

Miniaturizace v analytické chemii

Předpoklady pro miniaturizaci

- „**micromachining, microfabrication**“ (litografie, reactive plasma etching ...)
 - výroba „čipů“ („microfabricated devices“)
- **kapiláry, mikrokanálky, mikroviálky**
 - průměry $10^0 - 10^2 \mu\text{m}$
- **lasery**
 - možnost zaostřit na plošku o průměru $\sim \mu\text{m}$
 - kompatibilita s mikrokanálky a kapilárami
- **piezoelektrické mikropipetory, mikroelektrody, mikroreaktory, mikročástice atd.**

Výhody miniaturizace

- **menší objemy vzorků**
- **rychlejší analýza** (redukce délky transportu, optimalizovaný transport hmoty pro chem. reakce a separace) – vysoký výkon, snížená cena na analýzu jednoho vzorku
- **redukce spotřeby činidel** - redukce odpadu, vyšší reakční výtěžek – redukce reakčního času + příprava vzorku
- **možnost integrace a spojování**
“Lab-on-chip”, miniaturizované systémy totální analýzy („ μ -TAS“)
- **paralelní analýzy**
opakující se motiv na jednom čipu, vysoký výkon
- **snadná přenositelnost**
- **masová výroba = nízká cena**
- **mikroanalýzátory na jedno použití**



Lab on a Chip

Základní typy čipů

- 1. 2D pole**
 - afinitní pole: nejprve zakoncentrování specifických látek, poté MALDI MS přímo z čipu
 - pole vialek se špičkami pro ESI: zvýšení reprodukovatelnosti analýzy
- 2. Fluidní systém (čipy s kanálky)**
 - systémy pro paralelní analýzu– pro ESI, MALDI ...
 - systémy integrující několik kroků (“Lab-on-chip”)
dávkování, úprava vzorku, reakce, separace, detekce ...

Spojení mikro + makro?

např. čip a hmotnostní spektrometr

Trendy

TAS (total analysis system) ... systémy, kde vzorkování, úpravy vzorku, reakce, separace, detekce aj. jsou integrovány do jednoho přístroje, nejlépe miniaturizovaného - μ TAS

Využití TAS

- env. chemie: kontrola znečištění a zdrojů kontaminace
- farmaceutický a agrochemický výzkum resp. biomedicínální
- screening
- umělá čidla, např. umělý nos

Rozměr nebo objem? Mikro – nano – piko?

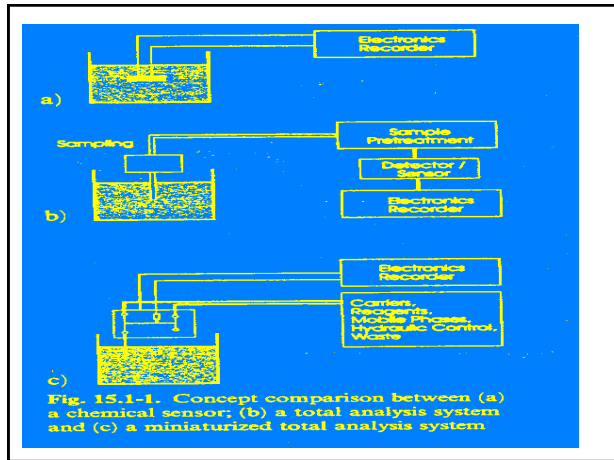
$$100 \mu\text{m} \times 100 \mu\text{m} \times 100 \mu\text{m} = 10^{-12} \text{m}^3 = 10^{-9} \text{l} = 10 \text{nl}$$

$$10 \mu\text{m} \times 10 \mu\text{m} \times 10 \mu\text{m} = 10^{-15} \text{m}^3 = 10^{-12} \text{l} = 10 \text{pl}$$

Nevýhody miniaturizace

- **změny poměrů** délky, plochy a objemu vedou ke změnám vlivu fyzikálních parametrů na signál např. kapilární efekty – TRIBO polarograf
- **zvýšený poměr plocha/objem** – sorpce, kontaminace
- **kratší doba analýzy**
- **zvýšený transport tepla a hmoty**

(uvedené parametry mohou být výhody i nevýhody)

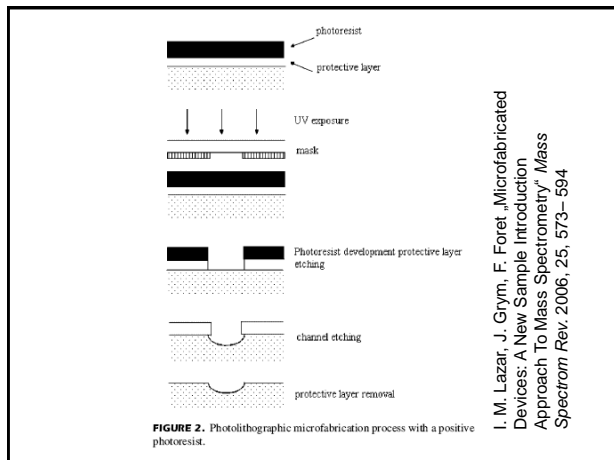


Technologie výroby čipů

D. Mijatovic, J. C. T. Eijkel and A. van den Berg „Technologies for nanofluidic systems: top-down vs. bottom-up – a review” *Lab Chip*, 2005, 5, 492–500

Procesy při výrobě čipů

- vytvoření vzoru (litografie – hv, e, ionty ...)
- mechanické obrábění
- leptání
- odlévání (silikonová guma, polyakrylát)
- tavení



GC na čipu

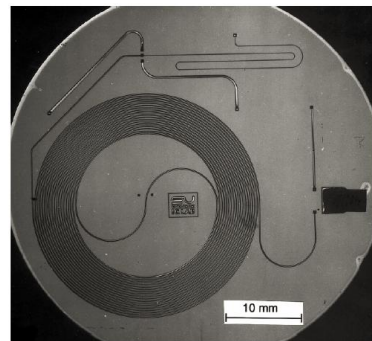
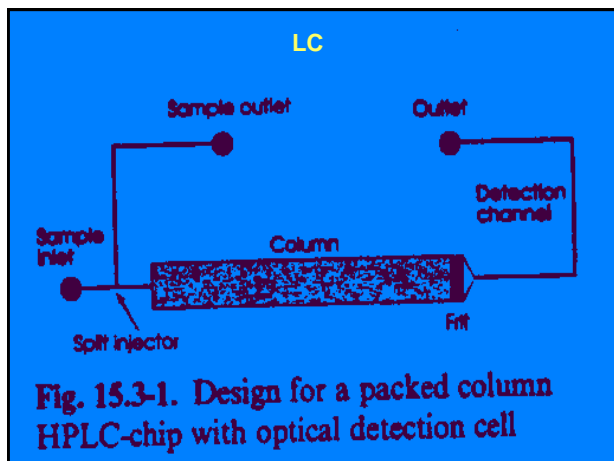
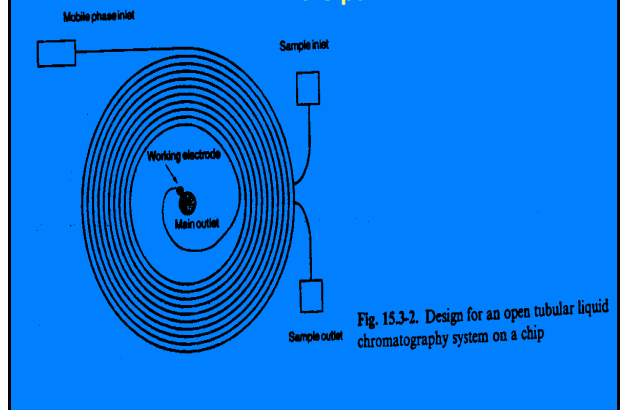


Fig. 1 Photograph of a gas chromatograph integrated on a planar silicon wafer fabricated by Terry and co-workers at Stanford University.

de Mello, *Lab Chip*, 2002, 2, 48N–54N



LC na čipu



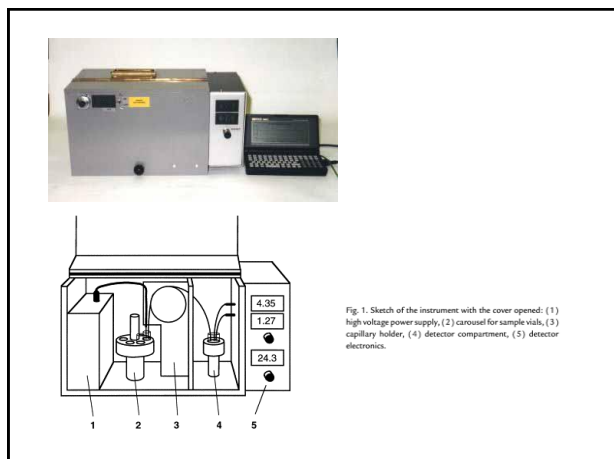


Fig. 1. Sketch of the instrument with the cover opened: (1) high-voltage power supply, (2) carousel for sample vials, (3) capillary holder, (4) detector compartment, (5) detector electronics.

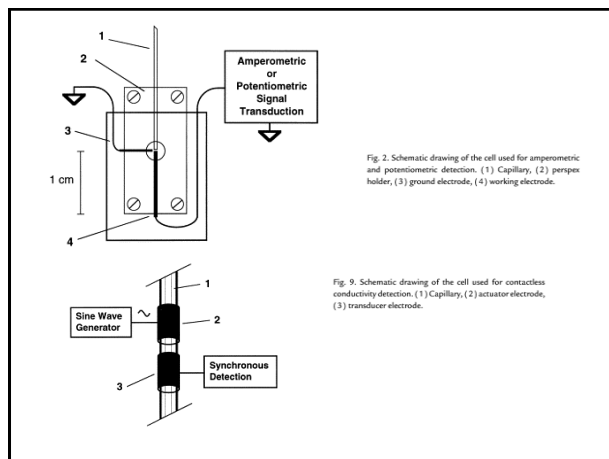


Fig. 2. Schematic drawing of the cell used for amperometric and potentiometric detection. (1) Capillary, (2) peristaltic pump, (3) ground electrode, (4) working electrode.

Fig. 9. Schematic drawing of the cell used for contactless conductivity detection. (1) Capillary, (2) actuator electrode, (3) transducer electrode.

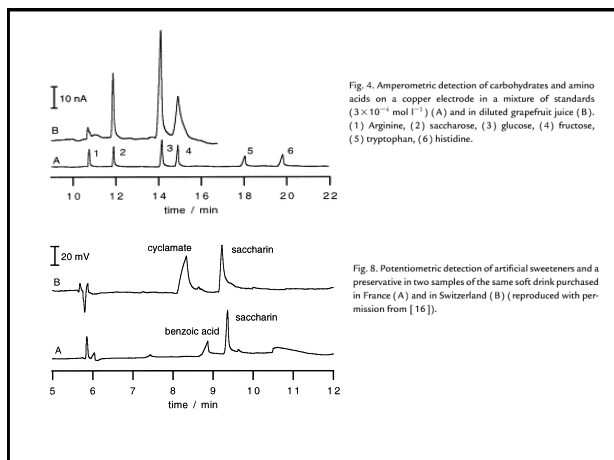
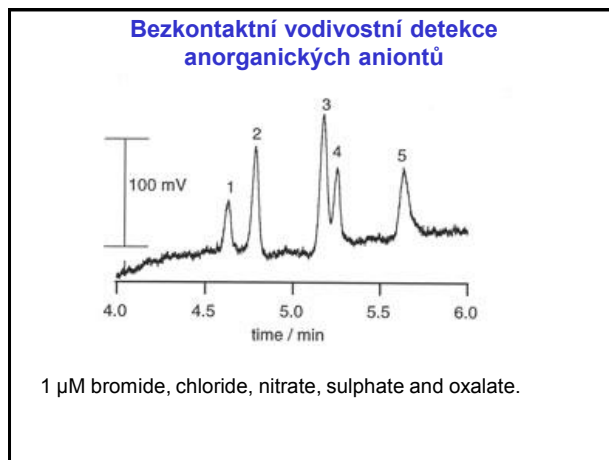


Fig. 4. Amperometric detection of carbohydrates and amino acids on a copper electrode in a mixture of standards ($3 \times 10^{-4} \text{ mol l}^{-1}$) (A) and in diluted grapefruit juice (B). (1) Arginine, (2) saccharose, (3) glucose, (4) fructose, (5) tryptophan, (6) histidine.

Fig. 8. Potentiometric detection of artificial sweeteners and a preservative in two samples of the same soft drink purchased in France (A) and in Switzerland (B) (reproduced with permission from [16]).



1 μM bromide, chloride, nitrate, sulphate and oxalate.

LAB ON A CHIP
Review

Recent developments in detection methods for microfabricated analytical devices

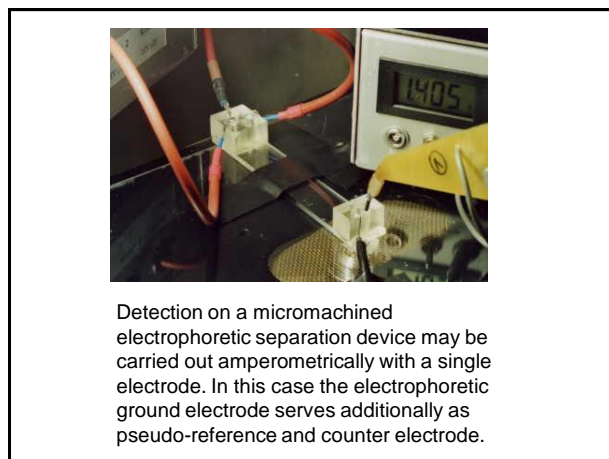
Maria A. Schwarz and Peter C. Hauser*

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

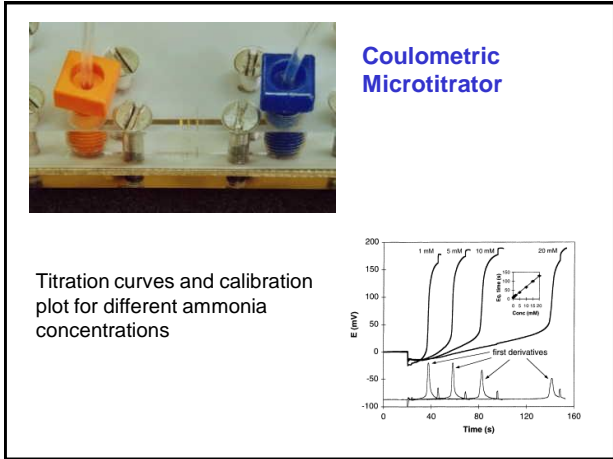
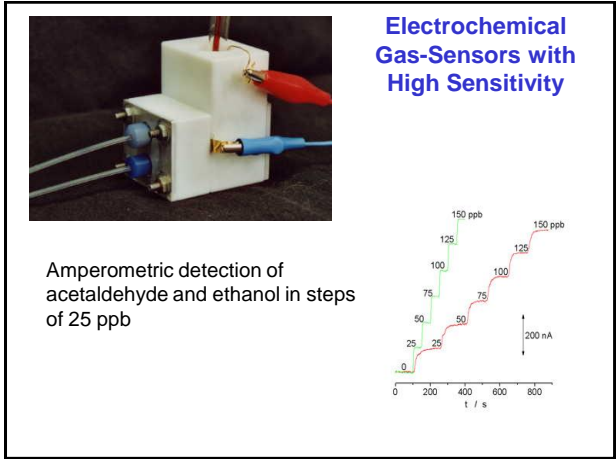
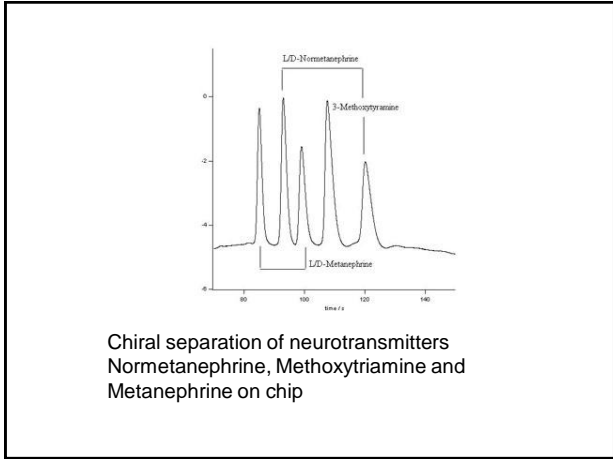
Received 26th April 2001, Accepted 13th June 2001
First published as an Advance Article on the web 9th August 2001

Sensitive detection in microfluidic analytical devices is a challenge because of the extremely small detection volumes available. Considerable efforts have been made lately to further address this aspect and to investigate techniques other than fluorescence. Among the newly introduced techniques are the optical methods of chemiluminescence, refraction and thermooptics, as well as the electrochemical methods of amperometry, conductivity and potentiometry. Developments are also in progress to create miniaturized plasma-emission spectrometers and sensitive detectors for gas-chromatographic separations.

Lab on a chip, 1, 1-6 (2001)

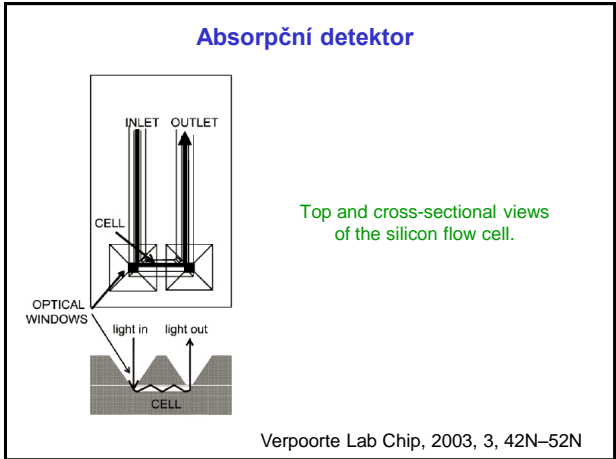
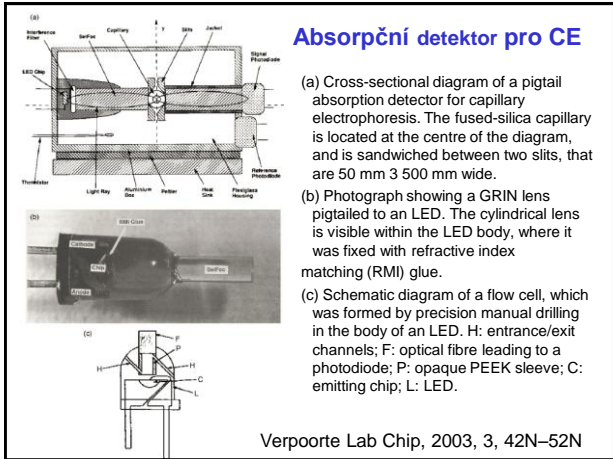


Detection on a micromachined electrophoretic separation device may be carried out amperometrically with a single electrode. In this case the electrophoretic ground electrode serves additionally as pseudo-reference and counter electrode.

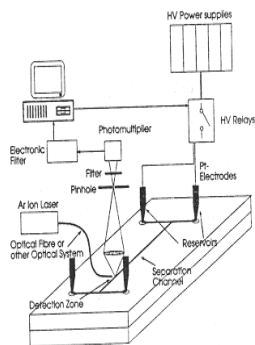


Optické detektory pro separace na čipu

Verpoorte „Chip vision – optics for microchips“ *Lab Chip*, 2003, 3, 42N–52N



LIF na čipu pro CE



LIF = laser induced fluorescence

konfokální detekce

čip místo kapiláry

Fig. 15.3-8. Experimental setup for a CE experiment on a chip

LIF detektor pro čip

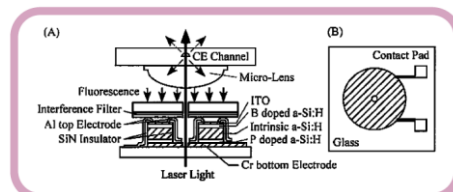


Fig. 9 Design of an amorphous integrated silicon photodiode and optical system for fluorescence detection in microchip electrophoresis chips. (A) Cross-sectional view. (B) Top view of the ring-shaped detector. (Reprinted with permission from ref. 67 Copyright 2002 Kluwer Academic Publishers).

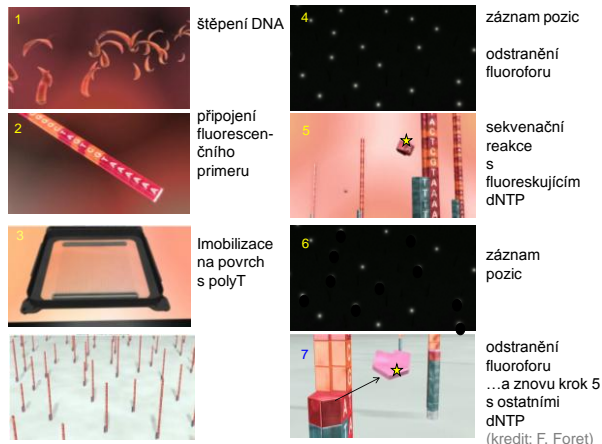
Verpoorte Lab Chip, 2003, 3, 42N–52N

Detekce LIF v sekvenování DNA

True single molecule sequencing
(sequencing by synthesis)



Heliscope™
www.helicosbio.com



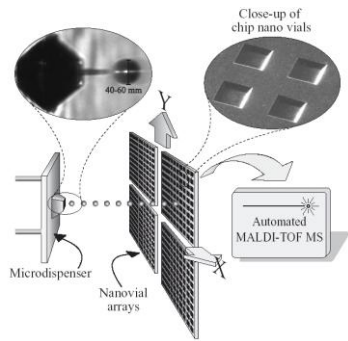
Prameny

Y. C. Lim, A. Z. Kouzani, W. Duan: *Lab-on-a-chip: a component view* Microsyst Technol 16,1995–2015, 2010

Čipy v hmotnostní spektrometrii

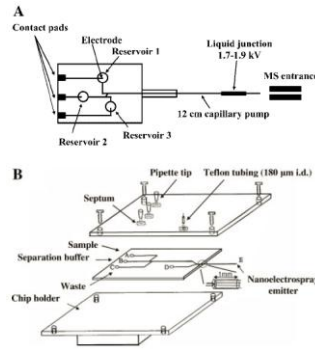
M. Lazar, J. Grym, F. Foret „Microfabricated Devices: A New Sample Introduction Approach To Mass Spectrometry“ *Mass Spectrom Rev.* 2006, 25, 573– 594

Mikrometody v hmotnostní spektrometrii MALDI



Laurell, T.; Nilsson, J.; Marko-Varga, G. *Trends Anal. Chem.* **2001**, *20*, 225-231.

Čipy pro ESI MS



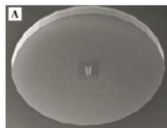
Microchip configurations with inserted ESI needles

(A) Schematic representation of the microchip configuration for MSinfusion with an inserted capillary that acts as an electroosmotic pump and transfer line between the chip and ESI interface. *Figeys et al. Anal. Chem.* **70**:3728-3734.;

(B) Schematic representation of the microchip configuration for CE separation and MS analysis using a capillary ESI emitter.

Li J. Anal. Chem. **2000**, *72*, 599-609.

Emitor pro ESI MS

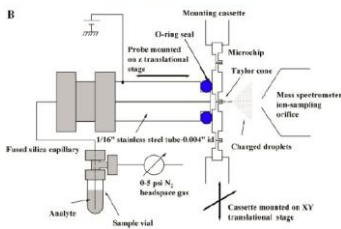


Microchip with integrated electro spray emitter.

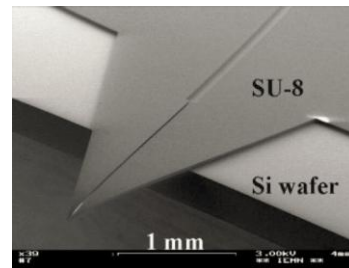
(A) SEM image of a microfabricated silicon electro spray nozzle;

(B) Schematic representation of a complete chip handling and fluid delivery system.

Schultz et al. Anal. Chem. **2000**, *72*, 4058-4063.



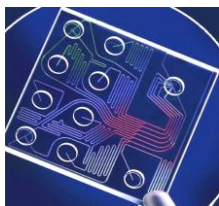
Emitor pro ESI MS



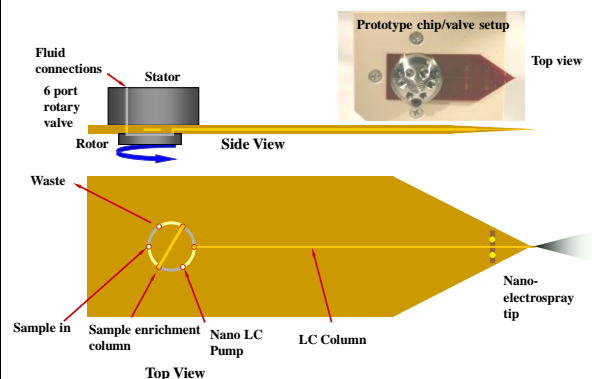
SEM image of a planar ESI emitter fabricated from negative SU-8 photoresist. *Le Gac S, Arscott S, Rolando C. Electrophoresis* **2003**, *24*, 3640-3647.

Příklady analytických čipů

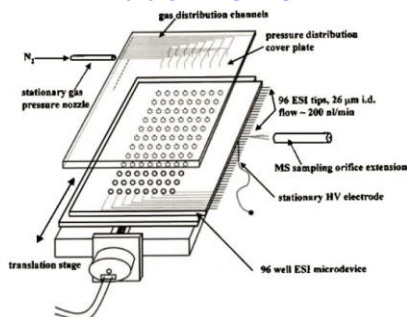
- Agilent/Caliper



Čip pro HPLC-MS: zakoncentrování vzorku, RPLC a ESI



Paralelní ESI MS



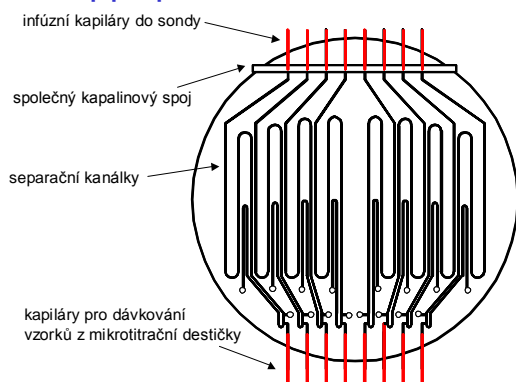
Liu H.H., Felten C., Xue Q.F., Zhang B.L., Jedrzejewski P., Karger B.L., Foret F. Development of multichannel devices with an array of electrospray tips for high-throughput mass spectrometry. *Anal Chem* 2000 72:3303–3310.

MALDI MS z čipu

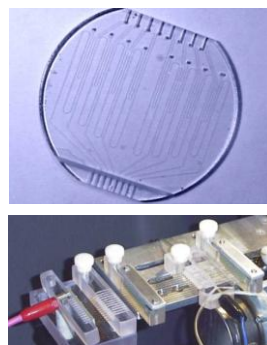


www.gyros.com

Čip pro paralelní CE – MALDI MS



Čip CE – MALDI MS vs. konvenční zařízení

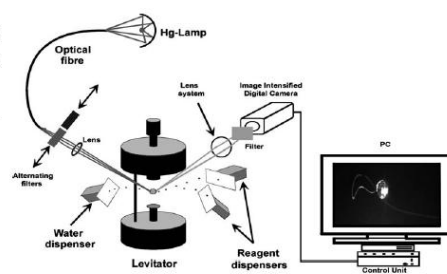


Techniky levitace

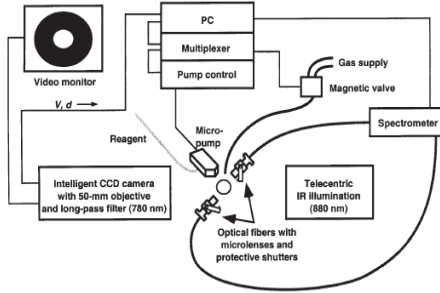
- slouží k **eliminaci „krusty“ kolem kapalného vzorku** za účelem minimalizace sorpce vzorku
- použití v bioanalytické chemii – biolog. kompatibilita a snadné ovládání, nízké náklady na pořízení přístroje a ovládání, snadný přístup ke vzorku, stabilní poloha vzorku – splňuje pouze akust. levitace
- **Druhy levitace:**
 - optická, elektrostatická, aerodynamická, akustická
 - hybridní (aerodynamická akustická, elektrostatická aerodynamická, elektrostatická akustická)
 - Výhody akust. levitace: nevyžaduje speciální vlastnosti vzorku (např. el. náboj event. index lomu vzorku,
 - Takřka všechny vzorky (pevné vzorky, kapaliny) mohou být levitovány.

Experimentální uspořádání

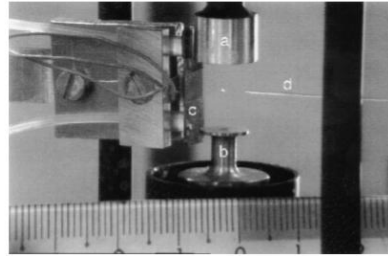
Instrumental set-up for airborne cell experiments using fluorescence imaging detection consisting of a Hg lamp, an optical fibre, two alternating interference filters (405 and 435 nm), a lens, an acoustic levitator, continuous flow-through droplet dispensers, a lens system, another interference filter (510 nm), a CCD camera and a computer



Experimentální uspořádání



Akustická levitace



Droplet levitated in the nodal points of a standing ultrasonic wave. (a) Reflector (b) ultrasonic transducer (c) flow-through liquid microdispenser and (d) capillary. The ruler displays the scale in centimetres of the experimental set-up. The levitated droplet is less than 1 mm in diameter.

Akustická levitace

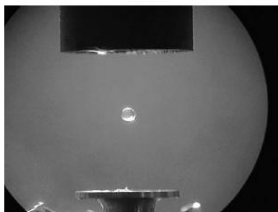


Fig. 1 A 500-µl water drop levitated in a node in a standing wave created between an ultrasonic transducer (bottom) and a solid reflector (top)

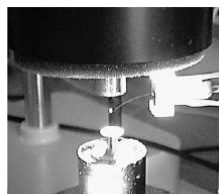


Fig. 2 Dispenser droplet addition to the levitated drop. The dispenser trajectory is seen as the thin white line connecting the dispenser nozzle on the right with the levitated drop

Extrakce v levitující kapse

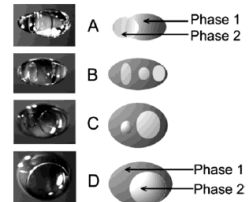
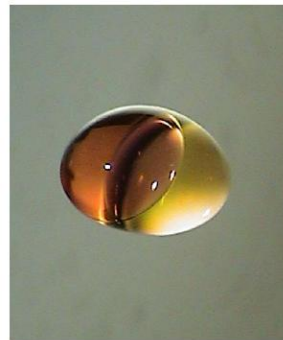
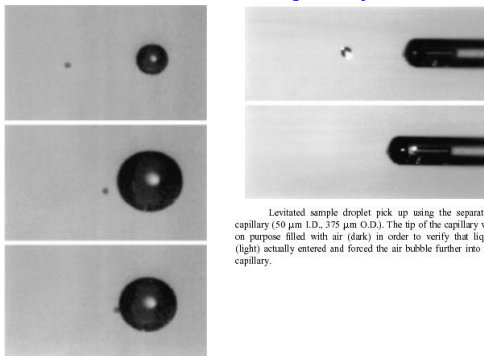


Figure 3. Two-phase formation in a levitated drop using two immiscible liquids (water and *n*-butanol). The levitated drop is mixed by disturbing the ultrasonic field. (A) A drop of each liquid was positioned together in the same ultrasonic node. (B) Phase separation starts when the ultrasonic field is readjusted to optimal conditions. (C) Smaller drops coalesce into fewer, larger ones. (D) Complete phase separation. Left, photographs; right, sketches. Phase 1 corresponds to the *n*-butanol phase and phase 2 to the aqueous phase (identified by HPTS).

Fig. 3 Two-phase (aq/org.) system in levitated drop. The aqueous phase is in yellow and the organic phase in orange

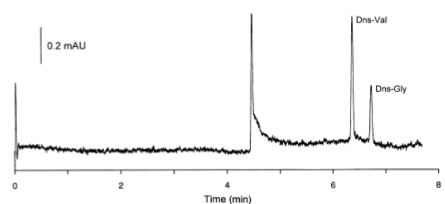
CE s detekcí v levitující kapse



Levitated sample droplet pick up using the separation capillary (50 µm I.D., 375 µm O.D.). The tip of the capillary was on purpose filled with air (dark) in order to verify that liquid (light) actually entered and forced the air bubble further into the capillary.

Stroboscopic images of a 65 µl droplet approaching the levitated drop. The images are from three experiments and therefore the levitated drop size varies.

CE s detekcí v levitující kapse



CE analysis of in all 36 000 sample droplets (2.3 µl) enriched in an acoustically levitated drop for 25 min. Droplets (6000) were added to the diminishing levitated drop every fourth minute. Capillary 45 cm x 50 µm I.D., electrolyte 100 mM borate buffer (pH 9.0), sample 0.1 µM Dns-Val and 0.05 µM Dns-Gly in ethanol, voltage 10 kV, current 14 µA, detection wavelength 200 nm.

**Akust.
Levitace
RTG
Analýza**



Figure 1
A 'Hing' droplet in the acoustical levitator.

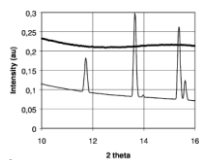


Figure 2
Two X-ray diffraction curves of $K_2[PCl_3]$ (200 mM). The upper curve was collected during 1 min and only diffuse scattering is observed, but in the lower curve, collected during 1 min, clear Bragg diffraction peaks are observed as the sample had crystallized during the evaporation process.

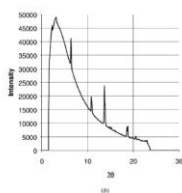


Figure 3
a) The X-ray pattern of $Zn(OHPO_4)$ in a suspended mixture with water. The sharp peaks of the beam stop is visible in the center of the diffraction image. b) The X-ray diffraction curve of $Zn(OHPO_4)$ in a suspended mixture with water.