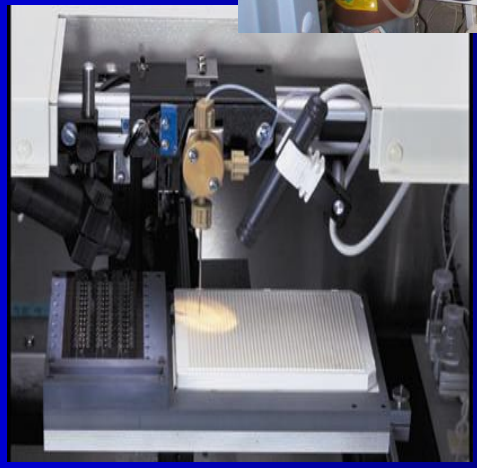
Clinical Proteomics:
A Technology to shape the future?



Zakopane, April, 2011

Protein Chemistry/Proteomics/Peptide Synthesis and Array Unit

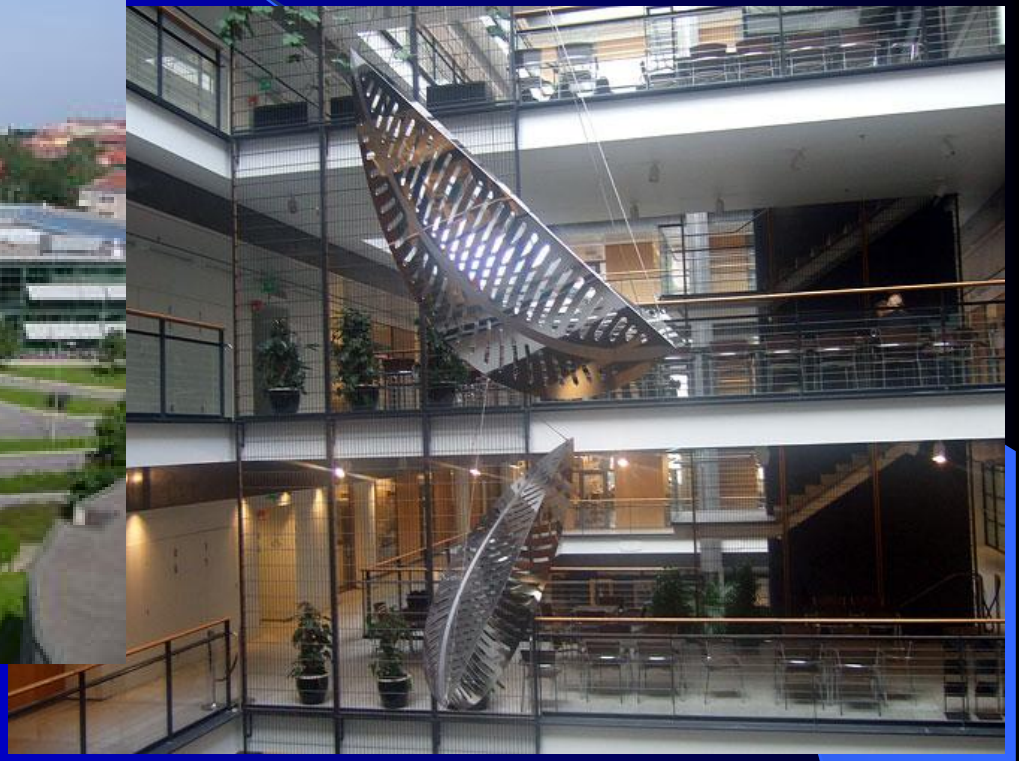
Institute of Biomedicine/Anatomy

Biomedicum Helsinki, 00014 University of Helsinki, Finland

E-Mail: marc.baumann@helsinki.fi

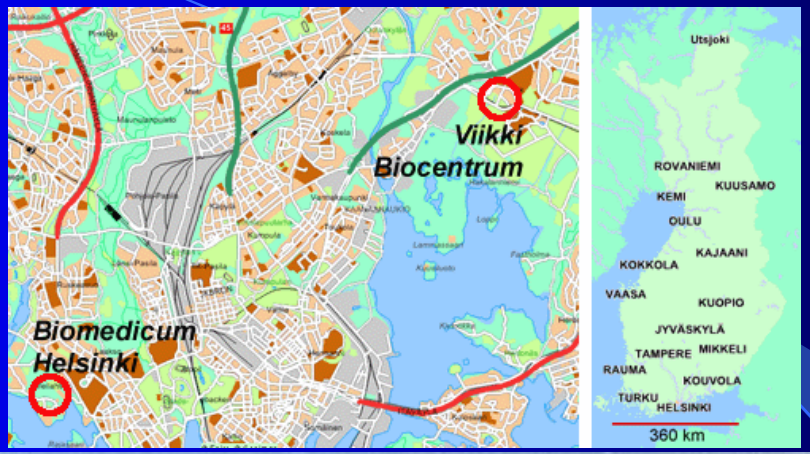
(**http://research.med.helsinki.fi/corefacilities/proteinchem**)

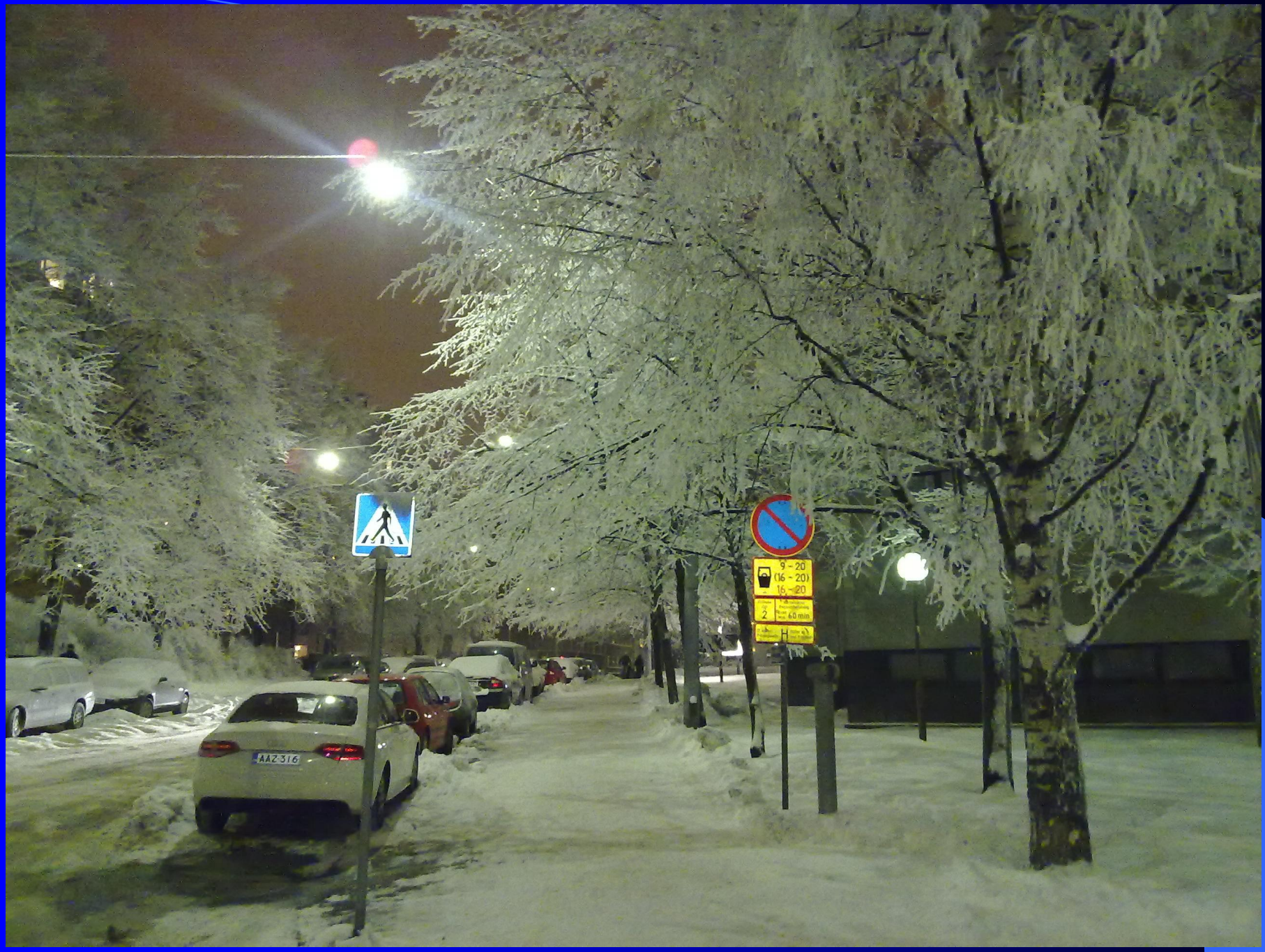
BIOMEDICUM



Over 1200 Researchers in only Medical Research
(Cancer, Genetics, Developmental Medicine, Neuroscience etc.)

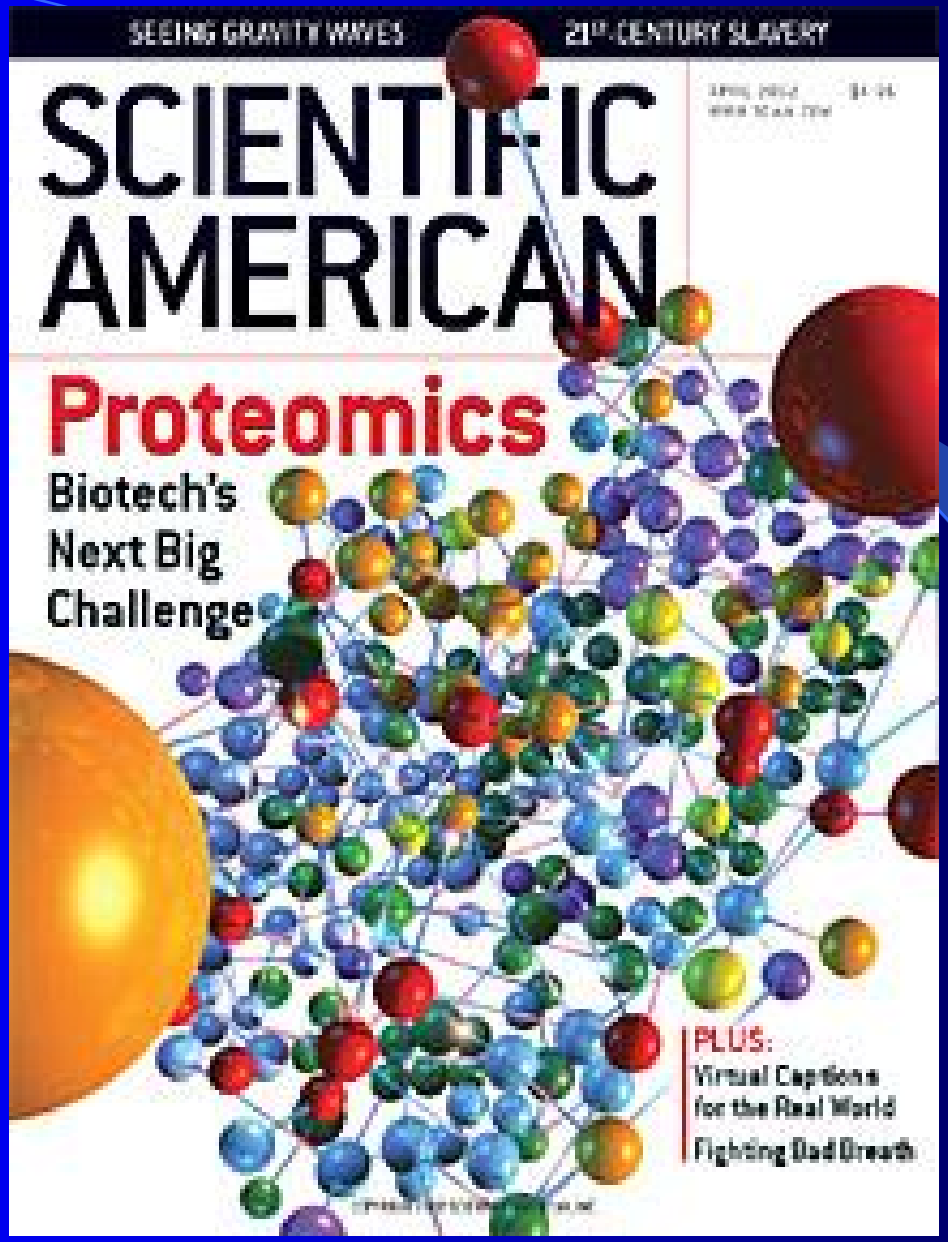
The Medical Faculty




AAZ-316
9 - 20
(16 - 20)
16 - 20
2
60 min

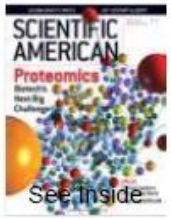
Proteomics

What is it all about??


SEEING GRAVITY WAVES
21ST-CENTURY SLAVERY
SCIENTIFIC AMERICAN
Proteomics
Biotech's Next Big Challenge
PLUS:
Virtual Captions for the Real World
Fighting Bad Breath

SCIENTIFIC AMERICAN™

Permanent Address: http://www.scientificamerican.com/article.cfm?id=proteins-rule



Proteins Rule

Biotech's latest mantra is "proteomics," as it focuses on how dynamic networks of human proteins control cells and tissues

By Carol Ezzell | Wednesday, April 24, 2002 | 0

Move over, human genome, your day in the spotlight is coming to a

close. Researchers are now concentrating

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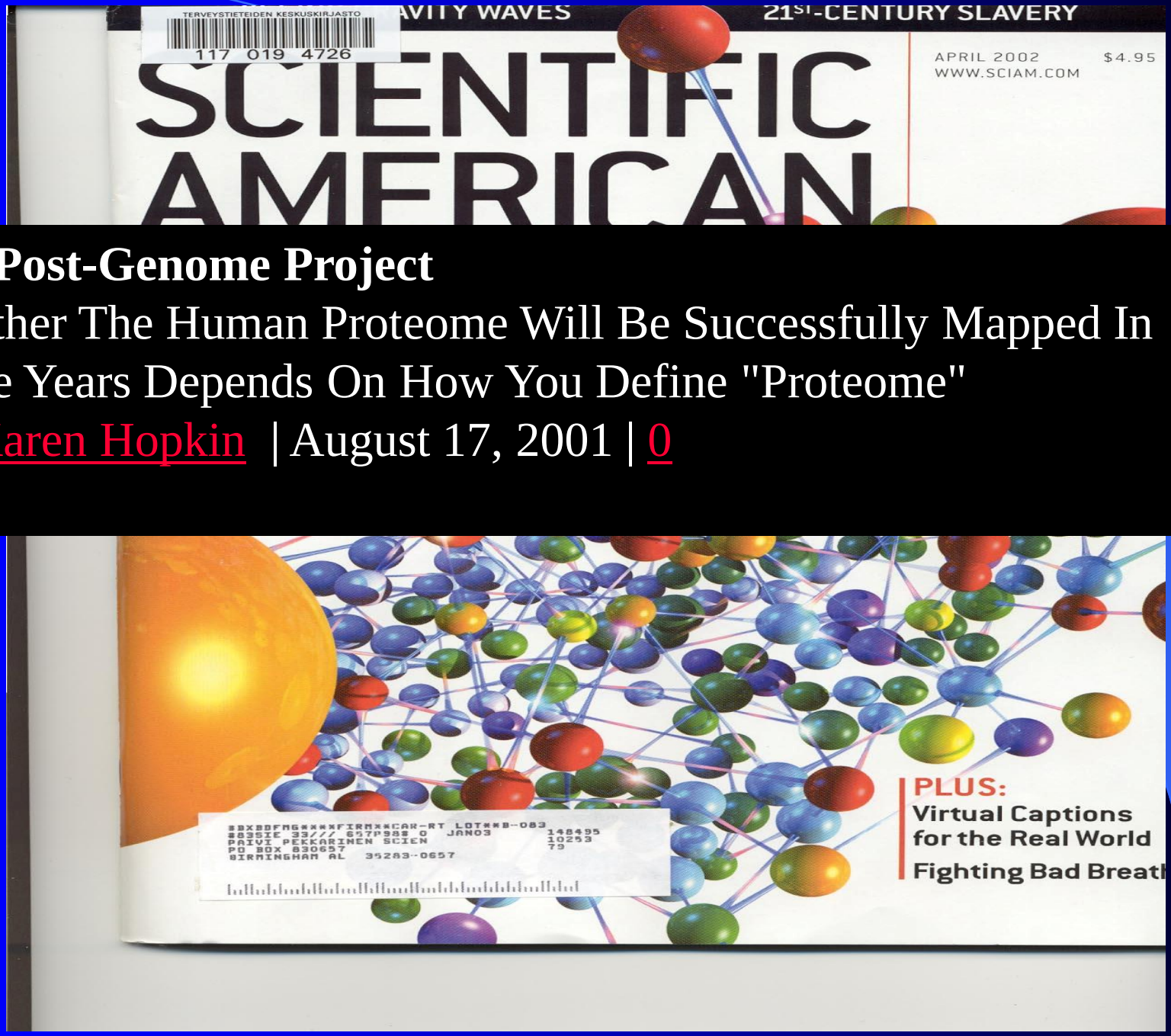


The Post-Genome Project

Whether The Human Proteome Will Be Successfully Mapped In Three Years Depends On How You Define "Proteome"

By Karen Hopkin | August 17, 2001 | 0

Vol. 14 No. 3

www.dddmag.com

Advantage Business Media

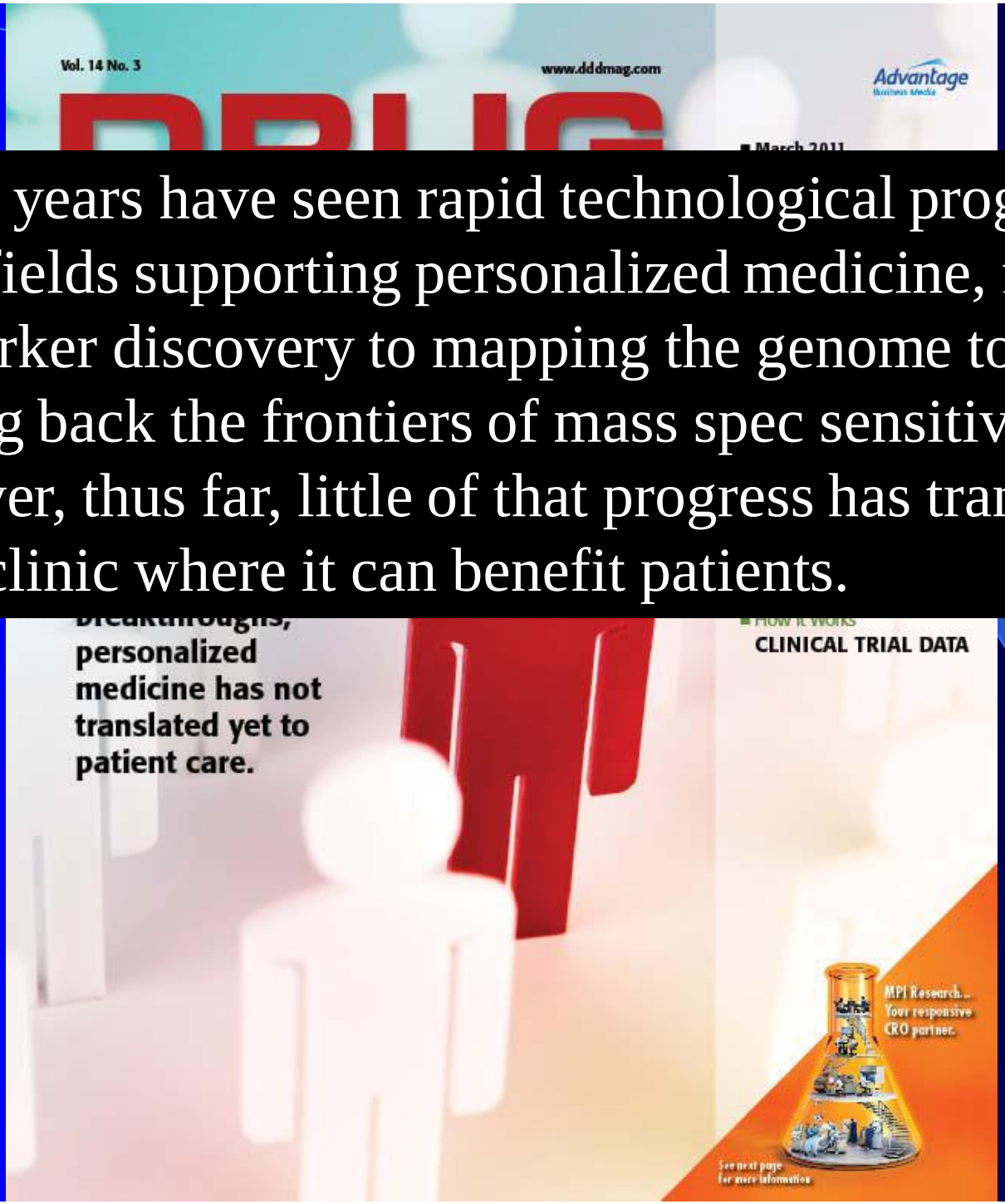
DRUG DISCOVERY & DEVELOPMENT

STRATEGIES & TECHNOLOGIES DRIVING DRUG DISCOVERY TO MARKET

■ March 2011

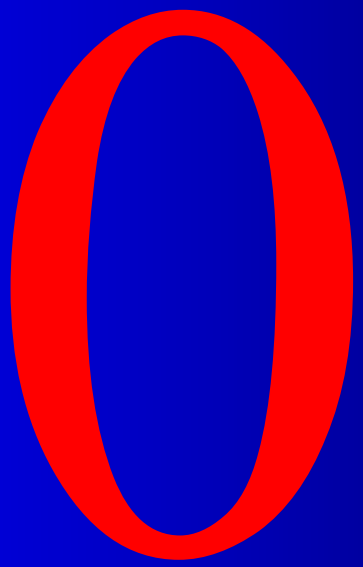
A Long Way to the Bedside

Despite many breakthroughs, personalized medicine has not translated yet to patient care.



Recent years have seen rapid technological progress in the fields supporting personalized medicine, from biomarker discovery to mapping the genome to pushing back the frontiers of mass spec sensitivity. However, thus far, little of that progress has translated to the clinic where it can benefit patients.

Success rate of biomarker search which would be in clinical use:



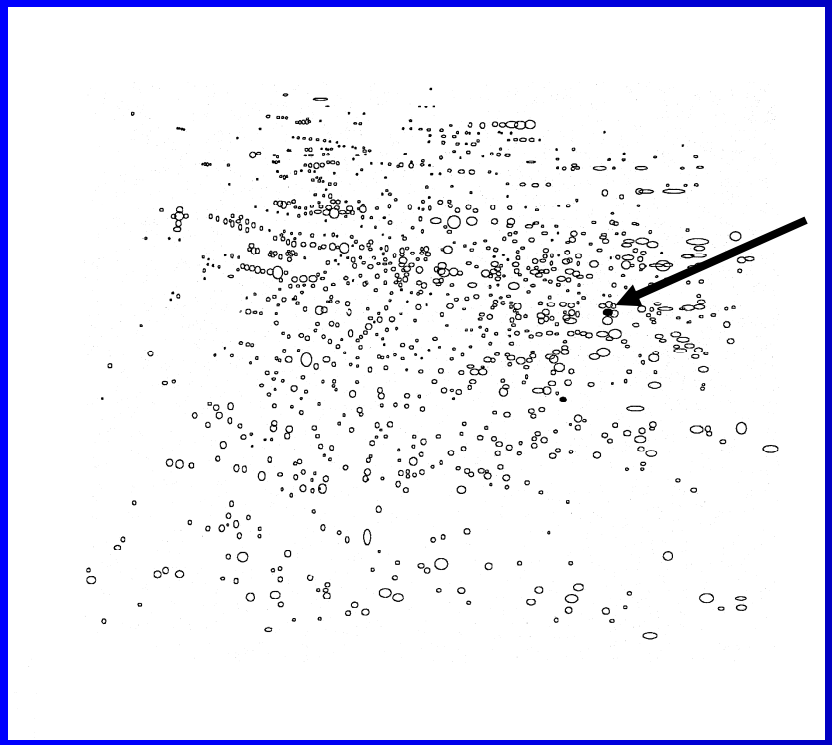
2010

How can we make Proteomics more suitable to the “real” life?

The technology...

2D gel electrophoresis
2D liquid chromatography
Micro arrays

We need a technology to find changes in Proteome



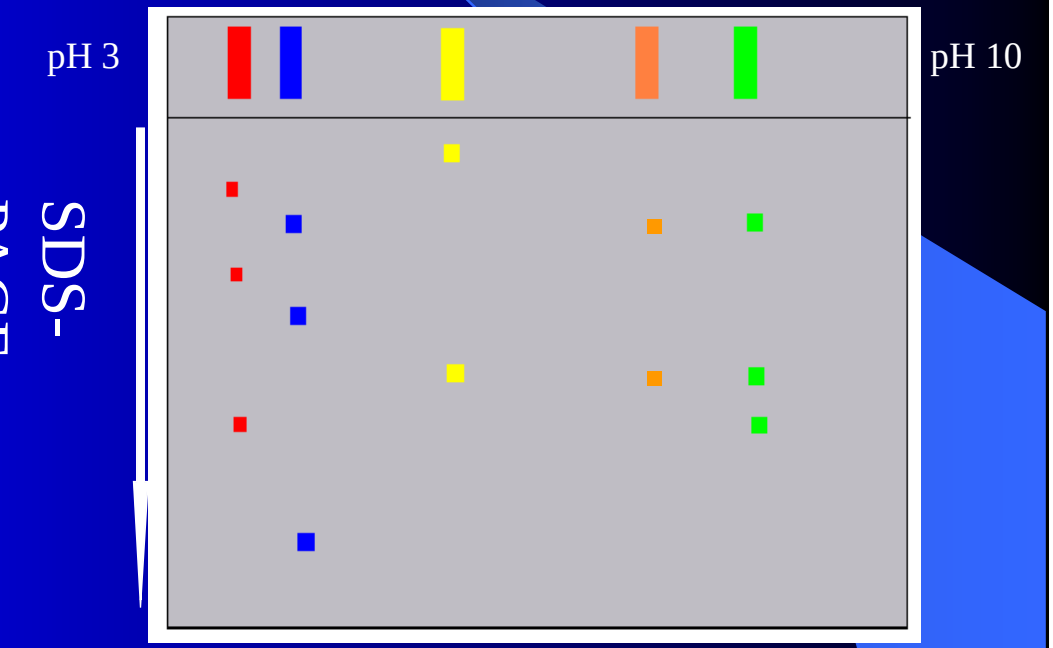
A protein Array

Administration of a drug known to bind to an orphan receptor

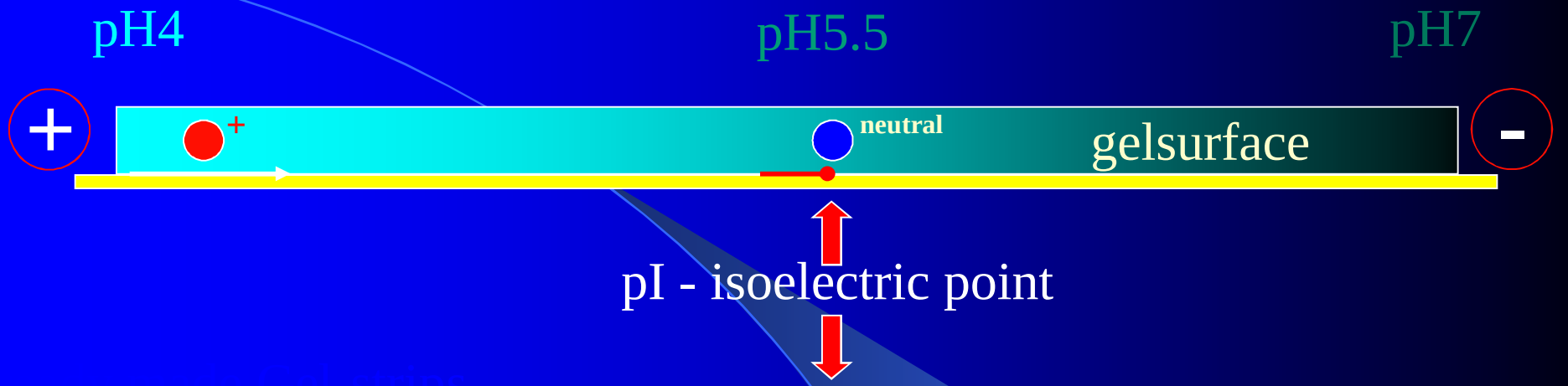
- **Changes in expression level of 23 proteins**

Two-dimensional gel electrophoresis (2D) could do it?

- 1st dimension, IEF, Proteins are separated
- according to their isoelectric point (IP)
- 2nd dimension, SDS-PAGE, Proteins are separated according to their molecular mass
- Efficient: More than a thousand proteins resolved in E-Coli cell lysates and ~8000 in brain lysates

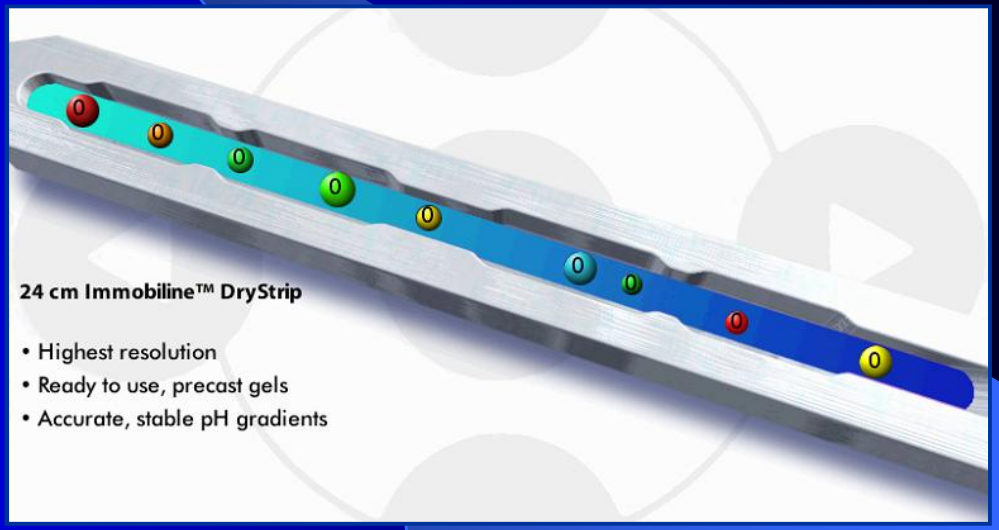


1st Dimension - Isoelectric Focusing



pI - isoelectric point

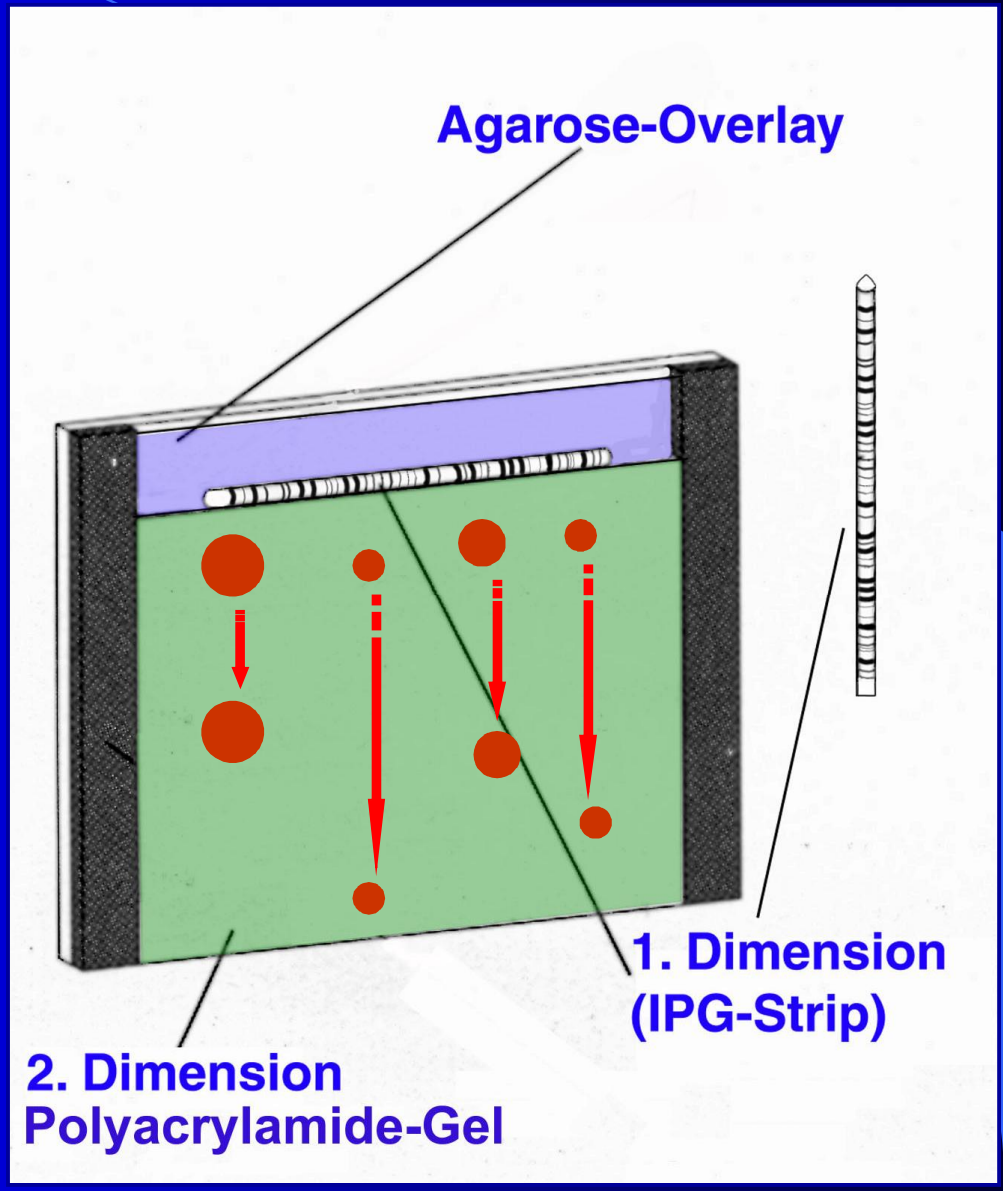
IPG-strips



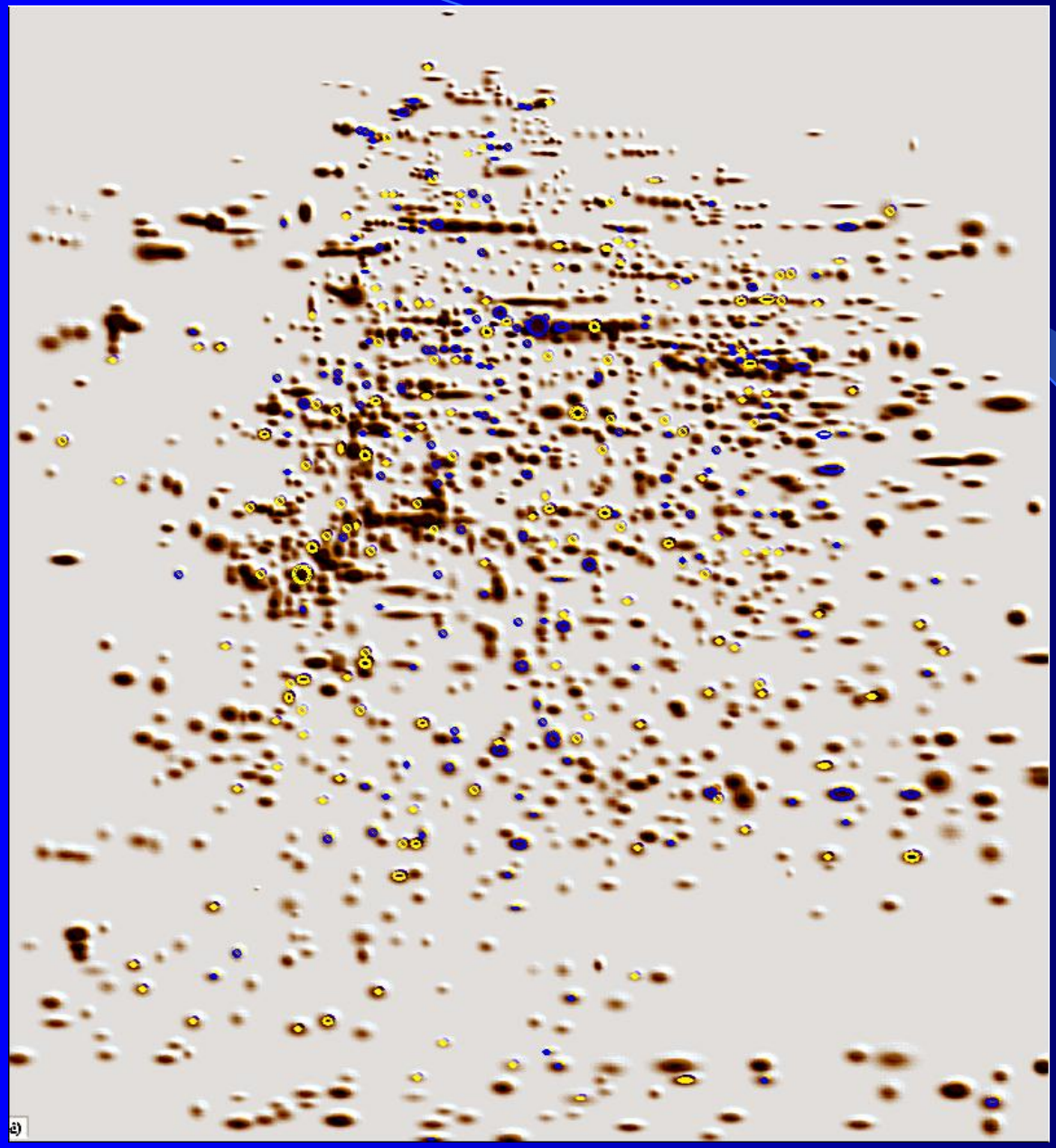
2nd Dimension - Isoelectric Focusing

2DE

Mw

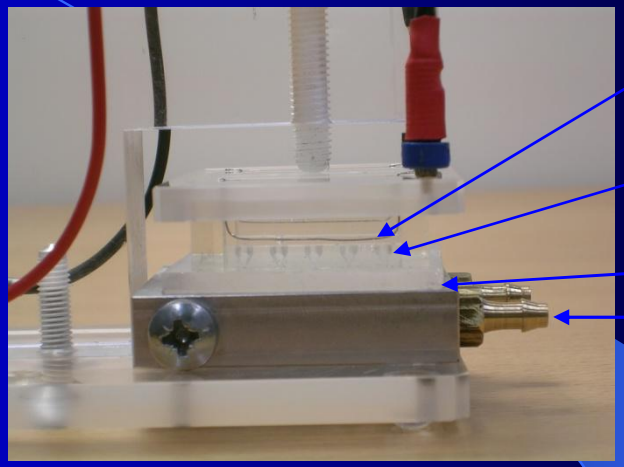


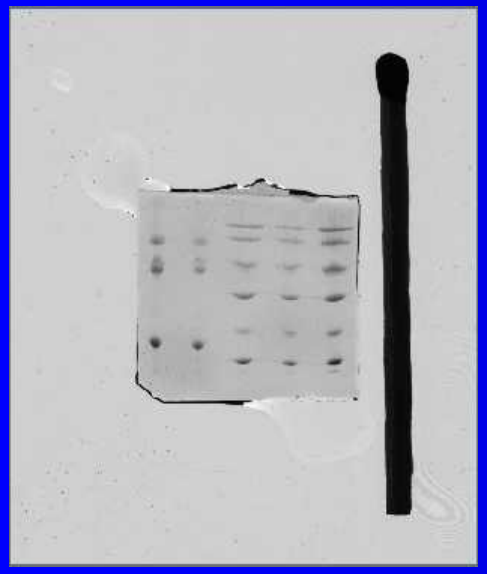
Protein Fingerprint: 2-DE

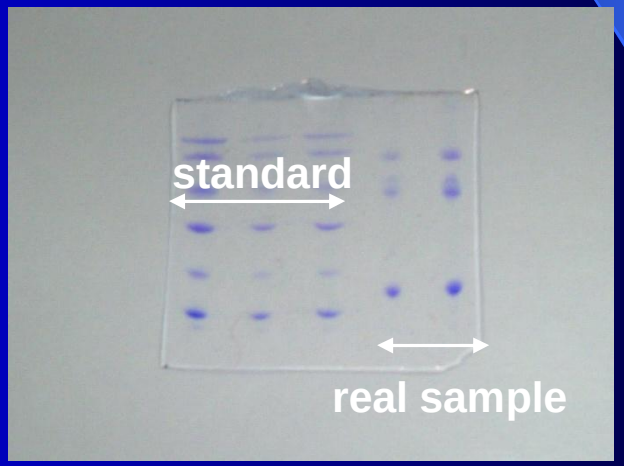


About 2000 proteins

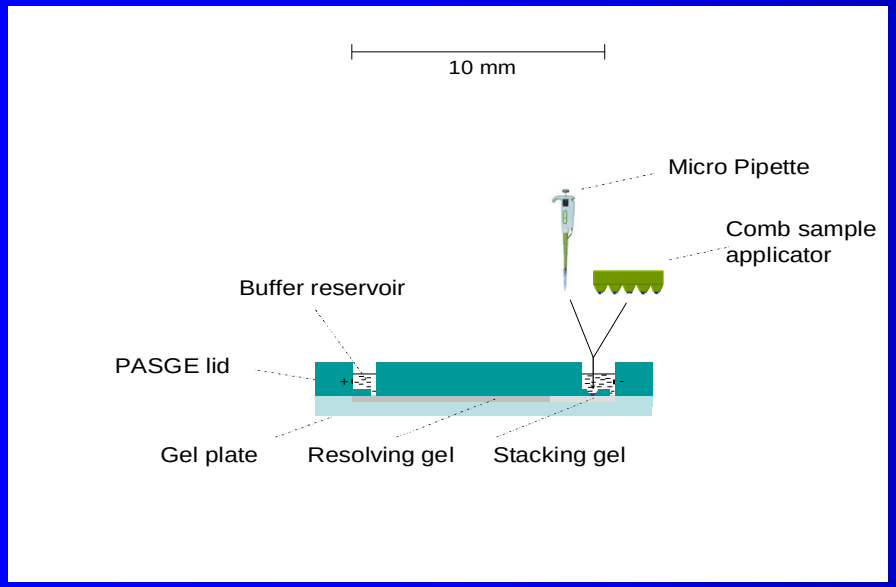
Micro gel devices

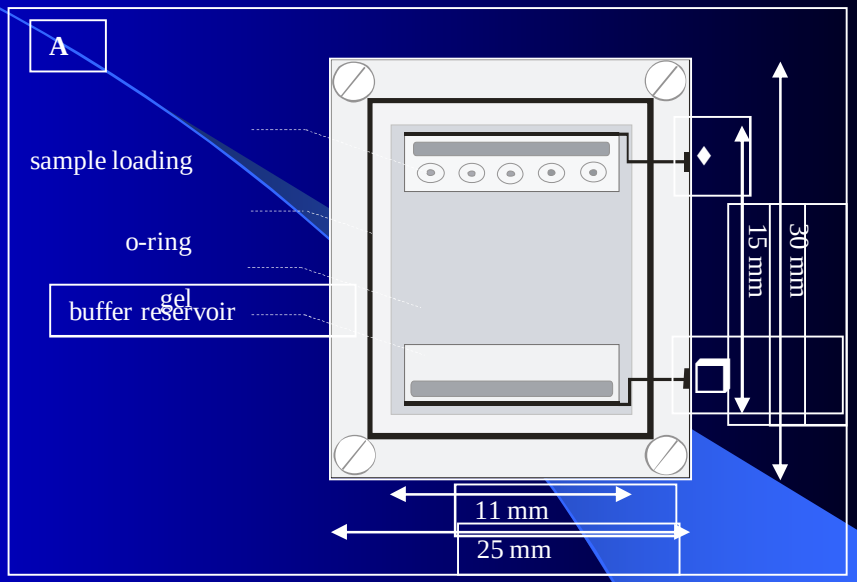


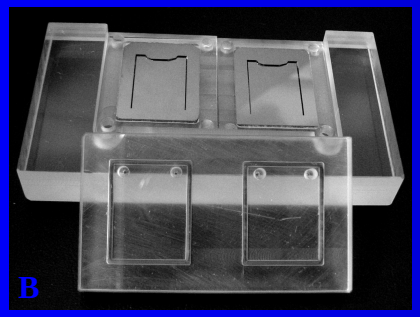


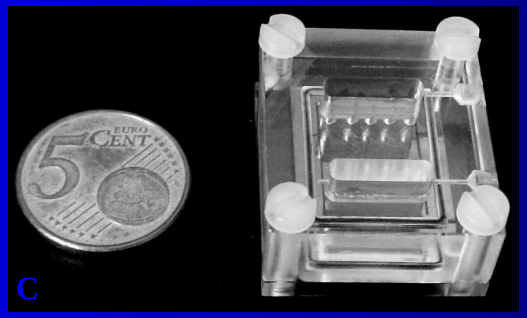


Running time 10 minutes


10 mm
Micro Pipette
Comb sample applicator
Buffer reservoir
PASGE lid
Gel plate
Resolving gel
Stacking gel


A
sample loading
o-ring
gel
buffer reservoir
15 mm
30 mm
11 mm
25 mm


B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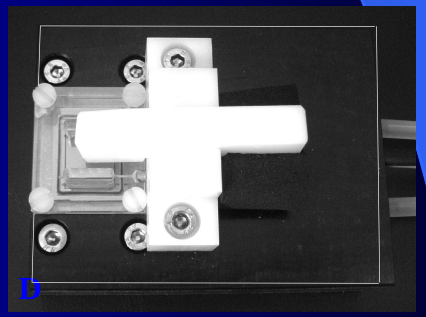
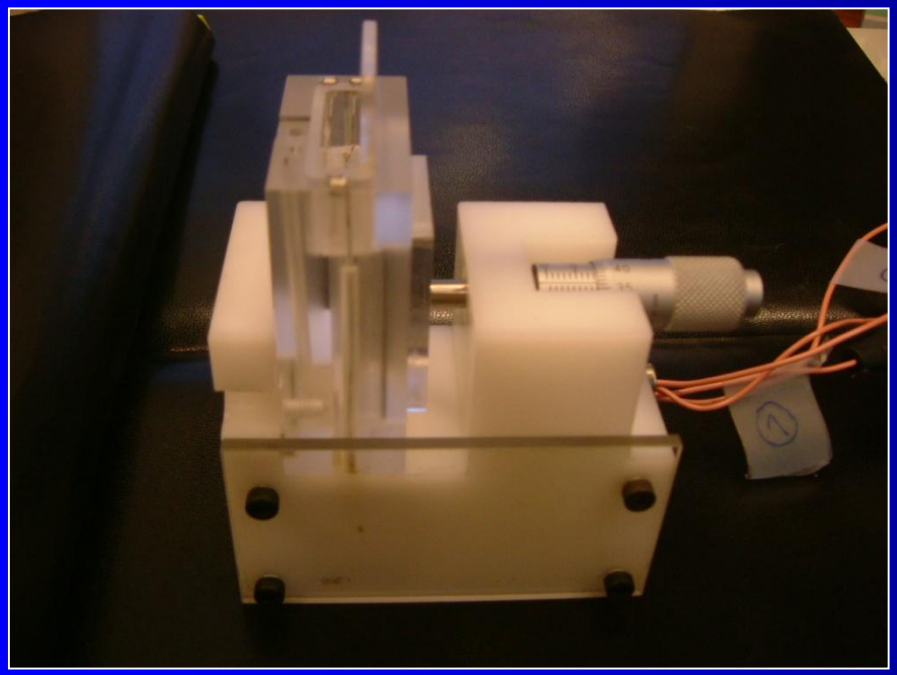
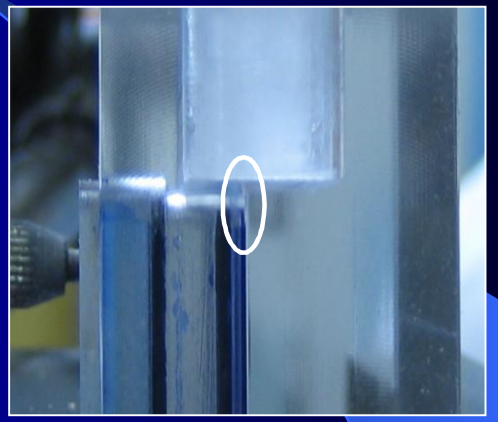
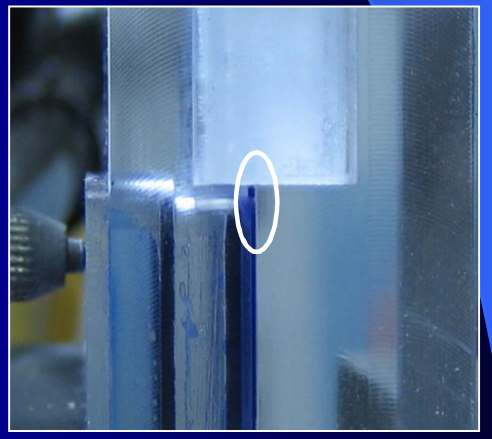
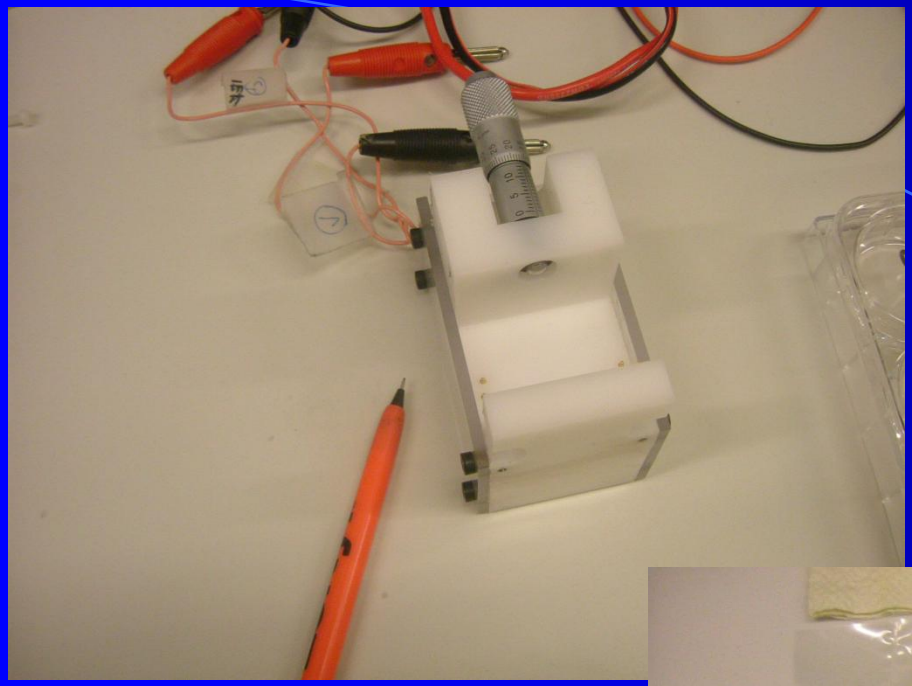

D

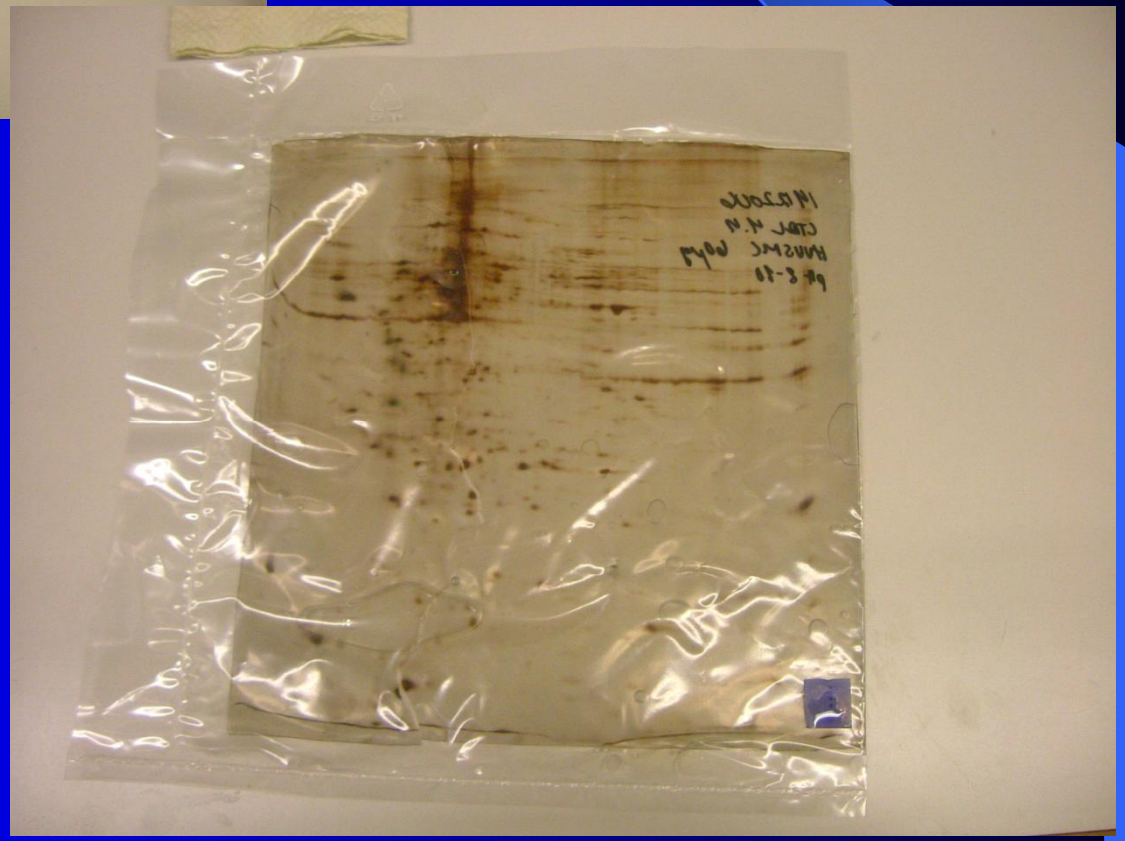
Automated 2D devices



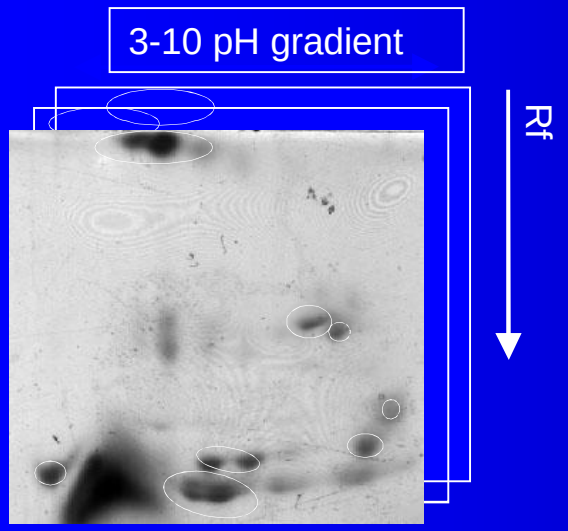








2-D map of IEF standards



Repeatability of 2-DE runs

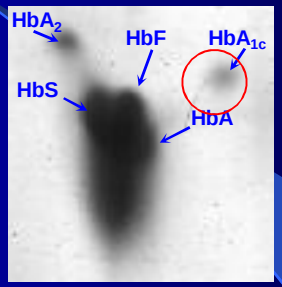
Rf values (%)*		**pI position errors(%)***	
STDV	6,1	STDV	2,5
max	15	max	6
min	0,8	min	0,6

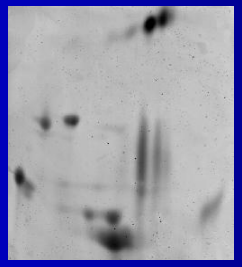
* comparison of 3 gels

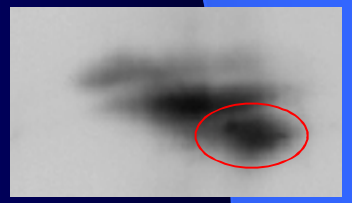
- 2-DE separation completed in approx. 80 min
- Limit of detection is approx. 65 ng

Running time 20-30 minutes

- Performance
  - Native IEF and native PAGE
    - 5 variants of hemoglobin
    - pH 6.7 -7.7
  - Native IEF and SDS-PAGE
    - standard IEF proteins
    - pH 3-10
  - Denatured IEF and SDS-PAGE
    - GFAP protein variants expression differences
    - in control and Alzheimer diseased patients
    - pH 4-6



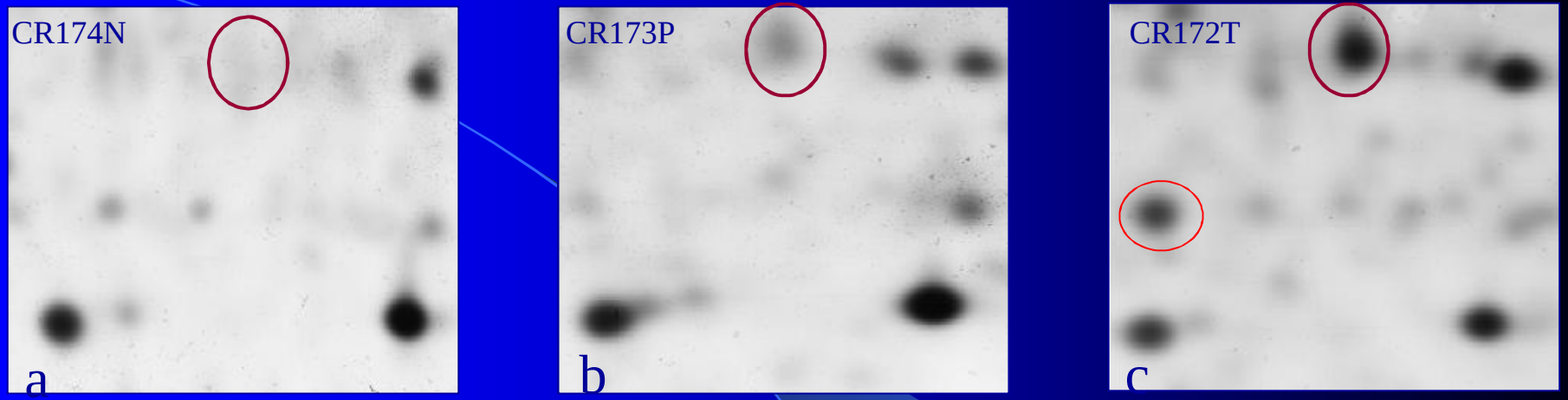




control

AD

Intra-individual expression differences of Cytokeratin 20 in patient 14.

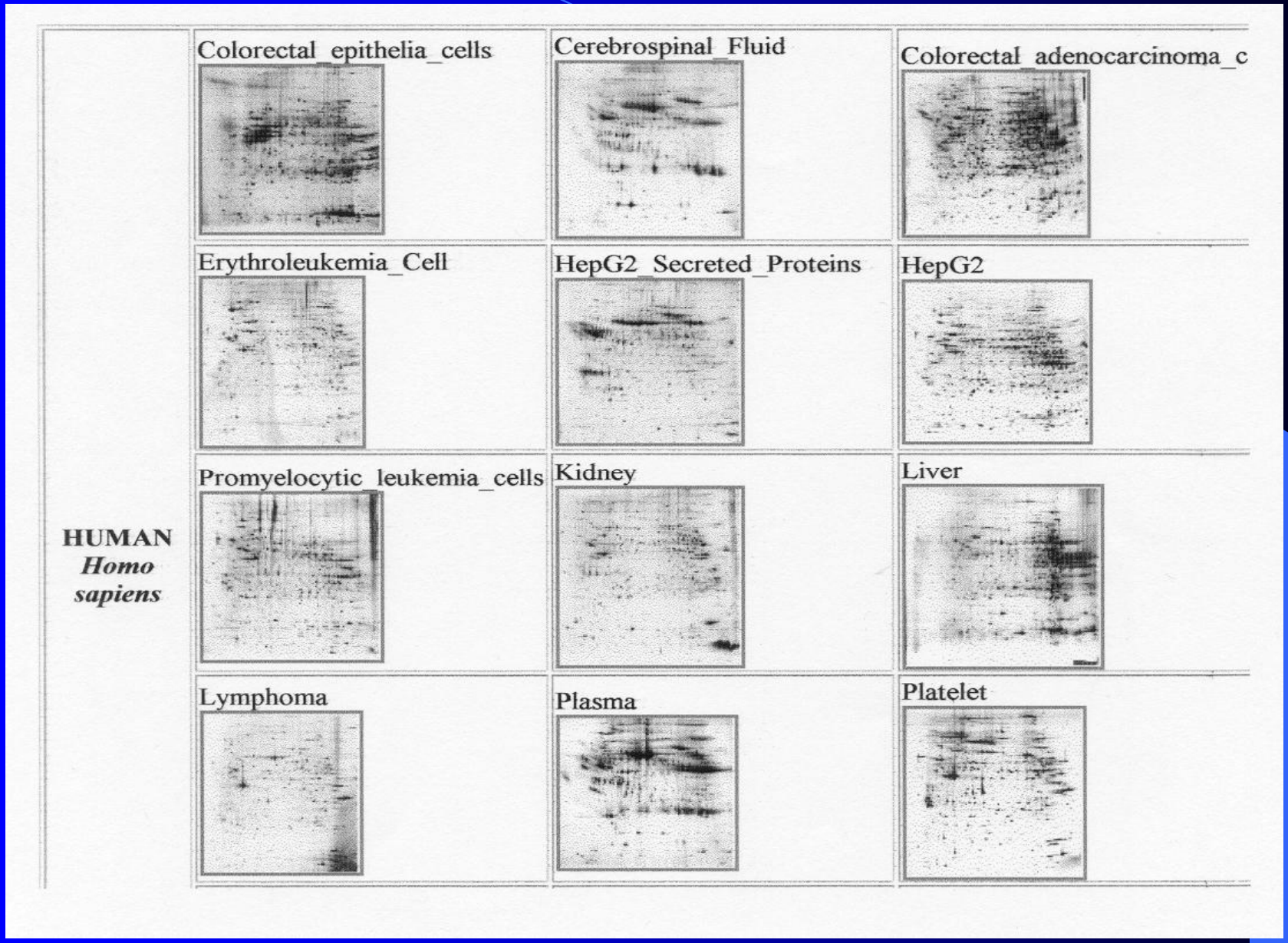


The left gel segment (a) is zoomed from the normal mucosa,

gel b represents the patients polyp and

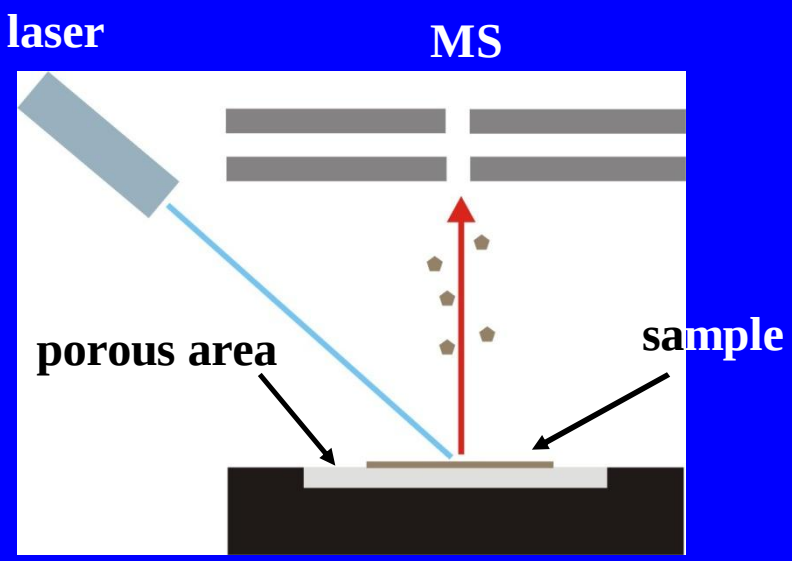
gel c is the corresponding segment of the same patients adenocarcinoma

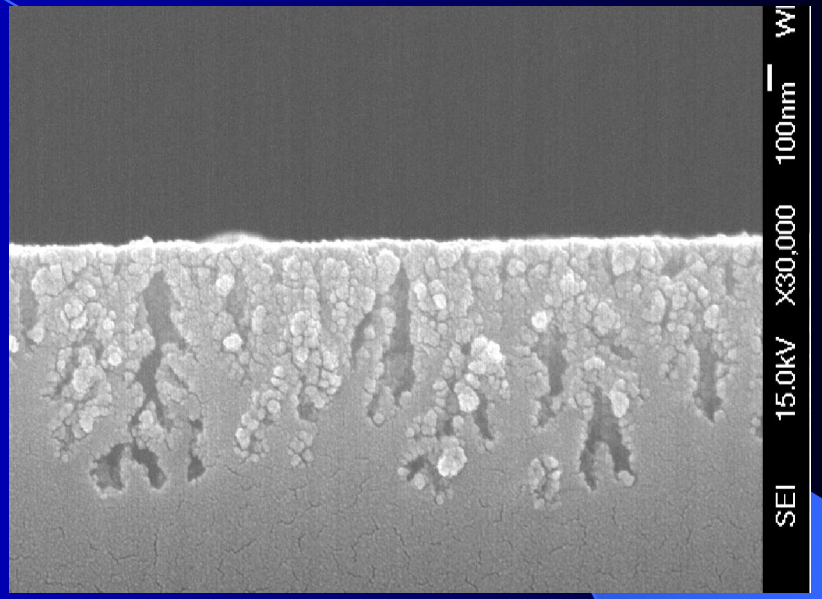
Also 2D Databases exist!

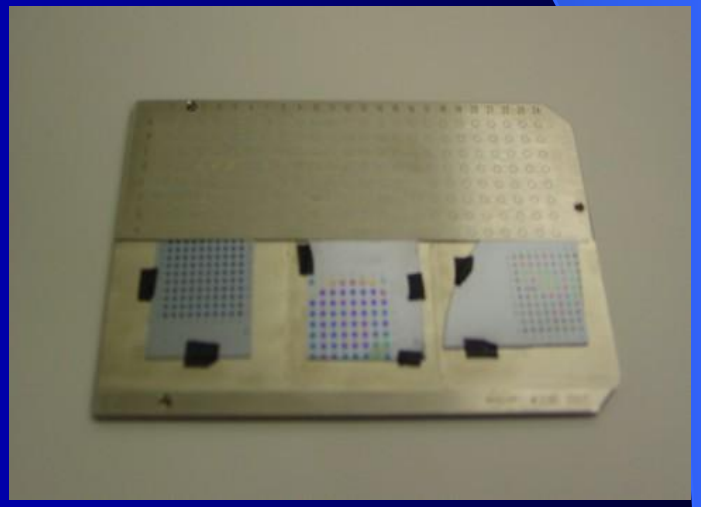


What about chip-based proteomics: DIOS-MS

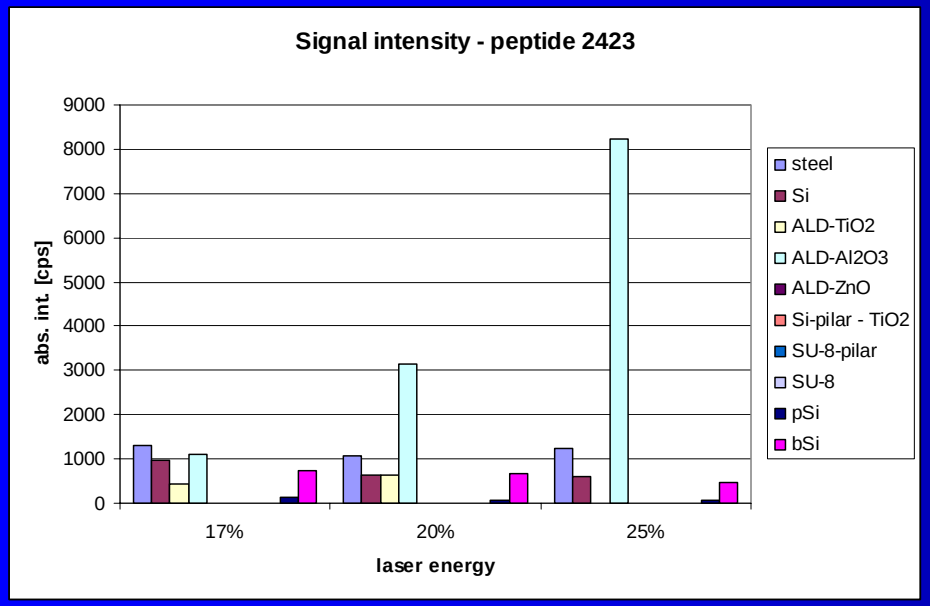
Desorption Ionization On Silicon (DIOS)

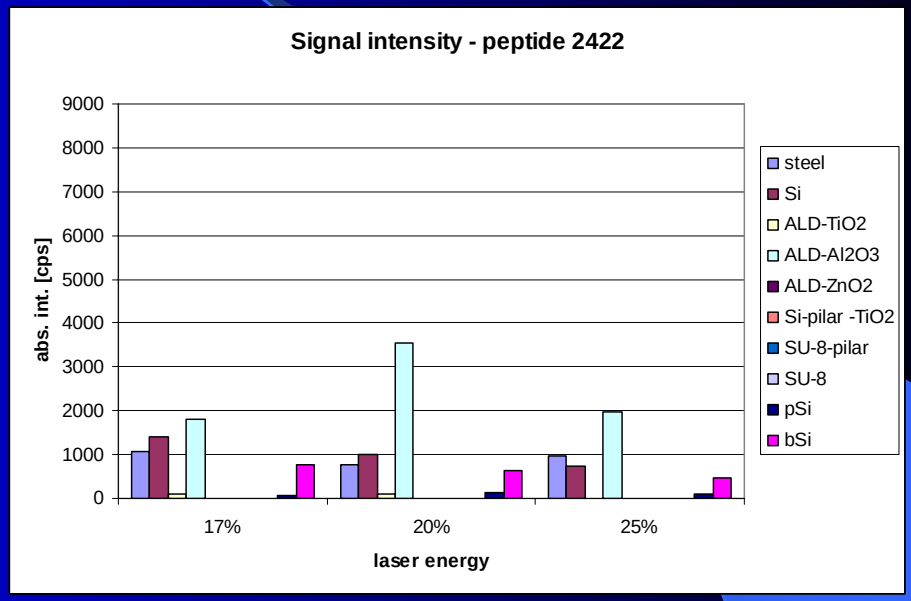






Testing the functionality of various surfaces for proteomics

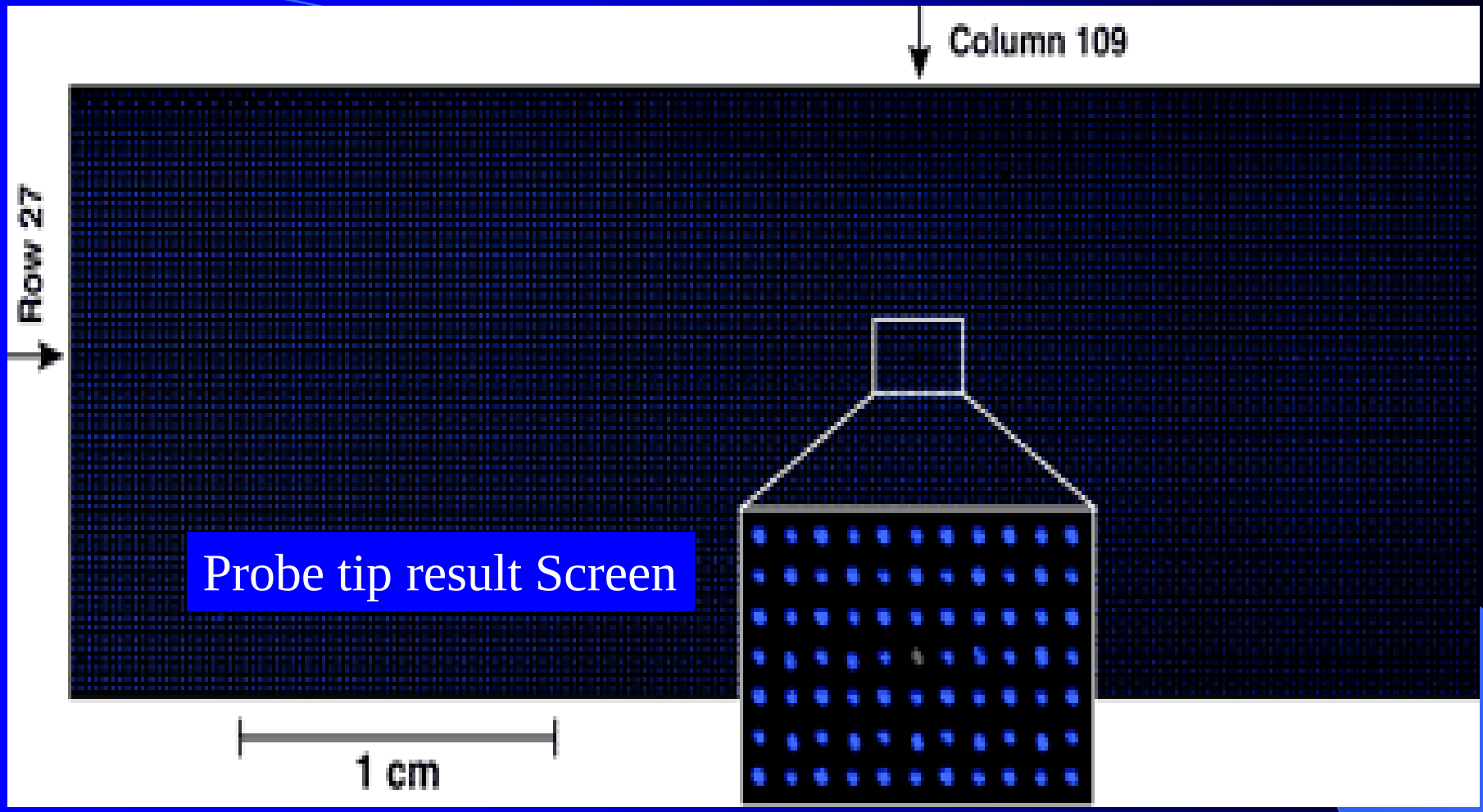




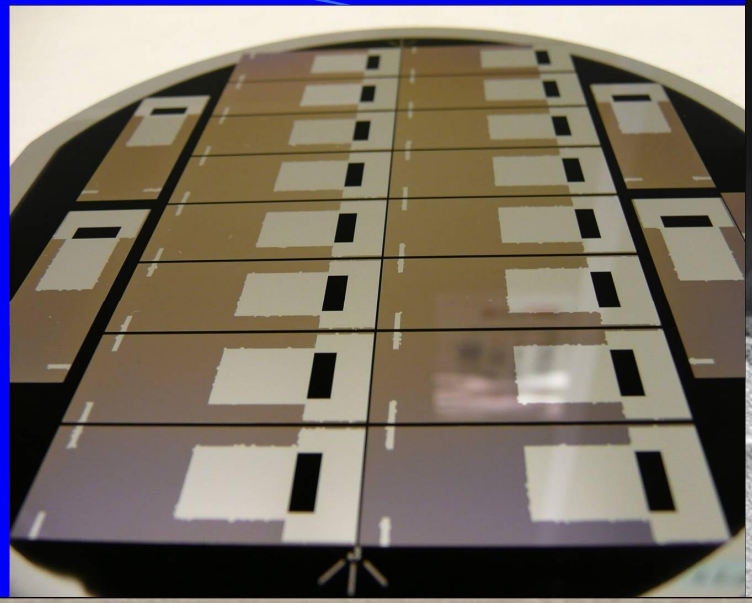
Signal intensity of unphosphorylated peptide 2423 (left) and phosphorylated peptide 2422 (right) from different surfaces.

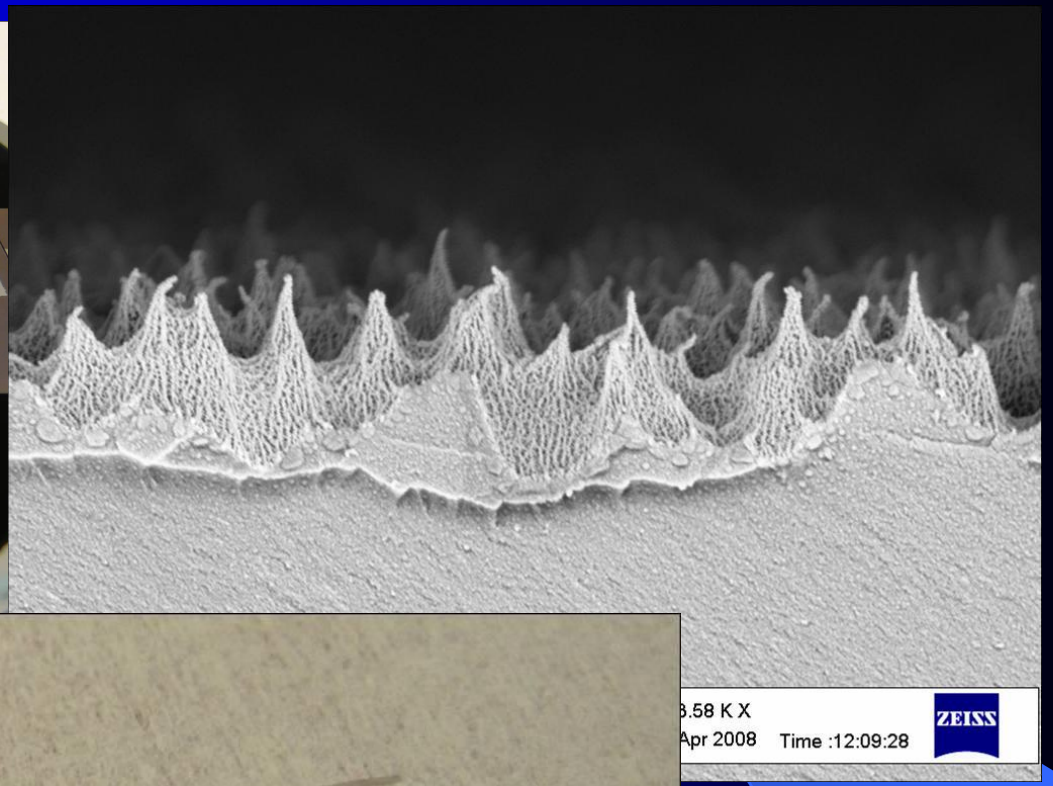
Black Silica chip

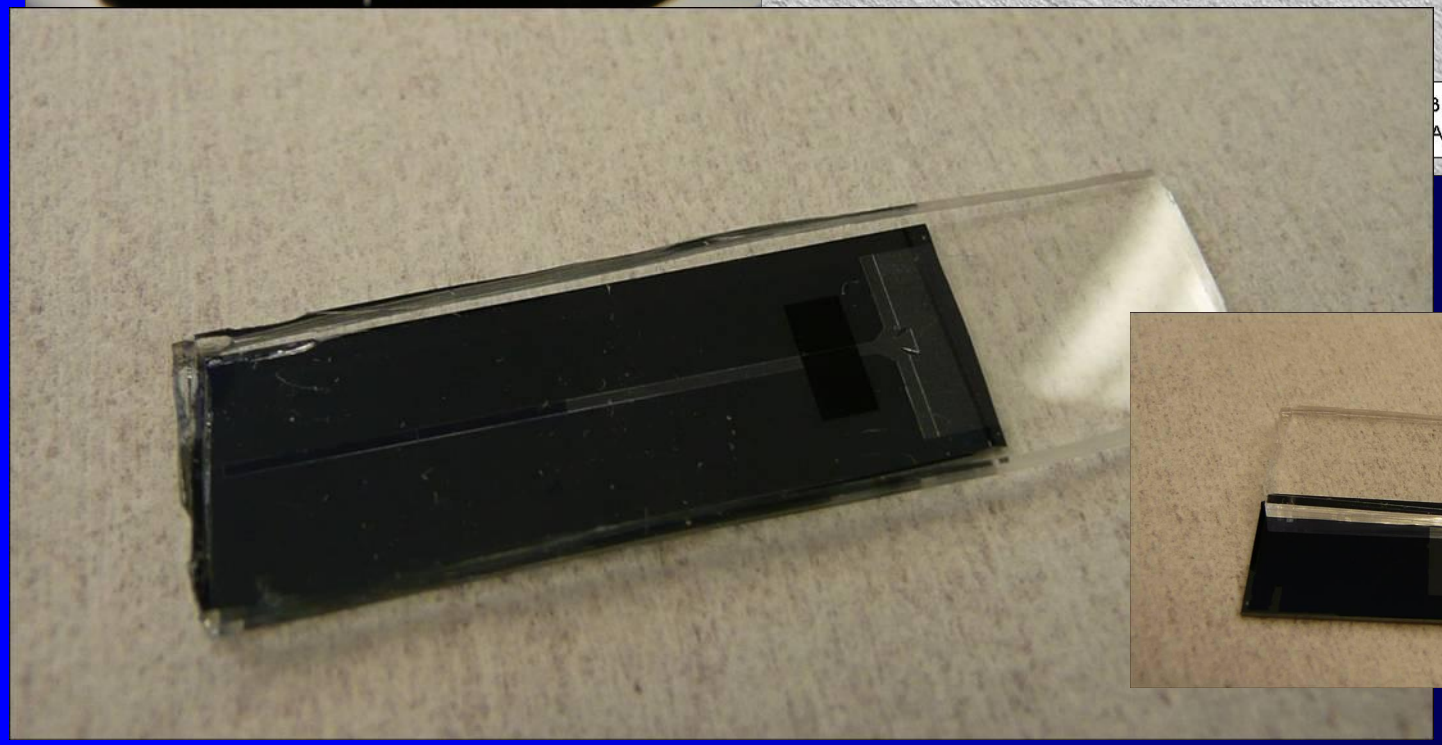
384 samples (e.g. phosphopeptides)

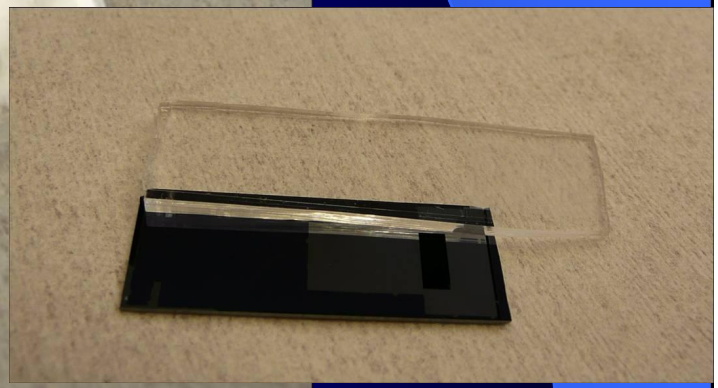


10800 samples on the chip



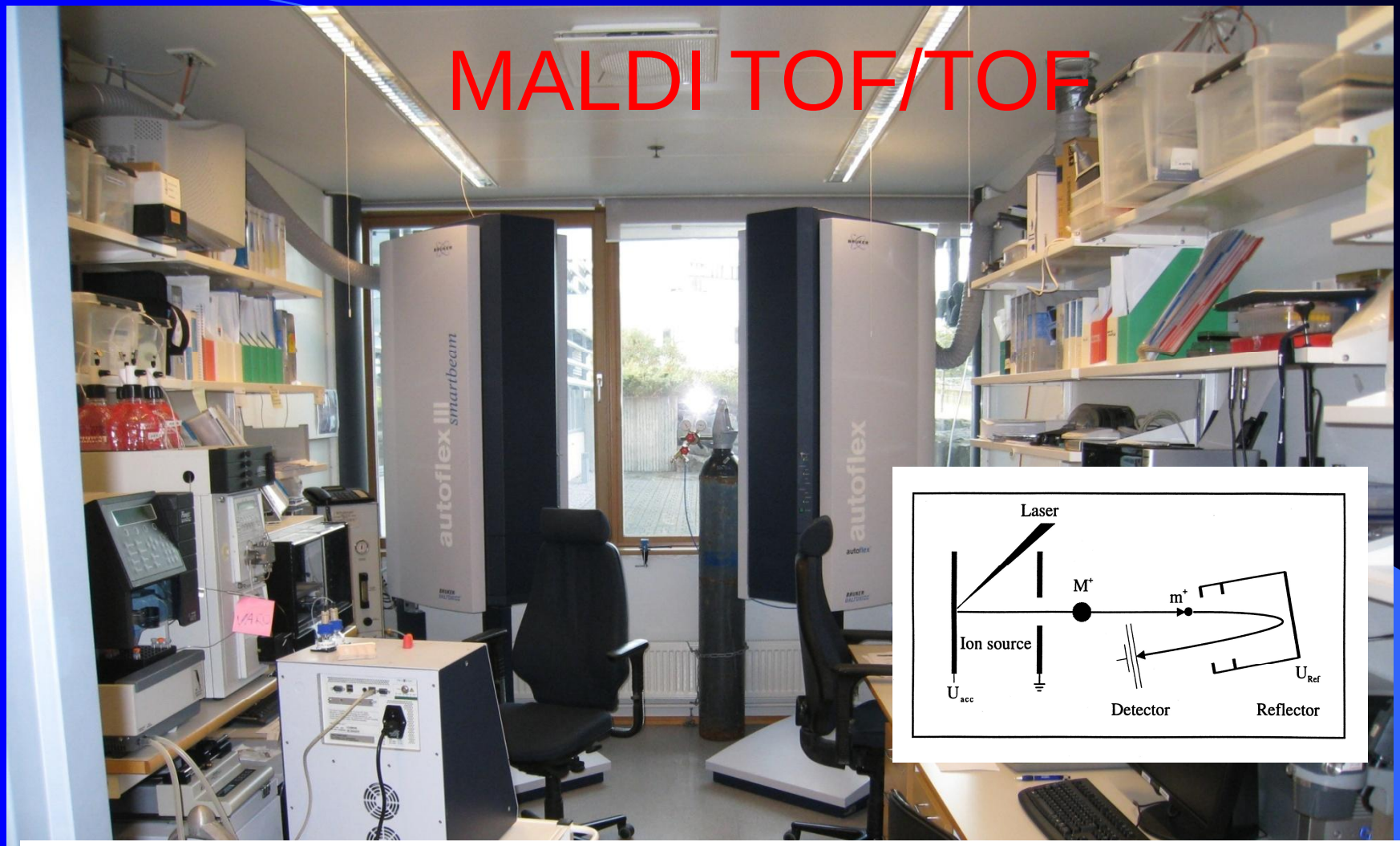

.58 K X
Apr 2008
Time :12:09:28
ZEISS





High Sample Throughput for the Post-Genomic Era

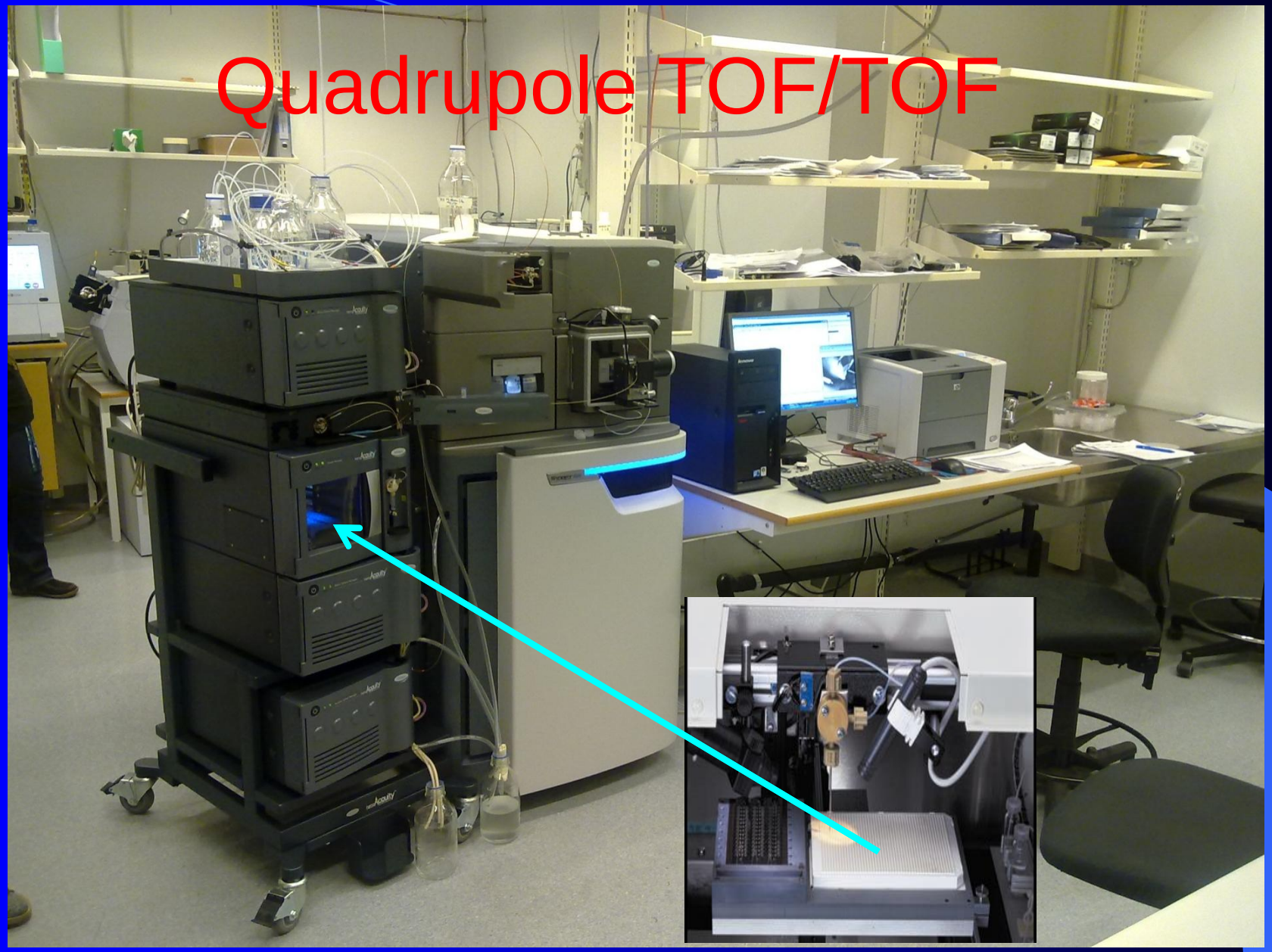
MALDI TOF/TOF



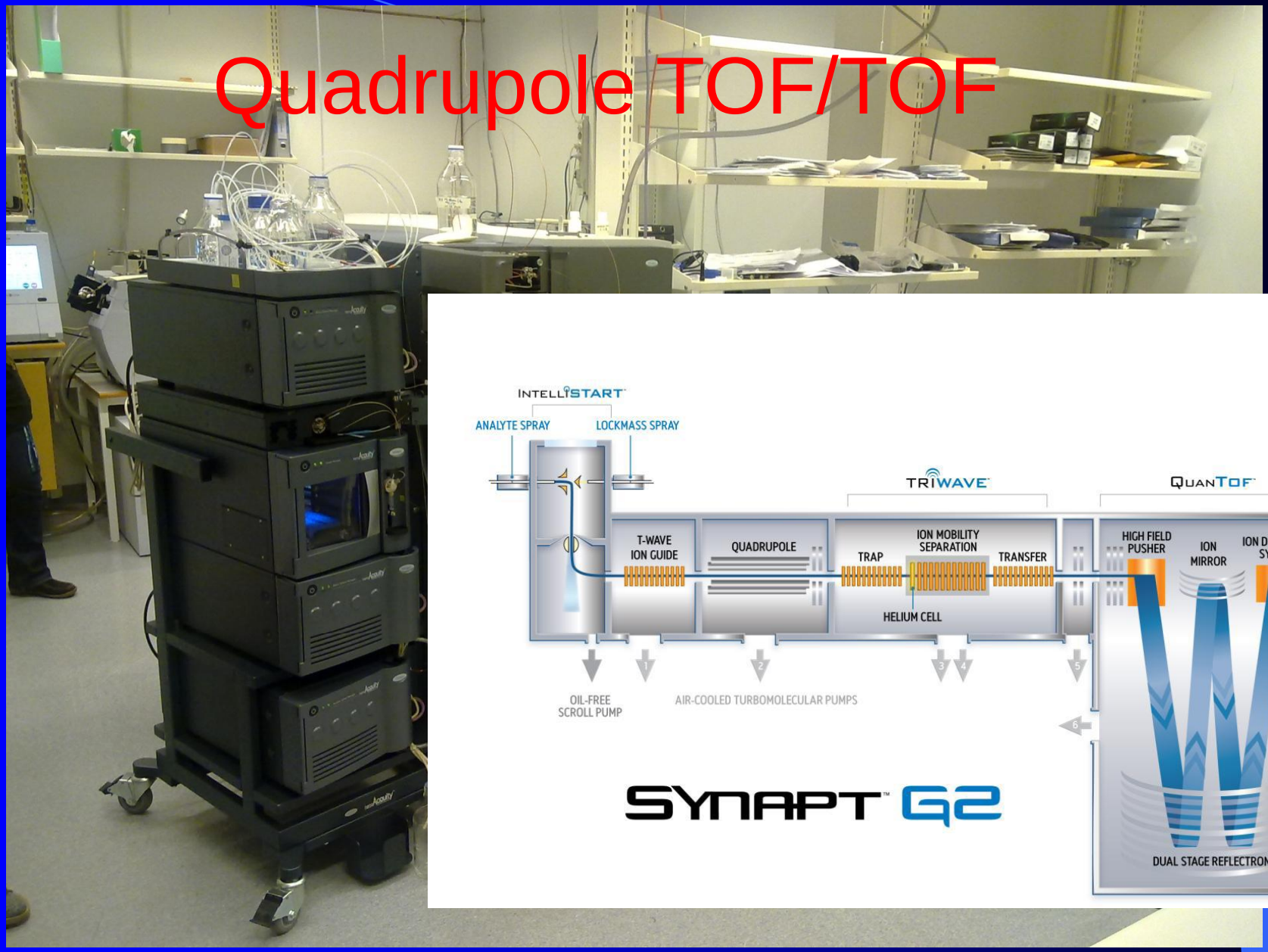
Schematic diagram of a Reflector MALDI-TOF mass spectrometer.

MALDI-TOF = Matrix Assisted Laser Desorption/Ionization - Time Of Flight

Quadrupole TOF/TOF



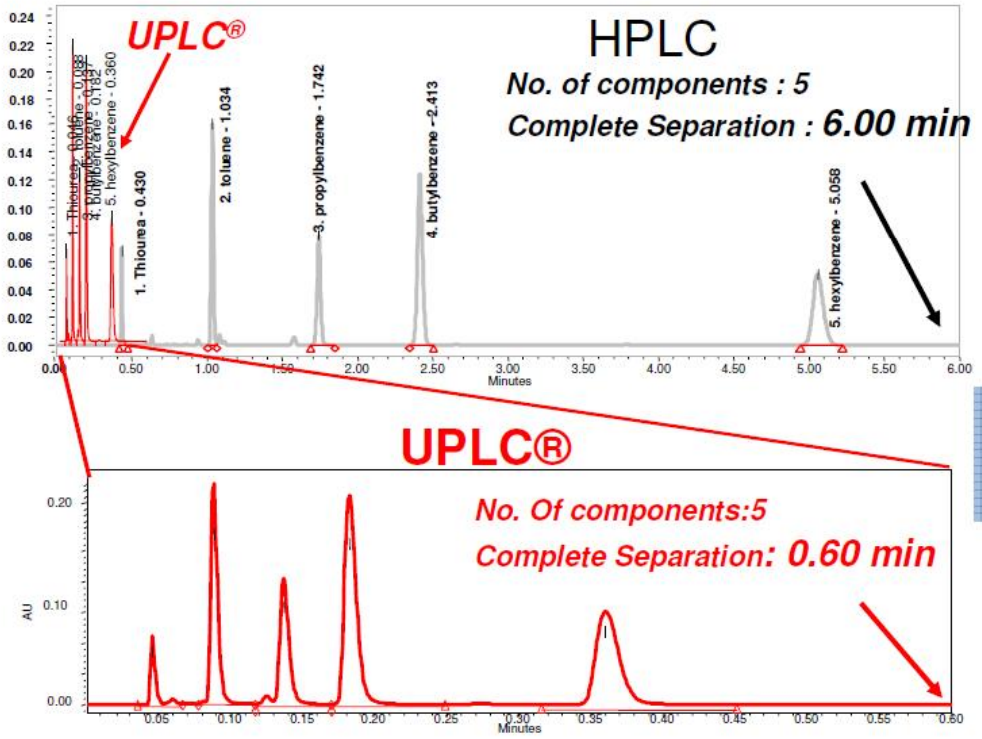
Quadrupole TOF/TOF



Even with the most sophisticated LC-based MS instruments of today we will possibly not be able to use them for high-throughput clinical screening?

Although the time for one analysis is fast…

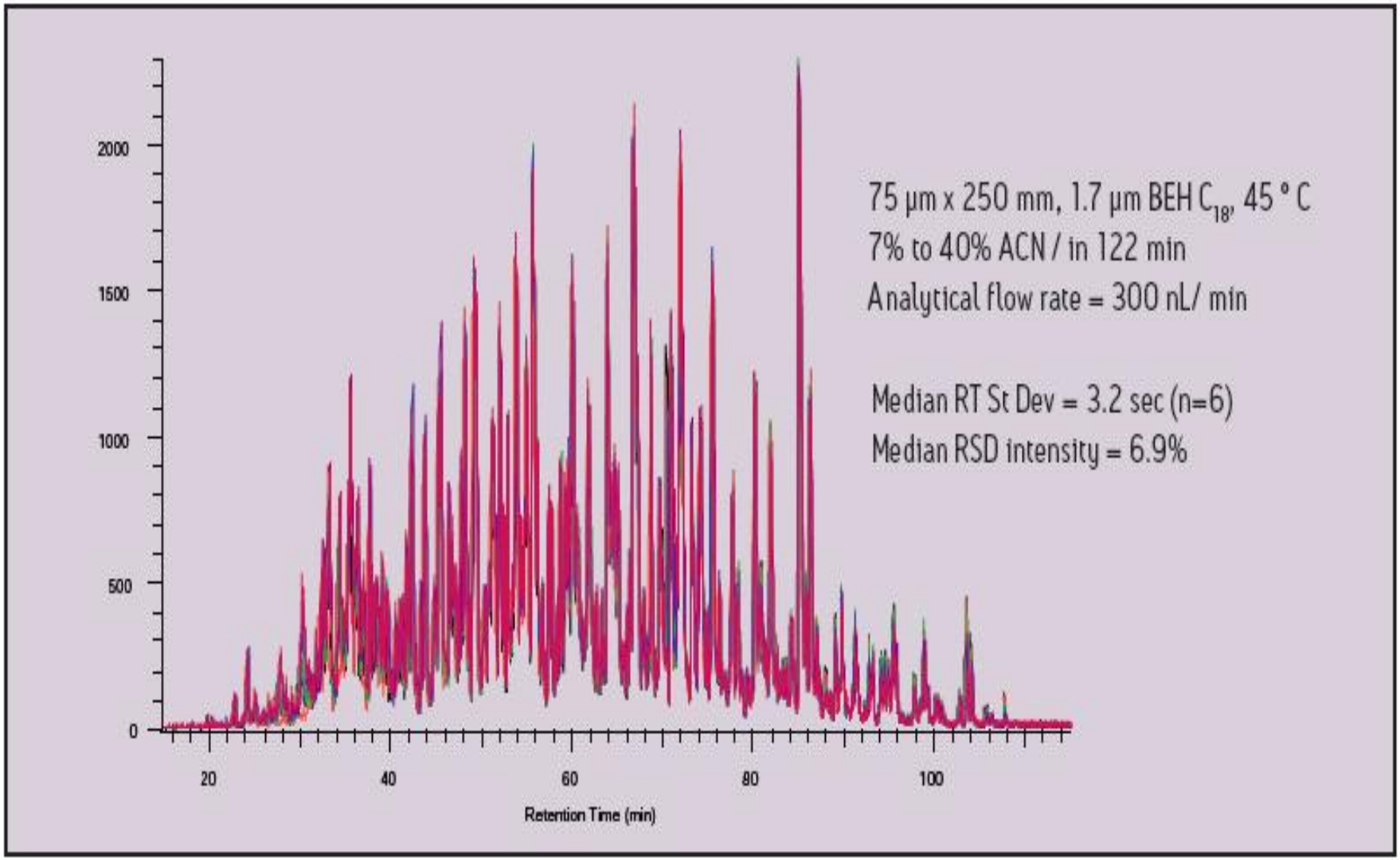
UPLC™ increases Speed by 9X (900%)



Increased sample throughput

©2009 Waters Corporation

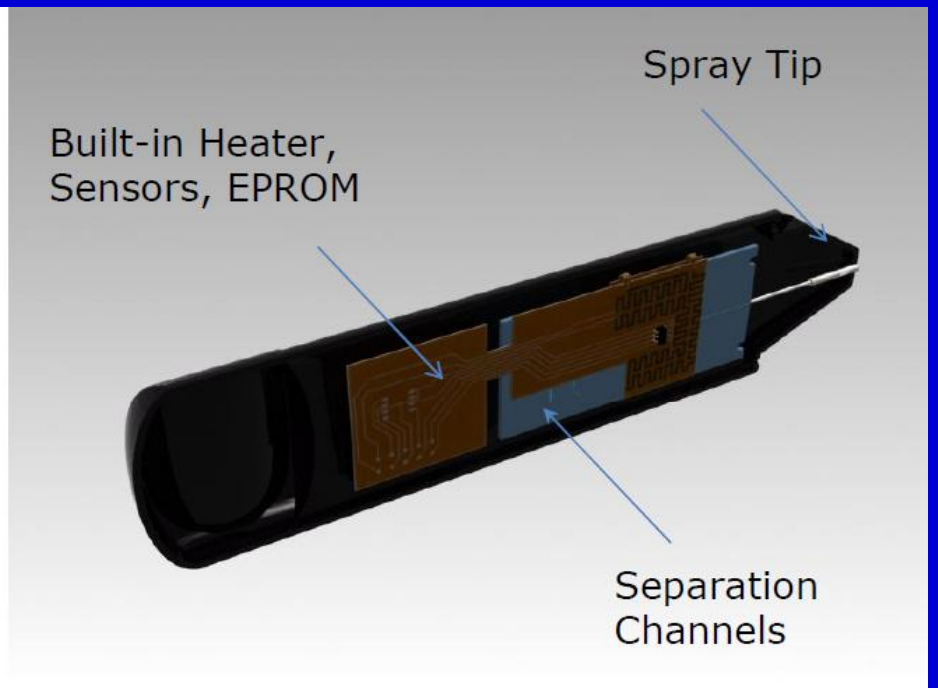
And you can analyze thousands of compounds in a few hours...



Proteomics chip technology

The whole 2D LC can be done in a chip

- Sub-2-micron chromatographic performance
- Eliminates manual connections
- Low system volumes
- Integrated emitter and electronic components



TRIZAIC™
UPLC SYSTEM

The whole 2D LC can be done in a chip

- Sub
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Tip

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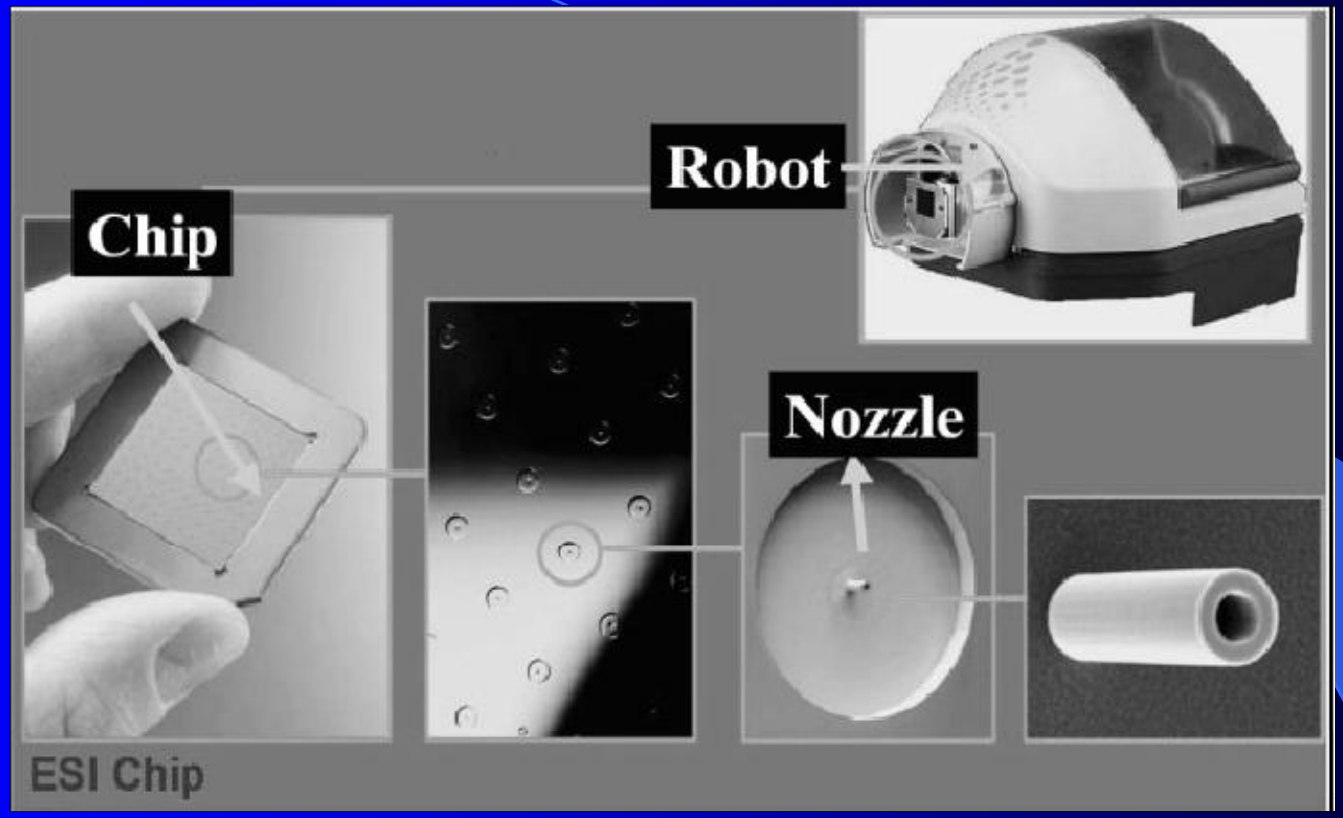
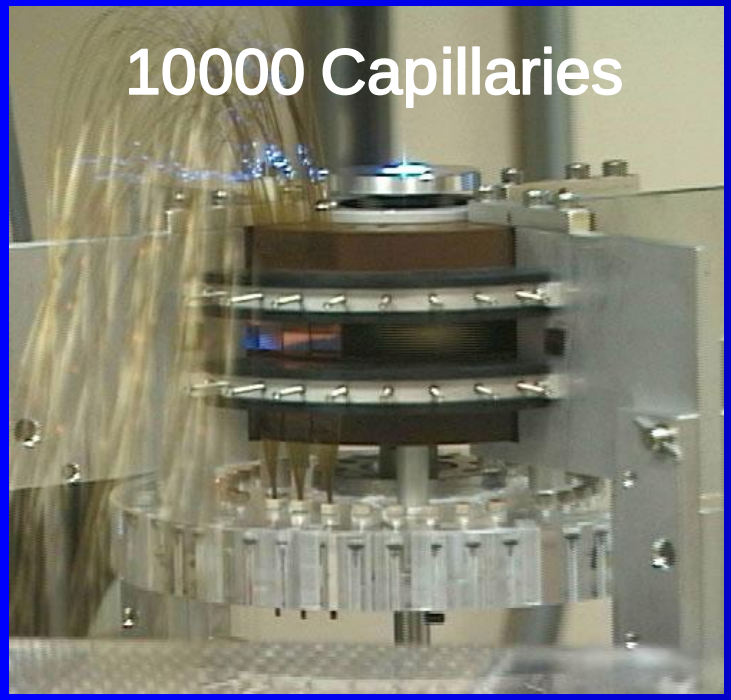
ls

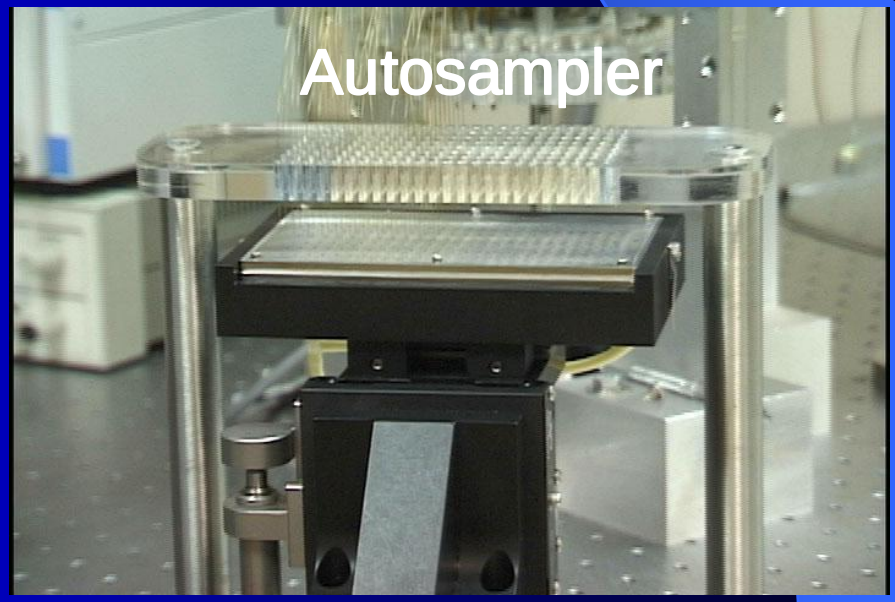
NanoSpray LC/MS



High throughput CE



LED detection (MIT, Forest et al, 2006)



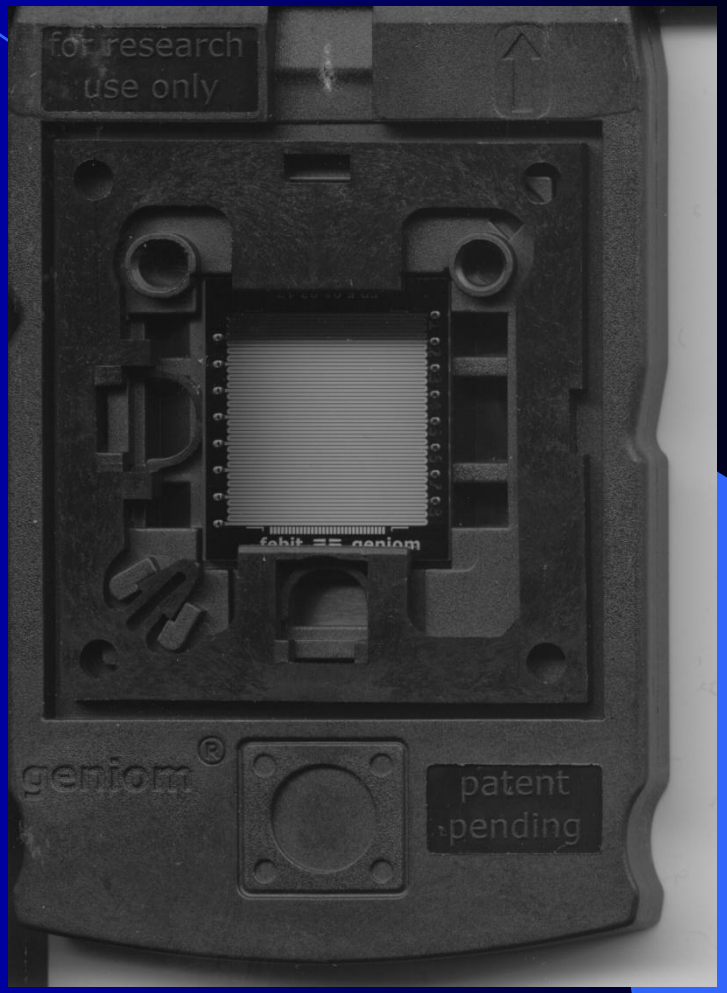
Ultra-high throughput CE chips for proteomics

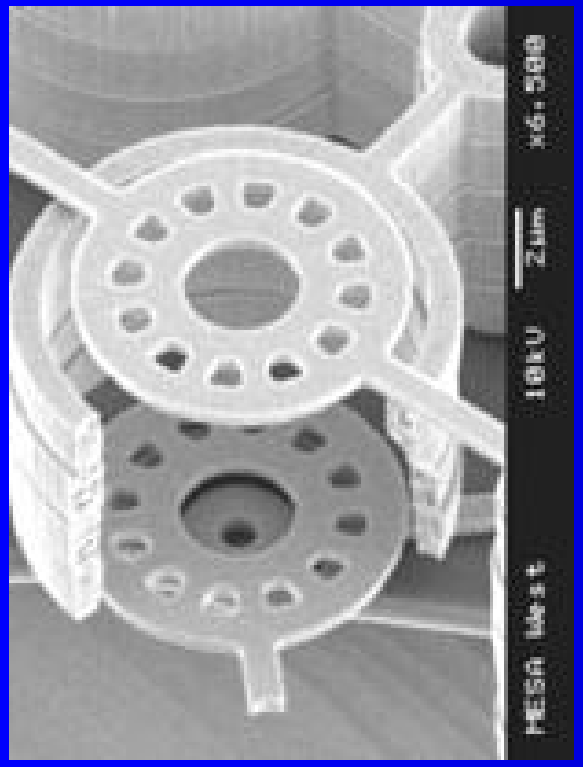
Geniom chips

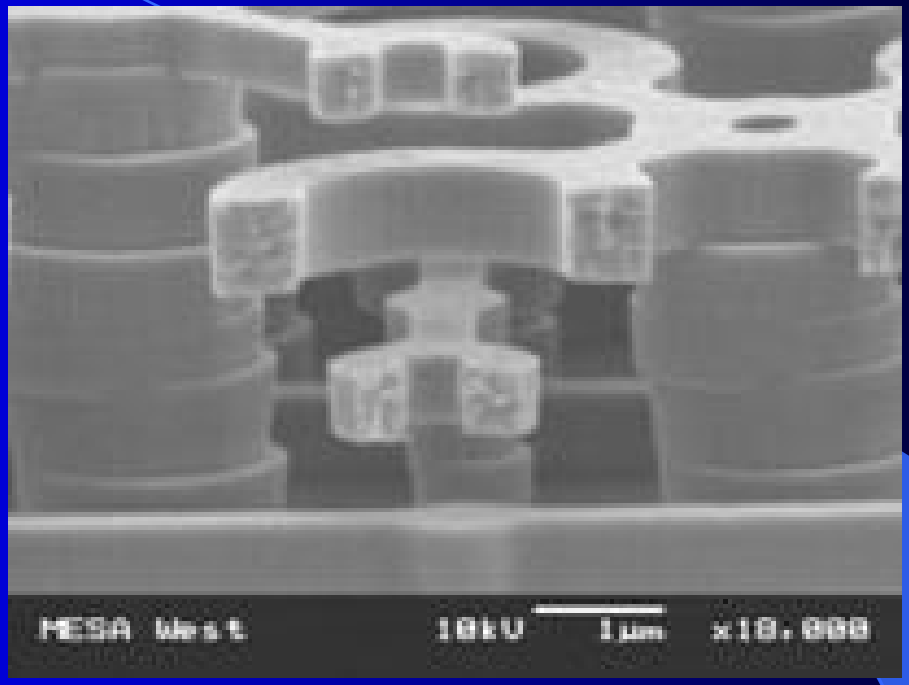
-Currently for DNA/RNA analysis

(Development ongoing for proteome analysis)

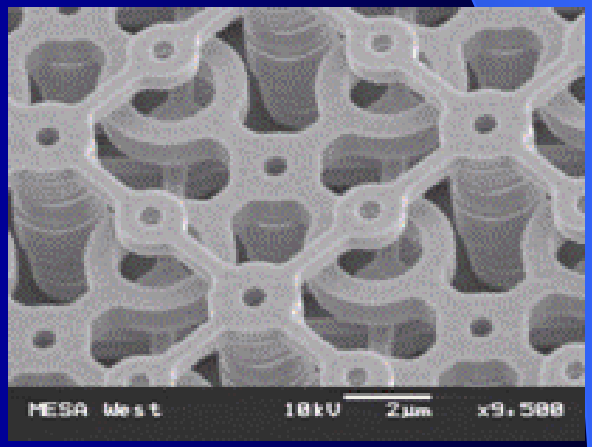
Capacity approx. 40000 ligands







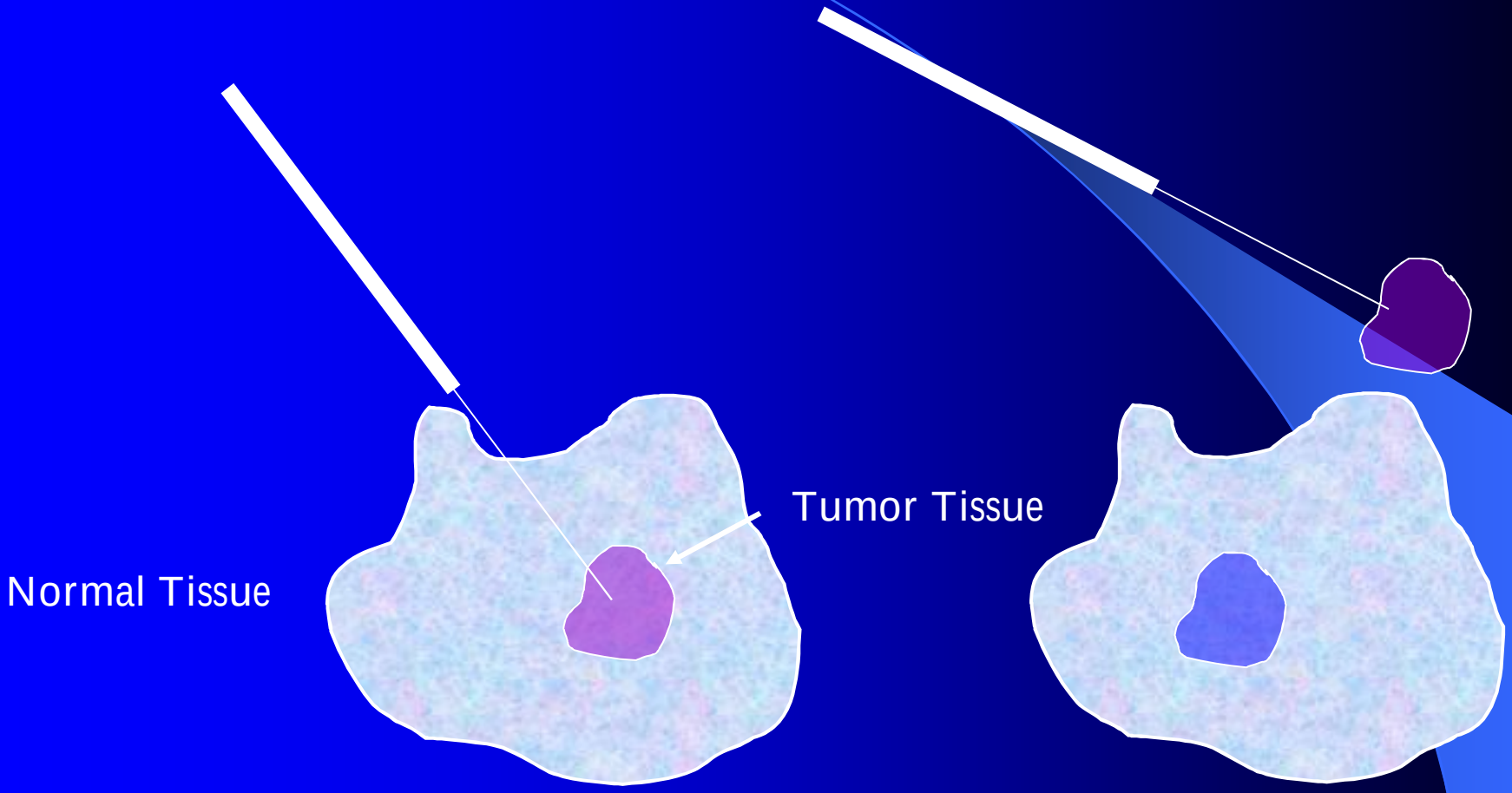
A set of thousands of Mass Spectrometers in one chip



Other techniques to help in proteomics?

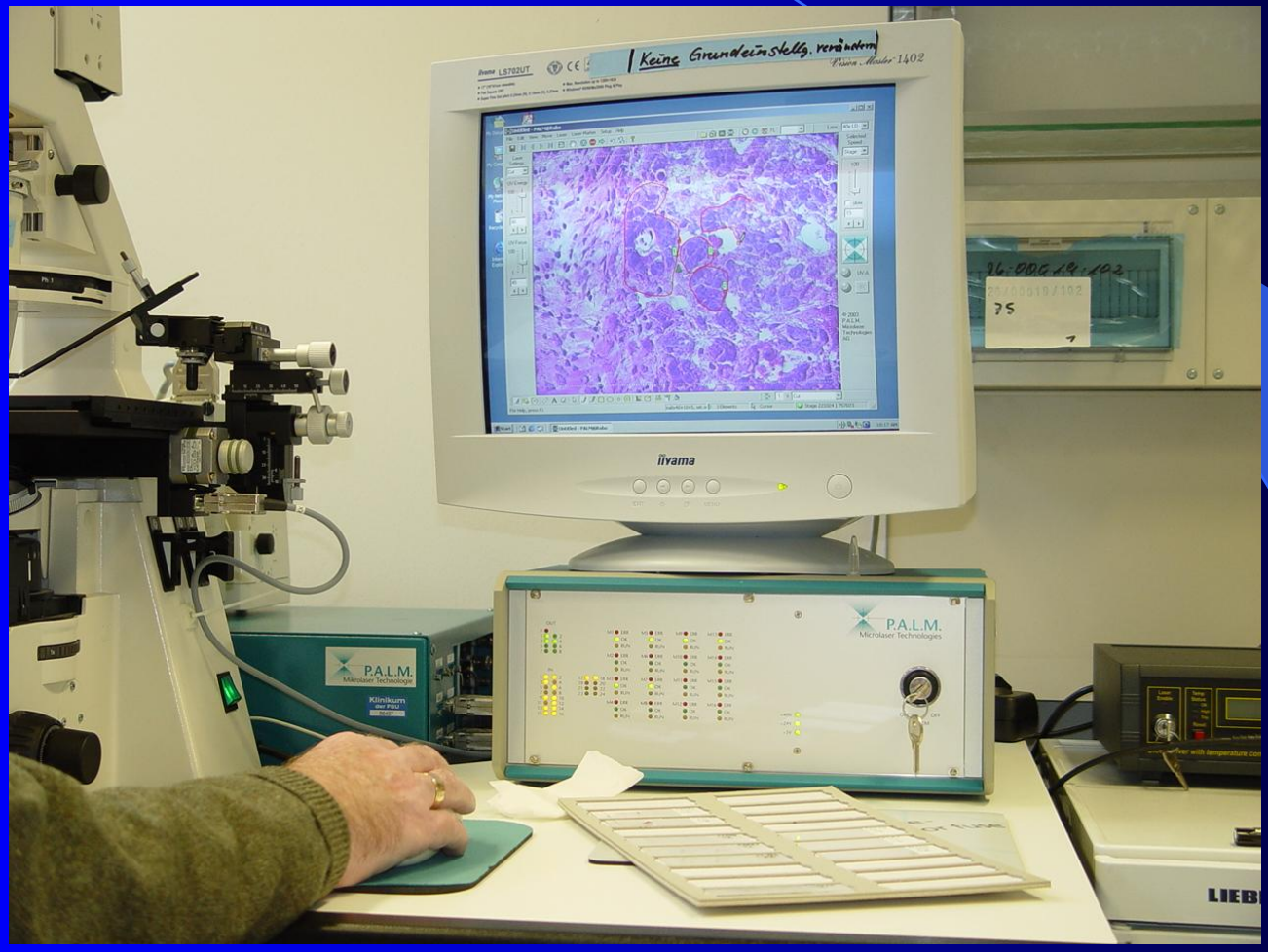
- Tissue microdissection
- Imaging MS

Why Tissue Microdissection?



You would like to isolate only the targeted diseased tissue

Laser-based microdissection:

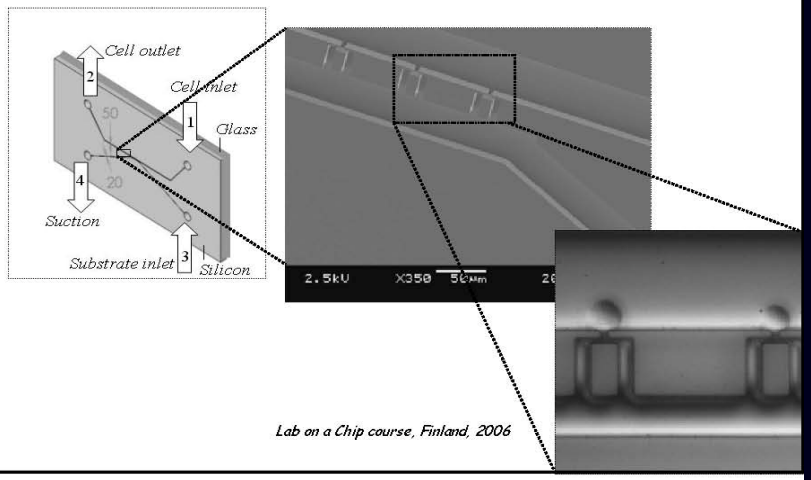


Capturing of the vessels in the control brain slide

MALDI MS analysis directly from the tissue captured on the cap membrane

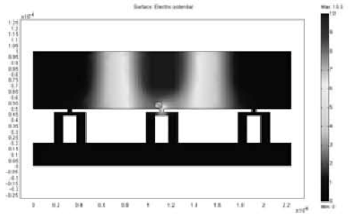
Whole Cell and Protein Microarray Chip structures

Electroporation on a chip: Cell trapping



Lab on a Chip course, Finland, 2006

Single cell electroporation

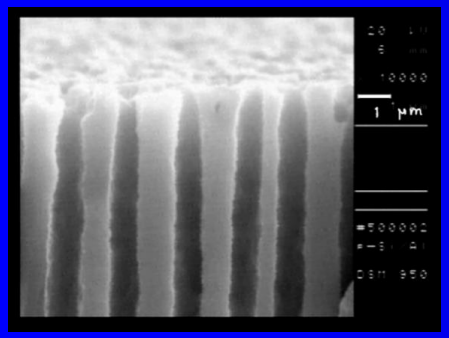


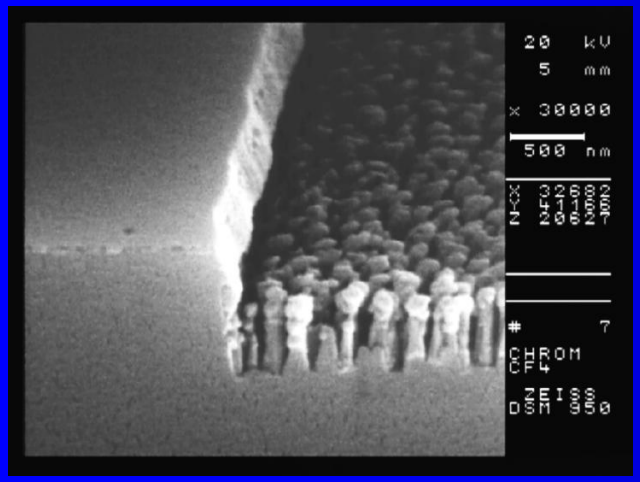
Gene transfection → GFP expression

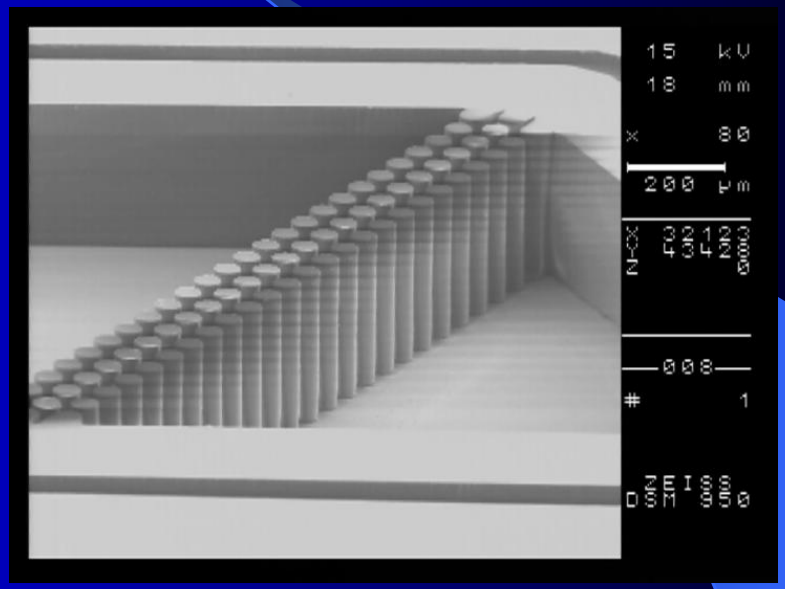
H. Andersson and A. van den Berg, "Microfluidic devices for cellomics: a review", *Sensors and Actuators B*, **92 (3)**, (2003), 315-325.

Lab on a Chip course, Finland, 2006

Cell trapping and - lysis Chip structures for single Cell Proteomics?



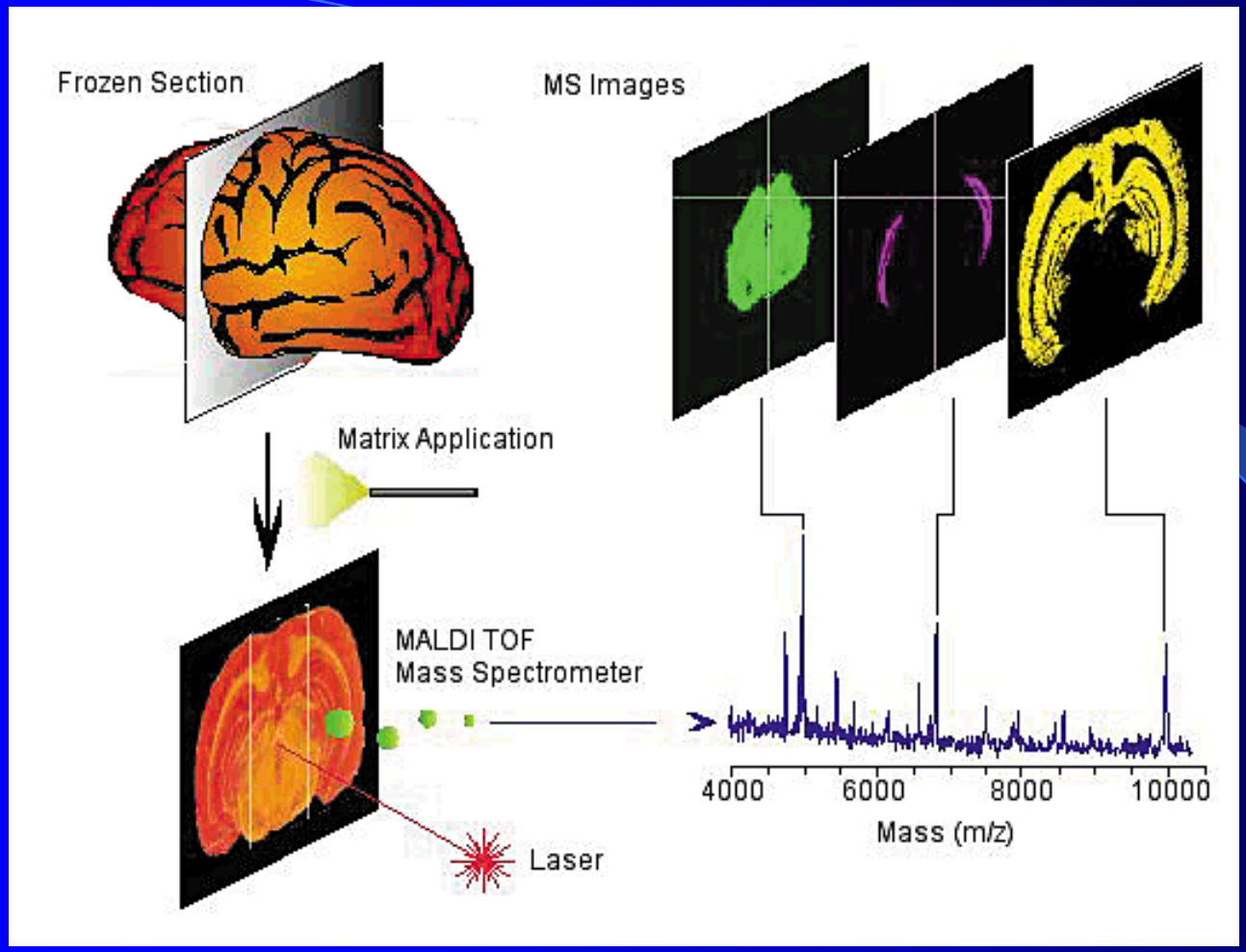


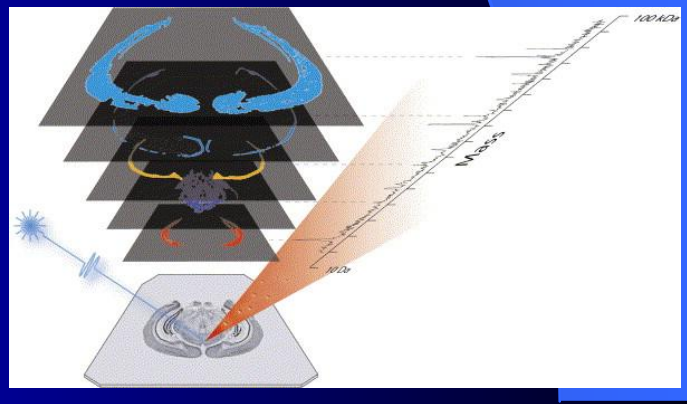


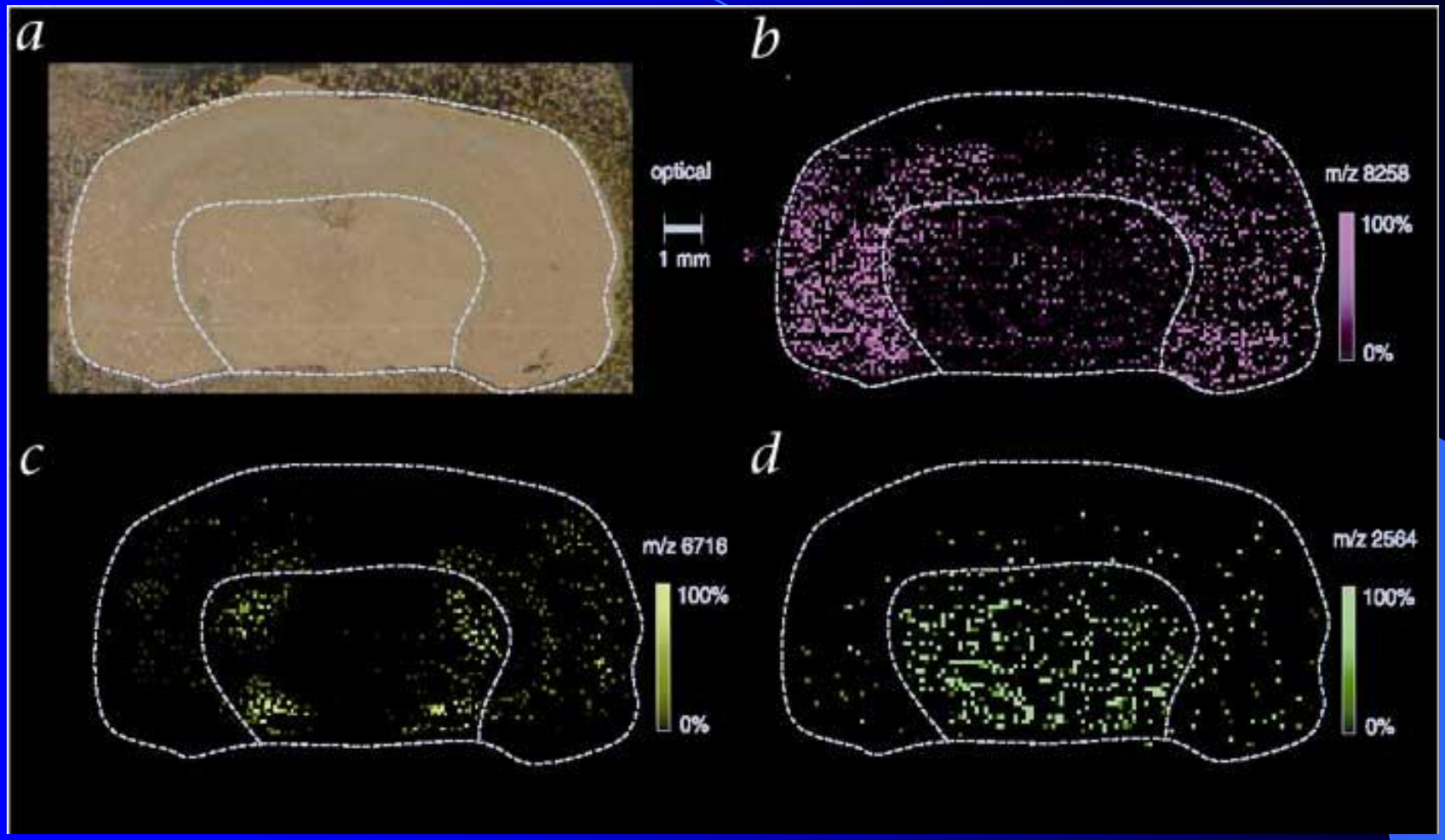
The TIME component!

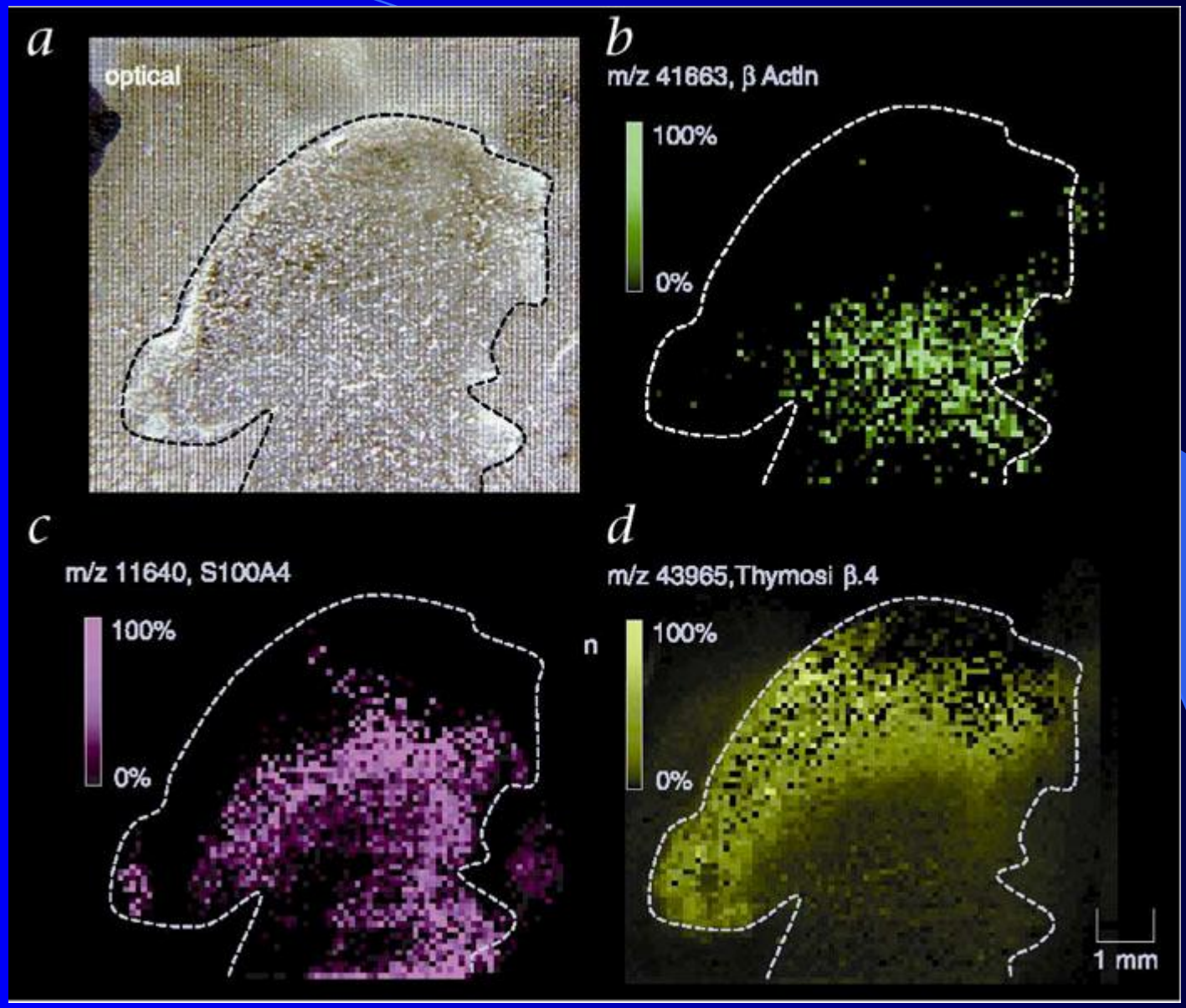
Development of Integrated Nanoliter Analysis Devices (DDTC-Viikki, Microtechnology Center-HUT, Biomedicum Helsinki)

MALDI mass spectrometric imaging of biological tissue sections for protein imaging


Frozen Section
MS Images
Matrix Application
MALDI TOF
Mass Spectrometer
Laser
4000
6000
8000
10000
Mass (m/z)




a
optical
1 mm
b
m/z 8258
100%
0%
c
m/z 6716
100%
0%
d
m/z 2564
100%
0%


a
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m/z 41663, β Actin
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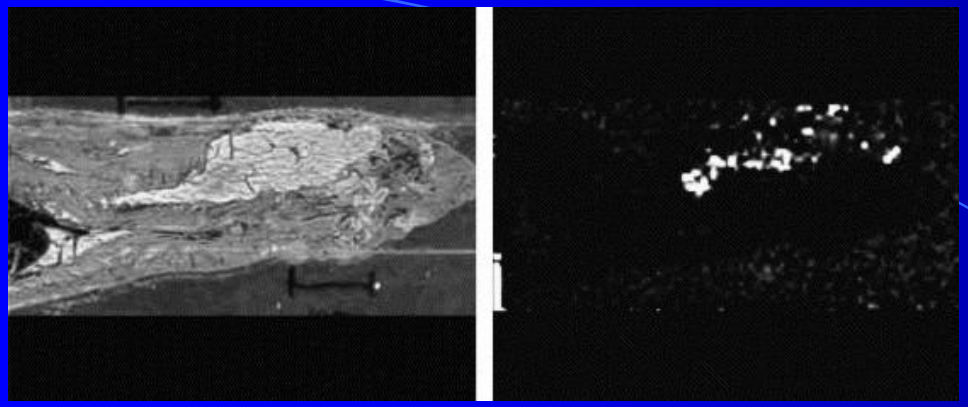
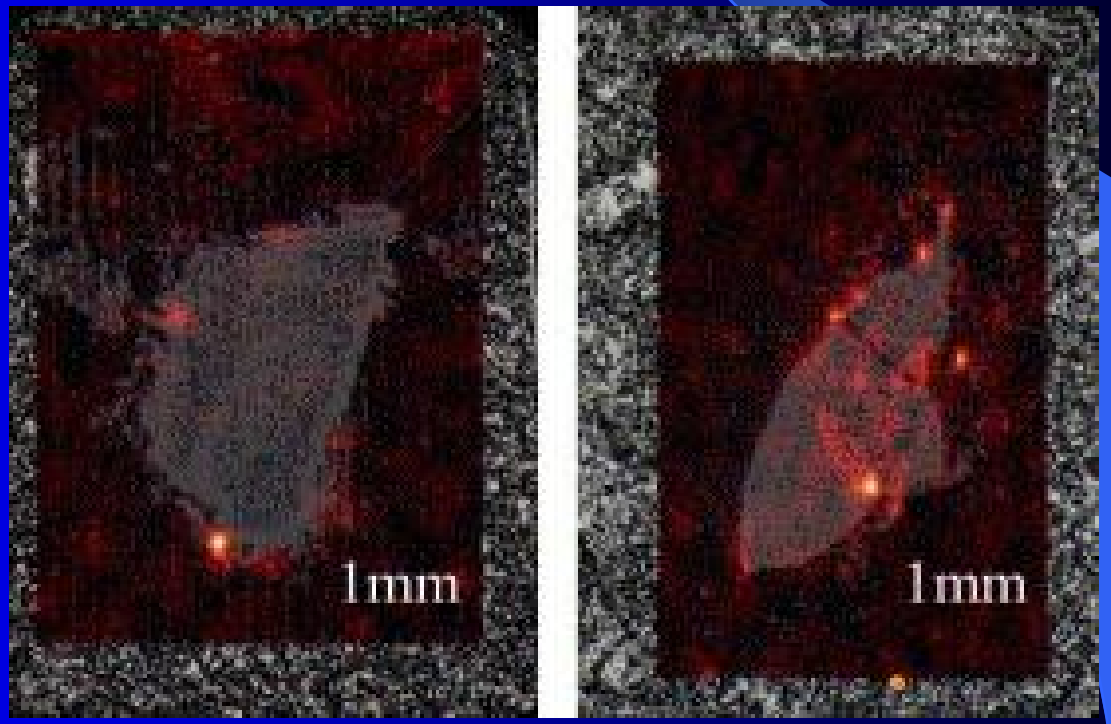


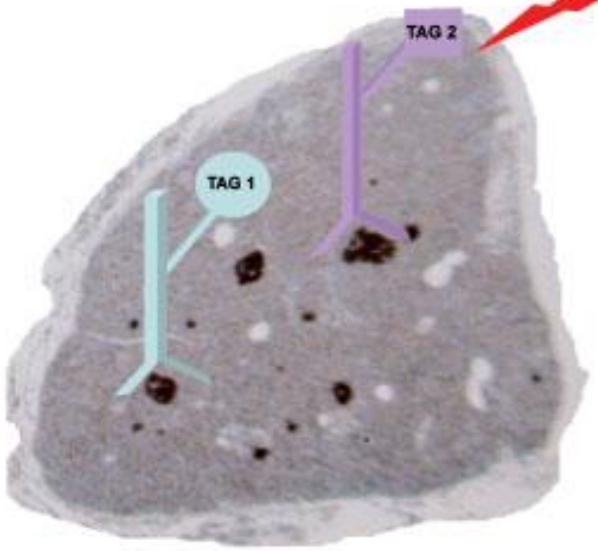
A whole mouse

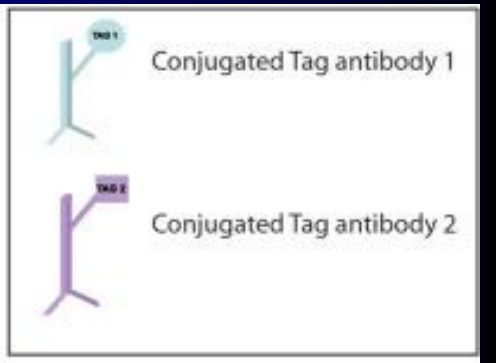


Penetration of the drug into the tissue

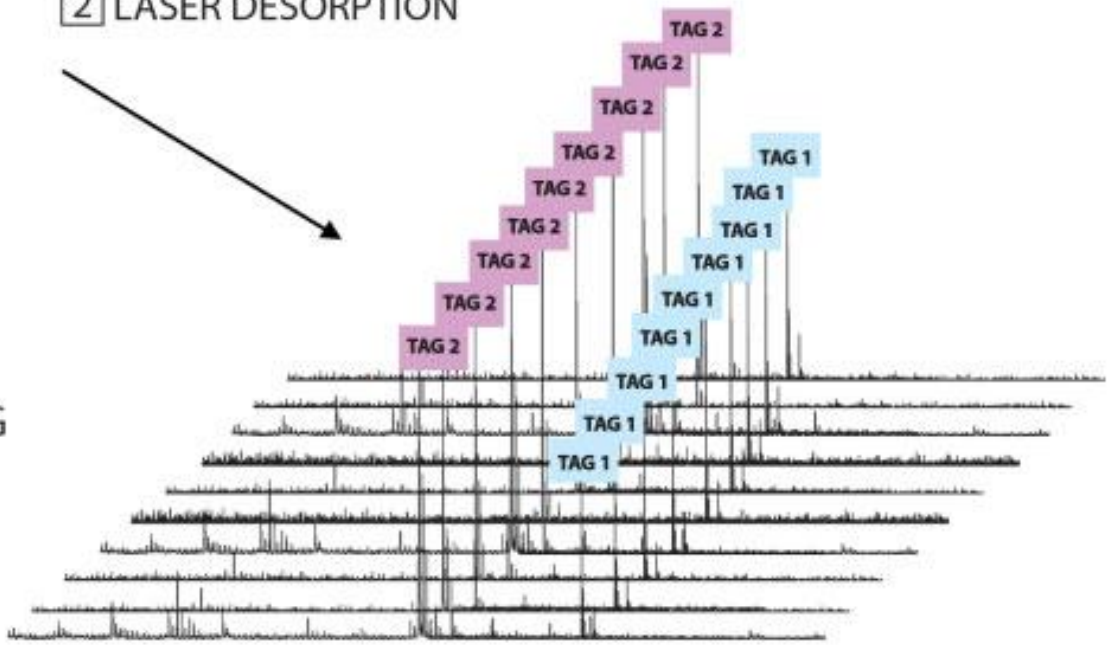
1 DIRECT IMMUNOHISTOCHEMISTRY

MALDI UV LASER

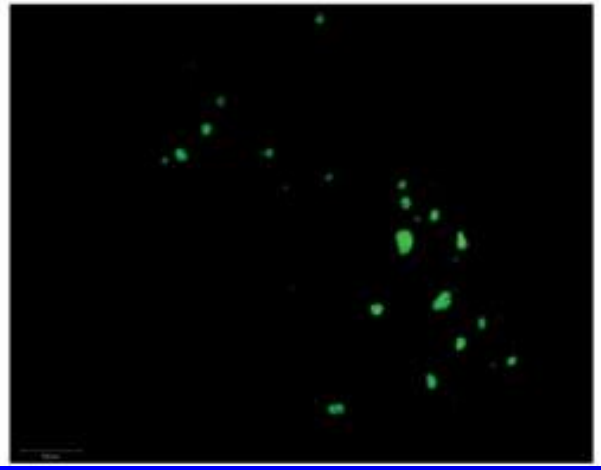




2 LASER DESORPTION



3 SPECIFIC MASS SPECTROMETRY IMAGING



Proteomic Research

The main objectives today for clinical and general Proteomics:

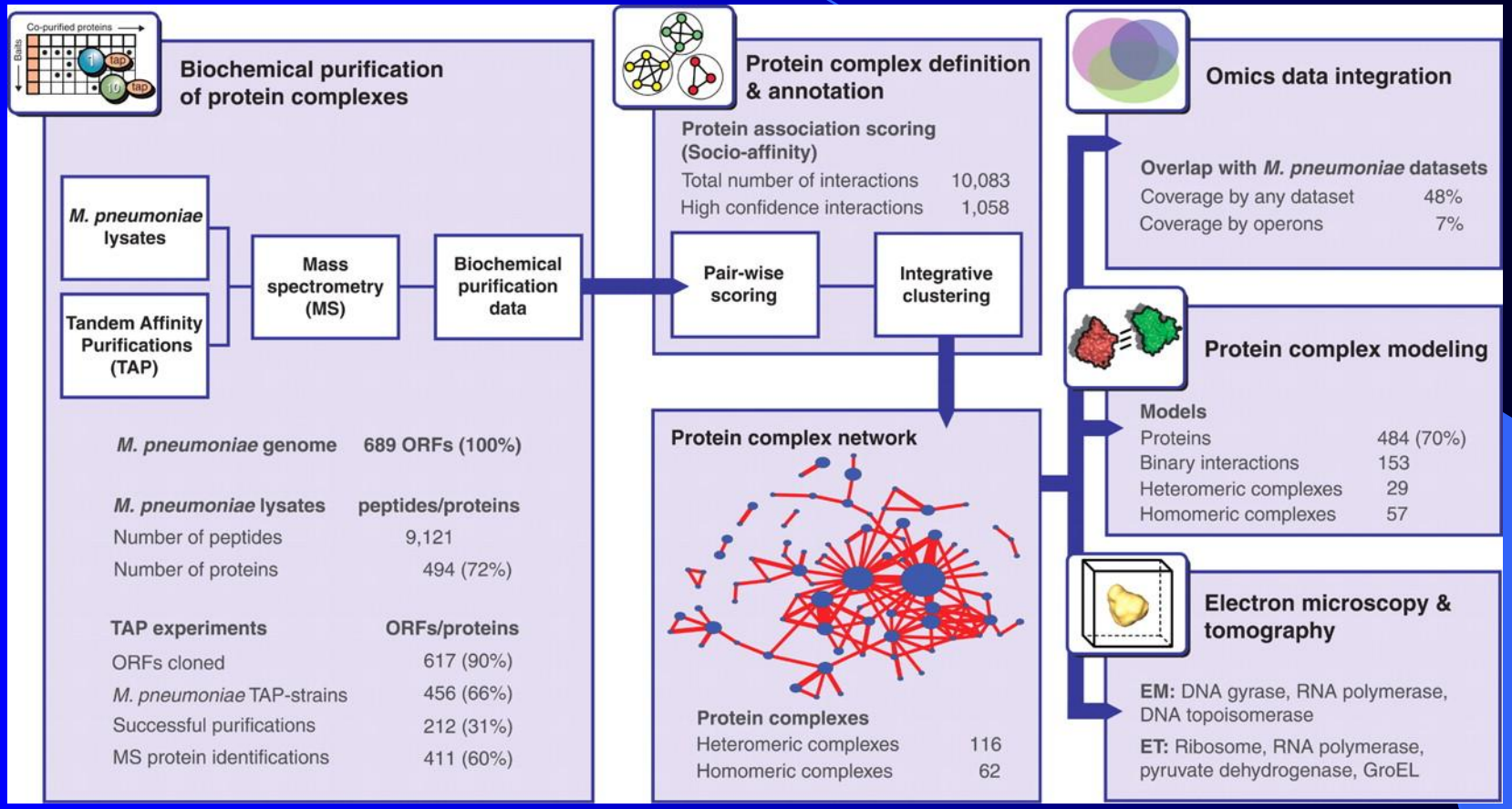
- Quantification of all the proteins expressed in a cell or tissue proteome, body fluids e.g. blood, CSF etc. Searching for Biomarkers!

- Functional study of thousands of proteins in parallel, which protein is in contact to another protein and where? Searching for functionality!

For quantification purposes, standard method is 2DE electrophoresis or MudPIT separation followed by MS identification

For protein function studies, microarray based assays are used to study protein-protein and protein-ligand interactions

Synopsis of the genome-wide screen of complexes in M. pneumoniae



S Kühner et al. Science 2009;326:1235-1240

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