

Mikrofluidika a nanotechnologie v chemické analýze

František FORET
Ústav analytické chemie
AV ČR Brno

Trends in (bio)analytical instrumentation

DNA - Proteins
Carbohydrates-Metabolites
Sequence
Structure
Quantitative differences

ANALYTICAL TECHNOLOGY

DNA

Separation (CE)

Fluorescence

Miniaturization
(arrays)

PROTEINS

Separation (2D PAGE)

ESI - MALDI

Miniaturization
(arrays, microfluidics)



Informatics

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)

*Information on a small scale, Miniaturization by evaporation
Miniaturizing the computer, Better electron microscopes
Problems of lubrication, The marvelous biological system
A hundred tiny hands, Rearranging the atoms*

\$1,000 to the first guy who makes an operating electric motor---a rotating electric motor which can be controlled from the outside and, not counting the lead-in wires, is only 1/64 inch cube

reality

Data storage
Microcomputers
Rearranging the atoms

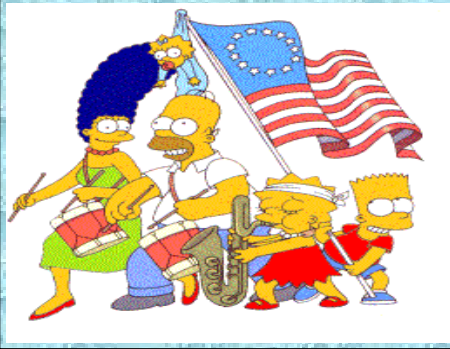
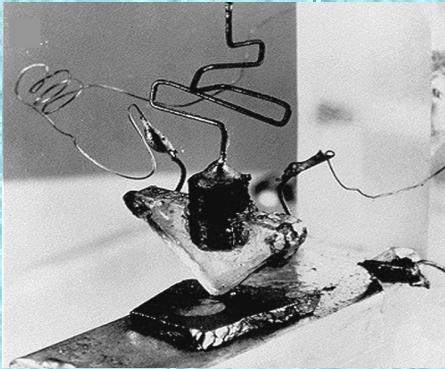
not quite yet

perfect microscope
marvelous biology systems
...

CD, DVD, HDD, DLP, accelerometers, CCD, AFM, ...

Microelectronics

electric current



Technology



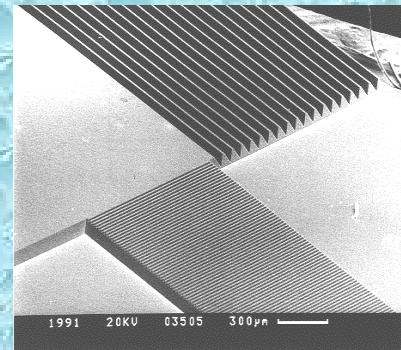
Products



Consequences

Microfluidics

solution flow



space
savings

cost
savings

mass
production

Advion

Agilent

Caliper

Diagnoswiss

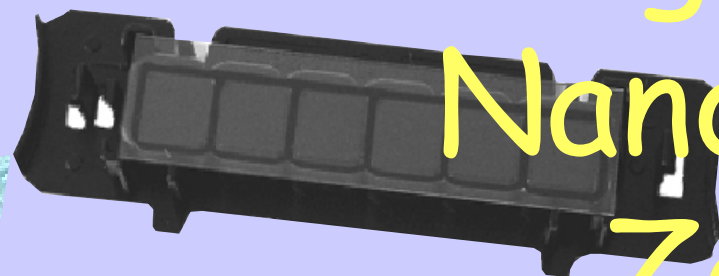
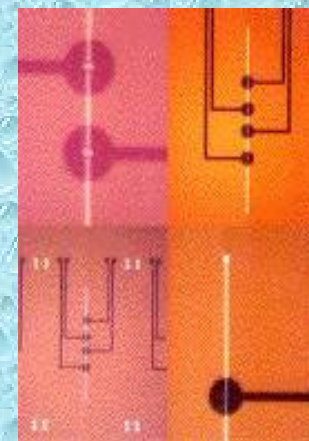
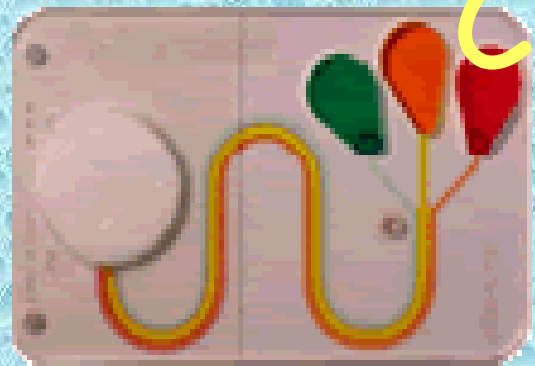
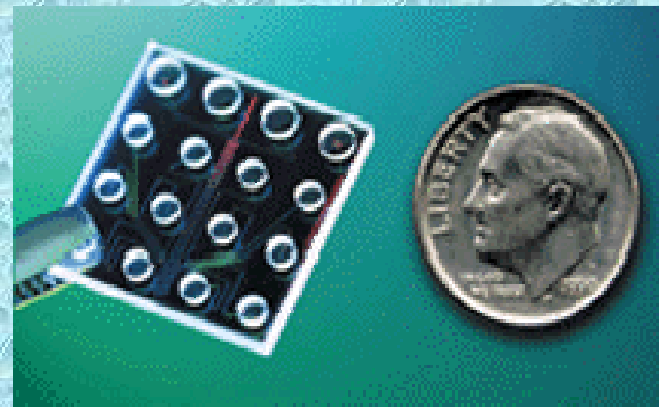
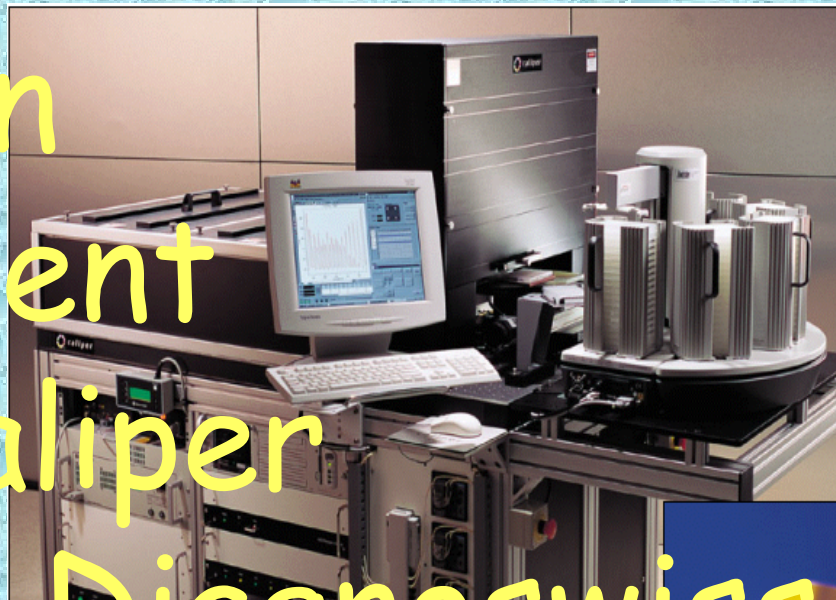
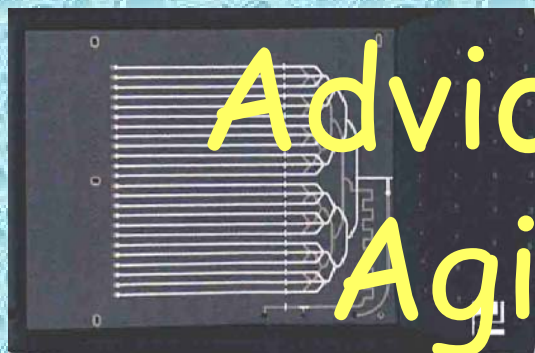
Gyros

Micronics

Nanogen

Nanostream

Zeptosyns



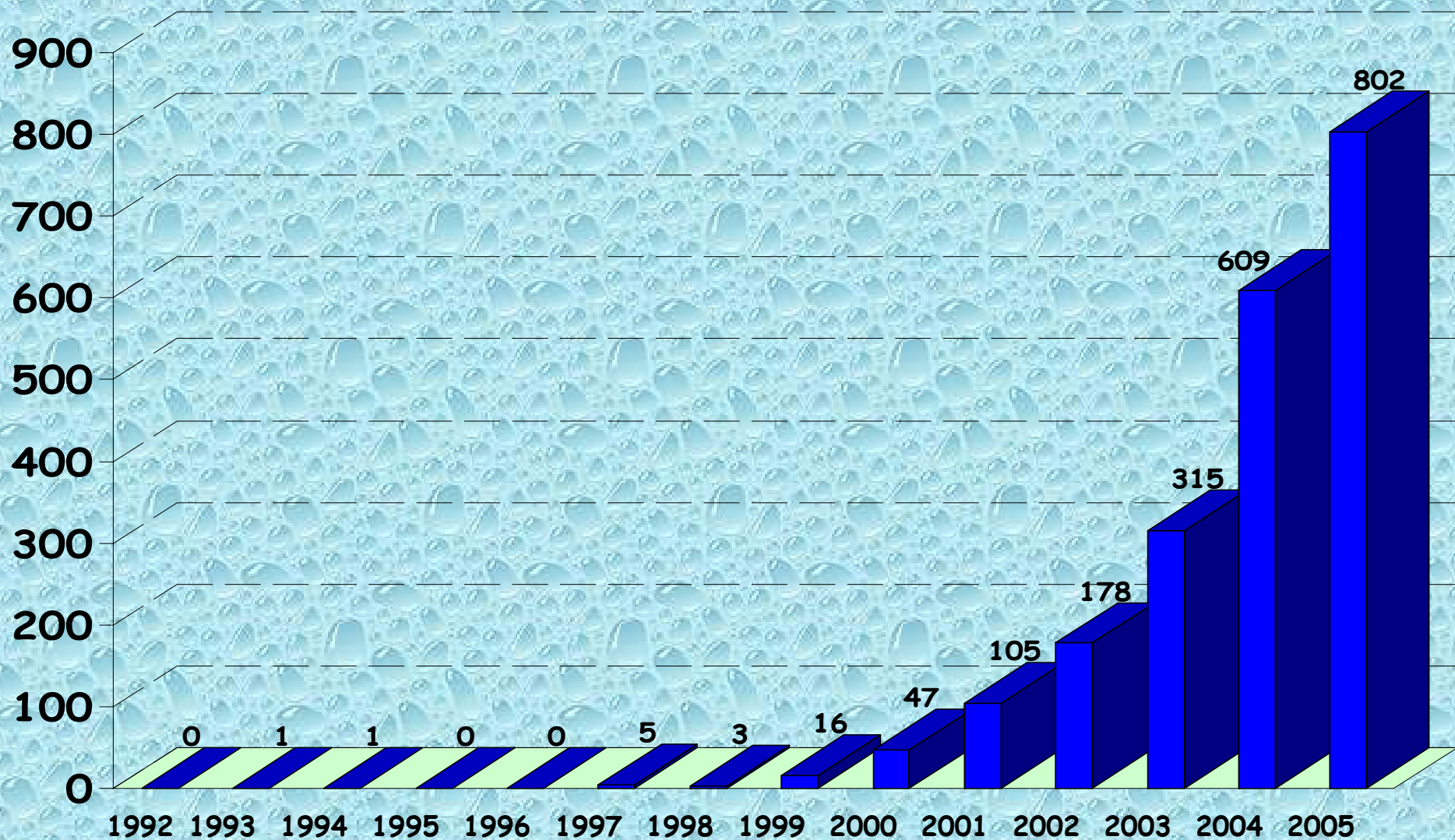
Microfluidics

"... microfluidics might just be too powerful a concept not to succeed eventually."

"... there's a frantic search for the one application that will force reluctant customers in academia, biotech, and big pharma to go whole hog on microfluidics."

John S. MacNeil, GenomeWeb News 5/14/03

Incidence of the word "microfluidic" in PubMed

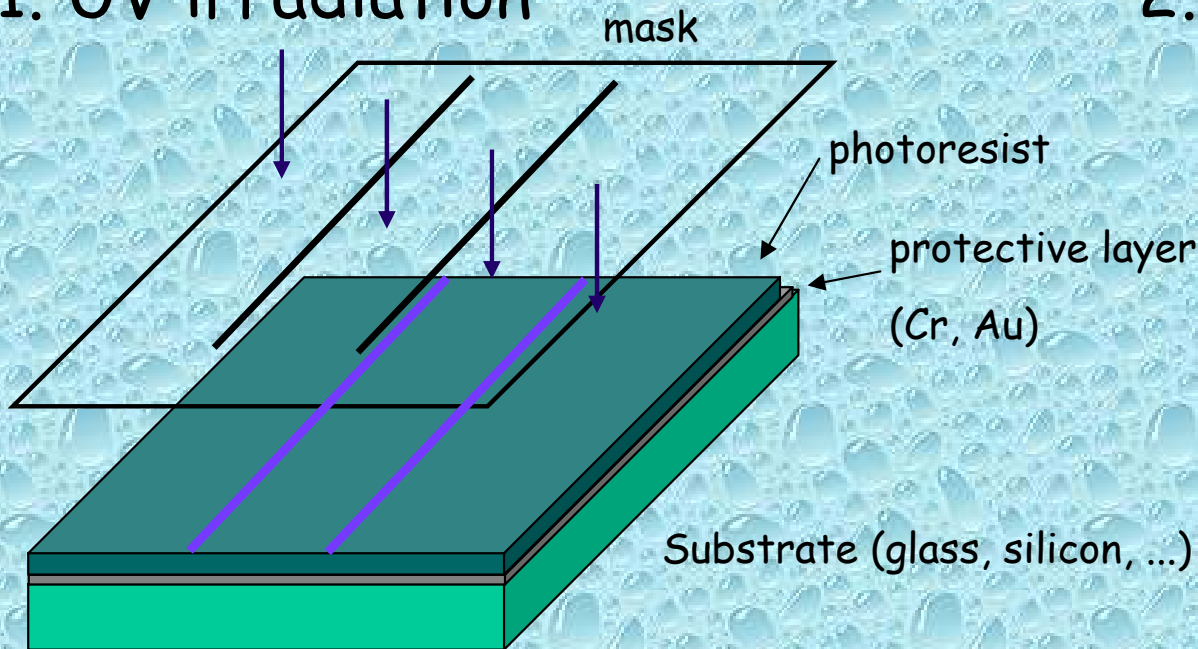


(as of April 4, 2006)

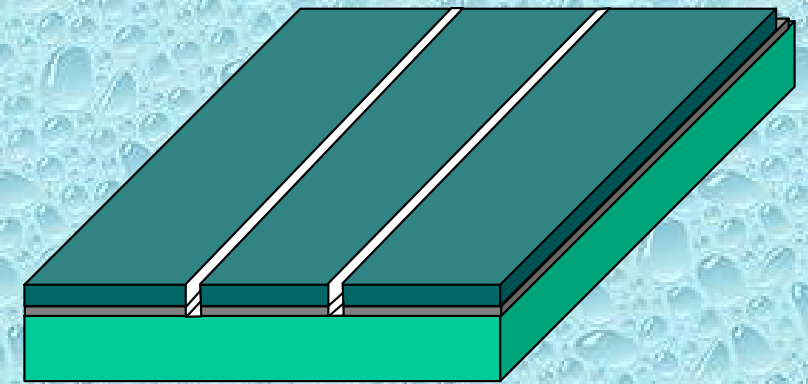
<http://www.ncbi.nlm.nih.gov/PubMed>

Microfabrication by Photolithography

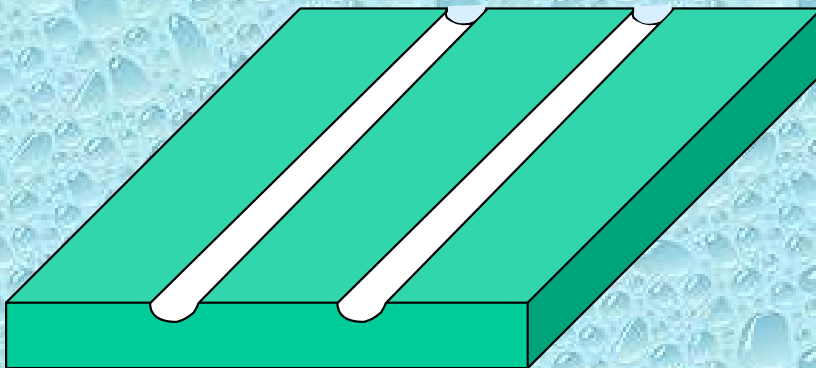
1. UV irradiation



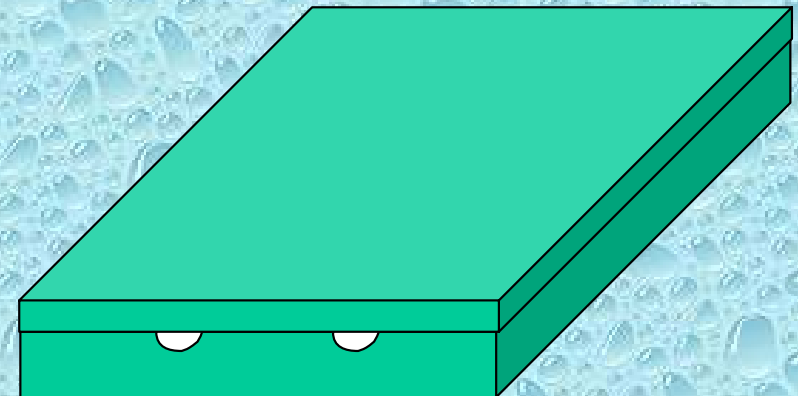
2. Development and etching



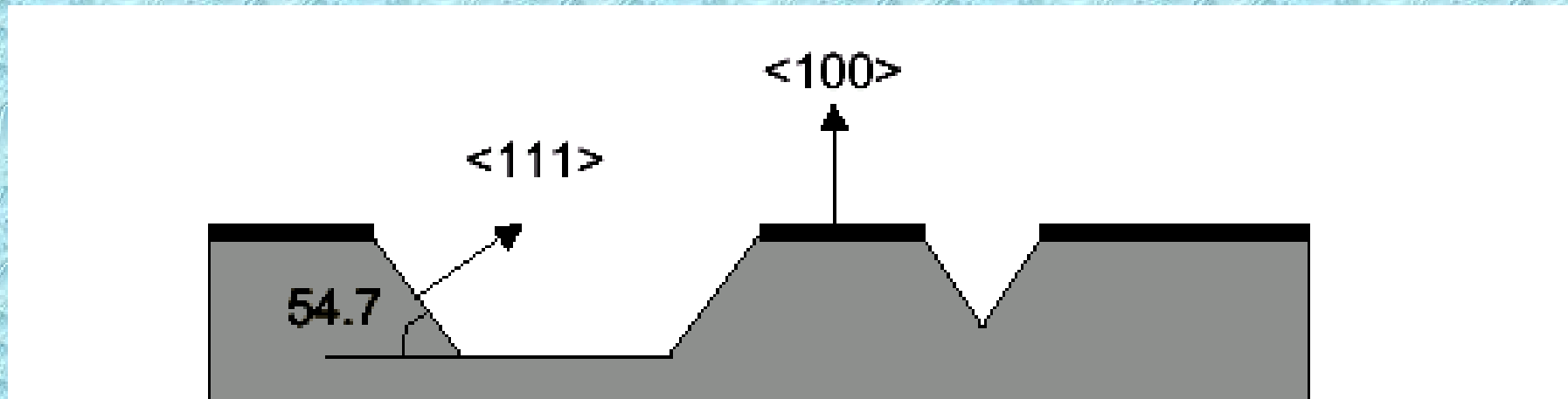
3. Protective layer removal



4. Cover bonding



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

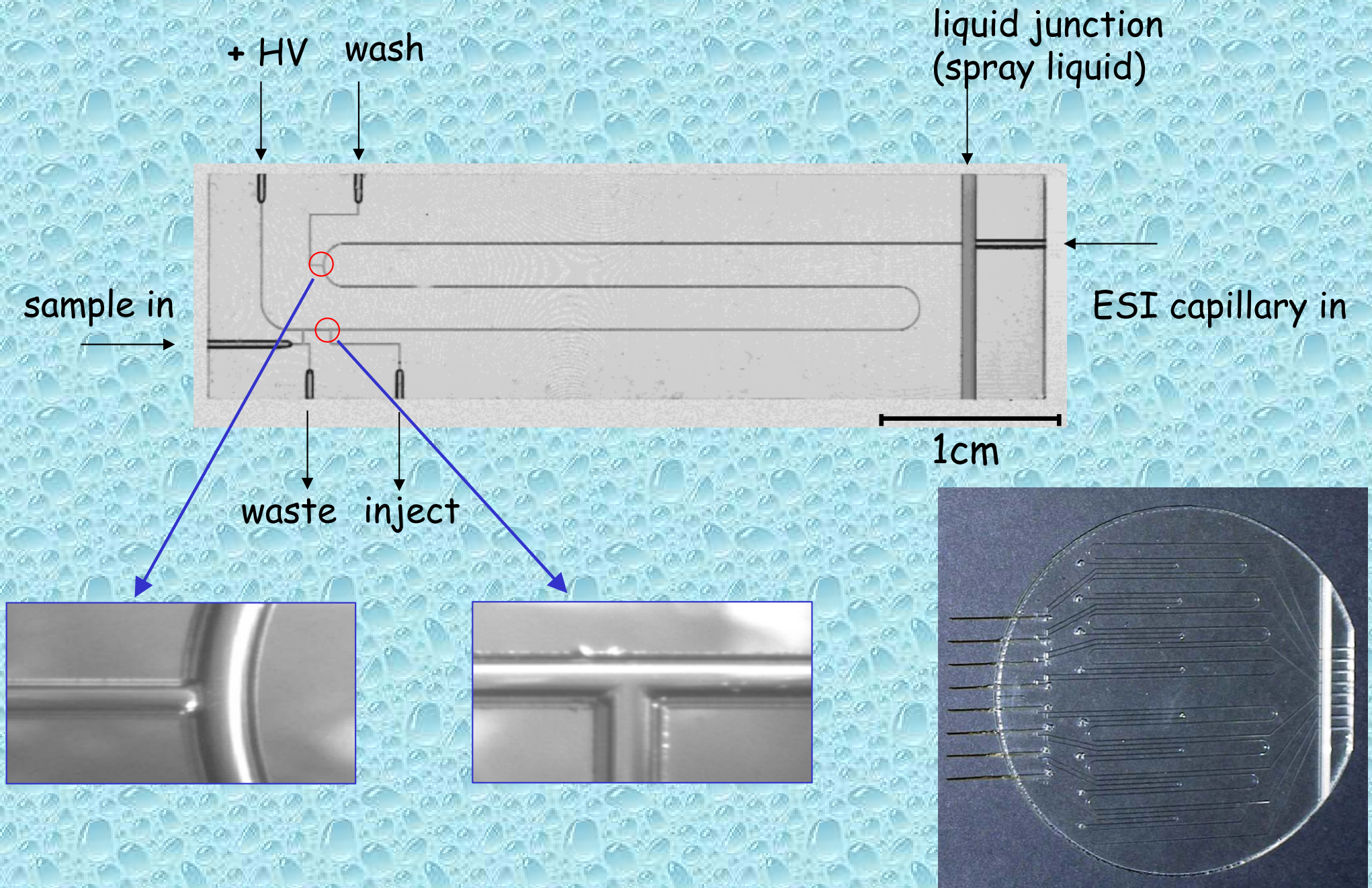
Injection molding - production scale

Casting - polymeric resins, PDMS

Plasma etching

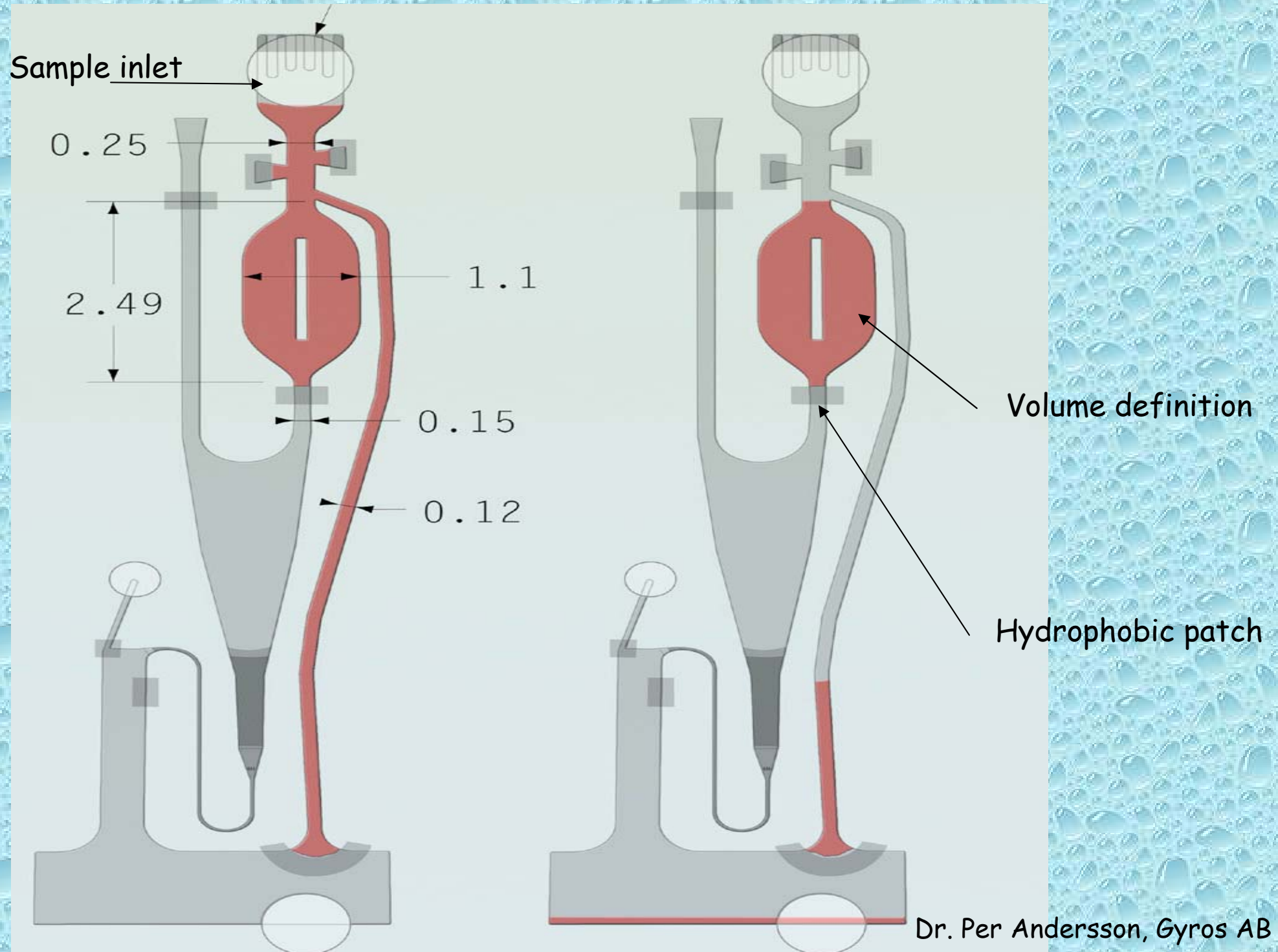
Laser machining

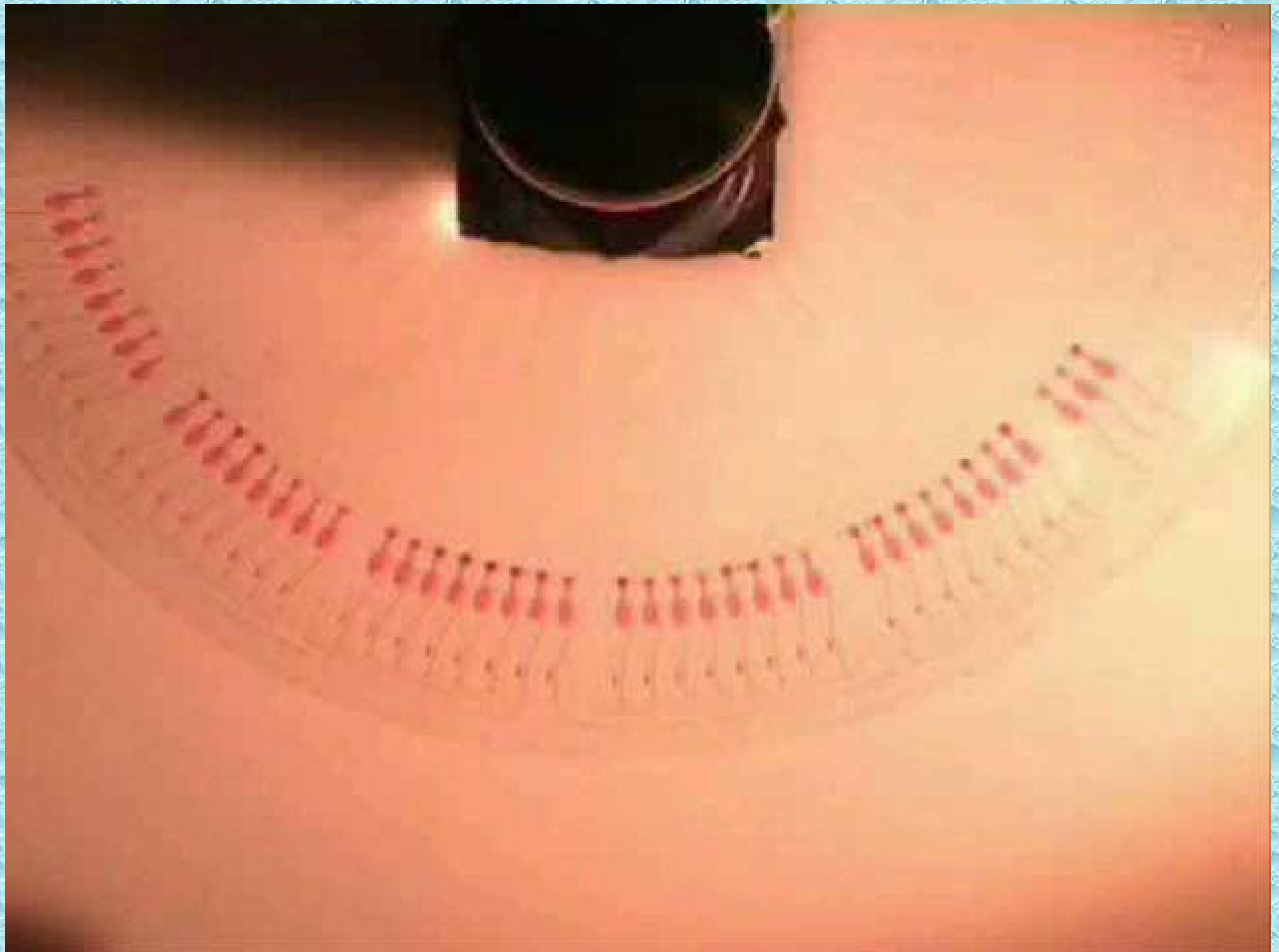
System integration



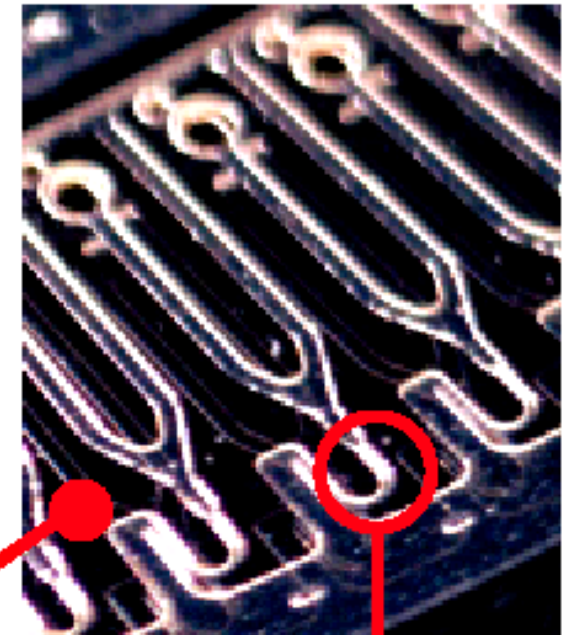
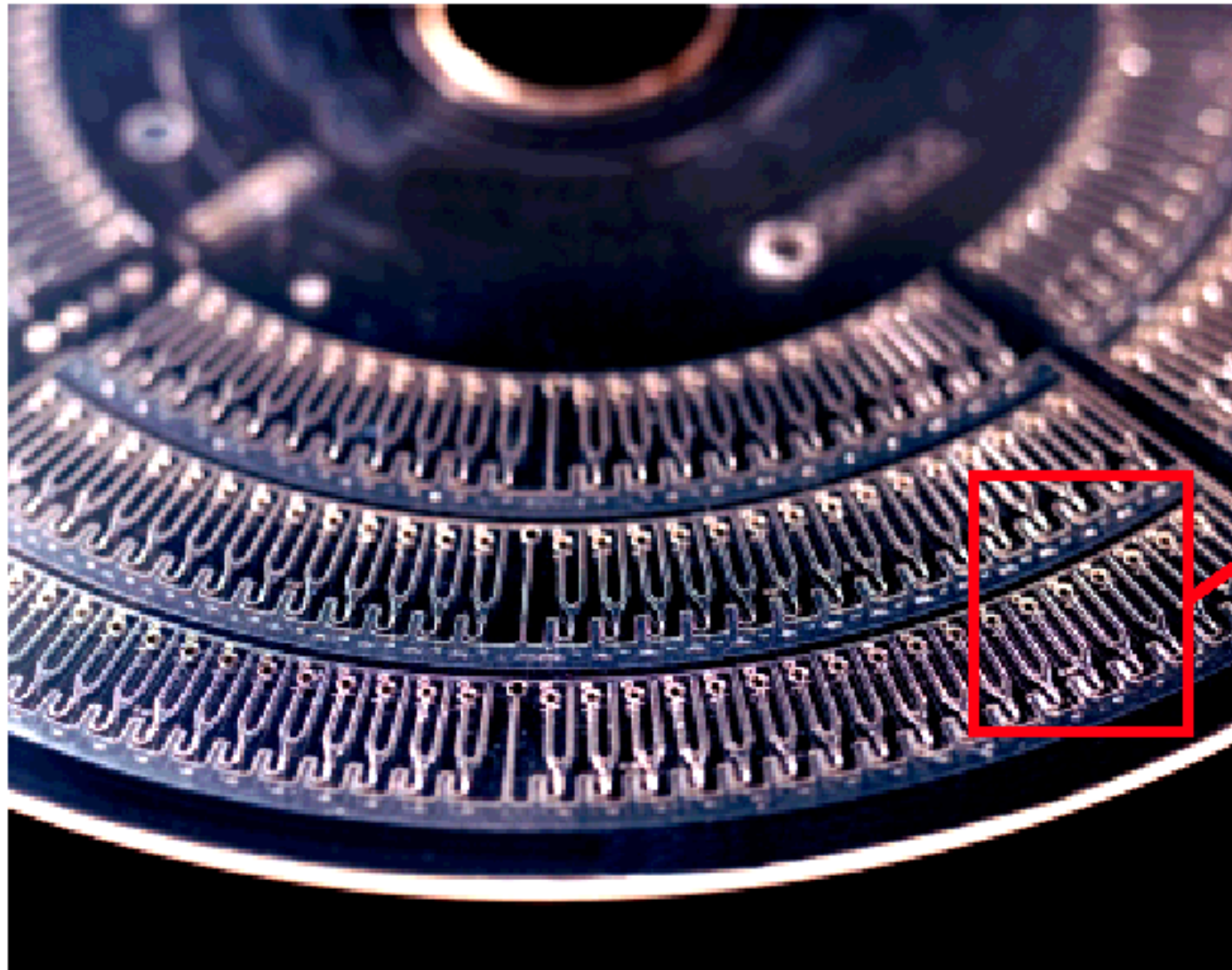


Volume metering in the nL range





The CD microdevice concept



A CD can contain 400 identical structures each with individual sample inlet, common distribution channel for reagents, reaction chamber and common waste channel. Columns can be packed reproducibly, in parallel within the CD. Volumes depend on the application, but 10-20 nl, RSD 2-4%, is a typical range.

Gyrolab Bioaffy

Detection
reagent

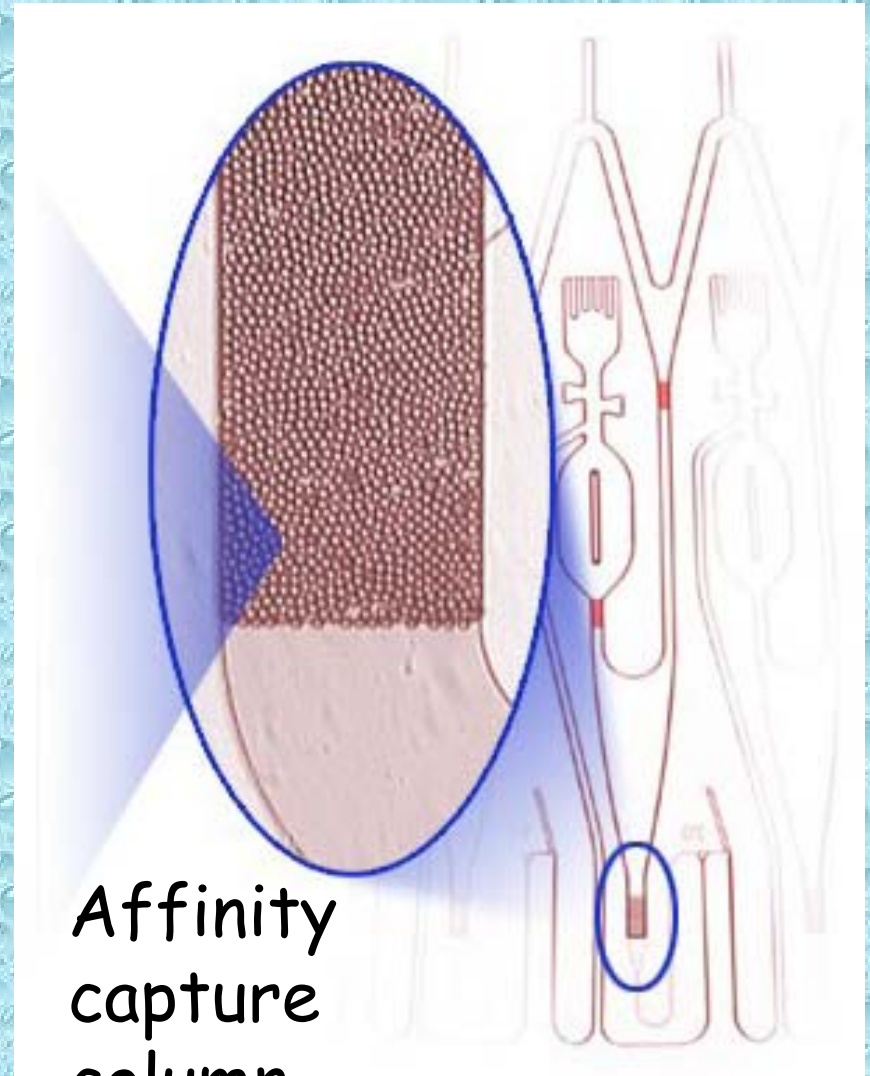
Protein

Biotinylated
capture
reagent

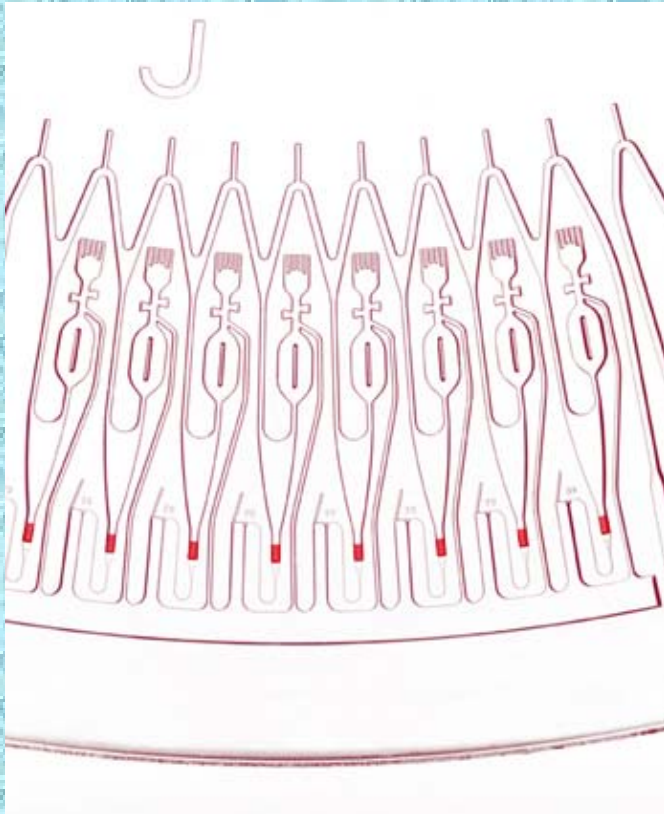
Streptavidin-
coated
particle



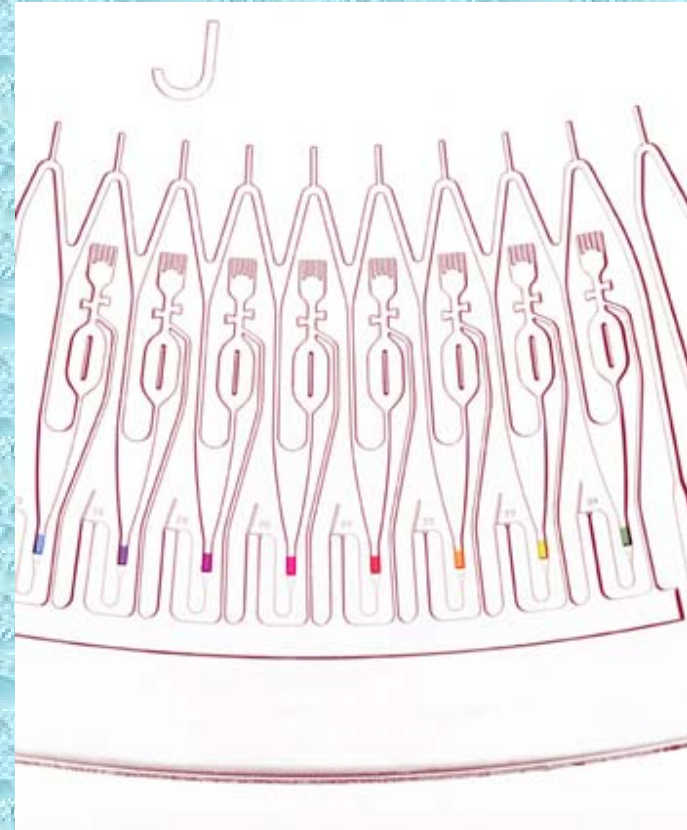
Affinity
capture
column



Open solution: Assay flexibility



Single protein
Many samples



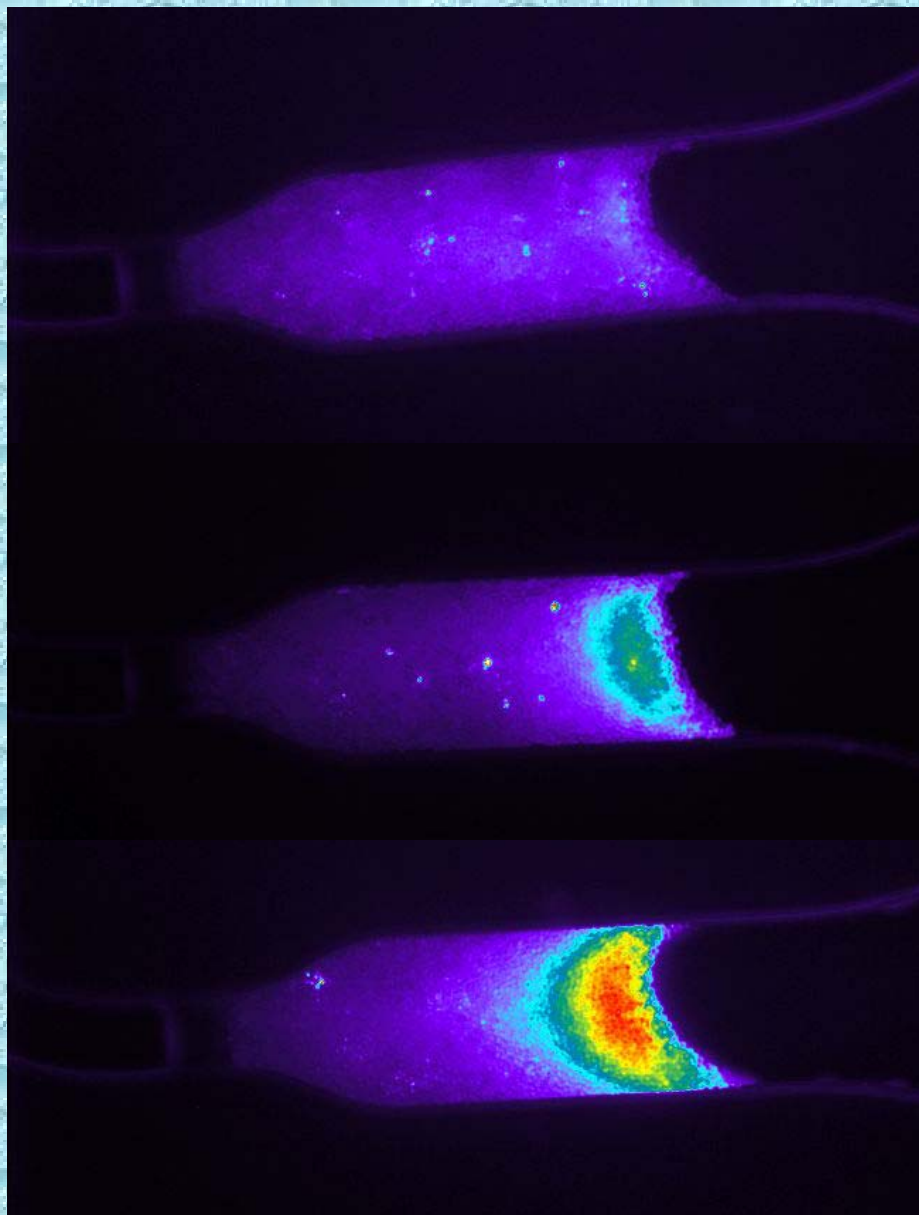
Different proteins
Few samples

Concentration effect and broad dynamic range

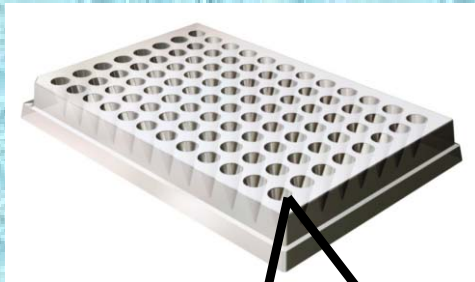
Column 1; 20 ng/mL

Column 2; 400 ng/mL

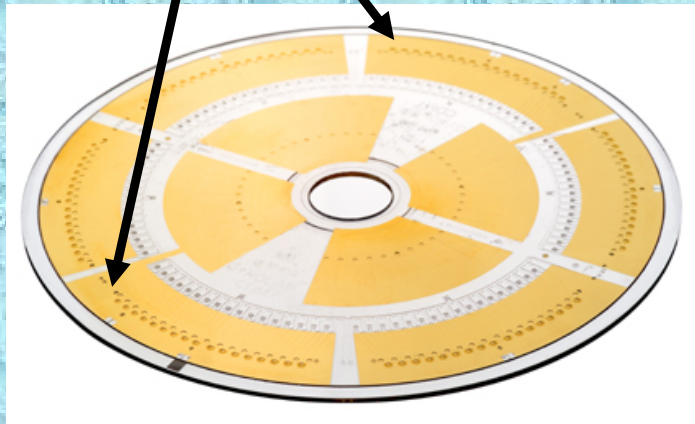
Column 3; 1600 ng/mL



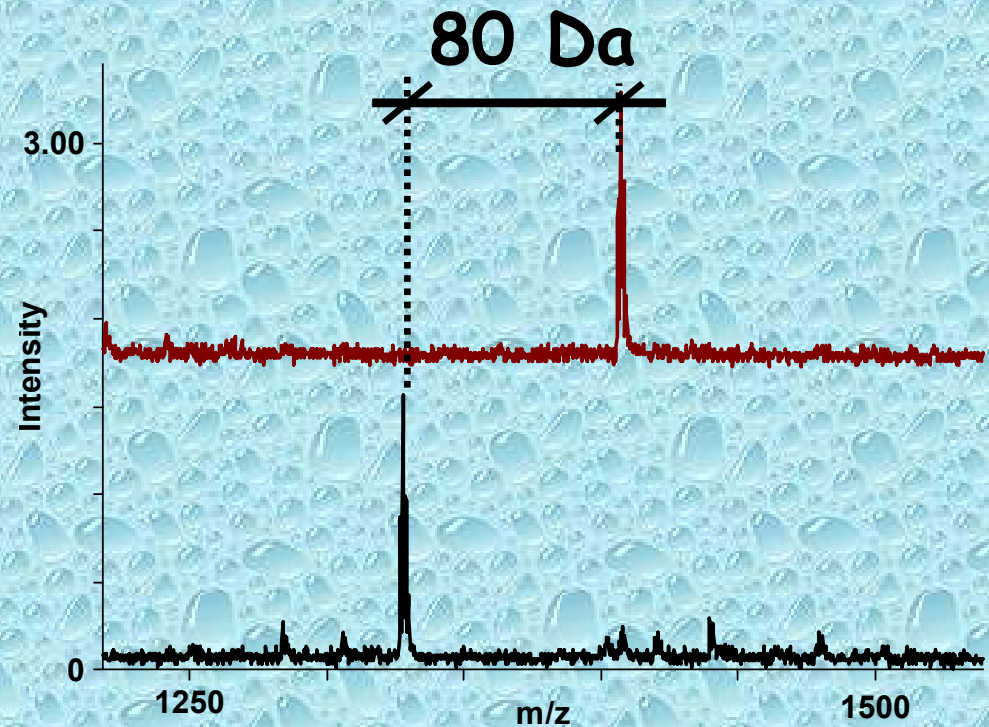
Phosphopeptide verification



Samples prepared in duplicate



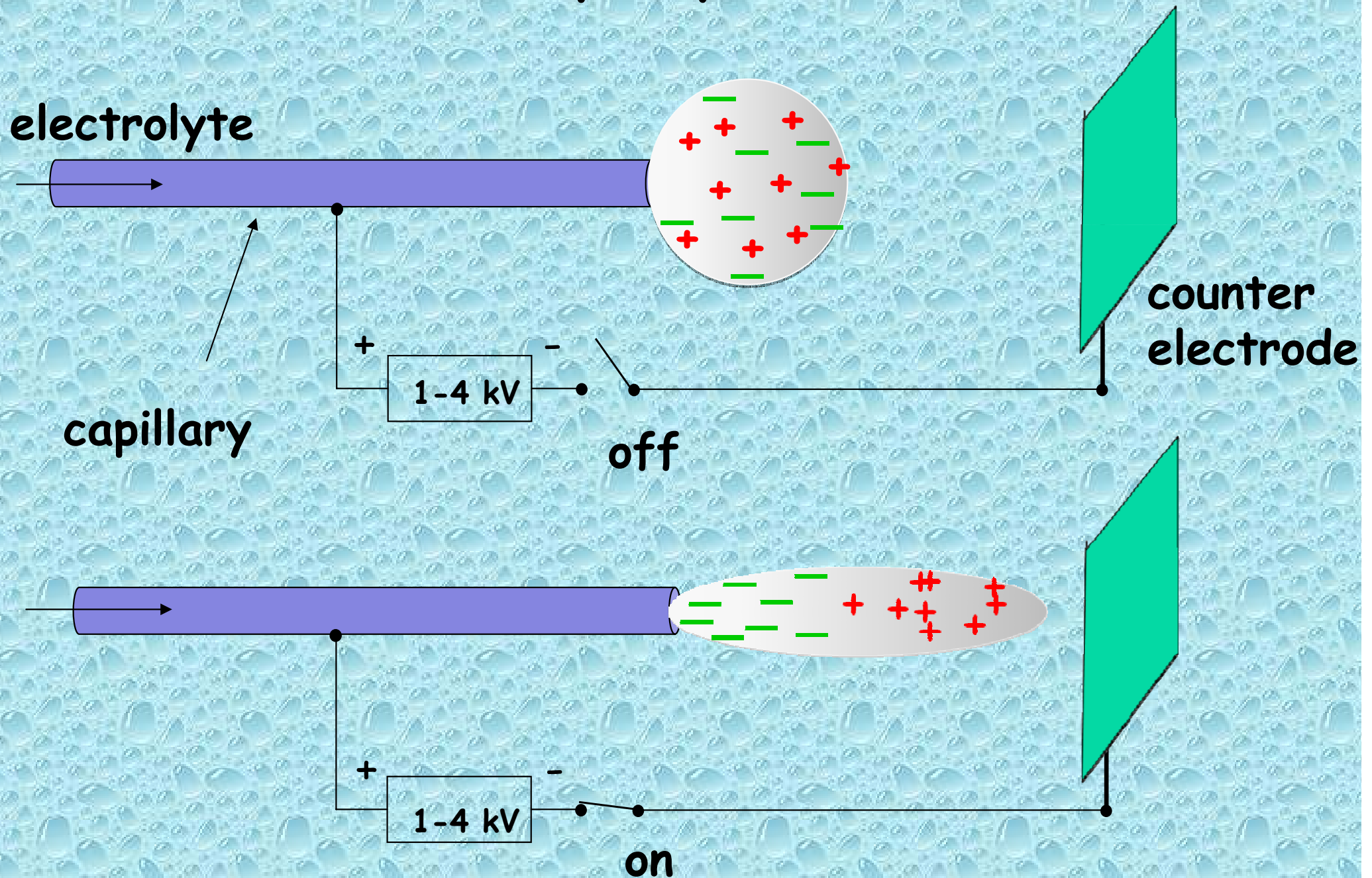
One set of the sample is IMAC enriched and eluted



One set of the sample is IMAC enriched, dephosphorylated and eluted

A mass difference of 80 Da (or multiples of 80 Da) verifies phosphopeptide presens

Electrospray ionization



Electrospray

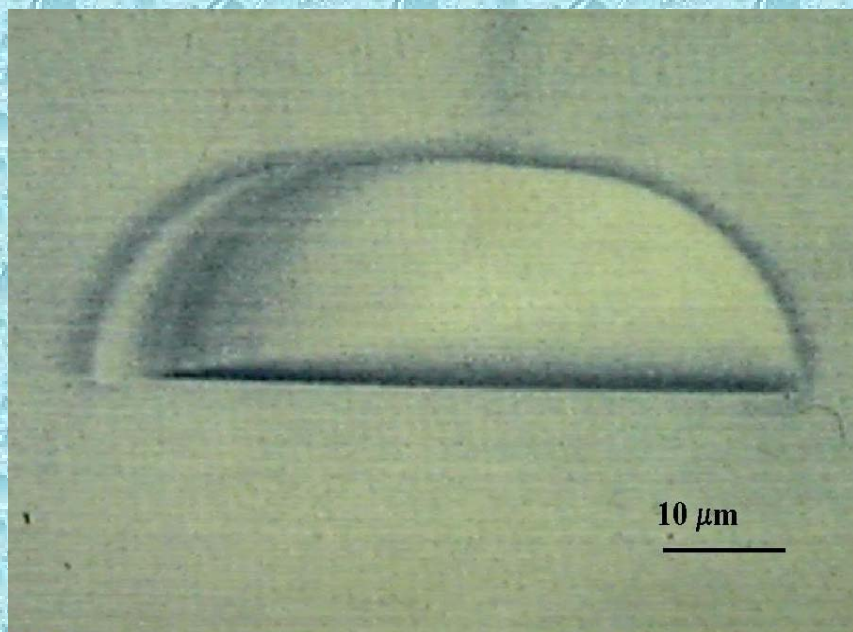
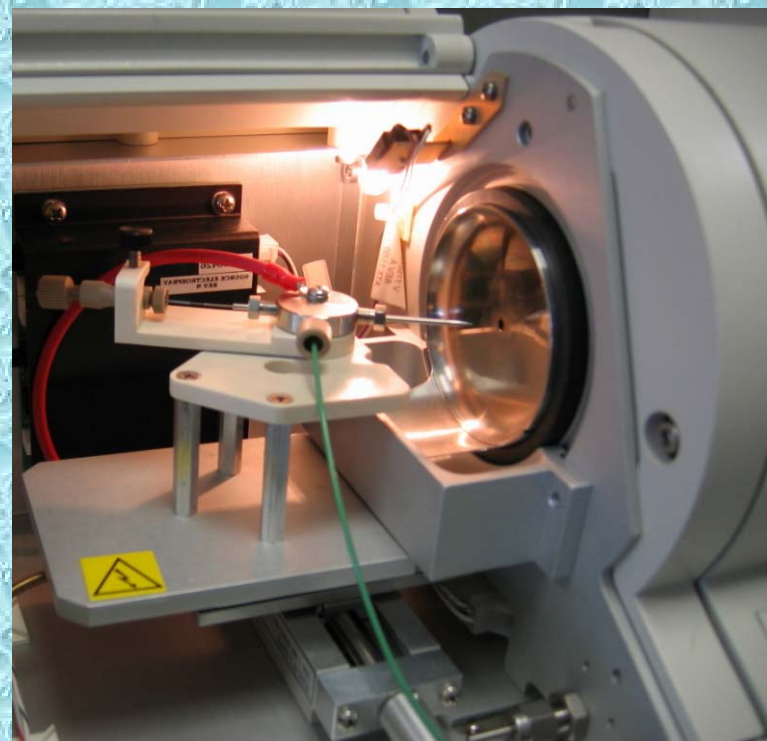
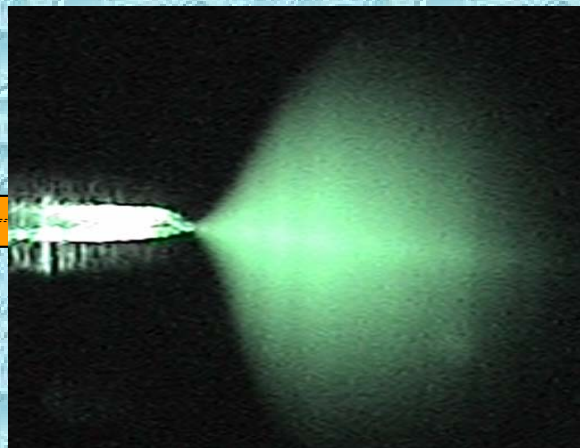


Electrospray - mass spectrometry coupling

3000 V



sample



Microfluidic channel



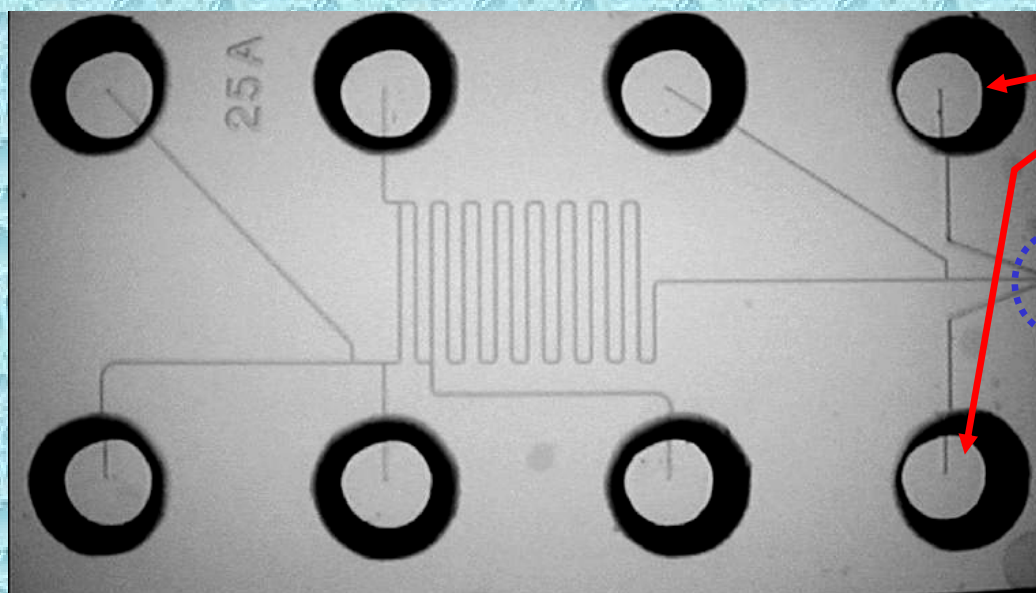
Integrated Nebulizer

sample

spray fluid

nebulizer gas

ESI exit

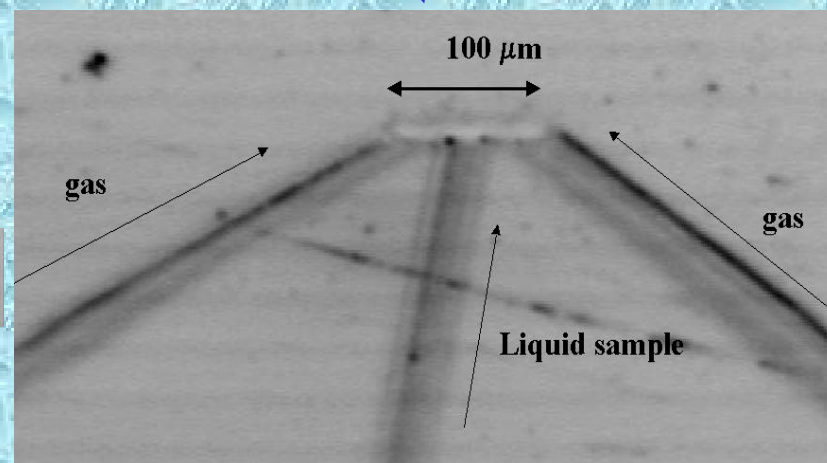


BGE

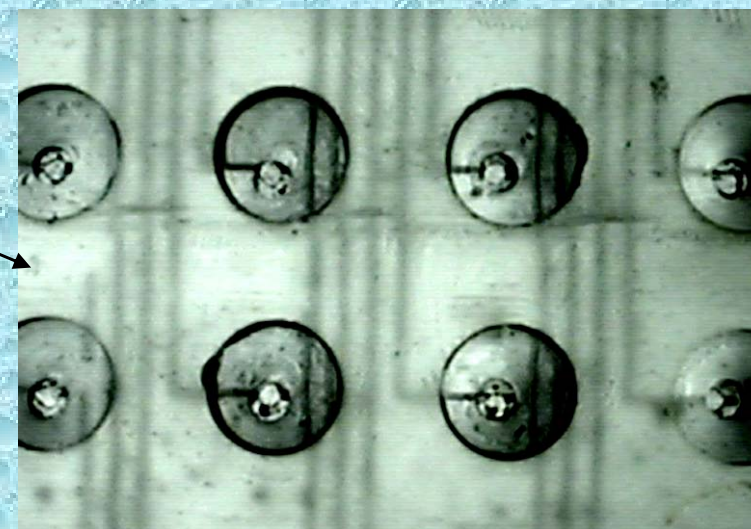
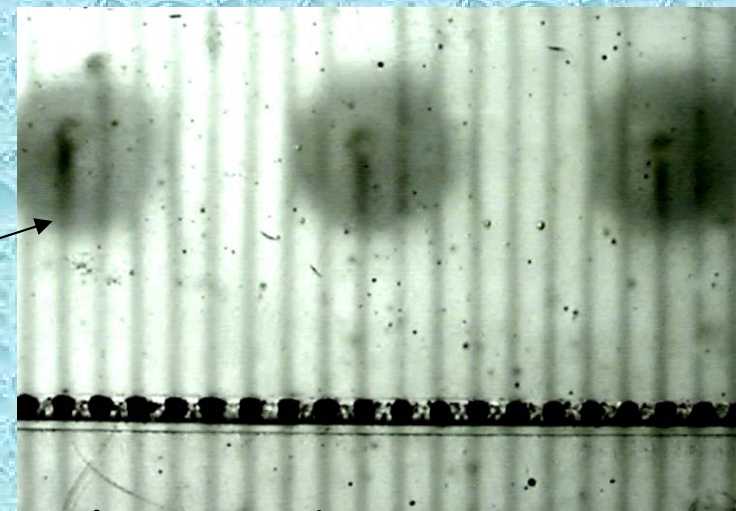
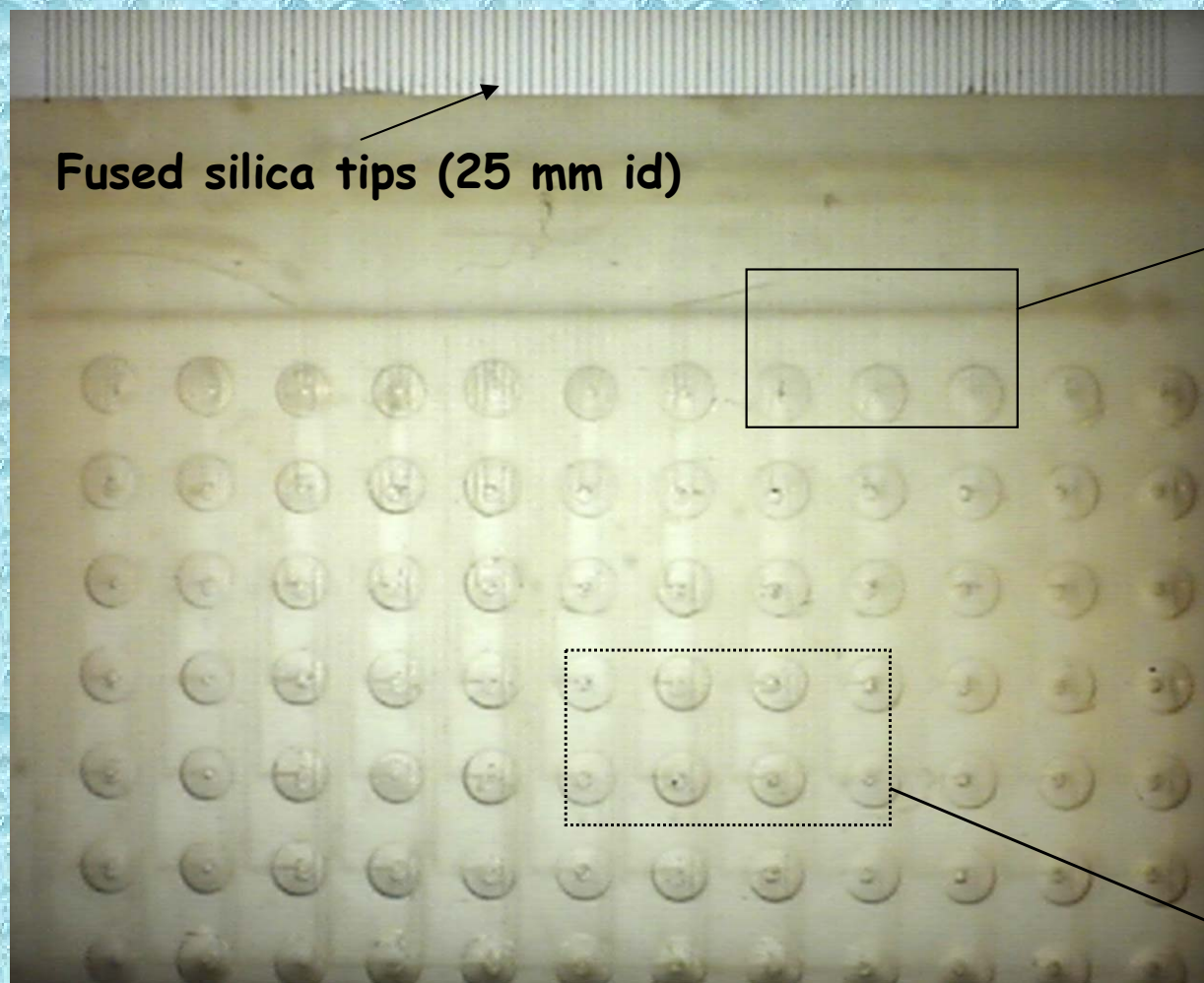
waste



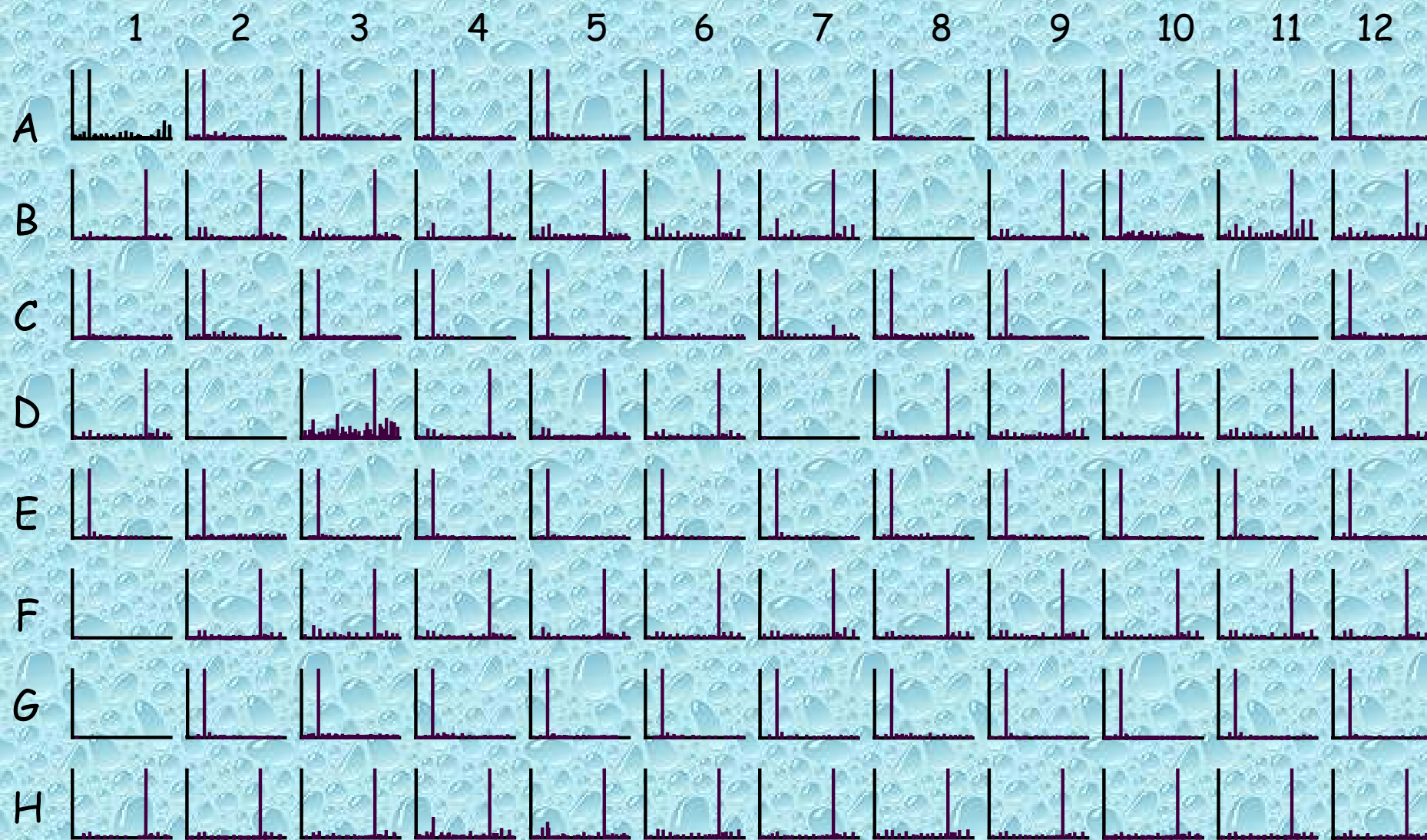
10 mm



96 tip plastic microdevice



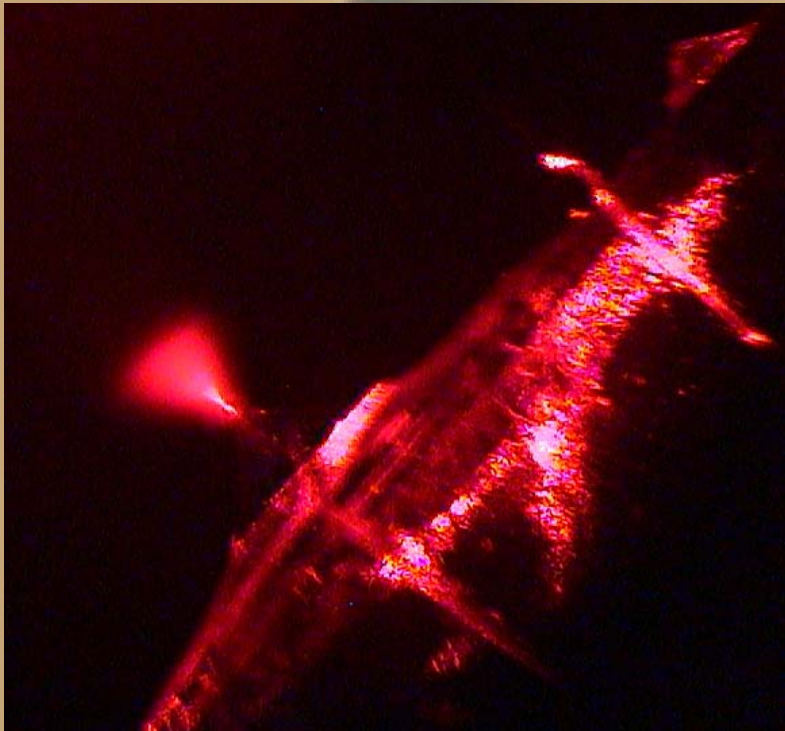
High Throughput Analysis with 96 ESI Tips Microdevice



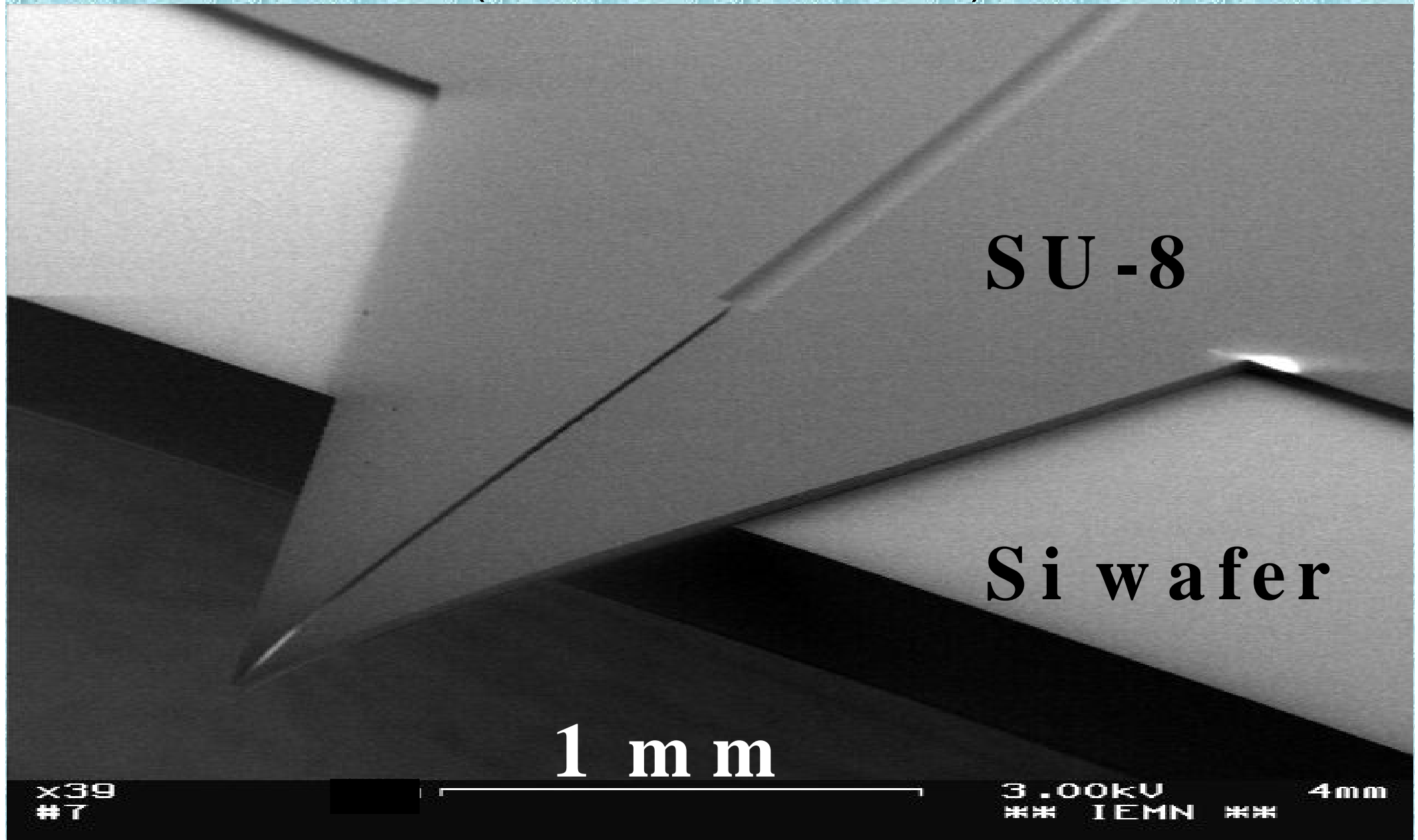
Total analysis time = 480 s

Microchannel patterned with gold

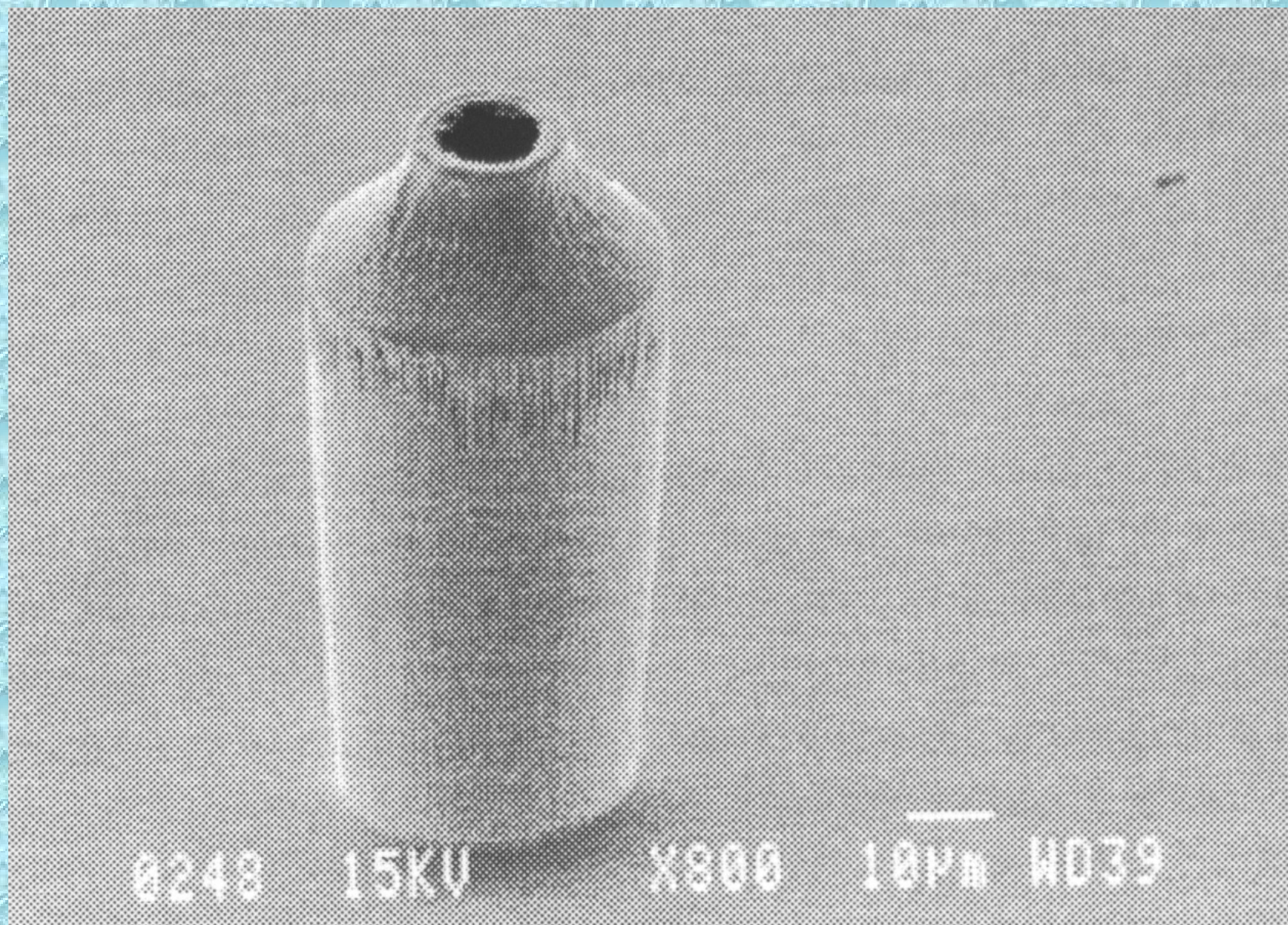
100 μm



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

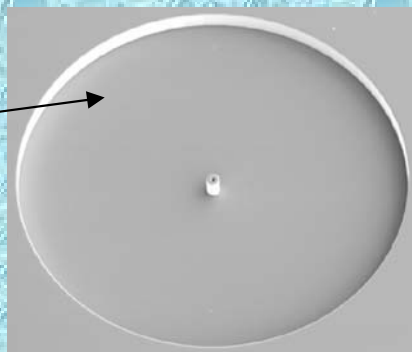
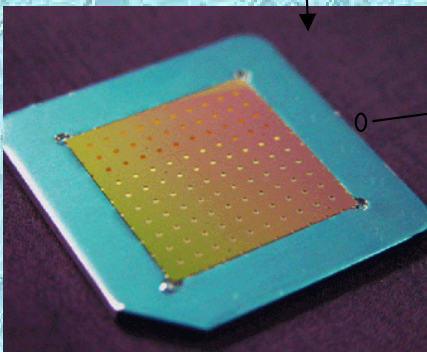
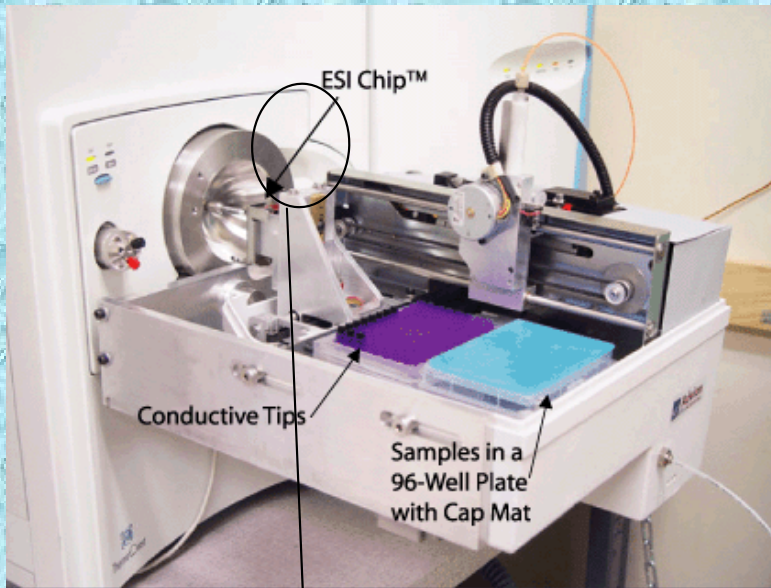


ESI tips produced by DRIE in silicon



Commercial products

DRIE in silicone



www.advion.com

Molded plastics

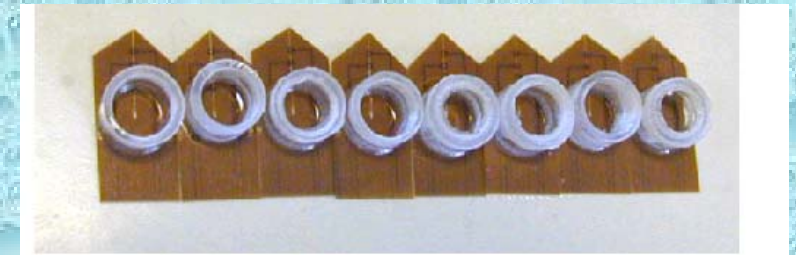


www.phoenix-st.com



www.agilent.com

Plasma etched in polyimide



www.diagnoswiss.com

ESI - concentration sensitive

(10 nL/min or 10 μ L/min - similar sensitivity)

Charge competition
Different proton affinity
in the gas phase

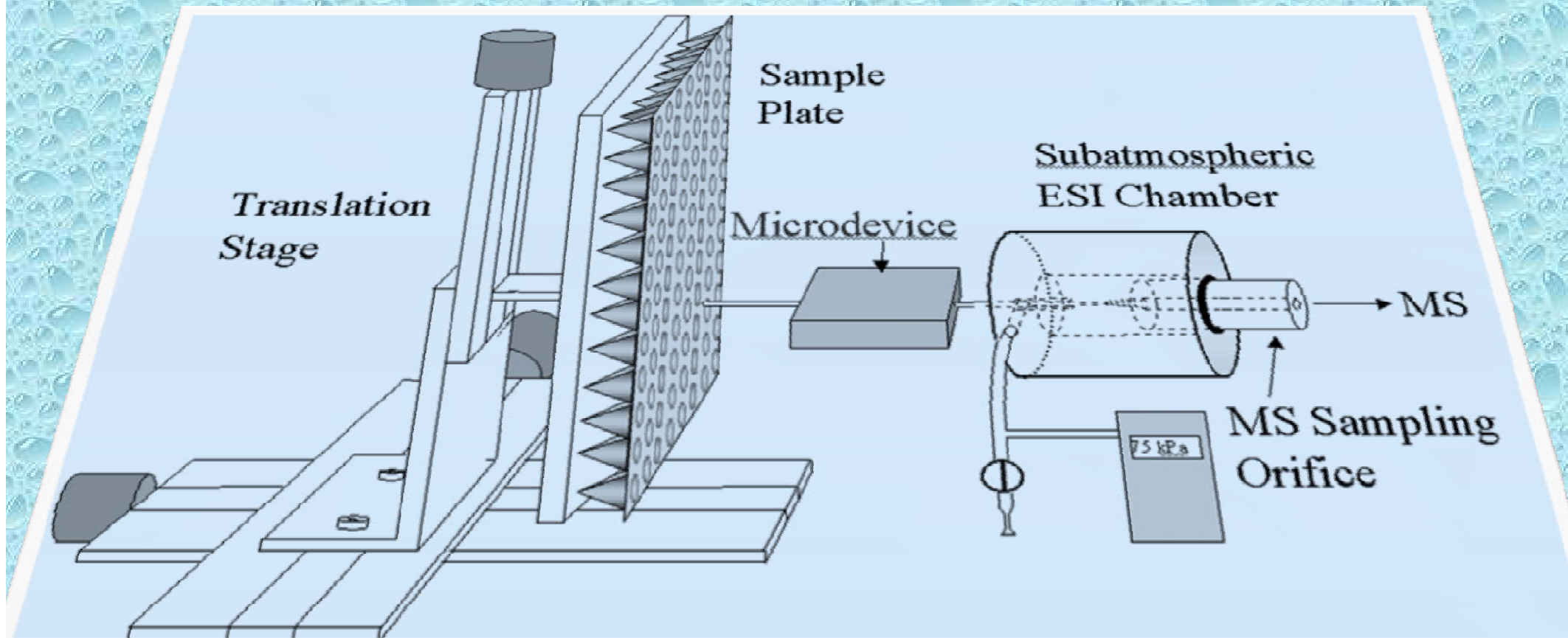


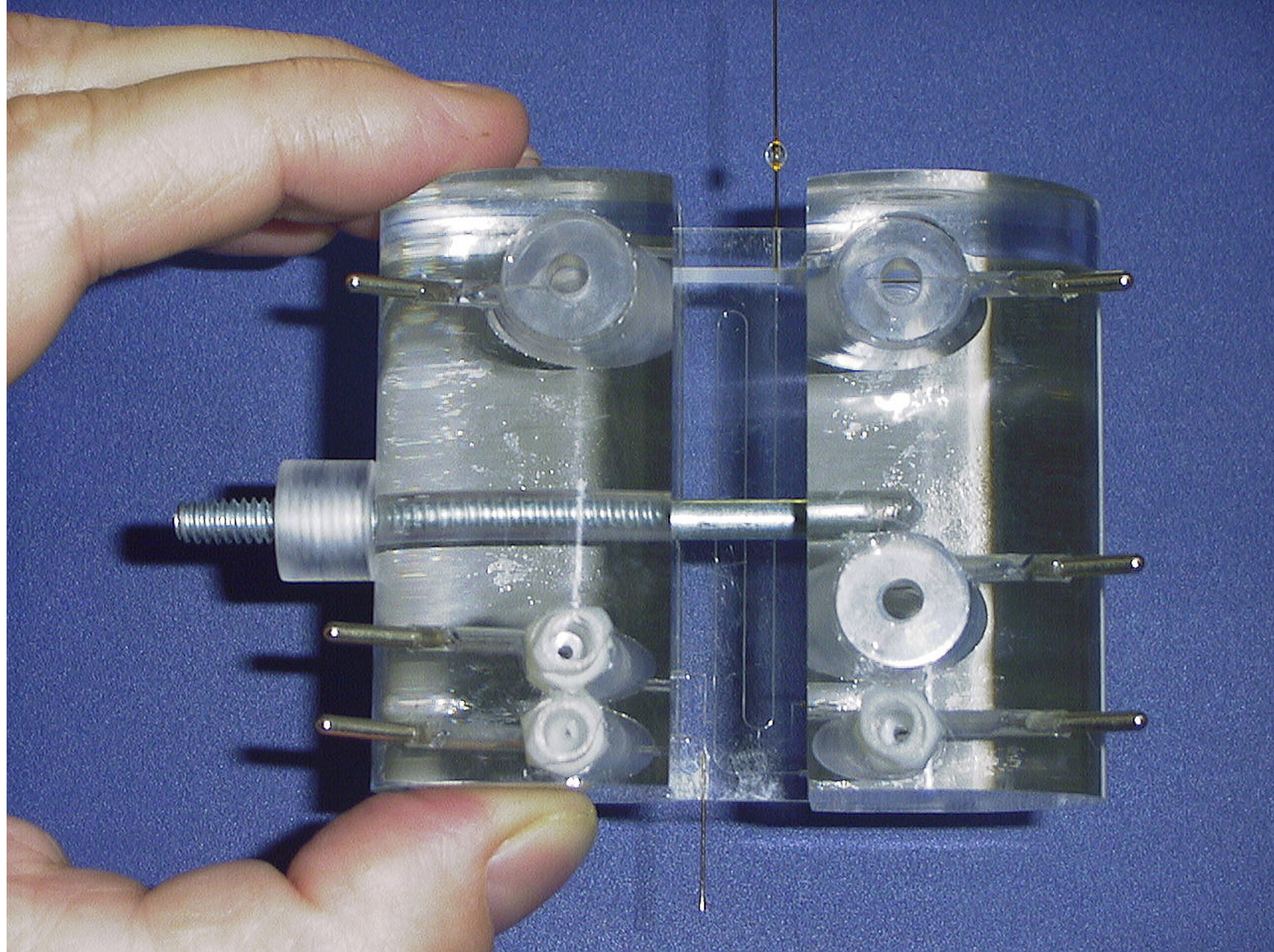
Signal suppression



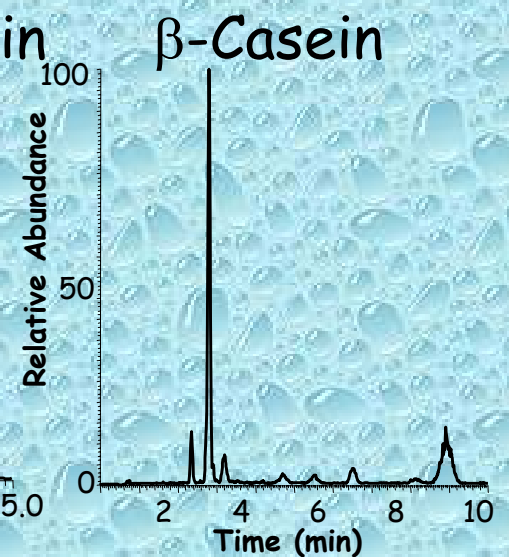
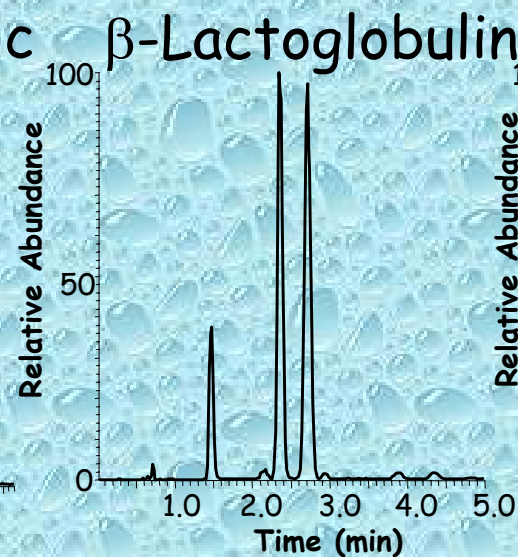
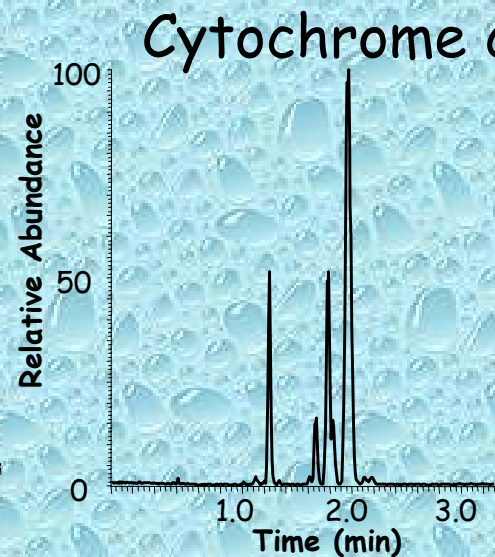
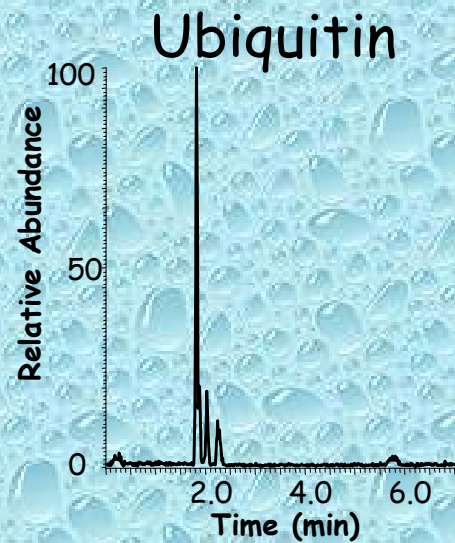
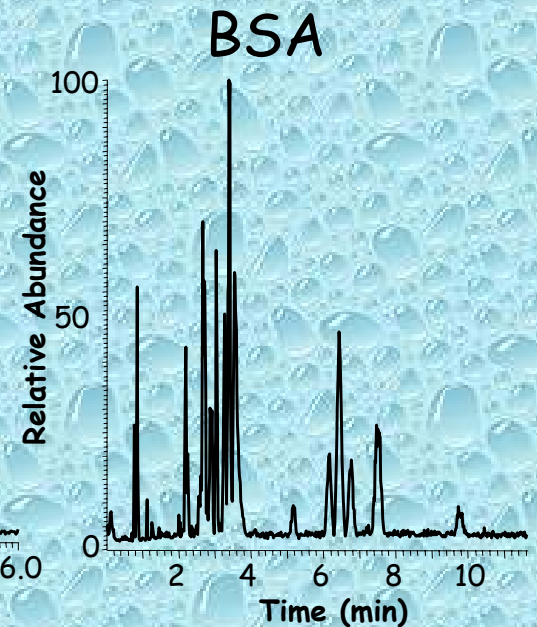
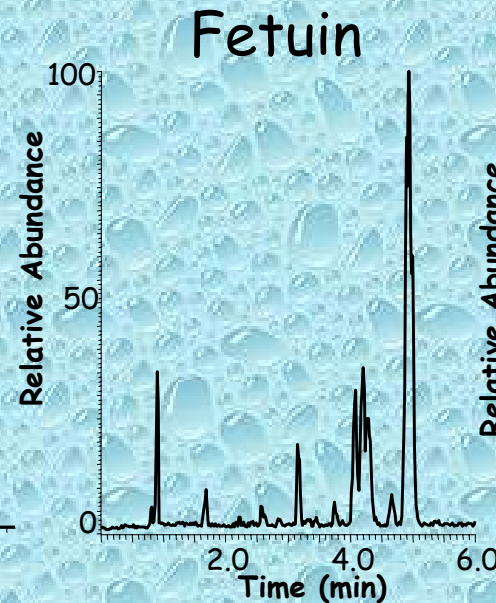
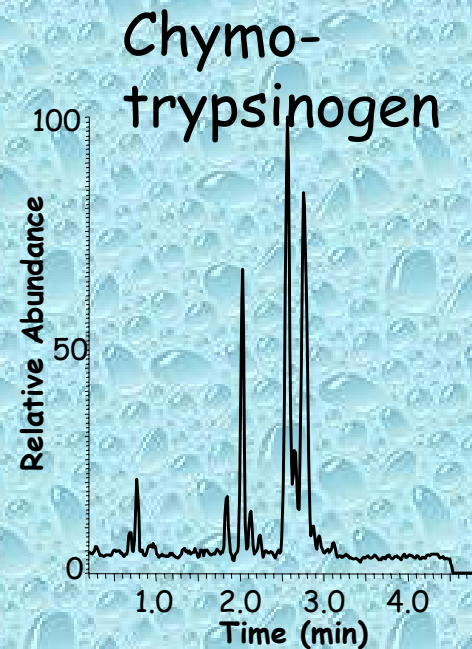
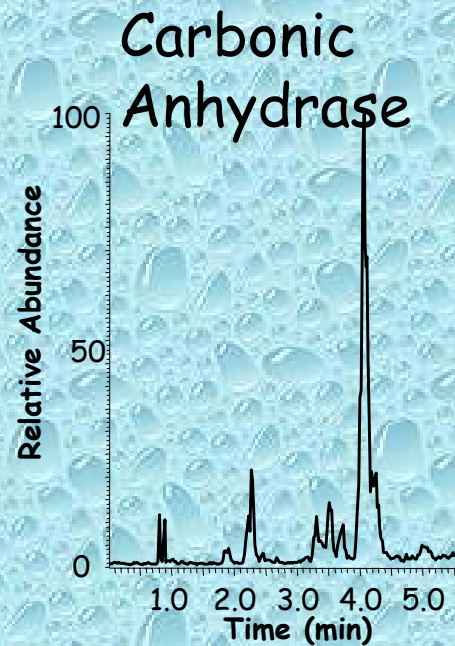
SEPARATION

External ESI interface

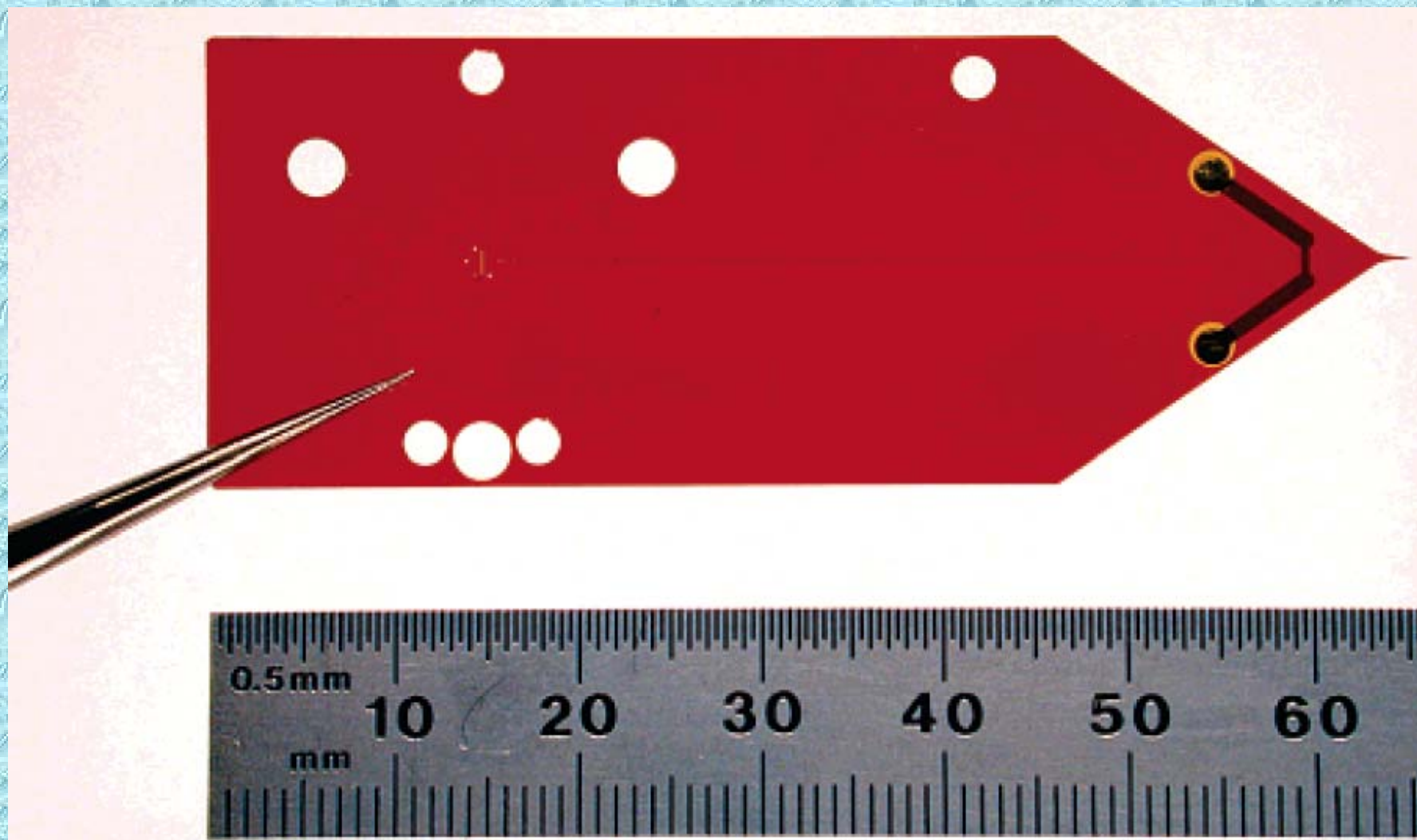




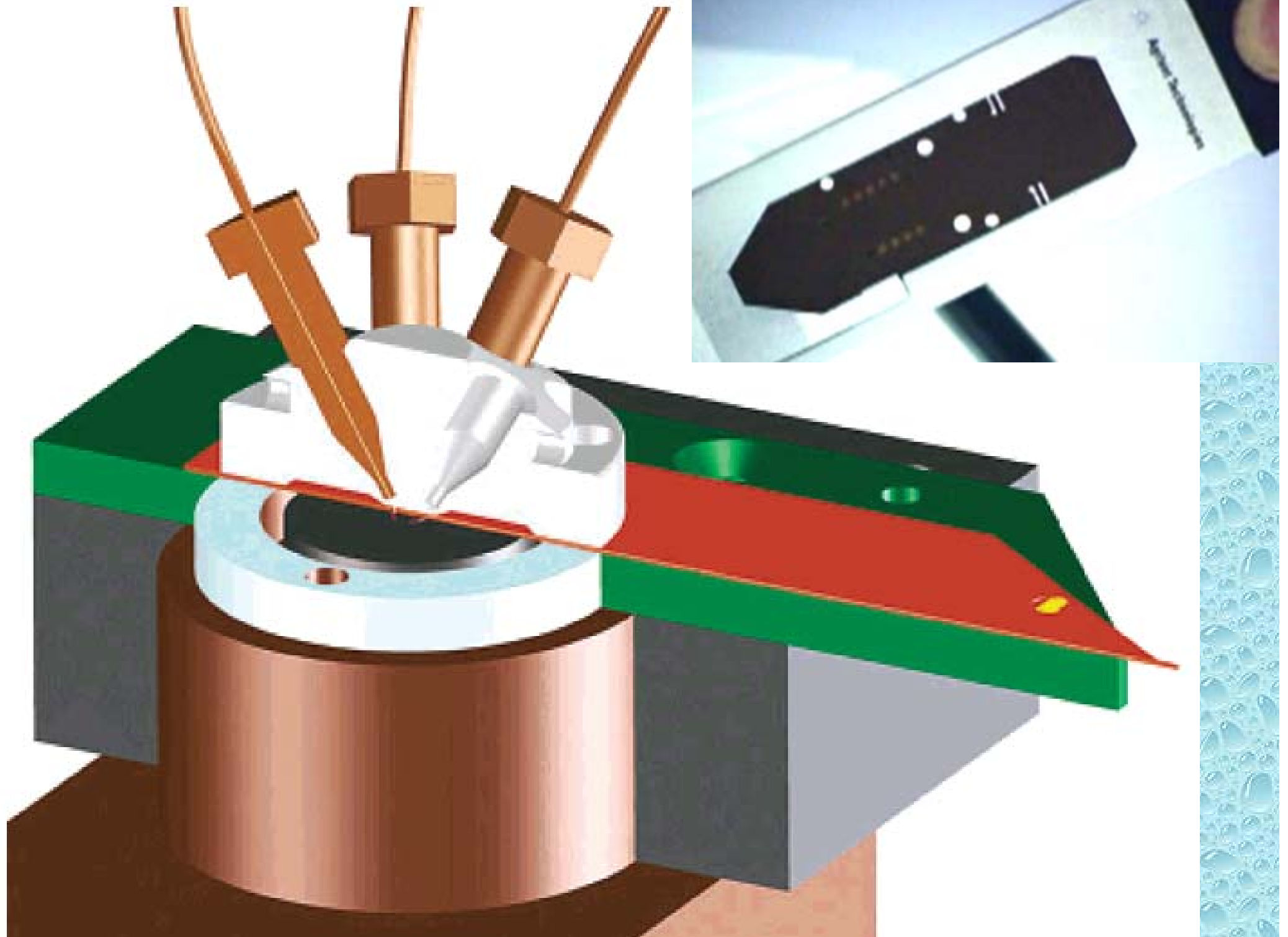
PROTEIN TRYPTIC DIGESTS

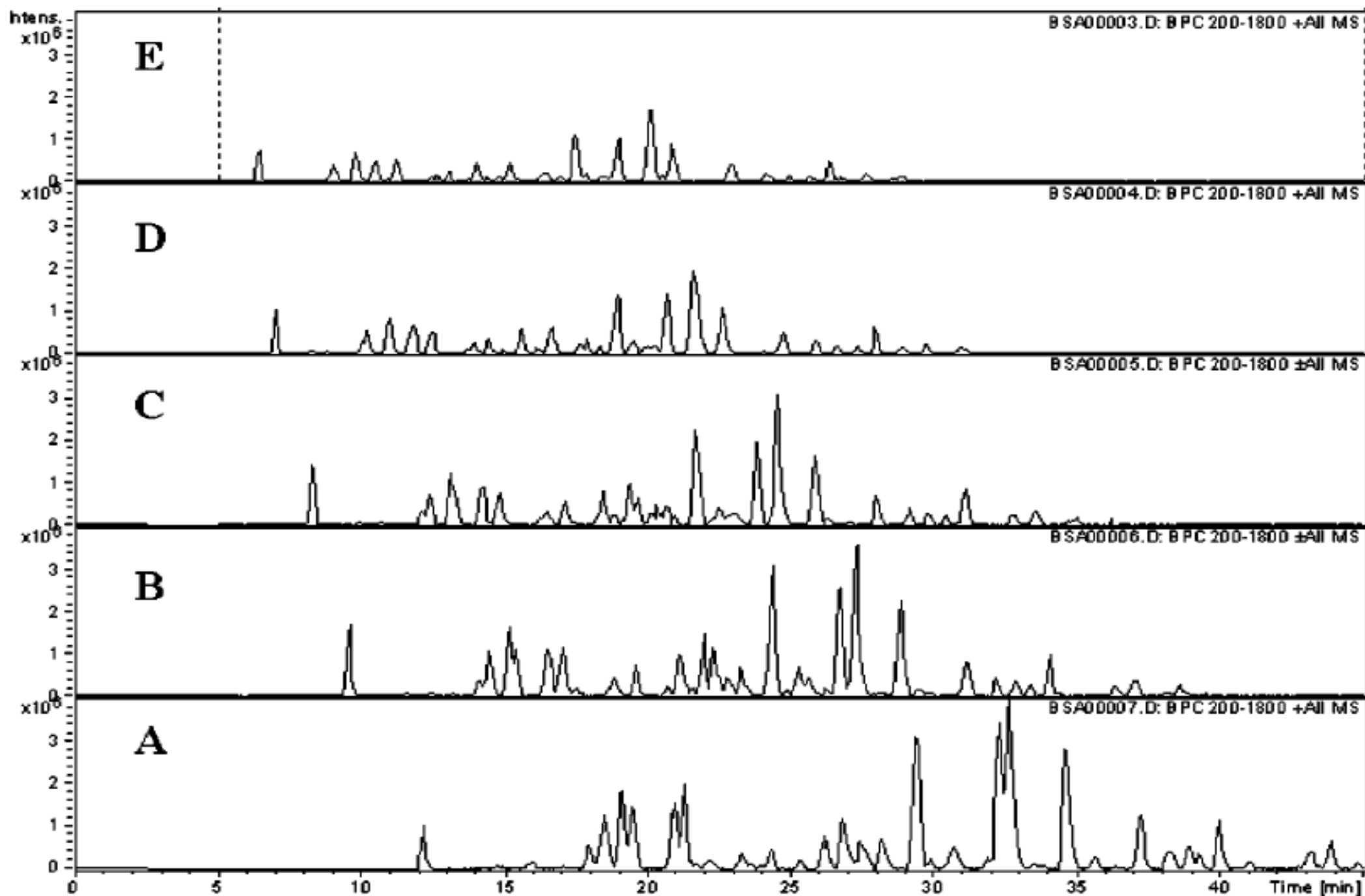


Microfluidic Chip for Peptide Analysis with an Integrated HPLC Column, Sample Enrichment Column and Nanoelectrospray Tip



van de Goor et al. Anal. Chem. 2005, 77, 527-533

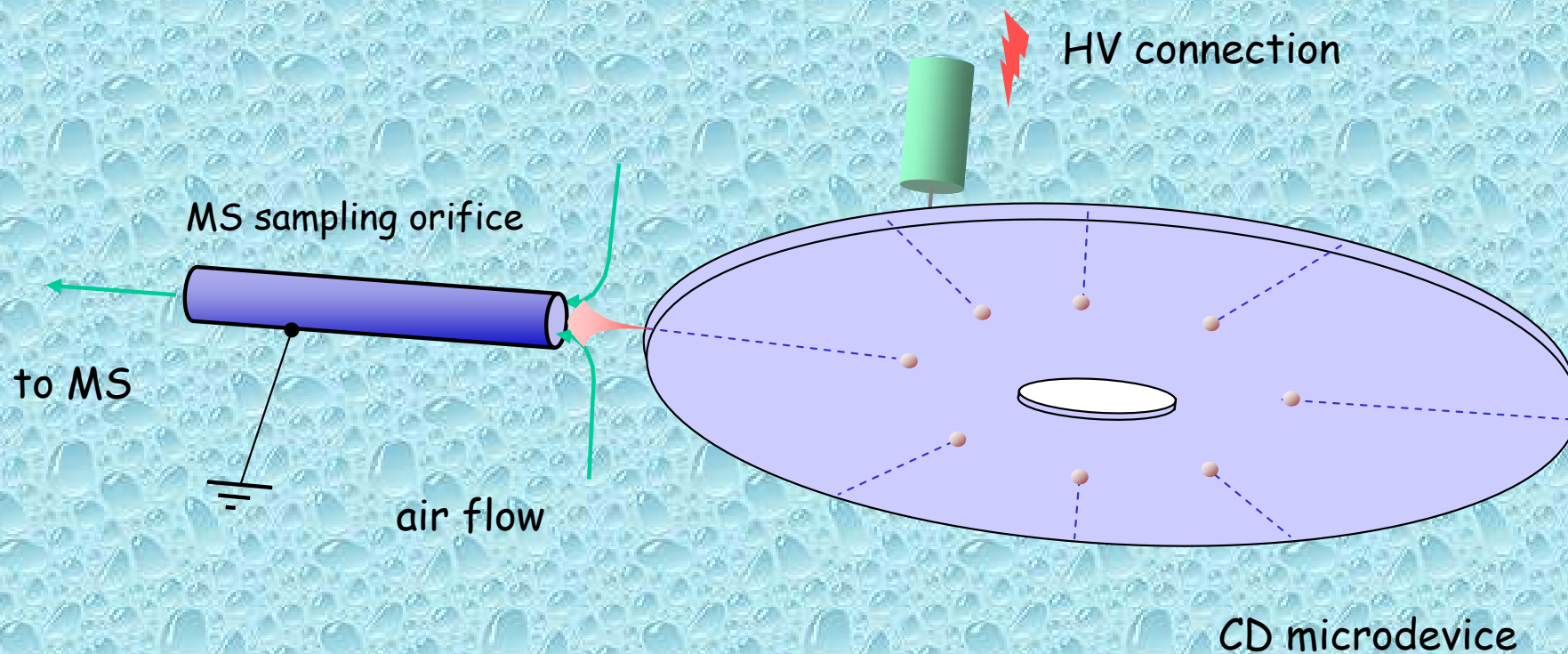


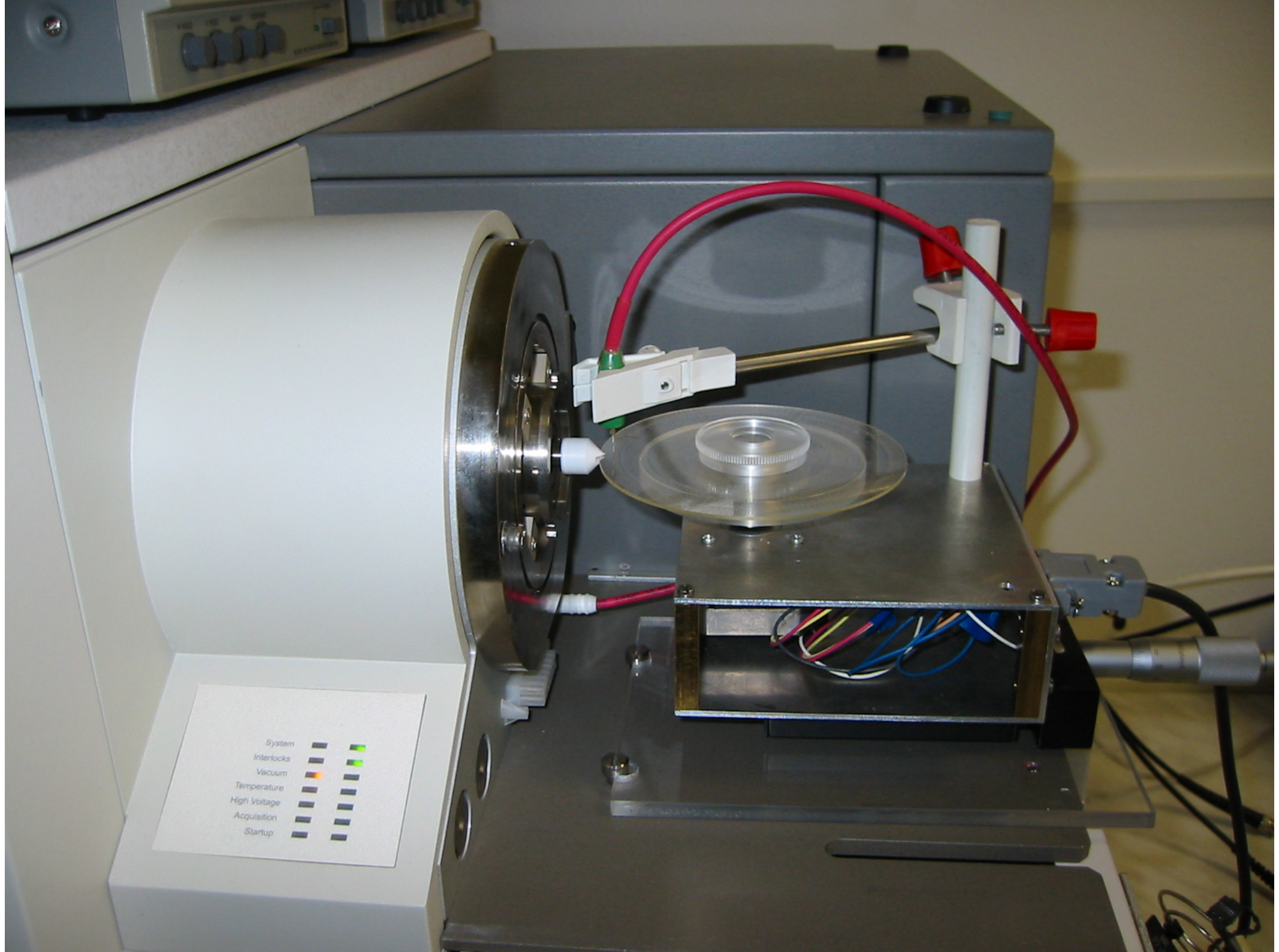


Base peak chromatograms of BSA digest running under different LC flow rate: (A) 100, (B) 150, (C) 200, (D) 300, and (E) 400 nL/min.

External nebulizer

- * vacuum suction
- * flow control





System ☐ ☒

Interlocks ☐ ☒

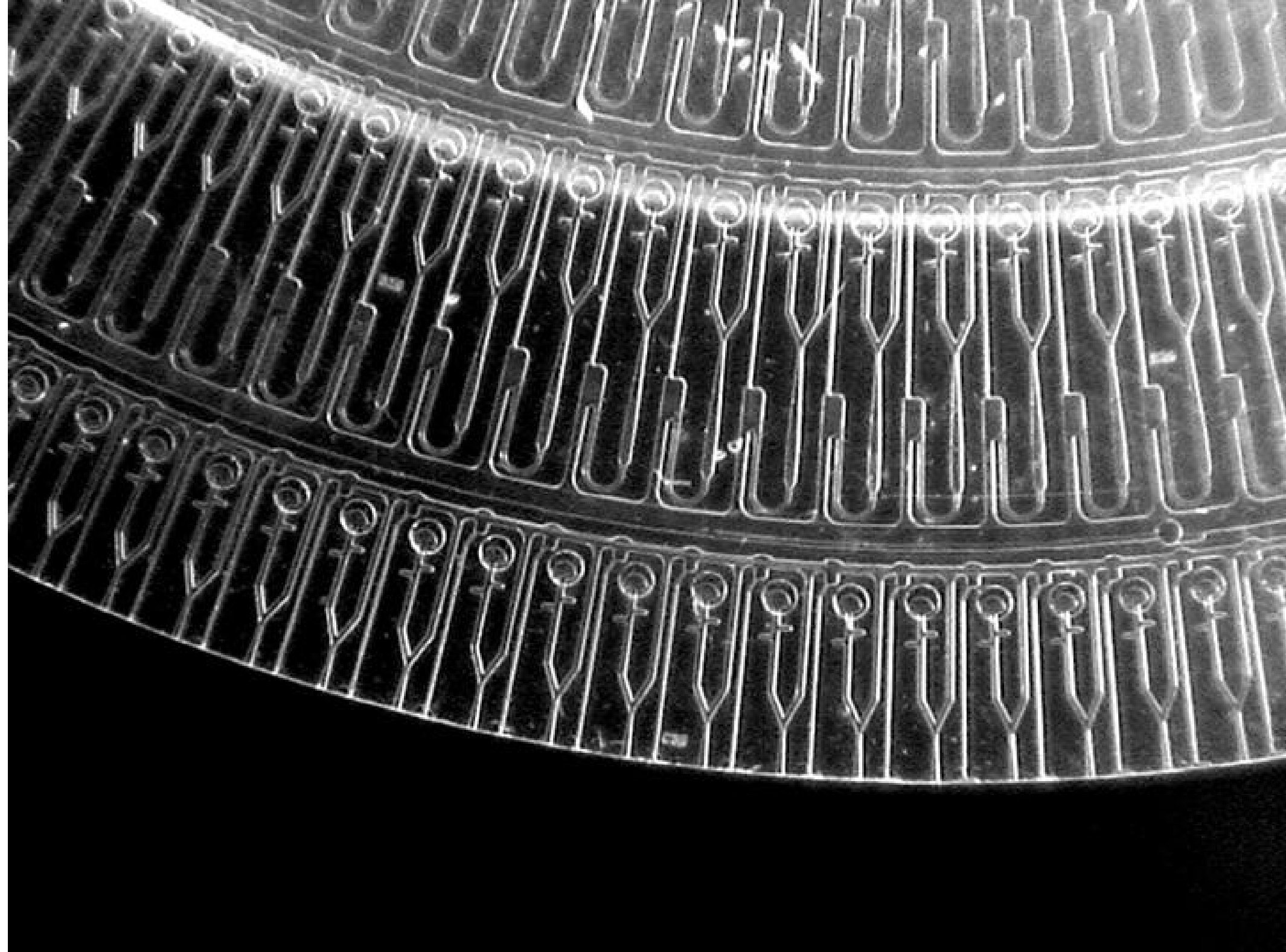
Vacuum ☒ ☒

Temperature ☐ ☐

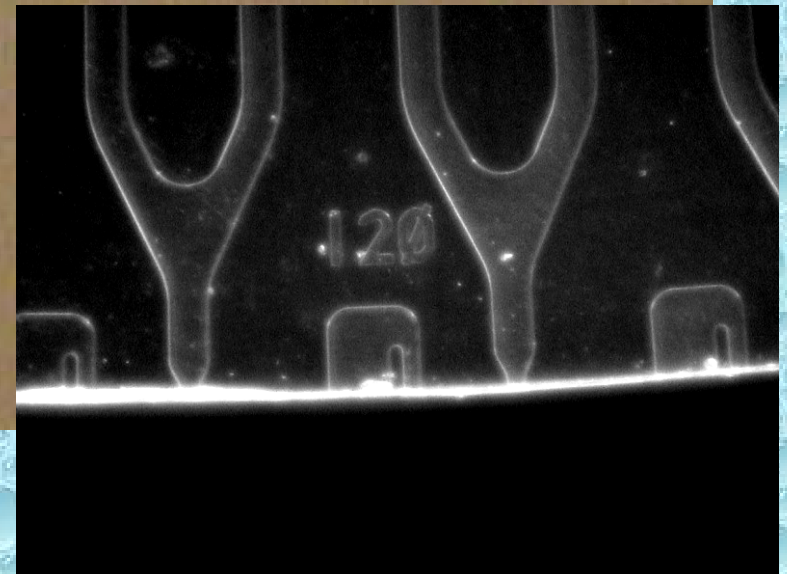
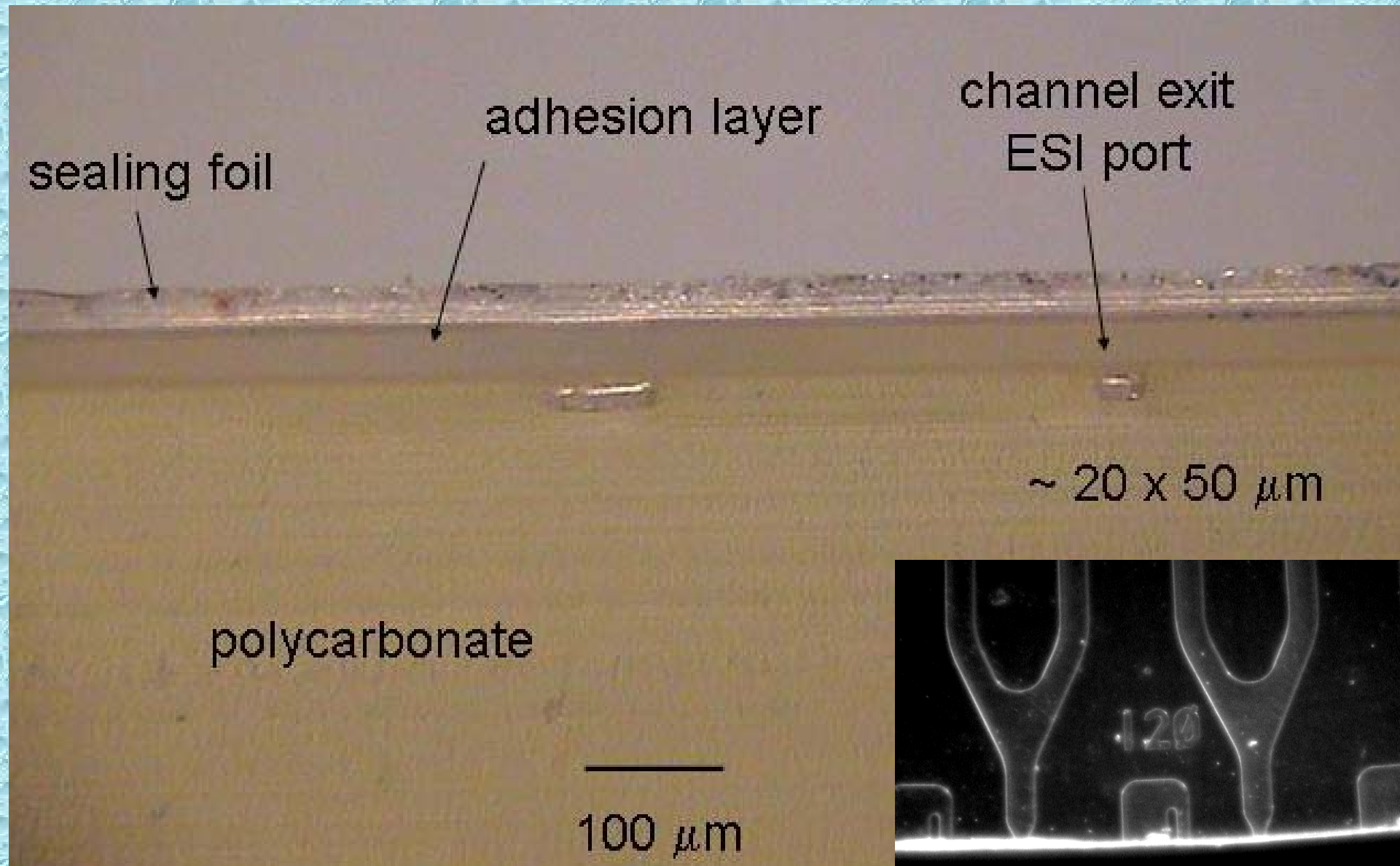
High Voltage ☐ ☐

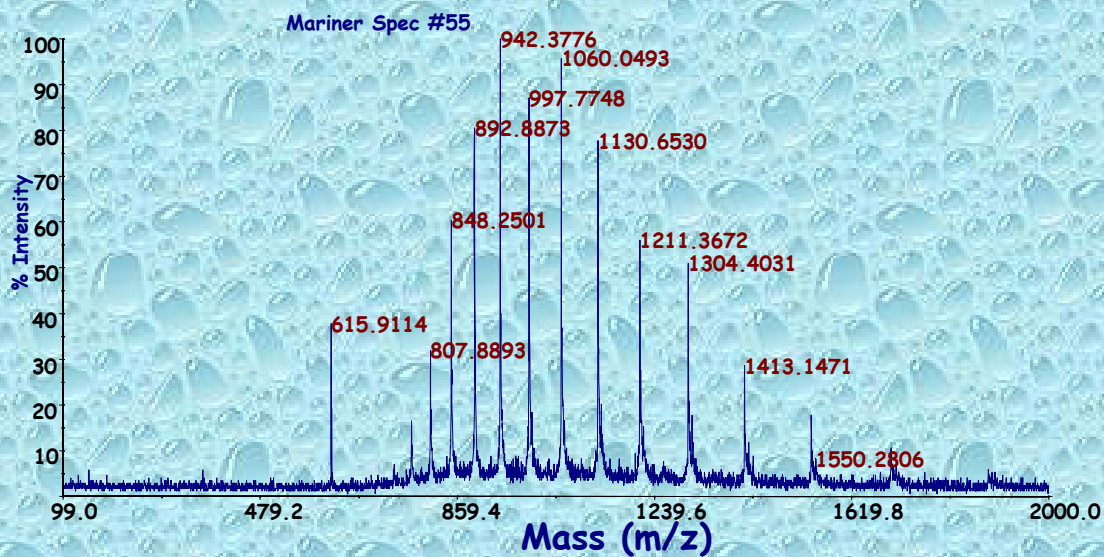
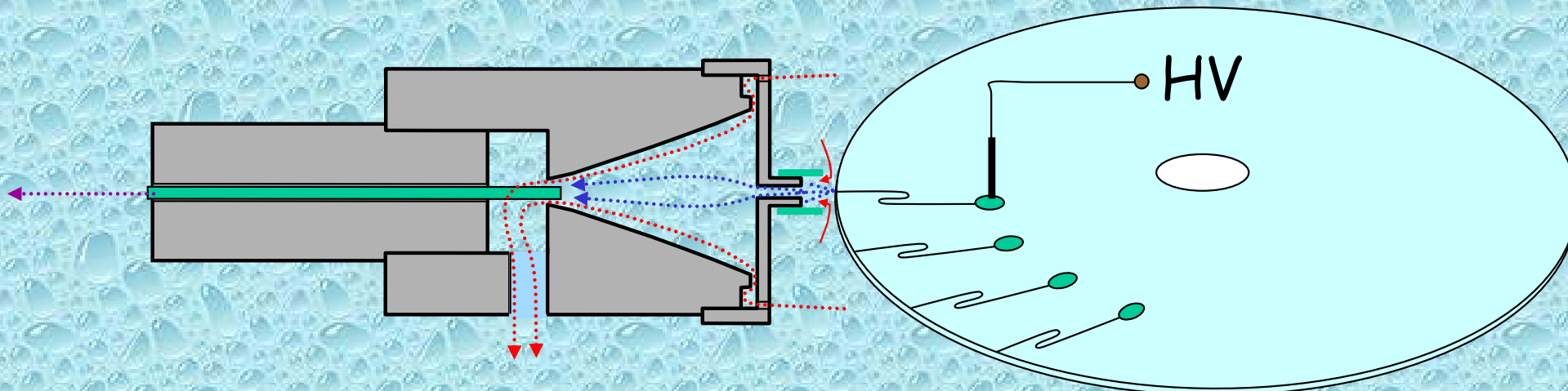
Acquisition ☐ ☐

Startup ☐ ☐

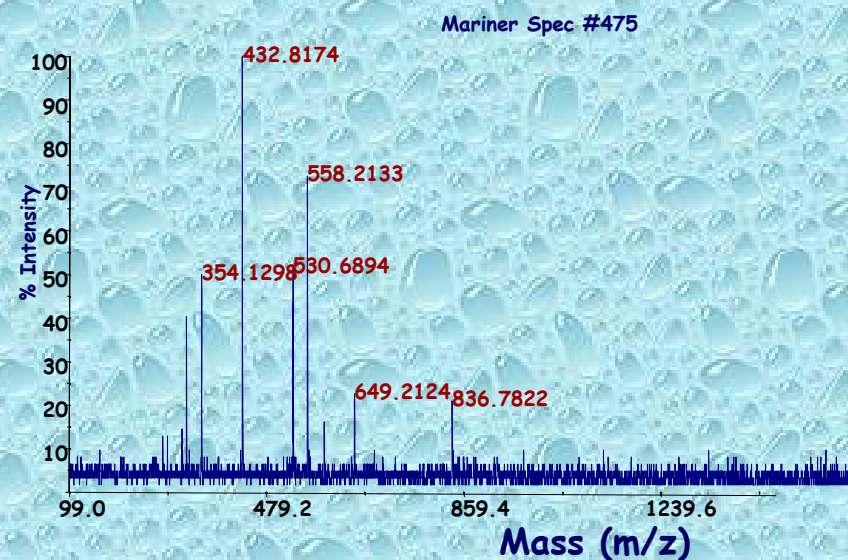


CD structure



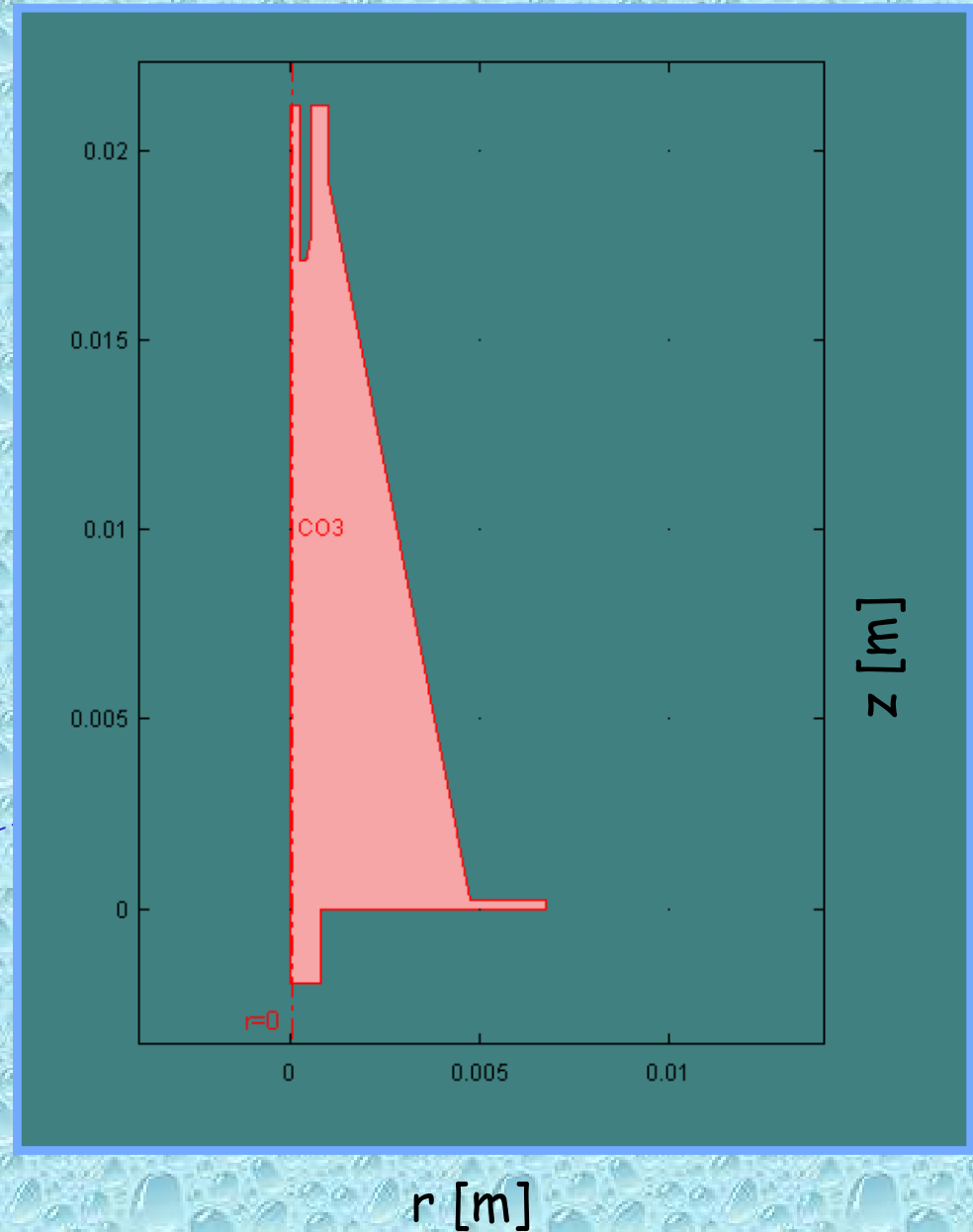
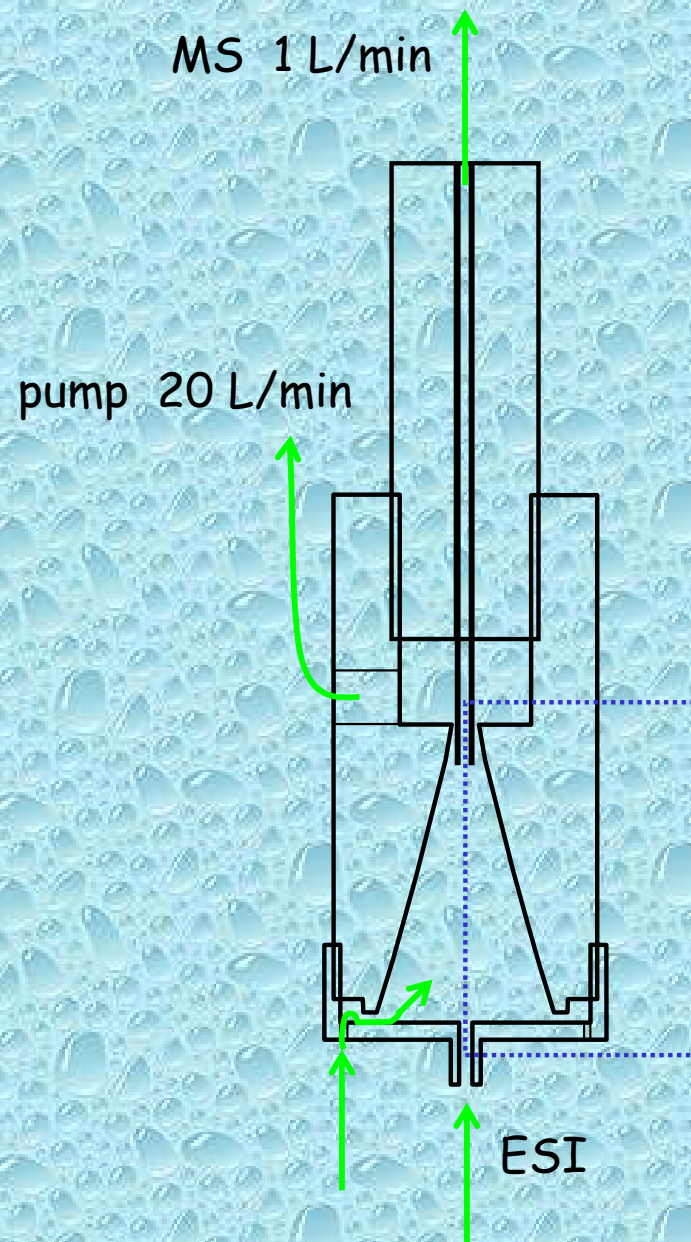


10^{-6} M myoglobin
in 50% IPA, 1% HAc



10^{-5} M Bradykinin, Angiotensin, Neurotensin
in 50% ACN, 1% HAc

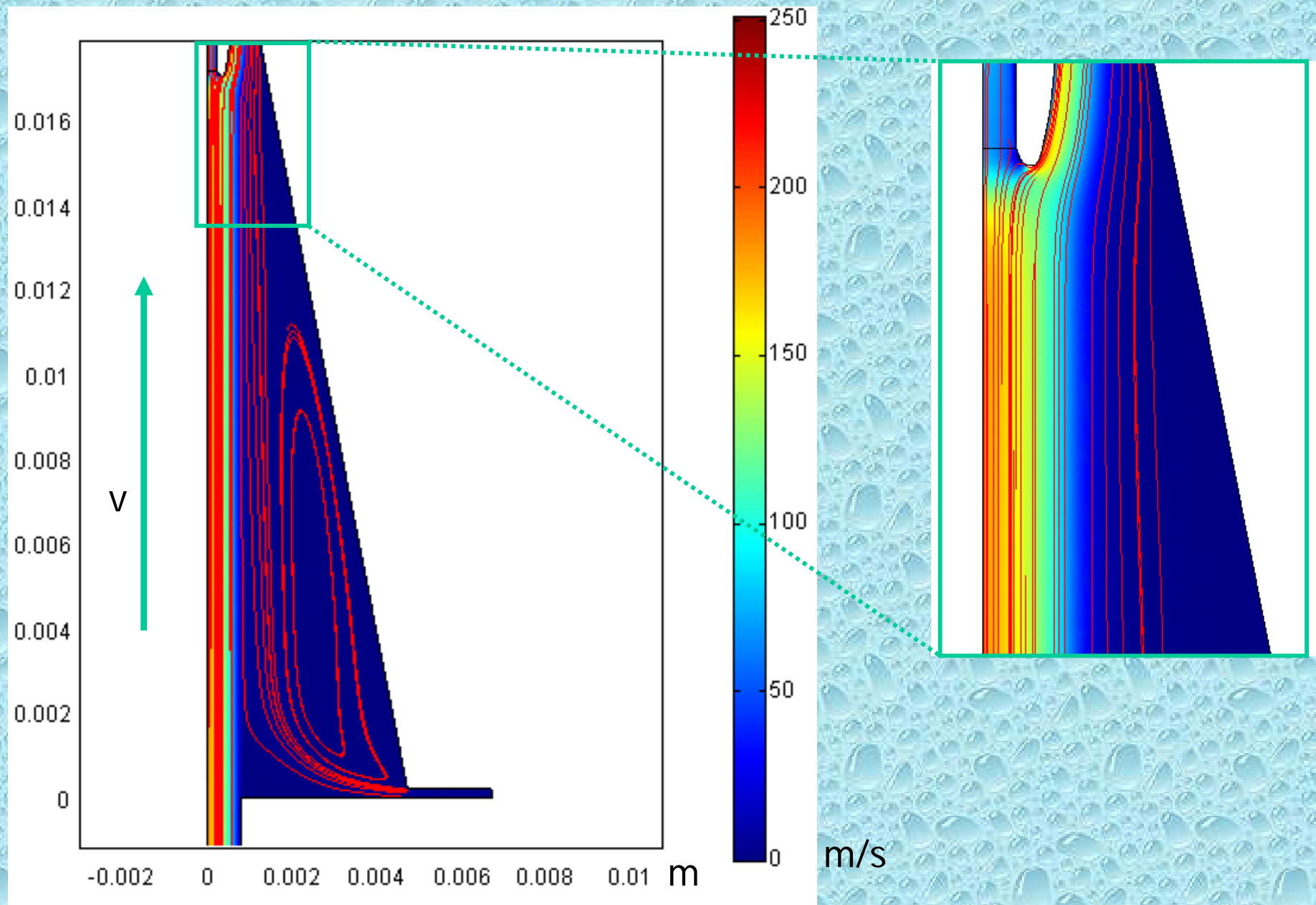
Computer simulation



Air velocity field

- Stationary Navier-Stokes equation
- Finite elements method
- FEMLAB 3 - the "incompressible Navier-Stokes" application

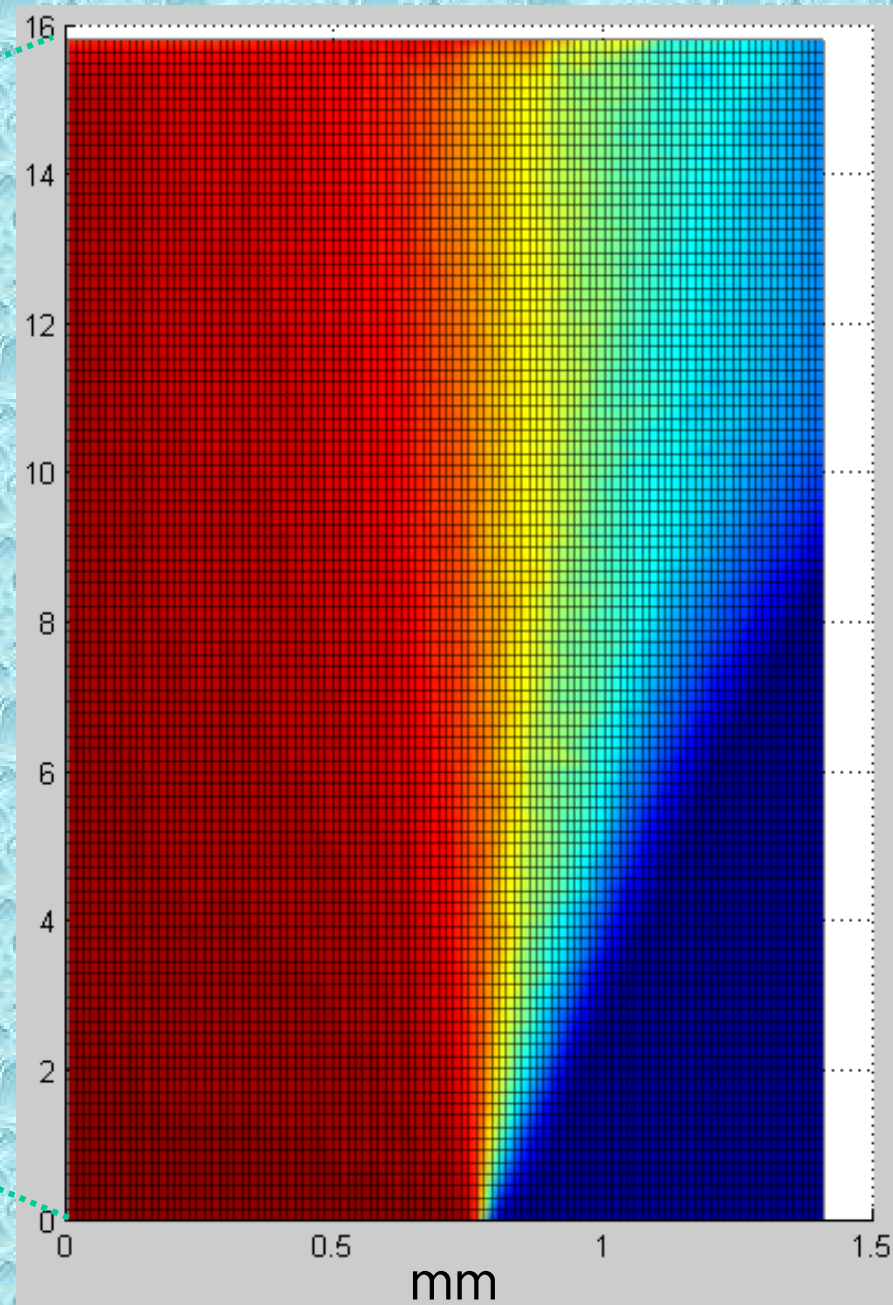
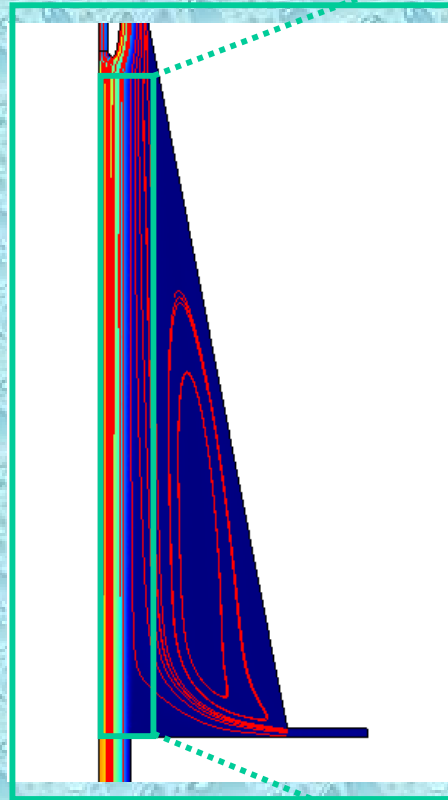
Air velocity field



Charged particle motion

- ESI current: 100nA
- ESI flow rate: 100nl/min
- Droplet diameter: 200 nm
- Droplet charge: at Rayleigh-limit

Mass transport at the MS entrance



Problems to solve...

- Velocity field of air - (in)compressible viscous liquid - in the device.
- Droplet evaporation
-



NANOTECH was officially established on August 13, 2003 by the Thai Cabinet's decree in response to the fast growing development of nanotechnology and its applications to future manufacturing.

Nanoscience and nanotechnologies

structures on 100 nm scale

Applications :

- Electronics
 - Material science and mechanical engineering
 - Optics
 - Medicine
 - Cell and Molecular biology
 - Chemistry
 - Novel functional materials and devices

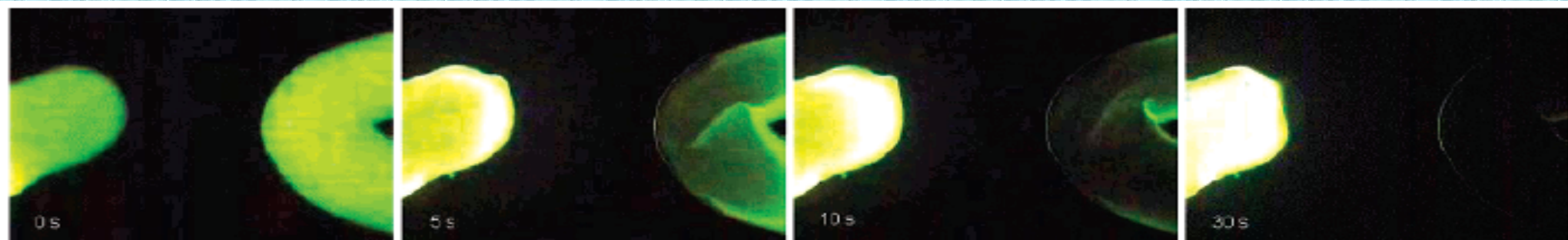
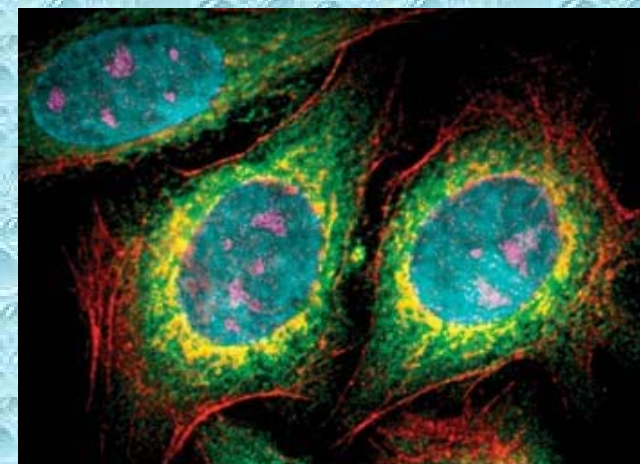
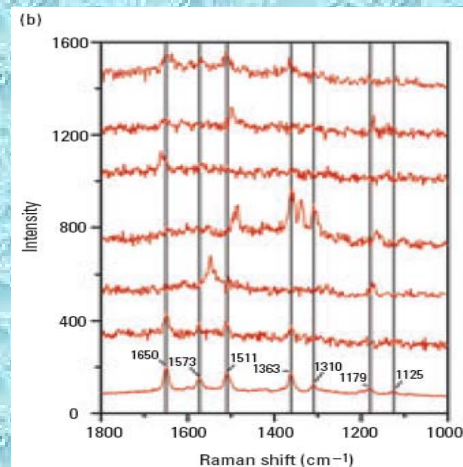
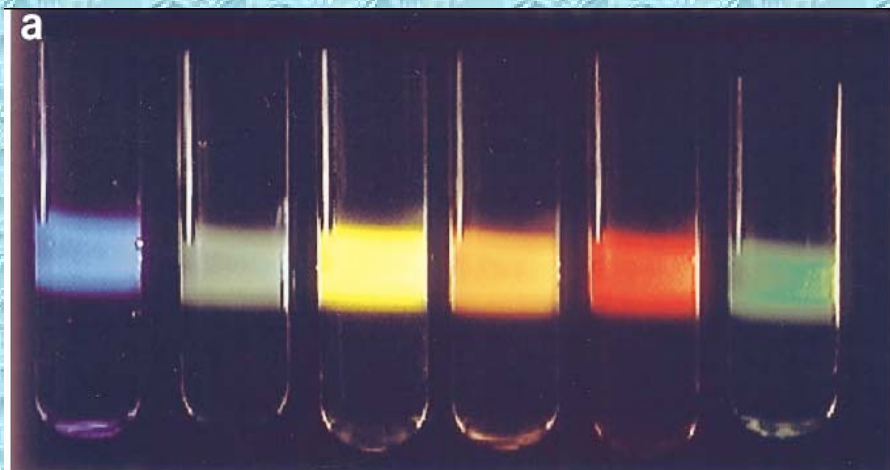
Properties of nanoparticles

sub-100 nm dimensions provide new (unexpected) properties:

- surface plasmon resonance,
- surface enhanced Raman scattering
- biocompatibility - no denaturing of enzymes, repulsive electrostatic forces - stable colloids, high surface to volume ratio
- magnetic

Nanotechnologies in analytical chemistry

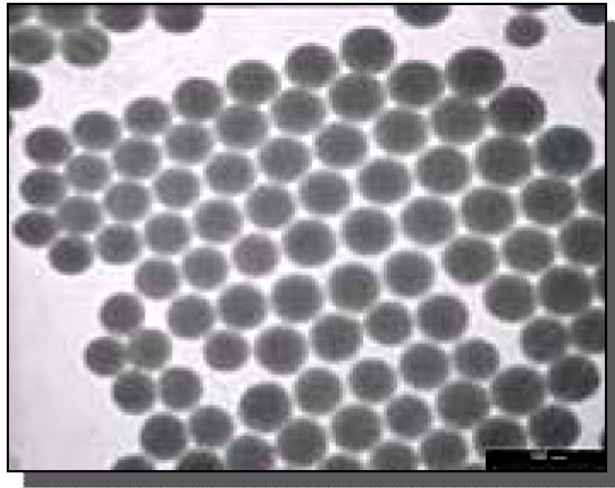
- chemically modified probes - *in vitro* imaging, *in vivo* targeting
- chemically modified fluorescence labels - quantum dots
- contrast agents for electron microscopy and magnetic resonance imaging
- modified superparamagnetic particles (Fe_3O_4) for cell separation
- nanochannels - separations controlled by electric double layer
- immunoagglutination - 3D linkage of latex particles by antigens



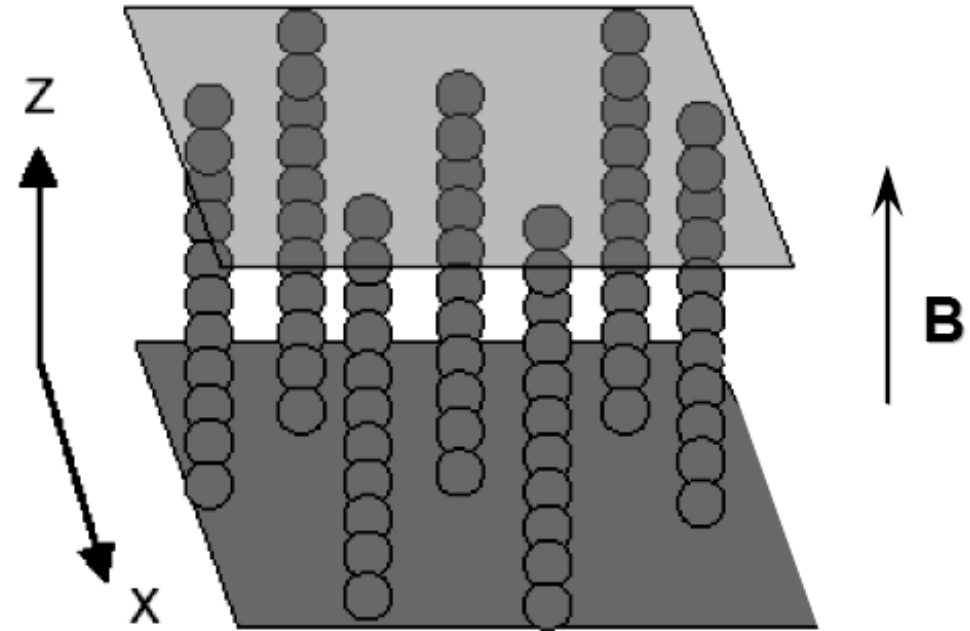
Enzymatic microreactor with magnetic nanoparticles



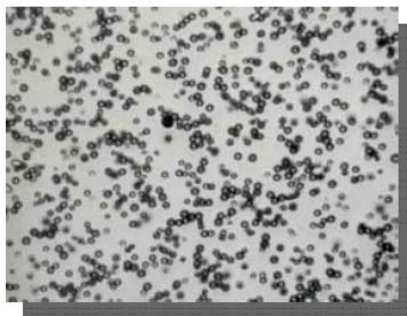
Self assembly of magnetic nanoparticles




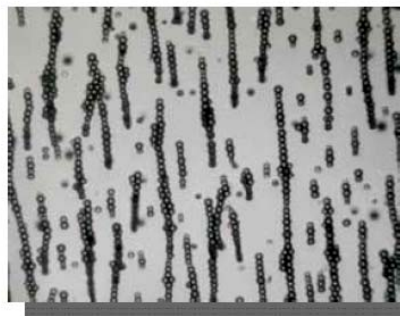
TEM micrograph of 300 nm magnetic particles



□ Self-organization under Magnetic Field

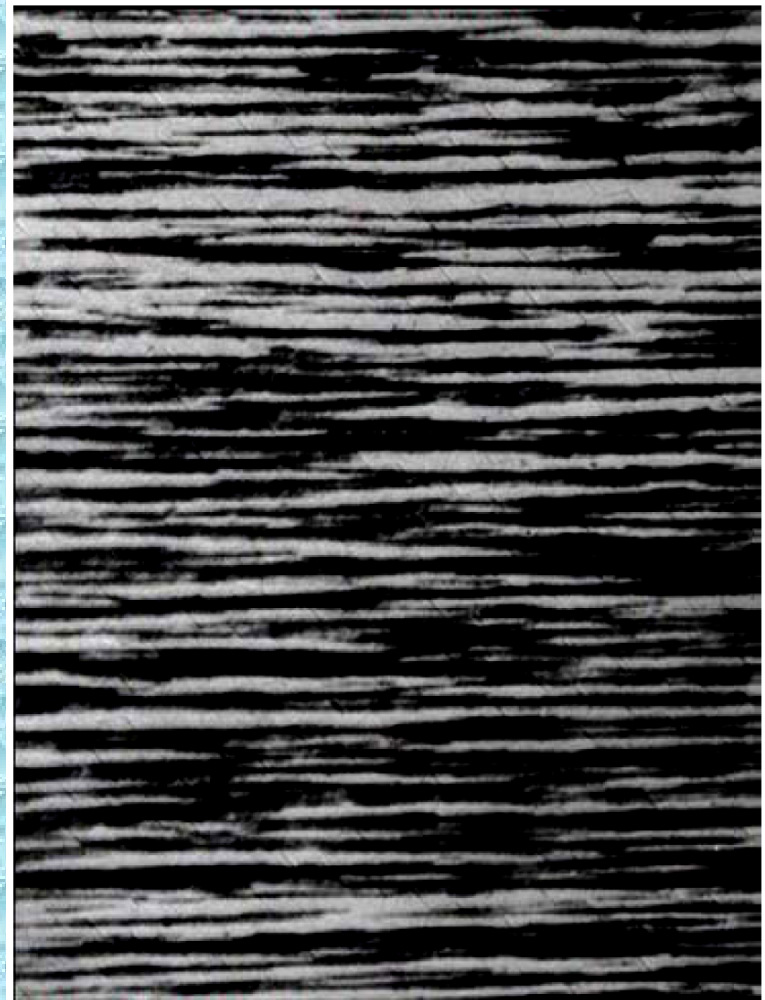
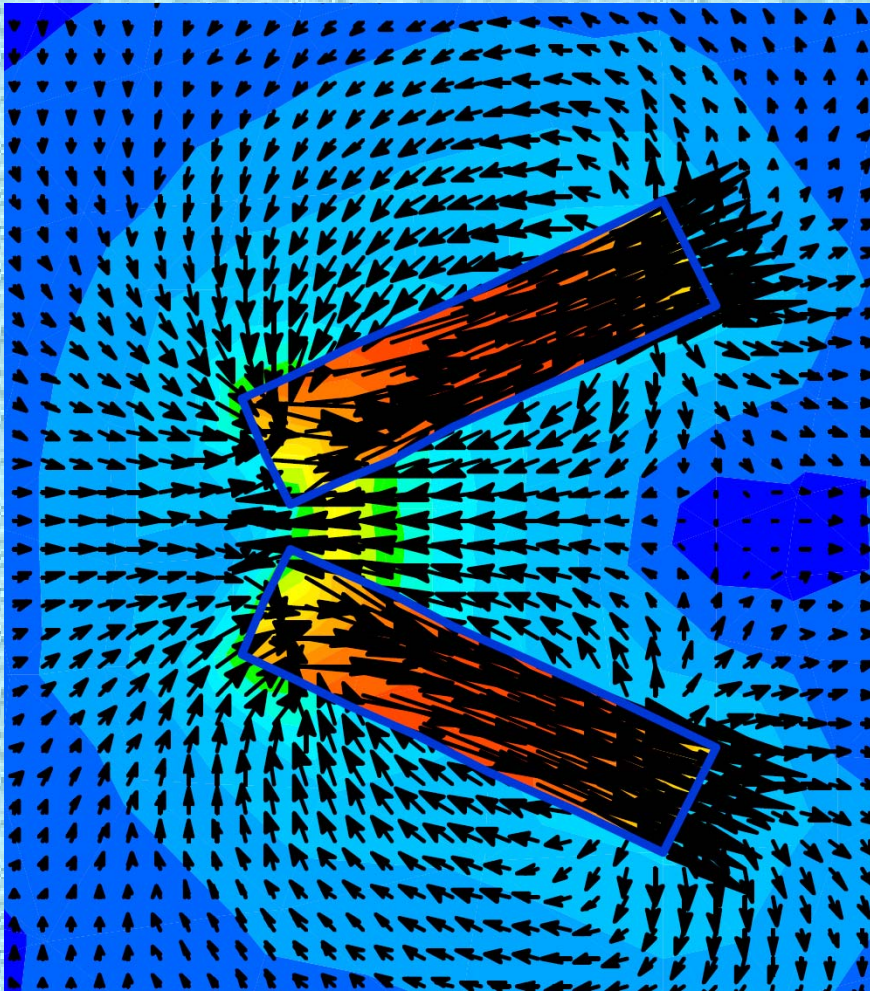



Magnetic field

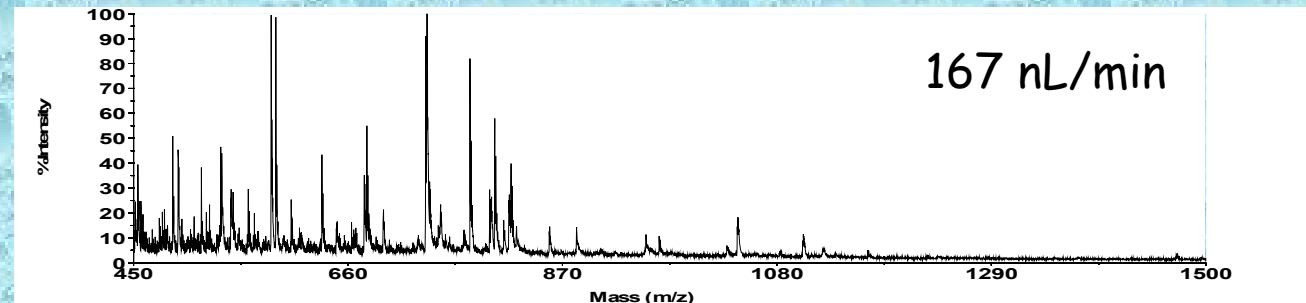
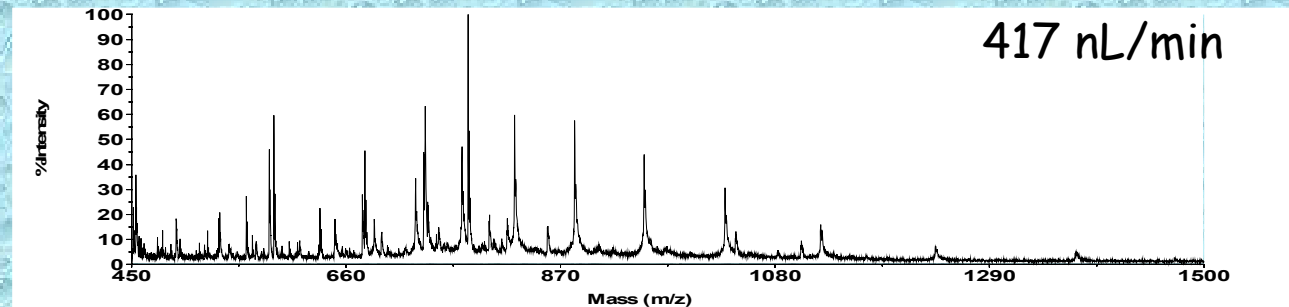
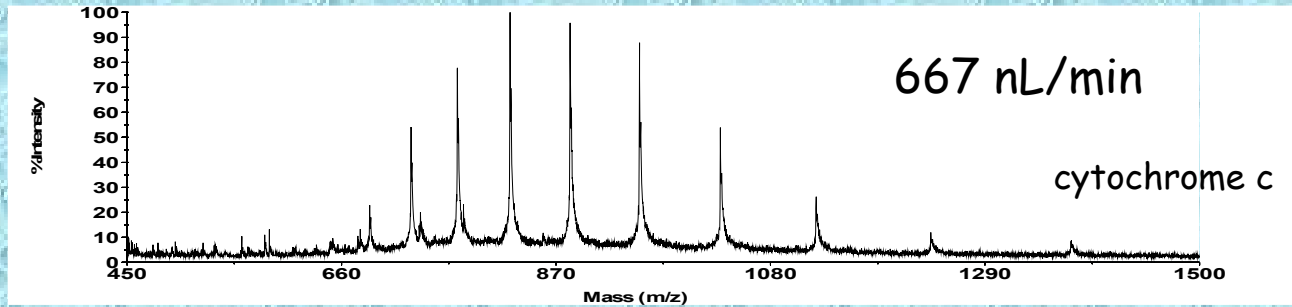


Jean-Louis Viovy, Institut Curie

Self assembly of magnetic nanoparticles



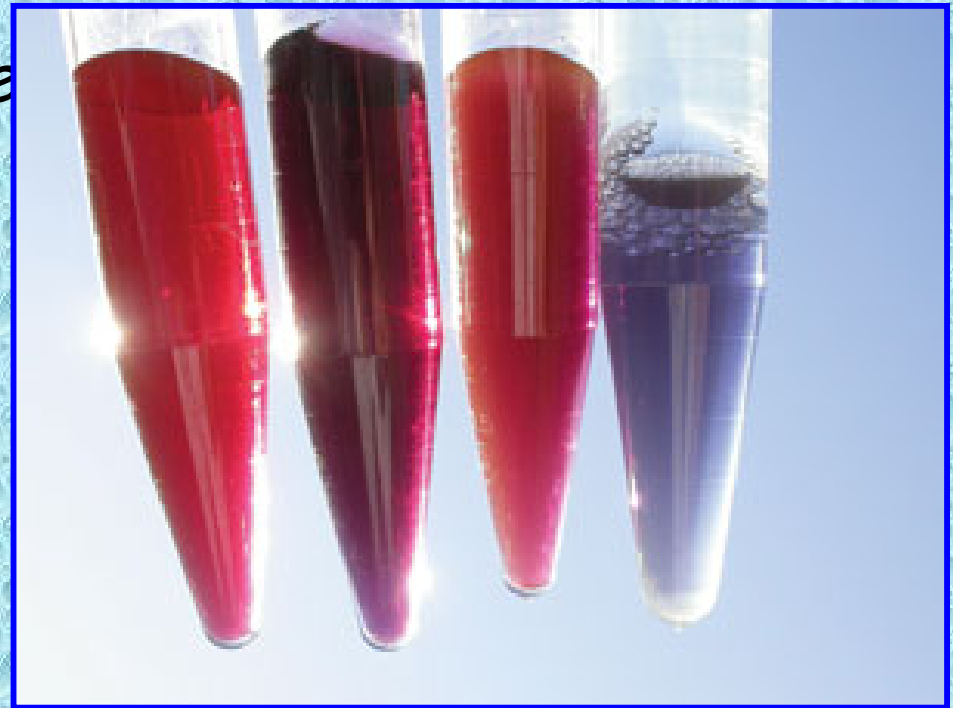
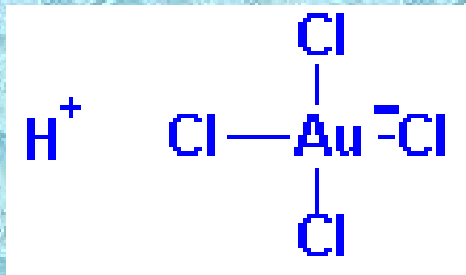
On-line ESI/MS with magnetic nanoparticles based immobilized trypsin microreactor



Au - nanoparticle preparation

Citrate reduction of HAuCl_4

Hydrogen tetrachloroaurate

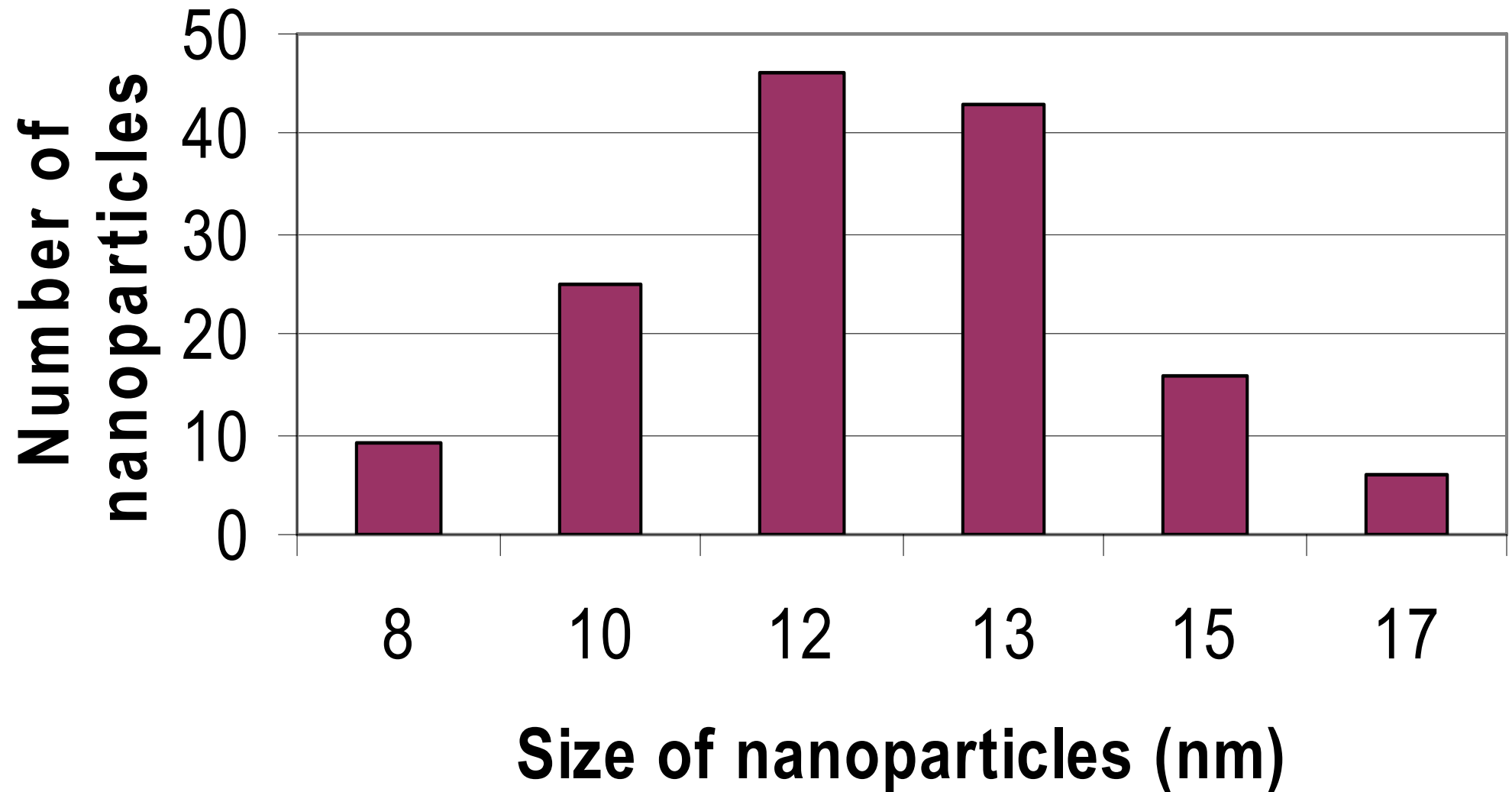


Mixing of 5 ml 1 mM HAuCl_4 + 0.5 ml 2.9 mM citrate at 85 °C

Colored gold nanoparticles „*Purple of Cassius*“

Histogram of gold nanoparticles

Transmission electron microscopy



Au nanoparticle DNA extraction

CE separation conditions:

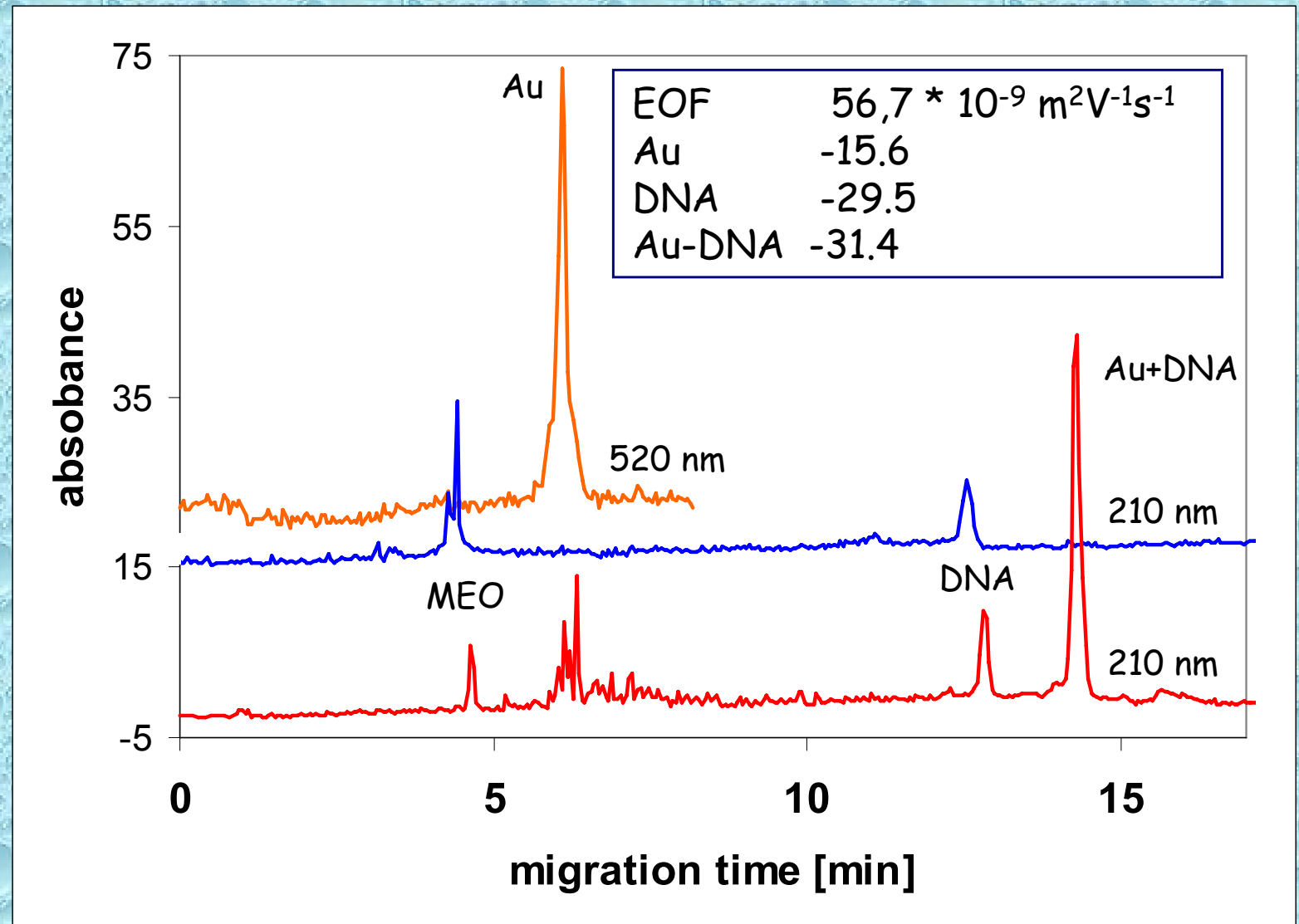
Capillary: uncoated, ID 75 μm , length 30/50 cm; **Electrolyte:** 6 mM acetate buffer pH 5;

Electrophoresis: 10 kV; **Injection:** 10 s; **MEO:** marker of electroosmosis

DNA poly (A)₉

Au ~ 12 nm

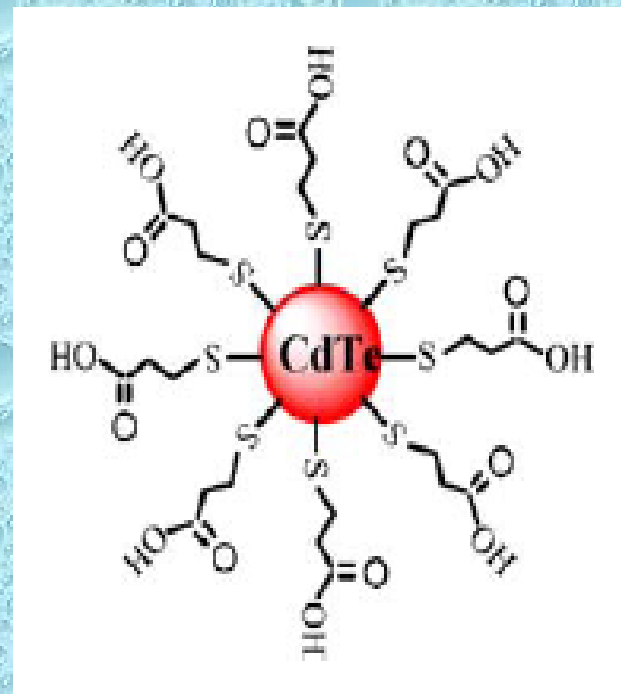
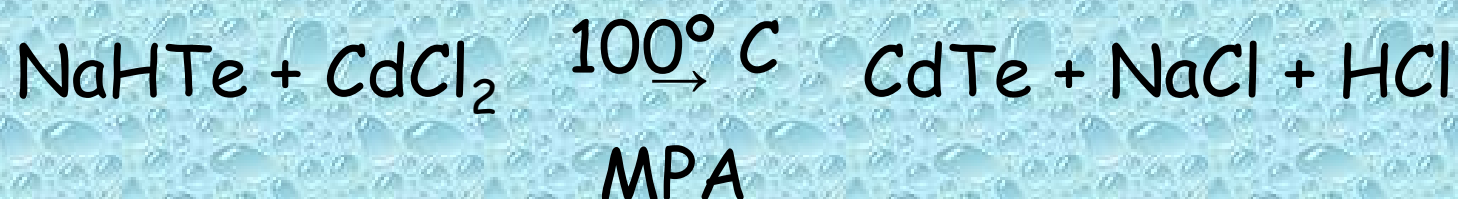
Capillary: SiO⁻



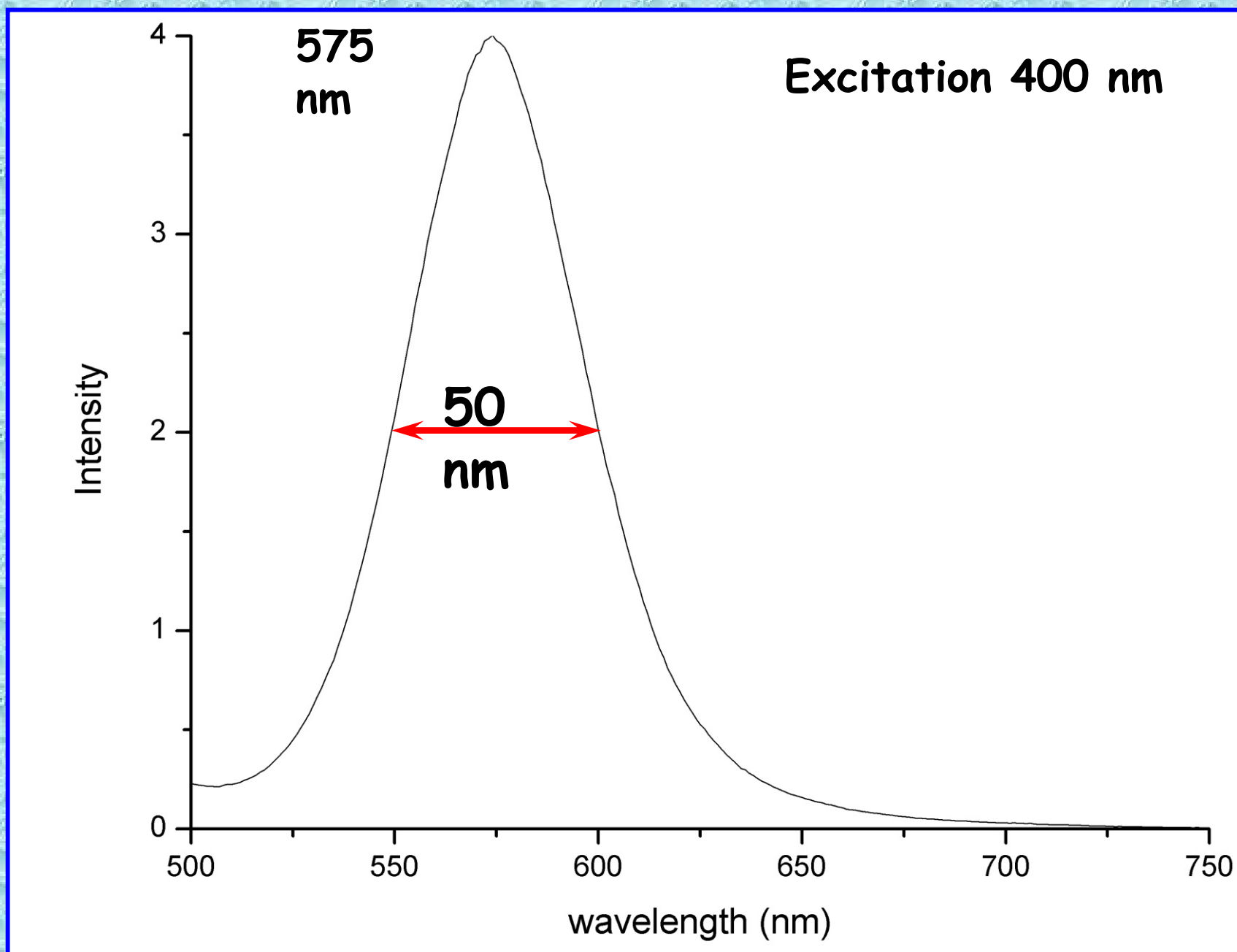
Noncovalent interactions of DNA with gold nanoparticles

- Elimination of anomalous migration due to hairpin conformations
- Increase in detection sensitivity
- Free solution separation

Water soluble quantum dots preparation



CdTe QDs emission spectra



QD transport into cells

Saccharomyces cerevisiae cells

CdTe fluorophor

30 hours uptake

Excitation BP 515 - 560

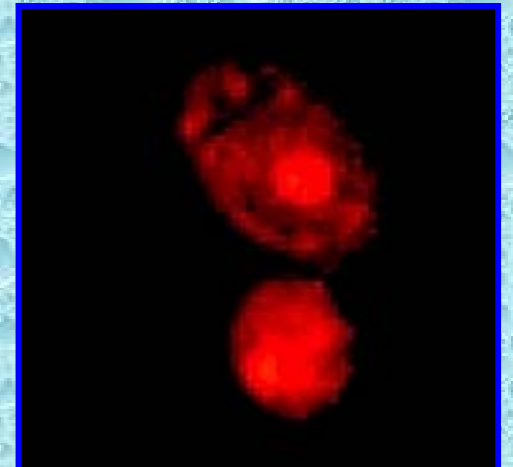
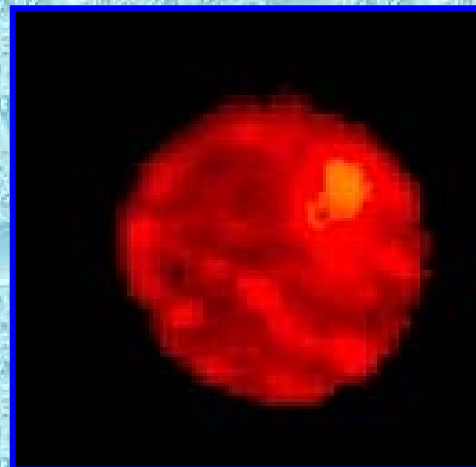
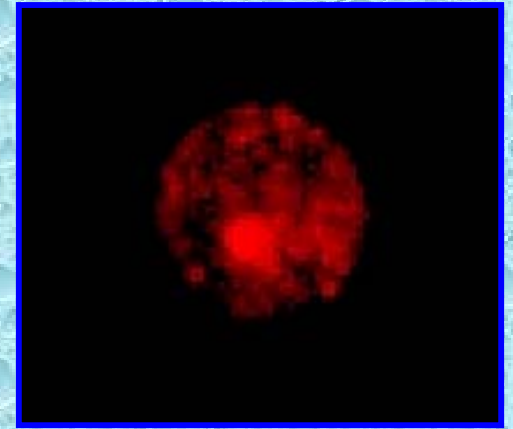
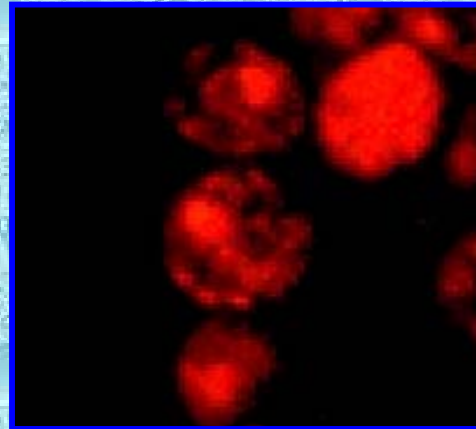
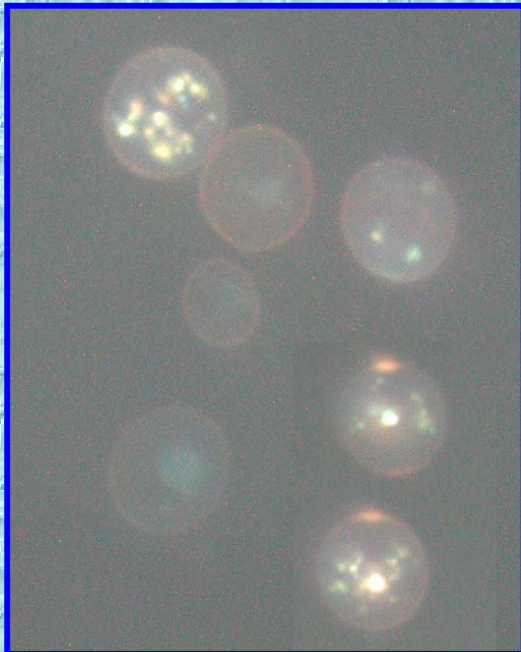
Emission LP 590 nm

Magnification 400 x

Excitation BP 450 - 490

Emission LP 515 nm

Magnification 2000 x



CdTe quantum dots

- Broad excitation curve
- Narrow emission spectra
- High photo-stability
- Effective uptake into living cells



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Kusý



Applied Biosystems
Gyros
GAČR
GAAV

CECE 2007

November 15 - 16, 2007

Hotel Continental, Brno, Czech Republic

<http://www.iach.cz/cece/>



Invited speakers:

Silvija Abele, DCU, Dublin, Ireland

Salvatore Fanali, CNR, Rome, Italy

Bohuslav Gas, Charles University, Prague, CZ

Andras Guttman, University in Innsbruck, Innsbruck, Austria

Ernst Kenndler, University of Vienna, Vienna, Austria

Jaroslav Koca, Masaryk University, Brno, CZ

Stanislav Kozubek, Institute of Biophysics, ASCR, v.v.i., Brno, CZ

Alexander Leitner, University of Vienna, Vienna, Austria

Pavel Neuzil, Institute of Microelectronics, Singapore

Frantisek Svec, UC at Berkeley and LBL, Berkeley, USA.

Karel Slais, Institute of Analytical Chemistry ASCR, v.v.i., Brno, CZ.

Pavel Zemanek, Institute of Scientific Instruments ASCR, v.v.i., Brno, CZ