# 7. 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

pre-treatment

incubation

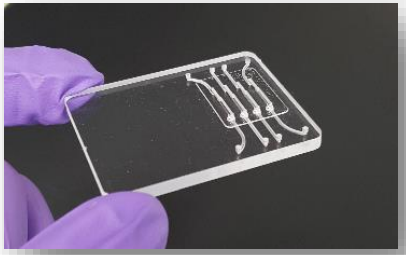
analysis

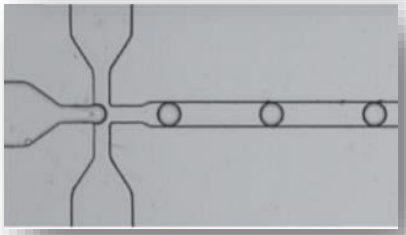
preparation

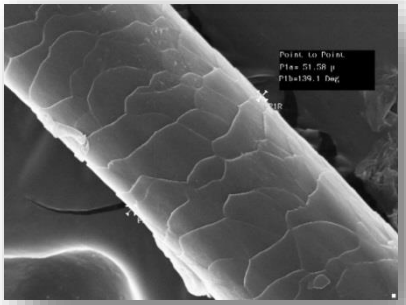
collection

# 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



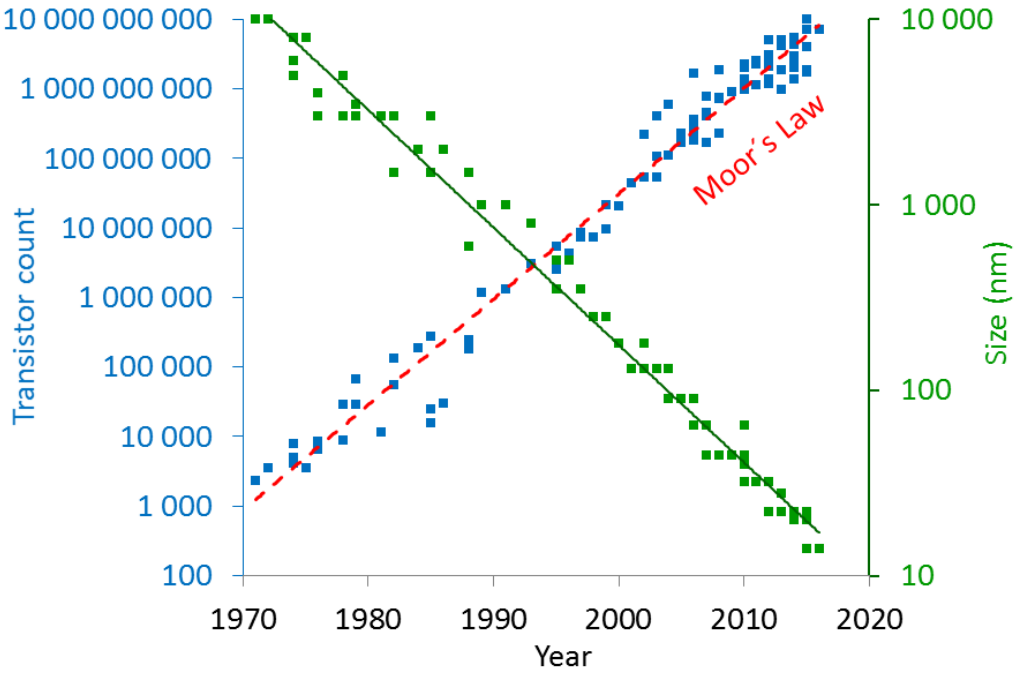




*Nature 507, 181 (2014)*

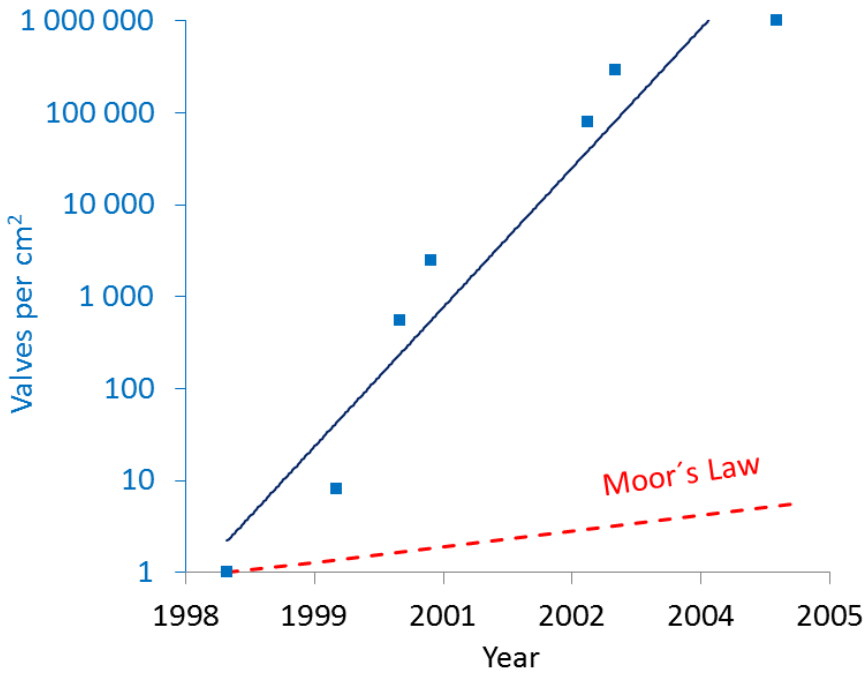
# Revolution in Electronics

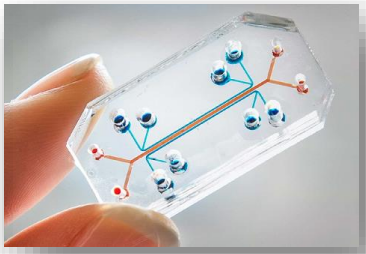
| | Size (nm) | Price (USD) |
|---|---|---|
| Vacuum tube | 100 | 10 |
| Trasistors | 10 | 1 |
| Microchip | 0.000 010 | 0.000 000 100 |

# 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 |

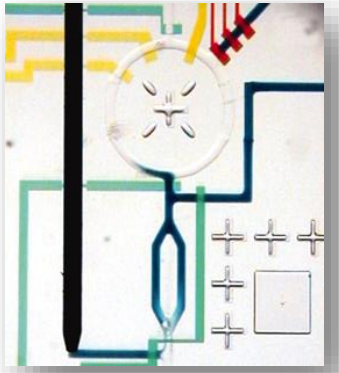



*Nature Biotechnol. 21, 1179 (2003)*

# Concepts in microfluidics

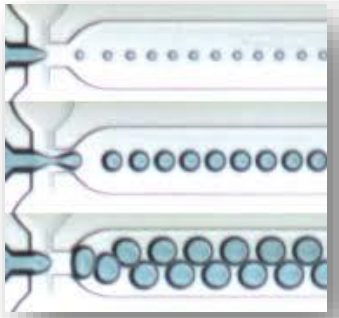
- **continuous-flow microfluidics**

  manipulation of continuous liquid flow through micro-fabricated channels



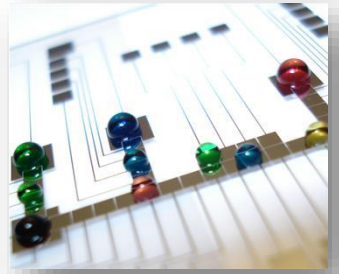
- **droplet-based microfluidics**

  manipulating discrete volumes of fluids in immiscible phases



- **digital microfluidics**

  droplets manipulated on a substrate using electro-wetting



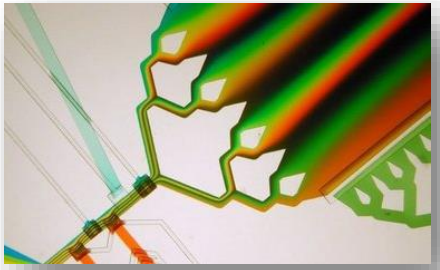
# Novel Physics of Micro-Scale

- viscosity, surface tension and capillary forces dominate

  - **lack of turbulent phenomena**

    + nontrivial chemical gradients

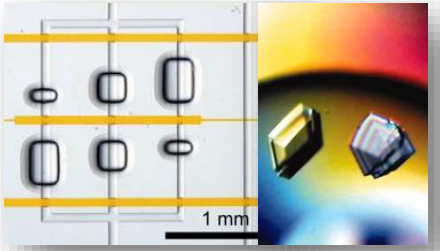
    to study chemotaxis



  - **absence of density-driven convection**

    + free interface diffusion, efficient

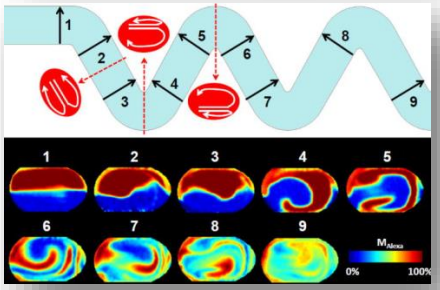
    protein crystallization kinetics




  - **strong shearing forces**

    + fast mixing kinetics of protein

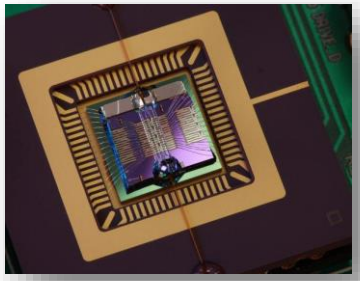
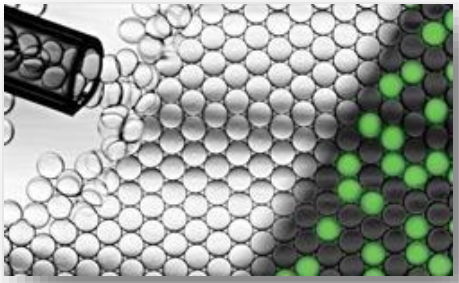
    folding and/or catalysis

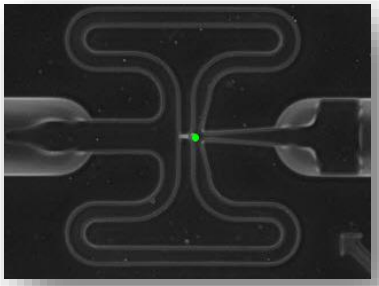
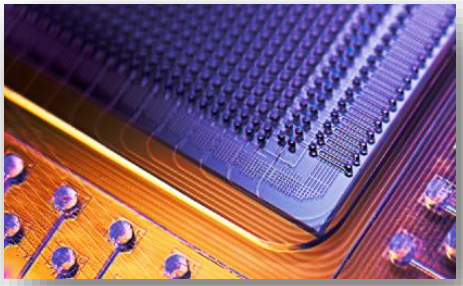



*Nature Biotechnol. 20, 826 (2002)* *Appl. Phys. Lett. 83, 4664 (2003)* *PNAS 99, 16531 (2002)*

# 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

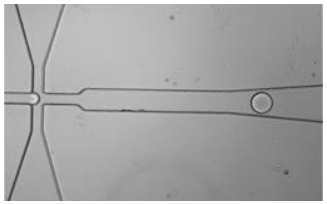
- **classical PCR**
  - slow heating/cooling cycles
  - PCR tubes (strips), 96-well MTP
  - volume 50 to 500 µL

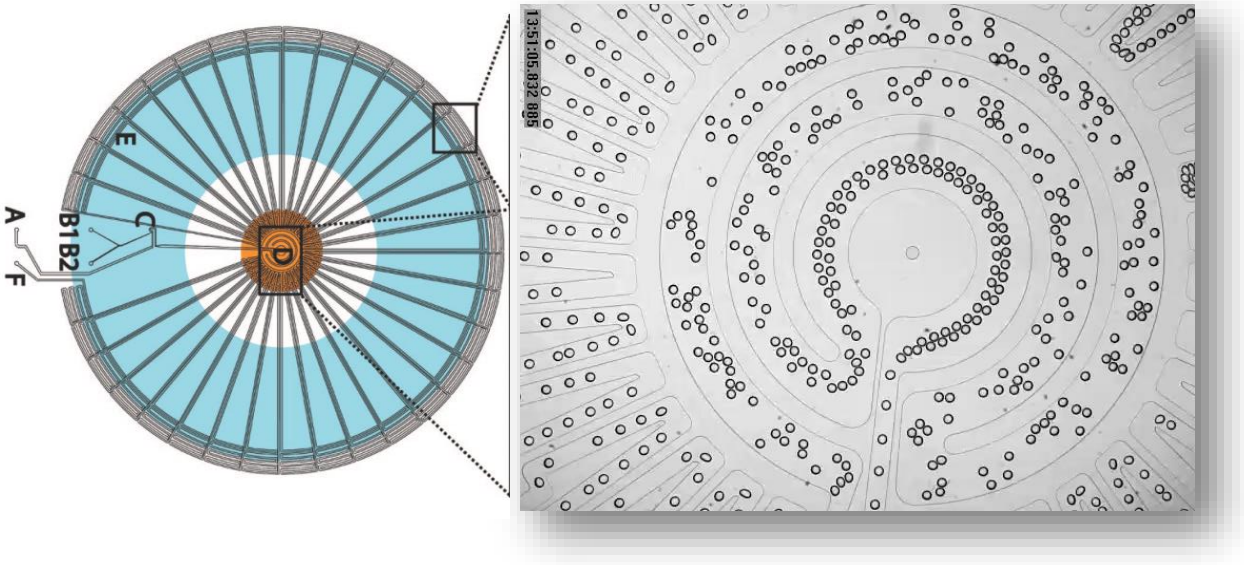
**Kary Mullis**

Nobel Prize in 1993

# Polymerase chain reaction

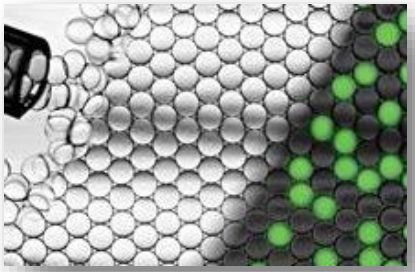
- **PCR in microfluidic droplets**
  - 500 droplets per second
  - volume 50 to 100 pL
  - 10 to 20 s per heating/cooling cycle





# Digital polymerase chain reaction

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

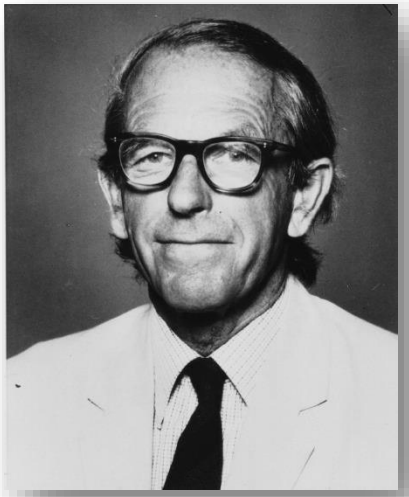


# Next-generation sequencing

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

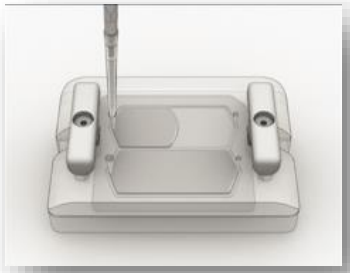
  water in oil droplets 1 picoliter ($10^{-12}$ liters)

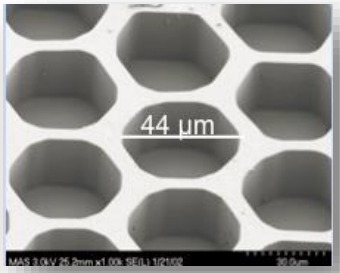
  1 mil. reads/run, 10 USD/Mbase

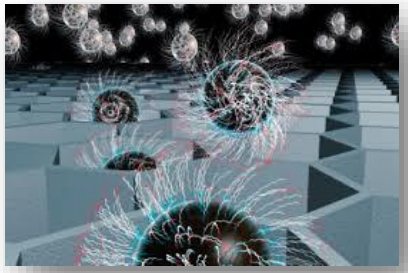


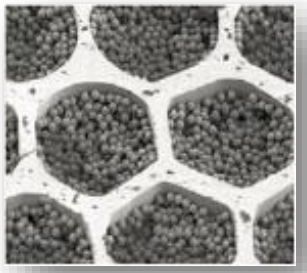
**Frederick Sanger**

Nobel Prize in 1980

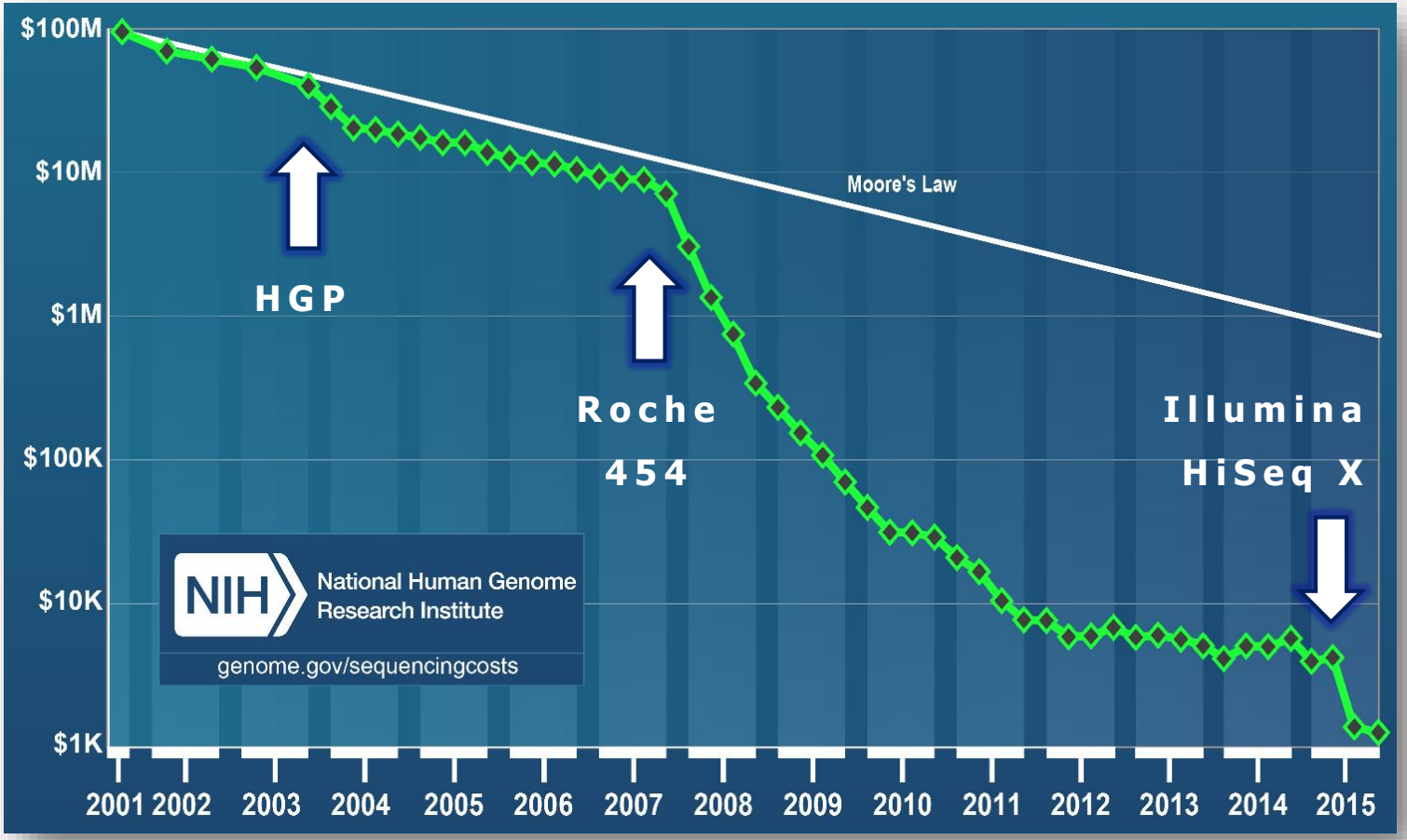






# Revolution in Science?

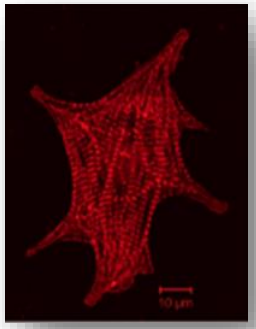


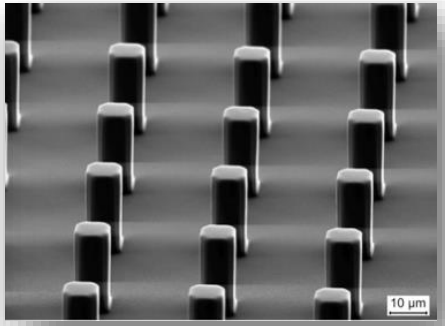

- 2003: 13 years, 3 billion USD
- 2016: days, < 1,000 USD

# Organs on chip

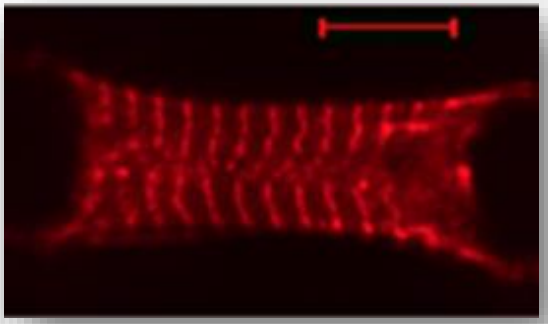
- 3D chips **mimicking human's physiological responses** (e.g., pathological, pharmacokinetic, toxicological)
- realistic *in vitro* model **closer to *in vivo* cell environment** (e.g., mechanical strain, patterning, fluid shear stresses)
- **can replace** expensive and controversial **animal testing**

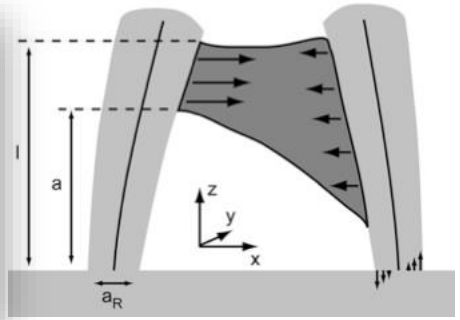
**flat surface**






**micropillar**

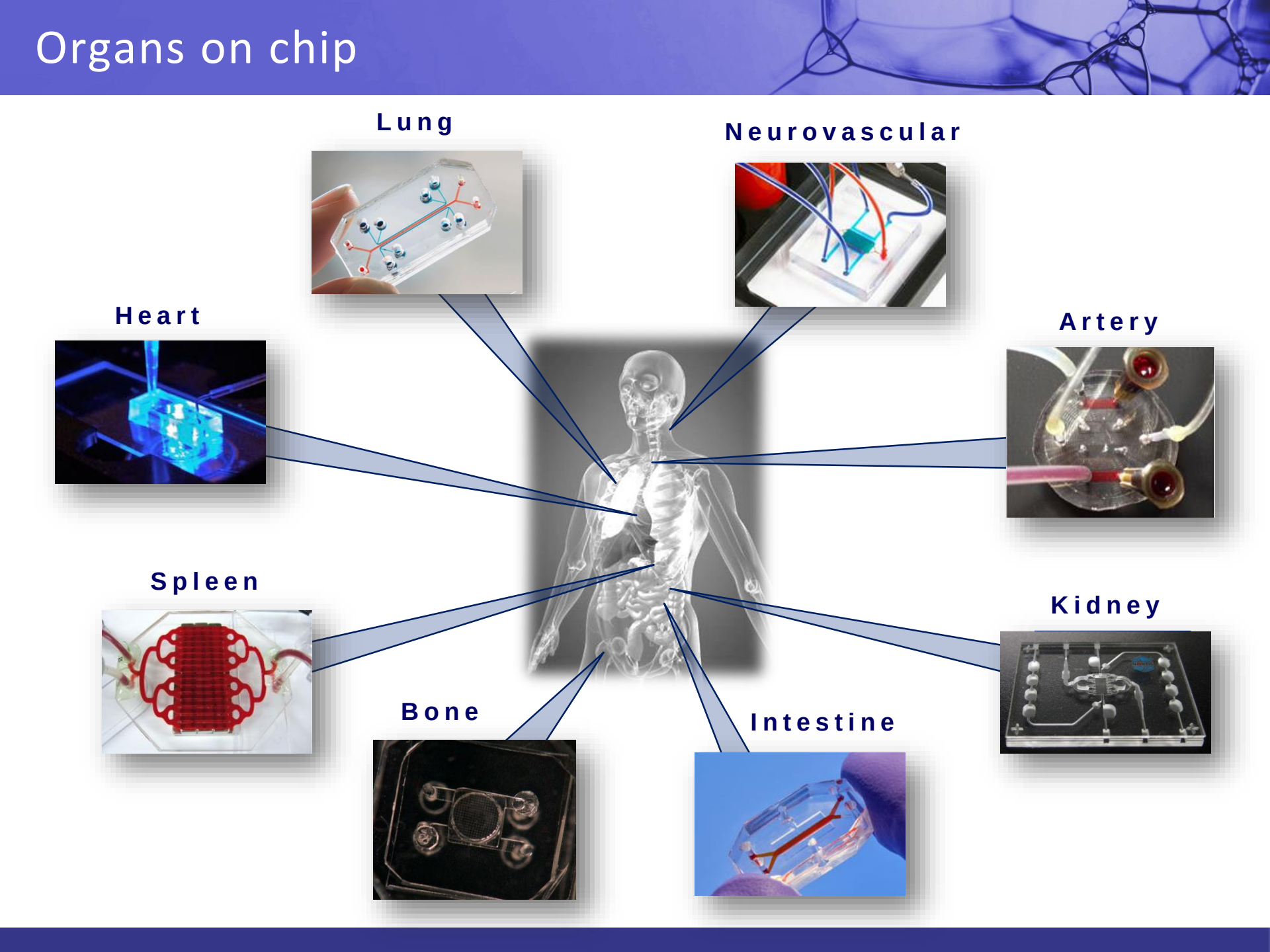




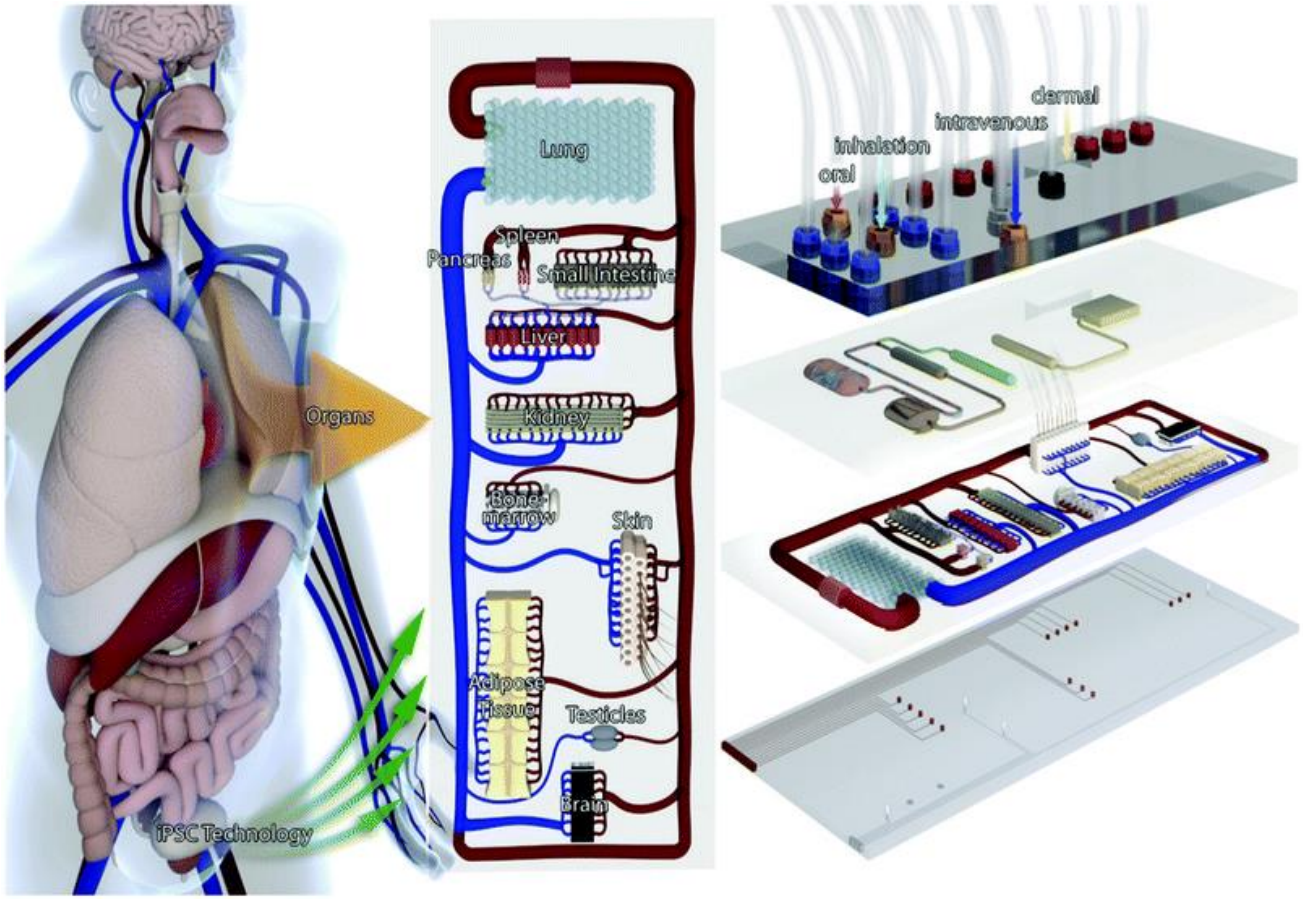

*Nature 471, 661–665 (2011)*

*Biophysical Journal 94(5) 1854–1866*

# Organs on chip

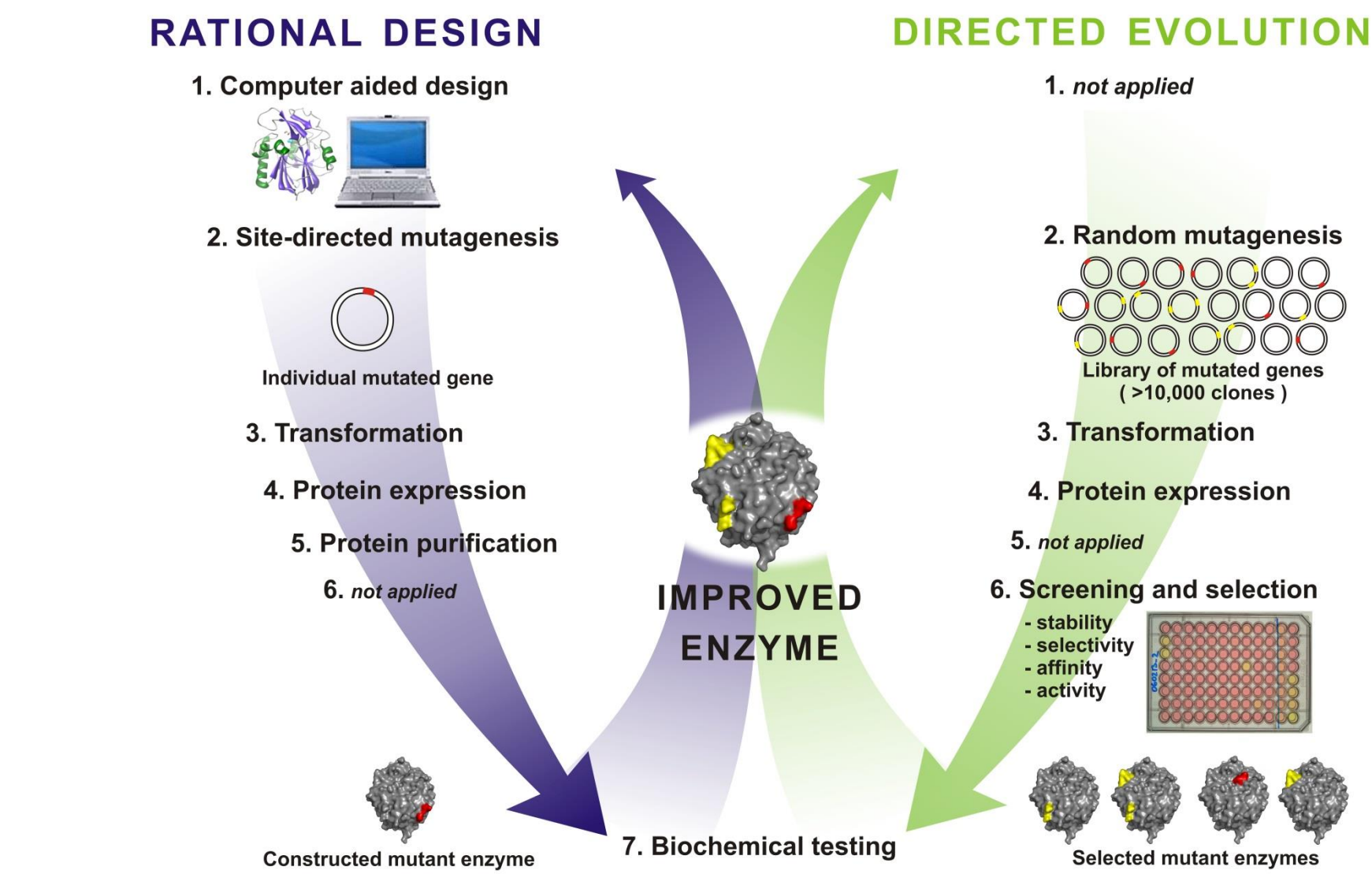


# Human on chip concept



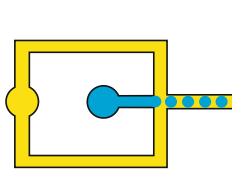
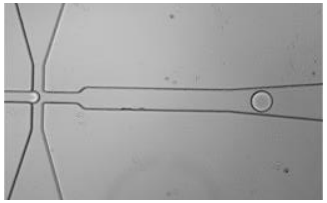

- correct limitations of organs isolation
- whole body biomimetic devices

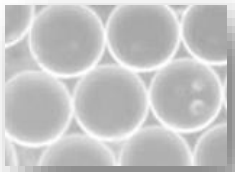
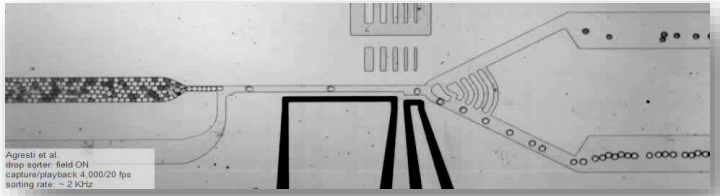
# Protein Engineering



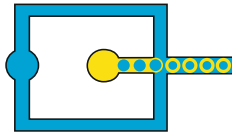
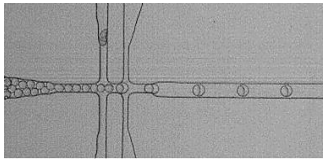
# High Throughput Screening

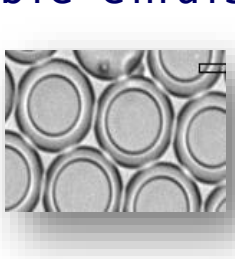
primary emulsion

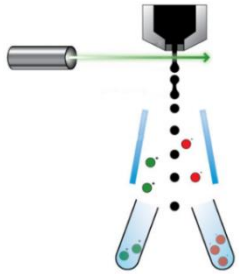



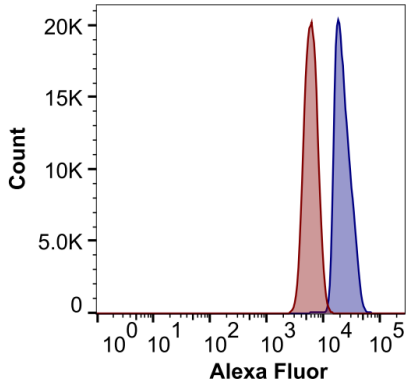

double emulsion

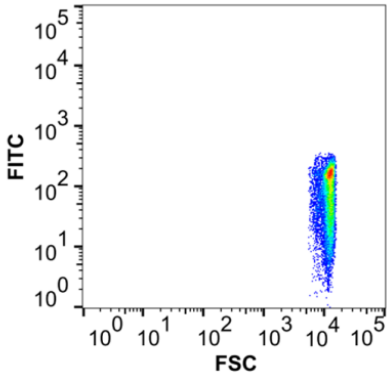







- **single cell analysis**
- 1 pL droplet volume
- 1 000 000 assays/hour

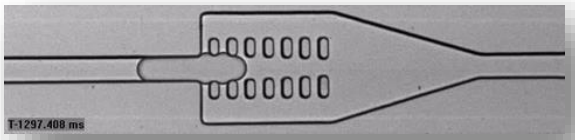


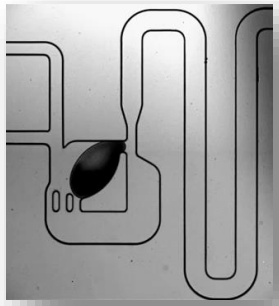




*PNAS 107: 6550 (2011)*

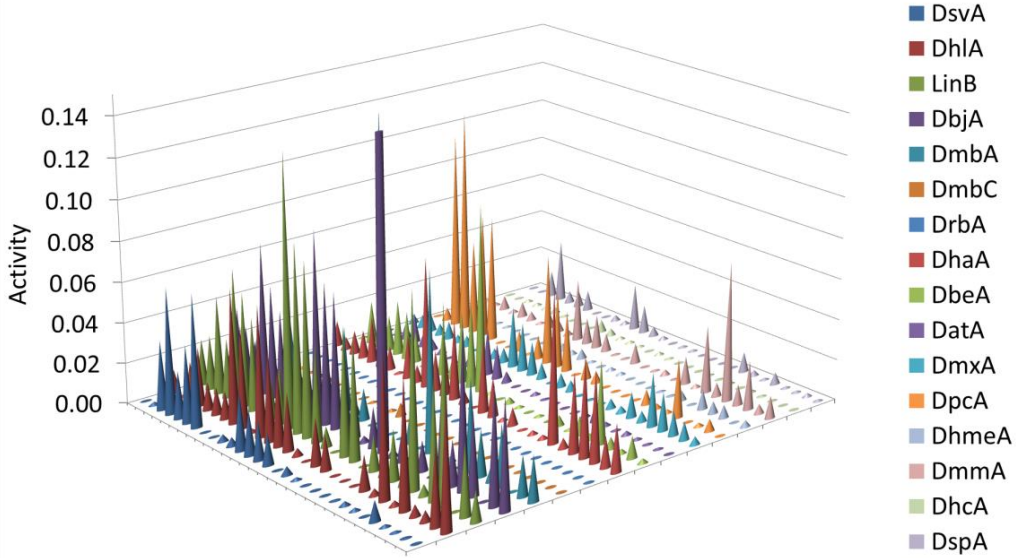
*Anal. Chem. 86: 2526 (2014)*

# Substrate Specificity

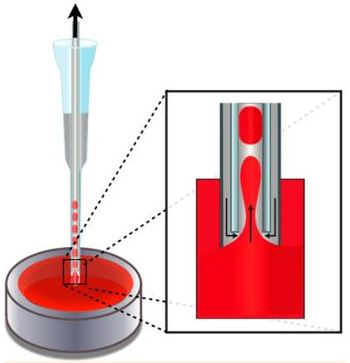


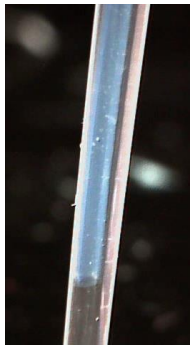



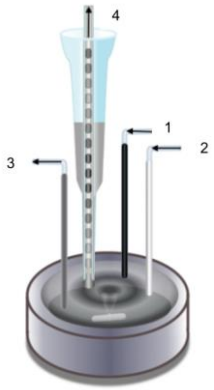
- 15 µL sample volume
- 50 nL droplet volume
- 1 000 reactions/hour

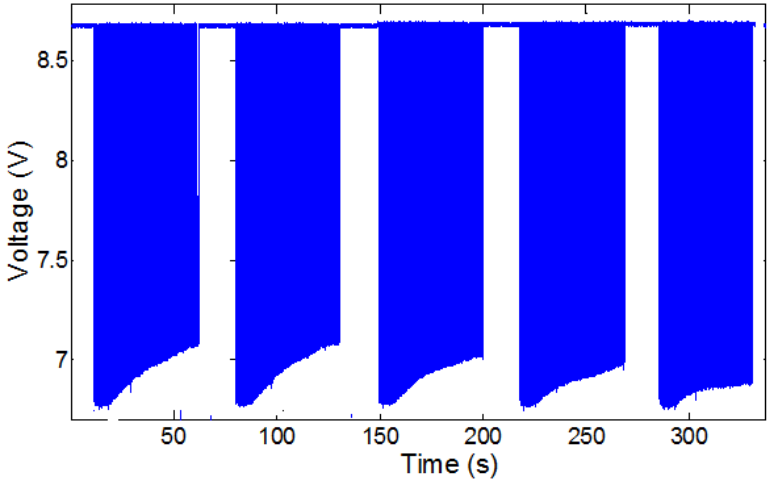


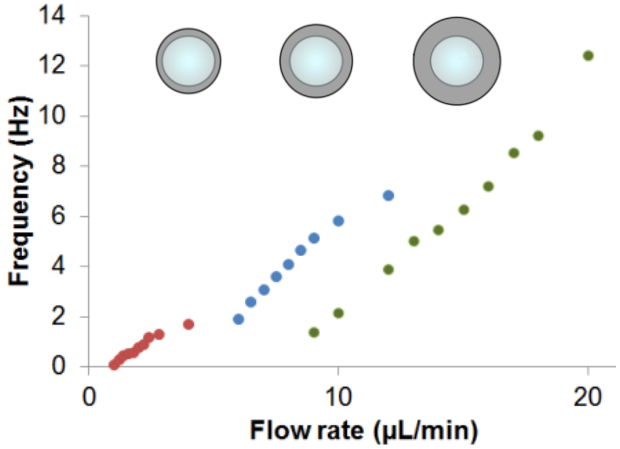
# Enzyme Kinetics

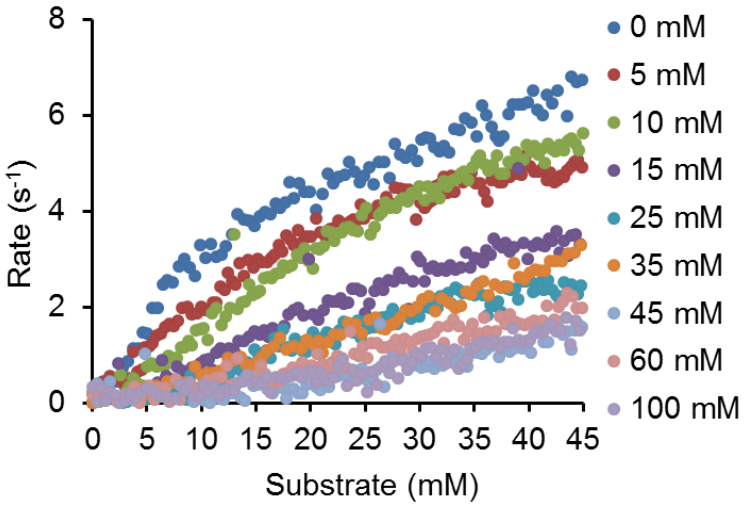






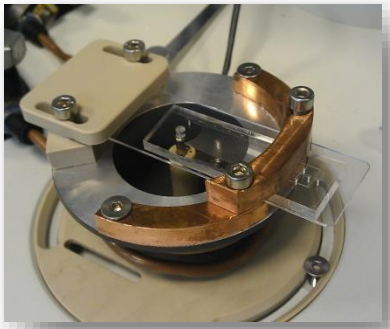



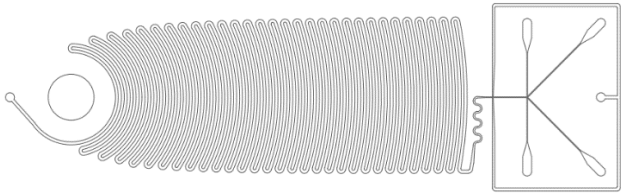



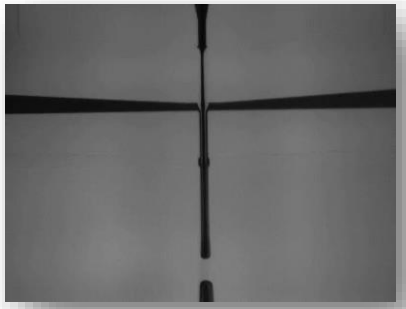

- 10 nL droplet volume
- 10 000 reactions/hour

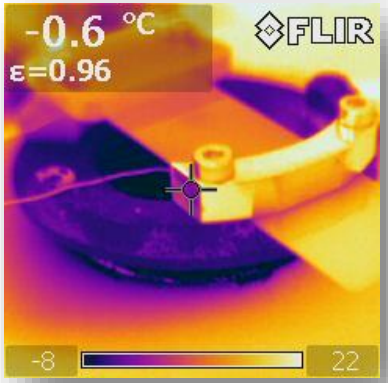
*Anal. Chem. 87: 624 (2015)*

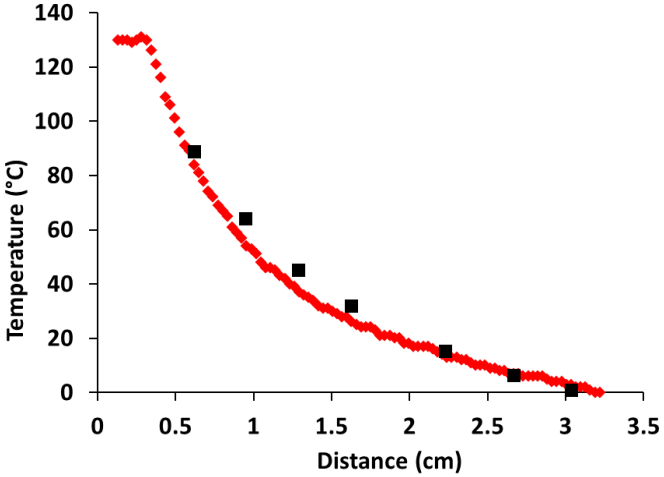
# Thermodynamics

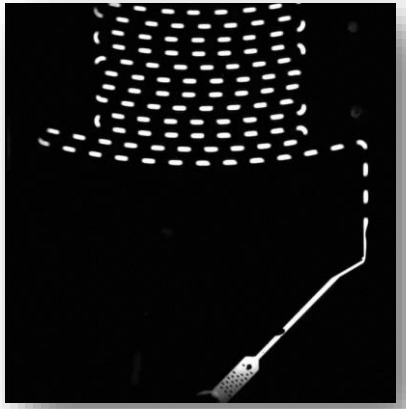




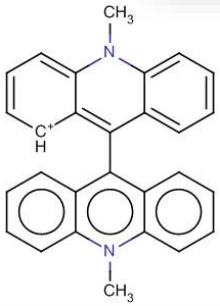


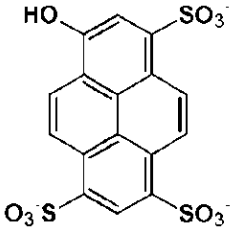


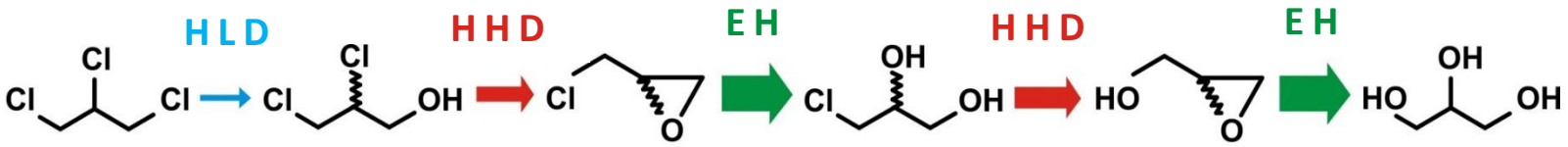



- 1 nL droplet volume
- 100 000 assays/hour

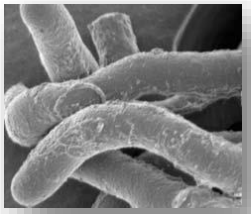


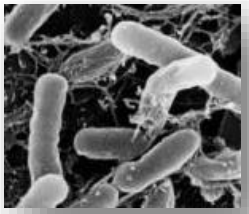
# Multienzyme Systems




*Rhodococcus*



*Agrobacterium*



$$\frac{dc_{TCP}}{dt} = -\frac{k_{cat,TCP,(R)-DCP} \times c_{DhaA} \times c_{TCP}}{(c_{TCP} + K_{m,TCP})} - \frac{k_{cat,TCP,(S)-DCP} \times c_{DhaA} \times c_{TCP}}{(c_{TCP} + K_{m,TCP})}$$

$$\frac{dc_{(R)-DCP}}{dt} = \frac{k_{cat,TCP,(R)-DCP} \times c_{DhaA} \times c_{TCP}}{c_{TCP} + K_{m,TCP}} - \frac{k_{cat,(R)-DCP} \times c_{HheC} \times c_{(R)-DCP}}{c_{(R)-DCP} + K_{m,(R)-DCP}}$$

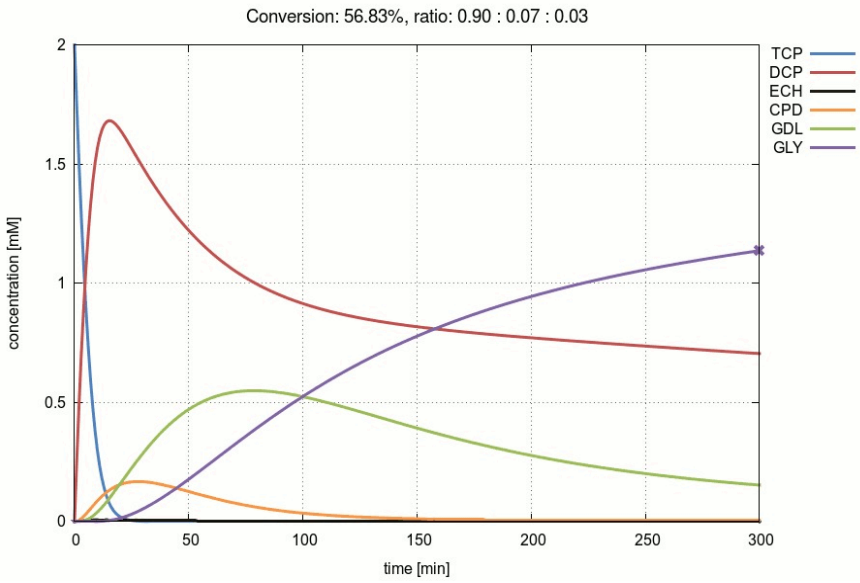
$$\frac{dc_{(S)-DCP}}{dt} = \frac{k_{cat,TCP,(S)-DCP} \times c_{DhaA} \times c_{TCP}}{c_{TCP} + K_{m,TCP}} - \frac{k_{cat,(S)-DCP} \times c_{HheC} \times c_{(S)-DCP}}{c_{(S)-DCP} + K_{m,(S)-DCP}}$$

$$\frac{dc_{ECH}}{dt} = \frac{k_{cat,(R)-DCP} \times c_{HheC} \times c_{(R)-DCP}}{c_{(R)-DCP} + K_{m,(R)-DCP}} + \frac{k_{cat,(S)-DCP} \times c_{HheC} \times c_{(S)-DCP}}{c_{(S)-DCP} + K_{m,(S)-DCP}} - \frac{k_{cat,ECH} \times c_{EchA} \times c_{ECH}}{c_{ECH} + K_{m,ECH}}$$

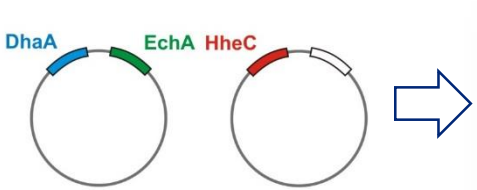
$$\frac{dc_{CPD}}{dt} = \frac{k_{cat,ECH} \times c_{EchA} \times c_{ECH}}{c_{ECH} + K_{m,ECH}} - \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}}$$

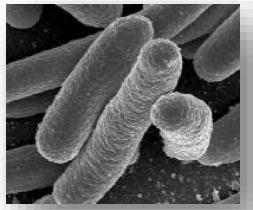
$$\frac{dc_{GDL}}{dt} = \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}} - \frac{k_{cat,GDL} \times c_{EchA} \times c_{GDL}}{c_{GDL} + K_{m,GDL} \times \left(1 + \frac{c_{GLY}}{K_i} + \frac{c_{TCP}}{K_c}\right)}$$

$$\frac{dc_{GLY}}{dt} = \frac{k_{cat,GDL} \times c_{EchA} \times c_{GDL}}{c_{GDL} + K_{m,GDL} \times (1 + \frac{c_{GLY}}{K_i} + \frac{c_{TCP}}{K_c})}$$




*Escherichia*

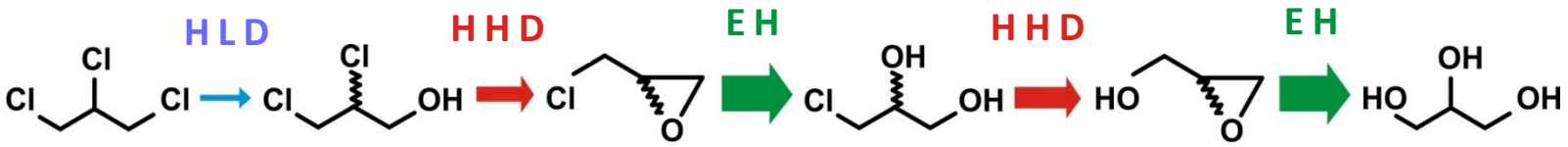


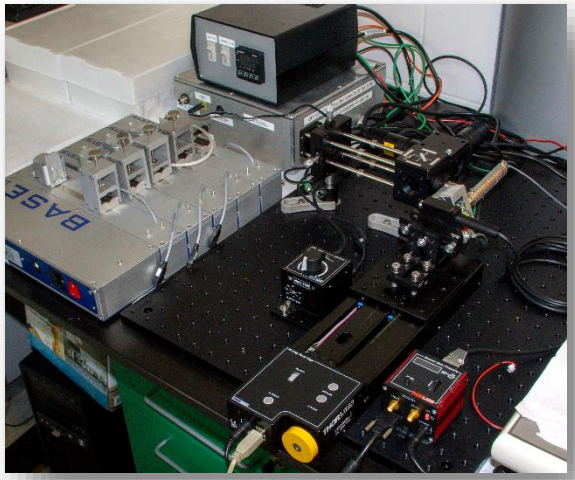



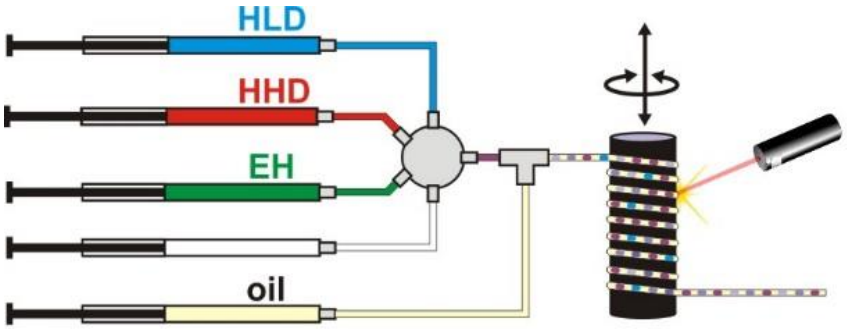
*ChemBioChem 15: 1891 (2014)*

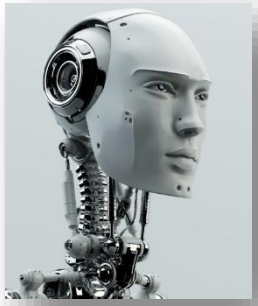
*ACS Synth. Biol. 3: 172 (2014)*

# Multienzyme systems

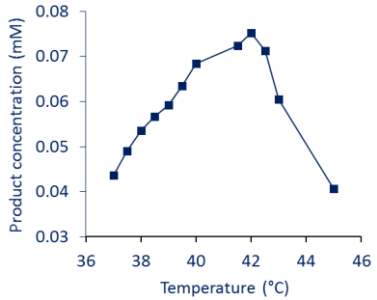


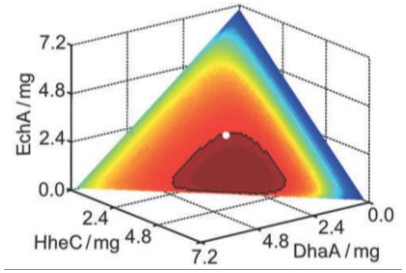





robot scientist

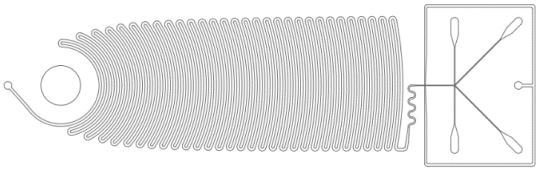


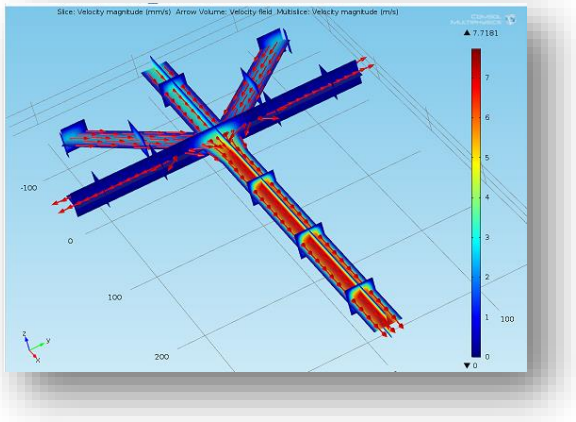




- 1 nL droplet volume
- 10 000 assays/hour

# Design and fabrication

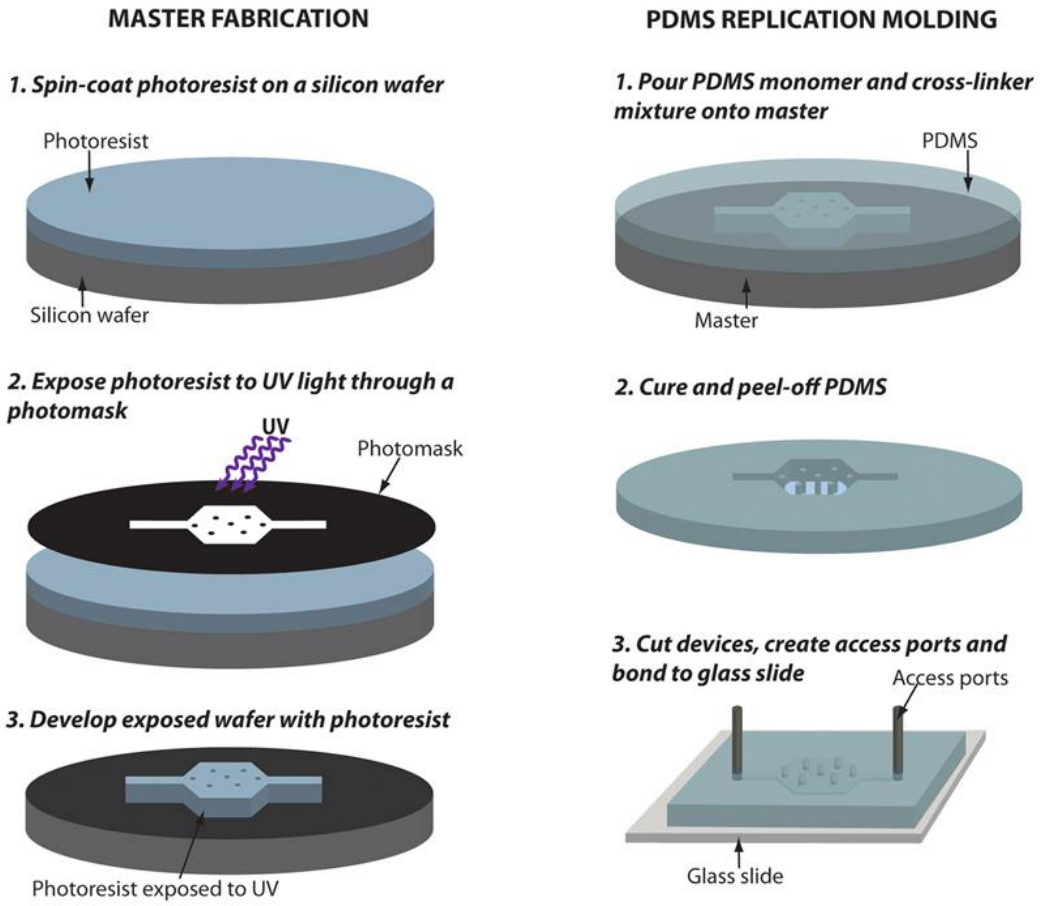
- **design**
  - engineering software (e.g., AutoCAD, DraftSight)
  - modelling (e.g., COMSOL, MatLab)

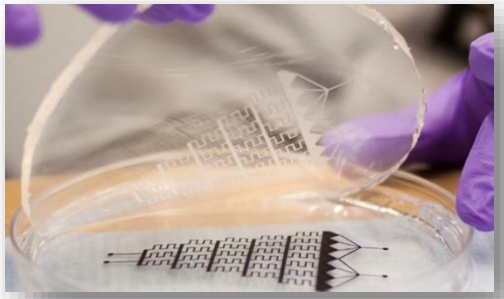




# Design and fabrication

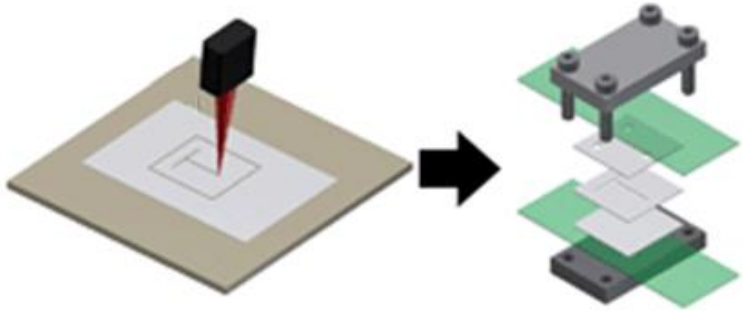
- **fabrication**
  - soft photolithography

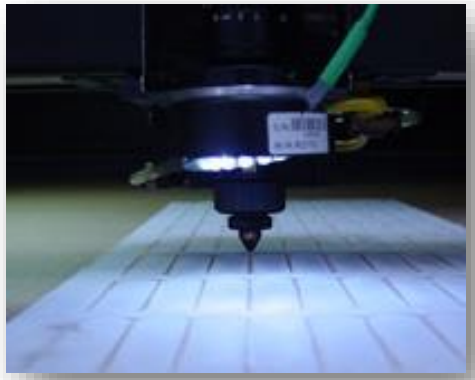


# Design and fabrication

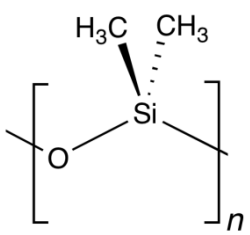
- **fabrication**
  - direct fabrication methods
    - 3D printing
    - CNC micro-milling
    - laser cutting
    - cutting plotters

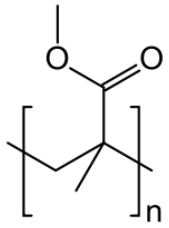


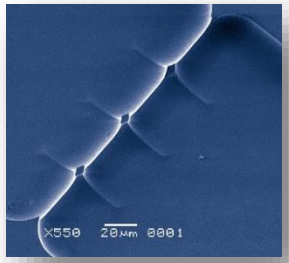


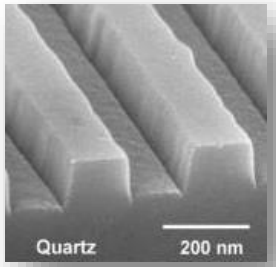
# Design and fabrication

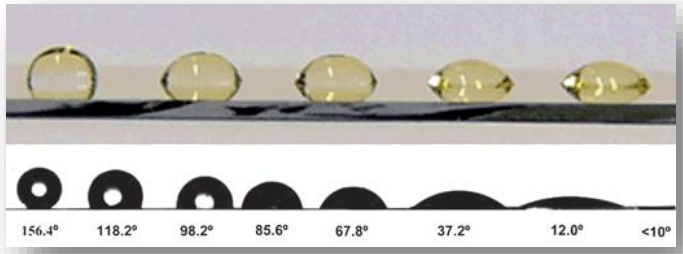
- **materials**
  - inert and transparent
  - PDMS - poly(dimethyl siloxane)
  - PMMA - poly(methyl methacrylate)
  - fused silica, quartz and glass
- **surface modification**
  - plasma treatment
  - silanization
  - sol–gel coating

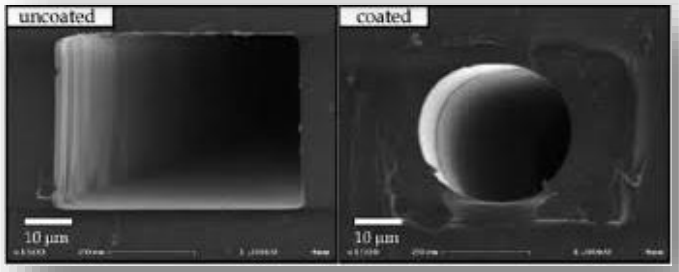


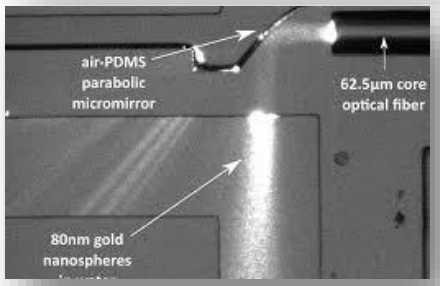


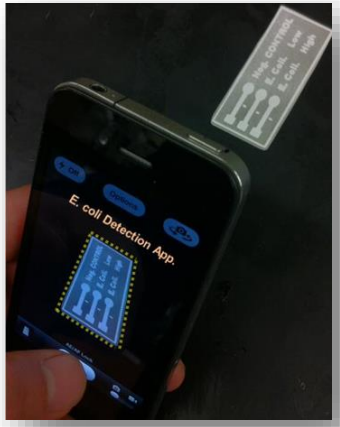



# Sensing and detection

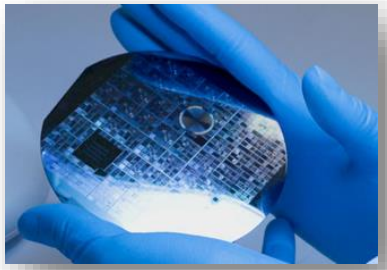
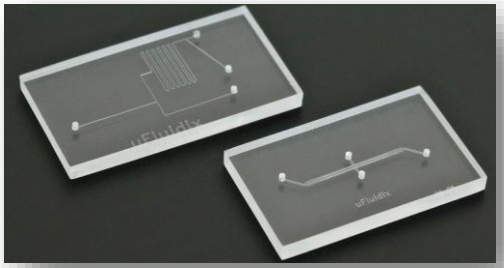
- processing of **small reagent volumes**
- **analytical timescale** and performance
- **on chip detection**
  - fluorescence (LSM, FCS, FLIM)
  - UV/VIS absorbance
  - IR spectroscopy
  - Raman scattering
  - (chemo/electro) luminescence
  - thermal conductivity
  - RI variation
- **off chip detection**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