Electronic Supplementary Information

Self-generation of two-dimensional droplet array using oil-water immiscibility and replacement

Hiroki Yasuga, Koki Kamiya, Shoji Takeuchi and Norihisa Miki

Experiment S1. Preparation of calibration curves for the determination of actual concentration.

We prepared calibration curves by investigating the relationship between concentration of molecules and fluorescence intensity. It is reported that fluorescence intensities linearly increase with molecular concentration [1]. The linear calibration curves were prepared as follows. First, 1 µl of rhodamine B solution was placed on a glass slide. The concentration was 0, 1, 2, 3, 4, 4.2, 4.3, 4.4, and 4.6 µM. Then, an acrylic square frame of 0.5 mm thickness was attached on another glass slide. Next, the slide with solution was placed with flipping onto the other glass slide. Finally, the container surrounded by the frame was immersed with n-decane as the solution were formed as half-sphere droplets. The droplets were laser-scanned with a confocal microscope. Fluorescence intensities on each concentration were obtained from the scanned images by using ImageJ. The experiments were conducted three times. Same experiment and analysis were conducted on using fluorescein with the concentration of 0, 5, 10, 15, and 20 µM.

ESI Figures S2A and B show the relationship between concentration and fluorescence intensity on rhodamine B and fluorescein, respectively. The calibration curves shown in the graphs were prepared as the linear least-squares fit of mean values. The obtained equation is used for the determination of concentration from fluorescence intensity. For the rhodamine B, because the increase in fluorescence intensity with respect to concentration was drastically changed at approximately 4 µM, two calibration curves were prepared as set 4 µM as the boundary. We consider the change originated from the tendency for rhodamine B to gather at oil-water interface [2]. To calibrate fluorescence intensity into concentration, the boundary of the fluorescence intensity was set as 9.64 on rhodamine B: On a lower value than the boundary, the equation of $I = 1.57\,C + 3.35$ (where I is fluorescence intensity and C is concentration) was used while $I = 100.12\,C - 395.40$ was used otherwise.

[1] B. Vazquez, N. Qureshi, L. Oropeza-Ramos and L. F. Olguin, *Lab Chip*, 2014, **14**, 3550–3555.
[2] J. H. J. Thijssen, A. B. Schofield and P. S. Clegg, *Soft Matter*, 2011, **7**, 7965.

Table S1. Parameters of dimension (width, step height, A_g, and A_p). Components of this table show the step heights. Gray area shows no fabricated parameters.

		A_p				
Width	A_g	0	2	4	6	8
100 µm	5	0 µm	200 µm	400 µm	600 µm	800 µm
50 µm	10	0 µm	100 µm	200 µm	300 µm	400 µm
33 µm	15	0 µm	66 µm	133 µm	200 µm	266 µm
25 µm	20	0 µm	50 µm	100 µm	150 µm	200 µm
20 µm	25	0 µm	40 µm	80 µm	120 µm	160 µm
		Step height				

Table S2. Volume of injected solvent with respect to A_g and A_p. Gray area shows no fabricated parameters.

	A_p				
A_g	0	2	4	6	8
5	4.5 µl	6.3 µl	8.1 µl	9.9 µl	11.7 µl
10	4.5 µl	5.4 µl	6.3 µl	7.2 µl	8.1 µl
15	4.5 µl	5.1 µl	5.7 µl	6.3 µl	6.9 µl
20	4.5 µl	5.0 µl	5.4 µl	5.9 µl	6.3 µl
25	4.5 µl	4.9 µl	5.2 µl	5.6 µl	5.9 µl

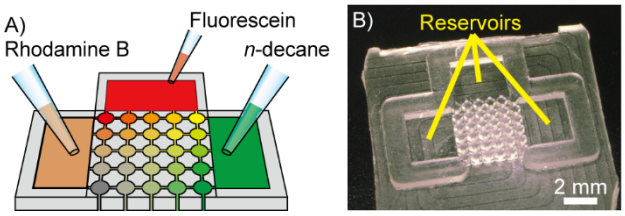

Figure S1. A) Formation of concentration gradient of two fluorescence molecules and B) An image of device.

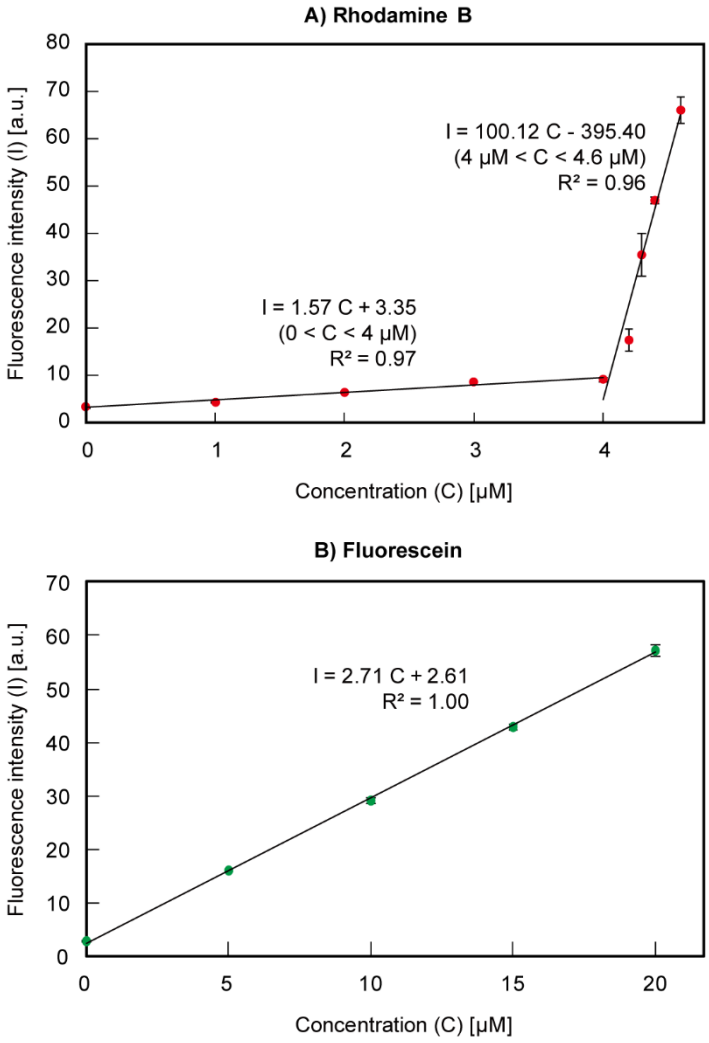

Figure S2. Calibration curves between concentration of fluorescent molecules and fluorescence intensity. A) Rhodamine B and B) fluorescein.

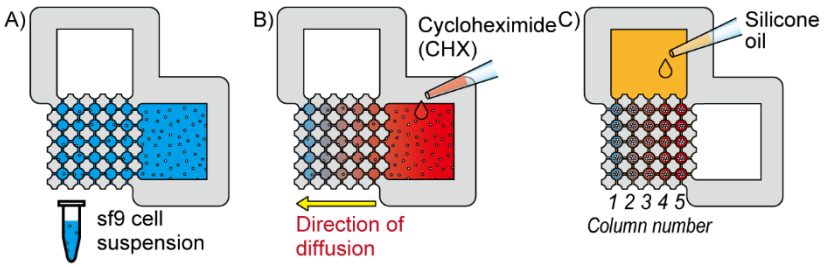

Figure S3. Procedures to prepare sf9 cell and form cycloheximide (CHX) concentration gradient into droplet array. A) Impregnation of sf9 cell suspension. B) Formation of CHX concentration gradient by diffusion. C) Droplet generation by silicone oil injection.

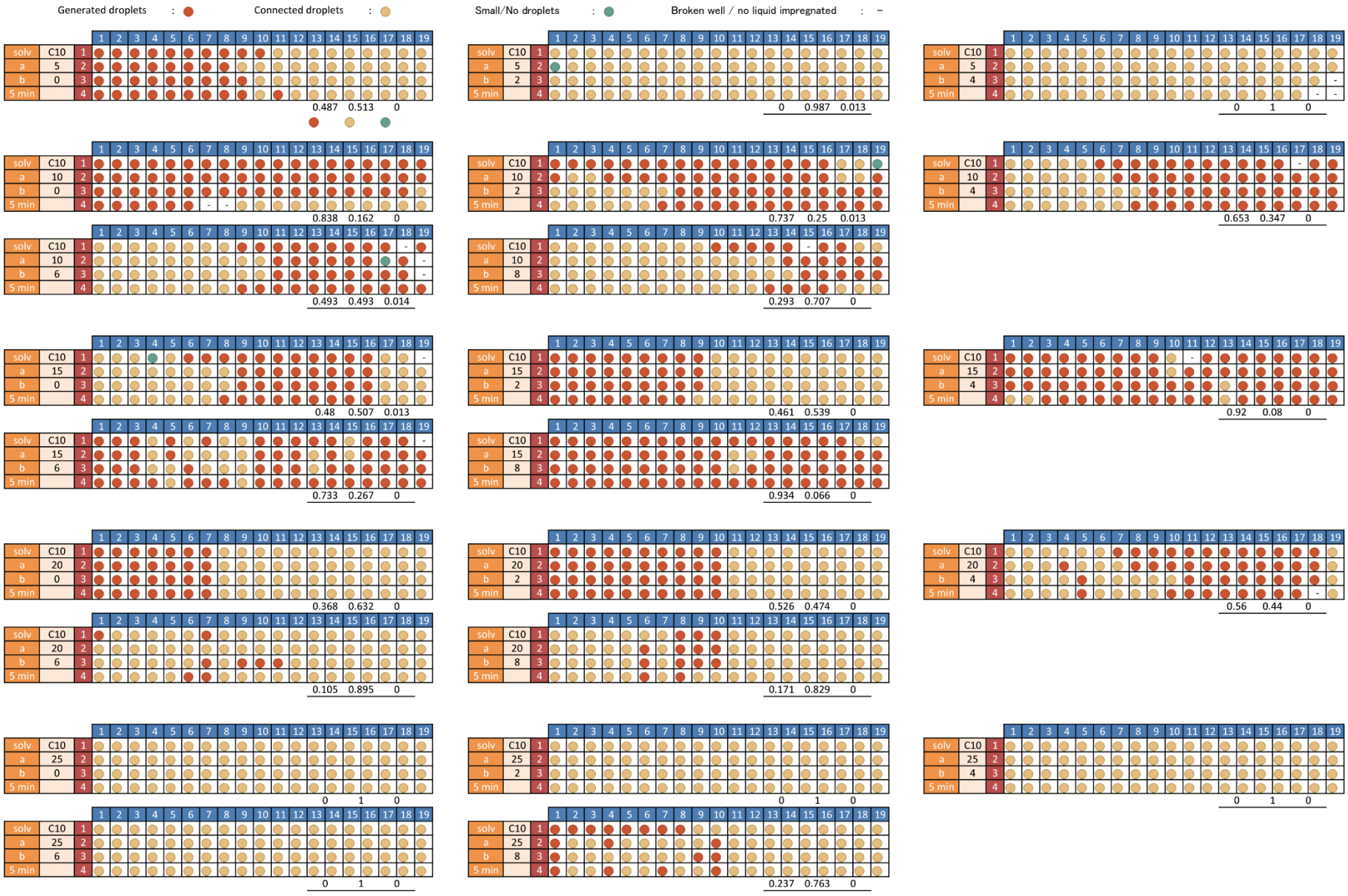

Figure S4 Details of droplet states on using *n*-decnae

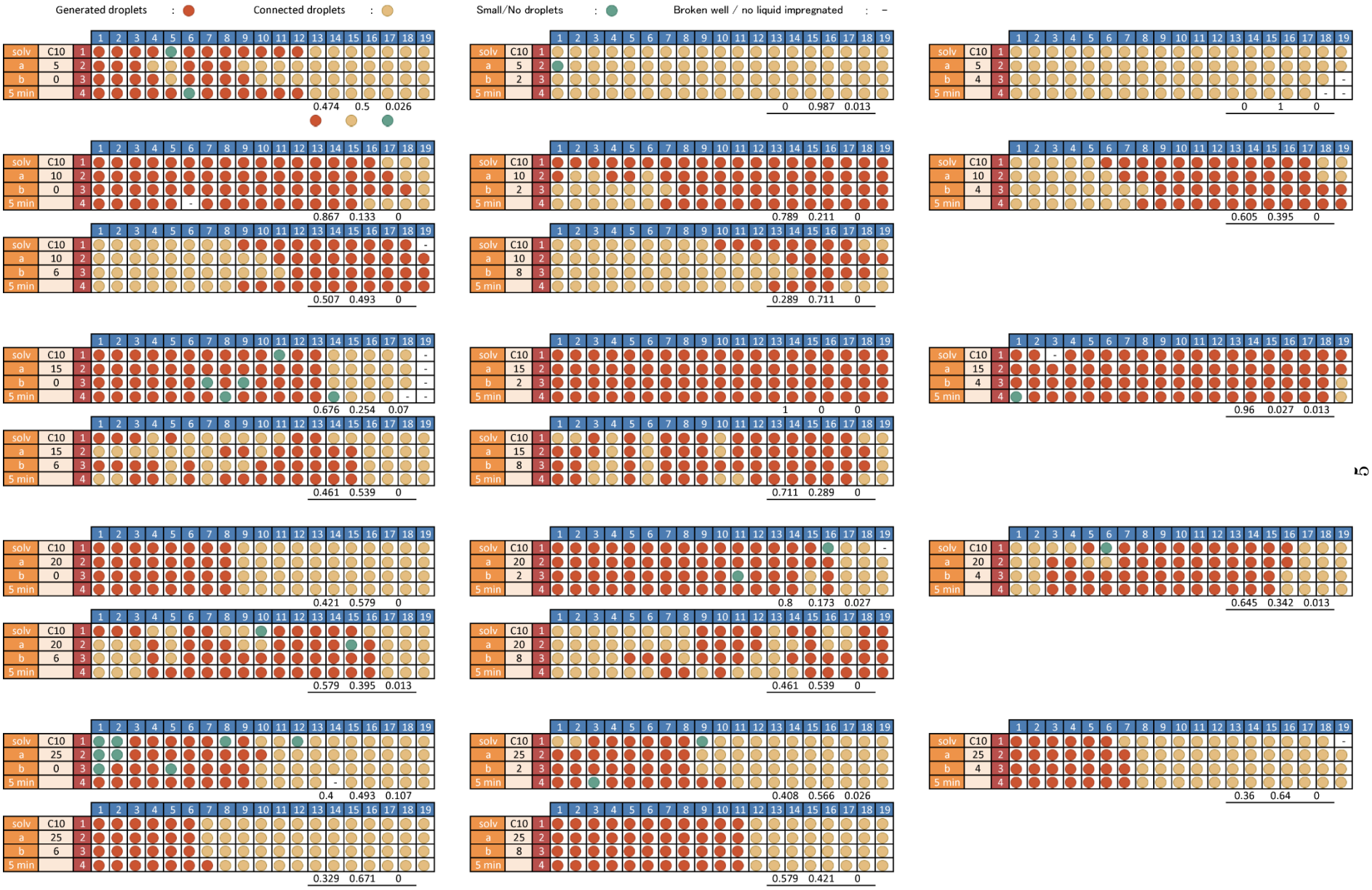

Figure S5 Details of droplet states on using *n*-hexane

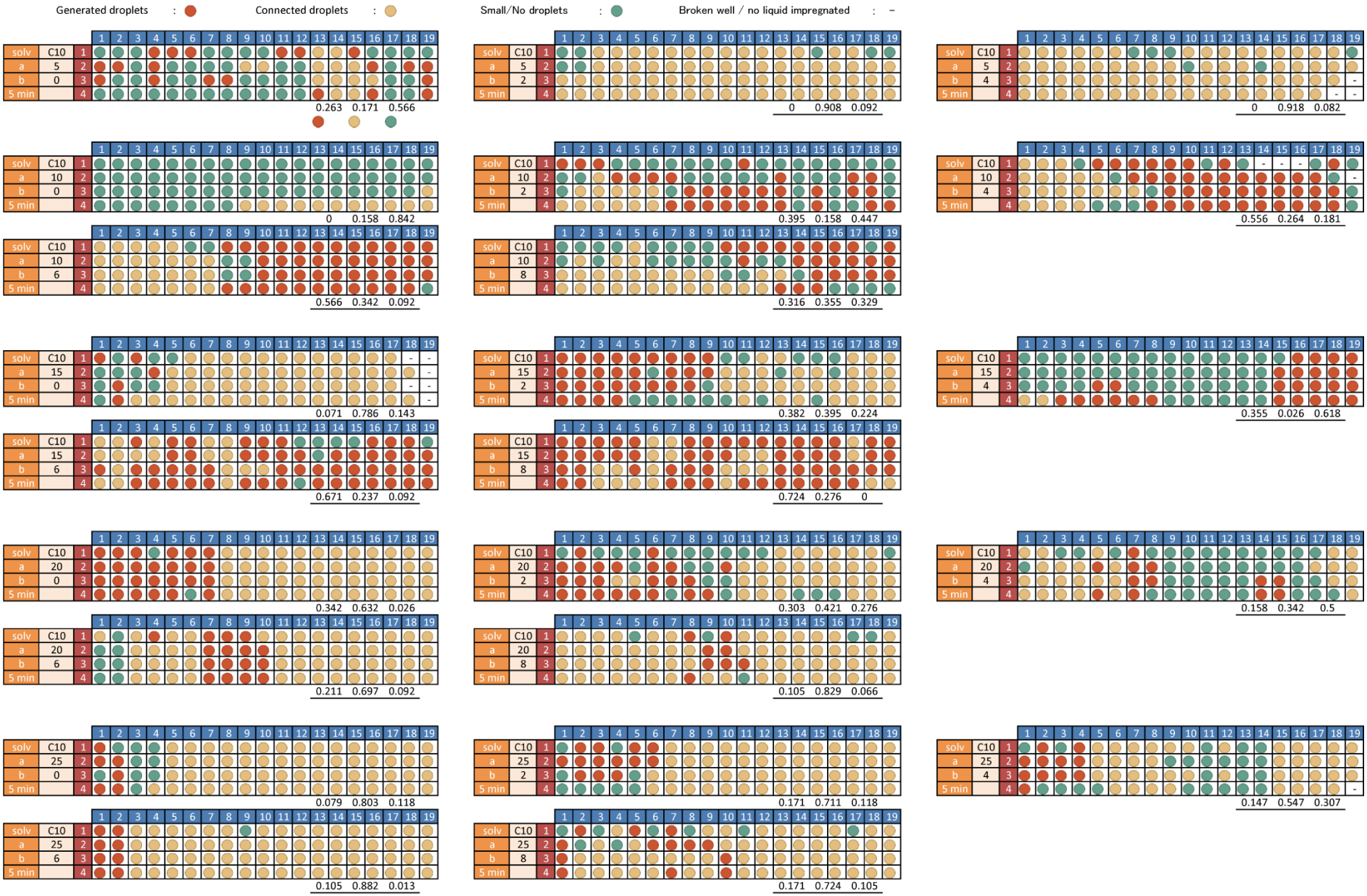

Figure S6 Details of droplet states on using *n*-hexadecane

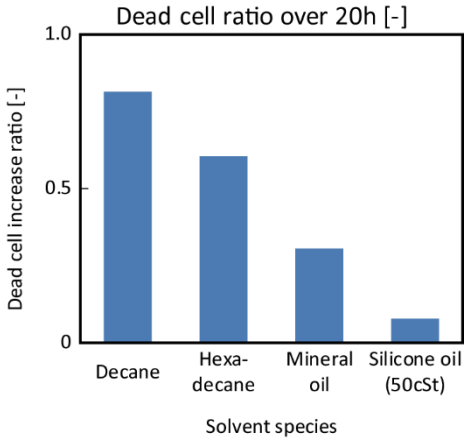

Figure S7. Dead cell increase ratio in a droplet generated in proposed well array. The cells were kept over 20 h in a droplet of a 5 x 5 well array. Here, the cells which had been died during droplet generation process was not counted. (n = 1)

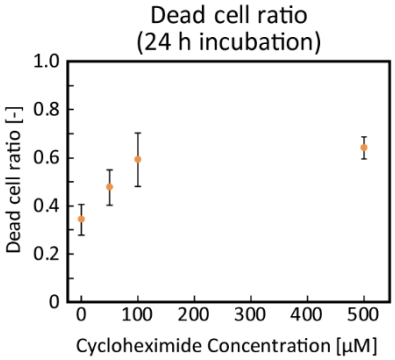

Figure S8. Dead cell ratio in wells of a 96-microtiter plate after 24 h incubation. (n = 3)