IATP Annual Business meeting

Tuesday June 10th 2025

Point to consider on
Mass Spectrometry Imaging (MSI) in Pathology for Detection of Molecules
according to the
ESTP Pathology 2.0 Mass Spectrometry Imaging Group

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Toxicologic Pathology
1–29

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This "Points to Consider" article is a product of a European Society of Toxicologic Pathology (ESTP) Pathology 2.0 Working Group. It has been reviewed and approved by the Committee of Regulatory and Scientific Standards (CRSS) and Executive Committee of the ESTP and endorsed by the Executive Committees of the British Society of Toxicological Pathology (BSTP) and Society of Toxicologic Pathology (STP), but it does not represent a formal best practice recommendation of the Societies; rather, it is intended to provide key "points to consider" for the toxicologic pathology community. The opinions expressed in this document are those of the authors and do not reflect views or policies of the employing institutions. Readers of Toxicologic Pathology are encouraged to send their thoughts on these articles or ideas for new topics to the editor.

European Society of Toxicologic Pathology—Pathology 2.0 Mass Spectrometry Imaging Special Interest Group: Mass Spectrometry Imaging in Diagnostic and Toxicologic Pathology for Label-Free Detection of Molecules—From Basics to Practical Applications

Enrico Vezzali[1], Michael Becker[2], Fernando Romero-Palomo[3], Marjolein van Heerden[4], Caroline Chipeaux[5], Gregory Hamm[6], Dinesh S. Bangari[7], Thomas Lemarchand[8], Barbara Lenz[3], Bogdan Munteanu[5], Bhanu Singh[9], Celine Thuilliez[10], Seong-Wook Yun[2], Andrew Smith[11], and Rob Vreeken[12]

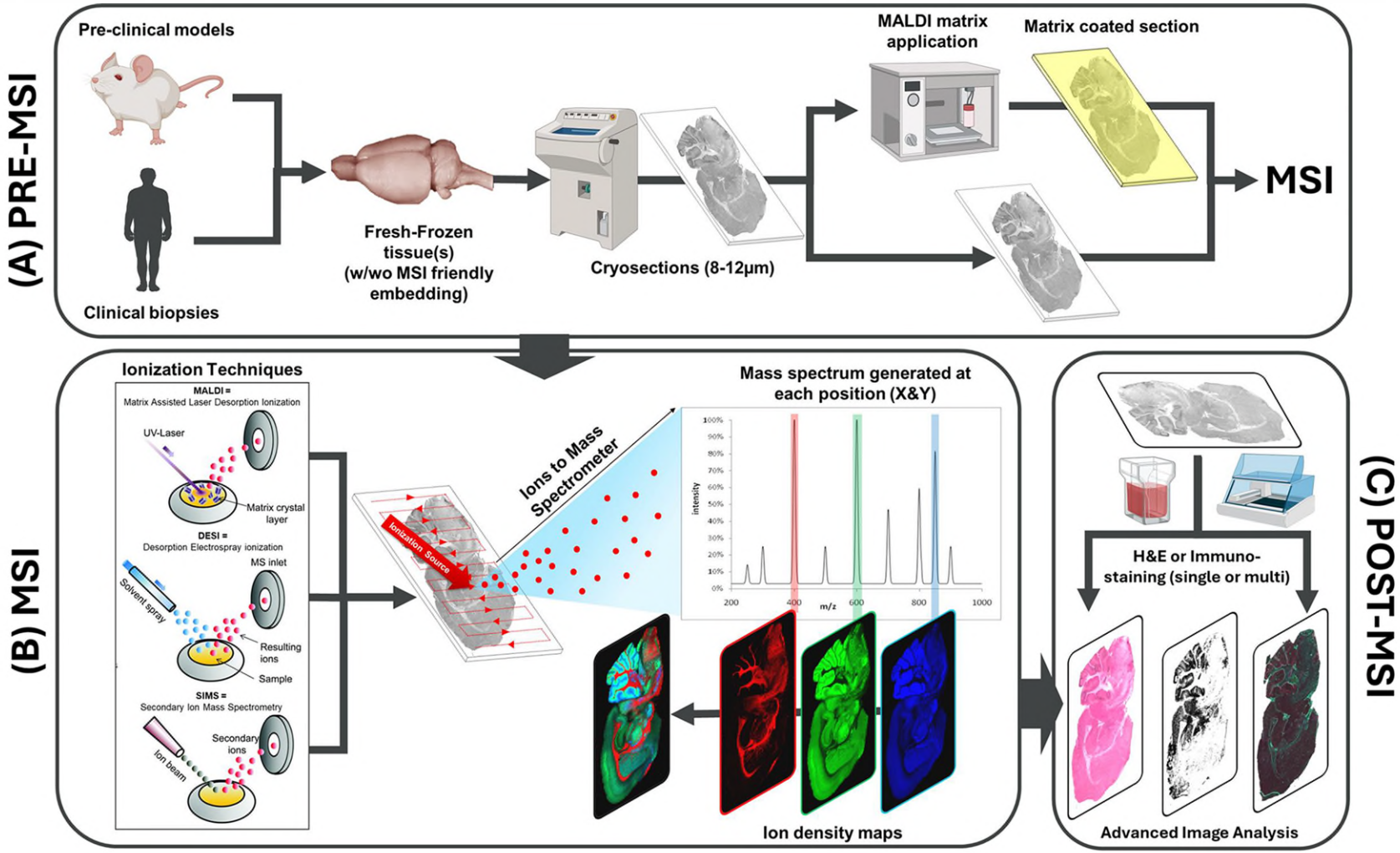
Abstract

Mass Spectrometry Imaging (MSI) is a powerful tool to understand molecular pathophysiology and therapeutic and toxicity mechanisms, as well as for patient stratification and precision medicine. MSI, a label-free technique offering detailed spatial information on a large number of molecules in different tissues, encompasses various techniques including Matrix-Assisted Laser Desorption Ionization (MALDI), Desorption Electrospray Ionization (DESI), and Secondary Ion Mass Spectrometry (SIMS) that can be applied in diagnostic and toxicologic pathology. Given the utmost importance of high-quality samples, pathologists play a pivotal role in providing comprehensive pathobiology and histopathology knowledge, as well as information on tissue sampling, orientation, morphology, endogenous biomarkers, and pathogenesis, which are crucial for the correct interpretation of targeted experiments. This article introduces MSI and its fundamentals, and reports on case examples, determining the best suited technology to address research questions. High-level principles and characteristics of the most used modalities for spatial metabolomics, lipidomics and proteomics, sensitivity and specific requirements for sample procurement and preparation are discussed. MSI applications for projects focused on drug metabolism, nonclinical safety assessment, and pharmacokinetics/pharmacodynamics and various diagnostic pathology cases from nonclinical and clinical settings are showcased.

Keywords

mass spectrometry imaging, label-free, multiplex, diagnostic pathology, drug development

	Usefulness	Limitation
Intrinsic	**Strengths** Label-free Spatially resolved Multiplexed Compatible with histology High specificity (targeted experiments)	**Weaknesses** Limited sensitivity No chromatographic separation Molecular identification challenges No sub-cellular resolution Limited coverage (untargeted experiments) Limited standardization
Extrinsic	**Opportunities** Ion Mobility Separation Spatial 'Omics' Multimodal applications	**Threats** Incongruent new information



Vaysse PM, **Heeren** RMA, Porta T, Balluff B. Mass spectrometry imaging for clinical research - latest developments, applications, and current limitations. Analyst. 2017;142(15):2690-2712. doi:10.1039/c7an00565b.

Porta Siegel T, **Hamm** G, Bunch J, Cappell J, Fletcher JS, Schwamborn K. Mass Spectrometry Imaging and Integration with Other Imaging Modalities for Greater Molecular Understanding of Biological Tissues. Mol Imaging Biol. 2018;20(6):888-901. doi:10.1007/s11307-018-1267-y.

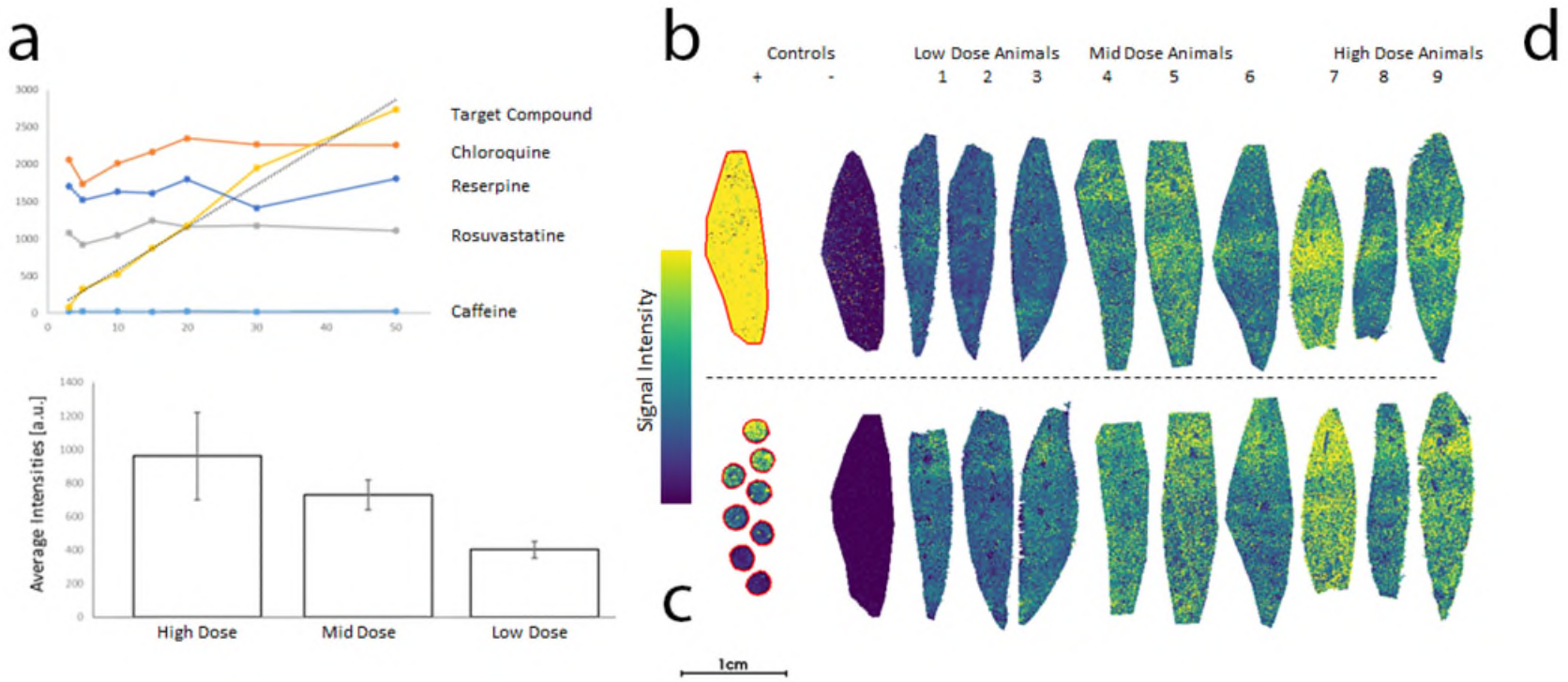
	Interface	Type of ionization	Matrix	Ambient or Vacuum	Analytes	Molecular weight (kD)	Spatial Resolution
MALDI	(UV) Laser beam	Soft	Yes	Vacuum	Small molecules, drugs , lipids, peptides, proteins, sugars	< 5000	5-10µm
DESI	Solvent Spray	Soft	No	Ambient	Small (polar) molecules, lipids, small peptides	< 2000	30-40 µm
SIMS	Ion gun	Hard	No	High Vacuum	Elemental ions, small molecules, lipids	< 1000	< 1 µm
LESA	Solvent droplet	Soft	No	Ambient	Proteins, small molecules, drugs	<20000	> 200 µm
LAESI	Laser beam	Medium	No	Ambient	Small molecules and Drugs	< 1000	150-400 µm

MSI in toxicologic pathology applied to drug development

Test item (e.g small molecule or bioproduct) or biomarker	MSI method (e.g. MALDI, SIMS, …)	Organ/tissue	Tissue preparation (e.g. frozen vs FFPE)	Reference (otherwise N.A. – not available)
Drugs and Drug-Related Metabolites	MALDI-MS	Multiple organs	Frozen	N.A.
Crystal Deposits in Tissue Samples	MALDI-MS	Harderian gland (mouse)	FFPE	N.A.
Biomarker for Drug-induced Phospholipidosis (DIPL)	MALDI-MS	Multiple organs (rat)	Frozen	N.A.
LNA-Containing Antisense Oligonucleotides	MALDI-FTICR-MS	Kidney and Liver (rat)	Frozen	(Romero-Palomo et al., 2021)
Needle-Shaped Crystalline Material in Rat Intestine	MALDI-MS	Small Intestine	Frozen	N.A.
Tissue biomarkers in healthy livers	MALDI-FTICR-MS	Liver	Frozen	(Flinders et al., 2018)
Long-Acting Injectable (LAI) Formulations	MALDI-MS	Injection site (muscle)	Frozen	N.A.
Mature Minipig Ovaries	TimsTOF flex MALDI-2	Ovaries	Frozen	N.A.
ADC toxicity in lungs	AP-SMALDI QExactive Plus	Lung	Frozen	N.A.
Eye pathology	MALDI-MS	Eye	Davidson's-Fixed PE	(Hamm et al., 2022)

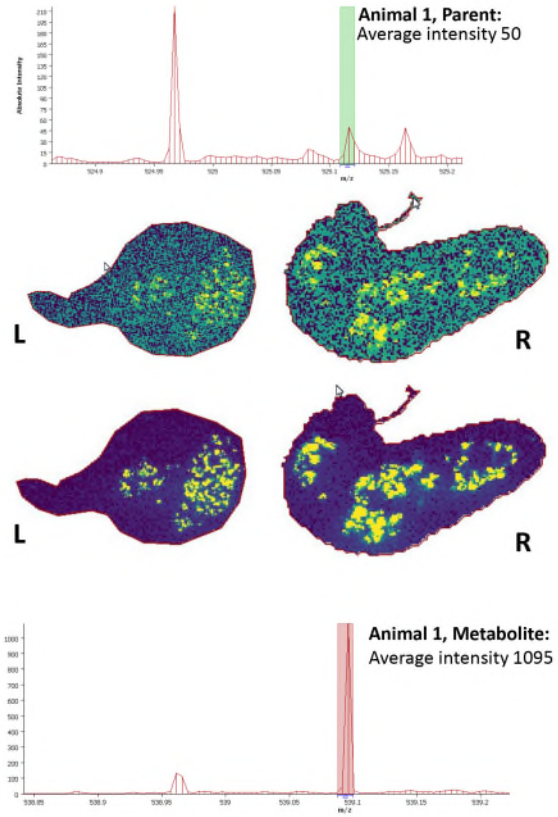
MALDI MS imaging of drugs and drug-related metabolites (Becker M.)

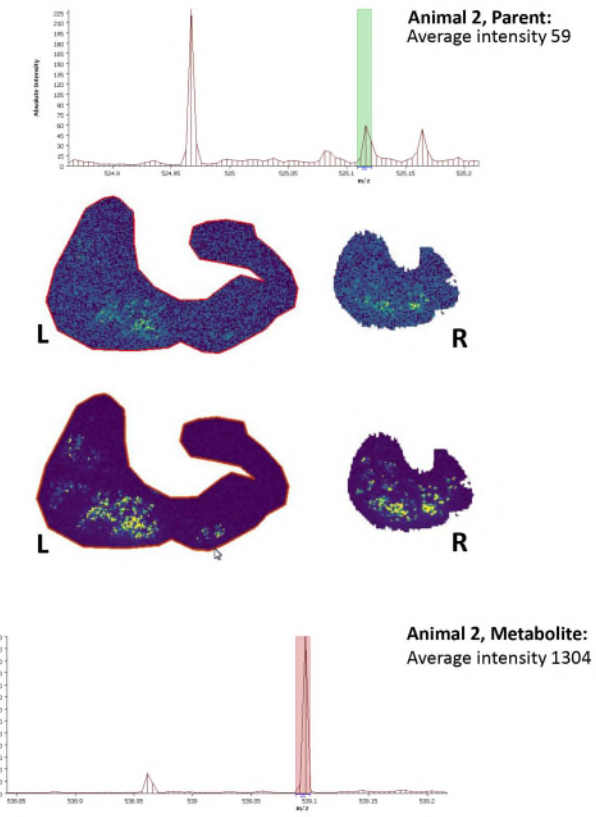
- Example workflow highlighting **limit of detection** and **dynamic range** for the target compound, **potential ion suppression effects** of specific tissue areas, **interference of endogenous background signals** and **slide-to-slide variability**.
- **Semi-targeted** approach using a list of drug metabolites with multiplexed capabilities.



Analyzing crystal deposits in FFPE-tissue samples (Becker M.)

- FFPE common specimen in histopathological investigations, processing can be detrimental for MSI.
- **Reference compounds** allow **optimization** of experimental conditions and enable **comparisons** by ionization efficiencies.
- **Interpretation of MSI results in the context** of additional results (microscopy or pharmacokinetics).





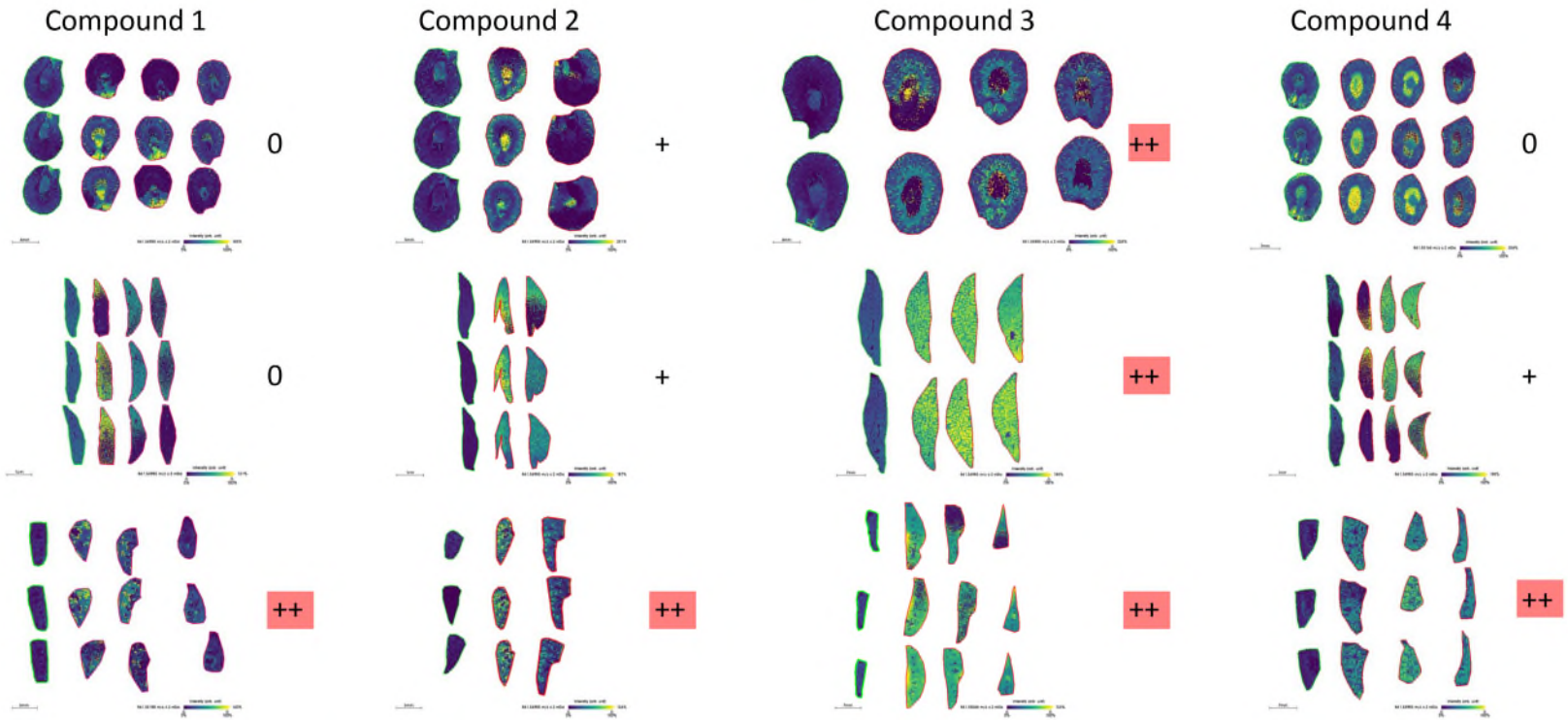
Biomarker elucidation for drug-induced phospholipidosis (Becker M.)

- **Non-targeted** discovery MSI.

- Unequivocal **identification** of markers **require additional methodology (LC-MS/MS)**.

- **Time course studies difficult** when using only necropsy samples.

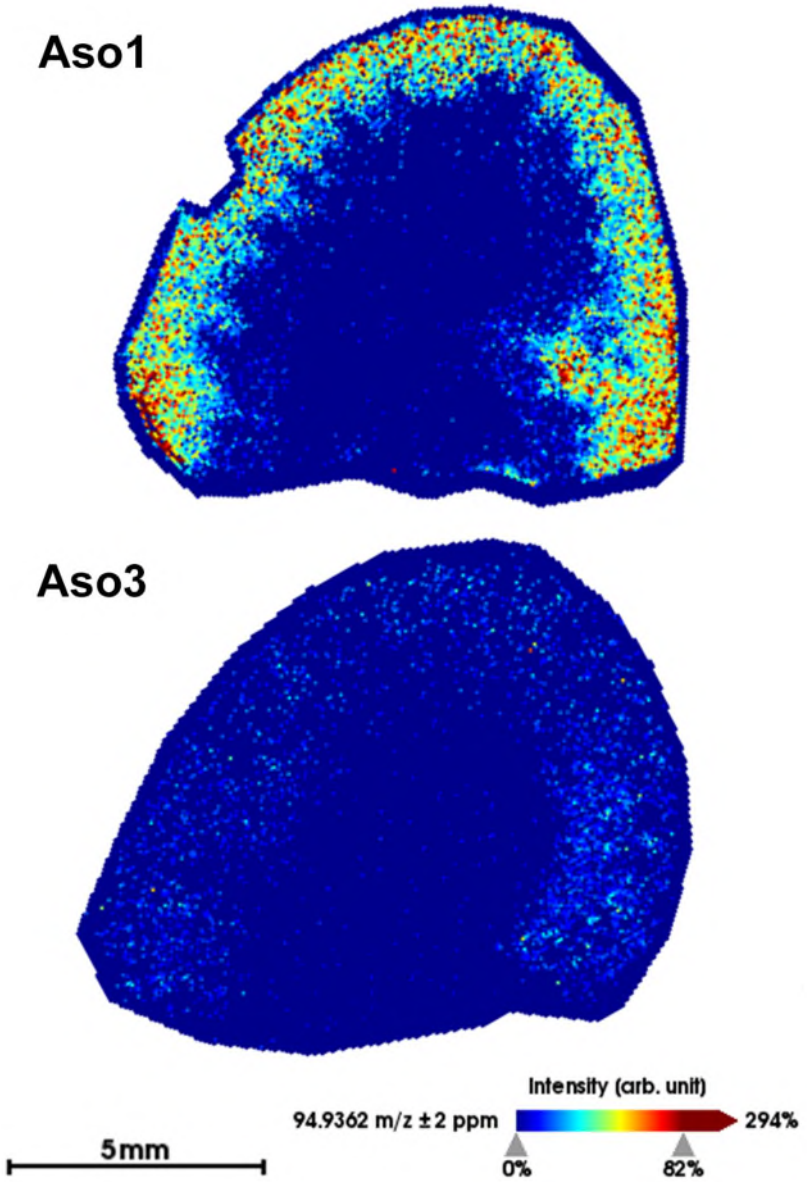
- **Automated analysis of many samples is challenging** due to the biological variation.



Imaging of phosphorothioate-containing antisense oligonucleotides in rat kidney and liver with MALDI-MS (Romero-Palomo F., Lenz B.)

- **ASOs** mechanisms of toxicity tracing metabolism: accumulation not associated with safety profile.
- **No gold-standard** technique for quantifying ASOs; to be considered when selecting the appropriate techniques:

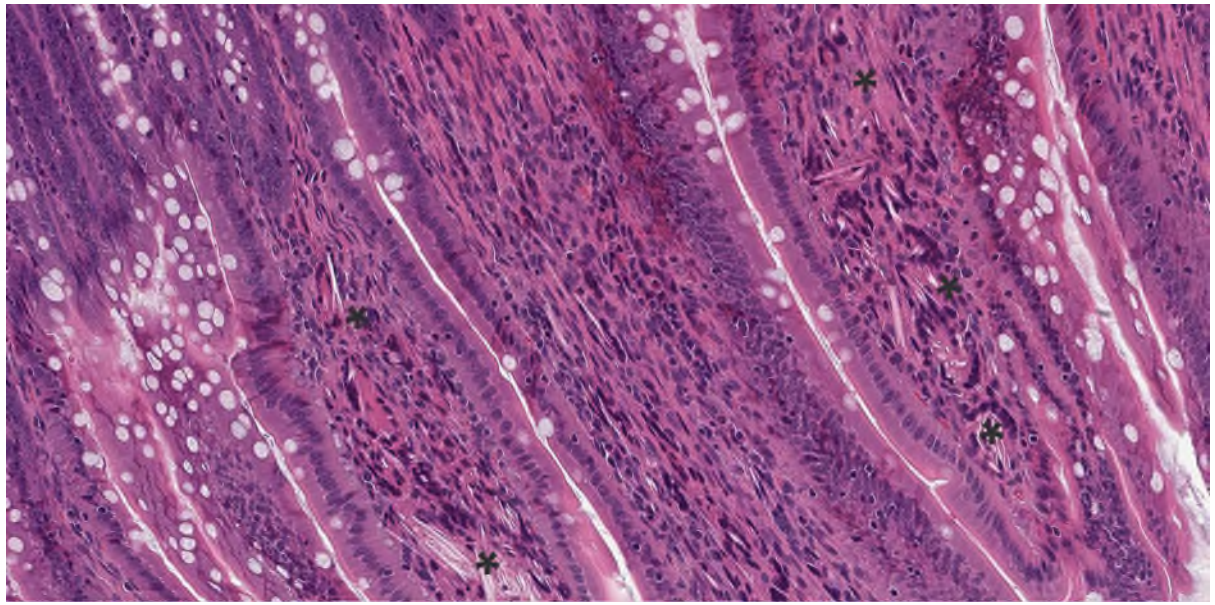
- spatial tissue resolution
- Sensitivity
- differentiation between parent and metabolized compounds



Romero-Palomo F, Festag M, **Lenz B**, et al. Safety, Tissue Distribution, and Metabolism of LNA-Containing Antisense Oligonucleotides in Rats. Toxicol Pathol. 2021;49(6):1174-1192. doi:10.1177/01926233211011615.

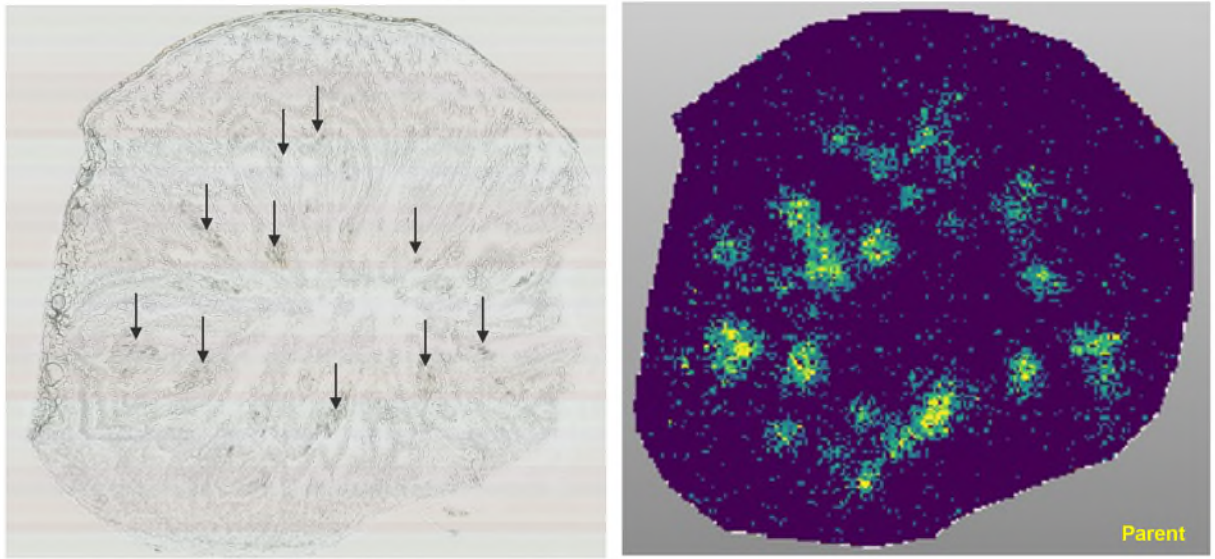
MALDI-MSI of needle-shaped crystalline material in rat intestine (van Heerden M., Vreeken R.)

MALDI-MSI demonstrated a **direct link** between the (spray dried) compound and the needle shaped crystalline material indicating intestinal absorption and precipitation of compound in the lamina propria.

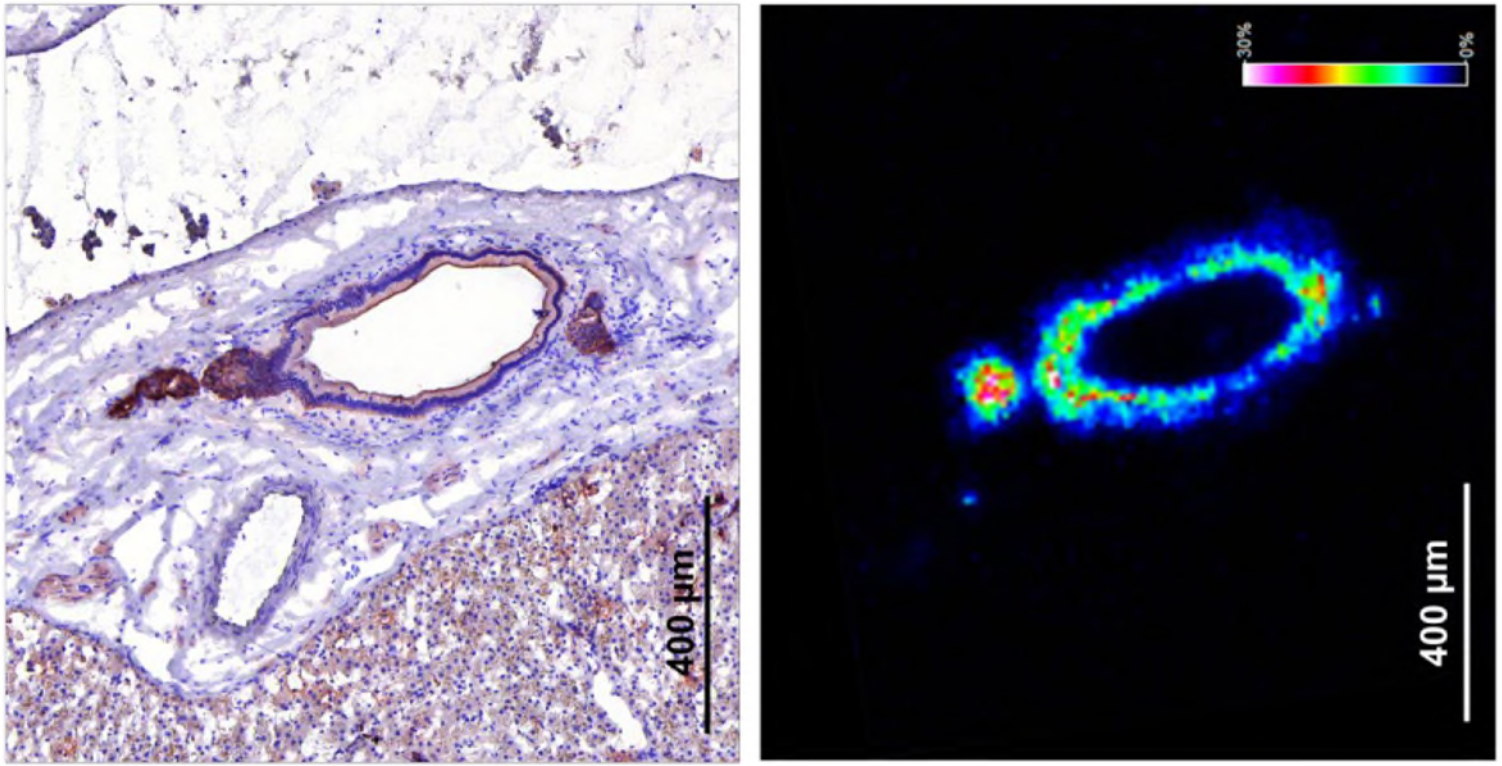


Optical image

MALDI MSI



Multimodal imaging of healthy livers to identify tissue markers and their inclusion in livers from Primary Sclerosing Cholangitis (PSC) patients (van Heerden M., Vreeken R.): MALDI-MSI allowed the identification of healthy liver tissue markers and a specific hydroxylated-sulfatide biomarker for differentiated bile ducts.



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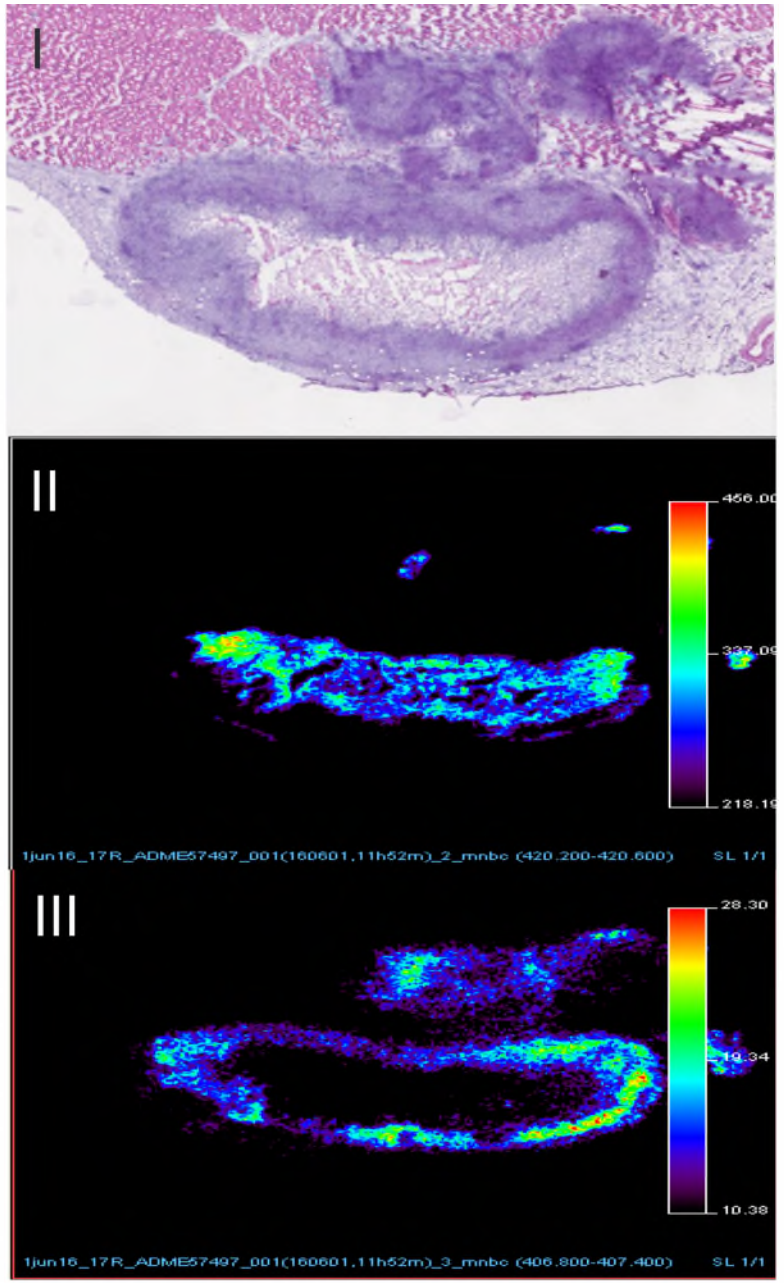
MALDI-MSI of long-acting injectable formulations

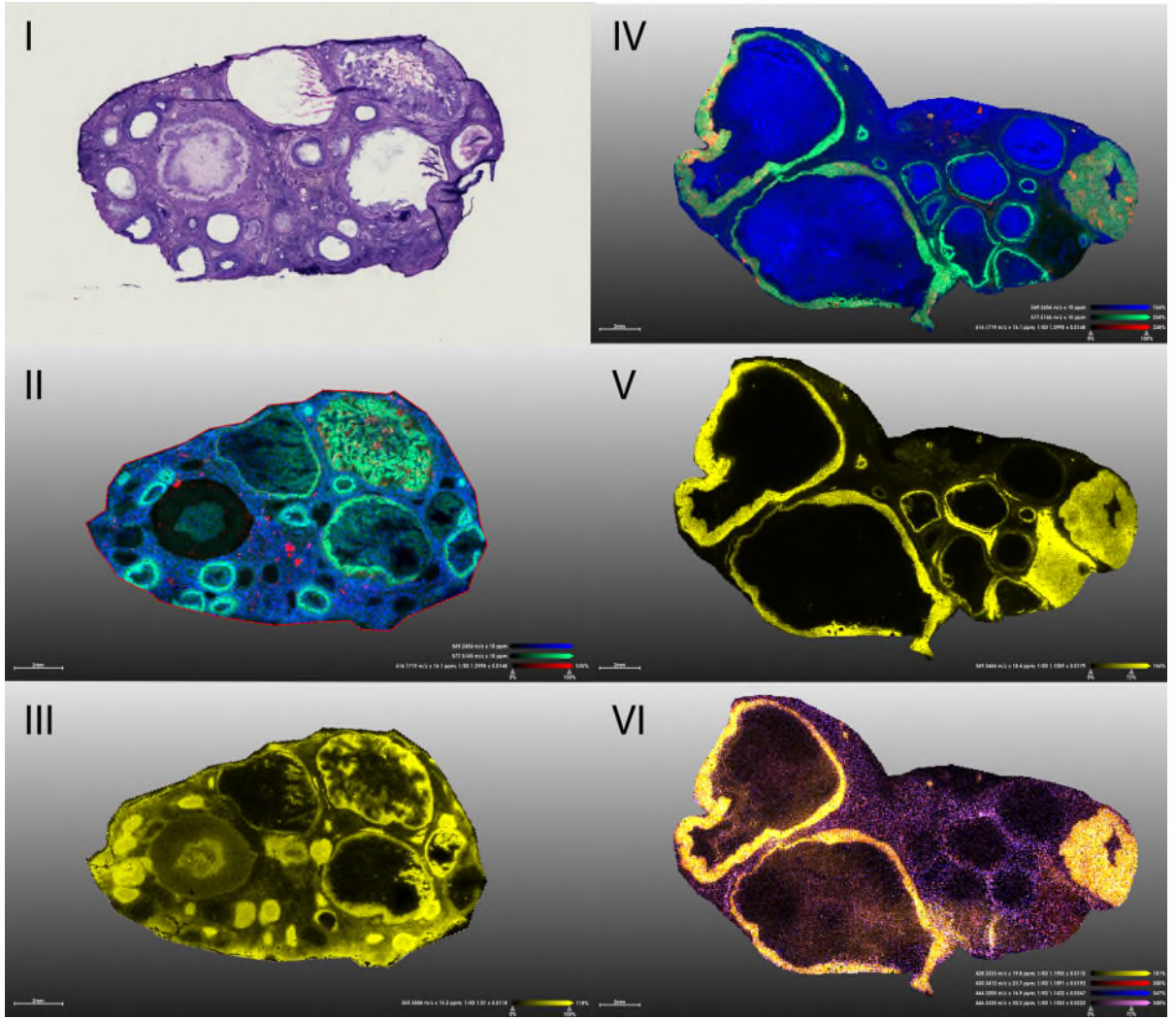
(van Heerden M., Vreeken R.)

MALDI-MSI demonstrated that the histopathologically observed central amorphous material in the injection site after one week, corresponded to the delivery material (prodrug) and that conversion to the active drug mainly occurred in the surrounding granulomatous rim, indicating an important role for the macrophages in the conversion of the pro-drug to the active drug.



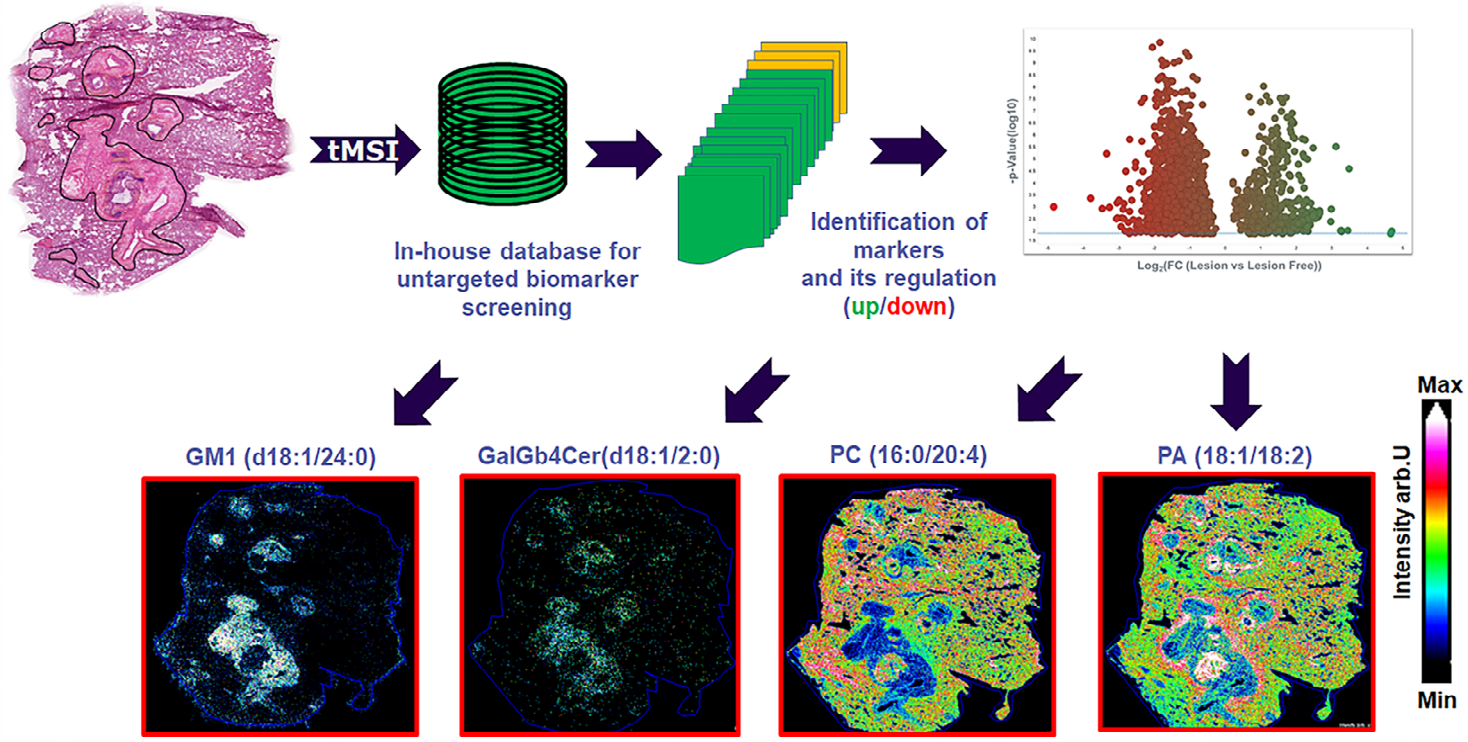
MALDI-MSI on mature minipig ovaries (van Heerden M., Vreeken R.)

TimsTOF flex MALDI-2 analysis on minipig ovaries confirmed the ability to detect and localize certain steroid hormones and their precursor cholesterol in this proof-of-concept study.



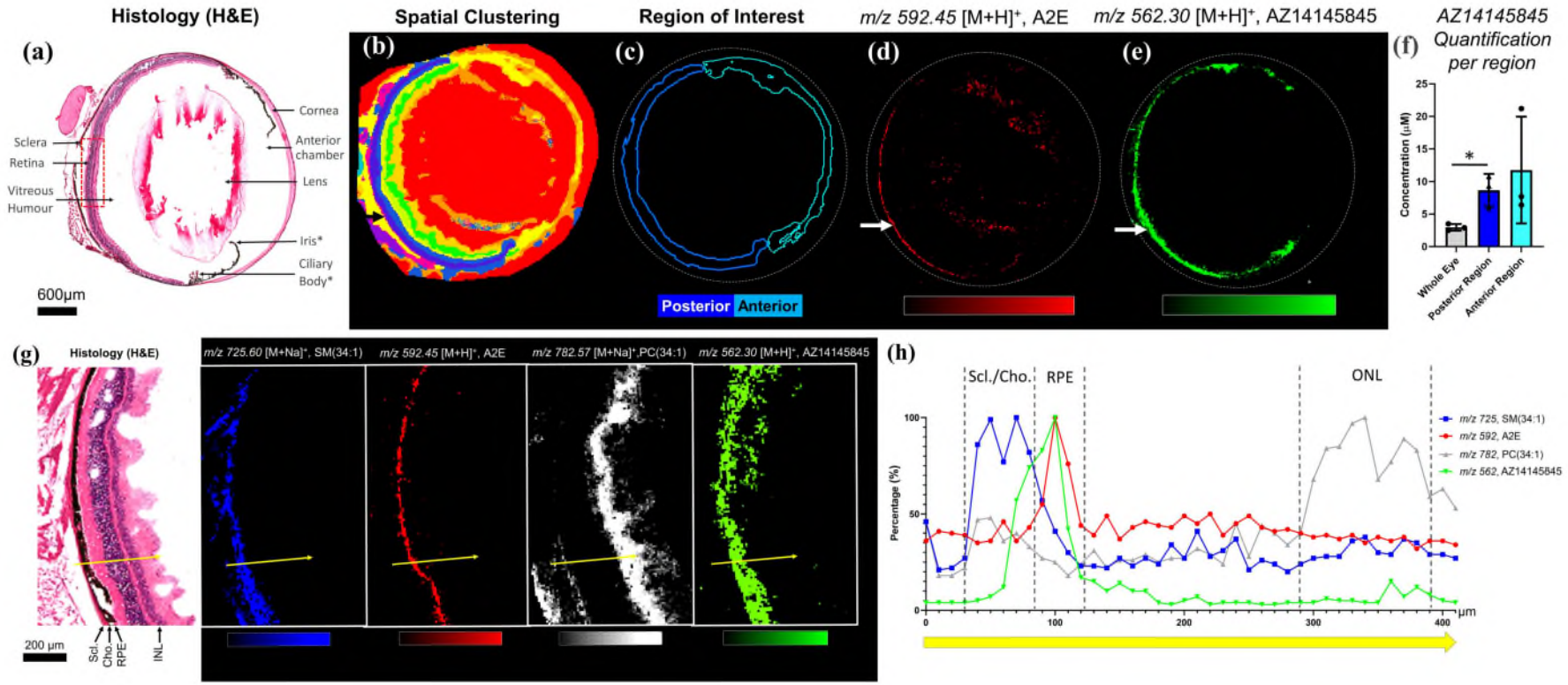
Untargeted biomarker analysis for lung toxicity of intravenous ADC in cynomolgus monkeys by Ultrahigh-Mass Resolution (UHR)-MALDI-MSI (Chipeaux C., Munteanu B.)

- **Standardization** and **quality control**
- **Distribution of drugs and metabolites**
- Spatial resolution
- **Untargeted biomarkers** driven by database.
- Single measurement detecting thousands of metabolites, assigned to metabolic pathways.
- ADC lung toxicity findings in cynomolgus monkeys correlated with biomarker distributions, demonstrating accumulation/depletion of metabolites.



MSI for ocular toxicity assessment (Hamm G.)

- DESI MSI is **non-destructive,** important in ocular studies, with limited sample availability.
- The compartmentalized eye **has non-standard vascularization, with peculiar intra-organ biodistribution**.
- MSI applied to the eye offers spatial resolution, detection sensitivity, wider range and optimized data analysis solutions.



Hamm G, Maglennon G, Williamson B, et al. Pharmacological inhibition of MERTK induces in vivo retinal degeneration: a multimodal imaging ocular safety assessment. Arch Toxicol. 2022;96(2):613-624. doi:10.1007/s00204-021-03197-8.

THANKS !!!

Questions ?

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Keywords

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Backup slides

MSI in diagnostic pathology

Test item (e.g small molecule or bioproduct) or biomarker	MSI method (e.g. MALDI, SIMS, …)	Organ/tissue	Tissue preparation (e.g. frozen vs FFPE)	Reference (otherwise N.A. – not available)
Renal amyloid deposits	MALDI-TOF_MSI	Kidney (human)	FFPE	(Bindi et al., 2024)
Lipids, N-Glycans, and Tryptic Peptides	MALDI-TOF-MSI	Brain (mouse) and kidney (human)	FFPE	(Denti et al., 2022)
thyroid cytopathology	MALDI-TOF-MSI	Thyroid (human)	Frozen	(Nobile et al., 2023)

Case examples of MALDI-MSI applications in diagnostic pathology (Smith A.)

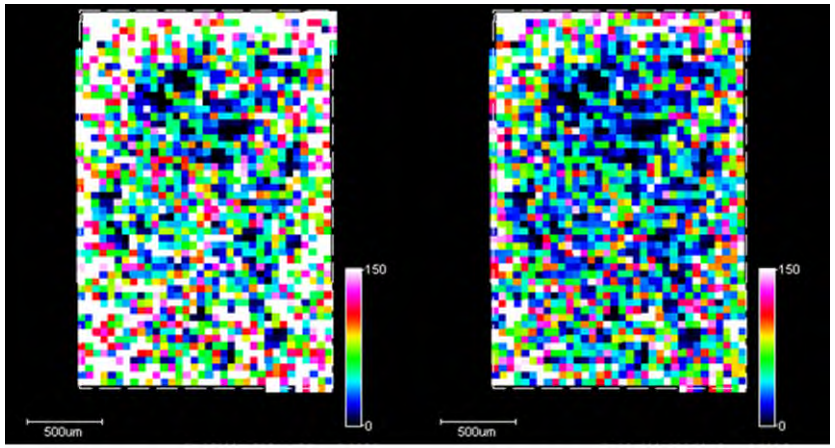
MSI-based proteomics for the in situ detection and typing of renal amyloid deposits. MALDI-MSI represents a feasible tool for the spatially resolved detection and subtyping of renal **amyloid** deposits, even in instances with **minimal material available and at the earlier stages** of formation. This has the potential to offer distinct advantages with respect to traditional histology and MS-based techniques.

Spatial multi-omics of lipids, N-glycans, and tryptic peptides on a single section of FFPE tissue. The possibility to **ingrate spatial multi-omics data**, obtained from one single FFPE tissue section, can help to generate a more complete molecular overview and better discriminate cells of diverse pathological states. Moreover, the recent development of the MALDI-HiPLEX-IHC workflow has opened new avenues within the realm of clinical diagnostics and has the **potential to serve as a high-throughput** alternative for performing routine clinical IHC assays.

Implementation of MSI-based proteomics for the classification of cytological thyroid specimens. MALDI-MSI can also be applied within **cytological** contexts, for the classification of **thyroid** nodules. Recent works have demonstrated a **specificity** and **sensitivity** and work is ongoing to provide more clear classification guidelines when seeking to define nodules based upon the percentage of malignant and benign pixels in the specimen.

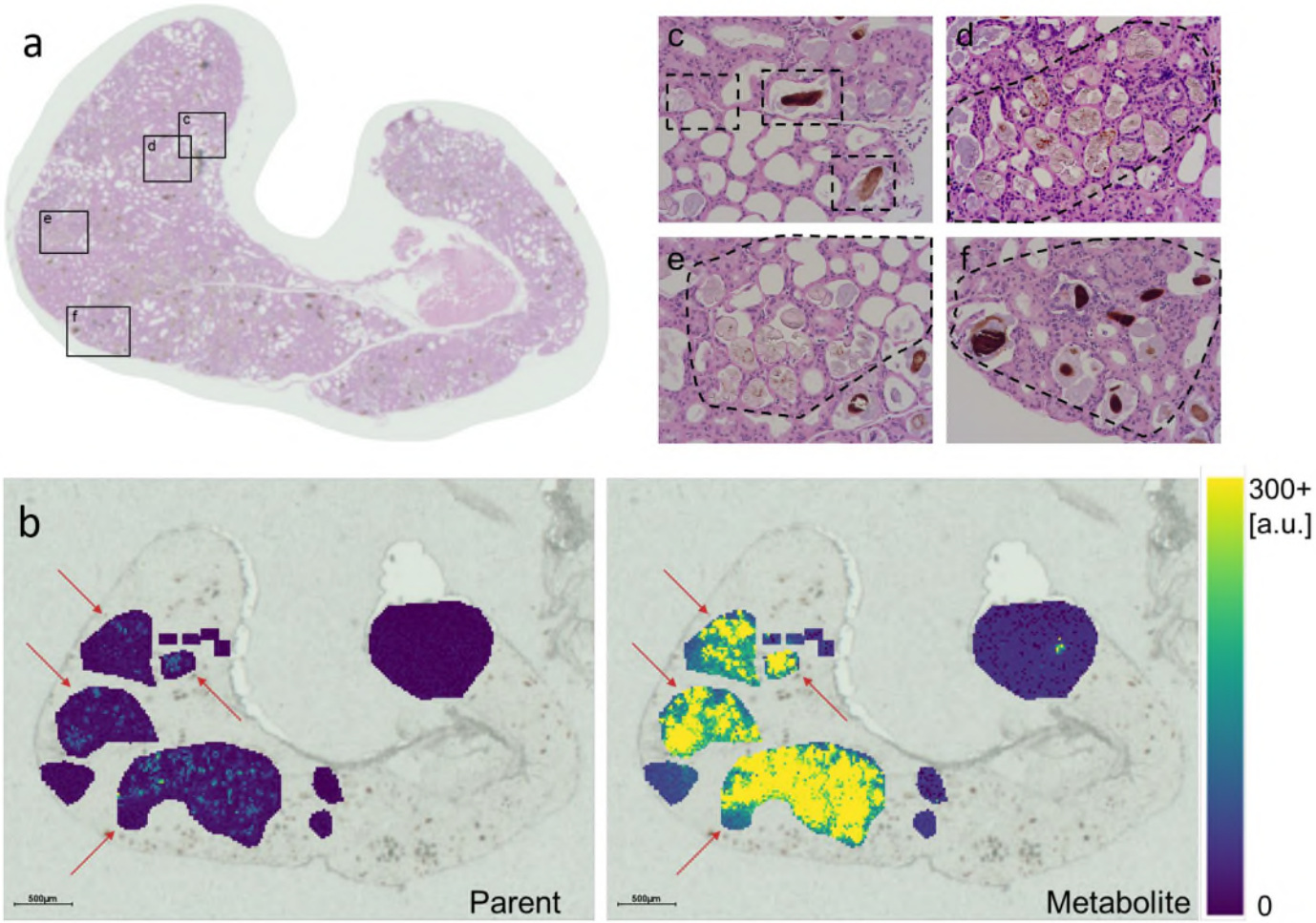
Analyzing crystal deposits in FFPE-tissue samples (Becker M.)

Intensity correction

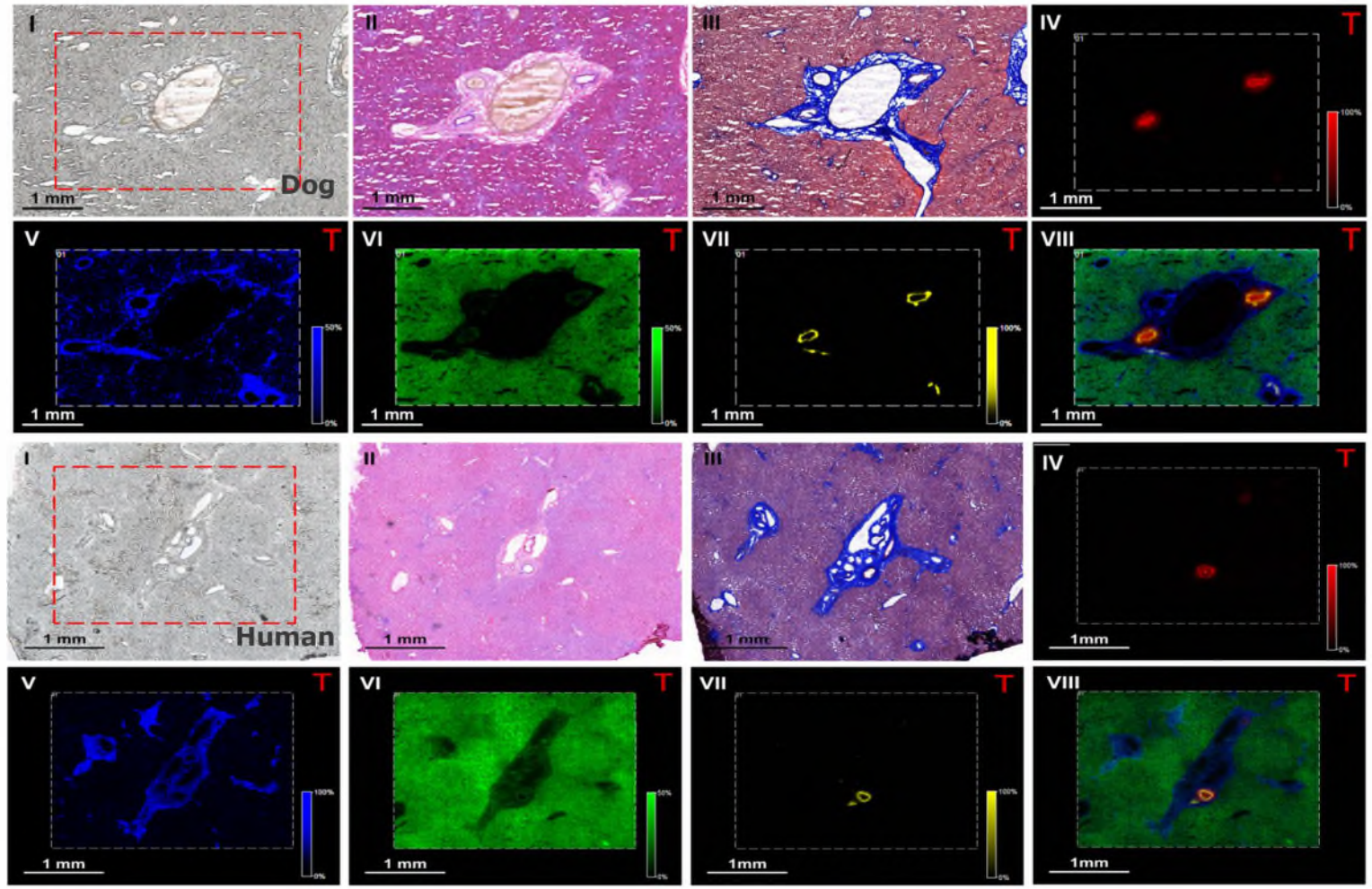


Parent [10 µg/ml]		Metabolite [10 µg/ml]
1.27	Intensity ratio	1.00
0.97	Molecular weight ratio	1.00

Histology-guided MSI acquisition.



Multimodal imaging of healthy livers to identify tissue markers and their inclusion in livers from Primary Sclerosing Cholangitis (PSC) patients (van Heerden M., Vreeken R.)



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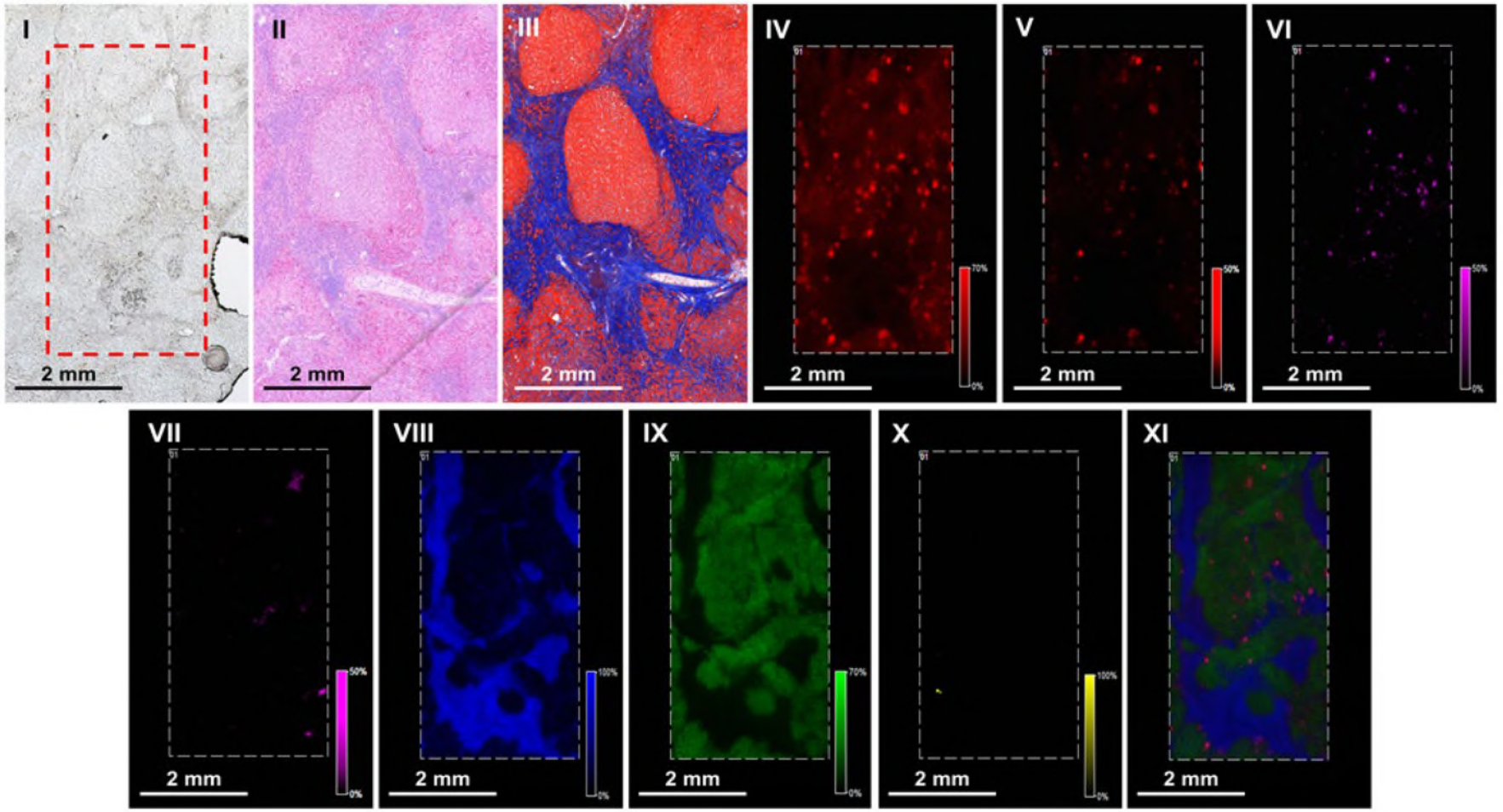

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