

# Droplet Microfluidic System for High Throughput and Passive Selection of Bacteria Producing Biosurfactants

Klaudia Staskiewicz <sup>a,c</sup>, Maria Dabrowska <sup>a</sup>, Lukasz Kozon <sup>b,c</sup>, Zofia Olszewska <sup>b</sup>, Lukasz Drewniak <sup>a</sup>, Tomasz S. Kaminski <sup>a,b</sup>

<sup>a</sup> Department of Environmental Microbiology and Biotechnology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Poland;

<sup>b</sup> Department of Molecular Biology, Institute of Biochemistry, Faculty of Biology, University of Warsaw, Poland;

<sup>c</sup> Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

## Supplementary information

### PROTOCOLS FOR BACTERIA CULTIVATION

We optimized the method using two strains of bacteria: i) *Bacillus* sp. ANT\_WA51<sup>1</sup> strain for the generation of “positive” microdroplets, and ii) *Escherichia coli* BL21 strain as a negative control strain, as it does not produce biosurfactants. An overnight culture was prepared by inoculating 25 mL of rich broth liquid LB medium (Miller, BioMaxima) with a single colony and incubating the culture at room temperature with shaking at 180 rpm. OD<sub>600</sub> of the overnight culture was measured using 200 µL of cell culture with a Tecan plate reader (Sunrise TECAN, Tecan Trading AG) and Magellan<sup>TM</sup> software. For encapsulation, 1 mL of the overnight culture was centrifuged at 4000 rpm for 8 min, and the bacterial pellet was rinsed with 1 mL of sterile 0.9% NaCl saline solution. The activity was repeated twice to remove the medium containing biosurfactants. After the third centrifugation, the pellet was diluted in the saline solution and suspended in LB medium (at a volumetric ratio of 1:9 of saline solution to LB) to achieve the expected number of bacterial cells per droplet. The concentration of cells was estimated based on the OD<sub>600</sub> value for the overnight culture. The remaining volume of the overnight culture was saved for measurement of surface tension.

### SURFACE TENSION MEASUREMENT

Around 20 mL of the overnight culture was centrifuged (4000 rpm, 8 min), and the supernatant was further used to measure surface tension using the Wilhelmy Plate method. This method included the preparation of an appropriate volume of liquid sample in a small beaker, before placing it on a dedicated table of a Kruss Tensiometer K20 (Kruss GmbH) and waiting till the liquid surface and the setup parts were static. The tensiometer table with the sample was slowly elevated until the tip of the measuring plate touched the surface of the liquid sample. The plate was gently immersed in the sample as described by the tensiometer manual, and then the measurement was carried out. We used a surfactin (Sigma-Aldrich) diluted in LB broth for surface tension measurement.

### FABRICATION OF MICROFLUIDIC DEVICES

The microfluidic devices used for droplet generation and sorting (Fig. 1C) were fabricated following standard photolithography and soft lithography procedures using high-resolution acetate masks and SU-8 photoresist patterning.<sup>2,3</sup>

#### Photolithography of microfluidic molds

The channel layout for the microfluidic chips was designed using AutoCAD (Autodesk) and printed out on a high-resolution film photomask (Micro Lithography Services). CAD drawings with designs of a flow-focusing droplet generation device and droplet sorter are attached as a supplementary .dxf file.



The microfluidic devices were fabricated following standard hard lithography protocols that can be performed in local cleanrooms or outsourced to contract manufacturing companies. First, microfluidic molds were patterned on 3" silicon wafers (Microchemicals) using high-resolution film masks (Microlithography Services Ltd) and SU-8 2025 and 2050 photoresists (Kayaku Advanced Materials). A MJB4 mask aligner (SÜSS MicroTec) was used to UV expose all the SU-8 spin-coated wafers. The thickness of the structures (corresponding to the depth of channels in the final microfluidic devices) was measured using a GT-Contour profilometer and 5x objective (Bruker). The profile of the droplet sorting module is presented in Fig. S1.

**Table S1. Settings for the photolithography of the flow focusing chip.**

	Fabrication step (no. of a layer)	
	1 <sup>st</sup> layer	
Nominal thickness	50 $\mu\text{m}$	
Resist used	SU-8 2050	
Spin coating speed	1 <sup>st</sup> step: 10 sec, 500 rpm 2 <sup>nd</sup> step: 30 sec, 4000 rpm	
Pre-baking	3 min at 65°C 6 min at 95°C	
Exposure (at $\sim 10 \text{ mW cm}^2$ )	2 x 8.5 sec	
Post-baking	1 min at 65°C 6 min at 95°C	
Development in the beaker filled with 30-50 mL of PGMEA (propylene glycol methyl ether acetate, Sigma-Aldrich)	Approximately 5 minutes until all uncured SU-8 was removed from the wafer	
Measured range of thicknesses	46–48 $\mu\text{m}$	

**Table S2. Settings for the photolithography of the passive sorting chip.**

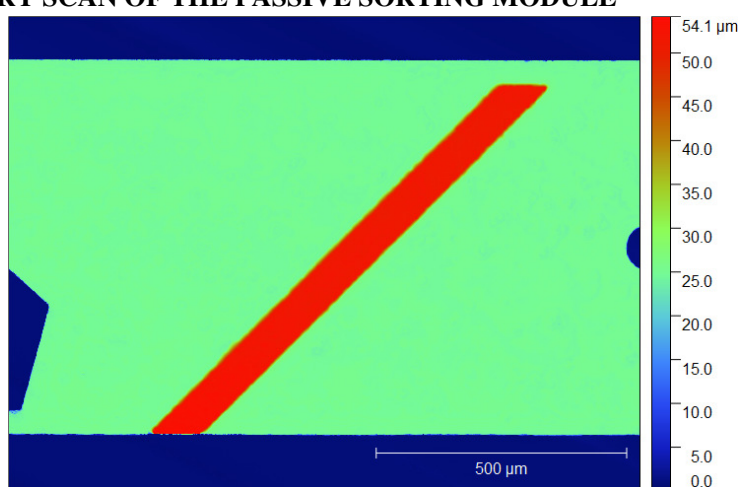
	Fabrication step (no. of a layer)	
	1 <sup>st</sup> layer	2 <sup>nd</sup> layer
Nominal thickness	25 $\mu\text{m}$	25 $\mu\text{m}$ 2 <sup>nd</sup> layer (50 $\mu\text{m}$ final thickness)
Resist used	SU-8 2025	SU-8 2025
Spin coating speed	1 <sup>st</sup> step: 10 sec, 500 rpm 2 <sup>nd</sup> step: 30 sec, 4000 rpm	1 <sup>st</sup> step: 10 sec, 500 rpm 2 <sup>nd</sup> step: 30 sec, 4000 rpm
Pre-baking	5 min at 95°C	5 min at 95°C
Exposure (at $\sim 17 \text{ mW cm}^2$ )	9 sec	9 sec
Post-baking	5 min at 95°C	5 min at 95°C
Development in the beaker filled with 30-50mL of PGMEA (Sigma-Aldrich)	n.a.	Approximately 5 minutes until all uncured SU-8 was removed from the wafer
Measured range of thicknesses	24 $\mu\text{m}$	51 $\mu\text{m}$



### Soft lithography of PDMS microfluidic devices

To fabricate PDMS microfluidic devices, 20–30 grams of silicone elastomer base and curing agent (Sylgard 184, Dow Corning) were mixed at a 10:1 (w/w) ratio in a plastic cup and degassed in a vacuum chamber for 30 minutes. PDMS was then poured on a master wafer with SU-8 structures and cured in the oven at 65 °C for at least 4 hours. Next, the inlet holes were punched using a 1 mm biopsy puncher with a plunger (Kai Medical). The PDMS chip was then plasma bonded to a 52 mm x 76 mm x 1 mm (length x width x thickness) glass slide (VWR) in a low-pressure oxygen-automated plasma generator (Zepto, Diener Electronics). Finally, the hydrophobic modification of microfluidic channels was performed by flushing both types of devices with 1% (v/v) trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma-Aldrich) in HFE-7500 (3M) and baked on a hot plate at 75 °C for at least 30 minutes to evaporate the fluorocarbon oil and silane mix.

### PROFILOMETRY SCAN OF THE PASSIVE SORTING MODULE



**Fig. S1. Profilometry scan (recorded by Bruker ContourGT-K, USA) of the mould depicting the architecture of the passive sorting module.**

### FABRICATION OF THE CHAMBERS FOR DROPLET INCUBATION

The chambers were made according to the protocol published by Neun et al.<sup>4</sup> First, the holes on the bottom tip and the side of the closed Eppendorf tube were made using a 1-mm wide biopsy puncher (Kai Medical). Next, the lid of the Eppendorf tube was glued with PR1500 instant adhesive (3M) to the 1-mm-thick glass slide. In the final step, the 30–50 cm long pieces of polyethylene tubing (0.38 mm I.D. x 1.09 mm O.D., Smiths Medical) were inserted into both holes in the tube and next glued with PR1500 instant adhesive (3M). During droplet generation, the top tubing was connected to a flow-focusing chip, and droplets were trapped in the upper part of the tube by their intrinsic buoyancy. For sorting, the flow direction was reversed: the oil was pumped through the side tubing, and the droplets moved to the sorting chip via the top tubing.

### SUPPLYING THE EMULSION WITH OXYGEN

The top chamber tubing of the incubation chamber was placed into 5 mL of 2% RAN fluorosurfactant in a filtered Novec HFE-7500, and bubbles were gently removed from the tubing. The bottom chamber tubing was attached tightly, with the support of adhesive glue, to the Tygon tubing (0.38 mm I.D., 0.9 mm O.D, Ismatec) of a peristaltic pump (Reglo ICC, Ismatec). Another end of the Tygon tubing was inserted into a piece of PTFE tubing connected to a tube with 2% RAN in oil stock to ensure a constant flow of continuous phase in a closed system. The chamber containing the emulsion was placed in an incubator set to 25 °C, and the peristaltic pump was set to a flow rate of 20 μL/min in a direction that caused oil to flow into the chamber through the upper chamber tubing, and as a consequence, the emulsion was trapped by buoyancy and did not leak from the chamber to the oil stock (Fig. S2).



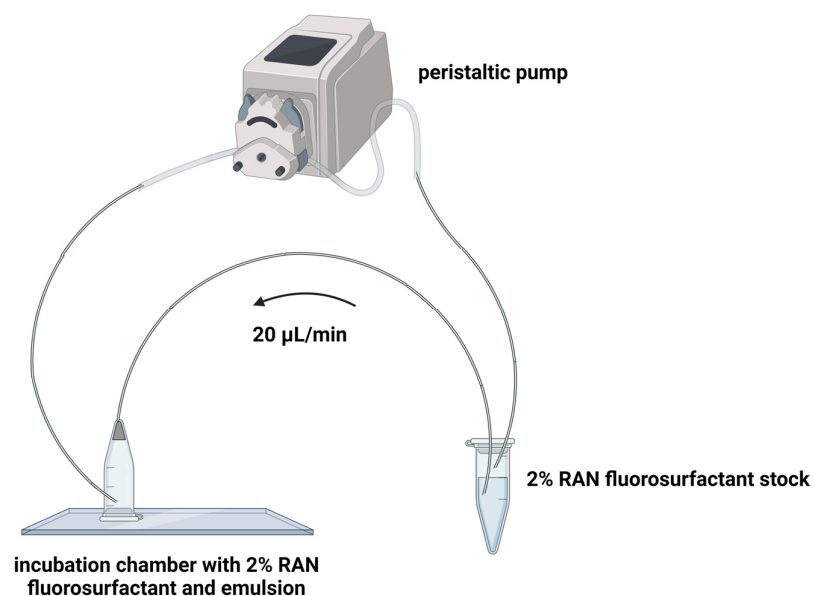


Fig. S2. Schematic drawing with an overview of a system for emulsion oxygenation.

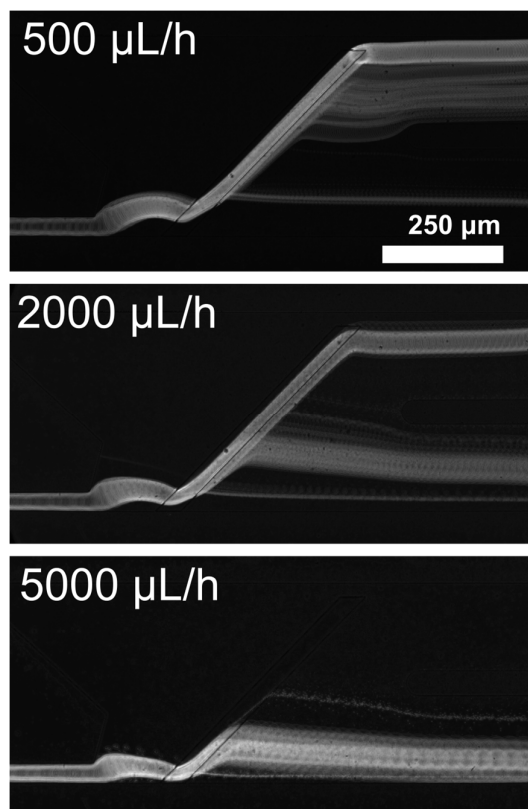


Fig. S3. Standard deviation-based stack images made of videos captured during the passive sorting using various flow rates of sorting oil. Passive sorting process applied to an emulsion composed of 1:1 mix of positive droplets with *Bacillus* sp. ANT\_WA51 and negative droplets with pure LB medium. Depending on the flow rate of the sorting oil, all the droplets can be directed either to positive or negative channel or only positive droplets can be sorted.



## FORMULA FOR CALCULATION OF DROPLET VOLUME

Diameters for the microdroplets obtained by our group were calculated using the following formula for a rotational ellipsoid:

$$V = \frac{4}{3} \pi \cdot a^2 \cdot b$$
$$d = 2 \sqrt{\frac{3V}{4\pi b}}$$

V – microdroplet volume [pL]

a, b – ellipsoid radii [μm] (a – radius of squeezed droplet, b – half of the channel height)

d – microdroplet diameter [μm]

## IMAGE ANALYSIS PROTOCOL

To determine the values of the displacement factor, slow-motion videos with droplet sorting were analyzed using ImageJ software. First, an averaged background of a video was generated as an image using the “Image/Stack/Z Project” tool with the “Average Intensity” option. Then, the background image was subtracted from each frame of the video using the “Process/Image Calculator” function. Next, the function “Image/Adjust/Threshold” was used to indicate droplets in the resulting “background-subtracted” video. The coordinates of centers of sorted droplets for each frame of the differential video slice were extracted using the functions “Set Measurements” and “Analyze Particles”. Obtained data were used to generate density histograms in RStudio. Heatmaps were generated using Origin Software. To generate stack images presented in Figure 3a and Supplementary Figure S3 we used the function “Image/Stack/Z Project” tool with the “Standard Deviation” option.



## TECHNICAL PARAMETERS OF THE SYSTEMS

**Table S3. Comparison of the parameters of the SIFT method and the system presented herein:**

	SIFT system (Abbyad group)		this work
	Pan et al. 2019 <sup>5</sup> Horvath et al. 2019 <sup>6</sup>	Dobson et al. 2020 <sup>7</sup> Zielke et al. 2020 <sup>8</sup> Zielke et al. 2022 <sup>9</sup>	
Max. frequency of passive sorting [Hz]	30	30	250
<b>Dimensions of the sorting device</b>			
Sorting module width [ $\mu\text{m}$ ]	3000	1500	750
Distance of the rail from the chamber entrance [ $\mu\text{m}$ ]	700 or 1500	600	240
Sorting rail length [ $\mu\text{m}$ ]	3800 or 4600	2000	1000
Sorting rail width [ $\mu\text{m}$ ]	150	150	75
Sorting rail tip	sharp	sharp	flat
Droplet inlet width [ $\mu\text{m}$ ]	100	125	75
Top layer depth (rail) [ $\mu\text{m}$ ]	15	15	24
Bottom layer depth (chamber) [ $\mu\text{m}$ ]	25 (Pan et al.) 50 (Horvath et al.)	25	27
Total height [ $\mu\text{m}$ ]	40 (Pan et al.) 65 (Horvath et al.)	40	51
<b>Flow rates</b>			
Sorting oil [ $\mu\text{L}/\text{h}$ ]	1500-3600	480-1380	2000-3000
Spacing oil [ $\mu\text{L}/\text{h}$ ]	not given	not given	480
Diluting oil [ $\mu\text{L}/\text{h}$ ]	not applicable	not applicable	20
<b>Droplet parameters</b>			
Approximate droplet volume [pL]	35-85 (Pan et al.) 170-590 (Horvath et al.)	35-105	100
Diameter [ $\mu\text{m}$ ] of confined droplets	50-80 (Pan et al.) 80-180 (Horvath et al.)	50-90	85
Droplet incubation	on-chip	on-chip	off-chip



## PASSIVE SORTING PROCESS CHARACTERISTICS DEPENDING ON DROPLET VOLUMES

**Table S4. Passive sorting process characteristics: frequency, sensitivity, specificity, and accuracy determined for microdroplet volumes 80 pL, 100 pL, and 120 pL.**

Droplet volume [pL]	Flow rate of sorting oil 2500 $\mu\text{L/h}$				Flow rate of sorting oil 3000 $\mu\text{L/h}$			
	Frequency [Hz]	Sensitivity	Specificity	Accuracy	Frequency [Hz]	Sensitivity	Specificity	Accuracy
80	134	1.00	1.00	1.00	111	1.00	1.00	1.00
	261	1.00	0.913	0.917	264	1.00	0.959	0.963
	317	1.00	0.594	0.639	325	1.00	0.562	0.606
100	123	1.00	1.00	1.00	114	1.00	1.00	1.00
	253	1.00	0.993	0.995	263	1.00	1.00	1.00
	344	1.00	0.723	0.768	357	1.00	0.675	0.712
120	119	0.800	1.00	0.988	109	0.800	1.00	0.986
	227	1.00	1.00	1.00	227	1.00	1.00	1.00
	301	1.00	0.855	0.869	303	1.00	0.903	0.910

## BULK SURFACE TENSION MEASUREMENTS

**Table S5. The time-course bulk surface tension measurements of *Bacillus* sp. ANT\_WA51 supernatant from a bulk culture incubated in aerobic and microaerobic conditions. Aerobic conditions were provided by constant flask shaking for bacterial cell suspension. The static culture was carried out in a closed falcon that was not shaken to emulate microaerobic conditions.**

time [h]	surface tension [mN/m]	
	static culture	oxygenated culture
0	58.10 $\pm$ 0.00	58.10 $\pm$ 0.00
4	56.00 $\pm$ 0.10	28.40 $\pm$ 0.02
8	53.40 $\pm$ 0.12	27.60 $\pm$ 0.00
12	49.70 $\pm$ 0.11	27.30 $\pm$ 0.00
16	33.20 $\pm$ 0.10	27.80 $\pm$ 0.00
20	30.50 $\pm$ 0.02	27.80 $\pm$ 0.00
24	30.20 $\pm$ 0.06	27.80 $\pm$ 0.00



## CALCULATION OF ENRICHMENT FACTORS

**Table S6. Enrichment values obtained with the passive sorting method. The bacterial suspension was plated in 100x dilution for not-sorted emulsion and for emulsion after sorting.**

$\lambda$	$\varepsilon_0$	$\varepsilon_0'$	Number of colonies on a Petri dish from cell suspension before sorting		Number of colonies on a Petri dish cell suspension collected from positive channel		Baret <i>et al.</i> <sup>10</sup>		Zinchenko <i>et al.</i> <sup>11</sup>	
			Negative ( <i>E.coli</i> BL21)	Positive ( <i>Bacillus</i> sp. ANT_WA51)	Negative ( <i>E.coli</i> BL21)	Positive ( <i>Bacillus</i> sp. ANT_WA51)	$\varepsilon_1$	$\eta$	$\varepsilon_1'$	$\eta'$
0.1	$8.23 \cdot 10^{-3}$	$8.16 \cdot 10^{-3}$	121	1	0	36	35	<b>4250</b>	0.972	<b>119</b>
			122	1	2	34				

Values of these enrichment factors differ, as the Baret method determines enrichment ( $\eta$ ) by dividing the final ratio of 'positive' to 'negative' colony-forming units ( $\varepsilon_1$ ), divided by the initial ratio ( $\varepsilon_0$ ) before sorting. The enrichment factor ( $\eta'$ ) in the Zinchenko method was counted in a similar way, but this time the percentage of 'positive' colony-forming units to the total number ( $\varepsilon_1'$ ), was divided by the initial percentage before sorting ( $\varepsilon_0'$ ).



**Table S7. The values measured during optimization of the flow parameters for the most efficient sorting, and subsequently used for the preparation of Figure 2.**

Droplet flow rate [ul/h]	Sorting oil [ul/h]	Total oil [ul/h]	Frequency [Hz]	sensitivity	specificity	accuracy	all droplets	true negative	true positive	false negative	false positive
50	1000	1550	84	0.500	1.000	0.976	42	40	1	1	0
50	1500	2050	100	0.667	1.000	0.982	55	52	2	1	0
50	2000	2550	119	0.571	1.000	0.950	60	53	4	3	0
50	2500	3050	108	1.000	1.000	1.000	53	50	3	0	0
50	3000	3550	93	0.833	1.000	0.980	50	44	5	1	0
50	3500	4050	111	1.000	1.000	1.000	60	53	7	0	0
50	4000	4550	103	1.000	1.000	1.000	54	52	2	0	0
75	1000	1575	182	0.833	1.000	0.981	106	94	10	2	0
75	1500	2075	154	1.000	1.000	1.000	89	84	5	0	0
75	1500	2075	167	1.000	1.000	1.000	93	84	9	0	0
75	2000	2575	154	1.000	1.000	1.000	76	74	2	0	0
75	2000	2575	165	1.000	1.000	1.000	85	73	12	0	0
75	2500	3075	154	1.000	1.000	1.000	82	76	6	0	0
75	3000	3575	152	1.000	1.000	1.000	77	68	9	0	0
75	3000	3575	156	1.000	1.000	1.000	87	83	4	0	0
75	3500	4075	135	1.000	1.000	1.000	76	64	12	0	0
75	3500	4075	159	1.000	1.000	1.000	82	76	6	0	0
75	4000	4575	157	1.000	1.000	1.000	90	83	7	0	0
100	1000	1600	225	1.000	0.900	0.905	105	90	5	0	10
100	1500	2100	211	1.000	1.000	1.000	112	104	8	0	0
100	2000	2600	211	1.000	1.000	1.000	106	98	8	0	0
100	2500	3100	219	1.000	1.000	1.000	108	103	5	0	0
100	3000	3600	200	1.000	1.000	1.000	115	107	8	0	0
100	3000	3600	215	1.000	1.000	1.000	125	116	9	0	0
100	3500	4100	212	1.000	1.000	1.000	117	107	10	0	0
100	4000	4600	206	1.000	1.000	1.000	110	106	4	0	0
100	4000	4600	220	1.000	1.000	1.000	125	115	10	0	0
125	1000	1625	260	0.786	0.722	0.727	165	109	11	3	42
125	1500	2125	261	0.895	0.799	0.810	158	111	17	2	28
125	2000	2625	250	1.000	0.970	0.973	147	130	13	0	4
125	2000	2625	260	0.846	0.935	0.928	152	130	11	2	9
125	2500	3125	256	1.000	0.945	0.948	155	137	10	0	8
125	3000	3625	255	1.000	0.993	0.993	151	140	10	0	1
125	3500	4125	263	0.950	0.938	0.939	148	120	19	1	8
125	3500	4125	265	0.909	0.965	0.956	135	109	20	2	4
125	4000	4625	276	0.853	0.853	0.853	156	104	29	5	18
150	1000	1650	360	0.722	0.661	0.667	192	115	13	5	59
150	1500	2150	315	0.750	0.657	0.665	188	113	12	4	59
150	1500	2150	348	0.818	0.678	0.693	202	122	18	4	58
150	2000	2650	342	0.955	0.747	0.770	196	130	21	1	44
150	2500	3150	360	1.000	0.677	0.688	173	113	6	0	54
150	3000	3650	351	1.000	0.728	0.756	176	115	18	0	43
150	3500	4150	333	0.966	0.674	0.716	201	116	28	1	56
150	4000	4650	374	1.000	0.635	0.668	187	108	17	0	62



## VIDEO CAPTIONS

**Video S1.** Passive sorting process applied to two 100 pL droplet emulsions of LB medium and bulk culture of *Bacillus* sp. ANT\_WA51 encapsulated at an average cell concentration of 5 cells per droplet ( $\lambda = 5$ ). The effective ratio during sorting was respectively 9:1, adjusted by proportional flow rates for emulsions. Oil flow was directed from left to right. “Positive” droplets containing bacteria, distinct by visible granularities, fell off the rail immediately, and their flow trajectories were only slightly deflected on the rail. On the contrary, “negative” droplets containing pure medium followed the rail upwards. The video speed was 50 times slower than the actual run speed. The sorting frequency equaled 153 Hz.

**Video S2.** Passive sorting process applied to a 2-strain mix of *Bacillus* sp. ANT\_WA51 and *E. coli* BL21 at a ratio of 1:100, encapsulated with  $\lambda = 0,1$ . Only 1 in 1000 droplets was “positive” (containing *Bacillus* strain), and one such droplet was captured in this video. All other droplets followed the rail upwards.

**Video S3.** Excessive droplet frequency leading to collisions of droplets and sorting errors in the narrow device. The passive sorting was applied to 100 pL droplets containing LB medium and *Bacillus* sp. ANT\_WA51 bulk culture. The effective ratio during sorting was respectively 9:1, adjusted by proportional flow rates for emulsions. The frequency of sorting was approximately 370 Hz.

## SUPPLEMENTARY REFERENCES

- (1) Styczynski, M.; Biegniewski, G.; Decewicz, P.; Rewerski, B.; Debiec-Andrzejewska, K.; Dziewit, L. Application of Psychrotolerant Antarctic Bacteria and Their Metabolites as Efficient Plant Growth Promoting Agents. *Front. Bioeng. Biotechnol.* **2022**, *10*, 772891. <https://doi.org/10.3389/fbioe.2022.772891>.
- (2) Qin, D.; Xia, Y.; Whitesides, G. M. Soft Lithography for Micro- and Nanoscale Patterning. *Nat Protoc* **2010**, *5* (3), 491–502. <https://doi.org/10.1038/nprot.2009.234>.
- (3) Jenkins, G. Rapid Prototyping of PDMS Devices Using SU-8 Lithography. In *Microfluidic Diagnostics*; Jenkins, G., Mansfield, C. D., Eds.; Methods in Molecular Biology; Humana Press: Totowa, NJ, 2013; Vol. 949, pp 153–168. [https://doi.org/10.1007/978-1-62703-134-9\\_11](https://doi.org/10.1007/978-1-62703-134-9_11).
- (4) Neun, S.; Kaminski, T. S.; Hollfelder, F. Single-Cell Activity Screening in Microfluidic Droplets. In *Methods in Enzymology*; Elsevier, 2019; Vol. 628, pp 95–112. <https://doi.org/10.1016/bs.mie.2019.07.009>.
- (5) Pan, C. W.; Horvath, D. G.; Braza, S.; Moore, T.; Lynch, A.; Feit, C.; Abbyad, P. Sorting by Interfacial Tension (SIFT): Label-Free Selection of Live Cells Based on Single-Cell Metabolism. *Lab Chip* **2019**, *19* (8), 1344–1351. <https://doi.org/10.1039/C8LC01328D>.
- (6) Horvath, D. G.; Braza, S.; Moore, T.; Pan, C. W.; Zhu, L.; Pak, O. S.; Abbyad, P. Sorting by Interfacial Tension (SIFT): Label-Free Enzyme Sorting Using Droplet Microfluidics. *Analytica Chimica Acta* **2019**, *1089*, 108–114. <https://doi.org/10.1016/j.aca.2019.08.025>.
- (7) Dobson, C.; Zielke, C.; Pan, C.; Feit, C.; Abbyad, P. Method for Passive Droplet Sorting after Photo-Tagging. *Micromachines* **2020**, *11* (11), 964. <https://doi.org/10.3390/mi11110964>.
- (8) Zielke, C.; Pan, C. W.; Gutierrez Ramirez, A. J.; Feit, C.; Dobson, C.; Davidson, C.; Sandel, B.; Abbyad, P. Microfluidic Platform for the Isolation of Cancer-Cell Subpopulations Based on Single-Cell Glycolysis. *Anal. Chem.* **2020**, *92* (10), 6949–6957. <https://doi.org/10.1021/acs.analchem.9b05738>.



- (9) Zielke, C.; Gutierrez Ramirez, A. J.; Voss, K.; Ryan, M. S.; Gholizadeh, A.; Rathmell, J. C.; Abbyad, P. Droplet Microfluidic Technology for the Early and Label-Free Isolation of Highly-Glycolytic, Activated T-Cells. *Micromachines* **2022**, *13* (9), 1442. <https://doi.org/10.3390/mi13091442>.
- (10) Baret, J.-C.; Miller, O. J.; Taly, V.; Ryckelynck, M.; El-Harrak, A.; Frenz, L.; Rick, C.; Samuels, M. L.; Hutchison, J. B.; Agresti, J. J.; Link, D. R.; Weitz, D. A.; Griffiths, A. D. Fluorescence-Activated Droplet Sorting (FADS): Efficient Microfluidic Cell Sorting Based on Enzymatic Activity. *Lab Chip* **2009**, *9* (13), 1850. <https://doi.org/10.1039/b902504a>.
- (11) Zinchenko, A.; Devenish, S. R. A.; Kintses, B.; Colin, P.-Y.; Fischlechner, M.; Hollfelder, F. One in a Million: Flow Cytometric Sorting of Single Cell-Lysate Assays in Monodisperse Picolitre Double Emulsion Droplets for Directed Evolution. *Anal. Chem.* **2014**, *86* (5), 2526–2533. <https://doi.org/10.1021/ac403585p>.