Incorporating Multifunctionality into Microchip Separations

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Microfluidics: Working definition

Microfluidics:

- ***control of small volumes of liquid (fL to μL) in microscopic channels (1 to 1000 μm)***

  → ultra-small-volume liquid handling

- ***Inherent to microfluidics*:**

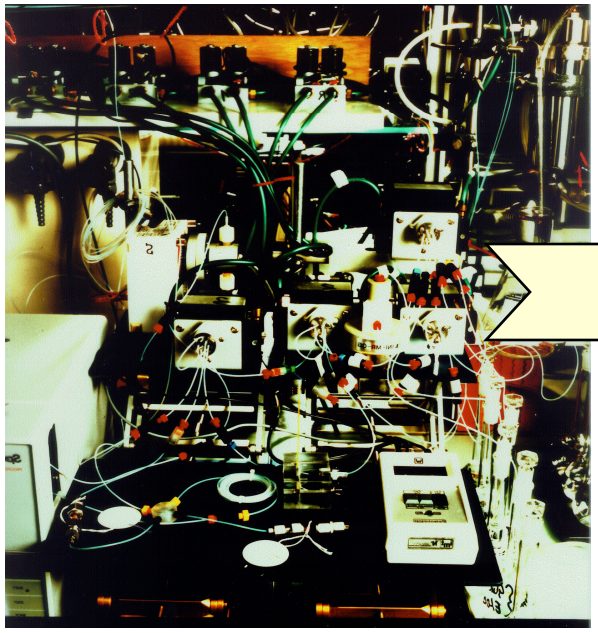
(1) fundamental study of fluid flows in microchannels

(2) design, fabrication, and implementation of suitable pumps, valves and mixing elements for liquid handling

(3) application to chemical analysis, synthesis; biochemistry, biology

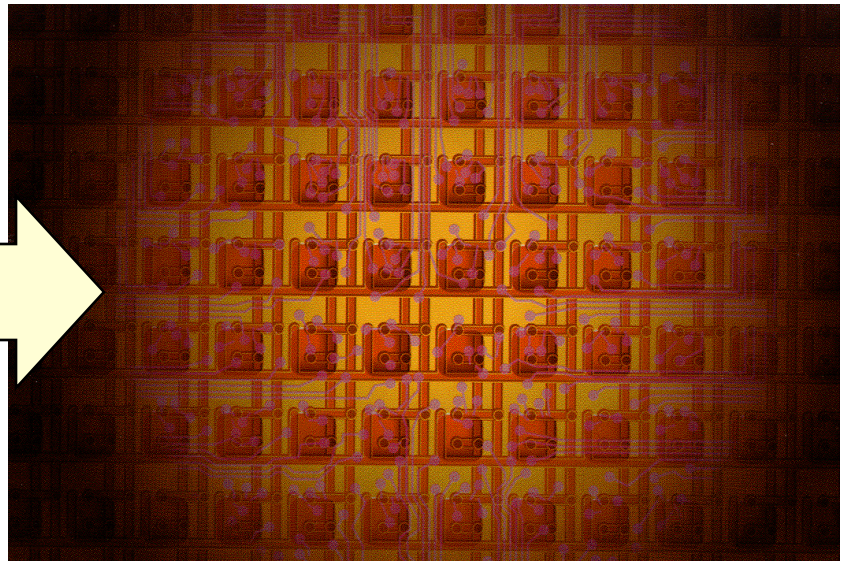
(Duffy et al., Anal. Chem. 1999, 71, 4669-4678)

From tubes to microchannels…
From microelectronics to microfluidics...



Tubing-based flow system for monitoring glucose and ethanol in a bioreactor

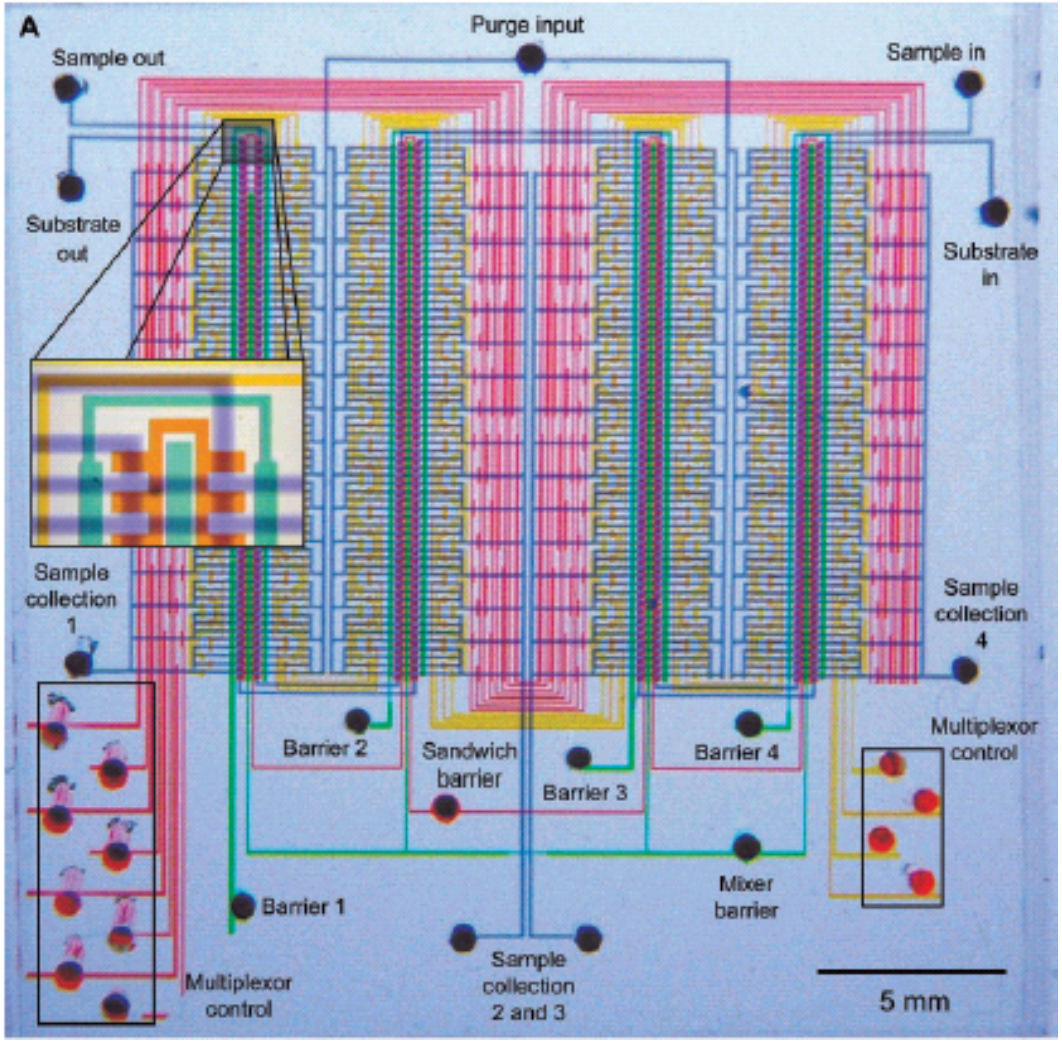
Source: A. Spielmann, S. Haemmerli, et al., Ciba Geigy AG



Chip: Microchannels and wells (diameters of micrometers / volumes of pL → nL) replace tubing and beakers for combinatorial chemistry

Source: Orchid Biocomputer Inc. chip, *Photonics* (Jan. 1998), News & Analysis

Forbes, Dec. 23, 2002: “Science beyond belief”



“Just one square inch, the chip contains 2,056 microvalves that can … mix reagents together… in 256 reaction chambers, each one holding less than one-billionth of a liter…

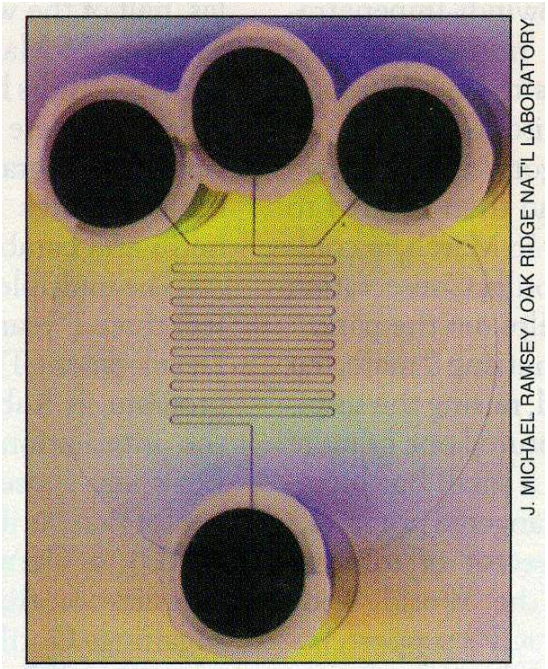
Twenty years from now, the implications will be seismic.”

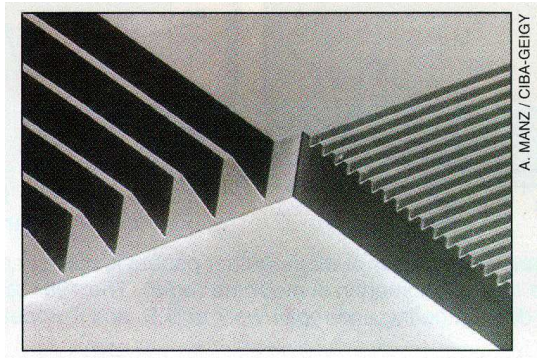
- R. Langreth

(left) Thorsen et al., *Science* **2002**, *298*, 580-84.

Microfluidic chips for chemistry: Why?

- very low volume
  - (a) reduced consumption
  - (b) excellent control of ultra small volumes
  - (c) increased surface-to-volume ratios
    - –improved heat transfer
    - –better chemical control
- enhanced performance
  - – high speed
- automation
  - – synthesis /sample prep'n / analysis on a chip
- dimensions in biological regime
- parallel systems
  - – high throughput applications



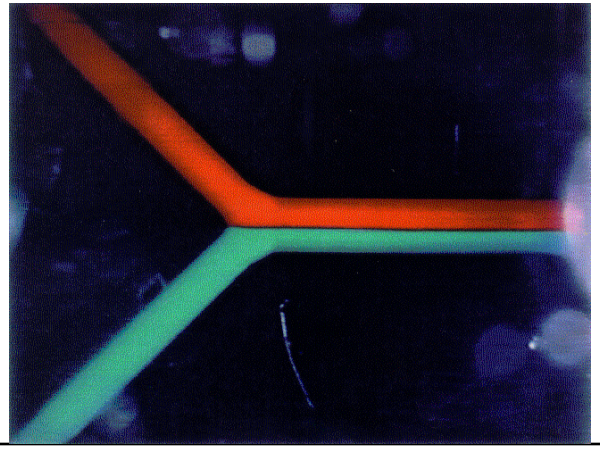


This talk will explore the added value of using microfabricated separation devices when striving for optimal analytical functionality and performance.

First: Chips are small…

…that is, the microchannels that guide fluid flows are generally small

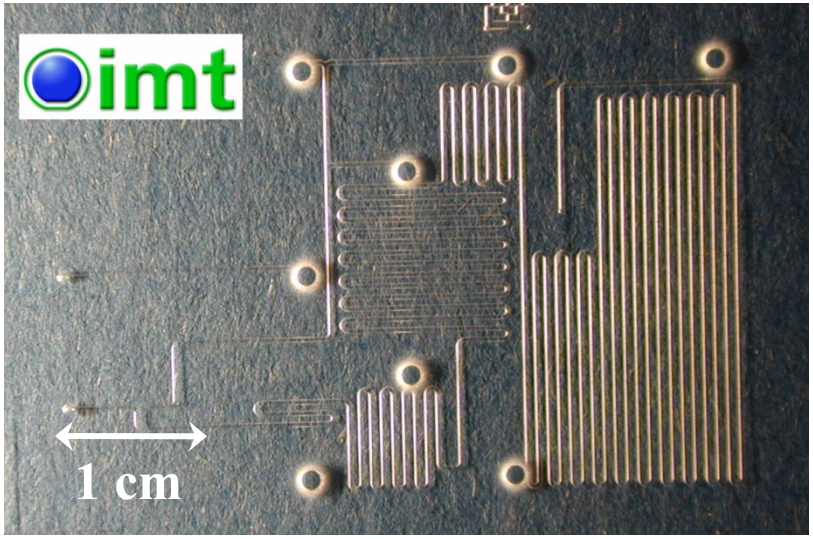
- channel cross-sections measurable in μm or even nm
- lengths from μm to cm
- flow regime: viscous forces dominate, streamlines are well defined (low Reynold's number)



Microchannels: Laminar flow rules!

Second: Chips mean integrated networks…

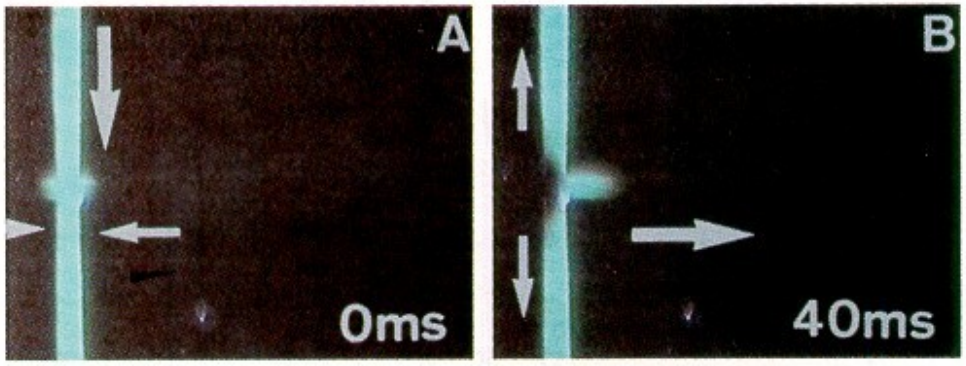
- fabrication processes used allow easy formation of interconnected microchannel networks
- route to more functional devices, fluidicly speaking



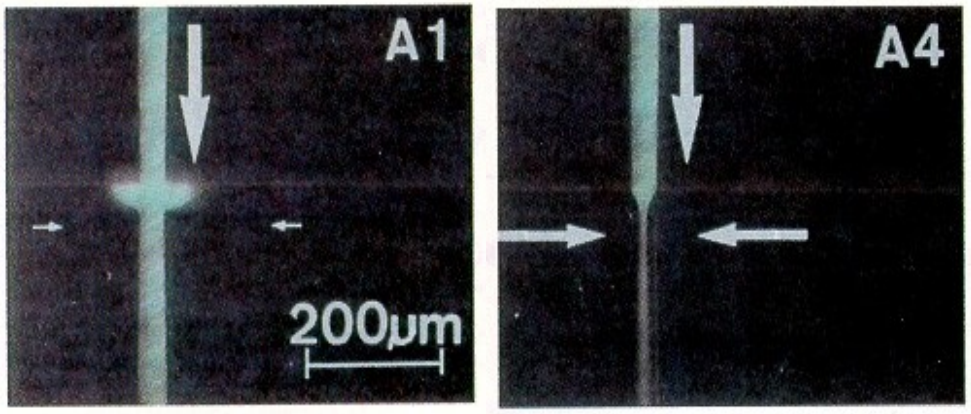
e.g. volume-defined injection, post-column derivatization

Micro liquid handling

define a volume and inject…



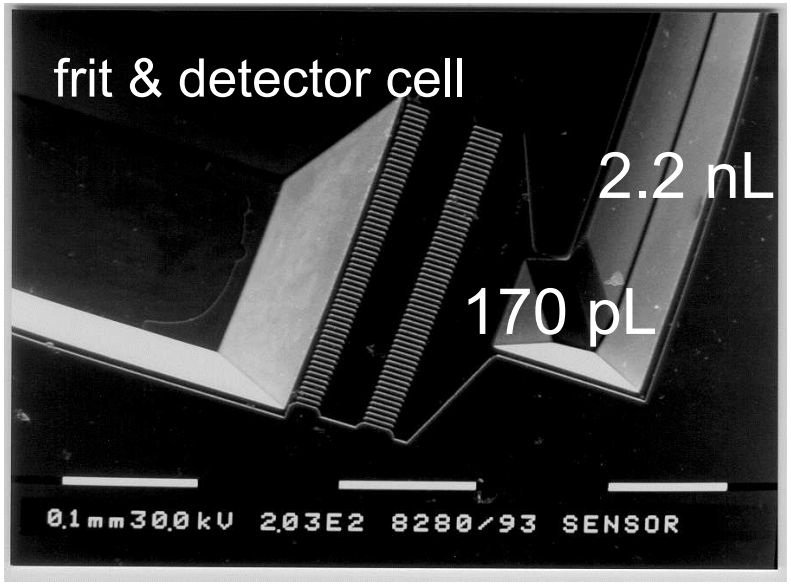
precise volume control / dilution…

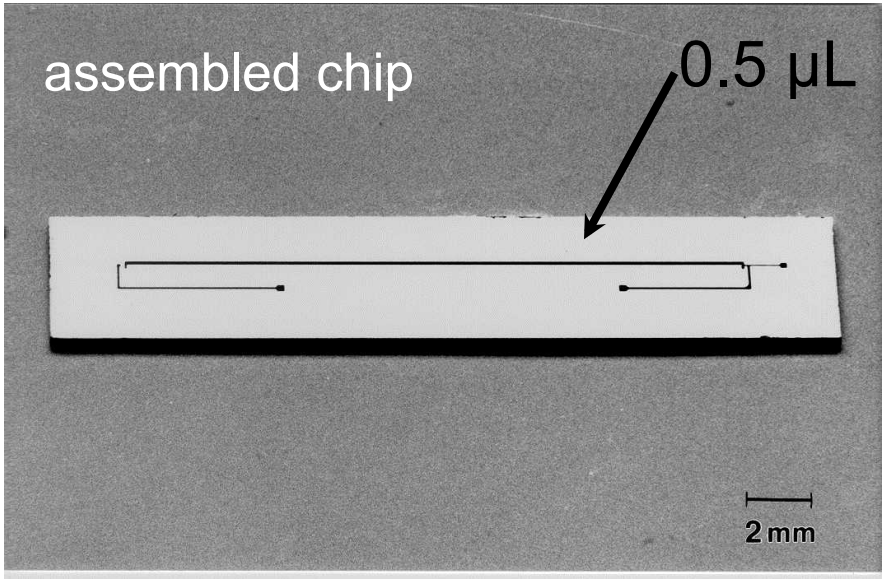


- *all common liquid handling done manually or robotically is possible on chip*
  - *e.g. mixing, aliquoting, reactions, etc.*

Ref: F. von Heeren et al. *Anal. Chem.* **68(13)** (1996), 2044-2053.

Minimization of dead volumes





- When the column shrinks, the detector must shrink accordingly
- Chip technology makes dramatic reduction in system dead volumes possible

Source: *Analytical Methods and Instrumentation* **2** (1995), 74-82. (Chips from ICSensors, Milpitas, CA)

Third: Chips mean high tech…

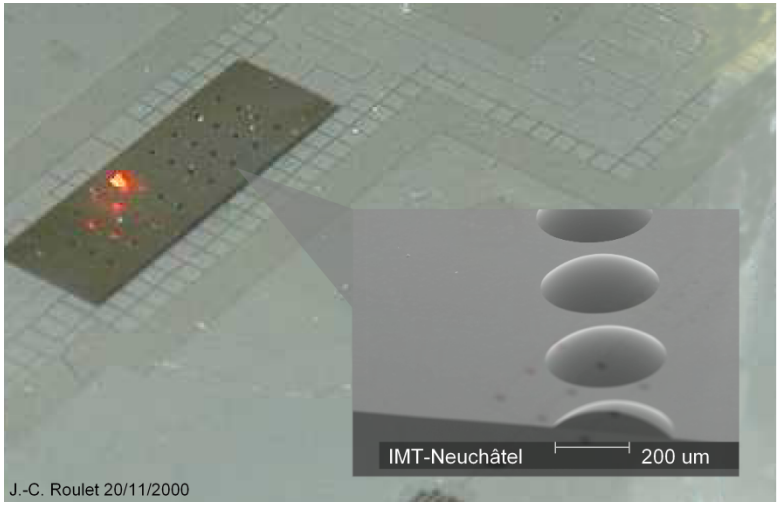
From the micro electro mechanical systems (MEMS) perspective:

- micromachining technology required for glass (and other) microfluidics is simple (at 1st glance, anyway)
- making microchannels not really interesting…

Why?

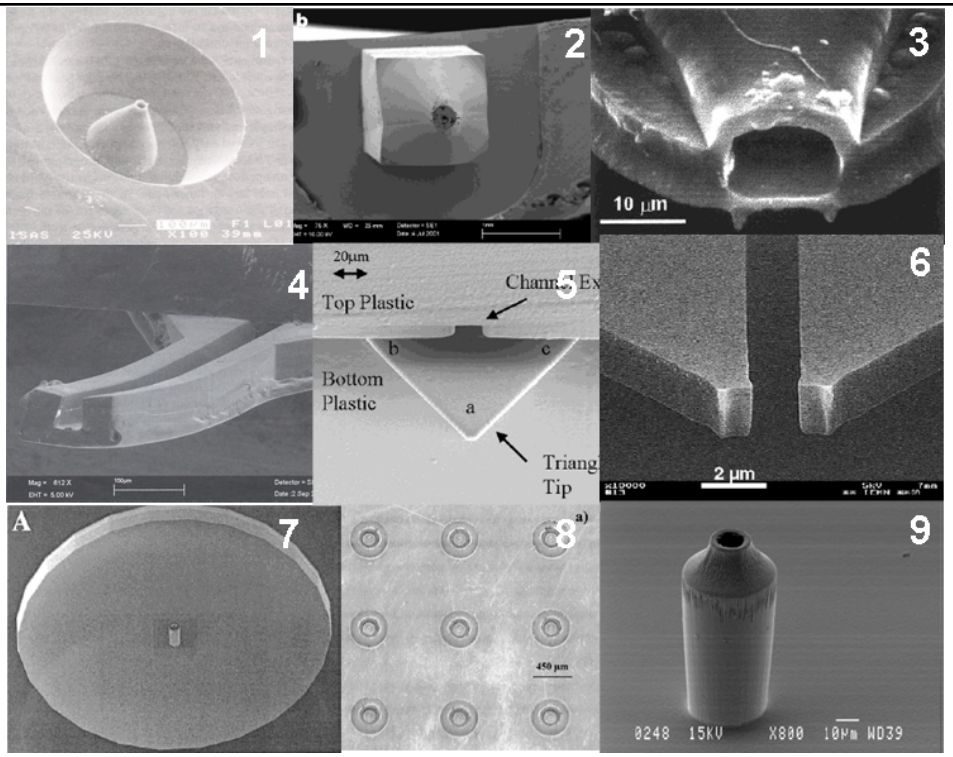
- can do lots more with MEMS than just make channels:

  deposit & pattern metals / make moving surface structures / build in electronics, optics / etc.



Microlenses made in photoresist (courtesy of J.-C. Roulet, Applied Optics Group, IMT, Neuchatel, CH, 2000)

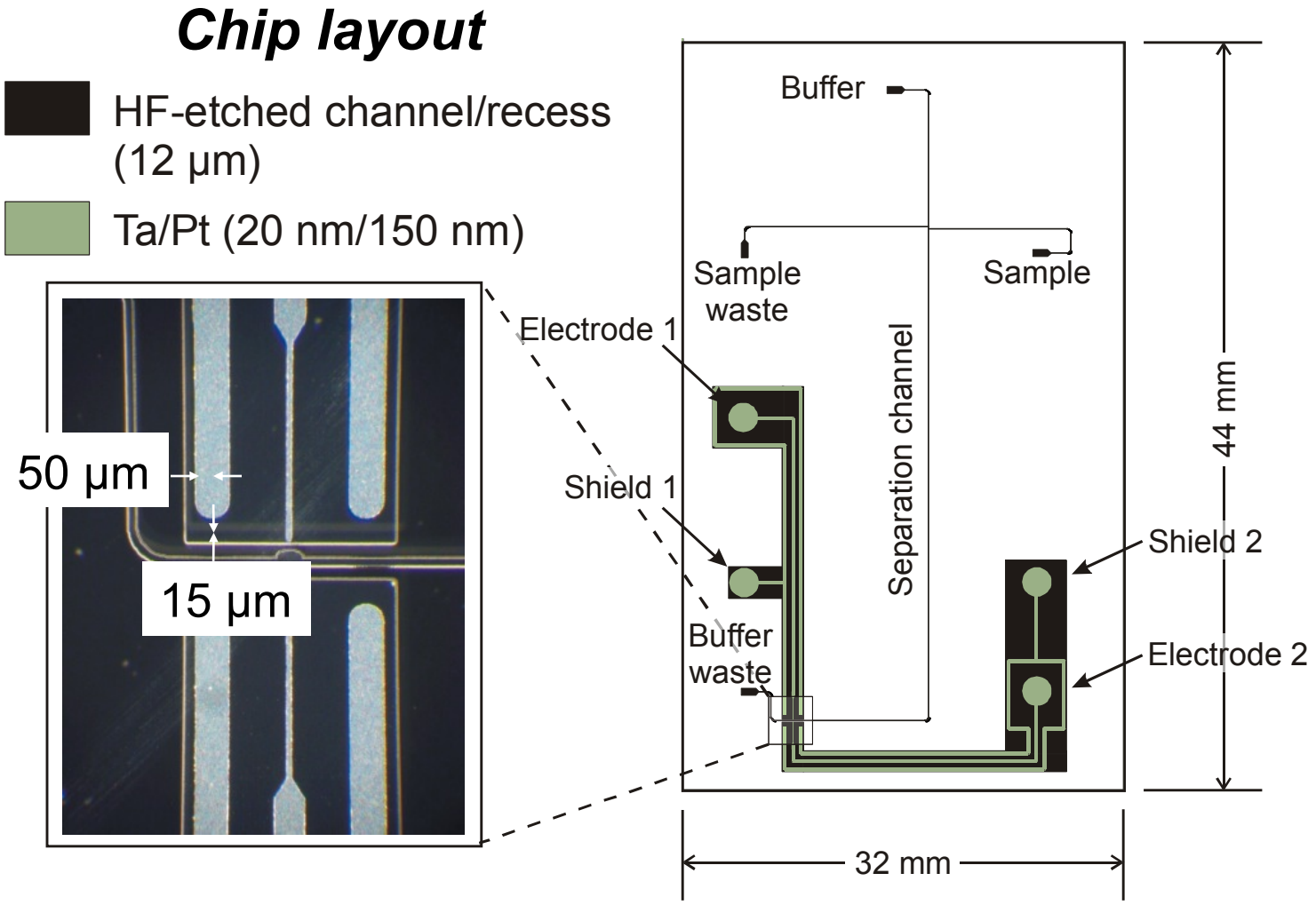
Coupling microfluidics to ESI-MS: Integrated emitters



A selection of integrated emitters. Emitter 1 is made in polymethyl methacrylate by micro milling, 2 is made in polycarbonate/polymethyl methacrylate by injection moulding, 3 is a parylene emitter, 4 is a PDMS emitter made by replica moulding, 5 is an emitter formed from a triangular piece of parylene sandwiched between two Zeonor substrates structured by hot embossing, 6 is an emitter made in SU-8 using standard cleanroom processes, 7 is an emitter made in silicon using deep-reactive ion etching, 8 are nozzles made in polycarbonate using laser ablation,9 is made in silicon dioxide using deep reactive- ion etching.

Koster, Verpoorte *Lab Chip* **2007**, *7*, 1394-1412.

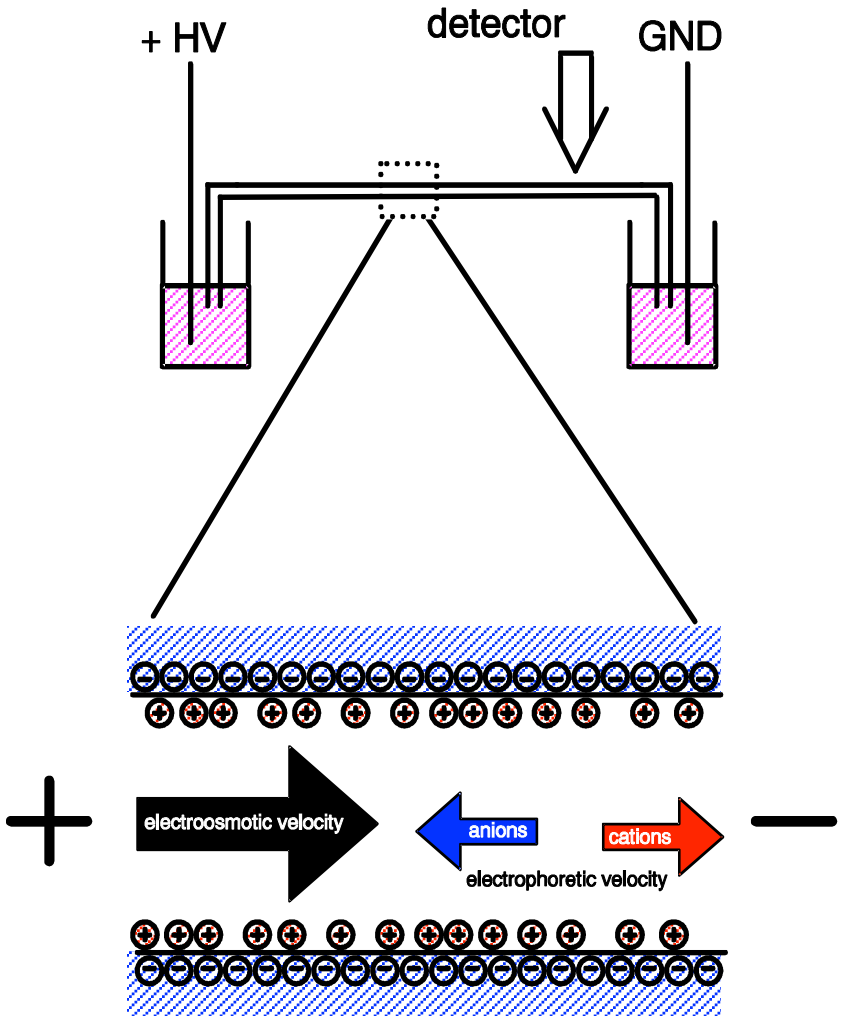
Integrated electrodes for electrochemical detection



J. Lichtenberg *et al.*, *Electrophoresis* **2002**, *23*, 3769-3780.

Integrating capillary electrophoresis onto chip

Capillary electrophoresis: Standard



Standard conditions:

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

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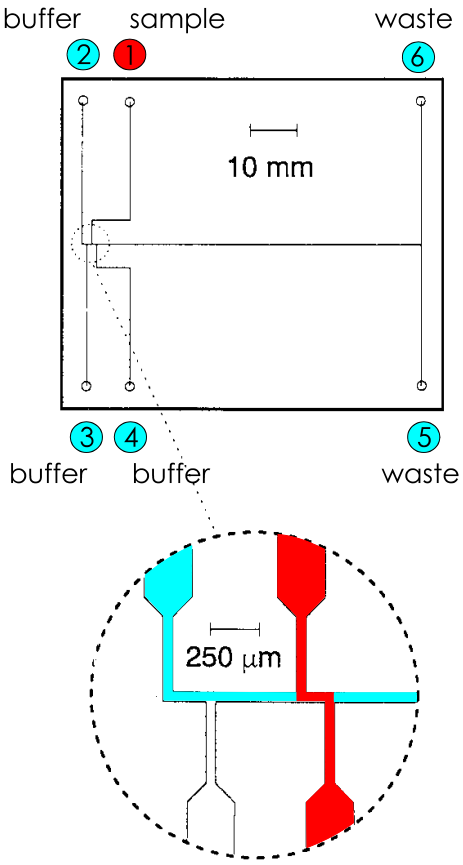
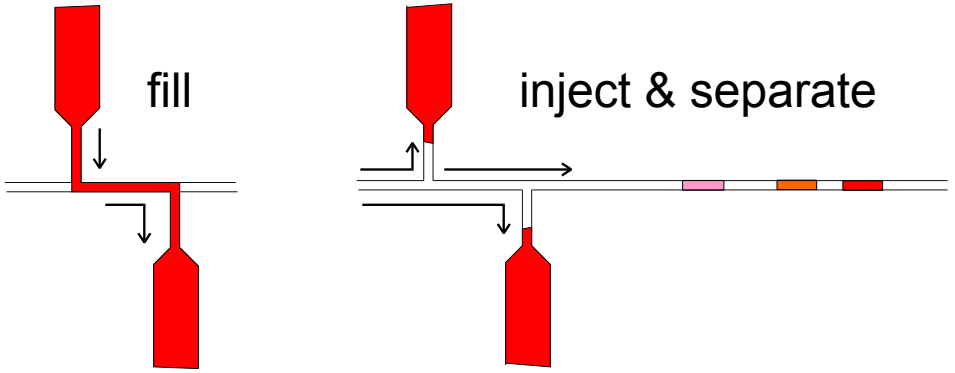
* *drawing adapted from diagram by N. Burggraf, CIBA/IMT 1995*

CE-on-chip: 1992

Chip layout



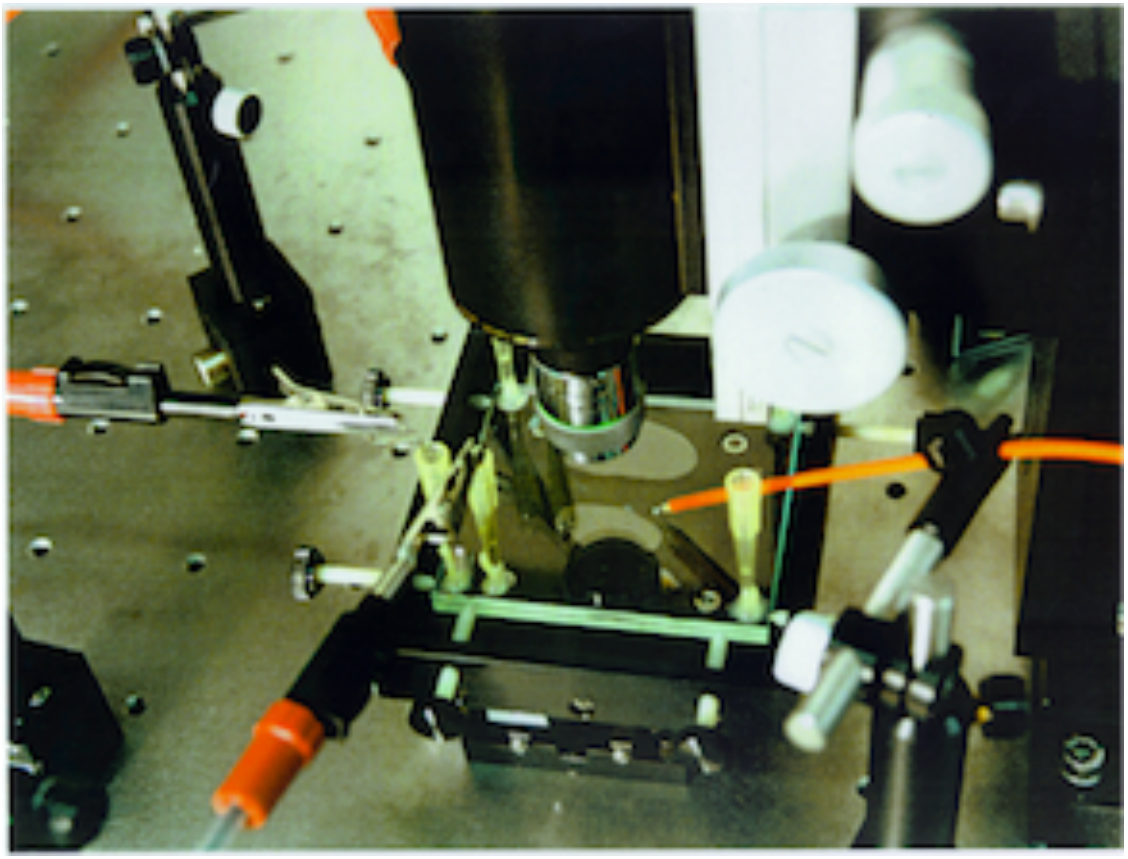
Schematic of separation



New:

- volume defined injection
  - 100 pL (150 µm plug)
- capillary length: ca. 7 cm
- capillary width at top: 50 µm; depth: 12 µm
- E = 1100 V/cm
- footprint: 80 × 70 × 3 mm

Chip-based CE: Experimental set-up



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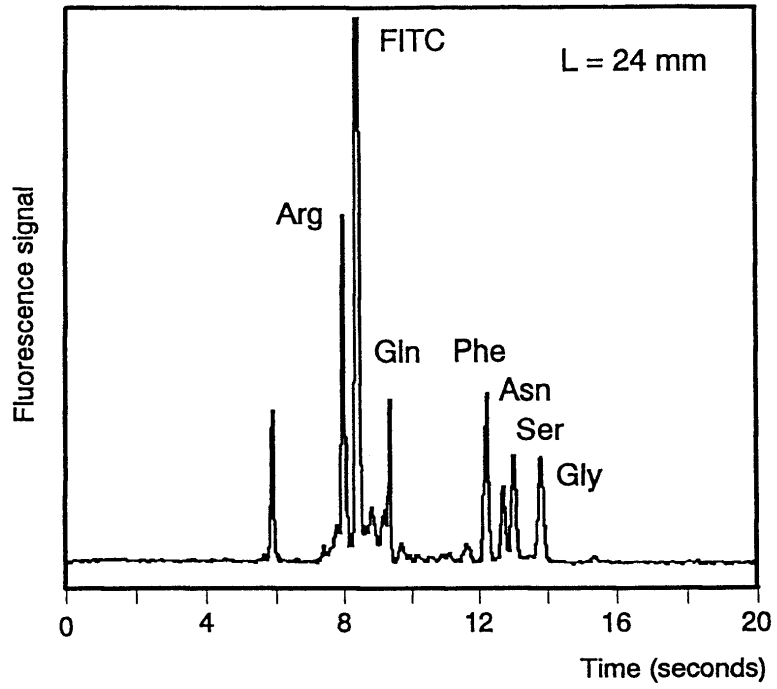
Source: C. S. Effenhauser, Ciba AG

Chip CE: Amino acid separation

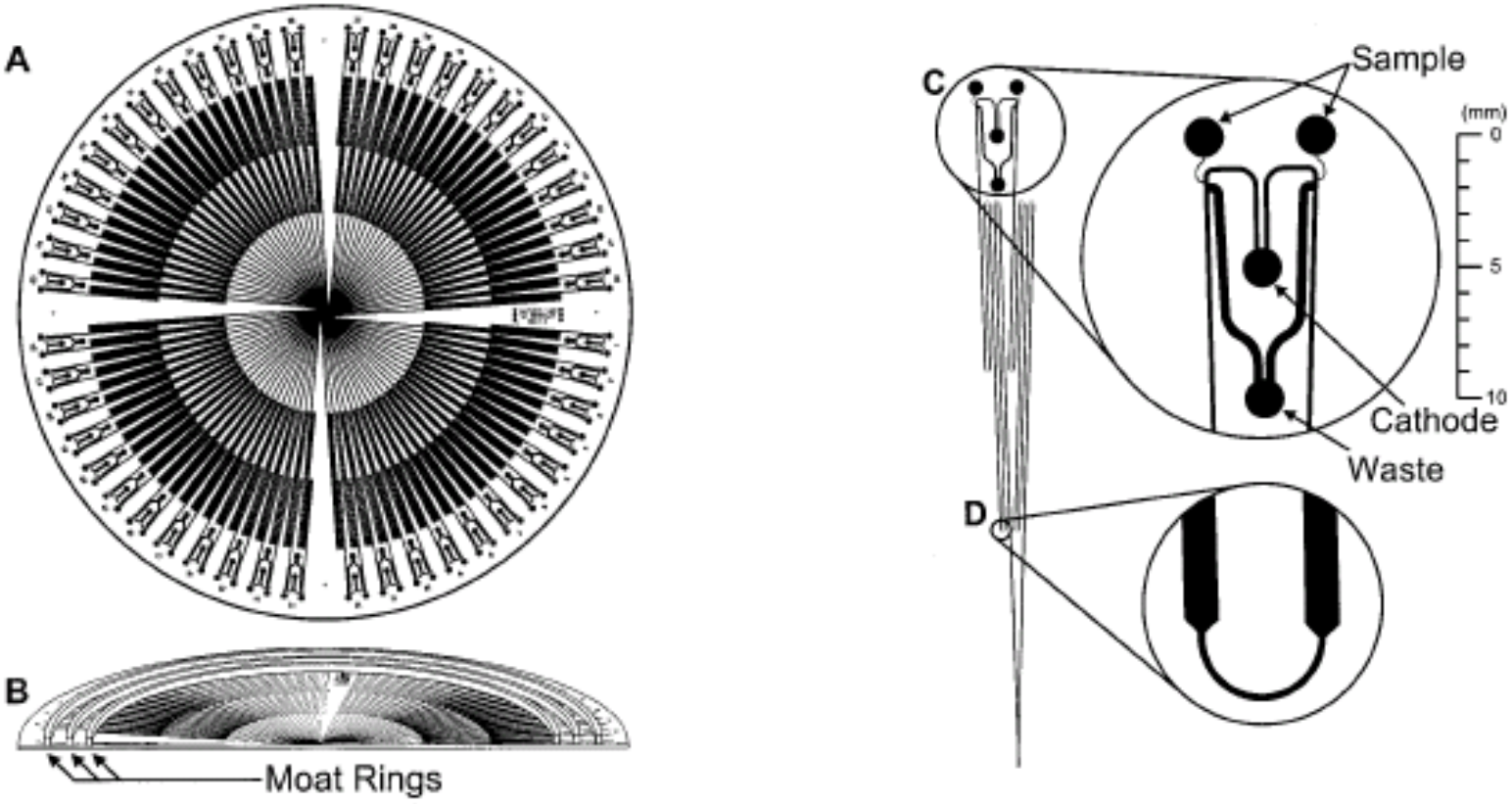


Concentration: 10 μM
Injected amount: 1 fmol
Detection limit: 20 amol

- mixture of 6 labelled amino acids were separated in seconds rather than minutes
- this early result showed the promise of electrokinetically driven chips for liquid handling on a nL to pL scale, as well as for separation

Ref: C.S. Effenhauser et al., *Anal. Chem.* **65** (1993) 2637-2642.

High-speed DNA sequencing in parallel channels



96-lane device; common anode in center; linear polyacrylamide sieving matrix; separation channel 200 μm wide, 30 μm deep, 15.9 cm long

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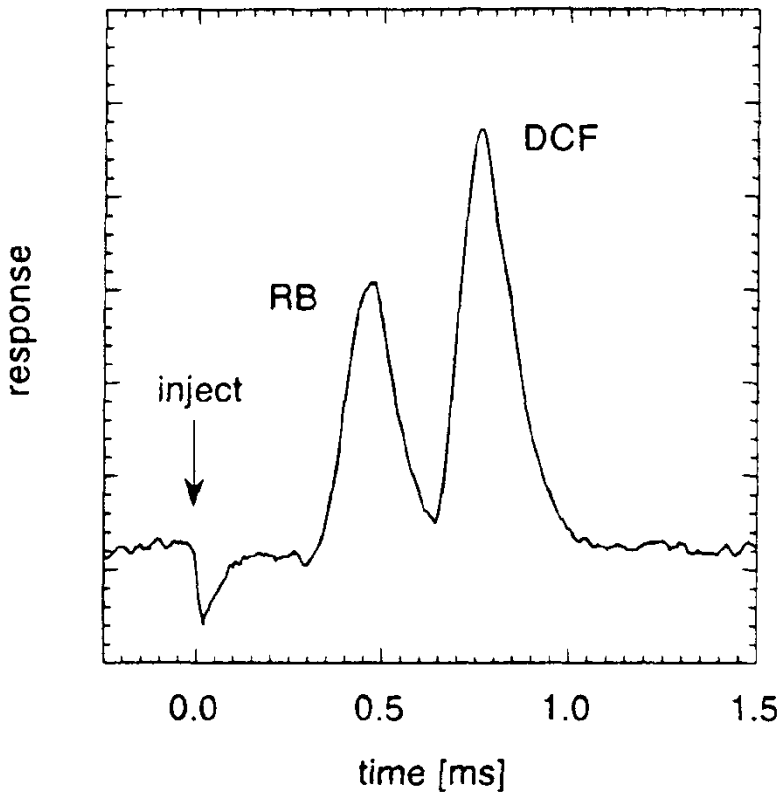
Ref: Paegel *et al.*, *PNAS* **2002**, *99*, 574

Fast separations pushed to the limit…



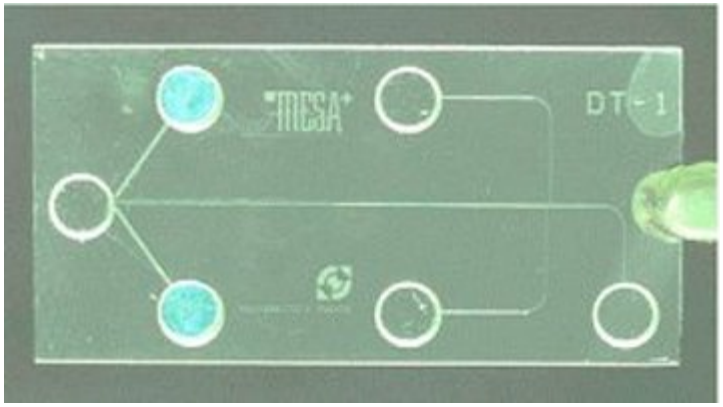
1998: ms CE demonstrated

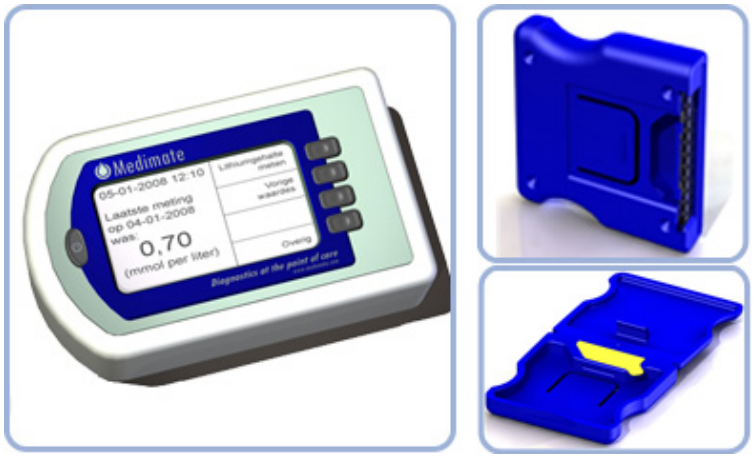
- *sample:* RB = rhodamine B (8 μM)
  DCF = dichlorofluorescein (4 μM)
- E_{sep} = 53 kV/cm (ΔV = 2.8 kV)
- separation length = 200 μm

Where do we go from here??

Ref: S.C. Jacobsen et al., *Anal. Chem.* **1998**, *70*, 3476-3480

Fast chip CE for Li^+ analysis in blood





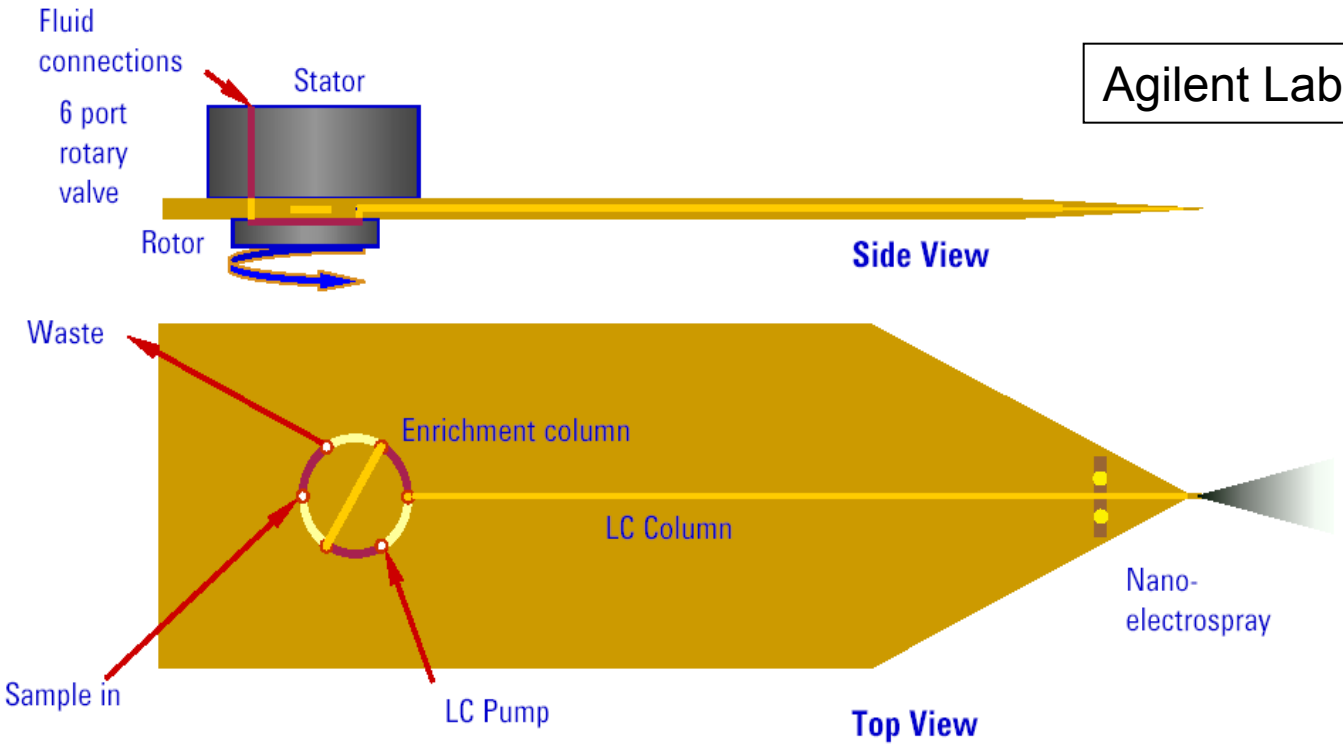
Glass capillary electrophoresis chip (3 x 1.5 cm). Ions detected by conductivity detection in seconds. Research prototype: 2004

Product: Medimate, 2009. Medimate is a spin-out of the University of Twente, NL

A new application for CE, made possible by miniaturization

- Point-of-care diagnostics is a growing market
- issues like user-friendliness and market acceptance could make or break products

Integrated HPLC, sample enrichment, ESI tip



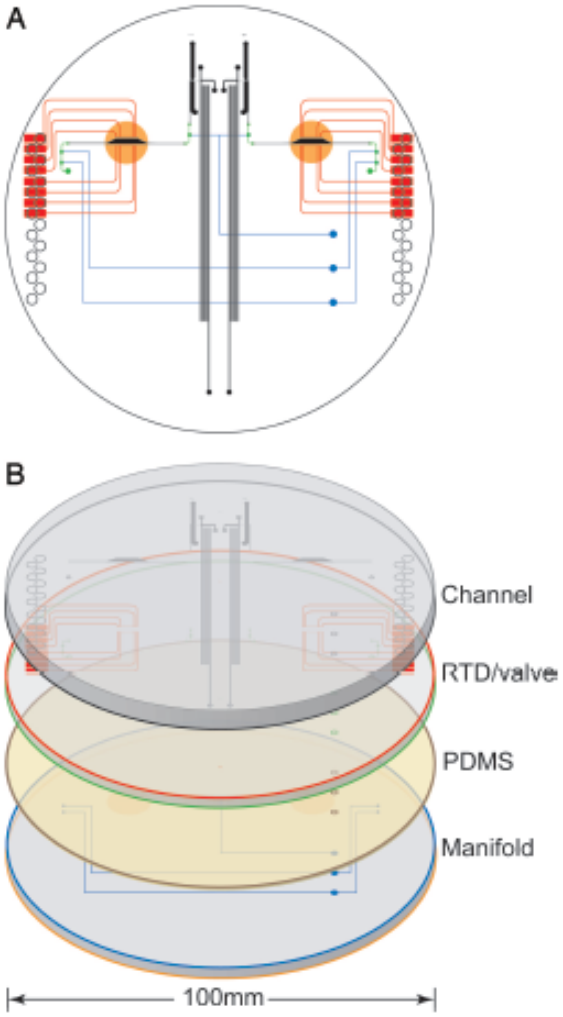
Agilent Laboratories

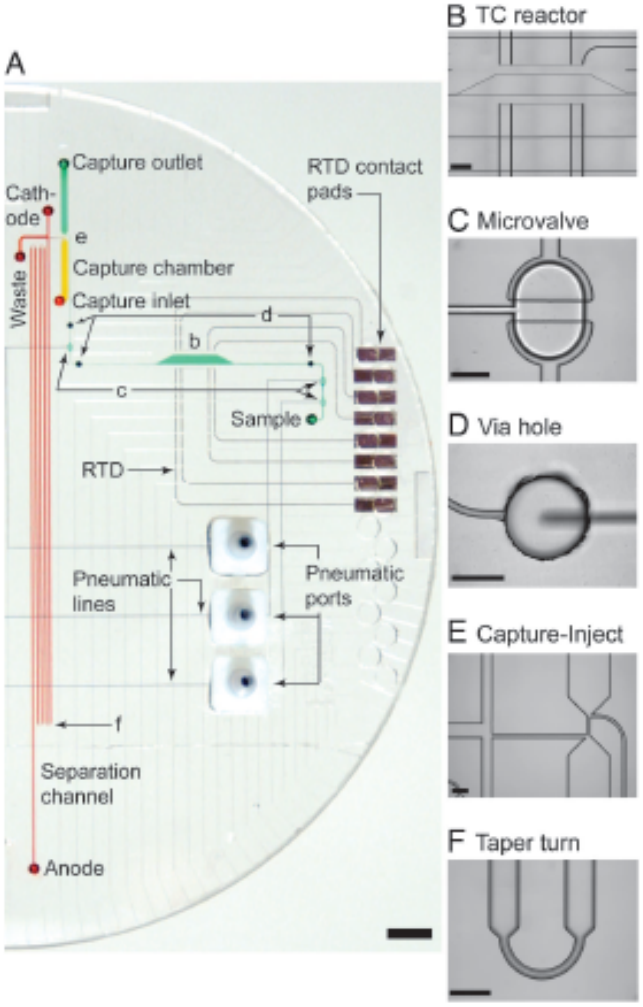
- Chip made by laminating polyimide films with laser-ablated channels, ports
- ***Ref:*** Yin et al., *Anal. Chem.* **2005**, *77*, 527-533.

Advantages:

1) minimal carryover, analyte losses (all processing in one device)
2) subfemtomole detection sensitivity for peptides
3) robust, stable electrospray

Towards personalized medicine...nL-scale Sanger sequencing





Ref: Blazej *et al.*, *PNAS* **2006**, *103*, 7240.

Other developments: Alternatives to packing Monolithic stationary phases

In-situ polymerization to form a continuous bed in a chip. The technique was used for both CEC and HPLC applications.

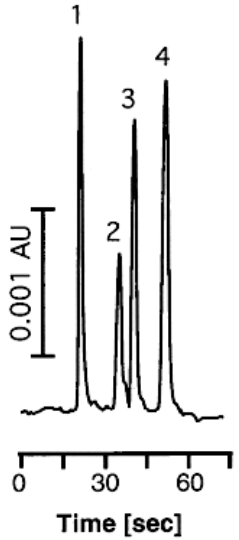


Figure 9. Fast anion-exchange chromatography of four standard proteins in a chip column filled with a continuous bed derivatized with ammonium groups. Straight column design, 6.2 cm (4.5-cm effective length) × 40-μm i.d.(width); mobile phase, (A), 20 mM sodium phosphate, pH 7.8, (B) 20 mM sodium phosphate, pH 7.8, containing 0.4 M sodium chloride; linear gradient 0−100% B in 1 min; flow rate through the inlet reservoir, 0.1 mL/min; flow rate through the chip column, 0.4 μL /min; pressure, 22 bar; injection by pressure, 3 bar, 20 s. UV detection at 230 nm; sample concentration, 0.5−1.5 mg/mL. Sample: 1, myoglobin (horse); 2, conalbumin; 3, ovalbumin (chicken); 4, trypsin inhibitor (soybean).

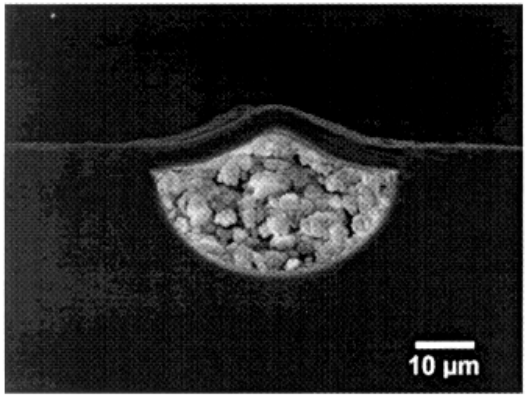


Figure 5. Continuous bed polymerized in situ in the chip channel. This bed is synthesized for anion-exchange chromatography and has, compared with the electrochromatographic bed in Figure 4, relatively large aggregates of polymer particles to ensure low flow resistance (SEM).

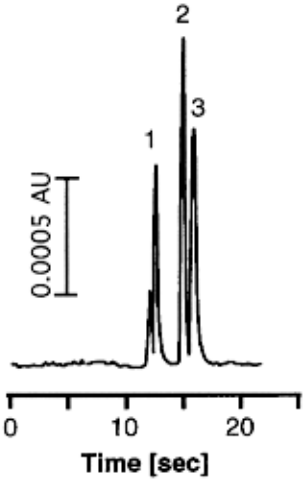
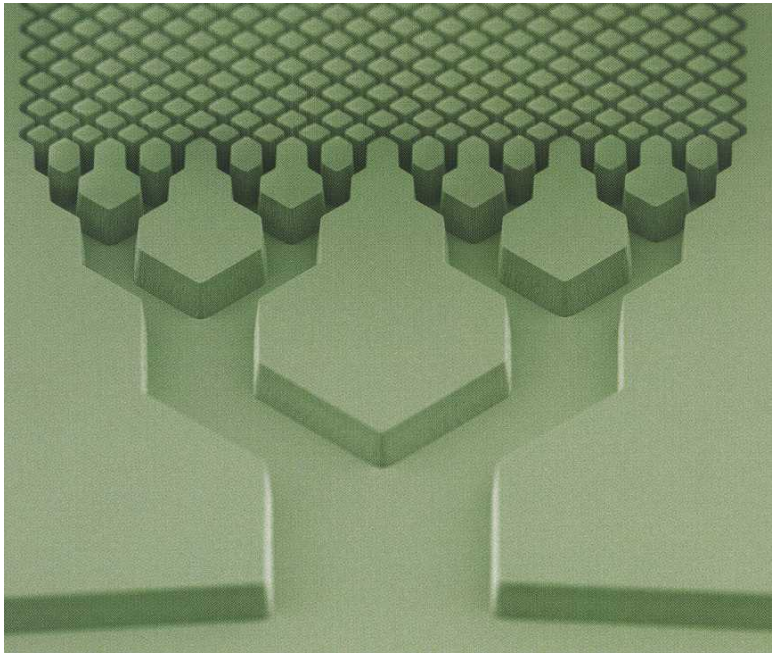


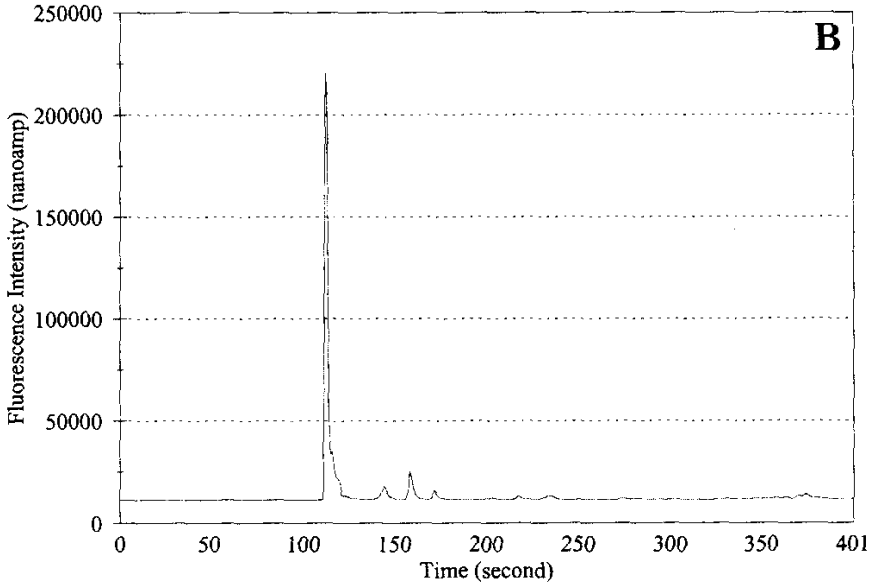
Figure 10. Fast electrochromatography on chips using a C_3^- bed with high EOF velocity. Straight column (18-mm effective length) × 40-μm i.d. (width); mobile phase, 5 mM sodium phosphate, pH 7.4, containing 15% acetonitrile (v/v); applied voltage, 2.4 kV (500 V/cm); EOF velocity, 2.1 mm/s. UV detection at 254 nm; electrokinetic injection, 100 V, 2 s. Sample: 1, uracil; 2, phenol; 3. benzyl alcohol. Separation efficency of uracil was 350 000 plates/m (650 plates/sec).

Ref: Ch. Ericson et al., *Anal. Chem.*, **2000**, *72,* 81-87.

Other developments: Alternatives to packing
Microfabricated packing



SEM of the microfabricated inlet structure of a nanocolumn etched in quartz. Diamonds are 10 x 10 μm, and are separated by 1.5 μm.



Capillary electrochromatographic separation of rhodamine 123.
L = 4.5 cm, E = 1700 V/cm
N = 35'000

Source: B. He, N. Tait, F. Regnier *Anal. Chem.* **70** (1998), 3790-3797.

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Ref: Ch. Ericson et al., *Anal. Chem.*, **2000**, *72,* 81-87.

Conclusions

Benefits of microfabrication for separations

- Chips are small
  - Controlled flows, faster separations
- Chips mean integrated networks
  - Crucial for integration of multiple sequential or parallel operations
- Chips mean high tech
  - Integration of detection elements

Further developments will rely on adjacent developments in the areas of stationary phases (e.g. monolithic phases), new chip materials, integrated detection, etc.

μTAS Team (University of Groningen)