Proteomics goes Clinical, or does it?

Salerno, July 2012

Meilahti Clinical Proteomics Core Facility

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

Services

Services

- Proteomic services from Bedside-to-Bench
- High-throughput analysis of Clinical Samples (CSF, Saliva, Urine, Blood, Ascites, Tears etc.)
- Validation of Clinical Samples
- Validation of Clinical Biomarkers

- Basic Proteomics Analyses (1-2D-GE, 1-2D-LC)
- Ion Mobility Structural Analyses

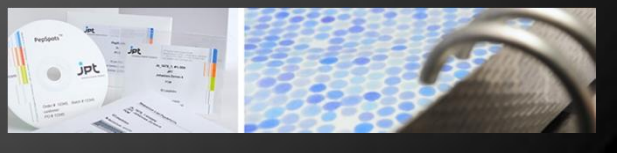
- Array-based epitope mapping (Pep/Prot-Arrays)
- Pep-Array synthesis
- Imaging Mass Spectrometry (IMS) from tissue to cells
- Glycoproteomics
- Glycopeptidomics
- LC-MS/MS2 analysis of carbohydrates
- N-glycopeptide spectra analysis
- Core fucosylation analysis

University of Helsinki • Haartman Institute

Biocentrum Helsinki

BF A member of the Finnish Proteomics and Metabolomics technology platform

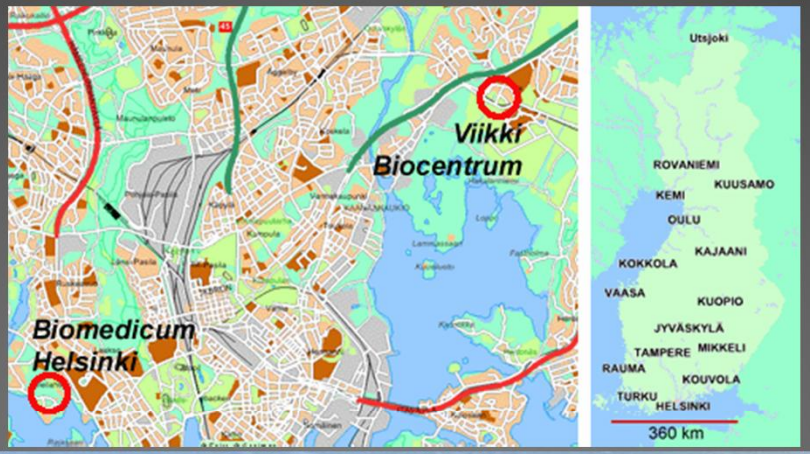
ProtMet.net

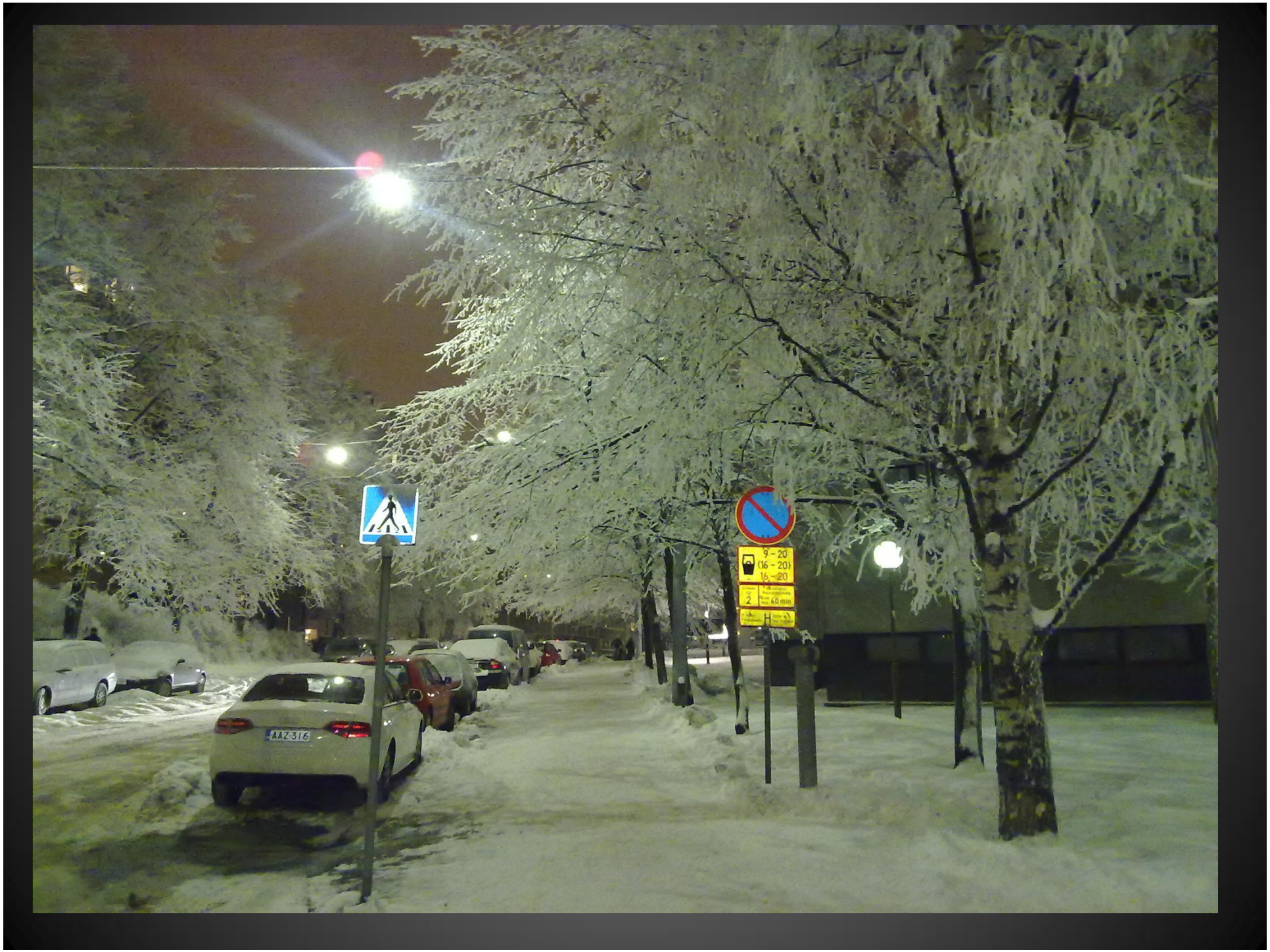


BIOMEDICUM

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??

Proteomic Research

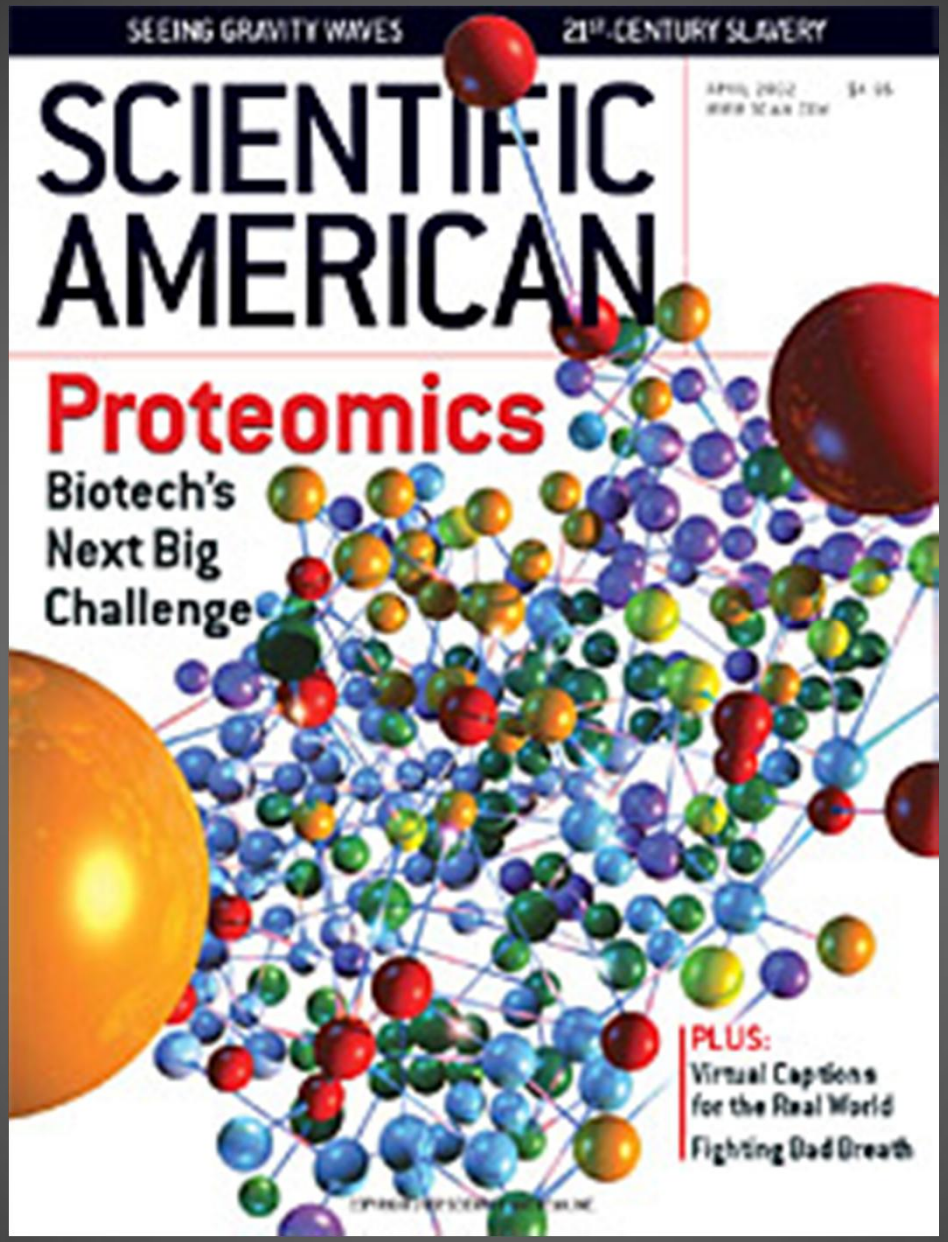
The main objectives today for clinical and general Proteomics:

- 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!

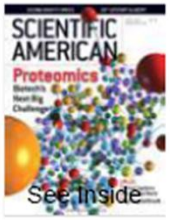
For quantification purposes, standard method is 2DE electrophoresis or MudPIT separation followed by MS identification

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


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



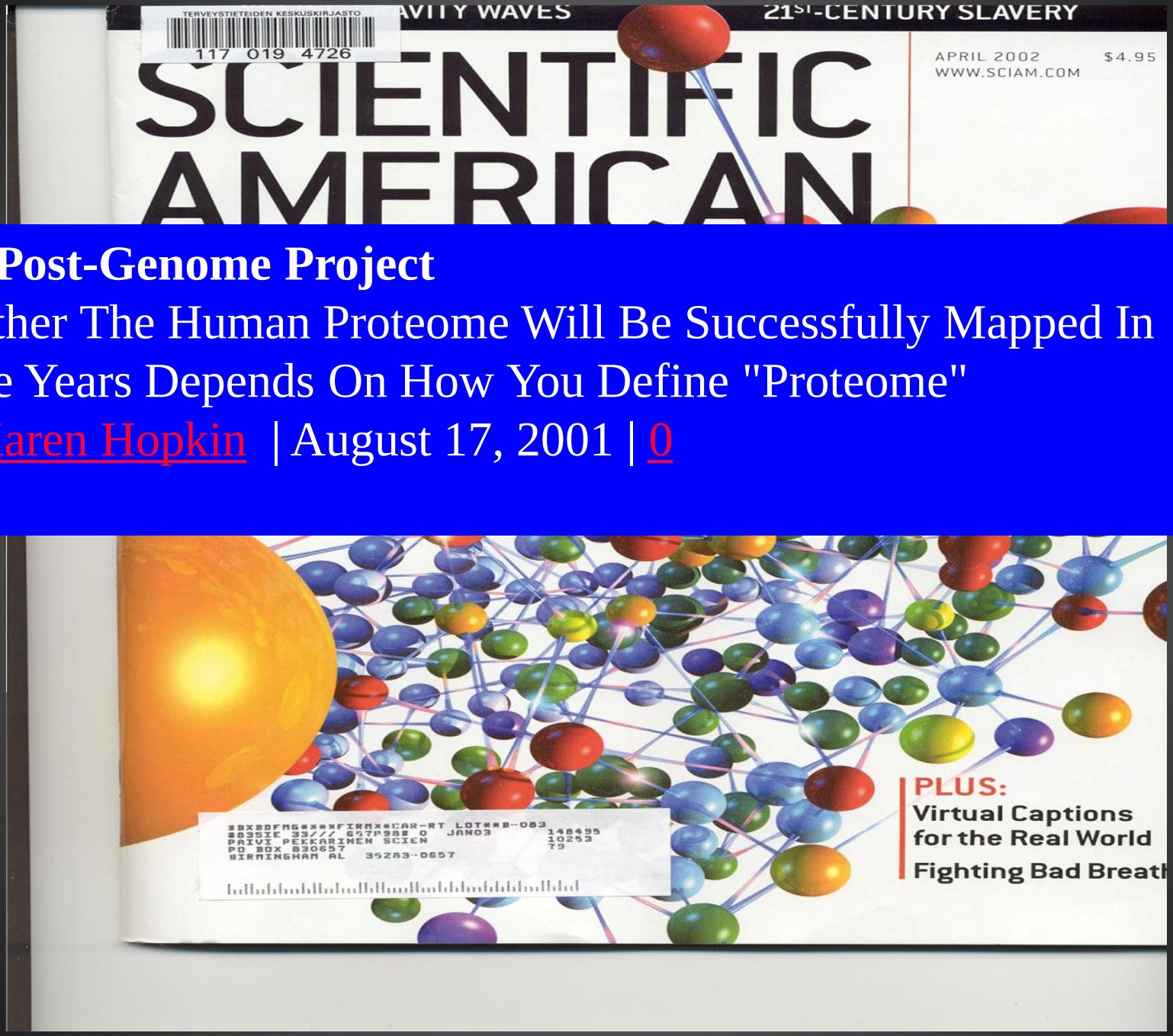
Proteins Rule

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

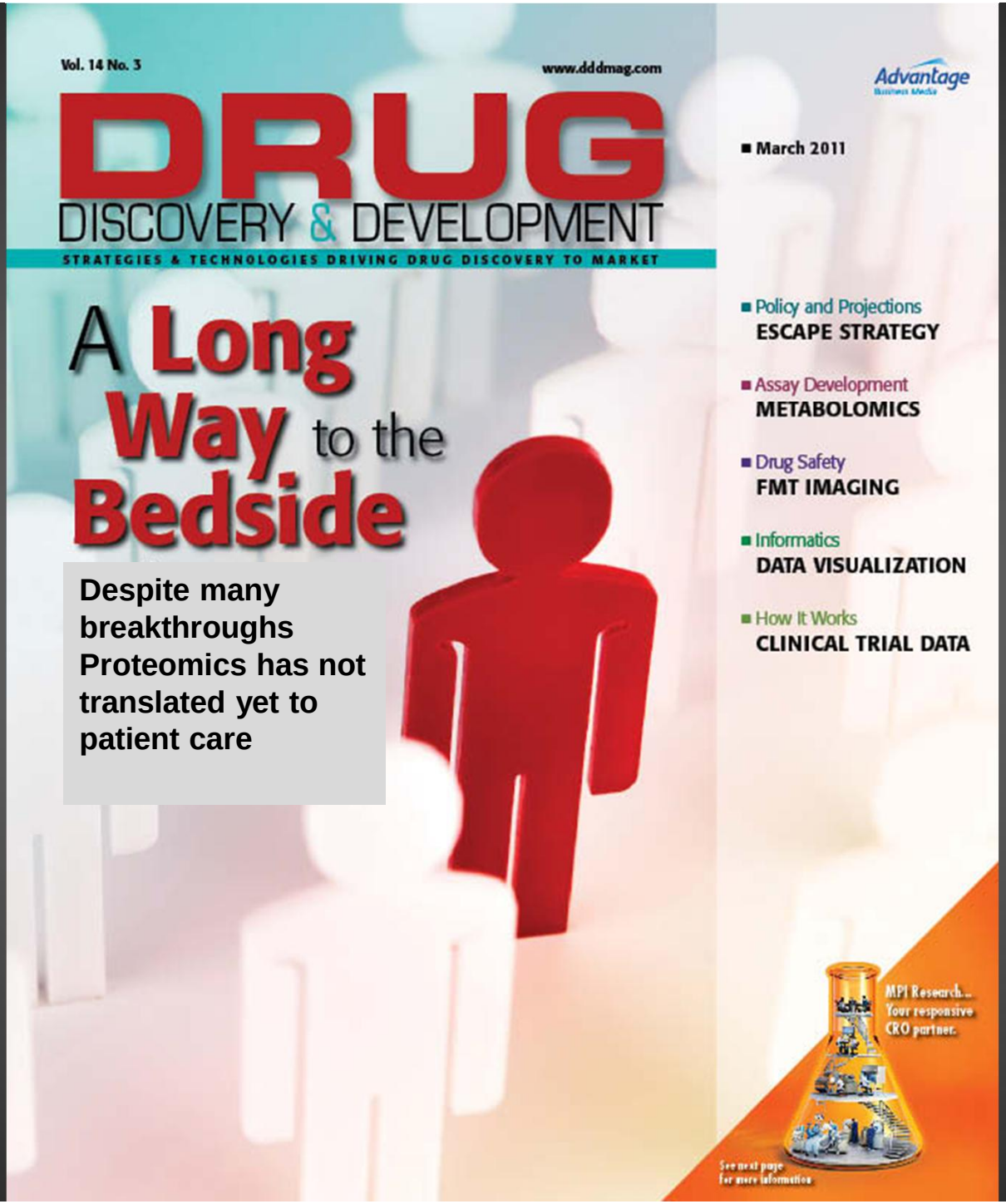
close. Researchers are now concentrating



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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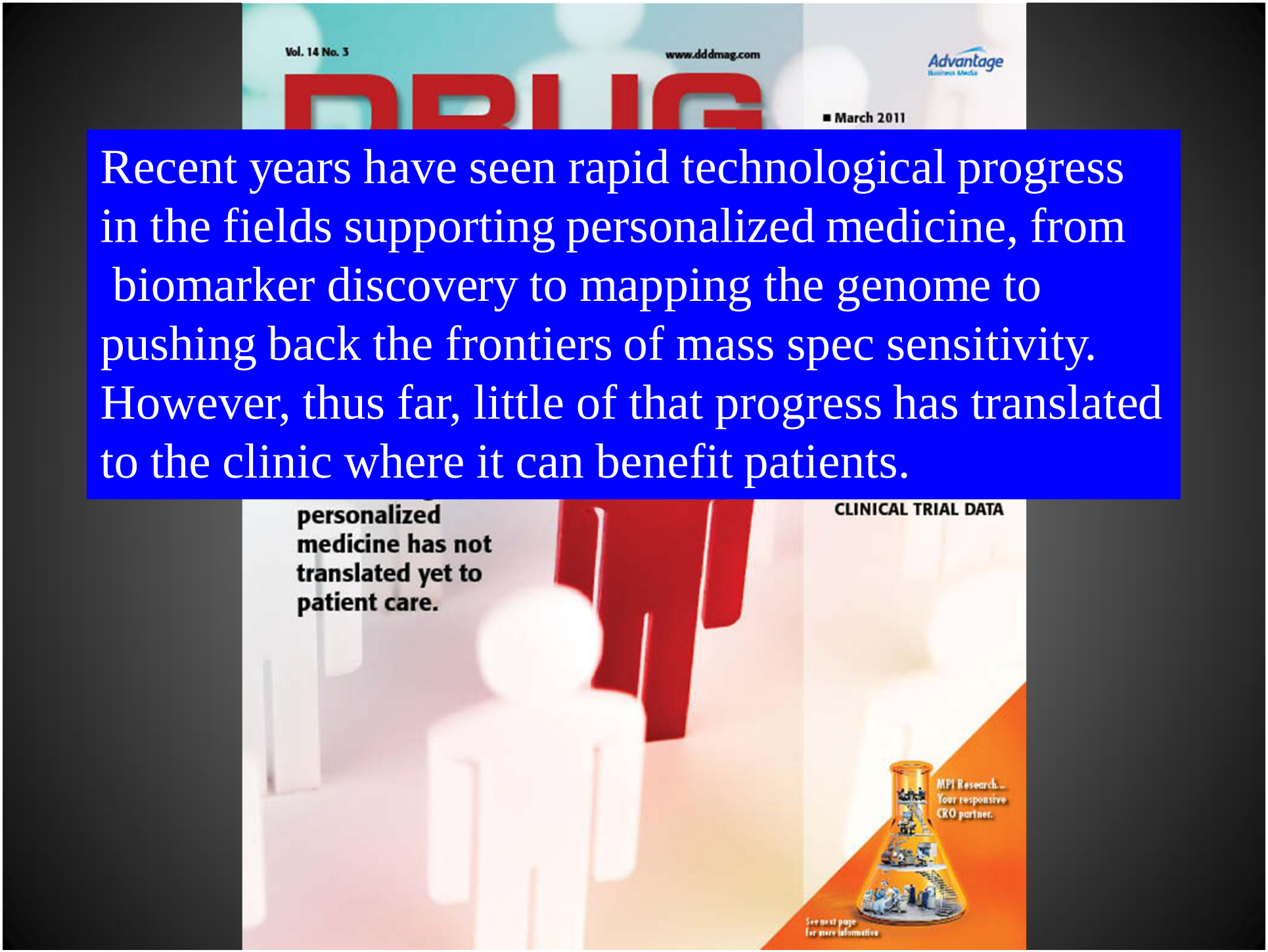
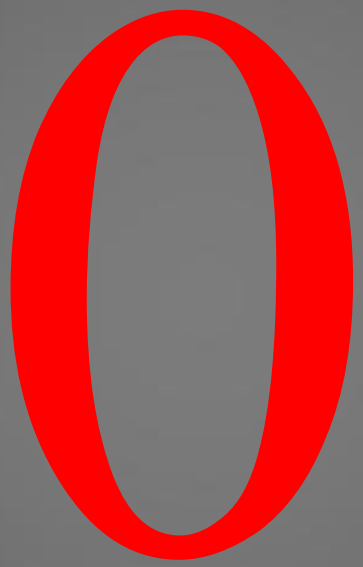
Advantage
March 2011
A Long Way to the Bedside
Despite many breakthroughs Proteomics has not translated yet to patient care
Policy and Projections
ESCAPE STRATEGY
Assay Development
METABOLOMICS
Drug Safety
FMT IMAGING
Informatics
DATA VISUALIZATION
How It Works
CLINICAL TRIAL DATA
MPI Research...
Your responsive
CRO partner.
See next page
for more information



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:



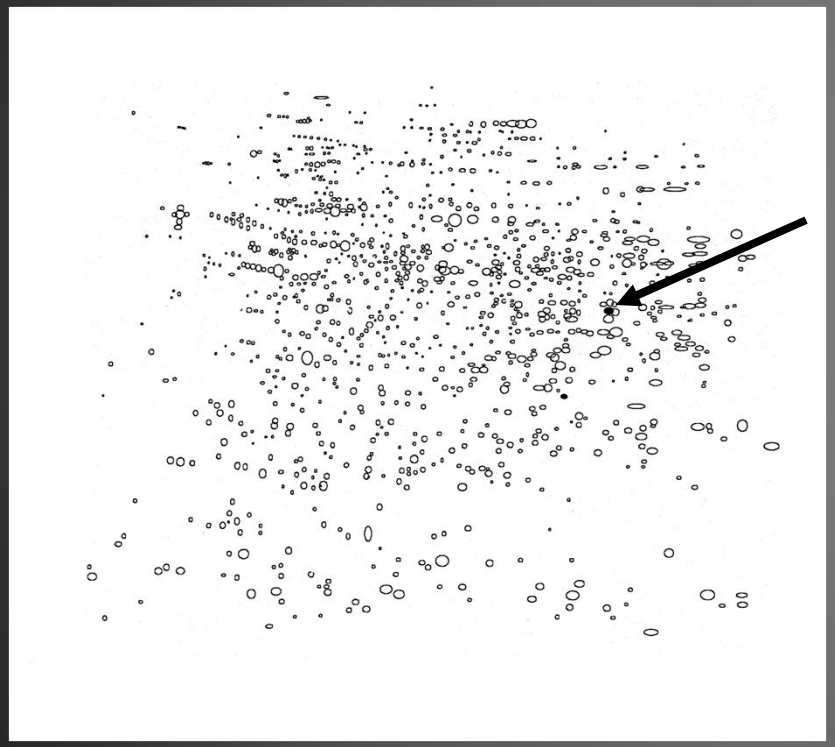
2010

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



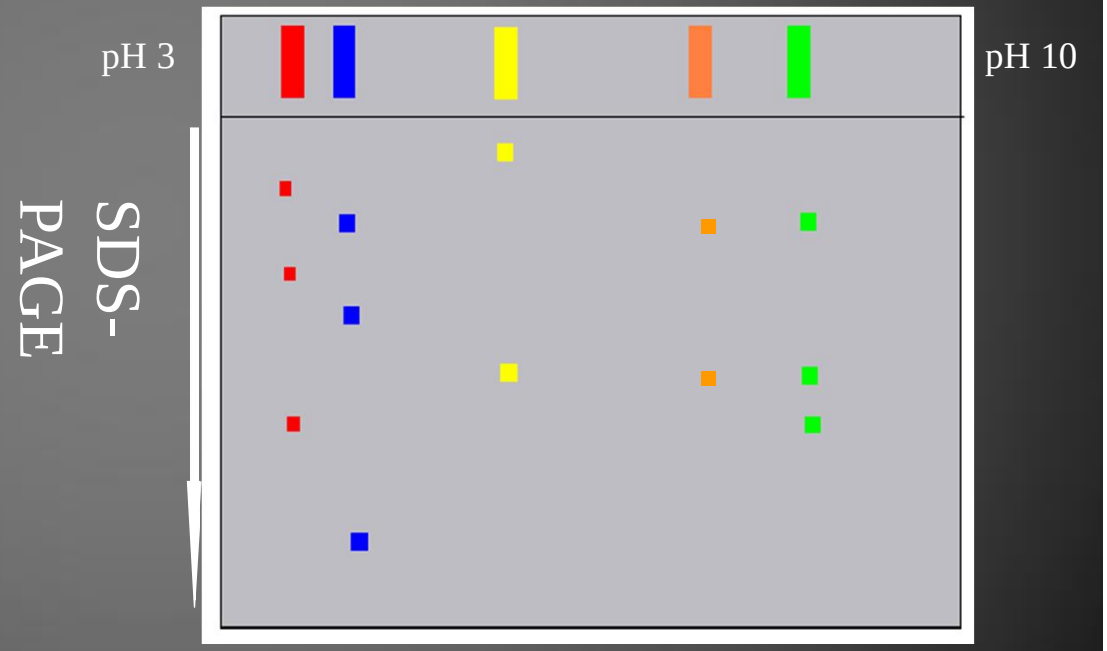
A protein Array

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?

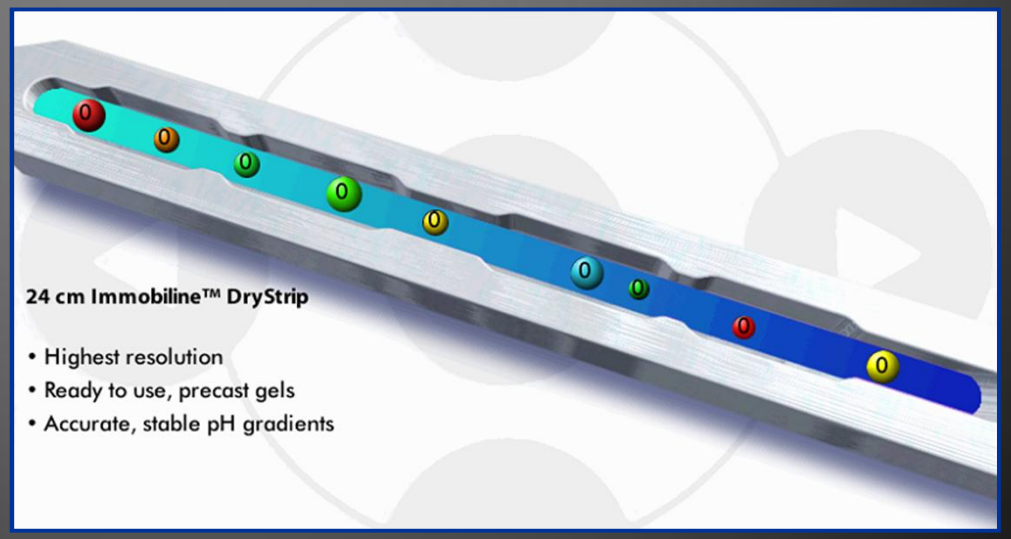
- 1st dimension, IEF, Proteins are separated
- according to their isoelectric point (IP)
- 2nd dimension, SDS-PAGE, Proteins are separated according to their molecular mass
- Efficient: More than a thousand proteins resolved in E-Coli cell lysates and ~8000 in brain lysates



1st Dimension - Isoelectric Focusing

pI - isoelectric point

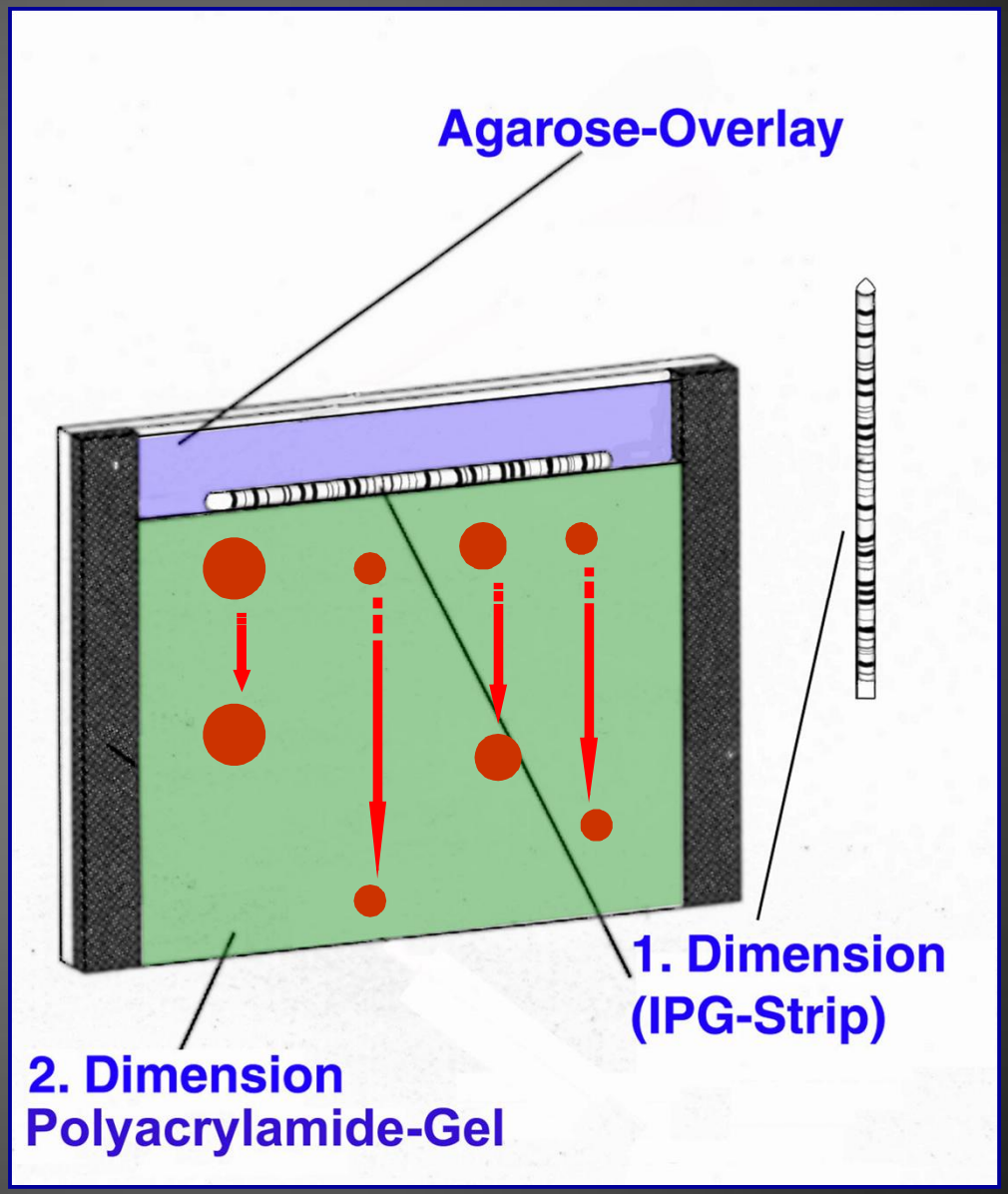
ready made Gel-strips



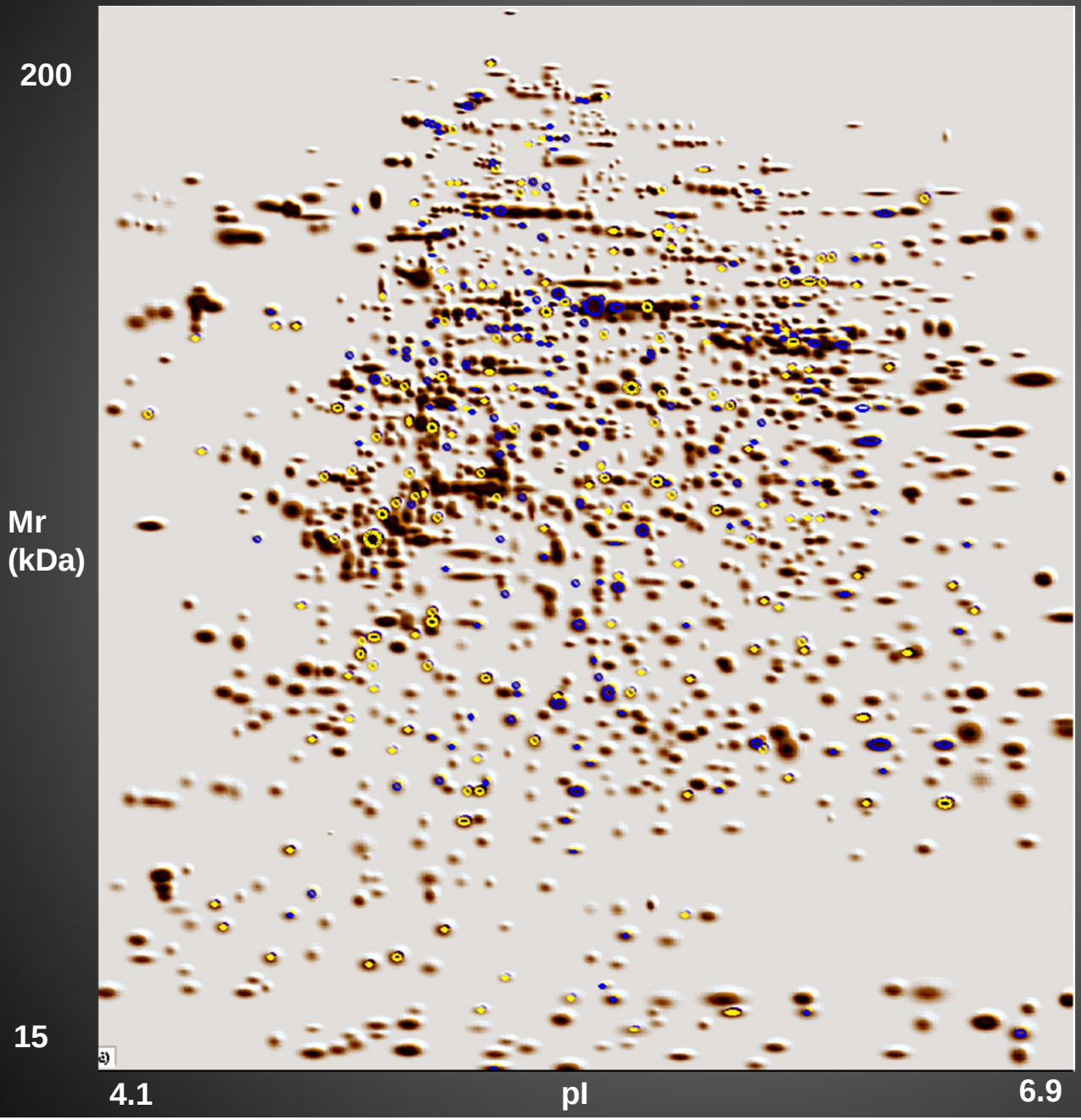
2nd Dimension - Isoelectric Focusing

2DE

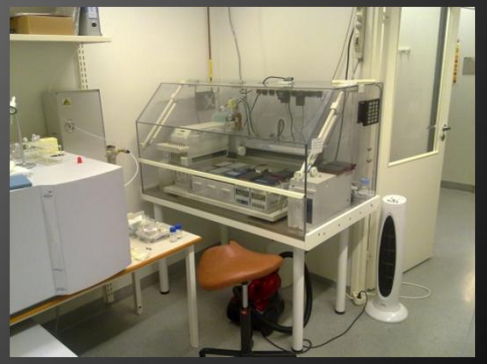
Mw



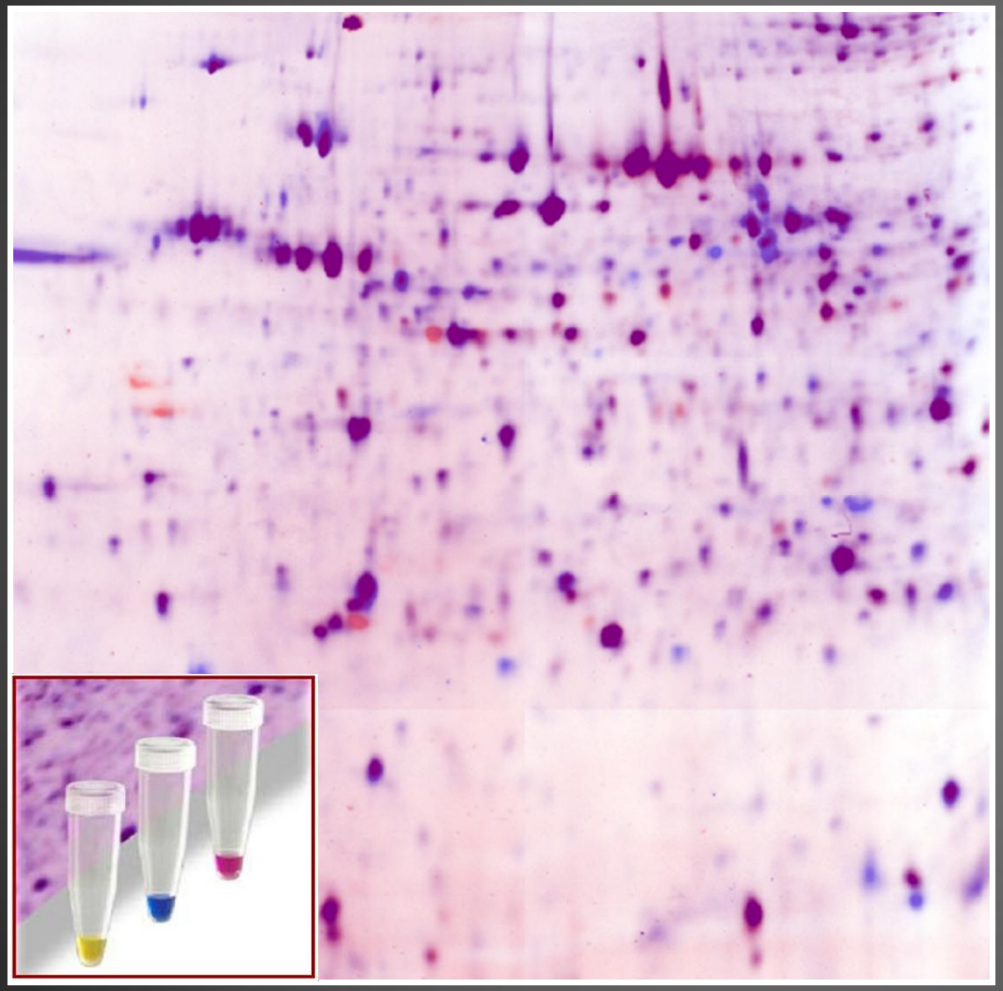
Protein Fingerprint: 2-DE

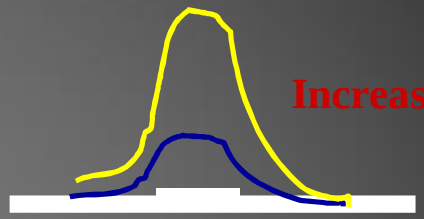


About 2000 proteins

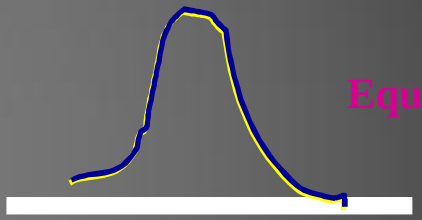


Overlay of normal and patient protein samples

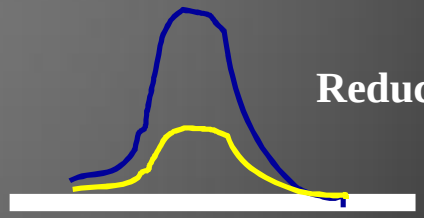




Increased abundance



Equal abundance



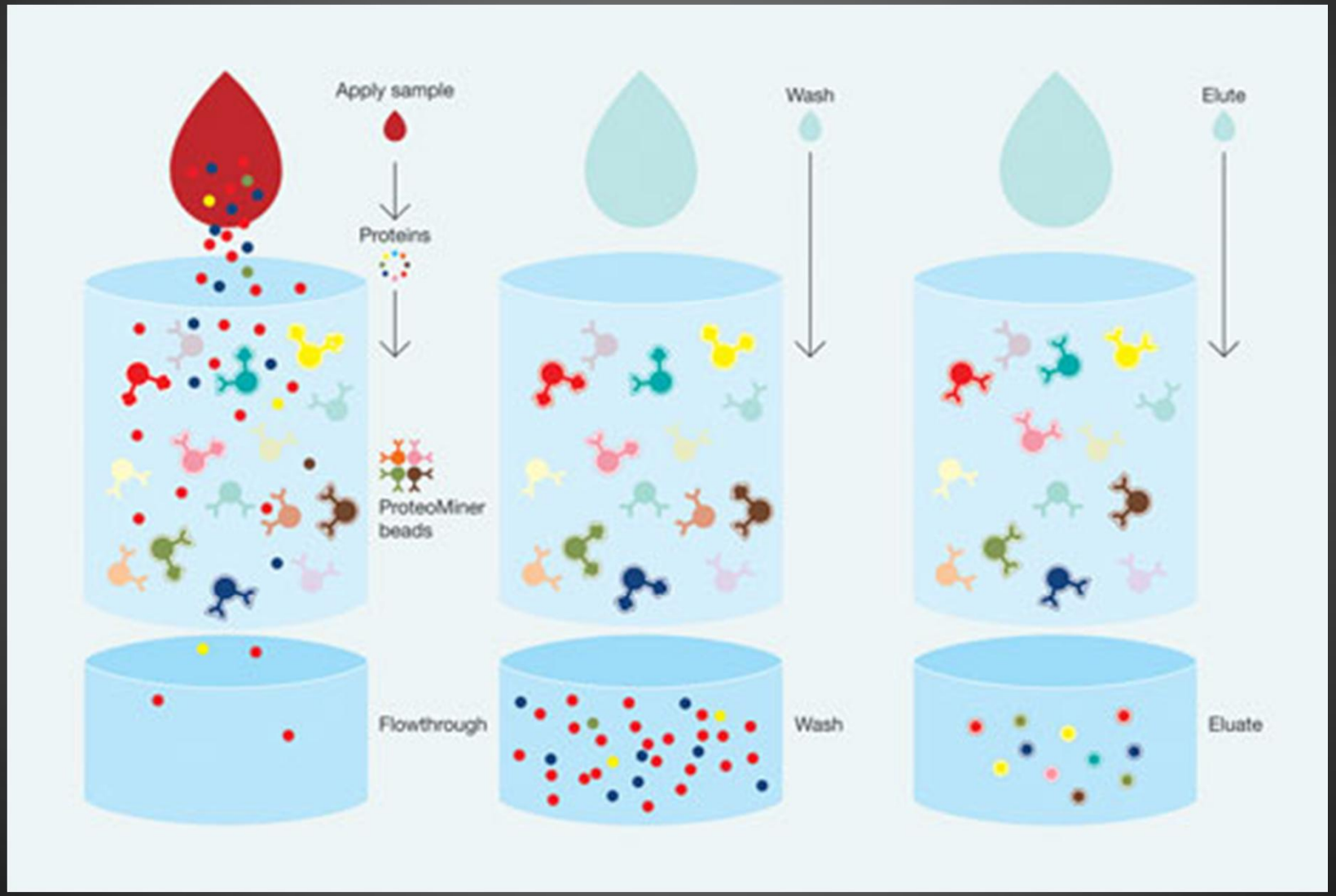
Reduced abundance

Normal control = Cy™3 labelled - Blue

Patient A sample = Cy5 Labelled - Red

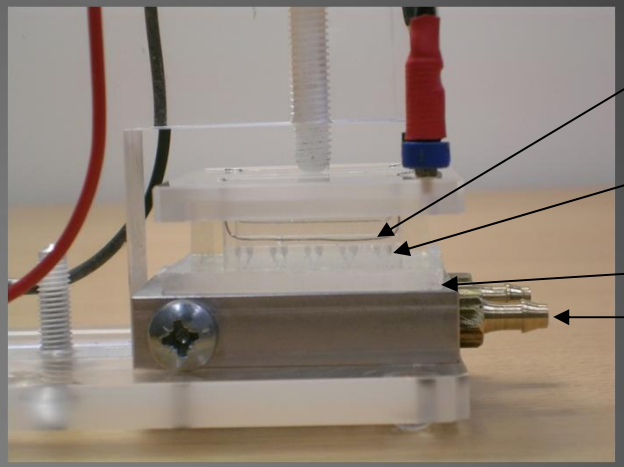
Ettan™ DiGE
the quantitative approach to do Proteomics

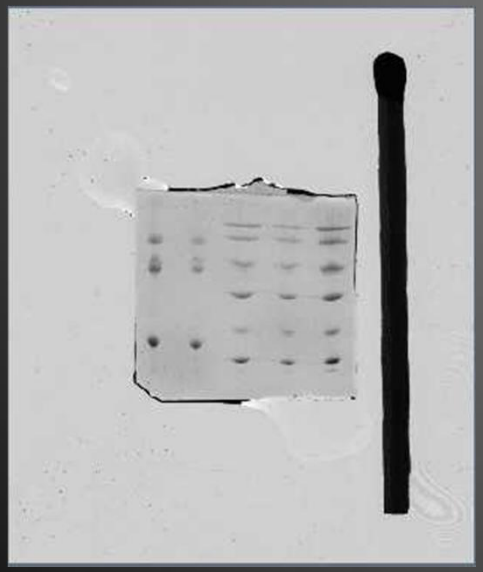
Low-Abundance Protein Enrichment
Hexapeptide Kits Useful for Many Sample Types

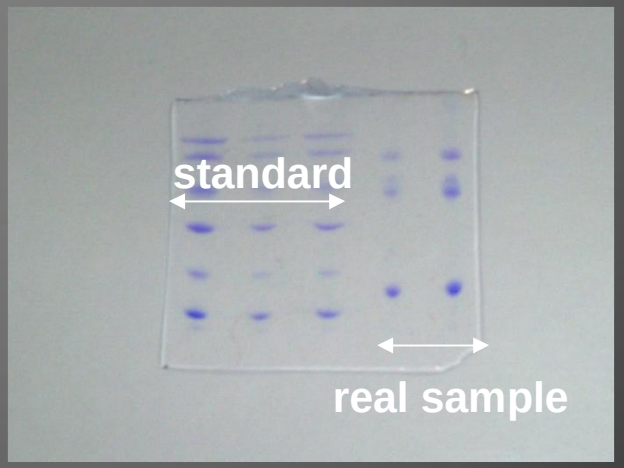


Proteominer BioRad

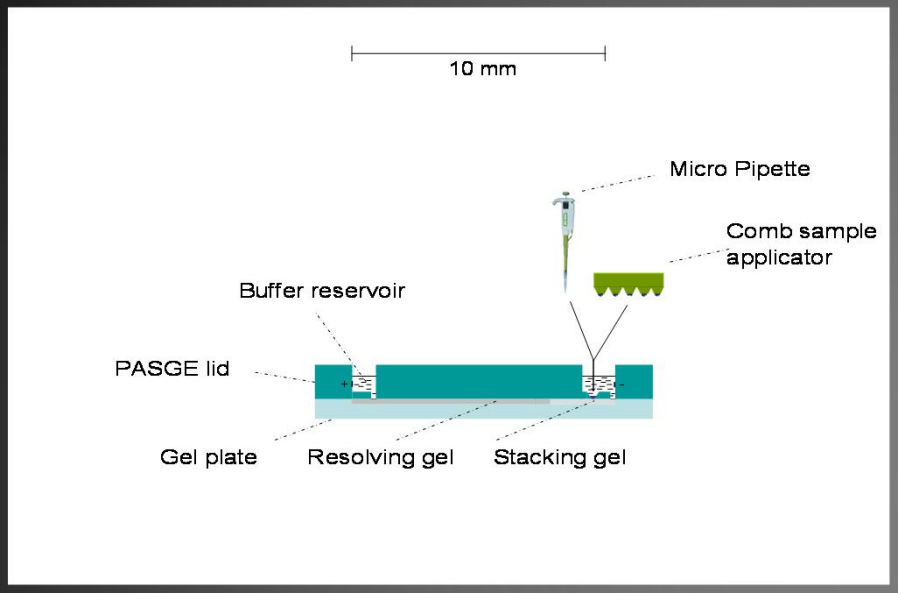
Micro gel devices

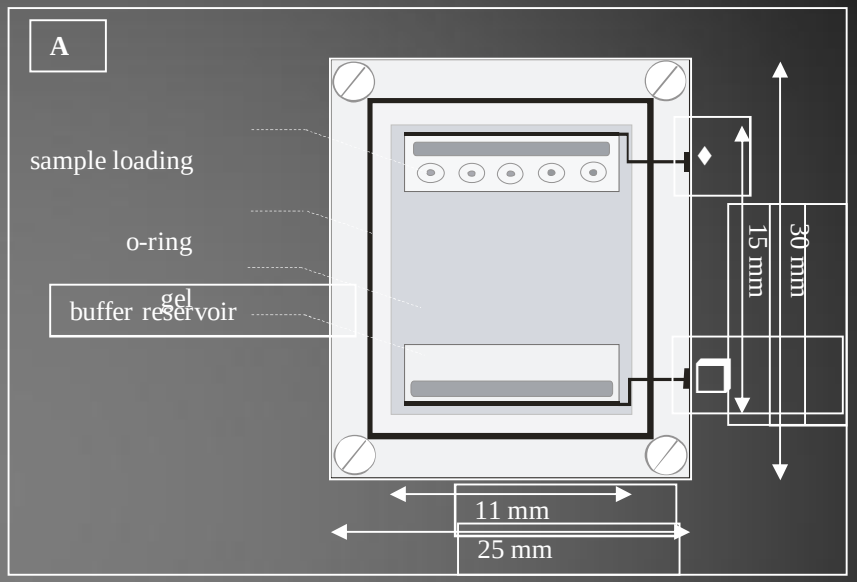


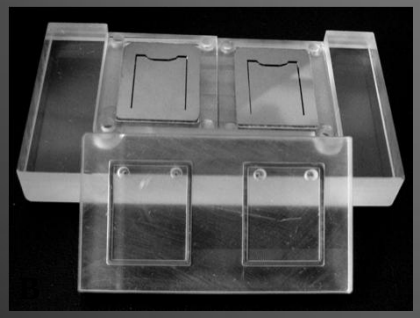


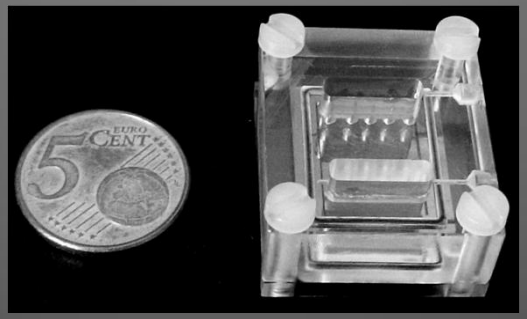


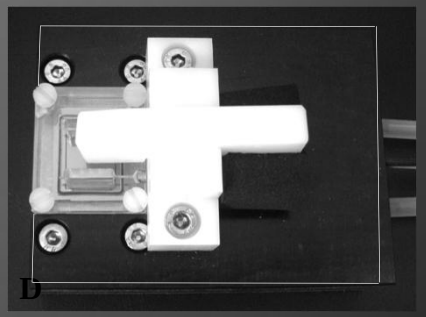
Running time 10 minutes


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

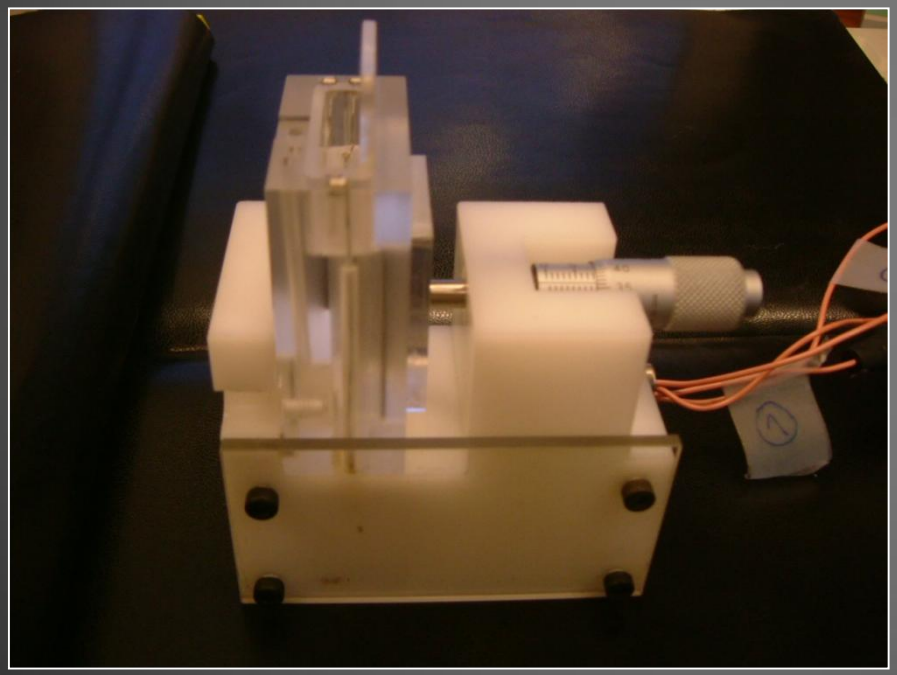


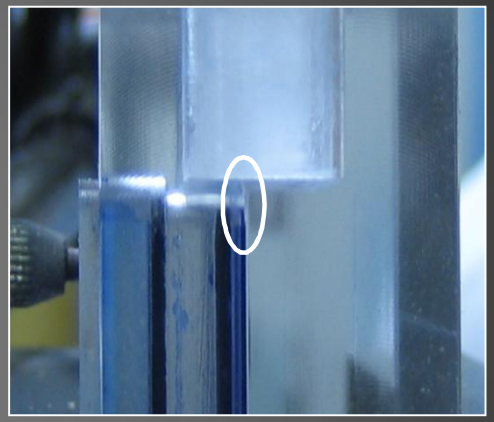


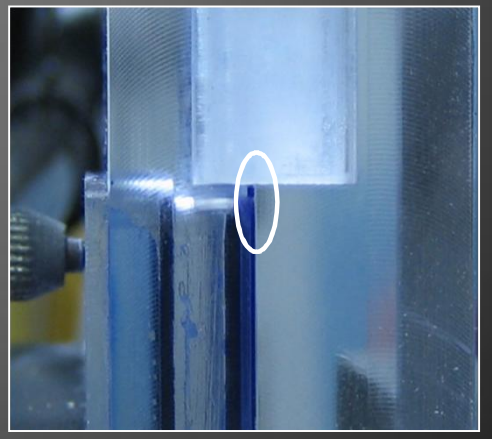

D

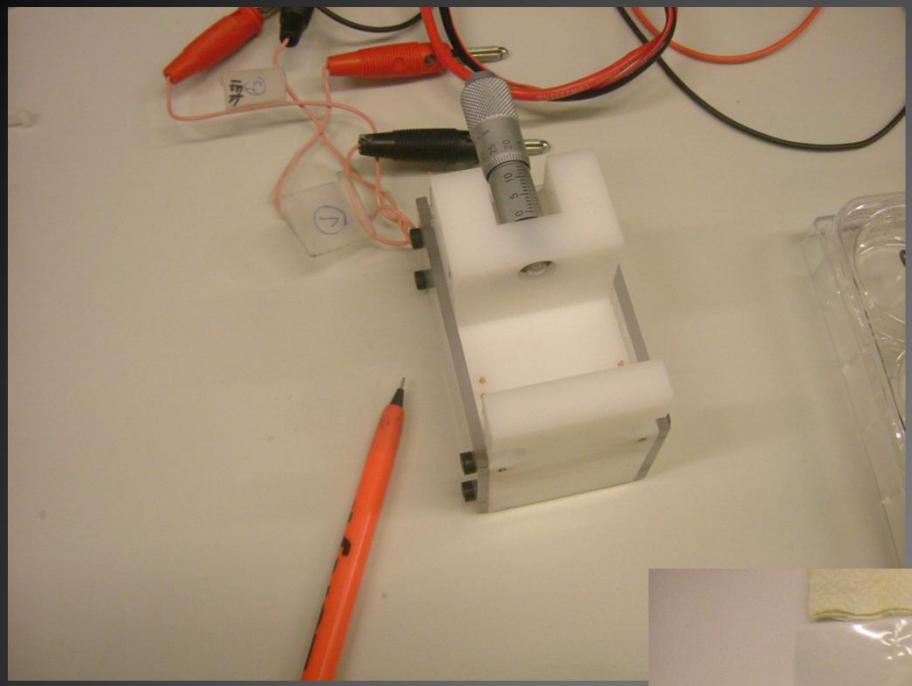
Automated 2D devices

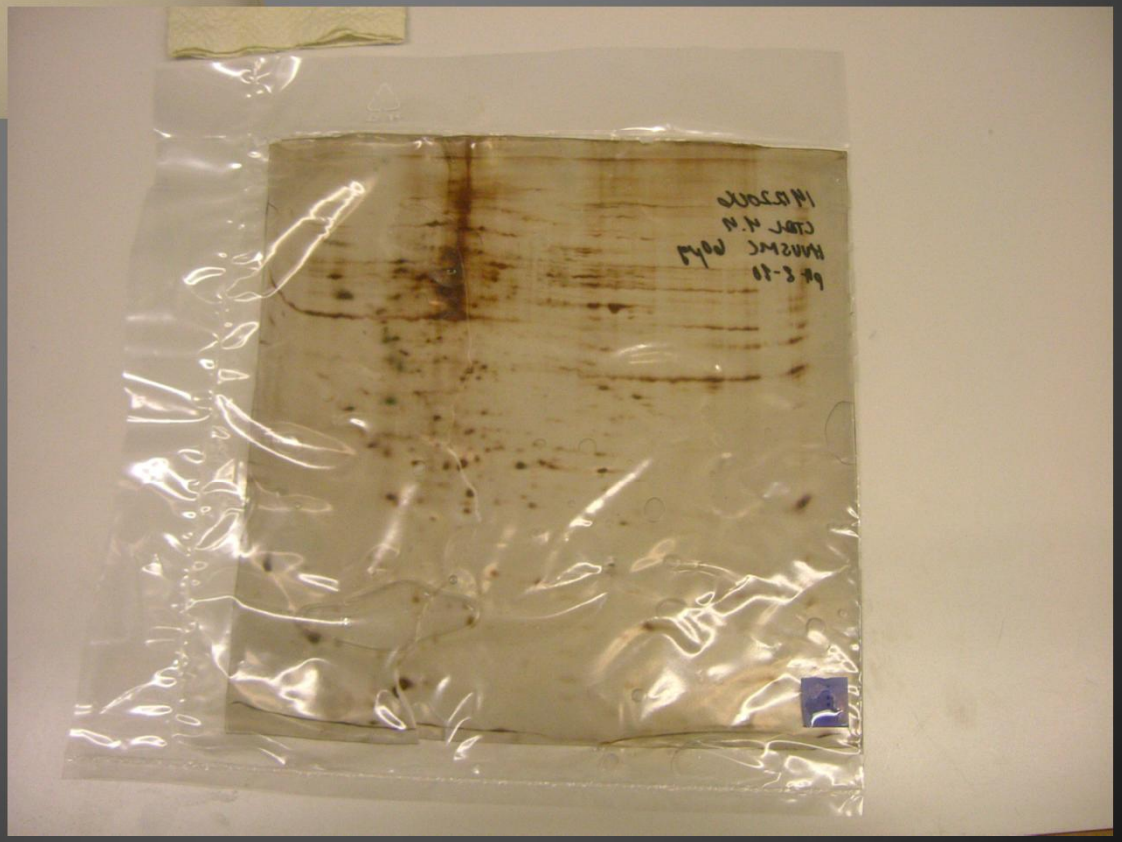
The compress system



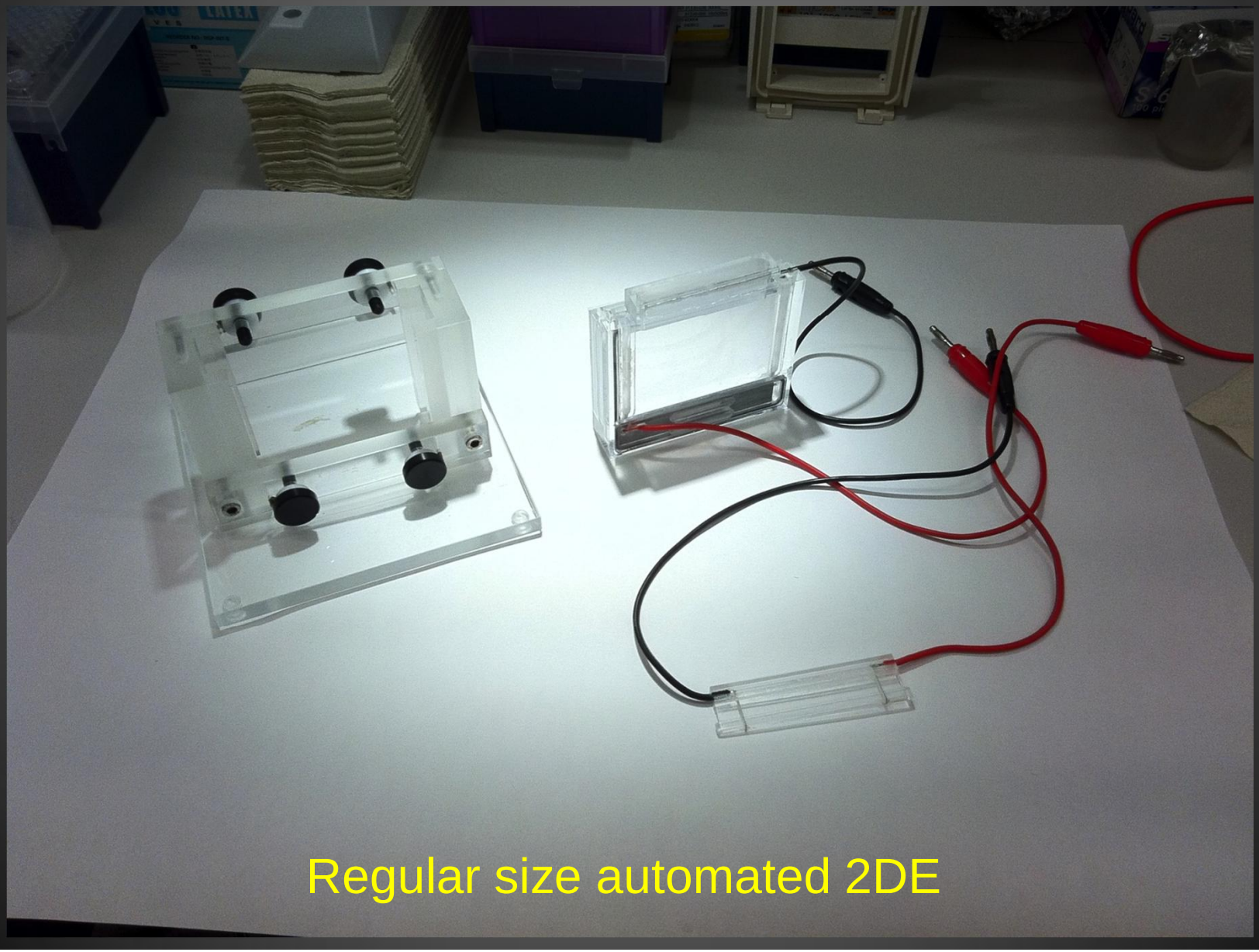






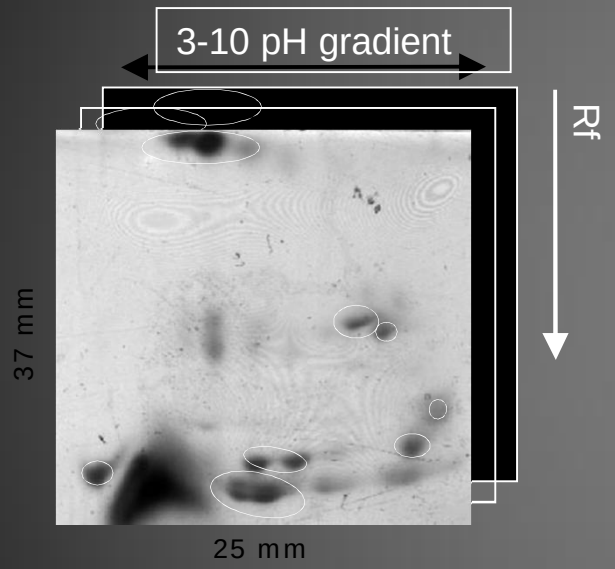


And the big brother (2011)



Regular size automated 2DE

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

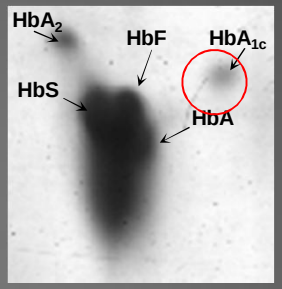
* comparison of 3 gels

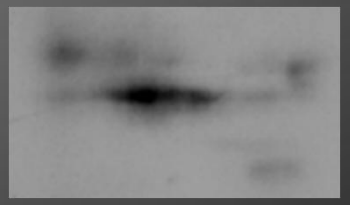
- 2-DE separation completed in approx. 80 min
- Limit of detection is approx. 65 ng

Running time 20-30 minutes

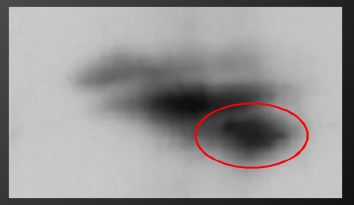
Examples for the Clinics

- 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





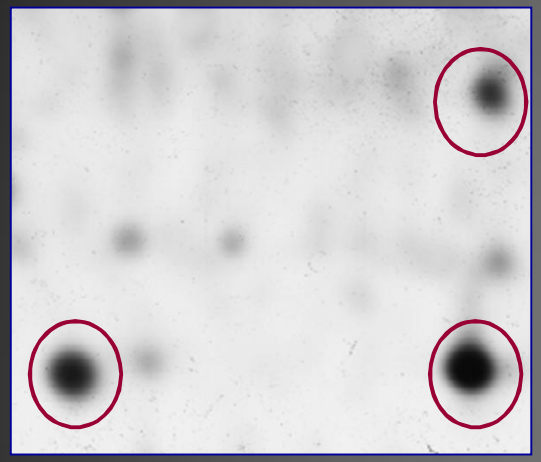
control



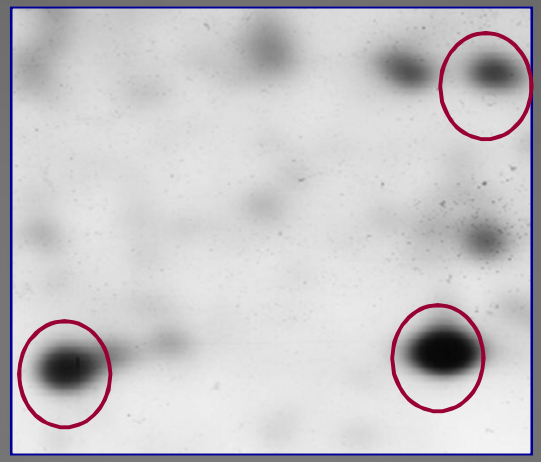
AD

TARGETED measurements of unknown protein expression diffrences:

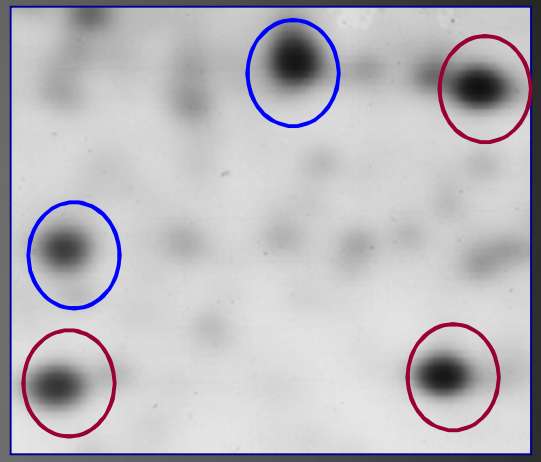
Application: Screening of protein variations of viruses or bacteria



Normal virus

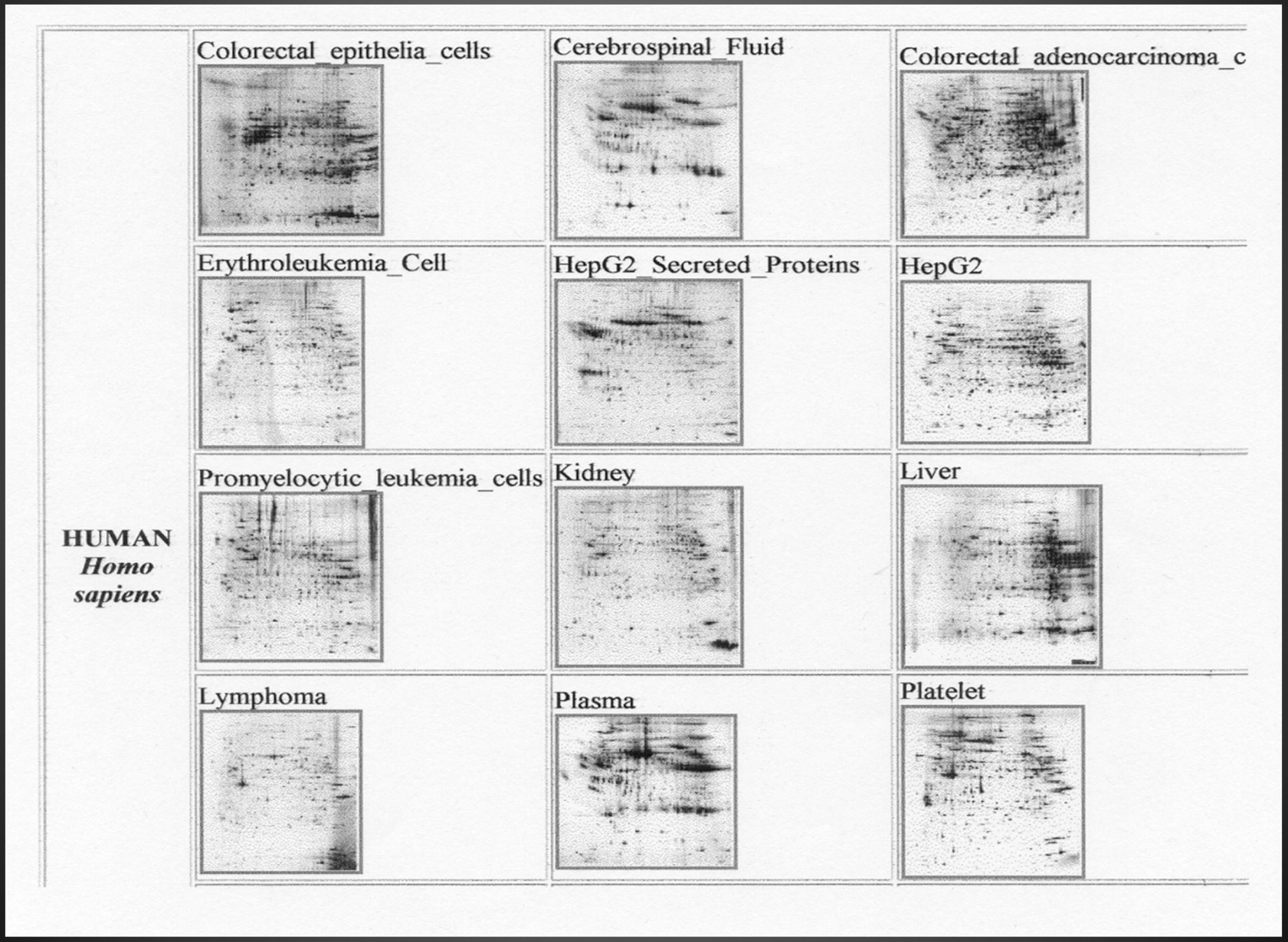


Normal virus + mutated virus (LT)



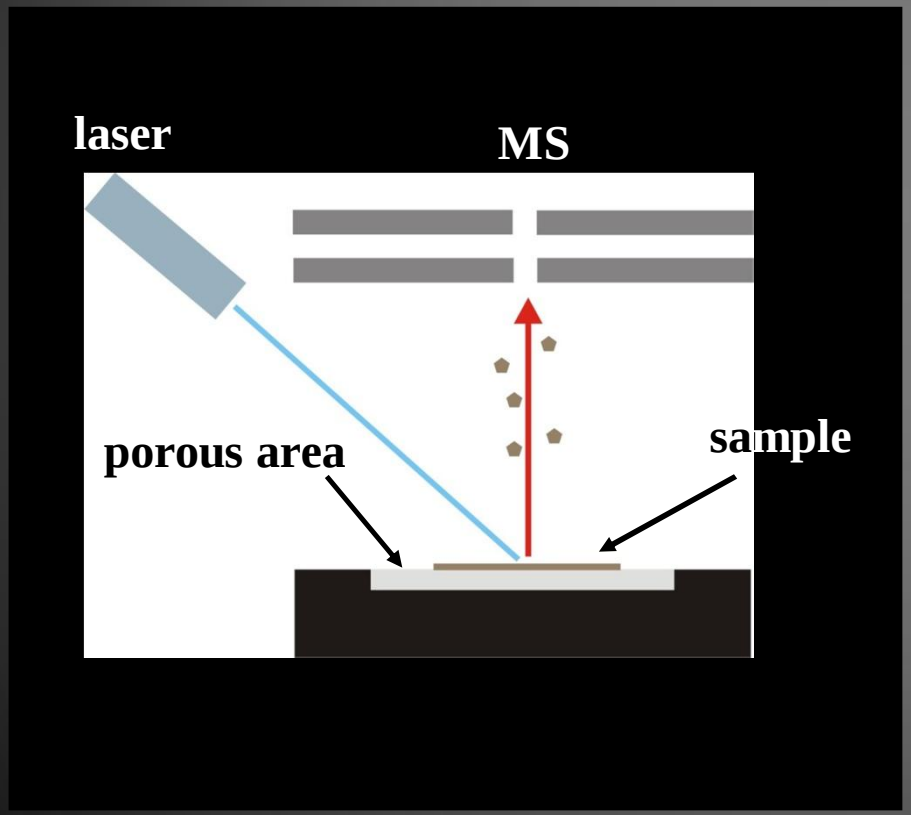
Normal virus + mutated virus (HT)

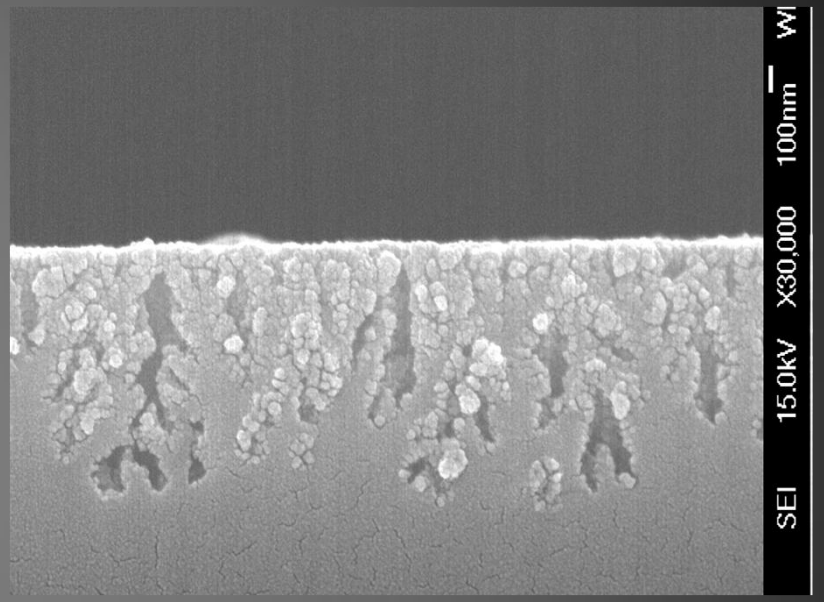
Also 2D Databases exist!

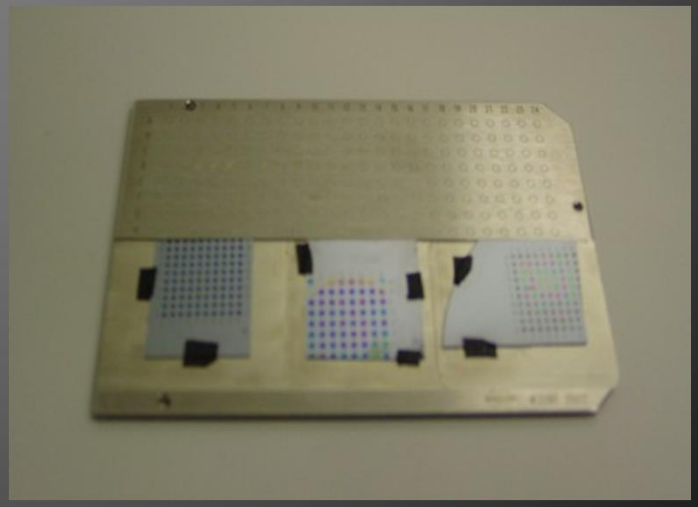


What about chip-based proteomics: DIOS-MS

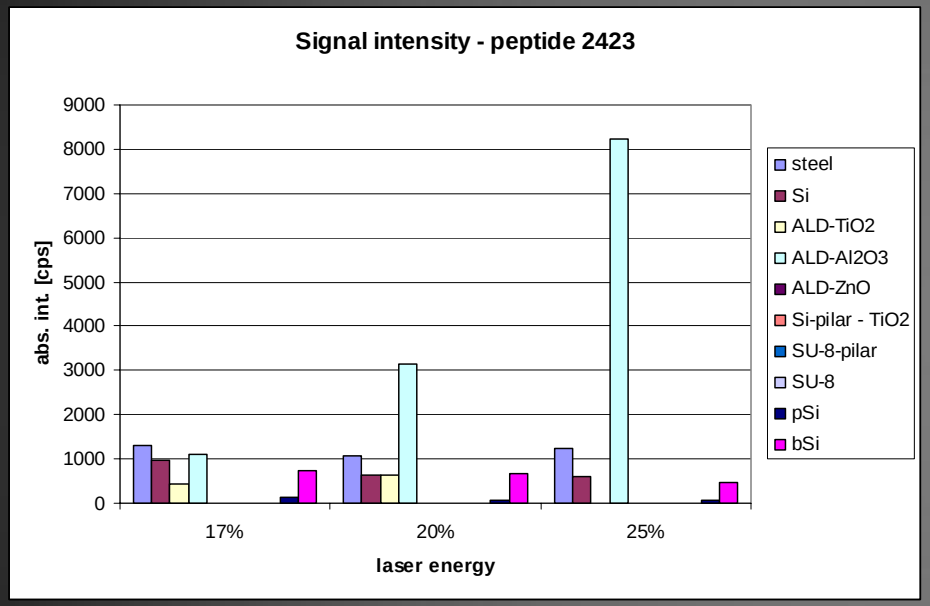
Desorption Ionization On Silicon (DIOS)

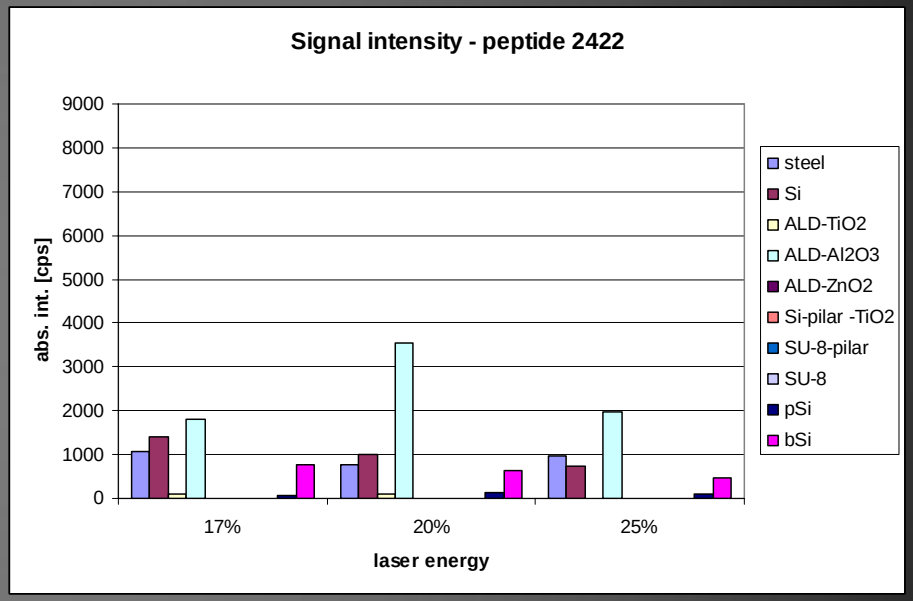






Testing the functionality of various surfaces for proteomics





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

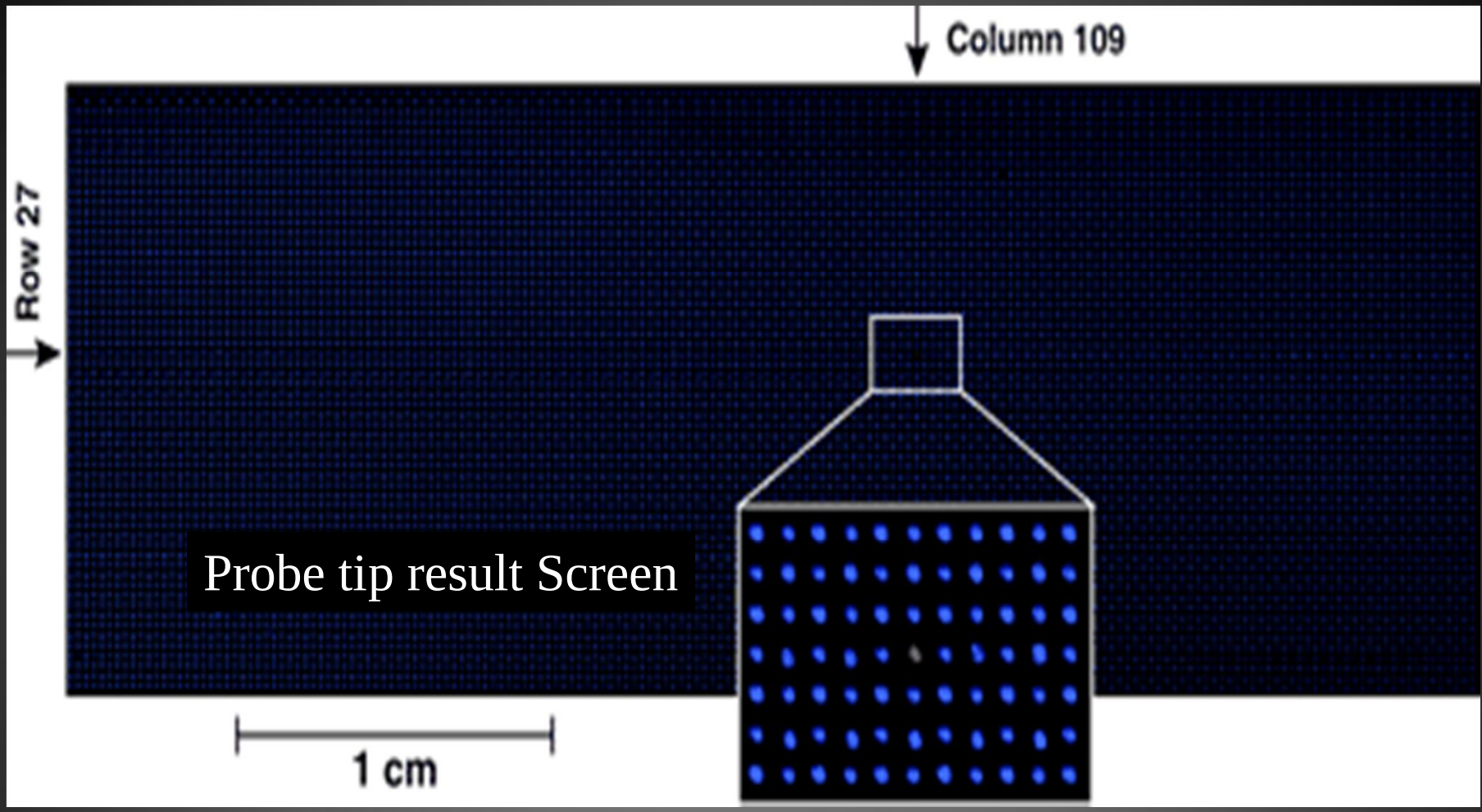
Application: Phosphoproteomics, determination of phosphorylation states (kinasome)

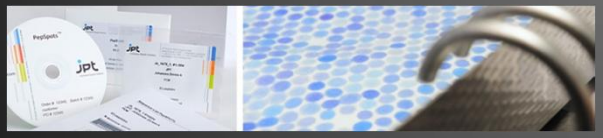
The μMOTC-nESI Lab-on-a-Chip

In collaboration with Prof. Evangelos Gogolides, Athens

Black Silica chip

384 samples (e.g. phosphopeptides)





10800 samples on the chip

Application: Array based Proteomics

UNIVERSITY OF HELSINKI

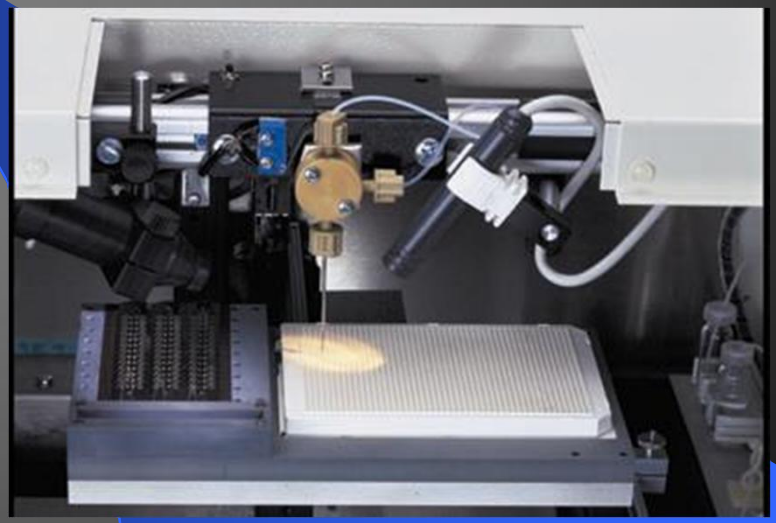
MEILAHTI CLINICAL PROTEOMICS CORE FACILITY
FACULTY OF MEDICINE

Array-based epitope mapping

1. Peptide synthesis on membrane

2. Peptide/Protein spotting on glass slide

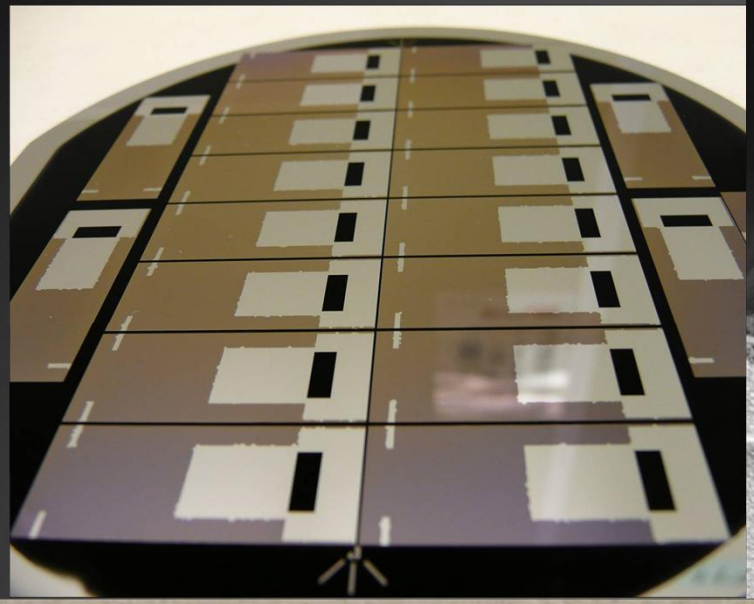
3. Analysis by AB, dyes or MS

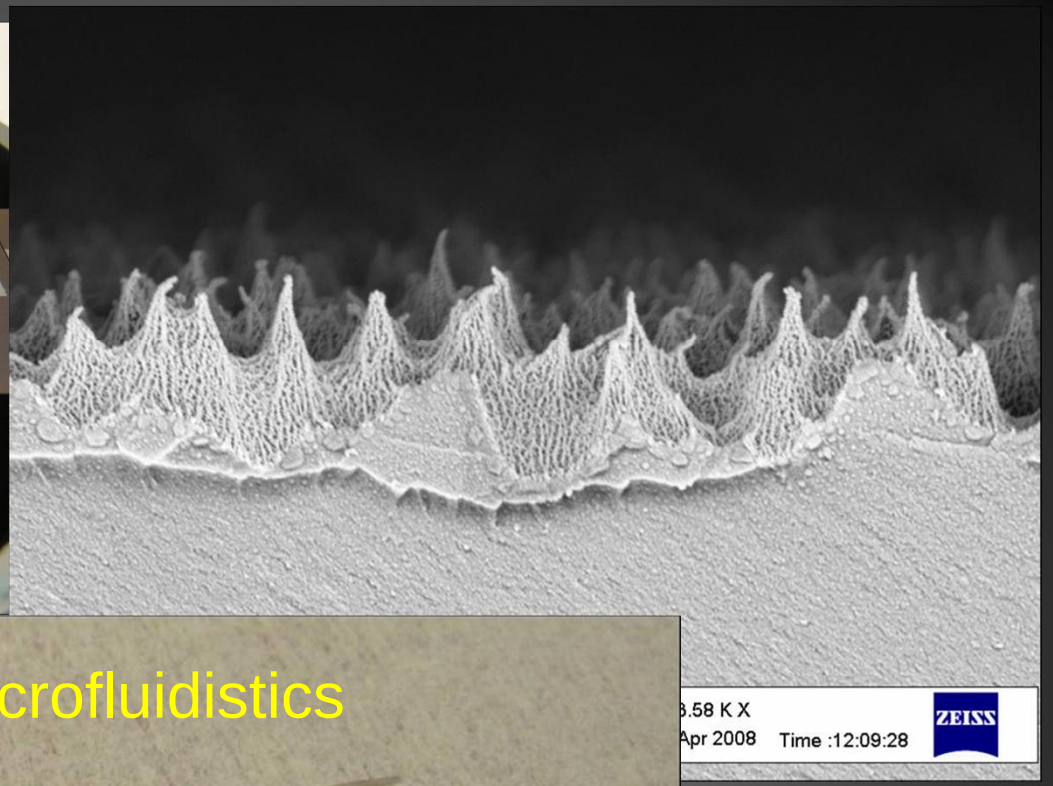


ProtMet.net

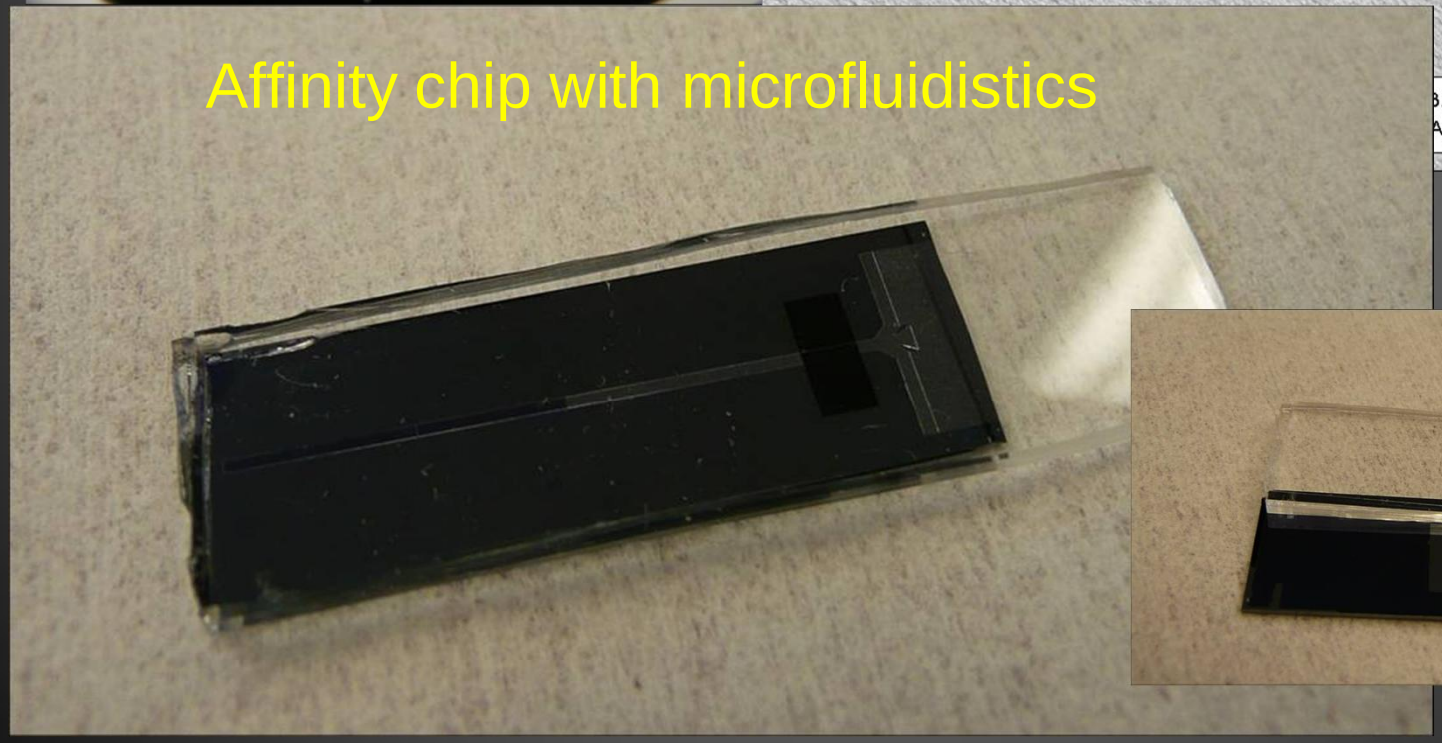
BIOCENTRUM HELSINKI

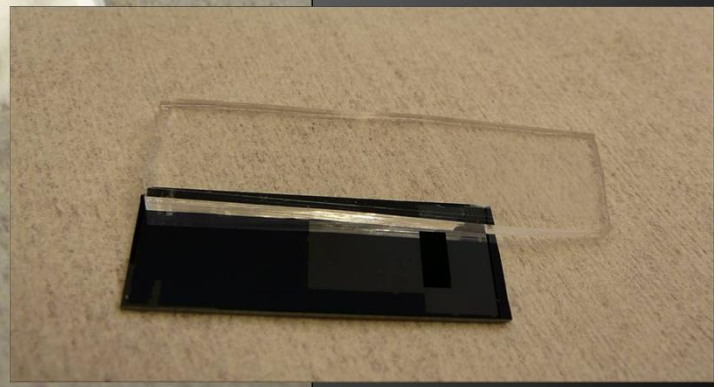
BF A member of the Finnish Proteomics and Metabolomics technology platform



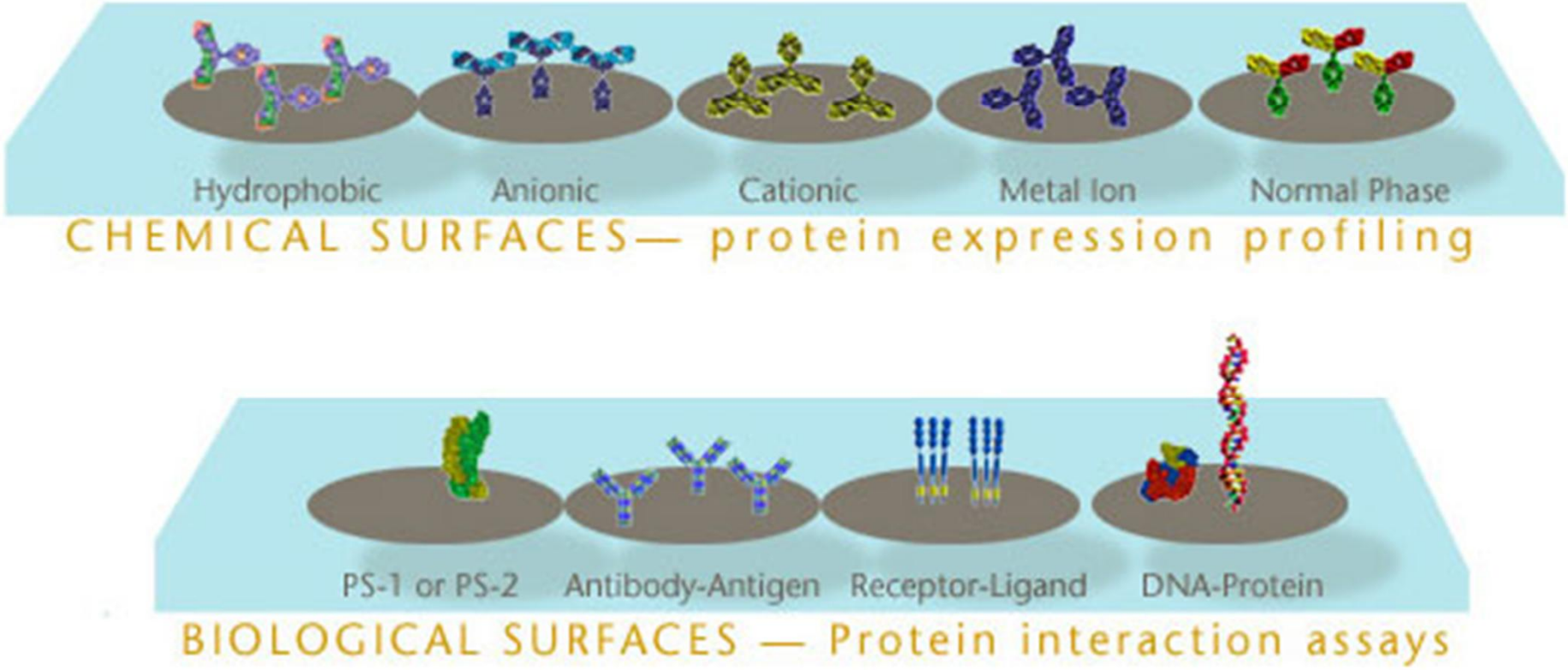


Affinity chip with microfluidistics





Several affinities to choose from



High Sample Throughput for the Post-Genomic Era and Clinical use

(35000 samples/ week)

MALDI-TOF(TOF)-MS

Twister

Prespotted AnchorChip targets
dedicated for Clinical Proteomics

> no cross contamination

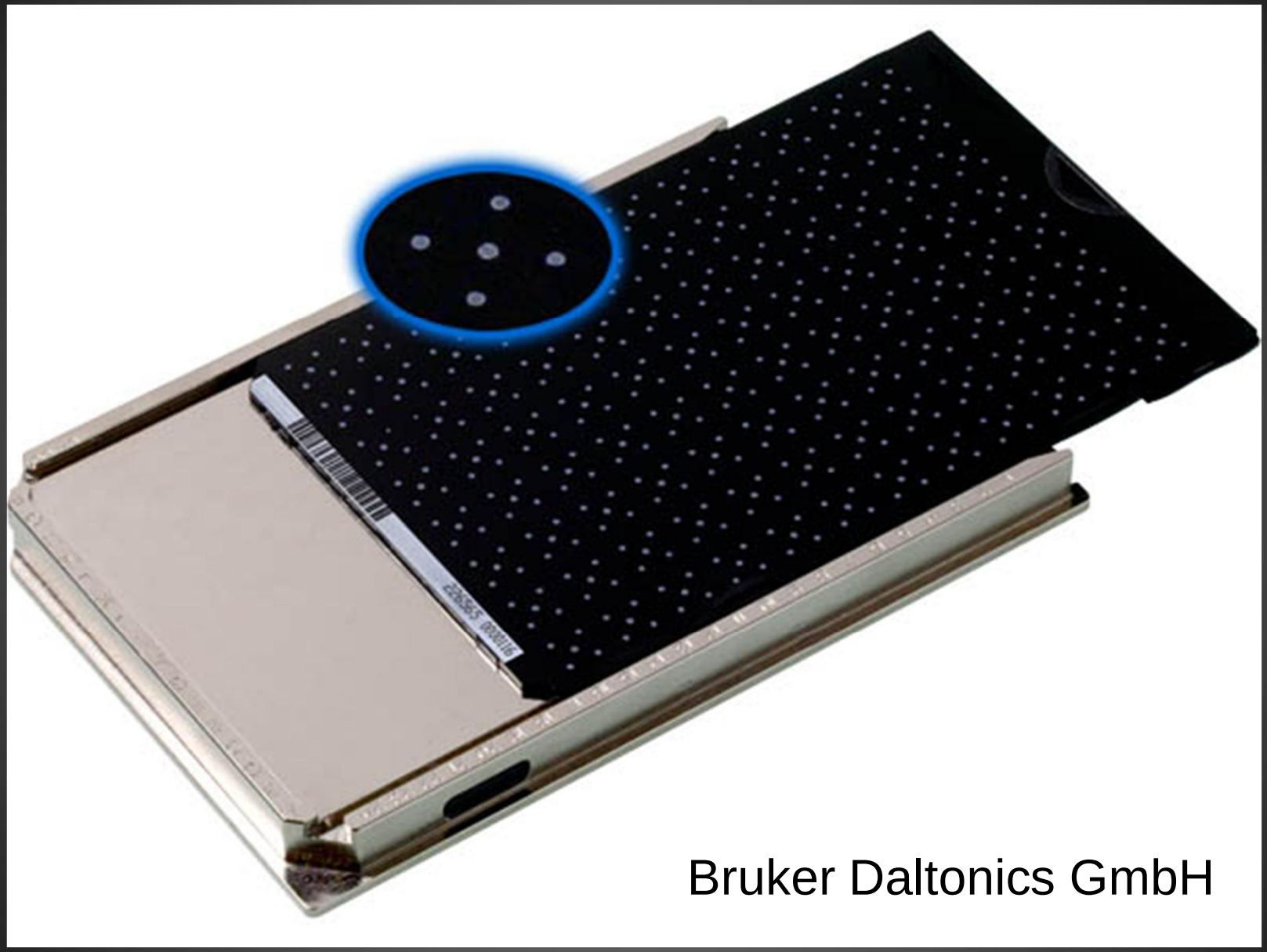
> no memory effects

> Easy storing and archiving of targets

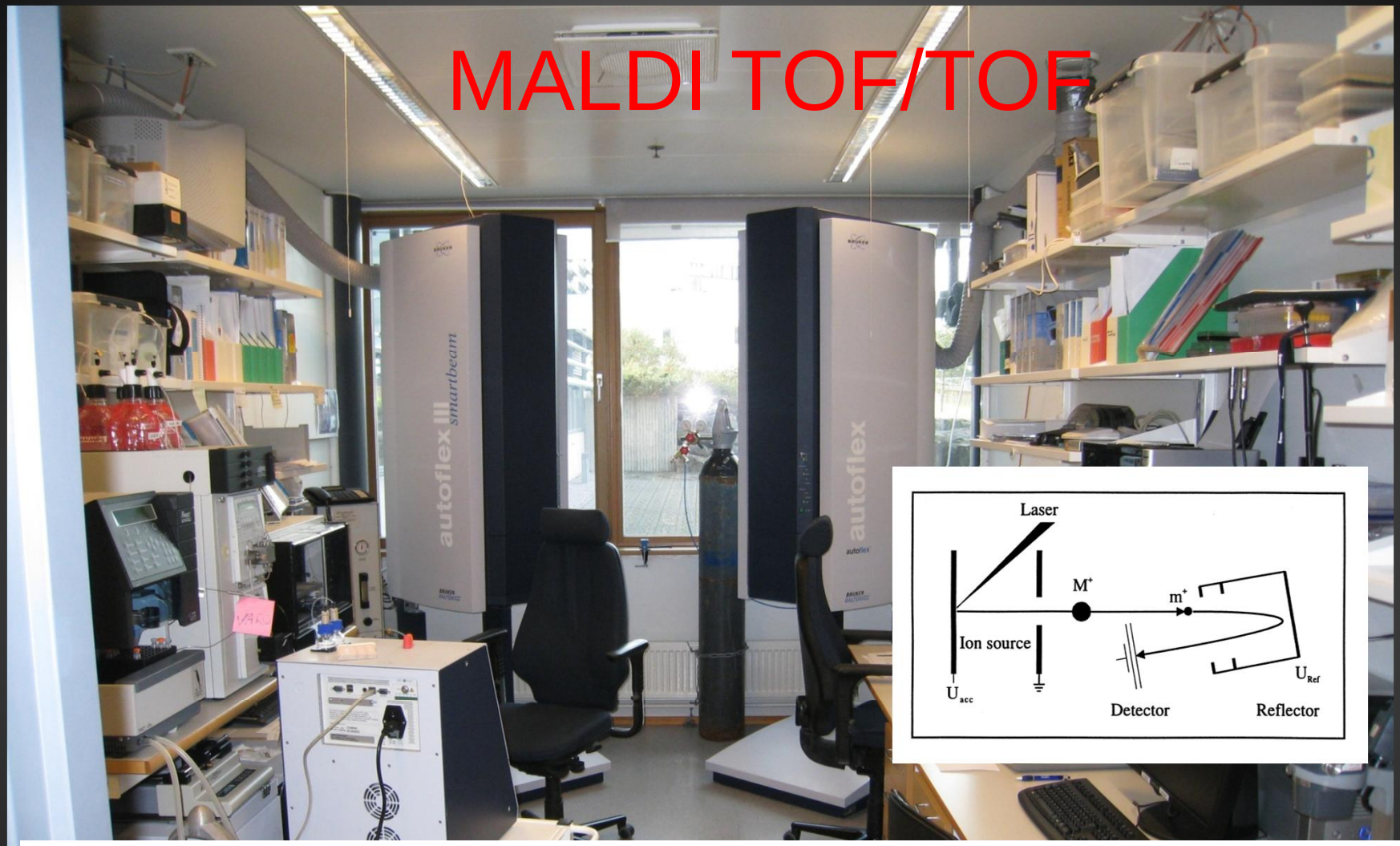
> Re-visiting of samples

> One calibrant, four matrix spots for samples

> 10-100 fold increased sensitivity, in situ sample purification


Bruker Daltonics GmbH

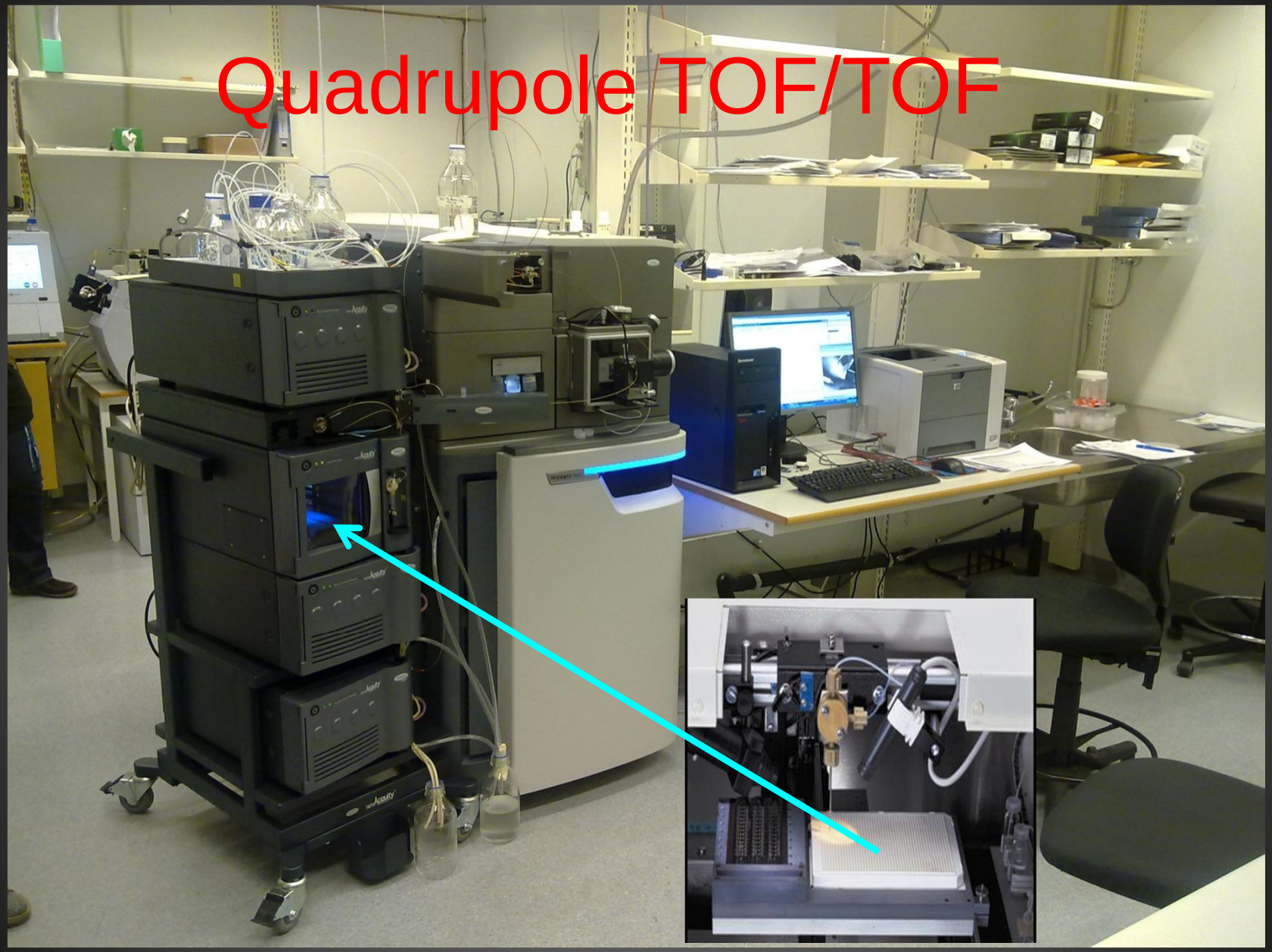
MALDI TOF/TOF

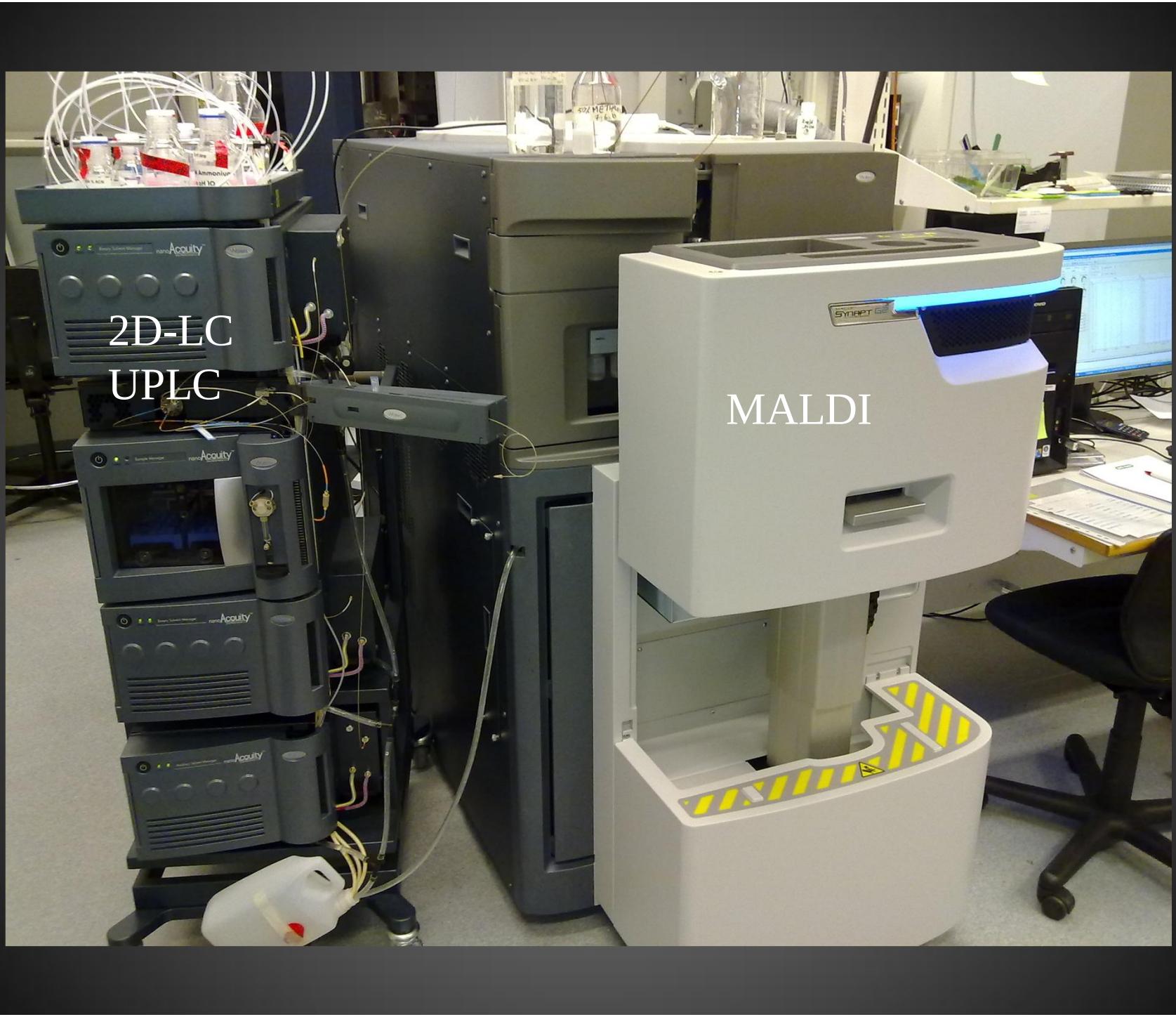


Schematic diagram of a Reflector MALDI-TOF mass spectrometer.

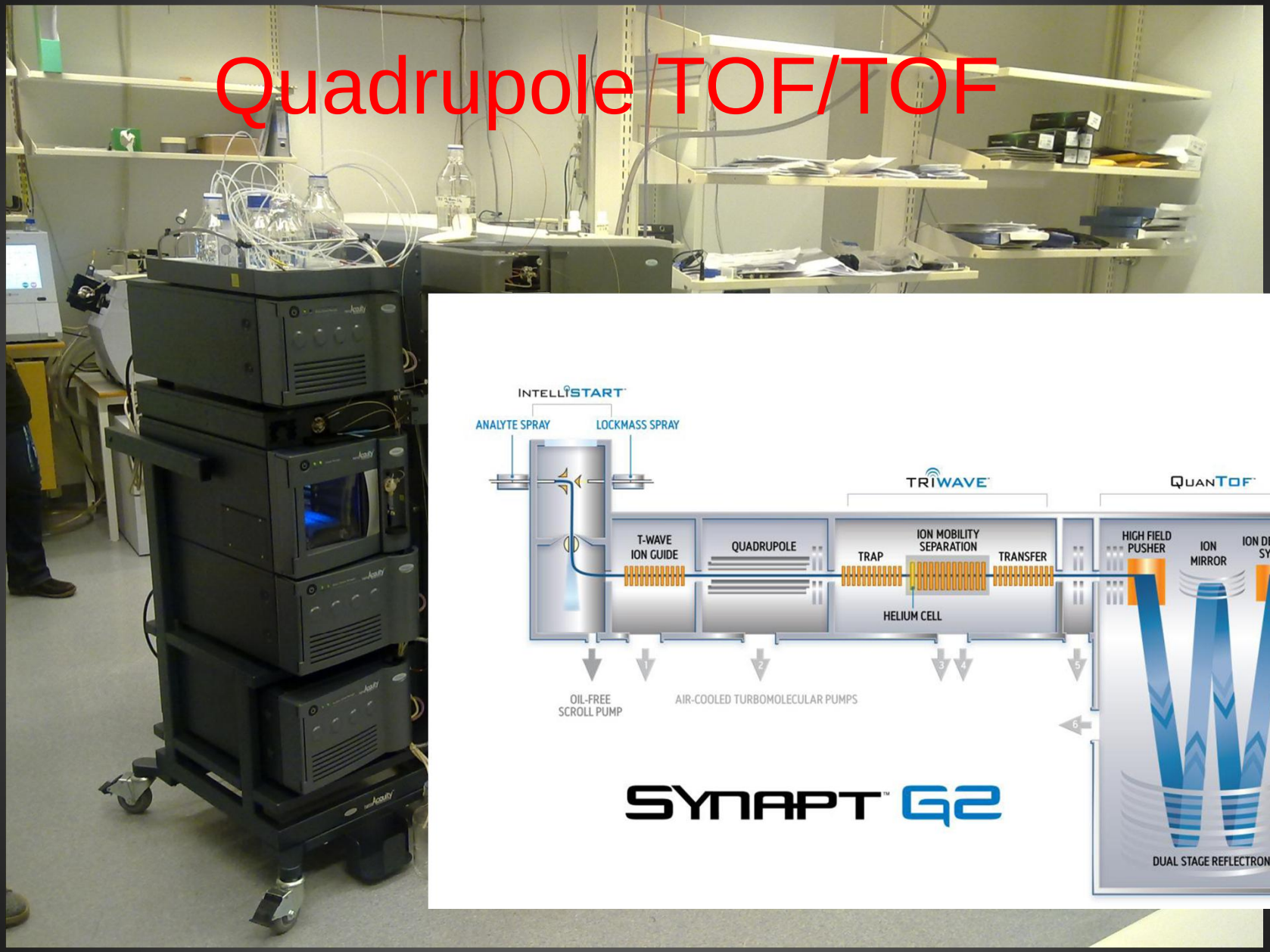
MALDI-TOF = Matrix Assisted Laser Desorption/Ionization - Time Of Flight

Quadrupole TOF/TOF

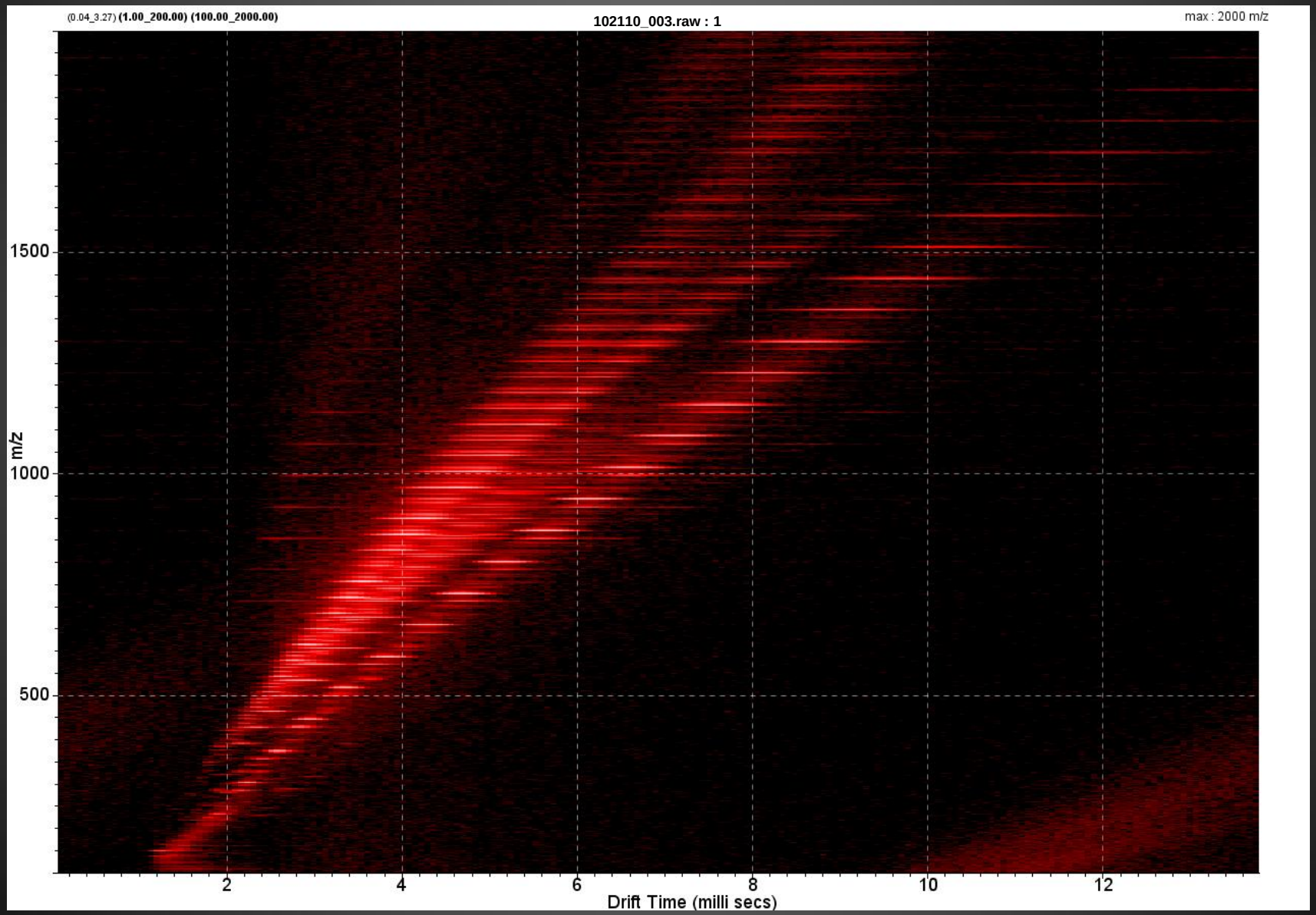



2D-LC
UPLC
MALDI

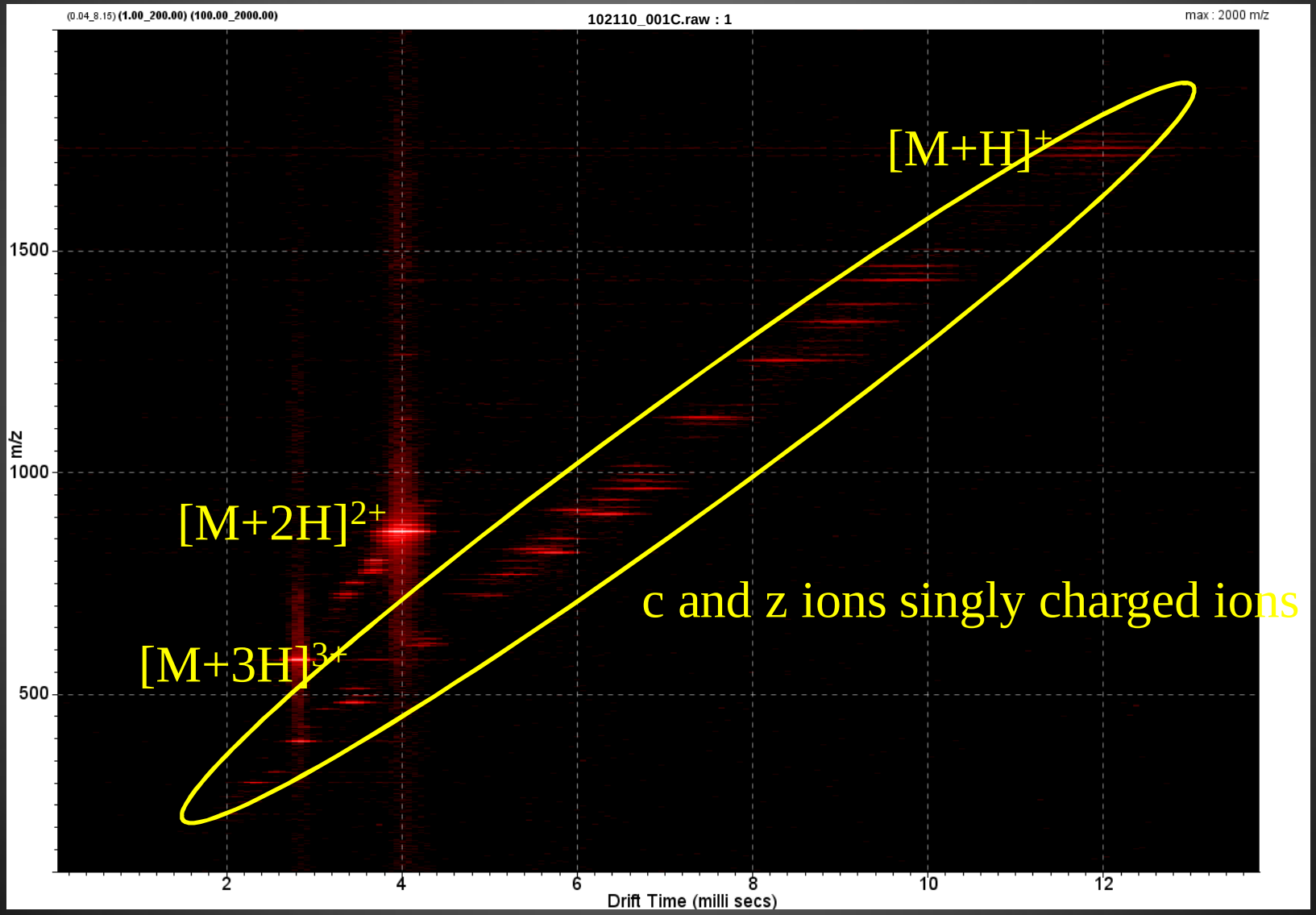
Quadrupole TOF/TOF



Ion Mobility driftscope view



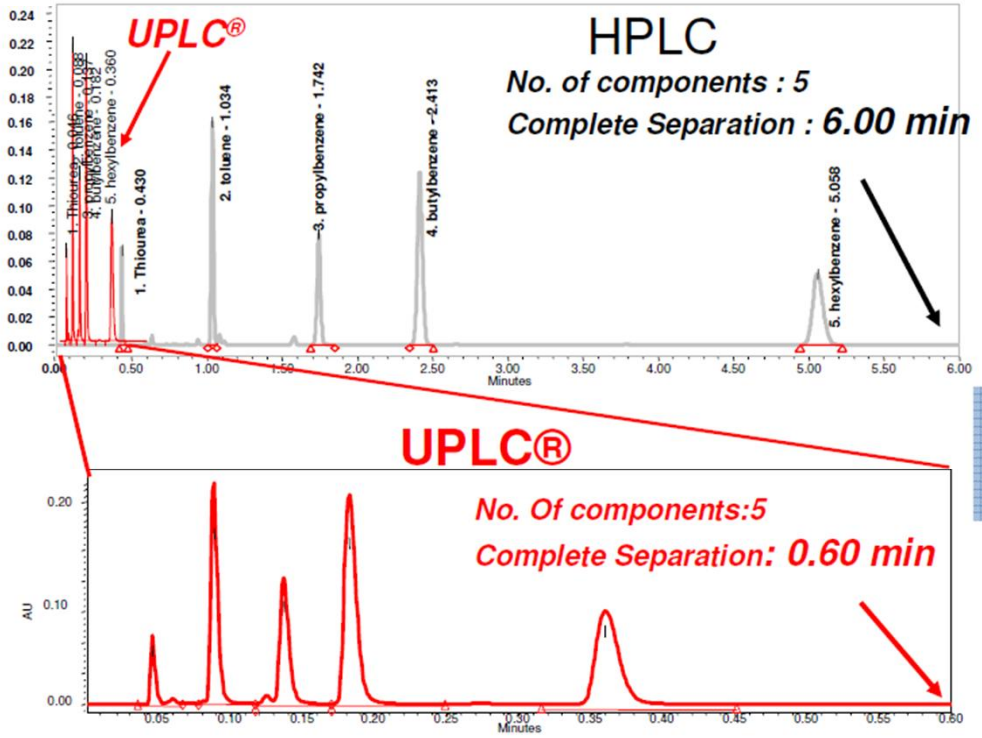
Ion Mobility driftscope view



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

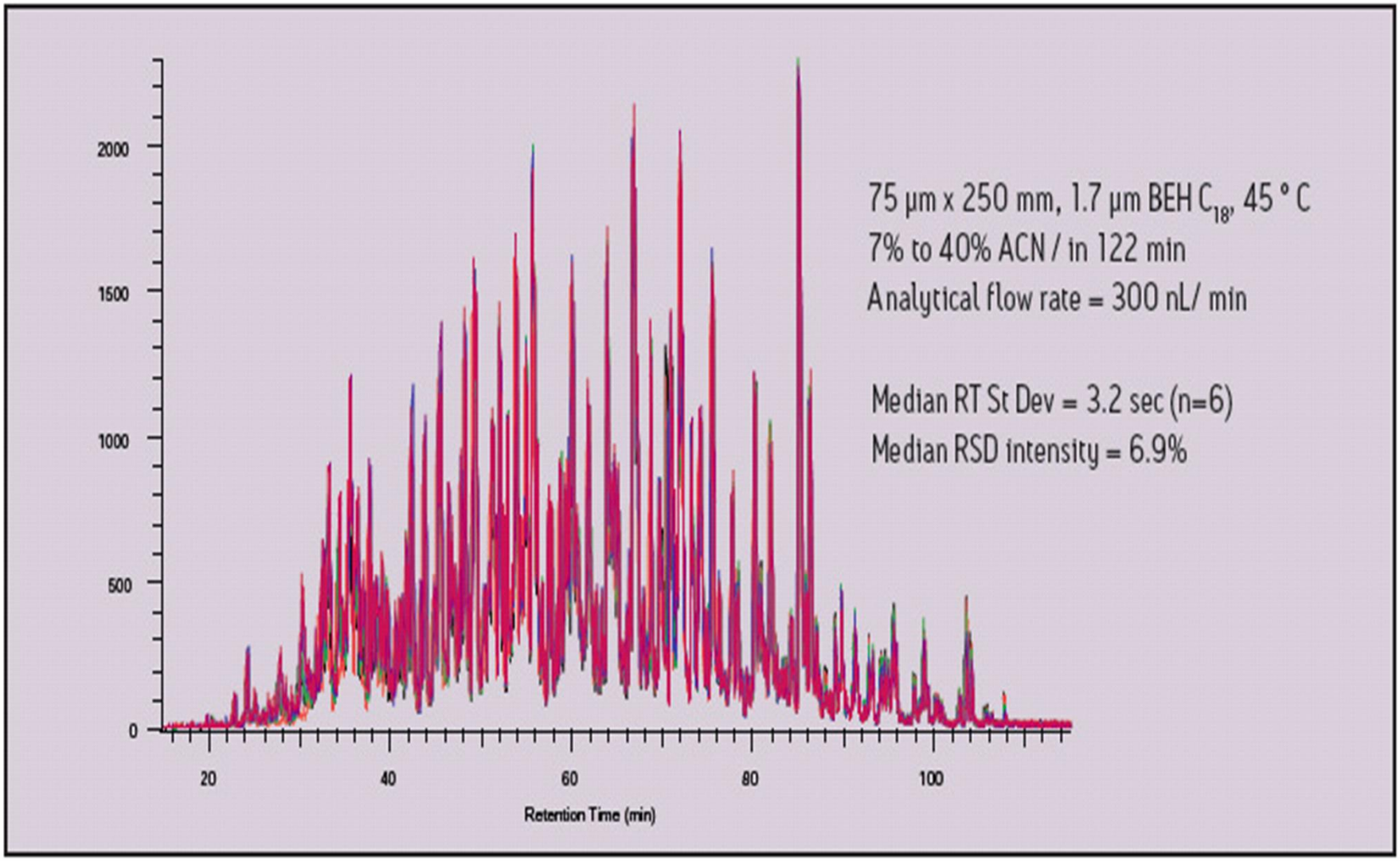
Although the time for one analysis in theory is fast…

UPLC™ increases Speed by 9X (900%)

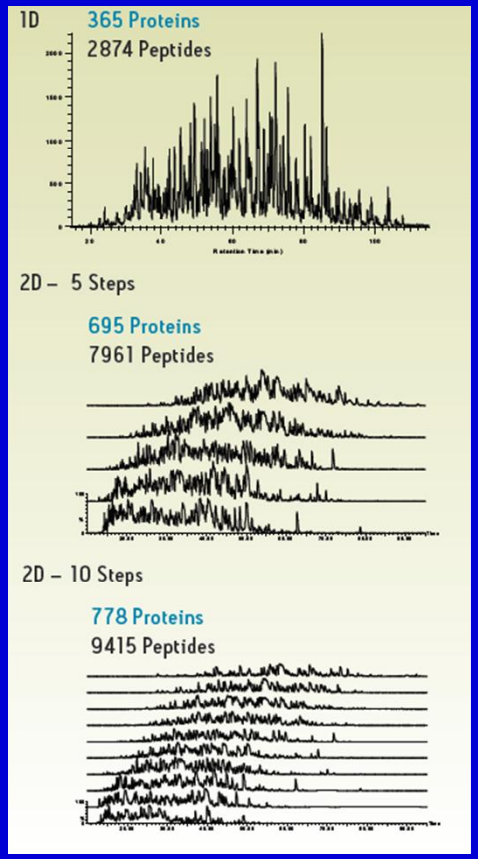


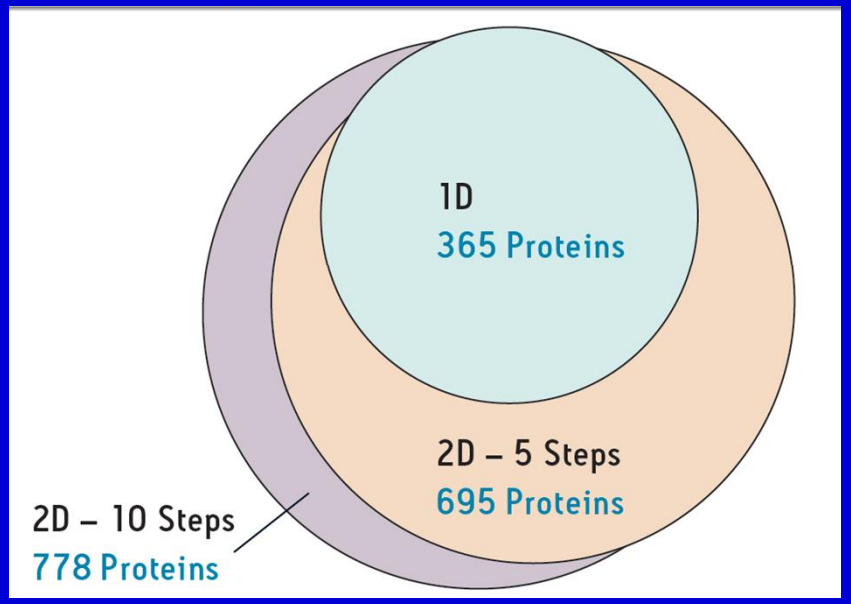
Increased sample throughput

In the reality you can analyze a few hundred compounds in a few hours...



You can then combine several analyzes to get thousands of compounds measured





If you want to quantify those, you need to run several serial analyses of each sample (label-free quantification)

Or use isotopic labeling (ITRAQ, ICAT, SILAC)

In any case

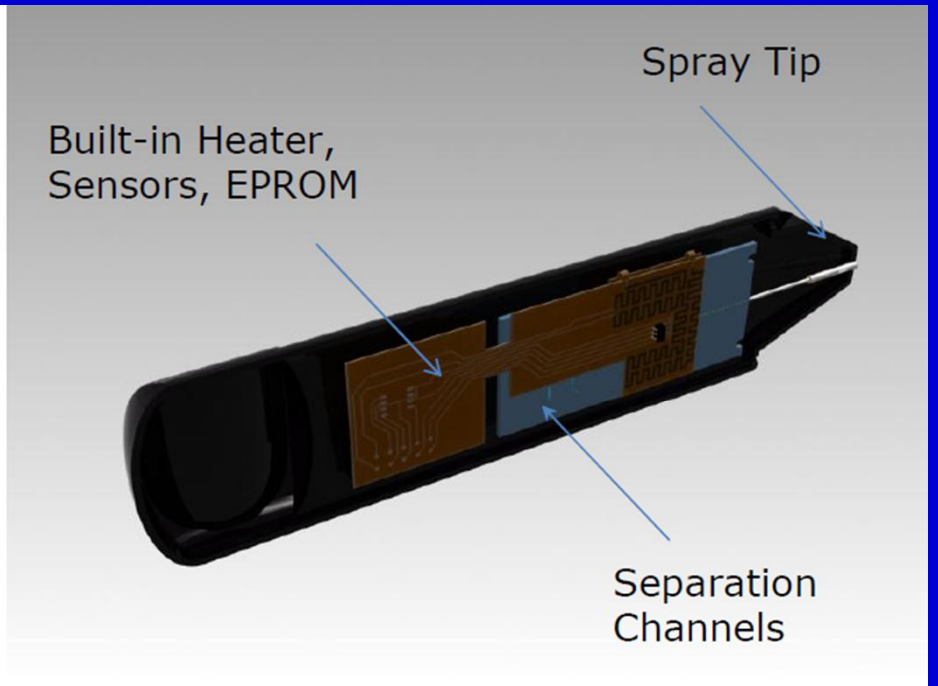
One sample needs approx. 24-38 hours of instrument time!

>>> One instrument can handle a few clinical sample series a year

Proteomics chip technology for LC

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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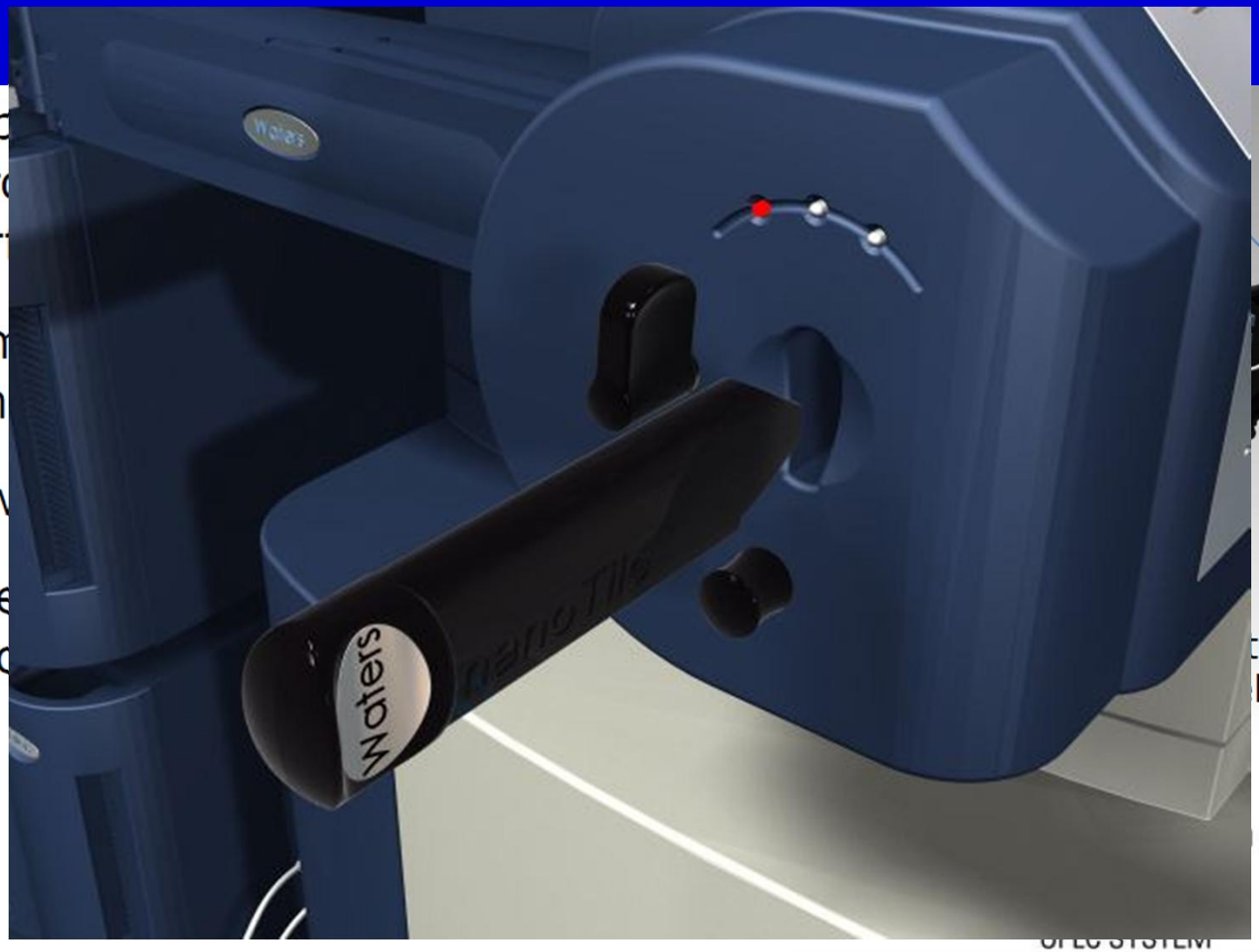
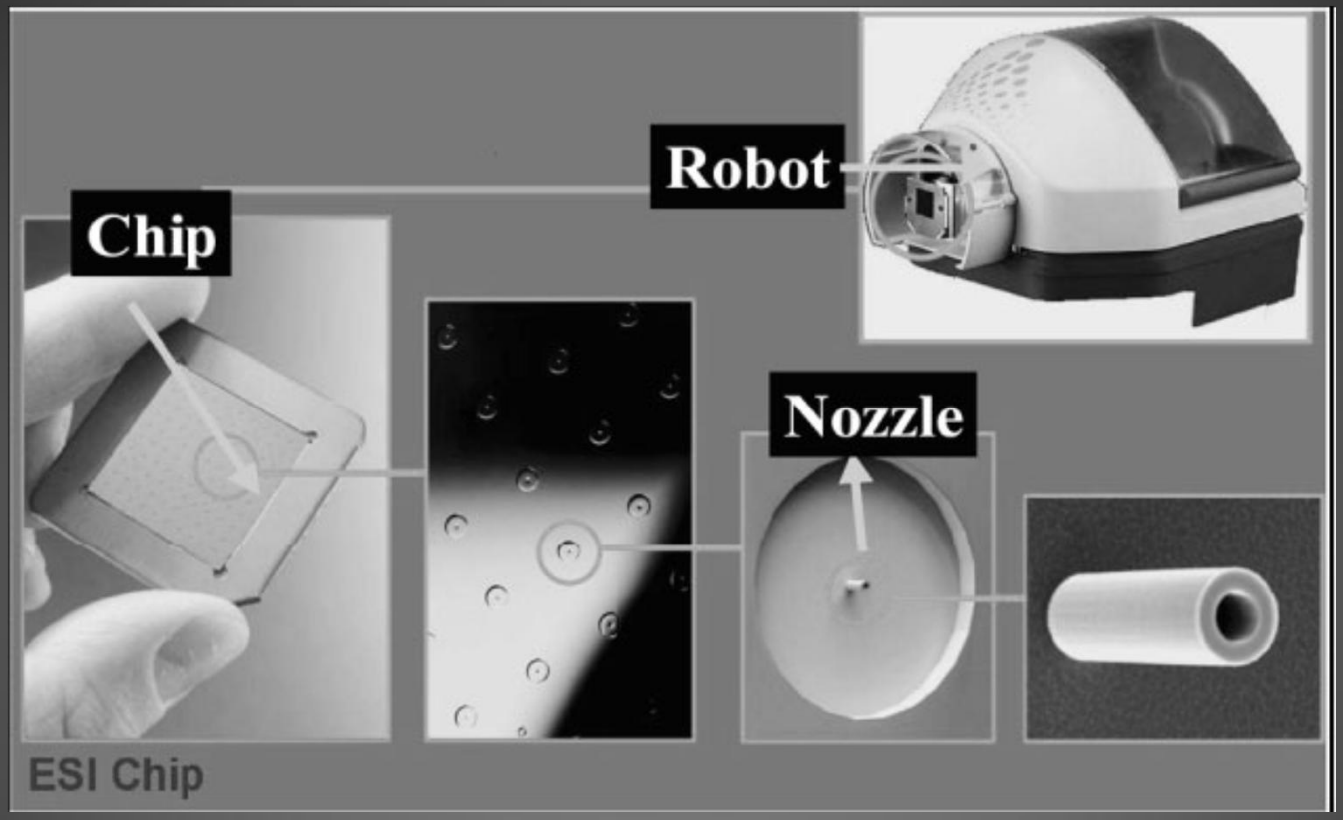
  per
- Elim
  con
- Low
- Inte
  elec



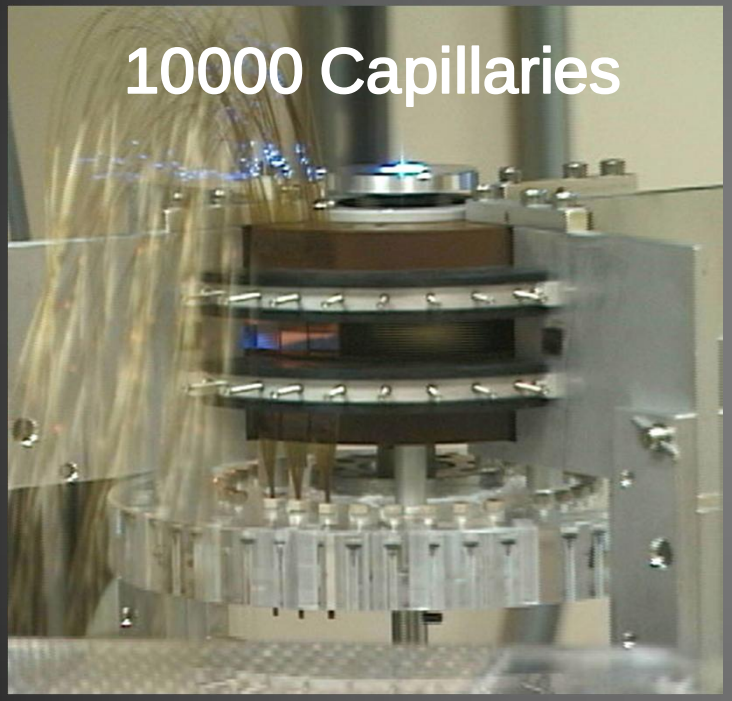
Tip

tion
ls

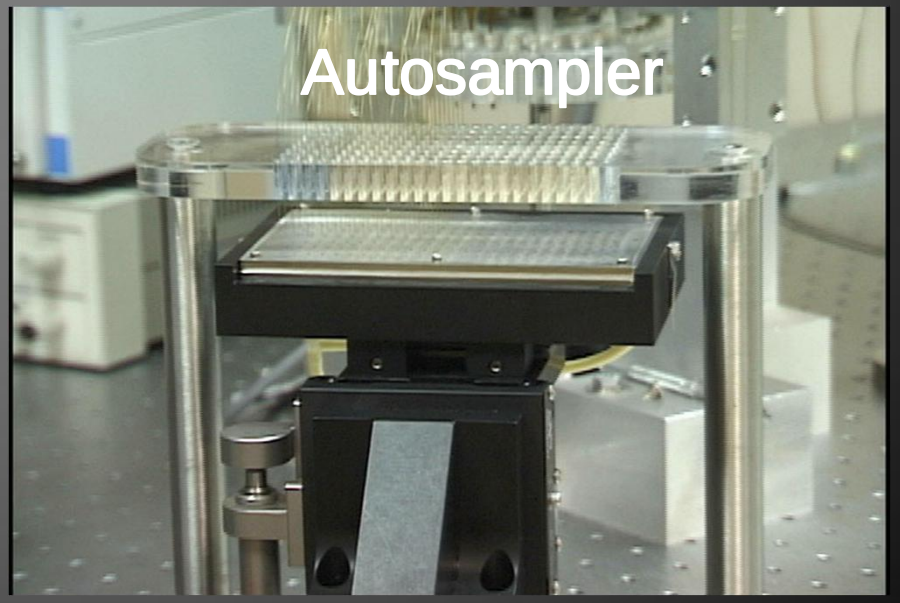
NanoSpray LC/MS



High throughput CE



LED detection (MIT, Forest et al, 2006)



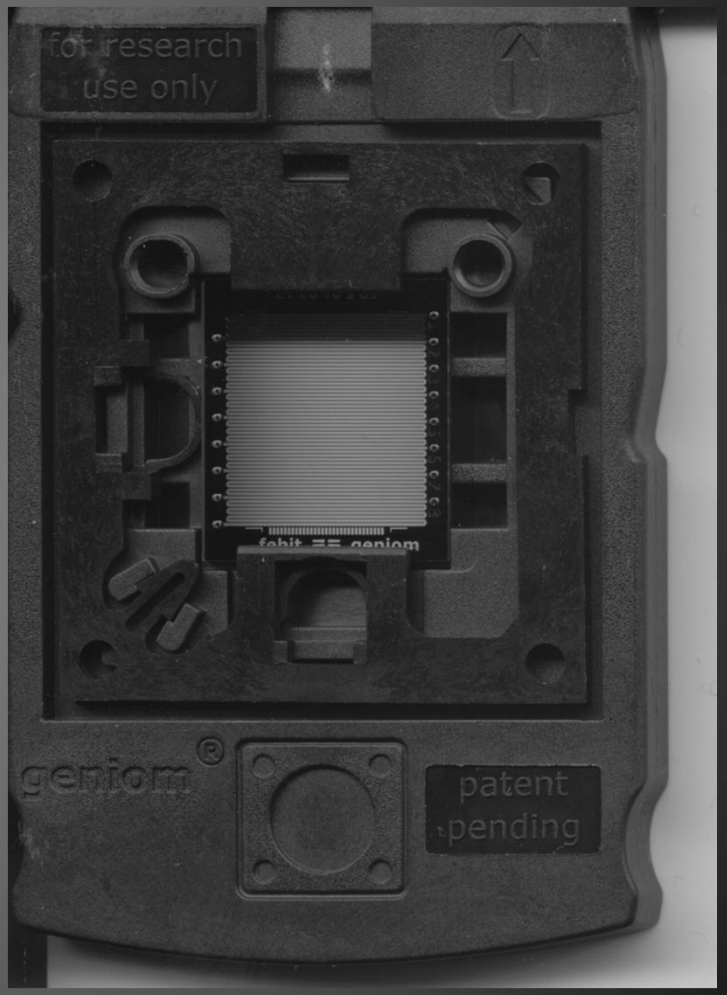
Ultra-high throughput CE chips for proteomics

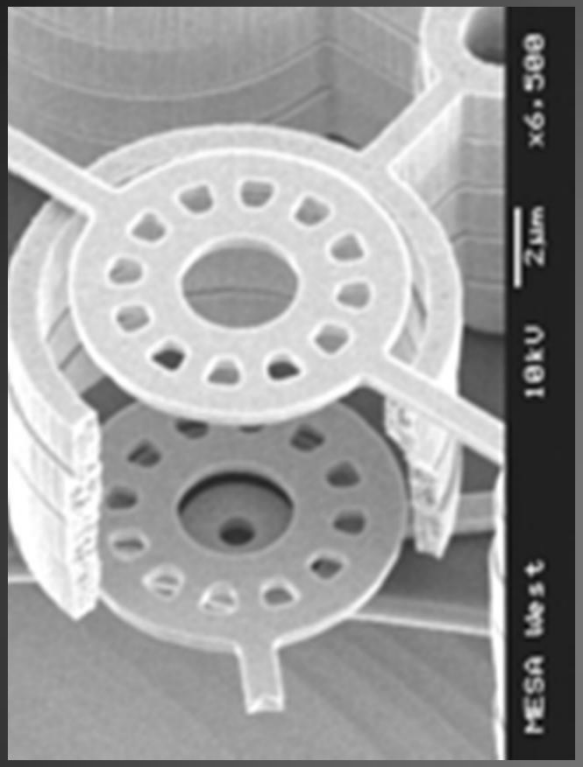
Geniom chips

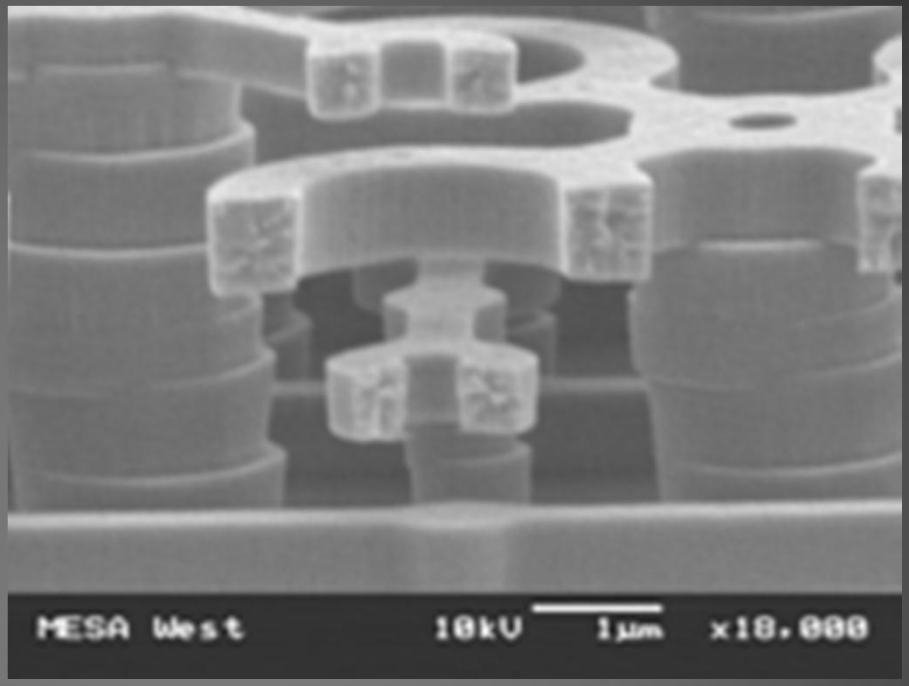
-Currently for DNA/RNA analysis

(Development ongoing for proteome analysis)

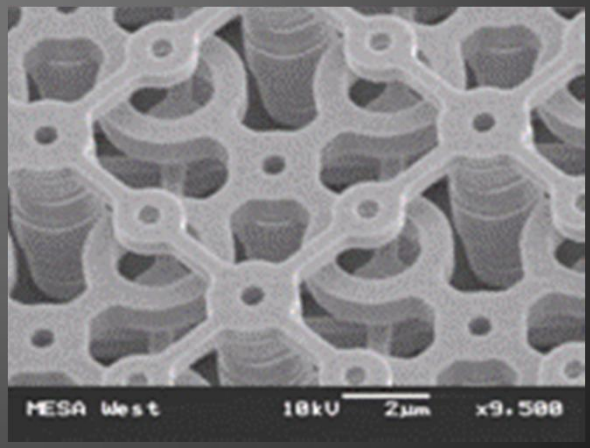
Capacity approx. 40000 ligands







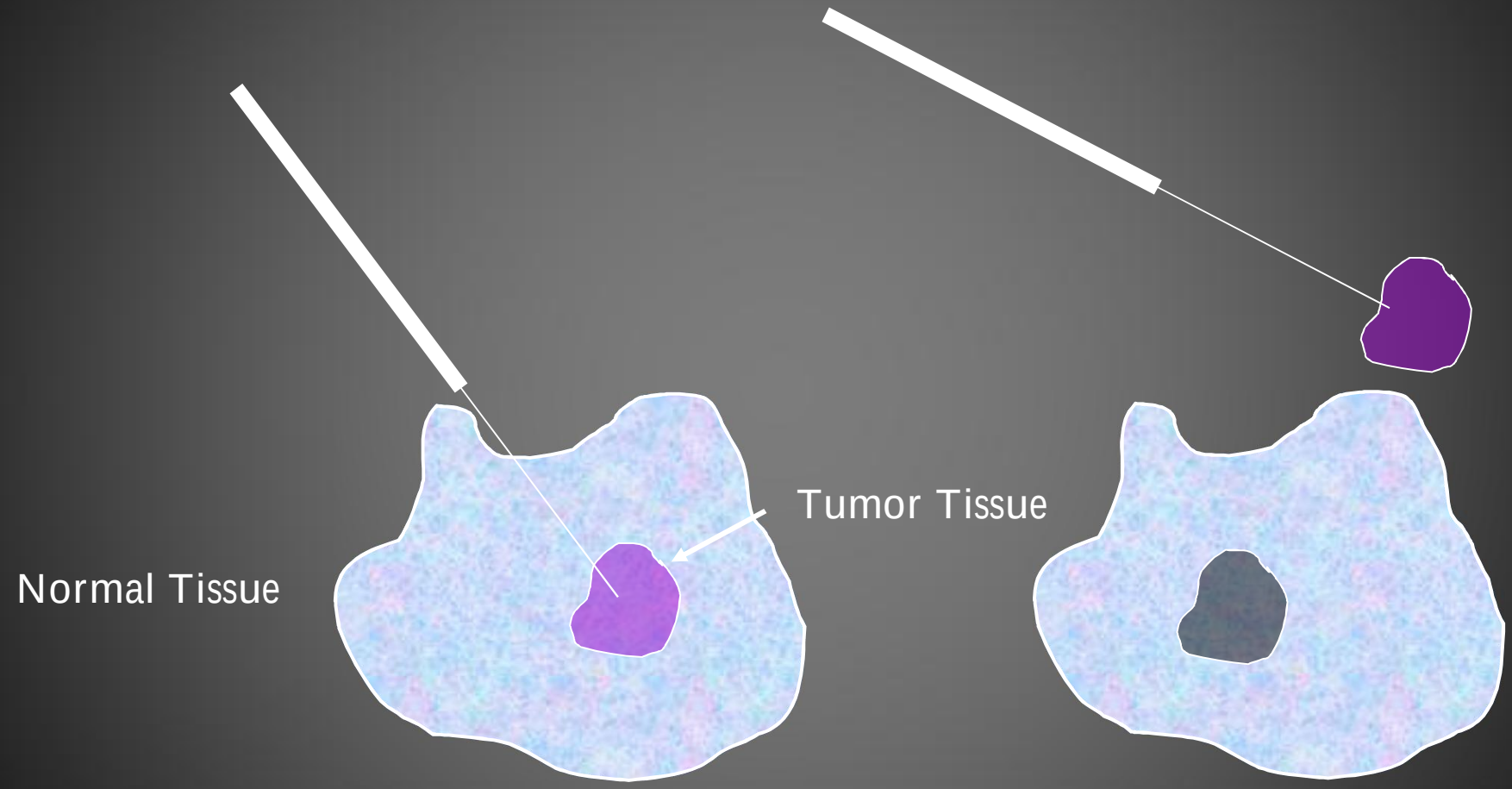
A set of thousands of Mass Spectrometers in one chip



Other techniques to help in proteomics?

- Tissue microdissection
- Imaging MS

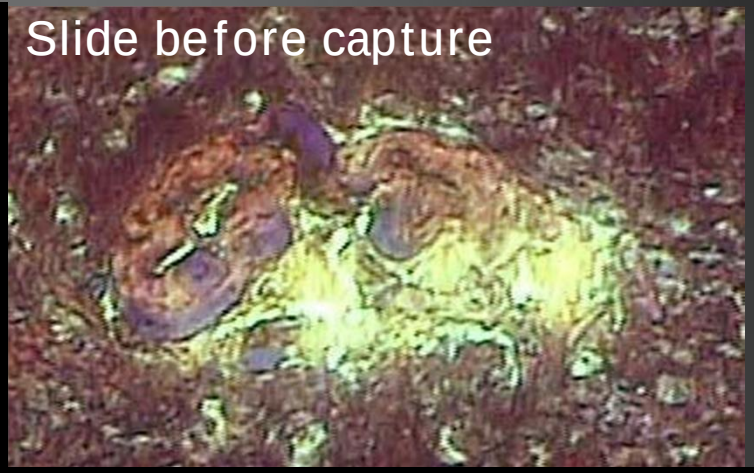
Why Tissue Microdissection?



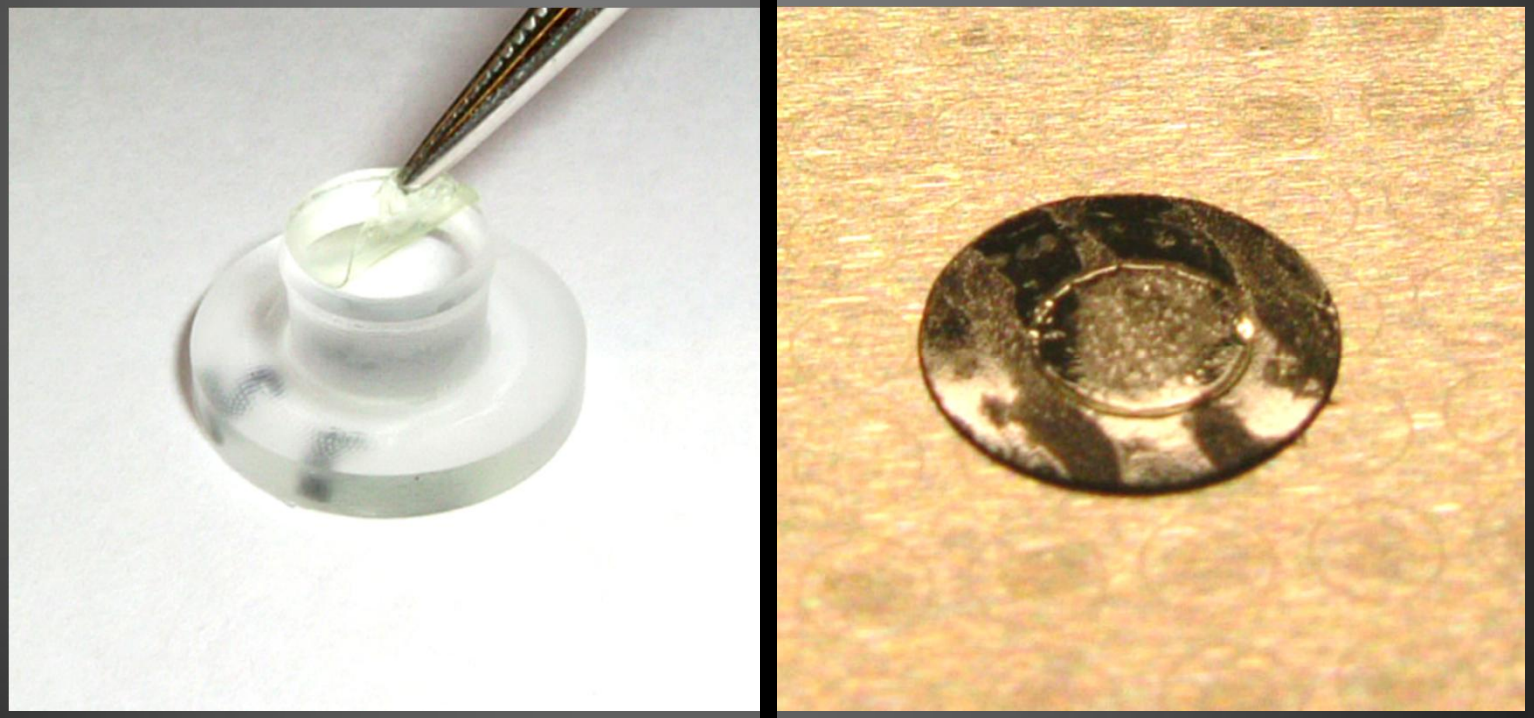
You would like to isolate only the targeted diseased tissue

Laser-based microdissection:

Capturing of the vessels in the control brain slide



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



Filter Aided Sample Preparation (FASP)

Whole tissue homogenated in SDS

>> centrifuge in a 10 kD co filter

Digest with trypsin

Change SDS to Urea

Filter again

>> run MS (approx 30-40% more proteins)

TECHNICAL BRIEF

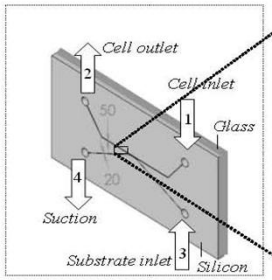
Comparison between procedures using SDS for shotgun proteomic analyses of complex samples

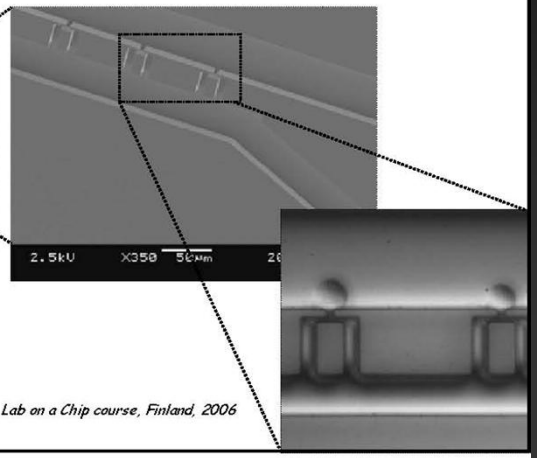
Michael S. Bereman, Jarrett D. Egertson and Michael J. MacCoss

Proteomics 2011, 11, 2931–2935 DOI 10.1002/pmic.201100045 2931

Whole Cell and Protein Microarray Chip structures

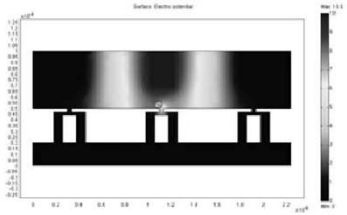
Electroporation on a chip: Cell trapping





Lab on a Chip course, Finland, 2006

Single cell electroporation

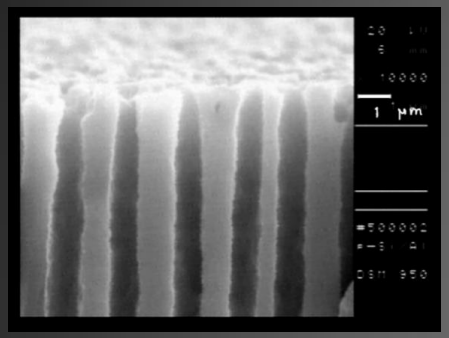


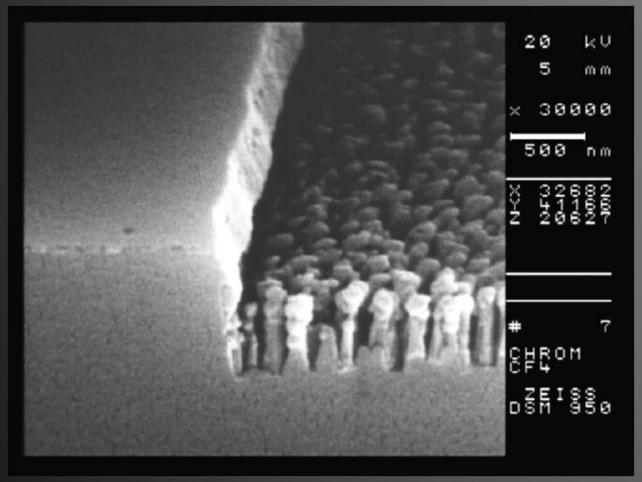
Gene transfection → GFP expression

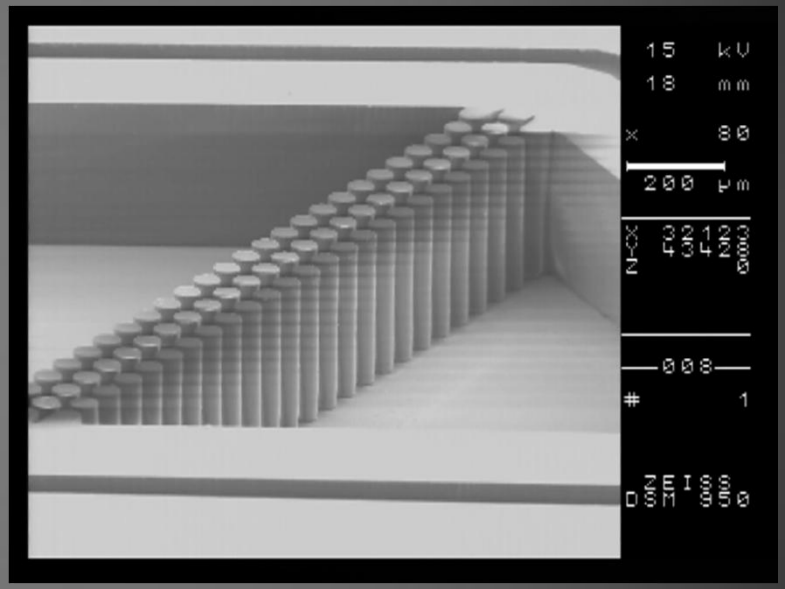
H. Andersson and A. van den Berg, "Microfluidic devices for cellomics: a review", *Sensors and Actuators B*, **92 (3)**, (2003), 315-325.

Lab on a Chip course, Finland, 2006

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





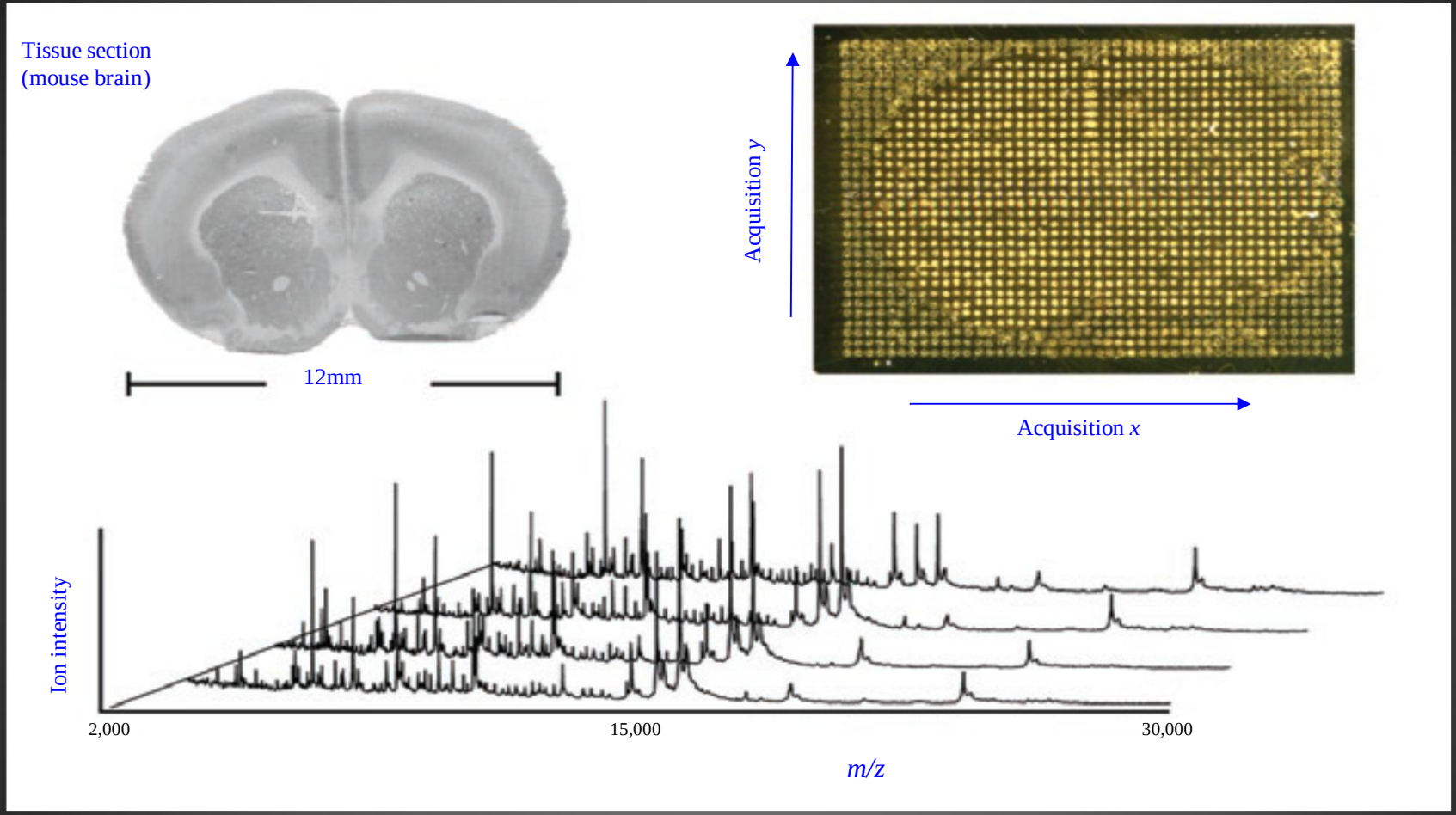


Development of Integrated Nanoliter Analysis Devices (DDTC-Viikki, Microtechnology Center-HUT, Biomedicum Helsinki)

The TIME component!

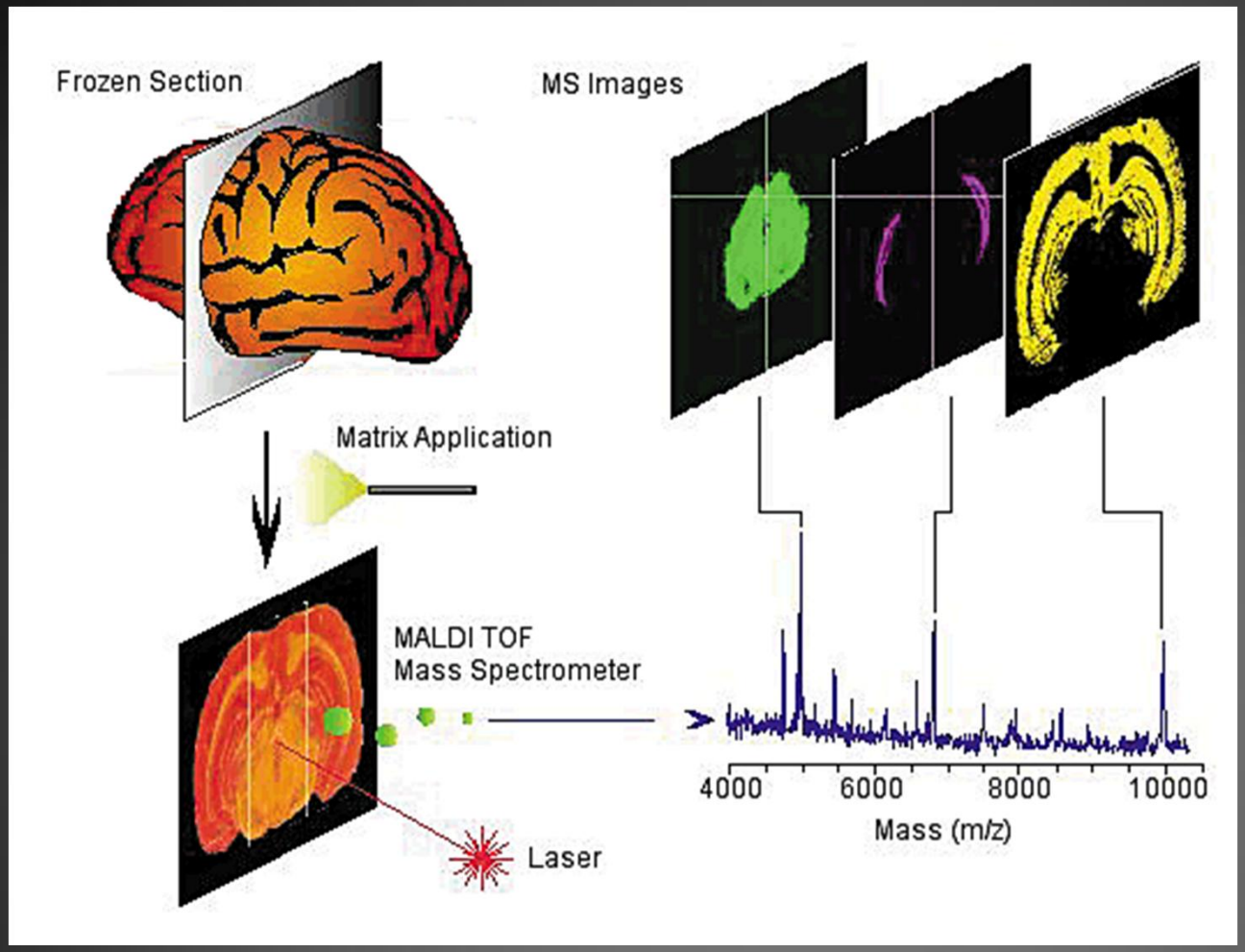
MALDI mass spectrometric imaging of biological tissue sections for protein imaging

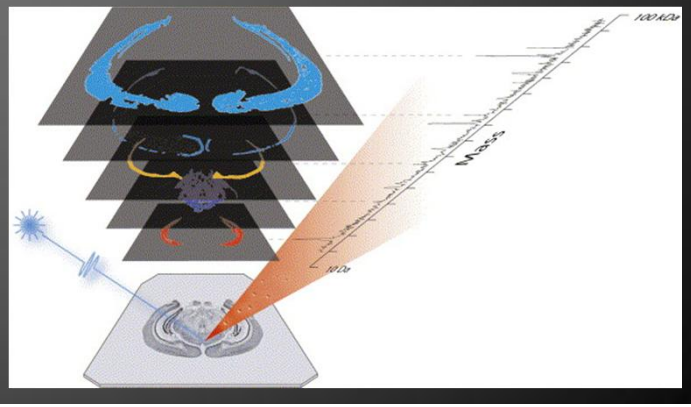
Principles



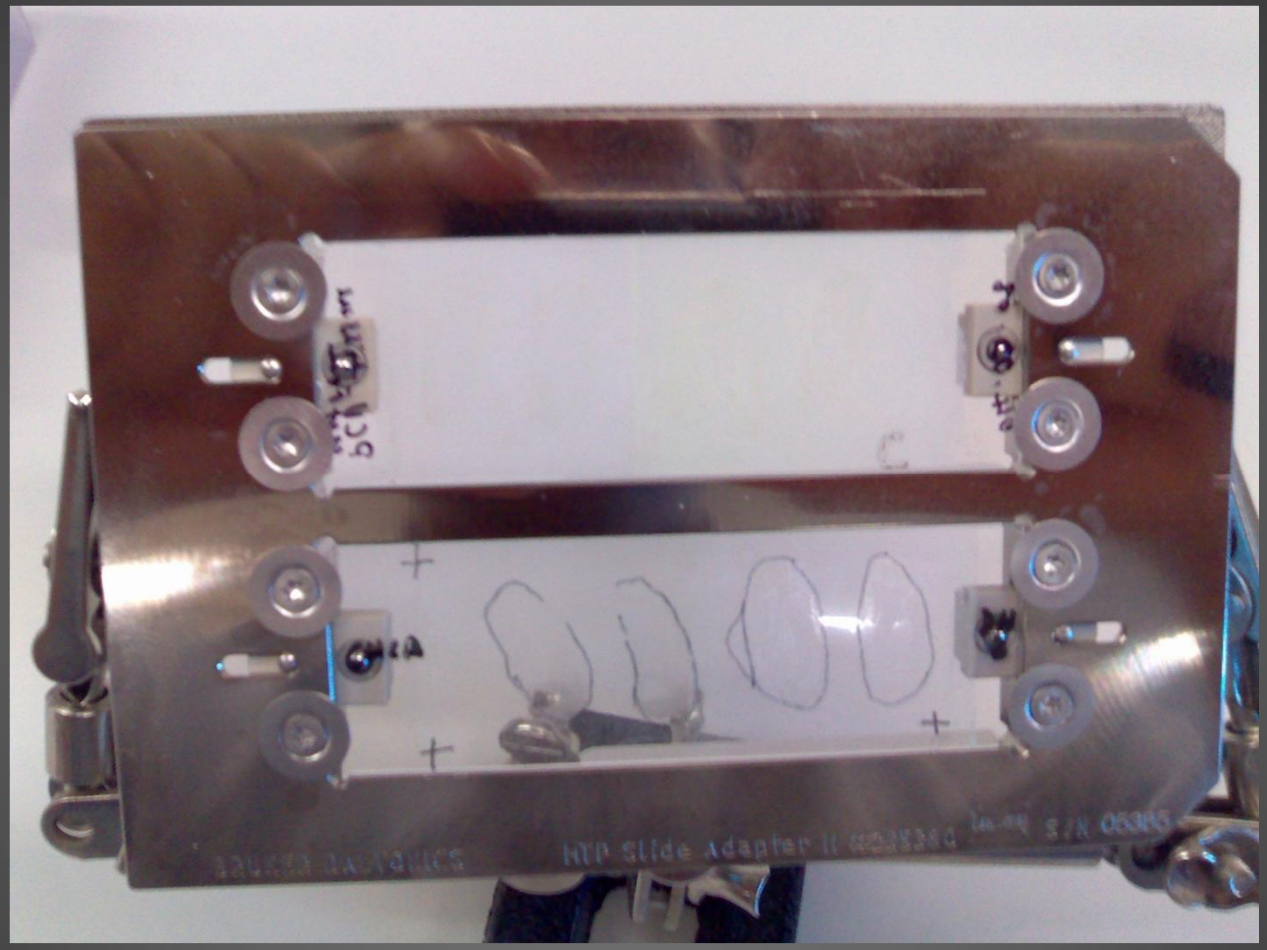
- A laser is rastered over a defined area while acquiring a complete mass spectrum from each position, resulting in molecular images for multiple analytes

  Cornett, et al., Nature Methods 2007


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


Mass

Tissue slide for IMS



research articles Journal of proteome research

MALDI Imaging of Formalin-Fixed Paraffin-Embedded Tissues: Application to Model Animals of Parkinson Disease for Biomarker Hunting

J. Stauber,[ζ,†] R. Lemaire,[ζ,†] J. Franck,[†] D. Bonnel,[†] D. Croix,[†] R. Day,[‡] M. Wisztorski,[†] I. Fournier,[*,†] and M. Salzet[*,†]

Clathrate nanostructures for mass spectrometry

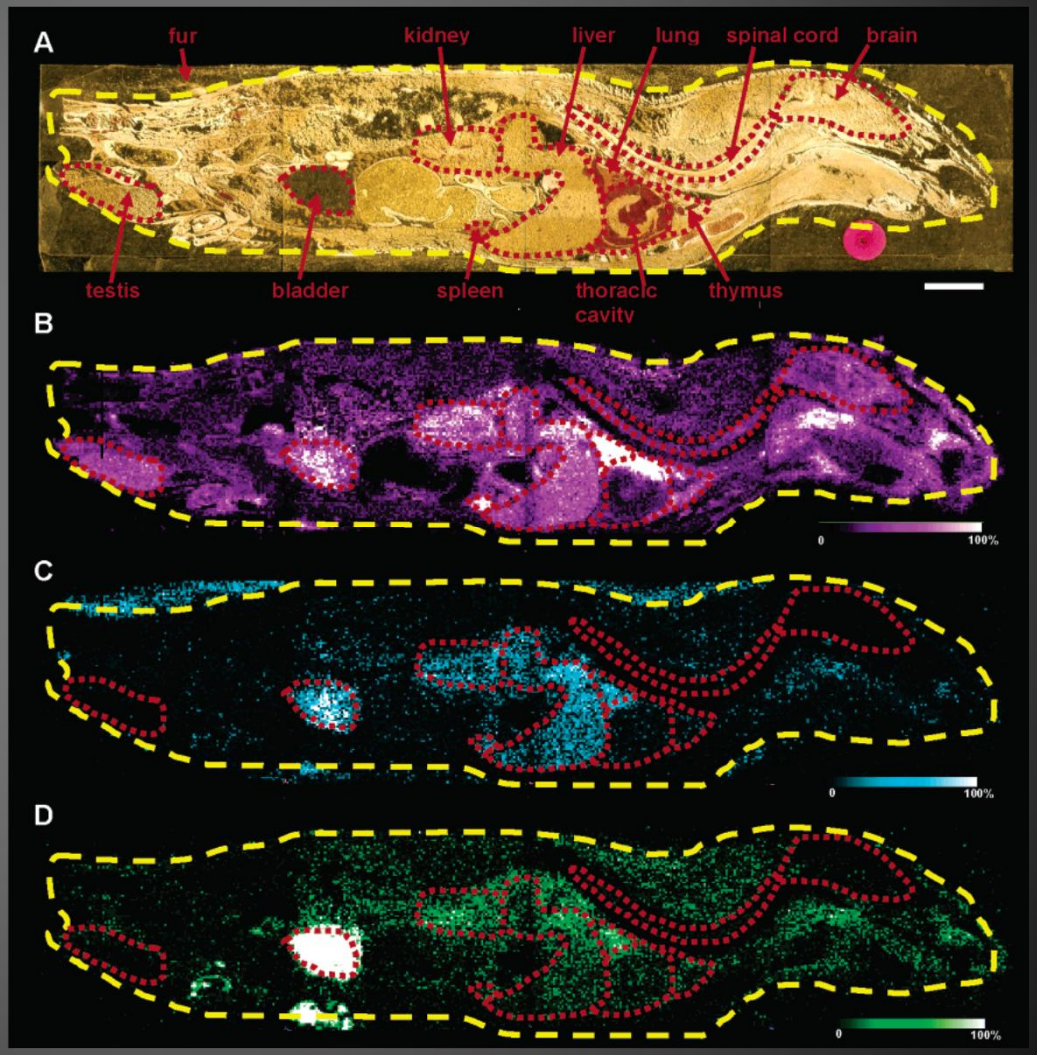
Trent R. Northen[1,2*], Oscar Yanes[1,2*], Michael T. Northen[4], Dena Marrinucci[3], Winnie Uritboonthai[1,2], Junefredo Apon[1,2], Stephen L. Golledge[5], Anders Nordström[1,2] & Gary Siuzdak[1,2*]

MALDI imaging mass spectrometry: molecular snapshots of biochemical systems

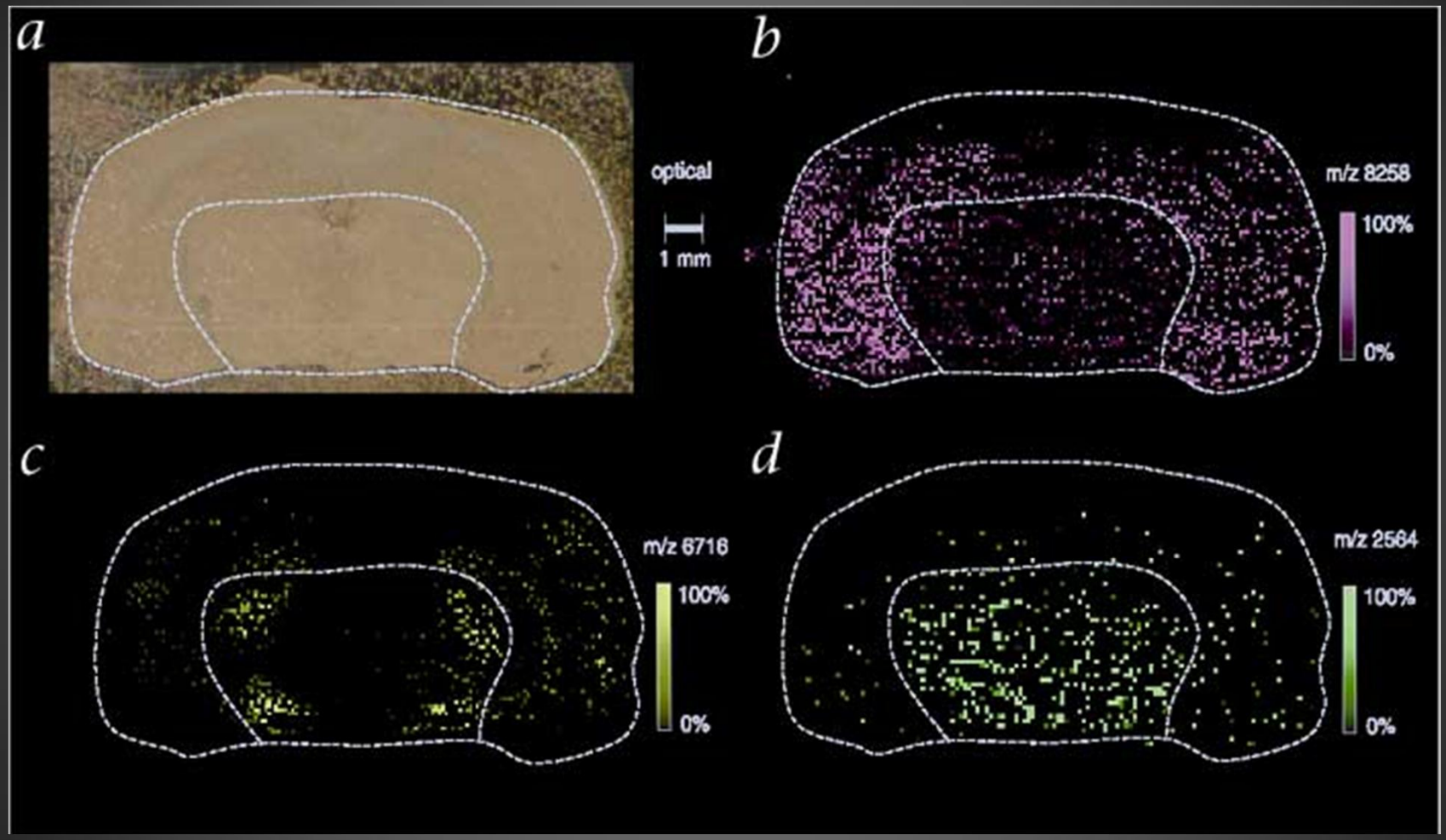
Dale S Cornett, Michelle L Reyzer, Pierre Chaurand & Richard M Caprioli

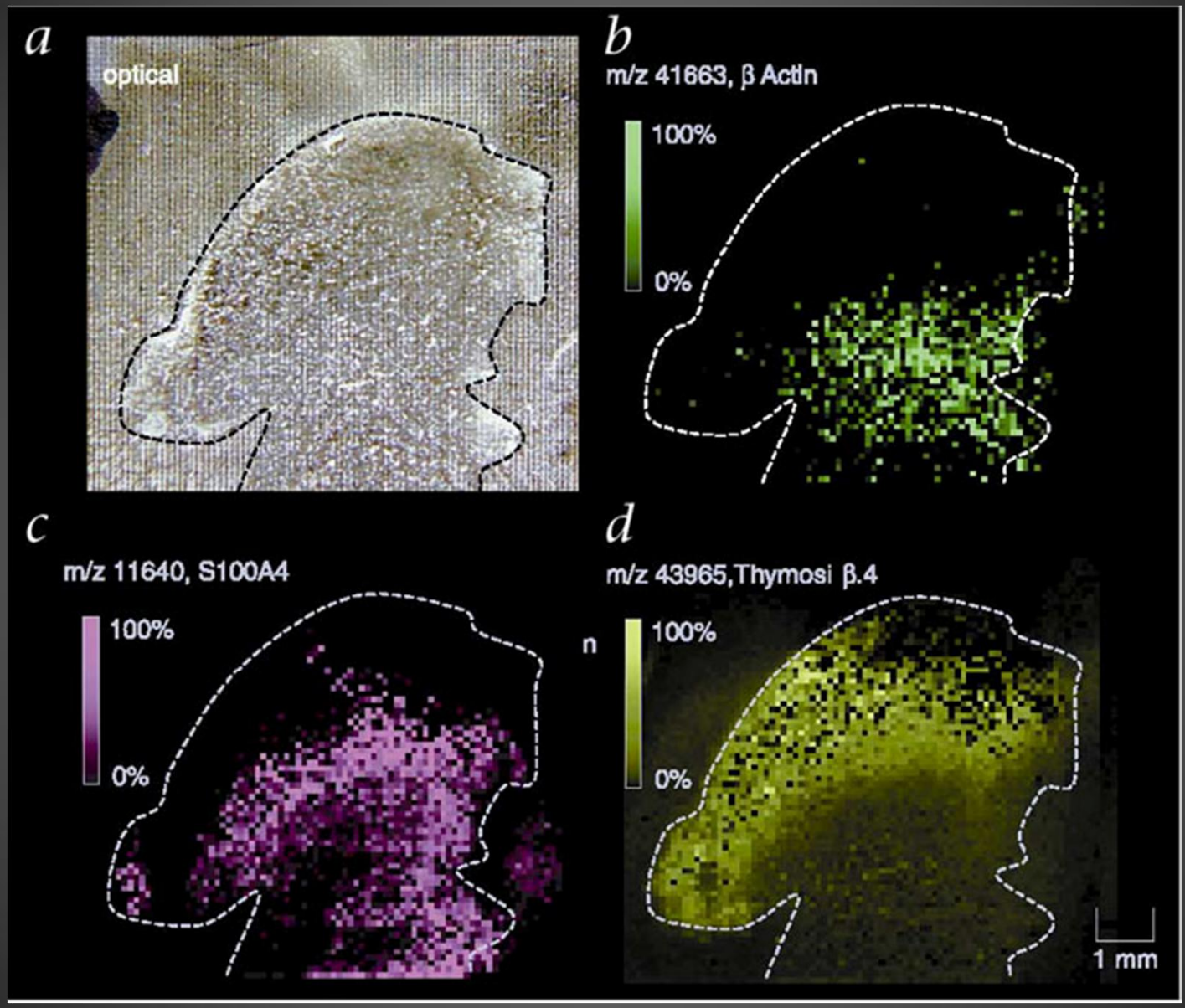
Benefits of MALDI-IMS

- Analysis of entire sample in one reading
- Previous knowledge of molecular composition is not necessary
- Allows for investigation of disease formation, progression, and treatment

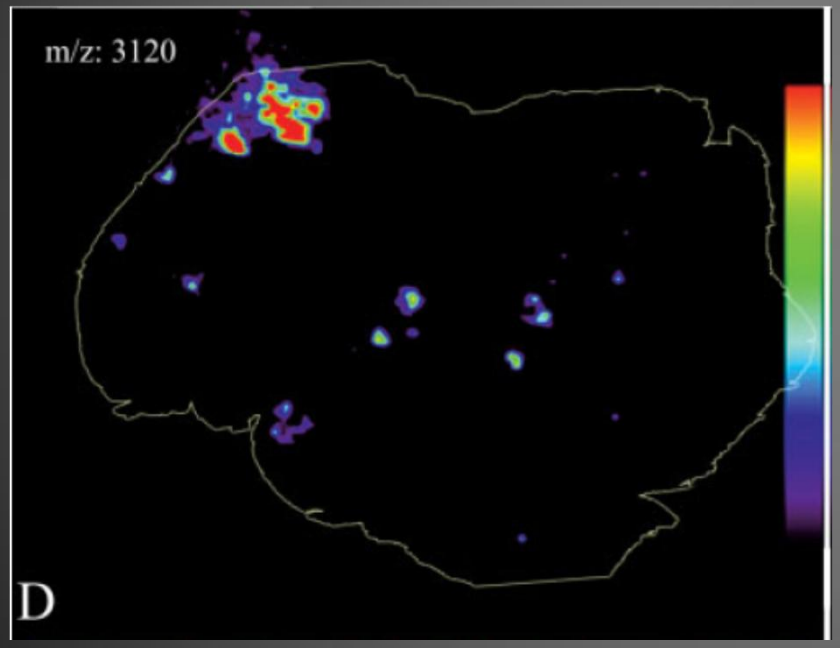


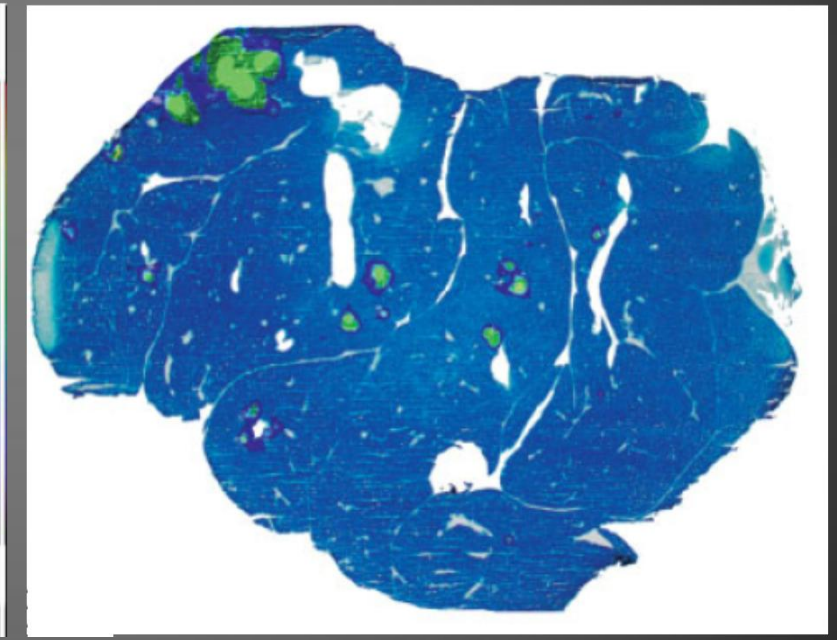
www.maldi-msi.org


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

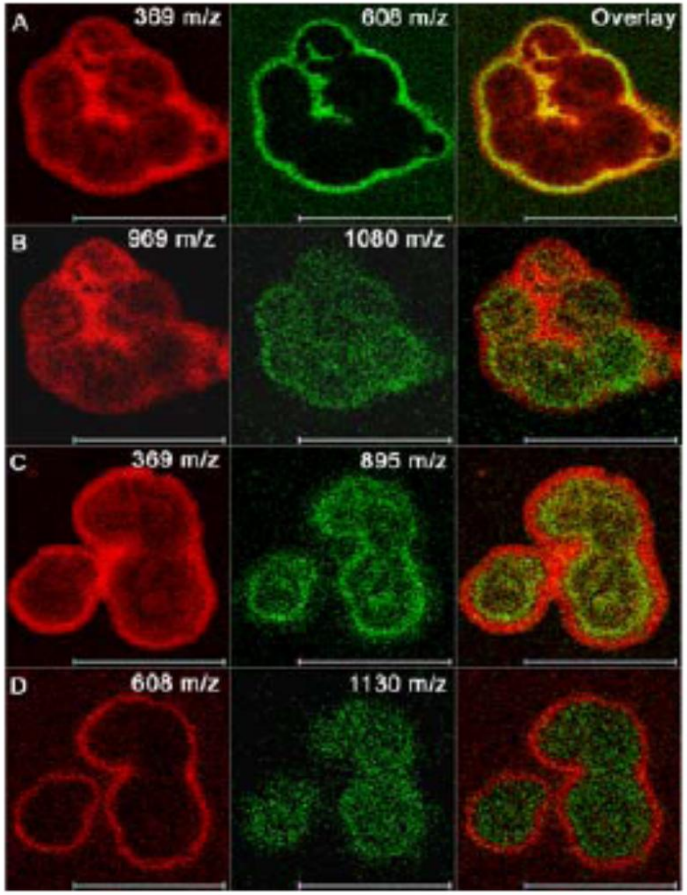

a
optical
b
m/z 41663, β Actin
100%
0%
c
m/z 11640, S100A4
100%
0%
d
m/z 43965,Thymosi β.4
100%
0%
1 mm

IMS vs. histochemical stain

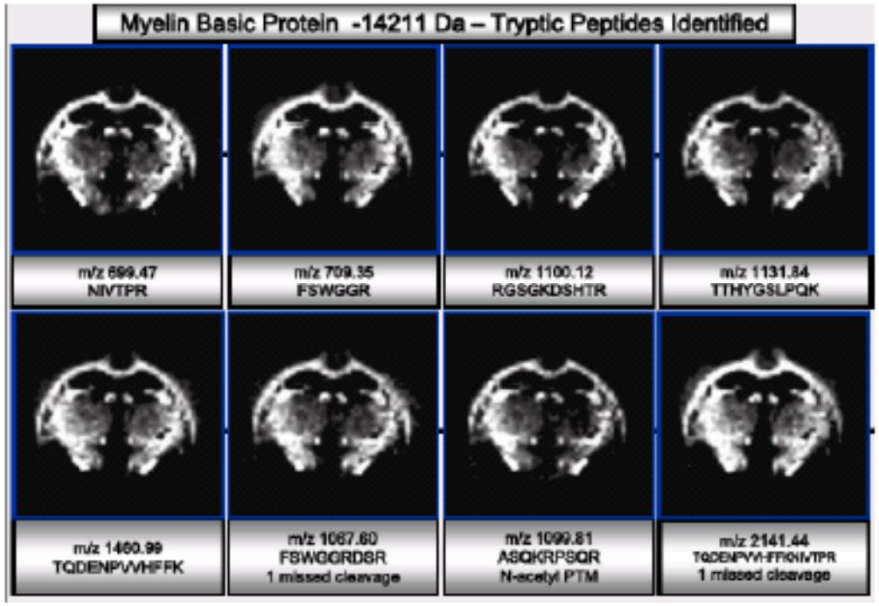




Imaging Lipidomics



Imaging Proteomics



M. Reid Groseclose, Malin Andersson, Richard M. Caprioli ASMS 2006, poster presentation

Localization of water-soluble carbohydrates in wheat stems using imaging matrix-assisted laser desorption ionization mass spectrometry

Sarah Robinson[1], Karen Warburton[3], Mark Seymour[2], Malcolm Clench[3] and Jane Thomas-Oates[1]

[1]Department of Chemistry, University of York, Heslington, York YO10 5DD, UK; [2]Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK; [3]Biomedical Research Centre, Sheffield Hallam University, Howard Street, Sheffield S1 1WB, UK

New Phytologist (2007) **173** : 438 –444

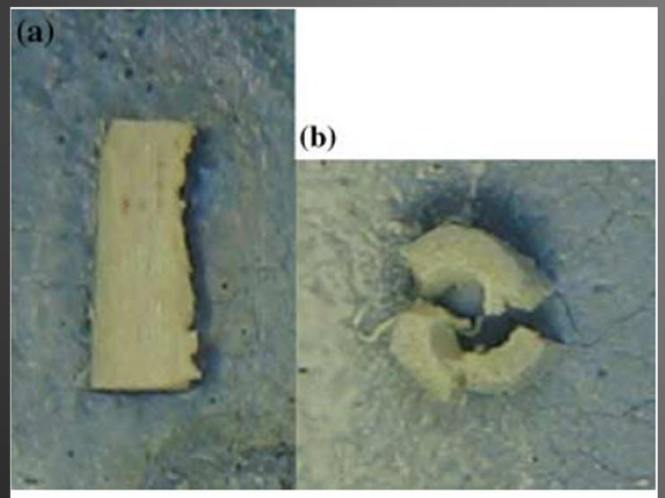
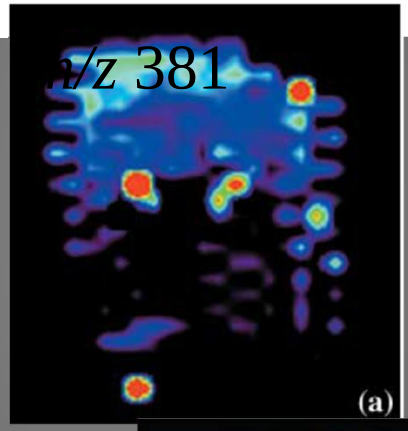
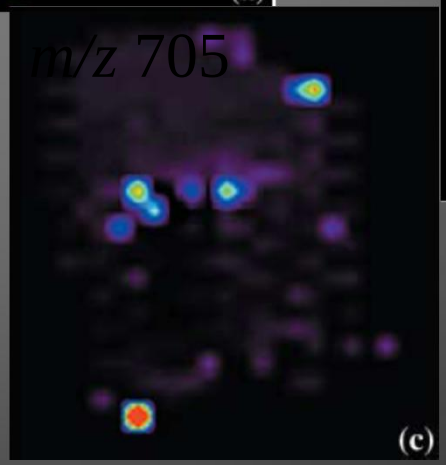
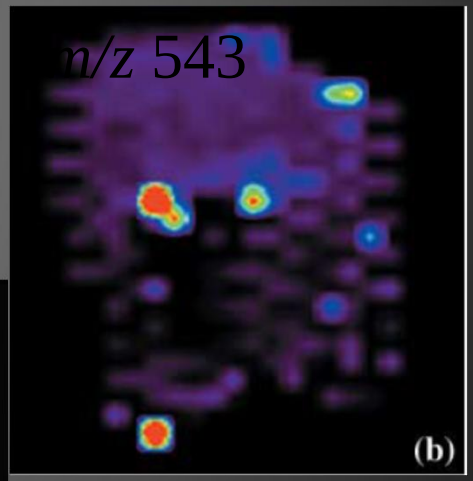


Fig. 2 Example photographs of (a) a longitudinal section and (b) a cross section through a piece of wheat (*Triticum aestivum*) stem before positioning in the matrix-assisted laser desorption ionization (MALDI) source of the mass spectrometer.

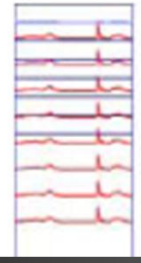


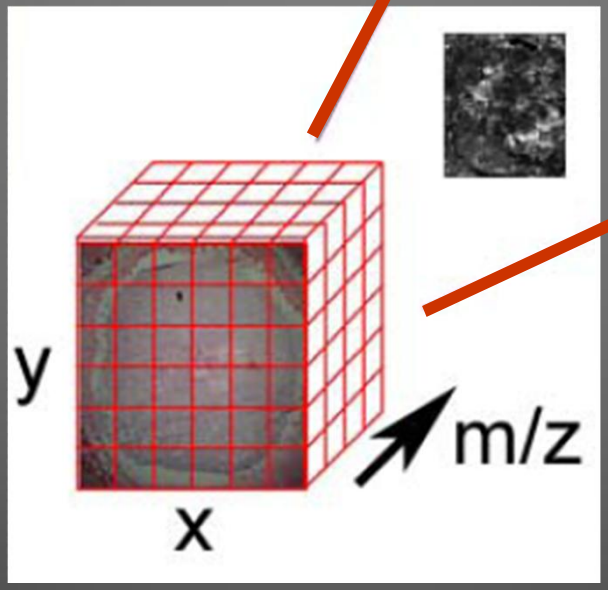


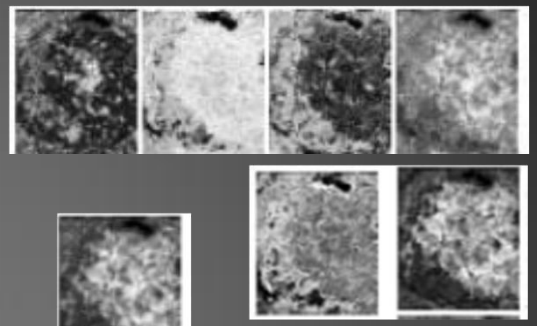


3D IMS-MS

a spectrum per pixel







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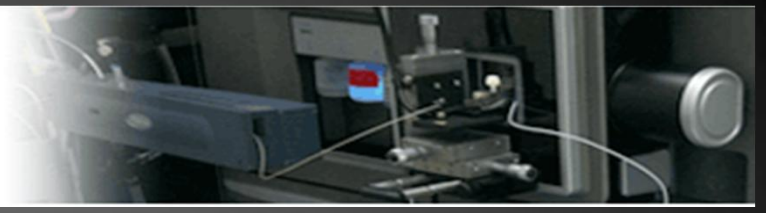
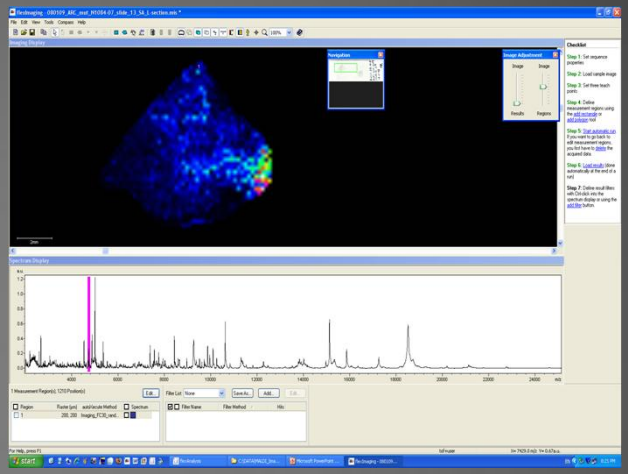
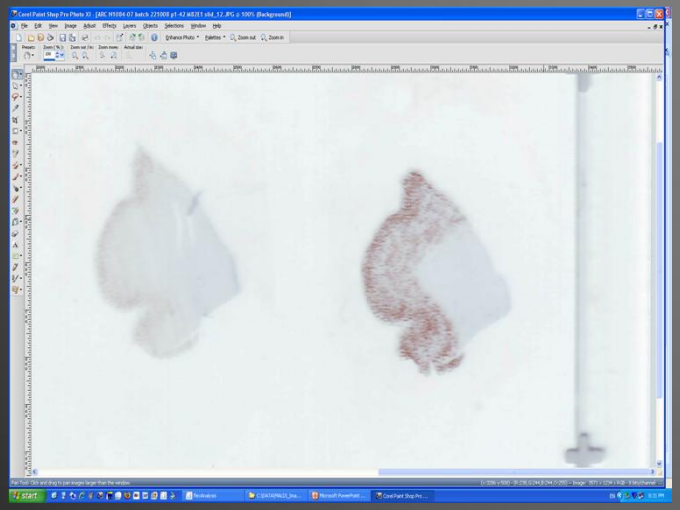
FACULTY OF MEDICINE

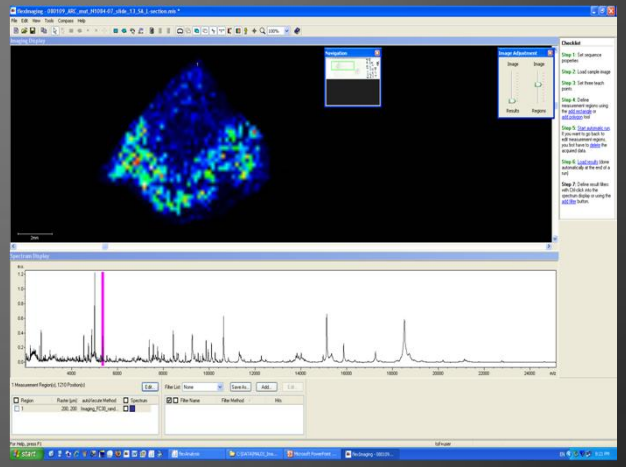


Spatial distribution of AD amyloid beta peptide In brain tissue

= not detectable by AB staining







ProtMet.net

BIOCENTRUM HELSINKI

BF A member of the Finnish Proteomics and Metabolomics technology platform

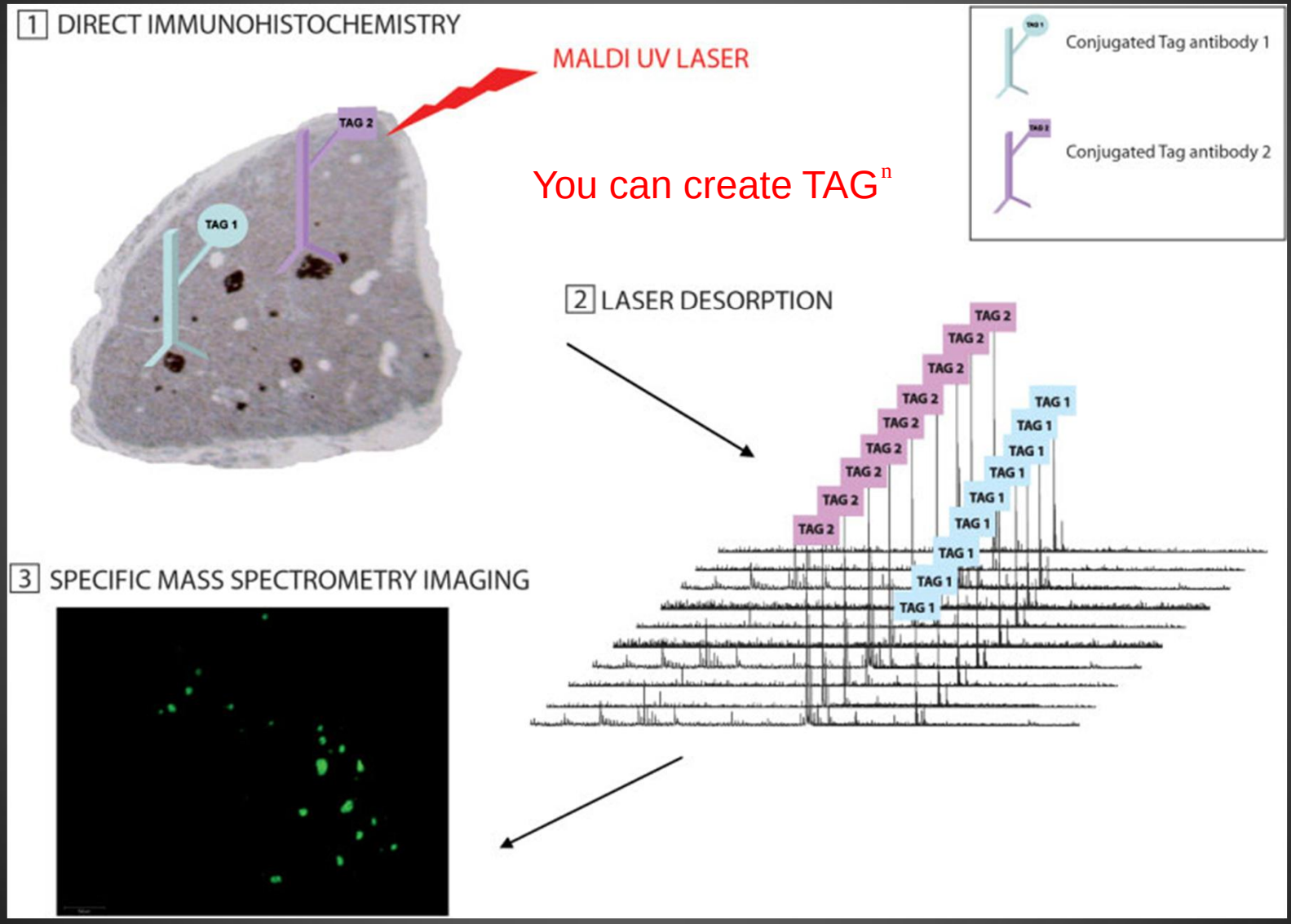
Diagnostic value?

Imaging Mass Spectrometry (IMS) of a Specific Fragment of Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Kinase Kinase 2 Discriminates Cancer from Uninvolved Prostate Tissue

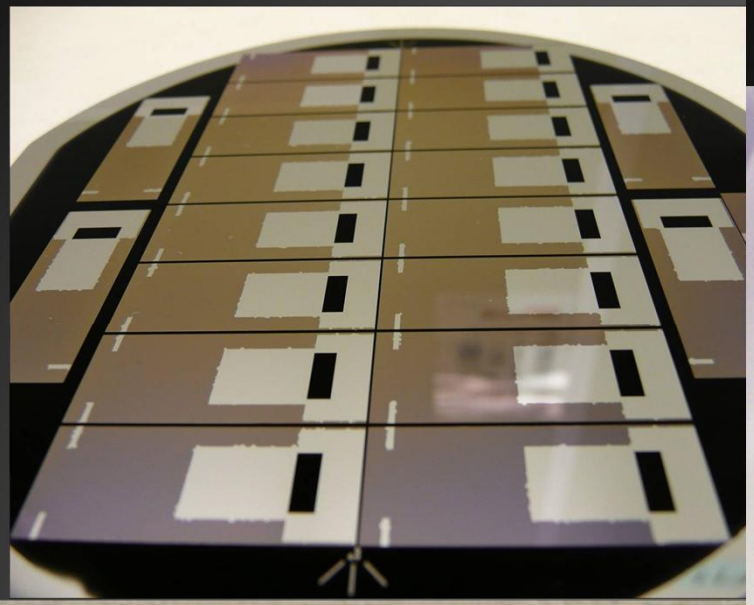
Lisa H. Cazares,1,2,3 Dean Troyer,1,3 Savvas Mendrinos,3 Raymond A. Lance,3,5, Julius O. Nyalwidhe,1,2,3 Hind A. Beydoun,4 Mary Ann Clements,1,2,3, Richard R. Drake,1,2,3 and O. John Semmes1,2,3

Clin Cancer Res 2009;15(17) September 1, 2009

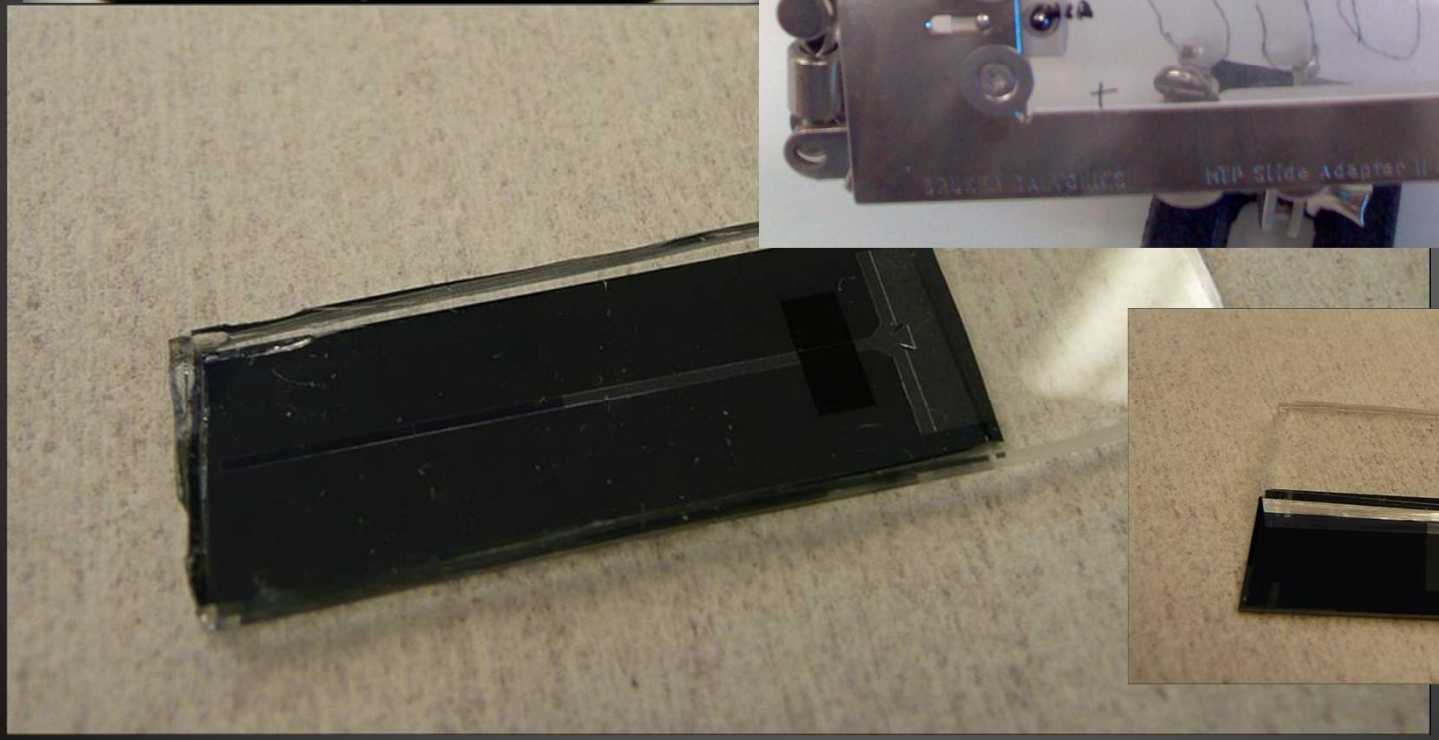
IMS and protein arrays

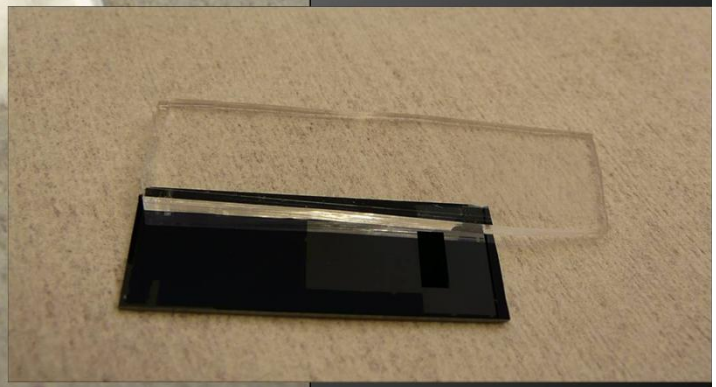


Thiery *et al*. *Proteomics* **2008**, *8*, 3725–3734




HTP Slide Adapter II





Proteomics goes Clinical, or does it?

Hopefully ☺

same Genome - different ***Proteome***


BIOMEDICUM HE
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