ATMOSPHERIC PRESSURE MALDI COUPLED TO ORBITRAP(S), PRINCIPLE, APPLICATIONS AND CURRENT DEVELOPMENTS

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MassTech

KR ANALYTICAL

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AP/MALDI Principle

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MASSTECH AP/MALDI UHR ION SOURCE

Benefits

- Compatible with various high-resolution LC/HRMS models

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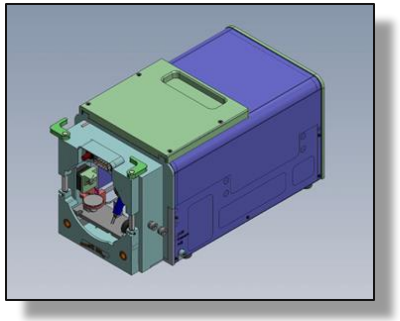
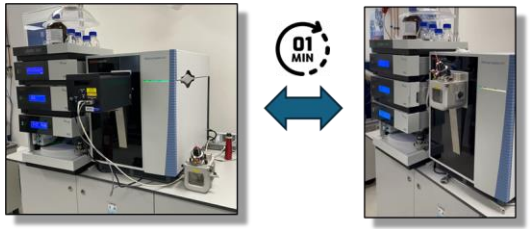


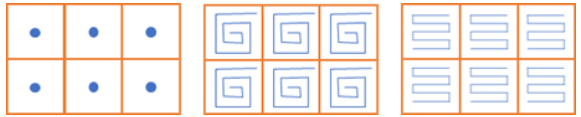
MASSTECH AP/MALDI UHR ION SOURCE

Benefits

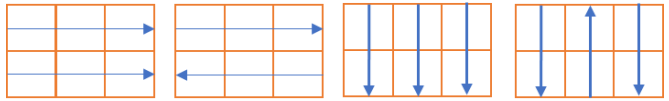
- Compatible with various high resolution LC/HRMS models
- Fully integrated design
- Swap ESI to AP/MALDI within a minute
- Several modes of operation (pixel map mode or continuous mode)



- Pixel Map without or with in-pixel motion)



- Constant Speed Raster (Flyback/Meandering, horizontal/vertical)



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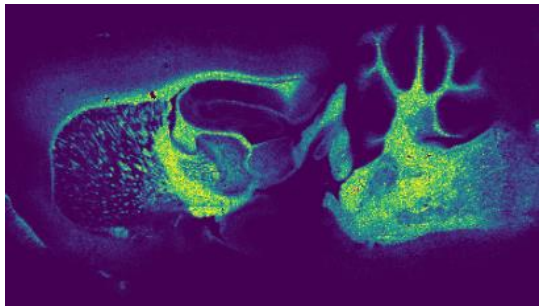
- Hgh tolerance to topography
- Analysis of biological samples in their native form



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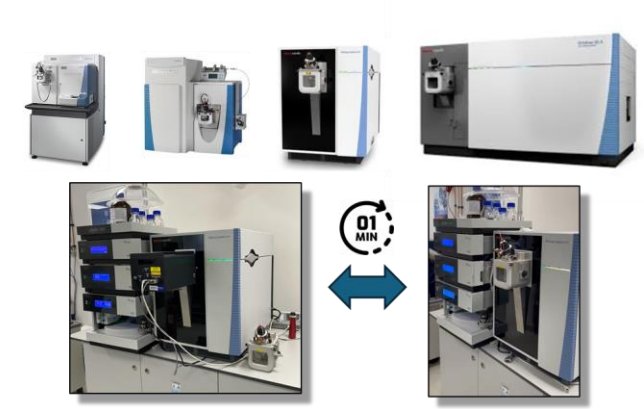
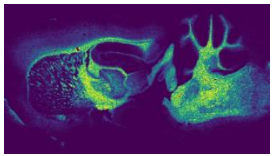
- Analysis of biological samples in their native form
- User friendly control and conversion software, allowing sequences of multiple images (any shape)
- Vast range of third-party data handling solutions





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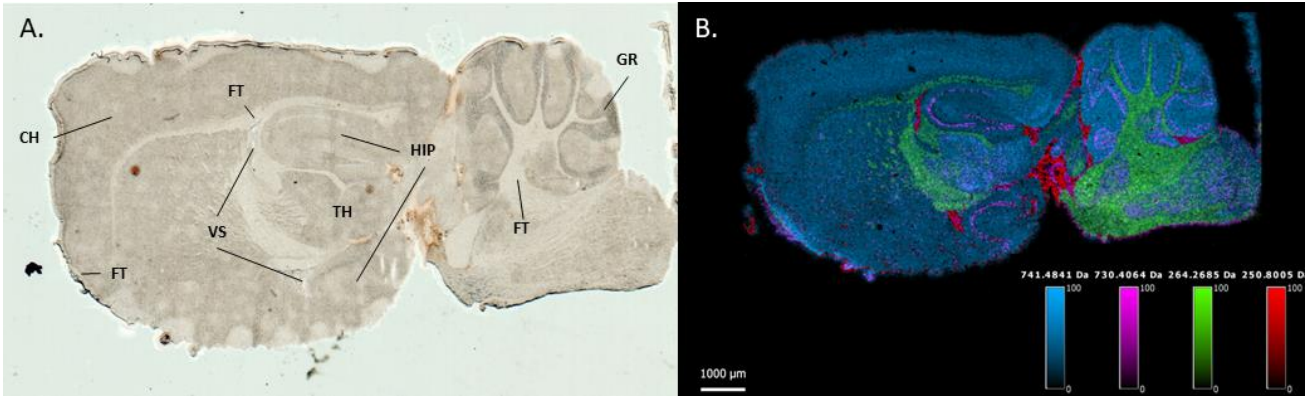
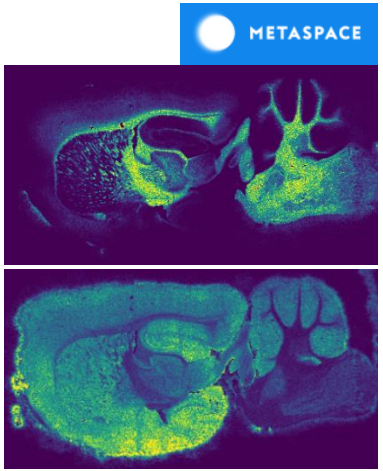
AP/MALDI Applications

AP/MALDI HRMS IMAGING OF LIPIDS IN A MOUSE BRAIN SECTION

Spatial lipidomics





AP/MALDI imaging on sagittal mouse brain section. (A) Light microscopy of sagittal mouse brain section using a PrimeScan microscope slide scanner (GT Vision Ltd., Newmarket, UK). CH: Cerebrum, FT: Fiber tracts, GR: Granular layer, HIP: Hippocampal region, TH: Thalamus, VS: Ventricular systems. (B) Full-brain AP/MALDI-image, 25 µm spatial resolution (blue: *m/z* 741.4841, purple: *m/z* 730.4064, green: *m/z* 264.2685, red: *m/z* 250.8005).

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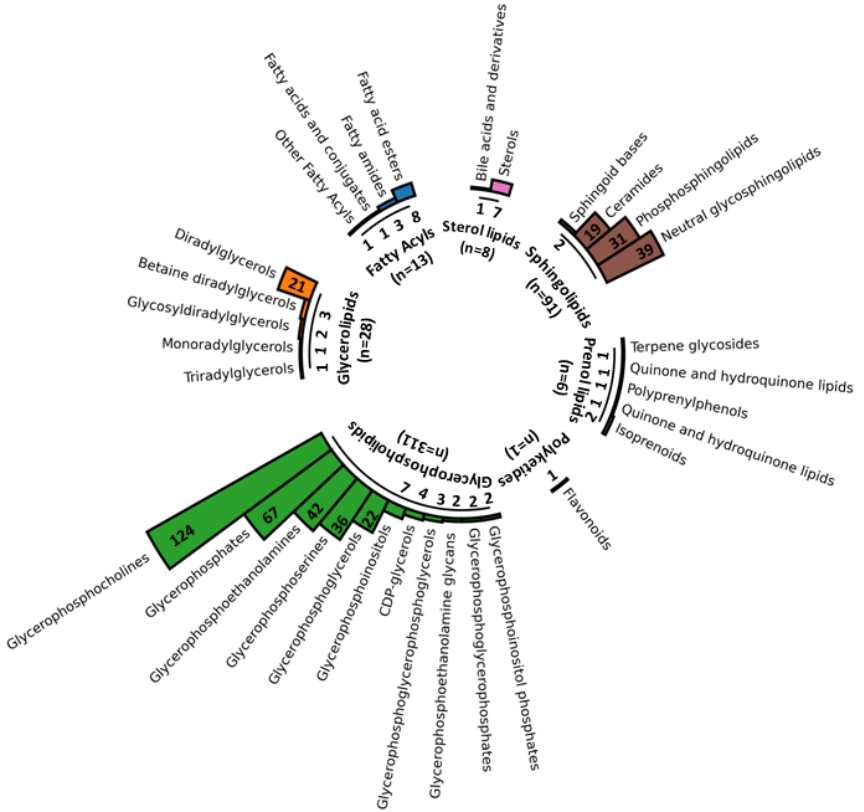
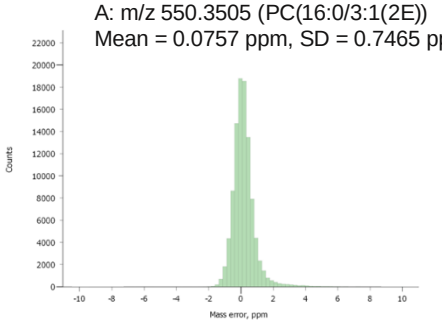
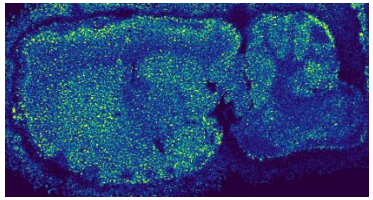
AP/MALDI HRMS IMAGING OF LIPIDS IN A MOUSE BRAIN SECTION (R=480.000 AT 200 M/Z)



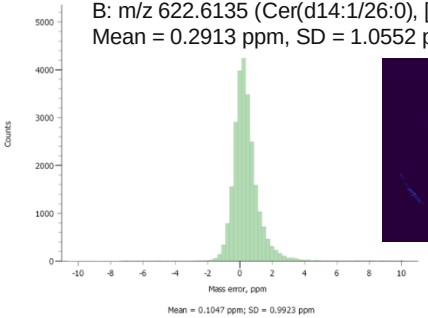
Repartition of lipid categories and classes identified within the sagittal mouse brain section based on the chemical formulae (tolerance: 5 ppm) identified using the Lipostar MSI software

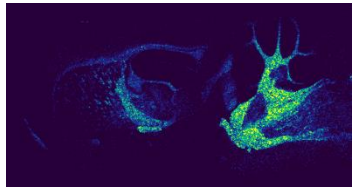
A: m/z 550.3505 (PC(16:0/3:1(2E))
Mean = 0.0757 ppm, SD = 0.7465 ppm.



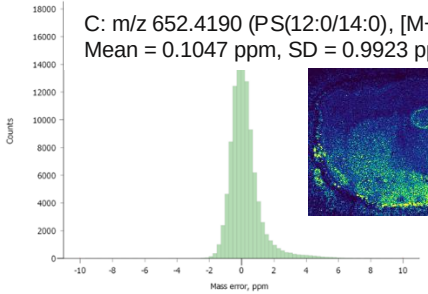


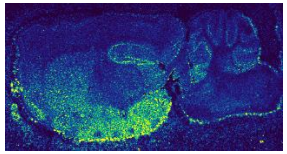
B: m/z 622.6135 (Cer(d14:1/26:0), [M+H]+).
Mean = 0.2913 ppm, SD = 1.0552 ppm.





C: m/z 652.4190 (PS(12:0/14:0), [M+H]+).
Mean = 0.1047 ppm, SD = 0.9923 ppm.





Mass error distribution histograms for mass spectra acquired with the EASY-IC™ lock mass.
The bin width is 0.25 ppm.

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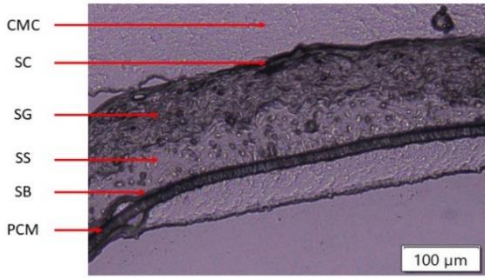
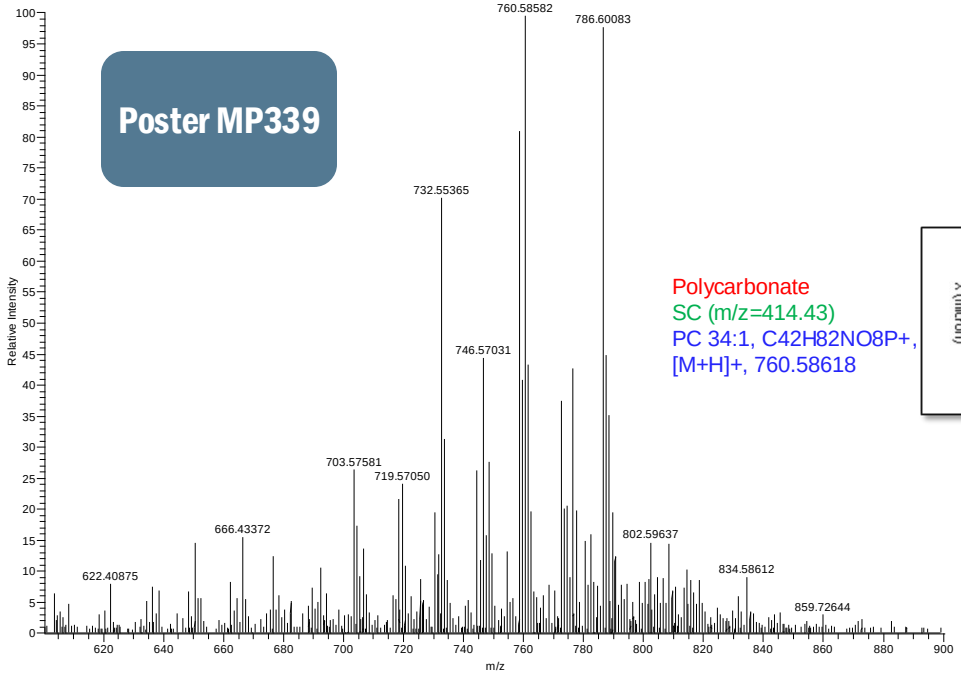
AP/MALDI EXPLORIS 480

Reconstructed Human Epidermis (RHE)



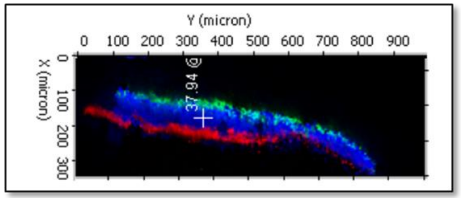
#5981 uS: 1 NL: 1.30E4
Click to insert source



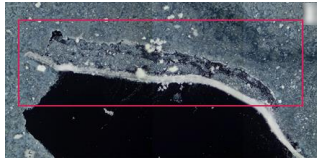
Poster MP339

First AP/MALDI imaging on Exploris 480 at 5um lateral resolution

Polycarbonate
SC (m/z=414.43)
PC 34:1, C42H82NO8P+, [M+H]+, 760.58618

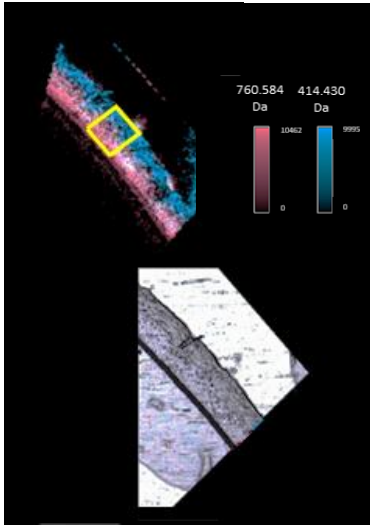


m/z images



Light Microscopy

Optimized AP/MALDI imaging [Feucherolles et al., submitted]



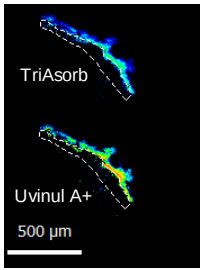
Maureen Feucherolles et al., https://pubs.acs.org/action/showCitFormats?doi=10.1021/jasms.3c00185&ref=pdf

AP/MALDI IMAGING OF SKIN CROSS SECTION

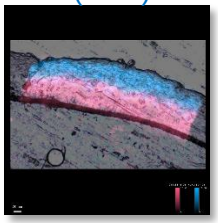
(Exploris 480, 5µm/pixel)

From dermo-cosmetics to dermatology

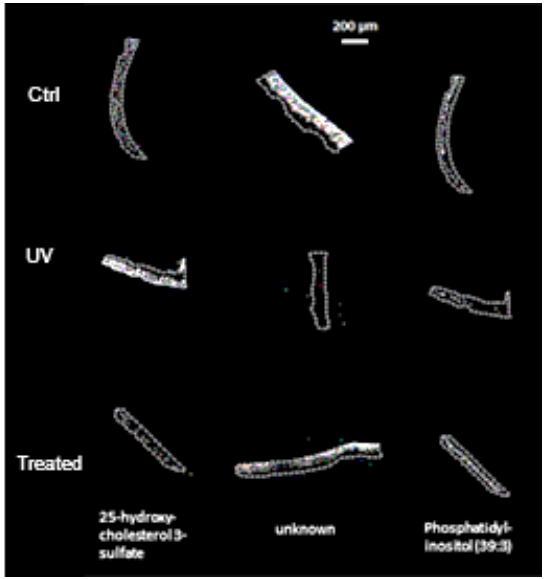
Detection of UV filters on RHE



Spqtiql lipidomics in Skin models (RHE)



Detection of inflammation biomarkers



Detection and localization of biomarkers showing:

- Reduced photo-inflammation (CholS) for treated samples
- Differentiation of keratinocyte in stratum basale (PIs), altered by UV
- Modulation of coenzymes, vitamins, all classes of lipids
- **Assessment of the efficiency of formulations**

Replacement of human skin biopsies with reconstructed Human Epidermis

Biomarker discovery

Cross-validation of identified metabolites with established LC/MS data from extracts

Dermatological interpretation

Spatial metabolomics (lipidomics)

Skin inflammation - Atopic Dermatitis

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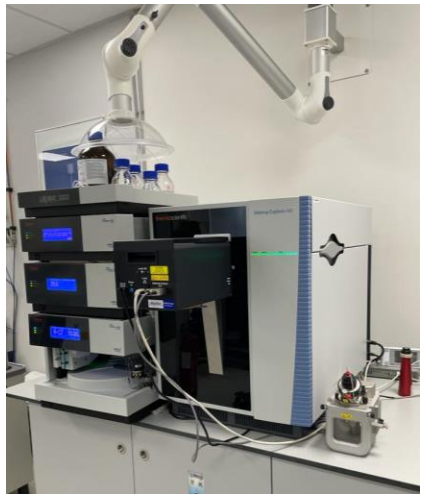
CONCLUSIONS

AP/MALDI HRMS Imaging

- **high performances** MALDI analysis and imaging (high sensitivity and lateral resolution down to 5 µm)
- **cost-effective** add-on module for LCMS instruments, ideally coupled to high-end HRMS instrument
- **flexible**: ESI-AP/MALDI swap is done within a minute.
- Compatible with Thermo Orbitraps, including QEs, Exploris or tribrids

Sample prep. / Method development is the key for a successful MALDI MS imaging experiment!

Some lipids are detected by LCMS but not in MSI…



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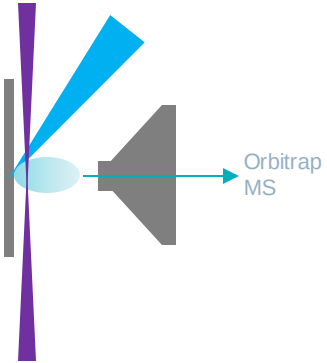
AP/MALDI Developments

AP/MALDI-2

AP/MALDI with laser post-ionization

- ESI / AP/MALDI-2 swap within 1 minute
- Fully integrated design
- Laser desorption/ionization (355nm) + post-ionization (266nm)
- Synchronization of 2 lasers



Masstech AP/MALDI source

AP/MALDI-2 Add-on (patent LU505964)

Exploris 480 ThermoFisher Orbitrap high resolution mass spectrometer

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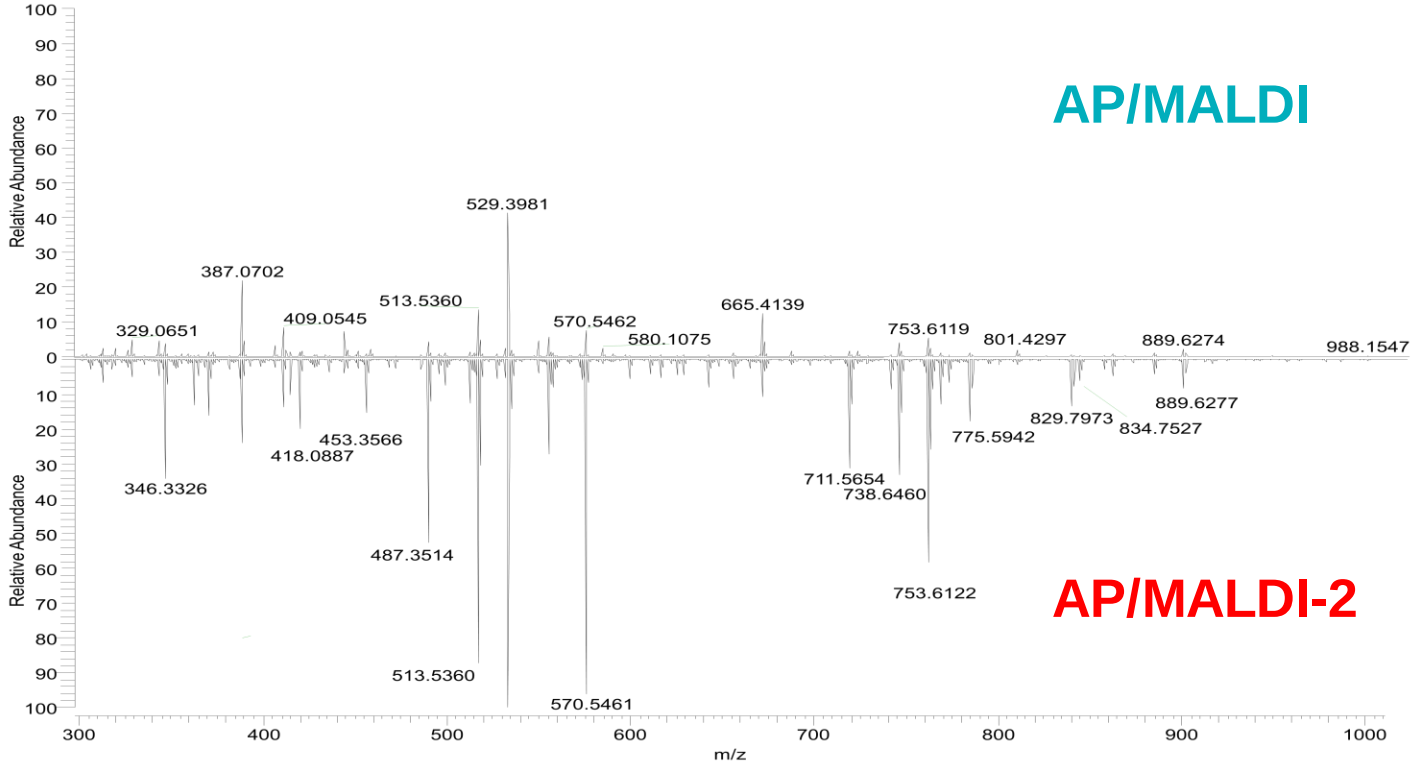
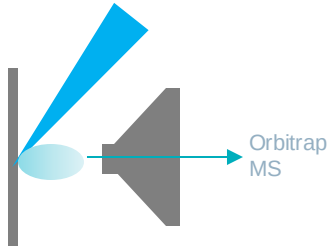
AP/MALDI-2

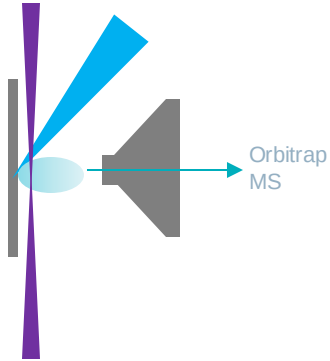
Equisplash deuterated lipid mixture



AP/MALDI



AP/MALDI-2



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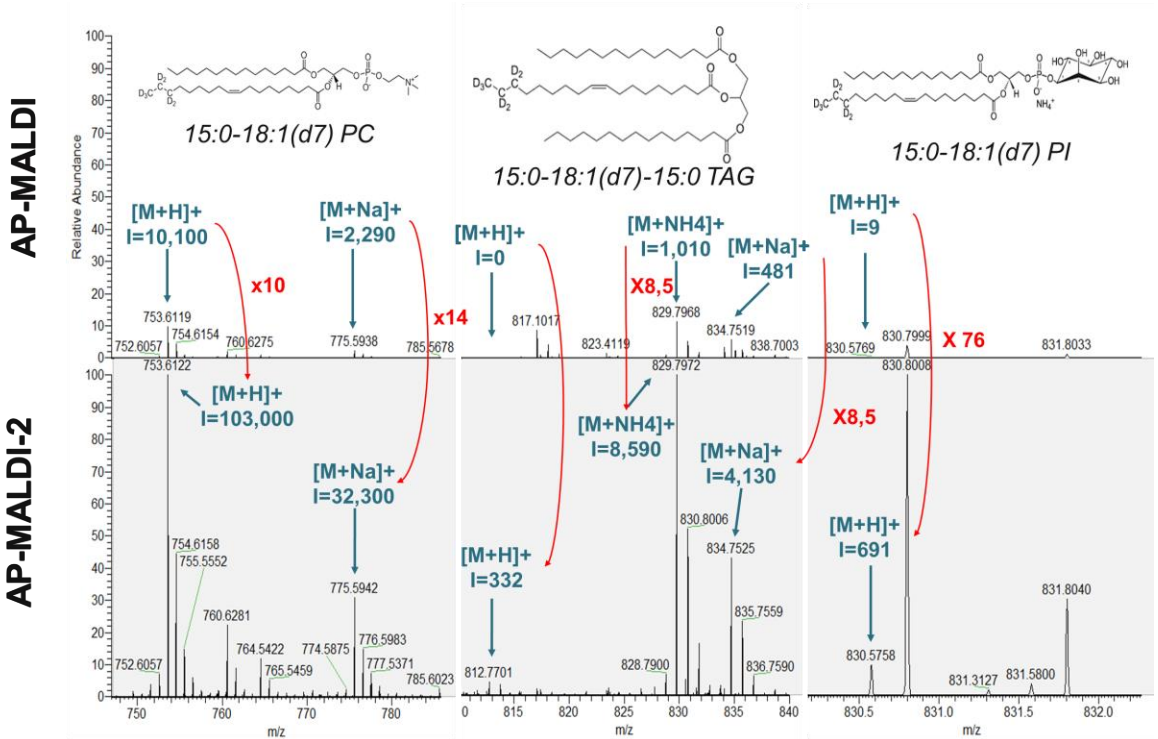
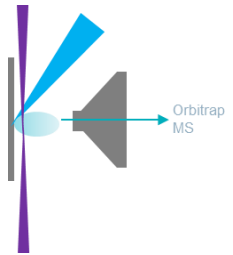
AP/MALDI-2

Increased sensitivity for lipids (Equisplash deuterated lipid mixture)



- substantial enhancement in lipid detection with the AP/MALDI-2 prototype.
- Detection of triacylglycerols (TAG), not achieved with conventional AP/MALDI sources under the tested conditions.



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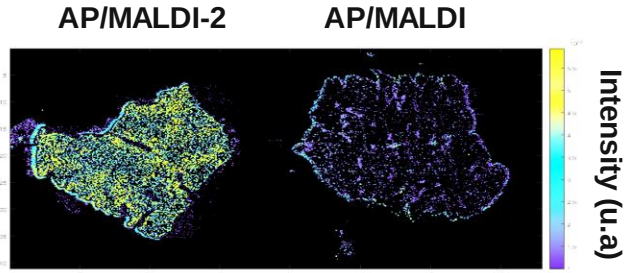
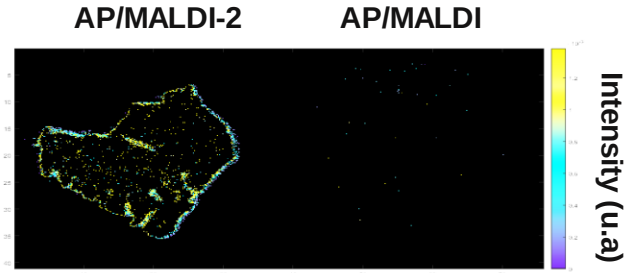
AP/MALDI-2 IMAGING

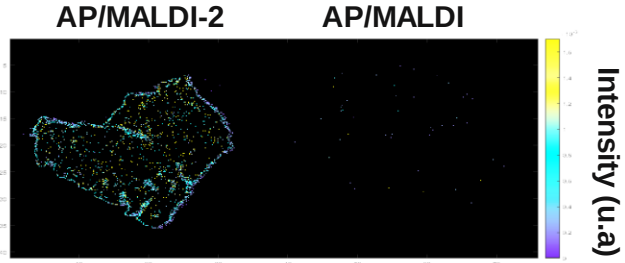
Liver section



PE 38:6 m/z=764.522



PI(40:0) m/z=943.6254



TG(57:12)
m/z=947.6530

→Sensitivity increase by up to two orders of magnitude in lipid imaging applications using AP/MALDI-2

→ New detected molecules (different ionization mechanisms)

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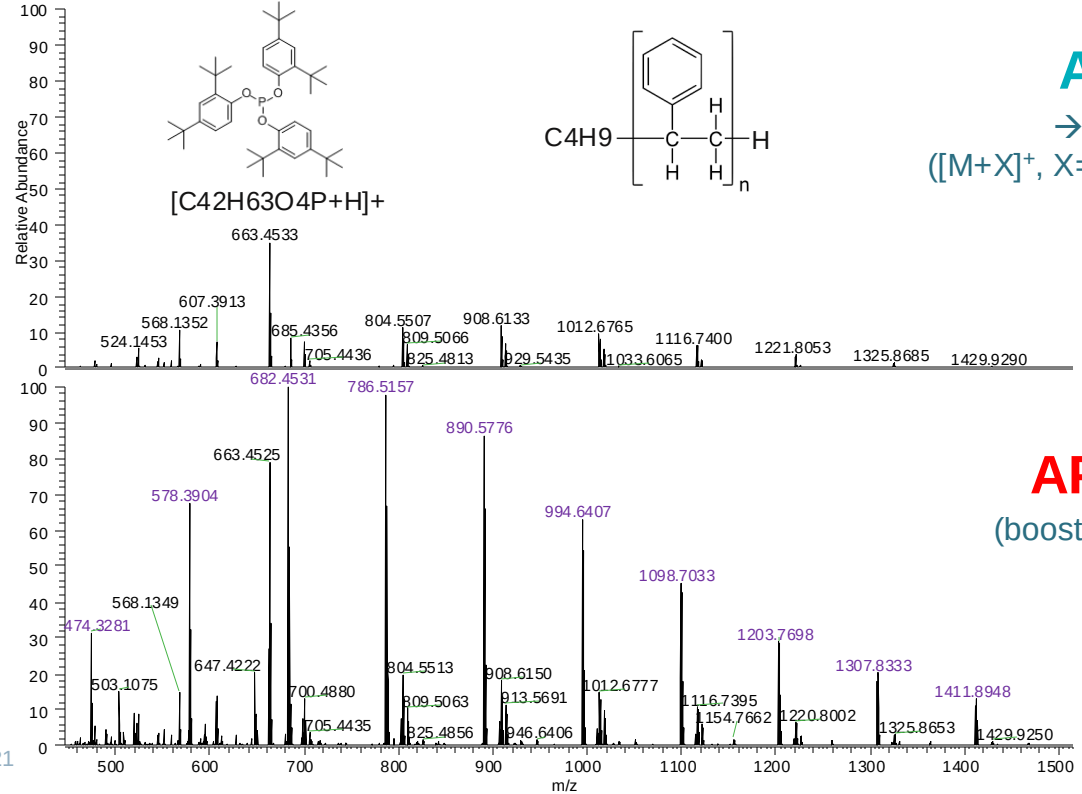
AP/MALDI-2

Polystyrene 700 + Irgafos 168



AP/MALDI

→ MALDI like ions

([M+X]$^+$, X= H, Na, NH_4, K, Li, Ag…)

AP/MALDI-2

(boosted) MALDI like ions

REMPI [M]$^{\bullet+}$

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AP/MALDI-2 OF SYNTHETIC POLYMERS

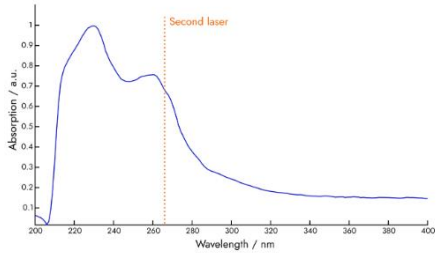


Figure S8. UV-Vis spectrum of PS standard in THF solution featuring broad absorption bands at 230 and 256 nm. At the wavelength of the MALDI-2 laser (266 nm, highlighted in orange), PS absorbs strongly. Thus, it is plausible that upon laser irradiation, PS in the gas phase can ionize directly via 1+1 REMPI.

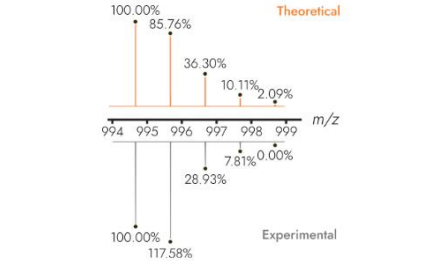
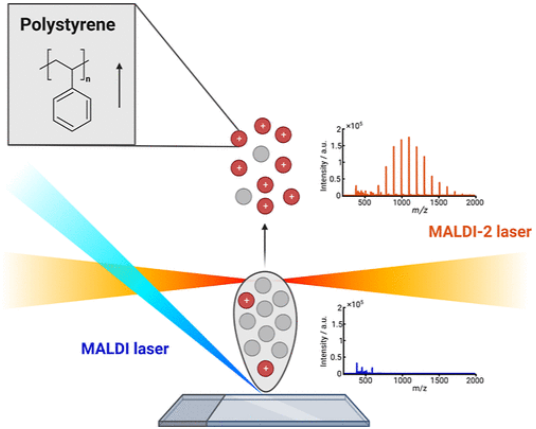


Figure S9. Theoretical (top, orange) and experimental (bottom, grey) isotopic distributions of the PS oligomer $[C_{71}H_{80}]^{+\bullet}$ at m/z 994.64. The higher abundance in $[C_{71}{}^{13}CH_{80}]^{+\bullet}$ (m/z 995.64) reveals the presence of protonated species when analyzing PS with DT matrix and Ag(TFA with MALDI 2.



MALDI-2 Mass Spectrometry for Synthetic Polymer Analysis, Lidia Molina-Millán, Aljoscha Körber, Bryn Flinders, Berta Cillero-Pastor, Eva Cuypers, and Ron M. A. Heeren*, Macromolecules 2023, 56, 19, 7729–7736

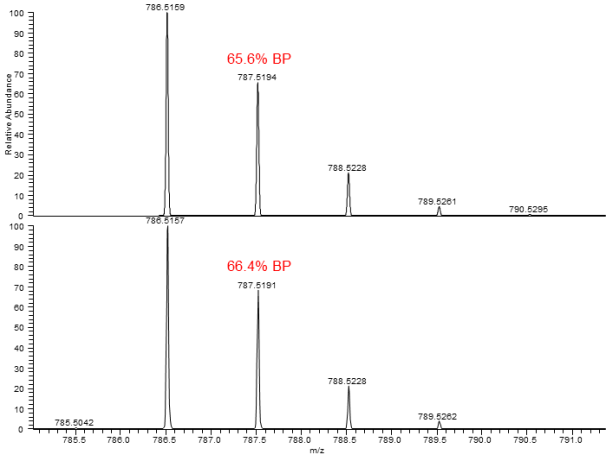
Publication Date: September 22, 2023

https://doi.org/10.1021/acs.macromol.3c01401

AP-MALDI-2

(boosted) MALDI like ions

REMPI 1+1 $[M]^{\bullet+}$



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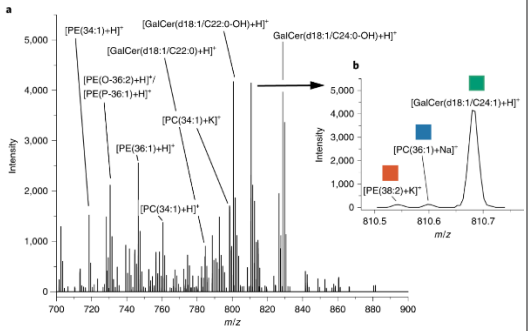
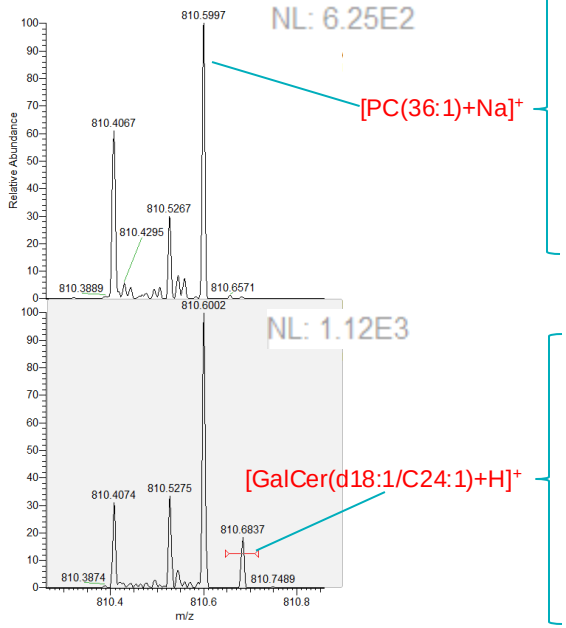
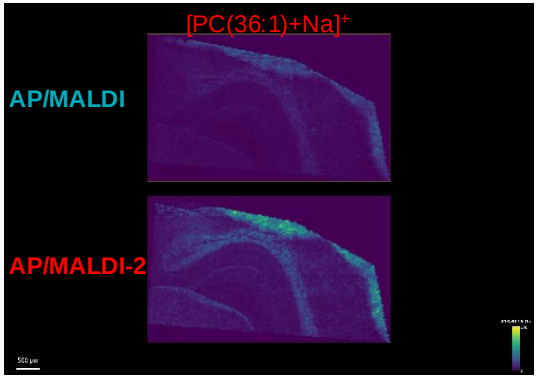
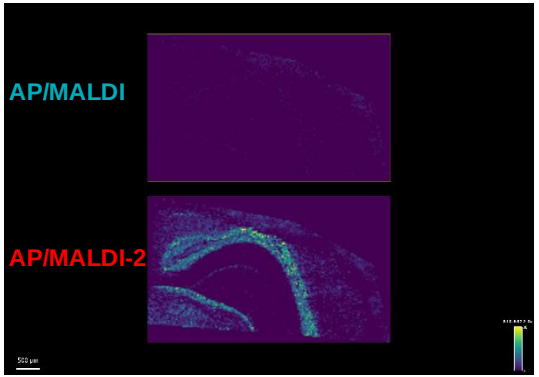
AP/MALDI-2



Niehaus, M., Soltwisch, J., Belov, M.E. et al. Transmission-mode MALDI-2 mass spectrometry imaging of cells and tissues at subcellular resolution. Nat Methods 16, 925–931 (2019). https://doi.org/10.1038/s41592-019-0536-2







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CONCLUSION S AND OUTLOOKS

Improvements and new challenges

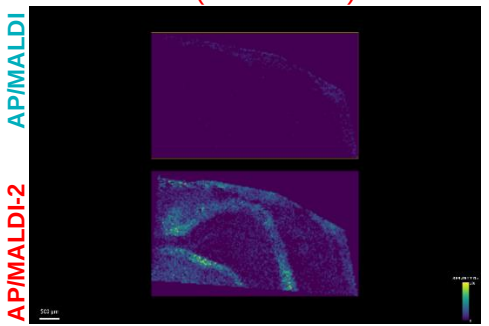
- **First plug-and-play AP/MALDI-2 module for Orbitrap**

- +30% of detected peaks
- 3x more annotations in Metaspace
- Boosted MALDI ionization + additional mechanism of ionization (REMPI)
  → benefit for e.g. pharmaceutical research, polymers…
- Compact/plug-and-play design

Perspectives

- ***Alignment and synchronization***
- ***Ion transmission***
- ***Optimization (lateral resolution)***

C27H45+ (Cholesterol)



AP/MALDI

Database	5%	10%	20%	50%
ChEBI-2018-01	1	3	44	174
KEGG-v1	1	1	2	27
HMDB-endogenous-v4	4	8	87	311
HMDB-v4	2	2	10	376
Total Annotations	8	14	143	888

AP/MALDI-2

Database	5%	10%	20%	50%
ChEBI-2018-01	3	4	5	1398
KEGG-v1	0	0	0	11
HMDB-endogenous-v4	4	5	8	475
HMDB-v4	5	7	7	832
Total Annotations	12	16	20	2716

Thank you for your attention

European Application & demo lab for MassTech AP/MALDI

Contact:
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AP/MALDI
European distributor

Venkat Panchagnula
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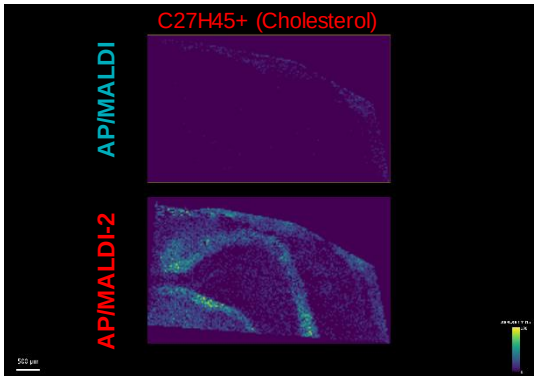
Konstantin Novoselov

Maureen Feucherolles,
Mathieu Gerard,
Olivier Bouton

Pr. Peter Verhaert

AP/MALDI VS AP/MALDI-2 IMAGING

Improvements and new challenges



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- ***Optimization (lateral resolution)***

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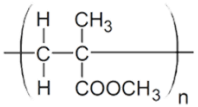
AP/MALDI HRMS ANALYSES FOR HIGH MW POLYMER POLYMERS

Case of poly(methylmethacrylate) MW=200kg/mol

Matrix= Unusual sample prep. for high MW polar polymers: nanomaterials!

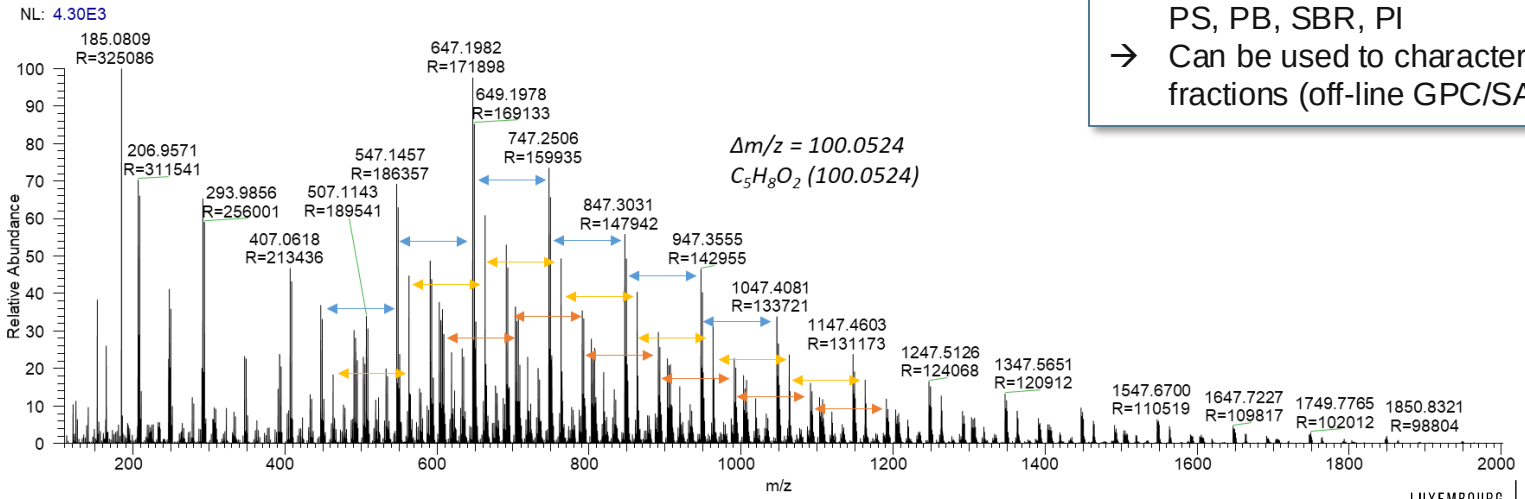
PMMA 200k

1 min. acquisition
BG subtract
240k reso.



AP/SALDI provides fragmentation fingerprint for High MW polymers by laser-induced photocatalytic degradation

- → Successfully tested on PLA, PMMA, PS, PB, SBR, PI
- → Can be used to characterize GPC fractions (off-line GPC/SALDI-MS)



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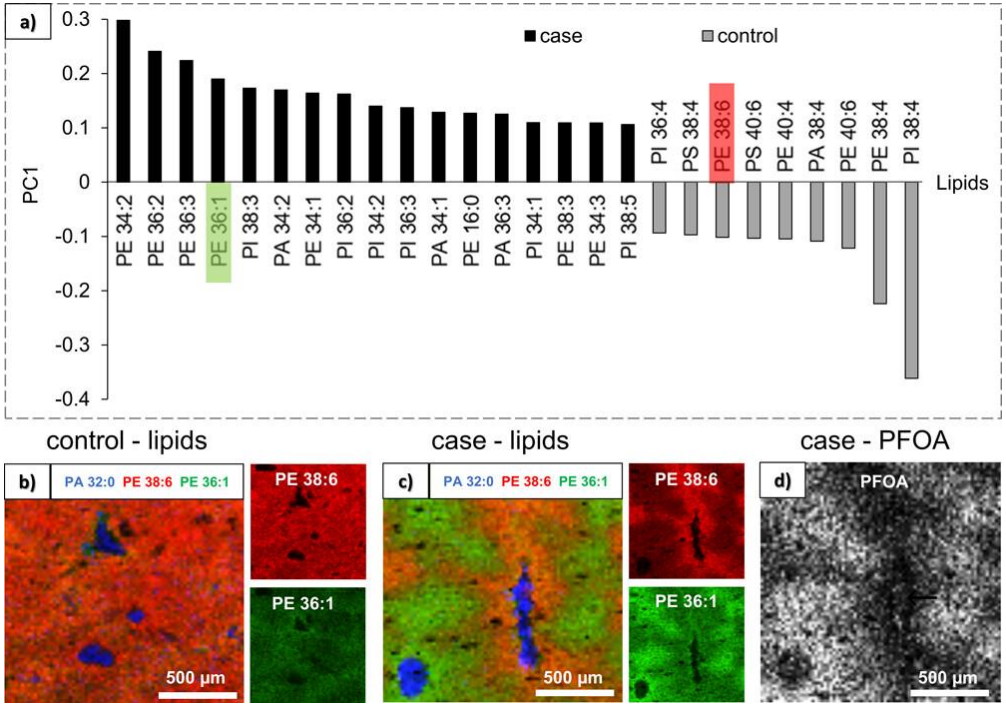
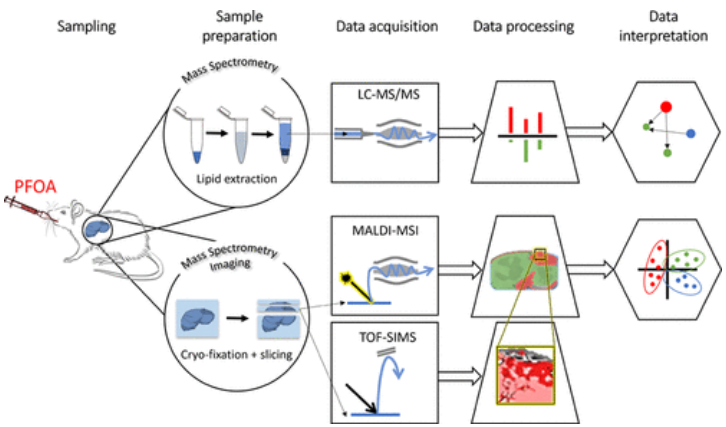
TOXICOLOGY STUDY

Perfluorooctanoic acid accumulation in liver





- **Lipid profile alteration co-localize with bioaccumulated PFOA**
- Good correlation between AP/MALDI imaging and LCMS analyses from extracts
- [10.1021/acs.analchem.2c05470]

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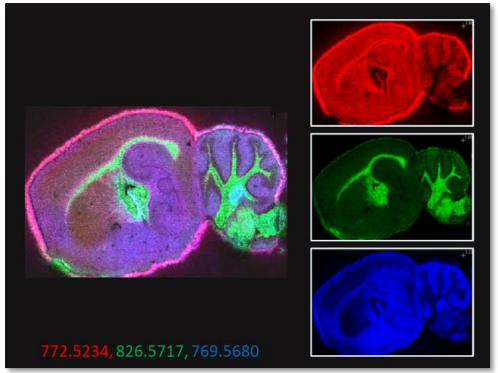
AP/MALDI HRMS imaging of lipids in a mouse brain section

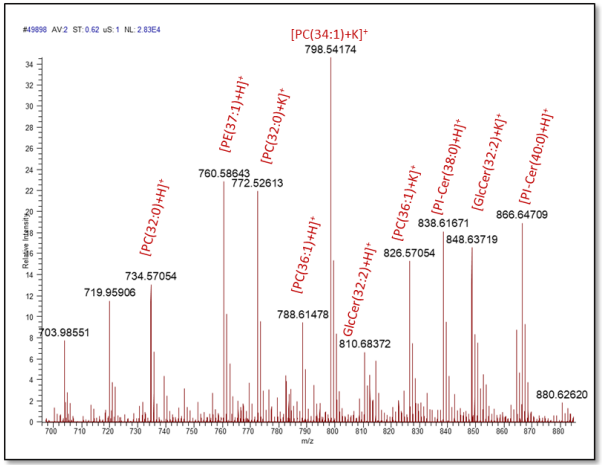
Usual sample prep. for lipid imaging = spray of α-cyano-hydroxycinnamic acid



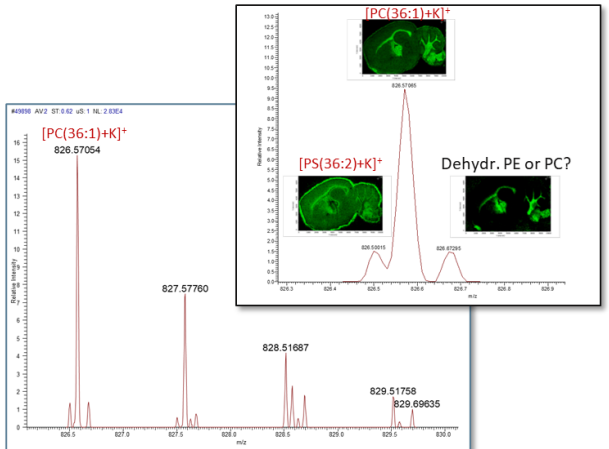
- WT mouse brain
- CSR imaging 20µm laser spot size

- AP/MALDI / Orbitrap:

→ High S/N
→ Resolution >60k at m/z400
→ +/-0.004 Da tolerance
→ 772.5234 = [PC(32:0)+K]+
→ 826.5717 = [PC(36:1)+K]+
→ 769.5680 = [PA 39:0+Na]+



Single pixel mass spectrum



- AP/MALDI / Orbitrap Elite :

→ Multiple lipids per mass unit
→ Separation and localization of isobaric lipids from various lipid classes

Sample courtesy Pr. William Griffiths, Swansea University

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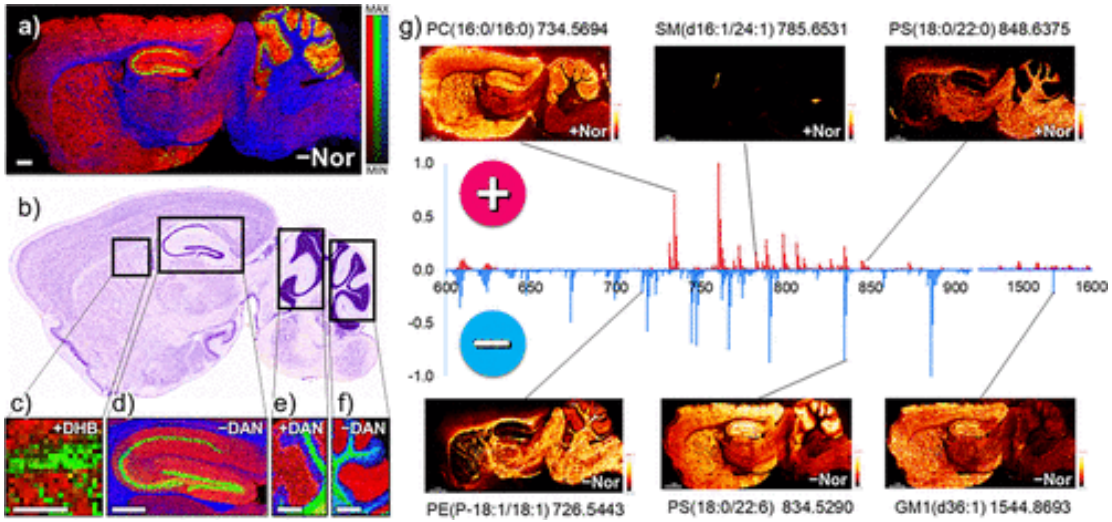
WHOLE BRAIN IMAGING

Sample preparations

Optimization of sample prep. for lipid imaging : CHCA, norharmane, DHB, DHAP, THAP, and DAN in combination with tissue washing and matrix additives



40 µm AP/MALDI imaging of sagittal brain sections (Norharmane matrix) in positive (red) and negative (blue) ion mode.

Spectral lipid region and MS images of various single lipid species displayed. (6mDa tolerance)

Optimized sample prep for positive and negative ion modes. Evaluation of wash/salts/matrices.

“Evaluation of 6 MALDI-Matrices for 10 µm Lipid Imaging and On-Tissue MSn with AP/MALDI-Orbitrap”, T.B. Angerer, J.Bour, J.-L. Biagi, E. Moskovets, and G. Frache, J. Am. Soc. Mass Spectrom. 2022, *33*, 5, 760–771

https://doi.org/10.1021/jasms.1c00327

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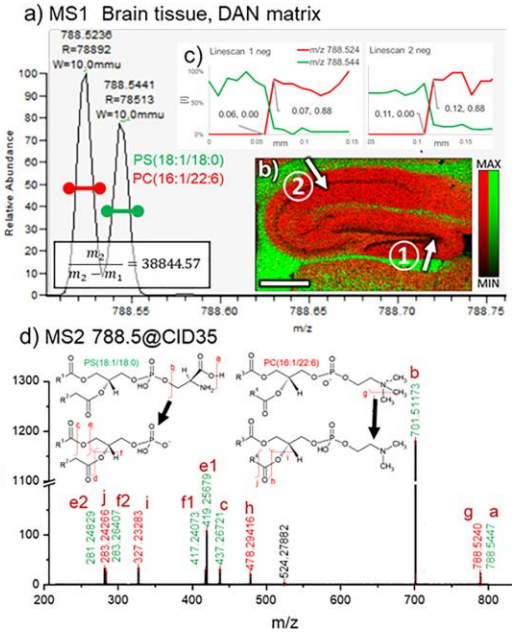
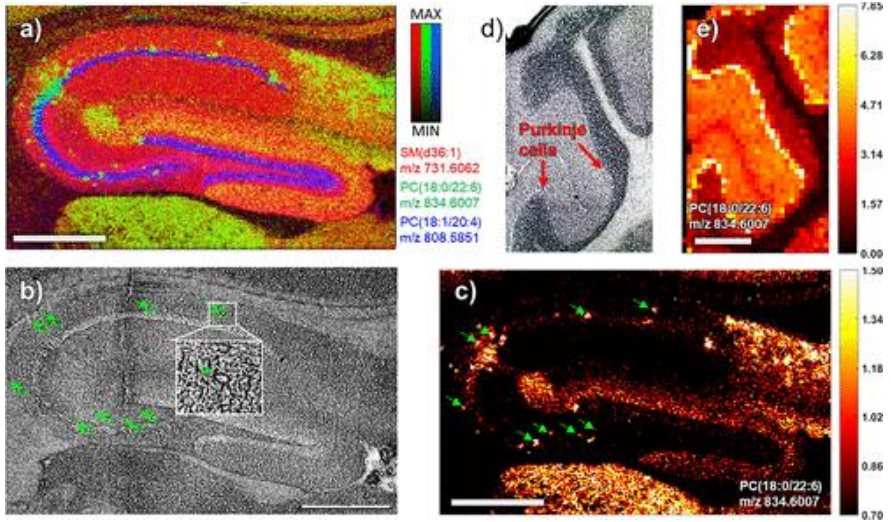
HIGH LATERAL RESOLUTION IMAGING

10um pixel size (Orbitrap Elite)

- (a) MS1 scan on brain tissue (matrix: DAN70, negative ion mode, showing *m/z* 788.5447 and *m/z* 788.5236.
- (b) Distribution of PS(18:1/18:0) (green) and PC(16:1/22:6)–CH_3 (red) in a **10 µm** AP/MALDI image, white arrows show the positions of linescans in (c) scale bar: 500 µm.
- (c) Two linescans, normalized to their individual maximum intensity (100%).
- (d) **On-tissue tandem-MS** analysis of *m/z* 788.5 ± 0.5 Da, containing PS (green) and PC (red) fragments.





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Range of MSI techniques techniques

Atmospheric Pressure Matrix Assisted Laser Desorption/Ionization (AP/MALDI) / Orbitrap

Time-Of-Flight Secondary Ion Mass Spectrometry IonTOF TOFSIMS.5

Dynamic Secondary Ion Mass Spectrometry Cameca NanoSIMS 50

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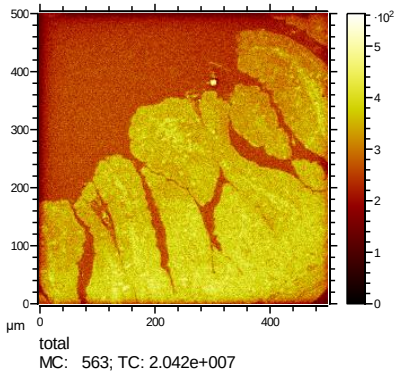
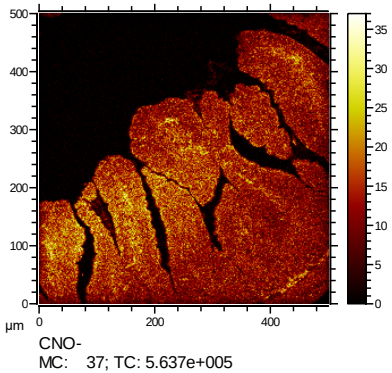
TOFSIMS imaging

Jejunum sections

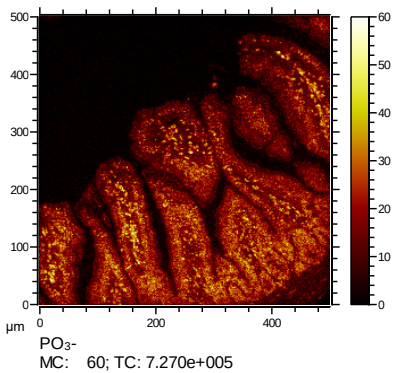
Label-free TOFSIMS imaging of FFPE



total
MC: 563; TC: 2.042e+007



CNO-
MC: 37; TC: 5.637e+005



PO_3^-
MC: 60; TC: 7.270e+005

Limitations of TOFSIMS for biological tissue imaging (label-free approach):

- high fragmentation rate of molecules,
- low mass resolution

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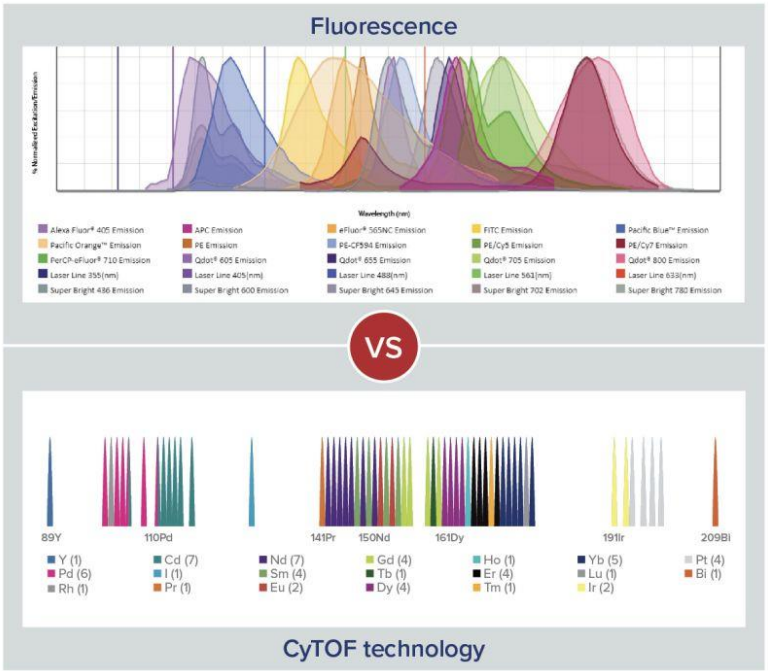
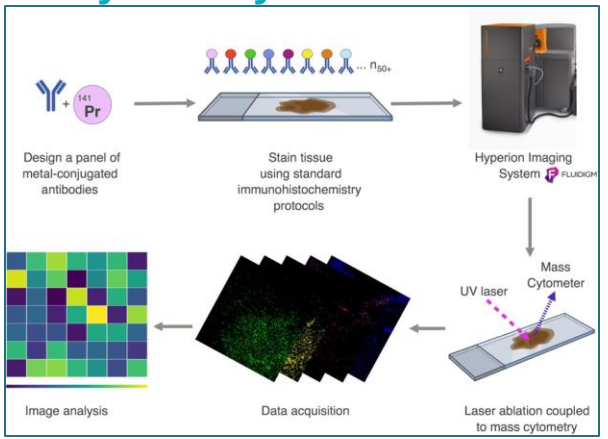
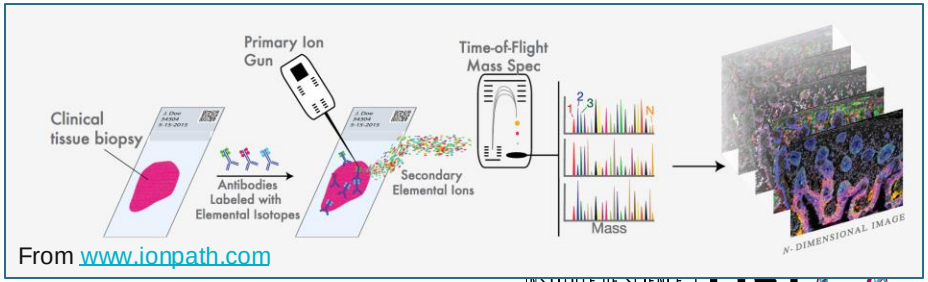
NEXT STEP

SIMS-based Imaging Mass Cytometry

Laser-based or SIMS-based Imaging Mass Cytometry







From www.ionpath.com

EXCELLENCE FOR IMPACT

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