1st Italian MS-Imaging Symposium

Event Schedule

Morning session	
08:30 - 09:00	**Registration**
09:00 – 09:15	**Welcome & Introduction** **Enrico Davoli, Alice Passoni, Silvio Garattini**
09:15 – 10:00	**Plenary Talk #1** **prof. Simona Francese (Sheffield Hallam University, UK)** Fingerprints, from catching criminals to catching cancer
10:00 – 10:20	**Oral #1** **Marcello Ziaco (University of Naples & CNR-ICB of Naples)** Integrated approach based on LC-MS/MS and DESI-MSI for identification and mapping of sulfolipids in brain tissue
10:20 – 10:40	**Oral #2 - Davide Franchina (Stanford University, CA, USA)** MnM: co-spatial tissue imaging using MALDI and MIBI
10:40 – 11:00	**Sponsor Talk #1 - Thermo Scientific** **Giuseppe Martano (IRCCS Humanitas Research Hospital, Milan)** Mapping metabolomic landscapes in ulcerative colitis
11:00 – 11:30	**Coffee Break**
11:30 – 11:50	**Sponsor Talk #2 - Shimadzu** **Matteo Calligaris & Antonio Paolo Beltrami (University of Udine)** Molecular Insights from MALDI Imaging in Cardiovascular Diseases
11:50 – 12:10	**Oral #3** **Peter Verhaert (University of Leuven, BE)** Towards direct MS histochemistry of monoamine neurotransmitters, precursors & metabolites in FFPE tissues
12:10 – 12:30	**Oral #4** **Marta Berzaghi (University of Parma)** Optimizing DESI-MSI parameters for the detection of (poly)phenol metabolites in mimetic model tissue
12:30 – 12:50	**Sponsor Talk #3 - AmberGen** **Greta Bindi (University Bicocca, Milan)** Sequential MALDI-HiPLEX-IHC and untargeted protein imaging to map the tumour-immune environment: Academic researcher experience
12:50 – 13:30	**Lunch time & Networking**

1st Italian MS-Imaging Symposium

Event Schedule

Afternoon Session	
13:30 – 14:00	**Poster session**
14:00 - 14:10	**Presenting the International Mass Spectrometry Imaging Society (IMSIS)** **Liam McDonnell**
14:10 – 14:40	**Plenary Talk #2** **dr. Rita Casadonte (Proteopath GmbH, Trier, DE)** MALDI imaging for in situ analysis of tryptic peptides in clinical tissues
14:40 – 15:00	**Sponsor Talk #4 - Waters** **Andrea Perissi & Angelo Palmese** The Evolution of Imaging: DESI Source
15:00 – 15:20	**Oral #5** **Emanuela Salviati (University of Salerno)** Seeing in the dark: visualizing endocannabinoids spatial distribution by MALDI-2 mass spectrometry imaging
15:20 – 15:40	**Sponsor Talk #5 - Bruker** **Bram Heijs** iprm-PASEF, an integrated workflow to identify your images with confidence
15:40 – 16:00	**Oral #6** **Georgia Millard (Sheffield Hallam University, UK)** Metabolomic distribution in uveal melanoma multi-cellular tumour spheroids using MSI
16:00 – 17:00	**Final Remarks & Networking**

1st Italian MS-Imaging Symposium

Poster Contribution

13:30 - 14:00 Poster session	
Poster #1	**Alex Affricano (University of Turin)** First application of conductive adhesive films for MALDI-MSI analysis of purine metabolism in beans
Poster #2	**Chiara Calabretta (Istituto Neurologico Carlo Besta IRCCS, Milan)** MALDI imaging mass spectrometry: a novel approach to molecular histology in epilepsy surgery
Poster #3	**Giulio Calza (Bruker)** A spatial multiomics workflow on a new benchtop MALDI-TOF instrument deciphering the lipid & protein landscape
Poster #4	**Stefano Mauro Carabellese (Istituto Mario Negri IRCCS, Milan)** Uncovering metabolic responses to candidatus phytoplasma vitis infection in grapevine leaves using mass spectrometry imaging
Poster #5	**Barbara Cardinali (Hospital San Martino IRCCS, Genova)** MALDI imaging mass spectrometry for sub-typing; a pilot study unrevealing breast cancer heterogeneity
Poster #6	**Coronel Vargas Gabriela (Hospital San Martino IRCCS, Genova)** Optimization of pre-processing for hyperion cyTOF's multi-channel tiff data analysis
Poster #7	**Sophie M. Pearce (Sheffield Hallam University, UK)** Chemotherapy resistance investigated by multimodal MSI of osteosarcoma multicellular tumour spheroid models
Poster #8	**Andrea Perissi & Angelo Palmese (Waters)** high resolution low flow DESI imaging using a commercial desi source
Poster #9	**Daniele Stefanizzi (University of Udine)** MALDI imaging analysis of thoracic aortic aneurisms shows differentially expressed molecular species associated with disease severity
Poster #10	**Riccardo Zecchi (University of Florence)** MS-imaging: in situ investigation of secondary metabolites of oryza sativa varieties before and after cooking
Poster #11	**Samuele Zoratto (Technical University of Wien, AU)** Mapping epidermal lipids in 3D skin models and human biopsies

1st Italian MS-Imaging Symposium

Book of Abstract

Plenary Talk #1

FINGERPRINTS, FROM CATCHING CRIMINALS TO CATCH CANCER

Simona Francese

Sheffield Hallam University, Howard Street , Sheffield, UK

In the last decade fingerprint analysis by mass spectrometry and mass spectrometry imaging has brought about significant forensic opportunities to both profile and identify a suspect. Particularly Matrix Assisted Laser Desorption Ionisation profiling and Imaging (MALDI MSP and MSI) have been investigated to provide chemical and biometric information on an individual from their fingerprints1.

The overall method that we called "molecular fingerprinting" has been reported in the Fingermark Visualisation Manual, edited by the Home Office and the Defense Science and Technology, Laboratory UK, and has been already used in casework Nationally2 and Internationally.

However, MALDI MSI has also shown significant potential to use fingerprints and fingerprint smears to non-invasively detect pathologies3,4, thus locating fingerprints at the interface between forensic science and clinical diagnostics.

In this presentation, both these applications will be discussed through the journey that has led to bridge the gap between these two fields.

References

1. Francese S, Criminal profiling through MALDI MS based technologies – breaking barriers towards border free forensic science, 2019, Australian Journal of Forensic Sciences, DOI: https://doi.org/10.1038/s41598-023-29036-710.1080/00450618.2018.1561949

2. Bradshaw R., Denison N., Francese S., Implementation of MALDI MS profiling and imaging methods for the analysis of real crime scene fingermarks, 2107, Analyst, 142:1581

3. Heaton C, Witt M, Cole L, Eyre J, Tazzyman S, McColm R, Francese S " Detection and mapping of haemoglobin variants in blood fingermarks by MALDI MS for suspect “profiling”, 2021, Analyst, 46:4290

4. Russo, C., Wyld, L., Da Costa Aubreu, Bury, C., Heaton C., Cole, L.M., Francese S. Non-invasive screening of breast cancer from fingertip smears—a proof of concept study, 2023, https://doi.org/10.1038/s41598-023-29036-7

Oral Talk #1

INTEGRATED APPROACH BASED ON LC-MS/MS AND DESI-MSI FOR IDENTIFICATION AND MAPPING OF SULFOLIPIDS IN BRAIN TISSUE

Marcello Ziaco[1], Giovanni Andrea Vitale[2], Giusi Barra[1], Carmela Gallo[1], Genoveffa Nuzzo[1], Daniela Castiglia[1], Emiliano Manzo[1], Ivan Conte[2], Maria G. Miano[3], Luigia Cristino[1], Giuliana d'Ippolito[1] and Angelo Fontana[1,2]

[1]*Institute of Biomolecular Chemistry ICB, National Research Council CNR, Via Campi Flegrei 34, 80078, Italy*

[2]*Department of Biology, University of Napoli "Federico II", Via Cupa Nuova Cinthia 21, 80126, Italy*

[3]*Institute of Genetics and Biophysics, National Research Council CNR, Via Pietro Castellino, 111, 80131, Italy*

**Email: m.ziaco@icb.cnr.it*

Sulfolipids are a class of lipids characterized by the presence of a sulfur-containing functional group. In plants, sulfoquinovosyl diacylglycerides (SQDG) play a role in the photosynthetic electron transport chain [1]. In mammals, sulfated glycolipids have been identified as regulators of aging and inflammatory processes within the central nervous system [2]. Recently, our research demonstrated that Sulfavant A, a synthetic analogue of SQDG, acts as a potent ligand for TREM2 regulating innate immune response [3,4]. To further explore functional roles of sulfolipids, we developed a mass spectrometry-based approach to identify and map these molecules within the mouse brain. Our workflow includes deconvolution of Orbitrap LC-MS/MS data using MZmine to select metabolites with diagnostic fragments related to sulfate and sulfonic groups. Sulfolipid identity has been established by analysis of MS/MS and NMR data. Localization of these molecules in the mouse brain was achieved through mass spectrometry imaging (MSI), employing a DESI-XS Synapt XS system (Waters) and an embedding matrix to enhance analysis precision. The lock mass correction, a key challenge in MSI, was addressed by using an intrinsic component of the matrix to successfully correct m/z values along the experiments. This approach allowed us to achieve accurate localizations (<5 ppm error) of metabolites within their native environment, eliminating the need for exogenous standards.

References:

[1] Dörmann, Benning. "Galactolipids rule in seed plants." TRENDS in Plant Science Vol.7 No.3 March 2002.
[2] Tan et al. "A class of anti-inflammatory lipids decrease with aging in the central nervous system." Nature Chemical Biology 19.2 (2023), 187-197.
[3] Gallo et al. "Sulfavant A as the first synthetic TREM2 ligand discloses a homeostatic response of dendritic cells after receptor engagement." Cellular and Molecular Life Sciences (2022), 79 (7), 369.
[4] Manzo et al. "Chemical synthesis of marine-derived sulfoglycolipids, a new class of molecular adjuvants." Marine Drugs 15.9 (2017), 288.

Acknowledgements:
Progetto PNRR-MAD-2022-12376849 "Rescuing the function of Trem2 variants associated with Alzheimer's Disease via a novel class of small molecules", M6/componente: C2, Investimento: 2.1

Oral Talk #2

MnM: CO-SPATIAL TISSUE IMAGING USING MALDI AND MIBI

Davide G. Franchina[1*], Albert G. Tsai[1], Michael R. Angelo[1], Sean C. Bendall[1]

[1]*Department of Pathology, Stanford University, Stanford, CA, 94305, USA.*
**Email: dgf@stanford.edu*

Spatial mapping tools offer an approach to study tissue complexity and heterogeneity in (patho)physiology. However, due to specific platform-to-platform incompatibility (*i.e.* slide type, tissue processing and preparation), protocols that maximize output from a single tissue section are lacking. Here, we present a workflow for the sequential imaging of archival material using matrix assisted laser desorption ionization (MALDI) and multiplexed ion beam imaging (MIBI). MnM enables the acquisition of untargeted molecular composition (MALDI) and targeted single-cell phenotype (MIBI) from a single tissue section. FFPE sections from lymphatic tissues were sectioned onto an organic polymer-coated slide (MIBIblue). The tissue was first imaged with a MALDI timsTOFflex. The matrix was washed off and sections were stained with a mix of metal-labelled antibodies targeted to immune phenotypic markers. Data was acquired with a MIBIscope and sections were counterstained with H&E. Bilinear interpolation was used for pixel up-sampling of the MALDI dataset to match the MIBI pixel size. Alignment of the two modalities into a shared coordinate system was achieved by registration between MALDI-H&E and MIBI-H&E using the H&E as ground truth. Registered MnM imaging data were combined into a single dataset where each pixel retains bimodal information from N-glycans and MIBI probes. We present a bimodal imaging strategy enabling mass spectrometry imaging (MALDI), high-definition spatial proteomics (MIBI), and H&E on the same tissue area, demonstrating the proof of concept of human lymphatic tissues. The number of ionized species and N-glycan annotations were comparable between the two slides, indicating that MIBIblue can be used for N-glycan MALDI imaging. Then, we used MIBI to measure the expression of metal-tagged immune markers across the tissue area previously rastered with MALDI. MIBI imaging data from MALDI-treated regions were comparable to MALDI-free areas, suggesting that laser ablation by MALDI does not affect MIBI acquisition. Our workflow allows for integration of spatial modalities and analysis of N-glycans that colocalize with specific cell types or microanatomical areas as defined by protein expression acquired by MIBI. We show that MnM phenotypic marker-to-ion correlations can be rapidly achieved from different datasets.

Sponsored Talk #1 - Thermo Scientific

MAPPING METABOLOMIC LANDSCAPES IN ULCERATIVE COLITIS

Faris Hrvat[1], **Giuseppe Martano[2]**, Giuseppe Converso[1], Marek Wozny[1], Sara Timo[2], Lavinia Morosi[2], Samuel Pineda[3], Alejandra Angela Carriles[3], Giada Mori[3], Giulia Rizzo[3,4], Lotte Oldenburg[5], Geert D'Haens[5], Florian Tran[6,7], Phillip Rosenstiel[6,7], Stefan Schreiber[6,7], Alessandro Armuzzi[1,8], Stefania Vetrano[1,2,3]

1 - Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, 200972, Milan, Italy.
2 - IRCCS Humanitas Research Hospital, Via Manzoni 56, Rozzano (MI), Italy
3 - Laboratory of Gastrointestinal Immunopathology, Department of Gastroenterology, IRCCS Humanitas Research Hospital, Rozzano, 20089 Milan, Italy
4 – Gastroenterology Unit, Department of Medical Science and Public Health, University of Cagliari, Cagliari, Italy
5 – Department of Gastroenterology, Amsterdam University Medical Centers, Amsterdam, The Netherlands
6 – Department of Internal Medicine I, Christian Albrechts University and University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
7 - Institute of Clinical Molecular Biology, Christian Albrechts University and University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
8- IBD Unit, Department of Gastroenterology, IRCCS Humanitas Research Hospital, Rozzano, 20089 Milan, Italy
**Email: giuseppe.martano@humanitasresearch.it*

Ulcerative Colitis (UC) is a debilitating disease without a cure. The step-up therapy approach aims to control and alleviate intestinal inflammation by achieving and maintaining clinical and endoscopic remission. However, the rate of primary non-response or loss of response to therapies is high. Predictive markers to guide therapeutic decisions are needed. This study aims to determine the intestinal metabolic profiles of UC patients and explore differences in response groups to improve patient stratification. Mucosal biopsies from 26 active UC patients were collected at weeks 0 and 14 after pharmacological treatment. Patients were classified as non-responders (NR), responders (R), and super responders (SR) based on the Mayo score. Biopsies were processed using MALDI coupled with Orbitrap Exploris™ 120 Mass Spectrometer. The MALDI method was developed for spatial untargeted metabolomics analysis, allowing for detailed spatial mapping of metabolites within tissue sections. Data was analyzed for metabolite quantification and annotation using MSInside. Statistical analyses included metabolite distributions, Moran's I spatial correlations, and pathway enrichment analysis. Spatial metabolomic analysis revealed different metabolite patterns between R and NR across treatments. All non-responders exhibited dysregulated lipid metabolism. NR showed fragmented metabolite patterns. SR showed significant tissue reorganization with increased metabolites involved in anti-inflammatory pathways, glutathione, and purine metabolism. SR also showed histamine- and IgE-related pathways supporting cellular regeneration. The alteration of mucosal lipid metabolism is a hallmark of non-response to therapies. The effectiveness of specific treatments in UC is influenced by the initial mucosal metabolic state, conditioned by diet and medications. Validation in a larger sample size will aid in predicting therapy response.

Fundings:
This project has received funding from the Innovation Medicines Initiative 2 Joint Undertaking (JU) under grant agreement no. 853995.

Sponsored Talk #2 - Shimadzu

MOLECULAR INSIGHTS FROM MALDI IMAGING IN CARDIOVASCULAR DISEASES

Matteo Calligaris [1], Daniele Stefanizzi [1], Francesco Curcio [1], **Antonio Paolo Beltrami** [1]

[1] *Department of Medicine, University of Udine, Udine, Italy;*

**Email: matteo.calligaris@uniud.it, antonio.beltrami@uniud.it*

Human-induced pluripotent stem cells (h-iPSCs) have revolutionized disease modeling and therapeutic research, particularly in cardiology. However, despite advancements in differentiation protocols, h-iPSC-derived cardiomyocytes (h-iPSC-CMs) remain distinct from mature heart cells. A critical aspect of this discrepancy lies in mitochondrial function, particularly cardiolipin metabolism. MALDI imaging of h-iPSC-CMs and failing heart tissues revealed cardiolipin imbalances, with a shift toward higher-molecular-weight species in end-stage failing hearts, highlighting potential metabolic dysfunctions in disease pathogenesis (Figure 1).

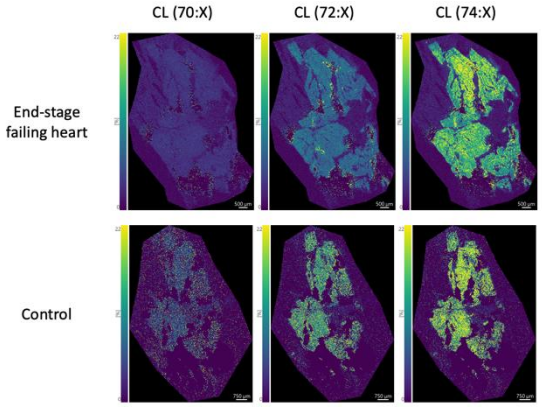


Figure 1: Profile of cardiolipins in control and end-stage failing heart tissue.

Similarly, thoracic aortic aneurysms and dissections (TAAD) represent a spectrum of vascular disorders with significant molecular alterations. MALDI imaging of aortic biopsies from patients with varying disease severity identified key metabolic markers, including phosphatidylethanolamines and diacylglycerols, with distinct spatial distributions. Notably, a molecular species (PE-NMe(18:4/22:1), 798 m/z) was significantly elevated in severe cases, suggesting its potential as a disease severity marker (Figure 2).

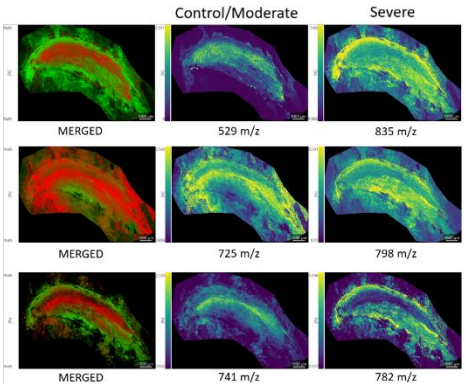


Figure 2: Spatial localization of masses found in positive ion mode.

These findings underscore the power of MALDI imaging in uncovering metabolic signatures of cardiovascular diseases, offering insights into mitochondrial dysfunction and lipid remodeling that could inform future therapeutic strategies.

Oral Talk #3

TOWARDS DIRECT MS HISTOCHEMISTRY OF MONOAMINE NEUROTRANSMITTERS, PRECURSORS & METABOLITES IN FFPE TISSUES

Peter Verhaert[1,2*], Ling Shan[3], Dick Swaab[3], Per Andrén[4], Dietmar Thal[1], Raf Sciot[1], Gilles Frache[5]

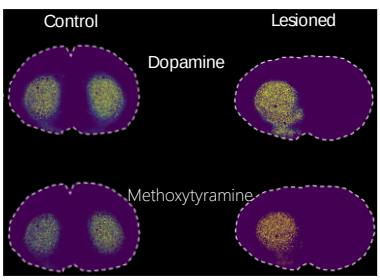
[1]University of Leuven, Department of Imaging and Pathology, Leuven (Belgium); [2]ProteoFormiX BV, Vorselaar (Belgium); [3]Netherlands Institute for Neuroscience, Amsterdam (Netherlands); [4]University of Uppsala; Science for Life Labs, Uppsala (Sweden); [5]Luxembourg Institute for Science and Technology, Belvaux (Luxembourg)

**Email: peter.verhaert@proteoformix.com*

Formaldehyde-fixation and paraffin-embedding (FFPE) are generally considered not to be appropriate as sample preparation of biological samples for mass spectrometry imaging (MSI). Particularly tissue imaging ion signals of lipids are significantly higher in intensity from fresh/frozen material than those obtainable from comparable FFPE samples. The high abundancy of lipids in fresh biological tissues, in combination with the considerable lipid extraction during tissue dehydration and clearing necessary prior to paraffin-embedding are mainly responsible for this typical observation in MSI. On the other hand, MSI of less abundant chemically labile compounds with limited ionizability in comparison to many lipids has proven very challenging in fresh/frozen tissues, which in our opinion reflects the fact that freezing only partially blocks analyte modifying/catabolizing enzymes. Well-known examples are neurotransmitters: small monoamine biomolecules, which are known to require on tissue chemical derivatization (OTCD) to raise their ion signal intensities to a level that makes them observable in a typical background of plentiful lipids. We hypothesize that formaldehyde-fixation (FF) efficiently blocks enzymatic postmortem breakdown of neurotransmitters (NTs) and other biomolecules that are extracted to a lesser extent during the paraffine-embedding (PE) process.

We show that atmospheric pressure (AP)/MALDI in combination with FT (e.g., orbitrap Exploris 480^{TM}) analysis allows for high accuracy on-tissue mass measurement of neurotransmitter, neurotransmitter precursors and neurotransmitter metabolite ions, suggesting that direct MS histochemistry (HC) of NTs is achievable in FFPE samples. We will discuss various experiments in progress to validate our MSHC detections and annotations of NTs, as well as some of their precursors and metabolites. These include comparing FFPE MSHC data of unilaterally lesioned rodent brain sections with well-known NT data of corresponding on-tissue OTCD fresh/frozen samples (FMP-10 [www.tag-on.se]; Fig. 1) and comparing different areas of the human brain rich in specific NTs.

Additionally, targeted high-sensitivity on-tissue AP/MALDI MRM analyses are evaluated and correlations between MSHC and MSIHC "proteomic" images [www.ambergen.com] are investigated.



Oral Talk #4

OPTIMIZING DESI-MSI PARAMETERS FOR THE DETECTION OF (POLY)PHENOL METABOLITES IN MIMETIC MODEL TISSUE

Marta Berzaghi[1*], Marcello Ziaco[2], Giovanni Andrea Vitale[3], Iolanda Bilotti[4], Pedro Mena[1], Giuliana D'Ippolito[2], Angelo Fontana[2,3], Monia Savi[4], Daniele Del Rio[1], Letizia Bresciani[1]

[1] *Human Nutrition Unit, Department of Food and Drugs, University of Parma, Via Volturno 39, Parma, 43125 (Italy);*

[2] *Bio-Organic Chemistry Unit, CNR-Institute of Biomolecular Chemistry, Via Campi Flegrei 34, Pozzuoli, Napoli*

[3] *Bio-Organic Chemistry, Department of Biology, University of Naples Federico II, Via Cinthia, Napoli, 80126 (Italy);*

[4] *Life Sciences and Environmental Sustainability, Department of Chemistry, University of Parma, Parco Area delle Scienze 11/A, Parma, 43124 (Italy)*

**Email: marta.berzaghi@unipr.it*

Desorption electrospray ionization mass spectrometry imaging (DESI-MSI) is an analytical tool for spatially mapping metabolites directly on tissue surfaces. However, the desorption and detection of compounds are affected by multiple instrumental parameters. This work focuses on optimizing the capillary voltage, the temperature of the heat transfer line, and the solvent used on a Waters™ DESI XS source mounted on a Synapt XS to enhance the desorption and ionization of (poly)phenol metabolites detected in a mimetic model prepared with commercially available liver tissue. The model was developed by homogenizing the organ and adding a pool of (poly)phenol metabolite standards, choosing among those associated with different metabolic phenotypes (metabotypes), at increasing concentrations to various aliquots, allowing us to consider the matrix effect of the tissue in the desorption of the compounds. The parameter combination yielding the highest compound intensities was then applied for DESI-MSI analysis of rat tissues treated intraperitoneally with the same molecules. Liquid chromatography (LC) analysis coupled with MS was also performed on the mimetic model tissues, on rat organs, and on slices of the same organs scraped from the glass slide using an Acquity UPLC™ system coupled with a Xevo TQ-S mass spectrometer. The results highlight the importance of optimizing DESI-MSI parameters for (poly)phenol metabolites detection to achieve improved sensitivity under specific conditions. Comparisons with LC-MS data helped to overcome DESI detection limits, enhance quantitative accuracy and confirm molecular identification.

Acknowledgements:

Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods".

This study has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 950050, PREDICT-CARE project).

Sponsored Talk #3 - AmberGen

MIRALYS™ AMBERGEN. SEQUENTIAL MALDI-HIPLEX-IHC AND UNTARGETED PROTEIN IMAGING TO MAP THE TUMOUR-IMMUNE ENVIRONMENT: ACADEMIC RESEARCHER EXPERIENCE

Greta Bindi[1*], Nicole Monza[1], Glenda Santos de Oliveira[1], Vanna Denti[1], Farnaz Fatahian[2], Seyed-Mohammad Jafar Seyed-Golestan[2], Vincenzo L'Imperio[3], Fabio Pagni[3], and Andrew Smith[1]

[1] *Proteomics and Metabolomics Unit, University of Milano-Bicocca, Vedano al Lambro 20854, Italy*

[2] *Medicinal Plants and Drug Research Institute, Shahid Beheshti University, TehranIR 00989034563002, Iran*

[3] *Pathology Unit, Fondazione IRCCS San Gerardo dei Tintori, University of Milano-Bicocca, Monza 20900, Italy*

**Email: g.bindi@campus.unimib.it*

Spatial multi-omics technology represents a powerful approach to map diverse classes of biomolecules within tissue sections, enabling the comprehensive and multilayered study of the Tumor Microenvironment (TME). In immunogenic tumors, such as clear cell Renal Cell Carcinoma (ccRCC), the spatial organization and phenotype of Tumor-Infiltrating Lymphocytes (TILs) are critical to our understanding of tumor progression and response to treatment, including resistance to Immune Checkpoint Inhibitors (ICIs). This work focuses on developing a flexible workflow leveraging Ambergen's MALDI-HiPLEX-IHC and untargeted spatial omics approaches to explore the spatial heterogeneity of immune cell populations and the molecular mechanisms driving immune suppression and therapeutic resistance in the TME. In the workflow, spatial lipidomics is followed by multiplexed mass spectrometry-based IHC[1], enabling the identification and spatial mapping of immune cell subpopulations, including immunosuppressive T regulatory cells and other TILs, along with the lipidome of the connected microenvironment. The untargeted spatial insights are extended through spatial proteomics[2] to finally correlate immune cell distributions with both proteomic and lipidomic signatures. The integration of these insights gained from several molecular perspectives enables a multi-layered exploration of cell-cell interactions and molecular mechanisms underlying immune suppression. This integrated methodology provides a robust approach for the integration of diverse spatial layers, and may offer new insights into the molecular architecture of the TME and its mechanisms of therapeutic resistance; however, the versatility of the workflow facilitates its application to a range of experimental contexts.

References

1 Yagnik G, Liu Z, Rothschild KJ, Lim MJ. Highly Multiplexed Immunohistochemical MALDI-MS Imaging of Biomarkers in Tissues. J Am Soc Mass Spectrom. 2021 Apr;32(4):977–88.

2 Bindi G, Monza N, de Oliveira GS, Denti V, Fatahian F, Seyed-Golestan S-MJ, et al. Sequential MALDI-HiPLEX-IHC and Untargeted Spatial Proteomics Mass Spectrometry Imaging to Detect Proteomic Alterations Associated with Tumour Infiltrating Lymphocytes. J Proteome Res. 2025 Jan DOI: 10.1021/acs.jproteome.4c00914

Acknowledgements:

This work was funded by the National Plan for NRRP 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 by Regione Lombardia, regional law no. 9/2020, resolution no. 3776/2020": PROGRAMMA DEGLI INTERVENTI PER LA RIPRESA ECONOMICA: SVILUPPO DI NUOVI ACCORDI DI COLLABORAZIONE CON LE UNIVERSITÀ PER LA RICERCA, L'INNOVAZIONE E IL TRASFERIMENTO TECNOLOGICO: NephropaThy. This work reflects only the authors' views and opinions, neither the Ministry for University and Research nor the European Commission can be considered responsible for them. Moreover, funding was also provided by Fondazione Roche per la ricerca indipendente 2023. G.B. would like to thank Milan Rotary Clubs for partially supporting her with the "Borsa di Studio Rotary Dott. Gabriele Corbelli". The authors would also like to thank AmberGen Inc. for kindly providing tonsil tissue for the analyses.

Plenary Talk #2

MALDI IMAGING FOR *IN SITU* ANALYSIS OF TRYPTIC PEPTIDES IN CLINICAL TISSUES

Rita Casadonte[1*]

[1] *Proteopath GmbH, Max-Planck-Str. 17, Trier, D-54296 (Germany)*
**Email: rita.casadonte@proteopath.de*

The ability of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging to release peptides from clinical formalin-fixed paraffin-embedded (FFPE) tissues has represented a significant milestone in proteomics with a great advantage to provide targeted and untargeted detection as well as multiplexed mapping of hundreds of peptides from a single analysis. Using vast archives of FFPE samples in many cases from patients with known clinical outcomes allows us to use large cohorts of samples for retrospective studies with the aim to establish diagnostic/prognostic molecular signatures driving health and disease.

MALDI imaging analysis of FFPE tissue follows the bottom-up principle. Under proper sample preparation conditions for on-tissue protease digestion proteolytic peptides are released from areas of interest and spatially analyzed by MALDI imaging. With this *in-situ* digestion approach, MALDI imaging can connect peptide profiles with specific histological features, without preliminary step of molecular extraction or labeling.

Different applications in the field of pathology/oncology will be presented, showing the strengths and prerequisites of this technology to be used for supporting routine diagnostic, in particular tumor typing and biomarker identification.

Sponsored Talk #4 - Waters

THE EVOLUTION OF IMAGING: DESI SOURCE

Andrea Perissi[1*], **Angelo Palmese**[1*],

[1] *Waters Corporation, Viale Edison 110, Sesto San Giovanni - MI, 20099 (Italy).*

** Andrea_Perissi@waters.com, Angelo_Palmese@waters.com*

DESI (Desorption ElectroSpray ionization).

Developed in 2004 by Professor R. Graham Cooks, Dr.Zoltan Takats, and Dr.Justin Wiseman from Purdue University, who founded Prosolia (Indianapolis, USA). Prosolia launched its first DESI molecular imaging product in 2008. The Prosolia DESI 2D source option was one of several DESI sources invented later, to fit most contemporary mass spectrometers, including Waters™Tof instruments. In July 2018, Waters acquired the DESI-MS technology from Prosolia and the Purdue Research Foundation. Since 2019, Waters has been developing an in-house source -the DESI XS. This source option enables the direct analysis of materials in atmospheric conditions, with minimal sample preparation and without the need fora matrix. An ionized solvent is sprayed at high velocity, directly onto the sample surface, to create a charged plume of droplets. The generated ions are drawn into the MS by the source vacuum.

Key features include:

- Direct analysis of a sample at atmospheric pressure
- Characteristic of an ESI mass spectra
- Minimal sample preparation
- Mainly suitable for small molecule analysis

Recent technical improvement released by Waters as an enhanced sprayer for the DESI XS source and Heated Transfer line (HTL) option give additional advantaged

- Improved tuning robustness and reproducibility between sprayers and emitters
- Simplified optimization routines
- Simplified maintenance procedures

DESI-MSI offers several benefits in comparison to the conventional imaging techniques as it enables a direct analysis, immediately after a routine in vitro experiment, with no need of time-consuming sample preparation or derivatization of the molecule of interest. Compared to the other ionization sources used in MSI analysis, DESI has also the advantage to be considered as a “soft” ionization technique as it allows the detection of intact molecules with a high molecular weight and avoids any addition of matrix during sample preparation as is the case for MALDI

Fig. 1: DESI XS Source

Oral Talk #5

SEEING IN THE DARK: VISUALIZING ENDOCANNABINOIDS SPATIAL DISTRIBUTION BY MALDI-2 MASS SPECTROMETRY IMAGING

Emanuela Salviati[1], Eduardo Sommella[1], Fabiana Piscitelli[2], and Pietro Campiglia[1]

[1] *University of Salerno, Department of Pharmacy, Fisciano (SA) 84084*

[2] *Institute of Biomolecular Chemistry, National Research Council of Italy, Pozzuoli (NA), 80078, Italy*

**Email: esalviati@unisa.it*

Endocannabinoids (eCBs) are endogenous lipid messengers that primarily bind cannabinoid receptors CB1/CB2 and together with the enzymes that regulate their biosynthesis and degradation define the endocannabinoid system. The eCB signaling system plays a key role in the central nervous system, resulting altered in most neurological disorders. The analysis of eCBs is challenging due to their low concentration in biospecimens, and this is exacerbated in Mass Spectrometry Imaging (MSI) where low sensitivity and tissue dependent ion suppression obscure their spatial visualization. In this work we address this limitation by the application of laser-induced post-ionization (MALDI-2) approach. Herein we demonstrate that MALDI-2 boosts the detection of 2-arachidonylglycerol (2-AG) and N-acylethanolamines (AEA, PEA, OEA) with respect to MALDI, and that eCBs can be visualized in brain at endogenous concentration only by MALDI-2-MSI. Both root-mean-square (RMS) and internal standards (I.S.) normalization were evaluated, with I.S. normalization providing improved pixel to pixel variation and more uniform distribution in specific brain regions, especially for 2-AG and PEA. Furthermore, high lateral spatial resolution up to 5 µm pixel size was evaluated, resulting in the detection of all eCBs and confirming the MALDI-2 potential even reducing the ablated tissue amount. As proof of concept, the method was applied to map eCBs in a mouse model of mild traumatic brain injury, the APP-SWE mice, highlighting differences in the modulation of eCBs in Cortex, Hippocampus and Hypothalamus, suggesting the ability to reveal valuable biological insights for neuropharmacology.

Acknowledgements:

This work was supported by Ministero dell'Università e della Ricerca (MIUR) project PIR01_00032 BIO OPEN LAB BOL "CUP" J37E19000050007, project CIR01_00032 – BOL "BIO Open Lab - Rafforzamento del capitale umano", project "Pathogen Readiness Platform for CERIC ERIC upgrade" - PRP@CERIC CUP J97G22000400006, and project PNC0000001 "D34 Health—Digital Driven Diagnostics, prognostics and therapeutics for sustainable Health care" CUP B53C22006090001. Part of this study was funded by USAMRDC Peer Reviewed Alzheimer's Research Program Convergence Science Research Award Program Announcement (Funding Opportunity Announcement Number W81XWH-19-PRARP-CSRA), Award number: W81XWH1810000.

Sponsored Talk #5 - Bruker

IPRM-PASEF, AN INTEGRATED WORKFLOW TO IDENTIFY YOUR IMAGES WITH CONFIDENCE

Bram Heijs[1*], Arne Behrens[2], Tobias Boskamp[2], Sören-Oliver Deininger[2], Nikolas Kessler[2], Arne Fütterer[2], Nadine T. Smit[2], Kate Stumpo[3]

[1] *Bruker Nederland B.V., Elisabethhof 15, 2353 EW, Leiderdorp, The Netherlands*
[2] *Bruker Daltonics GmbH & Co. KG, Fahrenheitstraße 4 28359 Bremen, German*
[3] *Bruker Scientific LLC, 40 Manning Road, Billerica MA 01832, USA*
**Email: bram.heijs@bruker.com*

Spatial tandem-MS-based molecular identification remains a challenge in mass spectrometry imaging. While several automated data acquisition and annotation approaches exist, they mostly rely on "profiling" where the spatial relationship between precursors and fragments is severely reduced. Here we present iprm-PASEF, a novel workflow exclusively available on timsTOF fleX platforms, that integrates CCS-enabled MALDI Imaging with a targeted tandem-MS imaging approach that allows the fragmentation and analysis of up to 25 precursors in a single acquisition, and which comes with a fully integrated computational workflow to generate accurate identifications with spatial fidelity.

Here, CCS-enabled MALDI Imaging of lipids was performed on several rat kidney and brain cryosections using a timsTOF fleX. Data was imported into SCiLS Lab and T-ReX^3 feature finding was performed. A selection of CCS features was exported as precursor list for the iprm-PASEF acquisition. Tandem-MS data were imported into SCiLS Lab. Using MetaboScape-powered rule-based Lipid Species Annotation, fully integrated into SCiLS Lab, fragments and precursors were annotated and identified respectively. Several chimeric tandem-MS spectra were observed, since evidence for multiple explanations at the molecular species level were found in a single tandem-MS spectrum. For example, in the kidney m/z 766.54 was identified as PE(38:4) at the species level based on the headgroup fragment. However, additional side chain fragments confirm the presence of PE(18:0_20:4), PE(16:0_22:4), PE(18:1_20:3) and PE(18:2_20:2). Based on fragment intensities, spatial fragment distributions and MS/MS score, PE(18:0_20:4) was found to be predominant.

This workflow allows for a complete and comprehensive analysis of spatial tandem-MS data in an intuitive and integrated manner and enables confident tandem-MS-based molecular annotation directly from tissue.

Oral Talk #6

METABOLOMIC DISTRIBUTION IN UVEAL MELANOMA MULTI-CELLULAR TUMOUR SPHEROIDS USING MSI

Georgia Millard [1]*, Neil Cross [1], Helen Kalirai [2], Karen Aughton [2] and Laura Cole [1]

[1] Affiliation 1: Centre for Mass Spectrometry Imaging (CMSI), Biomolecular Sciences Research Centre (BMRC), Sheffield Hallam University, Howard St., Sheffield City Centre, Sheffield, S1 1WB (England);

[2] Affiliation 2: Liverpool Ocular Oncology Research Group, William Henry Duncan Building, University of Liverpool, 6 West Derby Street, Liverpool, L7 8TX (England)

*Email: G.Millard@shu.ac.uk

Uveal melanoma (UM) is an intraocular disease that occurs due to mutations in *GNAQ, GNA11*, *PLCB4* and *CystLR2*. [1] Multimodal mass spectrometry imaging (MSI) and multi-cellular tumour spheroids (MCTS) can be used to understand the changes in metabolite and N-glycan distribution which may aid in the development and metastasis of cancerous cells. [2]. Metabolomics data was collected using the desorption electrospray ionisation (DESI) source coupled to a multi-reflecting time of flight mass spectrometer (Waters). For glycomic analysis, data acquisition was performed using the matrix assisted laser desorption ionisation (MALDI) source coupled to the Synapt G2 mass spectrometer (Waters). HDI 1.8, Waters Mass Lynx 4.2 and Waters segmentation 1.2.1 (Waters) were used for data analysis. The DESI-MSI results indicate that MCTS are excellent models of mimicking the tumour microenvironment.[3] The distribution of metabolites varied across different MCTS of different cell lines, n-acetyl aspartic acid (m/z 174.04) was found in the core whereas oleic acid (m/z 281.24) and uridine were found in the proliferating region (Figure1). MALDI-MSI data reveals the distribution of N-glycans across MCTS for different cell lines. Future work includes analysing UM patient tissue samples to correlate the metabolomic and glycomic findings with patient data to inform future medicinal work.

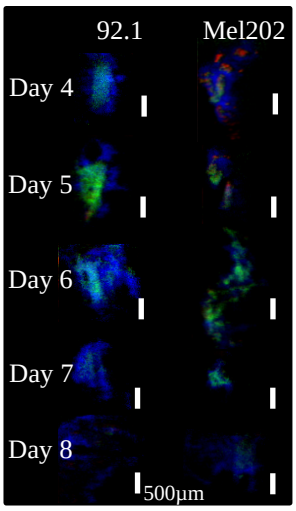


Fig. 1: Ion images of uveal melanoma MCTS showing the distribution of N-acetyl aspartic acid- m/z 174.04 (green), Oleic Acid – m/z 281.24 (red) and Uridine- m/z 606.07.

References:

[1] J. Ma, L. Weng, B. C. Bastian and X. Chen, *Oncogene*, **40 (**2021), 806-820

[2] L. E. A. Young, P. J. Nietert, R. Stubler, C. G. Kittrell, G. Grimsley, D. N. Lewin, A. S. Mehta, C. Hajar, K. Wang, E. C. O'Quinn, P. M. Angel, K. Wallace and R. R. Drake, *Front Pharmacol.* 11:14 (2024)

[3] L. E. Flint, G. Hamm, J. D. Ready, S. Ling, C. J. Duckett, N. A. Cross, L. M. Cole, D. P. Smith, R. J. A. Goodwin and M. R. Clench, *Metabolites*, **11 (**2021), 506

Acknowledgements:

The authors acknowledge Liverpool Ocular Oncology Research Group for their supply of UM cell lines and their support. With thanks to Sheffield Hallam University for funding the PhD studentship.

Poster #1

FIRST APPLICATION OF CONDUCTIVE ADHESIVE FILMS FOR MALDI-MSI ANALYSIS OF PURINE METABOLISM IN BEANS

Alex Affricano[1], Claudio Medana[1], Komei Kawamoto[2], Tadafumi Kawamoto[2], Daisuke Saigusa[3]

[1] *Department of Molecular Biotechnology and Health Sciences, University of Turin, Via Nizza 52, 10125, Torino, Italy*

[2] *School of Dental Medicine, Tsurumi University, 2–1–3 Tsurumi, Tsurumi-ku, Yokohama, Kanagawa 230–8501, Japan*

[3] *Laboratory of Biomedical and Analytical Sciences, Faculty of Pharma-Science, Teikyo University, 2–11–1 Kaga, Itabashi-ku, Tokyo 173–8605, Japan*

**Email: alex.affricano@unito.it*

Mass spectrometry imaging (MSI) is a powerful tool for visualizing the spatial distribution of metabolites in tissues, enabling insights into biochemical pathways without requiring labels [1]. In plant metabolomics, MSI has an important role in mapping key metabolites, enhancing our understanding of plant biochemistry and responses to environmental or processing conditions. For beans and other crops, spatial metabolite analysis can uncover biochemical adaptations and processing effects, offering valuable insights for food science and agricultural research.

Starting from the previous work of the group who demonstrated the advantages of conductive adhesive films for enhancing MSI signals in animal tissues [2], we successfully adapted this innovative approach to plant matrices. Specifically, we applied the conductive adhesive film to study purine metabolism in beans, marking the first application of this technique in plant tissues.

Optimization of instrumental parameters, particularly laser power and spatial resolution, was critical to ensuring robust and reproducible results. Focusing on the purine metabolic range (m/z 100–280), we analyzed dried, wet, and cooked (natto) beans, revealing distinct spatial distributions of purines, particularly adenosine and guanosine.

Our results highlighted metabolic shifts associated with different preparation states, providing new insights into biochemical adaptations and processing effects. This study underscores the potential of this methodology to address critical questions in food science and plant metabolomics, offering a novel tool for characterizing metabolite distributions in complex plant matrices.

References:

[1] R.M. Caprioli, T.B. Farmer, J. Gile. Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS. Anal Chem. 1997 Dec 1;69(23):4751-60. doi: 10.1021/ac970888i. PMID: 9406525.

[2] D. Saigusa, R. Saito, K. Kawamoto, A. Uruno, K. Kano, S. Shimma, J. Aoki, M. Yamamoto, T. Kawamoto. Improving the signal intensity of cryosections using a conductive adhesive film in matrix-assisted laser desorption/ionization mass spectrometry imaging. Mass Spectrom (Tokyo). 2023;12(1):A0137. doi:10.5702/massspectrometry.A0137.

Poster #2

MALDI IMAGING MASS SPECTROMETRY: A NOVEL APPROACH TO MOLECULAR HISTOLOGY IN EPILEPSY SURGERY

Chiara Calabretta[1*], Dalia De Santis[1], Rita Garbelli[1], Marco de Curtis[1], Cinzia Cagnoli[1].

[1] *Clinical and Experimental Epileptology Unit, Fondazione IRCCS Istituto Neurlogico Carlo Besta, via Amadeo 42, Milano, 20133 (Italy);*

**Email: chiara.calabretta01@gmail.com*

Epilepsy is a severe neurological syndrome characterized by recurrent seizures which affects over 70 million people worldwide; the causes underlying epilepsy can be different. Antiseizure medications (ASM) are effective in about 70% of epileptic patients. In a subset of patients with focal seizures resistant to ASM, epilepsy surgery is a safe and effective treatment. Histopathological diagnosis of brain tissue samples obtained after therapeutic epilepsy surgery is based on microscopic assessment of specific staining and multiple immunohistochemistry by expert pathologists. However, several interobserver evaluations highlighted the problem of uncertain or discordant diagnoses, particularly for some pathologies. These discrepancies can potentially impact prognosis and decision-making during patient follow-up. The aim of this work was to assess the potential value of mass spectrometry imaging on post-surgical tissue samples to generate additional molecular insights to integrate the histopathology diagnosis. We present preliminary experiments of MALDI IMS analysis of Formalin Fixed Paraffin Embedded (FFPE) tissue sections from two patients with distinct histological diagnosis: Focal Cortical Dysplasia IIB (FCDIIB) and Temporal Lobe Epilepsy due to Hippocampal Sclerosis (TLE-HS). We show a good correlation between peptide intensity map and immunohistochemistry of the correspondent protein. Unsupervised classification of the spectra, achieved by hierarchical clustering or by principal component analysis, is able to differentiate distinct histopathological features, such as gray matter and white matter, the core of the lesion and the adjacent perilesional area, as validated on immunoreacted-adjacent sections. We also show that PCA, analysis applied on both datasets, displays the relative scores in two separate clusters (Figure 1). We suggest that tissue analysis with MALDI IMS could improve the characterization/identification of the different histopathological entities encountered in epilepsy surgery, particularly those poorly understood in diagnostic routine practice and with low inter-observer agreement.

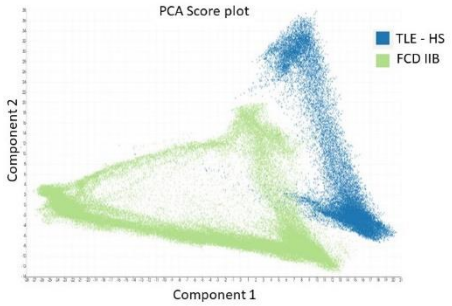


Fig. 1: PCA score plot of two samples from epilepsy surgery with different diagnosis.

Acknowledgements: This work was supported by Next generation EU project PNRR-MCNT2-2023-12377819.

Poster #3

A SPATIAL MULTIOMICS WORKFLOW ON A NEW BENCHTOP MALDI-TOF INSTRUMENT DECIPHERING THE LIPID & PROTEIN LANDSCAPE

Janina Oetjen[1], **Giulio Calza[2]**, Corinna Henkel[1], Dagmar Niemeyer[1], Ulrike Schweiger-Hufnagel[1], Tanja Bien[1], Sebastian Böhm[1], Christoph Nordmann[1], Andree Schuster[1], Katherine Stumpo[3], Michael Easterling[3], Andras Kiss[4], Andrea Tannapfel[5], Jens Christmann[5]

[1] *Bruker Daltonics GmbH & Co. KG, Fahrenheitstraße 4, Bremen, 28359 (Germany);*
[2] *Bruker Italia, Via Cluentina 26, Macerata, 62010 (Italy);*
[3] *Bruker Scientific, 40 Manning Road, Billerica, 01821 (USA);*
[4] *Bruker Austria GmbH, Lemböckgasse 47b, Wien, 1230 (Austria);*
[5] *Institute for Pathology, Ruhr-University Bochum, Bürkle-de-la-Camp-Platz 1, Bochum, 44789 (Germany);*
**Email: giulio.calza@bruker.com*

Spatial biology is an emerging field combining spatial cellular information with molecular information of biological tissues, and as such is perfectly complementary to MALDI mass spectrometry imaging (MALDI Imaging). Here we present a spatial multi-omics workflow combining MALDI Imaging of lipids and spatial proteomics through MALDI HiPLEX-IHC on a new benchtop axial MALDI-TOF mass spectrometer using a clinically relevant research sample (Figure 1).

Fig. 1: Spatial Multi-omics Workflow

Formalin-fixed and paraffin-embedded (FFPE) colorectal cancer sections were processed for MALDI Imaging of lipids. Briefly, paraffin was removed by xylene washes and DHB matrix was sprayed using a M3+ sprayer (HTX-Technologies). MALDI Imaging data was acquired on a neofleX Imaging Profiler in positive reflector mode with 20 µm raster width at m/z 500-1100. A consecutive tissue section was measured on the timsTOF fleX to obtain a reference list of annotated lipids for this sample. A 14-plex MALDI HiPLEX-IHC experiment, using technology from AmberGen, Inc. was conducted on the same section as used before for lipid imaging on the neofleX: after HCCA matrix application, data was acquired on the neofleX using a default acquisition method with 20 µm raster width. An OME-TIFF file was generated automatically for visualizations in SCiLS Scope. The section was stained with H&E post MALDI, and microscopy images scanned on Hamamatsu nanozoomer. Multimodal data integration, statistical analysis and visualizationns were done in SCiLS™ Lab 2024b.

Poster #4

UNCOVERING METABOLIC RESPONSES TO *CANDIDATUS PHYTOPLASMA VITIS* INFECTION IN GRAPEVINE LEAVES USING MASS SPECTROMETRY IMAGING

Stefano Mauro Carabellese[1*], Alice Passoni[1*] and Enrico Davoli[1]

[1] *Laboratory of Mass Spectrometry, Department of Environmental Health Sciences, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy*

**Email: stefano.carabellese@marionegri.it*

The phytoplasma Candidatus Phytoplasma vitis is a unicellular prokaryotic microorganism responsible for Flavescence dorée (FD), a yellow disease affecting grapevines (FD) [1/2]. This quarantine pest, transmitted by the vector *Scaphoideus titanus*, leads to high problems in European grapevine yield, causing a reduction in the size and abundance of the fruits, drying of the plant, particularly the leaves, and an increase in the use of pesticides. These factors lead to higher economic costs [1/3].

In this work, We used a high-resolution imaging mass spectrometry approach to characterize the metabolic profiles of the lymphatic vessels in leaves from healthy and FD-affected plants of the Barbera cultivar

Different types of matrices were applied using the SunCollect Sprayer. Samples were analyzed using an AP-MALDI Orbitrap Q-Exactive, and metabolite spatial distribution was visualized with MSiReader software.

We monitored quercetin 3-glucuronide, resveratrol, and piceide, three metabolites whose concentration increases in response to stress factors such as pathogen infections and oxidative stress.

Our results confirmed the studies present in the literature, highlighting the accumulation of specific metabolites in response to biotic and abiotic stress, such as quercetin 3-glucuronide, resveratrol, and piceide. All identified metabolites in the lymphatic vessels of plants present a protective role against inflammation and oxidative damage [4].

In conclusion, our mass spectrometry imaging approach proved to be an effective tool for detecting metabolic alterations in the lymphatic vessels of leaves infected with Candidatus Phytoplasma vitis, paving the way for future spatial metabolomics studies using an untargeted approach.

References:

[1] Jarausch B, Biancu S, Kugler S, Wetzel T, Baumann M, Winterhagen P, Jarausch W, Kortekamp A, Maixner M. First report of Flavescence dorée-related Phytoplasma in Grapevine in Germany. Plant Dis. 2021 Apr 6. doi: 10.1094/PDIS-02-21-0330-PDN. Epub ahead of print. PMID: 33823613.

[2] Teixeira A, Martins V, Frusciante S, Cruz T, Noronha H, Diretto G, Gerós H. Flavescence Dorée-Derived Leaf Yellowing in Grapevine (Vitis vinifera L.) Is Associated to a General Repression of Isoprenoid Biosynthetic Pathways. Front Plant Sci. 2020 Jun 17;11:896. doi: 10.3389/fpls.2020.00896. PMID: 32625230; PMCID: PMC7311760.

[3] Arricau-Bouvery N, Dubrana MP, Canuto F, Duret S, Brocard L, Claverol S, Malembic-Maher S, Foissac X. Flavescence dorée phytoplasma enters insect cells by a clathrin-mediated endocytosis allowing infection of its insect vector. Sci Rep. 2023 Feb 7;13(1):2211. doi: 10.1038/s41598-023-29341-1. PMID: 36750707; PMCID: PMC9905606.

[4] Lee MS, Chyau CC, Wang CP, Wang TH, Chen JH, Lin HH. Flavonoids Identification and Pancreatic Beta-Cell Protective Effect of Lotus Seedpod. Antioxidants (Basel). 2020 Jul 24;9(8):658. doi: 10.3390/antiox9080658. PMID: 32722185; PMCID: PMC7466071.

Poster #5

MALDI IMAGING MASS SPECTROMETRY FOR SUB-TYPING; A PILOT STUDY UNREVEALING BREAST CANCER HETEROGENEITY

Barbara Cardinali[1], Thomas W. Powers[2], Benjamin A. Neely[2], Richard R. Drake[2], Franca Carli[3], Aldo Profumo[4], Annalisa Salis[5], Gianluca Damonte[5] and Lucia Del Mastro[1]

[1] *Clinica di Oncologia Medica, IRCCS Ospedale Policlinico San Martino, Genoa, Italy;*
[2] *MUSC Proteomics Center, Medical University of South Carolina, Charleston , SC USA;*
[3] *Surgical Pathology Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy;*
[4] *Proteomics and Mass Spectrometry Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy;*
[5] *Center of Excellence for Biomedical Research, University of Genoa ,Genoa, Italy*
Email: barbara.cardinali@hsanmartino.it

Breast Cancer (BC) proteomic profiling of could serve as a complementary tool to the routinely used histopathological markers in refining subgroups and guiding treatment strategies. This approach is particularly important for characterizing hormone receptor-positive (HR+), G2, HER2 negative BC, which are currently considered to be at intermediate risk of recurrence, since in nearly 40% of these cases, even genomic tests – that provide a more accurate clinical classification—fail to fully address the complexity of these tumors and do not provide sufficient guidance for clinicians in selecting the appropriate therapy. This study aims to classify BC into distinct risk categories using MALDI Imaging Mass Spectrometry (MSI). A set of formalin-fixed paraffin-embedded (FFPE) HR+, HER2-, G2 BC samples from patients enrolled in selected clinical trials at IRCCS Policlinico San Martino was characterized at the MUSC Proteomics Center, Medical University of South Carolina. After optimizing sample preparation protocol (antigen retrieval, in situ trypsinization, and matrix coating), a training set of 30 FFPE samples (25 G2 and 5 G3, which are more aggressive) was analyzed on an AutoflexIII Smartbeam (Bruker Daltonics) mass spectrometer. Statistical analysis revealed 55 significantly different signals and grouped samples into two clusters. Notably, samples from patients who relapsed were classified in the same group, suggesting that IMS proteomic analysis has the potential to categorize patients based on their risk of recurrence. The performance of the classifier was tested on a validation set of 11 samples collected in the same trials: samples from patients with recurrence were correctly classified into the high-risk group. To further enhance classification accuracy, additional analyses were carried out on a larger training set of 70 FFPE samples using a Bruker Solaris FT mass spectrometer. Statistical and MS/MS analyses of the tryptic digests for protein identification are ongoing. This study could provide new insights into BC heterogeneity offering a potential proteomic signature able of predicting risk of recurrence for patients with a BC subtype for which therapy choices are still controversial.

Acknowledgements: BC Thanks Fondazione Umberto Veronesi

Poster #6

OPTIMIZATION OF PRE-PROCESSING FOR HYPERION CYTOF'S MULTI-CHANNEL TIFF DATA ANALYSIS

Coronel Vargas Gabriela [1], Altosole Tiziana[1], Berra Silvia [1], Mariani Maria Cristina[2], Damele Laura[1], Vigo Tiziana[2]

[1] Direzione Scientifica, IRCCS Ospedale Policlinico San Martino, [2] Laboratorio di Neuroscienze Sperimentali, IRCCS Ospedale Policlinico San Martino

Abstract: Hyperion CyTOF imaging system, also known as Imaging Mass Cytometry (IMC), enables simultaneous measurement of over 40 parameters at subcellular resolution. While this capacity provides unprecedented insights into tissue architecture and cellular phenotypes, it also generates vast and complex datasets that require sophisticated computational strategies to obtain single cell data. The integration of tools and workflows into a cohesive, accessible pipeline remains an unmet need, highlighting the importance of interdisciplinary collaborations between biologists, bioinformaticians, and software developers. Addressing these challenges will facilitate broader adoption of CyTOF imaging and unlock its full potential in answering pressing biological questions. Tools, and gaps in the pipeline, were improved for tackle novel strategies to overcome these challenges. Denoising is a critical step in the analysis of CyTOF imaging data, particularly for Imaging Mass Cytometry (IMC), where noise from multiple factors, including sample preparation quality, variations in conjugated metal isotopes, antibody concentration and arrangement can obscure biologically meaningful signals. Effective denoising enhances data clarity and improves downstream analyses such as cell segmentation, phenotyping, and spatial mapping.

Figure 2 adaptation of the bioinformatic tool IMC-denoise© for the creation of training sets to improve Imaging Mass Cytometry data using Deep Neural Network

References: [1] Windhager J, et al.. An end-to-end workflow for multiplexed image processing and analysis. Nat Protoc. 2023 Nov;18(11):3565-3613. doi: 10.1038/s41596-023-00881-0. Epub 2023 Oct 10. PMID: 37816904. [2] Lu P, et al.. IMC-Denoise: a content aware denoising pipeline to enhance Imaging Mass Cytometry. Nat Commun. 2023 Mar 23;14(1):1601. doi: 10.1038/s41467-023-37123-6. PMID: 36959190; PMCID: PMC10036333. [3] Schapiro D, et al.. histoCAT: analysis of cell phenotypes and interactions in multiplex image cytometry data. Nat Methods. 2017 Sep;14(9):873-876. doi: 10.1038/nmeth.4391. Epub 2017 Aug 7. PMID: 28783155; PMCID: PMC5617107.

Poster #7

CHEMOTHERAPY RESISTANCE INVESTIGATED BY MULTIMODAL MSI OF OSTEOSARCOMA MULTICELLULAR TUMOUR SPHEROID MODELS

Sophie M. Pearce[1*], Neil A. Cross[1], David P. Smith[1], Malcolm R. Clench[1], Lucy E. Flint[2], Gregory Hamm[2], Richard Goodwin[2], James I. Langridge[3], Emmanuelle Claude[3], Laura M. Cole.[1].

[1] *Centre for Mass Spectrometry Imaging, Sheffield Hallam University, Howard Street, Sheffield S1 1WB, UK;*

[2] *Imaging and Data Analytics, Clinical Pharmacology and Safety Sciences, BioPharmaceuticals R&D, AstraZeneca, The Discovery Centre (DISC), Biomedical Campus, 1 Francis Crick Ave, Trumpington, Cambridge CB2 0AA, UK;*

[3] *Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, Cheshire SK9 4AX, UK.*

**Email: s.pearce1@shu.ac.uk*

A Multimodal Mass Spectrometry Imaging (MSI) approach was used to investigate the chemotherapy drug-induced response of a Multicellular Tumour Spheroid (MCTS) 3D cell culture model of osteosarcoma (OS). Addressing the need for enhanced translatable early drug discovery methods, this work demonstrates spatially resolved analysis of tumour models following chemotherapeutic intervention [1]. MSI revealed characteristic distributions to that of an in-vivo tumour and previously unknown doxorubicin (DOX)-induced metabolite upregulation was identified by DESI-MSI. The first application of MALDI-IHC to 3D cell culture models, complemented by Imaging Mass Cytometry (IMC), explored protein localisation and expression following acute dosage. This multimodal approach revealed novel insights into region-specific tumour survival mechanisms and cell cycle-specific drug responses. Additionally, chemotherapy-resistant models derived from treatment-naive counterparts offered further understanding of the metabolic reprogramming underlying chemotherapy resistance.

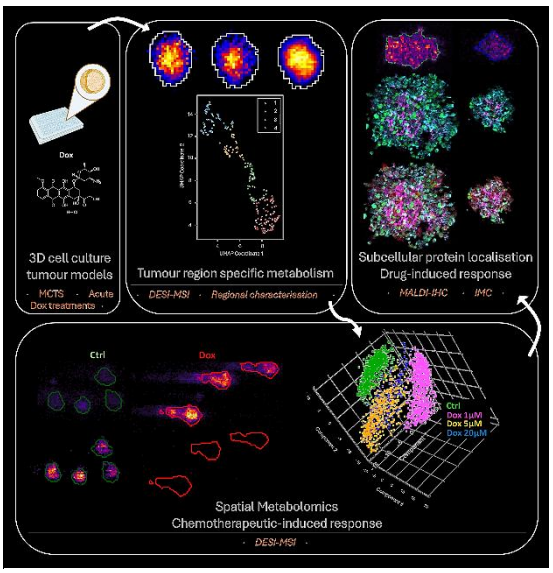


Fig. 1: A multimodal MSI approach to investigate chemotherapy resistance.

References:

[1] S. M. Pearce, N. A. Cross, D. P. Smith, M. R. Clench, L. E. Flint, G. Hamm, R. Goodwin, J. I. Langridge, E. Claude and L. M. Cole, Metabolites 2024, Vol. 14, Page 315, 2024, 14, 315.

Acknowledgements:

This work is funded by a Waters Corporation Ph.D. Studentship and a Sheffield Hallam University Vice-Chancellor Award. Astra Zeneca are greatly acknowledged for their collaboration and facilitation of student research placements.

Poster #8

HIGH RESOLUTION LOW FLOW DESI IMAGING USING A COMMERCIAL DESI SOURCE

Mark Towers[1], Emrys Jones[1], Lisa Reid[1], Sheba Jarvis[2], Joanne Ballantyne[1], **Andrea Perissi[3]*, Angelo Palmese[3]***

[1] Waters Corporation, Wilmslow UK;[2] Imperial Collage London, Hammersmith Hospital, London UK;
[3] Waters Corporation, Sesto San Giovanni Italy
**Email: Andrea_Perissi@waters.com; Angelo_Palmese@waters.com*

Desorption electrospray ionization (DESI) is an established imaging technique with a continuous drive towards smaller pixel sizes and higher spatial resolution. This study demonstrates a low flow DESI technique for high image resolution using Waters commercially available DESI XS. A time-saving, data-driven re-acquisition technique is described to achieve detailed high-resolution images after quick low-resolution initial passes.

Methods:

Waters commercially available DESI XS source with a high-performance sprayer, heated transfer line (HTL), and µBinary solvent manager fluidics system (M-class ACQUITY BSM) was used for analyzing porcine liver, human adrenal gland, and rat brain tissue. Modifications included adding a 1.7µm ACQUITY C18 column and replacing solvent delivery tubing to increase back pressure. DESI settings included a solvent flow rate of 200-250 nL/min, nebulizing gas pressure of 1.35 bar, and capillary voltage of 0.79-0.85 kV. Images were acquired at 10-20 pixels/second.

Discussion:

Reducing the solvent flow rate to 250 nL/min allowed for reduction in spray beam diameter to <10 µm, increasing imag resolution to the cellular level. The low flow DESI setup wa stable across multiple tissues and suitable for long acquisition (>35 hrs). A data-driven microscope approach enabled high resolution re-acquisition of targeted sub-regions after an initia low-resolution survey scar

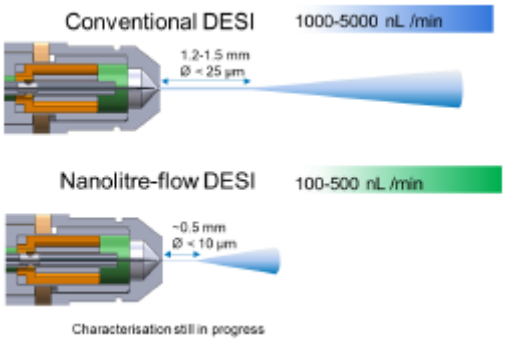


Fig. 1: Schematic diagram of the nanoliter flow DESI compared to the conventional flow DESI.

Conclusion:

Sub-10 µm image resolution is achievable using the commer modification. The low flow DESI setup is stable and suitable resolution imaging with minimal detriment to image or signal quality.

Poster #9

MALDI IMAGING ANALYSIS OF THORACIC AORTIC ANEURISMS SHOWS DIFFERENTIALLY EXPRESSED MOLECULAR SPECIES ASSOCIATED WITH DISEASE SEVERITY

Daniele Stefanizzi[1], Matteo Calligaris[1], Pierluigi Visentin[2], Daniela Cesselli[1-2], Antonio Paolo Beltrami[1-2], Francesco Curcio[1-2]

[1]*Università di Udine, Department of medicine, Italy;*
[2]*Institute of Clinical Pathology, ASUFC, Udine, Italy*

Thoracic aortic aneurisms (TAA) and dissection (TAAD) are a heterogeneous spectrum of disorders caused by the degeneration of the Intima, Media and Adventitia layers. Approximately 20% of patients with TAAD show an autosomal dominant pattern of inheritance. TAAD patients are treated with prophylactic surgery once the aneurism reaches 5-5.5 cm in diameter; however, up to 60% of patients with dissection show an aortic diameter <5 cm [1]. Therefore, the aim of this study was to search for possible molecular and morphological markers using MALDI-IMS. Twenty aortic biopsies, including controls (n=4), moderate TAA (n=5), and severe TAA (n=11), were included in carboxymethylcellulose (CMC) and frozen at -80 °C. 10 µm thick sections were cut, placed on indium tin oxide (ITO) slides, and kept at -20 °C until testing. Each biopsy was analyzed employing both α-Cyano-4-hydroxycinnamic acid (CHCA) and IR-780 in both positive and negative ion modes, respectively. The matrix was deposited by spraying, and images were acquired using MALDI 7090 (Shimadzu, Japan). The resulting spectra were analyzed using ImageREVEAL (Shimadzu, Japan), peaks were identified using the human metabolome database. One molecular species, 798 m/z (PE-NMe(18:4/22:1)), was found to be significantly increased in severe patients vs. controls in the positive ion mode, while in the negative ion mode, four molecules were found to be significantly altered: 684.5 m/z (PE (14:10/18:3)) and 687.068 m/z (2-O-Galloyl-1,4-galactarolactone) were more expressed in moderate patients than in severe ones, and 775.5 m/z (PA(22:4/20:2)) and 665.5 m/z (DG(18:1/0:0/22:6)) were more expressed in controls than in severe patients. Lastly, we observed a different spatial distribution of some molecular species that changed in relation to the disease state of the patient, Figure 1. In conclusion, imaging analysis of 20 patients with TAAD identified five potential markers of disease severity.

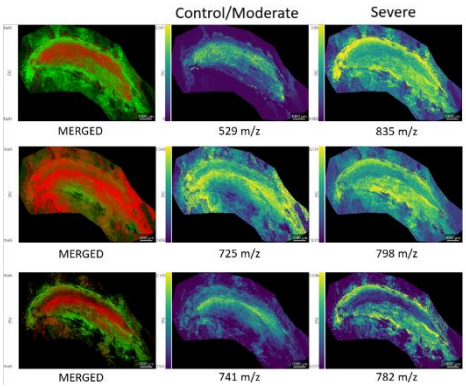


Fig.1: Spatial localization of masses found in positive ion mode

Reference

[1] Pinard A, Jones GT, Milewicz DM. Genetics of Thoracic and Abdominal Aortic Diseases. Circ Res. 2019 Feb 15;124(4):588-606.

Poster #10

MS-IMAGING: IN SITU INVESTIGATION OF SECONDARY METABOLITES OF ORYZA SATIVA VARIETIES BEFORE AND AFTER COOKING

Riccardo Zecchi[1]*, Antonella Di Napoli[1], Riccardo Romoli[2], Giuseppe Pieraccini[2], Giancarlo la Marca[2], Marzia Innocenti[1], Nadia Mulinacci[1]

[1] *Department of NEUROFARBA (Nutraceutical section) University of Florence, Via Ugo Schiff, 6, 50019, Firenze, Italy*
[2] *Mass spectrometry service centre (CISM), University of Florence, V.le G. Pieraccini 6, 50139, Firenze, Italy*
** Presenting Author: riccardo.zecchi@unifi.it*

The processing steps to which rice (*Oryza sativa*) is subjected after harvesting could result in the depletion of micronutrients that are concentrated in the germ and bran[1]. Parboiling is suggested as an effective hydro-thermal treatment able to reduce cooking time and retain micronutrients of the bran inside the caryopsis[2].

Our aim was to define the best procedure for the application of MALDI-MSI to investigate the migration of two classes of rice micro-components after industrial processing (parboiling), and domestic cooking (water boiling and steam cooking): steryl ferulates and anthocyanins[3], which are known to provide positive physiological effects on human health[4]. The following rice samples were analysed: 'Carnaroli' (white rice), 'Oro' (white parboiled rice), 'Nerone', (black pigmented rice), and 'Venere', (black pigmented parboiled rice).

Spectral acquisitions were conducted with full scan high mass resolution analysis in both polarities at 50 µm lateral resolution. Through Shiny Cardinal software[5,6] we were able to implement ROIs annotation method based on spatial shrunken centroid clustering algorithm (SSCCA). This procedure was fundamental for reliable statistics of target ion intensities in the different seed compartments even after cooking procedures that greatly change histology features.

As regards steryl ferulates, we highlight a negligible migration towards the endosperm in the parboiled rice. Regards anthocyanins in pigmented rice, it was found that steaming or boiling provides a large migration of these compounds in the endosperm. The proposed method and the obtained results provide a solid basis for future experimental designs for the study of rice metabolites by MS-imaging.

References:

[1] Paiva F.F., et al. (2014). J. Food Comp. & Anal. 35, 10–17.
[2] Bhattacharya K. R. (2004). Rice chemistry and technology (3rd ed., pp. 329–404).
[3] Ciulu, M., et al. (2018). Molecules, 23(11), 1–20.
[4] Goffman F. D., et al. (2004). J. Sci. Food &Agric., 84, 1235–1240
[5] Yonghui D., et al. (2024) PREPRINT (Version 1) available at Research Square
[6] Bemis K.A., et al. (2023) Nat Methods. 20(12):1883-1886.

Poster #11

MAPPING EPIDERMAL LIPIDS IN 3D SKIN MODELS AND HUMAN BIOPSIES

Samuele Zoratto[1,2], Christopher Kremslehner[2,3], Michela Schirato[2,3], Gaelle Gendronneau[2,4], Agnes Tessier[2,4], Florian Gruber[2,3,5], Martina Marchetti-Deschmann[1,2,5,6]

1) Institute of Chemical Technologies and Analytics, TU Wien, Vienna, Austria, 2) Christian Doppler Laboratory for Multimodal Imaging of Aging and Senescence - SKINMAGINE, Vienna, Austria, 3) Department of Dermatology, Medical University of Vienna, Vienna, Austria, 4) Chanel PB, Pantin, France, 5) ViCEM, Vienna Center for Engineering in Medicine, 6) ACTR Austrian Cluster for Tissue Regeneration, Vienna, Austria

**Email: samuele.zoratto@tuwien.ac.at*

Developing an *in vitro* skin model that accurately mirrors the molecular and biological events of human skin *in vivo* is a challenging task. Here, we present lipid profile data from *in vitro* skin equivalent model (SE), and compare them with profiles observed in human skin biopsy (HS). We investigate the distribution and composition of lipids in these tissues.

Spatial lipid profiling of HS and SE was performed by MALDI MSI using a 7T FTICR instrument. A reference dataset from fibroblast- and keratinocyte-based cultures, obtained by HPLC-MS/MS, served as a reference for the MSI analyses. MALDI FTICR MSI was employed in both positive and negative ion modes, allowing for the detection of diverse lipid classes, from ceramides to glycerophospholipids, thus providing comprehensive coverage for an in-depth comparative analysis. This setup enabled visualization and unambiguous identification of lipids at 10 µm lateral resolution in tissue sections. H&E staining was used to correlate lipid distributions with morphological features of HS and SE.

In total, more than 300 annotated lipids were detected. Ceramides, important for skin barrier function and moisture control, were predominantly localized in the upper parts of the suprabasal layers of the epidermis. Other lipid classes, including sphingoid-based, lysophospho-, and phospholipid species, showed distinct distribution patterns in the epidermal and dermal layers. In total, 135 lipids were shared between HS and SE with a similar distribution, while some lipids appeared exclusively in native tissue, reflecting the complexity of human skin.

These results demonstrate that the SE model can reflect many key features of HS, making it an effective tool for detailed molecular investigations under controlled conditions. Although reproducing the full complexity of native human skin remains an ongoing challenge, the substantial overlap in lipid composition and distribution supports SE as a reliable model for studying and understanding how lipid profiles change during skin development and aging processes.