Miniaturization of Bioanalytical Instrumentation

DNA analysis – genomics

Protein analysis – proteomics

Metabolite analysis – metabolomics/metabonomics

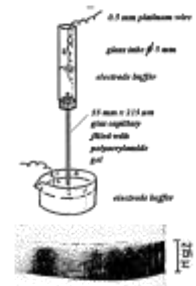
Glycomics, …

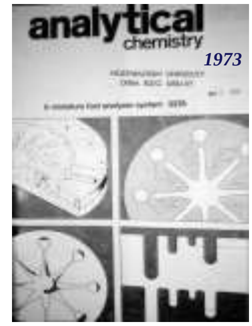
New technologies

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. Hydén et al. Anal.Biochem, 17, 1-15, 1966.

Microfluidics?

Microelectronics

Control of electric current

Technology

↓

Producst

↓

Consequences

Microfluidics

control of fluid flows

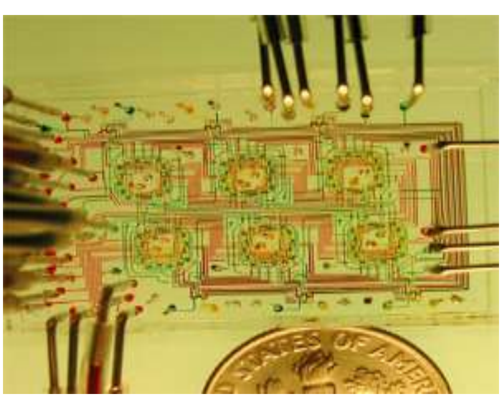
Speed of analysis

Space saving

Cost cutting

Mass production


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



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

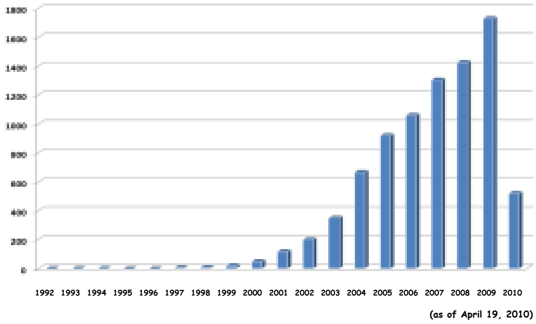
MICROFABRICATED DEVICES

* Sensors - accelerometers, glucose monitors, ...

* Genomics - first commercial applications

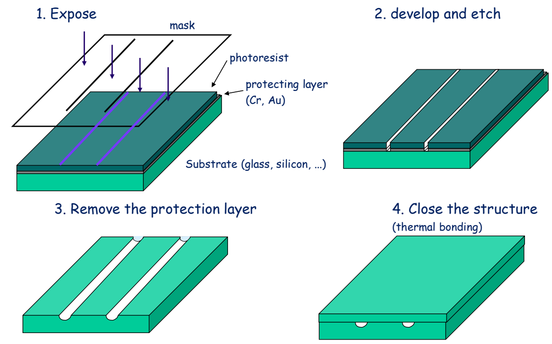
* Proteomics - sample processing separation

Incidence of the word "MICROFLUIDIC" in PubMed



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

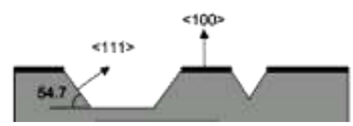
Photolithography



µPG 101 Tabletop Laser Pattern Generator

- Substrates up to 100 x 100 mm2
- 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

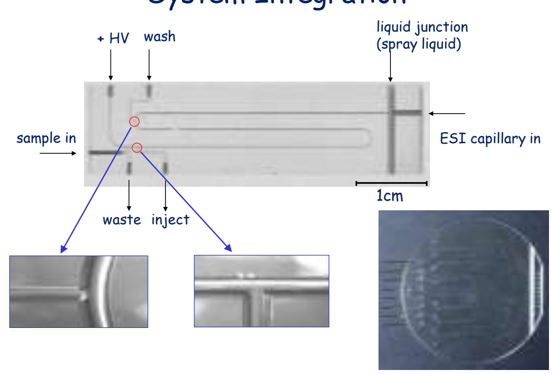
* Etch rate slower perpendicularly to the crystalline planes with the highest density

* Typical etches: KOH, Tetramethyl Ammonium Hydroxide (TmAH) Ethylene Diamine Pyrocatechol (EDP)

Alternative technologies

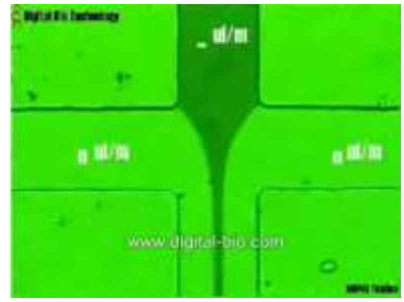
Hot embossing
Injection molding - production scale
Casting - polymeric resins, PDMS
Plasma etching
Laser machining

System Integration



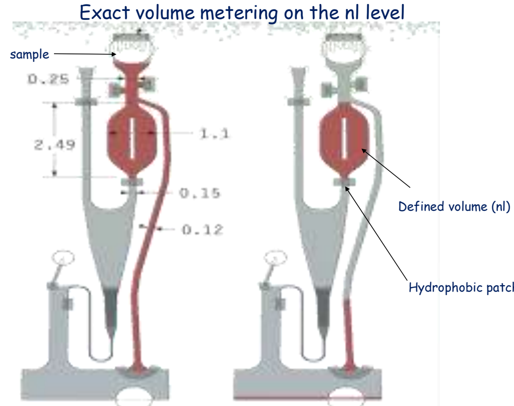
Diffusion limited mixing

Spatial flow focusing

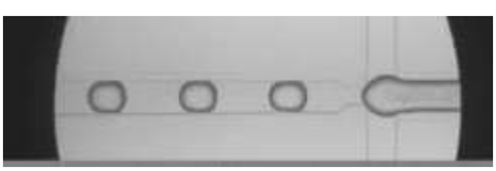


Capillary force filling

Exact volume metering on the nl level



Droplet deneration in nl-pl volumes



Seth Fraden 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

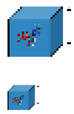
Disposable parts

Phenomena unimportant on a macro scale become dominant

Space saving

Small volume problem

Example: LOD = 100 molecules



2.15 mm	->	10 μl ~ 10^{-15} M
1 mm	->	1 μl ~ 10^{-14} M
0.1 mm	->	1 nl ~ 10^{-11} M
0.001 mm	->	1 fl ~ 10^{-5} M

Point-of-care analysis

i-Stat -> Heska -> www.abbottpointofcare.com


www.gyros.com

Examples

New approaches for DNA analysis based on:

massively parallel PCR and pyrosequencing

(www.454.com)

or

microfluidics

and

high sensitivity (single molecule) detection

Human genome for $ 1000?

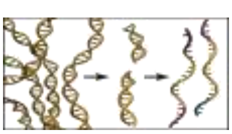
J. Craig Venter

At present ~1000 x more

Microfluidics necessary

First system - 454.com (www.454.com, Roche)

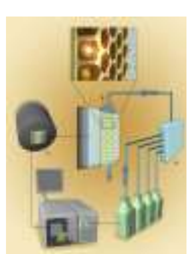
1 454 Massively Scaleable Sequencing in Picoliter Volumes



1) Random DNA fragments

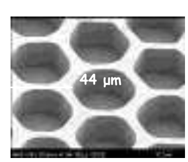
2) Emulsion amplification on 28 μm beads

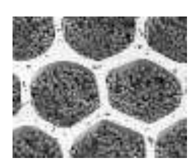
3) Loading of the beads into 100 000 microchannel glass plate



4) Sequencing using microbeads with immobilized enzyme and chemiluminiscence detection

Bead Loading - 454 PicoTiterTM Plate



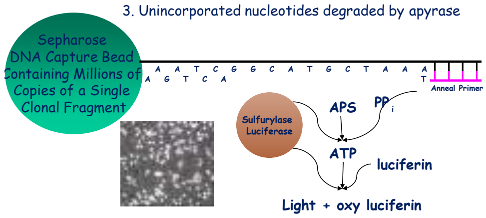


Each Well contains a single DNA Bead & hundreds of enzyme beads

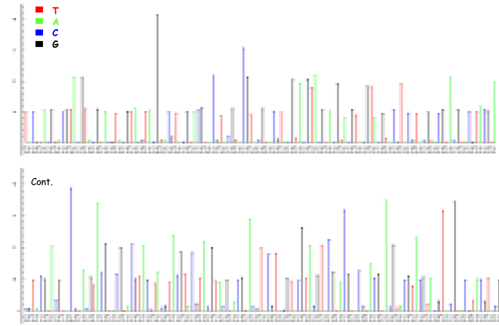
Three current plate sizes:
300K Wells (25x75 mm^2)
860K Wells (40x75 mm^2)
1.6M Wells (70x75 mm^2)

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



191bp Perfect Read on 454 System



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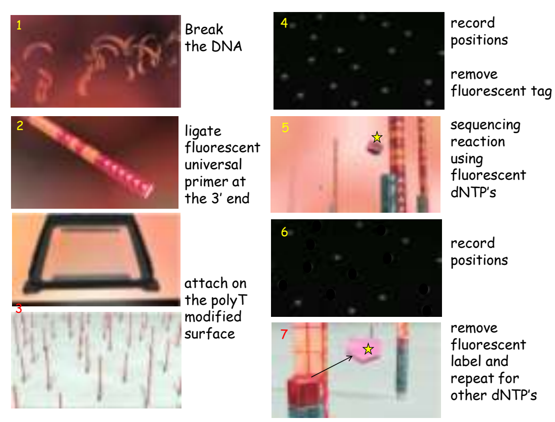
HeliScope™

tSMS -true Single Molecule Sequencing

www.helicosbio.com



3

SMRT™ single molecule, real-time sequencing

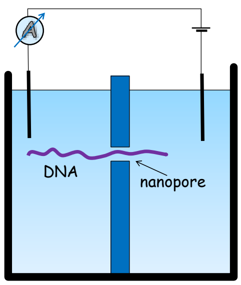
www.pacificbiosciences.com

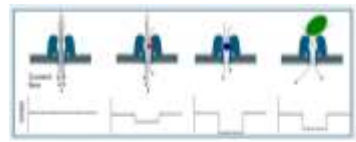
3 Ion Torrent

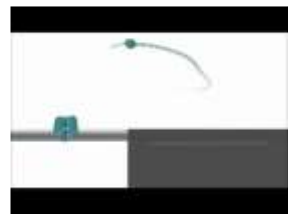
Direct electrical detection of DNA synthesis
Nader Pourmand, Miloslav Karhanek, Henrik H. J. Persson, Chris D. Webb, Thomas H. Lee, Alexandra Zahradníková, and Ronald W. Davis, PNAS 2006 vol. 103 6466-6470

3

Nanopore DNA sequencing







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 µL/min – similar sensitivity)

Charge competition
Different proton affinity
in the gas phase

↓

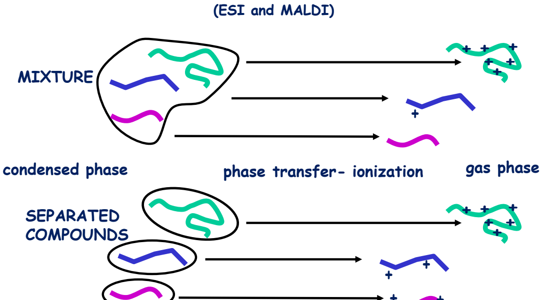
Signal suppression

↓

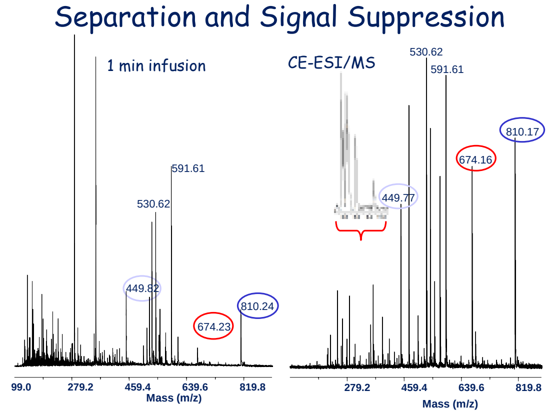
SEPARATION

MS IONIZATION - SIGNAL SUPRESSION

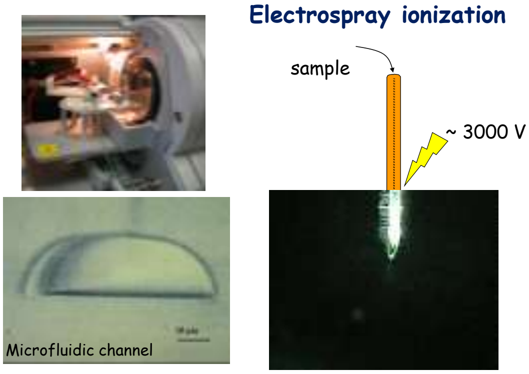
(ESI and MALDI)



Separation and Signal Suppression



Electrospray ionization

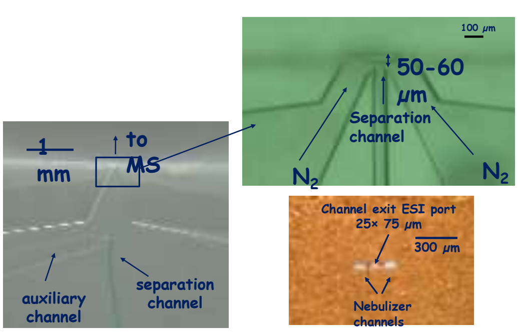


CHIP ESI/MS COUPLING

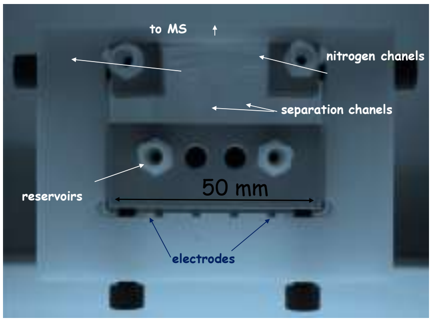
* flat surface electrospray
* microfabricated tips
* external (inserted) tips
* external interface with a transfer capillary
* integrated pneumatic nebulizer
* integrated liquid junction

ESI for Microfluidics

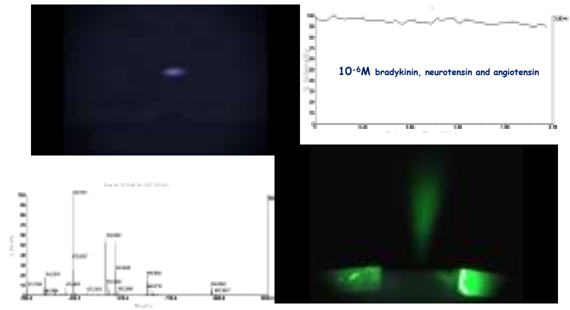
Microfabricated nebulizer



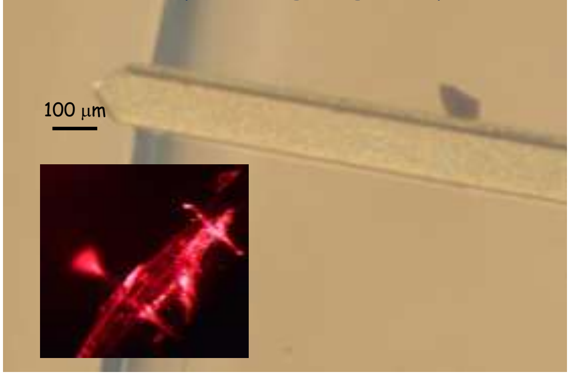
Pneumatic nebulizer



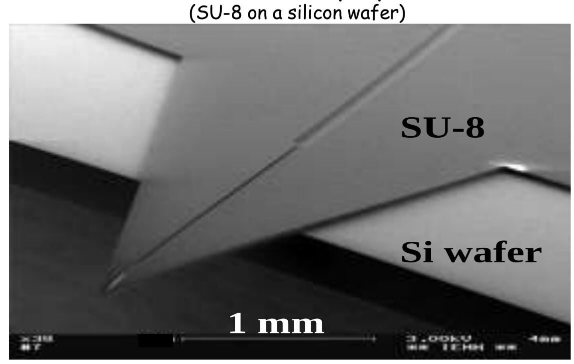
Pneumatic nebulizer



Sputtering of gold tips

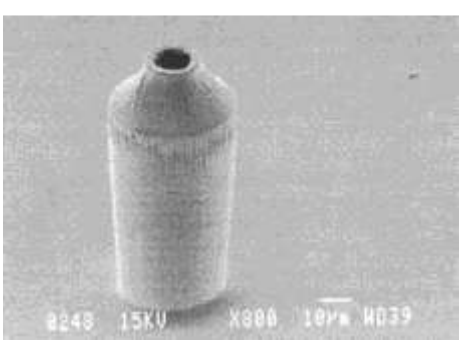


Micro-nib electrospray source
(SU-8 on a silicon wafer)



Le Gac S., Arscott S., Rolando C. ELECTROPHORESIS 2003 , 24, 3640-3647.

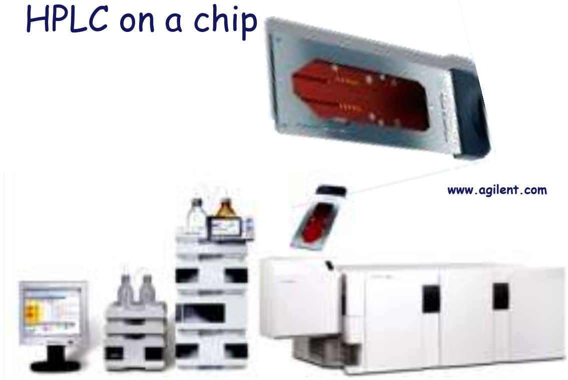
ESI tips produced by DRIE in silicon



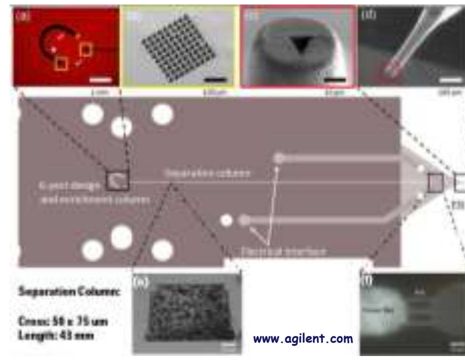
Sjödahl, J., Melin, J., Griss, P., Emmer, A., Stemme, G., Roeraade, J. Rapid Commun. Mass Spectrom. 2003, 17, 337–341.

ESI tip array

HPLC on a chip

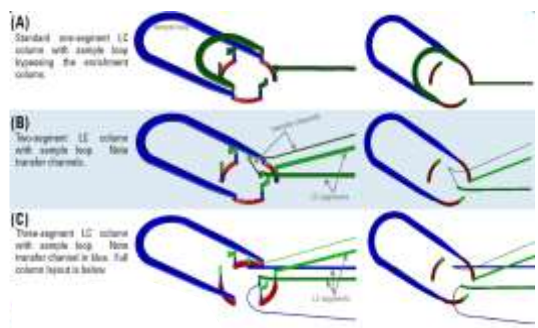


Polyimide HPLC-chip, integrating an enrichment column, frits, a laser ablated ESI tip and trapazoidal separation column

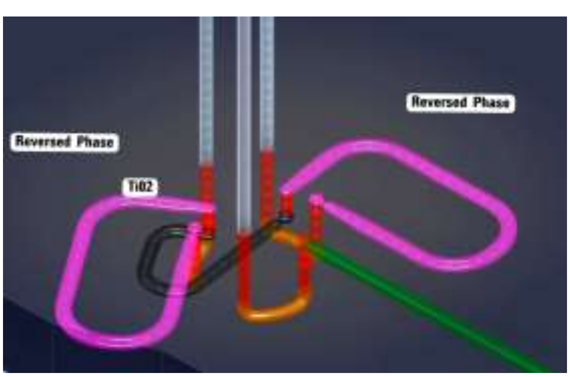


http://www.youtube.com/watch?v=oBXK29YsplU

Segmented column HPLC/chip



www.agilent.com

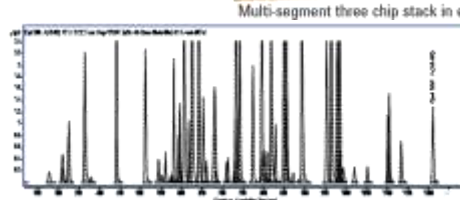


www.agilent.com

Segmented column HPLC/chip

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

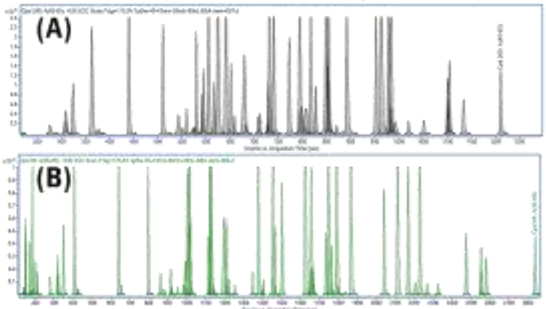
Multi-segment three chip stack in enclosure.



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

www.agilent.com

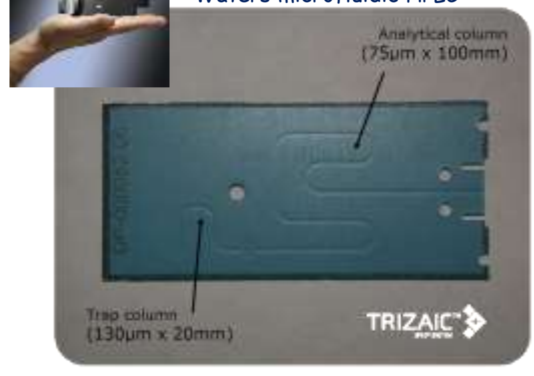
Peak capacity with a two-segment chip



(A) 30min total run time
(B) 70min total run time

www.agilent.com

Waters microfluidic HPLC



Green tape

Al_2O_3-MgO-SiO_2 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:

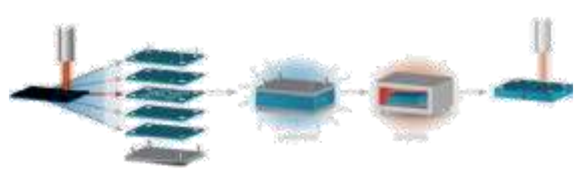
- Multichip modules
- Single chip packages
- Ceramic printed wiring boards
- RF modules

http://www.dupont.com/mcm

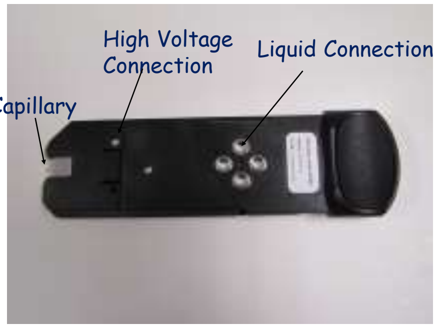
Trizaic System

Manufacturing process

The stack of green tape is laminated at elevated temperature and pressure



nanoTile Assembly



Microfluidics

NanoTechnology

There's Plenty of Room at the Bottom

An Invitation to Enter a New Field of Physics

by Richard P. Feynman

December 29th 1959 at the annual meeting of the American Physical Society at the California Institute of Technology (Caltech)

Fluorescent quantum dots

semiconductor inorganic crystals (1-10 nm)
core from elements of II. and VI. or III. and V. group
(ZnS; ZnSe; PbS; CdSe; CdTe)

Surface groups -COOH, $-NH_2$

Preparation

1st step: preparation of hydrogen telluride

$4\ NaBH_4 + 2\ Te + 7\ H_2O \rightarrow 2\ NaHTe + Na_2B_4O_7 + 14\ H_2$

2nd step: quantum dots formation

$CdCl_2$ + NaHTe + MPA or MA + heating

MPA: 3-mercaptopropionic acid: $HS-CH_2-CH_2-COOH$
MA: 2-mercaptoethylamin : $HS-CH_2-CH_2-NH_2$

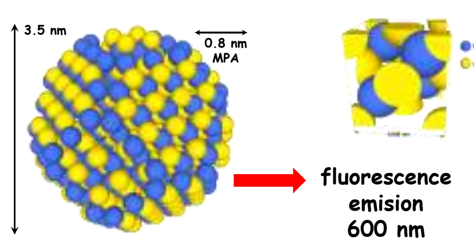
3rd step: coating

CdS: CdTe + $CdCl_2$ + Na_2S + MPA or MA + heating

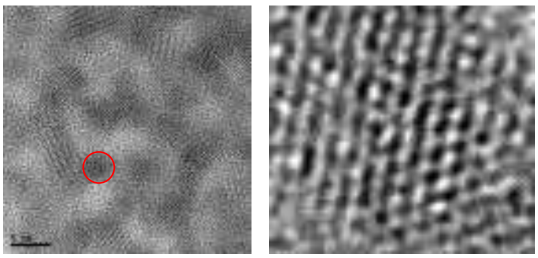
ZnS: CdTe/CdS + $ZnCl_2$ + Na_2S + MPA or MA + heating

Quantum dot CdTe nanocrystal

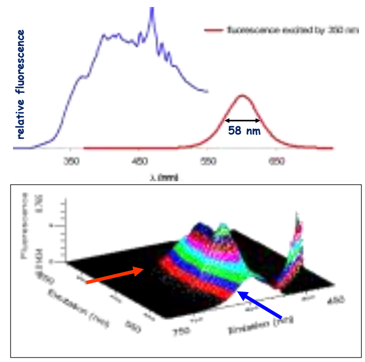
36 crystal elements, 650 atoms, 78 000 Da



High resolution transmission electron microscopy



CdTe QDs excitation and emission spectra

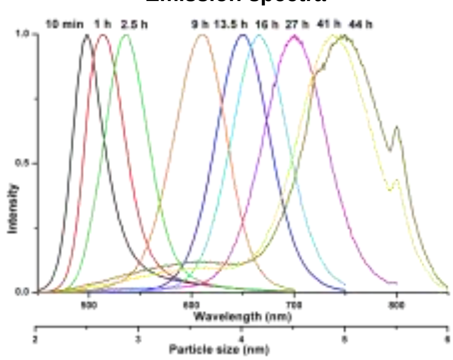


wide excitation spectra

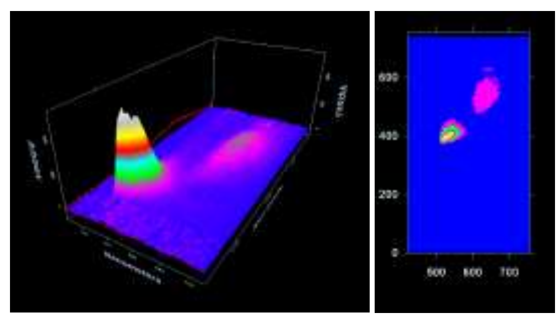
narrow and symmetric emission spectra with maximum at 600 nm

bandwidth 58 nm at half height

Emission spectra

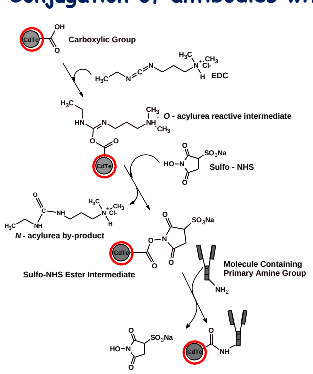


Electrophoresis in replaceable sieving media



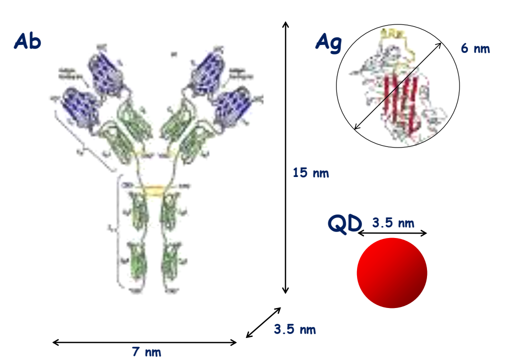
PVA coated capillary 20/30 cm, i.d. 75μm, separation buffer 3% LPA 10 MDa in 50 mM TRIS/TAPS buffer, pH = 9, QD 2.8 and 3.7 nm (525 and 610 nm 1:1), injection time 10 s, separation voltage 3 kV

Conjugation of antibodies with quantum dots



- carboxylic group on surface
- zero length linkers: (carbodiimide) EDC (succinimide) HNS
- amine group from antibody
- formation of peptide bond

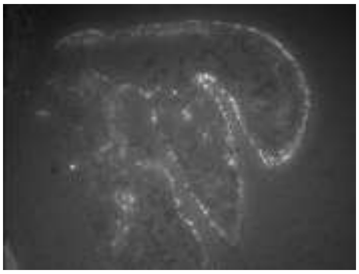
Size comparison of antiovalbumin, ovalbumin and CdTe QD



Conjugation of CdTe to Ab PCNA protein

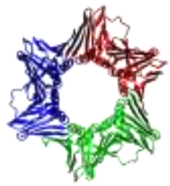
(Immunofluorescence microscopy of cultivated mouse embryo tissues)

Fluorescence microscope Leica
20 x objective
100 W Hg lamp
530nm 600 nm
Luca Andor EMCCD camera



Proliferating **C**ell
Nuclear **A**ntigen

DNA repair and
replication
protein



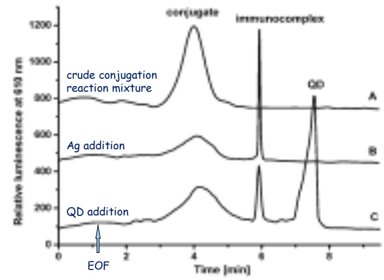
CE immunoassay

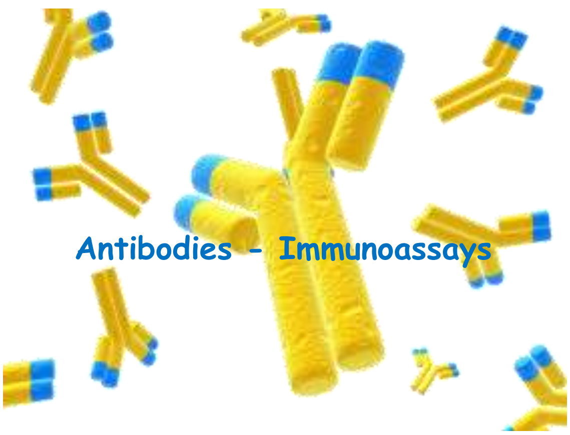
LIF detection 488/610 nm
uncoated capillary length15/20 cm, i.d. 75 μm
buffer 100 mM TRIS/TAPS, pH 8.3
voltage 6 kV, eof mobility 66 .10^{-9} $m^2/(Vs)$

Ab - anti-ovalbumin

QD - CdTe (610nm)

Ag - ovalbumin





Spectroscopic methods

- UV-vis absorbance spectroscopy
- Laser Induced Fluorescence
  Total Internal Reflection Fluorescence (TIRF)
  Multiple Photons Absorption
- NMR best structure information – miniaturization limits
- IR spectroscopy
- Scanning Probe Microscopy (SPM), Near Field Microscopy

....

Raman Spectroscopy

Structure related spectra

Examination of minerals
Objects of art
Proteins, cells and organs ...

Inherently insensitive

Surface Enhanced Raman Scattering (SERS)
Surface Enhanced Resonance Raman Scattering (SERRS)
Coherent Anti-Stokes Raman Scattering (CARS) ...

Surface-enhanced Raman scattering (SERS)

Martin Fleischmann
Van Duyne in 1970's

Silver or gold surface – typically colloid
Surface plasmons of the metal excited by laser
Increase in the electric fields surrounding the metal
Raman intensities proportional to the electric field

Signal enhancement over 10^{11}

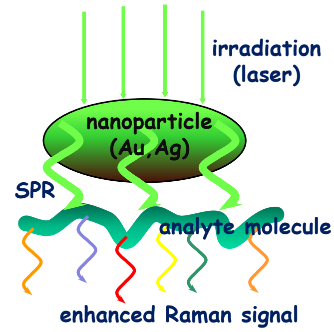
Surface-enhanced Raman scattering

1. Molecule in contact with metal (colloid, nanoparticle …)

2. Irradiation

3. Surface Plasmon Excitation

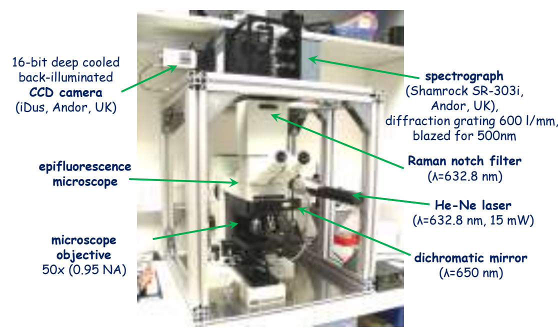
4. Raman scattering



Surface-Enhanced Raman Scattering (SERS)

- qualitative analysis – vibrational bands unique to molecular structures
- quantitative analysis
- metal nanomaterial:
  metal nanoparticles (NPs),
  roughened surfaces,
  metal tips,...
- enhancement factor 10^6 to 10^{12} => high sensitivity

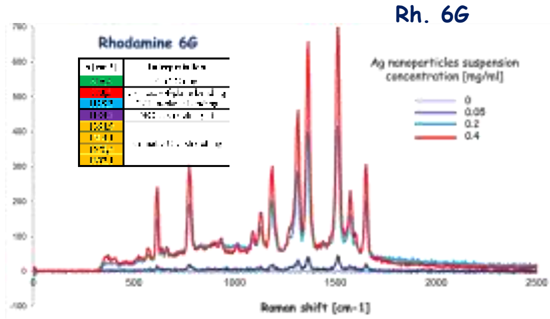
Laboratory Detection System



Dependence of SERS on NP's concentration

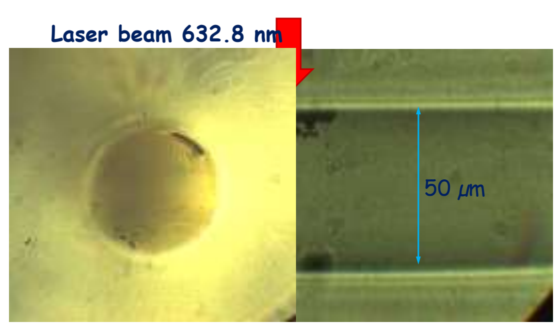
Laser 632.8 nm, Ag particles 50 - 100 nm

LOD ~ $2\cdot10^{-8}$ M
Rh. 6G



Detection window

Ag nanoparticles deposited on inner fused-silica surface

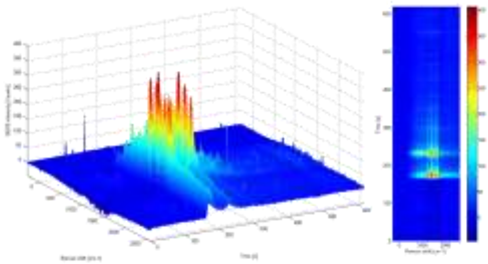


CE with SERS detection

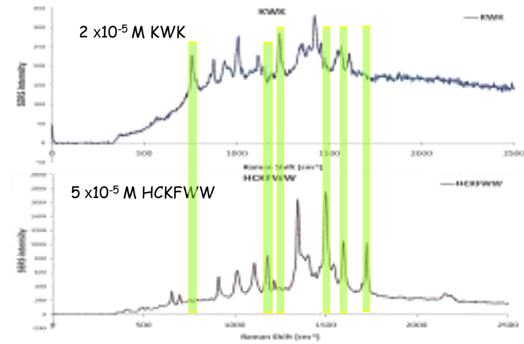
2D analysis of rhodamines 123 and B as model samples

CZE:
fused-silica capillary ID 50 µm, 15/25 cm
BGE: 40 mM CAPS with 20% methanol, pH 10
Voltage 6 kV
Sample: 5×10^{-5}M Rhodamine 123
2.5×10^{-5}M Rhodamine B

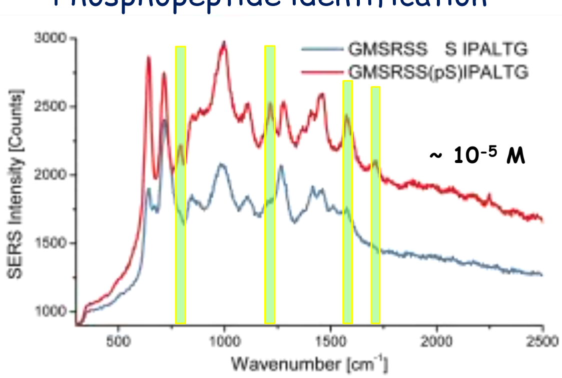
SERS:
exposure time 0.5 s



SERS Spectra of Peptides



Phosphopeptide identification



Structure information

Very small detection volume

Very good mass sensitivity
– fg – ag
(significant potential for improvement)

Concentrating techniques
– IEF, ITP

Conclusions

Never ending story

CECE 2012 Brno
www.ce-ce.org

"… 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/

Companies offering microfluidics solutions

Abbott Laboratories	http://www.abbott.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/
Biacore	http://www.biacore.com
Bioident	http://www.bioident.com/
Bioprocessors	http://www.bioprocessors.com/
Bio-Rad	http://www.bio-rad.com
Biotrove	http://www.biotrove.com
Caliper Life Sciences	http://www.caliperlifesciences.com/
Cellix	http://www.cellixltd.com
Cepheid	http://www.cepheid.com
Ciphergen	http://www.ciphergen.com
Cole-Parmer	http://www.coleparmer.com
Dionex	http://www.dionex.com
Eksigent Technologies	http://www.eksigent.com
Erie Scientific Company	http://www.eriemicrofluidics.com/
Evotec Technologies	http://www.evotec-technologies.com/
Fluidigm	http://www.fluidigm.com
Gyros	http://www.gyros.com
Handy Lab Inc.	http://www.handylab.com
Helicos Biosciences Corporation	http://www.helicosbio.com
Hewlett-Packard	http://www.hp.com
Ibidi Integrated BioDiagnostics	http://www.ibidi.de/
Invitrogen	http://www.invitrogen.com
Iq Micro Inc.	http://www.iq-micro.com/
Liquidia Technologies	http://www.liquidia.com/
Micralyne Inc.	http://www.micralyne.com/
Microchip Biotechnologies Inc	http://www.microchipbiotech.com
Microfluidics	http://www.microfluidicscorp.com/
Micronics	http://www.micronics.net
Micronit Microfluidics BV	http://www.micronit.com/
Monogram Biosciences	http://www.monogrambio.com/
Nanogen	http://www.nanogen.com
Nanostream	http://www.nanostream.com
Nanoterra	http://www.nanoterra.com/
Network Biosystems	http://www.networkbiosystems.com
Orchid Biosciences	http://www.orchid.com
Pyrosequencing AB	http://www.pyrosequencing.com
Roche 454	http://www.roche.com
Spin X Technologies	http://www.spinx-technologies.com/
Surface Logix	http://www.surfacelogix.com
Tecan	http://www.tecan.com
Tronics Microsystems	http://www.tronics-mst.com/