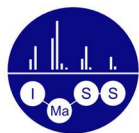


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2nd Italian MS-Imaging Symposium Event Schedule

March 5 th , 2026	
12:00-14:00	Registration & Welcome Coffee/Snack
14:00-14:15	Welcome & Introduction Giuliana d'Ippolito, Paola Montoro, Alice Passoni
SESSION 1 14:15-16:00	Title: Multi-imaging and interfacing with other analytical methods Chair: Andrew Smith, Fabiana Piscitelli
14:15-15:00	Invited Talk #1: Gregory Hamm (Clinical Pharmacology & Safety Sciences R&D, AstraZeneca, UK) Mass Spectrometry Imaging Integration Within Spatial Biology: A Pharmaceutical Perspective
15:00-15:20	Oral #1: Vanna Denti (Proteomic and Metabolomic Unit, University of Milano-Bicocca, IT) Exploring The Multi-Omic Landscape of Thyroid Nodules: A Retrospective Study
15:20-15:40	Sponsor Talk #1: Giulio Calza (Bruker Italia S.r.L.) Multiomics Analysis for Advanced Tumor Typing of Lung Cancer Using 116plex MALDI Hiplex-IHC And Released N-Glycans on the Neoflex
15:40-16:00	Oral #2: Nicole Monza (Proteomic and Metabolomic Unit, University of Milano-Bicocca, IT) Spatial Multi-Omics Maldi-Msi Reveals Molecular Signatures Across Breast Cancer Subtypes
16:00-16:30	Coffee Break & Poster Session

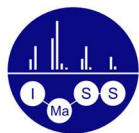


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2nd Italian MS-Imaging Symposium Event Schedule

March 5 th , 2026	
SESSION 2 16:30-18:15	Title: Environmental, animal, plant applications and bioinformatics Chair: Alice Passoni and Genoveffa Nuzzo
16:30-17:15	Invited Talk #2: Roberto Angelini (Medical School, Swansea University, UK) Imaging Cholesterol Metabolism in the Brain: Capabilities, Constraints, and Biological Interpretation
17:15-17:35	Oral #3: Riccardo Zecchi (Department of Neuroscience, University of Florence, IT) Quantitative And Spatial Distribution of Hormones in Kiwifruits Buds
17:35-17:55	Sponsor Talk #2: Angelo Palmese (Waters Corporation) Desi MS-Imaging – High Mass And Image Resolution at Speed on a Bench Top Mass Spectrometer
17:55-18:15	Oral #4: Vasco Coelho (Department of Informatics, University of Milano-Bicocca, IT) Co-Registration of Mass Spectrometry and Optical Whole Slide Histology Images for Computational Pathology
18:30	Transfer from Centro Congressi UniNa to Hotel Royal Continental/Borgo Marinari
20:15	Social Dinner at “Club Nautico della Vela”, Borgo Marinari



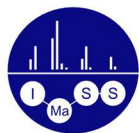
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2nd Italian MS-Imaging Symposium Event Schedule

March 6 th , 2026	
8:30	Transfer from Hotel Royal Continental to Centro Congressi UniNa Monte Sant'Angelo
9:15-9:30	Presenting the International Mass Spectrometry Imaging Society (IMSIS) Liam McDonnell (Fondazione Pisana per la Scienza, IT)
SESSION 3 9:30-11:15	Title: Emerging and innovative technologies, platforms and applications (part I) Chairs: Liam Mc Donnell, Barbara Cardinali
9:30-10:15	Invited Talk #3: Zoltan Takats (Imperial College London-UK and University of Regensburg-DE) LA-REIMS: A Promise for Quantitative Imaging at Subcellular Resolution
10:15-10:35	Oral #5: Marcello Ziaco (CNR, Institute of Biomolecular Chemistry, Naples, IT) Brain Lipid Distribution Under Different Dietary Conditions Using DESI-MSI
10:35-10:55	Sponsor Talk #3: Maria Grazia Di Lago (Shimadzu) Characterization Of Diabetic Encephalopathy Via Mass Spectrometry: Integration Between LC-MS and MS Imaging
10:55-11:40	Coffee Break & Poster Session
SESSION 4 11:40-13:00	Title: Emerging and innovative technologies, platforms and applications (part II) Chairs: Liam Mc Donnell, Barbara Cardinali
11:40-12:00	Oral #6: Emanuela Salviati (Department of Pharmacy, University of Salerno, IT) Breaking BMP/PG Ambiguity in Spatial Lipidomics by Ion Mobility MALDI-MSI
12:00-12:20	Sponsor talk #4: Vanna Denti (Ambergen) Single-Slide Detection and Typing of Renal Amyloidosis: A Step Toward the Clinic Application of MALDI-MSI"
12:20-12:40	Oral #7; Marta Berzaghi (Department of Food and Drugs, University of Parma, IT) Mapping The In Vivo Metabolic Fate of Deuterated (+)-Catechin-D ₄ in Mice Using DESI-MSI and LC-MS/MS
12:40-13:00	Sponsor Talk #5: Une Kontrimaite (KR Analytical) Mapping Metabolic Crosstalk Between Glioblastoma and Astrocytes Using Integrated Single-Cell AP-Maldi and LC-MS Metabolomics
13:00-14:00	Lunch

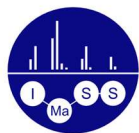


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March 6 th , 2026	
SESSION 5 14:00-15:25	Title: Medical, diagnostic and pharmaceutical applications Chairs: Giuliana d'Ippolito and Riccardo Zecchi
14:00-14:45	Invited Talk #4: Nicole Strittmatter (Department of Biosciences, Technical University of Munich, DE) Studying Microbial Communities Using Mass Spectrometry Imaging
14:45-15:05	Oral #8 Alessia Di Noi (Department of Chemistry, "Sapienza" University of Rome, IT) Repurposing PBS-PFA-Perfused/OCT-Embedded Glioblastoma Mouse Brain Sections for Mapping Lipid Heterogeneity By AP-MALDI-MSI
15:05-15:25	Oral #9 Lucia Martin Saiz (DMPK, Institut de R&D Servier Paris-Saclay, FR) Quantitative Imaging Mass Spectrometry of Glutamate in Rat Brain
15:25-15:45	Final Remarks and Congress closure Giuliana d'Ippolito & Scientific Committee



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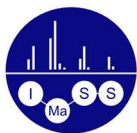


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2nd Italian MS-Imaging Symposium

Poster contributions

Poster session	
Poster #1	Eugenia Pechkova (University Genova, IRCCS) Maldi Imaging Mass Spectrometry at IRCCS Azienda Ospedaliera Metropolitana (AOM), Genova
Poster #2	Giusi Barra (Institute of Biomolecular Chemistry ICB, National Research Council CNR) Single-Section Multimodal Analysis by Desi-MSI and Imaging Mass Cytometry
Poster #3	Amelia Locatelli (Department of Environmental Health Sciences, Istituto di Ricerche Farmacologiche Mario Negri IRCCS) Surface-Specific Metabolic Signatures of Flavescence Dorée in Grapevine Leaves Revealed by MSI
Poster #4	Giovanni Andrea Vitale (Institute of Biomolecular Chemistry ICB, National Research Council CNR) An Integrated LC-MS and DESI-MSI Workflow for Spatial Metabolomics in Medaka
Poster #5	Andrew Smith (University of Milano-Bicocca) Mapping Drought-Driven Metabolic Adaptations in Coffee Fine Roots by MALDI-MS Imaging
Poster #6	Natalia Shelly Porto (University of Milano-Bicocca) Mass Spectrometry Applications for the Characterization of Kras Mutations in Lung Cancer
Poster #7	Chiara Calabretta (IRCCS Milano) Molecular Histology in Epilepsy Surgery
Poster #8	Giulio Calza (Bruker) Multiomics Analysis for Advanced Tumor Typing of Lung Cancer Using 116plex MALDI Hplex-IHC and Released N-Glycans on the Neoflex



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Mass Spectrometry Imaging integration within spatial biology: A Pharmaceutical perspective

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Mass spectrometry imaging (MSI) provides label-free, spatially resolved molecular maps that connect pharmacology to pathology. In pharmaceutical R&D, MSI complements spatial biology by simultaneously visualizing drugs, metabolites, and endogenous molecules at micrometer resolution, delivering integrated insights into biodistribution, efficacy, safety, and disease biology within the same tissue. A focused case study in prostate cancer (1,2,3) will demonstrate how MSI resolves spatial complexity across disease grades. Integrating MSI with digital pathology uncovers grade-dependent metabolic rewiring, forming distinct molecular neighbourhoods. We show how distributions metabolites intersect with glandular architecture, and how these features correlate with response patterns and resistance phenotypes. This case underscores MSI's ability to unify pharmacology with tumour ecology, guiding biomarker strategies, refine patient selection and rational combination. Looking ahead, we will discuss multi-omics integration with spatial transcriptomics notably (4), MSI in advanced cellular models (in-vitro cell culture using SpaceM approach), and AI for ionization prediction, advanced MS topographic images analysis and atlas generation.

References:

1. Imaging tumor lactate is feasible for identifying intermediate-risk prostate cancer patients with postsurgical biochemical recurrence. Sushentsev, N., et al., Proc Natl Acad Sci U S A, 2023..
2. Metabolic imaging across scales reveals distinct prostate cancer phenotypes. Sushentsev N, Hamm G, et al. Nature Communications 2024
3. Spatial metabolomics informs the use of clinical imaging for improved detection of cribriform prostate cancer. Sushentsev, N. et al., Proc Natl Acad Sci U S A, 2025.
4. Williams, E. C., et al. (2026). Nature communications 17(1): 205.

EXPLORING THE MULTI-OMIC LANDSCAPE OF THYROID NODULES: A RETROSPECTIVE STUDY

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² Department of Informatics, Systems and Communication, University of Milano-Bicocca, Milan, (Italy);

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Indeterminate lesion diagnosis in thyroid nodules remain an open problem for the risk of patient over-treatment [1]. In this context multi-omic analyses able to maintain the native spatial information could represent an unprecedented source of molecular information to assist clinicians in patients' stratification. Here we analysed two archival Tissue Microarrays (TMA) including thyroid nodules from around 80 patients with different diagnosis. Sequential MALDI-MSI of N-glycans and tryptic peptides were acquired with a timsTOF flex operating in positive ion mode and at a spatial resolution of 20 $\mu\text{m}/\text{px}$. Following MSI acquisition, slides were H&E stained and whole-slide images were used to automatically define regions of interest (ROIs) through a pixel-based classifier, as described in Coelho et al [2]. N-glycans digestion significantly enhanced spectral quality by minimizing colloid-derived interference, allowing more robust spatial molecular profiling. The tryptic peptides observed in the second molecular layer were more abundant in the stromal component of each core. Additionally, the automated ROI selection based on histological features ensured consistent and reproducible data extraction. The integration of N-glycans and tryptic peptides revealed distinct molecular signatures associated with different thyroid lesion types, highlighting alterations in glycosylation patterns alongside proteomic changes. Despite the need for further investigations, the proposed workflow opens new avenues to explore molecular heterogeneity in tissue neoplasms, enhancing the diagnostic potential of challenging thyroid lesions.

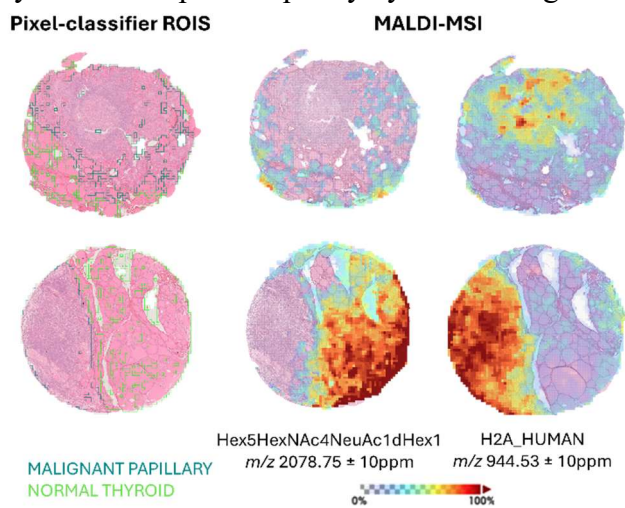


Fig. 1: The spatial distribution of one N-glycan and a tryptic peptide is shown. The first co-localised in the normal tissue, the last in the tumor area.

References:[1] Nikiforov, Y. E. et al. JAMA oncology, 2.8 (2016), pp. 1023-1029; [2] Coelho, V. et al., Journal of Proteome Research, (2026).

Acknowledgements: Funding: Fondazione Cariplo (2023-1804).

Getting the most out of Spatial Multiomics Data with SCiLS Lab: iprm-PASEF and a case study on human squamous cell carcinoma of the lung

Giulio Calza^{1*}

Janina Oetjen¹, Corinna Henkel¹, Giulio Calza^{2*},

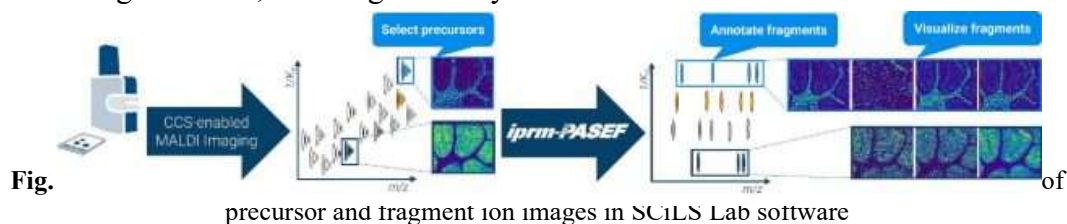
Nannan Tao³, Mark Lim³, Gargey Yagnik⁴, Andrew Yatsuhashi⁴, Kenneth Rothschild^{4,5},
Mengze Stettler-Zhang^{6,7}, Konrad Steinestel⁸, Bernd Bodenmiller^{6,7}

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⁴ AmberGen Inc., 44 Manning Road, Billerica, Massachusetts 01821; ⁵ Department of Physics and Photonics Center, Boston University, Boston, Massachusetts 02215; ⁶ University of Zurich, Zurich, Switzerland; ⁷ ETH Zurich, Zurich, Switzerland; ⁸ Bundeswehrkrankenhaus Ulm, Ulm, Germany;

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Spatial multiomics approaches are becoming more prevalent in probing molecular mechanisms underlying diseases and therapies. MALDI Imaging plays a pivotal role by uncovering spatial patterns across various omics layers within tissue samples. To improve precision in molecular identification with MALDI MSI, iprm-PASEF® (imaging parallel reaction monitoring Parallel Acquisition Serial Fragmentation) is a new tool that brings confidence to assigning molecular structures to features. The addition of MS/MS data to m/z values, isotope patterns, and ion mobility-derived CCS values, makes spectral matching available, allowing for analysis on the SCiLS Lab Software suite.



An example of data integration for human squamous cell carcinoma samples utilizing SCiLS Lab is also presented: by integrating spatial proteomics (HiPlex-IHC, 116-plex) with Spatial Glycomics and H&E staining it was possible to define the clustering based on the HiPlex markers and characterize the glycan environment within those clusters, differentiating the glycan signature of tumor microenvironment (TME) from the metabolically active cancer, driving hypothesis on the effect of sialylation and fucosylation in immune evasion, tumorigenesis and progression.



SPATIAL MULTI-OMICS MALDI-MSI REVEALS MOLECULAR SIGNATURES ACROSS BREAST CANCER SUBTYPES

Nicole Monza¹, Seyed M. J. Seyed Golestan², Farnaz Fatahian², Lisa Pagani¹, Greta Bindi¹, Mohammad A. AS`habi², Hossein Behboudi², Andrew Smith¹, Alireza Ghassempour², and Vanna Denti¹.

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The pronounced molecular heterogeneity of breast cancer (BC) represents a major challenge for accurate classification and biological characterisation [1]. Despite extensive efforts, conventional immunohistochemical approaches and traditional mass spectrometry (MS) techniques have not achieved a comprehensive characterisation of tumour subtypes, largely due to the loss of spatial information that is critical for data interpretation [2-3].

In this study, we present a Matrix-Assisted Laser Desorption/Ionisation Mass Spectrometry Imaging (MALDI-MSI) strategy that spatially integrates three complementary omics layers—lipids, N-glycans, and tryptic peptides—on the same tissue microarray (TMA) section containing BC and normal tissue cores, for a total of 16. Analysis of both individual molecular layers and their integrated datasets demonstrate the ability of multi-omics MALDI-MSI to clearly discriminate between healthy and tumour tissues and to capture molecular features associated with distinct BC subtypes.

Notably, lipidomic and glycomic profiles were significant in classifying breast cancer subtypes, while the proteomic layer offered additional information on tumour proliferation and biological variability, successfully differentiating neoplastic from non-neoplastic tissue.

This proof-of-concept study demonstrates the promise of spatial multi-omics MALDI-MSI as a robust instrument for the comprehensive characterisation of BC subtypes, facilitating future applications in larger and clinically significant cohorts.

References:

[1] Cheung AM, Wang D, Quintayo MA, et al. *Breast Cancer Res.* 2025;27(1):88. doi:10.1186/s13058-025-02038-1 N.

[2] Doud EH, Yeh ES. *Technol Cancer Res Treat.* 2023; 22:15330338221148812. doi:10.1177/15330338221148811

[3] Raj-Kumar PK, Lin X, Liu T, et al. *Breast Cancer Res.* 2024;26(1):76. doi:10.1186/s13058-024-01835-4

Acknowledgements:

This work was supported by the National Plan for PNRR Complementary Investments (PNC, established with the decree-law 6 May 2021, no. 59, converted by law no. 101 of 2021) in the call for the funding of research initiatives for technologies and innovative trajectories in the healthcare sectors (Directorial Decree no. 931 of 06 – 06 – 2022) - project no. PNC0000003 - Advanced Technologies for Human-centred Medicine (project acronym: ANTHEM) and the Associazione Italiana Ricerca sul Cancro Associazione Italiana Ricerca sul Cancro Grant -AIRC- MFAG 2024 ID. 31092, by the Iran National Science Foundation (INSF, project No. 4026064) and by the Ministry of Science, Research and Technology of Iran through its program supporting PhD research initiatives and international sabbatical activities.



Imaging Cholesterol Metabolism in the Brain: Capabilities, Constraints, and Biological Interpretation

Roberto Angelini¹

1 Institute of Life Sciences, Faculty of Medicine, Health and Life Sciences, Swansea University, Swansea, UK.

Cholesterol plays a central role in brain structure and function, yet its spatial analysis by mass spectrometry imaging (MSI) has long been limited by poor ionisation and analytical artefacts. As a result, multiple chemical and instrumental strategies have emerged, each involving trade-offs between sensitivity, specificity, spatial resolution, and biological interpretability. In this lecture, I will discuss complementary MSI approaches developed to study cholesterol metabolism in brain tissue. I will first present an enzyme-assisted, on-tissue derivatisation MALDI-MSI workflow that enables quantitative imaging of cholesterol with preserved neuroanatomical fidelity (1), providing a robust readout of pathway activity despite limited ability to resolve individual cholesterol precursors. I will then introduce liquid extraction surface analysis (LESA) as an orthogonal strategy that sacrifices spatial resolution in exchange for chemical specificity (2), enabling quantitative separation of cholesterol precursors and low-abundance oxysterols. Finally, I will place these methods in the context of recent high-resolution, label-free sterol imaging approaches (3), including MALDI-2 post-ionisation (4), highlighting both their potential and their interpretative limitations arising from cholesterol-derived in-source fragmentation. Overall, the presentation will emphasise that meaningful biological insight into cholesterol metabolism requires a clear understanding of methodological constraints and the deliberate integration of spatial and chemical information.

References:

- [1] Angelini, R., et al. (2021). Visualizing Cholesterol in the Brain by On-Tissue Derivatization and Quantitative Mass Spectrometry Imaging. *Analytical chemistry*, 93(11), 4932–4943.
- [2] Yutuc, E., et al. (2020). Localization of sterols and oxysterols in mouse brain reveals distinct spatial cholesterol metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, 117(11), 5749–5760.
- [3] Griffiths, L., et al. (2025). Sterol imbalances and cholesterol-24-hydroxylase dysregulation is linked to the underlying progression of multiple sclerosis. *Brain pathology (Zurich, Switzerland)*, 35(5), e70001.
- [4] Sidorov, I., et al. (2026). In-Source Fragmentation Annotation in Sterol Mass Spectrometry Imaging. *Analytical chemistry*, 98(1), 509–518.

QUANTITATIVE AND SPATIAL DISTRIBUTION OF HORMONES IN KIWIFRUIT BUDS.

Riccardo Zecchi^{*1,2}, Alessandra Francini³, Andrea Raffaelli³, Giuseppe Pieraccini², Giancarlo la
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Phytohormonal regulation is central for floral bud development, integrating environmental and endogenous cues through a complex interplay^[1]. Bud development in plants, including kiwifruits (*Actinidia chinensis*), depends on the balance between growth-promoting signals, such as auxins, cytokinins, and gibberellins, and inhibitory signals, such as abscisic acid, whose integration establishes the developmental fate of buds. This

study aims to investigate the possibility offered by HPLC-MS/MS and MALDI-MSI in investigating the phytohormone landscape in kiwifruits bud. To this aim, buds at initial (IN) and late (LT) phase of development were used to prove if there is a spatiotemporal modification of key hormones. Chemical derivatization with Girard's reagent T^[2] and 4-APEBA^[3] assured detection of several phytohormones and related biosynthesis precursors and metabolites. Results confirm the presence of abscisic acid and its decreasing trend from the IN to the LT phases, and the presence of jasmonates and salicylates and their increase from the IN to the LT phases.

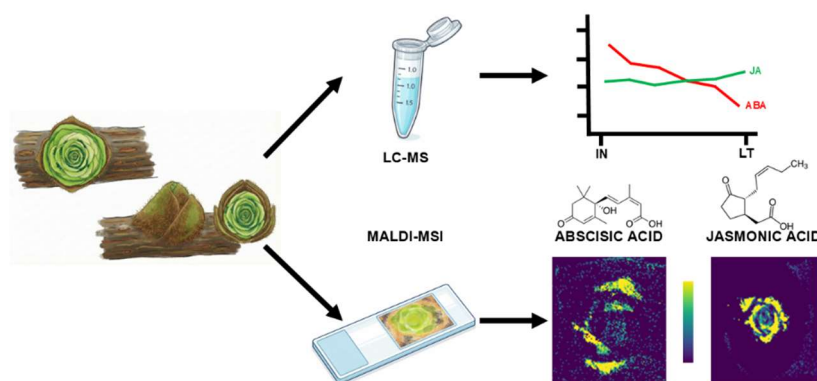


Fig.1: Experimental workflow for the phytohormone analysis of kiwi buds. Quantification was carried out with HPLC-MS/MS and MALDI-MSI provide their localization.

References:

- [1] Horvath D. P., Anderson J. V., Chao W. S., Foley M. E. (2003) Trends in Plant Science, Vol. 8, Issue 11, pp 534-540.
[2] Enomoto H., Sensu T., Yumoto E., Yokota T., Yamane H. (2018). Rapid Comm in Mass Spec, 32(17), pp 1565–1572.
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DESI MS-IMAGING – HIGH MASS AND IMAGE RESOLUTION AT SPEED ON A BENCH TOP MASS SPECTROMETER

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Mouse brain and porcine liver sections (18 μm) were imaged using the Waters Imaging App on a XEVO™ MRT Q-ToF mass spectrometer within the waters_connect™ environment. Acquisition speeds ranged from 5–100 Hz, demonstrating retention of mass resolution and accuracy in untargeted and semi-targeted (EDC) modes. For high-resolution imaging, an ACQUITY M-class Binary Solvent Manager with a low-flow kit enabled <250 nL solvent flow, achieving 5 μm pixel size. This low flow supports multiple passes over the same region, as shown in microscope mode: a survey image at low resolution followed by targeted high-resolution imaging.

The XEVO™ MRT Q-ToF, a bench-top folded flight path instrument, routinely delivers up to 100,000 FWHM mass resolution and sub-500 ppb mass accuracy. Coupled with a DESI XS source, it facilitates MS imaging workflows. Its electronic architecture minimizes inter-scan delay to 2 ms, maintaining a 90% duty cycle at 50 Hz. Mass resolution remains unaffected by scan speed, and the DESI source's continuous raster ensures pixel size does not impact acquisition speed. Imaging at 100 Hz for MS and MS/MS was demonstrated, alongside high image resolution at 5 μm pixel size, achieving <10 μm spatial resolution.

The DESI source is integrated via a new software interface compatible with waters_connect™, enabling seamless LC-MS and DESI XS imaging workflows. These capabilities allow rapid, high-resolution imaging without compromising mass accuracy or resolution, supporting advanced untargeted and targeted analyses across diverse tissue types.



Fig. 1:
DESI XS and Xevo MRT Mass
Spectrometer



CO-REGISTRATION OF MASS SPECTROMETRY AND OPTICAL WHOLE SLIDE HISTOLOGY IMAGES FOR COMPUTATIONAL PATHOLOGY

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Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) enables interrogating the molecular composition of tissue with ever-increasing resolution and sensitivity. The output of a MALDI-MSI analysis is a sparse 3D tensor depicting the spatial distribution and relative ion intensity of molecular features differentiated by mass-to-charge ratio. Optical whole slide images (WSIs) can provide morphological characteristics of the same tissue section, due to the non-destructive nature of MALDI-MSI. The bimodal analysis of the morpho-molecular signal might enhance downstream diagnostic tasks and offer crucial support to pathologists [1, 2]. We propose an operator-free and data-efficient co-registration toolbox using the ANTsX ecosystem [3]. We develop and validate an intensity-based method, inspired by the work of W.M. Abdelmoula et al. [4], to estimate the affine transform parameters linking the coordinate systems of the two spatially-resolved modalities: the MALDI-MSI (.imzML) and the WSI (e.g., .tif, .svs); additionally, we also linked them to the SCiLS Lab closed-source software (Bruker Daltonics, Germany). We tested the method on more than 50 image pairs from FFPE tissue sections, comprising lung and thyroid tissue microarrays (TMAs), kidney and thyroid resections, and organoids. The molecular data is acquired with *tims TOF fleX* and *rapifleX* instruments at a spatial resolution of 20 and 50 μm . Compared to manual co-registration, in two thyroid TMAs of 86 patients, we achieved higher cell coverage (95 wins out of 132 cores) within histone-expressing regions and higher connective cells coverage (78 wins out of 118 cores) within collagen-expressing regions.

References: [2] B. Balluff, et al. *An overview of image registration for aligning mass spectrometry imaging with clinically relevant imaging modalities*. *JMSACL*, 23 (2022) 26-38; [1] V. Coelho, et al., Improving the Annotation for Spatial Proteomics: A Computational Approach to Enhance Molecular Characterization of Thyroid Nodules, *J. Proteome Res.*, (2026); [3] <https://github.com/ANTsX/ANTs>; [4] W.M. Abdelmoula, et al. *Automatic Generic Registration of Mass Spectrometry Imaging Data to Histology Using Nonlinear Stochastic Embedding*. *Anal. Chem.*, 86 (2014), 9204-9211.

Acknowledgements: The proteomics and metabolomics unit at UniMiB, guided by Prof. Fulvio Magni. The cancer molecular pathology unit at Fondazione IRCCS San Gerardo dei Tintori, guided by Prof. Fabio Pagni. Funding: Fondazione Cariplo (2023-1804).



LA-REIMS: A Promise for Quantitative Imaging at Subcellular Resolution

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Laser ablation Rapid Evaporative Ionisation Mass Spectrometry (LA-REIMS) was originally developed as an analytical technology to monitor cancer surgery interventions using surgical lasers. The technique was quickly developed into a mass spectrometric imaging platform due to the need for histologically annotated datasets. These datasets are used for building statistical classifiers enabling real-time tissue classification during surgical interventions. While the spatial resolution and sensitivity of the imaging LA-REIMS was limited using standard surgical CO₂ lasers, both parameters showed significant improvement by using short pulse width (nanosecond – picosecond) lasers operating at or close to 2.94 μm . Good beam quality picosecond lasers were found to provide sensitivity and spatial resolution values competitive with commercially available MALDI systems. Data covering murine central nervous system and human cancers will be presented and discussed. Unlike DESI (and to some extent MALDI), LA-REIMS is able to completely ablate the tissue off the support, leaving no residue, enabling a number of experimental approaches including oversampling. Using significant oversampling with a beam diameter of 5-8 μm , <3 μm spatial resolution was achieved, allowing the clear visualisation of cellular nuclei in various tissues. The exhaustive ablation capability also allows the ablation of known amounts of tissue. Since the analyte is not distributed across tissue and matrix layers, this setup – in principle – can also be used for quantitative imaging. Initial attempts to test the linearity of the method will be presented and necessary steps towards global quantitative imaging will be discussed.



BRAIN LIPID DISTRIBUTIONS UNDER DIFFERENT DIETARY CONDITIONS USING DESI MASS SPECTROMETRY

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Lipids play fundamental roles in brain structure and function, contributing not only to membrane architecture but also to cellular signaling, energy metabolism, and neuroimmune regulation. Alterations in lipid homeostasis have been increasingly associated with metabolic disorders and their impact on brain physiology [1]. However, understanding how dietary interventions influence lipid organization within specific brain regions remains a significant analytical challenge, particularly in preserving the spatial context of molecular changes [2].

Mass spectrometry imaging (MSI) has emerged as a powerful tool for the investigation of molecular distributions directly in tissue sections [3]. In this context, we have developed an optimized tissue preparation workflow enabling reliable detection and spatial characterization of diverse lipid species in brain sections using desorption electrospray ionization (DESI) MSI [4].

Building upon this analytical platform, we applied DESI-MSI to investigate the brain lipid landscape in mice subjected to different dietary regimens, comparing standard diet and high-fat diet conditions. This approach enables the spatial visualization of lipid species across anatomically defined brain regions while preserving their native molecular context. These analyses provide a foundation for ongoing and future studies integrating spatial lipidomics with complementary multimodal approaches, including mass cytometry, to explore lipid-associated processes in metabolically relevant brain models.

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CHARACTERIZATION OF DIABETIC ENCEPHALOPATHY VIA MASS SPECTROMETRY: INTEGRATION BETWEEN LC-MS AND MS IMAGING

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Neurological complications targeting the central nervous system (CNS) in diabetic patients are collectively referred to as diabetic encephalopathy (DE), a condition encompassing characteristic biochemical, neurophysiological, morphological, and cognitive deficits. Impairments in learning, memory, problem-solving, and mental and motor speed are significantly more common in diabetic patients than in the general population. Notably, these diabetes-induced neurological changes are associated with an increased risk of dementia, seizures, stroke, and Alzheimer's disease, which is twice as prevalent in the diabetic population as in non-diabetic subjects. It is well established that specific brain regions (i.e. the hypothalamus, cerebellum, hippocampus, and cerebral cortex) are extremely sensitive and exhibit differential responses to glucose homeostasis alterations, given their involvement in distinct cognitive, motor, and neuroendocrine activities. We performed a longitudinal analysis (i.e. short and long term) in a preclinical model of Diabetes, of the four brain regions (i.e. hypothalamus, cerebellum, hippocampus and cerebral cortex). Targeted LC-MS/MS quantification of a panel of 180 metabolites revealed region-specific metabolic signatures associated with the disease, thereby highlighting differential vulnerability and adaptive responses within the CNS. To build on these findings and map these localized biochemical alterations, the current study employs high-resolution mass spectrometry imaging (MSI) using the iMScope QT platform. Integrating targeted LC-MS quantification with spatial metabolomics via MSI represents a powerful, multilevel strategy for characterizing diabetic encephalopathy. This combined approach enables us to bridge the gap between absolute concentration variations and their precise anatomical distribution, providing novel spatial insights into the region-specific pathophysiology of the diabetic brain.

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BREAKING BMP/PG AMBIGUITY IN SPATIAL LIPIDOMICS BY ION MOBILITY MALDI-MSI

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Bis(monoacylglycero)phosphates (BMPs) are a rare glycerophospholipid class involved in lysosomal function and lipid sorting. Their low abundance and structural similarity to phosphatidylglycerols (PGs) make confident in situ detection and spatial characterization challenging. Accurate mapping of BMPs in tissues is therefore essential for understanding lipid dysregulation in lysosomal disorders and related pathologies.

Here, we present an ion mobility-enabled MALDI-MSI strategy for the spatially resolved discrimination of BMPs and PGs directly in tissue. Negative-ion mode and optimized matrices were used to enhance BMP signals, and tissue preparation strategies were tested to minimize interference from co-localized lipids. A targeted iPRM-PASEF acquisition strategy was employed, based on prior HILIC-DDA PASEF characterization, to selectively isolate and fragment specific BMP and PG pairs, providing higher confidence in precursor assignment and structural annotation. Preliminary data show that BMP and PG precursors can be selectively isolated and fragmented using mobility-constrained iPRM windows, which is not possible by MALDI-TIMS-MSI alone. This approach ensures high-quality MS/MS spectra on tissue, further resolving type-II isobaric overlap, and allows confident annotation of low-abundance BMPs. On-tissue standard spotting experiments confirmed reproducibility and specificity of precursor selection.

Overall, this workflow demonstrates the potential of ion mobility-enhanced targeted MALDI-MSI to provide spatially resolved insights into rare lipid species. These results establish a methodological framework for future studies of lysosomal lipid metabolism and may be extended to other challenging lipid classes in situ.



MAPPING THE *IN VIVO* METABOLIC FATE OF DEUTERATED (+)- CATECHIN-D₄ IN MICE USING DESI-MSI AND LC-MS/MS

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Despite extensive studies on flavan-3-ol plasma pharmacokinetics in humans and rodents, there is scarce quantitative data on their organ-specific tissue distribution and spatial localization. This study aimed to map the gastrointestinal transit, microbial and enzymatic transformation, and systemic distribution of orally administered deuterated (+)-catechin-d₄ in mice.

Quantitative LC-MS/MS analysis of tissue extracts was combined with DESI-MSI on stomach, small intestine, colon, liver, and kidney tissues collected at different time points. Stability experiments assessed how pH levels affect de-deuteration, leading to the formation of (+)-catechin-d₂ in the acidic environment of the stomach. The administered compound showed rapid gastrointestinal transit followed by phase II metabolism and microbial transformation in the colon to phenyl- γ -valerolactones. DESI-MSI revealed the metabolite distribution *in situ* at time points and organs corresponding to the highest or near-highest concentrations quantified by LC-MS/MS, while the traditional LC approach reconstructed organ-specific metabolic and kinetic profiles.

This study provides new information on how metabolites are distributed in specific organs, adding depth to the understanding of flavan-3-ol pharmacokinetics.

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MAPPING METABOLIC CROSSTALK BETWEEN GLIOBLASTOMA AND ASTROCYTES USING INTEGRATED SINGLE-CELL AP-MALDI AND LC-MS METABOLOMICS

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Glioblastoma is an aggressive brain tumour that, despite multimodal treatment, almost always recurs. Infiltrative tumour cells within the brain microenvironment are thought to drive recurrence, supported by surrounding non-cancerous brain cells. Among these, astrocytes, a type of glial cells that maintain neuronal and metabolic balance in the brain, have been implicated in promoting tumour progression. However, their metabolic interactions with glioblastoma cells remain poorly understood due to the difficulty of resolving cell-specific metabolism.

Here, we combined microscopy-guided atmospheric pressure matrix-assisted laser desorption/ionisation mass spectrometry imaging (AP-MALDI MSI) with liquid chromatography–MS (LC–MS) to investigate metabolic relationship between patient-derived glioblastoma invasive margin (GIN) cells and human astrocytes. AP-MALDI coupled with high-resolution MS at 10 μm spatial resolution enabled untargeted single-cell metabolic profiling, revealing distinct biochemical signatures and spatially localised metabolites indicative of glioblastoma–astrocyte metabolic exchange. Complementary LC–MS analysis of cell extracts provided broader pathway coverage and supported metabolite trends identified in the single-cell imaging data. Integrated analysis revealed distinct alterations in metabolic pathways including metabolism and lipid-associated signalling in glioblastoma cells under co-culture conditions.

This multi-modal metabolomics workflow bridges spatially resolved and bulk analyses, offering new insights into glioblastoma–astrocyte metabolic communication and mechanisms of tumour recurrence.

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STUDYING MICROBIAL COMMUNITIES USING MASS SPECTROMETRY IMAGING

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Mass Spectrometry Imaging (MSI) is a powerful technique to characterise complex biological samples such as tissues or biofilms, enabling the mapping of hundreds of chemical constituents simultaneously. In the Strittmatter lab, we are using MSI to map changes in the metabolome (spatial metabolomics) of biomedical specimen ranging from microbial cultures to clinical tissue specimen using Desorption Electrospray Ionisation (DESI) MSI, an ambient technique operating under atmospheric conditions and enabling analysis without prior sample preparation. This presentation will discuss several applications of DESI-MSI to study microbial biofilms, metabolism and taxon-specific markers, bacterial biomarkers that can be used to map bacteria in complex specimen such as complex mixtures, biofilms and tissues.



Repurposing PBS-PFA-Perfused/OCT-Embedded Glioblastoma Mouse Brain Sections for Mapping Lipid Heterogeneity by AP-MALDI-MSI

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Glioblastoma (GBM) is the most aggressive primary brain tumor, marked by profound metabolic reprogramming and a highly infiltrative growth pattern [1]. In this study, we demonstrate the feasibility of repurposing archived PBS-perfused, PFA-fixed, and Optimal Cutting Temperature (OCT)-embedded murine brain sections for spatial analysis of lipids using atmospheric-pressure MALDI mass spectrometry imaging (AP-MALDI-MSI) coupled to a high-resolution Orbitrap Exploris 120 analyzer. To enable MSI analysis, archived 20 μm coronal brain sections were gently recovered from classic microscope glass slides, flotted in water to remove residual OCT, and transferred to conductive indium tin oxide (ITO)-coated slides. This repurposing strategy aligns with the 3Rs principles, allowing the reuse of existing biological material and reducing the need for additional animal experiments [2]. Matrix deposition by vacuum sublimation of 2,5-dihydroxybenzoic acid (DHB) and 1,5-diaminonaphthalene (DAN) produced homogeneous coatings while preserving spatial integrity [3]. AP-MALDI-MSI acquisition generated molecular images with 40 μm spatial resolution and mass accuracy below 2 ppm, allowing reliable detection of tumor-associated lipids despite known interferences. Accurate mass matching against open-access databases revealed a clear biochemical distinction between the tumor core and surrounding healthy tissue [4]. Ongoing studies will compare treated and untreated tumors to elucidate therapy-induced metabolic changes.

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QUANTITATIVE IMAGING MASS SPECTROMETRY OF GLUTAMATE IN RAT BRAIN

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Introduction

Glutamate is the main excitatory neurotransmitter in the mammalian brain and plays a central role in synaptic transmission, plasticity, and neurodegenerative and neuropsychiatric disorders. Despite its importance, absolute and spatially resolved quantification of glutamate in brain tissue remains challenging due to its small size, high polarity, and strong matrix-dependent ionization effects. Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) offers unique advantages for spatial metabolomics, but robust quantitative workflows for small neurotransmitters have to be more extensively validated.

Methods

Here, we developed a quantitative MALDI-IMS workflow for absolute determination of glutamate in rat brain sections. The method combines an on-tissue calibration curve generated using isotopically labelled glutamate with pixel-wise normalization to isotopically labelled aspartate co-deposited with the matrix.

Coronal rat brain sections were analyzed by high-resolution FT-ICR MALDI-IMS, and glutamate concentrations were extracted from atlas-defined brain regions. Quantitative performance was assessed in terms of linearity, limits of quantification, and reproducibility. Results were validated by comparison with targeted HPLC-MS analysis performed on anatomically matched tissue homogenates blocks.

Results

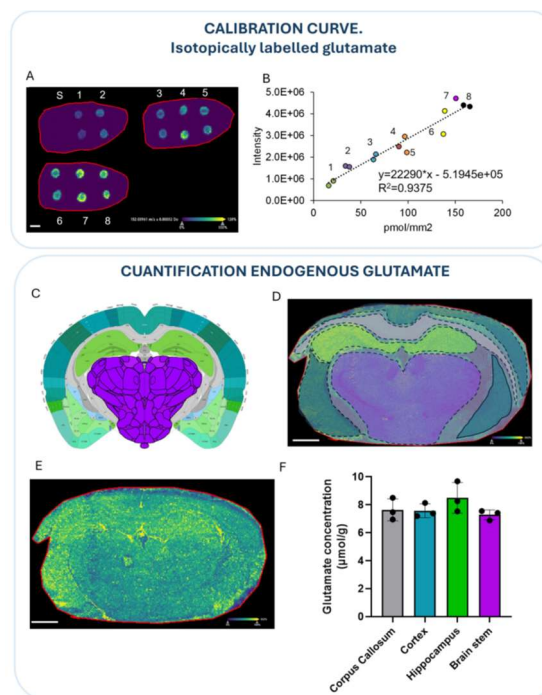
The workflow showed a linear response across the calibrated range, suitable with endogenous glutamate levels, and low technical variability (CV $\leq 10\%$ intra-animal and $\leq 15\%$ inter-animal). Absolute quantification revealed region-specific endogenous glutamate concentrations, with the highest levels detected in the hippocampus, ranging from 8.26 to 10.43 $\mu\text{mol/g}$ across animals, while lower and more stable concentrations were observed in cortex, corpus callosum, and brainstem. Clear rostro-caudal variations in glutamate distribution were resolved, consistent with known glutamatergic architecture. Quantitative values obtained by MALDI-IMS were in good agreement with HPLC-MS measurements, with no significant differences between methods.

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MALDI IMAGING MASS SPECTROMETRY AT IRCCS “AZIENDA OSPEDALIERA METROPOLITANA (AOM), GENOVA

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The Proteomics and Mass Spectrometry Unit of the Azienda Ospedaliera Metropolitana (AOM) has recently established a state-of-the-art MALDI Mass Spectrometry Imaging (MALDI-MSI) platform based on a Bruker UltrafleXtreme MALDI-TOF/TOF instrument coupled with an HTX Imaging M3+ Sprayer, enabling spatially resolved molecular analyses. This newly implemented platform represents a significant technological advancement and is currently being integrated into ongoing research programs in oncology and neuroscience, and dedicated methodological optimization is ongoing across multiple projects:

- **Proteomics – Characterization of Tumor Tissue and Microenvironment** in different cancer including: proteomic profiling of Hormone receptor-positive, HER2-low breast cancer; comparative analysis of differential protein and lipid expression between the tumor core and invasive margin in high-grade gliomas; investigation of tissue architecture and the proteomic landscape of the tumor and peritumoral microenvironment in well-differentiated small intestinal neuroendocrine tumors, with particular emphasis on tumor-induced fibrosis.

- **Drug Discovery and Spatial Pharmacology**: MALDI-MSI analyses are in progress to investigate the spatial effects of Fraisinib [1], a novel experimental compound, in murine NSCLC models, enabling the assessment of tumor microenvironment remodeling and treatment-induced molecular changes.

These activities are embedded within a comprehensive multi-omics framework integrating quantitative LC-MS/MS-based proteomics, lipidomics, metabolomics, and systems-level data analysis. This integrative approach aims to generate spatially resolved molecular signatures with direct relevance to translational oncology and drug development.

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SINGLE-SECTION MULTIMODAL ANALYSIS BY DESI-MSI AND IMAGING MASS CYTOMETRY

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The integration of molecular and cellular spatial information remains a key challenge in tissue imaging. Desorption electrospray ionization mass spectrometry imaging (DESI-MSI) enables label-free mapping of small molecules, while imaging mass cytometry (IMC) provides highly multiplexed protein localization at single-cell resolution. However, their integration is constrained by incompatible sample preparation workflows and potential effects on tissue integrity and antigen preservation [1–3]. Here we present a multimodal workflow enabling sequential DESI-MSI and IMC on the same tissue slice using a sucrose-gelatin embedding matrix optimized for DESI analysis [4]. The matrix preserves morphology and antigenicity while maintaining a low chemical background, allowing immunolabeling without compromising molecular readouts. Mouse wild-type brain and spleen tissues were analyzed by sequential DESI-MSI and IMC on the same tissue section, while adjacent serial sections, analyzed separately, were used as controls. DESI acquisition preserved antibody staining quality, tissue morphology and IMC signal distribution with spatial patterns and quantitative metrics showing high concordance with control sections.

This workflow establishes a robust platform for multimodal spatial analysis, enabling direct co-localization of small molecules and protein markers within a single slice, offering a powerful framework for linking metabolite distributions to cellular phenotypes and tissue architecture [5].

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SURFACE-SPECIFIC METABOLIC SIGNATURES OF FLAVESCENCE DORÉE IN GRAPEVINE LEAVES REVEALED BY MSI

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Flavescence dorée (FD) is a serious grapevine disease spread by leafhoppers and caused by a phytoplasma. This phytoplasma is considered a quarantine organism and poses a serious threat to European viticulture. FD infection alters host metabolism, making the identification of disease-associated metabolites essential for early diagnosis and mitigation of economic losses [1]. Current diagnostic approaches remain limited, and conventional bulk analyses mask metabolic differences between the adaxial and abaxial leaf surfaces, which perform distinct physiological functions. Mass spectrometry imaging (MSI) overcomes this limitation by enabling spatially resolved metabolite visualization directly within tissues. Here, we applied a high-resolution, MALDI MSI untargeted approach to investigate surface-specific metabolic changes in FD-infected *V. vinifera* cv. Barbera leaves to identify potential early biomarkers. Grafted cuttings were exposed to FD-infected insect vectors, and leaves were sampled three months post-exposure at symptom onset. Non-exposed controls, exposed asymptomatic, and exposed symptomatic plants were analyzed separately for adaxial and abaxial leaf surfaces (four biological replicates per condition). Samples were coated with α -cyano-4-hydroxycinnamic acid and analyzed using AP-MALDI coupled to a high-resolution Orbitrap Q-Exactive mass spectrometer. Across all groups, 5,100 features were detected after data processing with MSiReaderPro software. Principal Component Analysis revealed clear metabolic separation of symptomatic leaves from controls and asymptomatic plants, with surface-specific differences in infected tissues. Remarkably, asymptomatic leaves already displayed localized metabolic contrasts absent in controls, suggesting early surface-dependent metabolic reprogramming. Aligned rank transform ANOVA identified 71 features with significant interactions between infection status and leaf surface, 54 of these were tentatively annotated using an in-house grapevine metabolite database and are currently undergoing MS/MS validation. Overall, this study demonstrates that high-resolution MSI enables the detection of spatially resolved metabolic alterations induced by FD infection prior to visible symptom development. This approach provides a powerful framework to investigate functional heterogeneity in plant–pathogen interactions and supports the development of more sustainable strategies for grapevine disease management.

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An Integrated LC–MS and DESI-MSI Workflow for Spatial Metabolomics in Medaka

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Desorption electrospray ionization mass spectrometry imaging (DESI-MSI) is a powerful platform for spatially resolved analysis of metabolites and lipids under ambient conditions [1]. However, analysing extremely small and fragile biological specimens by MSI requires optimized sample preparation strategies to preserve morphology, prevent analyte delocalization, and maintain high mass accuracy.

To address these challenges, we employ a multifunctional sucrose-gelatin hydrogel embedding matrix integrating its use in DESI-MSI [2]. The matrix provides mechanical stability during cryosectioning while generating minimal spectral background. Importantly, sucrose-derived adduct ions act as intrinsic lock-mass references, enabling continuous mass recalibration throughout acquisition without the need for exogenous standards. This approach enhances mass accuracy and signal stability while preserving native molecular distributions, even in extremely small specimens. Here we used it to study medaka fish (*Oryzias latipes*), a powerful vertebrate model widely used in developmental biology, toxicology, and disease research due to its genetic conservation with humans.

Building on this optimized sample preparation strategy, we implemented an integrated analytical workflow combining global LC–HRMS-based metabolomics and lipidomics with high-resolution DESI-MSI. Untargeted LC–MS profiling is first performed to define the molecular landscape of the model system. Molecular networking, MS/MS spectral matching, and chemoinformatic tools support confident metabolite and lipid annotation [3, 4, 5]. The resulting molecular information guides targeted spatial interrogation by DESI-MSI, enabling localization of annotated species within medaka tissues. This workflow bridges comprehensive molecular characterization and spatially resolved imaging, establishing a robust analytical framework that positions medaka model as a versatile in vivo platform for future bioactivity and functional studies.

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MAPPING DROUGHT-DRIVEN METABOLIC ADAPTATIONS IN COFFEE FINE ROOTS BY MALDI-MS IMAGING

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The breeding of fruit tree crops, such as coffee, is threatened by challenging climate conditions, including those induced by climate change, such as drought conditions. Based on these premises, in 2022, the European Union funded the BOLERO project “to create resilient rootstock varieties for fruit tree crops to cope with climate change threats” (www.bolero-project.eu). In this work, we employed MALDI-MSI to map the metabolic alterations occurring in the fine roots of the *Coffea canephora* species following drought conditions in order to address the BOLERO project’s objective to “understand root-based traits”. A total of 40 root sections, 20 from a root exposed to drought conditions (WS) and 20 from a control (WW), were cut at a thickness of 20 μm and either Norharmane or 6-aza-thiothymine (ATT) were deposited using the HTX TM Sprayer™ M3. All imaging analyses were performed with a Bruker timsTOF fleX™ instrument operating in negative-ion mode within the m/z 0-1000 range and utilising a lateral resolution of 20 μm . Subsequently, all sections were stained with toluidine blue in order to annotate the epidermis/exodermis, cortex, and secondary xylem of each root. Metabolic alterations between the WS and WW roots were observed within all tissues, however, the most notable adaptations were observed within the epidermis/exodermis regions that are more greatly exposed to the external environmental conditions.

In particular, there appeared to be a notable reduction in metabolic activity and, whilst speculative, may be indicative of the roots seeking to facilitate greater water passage and conserve energy under such conditions. This finding was also supported by the increased presence of *lacunae* in the cortical area the WS samples. Whilst work is continuing to perform more extensive molecular annotation, these preliminary results indicate the contribution that spatial metabolic profiling can provide when seeking to better understand the metabolic adaptations occurring in *Coffea* plant roots following exposure to drought and how these may be targeted to render such crops more capable of coping with climate change threats.

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MASS SPECTROMETRY APPLICATIONS FOR THE

CHARACTERIZATION OF KRAS MUTATIONS IN LUNG CANCER

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Lung cancer represents about 12% of all cancer cases and remains one of the leading causes of cancer-related mortality worldwide [1]. The two main forms are non-small cell lung cancer (NSCLC, 80–85% of cases) and small cell lung cancer (SCLC, 10–15%) [2]. From a histological point of view, NSCLC is classified into three main subtypes: adenocarcinoma (nsqNSCLC), squamous cell carcinoma (sqNSCLC), and large cell carcinoma [3]. KRAS is the most common oncogenic driver in NSCLC, detected in up to 30% of nsqNSCLC and approximately 3% of sqNSCLC. Notably, distinct KRAS variants can influence the specific biology of a given tumour [4]. The prognosis of KRAS G12C patients has improved with inhibitors such as sotorasib and adagrasib [5], although resistance and variable responses remain major challenges, highlighting the need for improved molecular profiling and predictive biomarkers [6]. In this context, by using mass spectrometry imaging (MSI), we were able to proteomically characterize different subtypes of nsqNSCLC carrying distinct KRAS mutations. Briefly, formalin-fixed paraffin-embedded (FFPE) resections were trypsin digested and investigated with a timsTOF flex at a spatial resolution of 20µm/px, for a total number of 9 patients analysed (3 resections for each KRAS mutation type). This approach allowed us to discriminate proteomic profiles specific for the 3 different KRAS mutations under investigation, as well as to detect differences among patients who share the same KRAS driver mutation. These results are currently being integrated with the clinical information available for each patient, including responder status, smoking history, age, sex, and other relevant variables. These findings suggest that proteomic signatures may complement genomic KRAS classification by either identifying shared biological phenotypes across different KRAS variants or stratifying patients carrying the same mutation into distinct subgroups. This MSI approach may ultimately improve prediction of therapy response and clinical outcomes.

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MOLECULAR HISTOLOGY IN EPILEPSY SURGERY

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Introduction: Epilepsy affects over 70 million people worldwide, and while antiseizure medications control about 70% of cases, focal drug-resistant epilepsy often requires surgical treatment. Among its most frequent causes are Focal Cortical Dysplasias (FCDs), malformations of cortical development; which are defined in different subtypes by architectural and cytoarchitectural abnormalities. About 8% of patients instead show “cryptogenic epilepsy,” with no detectable histopathological changes in resected tissue. Post-surgical outcomes depend on histological diagnosis, which remains challenging for some pathologies. This study investigates the potential of mass spectrometry imaging to provide additional molecular insights to routine histopathology. **Methods:** Spatial peptide profiling was carried out using MALDI imaging (ultrafleXtreme, Bruker) on post-surgical paraffin embedded tissue sections following deparaffinization, antigen retrieval, tryptic digestion, and matrix application. The resulting MALDI MSI datasets were co-registered with the corresponding post MALDI H&E stained slides to ensure accurate anatomical alignment. Validation was performed using adjacent tissue sections analysed through immunohistochemistry or conventional histological stains. Data processing and statistical analyses were conducted using SCiLS Lab (Bruker) and R Studio. **Results:** Multivariate analyses (spatial segmentation, PCA, pLSA) showed a consistent separation between grey and white matter across samples, independent of aetiology. MALDI MSI successfully detected distinct lesional peptide profiles in most cases; however, small fragmented samples did not allow clear lesion identification, and required an optimization of the analytical workflow. Discriminative m/z peaks that differentiated lesional from perilesional regions were obtained. Preliminary PCA analysis on a small group of dataset of different aetiology showed a good clusterization among groups. This suggests that MALDI MSI could extract molecular differences across aetiologies.

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Multimomics analysis for advanced tumor typing of lung cancer using 116plex MALDI HiPLEX-IHC and released N-glycans on the neoflex

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In this proof-of-concept study, MALDI HiPLEX-IHC with 116 antibody probes was used to analyze human lung adenocarcinoma and squamous cell carcinoma, compared to healthy lung tissue. The high-plexing enables simultaneous assessment of multiple markers in a single experimental setting, coupled with the multimomics integration of released N-glycans from the same sections.

Samples were processed using 116 photocleavable mass tag imaging probes and data was acquired on a neoflex Imaging Profiler with 30 µm pixel size in positive ion mode. Post-acquisition, MALDI matrix was removed, and N-glycans were released by a PNGase digest. Afterwards, sections were stained with hematoxylin and eosin (H&E), and all data was integrated in SCiLS™ Lab 2025b.

The adenocarcinoma tumor region was distinguished from the squamous cell carcinoma tumor area, indicating distinct protein expression profiles. The segmented regions matched the manual annotation of the corresponding H&E image by a pathologist.

Our antibody panel contained markers used in routine diagnostic settings (TFF-1, Napsin A, PD-L1, Ki67, HIF-1,...) which were able to be assessed in a single analysis.

Proteins of signaling cascades like phosphorylated mTOR, phosphorylated ERK and PTEN were covered in our panel as well, providing information on spatially aware pathway activity.

The multimomic integration of enzymatically released N-glycans added another layer of information: we detected cell accumulations expressing the cell adhesion glycoprotein CEA/CD66e in both tumor areas. Interestingly, these cells exhibited distinct N-glycosylation patterns, which remain currently unexplained but could potentially provide further insights into cellular characterization in the future.

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