7. Microfluidics – „Lab on a Chip“

Outline

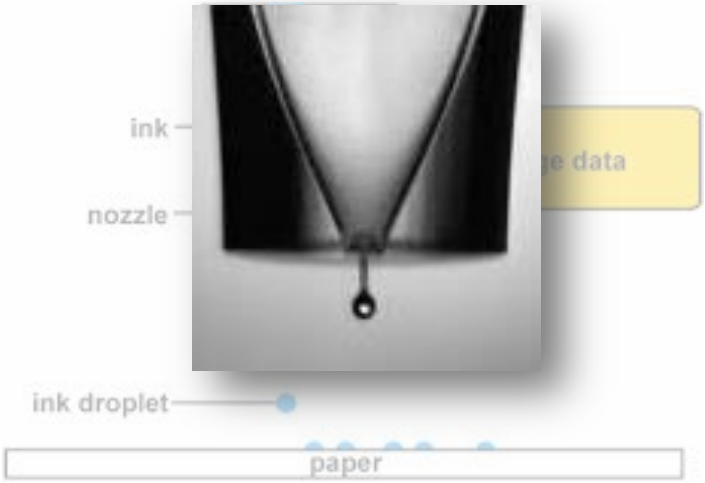
- Introduction to microfluidics
- Physics of micro-scale
- Design and fabrication
- Sensing and detection
- Lab on a chip (LOC) concept
- Examples of LOC applications

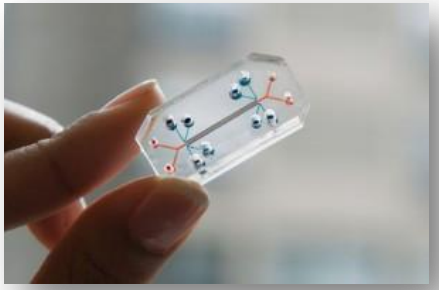
Introduction to microfluidics

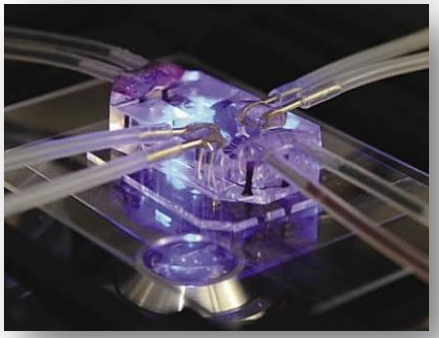
- developed in the 1980s (IBM)
- **multidisciplinary field**

  engineering, physics, chemistry,

  material science, nanotechnology
- **integrate processes on chip**
  - miniaturization and automation
  - (ultra)fast throughput
  - high precision
  - low energy and sample consumption
  - less waste production



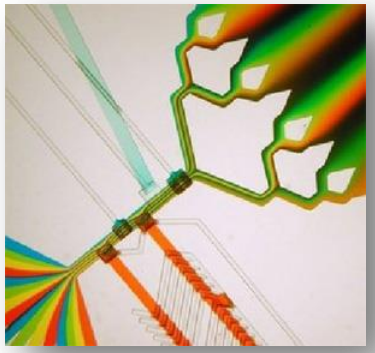




Introduction to microfluidics

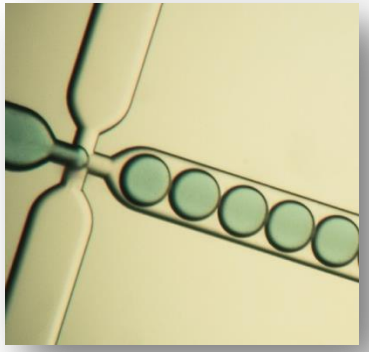
- **continuous-flow microfluidics**

  manipulation of continuous liquid flow through micro-fabricated channels



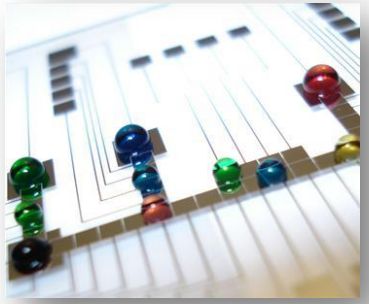
- **droplet-based microfluidics**

  manipulating discrete volumes of fluids in immiscible phases

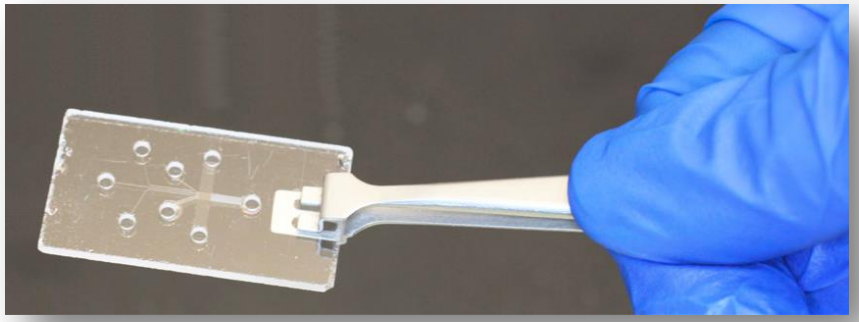


- **digital microfluidics**

  droplets manipulated on a substrate using electro-wetting



Physics of micro-scale

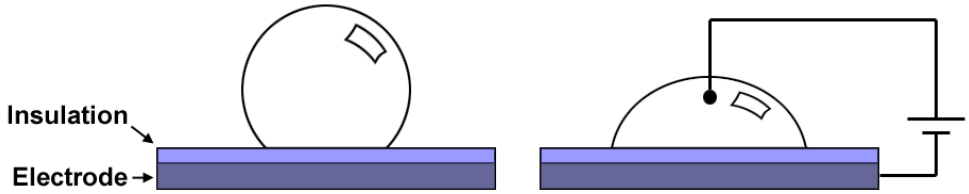


- **micro domain** differs greatly from macroscopic fluids
  - small volumes (nL, pL, fL)
  - reduce dimensions (mm, μm)
  - large surface area-to-volume ratio
  - highly efficient mass and heat transfer

Physics of micro-scale

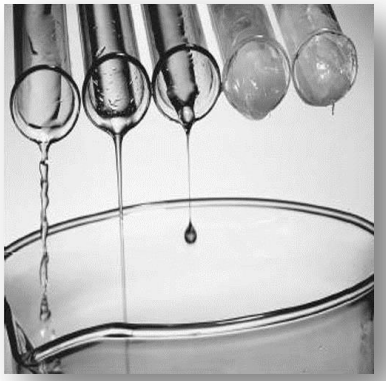
- **surface tension**
  - stretch force along the material interface
  - **Capillary number** (Ca) ratio between viscous force to surface tension
  - **Ca <<1** in microfluidics, fluid dominated by surface tension
  - **wetting** on (hydrophilic) surfaces
  - **electrowetting** - electrical modulation of the solid-liquid surface tension

$$Ca = \frac{\mu U}{\sigma}$$

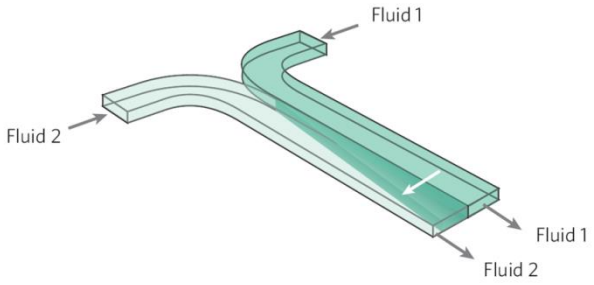


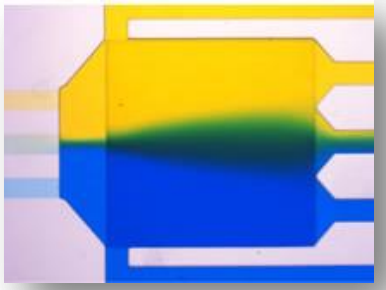
Physics of micro-scale

- **viscosity**
  - **Reynolds number** (Re) ratio between inertial force to viscous force
  - **Re<1** in micro-fluidics, fluids influenced by viscosity rather than inertia
  - **laminar flow** and **diffusion** dominant
  - mixing in microscale challenging



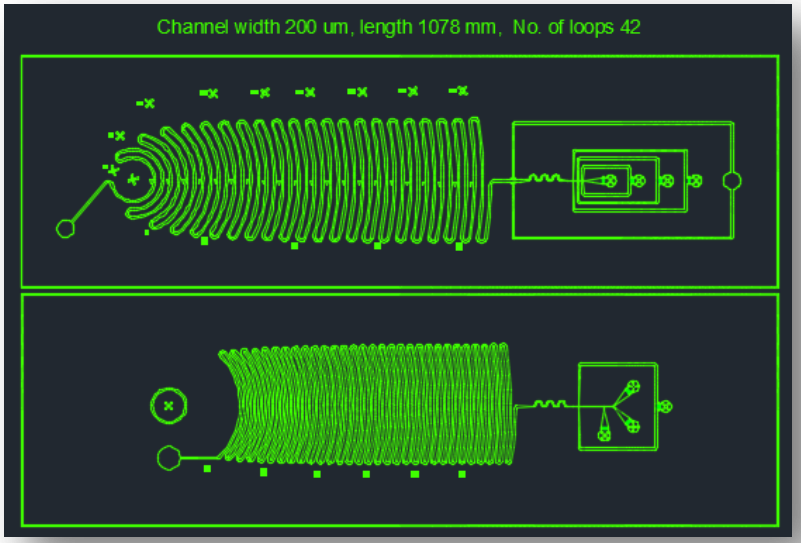
$$\mathrm{Re} = \frac{\rho U L}{\mu}$$

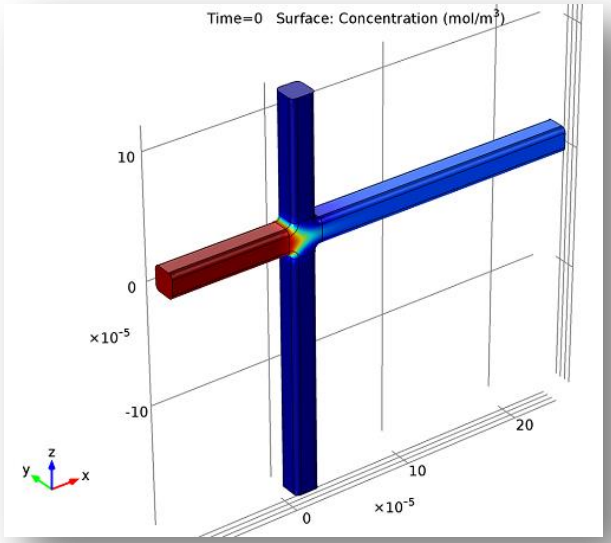




Design and fabrication

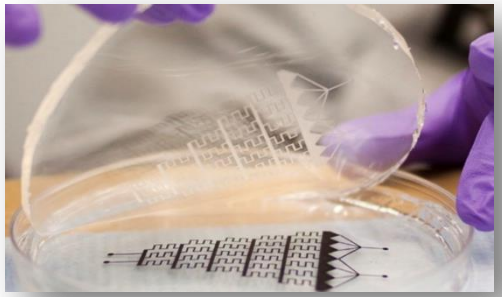
- **design**
  - engineering softwares (e.g., AutoCAD, DraftSight)
  - modelling (e.g., COMSOL, MatLab)
  - printing the mask

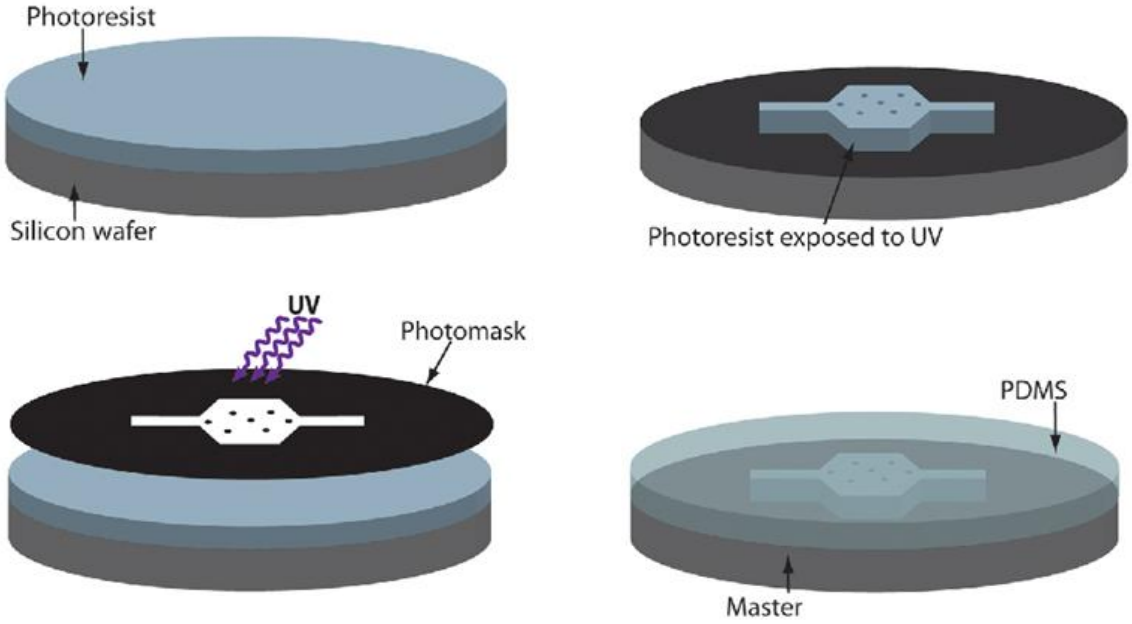




Design and fabrication

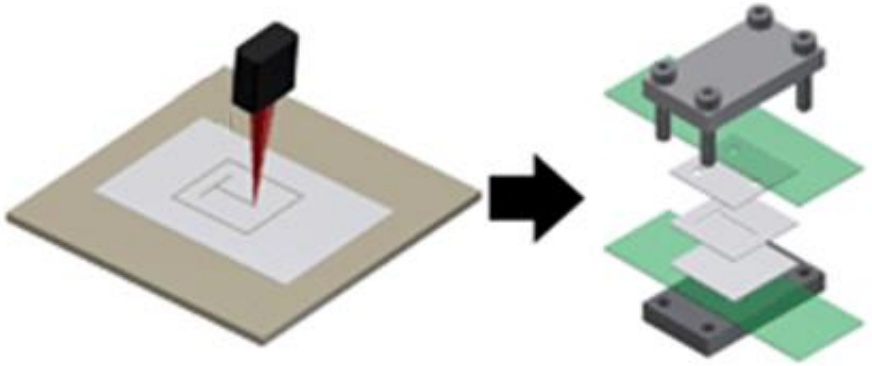
- **fabrication**
  - soft photolithography
  - negative/positive photoresists
  - PDMS molding

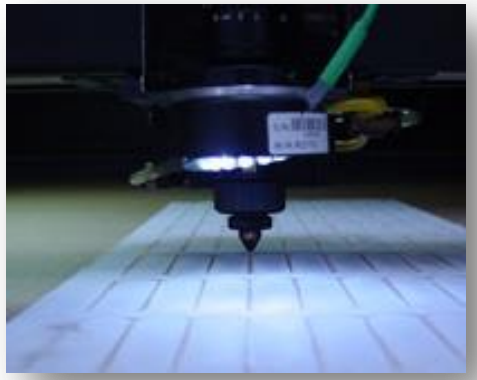




Design and fabrication

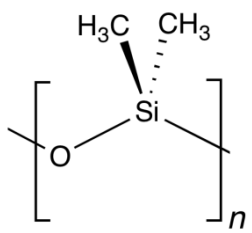
- **fabrication**
  - direct fabrication methods
    - 3D printing
    - CNC micro-milling
    - laser cutting

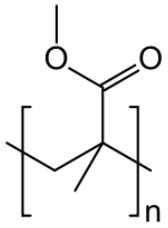


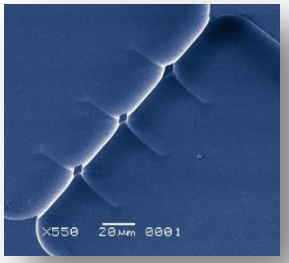


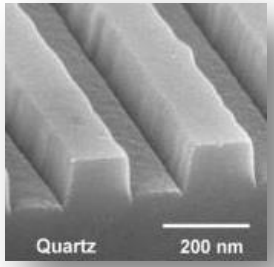
Design and fabrication

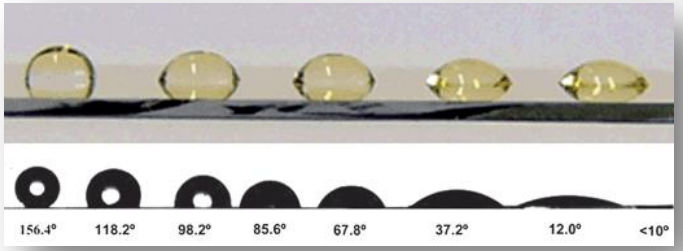
- **materials**
  - inert and transparent
  - PDMS - poly(dimethyl siloxane)
  - PMMA - poly(methyl methacrylate)
  - fused silica, quartz and glass
- **surface modification**
  - plasma treatment
  - silanization
  - functionalization
  - sol–gel coating

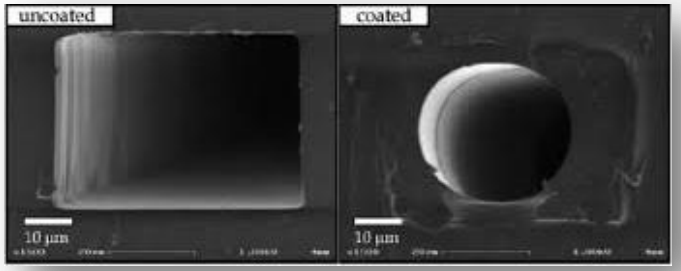






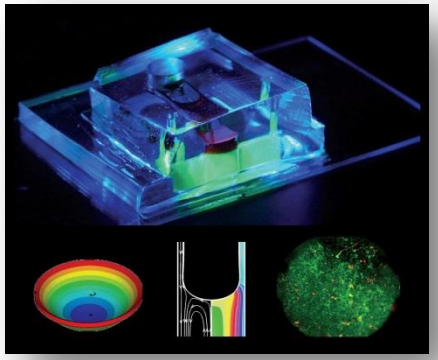




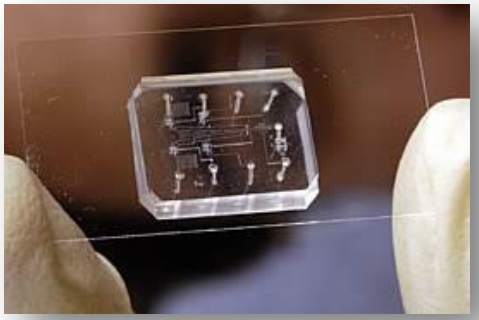


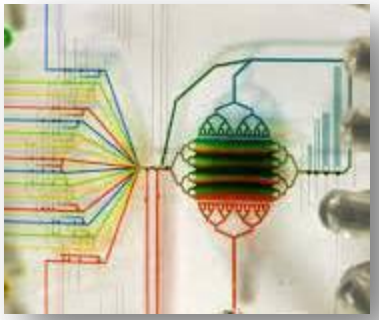
Sensing and detection

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



Microfluidics





- benefits of miniaturisation
  - **superior performance** (speed, efficiency and control)
  - **reduced consumption** of sample/reagent and power
  - **cost economies** through micromachining
  - **portability** (point-of-care/use applications)
  - facile process **integration and automation**
  - high analytical **throughput**

Lab on a Chip (LOC) concept

Incubation

Pre-treatment

Analysis

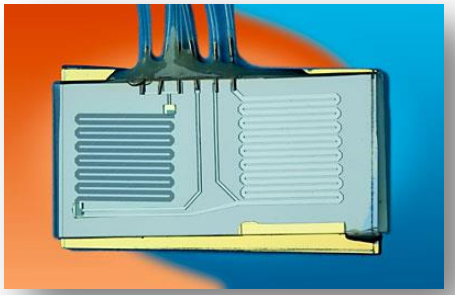
Preparation

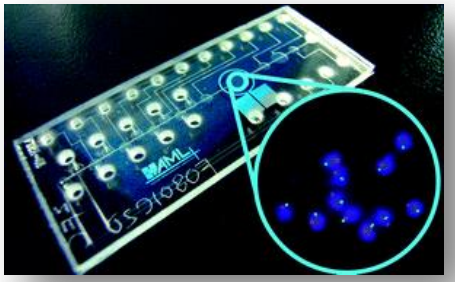
Collection

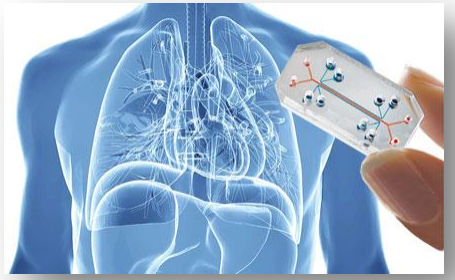
on a chip integration of laboratory processes

Life science and medical application

- analytics and synthesis
- PCR and sequencing
- diagnostics
- pharmacology
- proteomics
- (ultra)high-throughput biology
- clinical studies

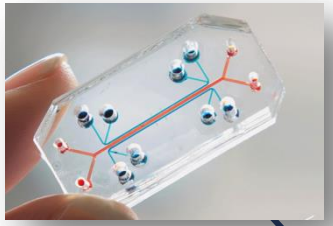




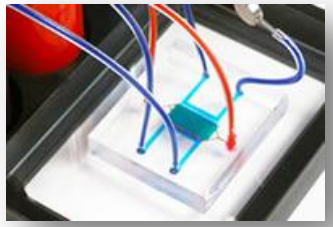


Organs (human) on chip

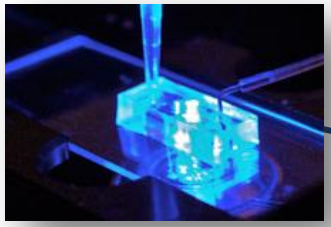
Lung

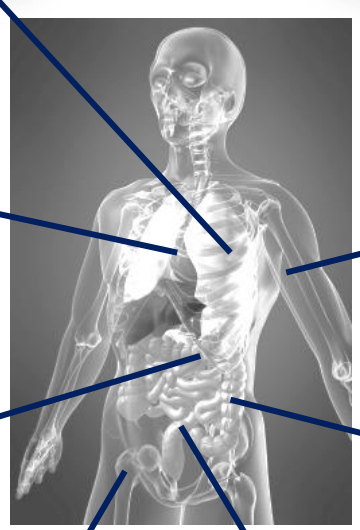


Neurovascular

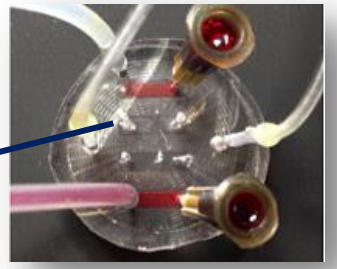


Heart

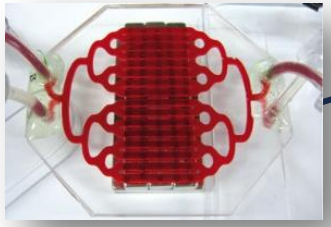




Artery

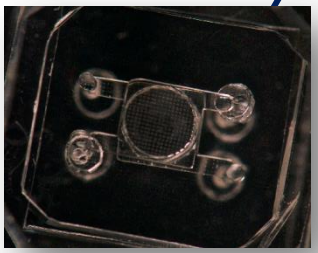


Spleen

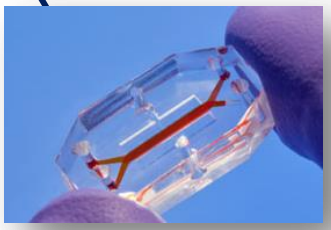


Kidney

Bone



Intestine



Organs (human) on chip

- **organs-on-a-chip**
  - multi-compartmental 3D microfluidic cell culture chips
  - simulates **activities, mechanics and physiological response**
  - realistic *in vitro* model closer to ***in vivo*** **cell environment**
  - mimicking human's physiological responses

    (e.g., pathological responses, pharmacokinetic, toxicology)
- **human-on-a-chip**
  - interactions under near-physiological fluid flow conditions
  - simulating multi-organ metabolic interactions
  - synergistic drug interactions
- can replace expensive and controversial animal testing

(Ultra)High-throughput biology

RATIONAL DESIGN

1. Computer aided design

2. Site-directed mutagenesis

Individual mutated gene

3. Transformation

4. Protein expression

5. Protein purification

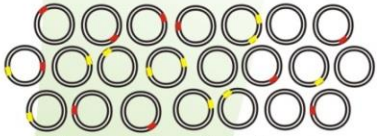
6. *not applied*

Constructed mutant enzyme

DIRECTED EVOLUTION

1. *not applied*

2. Random mutagenesis



Library of mutated genes
(>10,000 clones)

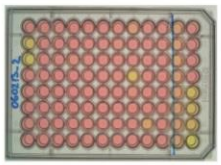
3. Transformation

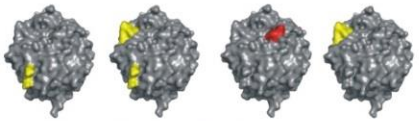
4. Protein expression

5. *not applied*

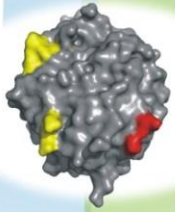
6. Screening and selection

- stability
- selectivity
- affinity
- activity





Selected mutant enzymes



IMPROVED ENZYME

7. Biochemical testing

(Ultra)High-throughput biology

STANDARD DESIGN

- random mutagenesis (2-3 positions)
- library of **10^4 clones**

volume: 100´ µL
assays/day: 10^3

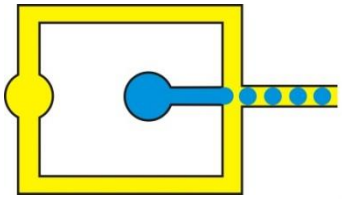
ADVANCED DESIGN

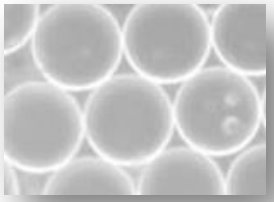
- random mutagenesis (5-7 positions)
- library of **$>10^6$ clones**

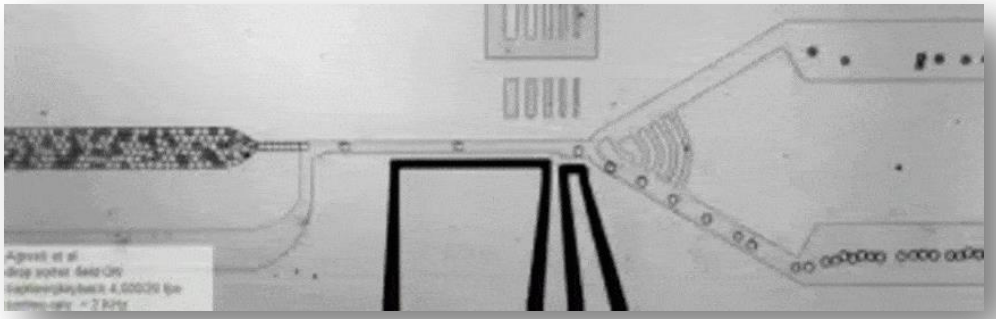
volume: 10´ pL
assays/day: 10^7

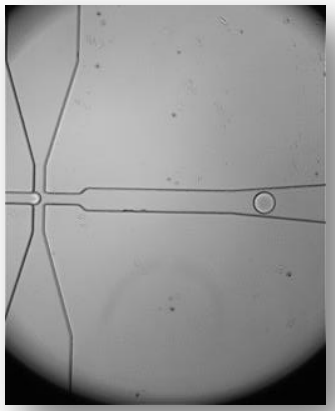
(Ultra)High-throughput biology

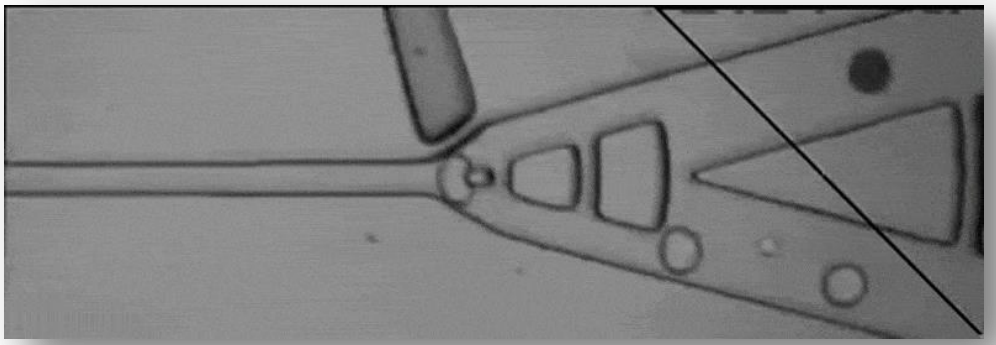
- monodisperse **emulsion** (2 pL, **10^7 droplets/hour**)
- fluorescence-activated **on-chip droplet sorting** (FADS)
- **10^3 events/hour**









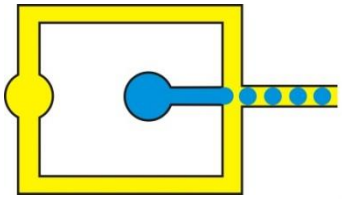


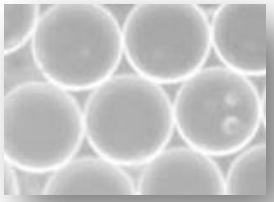
Baret et al. 2009. Lab Chip 9: 1850-1858

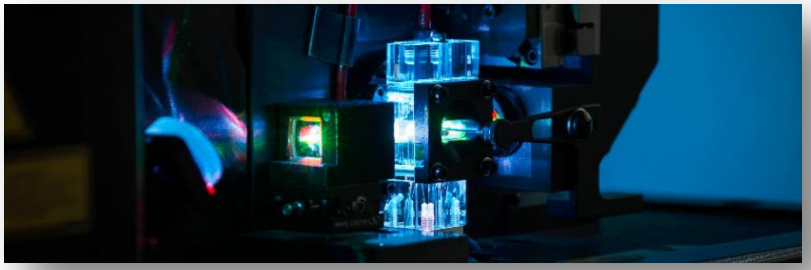
Abate et al. 2010. Appl. Phys. Lett. 96: 203509

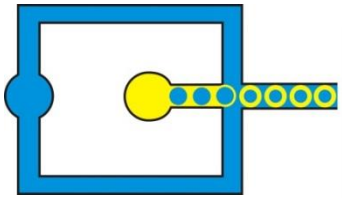
(Ultra)High-throughput biology

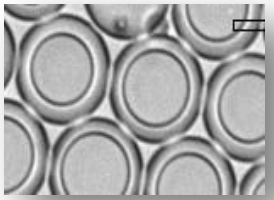
- fluorescence-activated **off-chip cell sorting** (FACS)
- **10^8 events/hour**
- monodisperse **double emulsion** (2 pL, **10^7 droplets/hour**)

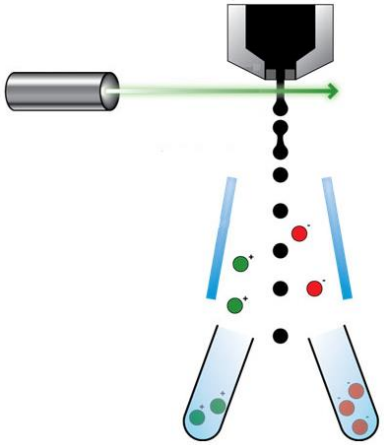


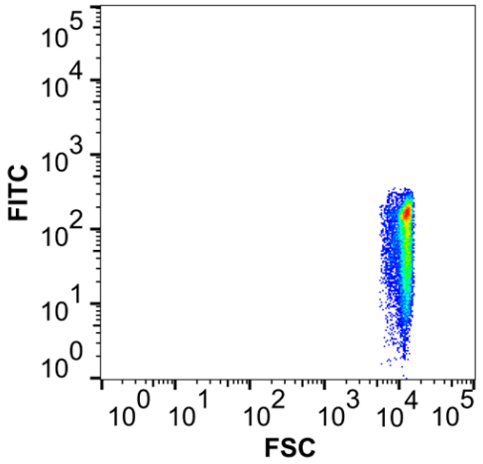












Polymerase chain reaction

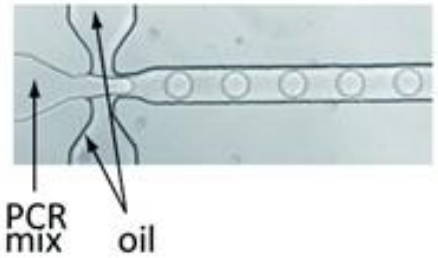
- **clasical PCR**
  - 96-well micro-titre plates
  - volume 50 to 500 µL
  - slow heating/cooling cycles

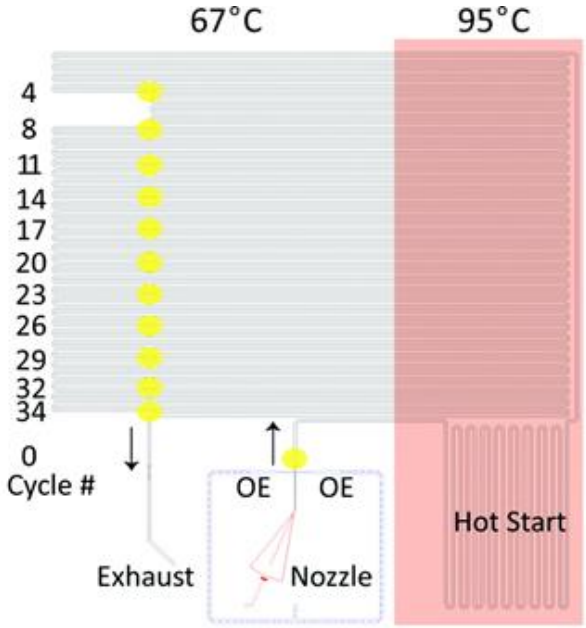
Kary Mullis

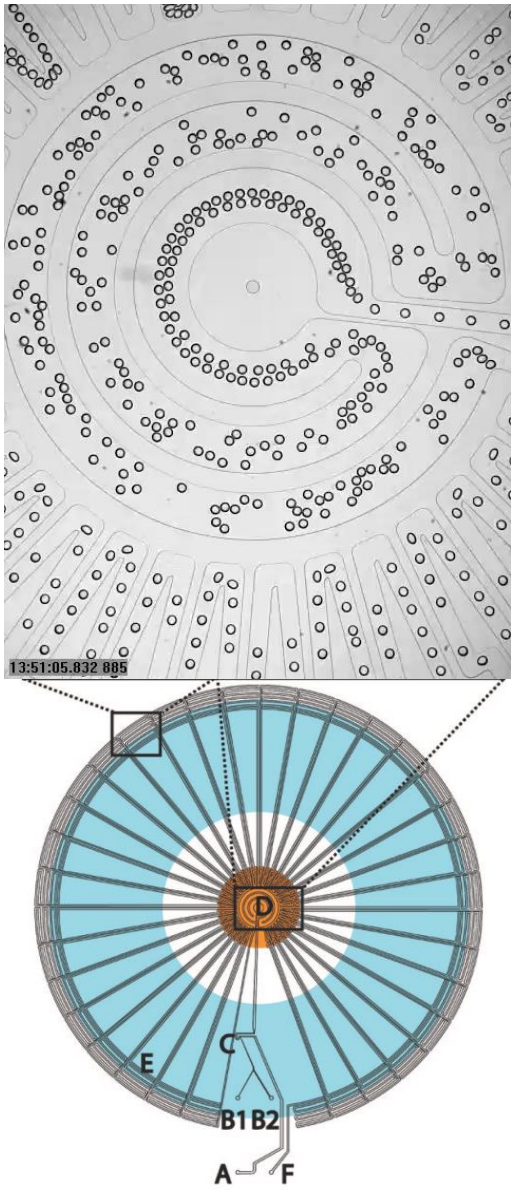
Nobel Prize in 1993

Polymerase chain reaction

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

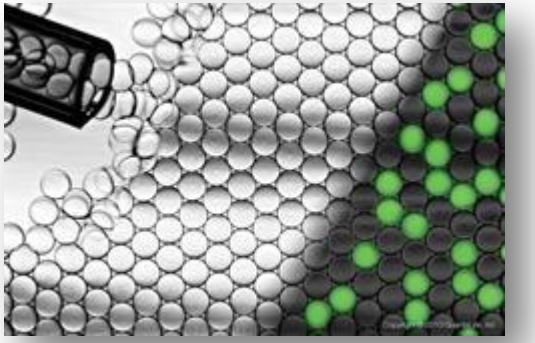






Digital PCR

- **“QX100” Droplet Digital PCR** (BioRad)



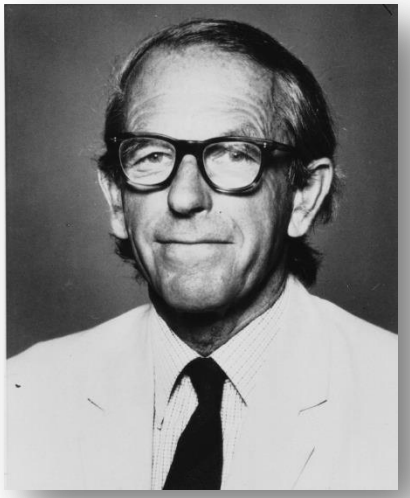
- **“Raindrop” Digital PCR** (Raindance)

Next-generation sequencing

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

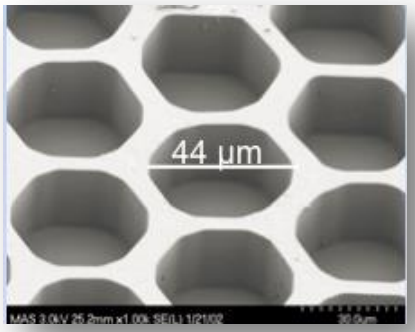
  detection volume 1 picoliter (10^{-12} litres)

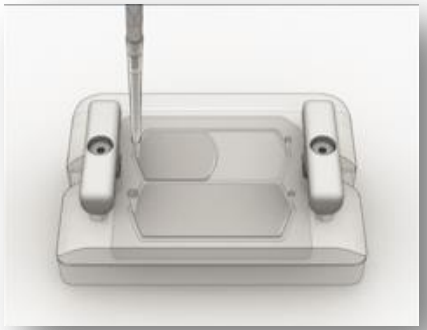
  1 mil. reads per run, 10 USD per Mbase

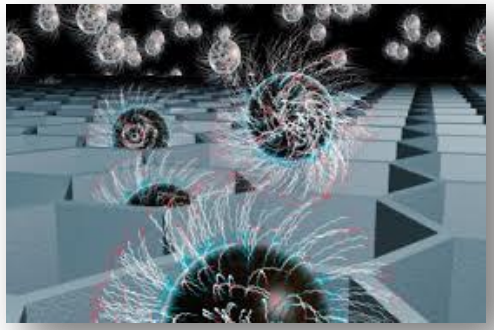


Frederick Sanger

Nobel Prize in 1980





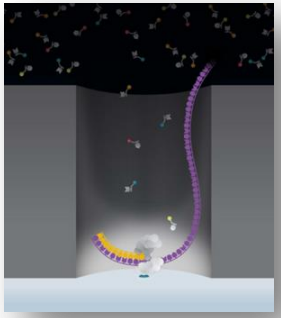


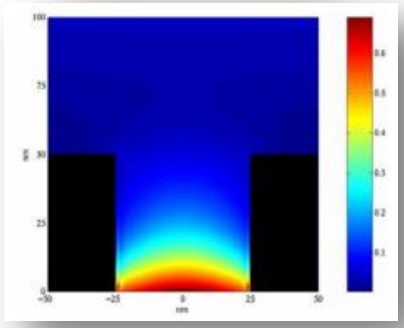
Next-generation sequencing

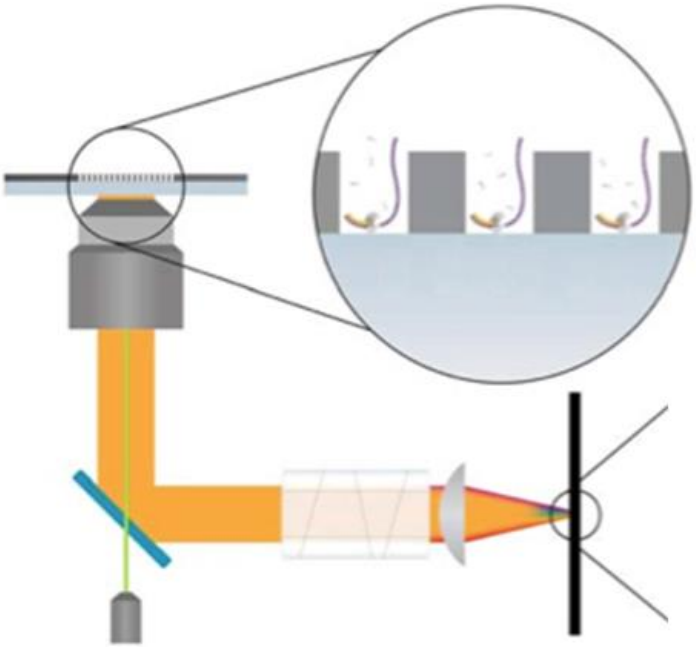
- parallelization of **single molecule** sequencing
- **SMRT sequencing** (Pacific Biosciences)

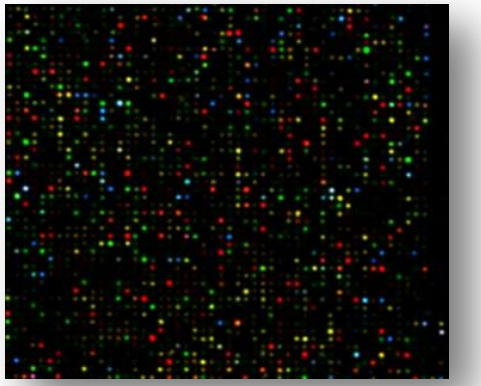
  detection volume 20 zeptoliters (10^{-21} litres)

  0.1 mil. reads per run, 0.5 USD per Mbase



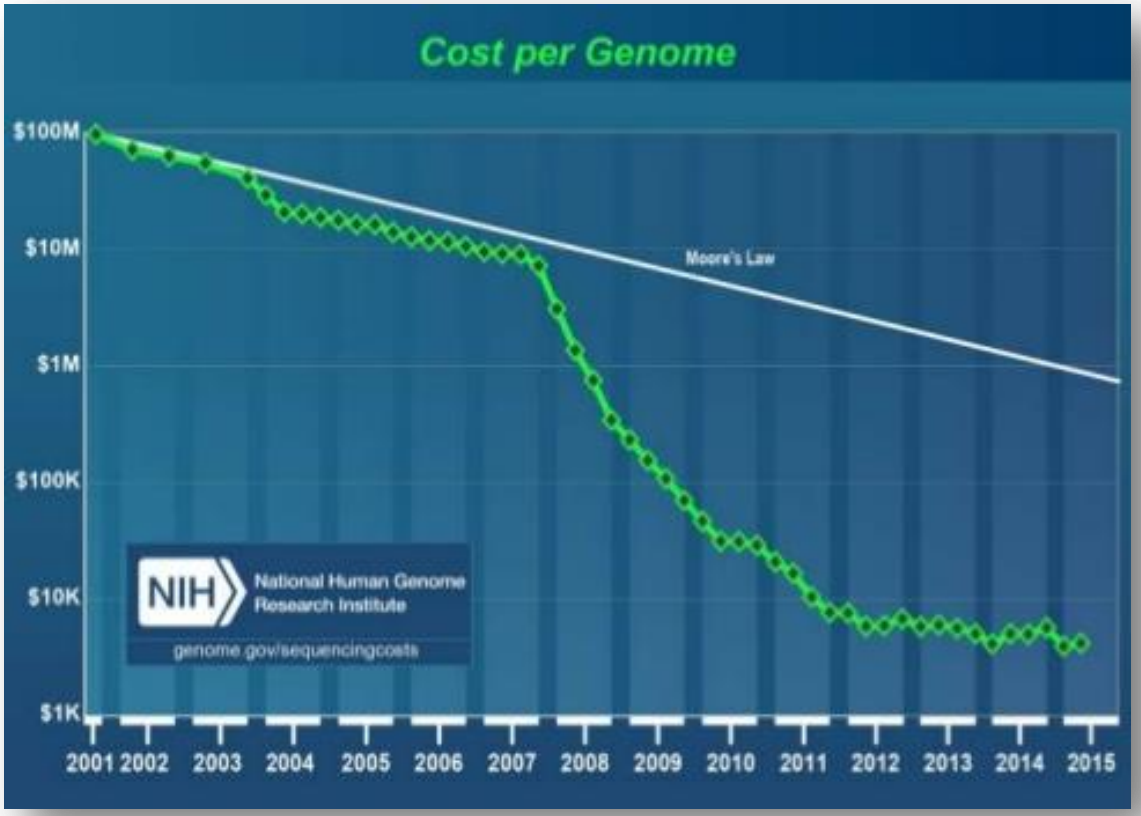






Next-generation sequencing

- Human Genom Project - 10 years, 3 billion USD
- genome sequencing today in 10 to 15 hrs



Plants on a chip

- **efficient control** over several **simultaneous experiments**
- observe developing roots in parallel
- fluoresce-labeled metabolite activity
- interaction with symbionts/parasites