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

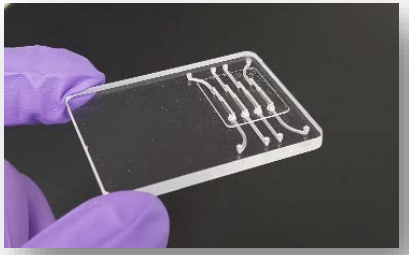
analysis

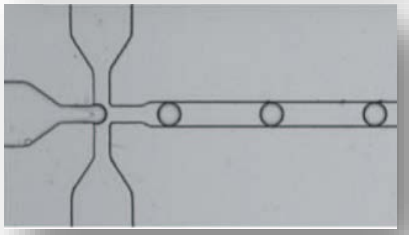
preparation

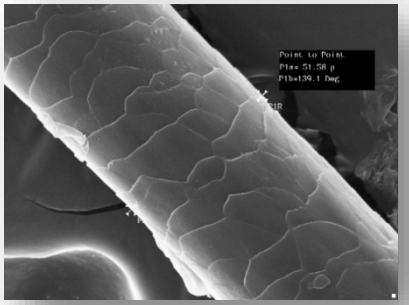
collection

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



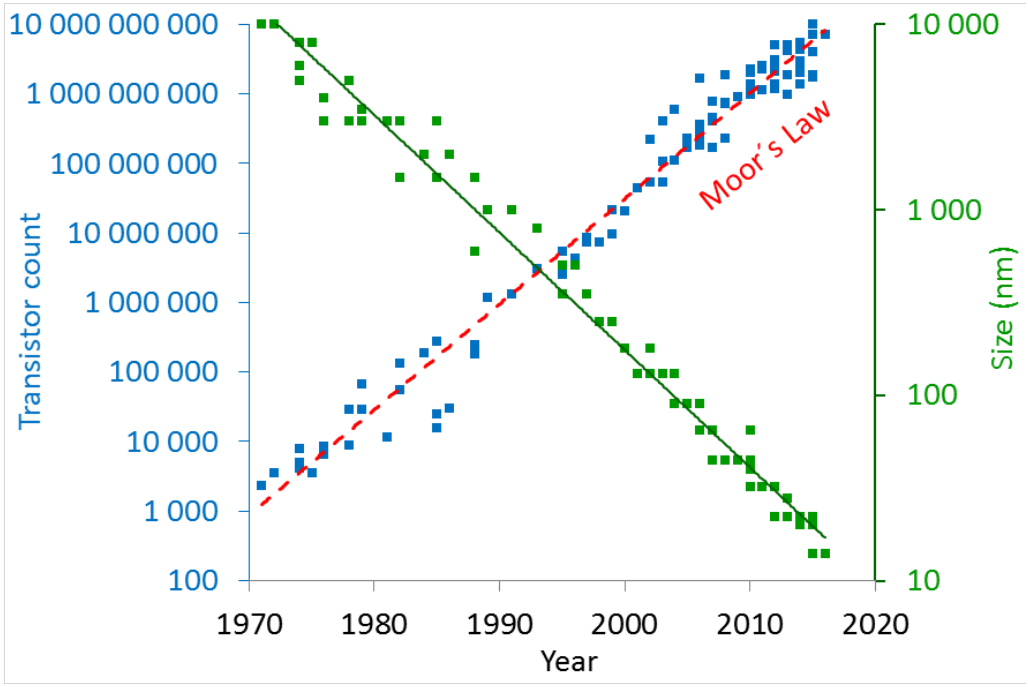




Nature 507, 181 (2014)

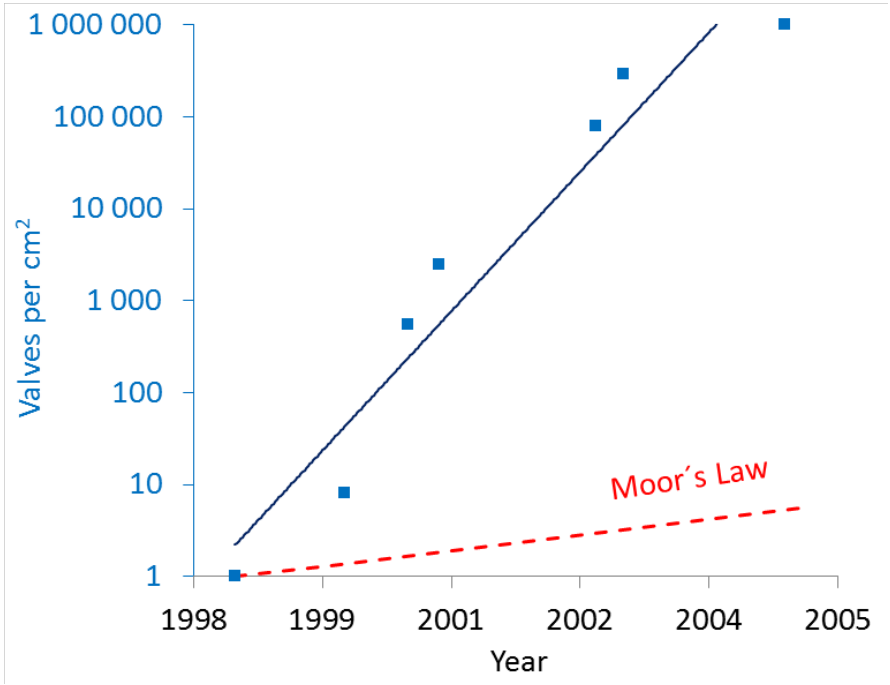
Revolution in Electronics

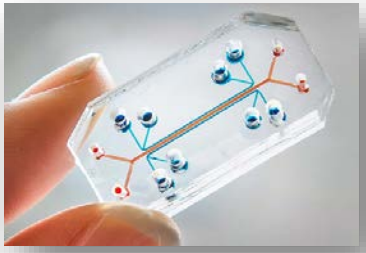
	Size (nm)	Price (USD)
Vacuum tube	100	10



Revolution in Science?

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



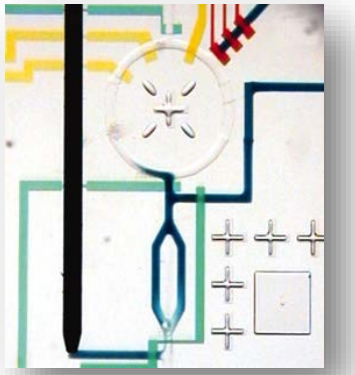


Nature Biotechnol. 21, 1179 (2003)

Concepts in microfluidics

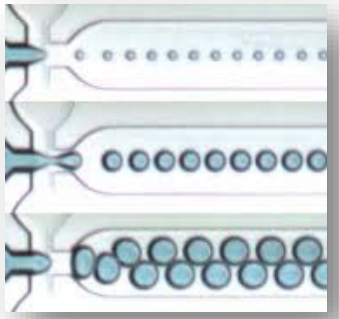
- **continuous-flow microfluidics**

  manipulation of continuous liquid flow through micro-fabricated channels



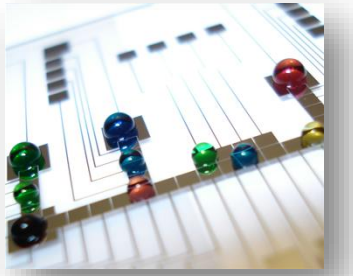
- **droplet-based microfluidics**

  manipulating discrete volumes of fluids in immiscible phases



- **digital microfluidics**

  droplets manipulated on a substrate using electro-wetting



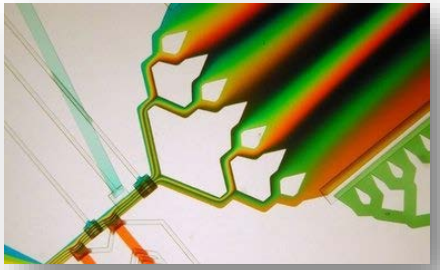
Novel Physics of Micro-Scale

- viscosity, surface tension and capillary forces dominate

  - **lack of turbulent phenomena**

    + nontrivial chemical gradients

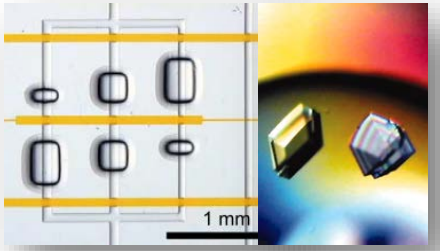
    to study chemotaxis



  - **absence of density-driven convection**

    + free interface diffusion, efficient

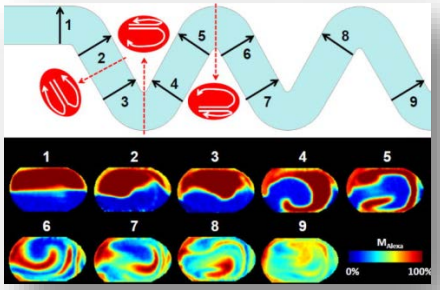
    protein crystallization kinetics



  - **strong shearing forces**

    + fast mixing kinetics of protein

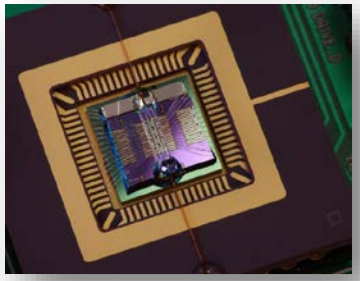
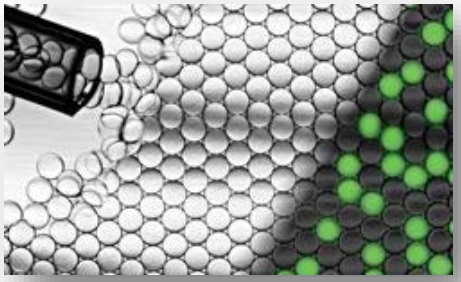
    folding and/or catalysis

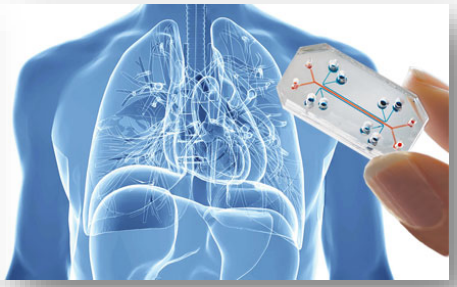
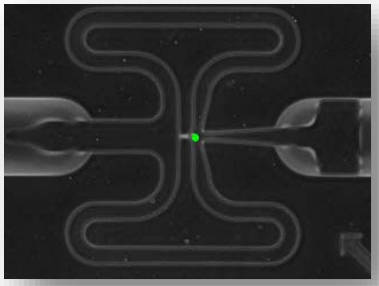
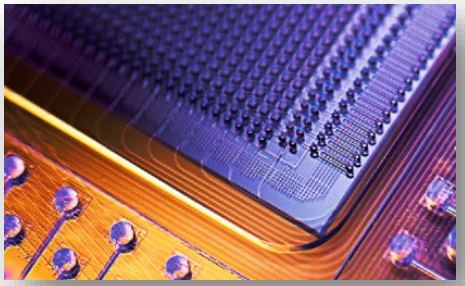


Nature Biotechnol. 20, 826 (2002) *Appl. Phys. Lett. 83, 4664 (2003)* *PNAS 99, 16531 (2002)*

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

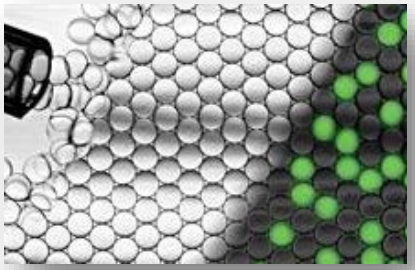
- **classical PCR**
  - slow heating/cooling cycles
  - PCR tubes (strips), 96-well MTP
  - volume 50 to 500 µL

Kary Mullis

Nobel Prize in 1993

Digital polymerase chain reaction

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

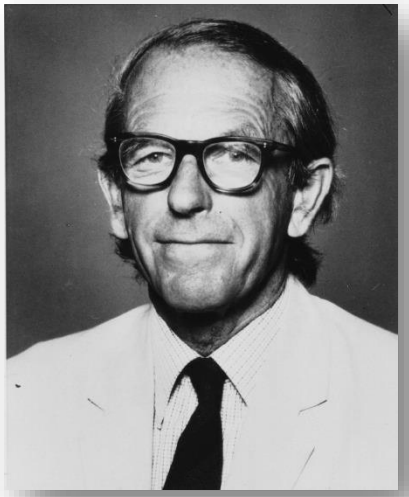


Next-generation sequencing

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

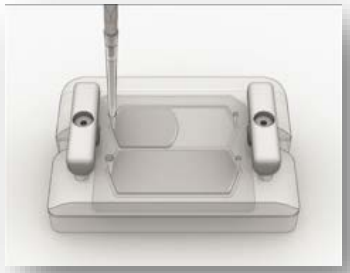
  water in oil droplets 1 picoliter (10^{-12} liters)

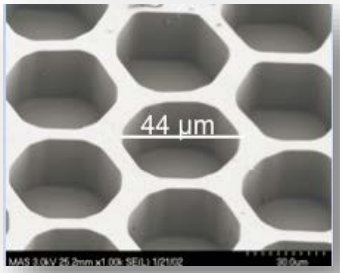
  1 mil. reads/run, 10 USD/Mbase

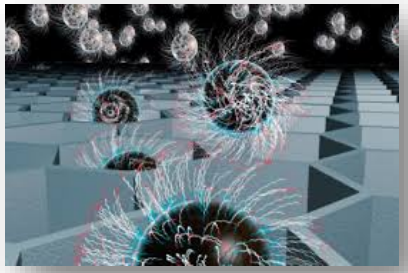


Frederick Sanger

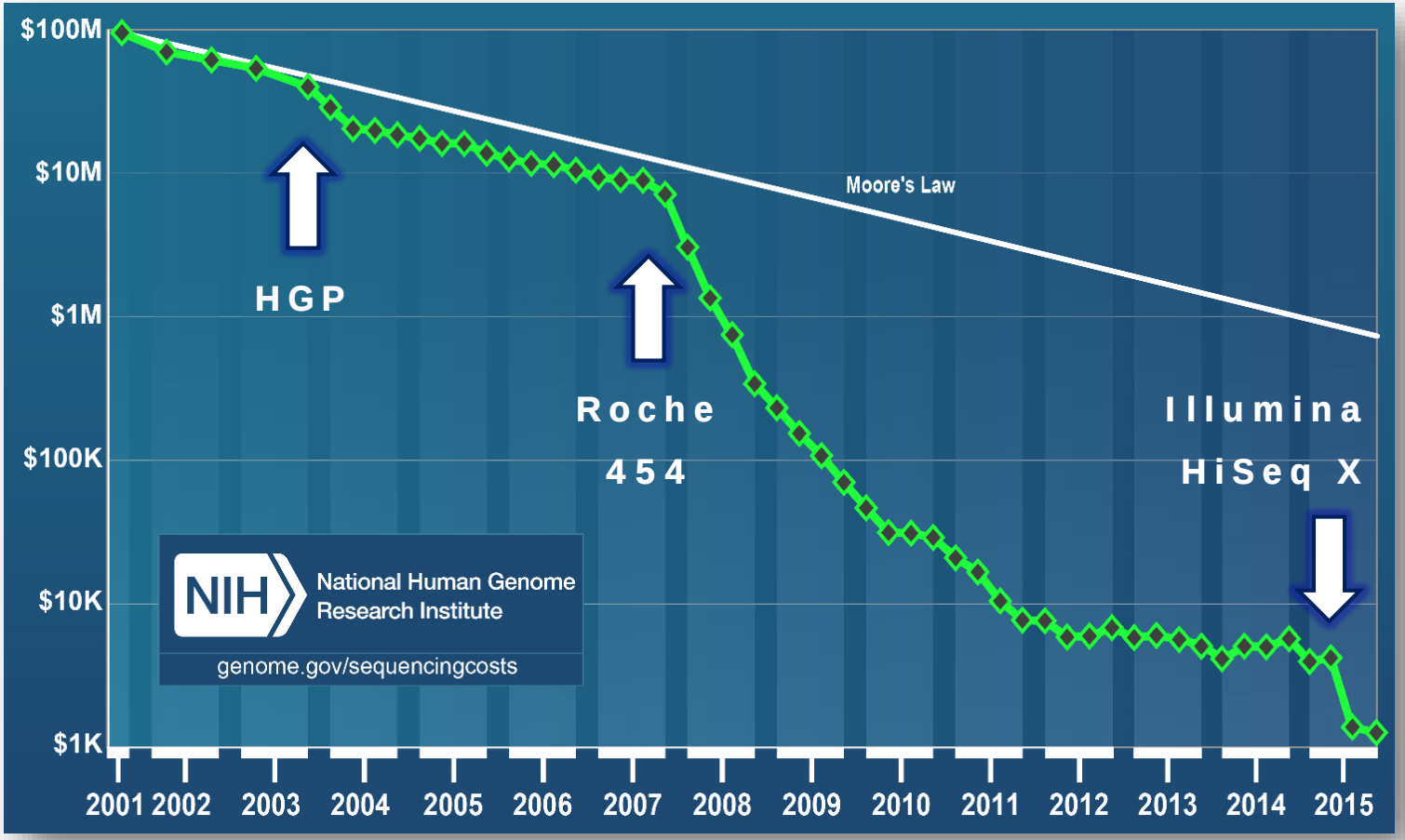
Nobel Prize in 1980







Revolution in Science?



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

Organs on chip

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

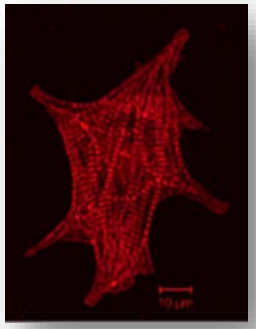
  (e.g., pathological, pharmacokinetic, toxicological)

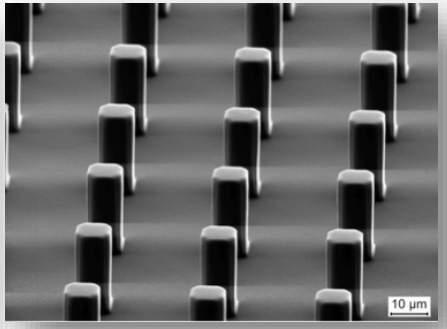
- realistic *in vitro* model **closer to *in vivo* cell environment**

  (e.g., mechanical strain, patterning, fluid shear stresses)

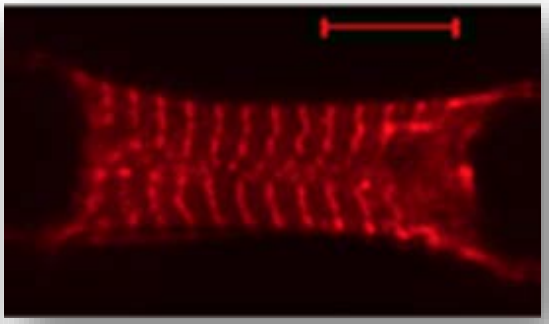
- **can replace** expensive and controversial **animal testing**

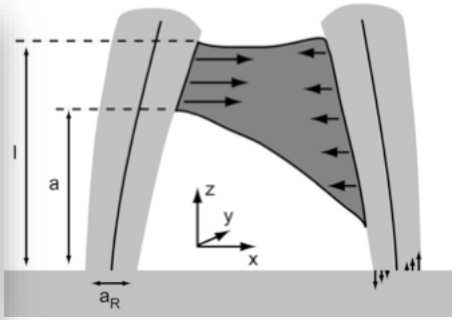
flat surface





micropillar

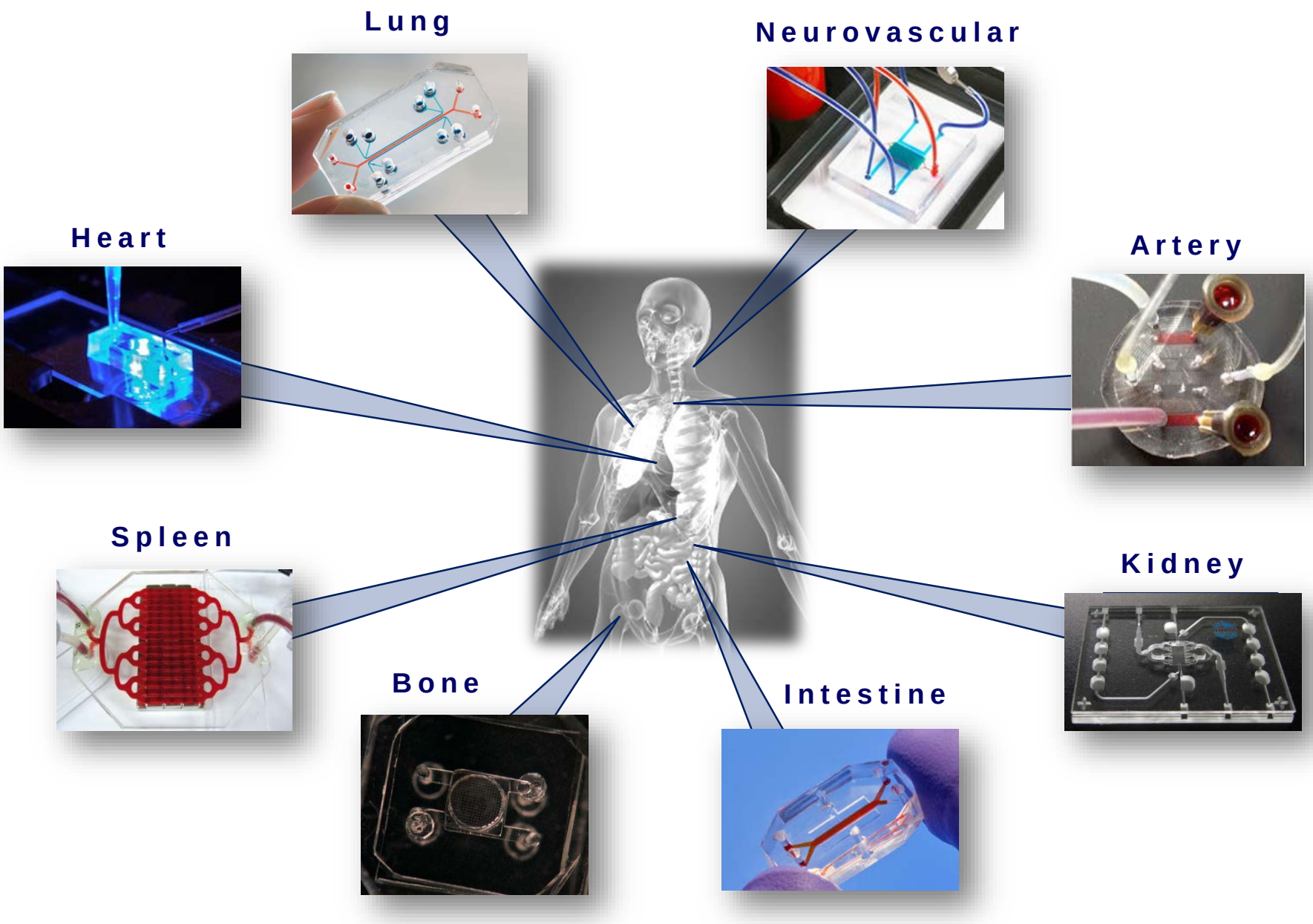




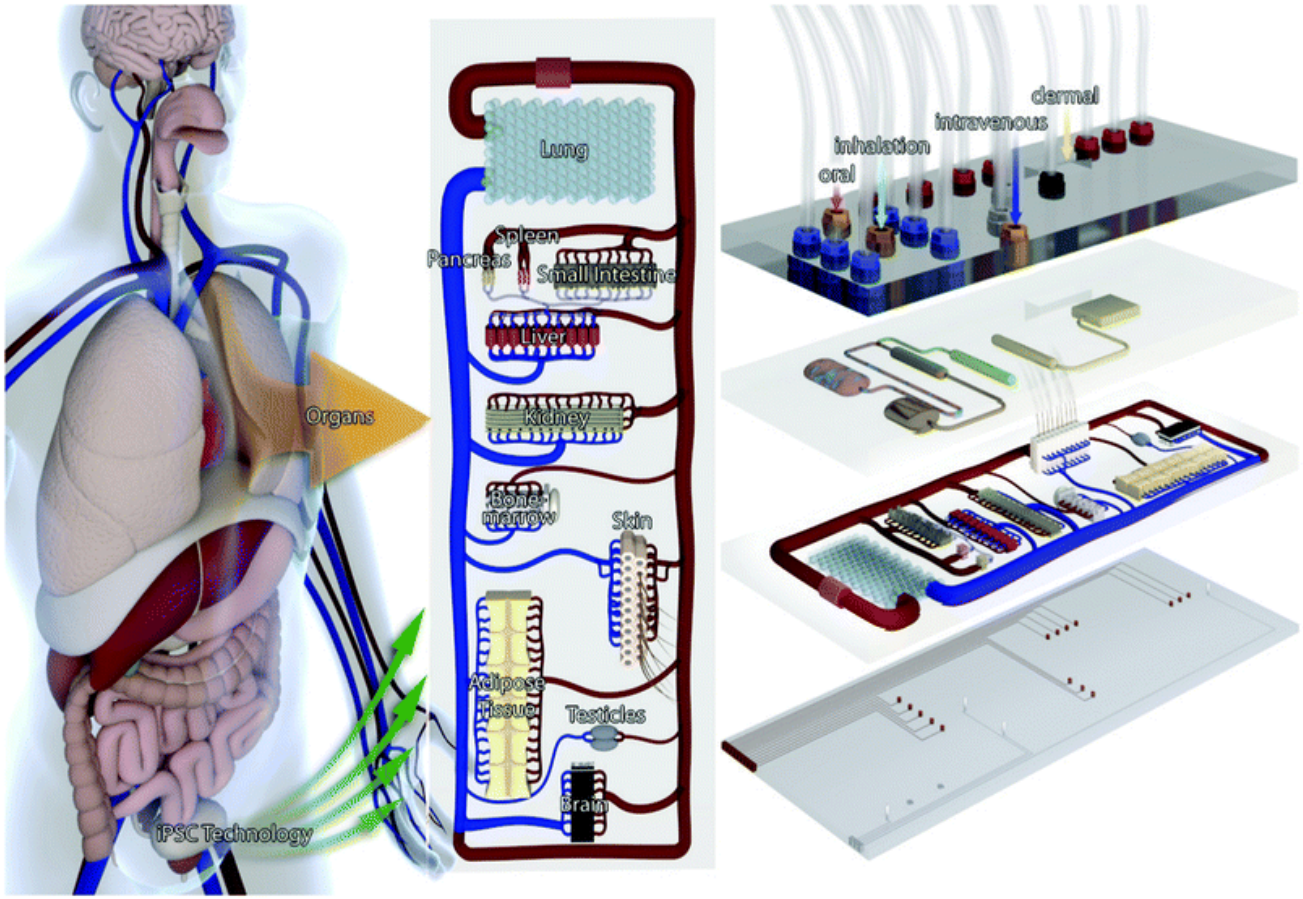
Nature 471, 661–665 (2011)

Biophysical Journal 94(5) 1854–1866

Organs on chip

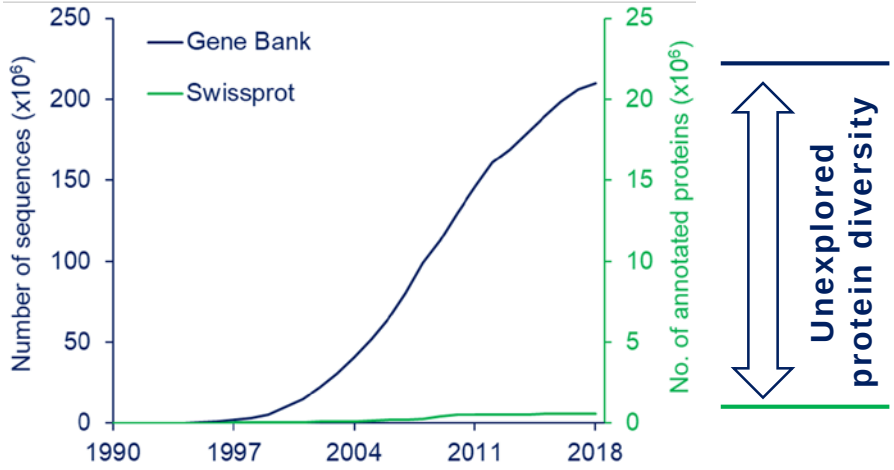


Human on chip concept



- 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

```
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 51-TGTNVEVCTTDDFTRGVDALVYEVDEARELTKIAYPVALFDRNITDGKAM-100
101-IASFLTLTMGNNQGMGDVEYAKMHDFYVPEAYRALFDGPSVNISALWKVL-150
151-GRPEVDGGLVVGTIIKPKLGLRPKPFAEACHAFWLGGDFIKNDEPQGNQP-200
201-FAPLRDTIALVADAMRRAQDETGEAKLFSANITADDPFEIIARGEYVLET-250
251-FGENASHVALLVDGYVAGAAAITTARRRFPDNFLHYHRAGHGAVTSPQSK-300
301-RGYTAFVHCKMARLQGASGIHTGTMGFGKMEGESSDRAIAYMLTQDEAQG-350
351-PFYRQSWGGMKACTPIISGGMNALRMPGFFENLGNANVILTAGGGAFGHI-400
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451-WRKALGVEDTRSALPA-466
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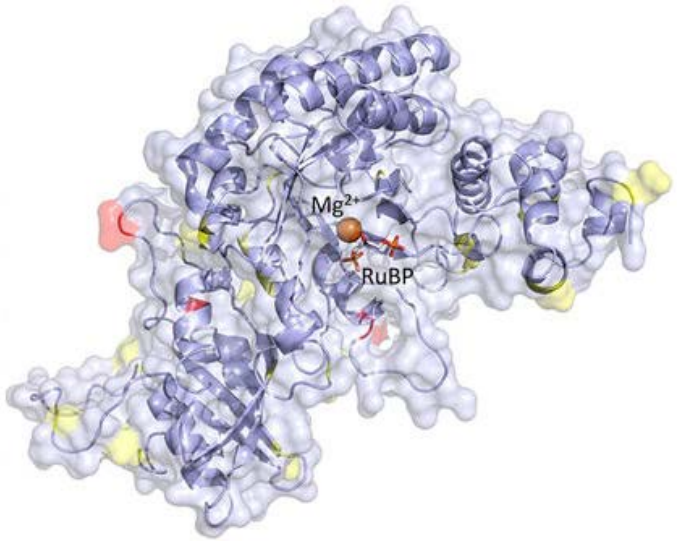
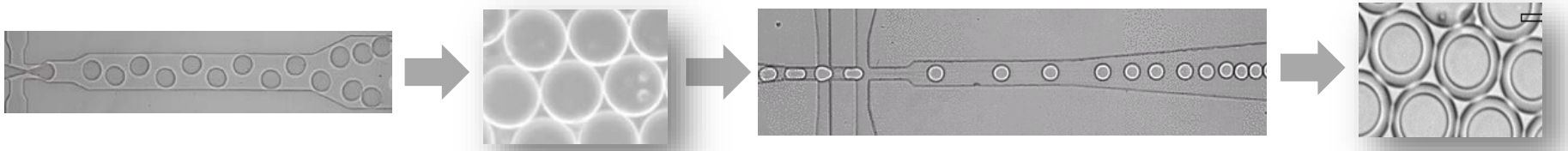
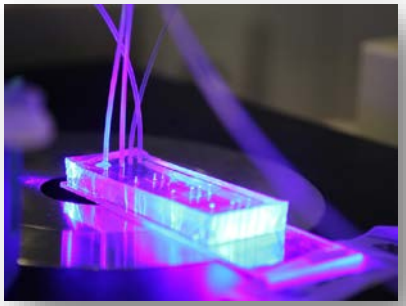
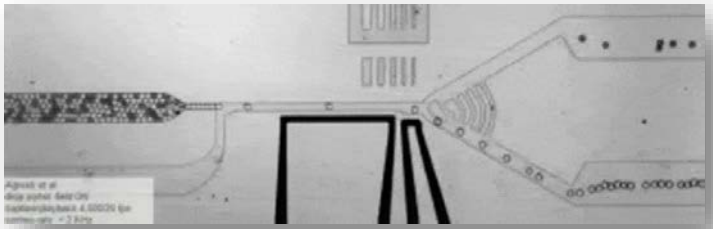
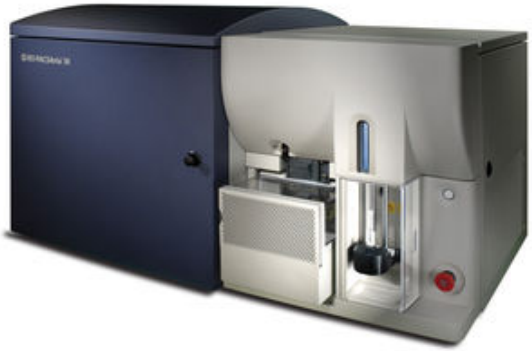


(Ultra)High-throughput screening

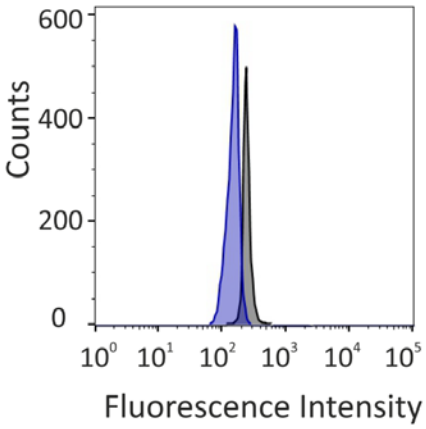




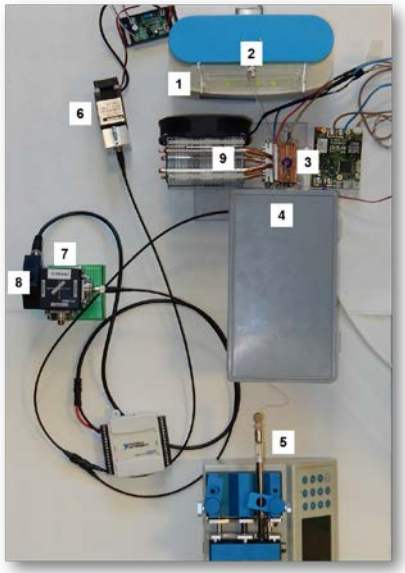


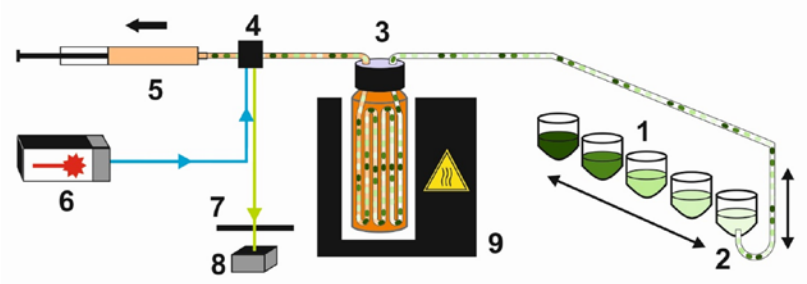


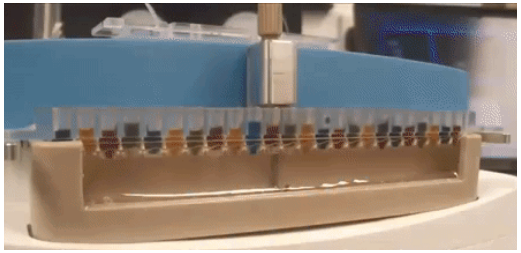
	Robotic	µFluidic
Reaction volume	100 µL	**5 pL**
Reactions / day	50 000	**$1 . 10^8$**
Total time	5 years	**3 days**
Total volume	5 000 L	**150 mL**
No. of plates / devices	250 000	**2.0**
No. of tips	28 000 000	**10**



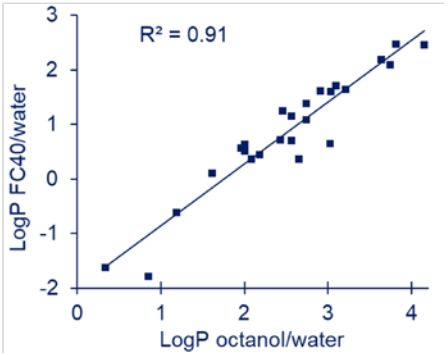
Enzyme specificity profiling

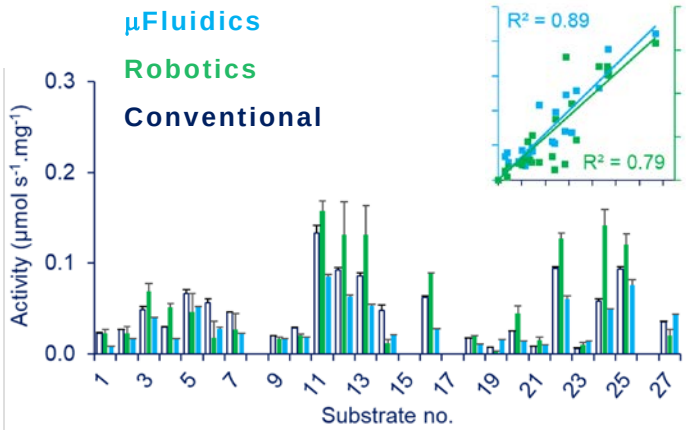


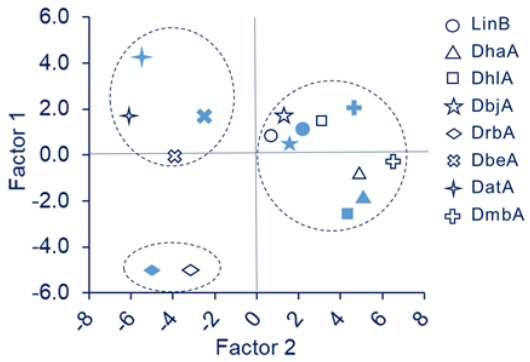


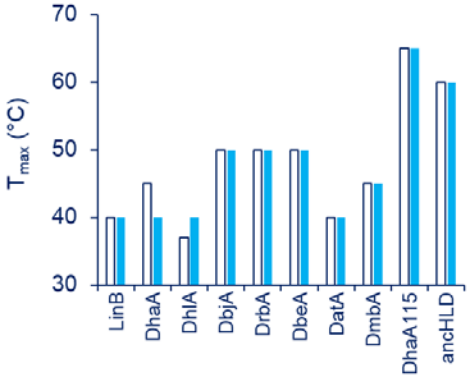


	Conventional	Robotic	μFluidic
Reaction volume (mL)	10	1	0.00015
Total enzyme (mg)	540	54	0.5
Total time (days)	100	30	5

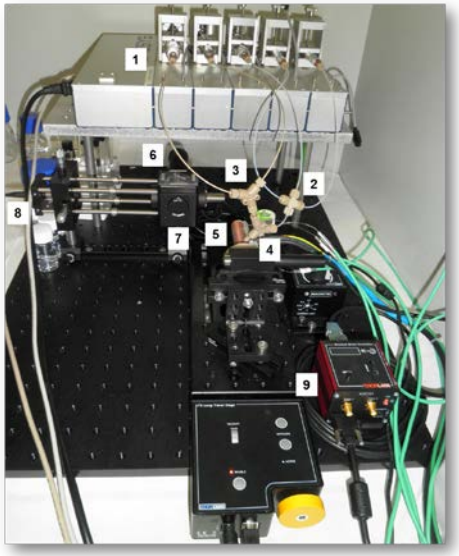


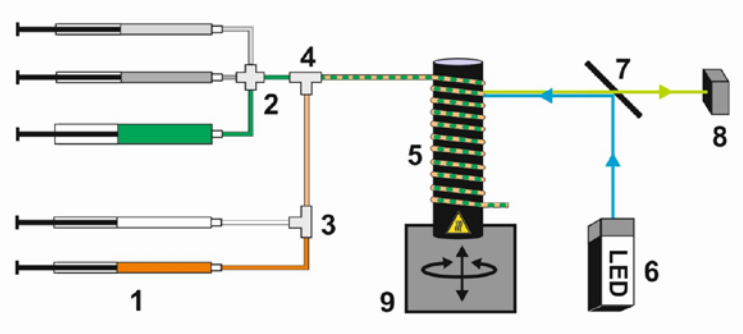




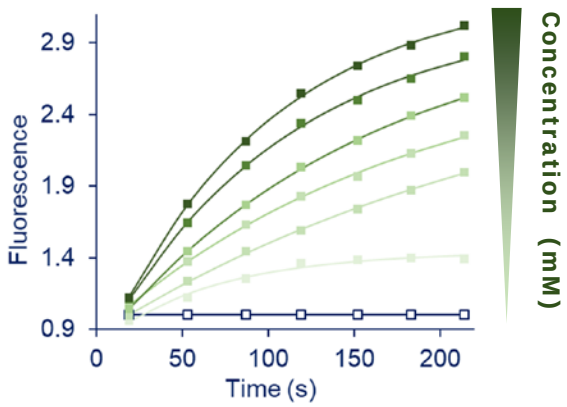


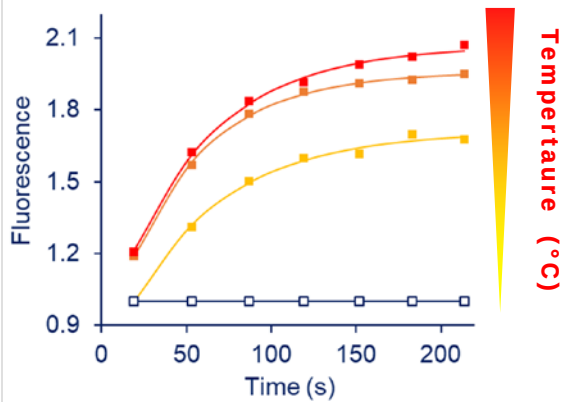
Steady-state kinetics

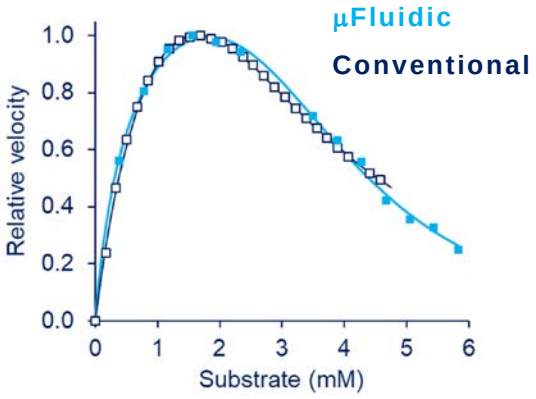




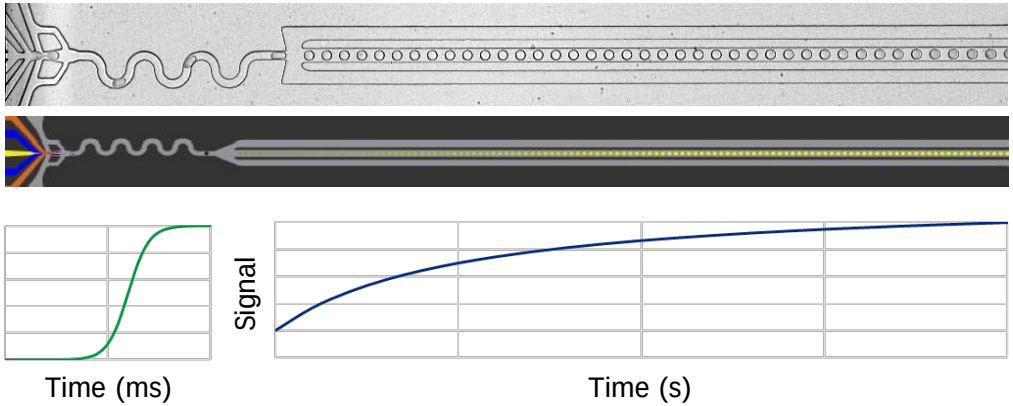
	Conventional	µFluidic
Reaction volume (mL)	2	0.00010
Total enzyme (mg)	1	0.01
Throughput per hour	5	10 000

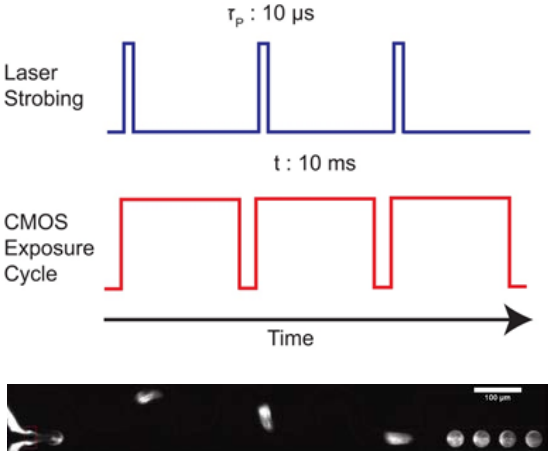


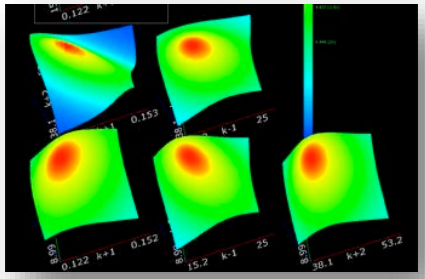




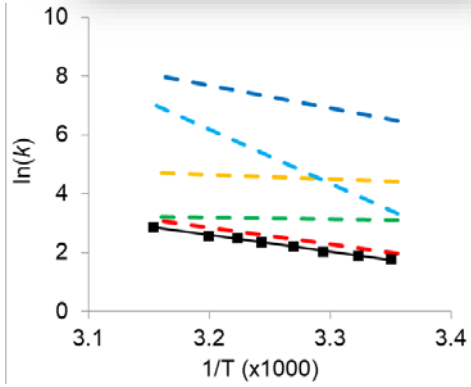
Mechanism and thermodynamics

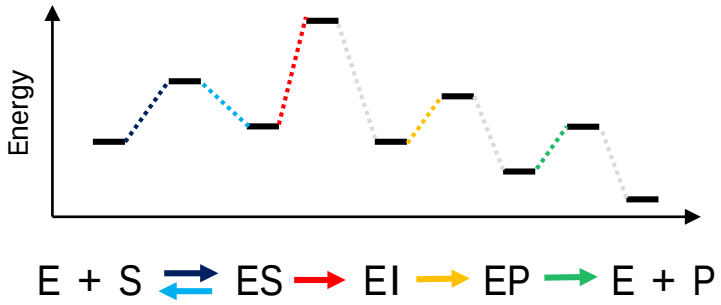


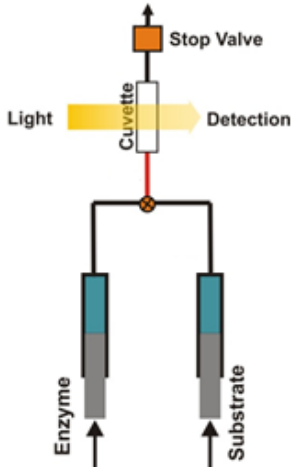




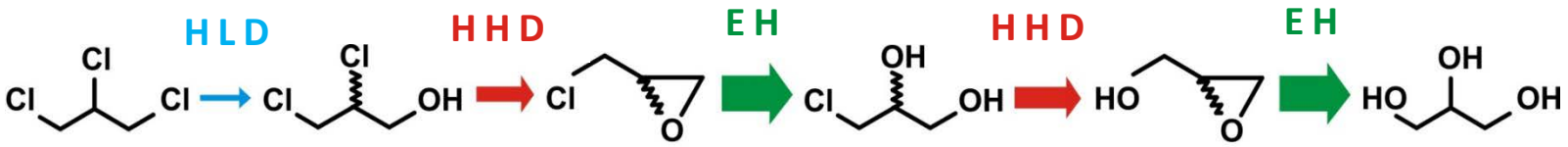
	Stopped-flow	µFluidic
Dead time	0.3 ms	0.7 ms
Reaction volume	100 µL	10 pL
Temp. equilibration	10 min	50 ms
Signal integration	0.5 ms	no limit



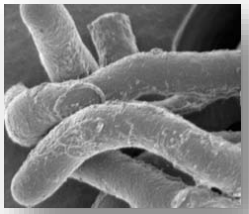




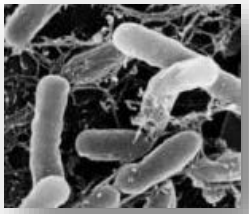
Multienzyme Systems



Rhodococcus



Agrobacterium



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

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

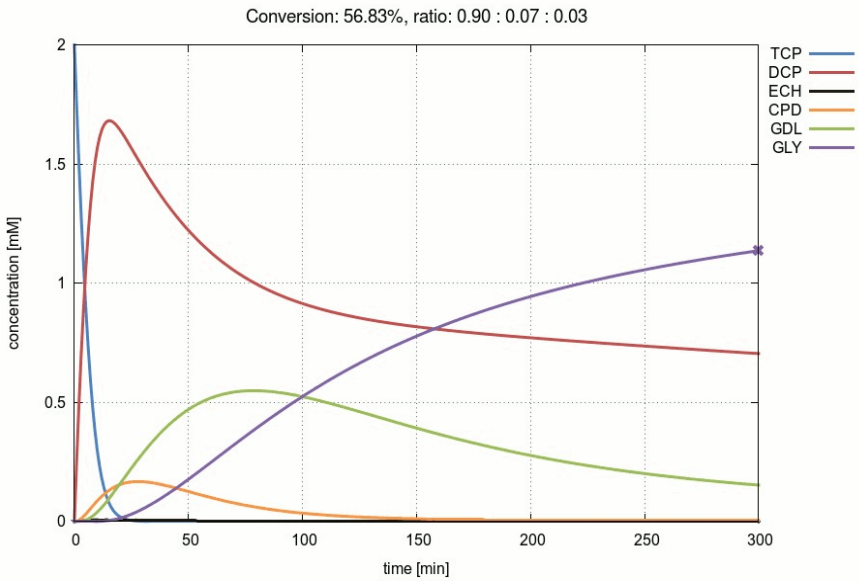
$$\frac{dc_{(S)-DCP}}{dt} = \frac{k_{cat,TCP,(S)-DCP} \times c_{DhaA} \times c_{TCP}}{c_{TCP} + K_{m,TCP}} - \frac{k_{cat,(S)-DCP} \times c_{HheC} \times c_{(S)-DCP}}{c_{(S)-DCP} + K_{m,(S)-DCP}}$$

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

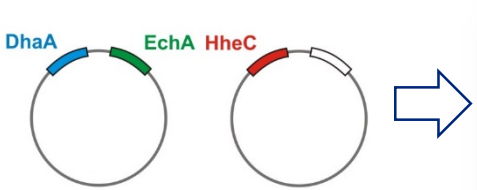
$$\frac{dc_{CPD}}{dt} = \frac{k_{cat,ECH} \times c_{EchA} \times c_{ECH}}{c_{ECH} + K_{m,ECH}} - \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}}$$

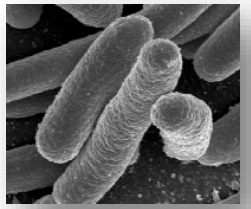
$$\frac{dc_{GDL}}{dt} = \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}} - \frac{k_{cat,GDL} \times c_{EchA} \times c_{GDL}}{c_{GDL} + K_{m,GDL} \times \left(1 + \frac{c_{GLY}}{K_i} + \frac{c_{TCP}}{K_c}\right)}$$

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



Escherichia

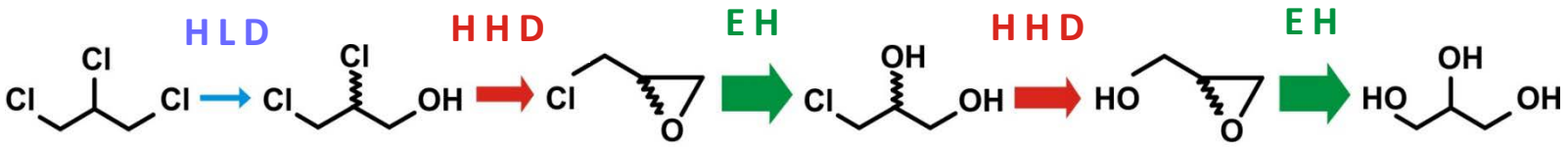


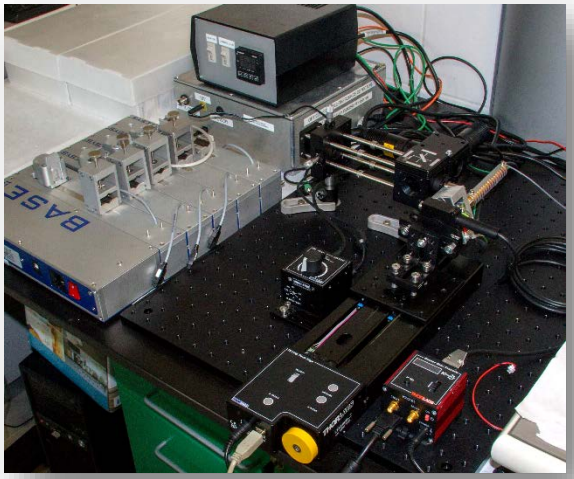


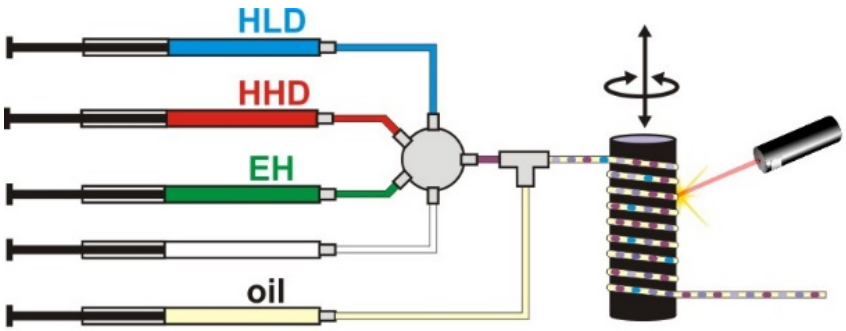
ChemBioChem 15: 1891 (2014)

ACS Synth. Biol. 3: 172 (2014)

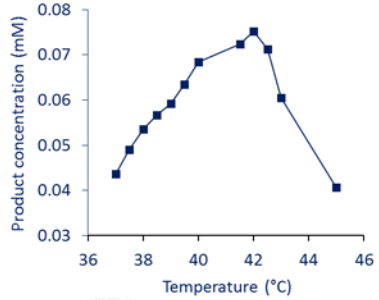
Multienzyme systems

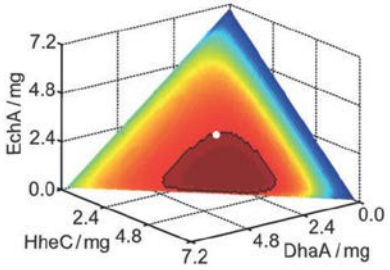






robot scientist



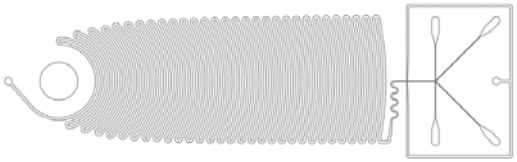


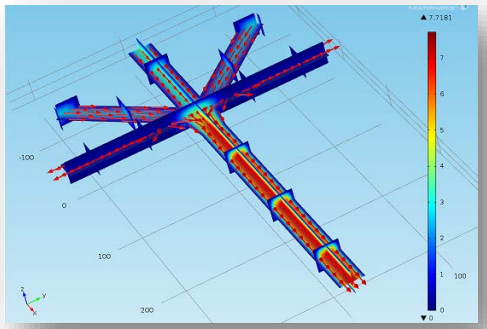
- 1 nL droplet volume
- 10 000 assays/hour

Design and fabrication

- **soft lithography** originates from semiconductor industry

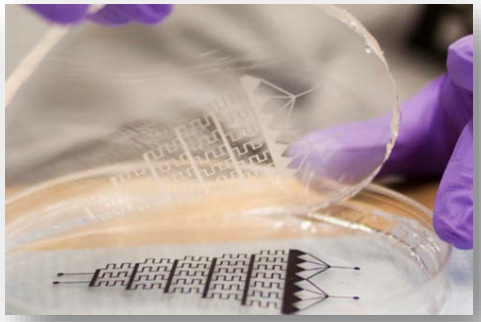
DESIGN / MODELING





MASK / MOLD

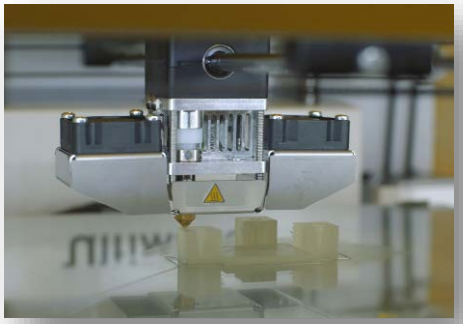
CASTING / BONDING

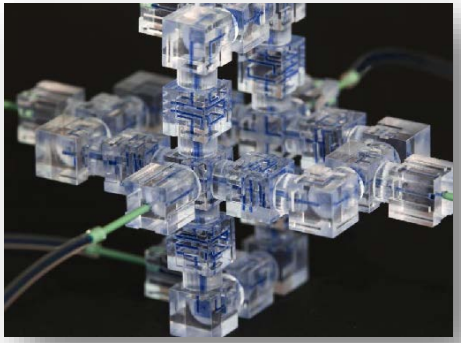


Design and fabrication

- **direct fabrication methods**

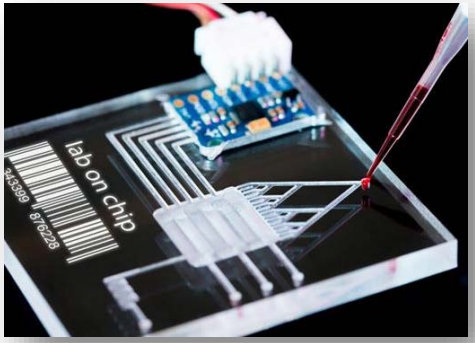
3D PRINTING





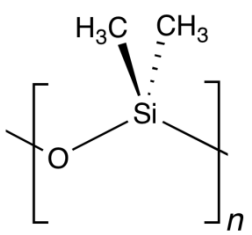
LASER CUTTING

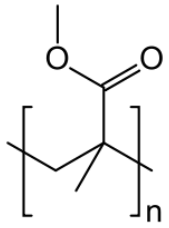
CNC μ-MILLING

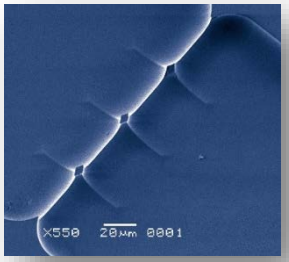


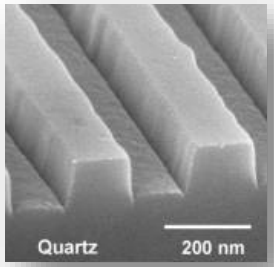
Design and fabrication

- **materials**
  - inert and transparent
  - PDMS - poly(dimethyl siloxane)
  - PMMA - poly(methyl methacrylate)
  - fused silica, quartz and glass

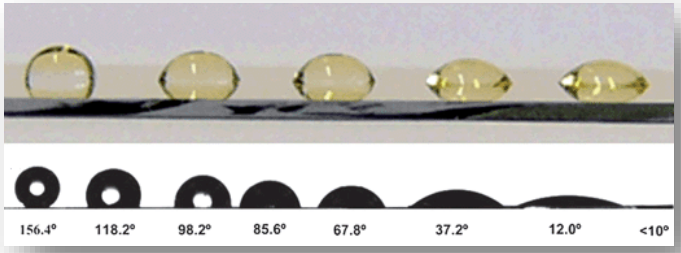


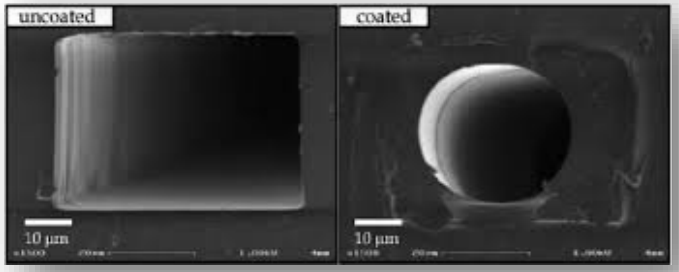






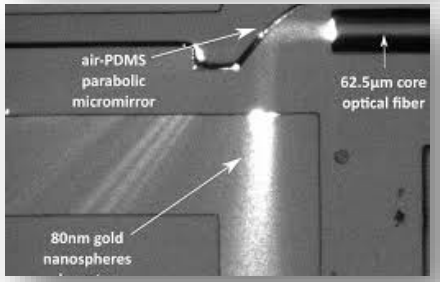
- **surface modification**
  - plasma treatment
  - silanization
  - sol–gel coating

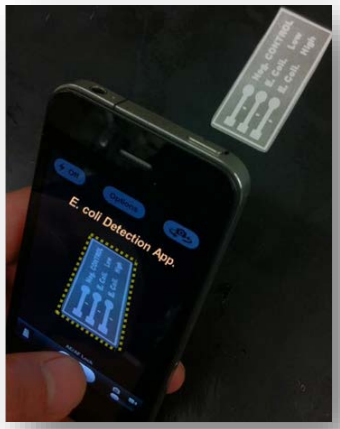




Sensing and detection

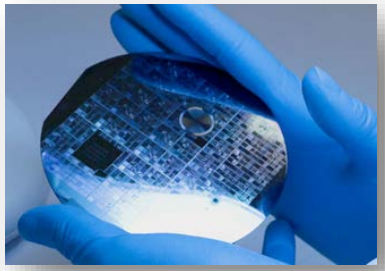
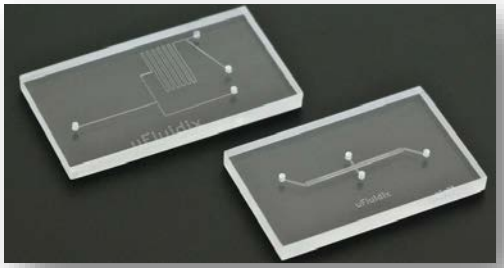
- processing of **small reagent volumes**
- **analytical timescale** and performance
- **on chip detection**
  - fluorescence (LSM, FCS, FLIM)
  - UV/VIS absorbance
  - IR spectroscopy
  - Raman scattering
  - (chemo/electro) luminescence
  - thermal conductivity
  - RI variation
- **off chip detection**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





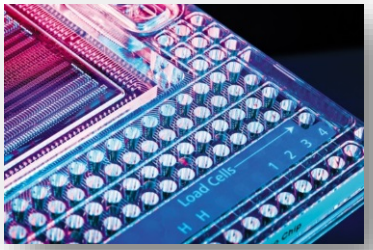
Commercial Solutions

- 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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[2] Department of Materials Science and Engineering, University of Texas, Arlington, TX, USA