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SMR.1670 - 27

INTRODUCTION TO MICROFLUIDICS

8 - 26 August 2005

Capillary Electrophoresis (CE) on a Chip

R. Luttge
University of Twente, Enschede, The Netherlands

Topics in this lecture

How did CE develop?
We will look into the development of CE as a leading microanalysis technique. Different fields of applications are addressed.

Miniaturization
Chip systems today often still contain several external component. We will evaluate why systems are installed as hybrid systems and if there is reason to gain yet advantage by efforts spent to miniaturize.

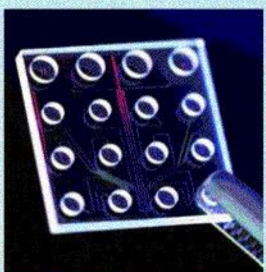
Applications
Gel electrophoresis has played an important role in Genomics. Next to this method also very fast open channel CE exists, e.g., point-of-care blood analysis.

7. Capillary Electrophoresis (CE) on a chip

- Introduction
- Progression of microchip CE systems
  - From modular integration by assembly vs. integration by technological strategy
- Tackling integration
- Blood analysis
- Outlook: Future developments
- Summary

Topics in this section

Leading companies go microfluidic analysis



Caliper & Agilent

From probes to systems

Introduction

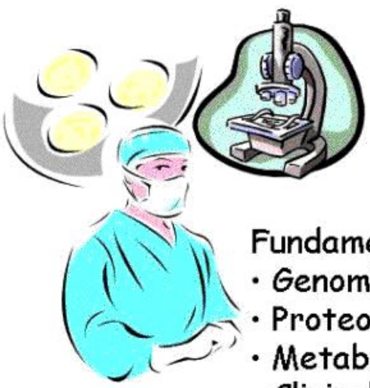
- Analytical requests
- Systems development for microchip CE
  - Historical development
  - Efforts of *big players.*
  - High-throughput development
  - New research activities within the established analytical field of separation science.

Objectives of analytical chemistry

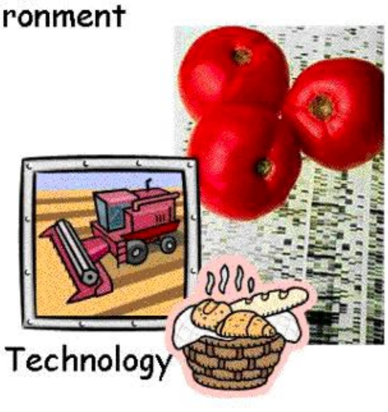
- Verification of measurement protocols (system evaluation).
- Conducting research leading to new measurement techniques (method development) including requirements of sample preparation, i.e. identifying interference.
- Design and development of new analytical systems. Capillary Electrophoresis (CE) played a large role in the fundamental research activities of Genomics (technology pull !!).
- Where to go now?
  - New frontiers of CE in analytical chemistry:
    - Proteomics
    - Metabolomics

Fields of analytic requests

Environment

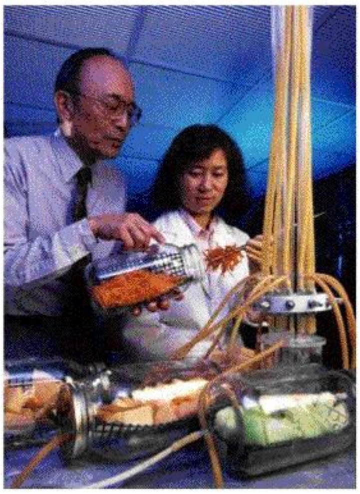


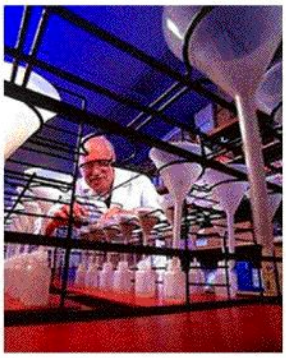
Fundamental R&D
- Genomics
- Proteomic
- Metabolomics
- Clinical



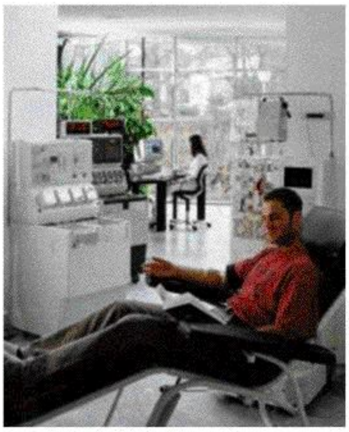
Food Technology

"Plenty of room at the bottom" in analytics for agricultural & clinical





Chemist extracts nitrogen from soil samples.



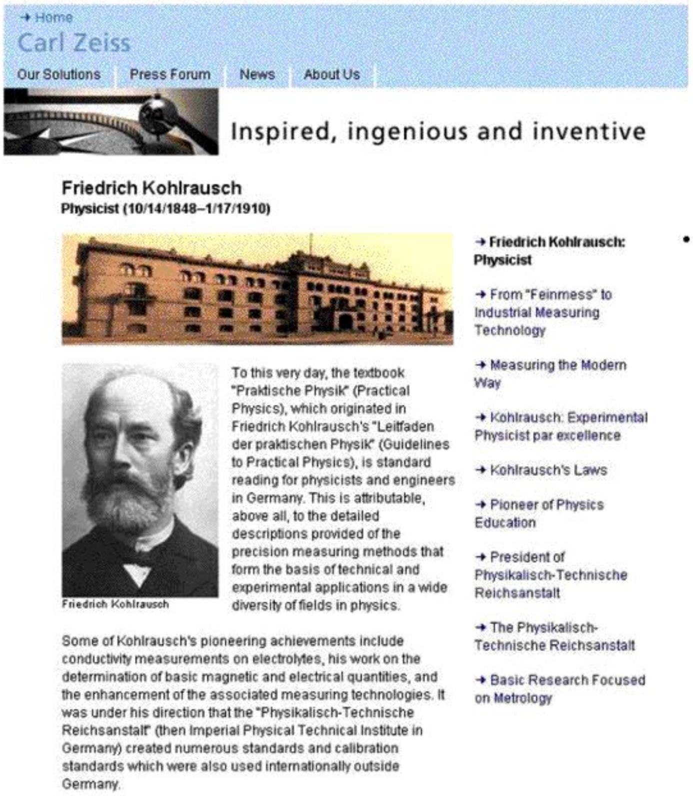
Dialysis patient

(Source: Fresenius)

Food technologist prepare e.g. shredded carrots for automated measurement of respiration rate and ethylene production.

www.ars.usda.gov

A historical figure



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Inspired, ingenious and inventive

Friedrich Kohlrausch
Physicist (10/14/1848–1/17/1910)

Friedrich Kohlrausch

To this very day, the textbook "Praktische Physik" (Practical Physics), which originated in Friedrich Kohlrausch's "Leitfaden der praktischen Physik" (Guidelines to Practical Physics), is standard reading for physicists and engineers in Germany. This is attributable, above all, to the detailed descriptions provided of the precision measuring methods that form the basis of technical and experimental applications in a wide diversity of fields in physics.

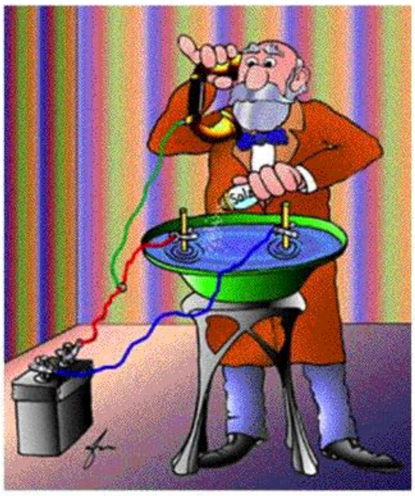
Some of Kohlrausch's pioneering achievements include conductivity measurements on electrolytes, his work on the determination of basic magnetic and electrical quantities, and the enhancement of the associated measuring technologies. It was under his direction that the "Physikalisch-Technische Reichsanstalt" (then Imperial Physical Technical Institute in Germany) created numerous standards and calibration standards which were also used internationally outside Germany.

→ **Friedrich Kohlrausch: Physicist**
→ From "Feinmess" to Industrial Measuring Technology
→ Measuring the Modern Way
→ Kohlrausch: Experimental Physicist par excellence
→ Kohlrausch's Laws
→ Pioneer of Physics Education
→ President of Physikalisch-Technische Reichsanstalt
→ The Physikalisch-Technische Reichsanstalt
→ Basic Research Focused on Metrology

- Mr. Kohlrausch, an important historical figure of the developments of capillary electrophoresis microanalysis.

www.zeiss.com

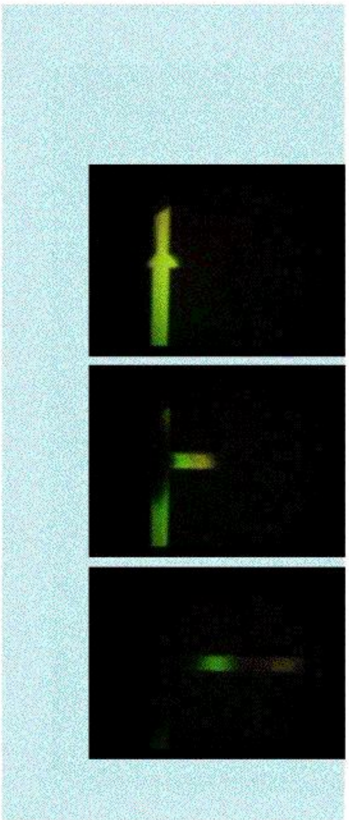
Ions on the move



- What's special about electrophoresis?
  - It's a method to separate ions on their mobility within an electrolyte solution thus it can be used to pull apart the different constitutes of a complex sample and determine with a suitable detector their concentrations.

(see also applications of electrical and optical detection and chip examples during the fabrication session…)

www.zeiss.com

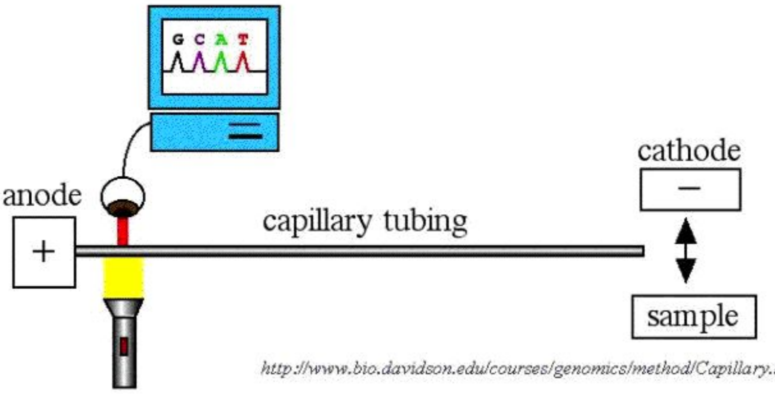


Progression of microchip CE systems

- Impact in Genotyping
- Birth of μTAS, here, microchip CE
- From modular integration by assembly vs. integration by technological strategy

DNA separation

- Most biological analytes of interest are positively charged and are therefore separated in the, so called, anodic mode.



http://www.bio.davidson.edu/courses/genomics/method/Capillary.html

Example: Running DNA electrophoresis on a MegaBASE Sequencer.

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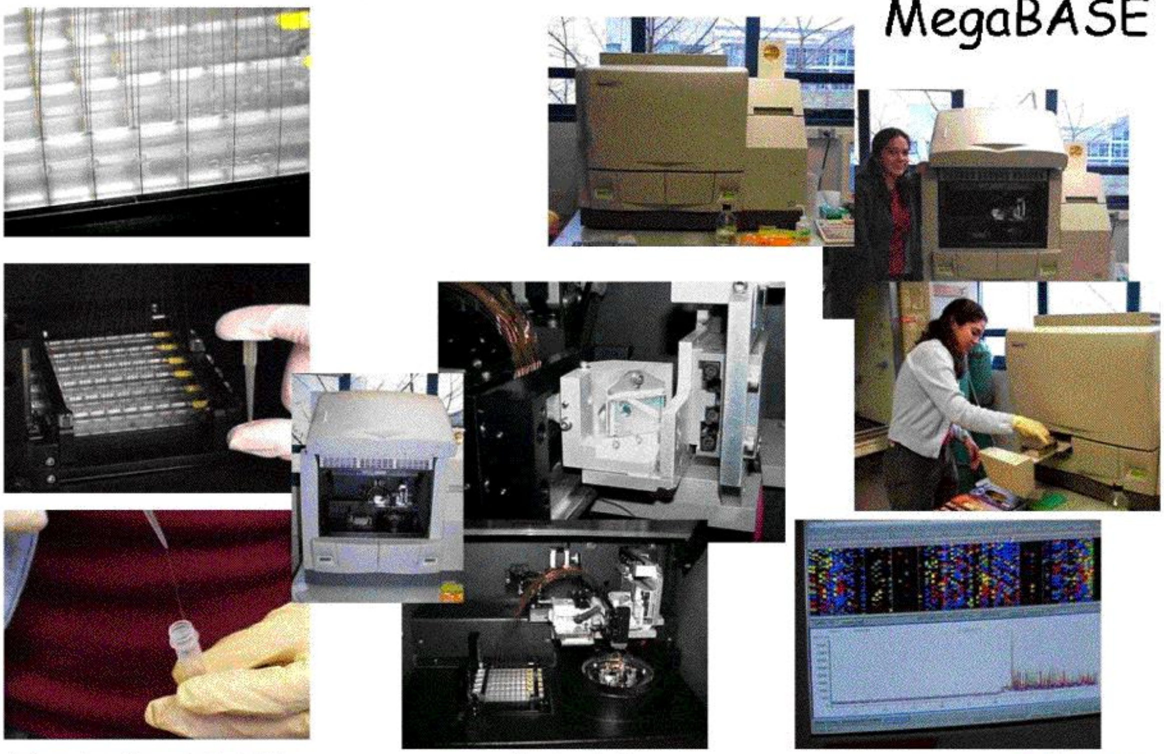
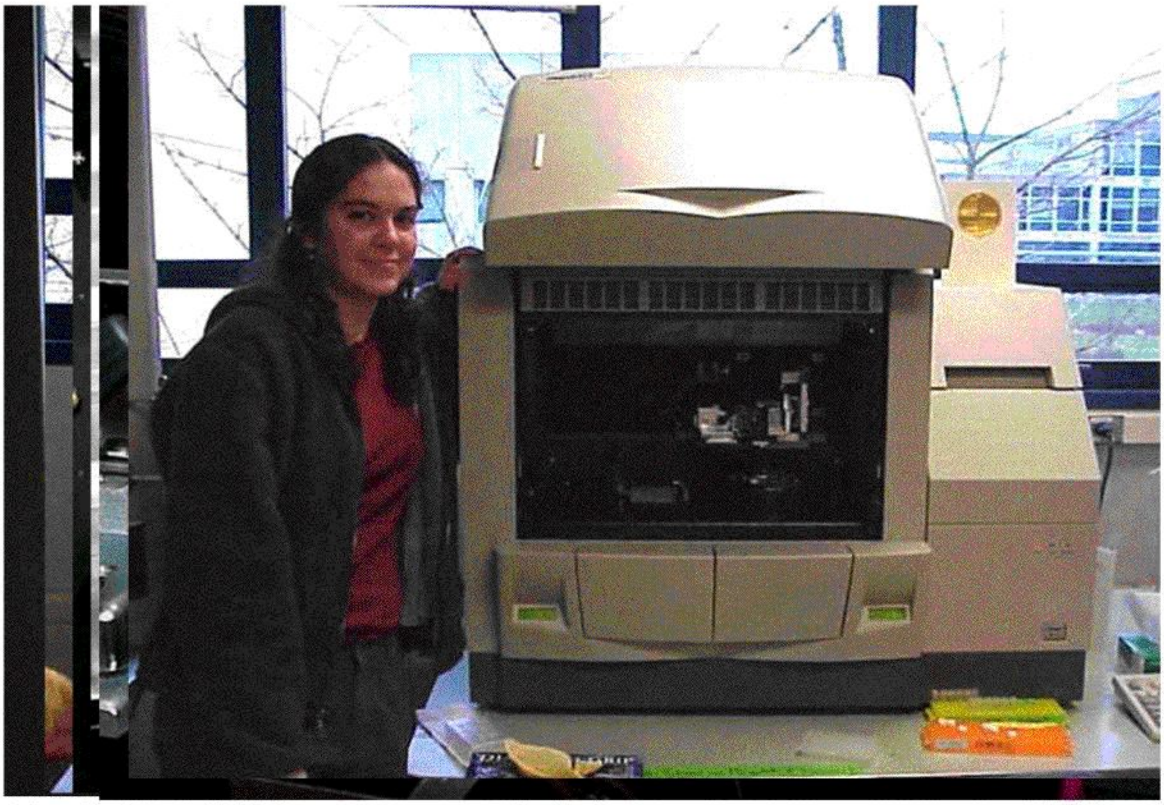
http://www.bio.davidson.edu/courses/genomics/method/Capillary.html

MegaBASE





http://www.bio.davidson.edu/courses/genomics/method/Capillary.html

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7.2. Progression of microchip CE systems

Capillary Electrophoresis goes planar

1926 *Anal. Chem.* **1992,** *64,* 1926–1932

Capillary Electrophoresis and Sample Injection Systems Integrated on a Planar Glass Chip

D. Jed Harrison,[*,†,‡] Andreas Manz,[*,§] Zhonghui Fan,[‡] Hans Lüdi,[§] and H. Michael Widmer[§]

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2, and Forschung Analytik, Ciba Geigy, CH 4002 Basel, Switzerland

- "Most successful analyses in the laboratory involve a complete ***system*** of sample treatment, separation, and analysis, designed to circumvent the complexities of a sample and its matrix. These methods are often time consuming or labor intensive".
- "To overcome this, the analysis process may be automated, increasing its speed, precision, and reproducibility. The use of flow injection analysis (FIA), and its coupling to separation methods such as gas or liquid chromatography, or selective chemical sensors is one route to achieve this."
- "High levels of automation have resulted in total chemical analysis systems (TAS) that can be used to monitor chemical concentrations continuously in industrial chemical and biochemical processes."

The birth of a μ-TAS

More then just the miniaturization of a TAS!

- "Such a device could be configured as a dip-type probe, giving out a reading for the analyte of interest, so that it behaved as a sensor from the perspective of the user."
- "Separation methods such as ***liquid chromatography*** and ***capillary electrophoresis***, as well as other bench-top analytical approaches such as FIA may also benefit from the μ-TAS approach."
- But why? → "Smaller dimensions result in improved performance for these analytical methods.[6-9]"

Essential background references

(6) Small Bore Liquid Chromatography Columns: Their Properties and Uses; Scott, R. P. W., Ed.; Wiley: New York, 1984.

(7) Micro-Column High Performance Liquid Chromatography; Kucera, P., Ed.; Elsevier: Amsterdam, 1984.

(8) Microcolumn Separations: Columns, Instrumentation and Ancillary Techniques., J. Chromatog. Libr. 1985, 30.

(9) van der Linden, W. E. Trends Anal. Chem. 1987,6, 37–40.

20 years of research and development: microchip CE gets finally "grown-up" (a bit)

- "Capillary electrophoresis (CE) is a separation method that could be coupled with FIA on a planar substrate to explore the μ-TAS concept, and this paper *(Harrison et al.)* examines the feasibility of doing so."
- Electroosmotic pumping is well suited to the μ-TAS concept, since the flow rate of solvent is controlled by electrokinetic effects that are approximately independent of capillary dimensions. In contrast, methods utilizing more conventional pumps develop extremely high back-pressures with small capillary dimensions and are not well suited to delivery of such low volume. [4, 5]"
- "By micromachining a complex manifold of flow channels in a planar substrate, it is possible to fabricate a network of capillaries capable of sample injection, pretreatment, and separation. We *(Harrison et al.)* have recently described the design of such a system.[5]"

Essential background references

(4) Manz, A.; Graber, N.; Widmer, H. M., Sens. Actuators 1990, Bl, 244-248.

(5) Manz, A.; Fettinger, J. C.; Verpoorte, E.; Ludi,H.; Widmer, H. M.; Harrison, D. J. , Trends Anal. Chem. 1991, 10, 144-149.

First attempt to microchip CE

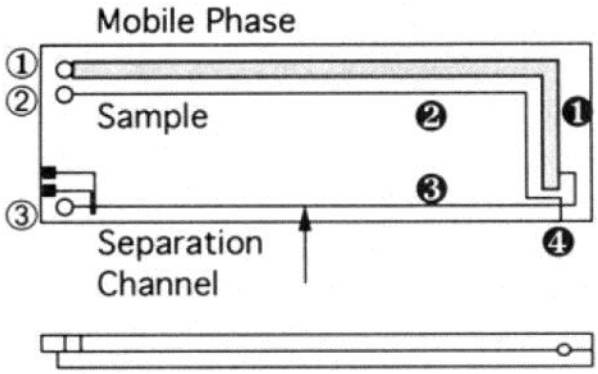


Figure 1. Layout of the channels in a planar glass substrate. Channels referred to in the text are identified by number (filled circles), as are the inlet points (reservoirs) to each channel (open circles). Each channel is labeled with its content or its function. Overall dimensions are 14.8 cm × 3.9 cm × 1 cm thick. The location of one pair of Pt electrodes is also shown; for clarity the others are not. The point of fluorescence detection is marked by an arrow.

7.2. Progression of microchip CE systems

Cross-injector structure in glass

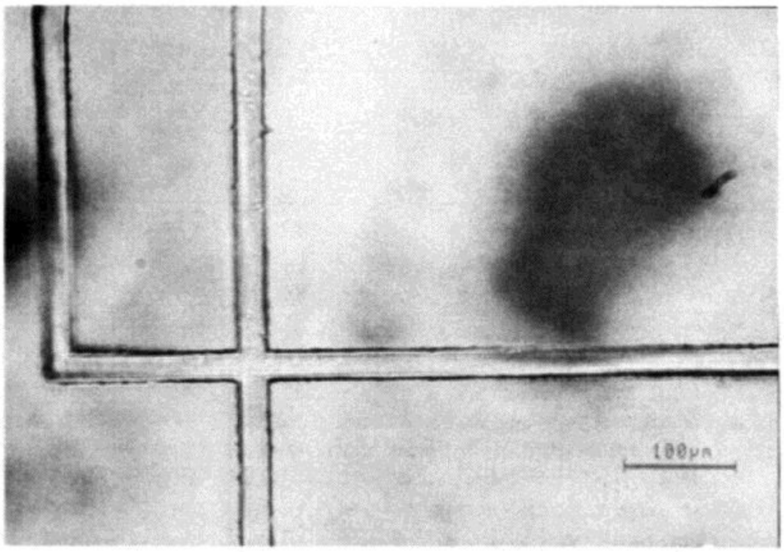
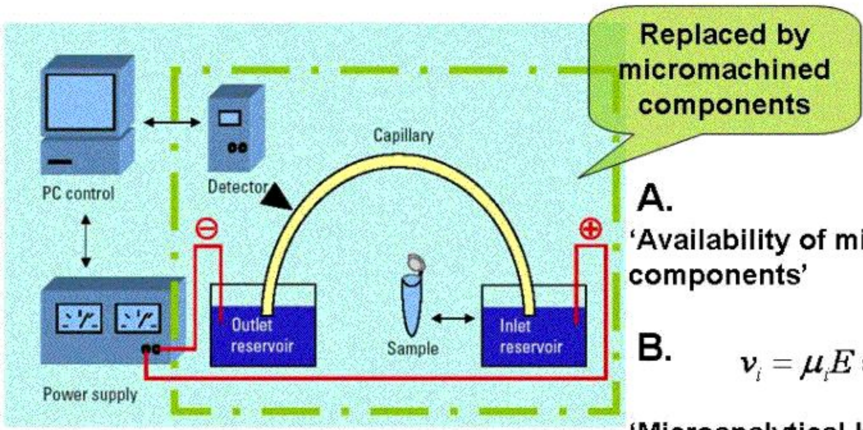


Figure 2. Photomicrograph of the intersection point of the four channels shown in Figure 1 after the glass plates have been bonded together. The channel width is 30 µm. Channel depth is 10µm.

Feasibility of separation and valveless liquid handling

- "In this work we *(Harrison et al.)* have demonstrated the feasibility of using electroosmotic pumping and electrophoretic separation methods within a planar structure fabricated in glass."
- "The effectiveness of the glass substrate for electrophoretic separation has been compared to more conventional fused-silica capillaries."
- "In addition, the valveless switching of fluid flow between channels in a multichannel manifold has been studied and the limits of the approach explored."
- "The results show that the combination of FIA and CE in a μ-TAS environment is possible, opening up exciting possibilities for this approach."

Status of CE miniaturization

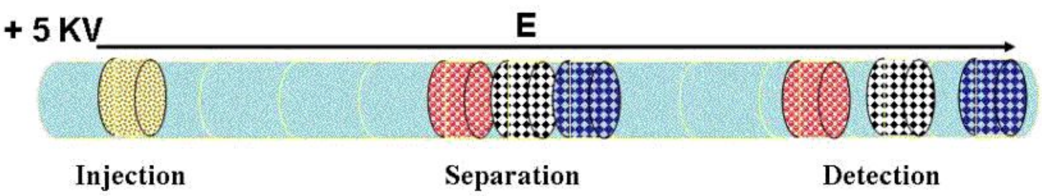


A.

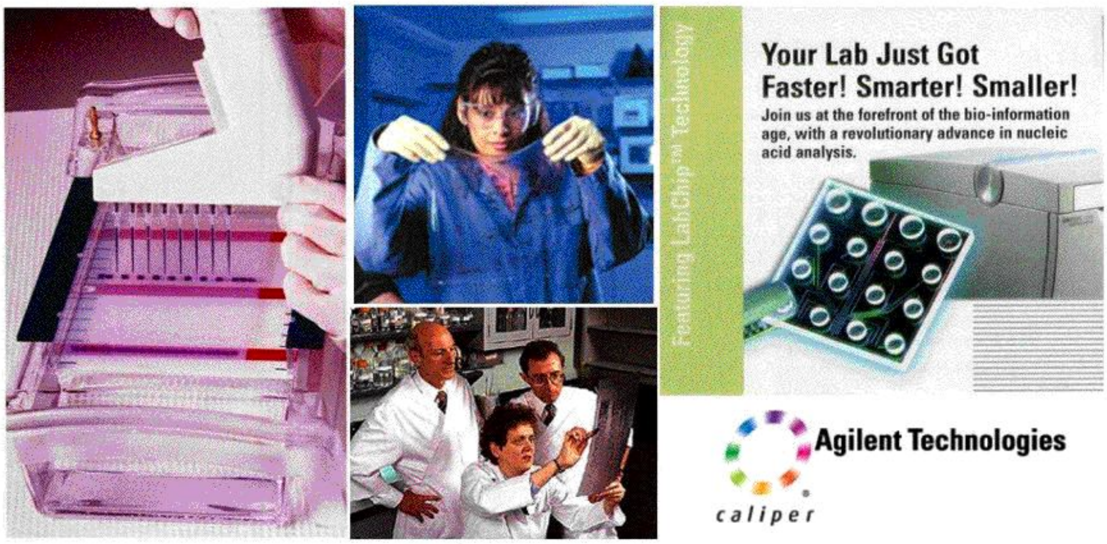
'Availability of miniaturized components'

B. $$v_i = \mu_i E \approx \frac{q_i}{6\pi r_i \eta} E$$

'Microanalytical know-how'

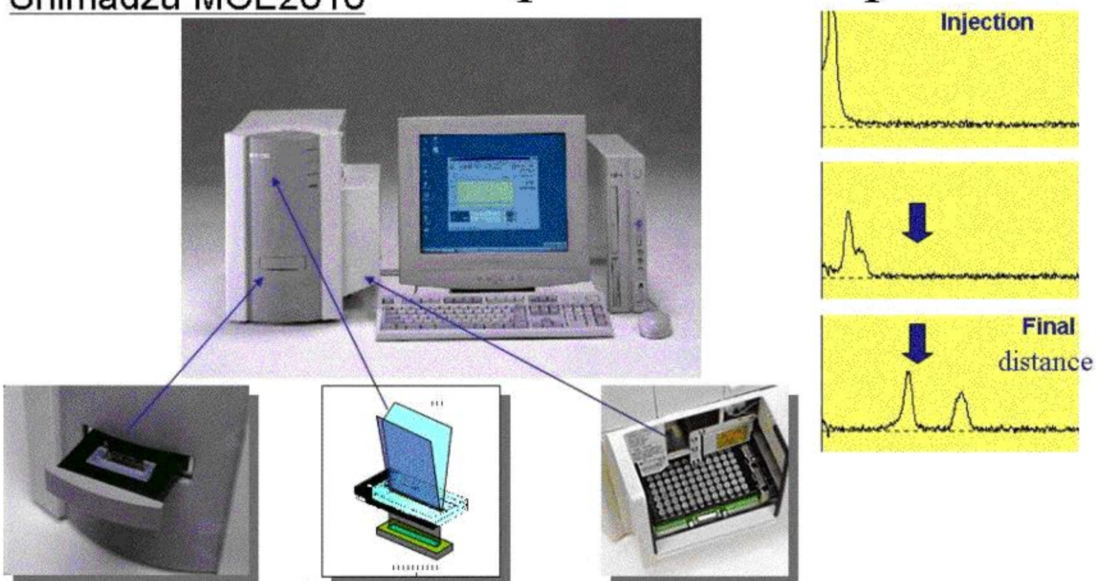


…going to lab automation and smaller and smaller operating apparatus footprint



More of a platform for chip-based electrophoresis

Shimadzu MCE2010

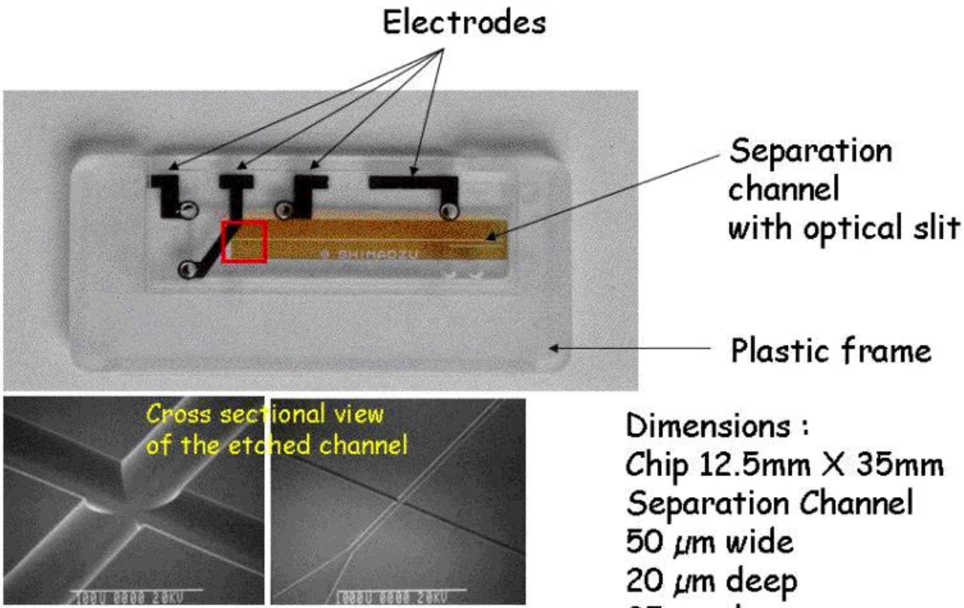


Quartz Chip

Linear Imaging UV Detector

Autosampler

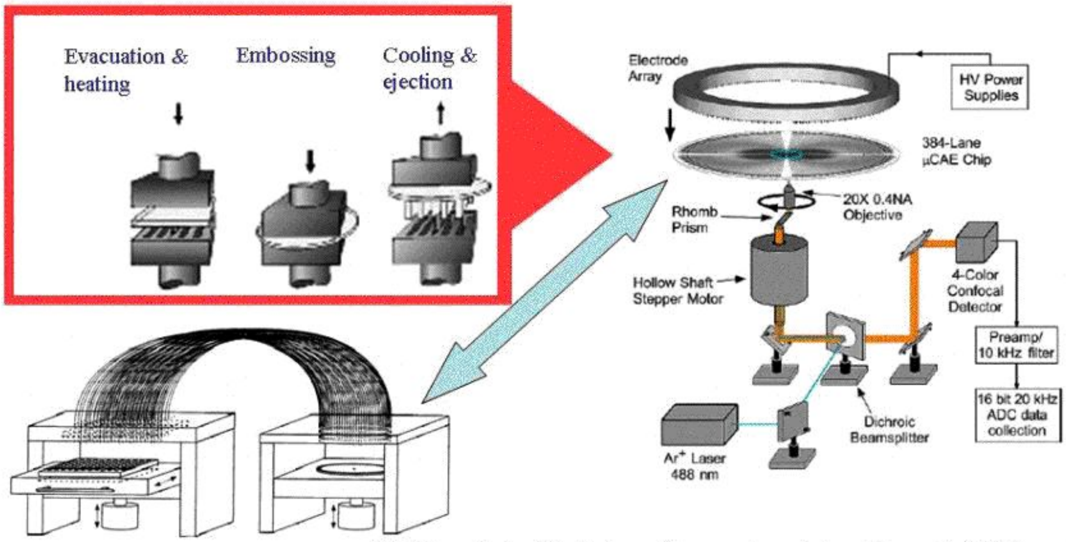
Shimadzu MCE Chip



Dimensions :
Chip 12.5mm X 35mm
Separation Channel
50 µm wide
20 µm deep
25 mm long

Massive parallel analysis

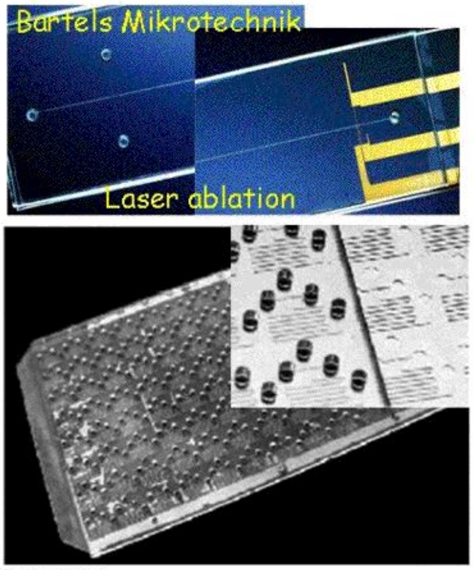
Advanced modular integration: 'Off-chip versus On-chip'



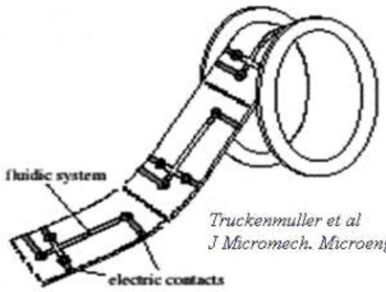
Mathies et al., 'Radial capillary microplate with up to 384-lanes'

7.2. Progression of microchip CE systems

Fabrication…faster and cheaper ?



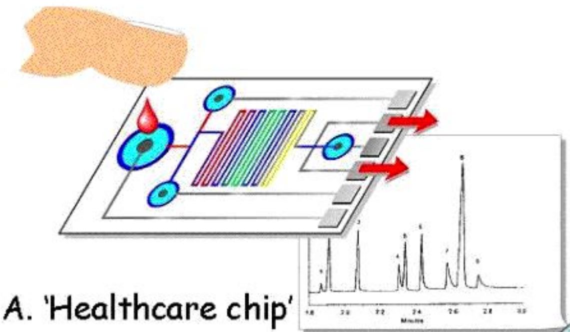
Gerlach et al.,
Microsystems Technologies 7 (2002), 265-268



Truckenmuller et al
J Micromech. Microeng. 12 (2002) 375

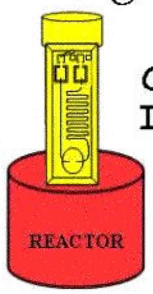
7.2. Progression of microchip CE systems

New research activities and further efforts of integration?

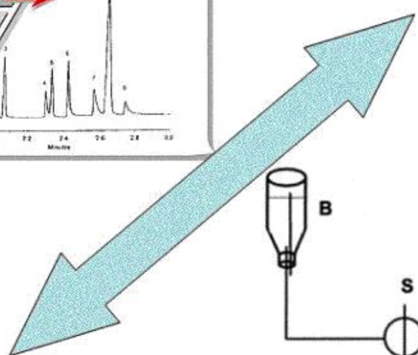


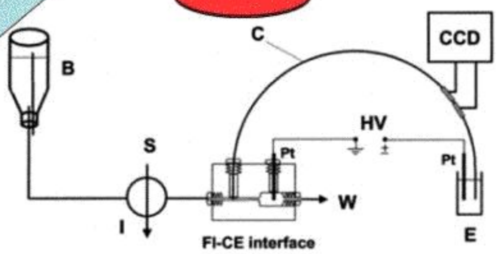
A. 'Healthcare chip'

E. Vrouwe et al., University of Twente



On-chip Integration





Off-chip modular integration

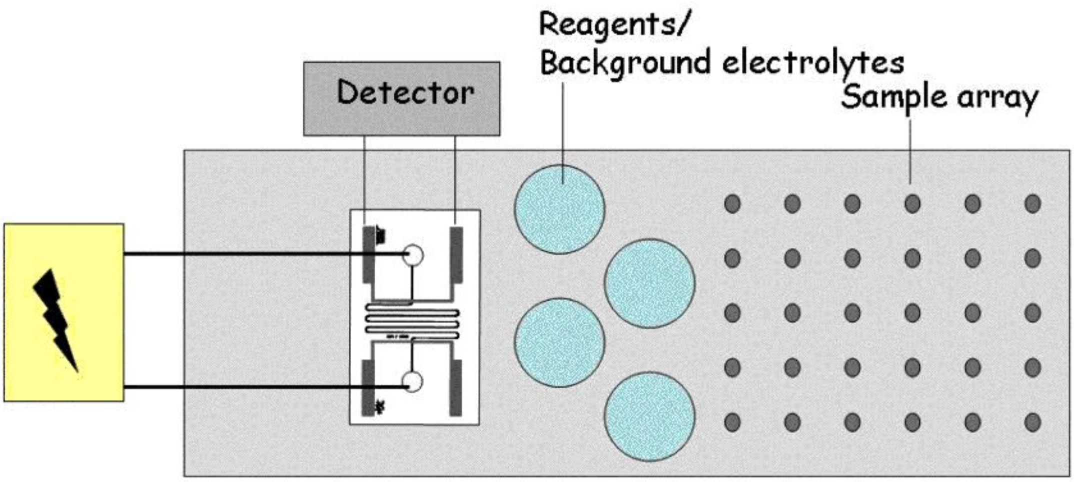
B. 'Flow injection-capillary electrophoresis (FI-CE) system'

Electrophoresis 2003, 24, 1935–1943

Tackling integration

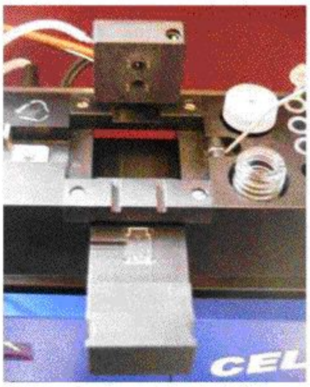
- Packaged microchip CE
  - CE microchip fabrication issues
  - Modular approach
  - Fully integrated
- Probe-type system
  - Chip design requirements.
  - Case study

Microchip CE platform

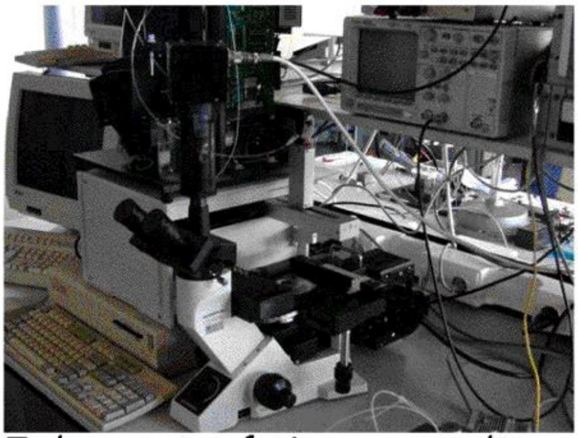


'Celine- concept'
Courtesy: Richard Schasfoort, University of Twente

Microchip research at the bench



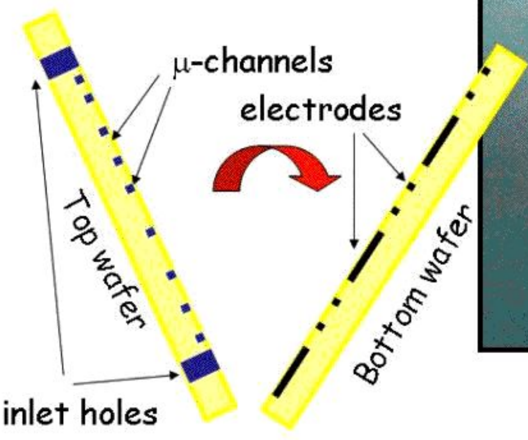
Celine-chip holder
"blind run"

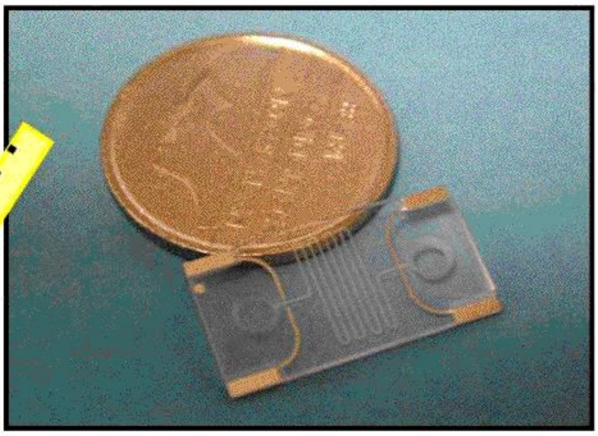


Tadem set-up of microscope and autosampler (Celine concept) allows visualization of fluidic flow profiles

Courtesy: Richard Schasfoort, University of Twente

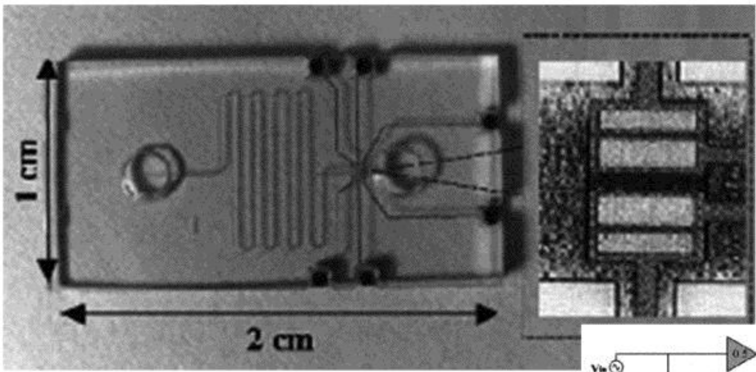
Glass chip

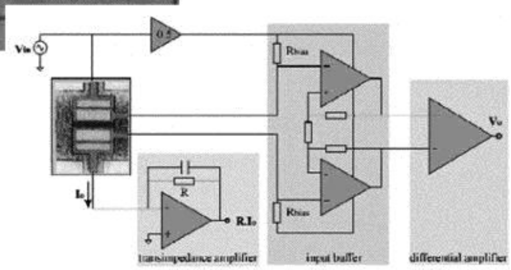




Stefan Schlautmann, STW-BIOMAS project (finalized)

Packaging: design varieties of conductivity detection cell

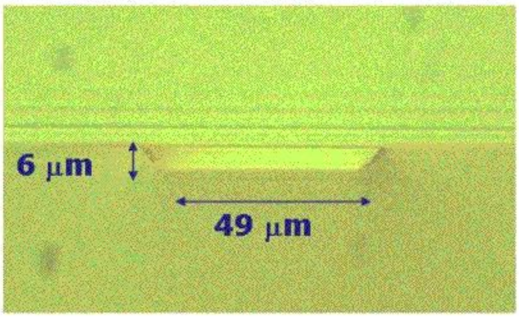




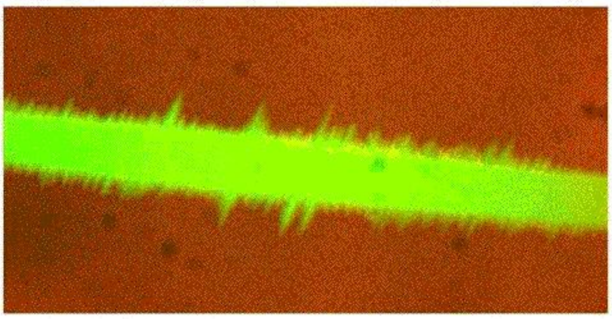
F. Laugere et al./ S&A B 83 (2002) 104-108

Influence of fabrication

"Rough edges"



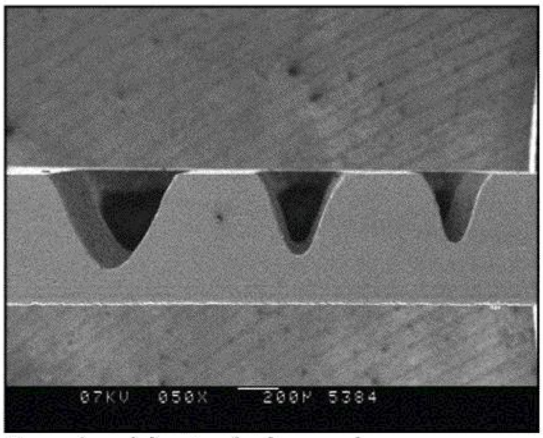
End view of hydrofluoric acid etched channel



Fluorescent dye inspection of filled channel. Etching defects.

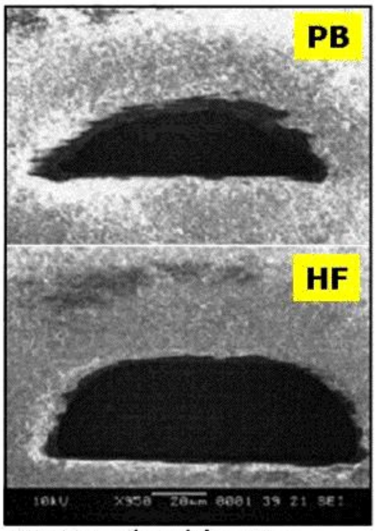
Channel definition

“Cross sectional profiles”



Powder blasted channels;
profile characterization

Q.-S. Pu et al., Electrophoresis 2003, 24,162–171

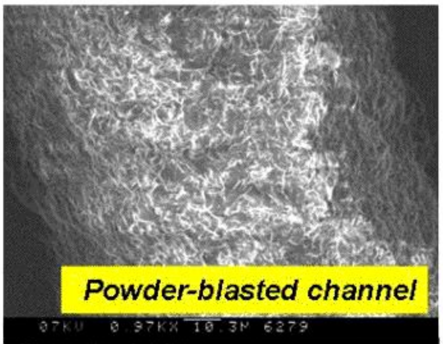


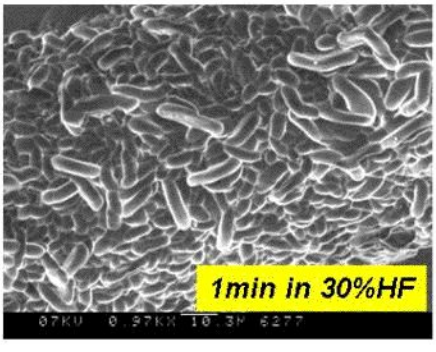
PB: Powder blasting,
HF: Hydrofluoric acid etching

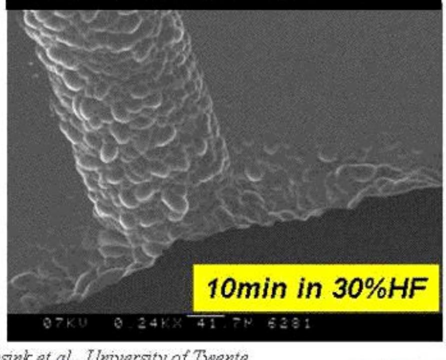
Channel characterization

“Surfaces”

Effects on separation performance due to surface topology are expected therefore wall coatings are strongly investigated.

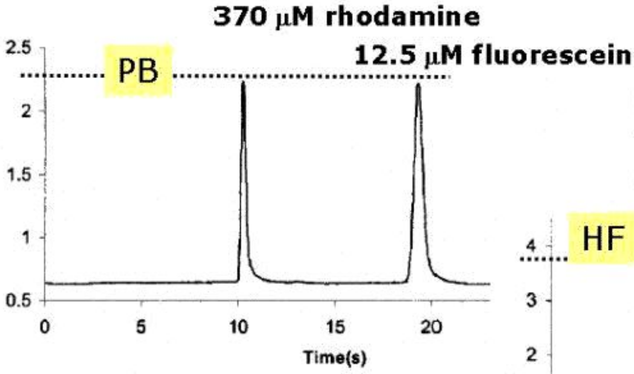




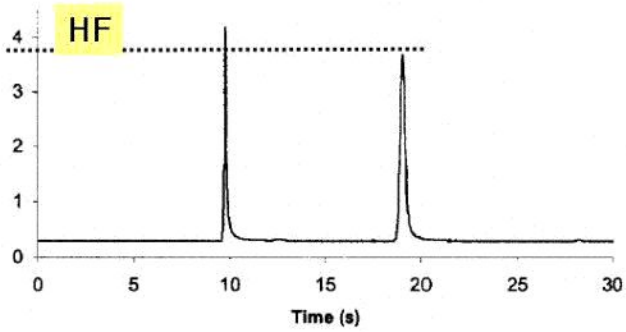


Wensink et al., University of Twente

Powderblasting versus HF etched channels



Running buffer: 10 mM $NaHCO_3$; field strength: 664 V/cm; migration length: 50mm



Fluorescent detection.

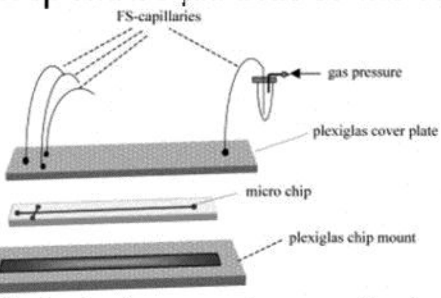
Vertical scale: arbitrary units.

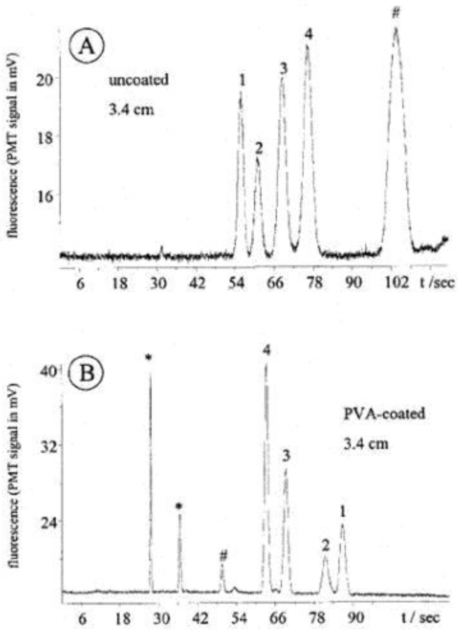
Q.-S. Pu et al., Electrophoresis 2003, 24,162–171

Chip wall surface coating

Micralyne (Edmonton, Canada). 16 × 95 × 2.2 mm, simple cross injector design.

Separation length is 85mm. All channels are 20 μm deep and 50 μm wide at the top.





Poly(vinyl alcohol)-coated microfluidic device for high-performance microchip electrophoresis

D. Belder et al., Electrophoresis 2002, 23, 3567–3573

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Optimization by *choice* of materials

- UV Laser machined polymer substrates for the development of microdiagnostic systems.

Requires not only optimization of fabrication technology but also dedicated **analytical characterization** of performance!

Table 1. Surface and Electroosmotic Flow Characteristics of Photoablated Polymers with and without Protein Coating

substrate	rugosity (μm)	ζ potential (mV)	v_{EOF} (mm·s^{-1})	μ_{EOF} ((cm^2·V^{-1}·S^{-1}) × 10^4)
unablated polymer	0.01	na[a]	na[a]	na[a]
polycarbonate	0.13	−52.75	0.91	4.20
BSA-coated PC	0.13	−30.47	0.53	2.42
polystyrene	0.13	−56.22	0.97	4.47
BSA-coated PY	0.13	−35.71	0.62	2.84
cellulose acetate	0.27	−59.65	1.03	4.74
BSA-coated CA	0.22	−29.01	0.50	2.31
PET	0.39	−72.85	1.26	5.79
BSA-coated PET	0.4	−39.83	0.69	3.17

[a] Not applicable.

M. A. Roberts et al., Anal. Chem., 1997, 69, 2035-2042

7.3. Tackling integration

Detection system

Principles:

- Conductivity detection, amperometric detection (EC methods),
- Fluorescent detection, UV absorption (optical methods)
- Mass spectrometry

See sessions electrical, optical an MS Detection

There are two aspects of detection:

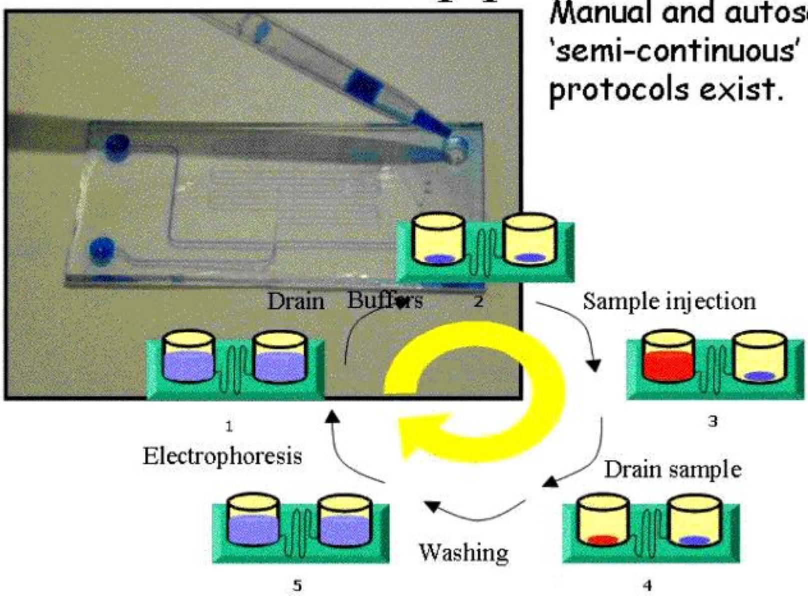
1) Peak identifying
2) Quantification

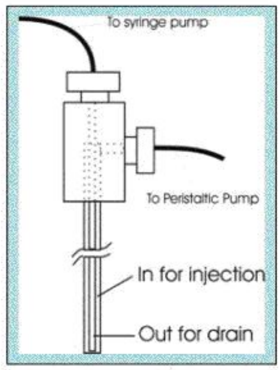
Most *Capillary Electrophoresis Experiments* (so far described in microsystems literature) are concerned with identification of compounds instead of quantified results !!

Thursday, August 18, 2005 Regina Luttge MESA+

Putting autosampling liquid handling into CE microchip practice: head-end injection

Manual and autosampler 'semi-continuous' liquid handling protocols exist.





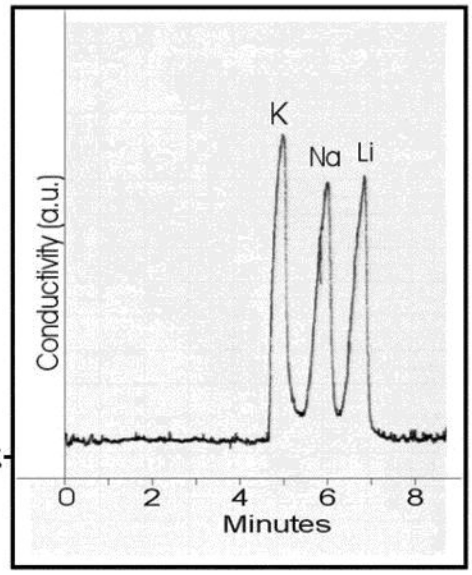
Double needle

Richard Schasfoort, University of Twente, STW-BIOMAS project (finalized)

First separation results

Cations

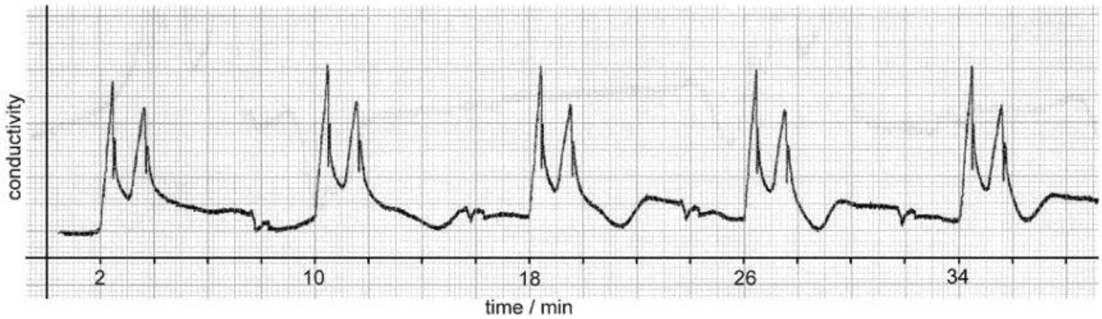
- Sample: 0.5 mM K, Na, Li
- Tris/MES 25 mM, pH 7.4
- V=500 V, 6 cm, 50x27 μm
- Head-end EK injection, 10 s, 500V
- I=0.6 μA
- Conductivity detection at 1 kHz, lock-in amplifier electronics (Sprenkels Consultancy)



Richard Schasfoort, 2000, University of Twente, STW-BIOMAS project (finalized),

First separation results

Anions



Repetitive injection and separation of nitrate and phosphate in a CE system: 6 cm powderblasted channel 85x22 μm; sample: 1 mM Nitrate, Phosphate; Tris/MES 20 mM, pH 7.4, CTAB, 30 μM,; EK injection, 5s, -1000V; Separation V= -1000 V, I=-1μA; Conductivity detection 1 kHz, lock in amplifier electronics.

Richard Schasfoort, 2000, University of Twente, STW-BIOMAS project (finalized),

---

Further reading

Electrophoresis 2001, *22*, 235–241 235

Rosanne M. Guijt[1]
Erik Baltussen[1]
Gert van der Steen[1]
Richard B. M. Schasfoort[2]
Stefan Schlautmann[2]
Hugo A. H. Billiet[1]
Johannes Frank[1]
Gijs W. K. van Dedem[1]
Albert van den Berg[2]

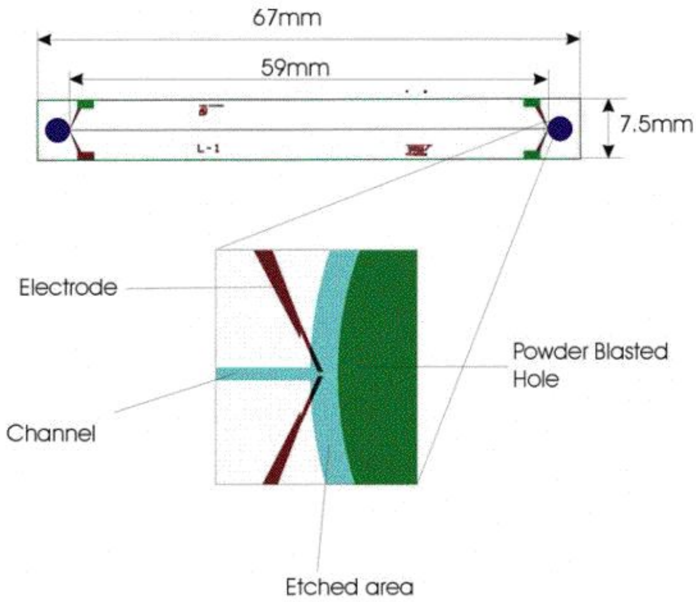
[1]Kluyver Laboratory for Biotechnology, Delft University of Technology, Delft, The Netherlands
[2]MESA Research Institute, University of Twente, Enschede, The Netherlands

New approaches for fabrication of microfluidic capillary electrophoresis devices with on-chip conductivity detection

In practice, microfluidic systems are based on the principles of capillary electrophoresis (CE), for a large part due to the simplicity of electroosmotic pumping. In this contribution, a universal conductivity detector is presented that allows detection of charged species down to the μM level. Additionally, powderblasting is presented as a novel technique for direct etching of microfluidic networks. This method allows creation of features down to 50 μm with a total processing time (design to device) of less than one day. The performance of powderblasted devices with integrated conductivity detection is illustrated by the separation of lithium, sodium, and potassium ions and that of fumaric, malic, and citric acid.

Keywords: Micro-total analysis systems / Micromachining / Capillary electrophoresis / Conductivity detection EL 4255

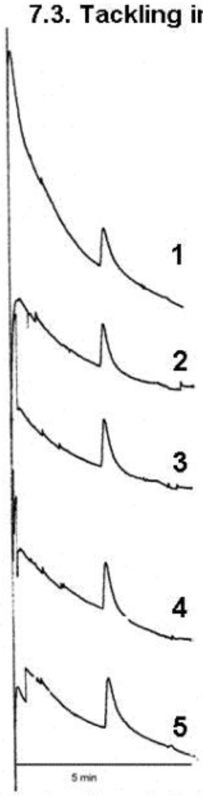
Chip design for CE-EC detection



- HF etched channel and two pairs of electrodes.
- electrodes were placed outside the channel and etched compartments were added for accurate alignment.
- Channel dimension 55μm. and 37μm wide, 9μm deep.

QS Pu, 2000, University of Twente, STW-BIOMAS project (finalized)

Integrated Pt-thin-film electrode



Electrochemical detection

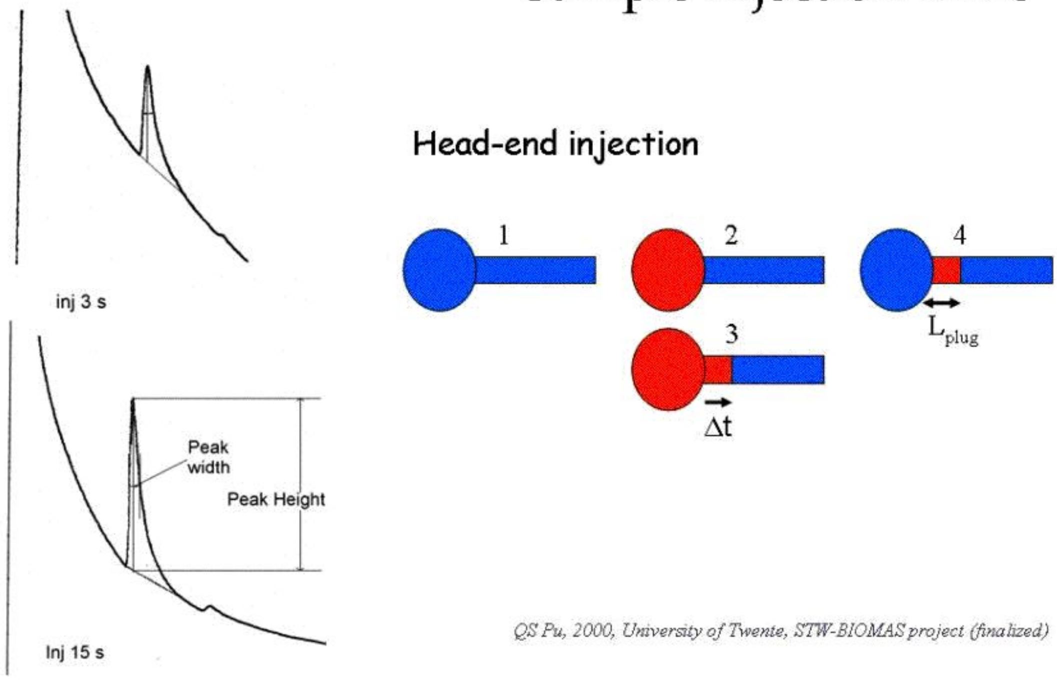
- *Dopamine* at Pt electrode, 2 electrode configuration.
- Smooth baseline was obtained.
- Both migration time and peak height were reproducible.
- Peak height RSD: 8.4% (n=5)

See also session: electrical detection

0.6 mM dopamine in water; phosphate buffer, pH 7.4;
Separation voltage: 500V (84.7V/cm);
Detection potential: 1.8V vs. CE-counter electrode

QS Pu, 2000, University of Twente, STW-BIOMAS project (finalized)

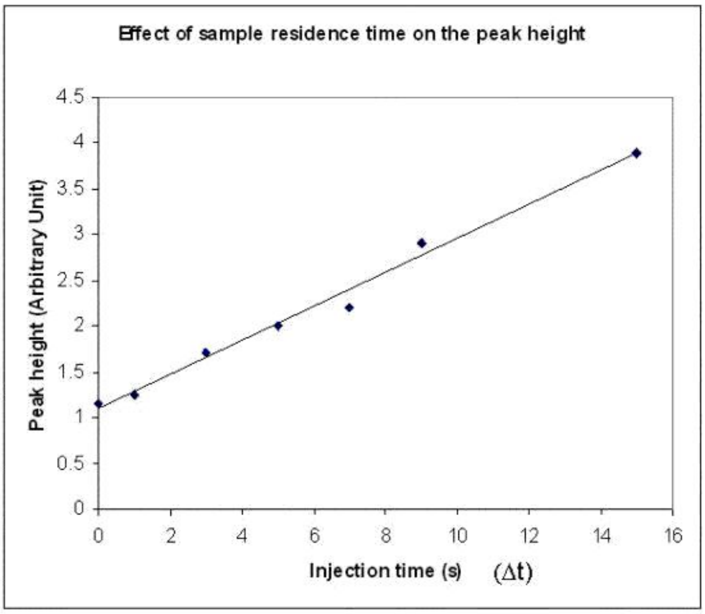
Sample Injection Time



QS Pu, 2000, University of Twente, STW-BIOMAS project (finalized)

Peak height linearity

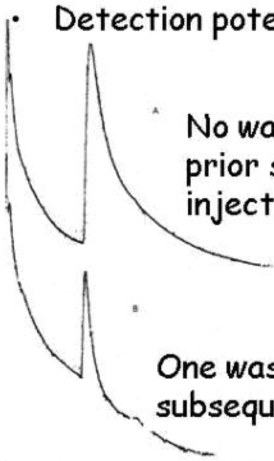
- Longer injection time results in a longer plug length = greater sample volume, greater peak height.
- Concentration, however, is dependent on peak area.
- Intercept:
  - Sampling response time
  - Response on hydrodynamic flow
  - Dead volume



QS Pu, 2000, University of Twente, STW-BIOMAS project (finalized)

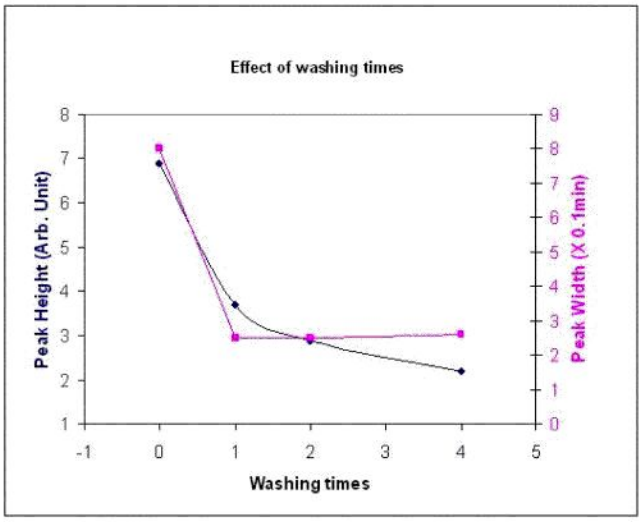
Side effect due to liquid handling procedure

- 0.6mM dopamine on Pt;
- Injection time:15s;
- Phosphate buffer, pH 7.4;
- E-field: 84.7 V/cm;
- Detection potential: 1.8V



No wash cycle prior subsequent injection

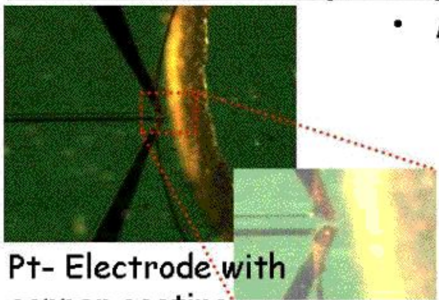
One wash cycle prior subsequent injection



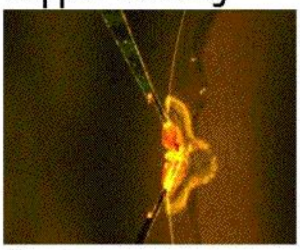
QS Pu, 2000, University of Twente, STW-BIOMAS project (finalized)

Modifying electrode surface

Localized Electroplating



Pt- Electrode with copper coating



During use copper diffusion takes place.

- After chip fabrication
  - Electroplating using $CuSO_4$ solution with platinum electrode as anode, process is difficult to control.
  - Copper diffusion during use of chip was observed (spreading of the copper, see picture).
  - Freshly plated electrodes could not be used immediately after plating.
  - Aging at 60°C under air can increase its stability.
  - Plating process with copper anode and commercial plating solution are more stable during use!

QS Pu, 2000, University of Twente, STW-BIOMAS project (finalized)

Further reading

THE ANALYST
FULL PAPER
www.rsc.org/analyst

A two-electrode configuration for simplified amperometric detection in a microfabricated electrophoretic separation device

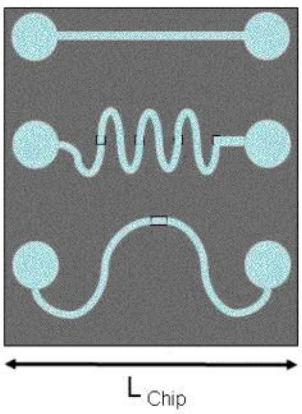
Maria A. Schwarz, Benedikt Galliker, Karl Fluri, Thomas Kappes and Peter C. Hauser*

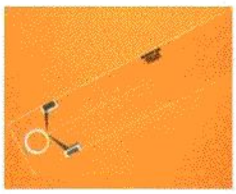
The University of Basel, Department of Chemistry, Spitalstrasse 51, 4056 Basel, Switzerland

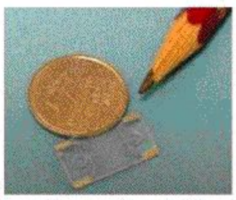
Received 12th September 2000, Accepted 20th November 2000
First published as an Advance Article on the web 5th January 2001

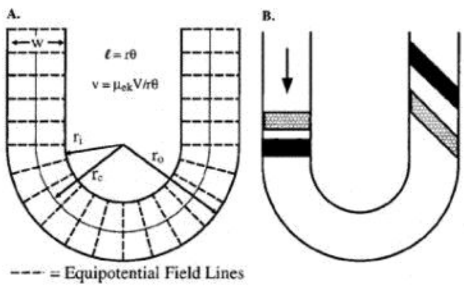
The simplified amperometric detection scheme demonstrated is based on the amperometric working and electrophoretic ground electrodes only. The latter serves as counter and pseudo-reference as well. It is shown *via* the successful determination of neurotransmitters, ascorbic acid and phenols on gold or platinum working electrodes that this approach is feasible for detection on a channel based electrophoretic separation device. Also presented is the detection of carbohydrates and amino acids with copper electrodes. The results were found to be similar to those obtained with conventional capillary systems with amperometric detection, albeit at much reduced analysis times.

Chip design



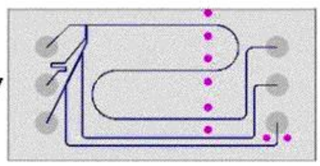


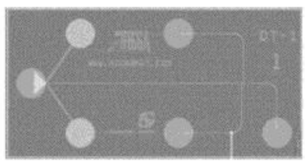




C.T. Culbertson et al.,
Anal. Chem., 1998, Vol. 70, 3781-3789

Increasing complexity of the flow pattern



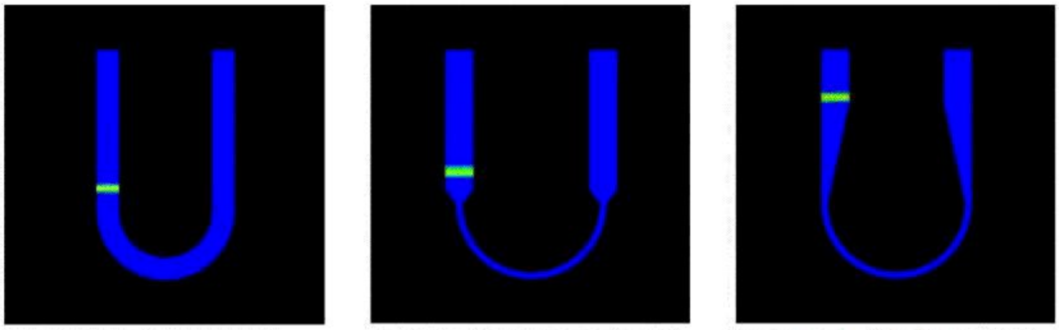


Turn designs

MICROCOSM TECHNOLOGIES
Enabling the Future with Microsystems
(Mathies and Paegel)

Examples of design optimization

- Optimization aided by FlumeCAD
- Avoids time consuming experiments
- Results enabled first high quality sequencing on a chip for ~ 500 bp DNA



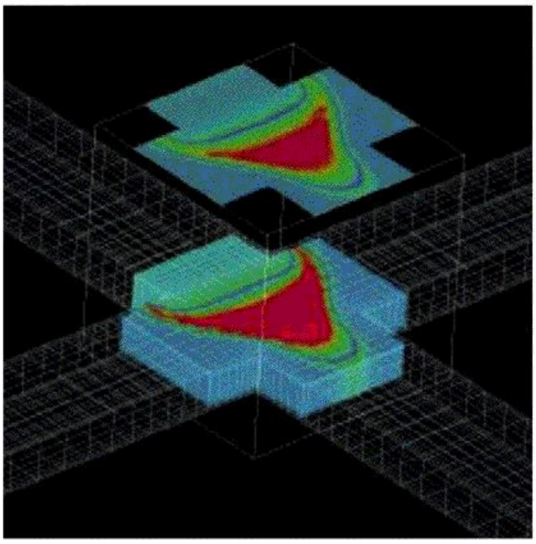
Straight turn → Optimized turn

Pull back injection scheme

Examples of design optimization

- Microcosm can help optimize a design already created experimentally
- Here a case study is presented that began with design optimization from experiment, but which ended up with simulation leading the innovation process.

MICROCOSM TECHNOLOGIES
Enabling the Future with Microsystems

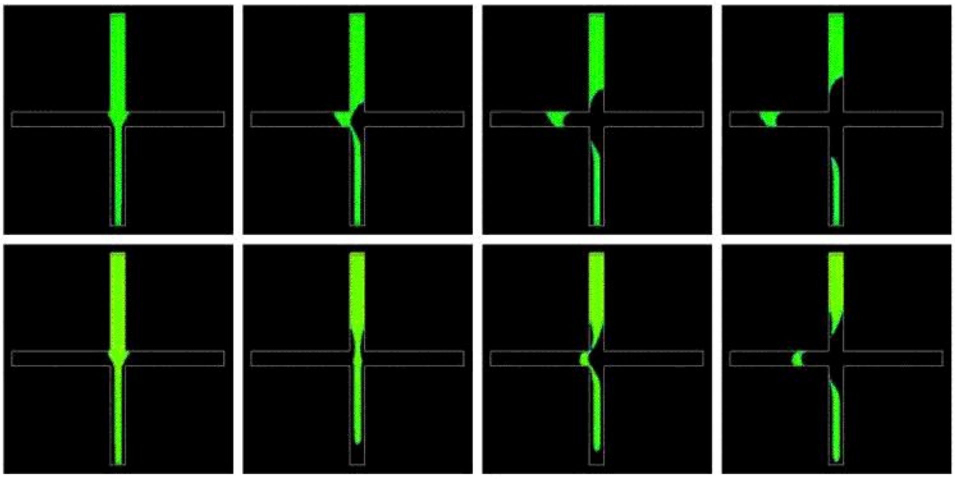


Field-reversal just before injection

MICROCOSM TECHNOLOGIES
Enabling the Future with Microsystems

Top row: Normal injection

Bottom row : Reversal added before injection



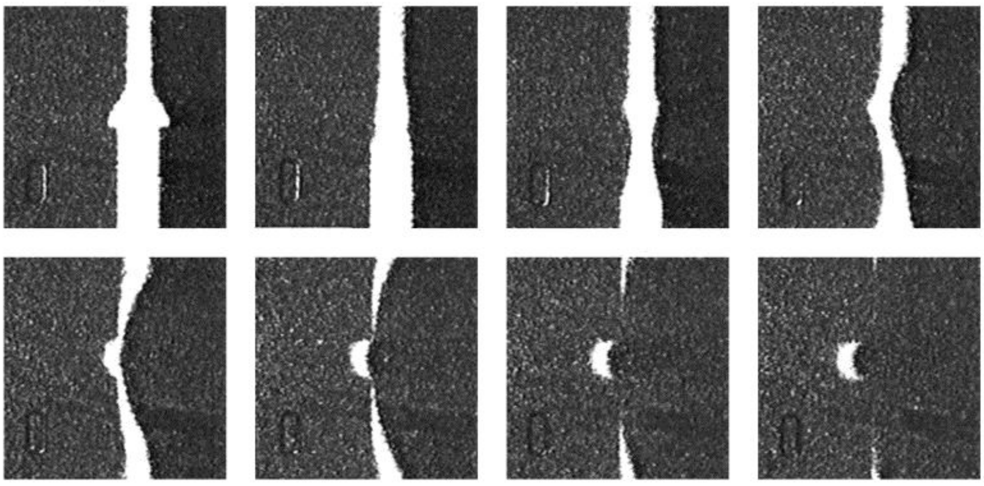
MESA+

7.3. Tackling integration

Experimental implementation by Caliper

MICROCOSM TECHNOLOGIES
Enabling the Future with Microsystems

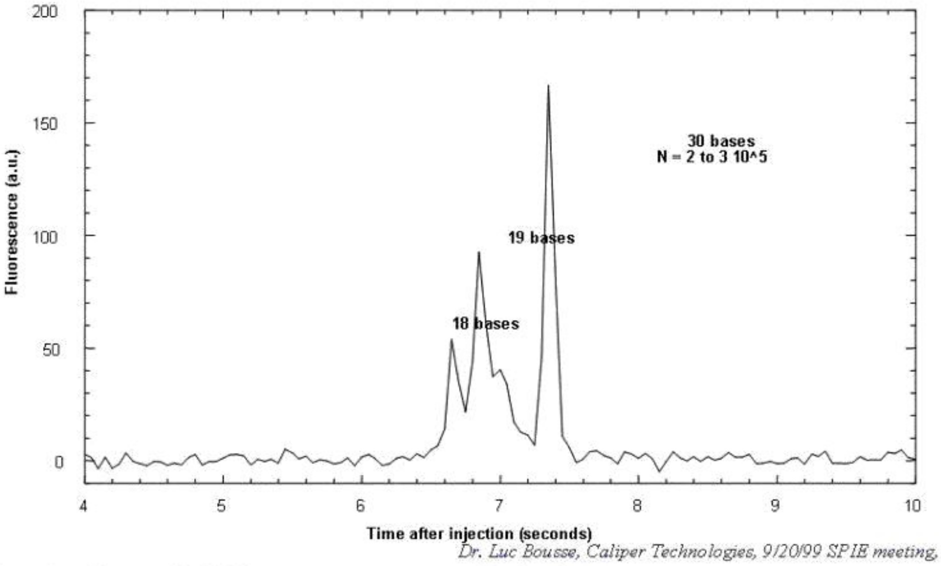
- Images shown here are at 100 ms intervals



MESA+

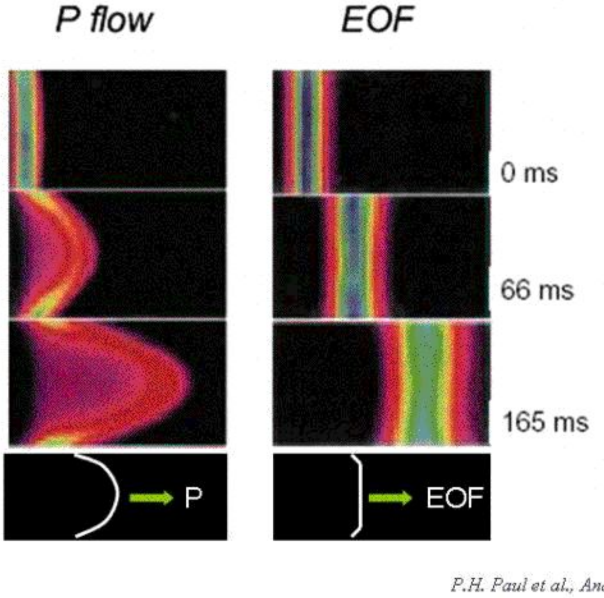
World's fastest DNA separations

MICROCOSM TECHNOLOGIES
Enabling the Future with Microsystems



Dr. Luc Bousse, Caliper Technologies, 9/20/99 SPIE meeting, Santa Clara CA

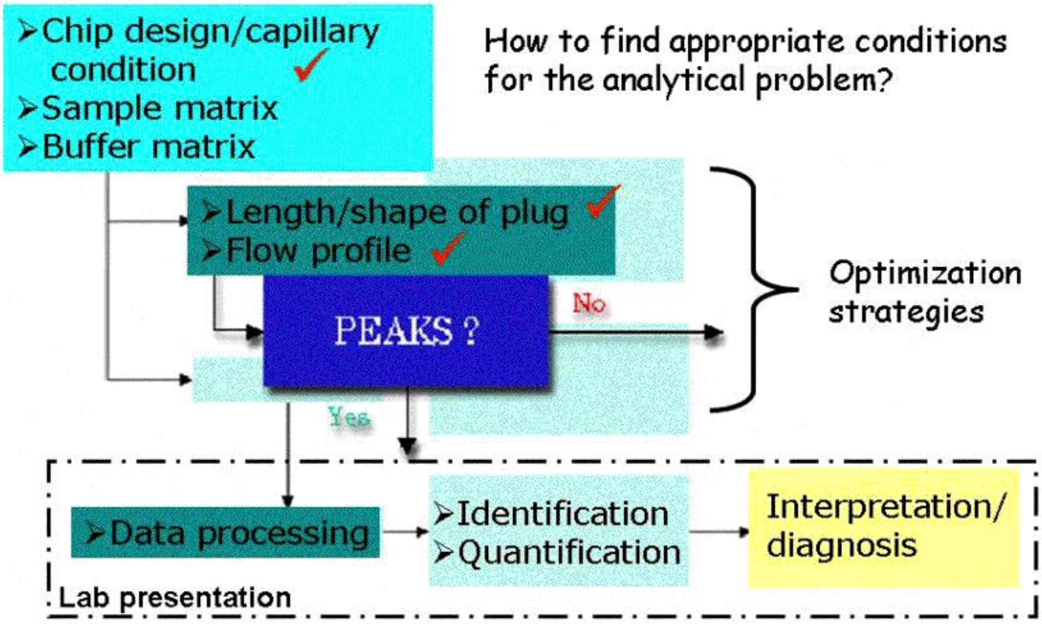
EOF versus Poisseuille (laminar) flow

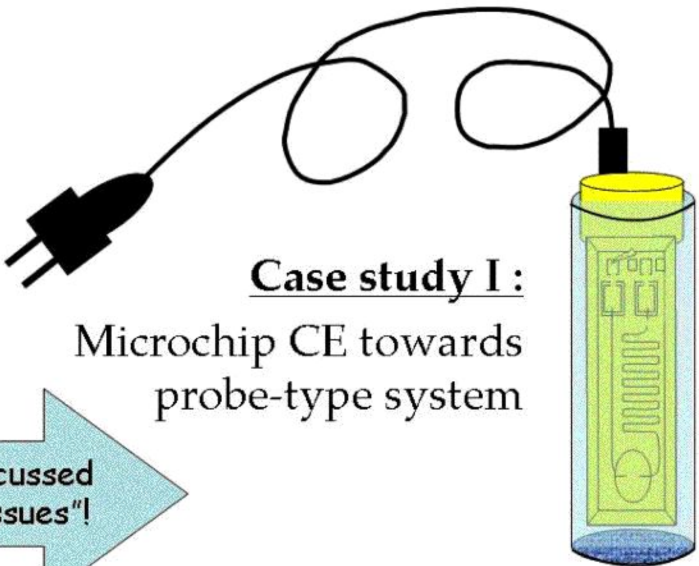


- fluorescent dye visualizes distortion of a fluid volume in EOF and P flow
- greater distortion in P flow
  - ⇒ greater dispersion of sample plugs
  - ⇒ dilution (bad for detection)
  - ⇒ bandbroadening (bad for sample throughput)

P.H. Paul et al., Anal. Chem. 1998, 70, 2459-2467

System method development



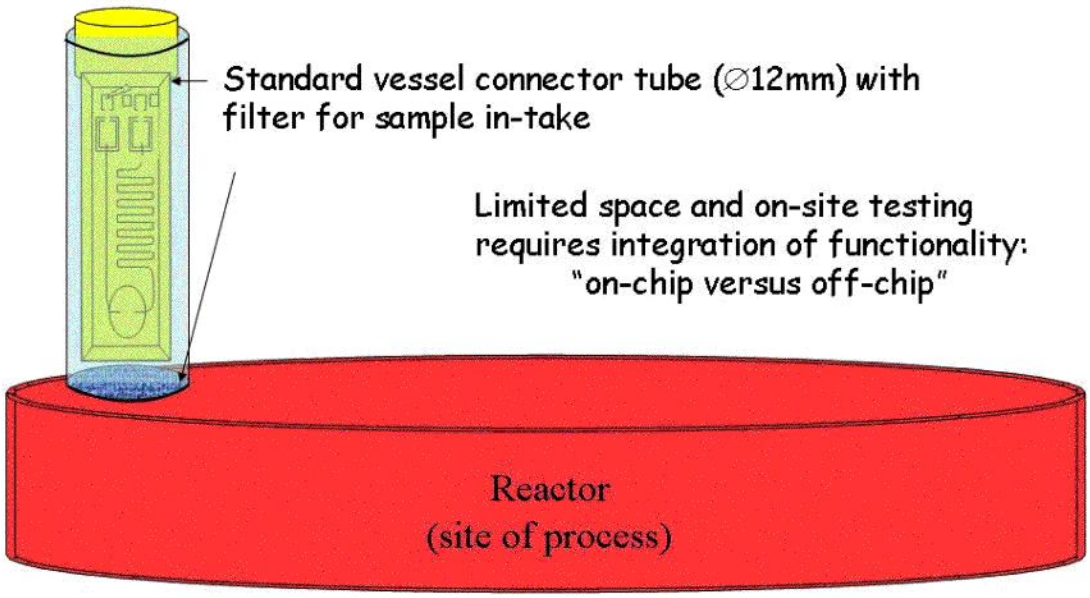


Case study I :

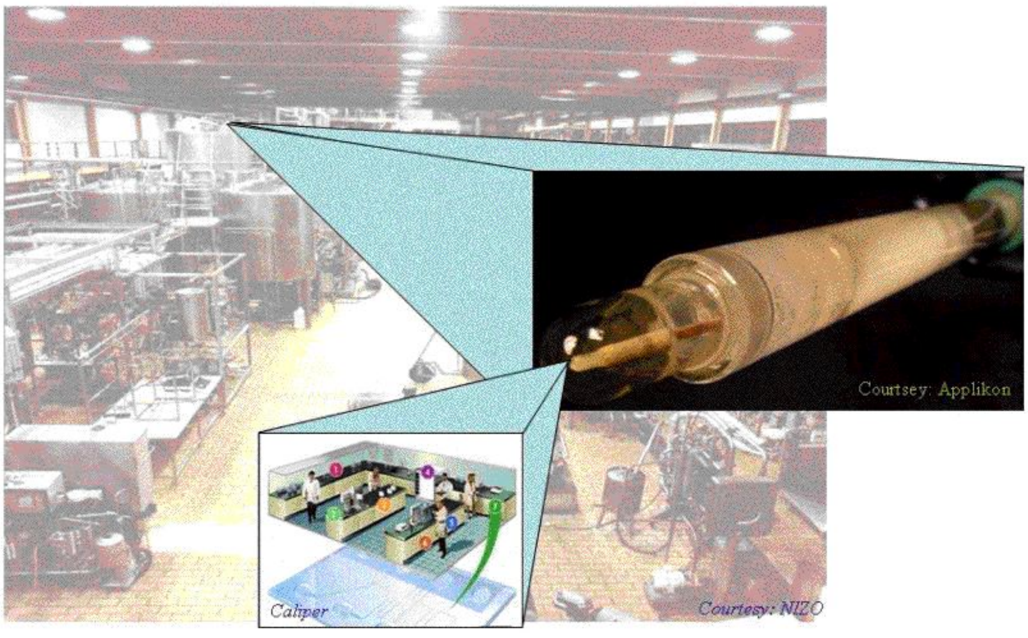
Microchip CE towards probe-type system

Case will be further discussed in last session "design issues"!

Towards full integration

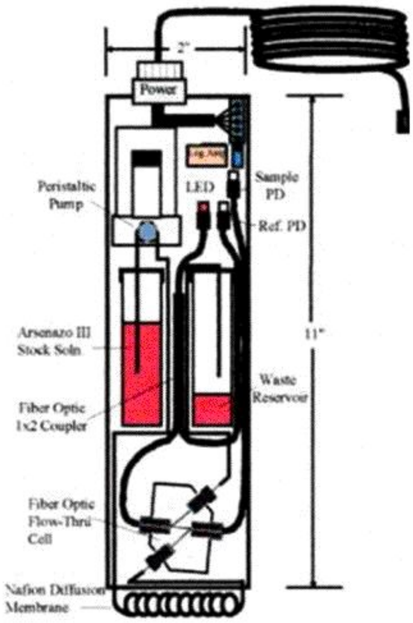


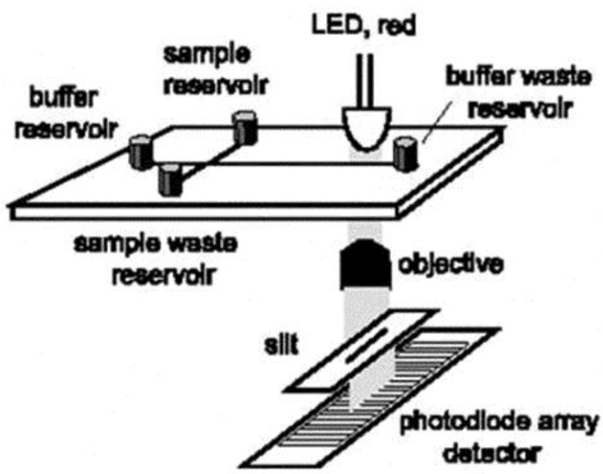
Vision of process control



Portable CE system

IR detection





G.E. Collins et al., Metal ion analysis using nearinfrared dyes and the "laboratory-on-a-chip", final report; http://www.osti.gov/em52/final_reports/64982.pdf

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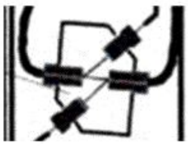
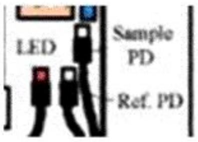
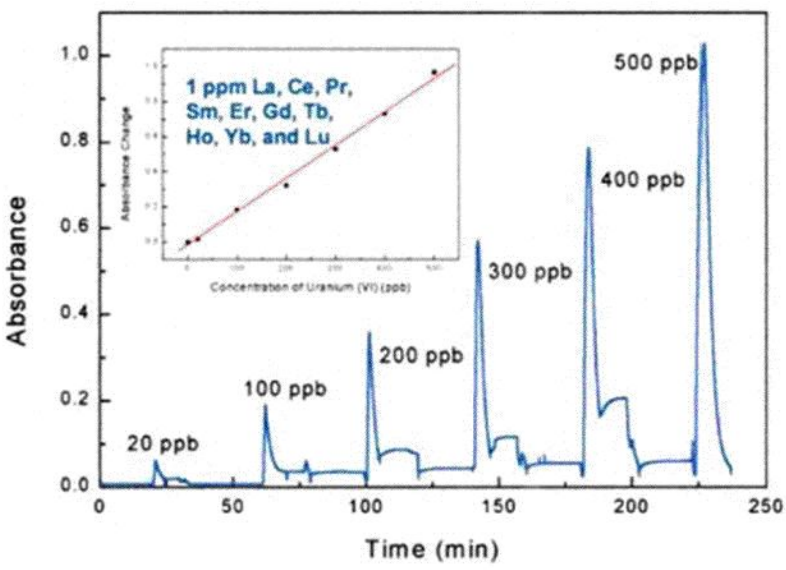
Calibration results

- Fibre-optic pick up of signal

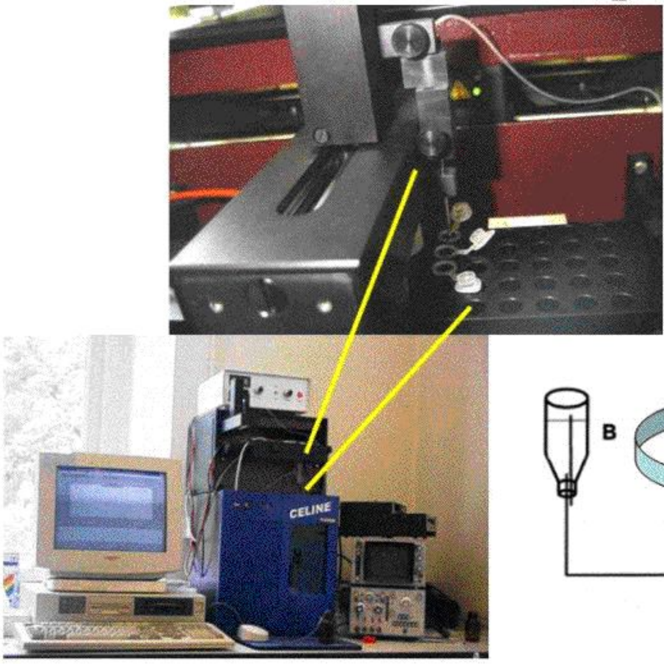


- IR detection

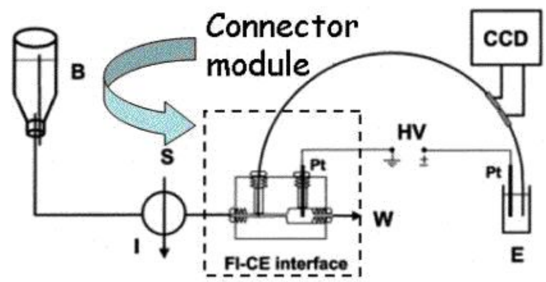




Sample reservoir-to-CE



Conventional FIA

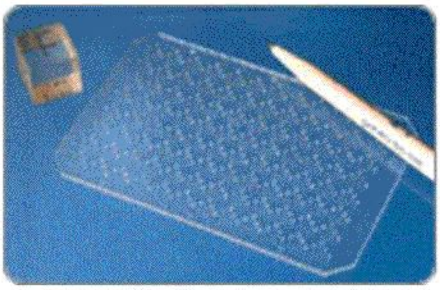


Electrophoresis 2003, 24, 1935–1943

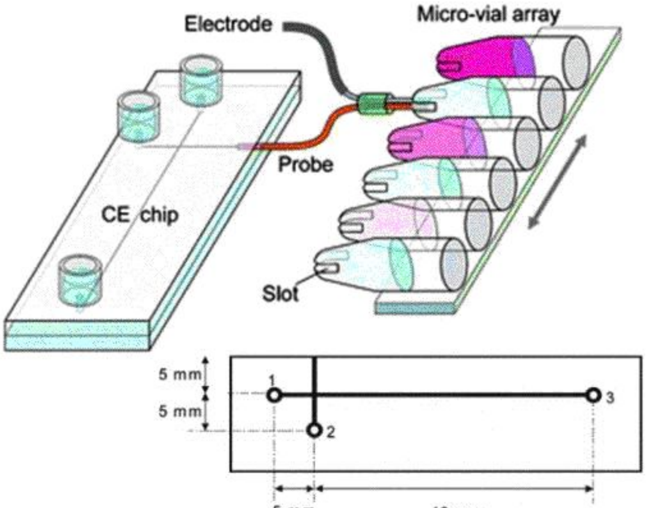
Sample reservoir-to-CE

- Titer plate integrated electrophoresis devices vs. coupled array

96-well configuration



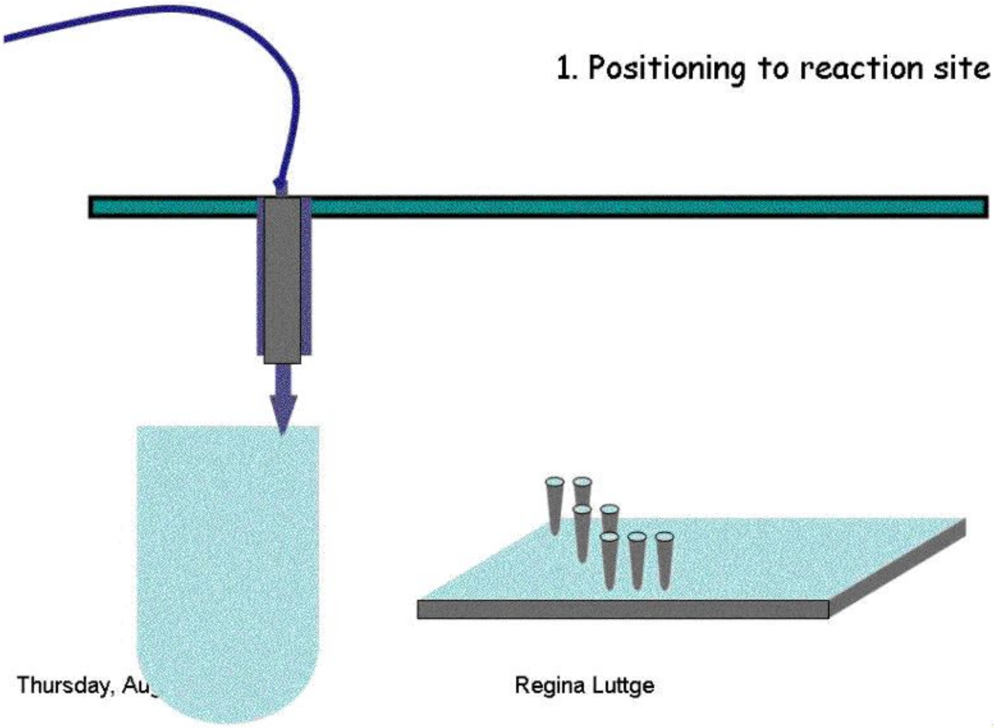
Sources:
Forschungszentrum Karlsruhe (FZK), www.fzk.de
ACLARA BioSciences



Q.-H. He et al., Analyst, 2005, 130, 1052–1058

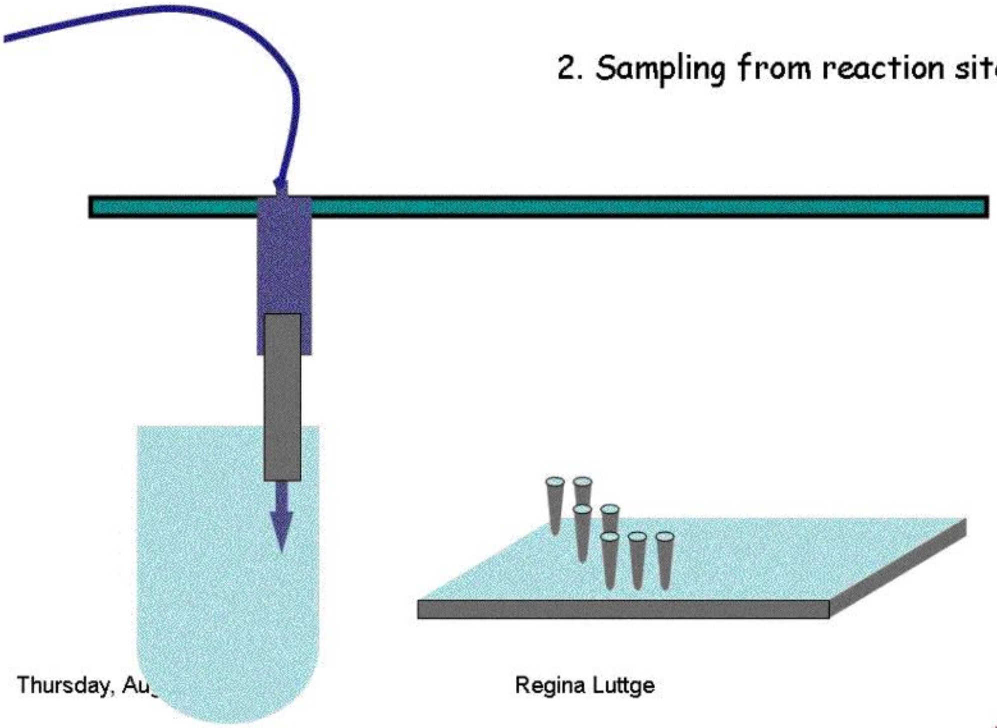
World-to-sample reservoir

1. Positioning to reaction site

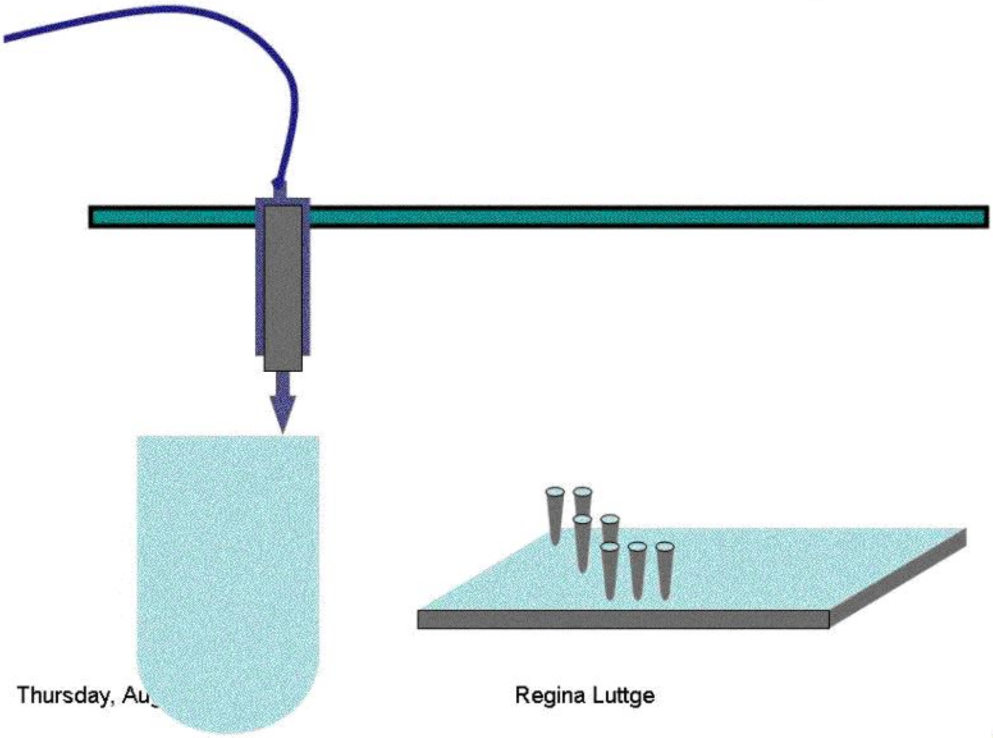


World-to-sample reservoir

2. Sampling from reaction site

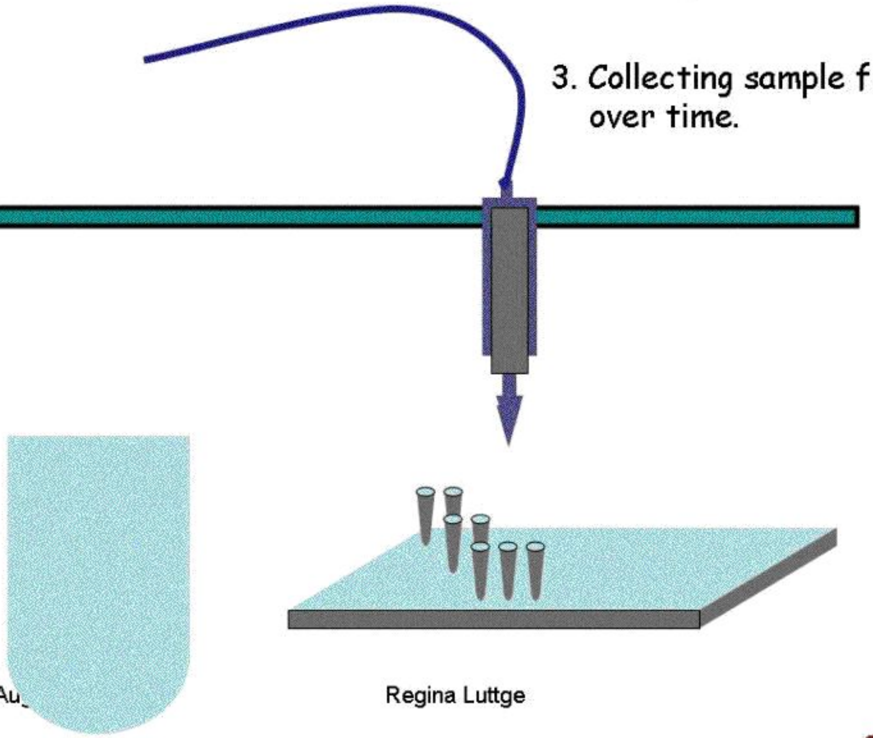


World-to-sample reservoir



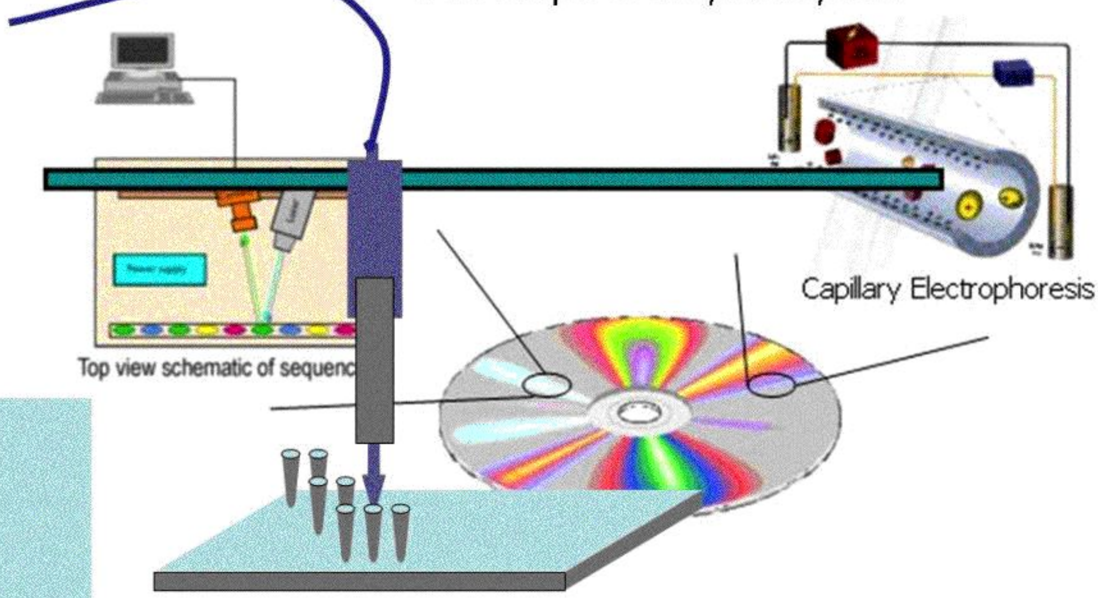
World-to-sample reservoir

3. Collecting sample fractions over time.

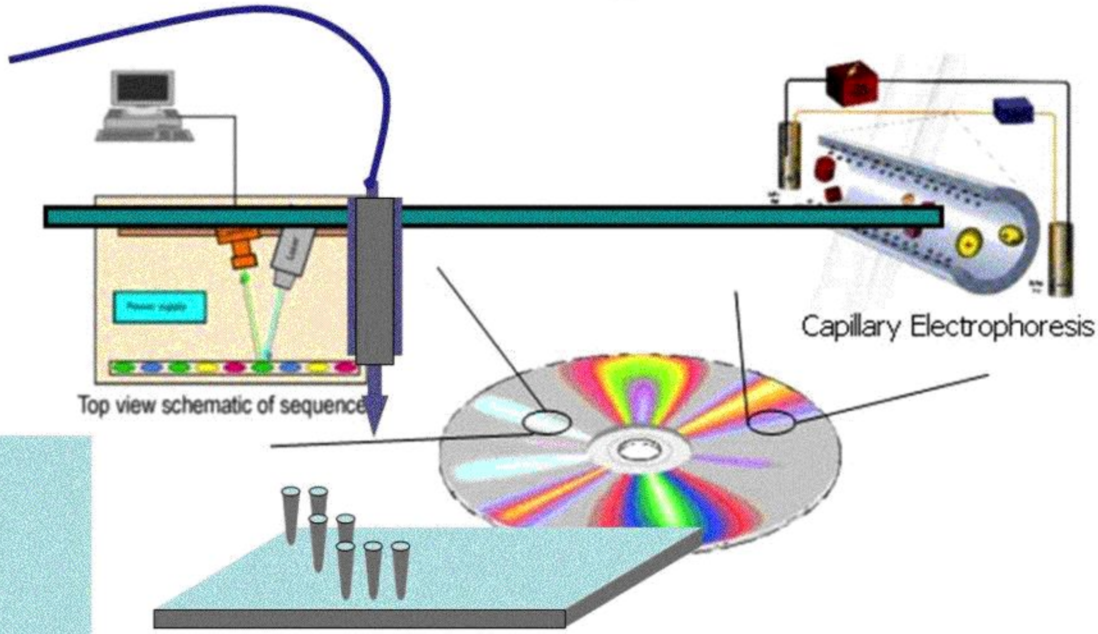


Sample reservoir-to-CE

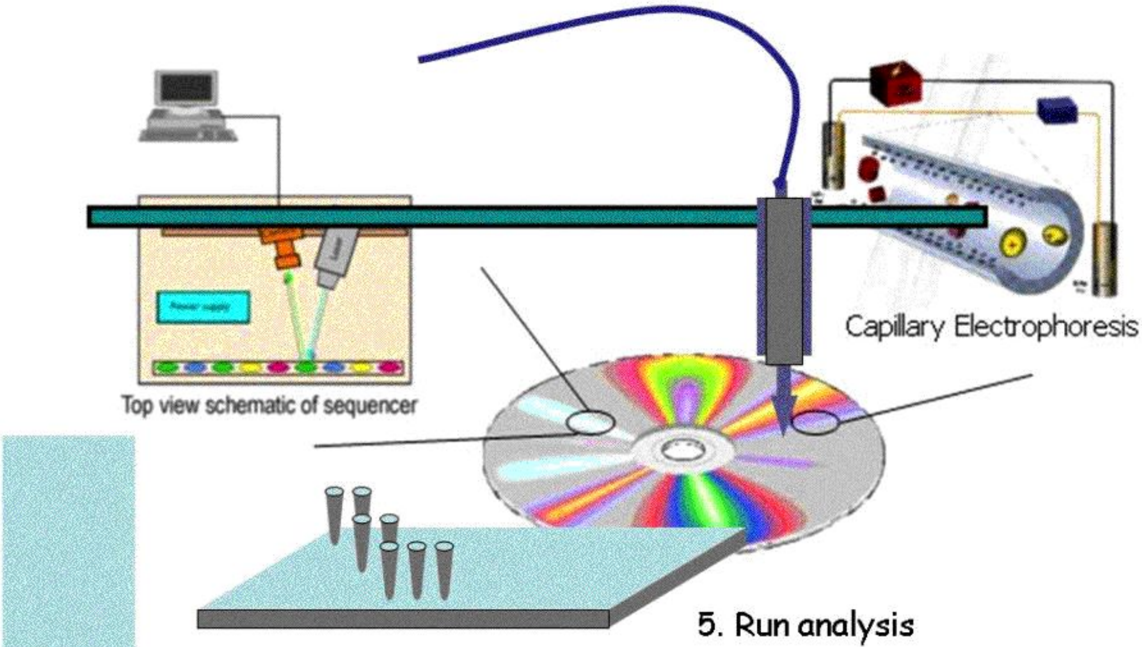
4. Re-sample to analytical system



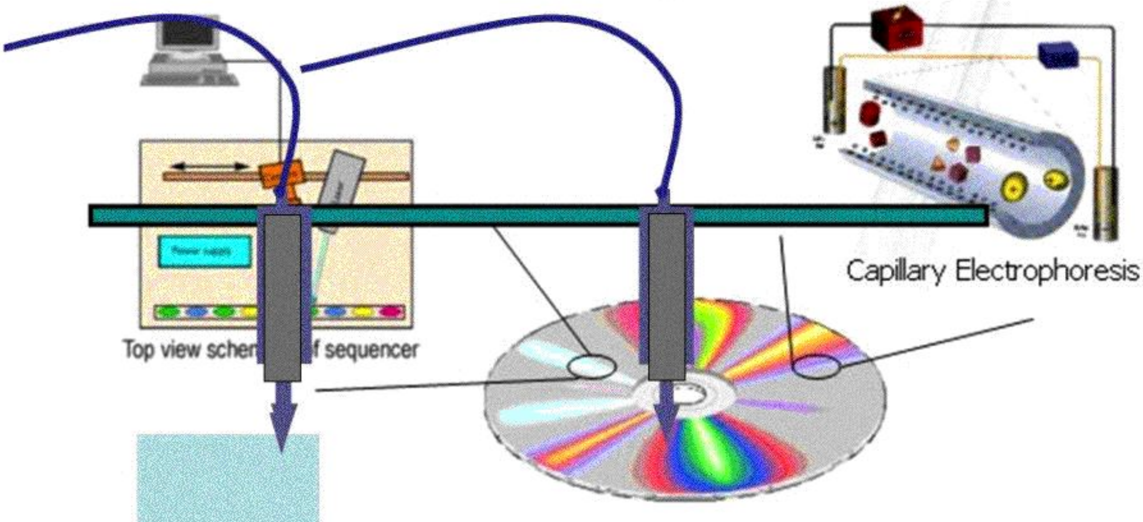
Sample reservoir-to-CE



Sample reservoir-to-CE



Sample reservoir-to-CE



- Autosampling
- Drinking from a waterhose ??
- FIA for CE (Rose μTAS-2001)
- Falling droplet (Fang)

Topics in this section

Smaller equipment?
Less restricting and scary.

Health care
A nurse and a doctor at the same job. Microfluidics could help. Error reduction, faster results and documentation

Blood analysis

- Health care chip
- System requirements: from the lab to clinical trial protocol.
- Case study: point-of-care microchip CE
  - Developments at MESA+

Does "one sensor" fits all applications?

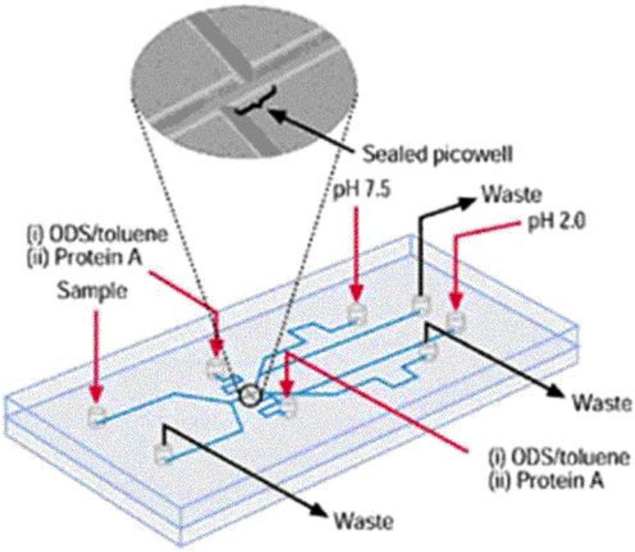
- Commercial examples show that there is a strong trend to integrate a divers number of biofunctionalities with passive microfluidic systems leading also to the merging with array technology.
- Biosensors can be very specific and sensitive but often the biomarker is not sufficiently known.
- Lab-on-a-Chip examples demonstrate that chemical separation techniques can be applied to a variety of substances of medical interest using very little sample volumes.

Point-of-Care applications can benefit from integrated microfluidics; Multiparameter analysis from a drop of blood in fully automated fashion.

Health care microchips

Spin-offs of microchip CE developments

- Immunoassay
- Cell chip
- CE – PCR
- Etc.

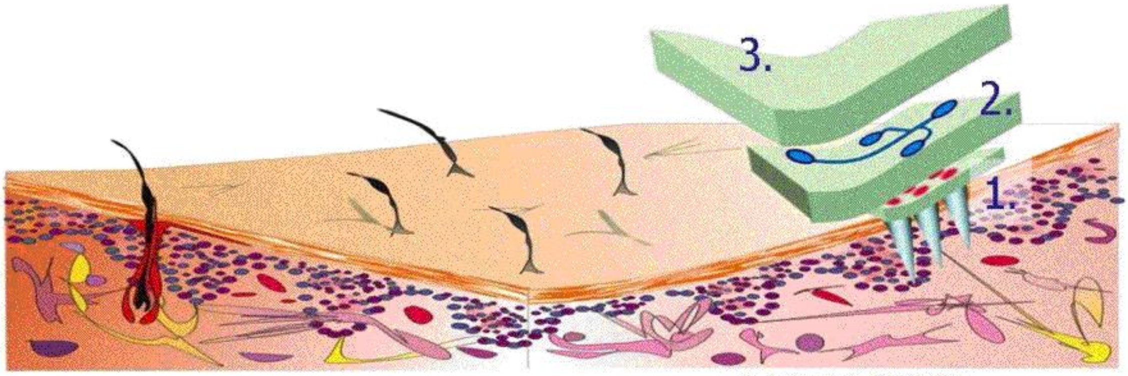


Case study :

Point-of-care cartridge system –
A microchip CE system for lithium determination in whole blood.

Microfluidics at the point of care

- Multifunctional diagnostic Point-of-Care system's components:
  - Miniscule small, painless blood sampling.
  - Analytical component (chemical separation technique, non-selective sensor element).
  - Fluidic handling in an integrated cartridge.



R. Luttge et al, μTAS 2003

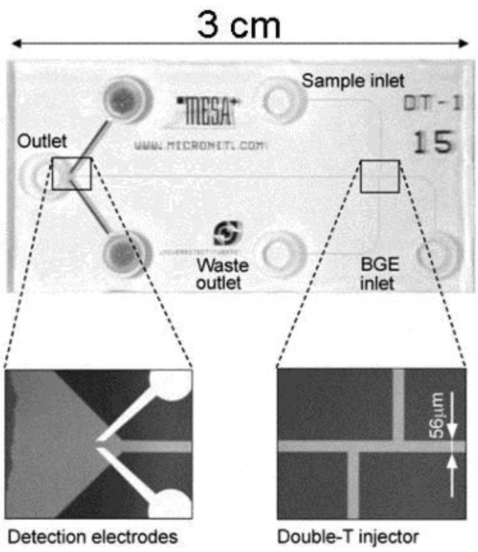
Mood disorder therapy

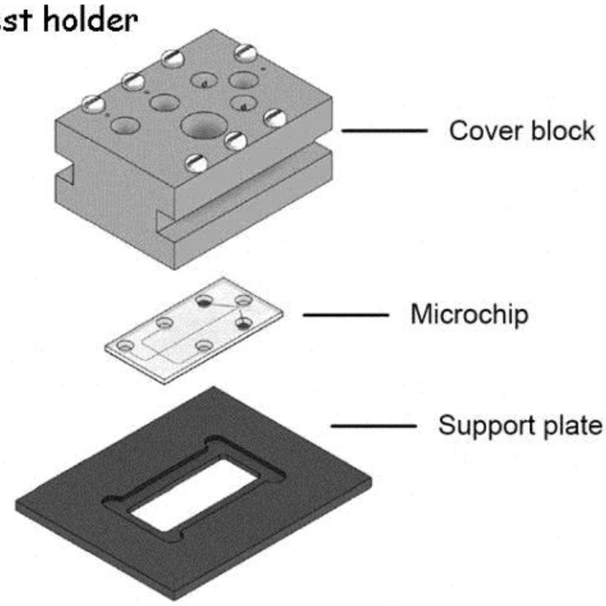
- 1 - 2% of population.
- Lithium can help.
- 0.5 - 1 million users of lithium worldwide.
- Frequent blood tests in clinical lab required.
- Therapeutic level: 0.4-1.2 mM, above 1.5 mM toxic!

Good reasons to work on a portable chip device for Li-monitoring at *point-of-care*.

Capillary Electrophoresis on Chip

- Chip design and laboratory test holder

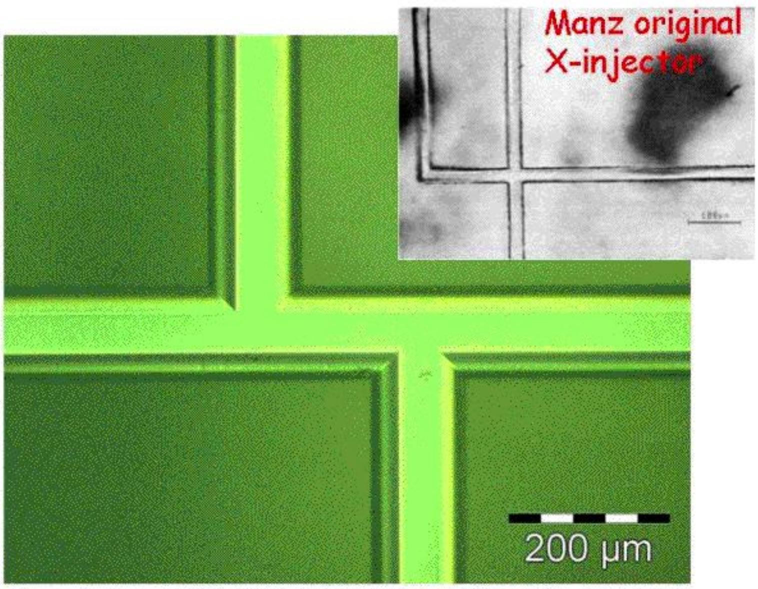




E.X. Vrouwe, PhD-Thesis, University of Twente

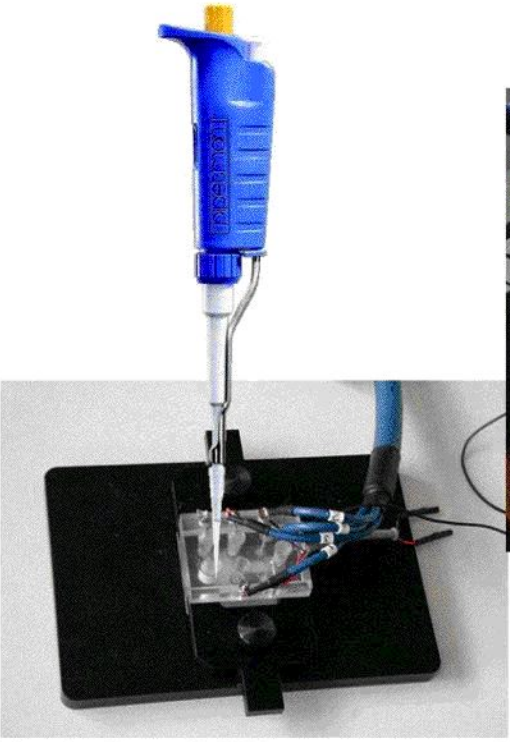
CE chip T-injector

- Borofloat® glass
- HF etched
- Integrated electrodes for conductivity detection



E.X. Vrouwe, PhD-Thesis, University of Twente

Type of holders



E.X. Vrouwe, PhD-Thesis, University of Twente

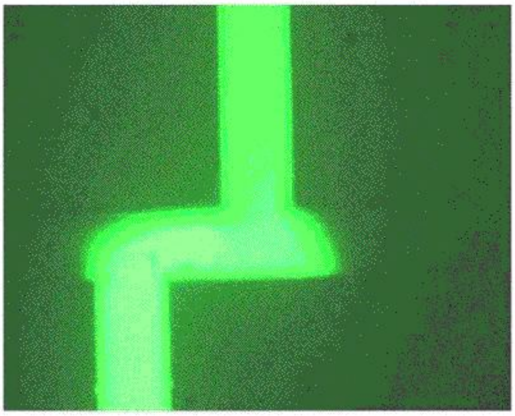
Electrokinetic microfluidic control

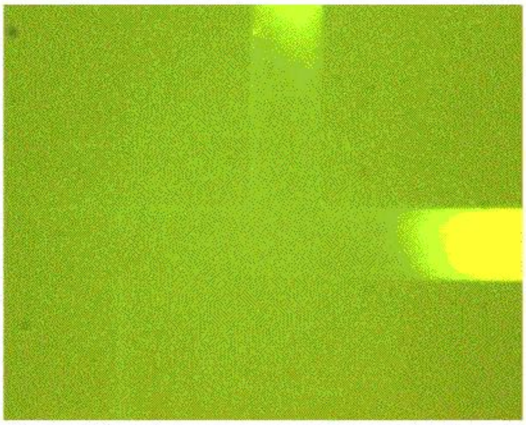
- 4- channel HV-power supply (IBIS) 1000V

E.X. Vrouwe, PhD-Thesis, University of Twente

Plug forming

- CE performance is volume sensitive.





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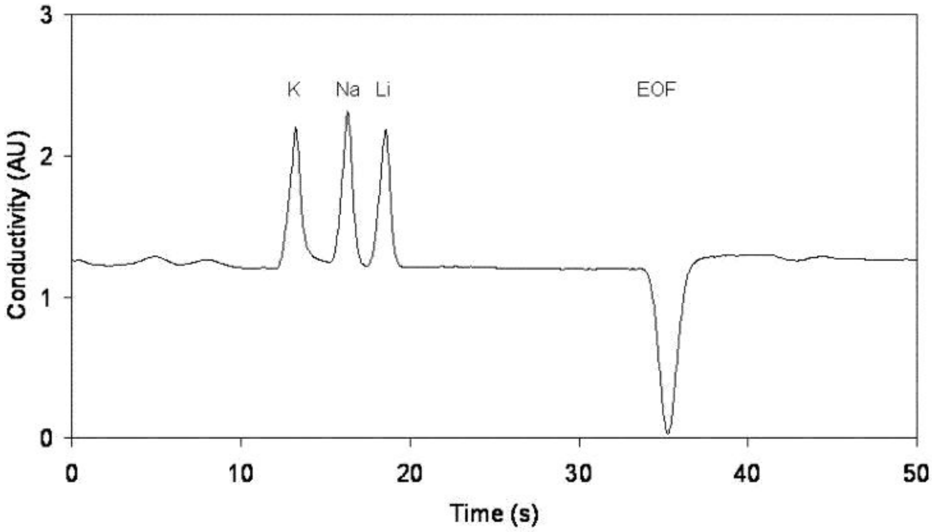
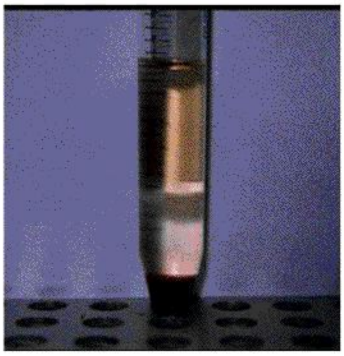
Chip performance

- Equimolar mixture of three cations in water



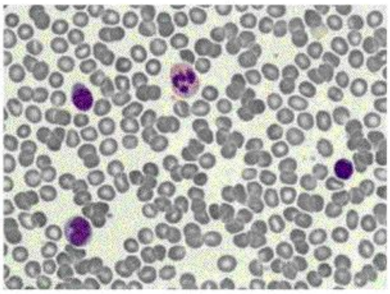
E.X. Vrouwe et al, Electrophoresis 2004, 25, 1660

Blood: a complex fluid



- On-going work in strong collaboration with Hospital Group, Enschede.

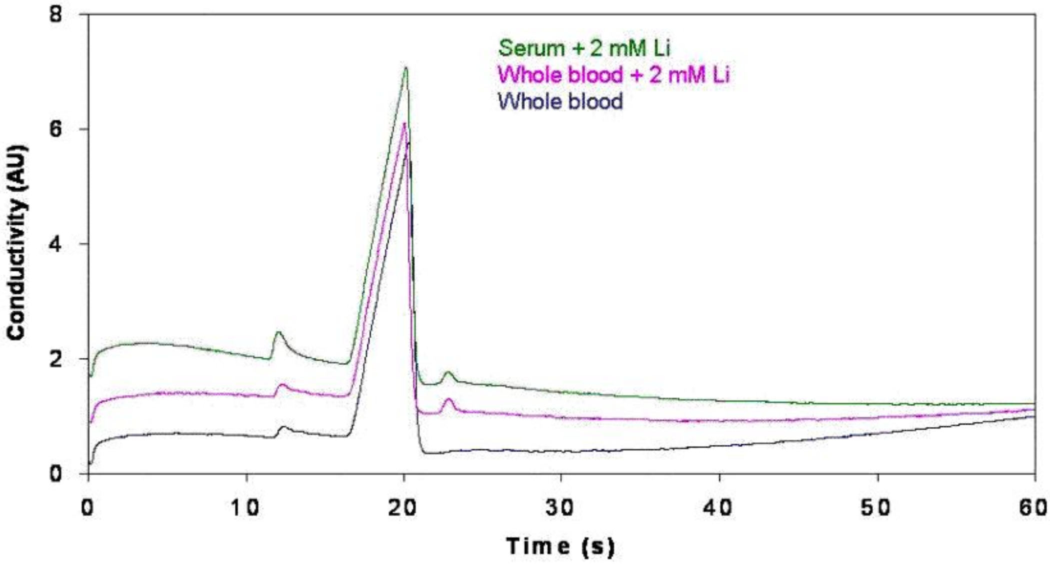
Courtesy: prof. I. Vermes
Medisch Spectrum Twente



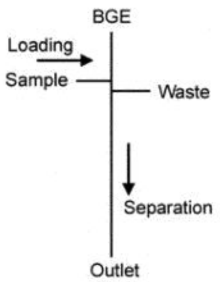
	Cations (mM)		Anions (mM)
Na^+	142	Cl^-	103
K^+	4	HCO_3^-	27
Ca^{2+}	5	HPO_4^{2-}	2
Mg^{2+}	2	SO_4^{2-}	1
Trace	1	Organic acids$^-$	5
		Proteins$^-$	16

Therapeutic concentration lithium 0.4-1.2 mM

Lithium serum & blood level



E.X. Vrouwe et al, Electrophoresis 2004, 25, 1660



Quantitative sampling on chips with EOF inhibition

- Dilution on chip occurs during sampling

ω^{sample}	ω^{BGE}
140 Na 5 Li 120 Cl 25 HCO_3	10 NH_4 20 Ac

α	β	γ	δ	ε	φ
140 Na 5 Li 120 Cl 25 HCO_3	114 Na 4.2 Li 117 HCO_3	109 Na 4.1 Li 113 Ac	8.2 Na 0.30 Li 18.5 Ac	8.6 Na 18.6 Ac	10 NH_4 20 Ac

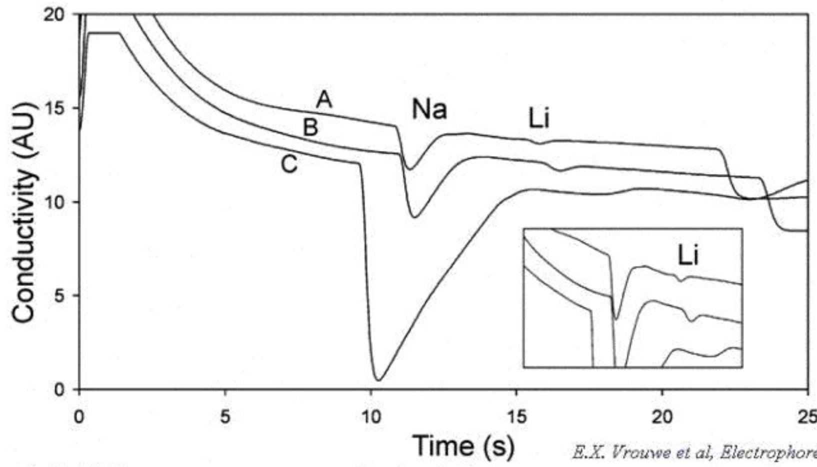
$\longleftarrow v^{\alpha\beta}$ $\longleftarrow v^{\beta\gamma}$ $v^{\gamma\delta}=0$ $v^{\delta\varepsilon}\longrightarrow$ $v^{\varepsilon\phi}\longrightarrow$

E.X. Vrouwe et al, Electrophoresis 2005, in press

7.4. Blood analysis

Zone selection by loading time

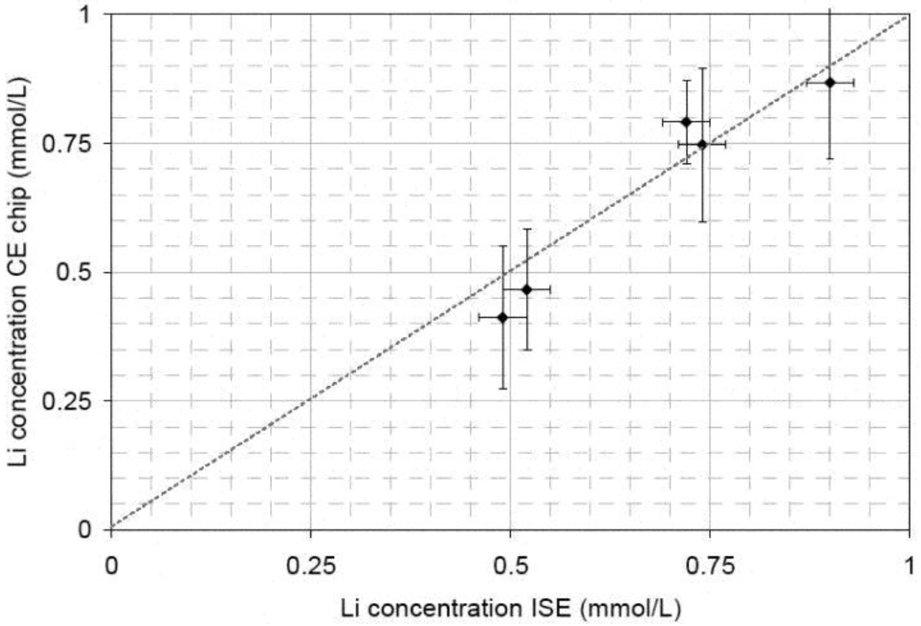
- Electropherograms as function of the loading duration. (A) 30s, (B) 240s and (C) 300s sample loading time.
- Sample 150 mmol/L sodium, 5 mmol/L lithium.
- BGE 10 mmol/L ammonium acetate/acetic acid with EOF modifier.



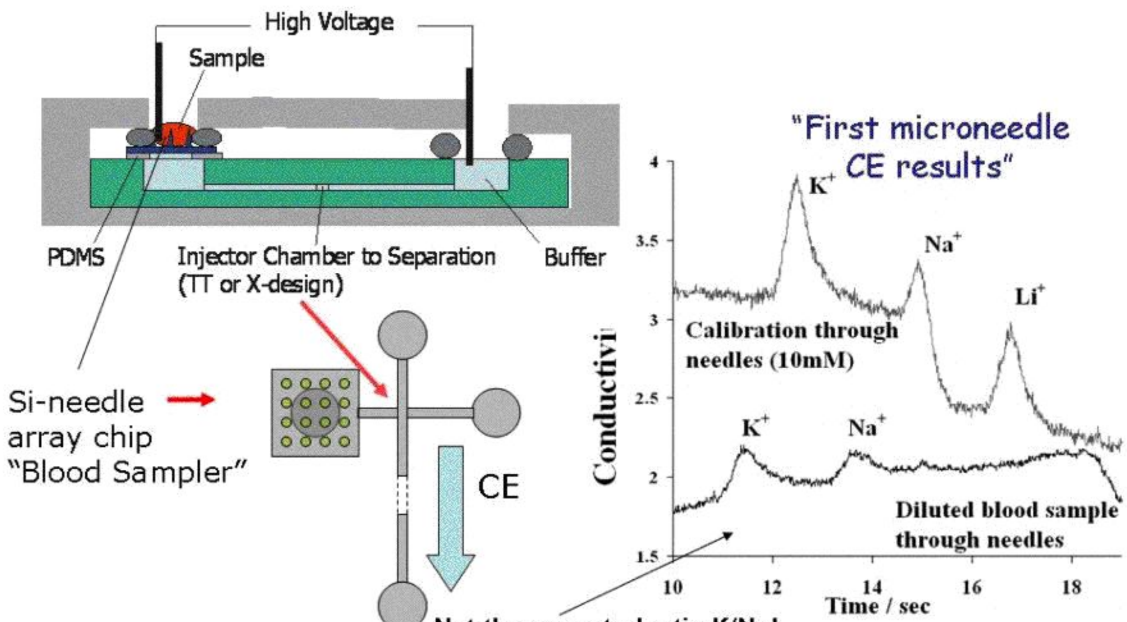
E.X. Vrouwe et al, Electrophoresis 2005, in press

Thursday, August 18, 2005 Regina Luttge

5 patient samples (serum): correlation plot



Integration of CE System



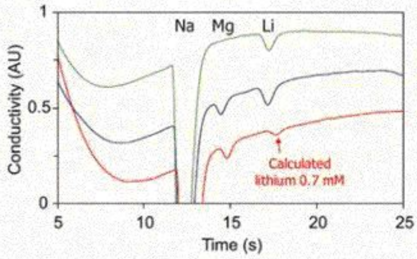
Not the expected ratio K/Na!

More care on sample prep must be taken!

Gardeniers et al, Journal of Microelectromechanical Systems, Vol. 12, No. 6, 855-862, 2003

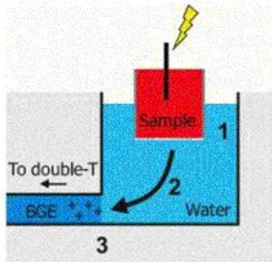
μ–needle μ – CE: optimized

The inorganic cations in the blood samples are separated without any sample pretreatment:

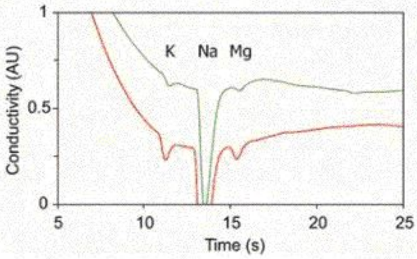


- Calibration
- Whole blood (spiked)
- Plasma from patient

Separation conditions: background electrolyte 30 mM ammonium acetate/acetic acid. Field 330 V/cm.

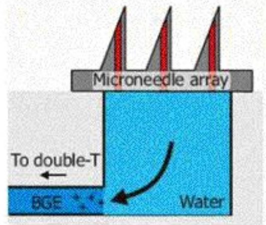


A microneedle array sandwiched on top of the CE chip can also be used for this type of analysis:



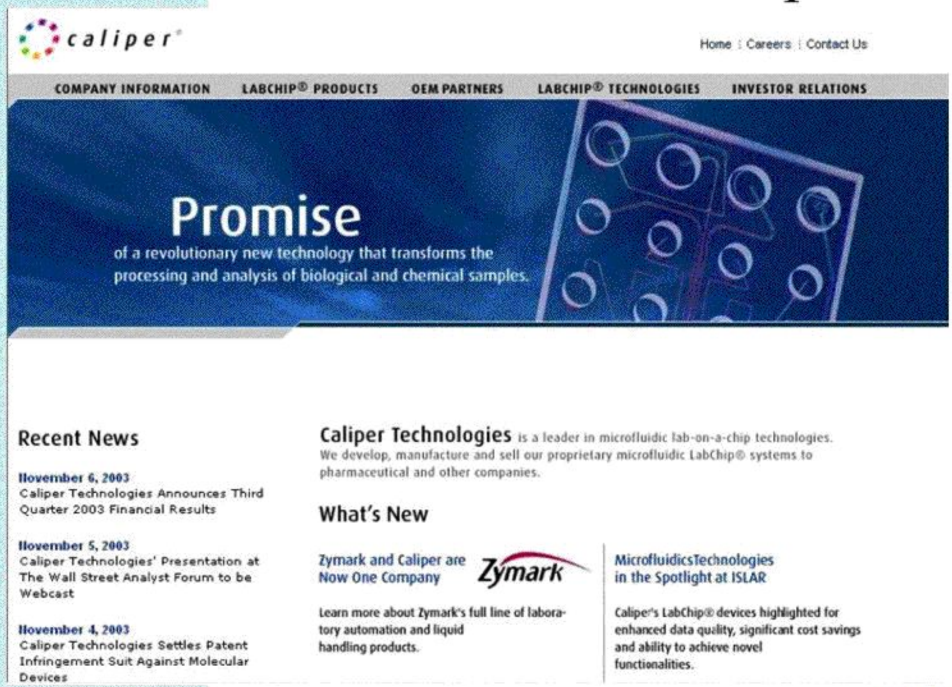
- Blood diluted (1:225)
- Blood diluted through microneedles

Separation conditions: background electrolyte 30 mM ammonium acetate/acetic acid, 5 mM 18-crown-6.

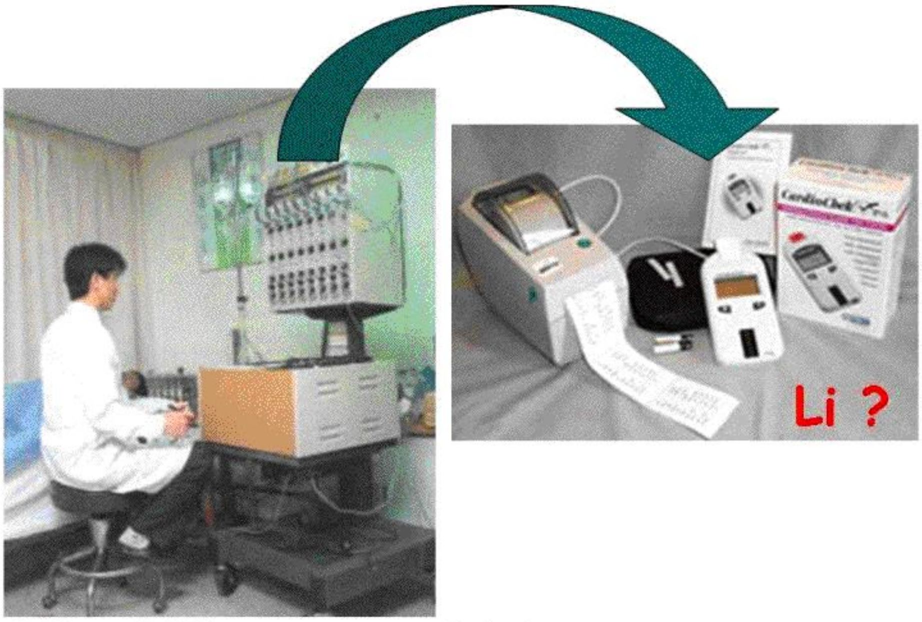


E.Vrouwe et al, μTAS 2004

Outlook: Future developments



From bedside systems to patient self-test devices



Further reading: sample preparation, CE, and other microassays

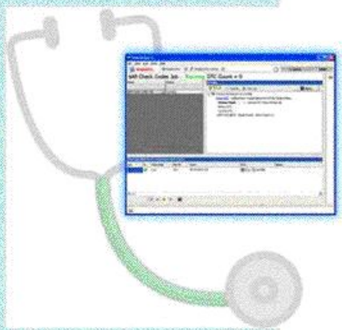
Wen-Tso Liu and Liang Zhu
Department of Civil Engineering, National University of Singapore
"Environmental microbiology-on-a-chip and its future impacts"

Opinion TRENDS in Biotechnology Vol.23 No.4 April 2005 175

Table 1. Laboratory-on-a-chip devices for sample preparation, biochemical reactions and detection[a]

LOC for cells and DNA analyses	Sample preparation				Biochemical reaction						Detection		Potential environmental applications			
	Concentraton/ trapping	Separation/ purification	Cell lysis/nucleic acid extraction	Cell sorting	Immunological assay	PCR	Restriction enzyme digestion	DNA sequencing	DNA separation	DNA hybridization	Optical methods	Electrochemical methods	Pathogen detection	Community fingerprinting	Clone library	Metagenomics
Sample concentration [6–8]	✓	✓														
DNA extraction [10,12]	✓	✓	✓													
μFACS [13]		✓		✓												
CE chip [14,15]									✓							
DNA microarray [16]										✓	✓		✓	✓		
Immunological assay [8,9]	✓	✓			✓						✓		✓			
PCR [11,21]			✓			✓					✓		✓			
PCR and CE [18,27]						✓			✓		✓		✓			
PCR and DNA microarray [20]						✓				✓	✓		✓			
Pathogen detection-on-a-chip [19]	✓		✓			✓				✓		✓	✓			
Sequencing factory-on-a-chip [17]				✓		✓		✓			✓				✓	✓
Community fingerprinting chip			✓				✓		✓		✓			✓		

[a]Abbreviations: CE, capillary electrophoresis; μFACS, microfabricated fluorescence accelerated cell sorting.



Summary

- Strong progress on components and devices.
- Early adapters are to find in clinical research.
- So far, there is little on complete analytical systems for self-testing *(means: commercial, FDA approved)* based on integrated microfluidics but patch-type patient monitoring and treatment devices are strongly attempted in microfluidic applications and research there of.