Mass spectrometry imaging

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The analytical chemist goal:

The full characterization of several (all?) analytes in a complex sample

- Identify precisely with descriptors: retention time, mass, connectivity's, shape
- Quantify: relative or absolute quantification
- Partners in interaction
- Locate/colocate: where in the sample and who is at the same place

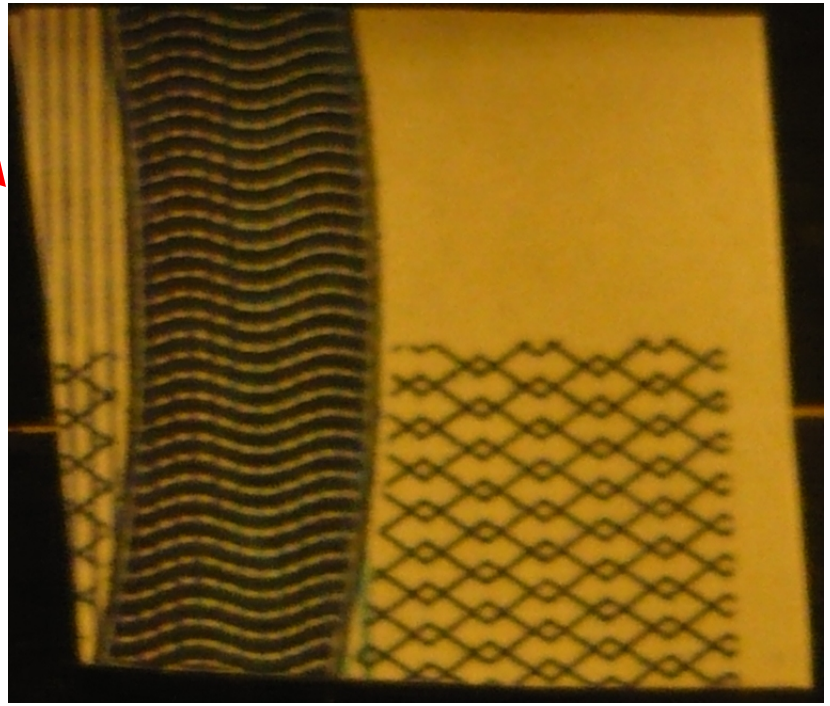
Mass spectrometry imaging

Outline

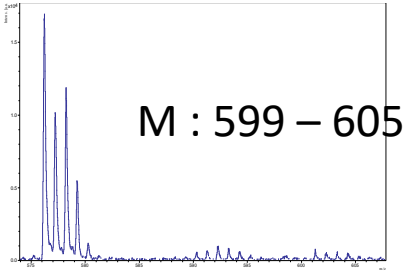
1. Two introductory examples
2. Ionization methods
3. MALDI, the matrix
4. From identification to quantification
5. Open questions
6. Ion mobility

Why Imaging with mass spectrometry ?

EU FT-ICR MS



A comparison with other molecular methods

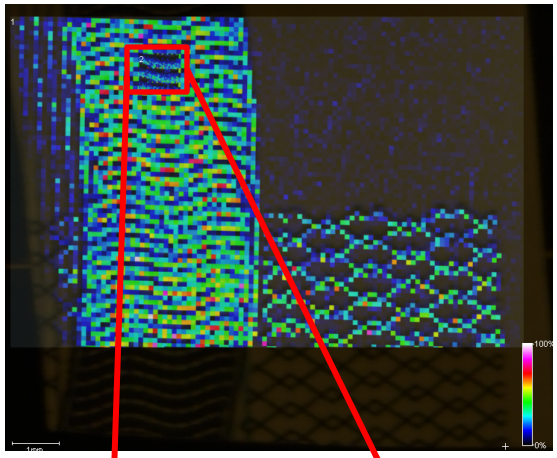


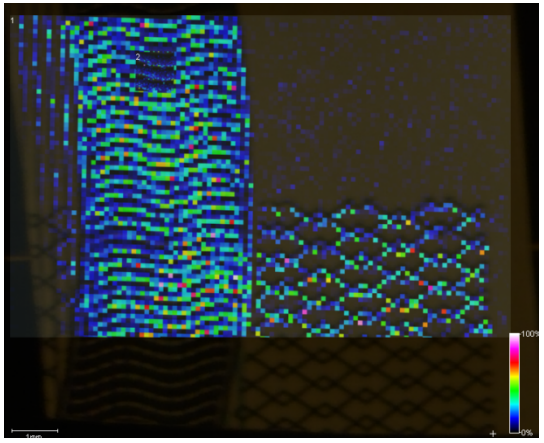
M : 599 – 605

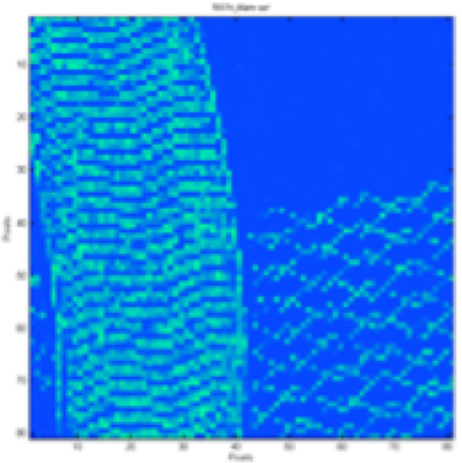
RAMAN, exc 633nm

REGION 1 :

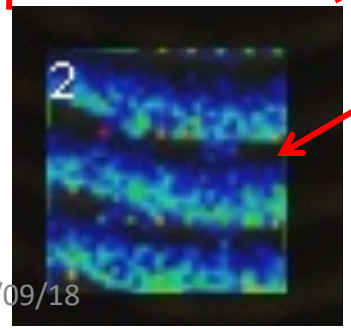
M : 574,5 – 580,5



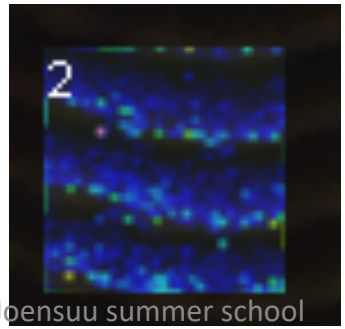


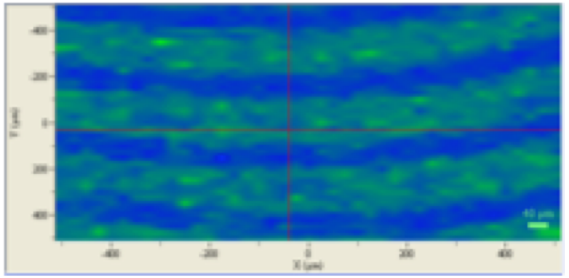


REGION 2 :

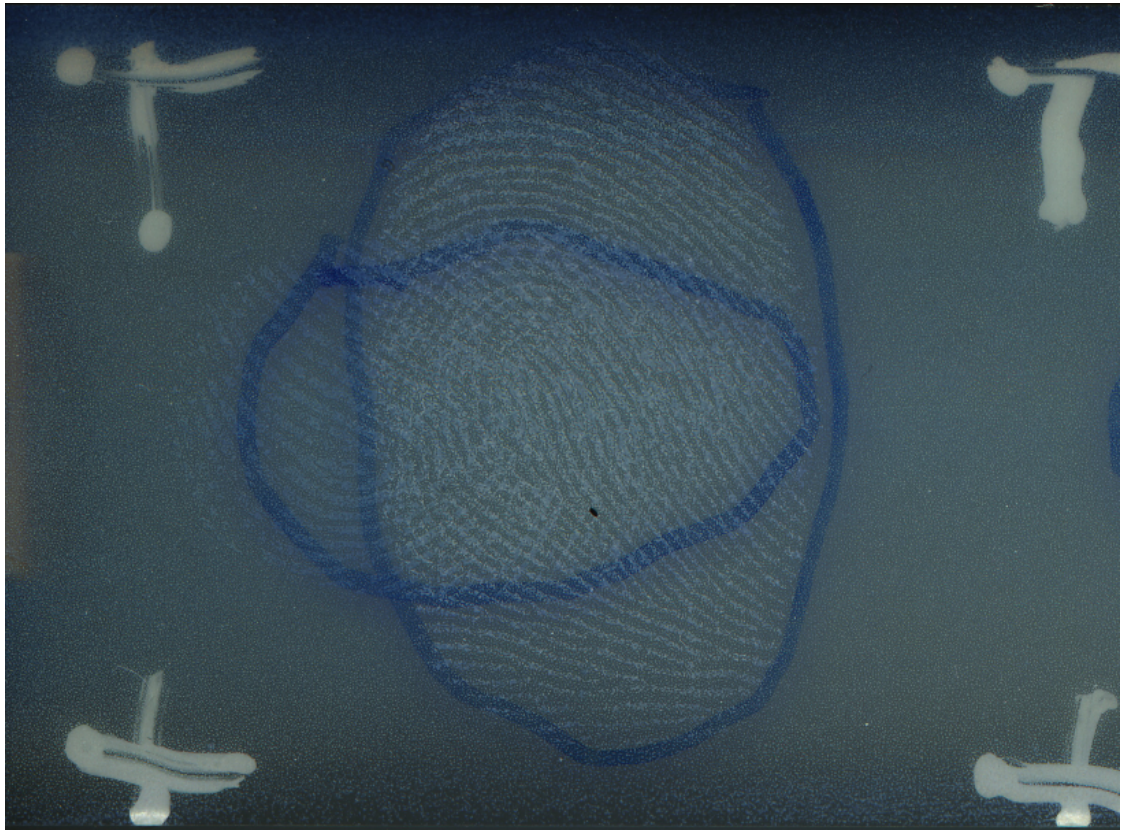


gap: ~ 50 µm





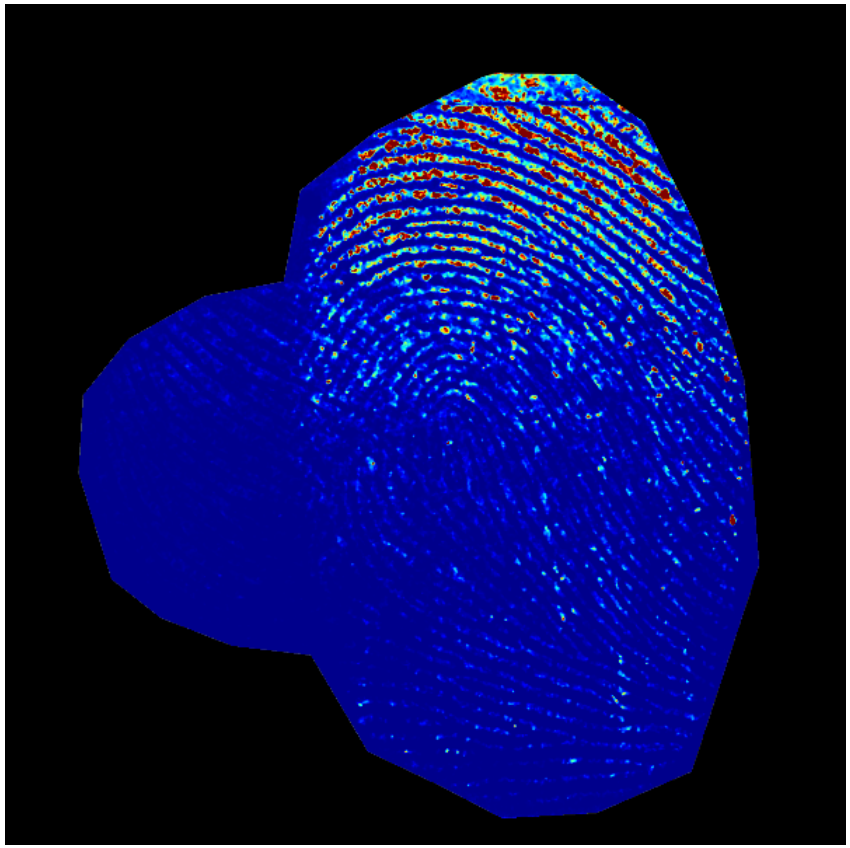
Two superimposed fingerprints optical image



α-Cyano-4-hydroxycinnamic acid

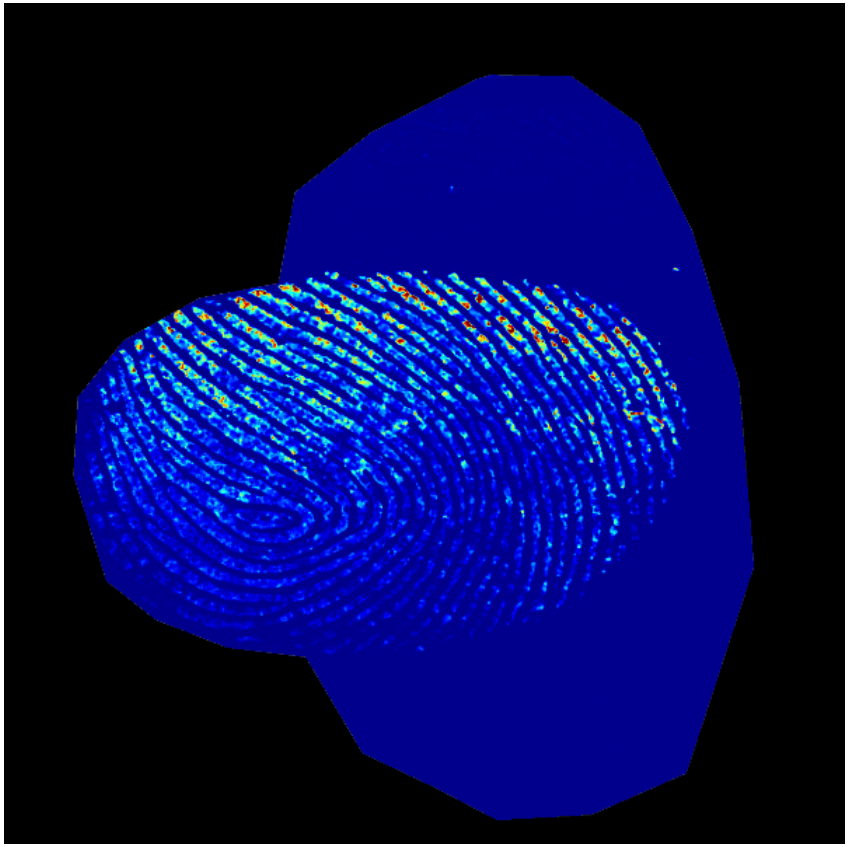
Mass resolved images

Fingerprint below



494,578 ± 0,06 Da

Upper fingerprint

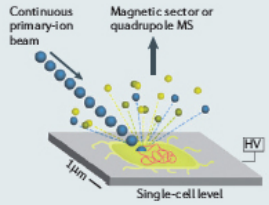
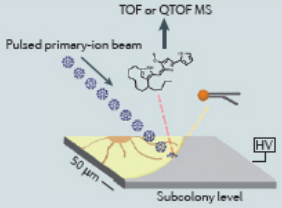
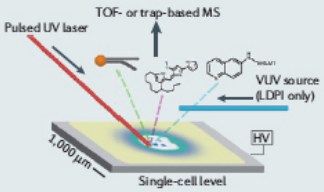
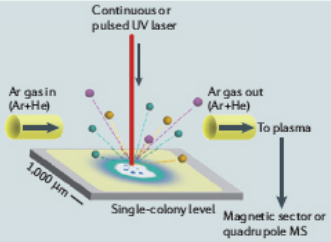
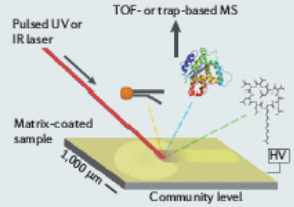


283,314 ± 0,04 Da

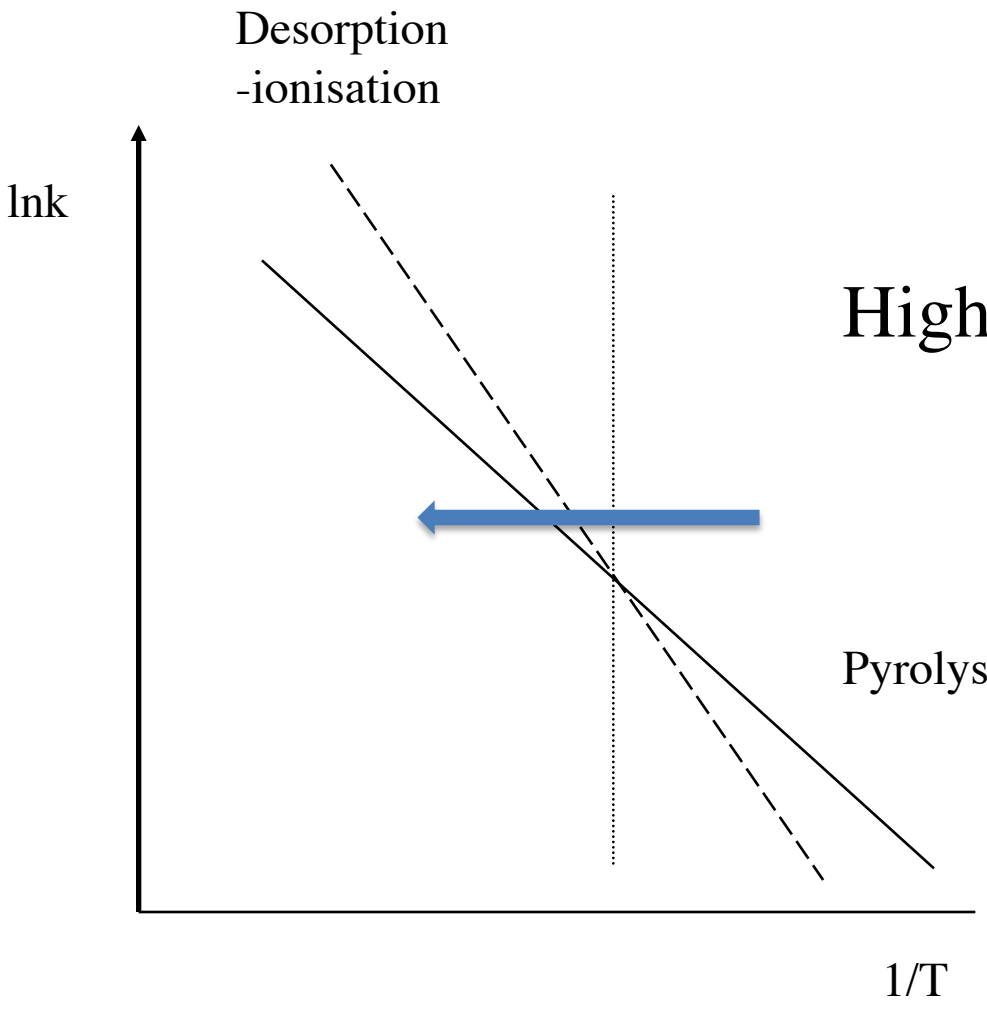
Ionization methods

From Dorrestein,
Nature Reviews Microbiology
sept 2011

Table 1 | **Overview of various scanning probes used for imaging mass spectrometry analysis of microbial samples***

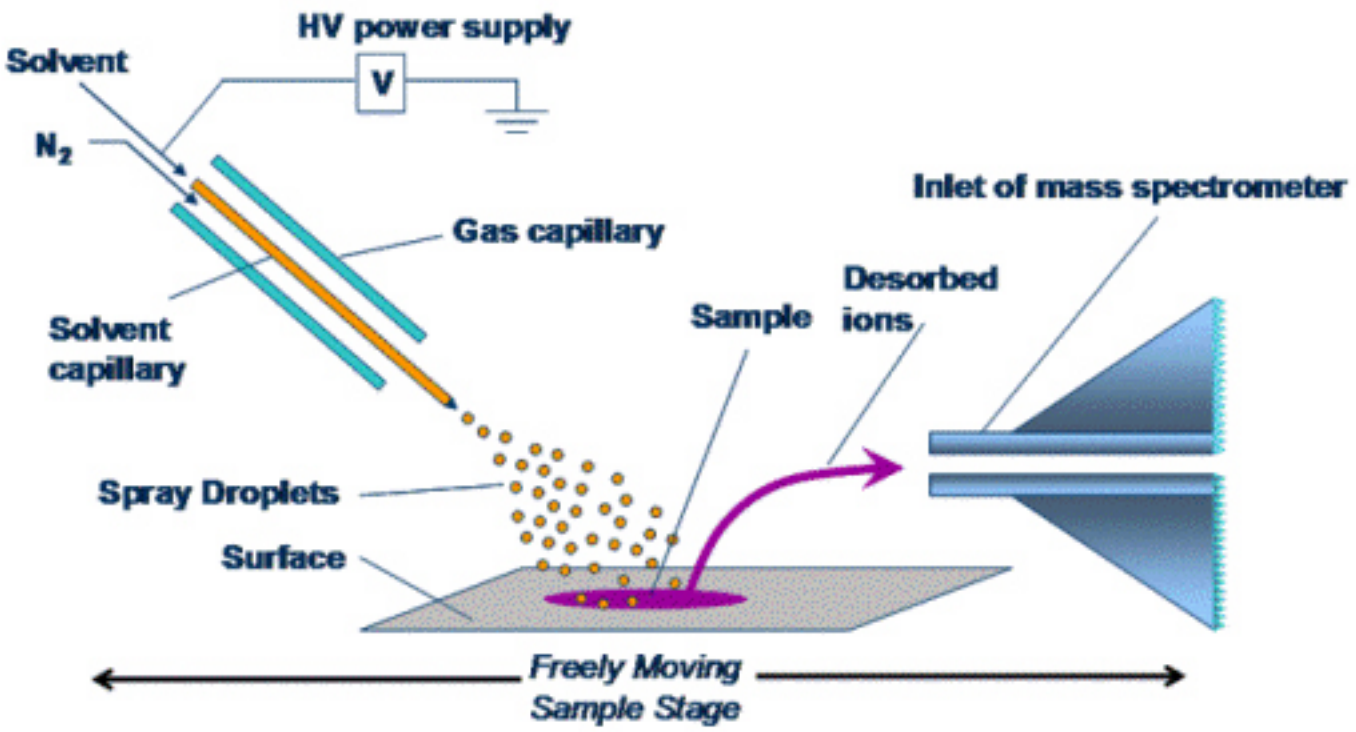
Technique	Schematic	Benefits	Challenges	Usage
Dynamic SIMS‡		• Highest spatial resolution available • ppm-level elemental analysis • Quantitative imaging possible • Depth profiling possible	• Lack of molecular information • Small sampling area (few mm²) • Mounting surface must be conductive • Samples should be flat • Samples must be stable under UHV • Only a few signals can be monitored simultaneously	• Surface and subsurface analysis of single elements and small atomic clusters at the cellular and subcellular levels
Static SIMS		• High spatial resolution • Molecular information for sizes up to *m/z* ~ 1,500§ • Low amounts of material consumed • Wide variety of primary-ion guns available	• Source-induced fragmentation • Small sampling area (few mm²) • Mounting surface must be conductive • Samples should be flat • Samples must be stable under UHV • Quantitative imaging currently not possible	• Near-surface analysis of low molecular-mass compounds at the multicellular and single-colony levels
LDI		• Molecular information up to small proteins • Available at AP, IP and high vacuum • LDPI configuration offers better sensitivity • High sample tolerance (size and shape)	• Analyte must have a chromophore • Mounting surface must be conductive • Quantitative imaging currently not possible • Source-induced fragmentation (extensive CO loss) • Lack of elemental and atomic-cluster information	• Monitoring of molecular species (containing a chromophore compatible with the laser used) at the colony and community levels, as well as complex biological samples
LA-ICP		• ppm- to ppb-level elemental analysis • Depth profiling possible • Quantitative imaging possible • Excellent for metal analysis	• Lack of molecular and atomic cluster information • Common matrix, diatomic and multiply charged elements can interfere with analysis • Only one ion can be imaged at a time	• Surface and subsurface elemental, especially metal, analysis (7–250 Da) at the colony and community levels
MALDI		• Widest mass range available • Robust protocols in place for imaging of microbial colonies on solid media • AP and IP sources available • High sample tolerance (size and shape)	• Samples must be covered with an organic matrix (i.e. DHB or CHCA) • Mounting surface must be conductive • Quantitative imaging currently not possible • Lack of information below *m/z* ~ 300	• Analysis of intact molecules (*m/z* ~ 300 to >5,000) from individual microbial colonies, microbial communities and complex biological samples.

Ion production from non volatile chemicals: impact methods



High density of energy
SIMS, LDI, MALDI

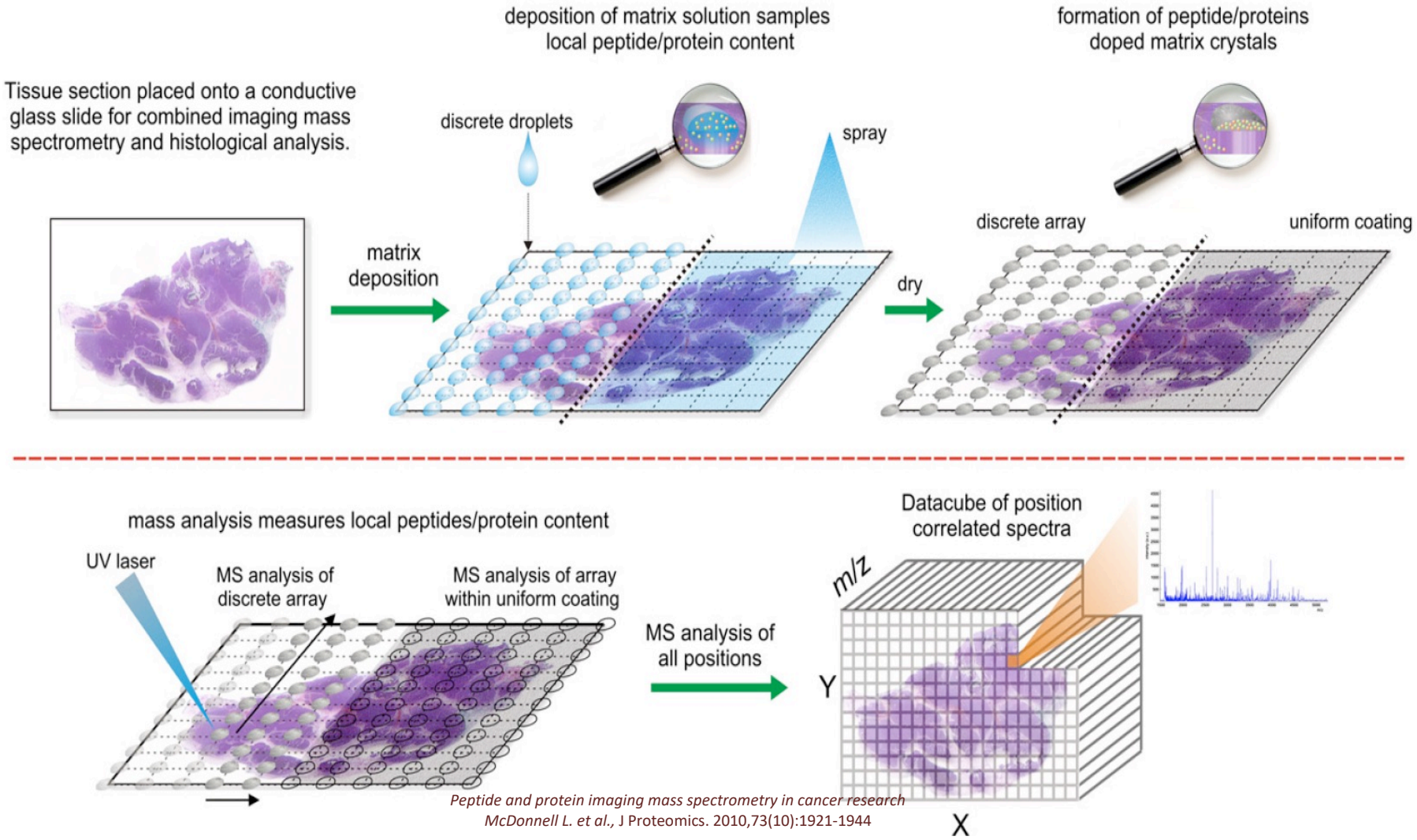
Ion production from non-volatiles: spray methods



What are the key points!

- Ionization efficiency
- Mass resolution ($M/\Delta M$)
- Mass accuracy (ppm)
- Lateral resolution
- MS/MS
- Adding descriptors

2. MALDI imaging mass spectrometry, the experimental flow chart



Peptide and protein imaging mass spectrometry in cancer research
McDonnell L. et al., J Proteomics. 2010,73(10):1921-1944

2.

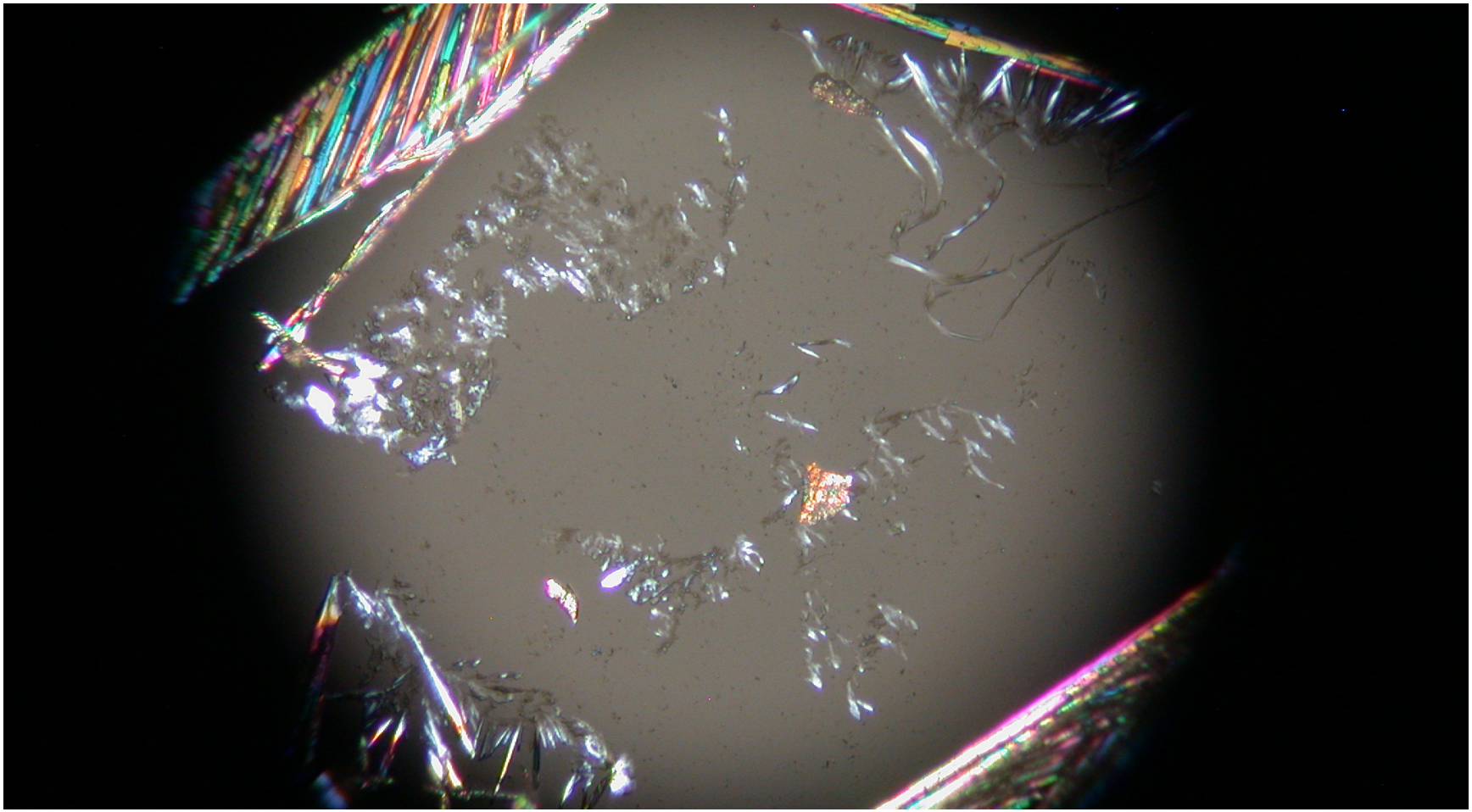
Energy transfert, ion production, controlled chemical reactions

2. Matrices for MALDI imaging

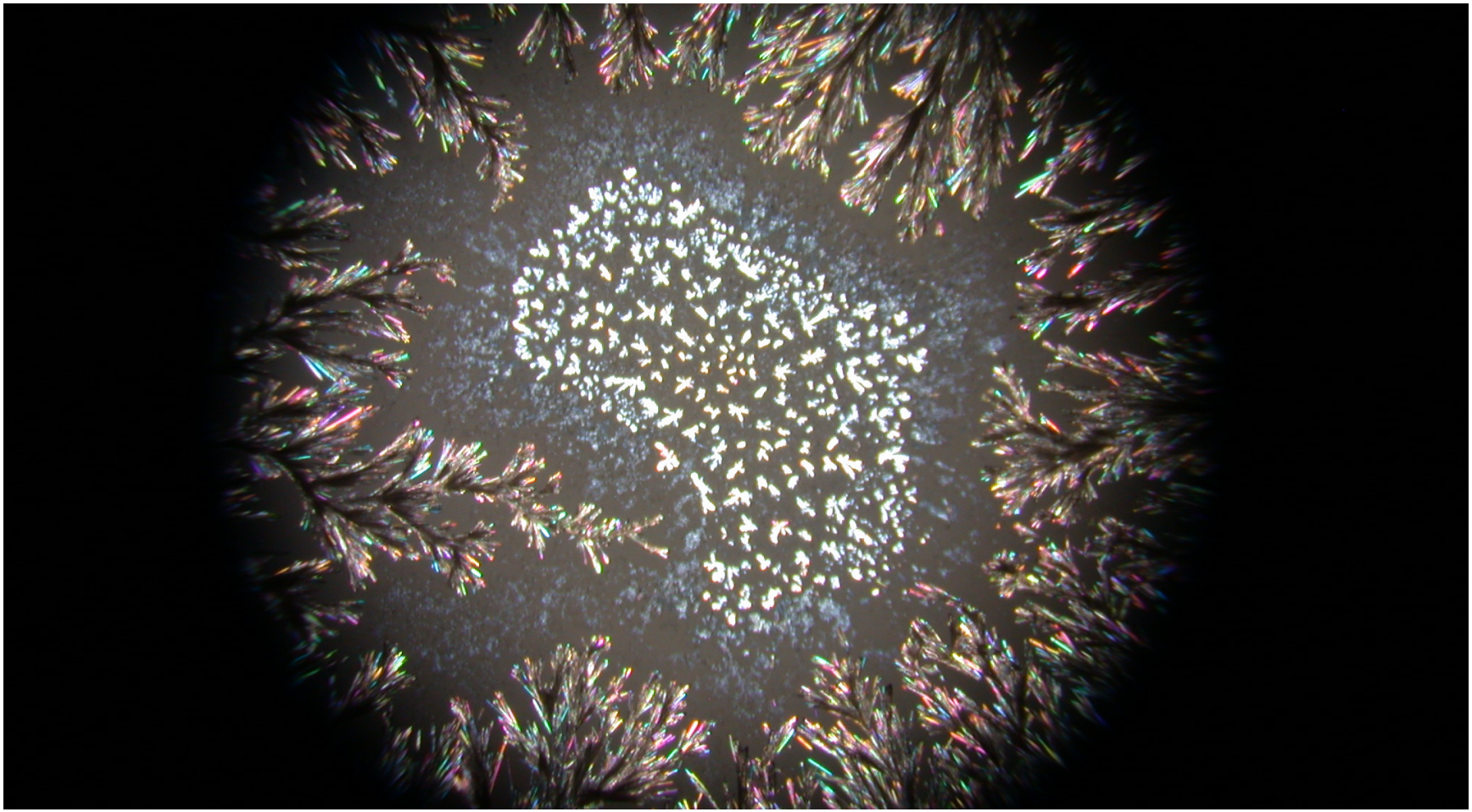
Analyte class	Effective Matrices
Peptides	α-cyano-4-hydroxycinnamic acid (CHCA)
Proteins	3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA) 2,5-dihydroxyacetophenone (DHA)
Lipids	1,5-diaminonapthalene (DAN) 2,5-dihydroxyacetophenone (DHA) 2,5-dihydroxybenzoic acid (DHB)
Small molecules	2,4,6-trihydroxyacetophenone (THAP) α-cyano-4-hydroxycinnamic acid (CHCA) 2,5-dihydroxybenzoic acid (DHB)

Co-crystallisation with the analytes

2. Binary matrices



2. Binary matrices



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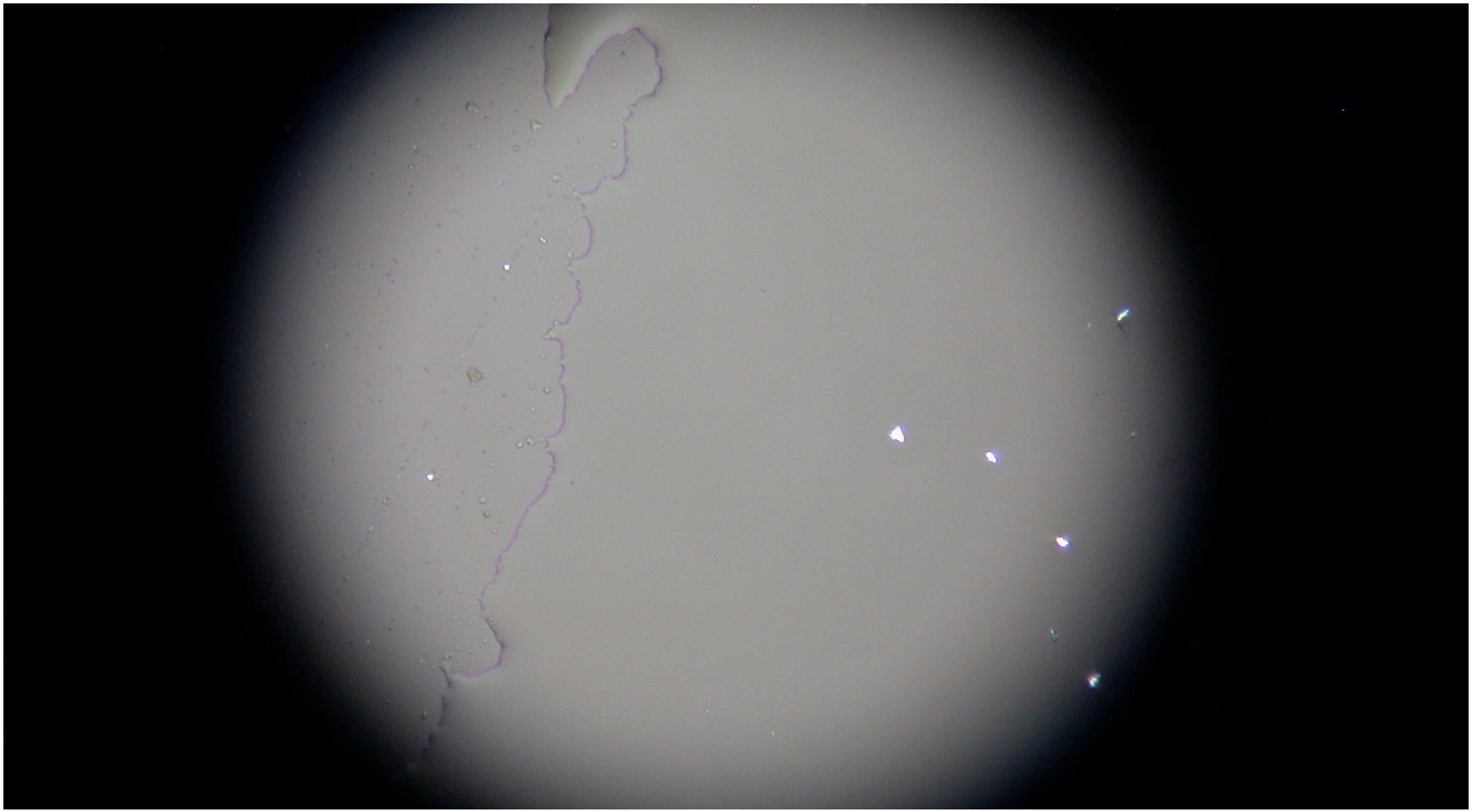
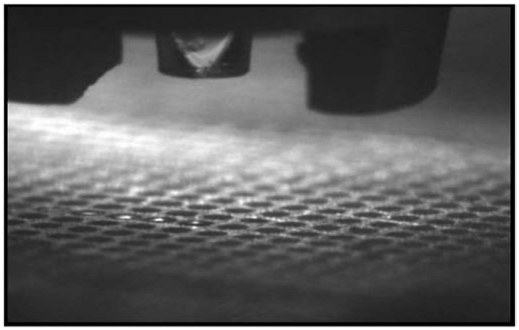
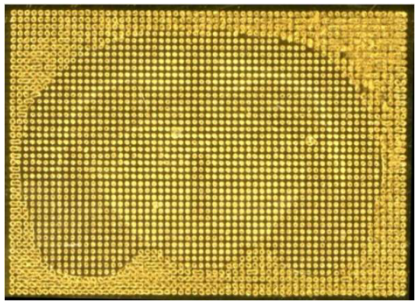


2. Matrix deposition

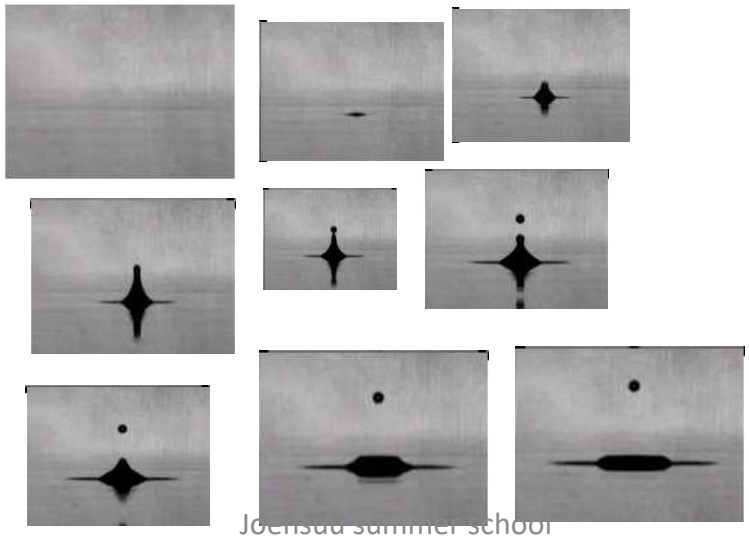
Shimadzu Chemical Inkjet Printer – Piezo printer

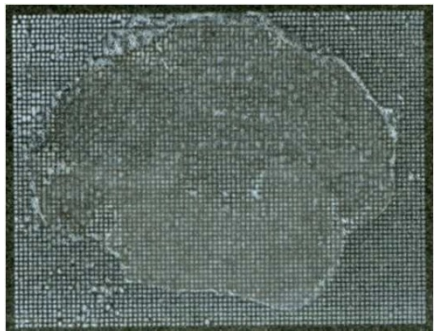




Résolution de 100 à 300 µm

Labcyte Portrait 630 MALDI reagent multi-spotter –acoustic ejection

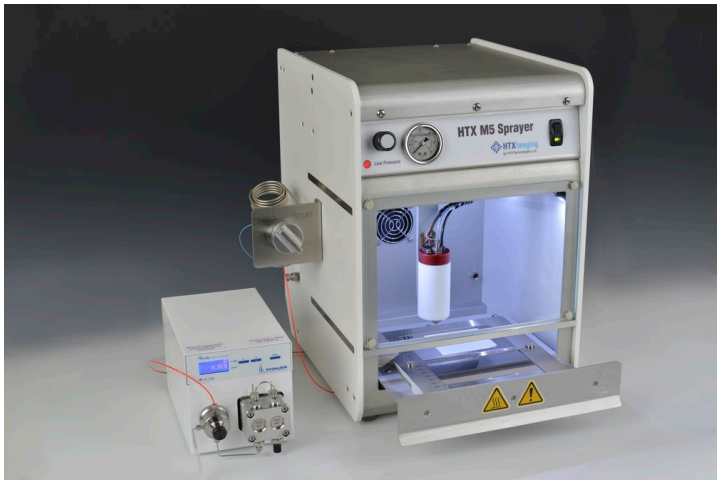


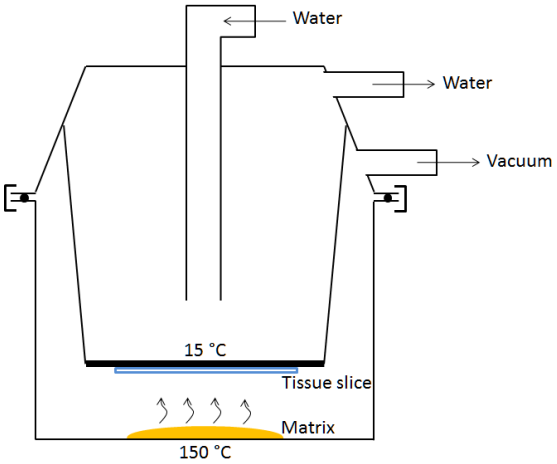


Résolution 40 à 300 µm

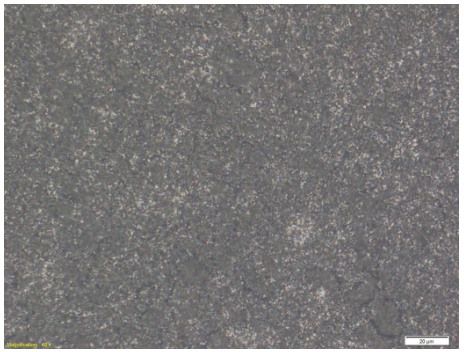
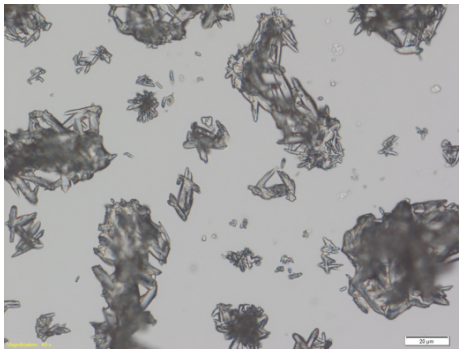
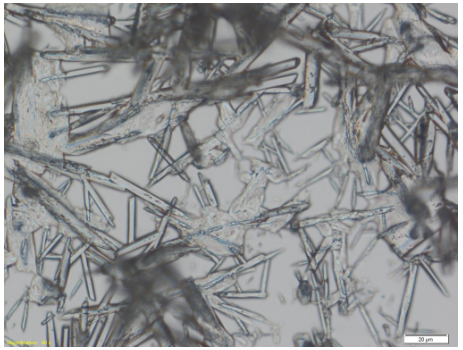
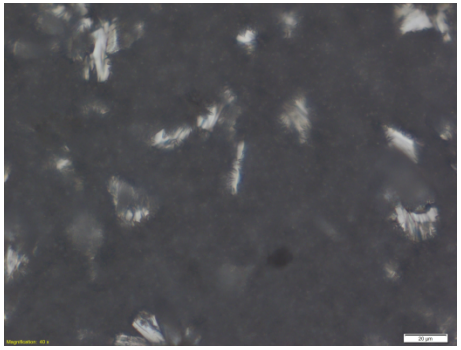
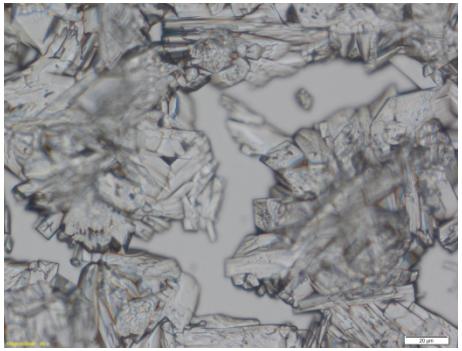
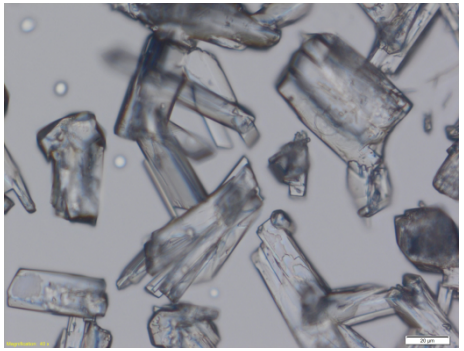
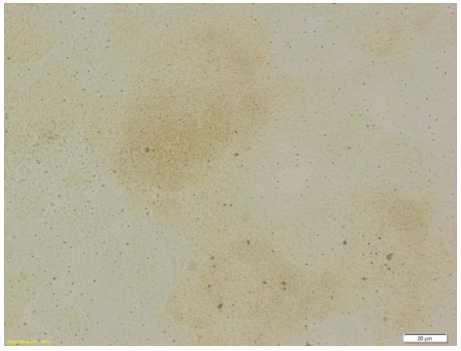
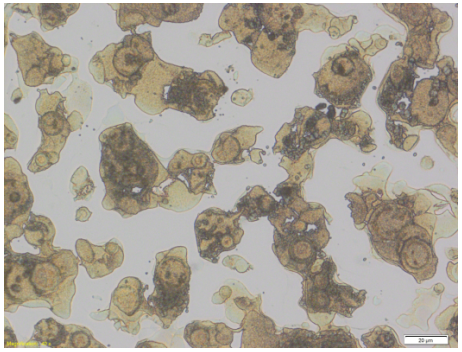
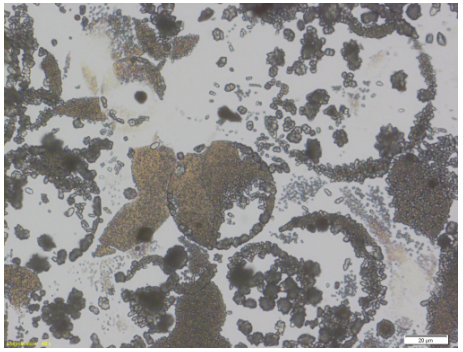
2. Matrix deposition

- Sample nebulisation
  - Spray deposition
- Sublimation
  - Very thin crystals, homogeneous



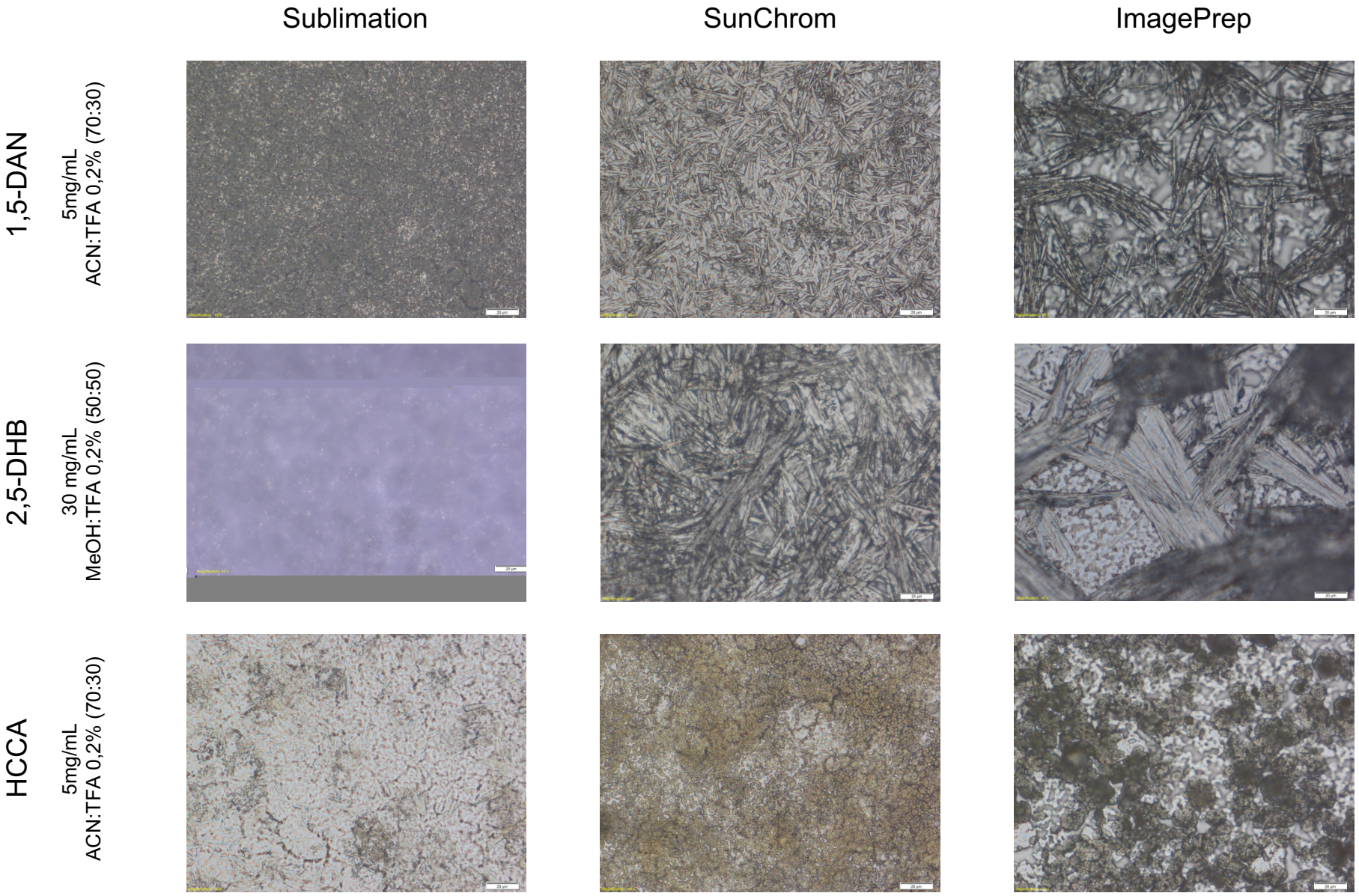


2. Shapes of the crysrals 1,5-DAN, 2,5-DHB and HCCA on ITO

	Sublimation	SunChrom	ImagePrep
1,5-DAN 5mg/mL ACN:TFA 0,2% (70:30)			
2,5-DHB 30 mg/mL MeOH:TFA 0,2% (50:50)			
HCCA 5mg/mL ACN:TFA 0,2% (70:30)			

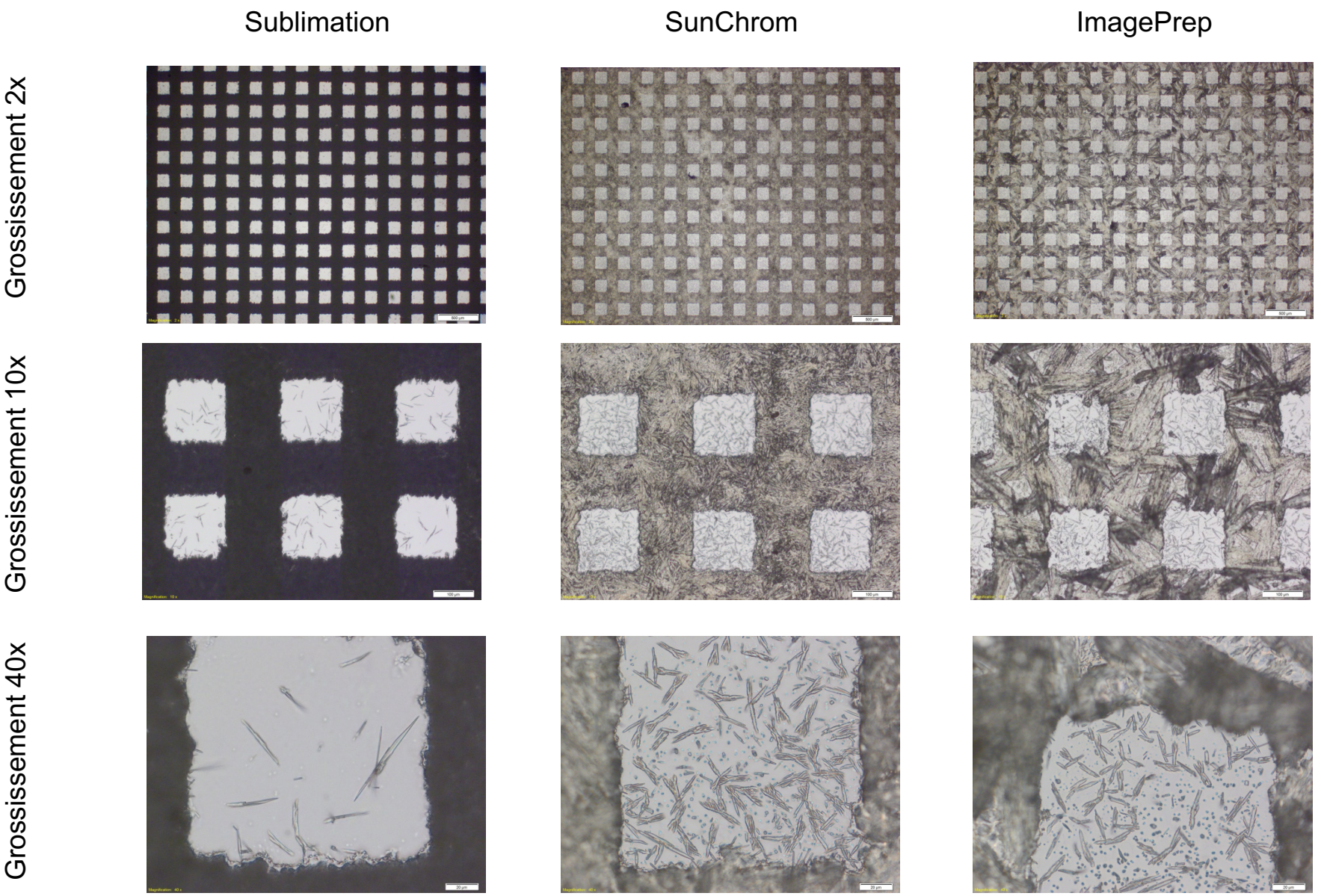
Enregistrements photographiques effectués avec le microscope inversé Olympus IX81, BF, 40x

2. 1,5-DAN, 2,5-DHB et HCCA on brain homogenates



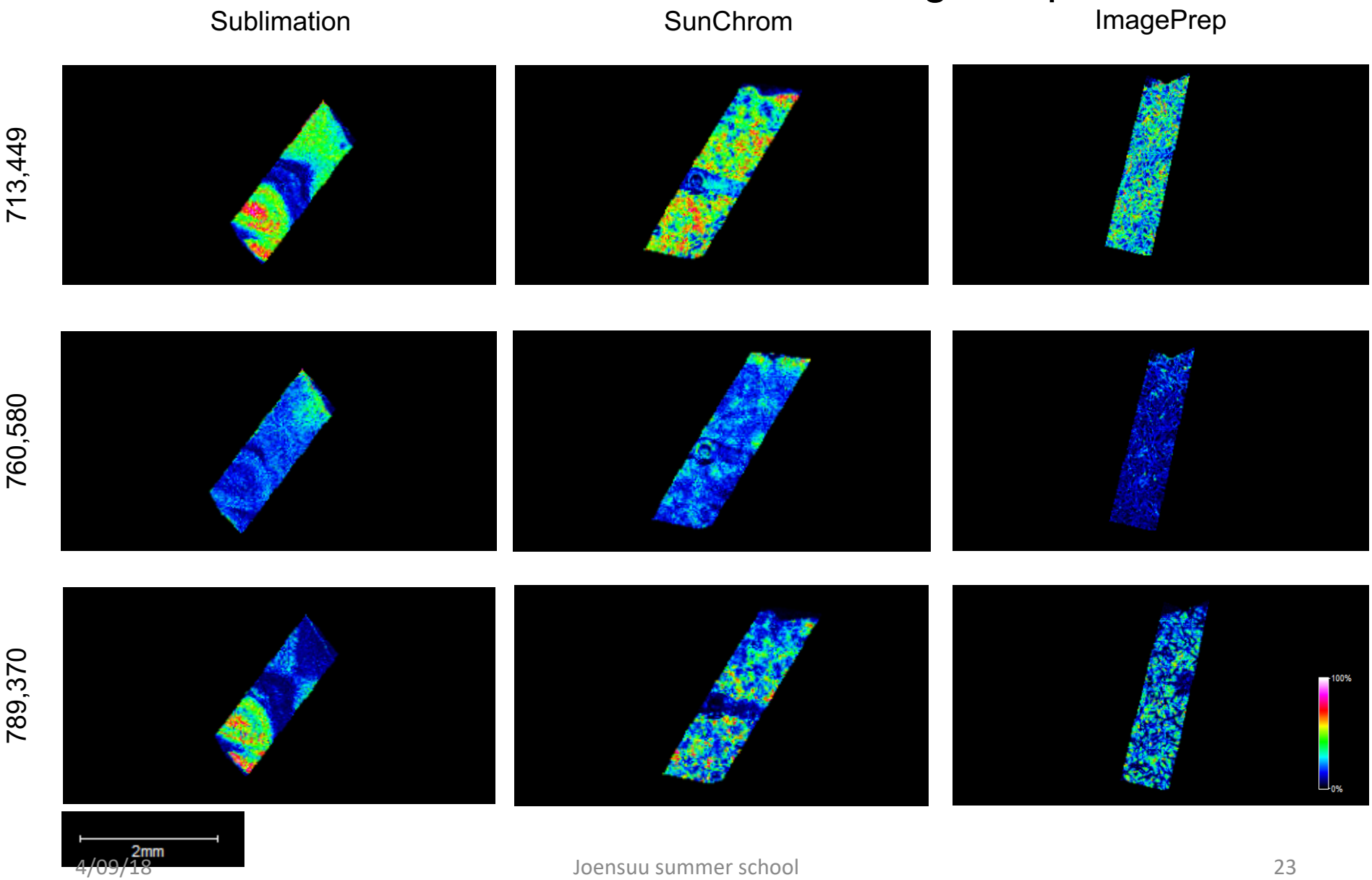
Enregistrements photographiques effectués avec le microscope inversé Olympus IX81, BF, 40x

Laser ablation 2,5-DHB on brain homogenate

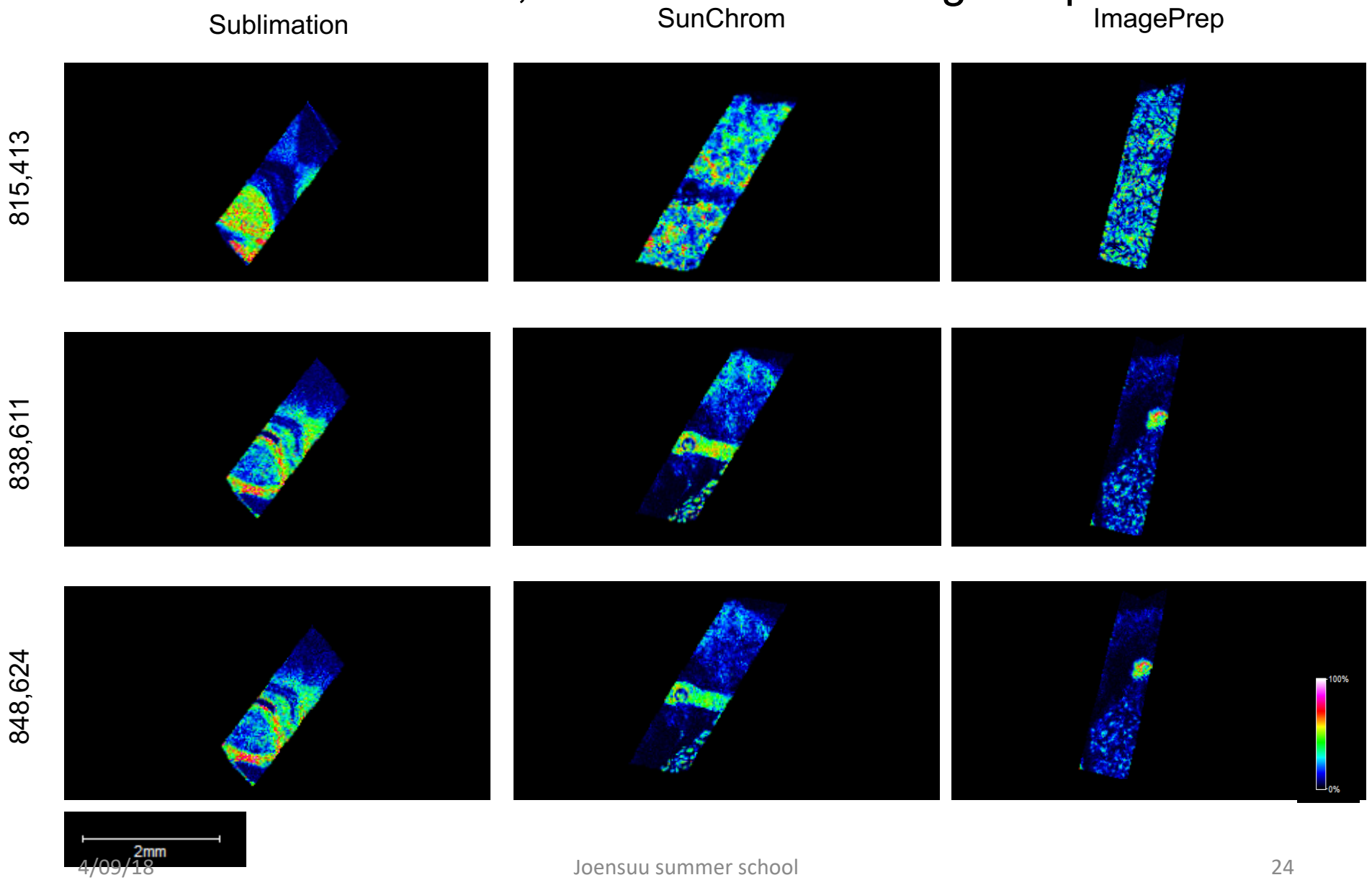


Enregistrements photographiques effectués avec le microscope inversé Olympus IX81, BF,

LR 20 microns - 2,5-DHB (30 mg/mL, MeOH:TFA 0,2% (50:50)) sublimation, SunChrom et ImagePrep



20 microns - 2,5-DHB (30 mg/mL, MeOH:TFA 0,2% (50:50)) sublimation, SunChrom and ImagePrep



Instruments used for MS imaging

- Any MALDI type instrument precise motion of the Important point: speed!!!
- One image can take hours
- Dual strategy: a fast first line instrument
- Fast RAPIFLEX watch at 1:40’
- Slower FTICR-MS

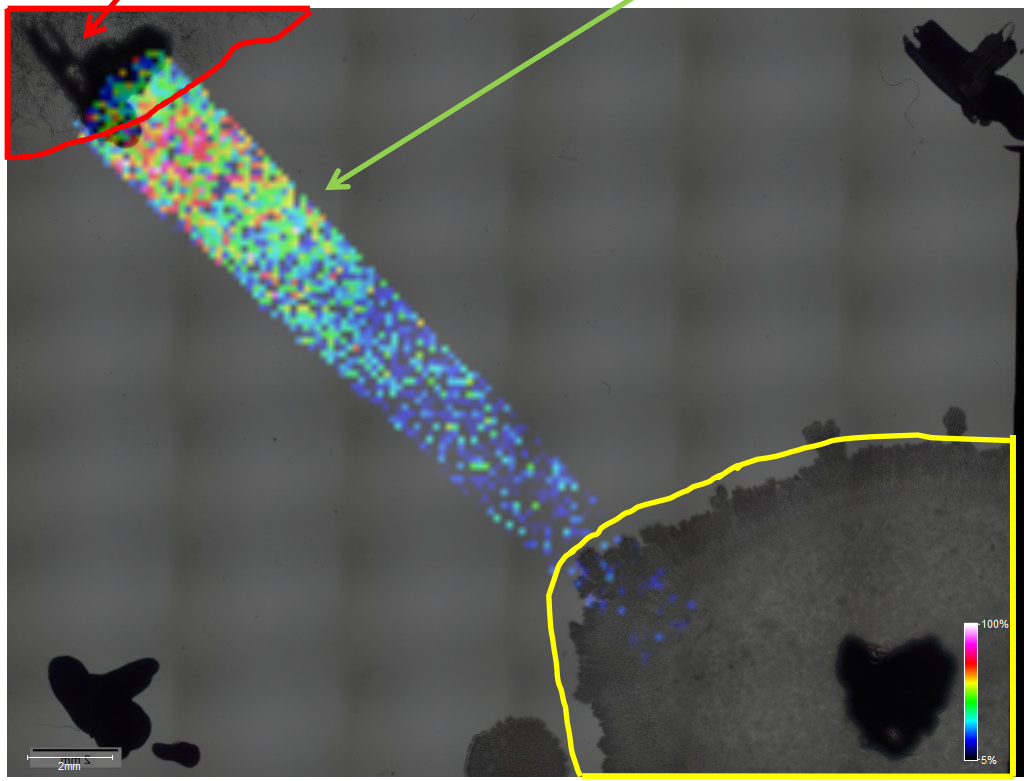
Examples of small molecules imaging

- Chemical ecology
- Forensic
- Microbiology
- Metabolism

Chemical ecology

Pathogen

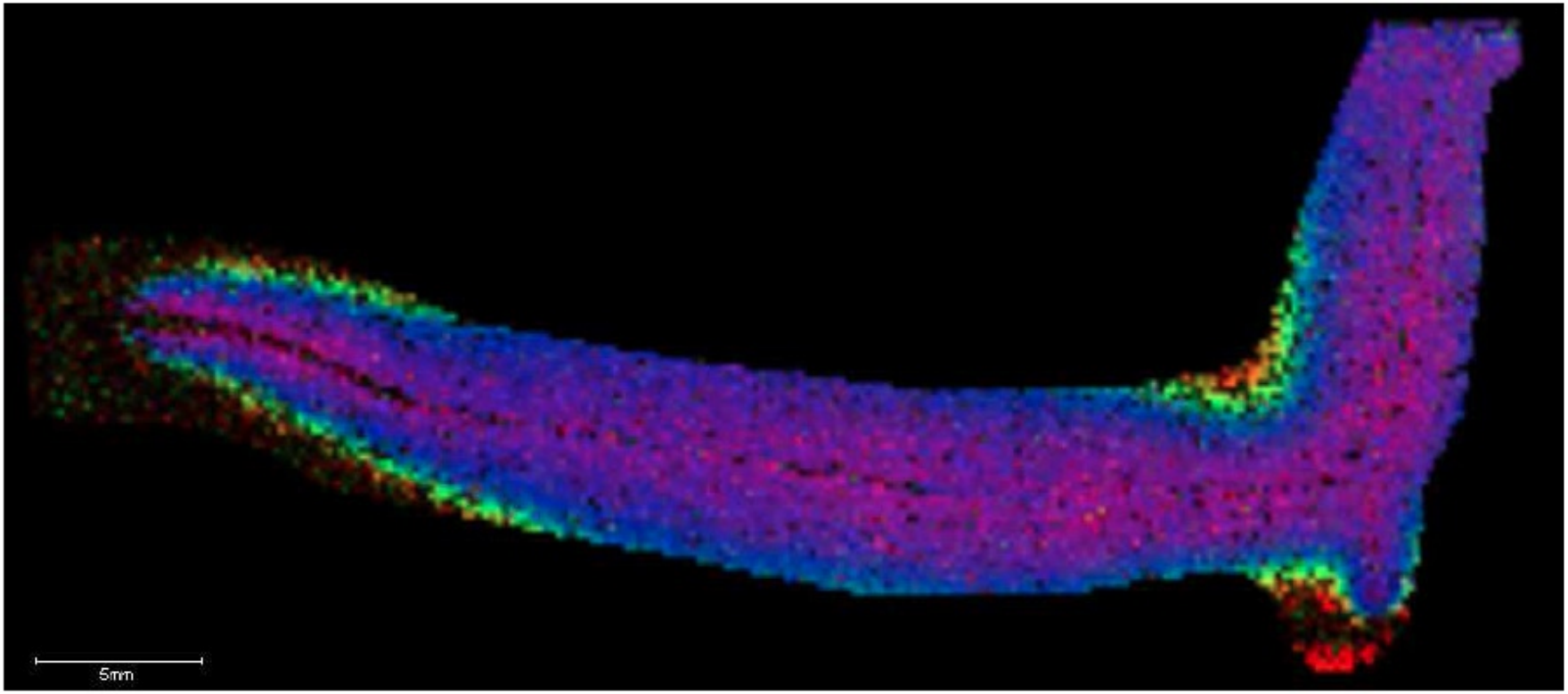
Distribution of an antibiotic molecule: surfactin



Bacterial colony

Sample directly grown on agar support

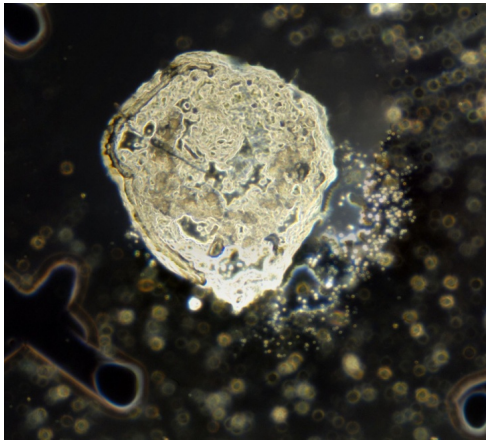
Chemical ecology: symbiotic activity



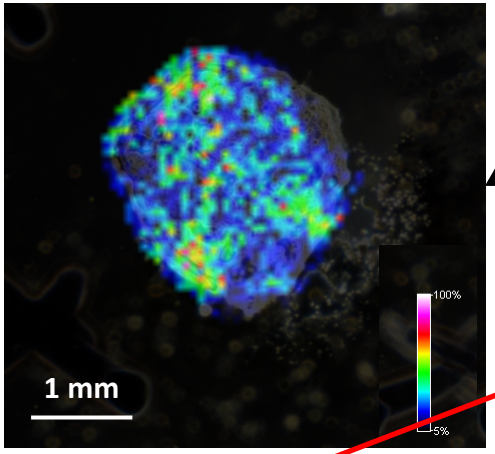
Superposition des images des quatre surfactines. Surf C12 en rouge sur les bords (éloignée de la racine), Surf C13 en vert, Surf C14 en bleu, très abondante et Surf C15, en rouge aussi, présente au voisinage direct de la racine.

Example of MS identification in forensics

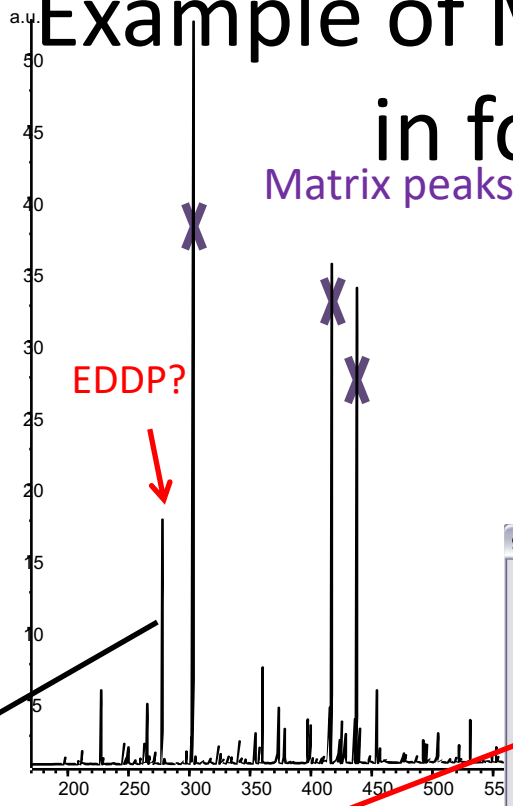
EU FT-ICR MS



Picture of the « tox » larvae
Magnification x2.5

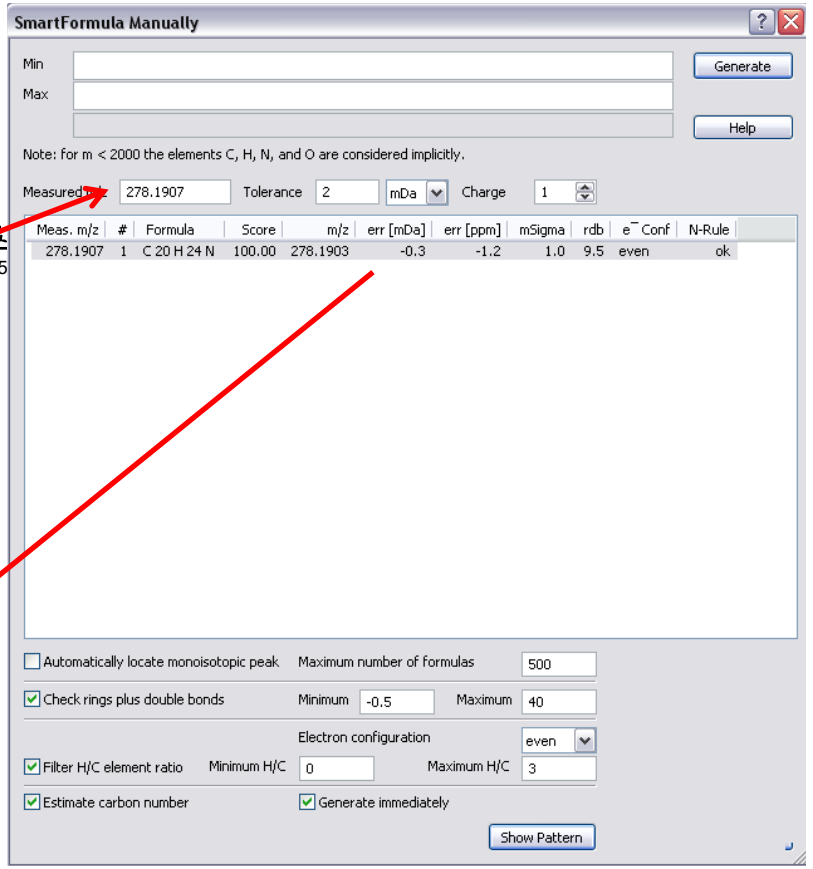


m/z 278.1907



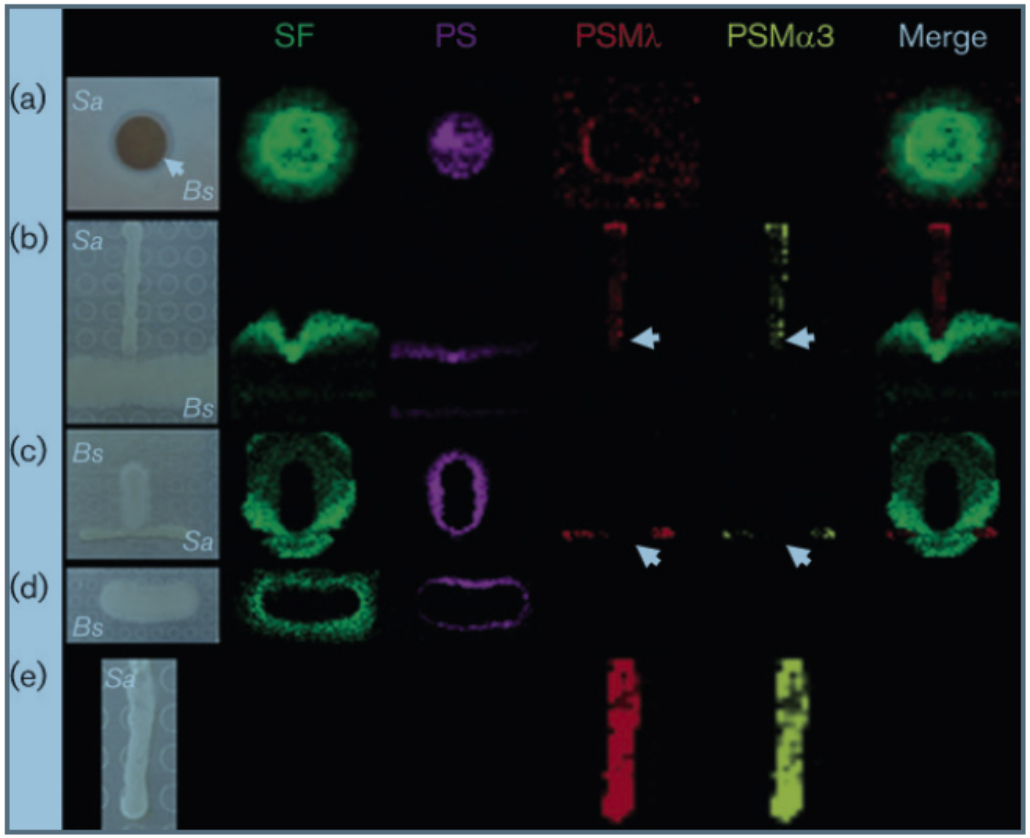
Average mass spectrum of the « tox » larvae

Software interrogation for the exact m/z



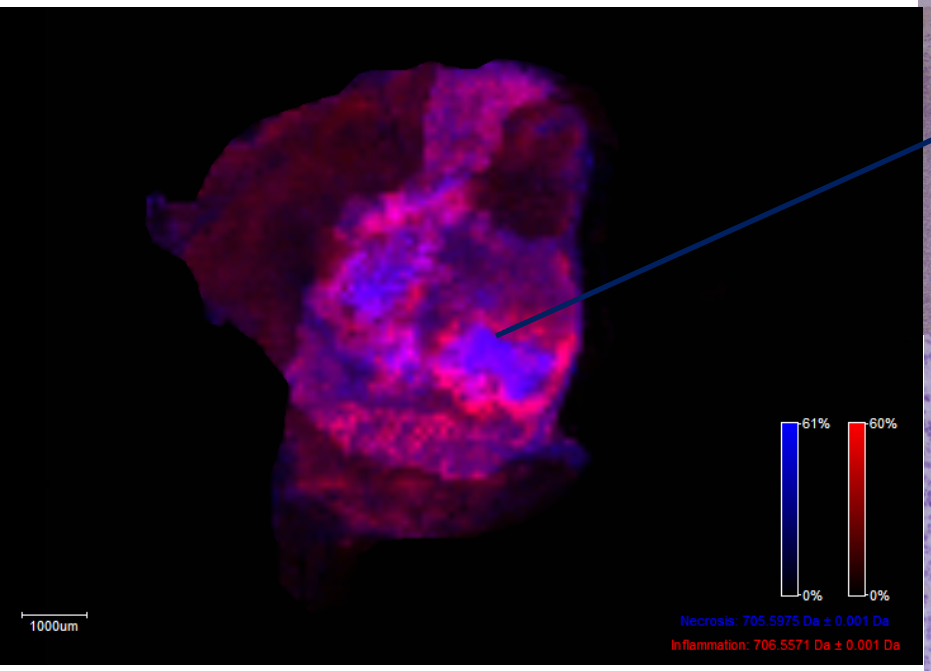
Identification of EDDP with the exact mass! Score: 100%, only one candidate **(2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)**

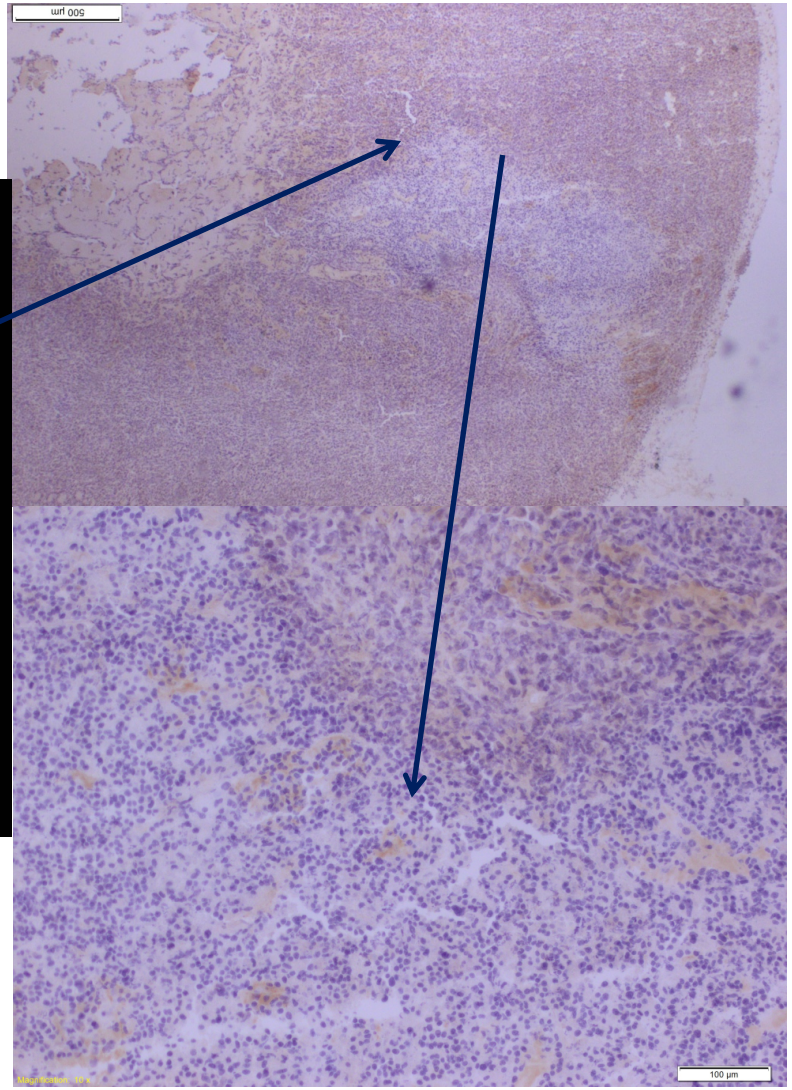
Microbiology (chemical communication)



IMS of the interaction between B. subtilis (Bs) and S. aureus (Sa). (a) Zone of inhibition; (b) T-shape experiment; (c) converse of (b); (d) B. subtilis alone; (e) S. aureus alone. Ion distributions are represented by colour: surfactin (SF; green), plipastatin (PS;magenta), PSMl (red) and PSMa3 (yellow).Arrows indicate areas of toxin suppression. (Dorrestein and al Microbiology **(2011),** 157, **2485–2492**)

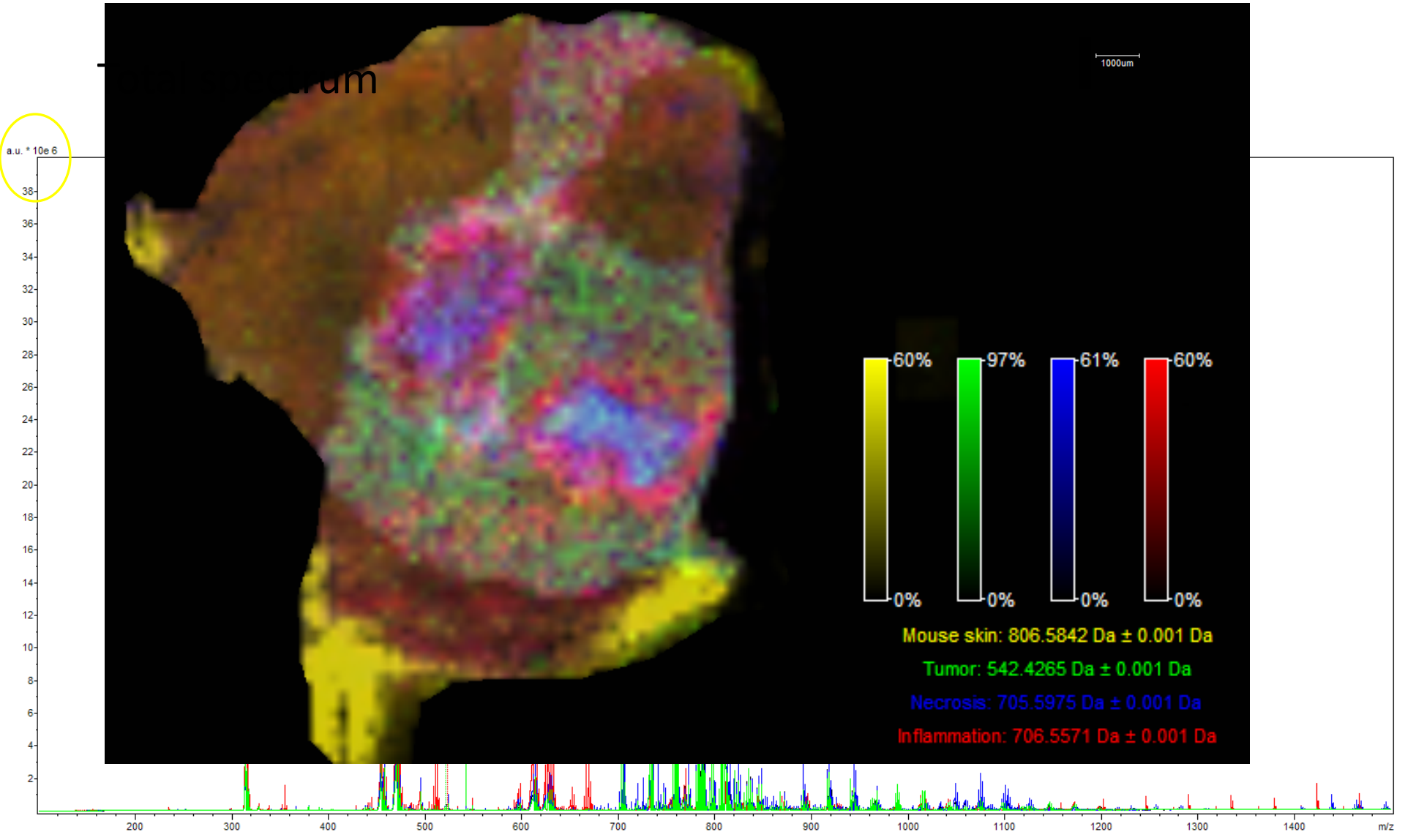
Molecular histology



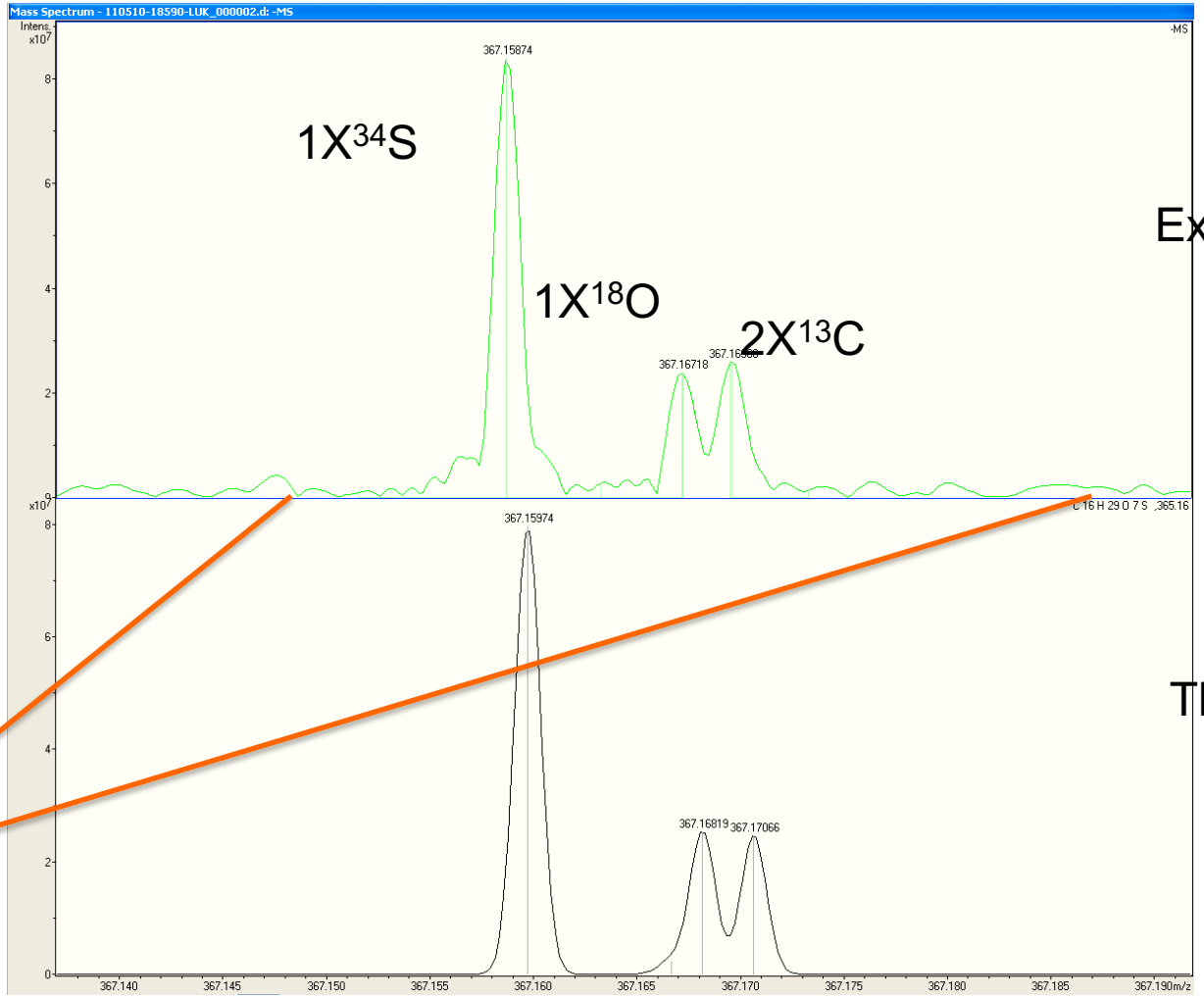


705,59 m/z (necrosis) and 706,55 m/z (inflammation) ?

Molecular histology



High resolution and exact mass



Expériment

Isotopes @ M+2

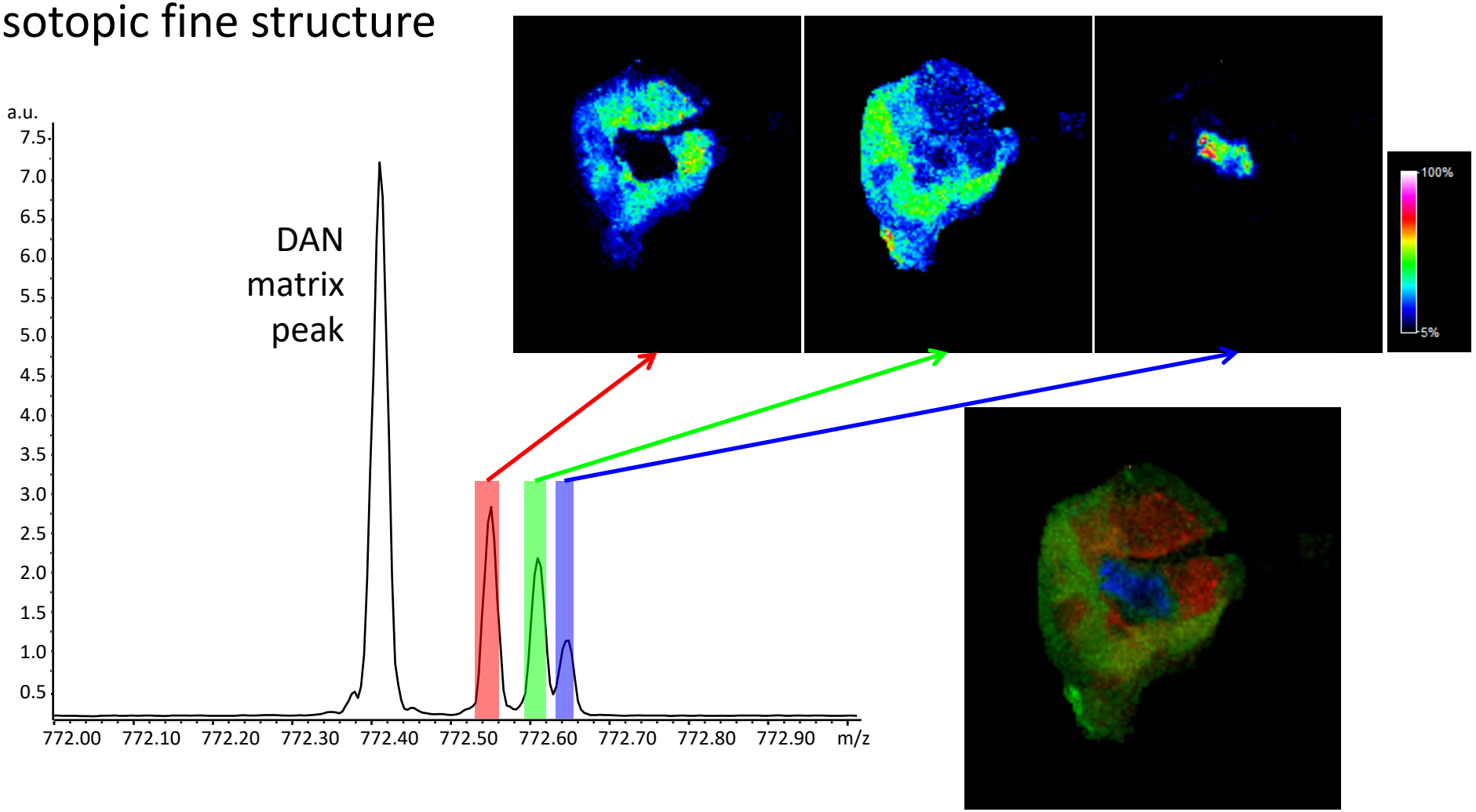
MH^+

Theory

What FTICR-MS Brings to MALDI IMS

Higher mass resolution

Isotopic fine structure

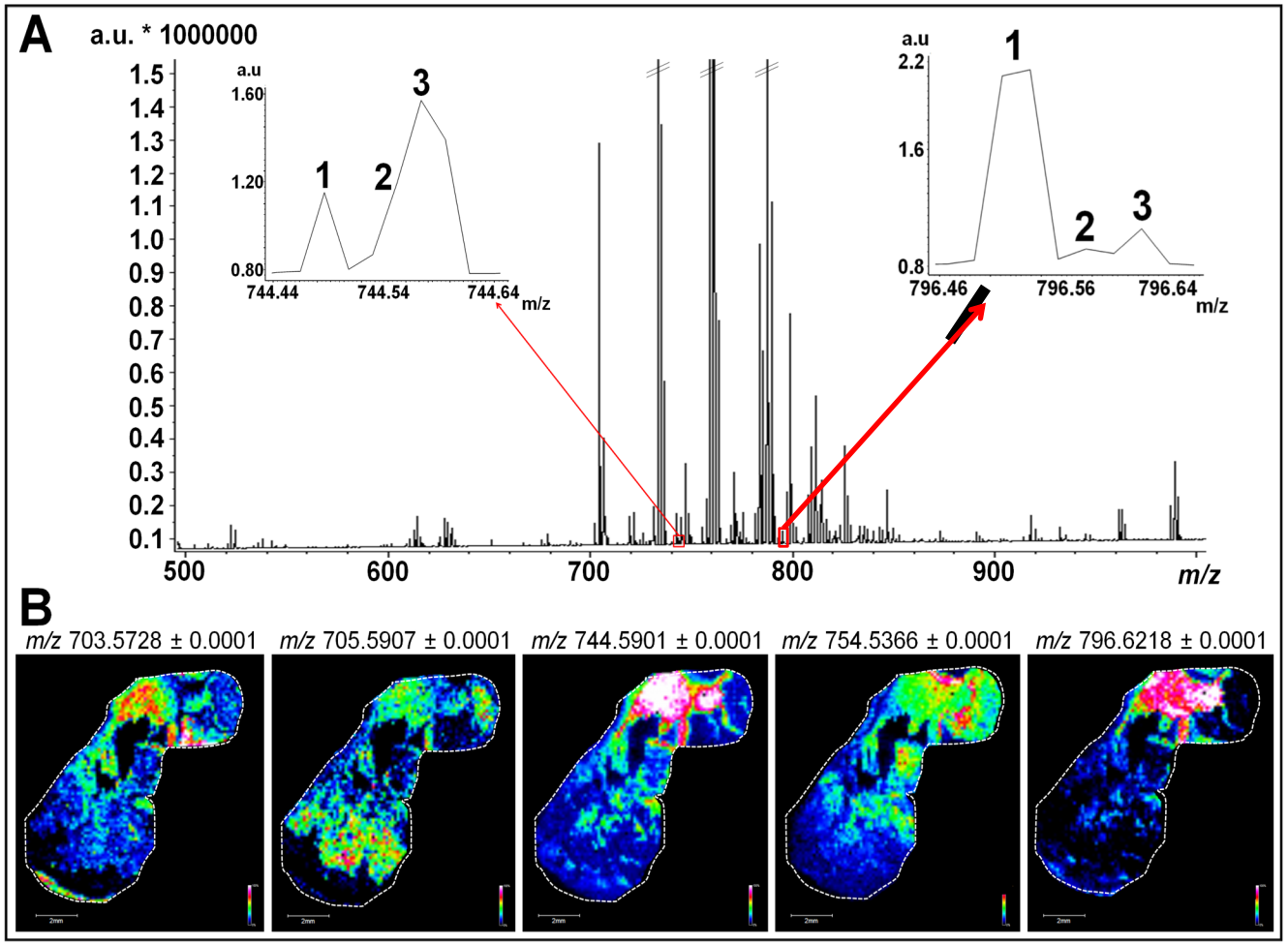


Lipidomics of Low-Abundant Species for Exploring Tumor Heterogeneity Guided by High-Resolution Mass Spectrometry Imaging

J. Cimino, D. Calligaris, J. Far, D. Debois, S. Blacher, N E. Sounni, A. Noel and E. De Pauw

doi:10.3390/ijms141224560

1- MALDI Imaging acquisition in broadband detection mode



Broadband average mass spectrum of a simultaneous MALDI MSI analysis on sections of tumors derived from MCF-7, MDA-MB-231 and MDA-MB-435 cells.

MALDI MSI ion image representing the repartition of five low-abundant PLs) in a MDA-MB-435 tissue section.

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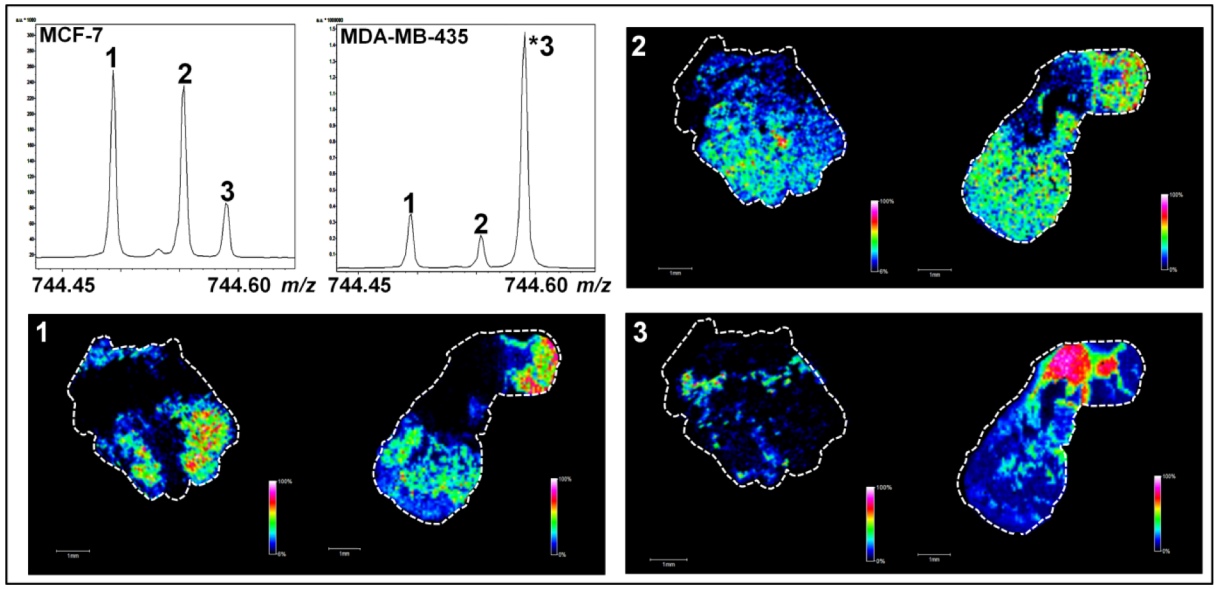
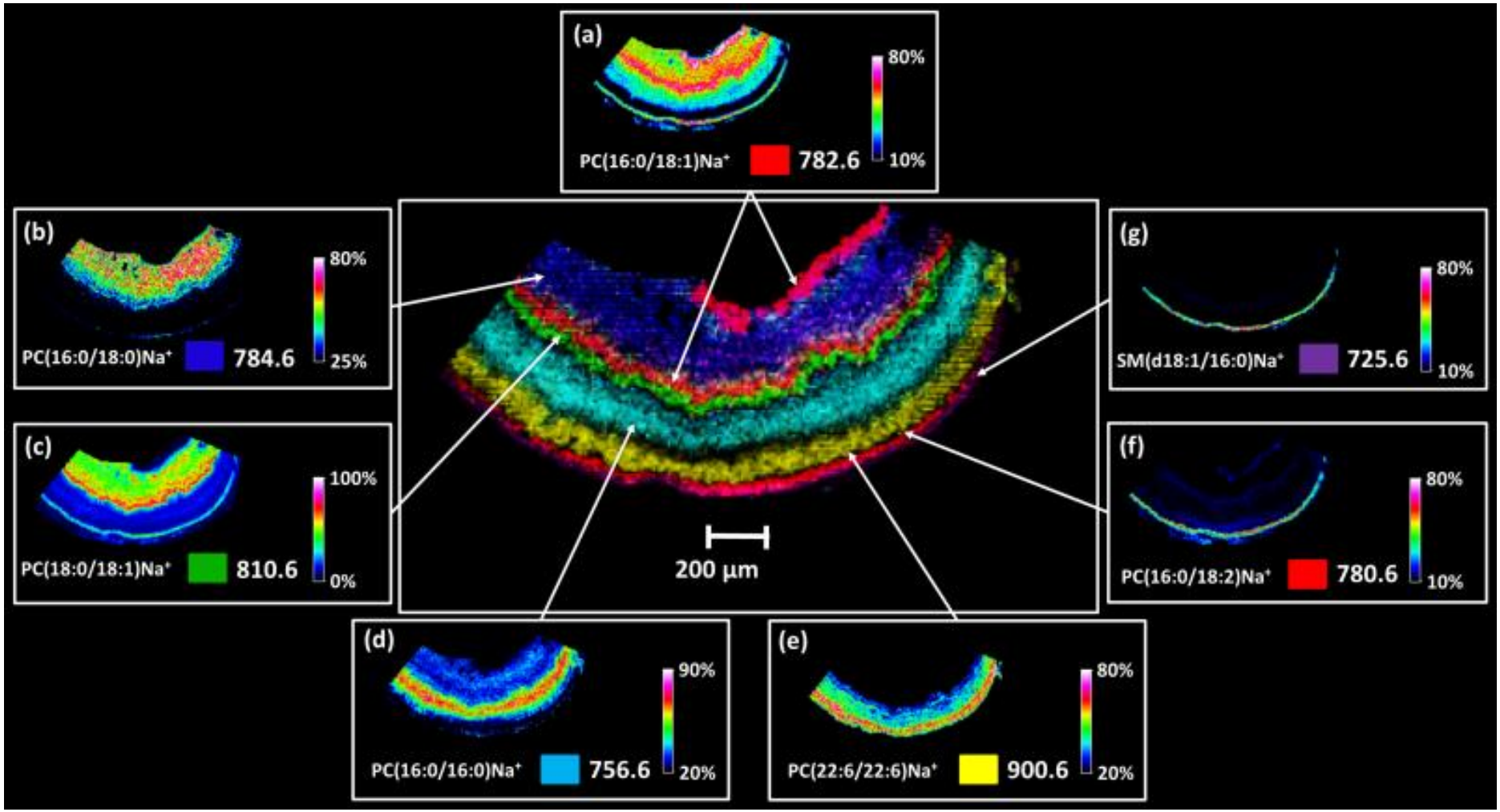
EU FT-ICR MS

2- MALDI Imaging acquisitions in narrowband detection mode

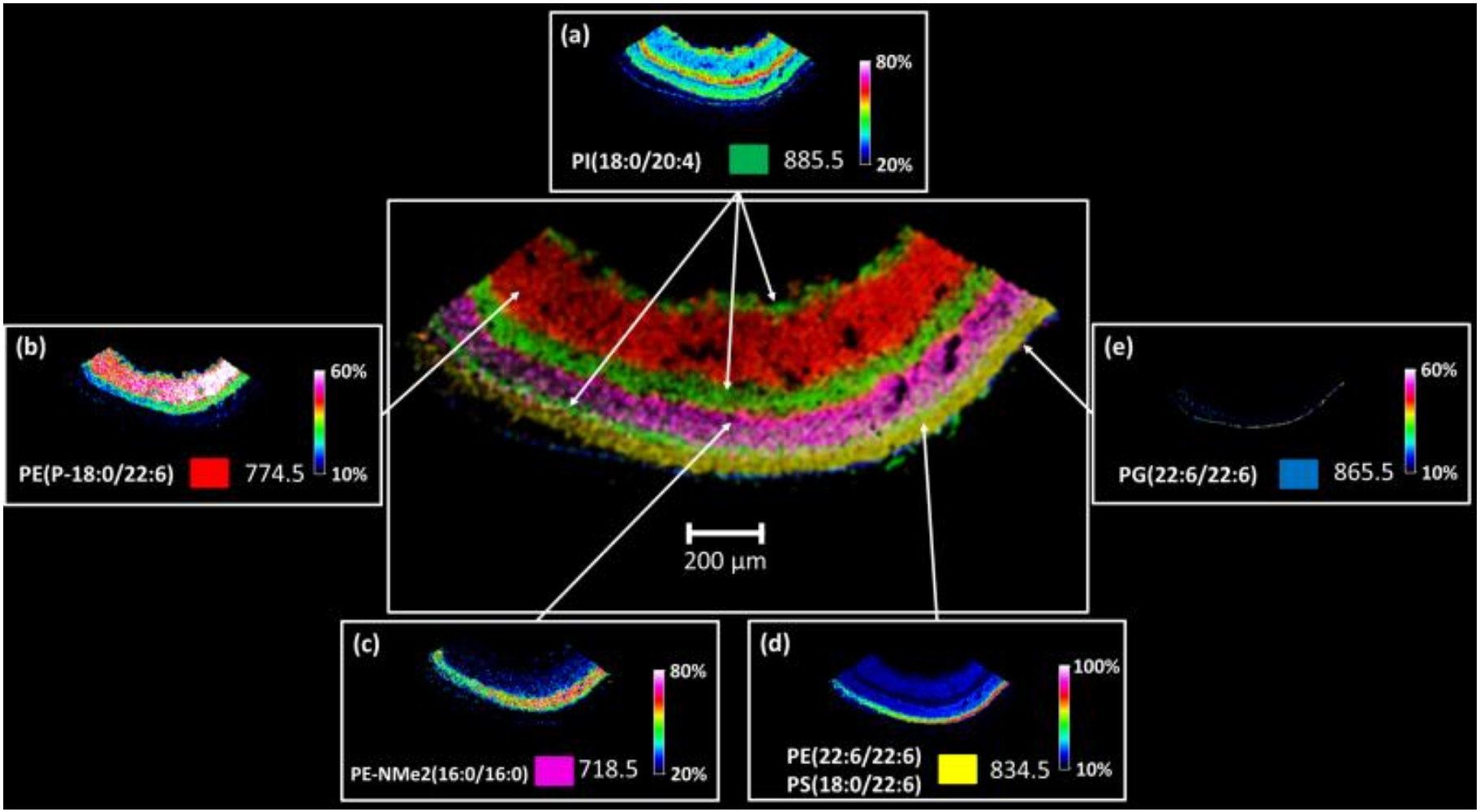


m/z_{obs}	Elemental formula	m/z_{th}	Error (ppm)	
744.49419	$C_{38}H_{76}NO_8P$ ($[M+K]^+$)	744.49401	0.24	**12 PC/11 PE !!**
744.55385	$C_{41}H_{79}NO_8P$ ($[M+H]^+$)	744.55378	0.09	**12 PC/17 PE !!**
744.59019	$C_{42}H_{83}NO_7P$ ($[M+H]^+$)	744.59017	0.04	**11 PC/3 PE !!**

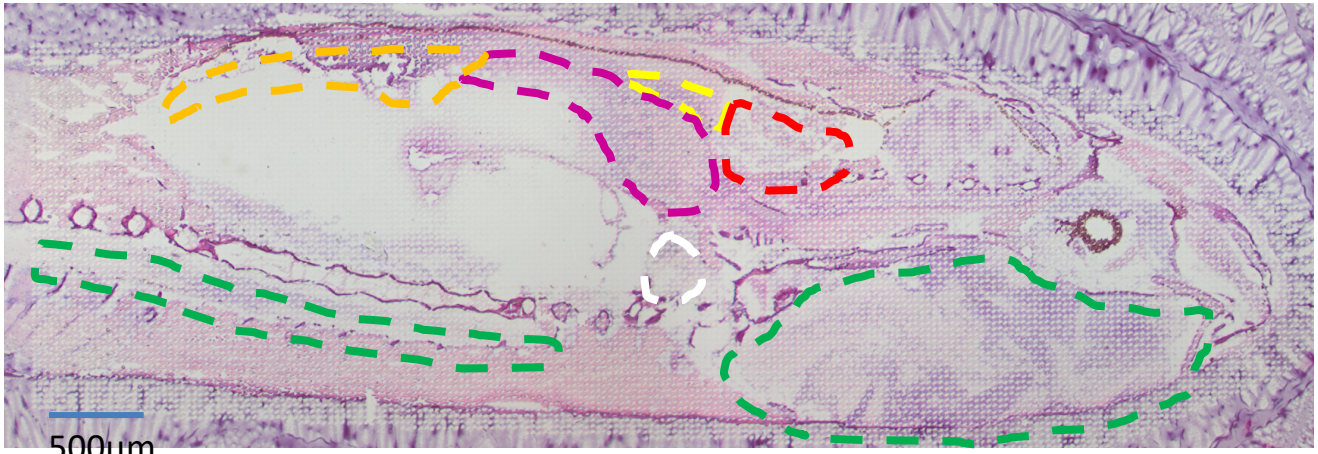
Fine structure of structure of organs base on specific lipids positive mode (From Spengler J Am Soc Mass Spectrom. 2014 Aug; 25(8): 1394–1403.)



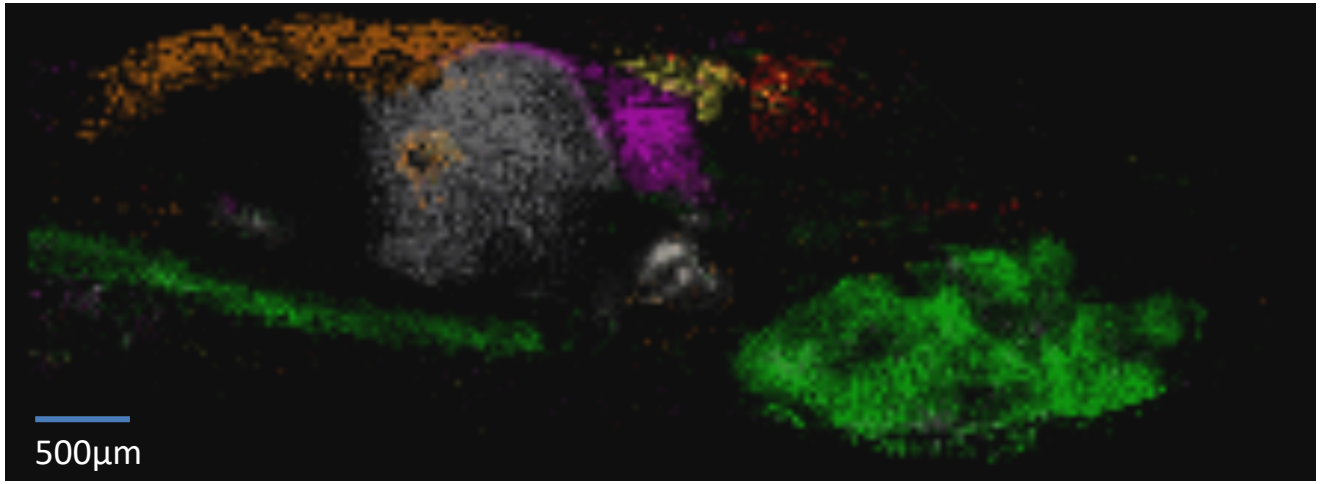
Fine structure of structure of organs base on specific lipids negative mode (From Spengler J Am Soc Mass Spectrom. 2014 Aug; 25(8): 1394–1403.)



Whole zebrafish organs visualization with specific ions

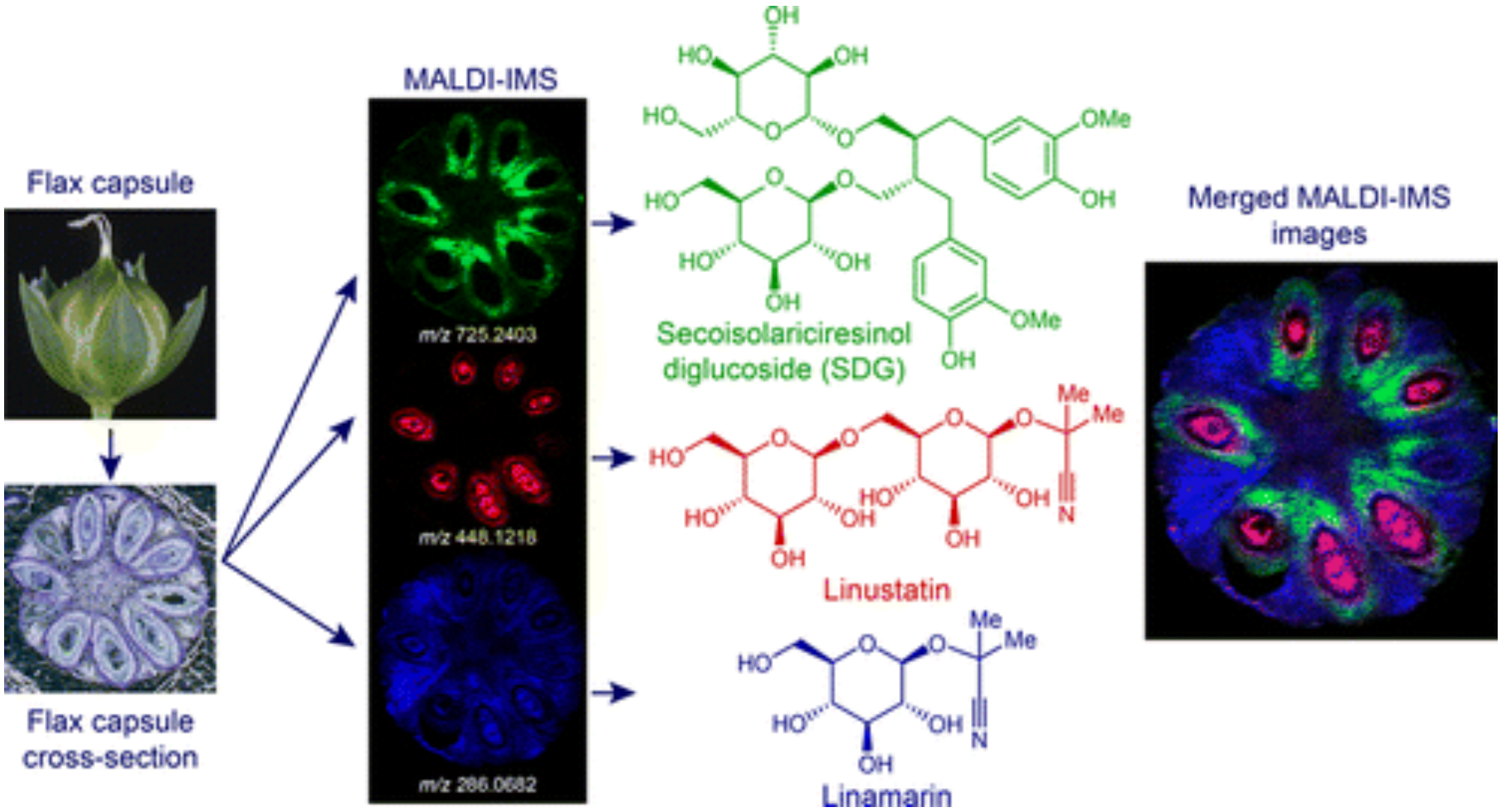


histological staining of the slice used for MSI.



organ-specific *m/z* localizations selected with a 0.001% mass range. 549.2546 (gall bladder - uncertain); 571.4811 (intestine); 587.562 (kidney); 601.0739 (liver); 732.9773 (heart); 789.40617; 880.4465 (intestinal bulb).

Metabolites distributions



Doralyn S. Dalisay and al, J. Nat. Prod. 78, 6, 1231-1242

Large molecules identificaion

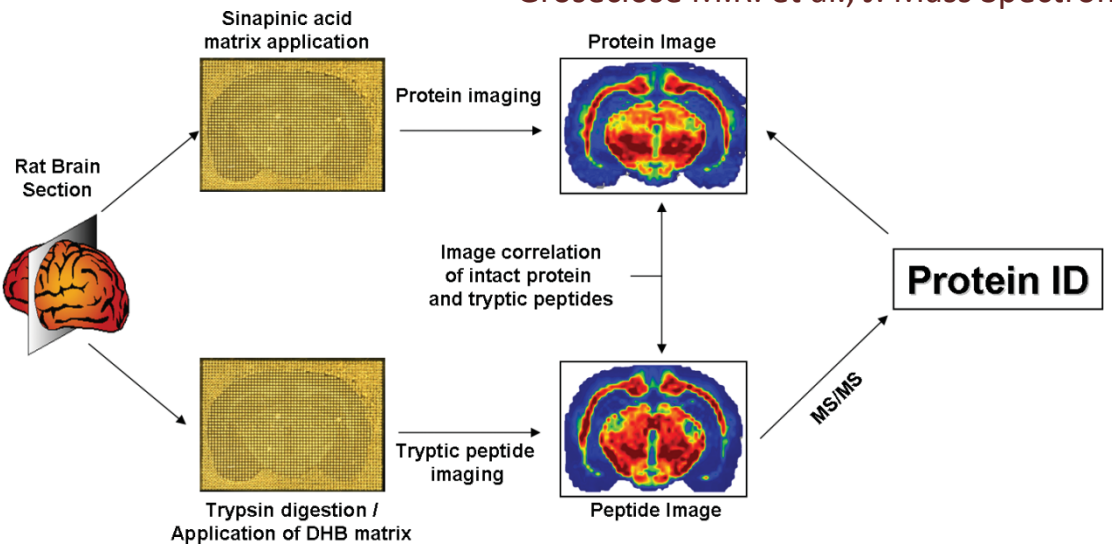
- Two strategies
  - In situ identification
  - Microsampling: laser ablation, lasermicrodissection of region of interest and microproteomics. The ROI can also be based on small molecules

Longuespée, R., Alberts, D., Pottier, C., Smargiasso, N., Mazzucchelli, G., Baiwir, D., Kriegsmann, M., Herfs, M., Kriegsmann, J., Delvenne, P., & De Pauw, E. (2016). A laser microdissection-based workflow for FFPE tissue microproteomics: important considerations for small sample processing. Methods, 104, 154-162.

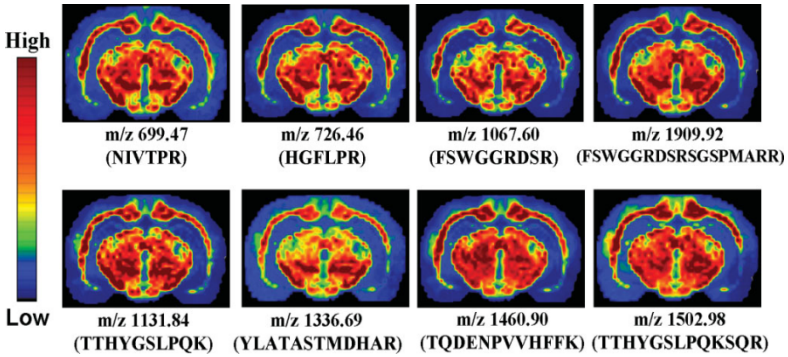
In situ digestion

Identification of proteins directly from tissue: in situ tryptic digestions coupled with imaging mass spectrometry

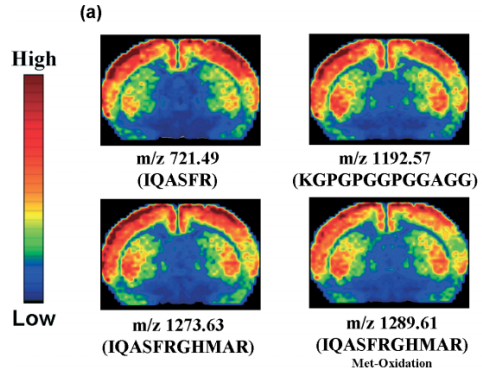
Groseclose M.R. et al., J. Mass Spectrom.,2007,42:254-262

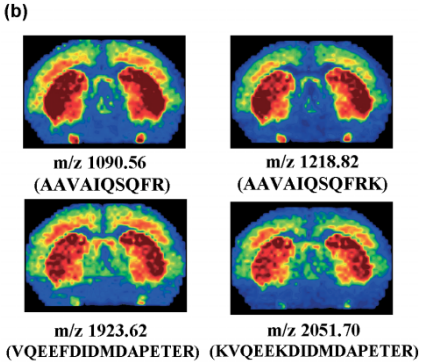


- Myelin Basic Protein (14211 Da)



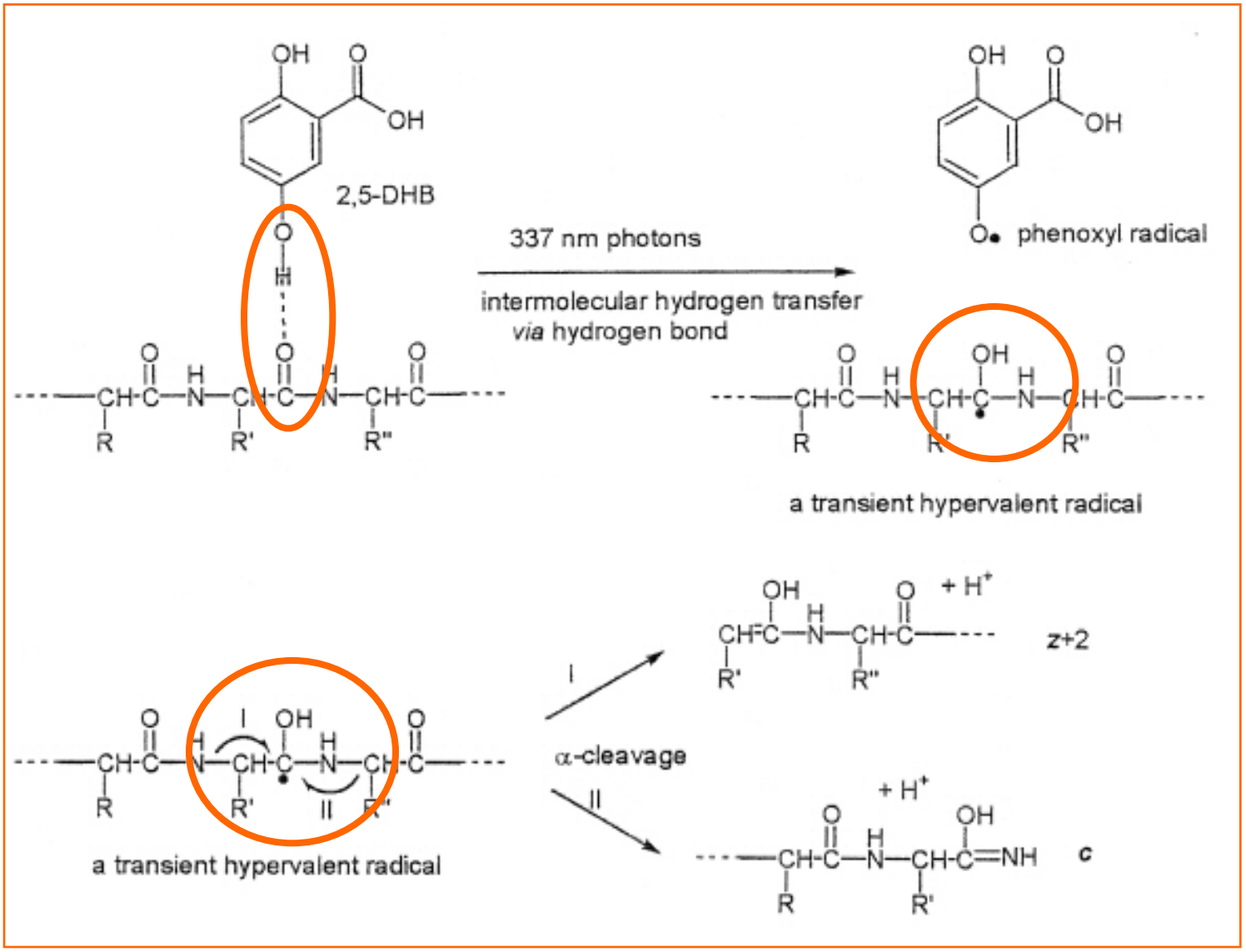
- Brain-specific polypeptide (PEP-19)





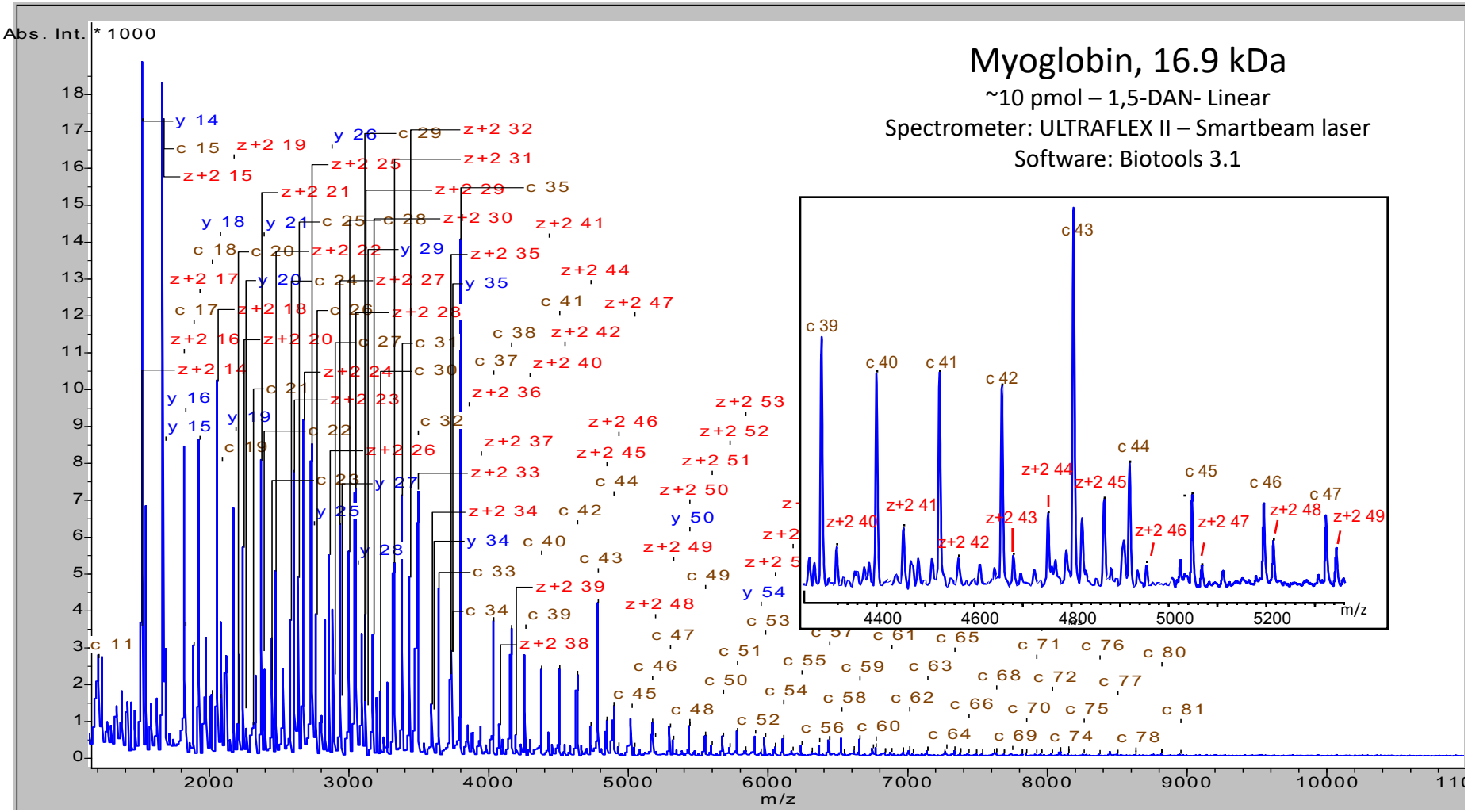
- Neurogranine

Chemically induced In-Source Decay



Takayama M., 2001, JASMS, 12, 420-427.

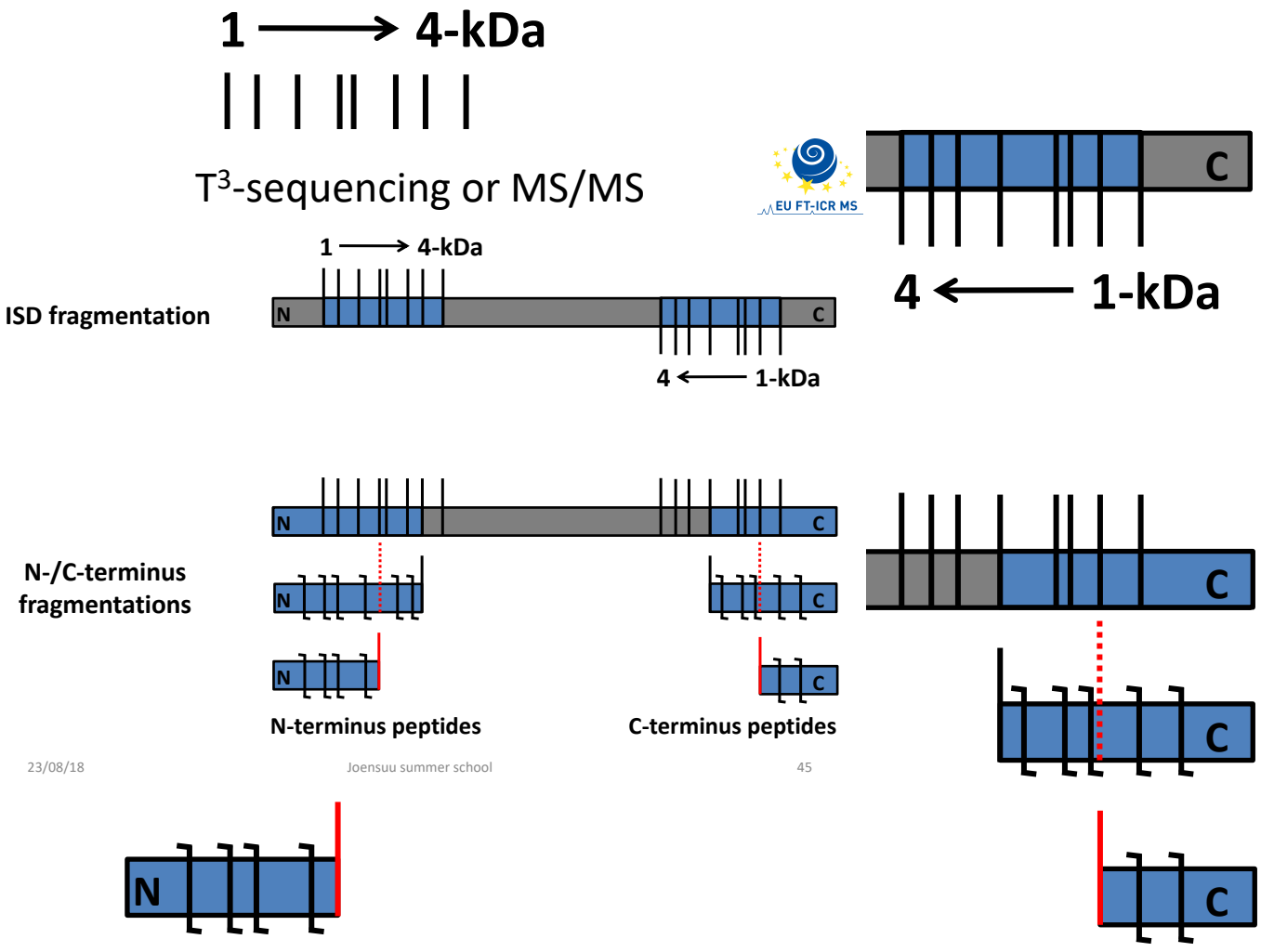
ISD top down sequencing using c- and z- ions



Large series of c- and z-ions are effectively generated allowing TOP-DOWN sequencing

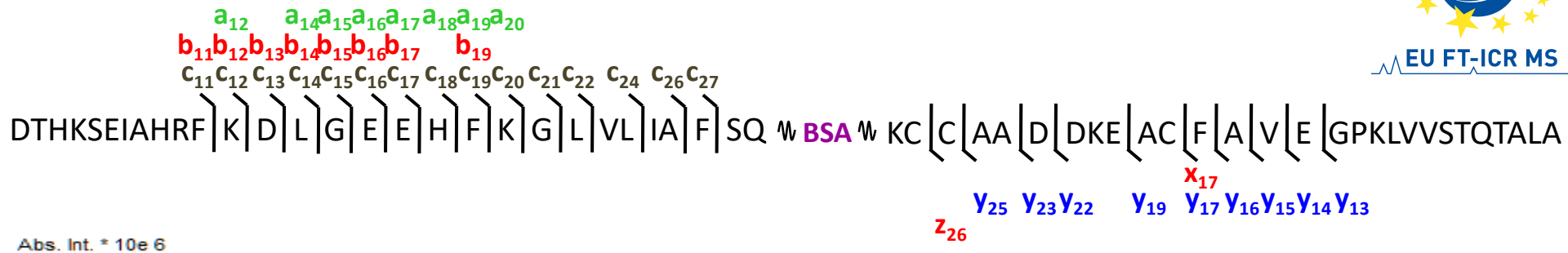
T^3-sequencing or MS/MS

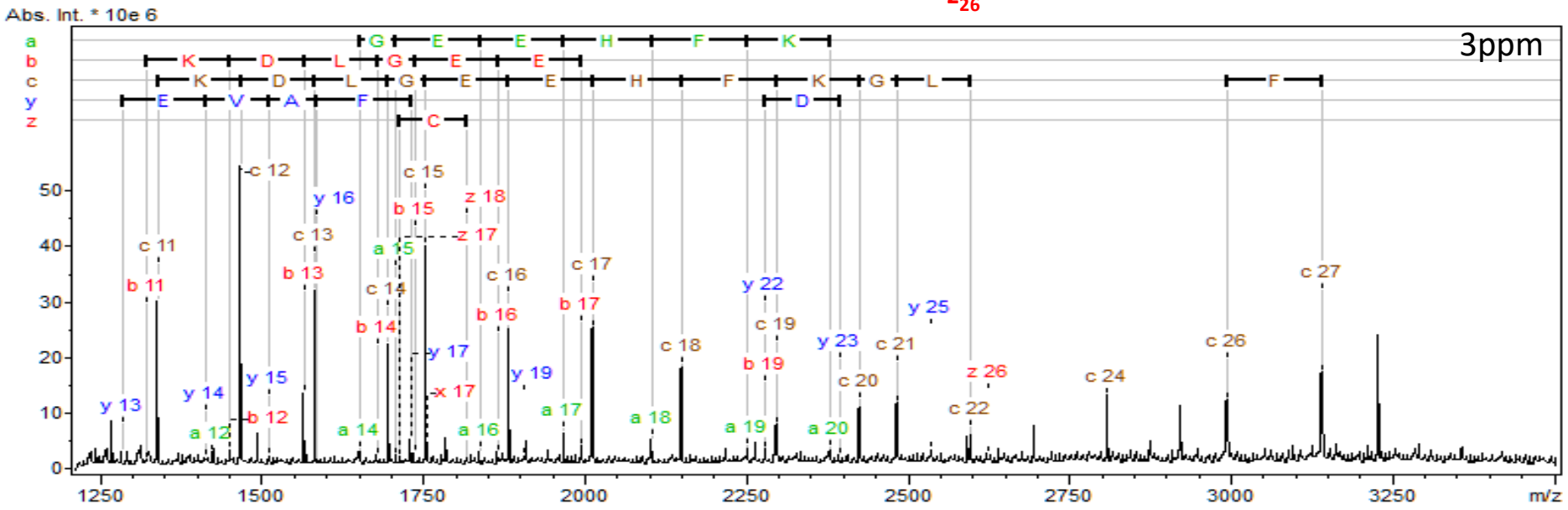
ISD fragmentatio



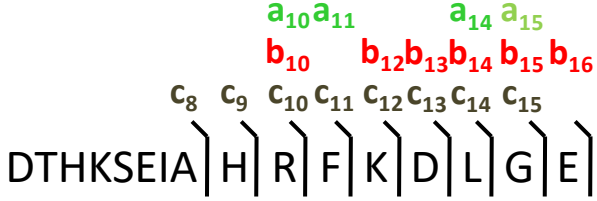
N-/C-terminus fragmentations

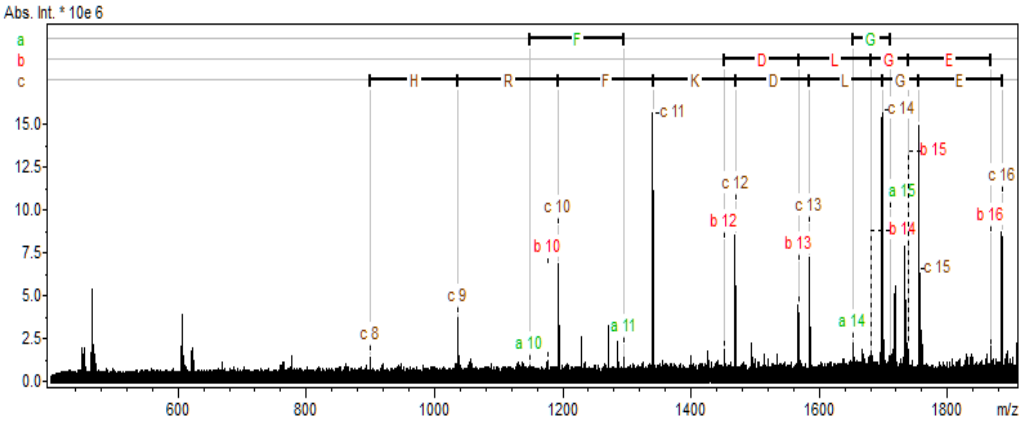
MALDI-ISD of Pure Protein Solution





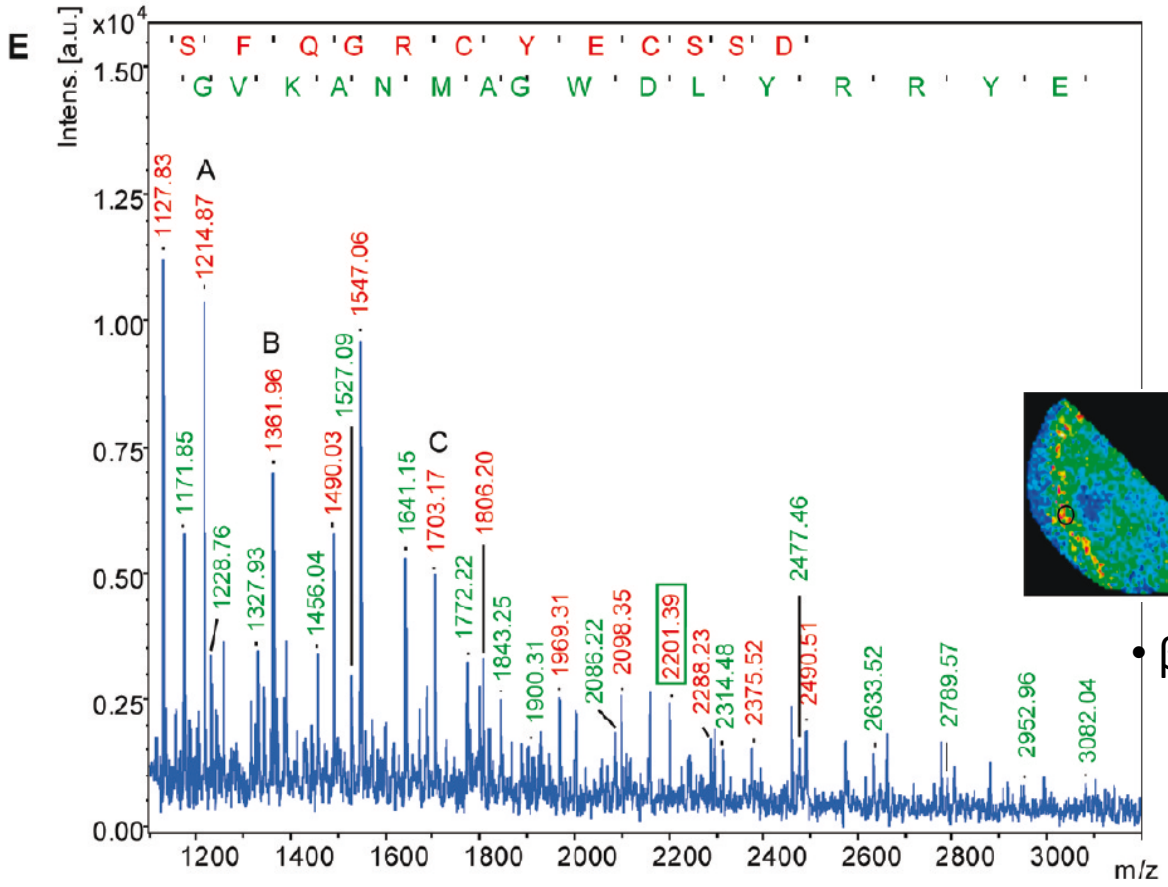
MS/MS c_{16} fragment ion (m/z 1882.94 Da)



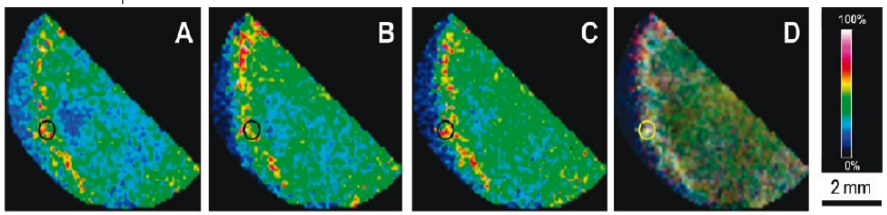


Proteins identification : *in situ* top-down ISD

MALDI – In Source Decay Applied to Mass Spectrometry Imaging : A new toll for protein identification
Debois D. et al., Anal. Chem.,2010,82(10):4036-4045



- Direct β-cristallin B2 identification by MALDI-ISD



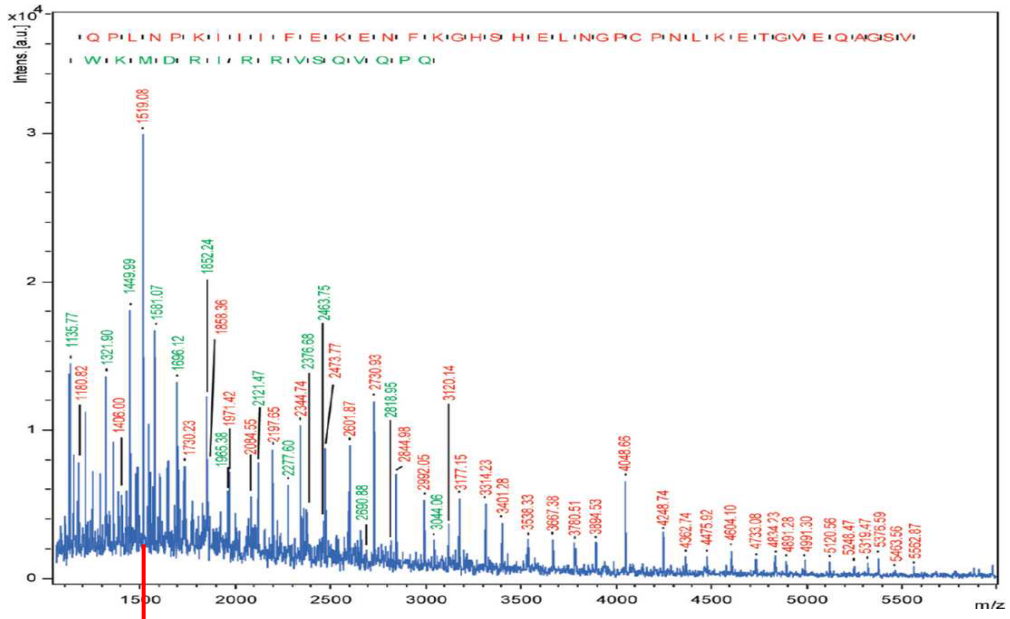
- β-cristallin B2 localization in eye lens

Possibility to identify proteins by ISD
without tissue slice treatment (use of matrix such as 1,5 DAN) and selection of precursor ion

β-cristallin B2 T³-sequencing

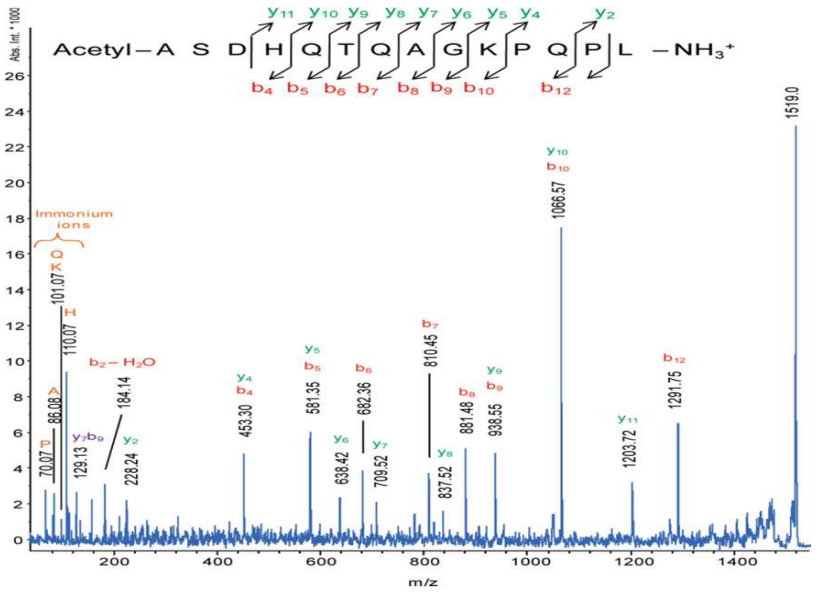
MALDI – In Source Decay Applied to Mass Spectrometry Imaging : A new toll for protein identification
Debois D. et al., Anal. Chem.,2010,82(10):4036-4045

EU FT-ICR MS



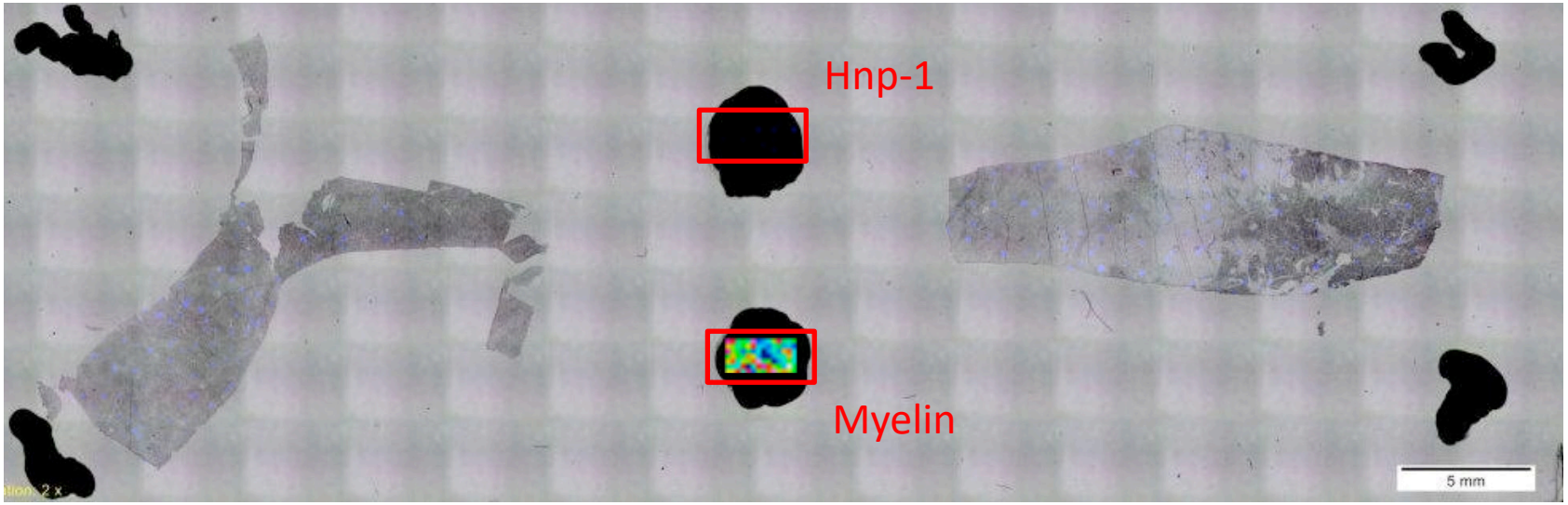
ISD spectrum

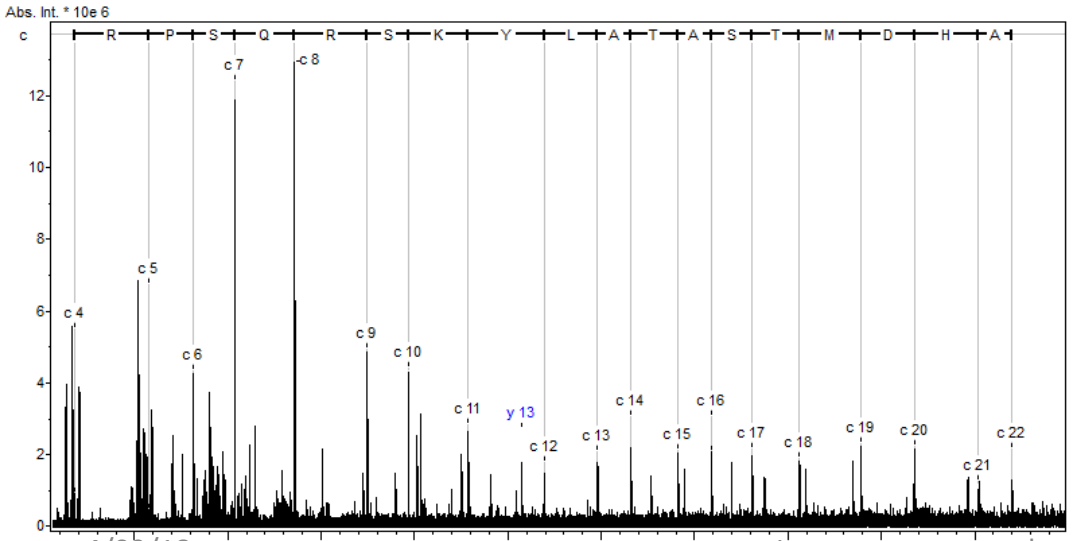
T³ Sequencing

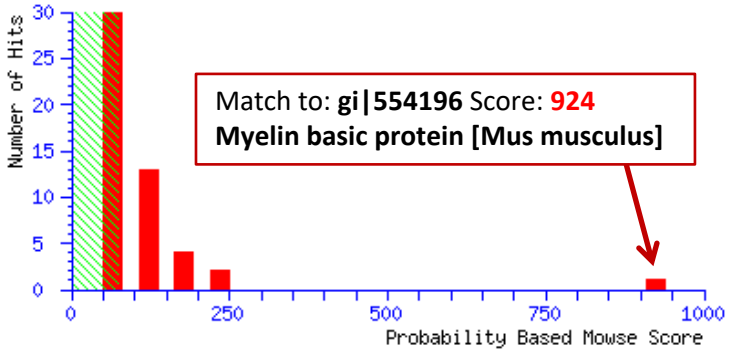


N-Terminal characterization of β-cristallin B2 by T³ sequencing

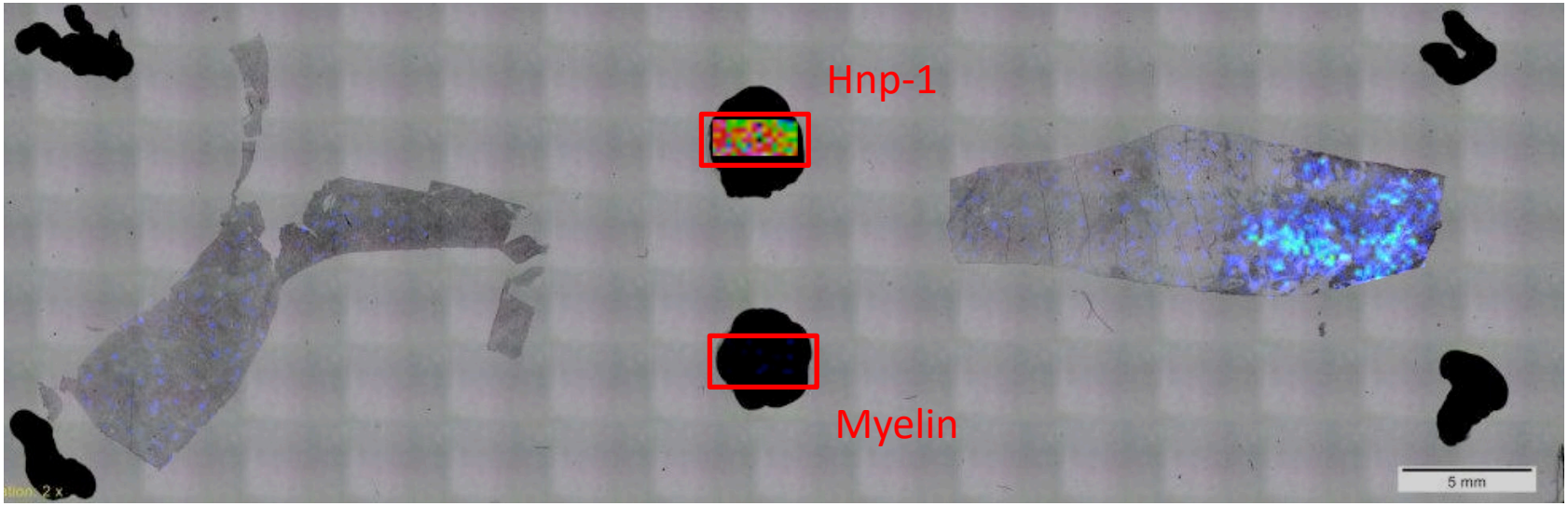
Proteins identification by localization and MALDI-ISD profile matching

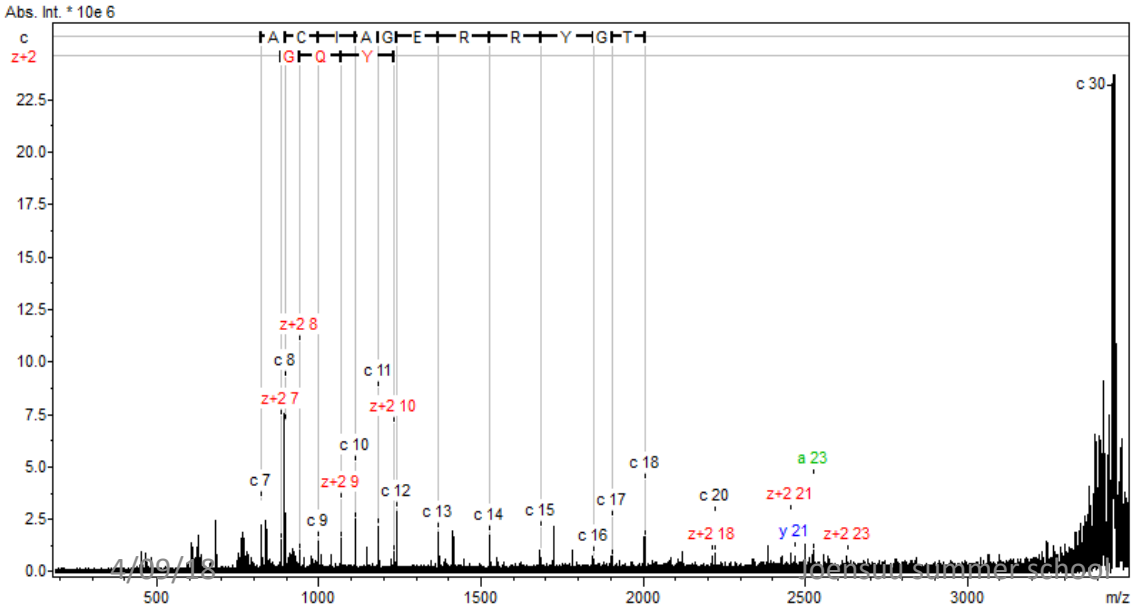


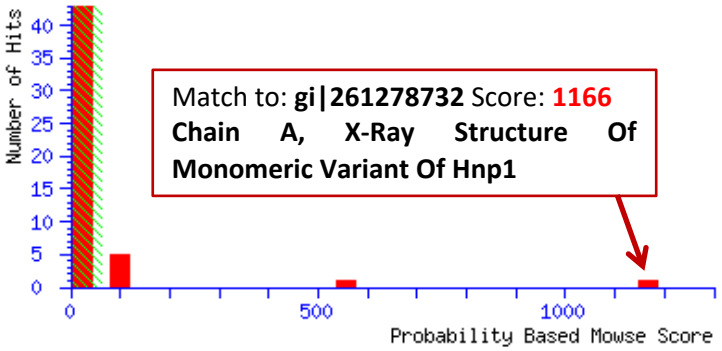




Proteins identification by localization and MALDI-ISD profile matching



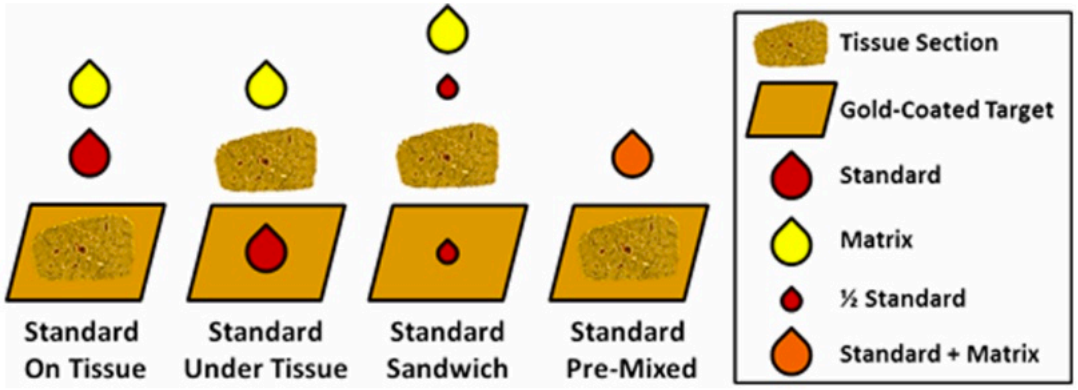




Quantification

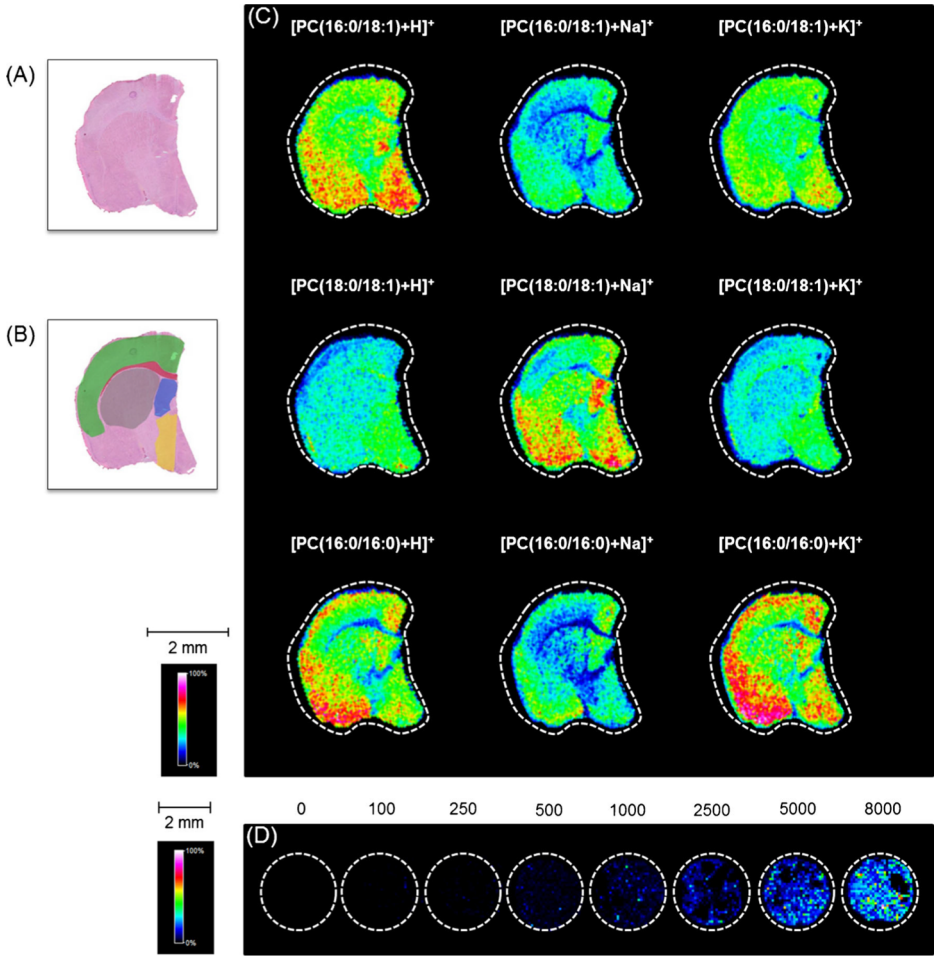
- Suppression effects
- Adducts
- Matrix interactions

But, we have MS, working wity standards



FromCaprioli: Anal.Chem. 2016, 88, 2392−2398

Spiked Tissue method

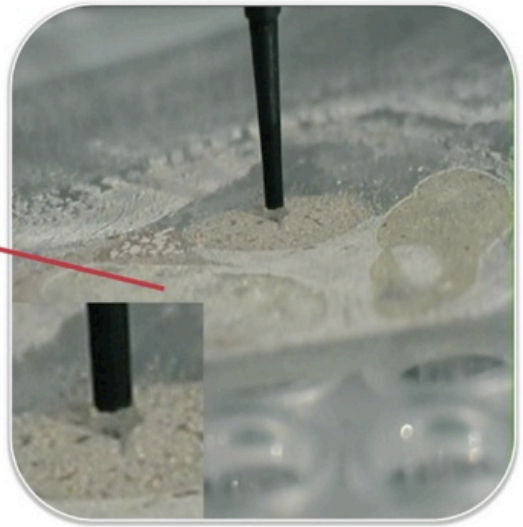


Normalization against a similar tissue spiked

Jadoul, Anal Bioanal Chem (2015) 407:2095–2106

In situ sampling (and LESA system)

LESA kit (software, custom solvent reservoirs, special sample plate, lightbox and TriVersa NanoMate)



Surface sampling of thin tissue section via LESA and the TriVersa NanoMate

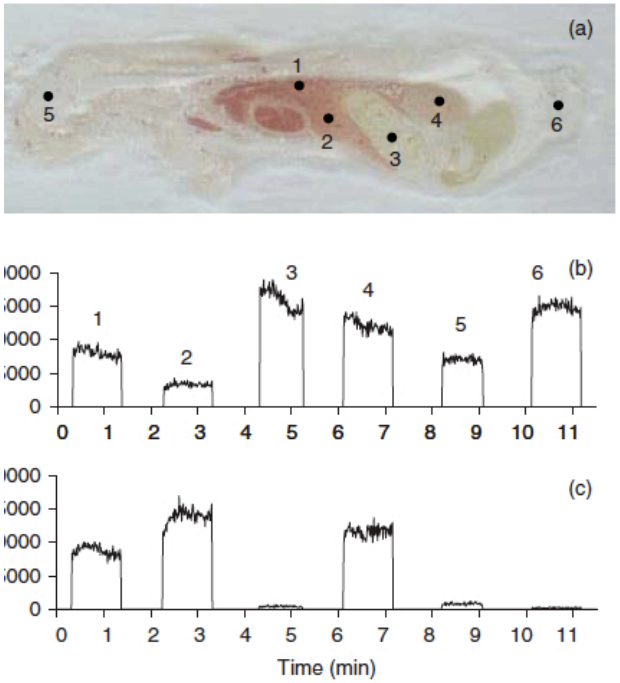


Figure 5. (a) Photograph of a propranolol dosed mouse (7.5 mg/kg, I.V. dosed, sacrificed 1 h after dose) whole-body thin tissue section showing six discrete points analyzed using the robotic nanospray system. Signal levels for (b) propranolol (*m/z* 260 → 183) and (c) hydroxypropranolol glucuronide (*m/z* 452 → 276) were recorded during 1-min spraying of samples taken at each point. Dwell time was 50 ms for each transition monitored. Extraction solvent was 80/20/0.1 (v/v/v) ACN/water/FA. Aspirated and dispensed solvent volumes were 3 and 2 µl, respectively, and aspirated sample volume was 2 µl. Sampling locations: 1 = lung; 2 = liver; 3 = stomach/contents; 4 = kidney; 5 = brain; 6 = muscle.

Injection in ESI

NanoHPLC possible

Trapped ion mobility

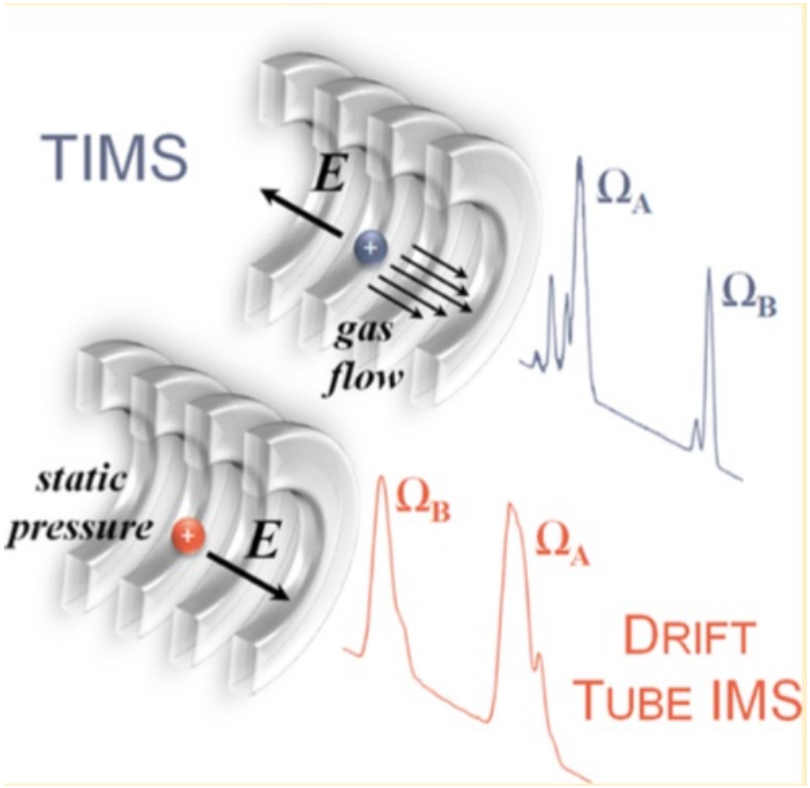
Concept: stopping the ions according to their cross section, time scale compatible with FTICR

Experimental differences

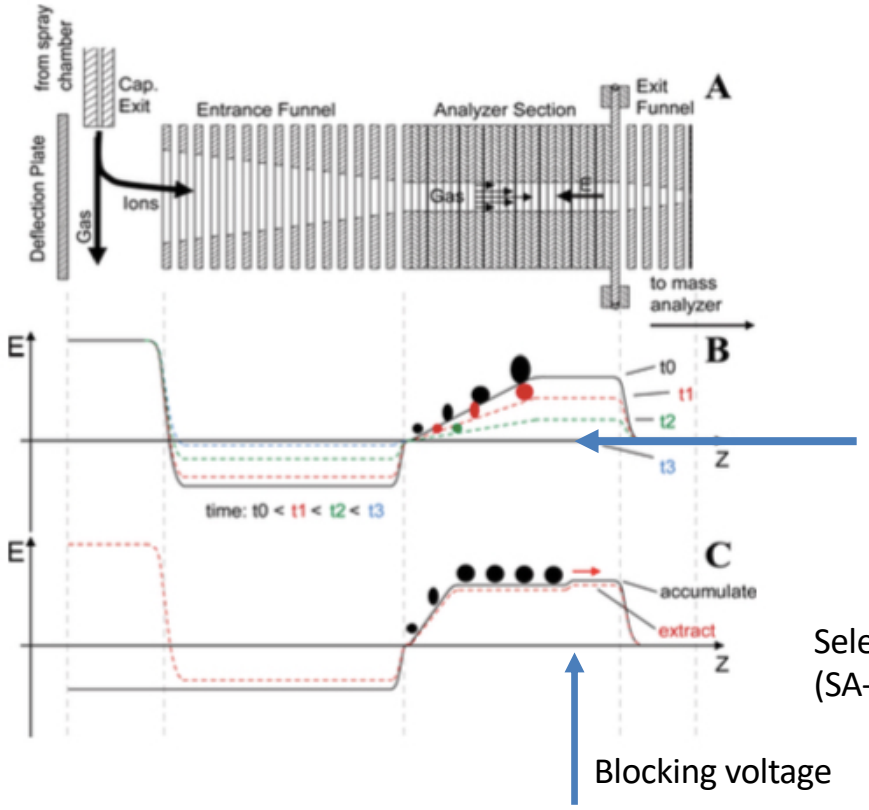
- Constant field, static gas drift tube
- Wave type field, static gas TWAVE
- Increasing field, gas counterflow Trapped ion mobility

Basics of TIMS

The concept



Basics of TIMS



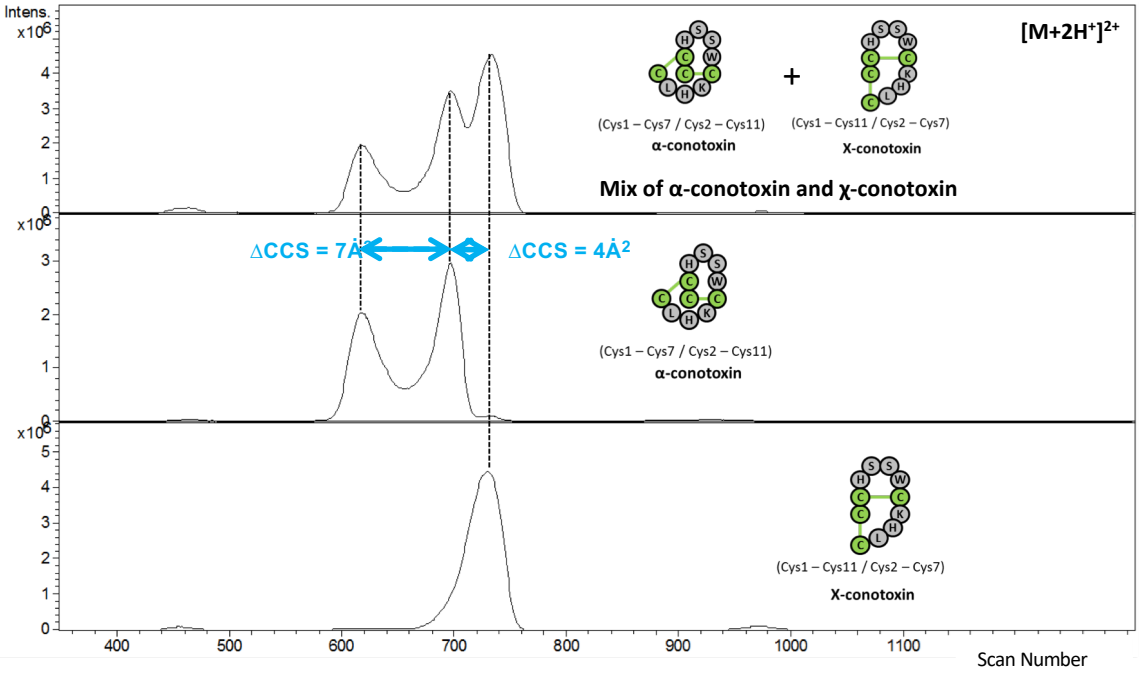
v_{drift} =K E

$v_{drift} + v_{gas} = 0$

Selected accumulationTIMS (SA-TIMS) or SAIMS

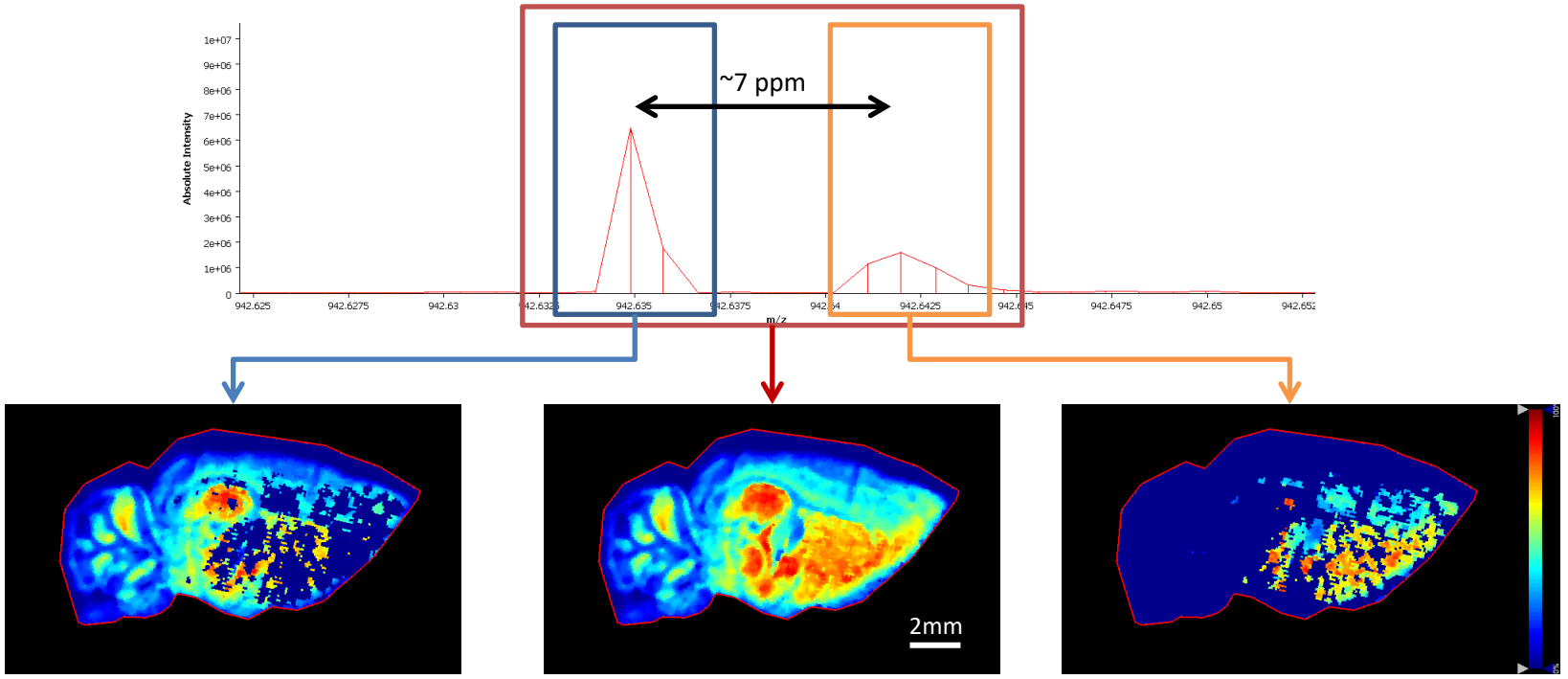
Blocking voltage

Ion Mobility TIMS (Bruker) Conotoxins



Large improvment in resolution

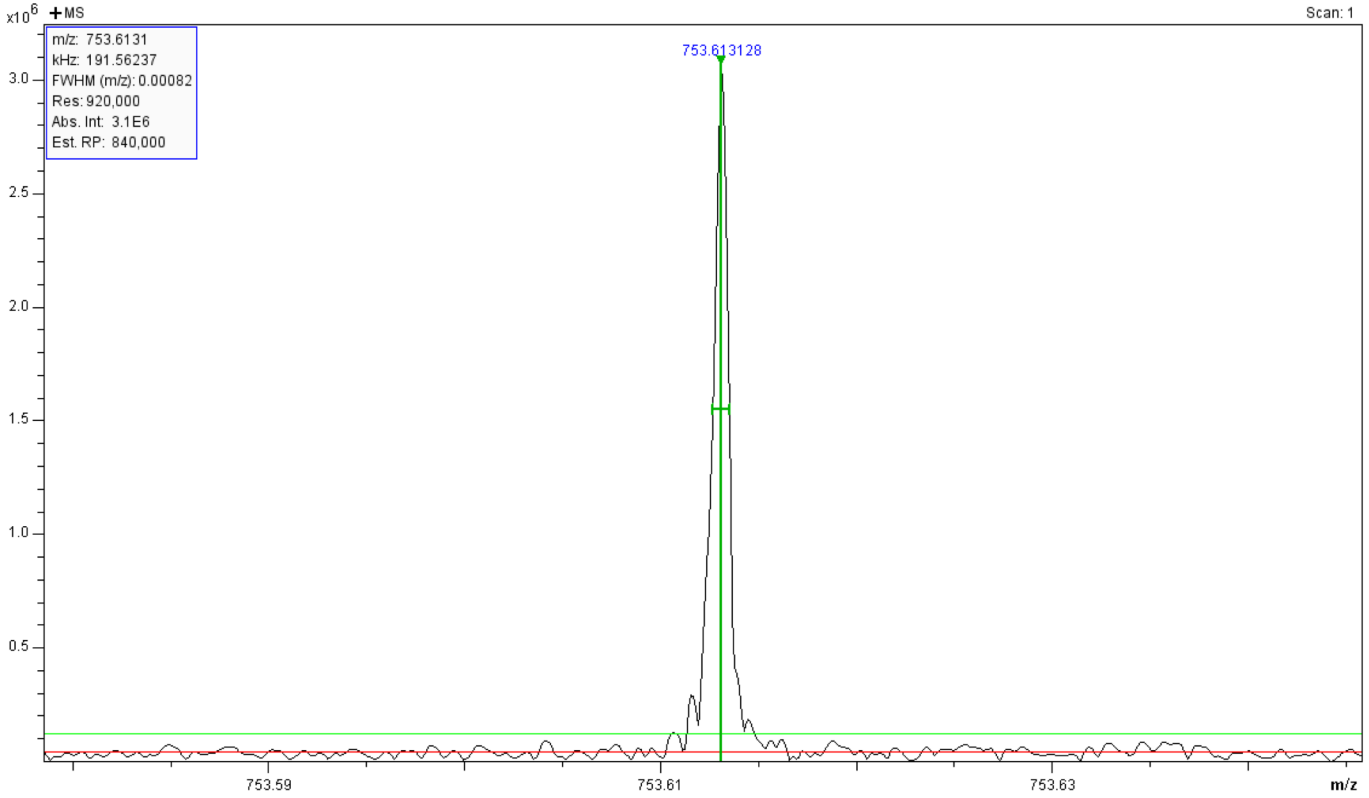
Problems with HiRes imaging: Mass shift in MSI



In this example the two peaks shown correspond in reality to only one ion. As it can be seen, images from the blue and orange windows do match together to create the image generated by the red window. Thus those signal should be combined.

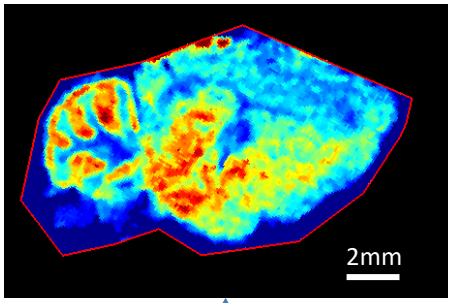
Control of the TIC fluctuations

- Method tested on lipid standards
  - Mass accuracy : 0,32ppm (ref. : 753,61337 *m/z*)
  - Peak resolution : 920.000



Mass shift in MSI

- A new acquisition method adapted to MSI solves the mass shift



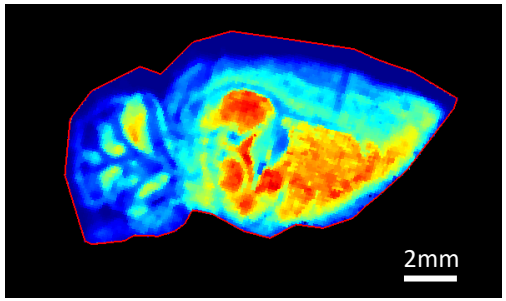
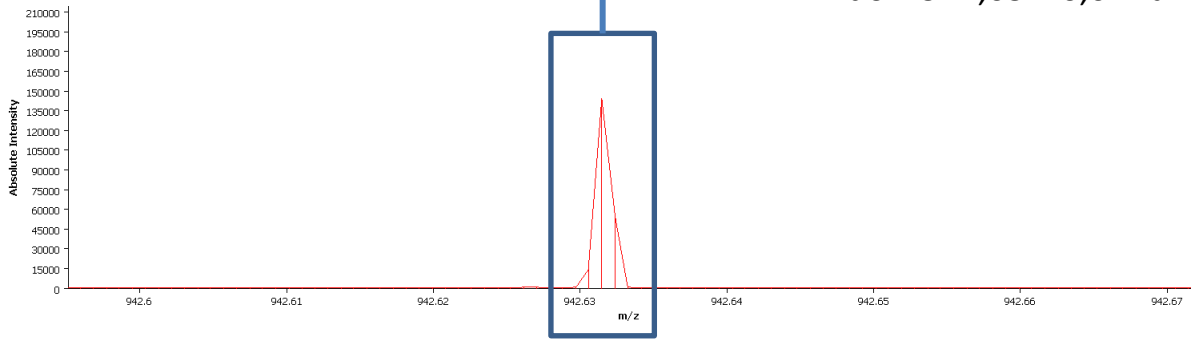


Image from the « classic » method
Window 942,63 ± 0,02Da



Thanks to

- Mathieu Tiquet
- Delphine Debois
- Remi Longuespée
- Deborah Alberts

- Johan Far
- Christopher Kune
- Jean Hallert

- Gabriel Mazuchelli
- Nicolas Smargiasso
- Dominique Baiwir

- The FNRS
- The EU
- Interreg
- The Walloon region
- The University of Liege
- The “constructors”