Nanostructured Liquid Chromatography Systems

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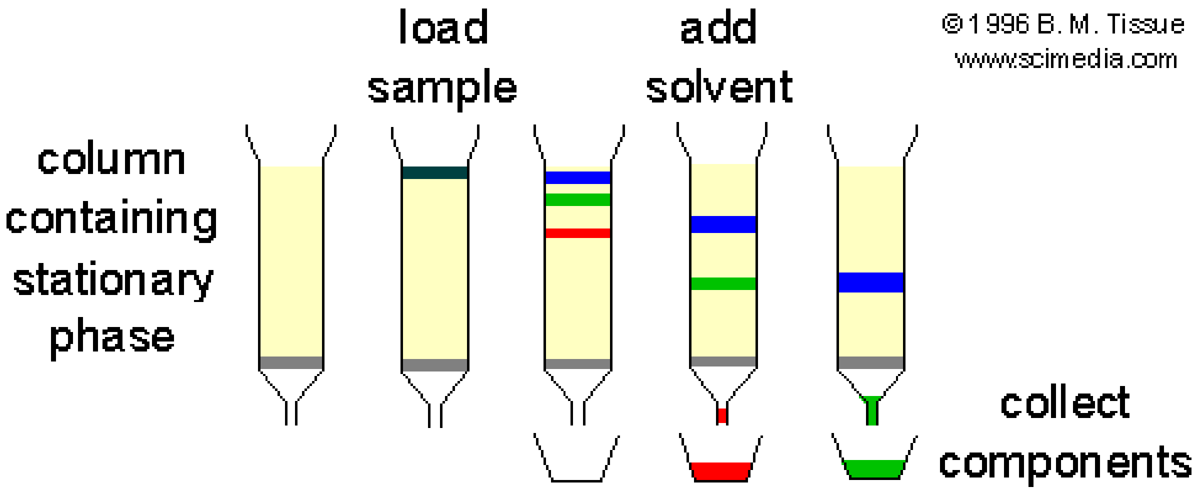
The concept of chromatography

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 position difference, which enables sorting

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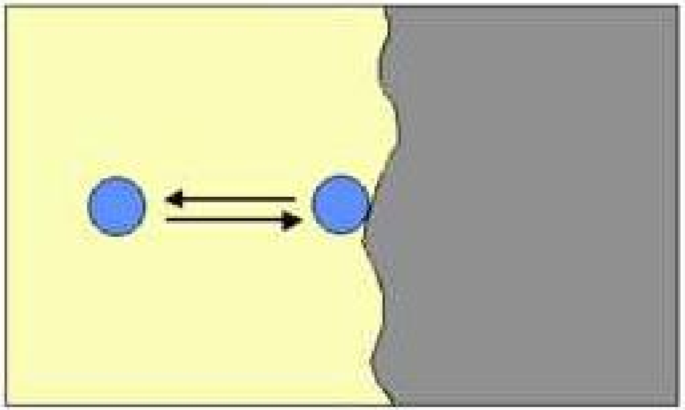
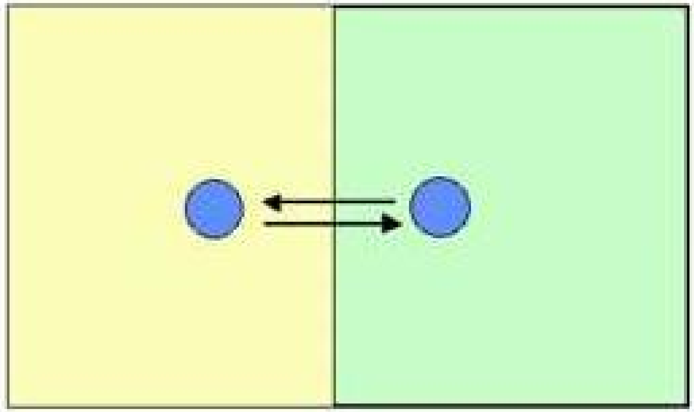
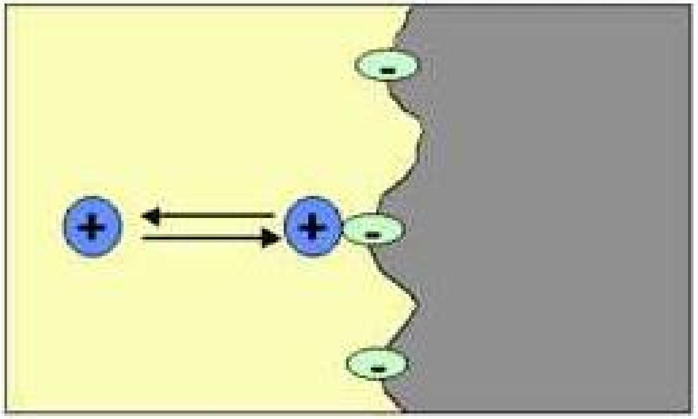
Major categories of LC



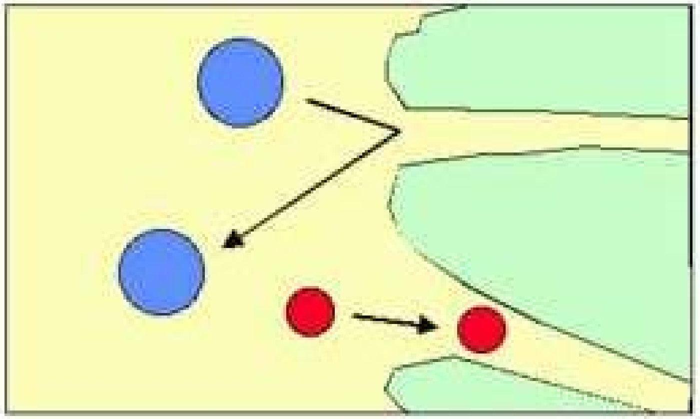
adsorption



partition (solvent polarity)



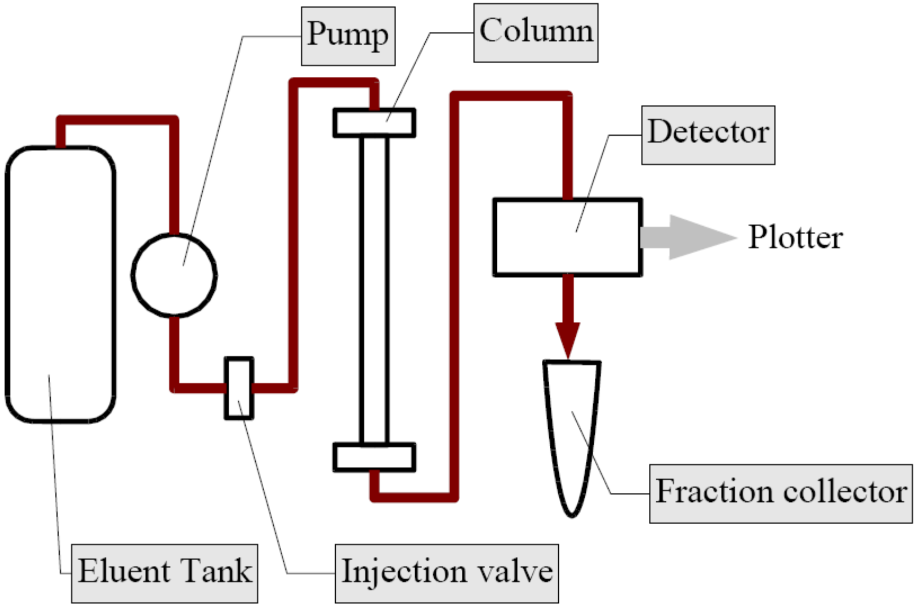
ion exchange

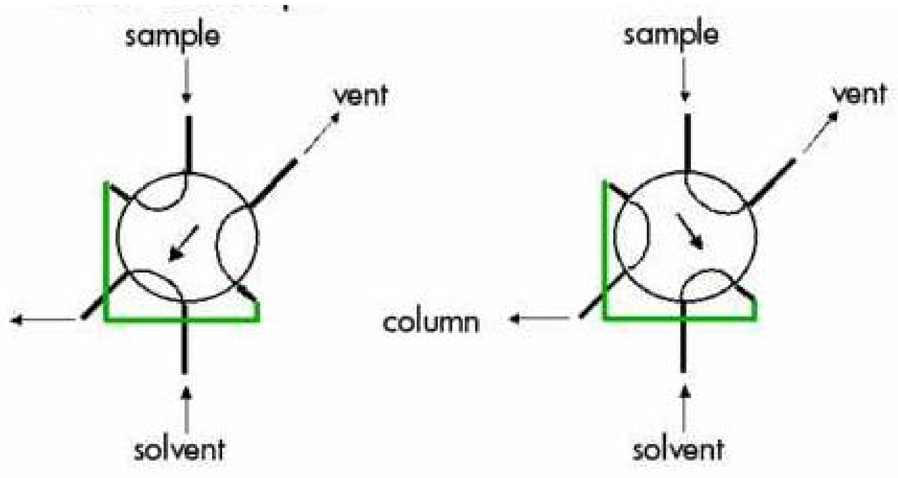


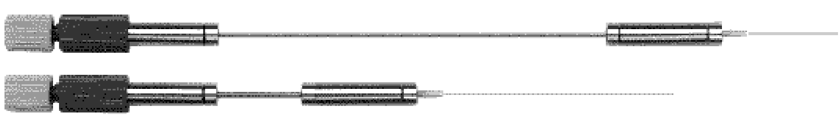
size exclusion (gel permeation)

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

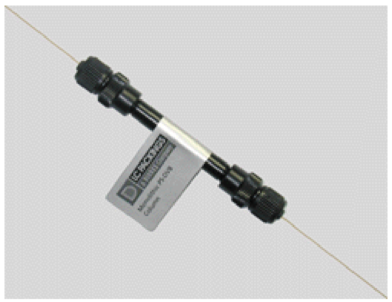
Basic LC setup







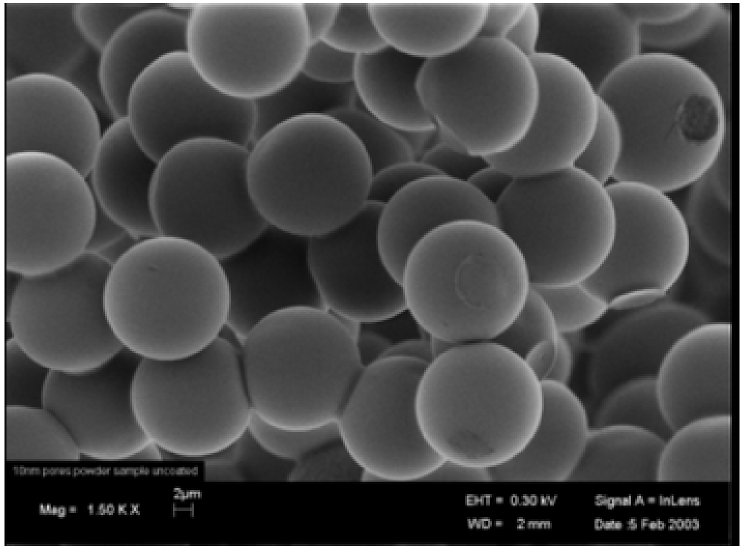
300 μm i.d. column (the standard)

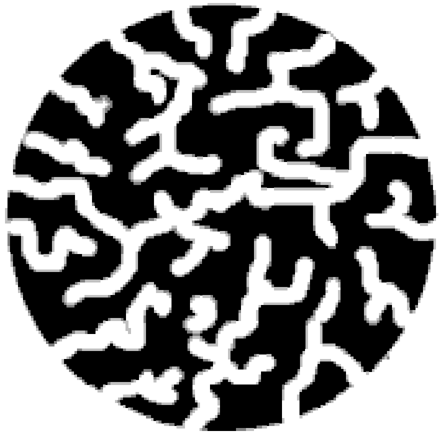


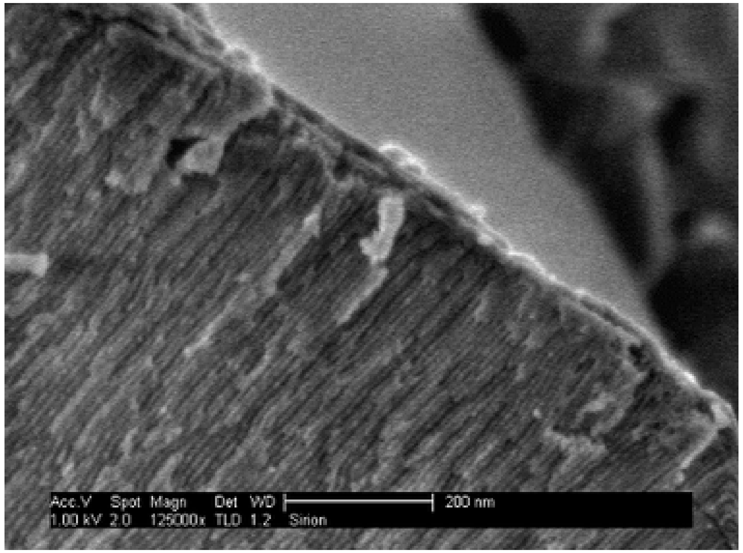
100 μm i.d. monolith column

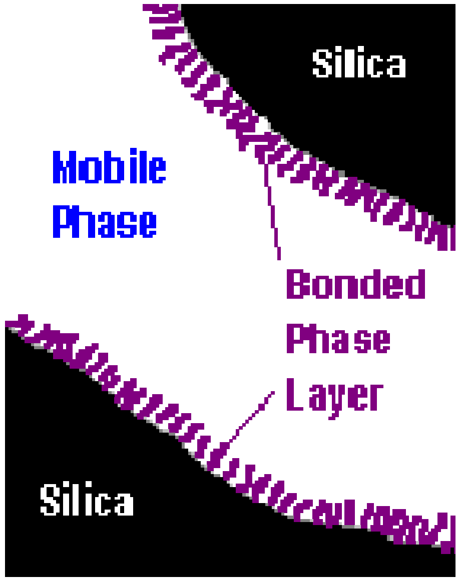
setup drawing from: http://www.tut.fi/units/ymp/kem/opintojaksot/3500820/L2.pdf

Column materials: Particles

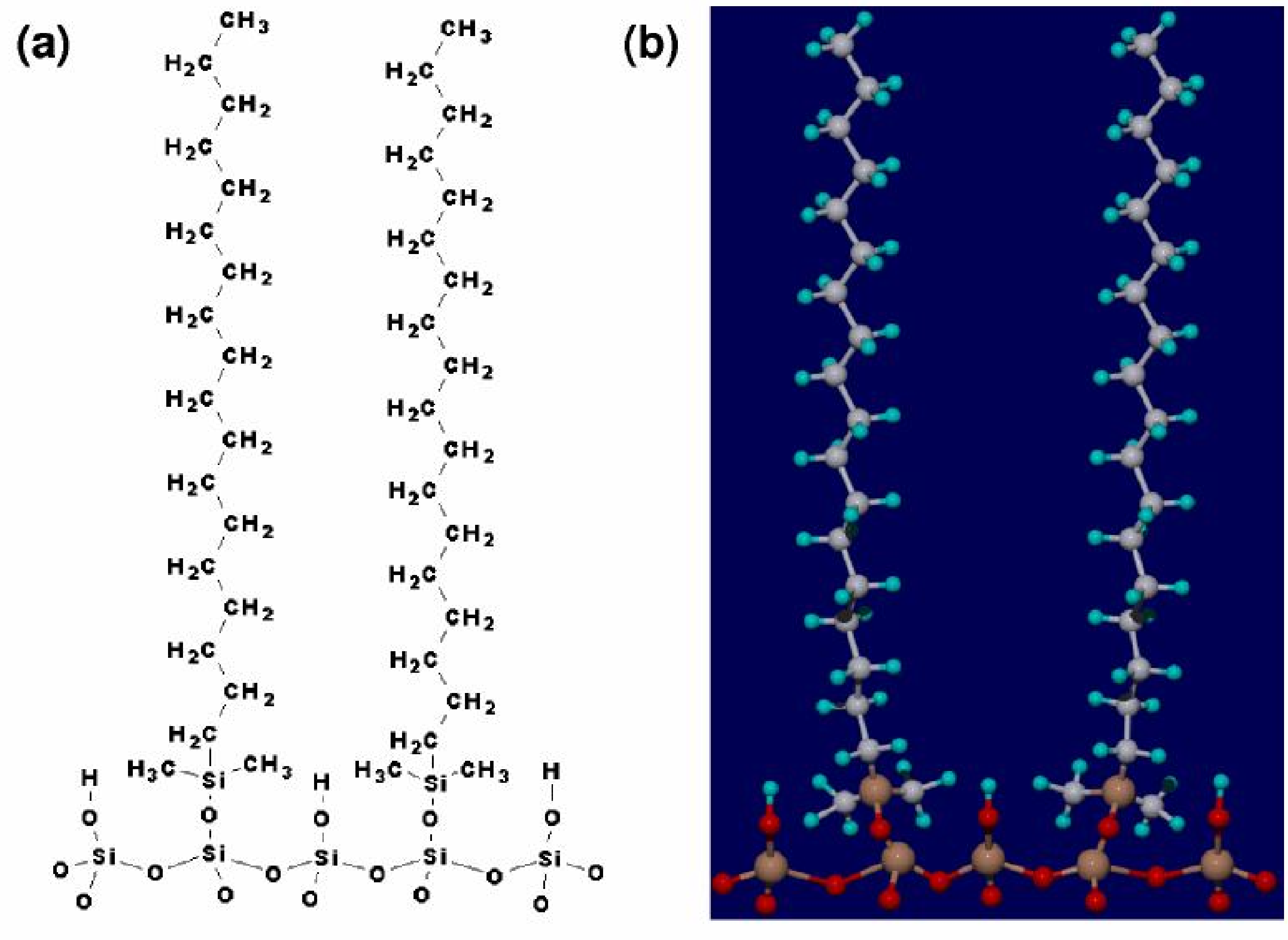






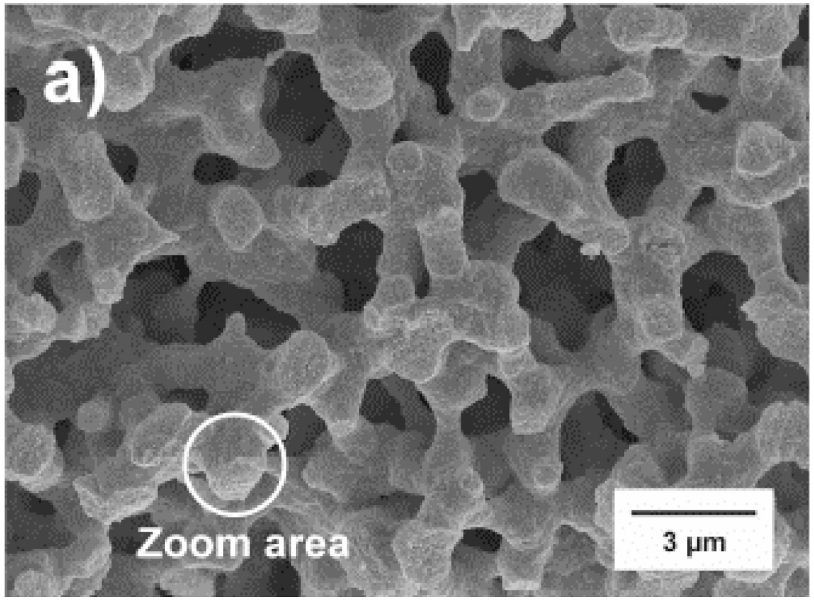


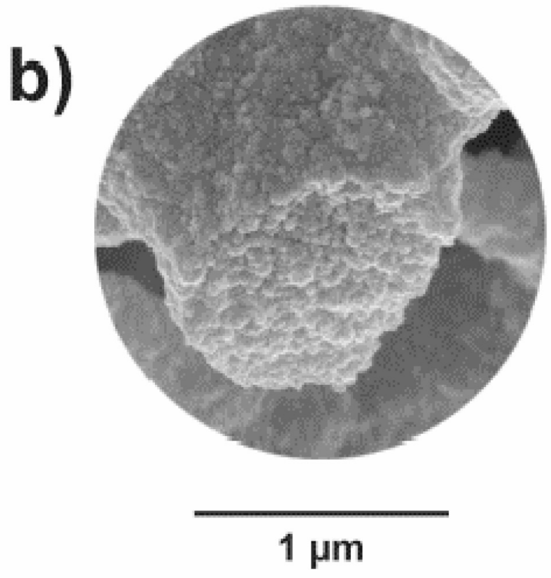
SEM pictures from S.B. Rathod et. al., Mat. Res. Soc. Symp. Proc. 775, 2003; p. P1.11.1



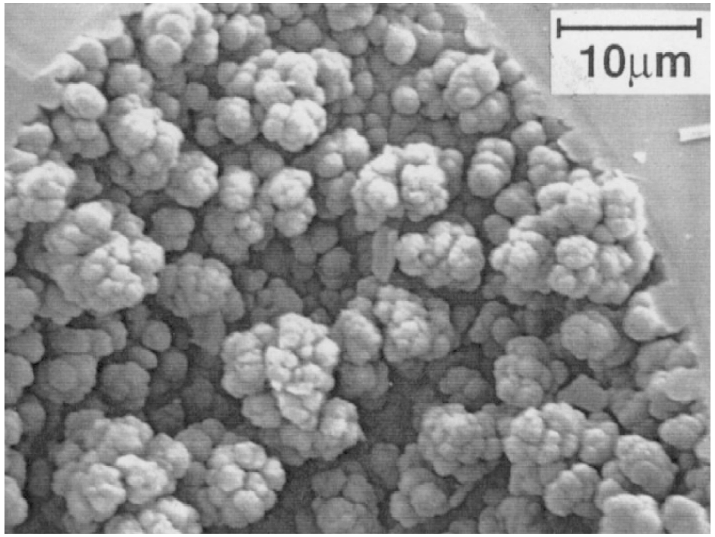
Chemical structure and 3D illustration of C_{18} functional groups on silica

Column materials: Monoliths





SEM cross-section of a silica monolith showing: a) the macropores (1-3 µm), and: b) the mesopores (10-20 nm).
From U. Tallarek e.a. Chem. Eng. Technol. 25, 2002, p.1177



Polystyrene-based monolith.
From I. Gusev e.a. J. Chrom. A 855, 1999, p.273

Trends in liquid chromatography

- narrower columns
- smaller particles (and -thus- higher pressures)
- monoliths replace particles
- microfluidic systems

Because of:

- higher separation "quality"
- higher separation speed
- lower sample and solvent consumption

Trends in particle size

Figure 3: History of HPLC particle development.

Years of acceptance	Particle size	Most popular nominal size	Plates / 15 cm (approximate)
1950s	Irregular-Shaped	100 µm	200
1967	Glass bead	50 µm (pellicular)	1000
1972		10 µm	6000
1985		5 µm	12000
1992		3–3.5 µm	22000
1996*		1.5 µm (pellicular)	30000
1999		5.0 µm (poroshell)	8000**
2000		2.5 µm	25000
2003		1.8 µm	32500

*Non-porous silica or resins ** For protein MW 5700

From: R.E. Majors, "Fast and Ultrafast HPLC on sub-2 µm Porous Particles — Where Do We Go From Here?" LC GC Europe, June 2006, p. 352

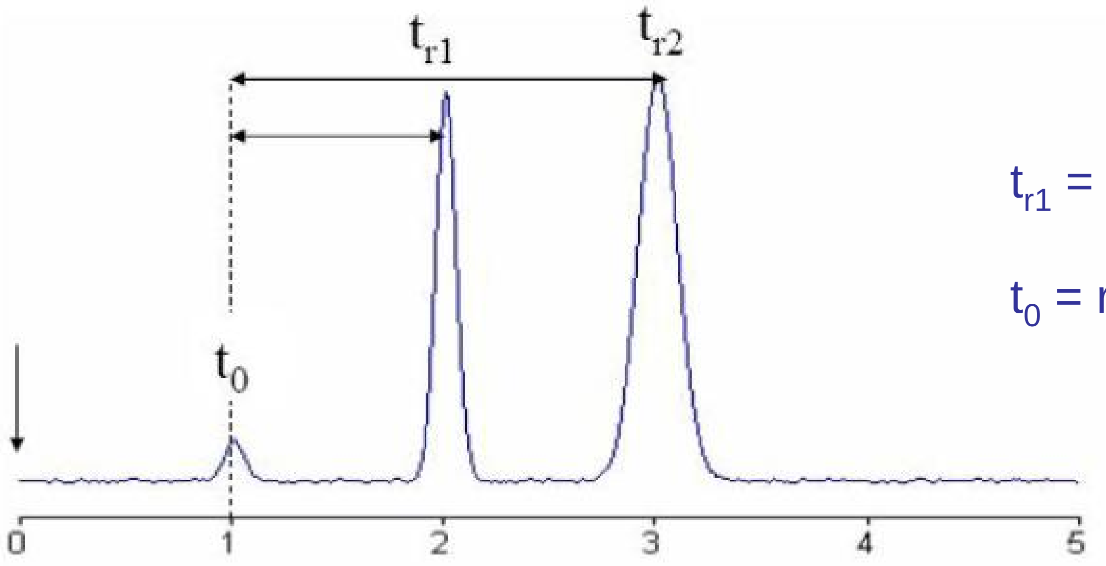
Table 1: Commercial Sub-2 µm HPLC Columns. status: April 2005

Manufacturer	Family name(s)	Average particle size µm
Agilent	Zorbax RR	1.8
Alltech	Altima, Platinum and ProSphere	1.5
Bischoff	ProntoPEARL sub–2 TPP Ace	1.8
Thermo	Hypersil GOLD 1.9µm	1.9
Waters	ACQUITY	1.7

Retention and partitioning

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

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



Detector signal in time

t_{r1} = retention time analyte 1

t_0 = retention solvent (unretained)

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

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

Furthermore: $t_r = (1+k)t_0$

Chromatography parameters

Column consists of a number of ***plates***, in analogy to distillation column. On each plate, equilibration of partioning of the solute between mobile and stationary phases occurs.

height equal to a theoretical plate H (or HETP):

$$H = \frac{s_x^2}{L} = L\left(\frac{s_t}{t_r}\right)^2$$

plate number:

$$N = \frac{L}{H}$$

Higher ***N*** or smaller ***H*** means a more efficient column

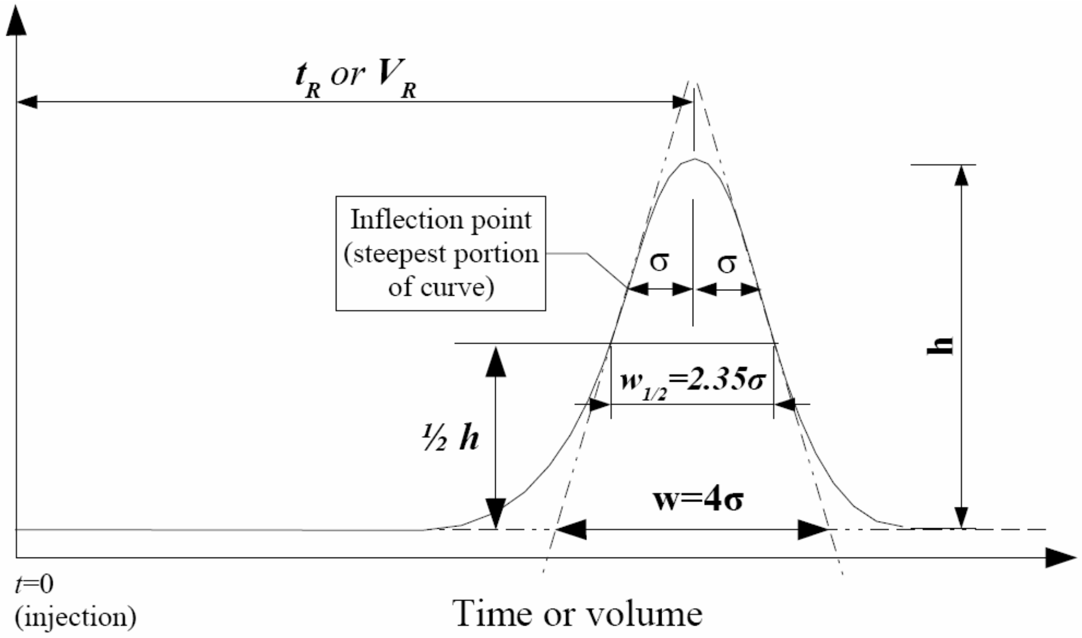


Plate height: Van Deemter equation

$$H/d_p = BD_m/(ud_p) + A_1/\{1 + 1/D(ud_p/D_m)^{1/2}\}$$
$$+ A_2\{k'/(1 + k')\}^2\{ud_p/D_m\}^{1/2}$$
$$+ Ck'/(1 + k')^2\{ud_p/D_s\}$$

d_p = particle diameter
k' = phase capacity ratio
D_m D_s = diffusion coefficient in mobile or stationary phase
u = linear flow velocity
A_1 A_2 B C D = constants

J. Knox, J. Chrom A 960, 2002, 7

$$H = \frac{BD_m}{u} = \frac{BD_m}{L/t} = B\frac{2D_m t}{2L} = \frac{B}{2}\frac{s_x^2}{L}$$

so this term = axial diffusion
(and B = 2)

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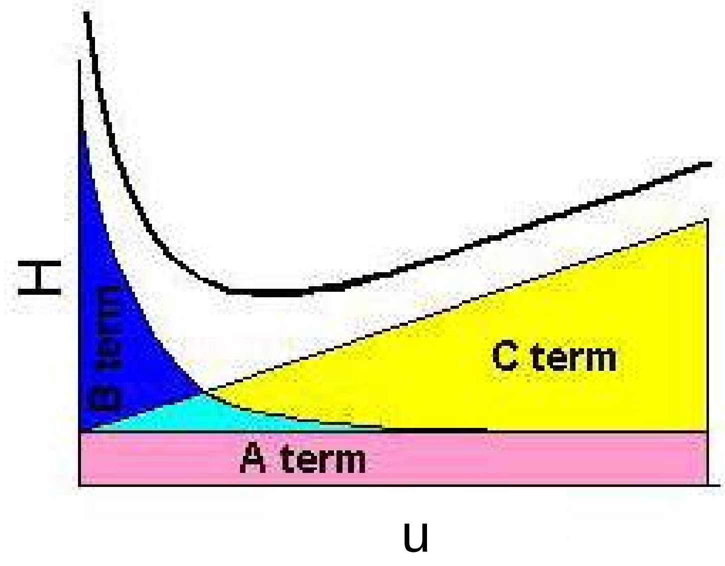
Parameters in Van Deemter equation

Basic form: $H \approx \frac{B}{u} + Au^{1/n} + Cu$ (n=1,2,3)

A: multiple passes through the column packing

B: molecular diffusion

C: equilibration between phases



A

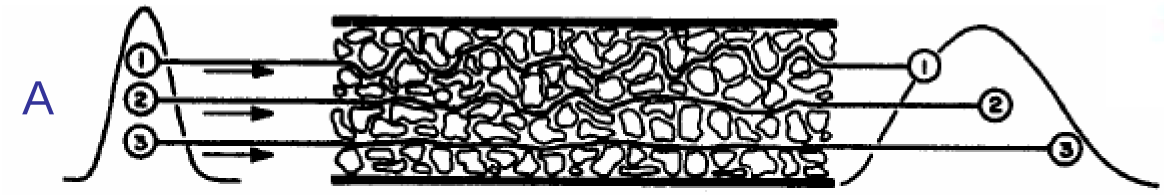
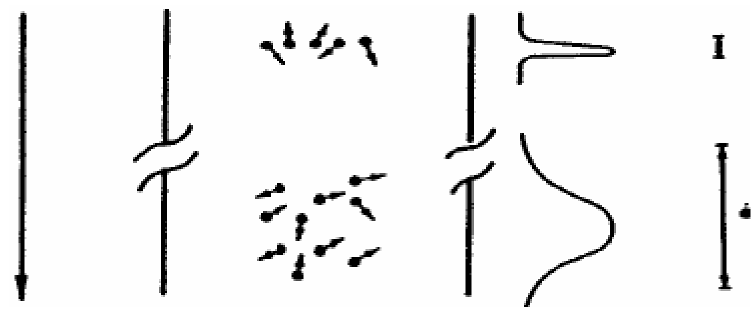
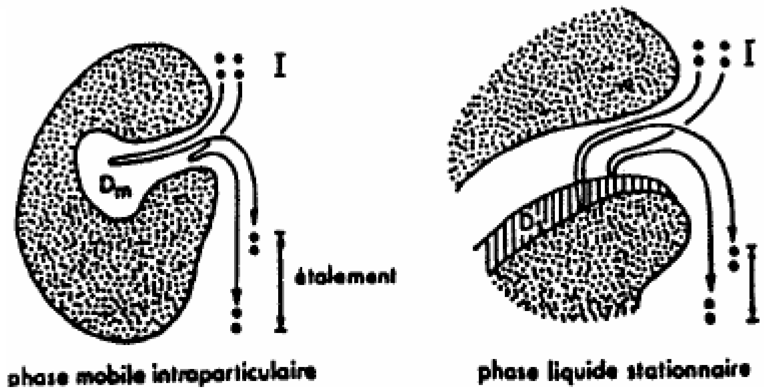


Illustration of eddy diffusion.

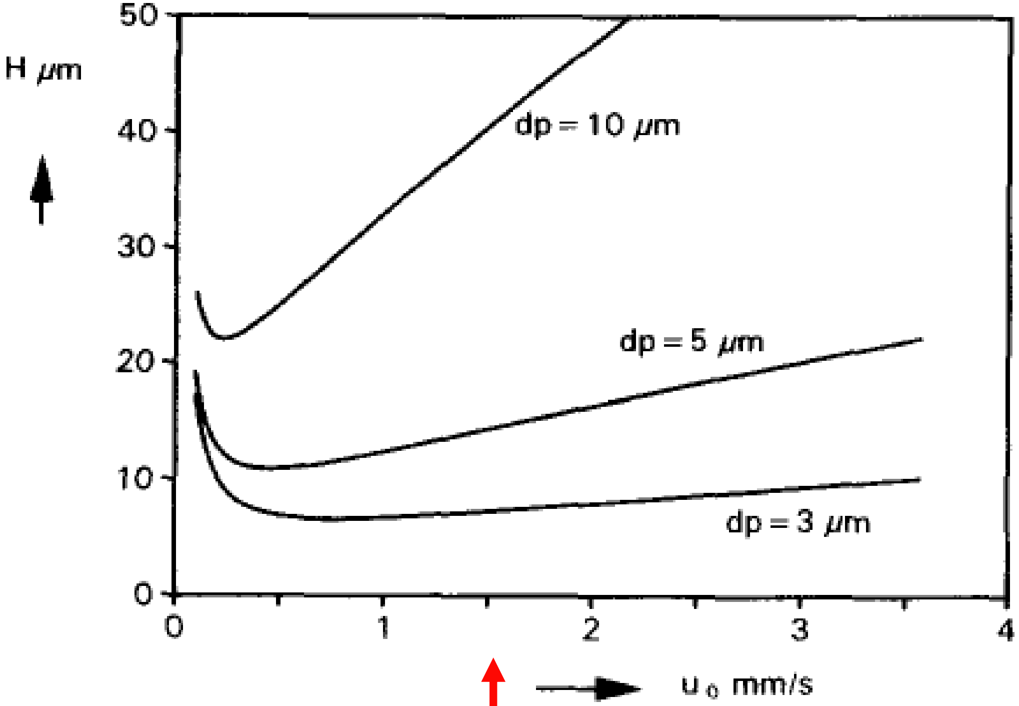
B



C



Van Deemter for different particle diameter



With reduced plate height:

$$h = H / d_p$$

and reduced velocity (= Peclet number):

$$\nu = u d_p / D_m$$

we obtain the reduced equation:

$$h = B/\nu + 1/\{1/A_1 + 1/D\nu^{1/2}\}$$
$$+ A_2\{k'/(1+k')\}^2 \nu^{1/2}$$
$$+ C\{k'/(1+k')^2\}\{D_m/D_s\}\nu$$

And all these curves fall on one and the same curve !

J. Knox, J. Chrom A 960, 2002, 7 H. Poppe, J. Chrom A 778, 1997, 3

Miniaturization in chromatography

Standard LC formats are already micro/nano-nized:

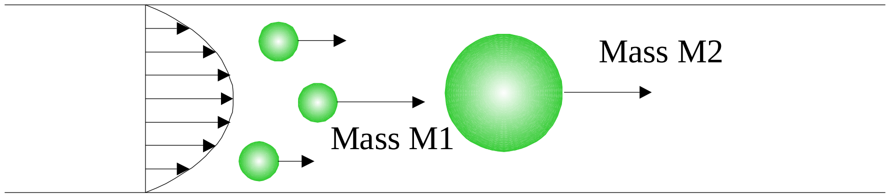
- 75 µm capillaries
- packed columns with (sub)micron flow passages
- mesoporous materials (particles and monoliths)
- surface processes
- low-volume detectors, low sample volumes and low flow rates

"nano-LC"

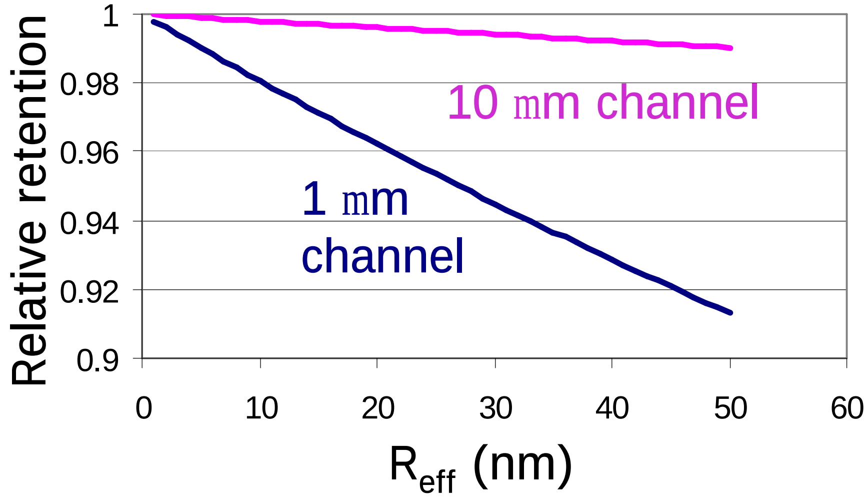
Micro/nanofabrication benefits

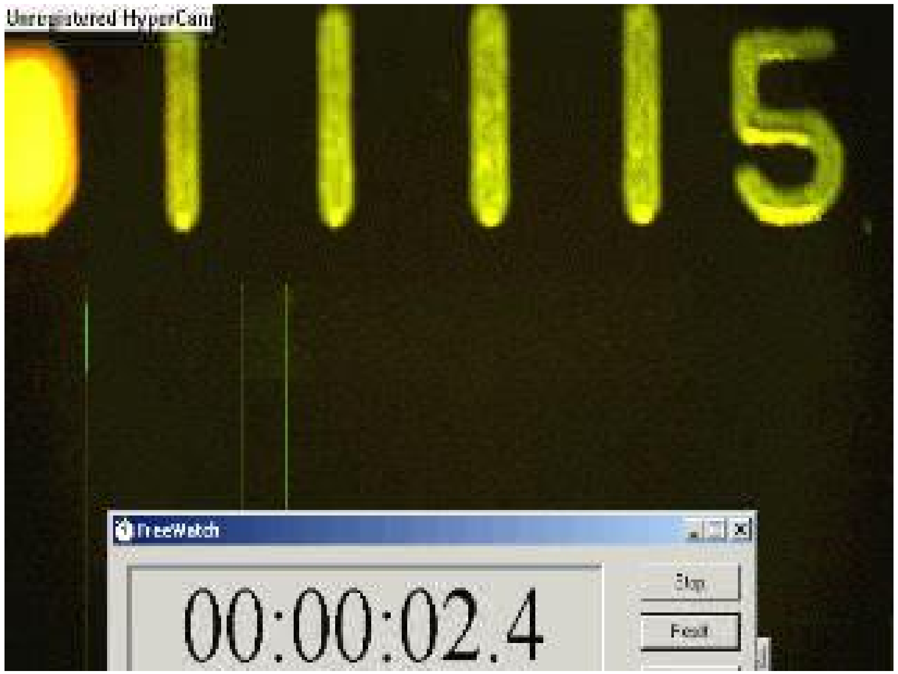
- integration reduces dead volumes (injector and detector, connections)
- parallel separations (e.g. 96-channel electrophoresis chips)
- new types of (size) separation (see Bob Austin's lectures)
- high-order columns!

Hydrodynamic "chromatography"



side view (smallest dimension)





Top view (perpendicular to smallest dimension).

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

Channel height 1 μm, channel width 500 μm

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E. Chmela e.a. Anal. Chem. 74, 2002, p. 3470

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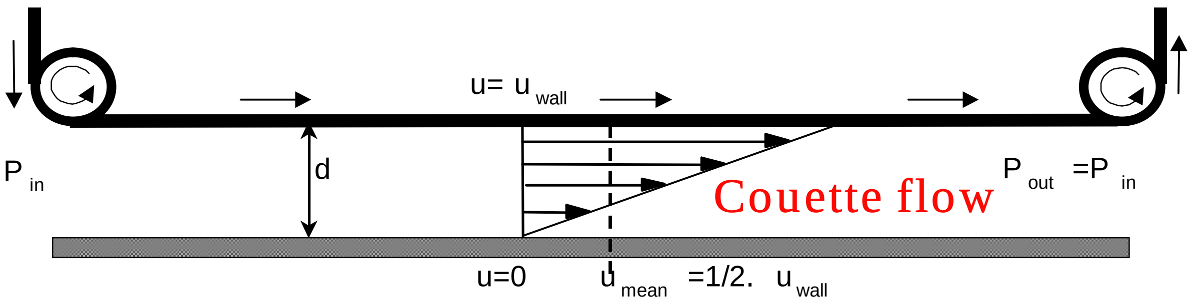
Shear-driven chromatography: basics

Limitations in pressure-driven chromatography: pressure drop: $\Delta P = f\,\frac{hLu_m}{d^2}$

with η viscosity, ***L*** length, ***d*** column or particle diameter, u_m average linear velocity of mobile phase, ***f*** flow resistance parameter (32 for open and 500-1000 for packed columns)



Darcy's equation:

$$u = \frac{\Delta P d_p^2}{\phi \eta L}$$

1/ϕ=specific permeability

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 (Note: no A-term)

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Desmet e.a. Anal.Chem. 72, 2000, p.2160

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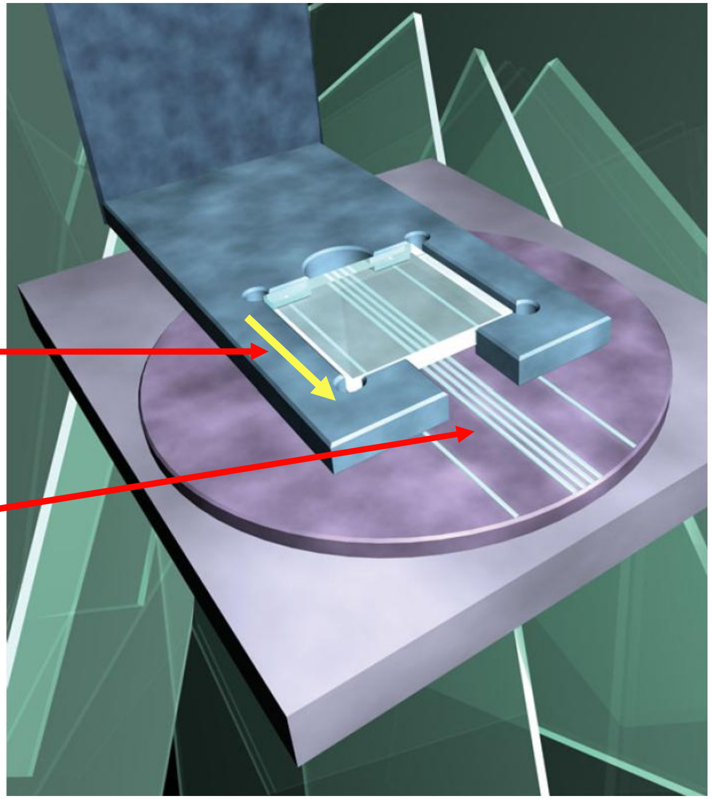
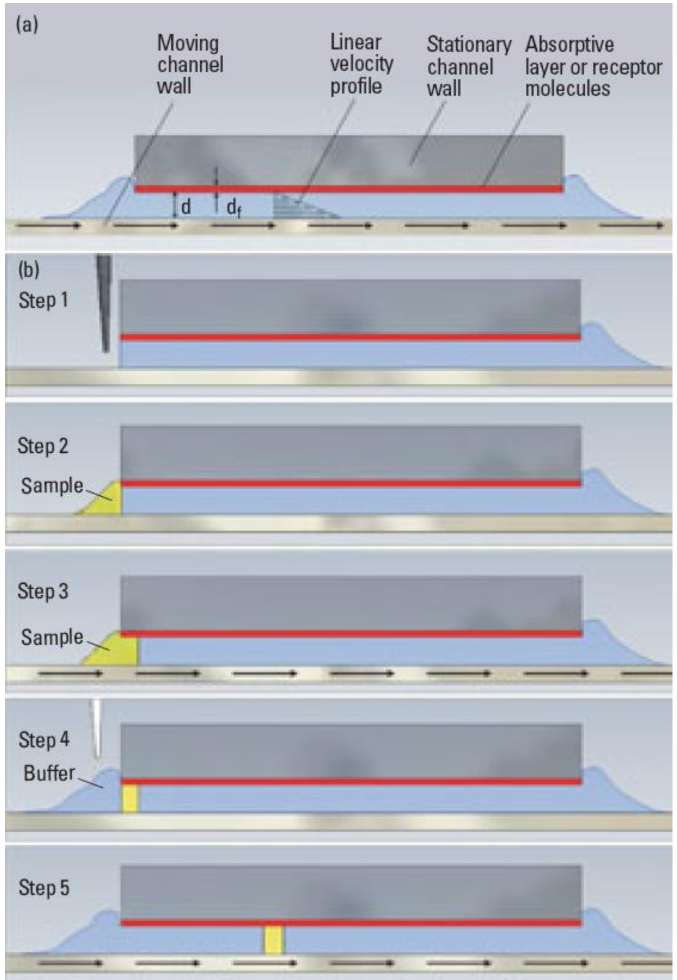
Shear-driven chromatography experimental

sliding direction

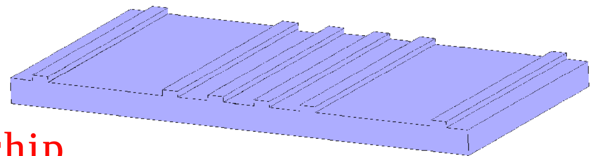
nanochannels 50 -300 nm



high-speed separation of 4 coumarin dyes



Old injection procedure



SDC chip

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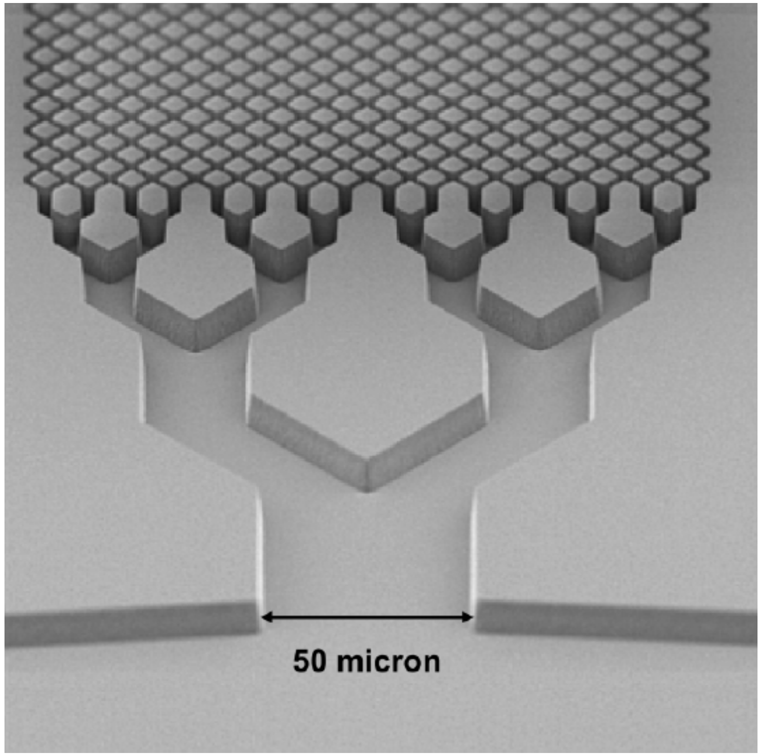
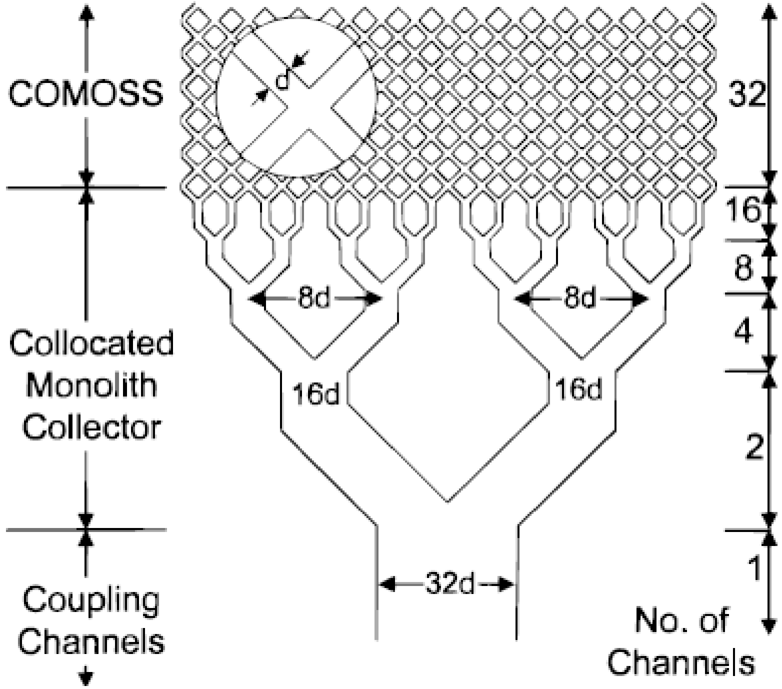
Clicq e.a. Anal.Chem. Dec.1, 2004, 430A-438A


Microfabricated high-order columns for LC
3.0kV
X370
50µm
17 14 SEI

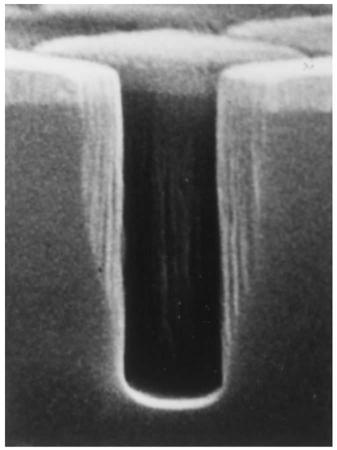
Original idea by Regnier e.a.





Used for electro chromatography,
disappointing performance

pillars: 5 x 5 x 9 μm^3



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He e.a. Anal.Chem. 70, 1998, 3790
He e.a. J. Pharm. Biomed. Anal. 17, 1998, 925

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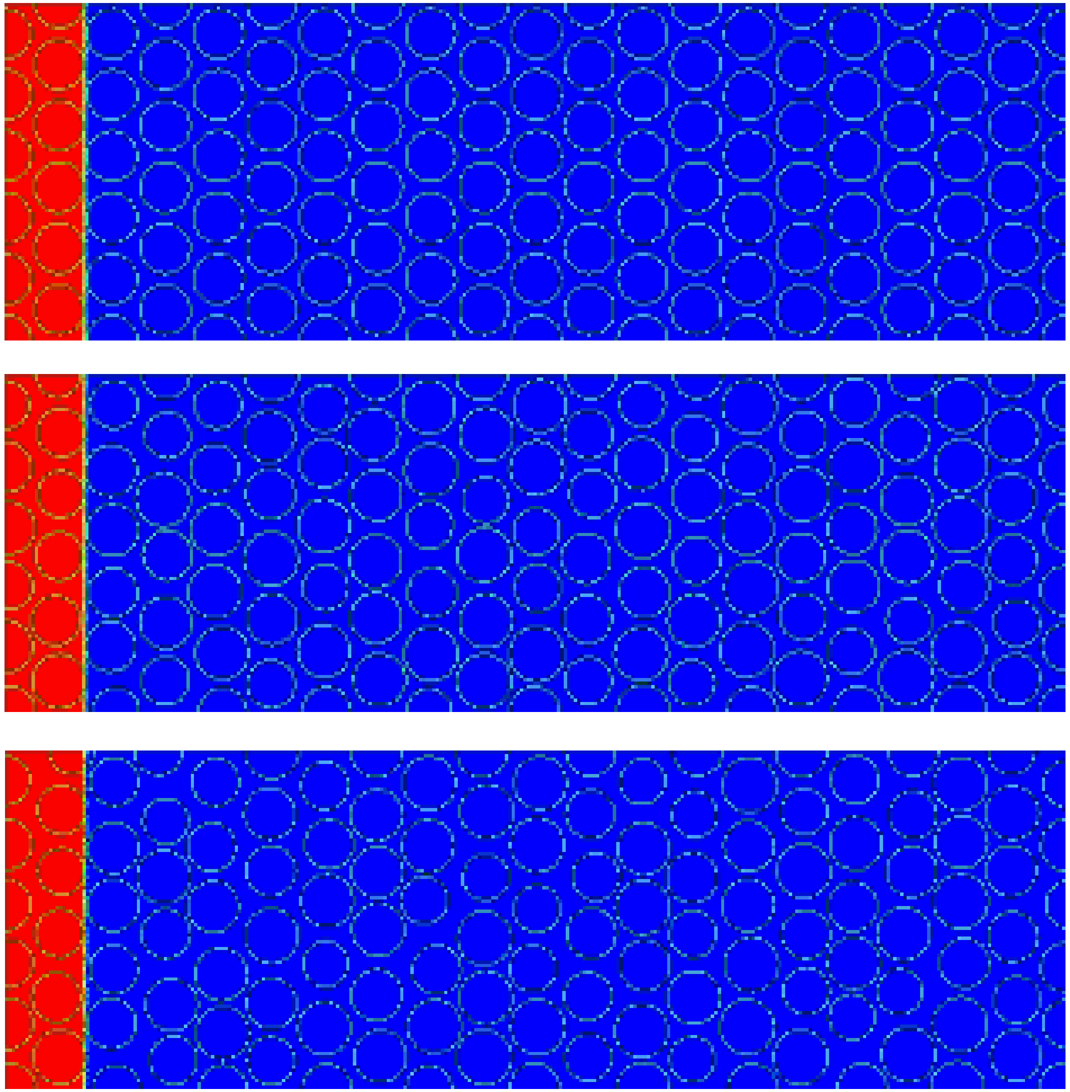
Why order?

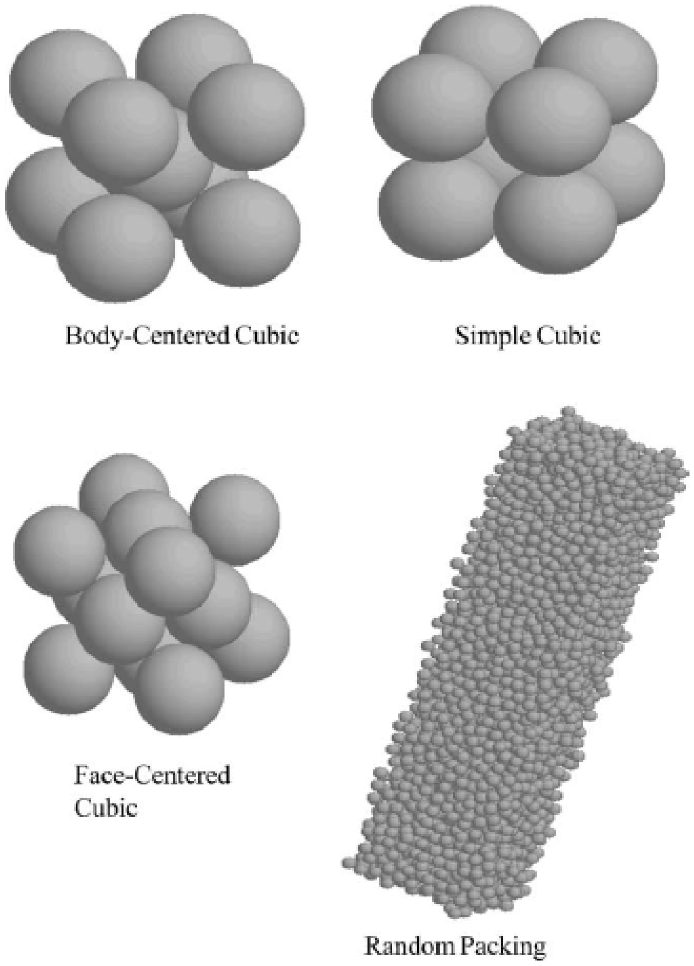


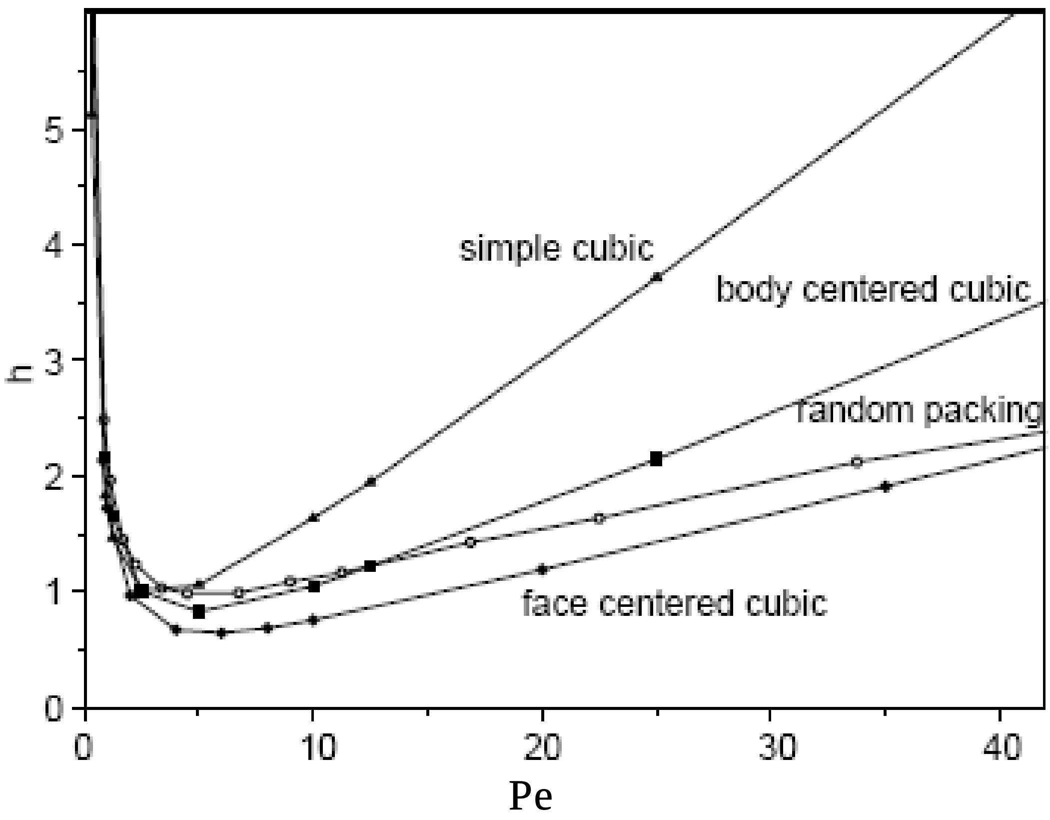
Comparison of band broadening in a two-dimensional mimic of a chromatographic column with increasing degree of heterogeneity;

Note: this is part of the A-term in the Van Deemter equation

source: J.Billen, VU Brussels, B

Ordered packed columns





The pressure drop, dp/dz for 5 μm spheres, the permeability, and the flow resistance for the three ordered packs and the random pack

Pack	Pressure drop dp/dz (kg/m^2/s^2)	Permeability k (m^2)	Flow resistance ϕ (nondimensional)
sc	7.76×10^7	6.14×10^{-14}	194
bcc	2.58×10^8	1.24×10^{-14}	645
fcc	6.10×10^8	4.25×10^{-15}	1520
Random	2.23×10^7	1.61×10^{-14}	559

Lattice-Boltzmann simulations
M.R. Schure e.a. J.Chrom.A. 1031, 2004, 79

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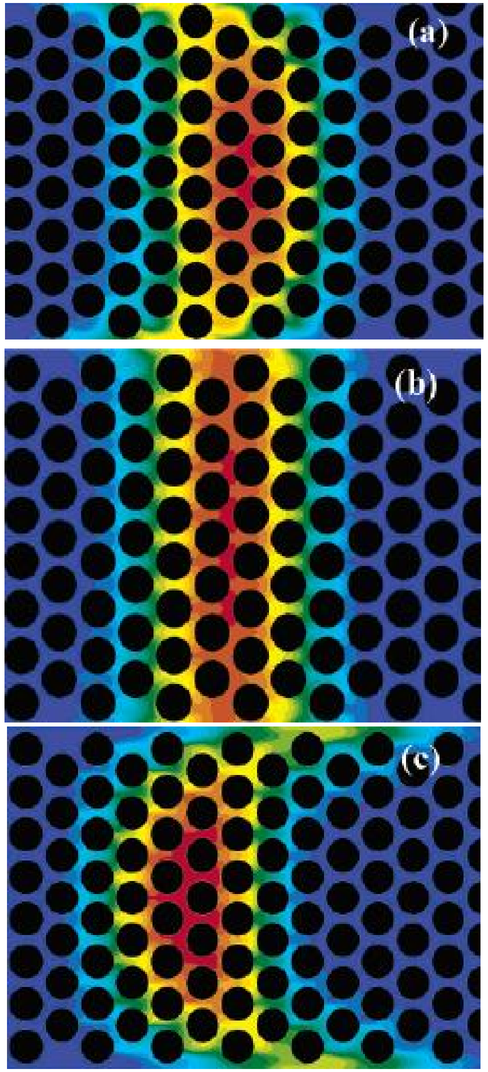
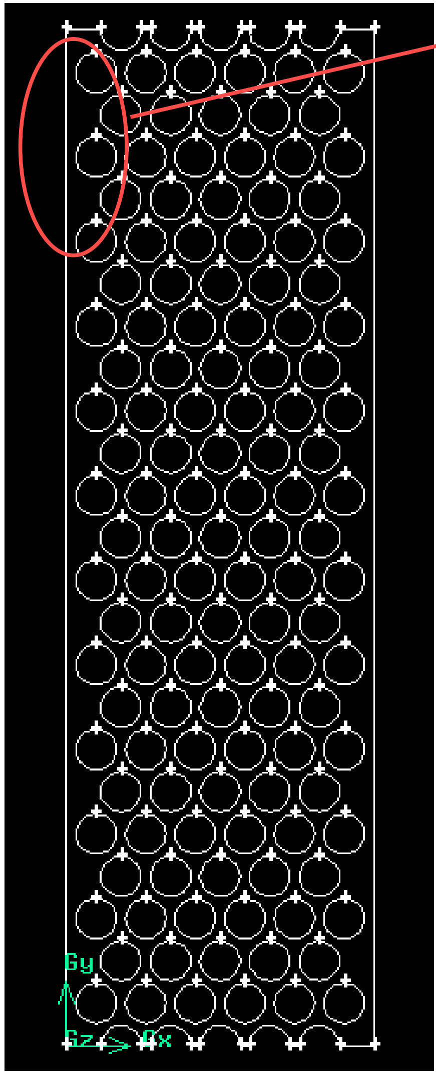
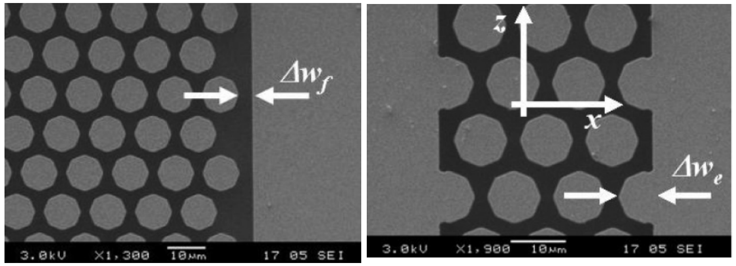
Wall effects





Distance between last pillar row and sidewall is very critical



$$h = \frac{s_x^2}{L d_p} = \frac{L}{d_p}\left(\frac{s_t}{t_{mean}}\right)^2 \qquad \Delta w = \frac{\Delta w}{d_p}$$

Table 1. Geometrical Parameters and Performance of the Channels

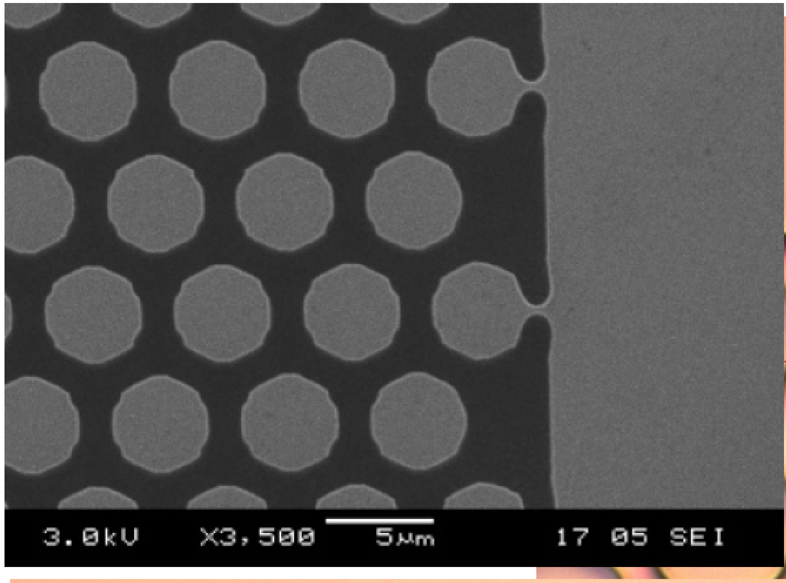
channel	width (μm)	d_p (μm)	wall type	$\Delta\omega$	h_{min}[a]
1	1000	10	flat	0.14	0.29 ± 0.06
1C[b]		10			0.36 ± 0.06
2	1000	5	flat	0.28	0.72 ± 0.14
2C[b]		5			0.32 ± 0.08
3	36	10	flat	0.07	0.66 ± 0.19
4	60	10	embedded	0.43	0.49 ± 0.03
5	100	10	embedded	0.59	0.27 ± 0.02
6	40	10	embedded	0.66	0.16 ± 0.03

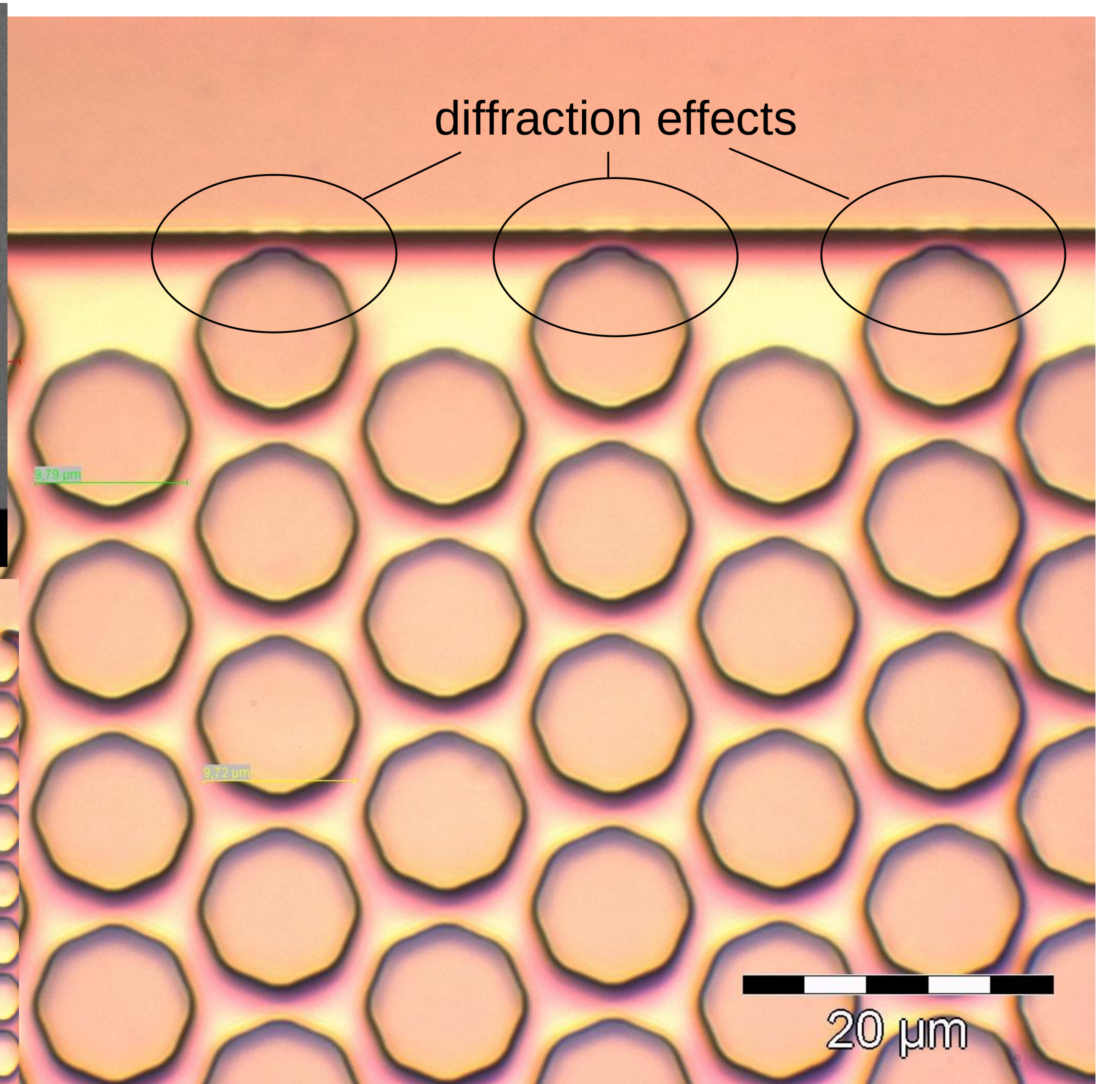
[a] Mean ± 95% confidence interval. [b] Center strip of the channel.

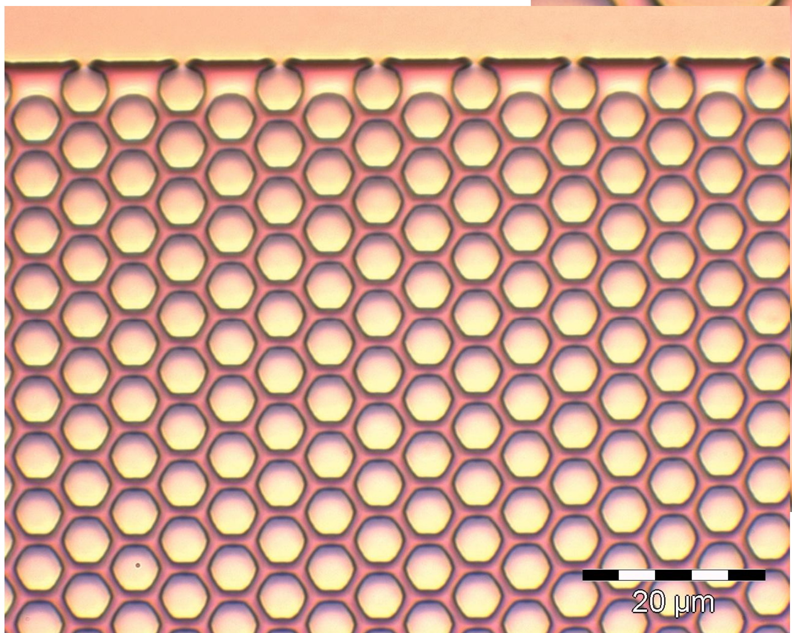
N.B. Also the top and bottom wall of the channel add to h, and shift h(Pe) minimum to lower Pe (H. Eghbali e.a. J. Sep. Sci. 2007 in press)

Experiments: M. De Pra e.a. Anal. Chem. 78 (2006) 6519
Modelling: N. Vervoort e.a. Anal. Chem. 76 (2004) 4501

UV photolithography limitation (~1 µm)







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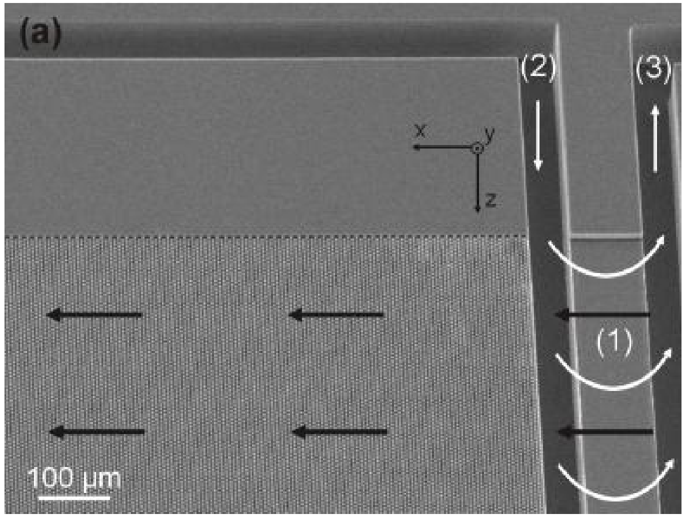
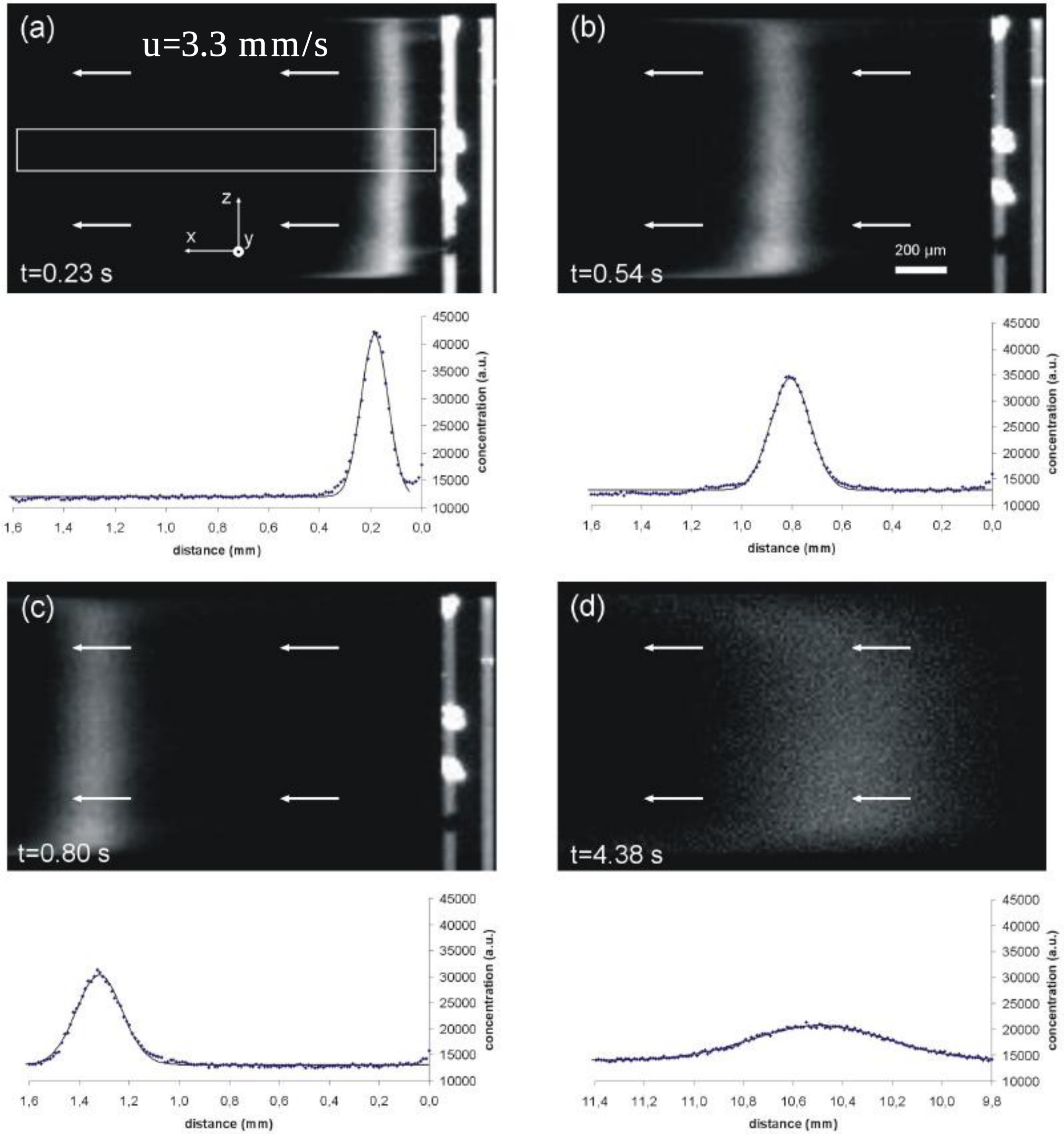
Tracer dispersion analysis



Injection system & principle



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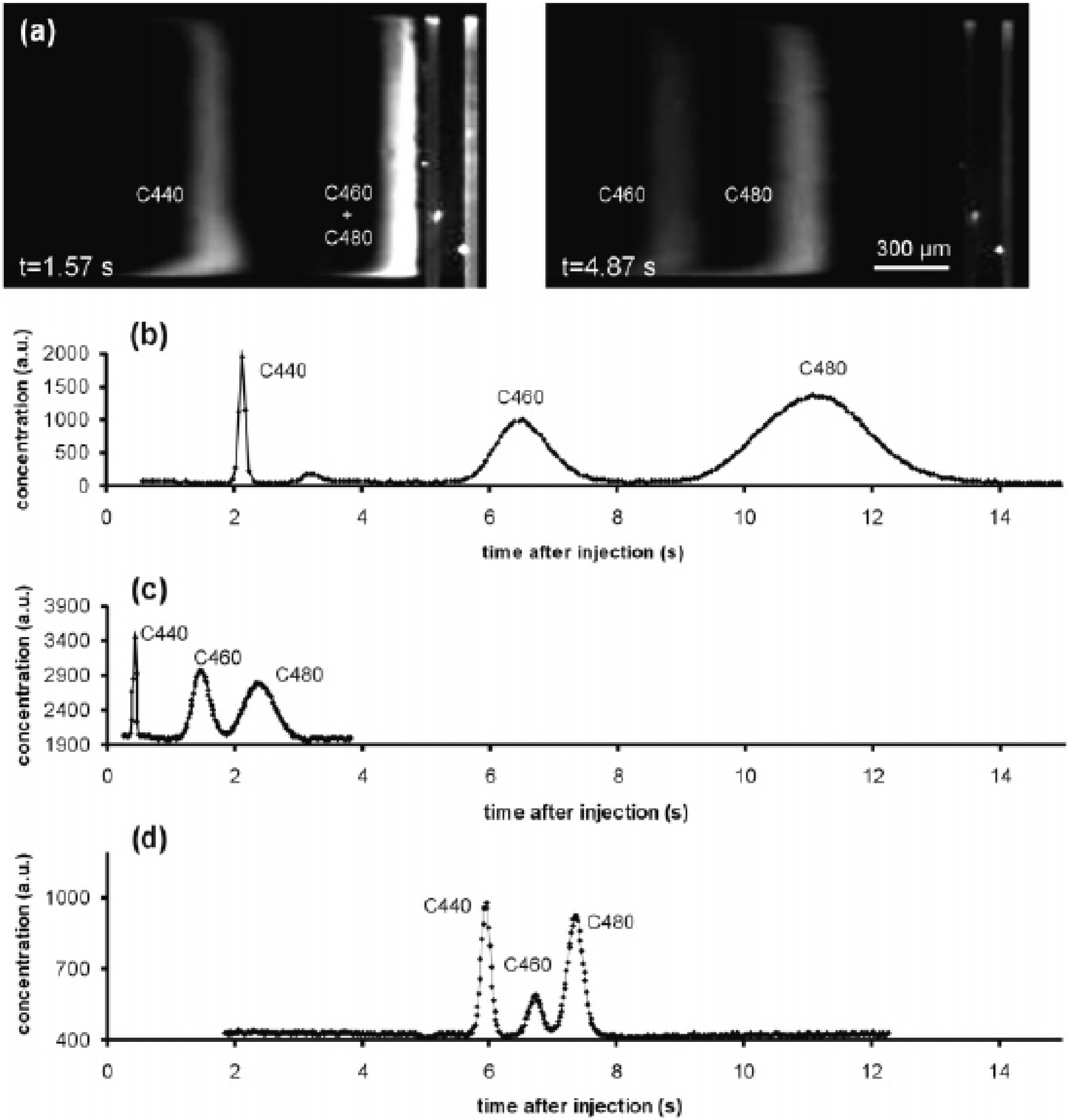
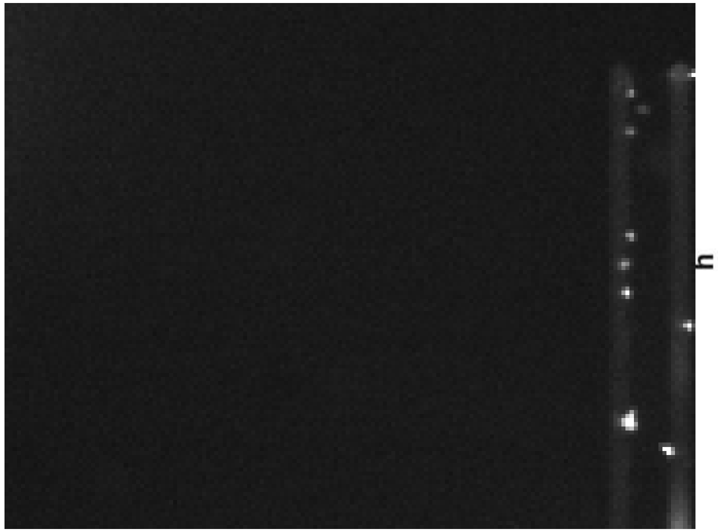
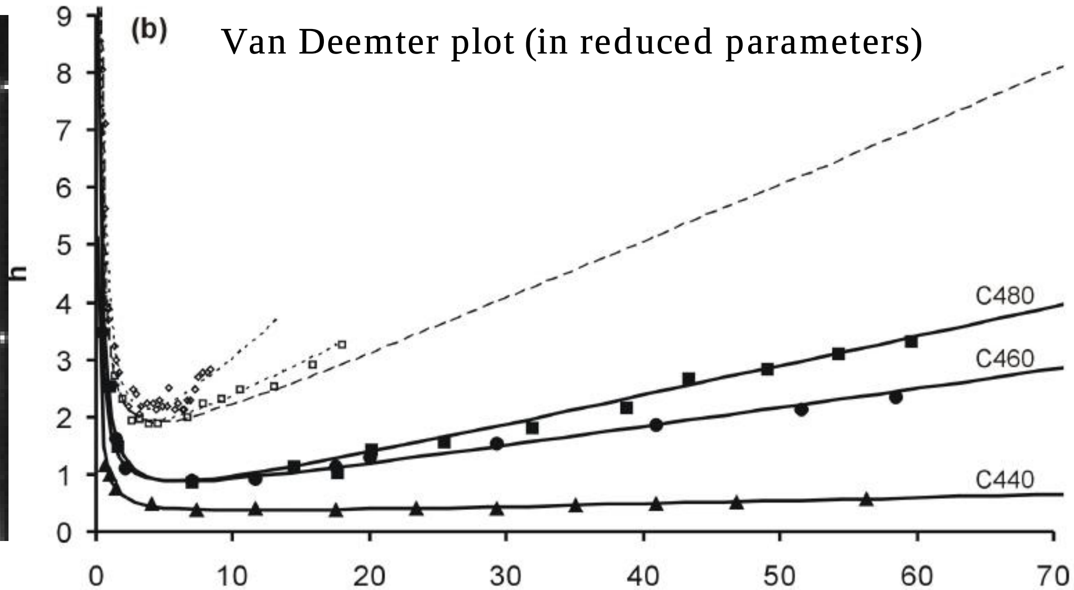


W. De Malsche e.a., Anal. Chem. 79 , 2007, 5915

Micromachined liquid chromatography columns



Separation of 0.25 mM coumarin dyes on C8-coated pillar column, in mobile phase of (20/80, v/v) methanol-water

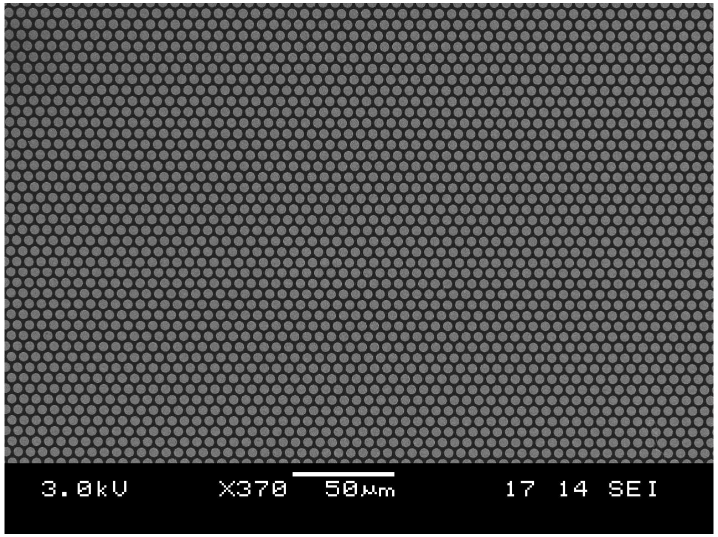


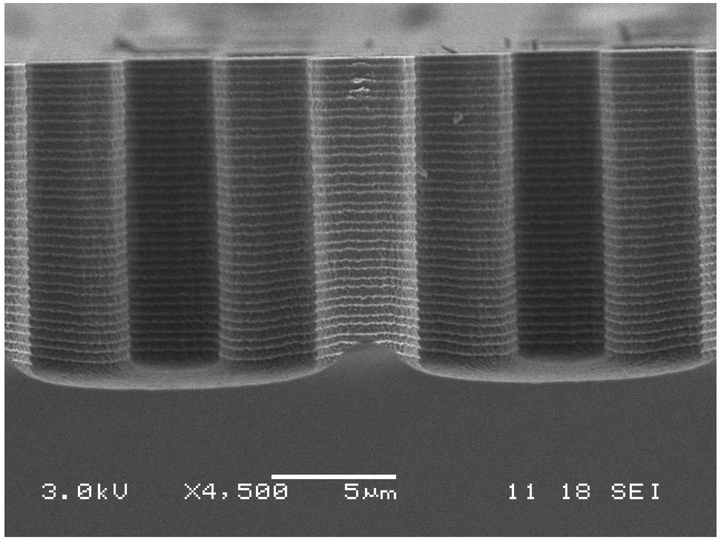
$$Pe = \frac{ud_p}{D}$$

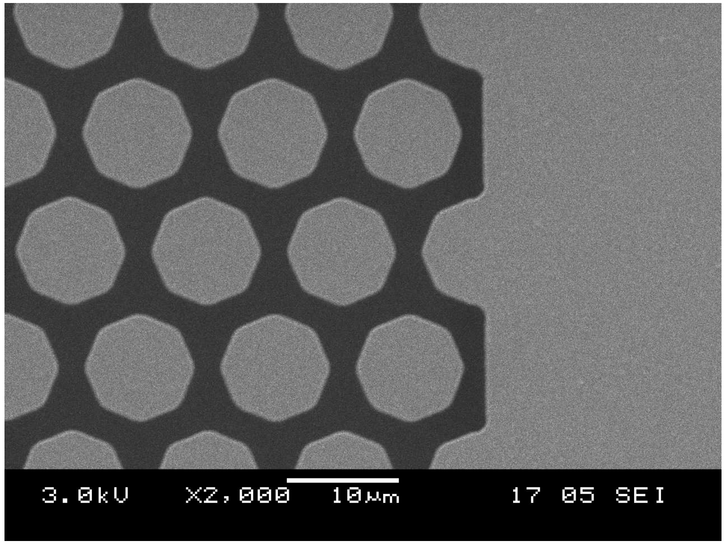
Van Deemter curves of coumarin dyes in mobile phase of (30/70, v/v) methanol-water and comparison with experimental values for 1.5 (◊) and 3 μm (□) non-porous particle packing (Wu et al.). Dashed curve: typical curve for packed bed

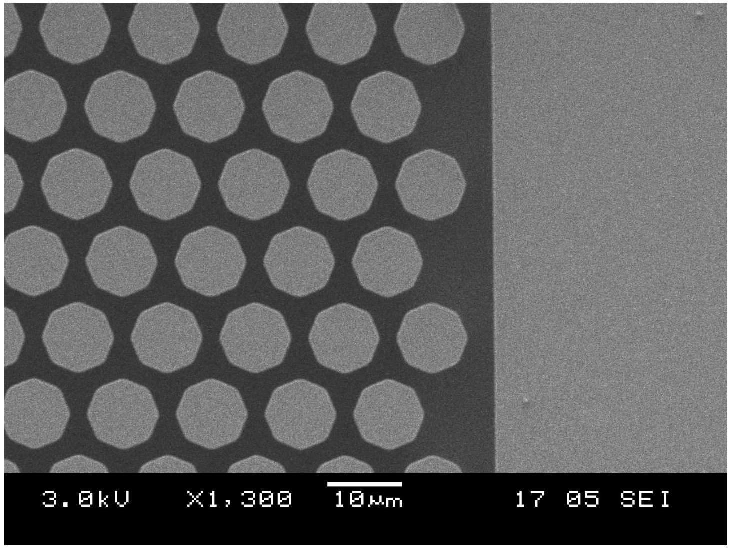
W. De Malsche e.a., Anal. Chem. 79 , 2007, 5915

Pictures of the column ("Bosch" etched)

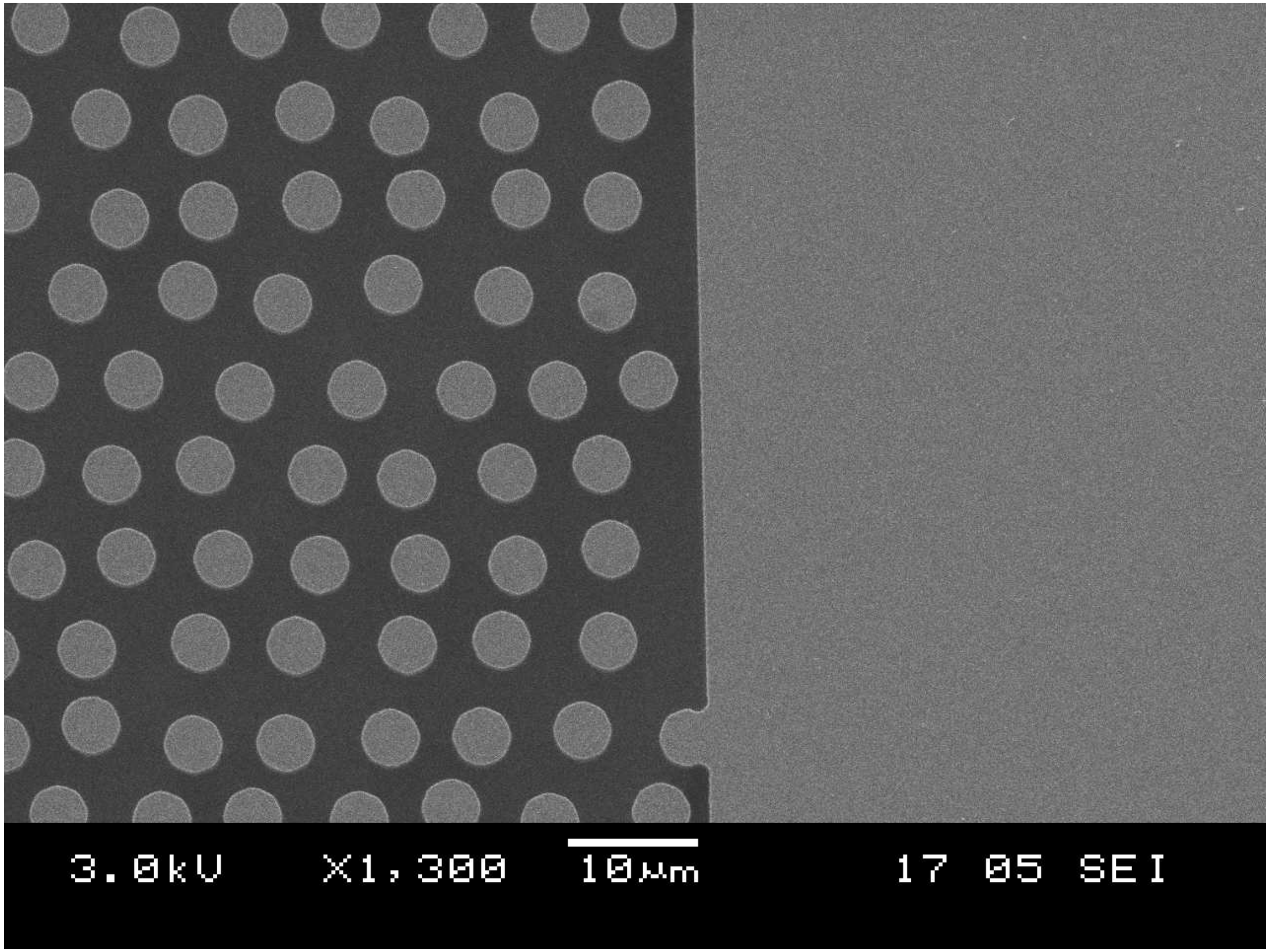




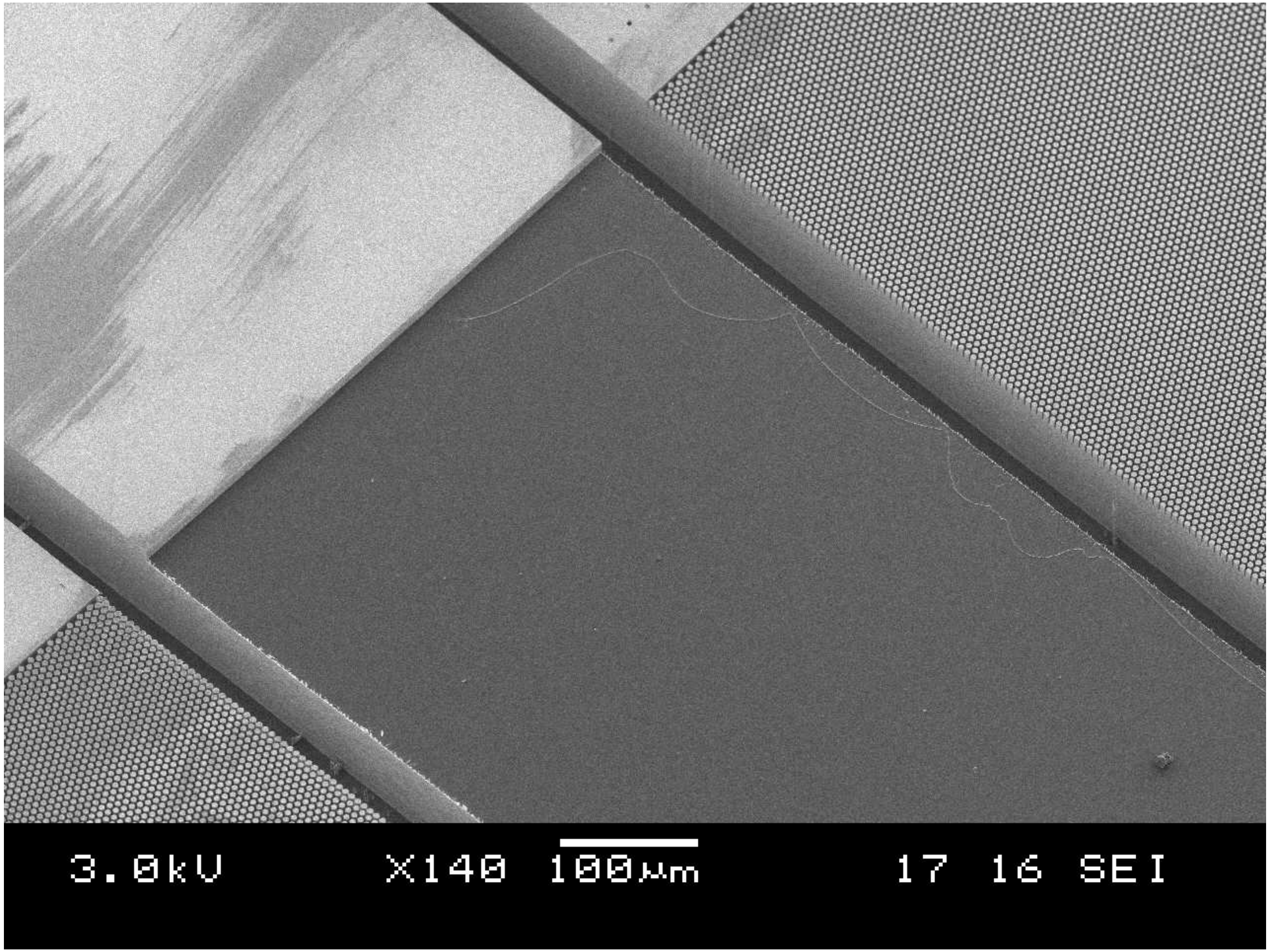




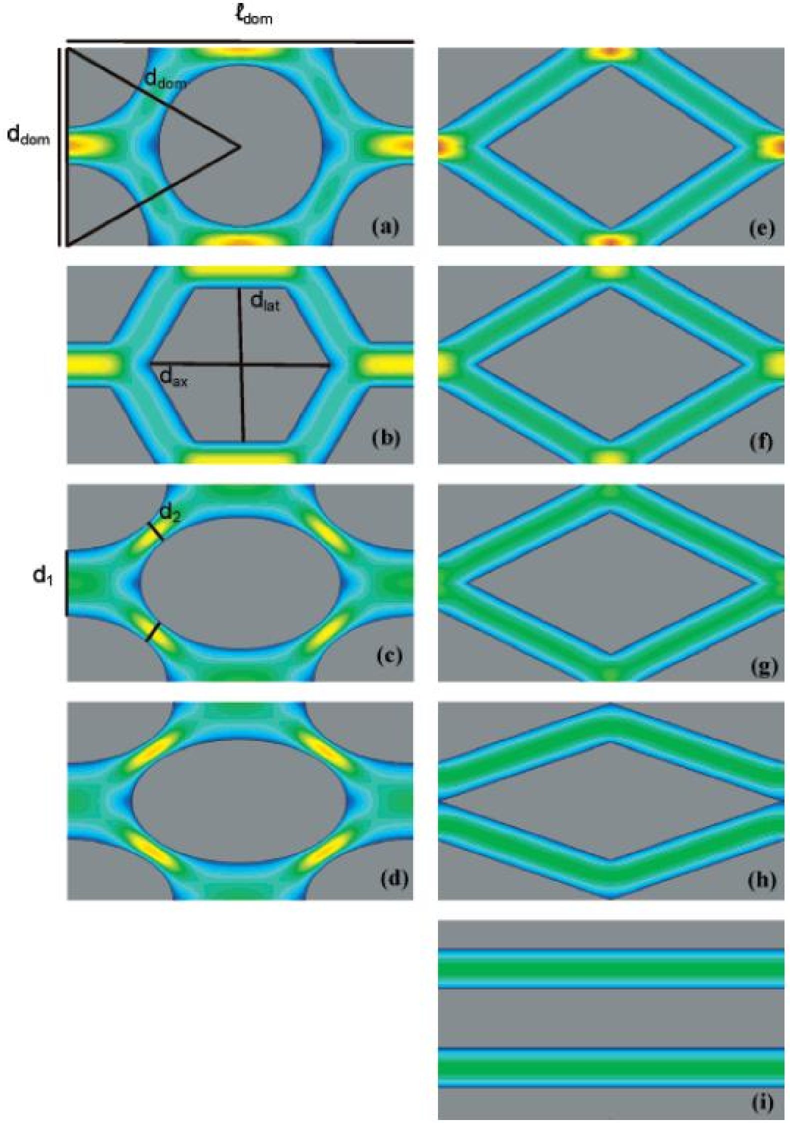
Etched microcolumns -random pillars



Etched microcolumn -injector



Different pillar shapes



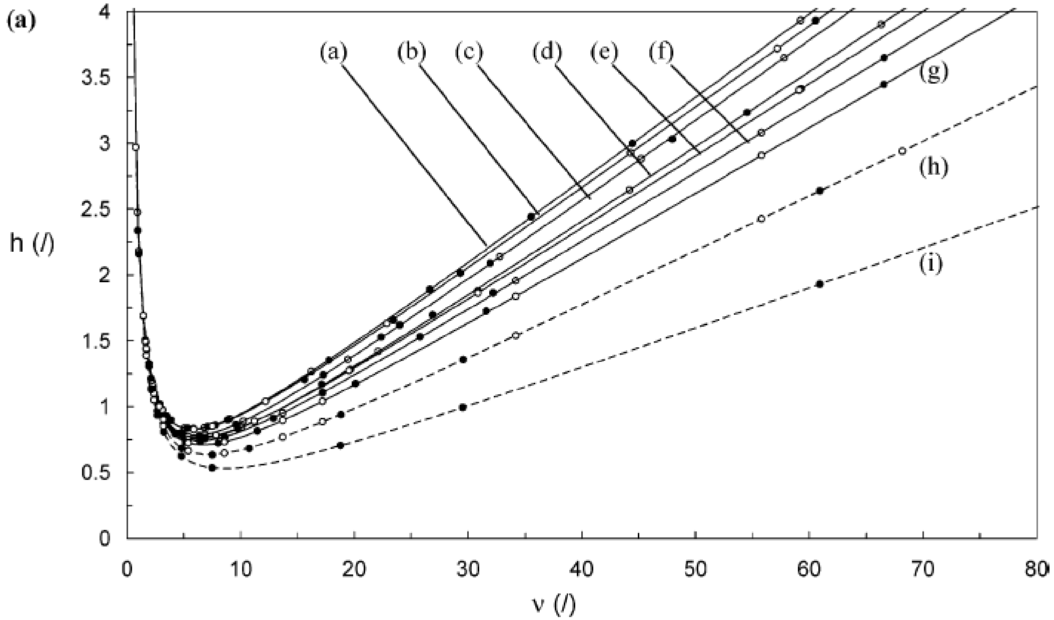
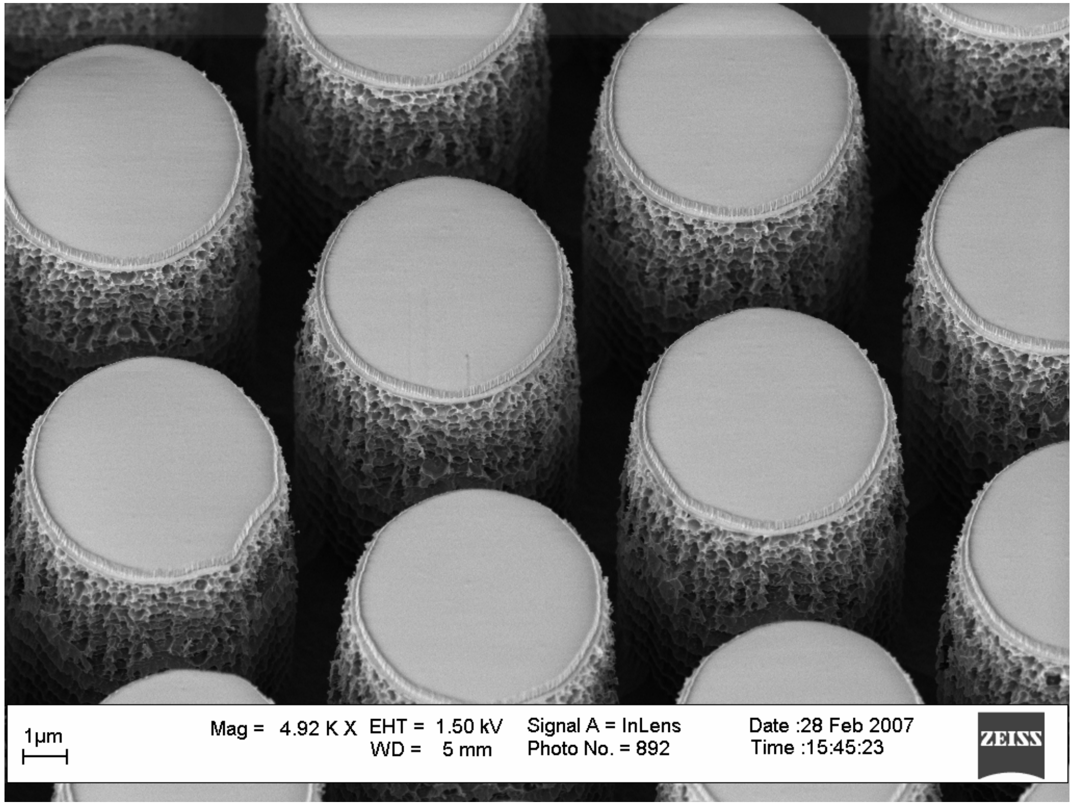
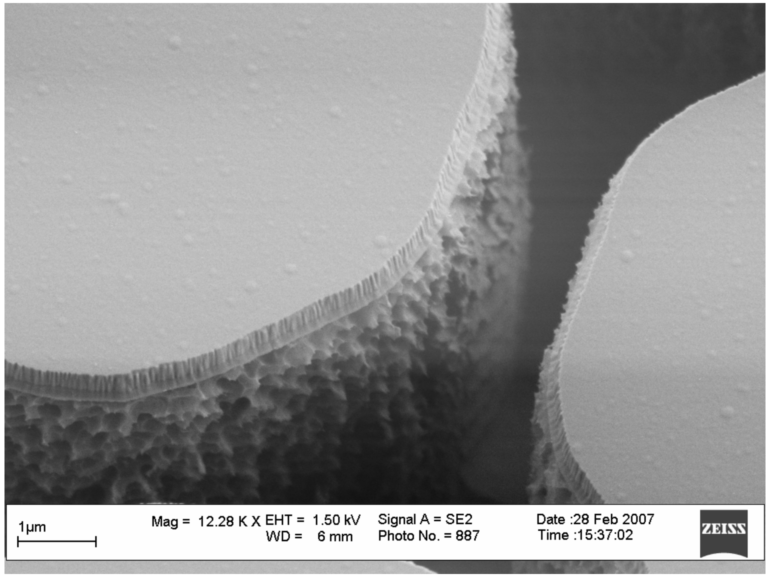


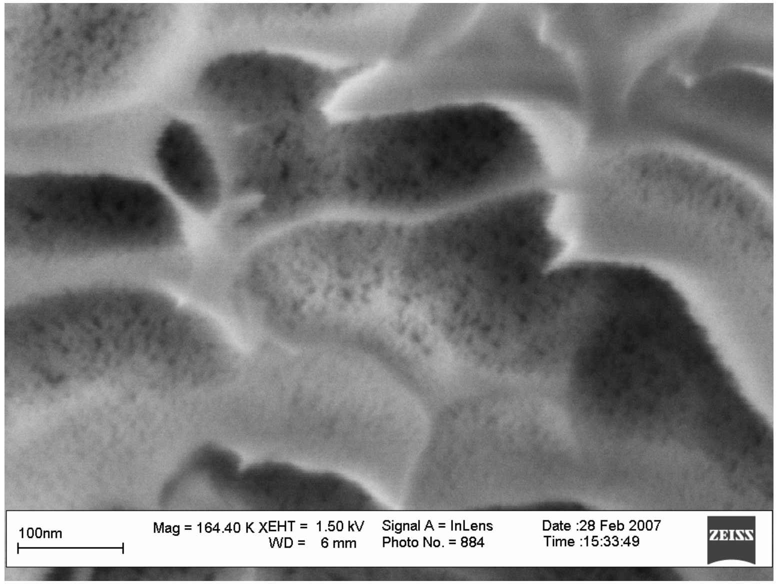
Plate heights (equivalent diameter: 1 μm)

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

Porous pillars







Material: fused silica on silicon
Method: anodization of silicon

(W. De Malsche & V. Verdoold, to be presented at µTAS conf. Oct. 2007, Paris France)

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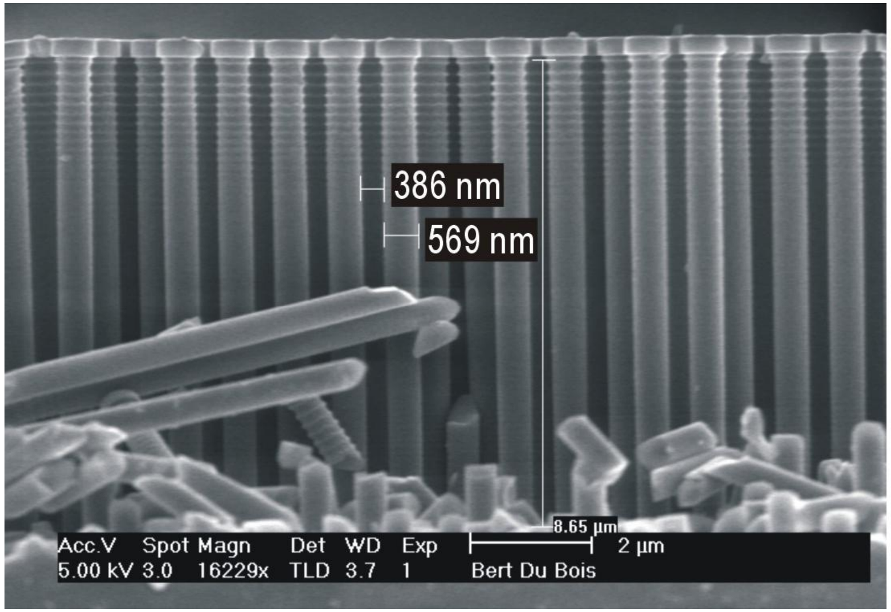
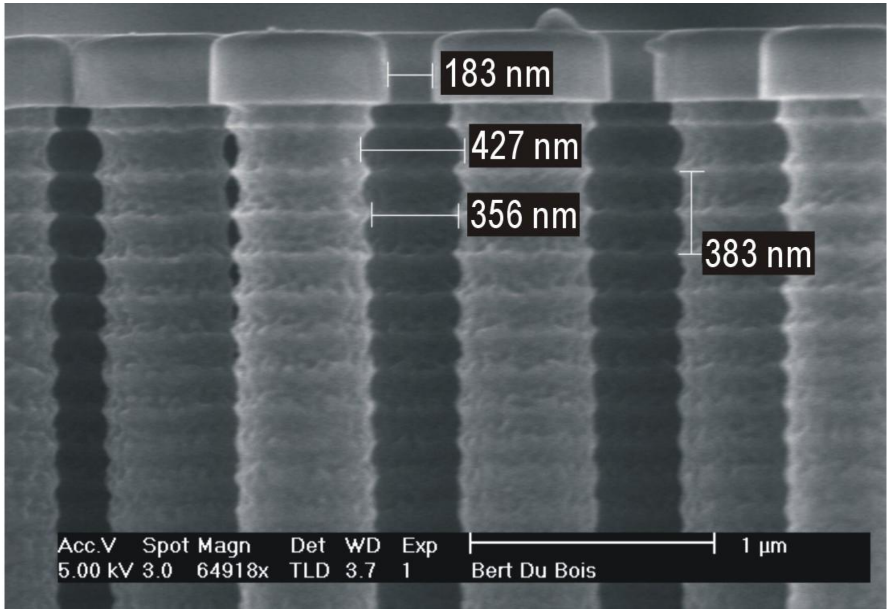
Nano pillars

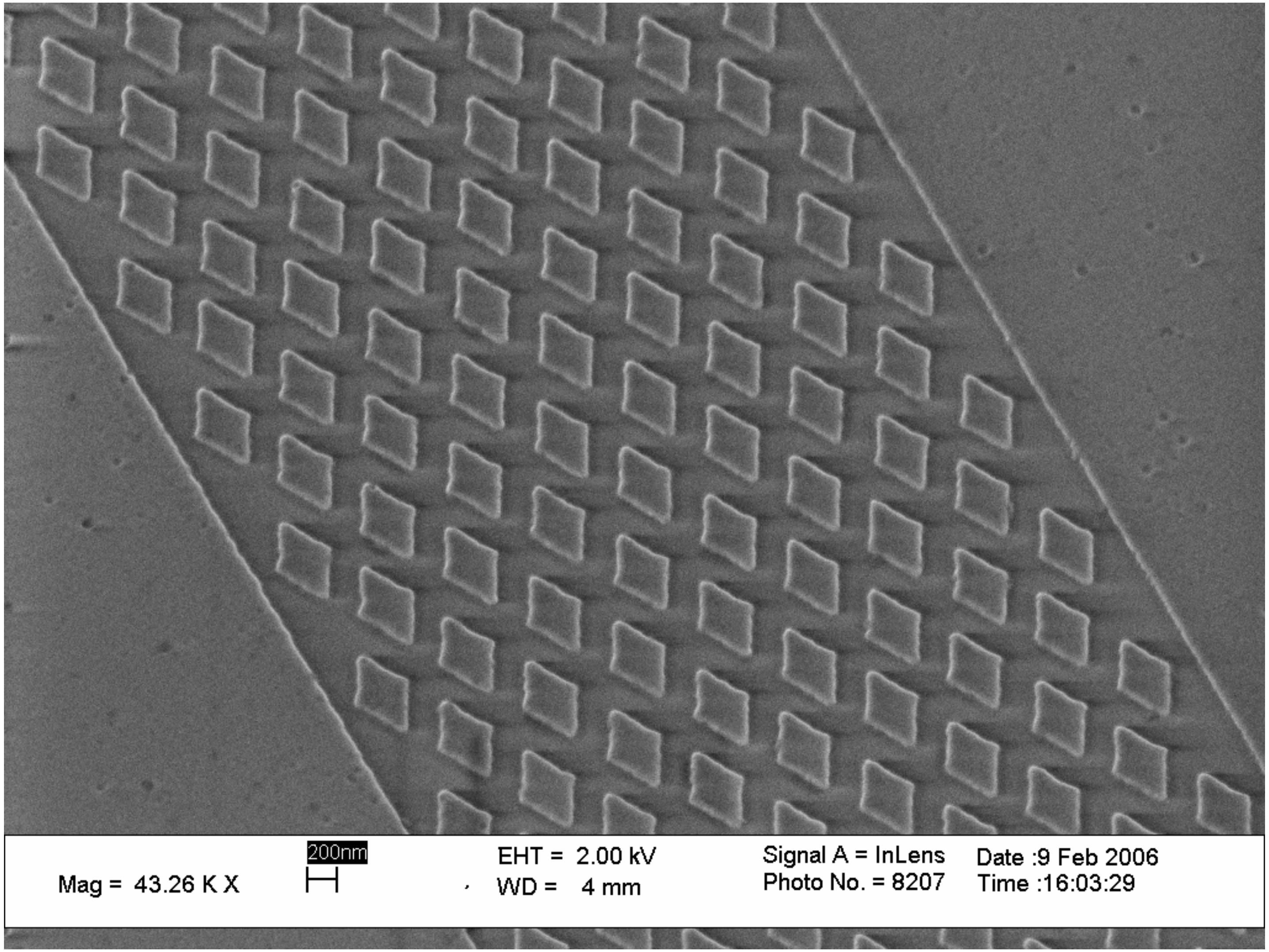




Silicon pillars with diameter of 570 nm by Bosch-type deep reactive ion etching. Depth is 8.7 µm. The SiO_2 hard mask is still present on top of the pillars. Photolithography was done with a deep-UV wafer stepper (λ=193 nm) at the IMEC institute, Leuven, Belgium

W. De Malsche, to be presented at µTAS conf. Oct. 2007, Paris France

Nano pillars by nano imprint lithogr.



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Addendum: Viscous fingering

"Saffman-Taylore instability"
resembles Rayleigh-Taylor instability (lecture Asinimov)

P.G. Saffman & G. Taylor, The penetration of a fluid into a porous medium or Hele-Shaw cell containing a more viscous liquid, Proc. Royal Soc. London A 245, 1958, p.312

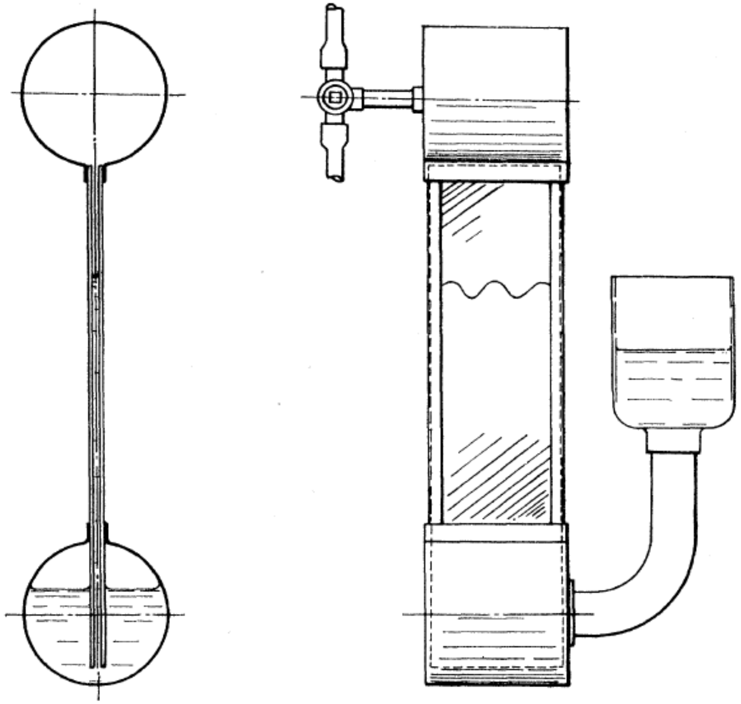

FIGURE 1. Sketch of Hele-Shaw cell.

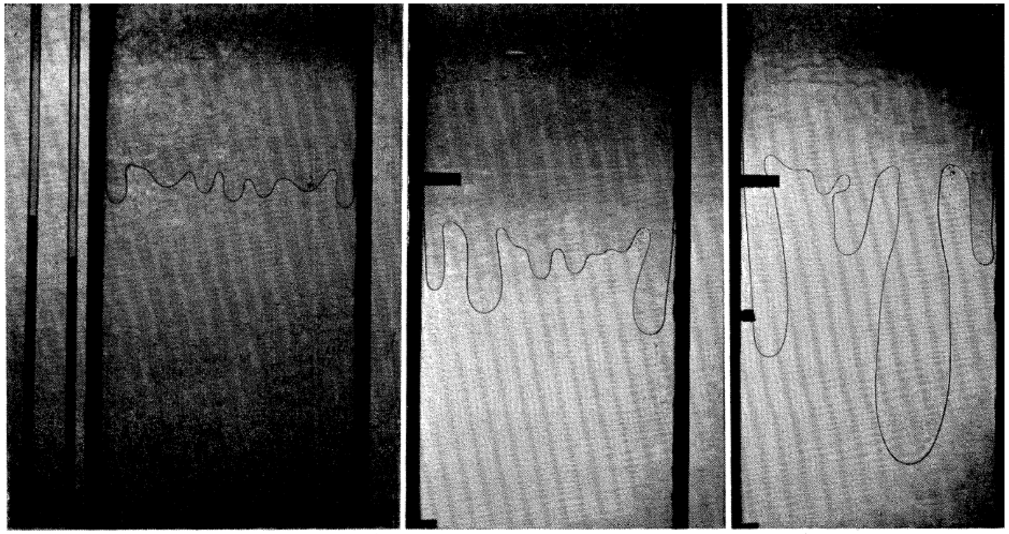

FIGURE 2 FIGURE 3 FIGURE 4

FIGURE 2. Interface between air and glycerine at an early stage of the instability.

FIGURE 3. Development of instability.

FIGURE 4. Inhibiting effect of a finger which gets ahead of its neighbour.

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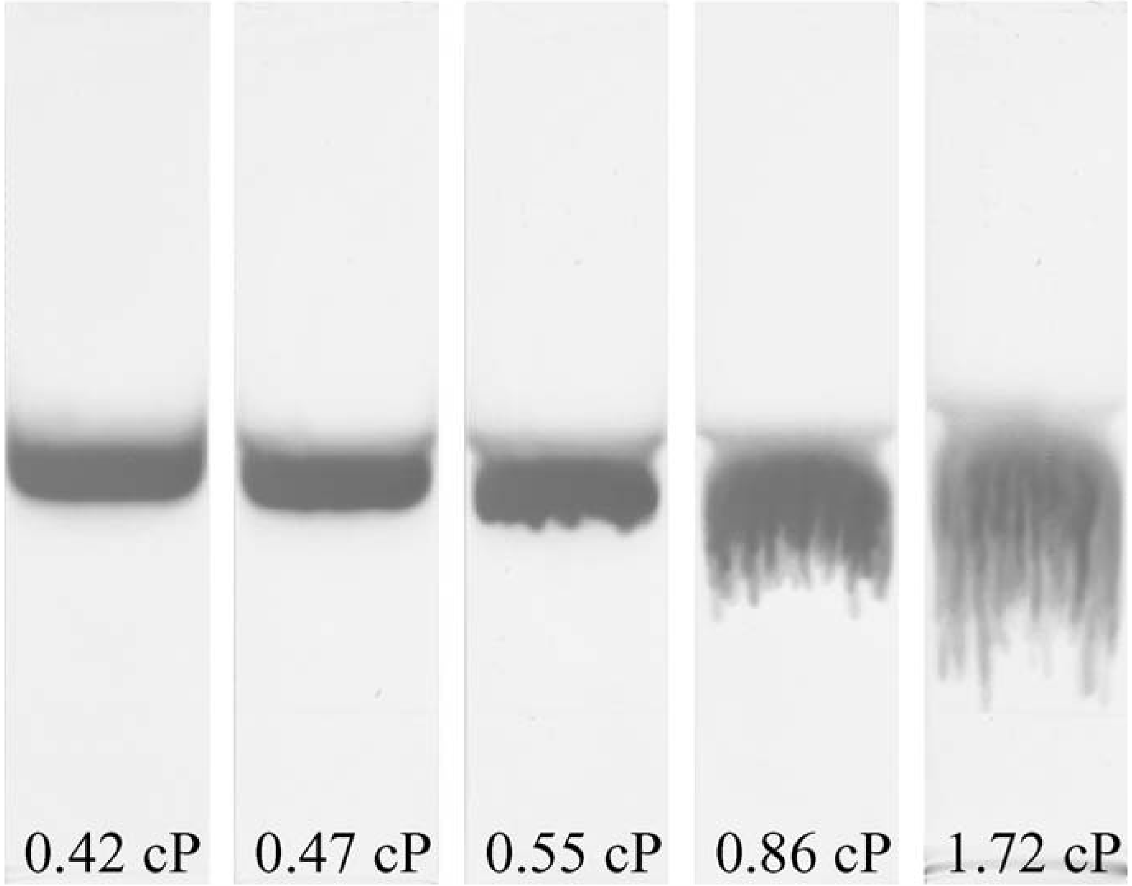
Viscous fingering in chromatography



viscous fingering when the viscosity of the solute injection plug is less than the mobile phase. Solute injection plug viscosity was 0.38 cP, the mobile phase viscosity is noted below each photograph

From: H.J. Catchpoole e.a., J. Chrom. A 1117, 2006, p. 137

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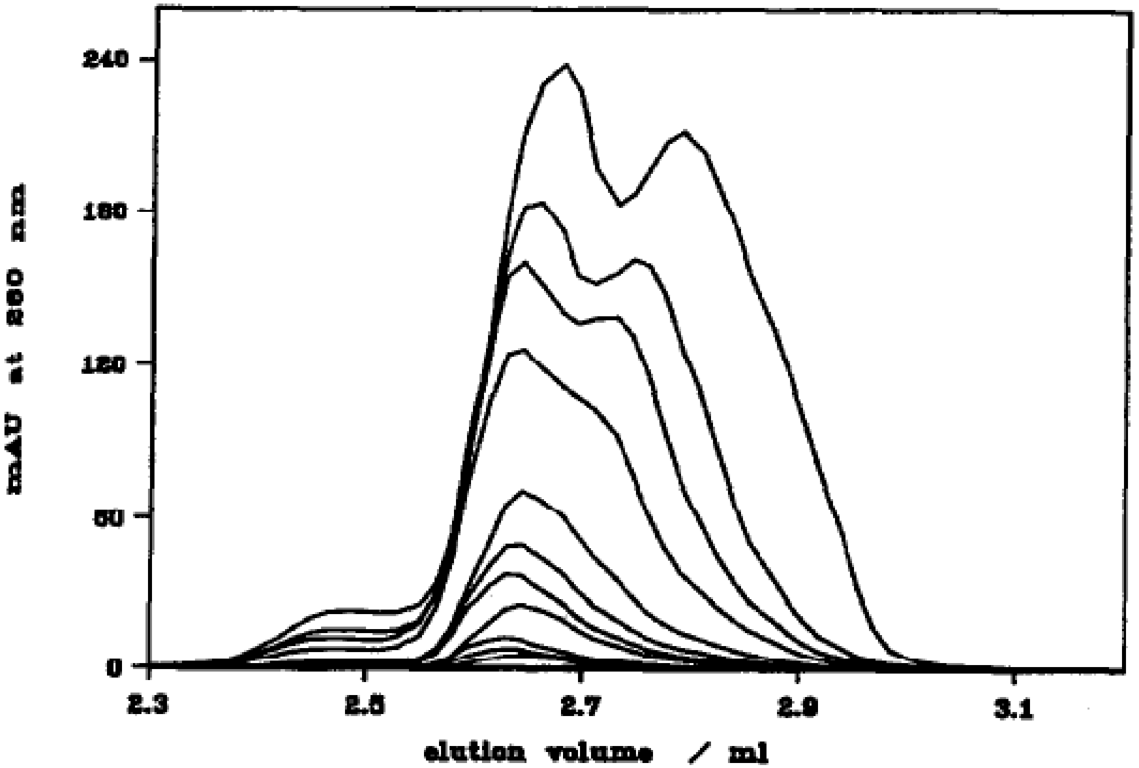
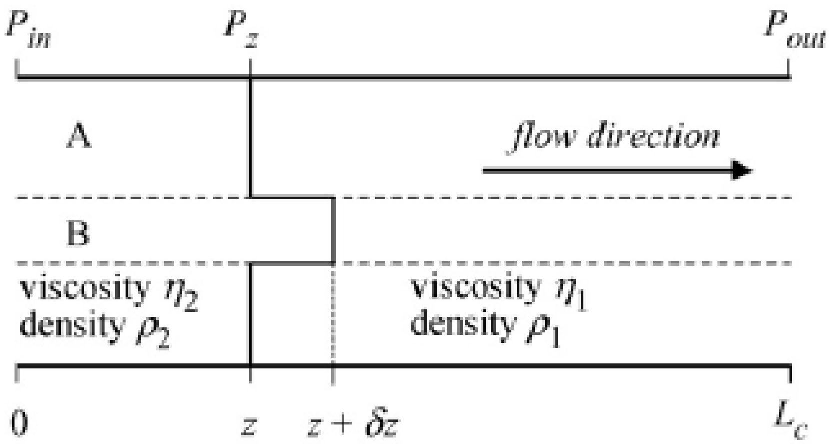
Peak distortion by viscous fingering



Elution profiles of chicken ovalbumin at increasing concentrations.
"Overloading"

From: M. Czok e.a., J. Chrom. 550, 1991, p. 705



basic flow equation for porous medium:

$$u = \frac{k}{h}\left(-\frac{dP}{dz} + r\,dg\right) = \frac{k}{h}\left(\frac{P_{in} - P_{out}}{L_c}\right)$$

$$u_A = \frac{k}{h_2}\left(\frac{P_{in} - P_z}{z}\right) = \frac{k}{h_1}\left(\frac{P_z - P_{out}}{L_c - z}\right)$$

with: $\Delta P = P_{in} - P_{out}$

$$\frac{\Delta P}{L_c} = u_A\left[h_2\frac{z}{L_c} + h_1\left(1 - \frac{z}{L_c}\right)\right]$$

similarly:

$$\frac{\Delta P}{L_c} = \frac{u_B}{k}\left[h_2\frac{z}{L_c} + h_1\left(1 - \frac{z}{L_c}\right)\right] + \frac{dz}{L_c}\left[\frac{u_B}{k}(h_2 - h_1)\right]$$

leads to:

$$u_A - u_B = k\frac{dz}{L_c}\left[\frac{u_B}{k}(h_2 - h_1)\right]\left[h_2\frac{z}{L_c} + h_1\left(1 - \frac{z}{L_c}\right)\right]^{-1}$$

stability criterion: $\boldsymbol{u_A > u_B}$ for $\delta \boldsymbol{z > 0}$ and $\boldsymbol{u_A < u_B}$ for $\delta \boldsymbol{z} < 0$, i.e. the flow remains stable if:

$$\eta_2 > \eta_1$$