Microfluidics – „Lab on a Chip“

Outline

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

Lab on a Chip Concept

pre-treatment

incubation

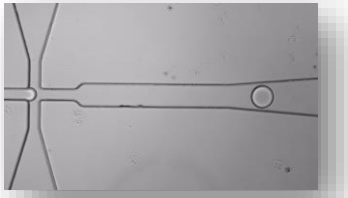
analysis

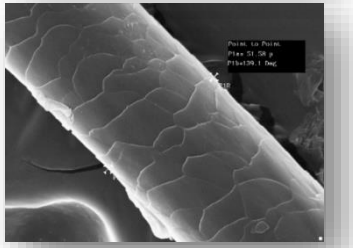
preparation

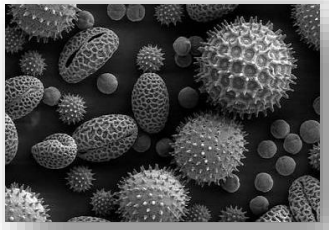
collection

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

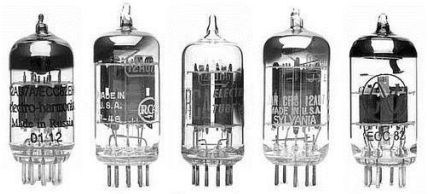


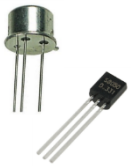




Nature 507, 181 (2014)

Miniaturization & integration

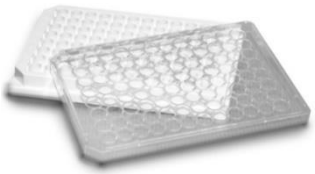


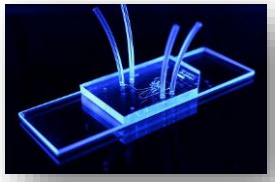


	vacuum tubes	transistors	micro chips
size (mm)	100	10	0.000 01
price (USD)	10	1	0.000 000 1

	test tube	microtiter plate	µ-fluidic chip
volume (µL)	1 000	10	0.000 001
throughput (assays/day)	10	1 000	1 000 000

?

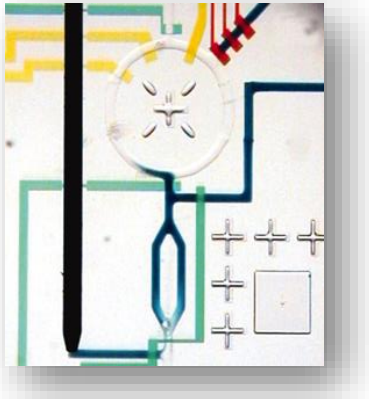




Concepts in microfluidics

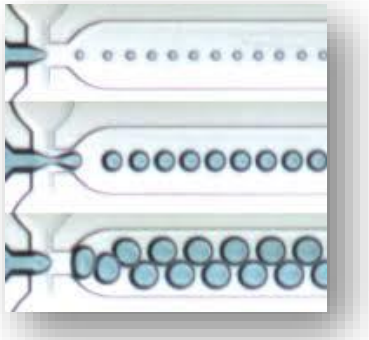
- **continuous-flow microfluidics**

  manipulation of continuous liquid flow through micro-fabricated channels



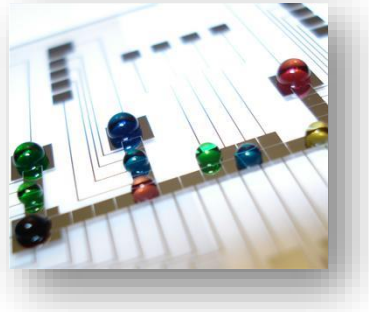
- **droplet-based microfluidics**

  manipulating discrete volumes of fluids in immiscible phases



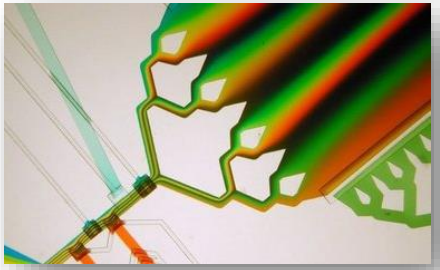
- **digital microfluidics**

  droplets manipulated on a substrate using electro-wetting

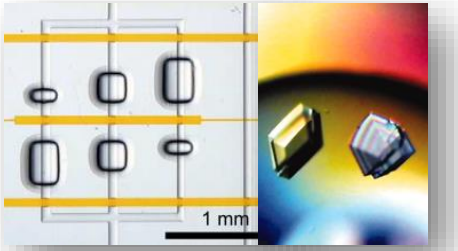


Novel Physics of Micro-Scale

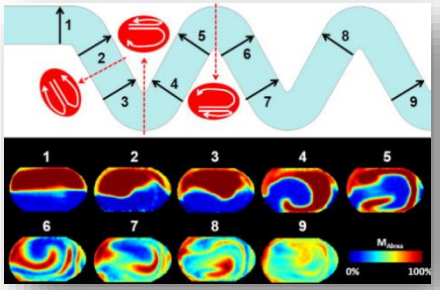
- viscosity, surface tension and capillary forces dominate
  - **lack of turbulent phenomena**



  - **absence of density-driven convection**



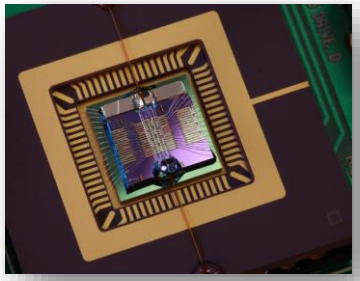
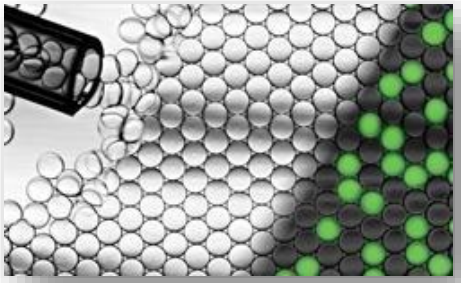
  - **strong shearing forces**

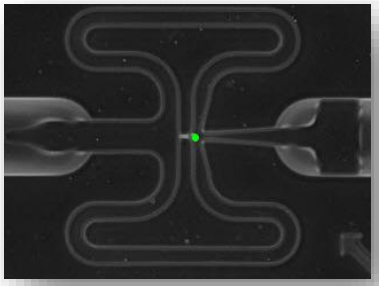
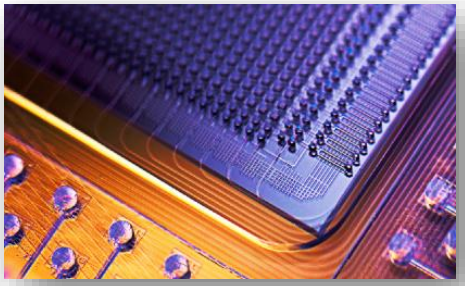


Nature Biotechnol. 20, 826 (2002) *Appl. Phys. Lett. 83, 4664 (2003)* *PNAS 99, 16531 (2002)*

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

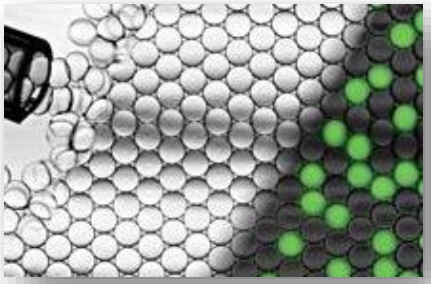
- **classical PCR**
  - slow heating/cooling cycles
  - PCR tubes (strips), 96-well MTP
  - volume 50 to 500 µL

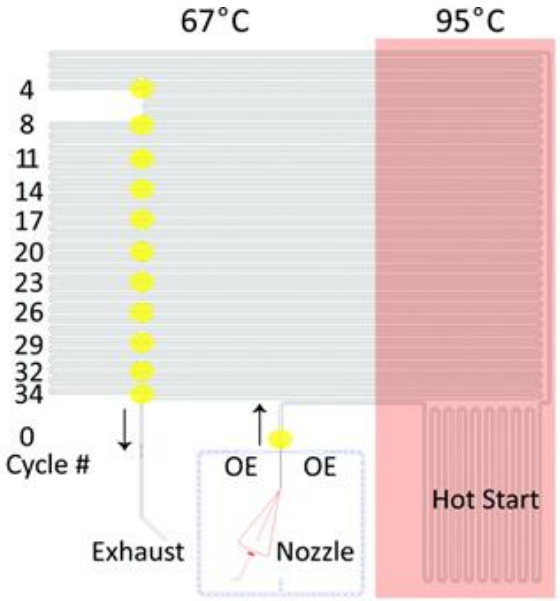
Kary Mullis

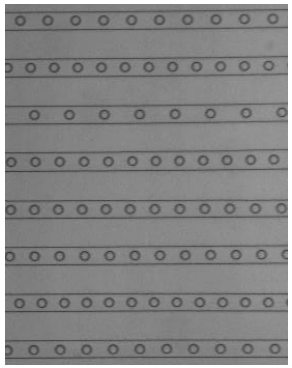
Nobel Prize in 1993

Digital polymerase chain reaction

- **digital PCR (single molecule)**
  - 1 nanoliter droplets
  - 20 000 droplets per run
  - fast heat transfer





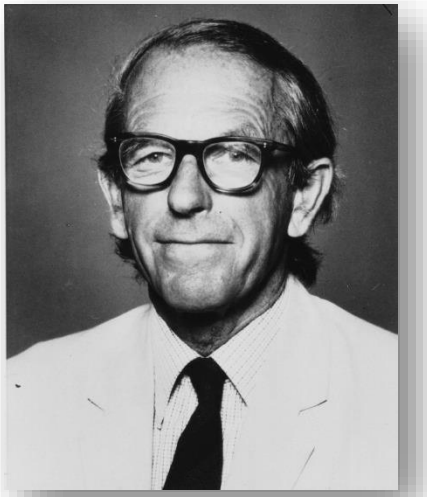


Next-generation sequencing

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

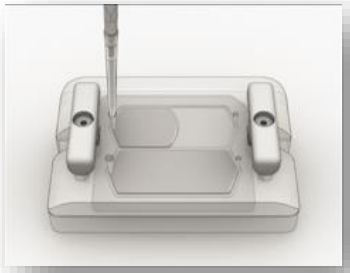
  water in oil droplets 1 picoliter (10^{-12} liters)

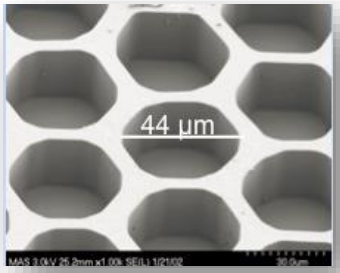
  1 mil. reads/run, 10 USD/Mbase

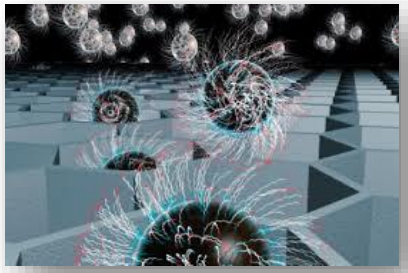


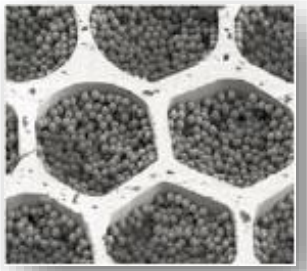
Frederick Sanger

Nobel Prize in 1980

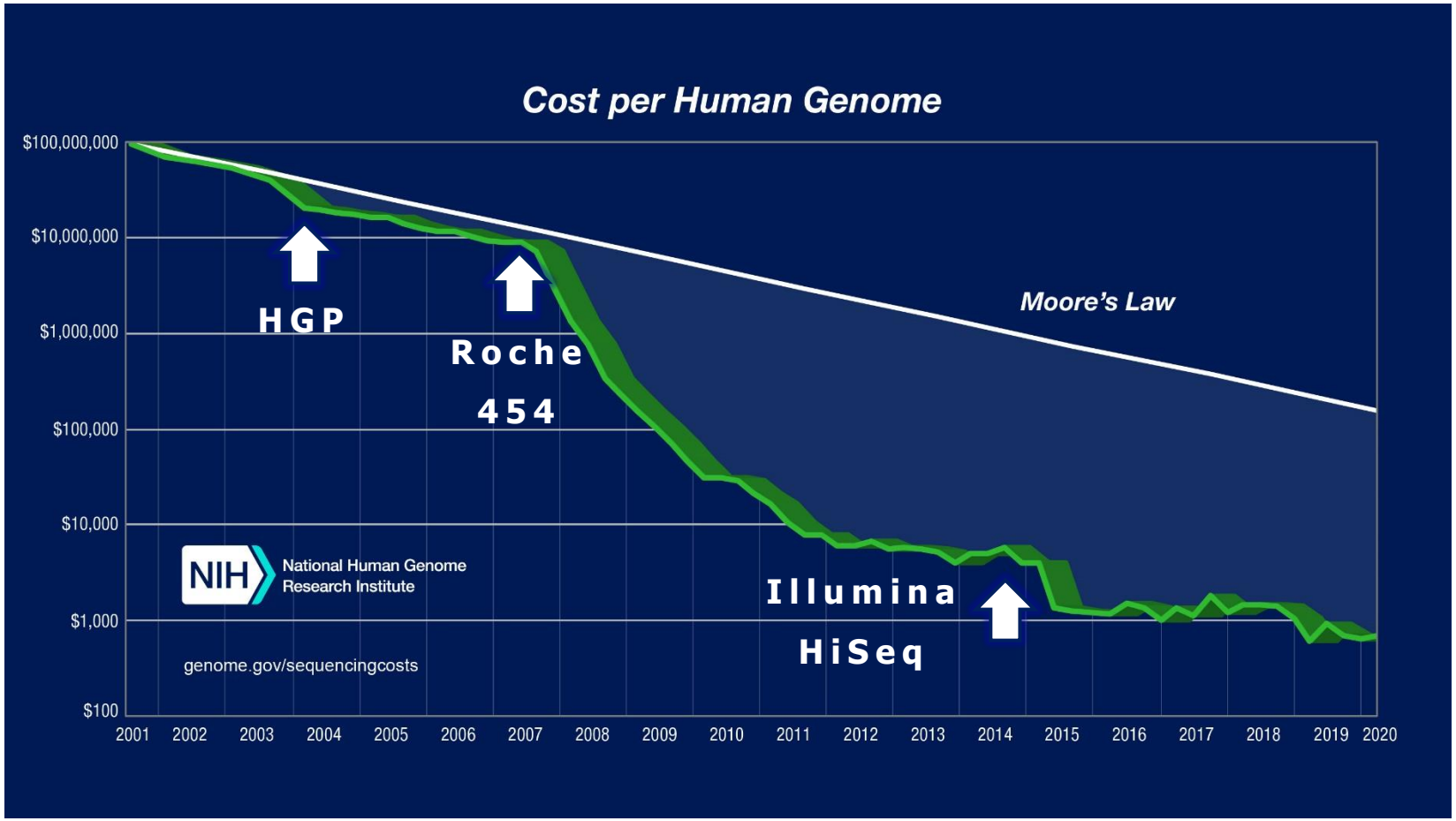








Revolution in DNA analysis



- 2003: 13 years, 3 billion USD
- 2018: days, < 1,000 USD

Organ(oid)s on chip

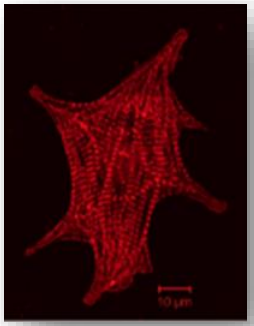
- 3D chips **mimicking human's physiological responses**

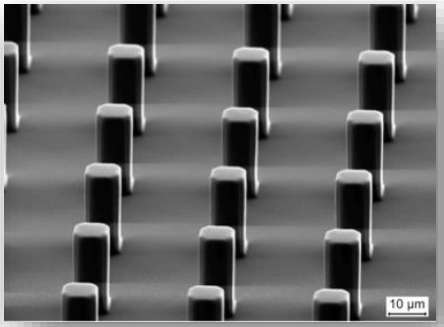
  (e.g., pathological, pharmacokinetic, toxicological)

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

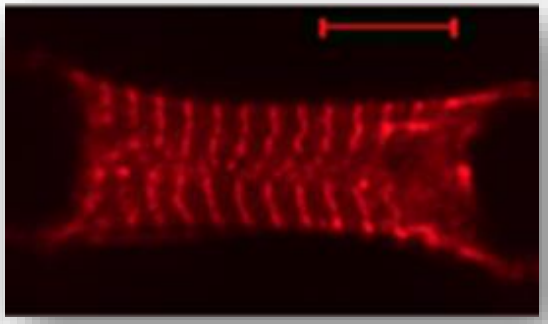
  (e.g., mechanical strain, patterning, fluid shear stresses)

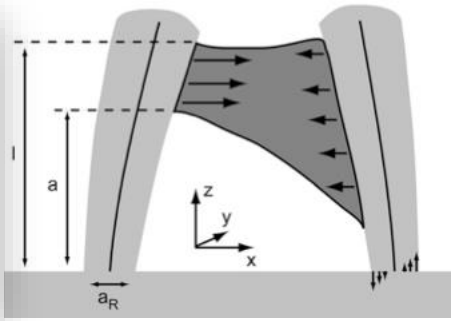
flat surface





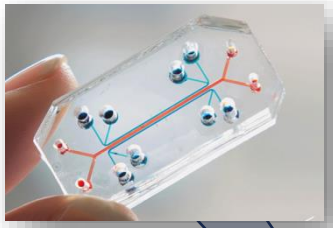
micropillar



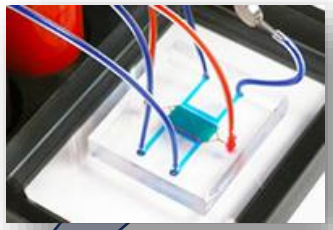


Organs on chip

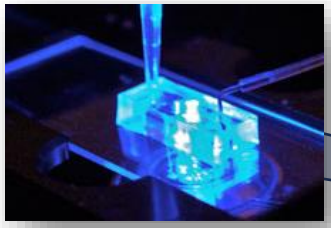
Lung

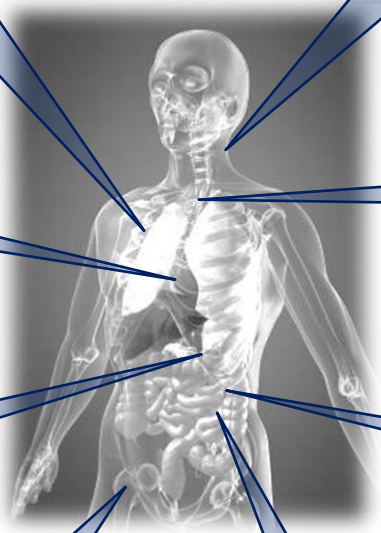


Neurovascular



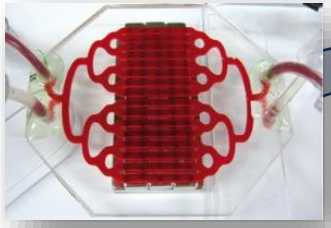
Heart





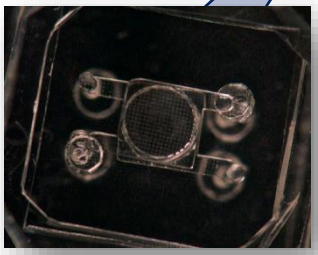
Artery

Spleen

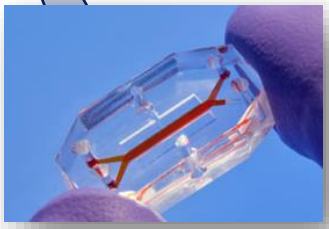


Kidney

Bone



Intestine



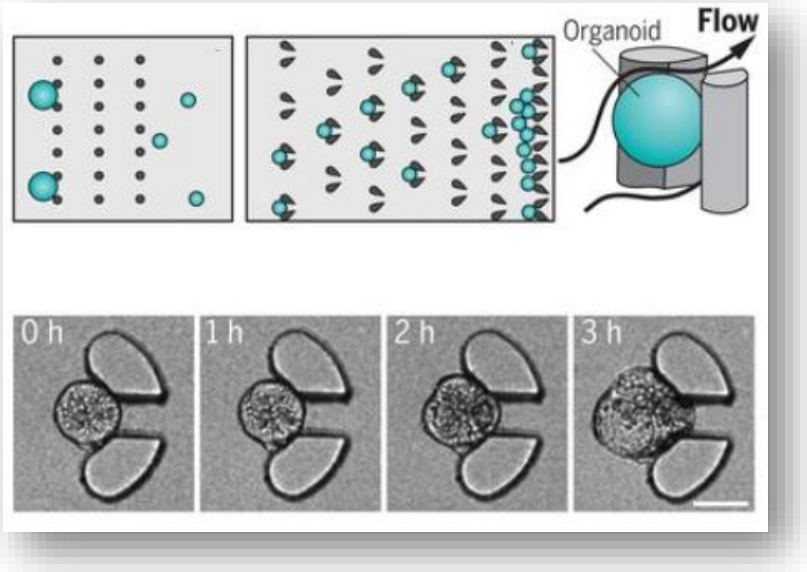
Organ(oid)s on chip

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

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

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

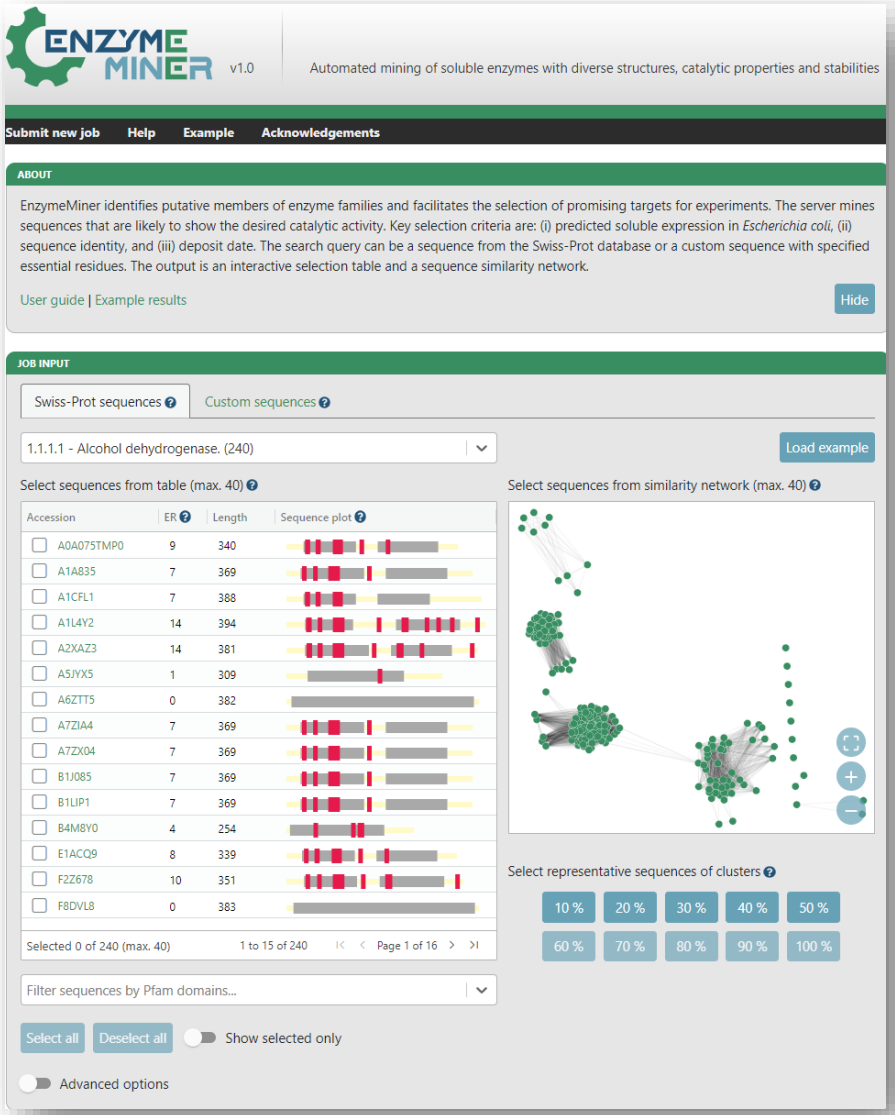
  (e.g., mechanical strain, patterning, fluid shear stresses)



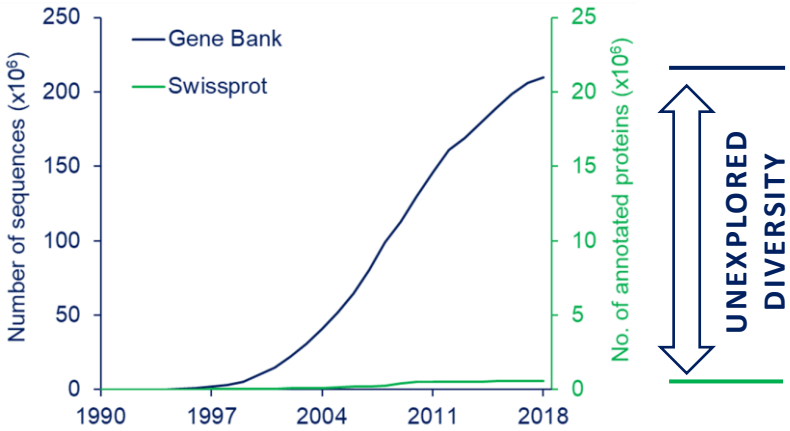
- **can replace** expensive and controversial **animal testing**

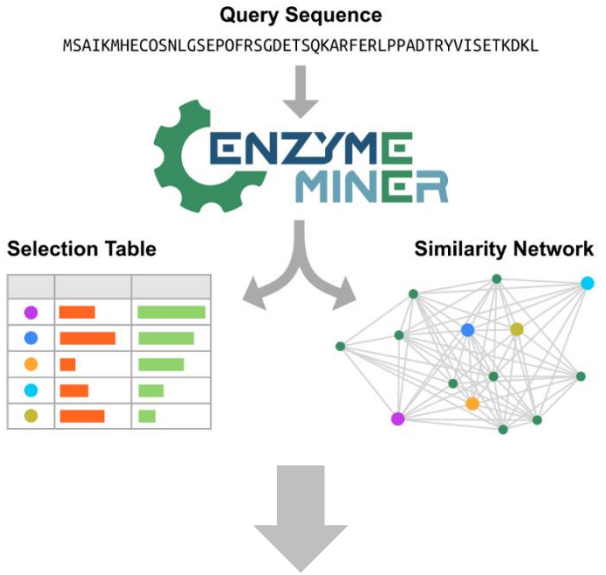
Nature 471, 661–665 (2011) *Science 364, 960-965 (2019)*

Protein Discovery and Engineering



Nucleic Acids Res. 48, W104–W109 (2020)





FUNCTIONAL CHARACTERISATION

Protein Discovery and Engineering

```
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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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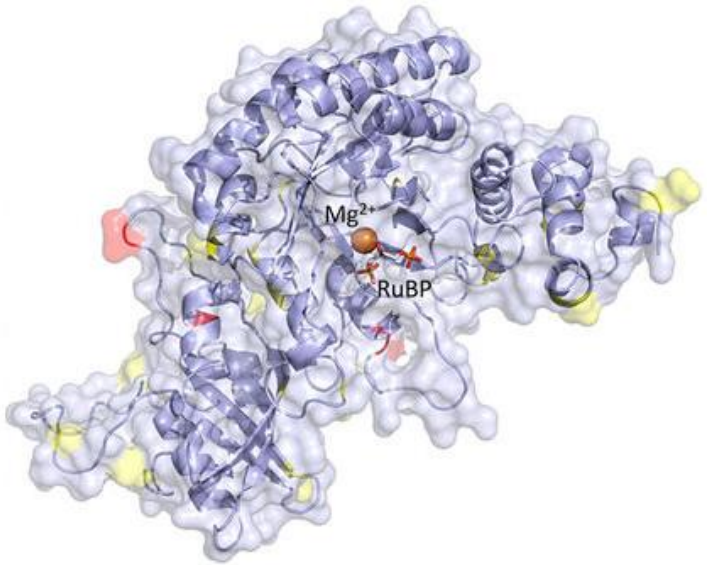
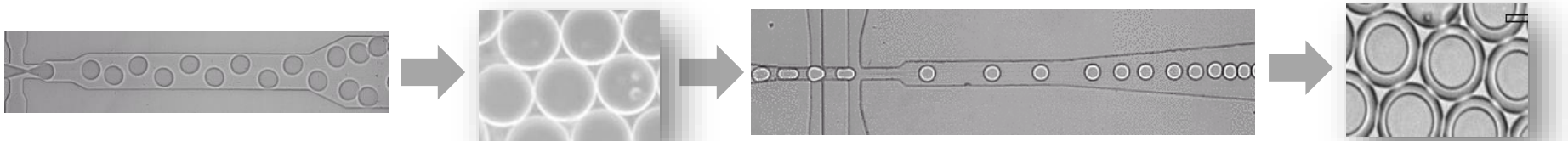


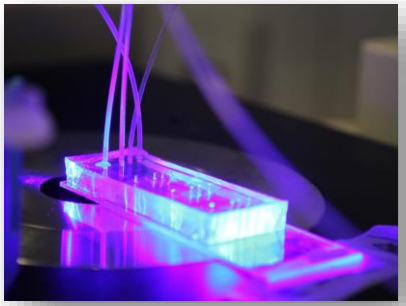
No.	Coverage (95%)
1	94
2	3 066
3	98 163
4	3 141 251
5	100 520 093

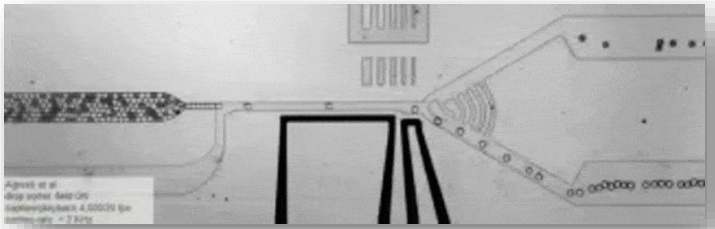
SCREENING AND IDENTIFICATION OF POSITIVE HITS

FUNCTIONAL CHARACTERISATION

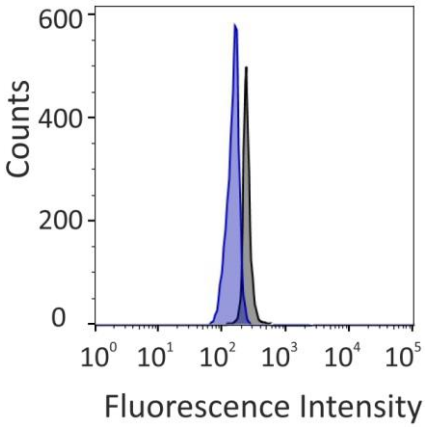
(Ultra)High-throughput screening







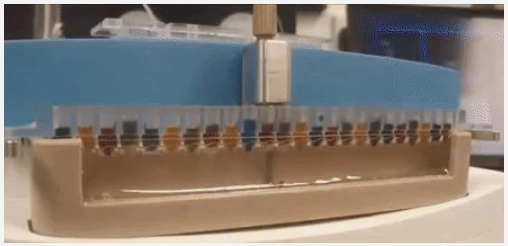
	Robotic	µFluidic
Reaction volume	100 µL	**5 pL**
Reactions / day	50 000	**$1 . 10^8$**
Total time	5 years	**3 days**
Total volume	5 000 L	**150 mL**
No. of plates / devices	250 000	**2.0**
No. of tips	28 000 000	**10**

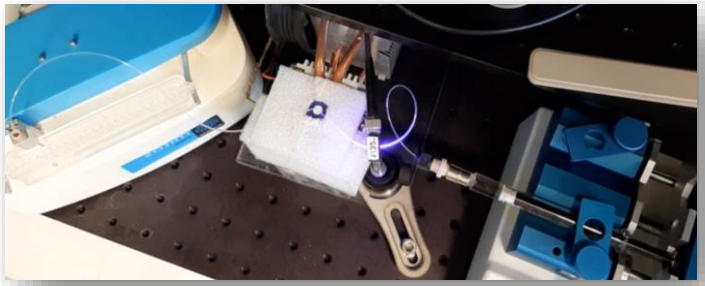


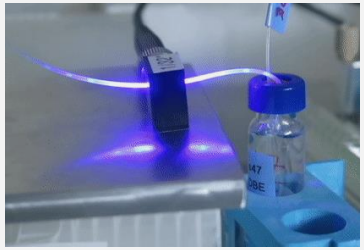
Lab Chip 2009, 9: 1850

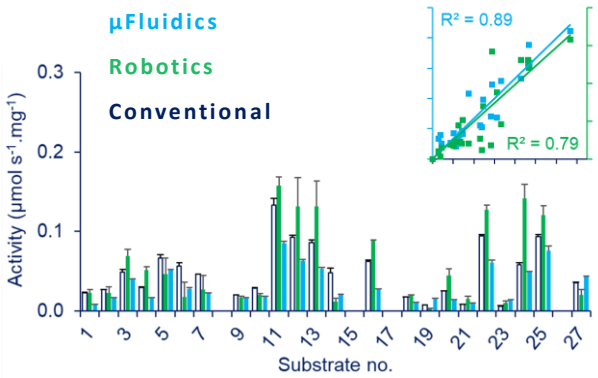
Anal. Chem. 2014, 86: 2526

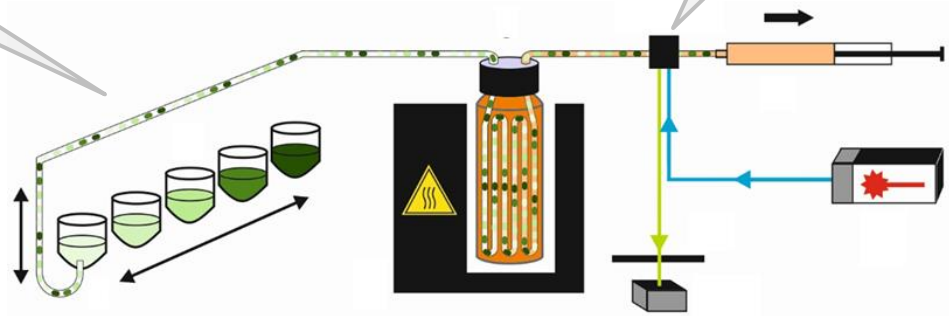
Activity and specificity in µ-droplets

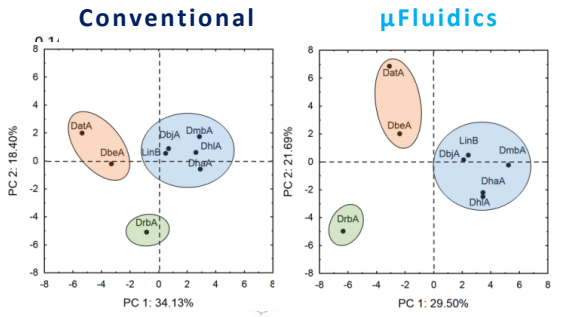




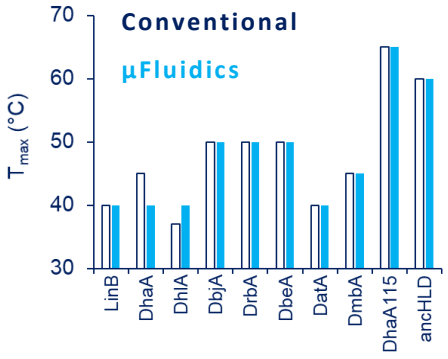








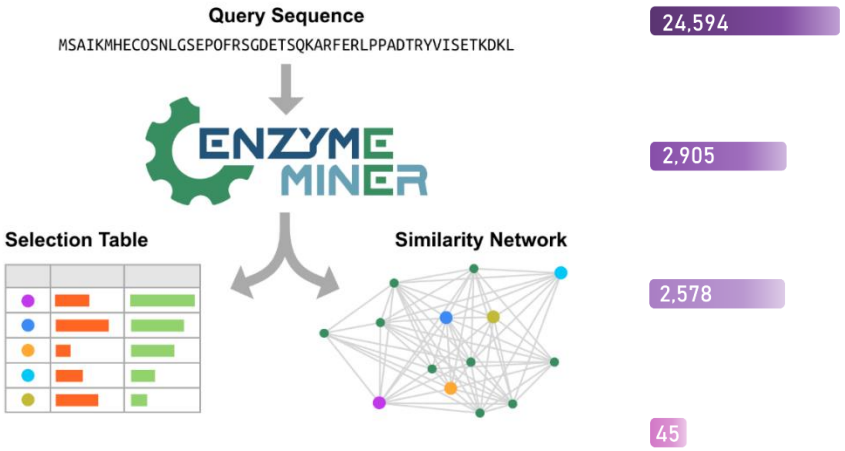
	Test tube	Robotic	µFluidic
Reaction volume (mL)	10	1	0.000 15
Total enzyme (mg)	540	54	0.5
Total time (days)	100	30	5



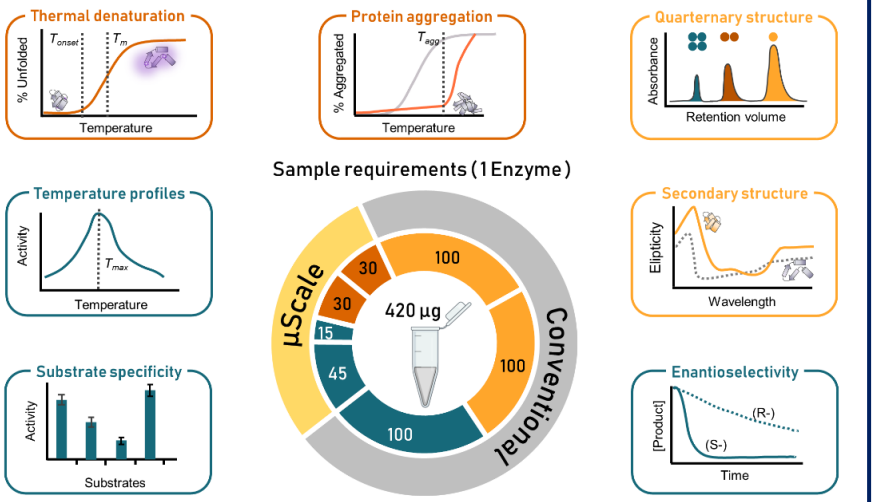
Anal. Chem. 2019, 91: 10008-10015

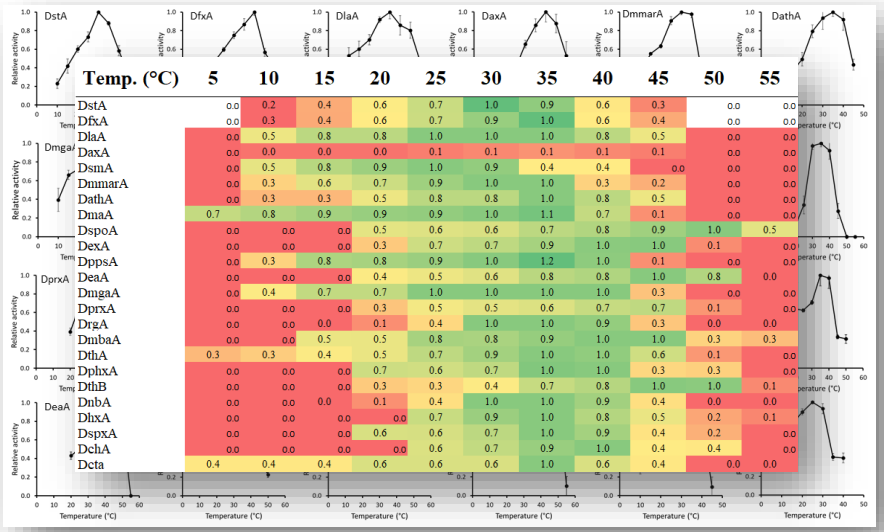
Activity and specificity in µ-droplets

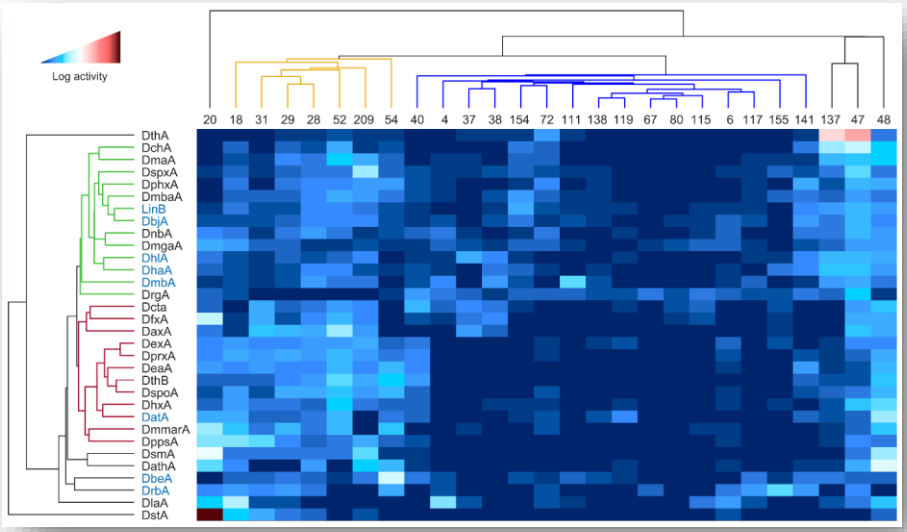
IN SILICO SCREENING



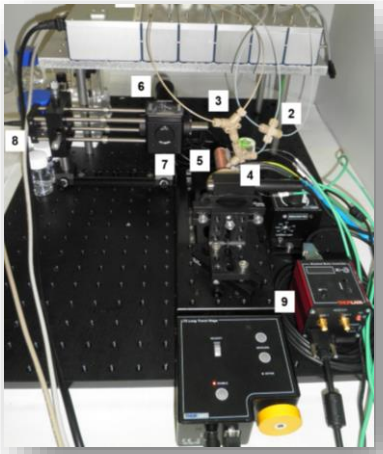
HIGH-THROUGHPUT CHARACTERISATION

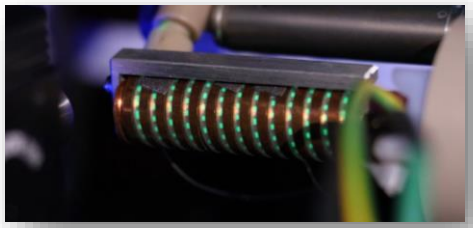


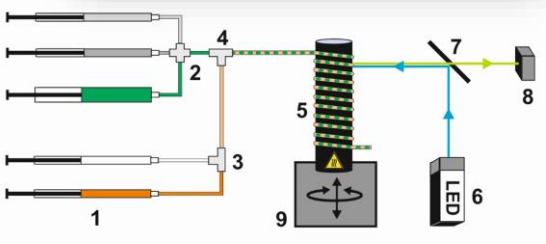


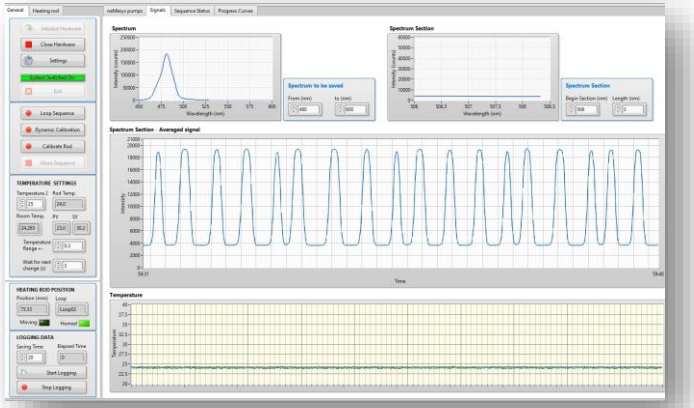


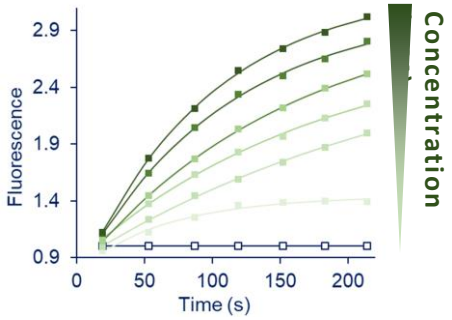
Steady-state kinetics in µ-droplets



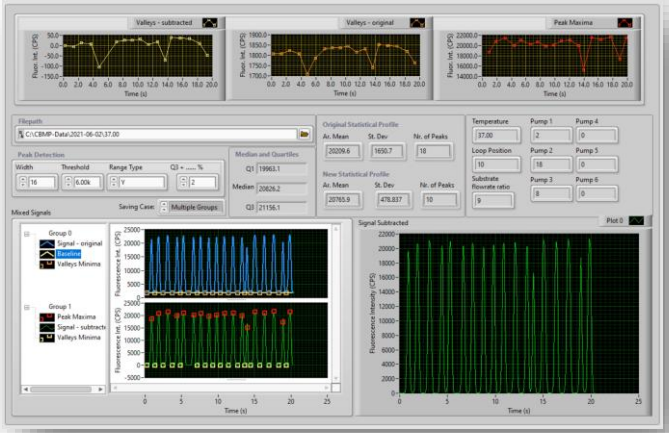


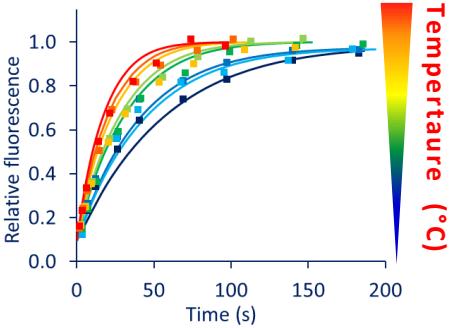


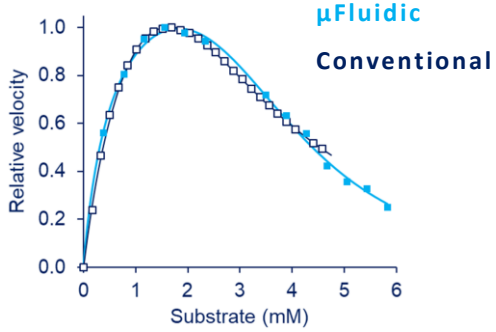




$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} E.S \xrightarrow{k_{cat}} E + P$$
$$E.S + S \rightleftharpoons S.E.S \quad (K_i)$$

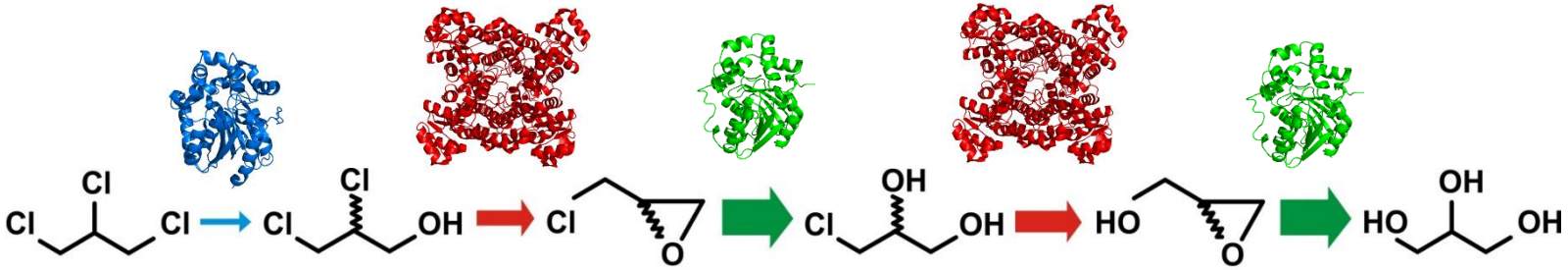




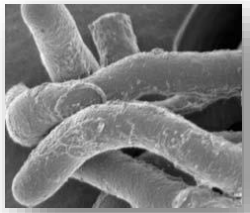


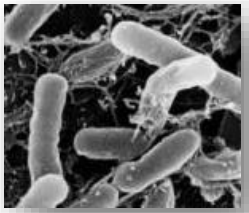
	Test tube	µFluidic
Reaction volume (mL)	10	0.000 1
Total enzyme (mg)	1	0.01
Throughput per hour	50	10 000

Synthetic biology



Rhodococcus *Agrobacterium*





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

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

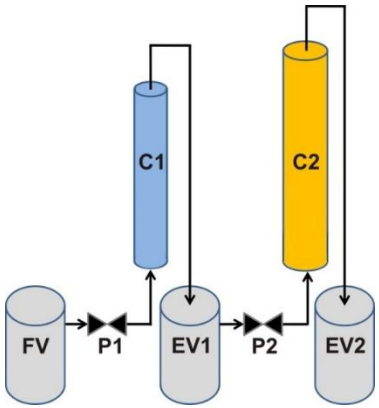
$$\frac{dc_{(S)-DCP}}{dt} = \frac{k_{cat,TCP,(S)-DCP} \times c_{DhaA} \times c_{TCP}}{c_{TCP} + K_{m,TCP}} - \frac{k_{cat,(S)-DCP} \times c_{HheC} \times c_{(S)-DCP}}{c_{(S)-DCP} + K_{m,(S)-DCP}}$$

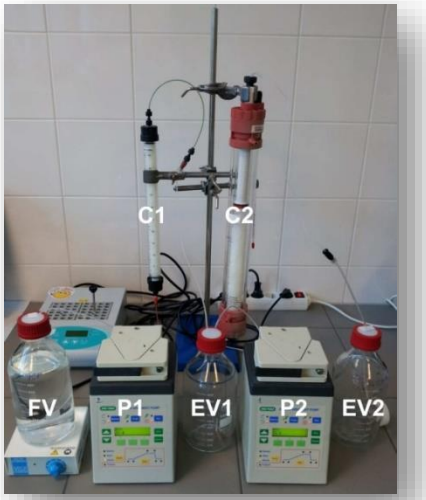
$$\frac{dc_{ECH}}{dt} = \frac{k_{cat,(R)-DCP} \times c_{HheC} \times c_{(R)-DCP}}{c_{(R)-DCP} + K_{m,(R)-DCP}} + \frac{k_{cat,(S)-DCP} \times c_{HheC} \times c_{(S)-DCP}}{c_{(S)-DCP} + K_{m,(S)-DCP}} - \frac{k_{cat,ECH} \times c_{EchA} \times c_{ECH}}{c_{ECH} + K_{m,ECH}}$$

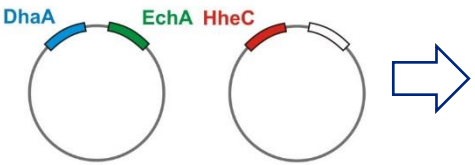
$$\frac{dc_{CPD}}{dt} = \frac{k_{cat,ECH} \times c_{EchA} \times c_{ECH}}{c_{ECH} + K_{m,ECH}} - \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}}$$

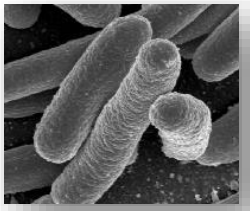
$$\frac{dc_{GDL}}{dt} = \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}} - \frac{k_{cat,GDL} \times c_{EchA} \times c_{GDL}}{c_{GDL} + K_{m,GDL} \times \left(1 + \frac{c_{GLY}}{K_i} + \frac{c_{TCP}}{K_c}\right)}$$

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

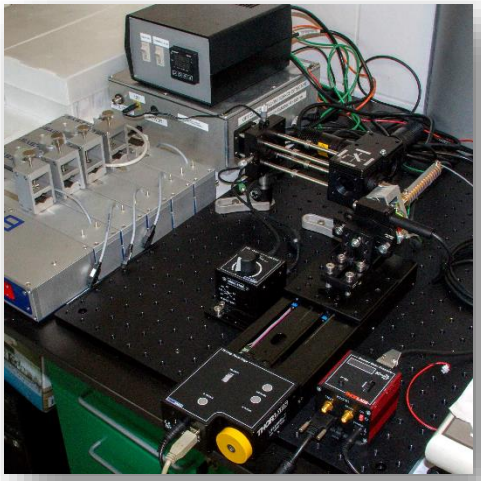


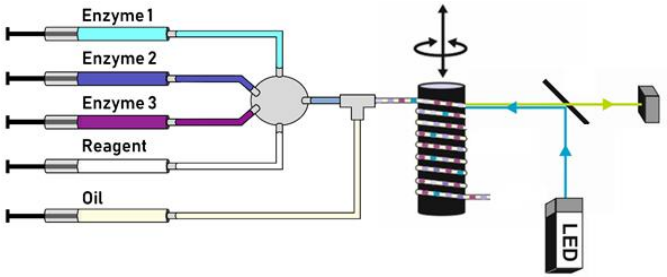


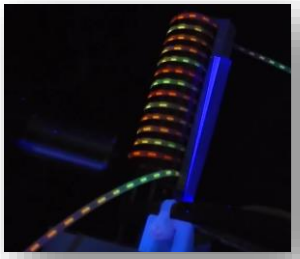


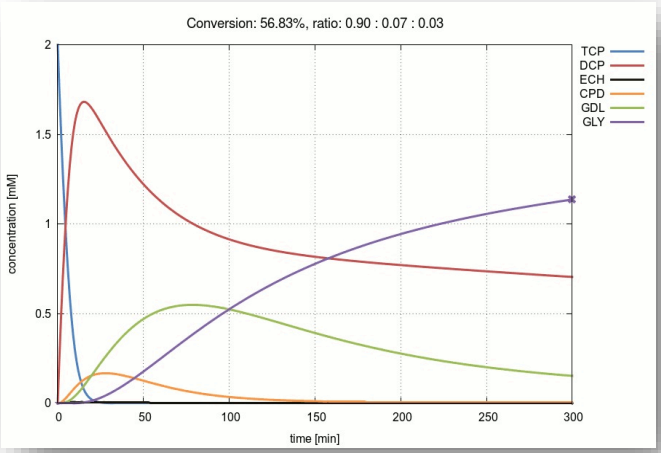


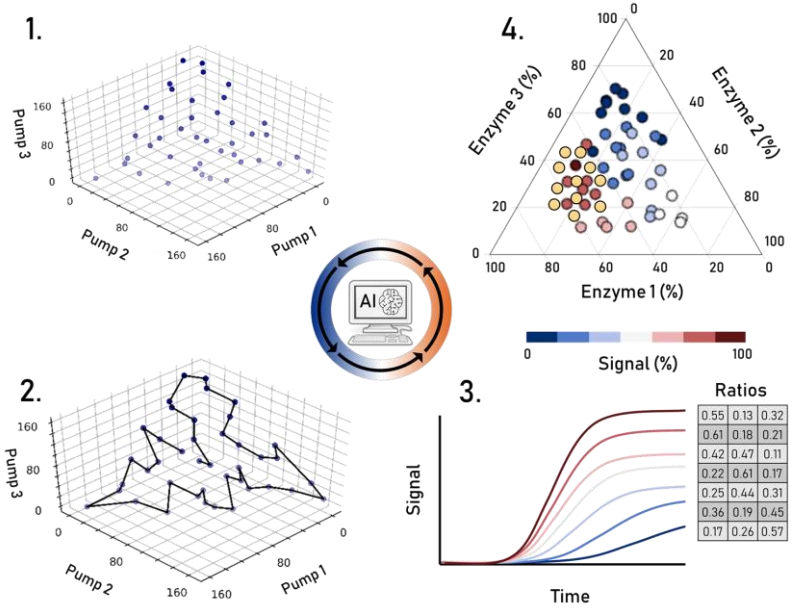
Enzyme cascades in µ-droplets











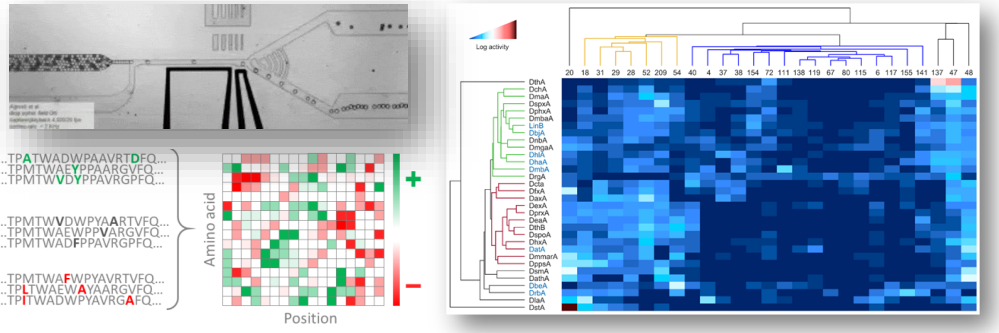
AI in protein engineering

ACS Catalysis
Perspective
Cite This: ACS Catal. 2020, 10, 1210−1223
pubs.acs.org/acscatalysis

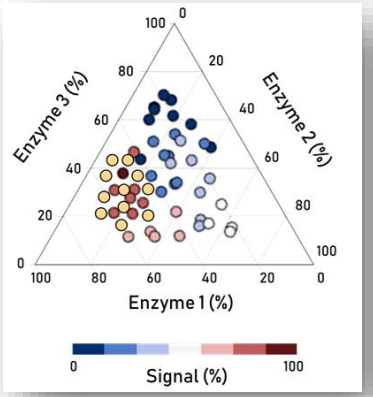
Machine Learning in Enzyme Engineering

Stanislav Mazurenko,*,† Zbynek Prokop,†,‡ and Jiri Damborsky†,‡

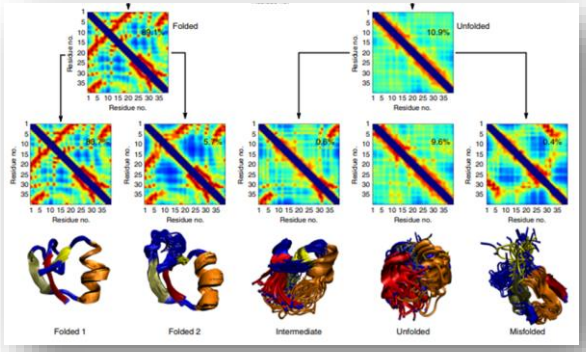
SEQUENCE BASED PREDICTION
supervised learning



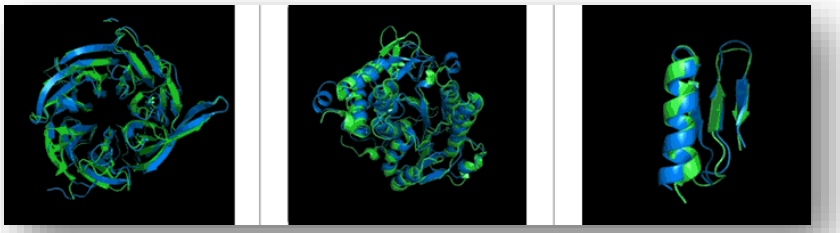
ITERATIVE OPTIMIZATION
Bayesian optimization



MOLECULAR DYNAMICS
unsupervised learning

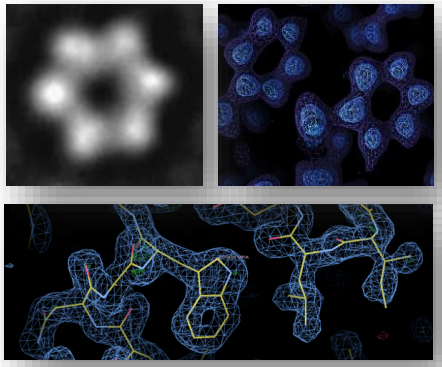


STRUCTURE PREDICTION
deep learning

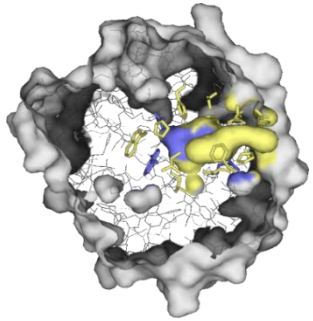


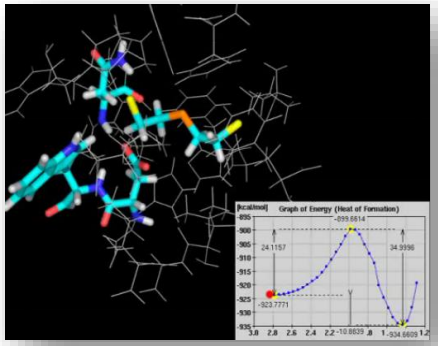
Multidisciplinary science

STRUCTURAL BIOLOGY

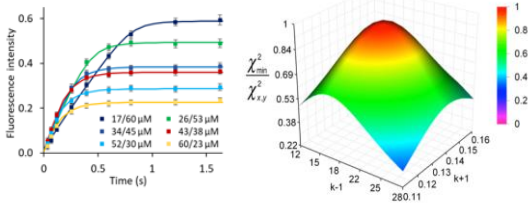


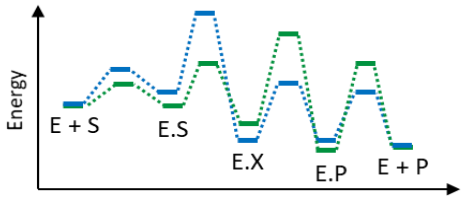
COMPUTATIONAL DESIGN



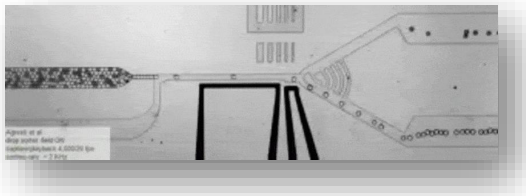


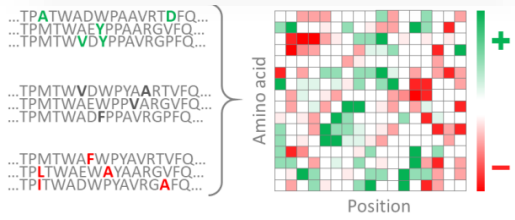
ENZYME KINETICS



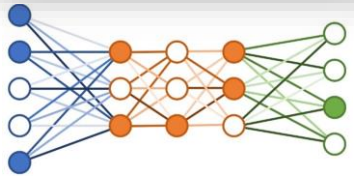


MICROFLUIDICS





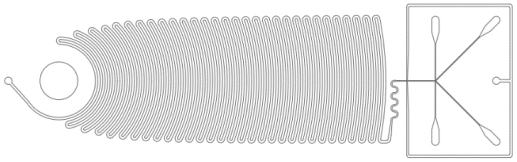
ARTIFICIAL INTELLIGENCE

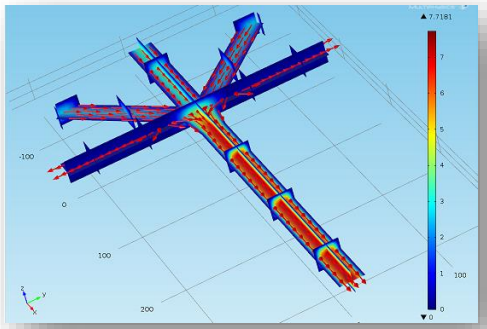


Design and fabrication

- **soft lithography** originates from semiconductor industry

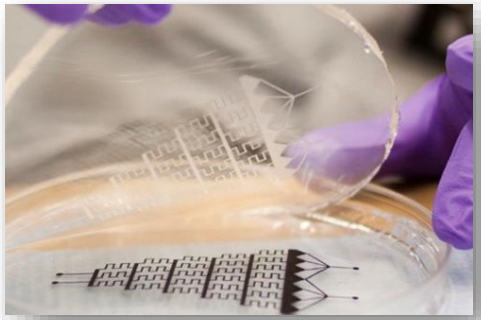
DESIGN / MODELING





MASK / MOLD

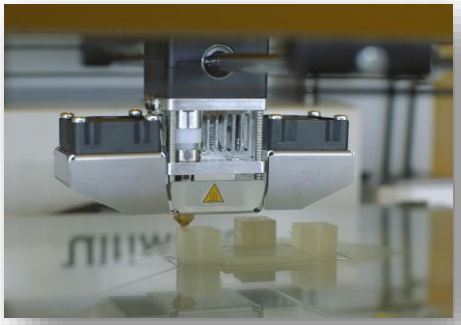
CASTING / BONDING

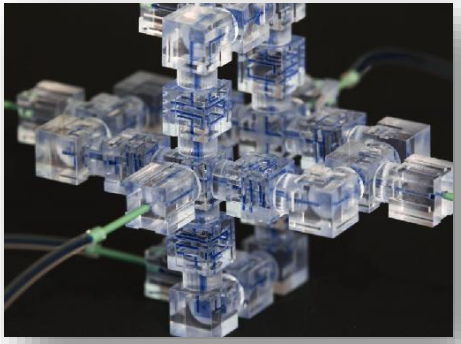


Design and fabrication

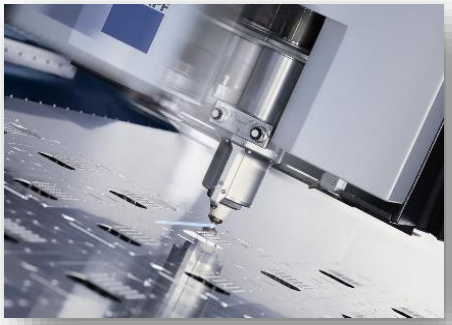
- **direct fabrication methods**

3D PRINTING

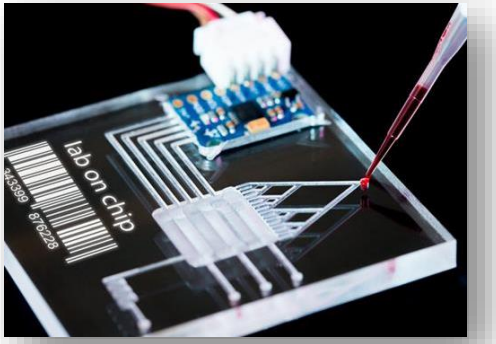




LASER CUTTING



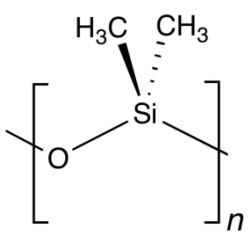
CNC μ-MILLING

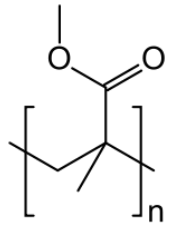


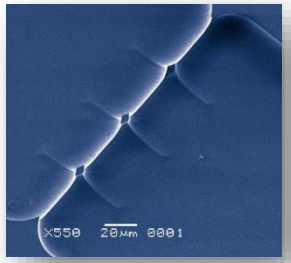
Design and fabrication

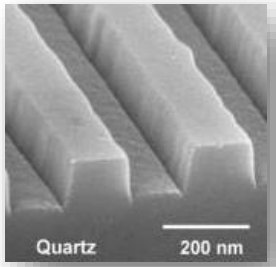
❑ materials

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



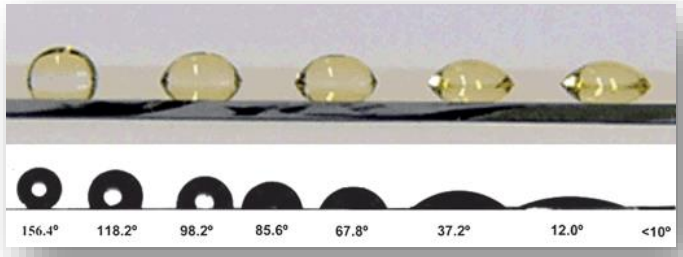


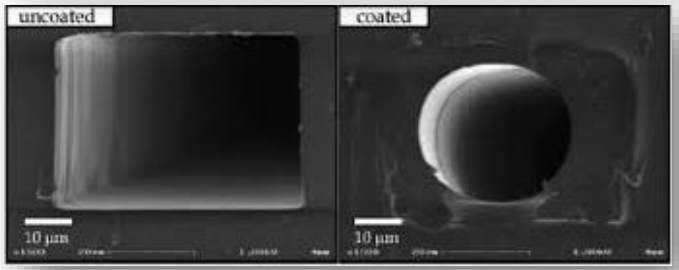




❑ surface modification

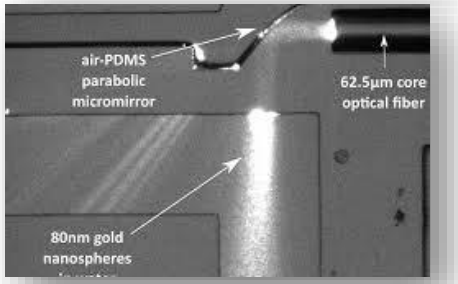
- plasma treatment
- silanization
- sol–gel coating

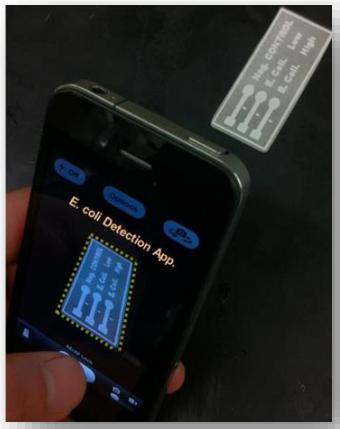




Sensing and detection

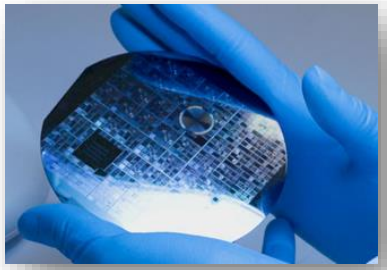
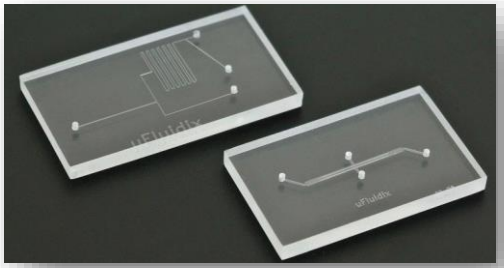
- processing of **small reagent volumes**
- **analytical timescale** and performance
- **on chip detection**
  - fluorescence (LSM, FCS, FLIM)
  - UV/VIS absorbance
  - IR spectroscopy
  - Raman scattering
  - (chemo/electro) luminescence
  - thermal conductivity
  - RI variation
- **off chip detection**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





Commercial instruments and services

- 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 & technology

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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