SUPPORTING INFORMATION FOR

Sequential Operation Droplet Array (SODA): An Automated Microfluidic Platform for Picoliter-Scale Liquid Handling, Analysis and Screening

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Chemicals. All solvents and chemicals were used as received unless stated otherwise, and demineralized water was used throughout. Recombinant human caspase-1 (BioVision, Mountain View, CA) and its fluorescence substrate (Z-YVAD-R110, Invitrogen, Carlsbad, CA) were prepared in a reaction buffer (pH 7.2) containing 50 mM HEPES, 5 mM EDTA, 100 mM sodium chloride, 10 mM DL-Dithiothreitol (DTT), and 10% glycerol. Six caspase inhibitors: Ac-Tyr-Val-Ala-Asp-CHO, Ac-(NMe)Tyr-Val-Ala-Asp-CHO, Ac-Tyr-(NMe)Val-Ala-(NMe)Asp–CHO, Boc-Asp(OBzl)-Chloromethyl Ketone, Ac-Trp-Glu-His-Asp-CHO, and Z-Val-Ala-Asp-FMK were purchased from Anaspec (Fremont, CA). The 5 mM stock solutions of the inhibitors were prepared in DMSO and were further diluted with water before use. Sodium fluorescein, Rhodamine 110, octadecyltrichlorosilane (OTCS), Span 80, and

mineral oil for molecular biology were purchased from Sigma-Aldrich (St. Louis, MO). Mineral oil containing 0.5% Span 80 (v/v) was used as droplet carrier and droplet array cover. Food dyes were purchased from Shanghai Dyestuffs Research Institute (Shanghai, China). A chemical library containing 5 known caspase inhibitors and 27 randomly picked small-molecule compounds (see Table S1 in Supporting Information) was assembled and stored in 384-well plate with a concentration of 100 μM for each compound.

Building of the SODA System. The SODA system is composed of four parts: a thin-wall capillary with a tapered tip for liquid aspirating, droplet assembling, and droplet depositing; a microchip with 2D picowell array for droplet storage and reaction (Figure S1); a high-precision syringe pump (PHD 2000, Harvard Apparatus, Holliston, MA) with a 1-μL syringe (7000 series, Hamilton, Reno, USA) for liquid metering; and an automated *x*-*y*-*z* translation stage (Zolix, Beijing, China) on which the droplet array chip and a 384-well plate (Corning Life Sciences, San Nicolas, NL) for sample and reagent storage were installed. A program written with Labview (Labview 8.0, National Instruments, TX) was used to control the syringe pump and the *x*-*y*-*z* translation stage simultaneously. Unless mentioned otherwise, the syringe pump was operated at a flow rate of 80 nL/min and the *x*-*y*-*z* translation stage moved with an initial velocity of 6 mm/s, an acceleration of 10 mm/s^2, and a uniform velocity of 10 mm/s. A stereomicroscope (SMZ 850T, Touptek, Hangzhou, China) equipped with a CCD camera (UMD 300, Glory Technology, Hangzhou, China) was used to monitor and record the droplet generation process.

The tapered tip of thin-wall capillary (100 μm i.d., 150 μm o.d., 12 cm length, Reafine Chromatography Co., Yongnian, China) was fabricated by heating its middle region (2 mm length) with a butane lighter and then pulling it to form two tapered capillaries. Using the manual fabrication method, the tip diameters commonly varied in the range of 10–30 μm (i.d.) and 20–40 μm (o.d.). Only the capillaries with tip size of *ca.* 20 μm i.d. and 30 μm o.d were chosen to use. The inner and outer wall surface of the capillary were silanized to be hydrophobic with 1% OTCS in isooctane (v/v).[27] The

capillary was connected to the syringe via a 10-mm-long Tygon tubing (130 µm i.d., 1.7 mm o.d., Saint-Gobain Performance Plastics, France). The microchip with picowell array was fabricated on glass substrate using standard photolithographic and wet etching technique as described previously.[28] With an etching time of 60 min in an etching solution ($HF/NH_4F/HNO_3$, 1.0/0.5/0.75 mol/L), a picowell array with each well size of *ca.* 60 µm in depth, 170 µm in diameter, 250 µm center-to-center distance between adjacent wells, and with a density of 1600 droplets/cm^2 was produced. The surface of the picowell chip was modified to be hydrophobic as described above. Finally, a 2-mm thick glass frame was glued on the microchip surrounding the picowell array for containing the cover oil.

A home-made fluorescence detector was used to measure the fluorescence intensity of the droplet array as described previously.[29] Briefly, two high-power LED lamps (460 nm, 3W, CREE, Durham, NC) were used to illuminate the droplet array from the top-left and top-right of the microchip, respectively. The fluorescence was collected with a camera lens (Computar MLM-3XMP, Daheng Image, Beijing, China) through a filter (535AF40, Omega Optical, Brattleboro, VT), and recorded with a high sensitive CCD camera (DH-SV1401FC/FM, Daheng Image, Beijing, China). The grayscale value of each droplet was extracted from image using ImageJ (NIH free software).

Safety Consideration The wet etching for glass picowell-array chips, and the silanization of the chips and capillaries should be performed in a well-ventilated hood, while wearing protective goggles and gloves.

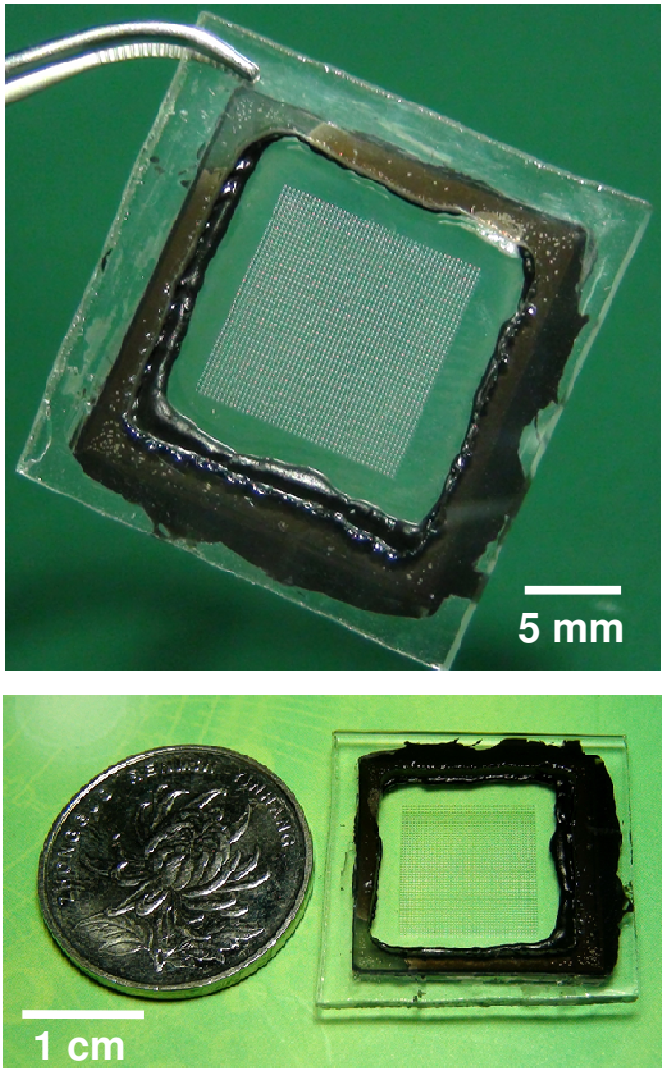


Figure S1. Images of the microchip with picowell array. The picowell array was fabricated at a density of 1600 droplets/cm^2.

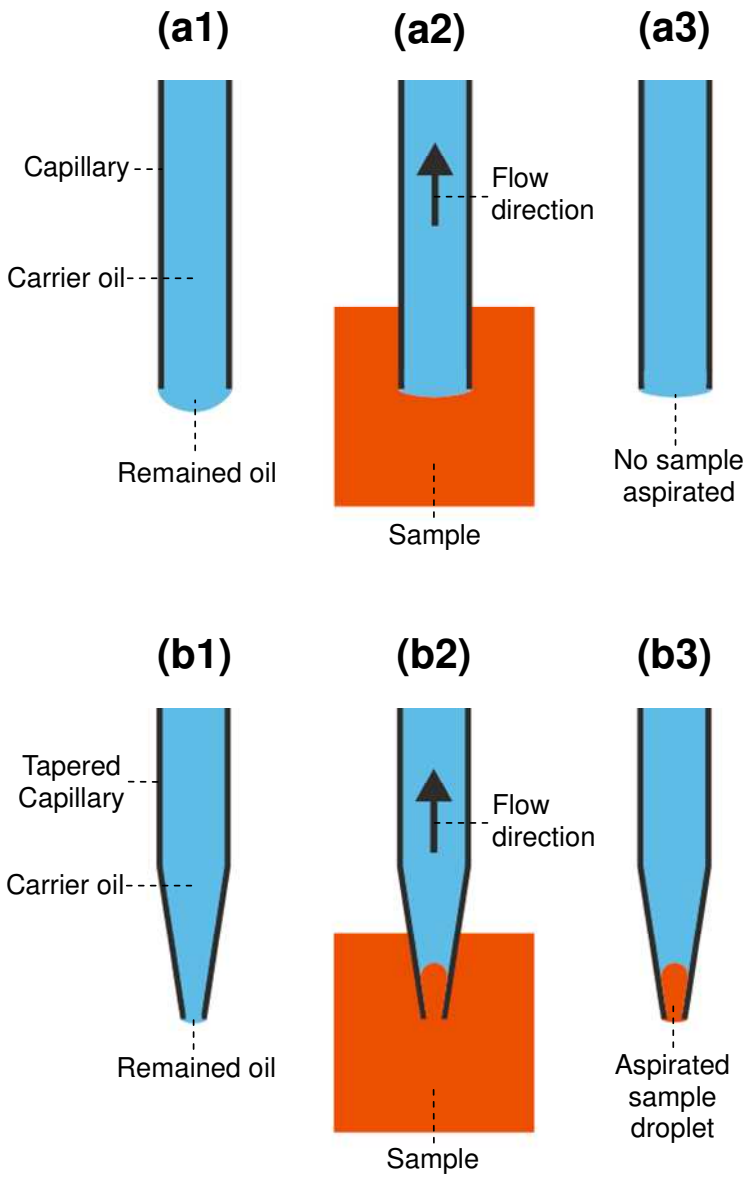


Figure S2. Schematic diagram of droplet aspiration process in two capillaries with different capillary tip shapes, flat tip (a) and tapered tip (b) (not to scale). With the flat tip capillary, larger volume (usually 200–300 pL for a capillary with an outer diameter of 150 µm) of oil is remained at the tip end of the capillary when it is removed from an oil well and inserted in a aqueous sample solution for sampling. If the set aspirating volume is lower than the remained oil volume, no sample solution will be aspirated into the capillary. With the tapered capillary, few oil (usually lower than 5 pL for a tapered tip with a diameter of 20 µm) is remained at the capillary tip end, thus it has no evident effect on picoliter-scale liquid aspirating.

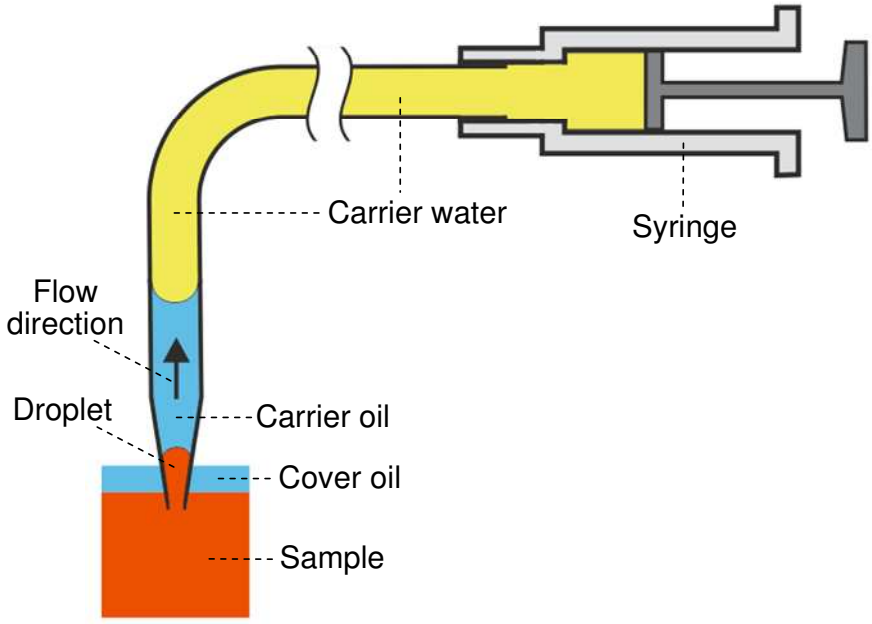


Figure S3. Schematic diagram showing the liquids in the capillary-syringe pump module. The syringe and capillary are filled with carrier water instead of oil to minimize the thermal expansion and contraction of the liquid filled in them. A 10 nL plug of carrier oil is filled in the tip of the capillary to segment the aspirated droplet from the carrier water.

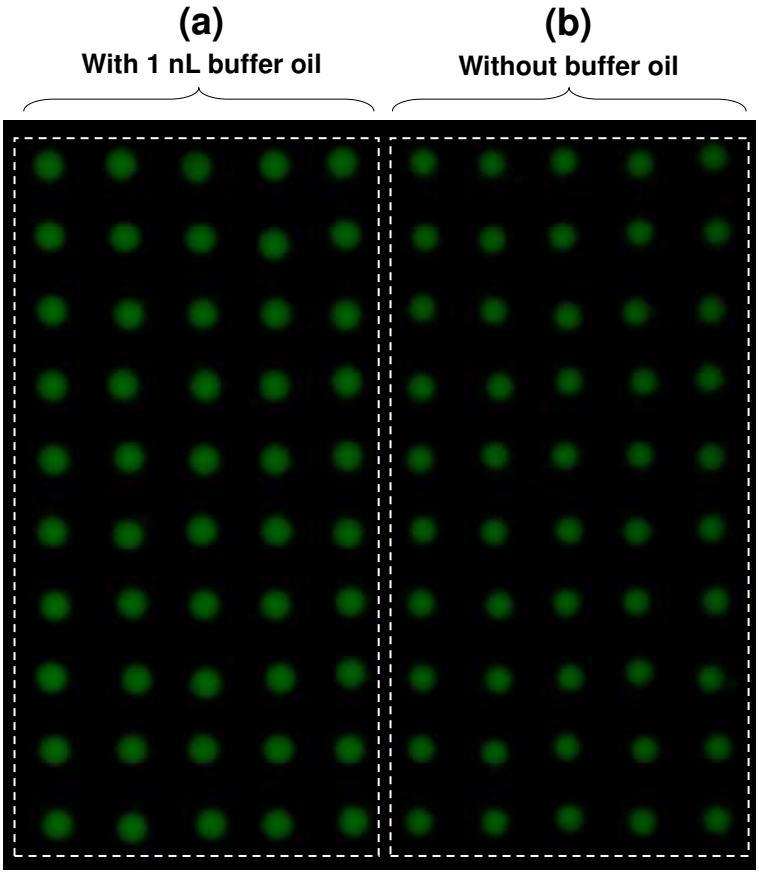


Figure S4. Fluorescent image of droplet array including 50 droplets generated with 1-nL buffer oil (a) and 50 droplets without the buffer oil (b), respectively. The set droplet volume in both (a) and (b) experiments is 990 pL. An evident decrease in droplet volumes (ca. 300 pL) can be observed in (b) compared with (a) due to the influence of the syringe pump mechanical backlash.

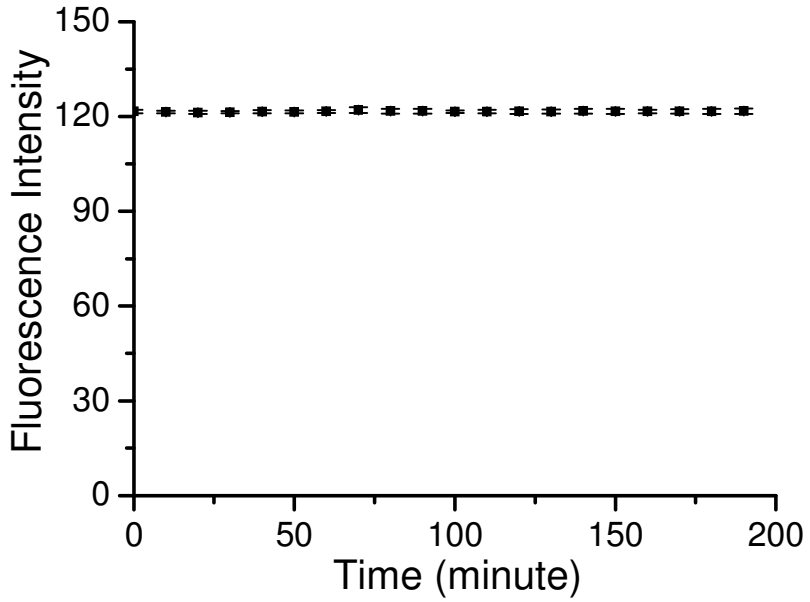


Figure S5. Testing of the effect of oil in reducing evaporation of water in droplets by monitoring the fluorescence intensity of three 480-pL droplets for over three hours using sodium fluorescein (10 μM) as a model sample.

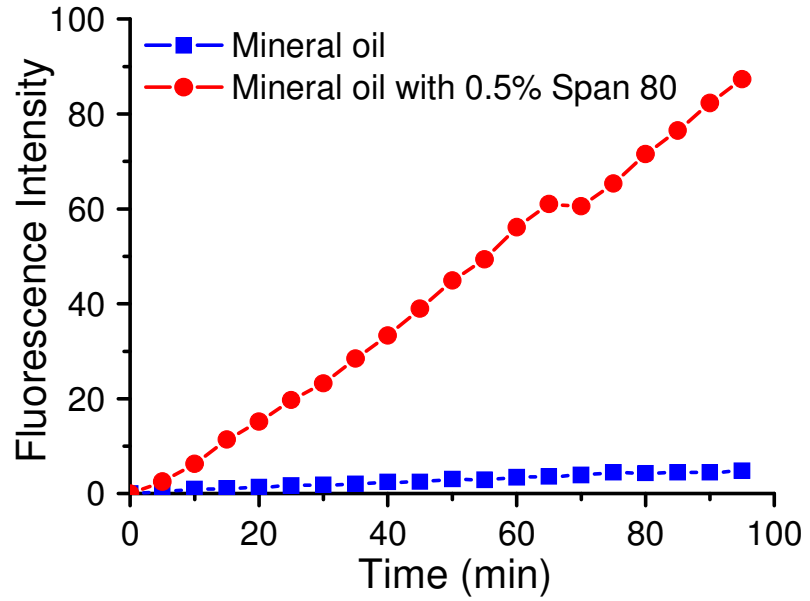


Figure S6. Results of enzyme activity measurement for caspase-1 in droplets using mineral oil with and without surfactant Span 80 (0.5%, v/v). Droplet volume: 1.98 nL; enzyme concentration: 6 mU/μL; substrate concentration: 20 μM.

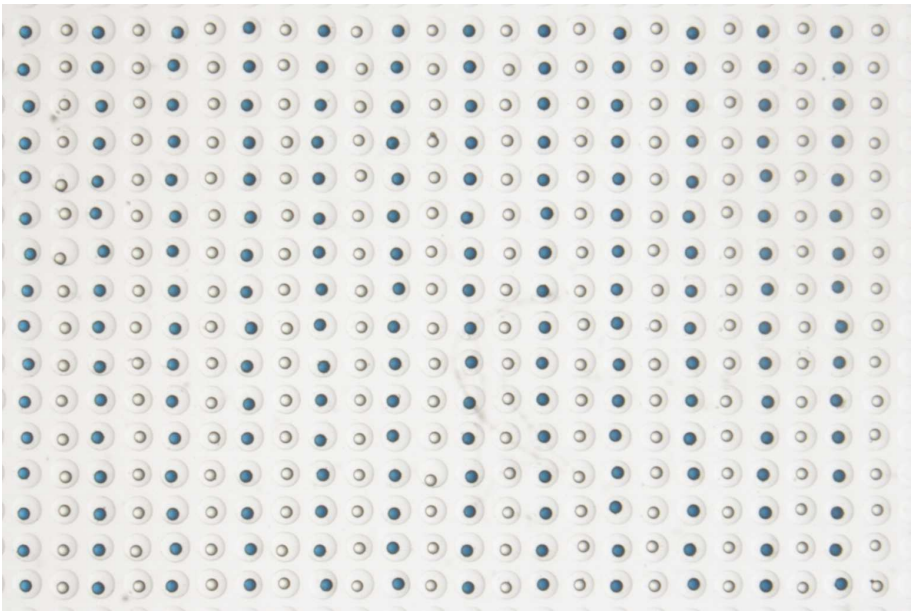


Figure S7. A CCD image of an array of 384, 480-pL droplets with different dyes for showing the ability of the SODA system in generation large number of droplets.

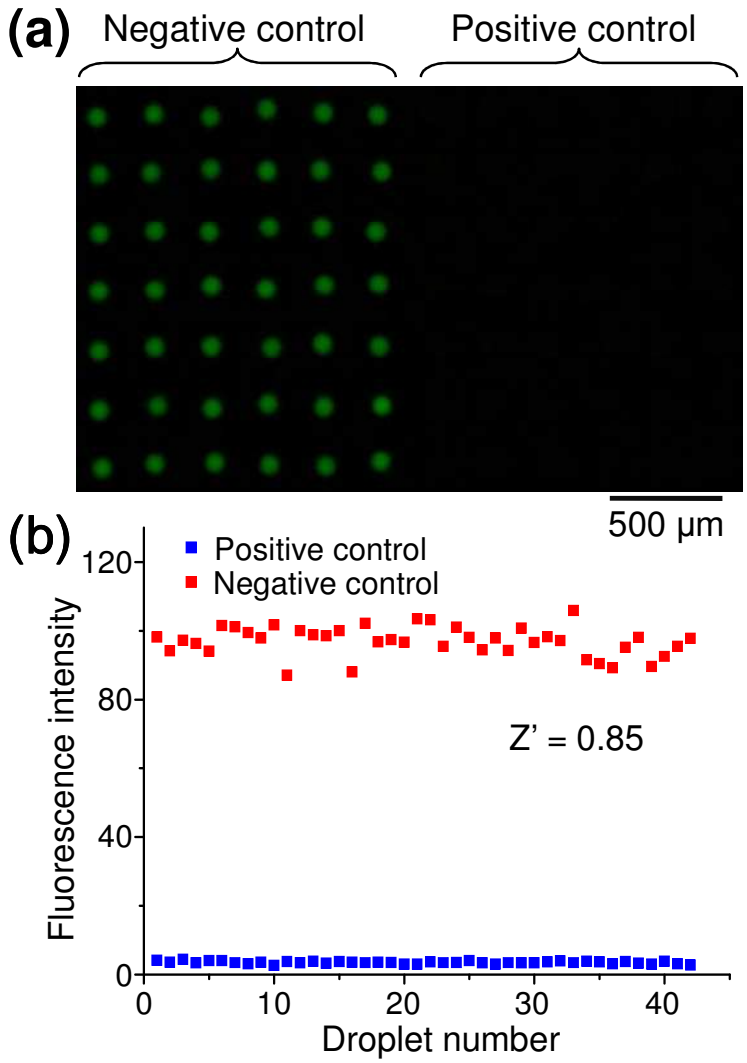


Figure S8. Evaluation of the repeatability of the SODA system for enzyme inhibition screening. (a) Fluorescent image of a droplet array for enzyme microreactions with 84 droplets including 42 droplets without inhibitor as negative control and 42 droplets with an known inhibitor (Ac-(NMe)Tyr-Val-Ala-Asp-CHO, 100 µM) as positive control. Each droplet contains 180-pL enzyme (caspase-1, 6 mU/µL), 180-pL substrate (20 µM), and 180-pL inhibitor (100 µM) solutions or water. (b) The fluorescence intensities of negative control droplets (red dots) in comparison with those of positive control droplets (blue dots). The *Z'*-factor value is calculated as 0.85 from these results.

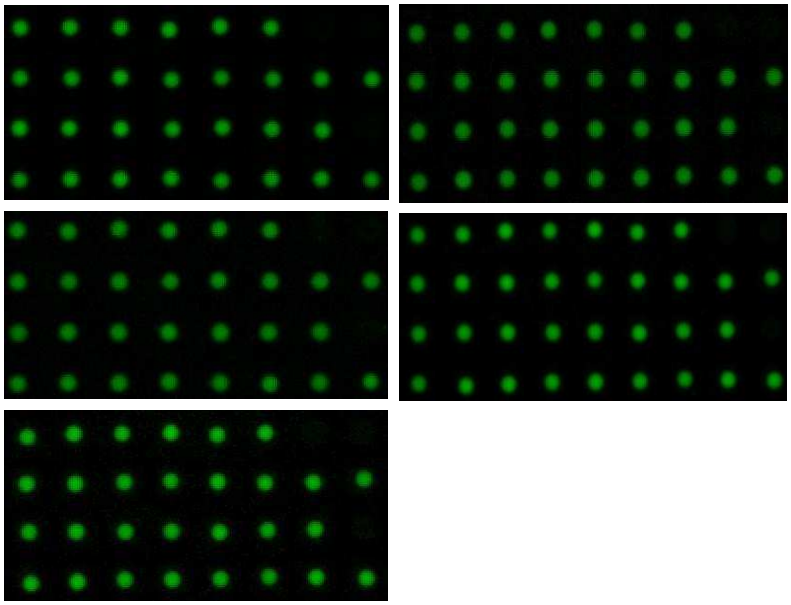
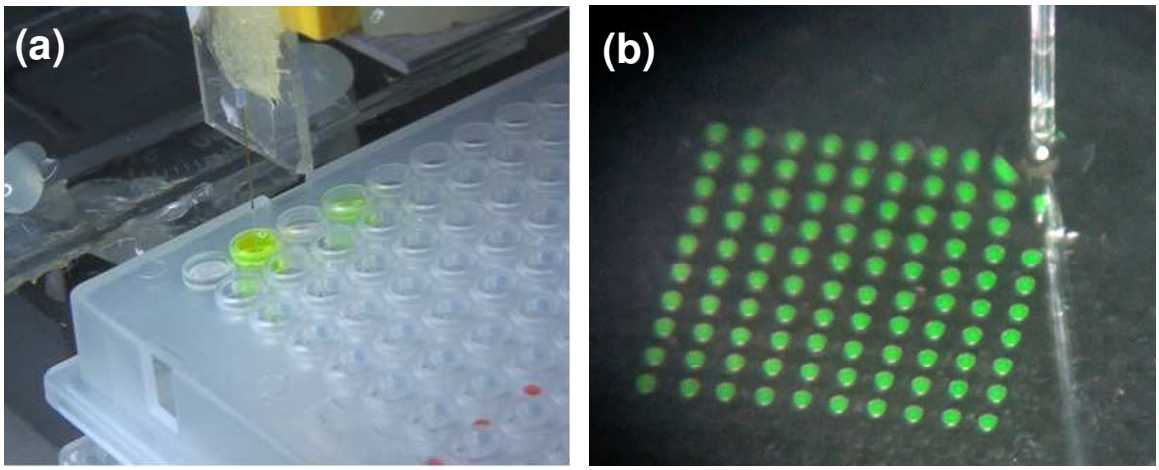


Figure S9. Fluorescent images showing the 32-compound droplets for inhibitor screening in five parallel experiments. Each droplet contains 180-pL enzyme (caspase-1, 6 mU/μL), 180-pL substrate (20 μM), and 180-pL compound solution (100 μM).

Table S1. Compositions of the compound library for screening.

	Compound name	Molecular weight
1	Water (negative control)	18
2	Glutamicacid (Glu)	147
3	Threonine (Thr)	119
4	Histidine (His)	155
5	Arginine (Arg)	174
6	Glutarnine (Gln)	146
7	Lysine (Lys)	146
8	Proline (Pro)	115
9	Cystine (Cys)	121
10	Serine (Ser)	105
11	Leucine (Leu)	131
12	Glycine (Gly)	75
13	Aspartic Acid (Asp)	133
14	Alanine (Ala)	89
15	Tyrosine (Tyr)	181
16	Tryptophan (Trp)	204
17	Isoleucine (Ile)	131
18	Valine (Val)	117
19	Phenylalanine (Phe)	165
20	Methionine (Met)	149
21	Reserpine	609
22	Angiotensin I	1296
23	Angiotensin II	1046
24	Lys-Cys-Asp-Ile-Cys-Thr-Asp-Glu-Tyr	1089
25	n-Butylamine	73

26	Cyclohexylamin	99
27	DMSO	78
28	Ac-(NMe)Tyr-Val-Ala-AspCHO	507
29	Ac-Tyr-(NMe)Val-Ala-(NMe)Asp-CHO	522
30	Ac-Trp-Glu-His-Asp-CHO	612
31	BOC-Asp(OBzl)-Chlomethyl Ketone	356
32	Ac-Tyr-Val-Ala-Asp-CHO	492

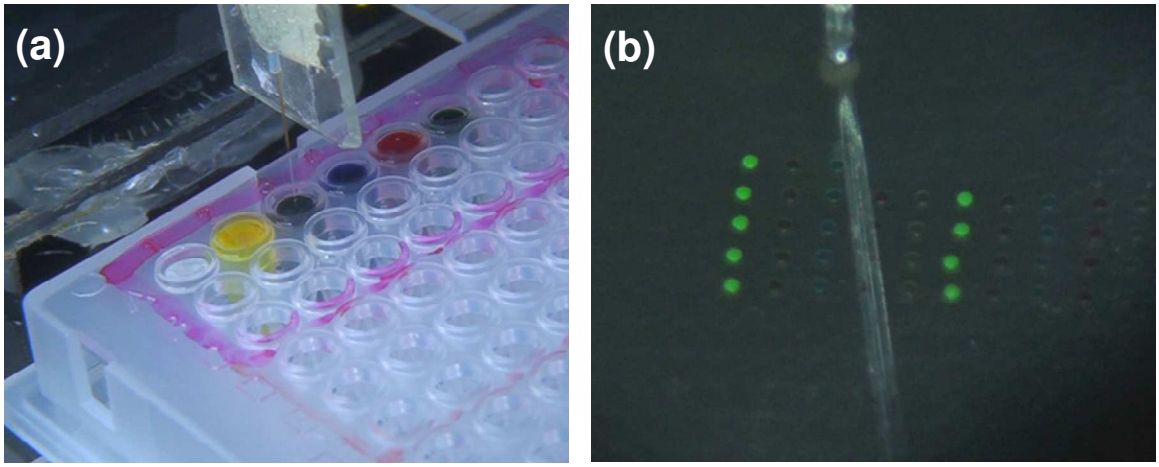


Movie S1. Recording of liquid aspirating (a) and depositing (b) process for generating a composite droplet.

Table S2. Operation program used in Movie S1 for generating a composite droplet

Step	Position	Action of syringe pump	Volume (nL)	Function
1	MWP (0, 0)	Aspiration	1.00	Aspirate 1-nL buffer oil from well (0, 0) in MWP.
2	MWP (0, 1)	Aspiration	0.18	Aspirate 0.18-nL sample from well (0, 1) in MWP.
3	MWP (0, 2)	Aspiration	0.18	Aspirate 0.18-nL reagent 1 from well (0, 2) in MWP.
4	MWP (0, 3)	Aspiration	0.18	Aspirate 0.18-nL reagent 2 from well (0, 3) in MWP.
5	PWC (9, 7)	Dispensing	1.54	Deposit the assembled droplet on well (9, 7) in PWC.

Microwell plate, MWP; picowell chip, PWC.



Movie S2. Recording of liquid aspirating (a) and depositing (b) process for generating different dye droplets.

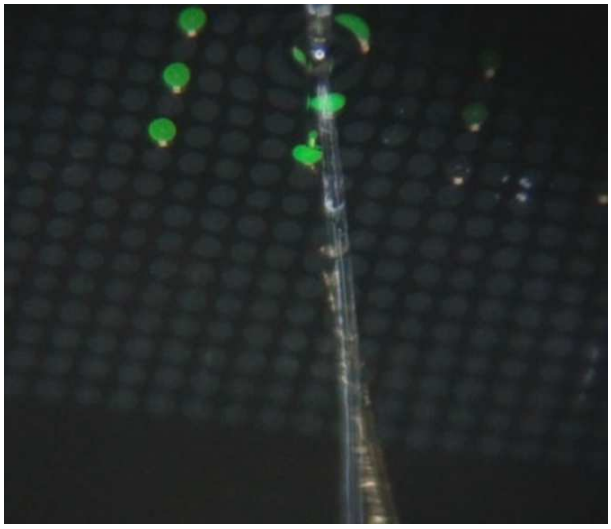
Table S3. Operation program used in Movie S2 for generating different dye droplets.

Step	Position	Action of syringe pump	Volume (nL)	Function
1	MWP (0, 0)	Aspiration	1.00	Aspirate 1-nL buffer oil from well (0, 0) in MWP.
2	MWP (0, 2)	Aspiration	0.48	Aspirate 0.48-nL sample 1 from well (0, 2) in MWP.
3	PWC (1, 4)	Dispensing	1.48	Deposit the droplet on well (1, 4) in PWC.
4	MWP (0, 0)	Aspiration	1.00	Aspirate 1-nL buffer oil from well (0, 0) in MWP.
5	MWP (0, 3)	Aspiration	0.48	Aspirate 0.48-nL sample 2 from well (0, 3) in MWP.
6	PWC (2, 4)	Dispensing	1.48	Deposit the droplet on well (2, 4) in PWC.

Microwell plate, MWP; picowell chip, PWC.

Table S4. Calculated inhibition percentage of the combined inhibitors in Figure 6.

	No. 28	*No.* 30	*No.* 32	Z-VAD-FMK
No. 28	60%	65%	63%	58%
No. 30	71%	79%	70%	70%
No. 32	64%	74%	62%	56%
Z-VAD-FMK	59%	72%	59%	56%



Movie S3. Recording of serial dilution process of nanoliter-scale droplets.

Table S5. Operation program used in Movie S3 for serial dilution of sodium fluorescein droplets.

Step	Position	Action of syringe pump	Volume (nL)	Function
1	PWC (0, 0)	Aspiration	1.00	Aspirate 1-nL buffer oil from well (0, 0) in PWC.
2	PWC (5, 8)	Aspiration	0.48	Aspirate 0.48-nL solution from droplet of 10 mM sodium fluorescein in PWC.
3	PWC (9, 8)	Dispensing	1.48	Deposit the droplet into a preformed 4.32 nL buffer droplet in PWC to form a droplet of 1 mM sodium fluorescein.
4	PWC (0, 0)	Aspiration	1.00	Aspirate 1-nL buffer oil from well (0, 0) in PWC.
5	PWC (9, 8)	Aspiration	0.48	Aspirate 0.48-nL solution from droplet with 1 mM sodium fluorescein in PWC.
6	PWC (13, 8)	Dispensing	1.48	Deposit the droplet into a preformed 4.32 nL buffer droplet in PWC to form a droplet of 0.1 mM sodium fluorescein.
7	PWC (0, 0)	Aspiration	1.00	Aspirate 1-nL buffer oil from well (0, 0) in PWC.
8	PWC (13, 8)	Aspiration	0.48	Aspirate 0.48-nL solution from droplet of 0.1 mM sodium fluorescein in PWC.
9	PWC (17, 8)	Dispensing	1.48	Deposit the droplet into a preformed 4.32 nL buffer droplet in PWC to form a droplet of 0.01 mM sodium fluorescein.

Picowell chip, PWC