

# QUANTITATIVE PHYSIOLOGY WITH ISOLATED SINGLE CELLS AND MICROPOPULATIONS IN CONTROLLED MICROENVIRONMENTS EMPLOYING A PICOLITER BIOREACTOR

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## ABSTRACT

We present a systematic investigation of microbial physiology at single cell level in controlled microenvironments employing the Envirostat 2.0 microfluidic single cell analysis system. The Envirostat represents a unique combination of contactless isolation of cells by dielectrophoresis with continuous cell perfusion. Thus, a chemically and physically steady extracellular environment is created, featuring unlimited nutrient supply, removal of secreted metabolites and avoidance of cell-surface interactions. By optimizing the electrode cage design, the Envirostat 2.0 now enables the manipulation of sub-micrometer bioparticles. Cytometric studies of trapped cells showed that the steady environment is reflected in higher specific growth rates as in populations.[1]

## KEYWORDS

Single cell analysis, nDEP, Envirostat 2.0, extracellular environment, physiology, contactless manipulation

## INTRODUCTION

Clonal microbial populations exhibit significant cell-to-cell differences that occur on all functional levels from genome to phenome. The extent of this physiological intrapopulation diversity was shown to be connected with frequency and strength of changes in the composition of the extracellular environment.[2] During conventional population-based cultivations, the extracellular environment is permanently changing because of inevitable concentration gradients caused by insufficient mixing and the metabolic activity of the cells. In consequence, these technologies do not allow for an adequate resolution of cell-cell differences and the analysis of specific phenotypes.[3] It can be deduced from these facts that the ability to cultivate and analyze single cells in controlled environments and hence reducing physiological oscillations is a fundamental prerequisite for the accurate description of the cellular biochemical network functionality.

We face the challenge of creating controlled extracellular environments for the analysis of single cells with the Envirostat 2.0 system.[4] The Envirostat principle combines cell perfusion with contactless single cell isolation and retention by negative dielectrophoresis (nDEP) in order to create controlled microenvironments.[5] By applying a continuous medium flow, the rapid removal of extracellular metabolites and unlimited availability of nutrients and oxygen is guaranteed. Moreover, the mass transfer in this system is greatly enhanced by the enforced convection. CFD simulations substantiated the assumption that the environment of the target cell has a steady and static composition (Fig. 1).[5] In contrast to most other microfluidic systems for single cell analysis, relying on mechanical retention of cells, contactless trapping excludes the induction of unknown phenotypes by cell-surface interaction.

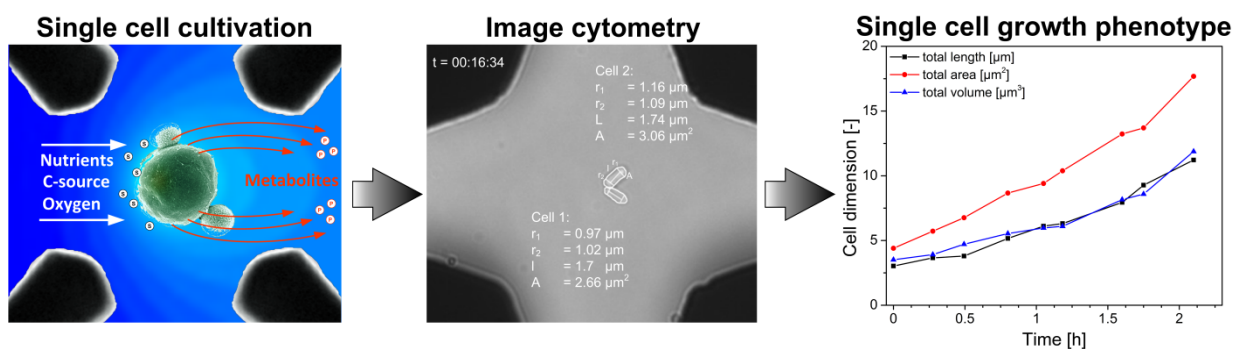


Figure 1: The Envirostat analysis principle. Single cell perfusion in electrode cage: A continuous medium flow ensures unlimited nutrient and oxygen availability, as well as fast removal of secreted metabolites during cultivation and analysis of the single cell(s). Cell growth can be precisely followed by image cytometry analysis. On the basis of the image cytometry data, the growth phenotype of can be determined.[1]

The Envirostat 2.0 extends the Envirostat principle to cover a wider range of cell-types. Through the optimization of the electrode cage geometry and the resulting enhancement of the dielectrophoretic force, the Envirostat 2.0 system now allows for the manipulation of polarizable bioparticles with sub-micrometer size and thus facilitates to venture into the minutest dimensions of single cell analysis, including small bacterial cells (Fig. 2).

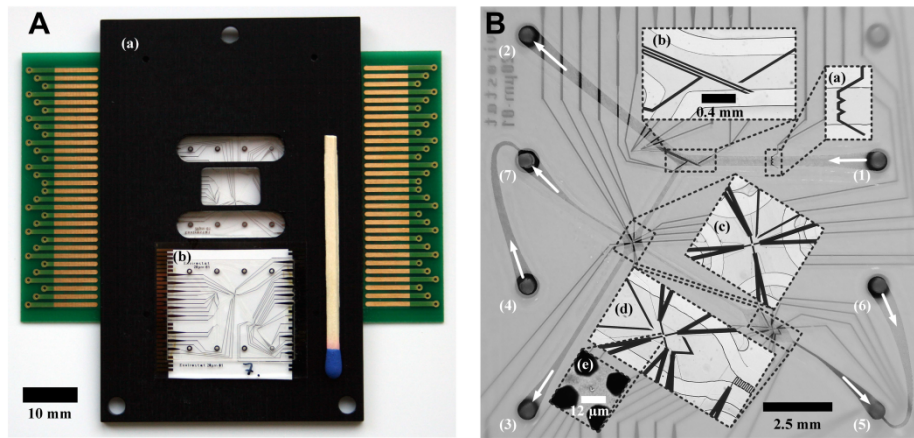


Figure 2: The Envirostat 2.0 chip: (A) (a) Topview of the microfluidic chip with metal carrying plate and the electrical contacting (b) topview of a non-assembled chip. (B) Microscopic picture of microchannel and microelectrode structures: (a) cell retention with Zig-Zag electrode and (1) cell suspension inlet, (b) single cell isolation section with (2, 3) waste outlet, (c) cross section with octode cage and (4, 7) cultivation medium inlet, (d) funnel electrode in cultivation microchamber with (5, 6) sampling outlets, (e) octode cage in cultivation microchamber.[4]

## RESULTS

With the Envirostat 2.0, a unique data set including specific growth rates of single cells and micropopulations that were cultivated under precisely defined (micro-) environmental conditions could be obtained (Fig. 3).[1] As representative eukaryotic and prokaryotic model organisms with high biotechnological relevance, two yeast strains (*Pichia pastoris* Mut<sup>S</sup> and *Hansenula polymorpha* RB11 conphys) and one bacterial strain (*Corynebacterium glutamicum* ATCC 13032) were investigated. It was found that isolated cells exhibited consistently faster volume growth rates in steady environments that were up to 2-fold higher than observed at population level (Fig. 4).[4] Noteworthy, this study reports the first contactless cultivation and analysis of a single bacterial cell, which could be achieved by the optimization of the electrode cage geometry. The cage electrodes were placed in a symmetrical octode arrangement with an electrode tip distance of 20  $\mu\text{m}$ , resulting in a reactor volume of 8 pL. The design of the microfluidic channel structure and electrode functionality facilitates the reliable separation of single cells from bulk samples

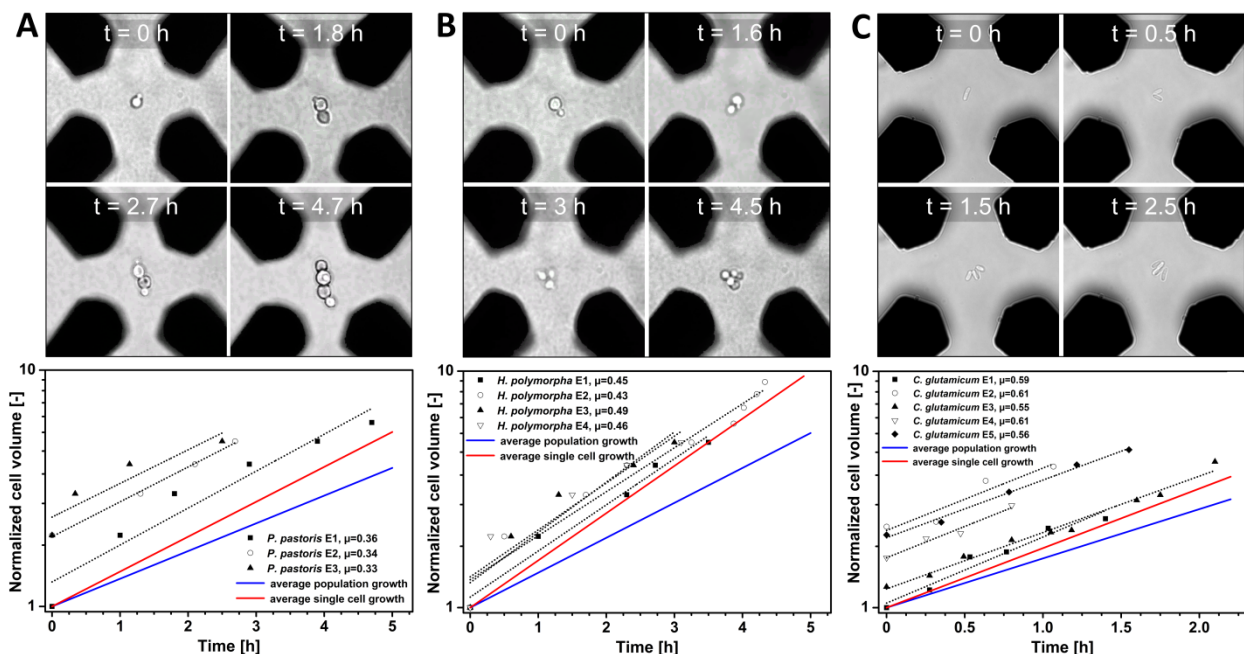


Figure 3: Volume growth analysis of single cells and micropopulations in the Envirostat 2.0 system. (A) The total cell volume of trapped cells, normalized to the smallest observed cells is shown on the y axis in a common logarithmic scale. Red lines represent the average growth rate at single cell level; blue lines show the growth rate at population level.[1]

In order to quantify the effect of inhomogeneous extracellular environments on microbial growth, the kinetic data of single cells in the Envirostat 2.0 were compared with shake flask cultivations and diluted populations grown in

