe two promising applications for microfluidic networks, namely fast and flexible drug screening and screening of waterborne pathogens. Finally, we discuss future research opportunities and challenges.

Keywords: Droplet-on-Demand, Microfluidic Networks, Passive Routing, Two-Phase Flow Microfluidics

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1. Introduction

Two-phase flow microfluidics, where the droplets flow in closed microchannels, is a promising platform for the realization of Labs-on-Chips (LoCs) devices, that perform different laboratory functions on a single chip [1, 2]. The devices are usually fabricated in glass, plastic or various polymers and extend in only a few square centimeters in size [3, 4]. In order to perform complex laboratory functions, a single LoC device integrates several microfluidic elements of which each element performs specific laboratory operations.

However, most of the corresponding microfluidic elements are, thus far, interconnected in a predefined order, which means that biological samples carried within the droplets always pass the same sequence of processing elements. This clearly limits the flexibility, effectiveness and reusability of LoC devices.

The flexibility of LoCs could be significantly increased if samples could selectively perform laboratory operations. This means that the sequence of microfluidic elements to be involved in the processing of droplets is in some way programmable, flexible and adaptive. In that case, it is possible to use the same LoC platform to perform different microfluidic operations.

While, in conventional microfluidic systems, this flexibility could not easily be achieved since microfluidic elements are interconnected and built into a fixed, static chip topology, a recently introduced concept called microfluidic networks offers a promising solution to this problem (cf. [5, 6] and references therein).

In particular, microfluidic networking targets to dynamically assign the droplets' path through a microfluidic network in order to perform specific analyses [5, 7]. Here, several microfluidic characteristics such that the droplets within a fluidic flow will always take the path with the smallest fluidic resistance and that positions, as well as sizes, of droplets may affect those resistances, are exploited. This allows for employing networking technologies which, in turn, allow for the realization of programmable and flexible LoC devices.

The key element in microfluidic networks is the microfluidic switch – the element inherited from conventional ICT systems [8]. Microfluidic switches interconnect microfluidic elements within the network and direct/control the path of a single or multiple droplets, just like switches in a computer network connect devices and forward the data to the destination device. This is accomplished by sending a controlling, so-called header droplet, in front of the droplet containing the biological/chemical samples, the so-called payload droplet. Just like in ICT terminology, header droplets are only used for signalling and switching of the payload droplet, and contains no sample [5, 9]. The targeted location of the payload droplet can be either encoded in the distance between payload and header droplet, yielding a so-called droplet by distance (DbD) switching [5], or in the size of the header droplet [10], a so-called droplet by size (DbS) switching. Translating principles from computer networks to the microfluidic domain by introducing microfluidic networks and microfluidic switches, enables realizing flexible LoC devices with improved effectiveness and reusability.

However, while those concepts have already been investigated (cf. [5, 6, 9, 11] and references therein), and even first methods towards the automatic design

of those microfluidic networks were proposed [12, 13, 14], rather few experimental works on microfluidic networks exists yet. This is mainly caused by the fact that experimental investigation of microfluidic networks, and concepts presented therein, requires a precise, passive, controllable and automated droplet generation element, which to this point has been poorly investigated element in microfluidics. Here, so called Droplet-on-Demand (DoD) element plays an important role.

Most of the reported DoD systems rely on active droplet control [15]. These systems require complex multilayer fabrication and introduce limitations in terms of biochemical compatibility of the device due to the effect of the electrical signals on biological samples, which leads to its limited applicability. Over the years, only a few passive DoD systems have been proposed [16, 17]. In this paper, we introduce a robust and simple passive DoD system, which is able to perform arbitrary protocols of droplet formation.

1.1. Contributions

The contribution of this paper can be summarized as follows:

1. We provide a comprehensive overview of state-of-the-art microfluidic networks for two-phase flow microfluidics, including switching principles, network topologies and simulation methods.
2. We propose a robust, simple and bio-compatible DoD system, which allows to generate droplets at the prescribed time and with prescribed volume, which is a crucial accomplishment in order to exploit the potential of microfluidic networks for practical purposes. Moreover, we verify its functionality by providing proof-of-concept experimental results.
3. We propose two promising applications for microfluidic networks: Fast and flexible drug screening and screening of waterborne pathogens.

1.2. Organization

The rest of the paper is organized as follows: In Sec. 2 we briefly review the basics of droplet-based microfluidics. In Sec. 3 we discuss various switching principles, and in Sec. 4 we introduce state-of-the art network topologies. Different methods for validating microfluidic networks are introduced in Sec. 5. In Sec. 6 we provide an overview of state-of-the-art DoD systems and propose a new DoD method. Some promising applications for microfluidic networks are presented in Sec. 7. In Sec. 8 we summarize the main results and discuss future research opportunities and challenges.

2. Basics of Two-Phase Flow Microfluidics

In this section, we briefly review the main principles in droplet-based microfluidics. We present requirements and limitations of the stable droplet forma-

tion process[1]. Moreover, we introduce hydrodynamic resistance principle used to design μfSwitches.

In order to create droplets (microliter to picoliter volumes of fluids), numerous droplet generation mechanism have been introduced [18]. Two most common geometries are the T-junction and the flow focusing geometry [19]. Although both geometries allow producing droplets in a controllable and reproducible manner with high frequencies, in this work, a T-junction configuration is used as it offers simplicity in its design.

In a typical T-junction configuration, as shown in Fig. 1a, two phases (continuous and dispersed phase) are brought into the system by externally controlled devices (usually pressure or syringe pump) [19]. There, the droplets are formed through a breakup of the dispersed phase, due to the competition between viscous shear stress and interfacial tension. Droplets are then carried throughout the microfluidic network by the continuous phase. Fig. 1b shows a practical realization of the T-junction geometry and droplet formation process.

The process of droplet formation depends on the geometry of the T-junction (width and height of channels, and volume of the junction), relative viscosities between the two phases, and flow rates of the two fluids [18, 19]. The influence of all these parameters is described with a Capillary number, Ca, that describes the relative effect of viscous shear forces versus interfacial tension, and can be calculated as [10]

$$C_a = \frac{\mu_c Q_C}{\sigma w h}, \tag{1}$$

where Q_C and μ_c are the input flow rate and the dynamic viscosity of the continuous phase, respectively. The interfacial tension coefficient between dispersed and continuous phase is denoted by σ and w and h are the width and height of the continuous phase channel. In order to enable precise and controllable droplet formation, the droplet generator needs to work in the squeezing regime, which requires $C_a < 10^{-2}$.

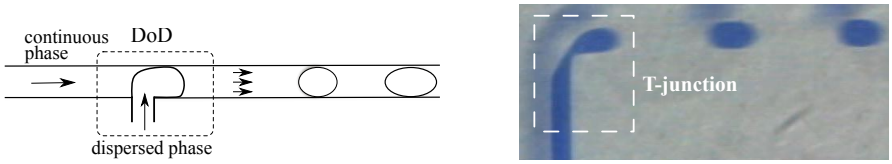
As droplets flow across the microfluidic networks, they encounter various physical obstacles (mostly due to fabrication defects, e.g., partly blocked/damaged channels) and channel junctions where the behaviour of the droplets may deviate from that expected [20]. In those cases, events such as droplet collision, coalescence or splitting can occur. In this work, we focus on microfluidic systems that preserve droplet integrity throughout the network. This is achieved by maintaining the droplets in the non-breakup regime [2] that limits maximum droplet length to

$$L_D < \chi w Ca^{-0.21} \tag{2}$$

where χ is a dimensionless parameter depending on the viscosity ration between dispersed and continuous phase ($\frac{\mu_d}{\mu_c}$).

[1] Please refer to [18] and [19] for more information.

[2] More details on non-breakup regime can be found in [21].



(a) Schematic illustration (b) Practical realization

Figure 1: Droplet generation with a T junction

When arriving at a junction, a droplet will follow the branch with maximum volumetric flow rate, or a minimum hydrodynamic resistance. For a microchannel with a rectangular cross-section of length L, height h, and width w, the resistance can be calculated as [5]

$$R = \frac{12\mu L}{wh^3(1 - 0.630h/w)} = \alpha\frac{L}{w}, \tag{3}$$

where, μ denotes the dynamic viscosity of the fluid passing the channel. It follows that daughter channels of different lengths $L_1 < L_2$, will have different hydrodynamic resistances where $R_1 < R_2$. Therefore, the first droplet entering and passing the T-junction will be steered to the channel of length L_1. Presence of the droplet in the channel of the length L_1 and corresponding resistance, R_1, increases the resistance of the channel by a factor R_D [5], making the total resistance of the channel $R_1 + R_D$. Once the next droplet arrives at the T-junction, it will flow to the channel with lower hydrodynamic resistance. If the increased resistance $R_1 + R_D$ is now greater than the resistance R_2, the payload droplet will be directed to the second daughter channel. Otherwise, if the header droplet has already left the daughter channel, there will be no increase in resistance R_1, and the payload droplet will also follow the path of lower resistance, L_1.

In the following section, we exploit the hydrodynamic resistance principles to introduce a device that is able to control the droplets' path at the junction-microfluidic switch.

3. Microfluidic Switches

In this section, we present the principle of microfluidic switches (μfSwitches), which are the main building blocks in microfluidic networks (cf. [5, 6] and references therein). Microfluidic switches control the path of a single, or multiple, droplets containing biological/chemical samples (payload droplet) by sending a controlling (header) droplet in front, or after, the payload droplet. We investigate the switching principle by means of a bus network[3], since only this architecture supports all switching mechanisms presented in the following.

[3]Please refer to Sec. 4 for more details on the topology of microfluidic networks.

3.1. Basic Switching Principle

As shown in Fig. 2, μfSwitches are realized as a T-junction where a central channel branches into two daughter channels [8]. The ends of the two daughter channels are connected through a *bypass* channel, used as a pressure shunt to equalize the pressure between the junction point and the two ends of the daughter channels [9]. Due to the pressure equalization, controlling (switching) of the droplets' path inside the T-junction is simplified and dependent only on the geometric characteristics of the two daughter channels.

The basic switching principle is based on the previously introduced hydrodynamic control of droplets' path (cf. Sec. 2). The principle states that, when arriving at the junction, a droplet will follow the branch with the maximum volumetric flow rate, or the minimum hydrodynamic resistance. For this reason, μfSwitch shown in Fig. 2 has two asymetric branches of lengths, L_1 and L_2 where $L_1 < L_2$ and consequently, $R_1 < R_2$.

It is important that L_2 is greater than L_1 in order to satisfy the switching principle. However, for the switching principle to hold, the limits for L_2 in terms of L_1 can be expressed as follows [22]:

$$0 < L_2 - L_1 < L_D \left(\frac{\mu_d}{\mu_c} - 1 \right), \tag{4}$$

where, $L_D = w(1 + Q_d/Q_c)$ denotes the droplet length, Q_d and Q_c correspond to the input flow rates for the droplet generation and μ_d and μ_c ($\mu_d > \mu_c$) are dynamic viscosities of dispersed and continuous phase.

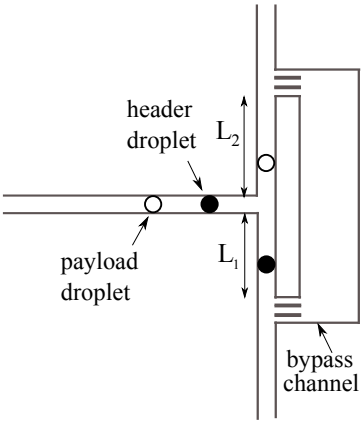
Therefore, under these geometry limitations, it is possible to design a μfSwitch that enables controlling the path of a single payload droplet. As shown in Fig. 2, header droplet arrives at the junction before the payload droplet. Having no droplets ahead of it, the header droplet is always steered to the branch 1, as it offers lower hydrodynamic resistance. The direction of the next, payload, droplet will depend on the position of the previous, header, droplet. If the header droplet is still in branch 1 once the payload droplet arrives, increase in resistance of the branch 1 will direct the payload droplet into the branch 2. However, if the header droplet has already left branch 1 (passed the bypass opening) once the payload droplet arrives at the junction, payload droplet will also flow along the branch 1.

3.2. Single Droplet Switching

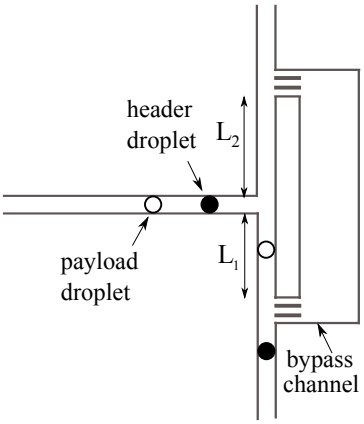
For single droplet switching, the path of a single *payload* droplet is controlled by sending a signaling/control droplet (header droplet) in front of the payload droplet. Two promising approaches have been proposed in the literature: *Droplet by Distance* [7] microfluidic switching and *Droplet by Size* [10] microfluidic switching. In the following we review these concepts.

3.2.1. Droplet by Distance Switching

The principle of the droplet by distance (DbD) switching was first introduced in [7]. For the purpose of DbD switching the previously introduced μfSwitch, shown in Fig. 3, is used.



(a) Due to the presence of header (black) droplet in branch 1, payload (white) droplet is steered to the branch 2



(b) The header droplet (black) has left branch 1, and, thus the payload droplet (white) follows the header droplet

Figure 2: A microfluidic switch for single droplet switching

In DbD switching, the header droplet arriving at the junction will always flow into branch 1, due to the lower hydrodynamic resistance. If the distance between the payload and header droplet (D_{HP}) is such that $D_{HP} < L_1$, the header droplet is still in branch 1, when the payload droplet arrives at the junction. Due to the presence of the header droplet in branch 1, the payload droplet will be directed to branch 2. However, if the distance between the two droplets is $D_{HP} > L_1$, the header droplet has already left branch 1 once the payload droplets arrives at the junction, and thus, the payload droplet flows into the branch 1.

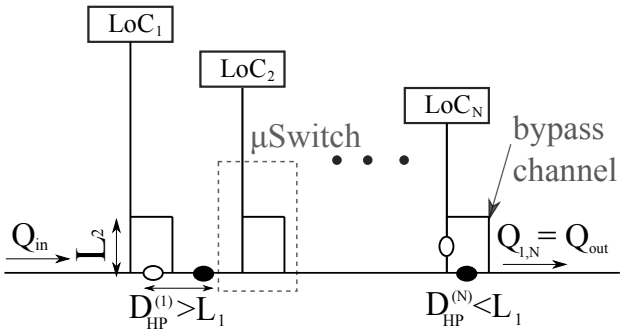


Figure 3: Droplet by distance switching. All droplets are of the same size, while the distance between header (black) and payload (white) droplet decides on the path of the payload droplet

Fig. 3, demonstrates droplet by distance switching. As it can be seen, μfSwitch$_1$ directs the payload droplet to the next switch since $D_{HP}^{(1)} > L_1$. In contrast, for droplets arriving at the $N-$th μfSwitch, $D_{HP}^{(N)} < L_1$, due to the distance reduction along the main bus, and payload droplet is directed to the LoC_N for processing. Therefore, in order to route the payload droplet to the nth LoC, the distance at the input must be set so that droplets arriving at the nth μfswitch satisfy $D_{HP} < L_1$.

3.2.2. Droplet by Size Switching

The droplet by size (DbS) switching principle was introduced in [10]. In contrast to DbD switching, DbS utilizes different sizes of header droplets to control the path of the payload droplet, while keeping the distance between header and payload fixed. Therefore, for every header-payload pair of droplets, the distance is a fixed parameter, while variable header size carries the information about the destination address of the payload droplet.

Fig. 4, shows the working principle of the DbS switching. In contrast to DbD switching, μfSwitches are no longer of the same geometrical characteristics. Here, the asymmetry between the branches is reduced when moving from the first to the last switch (left to right). This is because branch 2 of the μfSwitch needs to offer comparable resistance to branch 1 when there is a header droplet present in branch 1. In order to guarantee the correct switching functionality, the geometry of the droplet by size μfSwitch needs to satisfy [10]

$$L_{2,n} - L_1 < \frac{w}{\alpha} R_{D,n} < L_{2,n-1} - L_1, \tag{5}$$

$$L_{2,n} > L_{1,n} \tag{6}$$

for $n = 1, ..., N$ and $R_{D,n}$ being an increment in the resistance due to the presence of the header droplet in the nth channel.

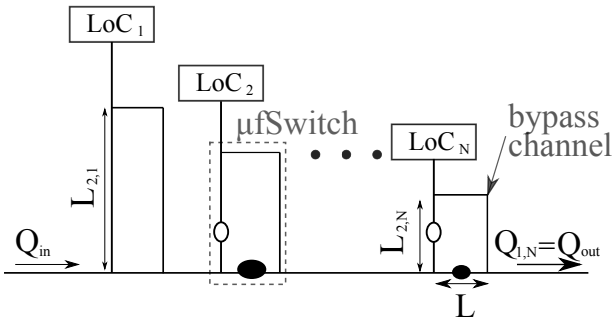


Figure 4: Droplet by size switching. Between all droplets there is the same distance, while the size of the header (black) droplet directs the payload (white) droplet to the next switch or to the LoC

3.3. Multiple Droplets Switching

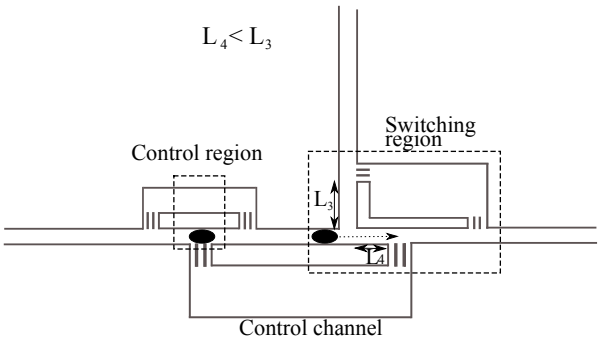
In Sec. 3.2, we have introduced the principle of single droplet switching, where a single payload droplet is routed to the target destination by means of a header droplet. However, some laboratory experiments require delivering multiple samples to a connected LoC [23] [24]. For those applications, single droplet switching would be time- and resource inefficient, since the large signal overhead of 50% would be present as a consequence of being able to route only one payload droplet per one header droplet.

Thus, a multidroplet switch (a μfSwitch for multiple droplets switching) was proposed in [6], which enables routing of multiple payload droplets using only a one controling droplet. The microfluidic multidroplet switch is shown in Fig. 5.

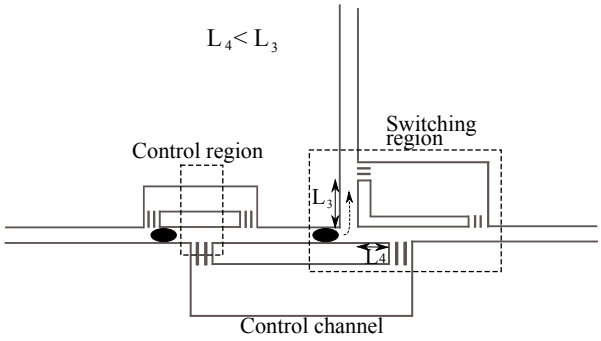
The switch utilizes DbD switching to control the droplets' path and has two important regions: control region and switching region. The basic idea is that a droplet in the control region controls the path of a droplet currently in the switching region by interrupting the flow through the control channel. If there is a droplet present in the control region, the switch is working in ON state. If there is no droplet present in the control region, the switch is in the OFF state. Assuming an asymmetric junction in the switching region ($L_4 < L_3$), the droplet flows into branch 3 or 4 if the switch is in OFF or ON state, respectively.

We consider a microfluidic bus network with a cascade of multidroplet switches, as shown in Fig. 6. To route multiple payload droplets to the nth LoC, a train of payload droplets and a single trailer droplet must be generated, where the distance between droplets must be set so that the nth switch is in the ON state for each droplet arriving at its switching region. The last payload droplet will be routed by the trailer droplet, and the trailer droplet will traverses through the cascaded switches to the output.

It is important to note that the proposed switch can also be used to route multiple payload droplets to different LoC devices. This can be accomplished through different distances between the individual droplets, where each distance indicates the address of the targeted LoC device.



(a) Control channel is clogged (ON state): payload droplet flows through the branch L_4 device.



(b) Control channel is not clogged (OFF state): droplet flows through the branch L_3

Figure 5: A microfluidic switch for multiple droplets switching

4. Microfluidic Networks

In this section, we provide an overview of network topologies used to design microfluidic networks. Three main network topologies can be identified: bus, ring and application specific network topologies. In this work, we focus on the bus and ring networks as most promising architectures for microfluidic networks. This is because the ring and bus networks are simple and generic topologies that can offer high scalability and flexibility and thus can be used as a basic physical template for any microfluidic application. On the other hand, the application specific network topology does not offer a generic microfluidic platform, but is designed to fit, and optimize, specific applications or experiments [4].

[4] Please refer to [12] for more details on application specific networks.

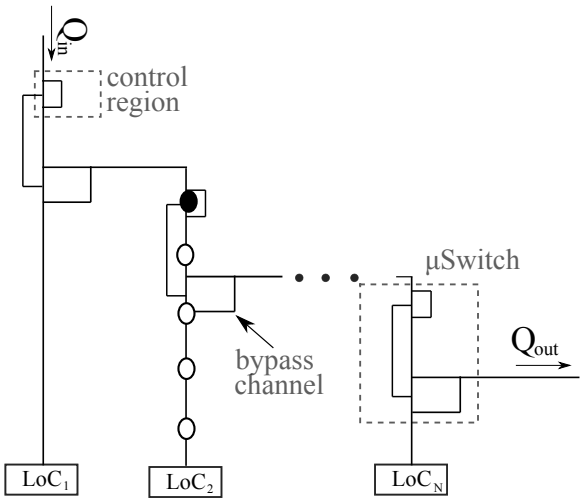


Figure 6: Bus network with multidroplet switching: a train of payload (white) droplets is directed towards the 2-nd LoC using trailer (black) droplet. The 2-nd multidroplet switch is working in ON state

4.1. Network topologies

In this section we discuss the bus and ring network topology, and introduce their basic working principles.

4.1.1. Bus network

The microfluidic bus topology was first introduced in [25]. The basic network consists of a cascade of μfSwitches interconnected on the main channel, where each μfSwitch connects one LoC device to the main channel (cf. Fig. 7).

The droplet flow in a bus network starts on the droplet generator site, where header-payload droplet pair is generated by the DoD system. Then, the droplets are injected into the network and carried further for processing by a continuous phase that is constantly supplied from the source (eg. pressure pump). Traversing the main bus, the droplets arrive at the microfluidic switch, where the payload droplet can be directed to the connected LoC or forwarded to the next switch, depending on the destination address encoded using the header droplet. If the μfSwitch recognizes the encoded address as the address of the connected LoC, the payload droplet is forwarded to the LoC for further processing, otherwise, the payload droplet is forwarded to the next μfSwitch where the address recognition process repeats. At the end of the experiment, payload and header droplet are delivered to the sink.

It is important to note that the μfSwitch used in the bus network can be either a single droplet switch (cf. Sec. 3.2) or a multiple droplet switch (cf. Sec. 3.3). Thus, the bus network allows for multiple payload switching.

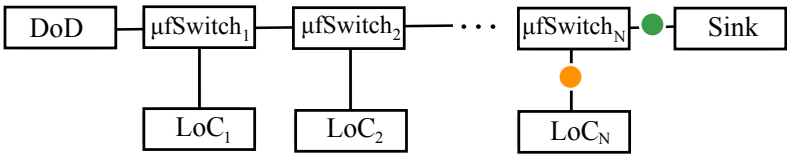


Figure 7: Microfluidic network with bus topology. Header (green) droplets are used to control the path of the payload (orange) droplets

4.1.2. Ring network

The basic ring network was first introduced in [7]. Here, the droplet flow throughout the network is different compared to the bus networks. However, similar to the bus network, the process starts by injecting a payload and header droplet. When droplets traverse the ring, they pass multiple μfSwitches in order to reach targeted LoCs and execute desired operations. Once the μfSwitch recognizes the destination address of the payload droplet, it delivers the payload droplet to the connected LoC for further processing and forwards the header droplet to the next μfSwitch. Once processed by an LoC, the payload droplet flows back to the ring. Finally, both header and payload droplets are delivered to the sink and forwarded to the DoD system where payload droplet, together with new header droplet, can be re-injected into the network for further processing, if needed. Therefore, unlike bus networks, in ring networks payload droplets can cyclically traverse the network depending on the application requirements.

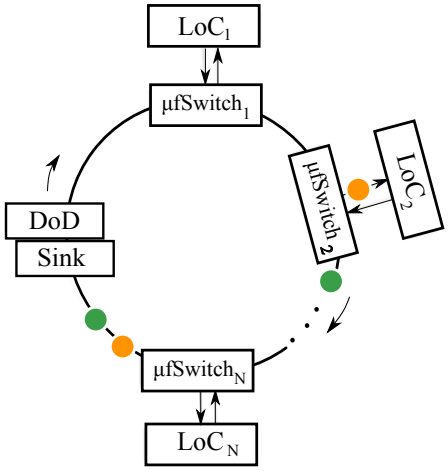


Figure 8: Microfluidic network with a ring topology. Header (green) droplet is used to control the path of the payload (orange) droplet. Once payload droplet is processed by LoC it is returned to the ring

It is important to note that the ring network does not support switching of multiple payload droplets, therefore the μfSwitch shown in Fig. 8 can only be single droplet switch.

4.2. Advanced network topologies

In the following, we present some extensions for the ring and bus network in order to increase their flexibility.

4.2.1. Advanced Bus Network

As discussed in Sec. 4.1.1, in a basic bus network, the droplets flow from the droplet generator, over a series of μfSwitches to the targeted LoCs or to the sink. Although the basic bus network offers simplicity in its architecture, it lacks the possibility to reuse the payload droplets that have already been procssed.

In order to increase reusability of reagents (droplets), an extended bus network was introduced in [5]. As shown in Fig. 9, it is possible to create a response channel, which allows the payload droplets to flow back to the droplet generator after bein processed by the LoC, in order to re-inject payload droplets back into the network at the source. For the system to work, it is necessary to ensure positive pressure difference between the main and response bus in order to initate the flow through LoCs.

Similar to the basic bus network, the advanced bus network supports switching of single and multiple payload droplets.

4.2.2. Advanced Ring Network

As discussed in Sec. 4.1.2, basic ring network already allows droplet reinjection. Once the payload droplet is processed by an LoC, it can be reinjected by the DoD module, together with a new header droplet. This means that in a basic ring architecture, the payload droplet can only be sent to a single LoC at a time and if payload droplet needs to be processed by multiple LoCs, it is re-injected multiple times at the source. The re-injection of droplets into the system can be a time demanding and complex task that potentially increases the risk of degrading the sample.

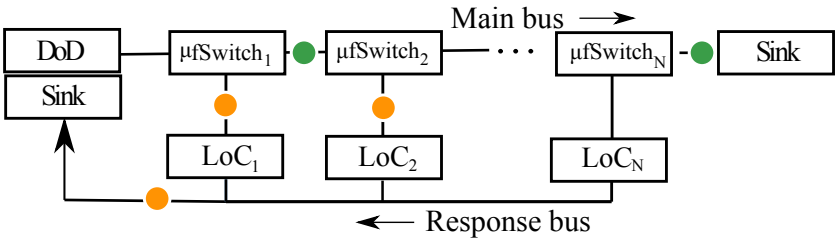


Figure 9: Advanced microfluidic bus network. Response bus is introduced to re-injected the payload (orange) droplets back into the network

The advanced ring network was introduced in [26] where an addressing scheme for targeting multiple LoCs without payload re-injection, and with using a small number of header droplets, is proposed. For this purpose, a new network switch (a combination of a single droplet switching and droplet by size sorter [27]) that allows payload droplets to execute or skip a specific LoC is introduced [5]. The idea is that for every LoC that the droplet needs to skip, new header droplet is inserted in front of the payload droplet. Since the header droplets carry no sample, generating multiple header droplets is a rather cheap and simple process compared to the payload re-injection.

The addressing scheme relies on the distance between header and payload droplet in order to address specific LoC, while newly introduced module for skipping specific LoC requires fine tuning and control over droplet size.

5. Validation of Microfluidic Networks

Microfluidic networks for two-phase flow microfluidics is a rather new research field. In order to establish this research it is necessary to validate the practicability of existing concepts (cf. Sec. 3 and 4). Two common ways of validating concepts in microfluidic networks involve simulations and experimental validation. Usually, simulations are used as a validation if system behaves as desired. The fabrication of the microfluidic system is carried out in order to obtain practical validation, demonstrating the realistic behavior of the system. Unfortunately, to this date, experimental validation of the microfluidic network concepts, and relevant switching mechanisms, is largely unexplored due to the lack of required passive DoD systems (cf. Sec 6).

In this section we first provide an overview of the state-of-the-art simulation tools available for modelling and simulating microfluidic networks on different levels of abstractions. We then present first steps towards the experimental validation of switching principles and required DoD mechanisms.

5.1. Simulations

In order to validate whether the respective microfluidic design works as intended, it can be fabricated and tested. However, as this usually is a costly process (which additionally has to be repeated in case of errors in the design), checking the design beforehand using simulations prior to first fabrications seems reasonable. For example, simulations can be used to validate designs even before the first prototype is fabricated. If, for example, a simulation predicts an unintended behavior, it is very likely that the fabricated prototype shows that behavior as well. Furthermore, simulations can also help to explore further design alternatives e.g. in order to make the design most robust, more efficient, etc. More precisely, with simulations a huge number of design variations/alternatives can be tested, which would be impossible using fabricated prototypes as they would cause too high costs (with respect to time as well as money).

[5] More details on the proposed module can be found in [26].

In principle two simulation approaches (conducted at different abstraction levels) can be applied in order to test the proposed design: Computational Fluid Dynamics (CFD) Simulations and simulations on the 1D Circuit Analysis Model.

5.1.1. Computational Fluid Dynamics Simulations

Tools like Comsol Multiphysics, Ansys, and OpenFoam provide algorithms for CFD simulations. Detailed reviews of these tools and also their algorithms are provided in [28, 29]. CFD simulations describe the fluid flow in the most accurate way [6] and hence, allow to simulate effects like droplet formation, droplet deformation, and coalescing of droplets. However, accounting for this high level of physical details requires a complex setup (e.g. generation of a simulation mesh) and results in high computational costs. For example, simulations of simple microfluidic chips can even take hours on typical computing hardware [30]. Therefore, this kind of simulation is most useful for simulating particularly complex subparts of a microfluidic network.

5.1.2. Simulations on the 1D Circuit Analysis Model

On this level, a microfluidic circuit is abstracted to 1D values (i.e. the duality between microfluidic networks and electrical circuits is employed [10]). For applying this model, the flow has to be laminar, viscous, and incompressible [31]. Furthermore, on this level the effects of droplets are modeled as additional resistances, which constantly change by their flow through the microfluidic network the flow rates and pressure gradients. Hence, this level allows for determining the path, position, and speeds of droplets as well as allows for design explorations (i.e. a parametric analysis). Furthermore, due to the applied abstractions, simulations can efficiently be conducted and enable the designer to simulate large-scale microfluidic networks as reviewed above (i.e. the bus, ring and application-specific architectures). Methods and algorithms used for 1D circuit analysis model can be found in [31]–[32].

(a) Multidroplet switch working in ON state

(b) Multidroplet switch working in OFF state

Figure 10: Multidroplet switch simulation using 1D Circuit Analysis Model

[6] CFD methods partition the simulation region in a dense volume grid which allows to solve systems of differential equations that express the interaction of liquids/gases with surfaces defined by boundary conditions.

Due to the low computation costs and high efficiency of 1D Circuit Analysis Model, we have employed an in-house developed 1D Circuit Analysis Simulator to verify the behaviour of the multidroplet μfSwitch. Using the developed simulator, we were able to verify the path, position and speed of droplets in a multidroplet switch. The results of a simulation are presented in Fig. 10.

Depending on the need of the designer, the respective simulation approach should be chosen. For example, to validate whether the droplets indeed take the intended paths and/or arrive on certain positions at the expected time, simulations based on the 1D model provide an efficient and fast method. If in contrast e.g. the deformation or stability of the droplets is of interest, the CFD approach is suitable at the expense that the simulation takes significantly longer and/or can only be conducted on parts of the design due to the resulting complexity.

5.2. Experimental Validation

In order to obtain a practical validation, that demonstrates the realistic behavior of the system, microfluidic designs are fabricated using one of the many available microfabrication techniques (e.g., [3, 4]). The most common method is microfabrication using a combination of photolithography and soft lithography in the polymer, often in PDMS
((Poly)dimethylsiloxane) [33].

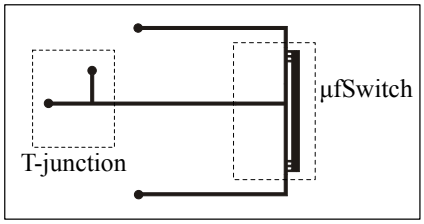


Figure 11: Simple microfluidic network design. The network consists of a droplet generator and one μfSwitch

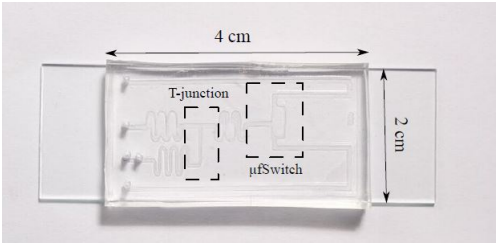


Figure 12: Physical realization of of the microfluidic design in Fig. 11

The design process starts by creating the footprint of microfluidic chip using one of the many drawing software applications (e.g., AutoCAD), as shown in Fig. 11. The design can then be realized using any fabrication technique and turned into a physical device, as shown in Fig. 12.

The device shown in Fig. 12, can then be used to practically verify basic mirofluidic switching principles (c.f. Sec 3). To this date, only one work reports on a practical investigation of microfluidic networks [9]. There, a similar design is reported where the microfluidic network, consisting of one droplet generator and one μfSwitch, was designed and experimentally tested. However, the behaviour of a μfSwitch was investigated using a train of droplets. Due to the coupling effects of droplets within the train, the reported work is not sufficient to truly validate the principles of microfluidic networks.

Therefore, prior to investigating practicability of μfSwitches and microfluidic networks, we focus our experimental validation on a droplet generation and delivering efficient, passive DoD system- a crucial element in microfluidic networks. Only once DoD method is introduced and experimentaly verified, it is possible to further investigate microfluidic networks.

6. Droplet-on-Demand Systems

To date, the true potential of microfluidic networks for two-phase microfluidics is largely unexplored, since it requires droplet generation at a prescribed time and with prescribed volume. Systems performing arbitrary protocols of droplet formation are referred to as Droplet-on-Demand systems [17]. Although, the necessity of DoD systems for microfludic networks was noticed [9], it was never truly investigated. Thus, experimental results on the concepts proposed for microfluidic networks (cf. Sec. 3 and 4) are lacking.

So far, droplets' routing, and proposed switching mechanisms [10, 9, 22], have been a strong focus of research on microfluidic networks. Here, as presented in Section 4, various switching mechanisms for controlling droplets' path have been proposed. However, a DoD, a crucial feature required for the desired functionality of the proposed switching mechanisms, was mentioned as a necessity for microfluidic networks but never truly investigated. Therefore, passive DoD systems are essential for practical investigation and further development of microfluidic networks and exploring the adaptation of these networks for numerous microfluidic applications *e.g.* high throughput drug screening systems [34].

Droplet-on-Demand systems have been reported in the literature and most of them focus on active control of the flow of the fluids on the chip[15, 35, 36, 37]. For this purpose, common solutions utilize integrated microvalves [15, 38] or electrowetting [39, 40]. Although active DoD systems enable high precision over the droplet generation process, the applicability of such systems is limited due to [17]: i) complex, multilevel fabrication, ii) external active equipment needed to control the valves; iii) negative impact of the electric fields on biological samples, iv) lower biocompatibility of the device. Due to the limitations of active DoD systems, we focus on investigating passive DoD systems that surpass

the limitations of active DoD systems and offer simplicity in their design and higher biocompatibility.

In this section, we review two promising passive DoD methods, introduce their basic functionalities, and address their applicability to microfluidic networks. Then, we present simple, passive DoD method and verify its functionality by providing proof-of-the-concept experimental results.

6.1. Passive DoD System using Negative Pressure

The work in [16], demonstrates an effective method to perform DoD using a flow-focusing droplet generator. The method utilizes a completely passive microfluidic chip with the flow-focusing droplet generator, connected to an external pressure controller. Since the flow-focusing geometry is used as droplet generator, the method requires three control channels, namely two input and one outlet channel, that are connected to the external pressure controller (cf. Fig.13).

The process of droplet generation starts by balancing the pressures of the dispersed and continuous phase to obtain a stable interface. Once the two phases are balanced, a negative pressure pulse is applied from the input pressure pump [7] at the outlet of the droplet generator to induce the droplet formation. Increasing the magnitude and time period of the negative pulse increases the size of the generated droplets, while applying the series of pulses enables generating multiple droplets of arbitrary sizes and interdroplet distances.

Although the method meets the main requirements for a DoD system in microfluidic networks (passive DoD generation, where individual and multiple droplets of various sizes and inter-droplet distances can be generated), it has limited applicability for microfluidic networks. This is due to the unpredictable flow distributions that would appear in the network due to the negative pressure applied at the outlet. However, the precision and simplicity of the technique make this method a promising candidate for many other microfluidic platforms (e.g., biochemical applications where arbitrary compartmentalizing of samples is of crucial importance [41]).

6.2. Passive DoD System using External Valves

The work presented in [17, 42] investigates a passive DoD method that utilizes a T-junction as a droplet generation mechanism. Here, two input channels are connected to two external valves that control the flow of the dispersed and continuous phase (cf. Fig. 14). Utilizing external, rather than integrated (on the chip) valves reduces the design and fabrication complexity and enables interfacing this DoD system with any microfluidic chip [17].

In this method, two external valves are used to switch on and off the flow of the dispersed and continuous phase. As shown in Fig. 14, a protocol where the flow of the continuous phase is normally open, and flow of the dispersed phase

[7] Pressure pump contains piezoelectric regulators and valves.

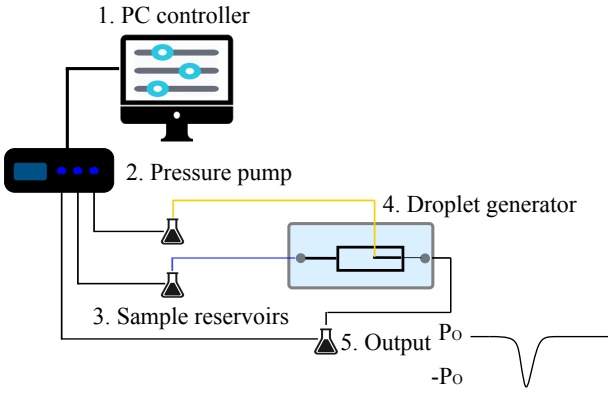


Figure 13: Passive DoD system using negative pressure method

is normally closed, is applied. Then, during the time period t_{open}, the dispersed phase is opened and continuous phase flow is closed. During this period droplet formation is induced and the droplet is growing in size. At the end of the time period t_{open}, the droplet breaks-off and is carried further downstream by the continuous phase which after t_{open} is again turned on while the dispersed phase is turned off.

Mutually switching on and off the two phases surpasses the limitation of conventional T-junction generators where droplet size/volume is limited by the mechanism of droplet formation. In a conventional T-junction generator, introduced in Sec. 2, both continuous and dispersed phase are constantly turned on. Once the dispersed phase obstructs the continuous phase, pressure builds up and eventually breaks-off the droplet after the time period that is proportional to the ration of the volumetric flow rate of the continuous phase and the volume of the junction [17]. Therefore, the break-off moment limits the volume of droplets generated by conventional T-junction generator. In this method, this limitation is surpassed by switching off the continuous phase during the period of droplet generation which reduces the pressure build-up inside the T-junction, allowing for bigger droplets to be generated. In order to generate bigger droplets, it is only necessary to increase the time period t_{open}, where the droplet size linearly increases when increasing t_{open} [42].

The method enables accurate and passive DoD technique that allows generating droplets of arbitrary volumes and inter-droplet distances using simple T-junction geometry. Moreover, the linear dependency between the time period t_{open} and droplet size enables easy control and fine tuning of the droplet size during the droplet formation process- a critical feature for employing this DoD method for microfluidic networks. Thus, this method can then be employed for practical realization of microfluidic networks.

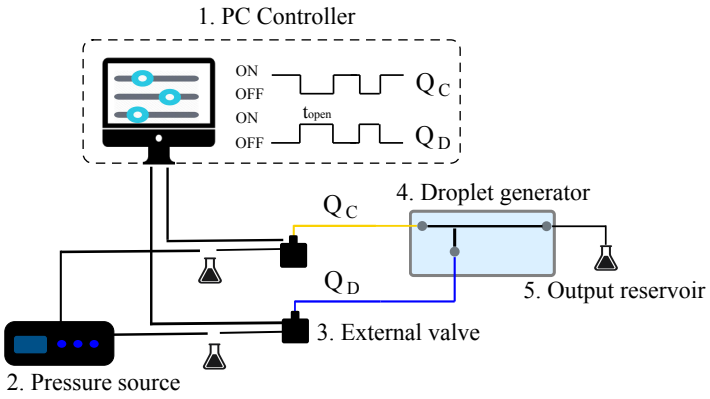


Figure 14: Passive DoD system using external valves

6.3. Low-Complexity Passive DoD System

Previously, we have introduced two promising passive DoD systems: DoD using negative pressure and DoD using external valves. Due to the unpredictable flow distributions caused by the negative pressure applied at the output of flow-focusing generator, the first method is not applicable for microfluidic networks. The second method, using two external valves, seems to be promising choice for microfluidic networks. However, the method requires two external valves for regulating both dispersed and continuous phase. This implies that synchronization between two valves is a crucial, and demanding, task.

In this work, we propose an alternative DoD system, which surpasses limitations of both previous methods. As shown in Fig. 15, the DoD system utilizes a simple T-junction geometry and applies a series of positive pulses on the dispersed phase, in order to generate droplets, while maintaining the continuous phase at constant input pressure. As the method applies positive pulses solely at dispersed phase input, the microfluidic network remains stable during the droplet formation process. Moreover, as the method utilizes solely one phase as a control phase, no synchronization issues need to be solved. Additionally, it is not necessary to have two high-precision devices with short response time, such as external valves, to obtain DoD. In this case, it is possible to connect the continuous phase to a simpler controller, such as a syringe pump, while using a high-precision device to control the dispersed phase. This can significantly reduce the total cost of laboratory equipment needed to carry out DoD.

The working principle of the proposed DoD system is as follows: during the initial stage of the DoD method, the system enters the equilibrium state (cf. Fig. 16a). In this state, the input pressure of the dispersed phase, $P_{D,eq}$, is finely tuned by the pressure pump to form a stable, equilibrium, interface with the continuous phase. Here, no droplets are formed due to the accurate balance

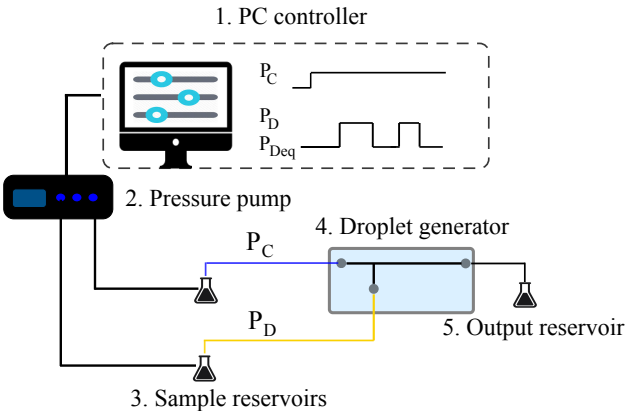


Figure 15: Proposed passive DoD system

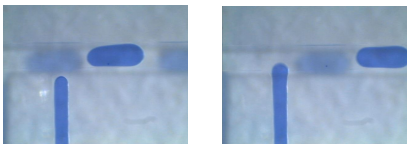
of input pressures[8]. Once the system is in an equilibrium state, the droplet generation is initiated on demand by rapidly overcoming the equilibrium forces, i.e. applying the positive pressure pulse of the duration T on the dispersed phase input and therefore increasing the dispersed phase pressure to $P_D > P_{D,eq}$. At this stage, during the period T, pressure of the dispersed phase is large enough to enable the dispersed phase to penetrate and obstruct the continuous phase channel. Depending on the required droplet parameters (droplet size and inter-droplet distances), the droplet is released at the end of period T (cf. Fig. 16b), simply by taking the system back to the equilibrium state, i.e. instantly reducing the dispersed phase pressure to $P_D = P_{D,eq}$.

Using this method, it is possible to create droplets of various sizes where the volume/size of generated droplets is proportional to the duration of the applied pulse, T, while the distance between consecutive droplets is proportional to the time between consecutive pulses, t, as shown in Fig. 17.

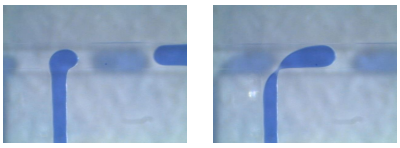
6.4. Practical validation of the proposed DoD method

We have fabricated the microfluidic chip with the proposed DoD system in *polydimethylsiloxane* (PDMS) polymer using standard soft lithography methods. We have used a pressure controller (Elveflow®, OB1MK3) to induce pressure to the continuous and dispersed phase. Moreover, the pressure controller is able to apply a sequence of positive pressure pulses either through the Elveflow® Smart Interface or by using the provided Matlab libraries. For the evaluation of the DoD system we have used a high-speed camera and an optical microscope. The geometry of the T-junction was chosen of $w_D = 150$ μm and $w_D/w_C = 0.5$ with rectangular cross-sections of the channels with a uniform height of $h = 40$ μ and

[8]The derivation of the equilibrium state is left for future work.



(a) DoD system in equilibrium (left) and applying positive pulse to initiate droplet generation (right)



(b) Droplet grows in size during the period T (left) and breaks-off at the end of this period (right)

Figure 16: Droplet formation process in DoD method

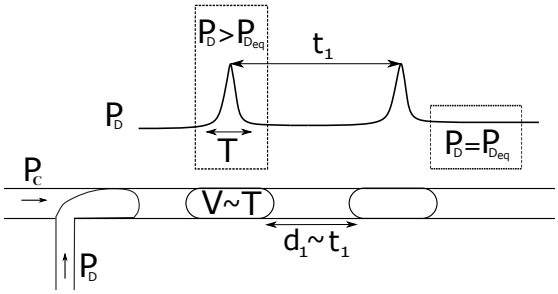


Figure 17: Working principle of the low-complexity passive DoD system

$h < w_C$ [43]. Moreover, we have ensured that $C_a << 0.01$. The experimental setup is shown in Fig. 18.

Fig. 19 shows the experimental results that verify the proposed DoD method. Here, duration of the positive pulse, applied on the dispersed phase, is reduced from $T = 1.75\,s$ to $T = 0.75\,s$ with $0.25\,s$ step, causing the droplet diameter to reduce linearly from $D = 400\,\mu m$ to $D = 170\,\mu m$, with the period reduction. The period between individual pulses was kept constant at 3 s which resulted in the same distance between all droplets. Moreover, we observe from Fig. 19, that between the applied pulses, the system returns to, and remains in, the equilibrium state.

The proposed method offers simplicity in design and reduces laboratory equipment needed to carry out DoD generation to a minimum. Moreover, applying positive pulses at only one inlet of the DoD system, maintains the network stability and eliminates the need for synchronization between the input channels.

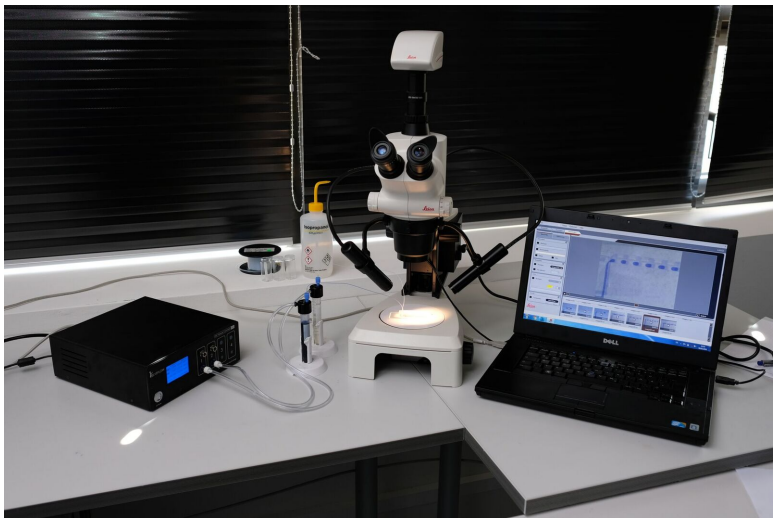


Figure 18: Experimental setup used for validating the proposed DoD method

(a) T=1.5s (left) and T=1.75s (right)

(b) T=1.25s (left) and T=1.5s (right)

(c) T=1s (left) and T=1.25s (right)

(d) T=0.75s (left) and T=1s (right)

Figure 19: Proposed DoD method: Applying positive pulses of various durations results in droplets of different sizes and inter-droplet distances

7. Applications of Microfluidic Networks

Enabling the integration of multiple LoCs on a single platform (i.e., microfluidic network) can offer parallelization, high throughput, increased efficiency and flexibility in performing laboratory experiments. Moreover, realizing DoD allows performing arbitrary protocols of droplet formations which is a critical feature in a number of microfluidic applications, such as, medical and pharmaceutical diagnostics, chemical synthesis/analysis and molecular biology [44, 45, 46].

In the following, we introduce two promising applications of microfluidic networks. First, we present microfluidic networks as a powerful tool for pharmaceutical diagnostics to perform fast and flexible drug screening[34]. Second, we investigate microfluidic networks in clinical pathology for the purpose of screening waterborne pathogens [47, 48].

7.1. Fast and Flexible Drug Screening

The pharmaceutical industry lacks insight into which bacteria are antibiotic resistant, or what exact amount of antibiotics is required for treating specific bacteria [49, 50]. Standard laboratory procedures are time demanding, expensive and lack of flexibility. For this reason, it is essential to have a platform, which allows fast delivery of different antibiotics and concentrations to the infected cells and allows to observe the reaction [51][52]. The concept of microfluidic networks allows realizing a microfluidic design, which serves as a disease model for human infections and is able to perform fast and flexible drug screening.

A concept of a microfluidic device realizing such a fast and flexible drug screening is shown in Fig. 20. It uses a microfluidic bus network as introduced in Sec. 4.1.1, with the DoD system proposed in Sec. 6.3. Either single or multiple droplet switching, as presented in Sec. 3.1 and Sec. 3.3, can be applied.

The droplet generator is able to generate droplets at the desired frequency, with desired size and interdroplet distances. For the purpose of drug screening, different antibiotics, or different antibiotic concentrations, are isolated inside the payload droplet (e.g., water droplet), which are carried throughout the network by a continuous phase (e.g., oil). The microfluidic switch directs single/multiple payload droplets to different microreactors for processing. Although it is possible to realize the design with both single- and multidroplet switching, it is recommended to use multidroplet switch in order to enable fast delivery and increase parallelism.

As shown in Fig. 21, microreactors incubate infected human cells (e.g., *Human Lung Epithelial* cells (HPAEpiC) infected by bacteria (e.g.,*Acinetobacter baumanii* bacteria) that are held in place by electrospun polymer nanofibers [53, 54]. Once a payload droplet enters the microreactor, the antibiotics inside the droplet initiate a biochemical reaction with the infected cells incubated inside the microreactor. Since the droplet volume is very small, antibiotics inside the droplets are able to initiate the reaction with lung cells and bacteria quickly and efficiently. The result of this reaction can be either observed inside

the microreactor or at the analysis stage, using, for example high-resolution microscopy tools.

Utilizing microfluidic networks, together with DoD, it is possible to carry out a large number of biochemical reactions for different types of infected human cells and for different types/concentrations of antibiotics in a short time. Moreover, the proposed microfluidic design can be fabricated at low cost, since no active elements are required on the chip. Such microfluidic design can enable gaining more insights into the behaviour of antibiotic resistant bacteria, which will ultimately help to produce more efficient drugs, in much shorter time and at much smaller costs.

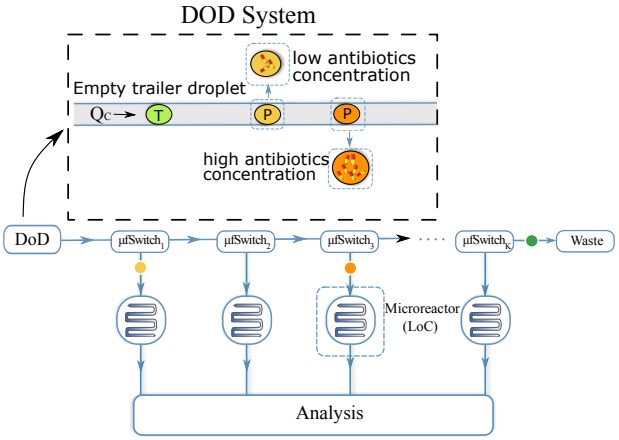


Figure 20: Microfluidic network design for fast and flexible drug screening [34]

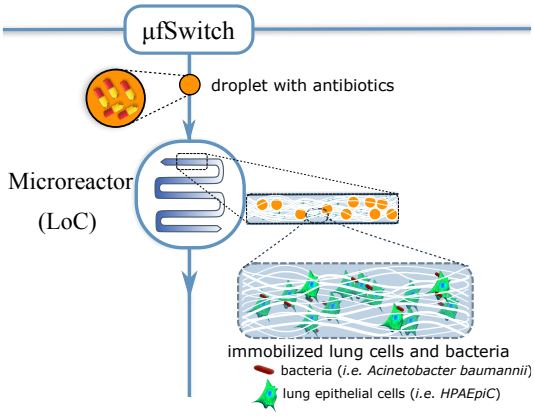


Figure 21: Inside of microreactor for fast and flexible drug screening

7.2. Screening of Waterborne Pathogens

Despite having modern water purification systems in place, waterborne pathogens are still a big threat to humans and livestock, especially in underdeveloped countries [47, 48, 55]. Being responsible for an estimated 1.9 million human deaths each year, protozoan parasites, such as *Cryptosporidium parvum*, are still one of the most important environmental concerns [55]. Such parasites are robust and highly resistant to commercial water disinfectants and filtration systems [55]. For this reason, recent research in the field of clinical pathology is focusing on finding new means of inactivating the water parasites.

Conventional water purification systems require extremely high concentration of pathogens in order to examine to which disinfectants are pathogens resistant, which disinfectants are effective and what is the precise dose of disinfectants needed to inactivate pathogen. However, some water pathogens, such as *Cryptosporidium parvum*, are of extremely small size (having only 5 μm diameter), are very difficult to detect and often not present in required concentrations [47][48]. For this reason, conventional water purification systems are limited in detecting these pathogens and finding efficient disinfectants to treat them.

For examining pathogens such as *Cryptosporidium parvum*, droplet-based platforms, such as microfluidic networks, seem to be a promising candidate for faster and more efficient detection. Due to their small size, droplets can serve as efficient microreactors for pathogens. Encapsulating pathogens, together with targeted disinfectants, inside these small droplets, it is possible to efficiently initiate a biochemical reaction between two the samples. This enables to quickly obtain first insights into behaviour and robustness of a specific pathogen. Moreover, employing microfluidic networks enables the parallelization of the experiments.

The proposed microfluidic design for the fast and efficient screening of waterborne pathogens is shown in Fig. 22. The system utilizes two DoD generators that work on the principle proposed in Sec. 6.3. The first DoD system is used to create droplets that precisely encapsulate desired concentrations of pathogens. The second DoD system is used to generate droplets with disinfectants. These two types of droplets flow down to targeted LoC where they merge insite the LoC and initiate the lysis process. The lysis process is carried out in order to break down the membrane of the pathogen and release the pathogen DNA [47, 48]. At the last stage, the Polymerase Chain Reaction (PCR) is carried out in order to amplify the released DNA. After the PCR stage, the droplets flow to the analysis stage where interaction between disinfectant and pathogen DNA can be observed using, for example phase-contrast light microscopy.

The proposed microfluidic design enables investigating the robustness of pathogens, such as *Cryptosporidium parvum*, which cannot be detected or deactivated using commercial water purification systems. Employing microfluidic networks, such pathogens can be tested for a number of disinfectants in a short time on a single microfluidic device. Moreover, since microfluidic networks are passive systems, the viability of pathogens during testing is not compromised.

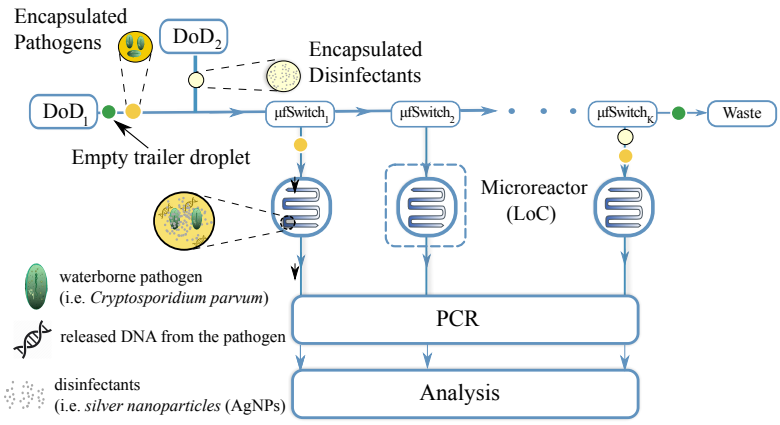


Figure 22: Proposed microfluidic networks for performing high screening of waterborne pathogens

8. Summary and Research Opportunities

In this work, we first provided a survey of the state-of-the-art in microfluidic networking: We presented different principles for passive switching of single/multiple payload droplets and discussed various network topologies and their applicability for microfluidic networks. We introduced various methods for validating microfluidic networks, including simulation at different abstraction levels and experimental validation. Second, we identified DoD as a crucial component in order to practically exploit the full potential of microfluidic networks. We proposed a simple and passive DoD system that meets all requirements of a DoD system for microfluidic networks and verified its functionality through experimental results. Finally, we discussed two promising applications, namely fast and flexible drug screening and screening of waterborne pathogens, which allows a faster analysis compared to conventional methods.

Although, various works have been devoted to microfluidic networking in the past, this research field is still in its infancy. Please find below a list of possible research opportunities:

- **Experiments:** Most of the theoretical work on microfluidic networking has only been validated through simulations (CFD or 1D circuit analysis), and, thus experimental validation is lacking[9]. With DoD systems (cf. Sec. 6) it is possible to experimentally validate the theoretical concepts (e.g., single/multiple droplet switching). Experimental work is a crucial step in order to fully exploit the potential of microfluidic networks for

[9] In [9], experimental results on the behavior of a single switch using a train of droplets have been presented. However, this is not sufficient to truly validate the principles of microfluidic networks due to the coupling effects of the droplets within the train.

practical purposes and to establish the research field.

- **Applications:** Since microfluidic networking aims at realizing programmable and flexibile LoC devices for chemical or biological analysis it is important to identify applications in these disciplines (cf. Sec. 7). Moreover, it is important to show that using microfluidic networking outperforms conventional methods (e.g., faster analysis).

- **Simulations:** Simulations based on the 1D circuit analysis model are a promising approach for simulating large microfluidic networks. However, the development of such simulation tools is still in its infancy, so far, only supporting a very limited number of components (e.g., trapping). Thus, the first step towards a more practical simulation tool would be to include many different components (e.g., mixing, splitting, ...) with different realizations. Moreover, it would be good to provide a graphical user interface for such simulation tools in order to increase their usage also from researchers without computer science background.

- **Fault-Tolerance:** The main source of failures on microfluidic chips are fabrication failures, resulting for example in blocked channels. This may lead to an undesired behavior of the microfluidic network. Thus, it is important to investigate the fault-tolerance of microfluidic networks and develop techniques to overcome this issue. Here, maybe techniques originating from conventional network theory can be used.[56].

- **Capacity:** So far, only the capacity of linear microfluidic channels [57] (channel without branches and section variations) was derived. Thus, a capacity analysis of more practical non-linear microfluidic channels is lacking in the literature. Such an investigation would be important in order to find the theoretical limits of microfluidic networks.

References

[1] H. Gu, M. H. G. Duits, F. Mugele, Droplets formation and merging in two-phase flow microfluidics, International Journal of Molecular Sciences 12 (4) (2011) 2572–2597.

[2] S. Hardt, T. Hahn, Microfluidics with aqueous two-phase systems, Lab Chip 12 (2012) 434–442.

[3] V. Silverio, S. Cardoso, Microfabrication techniques for microfluidic devices, in: Galindo-Rosales, F. Jose (Eds.), Complex Fluid-Flows in Microfluidics, Springer, 2018, Ch. 2, pp. 25–51.

[4] W. Wu, R. Rezai, H. Hsu, P. Selvaganapathy, Materials and methods for the microfabrication of microfluidic biomedical devices, in: X. J. Li, Y. Zhou (Eds.), Microfluidic Devices for Biomedical Applications, Woodhead Publishing Series in Biomaterials, Woodhead Publishing, 2013, Ch. 1, pp. 3 – 62.

[5] L. Donvito, L. Galluccio, A. Lombardo, G. Morabito, μ-net: A network for molecular biology applications in microfluidic chips, IEEE/ACM Trans. Networking 24 (4) (2016) 2525–2538.

[6] G. Castorina, M. Reno, L. Galluccio, A. Lombardo, Microfluidic networking: Switching multidroplet frames to improve signaling overhead, Nano Communication Networks 14 (2017) 48 – 59.

[7] E. D. Leo, L. Galluccio, A. Lombardo, G. Morabito, Networked labs-on-a-chip (NLoC): Introducing networking technologies in microfluidic systems, Nano Communication Networks 3 (4) (2012) 217–228.

[8] E. D. Leo, L. Donvito, L. Galluccio, A. Lombardo, G. Morabito, L. M. Zanoli, Design and assessment of a pure hydrodynamic microfluidic switch, in: 2013 IEEE International Conference on Communications (ICC), 2013, pp. 3165–3169.

[9] L. Donvito, L. Galluccio, A. Lombardo, G. Morabito, On the assessment of microfluidic switching capabilities in NLoC networks, in: Proc. Int. Conf. Nanoscale Computing and Communications, ACM, New York, NY, USA, 2007, pp. 19:1–19:7.

[10] A. Biral, D. Zordan, A. Zanella, Modeling, simulation and experimentation of droplet-based microfluidic networks, IEEE Transactions on Molecular, Biological and Multi-Scale Communications 1 (2) (2015) 122–134.

[11] A. Biral, D. Zordan, A. Zanella, Transmitting information with microfluidic systems, in: Proc. IEEE Int. Conf. Communications, 2015, pp. 1103–1108.

[12] A. Grimmer, W. Haselmayr, A. Springer, R. Wille, Design of application-specific architectures for Networked Labs-on-Chips, IEEE Trans. on Computer-Aided Design of Integrated Circuits and Systems 37 (1) (2018) 193–202.

[13] A. Grimmer, W. Haselmayr, A. Springer, R. Wille, A discrete model for Networked Labs-on-Chips: Linking the physical world to design automation, in: Proc. Design Automation Conference, 2017, pp. 50:1–50:6.

[14] A. Grimmer, W. Haselmayr, A. Springer, R. Wille, Verification of Networked Labs-on-Chip architectures, in: Proc. Design, Automation and Test in Europe, 2017, pp. 1679–1684.

[15] S. Zeng, B. Li, X. Su, J. Qin, B. Lin, Microvalve-actuated precise control of individual droplets in microfluidic devices, Lab Chip 9 (2009) 1340–1343.

[16] A. J. T. Teo, K.-H. H. Li, N.-T. Nguyen, W. Guo, N. Heere, H.-D. Xi, C.-W. Tsao, W. Li, S. H. Tan, Negative pressure induced droplet generation in a microfluidic flow-focusing device, Analytical Chemistry 89 (8) (2017) 4387–4391.

[17] K. Churski, P. Korczyk, P. Garstecki, High-throughput automated droplet microfluidic system for screening of reaction conditions, Lab Chip 10 (2010) 816–818.

[18] P. Zhu, L. Wang, Passive and active droplet generation with microfluidics: a review, Lab Chip 17 (2017) 34–75.

[19] M. F. Gu H., Duits M.H.G., Droplets formation and merging in two-phase flow microfluidics, International Journal of Molecular Sciences 12 (2011) 2572–2597.

[20] S. Protire, M. Bazant, D. A. Weitz, H. A. Stone, Droplet breakup in flow past an obstacle: A capillary instability due to permeability variations 92 (2010) 54002.

[21] A. M. Leshansky, L. M. Pismen, Breakup of drops in a microfluidic t junction, Physics of Fluids 21 (2) (2009) 023303.

[22] E. D. Leo, L. Donvito, L. Galluccio, A. Lombardo, G. Morabito, L. M. Zanoli, Communications and switching in microfluidic systems: Pure hydrodynamic control for networking labs-on-a-chip, IEEE Transactions on Communications 61 (11) (2013) 4663–4677.

[23] N. Shembekar, C. Chaipan, R. Utharala, C. A. Merten, Droplet-based microfluidics in drug discovery, transcriptomics and high-throughput molecular genetics, Lab Chip 16 (2016) 1314–1331.

[24] A. M. Kaushik, K. Hsieh, T.-H. Wang, Droplet microfluidics for high-sensitivity and high-throughput detection and screening of disease biomarkers (2018) e1522.

[25] A. Biral, A. Zanella, Introducing purely hydrodynamic networking mechanisms in microfluidic systems, in: 2013 IEEE International Conference on Communications Workshops (ICC), 2013, pp. 798–803.

[26] W. Haselmayr, A. Biral, A. Grimmer, A. Zanella, A. Springer, R. Wille, Addressing multiple nodes in Networked Labs-on-Chips without payload re-injection, in: Proc. Int. Conf. Communications, 2017, pp. 1–6.

[27] Y.-C. Tan, Y. Li Ho, A. Lee, Microfluidic sorting of droplets by size, Microfluidic Nanofluid 4 (2014) 67 – 114.

[28] T. Glatzel, C. Litterst, C. Cupelli, T. Lindemann, C. Moosmann, R. Niekrawietz, W. Streule, R. Zengerle, P. Koltay, Computational fluid dynamics (cfd) software tools for microfluidic applications a case study, Computers & Fluids 37 (3) (2008) 218–235.

[29] M. Wörner, Numerical modeling of multiphase flows in microfluidics and micro process engineering: a review of methods and applications, Microfluidics and Nanofluidics 12 (6) (2012) 841–886.

[30] J. Wang, V. G. J. Rodgers, P. Brisk, W. H. Grover, Instantaneous simulation of fluids and particles in complex microfluidic devices, PLOS ONE 12 (12) (2017) 1–14.

[31] M. Schindler, A. Ajdari, Droplet traffic in microfluidic networks: A simple model for understanding and designing, Phys. Rev. Lett. 100 (2008) 044501.

[32] O. Cybulski, P. Garstecki, Dynamic memory in a microfluidic system of droplets traveling through a simple network of microchannels, Lab Chip 10 (2010) 484–493.

[33] M. Dzebic, O. Kurikov, O. Dobroliubov, O. C. A. Nava, Design and fabrication of a pdms microfluidic device for titration of biological solutions, in: A. Badnjevic (Ed.), CMBEBIH 2017, Springer Singapore, Singapore, 2017, pp. 147–152.

[34] W. Haselmayr, M. Hamidović, A. Grimmer, R. Wille, Fast and flexible drug screening using a pure hydrodynamic droplet control, in: Proc. Euro. Conf. Microfluidics, 2018, pp. 1–4.

[35] A. R. Abate, J. J. Agresti, D. A. Weitz, Microfluidic sorting with high-speed single-layer membrane valves, Applied Physics Letters 96.

[36] J. J. Agresti, E. Antipov, A. R. Abate, K. Ahn, A. C. Rowat, J.-C. Baret, M. Marquez, A. M. Klibanov, A. D. Griffiths, D. A. Weitz, Ultrahigh-throughput screening in drop-based microfluidics for directed evolution, Proceedings of the National Academy of Sciences 107 (9) (2010) 4004–4009.

[37] C. N. Baroud, J.-P. Delville, F. m. c. Gallaire, R. Wunenburger, Thermocapillary valve for droplet production and sorting, Phys. Rev. E 75 (2007) 046302.

[38] B.-C. Lin, Y.-C. Su, On-demand liquid-in-liquid droplet metering and fusion utilizing pneumatically actuated membrane valves, Journal of Micromechanics and Microengineering 18 (11) (2008) 115005.

[39] F. Malloggi, H. Gu, A. G. Banpurkar, S. A. Vanapalli, F. Mugele, Electrowetting –a versatile tool for controlling microdrop generation, The European Physical Journal E 26 (1) (2008) 91–96.

[40] H. Gu, F. Malloggi, S. A. Vanapalli, F. Mugele, Electrowetting-enhanced microfluidic device for drop generation, Applied Physics Letters 93 (18) (2008) 183507.

[41] S. Jakiela, T. Kaminski, O. Cybulski, D. B Weibel, P. Garstecki, Bacterial growth and adaptation in microdroplet chemostats, Angewandte Chemie (International ed. in English) 52 (2013) 8908–8911.

[42] K. Churski, M. Nowacki, P. M. Korczyk, P. Garstecki, Simple modular systems for generation of droplets on demand, Lab Chip 13 (2013) 3689–3697.

[43] P. Garstecki, M. J. Fuerstman, H. A. Stone, G. M. Whitesides, Formation of droplets and bubbles in a microfluidic T-junction-scaling and mechanism of break-up, Lab Chip 6 (2006) 437–446.

[44] M. Tokeshi, Microfluidic devices for drug delivery systems, Advanced Drug Delivery Reviews 128 (2018) 1 – 2, microfluidic Devices for Drug Delivery Systems.

[45] N. Khalid, I. Kobayashi, M. Nakajima, Recent lab-on-chip developments for novel drug discovery, Wiley Interdisciplinary Reviews: Systems Biology and Medicine 9 (2017) e1381.

[46] D. Li, Microfluidic lab-on-a-chip devices for biomedical applications, in: S. Kakaç, B. Kosoy, D. Li, A. Pramuanjaroenkij (Eds.), Microfluidics Based Microsystems, Springer Netherlands, Dordrecht, 2010, pp. 377–397.

[47] H. Bridle, K. Jacobsson, A. C. Schultz, Sample processing, in: H. Bridle (Ed.), Waterborne Pathogens, Academic Press, Amsterdam, 2014, Ch. 4, pp. 67 – 114.

[48] H. Bridle, Optical detection technologies for waterborne pathogens, in: H. Bridle (Ed.), Waterborne Pathogens, Academic Press, Amsterdam, 2014, Ch. 5, pp. 115 – 118.

[49] J. W. Costerton, P. S. Stewart, E. P. Greenberg, Bacterial biofilms: A common cause of persistent infections, Science 284 (5418) (1999) 1318–1322.

[50] S. B. Levy, The challenge of antibiotic resistance, Scientific American 278 (3) (1998) 46–53.

[51] O. J. Miller, A. E. Harrak, T. Mangeat, J.-C. Baret, L. Frenz, B. E. Debs, E. Mayot, M. L. Samuels, E. K. Rooney, P. Dieu, M. Galvan, D. R. Link, A. D. Griffiths, High-resolution dose–response screening using droplet-based microfluidics, Proceedings of the National Academy of Sciences 109 (2) (2012) 378–383.

[52] G. Arrabito, C. Galati, S. Castellano, B. Pignataro, Luminometric sub-nanoliter droplet-to-droplet array (lumda) and its application to drug screening by phase i metabolism enzymes, Lab Chip 13 (2013) 68–72.

[53] C. H. Choi, J. S. Lee, Y. C. Lee, T. I. Park, J. C. Lee, Acinetobacter baumannii invades epithelial cells and outer membrane protein a mediates interactions with epithelial cells, BMC Microbiology 8 (1) (2008) 216.

[54] W. Li, Y. Guo, H. Wang, D. Shi, C. Liang, Z. Ye, F. Qing, J. Gong, Electrospun nanofibers immobilized with collagen for neural stem cells culture, Journal of Materials Science: Materials in Medicine 19 (2) (2008) 847–854.

[55] P. Cameron, B. Gaiser, B. Bhandari, P. Bartley, F. Katzer, H. Bridle, Silver nanoparticles decrease the viability of cryptosporidium parvum oocysts, Applied and Environmental Microbiology 82 (2) (2016) 431–437.

[56] S. Chouikhi, I. E. Korbi, Y. Ghamri-Doudane, L. A. Saidane, A survey on fault tolerance in small and large scale wireless sensor networks, Computer Communications 69 (2015) 22 – 37.

[57] L. Galluccio, A. Lombardo, G. Morabito, S. Palazzo, C. Panarello, G. Schembra, Capacity of a binary droplet-based microfluidic channel with memory and anticipation for flow-induced molecular communications, IEEE Trans. Commun. 66 (1) (2018) 194–208.