LOSCHMIDT LABORATORIES

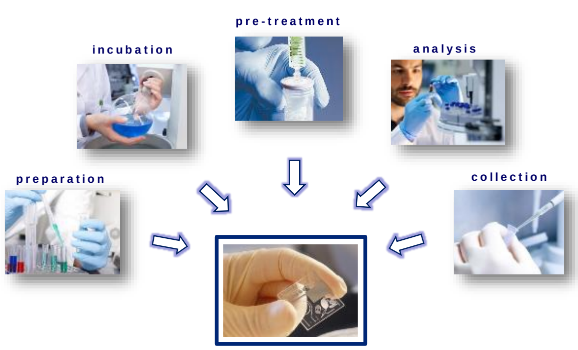
7. Microfluidics – „Lab on a Chip“

Bi7430 Molecular Biotechnology

Outline

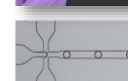
- introduction to microfluidics
- physics of micro-scale
- lab on a chip applications
  - life and medical science
  - **protein and metabolic engineering**
- design and fabrication
- sensing and detection

Lab on a Chip Concept



Microfluidics

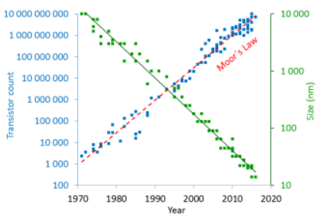
- „behavior, control and manipulation of fluids geometrically constrained to a small dimensions“
  - dimensions (1´-100´ µm)
  - volumes (nL, pL, fL)
  - unrivalled precision of control
  - (ultra)high analytical throughput
  - reduced sample and power consumption
  - facile process integration and automation



Nature 507, 181 (2014)

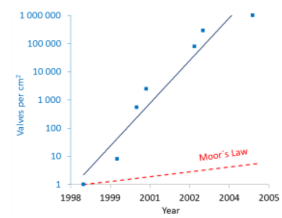
Revolution in Electronics

	Size (nm)	Price (USD)
Vacuum tube	100	10
Trasistors	10	1
Microchip	0.000 010	0.000 000 100



Revolution in Science?

	Volume (μL)	Throughput (assays/day)
Test tube	1 000	10
Microtiter plate	100	1 000
Microfluidic chip	0.000 001	1 000 000



Nature Biotechnol. 21, 1179 (2003)

Concepts in microfluidics

- **continuous-flow microfluidics**

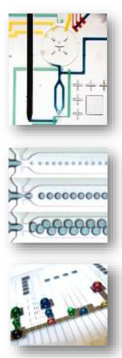
  manipulation of continuous liquid flow through micro-fabricated channels

- **droplet-based microfluidics**

  manipulating discrete volumes of fluids in immiscible phases

- **digital microfluidics**

  droplets manipulated on a substrate using electro-wetting



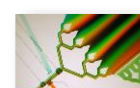
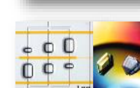
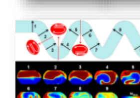
Novel Physics of Micro-Scale

- 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**

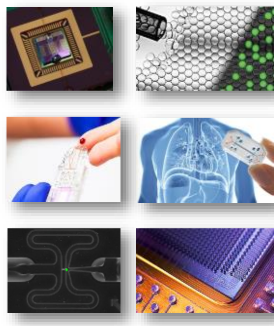
    + fast mixing kinetics of protein folding and/or catalysis

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
- biochemistry



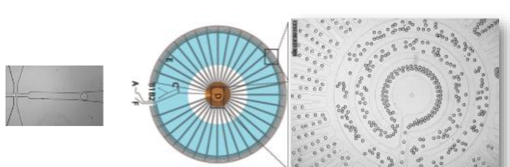
Polymerase chain reaction

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

Kary Mullis
Nobel Prize in 1993

Polymerase chain reaction

- **PCR in microfluidic droplets**
  - 500 droplets per second
  - volume 50 to 100 pL
  - 10 to 20 s per heating/cooling cycle



Schaerli et al. 2009. Anal. Chem. 2009, 81, 302–306

Digital polymerase chain reaction

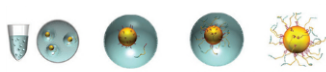
- **digital PCR**
  - 1 nanoliter droplets
  - 20 000 droplets per run

Next-generation sequencing

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

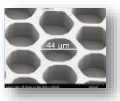
  water in oil droplets 1 picoliter (10^{-12} liters)

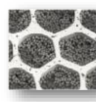
  1 mil. reads/run, 10 USD/Mbase



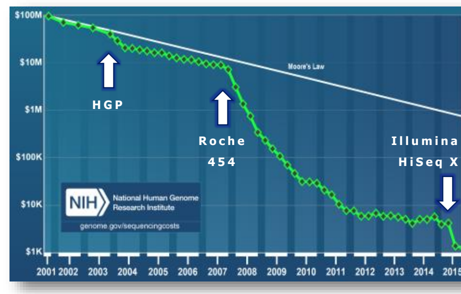
Frederick Sanger

Nobel Prize in 1980





Revolution in Science?



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

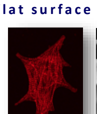
Organs on chip

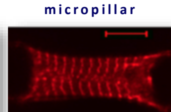
- 3D chips **mimicking human's physiological responses**

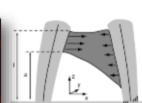
  (e.g., pathological, pharmacokinetic, toxicological)
- realistic *in vitro* model **closer to *in vivo* cell environment**

  (e.g., mechanical strain, patterning, fluid shear stresses)
- **can replace** expensive and controversial **animal testing**

flat surface **micropillar**

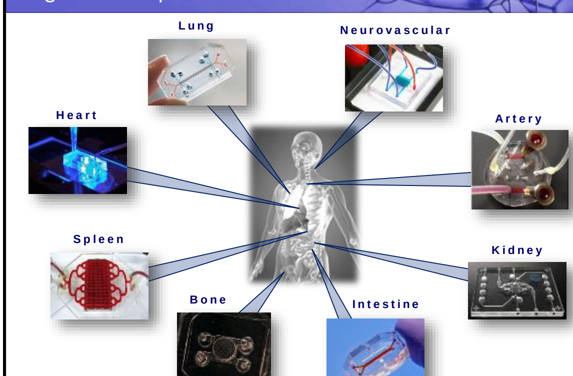




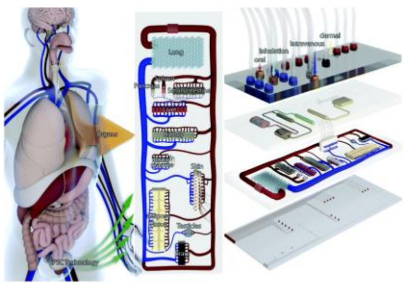


Nature 471, 661–665 (2011) *Biophysical Journal 94(5) 1854–1866*

Organs on chip

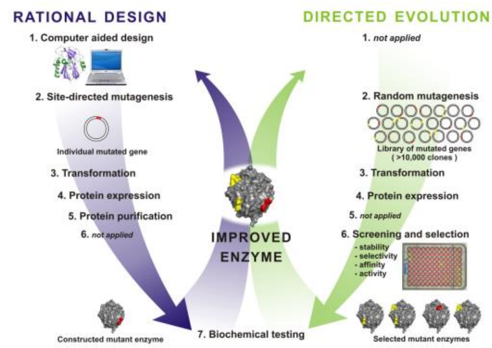


Human on chip concept

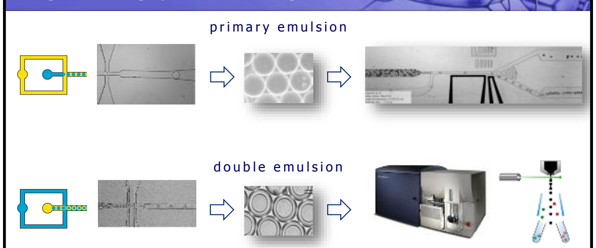


- correct limitations of organs isolation
- whole body biomimetic devices

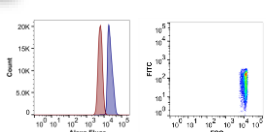
Protein Engineering



High Throughput Screening



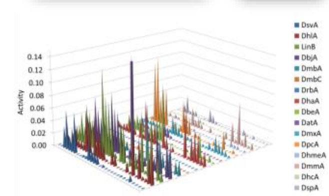
- **single cell analysis**
- 1 pL droplet volume
- 1 000 000 assays/hour



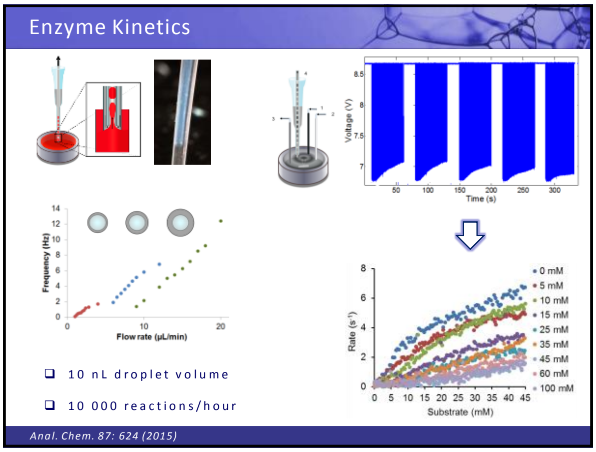
PNAS 107: 6550 (2011) *Anal. Chem. 86: 2526 (2014)*

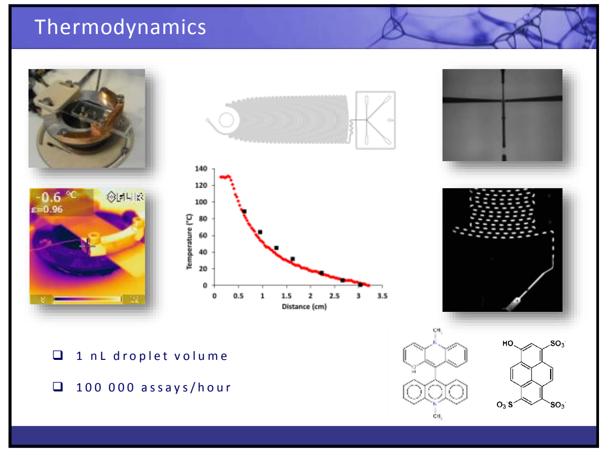
Substrate Specificity

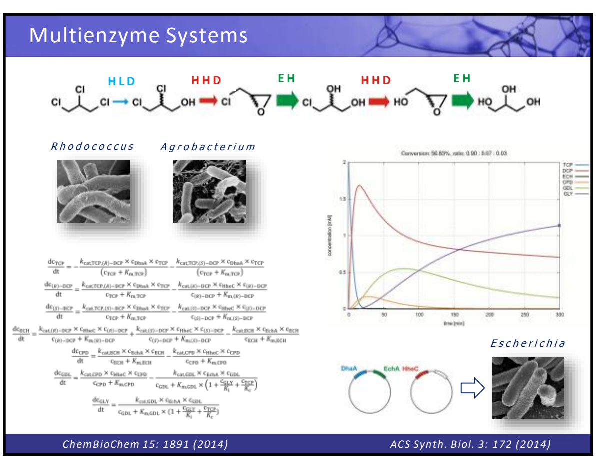
- 15 µL sample volume
- 50 nL droplet volume
- 1 000 reactions/hour

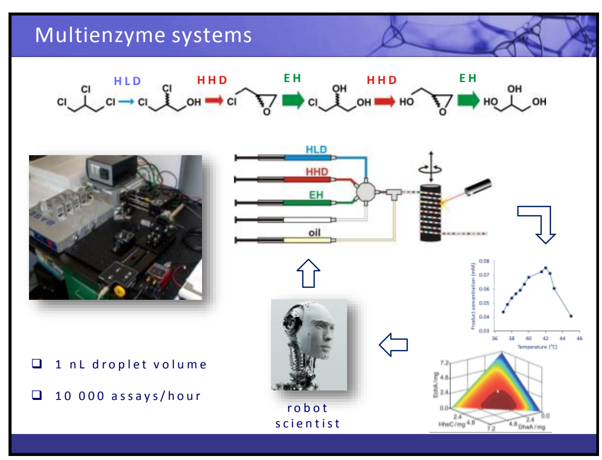


Anal. Chem. 85: 4761 (2013) *Lab Chip 8: 1837 (2008)* *Nature Chem. 3: 437 (2011)*


Enzyme Kinetics
10 nL droplet volume
10 000 reactions/hour
Frequency (Hz)
Flow rate (µL/min)
Voltage (V)
Time (s)
Rate (s⁻¹)
Substrate (mM)
0 mM
5 mM
10 mM
15 mM
25 mM
35 mM
45 mM
60 mM
100 mM
Anal. Chem. 87: 624 (2015)

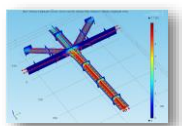

Thermodynamics
1 nL droplet volume
100 000 assays/hour
Temperature (°C)
Distance (cm)


Multienzyme Systems
HLD
HHD
EH
HHD
EH
Rhodococcus
Agrobacterium
Escherichia
ChemBioChem 15: 1891 (2014)
ACS Synth. Biol. 3: 172 (2014)


Multienzyme systems
HLD
HHD
EH
HHD
EH
HLD
HHD
EH
oil
1 nL droplet volume
10 000 assays/hour
robot
scientist

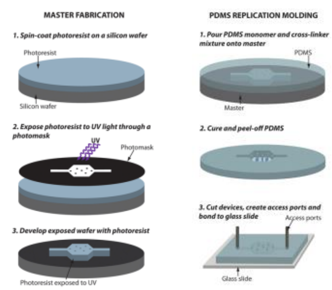
Design and fabrication

- **design**
  - engineering software (e.g., AutoCAD, DraftSight)
  - modelling (e.g., COMSOL, MatLab)



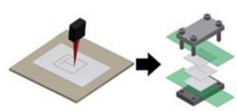
Design and fabrication

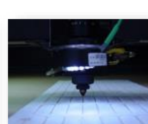
- **fabrication**
  - soft photolithography



Design and fabrication

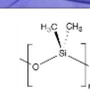
- **fabrication**
  - direct fabrication methods
    - 3D printing
    - CNC micro-milling
    - laser cutting
    - cutting plotters

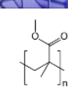


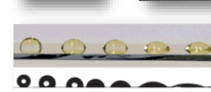


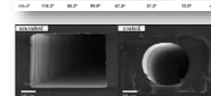
Design and fabrication

- **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







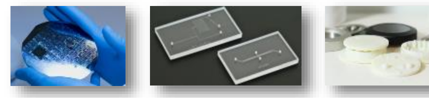


Sensing and detection

- 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