Biological Single Cell Analysis

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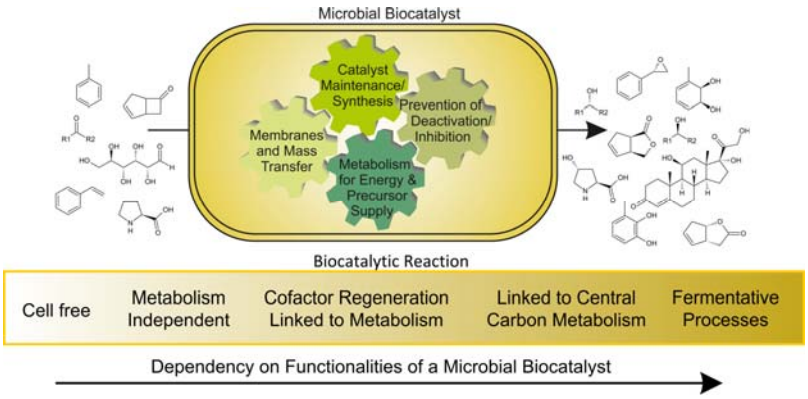
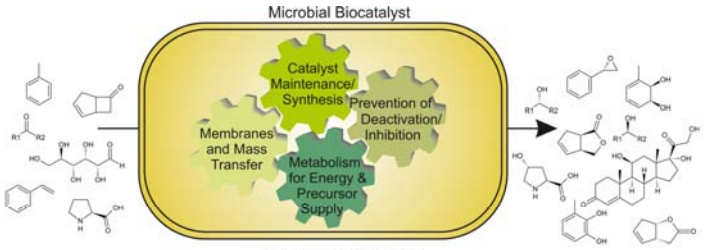
Single Cell Laboratory

Symposium – ITMO Molecular and Structural Bases of living organisms (BMSV)

Functional analysis of a (Bio-)catalyst



Functional analysis of a (Bio-)catalyst



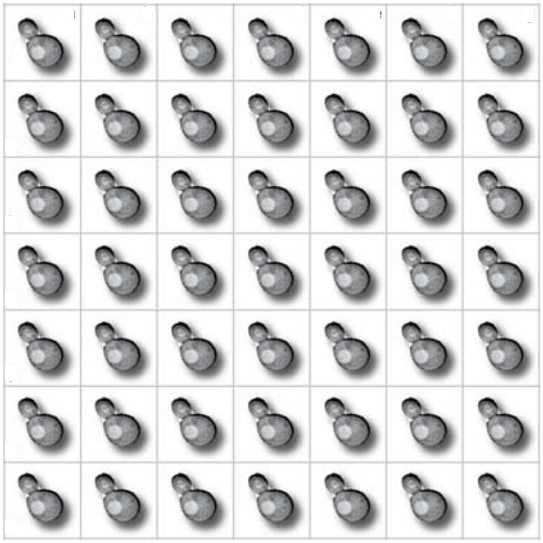
Cell free	Metabolism Independent	Cofactor Regeneration Linked to Metabolism	Linked to Central Carbon Metabolism	Fermentative Processes

Dependency on Functionalities of a Microbial Biocatalyst →

Enzymes	**Whole cells**
Explicit Mechanistic Analysis (e.g. X-Ray Christallography)	Implicit Black-Box Analysis at Population Level (e.g. Kinetics, Half-Life)
• Sequence • Conformation • Metabolic States • Active Site – Location, Reaction Mechanism	• Bulk Measurements **Lack of data about individual cellular activity and functionality → need for single cell analysis**

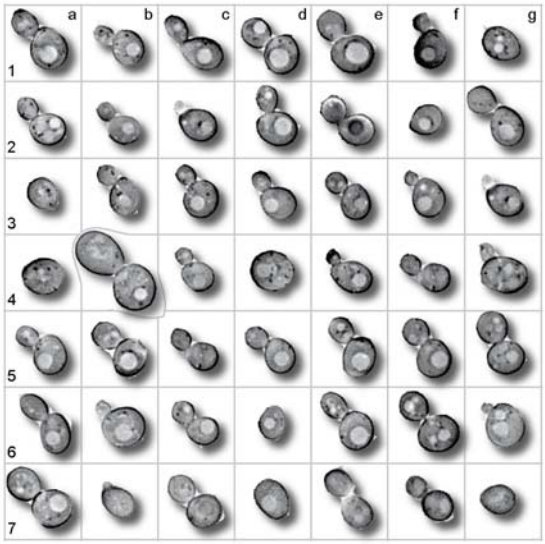
Structure of a microbial population

Text book biology – an isogenic population

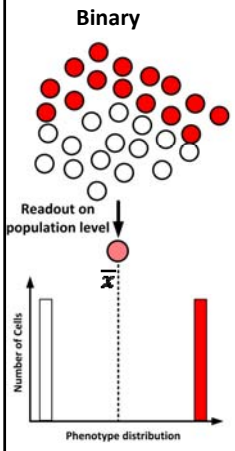


Structure of a microbial population

Reality – an isogenic population BUT different phenotypes



Why single cell analysis?



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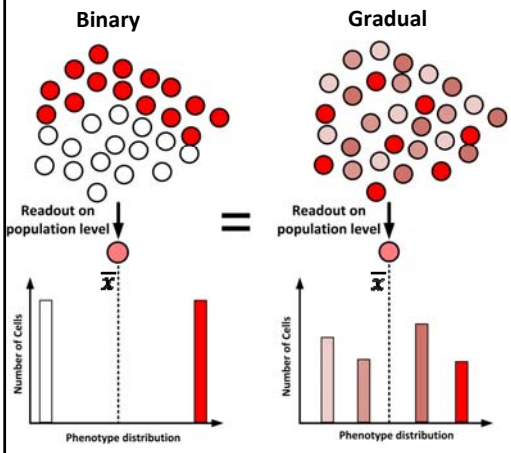

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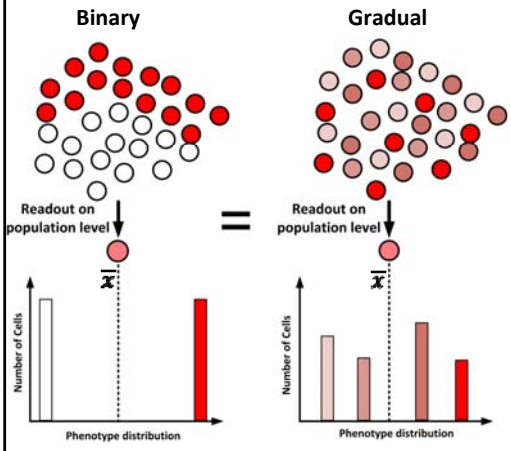
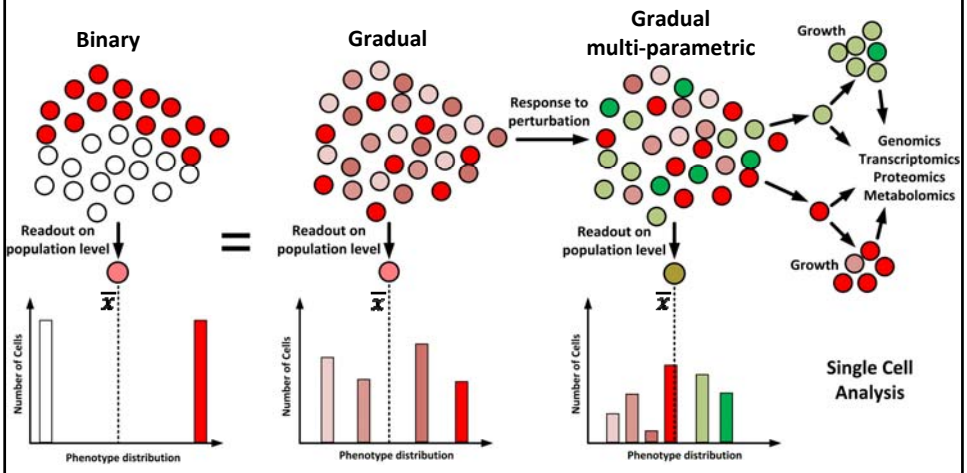


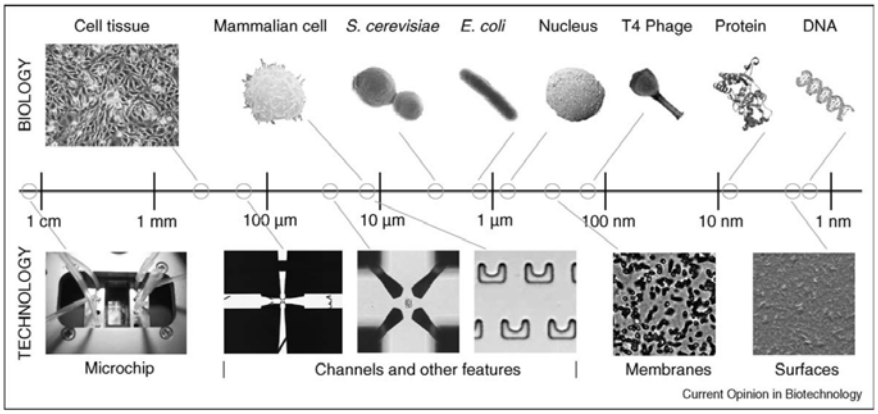
Same readout!

Fakultät Bio- und Chemieingenieurwesen
Andreas Schmid, Paris
May, 24th
technische universität dortmund

Why single cell analysis?

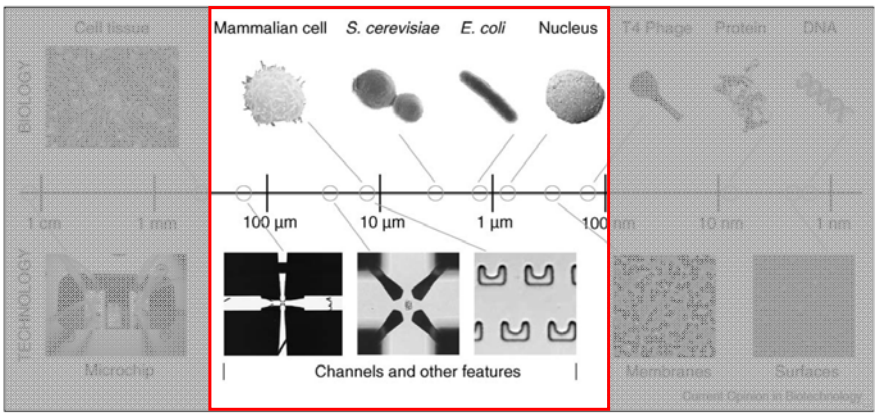


Size comparison of biological targets and microfluidics



Schmid *et al.* , *Curr. Opin. Biotechnol.* (2010)

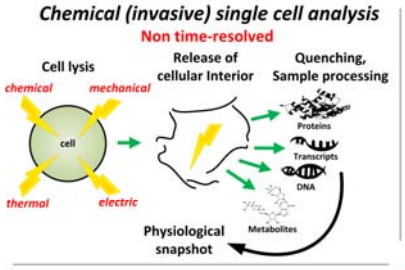
Size comparison of biological targets and microfluidics



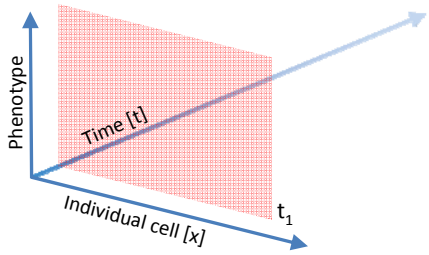
Focus of this talk

Schmid *et al.* , *Curr. Opin. Biotechnol.* (2010)

Concepts for single cell analysis

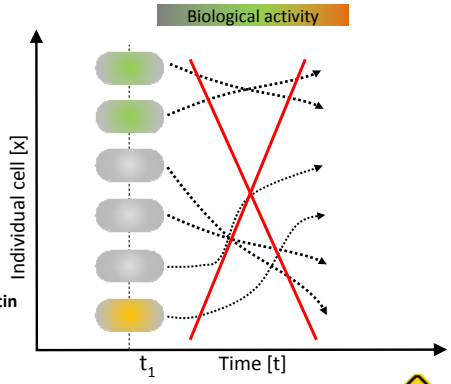


Single cell analysis – a multidimensional system



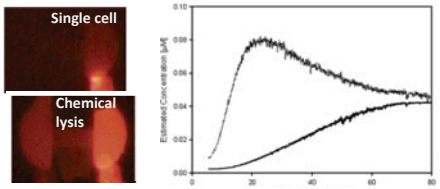
$$Phenotype = f\,(x, t)$$

Chemical single cell analysis -a physiological snapshot



High information content, but dynamic processes of individual cells cannot be resolved!

On-chip single cell lysis and quantification of insoluble actin

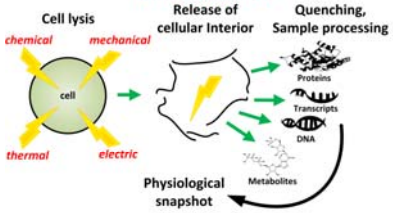


Irimia *et al.* , *Anal Chem.* (2004)

Concepts for single cell analysis

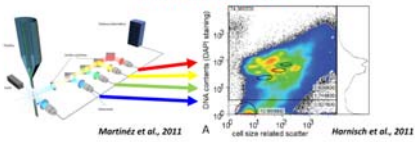
Chemical (invasive) single cell analysis

Non time-resolved



Biological (non-invasive) single cell analysis

Non time-resolved

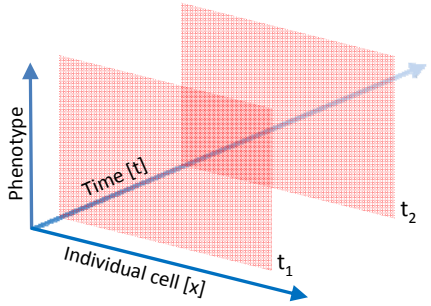


Flow cytomter (FACS)

+ high throughput, versatile

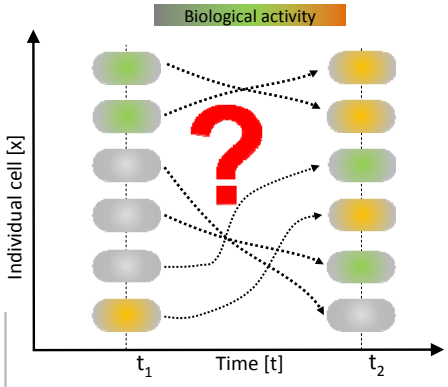
- snapshot analysis, no spatio-temporal resolution

Single cell analysis – a multidimensional system

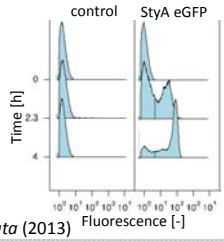


$$Phenotype = f\,(x, t)$$

Flow cytometry – a population snapshot



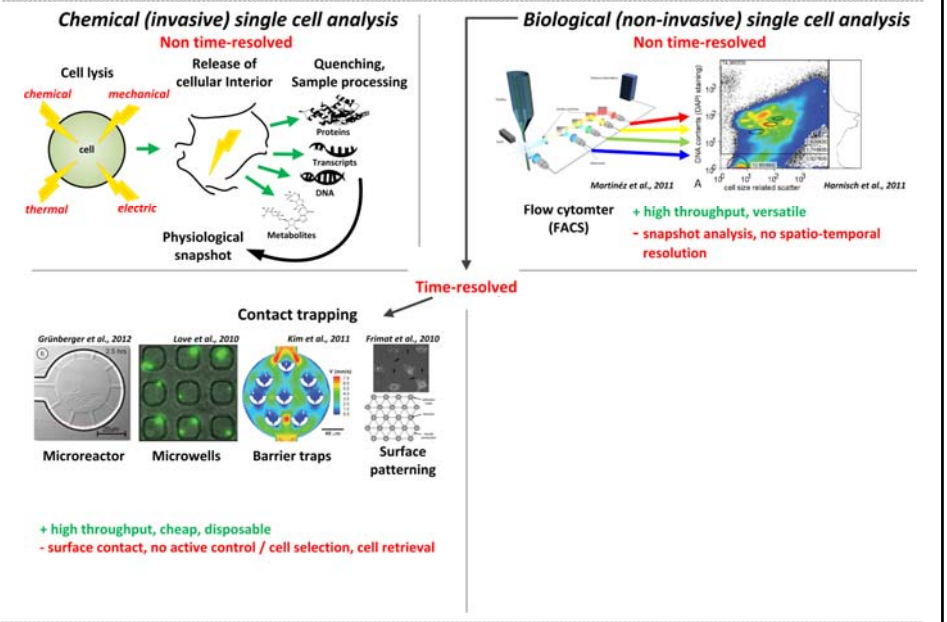
Assessment of gene expression profiles in *Pseudomonas* sp. VLB120 with fluorescent fusion protein



Lindmeyer *et al. , unpublished data* (2013)

Access to population dynamics, but not to fate/development of individual cells!

Concepts for single cell analysis



Concepts for single cell analysis

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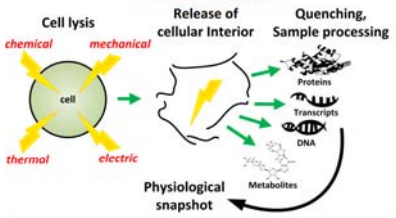
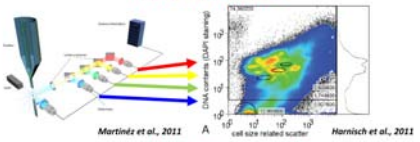


Biological (non-invasive) single cell analysis

Non time-resolved



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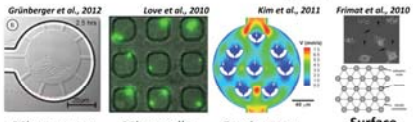
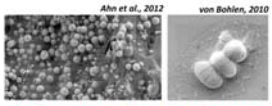
Time-resolved

Contact trapping



Microreactor | Microwells | Barrier traps | Surface patterning

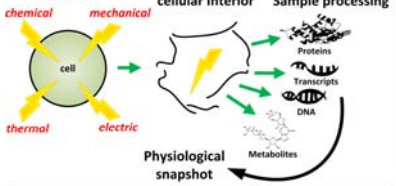


Surface contact-> change in phenotype!
(Biofilm / Activation)

Concepts for single cell analysis

Chemical (invasive) single cell analysis

Non time-resolved



Biological (non-invasive) single cell analysis

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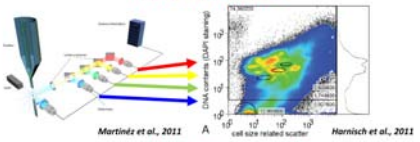

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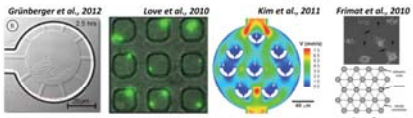
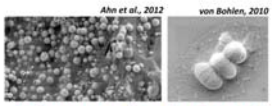


Microreactor | Microwells | Barrier traps | Surface patterning



Surface contact-> change in phenotype!
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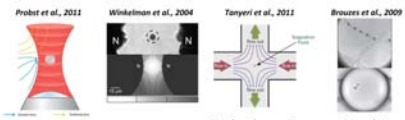
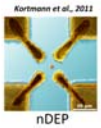
Contactless trapping



Optical tweezers | Acoustic traps | Hydrodynamic focusing | Droplet microfluidics



nDEP

+ cell perfusion, active control, contact-free, versatile, cell retrieval

- resistive heating, complex chip and periphery

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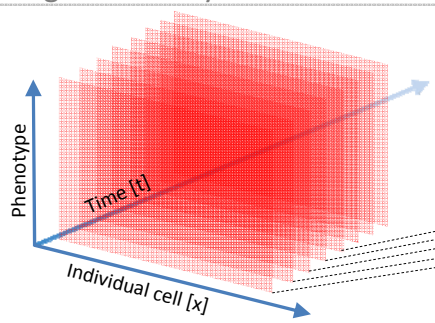
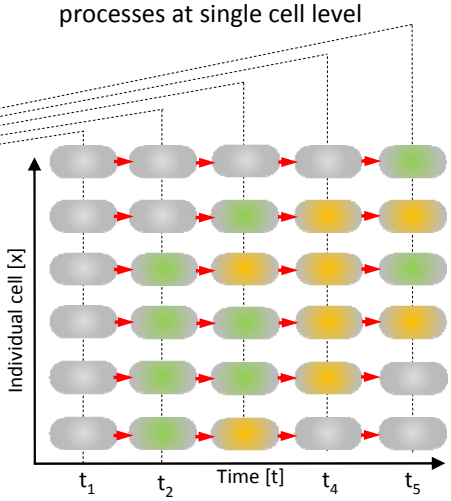
Single cell analysis – a multidimensional system

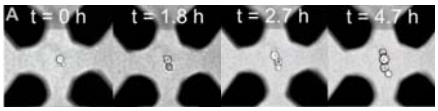


Single cell trapping – allows continuous analysis of dynamic processes at single cell level

$$\textit{Phenotype} = f\,(x, t)$$

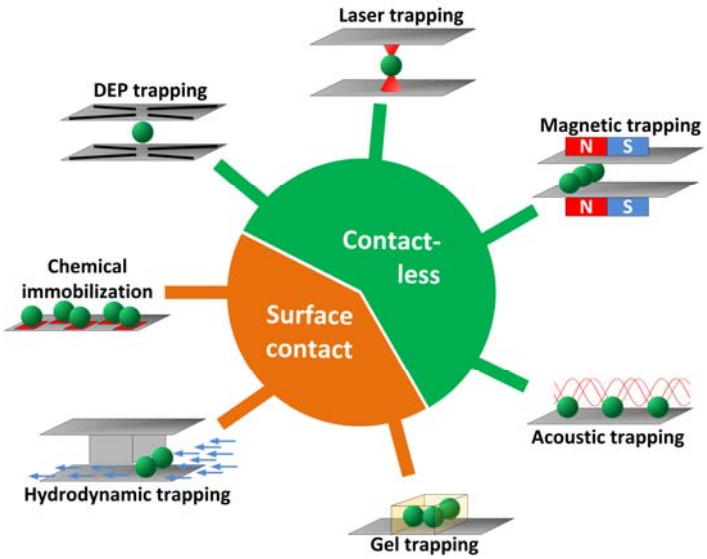


Trapping and cultivation of single *P. pastoris* cells with nDEP traps



Dusny *et al.* , *Appl. Environ. Microbiol.* (2012)

Trapping and cultivation for biological single cell analysis



adapted from Johann *et al.* , *Anal. Bioanal. Chem.* (2006)

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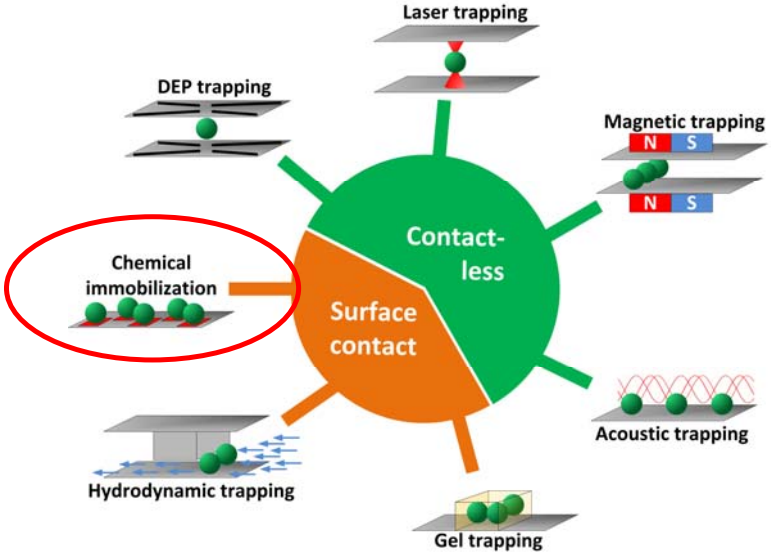
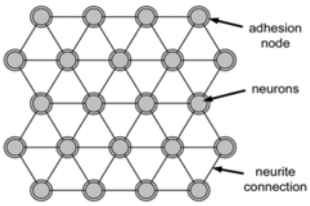


adapted from Johann *et al.* , *Anal. Bioanal. Chem.* (2006)

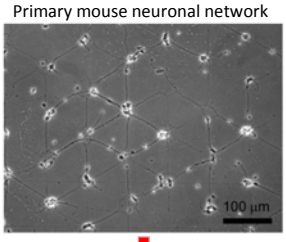
Surface functionalization for biological single cell analysis

An on-chip neuronal network display for sensitive neurotoxicity assessment

Neuronal micro patterning for controlled neurite outgrowth

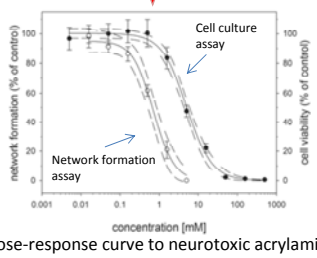


Primary mouse neuronal network



On-chip neuronal network formation assay:

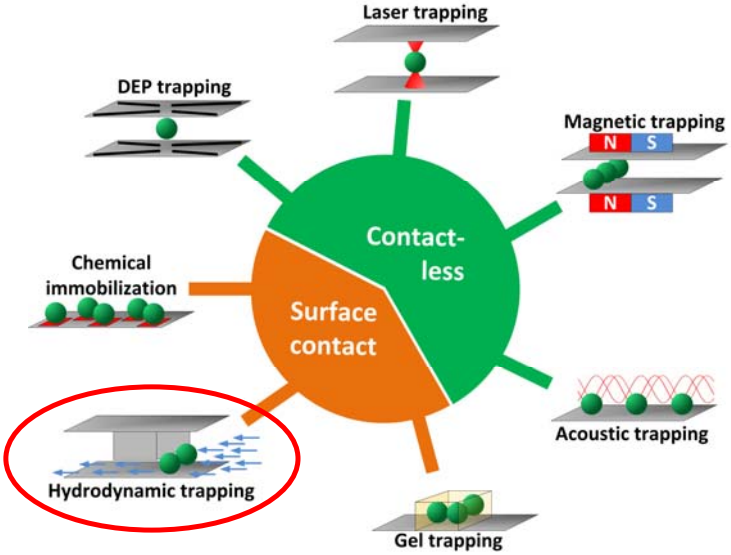
- Rapid and reproducible *in vitro* assay for the assessment of neurotoxic effects
- Neuronal outgrowth and interconnection as a measure for toxicity
- Higher sensitivity than cytotoxicity assays!
- Might replace animal experiments



Dose-response curve to neurotoxic acrylamide

Frimat *et al.* , *Lab. Chip.* (2010)

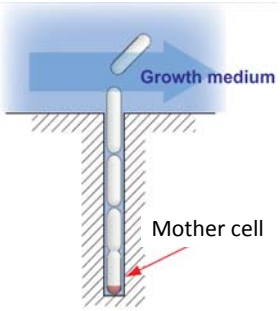
Trapping and cultivation for biological single cell analysis

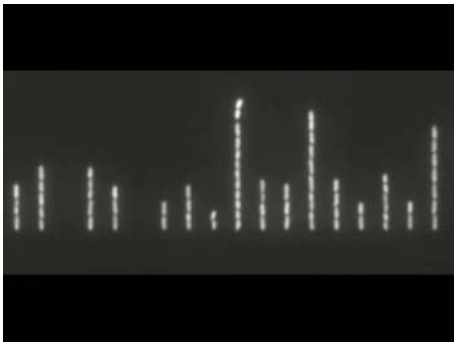


adapted from Johann *et al.* , *Anal. Bioanal. Chem.* (2006)

Hydrodynamic traps for biological single cell analysis

The "mother machine" – Robust growth of *E. coli*

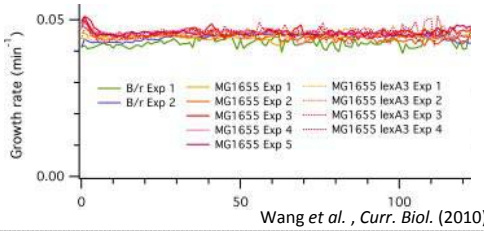




Continuous growth analysis of *E. coli* :

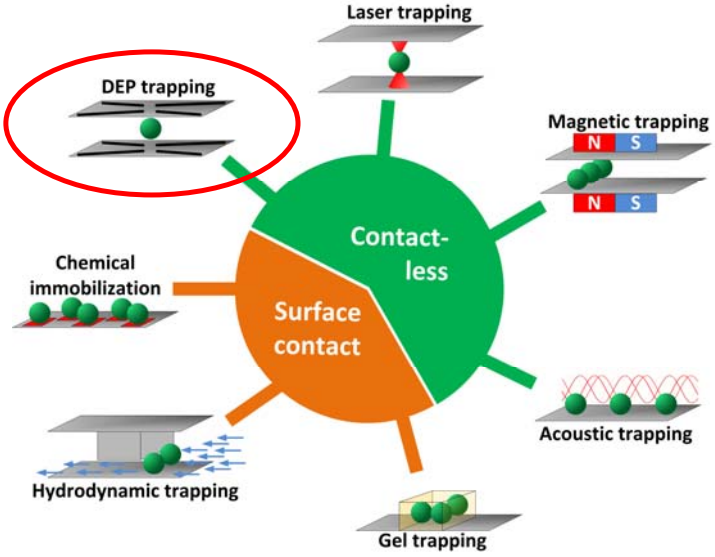
•Mother cells show stable rate of elongation over hundreds of consecutive divisions

•Replicative age of the cells is not connected to growth rate (as believed for decades!)
-> growth rate is homogeneous and robust



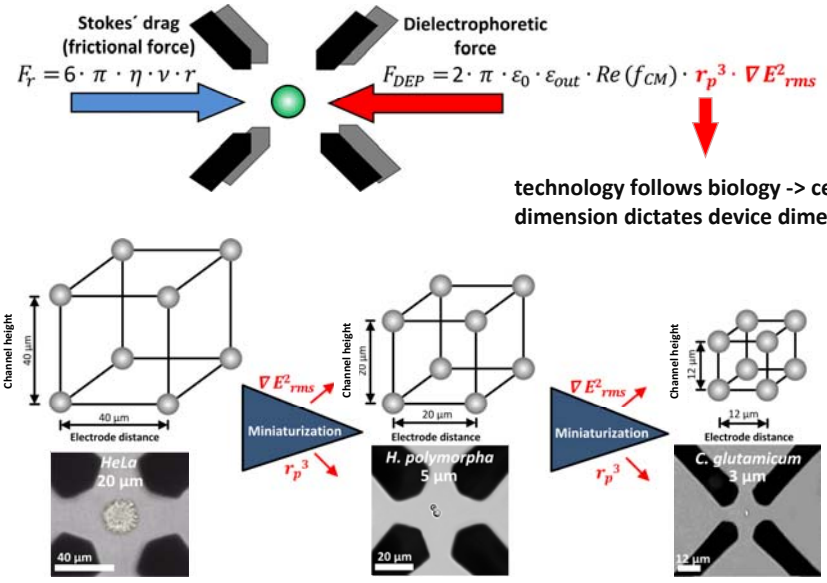
Wang *et al.* , *Curr. Biol.* (2010)

Trapping methods for biological single cell analysis



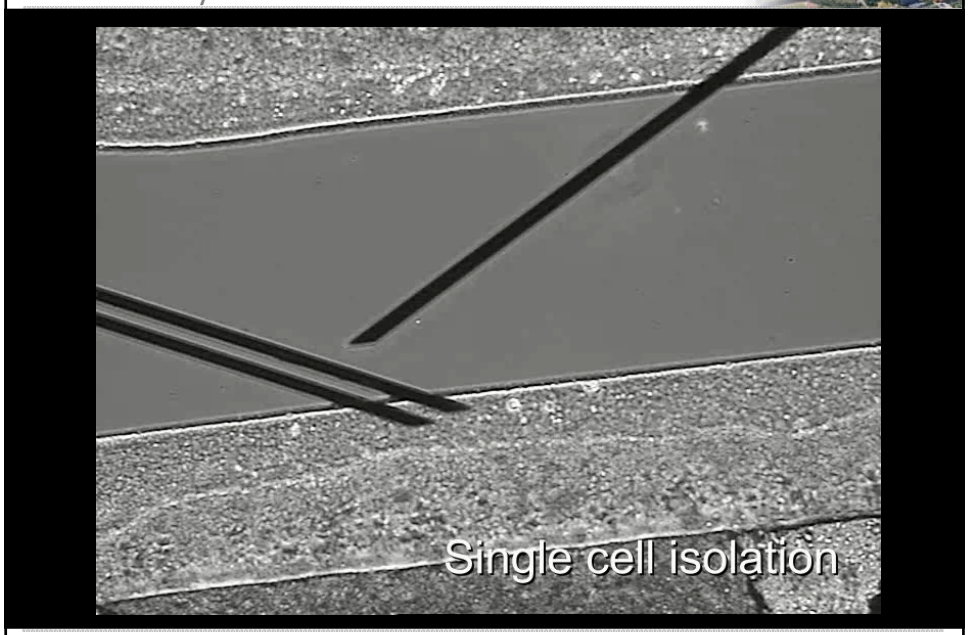
adapted from Johann *et al.* , *Anal. Bioanal. Chem.* (2006)

Negative dielectrophoresis for single cell trapping



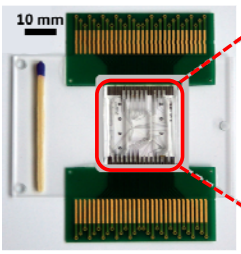
technology follows biology -> cell dimension dictates device dimension

Functionality of the Envirostat

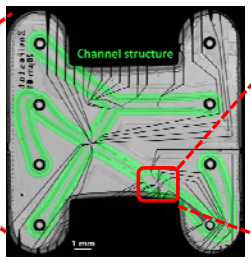


nDEP traps for biological single cell analysis

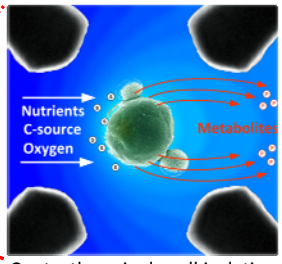
The Envirostat – a new bioreactor concept



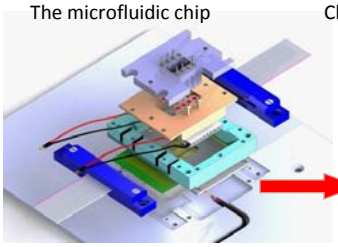
The microfluidic chip



Channel structure



Contactless single cell isolation, trapping and perfusion with nDEP



Temperature control and reliabe world-to-chip interface

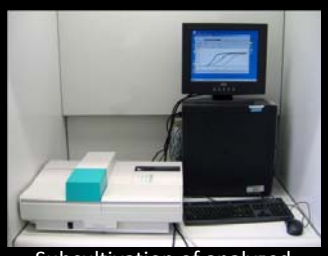
- Continuous supply with substrates and removal of extracellular metabolites -> **C = const.**
- Precise temperature control -> **T = const.**

-> Controllable and defined physico-chemical cellular microenvironment

Kortmann *et al.* , *Lab Chip* (2009)

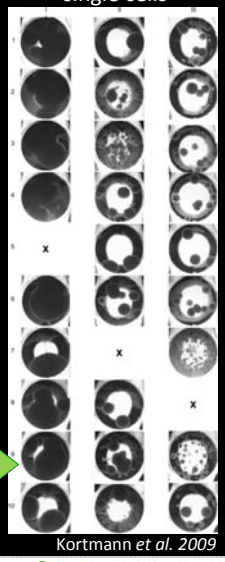
Analysis of subsequent cell generations

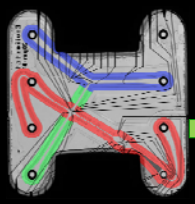
Deposition of cells with automated fraction collector

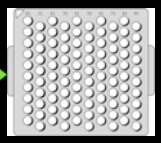


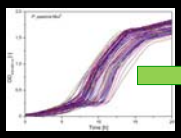
Subcultivation of analyzed single cells

Populations from analyzed single cells



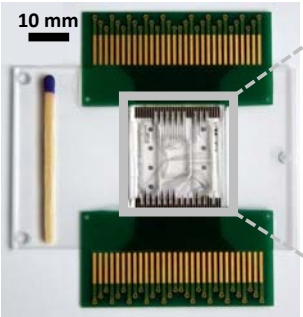




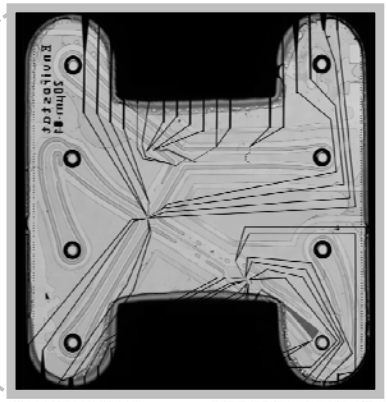


Kortmann *et al. 2009*

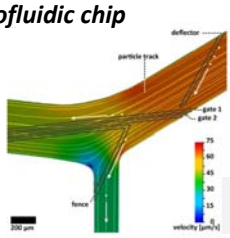
The Envirostat chip



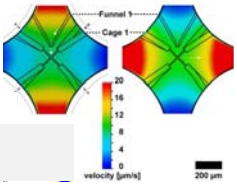
The microfluidic chip



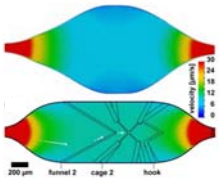
Design of microfluidics with CFD simulations



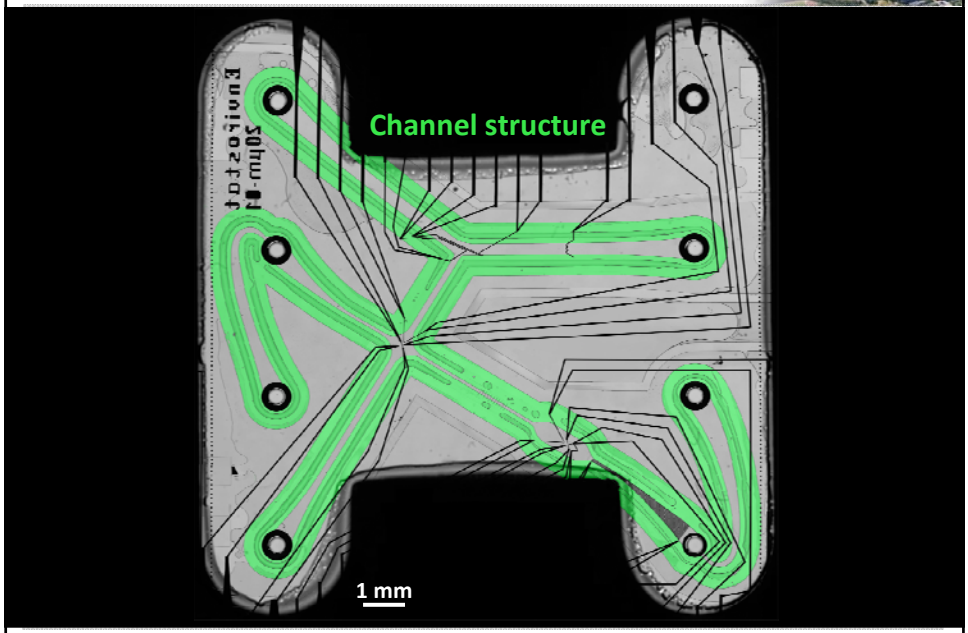
Single cell isolator



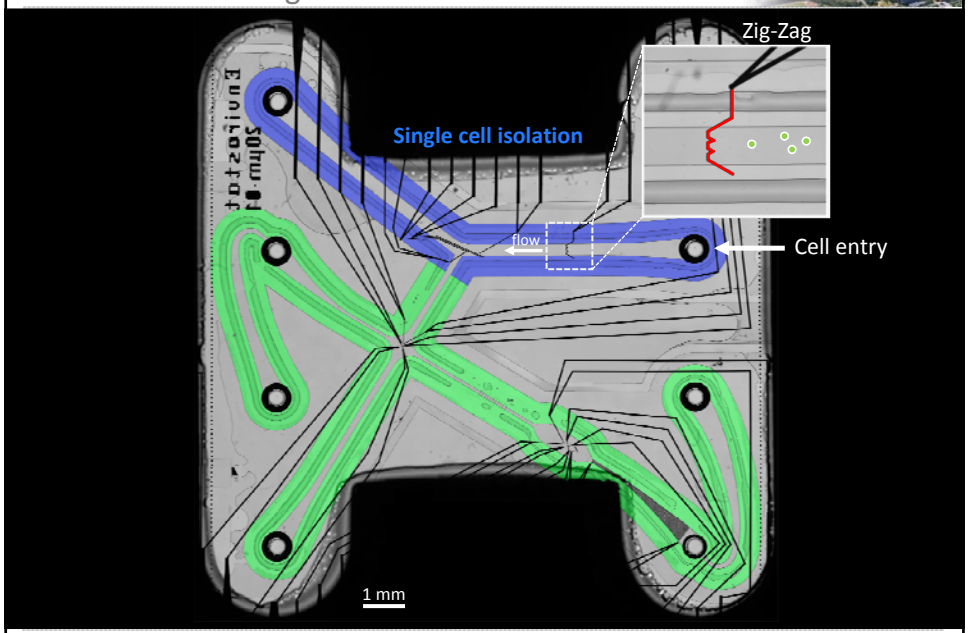
Single cell cultivation microchamber



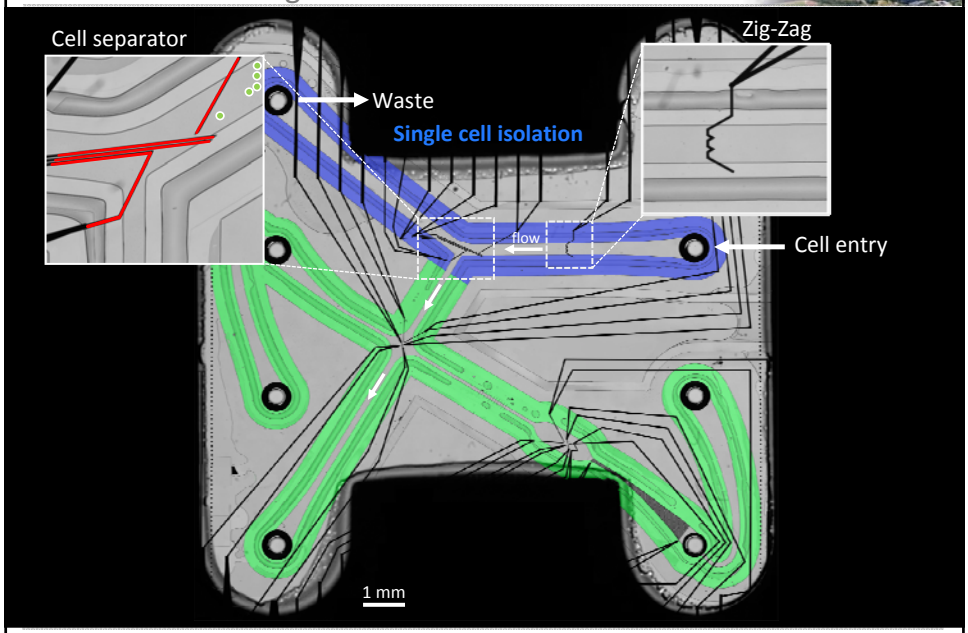
The Envirostat: microfluidic channel structure



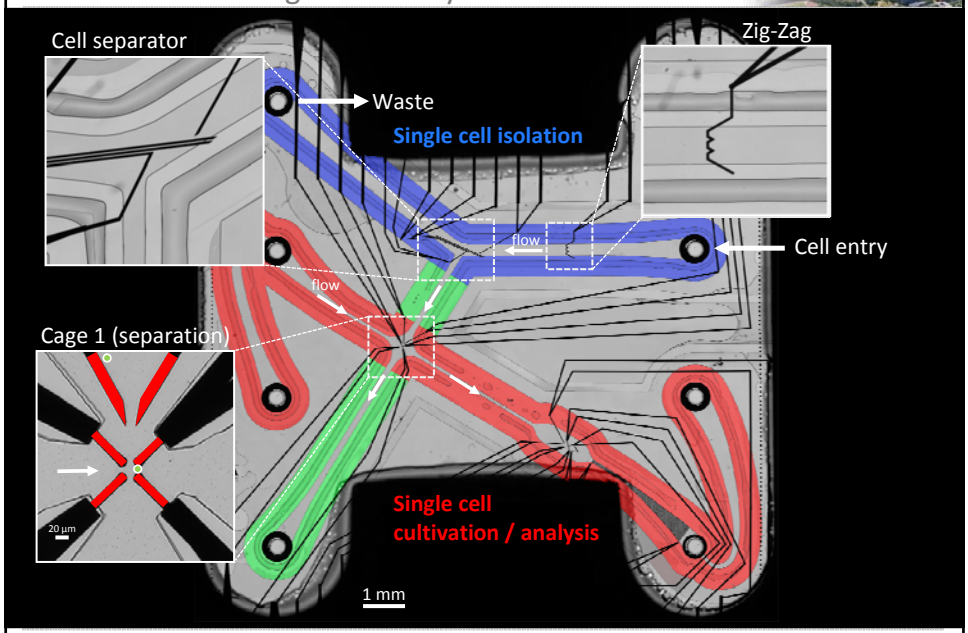
The Envirostat: single cell isolation



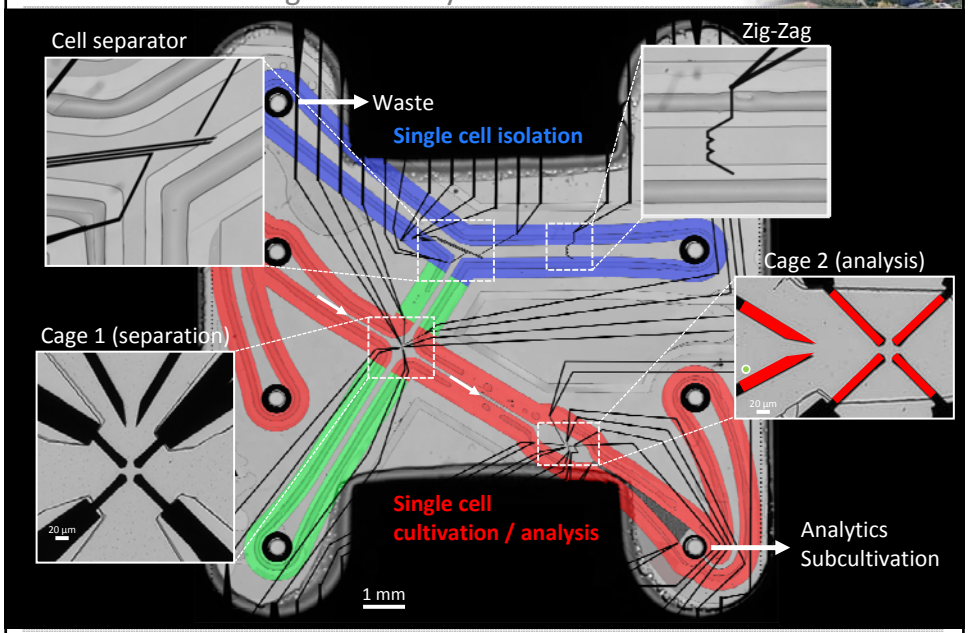
The Envirostat: single cell isolation



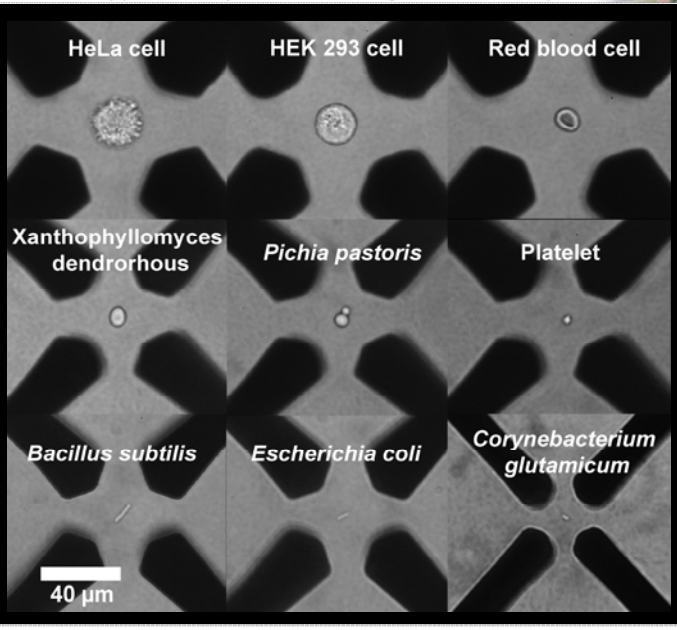
The Envirostat: single cell analysis



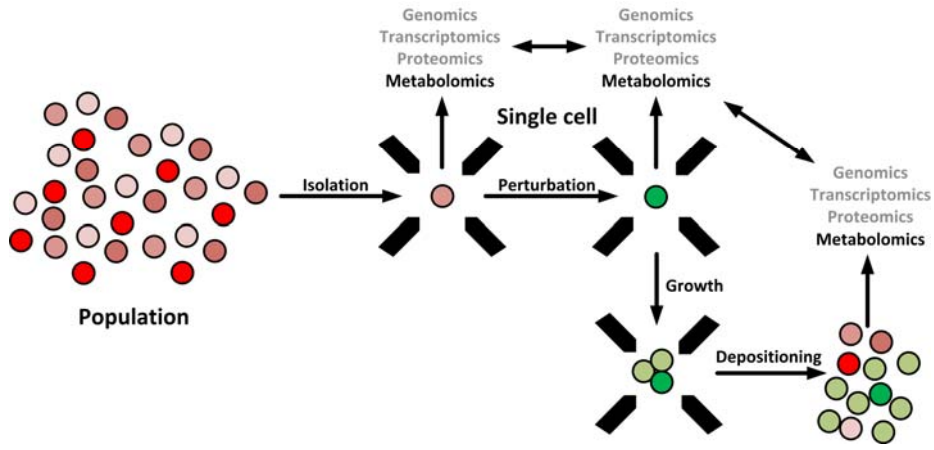
The Envirostat: single cell analysis



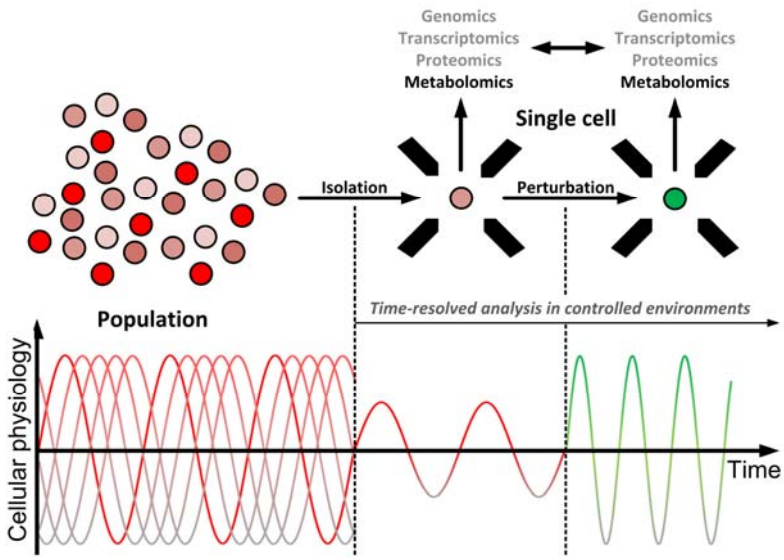
Envirostat: cell type-independent single cell analysis



Single cell analysis



Single cell analysis



„Single cell growth analysis… perhaps one of the simplest and most underexploited assays of cellular heterogeneity.“

Lecault *et al.*, *Curr. Opin. Chem. Biol.* (2012)

Microbial growth at single cell level

„Single cell growth analysis… perhaps one of the simplest and most underexploited assays of cellular heterogeneity.“

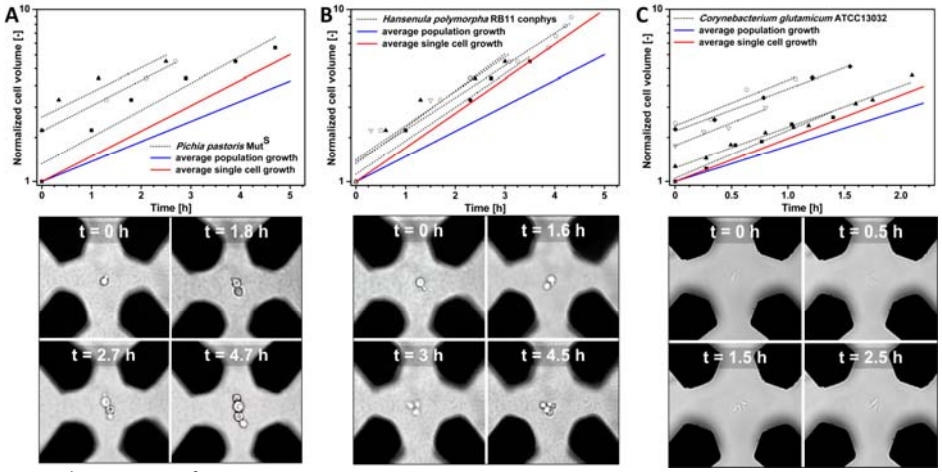
Lecault *et al.*, *Curr. Opin. Chem. Biol.* (2012)

… prime example for single cell analysis

Unambiguous determination at single cell level
-> what you see is what you believe!

Fakultät Bio- und Chemieingenieurwesen
Andreas Schmid, Paris
May, 24th
technische universität dortmund

Single cells grow faster in controlled environments



Pichia pastoris Mut^S
cell size: 5-8 µm

$\mu = 0.34\ h^{-1}$

Hansenula polymorpha RB11
cell size: 3-6 µm

$\mu = 0.46\ h^{-1}$

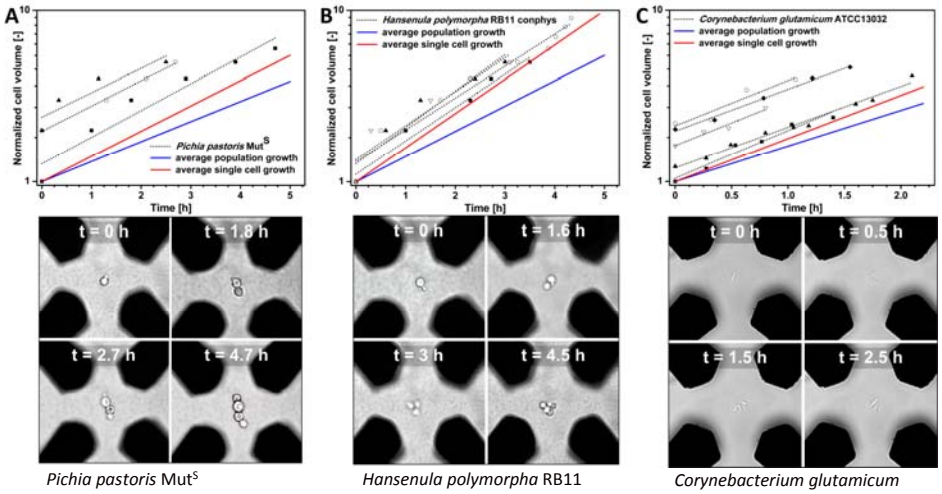
Corynebacterium glutamicum
ATCC 13032
cell size: 3-5 µm

$\mu = 0.58\ h^{-1}$

Cultivation conditions: 30°C, YPD medium for yeast (conductivity = 0.99 S m^{-1}), BHI medium for *C. glutamicum* (conductivity = 1 S m^{-1}), RotX field mode (V_{rms}= 2.5)

Dusny *et al.* , *Appl. Environ. Microbiol.* (2012)

Single cells grow faster in controlled environments



Pichia pastoris Mut^S | *Hansenula polymorpha* RB11 | *Corynebacterium glutamicum*

Extraordinarily fast and steady volume growth in controlled cellular environments!

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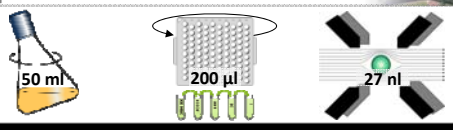
Andreas Schmid, Paris
May, 24th
technische universität dortmund

Shake flask vs. diluted population vs. Envirostat



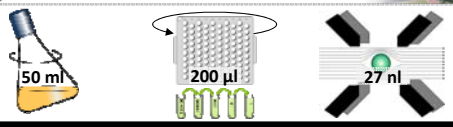
Microorganism	Specific growth rate µ [h⁻¹]		
	Shake flask	MTP	Envirostat
P. pastoris Mut^S	0.23 ± 0.01	0.27± 0.03	0.34 ± 0.01
H. polymorpha RB11 conphys	0.21 ± 0.01	0.34 ± 0.03	0.46 ± 0.02
C. glutamicum ATCC 13032	0.44 + 0.01	0.48 + 0.04	0.58 + 0.02

- Single cells show consistently higher specific growth rates compared to bulk cultivations
- Specific growth rates of single cells exceeded population growth rates by up to 120% !
- Extracellular environment results in steady-state physiology

-> Basal principle is valid for unicellular microbial eukaryotes and prokaryotes

Dusny *et al.* , *Appl. Environ. Microbiol.* (2012)

Shake flask vs. diluted population vs. Envirostat



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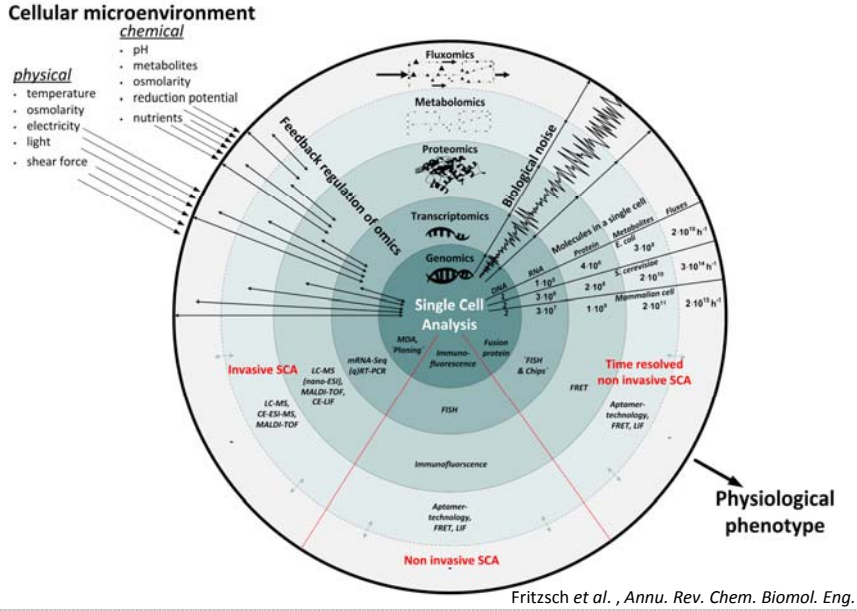
cellular environment is reflected in cellular physiology and can dictate growth rate

Dusny *et al.* , *Appl. Environ. Microbiol.* (2012)

Interrelation: extracellular environment and physiology



Fritzsch *et al.* , *Annu. Rev. Chem. Biomol. Eng.* (2012)

Content of a single cell

Average cell content of a mammalian, a *S. cerevisiae*, and an *E. coli* cell. Cells are sized to scale

	Mammalian cell	*S. cerevisiae*	*E. coli*	
Diameter	10	5	2.5×0.7	µm
Volume	500	65	1	$fL = \mu m^3$
Cell wet weight	5×10^5	7×10^4	1×10^3	fg
Cell dry weight	1×10^5	2×10^4	3×10^2	fg
Carbohydrates	1×10^4	8×10^3	2×10^1	fg
DNA	6×10^3	2×10^1	5	fg
DNA	2	1–2[a]	1[b]	molecules
RNA	2×10^4	1×10^3	6×10^1	fg
RNA	3×10^7	3×10^6	1×10^5	molecules
Lipids	1×10^4	1×10^3	3×10^1	fg
Lipids	1×10^{10}	1×10^9	2×10^6	molecules
Protein (total)	5×10^4	9×10^3	2×10^2	fg
Protein (total)	1×10^9	2×10^8	4×10^6	molecules
Different proteins	1×10^4	4×10^3	2×10^3	types
Protein (average)	5	2	9×10^2	fg
Protein (average)	1×10^5	5×10^4	2×10^3	molecules

Schmid *et al.* , *Curr. Opin. Biotechnol.* (2010)

Content of a single cell

Average cell content of a mammalian, a *S. cerevisiae*, and an *E. coli* cell. Cells are sized to scale

	Mammalian cell	*S. cerevisiae*	*E. coli*	
Diameter	10	5	2.5×0.7	µm
Volume	500	65	1	fL = µm^3
Cell wet weight	5×10^5	7×10^4	1×10^3	fg
Cell dry weight	1×10^5	2×10^4	3×10^2	fg
Carbohydrates	1×10^4	8×10^3	2×10^1	fg
DNA	6×10^3	2×10^1	5	fg
DNA	2	1–2[a]	1[b]	molecules
RNA	2×10^4	1×10^3	6×10^1	fg
RNA	3×10^7	3×10^6	1×10^5	molecules
Lipids	1×10^4	1×10^3	3×10^1	fg
Lipids	1×10^{10}	1×10^9	2×10^6	molecules
Protein (total)	5×10^4	9×10^3	2×10^2	fg
Protein (total)	1×10^9	2×10^8	4×10^6	molecules
Different proteins	1×10^4	4×10^3	2×10^3	types
Protein (average)	5	2	9×10^2	fg
Protein (average)	1×10^5	5×10^4	2×10^3	molecules

Low analyte amounts of single cells pose a considerable analytical challenge!

Schmid *et al.* , *Curr. Opin. Biotechnol.* (2010)

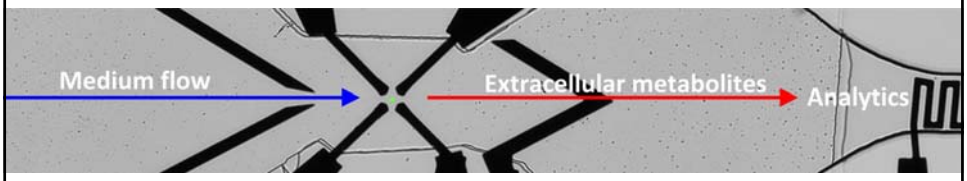
Single cell ethanol production: Can we monitor it?

Chip paramaters

Medium flow: 1 µL/h medium flow (25 µm/s)
Temperature: 30 °C

One cell 

Productivity: 20 pmol/cell/h EtOH



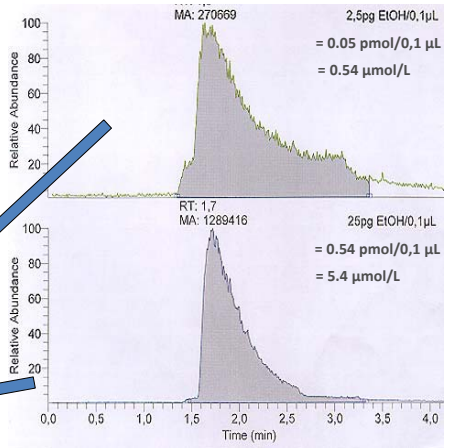
Single cell ethanol production: Can we monitor it?

Ethanol productivity
Population 20 mmol g^{-1} h^{-1}
Single Cell 20 pmol $cell^{-1}$ h^{-1}

Time [min]	Amount [pmol]
1	0.33
10	3.30
30	9.90
60	19.80

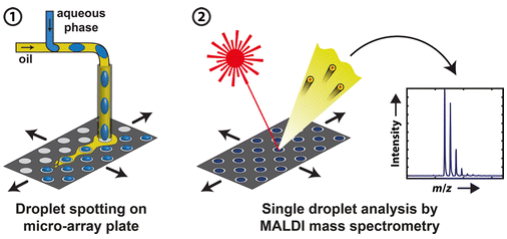
	Concentration [µmol/L]			
Vol. [µL]	0.01	0.1	1	10
1 min	33.000	3.300	0.330	0.033
10 min	330.000	33.000	3.300	0.330
30 min	990.000	99.000	9.900	0.990
60 min	1980.000	198.000	19.800	1.980

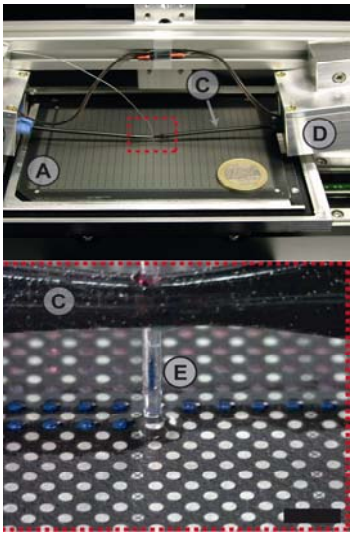
EtOH detection by GC-MS



Interfacing microfluidics and mass spectrometry

Automated spotting of microdroplets an nano-MALDI target





- label-free content analysis of single microdroplets (few nanoliters in volume)
- MS grants access to structural information about droplet content
- promising approach for single cell analysis

Küster *et al.* , *Anal. Chem.* (2013)

Interfacing microfluidics with mass spectrometry

Summary

- Numerous devices and analytical approaches have been developed to adress specific aspects and questions at a single cell level
- Microfluidic single cell trapping and analysis with total microenvironmental control has been demonstrated
- Analytical technologies for biological SCA at genomic, transcriptomic, proteomic, and metabolomic level with single cell resolution are available
- Mass spectrometry devices, recently reached the single cell threshold for sensitivity and specificity and will allow label-free structural information about single cell proteome and metabolome

-> Single cell analysis is broadly applicable and a key technology for systematic elucidation of the features and functions of life!

Future challenges for single cell analysis

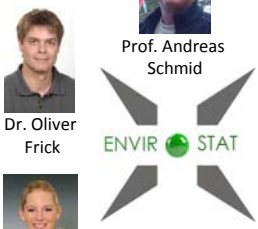
Devices for single cell analysis require:

- Integration of currently available LOC (Lab-on-a-chip) technologies for cell sorting, chemical and biological single cell analysis in defined microenvironments in one platform
- On-chip microfluidic sample processing and direct and loss-free coupling of single cell chips/µfluidics to analytical instruments
- Automation of LOC devices approaches for high throughput single cell analysis

Acknowledgement

The Single Cell Lab


Prof. Andreas Schmid



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Frederik Fritzsch


Verena Oehling

technische universität dortmund

EUROPÄISCHE UNION
Europäischer Fonds für Regionale Entwicklung

DSM

BE-Basic

SIM Gesellschaft für Silizium-Mikrosysteme mbH

S-BLOC
International Leibniz Graduate School

Ministry of Innovation, Science, Research and Technology of North-Rhine Westphalia.

CLIB Graduate Cluster
Industrial Biotechnology

Thank you for your attention!