Supplementary Information

A planar dielectrophoresis-based chip for high throughput cell pairing

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Supplementary methods

1. Detailed fabrication process of the planar chip

The glass substrates coated with indium-doped tin oxide (ITO) were purchased from Nanbo Display Technology Co. LTD (Shenzhen, China). The 100 nm sputtered ITO-coated glass had a sheet resistivity of 20 Ω/□. Prior to use, the substrates were cleaned with ultrasonication in acetone, deionized water, and 2-propanol for 20 min each. Then, the substrates were dried under a steam of nitrogen and subjected to the treatment of UV-Ozone for 30 min, which could help to significantly improve resist adhesion. Before use, the ITO conductive glass substrate was baked at 180 °C for 15 min.

The shape of pair A-interdigitated array (IDA) electrodes as well as all the separated fingers of pair B-IDA electrodes was patterned on the ITO-coated glass substrate by using conventional photolithography and wet etching processes. In detail, RZ 304 positive-type photoresist was firstly deposited onto the clean ITO glass using a spin coater (RCD8; Süss MicroTec, Germany). Here, the photoresist was spread onto the ITO glass at 500 rpm for 5 s and then increased to 3000 rpm for 30 s at an acceleration of 2000 rpm per second. The ITO glass substrate was then soft-baked at 95 °C for 2 min. After baking, a chromium photomask, which was patterned for pair A-IDA electrodes as well as all the separated fingers of pair B-IDA electrodes, was applied to the photoresist-coated ITO glass and exposed to ultraviolet light using a mask aligner (MA6/BA6; Süss MicroTec, Germany). Here, the photoresist was spread onto the ITO glass at 500 rpm for 5 s and then increased to 3000 rpm for 30 s at an acceleration of 2000 rpm per second. The ITO glass substrate was then soft-baked at 95 °C for 2 min. After baking, a chromium photomask, which was patterned for pair A-IDA electrodes as well as all the separated fingers of pair B-IDA electrodes, was applied to the photoresist-coated ITO glass and exposed to ultraviolet light using a mask aligner (MA6/BA6; Süss MicroTec, Germany). Subsequently, the resist was developed using a developer for 45 s, and the ITO glass was rinsed with deionized water and was dried under a steam of nitrogen. The ITO without cover of positive photoresist was etched with an etchant (Guangdong Huate Gas Co. LTD, China) for 2.5 min at 38 °C. Afterwards, the remaining positive photoresist was removed using acetone. Then, SU-8 2015 (MicroChem Co., USA) was spread onto the patterned ITO
electrode substrate at 500 rpm for 15 s and then raised to 4500 rpm at an acceleration of 500 rpm per second, to a thickness of 10.5 μm. The photoresist coated electrode substrate was then soft-baked at 95 °C for 2 min and let stand for 30 min. A chromium photo-mask patterned for the paired trap-well array was aligned with the patterned ITO electrodes and exposed to ultraviolet light. The substrate was then post-baked at 95 °C for 3 min and let stand for 10 min. Subsequently, it was developed for 2 min using an SU-8 developer (MicroChem Co., USA) and rinsed with isopropyl alcohol. Afterwards, the pad 2 and 3 of pair B-IDA electrodes was fabricated by direct-frequency magnetron sputtering (DFMS) a 1 μm-thickness copper layer. DFMS was performed with the TRP-450 system (SKY Technology Development Co., Ltd., CAS) in pure Ar gas (99.999%). In the sputtering process, the microwell-20 array and conductive pads of pair A-IDA electrodes parts were shaded by adhesive tape. Then, the second layer of SU-8 2015 was spread at 500 rpm for 15 s, which was then increased to 1600 rpm at an acceleration of 500 rpm per second, to a thickness of 17.9 μm. The substrate was then soft-baked at 95 °C for 4 min and let stand for 30 min. A chromium photo-mask patterned for microwell-160 array and microbaffle array was aligned with the under SU-8 layer and exposed to ultraviolet light. The substrate was then post-baked at 95 °C for 5 min and let stand for 10 min. Subsequently, it was developed for 4 min using an SU-8 developer (MicroChem Co., USA) and rinsed with isopropyl alcohol.

2. Preparation of HeLa cells in two different fluorescence colors

Human carcinoma (HeLa) cells were cultured as a monolayer in a 75 mm petri dish containing Dulbecco’s Modified Eagle Medium supplemented with 10 % (v/v) fetal bovine serum and 1 % penicillin/streptomycin at 37 °C in a 5 % CO₂ atmosphere. In preparation for an experiment, the cells were harvested at the log phase of growth by 0.25 % trypsin/EDTA from the petri dish for the following fluorescence labeling process. 5,6-Carboxy-fluorescein diacetate, succinimidyl ester (CFSE) stock solution was prepared at concentrations of 2 mM in dimethyl sulphoxide (DMSO) and was stored at -20 °C. CM-DiI stock solution was prepared at concentrations of 1 mM in DMSO and was stored at -20 °C. The working concentrations of CFSE and CM-DiI for cell labeling were 20 μM in phosphate buffered saline (PBS) buffer and 2 μM in Dulbecco’s phosphate buffered saline (D-PBS) buffer, respectively. Before usage,
cells were washed with PBS buffer and then incubated in the working solution for 5 min at 37 °C, following with an additional 15 min at room temperature. After labeling, cells were washed with PBS and resuspended in the low conductivity buffer with a concentration of $2 \times 10^6$ mL$^{-1}$.

3. Preparation of the sucrose-based low conductivity buffer

The low-conductivity cell suspension buffer was consisted of 10 mM HEPES, 0.1 mM CaCl$_2$, 50mM D-glucose and 236 mM sucrose. $^1$

**Supplementary discussion**

Mechanism of the positive dielectrophoresis (p-DEP) cell trapping

The cell trapping reported in this work is based on p-DEP which is the movement of cells towards the maximum of the non-uniform electric field $^2$. In detail, cells in dielectric medium and in the presence of non-uniform electric field experience a force, $F_{DEP}$, which can be calculated as following:

$$F_{DEP} = 2\pi \varepsilon_m a^3 \text{Re}[K(2\pi f)] \nabla E^2$$  

where $\varepsilon_m$ is the electrical permittivity of the medium, $a$ is the cell radius, $f$ is the frequency of the electric field and $E$ is the electric field. $K(2\pi f)$ is known as Clausius-Mossotti factor and is defined as:

$$K(2\pi f) = \frac{\varepsilon^*_c - \varepsilon^*_m}{\varepsilon^*_c + 2\varepsilon^*_m}$$  

where $\varepsilon^*_c$ is the complex electrical permittivity of cell and $\varepsilon^*_m$ is the complex electrical permittivity of the dielectric medium. Since the electrical permittivity depends on the frequency, the Clausius-Mossotti factor is also frequency dependent. When the real part of $K(2\pi f)$ is greater than zero, the cell moves towards the maximum of the electric field in a process known as p-DEP, and when the real part of $K(2\pi f)$ is less than zero, the cell moves towards the minimum of the electric field in a process known as negative DEP. In the present work, cells were trapped into the cell-size microwell array by applying ac signal to the paired interdigitated array electrodes. The confined electric field near the electrodes was also the maximum electric field, which proved a p-DEP trapping of cells. $^1,^3$
Supplementary Fig. 1

Scheme of a homemade fixture device

The picture exhibited that the cell pairing device was put in the fixture device. The fixture device was assembled by acrylic plate, copper pillar and wing nut.

Supplementary Fig. 2

The photos of the 3D printed mold and replicated polydimethylsiloxane (PDMS) channel
Supplementary Fig. 3

Oscilloscope measurement of electric potential of different connection mode for green cell trapping

When the sinusoidal ac signal was applied to pad 1 and 4 of pair A-IDA electrodes for green cell trapping, obvious induced electric potential at pair B-IDA electrodes could be seen if pad 2 and 3 were not grounded together with pad 1.

Supplementary Fig. 4

Fluorescence images of trapped HeLa cells stained with different colors. Scale bar: 500 μm
Supplementary Fig. 5

Photos of the PDMS at channel-80 before (left) and after (right) tightly enclosed to the planar chip by the fixture

Pink ink was used to clearly exhibit the channel-80 before and after closing by the fixture. From Fig. S5, we could see clearly that when the channel-80 for cell trapping and pairing was not closed, the own channel was full of pink ink (left image). However, pink color could only be seen in microwell-160 array after channel-80 was closed (right image).
References