Supplementary Information

Advancing in situ single-cell microbiological analysis through a microwell droplet array with gradual open sidewall

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1. Drawings of equipment shape and size, as shown in Fig. S1.

The mask plate is schematically depicted in Fig.S1(a) i, with the white section serving as the light-transmitting portion and the black portion acting as the mask. The mask plate contains 305 μm between the center of the small holes and 610 μm between the centers of the large holes. A photolithographic chrome plating procedure was used to create a circular chrome plating pattern on the glass substrate, and a wet etching procedure was used to create a microwell mold. To create a glass array mold with gradually open sidewalls, the etching solution is utilized to uniformly etch the glass substrate along each of the light-transmitting parts. Wet etching is used to create microwell arrays with a 100 μm depth. The ultimate bottom diameters of the tiny and large holes are 100 μm and 200 μm, respectively, due to isotropic etching. The PDMS microwell array chip was inverted from the glass mold. The structure of the sidewall of the microwell is readily visible in Fig.S1(a) ii, which is an enlarged version of the three-dimensional schematic from Fig. 1 of the main text. The gradually opening sidewall is seen between the microwell's bottom and top edges in Fig.S1(a) iii, which is a partial top view of the microwell array. Fig.S1(b) i shows the mask with widths of 50 μm. The channel with a thickness of 25 μm was fabricated by photolithography (See Fig. S2). The PDMS microchannel arrays were finally fabricated by the inverted molding process as shown in Fig.S1(b)iii. Fig. A2 displays the chip assembly diagram, we place it in Fig. 1b in the text.

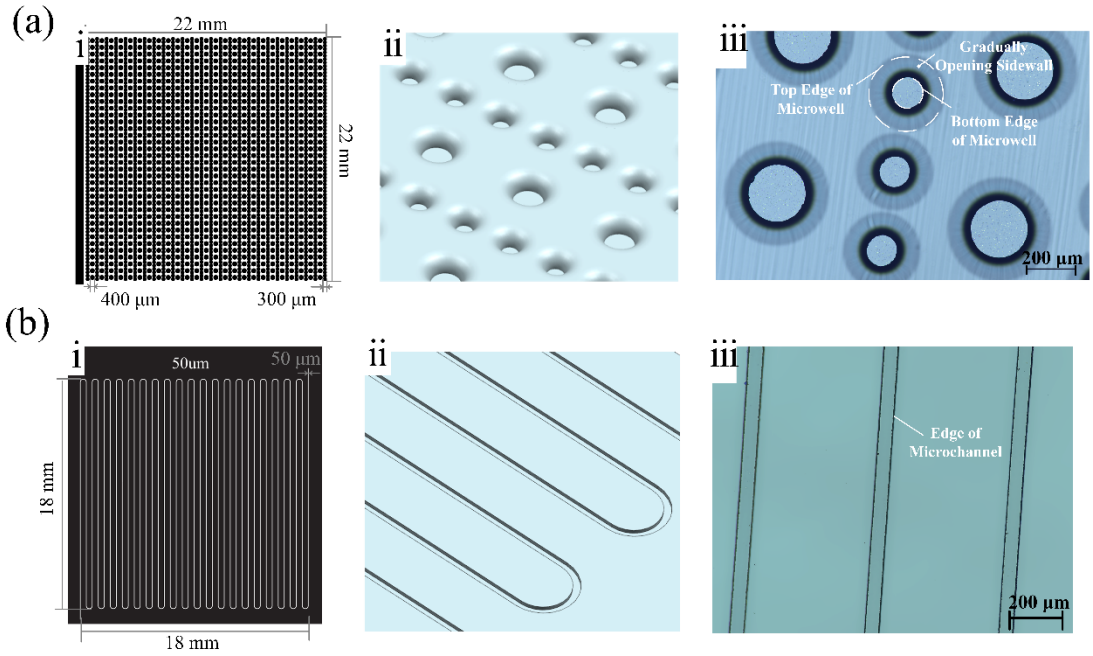


Fig.S1 (a) Shape of the microwells and the channel widths (b) of 50 μm in the (i) mask; (ii) 3D localized enlarged schematic; (iii) Localized top view of the PDMS mold.

2. We use the following steps to make channels, as shown in Fig. S2.

i. Substrate Preparation: Selection of a 4-inch wafer. The wafer should be cleaned with a piranha wet etch (using H_2SO_4 & H_2O_2) followed by a de-ionized water rinse.

ii. Coat: The SU-8 2025 was chosen to prepare channels with a thickness of 25 μm. The rotational speed was 3500 rpm.

iii. Soft Bake: Bake at 65°C for 3 min and 95°C for 6 min. To optimize the baking times/conditions, remove the wafer from the hotplate after the prescribed time and allow it to cool to room temperature.

iv. Exposure: The pattern on the mask was transferred to the SU-8 photoresist after UV radiation (MJB4, SUSS Micro-Tec, Germany).

v. Post Exposure Bake (PEB): the silicon wafer was baked at 65°C for 1min and 95°C for 6min.

vi. Development: the unexposed SU-8 was removed by rinsing with the SU-8 developer (Alfa Aesar, USA). The time was maintained for 5 min.

vii. Rinse and Dry: When using SU-8 developer, spray and wash the developed image with fresh solution for approximately 10 seconds, followed by a second spray/wash with Isopropyl Alcohol (Aladdin, Shanghai, China) for another 10 seconds. Air dry with filtered, pressurized air or nitrogen.

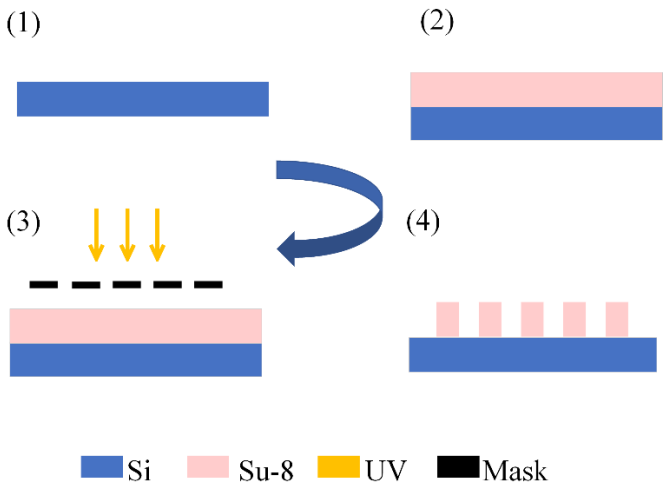
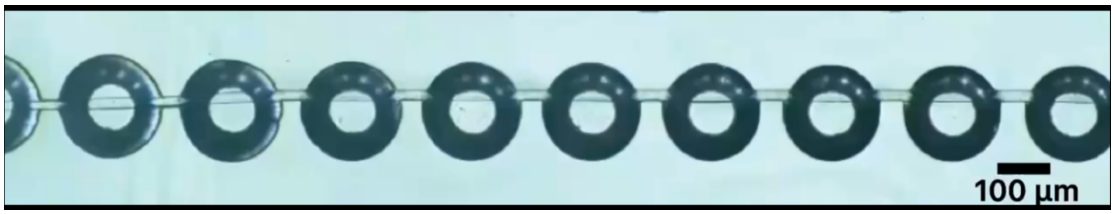


Fig. S2 Flow chart for the preparation of SU-8 channels with a width of 50 μm and a thickness of 25 μm.

3. Dynamic staining and washing procedure (Video)

The four reagent solutions included in the Gram kit were put through diffusion staining tests. The screenshots of crystal violet, iodine solution, 95% ethanol, and safranin solution flowing into the chip, respectively. The dyeing reagent solution entry microwells, the clear solution entry microwells, and the microwells following water washing are all shown in the screenshot as four rows of microwell arrays. The dynamic staining and washing procedure are shown in Video.



Video Staining experiment with Gram kit.

4. Stained images of bacteria

The bacteria are propagated in microwells in situ, with *S. aureus* and *E. coli* being stained as indicated in Fig. S3

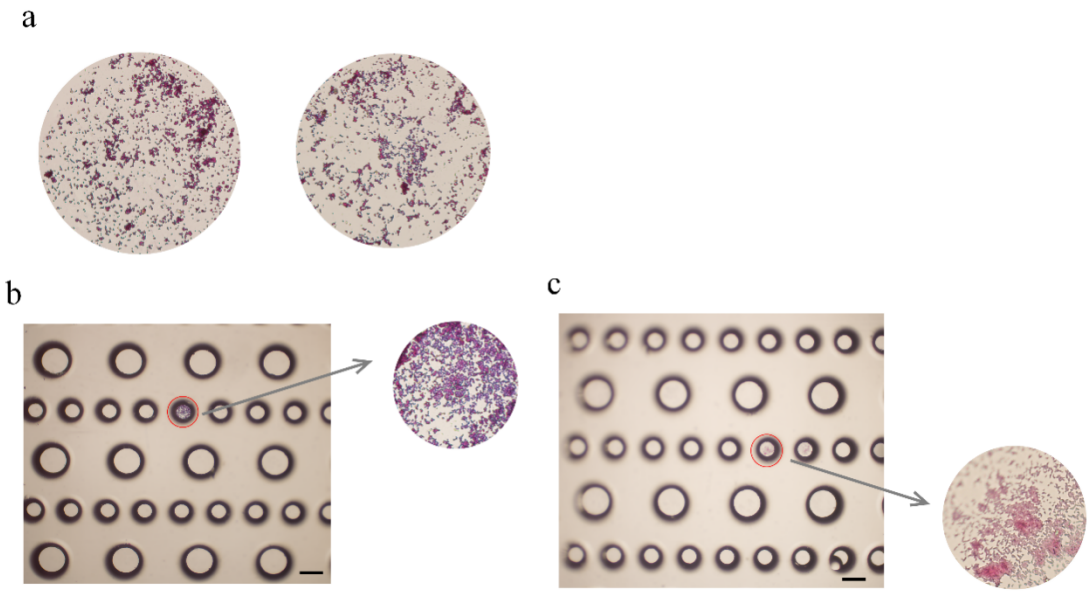


Fig. S3 Stained images of mixed bacteria and single bacteria. The morphology of mixed single cells cultured in situ in the microwell for 20 hours (a) Mixture of Staphylococcus aureus and Escherichia coli; (b) Staphylococcus aureus; (c) Escherichia coli. The entire image was captured using the 5× microscope objective. At a 10× magnification, the locally enlarged image of the micropores was taken. The scale bar is 200 μm.