High-throughput automated droplet microfluidic system for screening of reaction conditions†

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We demonstrate a new droplet on demand (DOD) technique and an integrated system for scanning of arbitrary combinations of 3 miscible solutions in ~1.5 μL droplets at 3 Hz. The DOD system uses standard electromagnetic valves that are external to the microfluidic chip. This feature makes up for modularity, simplicity of assembly and compatibility with virtually any microfluidic chip and yields an on-chip footprint of less than 1 mm². A novel protocol for formation of DOD enables generation of an arbitrarily large range of volumes of droplets at a maximum operational frequency of ~30 Hz. The integrated system that we demonstrate can be used to scan up to 10 000 conditions of chemical and biochemical reactions per hour using ~10 mL of solutions in total.

The current standard in high throughput screening (HTS) is set by the microtiter technology that provides a minimum reaction volume of ~2 μL and a minimum filling time of few seconds per well. From the very advent[1] of the ability to form droplets in microfluidic channels[2,3] the vision[4,5] emerged for the droplets to serve as reaction beakers. This technology offers several attractive features:[6] (i) lack of dispersion of time of residence, (ii) fast mixing, (iii) control over kinetics of reactions, (iv) improved statistics through repeated experiments, and (v) minute consumption of reagents. These characteristics have the potential of forming a basis for technology competitive with the microtiter platform.

To date, the true potential[4,7] of droplet microfluidics is largely unexplored, as it requires flexibility to perform arbitrary protocols of compositions of the reaction mixtures. Such integrated systems must allow for *e.g.* samples to be drawn from multiple sources, mixed, reacted and analyzed. These features are critical[8] for wide spread adaptation of microfluidic devices in *e.g.* drug discovery. A necessity in realization of this vision is to interface computer control. The most vital module in automation of droplet microfluidics is one for formation of droplets *on demand*: at a prescribed time of emission and with prescribed volume. Reported microfluidic DOD systems are few and most focus on integrated microvalves for the control of flow of the fluid to be dispersed. One common solution is to utilize an interfacial pressure blockade that can be actively overcome by lowering interfacial tension either upon heating[9] or application of an acoustic pulse.[10,11] An alternative is to use pneumatic microvalves. Lin and Su[12] and Zeng *et al.*[13] have recently reported DOD systems based on valves fabricated in polydimethylosiloxane (PDMS) on chip[14] or at the inlet port.[15] Other mechanisms of active control of fluids (*e.g.* electrowetting[16,17]) are also used.

Although elegant, the use of integrated microvalves presents difficulties: (i) assembly of valves requires multilevel fabrication and (ii) choice of materials amenable to such fabrication may introduce limitations in chemical compatibility (especially in the case of using PDMS[18]). In addition, integrated valves, besides their own footprint also need connections with either electrodes or pneumatic control channels, which all occupy space and complicate the design.

Here, we present a fully functional DOD system that utilizes *external* valves interconnected with the chip only *via* a small-diameter steel tubing. The technique is simple in that (i) it uses off-the-shelf valves, (ii) does not require complicated microfabrication and (iii) can be interfaced with any microfluidic chip and (iv) its on-chip footprint is as small as the inlet port. A similar approach (although a different technical solution) has been used by Vanapalli *et al.*[19] and Cybulski and Garstecki;[20] these authors used tapping or squeezing of the tubing that delivered the droplet phase to the chip to control the volume and time of emission of droplets.

Our system is based on a standard plunger-type solenoid valve (V165, Sirai, Italy) which defaults to a closed position. We first used the valve *as-is* on the tubing that supplied the aqueous phase from a pressurized (p_{water}) container to a T-junction chip fabricated in polycarbonate (PC). The continuous fluid (2% w/w solution of Span 80 (Sigma) in hexadecane (Sigma)) was fed from a syringe pump (Harvard Apparatus PHD2000). This setup did not provide for adequate control of formation of droplets: the flow was too large to set p_{water} to a low enough and stable value. In order to solve this problem we (i) inserted a steel capillary (O.D. 400 μm, I.D. 205 μm, length = 20 cm, Mifam, Poland) of a large hydraulic resistance (2.95×10^{12} kg m^{-4} s^{-1}) into the central cone of the valving chamber and filled the space between the capillary and the body of the valve with epoxy glue (Poxipol, Akapol S.A.) and (ii) glued an HDPE foil (thickness 200 μm, Folmad, Poland) on the upper surface of the core to reduce the stroke from ~250 to ~50 μm.

These modifications reduced the flow through the valve, lessened the buildup of pressure downstream of it, and provided control of the volume of droplets (V_{drop}) with p_{water}, and with the length of the interval (t_{open}) during which the valve was open. The frequency of this DOD system is limited to ~30 Hz by the responsiveness of the electro-magneto-mechanical construction of the valve (the delay between application of the current and opening of the valve is 24 ms, and 8 ms between stopping the current and shutting the valve). A dead volume of the modified valve is 70 nL, which sets the lower limit (V_{min}) on V_{drop}.

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Another limitation in formation of DOD comes from the mechanism of formation of droplets and bubbles in T-junction or flow-focusing geometries.[21,22] Once the growing droplet obstructs the cross-section of the main channel, pressure builds up and squeezes the droplet off within a time $O(Q_c/V_{junction})$, where Q_c is the rate of flow of the continuous phase, and $V_{junction}$ is the volume of the junction.[21] This limits the maximum volume of a droplet that can be formed for given Q_c, to a range of (1 to ~3)V_{min}. In order to circumvent this constraint we added an electrovalve on the line that supplied the continuous phase and applied a protocol in which the flow of oil is normally open, and flow of water is normally closed (Fig. 1a). Then, during the interval (t_{open}) of formation of the droplet, water is turned on, while oil is stopped. After t_{open}, the system defaults to oil open, water closed: droplet is broken off and flushed downstream. This protocol enables formation of droplets of arbitrarily large volumes. Fig. 1b illustrates ~15 fold increase of V_{drop} within the range of $t_{open} \in$ (25, 300) ms. Our system produces exactly the same volume of the droplets in PC and PDMS chips (Fig. 1b), and a perfectly linear relation between t_{open} and V_{drop}. This allows for independent control of the volumes of the droplets, intervals between them, and the speed of flow.

While merging of droplets has been proposed for *e.g.* synthesis of nanoparticles[23] or alginate beads,[24] or generally formation of reaction mixtures,[25] the ability to time emission of droplets of well controlled volumes over a wide range (*e.g.* 1 to 10 V_{min}) allows for construction of a system for high-throughput *screening* of chemical compositions by joining a number of calibrated doses of liquids of distinct chemical content. Fig. 2a shows a schematic representation of a system that integrates three T-junctions (of square cross-section of the channels 400 × 400 μm), each supplied with a different aqueous solution (of red and blue Parker ink, and clean water) and oil. Each of these junctions is controlled with two valves and executes a precoded

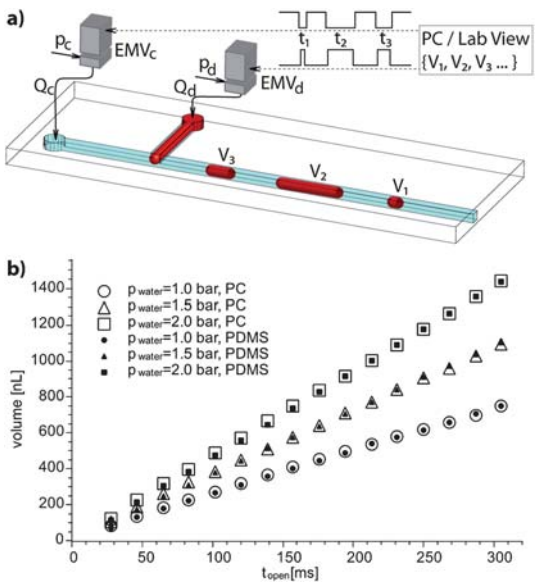

Fig. 1 (a) A schematic illustration of the DOD system with two electromagnetic valves controlling the flow of the continuous fluid (drawn from a reservoir maintained at a pressure p_c) and the droplet phase (p_d). (b) Volumes of the droplets generated by this system connected to polycarbonate (PC) or polydimethylsiloxane (PDMS) chips. The cross-section of the channels was 400 μm × 400 μm. Droplets generated at 3 Hz, $p_{oil} = 0.4$.

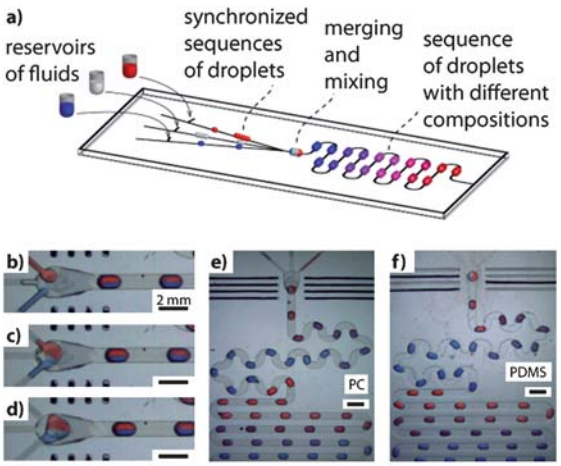

Fig. 2 (a) A schematic illustration of the integrated system for high-throughput scanning of reaction conditions. Three consecutive micrographs (b) through (d) recorded at 125 fps, show the process of merging of droplets into the reaction mixtures. Insets (e) and (f) illustrate sequences of concentrations formed in chips fabricated in PC and PDMS.

protocol of emission of droplets. The droplets are formed synchronously at the three junctions and flow down to a common chamber where they merge. The coalescence is stimulated by an alternating electrostatic potential (~1 kHz, ~500 V) applied across the merging chamber. Micrographs in Fig. 2b–d illustrate the process of merging and coalescence, and show two arrays of electrodes that end in proximity of the merging chamber (with a distance of ~2 mm between the opposite electrodes). After having merged, the droplets flow through a section of sinusoidally meandring channels (of cross-section of 1 × 1 mm) to mix and then through a series of parallel channels for facile visualization of the sequence of colors (Fig. 2e, f).

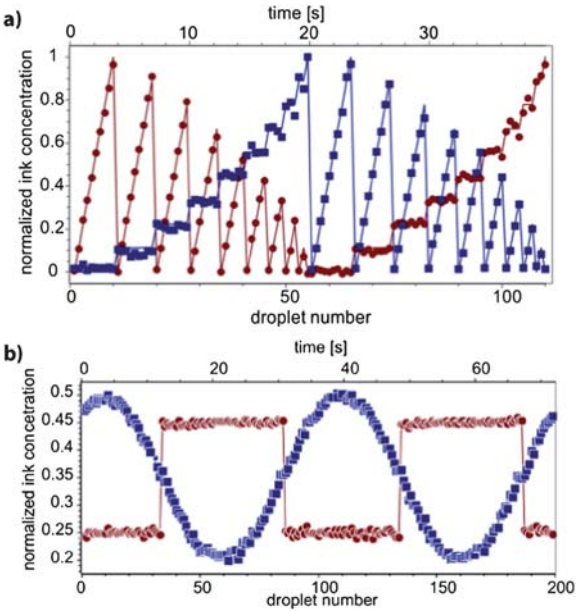

Fig. 3 The graphs show two sequences of concentrations of the reaction mixtures formed in the integrated systems (lines show expected concentrations, while symbols correspond to the values measured experimentally).

We used the integrated system for formation of sequences of packets of droplets drawn from the three different reservoirs. The droplets have volumes $V_{i,j}$, where i indexes the liquid (*e.g.* $i = 1$ for solution of the red ink, $i = 2$ for water, and $i = 3$ for blue ink) while j corresponds to the number of the packet, or—alternatively—to the number of the merged droplet. In the first demonstration, we programmed the sequence in such a way as to scan all the possible combinations of concentrations of the two inks, each concentration ranging from 0 to 100% in 9 steps. The volume of the droplets with clean water was adjusted for each packet to fill-up the final droplet to the same volume of 1.5 μL. The whole sequence comprises two scans of the combinations of concentrations of the two inks in 110 droplets and is executed within 41 s. Fig. 3a shows the predefined (expected) concentrations of inks together with those extracted from the experiment *via* analysis of the images of consecutive droplets and deconvolution of the RGB signal into the concentrations of the inks (see ESI for details†).

The same system is capable of producing smooth (with a resolution of ~250 levels in the example) variations and step changes of the concentration. Moreover, it is possible to produce these changes on two different components (here blue and red ink) independently and simultaneously (Fig. 3b).

In summary, we demonstrated a fully operational microfluidic DOD system that uses external valves. This system offers (i) small on-chip footprint, (ii) compatibility with chips fabricated both in elastic and stiff materials, (iii) wide range of volumes of droplets. An integrated system with three DOD lines produces arbitrary scans (at a rate of 3 Hz) of concentrations of two solutions with the third filling up the 'reaction' droplets to a constant volume of ~1.5 μL. This technique, as is, offers similarly small reaction volumes as microtiter robotics, and about 10 times faster creation of reaction mixtures, allowing for ~10^4 reactions to be tested within one hour. Improvements on the valving technology can further reduce the reaction volumes and further increase the operational speed. This system may form a basis for construction of automated chips for high-throughput screens in various areas of chemistry and biochemistry. For example, synthesis of nanoparticles often depends on many factors and could be optimized *via* a high-throughput screen. Similarly, in microbiology, the interactions between toxins are of great interest and demand technology for high throughput screening.

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