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Low-Cost Experimentation for the Study of Droplet Microfluidics

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Abstract

The continued growth of microfluidics into industry settings in areas such as point-of-care diagnostics and targeted therapeutics necessitates a workforce trained in microfluidic technologies and experimental methods. Laboratory courses for students at the university and high school levels will require cost-effective in-class demonstrations that instruct in chip design, fabrication, and experimentation at the microscale. We present a hand-operated pressure pumping system to form monodisperse picoliter to nanoliter droplet streams at low cost, and a series of exercises aimed at instructing in the specific art of droplet formation. Using this setup, the student is able to generate and observe the modes of droplet formation in flow-focusing devices, and the effect of device dimensions on the characteristics of formed droplets. Lastly, at ultra-low cost we demonstrate large plug formation in a T-junction using coffee stirrers as a master mold substitute. Our method reduces the cost of experimentation to enable intuitive instruction in droplet formation, with additional implications for creating droplets in the field or at point-of-care.

Keywords

Droplets; Education; Flow-focusing; Low-cost; Microfluidics

1 Introduction

In just over two decades of academic research, microfluidics has emerged as a powerful technology to manipulate fluids on the microscopic level. Evolving from technologies of the integrated circuits industry, microfluidic chips (also known as lab on a chip) have found applications in a broad array of fields, from point-of-care diagnostics to targeted therapeutics, to biological and chemical assays.[1–3] As commercial prospects mature, transitions of microfluidic methods from academia into industry settings will become more and more frequent[4], creating a need for highly-skilled workers trained in microfluidic processes and technologies, such as chip design, fabrication (e.g. soft lithography[5]), and experimentation at the microscale where viscous forces tend to dominate. Looking forward, student exposure to microfluidics at an early stage will be instrumental to the field's continued growth, in order to promote the utilization of microfluidics[6] both as a tool to simplify existing experiments and as an enabling method to drive new research and

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products[7]. Additionally, making microfluidic training available to students at younger ages will better prepare them for new careers in biotechnology, biomedicine and the life sciences, and will broaden awareness of this growing field.

In recent years a number of approaches have emerged to introduce microfluidics at the university or high-school levels in science, technology, engineering and mathematics (STEM) fields.[6–10] Yang *et al.*[8] for instance developed a method to teach microfluidics to young students and the general public using Jell-O as a casting material, producing three types of molds to model pressure-driven flow, a Y-channel, and a pH sensor. A great need for educational material to train in general microfluidics still exists, yet experiments designed for teaching microfluidics are few[10]. One particularly evident deficiency is a simple platform for instructing in the art of *droplet-based* microfluidics, referring to a subset of the field that involves the combining of two or more immiscible fluids to form discrete volumes of the dispersed phase within the continuous.[1,3] Using droplet microfluidics enables the researcher to create emulsions and microbubbles with high uniformity in size and content, and potentially complex structure, thereby expanding the usefulness of many droplet applications over traditional methods.[1] This article provides low cost methods to create droplets that will help students and trainees understand the basic principles of droplet formation in microchannels.

Few efforts have focused so clearly on training in droplet microfluidics. Najah *et al.*[11] did use a droplet-based microfluidic device to teach digital analysis, but at the cost of ~ 10,000 per workstation (consisting of an inverted microscope connected to a CCD camera and a set of two syringe pumps to inject fluids). Although students were asked to characterize the microfluidic device over a range of flow rates, hands-on experience with the various mechanisms of droplet formation and tuning of droplet size and generation rate were beyond the scope of the experiment, and hence instruction on the fundamental principles of droplets was limited. Other groups have notably expressed similar concerns that complex means of fabrication[10] and high cost of experimentation (e.g. Greener *et al.*[6] estimated a 50% increase in cost relative to a traditional system, in majority due to the price of syringe pumps) may limit the adaptation of microfluidics to the teaching laboratory.

We thus present a low-cost, simple method to form monodisperse droplets in a flow-focusing microfluidic chip, as overviewed in Figure 1, aimed at educators interested in the field of droplet-based microfluidics. The method utilizes handheld syringes to drive a homemade pressure pumping system in lieu of syringe pumps, affording control of droplet size and generation rate to the hands of the student. By varying the overall and relative pressures of the continuous and dispersed phases via hand inputs, we demonstrate the ability to modulate the mechanism of droplet formation from geometry-controlled mode to dripping to jetting.[12] Droplet volumes ranged from ~ 100 pL in geometry-controlled mode at rates of tens of Hz, to ~ 1 pL in dripping at rates of hundreds to over one thousand Hz. Alternatively, we narrow the channels and orifice of the flow-focusing region to generate small droplets. Students will be able to gain experience in chip design and PDMS fabrication, and learn fundamentals of droplet generation including the role of the capillary number on modes of instability leading to droplet breakup. Finally, we form large plugs in a T-junction molded

from coffee stirrers for use in classroom or at-home demonstrations to reach a less specialized or younger audience.

2 Experimental Overview

2.1 Learning Objectives and Basic Lessons

Table I summarizes the intent and learning objectives for each exercise, and provides sample post-activity questions to assess the students. To best demonstrate the pumping system's design, operation, and flexibility in forming monodisperse droplet streams, we describe the most advanced exercise first (Exercise I for graduate and advanced undergraduate students). Exercises II and III use the same hand-controlled pressure pumps but are intended to appeal more to entry-level students. Beginners to the field may wish to proceed directly to Section 6 after this Experimental Overview to foster understanding of the more basic material.

The educational exercises presented here demonstrate key subsets of the principles of droplet generation for applications in droplet-based microfluidics. These applications of uniform, picoliter to nanoliter size droplets range from drug delivery to point-of-care diagnostic chips, to organic synthesis and microreactors[1]; further, droplets of picoliter volumes as demonstrated here could be useful to provide compartmentalized volumes for the study of individual cells[3] and molecules[13]. Concepts to be introduced to the student in a session prior to the experiment or demonstration should cover device properties (e.g. dimensions and surface properties of the channel) and fabrication, and the various methods and principles of droplet formation. Special attention should be given to the effects of low Reynolds number flow in a microfluidic channel (typically well below 100[3]), where viscous forces dominate over inertial forces and laminar flow results.

High school teachers may especially wish to tailor the lecture with more basic concepts, as follows, to help students understand. A small volume of liquid held together by surface tension, a droplet forms when liquid accumulates at the end of a tube or other similar interface and is broken off when a pulling or shearing force exceeds the cohesive force of the liquid. For instance, in a leaking faucet the drop of liquid is suspended from the end of the tube by surface tension. As liquid accumulates, the force of gravity pulling on the droplet eventually exceeds the cohesive attraction holding the liquid together, and the drop breaks off from the main thread. Droplet formation in a microfluidic device differs slightly in that the continuous phase acts as the shearing force on the dispersed phase at the liquid interface; the drop forms as the dispersed phase accumulates until the shearing force exceeds the affinity of the dispersed phase for itself. Surfactants lower the interfacial tension between the two liquids to aid the process of droplet breakup, and thus are used in many droplet-based systems. Unlike in a faucet, where water forms a boundary with surrounding air, the interface in a droplet generator exists between two or more immiscible liquids, and so the surface properties of the microfluidic channel are essential in determining which liquid disperses in the other. Devices are fabricated such that the solid channel surface attracts the continuous phase to wet while repelling the dispersed phase to allow its breakup and travel as a discrete volume. For the oil droplets in Exercise I, the surface must be hydrophilic to support wetting of the aqueous phase and dispersion of the oil. On the other hand, the

hydrophobic surfaces of the devices in Exercises II and III support wetting of the oil phase and the breakup of water droplets.

2.2 Materials and Methods

Exercise I aims to present hand-on instruction to graduate and advanced undergraduate students in the complex dynamics of droplet formation. To demonstrate such dynamics we have chosen a flow-focusing droplet generator with expanding nozzle geometry.[14] Distribution channels measuring 38 μm in width direct continuous and dispersed fluid phases to a 25 μm wide orifice; post-orifice channel width measures 175 μm (Figure 1c). An oil-in-water emulsion was chosen to facilitate the transitions from geometry-controlled mode to dripping to jetting at low pressures. The continuous phase consists of an aqueous glycerol solution mixed with the lipids DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids) and DSPE-PEG2000 (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000], Avanti Polar Lipids) to enhance shell stability. DSPC and DSPE-PEG2000 were combined at a 9:1 molar ratio and dissolved in chloroform ($CHCl_3$, Sigma), evaporated and combined with water prior to sonication. Additional water, glycerol (Sigma) and nonionic surfactant (Pluronic F-68, Sigma) were added to make a 6:4:1 solution by volume, and the solution was placed on mild vortex and sonicated to ensure complete mixture.[15,16] Preparing this lipid solution provides students hands-on experience with formulation, valuable in industries such as pharmaceuticals; for simpler experiments, the continuous phase could be simplified to compose only water, glycerol, and surfactant. The dispersed phase consists of triacetin oil (Glyceryl triacetate, Sigma) mixed with Oil Blue N dye (Sigma) at a concentration of 0.01 mg mL^{-1} for visualization.[15] DI water was flowed through the device following fabrication to maintain hydrophilicity of the channels.

Exercise II accommodates undergraduate students with less experience in microfluidics or fluid dynamics, and teaches chip design by comparing characteristics of formed droplets in flow-focusing geometries of differing dimensions. We again utilize a flow-focusing droplet generator with expanding nozzle geometry; two versions of the generator are shown, with the first identical to the device in Exercise I. To generate smaller droplets, we narrow the orifice to 9 μm and post-orifice channel to 50 μm in order to increase the stresses and pressures acting on the dispersed phase at the flow-focusing region. A water-in-oil emulsion was chosen as a representative interface useful in the life sciences, consisting of DI water in light mineral oil (Sigma) mixed with 2% Span 80 nonionic surfactant (Sigma). The devices were oven baked following fabrication to maintain channel hydrophobicity.

Standard soft lithography techniques[5] were used to fabricate the microfluidic devices in Exercises I and II. Devices were designed in Illustrator (Adobe) and printed at 20000 DPI by CAD/Art Services. Negative channels were formed by pouring polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) at a ratio of 10:1 prepolymer to curing agent over positive SU8-25 (MicroChem) master molds. PDMS was cured overnight and individual devices were peeled from the hard masters in a laminar flow chamber. Inlets and outlet were punched using a blunt 18G needle. Devices were plasma treated in an air plasma machine

(Harrick Plasma) for 90 seconds at 200 milliTorr and bonded to cleaned glass slides. Surface properties of the channels were maintained as above.

Devices were mounted on an inverted microscope (IX71, Olympus) for experimentation and recorded using a high-speed camera (V310 Phantom, Vision Research). It should be noted that while a high-speed camera enables the recording of slow motion video detailing the formation process, informational changes to the shape and position of the interface could be observed through a standard microscope.

Exercise III facilitates exposure of droplet-based microfluidics to students at the high school level and to the general public. In lieu of lithography processes that require a cleanroom, potentially harmful chemicals, and expensive pieces of equipment, students are meant to design and fabricate a T-junction to form large plugs using coffee stirrers as a master mold substitute. Complete negative replicas can be made in hours by curing PDMS, or an appropriate alternate such as Jell-O[6], over the coffee stirrers, removing the cast, and bonding to a substrate. Coffee stirrers were obtained from a local coffee shop and measured to be 600 μm in width. Two lengths were cut and glued down in a Petri dish at a 90° angle to form the fluid junction. Soft lithography using PDMS proceeded as in Exercises I and II. DI water mixed with blue food dye for color was used as the dispersed phase, and light mineral oil was used as the continuous. Experiments were performed on the bench top and recorded using the video feature of a mounted iPhone (Apple).

Each exercise presented below may be tailored to the ability of the student and the intent of the class. For instance, students may be asked to fabricate the microfluidic devices in Exercises I and II if time and space permit in the laboratory in order to gain experience in soft lithography. ImageJ (NIH) was used to analyze all videos for generation rate (droplets per second, or Hz) and droplet diameter (μm, back calculated from area measurements); this program is free to download from NIH and can be included as follow-up analysis to the laboratory experiment.

2.3 Hand-Controlled Pressure Pumping

A homemade, handheld pressure pumping system lends control of the droplet generation to the hands of the student. Using pressure rather than syringe or peristaltic pumping provides a more stable flow with instantaneous response time and without the pulsing and hysteresis typical of mechanical pumps[17]. The student drives this system, shown in Figure 1a, by pushing the plunger of the syringe in hand (one each for the continuous phase and for the dispersed phase). Air is forced out of the syringe and into the sealed fluidic reservoir via Tygon tubing, compressing the gas occupying the headspace above the liquid phase; once pressurized, the liquid phase flows out of the reservoir via a second length of tubing and into the microfluidic device. The force exerted on each handheld syringe directly determines the flow rate into the device; as the student pushes harder or softer on the syringe the flow rate increases or decreases, respectively. Changes in this force are immediately reflected, allowing the student to perceive the effect on the system.

3 Hazards

Lab coats, gloves, and eye protection should be worn as a precaution during device fabrication. If lipids are to be used in Exercise I to add to the stability of the shell (e.g. to allow for droplet collection and study), chloroform should be handled with caution, as it can be hazardous in case of skin or eye contact, of ingestion, or of inhalation. In case of skin contact, immediately flush skin with water and cover with an emollient. In case of inhalation, remove to fresh air and give oxygen if breathing is difficult or artificial respiration if not breathing. Alternatively, the continuous phase in Exercise I can be prepared sans lipids by simply mixing water, glycerol, and nonionic surfactant in 6:4:1 ratio by volume.

4 Exercise I – Regimes of Droplet Formation in Flow-Focusing Devices

Exercise I is designed to appeal to graduate and advanced undergraduate students with some previous instruction in the basic principles of microfluidics. Still, a lecture on the principle of operation of flow-focusing droplet generators, discussed here, should be provided to students either before or after the laboratory experiment, depending on the intent of the investigation (i.e. to verify the dynamics of droplet formation or to explore them). The flow-focusing device in Figure 1b directs a central stream of one fluid and two side sheath flows of a second fluid to a small orifice, focusing the fluids at the orifice in order to cause the first fluid to be dispersed as a discrete volume in the second.[14] In forming the droplet, an interface is first created between the two immiscible fluids; energy is fed into the liquid interface from the system in order to reach an intermediate state with high interfacial free energy. This intermediate state, a saddle point representing the deformed interface, spontaneously decays into the final dispersed state.[3] This process then repeats to generate a stream of droplets.

Key in this process is the occurrence of an unstable mode of interface deformation causing the intermediate state, i.e. the saddle point. Various mechanisms of droplet formation therefore emerge based on the particular mode of surface induced instability, with the key regimes in flow-focusing microfluidic devices being geometry-controlled (or squeezing), dripping, and jetting[12,16]. Controlling the mode of droplet breakup effectively allows the student to tune the droplet size, generation rate, and monodispersity by an order of magnitude or more. A dimensionless parameter used to analyze the relative importance of viscous forces versus surface tension acting across a fluid interface, the capillary number plays the most determining role in droplet formation[18] and the transition between regimes. In basic form, the capillary number can be defined as

$$\mathrm{Ca}=\eta V/\gamma,$$

where η and V are the viscosity and superficial velocity of the continuous phase, and γ is the equilibrium surface tension between the fluid phases. Exercise I serves as a platform to engage the active student in such complex dynamics of droplet formation, as the student investigates these regimes by a hand-operated setup.

Figure 2 demonstrates the three droplet formation regimes characteristic of flow-focusing devices, accessed by modulating the force exerted by the handheld pressure pumping system. The student begins by applying a small pressure to each handheld syringe in order to first prime the channels of the device and then induce droplet formation. At low system pressures, a protrude-and-retract mechanism[19] distinguishes the geometry-controlled mode (Figure 2, images 1–3), wherein the dispersed phase finger elongates at the orifice and breaks off due to high shear caused by a pinch from the device geometry. More specific, as the dispersed phase passes through the orifice it comes into contact with the walls of the device, temporarily blocking the continuous phase flow. The dispersed phase necks at the orifice as the upstream pressure builds, until break off occurs. Droplets in this regime are monodisperse yet limited in minimum size by the width of the orifice; generation ranged from 52.7 ± 1.2 μm at 21 Hz to 37.4 ± 0.6 μm at 155 Hz as pressures of both the continuous and dispersed phases were increased. Intermediate to these stable zones of generation, satellite droplets occur as large droplets seemingly tail off into many smaller droplets.

The transition from geometry-controlled to dripping occurs as the capillary number is increased beyond a critical value (i.e. as the student pressurizes the system to increase the superficial velocity of the flow). In the dripping regime (Figure 2, images 4,6,8), the dispersed phase finger narrows to a fine tip and remains at a fixed location in the orifice; droplets break off at a high rate due to decay via Rayleigh-Plateau instability and may be much smaller in diameter than the minimum feature size of the device. The expanding nozzle geometry of our droplet generator may contribute to the stability of the dripping regime in the device, as the flow velocity reduces post-orifice creating a stabilizing backpressure in the expansion chamber. Jetting (Figure 2, images 5,7) is accessible at high capillary numbers once the dispersed phase no longer fulfills the detachment condition[3] at the orifice and extends far into the post-orifice channel. With a lesser focusing effect, the dispersed phase finger may fluctuate in both length and width. As in the dripping regime, droplets break off at the tip due to Rayleigh capillary instability, but the more dynamic nature of jetting may cause droplets to be large or polydisperse.[12,16] Because the mode of instability remains the same, the device can be easily fluctuated between dripping and jetting in part due to the instantaneous response of the hand-controlled pressure pumping. In particular, dripping can be induced from jetting by increasing the ratio of the continuous phase flow to the dispersed phase flow

$$\varphi = Q_C / Q_D$$

(where Q_C and Q_D are flow rates), and vice versa, indicating that the dimensionless flow rate ratio ϕ plays a role in addition to Ca in the transitions between dripping and jetting. Therefore, by alternatively adding pressure to the dispersed phase syringe and then the continuous phase syringe, the student can cycle between jetting and dripping formation while increasing the generation rate in each mode, as in Figure 2. Generation in the dripping regime ranged from 20.7 ± 0.4 μm at 633 Hz to 17.8 ± 0.5 μm at 1242 Hz, and was thus still highly monodisperse but with a much shorter breakup period than in geometry-controlled

mode. Generation in the jetting regime was less monodisperse with an intermediate breakup period, ranging from 28.3 ± 1.9 μm at 288 Hz to 21.1 ± 1.8 μm at 429 Hz.

The majority of groups in microfluidics have utilized the geometry-controlled mode to form droplets in the picoliter to nanoliter size range at rates in the hundreds or several thousands of Hz.[1,3] For applications in intravenous therapeutics, we have shown the dripping regime to be ideally suited, generating droplets of 3–6 μm in diameter at rates in excess of 10^5 Hz.[16] In a more extreme case tipstreaming can be induced to form sub-micrometer droplets at high rate to satisfy extravascular applications.[20] Training graduate and advanced undergraduate students in the numerous mechanisms to form droplets will play an essential role in the emergence of fine droplet applications such as site-targeted therapeutics and next generation sequencing as viable in clinical or industrial settings. Our hand-controlled pressure pumping method lends intuition to the complex modes of droplet formation, as the student senses the input and can immediately view the output.

5 Exercise II – Effect of Device Dimensions on Droplet Characteristics

Exercise II should appeal to undergraduate students in introductory-level microfluidics courses. The width of the orifice and geometry of the overall flow-focusing region are known to determine the flow field that acts on the dispersed phase finger. In the traditional mode of operation (geometry-controlled), as the droplet grows it obstructs the orifice, causing an increase of the dynamic pressure of the continuous phase upstream of the junction, and a necking of the dispersed phase leading to pinch off.[18] The size of the resulting droplet is thereby determined not only by the ratio of the flows but also by the width of the orifice and of the post-orifice channel (affecting the resistance downstream from the orifice and the pressure drop profile across the device). Exercise II serves to demonstrate these aspects of chip design through comparison of the generation characteristics between an original device and a device with narrowed orifice and post-orifice dimensions, suiting an audience of undergraduate students with less experience in microfluidics.

The effect of the device dimensions can be seen in Figure 3 using the hand-pressured pumping system as the input. Initially at low system pressure, the original generator (with a 25 μm wide orifice and 175 μm wide post-orifice channel) forms droplets 61.8 ± 1.2 μm in diameter at 56 Hz. Operating within the geometric constraints of this original device, the student can tune the generation by adding pressure to the continuous or dispersed phase syringe (Figure 3, images 1–4). A minimum droplet size of 29.3 ± 0.8 μm at 77 Hz is achieved by adding pressure to both syringes simultaneously to reduce the period between droplet breakup. Being both the smallest feature in the device and the cause of interface deformation, the orifice limits the droplet diameter to its approximate width, preventing the formation of smaller droplets. The orifice and post-orifice channel can be narrowed to extend this range for fine droplet applications. At pressure inputs judged to be similar by the operator, the narrowed generator (with a 9 μm wide orifice and 50 μm wide post-orifice channel) forms droplets 19.6 ± 0.8 μm in diameter at 72 Hz, reducing droplet diameter by one third. Further, the narrower post-orifice channel increases the flow velocity post formation in order to minimize contact amongst droplets when shell resistances are low.

Through this comparison between devices of differing dimensions, students will gain a sense for the design of droplet generators and the factors that contribute to characteristics of the droplet.

6 Exercise III – Coffee Stirrers as Fabrication Tools

In practice at the research university, microfluidics and its devices require complex means of fabrication, namely lithographic methods involving clean room facilities stocked with hazardous chemicals and expensive equipment. These facilities are used to fabricate a master mold (e.g. SU-8 photoresist patterned on a silicon wafer) that serves as the base for PDMS replica molding. Exercise III serves students at the high school level and the general public by replacing hard lithography with coffee stirrers as a means to rapidly prototype a T-junction to form large plugs of water dispersed in oil. Combined with hand-controlled pressure pumping and an iPhone for video recording, this setup vastly reduces the cost of microfluidic experimentation while demonstrating a simple-to-understand method of generating droplets (the T-junction). Additionally, though the PDMS device shown here is bonded to glass via air plasma, several alternative methods have been reported to seal PDMS in the absence of a plasma machine. Most similarly, a less expensive hand-held corona discharger may be substituted to activate the surface of the PDMS and glass prior to bonding, resulting in a temporarily hydrophilic surface.[21] More practical still, adhesive-based bonding requires only adhesive tape and a temperature-controlled oven to complete the bonding, and produces immediately hydrophobic channels.[22] This exercise and the previous two may thereby be replicated without the aid of air plasma, if needed. And while Exercise III has been designed with a high school setting in mind, we have used this exercise as an in-class demonstration for senior undergraduates and first-year graduate students with positive effect and feedback.

A sequence for producing T-junction chips using wooden coffee stirrers as the master mold substitute is presented in Figure 4. Coffee stirrers, 600 μm in width, are cut to unequal lengths using household scissors or blade, then glued flat to the base of a Petri dish at a 90° angle in order to form the junction. After allowing time to dry, PDMS is mixed at 10:1 base to curing agent, poured over the mold, and left to cure in a 70 °C temperature-controlled dry oven. The solidified chip is peeled off the mold and bonded to glass for experimentation.

The student begins by depressing the syringes a small amount in order to edge the continuous and dispersed phases to the fluidic junction. Adding pressure to each syringe – more so to the continuous bulk phase syringe to create the necessary shear force – increases the flow rate of each phase and induces plug formation, as in Figure 5. That the two fluids do not mix at the junction is a direct result of the length scale of the microfluidic device: having tiny dimensions, microfluidic devices exhibit decidedly different behaviors relative to at the macroscale, most notably laminar rather than turbulent flow. The flow of the fluids in the device can be characterized by the dimensionless Reynolds number relating inertial to viscous forces,

$$\mathrm{Re}=\rho V\mathrm{L}/\eta$$

where ρ is the density and η is the dynamic viscosity of the fluid, *V* is the characteristic velocity of the flow, and L is the characteristic length of the channel. In the low Reynolds number flow regime of the microfluidic device, viscous forces dominate and flow is completely laminar with no turbulent mixing.

To show an example of Reynolds number and capillary number calculations, we can take our device and method from Exercise III, forming W/O droplets (DI H_2O in light mineral oil) in a T-junction with L = 600 μm channel width. The density and viscosity of light mineral oil and its interfacial tension with water at room temperature are determined in the literature as ρ = 0.838 g/mL, η = 23.1 mPa-s = 23.1 cP, and γ = 49.3 mN/m.[23] By tracking the leading edge of the plug travelling in the channel over time, we can determine the approximate velocity of the flow as *V* = 0.014 cm/s. Using these values, we estimate the Reynolds number and capillary number for DI H_2O in light mineral oil in this T-junction, as:

$$\mathrm{Re}=\frac{\rho VL}{\eta}=\frac{(0.838g/mL)(0.014cm/s)(600\mu m)}{23.08cP}=0.003$$
$$Ca=\frac{\eta V}{\gamma}=\frac{(23.08cP)(0.014cm/s)}{49.3mN/m}=6.6\times10^{-5}$$

Thus, the low Reynolds number and capillary number predict laminar flow in the microfluidic channel and the slow breakup of plugs in the squeezing regime.[24]

As in Exercises I and II, the student gains an intuitive sense of the relative pressures needed to successfully disperse one phase in another. In this case, the dispersed phase is injected perpendicular to the continuous stream and experiences shear from the cross flow; this shear pulls on the dispersed phase causing a neck to form at the lagging end, and eventually break off occurs. Breakup of successive plugs was timed at ~ 20 s, indicating a generation rate of ~ 0.05 Hz. By means of ImageJ, a μm per pixel conversion factor can be obtained from the known width of the wooden coffee stirrer and the height of the channel in pixels; using this factor, the mean diameter of plugs formed in the device was back-calculated from the area as 811 ± 43 μm, and due to the low standard deviation relative to the mean the plugs are deemed monodisperse.

Using coffee stirrers as fabrication tools proves to be a simple and cost-effective strategy to demonstrate device manufacture and droplet generation to audiences not yet schooled in the field. Using Jell-O[8] to mold the T-junction may reduce the cost of experimentation even further, or alternatively the T-junction could be fabricated using shrink-film[21] or whole-glass[25], and likewise driven by hand-pressured pumping to widen the range of materials while maintaining low cost. We recently adapted Exercise III, for instance, to fit the capabilities of a small, local high school with limited access to PDMS. Students were first given an introductory lecture on microfluidics, with a focus on comparing natural microfluidic systems – such as capillary networks supplying nutrients in trees or the body – to man-made systems such as the T-junction. Under stepwise instruction, five students then created molds of a T-junction using either wooden coffee stirrers or flat toothpicks. A mix of Jell-O and unflavored gelatin powder was dissolved in boiling water, poured over the molds, and left in the refrigerator to cure overnight. The next day, students completed the fabrication process by peeling the Jell-O replica molds from the master and placing the

molds on aluminum foil to create a reversible seal. Finally, students demonstrated proof-of-principle operation by forming droplets using the PDMS T-junction and hand-controlled pressure pumping system of this paper. Students were successful not only in forming plugs in the channel but also in tuning the size of the plug and generation rate by adjusting the input pressures. Each student was required to write a mock scientific journal article, presenting a novel method to fabricate droplet generators for cancer therapeutics using low-cost materials (Jell-O/gelatin), to extend use of the technology to low socioeconomic communities and third-world countries.

We have since successfully generated oil-in-water droplets in a home setting by curing Jell-O and gelatin powder at high concentration[8] over flat toothpicks to form PDMS-like microfluidic devices. Mango-flavored Jell-O powder, unflavored gelatin, flat toothpicks, food-grade color dye, Krazy Glue, and aluminium foil were all obtained from a local grocery store. Concentrated Jell-O and gelatin solution was poured over the flat toothpicks (glued in the shape of a T-junction to the base of a square Petri dish) and allowed to cure for two or more days. Once peeled, the resulting mold bonded reversibly to aluminium foil and maintained its shape at room temperature sufficiently to run multiple experiments. As gelatin is naturally hydrophilic, we chose to form oil-in-water droplets using light mineral oil as the dispersed phase and blue-dyed water as the continuous phase. It should be noted that PAM Original no-stick cooking spray can help to release the mold from the plastic dish, but is hydrophobic and must be rinsed by the continuous phase to prime the surface for wetting of water rather than oil. Oil droplet formation in our Jell-O T-junction can be seen in the Electronic Supplementary Information (ESI) video file. Generation in this T-junction produced smaller droplets due to the tighter geometry of the flat toothpicks relative to the wooden coffee stirrers, at rates on the order of Hz to tens of Hz. That the experiment, and all preparation, was carried out at home using household materials demonstrates the flexibility of these low-cost microfluidic demonstrations to settings beyond the laboratory.

7 Conclusion

We have developed a series of educational modules suited to instruct in the art of droplet-based microfluidics. In lieu of costly syringe pumps, we use hand-operated syringes to drive pressurized fluidic reservoirs, thereby affording control of droplet generation to the hands of the student. In addition to being engaging and fun, the handheld pumps tap into the senses of the student to lend intuition to and insight into the dynamics of the experiment. Exercises I, II, and III are lower cost than traditional experimental setups, particularly useful for hands-on microfluidics education in teaching laboratories with limited resources. As the concepts build on each other, the exercises demonstrated here could be used as a series (III-II-I) to comprehensively demonstrate the fundamentals of droplet formation, from the basic to the complex. In addition, considering the portability of hand-controlled pumping, this system may be useful for creating monodisperse droplets in the field[26] and at point-of-care where resources and time are limited. Looking forward, microfluidics education remains in its infancy, and therefore it is critical that novel instructional exercises like the ones presented in this study need to be continually developed to demonstrate to students the growing technologies and applications of the field.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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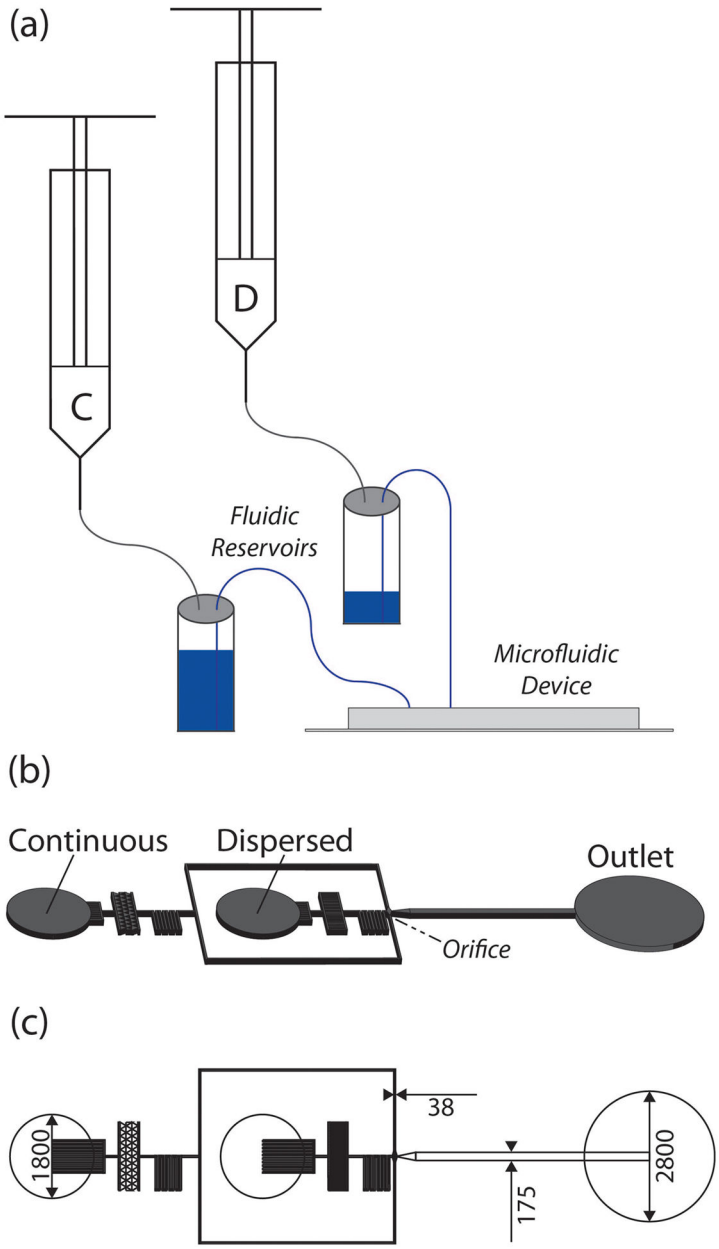

Figure 1.

(a) Hand-pressured pumping. Operation of the system is placed in the hands of the student, as depressing the continuous (C) and dispersed (D) phase syringes drives the fluids out of the fluidic reservoirs and into the microfluidic device. (b) Schematic view of the microfluidic flow-focusing device utilized in Exercises I and II. The device directs a central stream of dispersed phase to a small orifice, where it experiences a focusing effect from two side sheath flows of continuous phase and shear-based breakup. Expanding nozzle geometry, located immediately downstream of the orifice, supports break off of droplets. (c) Vector-based design of the droplet generator. Dimensions are shown in micrometers and are reduced in Exercise II as detailed.

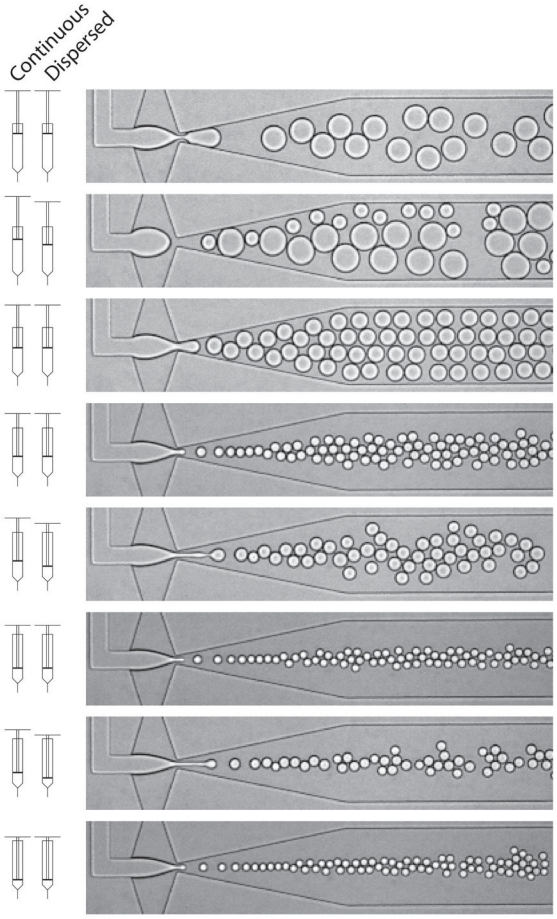

Figure 2.

Sequence of images showing the effect of continuous and dispersed phase pressures on droplet generation, in this case oil-in-water (O/W). By modulating the capillary number Ca and dimensionless flow rate ratio ϕ, droplet formation transitions from geometry-controlled (GC) to dripping (D) to jetting (J). The dripping regime yields droplets smaller than the minimum feature size – the orifice – at the shortest period between breakup. Top to bottom: GC, droplet diameter D = 52.7 ± 1.2 μm, generation frequency f = 21 Hz; GC, primary D = 61.4 ± 0.9 μm, satellite D = 32.1 ± 1.1 μm, combined f = 36 Hz; GC, D = 37.4 ± 0.6 μm, f = 155 Hz; D, D = 20.7 ± 0.4 μm, f = 633 Hz; J, D = 28.3 ± 1.9 μm, f = 288 Hz; D, D = 17.4 ± 0.4 μm, f = 795 Hz; J, D = 21.1 ± 1.8 μm, f = 429 Hz; D, D = 17.8 ± 0.5 μm, f = 1242 Hz. Supporting videos can be found in the Electronic Supplementary Information (ESI).

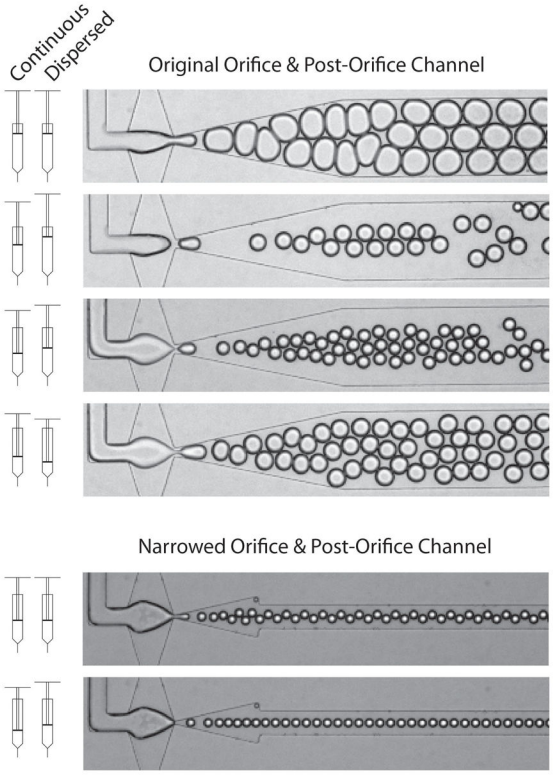

Figure 3.

Sequence of images showing the effect of device dimensions on droplet generation, in this case water-in-oil (W/O). Narrowing the orifice from 25 to 9 μm and post-orifice channel from 175 to 50 μm leads to the formation of much smaller droplets due to an increase in the shear-based force acting on the dispersed phase at the orifice. Top to bottom: droplet diameter $D = 61.8 \pm 1.2$ μm, generation frequency $f = 56$ Hz; $D = 37.5 \pm 1.0$ μm, $f = 12$ Hz; $D = 29.3 \pm 0.8$ μm, $f = 77$ Hz; $D = 38.6 \pm 1.3$ μm, $f = 120$ Hz; $D = 20.2 \pm 0.3$ μm, $f = 66$ Hz; $D = 19.6 \pm 0.8$ μm, $f = 72$ Hz.

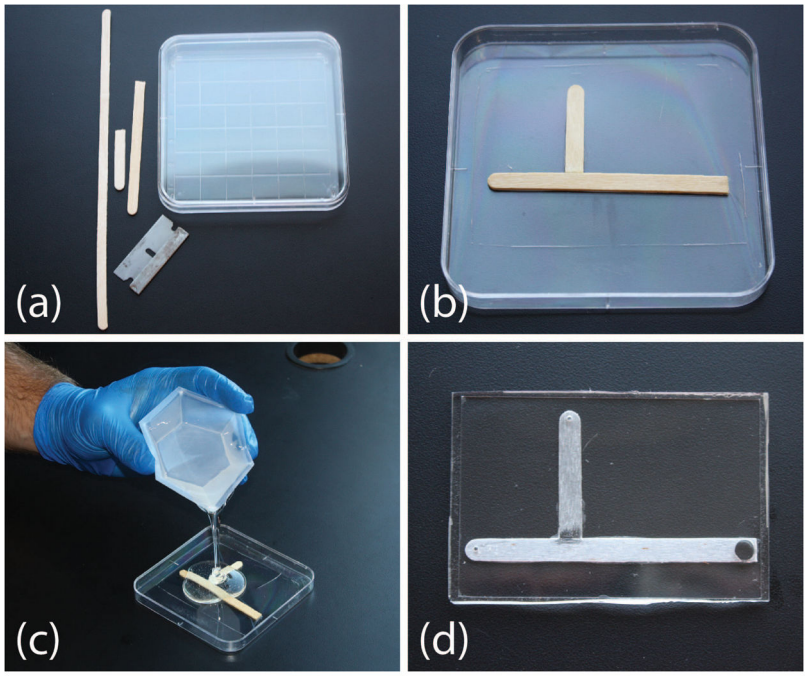

Figure 4.

Sequence for producing T-junction chips using coffee stirrers as a master mold substitute. (a) Materials include wooden coffee stirrers and a Petri dish. (b) Master mold is created by cutting and gluing coffee stirrers at a 90° angle. (c) Pouring PDMS over the master mold and curing overnight in a temperature-controlled oven forms the replica mold. (d) Completed replica mold in PDMS bonded to a glass slide.

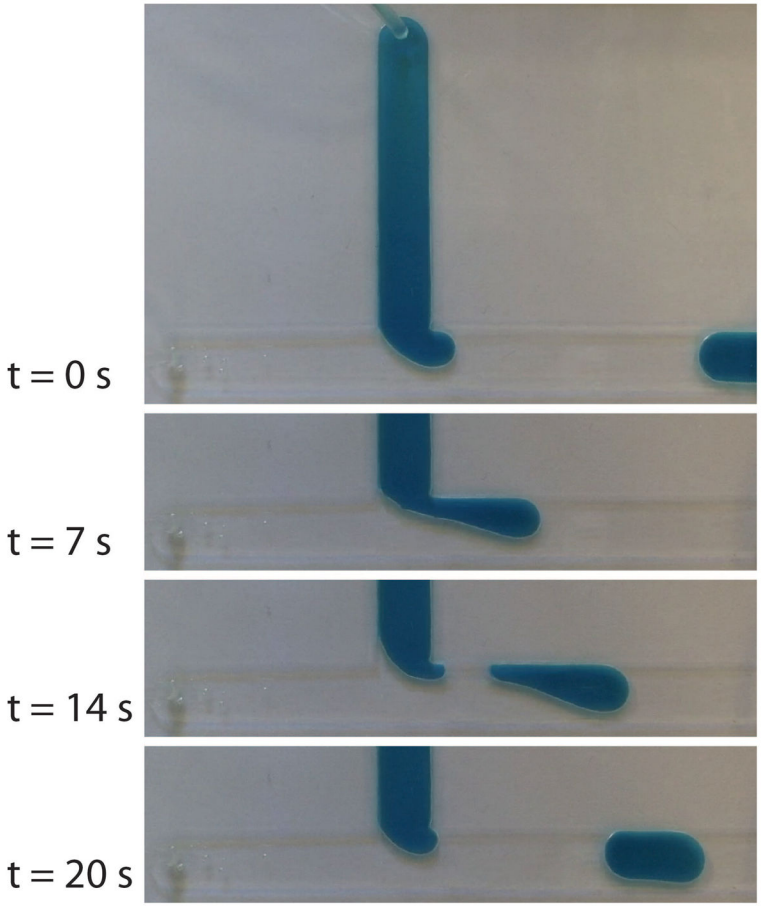

Figure 5.
Water-in-oil (W/O) droplets formed in the low-cost T-junction chip. Hand-operated pressure pumping provides immediate response and feedback to the student. Generation averaged 0.05 Hz in forming droplets 811 ± 43 μm in diameter.

Table I

Summary of learning objectives for each exercise. To illustrate the utility of our hand-controlled pressure pumping system in forming monodisperse droplet streams, the exercises are presented in decreasing order of difficulty. The learning objectives build upon each other, and thus these exercises may be used as a sequence of laboratory modules (Exercise III-II-I) to demonstrate droplet-based microfluidics to students of varying ages and abilities.

	Exercise I	**Exercise II**	**Exercise III**
Primary topic	Formation regimes	Device dimensions	Fabrication
Device	Flow-focusing	Flow-focusing	T-junction
Student level	Graduate and advanced undergraduate	Undergraduate	High school
Sample courses	Droplet-Based Microfluidics; Microsystems	Introductory Microfluidics	Physics; Chemistry
Students/groups	1–10	1–10	5–20
Time for experiments	2 hours (+ 1 hour over 2 days if fabricating)	1 hour (+ 1 hour over 2 days if fabricating)	30 minutes each over 2 days
Expendable cost per student/group	US$ 5.00+ (depending on formulation)	US$ 5.00	US$ 3.00
Learning objectives	**1** Inclusive of Exercises III and II **2** Capillary number **3** Flow rate ratio **4** Rayleigh capillary instability **5** Formation regimes **6** Formulation	**1** Inclusive of Exercise III **2** Design software **3** Soft lithography **4** Flow-focusing **5** Channel dimensions **6** Surface properties	**1** Design of microfluidic droplet generators **2** Pressure-driven flow **3** Length scale and laminar flow **4** Shear-based breakup **5** Data acquisition
Questionnaire	**1** What is the capillary number? How does the capillary number relate to the different regimes of droplet formation? **2** How does the mechanism of droplet breakup differ in dripping and jetting relative to geometry- controlled? **3** What factors might one consider in designing a device and formulating the continuous phase to induce dripping?	**1** How does the flow-focusing geometry differ from the T-junction? What are the advantages and disadvantages? **2** How might one tailor the dimensions of the device to produce smaller droplets? What specific features are key? **3** What would we need to change in order to form oil droplets instead?	**1** What is a droplet? How does a plug differ? **2** What causes a plug to break off at the junction? Why do the fluids not mix? **3** What happens to the size of the plug as the pressure on the continuous phase is increased? The dispersed phase?