Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information for:

Are droplets really suitable for single-cells analysis? A case study on yeast in droplets

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Supplementary Note 1: Proliferation of budding yeast cells cultured in droplets

To investigate whether the effect of the droplet size on cell activity can be seen based on probing the proliferation, we measured the biomass of budding yeast cells after they were encapsulated and cultured in 100-pL (~57 μm in diameter), 500-pL (~100 μm in diameter), and 1-nL (~125 μm in diameter) droplets. Here, we used the volume of cells that occupy the droplets as a readout of the biomass of cells after the culture droplets. As shown in Fig. S4, the biomass linearly increased with the increase in droplet volume i.e., the volume of cell medium.

The biomass was measured by estimating the volume of droplets occupied by cells as described below. First, incubated droplets were collected via glass capillary tubes (VitroTubes 3520-100, VitroCom, USA) and imaged using a microscope (IX73, Olympus, Japan). Two-dimensional bright-field images were recorded using a scientific complementary metal-oxide semiconductor (sCMOS) camera (ORCA-Flash4.0 C11440, Hamamatsu Photonics, Japan). All recorded images were processed with ImageJ as follows. First, the area within droplets occupied by cells was measured. Second, from the measured area, the radius and the volume were estimated under the assumption that the cells accumulated inside a droplet spherically. At least 185 cell-encapsulating droplets were analyzed for each droplet volume.

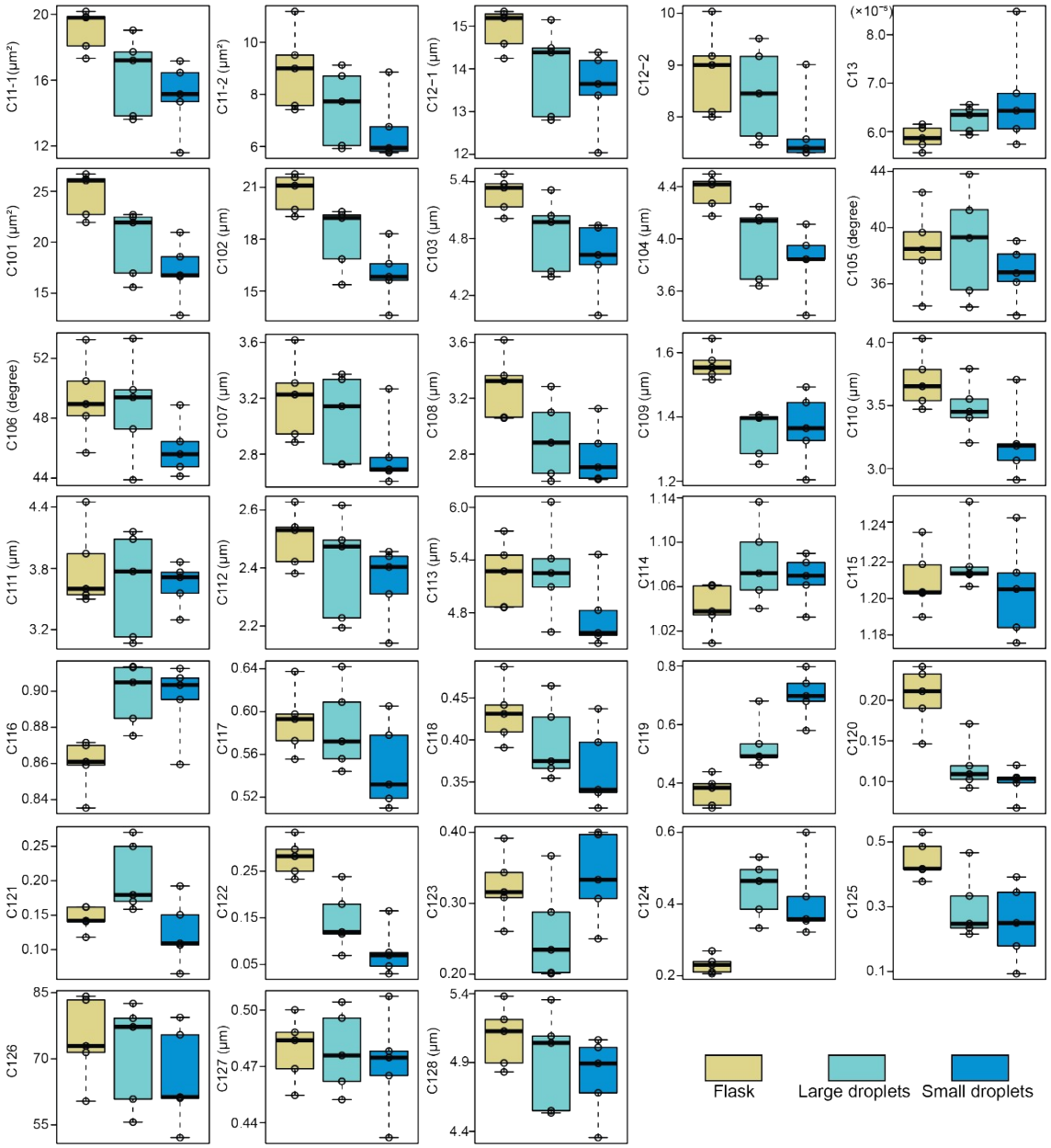

Fig. S1 Measured morphological features of cells cultured in a flask, large droplets, and small droplets. The descriptions of all parameters are shown in Table S1.

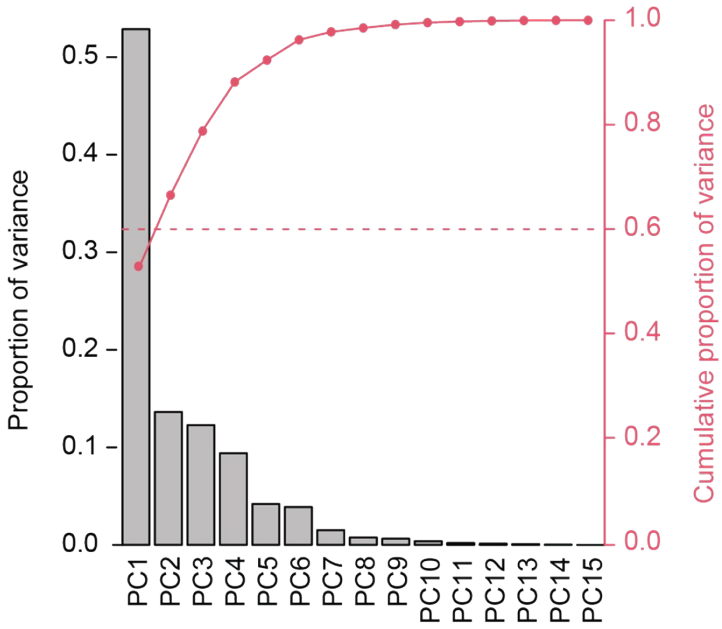

Fig. S2 Cumulative contribution ratio of principal components. The first 15 principal components are shown. PC1 and PC2, which accounted for the most significant contribution to variability, were used for further morphological analysis.

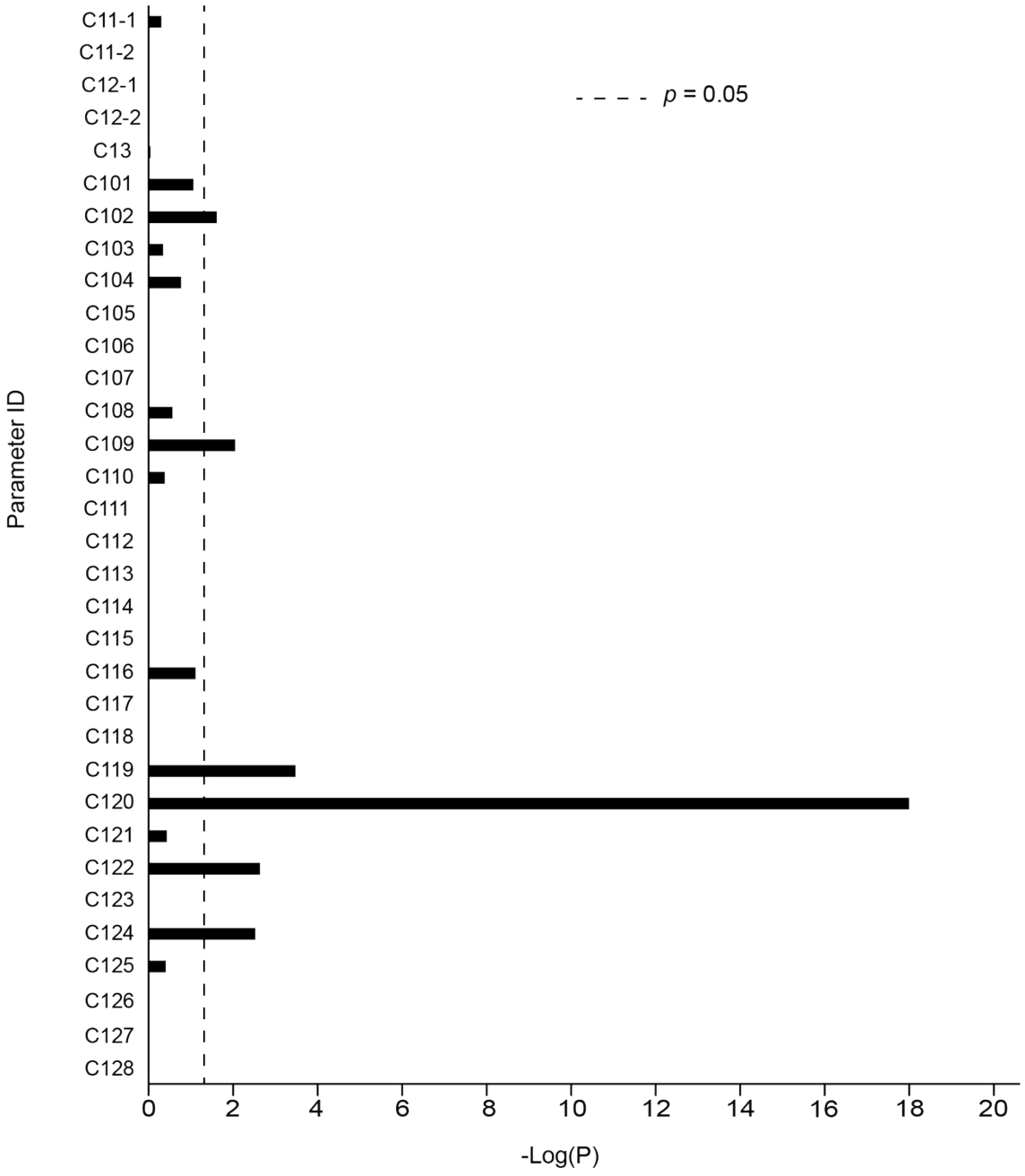

Fig. S3 p-values of the morphological parameters for the likelihood ratio test of ANOVA.

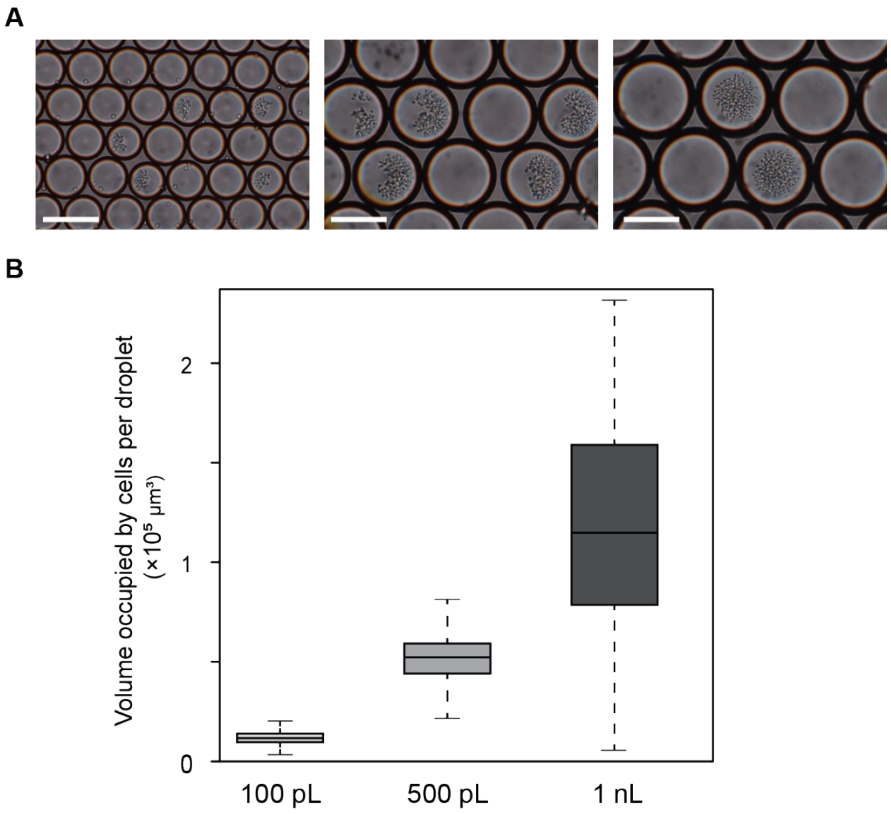

Fig. S4 Biomass of budding yeast cells cultured in different sizes of droplets. A Images of cells cultured for 15 hours in different sizes of droplets. Images of 100-pL, 500-pL, and 1-nL droplets are shown from the left. Scale bars, 100 µm. **B** Quantified biomass of cells cultured in 100-pL, 500-pL, and 1-nL droplets.

Table S1 Morphological parameters measured by CalMorph. A complete catalog of morphological parameters and their descriptions are available in a previously reported work[60].

ID	Descriptions
C11-1	The size of the mother cell
C11-2	The size of the bud
C12-1	The outline length of the mother cell
C12-2	The outline length of the bud
C13	The fitness of the mother cell for an ellipse
C101	The size of whole cell
C102	The length of the outline of whole cell
C103	The length of the long axis of the mother cell
C104	The length of the short axis of the mother cell
C105	The position of the neck
C106	The direction of the bud
C107	The length of the long axis of the bud
C108	The length of the short axis of the bud
C109	The width of the neck
C110	The distance between the bud tip and the long axis of the mother cell
C111	The distance between the bud tip and the short axis of mother cell
C112	The distance between the middle point of the neck and the mother cell
C113	The distance between the bud tip and the long axis of the mother cell passing through the middle of the neck
C114	The ratio of bud axes
C115	The ratio of the axes of the mother cell
C116	The ratio of the axis ratio of the mother cell and bud
C117	The ratio of the outlines of the mother cell and bud
C118	The ratio of the sizes of the mother cell and bud
C119	The ratio of unbudded cells to all cells
C120	The ratio of small-budded cells to all cells
C121	The ratio of medium-budded cells to all cells
C122	The ratio of large-budded cells to all cells
C123	The ratio of small-budded cells all budded cells
C124	The ratio of medium-budded cells to all budded cells
C125	The ratio of large-budded cells to all budded cells
C126	The brightness differences of the cell wall
C127	The thickness differences of the cell wall
C128	The distance between the middle point of the neck and the mother cell

Table S2 p-values of the morphological parameters for the likelihood ratio test of ANOVA.

ID	p-value after Bonferroni correction
C11-1	0.503
C11-2	1.00
C12-1	1.00
C12-2	1.00
C13	0.906
C101	0.087
C102	0.024
C103	0.455
C104	0.171
C105	1.00
C106	1.00
C107	1.00
C108	0.274
C109	0.009
C110	0.417
C111	1.00
C112	1.00
C113	1.00
C114	1.00
C115	1.00
C116	0.078
C117	1.00
C118	1.00
C119	3.35×10^{-4}
C120	1.01×10^{-18}
C121	0.371
C122	0.002
C123	1.00
C124	0.003
C125	0.393
C126	1.00
C127	1.00
C128	1.00

Table S3 Conditions for generating droplets of different sizes. Droplets were generated using flow-focusing devices previously reported[36].

Droplet size (pL)	Microchannel height (μm)	Cell concentration (cells/mL)	Flow rate of cell medium (μL/min)	Flow rate of oil (μL/min)
40	39-41	2.5×10^6	15	35
100	48-51	1.0×10^6	10	30
500	111-112	2.0×10^5	10	30
1000	111-112	1.0×10^5	30	80

Table S4. Probability distribution functions and link functions for 33 parameters. PDF: probability distribution function; asis: CalMorph parameter outputs were used as is; count-back: CalMorph parameter outputs were converted from the ratio to the number of cells; inverse: CalMorph parameter outputs were converted to the inverse.

ID	Transformation function	PDF	Link function η for location θ	Link function ε for scale σ
C11-1	asis	gamma	log	log
C11-2	asis	gamma	log	log
C12-1	asis	gamma	log	log
C12-2	asis	gamma	log	log
C13	asis	gamma	log	log
C101	asis	gamma	log	log
C102	asis	gamma	log	log
C103	asis	gamma	log	log
C104	asis	gamma	log	log
C105	asis	gamma	log	log
C106	asis	gamma	log	log
C107	asis	gamma	log	log
C108	asis	gamma	log	log
C109	asis	gamma	log	log
C110	asis	gamma	log	log
C111	asis	gamma	log	log
C112	asis	gamma	log	log
C113	asis	inverse gamma	log	log
C114	asis	gamma	log	log
C115	inverse	beta	logit	logit
C116	asis	gamma	log	log
C117	asis	beta	logit	logit
C118	asis	beta	logit	logit
C119	count-back	beta binomial	logit	log
C120	count-back	beta binomial	logit	log
C121	count-back	beta binomial	logit	log
C122	count-back	beta binomial	logit	log
C123	count-back	beta binomial	logit	log
C124	count-back	binomial	logit	-
C125	count-back	beta binomial	logit	log
C126	asis	gamma	log	log
C127	asis	gamma	log	log
C128	asis	gamma	log	log