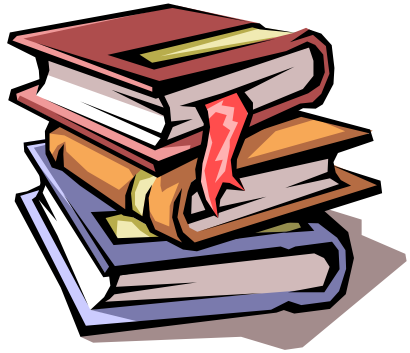
Contents

12. Analytical Chips

1. **Concept and History**
2. Analytical Separations
3. Microfluidic CD Technology
4. Flow Injection Analysis
5. Microfluidic Processors

12.1. Overview



- Integration on microchip
  - Microfluidic channels
  - Functionality
- Application: chemical analysis
- Pioneering work
  - Chip-based chromatography at Stanford in mid 1970s
  - Beginning of 1990s by Andreas Manz at Ciba-Geigy
- Technologies
  - Microtechnology
  - Microfluidics
  - Analytical chemistry
  - Biotechnology

12.1. Ideal: µTAS (micro Total Analysis System)

Integration on single chip

- Sample delivery
- Sample preparation
- Separation
- Detection
- Signal generation

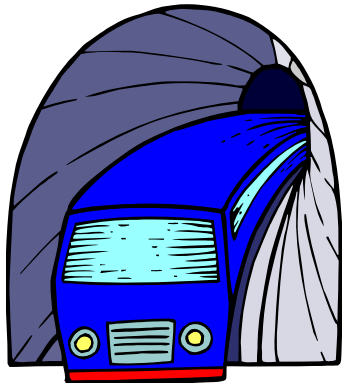
Advantages

- Process integration / automation
- High speed of analysis (min -> sec)
- Amenability for parallelism
- Small dimensions
  - Portability
  - Point-of-Care systems
- Small consumption of reagents and sample

Cost reduction

12.1. Transport of Fluids

- Pressure-driven flow
- Electroosmotic flow
- Capillary action
- Centrifugal forces



12.1. Transport of Fluids

- Capillary action
  - Passive mechanism
  - Surface chemistry
  - Only active during priming

$$p_{\mathrm{cap}} = \frac{2\sigma}{r}\cos\Theta$$

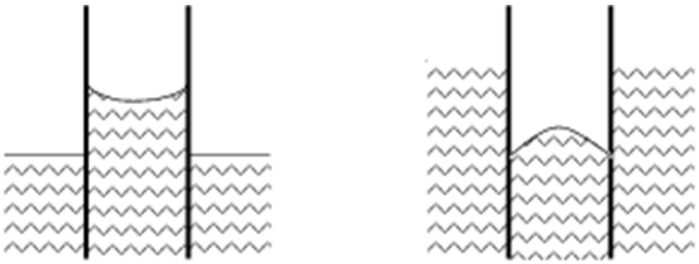


Fig. . Capillary pressure visualized in a tube whose inner surface is coated with a hydrophilic (left) and hydrophobic (right) layer

12.1. Transport of Fluids

- Pressure-driven flow
  - Pumps or pressure source required
  - Unfavorable flow profile for analytical applications
  - Chromatography

$$f_p = |\nabla p| \simeq \frac{\Delta p}{l}$$

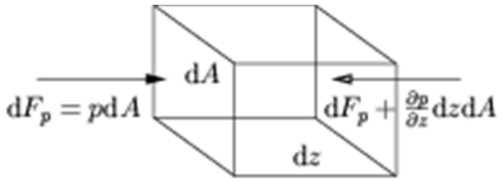


Fig. . Pressure force resulting from a pressure drop across an infinitesimal volume of width dz and cross section dA in the plane perpendicular to the x-axis

12.1. Transport of Fluids

- Electroosmotic pumping
  - Electrically controlled
  - Surface chemistry
  - Strongly depends on fluid-surface combination
  - Electrophoresis

$$v_{\mathrm{eof}} = \mu_{\mathrm{eof}} E$$

$$\mu_{\mathrm{eof}} = \frac{\varepsilon \varepsilon_0 \zeta}{\eta}$$

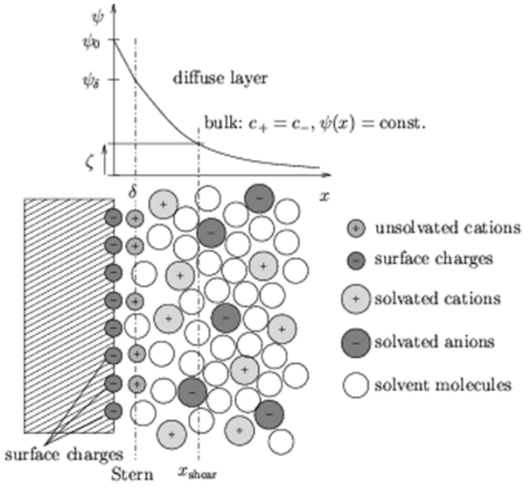


Fig. . Structure of the first fluid layers next to a (negatively) charged surface. The curve of the surface potential $\psi(x)$ reflects the transition from the surface over the immobilized Stern plane at $x = \delta$ and the diffuse layer to the bulk solution with $\psi(x \mapsto \infty) = 0$

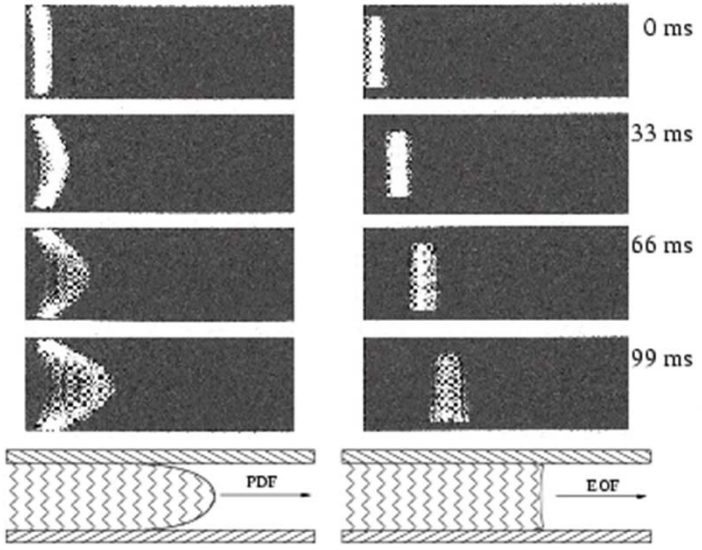
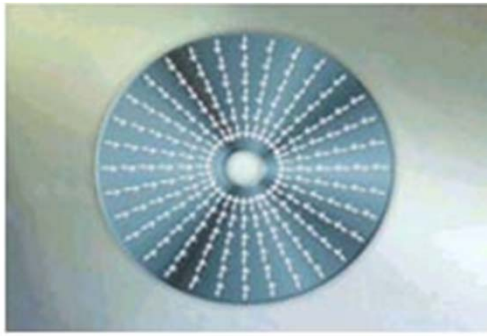


Fig. . Velocity profiles in pressure-driven and electroosmotic flow and experimental observations recorded in 33-ms time frames Paul98

12.1. Transport of Fluids

- Centrifugal forces
  - CD based technologies
  - Controlled by frequency of rotation
  - Volume force
  - Widely independent of fluid characteristics

$$\boldsymbol{f}_\omega = \varrho \omega^2 r \hat{e}_r$$



- Hydrophobic barriers
  - Blocking flow at low frequencies

$$\Delta p_{\mathrm{cap}} = 2\sigma \cos\Theta \left[\frac{1}{r_1} - \frac{1}{r_2}\right]$$

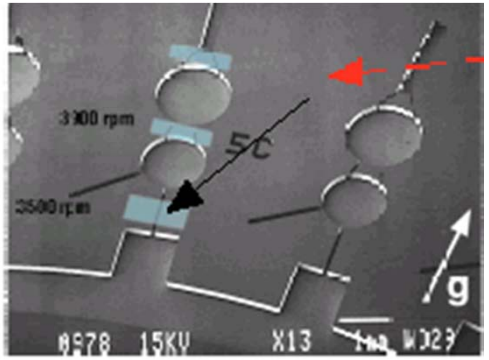


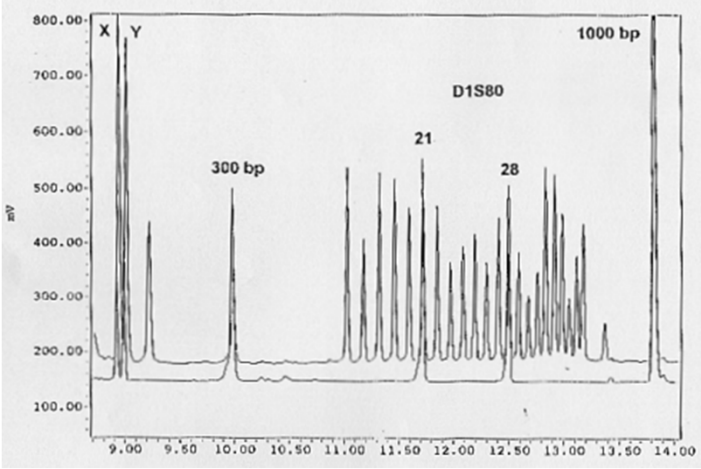
Fig. . Hydrophobic valve Gyros00

12. Analytical Chips

1. Concept and History
2. **Analytical Separations**
3. Microfluidic CD Technology
4. Flow Injection Analysis
5. Microfluidic Processors

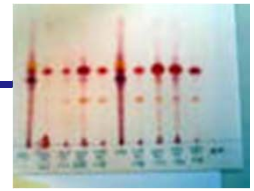
12.2. Introduction to Separations

- Initial situation
  - Solution / mixture containing different chemical substances
- Aim
  - Identification of individual components
- Method
  - Separation
  - Subsequent detection of individual components
- Simple procedures
  - Centrifugation
  - Crystallization
  - Distillation
  - Filtration
- Instrumental procedures
  - Chromatography
  - Electrophoresis

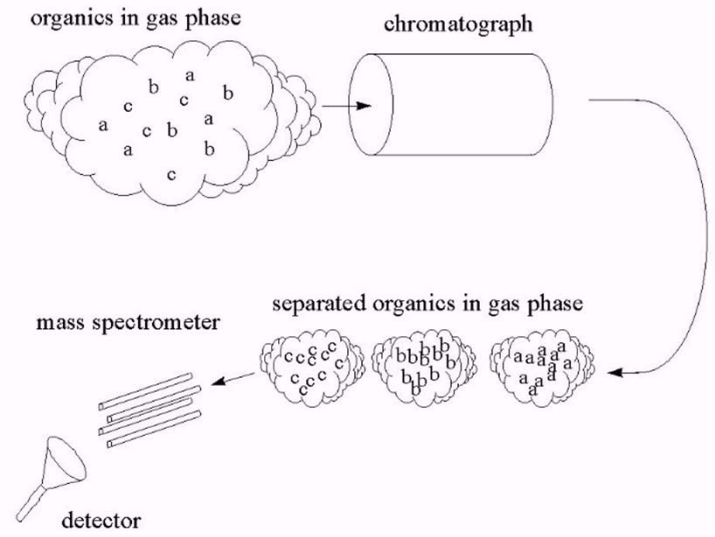


Source: E. Verpoorte: Short Course on Microfluidics at Nanotech´99 in Montreux

12.2. Components of Chromatography

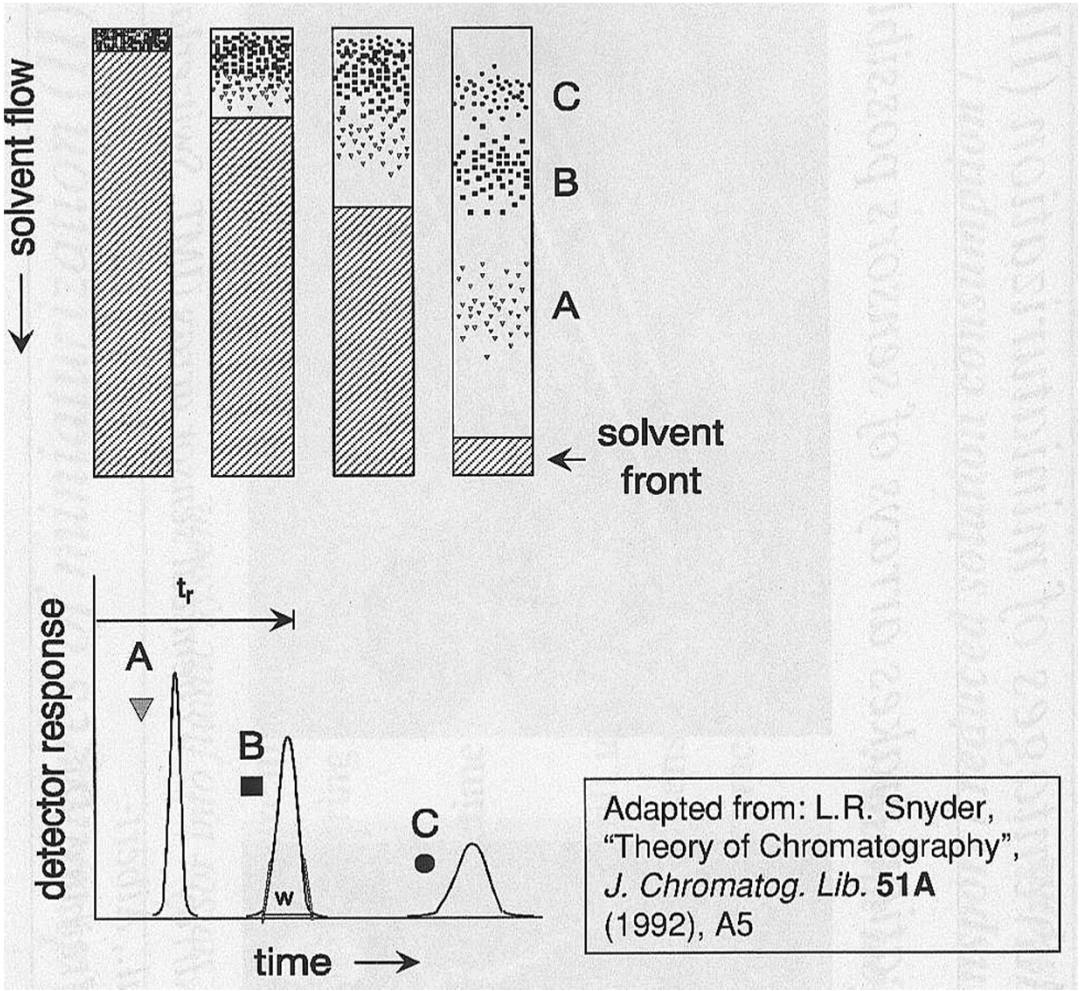


- Stationary phase
  - Attached to separation column
- Mobile phase
  - Liquid or gas
- Solvated / dispersed substances
- Injection
- Detection



- Operating principle
  - Interaction of physical or chemical nature of substances carried by mobile phase with stationary phase lead to diverging retention times

12.2. Chromatography: Principle

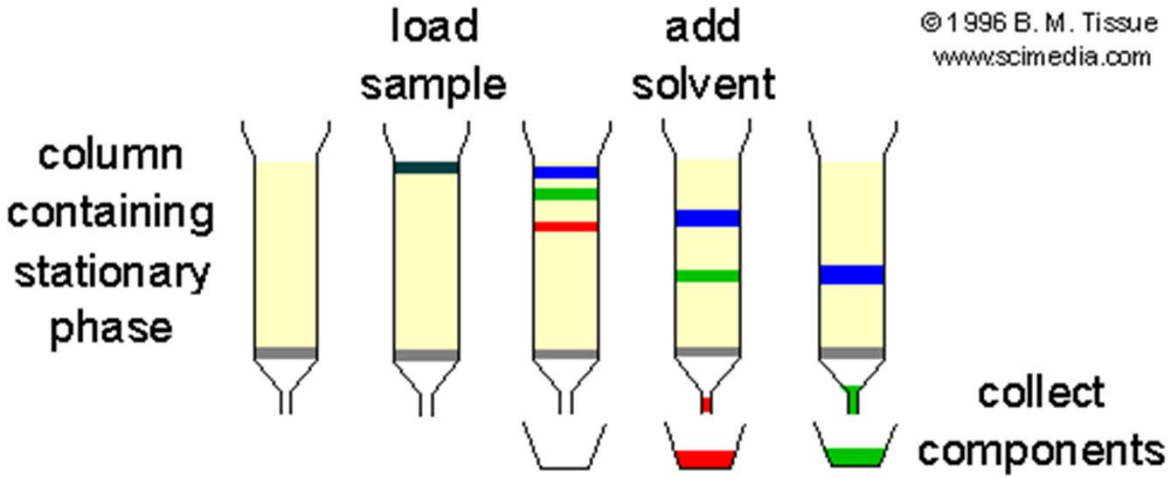


12.2. Types of Chromatography

- Gas chromatography (GC)
  - Applied to **volatile organic compounds**. The **mobile** phase is a **gas** and the **stationary** phase is usually a **liquid** on a **solid support** or sometimes a solid adsorbent.
- Liquid chromatography (LC)
  - Used to separate analytes in solution including **metal ions** and **organic** compounds. The **mobile** phase is a **solvent** and the **stationary** phase is a **liquid** on a solid support, a solid, or an ion-exchange resin.
- High-performance liquid chromatography (HPLC)
  - A variation of **liquid chromatography** that utilizes **high-pressure pumps** to increase the **efficiency** of the separation.
- Size-exclusion chromatography (SEC)
  - Also called gel-permeation chromatography (GPC), the mobile **phase** is a **solvent** and the **stationary** phase is a packing of **porous particles**.
- Thin-layer chromatography (TLC)
  - A simple and rapid method to monitor the extent of a reaction or to check the purity of organic compounds. The mobile phase is a solvent and the stationary phase is a solid adsorbent on a flat support.

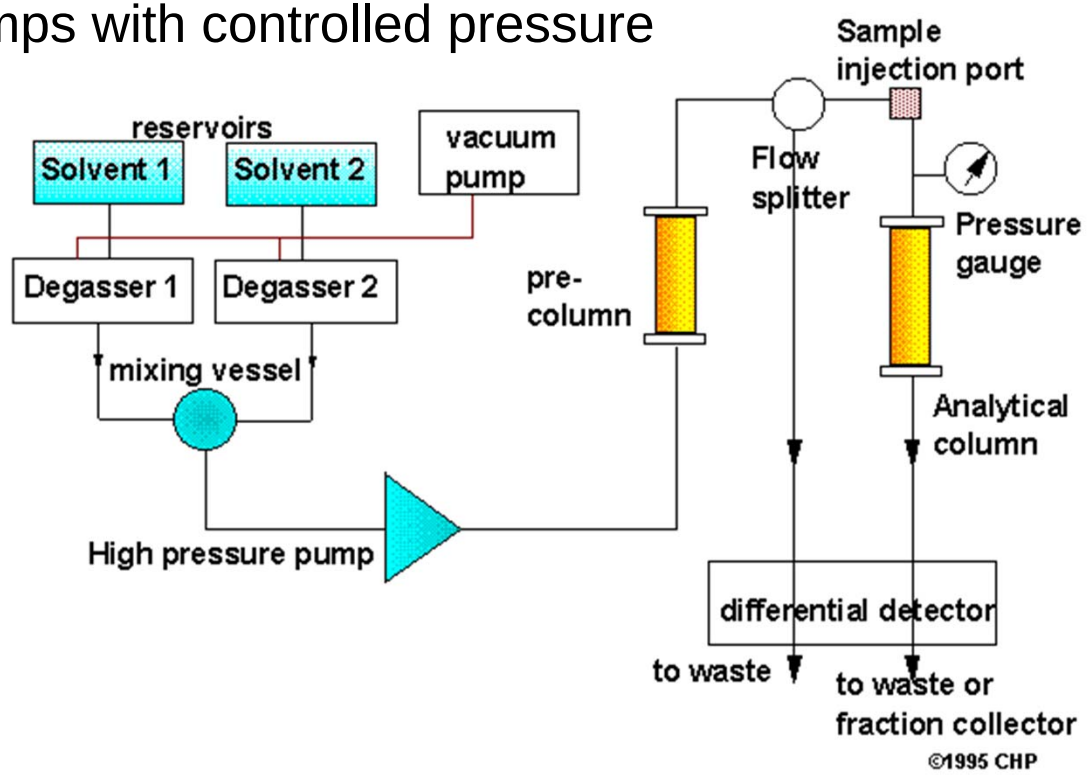
12.2. Liquid Chromatography (LC)

- Separation of ions and molecules in solvent
- Often used for preparation
  - ➢ Isolation of components of mixture



12.2. High-Pressure LC (HPLC)

- Analytical separations for detection and quantification
- Injection
- Degassing
- High-pressure pumps with controlled pressure

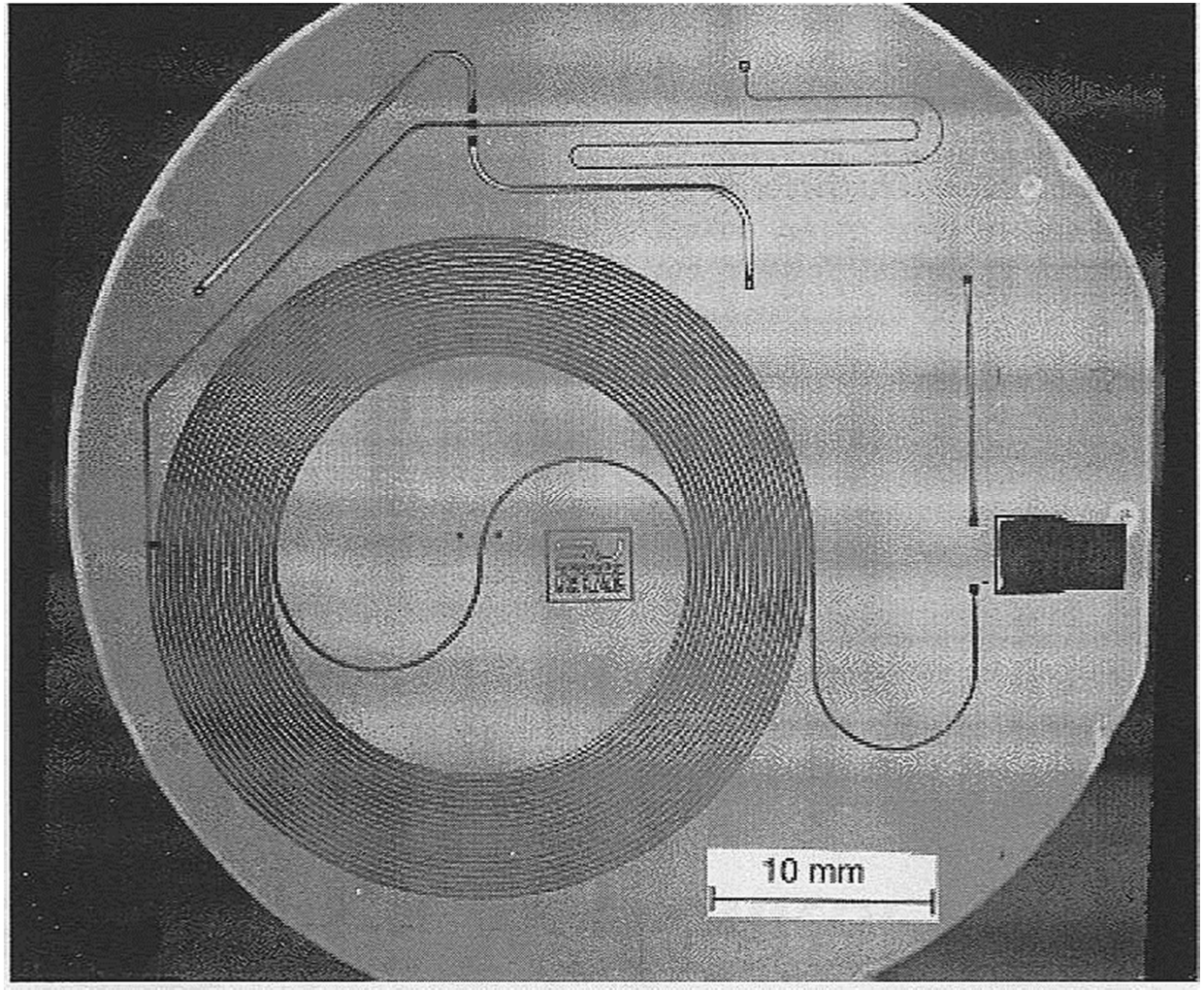


12.2. Micro-Column-HPLC: Advantages

- Reduction of column diameter
  - Improved efficiency due to smaller diffusion distances
- Faster separations
- Important applications
  - High-throughput analysis („High-Throughput Screening“)
  - Online monitoring

- Methods of miniaturization
  - Reduction of column diameter
  - Stationary phase made of micro-particles

12.2. Historical Example from 1975: Stanford Micro-Chromatograph (Terry et al.)



12.2. HPLC: Dead Volumes

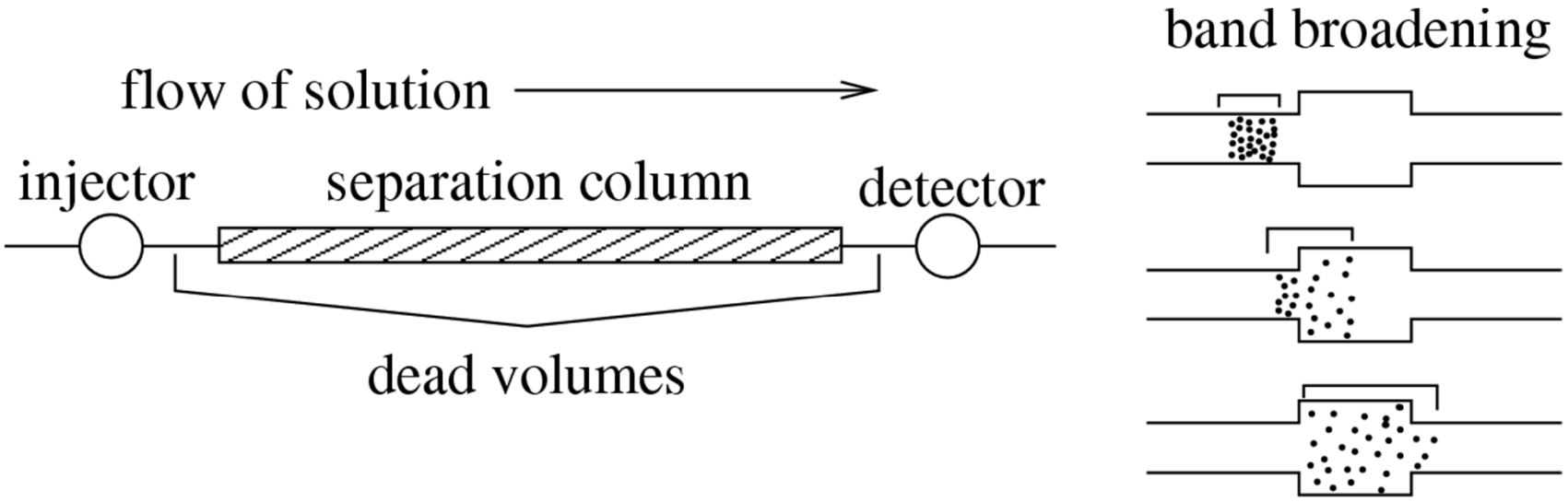
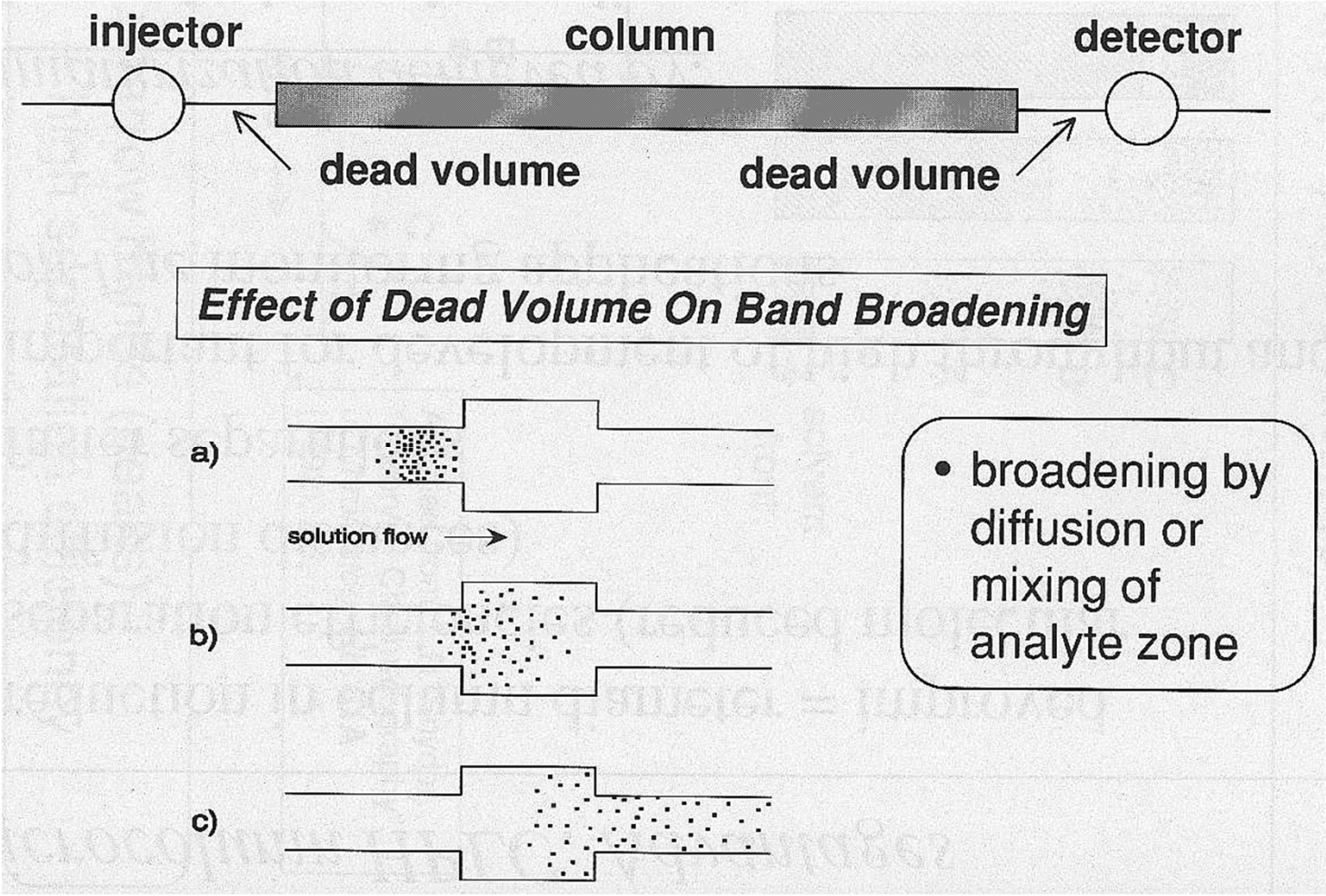


Fig. 12.6. Dead volumes in HPLC

12.2. Micro-Column-HPLC: Disadvantages (1)



12.2. Micro-Column HPLC: Disadvantages (2)

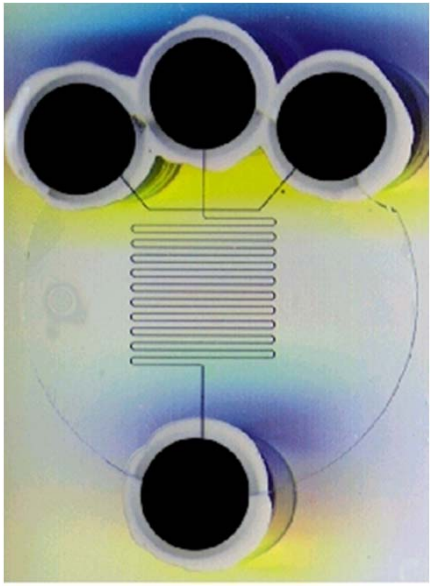
- Micro-LC bottleneck:
  - Reduction of column volume implies severe reduction in detection volume

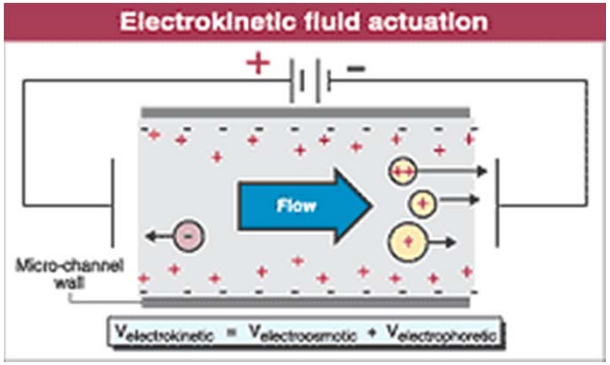
$$V_{\text{col}} = \mu\text{l} \;\; \text{means} \;\; V_{\text{det}} = \text{nl}$$

- Problems with efficiency
- Problems with detection of small probe volumes

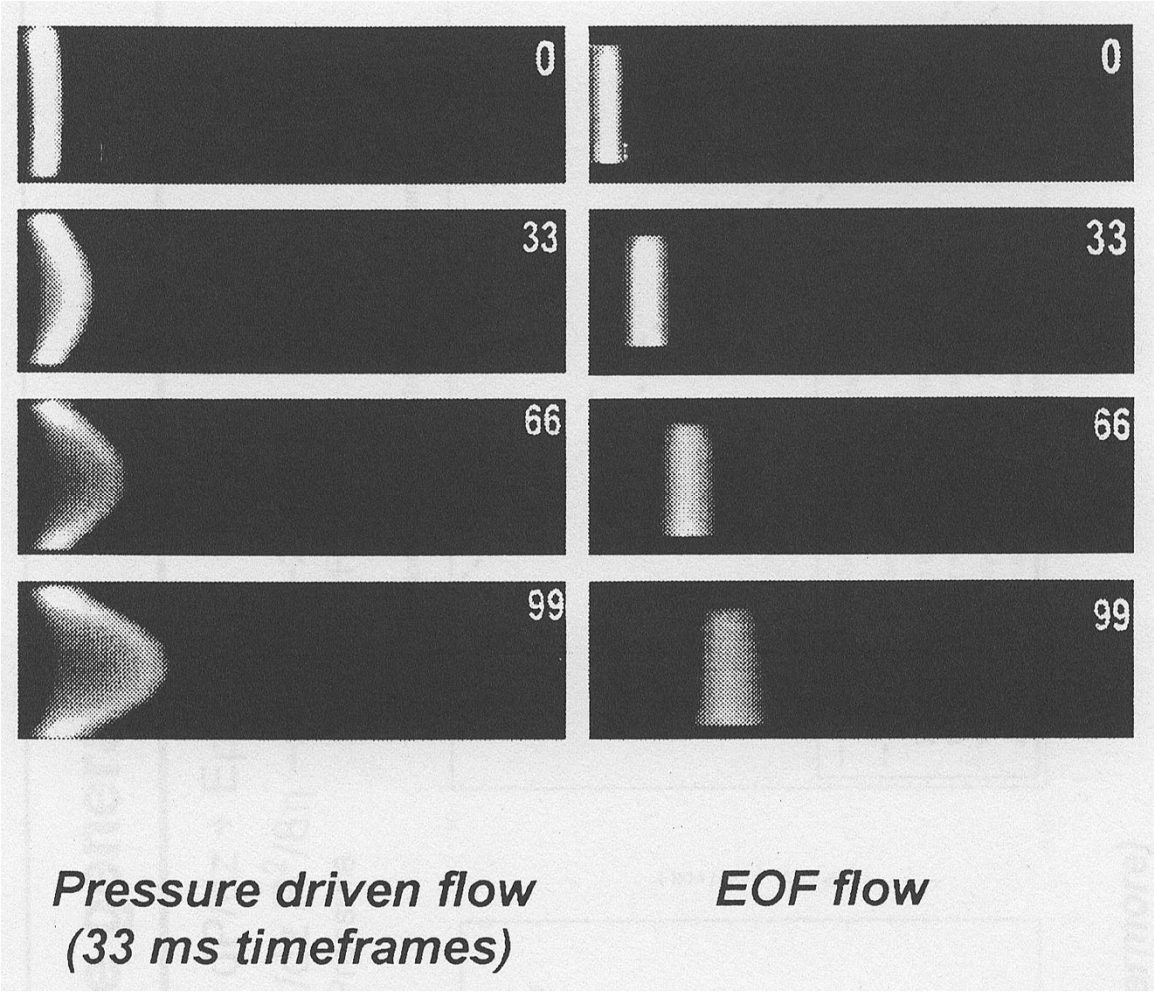
12.2. Electrokinetics

- Electroosmotic flow
- Electrophoresis



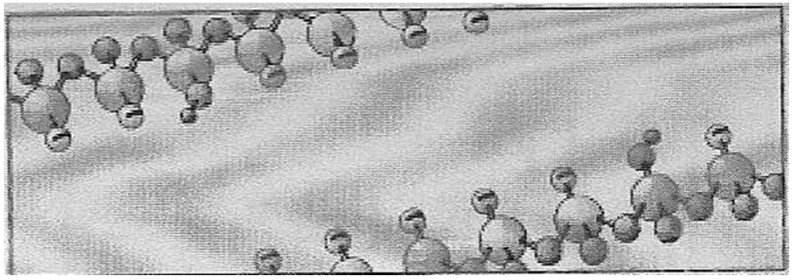


12.2. Flow Profiles: PDF and EOF

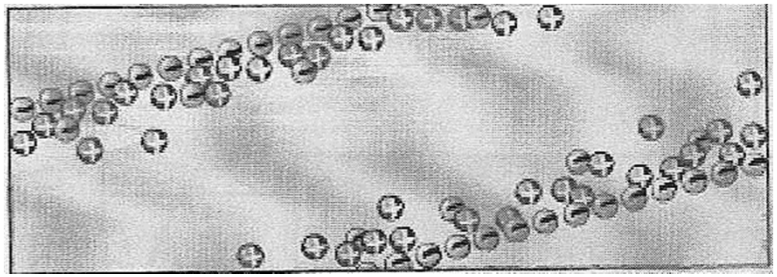


12.2. Electrokinetics: Electroosmotic Flow

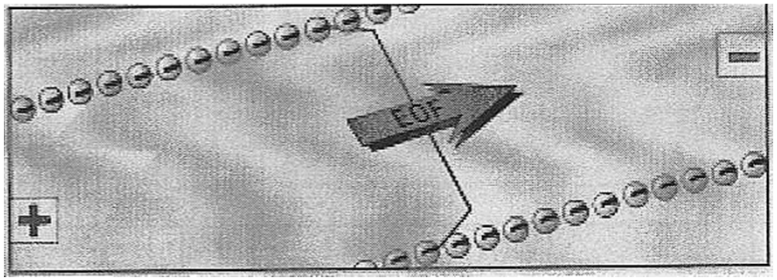
- Glass surface
  - Silanol groups
- pH-value above 2.5
  - Dissociation of protons in solution



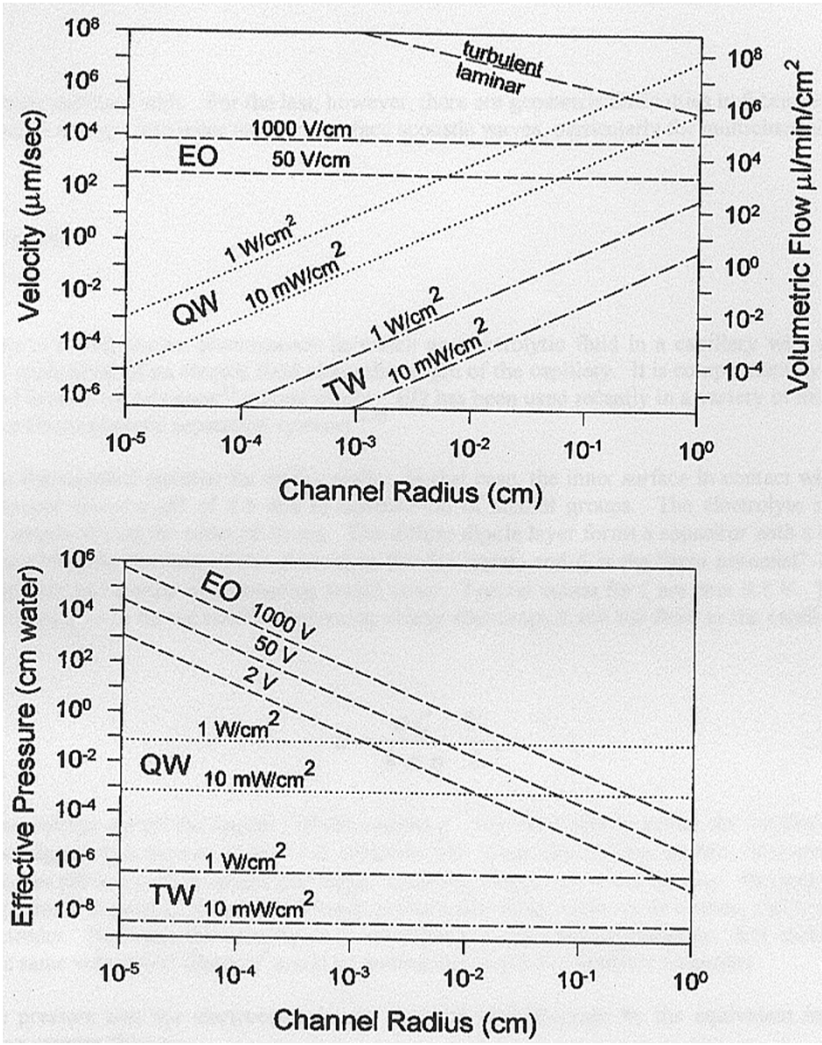
- Diffuse layer
  - Immobilized surface charges
  - Counteracted by
    - Solvated ions
    - Partially solvated ions



- Electric field
  - Cations migrate to cathode
  - Bulk liquid dragged



12.2. Electroosmotic Pumping: Characteristics



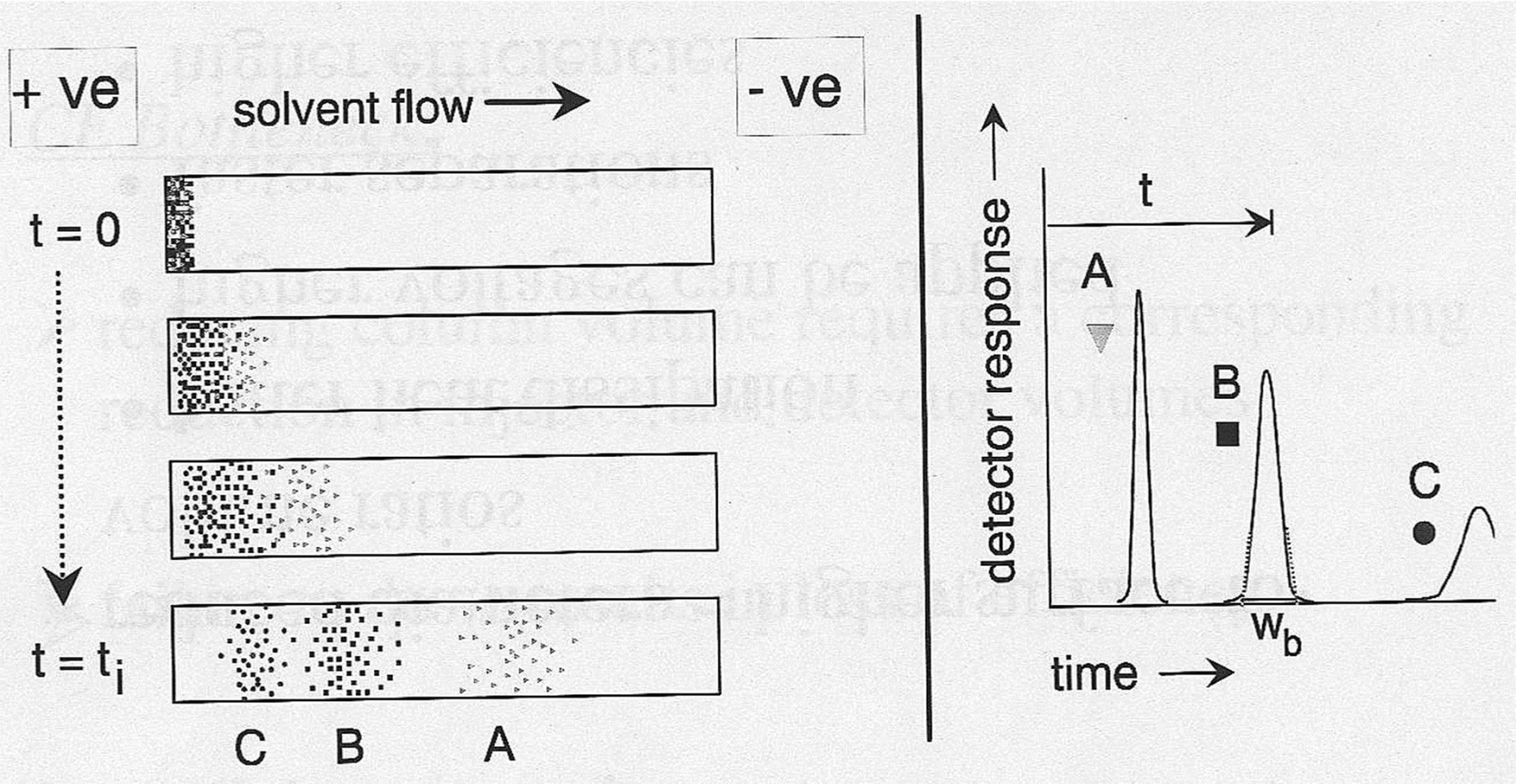
12.2. Electrophoresis (EP)

Definition: A technique for the analysis and **separation** of molecules based on their **migration** in an **electric field**; migration is generally dependent on both the **charge** and **size** of the molecule.

- Gel Electrophoresis
  - A technique for the analysis and separation of molecules based on their migration in an electric field; migration is generally dependent on both the charge and size of the molecule.

- Agarose Gel Electrophoresis
  - A technique for the analysis and separation of molecules using agarose gel as the stationary phase. Agarose gel electrophoresis is important in gene manipulation and sequencing, since it can separate DNA molecules on the basis of their molecular weights. The bands on the gel are detected using ethidium bromide, so that levels as low as 0.5 mg DNA can be detected by examination in ultraviolet light.

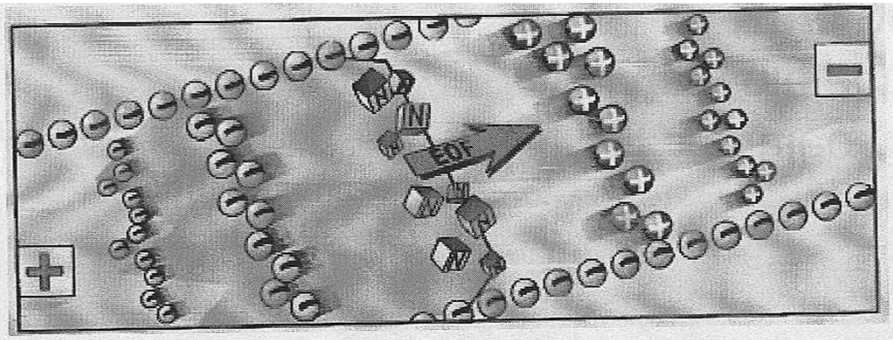
12.2. Electrophoresis: Principle



capillary electropherogram

12.2. Electrophoresis: Mechanism

- EP superimposed on EOF
- EOF prevails
- All ions (and neutrals) migrate to cathode



$v_E = \mu_E E$

$\mu_E = q / 6 \pi \eta r$

where: v_E = velocity
μ_E = electrophoretic mobility
E = electric field
q = ion charge
r = ion radius
η = viscosity

12.2. Gel Electrophoresis of Polyelectrolytes: Theory

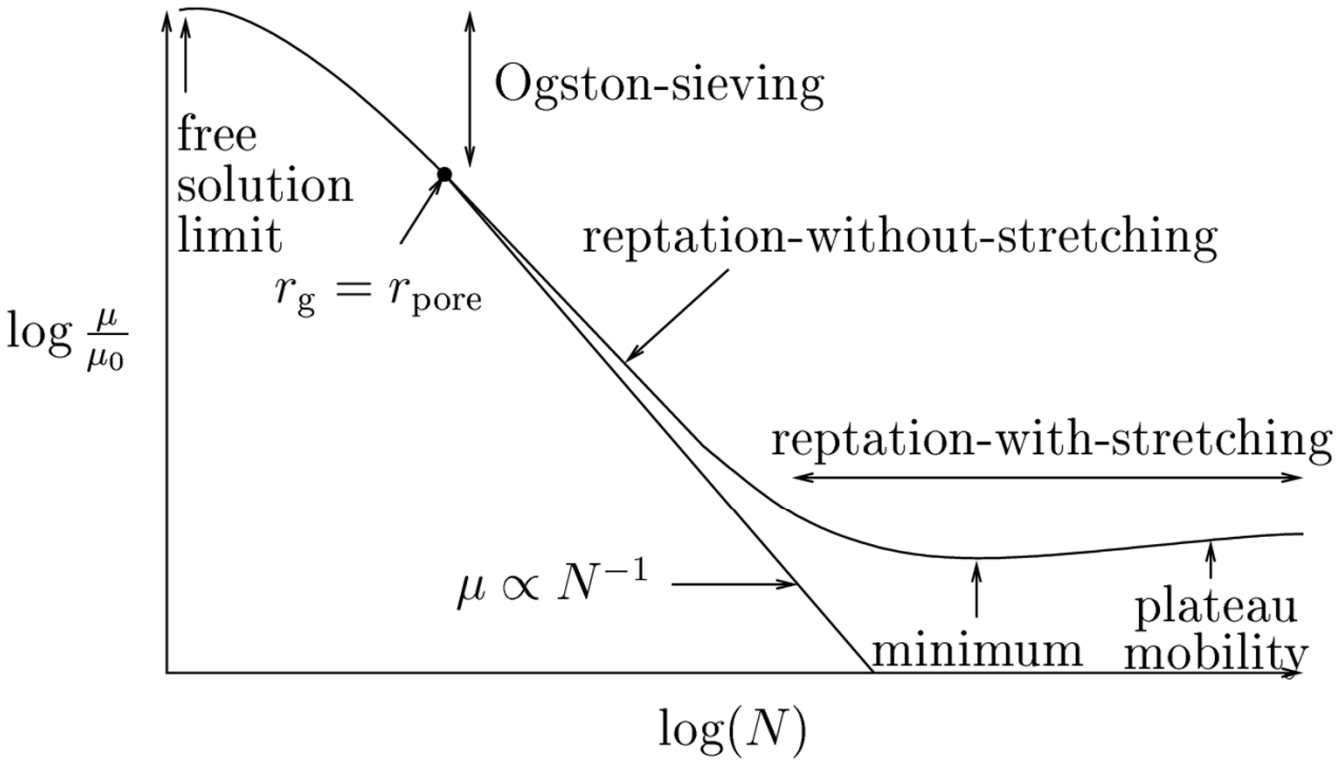


Fig. 12.7. Theory of capillary gel electrophoresis. In the log–log plot, the ratio of the electrophoretic mobility μ with respect to its mobility in free solution μ_0 is plotted versus the length of the polymer N. Three principal modes of electromigration can be distinguished: Ogston sieving, reptation–without–stretching and reptation–with–stretching

12.2. Gel Electrophoresis of Polyelectrolytes: Theory

Fundamental Modes

- Ogston sieving
  - Polyelectrolyte migrating through **sieving** matrix in electric field
  - Size smaller than pores of **sieving** matrix
  - Ogston model based on probability that undeformable spherical molecule of analyte enters pore
  - **Sieving** effect depends on
    - Pore size
    - Polyelectrolyte particle diameter
  - Probability for entering pore decreases with pore size
  - Modification for single-stranded DNA
    - applied electric field and gel matrix distort molecule to symmetric ellipsoid
    - Major axis of ellipsoid parallel to direction of migration
    - Gel pores interact primarily with circular cross-section
    - Ellipsoid functionally equivalent to sphere
- Reptation without stretching (orientation)
- Reptation with stretching (orientation)

12.2. Gel Electrophoresis of Polyelectrolytes: Theory

Fundamental Modes

- Ogston sieving
- Reptation without stretching (orientation)
  - Polyelectrolyte chain forms random coil larger than pore size
  - Migration as sphere not possible
  - Abandoning of coil shape
  - Migration by reptile-like motion through gel
  - Lateral motion blocked by entanglements
  - Mobility reciprocally proportional to polymer chain length *N*
- Reptation with stretching (orientation)
  - Above electric field strength and chain length *N*
  - Stretching of polyelectrolyte
  - Mobility independent of *N*

12.2. Electrophoresis: Setup

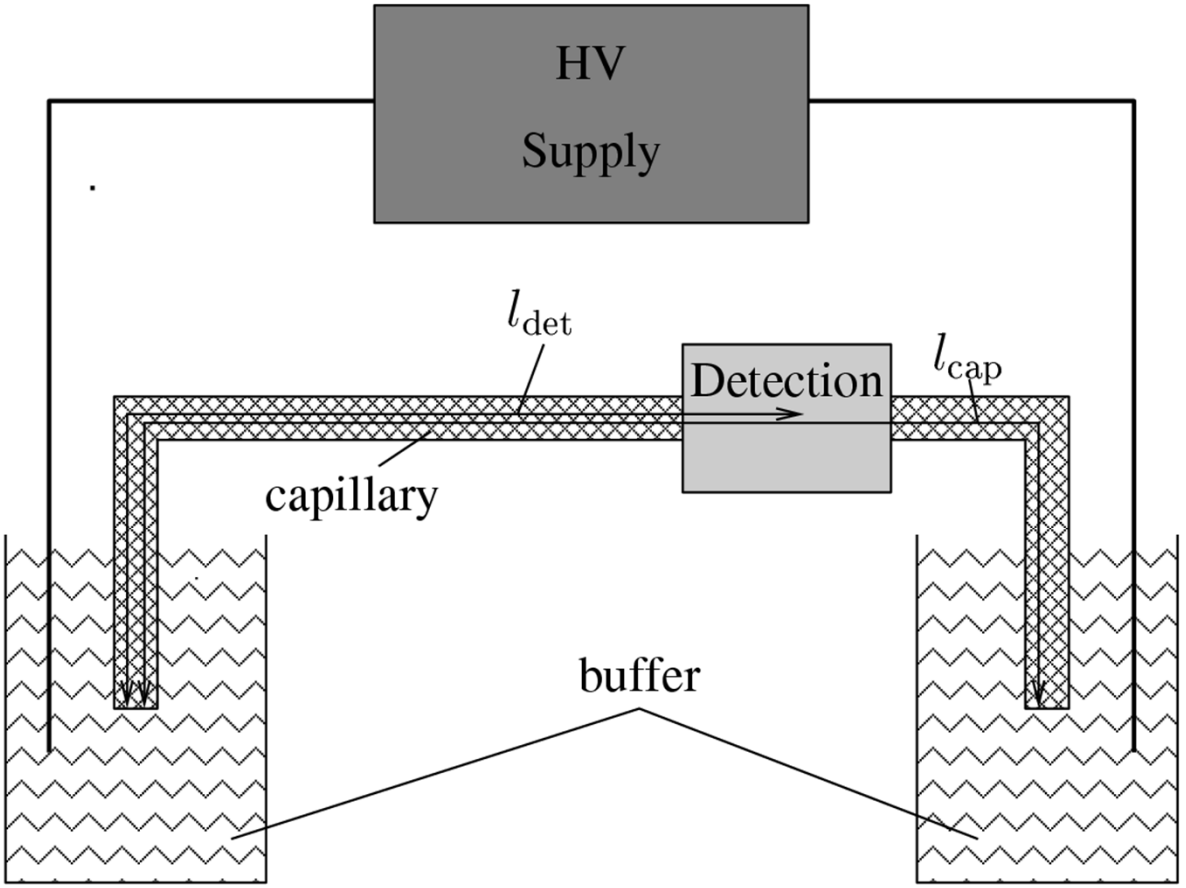


Fig. 12.9. Schematic diagram of capillary electrophoresis (CE). Upon application of the voltage, the sample migrates from the anode to the cathode. An electropherogram is recorded at the detection window

12.2. EOF and EP: Separation of Ions

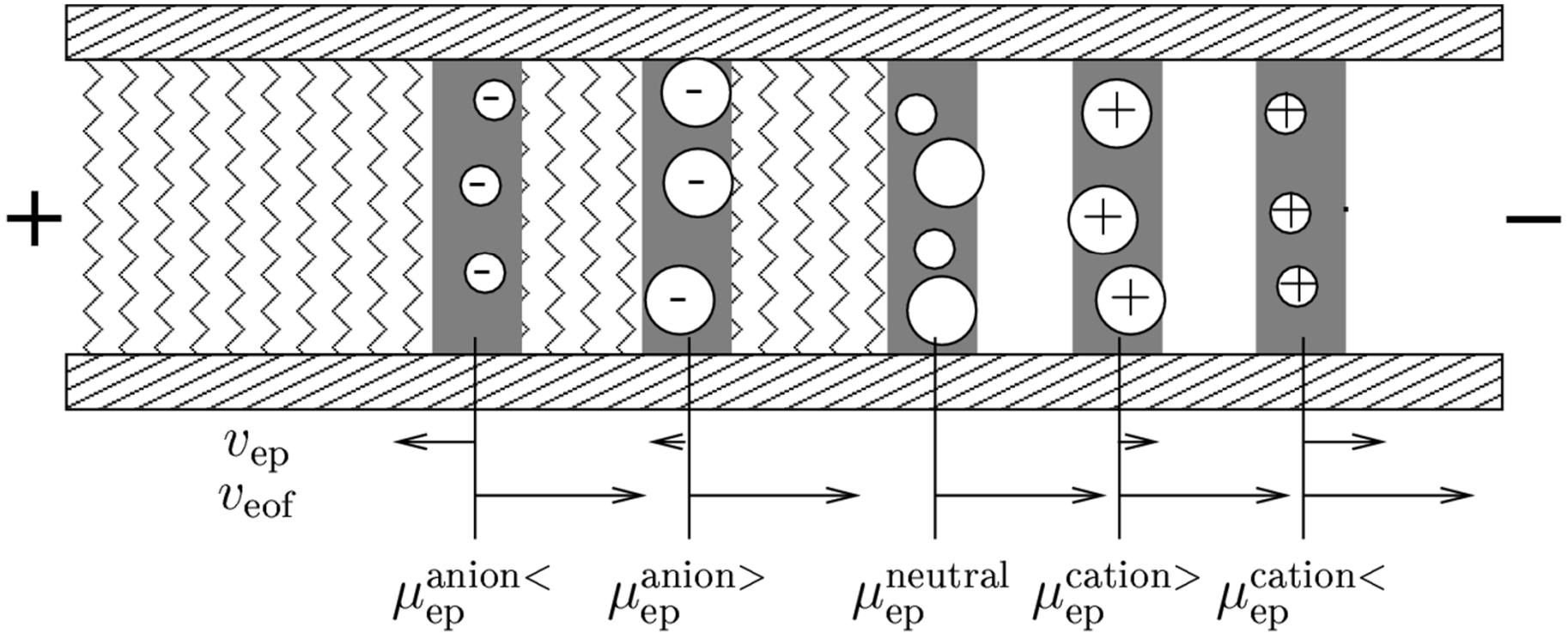


Fig. 12.10. Combined action of electroosmotic flow and electrophoretic separation of small and large ions. Neutrals follow the main front of EOF

12.2. Electropherogram

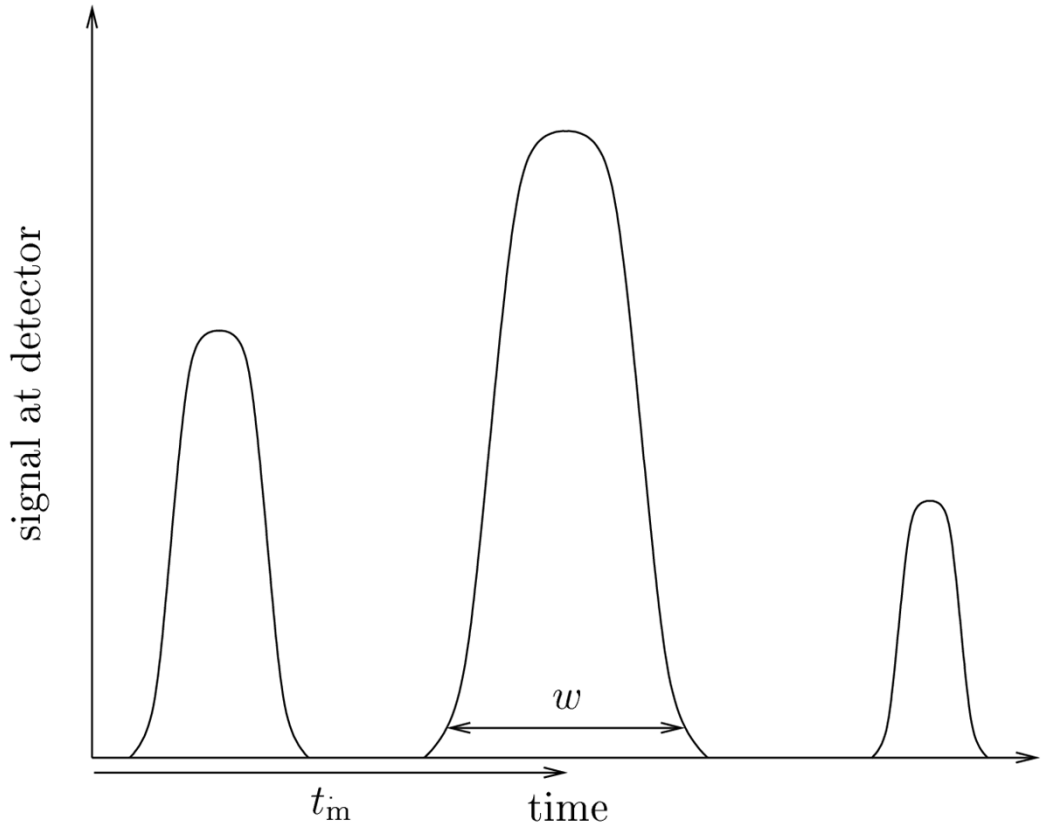


Fig. 12.11. Detector signal recorded in CE. For the central peak, the band width w and migration time t_{mig} are indicated

12.2. CE Modes

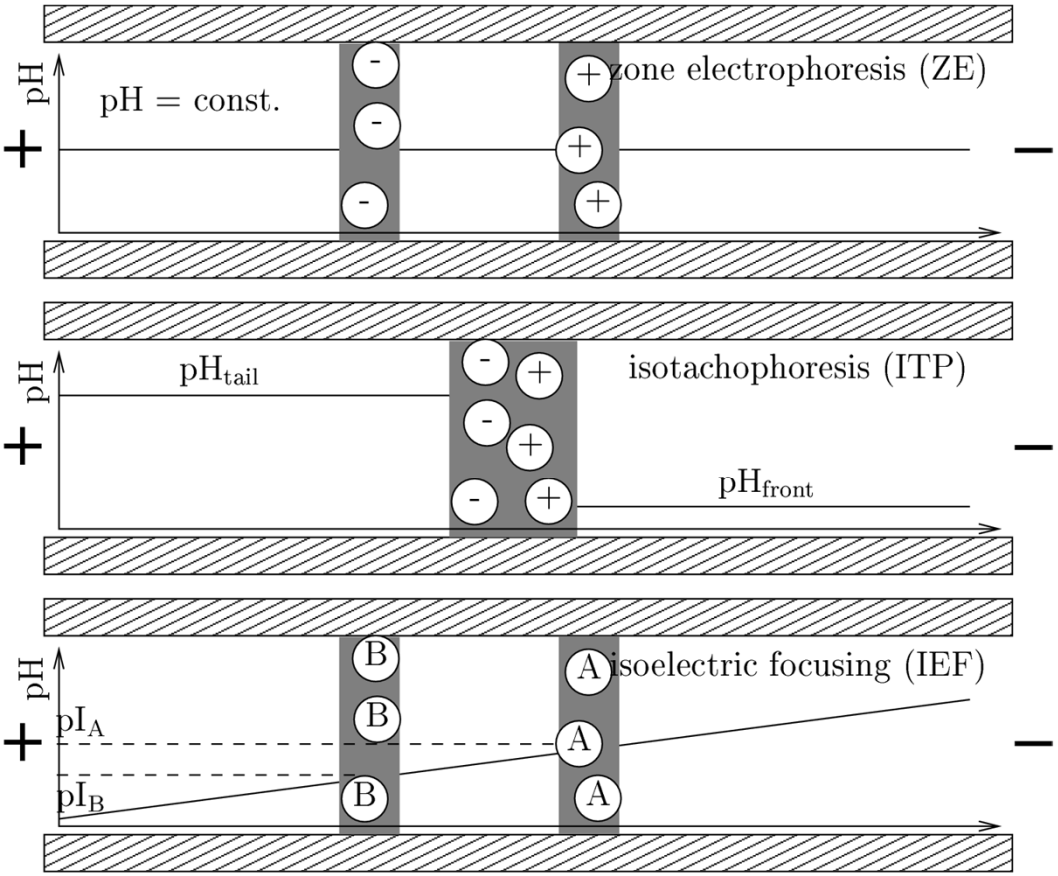
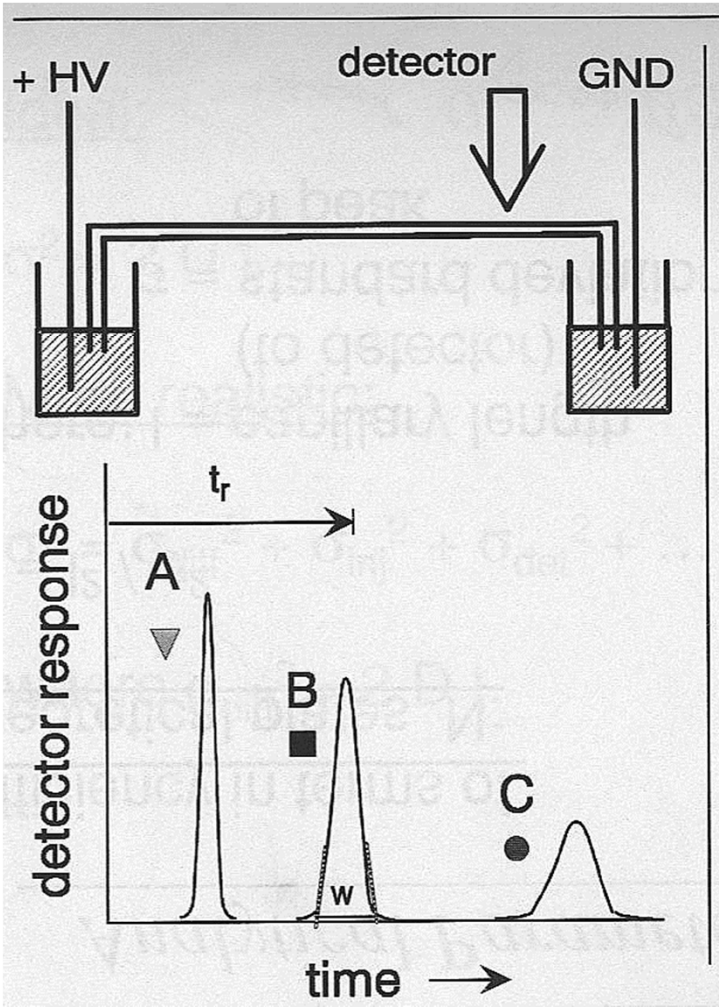


Fig. 12.12. Zone electrophoresis (ZE), isotachophoresis (ITP) and isoelectric focusing constitute the commonly employed modes of electrophoretic separations

- Distinction by pH-distribution along channel

12.2. Capillary Electrophoresis (CE)



GOAL:

narrow bands = high sep'n efficiency

[1] Bandwidth: $w = 4\,\sigma_L$

σ_L = st'd deviation of band (length)

[2] Migration time, t : $t = \dfrac{l}{\mu_a \; E}$

μ_a = apparent analyte mobility
l = capillary length (to detector)
E = electric field

$$\mu_a = \mu_E + \mu_{EOF}$$

**** large N (small H) = high efficiency***

12.2. CE: Efficiency

Definition: theoretical plate number *N*

$$N = l^2 / \sigma^2$$

- *l:* length of capillary to detector
- σ_{diff}: standard deviation of peaks
  - *D:* diffusion coefficient
  - *t:* time for EP
  - *L:* length of entire capillary
  - μ: ionic mobility

$$\sigma_{diff}^2 = 2Dt$$

$$t = \frac{l}{v} = \frac{l}{\mu E} = \frac{lL}{\mu V}$$

- thus:

$$N = \frac{\mu V l}{2DL}$$

$$\sigma^2 = \sigma_{diff}^2 + \sigma_{inj}^2 + \sigma_{det}^2$$

- Real:

$$\sigma_{inj}^2 = w_{inj}^2 / 12$$

  - w_{inj}: width of injection plugs

Goal: Minimization of width of injection plug w_{inj}

12.2. CE: Resistances

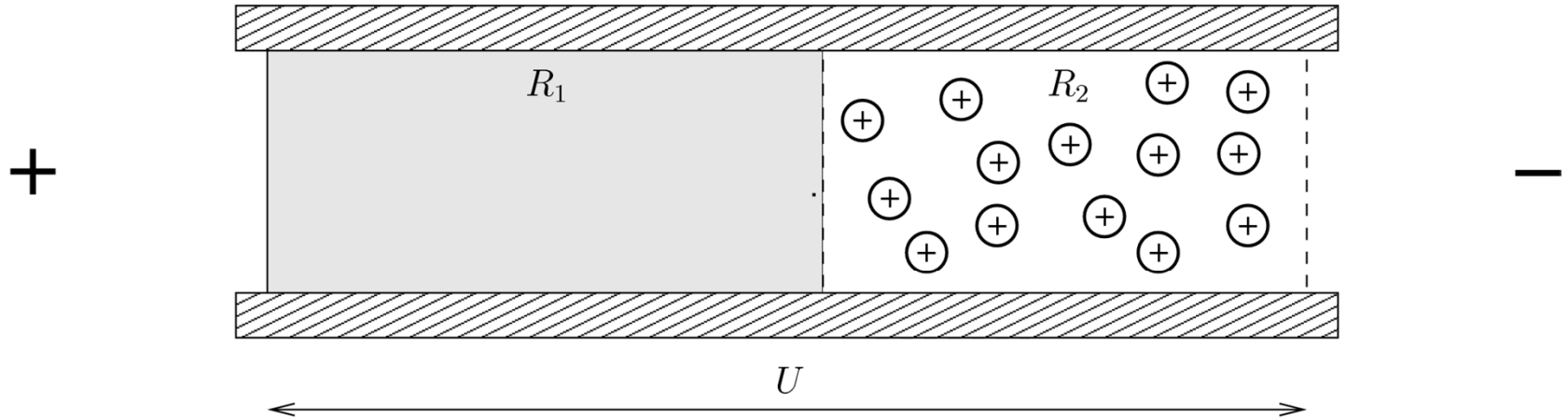
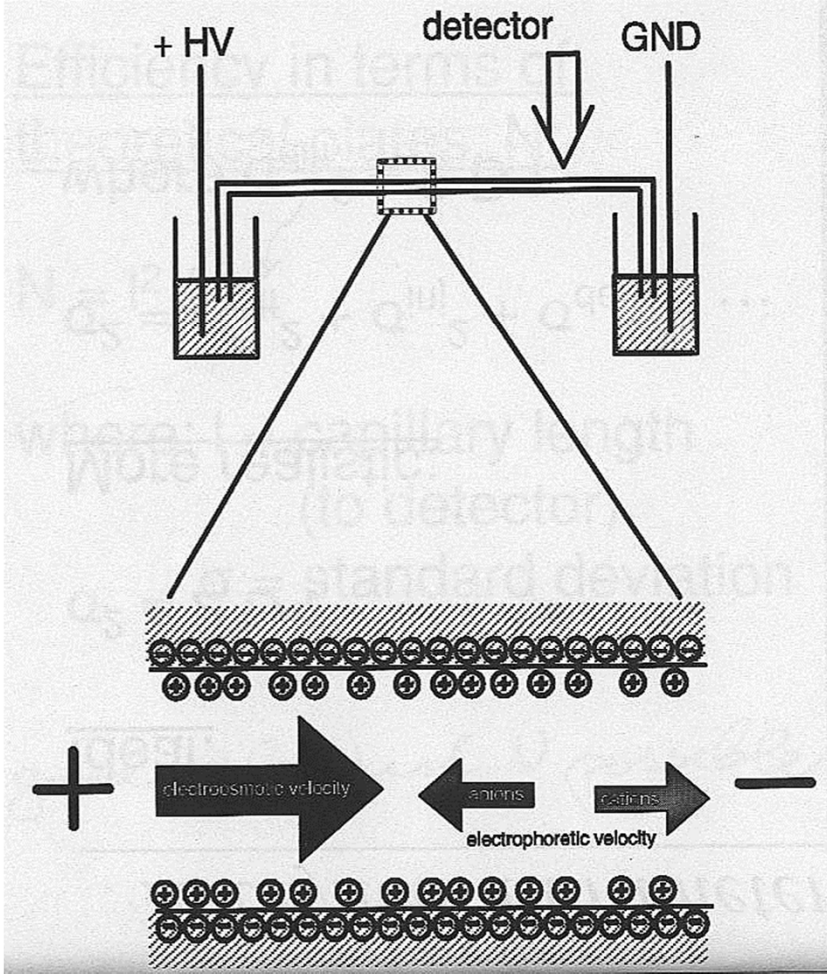


Fig. 12.8. Capillary aligns the resistances of the background electrolyte R_1 and injection plug R_2 to a series circuit. The overall voltage drop adds up to $U = I(R_1 + R_2)$

12.2. CE: Typical Parameters



Standard conditions:

capillary length:	0.3 - 1.0 m
capillary diameter:	25 - 75 µm
high voltage:	10 - 30 kV
electric field:	100 - 500 V/cm
sample volume:	1 - 50 nL
analysis time:	10 - 30 min
sep'n efficiency:	10^5 - 10^6 plates

12.2. CE: Pros and Cons

- Small capillary diameter => large surface-to-volume ratio
  - Improved dissipation of heat
  - Operation at higher voltages possible
  - Faster separations
  - Higher efficiency

- Disadvantages
  - Reduction of channel diameter
    - Reduction of injection and detection volumes
    - Otherwise loss of efficiency

12.2. Chip-Based CE

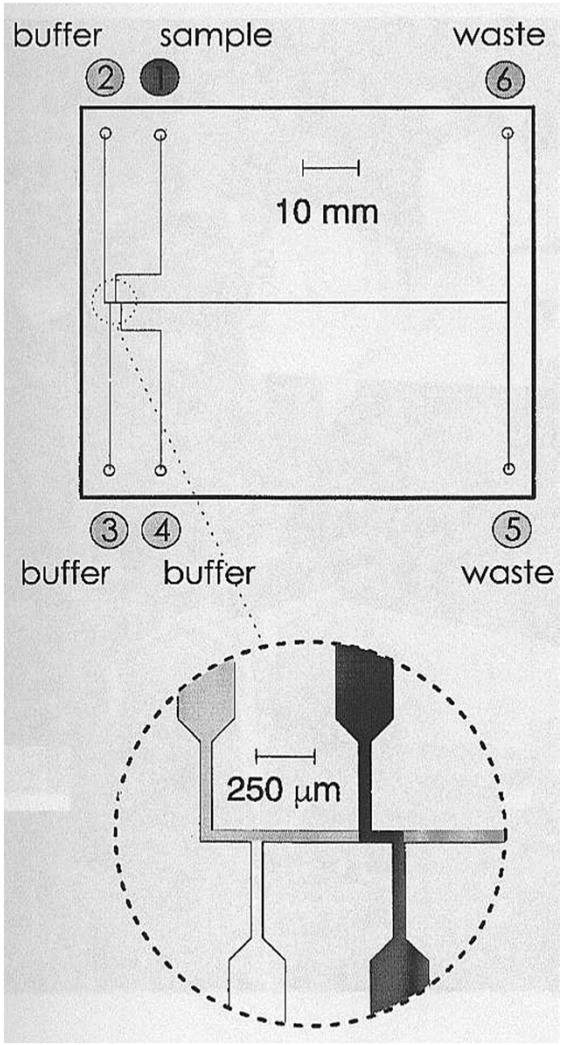
- Minimization of injection and detection volumes
  - Enhancing efficiency *N*
- Integration by microtechnology
  - Multiplexing
  - Parallelism
  - Concatenation / process automation

- CE and electrokinetic pumps are workhorses of lab-on-a-chip systems in analytical chemistry

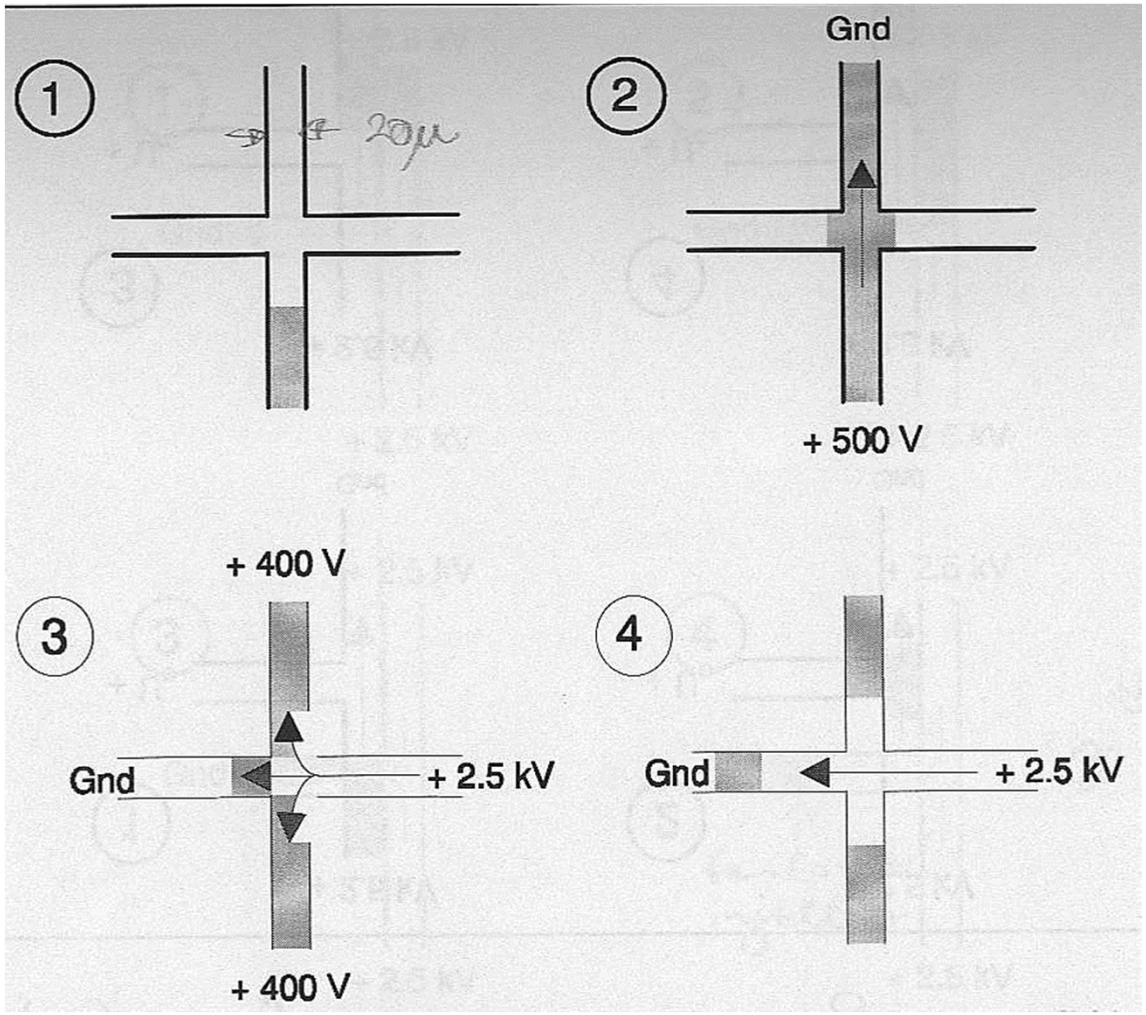
12.2. Chip-Based CE: Principle

Definition of sample plug

- EOF between 1 and 4
- EOF between 2 and 5

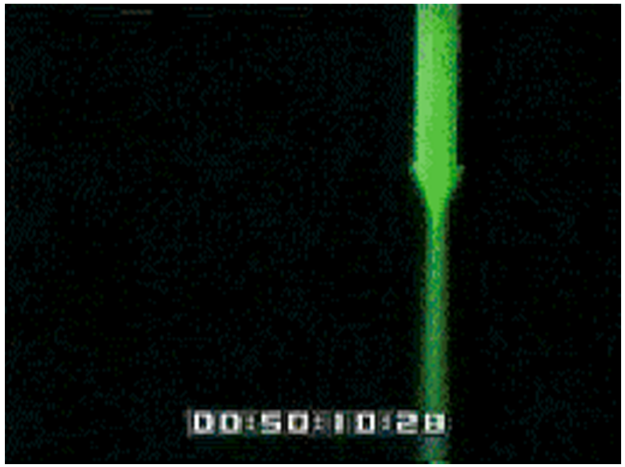


12.2. Chip-Based CE: Injection (1)

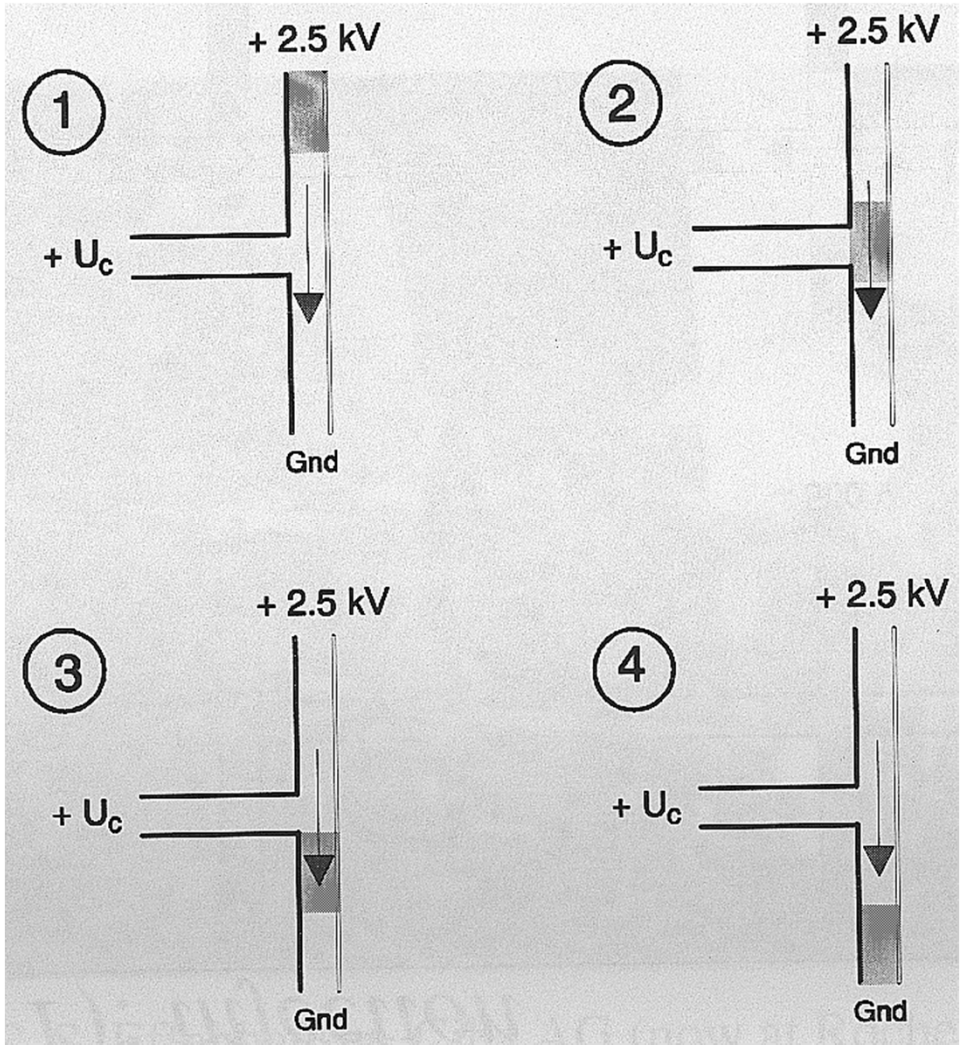


12.2. Chip-Based CE

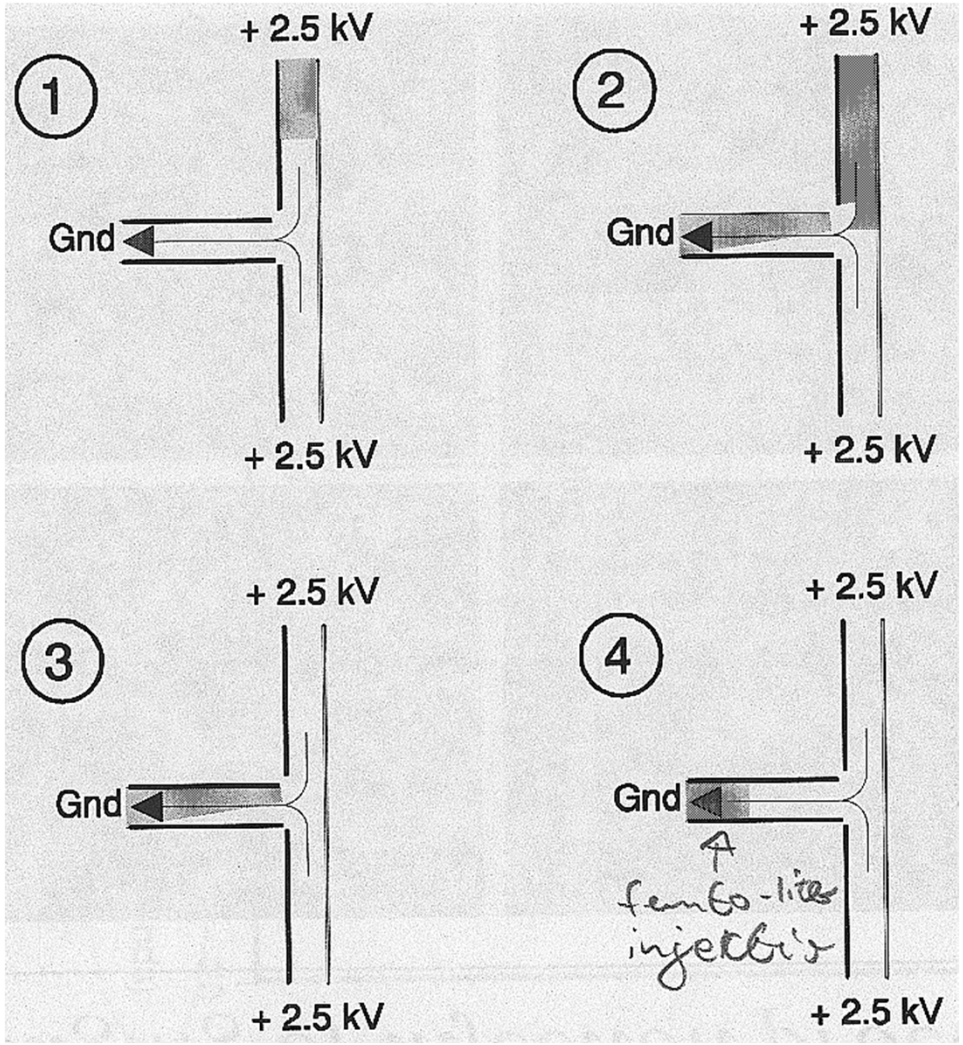
- EOF at 1 nl / s downwards
- Channel diameter 80 µm
- Volumes of 50 pl in channel intersection
- Sudden potential gradient in horizontal direction
  - ➢ Initiation of CE within 100 ms



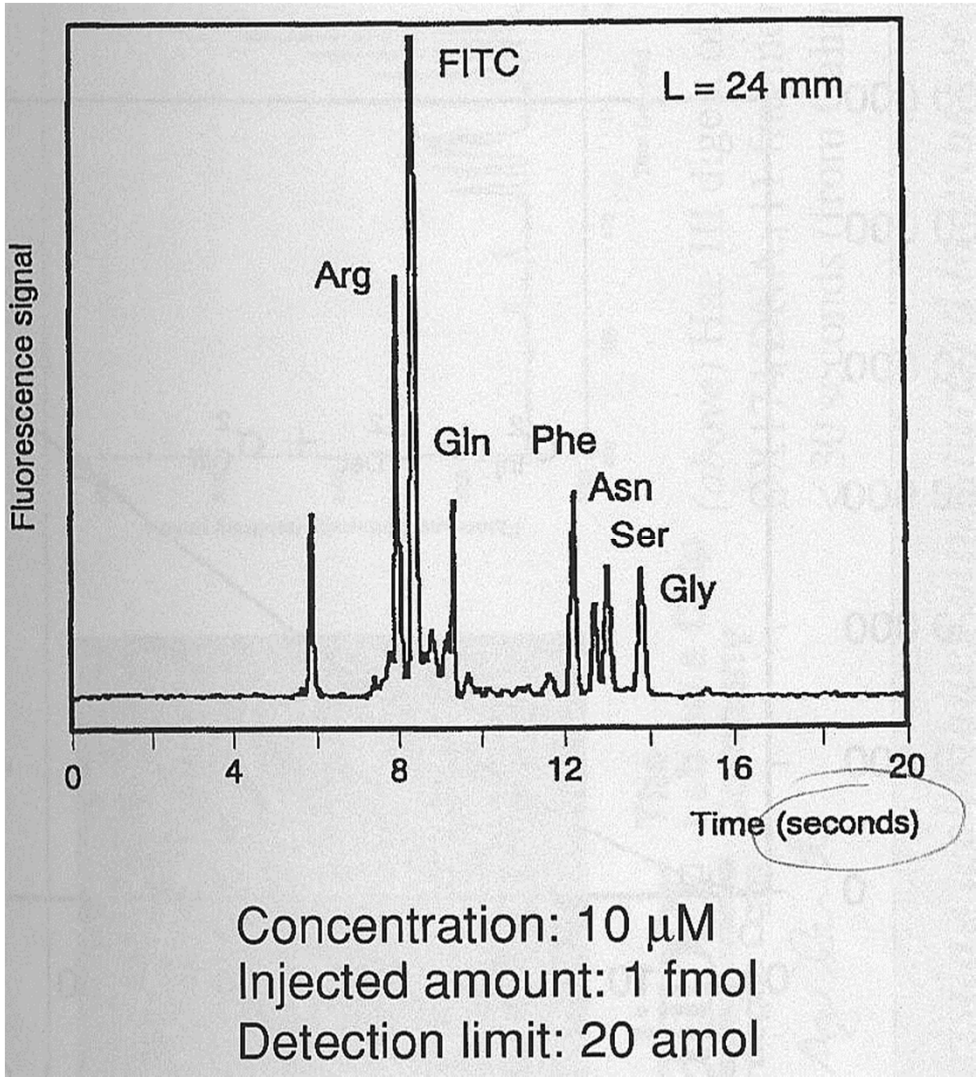
12.2. Chip-Based CE: Passage of T-Junction



12.2. Chip-Based CE: Injection at T-Junction



12.2. Chip-Based CE: Separation of Amino Acids



12.2. Submillisecond Chip-Based CE

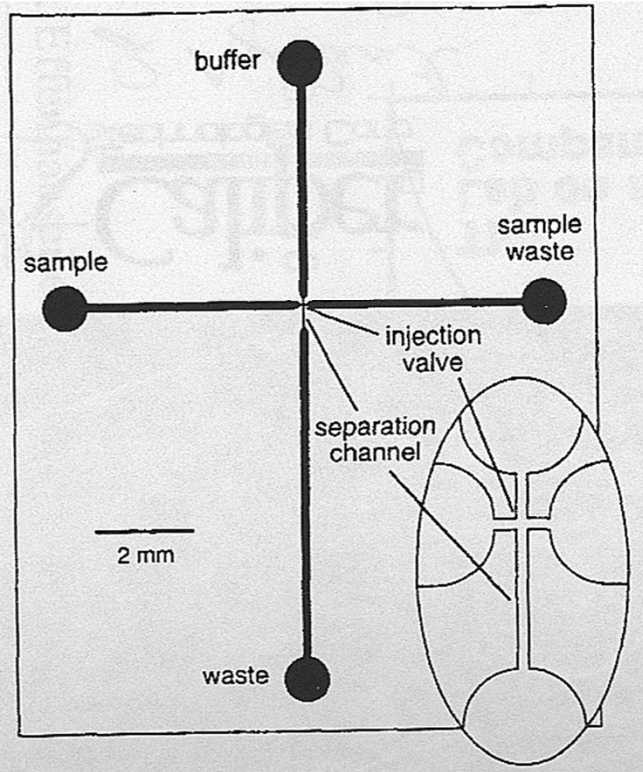


Figure 1. Schematic of microchip used for high-speed electrophoretic separations. (Inset) Enlargement of the injection valve and separation channel.

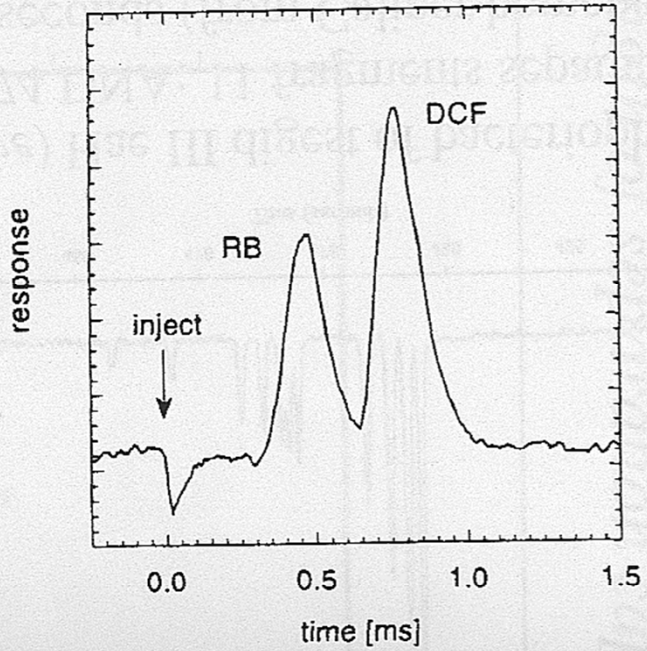
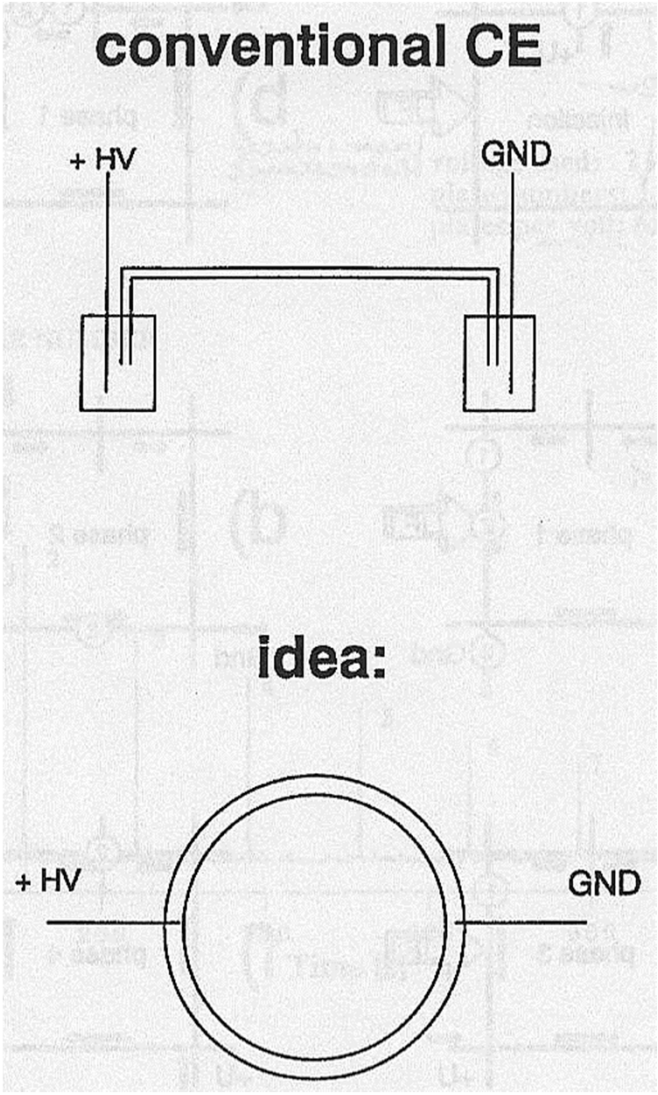
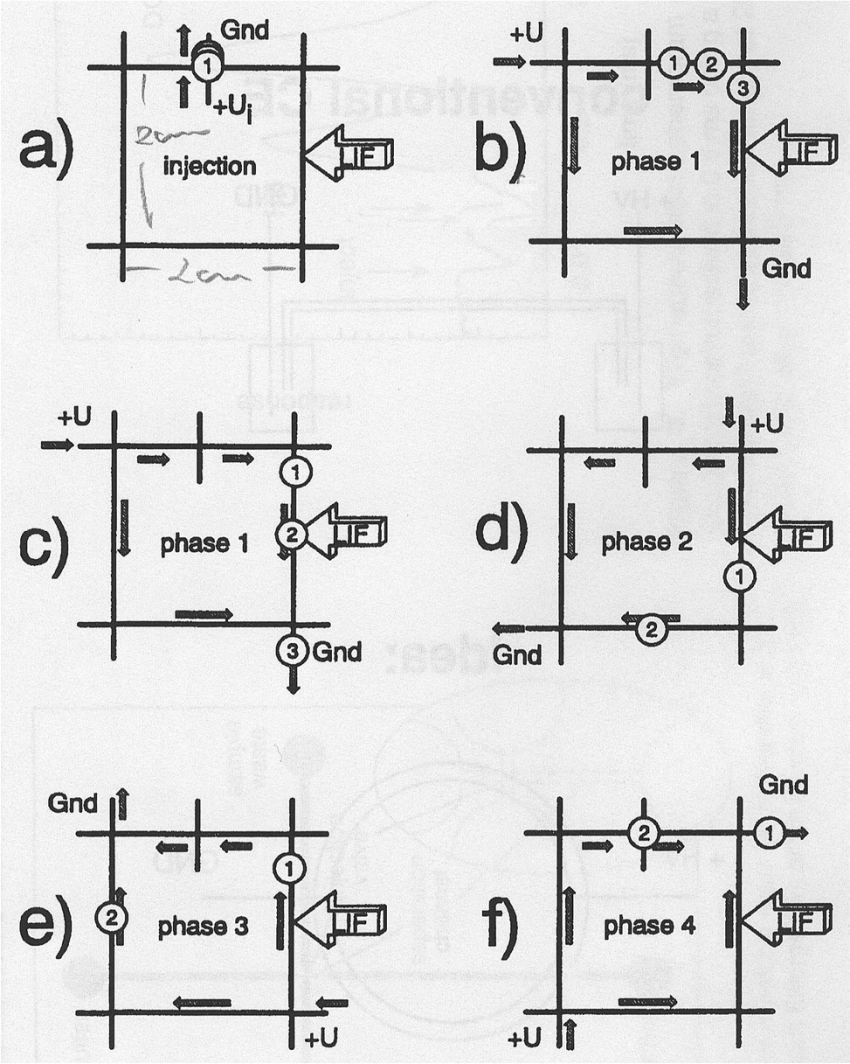


Figure 3. High-speed electropherogram of rhodamine B and dichlorofluorescein resolved in 0.8 ms using a separation field strength of 53 kV cm^{-1} and a separation length of 200 μm. The start time is marked with an arrow at 0 ms.

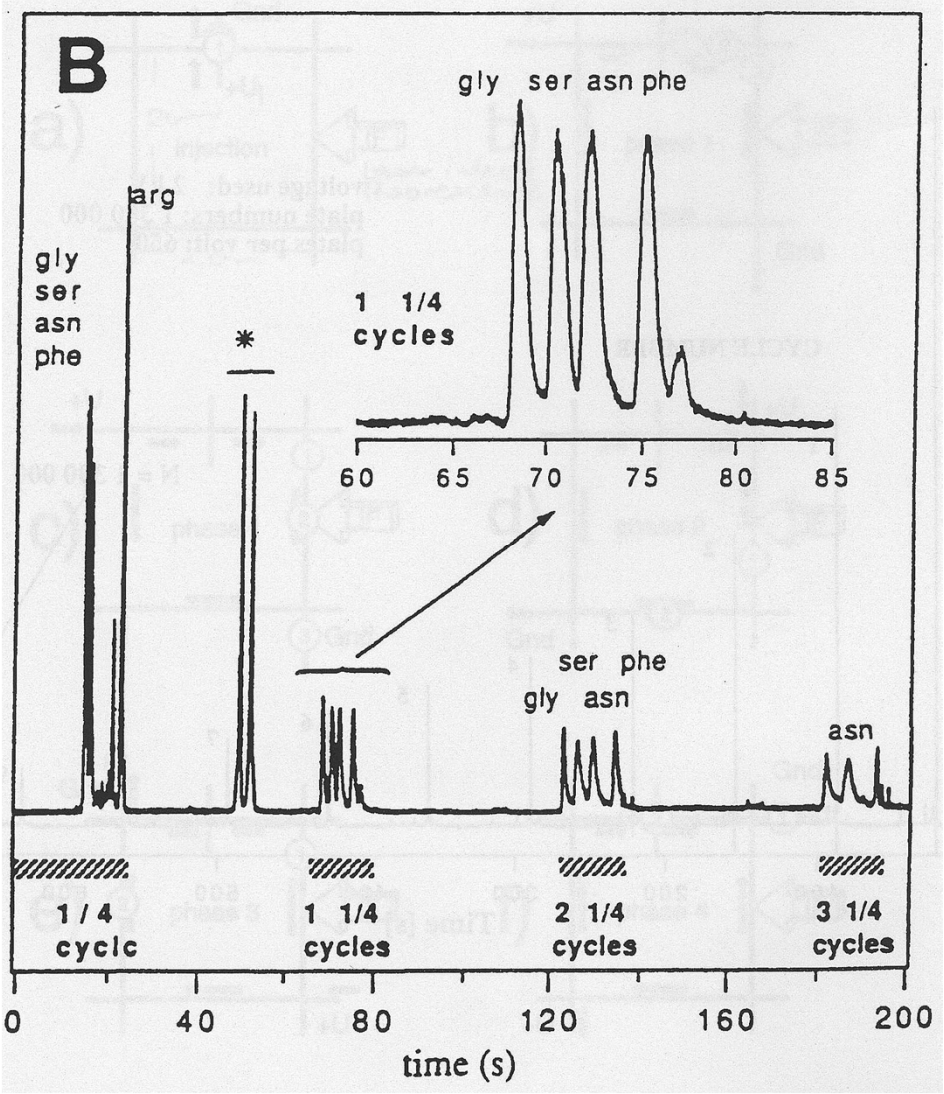
12.2. Synchronized Cyclic CE (SCCE): Idea



12.2. SCCE: Principle

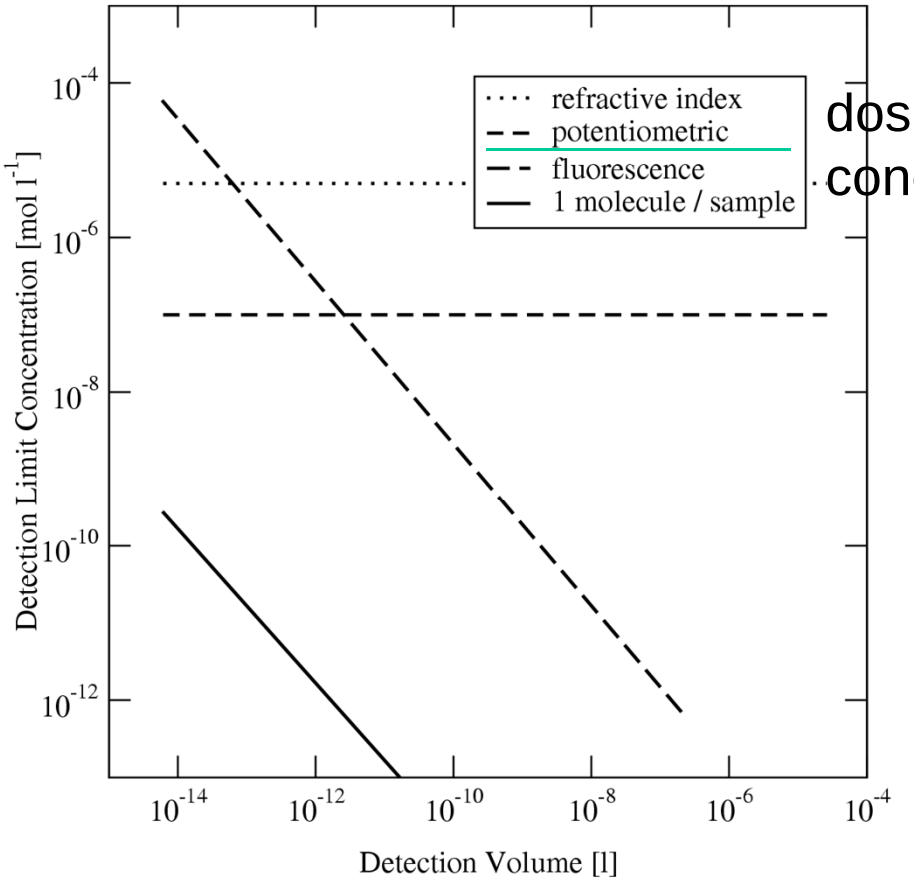


12.2. SCCE: Mixture of Amino Acids



12.2. Disadvantages of Miniaturization

- Dead volume
  - ➢ Injection and detection zone
  - ➢ Band broadening
    - Devastating effects on efficiency and resolution if in same order as injection volume
  - ➢ Integrated microfabrication
    - Potential for negligible dead volumes
- Detection limits
  - ➢ 1 µl injected sample
  - ➢ 1 nl detection volume



dosis

conc.

Fig. 12.1. Limits of various detection principles. In contrast to fluorescence which is linked to the total number of molecule in the sample, refractive index and potentiometric methods require a minimum concentration (adapted from)

12.2. Chip-Based CE: Racetrack Effect

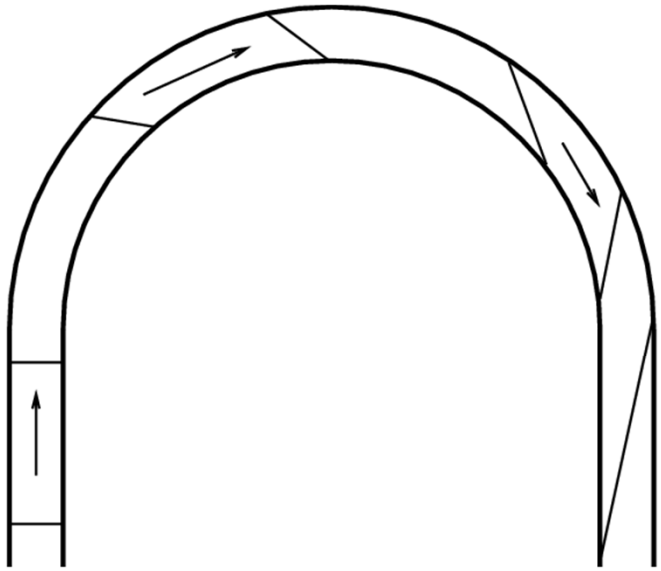
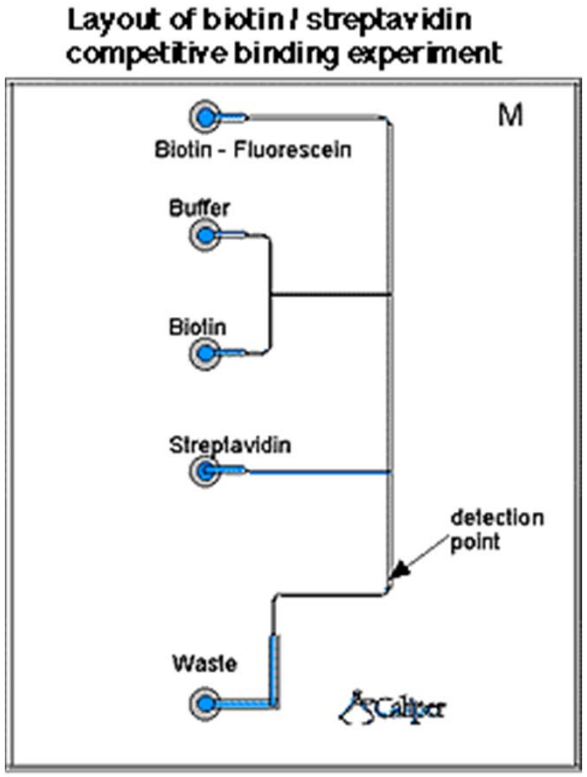


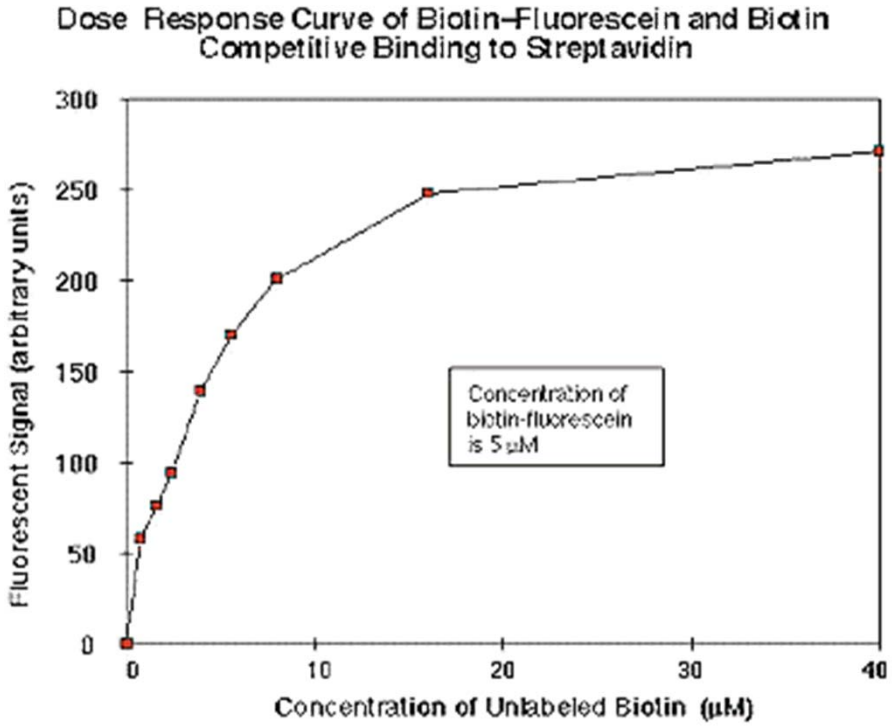
Fig. 12.13. Racetrack effect in bent channels

- Shorter path length on inner lane
- Higher electric field strength on inner lane
- Liquid moves completes turn on inner lane faster than on outer lane
- **Broadening of sample plug**

12.2. Application: Biochemical Reactions

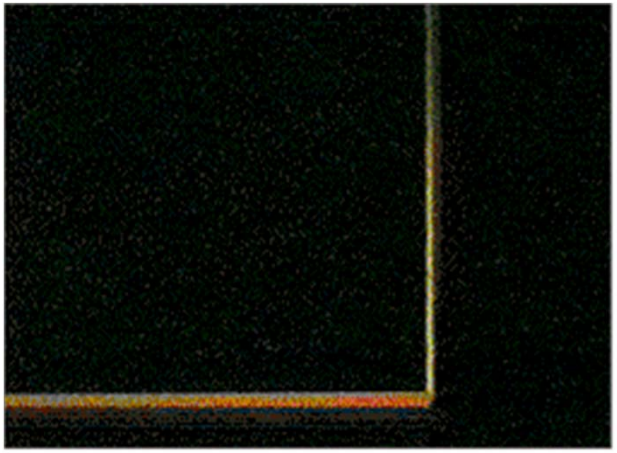
- Precise definition of biotin-concentration via EOF
- Streptavidin bound to biotin quenches fluorescence

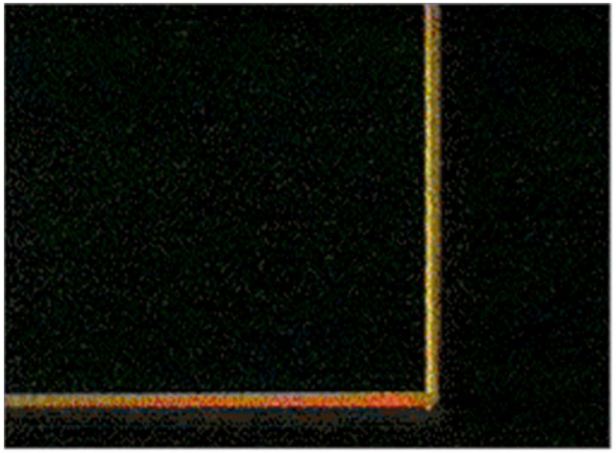




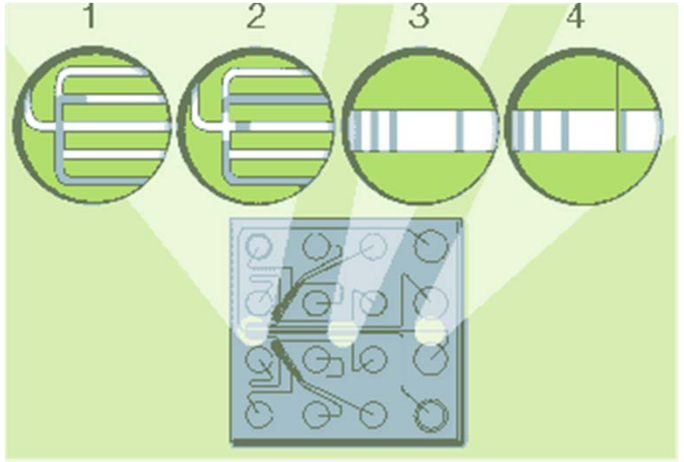
12.2. Application: Controlled Dilution

- Fluorescent flow from the left
- Non-fluorescent flow from right
- Mixing in perpendicular channel
- Accurate control over dilution by EP voltages
- Excellent reproducibility





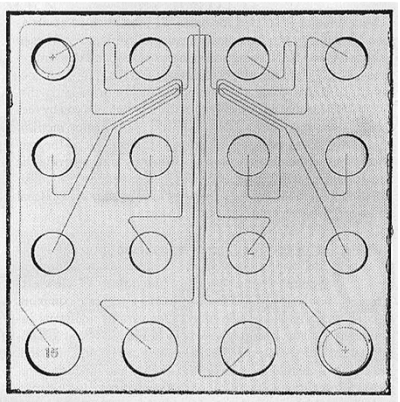
12.2. LabChip Technology

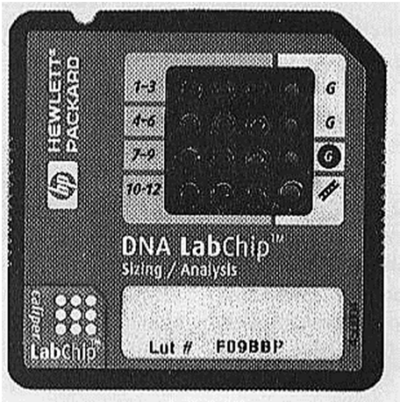


- **With Lab-on-a-Chip...**
  - Miniaturized fluid pathways shorten run times
  - Strong electrokinetic driving forces improve analytical resolution
  - Microfabricated chips yield better reproducibility than conventional technologies
  - Versatile chip design enables flexible experiment design on one system
  - Microscale format minimizes use of sample
- **Principles of electrophoretic separation on a LabChip**
  1. Sample moves through micro channels from sample well
  2. Sample injected into separation channel
  3. Electrophoretic separation of sample components
  4. Fluorescent detection
     - Gel-like images (bands)
     - Electropherograms (peaks)

12.2. Agilent / Caliper LabChip

- Nintendo strategy:
  - Fixed peripherals (ca. US$ 19,500)
  - Exchange of disposable, application-specific modules
- 1st commercial device: DNA-“Sizing“
  - RNA
  - Proteins
- Electrophoresis
  - Upper 12 wells contain samples
  - Lower 4 wells contain „working substances“
  - External electrodes supply voltage





12.2. Agilent / Caliper LabChip: Procedure

1. Load Sample

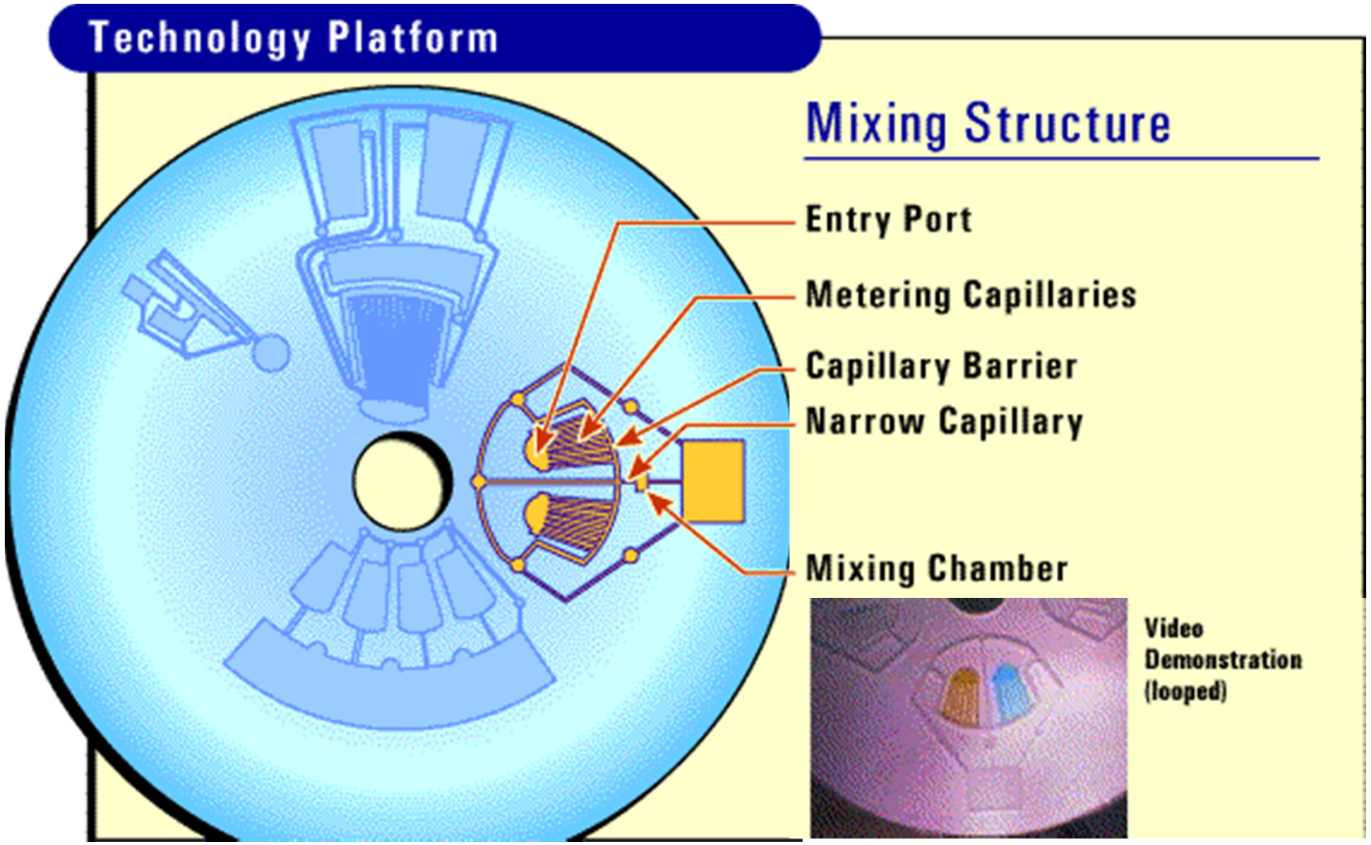
2. Run analysis

3. See data

12. Analytical Chips

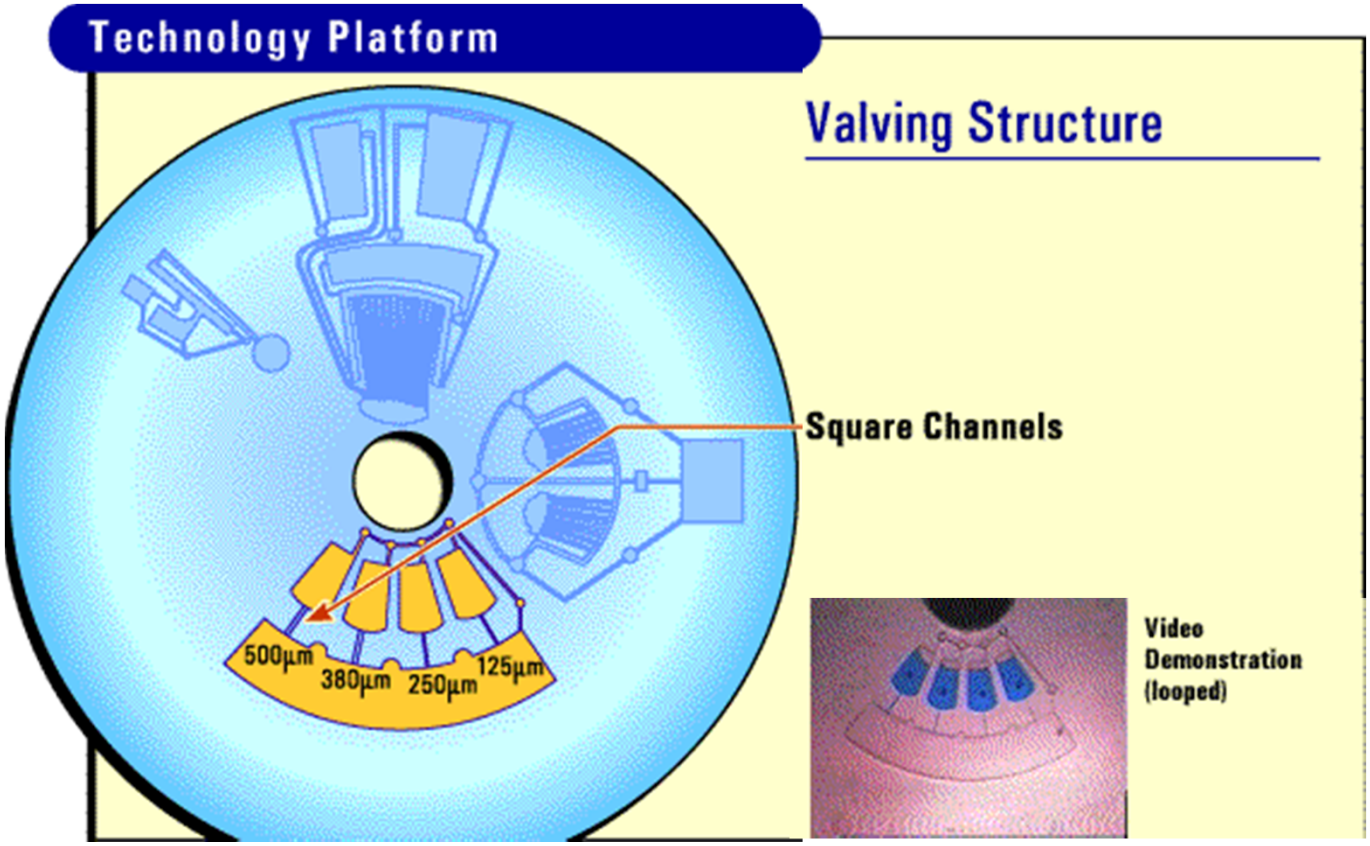
1. Concept and History
2. Analytical Separations
3. **Microfluidic CD Technology**
4. Flow Injection Analysis
5. Microfluidic Processors

12.3. LabCD™-Technology (1)



1. Two dye solutions in symmetric structures
2. Spin: solutions enter capillary barrier, meeting at junction with narrow capillary
3. Solutions flow in laminar sheets into narrow capillary
4. Turbulent flow in mixing chamber mixes solutions

12.3. LabCD™-Technology (2)

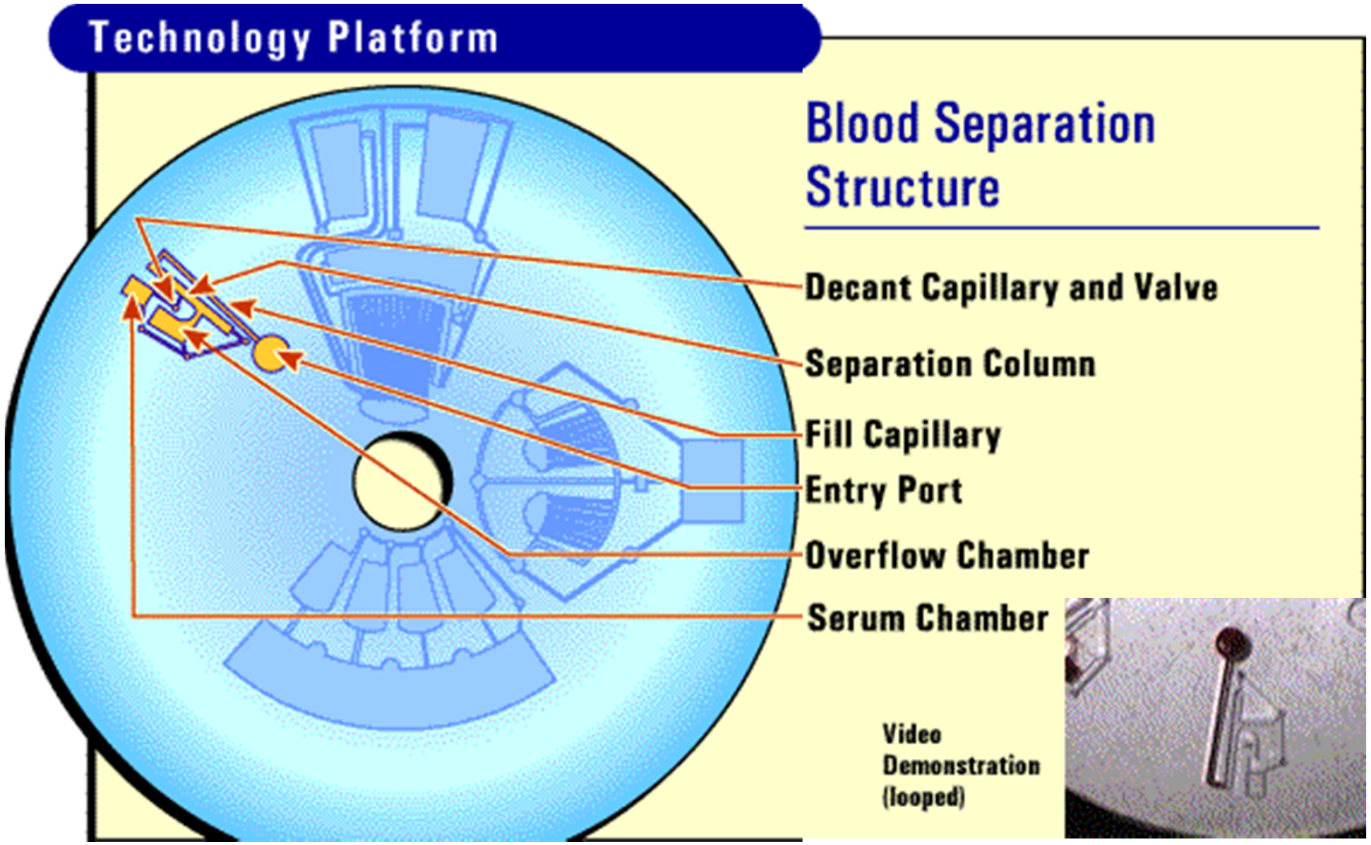


Capillary valving depends on capillary size, radial position, and geometry of the dispensing reservoir.

In this structure, the RPM at which fluid is gated increases with decreasing capillary size D:

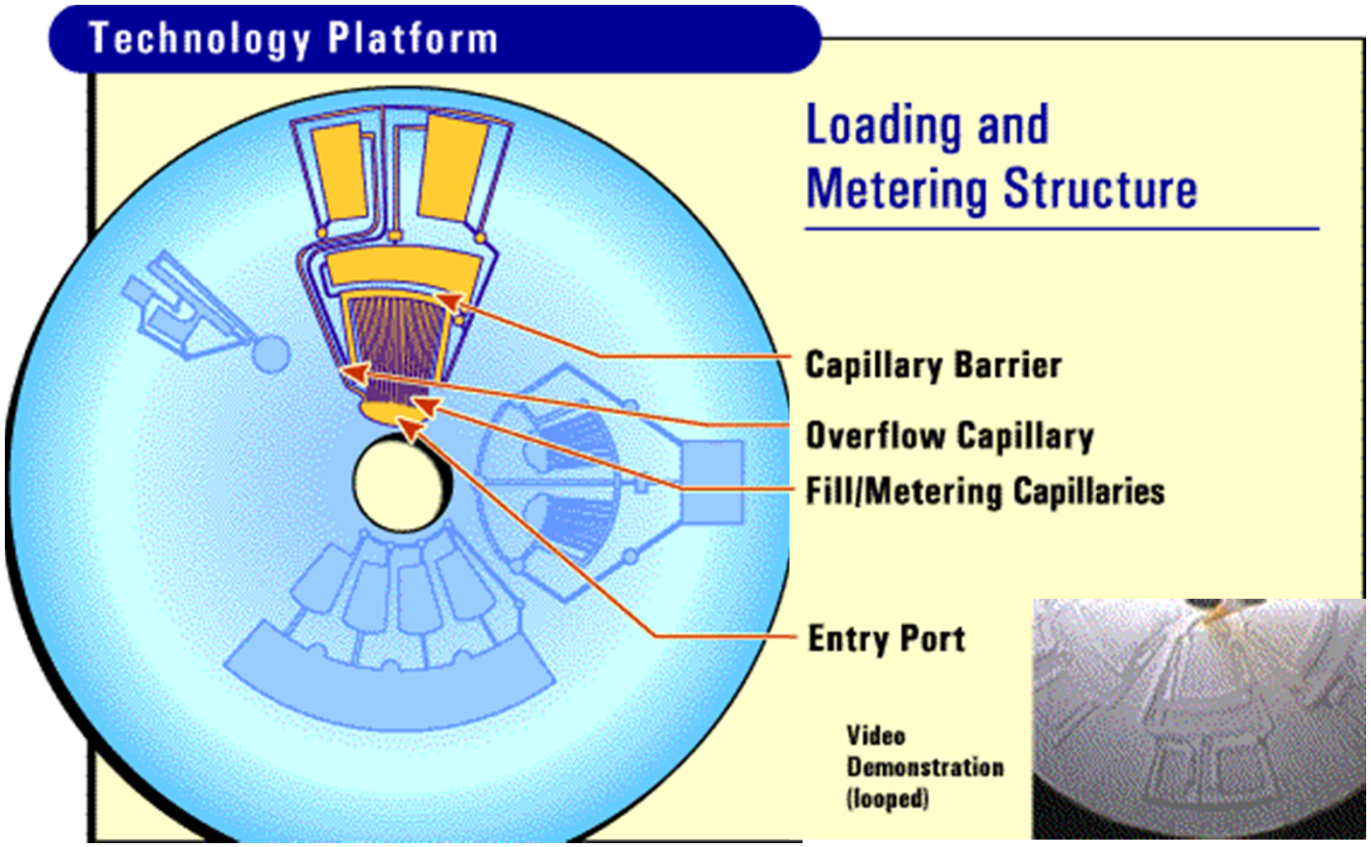
$$RPM \sim \frac{1}{\sqrt{D}}$$

12.3. LabCD™-Technology (3)



1. Blood added to port
2. Capillary fills to junction with separation column
3. Spin to first RPM: column fills, excess blood to overflow chamber
4. Centrifuge until serum separates
5. Spin at second, higher RPM: decant serum through capillary

12.3. LabCD™-Technology (4)



1. Sample applied to port
2. Metering/overflow capillaries fill to junctions
3. Spin at first RPM: excess to overflow chamber. 50µL retained in metering capillaries
4. Spin at second, higher RPM: fluid dispenses into 1st chamber


Bio-Disk
.com

Bio-Disk: Life Science Platform

- Centrifugal microfluidics

- Integrated & automated process
  - Simple handling
  - Fast results
  - „Sample in – push button – result out“

- Parallelization / multiplexing
  - Color-encoded beads
  - Optical detection

- Modular setup of “CD” components

Bio-Disk: Life Science Platform

Pilot application: Bio-Disk

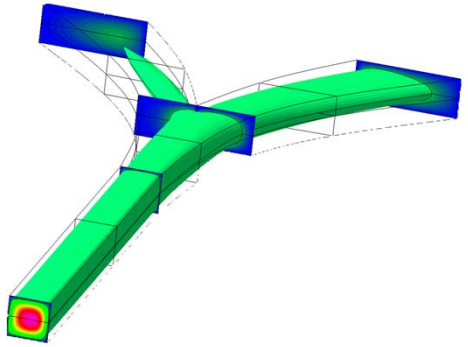
- Point-of-care diagnostics
- Whole blood
- Immune status of 20 antigens
- Markers in emergency medicine

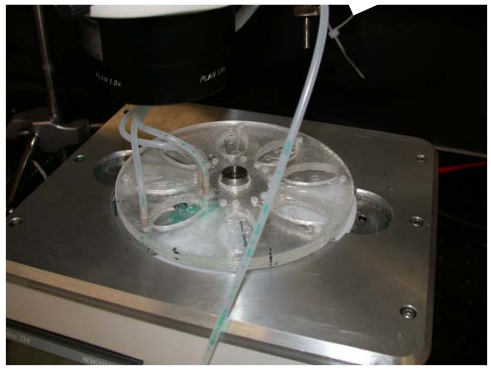
Further applications

- *Clinical diagnostics*
- *Sample preparation*
- Chemical synthesis
- Biochemical analysis
- …

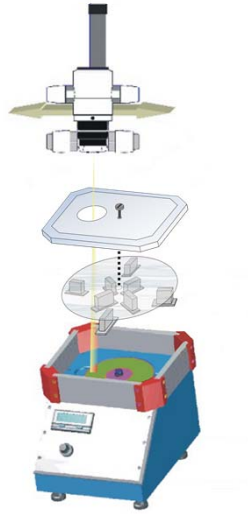
Bio-Disk – A Life Science Platform

- Process components and functions
  - Pumping
  - Valving
  - Metering
  - Centrifugation
  - Mixing
  - Detection



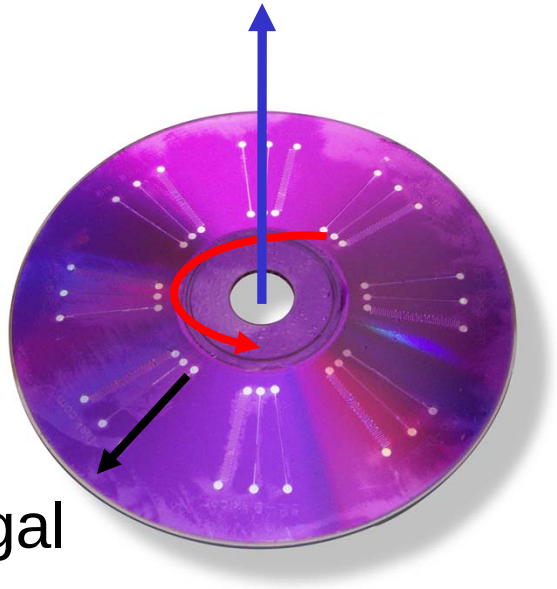


- Software
  - CAD
  - Simulation
- Process control
- Development platform
- Manufacturing



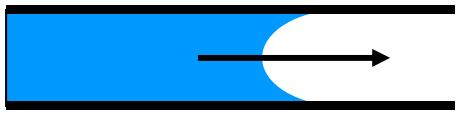
Pumping

- Centrifugal force
  - Spinning frequency
  - Radius



centrifugal force

- Capillary force
  - Priming
  - Interfacial surface



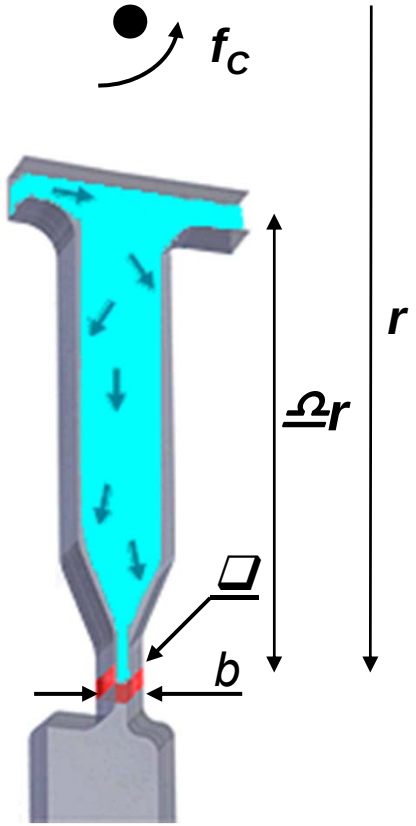
Passive Ventile

- **Flußkontrolle durch lokale Änderung des Kapillardrucks P❑**

$$P_\theta(\theta, b) = \frac{\gamma \cdot Cos(\theta)}{d_H(b,h)} \qquad ❑ > 90°$$

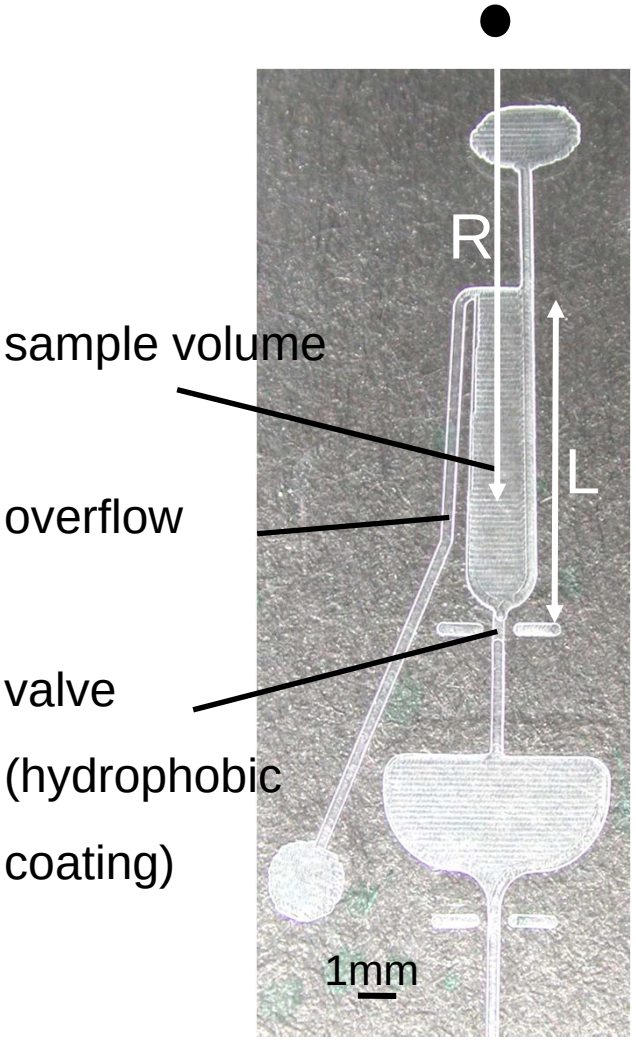
→ Durchbruchfrequenz **f_c**

$$f_c(\theta, d_H, r, \delta r) = \frac{1}{2 \cdot \pi} \sqrt{\frac{2 \cdot \gamma \cdot Cos(\theta)}{\rho \cdot r \cdot \delta r \cdot d_H}}$$

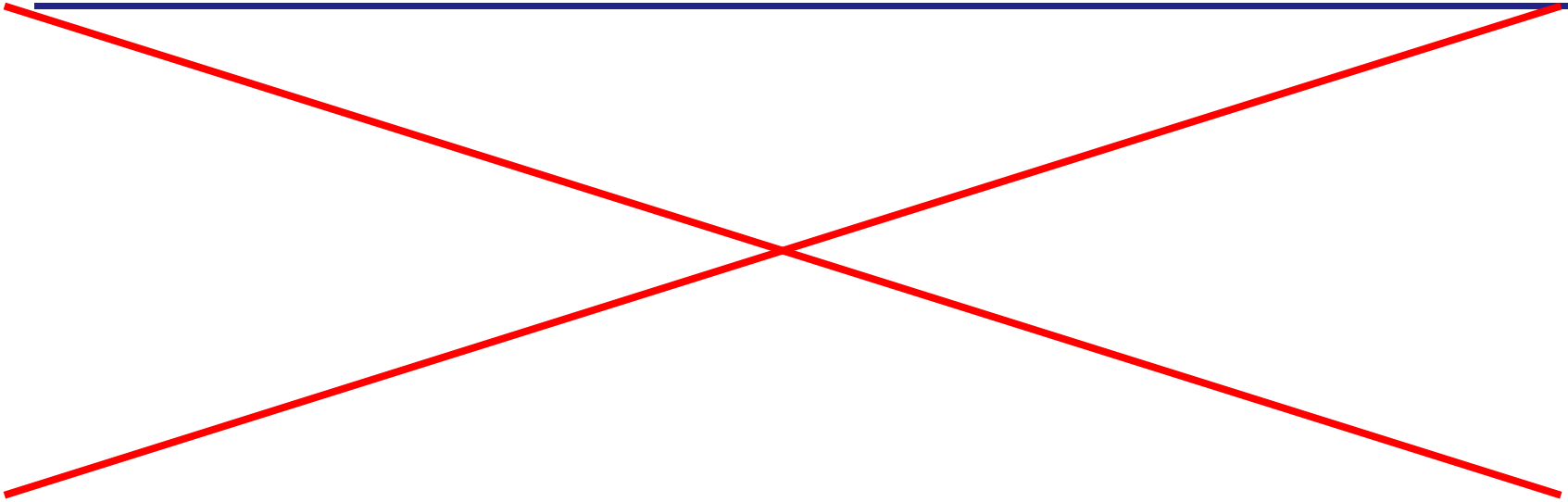


Switching & Metering

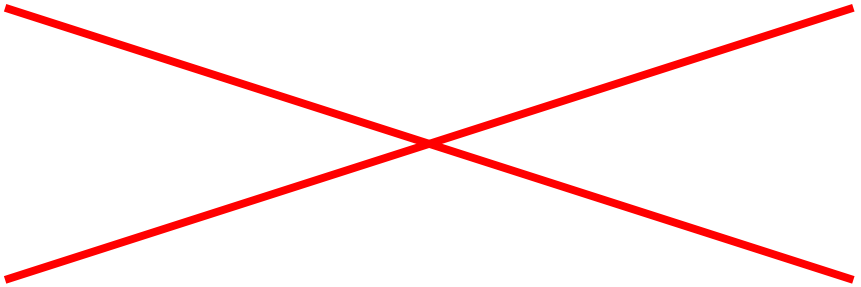
- Metering of nl-volumes
- Hydrophobic barriers
  - Overflow structures
  - Specific burst frequency
- Frequency protocol



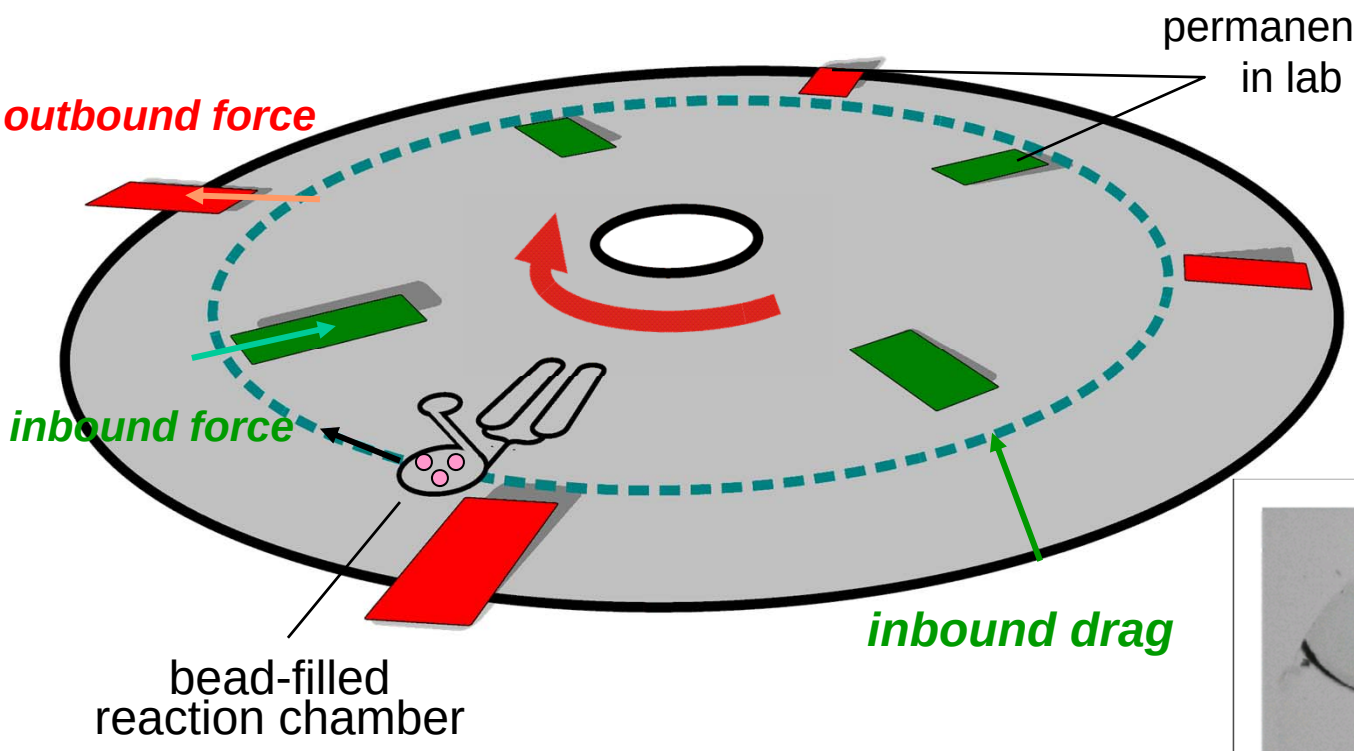
Sample Prep: Blood Separation



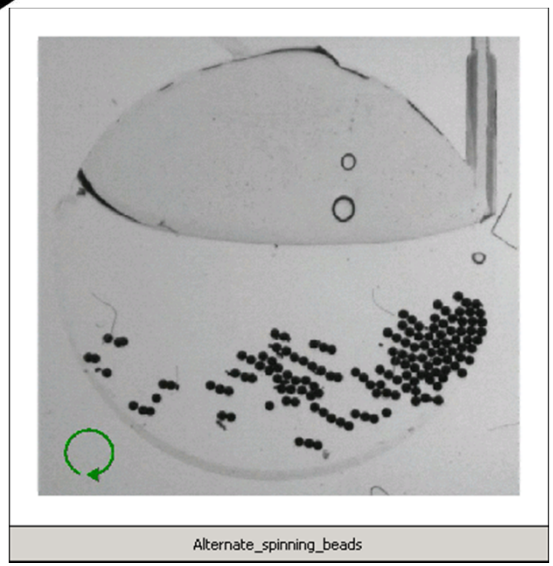
- Whole blood
- Cellular constituents interfere with detection
- „Bio-Disk centrifuge“
- Continuous flow separation
  - Time scale: 20 sec



Magnetic-Bead Enhanced Mixing



Real time video!



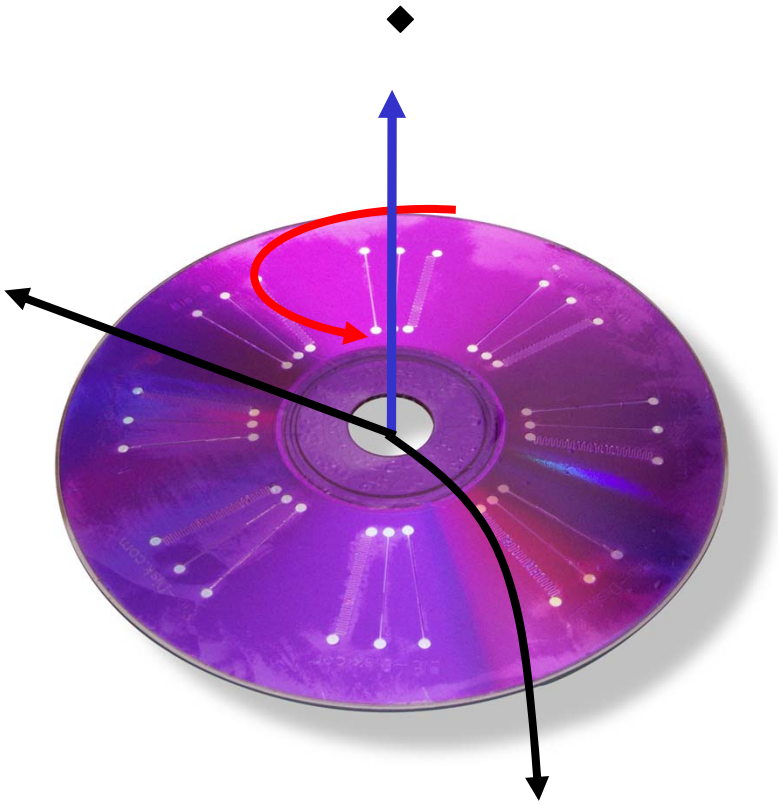
Diffusive mixing: **13 min**

Bead-enhanced: **1 s !!!**

Was bewegt auf der CD ?

- Zentrifugalkraft

$$F_Z = m \cdot \omega^2 \times r$$



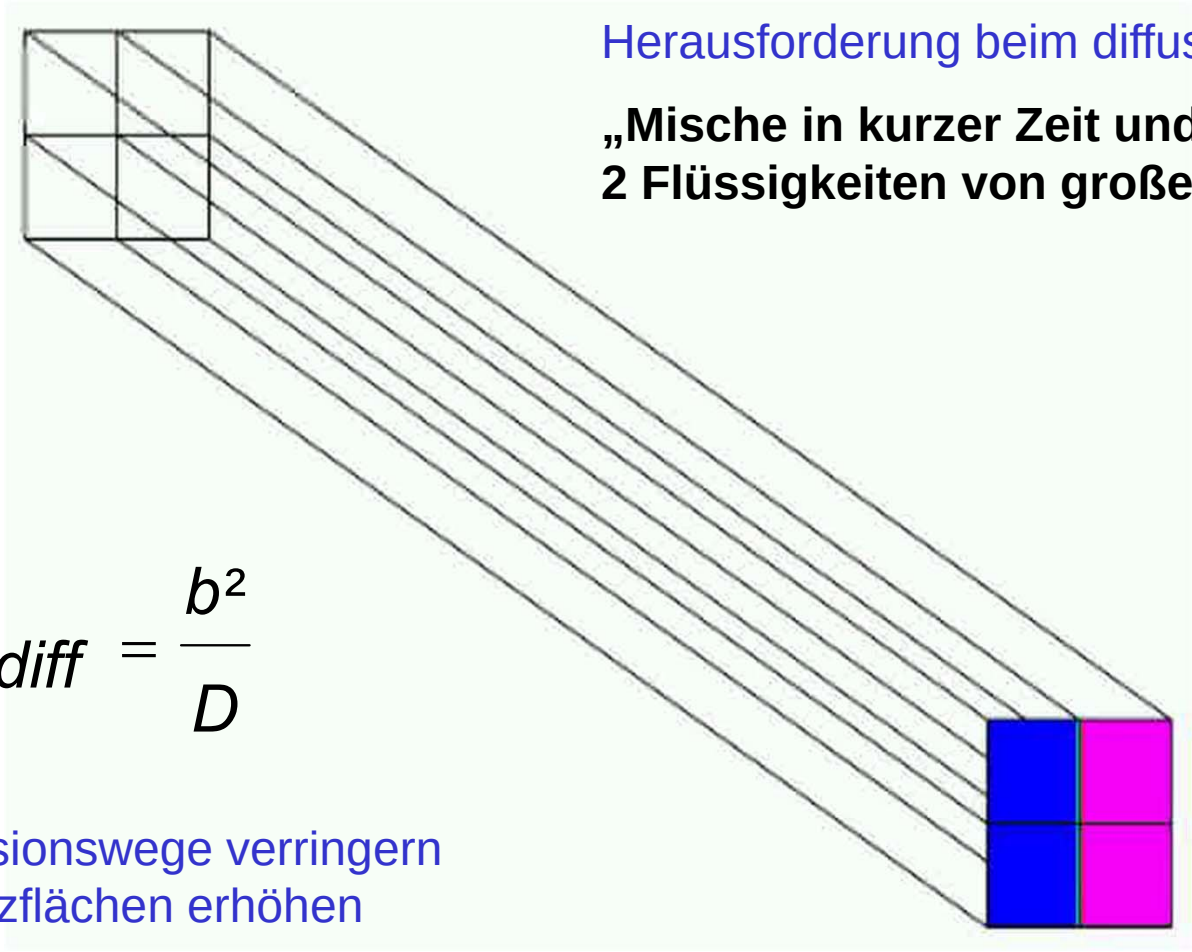
- Corioliskraft
  - Scheinkraft
  - Mitrotierendes Bezugssystem

$$F_C = m \cdot v \times w$$

Coriolis-Kraft

Herausforderung beim diffusiven Mischen

„Mische in kurzer Zeit und Wegstrecke 2 Flüssigkeiten von großem Volumen“

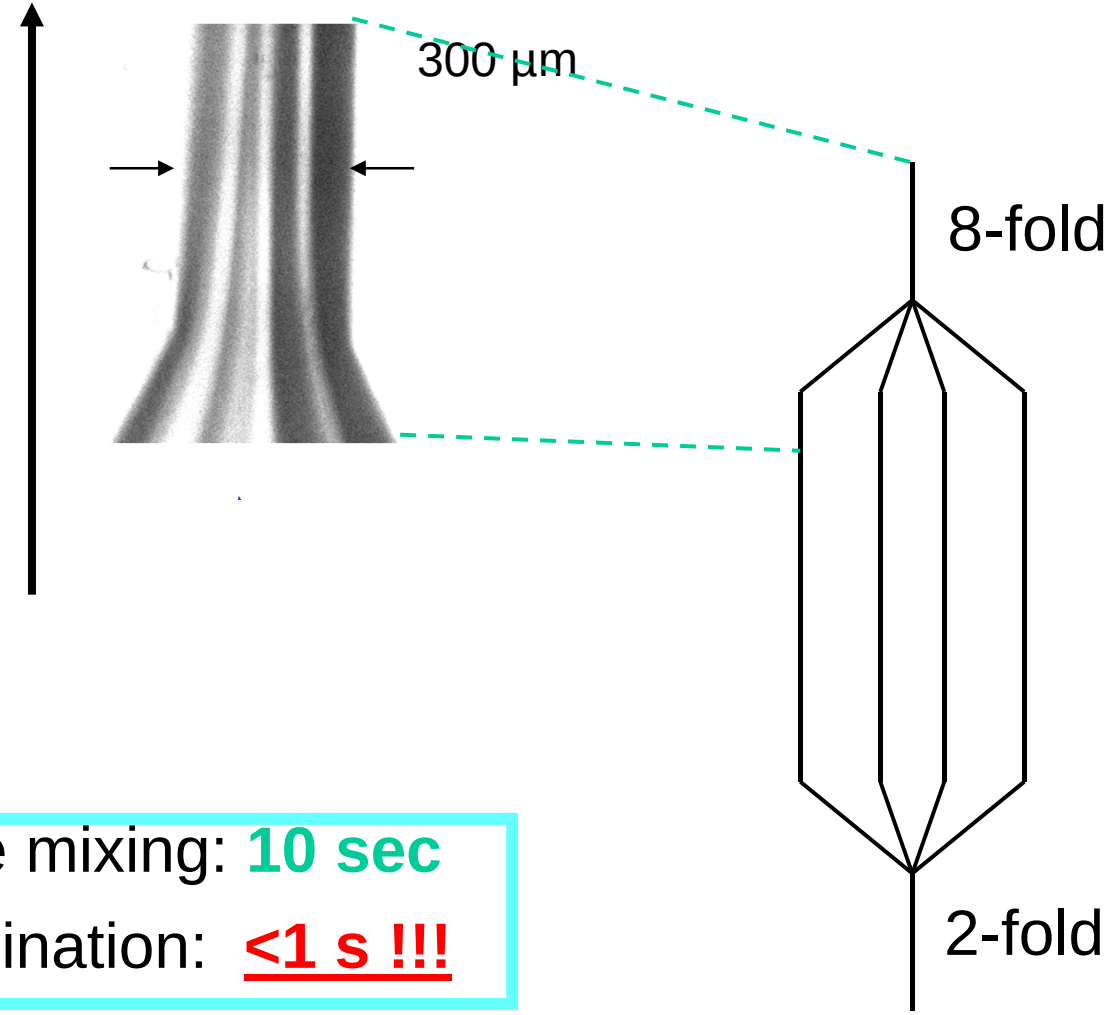


$$t_{diff} = \frac{b^2}{D}$$

Diffusionswege verringern
Grenzflächen erhöhen

Hydrodynamic Mixing: On-Disk Multilamination

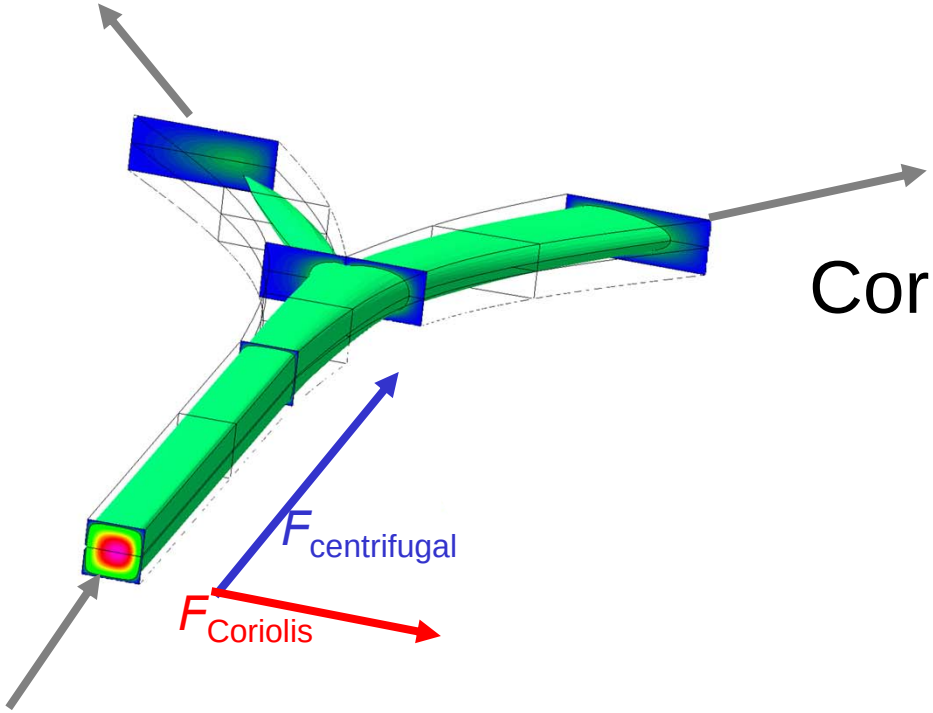
- Flow-through mixing
- Planar structure
  - 2-dim. micromachining
  - Low –aspect ratios
- Parallel channels
- Coriolis-induced stirring
- 8-fold lamination



Diffusive mixing: **10 sec**

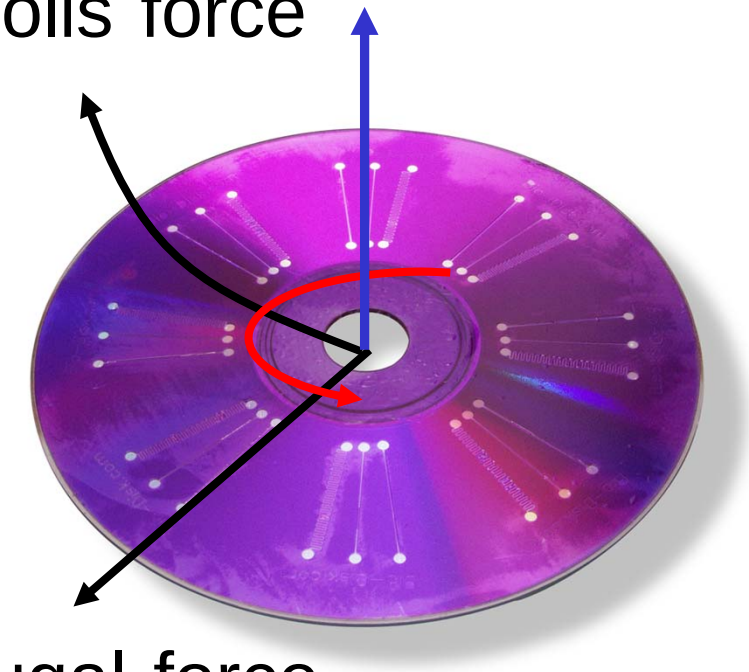
Multilamination: **<1 s !!!**

Switching



Simple channel structure

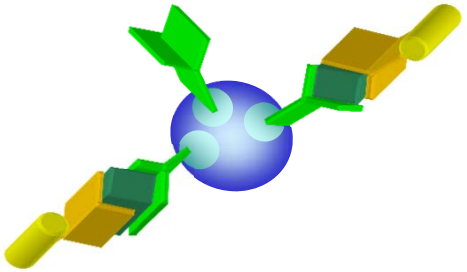
Coriolis force

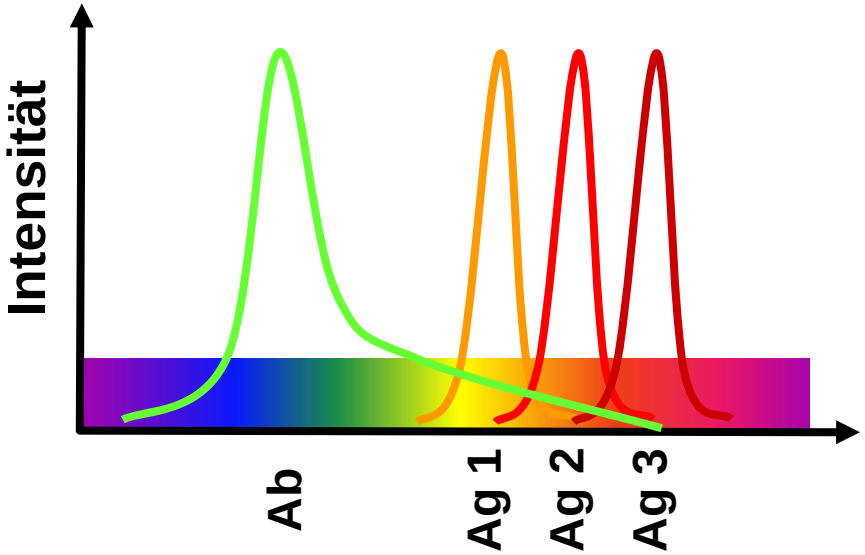


centrifugal force

Bead-Based Detection

- Bio-sensitive bead coating
  - Off-chip preparation
  - Introduction after sealing
- Parallel readout
  - Alignment in monolayer
  - Color-encoded beads



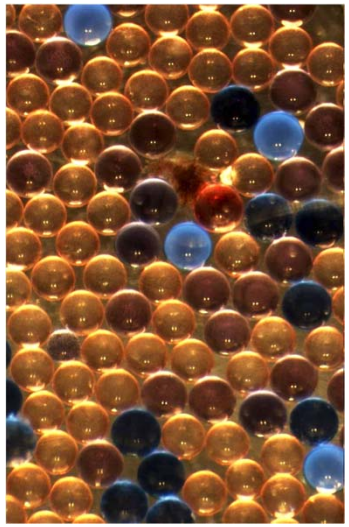


Antigens:

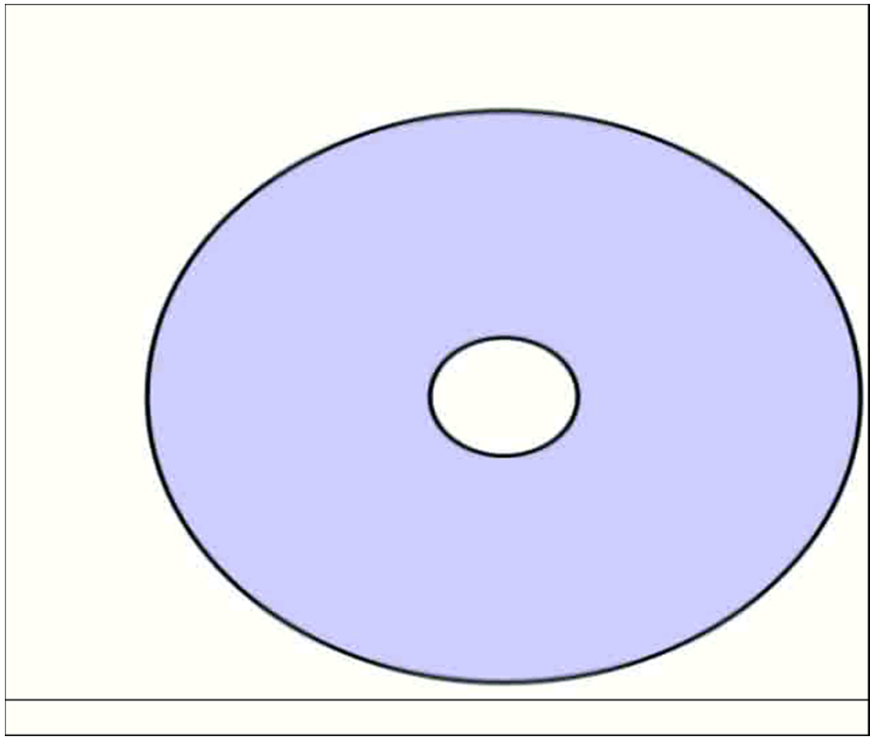
HIV

Hepatitis

...



Bio-Disk: Principle



12. Analytical Chips

1. Concept and History
2. Analytical Separations
3. Microfluidic CD Technology
4. **Flow Injection Analysis**
5. Microfluidic Processors

12.4. Flow Injection Analysis

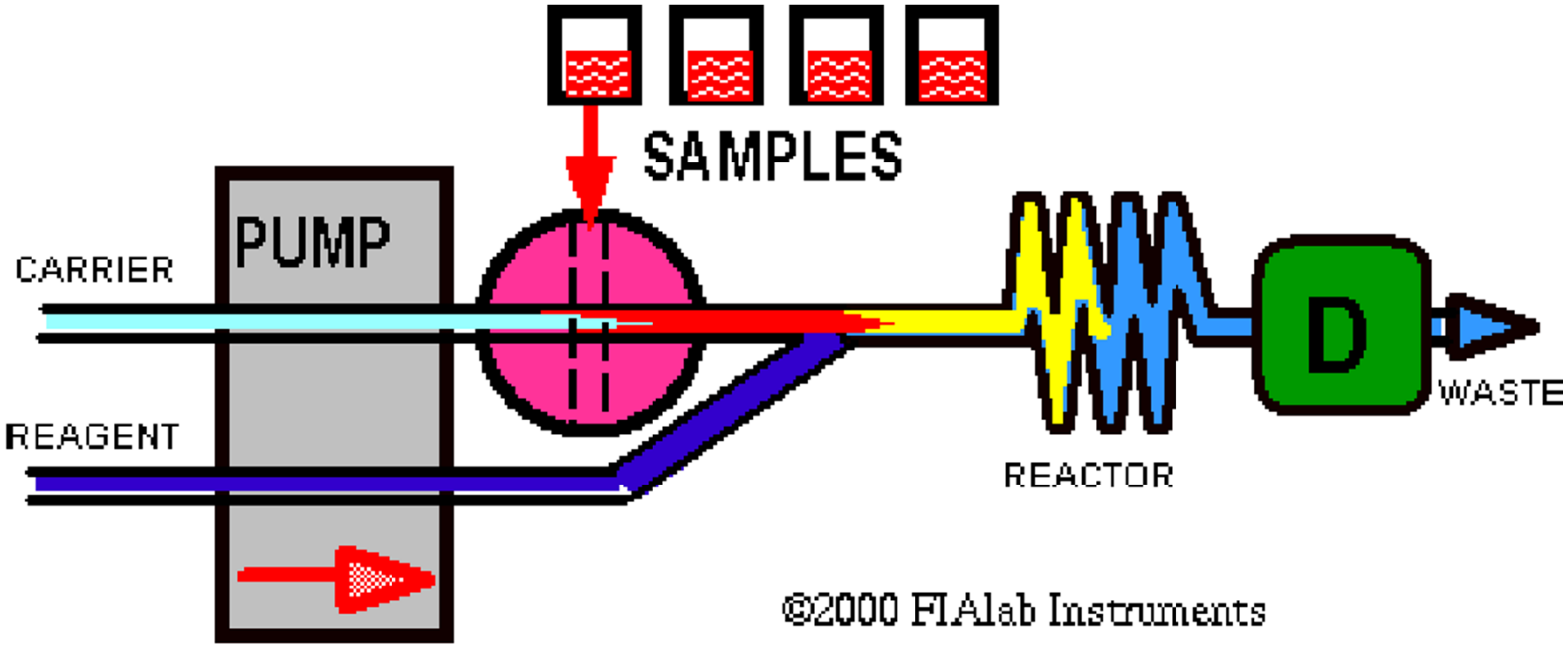


Fig. 12.17. Schematic of a flow injection analysis (FIA) system

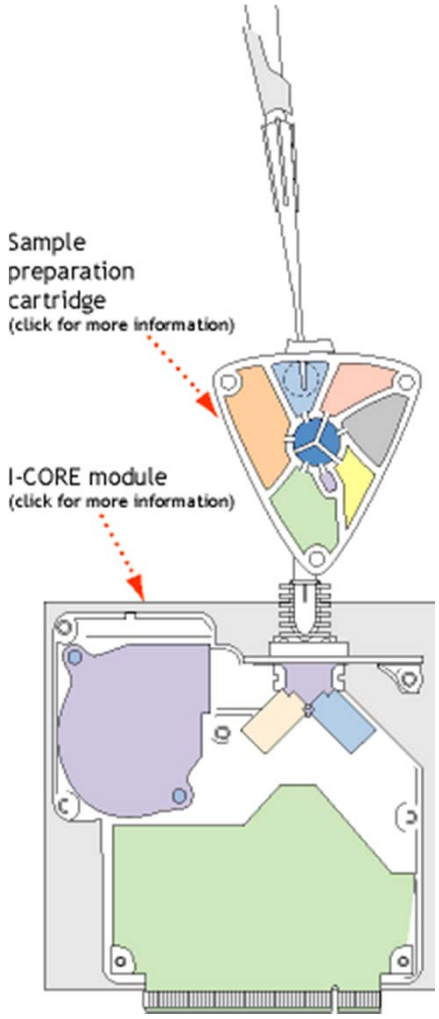
- Continuous flow of carrier and reagent
- Sequential injection of discrete sample volume in carrier stream
- Time-resolved detector signal

12. Analytical Chips

1. Concept and History
2. Analytical Separations
3. Microfluidic CD Technology
4. Flow Injection Analysis
5. **Microfluidic Processors**

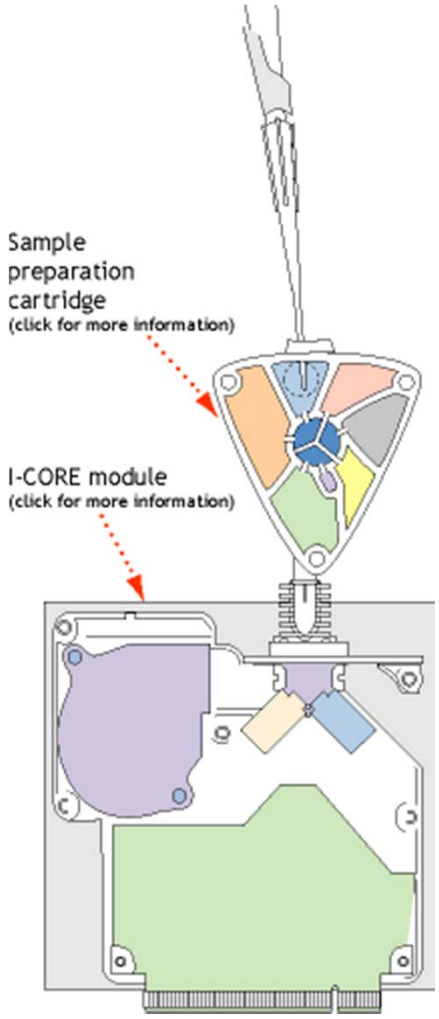
12.5. Cepheid Technology

- **Complex sample**
  - Blood
  - Biological tissues
  - Food
  - Soil
- **Sample preparation for PCR**
  - Extraction
  - Concentration
  - Purification
- **Process**
  - Multi-step
  - Time consuming
  - Skilled / trained personnel



12.5. Cepheid Technology

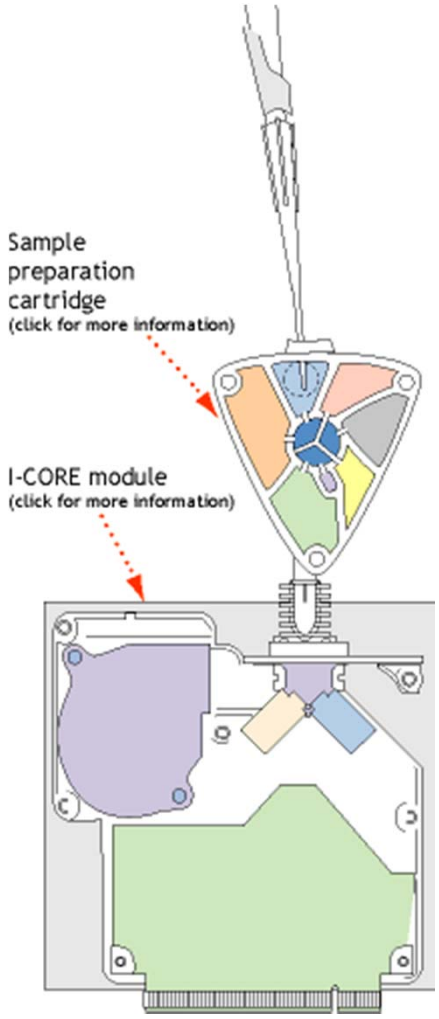
- **Process Steps of GeneXpert**
  - Ultrasonic disruption in 5-ml sample
    - Breaking cell membranes or spore coats
  - DNA extraction
    - Microfluidic channel
    - DNA immobilized on channel wall
    - Binding of bacterial DNA
    - Cellular debris flows over
    - Later release of bound DNA



12.5. Cepheid Technology

- **Time for PCR amplification**
  - Faster than culture
  - **Slow** (>90 min) with **traditional equipment**

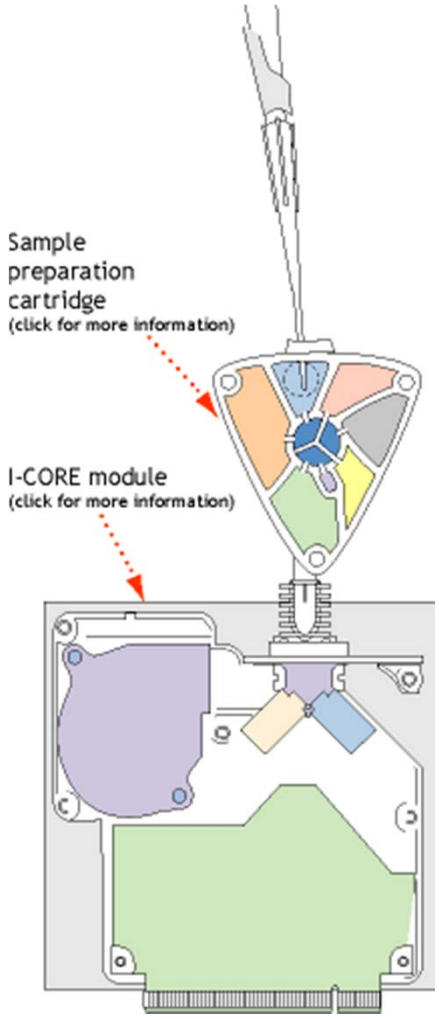
- **Detection in many amplification methods**
  - Separate,gel electrophoresis detection step
  - Amplified DNA target transferred to separate piece of equipment
  - Preparation and addition of reagents
  - Time consuming procedure



12.5. Cepheid Technology

Cepheid's Solution

- Target
  - Rapid, automated DNA based analyses
  - Wide range of settings (both lab and field)
  - Time-critical applications
- Simplification / integration of process
- Reduction of overall process time
  - **I-CORE module**
    - Rapid amplification and DNA detection
    - Carried out in single reaction tube
  - **Fluidic systems**
    - Preparation and processing of raw specimens and reagents
    - Rapid, automatic, hands-off processing
- Modular setup
  - I-CORE and fluidic systems
  - Integrable into wide range of system configurations



12.5. Other Real-Time PCR Devices

- Idaho Technology Inc. (Salt Lake City)
  - Ruggedized Advanced Pathogen Identification Device
- Lawrence Livermore National Laboratory
  - Hand-Held Advanced Nucleic Acid Analyzer
- Smiths Detection
  - Bio-Seeq detector

- For all of these commercial devices
  - No integrated sample prep
  - No integrated reagent-handling functions

12.5. O.R.C.A. Microfluidics

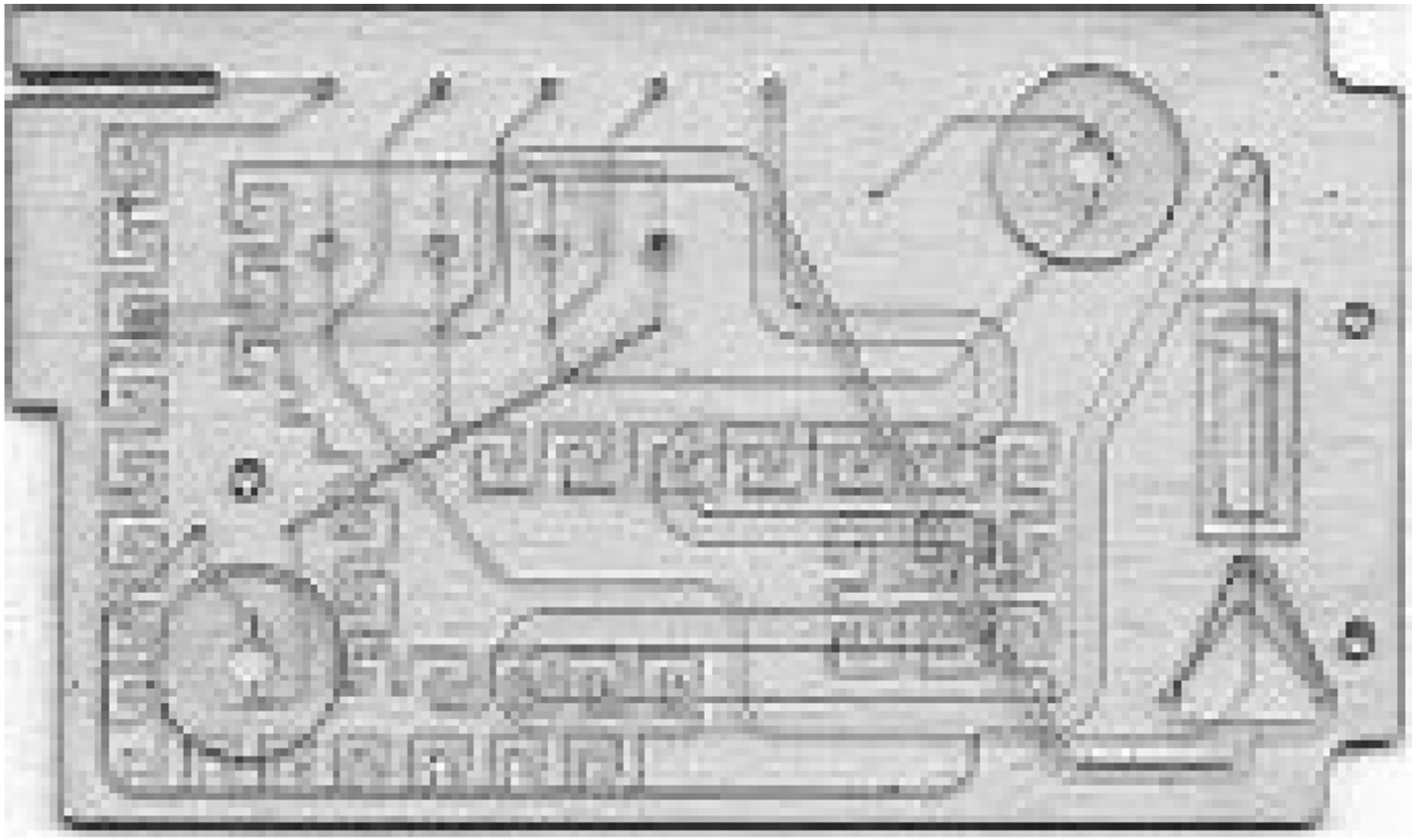
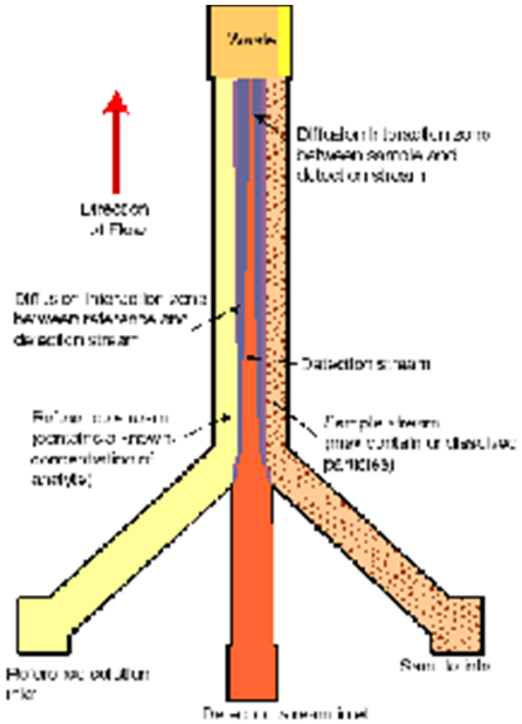


Fig. 12.18. O.R.C.A. μFluidics™ chip by Micronics Inc.

12.5. T-Sensor

MICRONICS

T-Sensor



- Reproducible evolution of interface
  - Laminar flow
  - Diffusion limited reaction
- Differential measurement
  - Center: reagent A
  - Left: reagent B
  - Right: reagent B’

12.5. Assay with T-Sensor

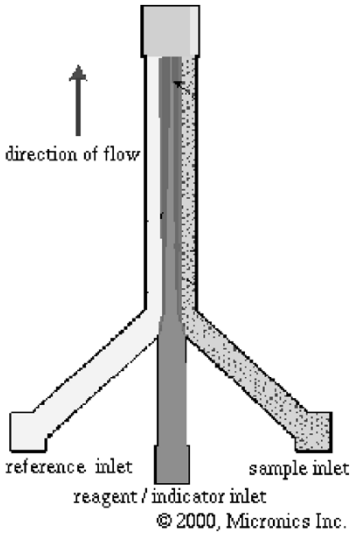


Fig. 12.20. Micronics T-sensor (JD: replace by graphics from)

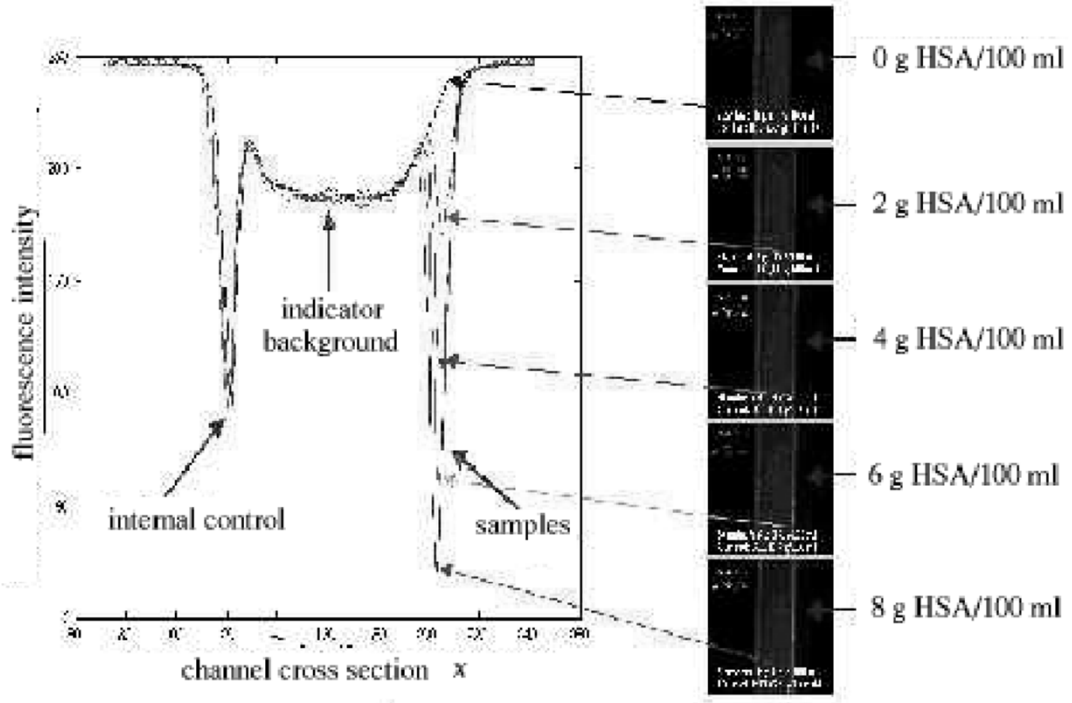


Fig. 12.21. Example T-Sensor assay. Fluorescence micrographs of a detection channel section containing a control, indicator, and sample streams during a determination of human serum albumin, and a graph displaying the corresponding light intensity profiles across the width of the channel