A Beginner's Guide to Mass Spectrometry Imaging

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A Beginner's Guide to Mass Spectrometry Imaging

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Mass Spectrometry Imaging (MSI) builds upon conventional mass spectrometry by allowing molecular distributions to be visualised directly from a sample surface. Unlike traditional approaches that require homogenisation and extraction, MSI captures mass spectra at defined spatial coordinates, building detailed molecular maps. Technological advancements in MSI have enabled very high spatial resolution and faster acquisition speeds. These developments have expanded MSI's applications across biomedical research, pharmaceutical development, and forensic analysis, establishing it as a powerful tool for spatial-omics and molecular imaging.

Introduction

Mass Spectrometry Imaging (MSI) is an analytical technique that provides label-free spatial mapping of molecular distributions across a wide range of sample types. It is used in biomedical research to study a range of molecular classes, including metabolites, lipids, and proteins. However, MSI also plays a crucial role in forensic science, materials analysis, and industrial applications. Unlike conventional mass spectrometry, where a sample is typically injected into the instrument, MSI works with a solid sample plane that is scanned over to map molecular distributions. Unlike light-based imaging, MSI builds molecular images by scanning the sample in an x/y grid, recording mass spectra at each pixel location, and then builds the image based on those ion intensities. The images that are generated can be of the distribution of a single *m/z* value or resulting from an overlay of multiple *m/z* values to highlight molecular co-localisation (Figure 1).

Evolution of MSI

Over the past four decades, MSI capabilities have significantly evolved to visualise complex biomolecular distributions in tissues, in a multiplexing and multi-modal fashion. Secondary Ion Mass Spectrometry (SIMS) was the first approach used for spatial molecular analysis, offering exceptional resolution but limited to small molecule analytes. A breakthrough came with the development of Matrix Assisted Laser Desorption Ionisation (MALDI) imaging, which enabled the detection of larger biomolecules such as peptides and proteins directly from tissue samples. Since then, advancements in ionisation techniques and mass analysers have further expanded MSI's capabilities, allowing the exploration of a wider range of molecular classes with greater sensitivity and spatial resolution.

Main applications of MSI

MSI plays a key role in understanding tissue microenvironments, disease pathology, and biomolecular distributions. It is widely applied in cancer biomarker discovery, drug distribution studies, and disease pathology, enabling the mapping of molecular changes in tissues, often in an untargeted manner. In pharmaceutical research, MSI aids in drug discovery and development by studying absorption profiles, pharmacokinetics, and the endogenous response to therapeutic exposure. Additionally, MSI has become an essential tool to investigate pharmacokinetics, pharmacodynamics and drug formulation. These MSI applications continuously deepen our understanding of biological processes, driving progress towards more effective treatments and therapeutic strategies.

Advancements in MSI

Advancements in MSI instrumentation have significantly enhanced its capabilities, enabling higher spatial resolution, improved mass accuracy, and faster data acquisition. Recent advancements have achieved sub-micron lateral spatial resolution, allowing for the visualisation of molecular distributions with unprecedented detail. Increased mass resolution has also improved confidence in molecular annotations, reducing spectral overlap and enabling more precise identification of analytes. Additionally, faster MS scan speeds have been achieved with fewer compromises on sensitivity, making large-area imaging more practical. However, as with all analytical techniques, MSI involves trade-offs. There is an inherent balance between spatial resolution, scan speed, and sensitivity. Higher spatial resolution results in smaller pixel sizes, but this often comes at the cost of longer acquisition times and potential sensitivity loss.

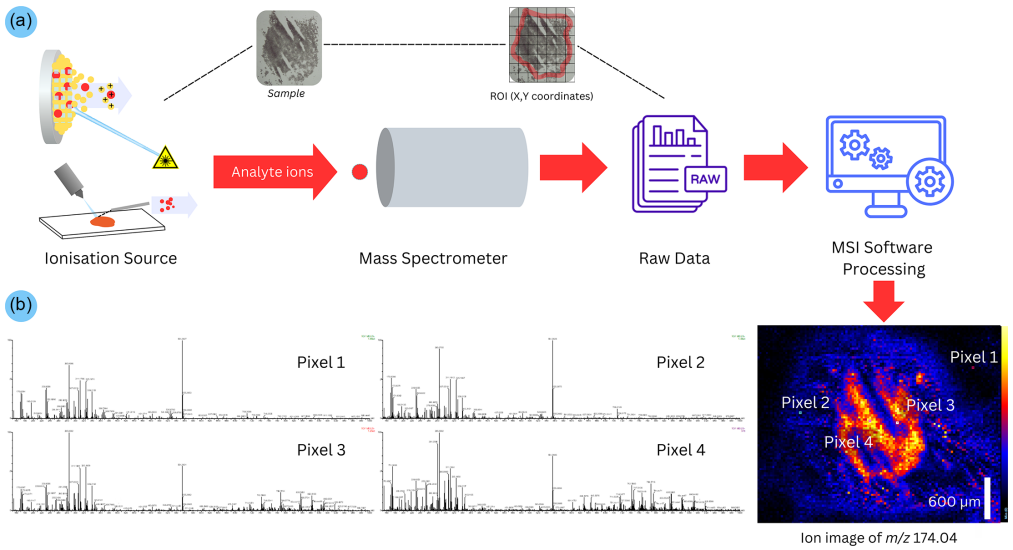

Figure 1. MSI Workflow. (a) A general mass spectrometry imaging workflow. (b) An ion image of a uveal melanoma spheroid with the extracted spectra of 4 individual pixels shown.

Overview of ionisation sources

MSI employs a range of ionisation sources, each having specific advantages and uses. MALDI and Desorption Electrospray Ionisation (DESI) sources are common types of ionisation sources which this guide will focus on. Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry Ionisation (LA-ICP-MSI) is used for elemental analysis and being a more destructive ionisation technique, it is employed as the last technique in a multimodal workflow. Its applications range from environmental to biomedical and forensics. SIMS delivers high spatial resolution between 50 nm and 10 µm, providing detailed elemental and molecular maps with the potential to analyse small organics, inorganic elements, and some biomolecules, but it is a "hard" ionisation technique, leading to the fragmentation of larger molecules such as proteins. Nano-DESI is a version of DESI analysis enabling high-resolution imaging. Unlike DESI, nano-DESI uses two capillaries which create a continuous liquid bridge on the sample surface, and desorption occurs to the top layer of the sample limiting analyte loss. Nano-DESI is similar to **Liquid Extraction Surface Analysis (LESA)**, in which a solvent drop is applied to the sample dissolving the surface ions before being extracted and transferred into the mass spectrometer.

Matrix-Assisted Laser Desorption Ionisation

MALDI is a soft ionisation technique that enables the analysis of a wide variety of molecules, such as lipids, peptides, proteins, drugs, and metabolites, with minimal fragmentation. In MALDI MSI, a sample is coated with an organic matrix, which absorbs the laser energy and facilitates ionisation of the analytes. The molecular information is collected at every pixel and used to generate a mass spectral image for every ion detected. Figure 2 represents the ionisation process and ion image generation process in MALDI. In certain systems, MALDI MSI can achieve cellular-level spatial resolution.

Matrix application

In MALDI MSI, the matrix is essential because it both facilitates ionisation and acts as an outer layer to protect the sample (the latter is achieved with a matrix-to-sample ratio of around 10,000:1). The matrix needs to co-crystallise with the analyte and effectively absorb the laser energy. Specific properties such as high UV absorbance, vacuum stability (for vacuum MALDI), low vapour pressure, and similar solubility to the analyte in the selected solvent are necessary for effective matrices. Common matrices that are suitable for a variety of compounds include 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), and 9-aminoacridine (9-AA).

Matrix application techniques

Matrix application techniques in MALDI analysis are critical for successful visualisation, each with distinct characteristics impacting analytical outcomes. Spray deposition, offering even coverage and high-throughput potential, is advantageous for rapid

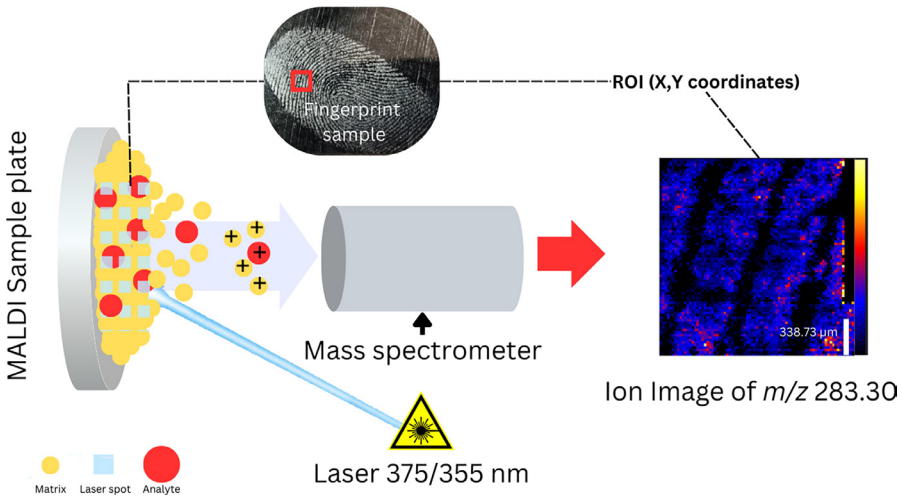

Figure 2. MALDI Schematics. The process of MALDI MSI and subsequent ion image generation, including an example ion image at m/z 283 from a fingermark sample.

screening. Sublimation, conversely, yields highly uniform and thin matrix layers, minimising analyte diffusion and enhancing spatial resolution, yet requires controlled and stable conditions, and it is only suitable for small molecules. Inkjet printing provides precision and reproducibility, ideal for quantitative studies and targeted applications, but it is limited by its slower processing speed.

Matrix Assisted Laser Desorption Ionisation- immunohistochemistry (MALDI-IHC)

MALDI MSI combined with immunohistochemistry (IHC) is an analytical approach that integrates molecular distribution analysis with targeted protein visualisation in tissue sections. This technique leverages MALDI MSI for targeted protein localisation by staining tissues with antibodies which are conjugated to unique mass reporters. Hundreds of antibody probes can be applied at the same time, each with unique mass reporters of a specific peptide and amino acid sequence, which can be easily resolved and detected by MSI. This technique, therefore, allows for a significantly highly multiplexed spatially resolved protein localisation experiment. MALDI-IHC overcomes the limitations of conventional targeted multiplexed imaging, as MSI bypasses the antibody multiplexing challenges associated with fluorophore excitation and emission spectral overlap, which typically restricts detection to only three to five antibodies. This enables detailed pathological assessment and molecular characterisation that would not be possible with traditional fluorescence-based methods.

Desorption Electrospray Ionisation

DESI utilises a charged stream of solvent to desorb molecules from the surface of a sample (Figure 3). The technique can be used to detect a wide variety of analytes including small metabolites, lipids, glycans, and drugs. DESI is a "soft" ionisation technique generating ions with minimal or no fragmentation.

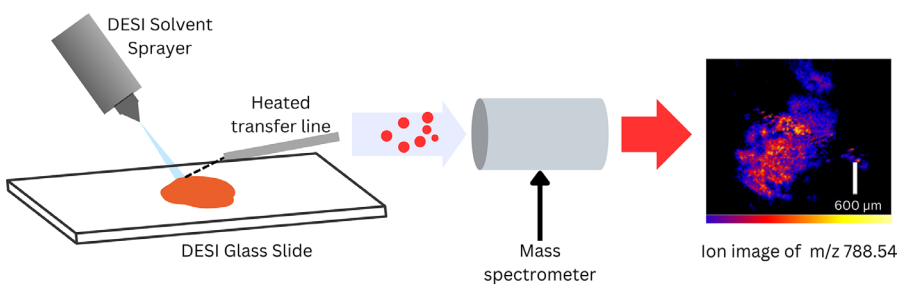

Figure 3. DESI Schematics. The process of DESI MSI and subsequent ion image generation, including an example ion image at m/z 788 from a uveal melanoma spheroid sample.

Unlike MALDI, DESI is a matrix-free ambient technique and thus it bypasses the potential of ion suppression or delocalisation due to the matrix application. Typical spatial resolution is 20–100 μm, but recent advances have permitted spatial resolution as low as 5 μm. Spatial resolution may be optimised by altering the solvent flow rate, step size, gas pressure, and the geometric orientation of the sprayer.

Due to minimal destructivity, biological tissue samples may be analysed twice in a multimodal workflow to maximise the molecular imaging information. MSI can also be performed in two stages for the purpose of achieving both broad coverage and high-resolution detail: first, at a lower spatial resolution to survey the entire sample and identify a region of interest (ROI), and then again at a higher spatial resolution to reanalyse the ROI, providing finer molecular localisation details within a smaller, targeted area. This approach allows us to balance coverage and resolution. However, this double analysis technique does not work for all samples, such as fingermarks, due to analyte delocalisation, a significant loss of certain analytes after the first acquisition, and due to the nature of the fingermark samples being a very thin layer of sweat.

Sprayer solvent composition

The solvent composition of the spray can have a large impact on the ionisation and desorption of analytes from a sample and the resulting molecular coverage. Often, 90–98% methanol solution is used, and a lock-mass compound such as leucine enkephalin is used for internal mass calibration purposes.

Sample preparation

DESI analysis requires minimal sample preparation compared with MALDI, but several critical factors must still be considered for successful execution. Typically, fresh frozen samples are preferred for DESI MSI of small metabolites and lipids, as harsh fixation may impact analyte detection. However, recent studies have demonstrated the detection of molecules in formalin-fixed paraffin-embedded (FFPE) tissues, which are typically lost, expanding detection capabilities and enabling the use of more widely available samples. Although FFPE samples require some sample preparation for DESI analysis, this is less laborious than for MALDI analysis. Regardless of the sample type, thin sectioning (<20 μm) is crucial to preserve spatial information. For fresh frozen tissue, samples must be sectioned, dried with nitrogen gas, vacuum packed, and promptly stored at −80°C to minimise analyte degradation and delocalisation. To prevent condensation-related analyte delocalisation, samples must be allowed to reach room temperature before analysis.

Applications of DESI and MALDI MSI

MALDI and DESI MSI have diverse applications across biomedical and pharmaceutical research. Key examples include:

- Cancer research: Identifying spatial distributions of proteins, n-glycans, lipids, and small metabolites in tumour tissues to understand disease progression, response to treatment, and identify key biomarkers.
- Pharmacokinetics and pharmacodynamics: Visualising drug distribution and metabolism in tissues whilst also analysing mechanisms behind therapeutic efficacy and toxicity.
- Neuroscience: Mapping neurotransmitters and metabolites in brain tissue to study mechanisms including neurodegeneration.
- Microbial analysis: Characterising bacterial colonies for species identification and antibiotic resistance studies.
- Forensics: Analysing trace evidence, drugs, hair, and fingermarks to aid criminal investigations.

Practical tips for DESI and MALDI beginners

- When analysing small molecules (>*m/z* 500) and lipids within the same method and on the same sample, optimise the method towards the lower *m/z* range. Lipids are often more abundant than small metabolites so that they will still be detected in the analysis.
- Always use the total ion count of a reliable endogenous ion as a reference when optimising parameters to achieve best sensitivity
- Slower scan speeds can enhance sensitivity by increasing the number of ions acquired per pixel.
- For successful MALDI analysis, the appropriate matrix must be selected: DHB for peptides and oligosaccharides, CHCA for small molecules, peptides and proteins, 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA) for larger proteins, 9-AA for phospholipids, and 1,5-diaminonaphthalene for lipids, each optimised for specific analyte desorption and ionisation.
- The fixation process and the embedding material of a sample should be carefully considered, depending on your target analytes and expected experimental output, taking into consideration ion suppression and interfering signal.

Conclusion

Mass Spectrometry Imaging (MSI) has revolutionised the way we visualise molecular distributions within samples. Its increasing impact is evident in the surge of publications, rising from 10 in 2005 to 319 in 2015 and 539 in 2024 (sourced from PubMed), reflecting its growing prominence in both academia and industry. MSI's expanding role is also recognised at the governmental level, with the UK Home Office and the Defence Science and Technology Laboratory (Dstl, UK) incorporating molecular fingerprinting by MALDI MSI into the international Fingermark Visualisation Manual, now classifying it as a Category B technique – advised for operational use in major crimes, when all the conventional avenues are exhausted.

The pharmaceutical industry has increasingly turned to MSI to visualise drug distribution and the biological response to therapeutic exposure within tissues, enhancing the translatability of drug discovery research and addressing the persistent challenge of high drug attrition rates. This capability is fundamental to understanding drug mechanisms of action and supporting the development of new medicines. As MSI continues to evolve, it is bridging the gap between exploratory discovery research and scalable clinical and multidisciplinary applications.

The integration of multimodal MSI enables researchers to simultaneously map a wide array of biomolecules within a single tissue section. By aligning these datasets with precision, MSI reveals how different molecular classes colocalise and interact, offering deeper insight into the biochemical interplay that governs cellular function and disease. In multimodal workflows, it is essential to perform the least destructive technique first such as DESI, MALDI, and then LA-ICP-MS. This multi-dimensional approach is driving a more comprehensive understanding of biological systems and expanding the horizons of molecular imaging. ■

Further Reading and Viewing

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Sophie Pearce *is a PhD student at Sheffield Hallam University working within the Biomolecular Sciences Research Centre, Sheffield Multimodal Imaging Centre and the Centre for Mass Spectrometry Imaging. Her work investigates chemotherapy resistance using 3D tumour spheroid models. By multimodal MSI, she explores drug distribution, metabolic reprogramming, and tumour survival mechanisms, aiming to enhance drug discovery and translational research. Email; S.Pearce1@shu.ac.uk Email: s.pearce1@shu.ac.uk*

Laura Cole *is an expert in multimodal mass spectrometry imaging. Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI-MSI) and Desorption Electrospray Ionisation Mass Spectrometry Imaging (DESI-MSI) have been the main techniques employed to investigate proteins, lipids and metabolites, followed more recently by Laser Ablation Inductively Coupled Plasma Mass Spectrometry Imaging (LA-ICP-MSI) which Dr Cole applies for the analysis of metals in a variety of biological samples (patient tissue and 3D spheroids/aggregoids). Recently, she and her ocular research group have begun to pioneer the multimodal analysis of pattern recognition receptors and metallomics in age related macular degeneration along with disease biomarkers, proteins and metabolites in patient uveal melanoma whole eye imaging. Ultimately her research centres around proteomics, lipids/metabolomics, biomarker discovery and how that can be translated and potentially linked to disease pathways and networks. Email; l.cole@shu.ac.uk Email: l.cole@shu.ac.uk*

Simona Francese *is Professor of Forensic and Bioanalytical Mass Spectrometry at Sheffield Hallam University (SHU), UK and a public speaker. She is the Lead of the Sheffield Multi-Modal Imaging Centre and Head of the Centre for Mass Spectrometry Imaging at SHU. Simona is an expert in the development of MALDI MS Imaging applications and has pioneered its development for the analysis of latent fingermarks and blood to profile offenders. More recently she has engaged in research at the interface between forensics and clinical diagnostics using blood and sweat in fingertip smears to detect cancer and other pathologies. Her research has been implemented in police casework in UK and Europe and has been partly funded by the Home Office, West Yorkshire Police and The Defence Science and Technology Laboratory, UK. Email; s.francese@shu.ac.uk Email: s.francese@shu.ac.uk*