Electronic Supplementary Material (ESI) for Analyst.

Supporting information

One-step molded microfluidic chip featuring two-layer silver-PDMS microelectrode for dielectrophoretic cell separation

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Contents

Important tips for fabrication

Here we elucidate more fabrication details as complementary for the fabrication process introduced in the main text that related to high reliability and yield of the device.

Tip a) and b) guarantee substantial filling of AgPDMS into the mold and successful release of the AgPDMS structure, which are critical to guarantee the continuity and intactness of the electrode structure, including the bulk electrode and the interdigitated digits. A good continuity of the AgPDMS structure ensures the effective delivery of the activation voltage from the bulk electrodes to the microelectrodes, and an intact microelectrode structure is determining factor for the distribution of the designated non-uniform electric field. The details are as below.

a) Careful cleaning and dry of the silver (Ag) particles before mixing. The purchased Ag particles upon delivery are cleaned in batch using acetone and isopropanol (IPA) in ultrasonic bath, followed by DI water rinse and freeze-dry in vacuum (LGJ-1A-50, YATAIKELONG, China). Then these Ag particles are vacuum-packed separately in plastic bags with smaller package (~20g/bag). The cleaning-dry and packaging process remove contaminations (e.g., oil stain and dust possibly induced during the manufacturing) and water moisture that potentially pose adverse effect for mixing of the Ag particles and the PDMS gel. It also protect the Ag particles from being oxidized during storage.

b) Critical directions of rubbing the mold during AgPDMS filling and peeling off of the device. When filling the AgPDMS composite into the mold cavities, to guarantee substantial filling, one should rub the mold against the copy paper in the direction orthogonal to the extending orientation of the narrow-long cavities (i.e., the cavities designating the electrode digits), as shown in Fig. S2a. When releasing the cured structure from the mold, on the contrary, one should gently peel the structure in the direction tangential to the narrow-long electrode digits, which help to protect the long digits from breaking, as illustrated in Fig. S2b.

Tip c) and d) stated below relate to the bonding between the AgPDMS layer and the glass slide.

c) To improve the bonding quality between the stacked PDMS-AgPDMS layers to the glass slide in plasma bonding, the PDMS-AgPDMS composite and glass slide were carefully cleaned with IPA in sonication followed by rinsing with DI water and baked drying before plasma bonding. The chip post plasma bonding is flipped over and pressed by a heavy object (~250g) put on the glass substrate for overnight to further improve the bonding quality.

d) We also developed an alternative bonding method by using a semi-cured PDMS layer as adhesive layer for the AgPDMS layer and the glass slide. It took advantage of PDMS cohesion between the semi-cured PDMS and the AgPDMS, as well as good bonding between the thin PDMS layer to the glass slide, and therefore exhibit good bonding quality. PDMS as an adhesive layer has been previously reported in [1-3]. In our approach, a thin layer (~12μm thick) of PDMS gel was spin-coated on the glass slide, followed by heating at 70^{o}C for 5 minutes on a hotplate to make the PDMS sticky. Then the stacked AgPDMS-PDMS structure was placed gently onto the semi-cured PDMS layer, being subsequently heated for another 30 minutes at 70^{o}C to completely cure the PDMS adhesive layer and thus form permanent bonding. Note that this approach was just an alternative, the plasma bonding described in the main text also yield leaky-free bonding for current applications using our device.

1. J. H. Koschwanez, R. H. Carlson and D. R. Meldrum, *PLoS one*, 2009, **4**.
2. N. J. Douville, Y. C. Tung, R. Li, J. D. Wang, M. E. El-Sayed and S. Takayama, *Anal. Chem.*, 2010, **82**, 2505-2511.
3. B. H. Chueh, D. Huh, C. R. Kyrtsos, T. Houssin, N. Futai and S. Takayama, *Anal. Chem.*, 2007, **79**, 3504-3508.

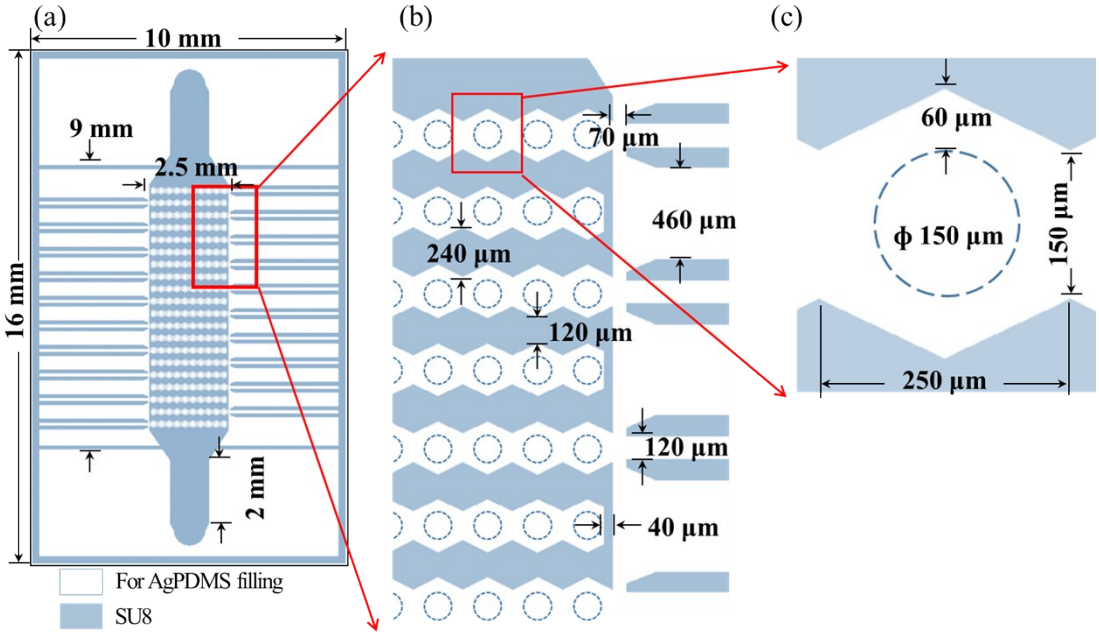

Fig. S1 Layout of the master mold with dimension parameters labeled on (a) the entire device, (b) close-up view for a part of the channel with key structures and (c) one of the repeating blocks as building unit for the electrode digits.

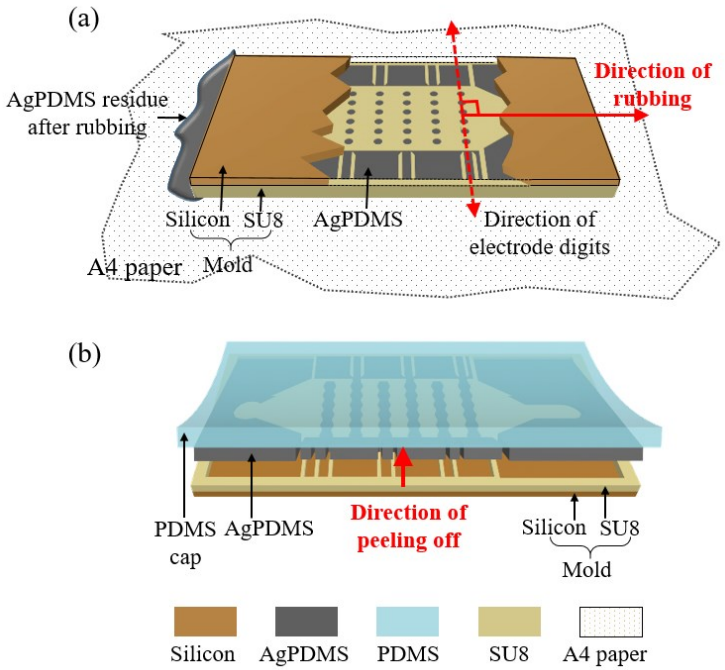

Fig. S2 Schematic illustration of the operation direction for (a) rubbing the mold against paper and (b) AgPDMS release from the mold.

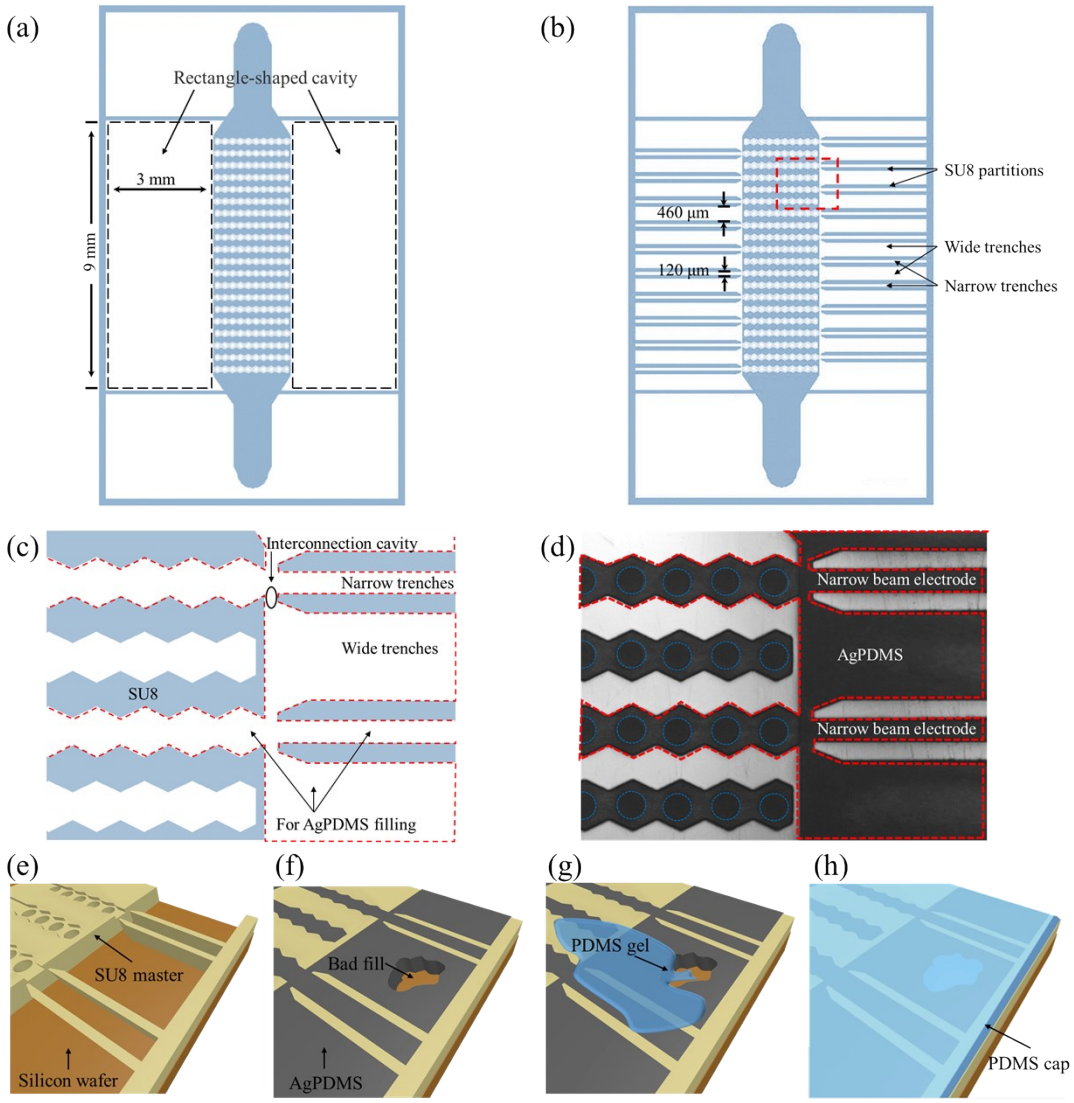

Fig. S3 (a-b) Master layout with (a) initially designed rectangle-shaped cavities featuring large bottom area and (b) improved design of rectangle-shaped cavities being divided into alternating wide and narrow trenches by narrow SU8 partitions for easier and more substantial filling of AgPDMS to mold the bulk electrodes. (c) Enlarged view of the region outlined by rectangle in (b). (d) Micrograph taken from the same region as (c) on a real AgPDMS structure peeled off from the mold. (e-h) Schematic illustration for the bulk electrode filling of the re-partitioned mold: (e) The re-partitioned mold with alternating wide and narrow cavity trenches. (f) Filling of AgPDMS into the mold with occasionally occurred issue of AgPDMS drop-off from a small portion of the wide trench which gives rise to a void on the mold. (g) Filling of the void in (f) during the PDMS pouring and onto the mold for the device cap formation. (h) Device with the PDMS cap layer and completely filled void by the PDMS.

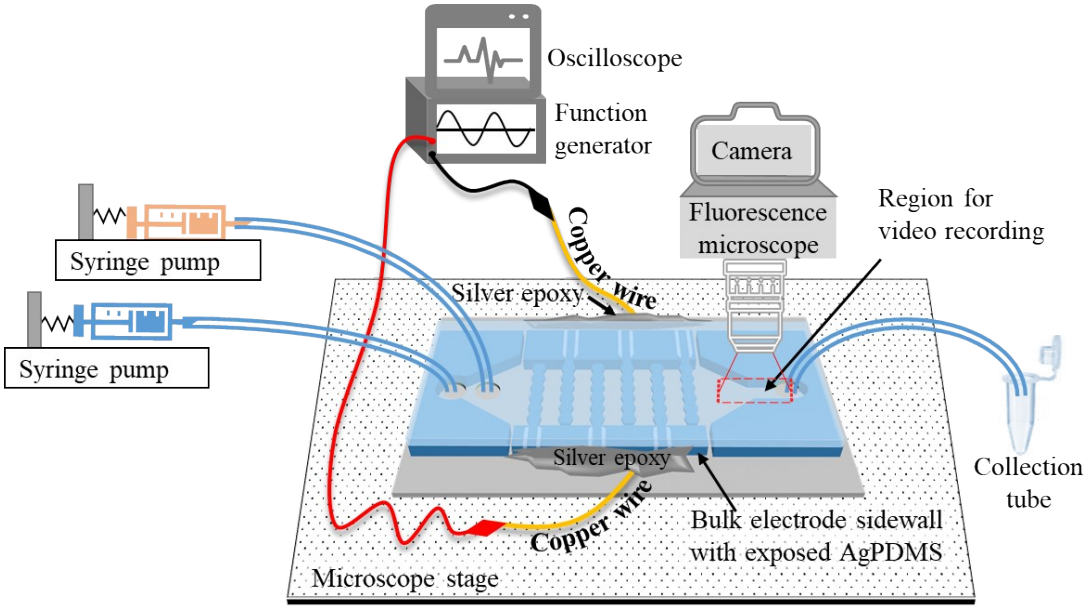

Fig. S4 Schematic of the experimental setup with a device diagram illustrating the connection of the copper wire electrode to the AgPDMS electrode sidewall of the device.

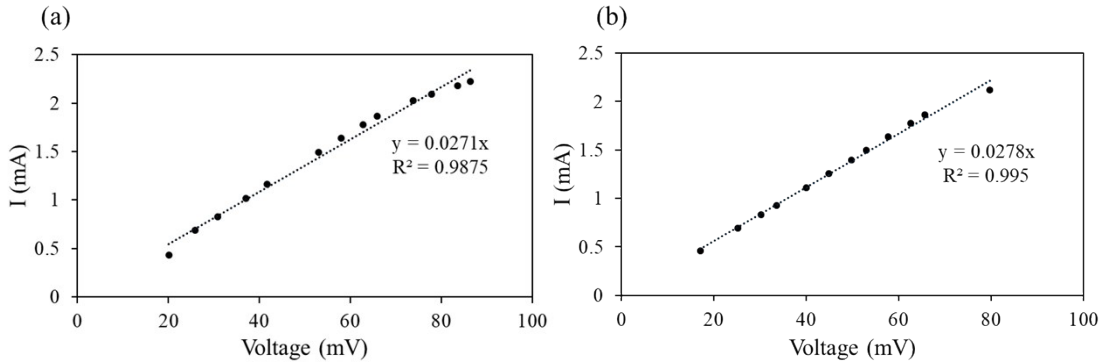

Fig. S5 I-V curves measured from two AgPDMS testing structures with the same design for conductivity assessment. In order to obtain the conductivity of the AgPDMS electrodes of the device, we fabricated simple testing structure through replica molding with the same AgPDMS composites as used for the device. The testing structure had a beam-like structure with 1 cm length and a cross-section measured 100 µm wide and 45 µm high. Such AgPDMS beam as a resistor was connected into the testing circuit with the two ends of its long side as the terminals. The current and its corresponding voltage values were measured using four-probe method by a high precision impedance spectroscope (HF2IS, Zurich, Switzerland). The I-V curves in (a) and (b) respectively measured for the two similar testing structures overlapped each other and the slope of each fitting line denoted the reciprocal of the resistance R of the AgPDMS beam. The conductivity σ was subsequently calculated with the law of resistance, $R= L/(\sigma \cdot S)$, where L and S were the length and cross-sectional area of the AgPDMS beam, respectively. The conductivity value averaged from both of the two test structures was 6.1e4 S/m for 85 wt% of Ag particles in AgPDMS composites.

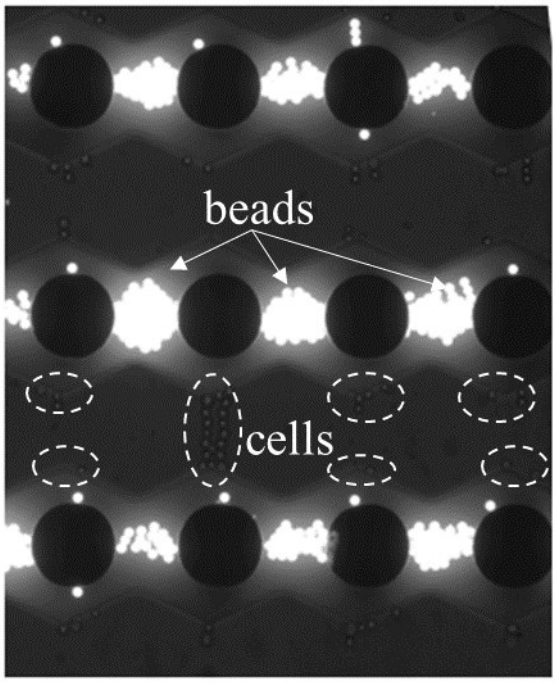

Fig. S6 Micrograph for DEP response of sample mixture with Hela cells and 15 µm beads in stagnant flow. Activation signal: 13 V_{pp} 400 kHz. DEP buffer conductivity: 100 µS/cm. The micrograph was taken by an inverted microscope (eclipse Ti2, Nikon, Tokyo, Japan) with illuminations of normal light and 488 nm fluorescence light respectively from top and bottom for simultaneous observation of the cells and fluorescent beads (excitation: 488 nm, emission: 525 nm).

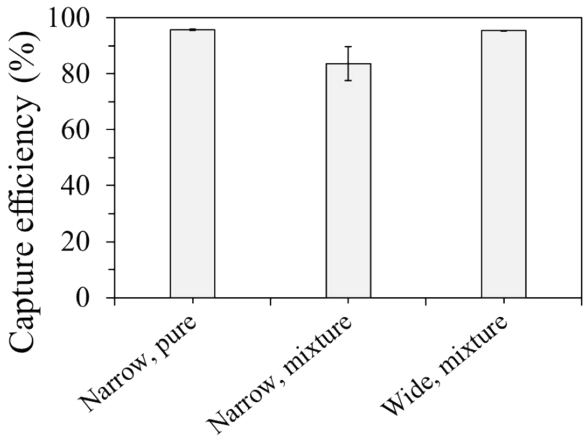

Fig. S7 Live cell capture efficiency respectively obtained by infusing pure live cells and sample mixture of live and dead cells into the narrow device, as well as injecting the sample mixture into the wide device. Activation signals: 13 V_{pp} and 400 kHz. Flow rate: 0.3 ml/h. DEP buffer conductivity: 100 µS/cm. Upper layer thickness: 28 µm. Data symbols and error bars denote mean ± s.d. (n = 3).

Movie S1

nDEP focusing experiments respectively carried out using 10 μm and 15 μm beads suspended in DEP buffer of 100 μS/cm and infused continuously into the device at 0.2 ml/h. The DEP activation was $13V_{pp}$ and 400 kHz. As displayed in the video, upon DEP onset, both the 10 μm and 15 μm beads are quickly focused to the center of each parallel flow path in pearl chain-like patterns, and meanwhile dragged by the fluid to effectively flow through the device in parallel streams, without any decrease or noticeable retention in the channel.

Movie S2

Video for separation of live (green) and dead (red) Hela cells mixed in DEP buffer of 100 μS/cm and infused continuously into the device at 0.3 ml/h. The DEP activation was applied at 13 V_{pp} and 400 kHz. The video displays a device section incorporating 5 electrode digits located in the midstream of the device with 8 parallel flow paths penetrating through. When DEP was off, both of the live and dead cells flowed across the digits. Upon the voltage onset, the live cells under strong pDEP got trapped onto the tip region of the electrode digits, whereas the dead cells under weak DEP remained in the original sample stream due to a dominating force from the flow drag.