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World Year of PHYSICS 2005

SMR.1670 - 30

INTRODUCTION TO MICROFLUIDICS

8 - 26 August 2005

Chip-based LC, Liquid Chromatography

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Chip-based LC, Liquid Chromatography

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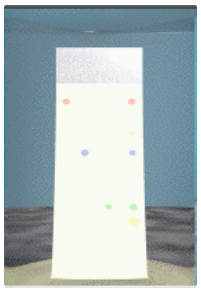

Methods of separation

Many variations exist, e.g.:
-distillation
-solvent extraction
-gas / liquid / supercritical fluid chromatography
-electrophoresis

The application of the methods can be either

-preparative (e.g. in oil refinery, food industry) - trend to larger
-analytical - trend to smaller

Basic idea

Separation is based on differences in:

- interaction/affinity (e.g. in solvent extraction)
- size/mass (e.g. in membrane filtering)
- charge (e.g. in ion-exchange chromatography)
- physical state/phase (e.g. in crystallisation)

These differences are translated in:

- residence time variation (retention) in a flow system
- accumulation in a batch system

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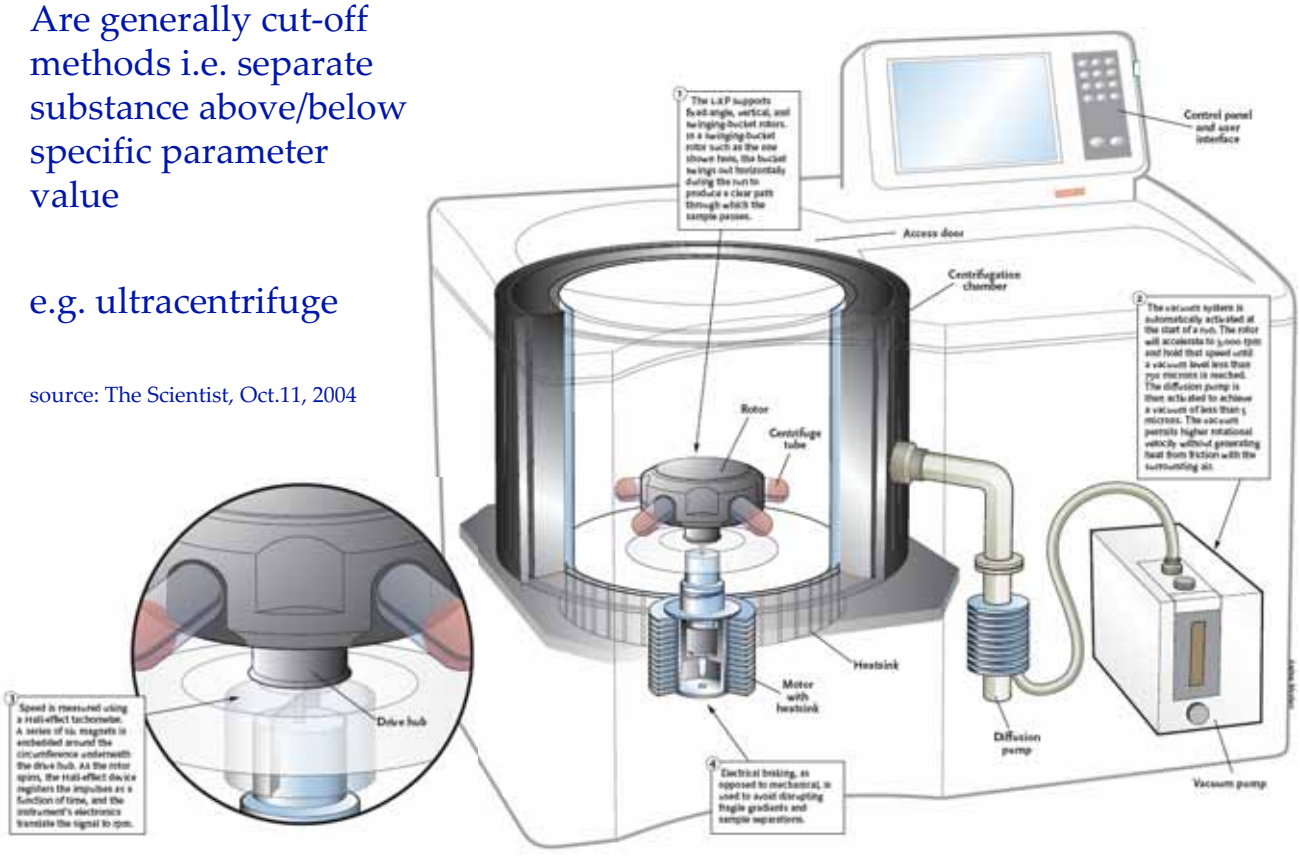
Filtering and related methods

Are generally cut-off methods i.e. separate substance above/below specific parameter value

e.g. ultracentrifuge

source: The Scientist, Oct.11, 2004

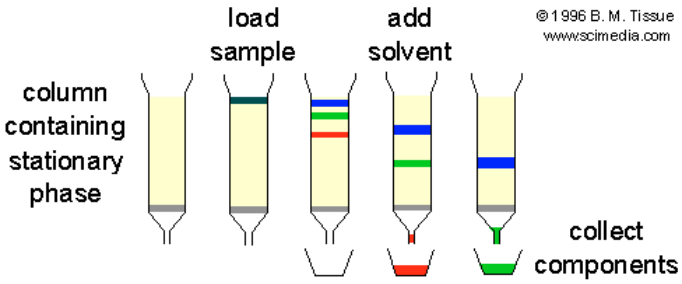


Chromatography and related methods

Substances are carried by a mobile phase along a stationary phase; individual species are retarded by the stationary phase

M. Tswett first observed separation of plant pigments as bands on chalk columns (1903) and named the phenomenon "chromatography" (in Greek "color writing")

L.S. Ettre and A. Zlatkis, Eds., 75 years of chromatography, Elsevier, Amsterdam, 1979



Note that a retention difference is translated in a location difference, which enables sorting

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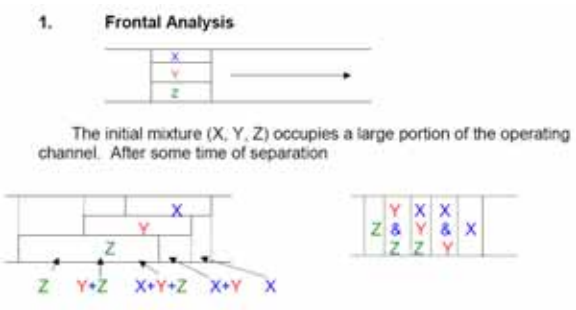
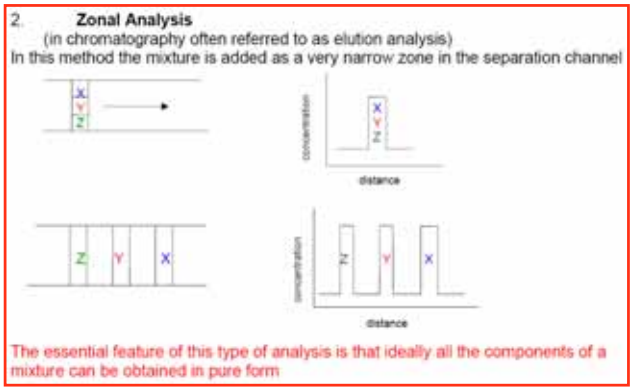
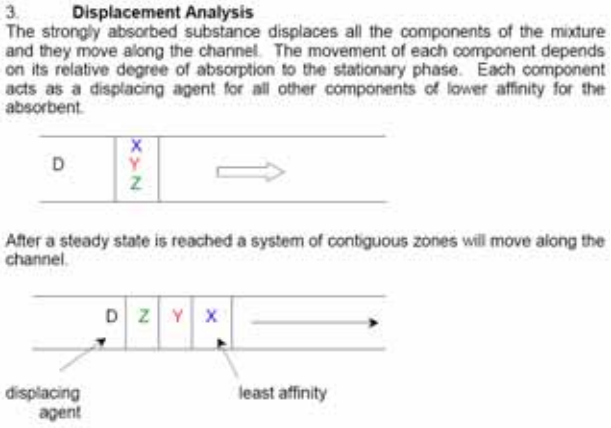
3 fundamental modes of operation







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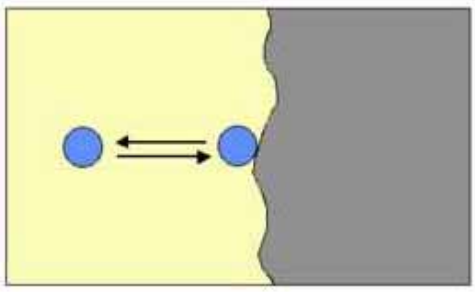
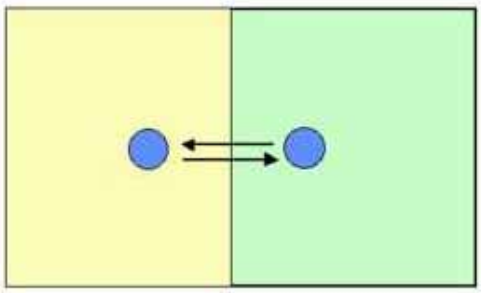
Chromatographic concepts

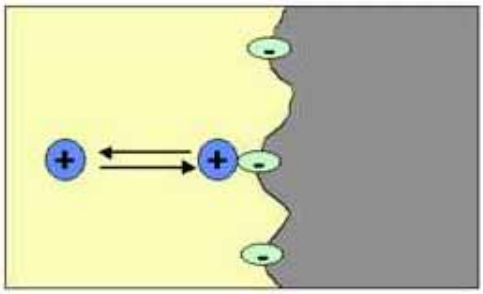
Major categories of LC



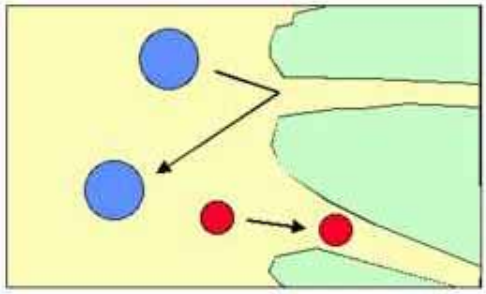
adsorption



partition



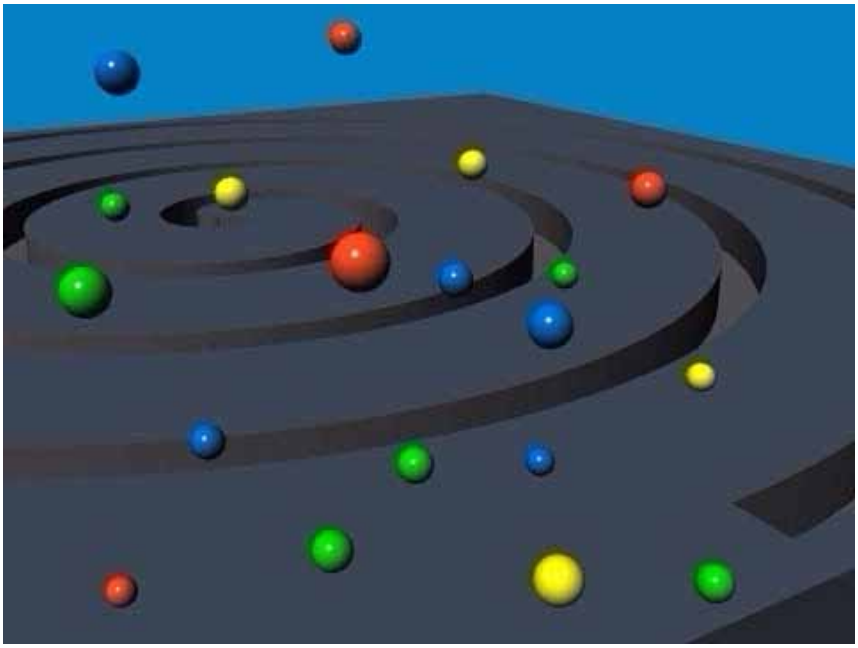
ion exchange



size exclusion (gel permeation)

pictures from: http://ull.chemistry.uakron.edu/chemsep/

Chromatography-animation



Movie courtesy of D. Williams, Sandia National Labs.

Partition chromatography

Normal phase: polar stationary phase and non-polar solvent
Reverse phase: non-polar stationary phase and polar solvent

Factors to consider in solvent selection:

Solvent strength: measure of relative solvent polarity (ability to displace a solvent) - scales are based on silica or alumina

Polarity index: index used for reverse phase methods

(NB these are only two of the many scales that are used to describe partitioning and solubility properties)

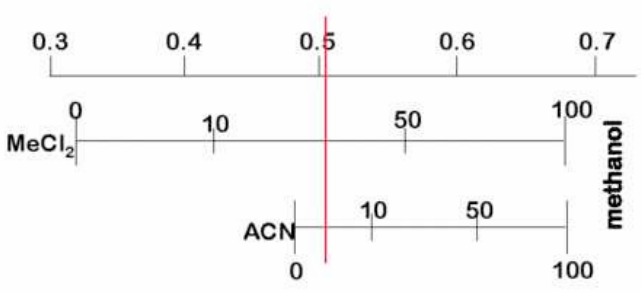
Solvent strength and polarity index

Solvent	ε^o	P'	viscosity	RI	UV cutoff
n-pentane	0.00	~0.0	0.23	1.36	210
CCl_4	0.18	1.6	0.97	1.47	265
toluene	0.29	2.4	0.59	1.50	285
ethyl ether	0.38	2.8	0.32	1.35	220
THF	0.45	4.0		1.41	220
MEK	0.51	4.7		1.38	330
acetonitrile	0.65	5.8	0.37	1.34	210
methanol	0.95	5.1	0.60	1.33	210

ε^o is for alumina.

Mixing solvents and gradient elution

Optimal solvent strength or polarity can be obtained by mixing solvents

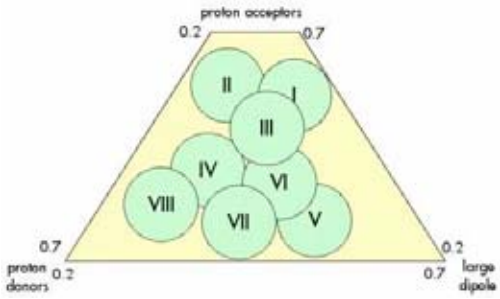


Gradient elution: stepwise or continuous change from one solvent to another during separation run
"Mixed stream": solvents are pumped together with turbulent mixing; total flow rate is kept constant, mixing is programmed

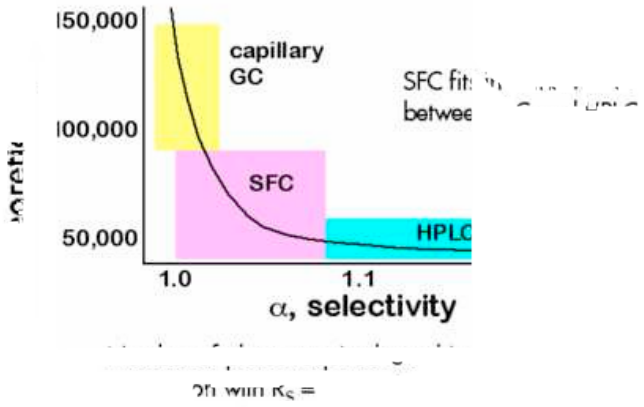
Solvent classes

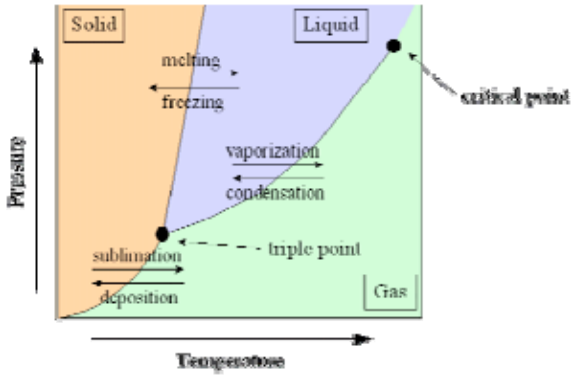
classification uses acid/base, dipole and chemical properties

Class	Partial solvent list
I	aliphatic ethers and alkyl amines
II	aliphatic alcohols
III	THF, pyridines, DMSO, amides
IV	formamide, acetic acid, glycols
V	CH_2Cl_2, 1,2-dichloroethylene
VI	alkyl halides, esters, ketones, nitriles
VII	benzene and derivatives
VIII	chloroform, m-cresol, water



Supercritical fluid chromatography





Pressure programming

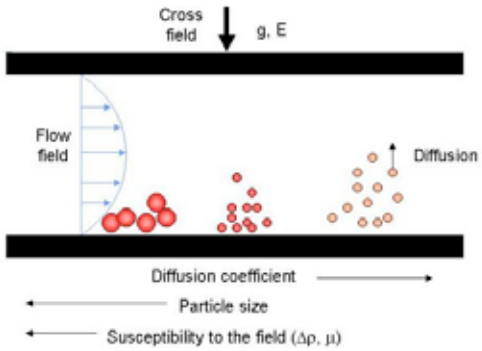
Density increases with pressure, which leads to increased solubility in mobile phase i.e. retention decreases

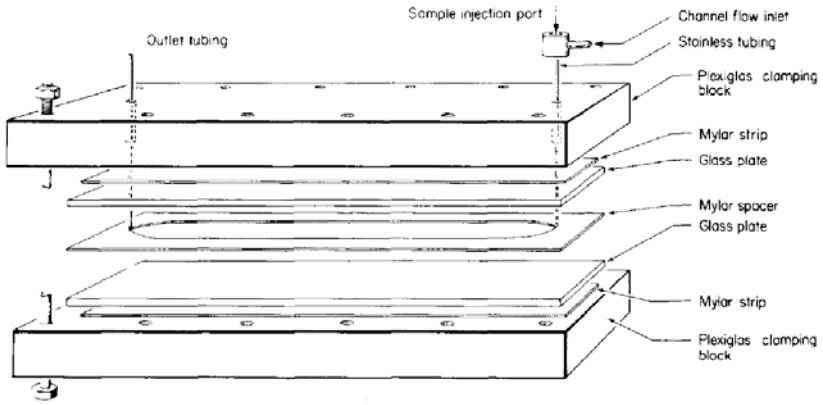
Field flow fractionation

Four common types of fields:

Sedimentation	density ~ M and d
Thermal	D/D_T ~ M and molecular conformation
Hydraulic (flow)	D ~ M
Electrical	μ and D

M: molecular weight
D: diffusivity
D_T: thermal diffusivity
μ: electrophoretic mobility
d: particle diameter





Giddings e.a. J.Chrom. 255, 359-379 (1983)

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Chromatographic theory

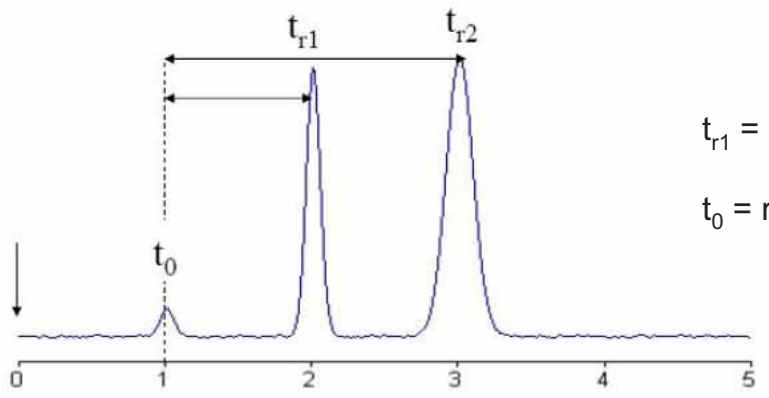
Plate theory (1941, Martin & Synge; based on analogy with distillation and counter-current extraction)

Rate theory (1956, van Deemter, dynamics of separation)

Retention and partitioning

Partitioning: $A_{mobile\ phase} \leftrightarrow A_{stationary\ phase}$

$$K = \left(\frac{c_s}{c_m} \right)_{eq}$$ distribution constant



t_{r1} = retention time analyte 1

t_0 = retention solvent (unretained)

capacity factor k gives ratio of amount of analyte in mobile : stationary phase:

$$k = \frac{V_s}{V_m} \left(\frac{c_s}{c_m} \right)_{eq} = \frac{V_s K}{V_m}$$

Relative retention times

fraction R of total analyte found in mobile phase:

$$R = \left(\frac{V_m c_m}{V_m c_m + V_s c_s} \right)_{eq} = \left(\frac{V_m}{V_m + K V_s} \right) = \frac{1}{1+k}$$

retention time of analyte zone: $t_r = \frac{L}{v} = \frac{L}{Ru}$

with *v* the average velocity of the zone and *u* the average velocity of the mobile phase. Obviously the unretained mobile phase has retention: $t_0 = \frac{L}{u}$

Note that: $k = \frac{t_r - t_0}{t_0}$

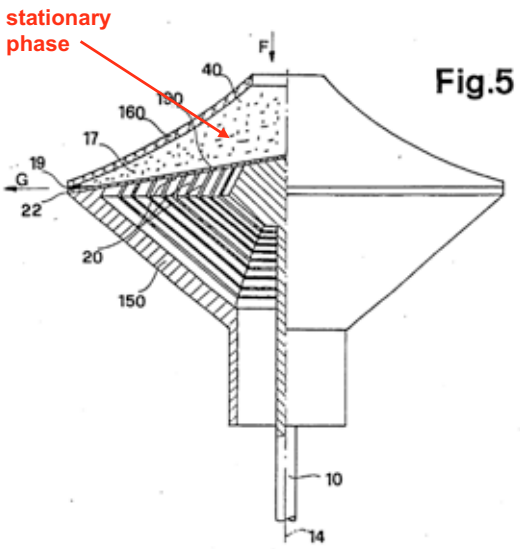
Thus, for different analytes: $\alpha_{2,1} = \frac{t_{r2} - t_0}{t_{r1} - t_0} = \frac{k_2}{k_1} = \frac{K_2}{K_1}$

Flow principles in separation science

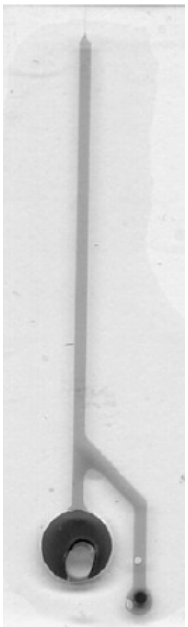
Pressure-driven	most common in LC and GC
Electrokinetic	Capillary Electrophoresis
Shear-driven	SDC, Rotational Planar Chromatography*
Centrifugal	RPC, Centrifugal Planar Column Chromatography
Gravity-driven	preparative LC
Capillary-force driven	Thin Layer Chromatography

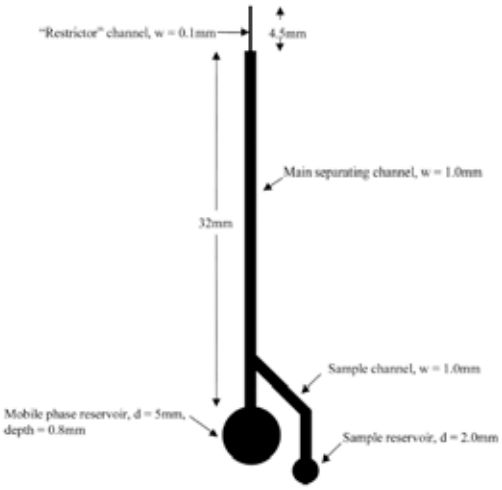
*RPC uses paper or TLC plates and centrifugal forces

Centrifugal chromatography, large & small



US Patent 4,678,570
Planar centrifugal chromatography device
Mèszàros e.a.; issued Jul.7, 1987



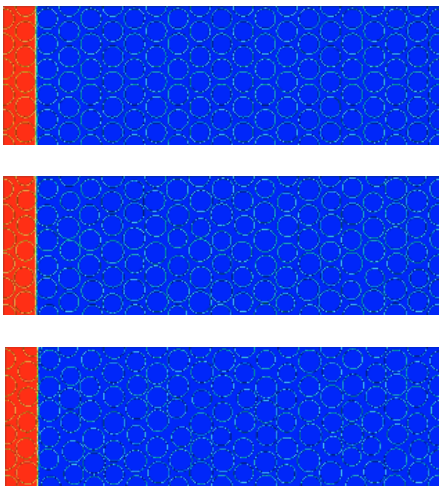


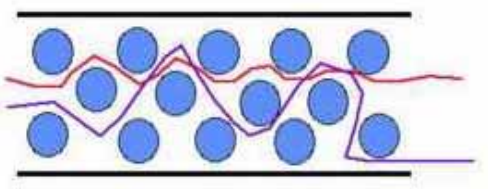
"Development and assessment of a miniaturised centrifugal chromatograph for reversed-phase separations in micro-channels" Penrose e.a. Analyst 129, 704-709 (2004)

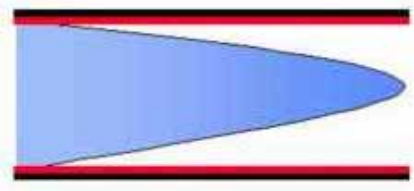
Why pressure-driven flow is preferred over EOF

1. more accurate flow control (independent of pH, electrolyte concentration, wall surface material, adsorption of large molecules onto the wall, composition of the sample matrix)
2. much broader range of applicable solvents
3. no interference in case of electrical detection methods
4. broader substrate material choice (allowing the use of silicon and the extended micromachining toolbox)

Packed vs. open-tubular

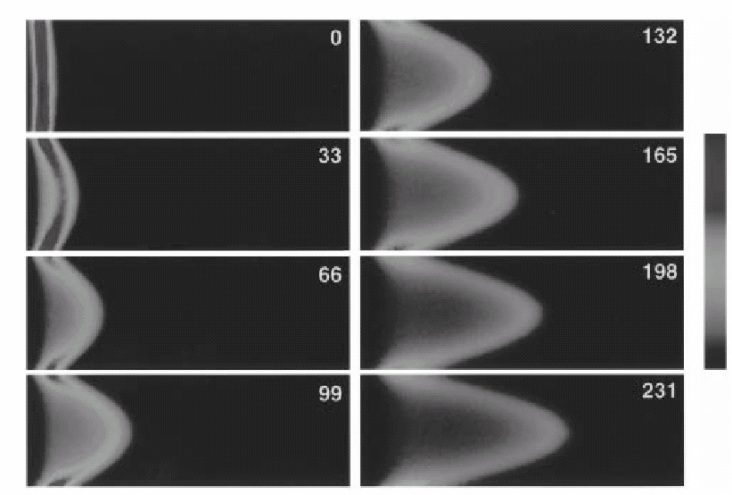






Comparison of the band broadening in a two dimensional mimic of a chromatographic column with increasing degree of heterogeneity;
source: J.Billen, VU Brussel, B
http://wwwtw.vub.ac.be/chis/Jeroen.htm

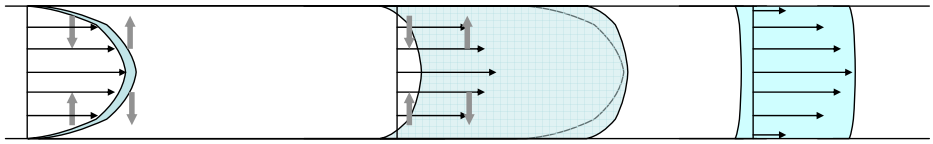
Zone spreading:



Parabolic flow profile in pressure-driven flow (short time-scale, numbers in ms)

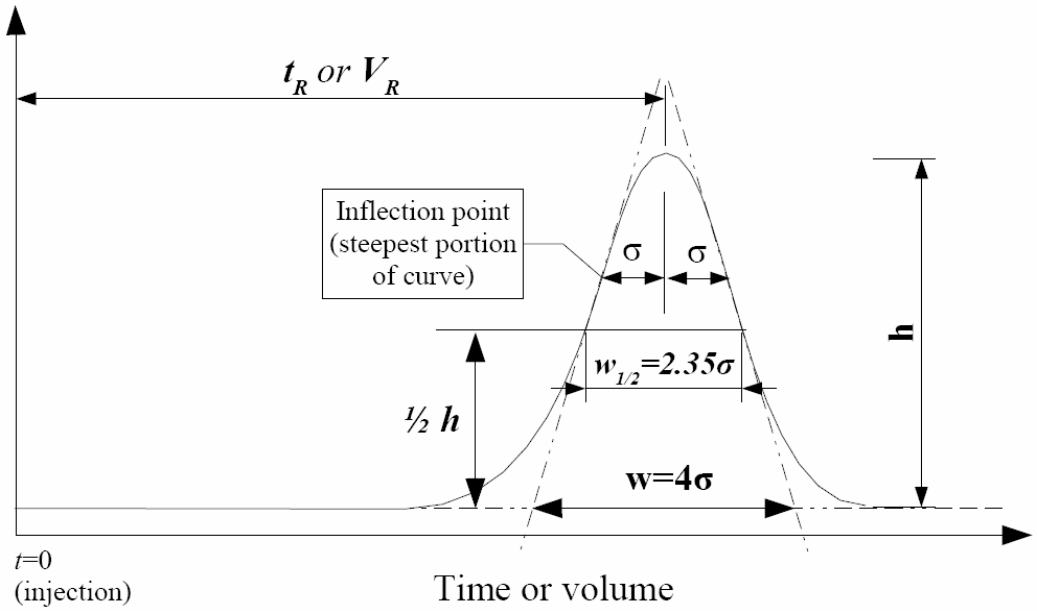
Paul e.a. Anal.Chem. 70, 2459-2467 (1998)

After longer times, diffusion "blurs" the parabolic shape



Drawing: Desmet (VUB), Chmela (UvA), Tijssen (UvA)

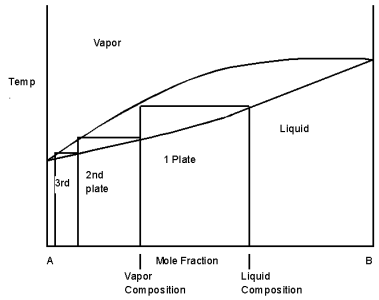
Gaussian peak dispersion due to diffusion



Standard deviation of band (infinitely small injection plug): $\sigma = \sqrt{2Dt}$
with *D* diffusivity of analyte

from: http://www.tut.fi/units/ymp/kem/opintojaksot/3500820/L2.pdf

Plate theory

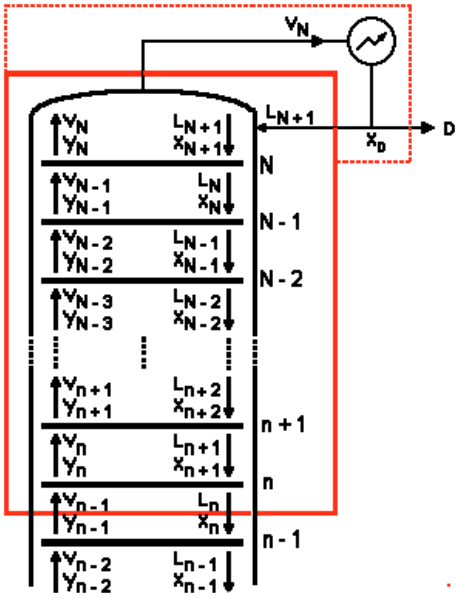


Column is considered to consist of a number of ***plates***, (in analogy to distillation) on which the equilibrium of the solute with the mobile and stationary phases occurs.
Length of column is divided by this ***number of theoretical plates N*** to give the ***height equal to a theoretical plate H (or HETP)***.
Higher *N* or smaller *H* means a more efficient column.

Plate height is introduced by the equation:

$$\sigma_x^2 = 2Dt = 2D\frac{L}{u} \equiv HL$$

or: $H = \frac{\sigma_x^2}{L} = L\left(\frac{\sigma_t}{t_r}\right)^2$



distillation column with plates

Resolution

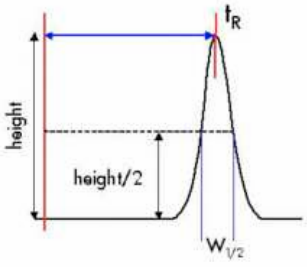
$$\text{Resolution} = \frac{\text{peak separation}}{\text{average peak width}}$$

It can be derived that: $R = \frac{t_{r2} - t_{r2}}{2(\sigma_1 - \sigma_2)} = \ln\left\{1 + \frac{k_2 - k_1}{1 + k_1}\right\}\sqrt{\frac{N}{4}}$

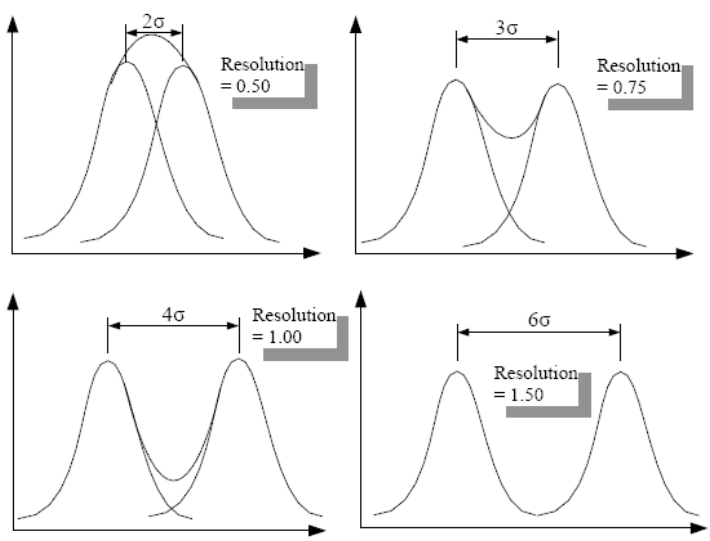
For $k_2 \approx k_1$: $R = \frac{k_2 - k_1}{1 + k_1}\sqrt{\frac{N}{4}}$

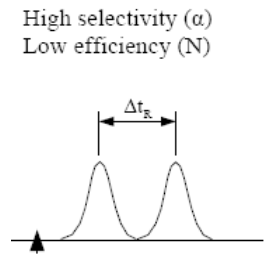
N = plate height, can be extracted from peak half-width:

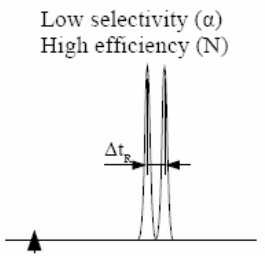
$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2$$



Resolution examples

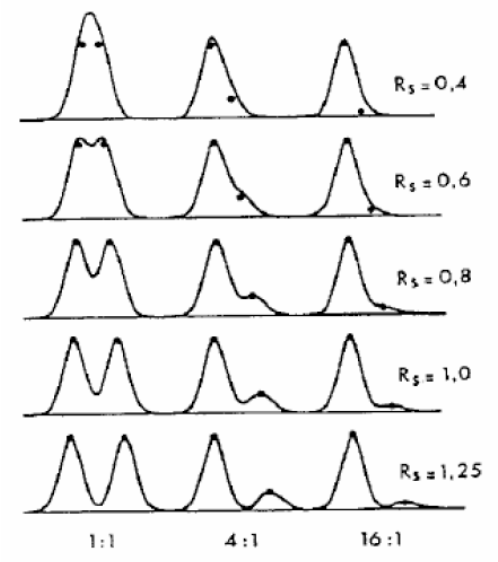






from: http://www.tut.fi/units/ymp/kem/opintojaksot/3500820/L2.pdf

Resolution limits

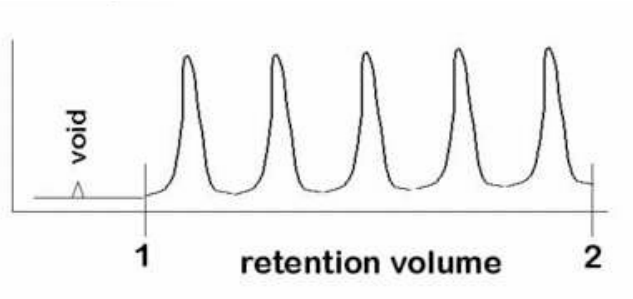


Resolution	Rel. impurity (%)
1.5	0.1
1.0	2.3
0.8	4.5
0.5	16

Source: A.Tudos, University of Twente,NL

Peak capacity

A measure of how many peaks can be totally separated between any two points on a chromatogram:



Band broadening outside the column

$$\sigma^2_{total} = \sigma^2_{column} + \sigma^2_{detector} + \sigma^2_{injector}$$

with $\sigma^2_{detector} = \frac{(\Delta t)^2}{12}$ and $\sigma^2_{detector} = \frac{(\Delta t)^2}{12}$

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Van Deemter equation

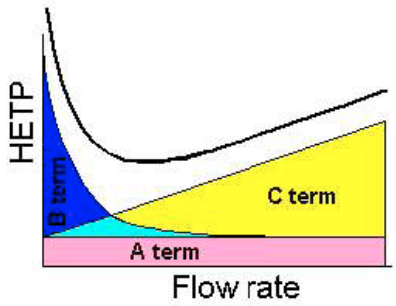
Proposed in 1956, has the basic form:

$$H \approx A + \frac{B}{u} + Cu$$

A: multiple passes through the column packing

B: molecular diffusion

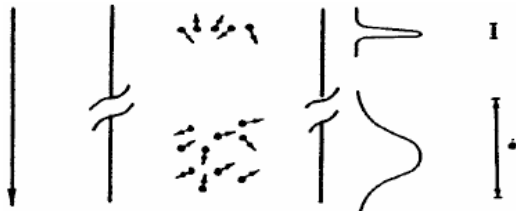
C: equilibration between phases



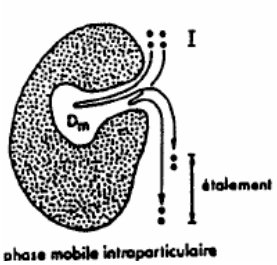
A

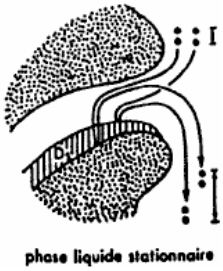
Illustration of eddy diffusion.

B



C





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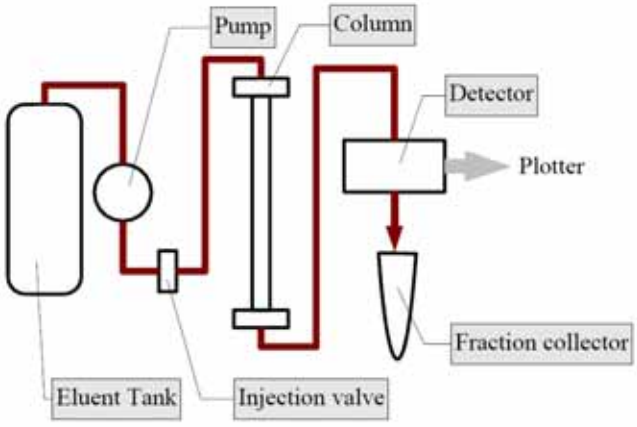
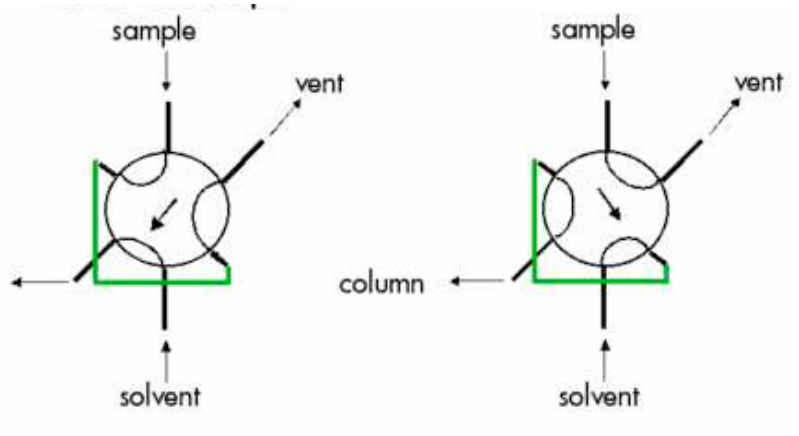
Instrumentation

Basic LC setup

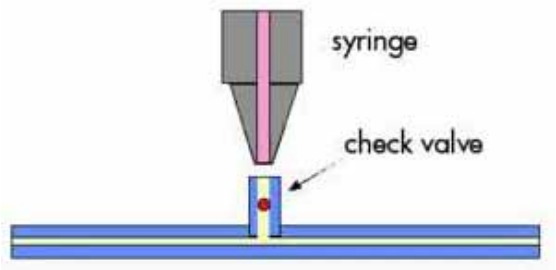


drawing from: http://www.tut.fi/units/ymp/kem/opintojaksot/3500820/L2.pdf

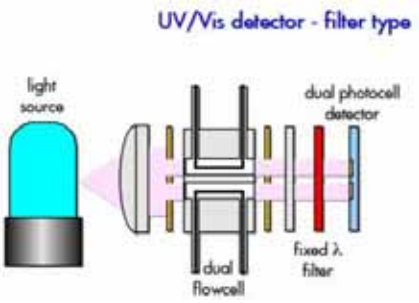
Injection valves and sampling loops

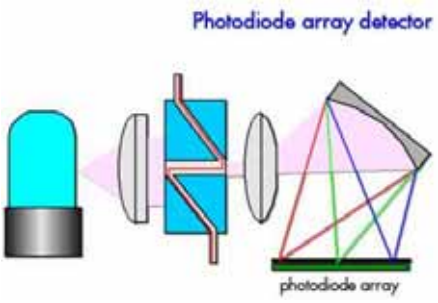


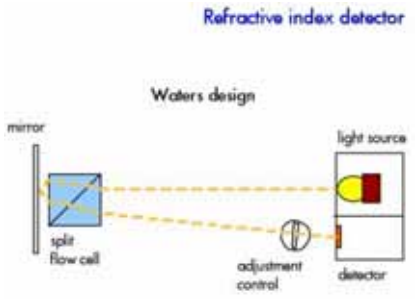
Injection by automated syringes

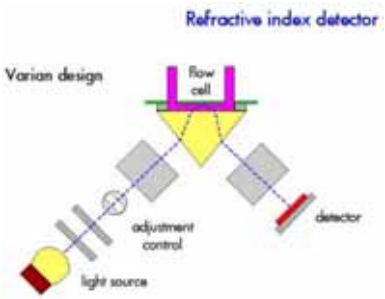


Optical detectors for LC

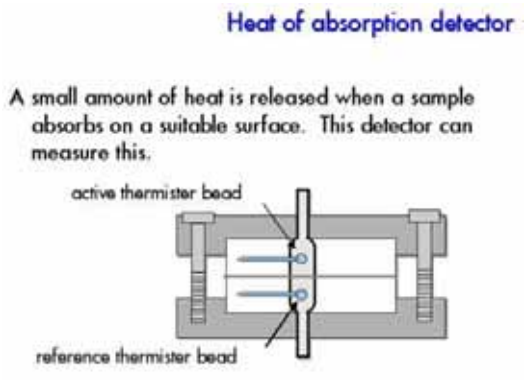








Other detectors for LC



Electrochemical detectors

Dielectric constant
Amperometric
Conductometric
Polarographic
Potentiometric

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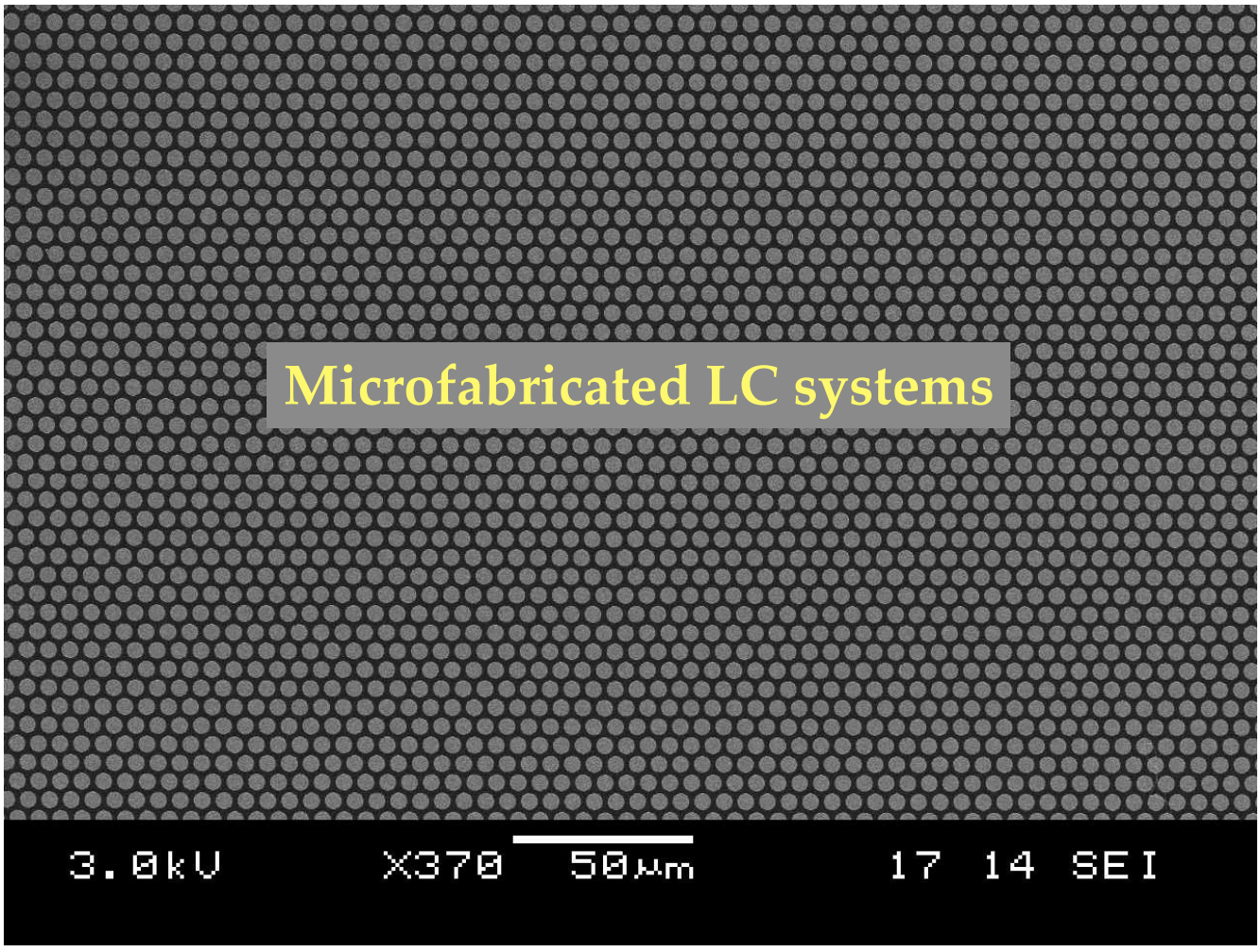
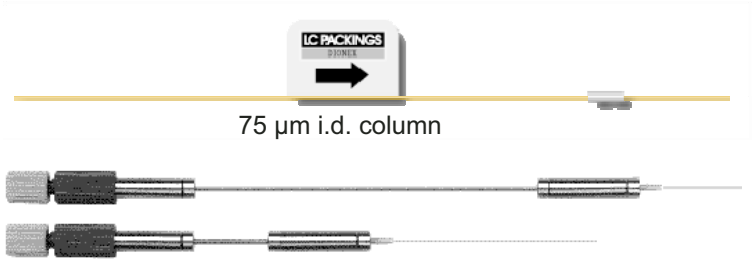


Why is miniaturisation important?

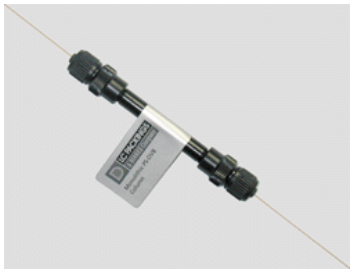
Standard LC formats are already miniaturised: 75 µm-1 mm capillaries; packed columns with (sub)micron flow passages; (nano)porous materials; surface processes; sensitive detectors; low sample volumes and flow rates

Micro/nanofabrication benefits: integration improves performance, parallel separations, new types of size separation, model systems for fundamental studies of pores



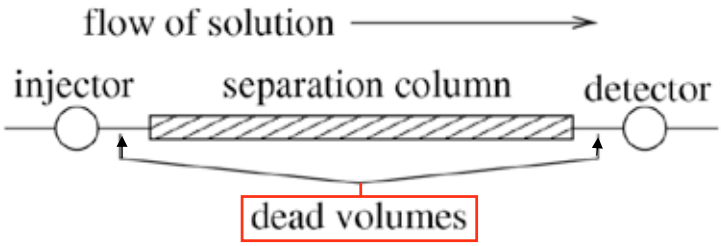
75 µm i.d. column

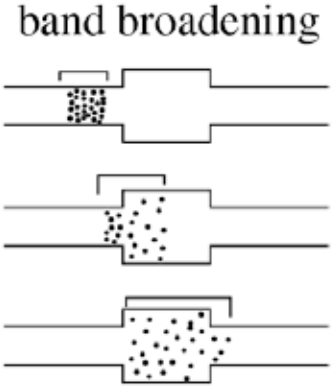
300 µm i.d. column (the standard)



100 µm i.d. monolith column

Dispersion by dead volumes





van Deemter equation

$$H = \frac{2D_m}{u_m} + 2\kappa\frac{u_m.d_H^2}{D} + \frac{2}{3}.\frac{k}{(1+k)^2}\frac{u_m.d_f^2}{D_s}$$

Knox equation: $H = A.u^{1/3} + \frac{B}{u} + Cu\frac{D_m}{D_s}$

Overview of κ-values

	κ (unretained solute)	κ (retained solute)
Plug flow	κ=0	$\kappa = \frac{k'^2}{6.(1+k')^2}$
Parabolic flow	$\kappa = \frac{1}{210}$	$\kappa = \frac{1+9k'+25.5k'^2}{210.(1+k')^2}$
Axisymmetric linear flow	$\kappa = \frac{1}{120}$	$\kappa = \frac{1+7k'+16k'^2}{120.(1+k')^2}$

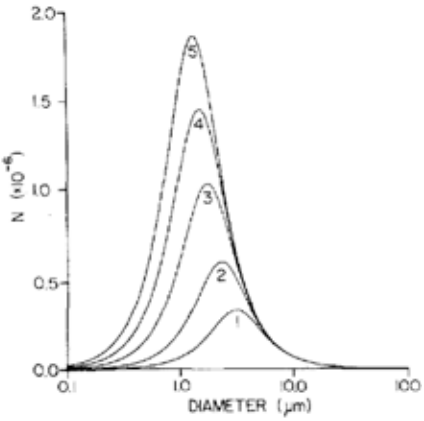
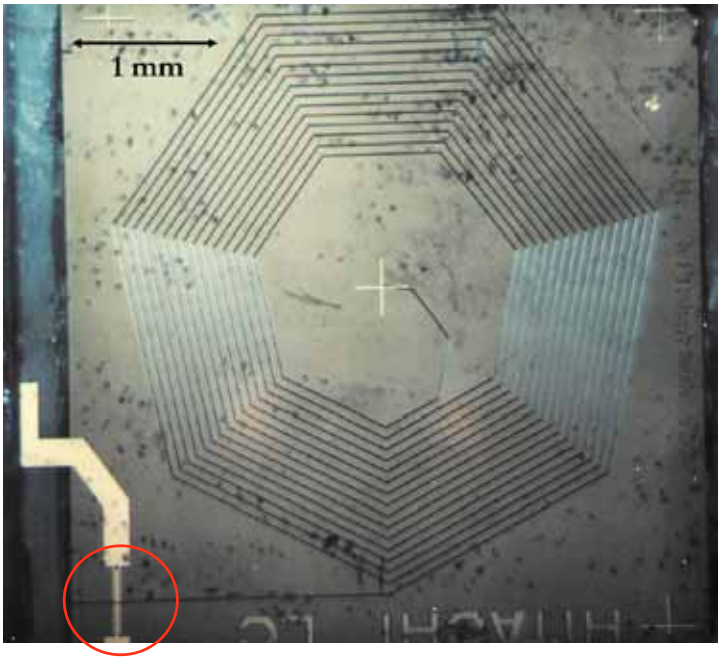
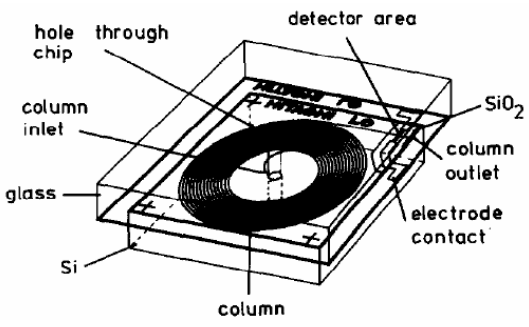


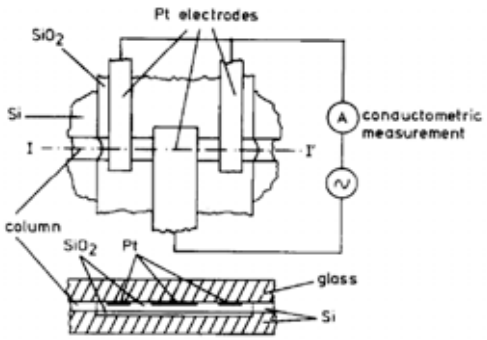
Fig. 1. Number of theoretical plates as a function of column diameter for five different pressures. $k' = 10$, $D = 1 \cdot 10^{-5}$ cm²/sec, $\eta = 5 \cdot 10^{-3}$ P, $t = 2$ h. 1 = 300 p.s.i.g. (21 bar); 2 = 1000 p.s.i.g. (69 bar); 3 = 3000 p.s.i.g. (210 bar); 4 = 6000 p.s.i.g. (420 bar); 5 = 10,000 p.s.i.g. (690 bar).

Desmet, 2005; figure from: Jorgenson e.a. J.Chrom. 255, 335-348 (1983)

First LC chip



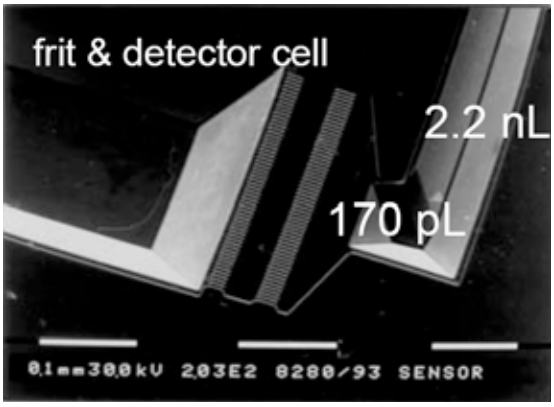


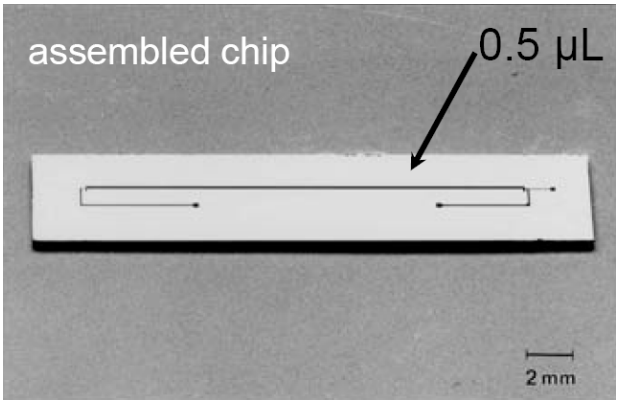


channel dimensions: 6 μm x 2 μm x 150 mm
silicon-glass combination; conductivity detector

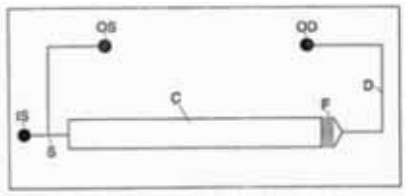
Manz e.a. Sens.Act.B 1, 249-255 (1990)

Packed LC chip





Channel dimensions: 20 mm, 313 µm x 102 µm
Stationary phase: C8 on 5 µm particles
mobile phase: methanol
detection: LIF
pump: conventional HPLC

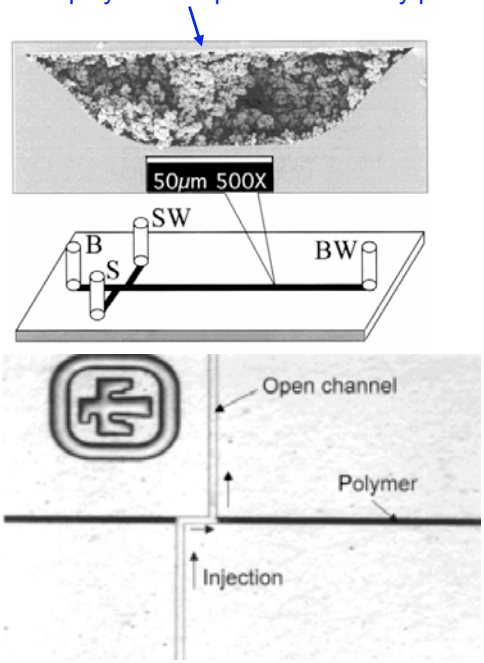


Ocvirk e.a. Anal.Methods & Instrum. 2, 74-82 (1995)

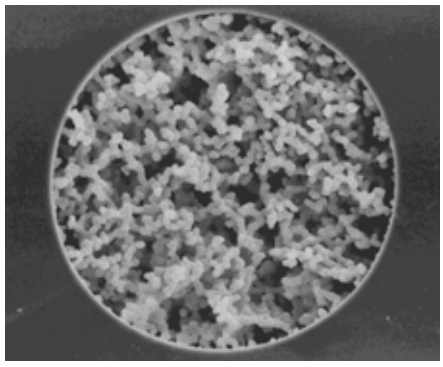
Monoliths for CEC

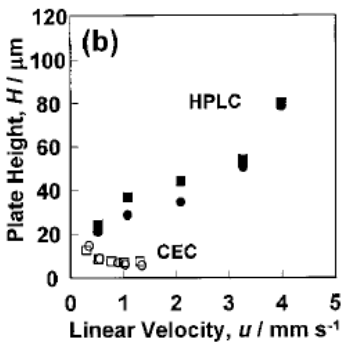
CEC: Capillary Electro Chromatography

Photopolymerised porous stationary phase



Silica monolith by in situ hydrolysis and polycondensation of alkoxysilane

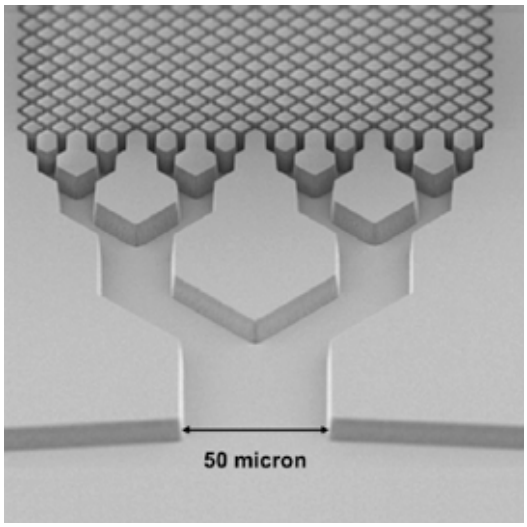


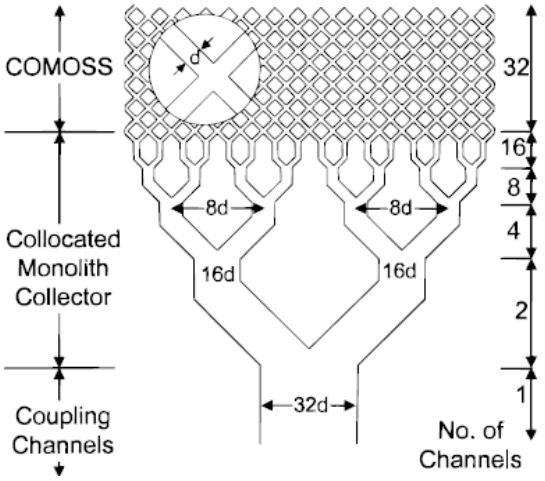


Ishizuka e.a. Anal.Chem. 72, 1275-1280 (2000)

Throckmorton e.a. Anal.Chem. 74, 784-789 (2002)

Micromachined LC chip





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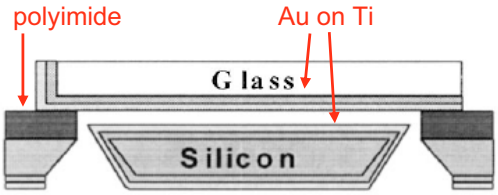
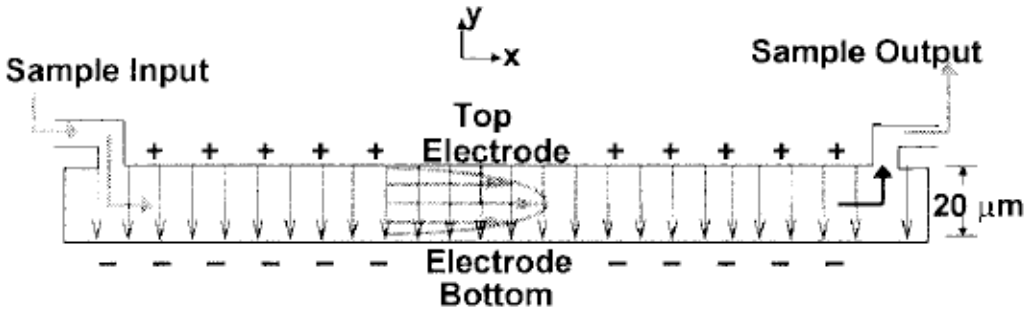
He e.a. Anal.Chem. 70, 3790-3797 (1998)

University of Twente
The Netherlands

Field-flow fractionation on a chip





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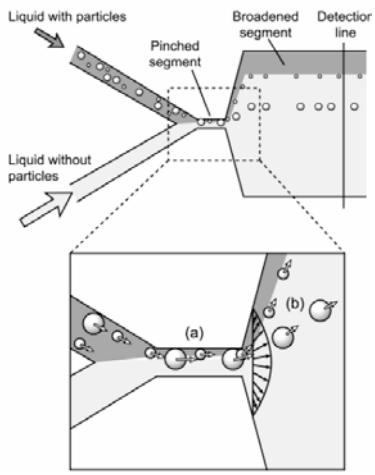
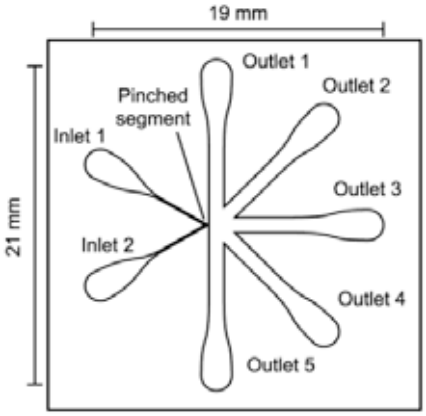
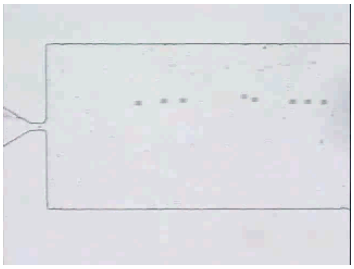
Gale e.a. IEEE Tr.Biomed.Eng. 4, 1459-1469 (1998)

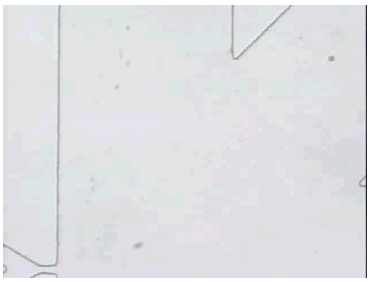
University of Twente
The Netherlands

Pinched flow fractionation



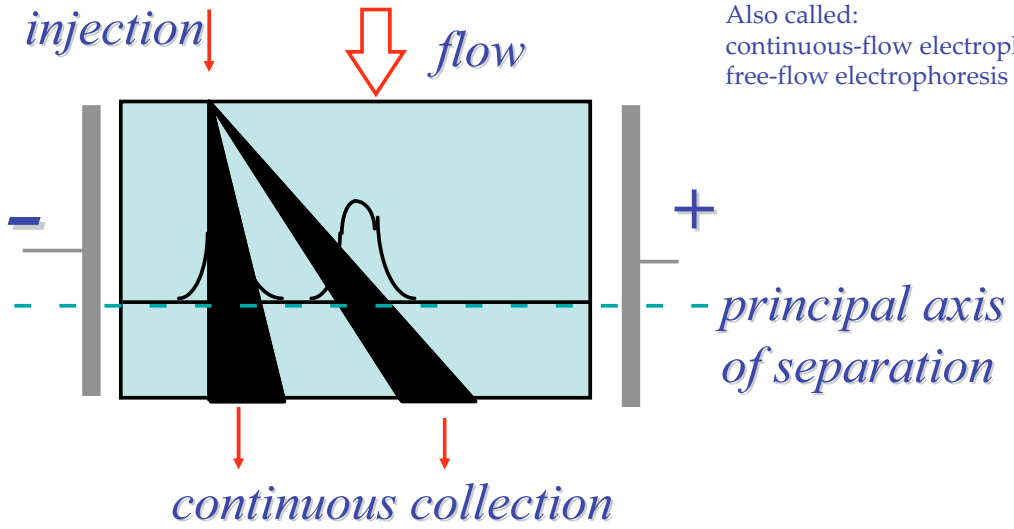






Yamada e.a. Anal.Chem. 76, 5465-5471 (2004)

Continuous Deflection Electrophoresis

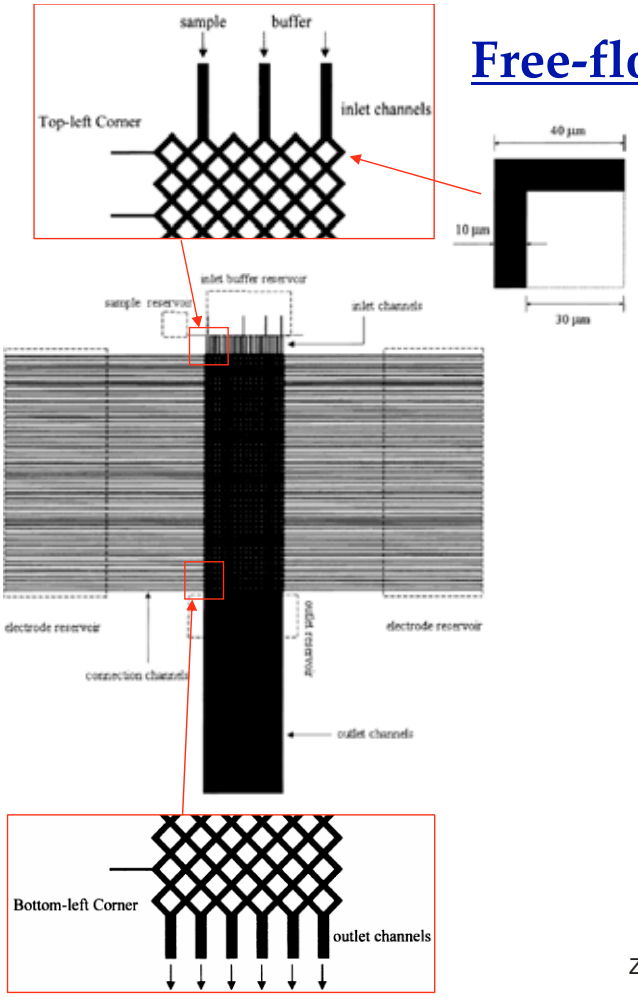


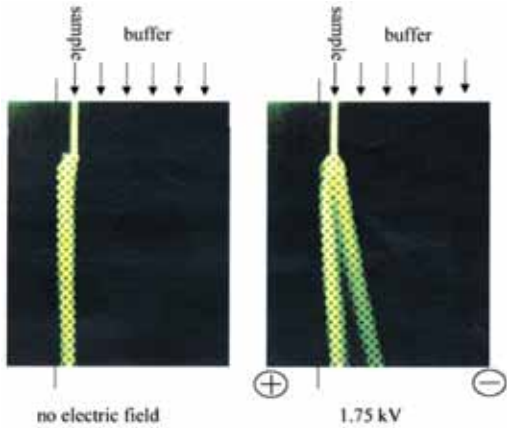
Also called:
continuous-flow electrophoresis
free-flow electrophoresis

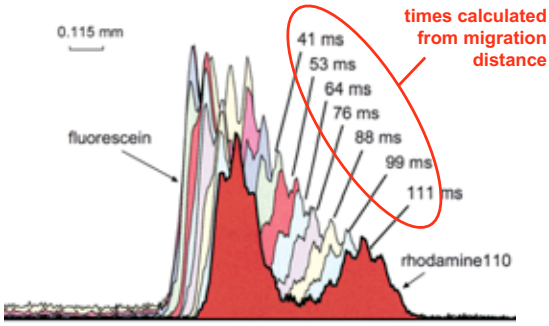
Flow down a paper sheet or between closely spaced plates carries different solutes, which are gradually separated by electrophoresis along a perpendicular axis, to different collection ports

Strickler, Sep.Sci.2, 335 (1967)

Free-flow electrophoresis on a chip



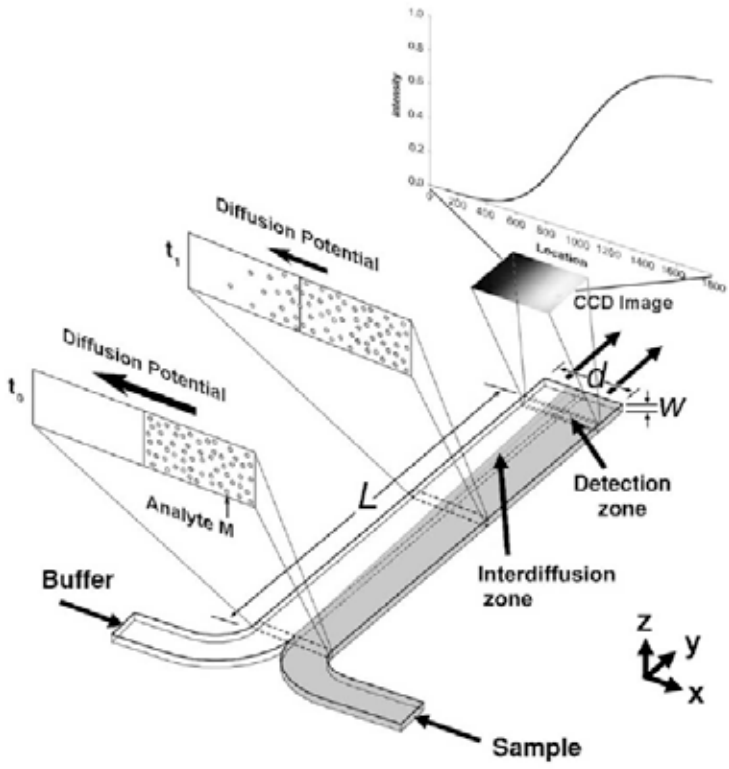




Zhang e.a. Anal.Chem. 75, 5759-5766 (2003)

University of Twente The Netherlands

T-sensor



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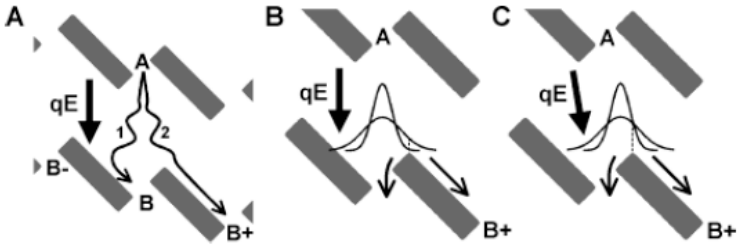
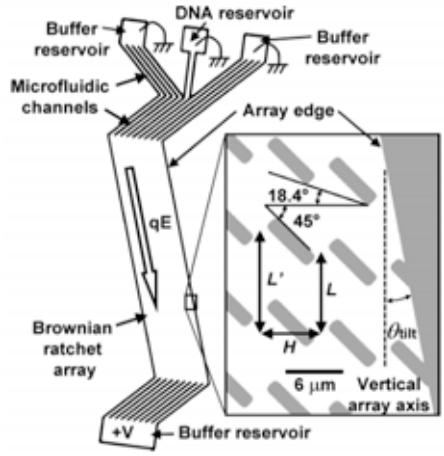
Hatch e.a. Proc.IEEE 92, 126-139 (2004)

University of Twente The Netherlands

Brownian ratchets for DNA separation

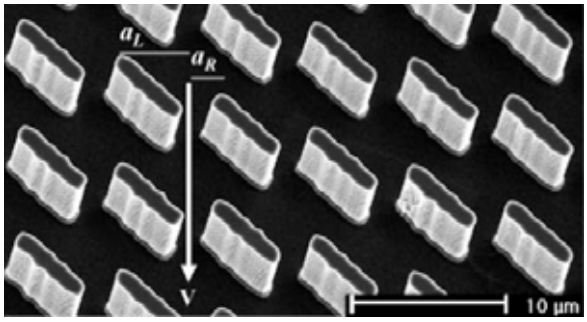


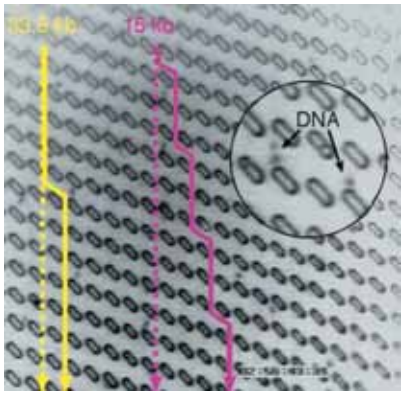


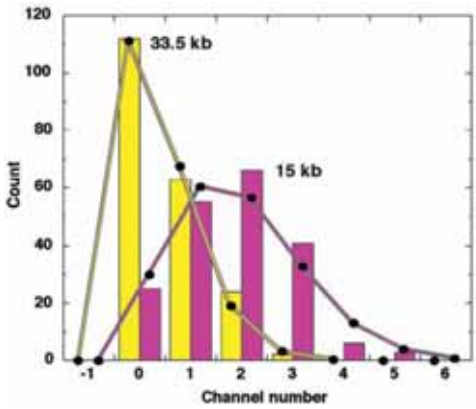
Basic principle of the Brownian ratchet array. Particles are driven through the array hydrodynamically or electrophoretically.
(A) Particles emerging from A and diffusing to the left (1) cannot reach B- but particles diffusing to the right (2) may reach B+.
(B) Particles of different size diffuse to different extents, resulting in different probabilities of reaching B+.
(C) Probability of reaching B+ is increased by tilting the flow at a small angle with respect to the vertical axis of the array.

Huang e.a. Anal.Chem. 75, 6963-6967 (2003)

Brownian ratchets



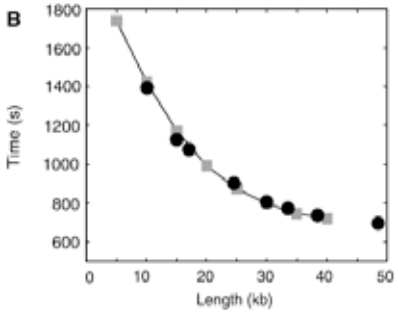


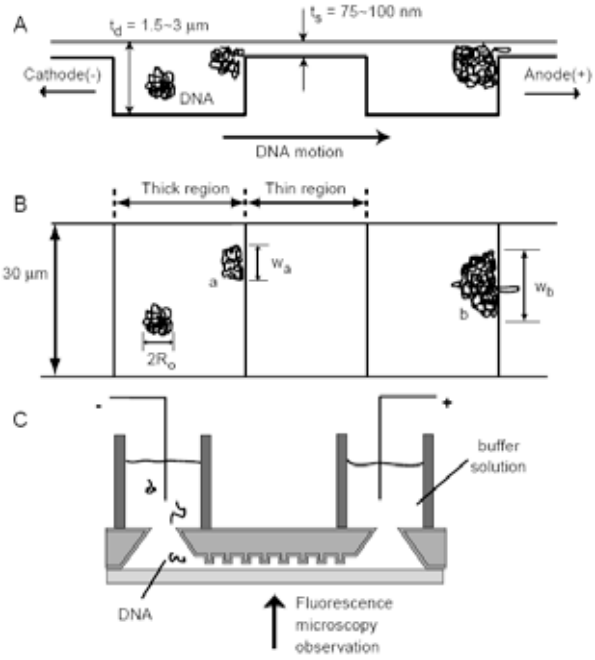


Obstacle dimensions: 0.35 μm high,1.5 μm x 6.0 μm. Gap between adjacent obstacles is 1.5μm.
Transverse Brownian motion causes molecule to skip one channel to the right if it diffuses through displacement a_R, or very rarely, one channel to the left if it diffuses through a_L

Chou e.a. PNAS 96, 13762-13765 (1999)

"Entropic traps"





Trapped DNA molecules escape with probability proportional to length (w_a,w_b) of slit that they cover

Han e.a. Science 288, 1026-1029 (2000)
Simulation; Streek e.a. J. Biotech. 112, 79-89 (2004)

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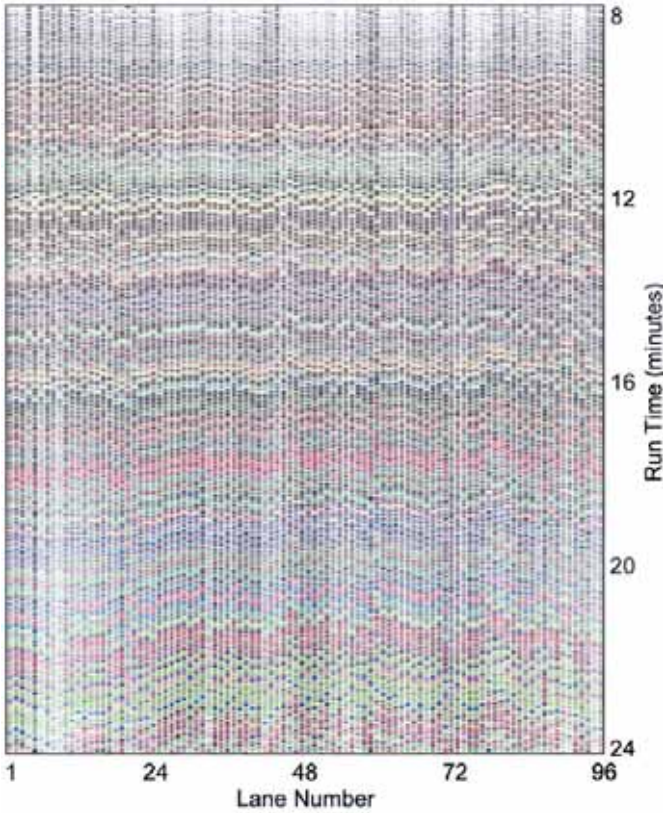
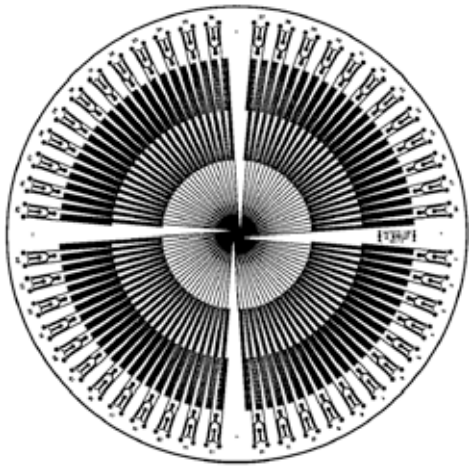
The Netherlands

Parallel separation channels: CE





Lane 72 failed due to photolithographic defect; Lane-to-lane variance in mobility is attributed to electrode placement as well as variability in coating and gel filling.

Paegel e.a. PNAS 99, 574-579 (2002)

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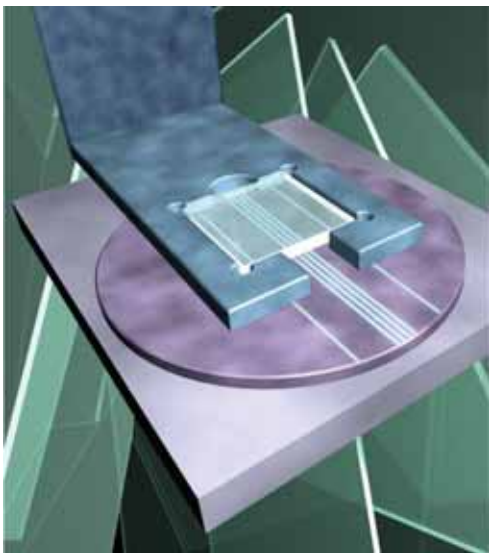
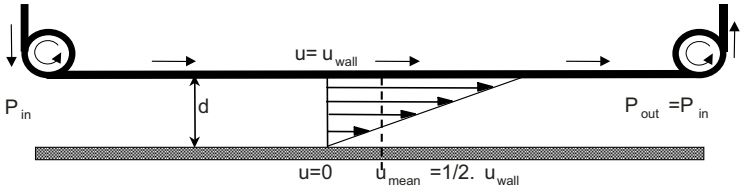
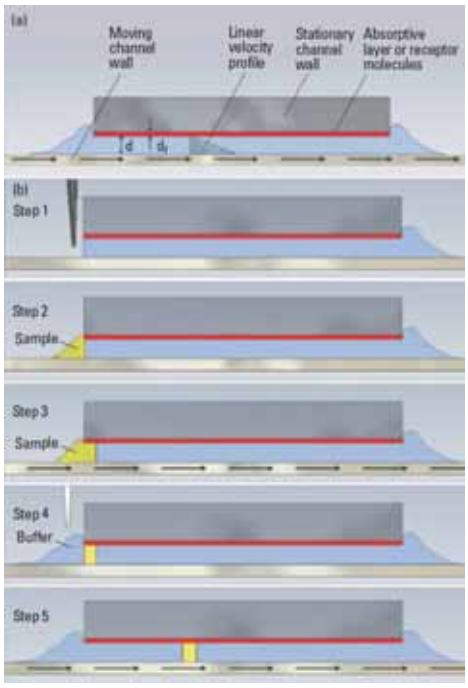
Case study 1
Shear-driven chromatography

Gert Desmet and co-workers
Free University of Brussels (VUB), Belgium
Wim de Malsche, Han Gardeniers
University of Twente, NL

Basics of shear-driven chromatography (SDC)







Injection procedure

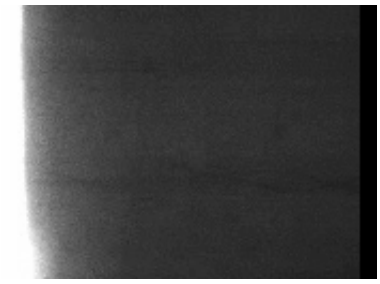
Clicq e.a. Anal.Chem. Dec.1, 2004, 430A-438A

Background and results

Limitations in pressure-driven chromatography:
pressure drop:

$$\Delta P = \frac{\psi \mu u_m L}{d^2}$$

with μ viscosity, L length, d column (or particle, in packed columns) diameter, u_m average linear velocity of mobile phase, ψ flow resistance parameter (32 for open and 500-1000 for packed columns).
Smaller d (for LC) or larger L (for GC) can not be used due to mechanical limitations of the system

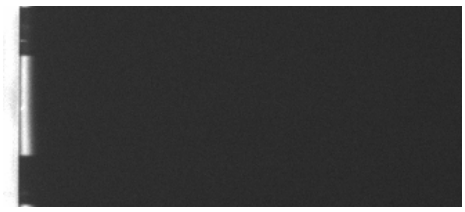


Shear-driven: $u_m = \frac{u_{wall}}{2}$

is basically unlimited. Plate height is given by:

$$H = 2\frac{D_m}{u_m} + \frac{2}{30}\left\{\frac{1+7k+16k^2}{(1+k)^2}\right\}u_m\frac{d^2}{D_m} + \frac{2}{3}\left\{\frac{k}{(1+k)^2}\right\}u_m\frac{d_t^2}{D_s}$$

with d_f thickness of stationary phase layer, D_s and D_m diffusivities in stationary and mobile phase, k retention coefficient, d thickness of mobile phase layer.



high-speed separation of 4 coumarin dyes

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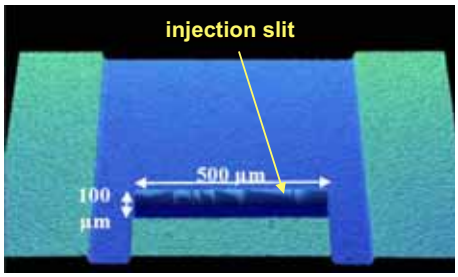
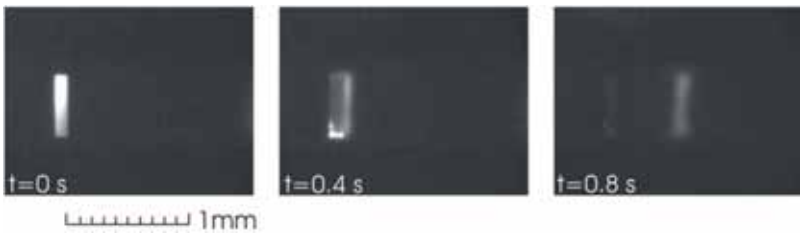
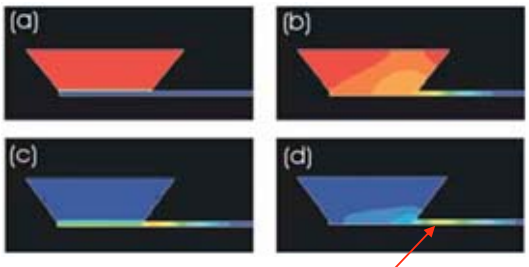
Desmet e.a. Anal.Chem. 72, 2160-2165 (2000)

University of Twente The Netherlands

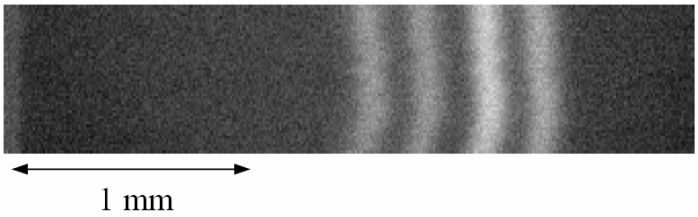
New features: micromachined injector







injected plug



CCD-camera image of separation of a mixture of 4 coumarin dyes in channel with d=280nm coated with monolayer of C8 (sample concentration 1 mM, separation time 1.44 s).

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de Malsche e.a. accepted for microTAS 2005, Boston, Oct.10-13

University of Twente The Netherlands

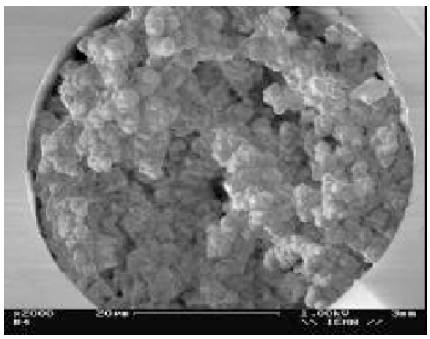
Case study 2
Micromachined separation columns

Peter Schoenmakers and co-workers,University of Amsterdam, NL

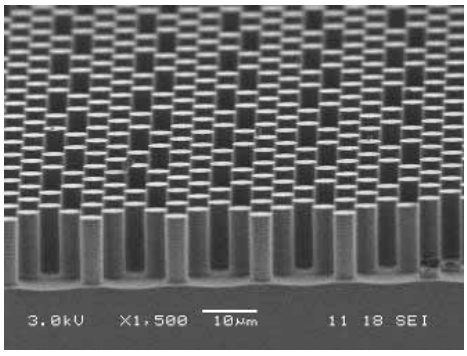
Gert Desmet and co-workers, Free University of Brussels, B

Wim de Malsche, Han Gardeniers, University of Twente, NL

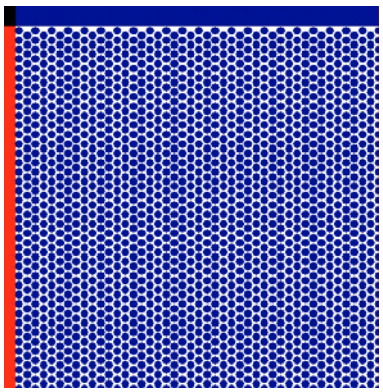
Chromatography on etched silicon columns



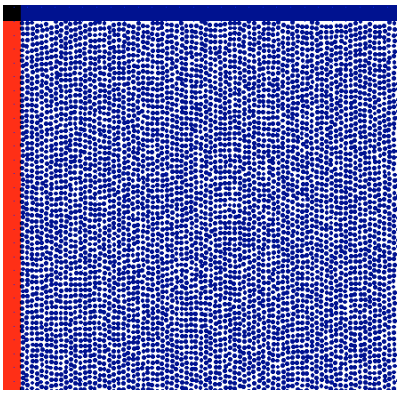
Packed chromatography column



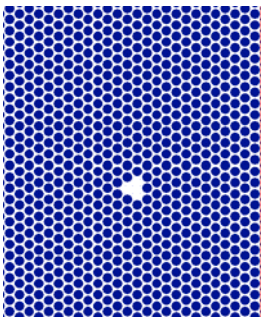
Etched chromatography column



Regular array of pillars

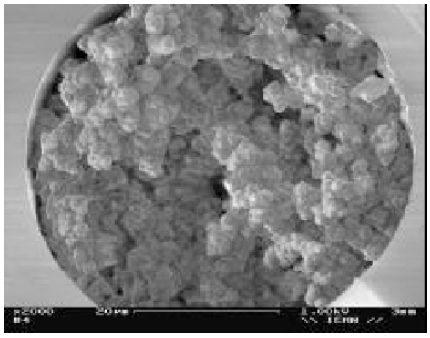


Random pillars

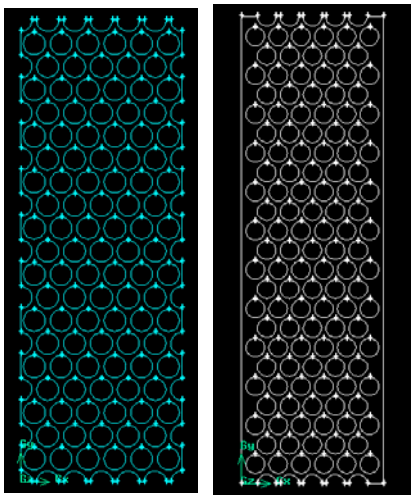


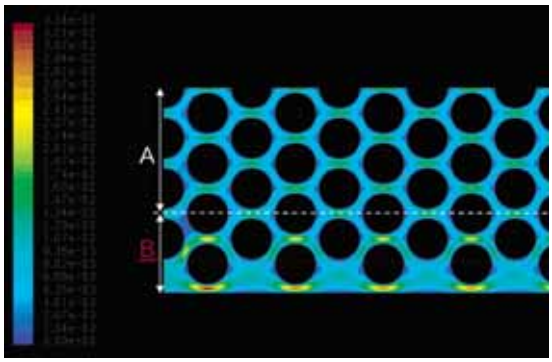
Regular array with defects

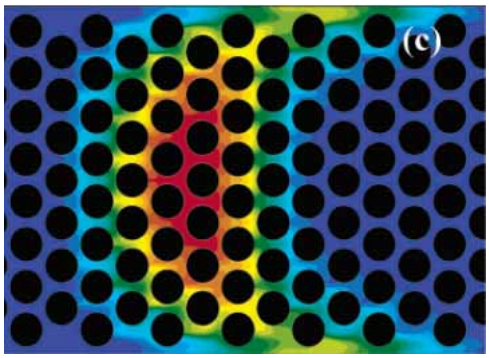
Wall effects



Packed chromatography column

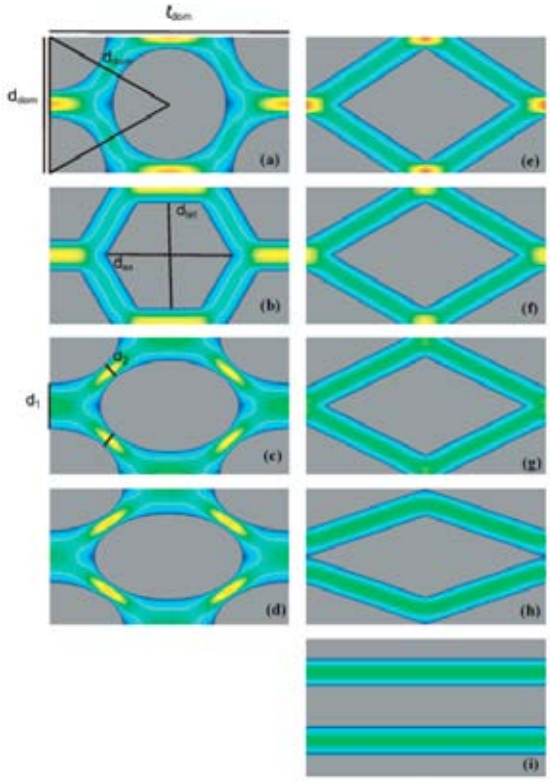






Left: velocity contour; right: plug shape near wall
Vervoort e.a. Anal.Chem. 76, 4501-4507 (2004)

Different pillar shapes



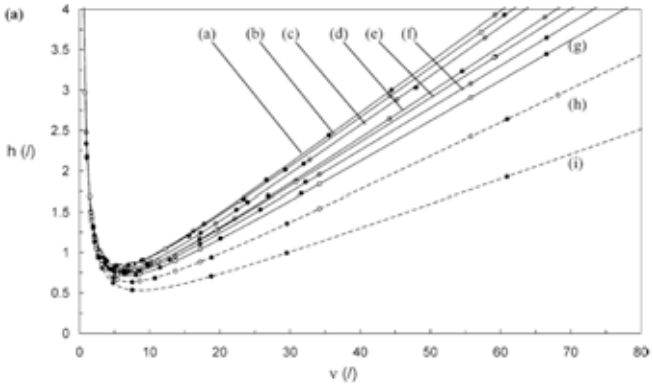
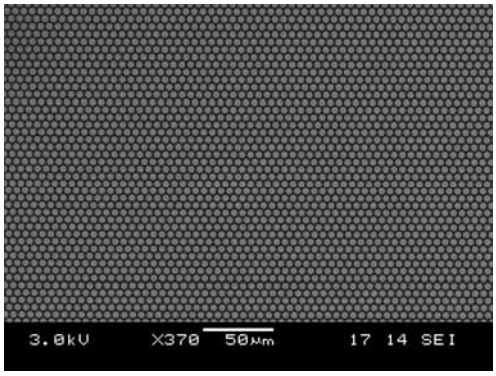
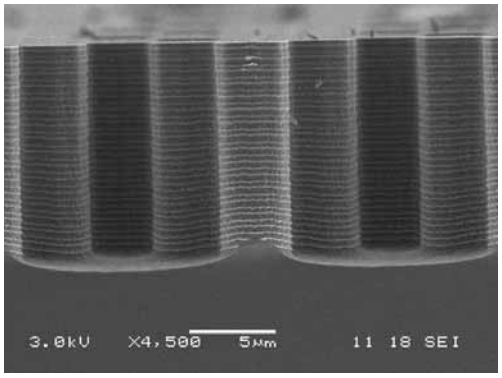


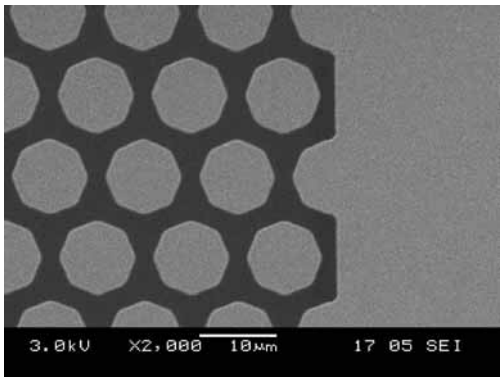
Plate heights (equivalent diameter: 1 µm)

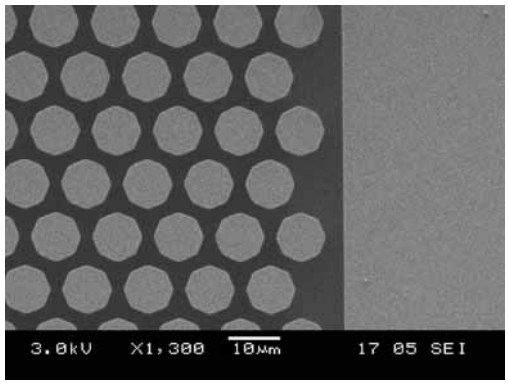
De Smet e.a. Anal.Chem. 76, 3716-3726 (2004)

Pictures of the column ("Bosch" etched)









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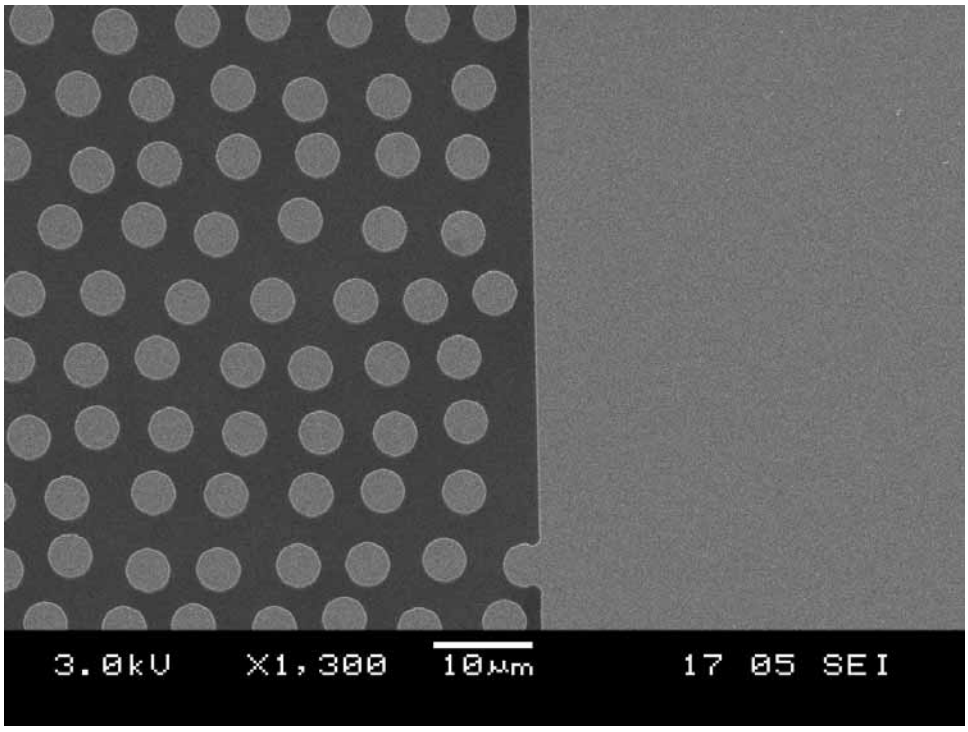
Etched microcolumns -random pillars



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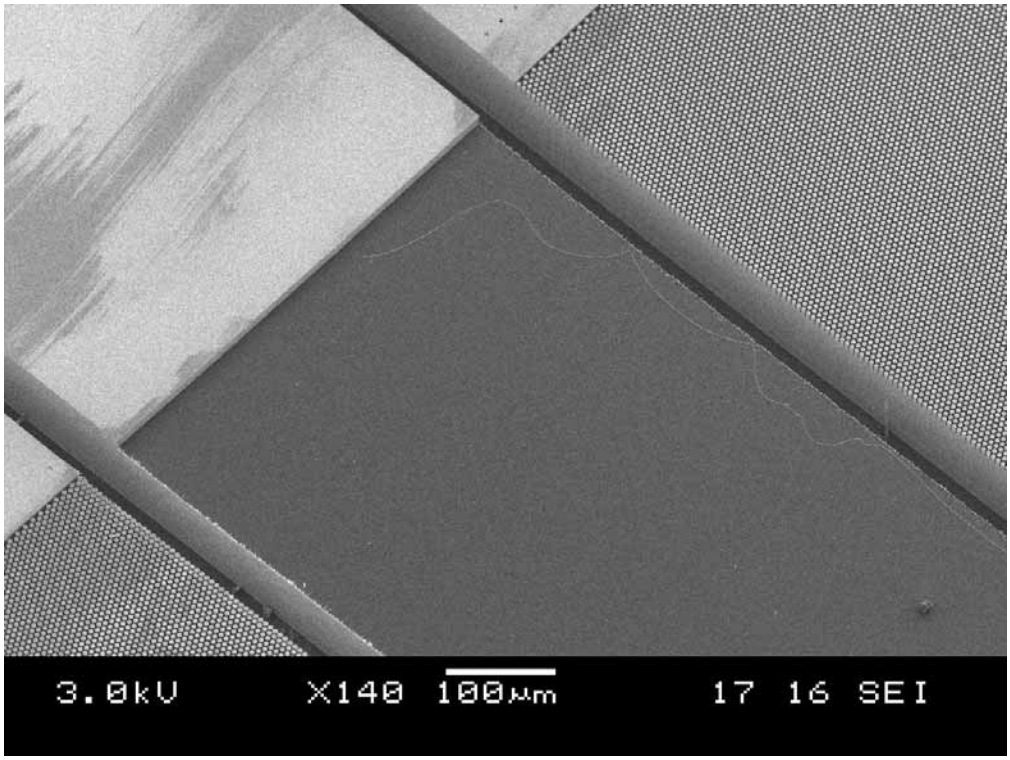
Etched microcolumn -injector



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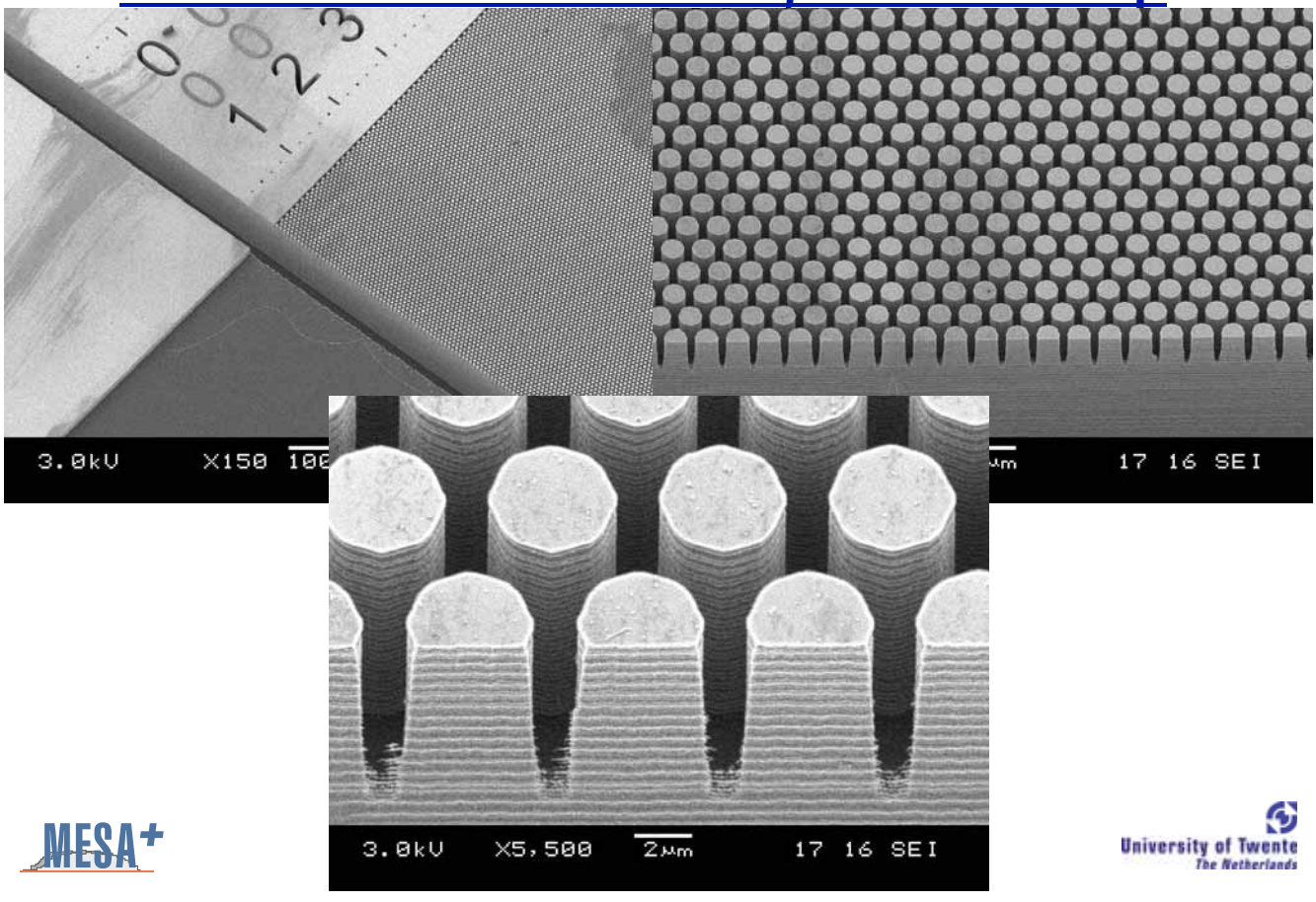
Etched microcolumns -injector close-up



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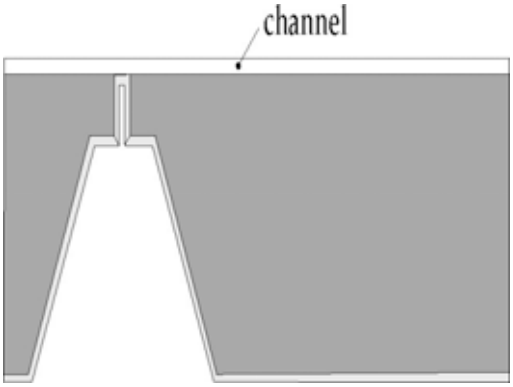
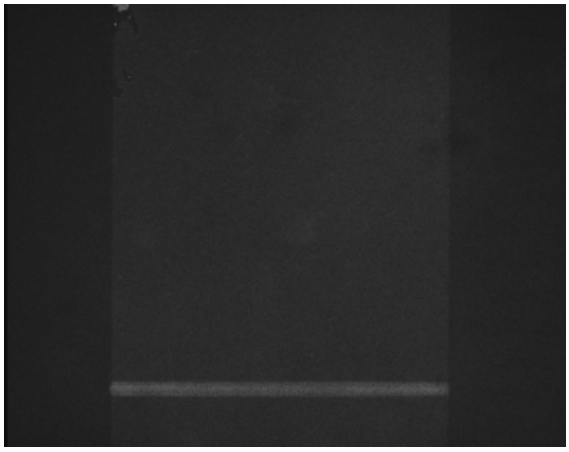
New injector design





first separation results (without new injector) : de Pra e.a. accepted for microTAS 2005, Boston, Oct.10-13

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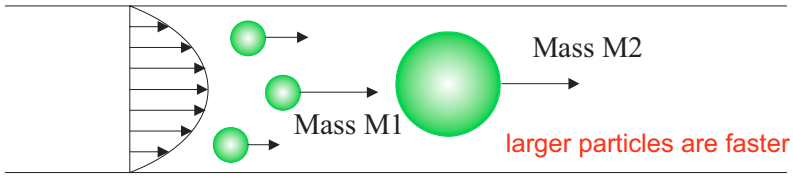
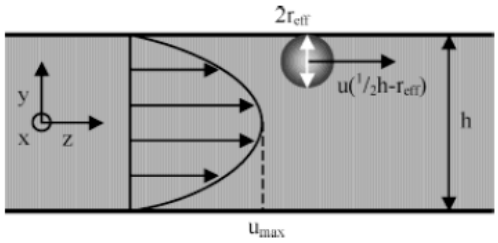
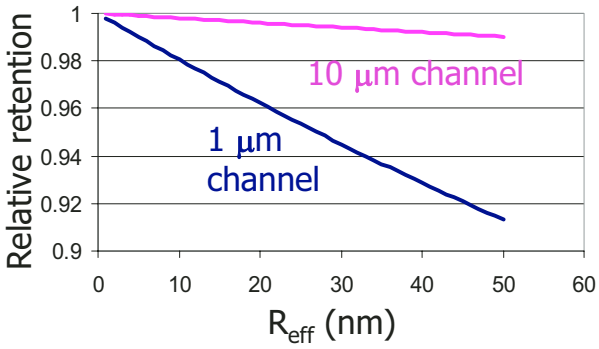
Case study 3:
Hydrodynamic chromatography chip

1998-2002, PhD work of
Emil Chmela (University of Amsterdam, NL) &
Marko Blom (University of Twente, NL)
Funding by Dutch Technology Foundation, STW

Principle of hydrodynamic chromatography







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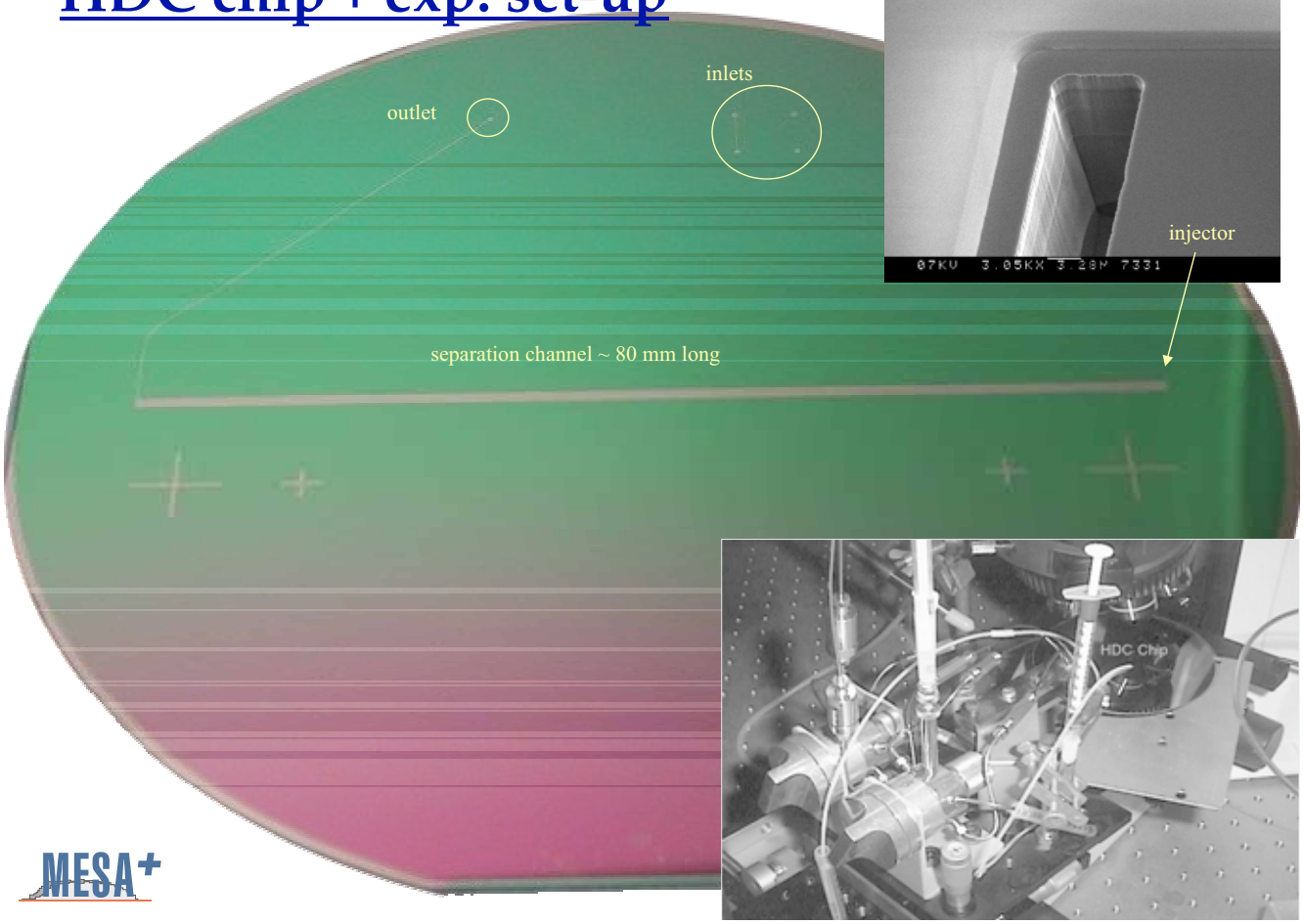
Chmela e.a. Anal.Chem. 74, 3470-3475 (2002)

University of Twente The Netherlands

HDC chip + exp. set-up



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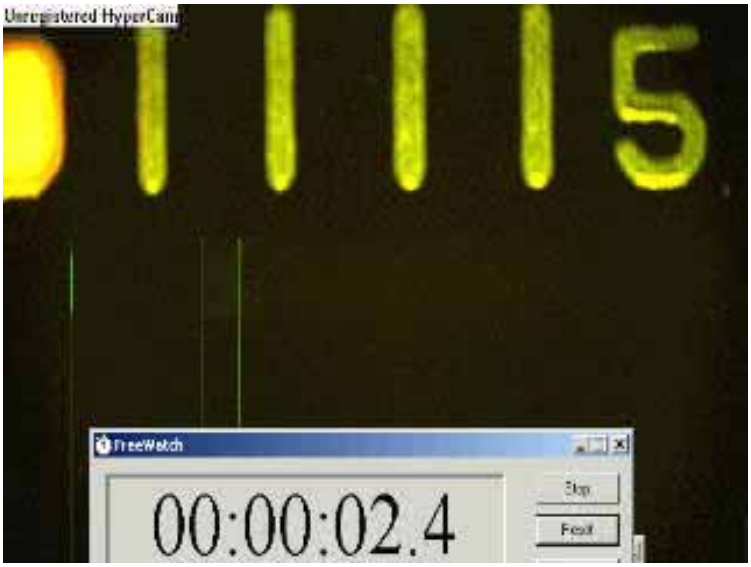
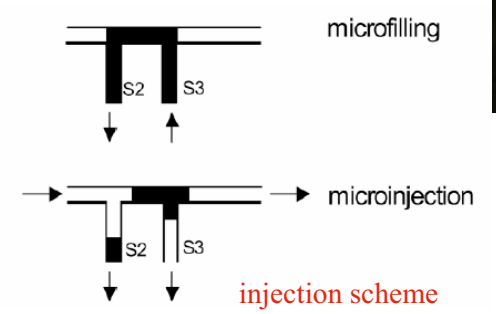
Separation results: Pyrex-silicon chip

Separation of
26, 44, 110, 180 nm
fluorescent polystyrene
nanoparticles and a marker





injection scheme

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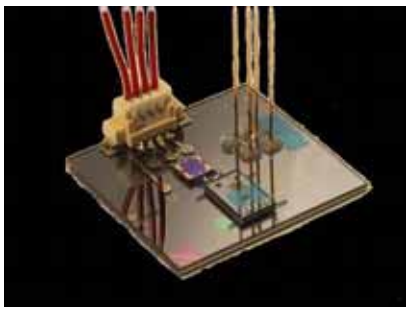
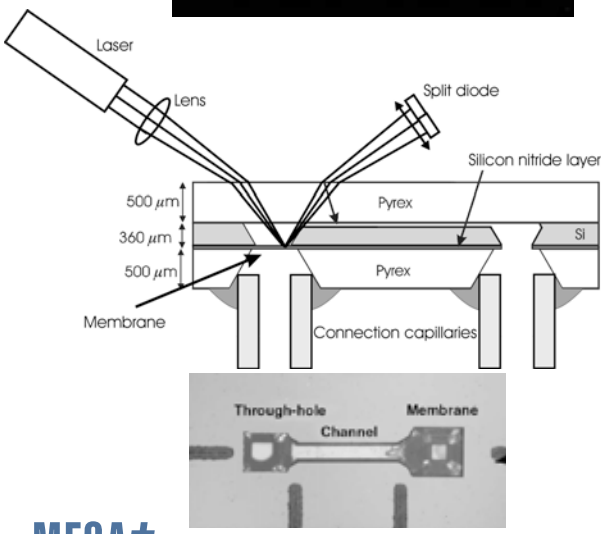
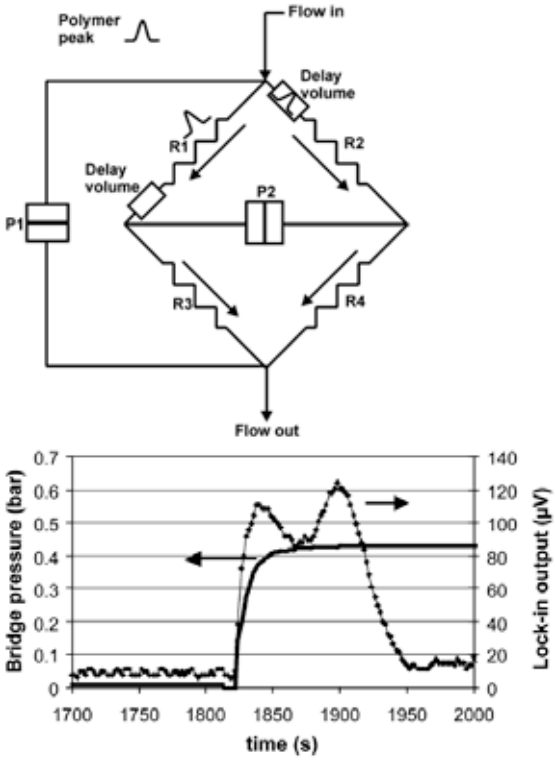
Planar HDC with integrated visco-detector







Injection of methanol in water

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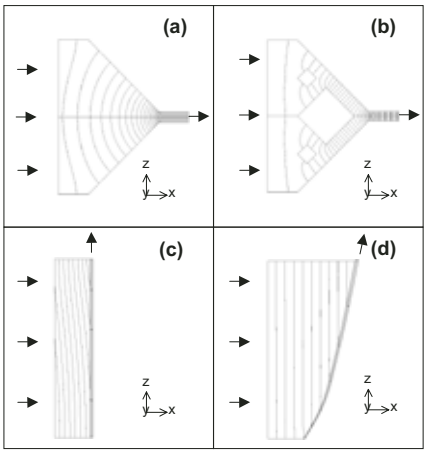
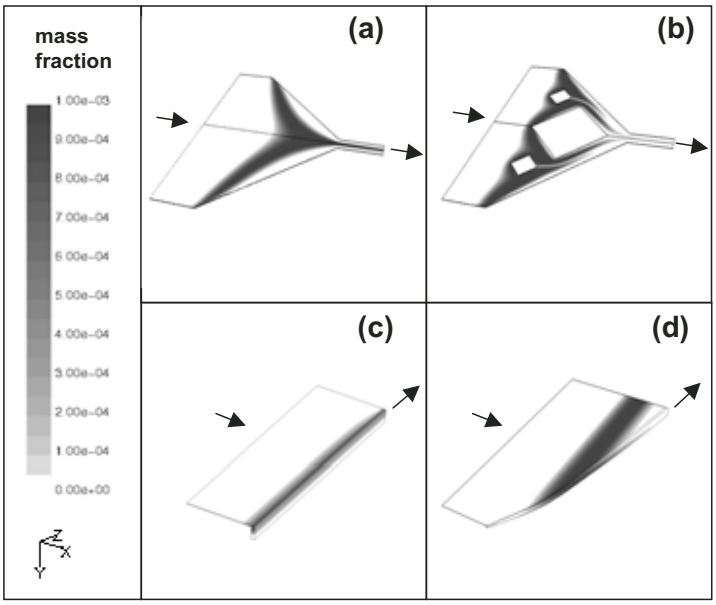
van der Heyden e.a. Sens.Act.B 92, 102-109 (2003); Blom e.a. JMEMS 14, 70-80 (2005)

Low dispersion outlet of chromatography chip (for UV detection)



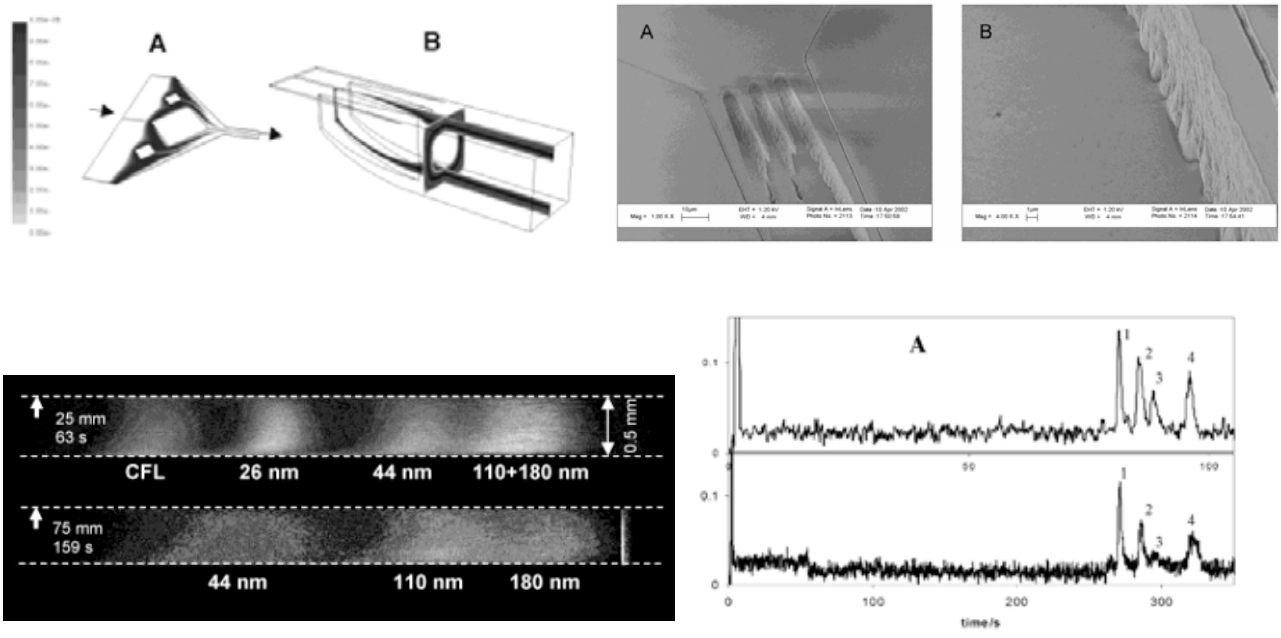
simulation of pressure distribution



simulation of the transport of a narrow zone

PhD Thesis Emil Chmela, University of Amsterdam, 2002

Hydrodynamic chromatography chip in fused silica



Separation of latex beads, UV absorption detection

Blom e.a. Anal.Chem.75, 6761-6768 (2003)

Recommended reading

- J. Calvin Giddings, "Unified separation science", John Wiley & Sons, Inc., New York, 1991

- J. Kutter and Y. Fintschenko, eds. "Separation Methods in Microanalytical Systems", CRC Press, Boca Raton, FL, USA, ISBN 0824725301; to appear September 2005.