Supplementary Information

Microfluidic static droplet arrays with tuneable gradients in material composition

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Section 1

Materials Solutions of black, blue, red and yellow dyes (McCormick) were diluted 20X by distilled water and used as reagent plugs. 0.1% (w/v) Span 80 (Fluka) in mineral oil (Sigma-Aldrich) was used as carrier fluid. 10^{-3}-10^{-8} M standard solutions of fluorescein sodium salt (Sigma-Aldrich) were freshly prepared in distilled water before experiment to assess the limits of dilution. 1 μm and 0.2 μm fluorescent beads (yellow-green and blue, Molecular Probe) were diluted 1000X yielding particle densities of 3.6×10^{7} and 4.5×10^{9} per mL. Human leukemia cancer cell line CCRF-CEM was maintained and grown in RPMI-1640 medium containing 10% fetal bovine serum and 0.1% Insulin-Transferrin-Selenium at 37 ºC in 5% CO_2. Cell density of 5×10^{5} cells/mL was used in the experiments.

Device Design The footprint of the 60-loop static droplet array is shown in Fig. S1. There are two flow paths in each loop – the bypass channel and the lower branch with a fluidic trap. The dimensions of the loop determine the ratio (R_B/R_T) of the hydrodynamic resistances of the two branches. Here R_B and R_T are the hydrodynamic resistance of the bypass channel and lower branch respectively. Except for Fig. 4b, all experiments reported in this study, the width and length of the bypass channel was 200 μm and 2000 μm respectively. In the lower branch, the diameter of the trap is 450 μm, and the width and length of the constriction are 40 μm and 100 μm respectively. The hydrodynamic resistance of each branch was calculated using the exact analytical solution of Poiseuille flow in a rectangular channel as shown in Eq. 1, where L, h and w are the length, height and width of the channel respectively, and μ is the viscosity of oil.

$$R = \frac{12\mu L}{h^3 w}\left[1 - \sum_{n,odd}^{\infty} \frac{1}{n^5} \times \frac{192}{\pi^5} \times \frac{h}{w} \tanh\left(\frac{n\pi w}{2h}\right)\right]^{-1} \quad \text{(Eq. 1)}$$

The calculated flow resistance ratio R_B/R_T is therefore 3.17.

We also used a design with R_B/R_T = 1.59 to trap a train of droplets generated by T-junction, which contain single cells (See Fig. 4b). In this case, the diameter of the trap is 320 μm, the width and length of bypass channel are 200 and 4000 μm, the width and length of the constriction channel are 40 and 100 μm respectively.

Dimensionless numbers The capillary number, Ca = μV/γ, where μ is the viscosity of carrier fluid (mineral oil, μ = 0.03 Pa·s); V is a characteristic velocity of the carrier fluid and γ is the surface tension between the two liquid phases. In our experiments V ranges from 0.2-2 μL/min (8.33×10^{-5}-8.33×10^{-4} m/s). In the concentration range of 0-2% Span 80, the interfacial tension for mineral oil-water system is 50-5 mN/m[S1]. Therefore Ca was in a range of 5×10^{-5}-5×10^{-3} in our experiments. The Reynolds number in our experiments, defined as ρVh/μ, ranges from 4.7×10^{-4}-4.8×10^{-3}. Here ρ (= 0.8390-0.8590 kg/m^3) is the density of mineral oil.

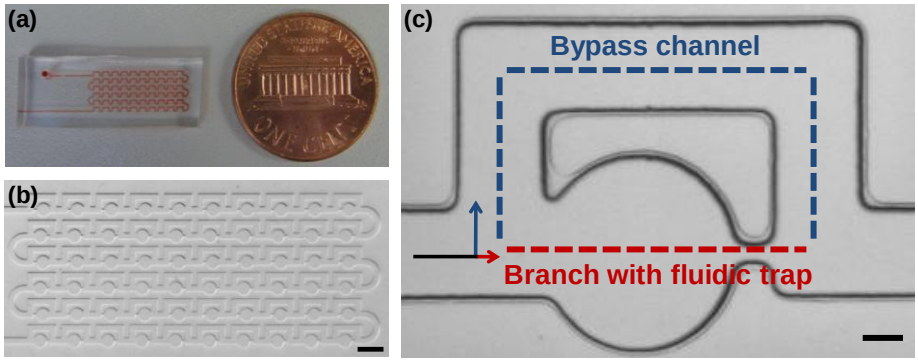

Fig.S1 (a) Image of the microfluidic chip versus a penny. (b) An enlarged view shows the 60 traps. Scale bar: 1 mm. (c) An enlarged view of the loop containing a bypass channel (blue dash line) and a branch (red dash line) with the hydrodynamic trap. Scale bar: 100 μm.

Device Fabrication Standard photolithography procedures were used to generate a mold (SU-8 2100, MicroChem) with uniform feature height of 200 µm. Devices were fabricated by pouring polydimethylsiloxane (PDMS) on the mold with subsequent curing. The PDMS replicas were bonded to another flat PDMS substrate using plasma treatment. The surface of the channels was modified by treatment with Aquapel (PPG Industries) followed by drying with air.

Microfluidic Experiments A PTFE Tubing (203 µm i.d., 356 µm o.d., Zeus, USA) was used as cartridge to supply samples to the microfluidic device through a Tygon tubing (250 µm i.d., 760 µm o.d., Saint-Gobain, USA) and a syringe (Gastight 1710, Hamilton, USA) on syringe pump (PHD2000, Harvard Apparatus, USA). Experiments were conducted on either a stereo microscope (SZX16, Olympus, Japan) or an inverted fluorescence microscope (IX71, Olympus, Japan). Images were recorded using CCD (StreamView-LR, SVSi, USA) and CMOS cameras (PL-B776F, PixeLINK, Canada). Images were analyzed using Image J v.1.43 (National Institutes of Health, USA). Both the capillary number and Reynolds number are less than 0.005 in our experiments.

Section 2

A video illustrating the movement of sample plug (2.2-µL) in the network that leads to the formation of a static droplet array of uniform composition (Movie S1). The plug is injected at 1 µL/min.

Section 3

A video illustrating the coalescence, mixing and dilution of the sample array by a diluting plug (Movie S2). The plug volume and injection flow rate are 2.2-µL and 0.5 µL/min respectively.

Section 4

A video showing a close-up view of the dynamics of dilution (Movie S3). The stationary droplet contains 10^{-3} M fluorescein solution. The diluting plug of volume 0.8 µL was injected at 0.5 µL/min.

Section 5

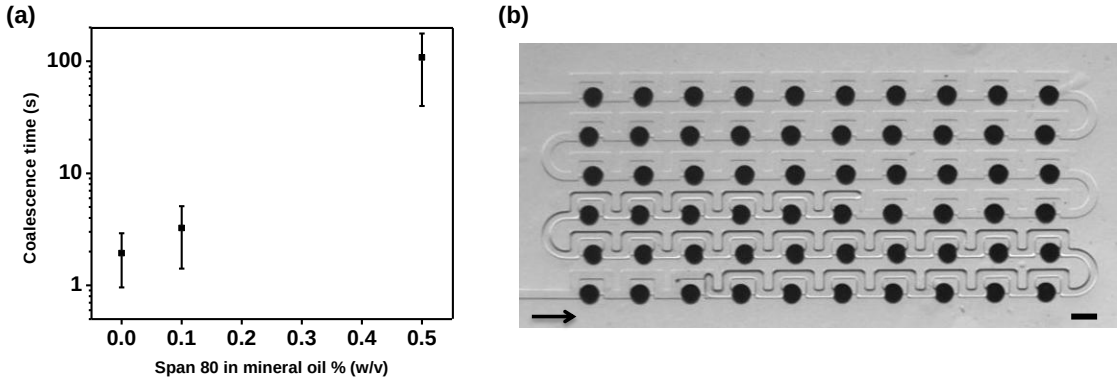

Fig. S2 (a) Effect of surfactant concentration on rupture time between diluting water plug and trapped black-dye droplets at flow rate of 0.5 µL/min. (b) Snapshot of a water plug flowing through the droplets array without mixing in 2% Span 80 in mineral oil (w/v). Scale bar: 500 µm.

We find that the concentration of the surfactant is important in regulating the coalescence between the stationary drop and the moving diluting plug. To quantify the effect of surfactant on coalescence we defined the 'coalescence time' as the time interval between the first (visual) contact of the moving plug with the immobilized drop and the instant at which coalescence occurs. As shown in Fig. S2a, the coalescence time increases with increase in surfactant concentration for a given moving plug flow rate (0.5 µL/min). This observation is consistent with other studies, which report that surfactants retard coalescence due to prolonged film drainage times[S2,S3]. In fact, at a concentration of 2% Span 80, we do not observe coalescence between the moving plug and immobilized droplets as shown in Fig. S2b. In addition, we believe that the noise in dilution concentration profiles (c.f. Fig. 2a-c) was caused by the heterogeneity of coalescence time (apparent from the standard deviation in Fig. S2a).

Section 6

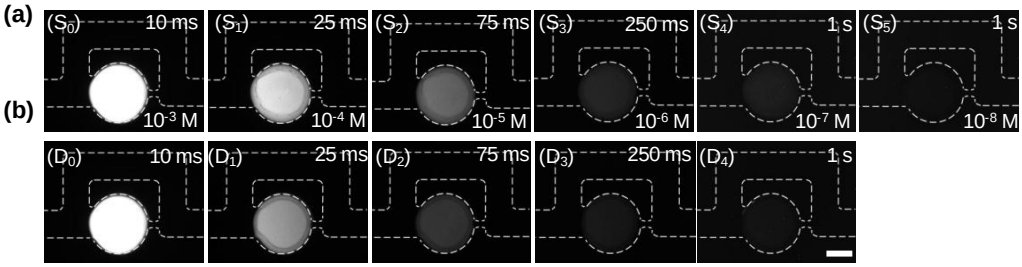

Fig. S3 Comparison images of standard and diluted fluorescein droplets. (S_0-S_5) Fluorescent images of the standard fluorescein droplets from 10^{-3} M to 10^{-8} M. (D_0-D_4) Fluorescent images of the diluted fluorescein droplets after each diluting by a 2.2-µL-water slug at flow rate of 0.5 µL/min; subscript n indicates the number of the diluting slugs. Continuous phase is 0.1% (w/v) Span 80 in mineral oil. Scale bar: 200 µm.

To assess the lower limit on the dilution generated by our method, we generated a static droplet array containing 10^{-3} M fluorescein and diluted it sequentially with 4 water plugs (2.2 µL) at a flow rate of 0.5 µL/min. To quantify the fluorescein concentration in the first drop in the array after each dilution we used the following procedure. First, we prepared a set of fluorescein solutions of known concentrations and generated static droplet arrays with each of the standard solution (Fig. S3a). Images were acquired of the array at various exposure times to ensure the intensity signal is within the dynamic range of the camera. Second, a similar image acquisition protocol was followed to image the first droplet after each dilution (Fig. S3b). Finally, we used the 'calibration images' from the fluorescein standards to estimate the fluorescein concentration in the first drop after each dilution. The resulting data is shown in Fig. 2d.

References

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