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

# Inertial microfluidic cube for automatic and fast extraction of white blood cells from whole blood

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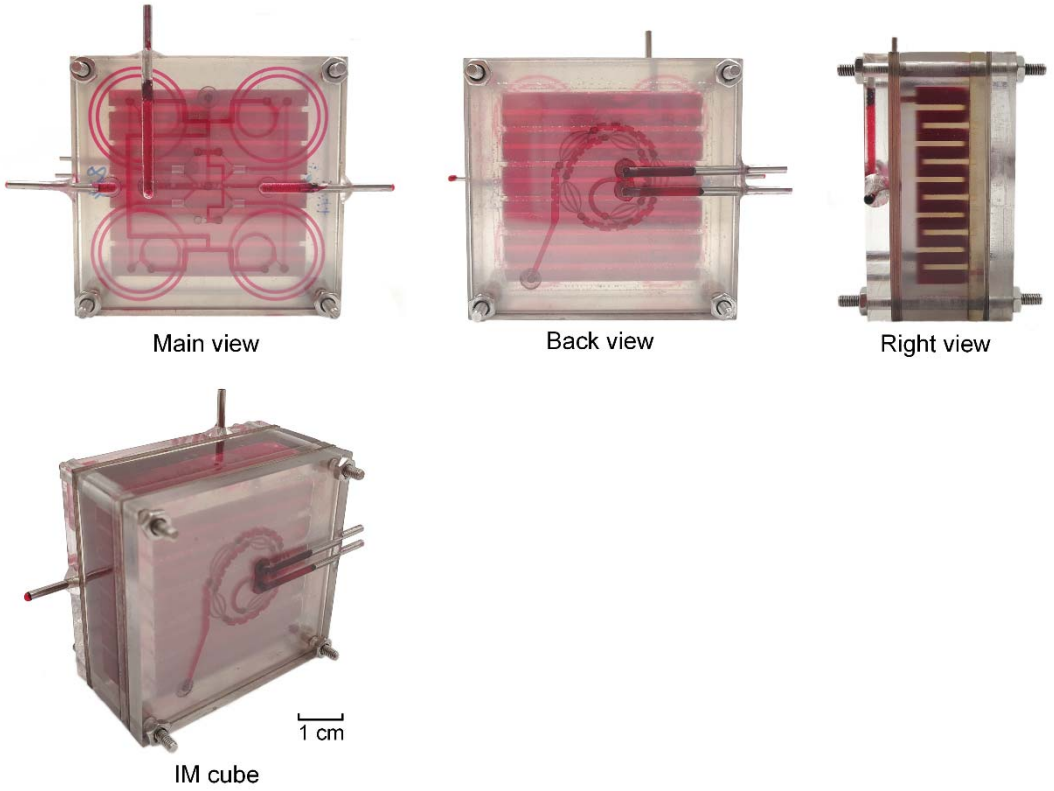


**Figure S1.** Photographs of the fabricated IM cube. The IM cube were filled with red ink for clear visualization.

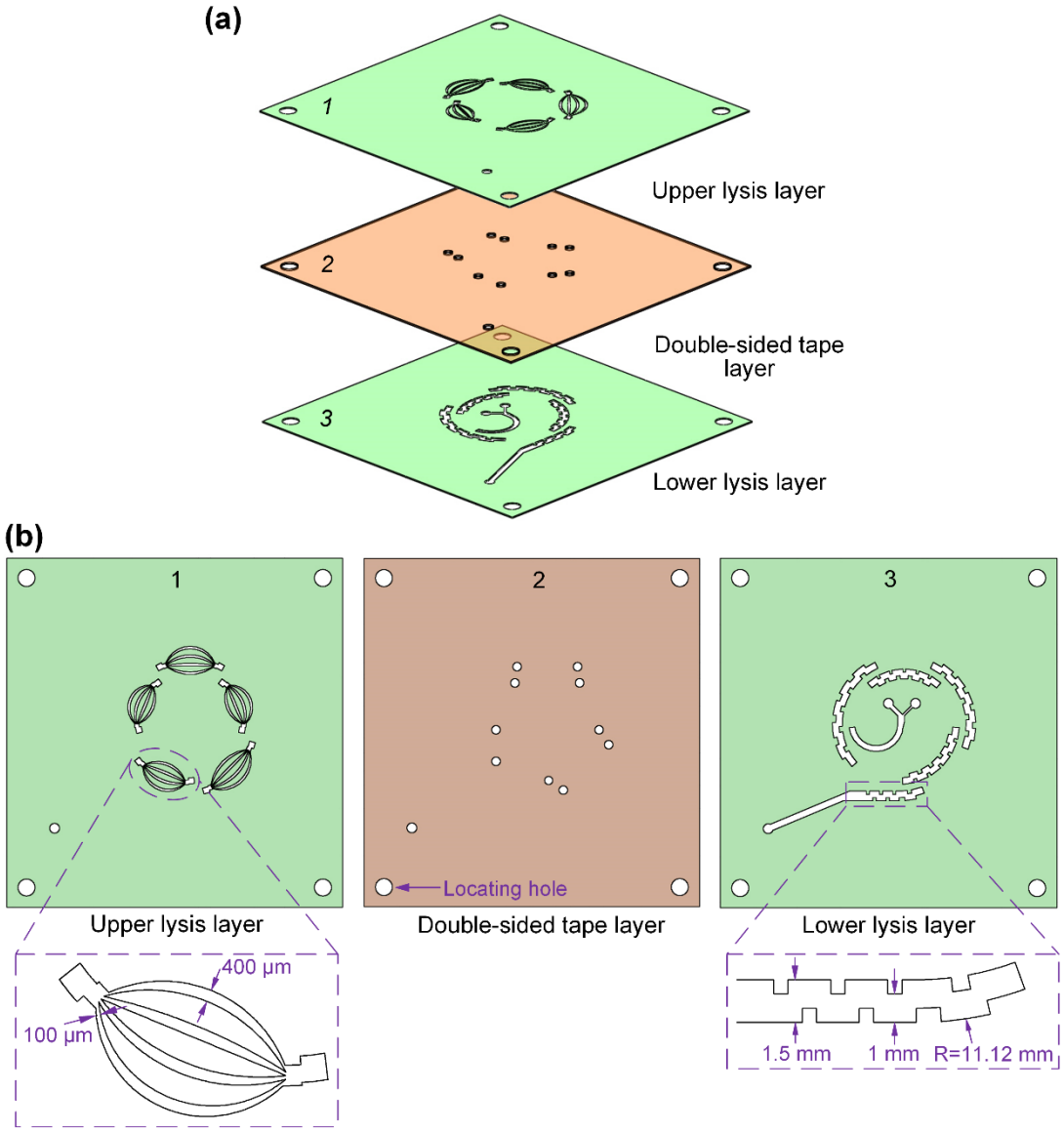


**Figure S2.** (a) Explosion diagram of the lysis module which was fabricated through adhering two channel layers (the upper lysis layer and the lower lysis layer) with a double-sided tape layer in the vertical direction. (b) Structure and dimension of each layer.

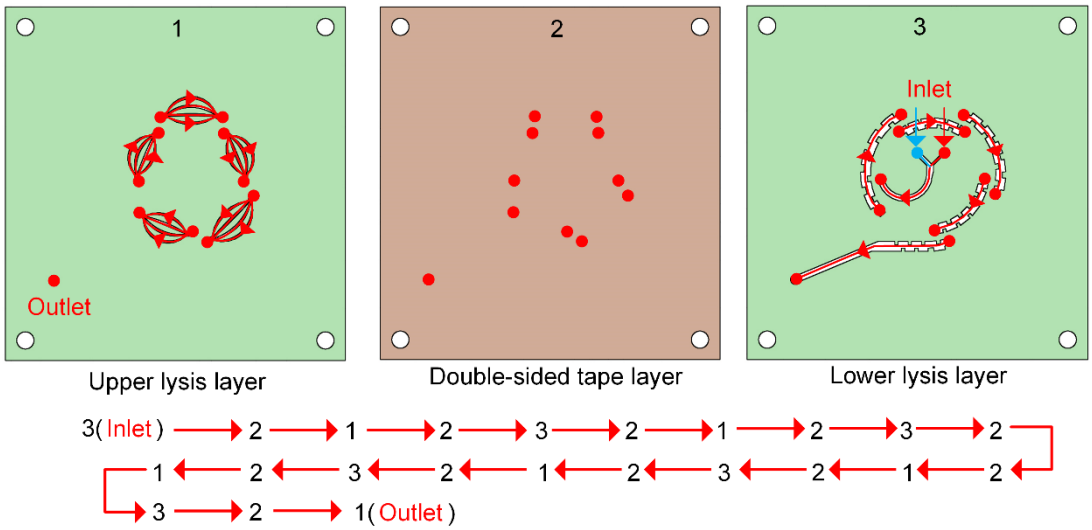


**Figure S3.** Flow paths in different layers of the lysis module. The two liquids were infused into lysis module through the inlets of the layer 3 and flow along a curved segment. Then, the liquids pass through the vertical holes in the layer 2 and flow into layer 1. In the layer 1, liquids are first divided into four channels and recombined in one channel, and then flow into the layer 3 through vertical holes in the layer 2 again. Next, the liquids reciprocate between layer 1 and layer 3 through the vertical holes in the layer 2. Ultimately, liquids flow out of the lysis module through the outlet in the layer 1.

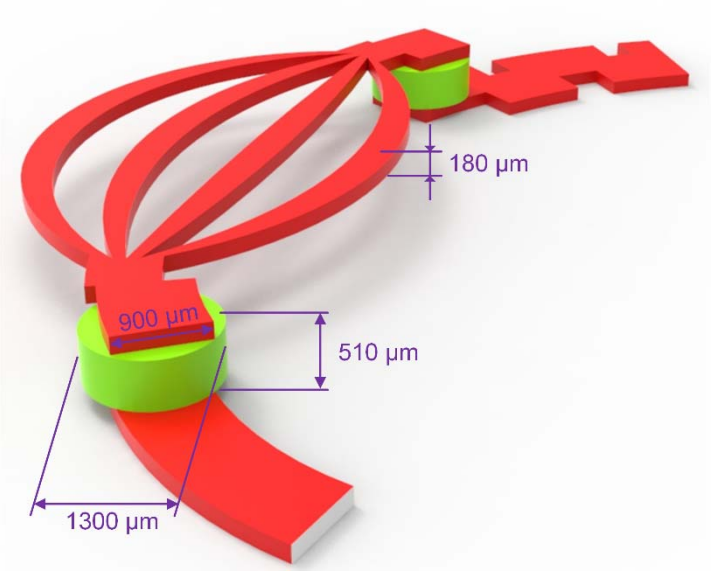


**Figure S4.** Concave-convex structures in the connection portions between the upper and lower lysis layers.

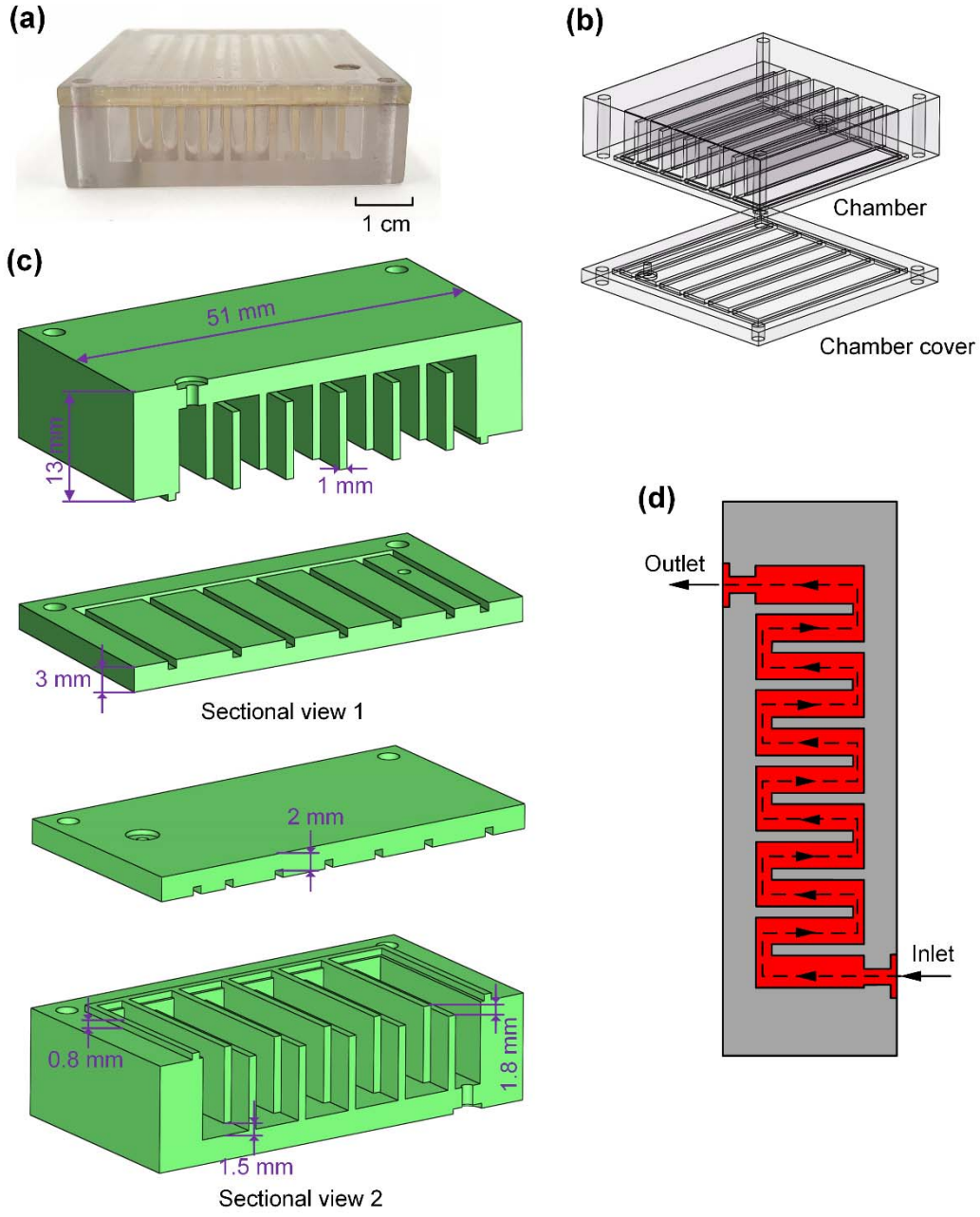


**Figure S5.** (a) Photograph of the storage module. (b) Structure of the storage module which was fabricated through assembling the two parts (chamber and chamber cover). (c) Sectional views of the storage module. (d) Flow path of the liquid in the storage module.

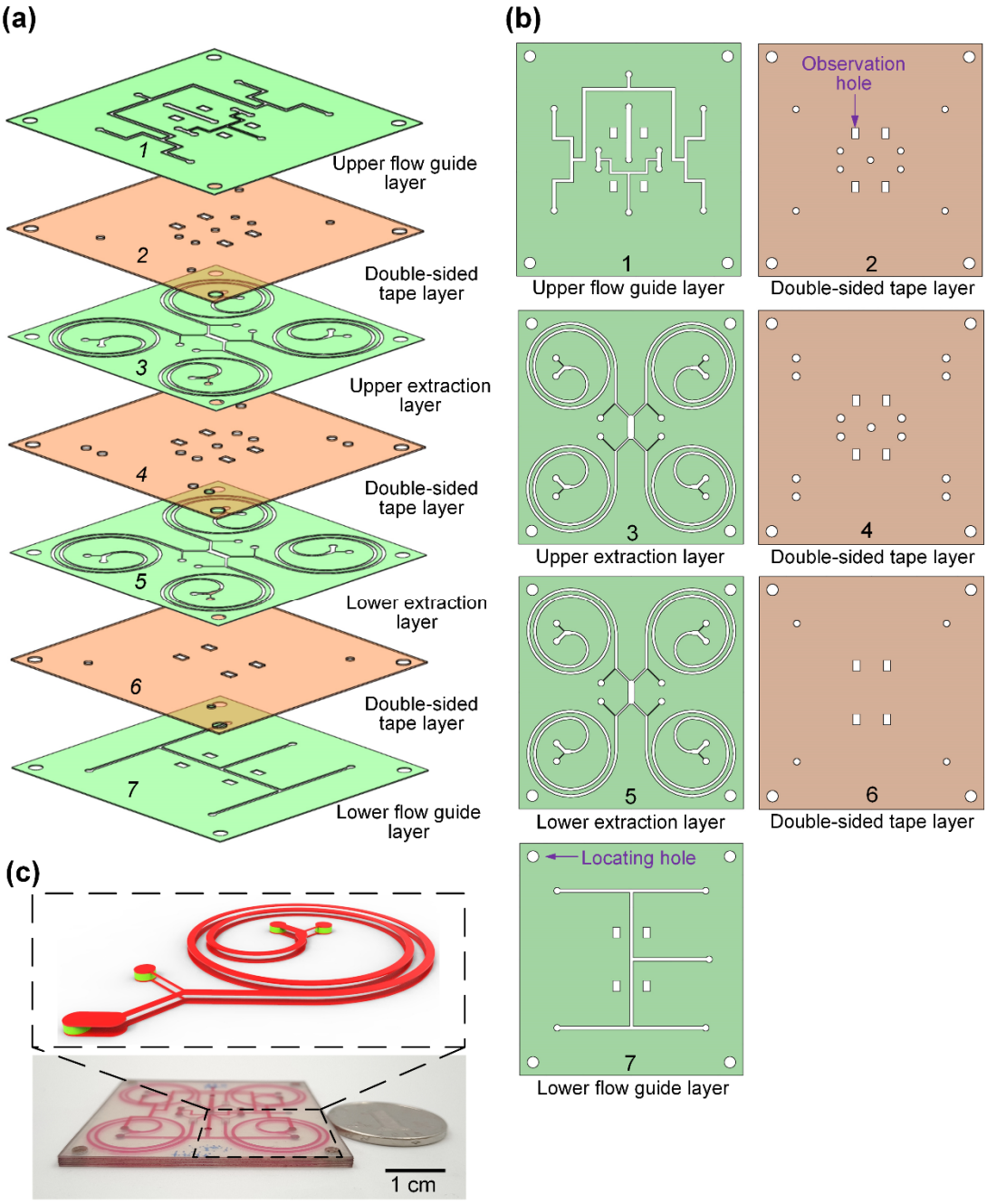


**Figure S6.** (a) Explosion diagram of the extraction module which was fabricated through stacking seven layers in the vertical direction. (b) Structure of each layer. (c) Two extraction layers (each layer contains four spiral channels) were stacked in the vertical direction to create an eight-unit structure.

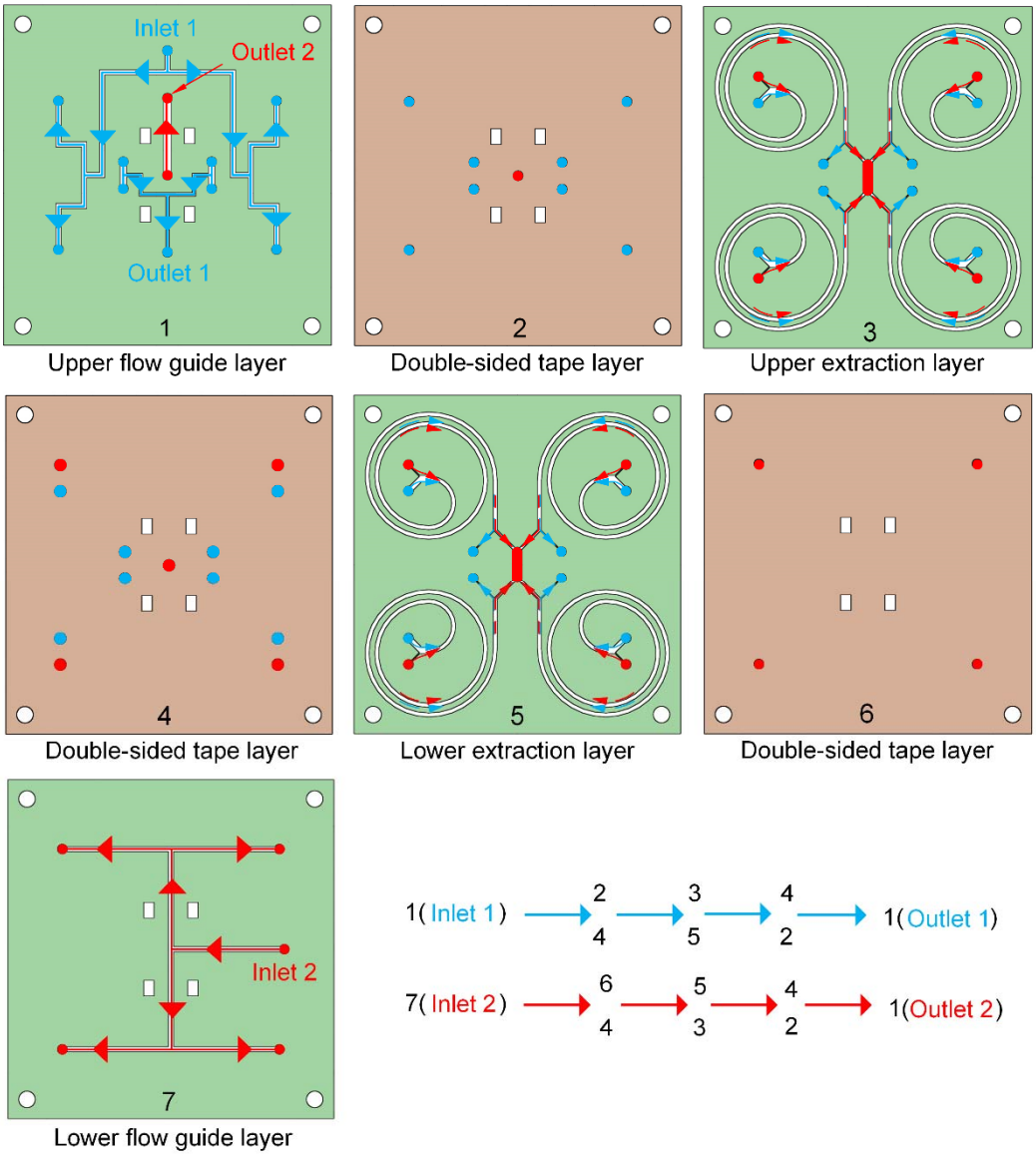


**Figure S7.** Flow paths in different layers of the extraction module. Specifically, the liquid (blue) infused through the inlet 1 is equally distribute into four channels in the layer 1, then flows into the layers 3 and 5 through vertical holes in the layers 2 and 4, and finally flows back to the layer 1. Then, the liquid is converged into one channel and exported from the outlet 1 in the layer 1. The liquid (red) infused through the inlet 2 is equally distributed into four channels in the layer 7, and flows into the layers 5 and 3 through the vertical holes in the layers 4 and 6, and then converges in the central region. Finally, the liquid flows into the layer 1 through the vertical holes in layers 2 and 4, and exports from the outlet 2.

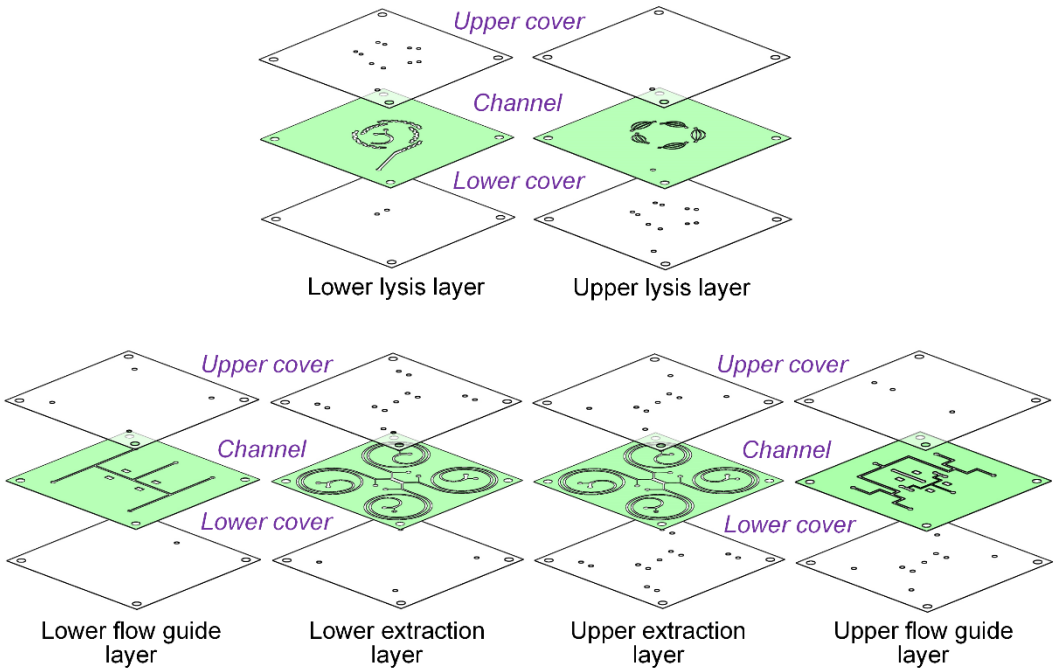


**Figure S8.** Three-sheet structures of the lysis layers, the flow guide layers, and the extraction layers. All these layers were fabricated by enclosing channels with the patterned upper and lower covers.

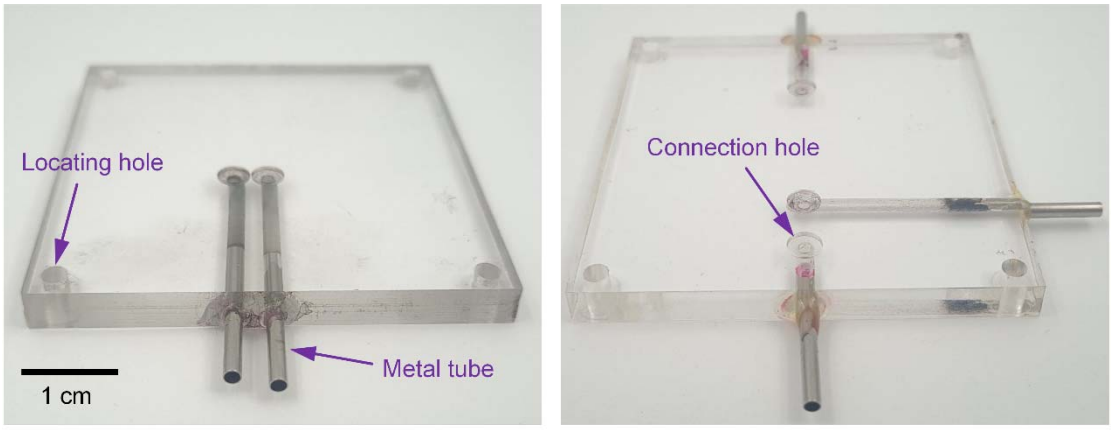


**Figure S9.** Photographs of lower and upper covers of our IM cube.

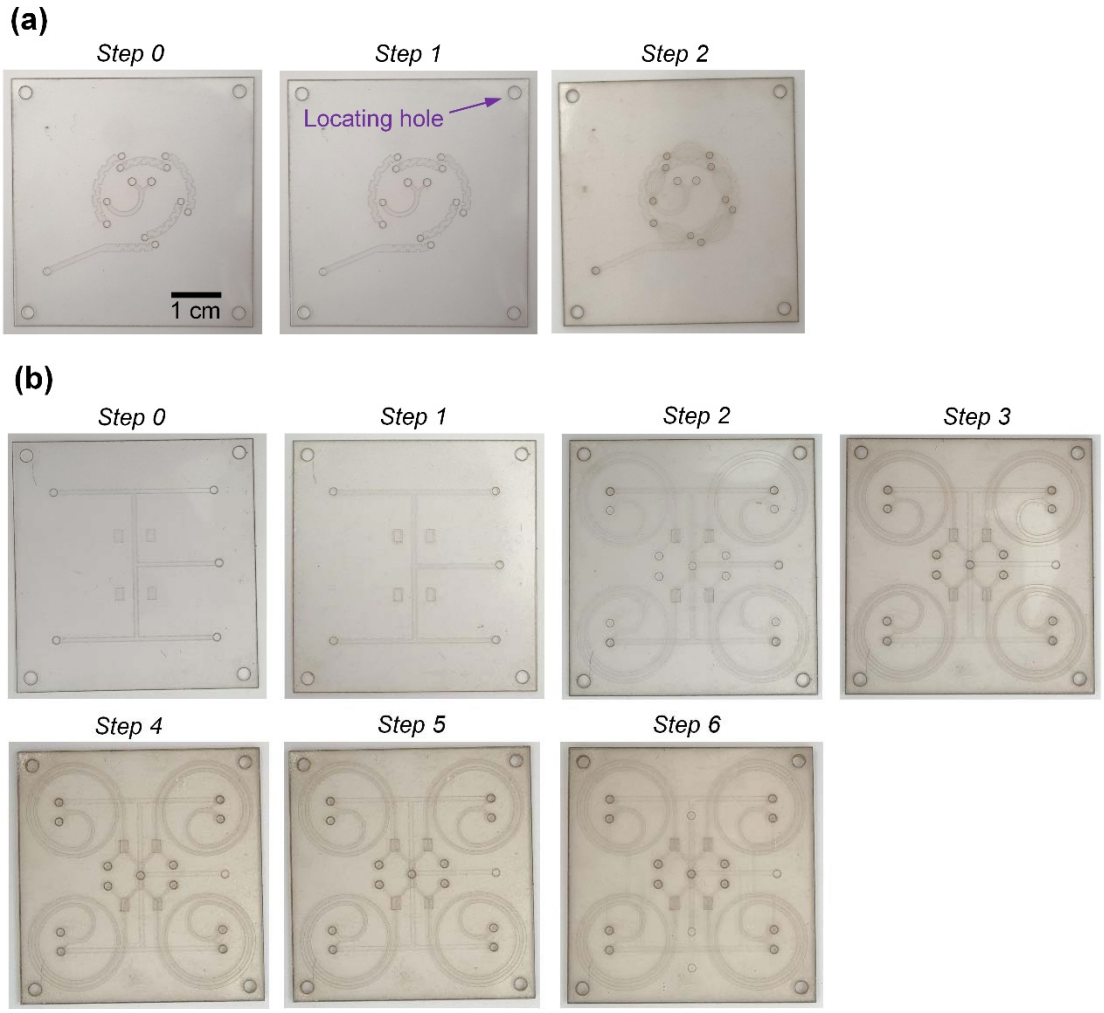


**Figure S10.** Assembly processes of the lysis module (a) and the extraction module (b).

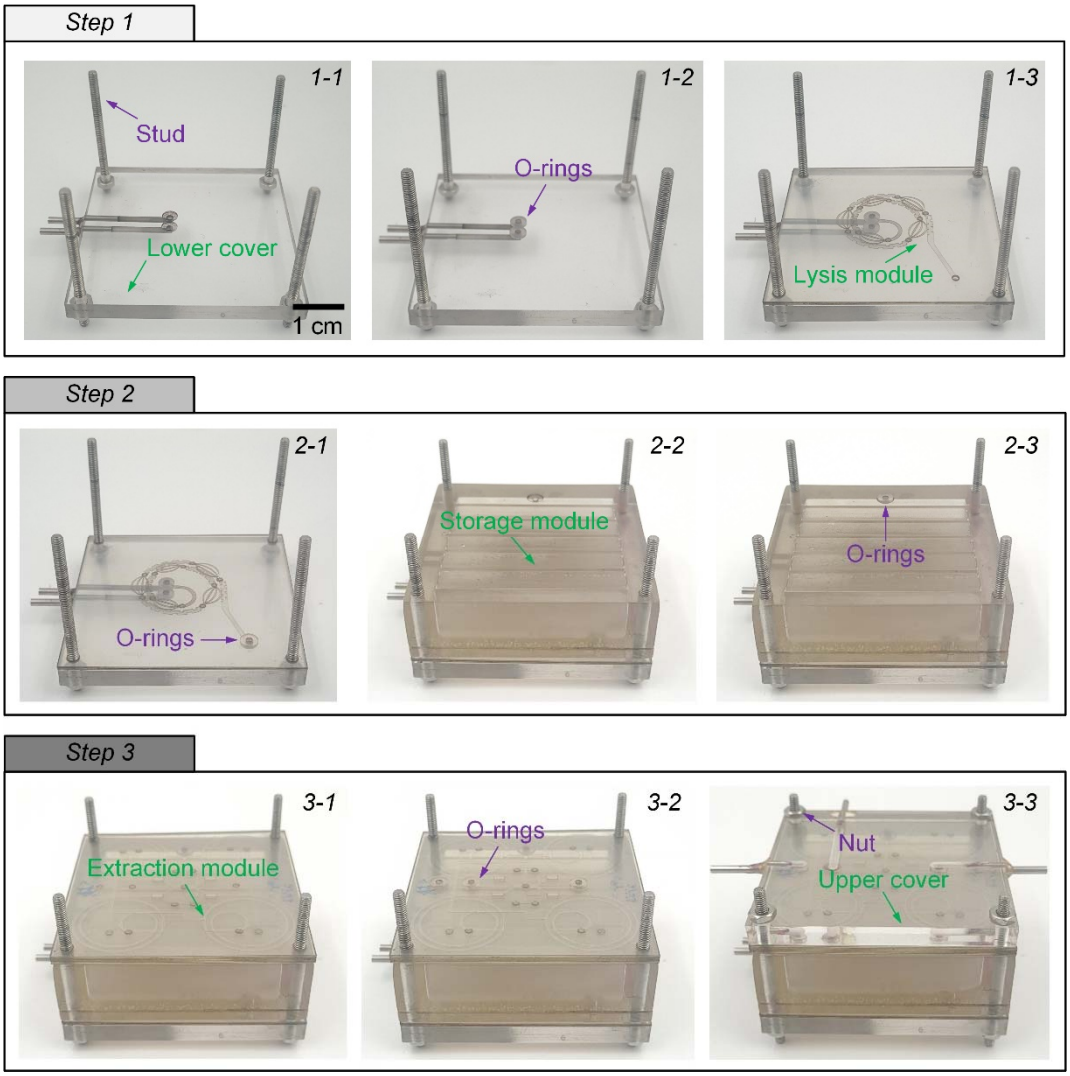


**Figure S11.** Assembly process of our IM cube after fabricating each modules and parts. The assembly of our IM cube can be accomplished within three main steps.

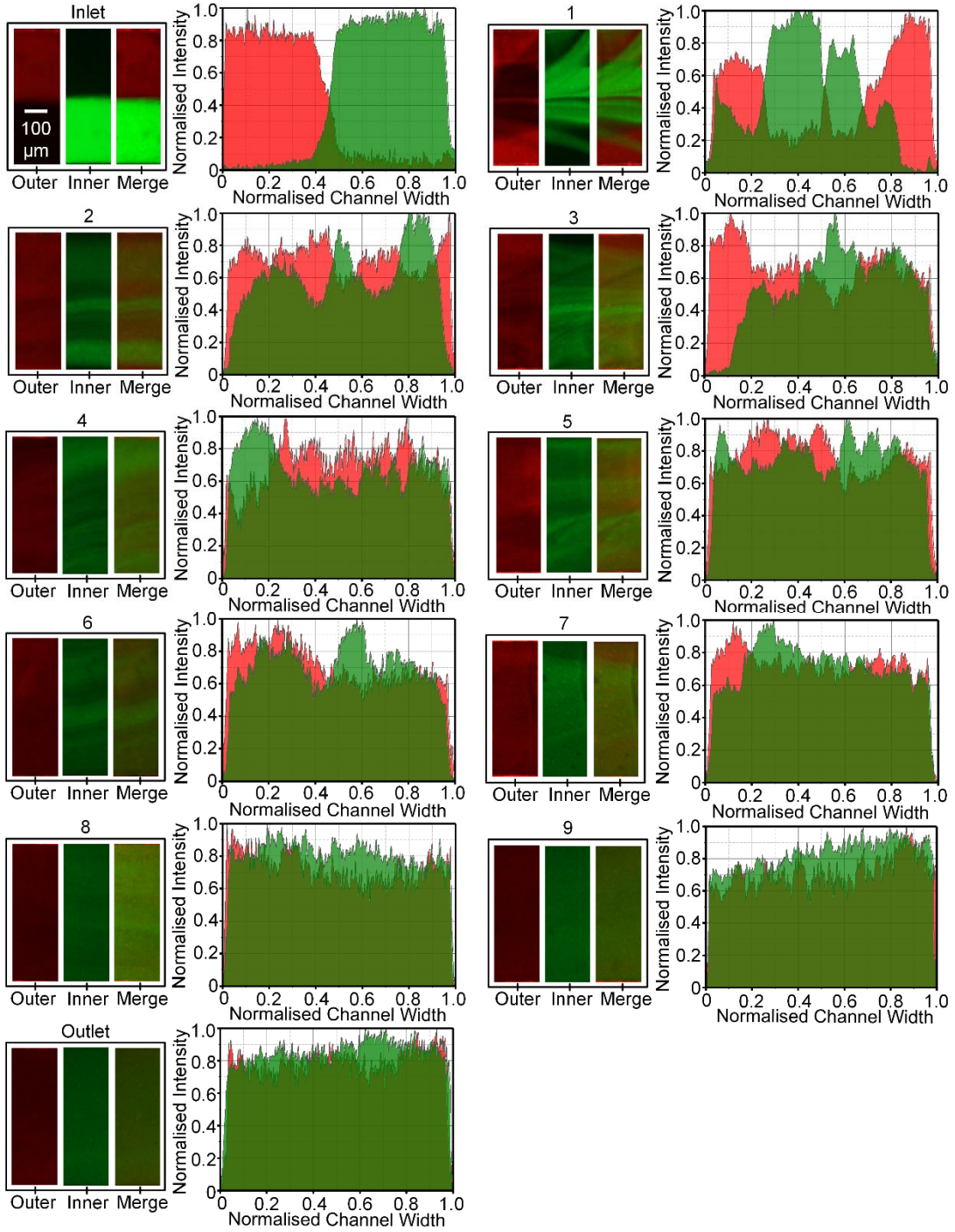


**Figure S12.** Mixing images and normalized intensity profiles of Rhodamine-B (red) and Fluorescein (green) solutions at different positions (inlet, outlet, and other nine positions marked in Figure 2a) of the lysis module.

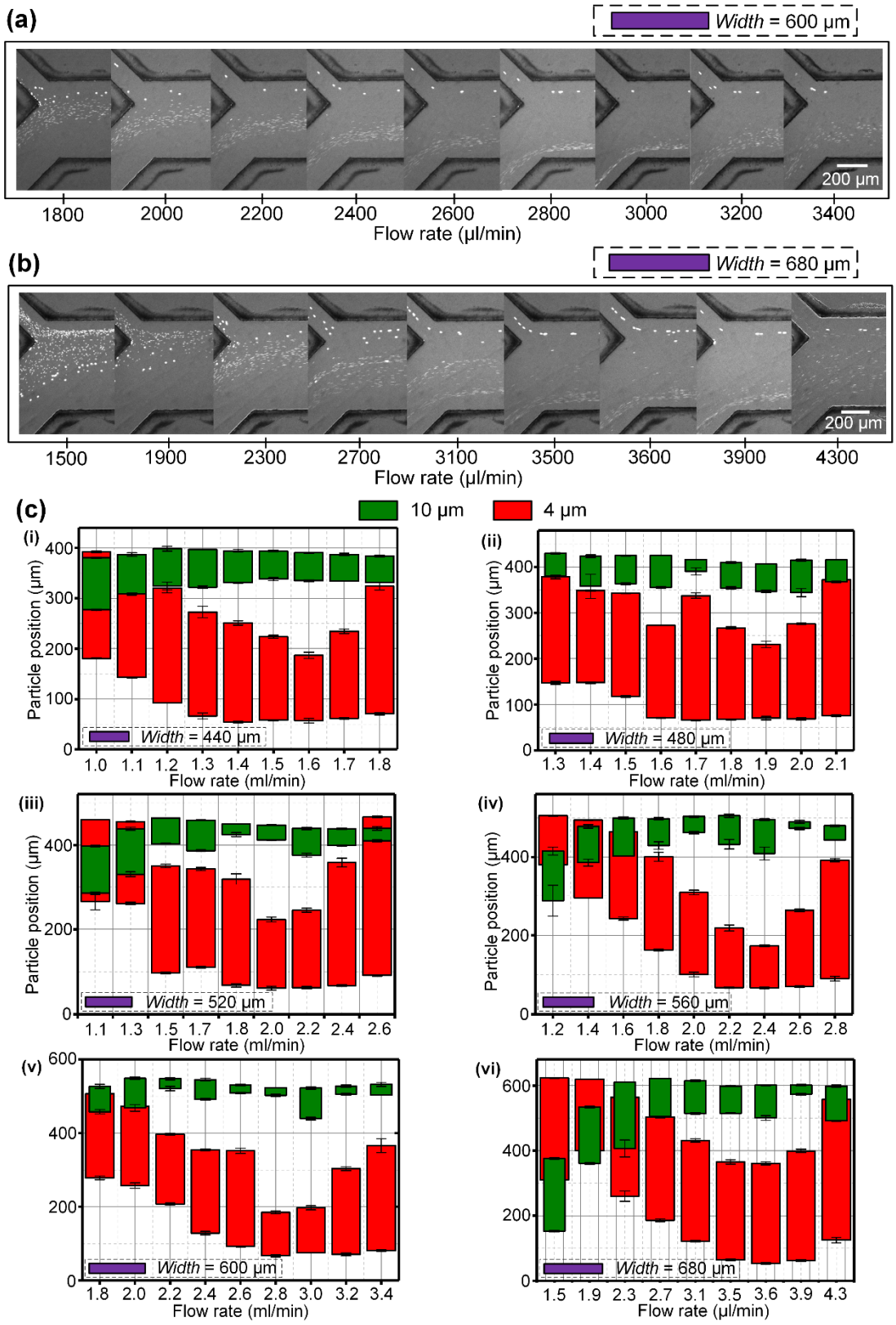


**Figure S13.** Images illustrating the distributions of 10 μm and 4 μm particles at different flow rates in the extraction channels with widths of 600 μm (a) and 680 μm (b). (c) The lateral positions of 10 μm and 4 μm particles in the extraction channels with width of 440 μm, 480 μm, 520 μm, 560 μm, 600 μm and 660 μm at different flow rates.

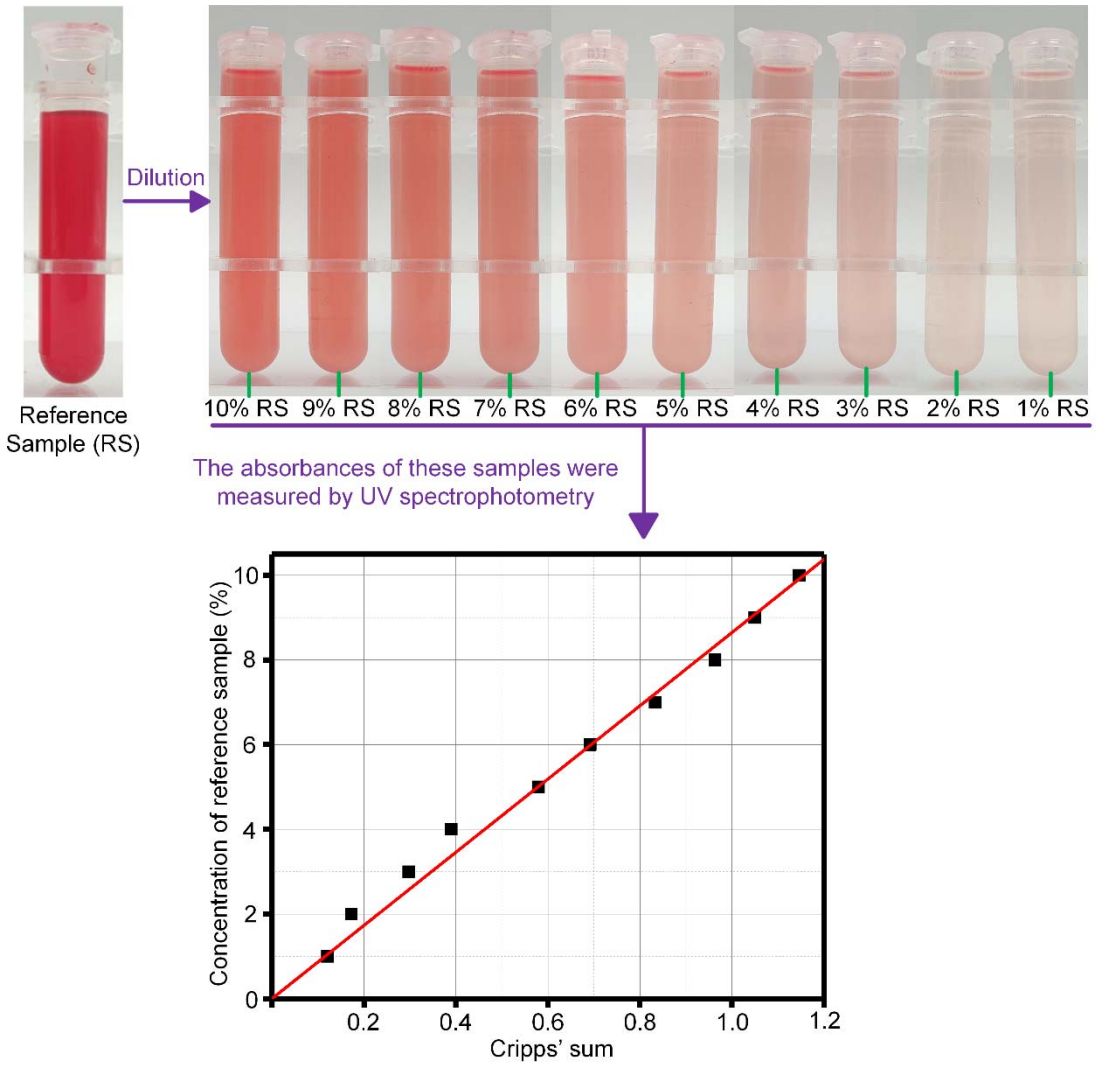


**Figure S14.** The successive dilution of the reference sample and the calibration curve illustrating the relationship between the concentration of reference sample and the Cripps' sum.

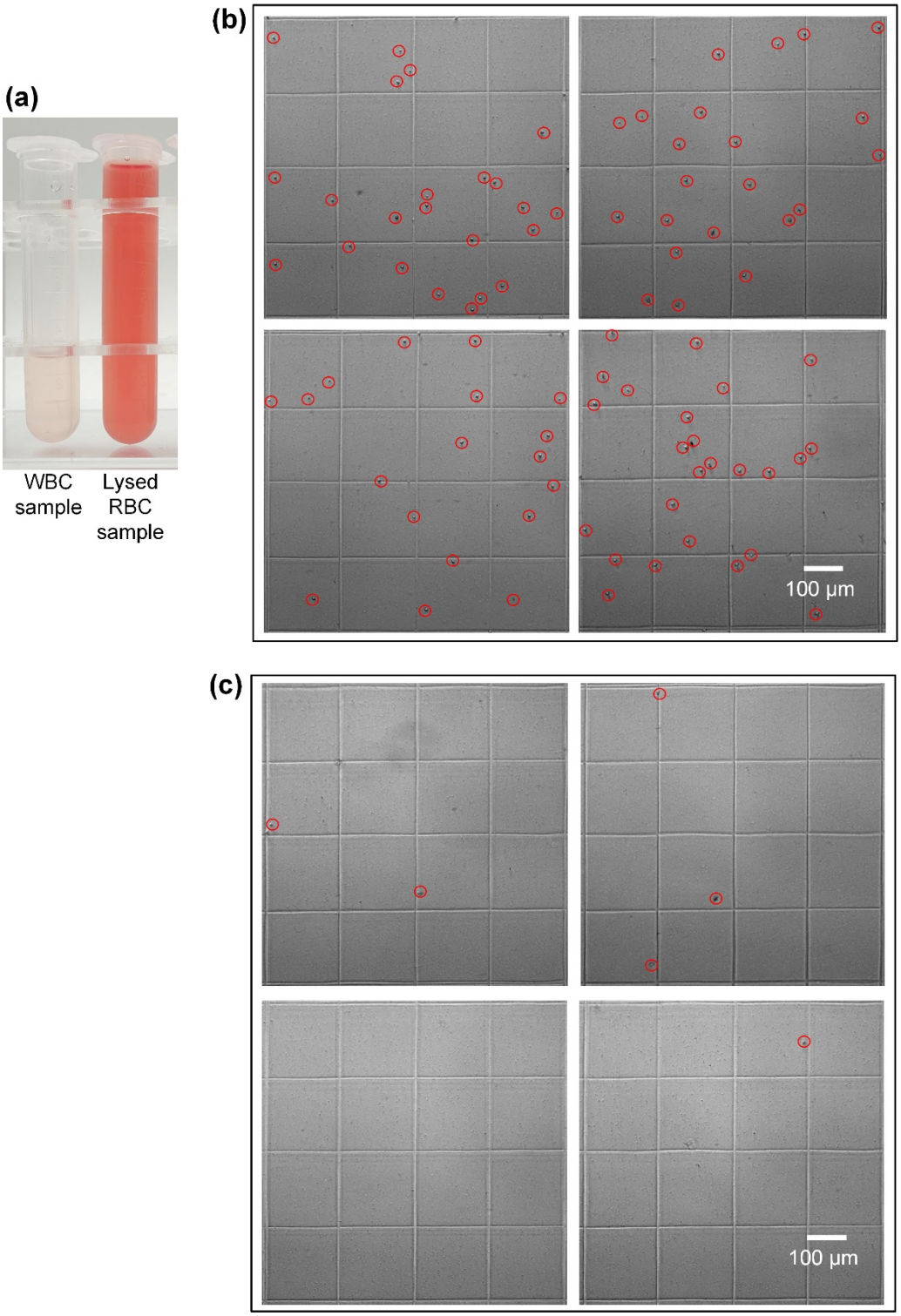


**Figure S15.** (a) Photograph of the collected WBC sample and the lysed RBC sample. (b-c) Microscopic images of the WBC sample (b) and the lysed RBC sample (c) collected from the two outlets of our IM cube.

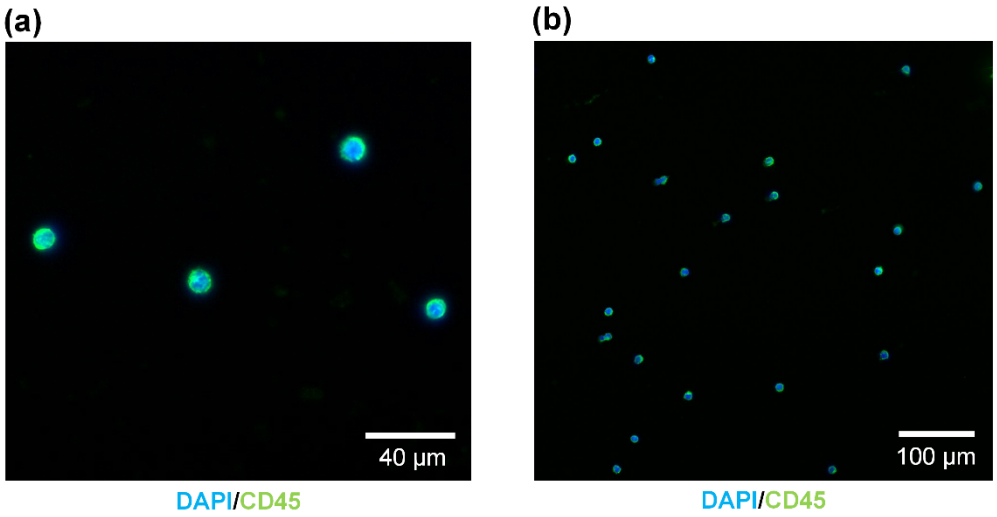


**Figure S16.** Immunofluorescence staining results of WBCs collected from IM cube. DAPI (blue) and CD45 (green) were used to stain the nucleus and membrane of WBCs, respectively. The merged fluorescent images in 50× (a) and 20× (b) microscopic images show that the WBCs have a good integrity.

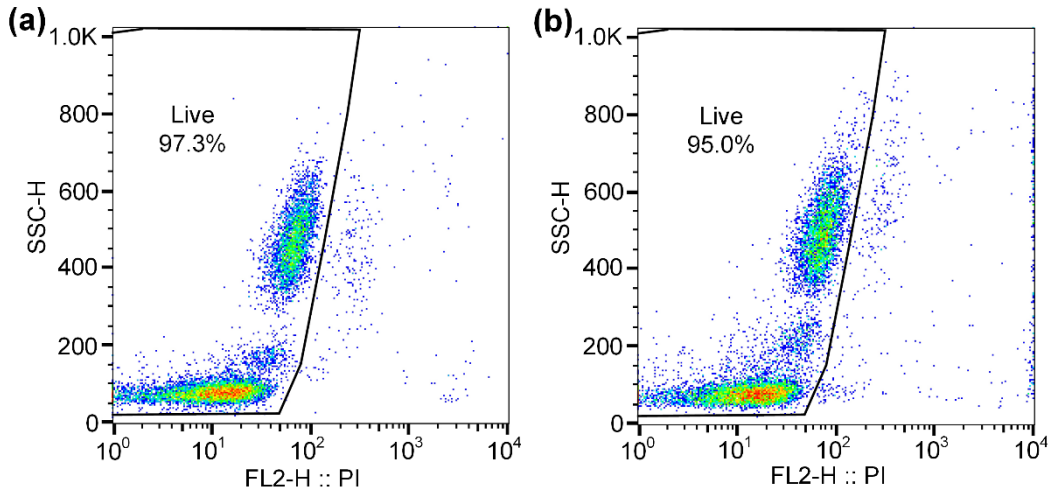


**Figure S17.** The result of cell viability analyzed by flow cytometry. The WBCs were first stained with propidium iodide (PI), and were analyzed using a flow cytometer. (a) The WBCs (10271 cells) acquired by our IM cube have a viability rate of 97.3%. (b) The WBCs (10289 cells) acquired by the centrifugation have a viability rate of 95%.

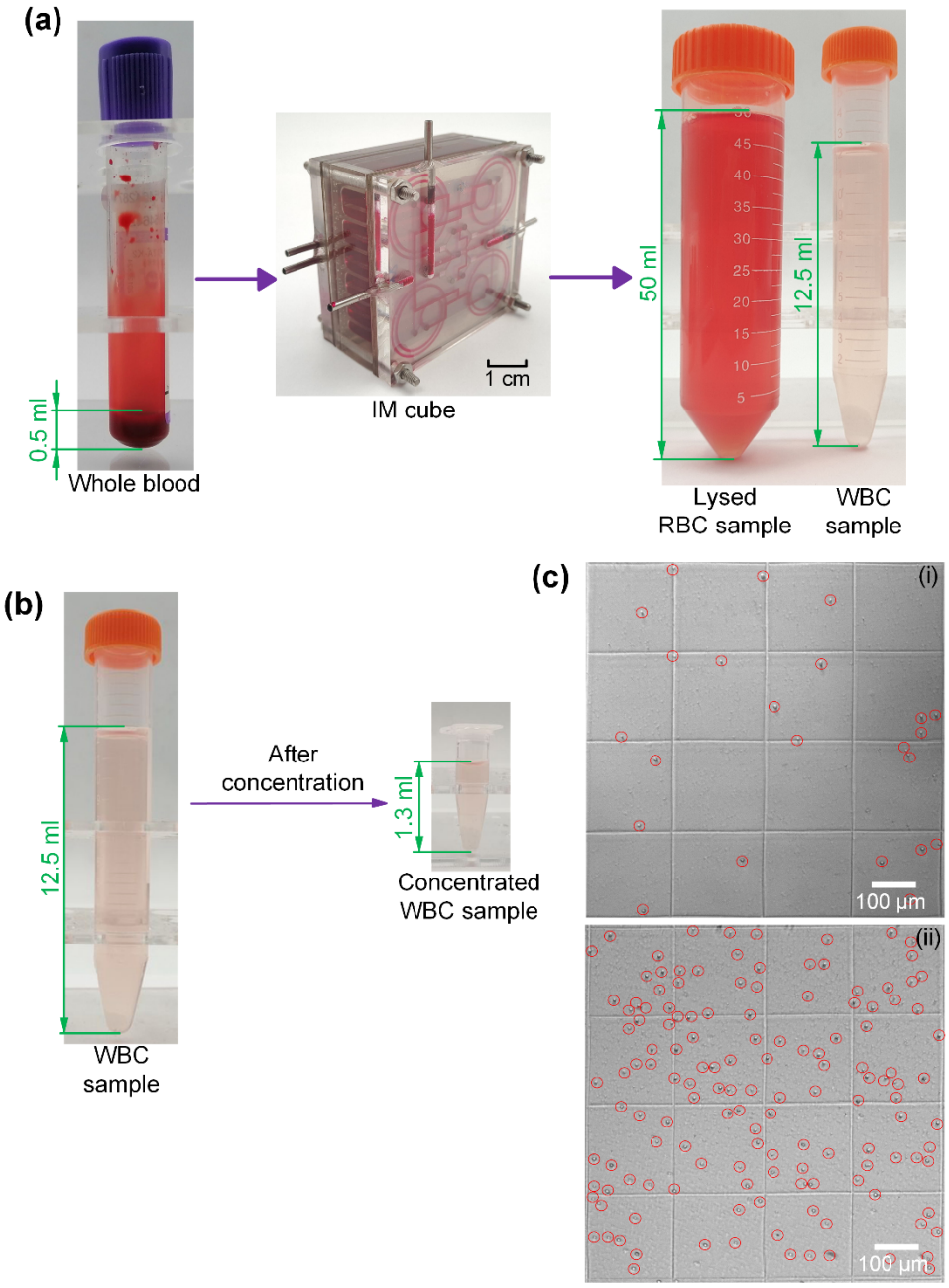


**Figure S18.** (a) Photograph illustrating the actual sample volume increase. (b) The WBC sample acquired by our IM cube was concentrated by our previously-developed inertial microfluidic concentrator. (c) The microscopic images of WBC samples before (i) and after (ii) concentration.

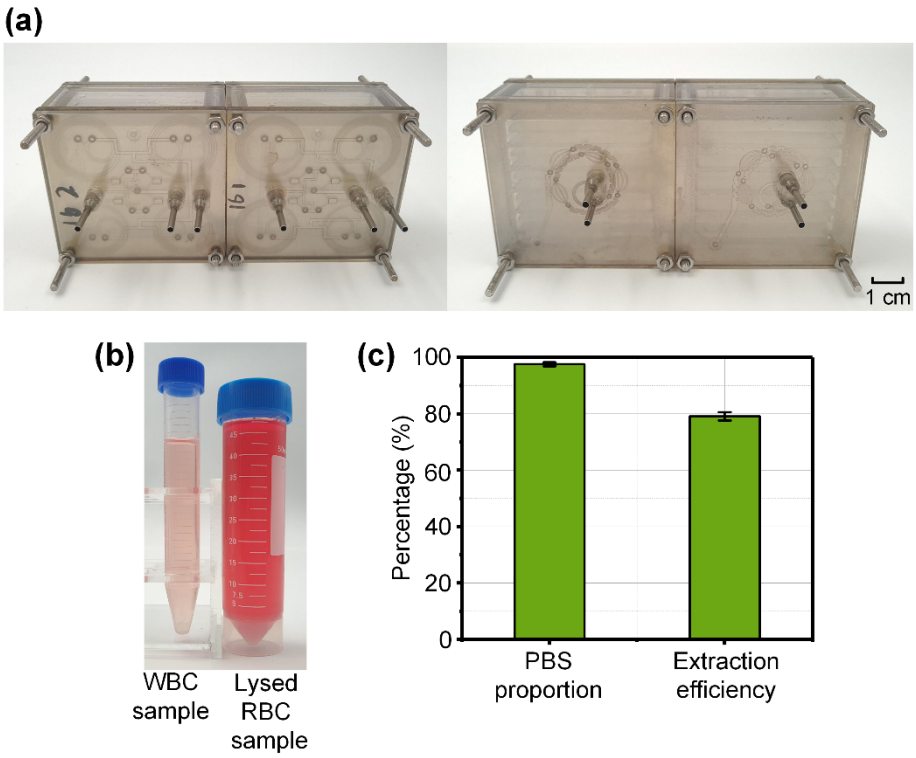


**Figure S19.** (a) The integrated device in which two IM cubes were paralleled and each cube was stacked with 16 spiral extraction units. The integrated device could achieve a throughput of 720 μl/min for whole blood. (b) The WBC and lysed RBC samples collected using the integrated device. (c) The PBS proportion of WBC sample collected by integrated device is about 97.6%, and the extraction efficiency of WBCs acquired by the integrated device is about 79.1%.

**Supplementary Video S1.** High throughput WBC extraction (720 μl/min) using the integrated device (two IM cubes were paralleled and each cube was stacked with 16 spiral extraction units).