

Miniaturization in sample preparation and separation: Part 2

Dr. Bert Wouters

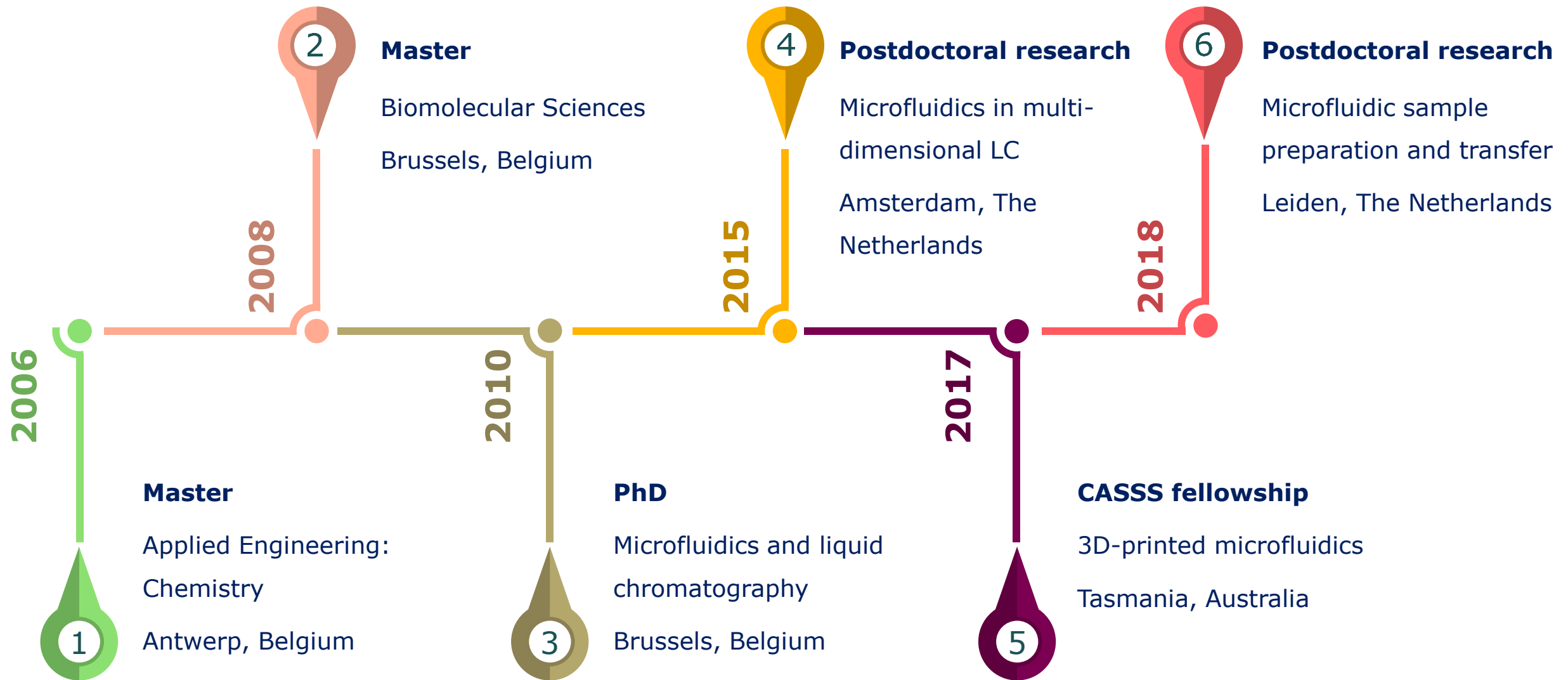
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Curriculum vitae



Overview

1. Performance limits of liquid chromatography

2. Microfluidics

1. Why microfluidics?
2. Applications
3. Microfabrication overview
4. 3D printing

3. Microchips for one-dimensional separations

1. Gas chromatography (GC)
2. Liquid chromatography (LC)

4. Microchips for multi-dimensional separations

1. Why multi-dimensional separations?
2. 2D spatial LC devices
3. 3D spatial LC devices



1. Performance limits of liquid chromatography

Current analytical strategies show only the tip of the iceberg

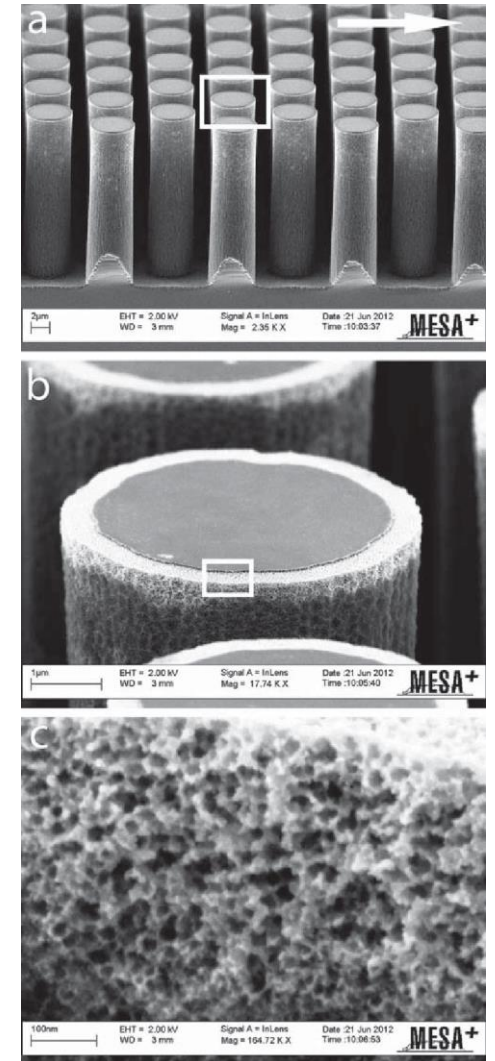
- **Major challenge:** analysis of low-abundant analytes in a complex mixture.
 - Examples: biomarkers in proteomics and metabolomics, contaminants in food matrices, and polymer analysis.
- **Golden standard** = high-performance one-dimensional **liquid chromatography** (HPLC) coupled to **mass spectrometry** (MS).
 - HPLC reduces **spectral complexity** and **minimizes ion-suppression effects**.



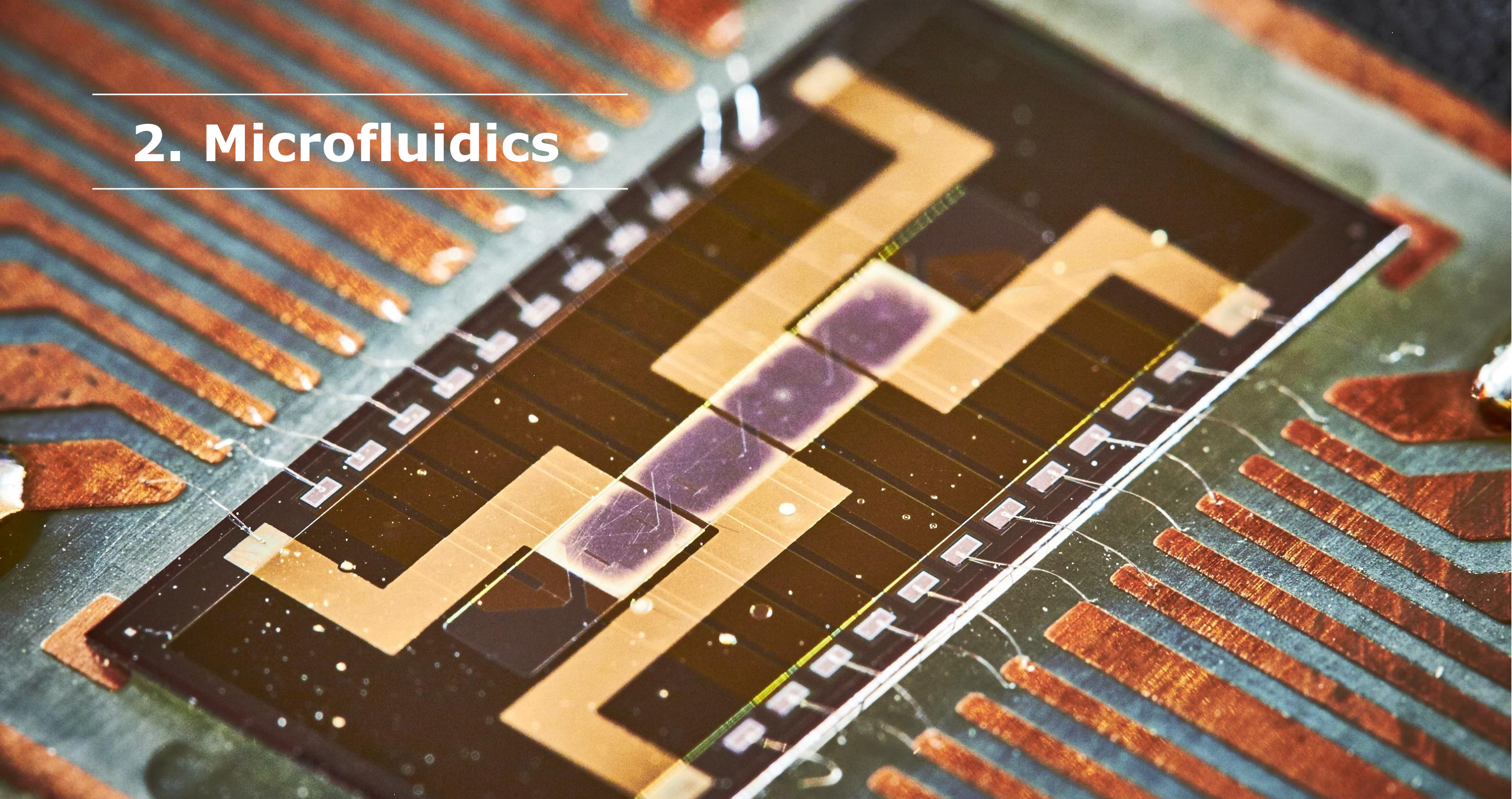
How to improve 1D-LC performance?

Trends:

1. Smaller **particle size** (d_p), *e.g.* sub-2 μm .
2. Decreasing mobile-phase **viscosity**:
 - Elevated mobile-phase temperature ($>60\text{ }^{\circ}\text{C}$).
 - Mobile phases with a high organic content (*e.g.* hydrophilic interaction chromatography, HILIC).
 - Low-viscosity (co-)solvent (*e.g.* CO_2 for supercritical fluid chromatography, SFC).
3. Optimisation of **column technology**, *e.g.* core-shell, pillar-array columns.

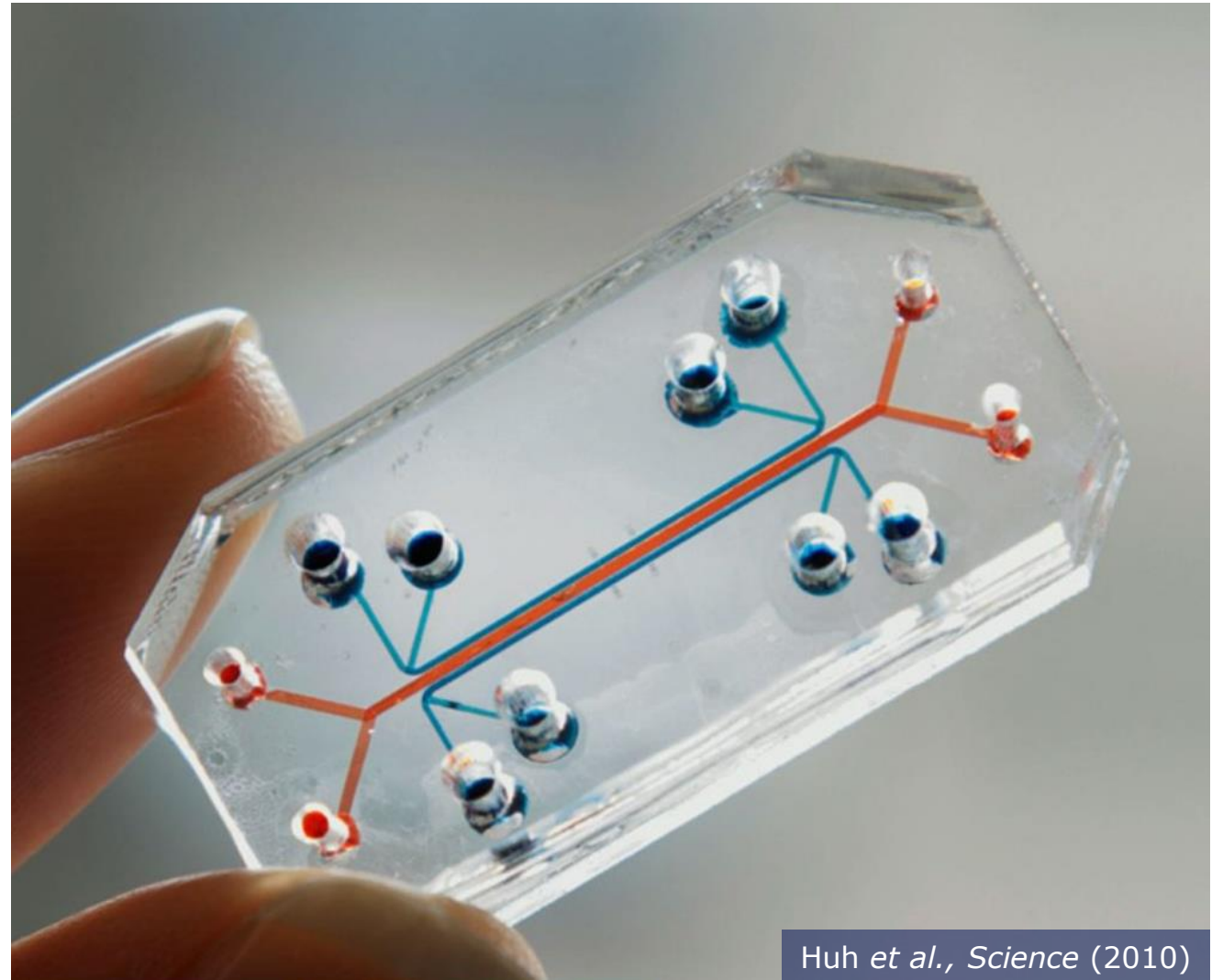


2. Microfluidics

A close-up photograph of a microfluidic chip. The chip is a dark, rectangular substrate with a complex network of gold-colored channels and reservoirs. A central channel is filled with a vibrant purple liquid. The chip is mounted on a larger, textured blue-green surface, which appears to be a carrier or a test platform. Several small, white, rectangular components are visible along the edges of the chip, possibly for electrical connection or fluid control.

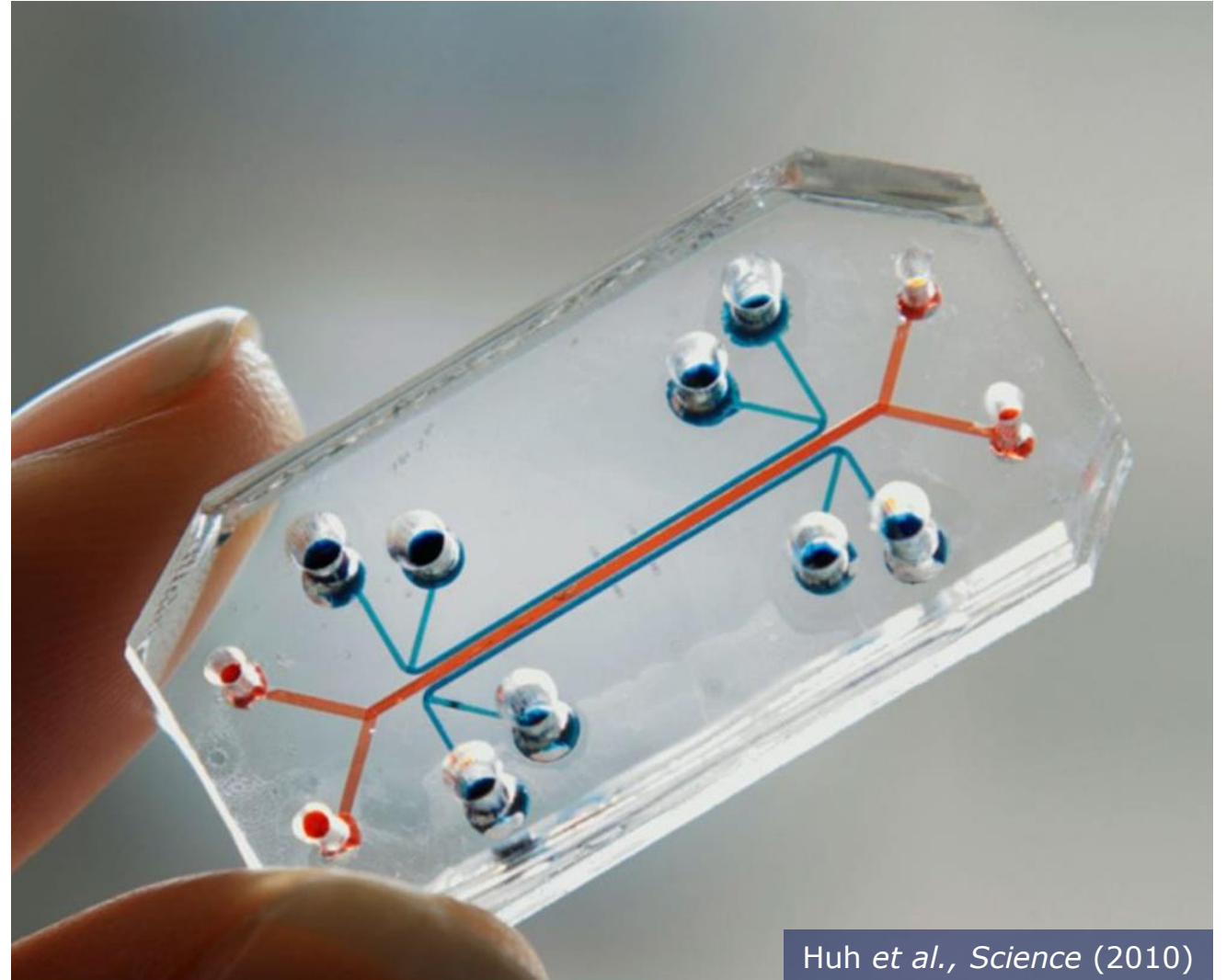
What is microfluidics?

- **Multidisciplinary** field intersecting engineering, physics, chemistry, biochemistry, nanotechnology, and biotechnology.
- “Science and technology of systems that process or manipulate **small amounts of fluids**, usually in the range of microliters (10^{-6}) to picoliters (10^{-12})”.
- Uses channels with **dimensions of tens to hundreds of micrometres**.



What is microfluidics?

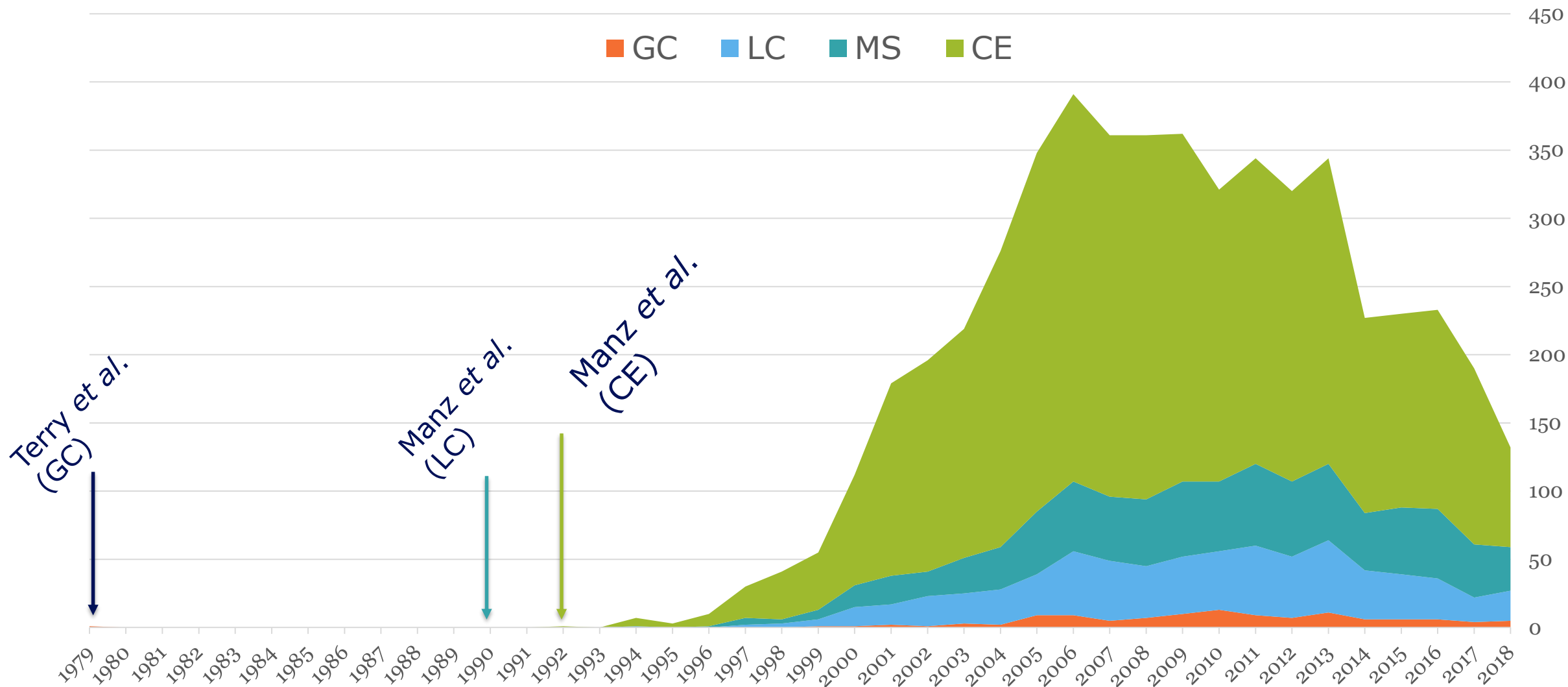
- **Drivers:** molecular analysis, biodefence, molecular biology, microelectronics.
- Started in the electronics industry using **semi-conductor technology** for fabricating computers.
- Dominated by high-powered technology centres (clean rooms, photolithography, metal deposition, SEM).



Why microfluidics?

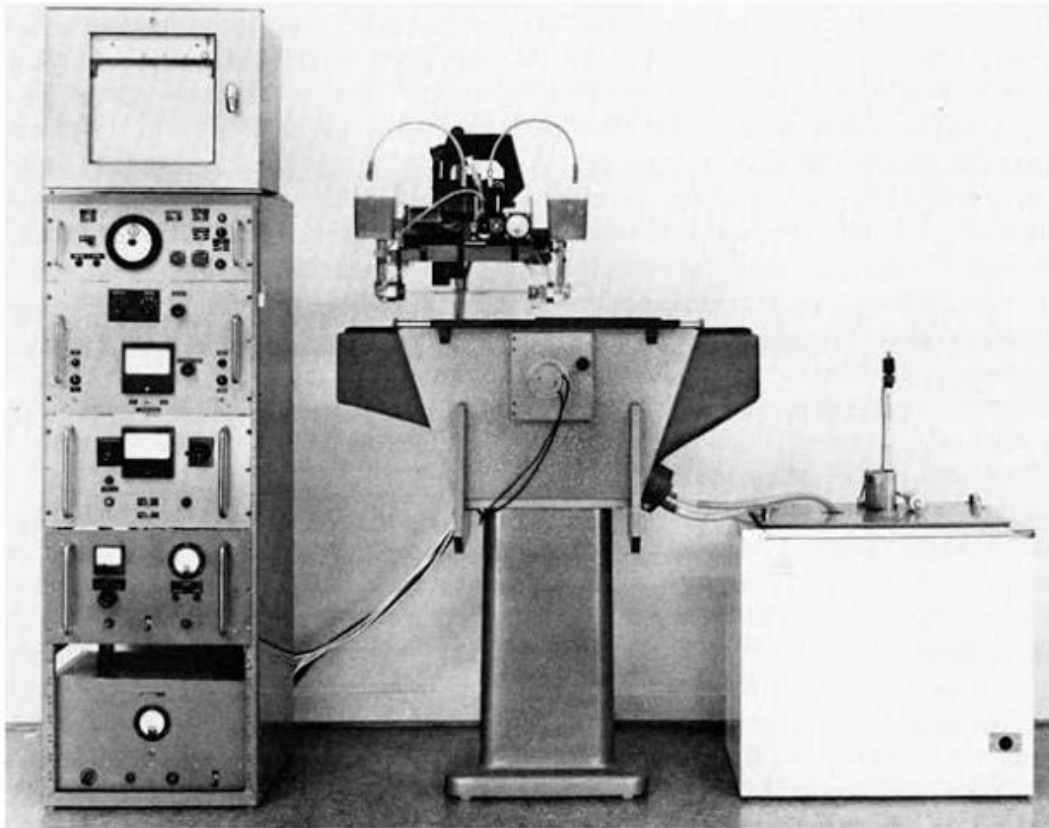
Microfluidic advantage	Description
Reduced sample size, reagent consumption, and waste	nL to pL volumes
Portability, small footprint	Point-of-care diagnostics, at-line measurements
Enhanced heat transfer	Higher surface-to-volume ratio
Unique physical properties lead to better separation performance and shorter analysis time	Laminar flow, reduced diffusion distances, electrophoretic and chromatographic separation proportional to L/d
Parallelisation	Multiple assays 'multiplexing' on a single chip

Microfluidics for separations



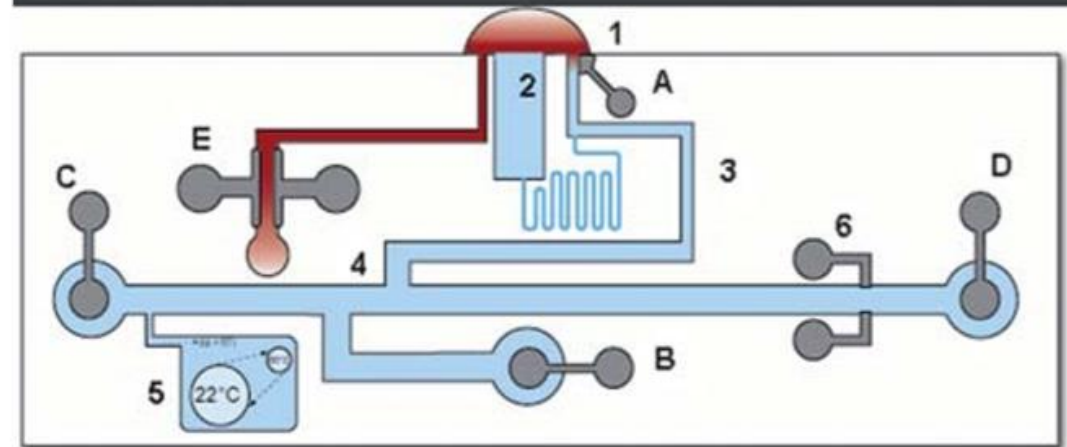
Miniaturization

1947 – capillary electrophoresis (CE) system designed by S. Hjertén



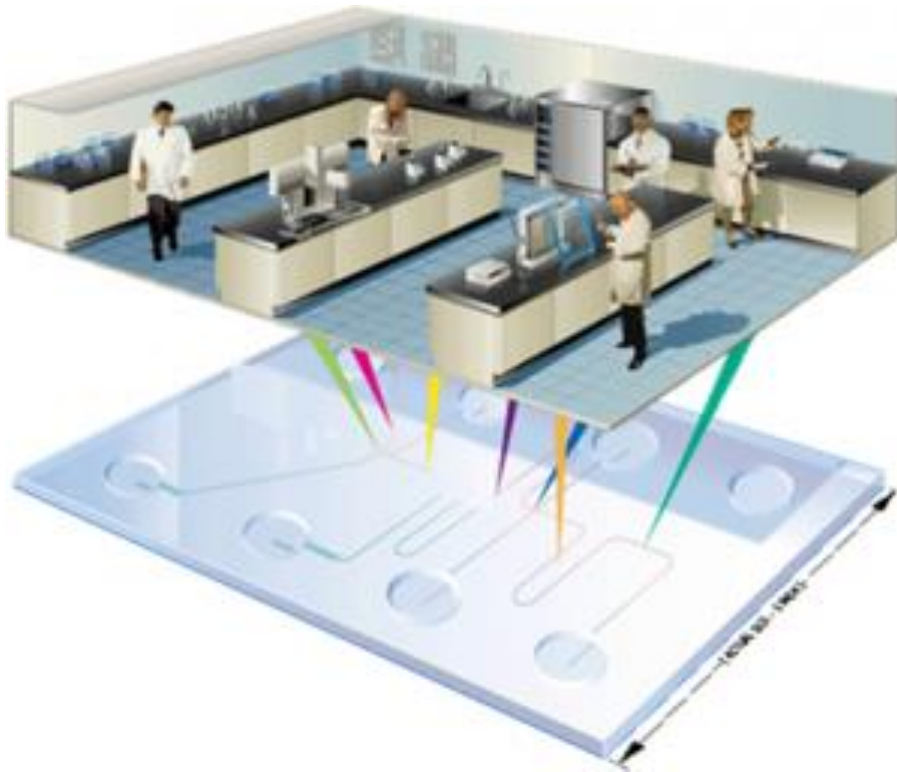
Handbook of Capillary and Microchip Electrophoresis and Associated Microtechniques, James P. Landers CRC Press, 2007.

2010 - Portable CE for lithium monitoring in blood



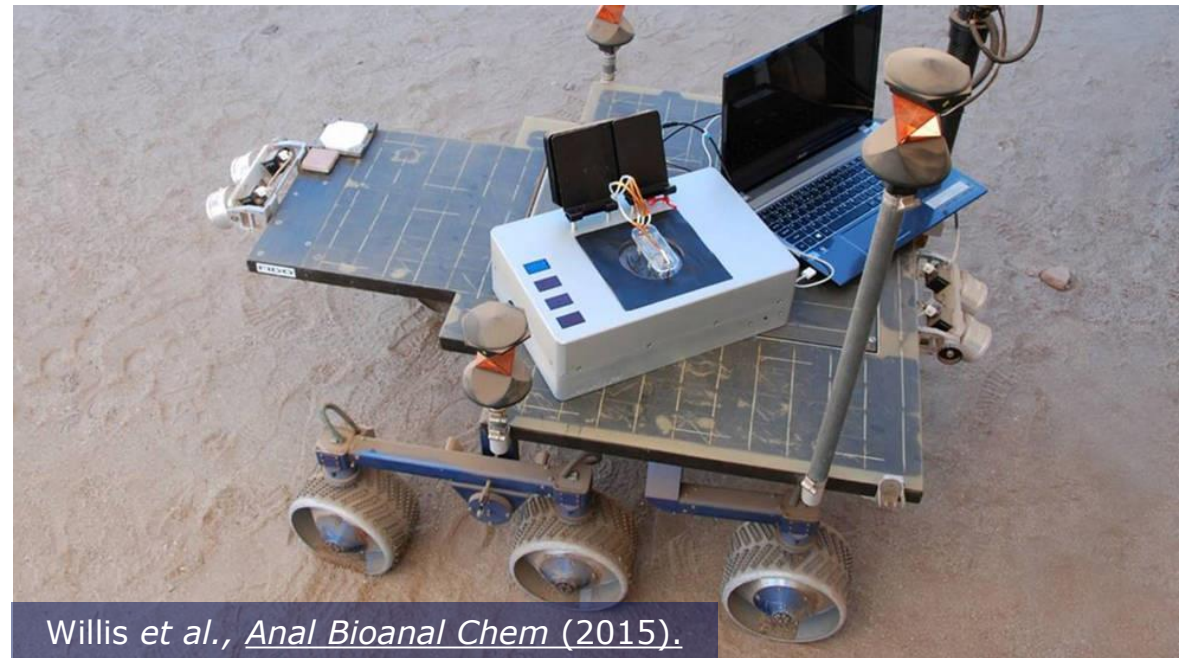
Floris *et al.*, Lab on a Chip (2010)

Lab-on-a-chip and μ TAS (Micro Total analysis system)



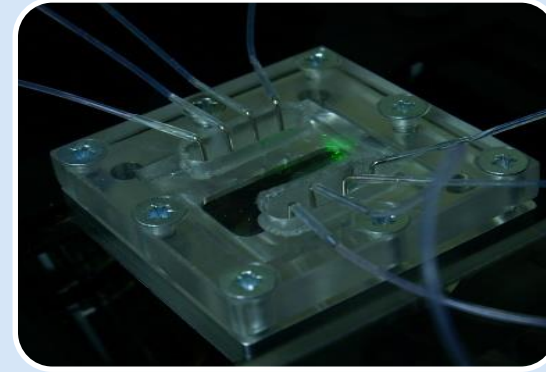
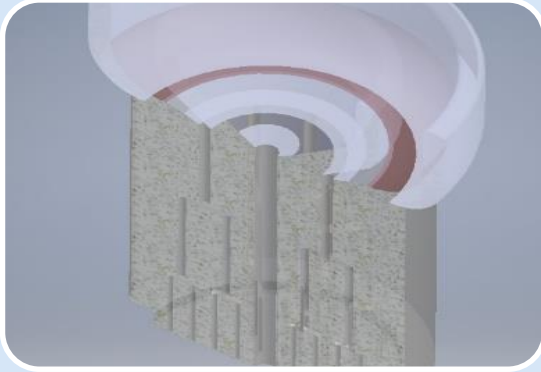
Chen *et al.*, Lab on a Chip (2012)

Rover-mounted system for analysis of liquid samples and detection of amino acids and fatty acids (NASA's Jet Propulsion Laboratory).



Willis *et al.*, *Anal Bioanal Chem* (2015).

Microfluidic device engineering



Design:

- CAD software (e.g. Inventor, Solidworks)
- Simulations (e.g. COMSOL, ANSYS)

Fabrication:

- Application specific (separations, cell culture)
- Materials required (glass, polymer)

Testing:

- Fluidic sealing, flow rates, pressure
- System integration (electronics, detection, pumps)

Microfabrication - overview

\$100+

Cleanroom



- Photolithography and etching (e.g. glass, silicon)
- Polymers (e.g. SU-8)
- Metal deposition

\$10+

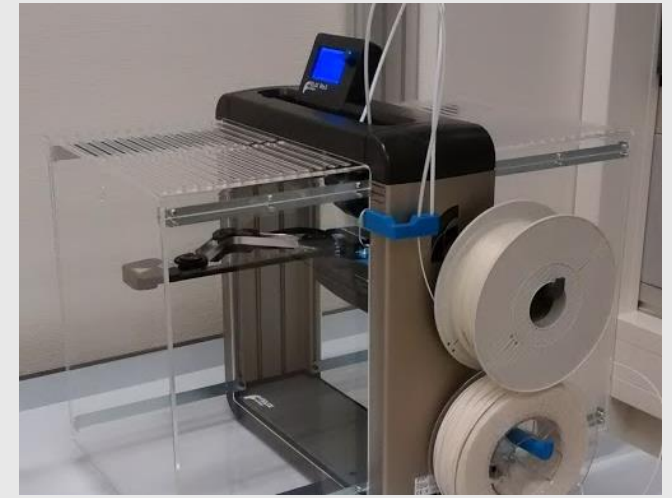
Workshop



- Injection molding and hot embossing
- Micromilling and 3D printing (e.g. selective laser sintering (SLS))

\$1+

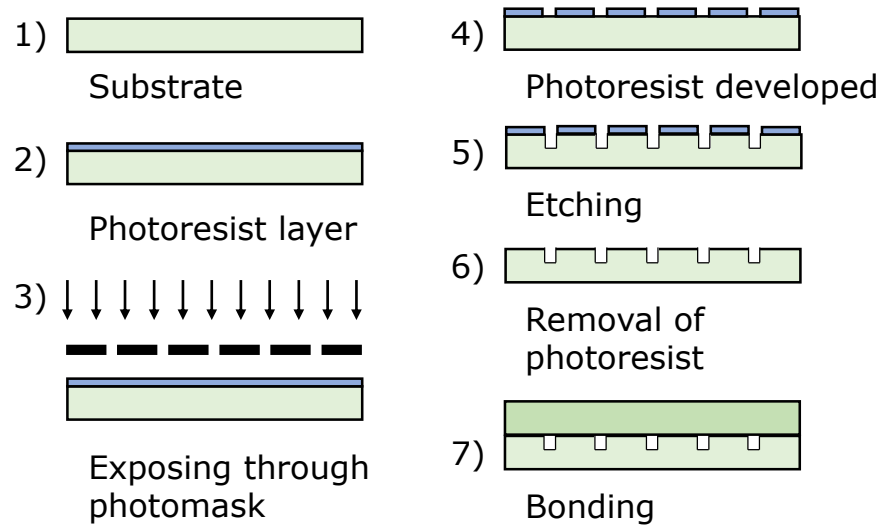
Lab



- 3D printing (e.g. fused deposition modeling (FDM))
- Soft lithography (e.g. with PDMS)

Microfabrication – Cleanroom

Photolithography

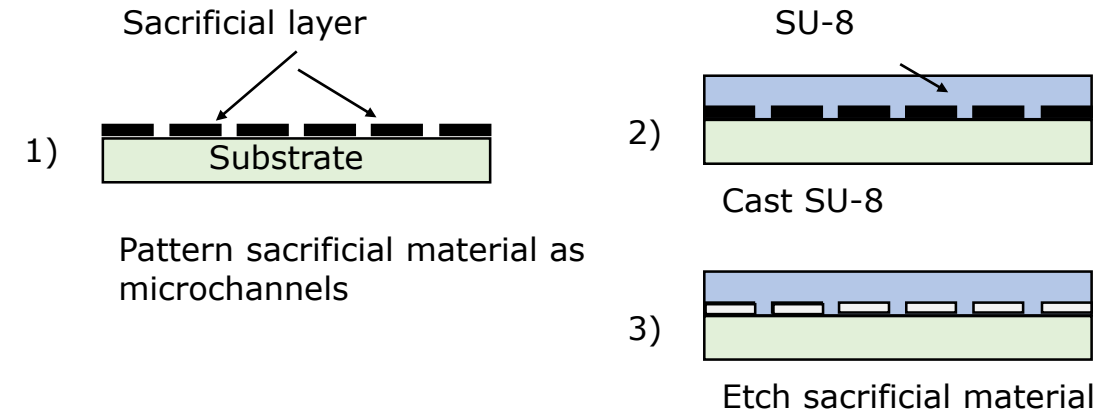


Advantages

- High resolution ($<1\text{ }\mu\text{m}$)
- Suitable for LC and GC
- Integrated functions
- High pressure
- Masks are expensive
- Time consuming (1 day+)
- High skill level
- Limited material choice

Disadvantages

Polymers (SU-8)



Advantages

- High resolution ($1\text{ }\mu\text{m}$)
- Electrode integration
- Multi-layer fluidics
- Integrated functions (valves, pumps, etc)

Disadvantages

- Polymer stability
- Low pressure
- Durability

Microfabrication – Workshop

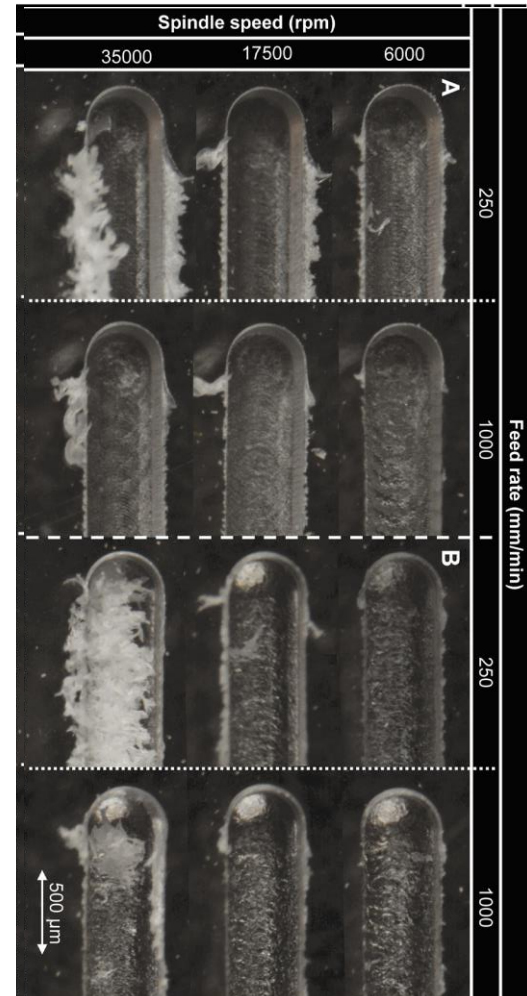
Micromilling

Advantages

- Mid resolution (100 μm)
- Cheap to make
- Repeatability
- Material choice

Disadvantages

- Resolution limitations
- Surface roughness
- Connections
- Bonding steps



Wouters *et al.*, *Journal of Chromatography A* (2017).

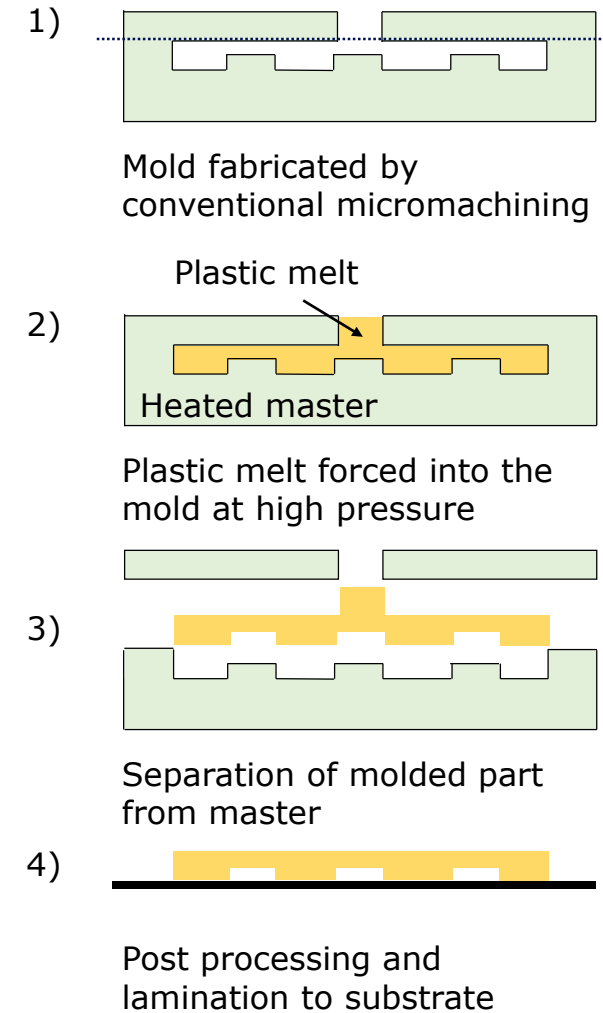
Injection moulding

Advantages

- Material selection
- High throughput
- Repeatability
- High resolution (depends on master)

Disadvantages

- Single material chips
- Expensive for prototypes
- Integrated functions



2009 - USA - Wako Diagnostics - L. Bousse - μ TASWako i30

Microfabrication – Lab

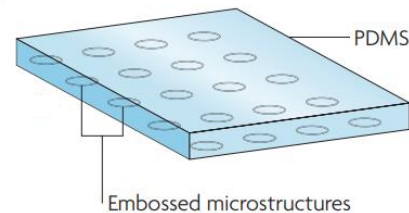
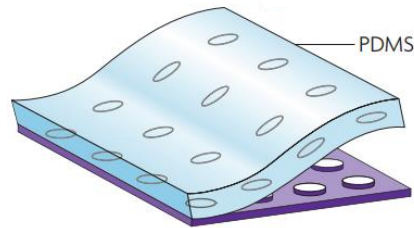
Soft lithography

Advantages

- High resolution (10 μm)
- Cheap to make
- Biocompatibility
- Gas permeable
- Flexibility (good for valves)

Disadvantages

- Biofouling
- Low-pressure systems
- Connection + sealing issues
- Requires a master for casting



Hong *et al.*, *Nat. Biotechnol.* (2004).

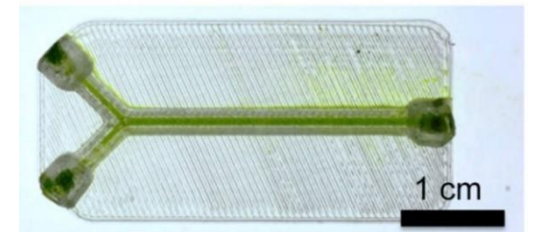
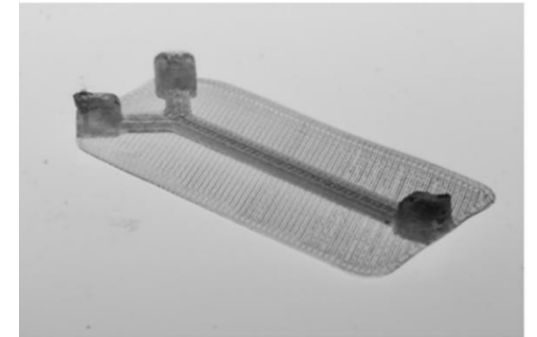
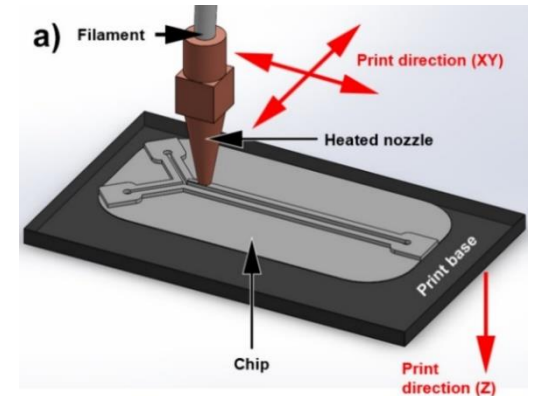
3D printing

Advantages

- Material selection
- Cheap to run
- Robust hardware and software
- Integrated functions

Disadvantages

- Low resolution (100-500 μm)
- Solvent compatibility
- Low throughput

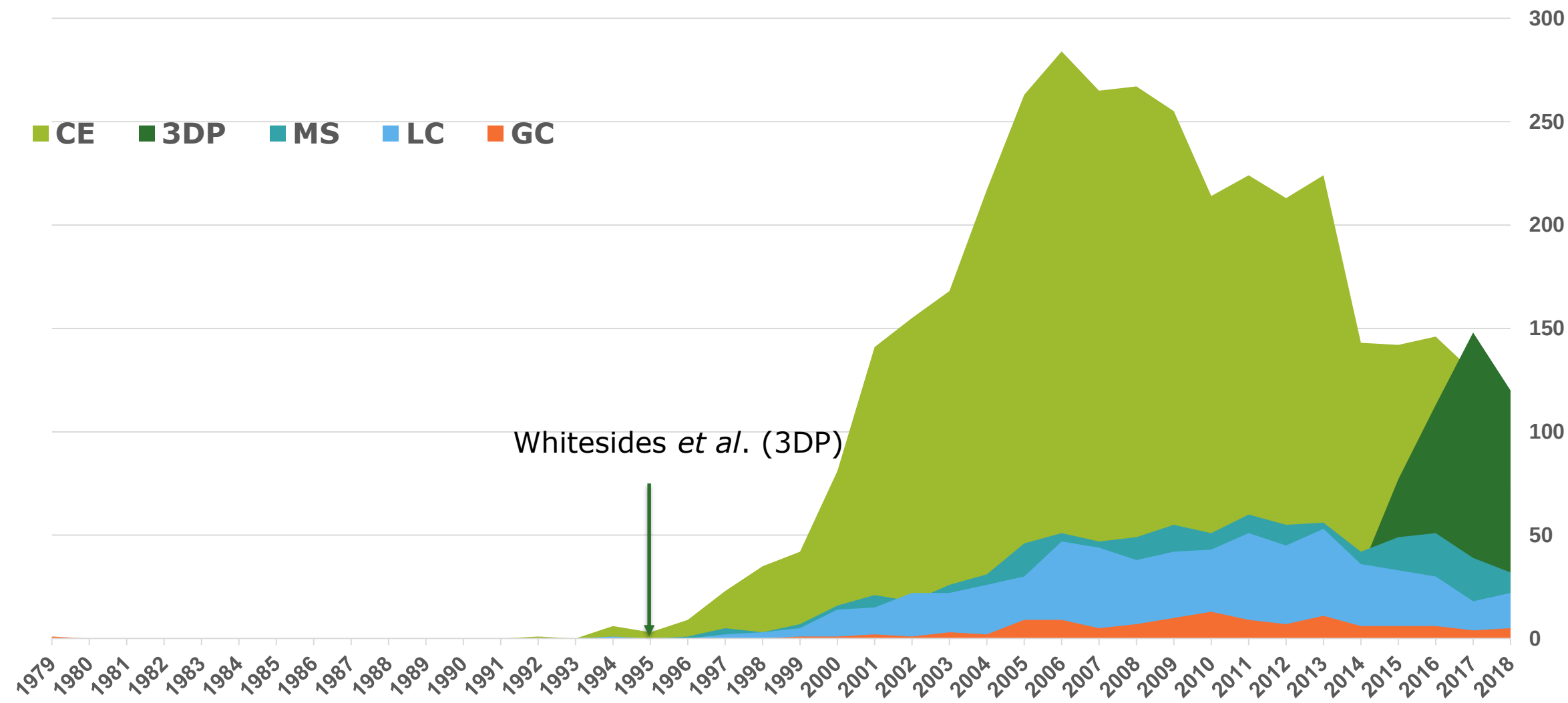


Macdonald *et al.*, *Analytical chemistry* (2017).

Microfabrication - summary

	Cleanroom	Workshop	Lab
Technologies	Photolithography and etching, metal deposition	Injection moulding, micromilling, hot embossing, vacuum casting, 3D printing	3D printing (<i>e.g.</i> FDM, stereo lithography (SLA)), soft-lithography (PDMS)
Resolution (channels)	$\cong 1\text{-}10\text{ }\mu\text{m}$	$\cong 100 - 500\text{ }\mu\text{m}$	$\cong 50 - 1000\text{ }\mu\text{m}$
Materials	- Soda lime, quartz, pyrex, silicon - Polymers (PMMA, SU-8 etc)	- Thermoplastic polymers, (COC, PEEK, ABS, PMMA) - Titanium, copper, steel	- Poly lactic acid (PLA), acrylonitrile butadiene styrene (ABS) - Polydimethylsiloxane (PDMS)
Cost	\$100+	\$10+	\$1+
Throughput	Medium volume	High volume	Low volume

3D-printed microfluidics



3D-printed chromatography

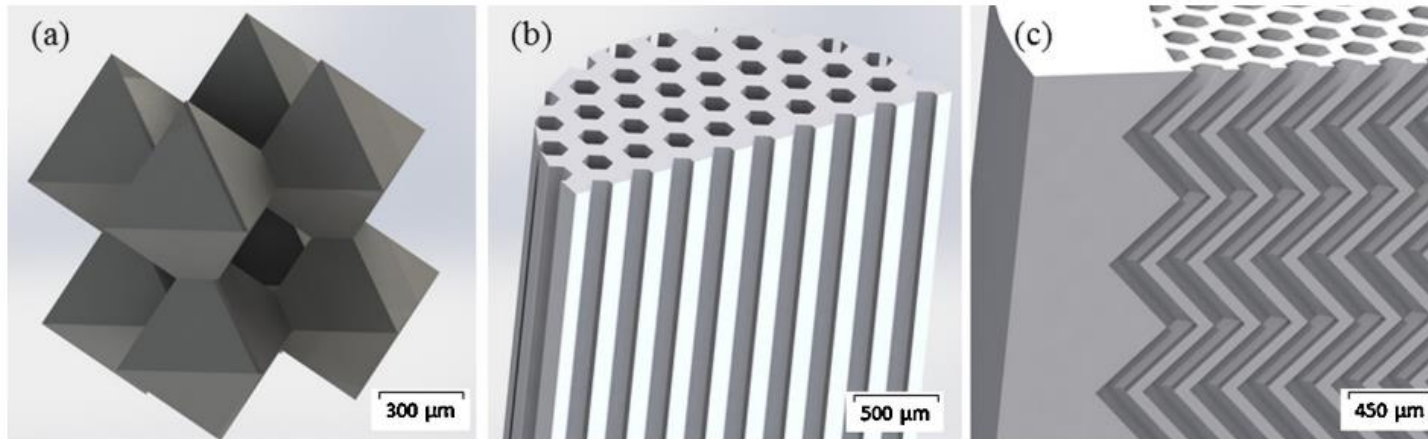


Fig. 1. The three bed geometric designs: (a) simple cubic beads, (b) straight channels, (c) herringbone channels.

Highlights

- Comparison between different packing and flow distributors.
- From design to prototype, close replication of CAD models
- Integrated flow distributors and connectors

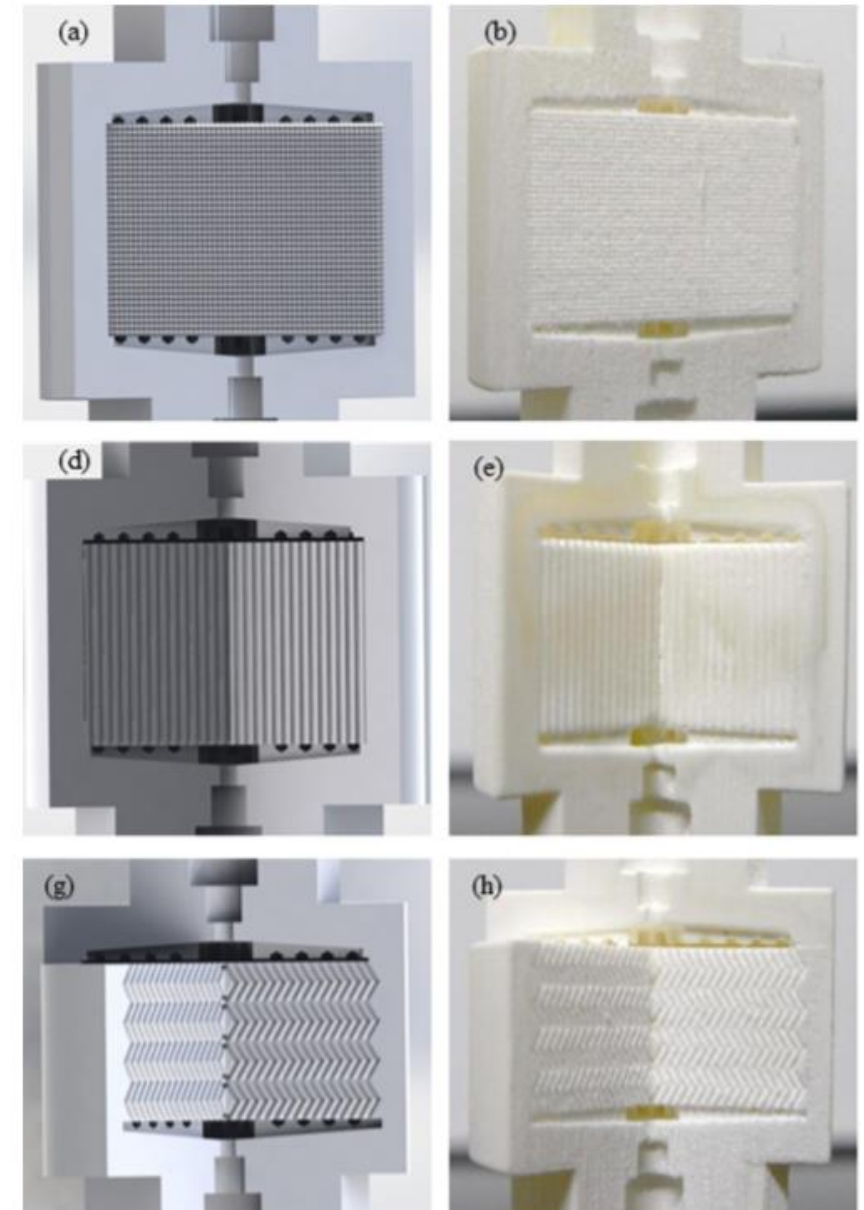
Tech details

Preparative LC

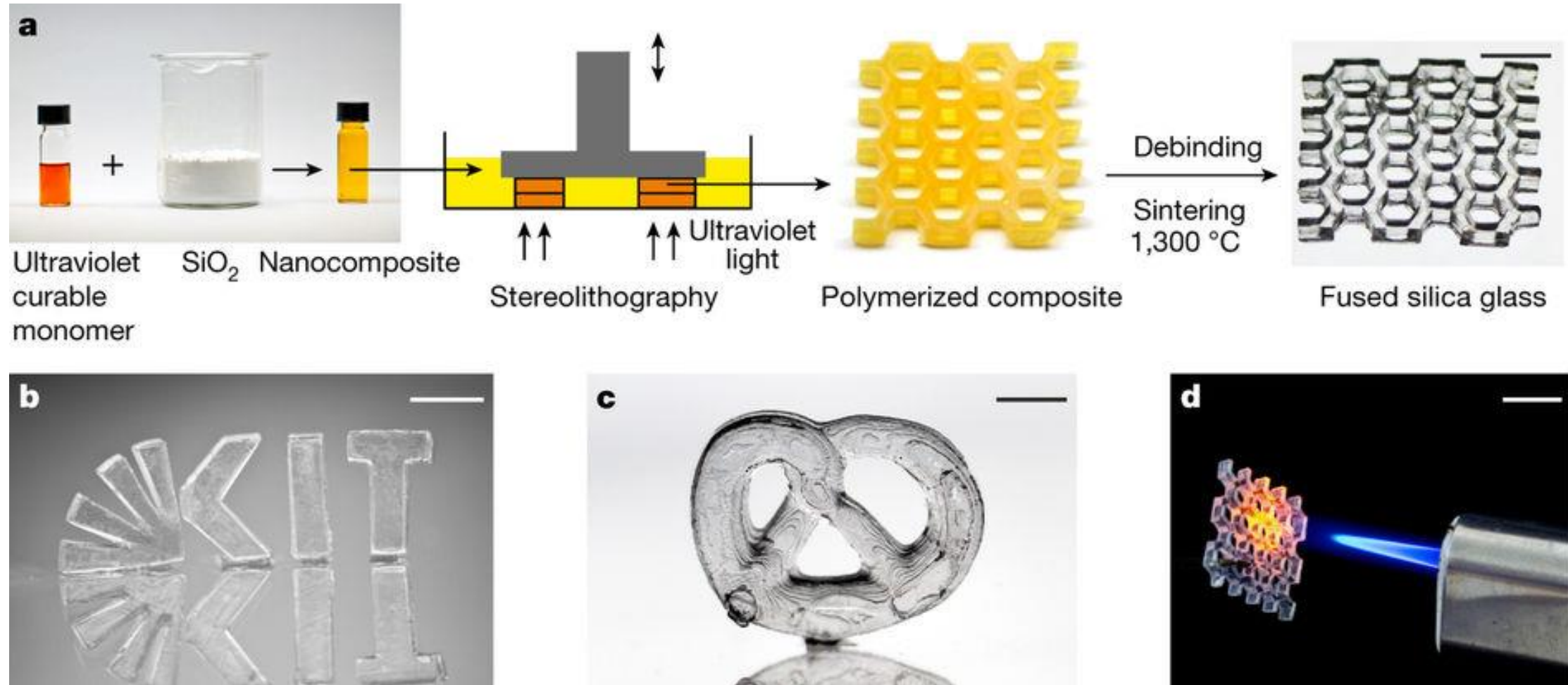
Acrylonitrile-butadiene-styrene (ABS)

3D printing

150 μm channels or 115 μm particles

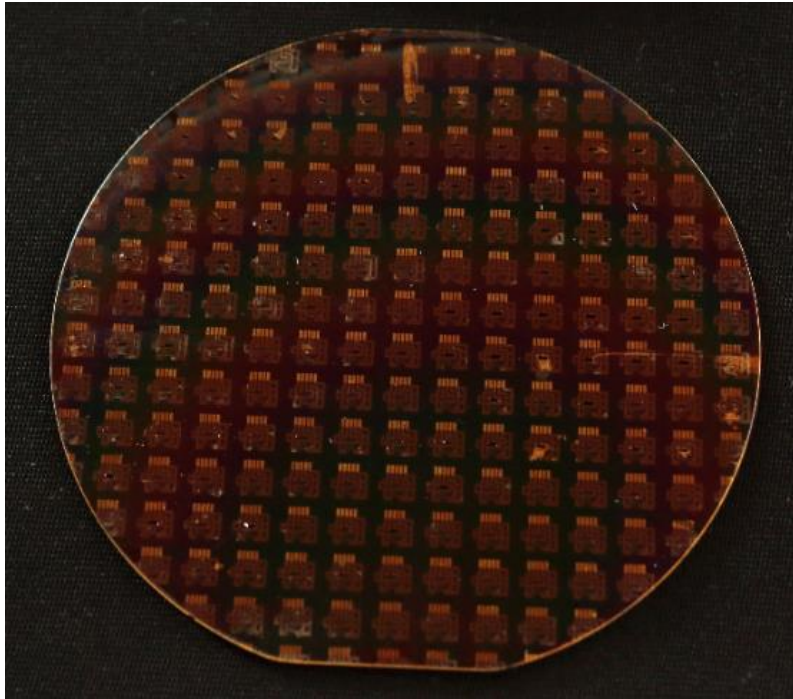


3D printing of transparent fused silica glass



**3. Microfluidic
technology in one-
dimensional liquid
chromatography**

Gas chromatography microchips



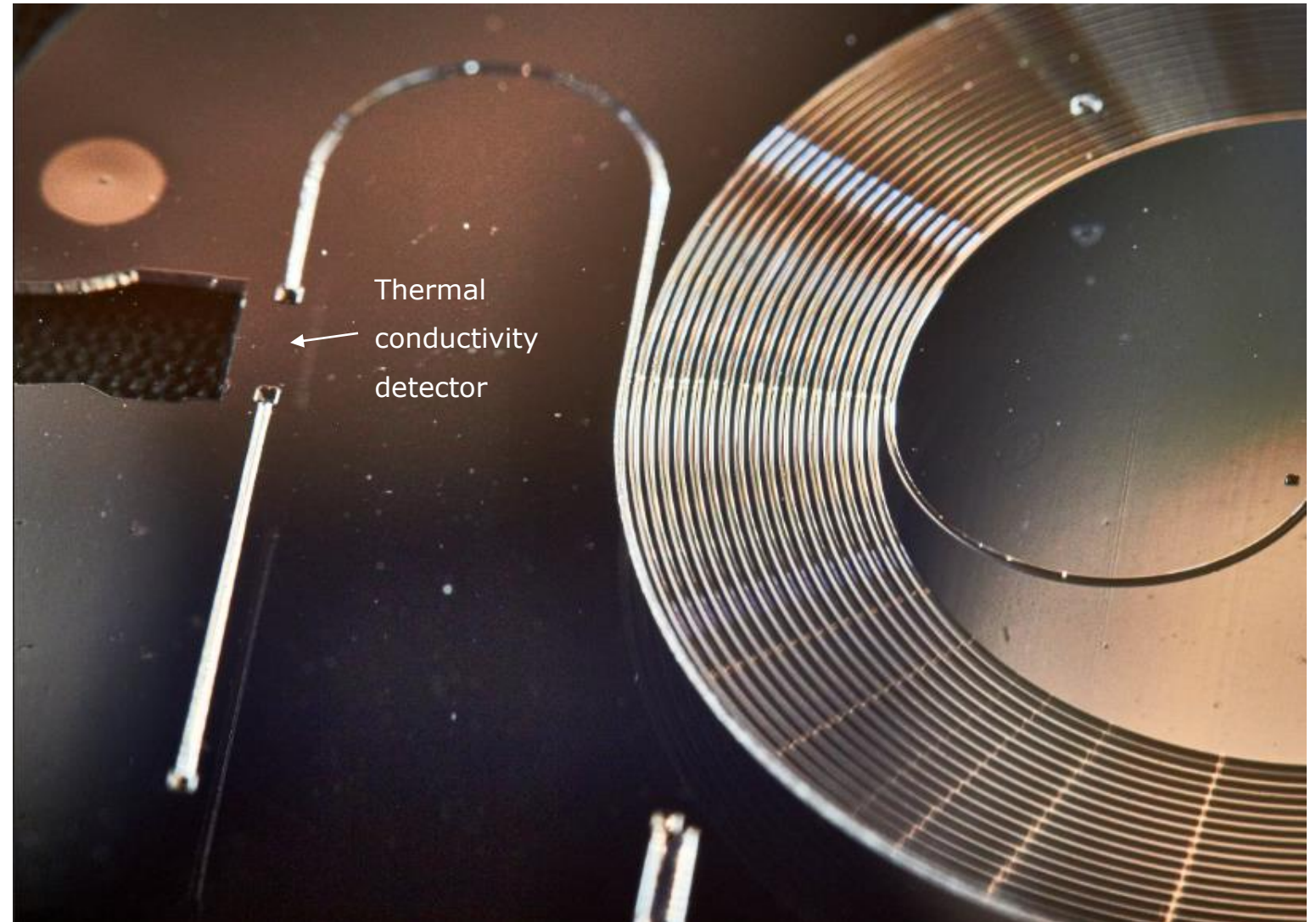
Tech details

Gas chromatography

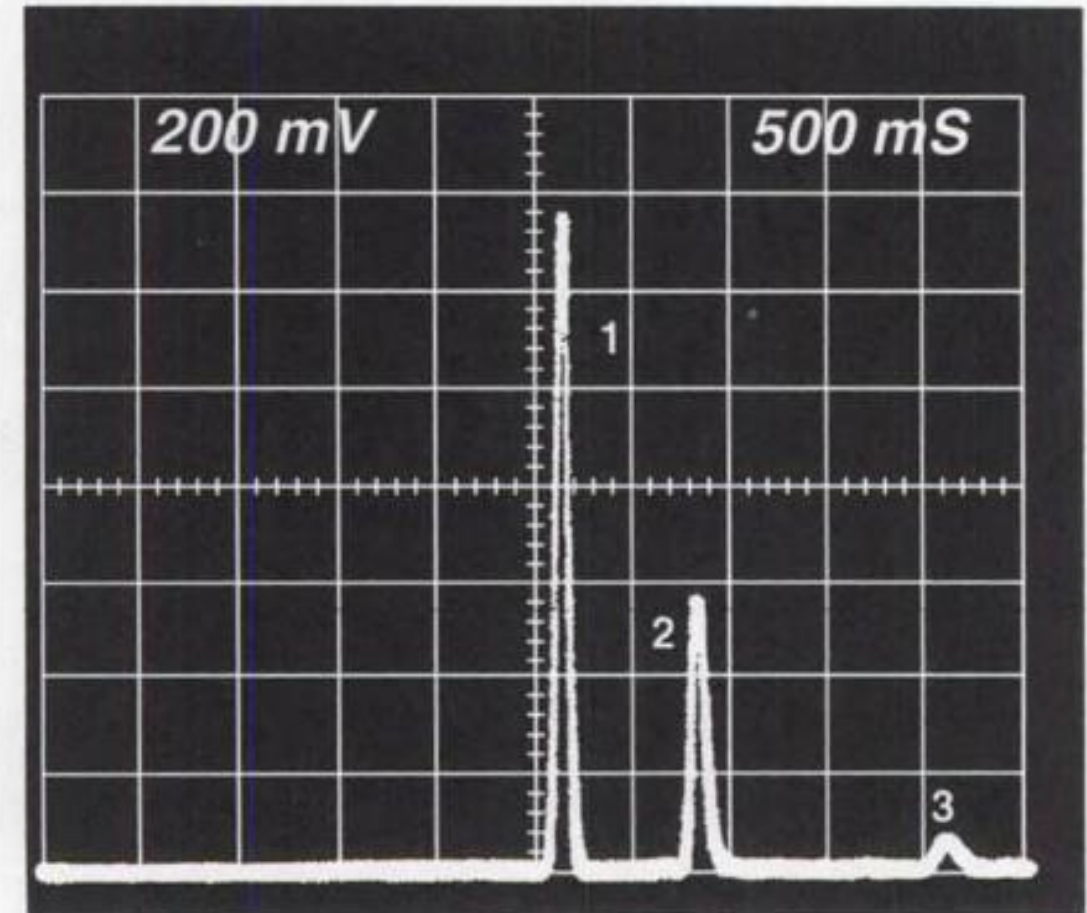
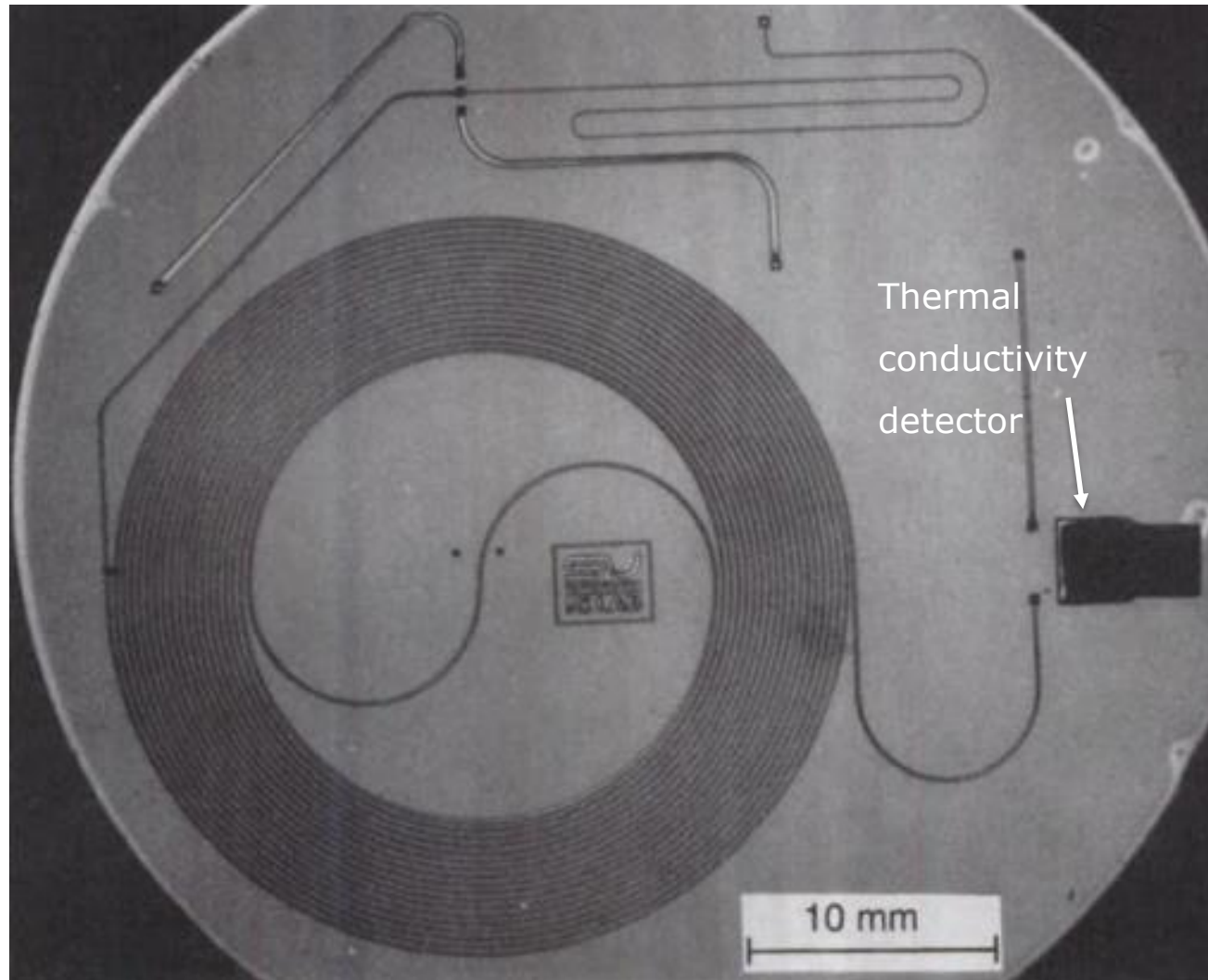
Silicon + glass

Lithography + etching

1.5 m long channel (200 x 30 μm , wxd)

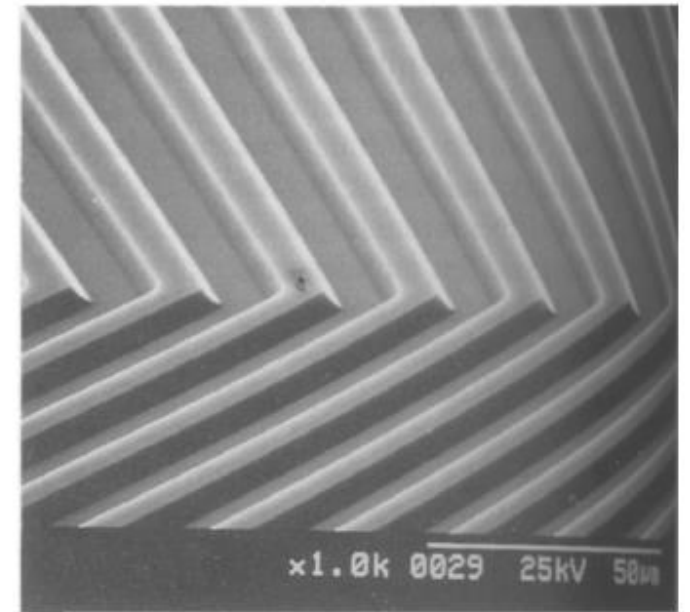
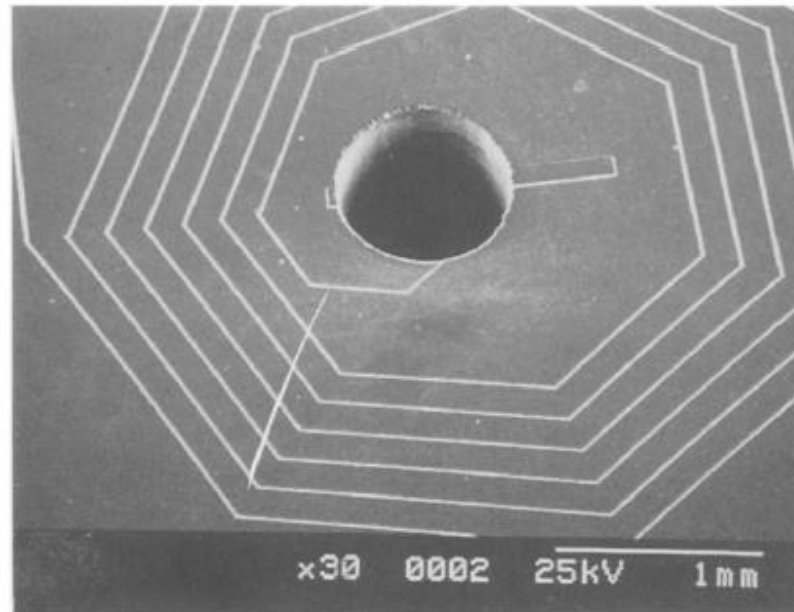
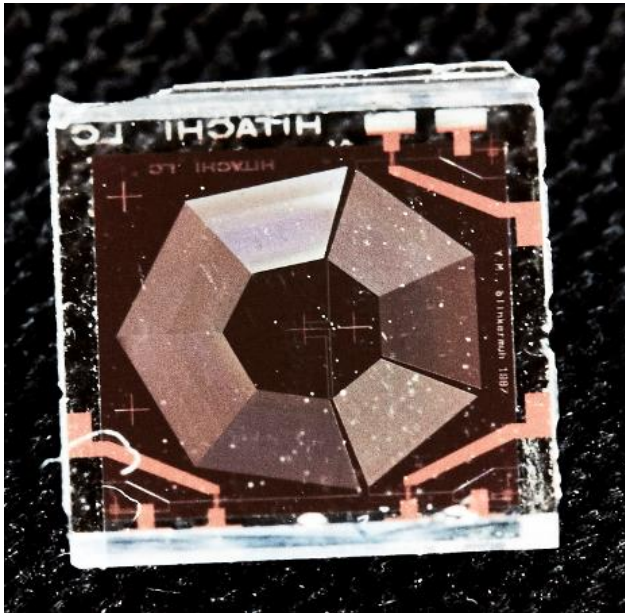


Gas chromatography microchips



(1) nitrogen 2.6 s, (2) pentane 3.3 s,
(3) hexane 4.6 s

First microchip open tubular liquid chromatography



Tech details

Open-tubular liquid chromatography

Silicon + Pyrex

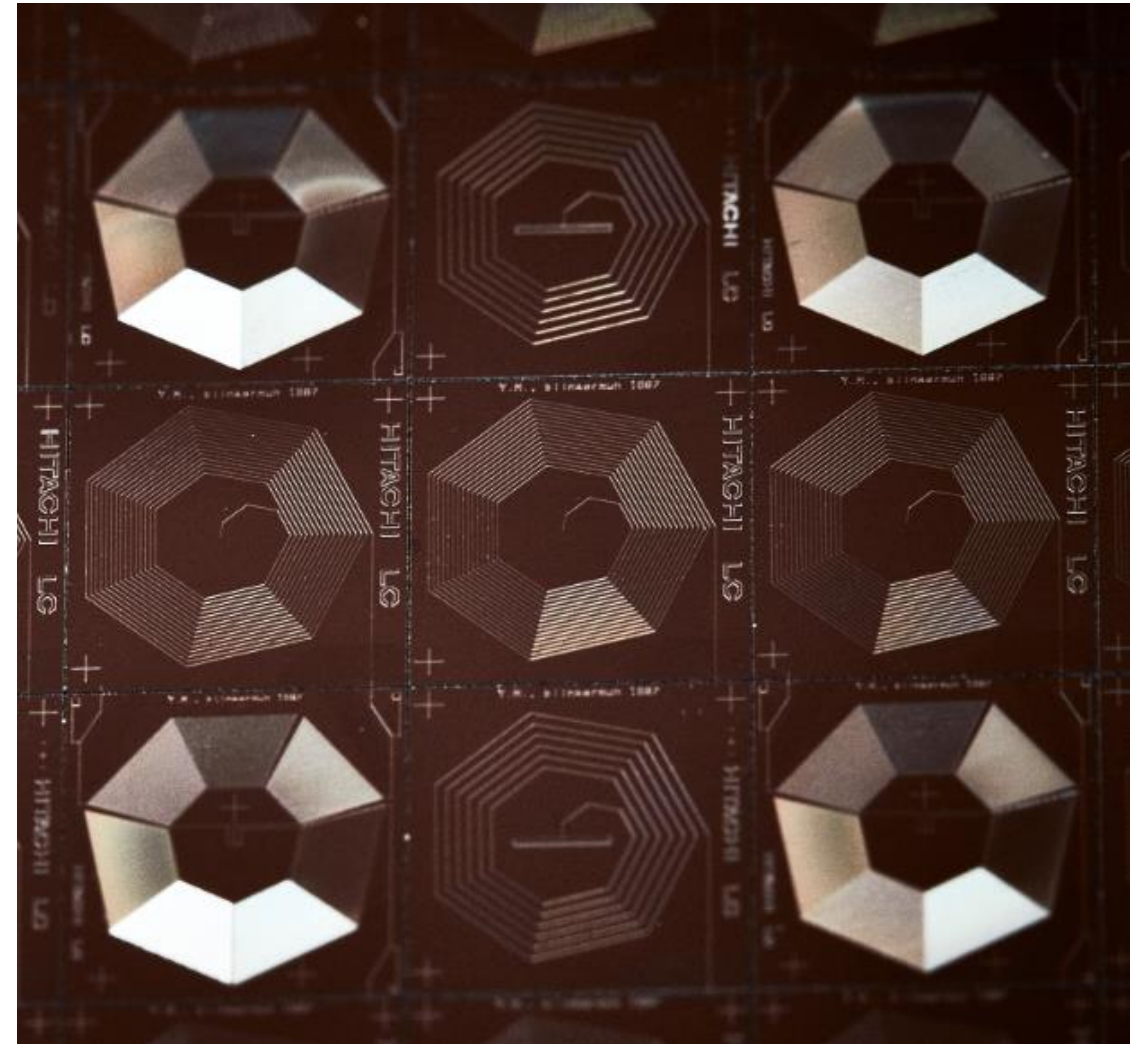
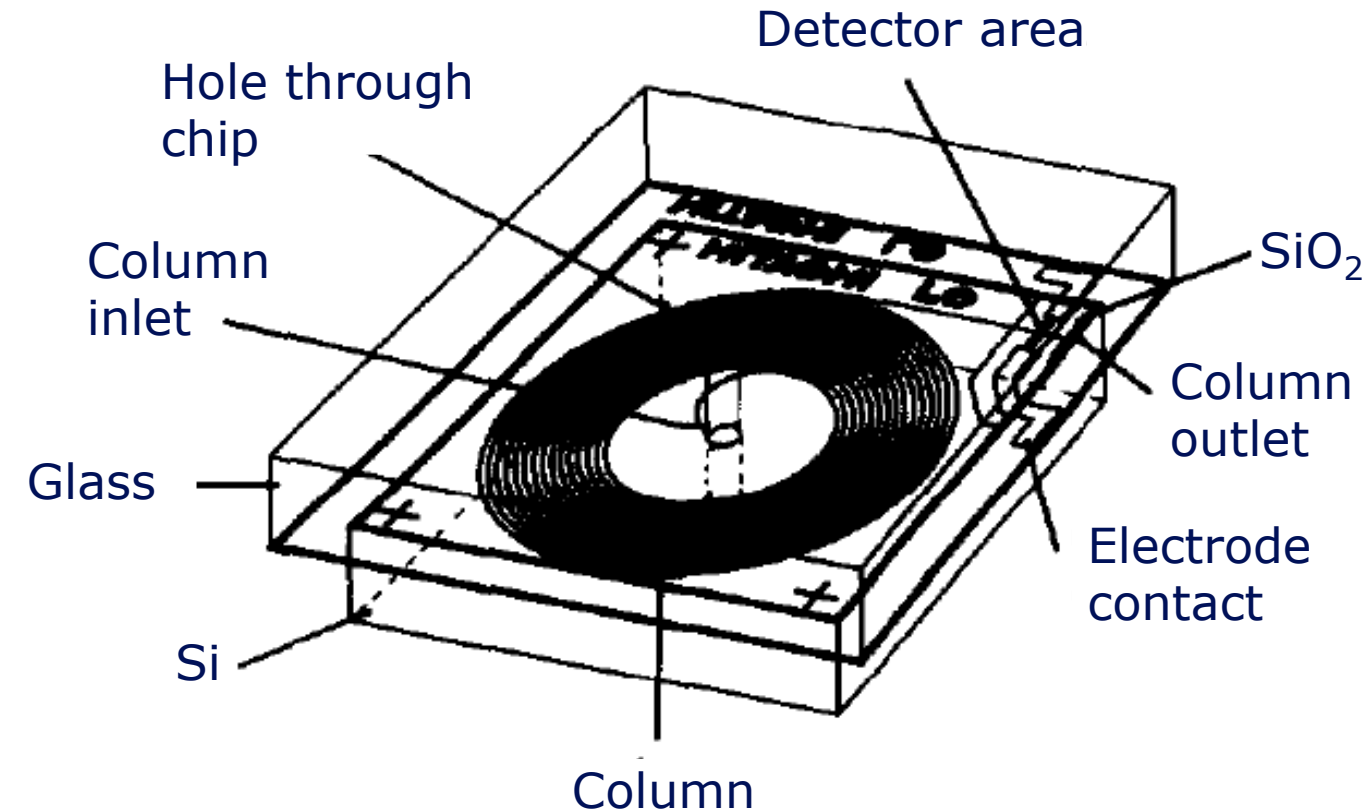
Etching, sputtering, bonding

15 cm long channel (6 x 2 µm, wxd)

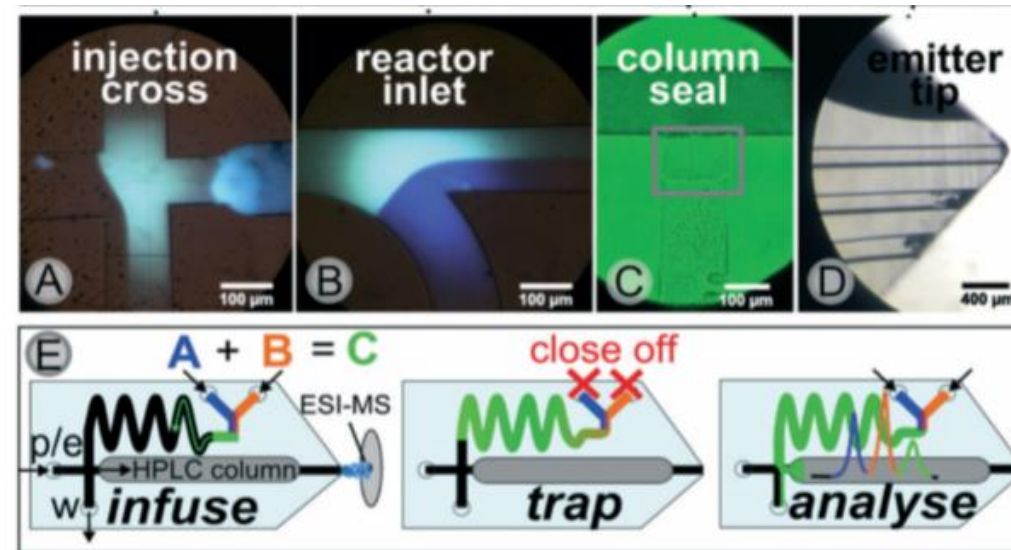
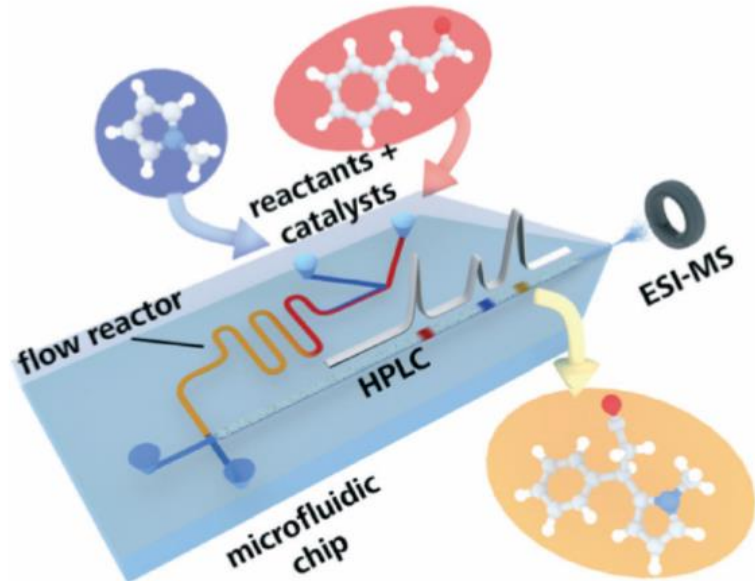
Highlights

- Conductometric detector, chip holder and pressure-pulse driven injector using a conventional LC pump and valves.
- Theoretical separation efficiencies of 8000 plates (1 min) and 25.000 plates in 5 mins.

First microchip open tubular liquid chromatography



Microchip HPLC-MS



Tech details

HPLC to MS

Glass

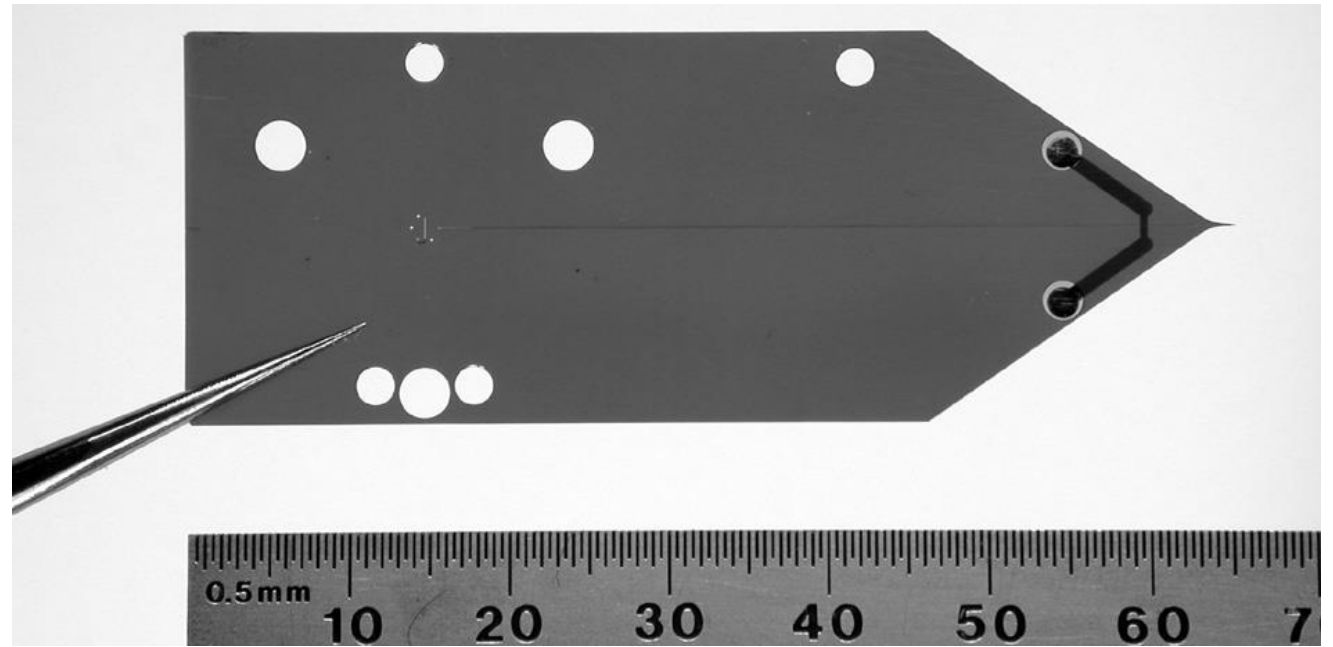
photolithography, wet etching, powder-blasting and bonding

130 or 155 x 45 µm, wxd

Highlights

- HPLC/nano-ESI-MS functionality on glass chip
- Nanoliter-range for studying reactions in quasi real-time
- Aminocatalyzed reactions, including asymmetric iminium-catalyzed Friedel–Crafts alkylations

Agilent HPLC-MS chip



Tech details

HPLC-MS

Polyimide

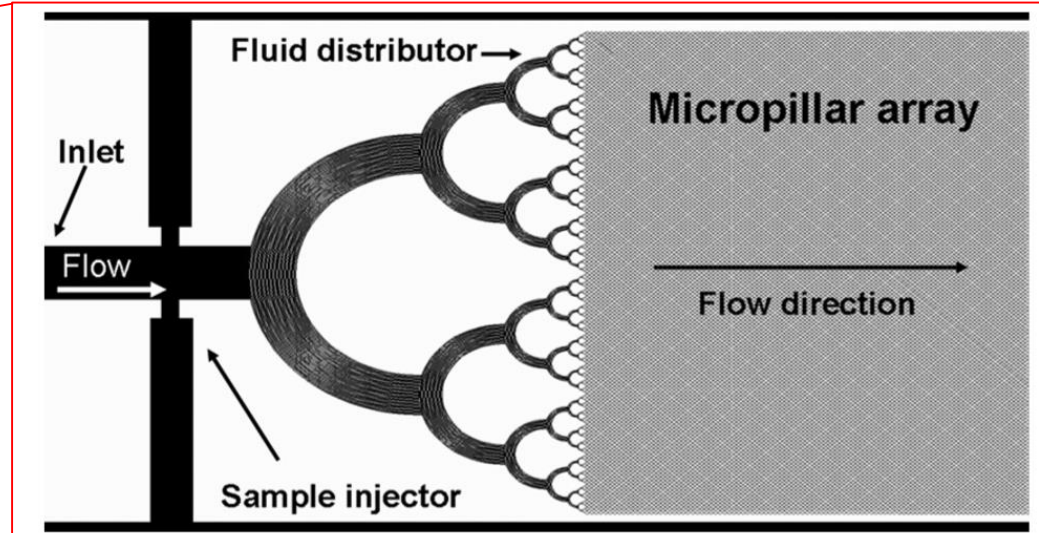
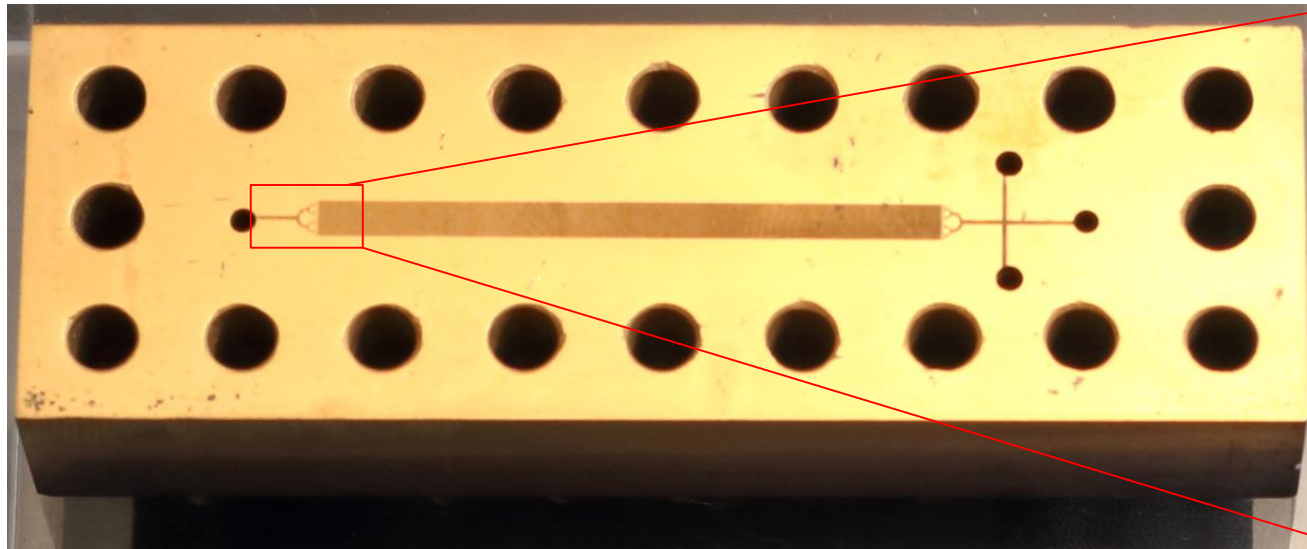
Laser ablation, lamination

1(50) mm long LC channel (75 x 40 μm , wxd) with 40 nL enrichment channel.

Highlights

- HPLC-chip with integrated nano-LC column, enrichment column and nanoelectrospray tip
- Additional sample processing can be integrated on same device.
- Transfer volume minimized by installing chip within LC rotary valve.

Microfabricated graphitic carbon HPLC



Tech details

HPLC

Graphite + stainless steel

Micromilling, 795 nm femtosecond laser ablation

~0.53 million micropillars over a length of 37 mm and 2.3 mm width

Highlights

- Significant difference in retention of the two eluting test species.
- Micropillar device increased the surface area by 250 times compared to an open-tubular duct.
- <1 min run times with low reagent consumption.

Micropillar-array columns (μ PAC)

Highlights

- Perfectly-ordered, reproducible pattern.
- Lower backpressure (high permeability).
- Longer columns (50-200 cm).

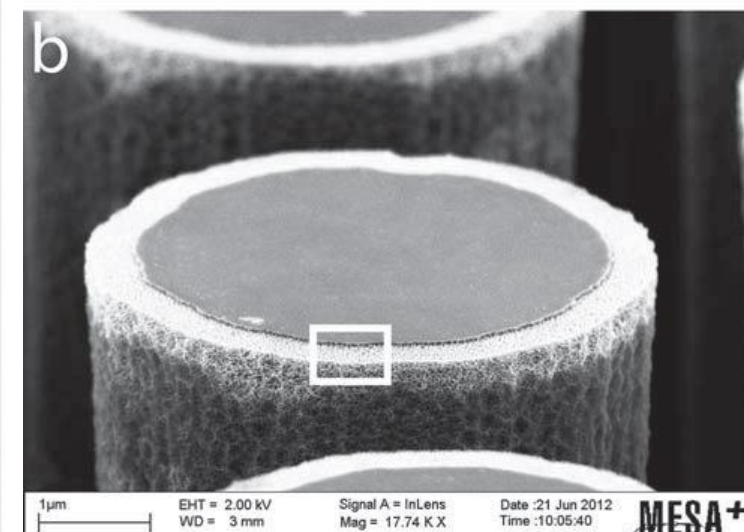
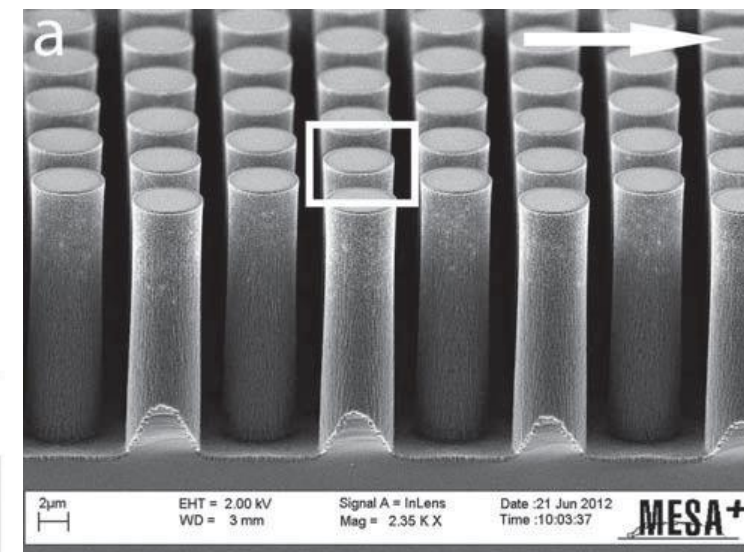
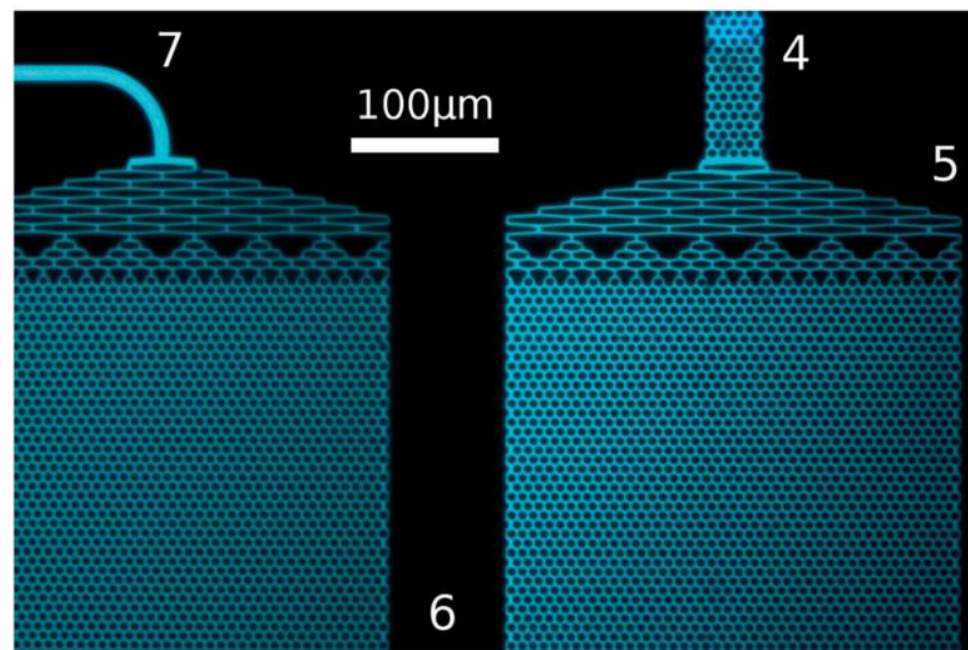
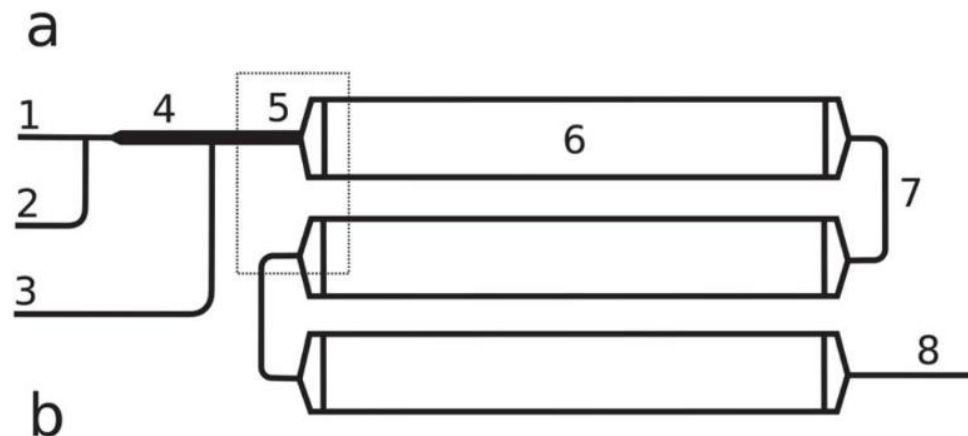
Tech details

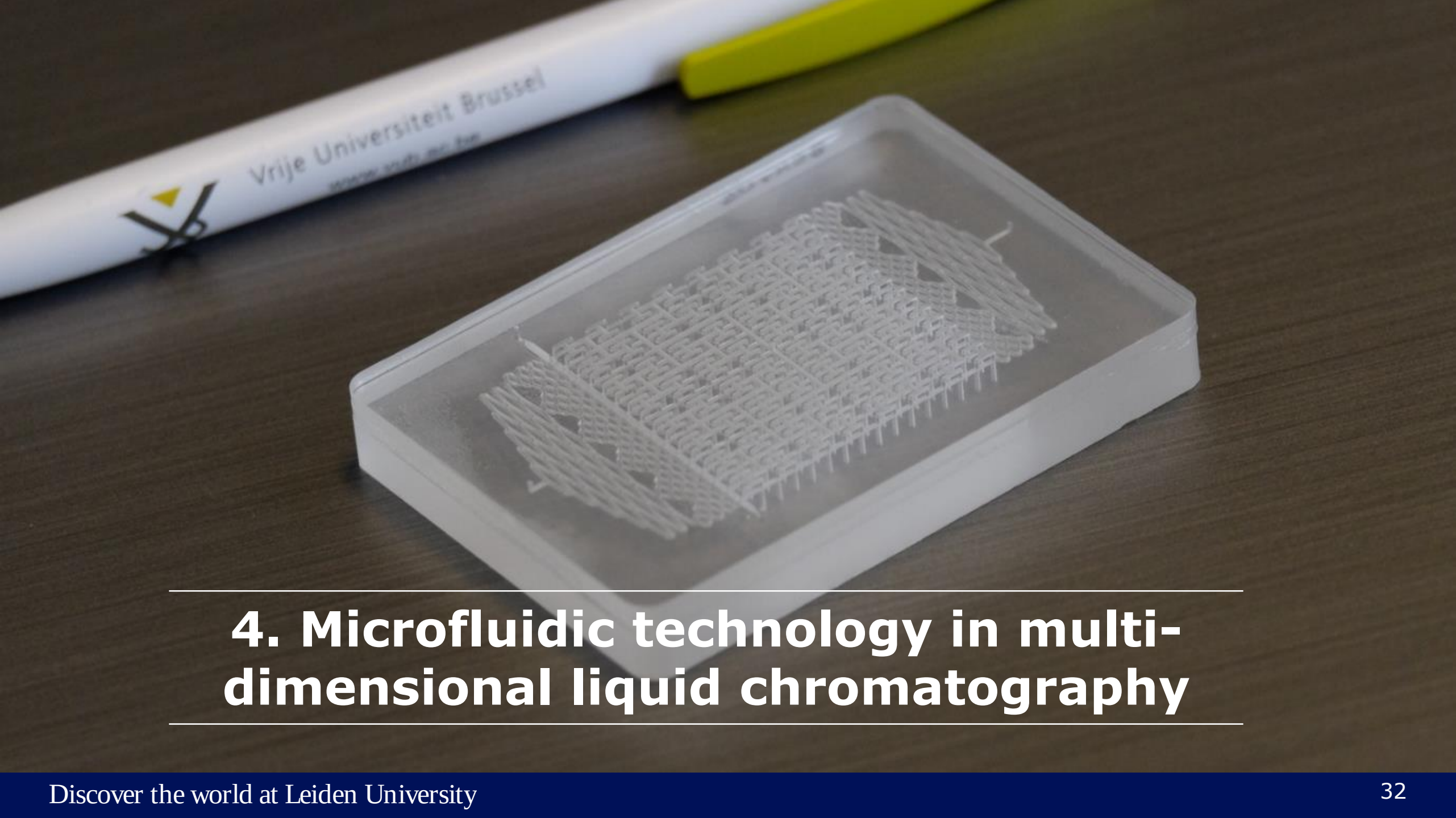
HPLC

Silicon wafer

photolithography

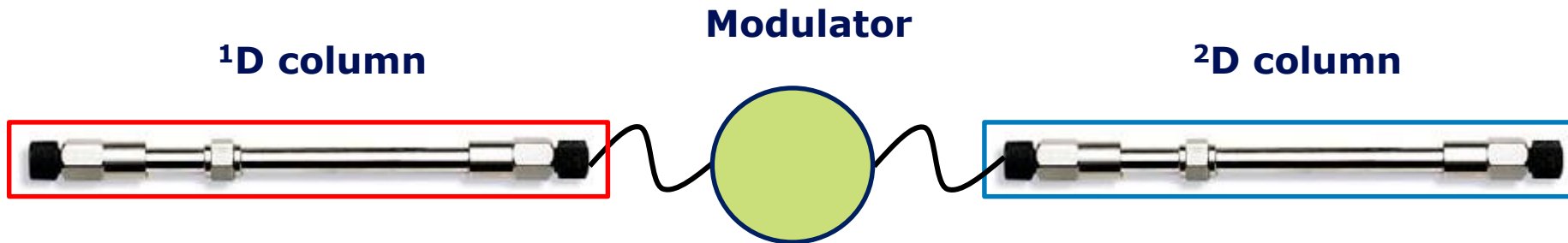
300 mm wide channels containing 5 μ m pillars with porous outer layer





4. Microfluidic technology in multi-dimensional liquid chromatography

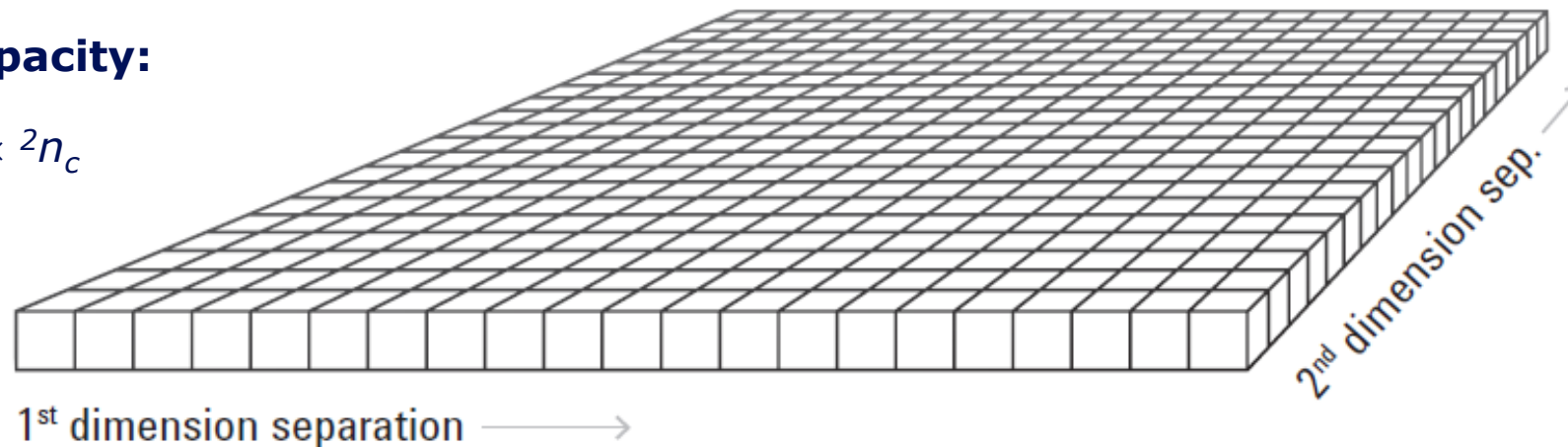
Two-dimensional chromatography



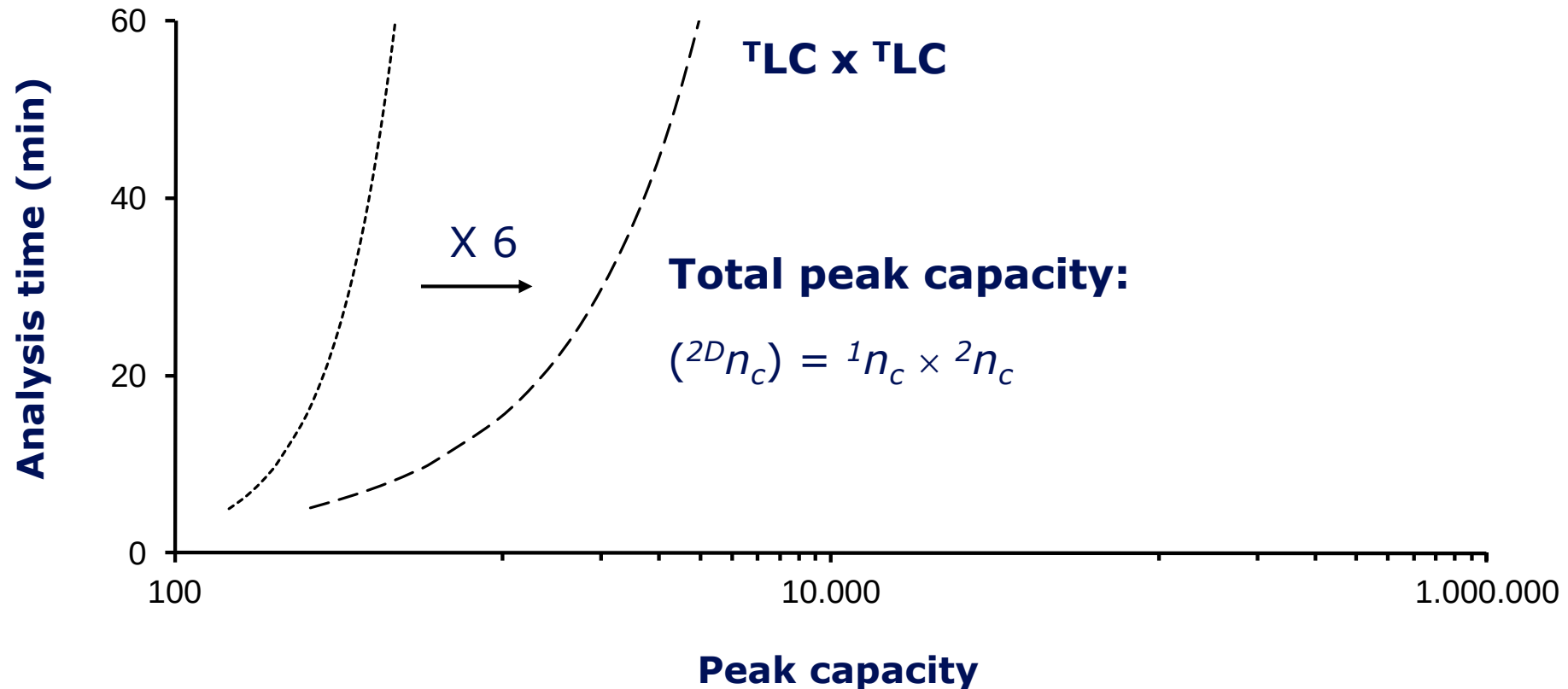
- **Multi-dimensional LC** = series of separation mechanisms which can occur in space or in time.

Total peak capacity:

$$({}^{2D}n_c) = {}^1n_c \times {}^2n_c$$



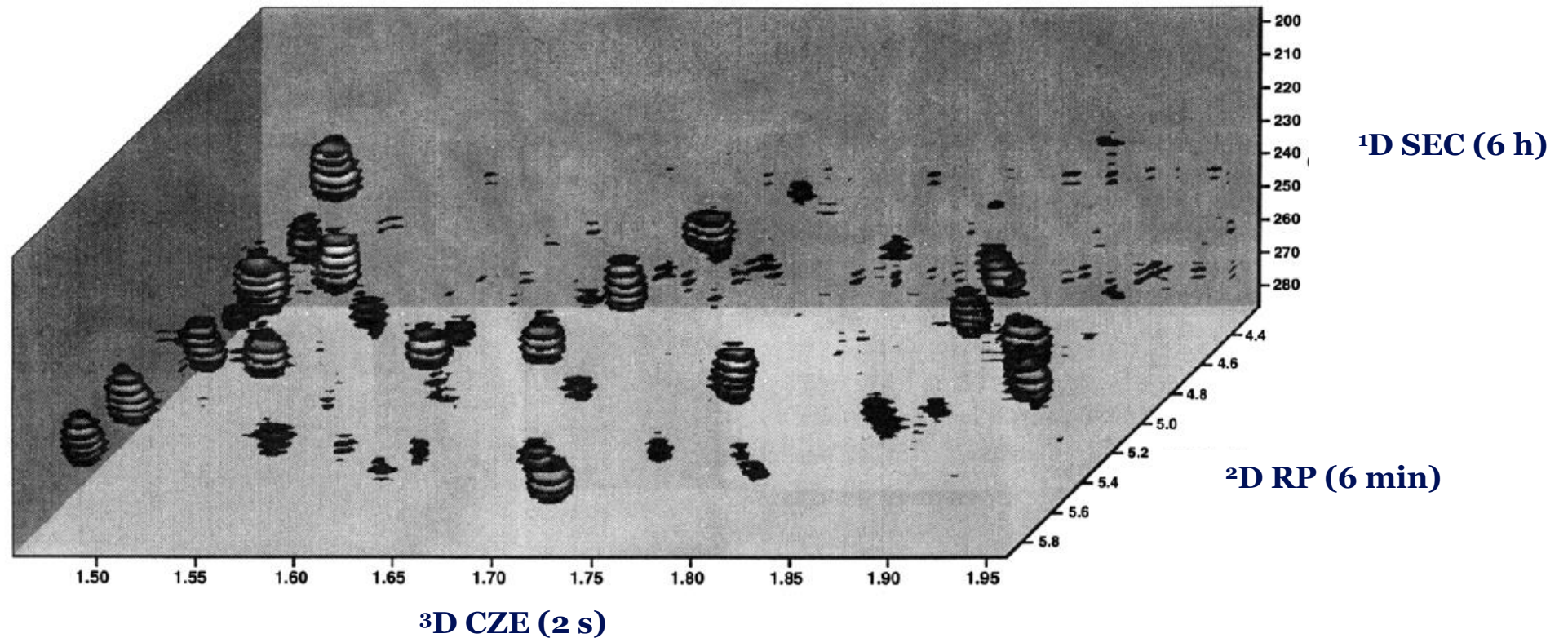
Trade-off between time and efficiency



$TLC \times TLC$: Peak capacities in the thousands, roughly a peak per second.

Three-dimensional LC: coupled-column approach

- 3D-LC separation of digest of ovalbumin (size-exclusion chromatography (SEC), reversed-phase chromatography (RP), and capillary zone electrophoresis (CZE))
- Peak capacity = 2800, analysis time = 400 min



Development of microfluidic devices for spatial two- and three-dimensional liquid chromatography

Brussels, Belgium

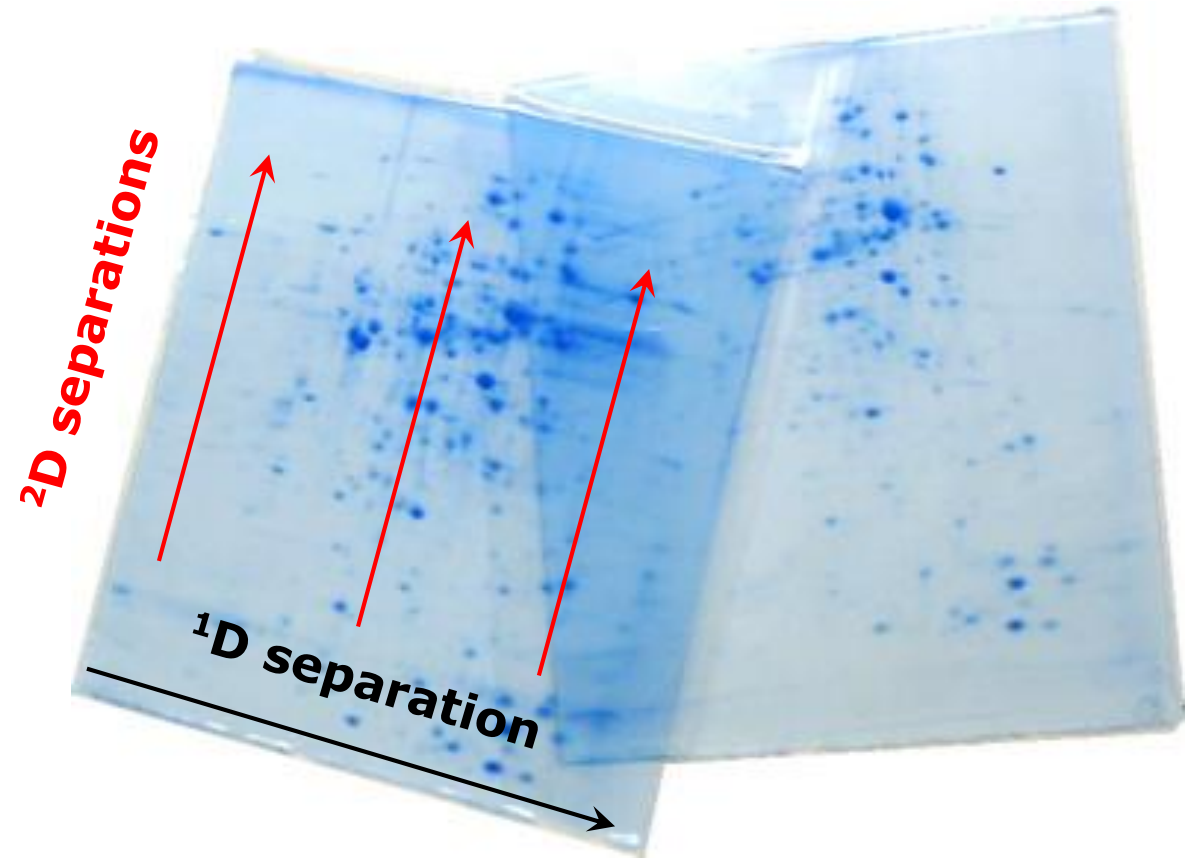
Spatial chromatography

Spatial 2D-LC

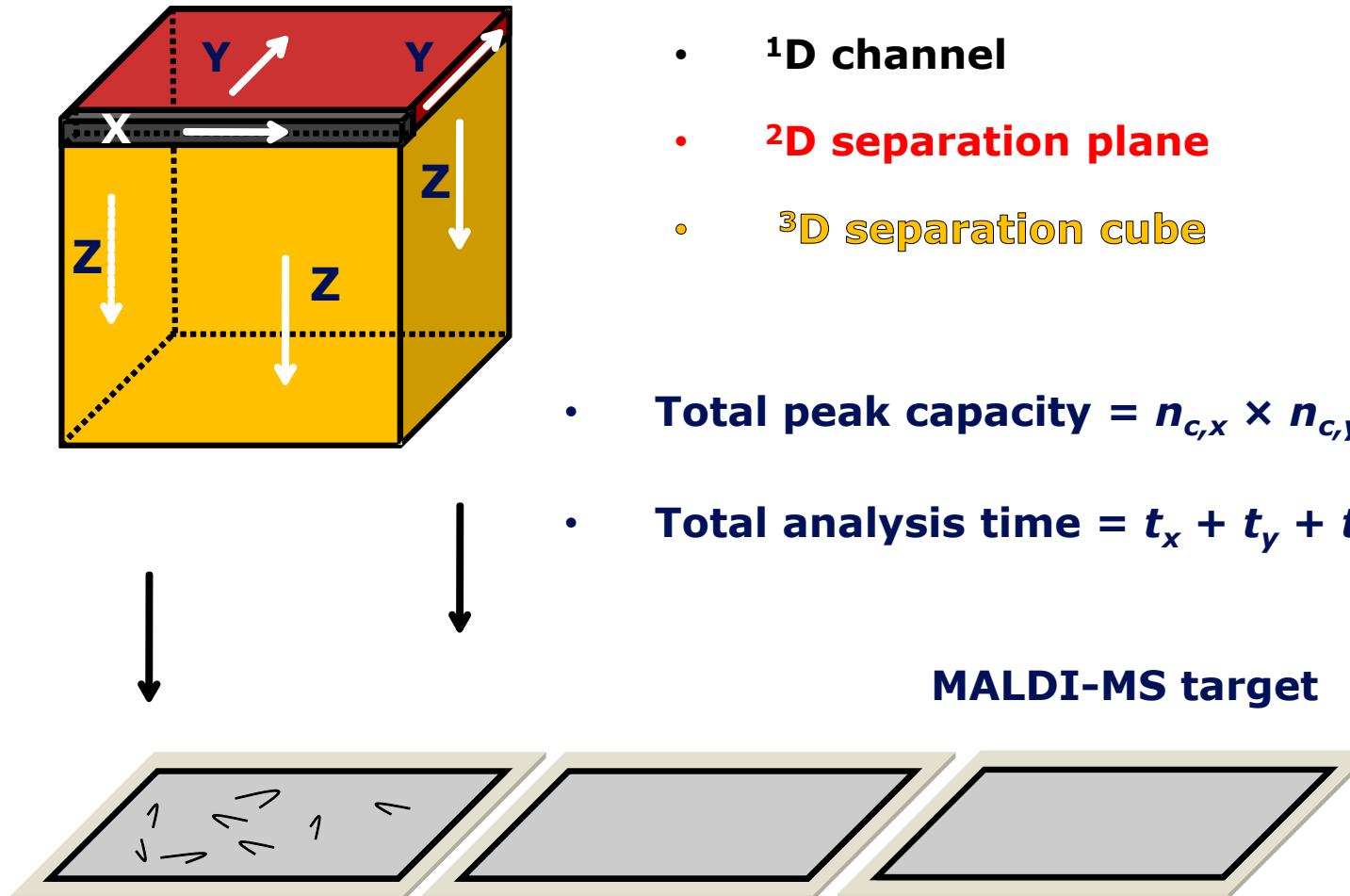
- Components separated in the space domain, with each peak being characterized by its x- and y-coordinates in the plane.
- Examples: thin-layer chromatography (**TLC**) and two-dimensional polyacrylamide gel electrophoresis (**2D-PAGE**).

Advantages:

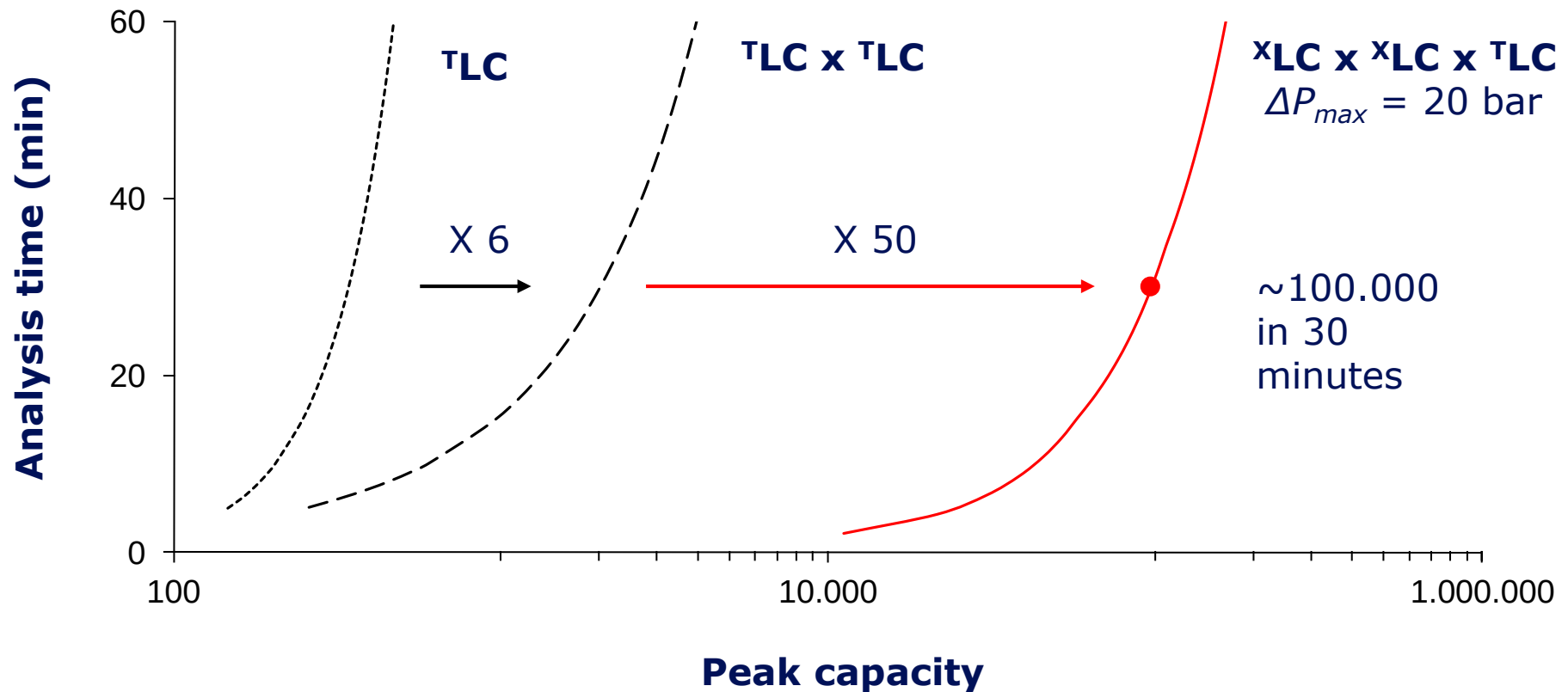
- Reduced analysis time and high peak-production rate due to **parallel 2D analysis**.



Spatial three-dimensional liquid chromatography



Column-based vs. spatial approaches



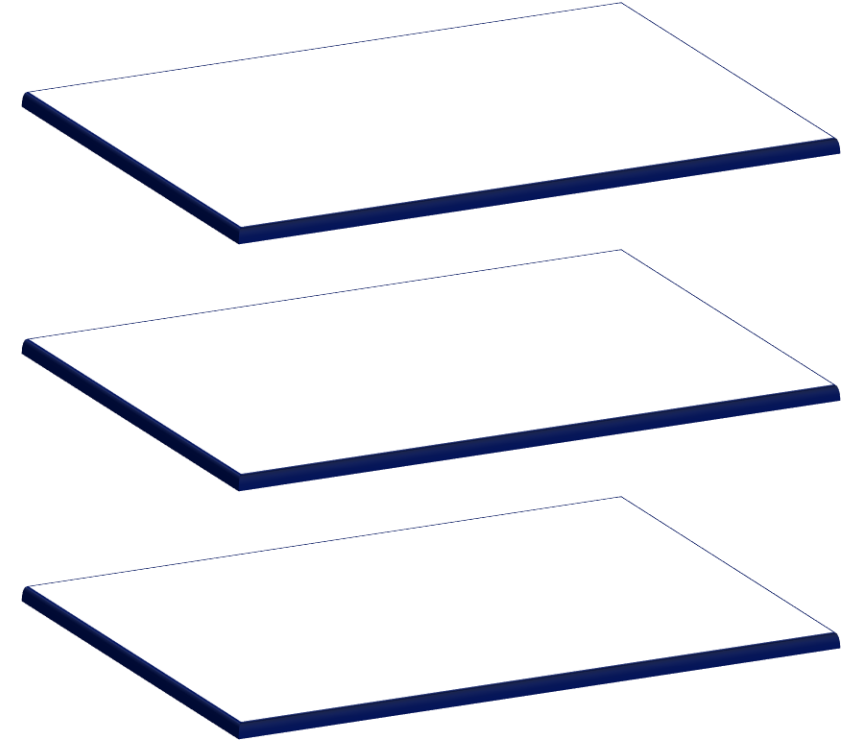
$\times LC \times \times LC \times \tau_{LC}$: Potential for unprecedented separation power.

Design challenges

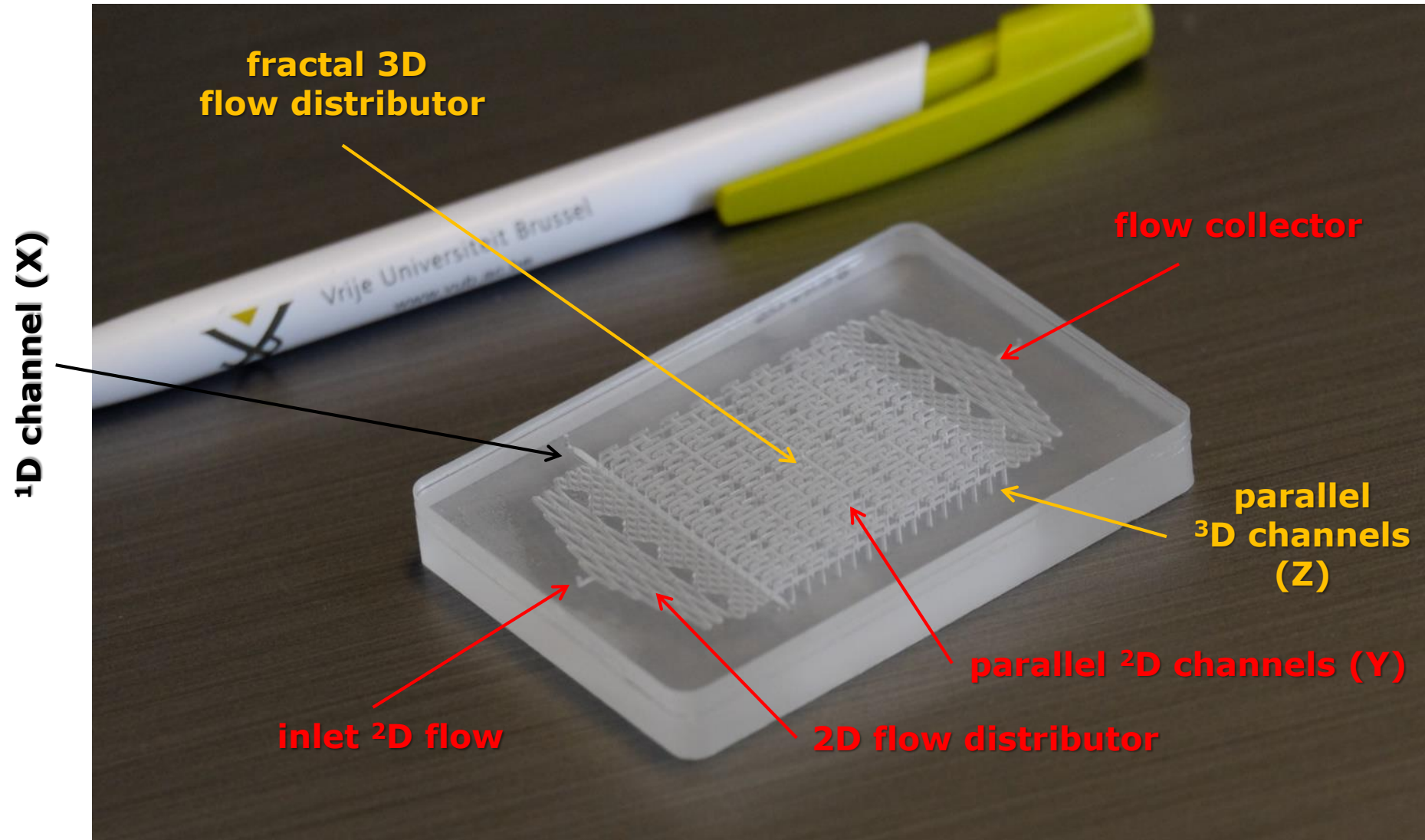
1. **Flow control** and **analyte transfer** between dimensions
2. Implementation of **stationary phases**
3. **Detection** of separated components
4. Macro-to-micro **interfacing**

First approach:

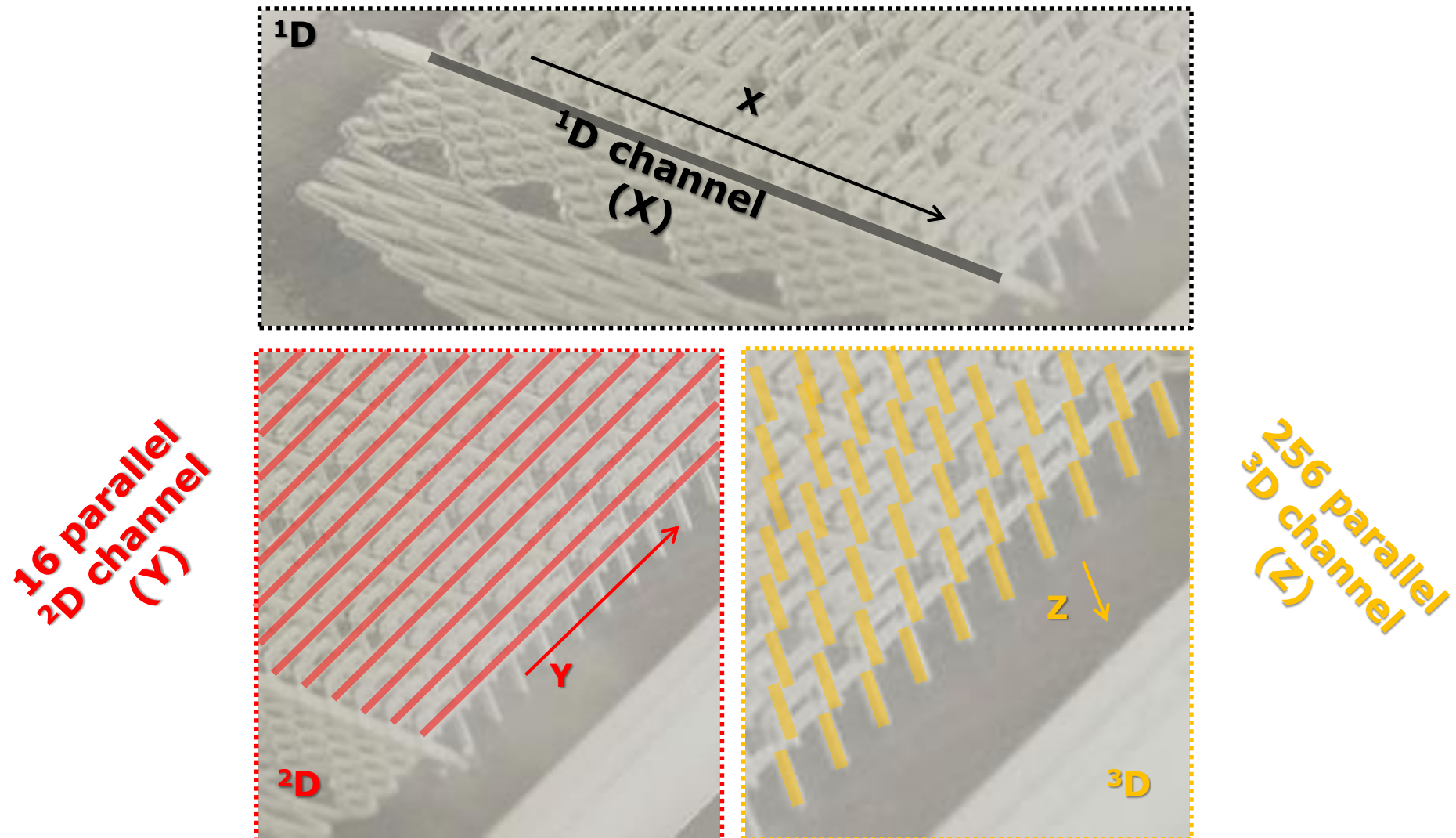
Stacked-layer microfluidic device with discrete channels and flow distributors.



A first prototype

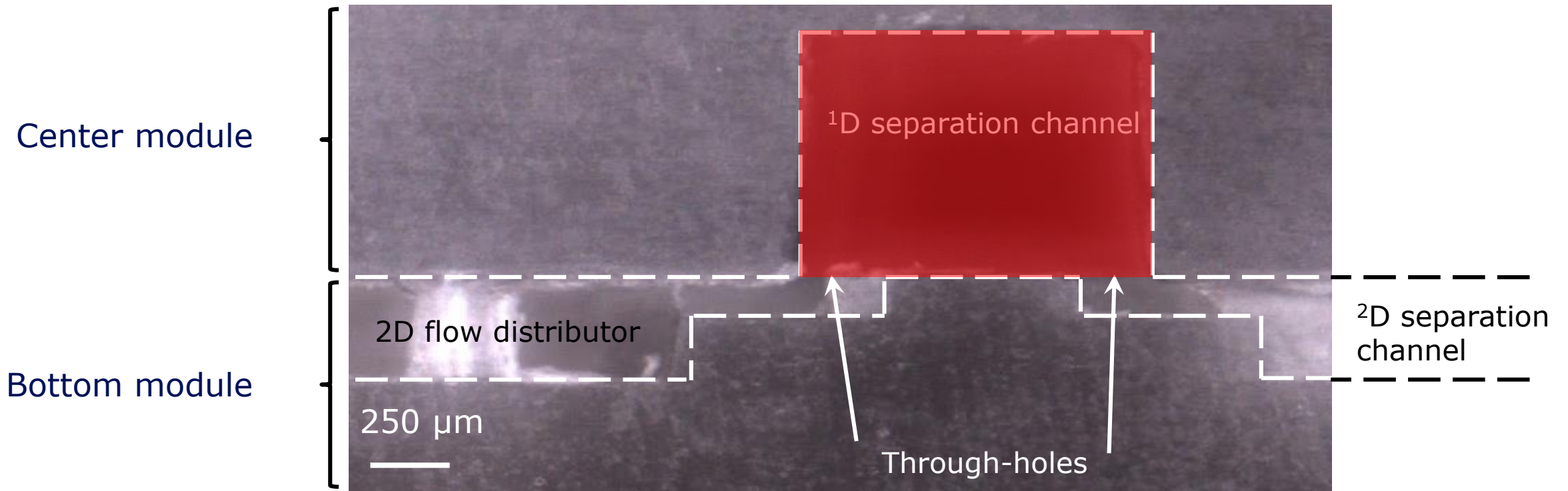


Stacked-layer approach

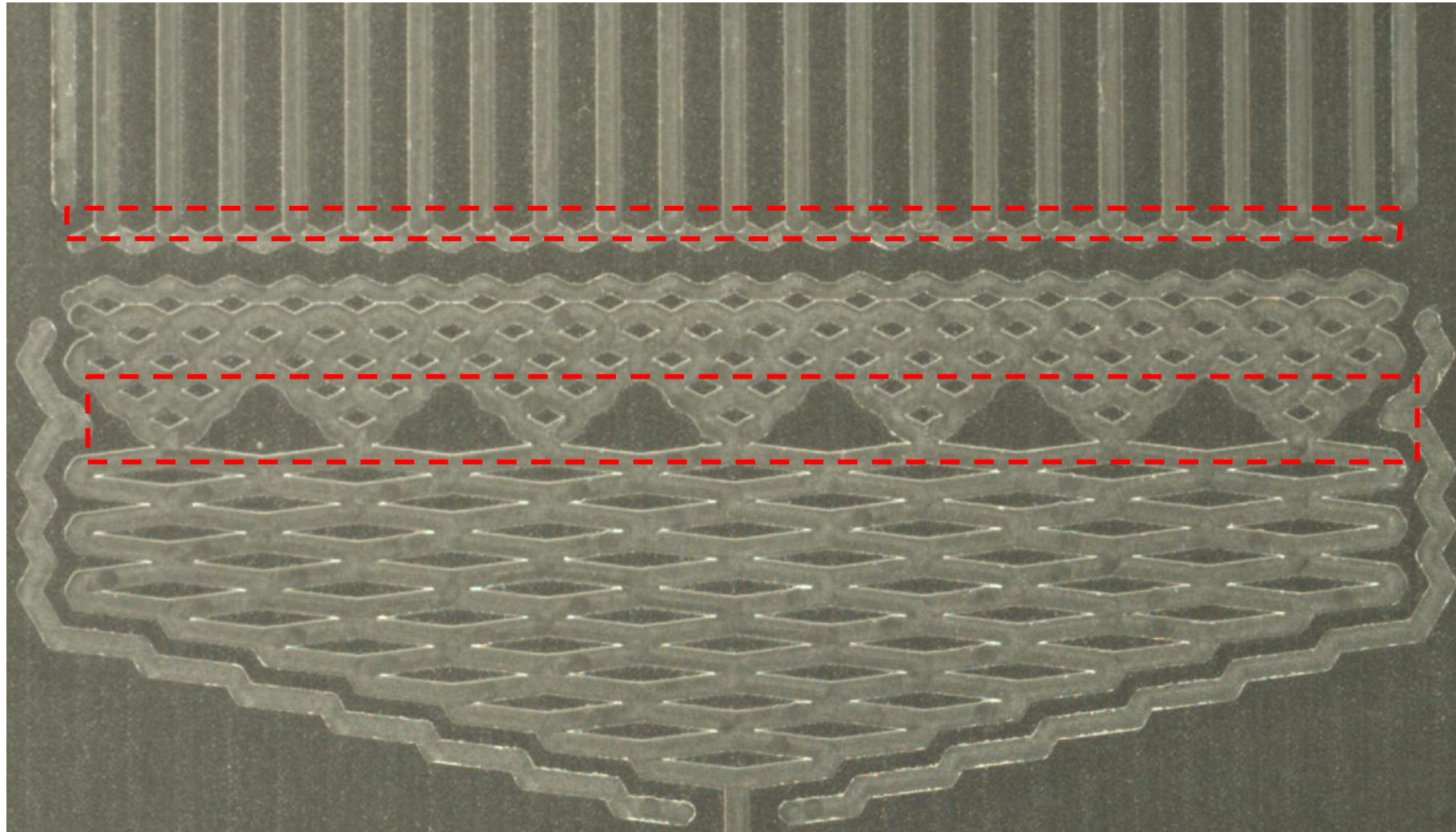


Flow control in first-dimension channel

- ^1D channel and ^2D channels placed in **different modules**, with connecting through-holes
- **Preferential flow path** of ^1D mobile phase



Second-dimension flow distributor



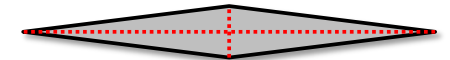
} Funneling wedges

AR = 2

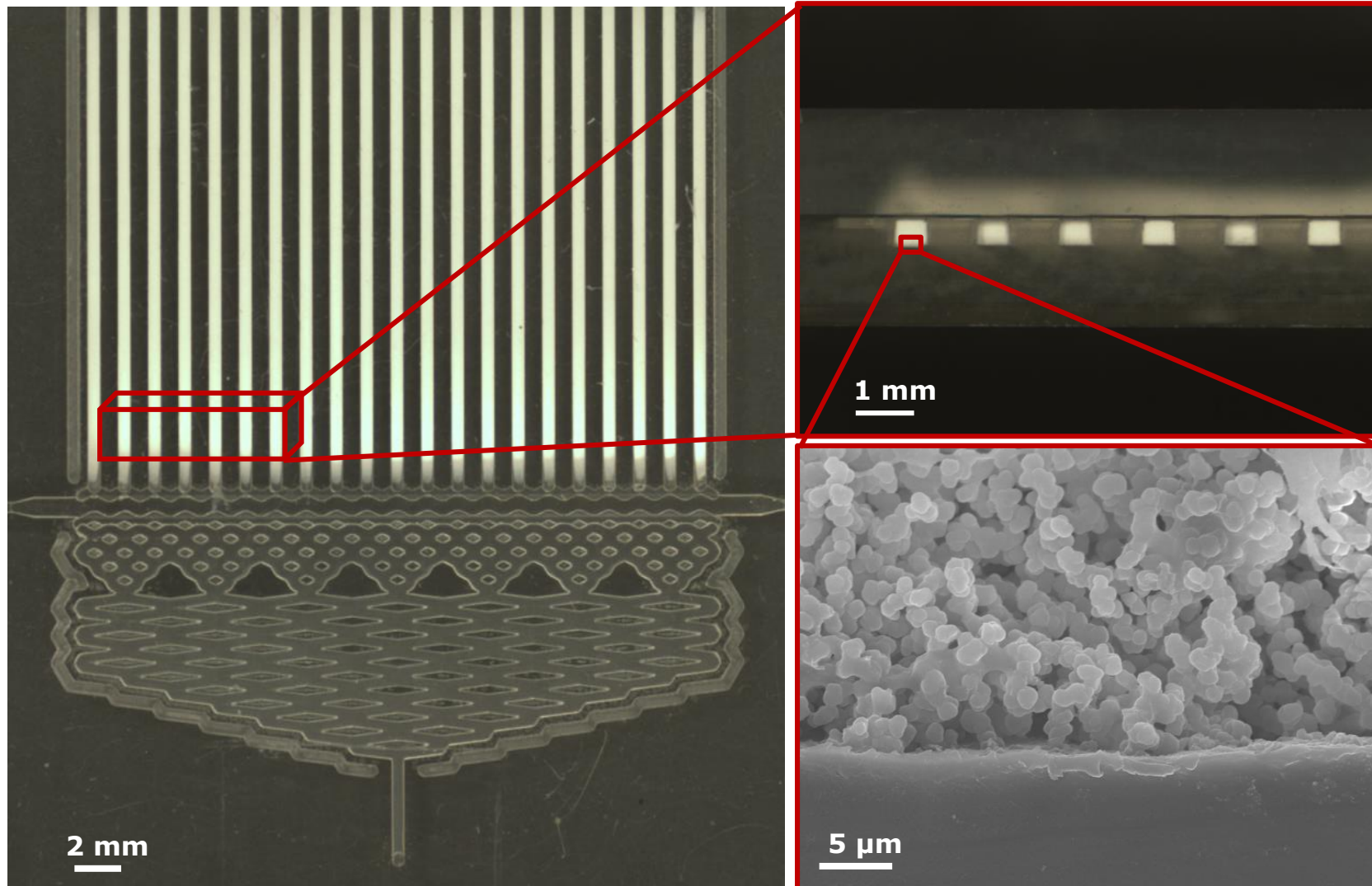


} Transitional
wedges

AR = 8

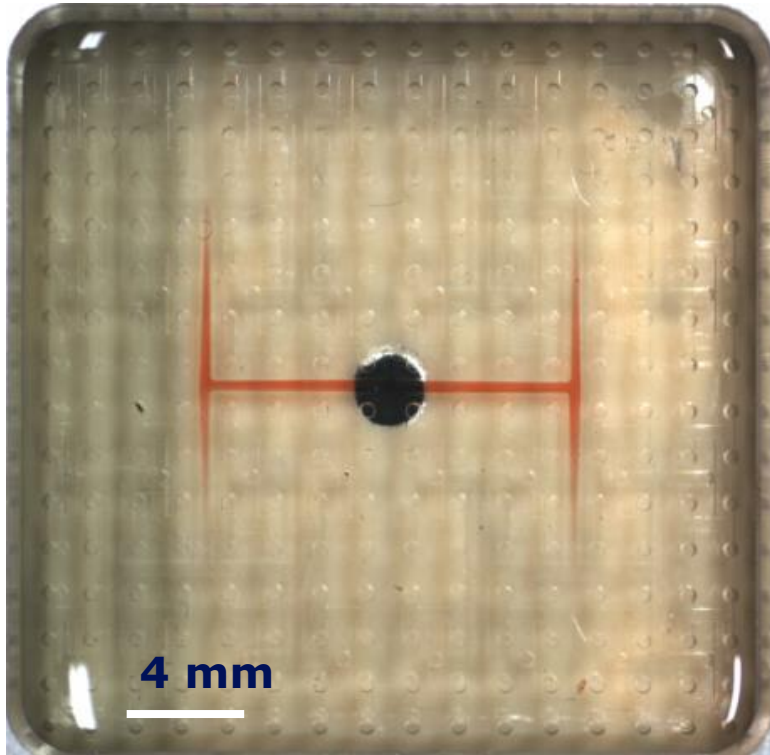


Polymer monolithic stationary phase



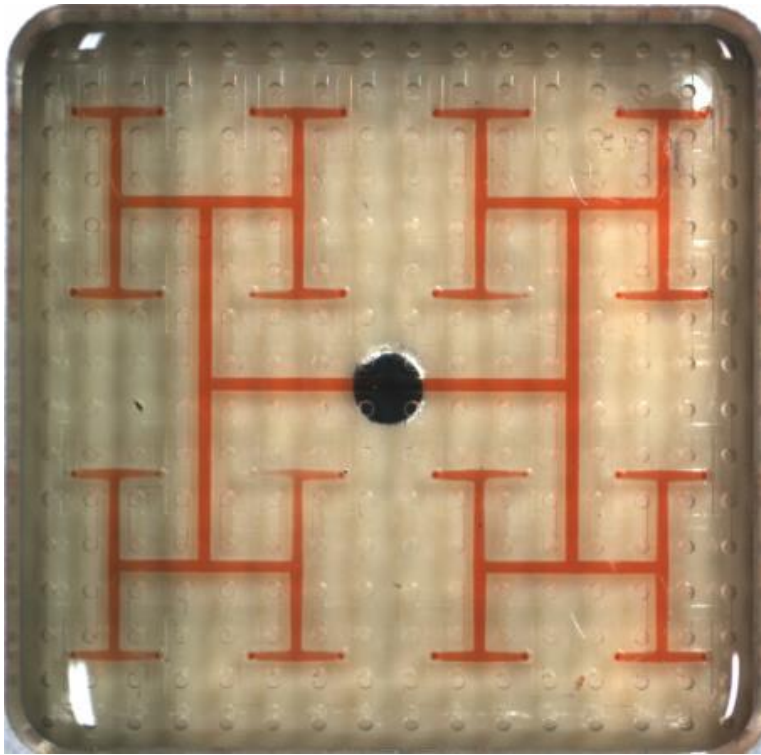
Third-dimension flow distributor

2 generations

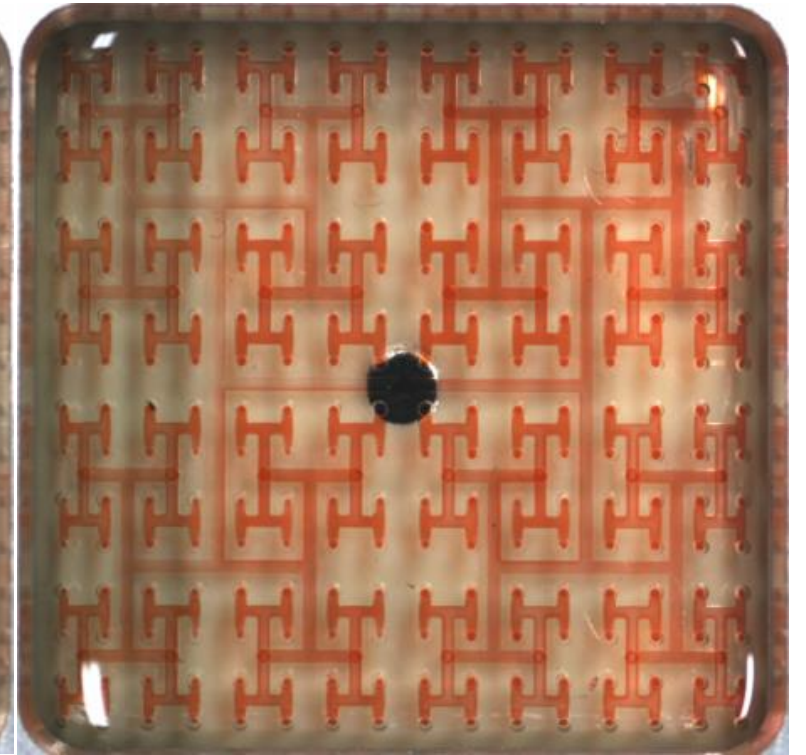


Single inlet

5 generations

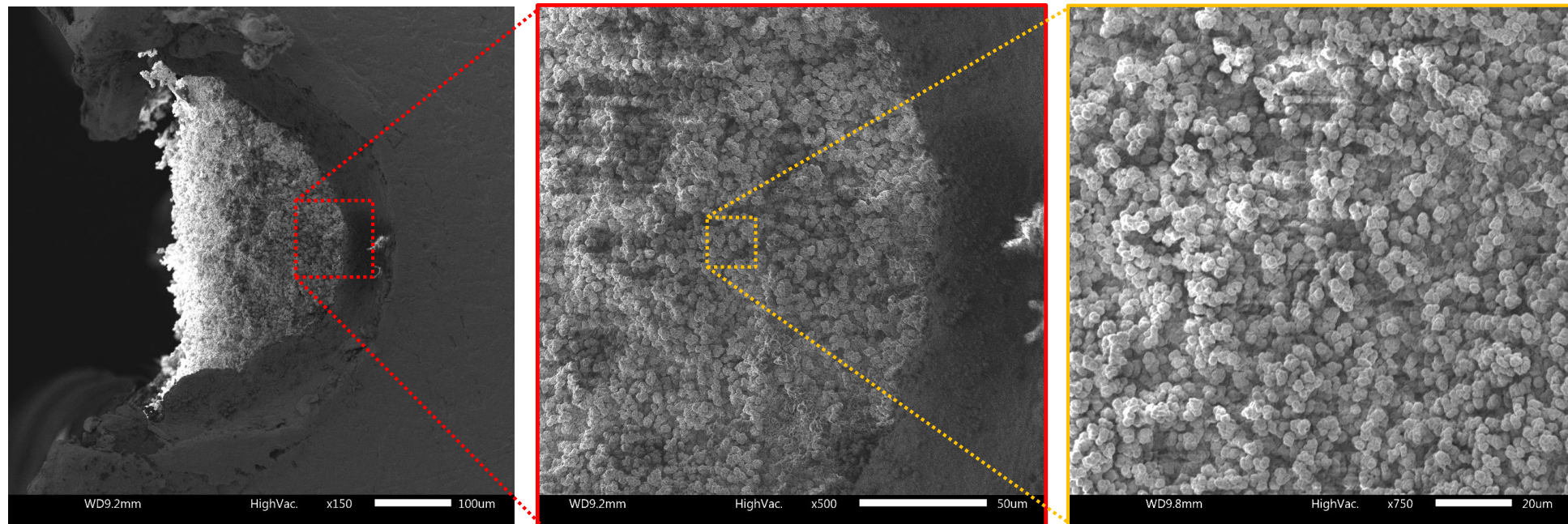
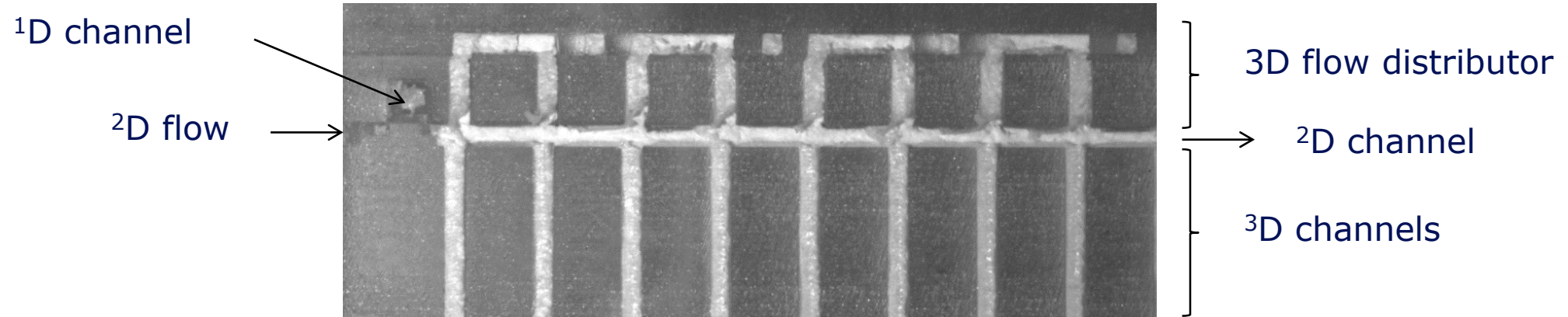


8 generations



256 outlets

Polymer monolithic stationary phase



Further Reading

Microfluidics

- Whitesides, George M., The origins and the future of microfluidics, *Nature* (2006).
- H. Becker, L.E. Locascio, Polymer microfluidic devices, *Talanta* (2002).

Spatial multi-dimensional chromatography:

- Wouters, Bert *et al.*, Towards ultra-high peak capacities and peak-production rates using spatial three-dimensional liquid chromatography, *Lab on a Chip* (2015).
- Adamopoulou, Theodora *et al.*, Experimental and numerical study of band-broadening effects associated with analyte transfer in microfluidic devices for spatial two-dimensional liquid chromatography created by additive manufacturing, *J. Chromatogr. A* (2019).

[illegible]

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scholar.google.nl/citations?user=EpfVC10AAAAJ&hl=en



www.mendeley.com/profiles/bert-wouters/

List of abbreviations

Thank you for listening!

μPAC: micropillar-array columns	CE: Capillary electrophoresis	MALDI: Matrix-assisted laser desorption/ionisation	SEC: Size exclusion chromatography
¹ D: First-dimension	CO ₂ : Carbon dioxide	MS: Mass spectrometry	SFC: Supercritical -fluid chromatography
² D: Second-dimension	COC: Cyclic olefin copolymer	n_c : Peak capacity	SLA: Stereolithography
2D-LC: Two-dimensional liquid chromatography	CZE: Capillary Zone Electrophoresis	nL: Nanolitre	SLS: Selective laser sintering
2D-PAGE: Two-dimensional polyacrylamide gel electrophoresis	d_p : Particle size	PDMS: Polydimethylsiloxane	t_G : Gradient time
3D-LC: Three-dimensional liquid chromatography	FDM: Filament-deposition modeling	PEEK: Polyether ether ketone	TLC: Thin-layer chromatography
ABS: Acrylonitrile butadiene styrene	GC: Gas chromatography	pL: Picolitre	W: Peak width
CAD: Computer-aided design	HPLC: High-performance liquid chromatography	PMMA: Poly(methyl methacrylate)	
	LC×LC: Comprehensive two-dimensional liquid chromatography	PS: Polystyrene	
		RP: Reversed phase	