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

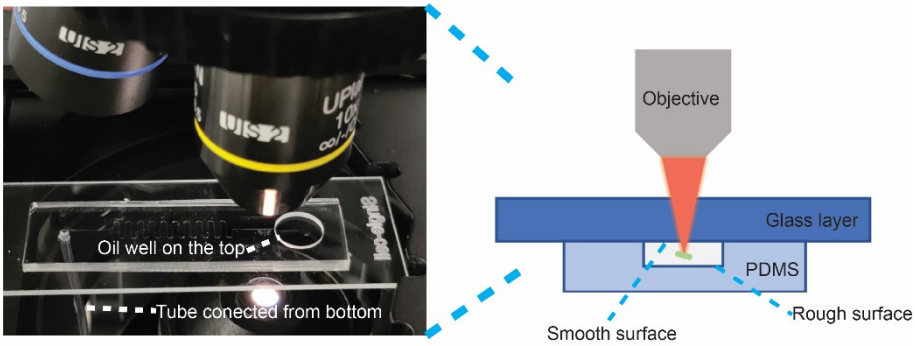

Fig. S1 The setup of the OPSI Chip on the EasySort stage. The laser would penetrate the upper glass layer and reach the cell in the channel on the PDMS layer. As the glass layer features a smoother surface than PDMS channel wall, the bottom set PDMS design can achieve a stronger optical tweezers force.

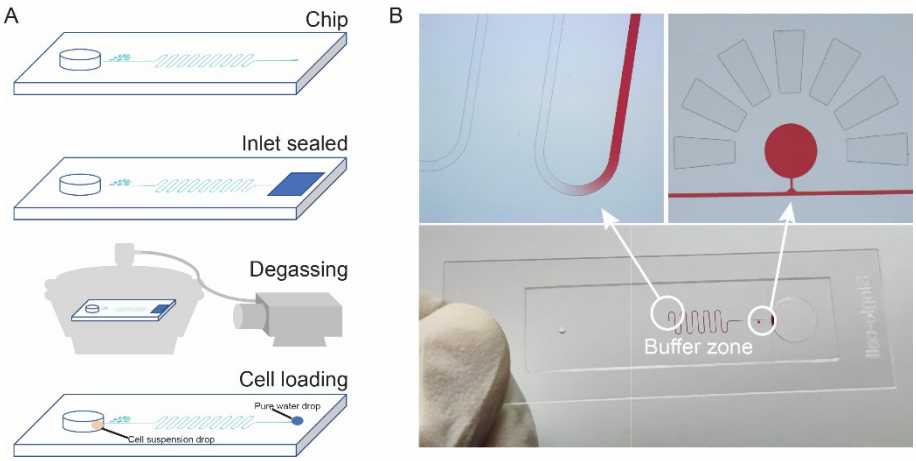

Fig. S2 Vacuum-driven chip loading indicated by amaranth solution. To verify the loading process, amaranth solution was used here for demonstration. Following operation scheme (**Fig. S2A**), 2 μL of amaranth was dropped near the channel outlet in oil well after removing the chip from vacuum desiccator. After 2 min, the amaranth would fill the whole cell chamber (**Fig. S2B**). With successful loading of sample into cell chamber, the redundant cell suspension can also fill the whole channel lately. To avoid the cell suspension entering inlet, and to avoid cells from sitting on the wall of inlet which can be difficult to washout from chip, a drop of pure water was added in inlet during sample loading. The pure water was also driven by the vacuum in channel and would reach the amaranth solution in the buffer zone. Thus, redundant cells were stopped in the buffer zone of the main channel and can be completely pumped out of the chip after the inlet was connected to the pure water phase. Only the cell chamber was filled with cells for later screening.

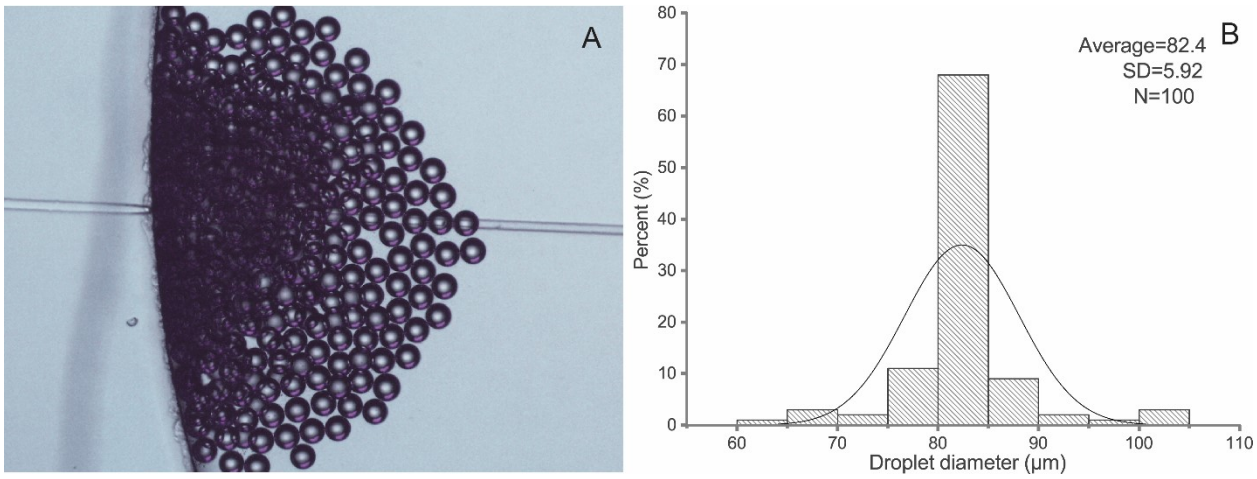

Fig. S3 Continuous droplet generation for the verification of size uniformity. (A) Droplets generated in the oil well. (B) Distribution of diameters of the 100 droplets generated.

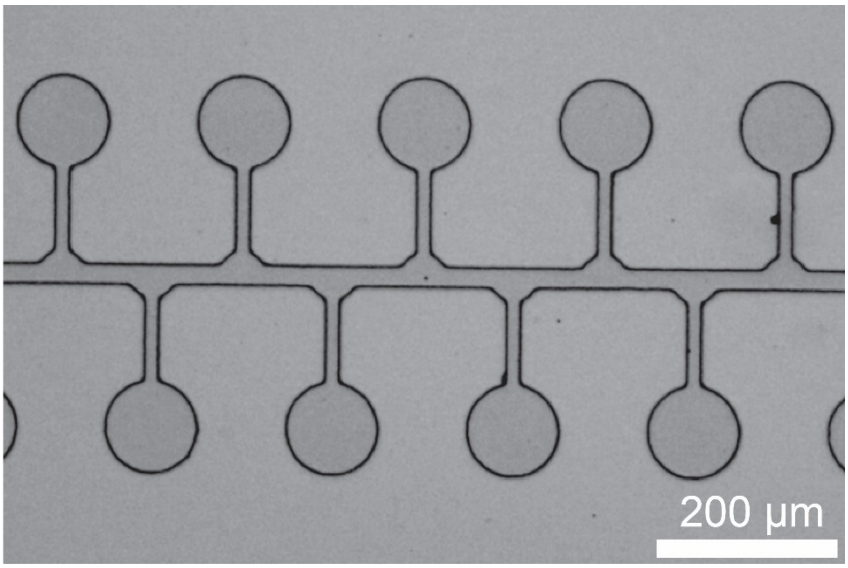

Fig. S4 The single-cell culture chip which includes multiple culture chambers.

Table S1. Comparison of OPSI with existing single-cell sorting devices.

Type	Cytometery[1,2]	DEPArray[3]	Cell injection[4]	Micromanipulation[5]	Optical tweezers		
					RAGE[6,7]	Open-well chip[8]	OPSI
Cell size	Depending on fluorescence strength	7~40 μm	1~5 μm	>3 μm	1~40 μm	1~40 μm	1~40 μm
Input sample volume	10^7cell/mL, >1 mL	>10^6cell/mL, >12 μL	>10^7cell/mL, >1 μL	>10^6cell/mL, 0.1~0.2 μL	>10^7cell/mL, >1 mL	2× 10^5 cells/mL, 0.5 μL	>10^6cell/mL, >0.5 μL
Target cell purity	Not 100%	100%	100%	100%	100%	~90%	100%
Throughput	>5000 events per second	107 s for a single cell or 192 s for a pool of 20 cells	~1s for a single cell	~3 min for a single cell	30~60s for a single cell	3 min for a single cell	3~6s for a single cell
Target cell imaging	No	Yes	Yes	Yes	Yes	Yes	Yes
Automated level	High	High	Manual	Manual	Manual	Manual	Manual

References

1. N. Navin, J. Kendall, J. Troge, P. Andrews, L. Rodgers, J. McIndoo, K. Cook, A. Stepansky, D. Levy, D. Esposito, L. Muthuswamy, A. Krasnitz, W. R. McCombie, J. Hicks and M. Wigler, *Nature*, 2011, **472**, 90-94.
2. R. Stepanauskas, E. A. Fergusson, J. Brown, N. J. Poulton, B. Tupper, J. M. Labonte, E. D. Becraft, J. M. Brown, M. G. Pachiadaki, T. Povilaitis, B. P. Thompson, C.

J. Mascena, W. K. Bellows and A. Lubys, *Nat. Commun.*, 2017, **8**, 84.

3. M. Di Trapani, N. Manaresi, G. Medoro, *Cytometry A*, 2018, **93**, 1260-1266.
4. X. Su, Y. Gong, H. Gou, X. Jing, T. Xu, X.-S. Zheng, R. Chen, B. Ma and J. Xu, *Anal. Chem.*, 2020, **92**, 8081-8089.
5. J. Frohlich, H. Konig, *FEMS Microbial. Rev.*, 2000, **24**, 567-572.
6. T. Xu, Y. Gong, X. Su, P. Zhu, J. Dai, J. Xu and B. Ma, *Small*, 2020, **16**, e2001172.
7. X. Jing, Y. Gong, T. Xu, Y. Meng and J. Xu, *mSystems*, 2021, e0018121.
8. T. Fang, W. Shang, C. Liu, J. Xu, D. Zhao, Y. Liu, A. Ye, *Anal. Chem*., 2019, **91**, 9932-9939.