Microfluidics, 10/11-2009

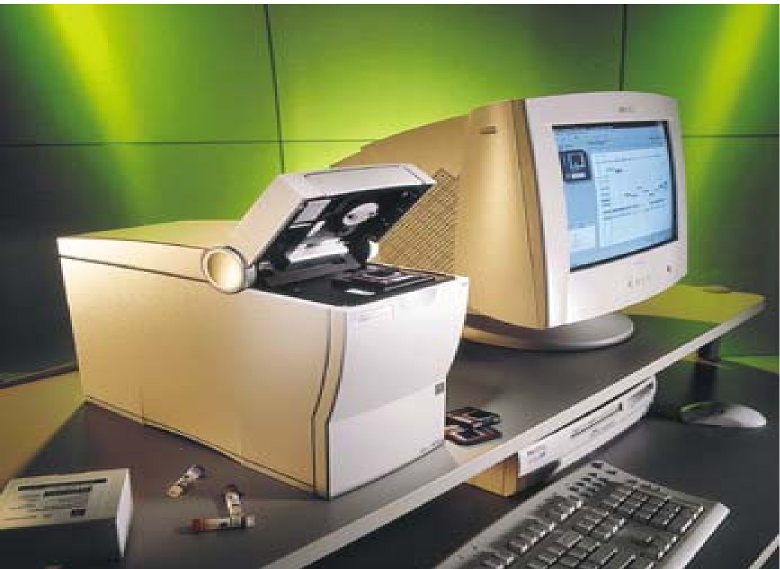
- Surface tension
- Capillary forces
- Ideal gas law
- Viscosity
- Navier Stokes equation
- Reynolds number
- Poiseuille flow
- Electroosmotic flow
- Electrophoresis
- Mixing

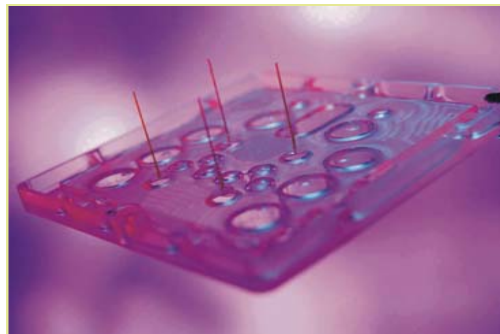
These topics are important for design of well-functioning fluidic microsystems.

A lab-on-a-chip is a miniature laboratory

integrate (multiple) laboratory functions on a single chip of only millimeters to a few square centimeters in size and that are capable of handling extremely small fluid volumes down to less than pico liters.

- Automatic analysis
- Quick: small heat capacities
- Less reagents and sample
- Closed chip – less pollution





Caliper/Agilent

Functions in:

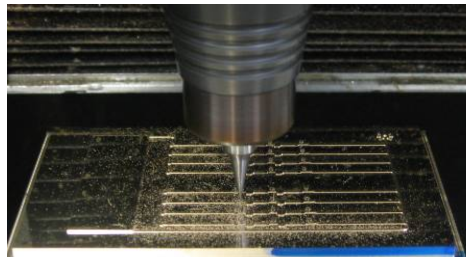
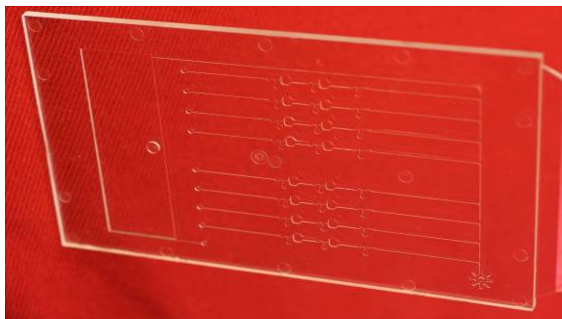
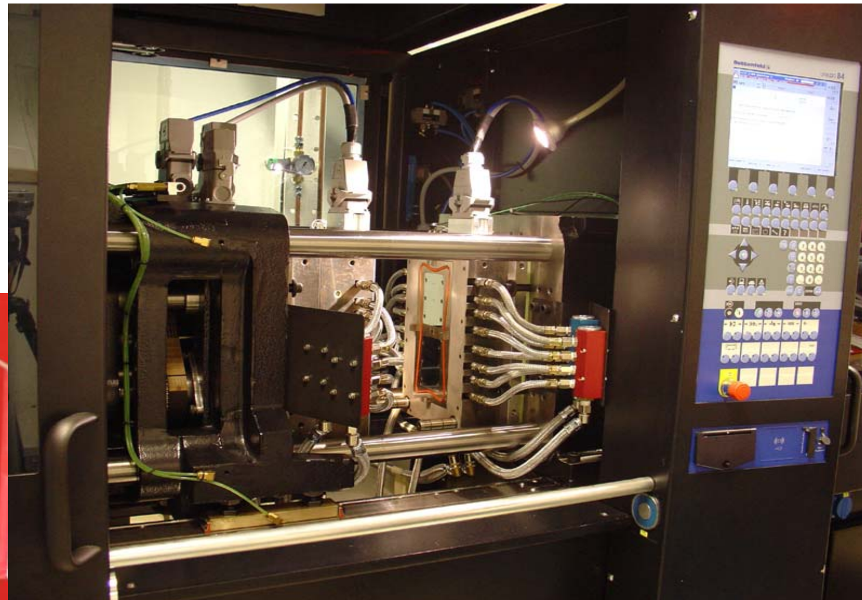
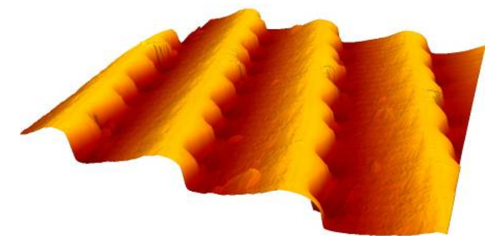
- Instrument

or

- Chip

Polymer components

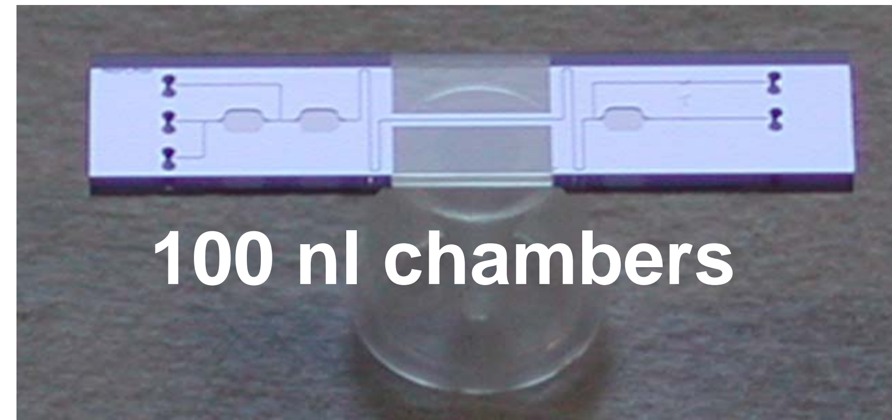
- Manufactured by micro-injection moulding

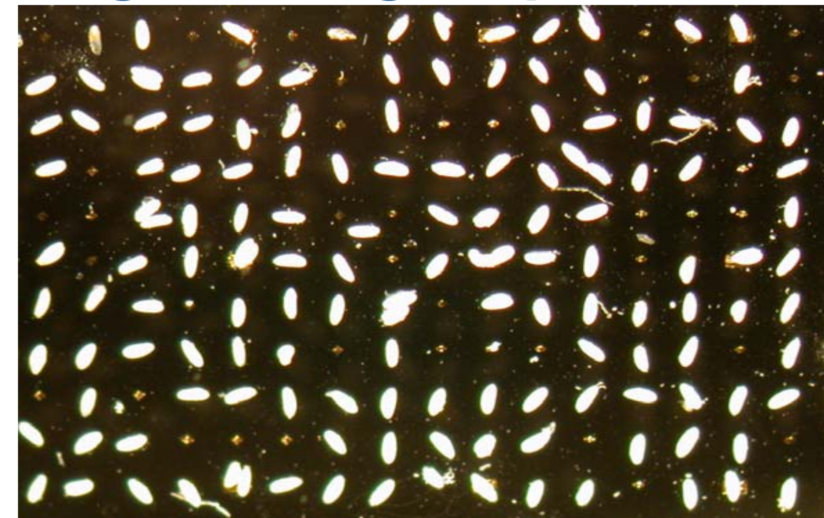
Fluidic channels and reaction chambers can be made in silicon

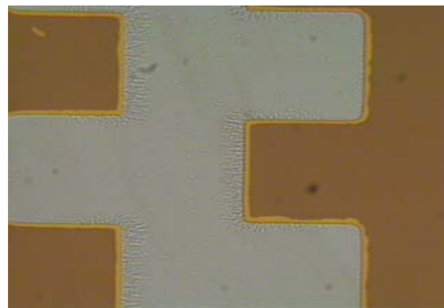
- DRIE etched in silicon
- Sealed by glass from above
- Holes for injection of reagents /extraction of products

- Heating elements
- Temperature sensors



Drosophila embryos aligned on gold pattern





Gold electrodes

di-electro phoresis

Yole 2009: Emerging markets for microfluidic applications

Microfluidics applications

	Analytical Devices	Clinical Diagnostics	Pharmaceutical Research	Point of Care	Industrial and Environment Testing	Drug Delivery
Micro Dispensing			Microdispensers, Flex-arrayers, inkjet dispensing…			Micropumps, Microneedles, Inhalers
Microfluidic Devices, Chips		Capillary Electrophoresis chips	Capillary Electrophoresis chips	Capillary Electrophoresis chips	Microfluidic devices for -industrial and environmental testing -outside laboratory -Homeland Security…	
	Microfluidic devices for MS/GC/HPLC Chips	Cartridges for clinical and veterinary diagnostics	Microfluidic devices for: -Proteomics analysis -PCR chips -Cell chips	Microfluidic devices for Point of Care, intensive care, doctor offices, near patient…		

SINT

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In-vitro diagnostics

- Sample taken out of the body
- Blood, salvia, urine, cell smear, biopsies

- Future: from the central laboratories to homes and doctors' offices
- Must be automatic: Sample in – answer out
- Reliable results
- Communication of results to doctor or hospital database

- Disposable chip
- Instrument
  - PC sized
  - Hand held

- Molecular diagnostics
  - Cancer
  - Infections (bacteria, virus, parasites)
  - Cardiovascular diseases
- Molecular markers
  - DNA, RNA
  - Proteins; antigens, enzymes, hormones
  - Low molecular compounds

- Sample preparation
  - filters, micro-pillars, magnetic beads, separation
- Washing
- (Amplification e.g. PCR)
- Reactions
  - Immunoreactions
  - Hybridization
- Detection
  - Labels (dye, fluorescent, radioactive)
  - Label-free (impedance, electrochemical, amperometric, cantilevers, evanescent fields)
- Choose methods for all steps:

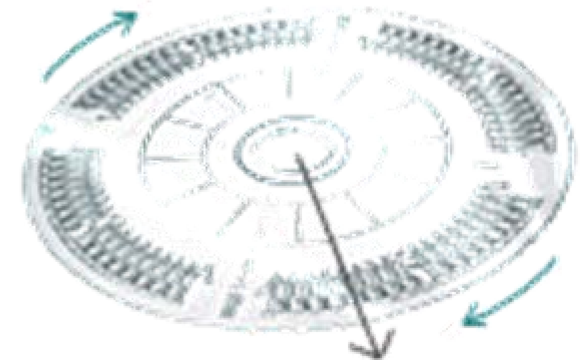
SENSITIVITY + SPECIFICITY

Cepheid GeneXpert technology

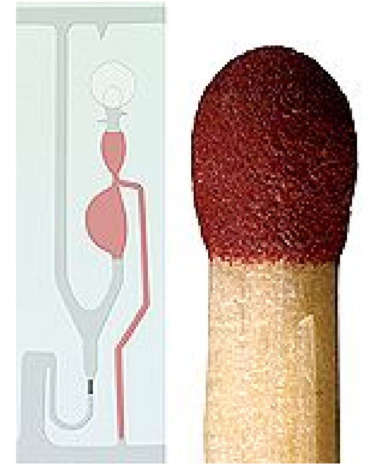
Roche AmpliChip Pharmacogenetic microarray based test

Gyros, Swedish life science company

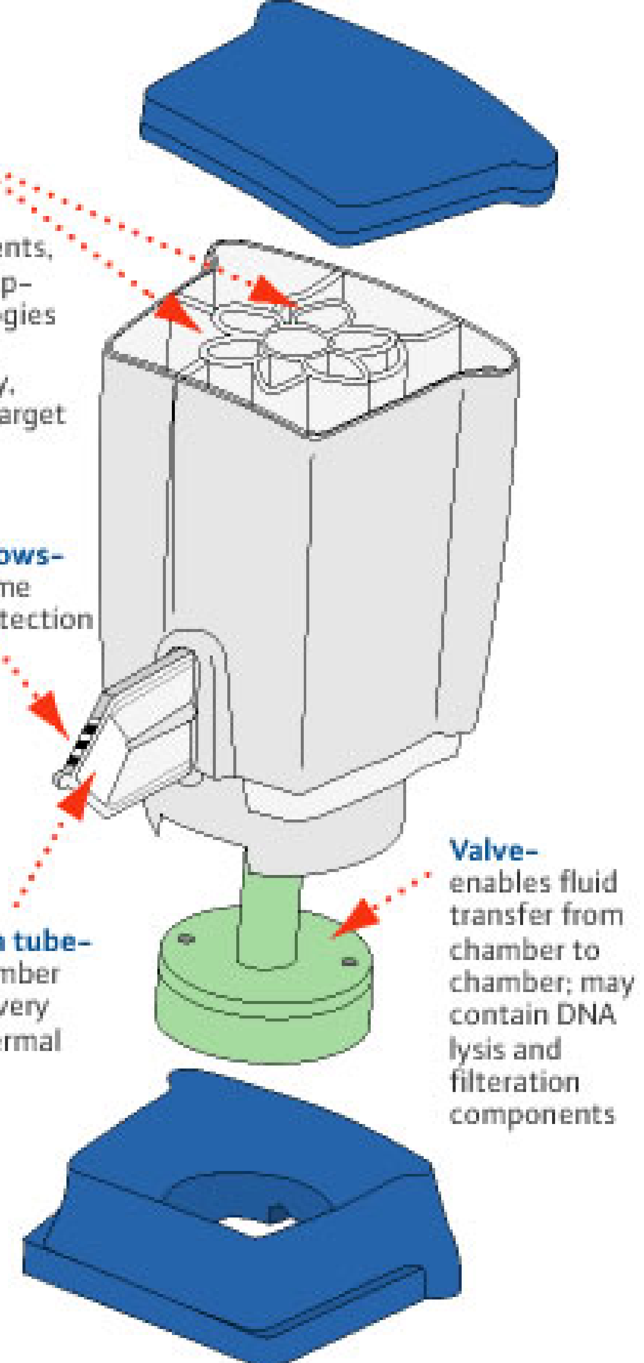
- Diagnosis
- Drug discovery



www.gyros.com



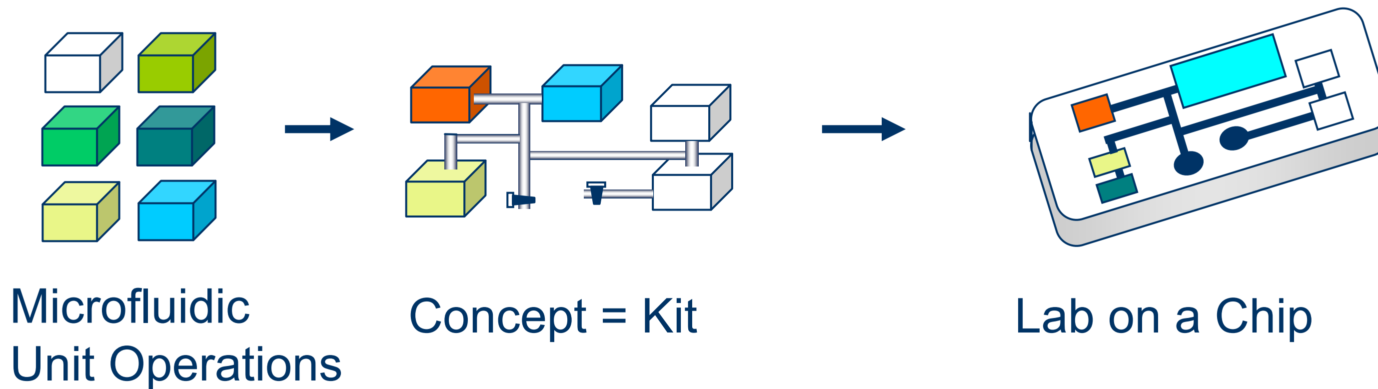
Cepheid GeneXpert



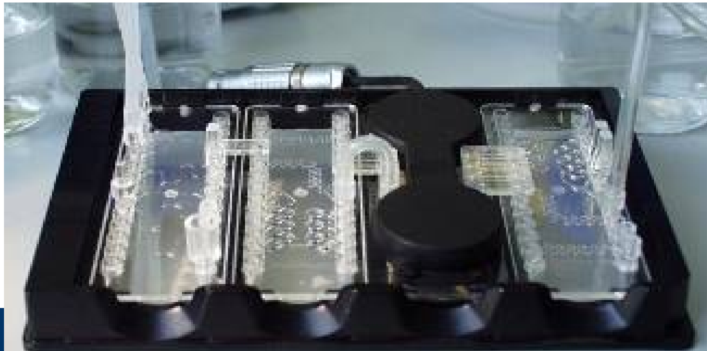
Microfluidic construction kit

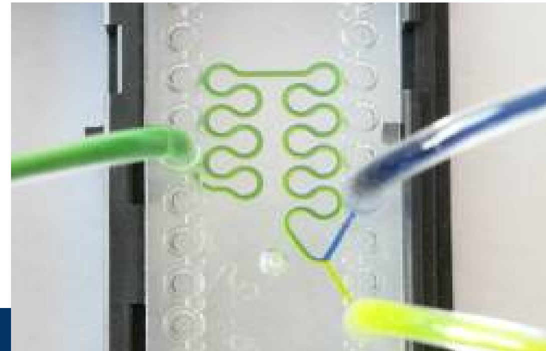
Integration of several polymer slides

<u>The idea:</u> modular concept as an intermediate step on the way to integrated systems



Library of standard slides: pumps, mixers, splitters



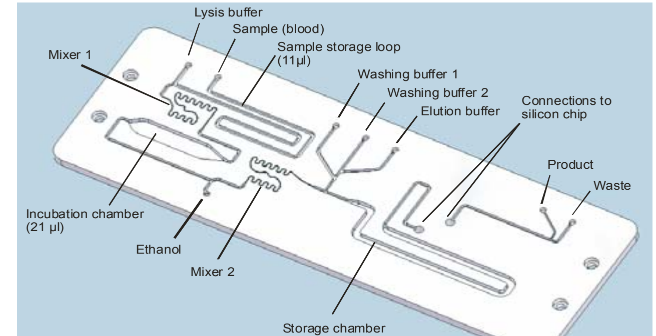


Microfluidics allow for controlled liquid handling:

ThinXXS design kit slides: microBUILDER

Splitting

Reagent integration
Excellent, liquid and gass barriers



Multifunctional slide for DNA extraction

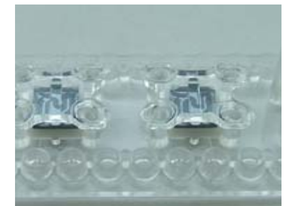
Mixing

Microfluidic functionalities are readily combined with a variety of sensor and actuator systems as well as a variety surface modifications (Bio-functional layers)

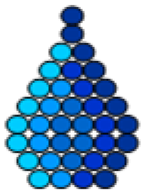
Exact metering

Filtering and active porous membranes

Cuvette cavities
COC: excellent optical features



Silicon based microchips

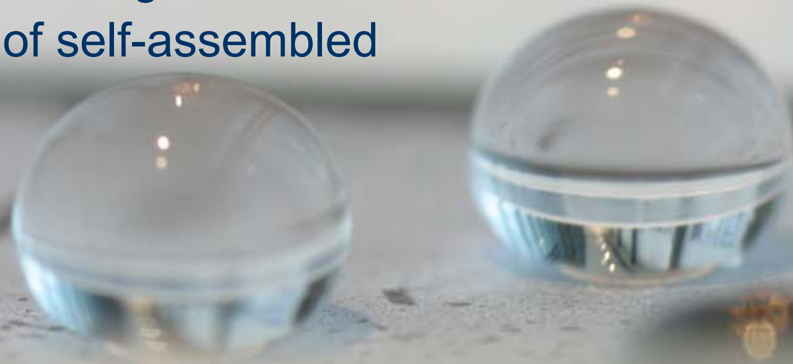


µBUILDER
www.microbuilder.org

Surface modification

- Hydrophilic / hydrophobic surfaces
- Wetting/non-wetting droplets
- Fuktende/ ikke-fuktende væsker

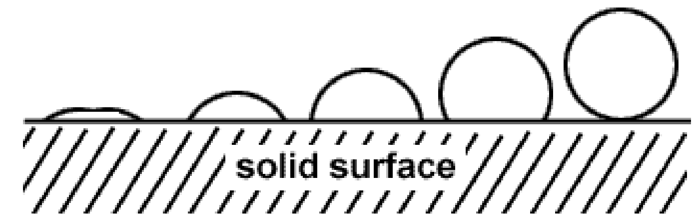
- Lithographic patterning
- E.g. deposition of self-assembled -monolayers

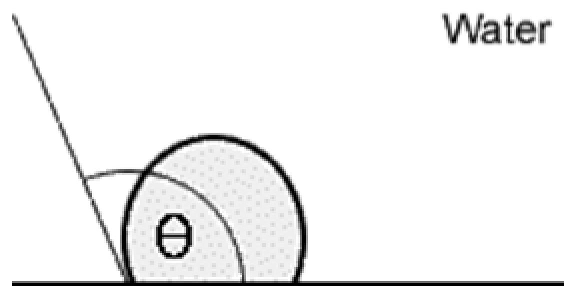


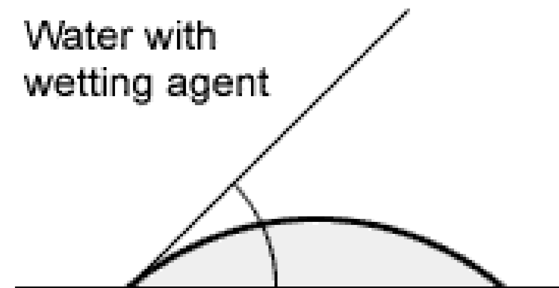
Wetting / Non-wetting

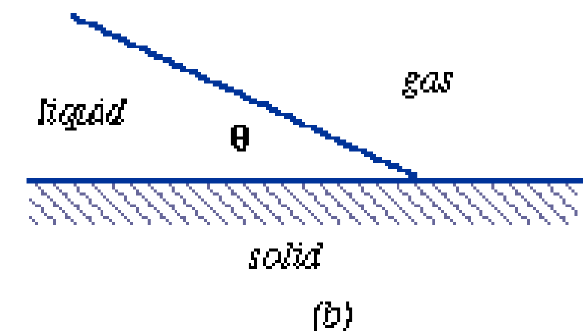
- Contact angle depends on the solid/liquid/gas that meet in one point

- Wetting fluid: Contact angle < 90
- Non-wetting: Contact angle > 90



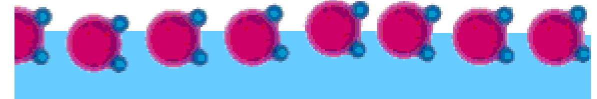






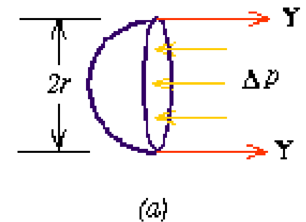
Surface tension

- Surface between two fluids
  - Gas-Liquid
  - Liquid-Liquid



- Energy per surface area

- Surface tension along periphery
- Pressure on section area



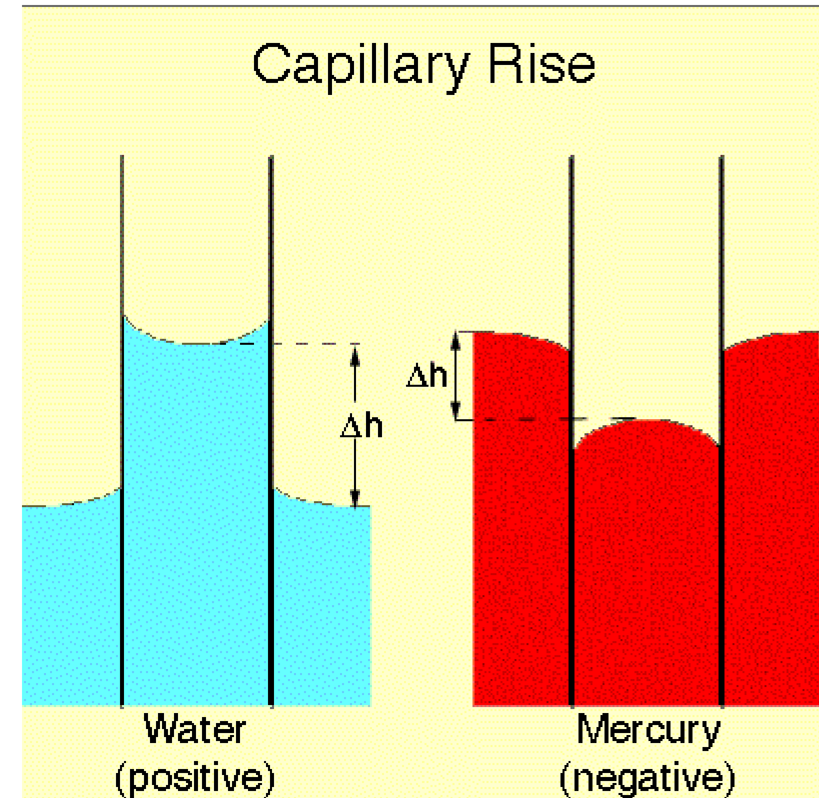
$$2\pi r\Gamma = \Delta P \pi r^2$$

Pressure difference outside/inside drop: $\Delta P = 2\Gamma / r$

Capillary rise Senturia 13.2.3

- A liquid that wets the walls will rise to a height *h* in a capillary tube
- Equilibrium is when weight of liquid column equals surface forces that pull meniscus up

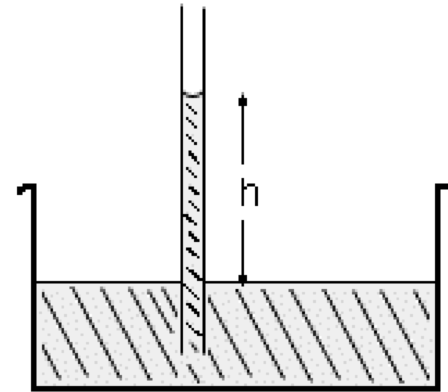
- Forces:
  - Surface forces pull meniscus up $2\pi r\Gamma\cos\Theta$
  - Gravity pull liquid down $\rho gh\ \pi r^2$

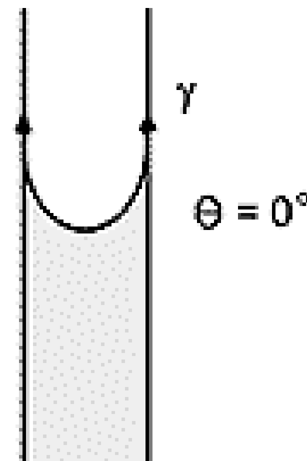


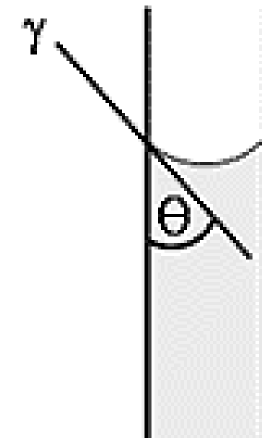
$$\rho g h \pi r^2 = 2\pi\Gamma\cos\Theta$$

Definition of wetting angle

- Can be modified by (chemical) surface treatment







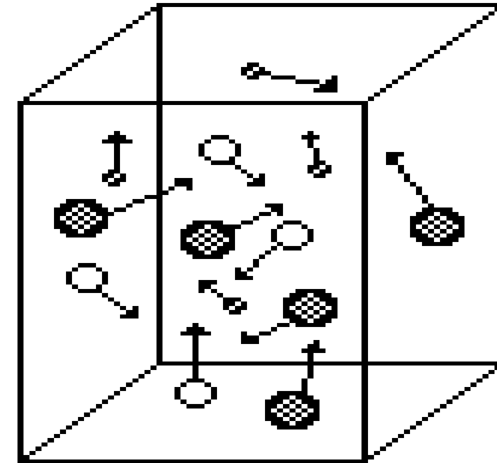
Ideal Gas Law

- Equation of state for (ideal) gases
- pV=NkT
- k=1.38 10^{-23} J/K, Boltzmann constant

- Senturia:

$$P = \rho_m \left(\frac{R}{M_W}\right) T$$

- R=8.31 J/(mol K), universal gas constant



State variables

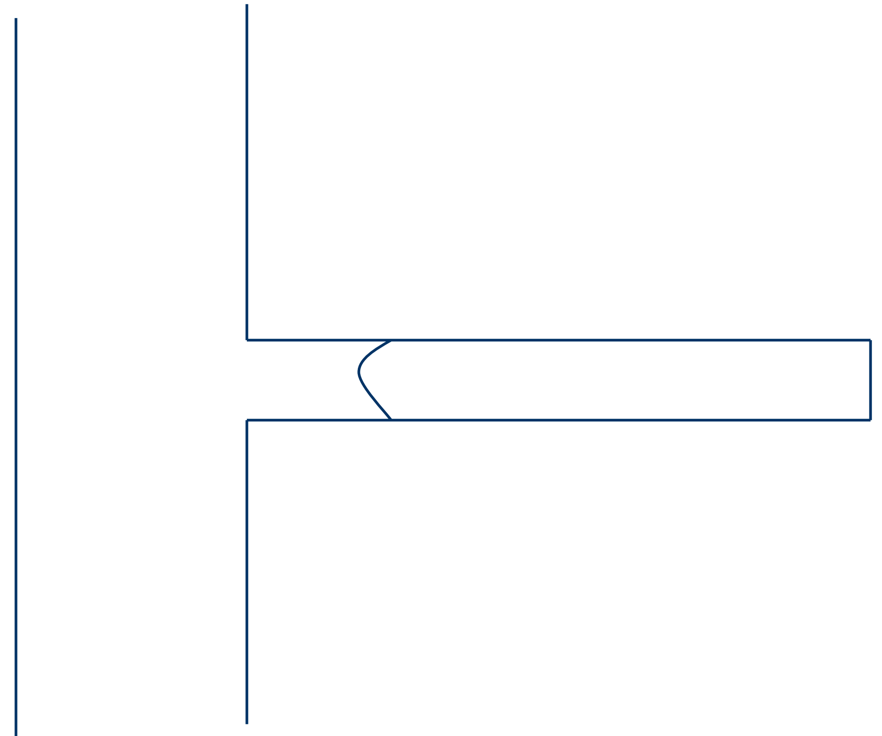
V volume

P absolute pressure

T absolute temperature

Exercise: Fluid volume in capillary “dead-end”

- Where does the meniscus stop?
- What is the volume that is pulled into the narrow capillary?
- What happens when temperature is increased?



Viscosity Senturia 13.2.1

- Deformation of fluids in the presence of shear forces
- The property of a fluid that resists the action of a shear force
- η[Pa s]

- Newtonian fluid:

$$\tau = \eta \frac{U}{h}$$

$$\tau = \eta \frac{\partial U_x}{\partial y}$$

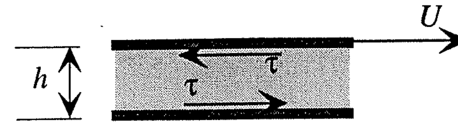
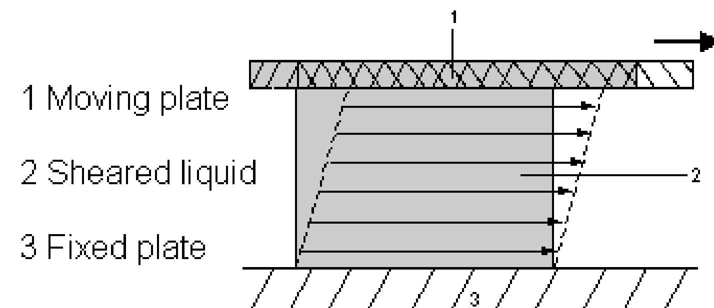


Figure 13.1. Fluid between two plates. The upper plate moves to the right with velocity U, setting up shear forces τ.



Navier-Stokes equation

- Conservation of mass

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{v}) = 0$$

- Newton's 2nd law for a fluid

$$\rho(\frac{\partial \vec{v}}{\partial t} + (\vec{v} \cdot \nabla)\vec{v}) = \nabla p + \eta \nabla^2 \vec{v}$$

Reynolds number

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

- Laminar or turbulent flow?
- Ratio of inertial forces to viscous forces
- Reynolds number:

  ratio of kinetic energy of a volume of fluid in the flow

  to

  the energy dissipated by the volume in the shear caused by interaction with its solid boundaries

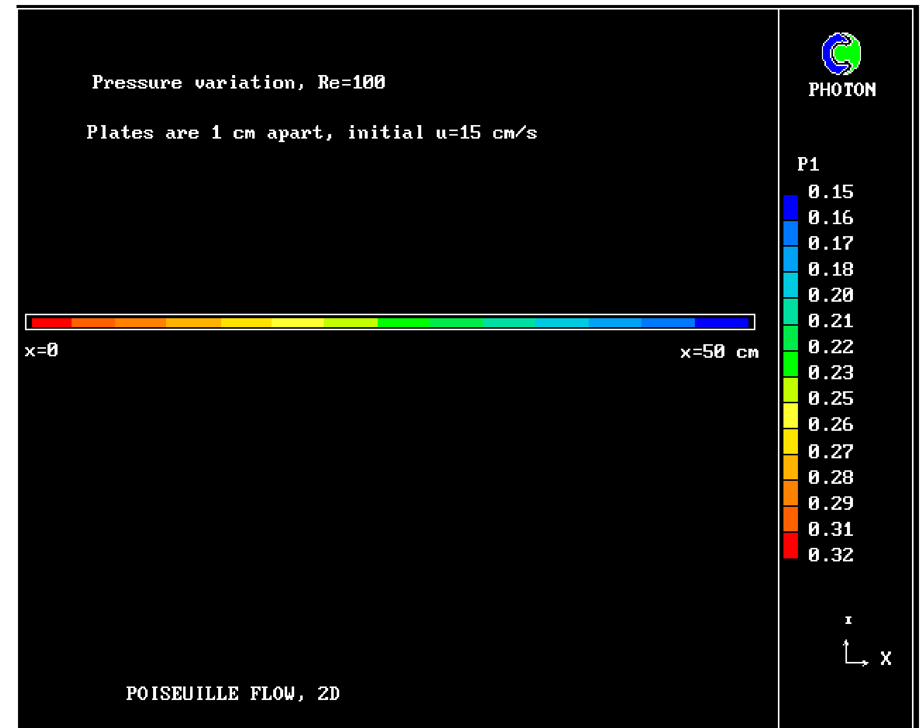
- Microchannel:
- 1 cm long
- 1 mm wide
- 100 μm deep

- L=50 μm
- ρ=1000 kg/m^2
- η=0.001 kg/ms

Laminar for flow speeds less than 10m/s

Poiseuille flow

- Pressure driven flow in channel
- Pressure drop along channel
- Steady flow
- Incompressible flow
- Flow in x-direction, only
- No-slip boundary equations



Poiseuille flow

$$\eta \frac{\partial^2 U_x}{\partial^2 y} + \frac{\Delta p}{L} = 0$$

Integrate twice:

$$U_x(y) = -\frac{1}{2\eta}\frac{\Delta p}{L} y^2 + c1y + c2$$

No slip boundary condition gives:

$$U_x(y) = \frac{1}{2\eta}\frac{\Delta p}{L}\left[(a/2)^2 - y^2\right]$$

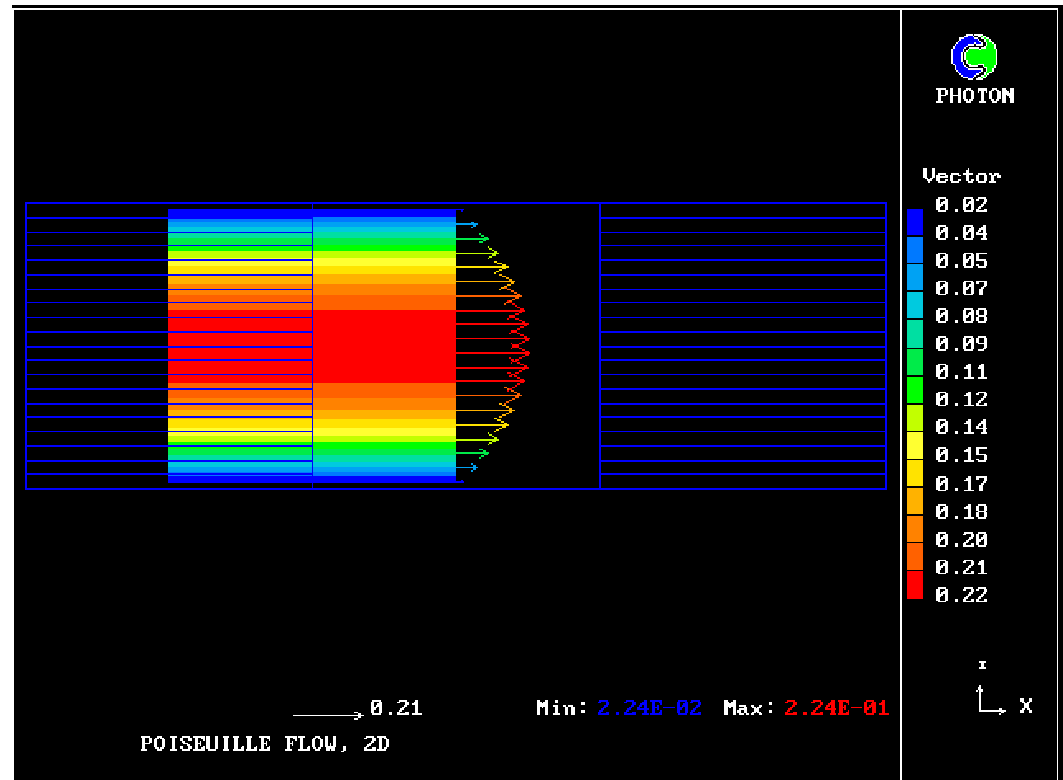
Flow rate:

$$Q = \int_0^{l_z} dz \int_{-a/2}^{a/2} U_x(y) dy$$

$$Q = \frac{l_z a^3}{12\eta}\frac{\Delta p}{L}$$

Circular pipe:

$$Q = \frac{\pi\ a^4}{8\eta}\frac{\Delta p}{L}$$



New Micro Flow Rate Sensor for Standardized Industrial Production

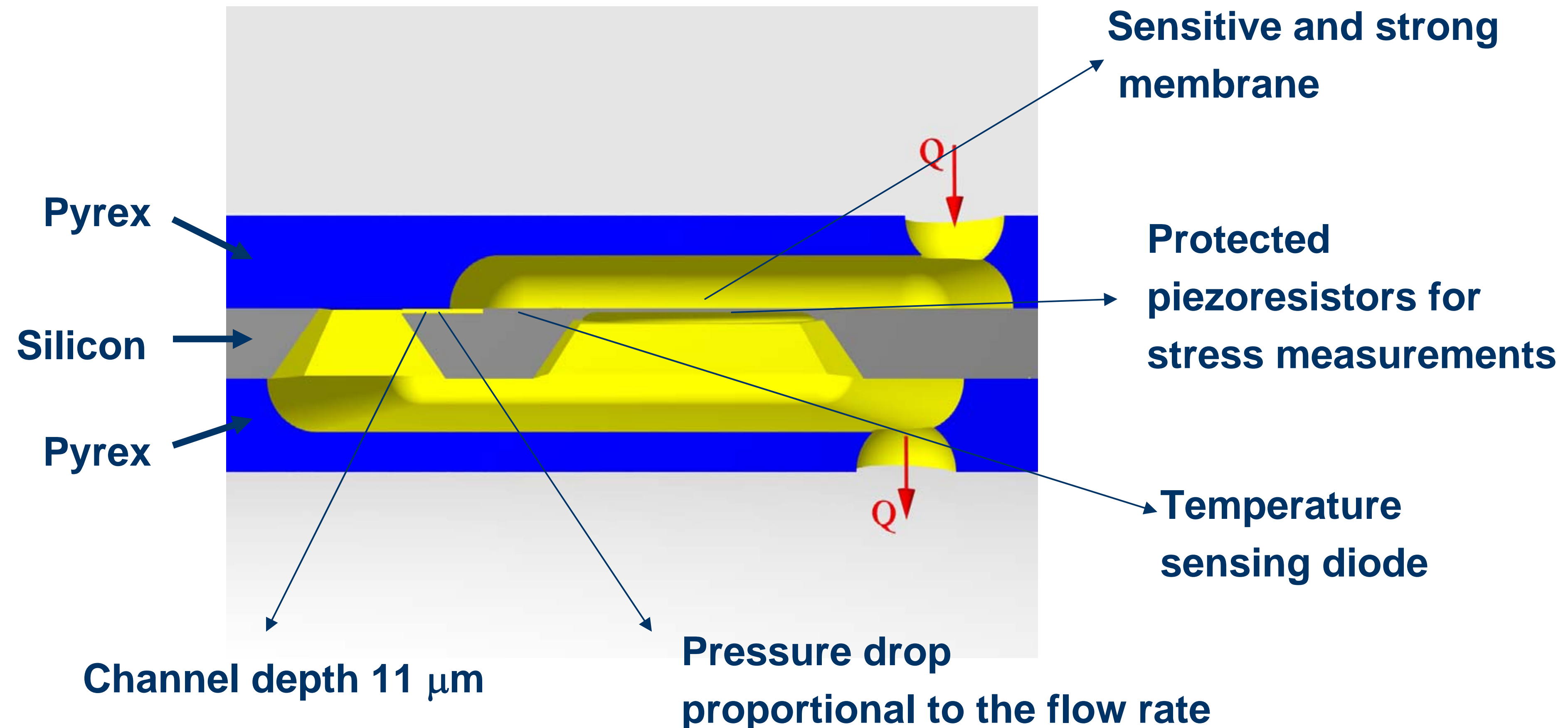
3 μm

6 mm

Liv Furuberg
Dag Wang
Andreas Vogl

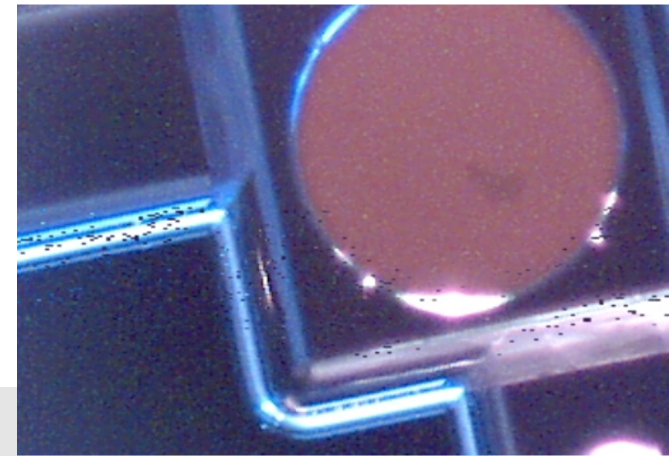
Microsystems and Nanotechnology
SINTEF Information and Communication Technology

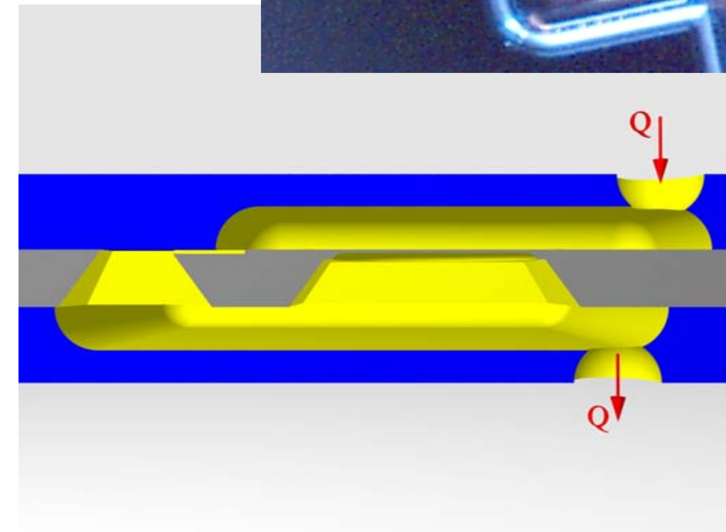
The new design suggests a low-noise, mechanically robust flow sensor



Flow rate sensor

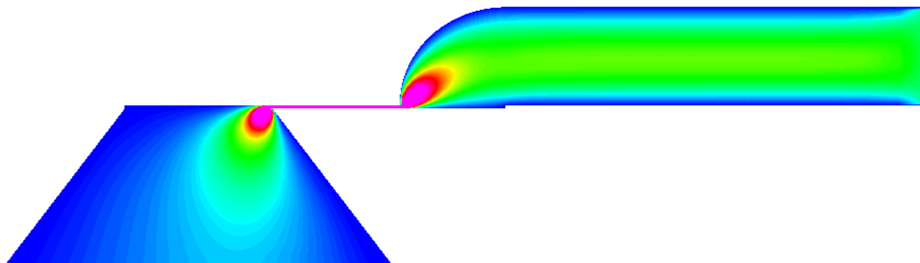
- Measure fluid flow through chip
- Glass-silicon-glass chip
- Laminar flow, low Re numbers
- Differetial pressure sensor (membrane + piezoresistors)
- Narrow channel with pressure drop, Pouseille flow
- Pressure drop ~ 100 -200 Pa
- Integrated thermometer





- Channel: 800x1500x10 μm
- Flow rate 2 μl/min

$$\Delta p = \frac{12 \cdot \eta \cdot l \cdot Q}{w \cdot h^3}$$



Electroosmotic Flow

- Flow driven by electric field
- Voltage applied between electrodes immersed in electrolyte

$$U_0 = \frac{\sigma_w E_x \lambda_D}{\eta}$$

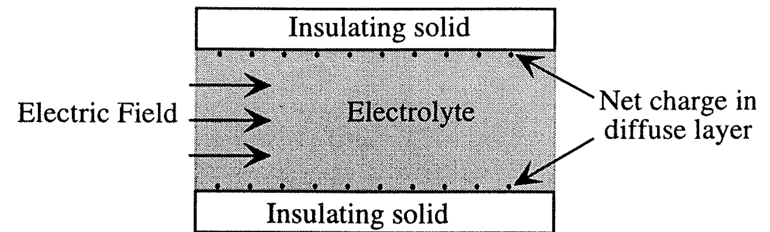


Figure 13.11. Illustrating electroosmotic flow

- Force on fluid near the boundaries, excess of charged particles
- Debye screening layer, typically 10nm

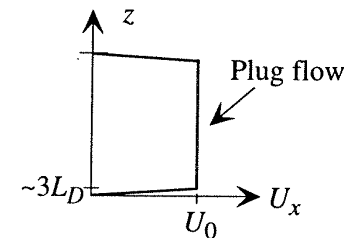


Figure 13.12. Electroosmotic flow profile.

- <u>Disadvantages:</u>

Sensitivity to impurities

Ohmic generation of heat

Need for high voltages

Solving Navier Stokes

Poiseuille flow vs. electroosmotic flow

Advantage in 3D visualization/detection

Three pictures after creation of fluorecent molecule:

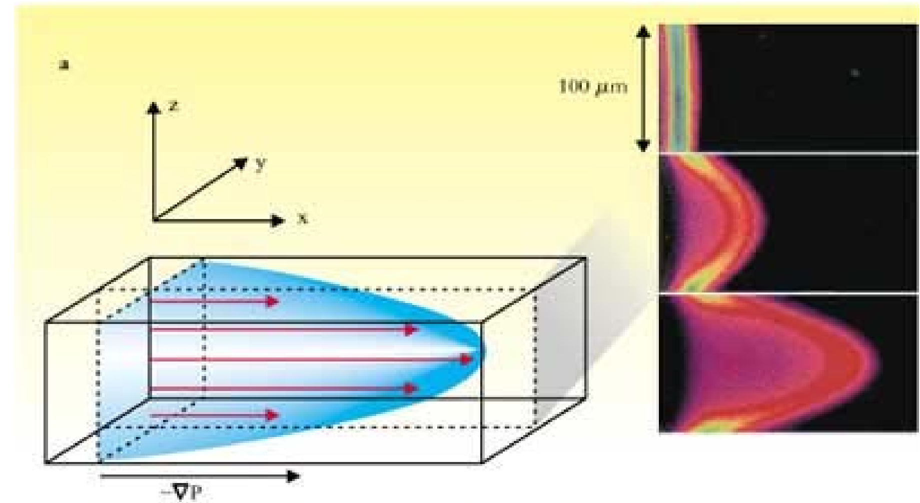
0s

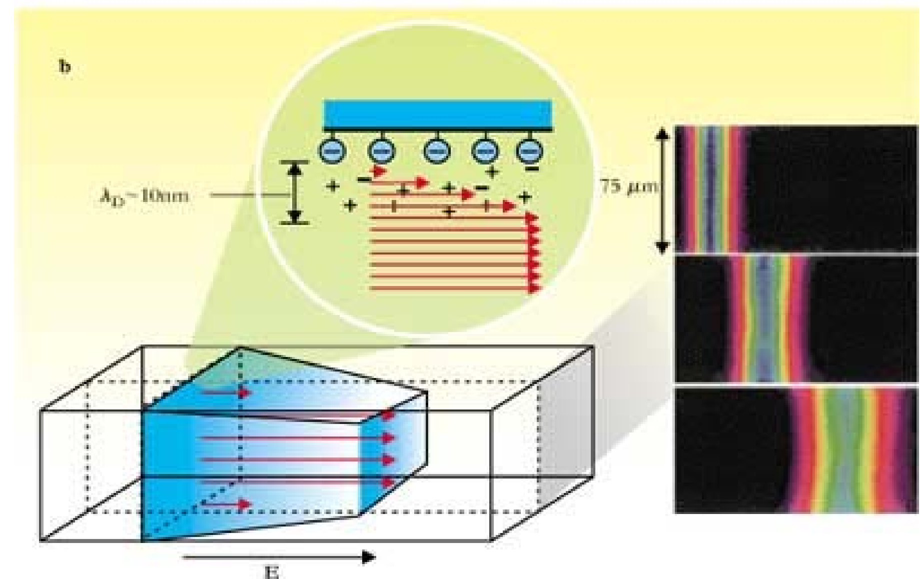
66ms

165ms

Separation based on charge-to-size ratio of molecules.

Separated bands of species





Electrophoresis

- Species carried along with electroosmotic flow
- Drift relative to the moving velocity:

$$v_{ep} = \mu_{ep} \mathcal{E}_x$$

  - Electrophoretic mobility

  - Apply voltages to channels
  - Create controlled plug of species
  - Separate molecules by charge and volume by electrophoresis

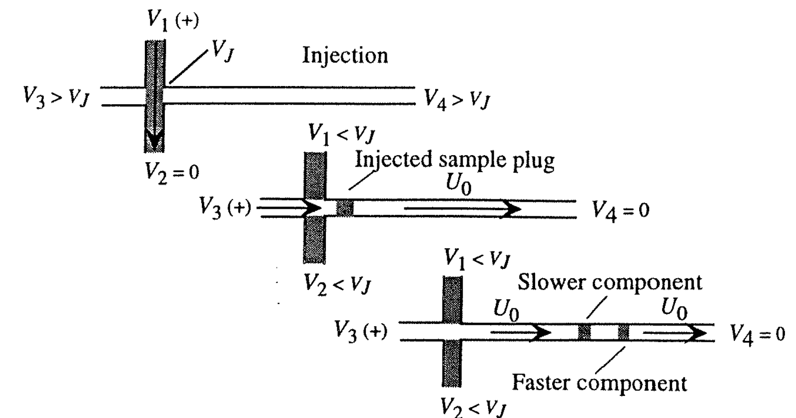


Figure 13.14. Illustrating electrophoretic separation with electroosmotic flow. The voltages used during the injection and separation sequence are described in the text.

Mixing

- Laminar flow
- Mixing by diffusion only

- Diffusion equation

$$\frac{\partial C(r,t)}{\partial t} = D\nabla^2 C(r,t)$$

  - Average displacement of diffusing particle:

$$l = \sqrt{4Dt}$$

  - Diffusion constant for water

$$D = 2.3 \cdot 10^{-9}\,\mathrm{m}^2 / s$$

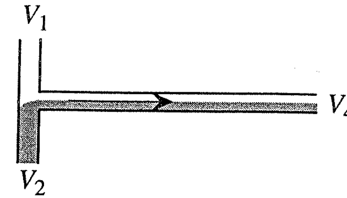


Figure 13.16. Illustrating laminar flow when two streams are combined. Mixing occurs only by diffusion.

- Water: Diffusion length after 1 s: 90µm

- On the other hand:
- Characteristic lines become blurred…
- What about larger molecules?

Mixing

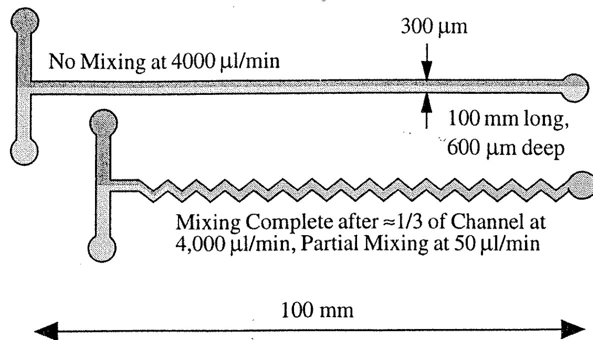
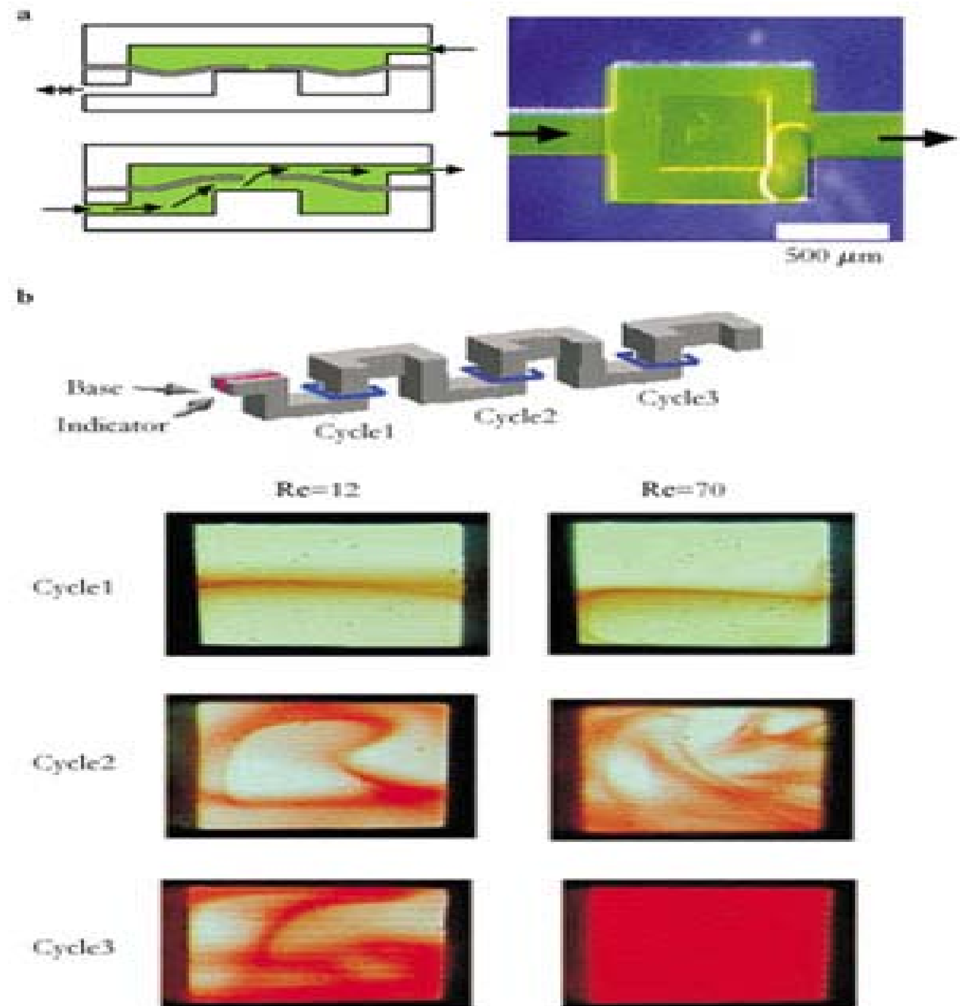
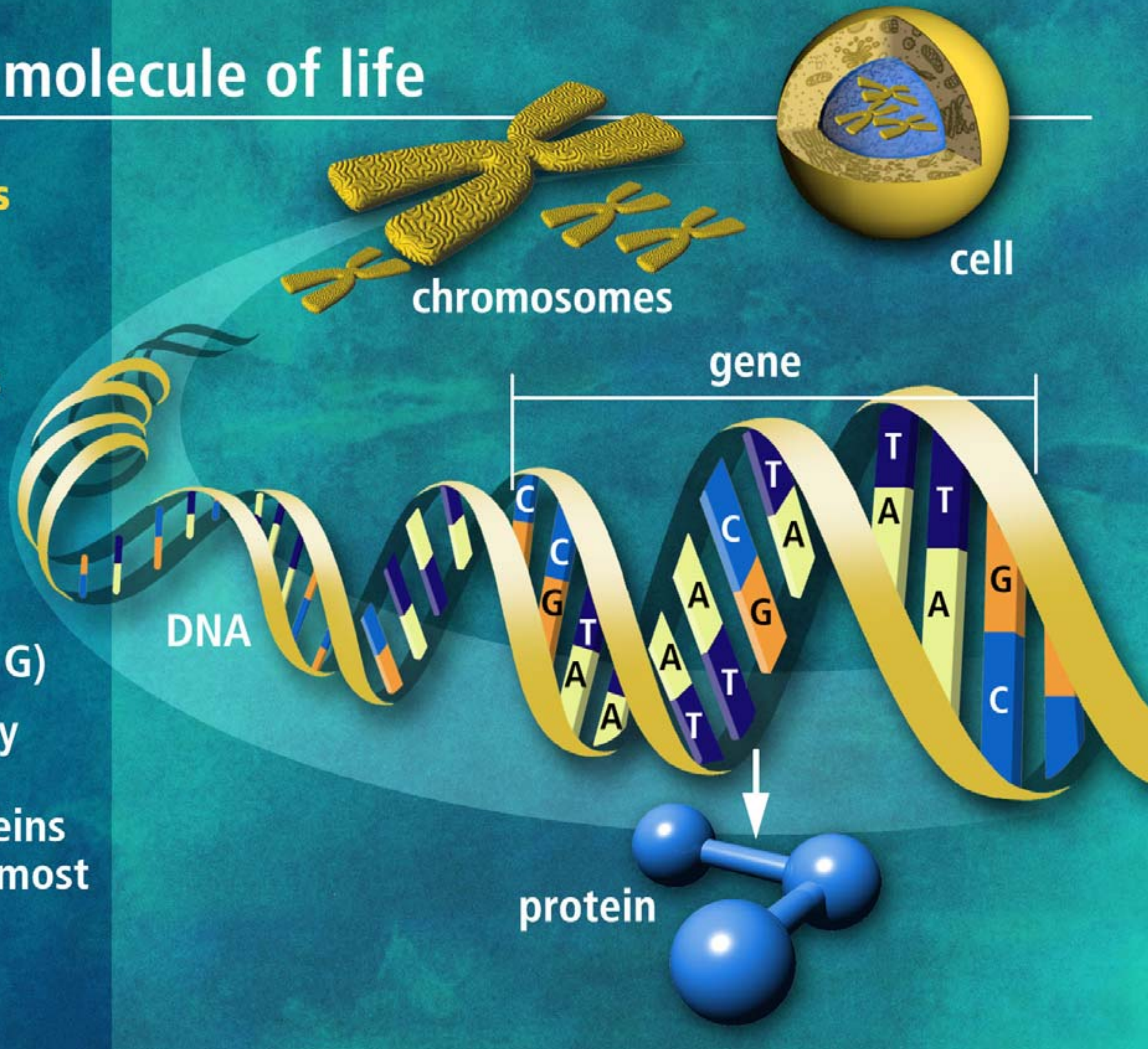
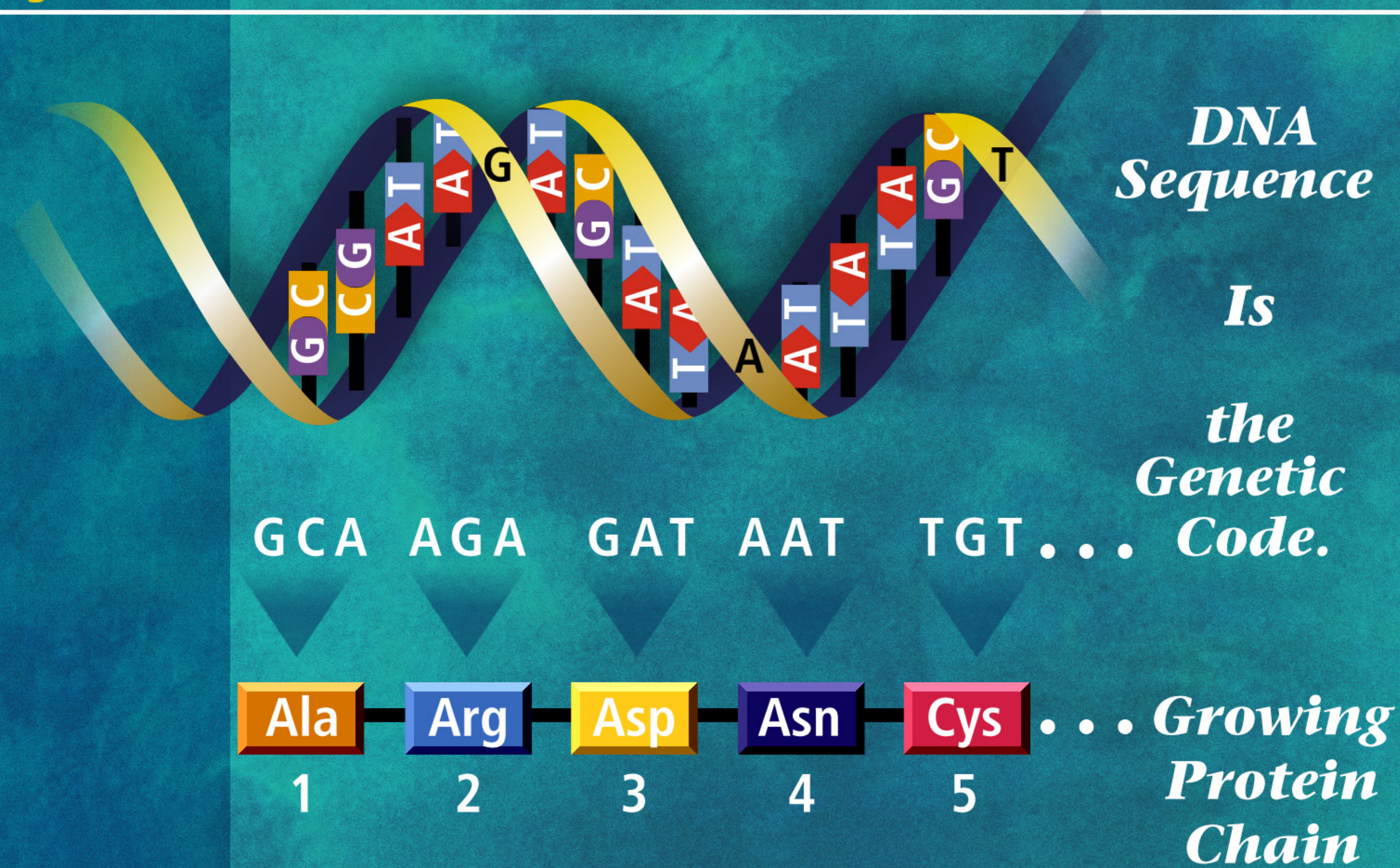


Illustration of miniature fluidic channels used to compare mixing in macroscopic and microscale fluidics. After Branebjerg, et al. (1994).




DNA the molecule of life
Trillions of cells
Each cell:
46 human chromosomes
2 meters of DNA
3 billion DNA subunits (the bases: A, T, C, G)
Approximately 30,000 genes code for proteins that perform most life functions
chromosomes
cell
gene
DNA
protein
Y-GG 01-0085

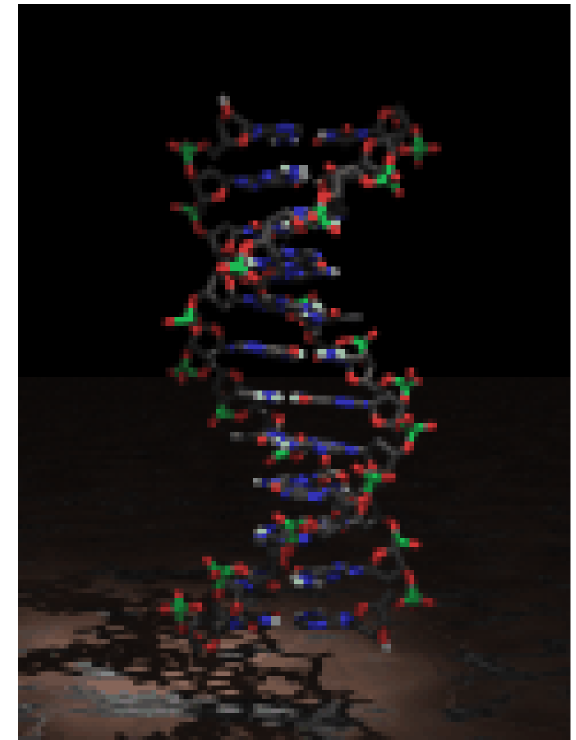
DNA Genetic Code Dictates Amino Acid Identity and Order

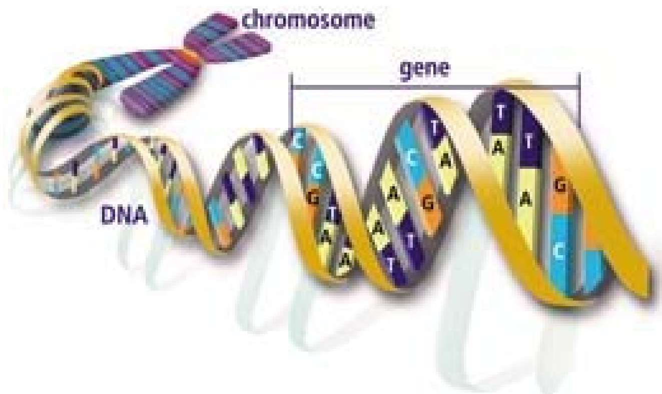


Nucleotides

- Sugar linkage
- Phosphate linking group
- Amino acid group BASE

- Nucleic acids: adenine A
  guanine G
  cytosine C
  thymine T





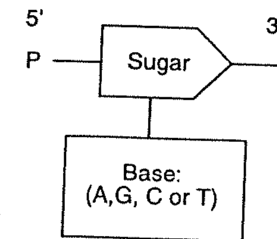
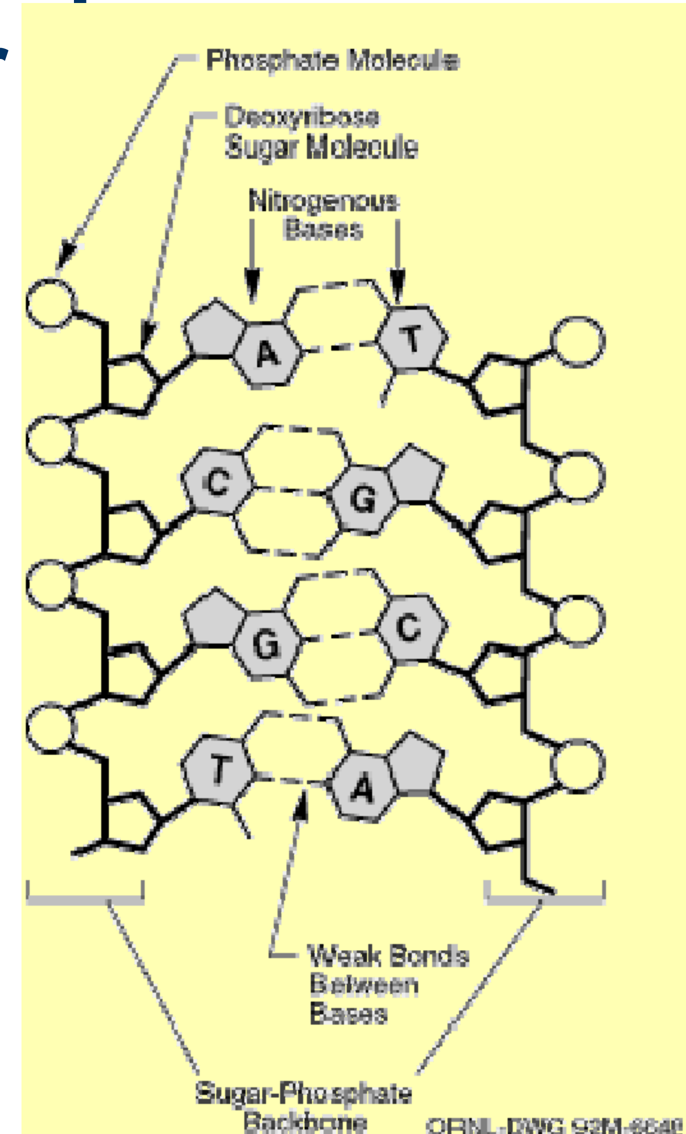
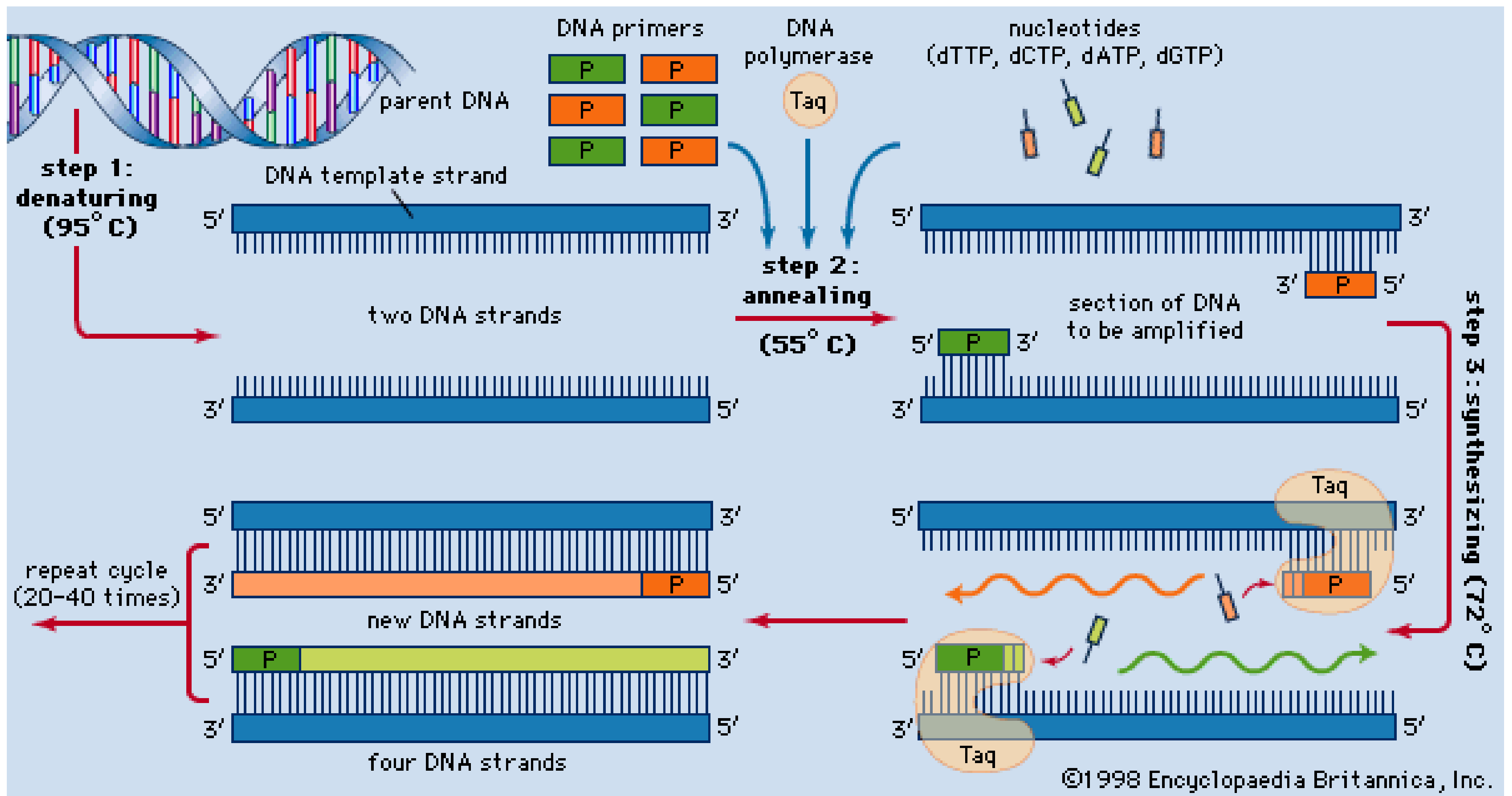


Figure 22.1. A cartoon representation of a nucleotide, consisting of a sugar linkage, a phosphate end-group (P) which serves to link to the next nucleotide, and an amino-acid group, the base. (After [131].)

The four nitrogenous bases of DNA are arranged along the sugar- phosphate backbone in a particular order





DNA primers
DNA polymerase
nucleotides (dTTP, dCTP, dATP, dGTP)
parent DNA
Taq
P
step 1: denaturing (95° C)
DNA template strand
5′
3′
step 2: annealing (55° C)
two DNA strands
section of DNA to be amplified
step 3: synthesizing (72° C)
repeat cycle (20-40 times)
new DNA strands
four DNA strands
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Polymerase Chain Reaction (PCR)

- Denaturing, separate strands, 95 °C
- Annealing,

  cool in presence of primer 65 °C
- Extension, in presence of

  nucleotides and enzymes

  (One enzyme is polymerase)

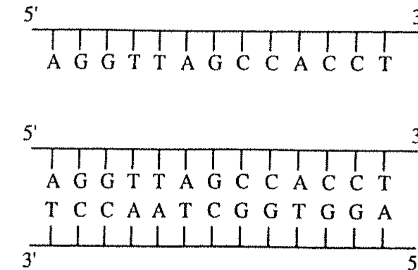


Figure 22.2. Single- and double-stranded DNA fragments.

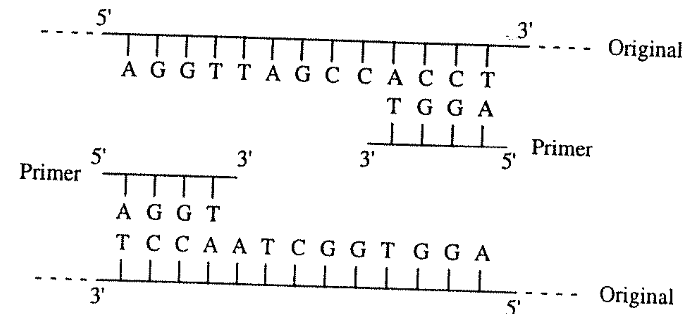


Figure 22.3. Annealing: the primer can attach to single-stranded DNA wherever the target sequence is complementary to the primer sequence.

PCR - cycles

- Copies start at a particular point of DNA chain, extend other way without limit
- Singly terminated chains
- Repeat denature-anneal cycle

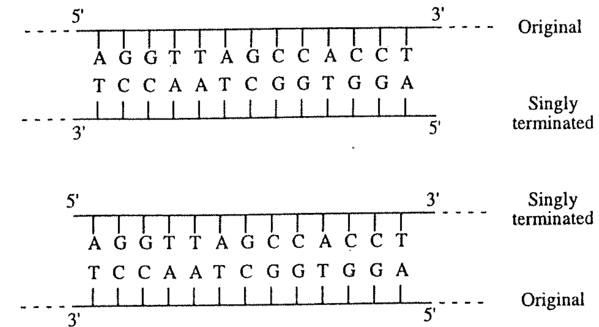


Figure 22.4. After the first extension reaction, the original DNA strands are copied as singly-terminated strands, each one starting from the point of attachment and extending from the 3' end.

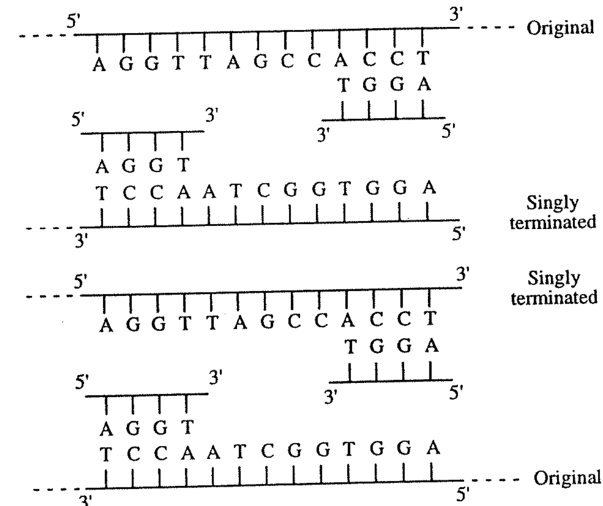


Figure 22.5. After the second anneal, primer can attach to all four strands.

Amplification after 20 cycles

- Start with N_0 DNA molecules
- N cycles
- $n * N_0$ singly terminated strands
- Number of doubly terminated strands more than doubles each cycle

$N_D(n) = 2\,N_D(n-1) + n\,N_0$

- 20 cycles: 2^{19} = 542000 doubly-terminated strands

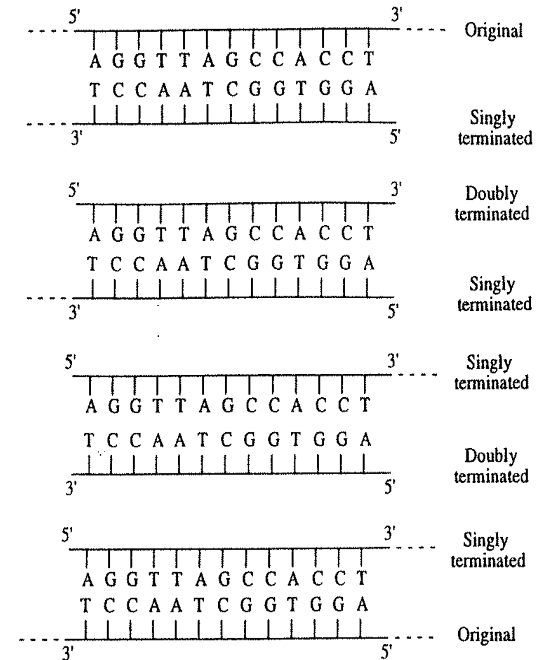


Figure 22.6. After the second extension cycle, there are original, singly-terminated, and doubly-terminated strands.

PCR reactor, batch system

- Thermal time constant of chamber

- Chamber 25 μl
- length: 10 mm height: 0.5 mm

- Heating 35°C/s

- Throughput 58 nl/s

- (Northrup et al)

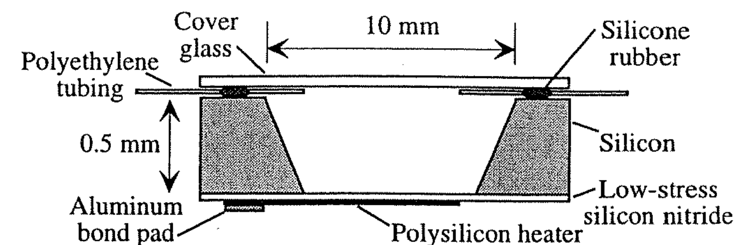


Figure 22.8. The miniaturized PCR chamber reported by Northrup [134].

PCR Batch system, with thermal isolation

- Thermal time constant of chamber
- Chamber 2 µl
- Heating 60-90°C/s
- Heating and cooling pr- cycle: 1.5s
- (Daniel et al.)

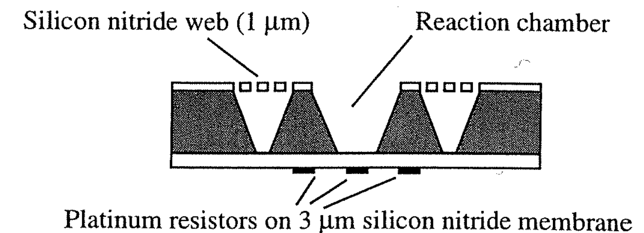
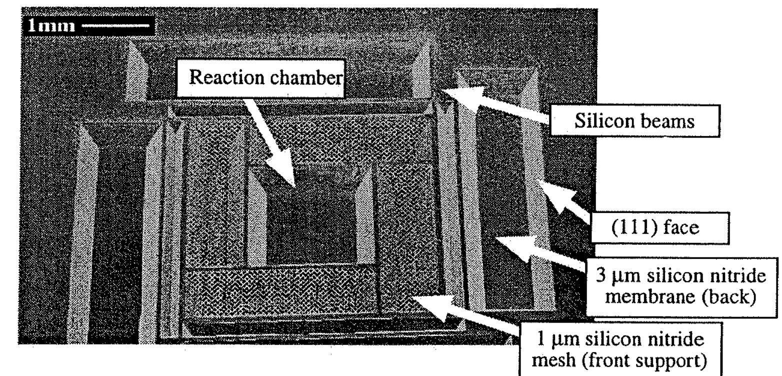


Figure 22.10. Cross-section of the Daniel design of a bulk-micromachined PCR reactor [135].



PCR flow system

- Continuos flow through channels
- Shaded areas at defined temperatures

- No ramping necessary
- Relative time spent in each zone is fixed

- Max flow rate through system: 72 nl/s
- Channel dimension: 40 μm x 90 μm

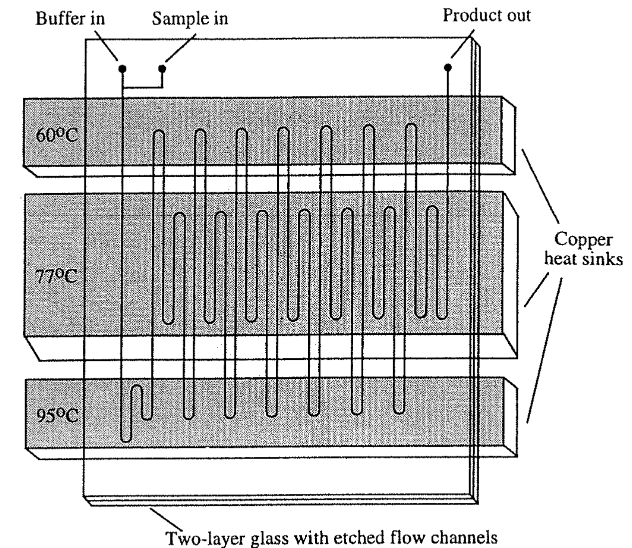
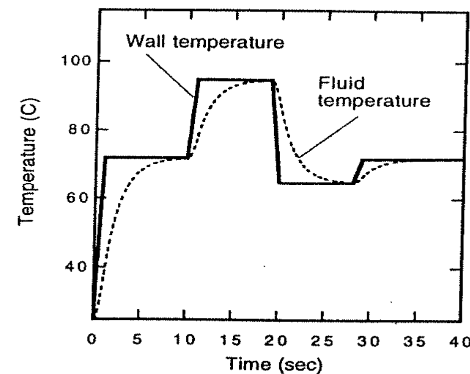


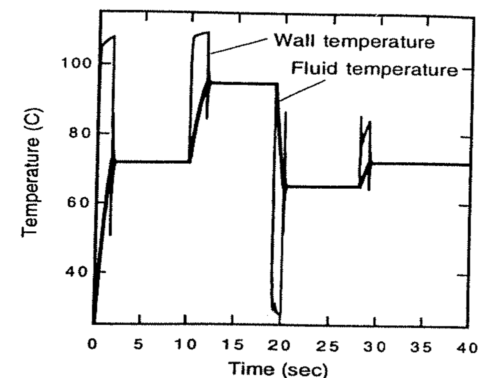
Figure 22.12. A continuous-flow PCR system [136]. A two-layer glass sample with flow channels etched into it is clamped to a support containing three copper heat sinks, each one controlled to a fixed temperature. As fluid flows through the channel, it encounters a typical PCR temperature cycle.

Temperature control in PCR chamber

a) Measure temperature in silicon wall

Feedback: Wall temperature tracks set-point temperature very closely. Fluid temperature lags with exponential responses.





b) Measure temperature in fluid cavity

Feedback: Wall temperature overshoots, not acceptable!