Electronic Supplementary Material (ESI) for Lab on a Chip.

Single-cell RT-LAMP mRNA detection by Integrated Droplet Sorting and Merging

Meng Ting Chung[a,c], Katsuo Kurabayashi[a,b*], Dawen Cai[c,d*]

[a] Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, 48105, USA

[b] Department of Electrical Engineering and Computer Sci., University of Michigan., Ann Arbor, MI, 48105, USA

[c] Department of Cell & Developmental Biology, University of Michigan, Ann Arbor, MI, 48105, USA

[d] Biophysics, College of LS&A, University of Michigan, Ann Arbor, MI, 48105, USA

[*]To whom correspondence should be addressed. E-mail: dwcai@umich.edu and katsuo@umich.edu

Supporting material

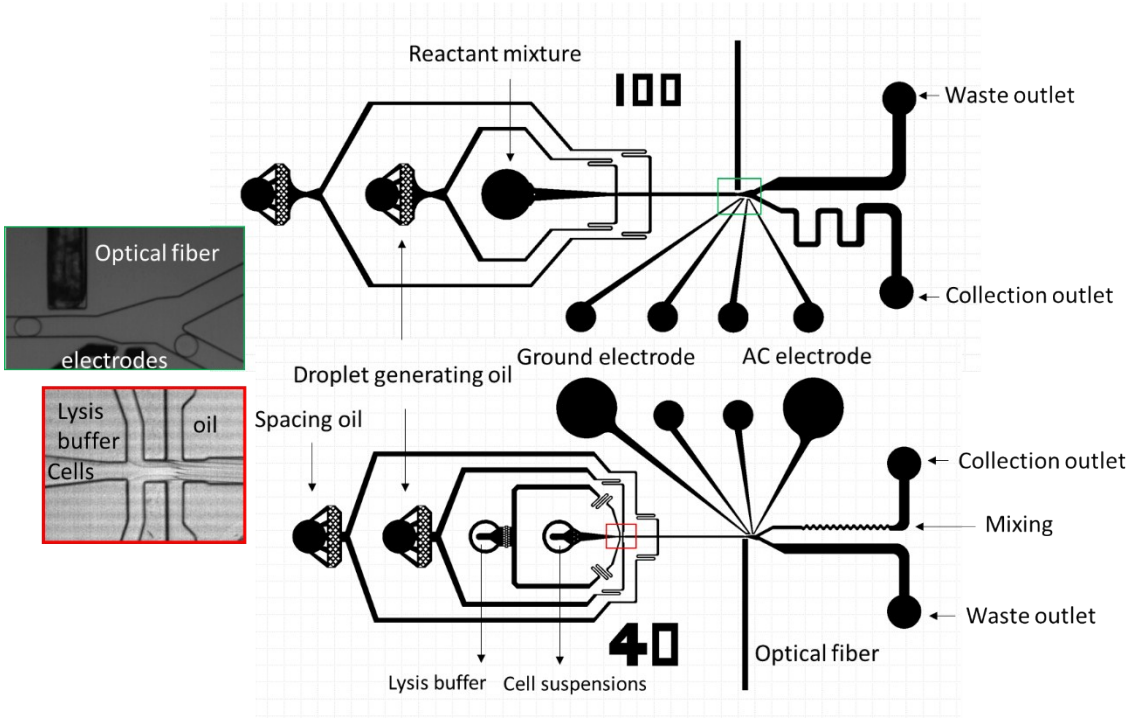

Figure S1. CAD layout of two similar droplet generating/sorting devices with different dimensions. Droplet Generator 1 (top) and Droplet Generator/Sorter 2 (bottom) were used to generate 125µm-diameter droplets with LAMP reactant mixtures (Droplet As) and 60µm-diameter droplets with single-cell lysates (Droplet Bs), respectively. Since sorting is not required for Droplet A, the design of Droplet Generator 1 has only two inlets for the droplet-generating oil and the LAMP reactant mixture and one outlet.

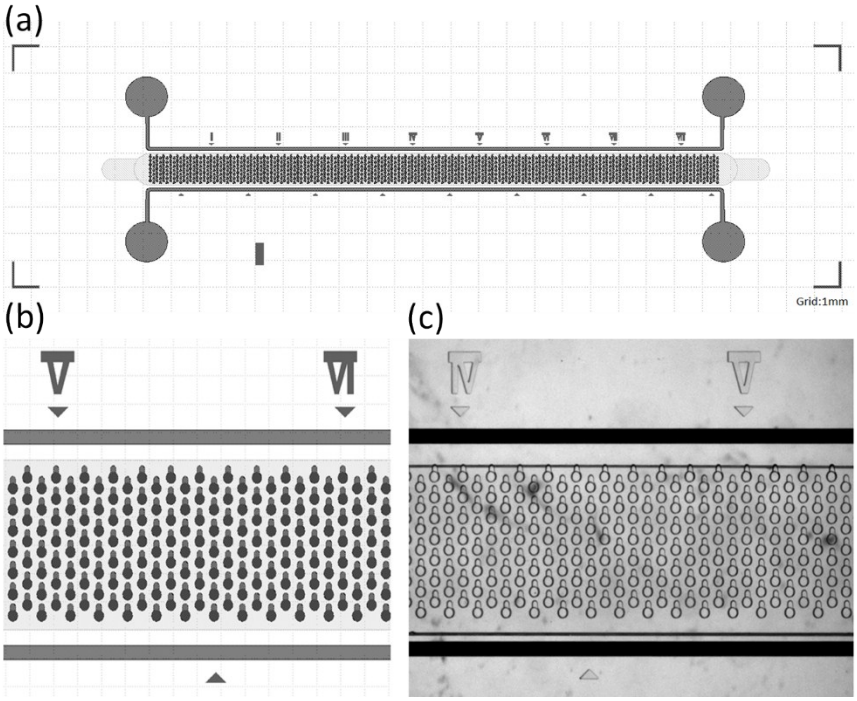

Figure S2. CAD layouts and device image of the storage device with pairing/merging arrays. (a) CAD layout of droplet pairing/merging device. (b) Zoom-in view of (a). (c) Optical microscopy image of (b). The two dark edge lines at the top and bottom of the array of microwells represent microelectrodes used for the electrocoalescence process.

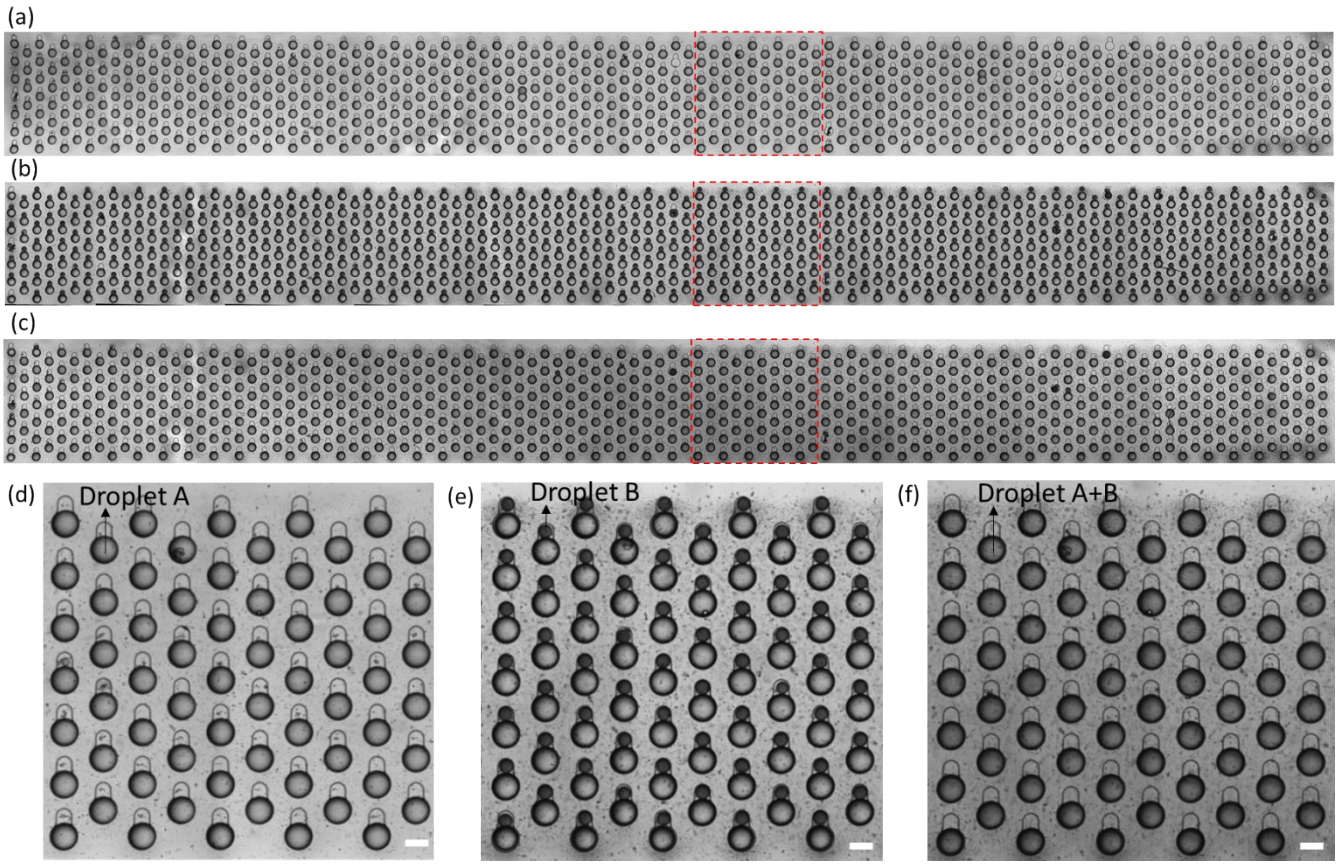

Figure S3. Droplet pairing/merging array containing 676 trapping microwells. (a) Droplets with a diameter of 125μm (gray colored) are first trapped. (b) The droplets in (a) are paired with droplets with a smaller diameter of 60μm (dark colored). (c) The droplets pairs are merged to form a single droplet in each chamber. (d)-(f) Zoom-in images of the red square regions taken from (a)-(c), respectively. Scale bars: 100μm.

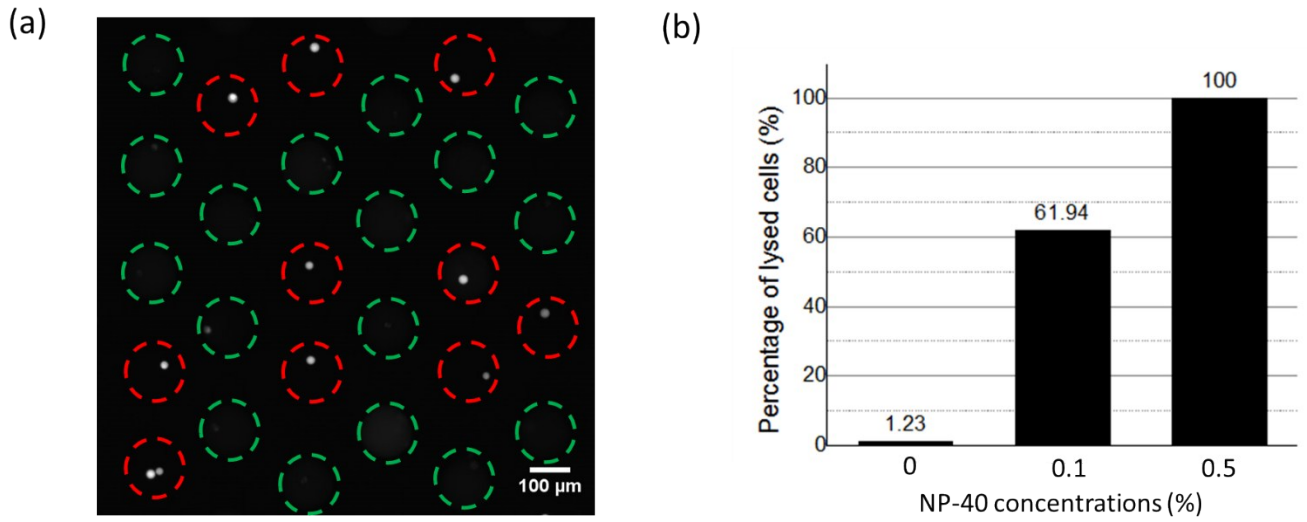

Figure S4. Effect of detergent concentration on cell lysing efficiency. (a) The florescence image shows that each stained Jurkat cell was encapsulated in a microdroplet with lysis buffer containing 0.1% of NP-40. The dashed circles indicate the positions of microdroplets. The cells remain fluorescent if they are not lysed in the droplets (red circles). The droplets showing dim and spatially uniform fluorescence indicate that the cells are lysed in the droplets (green circles). (b) Population (%) of droplets with lysed cells at different NP-40 concentrations (0%, 0.1%, and 0.5%). The number of droplets (cells) for each test are 500.

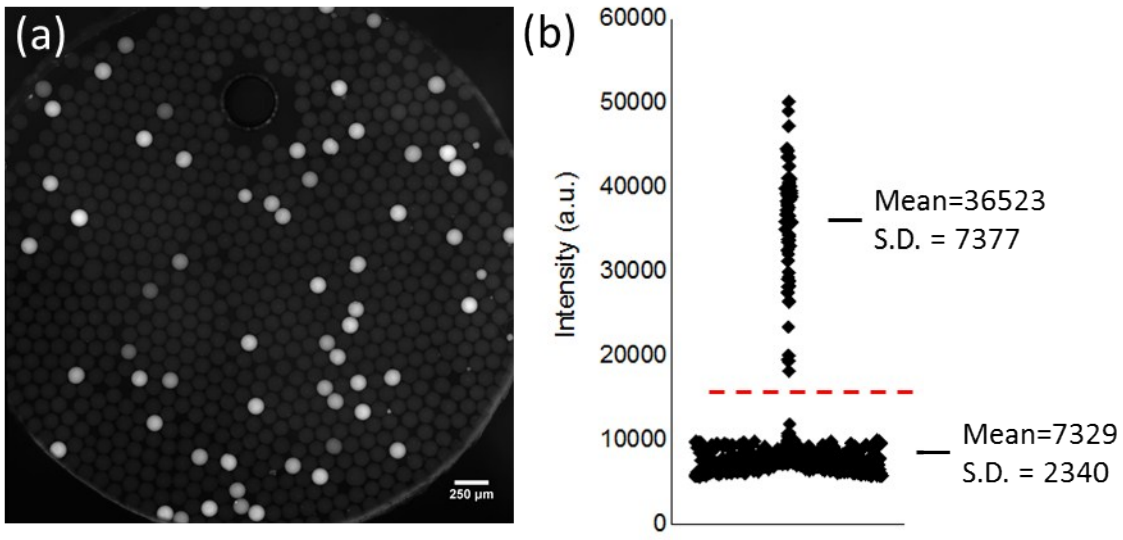

Figure S5. Quantification of the dynamic range of fluorescence change in our scRT-LAMP system. (a) Fluorescence image of droplets containing LAMP reactant mixtures and purified lambda DNA templates, as described in Ref [1]. The lambda DNA templates were diluted to the population of ~ 1 copy per 10 droplets. Thus, only ~10% of the droplets showed an increased fluorescence signal after incubated at 62°C for 30min. (b) Dot plot of fluorescence intensity distribution across 679 droplets, where each dot represents the fluorescence intensity of each droplet. The two clusters of droplets with and without lambda DNA template ("On" and "Off" droplets) can be clearly distinguished by the average values of fluorescence intensity of 36523 a.u. and 7329 a.u., respectively.

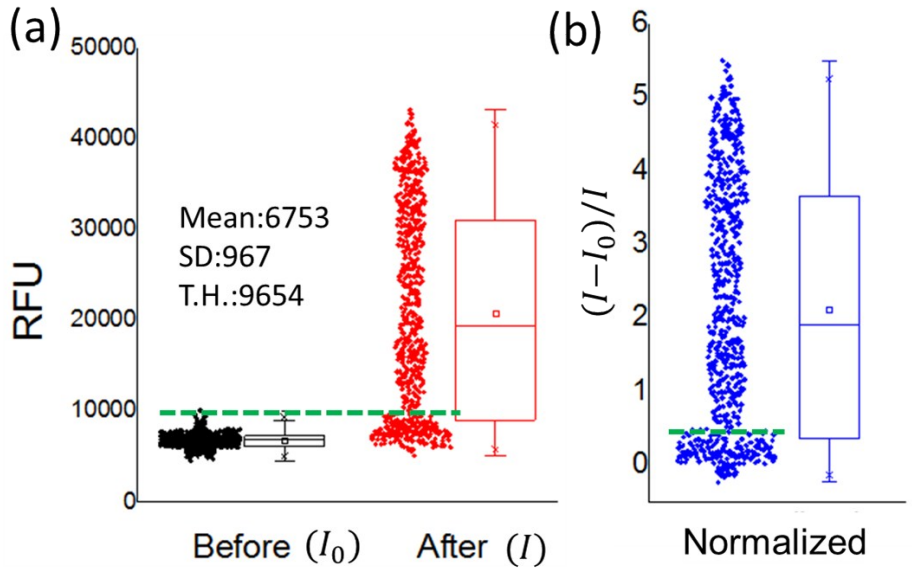

Figure S6. Illustration of normalizing fluorescence signal and thresholding the HMBS-positive/negative populations from scRT-LAMP assays with Jurkat cells. **(a)** I_0 is the initial intensity and I is the end-point intensity of each microwell containing Jurkat cells in Fig. 4a and 4b, respectively. The Threshold (green line) is determined by the mean value of I_0 plus its three standard deviations. The microwells with end-point intensity (I) greater than threshold value are categorized as the HMBS-positive group. (b) The normalized intensity change is defined as $(I - I_0)/I_0$.

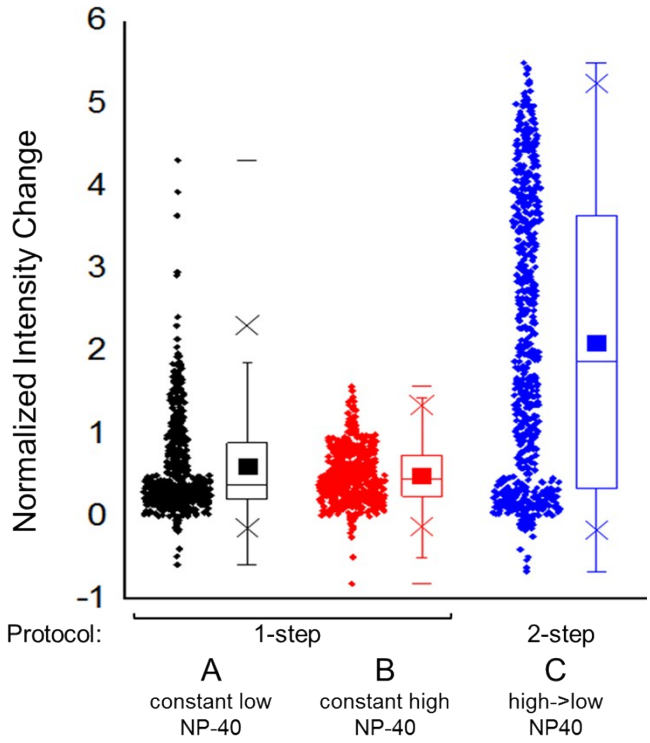

Figure S7. Effect of detergent concentration on droplet-based single-cell RT-LAMP efficiency. Box plot of scRT-LAMP result from three different protocols with Jurkat cells. Each dot represents the post-reaction fluorescence signal from each single cell, hence the expression level of each cell's HMBS gene. The normalized intensity change is defined as $(I - I_0)/I_0$, where I is the end-point intensity and I_0 is the initial intensity of each droplet. The experiment conditions are identical to those in Figure 2, except that the reaction volume is reduced from 25μL in bulk to 1.1nL in droplet. The numbers of data points for the A, B, and C tests are 607, 612, and 668, respectively.

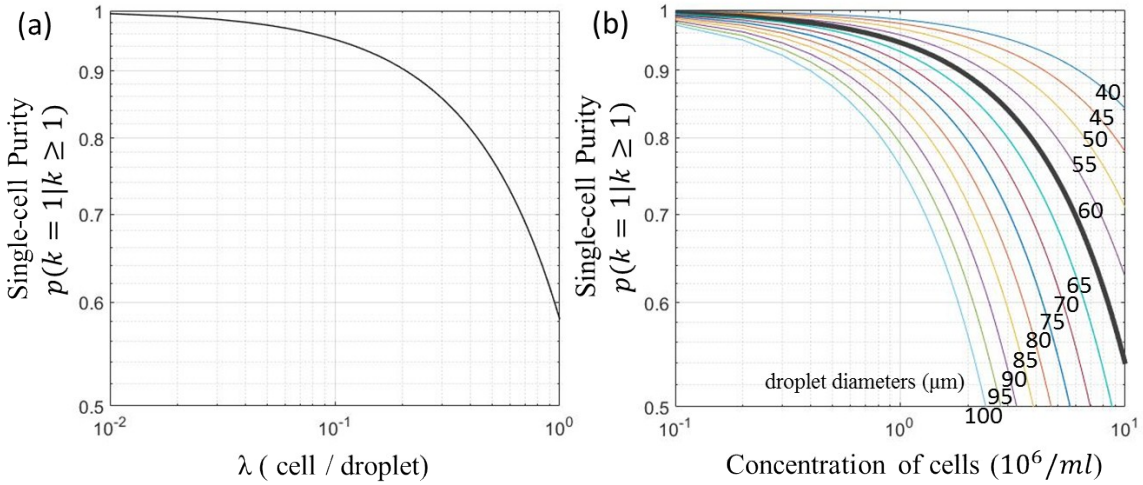

Figure S8. Theoretical predictions of in-droplet cell distribution based on *Poisson* distribution. (a) Fractional population of droplets exactly encapsulating a single cell among all sorted droplets (single-cell purity) predicted by the *Poisson* distribution at different cell suspension solution concentrations (λ = cells per droplet or cells / ml). (b) Effect of droplet size on single-cell purity. This study used droplets of 60μm in diameter and single-cell suspensions at a concentration of 1 million cells/mL.

	Jurkat	K562	Neuro-2A	NCC
Test cell number (n)	664	613	651	653
Detection rate (p)	0.7093	0.9299	0.0476	0.0429

Table S1. Calculation of statistical significance between different groups.

The test statistic of two binomial data sets can be determined by:

$$z = \frac{p_1 - p_2}{\sqrt{p(1-p)(\frac{1}{n_1} + \frac{1}{n_2})}},\ where\ p = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2}.$$

If $z > z_{0.975} = 1.96$ (for this two-tail test with 95% confidence), null hypothesis ($H_0: p_1 = p_2$) will be rejected. In our experiment, both Jurkat cells and K562 cells (HMBS-positive cells) show significant differences than Neuro-2A cells (HMBS-negative cells) with z=31.4 and 24.7, respectively. While the difference between Neuro-2A cells and no-cell-control is not significant (z=0.41).

Reference

1 M.Goto, E.Honda, A.Ogura, A.Nomoto andK.Hanaki, , DOI:10.2144/000113072.