ON DEMAND GENERATION OF DROP AND BUBBLE IN A MICROFLUIDIC CHIP

Jie Xu and Daniel Attinger, Columbia University, New York

Abstract

We introduce a novel technique, which consists in dispensing individual picoliter to nanoliter drops or bubbles on demand directly in the liquid-filled channels of a polymer microfluidic chip. The technique involves a PDMS chip, which is fabricated using photolithography, with one or several microliter-size chambers driven by piezoelectric actuators. Individual particles are dispensed at kHz frequencies from a chamber to a main transport channel filled with an immiscible fluid, in a process analogous to atmospheric drop on demand dispensing. The particle formation process is characterized with respect to critical dispense parameters such as the shape and duration of the driving pulse, and the size of both the fluid chamber and the nozzle. Several features of this technique with direct relevance to lab-on-a-chip applications are presented and discussed, such as the rapid mixing of reagents or the encapsulation of a biological cell. Finally, we show that the same piezoelectric technique can be used to generate a single gas bubble on demand in a microfluidic chip.

Introduction

The concept of lab on a chip, where a tiny fluid microprocessor performs complex analysis and synthesis tasks relevant to chemistry or biology, has been a subject of academic interest since the early 1990's [1, 2], and there are encouraging signs that industrial lab on a chip applications are growing, exemplified by microfluidic devices for DNA sequencing [3]. The operations needed for biological or chemical analysis are reagent dosing, transport, mixing, splitting, flushing, filtering, analysis, detection and monitoring [4], each operation requiring a precise control in space and time [5]. The microfluidics components needed do perform these operations are buffers, channels, valves, mixers, microheaters and sensors [4-6]. Several achievements, mostly in academia, have demonstrated that shrinking a chemical or biological laboratory into a microchip could have significant benefits such as increased sensitivity, fast response time, low reagent and sample consumptions, as reviewed in [2, 6-8]. The ability to dispense and control small liquid volumes in the microchannels is critical for the lab on a chip technology, and several techniques address, at least partially, this issue. For instance, the segmented flow technique, defined as the transport of two or more immiscible phases in the form of successive plugs in a microchannel, has been developed: it uses syringe pumps feeding two branches of a T-connection [5, 9, 10] or two concentric channels [11, 12]. In the T-connection process, drops or bubbles of the so-called *dispersed* phase are produced in the other liquid phase, the *continuous* phase, as a result of shear forces and interfacial tension at the fluid-fluid interface [13, 14]. The process using two concentric tubes, called *flow focusing*, injects the disperse phase from the smaller tube into the continuous phase that flows out of the larger tube, and breakup occurs in an analogous manner as the breakup of an atmospheric jet in atmosphere, which is due to the Plateau-Rayleigh-Savart instability

[15-17]. The segmented flow technique decreases mixing times by several orders of magnitude [5, 13], while ensuring an extremely low diffusion between the two immiscible phases [5, 18]. These two unique features are very attractive for studying and controlling the timing of chemical reactions [18].

The drawbacks of segmented flow techniques are that a setup might need a relatively long accommodation time before generating the train of particles (drops or bubbles) in a stable manner [19], the difficulty to reliably generate a single particle rather than a train of particles, and the fact that segmented flow techniques are not as flexible as digital microfluidics for processing multistep reactions [20]. There is therefore a need for a technique that can generate a single particle, on demand, in an immiscible fluid. This need is acknowledged by George Whitesides, who fathered soft microfluidics: "There is a particular bit of the puzzle that needs to be added, which will not be hard to do but it has not been done yet—that is, bubble on demand [21]". Recently, high-performance syringe pumps (microinjectors) [22, 23] or a high-voltage pulse have been used to induce the formation a single drop of liquid at a microfluidic T-junction [24, 25], or the thermal expansion of a gas meniscus that breaks into a single bubble when sheared by a perpendicular flow [26]. These techniques however rely on complex external actuation [22, 23, 26], with a timing and volumes accuracy that are either low [22, 24] or not quantified [23, 26]. In this paper, we present a novel technique to dispense a single drop or bubble on demand in a microfluidic chip by ways of piezoelectric actuation.

Design, fabrication and setup description

A typical design of our PDMS microfluidic chip is shown in Figure 1a: it involves one or several µL-volume reagent chambers such as *B* connected via a 25-100 µm nozzle to a main channel *A*, which is filled with immiscible oil such as hexadecane. The height of the channels is in the 50-100 µm range. The chip is sealed with a 180µm-thick PDMS membrane. A piezoelectric bimorph actuator taped on top of each chamber, modifies the chamber volume, and releases a drop (or bubble) on demand in the main channel as shown in Figure 1b.
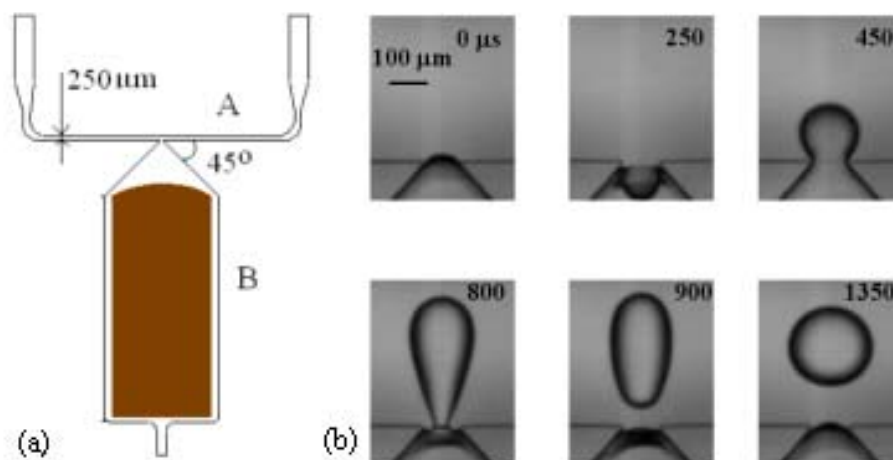

Figure 1: (a) Typical Geometry of a microfluidic chip for *drop on demand* dispensing. A piezoelectric bimorph actuator glued to the chamber allows the release of an aqueous drop on demand in the horizontal channel filled with an immiscible fluid. (b) Sequence of the dispensing of a 1nL drop.

The microfluidic chips are fabricated in the clean room of Columbia University using soft lithography [27]. First, a 10 μm thin base layer of *SU-8* resin (MicroChem) is spun and cured on a silicon wafer. On top of that layer, a 50-100 μm layer of *SU-8 2050* is cured with patterns transferred from a mask (CAD/Art Services Inc.). This base layer method presented in Carlier et al. [28] improves adhesion of *SU-8* to the wafer. The chip is then manufactured from the master using PDMS Sylgard 184 Kit (Dow Corning). The channels are sealed by a thin 180 μm membrane made from spin-coated PDMS. The piezoelectric actuators are commercially available bimorph actuators made of two PZT layers bonded on a thin brass layer, with a total thickness T 0.5 mm, with lengths and widths slightly smaller than the chamber dimensions as shown in Figure 1a. One actuator is then taped on top of each chamber, using a 90 μm layer of double-sided tape.

The experimental setup involves three subsystems: the microfluidic system, the actuation system and the sensing system. The microfluidic system involves the microfluidic chip described above. Syringes fill the main channel with hexadecane and control the subsequent injection of aqueous plugs in the dispensing chamber. The actuation system uses a 20MHz function generator (Agilent, 33120A) coupled to a 1MHz 17W amplifier (Krohn-Hite, 7600M), which generates high-voltage driving pulses for the actuators glued on the microfluidic chip. The sensing system is a high-speed high-resolution imaging system involving an Olympus IX-71 microscope and a high-speed camera (Redlake MotionXtra HG-100K, up to 100,000 frames per second).

Characterization

Characterization experiments reported in Figure 2a describe how the drop volume is influenced by the nozzle size, the pulse shape and the pulse duration.
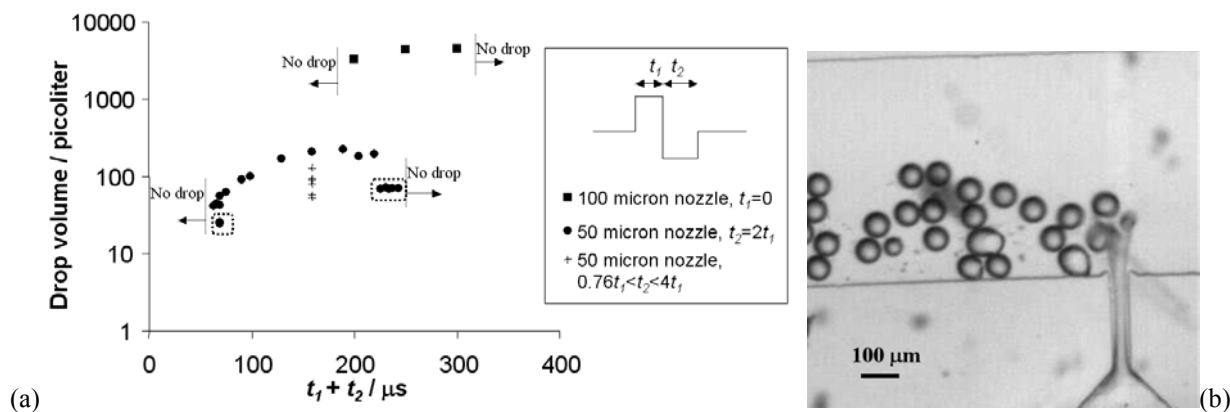

Figure 2: (a) Drop volumes vs. dispense parameters. The horizontal axis shows the total pulse length, involving the chamber expansion t_1 followed by compression t_2. The dotted zones show *doublet* dispenses, where two drops of smaller volume are generated simultaneously by a single pulse. (b) Dispense of individual drops by repeating the pulse at a rate of 2.5 kHz.

All the data in Figure 2a were obtained with an actuation voltage of +/- 200V. The chamber lengths used for the respective 50 and 100 μm nozzle case were 12mm and 20mm, respectively. Note that the chamber volume is about 10 nL, which is about 10^5 bigger than a 100 pL volume drop, so that a pre-filled chamber can generate a large amount of drops of interest before refilling. After each dispense, we observed that the meniscus typically comes to the initial location within a few milliseconds. The shape of the pulse corresponds to an initial expansion of the chamber for a time t_1

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followed by compression for a time t_2. Pulses with $t_1=0$ were also successful at generating a drop: they correspond to simple compression of the chamber. A quick look at the y-axis in Figure 2a shows that drops with volumes from 25 pL to 4.5 nL can be generated by varying the pulse shape and the nozzle size (corresponding to the channel height): this is a remarkable range, larger than two orders of magnitude. For a given nozzle geometry, Figure 2a also shows that the drop volume can be controlled smoothly by the pulse shape within one order of magnitude: for instance the 50 μm nozzle produces drops in the 40-300 pL range. Pulses with durations too different from an optimum duration will not produce any drop, as shown by the arrows in Figure 2a. The reason might be that surface tension forces are strong enough to pull back the meniscus in the case of a short pulse or that a given pulse duration is needed to generate and amplify an unsteady pressure wave in the chamber [29, 30]. For the 50 μm nozzle we observe some dual-dispense states for pulses close to the states where no drop is ejected: a dual-dispense state correspond to a case where two smaller drops are simultaneously produced, by the *doublet instability* process described in next section and shown in Figure 3c. Also, in the same plot, crosses demonstrate how the drop volume can be controlled by changing the ratio between the expansion time and the compression time, while keeping the total actuation time constant.

We also tried to quantify the maximum dispense rate by repeating the driving pulse with smaller and smaller time interval between pulses. Experiment shows that drops are still generated even if the time interval is reduced to 0s, which corresponds to applying the generation pulse continuously. Figure 2b shows a case where a 400 μs pulse, shaped as in Figure 2a with $t_2 = 2t_1$, is applied continuously and in turn generates a train of drops at 2.5 kHz. Note that, the nozzle is a 200 μm long and 70 μm wide straight channel. In addition, we also studied the uniformity of the drop volumes with the same nozzle. At a dispense rate of 6.2 Hz, 20 drops generated had an average volume of 1023 pL and a standard deviation of 16 pL, which corresponds to less than 2%.

Features and relevance to lab on a chip

The process described in this paper implements in a microfluidic chip a drop on demand technique with precise, reliable control of the drop volume and generating timing. The *in-chip drop on demand* technique has the potential to perform in-chip reagent mixing, transport and multistep reactions. Four features of the *in-chip drop on demand* technique with direct relevance to lab on a chip applications are presented in Figure 3, and are discussed in this section.

The potential of mixing different reagents into a single drop is illustrated in Figure 3a. The nozzle on the left generates a drop of ink, while a pure water drop is generated by the right nozzle simultaneously and hits the ink drop. Coalescence occurs then at $t=22$ms, starting the mixing of the ink and water through diffusion and the transient flow associated with coalescence and the drop transport along the channel. Interestingly, coalescence does not occur right after the drops hit each other, probably because of the thinning and breakup of an oil film between the two drops. This delay could be reduced by oppositely charging the two drops [31]. It is also worth mentioning that mixing and particle transport can be controlled by vibrating the liquid [32] with the same piezoelectric actuators that generate the drops. To some extent, the mixing process presented here can be compared to the impressive *airborne chemistry* technique, where a drop is immobilized in the air at the node of a high-power ultrasound field: this main drop acts then as an isolated reactor fed by smaller drops of reagent dispensed by atmospheric drop on demand nozzles. Airborne chemistry has been successful for screening the conditions for protein crystallization or for performing biological analyses [33-35]. Similarly, *in-chip drop on demand* also allows the drop dispensed in an immiscible fluid to function as an isolated reactor, fed by further reagent additions from neighboring nozzles. Major differences with

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the airborne chemistry technique are that optical measurements might be more difficult with the present technique, due to the presence of the hexadecane and the PDMS wall; however the surrounding hexadecane allows higher heat transfer and suppresses evaporation.
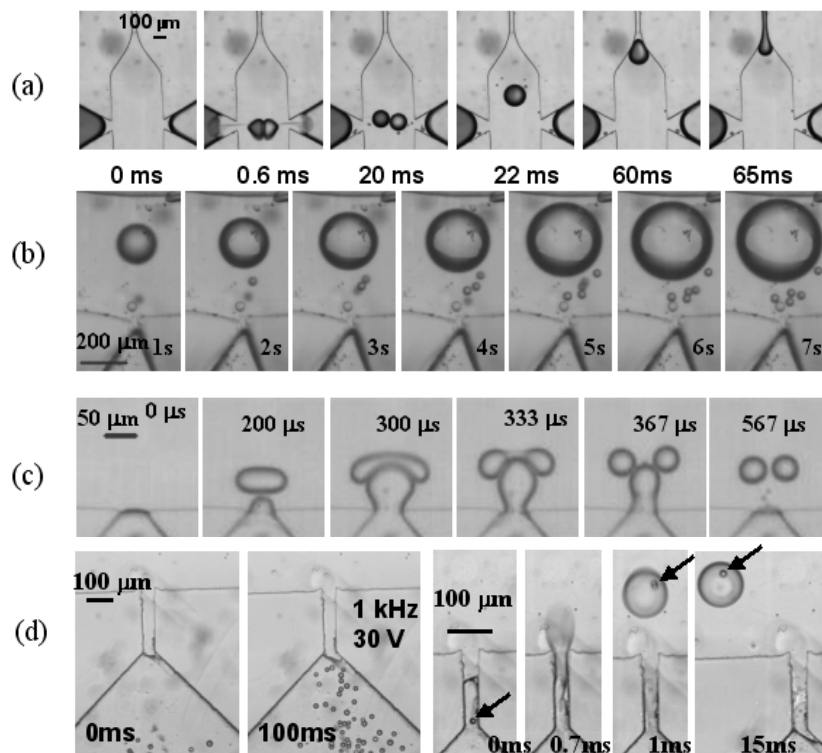

Figure 3: Feature of the drop on demand dispensing relevant to lab on a chip applications: (a) merging and mixing two drops of different reagents (b) digital control of drop volume (c) *doublet* dispense, where two drops are generated simultaneously by a single pulse (d) particle encapsulation in a single drop.

Figure 3b shows the second feature, which is the ability to digitally control the dispensed drop volume, by generating additional drops that coalesce with the original drop. The first frame shows a 500 pL drop, the volume of which increases to 3.5 nL by 6 successive increments of 500 pL. This coarse, digital way to control the drop volume can be coupled with the finer, analog volume control of modifying the pulse parameters, in order to exactly dispense the desired quantities over a wide range of volumes. While in-flight coalescence has been realized [31, 32] by *atmospheric* drop on demand, where drops are jetted in the air, the *in-chip drop on demand* technique allows a simpler realization of coalescence because the dispensed drop becomes immobile in the main channel after the kinetic energy of the dispense has been dissipated. The attentive reader probably noticed that the channel walls in Figure 3b are irregular: indeed these nozzles were manufactured during preliminary experiments where the master was simply a piece of electric tape applied to a glass slide and approximately cut to the desired geometry with a sharp cutter under the stereo microscope. Finally, it is worth mentioning that the large drops created in Figure 3b can be split into smaller drops by moving them into a T-shaped connection [33].

Figure 3c shows the third feature, which is the ability to generate a *doublet* of drops, while applying a single excitation pulse to the actuator. This occurs when an initially generated drop is hit by a strong subsequent excursion of the meniscus. During the process, the meniscus breaks the initial drop

into two half drops while briefly assuming the shape of a well-known cartoon character (367 μs). We call this type of dispense the *doublet* dispense. To the best of our knowledge, no *doublet* dispense has ever been realized with atmospheric drop on demand techniques because the dispensed drop quickly travels away from the nozzle area where the meniscus oscillates.

Figure 3d shows the fourth feature: the ability to encapsulate inside a single water drop a solid particle having the size of a biological cell. First, low amplitude vibrations of the actuator bring the particles close to the meniscus, before a stronger pulse generates a single drop encapsulating the particle. This encapsulation technique is well suited to handle and analyze single biological cells.
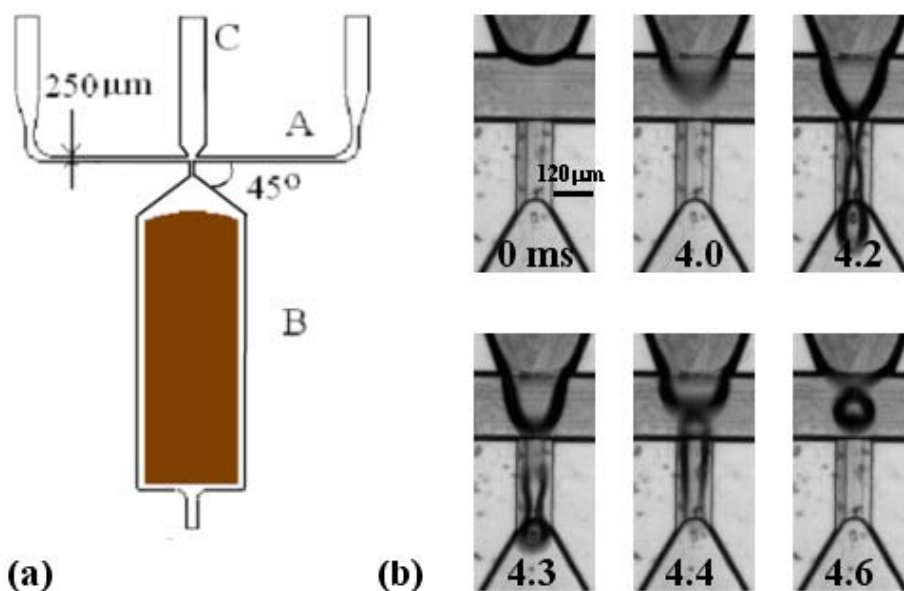

Figure 4: (a) Typical geometry of a microfluidic chip for dispensing a single bubble on demand. (b) Sequence of the dispensing of a 6.2 nL bubble.

In addition to dispensing drops, the piezoelectric actuation technique described here can be used to dispense a single bubble on demand in the main channel as shown in Figure 4. The bottom chamber and horizontal channel are filled with water, while the top channel is filled with air. Piezoelectric actuation of the bottom channel is used to modify the volume of the water chamber and pull the air out, which breaks up into a single bubble inside the nozzle.

Conclusions and outlook

The piezoelectric dispensing technique presented in this article generates individual drops and bubbles in a microfluidic chip with a temporal precision of one millisecond. The drop size can be precisely controlled from 40 pL to 4.5 nL. The technique allows drop merging and single cell encapsulation. The ability to precisely trigger the drop generation time will allow the coordination of the generation of drops with events occurring in the chip, such as the detection of chemical reaction or temperature changes, or the transit of biological cells and other particles.

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