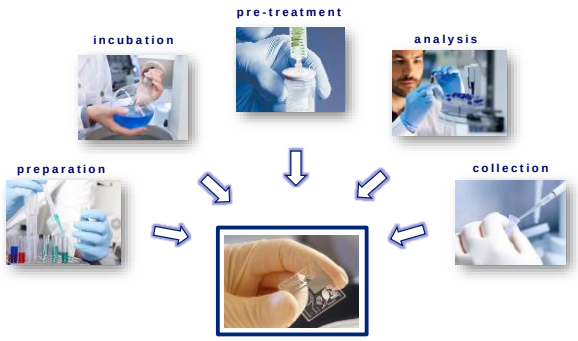
LOSCHMIDT LABORATORIES

Microfluidics – „Lab on a Chip"

Outline

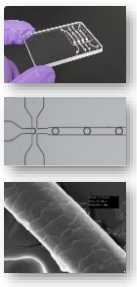
- introduction to microfluidics
- physics of micro-scale
- lab on a chip applications
  - life and medical science
  - **protein and metabolic engineering**
- design and fabrication
- sensing and detection

Lab on a Chip Concept



Microfluidics

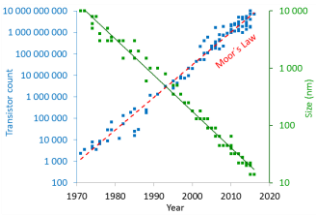
- „behavior, control and manipulation of fluids geometrically constrained to a small dimensions"
  - dimensions (1´-100´ µm)
  - volumes (nL, pL, fL)
  - unrivalled precision of control
  - (ultra)high analytical throughput
  - reduced sample and power consumption
  - facile process integration and automation



Nature 507, 181 (2014)

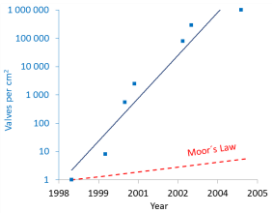
Revolution in Electronics

	Size (nm)	Price (USD)
Vacuum tube	100	10



Revolution in Science?

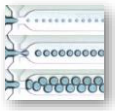
	Volume (µL)	Throughput (assays/day)
Test tube	1 000	10



Nature Biotechnol. 21, 1179 (2003)

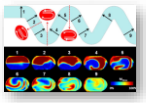
Concepts in microfluidics

- **continuous-flow microfluidics**
  manipulation of continuous liquid flow through micro-fabricated channels
- **droplet-based microfluidics**
  manipulating discrete volumes of fluids in immiscible phases
- **digital microfluidics**
  droplets manipulated on a substrate using electro-wetting



Novel Physics of Micro-Scale

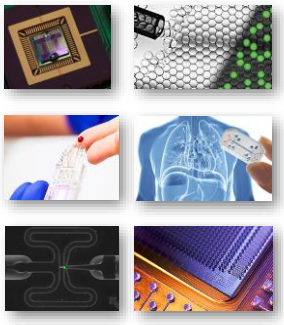
- viscosity, surface tension and capillary forces dominate
  - **lack of turbulent phenomena**
    + nontrivial chemical gradients to study chemotaxis
  - **absence of density-driven convection**
    + free interface diffusion, efficient protein crystallization kinetics
  - **strong shearing forces**
    + fast mixing kinetics of protein folding and/or catalysis



Nature Biotechnol. 20, 826 (2002) *Appl. Phys. Lett. 83, 4664 (2003)* *PNAS 99, 16531 (2002)*

Lab on a Chip applications

- analytics and chemistry
- PCR and sequencing
- point of care diagnostics
- pharmacology
- clinical studies
- single cell biology
- high throughput biology



Polymerase chain reaction

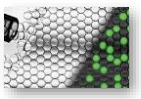
- **classical PCR**
  - slow heating/cooling cycles
  - PCR tubes (strips), 96-well MTP
  - volume 50 to 500 µL

Kary Mullis

Nobel Prize in 1993

Digital polymerase chain reaction

- **digital PCR**
  - 1 nanoliter droplets
  - 20 000 droplets per run

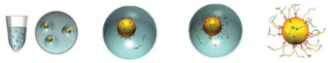


Next-generation sequencing

- **parallelization** of **single molecule** pyrosequencing
- **454 Pyrosequencing** (Roche)

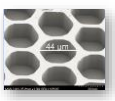
  water in oil droplets 1 picoliter (10^{-12} liters)

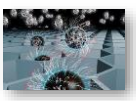
  1 mil. reads/run, 10 USD/Mbase

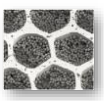


Frederick Sanger

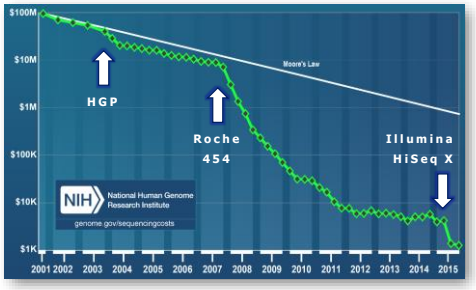
Nobel Prize in 1980







Revolution in Science?

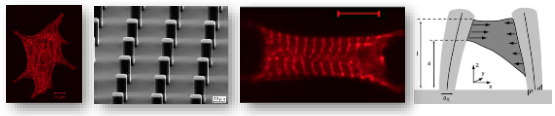


- 2003: 13 years, 3 billion USD
- 2016: days, < 1,000 USD

Organs on chip

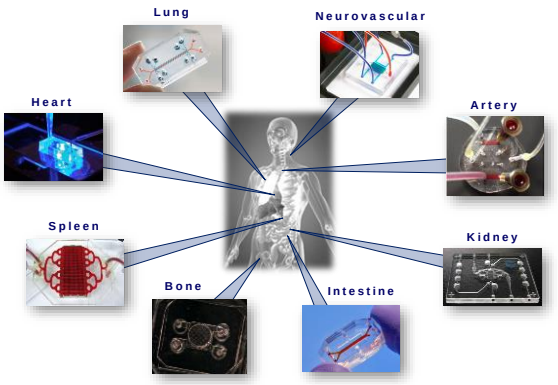
- 3D chips **mimicking human's physiological responses** (e.g., pathological, pharmacokinetic, toxicological)
- realistic *in vitro* model **closer to *in vivo* cell environment** (e.g., mechanical strain, patterning, fluid shear stresses)
- **can replace** expensive and controversial **animal testing**

flat surface **micropillar**

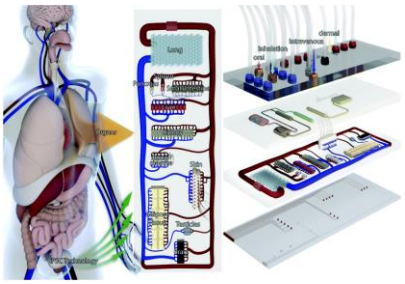


Nature 471, 661–665 (2011) *Biophysical Journal 94(5) 1854–1866*

Organs on chip

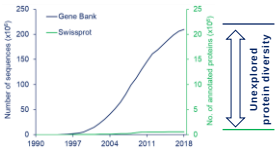


Human on chip concept

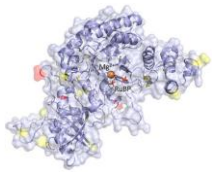


- correct limitations of organs isolation
- whole body biomimetic devices

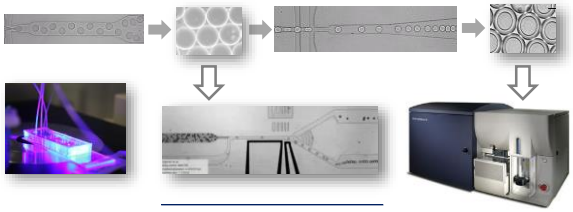
Sequence diversity



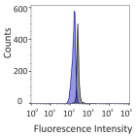
No.	Coverage (95%)
1	94
2	3 066
3	98 163
4	3 141 251
5	100 520 093



(Ultra)High-throughput screening

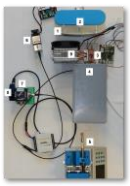


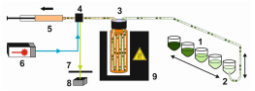
	Robotic	µFluidic
Reaction volume	100 µL	5 pL
Reactions / day	50 000	$1 \cdot 10^8$
Total time	5 years	3 days
Total volume	5 000 L	150 mL
No. of plates / devices	250 000	2.0
No. of tips	28 000 000	10

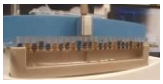


Lab Chip 2009, 9: 1850 *Anal. Chem. 2014, 86: 2526*

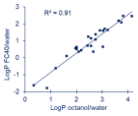
Enzyme specificity profiling

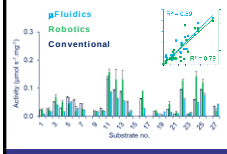


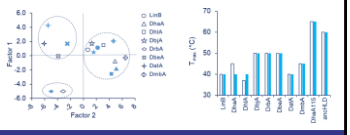




	Conventional	Robotic	µFluidic
Reaction volume (mL)	10	1	0.00015
Total enzyme (mg)	540	54	0.5
Total time (days)	100	30	5

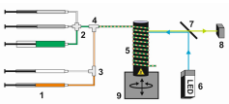




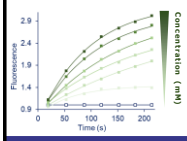


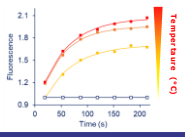
Anal. Chem. 2013, 85: 4761 ***Michal Vasina, CeCe Junior, Wednesday 10:15***

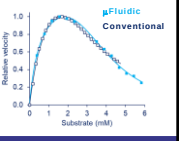
Steady-state kinetics



	Conventional	µFluidic
Reaction volume (mL)	2	0.00010
Total enzyme (mg)	1	0.01
Throughput per hour	5	10 000

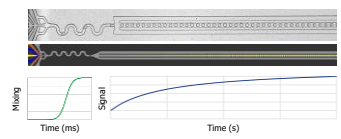


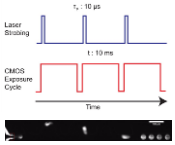


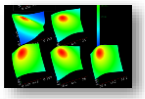


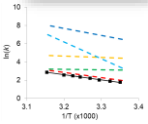
Small 2015, 11: 4009

Mechanism and thermodynamics

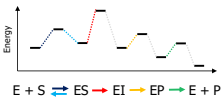




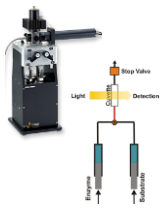




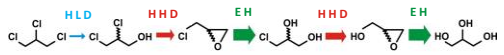
	Stopped-flow	µFluidic
Dead time	0.3 ms	0.7 ms
Reaction volume	100 µL	10 pL
Temp. equilibration	10 min	50 ms
Signal integration	0.5 ms	no limit



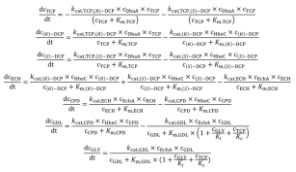
E + S ⇄ ES → EI → EP → E + P

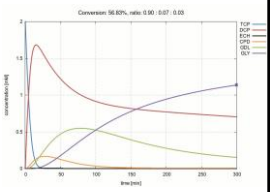


Multienzyme Systems

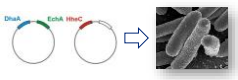


Rhodococcus *Agrobacterium*





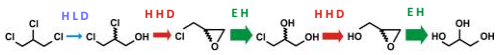
Escherichia

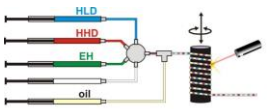


ChemBioChem 15: 1891 (2014)

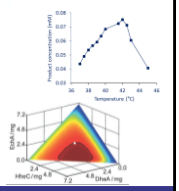
ACS Synth. Biol. 3: 172 (2014)

Multienzyme systems





robot scientist

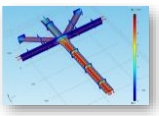


- 1 nL droplet volume
- 10 000 assays/hour

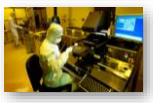
Design and fabrication

- **soft lithography** originates from semiconductor industry

DESIGN / MODELING



MASK / MOLD



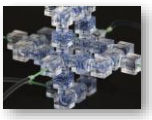
CASTING / BONDING

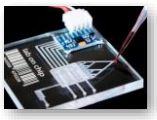
Design and fabrication

- **direct fabrication methods**

3D PRINTING | LASER CUTTING | CNC μ-MILLING



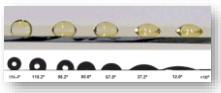


Design and fabrication

- **materials**
  - inert and transparent
  - PDMS - poly(dimethyl siloxane)
  - PMMA - poly(methyl methacrylate)
  - fused silica, quartz and glass
- **surface modification**
  - plasma treatment
  - silanization
  - sol–gel coating

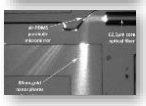


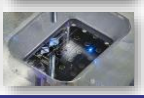





Sensing and detection

- processing of **small reagent volumes**
- **analytical timescale** and performance
- **on chip detection**
  - fluorescence (LSM, FCS, FLIM)
  - UV/VIS absorbance
  - IR spectroscopy
  - Raman scattering
  - (chemo/electro) luminescence
  - thermal conductivity
  - RI variation
- **off chip detection**
  - GC, HPLC, MS
  - NMR, X-ray




Commercial Solutions

- customized design and fabrication



- entire technologies


Nature Meth. 10, 1003 (2013)

Nature 499, 505 (2013)

Conclusions

- reduced sample/reagent/power consumption
- superior performance and novel physics
- applications in life and medical sciences
- in-house as well as commercial technologies

microfluidics revolutionize science

Reading

- Yum, K., 2014: **Physiologically relevant organs on chips**. *Biotechnol. J. 2014, 9, 16–27*
- *2. Key elements of microenvironments* (**page 18-22**)

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Review

Physiologically relevant organs on chips

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