Supplementary Information (SI) for Lab on a Chip.
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Supporting Information

Recovery of phenotypically sorted cells using droplet-digital microfluidics

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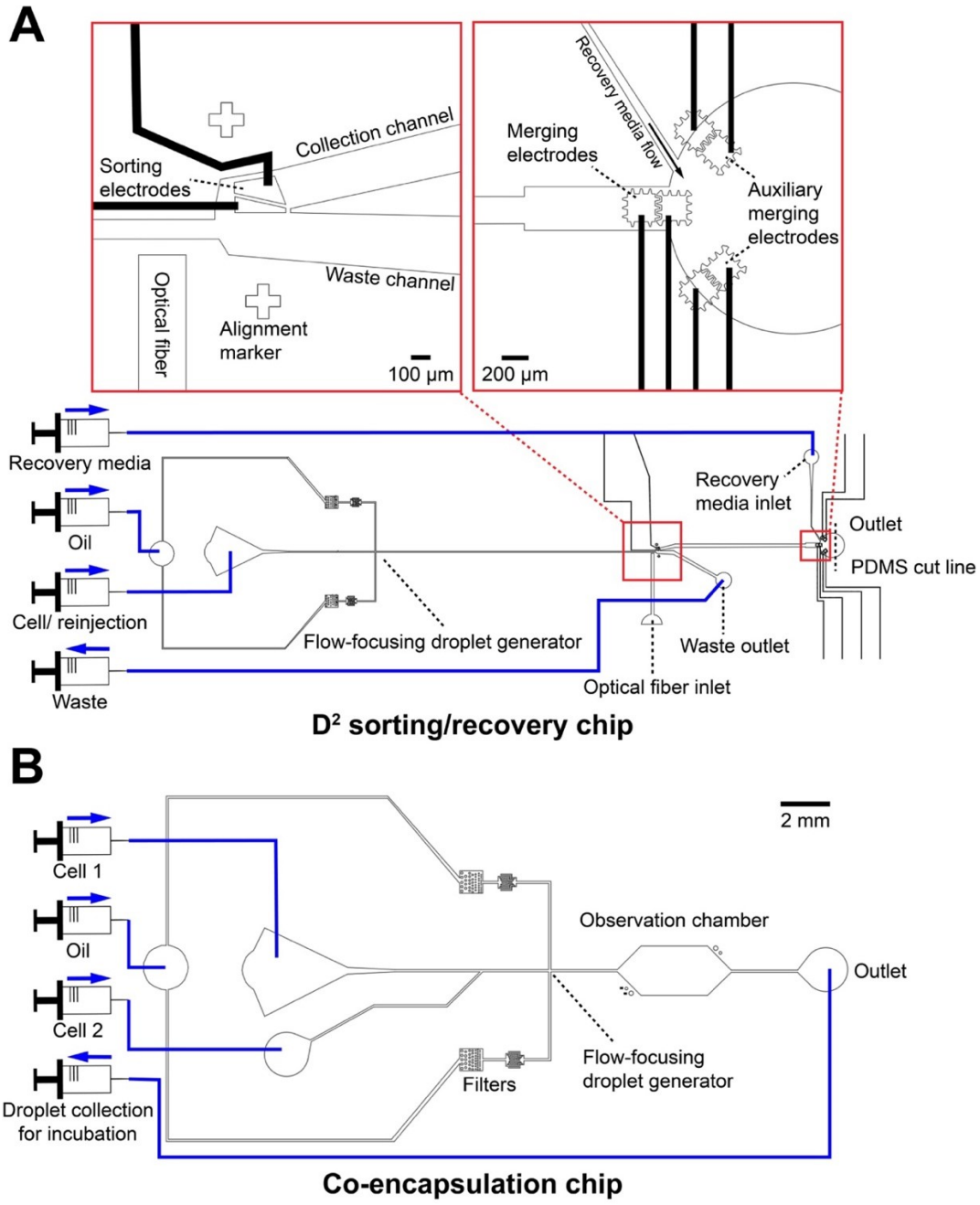

Figure S1. D^2 and co-encapsulation chip design.

(A) AutoCAD drawings of D^2 sorting/ recovery chip design. Overall view is shown on the bottom, DMF sorting electrodes and merging electrodes are highlighted in red boxes. **(B)** An AutoCAD drawing of a co-encapsulation chip design. All PDMS channels have a width of ~100 µm (except for collection - 210 µm, waste - 240 µm, outlet - 350 µm and observation chamber - 2000 µm) and a height of ~100 µm. The blue lines highlight the fluidic connections using syringes, and the blue arrows indicate the flow direction (dispense or withdraw).

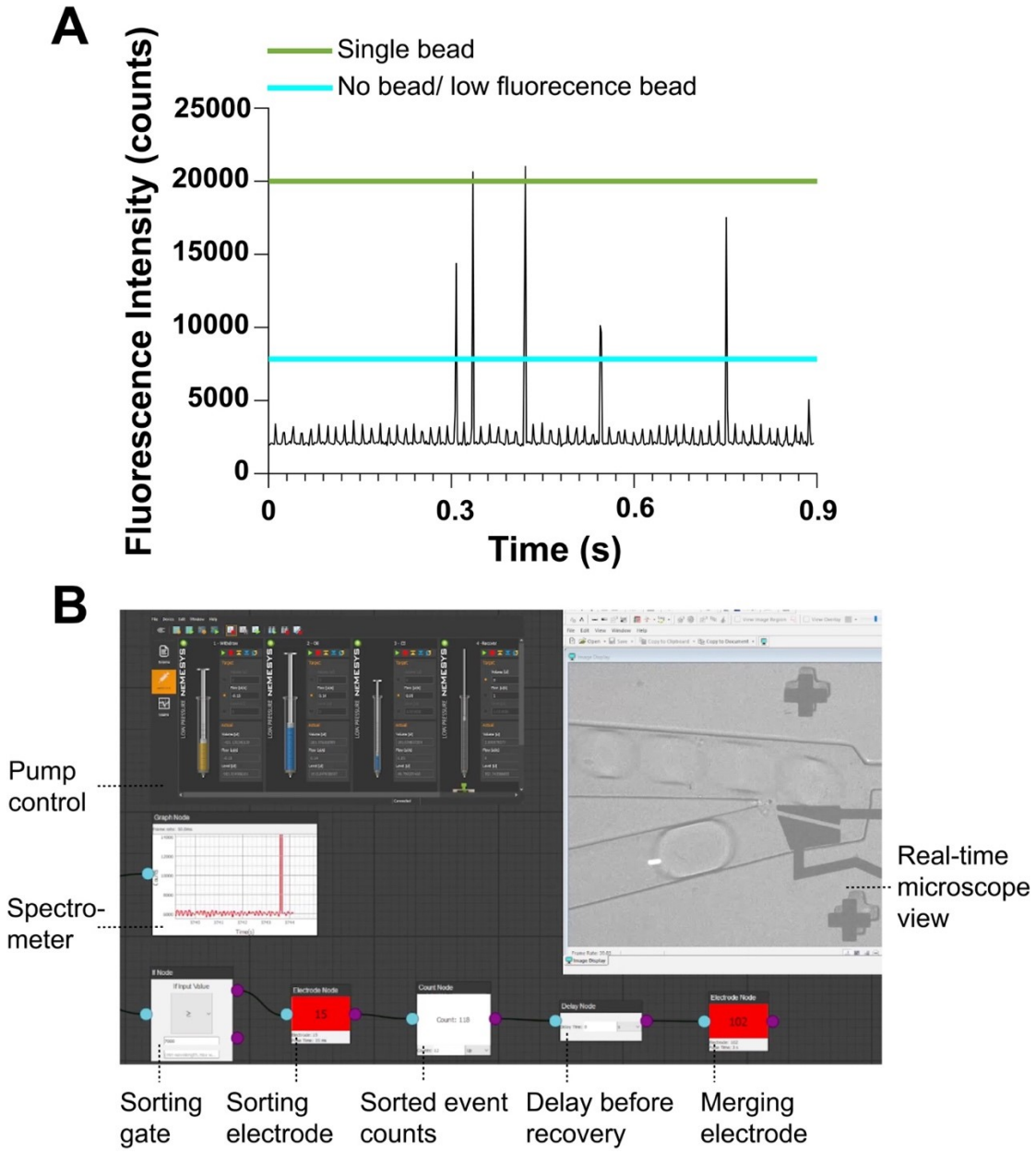

Figure S2. Fluorescent bead gating and D² sorting/recovery software interface.

(A) Graph showing detection of droplet with a frequency of about 50 Hz, using 0.02 µL/s droplet re-injection flow rate and 0.15 µL/s spacer oil flow rate. Empty or low-intensity-bead droplets < 7 x 10^3 counts – blue line, and multiple-bead droplets > 2 x 10^4 counts – green line. Droplets with counts between 7 x 10^3 and 2 x 10^4 were sorted as single-bead droplets. **(B)** Image showing real-time sorting/recovery software interface. A peak was detected/shown in the spectrometer graph, sorting electrode was actuated to sort the single-bead containing droplet to the collection channel, sorted event counts increased by 1, an 8-second delay was set for sorted droplet to travel to the collection site and the merging electrode was actuated after the delay to collect the content from the sorted droplet.

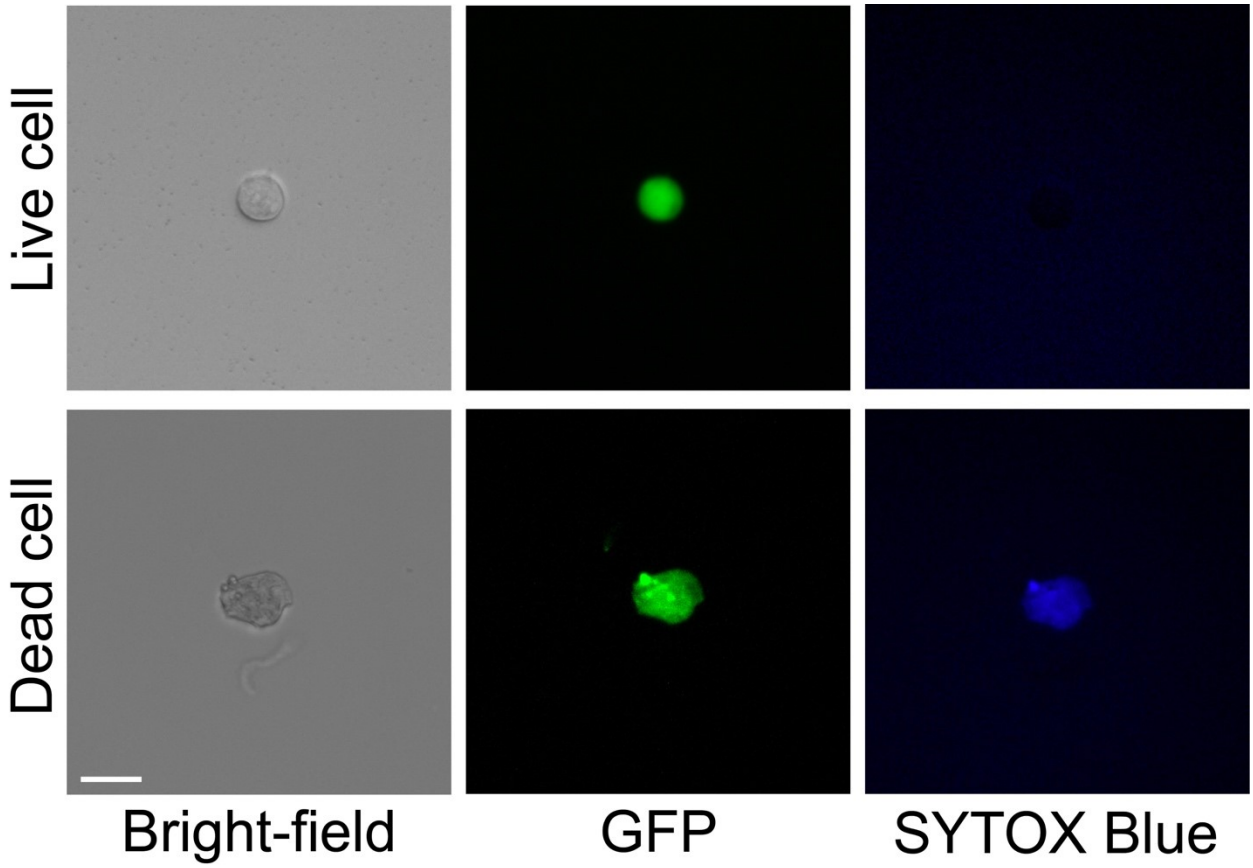

Figure S3. SYTOX Blue staining and single-cell proliferation after FACS.

Images showing SYTOX Blue staining of live and dead single HEK 293 cell 10 min after sorting/recovery. Live cell showed no blue fluorescence while a dead cell showed strong blue fluorescence under a excitation wavelength of 445 nm. Scale bar: 10 μm.

Supplementary Tables

Table S1. Comparison between existing systems and D^2 system.

	System accessibility	Output Format	Positive target ratios (%)	Starting cell number (x 10^6 cells)	Sorting throughput (Hz)	Recovery throughput (Hz)	Sorted hits recovery rate (%)
Nan et al.[30] (ODC)	Open	Microtube	1, 5, 10	N/A	200	~0.3	~98
Cole et al.[29] (PDM)	Open	Customized nano-well substrate	5	1	50-100	~1	N/A
Josephides et al.[32] (Cyto-Mine)	Closed	Well plate	2	0.1 - 40	300	N/A	~99
Pernod et al.[41] (LiveDrop)	Closed	Well plate	N/A	0.5	1000	N/A	N/A
This work (D^2)	Open	Well plate	0.25, 0.1	0.01, 0.1	50	~0.1	~90

NOTE: The preferred features for rare single-cell isolation/recovery are highlighted in red. ODC is on-demand droplet collection. PDM is printed droplet microfluidics. D^2 is droplet-digital microfluidics.

Table S2. Statistics for the recovery rate of 5, 10, 20, 50 beads using D^2 and tubing methods.

Recovery rate (%)					
Number of beads	Mean		Std. Deviation		P-value
	D^2	Tubing	D^2	Tubing	
5	93.33	80	11.55	0	0.1161
10	96.67	90	5.774	17.32	0.5614
20	88.33	90	12.58	17.32	0.8993
50	96	71.33	5.29	26.1	0.1839