

# Miniaturization of Bioanalytical Instrumentation

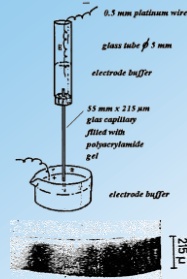
DNA analysis - genomics  
 Protein analysis - proteomics  
 Metabolite analysis - metabolomics/metabonomics  
 Glycomics, ...

New technologies  
**Microfluidics - Mass Spectrometry**

Frantisek Foret, Institute of Analytical Chemistry  
 Academy of Sciences of the Czech Republic, Brno

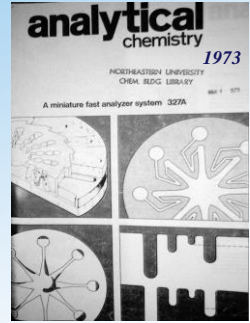


## Instrumentation Miniaturization



Capillary gel electrophoresis  
 Separation of nerve cell proteins

H. Hyddén et al. *Anal.Biochem.*, 17, 1-15, 1966.



## Microfluidics?

### Microelectronics

Control of electric current



Technology

Product

Consequences

### Microfluidics

control of fluid flows



Speed of analysis

Space saving

Cost cutting

Mass production

Making and inspecting semiconductor chips requires pushing laser techniques deeper into the ultraviolet.

by Hank Hogan, Contributing Editor



A semiconductor feature sizes shrink, manufacturers need a light touch — and at the right wavelength. A look at three areas — lithography, metrology and assembly — shows how photonic-based innovations are tackling some of the semiconductor industry's most pressing problems.

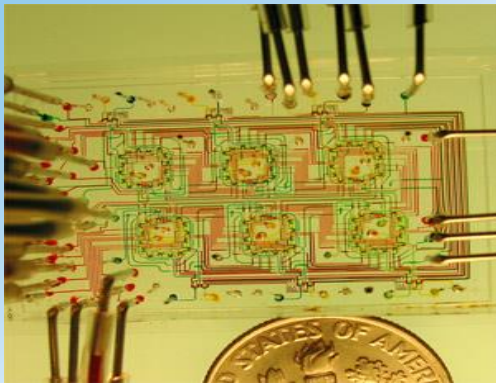
Scrapers are among the most critical tools used for semiconductor manufacturing and are at the heart of the photolithographic process, which transfers the features that are on a mask onto the photoresist material on a wafer. Subsequent processing reproduces that transferred layout in layers of conductors and insulators that eventually comprise a functioning integrated circuit. Today, state-of-the-art features are as small as 65 nm. Soon, they will be 45 nm, and the generation beyond that, 32 nm. The latter two scales are several years away, although the equipment needed for them is being ordered now.

Although designed for manufacturing on a microscopic scale, the latest lithography scraper from Carl Zeiss SMT AG of Oberkochen, Germany, is not small. The shielded 1000 weighs more than a metric ton, stands several feet tall and is as big around as a tree trunk. A catalytic beam consisting of reflecting mirrors and refractive optics, it enables volume semiconductor production.

The device pictured is a catalytic beam that, according to the manufacturer, can achieve 40-nm-resolution lithography on semiconductor chips. Courtesy of Carl Zeiss SMT.

82 PHOTONICS SPECTRA

DECEMBER 2005

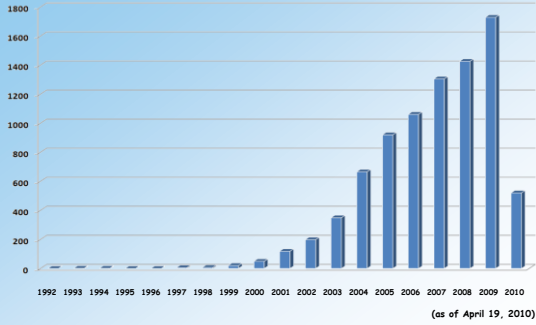


Stephen Quake, Dept. Bioengineering, Stanford University, <http://thebigone.stanford.edu/index.html>

## MICROFABRICATED DEVICES

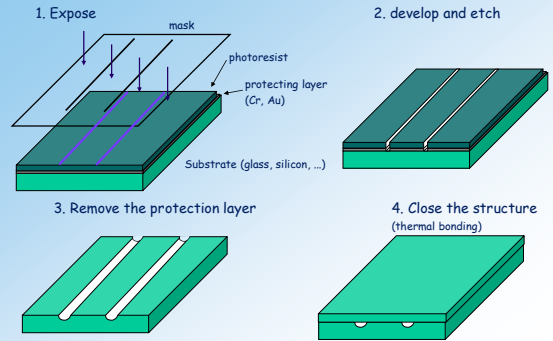
- \* **Sensors** - accelerometers, glucose monitors, ...
- \* **Genomics** - first commercial applications
- \* **Proteomics** - sample processing separation

### Incidence of the word "MICROFLUIDIC" in PubMed



<http://www.ncbi.nlm.nih.gov/PubMed>

### Photolithography



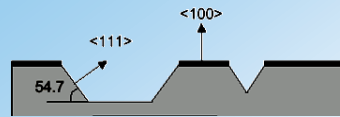
### μPG 101 Tabletop Laser Pattern Generator

HEIDELBERG INSTRUMENTS



- Substrates up to 100 x 100 mm<sup>2</sup>
- Structures down to 1 μm
- Address grid down to 40 nm
- 3D exposure mode
- Standard or UV laser source

### SILICON - ANISOTROPIC ETCHING

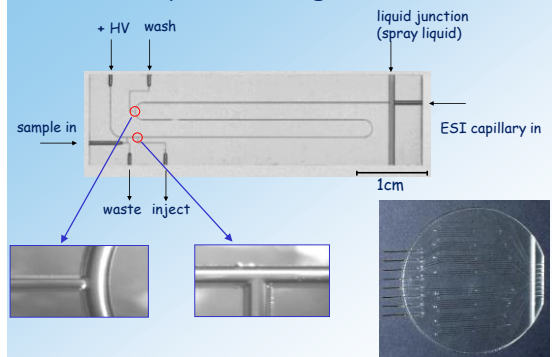


- \* Anisotropic etching - direction dependent etch rate
- \* Etch rate slower perpendicularly to the crystalline planes with the highest density
- \* Typical etches: KOH, Tetramethyl Ammonium Hydroxide (TMAH), Ethylene Diamine Pyrocatechol (EDP)

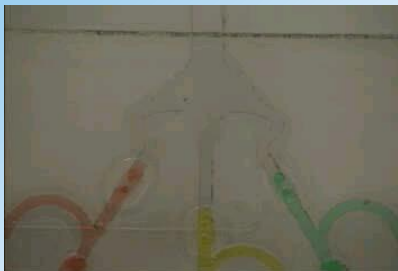
### Alternative technologies

- Hot embossing
- Injection molding - production scale
- Casting - polymeric resins, PDMS
- Plasma etching
- Laser machining

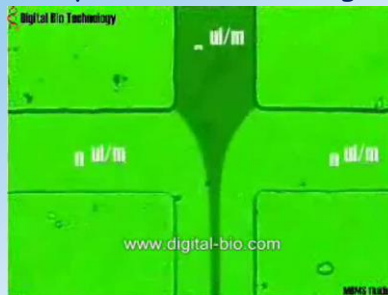
### System Integration



## Diffusion limited mixing



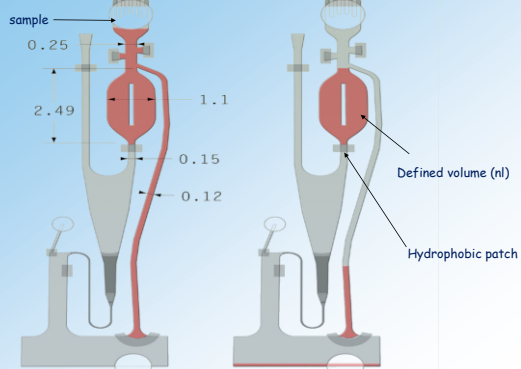
## Spatial flow focusing



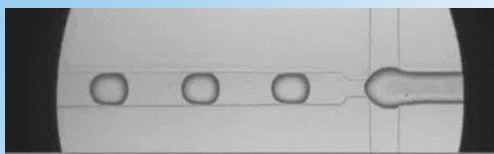
## Capillary force filling



## Exact volume metering on the nl level



## Droplet deneration in nl-pl volumes



Seth Froden et al., J. AM. CHEM. SOC. 2007, 129, 8825-8835.

## Microfluidics

Fluid (liquid) phase handling

Smaller size - faster analysis

Microchannel junctions without dead volume

Parallel systems for high throughput




Disposable parts

Phenomena unimportant on a macro scale become dominant

Space saving

## Small volume problem

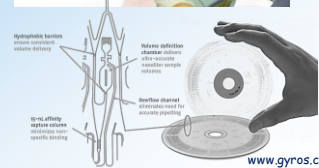
Example: LOD = 100 molecules

	2.15 mm	→	10 $\mu$ l $\sim$ 10 <sup>-15</sup> M
	1 mm	→	1 $\mu$ l $\sim$ 10 <sup>-14</sup> M
	0.1 mm	→	1 nl $\sim$ 10 <sup>-11</sup> M
	0.001 mm	→	1 fl $\sim$ 10 <sup>-5</sup> M

## Point-of-care analysis



i-Stat → Heska → [www.abbottpointofcare.com](http://www.abbottpointofcare.com)



[www.gyros.com](http://www.gyros.com)

## Examples

New approaches for DNA analysis based on:  
massively parallel PCR and pyrosequencing

([www.454.com](http://www.454.com))

OR

microfluidics  
and  
high sensitivity (single molecule) detection

## Human genome for \$ 1000?

J. Craig Venter

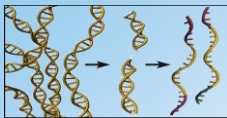
J. Craig Venter  
INSTITUTE

At present ~1000 x more

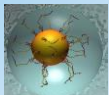
Microfluidics necessary

First system - 454.com  
([www.454.com](http://www.454.com), Roche)

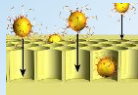
## 1 454 Massively Scaleable Sequencing in Picoliter Volumes



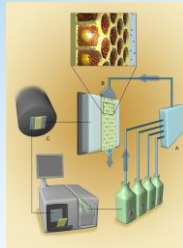
1) Random DNA fragments



2) Emulsion amplification on 28  $\mu$ m beads

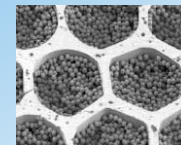
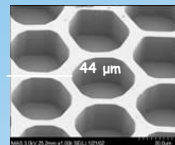


3) Loading of the beads into 100 000 microchannel glass plate



4) Sequencing using microbeads with immobilized enzyme and chemiluminescence detection

## Bead Loading - 454 PicoTiter™ Plate



Each Well contains a single DNA Bead & hundreds of enzyme beads

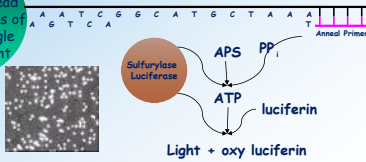
Three current plate sizes:  
300K Wells (25x75 mm<sup>2</sup>)  
860K Wells (40x75 mm<sup>2</sup>)  
1.6M Wells (70x75 mm<sup>2</sup>)



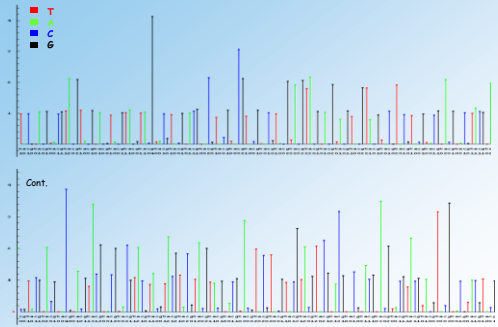
## Sequencing-By-Synthesis - Pyrosequencing

1. add one of the four dNTPs and DNA polymerase - pyrophosphate (PPi) released stoichiometrically
2. ATP sulfurylase converts PPi to ATP in the presence of adenosine 5' phosphosulfate; ATP-luciferase → luciferin to oxyluciferin. Light emission proportional to the amount of ATP.
3. Unincorporated nucleotides degraded by apyrase

Sepharose DNA Capture Bead containing Millions of Copies of a Single Clonal Fragment



## 191bp Perfect Read on 454 System



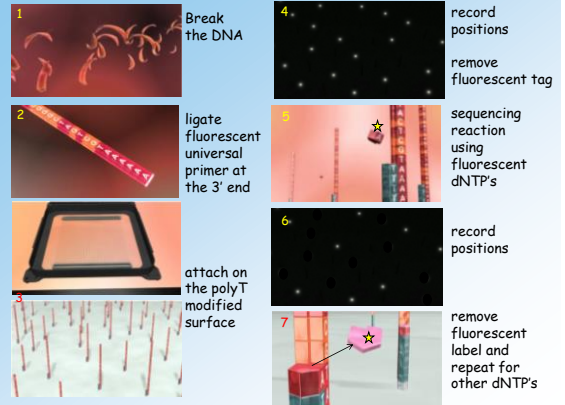
2

HeliScope™

†SMS - true Single Molecule Sequencing



www.helicosbio.com



3

SMRT™ single molecule, real-time sequencing



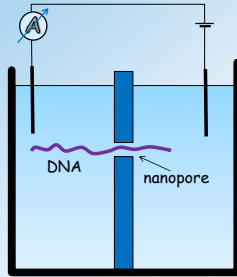
www.pacificbiosciences.com



Direct electrical detection of DNA synthesis  
Nadler Pourmand, Miloslav Karhaneck, Henrik H. J. Persson, Chris D. Webb, Thomas H. Lee, Alexandre Zahradnikova, and Ronald W. Davis, PNAS 2006 vol. 103 6466-6470

3

### Nanopore DNA sequencing



Oxford **NANOPORE** Read Long

The MinION is a miniaturised single molecule analysis device, designed for single use and to work through the USB port of a laptop or desktop computer.

<http://www.nanoporetech.com>

### Protein Analysis

Much more complicated than DNA  
 Posttranslational modifications  
 $10^{13}$  concentration range  
 No PCR

Separations + ESI/MALDI Mass Spectrometry

### Ionization in mass spectrometry

ESI - concentration sensitive  
 (10 nL/min or 10  $\mu$ L/min - similar sensitivity)

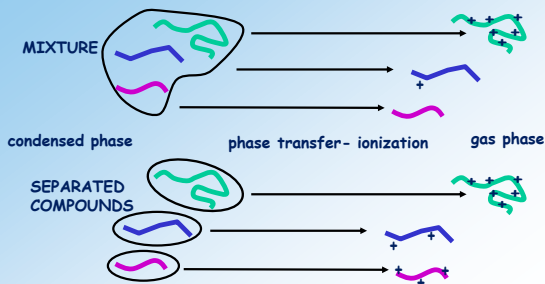
Charge competition  
 Different proton affinity  
 in the gas phase

↓  
 Signal suppression

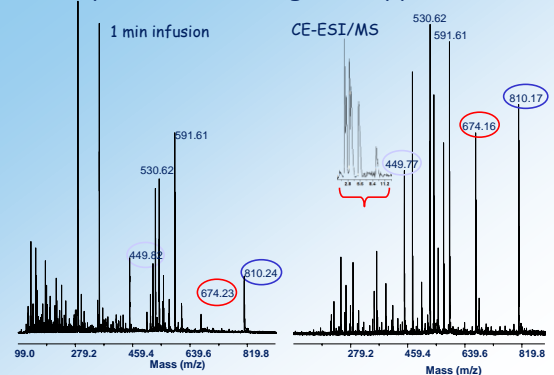
↓  
 SEPARATION

### MS IONIZATION - SIGNAL SUPPRESSION


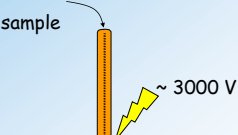
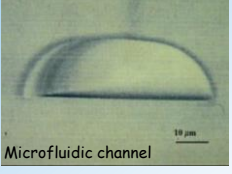

(ESI and MALDI)



### Separation and Signal Suppression

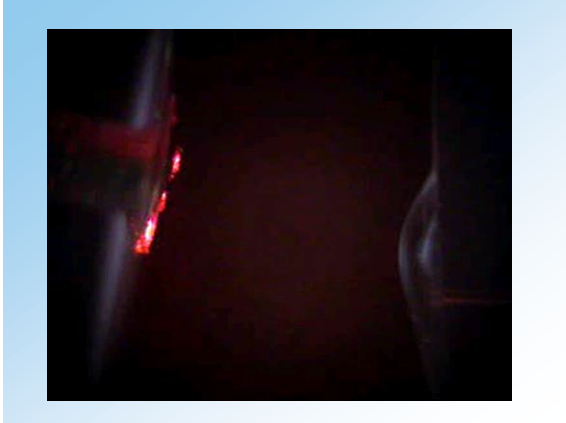


### Electrospray ionization

### CHIP ESI/MS COUPLING

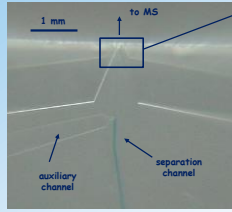
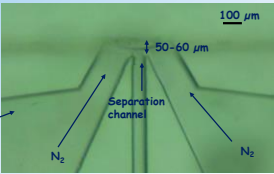
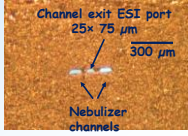
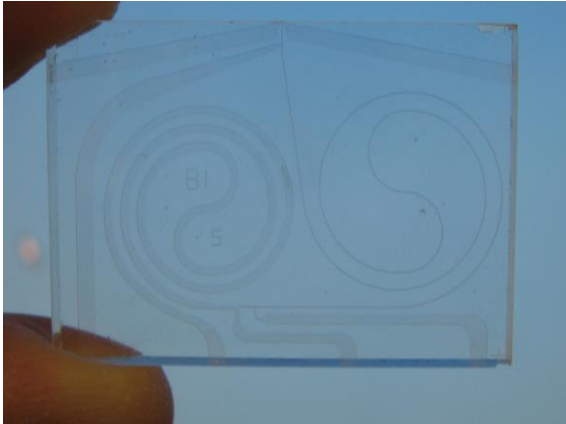
- \* flat surface electrospray
- \* microfabricated tips
- \* external (inserted) tips
- \* external interface with a transfer capillary
- \* integrated pneumatic nebulizer
- \* integrated liquid junction



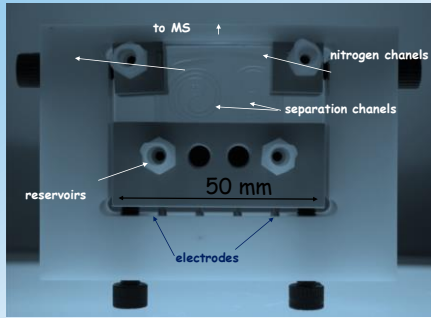
### ESI for Microfluidics



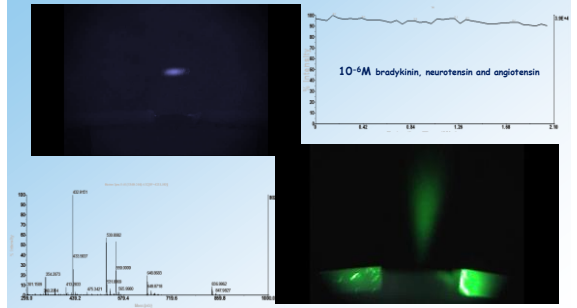
### Microfabricated nebulizer

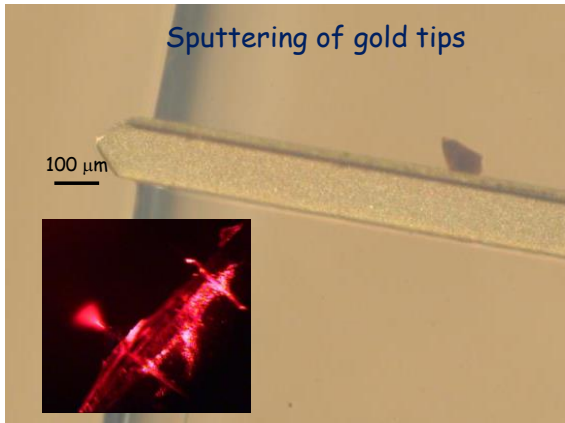
## Pneumatic nebulizer



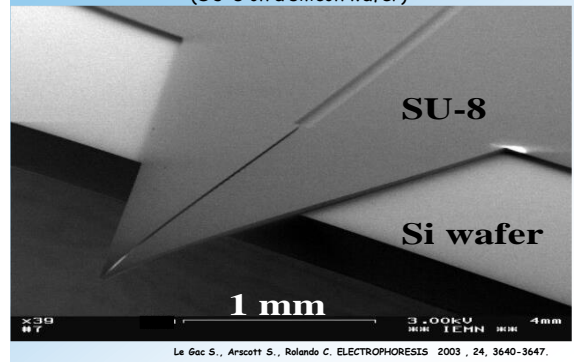
## Pneumatic nebulizer



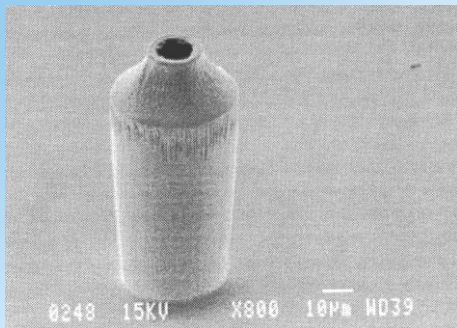
## Sputtering of gold tips



## Micro-nib electrospray source (SU-8 on a silicon wafer)



## ESI tips produced by DRIE in silicon



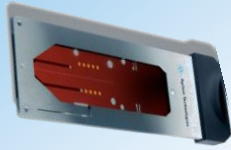
Sjodahl, J., Melin, J., Griss, P., Emmer, A., Stenme, G., Roerode, J. Rapid Commun. Mass Spectrom. 2003, 17, 337-341.

## ESI tip array





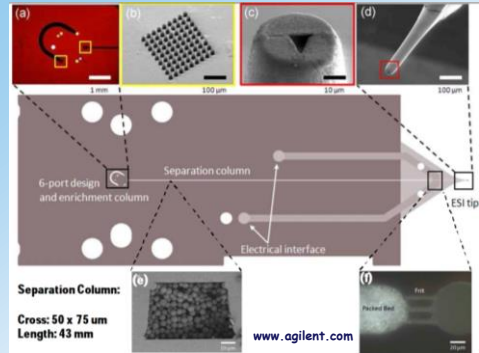
# HPLC on a chip



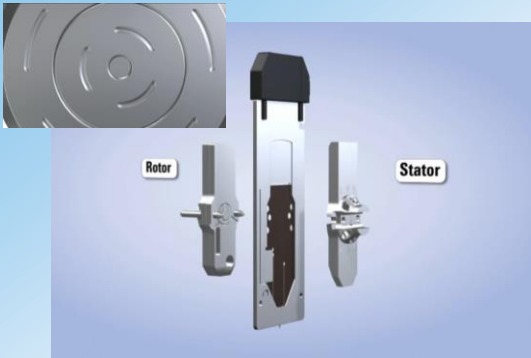
www.agilent.com



Polyimide HPLC-chip, integrating an enrichment column, frits, a laser ablated ESI tip and trapezoidal separation column

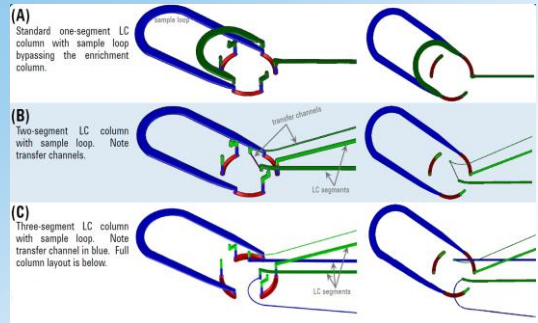


www.agilent.com

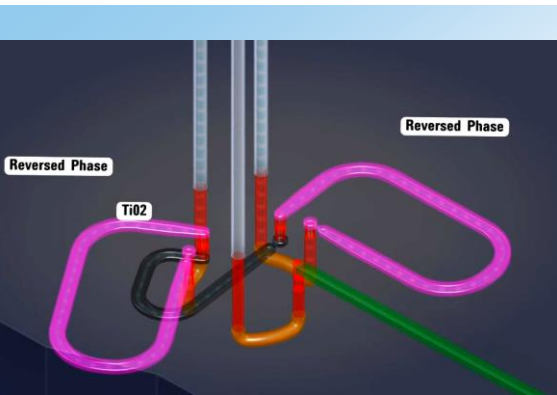


<http://www.youtube.com/watch?v=oBXK29YsplU>

## Segmented column HPLC/chip



www.agilent.com



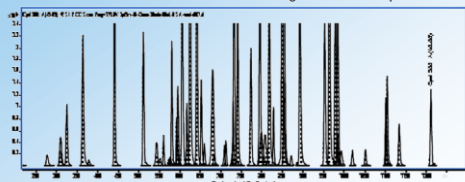
www.agilent.com

## Segmented column HPLC/chip

Three LC columns - length 130 mm  
Each segment individually packed.



Multi-segment three chip stack in enclosure.



BSA digest separated with a 30min gradient on a 2 column segmented chip, packed with 3.5μm particles

www.agilent.com

**TRIZAIC nanoTile UPLC System** Waters  
THE SCIENCE OF WHAT'S POSSIBLE.™

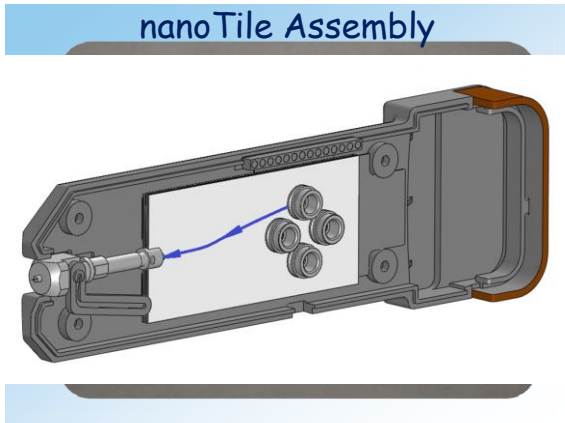
1.7µm BEH TRIZAIC UPLC SYSTEM

- UPLC Performance
- All fluidic connections are pre-made & factory tested
- Integrated ESI Emitter
- Low System Volumes
- Decreased Band Broadening
- Higher Sensitivity
- Incorporates:
  - Heater & Sensor
  - EPROM
- Increased Reproducibility

Built-in Heater, Sensors, EPROM

ESI Emitter

UPLC 'Column'



## Ceramic Microfluidic Fabrication

Cut Ceramic Material

Fired Wafer

Final Diced Part

Final Diced Part

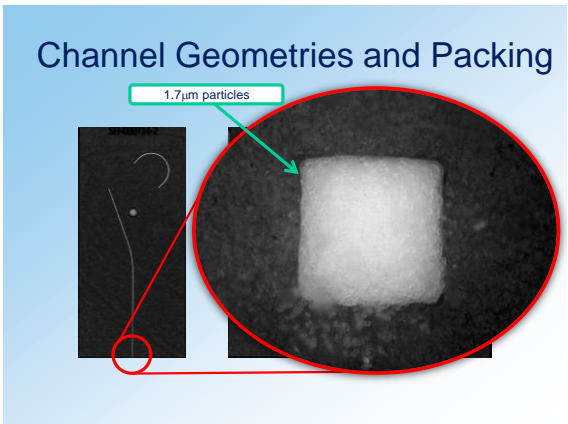
## Green tape

$Al_2O_3$ -MgO-SiO<sub>2</sub> glass particles mixed with organic binders and solvents to form glass ceramic

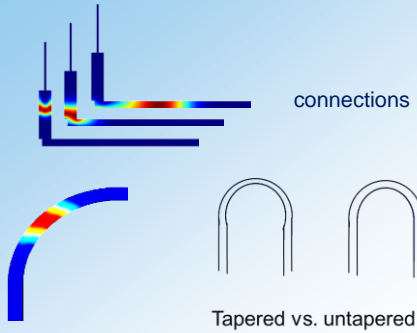
**Product Description**  
 951 Green Tape is a low-temperature cofired ceramic tape. The 951 system comprises a complete cofireable family of Au and Ag metallizations, buried passives, and encapsulants. 951 is available in multiple thicknesses for use as an insulating layer in:

- Multichip modules
- Single chip packages
- Ceramic printed wiring boards
- RF modules

<http://www.dupont.com/mcm>



## Channel shape effect

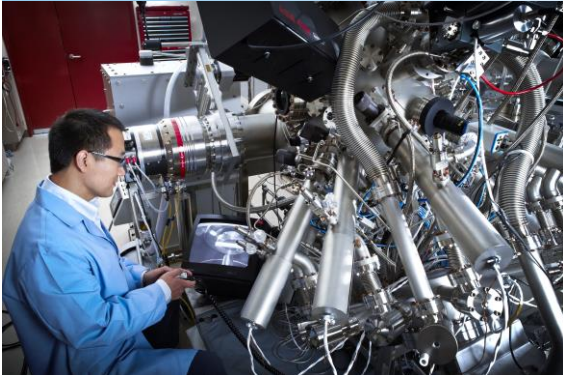


## Packed glass LC chip



Eksigent, part of AB SCIEX, www.eksigent.com

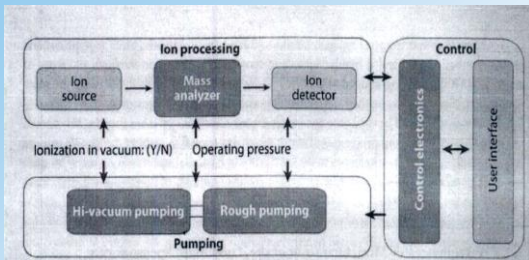
## Miniaturized (microfabricated) mass spectrometers



## Miniaturized (microfabricated) mass spectrometers



## Miniature Mass Spectrometers



Ouyang Z., Cooks, G.R., Annu.Rev.Anal.Chem. 2009, 2, 187-214.

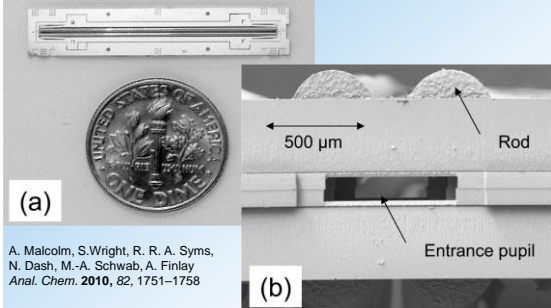
Table 1. Portable mass spectrometry analytical systems

System Developer	Self-sustainable portable systems					Portable systems without rough pumping		
	Mini 10/Mini 11 (3, 4)	ChemCube™ (115)	Guardion-7™ (5)	Suivease TOF (9)	Griffin 600™ (116)	Ion-camera MS (52) (117)	Palm-portable MS (52) (74)	HAPSITE™ (74)
Weight	10 kg/4 kg	14 kg	11 kg	N/A	13 kg	18 kg	1.5 kg	18 kg
Power	70 W/70 W	50 W	75 W	N/A	N/A	75 W	5 W	<150 W
Mass analyzer	Rectilinear ion trap	Quadrupole mass filter	Toroidal ion trap	TOF	Cylindrical ion trap	Matratch-Herzog sector	Cylindrical ion trap	Quadrupole mass filter
MS/MS	Yes	No	Yes	No	Yes	No	No	No
Sampling/ionization	MIMS, direct leak, GDEI, NCEI, ESI, DESI, LTP	SPMF, EI	SPMF, mini GCEI	MALDI	SPMF, MIMS EI	Direct gas leak EI	Pulsed gas leak EI	GCEI
Mass range/resolution	m/z 700, R = 700; m/z 1500, R = 750	m/z 600, R = 400	m/z 500, R = 500	m/z 70,000, R = 70	m/z 425, R = 400	m/z 300, R = 300	m/z 300, R = 150	m/z 300, R = 300

Abbreviations: NCEI, atmospheric pressure chemical ionization; DESI, desorption electrospray ionization; EI, electron impact; ESI, electrospray ionization; GCEI, gas chromatography electron impact; GDEI, glow discharge electron impact; LTP, low temperature plasma; MIMS, membrane introduction mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; NPMSE, solid-phase microextraction; TOF, time of flight.

Ouyang Z., Cooks, G.R., Annual Rev.Anal.Chem. 2009, 2, 187-214.

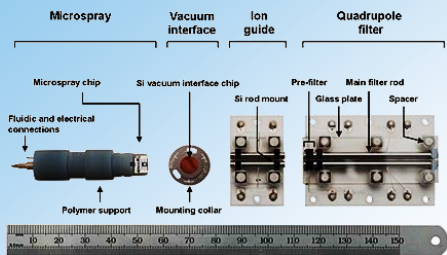
### Miniature Mass Spectrometer Systems based on a Microengineered Quadrupole Filter



A. Malcolm, S. Wright, R. R. A. Syms, N. Dash, M.-A. Schwab, A. Finlay  
*Anal. Chem.* **2010**, *82*, 1751–1758



### A miniature mass spectrometer for liquid chromatography applications



Rapid Communications in Mass Spectrometry  
Volume 25, Issue 21, pages 3281–3288, 3 OCT 2011 DOI: 10.1002/rcm.5230  
<http://onlinelibrary.wiley.com/doi/10.1002/rcm.5230/fulltext> fig-0002



Mass Analyzer ionchip® quadrupole mass spectrometer  
Mass Range  $m/z$  50-800 with ionchip®150  
Mass Accuracy  $\pm m/z$  0.3 in full scan  
Mass Resolution  $m/z$   $0.7 \pm 0.1$  FWHM  
Sensitivity 10pg of reserpine in SIM mode S/N ratio

**4000 MiD** Bringing mass spectrometry down to size  
[www.microsaic.com](http://www.microsaic.com)

**Advion expression Compact Mass Spectrometer**  
[www.advion.com](http://www.advion.com)

# Microfluidics

"... the potential of nanotechnology is so vast that it has been easy for researchers to get lost in the wilderness of possibilities."

Stephen Empedocles co-founder of Nanosys Inc., Palo Alto, CA

"... (the researchers) say the quantum dots can do amazing things. How do I make money from them?"

Edward K. Moran, Nanotech Industry Practice, Deloitte Services LP, NYC

## Patent? Patent!

What is a patent

Invention disclosure

Does it make sense to patent?

Patent search

Resources

## What Is a Patent?

A patent for an invention is the **grant of a property right to the inventor**, issued by the United States Patent and Trademark Office. Generally, the **term of a new patent is 20 years** from the date on which the application for the patent was filed in the United States or, in special cases, from the date an earlier related application was filed, subject to the **payment of maintenance fees**. U.S. patent grants are effective only within the United States, U.S. territories, and U.S. possessions. Under certain circumstances, patent term extensions or adjustments may be available. What is granted is not the right to make, use, offer for sale, sell or import, but the right to exclude others from making, using, offering for sale, selling or importing the invention. Once a patent is issued, the patentee must enforce the patent without aid of the USPTO.

There are **three types of patents**:

1) **Utility patents** may be granted to anyone who invents or discovers any new and useful process, machine, article of manufacture, or composition of matter, or any new and useful improvement thereof;

2) **Design patents** may be granted to anyone who invents a new, original, and ornamental design for an article of manufacture; and

3) **Plant patents** may be granted to anyone who invents or discovers and asexually reproduces any distinct and new variety of plant.

## Patentable subject

1. Does not fall under the laws of nature, natural phenomena or abstract ideas
2. Utility requirement - invention must be useful in association with machines, human-made products, compositions of matter or processing methods
3. Novelty the idea must not be presented to the public before the filing
4. Nonobviousness - it must be unrecognizable to a skilled person in the field of invention
5. Clarity of the description included in the application

## Patent je zákonná ochrana vynálezů zaručující vlastníkovi patentu výhradní právo k průmyslovému využití vynálezu.

V České republice udělování patentů upravuje zákon 527/1990. Podle něj se patenty udělují na vynálezy, které jsou nové, jsou výsledkem vynálezecké činnosti a jsou průmyslově využitelné.

Vynález se považuje za nový, jestliže není součástí stavu techniky.

Stavem techniky je všechno, co bylo zveřejněno přede dnem přihlášení patentu, ať již v České republice nebo v zahraničí.

Za vynálezy se naopak nepovažují zejména :

objevy, vědecké teorie a matematické metody,  
pouhé vnější úpravy výrobků,  
plány, pravidla a způsoby vykonávání duševní činnosti,  
programy počítačů,  
pouhé uvedení informace

Majitel patentu má výlučné právo vynález využívat (tj. výrobek vyrábět, uvádět do oběhu nebo upotřebit postup), dále poskytnout souhlas k využívání vynálezu jiným osobám (např. licenční smlouvou) a má právo převést patent na jinou osobu. Proto, aby patent zůstal v platnosti, je nutno platit tzv. udržovací poplatky, a to v každém státu zvlášť. Maximální možná délka patentové ochrany je 20 roků.

<http://cs.wikipedia.org/>

www.uspto.gov

http://www.epoline.org/

http://isdvapl.upv.cz

http://cz.espacenet.com/

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Did you mean: Kleparnik

Multichannel microscale system for high throughput preparative separation...  
US Pat. 6960149 - Filed Aug 24, 2000 - Beckman Coulter, Inc.  
--- Kleparnik ---

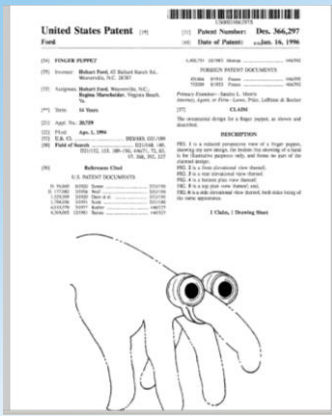
APPLICATION Multichannel microscale system for high throughput preparative separation...  
US Pat. App. 10593338 - Filed Aug 9, 2003 - NORTHEASTERN UNIVERSITY  
... Brighton, MA (US); Frantsek Foret, Maiden, MA (US); Marek Mnarik, Winthrop, MA (US); Karel Kleparnik, Brno (CZ) Correspondence Address: WEINGARTEN, ...

APPLICATION Multichannel microscale system for high throughput preparative separation...  
US Pat. App. 10592773 - Filed Aug 29, 2003 - NORTHEASTERN UNIVERSITY  
... Brighton, MA (US); Frantsek Foret, Maiden, MA (US); Marek Mnarik, Winthrop, MA (US); Karel Kleparnik, Brno (CZ) Correspondence Address: WEINGARTEN, ...

Methods and formulations for the separation of biological macromolecules  
US Pat. 6554985 - Filed Aug 13, 1999 - Corium Corporation  
There are a number of well known compressions in the sequence of M13 (see, eg. Kleparnik et al., supra). FIG. 9 shows a portion of the electrograms of ...

Fluorescence based nuclease assay  
US Pat. 6127124 - Filed Jan 20, 1999 - Isis Pharmaceuticals, Inc.  
Fol, SGP, Kleparnik et al., "Fast detection of a CA18 microsatellite ...

APPLICATION Dna separation using linear polymer solutions with dimethyl sulfoxide  
US Pat. App. 10592079 - Filed Aug 9, 2003  
[0077] Kleparnik, K., Foret, E., Berka, J., Goetzinger, W., Miller, AW and Karger, BL, Electrophoresis, 1990, 17, 1860-1866 ...  
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**SSC STŘEDISKO SPOLÉČNÝCH ČINNOSTÍ AV ČR**  
VÝZKUMNÁ VZDELÁVACÍ INSTITUCE

**Právní odbor**

**Právní odbor (PO)**

Právní odbor zajišťuje veškerou právní agendu ředitelství pro Institut SSC. Dále koordinuje a metodicky orientuje v praktické a teoretické oblasti AV ČR pracovníky AV ČR v oblasti výkonu státního práva v v.i. a provádí právní rady k případně rozhodnutí ve státní správě.

Právní odbor poskytuje právní konzultace a stanoviska mimo jiné:

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Společně s právními odděleními AV ČR poskytuje právní služby a stanoviska mimo jiné:

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**Companies offering microfluidics solutions**

- Abbot Laboratories <http://www.abbot.com/>
- Advanced Liquid Logic <http://www.liquid-logic.com/>
- Agilent Technologies <http://www.agilent.com/>
- Applied Biosystems <http://www.appliedbiosystems.com/>
- Aviva Biosciences <http://www.avivabio.com/>
- Biocore <http://www.biocore.com/>
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- Tronics Microsystems <http://www.tronics-met.com/>