SUPPORTING INFORMATION

Electric Triggering for Enhanced Control of Droplet Generation

[1,2]Daihyun Kim, [1,2]Austin Echelmeier, [1,2]Jorvani Cruz Villarreal, [1,2]Sahir Gandhi, [1,2]Sebastian Quintana, [1,2]Ana Egatz-Gomez, [1,2]Alexandra Ros*

[1] School of Molecular Sciences, Arizona State University, Tempe, Arizona 85287, United States
[2] Center for Applied Structural Discovery, The Biodesign Institute, Arizona State University, Tempe, Arizona 85281, United States

*Corresponding author. E-mail: Alexandra.Ros@asu.edu. Phone: +1-480-965-5323. Fax: +1-480-965-7954.

Keywords: microfluidic, droplet, oil, aqueous, crystallography, electrowetting, 3D printing

Table of Contents

Materials and Chemicals

IP-S photoresist was purchased from Nanoscribe GmbH (Eggenstein-Leopoldshafen, Germany) and SU-8 developer was purchased from Microchem (Westborough, MA, U.S.A.). Novec 1720 and double-sided tape were purchased from 3M (U.S.A.). 1H,1H,2H,2H-perfluorooctanol (PFO) was purchased from Alfa Aesar (Ward Hill, MA, U.S.A.). Gallium, perfluorodecalin (PFD), isopropyl alcohol, potassium phosphate monobasic, sodium phosphate dibasic, potassium chloride (KCl), tris hydrochloride (TRIS-HCl), poly(ethylene glycol)methylether (PEGME), 2-(n-morpholino)-ethanesulfonic acid (MES), n-dodecyl β-D-maltoside (β-DDM), Lysozyme (L68786-1G), sodium chloride (NaCl) and sodium acetate (CH_3COONa) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Double/Bubble® Red Extra Fast Drying Epoxy Adhesive was obtained from Hardman, Inc. (U.S.A.). Conductive silver epoxy was purchased from MG Chemical (U.S.A.). Ni-Cr wire was purchased from Omega Engineering Inc. (U.S.A.). Glass slides were obtained from Electron Microscopy Sciences (48 × 60 mm, No. 1; Hatfield, PA, U.S.A.). Ultrapure water was supplied from a Synergy purification system (Millipore, Burlington, MA, U.S.A.). Syringe filters (0.2 μm) were procured from Thermo Scientific (U.S.A.). Fused silica capillaries (Molex LLC, U.S.A.), fittings, ferrules (IDEX Health & Science LLC, Oak Harbor, WA, U.S.A.), PicoClear unions (New Objective, Inc., Woburn, MA, U.S.A.) and Polyetherether ketone (PEEK) tubings (Zeus, Orangeburg, SC, U.S.A.) were used for fluidic connections.

Droplet observation and data analysis

All droplet generation experiments were conducted on the x-y stage of an inverted microscope (IX71, Olympus, U.S.A.) equipped with a 100 W Halogen lamp power supply (TH4-100, Olympus, U.S.A.). A high-speed camera (Photron SA4, Photron, U.S.A.) and a CCD camera (QuantEM 512SC Photometrics, U.S.A.) captured images of droplets in the device. The Ni-Cr wires of the droplet generation device were connected via micro-clamps (LabSmith, U.S.A.) to a high voltage amplifier (AMT-3B20, Matsusada Precision Inc. U.S.A.) driven through a multifunction DAQ card (USB X Series, National Instruments, U.S.A.) programmed by LabVIEW 2014 (version 14.0, National Instruments, U.S.A.). For monitoring of the generated droplets, a custom-built droplet detector measured the transmittance of the oil and aqueous phases in the capillary as described elsewhere.[1] The droplet detector output signal was recorded with Powerlab 8/35 DAQ, coupled with LabChart Pro 8.1 (AD Instrument, Australia). Droplet images were acquired with Micro-Manager software (version 1.4.22), and analyzed with Image J (version 1.4.7, NIH, U.S.A.) and Matlab R2018a (Mathworks, U.S.A.) to determine droplet volume and droplet generation frequency. Reported droplet volumes were calculated by averaging 20 droplets in each case. The fittings and box plots were represented in a graph using Origin software (Origin Corp. Northampton, MA, U.S.A.).

Lysozyme crystallization

To prepare lysozyme crystals, three solutions were prepared. Lysozyme was prepared in DI water with a concentration of 100 mg/mL. A 1 M solution of CH_3COONa (@ pH 5) and a 30% (w/v) solution of NaCl were prepared with DI water. The CH_3COONa and NaCl solutions as well as the DI water used for the lysozyme solution were filtered with a 0.4 μm in-line syringe filter. For lysozyme crystallization, 345 μL of NaCl solution, 75 μL of CH_3COONa solution, 300 μL of the lysozyme solution and 280 μL of DI water were mixed. Crystal formation was apparent in the resulting white, milky suspension. This mixture was stored at 4˚C before use.

Crystal sizes were estimated by using a TS-M1 stage micrometer slide with a resolution of 10 μm. The slide was placed under a microscope and a small aliquot (~ 50 μL) of the crystal suspension

was pipetted on the glass slide covering the micrometer scale. Several images were obtained of the suspended crystals in reference to the micrometer scale and the size of these crystals were estimated using ImageJ. Lysozyme crystals sizes were determined to be in a range from 10 to 30 µm.

Table S1: *Summary of the electrical stimuli investigated, and the effect observed in the T-junction with respect to droplet generation.*

Mode	Initial Conditions	Signal Type	Amplitude (V)	Frequency of applied AC potential (Hz)	Duration of applied potential (time)	Outcome
1	No Droplets	DC	$210 \leq U_{DC} \leq 1000$	-	$300\ ms \leq t_{w,DC} < 60\ s$	Droplet on-demand
2	f_b = constant	AC	U_{AC} = 250	f_u = 100	$t_{w,AC}$ = 10 ms	Phase shift of f_b
3	f_b = constant	AC	$250 \leq U_{AC} < 400$	$100 \leq f_u \leq 400$	$100\ ms \leq t_{w,AC} < 5$ min	Base frequency acceleration (from $f_b$to f_t)

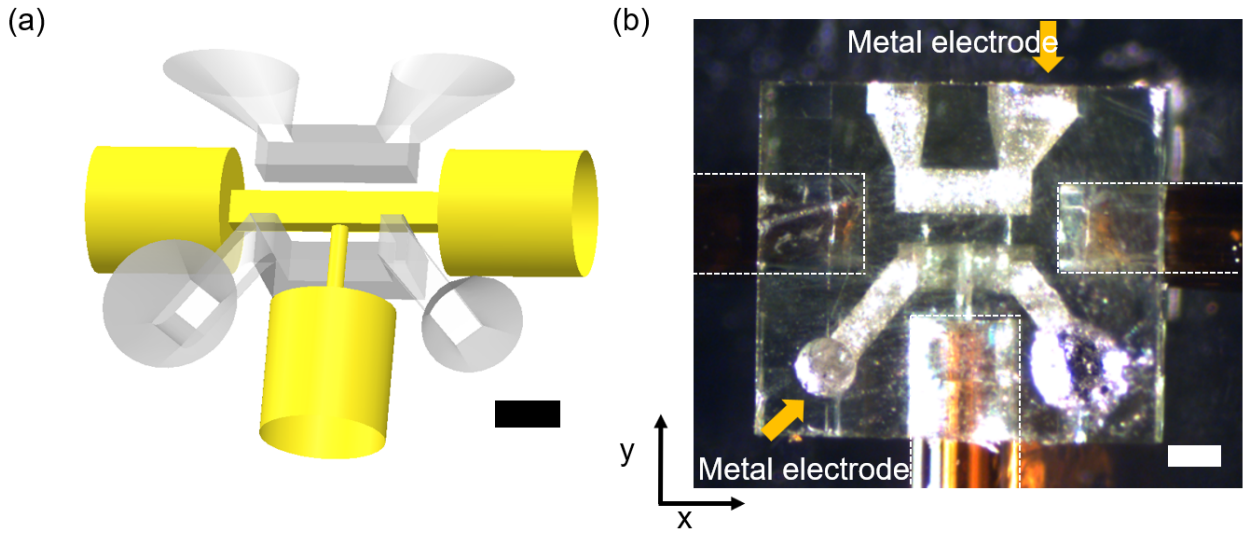

Figure S1. *(a) Computer-aided design of the device for printing shows the fluidic T-junction with capillary connection and two metal electrode channels. (b) Bright-field optical image of the two embedded metal electrodes (arrows) and three capillaries (OD=360 µm, white dotted). All scale bars represent 200 µm.*

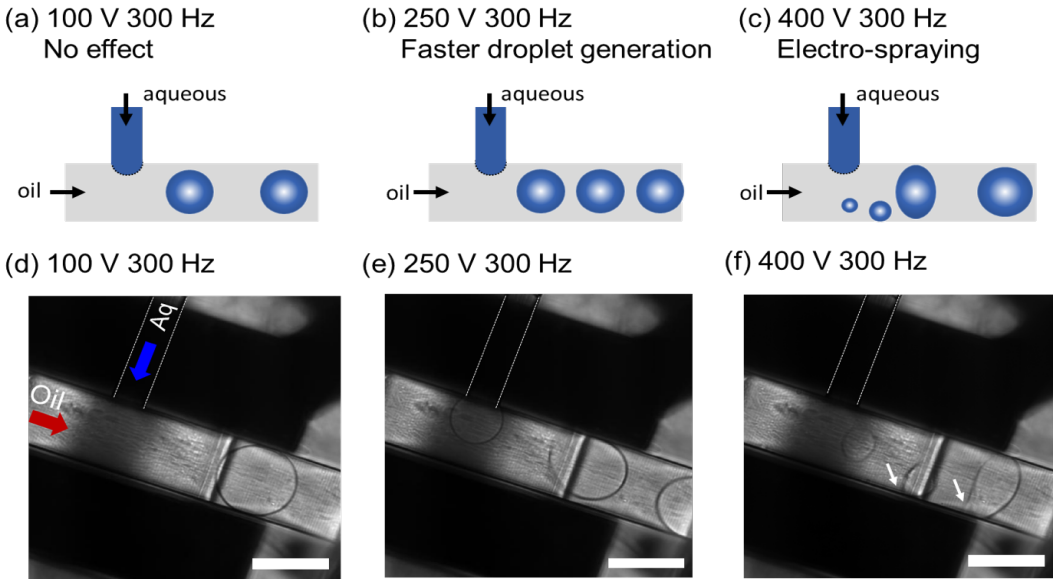

Figure S2. *Schematic representation of 3 regimes (a-c) at the T-junction with different electrical trigger signals and micrographs (d-f) depicting corresponding cases in the actual device. (a) No acceleration observed under the U_{AC} = 250 V. (b) Fast droplets generated under the electrowetting effect (250 ≤ U_{AC} < 400). (c) Higher potential induced a destabilization of droplets by creating satellite droplets. (d) Base frequency f_b observed at U_{AC}= 100 V, f_u= 300 Hz, $t_{w,AC}$ > 100 ms corresponding to case (a). (e) Acceleration of f_b observed for U_{AC} = 250 V at f_u = 300 Hz, $t_{w,AC}$> 100 ms. (f) Electro-spraying observed at higher applied potentials (U_{AC} = 400 V at f_u = 300 Hz, $t_{w,AC}$ > 1 s). Flow conditions were maintained at Q_a = 1 µL/min and Q_o = 10 µL/min for all three conditions described in this figure. Flow in the fluidic channel proceeds from left to right. All scale bars represent 100 µm.*

Video S-1 : Mode I shows a series of single droplet on-demand generation events using a DC trigger signal ($U_{DC,Th}$ = 210 V and $t_{w,DC}$ = 300 ms) in the device (d_{wall} = 5 µm).

Video S-2 : Mode II shows a difference in the droplet distance resultant from the triggering event (U_{AC}= 250 V at f_u = 100 Hz, $t_{w,AC}$= 10 ms) in the device (d_{wall} = 5 µm).

Video S-3 : Mode III shows acceleration of droplet frequency under various AC trigger signals in the device (d_{wall} = 50 µm).

References

(1) Echelmeier, A.; Cruz Villarreal, J.; Kim, D.; Gandhi, S.; Egatz-Gomez, A.; Quintana, S.; Coe, J.; Brehm, G.; Messerschmidt, M.; Meza-Aguilar, J. D.; Weinhaussen, B.; Mills, G.; Vagovic, P.; Kim, Y.; Schultz, J.; Döner, K.; Mancuso, A.; Weierstall, U.; Spence, J. C. H.; Chapman, H. N., et al. Segmented Flow Generator for Serial Crystallography at X-Ray Free Electron Lasers*, Manuscript in preparation.*