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Rapid Prototyping in Copper Substrates for Digital Microfluidics**

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Microfluidics, a technology based on enclosed, micrometer-dimension channels, first became popular in the early 1990s^[1,2] as a tool for miniaturizing chemical analyses. Recently, a new form of “digital microfluidics” (DMF) has emerged, in which droplets of liquid are manipulated on an array of electrodes by means of electrowetting^[3,4] and/or dielectrophoresis.^[5–8] There is currently much enthusiasm for DMF,^[9] as the device geometry seems a perfect match for array-based biochemical applications, such as enzyme assays^[10,11] and profiling proteomics.^[12–14] Despite this enthusiasm, the arduous procedure required to fabricate DMF devices, which typically requires metal deposition, photolithography, wet-etching, deposition or thermal growth of a dielectric layer, and deposition of a hydrophobic coating,^[3,4,11–19] is a barrier to its growth and development. Consequently, DMF devices are limited to use in a few laboratories worldwide.

The availability of an accessible microfabrication technique is critical for the growth and continued development of DMF. An analogy can be made to the state of conventional, channel-based microfluidics in the mid-1990s. A database search reveals that approximately eight articles per year were published on the subject of channel microfluidics in 1992–1998; this number exploded to approximately 53 articles per year in 1999–2000.^[20] This jump in popularity, which has greatly expanded the scope and range of applications for channel microfluidics, was driven in part by increased accessibility resulting from rapid prototyping microfabrication methods.^[21–23]

We report here, for the first time, two rapid prototyping techniques for the fabrication of DMF chips, making use of commercially available printed circuit board (PCB) substrates. In the first method, actuation electrodes were patterned on PCB substrates by using photolithography, in a manner similar to what has been reported for other applications.^[24,25] This method is fast, inexpensive, and easy relative to conventional microfabrication. In the second technique, actuation electrodes were patterned directly onto substrates using a desktop laser printer; this method enabled ultra-high-throughput fabrication. We anticipate that these methods will increase the accessibility of DMF, an effect that will significantly expand the impact of this promising technology.

The first rapid prototyping method, relying on photolithography, was used to form devices from two kinds of substrates: industrial-grade flexible sheets (9 μm thick copper on 50 μm polyimide) and low-grade inflexible boards (35 μm thick copper). Prior to use, devices were coated with Parylene-C (see Experimental section) or poly(dimethyl siloxane) (PDMS) as a dielectric layer, and then coated with Teflon-AF. Devices formed in this manner were used to actuate droplets sandwiched between two plates, as depicted in Figure 1a. This configuration is the most popular, as it is capable of performing all of the critical fluidic operations: dispensing droplets from reservoirs, and moving, merging, and splitting them.^[15] As shown in Figure 1b–f, devices formed by the new method were capable of performing each of the critical operations.

Actuation parameters for droplet movement and merging (Fig. 1b–d) were electric potentials of 300–400 V_{rms} (rms: root mean square) at 18 kHz for the actuation of 300 nL droplets of deionized (DI) water sandwiched between plates separated by a 150 μm spacer. Figure 1d demonstrates an on-chip reaction between droplets of HCl (120 mM) and methyl red indicator (0.9 mg mL⁻¹). Splitting and dispensing droplets (Fig. 1e and f) required application of higher potentials and/or smaller interplate spacings (for example, 400 V_{rms} and 150 μm spacing for flexible substrates, and 440 V_{rms} and 75 μm spacing for inflexible substrates). Dispensing (Fig. 1f) was achieved by actuating two to three electrodes in series to pull liquid into a column, and subsequently energizing the electrodes on either side of the column, while the electrode(s) between them was/ were allowed to float. The volume of dispensed droplets was a function of electrode size and interplate spacing (for example, ca. 300 nL for 150 μm spacing). Droplets dispensed from reservoirs were occasionally subject to a droplet–droplet repulsion that prevented merging. This phenomenon, which we attribute to charge accumulation on the contact line, has been observed previously.^[26–29] The repulsion could be overcome

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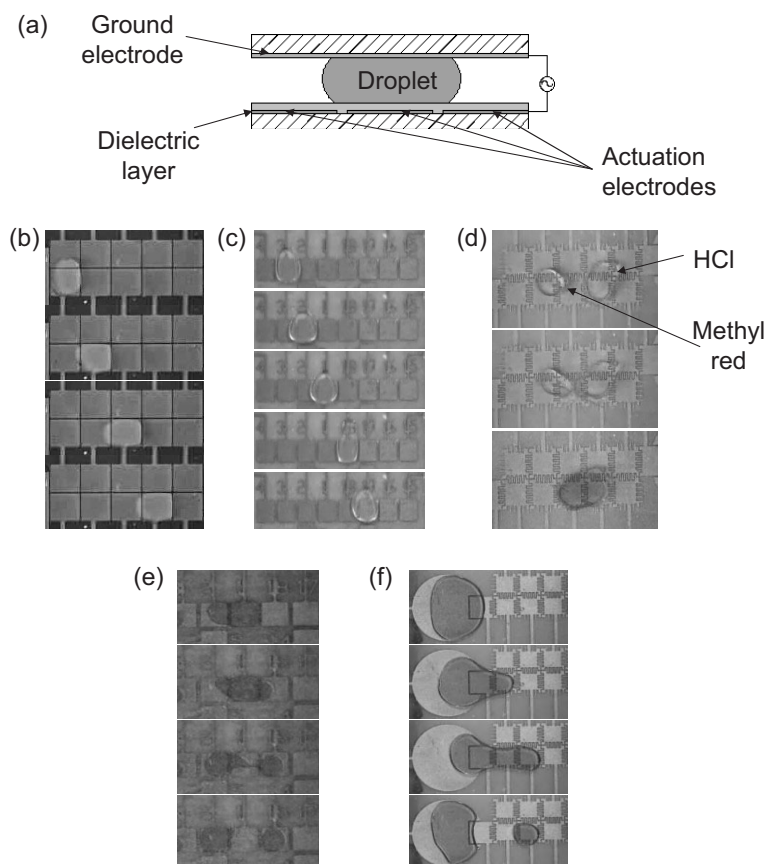


Figure 1. Two-plate DMF devices formed by using photolithography. a) A schematic of the two-plate configuration. b–f) Video sequences (top-to-bottom) depicting droplet movement, merging, splitting, and dispensing, respectively. In (d), droplets containing HCl (120 mM) and methyl red (0.9 mg mL^{-1}) were mixed to effect an on-chip reaction. Devices in (b), (d), and (f) were formed from flexible substrates; devices in (c) and (e) were formed from inflexible boards. The device in (b) was coated with $9 \mu\text{m}$ PDMS; devices in (d) and (f) were coated with $9 \mu\text{m}$ parylene; devices in (c) and (e) were coated with $1 \mu\text{m}$ parylene and $40 \mu\text{m}$ PDMS; all devices were coated with 50 nm Teflon-AF.

by allowing the dispensed droplets to remain still for a few moments prior to merging.

The first rapid prototyping method was also used to form devices in a single-plate configuration,^[17,30,31] depicted in Figure 2a. The advantage of this format is the capacity to move large droplets with a small footprint (for example, $1.5\text{--}3 \mu\text{L}$ droplets can be manipulated on $1 \text{ mm} \times 1 \text{ mm}$ electrodes); additionally, it has been reported that mixing is more efficient on a single-plate device compared to two-plate devices.^[17] As shown in Figure 2b and c, devices were used for droplet movement and merging; typical actuation parameters were $450 \text{ V}_{\text{rms}}$ at 18 kHz for $1.5\text{--}2 \mu\text{L}$ droplets on $1 \text{ mm} \times 1 \text{ mm}$ electrodes separated by $60 \mu\text{m}$.

Regardless of configuration (one or two plate), devices formed utilizing the rapid prototyping method were observed to be capable of facile and fast droplet actuation, with comparable performance to devices formed utilizing typical methods.^[3,4,11–16,18] The most significant difference is in the electrode thickness: the PCB substrates had copper thicknesses of

9 and $35 \mu\text{m}$, which stands in stark contrast to the ca. 100 nm gold layers used conventionally. In order to fabricate reliable DMF devices from substrates with thick electrodes, two challenges must be overcome: 1) loss of resolution when using isotropic etchants, and 2) rough device topography that can hinder droplet motion.

The first challenge is the lower limit on interelectrode spacing imposed by isotropic etching. For example, if actuation electrodes are formed from a $35 \mu\text{m}$ thick copper layer using a photomask pattern with a $5 \mu\text{m}$ gap between electrodes, the minimum possible gap after etching is $75 \mu\text{m}$ (in practice, the actual gap was often larger by a factor of about two because of variations in etch rate). These gaps are many times larger than those that are typical for devices formed by conventional means ($4\text{--}5 \mu\text{m}$).^[12,13,18] As large interelectrode gaps were occasionally observed to impede droplet movement, some devices were formed with interdigitated electrodes,^[3,15,16,19] which facilitated rapid smooth droplet movement, as shown in Figure 1d and f.

The second challenge related to copper layer thickness is the influence of the electrode pattern on device topography. Dielectric coatings on DMF devices are conventionally very thin ($\leq 2 \mu\text{m}$) in order to reduce the voltage required for droplet actuation.^[32] Initial experiments with thick electrodes and a thin dielectric layer (that is, $9 \mu\text{m}$ thick copper electrodes and $2 \mu\text{m}$ of Parylene-C) were unsuccessful; this phenomenon has been observed previously.^[33] Observations suggested that the droplets experienced actuation forces, but were not able to cross the deep trench between electrodes. A simple solution to this problem was to use a much thicker, $9 \mu\text{m}$ layer of Parylene-C, which enabled droplet movement between electrodes. Even so, the droplets on devices formed in this manner were still observed to occasionally get stuck at the boundary between electrodes. As shown in the optical profile in Figure 3a, this was likely caused by the trenches between electrodes that were observed even after coating with $9 \mu\text{m}$ of parylene.

Depositing thick layers of Parylene-C resulted in an increased fabrication time (ca. 6 h) and extra expense. While this is tolerable for $9 \mu\text{m}$ thick electrodes, this strategy was prohibitive for $35 \mu\text{m}$ thick electrodes. A solution to this problem was to use a dielectric layer of PDMS instead of Parylene-C. As shown in the optical profile in Figure 3b, spin-coated layers of PDMS were observed to form less conformal (i.e., more flat) surfaces than vapor-deposited parylene. This effect was enhanced by allowing substrates to sit for 10 min after spin-coating, to allow PDMS to reflow and form a flatter surface. Although this technique solved the problem of droplets getting stuck at interelectrode gaps, a disadvantage of using PDMS is the lower breakdown field ($21 \text{ V } \mu\text{m}^{-1}$)^[34] relative to

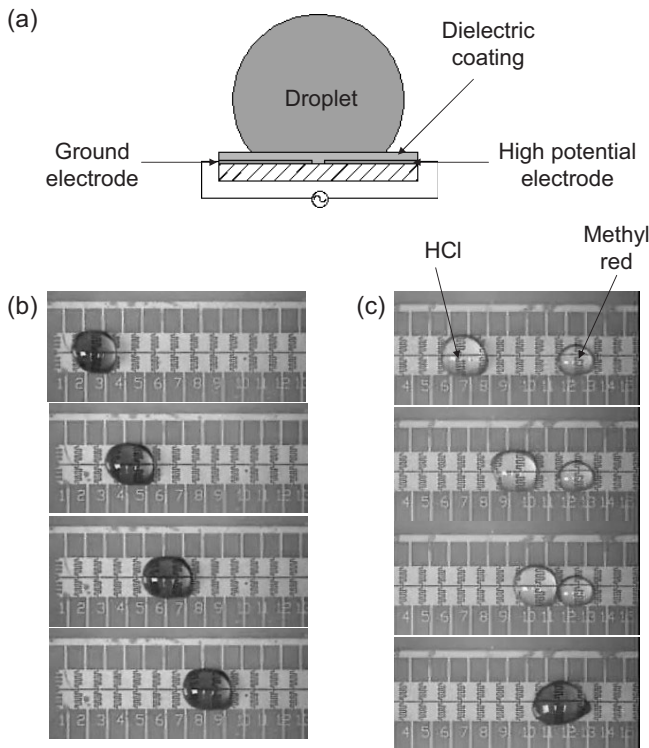


Figure 2. One-plate DMF devices formed by using photolithography. a) A schematic of the one-plate configuration. b,c) Video sequences (top-to-bottom) depicting droplet movement and merging. The devices were formed from flexible substrates coated with 9 μm PDMS and 50 nm Teflon-AF.

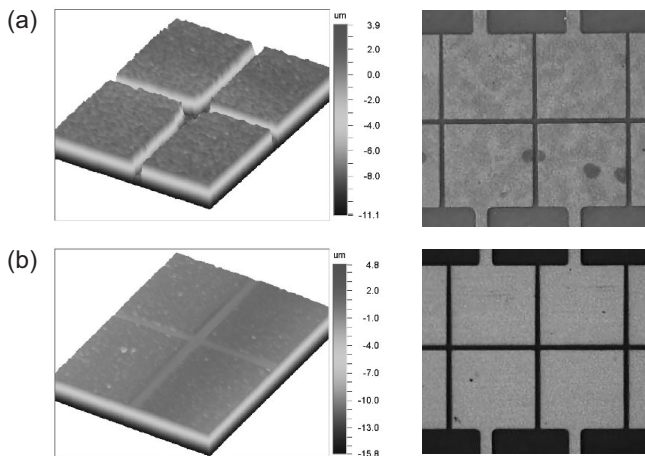


Figure 3. Optical profiles and pictures of devices coated with a) 9 μm parylene, and b) 9 μm PDMS. The gap between adjacent copper electrodes is 60 μm wide and 9 μm deep. The parylene coating maintains the original gap depth, whereas the PDMS coating reduces it considerably.

Parylene-C (220 $\text{V}\mu\text{m}^{-1}$).^[35] To reduce the probability of insulator breakdown, we sometimes used a combined dielectric layer of 9–40 μm PDMS (depending on the chip type) on top

of 1–3 μm of parylene. Regardless, devices formed with PDMS coatings (with and without parylene) were observed to enable droplet manipulation for all configurations and fluidic operations evaluated.

An additional benefit of using smooth PDMS layers was observed: such coatings were found to reduce the negative effects of large interelectrode gaps (caused by isotropic etching, as described above). As shown in Figure 1c, droplets on devices formed in this manner could be made to move between electrodes separated by gaps of up to 150 μm . As far as we are aware, this is the largest interelectrode gap that has been reported for DMF droplet actuation, without interdigitation.

We note that there are two previous reports^[31,33] of PCB-based DMF devices; a critical difference between the previous work and our own, however, is in the time required for fabrication. In these previous studies, chips were designed and submitted to commercial PCB vendors for fabrication in a process requiring many steps, including via-drilling through layers, electroplating to form vertical interconnects, and patterning of electrodes on the surface. This process is relatively expensive for small batches, and may require several days or weeks (including shipping times) from start to finish. This turnaround time is not convenient for rapid prototyping, that is, the fabrication of several iterations of devices quickly to optimize the design for a given application. In contrast to the previous work, our method is performed in house, requires only a single photolithography and etching step, and requires only a few hours of fabrication time, representing a significant advantage for rapid prototyping.

In an effort to form devices even faster, we built on previous work in the electronics industry^[36–41] to develop a second ultrarapid device-prototyping technique using a desktop laser printer. This method is capable of forming hundreds of devices in less than an hour, and is advantageous in that it does not require access to photolithography equipment. As shown in Figure 4a, device designs were printed directly onto a copper sheet by using a laser printer. The pattern subsequently served as a mask for copper etching, after which the devices were coated with a dielectric layer and used for DMFs. As shown in Figure 4b–d, using this method, we were able to create approximately eighty 2 cm \times 2 cm chips in less than 10 min (excluding coatings). Figure 4e depicts droplet actuation; typical conditions were 300 V_{rms} on a two-plate device with a 75 μm spacer between the plates.

The limitation on the ultrarapid prototyping method is resolution: for the 1200 \times 1200 dot per inch (dpi) printer that we used, the minimum interelectrode gap that could be resolved after etching was ca. 200 μm . A second limitation is that the amount of charge transferred by the laser printer's corona wire to the substrate, which controls toner density in the final print, depends on the type and condition of the substrate. We observed that if a small piece of copper-coated substrate was affixed to a carrier page, the charge transferred to the copper was not large enough to pull all of the toner particles from the drum to the substrate. This phenomenon was characterized by the appearance of a ghost image on the page below the pri-

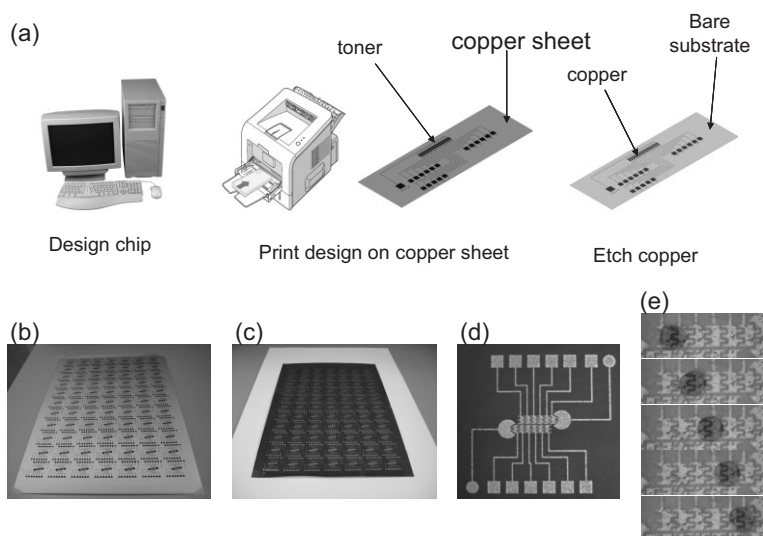


Figure 4. a) A schematic of ultrarapid prototyping through laser printing. b) Approximately 80 chips were printed on an A4-sized sheet ready for etching. c) The patterned sheet after copper etching and removal of toner. d) A close-up image of one of the patterned chips on the sheet. e) Video sequence (top-to-bottom) depicting droplet motion on a chip fabricated by using laser printing.

mary image on the copper. This effect, which reduced the robustness of the mask, was eliminated by using a letter-sized piece of copper-coated substrate (with no carrier page). We are currently optimizing the technique to overcome these limitations, and also intend to evaluate printers with higher resolution. Regardless, in preliminary experiments, droplet motion was comparable to that observed on chips formed from photolithography.

This new fabrication method relies on the property of laser-printed features to resist dissolution in copper etchants. Laser-printer toner is composed of iron oxide blended with a styrene-acrylate copolymer.^[42] When a substrate passes through a laser printer, toner is initially deposited as a powder, which is then melted as it passes through a heater. The melted toner adheres to the copper and is insoluble in ferric chloride solution, allowing it to serve as an etch mask for electrode fabrication. It should be noted that laser toner has been exploited previously for electronics applications as an etch mask,^[37–41] and as a substrate for lift-off and mask for UV exposure.^[36] These methods share the common requirement of a flexible substrate with suitable properties for electrode fabrication. Such substrates have only recently become widely available; these new techniques are so advantageous in fabrication ease and speed that we speculate that similar methods will soon become commonplace for many applications.

In conclusion, we have demonstrated two novel fabrication methods for digital microfluidic chips, using copper PCB substrates. The first method relies on photolithography to pattern the copper surface and form droplet-actuation electrodes, and is compatible with both industrial-grade copper sheets and low-cost copper boards of the type used by electronics hobbyists. Two-plate devices fabricated by using this method were demonstrated to be useful for dispensing, moving, splitting,

and merging 150–300 nL droplets; one-plate devices were capable of actuating droplets with volumes of up to 3 μL . Although both Parylene-C and PDMS were used as dielectric coatings, we found that PDMS-coated devices had flatter surfaces, which rendered droplet motion more facile and smooth. PDMS-coated devices were capable of moving droplets across interelectrode gaps (noninterdigitated) as large as 150 μm . A second, ultrarapid fabrication method made use of a desktop laser printer, and was demonstrated to be capable of producing as many as 80 chips in 10 min. We expect the methods presented here will make digital microfluidics accessible to any laboratory, which should enable development of a wide range of applications for this promising technology.

Experimental

Materials: Two PCB substrates were used: Pyralux AP double-sided copper-clad laminate AP7156E (DuPont Electronic Materials, Research Triangle Park, NC), and low-grade copper boards from Active Surplus Electronics (Toronto, ON). Photolithography chemicals included Shipley S1811 and MF-321 (Rohm and Hass, Marlborough, MA), hexamethyldisilazane (HMDS) (Shin-Etsu MicroSi, Phoenix, AZ), and AZ300T (AZ Electronic Materials, Somerville, NJ). Copper etchant CE-100 was from Transene Company Inc. (Danvers, MA). Device-coating materials included Sylgard-184 PDMS (Dow Corning, Midland, Michigan), Parylene-C (Specialty Coating Systems, Indianapolis, IN), Fluorinert FC-40 (Sigma-Aldrich, Oakville, ON), and Teflon-AF 1600 (DuPont Canada, Mississauga, ON). Indium tin oxide (ITO)-coated glass slides were from Delta Technologies (Stillwater, MN). The food-coloring dye was from McCormack Canada (London, ON), the methyl red indicator was from Sigma-Aldrich, and the HCl was from Fisher Scientific (Whitby, ON).

Electrode Fabrication Through Photolithography: One side of a copper substrate was etched in CE-100 while the other side was protected with dicing tape. Sheets were cut to size and attached to glass slides using double-sided tape, and then rinsed and dehydrated on a hotplate. Devices were primed by spin-coating HMDS (3000 rpm, 30 s) and then coated with Shipley-S1811 (3000 rpm, 30 s). After baking on a hotplate (100 $^{\circ}\text{C}$, 2 min), devices were exposed through a photomask using a Karl-Suss MA6 mask aligner. Photomasks were typically formed by high-resolution printing on transparency film (City Graphics, Toronto, ON); occasionally, chrome-on-glass masks were used, fabricated at the UCLA Nanofabrication facility (Los Angeles, CA). After exposure, substrates were post-baked on a hotplate (100 $^{\circ}\text{C}$, 1 min), developed in MF-321 (3 min) and etched in CE-100 (40 $^{\circ}\text{C}$, 2 min). Remaining photoresist was stripped in AZ300T in an ultrasound cleaner bath (5 min).

Electrode Fabrication Through Laser Printing: An AP7156E sheet was loaded into a desktop laser printer (Samsung ML-2250), and a pattern created in AutoCAD (Autodesk, San Rafael, CA) was printed onto the substrate. Electrodes were formed by immersing the substrate in CE-100 (40 $^{\circ}\text{C}$, 2 min), and the remaining ink was removed by wiping the substrate with an acetone-dampened tissue.

Device Coating: Patterned electrodes were coated with a dielectric layer formed from PDMS or Parylene-C. For PDMS coatings, Sylgard-184 monomer and curing agent were mixed (10:1 (w/w)), degassed under vacuum, and spin-coated (6000 rpm, 1 min on flexible substrates; and 2000 rpm, 1 min on copper boards). After spin-coating, the devices were allowed to sit for 10 min to allow the PDMS to reflow, and were then cured in an oven (typically 160 $^{\circ}\text{C}$, 12 h; how-

ever, coatings with bake times as short as 10 min were also operable). Parylene C was applied to flexible substrates only, using a vapor-deposition instrument (Specialty Coating Systems), for a thickness of 9 μm . In some cases, PDMS-coated substrates were first covered with a 2–3 μm parylene layer. The dimensions and profiles of all coatings were evaluated using a Wyko optical profilometer (Veeco Instruments Inc., Woodbury, NY). After deposition of dielectric layers, devices were spin-coated with Teflon-AF (Teflon-AF resin in Fluorinert FC-40, 1:100 (w/w), 2000 rpm, 1 min, forming a layer approximately 50 nm thick), and then baked on a hot plate (160 °C, 10 min). Unpatterned ITO-coated substrates were also coated with Teflon-AF (ca. 50 nm, as above).

Device Operation: Droplets were formed from aqueous solutions containing food-coloring dye (1:10 (v/v)), methyl red (0.9 mg mL⁻¹), or HCl (120 mM). Droplets were actuated by applying ac electric potentials (18 kHz, 300–500 V, depending on dielectric thickness) between electrodes, and monitored by using a Hitachi CCD camera mated to an imaging lens (Edmund Industrial Optics, Barrington, NJ) positioned over the top of the device. Two configurations were used, with droplets either a) sandwiched between a patterned substrate and an unpatterned ITO/glass slide [3, 4], or b) on a single-patterned substrate [17, 30, 31]. For two-plate experiments, droplets were actuated by applying potentials between the top electrode and sequential electrodes on the bottom plate; one or two pieces of double-sided tape (ca. 75 μm thick each) served as a spacer between plates. For single-plate experiments, droplets were actuated by applying potentials to adjacent pairs of electrodes.

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