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

Supplementary Information for:

Technical bias of microcultivation environments on single cell physiology

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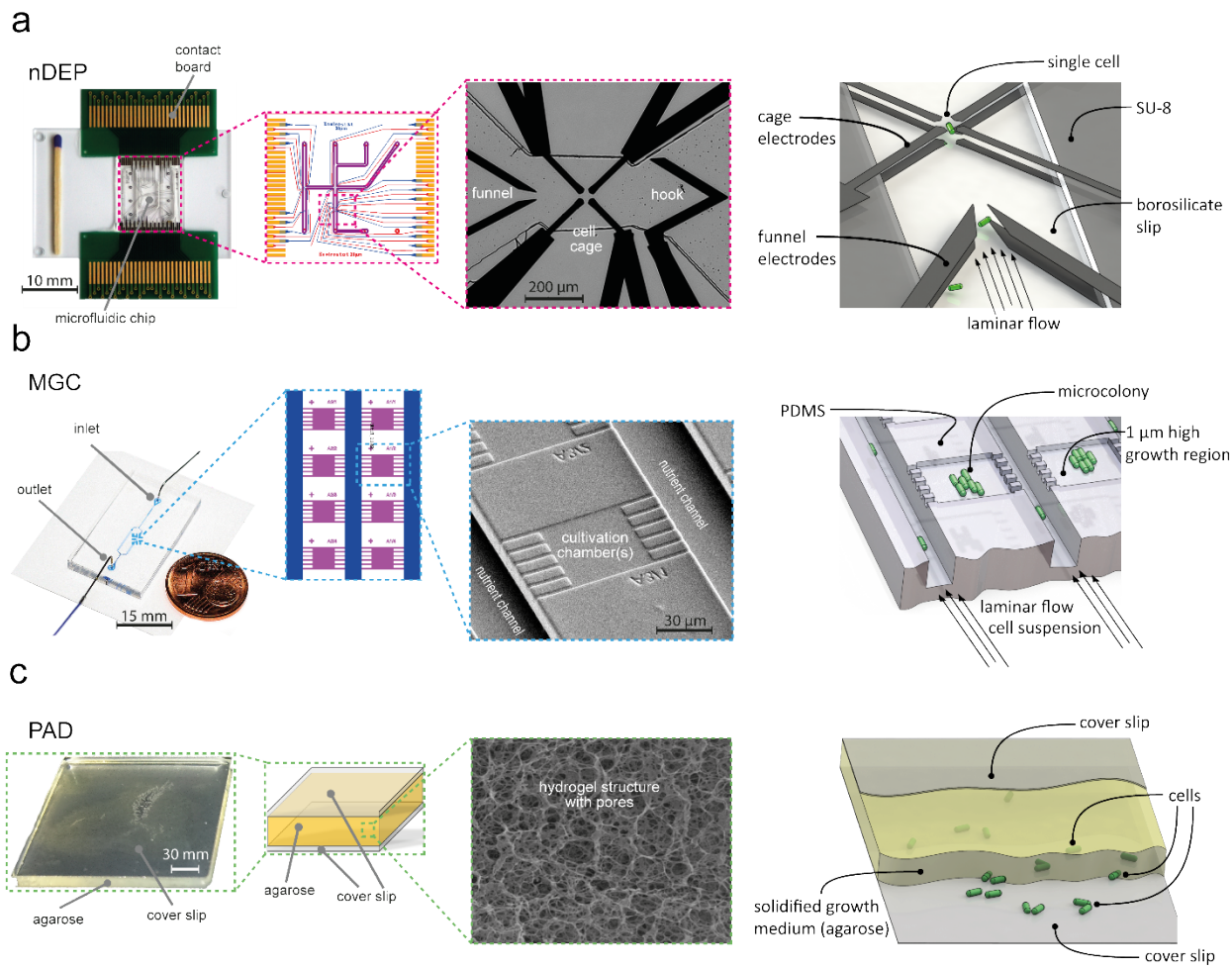
Evaluation of microcultivation technologies

The three different single cell microcultivation methods were compared with respect to functionality and applicability, revealing a distinct application window for each microcultivation technology. Cell trapping that can be triggered, like with nDEP systems, generally enables a highly selective isolation of specific phenotypes from whole populations. Such systems also prove their strengths if surface contact has to be excluded in order to prevent changes in the cellular phenotype. In addition, continuous cell perfusion allows an unprecedented control over the extracellular environment and enables rapid chemical perturbation of the cells. However, nDEP requires an external periphery for driving the electrodes and compensating joule heating. In contrast to this, the MGC system, stand out as they allow to perform experiments with high throughput while still enabling environmental control and chemical perturbation. Moreover, the system is not limited to the analysis of only a few cells, but allows tracking microcolonies of up to several hundred cells per chamber. Microcultivation systems like the MGC are usually easy to fabricate and allow for rapid prototyping within weeks.

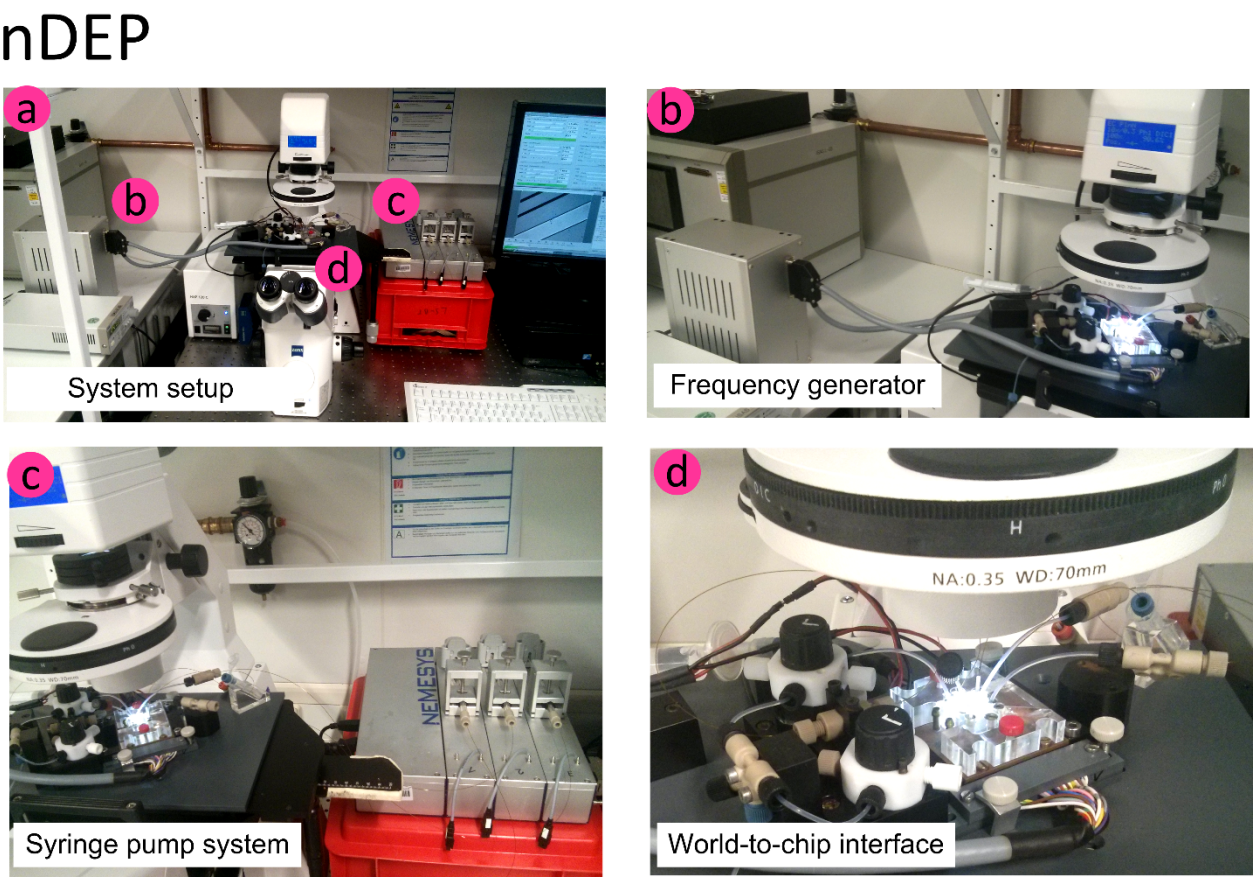
Agarose pads also enable high-throughput analyses of a large number of cells and resulting colonies in parallel, while precise control of the cellular environment and chemical short term-perturbation are not possible. Agarose pads are easy to fabricate and do not need any extra periphery. Therefore, this system provides an easy and efficient way to implement single-cell analyses into virtually every bio(techno)logy laboratory. A rational selection of a single cell cultivation system for specific experimental purposes is now possible.

Supplementary Figure 1|System design, functional cell trapping and cultivation principles of the compared microcultivation technologies



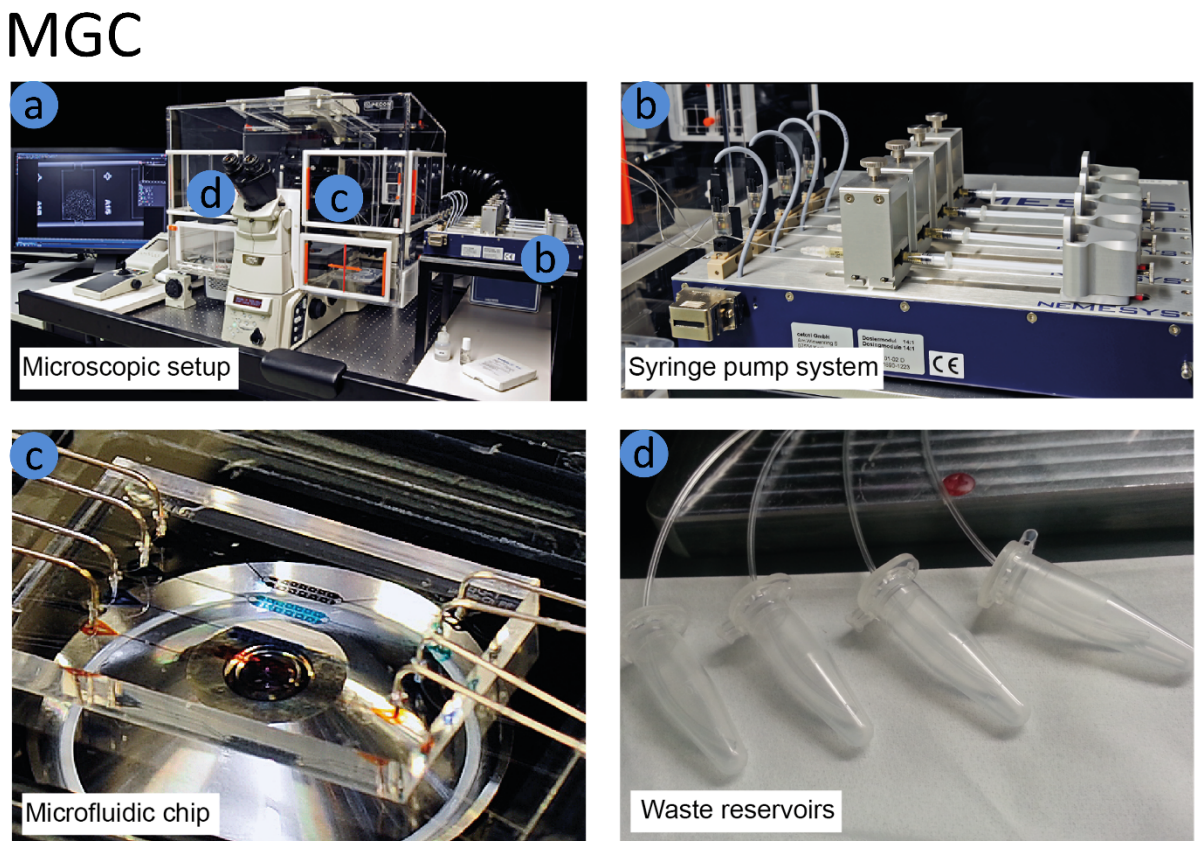
Design and functional principles of the compared single-cell cultivation systems from a macroscopic and microscopic point of view. **(a)** nDEP chips from a bench top view with fluidic and electric connections; channel and electrode design plans; microscopic image of the trapping area and functional electrode elements. Single-cell isolation and trapping with nDEP is schematically illustrated in the CAD drawing **(b)** PDMS-based MGC chip with fluidic connections; microchamber design; SEM image of the growth chamber with nutrient channels. Single-cell seeding and cultivation is schematically illustrated in the CAD drawing **(c)** Casted sandwich agarose pad with a layer of solidified growth medium between two glass cover slides; agarose pad setup as a schematic drawing; porous structure of a solidified agarose hydrogel (1.5% w/v) in a SEM image. Functional principle of cell cultivation with agarose pads on the basis of a CAD drawing.

Supplementary Figure 2| Experimental setup of the nDEP system



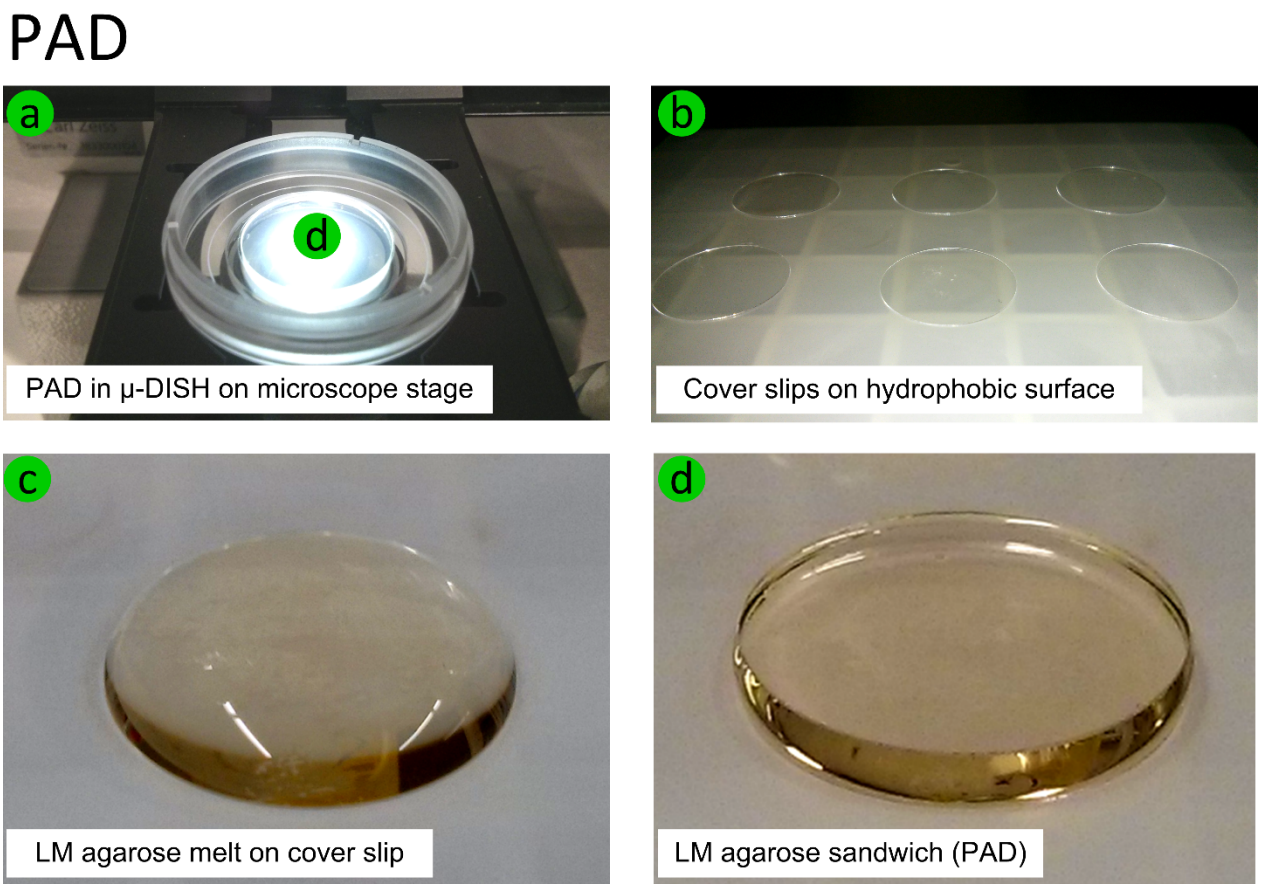
(a) Microfluidic nDEP chip with temperature control periphery and fluidic world-to-chip interface mounted to the stage of an inverted widefield fluorescence microscope. **(b)** Radio frequency generator for driving the octupole electrodes. **(c)** Syringe pump system connected with fused silica capillary to the fluidic interface. **(d)** Fluidic cassette with copper cooling block and electric connection of chip and generator via a 37 pin connection cable.

Supplementary Figure 3| Experimental setup of microfluidic MGC system



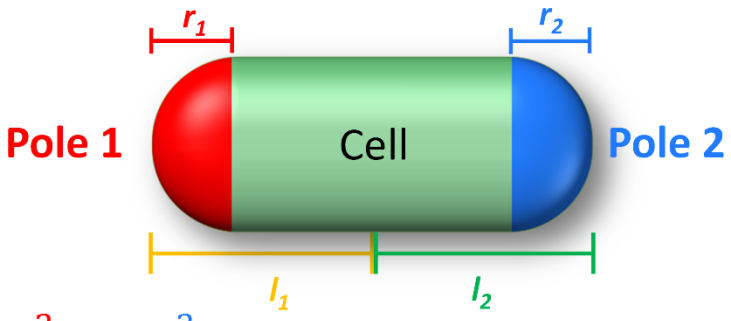
(a) Experimental setup of MGC system. **(b)** Syringe pump system for fluid delivery. **(c)** Microfluidic PDMS chip with curved connection needles. **(d)** Waste reservoirs for flow out medium.

Supplementary Figure 4| Preparation and experimental setup of agarose pads



(a) An agarose pad in a disposable glass bottom µ-dish and placed on the stage of an inverted fluorescence microscope. Preparation of agarose pads. **(b)** Glass cover slips placed on a hydrophobic surface for agarose melt deposition. **(c)** Fluid low melt (LM) agarose dissolved in cultivation medium is pipetted onto the glass slide. **(d)** After pipetting of the agarose melt, a second glass cover slip is placed on the agarose drop to form a sandwich. After solidification of the agarose melt, the top cover slip is removed and cells are seeded directly onto the agarose pad. For time-lapse microscopy, the inoculated pad is placed upside down in a glass-bottom µ-dish.

Supplementary Figure 5| Mathematical approximation of cell volume from time-lapse microscopy images



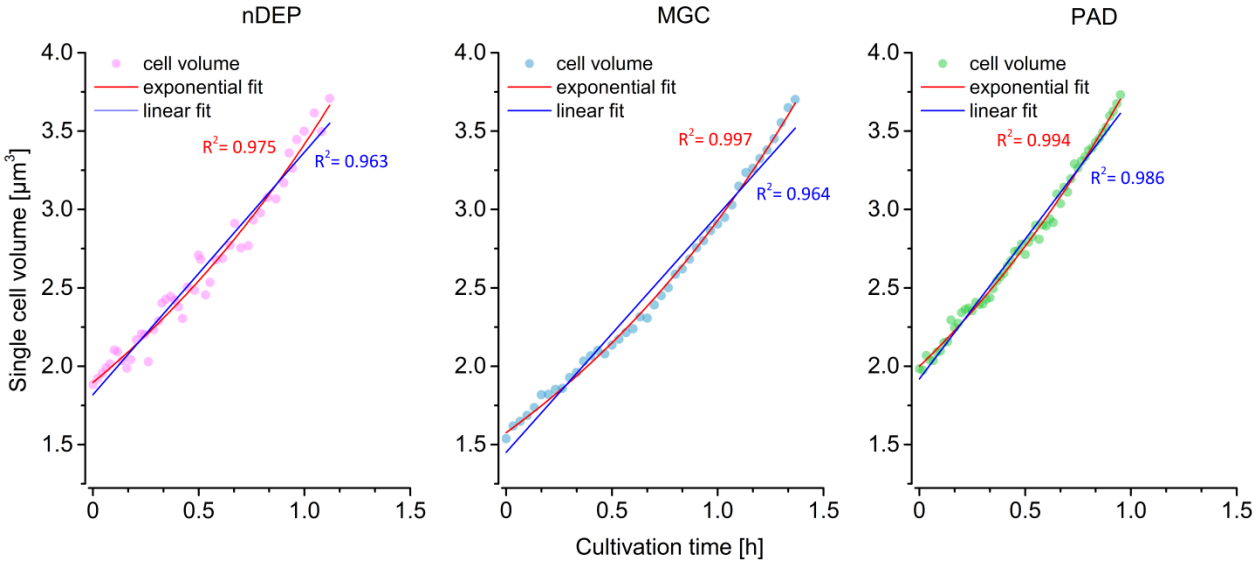
Central assumption:

$$\rho_{cell} = \frac{M_{cell}}{V_{cell}} = \text{const.}$$

$$V_{cell} = \frac{2}{3}\pi\, r_1{}^3 + \frac{2}{3}\pi\, r_2{}^3 + \pi r_1{}^2 \times (l_1 - r_1) + \pi r_2{}^2 \times (l_2 - r_2)$$

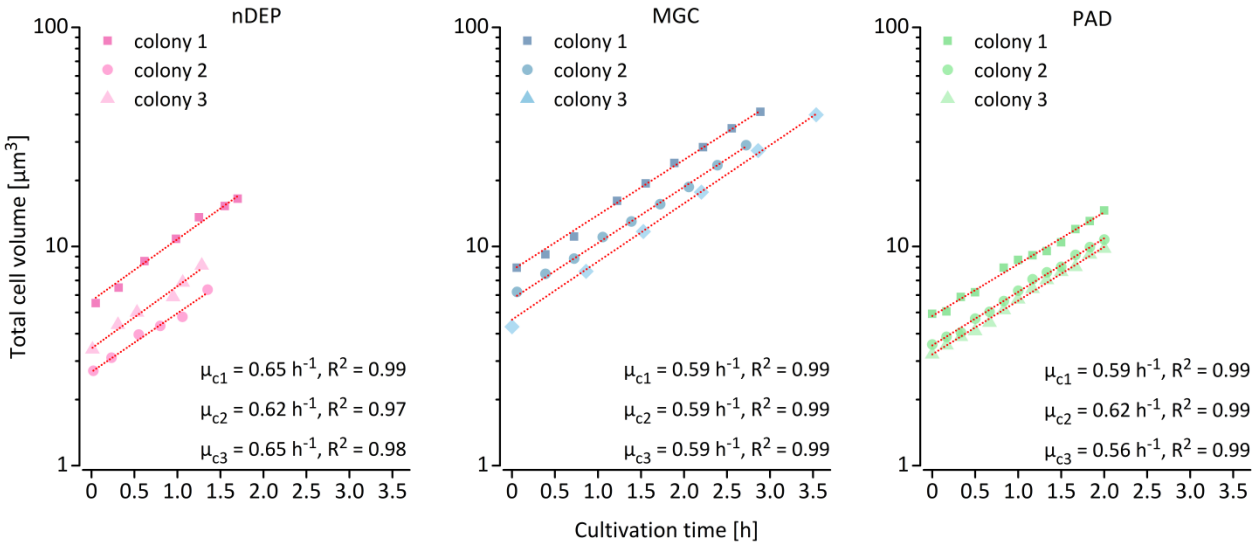
Cells were segmented into four solid bodies (two central cylinders and two hemispheres for the pole caps) to approximate the cell volume and account for the club shape of single *C. glutamicum* cells.

Supplementary Figure 6| Detailed analysis of specific single cell growth rates



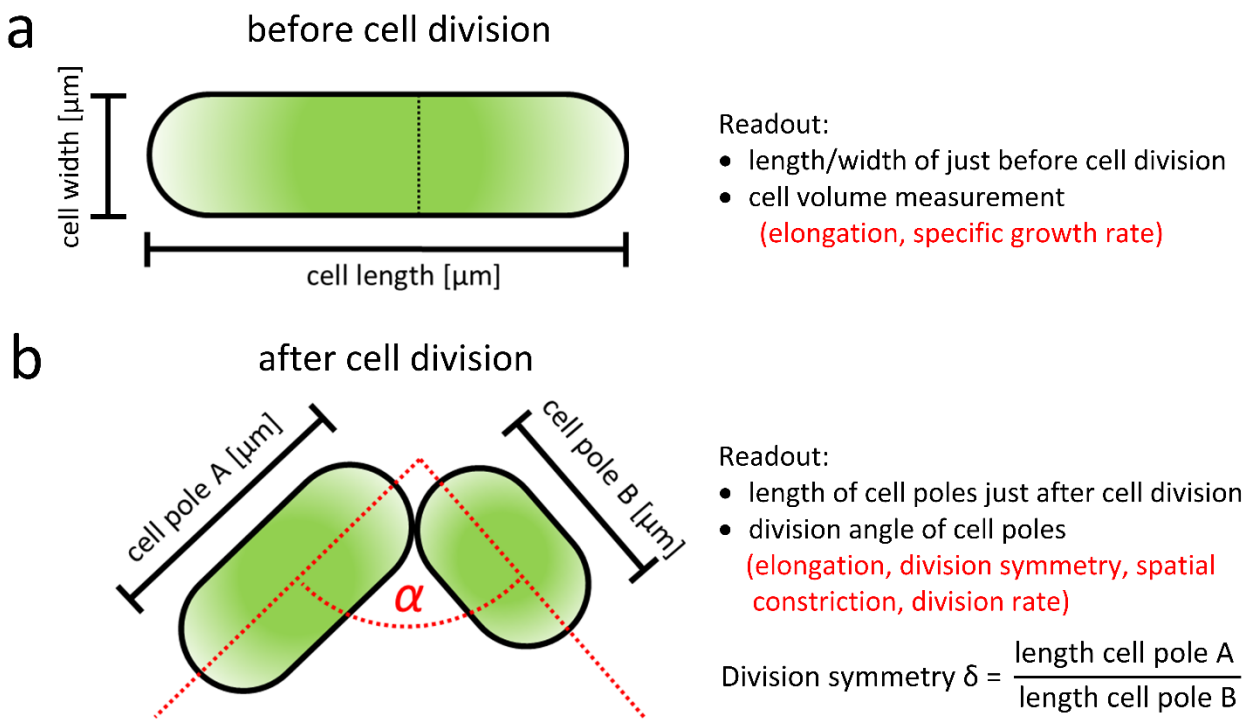
The increase of single cell volume during the cultivation can be best described by an exponential fit according to the coefficient of determination, indicating that biomass increase is a tightly controlled process in single *C. glutamicum* cells.

Supplementary Figure 7| Increase in total cell volume of *C. glutamicum* ATCC 13032 micropopulations in the respective trapping area of the three compared microcultivation systems



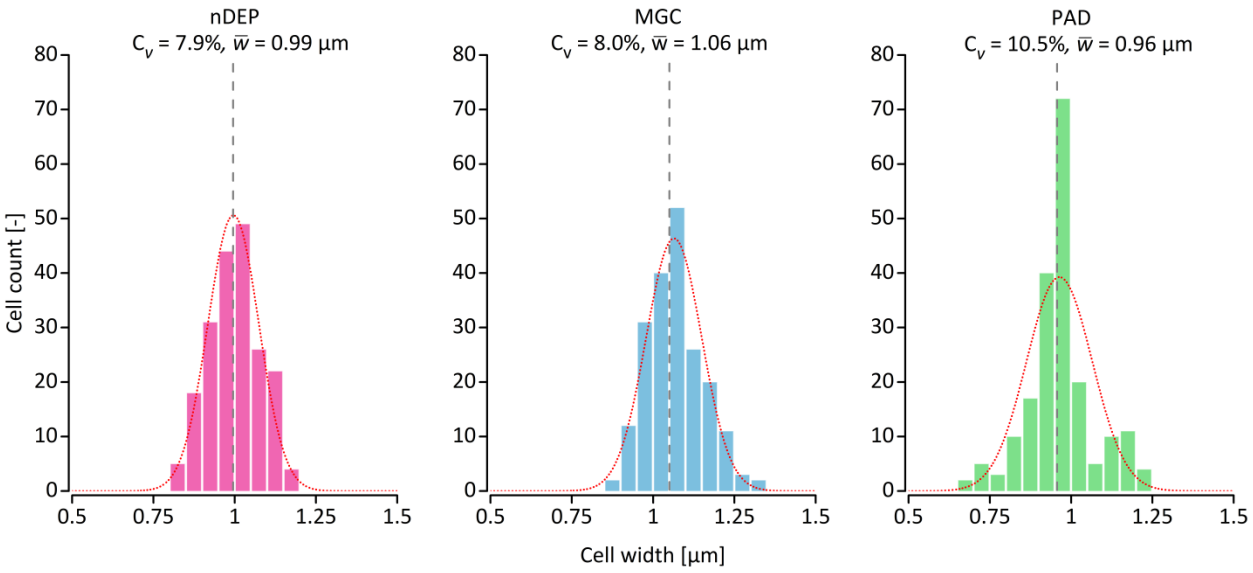
Representative growth curves of micropopulations with nDEP, MGC and PAD system. All observed micropopulations exhibited a strictly exponential increase in cell volume.

Supplementary Figure 8| Physiological and morphological readouts during single-cell cultivations



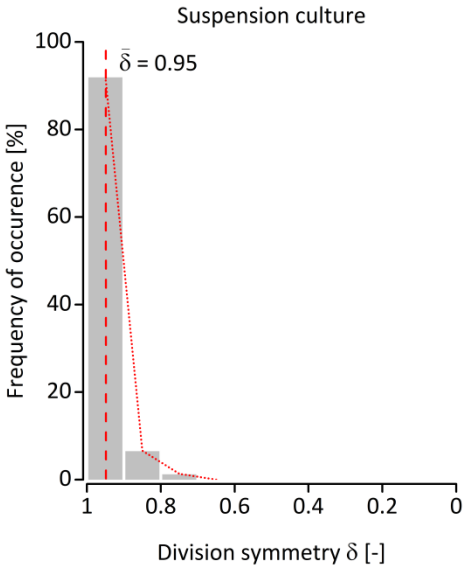
(a) During growth phase of a single cell, cell volume, cell length and cell width were continuously measured. From the gathered geometrical data, cell volumes were calculated in order to determine the specific growth rate of single cells. **(b)** Immediately after the snapping cell division event, cell lengths and division angles of the two newly emerged cell poles were determined in order to quantitatively evaluate cell elongation, division symmetry, division rates and spatial constriction inside the microcultivation device.

Supplementary Figure 9| Cell width distributions of *C. glutamicum* ATCC 13032 cultivated with the three microcultivation systems



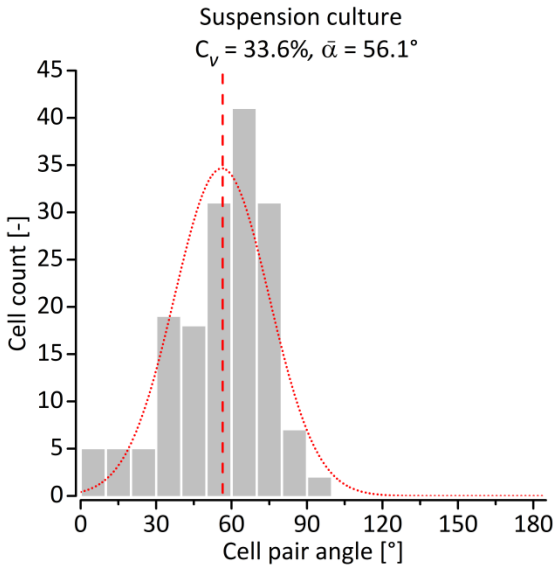
Cell width distributions of cells cultivated with nDEP, MGC and PAD system. The dotted lines represent the fitted normal distribution of the cell width data. The total number of measured cells was n= 200, respectively.

Supplementary Figure 10| Cell division symmetry of *C. glutamicum* ATCC 13032 cultivated in shaken liquid suspension



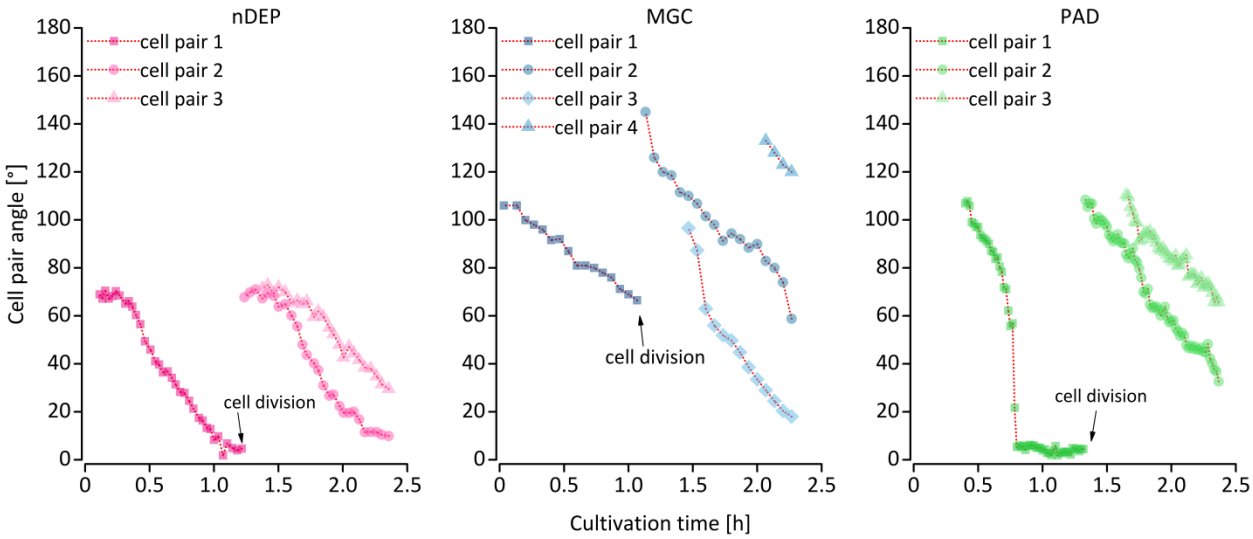
C. glutamicum cells feature highly symmetric division in shaken liquid suspension culture (BHI medium, 30°C, shaking frequency 250 rpm, 250 ml shake flask, 25 ml culture volume).

Supplementary Figure 11| Distribution of cell pair angles of *C. glutamicum* ATCC 13032 in shake flask cultivations



Cell pair angle distribution of *C. glutamicum* ATCC 13032 cultivated in a 250 ml shake flask (25 ml working volume) in BHI medium at 30°C and a shaking frequency of 250 min^{-1} (n=139). The dotted line represents a normal fit of the cell pair angle distribution. The vertical red line indicates the average cell pair angle of 56.1°. Cell pair angle data exhibited a coefficient of variation of 33.6%.

Supplementary Figure 12| Dynamics of cell pair division angles in single-cell cultivations of *C. glutamicum* ATCC 13032



Representative cell pair angle dynamics of *C. glutamicum* ATCC 13032 during cultivation with the three investigated microcultivation systems. Cells cultivated in the nDEP device typically displayed division angels between 70° and 80°, with a continually declining cell pair angle. Before the following cell division, the two cells poles originating from a common mother cell were typically aligned in parallel. Cells cultivated with the MGC displayed division and cell pair angles in a more random fashion compared to the nDEP system. Cells pair angles continuously declined during the cell growth. With the PAD system, the cell pair angles occasionally declined in a rapid and sudden fashion. This phenomenon was observed predominantly in the initial phase (first 45 min) of the cultivation. Following cell pair angles were comparable with nDEP and MGC cultivations.

Supplementary Table 1| System characteristics of nDEP, MGC and PAD

	nDEP	MGC	PAD
Trapping selectivity	Targeted trapping	Stochastic trapping	Stochastic trapping
Operation	Single cell isolation and analysis[1]	Single cell/microcolony trapping and analysis[2]	Single cell/microcolony trapping and analysis[3]
Fabrication	Complex fabrication process	Rapid prototyping and fabrication	Rapid fabrication
Reusability	Reusable	Disposable	Disposable
Cell types	Cell type-independent cultivation and analysis[1,4]	Cell type-dependent device design[5,6]	Cell type-independent cultivation and analysis[7,8]
Surface contact/ spatial confinement	No interaction between cell and surface, levitation in medium flow	Surface contact, spatial confinement dependent on cultivation chamber height	Surface contact, pronounced spatial confinement
Manipulation/ sensing	Targeted isolation and manipulation of single cells, multi-applicability of electrodes for: impedance sensing[9], electroporation and cell fusion[10], electrorotation[11]	Main focus on screening and analysis, no cell manipulation, focus on optical analysis[2,12]	No cell manipulation, focus on optical analysis[3]
Supernatant sampling	Total isolation from preculture metabolites enables sampling of extracellular metabolites from a single cell	Possible in theory, PDMS bleeding and unspecific adsorption is likely to hamper metabolite sampling	Not possible
Cell retrieval	Cell release and retrieval possible	Not possible	Not possible
Perturbation	Enables millisecond temperature perturbation, rather slow chemical perturbation since weak nDEP force limits flow rate	Rapid chemical perturbation possible	Slow chemical perturbation by diffusion through agarose pad
Cell number	Limited to few cells (up to 8 cells)	High cell densities possible (1000 cells per chambers)	Medium cell densities (spatial overlap at cell densities > 100 cells)
Cell localization	Cell switches focal planes during cultivation	Cells are fixed in one focal plane	Cells are fixed in one focal plane

Comparison of nDEP, MGC and PAD regarding application-specific characteristics comprising fabrication to application[1-12].

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