Supporting information

A Simple Three-Dimensional Microfluidic Platform for Studying Chemotaxis and Cell Sorting

Xiaobo Li [a#], Yanqing Song [a#], Andrew Glidle [a], Cindy Smith [b], William Sloan [b], Maggie Cusack[c] and Huabing Yin [a, *]

[a] James Watt School of Engineering, University of Glasgow, G12 8LT, UK

[b] Department of Infrastructure and Environment, James Watt School of Engineering, University of Glasgow, Glasgow, G12 8LT, UK

[c] Munster Technological University, Rossa Avenue, Bishopstown, Cork, T12 P928, Ireland.

* Corresponding Authors: Huabing Yin: E-mail: huabing.yin@glasgow.ac.uk

[#] L.X and Y.S contributed equally to this work.

1. Experimental section:

1.1. Ingredients of the mediums and buffers

Chemotaxis buffer (CB): 1× PBS, 0.1 mM EDTA, and 10 μM L-methionine, 10 mM DL-lactate. pH =7.0.

TB medium: 10 g/L tryptone and 8 g/L NaCl.

LB medium: 10 g/L tryptone, 10 g/L NaCl, and 5 g/L yeast extract.

CMC selective agar: 5.0 g/L CMC, 2.0 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 1.0 g/L K_2HPO_4, 0.05 g/L yeast extract, 20 g/L agar, pH 7.0.

Lignin selective agar: 1.0 g/L alkali lignin, 2.0 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 1.0 g/L K_2HPO_4, 0.05 g/L yeast extract, 20 g/L agar, pH 7.0.

1.2. Bacterial motility tests

The RFP-*E. coli* strains were grown overnight in either TB medium at 30°C or LB medium at 37°C supplemented with 50 mg/L kanamycin. Bacteria were harvested by centrifuging at 8000 rpm or 2000 rpm for 3 minutes and then resuspended in CB. Bacteria suspension, washed or unwashed, was diluted 10 folds in CB, and 10 μL of the solution was added to a glass slide covered with a coverslip. Before adding the bacteria suspension, the substrate and coverslip were treated with 1% Pluronic F-127 for 1 hour to reduce cell attachment to the glass surfaces. The motility of bacteria pre-treated in various conditions was monitored via time-lapse imaging.

1.3. 16S rRNA Sequencing

The selected strains were cultured to the log phase. The cells were harvested and subjected to the whole genome extraction using a MagaZorb® DNA Mini-Prep Kit. Polymerase chain reaction (PCR) was then employed to amplify the full-length 16S rRNA gene fragments using the universal primers of 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR was proceeded in a 50 μL reaction system containing 2 μL DNA template, 2 μL upstream primer (400 nM), 2 μL downstream primer (400 nM), 25 μL 2× Brilliant II SYBR Green QPCR Master Mix (Agilent technology, Catalog number: 600828), and 19 μL nuclease-free PCR-grade water. PCR amplification was performed as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 57.3 °C for 1 min, and 72 °C for 30 s. The target PCR products were confirmed through agarose gel electrophoresis and were purified with the QIAquick Gel Extraction Kit. The purified PCR products were Sanger sequenced by Eurofins. The sequences obtained were used for species identification via BLAST analysis with the NCBI database (http://blast.ncbi.nlm.nih.gov/), with the highest sequence similarity chosen for identified species. The 16S rRNA gene sequences have been deposited in the GenBank with Accession Numbers PP790382-790386.

2. Figures

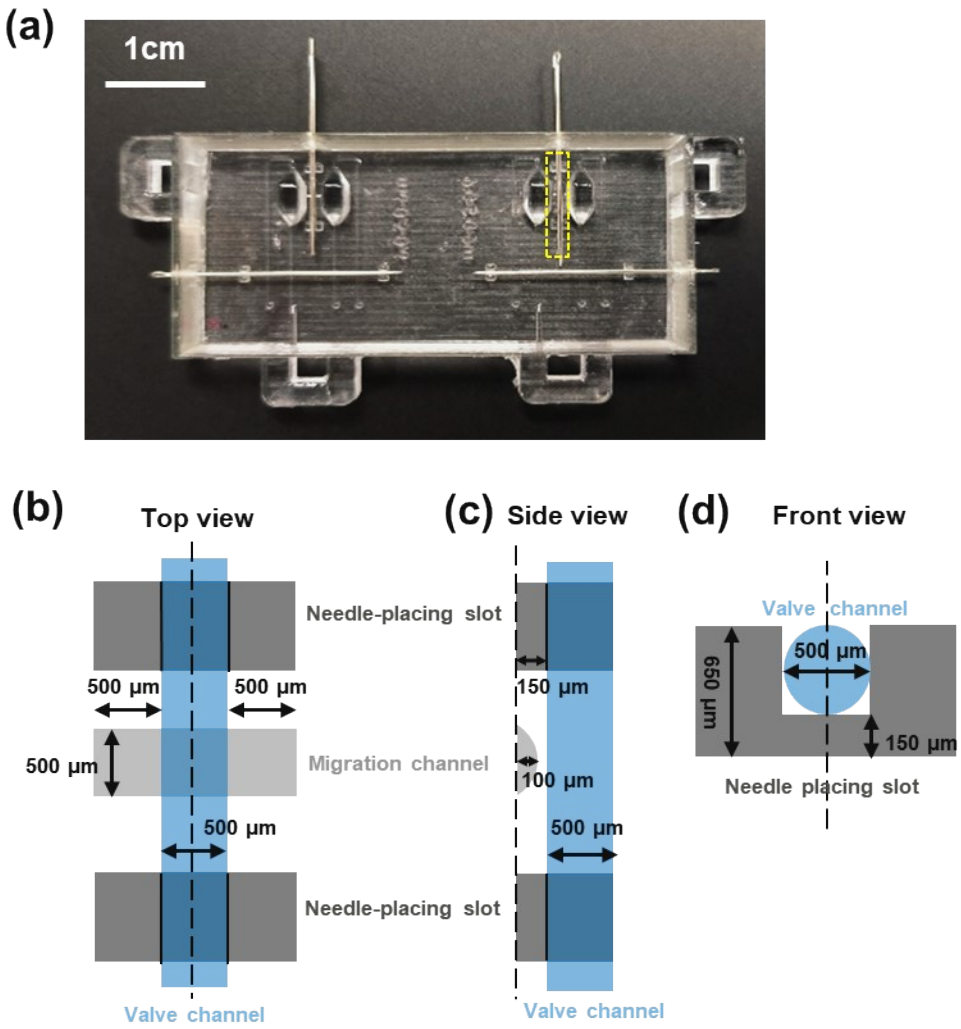


Figure S1. The structure and fabrication of valve channels. (a) Photograph of the 3D printed mould. Metal needles (0.5 mm diameter) are positioned in the designated slots to form the valve channel. The region highlighted with a yellow dotted frame in (a) is further illustrated with three-view schematics: (b) top view, (c) side view, and (d) front view. The 3D printed parts are shown in grey with dark grey representing the needle-placing slots and light grey representing the migration channel. The metal needles are depicted in bule.

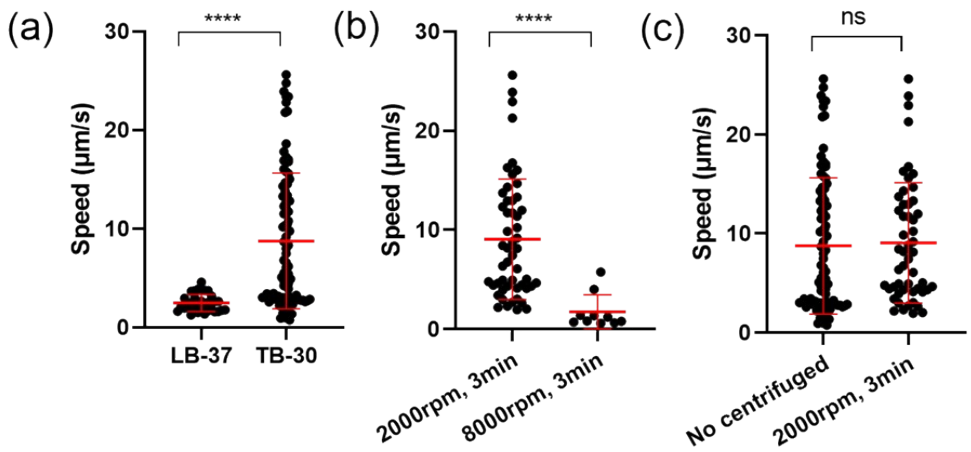


Figure S2. The speed of bacteria under different culture conditions. (a and b) The motile activity decreased when bacteria were cultured in a nutrient-rich medium with optical temperature (LB medium at 37 ℃) or harvested vigorously by centrifugation (8000rpm, 3min). (c) There was no difference in motile activity when bacteria were collected at a low centrifuge speed (2000 rpm, 3min).

Nutrient-rich media (e.g., LB medium), high-speed centrifugation and optimal culture temperature (e.g., 37° C) can reduce the motility of bacteria. Thus, to prepare samples for the chemotaxis assays, bacteria were cultured in the TB medium at 30 ℃ and harvested by centrifuging at 2000 rpm for 3 minutes.

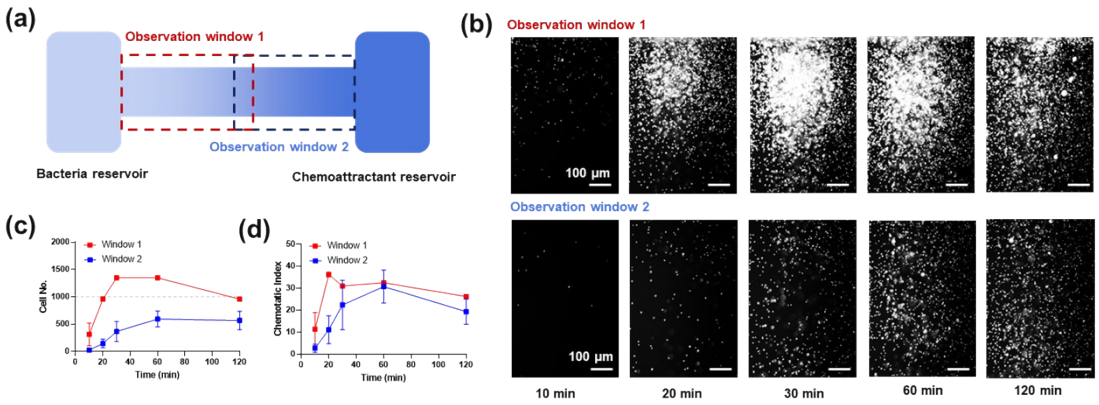


Figure S3. Observation window selection. (a) Schematic showing the positions of two observation windows, each spanning an area of 824 μm × 824 μm. Window 1 is located near the bacteria reservoir, while Window 2 is near the chemoattractant reservoir. (b) Time-lapse images capturing bacterial chemotaxis toward 2 mM ASP at the two observation window positions. A substantial number of bacteria were observed in Window 1 after 20 minutes, resulting in bacterial clusters. (c) Tracked swimming cell numbers under the two observation windows. The number of cells exceeded more than 1000 after 20 minutes in Window 1, effecting tracking accuracy. (d) Comparison of chemotaxis indices for bacteria chemotaxis toward 2 mM ASP at the two observation windows.

Positioning the observation window near the chemoattractant reservoir (Window 2) provides more reliable data by minimising tracking inaccuracies caused by bacterial clustering in Window 1. Additionally, motile bacteria could enter the Window 1 area but not the Window 2 area during the bacteria loading step. Therefore, Observation Window 2 was used for all tests.

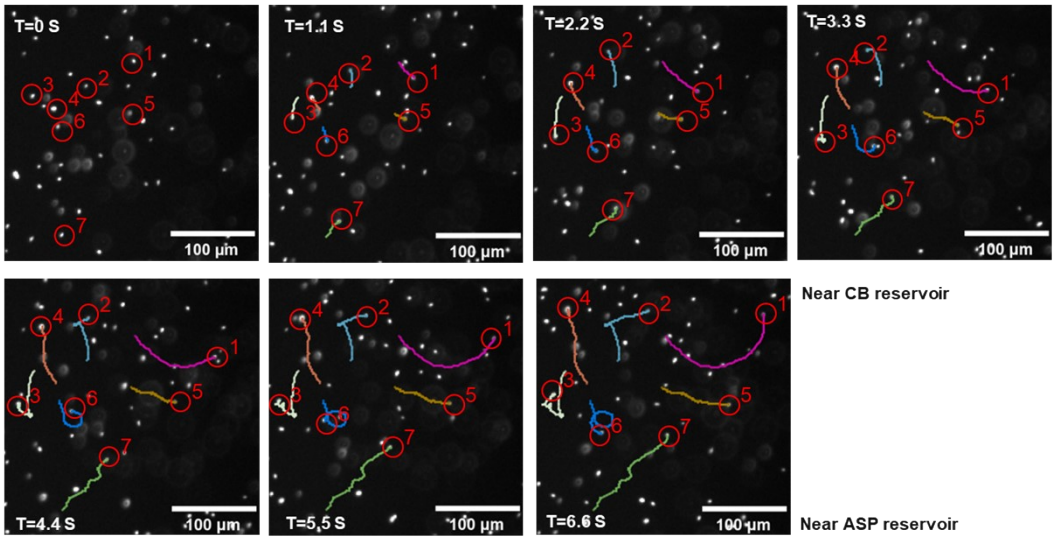
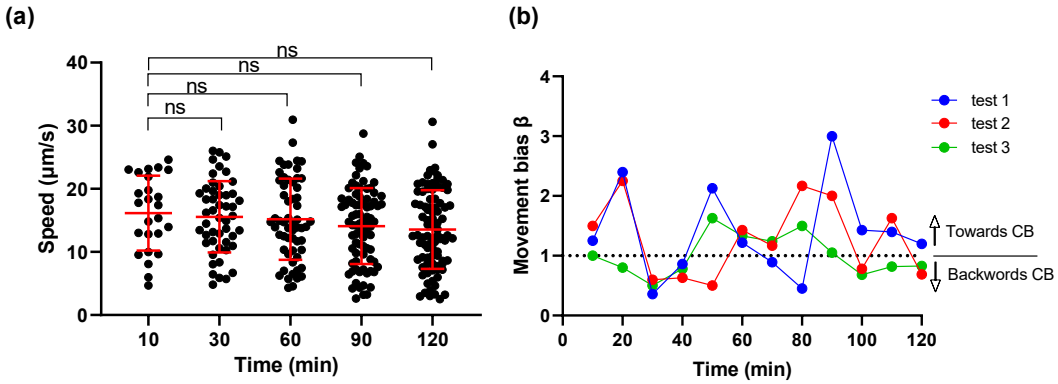


Figure S4. Time-lapse images of *E. coli* swimming towards ASP at the point of 30 minutes. Images were used to extract key parameters such as the number of swimming bacteria, their speeds, displacements, trajectories, movement bias, and swimming pathway. Each white dot in the image represents a single cell. The Fiji Trackmate system was employed for individual cell trajectory tracking, represented by red circles with unique identifiers and diverse colours indicating trajectories (a few cells were presented for illustrative purposes). Cell speeds were determined by calculating trajectory lengths (total travel lengths) over the entire recorded time (6.6 s). Displacement, defined as the straight-line distance between the start and end point, was also quantified. Movement bias was assessed by contrasting the final bacteria location with the starting point; for instance, cells 3, 5, and 6 exhibited movement towards ASP, while cells 1, 2, 4, and 7 moved in the opposite direction. Consequently, the movement bias for this bacterial population was 0.75 (3/4). Additionally, a comparison of displacement and trajectory enabled the identification of distinct swimming pathways. Notably, cells 4, 5, and 7 followed direct swimming pathways, while cells 1, 2, 3, and 6 exhibited run-tumble swimming pathways. CB: chemotactic buffer. ASP: aspartic acid.



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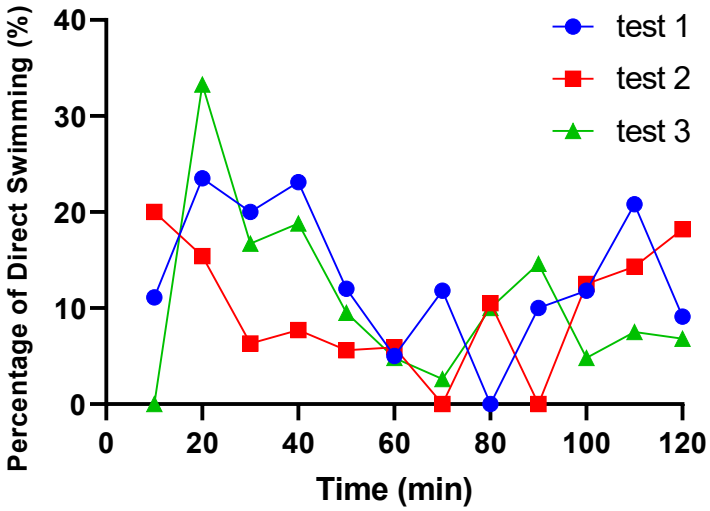
112 Figure S5. (a) The speed of bacteria in the control group (filled with CB in the
113 chemoattractant reservoir). (b) The movement bias of bacteria in the control group.
114 Movement bias is defined as the ratio of the number of cells swimming towards the
115 chemoattractant reservoir to the number of cells swimming away from the
116 chemoattractant reservoir. CB: chemotactic buffer.



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118 Figure S6. The percentage of bacteria that chose the direct swimming pathways in the
119 control (filled with chemotactic buffer in chemoattractant reservoir). No specific trend
120 was observed in this scenario, and the percentage of direct swimming pathways varied
121 with time in each test.

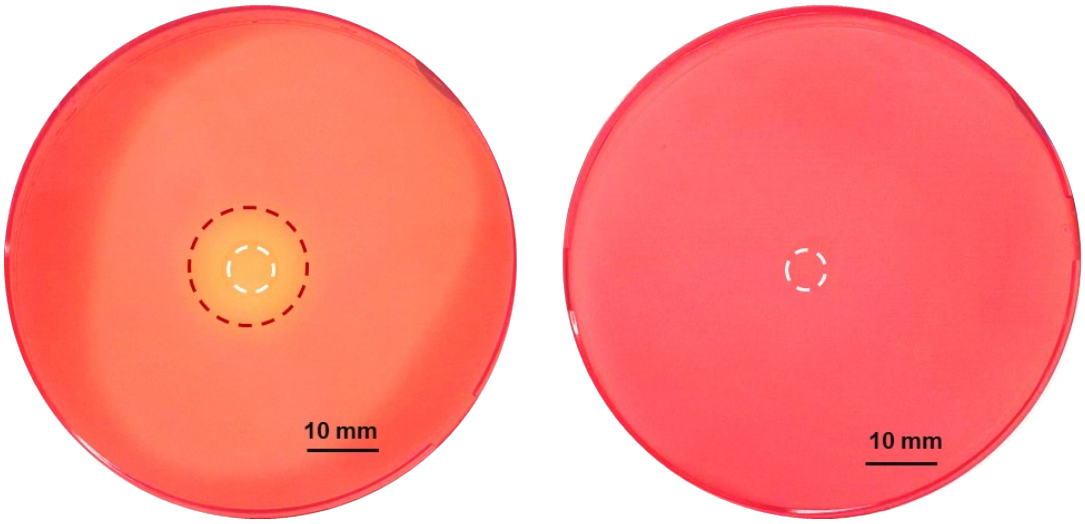
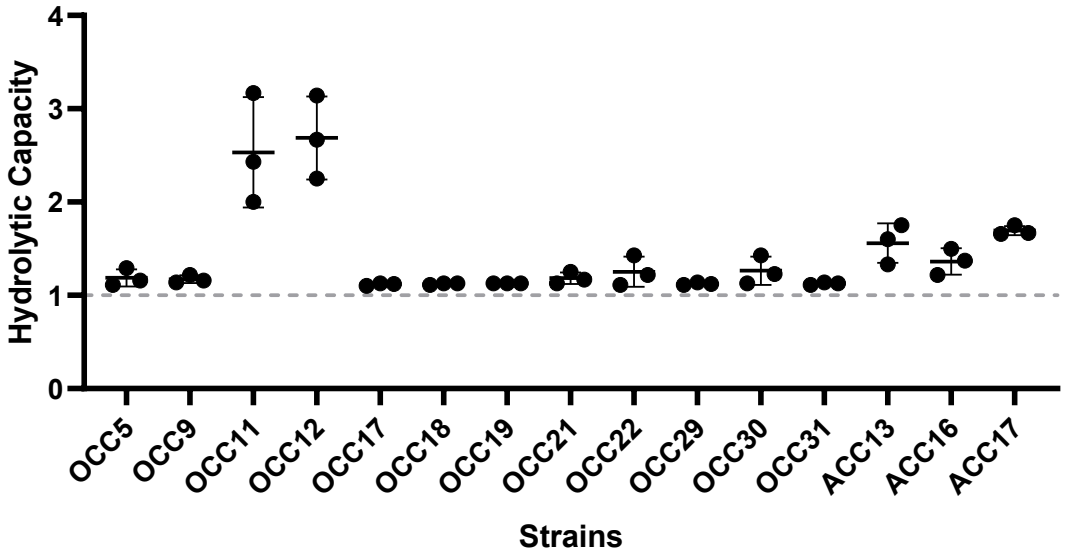


Figure S7. Congo red staining. (Left) The strain ACC13 exhibited a pale yellow halo (also referred to as a hydrolyzed circle, indicated by an open red circle) around the colony (indicated by a white open circle) after a 5-day incubation period, denoting the production of CMCase. (Right) The strain OCC10 did not display a hydrolyzed circle, suggesting the absence of CMCase production.



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130 Figure S8. Hydrolytic capacity in selected strains. Hydrolytic capacity is defined as the

131 ratio of the diameter of the hydrolysed circle to the diameter of the colony.

3. Video

Video 1. A typical time-lapse video of *E. coli* swimming towards ASP at 30 minutes. The individual bacterial trajectories were tracked using the TrackMate.

Video 2. The operation of Valve 2 involves opening and closing the migration channel by pushing or withdrawing water within the channel.