

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

The technology...

2D gel electrophoresis

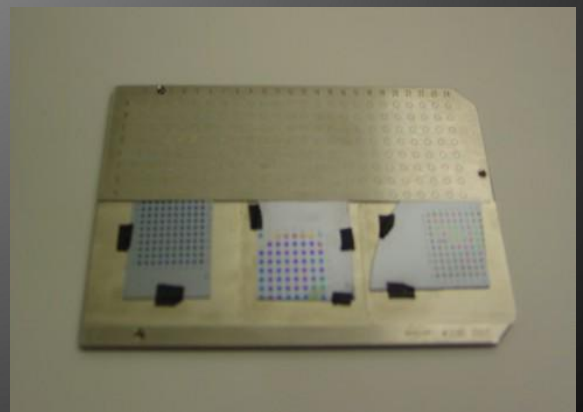
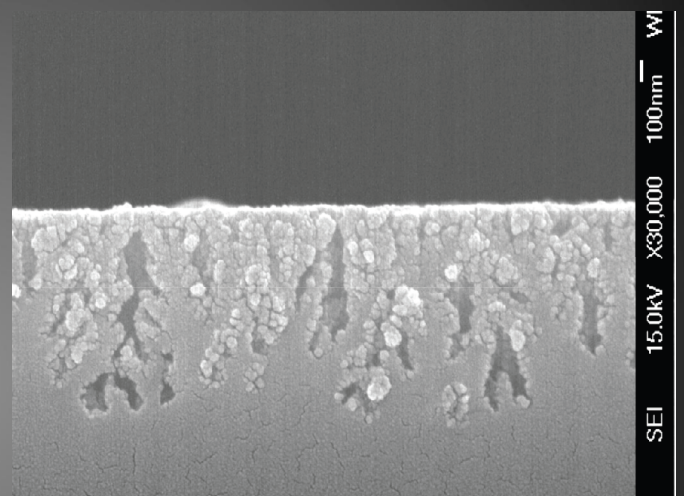
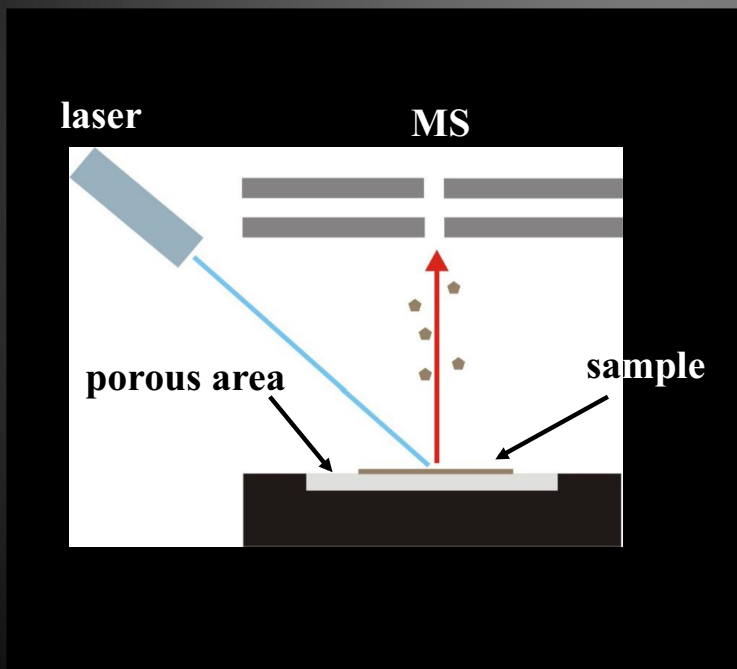
2D liquid chromatography

Micro arrays

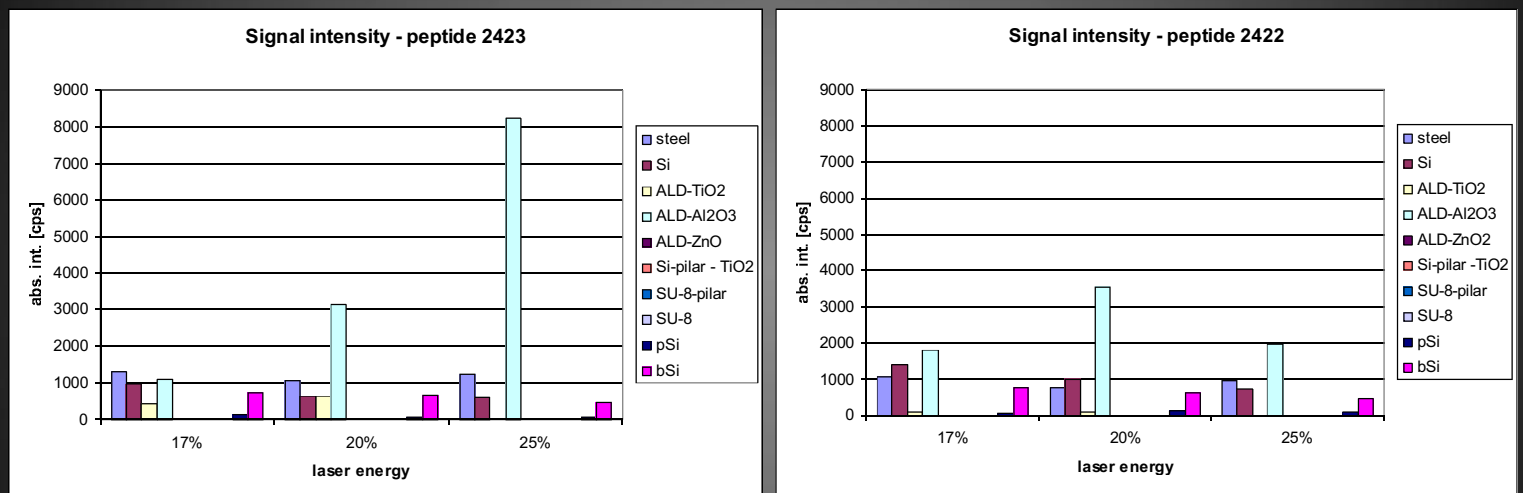
High-throughput protein/peptide Array technology

Chip-based proteomics: DIOS-MS

Desorption Ionization On Silicon (DIOS)



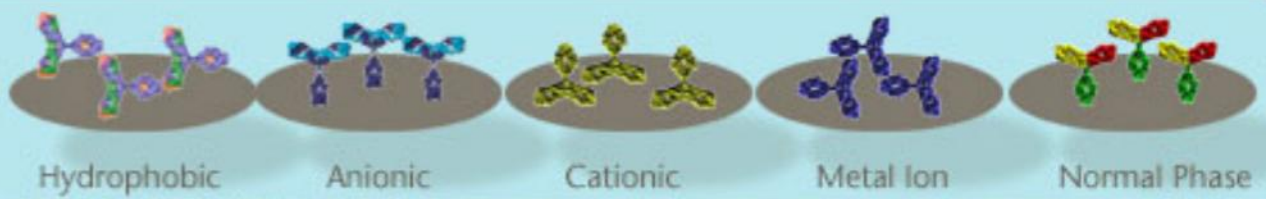
Testing the functionality of various surfaces for proteomics



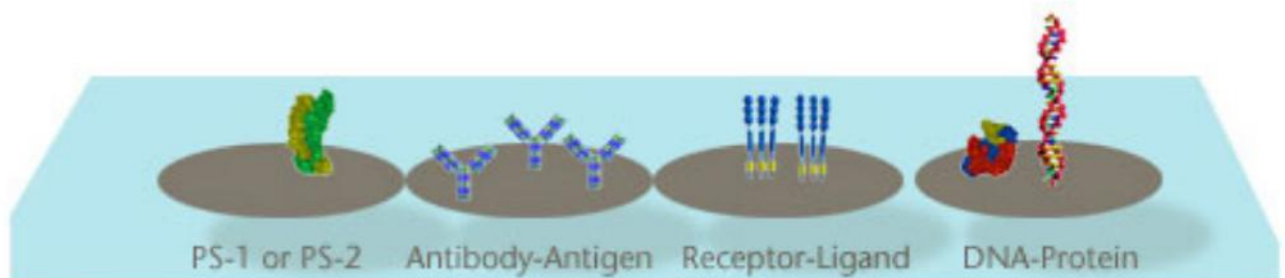
Signal intensity of unphosphorylated peptide 2423 (left) and phosphorylated peptide 2422 (right) from different surfaces.

Application: Phosphoproteomics, determination of phosphorylation states (kinasome)

Several affinities to choose from

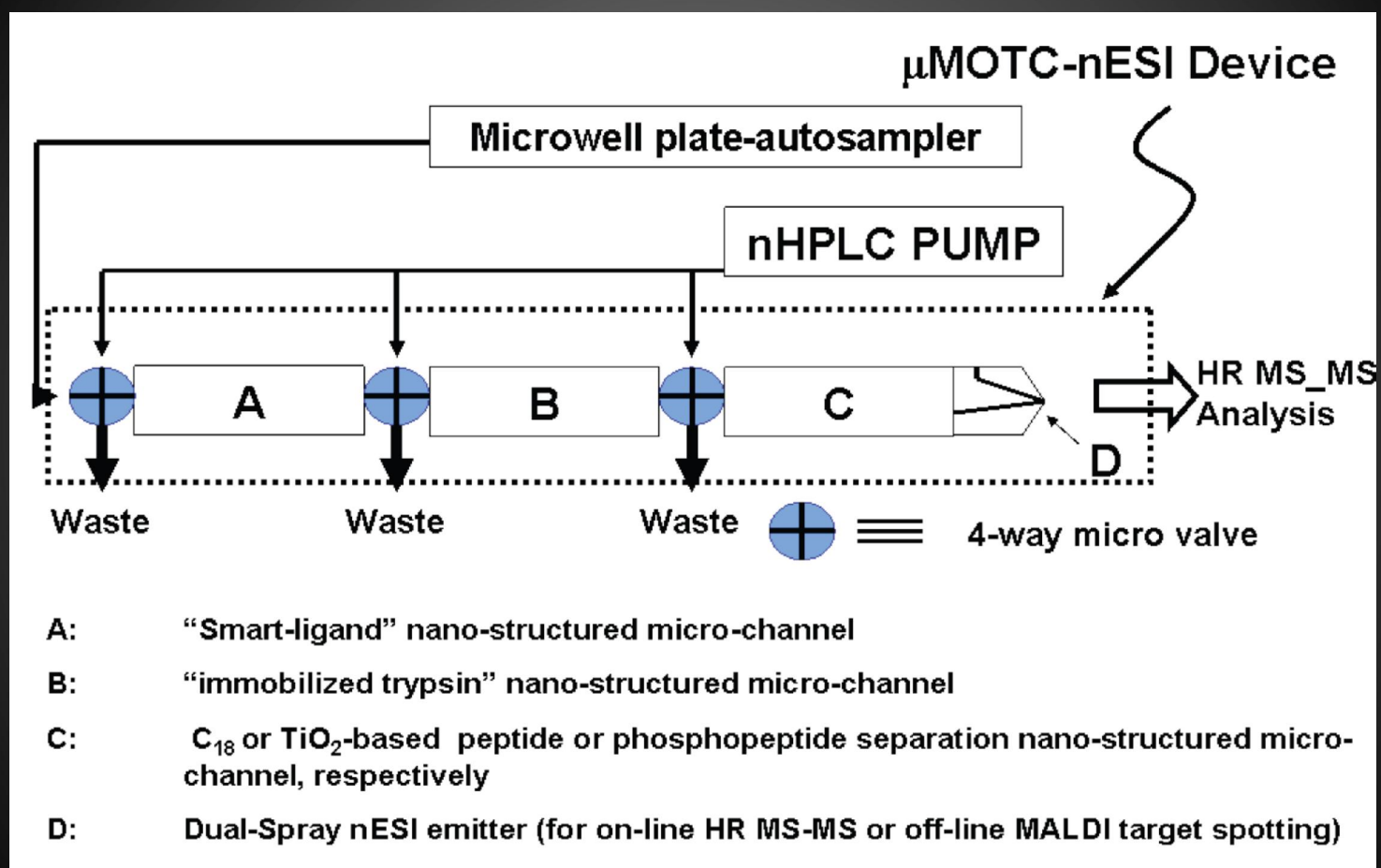


CHEMICAL SURFACES — protein expression profiling



BIOLOGICAL SURFACES — Protein interaction assays

The μ MOTC-nESI Lab-on-a-Chip



In collaboration with Prof. Evangelos Gogolides, Athens



MEILAHTI CLINICAL PROTEOMICS CORE FACILITY
FACULTY OF MEDICINE

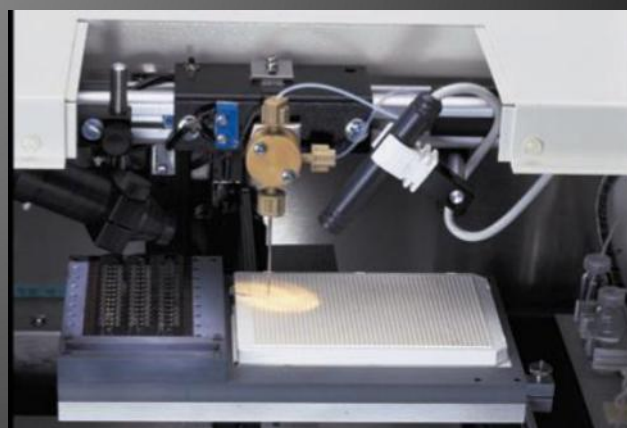


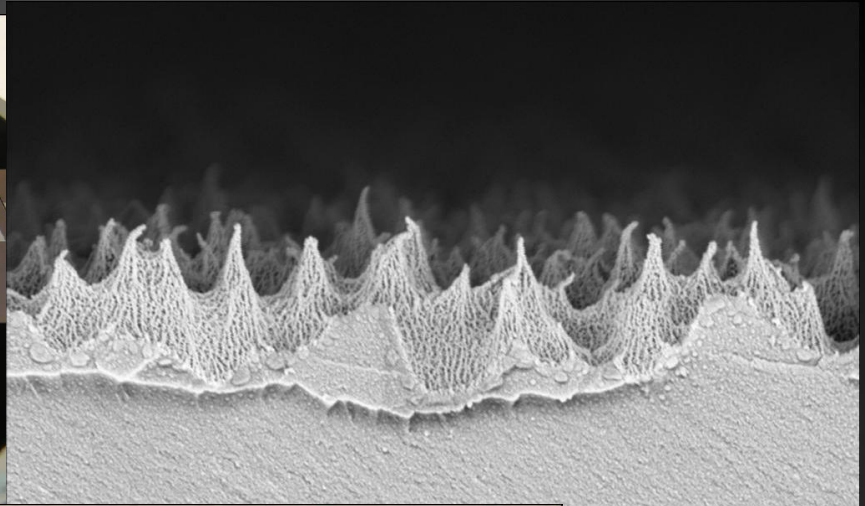
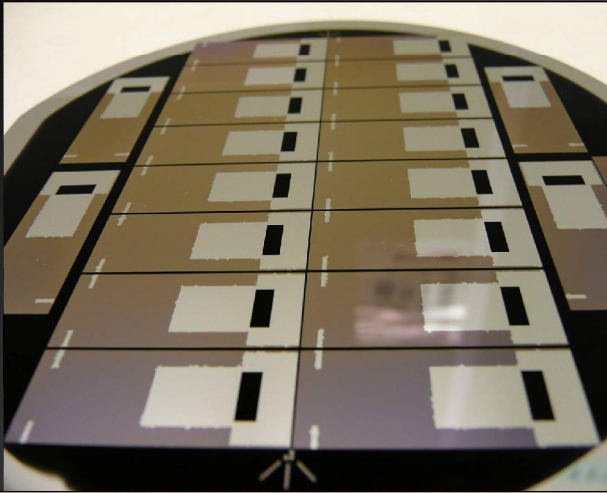
Array-based epitope mapping

1. Peptide synthesis on membrane

2. Peptide/Protein spotting on glass slide

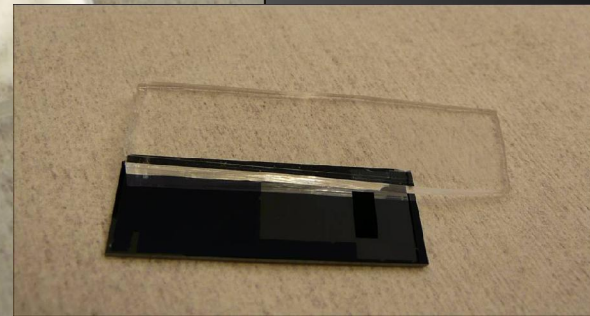
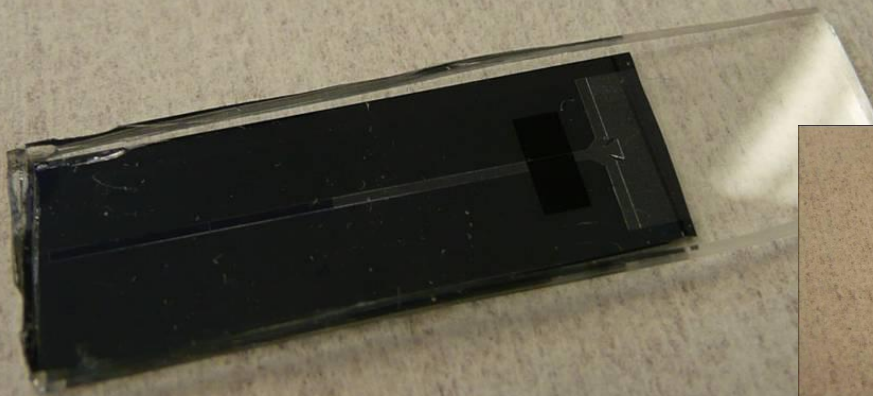
3. Analysis by AB, dyes or MS





Affinity chip with microfluidistics

9.58 K X
Apr 2008 Time :12:09:28



Microfluidics in a Chip for Proteomics

Biochip-IMB, Ltd.



A typical Chip and how to Make it...

Your Lab is Smarter when your slab gel is digital



The new **Agilent 2100 e-bioanalyzer** offers the same performance and tools for sizing and quantitation of RNA, DNA and proteins that more than 3,000 users have come to appreciate from the **Agilent 2100 bioanalyzer**.

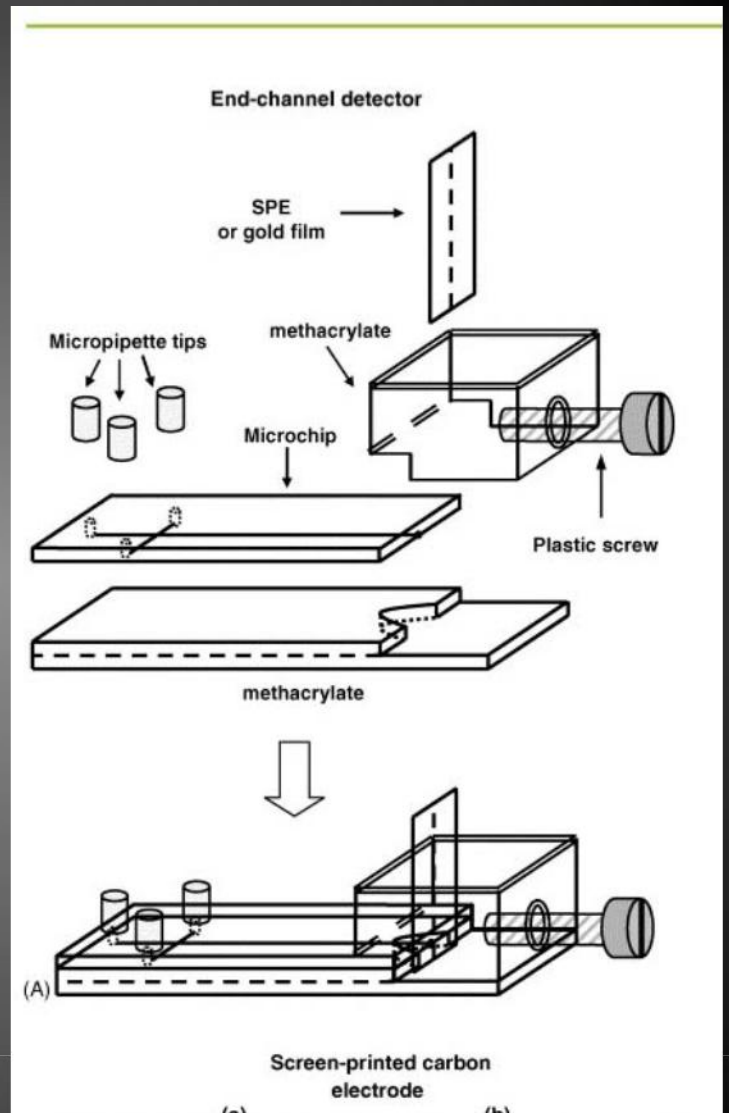


In addition to RNA, DNA and protein analysis, the Agilent 2100 bioanalyzer offers compatibility with flow cytometry assays and tools for 21CFR part 11 compliance.

New Price for the world's best selling Bioanalyzer for electrophoresis!



Experion Automated Electrophoresis Station



Screen-printed carbon electrode

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

The technology...

2D gel electrophoresis

2D liquid chromatography

Micro arrays

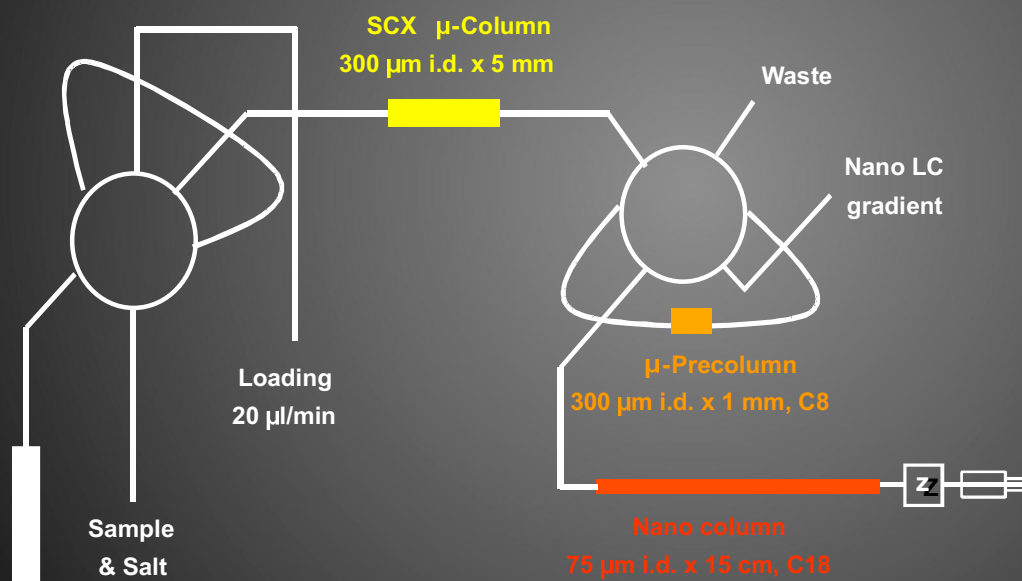
2D-LC/MS/MS

(2-dimensional liquid chromatography mass spectrometry)

Faster Protein Identification by MS/MS

Also called MudPIT or
Shotgun proteomics

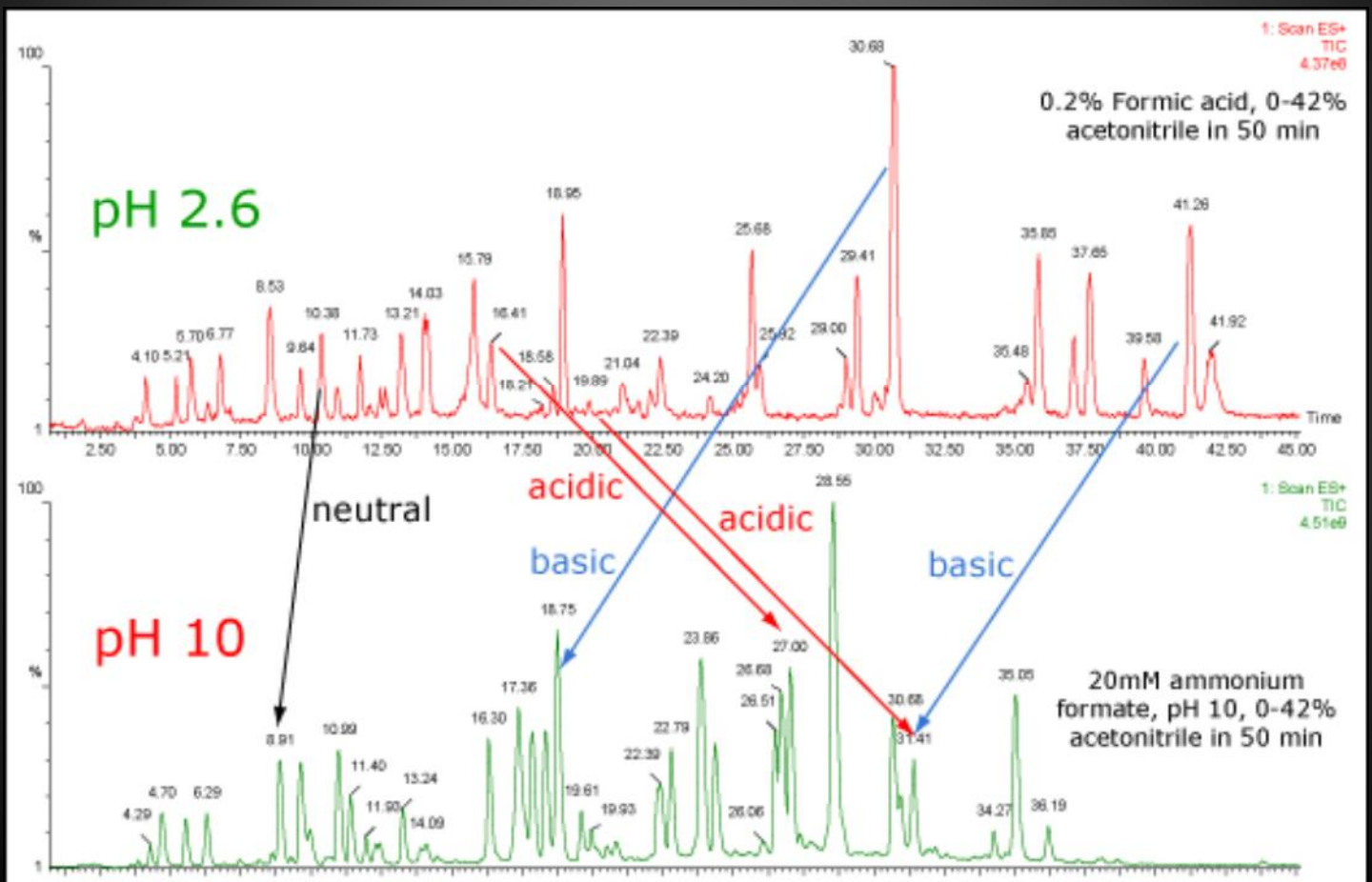
2D-LC/MS/MS Instrumental Set-up SCX/RP/RP



Waters 2D-nano UPLC aquity LC

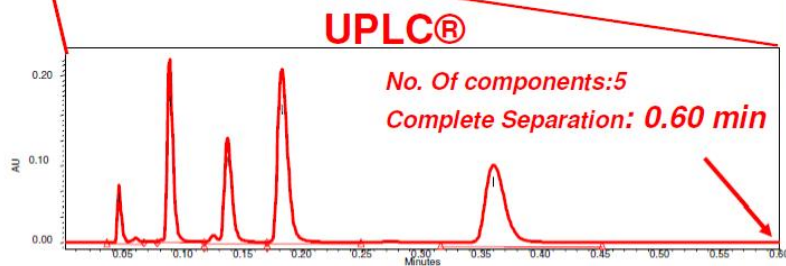
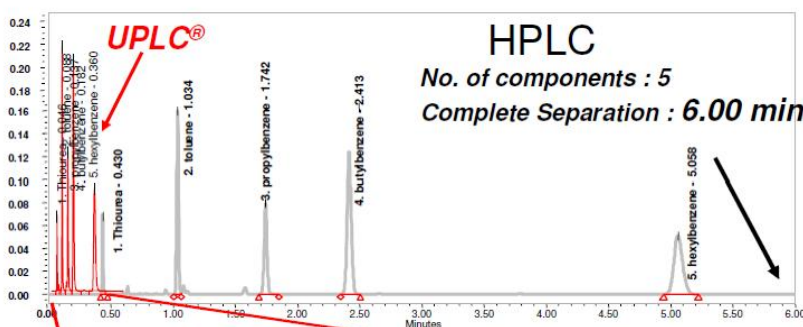


We use the property of proteins to behave differently in different pH



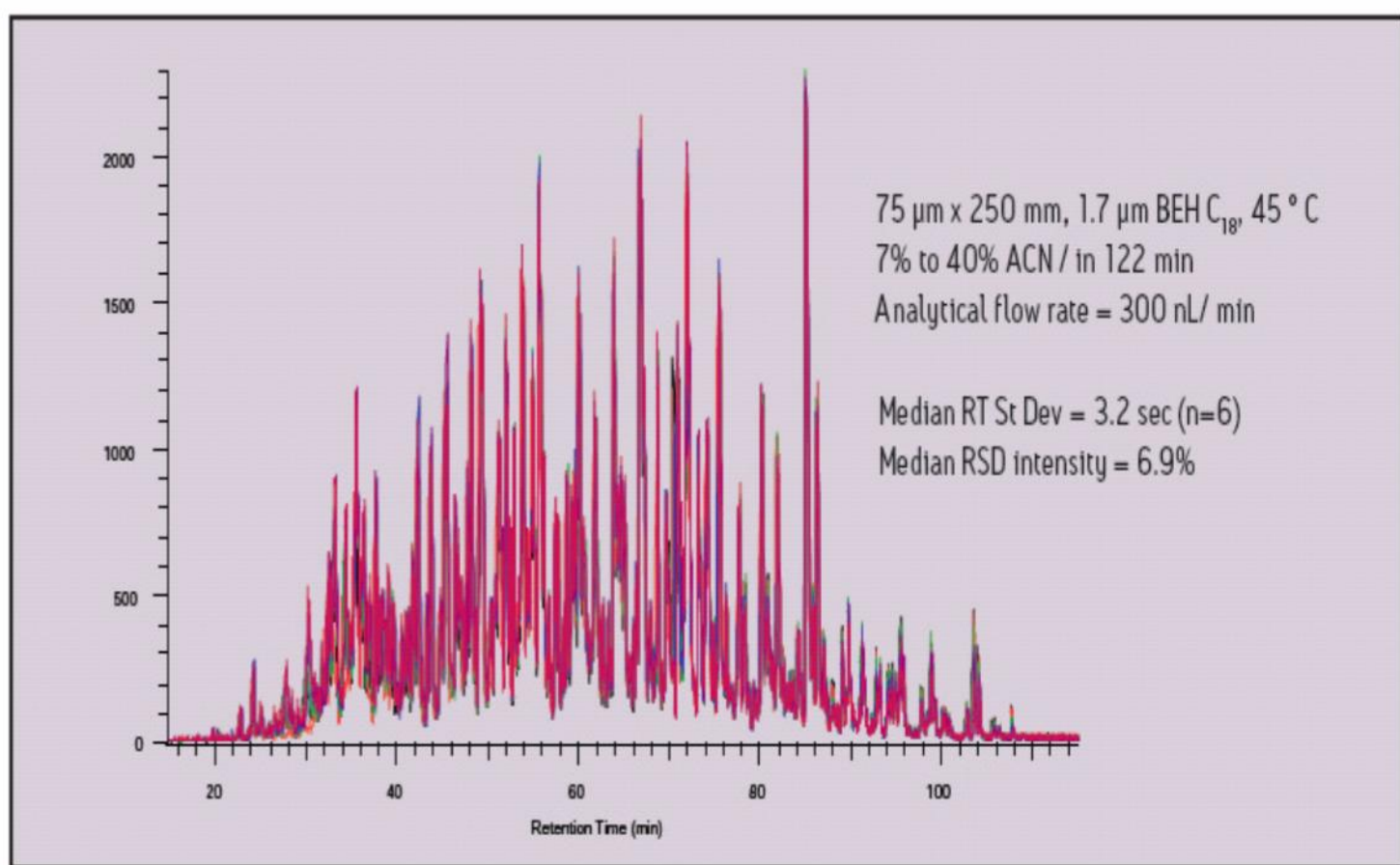
The time for one analysis is fast

UPLC™ increases Speed by 9X (900%)

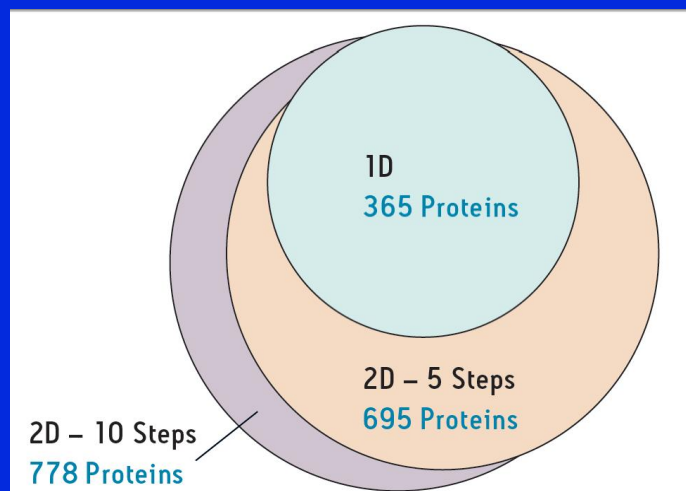
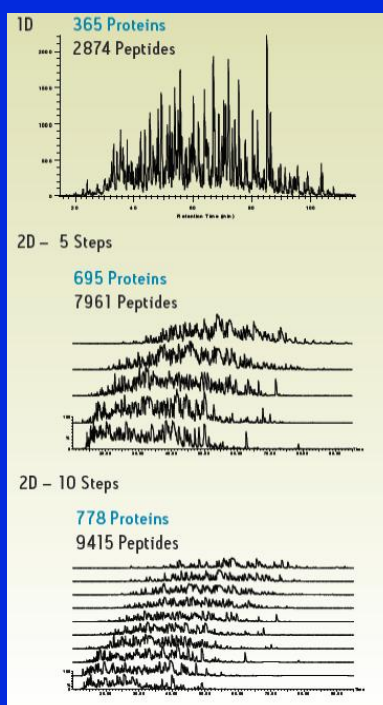


Increased sample throughput

You can analyze thousands of compounds in a few hours

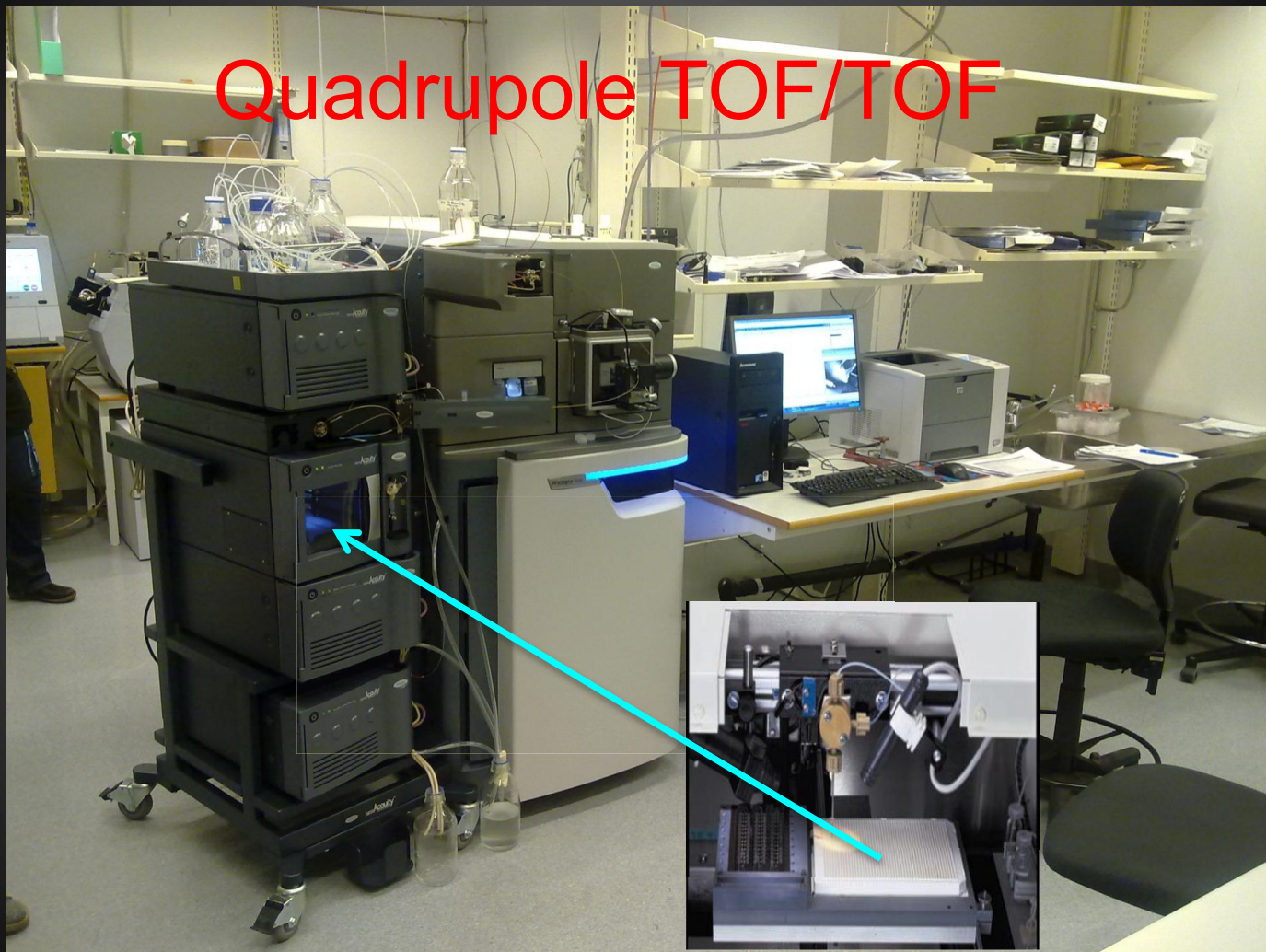


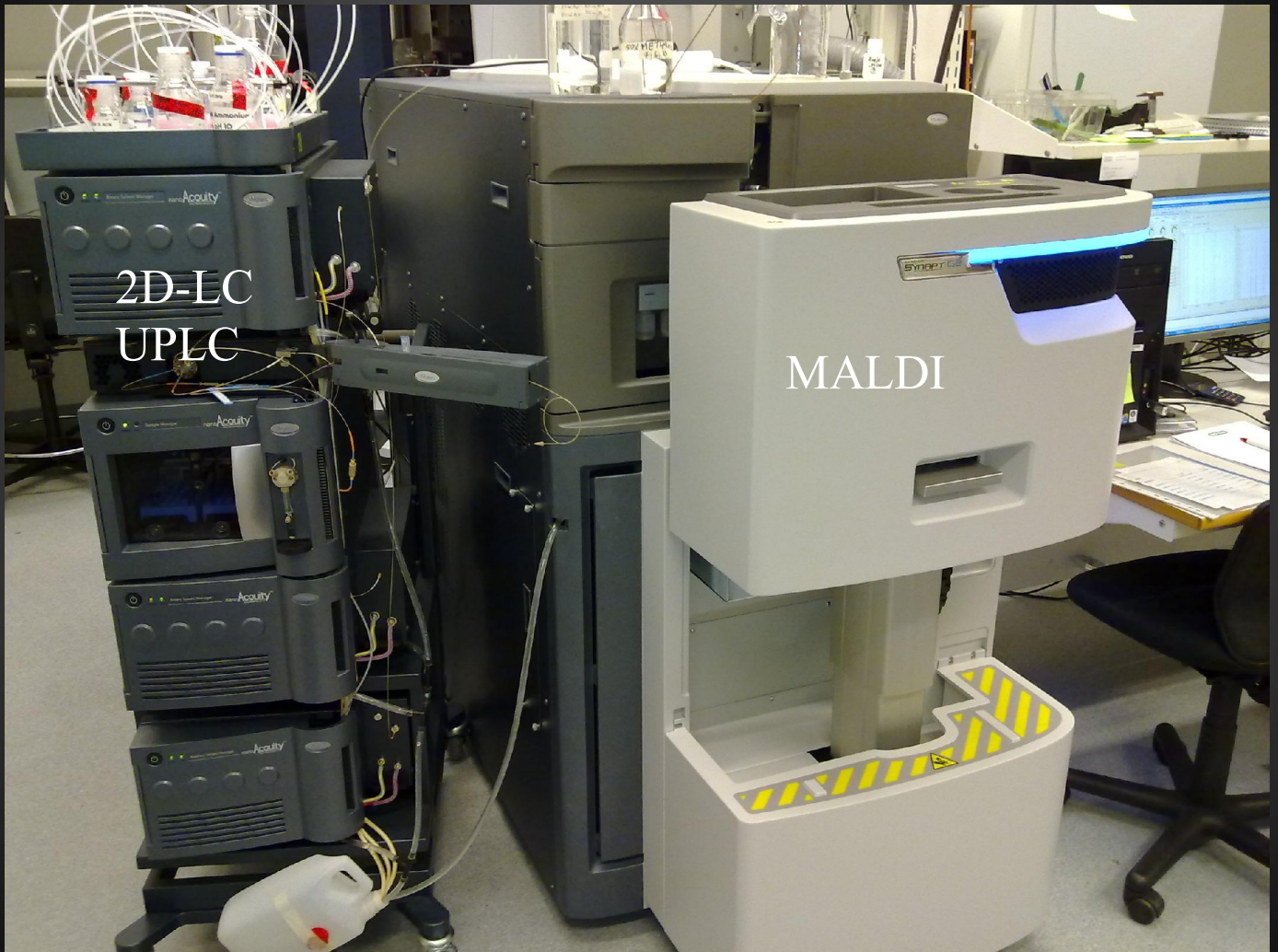
You can analyze thousands of compounds in a few hours



High end flexible mass spectrometers

Quadrupole TOF/TOF

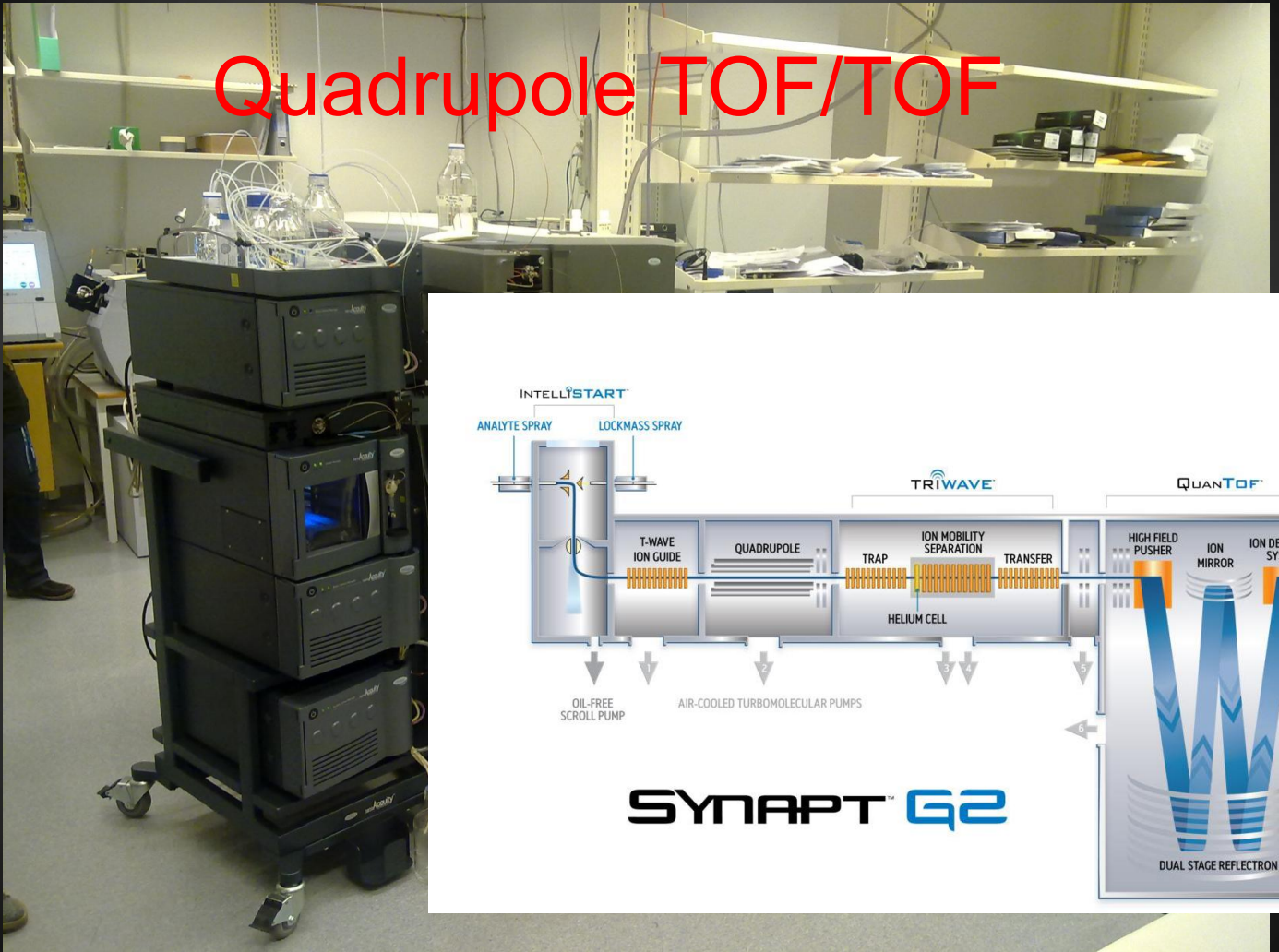




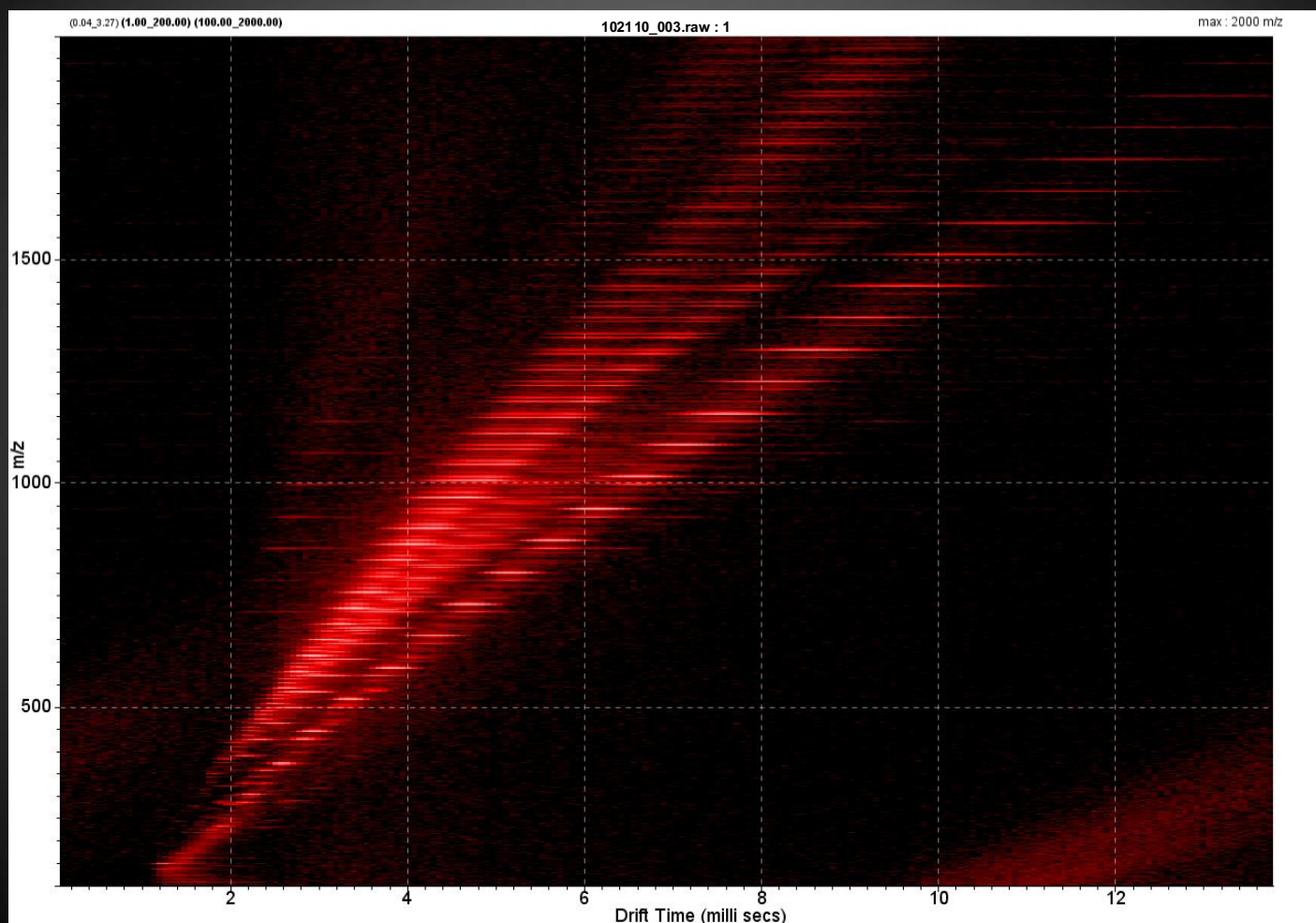
2D-LC
UPLC

MALDI

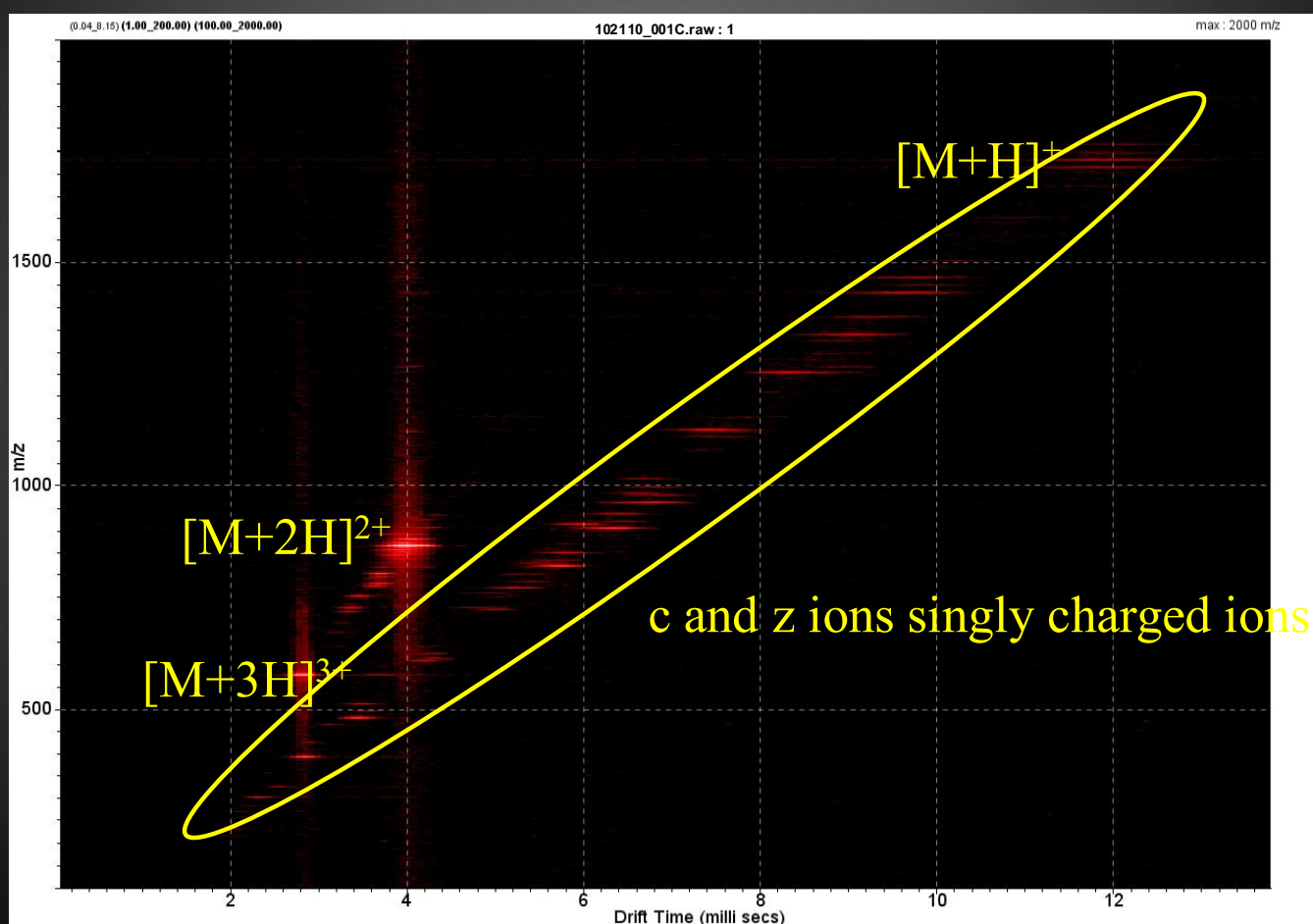
Quadrupole TOF/TOF



Ion Mobility driftscope view



Ion Mobility driftscope view



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

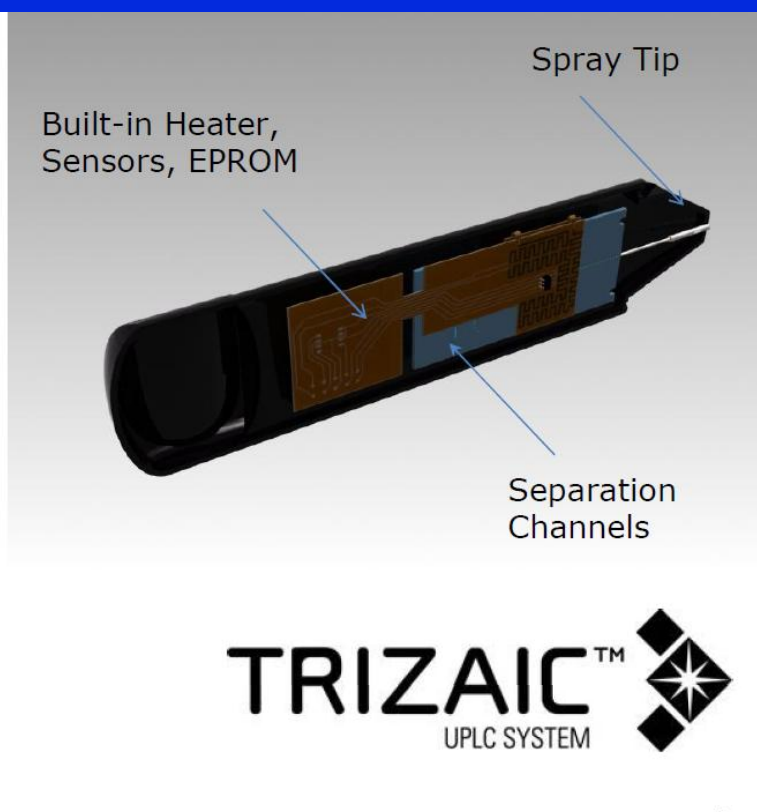
One sample needs approx. 24-38 hours of instrument time!

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

Proteomics chip technology for 2D-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



The whole 2D LC can be done in a chip

- Sub-
chroma-
per
- Elim-
con
- Low
- Inte-
elec



UPLC SYSTEM



© 2000 Waters Corporation

Other techniques to help in proteomics?

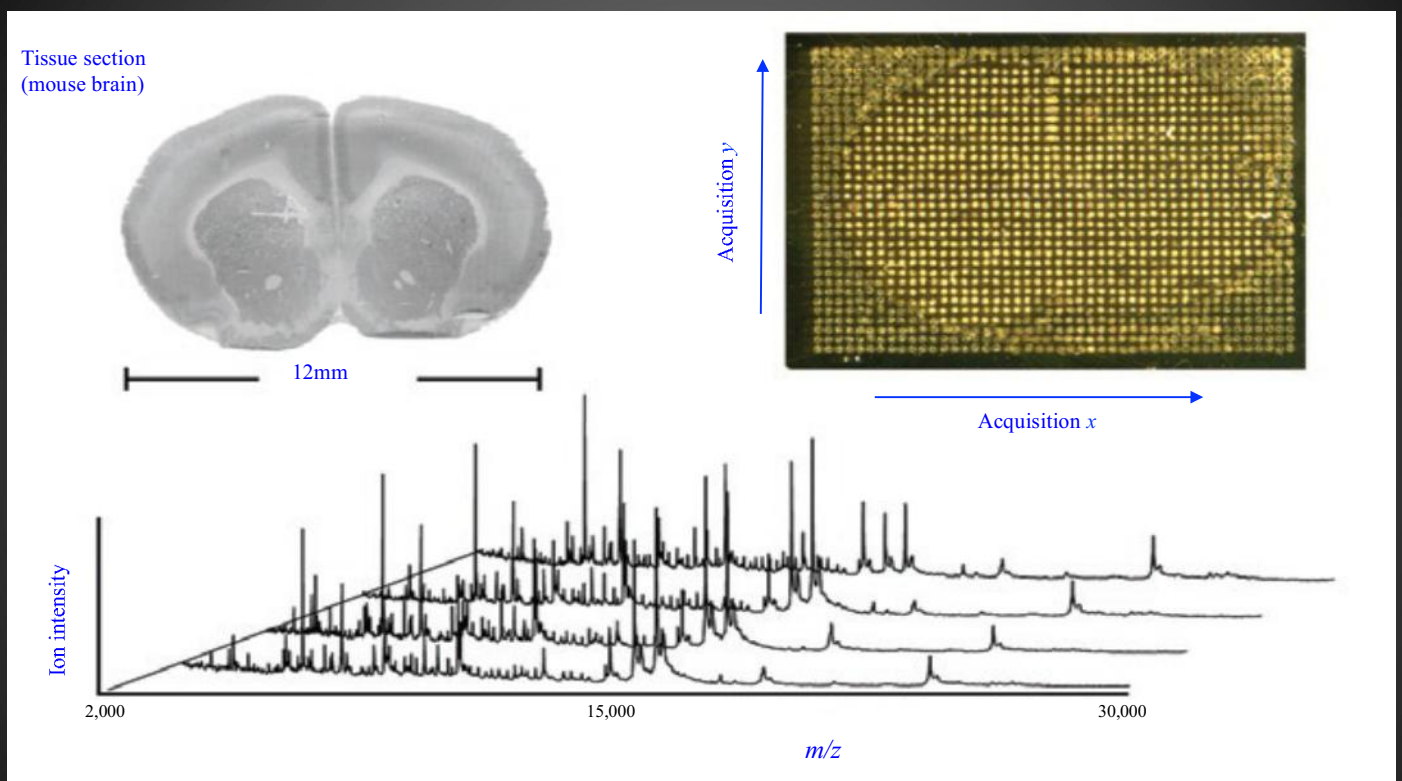
Imaging MS

Other techniques to help in proteomics?

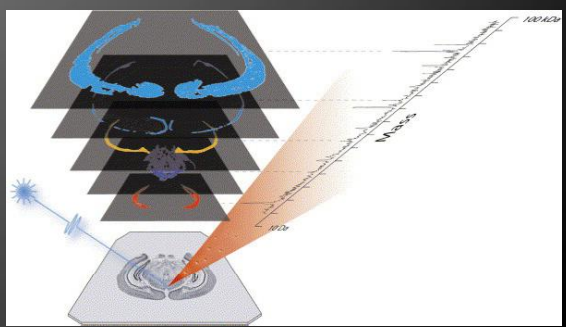
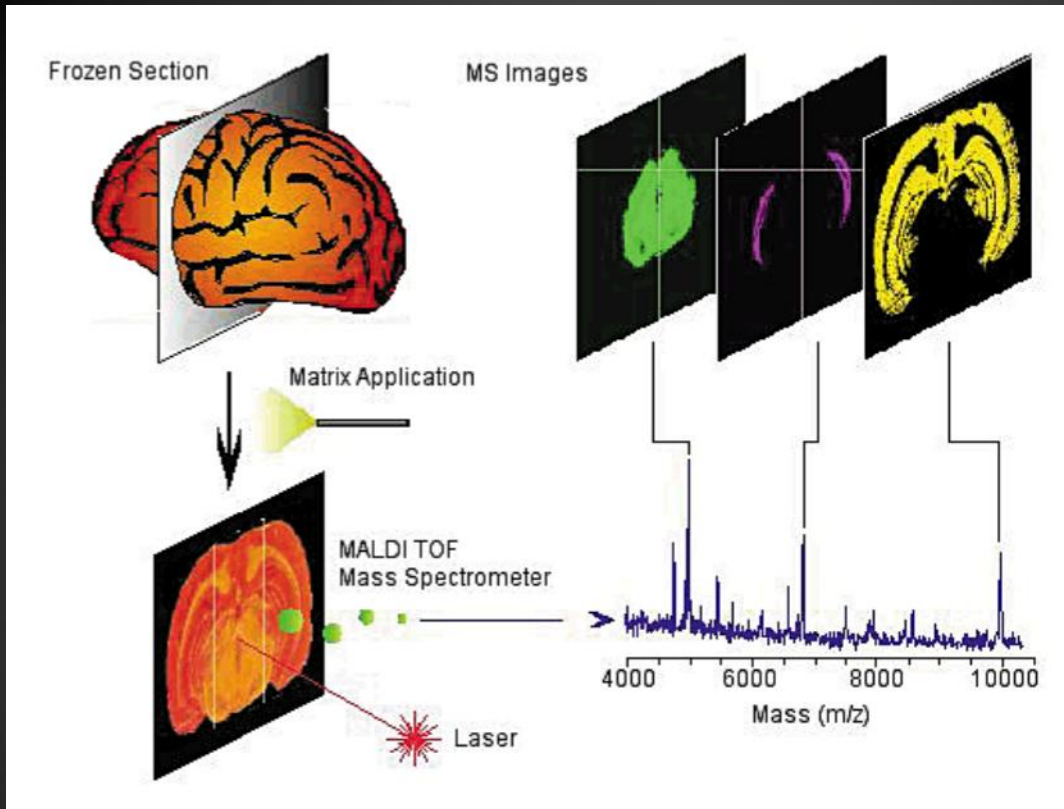
IMS

Imaging mass spectrometry

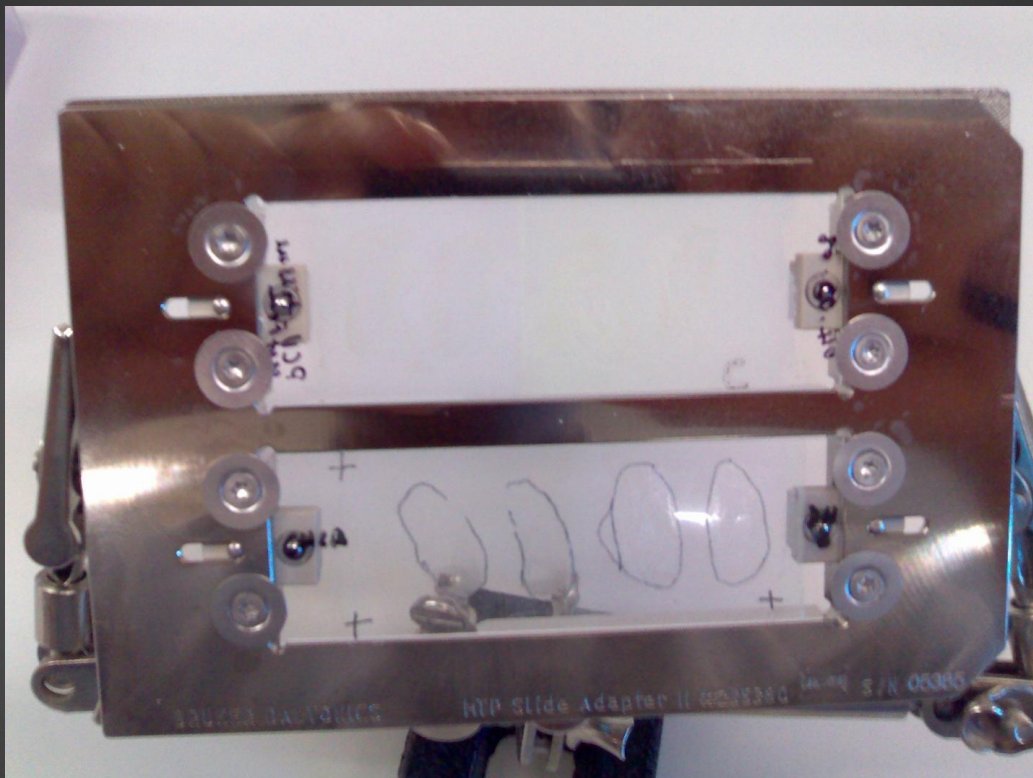
Principles



- 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

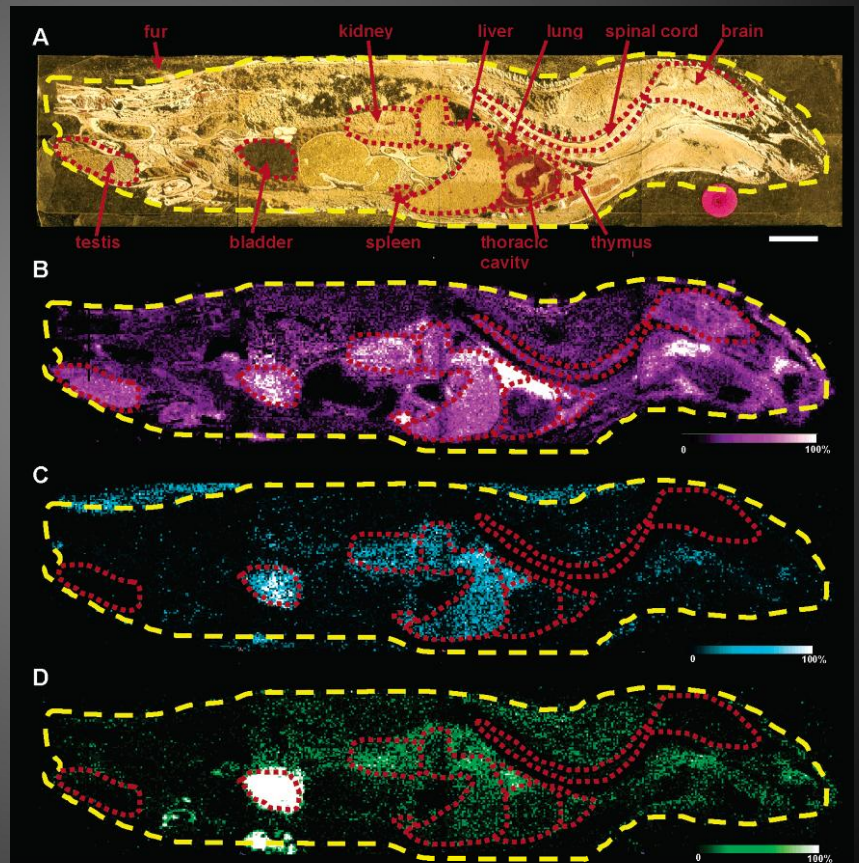


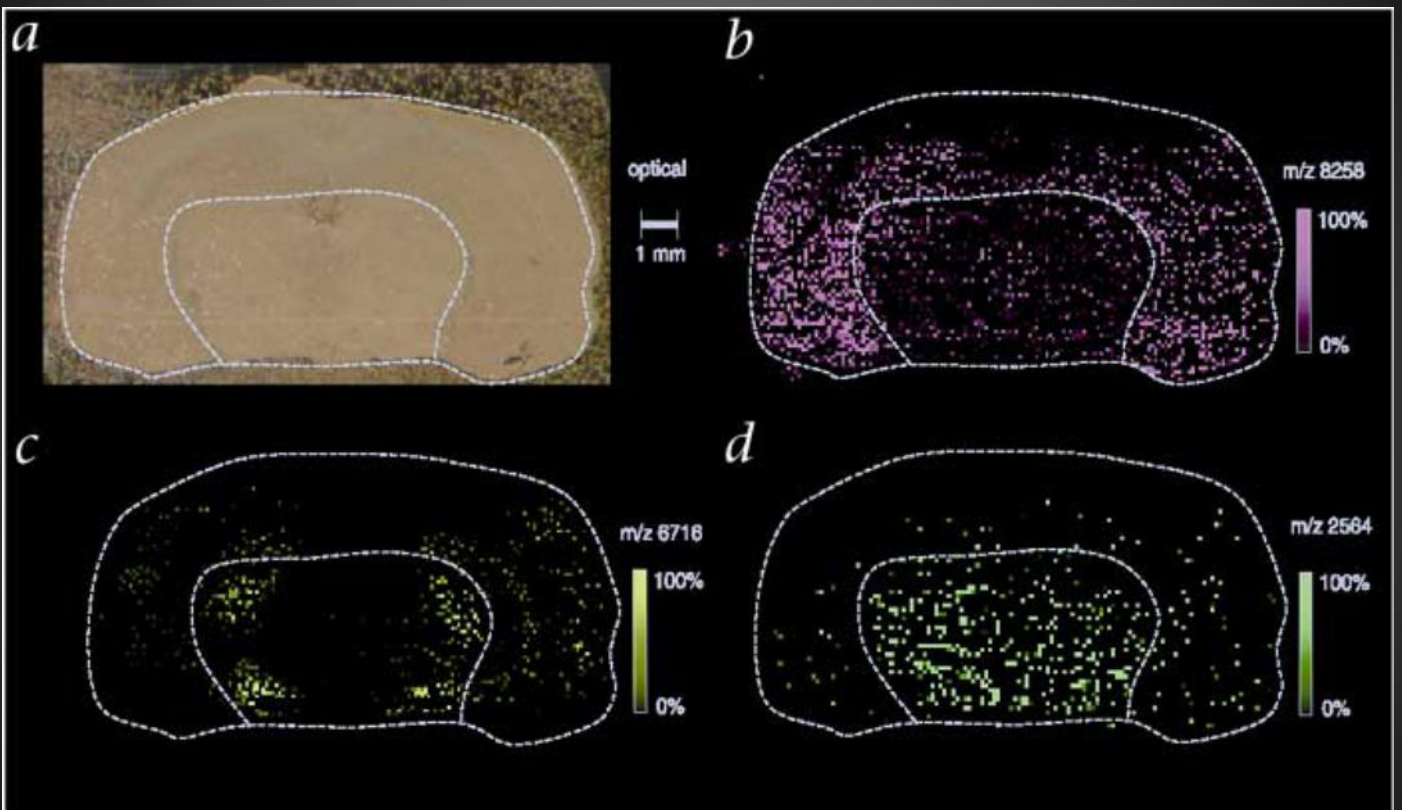
Tissue slide for IMS

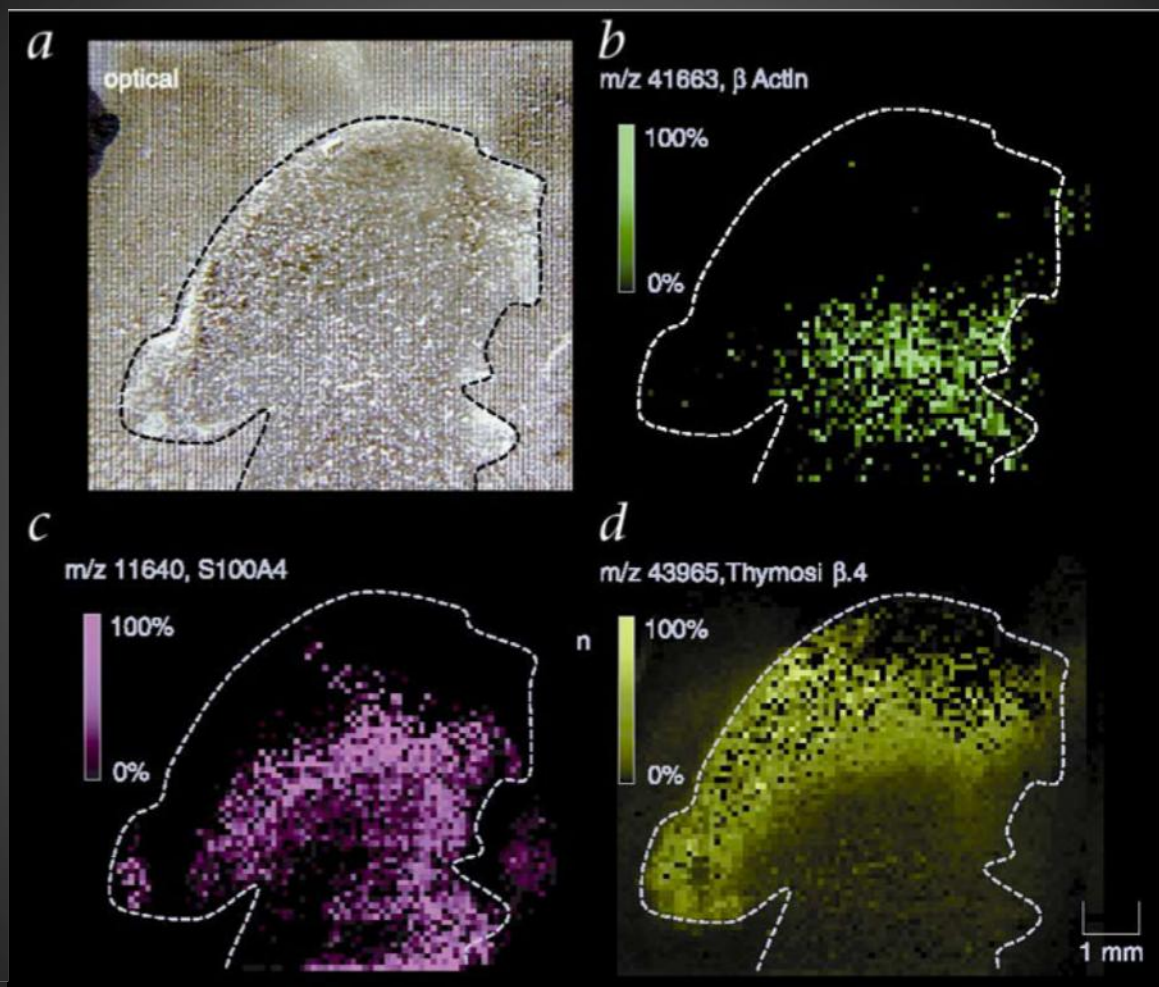


Benefits of MALDI-IMS

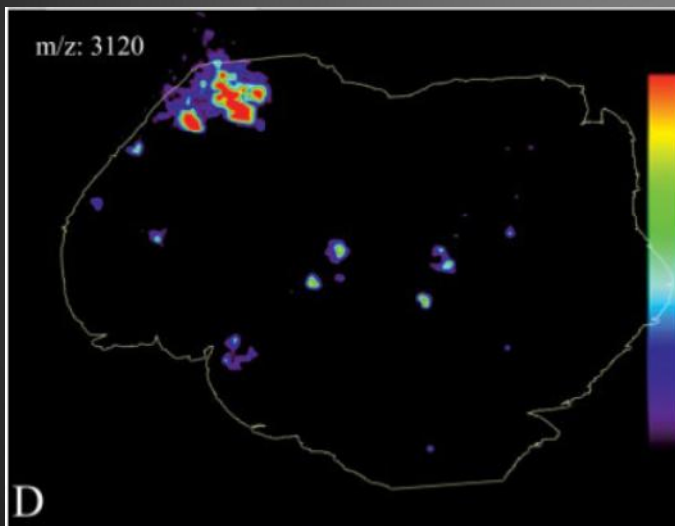
- Analysis of entire sample in one reading
- Previous knowledge of molecular composition is not necessary
- Allows for investigation of disease formation, progression, and treatment



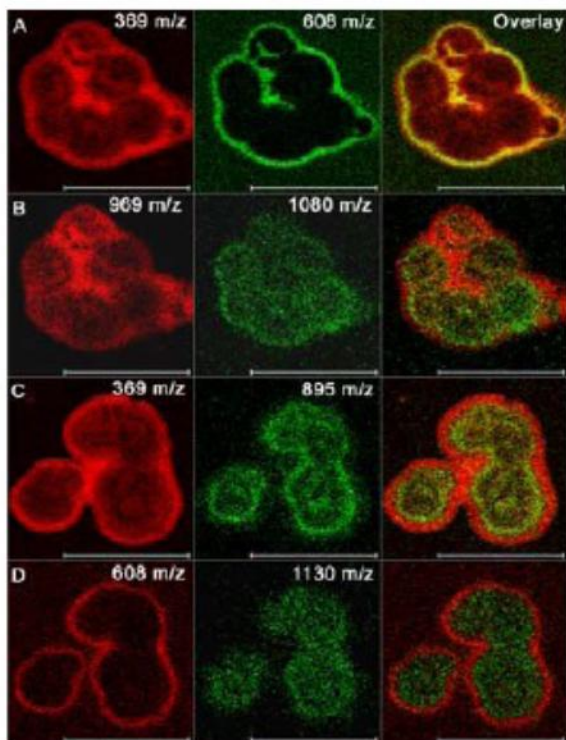




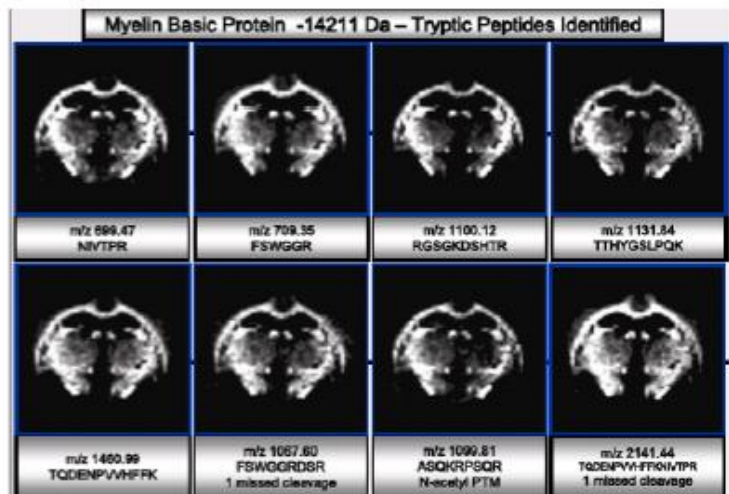
IMS vs. histochemical stain



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 Robinson¹, Karen Warburton³, Mark Seymour², Malcolm Clench³ and Jane Thomas-Oates¹

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

New Phytologist (2007) 173 : 438–444

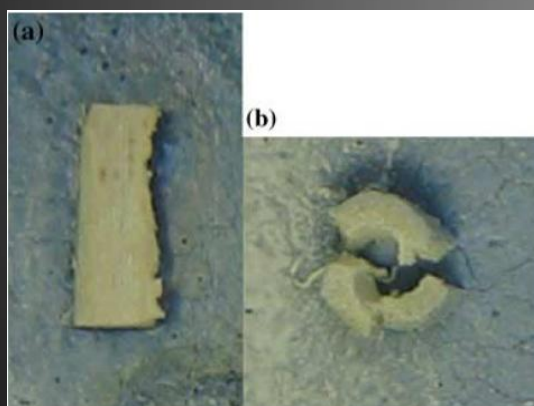
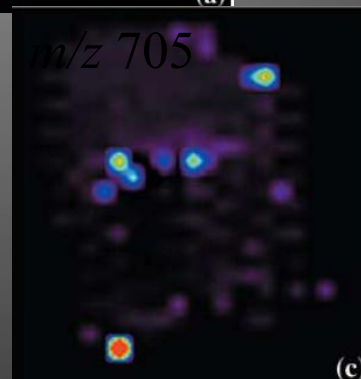
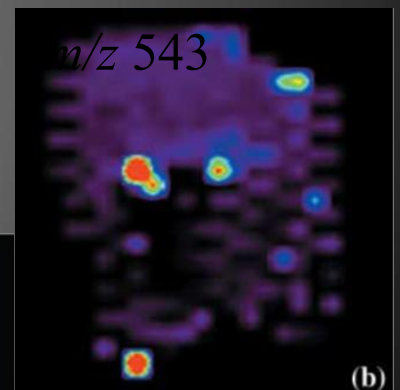
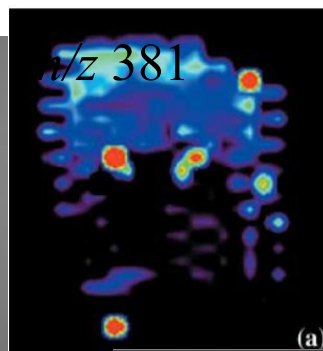
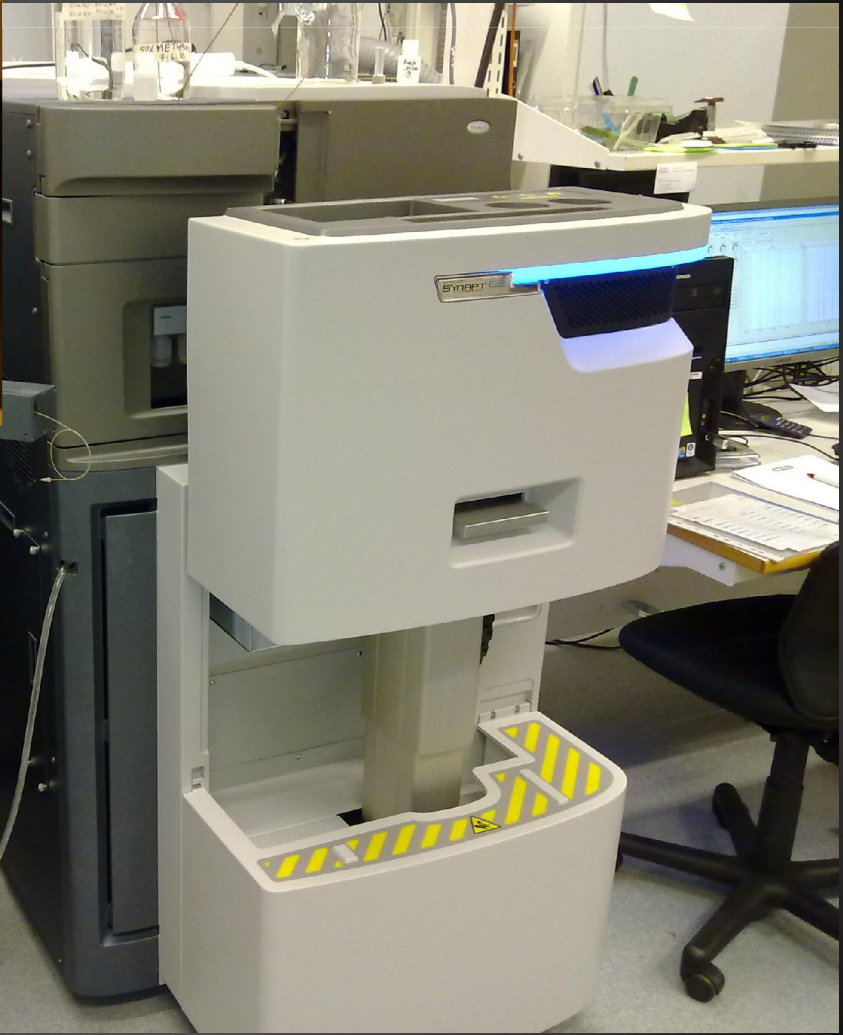
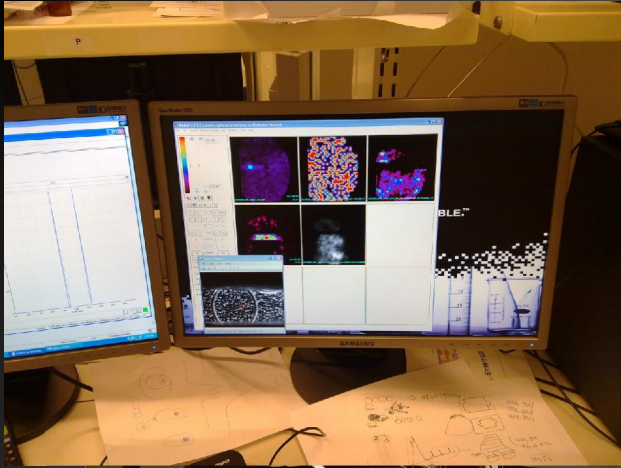
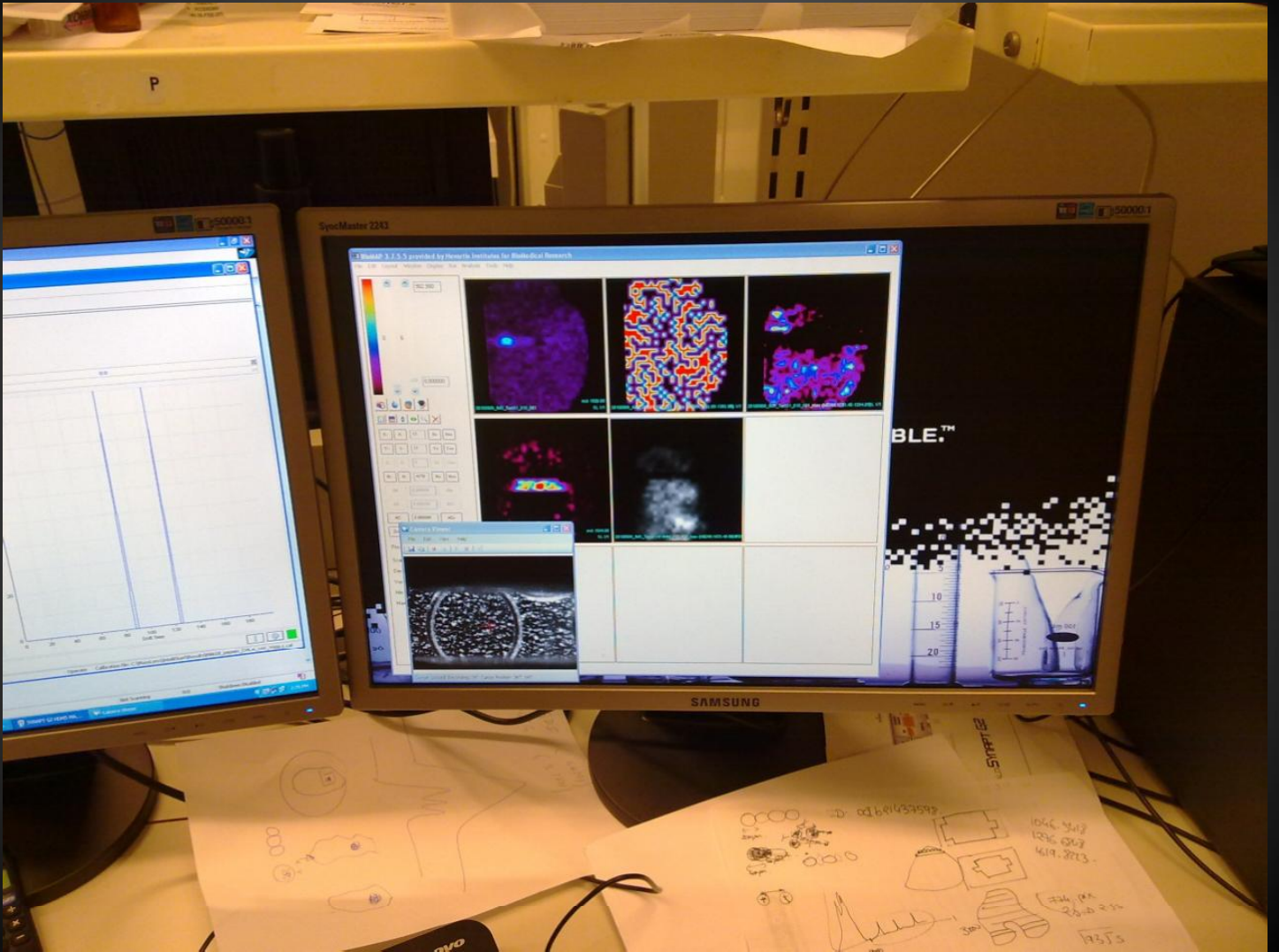


Fig. 2 Example photographs of (a) a longitudinal section and (b) a cross section through a piece of wheat (*Triticum aestivum*) stem before positioning in the matrix-assisted laser desorption ionization (MALDI) source of the mass spectrometer.

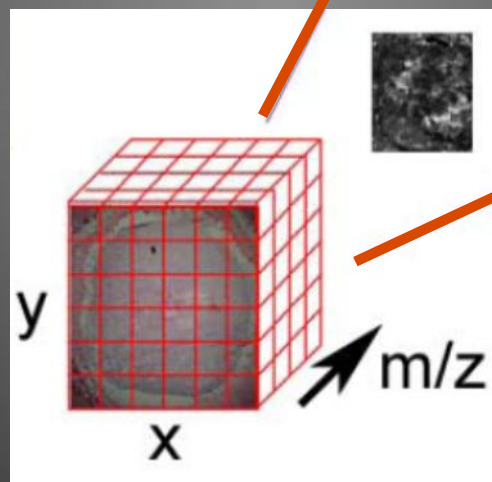
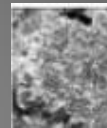
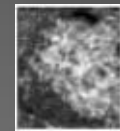
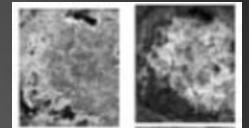
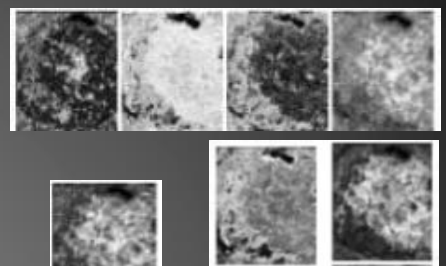
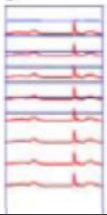






3D IMS-MS

a spectrum
per pixel



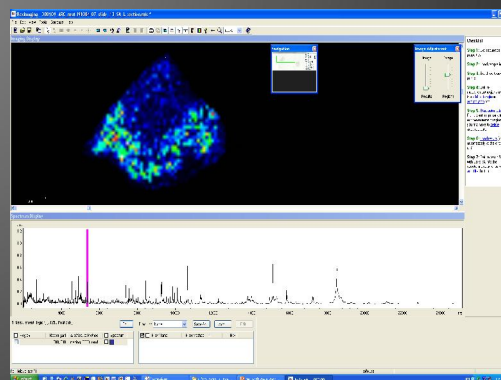
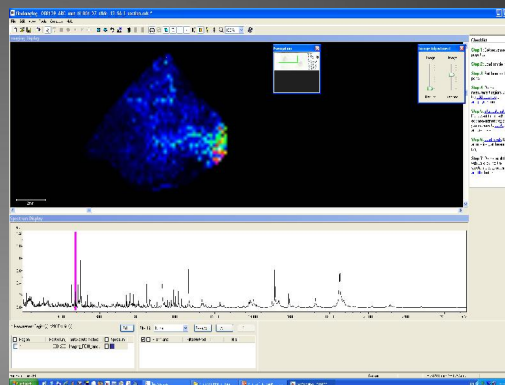
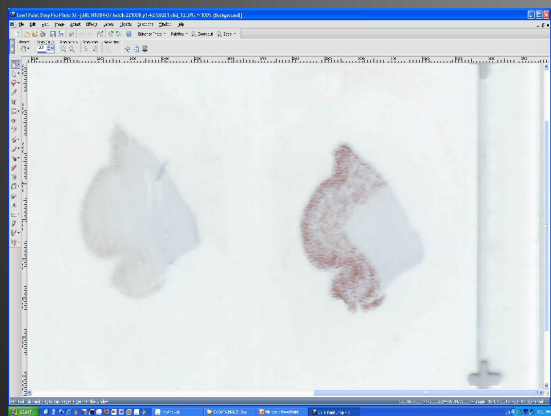


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Spatial distribution of AD amyloid beta peptide In brain tissue

= not detectable by AB staining



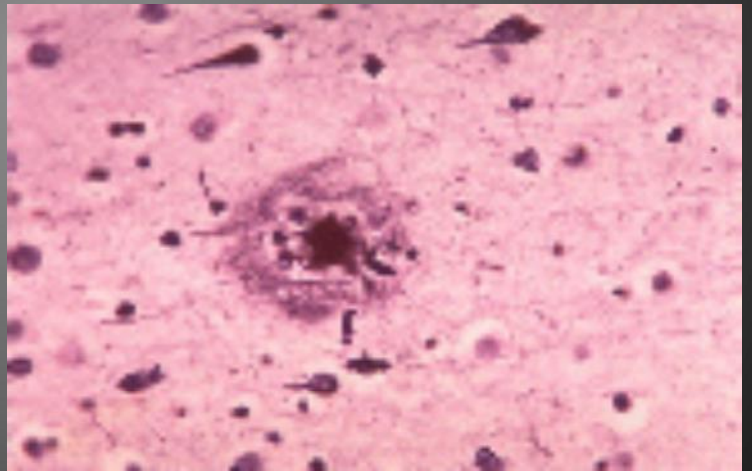
ProtMet.net

BIOCENTRUM HELSINKI

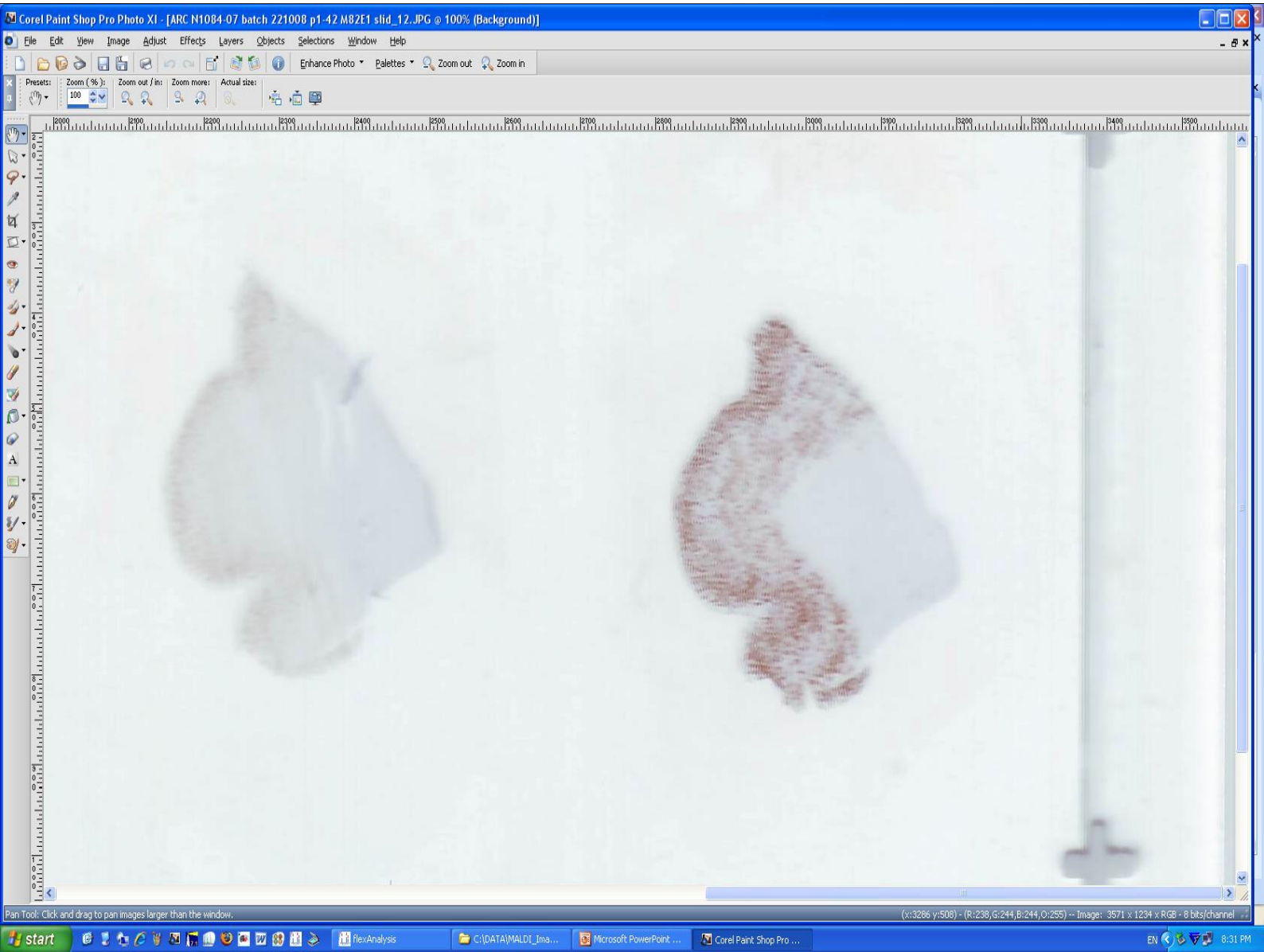


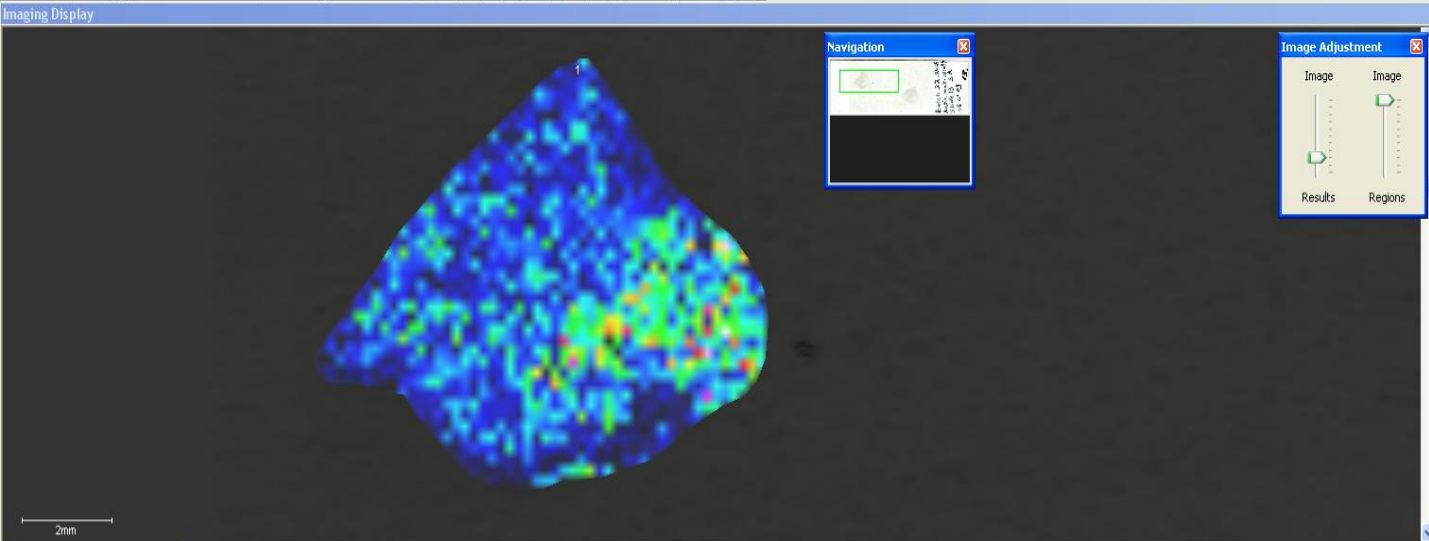
BC A member of the Finnish
Proteomics and Metabolomics
technology platform

Alois Alzheimer



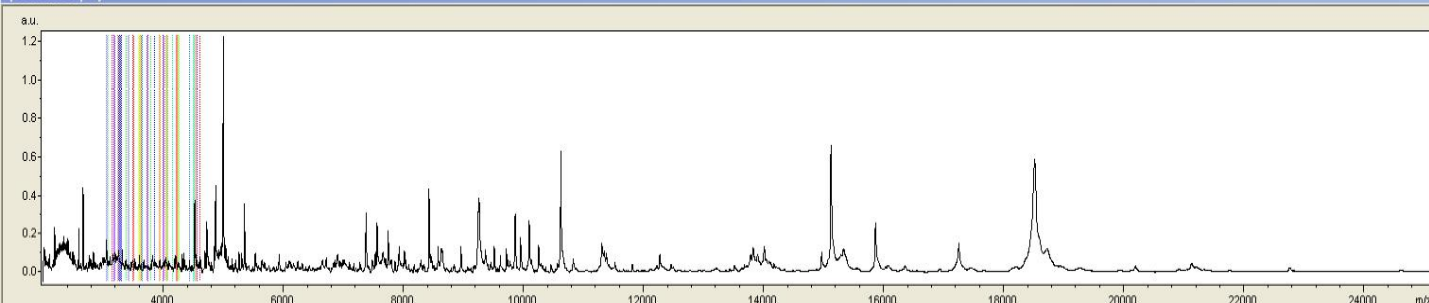
Alois Alzheimer's disease





- Checklist**
- Step 1:** Set sequence properties
 - Step 2:** Load sample image
 - Step 3:** Set three teach points
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 - Step 5:** [Start automatic run](#). If you want to go back to edit measurement regions, you first have to [delete](#) the acquired data.
 - Step 6:** [Load results](#) (done automatically at the end of a run)
 - Step 7:** Define result filters with [Ctrl-click](#) into the spectrum display or using the [add filter](#) button.

Spectrum Display

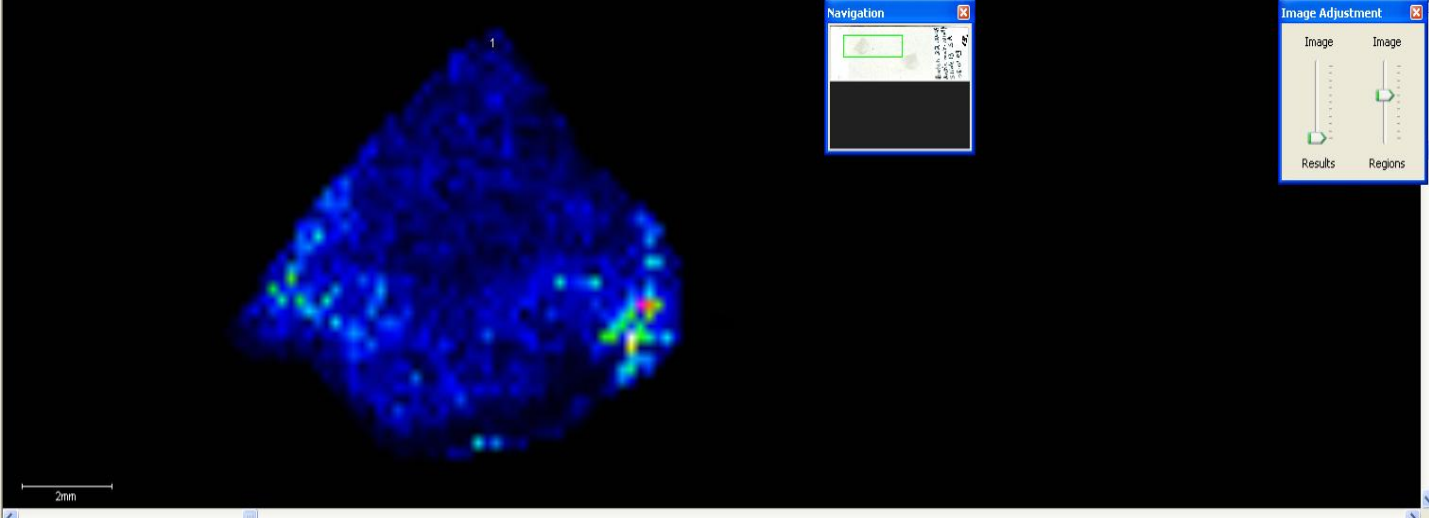


1 Measurement Region(s), 1210 Position(s)

Region	Raster (µm)	auto/teacute Method	Spectrum
<input checked="" type="checkbox"/> 1	200, 200	Imaging_FC30_rand...	<input checked="" type="checkbox"/>

Filter List: ABeta

Filter Name	Filter Method	Hits
<input checked="" type="checkbox"/> 4-43 Arc	4228.86 Da ± 5 Da	1207
<input checked="" type="checkbox"/> 1-40 Arc	4258.8 Da ± 5 Da	1208
<input checked="" type="checkbox"/> 1-42 Arc	4443.02 Da ± 5 Da	1207
<input checked="" type="checkbox"/> 1-42 vit	4515.1 Da ± 5 Da	1199



Navigation

Image

Results

Regions

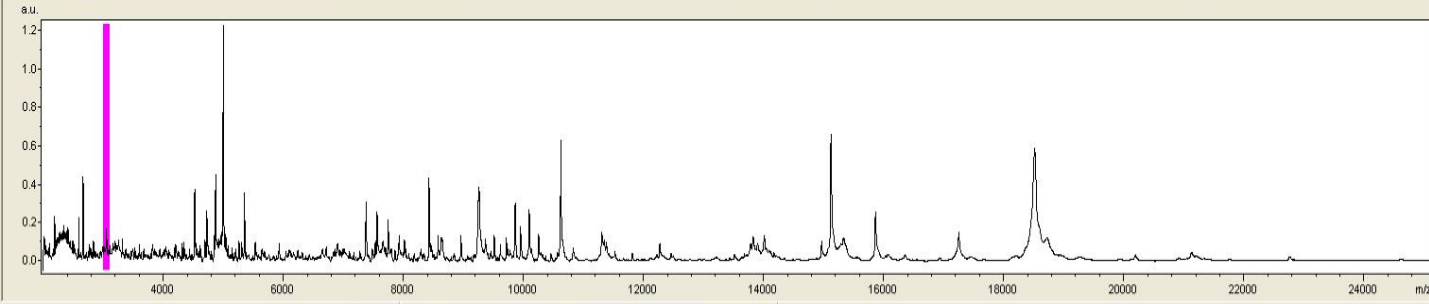
Image Adjustment

Image

Results

Regions

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- Step 1: Set sequence properties
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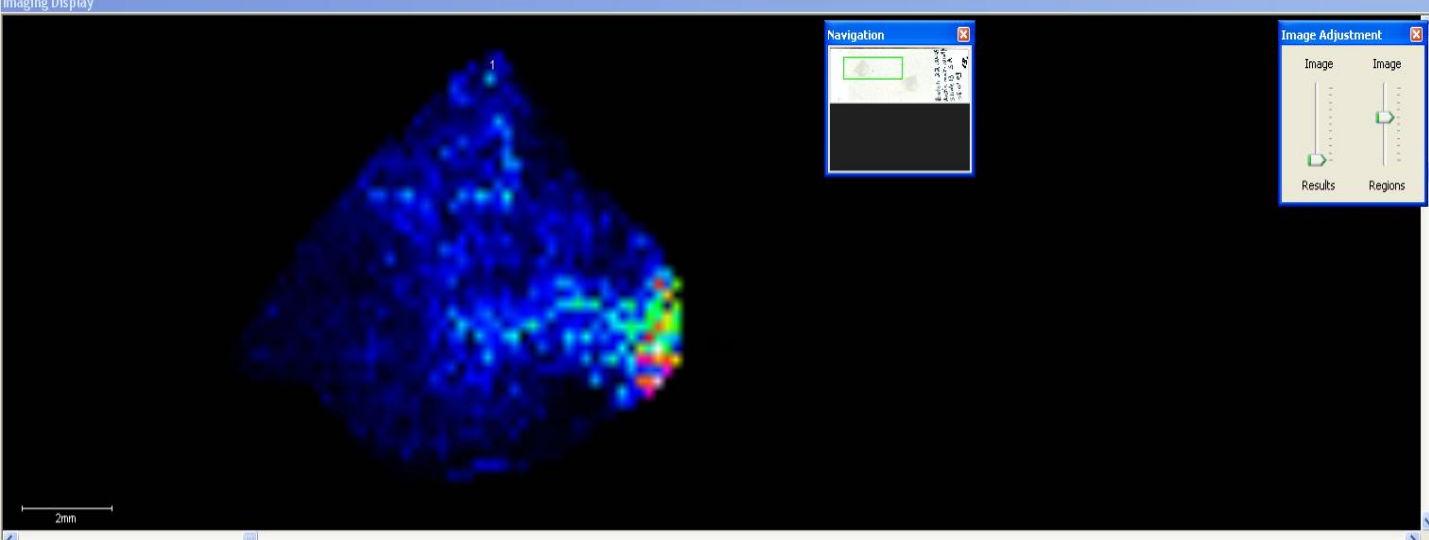


1 Measurement Region(s), 1210 Position(s)

Region: Region Raster (µm): 200, 200 auto/execute Method: Imaging_FC30_rand... Spectrum: Spectrum

Filter List: None

<input type="checkbox"/> Filter Name	Filter Method	Hits
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Navigation

Image

Results

Regions

Image Adjustment

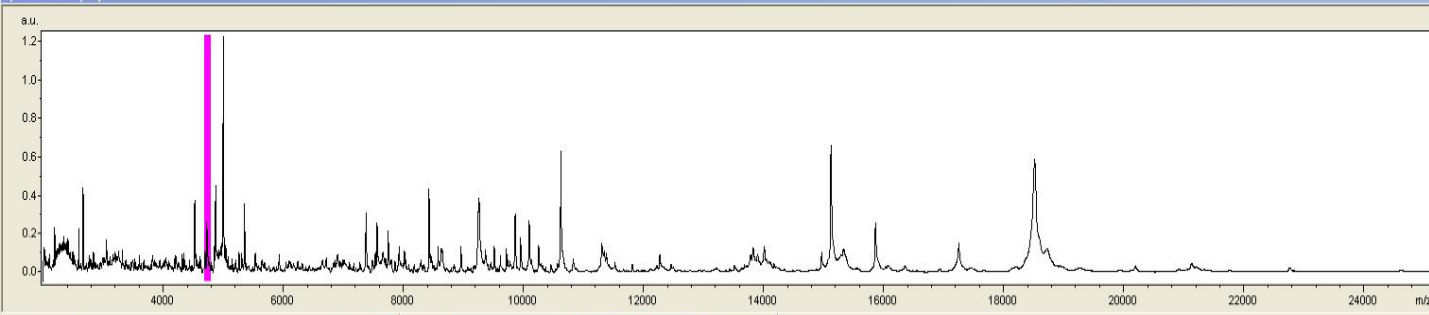
Image

Results

Regions

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Spectrum Display



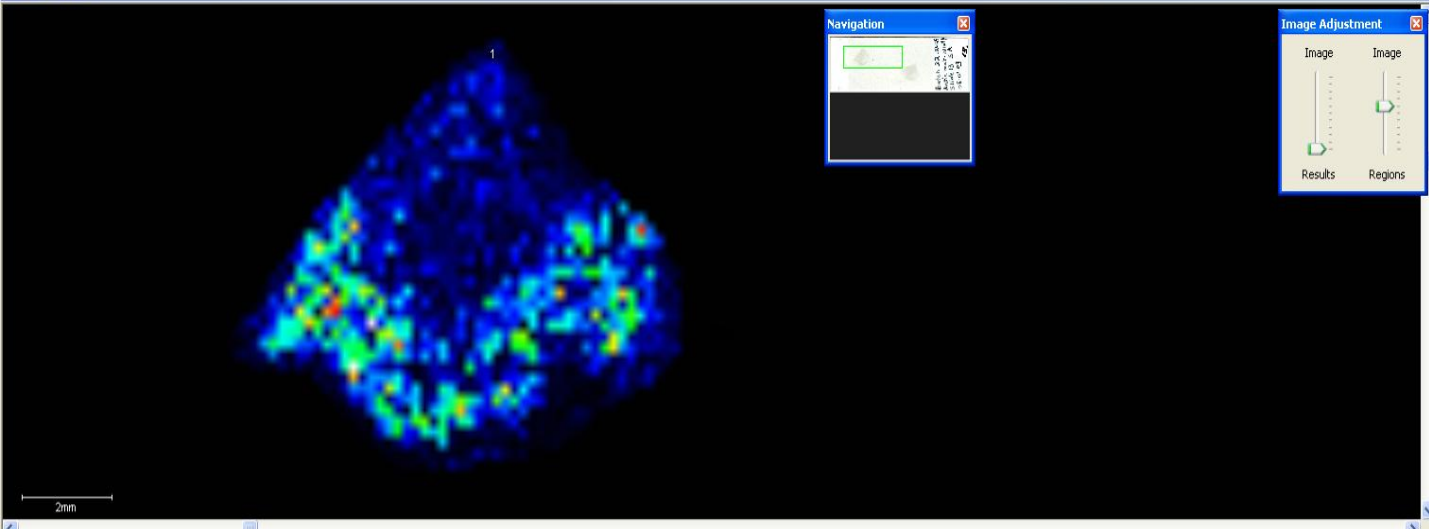
1 Measurement Region(s), 1210 Position(s)

Region Raster (µm) auto/ecute Method Spectrum

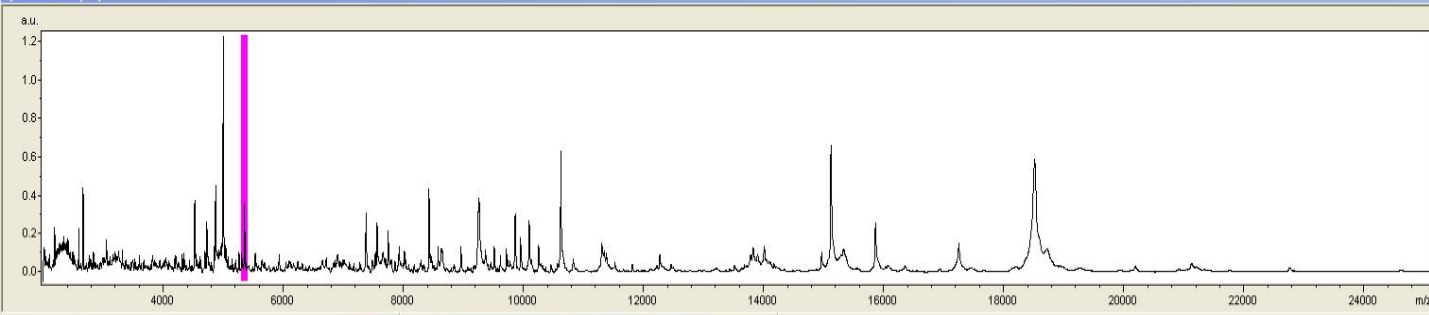
<input type="checkbox"/> 1	200, 200	Imaging_FC30_rand...	<input type="checkbox"/>
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Filter List: None Save As... Add... Edit...

<input type="checkbox"/> Filter Name	Filter Method	Hits
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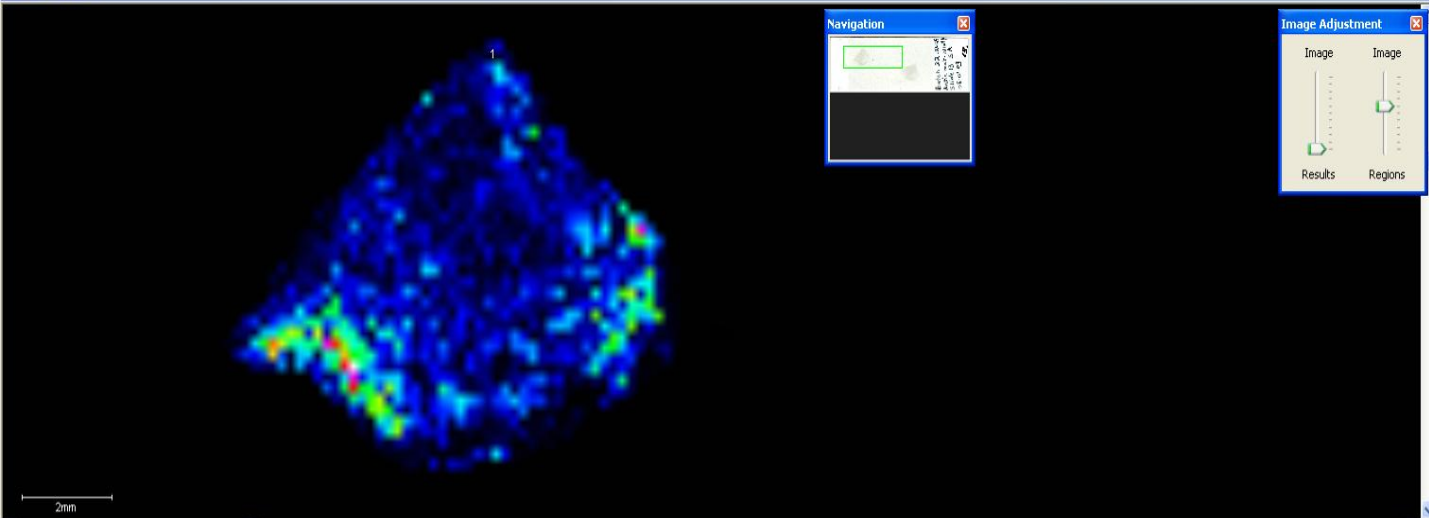
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1 Measurement Region(s), 1210 Position(s) Edit... Filter List: None Save As... Add... Edit...

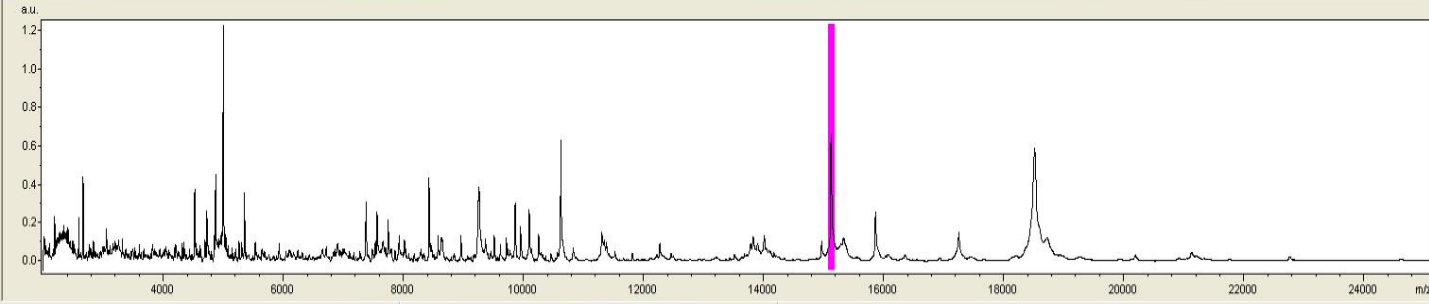
<input type="checkbox"/> Region	Raster (µm)	auto/tecut Method	<input type="checkbox"/> Spectrum
<input type="checkbox"/> 1	200, 200	Imaging_FC30_rand...	<input type="checkbox"/>

<input checked="" type="checkbox"/> Filter Name	Filter Method	Hits
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Spectrum Display



1 Measurement Region(s), 1210 Position(s)

Region	Raster (µm)	auto/ecute Method	Spectrum
1	200, 200	Imaging_FC30_rand...	<input checked="" type="checkbox"/>

Filter List: None

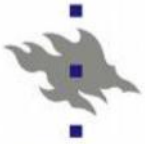
<input checked="" type="checkbox"/> Filter Name	Filter Method	Hits
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Diagnostic value?

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 Semmes^{1,2,3}

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



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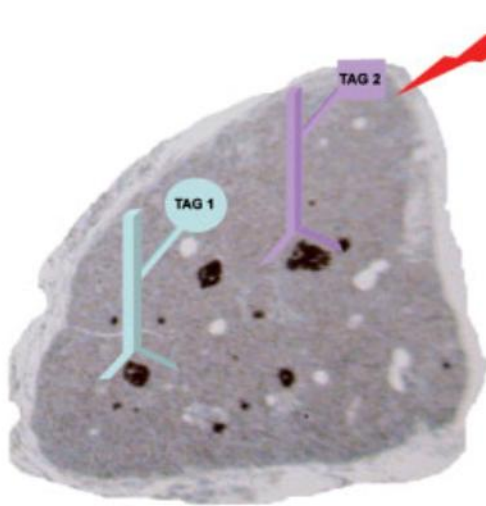
CHEMSEM

Chemical sensor arrays for application in mass spectrometry on surfaces and tissues

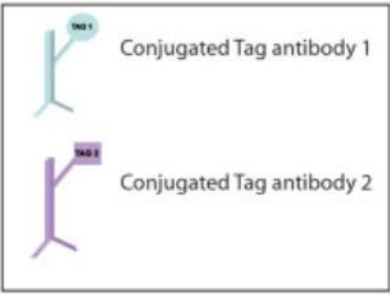
Multiplex Targeted Secondary Detection (MTSD)

- Targeting molecule of interest with specific antibodies which carry a photocleavable mass tag sensor
- The mass tag sensor is a small molecule of known mass easily detectable by MALDI MS (indirect detection)
- The tag is released just before detection step due to irradiation with MALDI laser (photolabile linker)

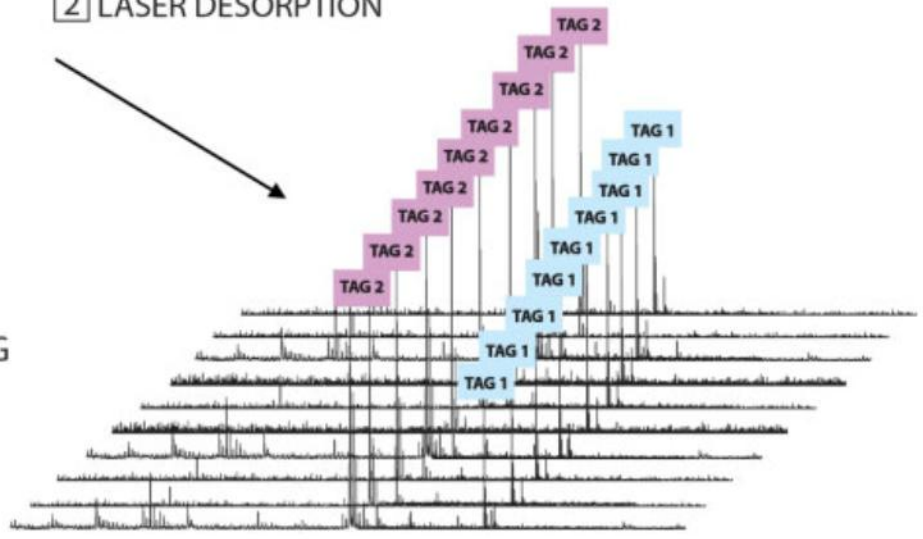
1 DIRECT IMMUNOHISTOCHEMISTRY



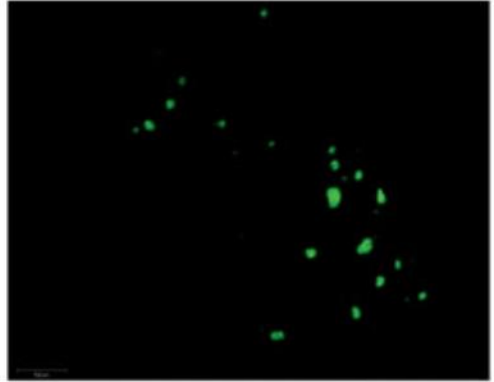
MALDI UV LASER



2 LASER DESORPTION



3 SPECIFIC MASS SPECTROMETRY IMAGING

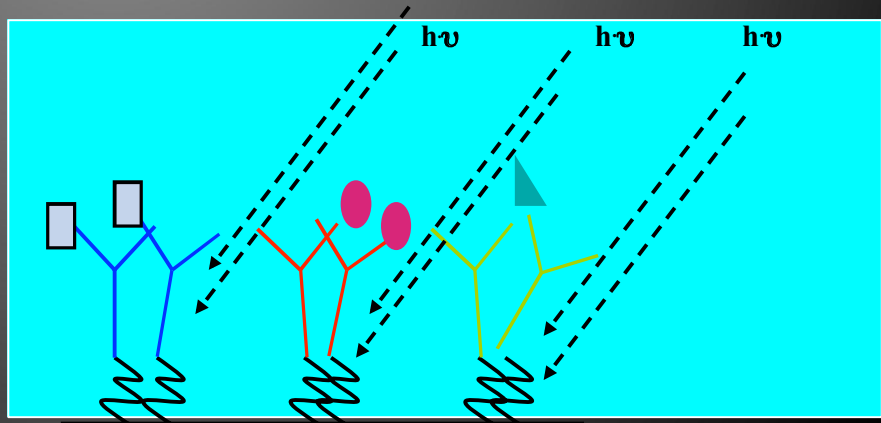


„normal“ MTSD Arrays

- Samples will be spotted on chip surface and immobilized
- Chip will be treated with mass tagged antibodies
- Matrix application
- Under MS conditions: sensor tags will be released and detected via TOF

Inverse MTSD Arrays

- Antibodies are attached to chip surface
- Sample is applied on chip, specific substrates bind to their antibodies
- Matrix application
- Substrate-antibody complex gets released and detected via TOF



B I O M E D I C U M H E
8

