

**Supplementary Materials for *Injection Molded Microfluidics for Establishing High-Density Single Cell Arrays in an Open Hydrogel Format***

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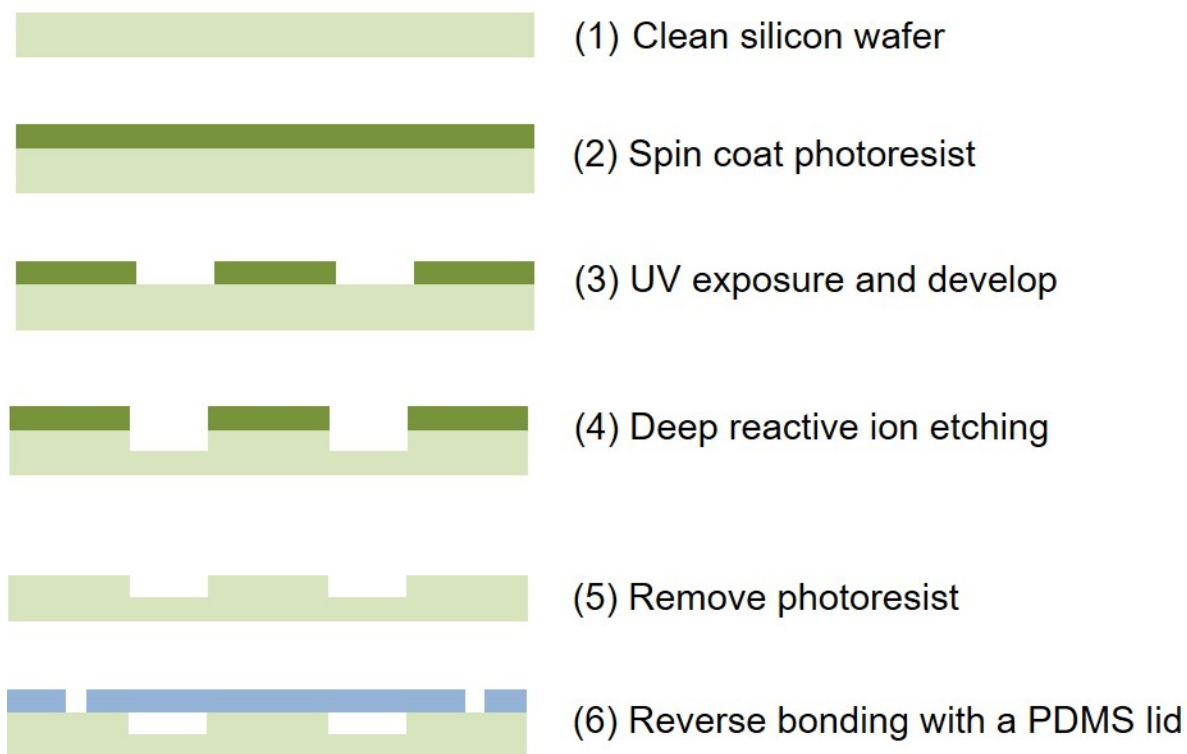
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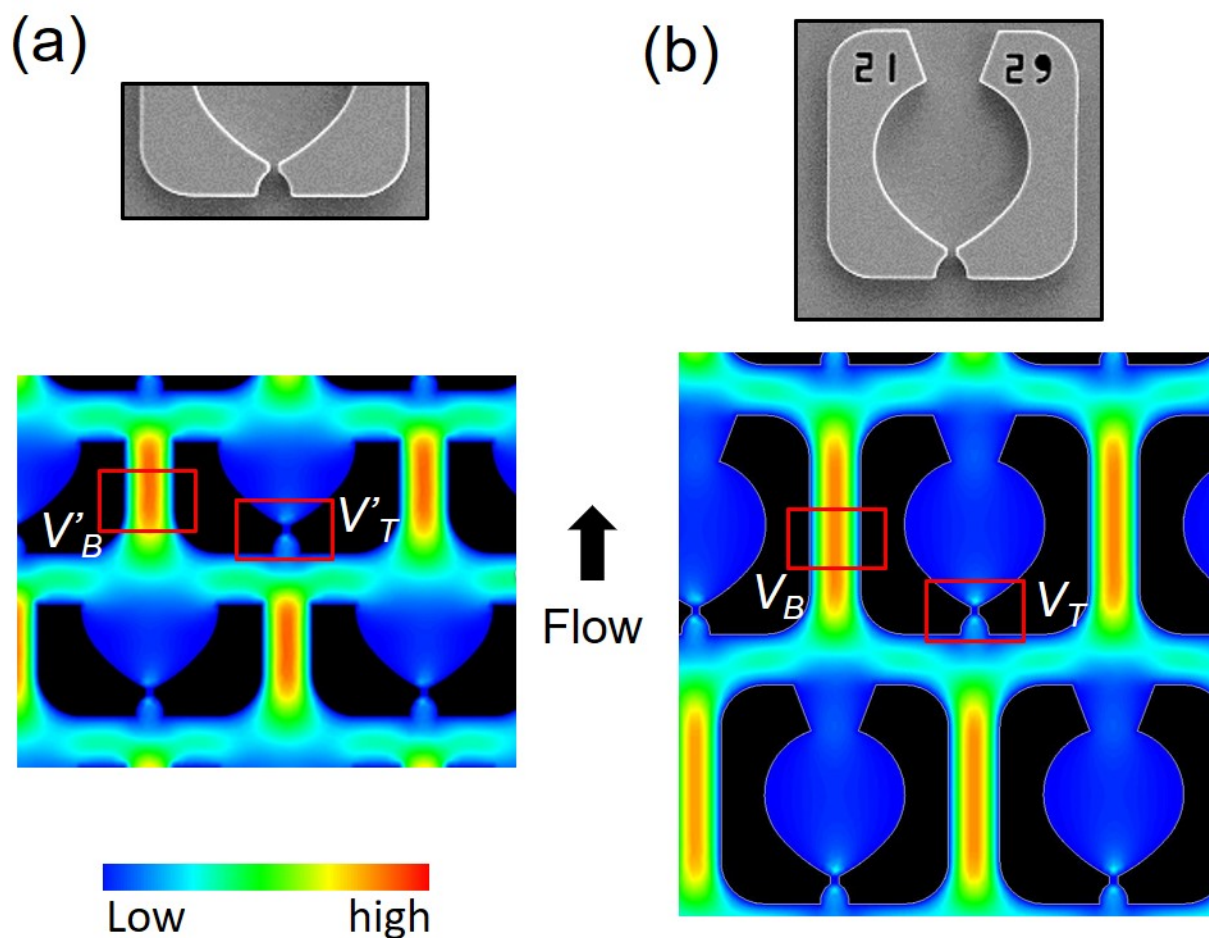
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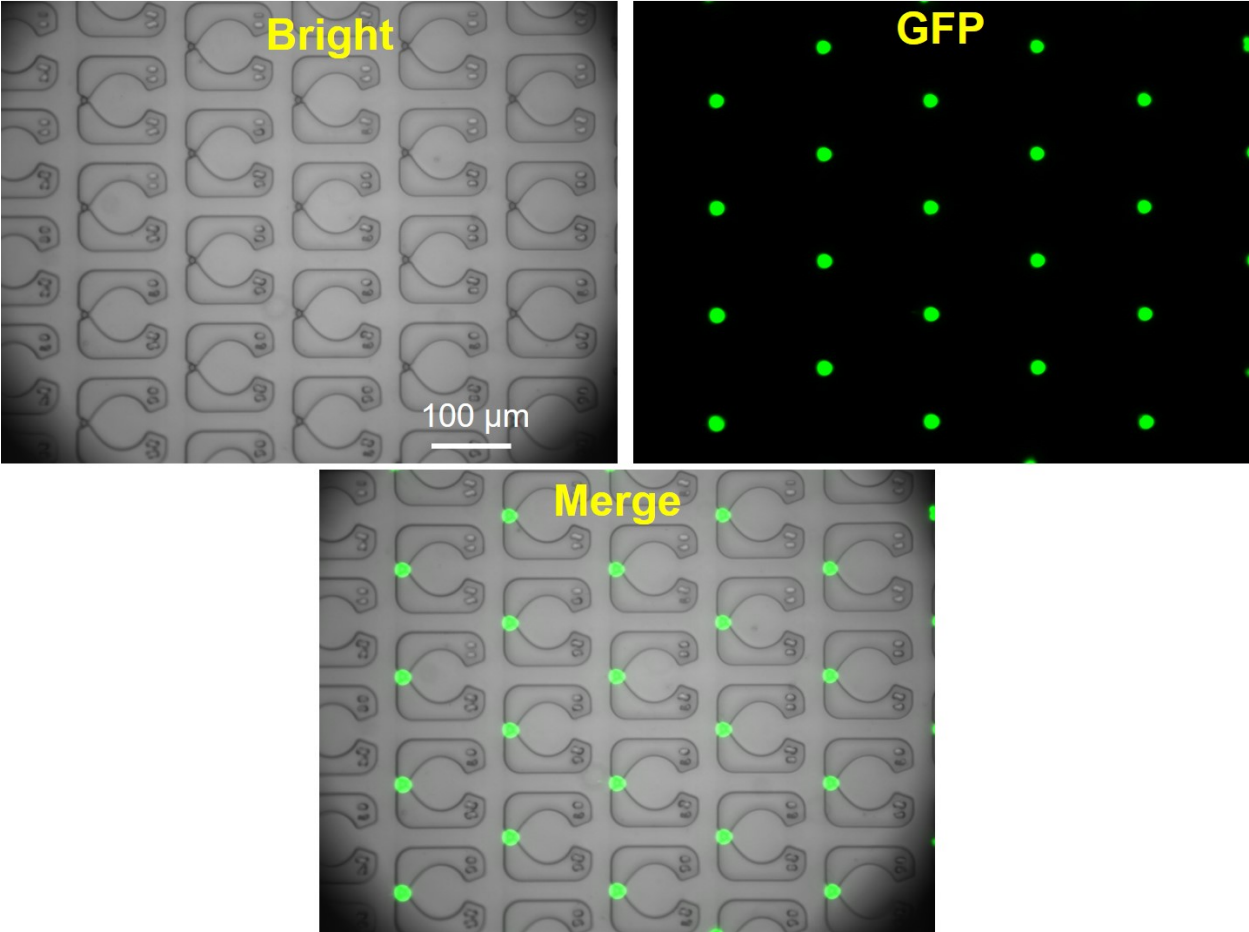
Email: [liying@wipm.ac.cn](mailto:liying@wipm.ac.cn); [yellen@duke.edu](mailto:yellen@duke.edu)



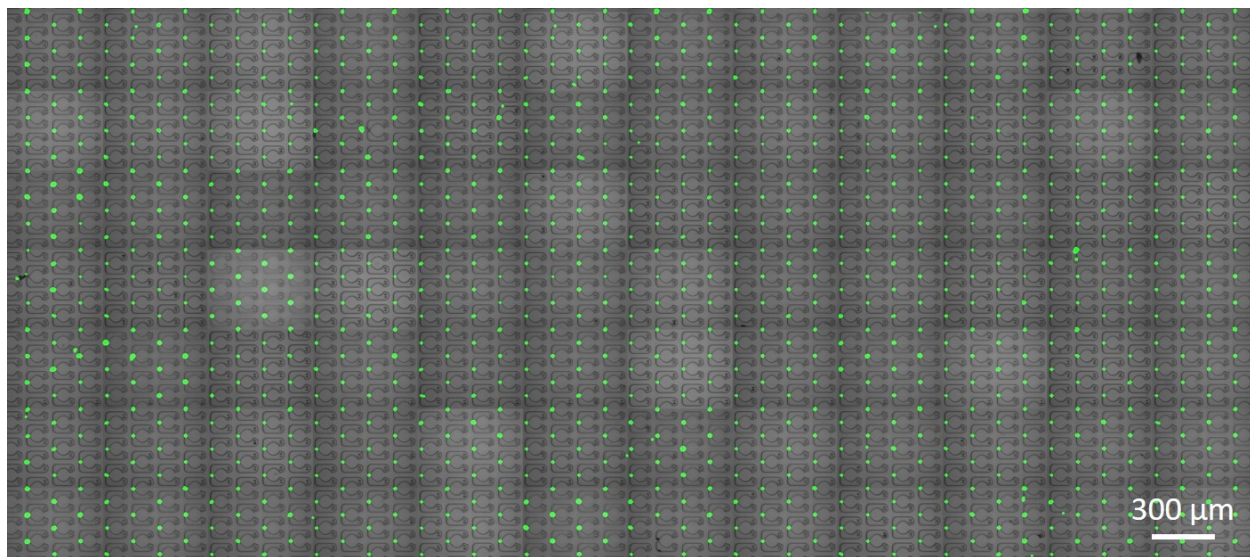
**Figure S1** Device Fabrication. The silicon chips are fabricated following standard protocols. The final device is built by attaching a PDMS lid to the silicon chip.



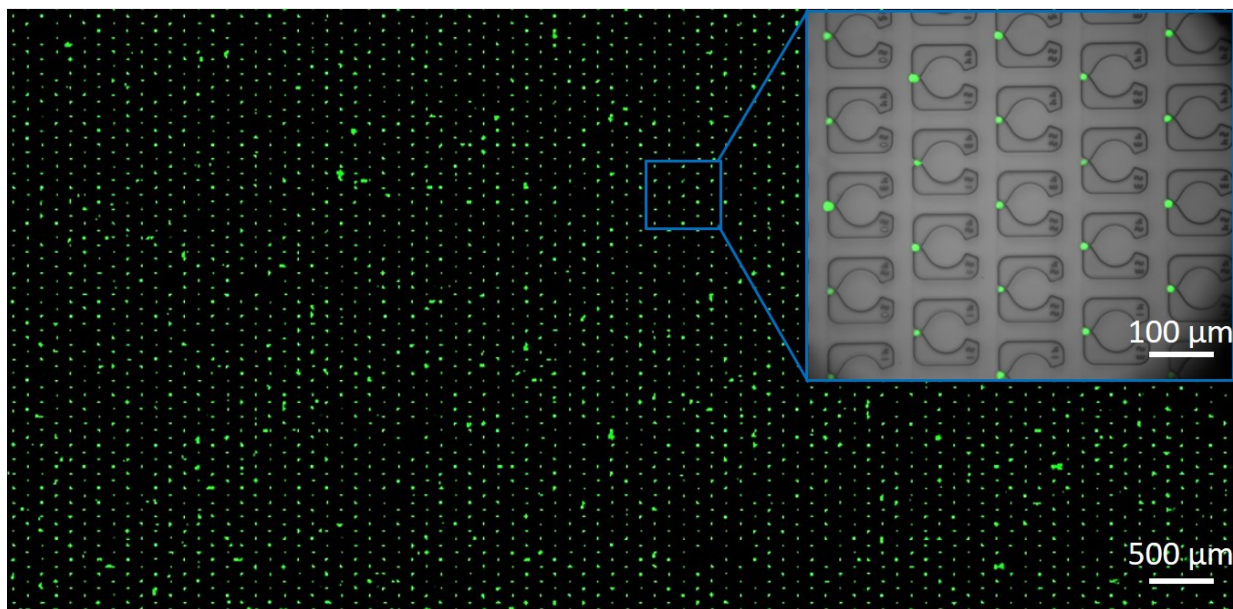
**Figure S2** Simulation results of the flow velocity profile for the two designs. The ratio of the flow velocity at the trap and the bypass  $V'_T / V'_B$  (a) is smaller than  $V_T / V_B$  (b), which means relatively more fluid flows through the trap in (b) than that flows through the trap in (a), which should facilitate a better trapping efficiency. The simulation was conducted using the simulation software Fluent 6.1. The simulation procedure was performed by solving the continuity equation, Navier-Stokes equation and diffusion-convection equation, similar to that described previously (Anal. Chem. 2002, 74, 4279-4286; Anal. Chem., 2014, 86, 4333-4339).



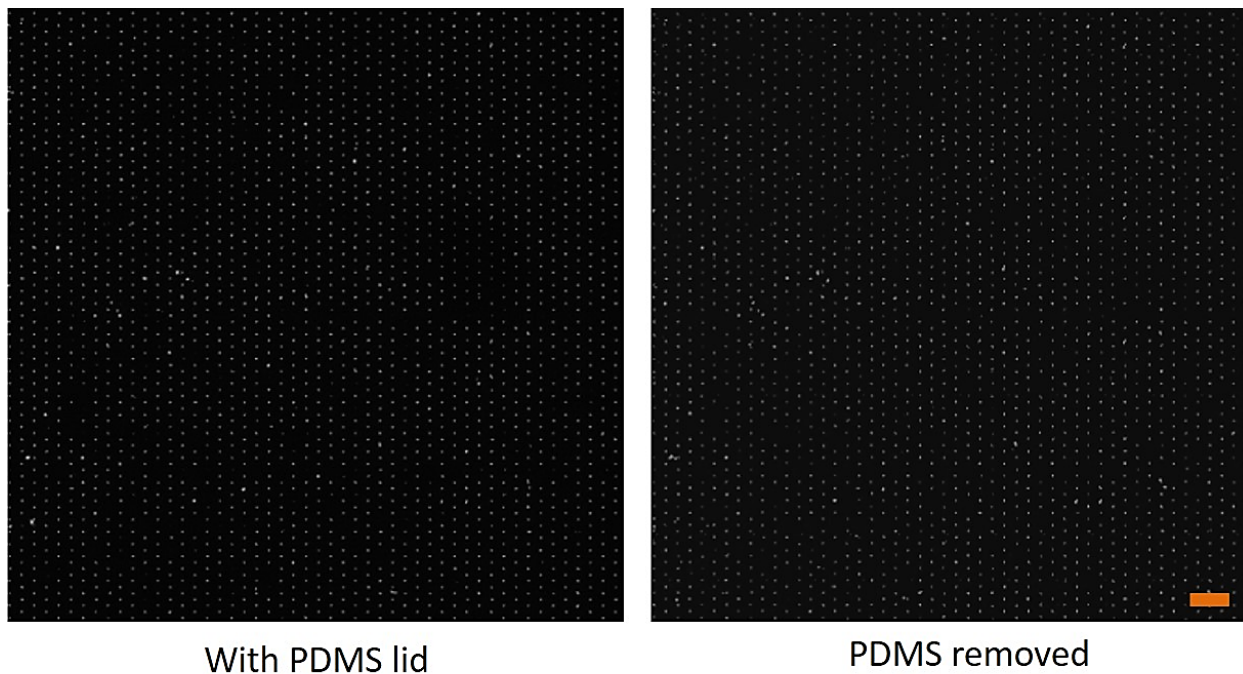
**Figure S3** Images to show the trapping efficiency for 10 μm fluorescent beads on the device.



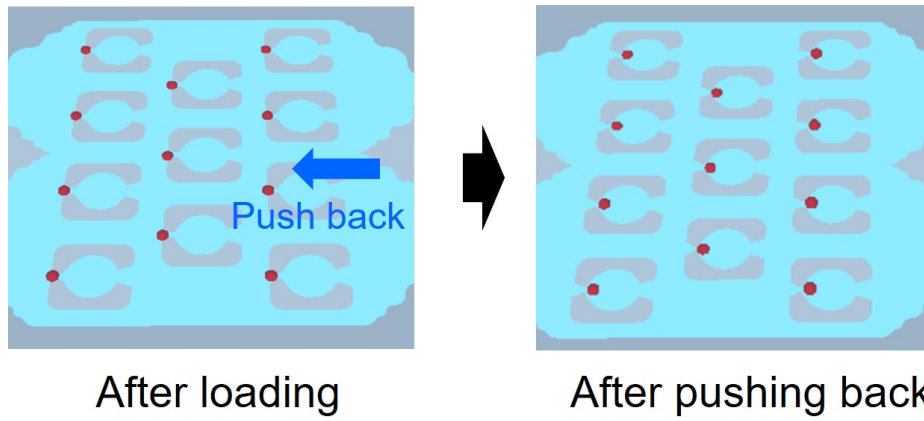
**Figure S4** A large area to show the high trapping efficiency for 10 μm fluorescent beads on the device.



**Figure S5** Stitched image to show high efficiency trapping of MDA-MB-231/GFP cells in the 100×100 device. The insert shows a single merged image of the bright-field and the green channel. The scale bar in the stitched image and the insert is 500 μm and 100 μm, respectively.

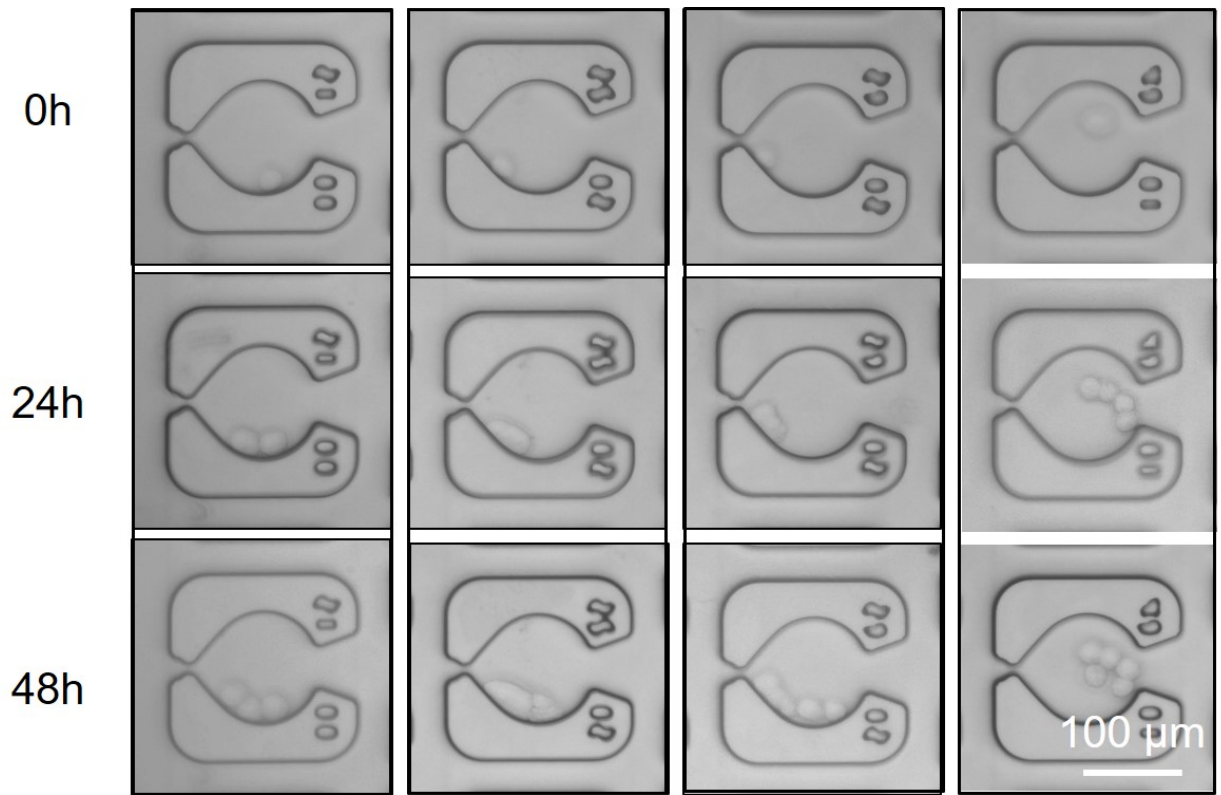


**Figure S6** A  $50 \times 50$  array of K562 cells trapped in hydrogel on the device with (left) and without (right) the PDMS lid. The results demonstrated that almost all of the cells were kept on the device after the removal of the lid. Scale bar, 500  $\mu\text{m}$ .

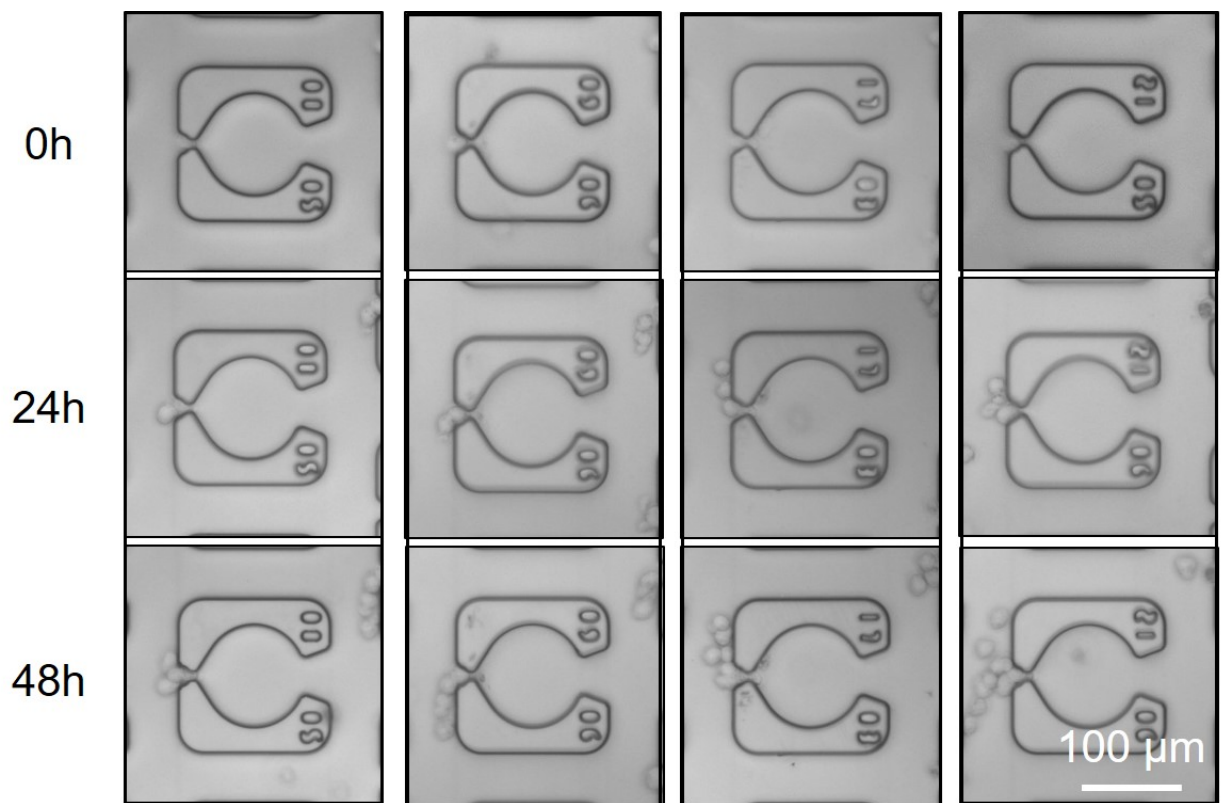


**Figure S7** Scheme to show how to push cells back into the front compartment.

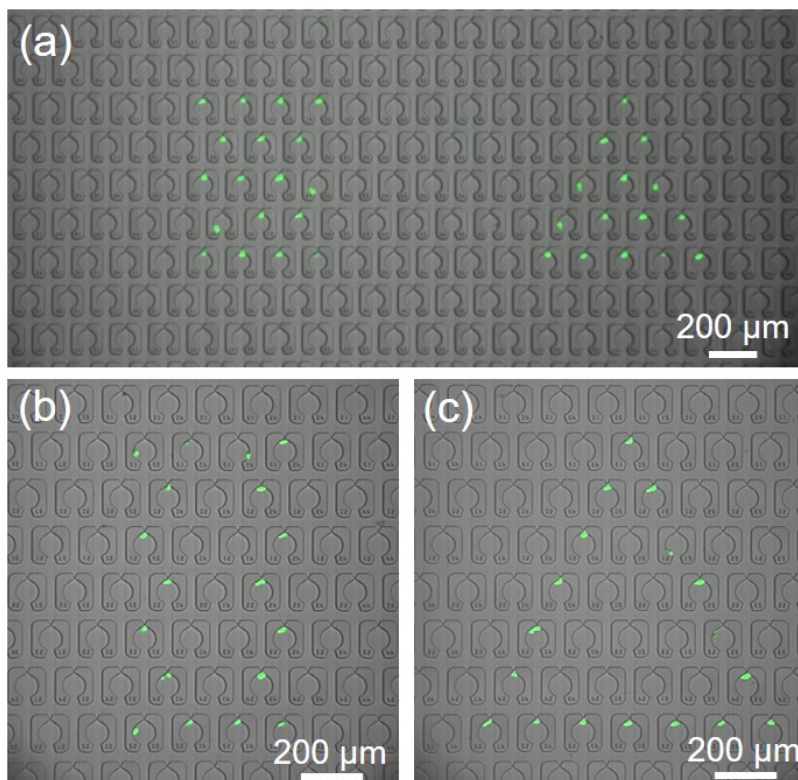




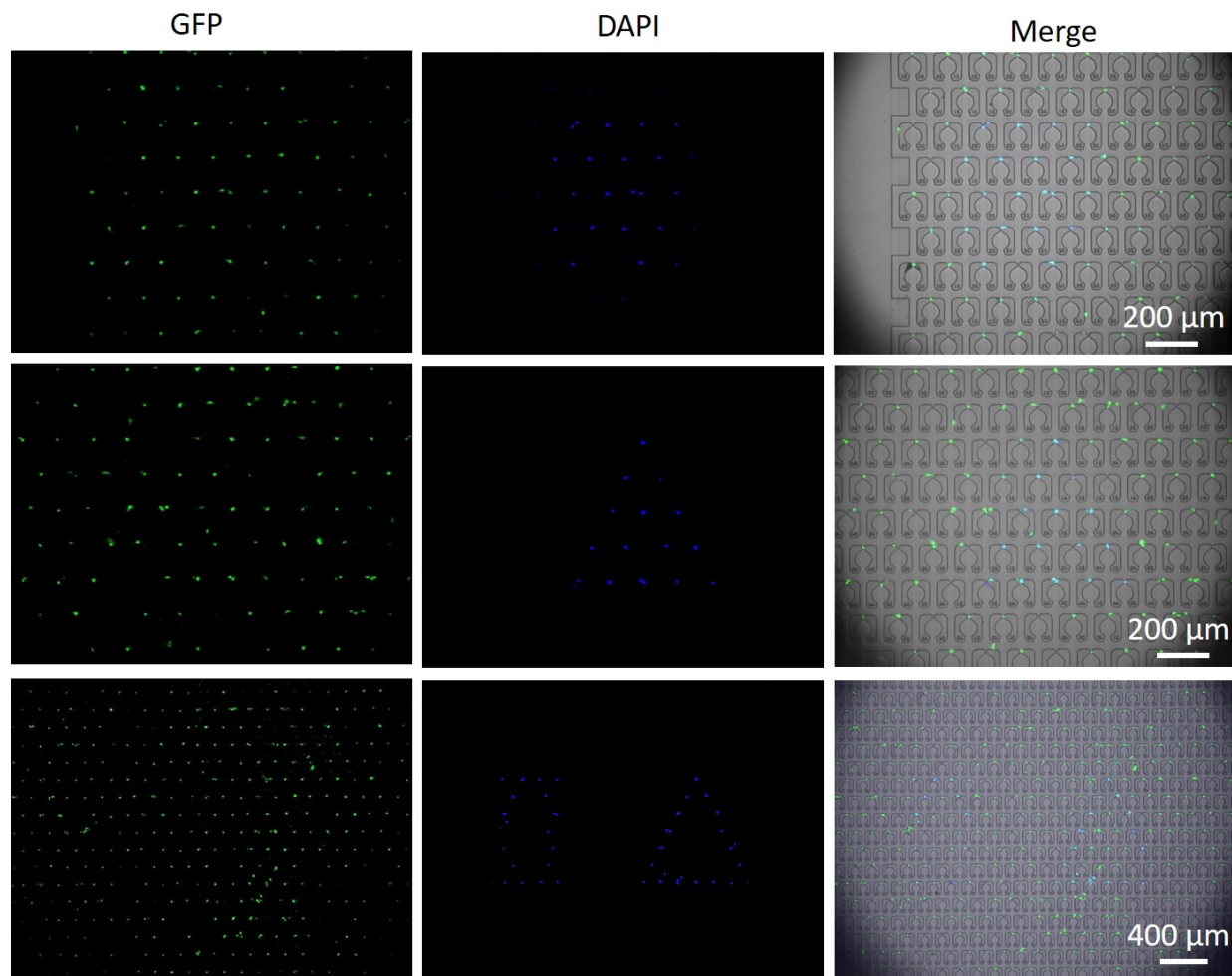
**Figure S8** On-chip culture of single K562 cells for 48 hours after pushing the cells back into the front compartment.



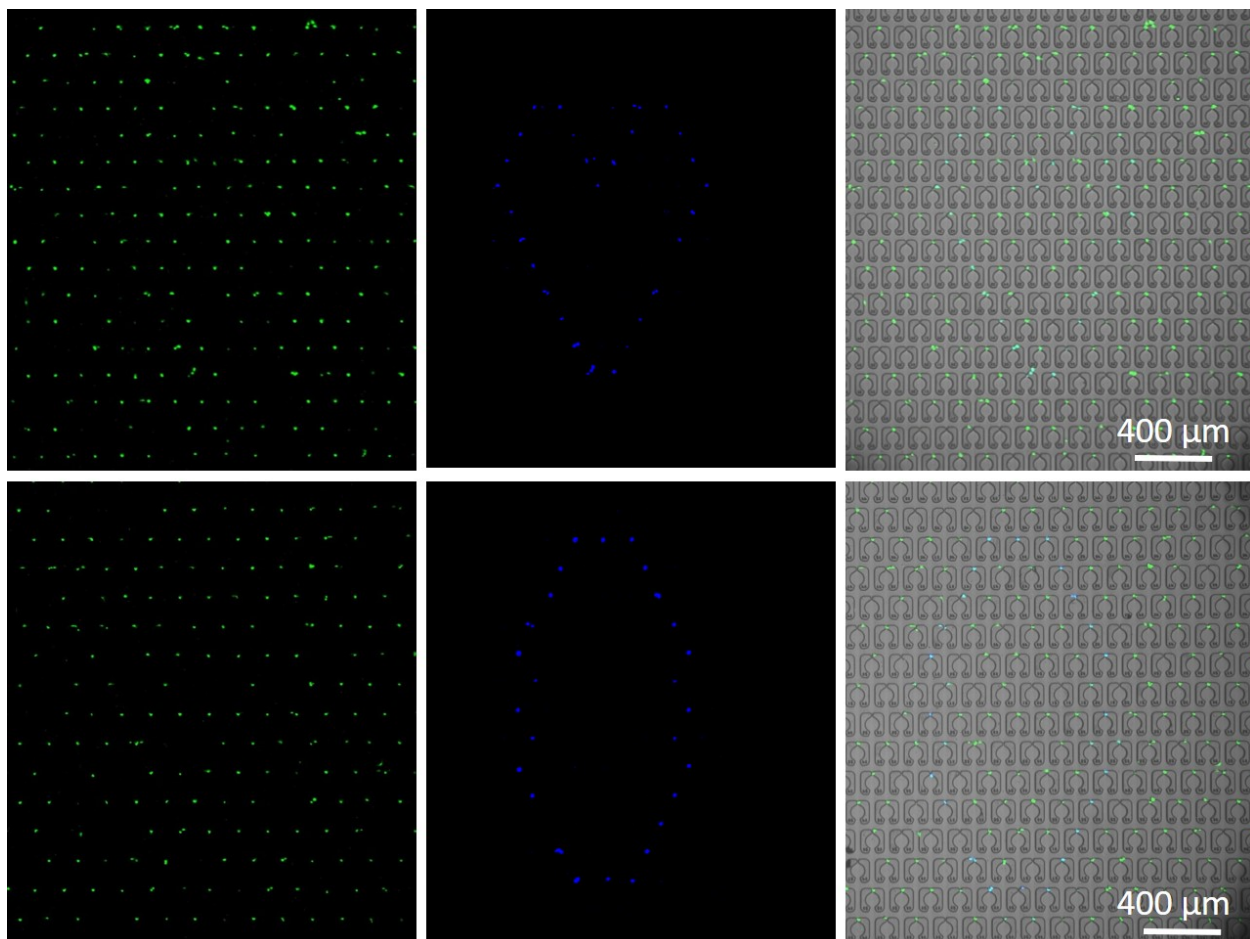
**Figure S9** On-chip culture of single HL60 cells in the hydrogel for 48 hours.



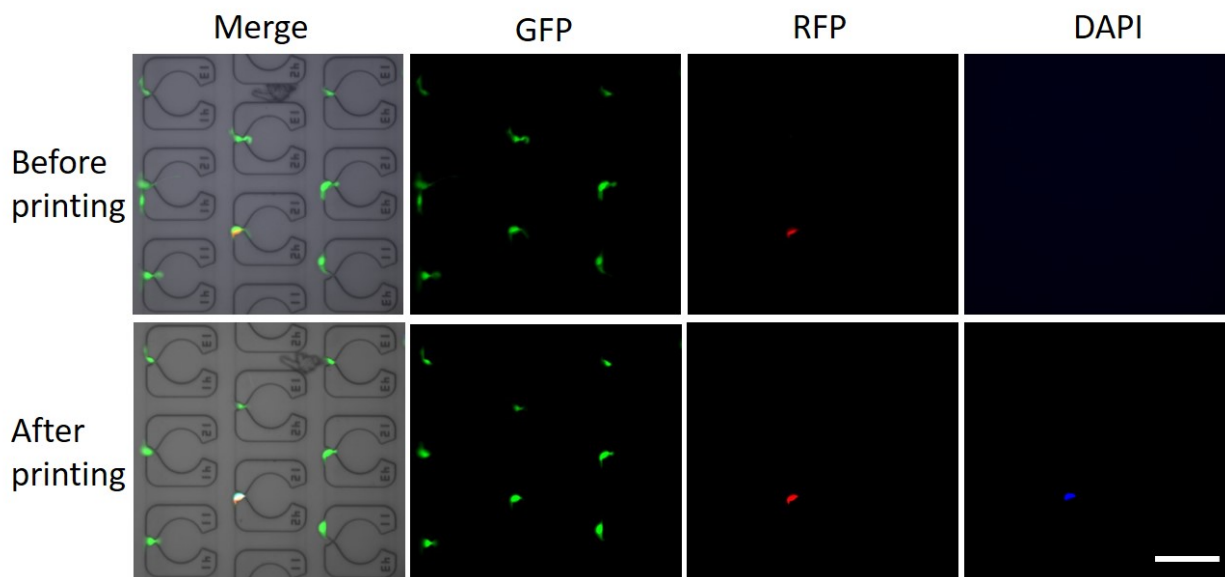
**Figure S10** Printed FITC patterns on a blank device. (a) A ‘filled’ rectangle and a triangle pattern. (b, c) An ‘unfilled’ rectangle and triangle pattern, respectively. The fluorescent spots in each apartment are clear and no contamination is found in the adjacent apartments, demonstrating the high accuracy and high resolution of this printing technique.



**Figure S11** Cell patterns with shapes of rectangle and triangle generated by chemical printing. The left green-channel images show the single cell array on the device; the middle blue-channel images show the cells that were printed DAPI; the right panel were merged images of bright-field, green and blue channel.



**Figure S12** Cell patterns with shapes of heart and diamond generated by chemical printing. The left green-channel images show the single cell array on the device; the middle blue-channel images show the cells that were printed DAPI; the right panel were merged images of bright-field, green and blue channel.



**Figure S13** Chemical printing on selected MDA-MB-231 cells. An example to show precise printing on one selected MDA-MB-231 cell. MDA-MB-231/GFP cells with or without DNA-red labelling were mixed at 1:10 and then were loaded and trapped as single cells. Cells showed both green and red fluorescence were selected as target cells to print Hoechst 33342. After the printing, the target cells showed green, red and blue fluorescence. Scale bar, 100  $\mu\text{m}$ .

**Movie S1** Repeated printing of picolitre droplets by the printer. The average volume of the droplets is 356 pL.

**Movie S2** Test of printing speed of the printer. The average printing speed is  $\sim 5$  spots/second.