AP-MALDI HRMS analyses and imaging at LIST

MASSTECH's European Demo and Application Lab

06/06/2019

Gilles Frache

LUXEMBOURG
INSTITUTE OF SCIENCE
AND TECHNOLOGY
LIST

LUXEMBOURG INSTITUTE OF SCIENCE AND TECHNOLOGY LIST

- LIST

  1. AP-MALDI coupled to LTQ/Orbitrap

  2. AP-MALDI HRMS analyses (applications and workflows)

  3. AP-MALDI HRMS imaging (applications and workflows)

- Conclusions & Take home messages:

LUXEMBOURG INSTITUTE OF SCIENCE AND TECHNOLOGY (LIST)

- RTO of 600+ employees, 80+ PhD students, 40 nationalities
- Three main RDI departments:
  - Environmental Research and Innovation (ERIN)
  - IT for Innovative Services (ITIS)
  - Materials Research and Technology (MRT)
    - 4 Main RDI activities:
      - Nanomaterials/Nanotechnology
      - Bio-based & Functional Polymers
      - Scientific Instrumentation & Coatings Process Engineering
      - Composites
    - One Transversal Platform: Materials Characterization, Testing, Prototyping and Composites manufacturing

→ European Application & Demo lab

ANALYTICAL IMAGING AT LIST

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From micro- to nano- scale

Molecular vibration
Crystal structure
Polymorphism

Topography, roughness evaluation,
local electrical properties

Molecular structures
Larger molecules

Molecular fragments
Small molecules

Trace elements
Isotopic enrichment

Topography,
morphology
Elemental composition

Correlative
SE+SIMS
imaging

100µm 10µm 1µm 500nm 50nm 2nm 1nm 0.5nm

MALDI TOF

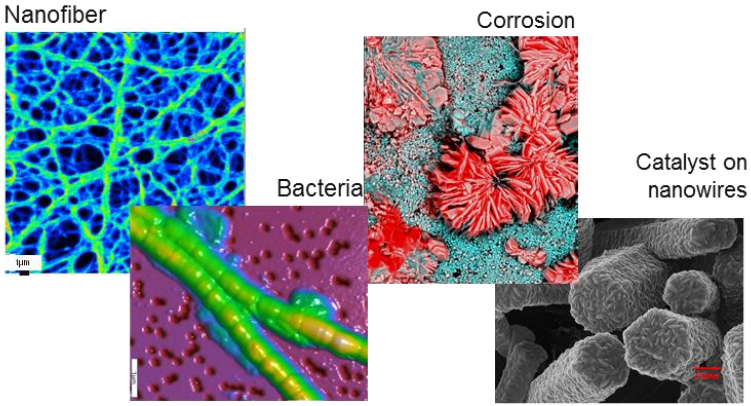
TOFSIMS imaging

Nano SIMS

AFM

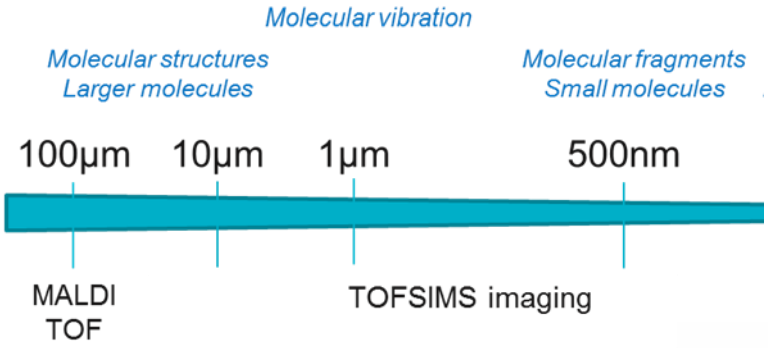
Electron and helium ion microscopy

Molecular imaging

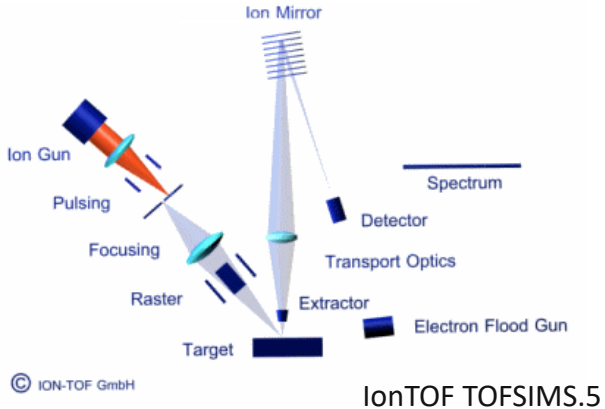


ANALYTICAL IMAGING AT LIST

Molecular imaging



- Pulsed primary ion gun focused down to 400nm
- Very fast TOF analyser (kHz)
- No sample preparation

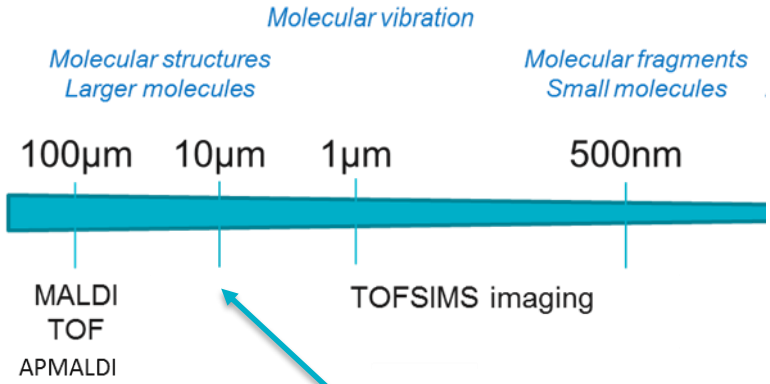


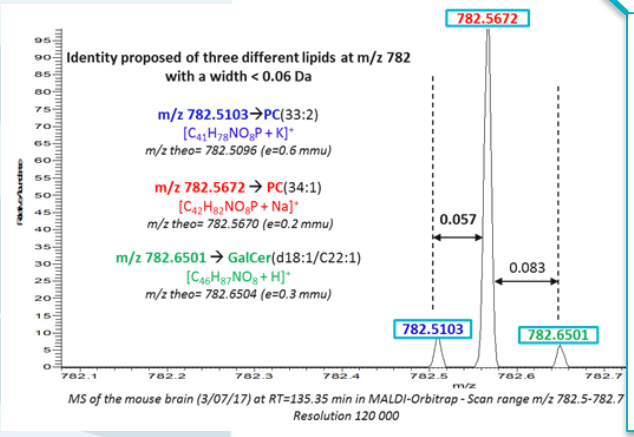
IonTOF TOFSIMS.5

- Strong ionization (high fragmentation rate)
- Moderate mass resolution (unreliable assignments of m/z signals)

ANALYTICAL IMAGING AT LIST

Molecular imaging





AP-MALDI Imaging with high resolution in mass and space

- Softer Ionization → MALDI
- Reliable identification
  - Exact mass
  - Structural analysis

→ LTQ/Orbitrap

- Keep LC/HRMS capability

→ Masstech AP-MALDI PDF+ (2012)

- AP-MALDI Imaging capability?

→ Masstech AP-MALDI PDF+ (2012)

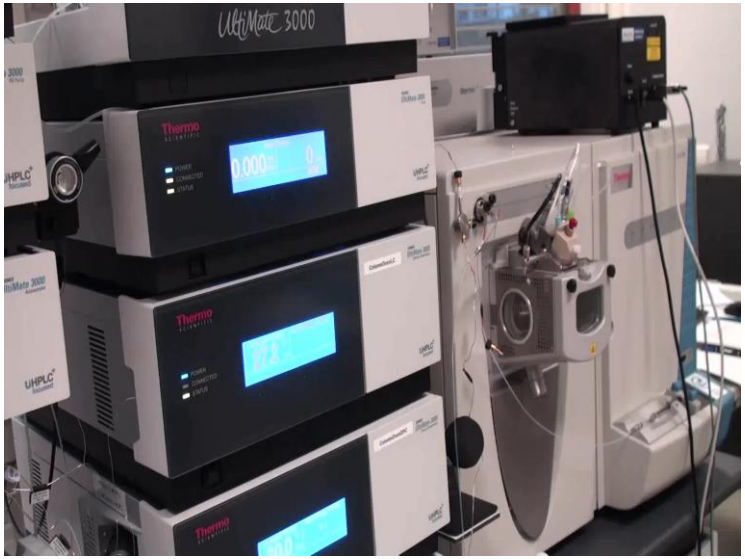
→ **Masstech AP-MALDI (ng) UHR**

AP-MALDI HRMS

- Benefits of LTQ/Orbitrap :
  - Hybrid mass spectrometer : 2 analyzers
    - Full scan Orbitrap → accurate (but slow)
    - Full scan LTQ → faster (but less accurate)
    - SIM, SRM, → targeted analysis
    - MS/MS, DDA, DIA, → highly informative!
  - AP interface (multiple ionization techniques (ESI, APCI, nanospray, DART…)

- Benefits of AP-MALDI :
  - Flexibility
  - Performances

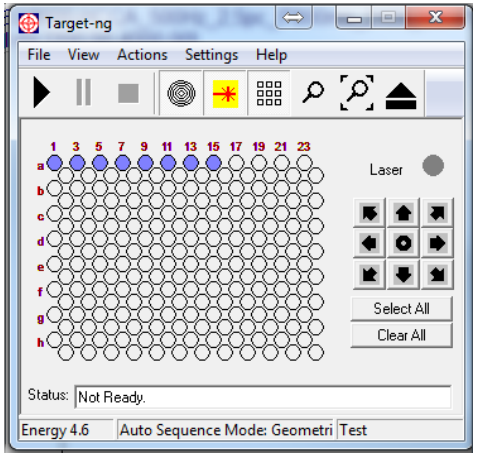
→MALDI analysis and imaging with HRMS analyzer



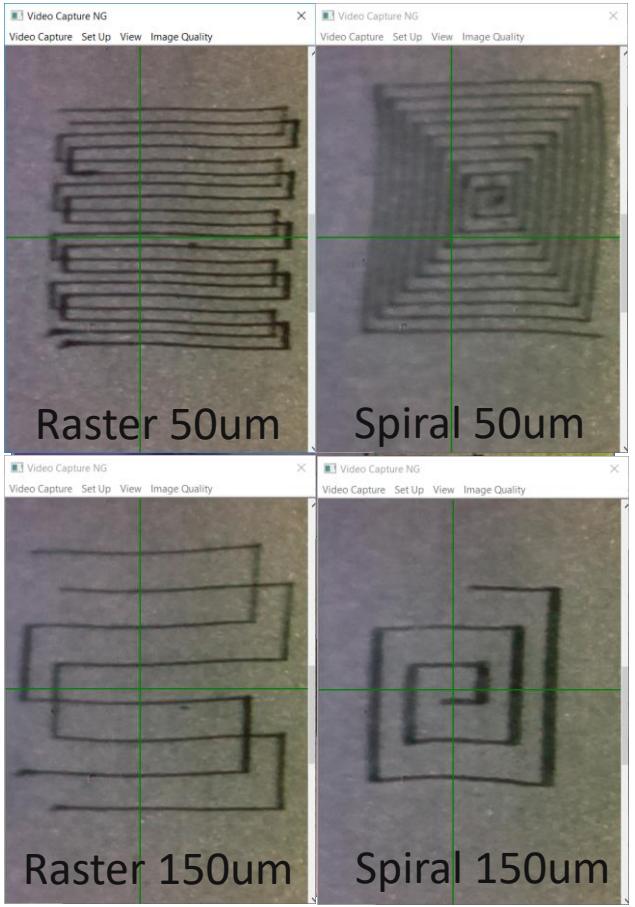
AP-MALDI HRMS ANALYSES

Software: Data acquisition

- Masstech Target®



→ Spiral or raster motion with tuneable parameters
→ Tuneable Laser frequency and Energy
→ Irradiation of each sample spot with internal or external timing with tuneable duration



AP-MALDI HRMS ANALYSES

Software: Data acquisition

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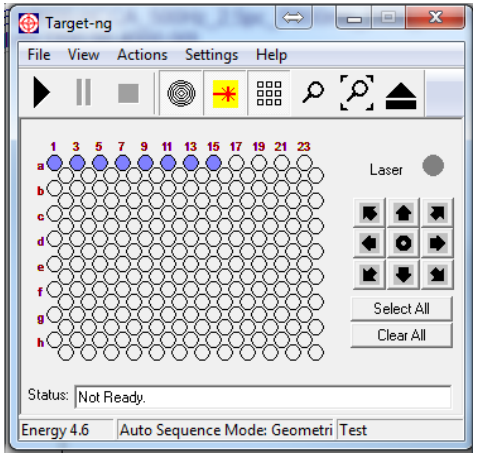

→ Spiral or raster motion with tuneable parameters
→ Tuneable Laser frequency and Energy
→ Irradiation of each sample spot with internal or external timing with tuneable duration

- Thermo Xcalibur:
  - Selection of the analyzer (LTQ or Orbitrap)
  - Single or multiple scan events (full scan, SIM, SRM) or method (DDA, DIA)
  - Mass range
  - Acquisition started
    - Either from Tune window (all sample spectra in one RAW file)
    - Or from Xcalibur sequence editor (each sample spectrum in one RAW file)

AP-MALDI HRMS ANALYSES

Applications : peptides

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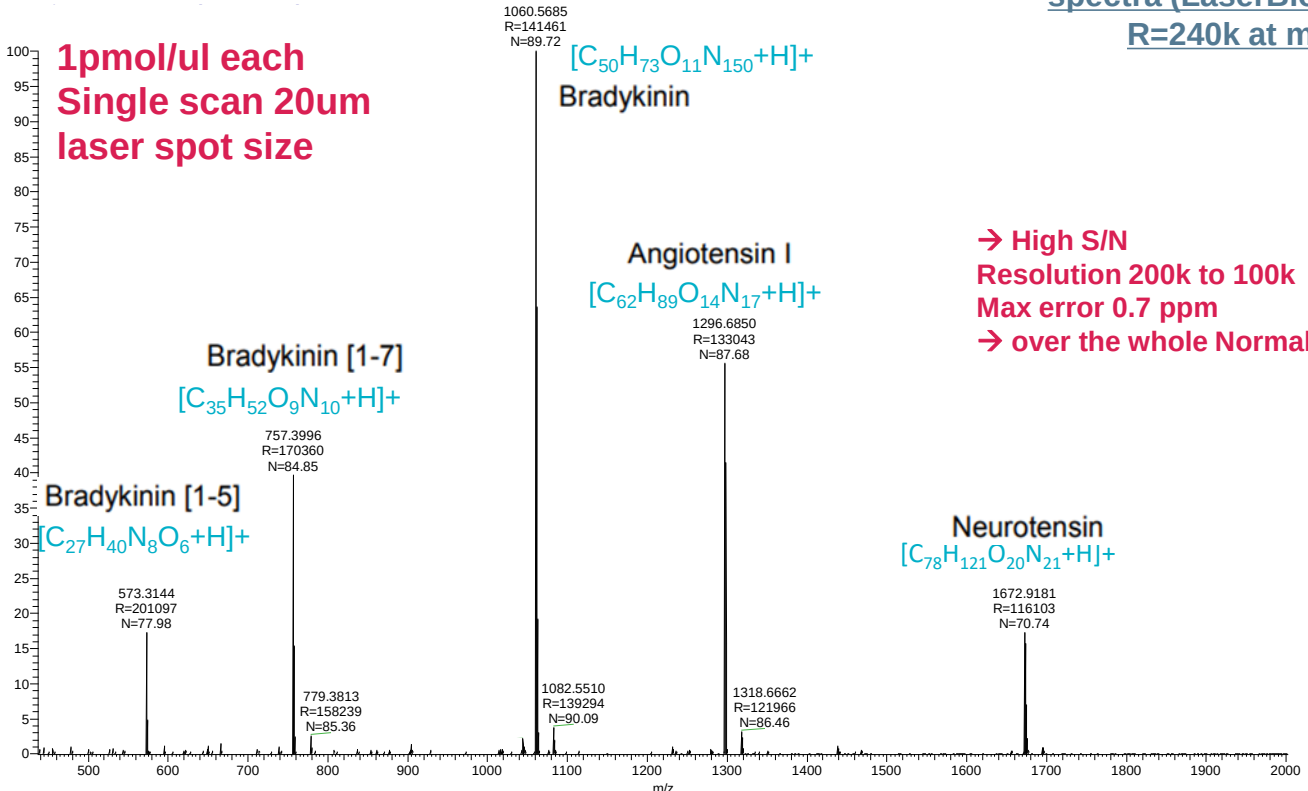
Experimental AP-MALDI HRMS spectra (LaserBioLabs Mix5) R=240k at m/z400:

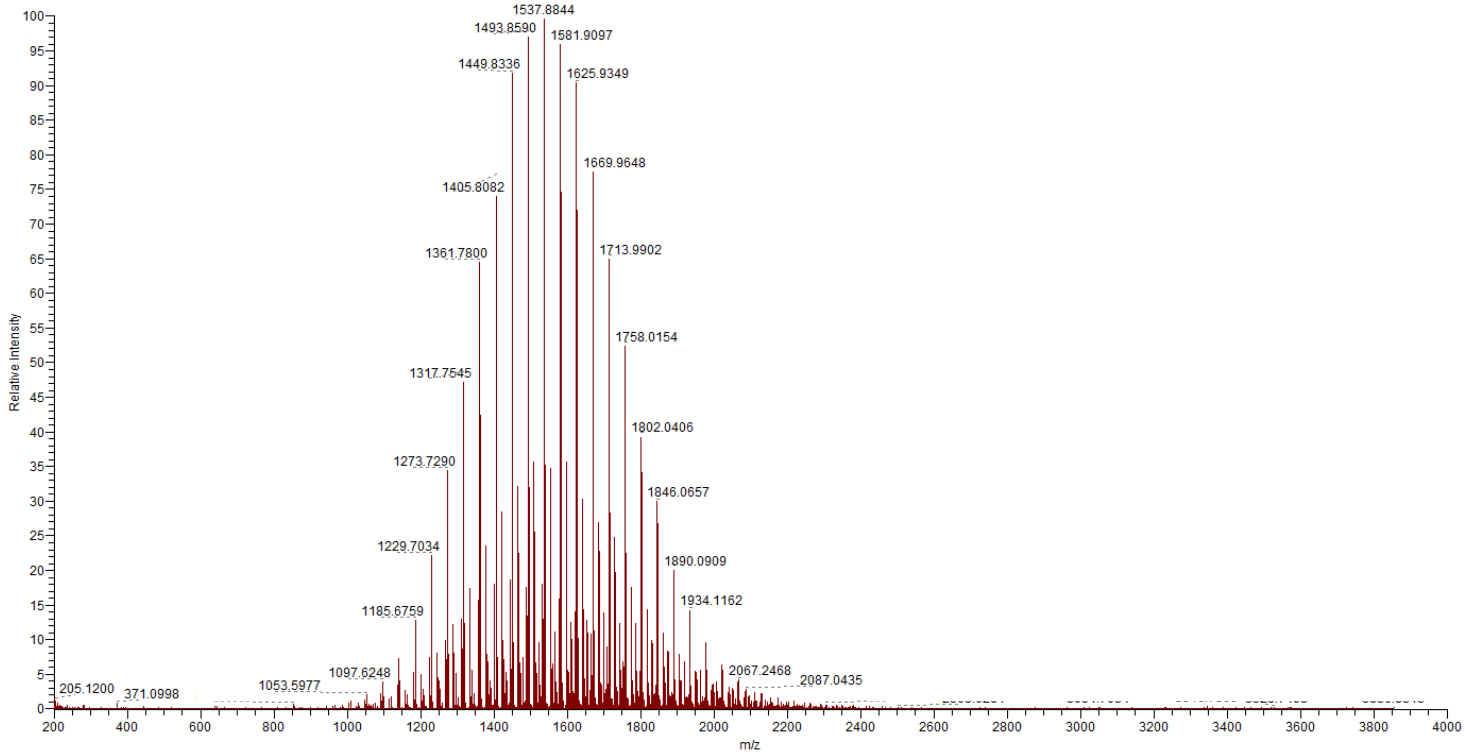
1pmol/ul each
Single scan 20um laser spot size



→ High S/N
Resolution 200k to 100k
Max error 0.7 ppm
→ over the whole Normal Mass Range

AP-MALDI HRMS ANALYSES

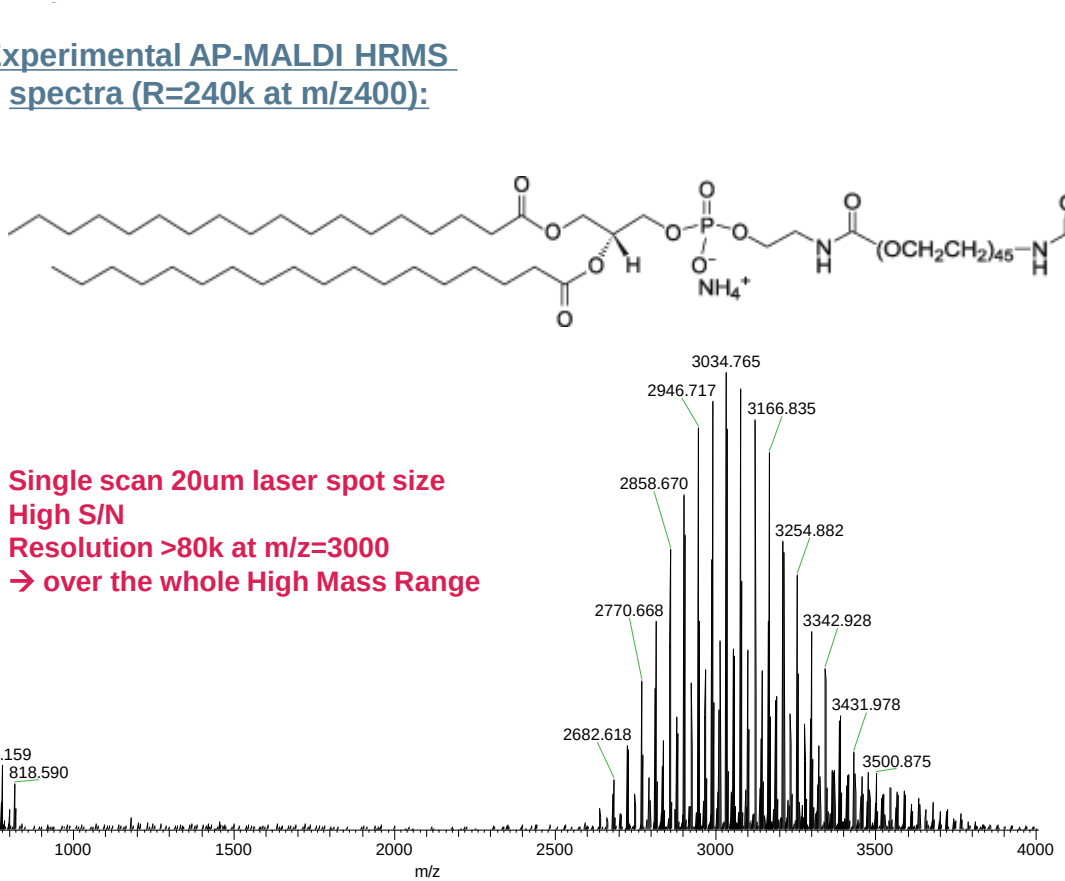
Applications: PEG-1500



AP-MALDI HRMS ANALYSES

Applications: PEGylated lipid / functionalized PEG

Experimental AP-MALDI HRMS spectra (R=240k at m/z400):

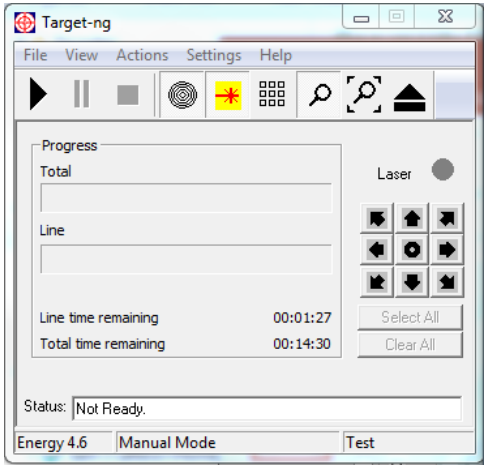


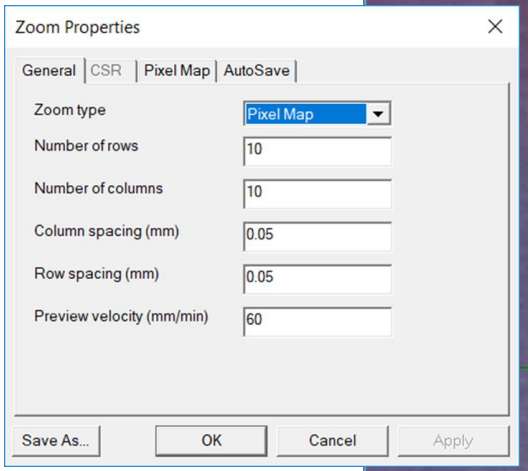
Single scan 20um laser spot size
High S/N
Resolution >80k at m/z=3000
→ over the whole High Mass Range

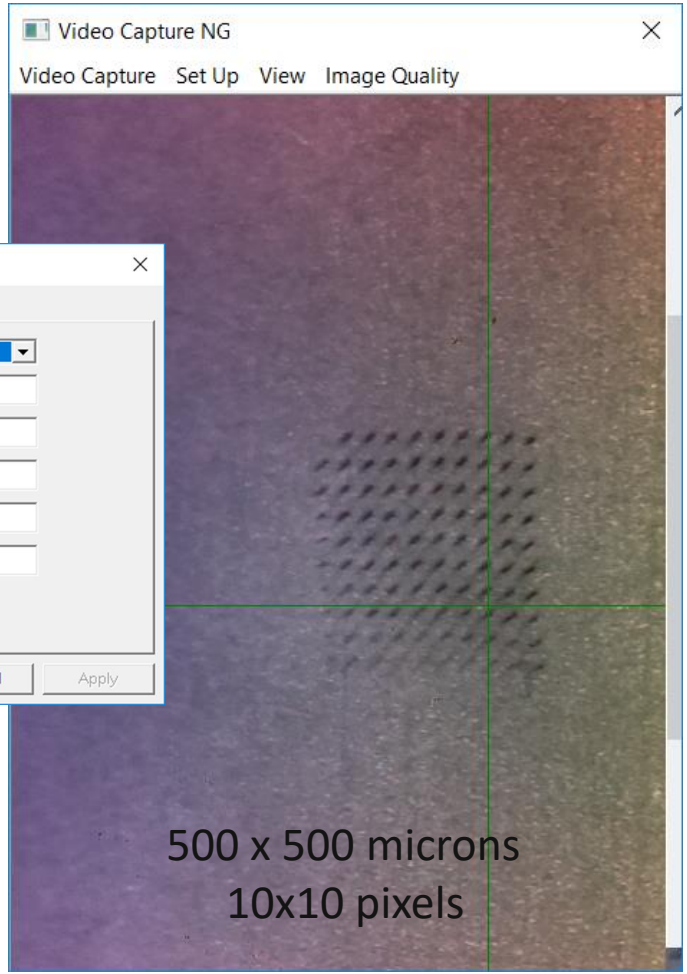
AP-MALDI HRMS IMAGING

Software: Image acquisition

- Masstech Target®







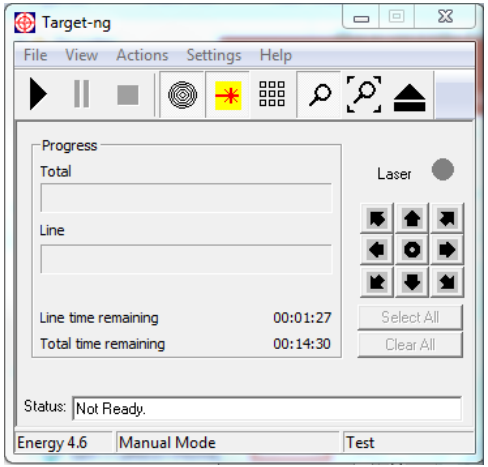
→ **PixelMap** : Irradiation of each pixel position

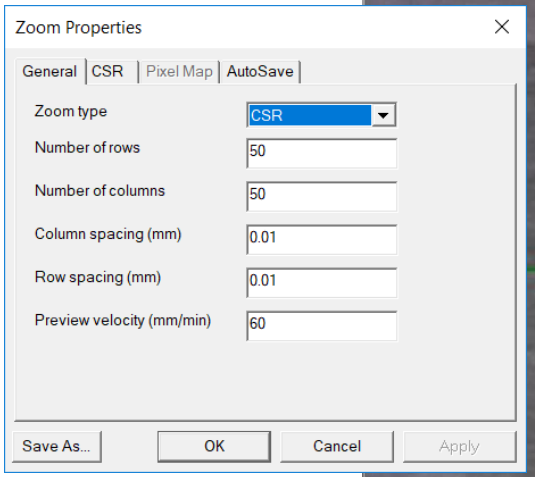
→ Constant Speed Raster (CSR) motion: Irradiation of continuous lines (sampled by the mass spectrometer into a given number of pixel per line)

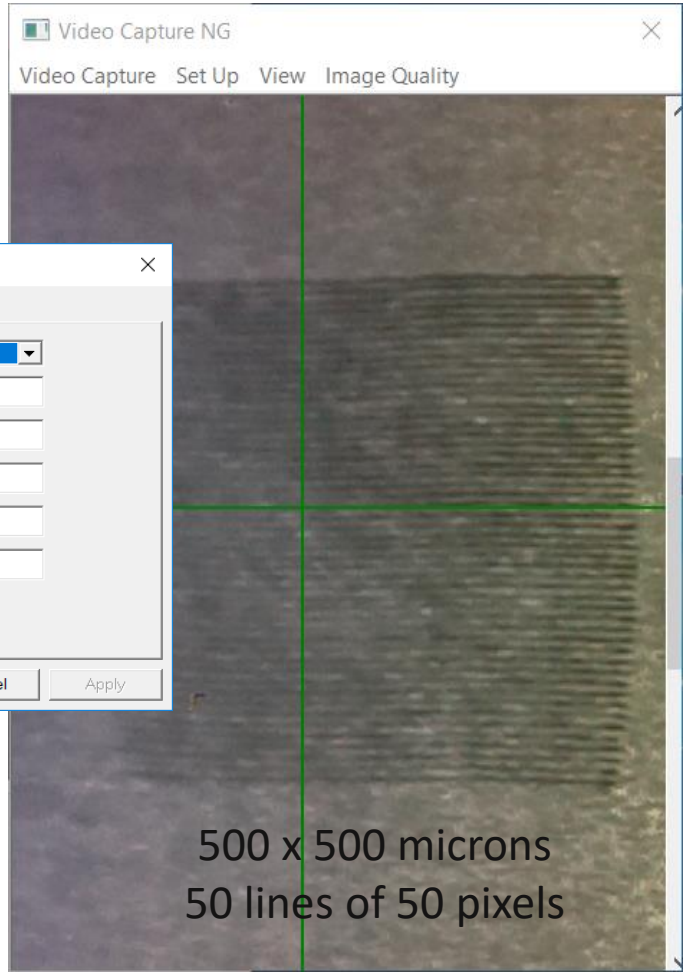
AP-MALDI HRMS IMAGING

Software: Image acquisition

- Masstech Target®







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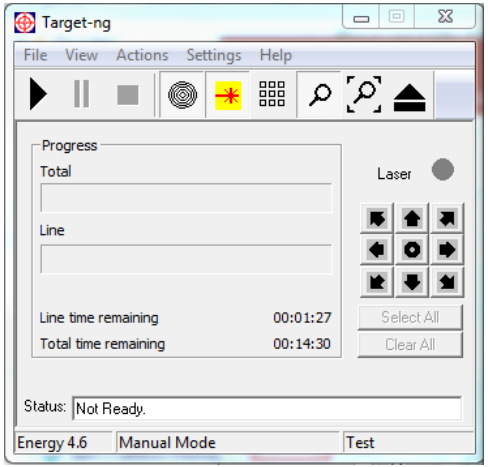
→ **Constant Speed Raster (CSR) motion:** Irradiation of continuous lines (sampled by the mass spectrometer into a given number of pixel per line)

AP-MALDI HRMS IMAGING

Software: Image acquisition

- Masstech Target®



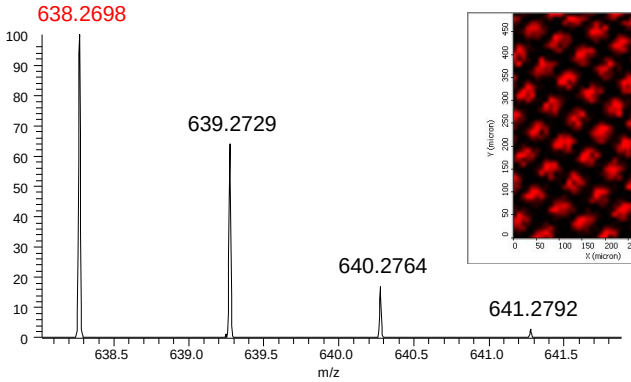
→ PixelMap : Irradiation of each pixel position

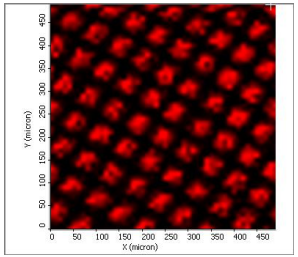
→ Constant Speed Raster (CSR) motion: Irradiation of continuous lines (sampled by the mass spectrometer into a given number of pixel per line)

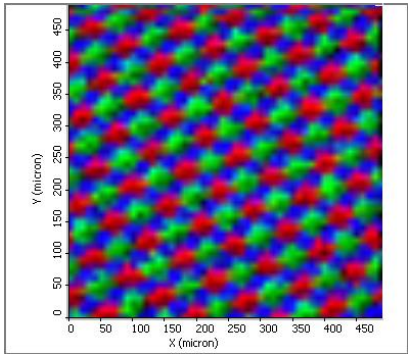
- Thermo Xcalibur:
  - Selection of the analyzer (LTQ or Orbitrap)
  - Single or multiple scan events (full scan, SIM, SRM) or method (DDA, DIA)
  - Mass range
  - Acquisition started using synchronization cable, and stopped after duration (estimated by Target software)
- Thermo ImageQuest:
  - Combines Raw files (+ Target xml position file) into MALDI images
  - Aternatively, imzML open source format can be used.
    → MSI Reader, DataCube Explorer, SCILS..

APMALDI HRMS IMAGING

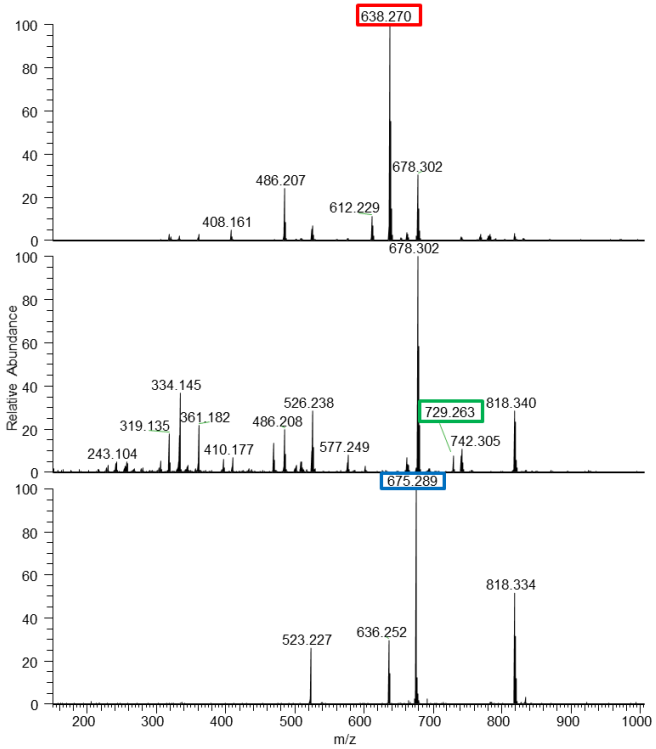
Application to OLEDs imaging







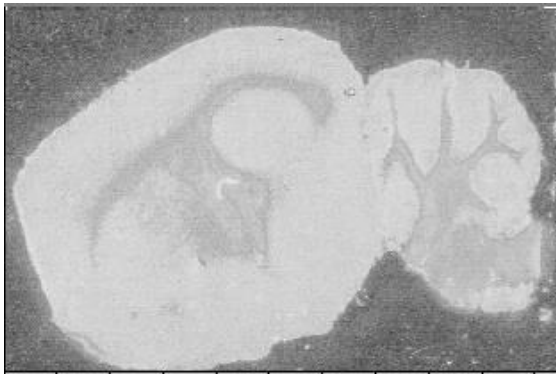
500um x 500um AP/MALDI(ng) UHR imaging of a portion of an OLED display (R: m/z=638.2685, G: m/z=729.2635, B: m/z=675.2890)



→ AP-MALDI HRMS imaging at 10 micron lateral resolution

APMALDI HRMS IMAGING

Whole brain imaging



Saggital mouse brain cryo-section
Sample preparation (Univ Swansea)
APMALDI imaging :

- CSR mode
- 30 micron resolution
- 350x230 = 80500 pixels

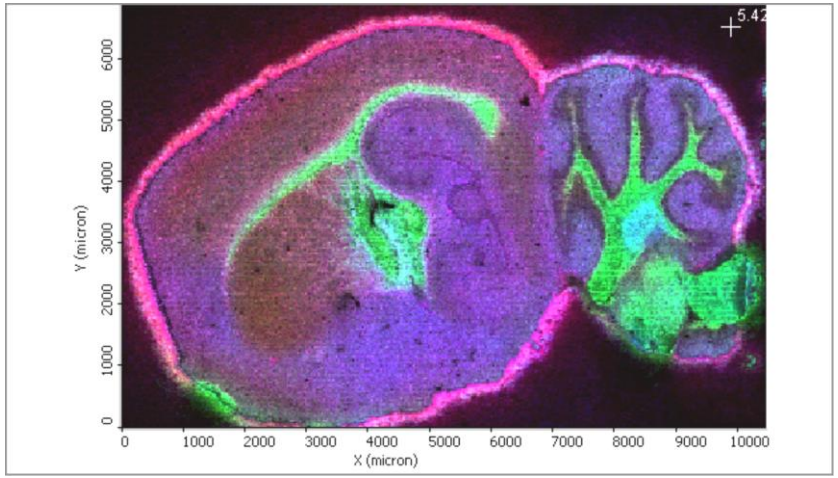
Orbitrap

- full scan (300-1200)
- 30k or 60k
- 1.5 to 2.5 pixels/second

6.5 GB, 15hrs @60k

APMALDI HRMS IMAGING

Whole brain imaging



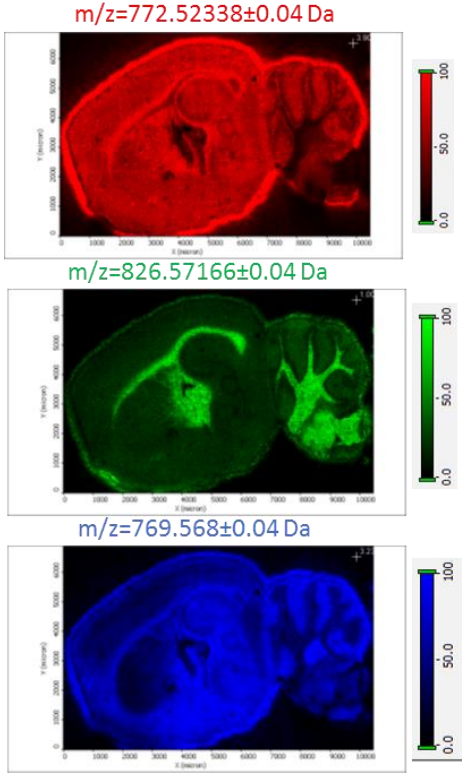


Fig.3: Overlaid AP-MALDI HRMS images of 3 lipids with a mass tolerance of 0.04Da (red: m/z=772.52338, green: m/z=826.57166, and blue : m/z=769.568) obtained using ImageQuest software

SINGLE SCAN / SINGLE PIXEL HRMS

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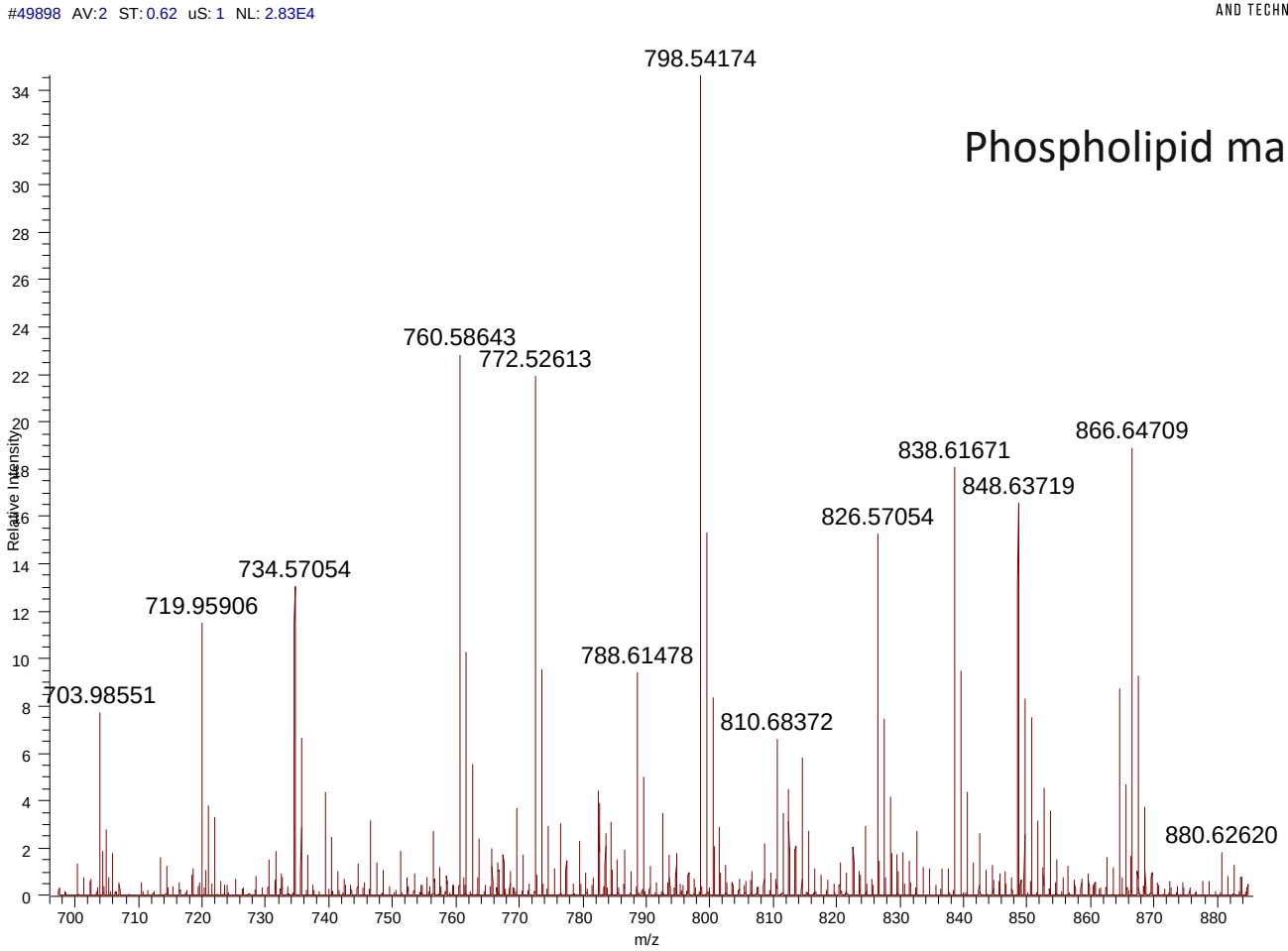

Phospholipid mass range

SINGLE SCAN / SINGLE PIXEL HRMS

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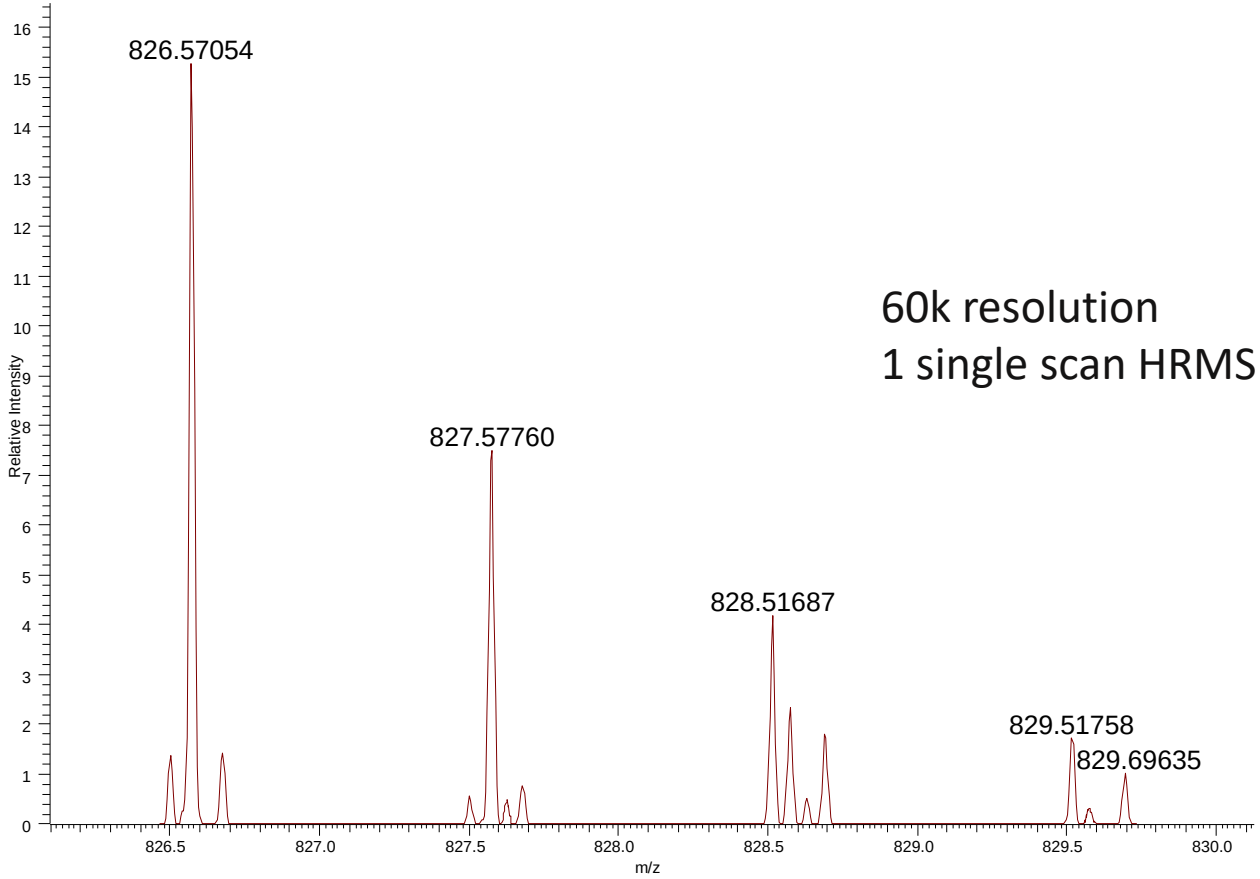
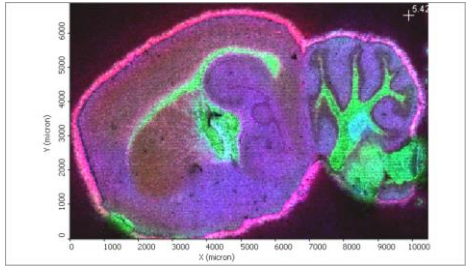


APMALDI HRMS IMAGING

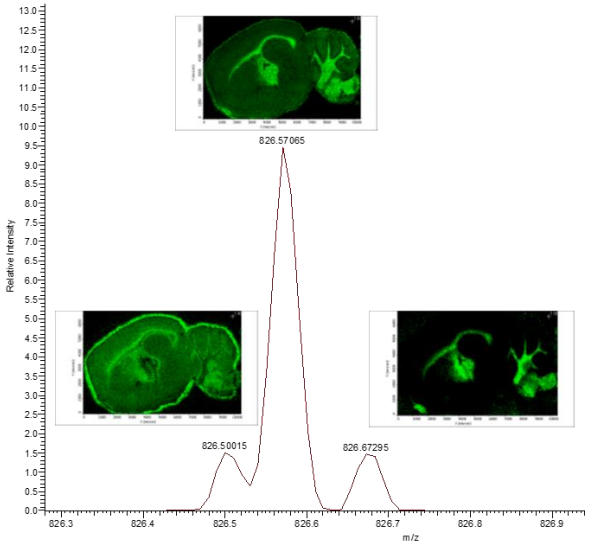
Whole brain imaging

LUXEMBOURG INSTITUTE OF SCIENCE AND TECHNOLOGY | LIST

Thermo RAW file + Target XML file



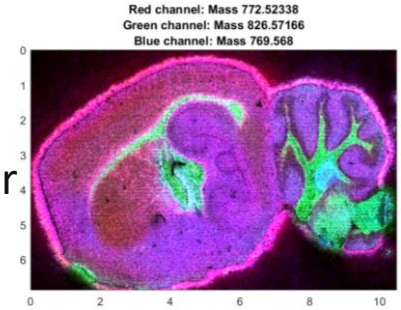
Thermo ImageQuest



Single pixel mass spectrum showing 3 intact lipids in a 0.3Da mass window

Thermo RAW file + Target XML file

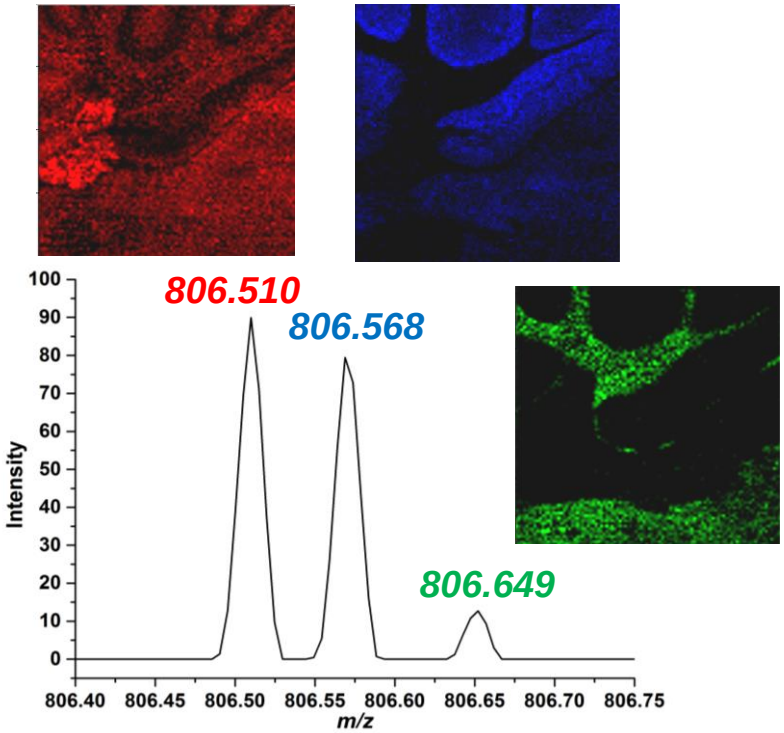
imzML converter

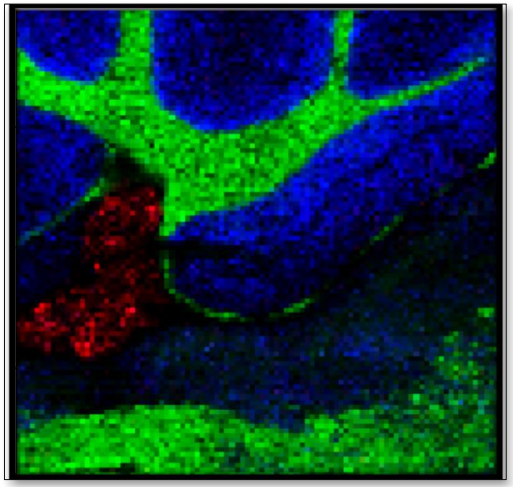


MSI Reader

APMALDI HRMS IMAGING

Down to 10 micron lateral resolution

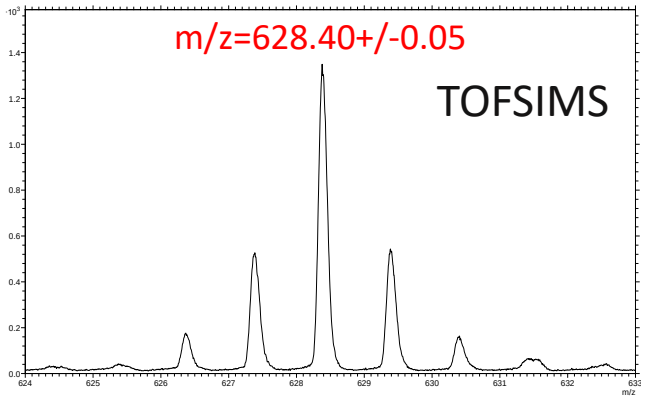


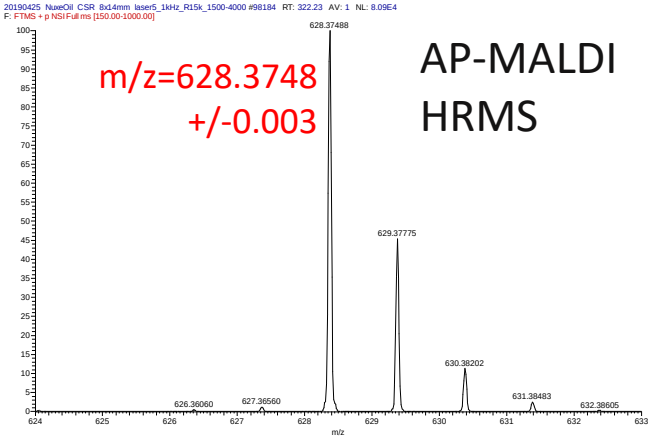


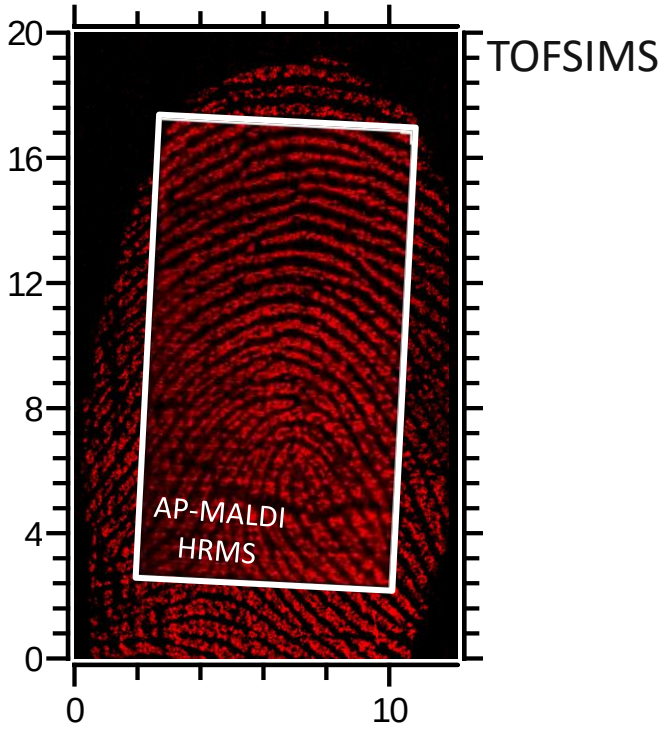
RGB Overlay

MULTIMODAL IMAGING

Skin applications

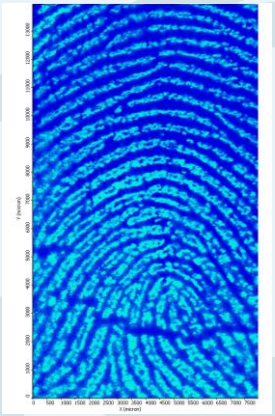


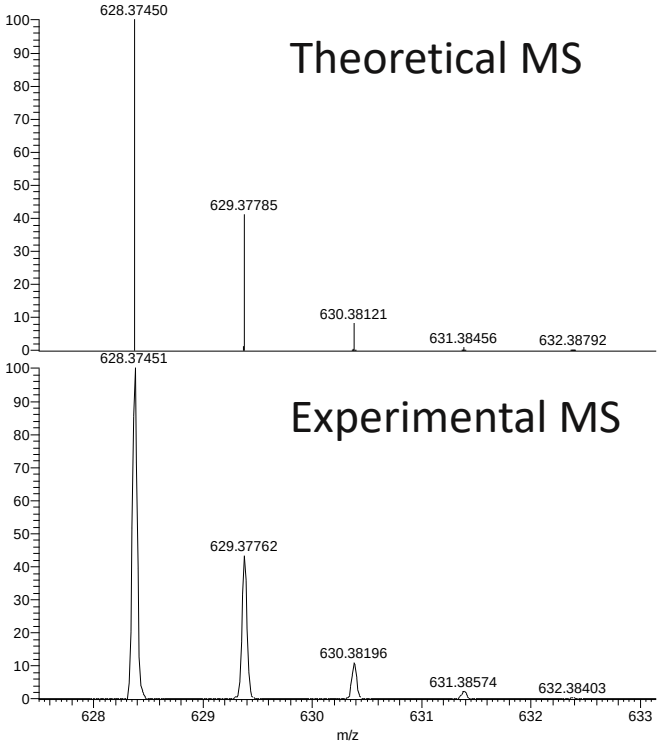




AP-MALDI HRMS IMAGING

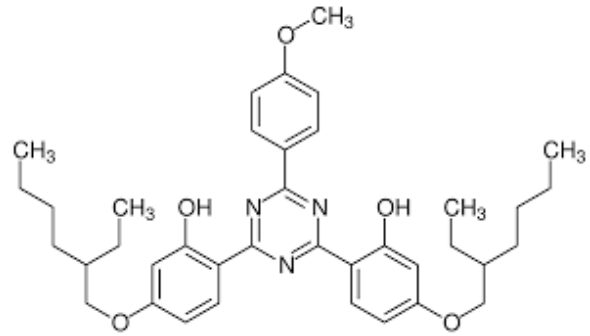
Accurate Formula assignment





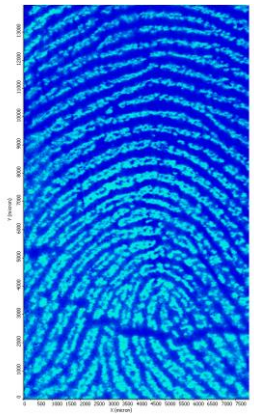
$628.37450=[C_{38}H_{49}N_3O_5+H]^+$

Bemotrizinol ?

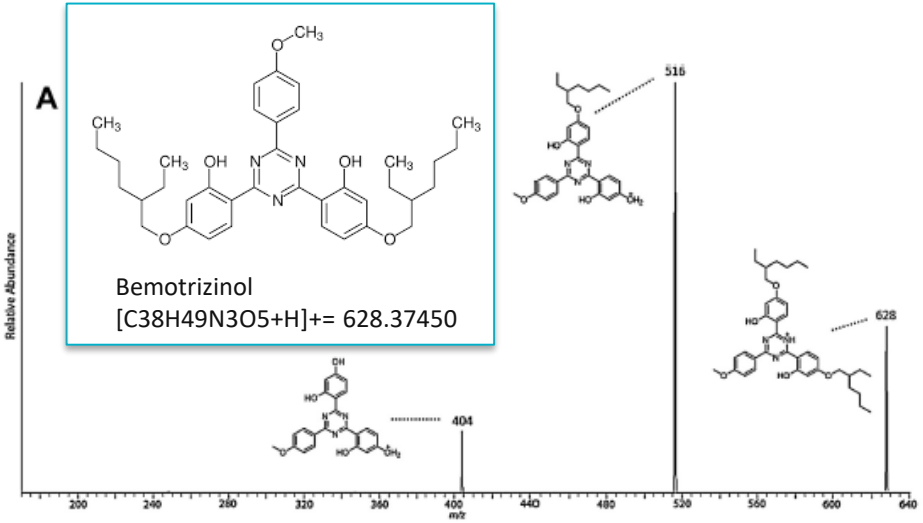


AP-MALDI MS/MS IMAGING

Structural confirmation and/or Targeted imaging



Bemotrizinol ?



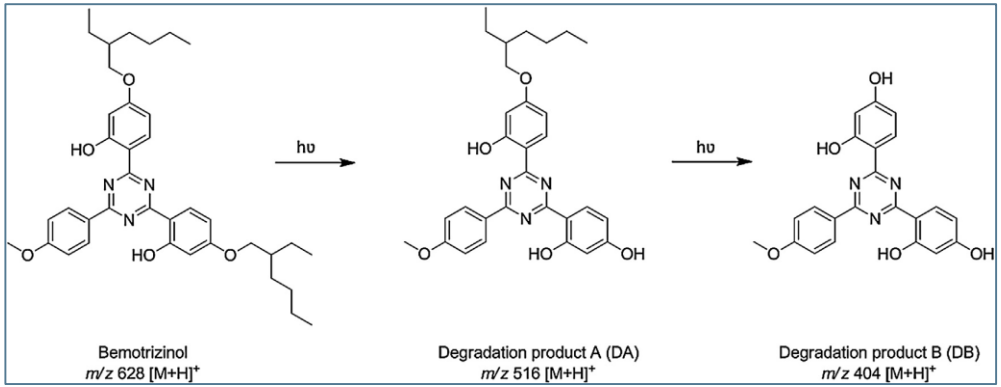
Collision-induced dissociation (CID) spectrum of (A) Bemotrizinol

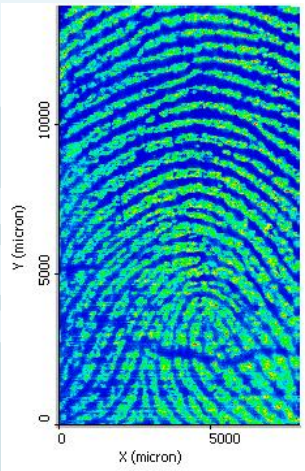
→ Possible application : Structural confirmation of active ingredient by Multiscan imaging (Fullscan + SRM)

D.N.d. Oliveira et al. /J.Chromatogr. B 988 (2015) 13–19

EFFECT OF AGING (UV)

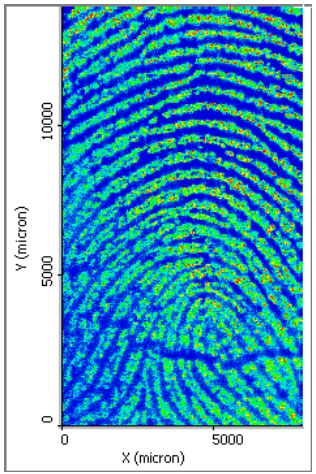
Degradation products

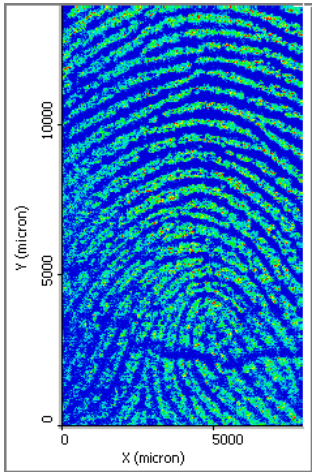




← Bemotrizinol
Max=2e5

Degradation pdt #1→
$[C_{30}H_{33}N_3O_5+H]^+$
m/z=516.24930
Max=1.6e3





←Degradation pdt #2
$[C_{22}H_{17}N_3O_5+H]^+$
m/z=404.12410
Max=1.6e3

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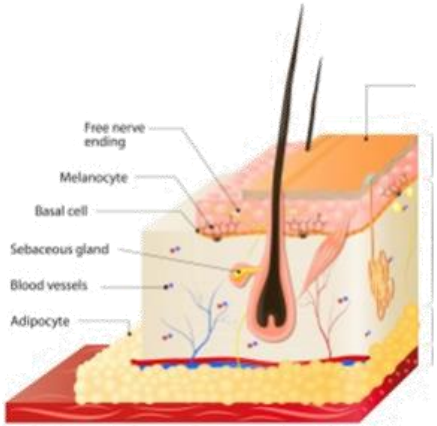
OUTLOOKS

Correlative molecular imaging HRMS on skin

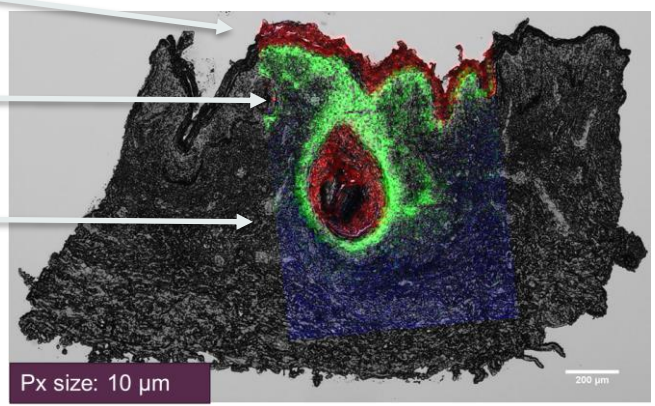


Stratum corneum

Epidermis

Dermis

Hypodermis

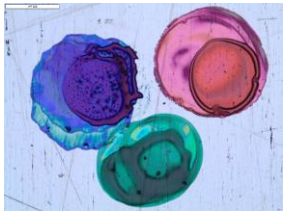


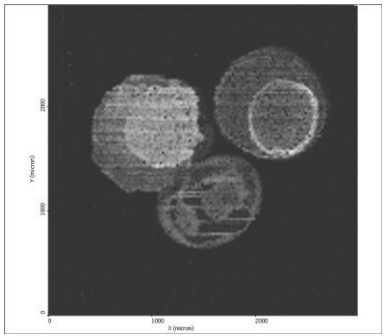
→ Application to penetration studies, skin diseases

- Ceramide m/z 386.399
- Phosphoethanolamine PE(36:2) m/z 744.553
- Peptide m/z 582.273

COMBINED MODE OF ANALYSIS

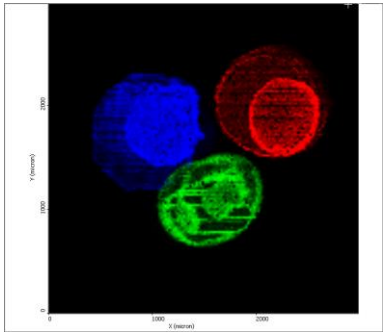
Fullscan (120k Orbitrap) + 3 MS/MS (LTQ)

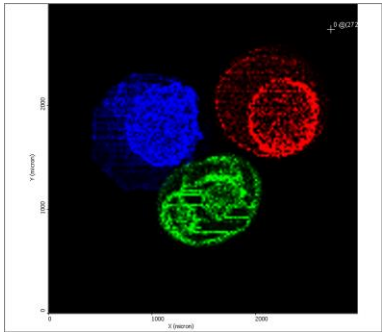




AP-MALDI HRMS imaging (Orbitrap@120k)
m/z=443.23257
m/z=385.26263
m/z=478.32063

AP-MALDI MS/MS imaging (LTQ-MSMS scan,@CID:60):
443.23, 385.26, 478.32
→structural confirmation





COMBINED MODE OF ANALYSIS

Fullscan (120k Orbitrap) + 3 MS/MS (LTQ)

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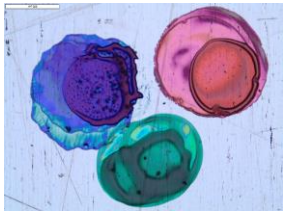
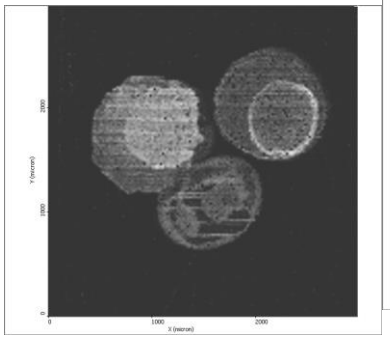
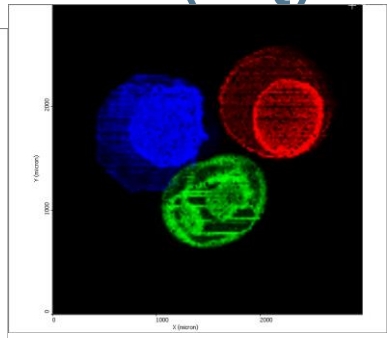
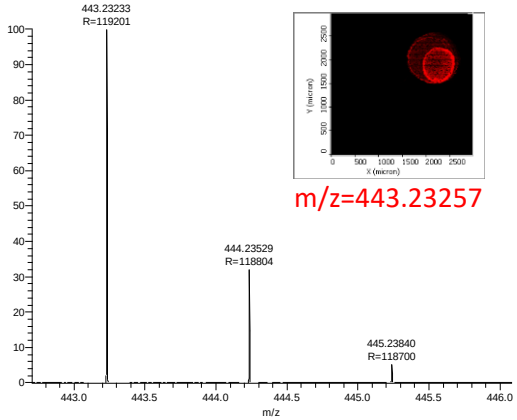


MR(443.23157)
MR(385.26263)
MR(478.32063)

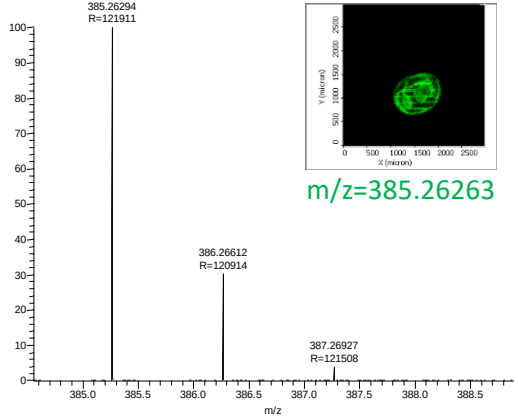




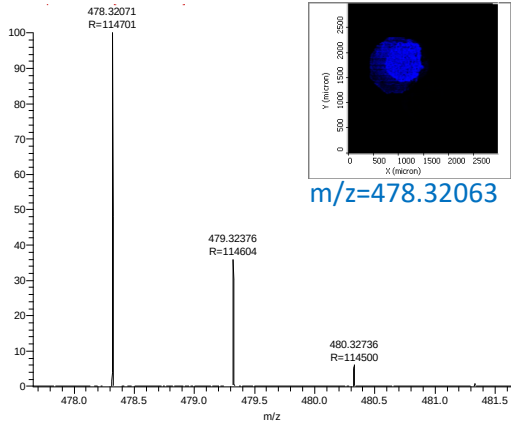
AP-MALDI HRMS imaging (Orbitrap@120k)
m/z=443.23257
m/z=285.26263
m/z=478.32063



m/z=443.23257



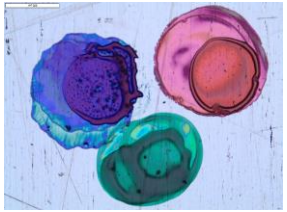
m/z=385.26263

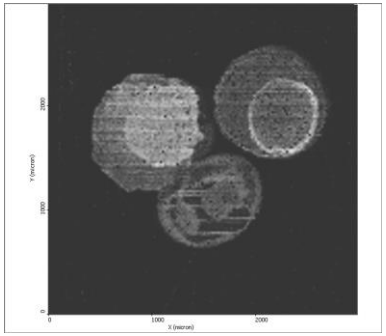


m/z=478.32063

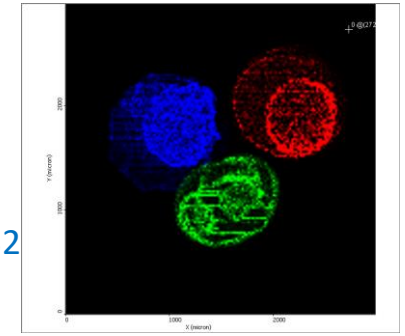
COMBINED MODE OF ANALYSIS

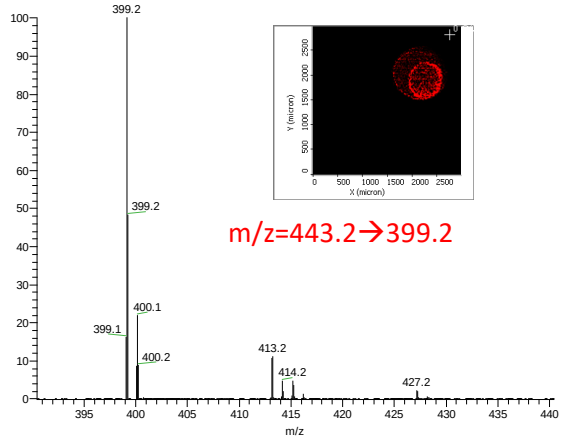
Fullscan (120k Orbitrap) + 3 MS/MS (LTQ)

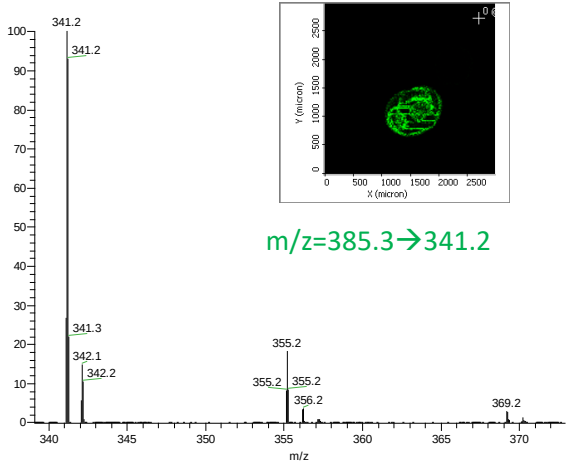


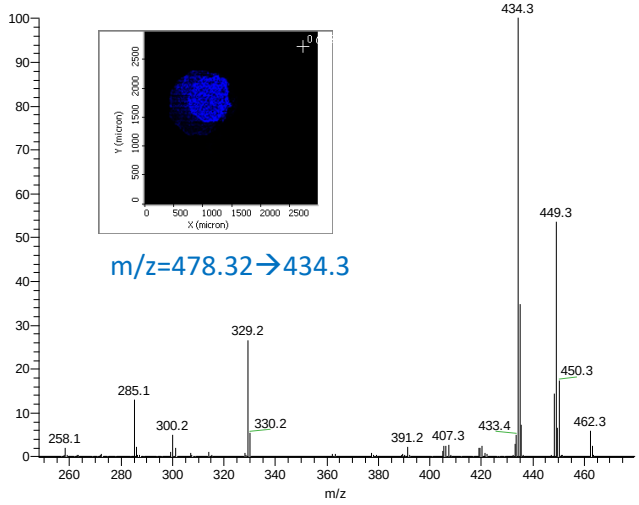


AP-MALDI MS/MS imaging (LTQ-MSMS scan,@CID:60):
443.23, 385.26, 478.32









CONCLUSIONS & TAKE HOME MESSAGES

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- **Masstech AP-MALDI (ng) UHR** source :
  - provides high performances MALDI analysis and imaging (sensitivity and lateral resolution)
  - cost-effective add-on module for LCMS instruments, ideally coupled to high-end HRMS instrument
  - flexible: ESI-APMALDI swap is done within a few minutes, the HRMS can still work in LC/HRMS configuration.

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