

Review

Spatial pharmacology using mass spectrometry imaging

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The emerging and powerful field of spatial pharmacology can map the spatial distribution of drugs and their metabolites, as well as their effects on endogenous biomolecules including metabolites, lipids, proteins, peptides, and glycans, without the need for labeling. This is enabled by mass spectrometry imaging (MSI) that provides previously inaccessible information in diverse phases of drug discovery and development. We provide a perspective on how MSI technologies and computational tools can be implemented to reveal quantitative spatial drug pharmacokinetics and toxicology, tissue subtyping, and associated biomarkers. We also highlight the emerging potential of comprehensive spatial pharmacology through integration of multimodal MSI data with other spatial technologies. Finally, we describe how to overcome challenges including improving reproducibility and compound annotation to generate robust conclusions that will improve drug discovery and development processes.

MSI technologies enable spatial pharmacology

Drug discovery and development typically involve (i) a pre-discovery phase of understanding dysregulated disease mechanism, biomarker discovery, and disease classification; (ii) a drug discovery phase of identifying targets and therapeutic agents that interfere with pathological processes; (iii) a pre-clinical phase of evaluating drug mechanism of action, efficacy, and toxicity; (iv) a clinical phase that investigates the effects of drugs on humans; and (v) a regulatory phase for approval of the drug [1]. Assessment of drug exposure at a target site using efficacious and safe drug concentrations is critical for advancing drugs through the development pipeline. In a pharmaceutical setting, liquid chromatography (LC)-coupled mass spectrometry (MS) and tandem mass spectrometry (MS/MS), and whole-body autoradiography (WBA) using radiolabeled compounds are key analytical techniques for quantitative assessment of the ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of drugs and their metabolites [2]. Although the sensitivity and specificity of LC-MS-based assays are valuable for evaluating the pharmacokinetic and toxicological properties of drugs in tissue homogenates, the heterogeneity of tissue and analyte distribution is not preserved in these bulk tissue analyses. On the other hand, although WBA provides a sensitive way to assess ADMET properties of drugs within a spatial context, the parent drug and its metabolites cannot be discriminated, and drugs require cumbersome and costly radiolabeling. New techniques and technologies to improve understanding of therapeutic molecules early in the drug development process are sought to improve attrition rate and accelerate time to market.

MSI unlocks new avenues for label-free spatial mapping of drugs and metabolites within tissues and cellular subcompartments, while simultaneously shedding light on biomarkers of drug efficacy and toxicity by capturing effects on endogenous biomolecules [measured as mass-to-charge (m/z) values] [3]. The distribution of small metabolites, lipids, glycans, proteins, and peptides can be visualized without a *priori* knowledge of the molecules of interest [4]. Using an array of MSI

Highlights

The spatial distribution of drugs, metabolites, and endogenous biomolecules within cells and tissues can be visualized by mass spectrometry imaging (MSI).

Targeted MSI can decipher the spatial pharmacokinetics, metabolism, and toxicology of drugs.

Untargeted MSI can elucidate disease stratification, disease subtyping, mechanism of pathophysiology, drug-related efficacy and toxicology, and associated biomarkers.

Machine learning and deep learning can reveal hidden structures within high-dimensional MSI data.

Multimodal imaging integrating complementary spatial biology information has the potential to provide improved biological insight and integrated biomarkers, thus aiding precision medicine.

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technologies, the spatial pharmacology of drugs can be determined at different scales – from whole-body to single-cell and subcellular compartments (Figure 1A).

Although the MSI field has mainly focused on technology development in recent decades, the latest advances in instrumentation and artificial intelligence (AI) for the analysis of high-dimensional data make MSI an emerging and valuable tool that will impact on the life cycle of drugs [5]. In this review, we provide a concise perspective on the most recent advances in MSI technologies over the past few years and their implications for different phases of drug discovery and development. We focus on the most commonly used ionization techniques, including matrix-assisted laser desorption ionization (MALDI) [6], desorption electrospray ionization (DESI) [7], and secondary ionization mass spectrometry (SIMS) [8] (Figure 1B). We discuss these technologies in terms of critical parameters in pharmaceutical research for obtaining reproducible spatial information with high sensitivity, **spatial resolution** (see [Glossary](#)), and assay scalability. We also discuss MSI-based quantitative spatial ADMET profiling, as well as emerging potential for discovery-based approaches for patient stratification and biomarker discovery. The high dimensionality of MSI data, although rich in information content, also brings data-analytic challenges. We also cover machine learning (ML) and deep learning (DL) approaches which provide insights into tissue heterogeneity for therapy selection and treatment response, as well as multimodal imaging and data integration for comprehensive spatial pharmacology analysis.

Developments in MSI technologies and their application in targeted drug and metabolite imaging

Diverse MSI technologies provide specific analytical capabilities to aid investigations with specific study objectives. Parameters of the highest priority in drug discovery and development include the sensitivity with which the compounds of interest can be detected, the spatial resolution, and the data acquisition speed (Table 1). These are determined by the combination of the ionization source and the mass analyzer on a mass spectrometer. Although it is generally desirable to achieve high spatial resolution, sensitivity, and throughput in a single experiment, each typically comes at the expense of another. Therefore, the critical parameters of drug evaluation need to be predetermined. Recent innovations in instrumentation allow (i) improved sensitivity for different classes of analytes, allowing the detection of multiple drugs, and also greater coverage of endogenous classes of molecules [4,9]; (ii) improved spatial resolution to detect compounds within smaller compartments, such as cellular and subcellular structures, for improved understanding of disease and drug mechanism of action [10]; and (iii) improved peak capacity and accuracy in the identification of drugs, metabolites, and biomarkers by coupling ion sources to ultra-high-resolution mass analyzers for better **spectral resolution** [11] and orthogonal **ion mobility** separation of complex mixtures [12]. High spatial resolution and ultra-high mass resolution, however, come at the cost of throughput, which is an important factor for time-sensitive decision-making in drug discovery and development pipelines. Instrumentation developments as well as advanced computational strategies for post-acquisition data processing can aid in addressing this issue [13].

DESI MSI for rapid imaging of drugs, metabolites, and their effects

DESI MSI is an ambient ionization method that enables rapid and direct analysis of samples without pretreatment, allowing high-throughput data acquisition. When a spatial resolution of 30–100 μm is sufficient to assess the distribution of analytes within tissue structures, DESI provides an effective means for rapid imaging of drugs, metabolites, and lipids across large areas with high sensitivity [7] (Table 1). Although a previous generation of DESI methods suffered from poor reproducibility, the latest solvent sprayer configuration reported in 2022 implements a controlled flow of solvent for increased sensitivity, spatial resolution, and robustness, enabling

Glossary

Ion mobility: a gas-phase technique where the speed at which a molecule travels in the presence of an inert gas depends on its 3D structure.

Ion suppression: the decrease in ionization efficiency and analyte detection caused by competing ions in the sample matrix in mass spectrometry (MS) analyses.

Isobaric compounds: molecules of different composition and structure that share exactly the same mass (or m/z value), resulting in overlapping spectra and confounding annotation.

LogP value: the hydrophobicity/lipophilicity of a compound is defined by its partition coefficient (P) between an organic phase and an aqueous phase. A lower or negative value of $\log P$ denotes more hydrophilicity (water-soluble), and a higher value denotes hydrophobicity (water-insoluble).

Matrix effect: suppression or enhancement of the ionization efficiency of the analyte based on other analytes that are present in the sample or biological matrix.

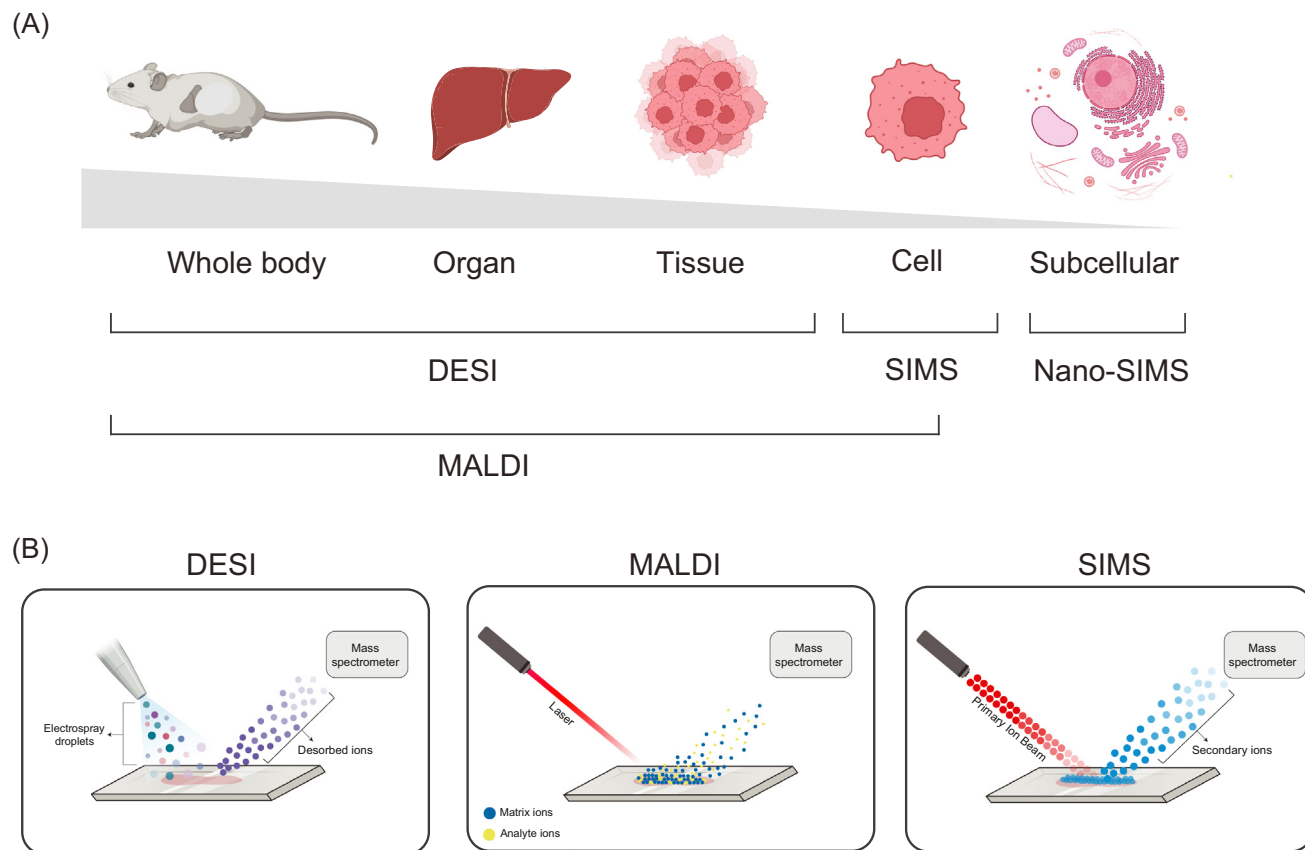
Multiple reaction monitoring: a highly specific and sensitive tandem MS/MS method performed on triple-quadrupole and linear ion trap MS in a multiplexed manner. Each predefined precursor ion is selected and subjected to fragmentation, and the product ion of predefined m/z is selected and detected.

Receiver operating characteristic (ROC) curve analysis: a graph-based analytical method for assessing the performance of the binary classifier as the discrimination threshold is changed over a range. The ROC curve is the plot based on true positive rate against false positive rate at each threshold setting.

Spatial metabolomics: profiling of the spatial distribution of metabolites, lipids, and small molecules.

Spatial resolution: a measure of the dimension of pixel size. For example, a pixel of $5\ \mu\text{m} \times 5\ \mu\text{m}$ is considered to have a spatial resolution of $5\ \mu\text{m}$. The smaller the pixel size, the higher the spatial resolution.

Spectral resolution: also termed mass resolution or resolving power, the ability of the mass analyzer to separate peaks of similar m/z values. The higher the spectral resolution, the higher the probability of accurately identifying ions based on accurate mass.



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Figure 1. DESI, MALDI, and SIMS mass spectrometry imaging (MSI) across spatial scales enable spatial pharmacology. Schematic illustration of (A) DESI, MALDI, and SIMS MSI demonstrating complementarity in spatial resolution. MSI allows imaging at the whole-body and organ level down to single-cell and subcellular locations, using small rodent body size as a reference. (B) DESI, MALDI, and SIMS ionization processes. DESI is an ambient ionization technique that uses charged solvent sprayed on a sample surface for desorption and ionization of surface molecules. MALDI ionizes molecules with a pulsed laser beam rastered across a crystallized matrix-coated tissue surface. SIMS uses a high-energy primary ion beam that sputters surface material. The secondary ions generated are accelerated, resolved, and measured by the mass spectrometer. Abbreviations: DESI, desorption electrospray ionization; MALDI, matrix-assisted laser desorption ionization; SIMS, secondary ionization mass spectrometry.

quantitative analysis of drugs [14,15]. In addition, nanospray DESI MSI allows high spatial resolution imaging and is amenable to detecting drugs and its metabolites [16].

MALDI MSI for versatile mapping of drugs and their effects

In MALDI imaging the choice of matrix and sample preparation is critical for the classes of analyte detected, spatial resolution, quantitation, and reproducibility [17] (Table 1). The low ionization efficiency of small molecules caused by matrix ion interferences occurring at low mass range, or a low amount of material ionized in high spatial resolution imaging, when using conventional MALDI, has been addressed to some extent with post-ionization enhancement using a second laser (MALDI-2). In the past 4 years MALDI-2 has shown improved ionization efficiency for drugs, small metabolites, glycans, and lipids by one to three orders of magnitude [18–20].

In addition, MALDI instrumentation developments have seen many prototype customizations for enhanced spatial resolution. The latest implementation of a combination of transmission-

Table 1. Comparison of the DESI, MALDI, and SIMS mass spectrometry imaging technologies^a

Technique feature	DESI	Nano-DESI	MALDI-ToF	AP-MALDI	SIMS-ToF	NanoSIMS
Ionization source	Electrospray of highly charged droplets	Electrospray of highly charged droplets	Laser beam	Laser beam	High-energy primary ion cluster beam such as Ar _n ⁺ , Bi ₃ ⁺ , C ₆₀ ⁺ , (H ₂ O) _n ⁺	High-energy primary ion beam such as Cs ⁺ and O ⁻
Molecular class detected	Drugs, lipids, metabolites	Drugs, lipids, metabolites, glycans, peptides	Drugs, lipids, metabolites, glycans, peptides, proteins	Drugs, lipids, metabolites, glycans, peptides, proteins	Drugs, lipids, metabolites, glycans, peptides	Stable isotope-labeled molecules
Spatial resolution (μm)	30–200 (lowest ~20 μm)	10–200 (lowest ~7 μm)	5–100 (lowest ~1 μm)	5 (lowest ~1.4 μm)	1–100 (lowest ~0.5 μm)	~0.05
Mass range (Da)	50–1200	50–1200	100–75 000	50–6000	100–10 000	1–400
Throughput	High	High	Medium-high	Medium-high	Low	Low
Temperature condition	Ambient	Ambient	Medium-high vacuum	Ambient	High vacuum	High vacuum
Sample preparation	No pretreatment	No pretreatment	Matrix coating	Matrix coating	No pretreatment	No pretreatment
Mass analyzers	q-ToF ^b TQ ^b MRT ^b FTICR Orbitrap LTQ	q-ToF Orbitrap	ToF ^b ToF/ToF ^b FTICR ^b MRT ^b MRMS ^b Orbitrap	Orbitrap ^b q-ToF ^b	ToF ^b Orbitrap	Magnetic sector ^b
Ion mobility	TWIMS ^b Cyclic-IMS ^b	TIMS	TIMS ^b			
Advantages	Minimal sample preparation High throughput	Minimal sample preparation High throughput	Broad class of molecules Medium to high spatial and spectral resolution Low to high throughput	Broad class of molecules High spatial and spectral resolution Medium to high throughput	Minimal sample preparation Single-cell resolution 3D depth profiling	Subcellular resolution
Limitations	Spatial resolution	Sensitivity In-house setup	Sample preparation is critical Matrix signal interference for low <i>m/z</i> region	Sample preparation is critical Matrix signal interference for low <i>m/z</i> region	Low mass resolution Low throughput	Maximum five to seven labeled analytes Complex sample preparation Low throughput
Commercial availability	Waters	No	Bruker, Waters	Thermo Fisher, Shimadzu	Ionoptika, Ionof	Cameca
Refs	[7, 11, 14, 66]	[16]	[6, 12, 21, 83]	[22, 23]	[8, 27, 54]	[29, 84]

^aAbbreviations: FTICR, Fourier transform ion cyclotron resonance; IMS, ion mobility spectrometry; LTQ, linear trap quadrupole; MRMS, magnetic resonance mass spectrometry; MRT, multi-reflecting ToF; q-ToF, quadrupole ToF; ToF, time of flight; TQ, triple quadrupole; TWIMS, traveling wave ion mobility spectrometry; TIMS, trapped ion mobility spectrometry.

^bCommercially available with corresponding MSI source.

mode geometry and MALDI-2 with an Orbitrap mass analyzer can achieve spatial resolution down to 600 nm, as well as increased sensitivity for small metabolites and lipids [21]. Similarly, an ambient atmospheric pressure (AP)-scanning microprobe MALDI (SMALDI) source coupled to a high-resolution Orbitrap MS can image drug and metabolite distribution at the high spatial resolution of 1 μm [22]. When combined with an optical microscope, it also allows high-resolution and high-speed imaging of drugs and their metabolites [23].

SIMS MSI for subcellular resolution imaging of drugs and their effects

Single-cell resolution imaging of biological samples can be performed using SIMS MSI, which uses gas cluster ion beams (GCIBs) to facilitate 2D imaging, as well as depth profiling of drugs, lipids, and peptides, within cells and tissues (Table 1) [24]. The molecular fragmentation caused by a high-energy ion beam and preferential sensitivity for compounds with high **logP value** had previously limited its use in drug imaging. The development of high-energy water GCIBs with cryogenic handling reduces fragmentation and enhances sensitivity for lipids, metabolites, and peptides [25], as well as for drugs with a low logP value [26]. Researchers developed a hybrid mass analyzer by combining the high acquisition rate of time of flight (ToF) and the high mass resolution of an Orbitrap detector [27] with a microscope-mode SIMS [28] to increase throughput. Isotope imaging using nanoscale SIMS (nanoSIMS) analyzes five to seven isotope-labeled analytes of interest, including drugs and metabolites, with a subcellular spatial resolution of 50 nm [29]. Spatial resolution can be improved to 15 nm by using a magnetic sector mass spectrometer combined with a scanning electron microscope [30]. Representative examples highlighting the use of DESI, MALDI, and SIMS MSI for different applications in the drug development pipeline, including pharmacokinetics, toxicology, and pharmacodynamics, are shown in Table 2.

Quantitative and targeted MSI of drugs and metabolites

Conventionally, tissue-based quantitative pharmacokinetic and toxicological studies are performed using LC-MS/MS assays. However, the analysis of bulk tissue samples obscures the heterogeneity of drug uptake and its metabolism within discrete histopathological and structural correlates. This can be overcome by implementing targeted and quantitative MSI during the early phases of drug discovery and development to provide critical insight into drug pharmacokinetics and metabolism. However, quantitative MSI of drugs and metabolites is challenging because of the **matrix effect** and **ion suppression**, resulting in heterogeneous analyte ionization efficiency within different tissue substructures. Normalization of ion suppression effects can be performed by correcting the intensity of drugs and metabolites to an isotope-labeled internal standard of the same drug or metabolite that is uniformly deposited on the sample surface [31]. However, this relies on the availability of isotope-labeled compounds, which might not be readily available or may be expensive to synthesize. Absolute quantification can be performed using a calibration curve with serial dilutions of compounds (i) spotted onto the slide, (ii) spotted onto control tissue, and/or (iii) in a mimetic model. Although more labor-intensive, the mimetic model, where the calibration curve is created by spiking drugs onto the tissue homogenate with the same background matrix, thus mimicking the ion suppression effects, is currently the most accurate technique for quantitative MSI of drugs and metabolites [32].

Quantitative MSI relies on instrumentation with high sensitivity and reproducibility, as well as on robust sample preparation, data normalization, and calibration measures [33]. Recently, a multi-center study designed to assess the reproducibility and accuracy of quantitative MSI of drugs normalized to internal standards has shown comparable results between centers, as well as correlation with results from LC-MS/MS assays [34]. This suggests that quantitative MSI is sufficiently robust to have potential as an effective method in the drug development pipeline. The sensitivity of LC-MS/MS assays, however, exceeds that of MSI for quantitative analysis. MSI systems offering high sensitivity for a variety of drugs are desired, especially for potent compounds administered and accumulating at low concentrations within a tissue. Assessing the limits of detection early in the design of MSI experiments is recommended to ensure that animals receive drug doses that provide sufficient sensitivity without inducing toxicity [35].

Recent studies showed higher sensitivity and speed for AP-MALDI MSI compared to DESI and MALDI for different classes of pharmaceutical compounds [23,36]. More MSI studies are

Table 2. Examples of quantitative and non-quantitative MSI methods used to understand drug distribution, pharmacokinetics, metabolism, toxicology, and mechanism

Small molecules	Tissue	Ion source	Mass analyzer	Quantitative calibration curve	Application/key findings	Refs
Ulixertinib	Mouse brain	DESI	q-ToF	Spotting on slide, tissue	Quantitative MSI for drug distribution analysis	[14]
STVNa and pirfenidone	Rat lung	DESI	q-ToF	Mimetics	Quantitative drug delivery of inhaled drugs in different regions of lungs	[85]
Citalopram	Mouse brain	MALDI	Tof/Tof and FTICR	Spotting on tissue	Quantitative MSI and cross-validation of pharmacokinetics and induction of serotonin and its metabolites	[86]
Clozapine and its metabolite	Rat liver	MALDI	FTICR	Spotting on tissue and mimetics	Quantitative pharmacokinetics and drug metabolism	[34]
Acetaminophen and its metabolite	Mouse kidney	AP-MALDI	q-ToF		Pharmacokinetics, drug metabolism, and nephrotoxicity of the drug performed without derivatization	[87]
Bleomycin	Skin	AP-MALDI	Orbitrap	Mimetics	Quantitative MSI for drug distribution analysis	[88]
¹³ C L-DOPA and ¹³ C dopamine	PC12 cells	NanoSIMS	Magnetic sector	Number of molecules counted by electrochemistry and imaging	Quantitative MSI of dopamine and its precursor in distinct subvesicular compartments, correlated with TEM images	[89]
¹⁴ C-labeled Climb-36 and its metabolites	Rat brain	DESI	LTQ (linear ion trap)	Spotting on tissue	Quantitative MSI of PET tracer and its correlation with autoradiography and PET	[74]
Amiodarone	Rat lung	DESI	q-ToF		Drug metabolism and organotoxicity through induction of markers of lipidosis	[90]
Efavirenz, tenofovir, and emtricitabine	Rat brain	MALDI	ToF	Spotting on tissue	Differential distribution of the antiretroviral drugs in brain suggesting potential benefit from combination therapeutics	[91]
Amiodarone	Rat alveolar macrophage cell	SIMS	Orbitrap		Mechanism of toxicity of the drug through increased phospholipidosis	[92]
Retigabine	Rat eye	MALDI	FTICR		Mechanism of toxicity of the drug through dimerization with its metabolite in melanin-containing layers of the eye	[93]
Doxorubicin	Varied	MALDI	Tof/Tof		Differential delivery of drug-loaded nanocarrier within tumor versus normal organs	[94]
Cisplatin	HeLa cells	NanoSIMS	Magnetic sector		Mechanism of drug resistance mediated through its nuclear localization within open and closed chromatin regions probed using metal-tagged antibodies	[95]
Aristolochic acids		Air flow-assisted (AFA) DESI			Nephrotoxicity related to arginine-creatinine, choline and lipid metabolism	[96]
Scopolamine	Rat brain	AFA-DESI			Spatially resolved metabolic alterations in drug-induced Alzheimer's disease model	[97]
Notoginsenoside R1 (NG-R1)	Rat brain	MALDI	Tof/Tof		Mechanism of action of drug through regulation of metabolic pathways	[98]
Tacrine	Mouse brain	MALDI	FTICR		Heterogeneous age-related metabolic perturbations and response upon tacrine treatment	[99]

necessary to benchmark classes of small molecules of interest in pharmaceutical research. Currently, several studies have shown quantitative and non-quantitative drug distribution for a variety of drugs and metabolites using DESI, MALDI, and SIMS, with different mass analyzers, for elucidating pharmacokinetics, metabolism, and toxicology [37] (Table 2). Although LC-MS/MS will still likely remain the primary means to assess quantitative pharmacokinetics, metabolism,

and toxicology, complementation with MSI could help to improve our understanding of drugs in early drug discovery and development.

MSI for discovery-based and biomarker-driven drug development

The high attrition rate in drug discovery and development necessitates new strategies to aid the process. One such approach is to implement biomarker-driven drug discovery and development. Biomarkers facilitate an adaptive drug development paradigm that impacts on different stages of drug development [38]. Biomarkers with high predictive power are essential to identify subgroups of patients who are likely to benefit from therapeutics in precision trial designs. Companion diagnostics that can reliably measure biomarkers are required, and spatial biology is currently being explored to support such endeavors [39]. In that regard, untargeted MSI data have potential to improve our understanding of the spatial relationships between biomolecules and cells for diagnostics and the discovery of spatially informed biomarkers.

An array of AI, ML, and DL tools are available to gain meaningful insight from high-dimensional [in both spectral (10^3 – 10^7 m/z spectral bins) and spatial (10^4 – 10^6 pixels) domains] MSI data. Details of the crucial components of MSI data analytics, including data preprocessing, normalization, dimensionality reduction, classification, statistical analysis, and post-acquisition data transformations, that are beyond the scope of this review, are discussed in [5,40–42] (Figure 2). This section discusses emerging developments within the cross-section of discovery-oriented MSI for clinical diagnostics and biomarker identification, challenges and strategies for implementing AI, ML, and DL algorithms, and multimodal imaging for comprehensive spatial profiling.

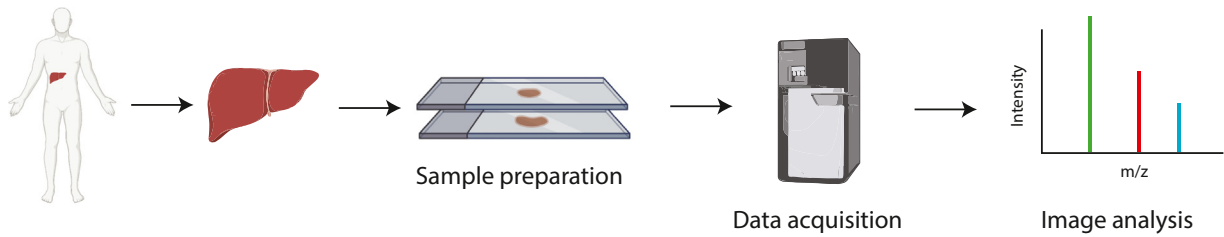
Computational approaches for tissue classification and biomarker discovery

The high-dimensionality of MSI data with non-linear relationships between features makes it difficult to manually identify and interpret underlying patterns. AI, ML, and DL strategies enable the identification of spatially informed biomarkers that capture metabolic heterogeneity and cellular interactions. Supervised and unsupervised ML algorithms for dimensionality reduction, classification, and visualization in the analysis of omic data have been translated to spatially aware MSI data analytics [5,43] (Figure 2C). In addition, architecture-based and multiple instance learning-based DL approaches have been successfully implemented in MSI data, enabling rapid distinction between tissue types and disease states [42]. Similarly, unsupervised ML methods, including matrix factorization, manifold learning, and clustering, are widely used for exploratory analysis to reveal underlying structure within MSI data [41]. More recently, DL methods such as variational autoencoder neural network for self-supervised peak learning without data preprocessing [44] and an algebraic topological framework [45] have been implemented for efficient denoising, data structure inference, and tissue subregion classification.

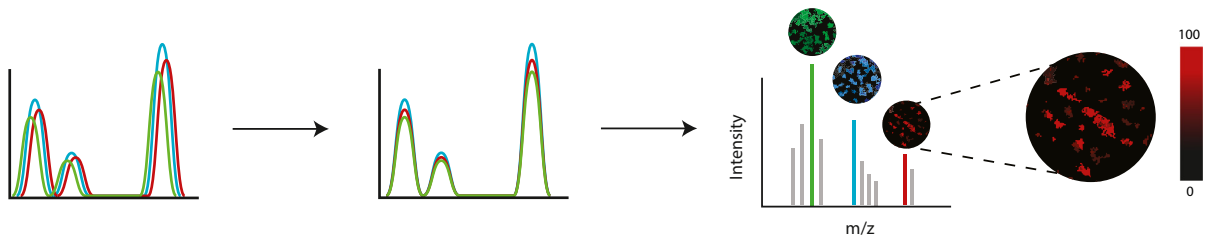
ML algorithms have been instrumental in diagnosing challenging tumors, in which pathologies of different disease etiologies exhibit histological similarities and lack discriminating markers for diagnosis [46]. For example, MALDI MSI of peptides discriminated pancreatic ductal adenocarcinoma (PDAC) versus cholangiocarcinoma [47], and DESI MSI of lipids and metabolites discriminated a benign renal tumor from renal cell carcinoma [48]. Classification of glioma samples based on SIMS MSI profiling of proteins and metabolites could identify clusters of cells indicative of pathophysiology [49]. Similar approaches could be implemented in preclinical study designs for tissue characterization and classification for therapeutic selection, as well as to understand the effect of therapeutics. A few examples are highlighted in Table 2.

Once intersample and intrasample heterogeneity is captured as distinct classes, statistical analysis to determine the biomarkers that are highly predictive of the classes of interest can be

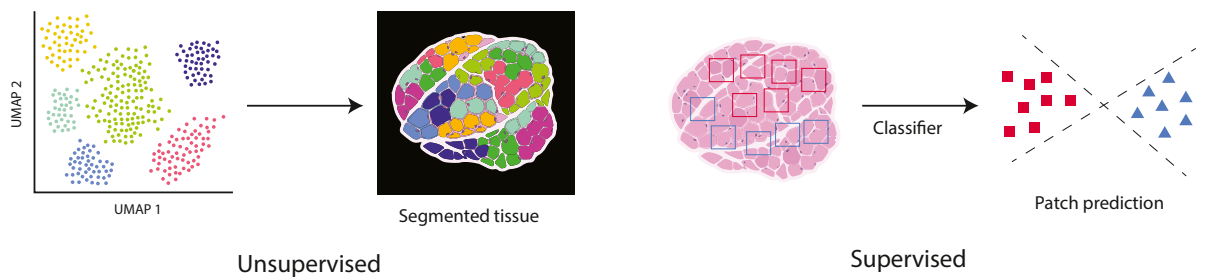
(A) Sample preparation and data acquisition



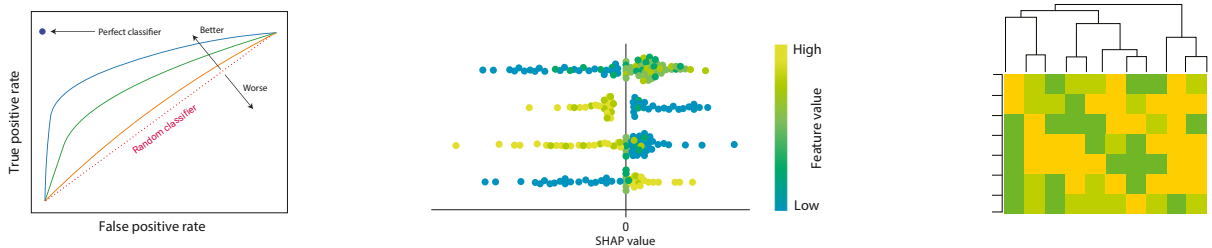
(B) Preprocessing



(C) Segmentation and classification



(D) Statistical analysis



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determined. **Receiver operating characteristic (ROC) curve analysis** has been the gold standard for evaluating the performance of a biomarker as a classifier [42], and Shapley additive explanations (SHAP) [50] are a recent addition. For example, classification of MALDI MSI data, followed by discriminatory biomarker analysis, showed that L-carnitine and short-chain acylcarnitines were significantly reprogrammed in breast cancer [51]. Multiple studies, including multicenter benchmarking and validation studies, have shown the potential of MSI in disease diagnosis as well as in biomarker identification [52].

Highly multiplexed antibody-based MSI to probe for spatial phenotypic biomarkers

In addition, a recent development with significant implications for drug discovery is the use of multiplexed antibody imaging to identify cellular phenotypes and interactions in health and disease. MSI is one of the new avenues being explored, and different antibody tags are amenable to ionization and detection with different MSI technologies. Highly multiplexed lanthanide metal-tagged antibodies have been used with SIMS [53,54], halide-tagged antibodies with nanoSIMS [55], photocleavable peptide-tagged antibodies with MALDI imaging [56], and boronic acid mass (BMT) tagged-antibodies with DESI [57]. With these tools, information concerning the cellular and biomarker distribution at the single-cell and cellular neighborhood level can now be obtained. For example, a panel of metal-tagged antibodies against subsets of tumor and immune cells using SIMS-multiplexed ion beam imaging enabled classification of triple-negative breast cancer tissues into subtypes associated with distinct spatial organizations of immune and tumor cells, which correlated with survival [53]. This new class of spatial phenotypic biomarkers can account for cell densities and their interactions, compared to classical biomarkers, and several are being tested in clinical trials as companion diagnostics [39].

Challenges in integrating biomarker-driven MSI studies in drug discovery and development

Most discovery-based MSI to examine **spatial metabolomics** is aimed at the classification of disease samples; few studies have evaluated the pharmacodynamic effects of drugs. Several considerations need to be made to generate reliable and biologically meaningful insights for such studies. First, highly reproducible results require robust study designs that have sufficient statistical power and incorporate methods to minimize sources of variability and artifacts. Second, the high dimensionality of untargeted MSI data, although rich in information content, could lead to spurious associations between features; only biomarkers with high accuracy and reproducibility that can be quantitatively validated and implemented in a time- and cost-effective manner have clinical utility. Third large-scale cross-cohort longitudinal studies are necessary for robust validation of methods and AI/ML/DL algorithm-derived biomarkers. Open-source and proprietary computational tools for MSI data analysis [42] require cross-validated pipelines and clear guidelines for use. One approach involves the integration of open-source MSI data analysis tools, such as Cardinal [58], into a cohesive framework that is coupled with quality control, visualization, preprocessing, and statistical analysis [59]. Repository infrastructures, such as those built for proteomics to store assay metadata, workflow details, experimental design, and data acquisition and analysis methods [60], should be implemented in the MSI field to improve rigor and reproducibility. Clinical validation of MSI as a clinical assay [61], combined with iterative training of classification models based on computational and expert evaluation,

Figure 2. Experimental and computational mass spectrometry imaging (MSI) workflows related to pharmacology. (A) A typical workflow of MSI sample processing includes cryosectioning of the tissue and placing it on the modality-specific substrate. In the case of MALDI, a uniform matrix coating is applied on the tissue surface. Data are acquired in the mass spectrometer. (B) Sample preprocessing includes baseline subtraction, smoothing, peak picking, peak alignment, and normalization to retrieve highly informative peaks. (C) Unsupervised (left panel) or supervised (right panel) machine learning and deep learning approaches can be used to derive a segmentation map within the tissue or for classification of tissues. (D) Multivariate statistical analysis allows the identification of biomarkers of spatial clusters or classes obtained from panel (C). Abbreviations: MALDI, matrix-assisted laser desorption ionization; SHAP, Shapley additive explanations; UMAP, uniform manifold approximation and projection.

will help to integrate MSI into clinical diagnostics and drug discovery workflow. Other critical components for annotating and identifying the hits from these studies are described in the following section.

Strategies to improve molecular annotation and identification in untargeted MSI

To derive meaningful biological insights and accurate biomarker annotations from untargeted MSI spatial metabolomic studies, the m/z values need to be accurately assigned to unique biomolecules such as lipids and metabolites. Several factors make accurate annotation challenging, including lack of additional separation capabilities during MSI data acquisition, the mass resolution of instrument, in-source fragmentation, and the influence of sample preparation on native compound modifications [62]. Orthogonal validation of ions by LC-MS/MS on the same sample is feasible [63], although the lack of chromatographic separation in MSI experiments could lead to inaccurate assignments of ions between the methods. The gold standard is to perform *in situ* MS/MS analysis to confirm the fragmentation patterns for verification of molecular identity. However, MS/MS is feasible only for targeted studies by performing single ion fragmentation on commonly used ToF instruments. Recent instrument configurations, such as targeted DESI MSI using **multiple reaction monitoring** of multiple ions on triple quadrupoles in imaging mode, enable quantitative imaging of drugs, metabolites, and lipid biomarkers of interest [31,64]. Adding another dimension of analysis, such as ion mobility separation, allows **isobaric compounds** to be resolved, thus aiding confidence in annotations [65,66]. Lipid class, chain length, and degree of unsaturation prediction using computational methods, such as Kendrick mass defect (KMD) analysis, can also complement these analyses [67]. Proprietary and open-source software are available to assist with annotation and identification [62]. However, clear guidelines for reporting MSI ion annotations are still lacking. A standardized way to report how annotations are performed along with the degree of confidence would provide transparency within the MSI community and increase confidence in the findings of the studies.

Next-generation multimodal imaging for integrated biomarker discovery and biological insights

The implementation of discovery-based approaches in the drug development pipeline also depends on whether mechanistic insights can be gleaned from these studies. Multi-omic ML-driven network-based approaches have shown promise for providing holistic views of systems to infer drug–target interactions, patient stratification, biomarkers, and molecular drivers of phenotypes [68]. Similar spatial multimodal studies implementing developments in MSI technologies, as well as other spatial technologies including spatial transcriptomics [69] and medical imaging [70], open an opportunity for multimodal imaging to capture complex dynamics in tissues (Figure 3).

Multimodality can be implemented both in the MSI field and in connection with other imaging fields. The complementarity of different MSI technologies in terms of the classes of molecules detected and their spatial resolution makes them ideal for combining to acquire a comprehensive map of tissues at different scales. For example, multimodal imaging using the same sample and instrument, such as MALDI and DESI [71], or MALDI and SIMS [72], can reveal metabolically complementary classes of small metabolites and lipids at different scales. Similarly, different primary ion beams allow lipids and metabolites, as well as multiplexed antibody imaging, to be acquired to derive single-cell metabolomics by SIMS on the same tissue section [54]. Correlative MSI using different instruments on the same or consecutive tissue sections using SIMS and DESI [63] has allowed researchers to dissect tissue organization at different scales.

Integrating other methods, such as *in situ* hybridization probes against mRNA markers, multiplexed antibody imaging [63,73], positron emission tomography (PET) [74], surface-enhanced Raman

spectroscopy (SERS) [75], and magnetic resonance imaging (MRI) [76], with different MSI modalities has allowed researchers to correlate metabolic maps to anatomical and molecularly defined structures. Multimodality can also be used as an additional validation tool. For example, the detection of some classes of metabolites that are laser-sensitive benefit from IR-assisted SERS imaging for visualization of cancer biomarkers without autooxidation [75]. In addition, recent advances in other imaging modalities such as spatial transcriptomics and epigenomics [69] create new avenues to examine the mechanistic relationship between omic layers. Fresh-frozen tissue sections are amenable to spatial molecular imaging techniques, including MSI, spatial transcriptomics, and multiplexed antibody imaging, allowing multimodal data acquisition and analysis of the same or consecutive tissue sections. By implementing complementary technologies, the limitations of each technology can be overcome to provide an integrated and systems perspective of the tissues under study. The biological insights gained from these studies can aid in precision medicine (Figure 3).

Multimodal MSI is in the early phases of development. Challenges in data integration and analysis using images acquired with different spatial resolutions, data structures, and features are currently being explored [77]. Lessons learned from non-spatial integrated omic research could be tested within the spatial domain [78]. Network and DL-based integrative approaches for multiomic data are emerging to gain comprehensive views of systems [68,79]. Multimodal imaging is being explored in drug discovery and development [70], although challenges in multimodal image registration and guidelines are becoming evident [77].

Concluding remarks and future perspectives

Spatial pharmacology has the potential to dramatically impact human health by decoding molecular/cellular contents and interactions in health and disease, thus providing new strategies for

Outstanding questions

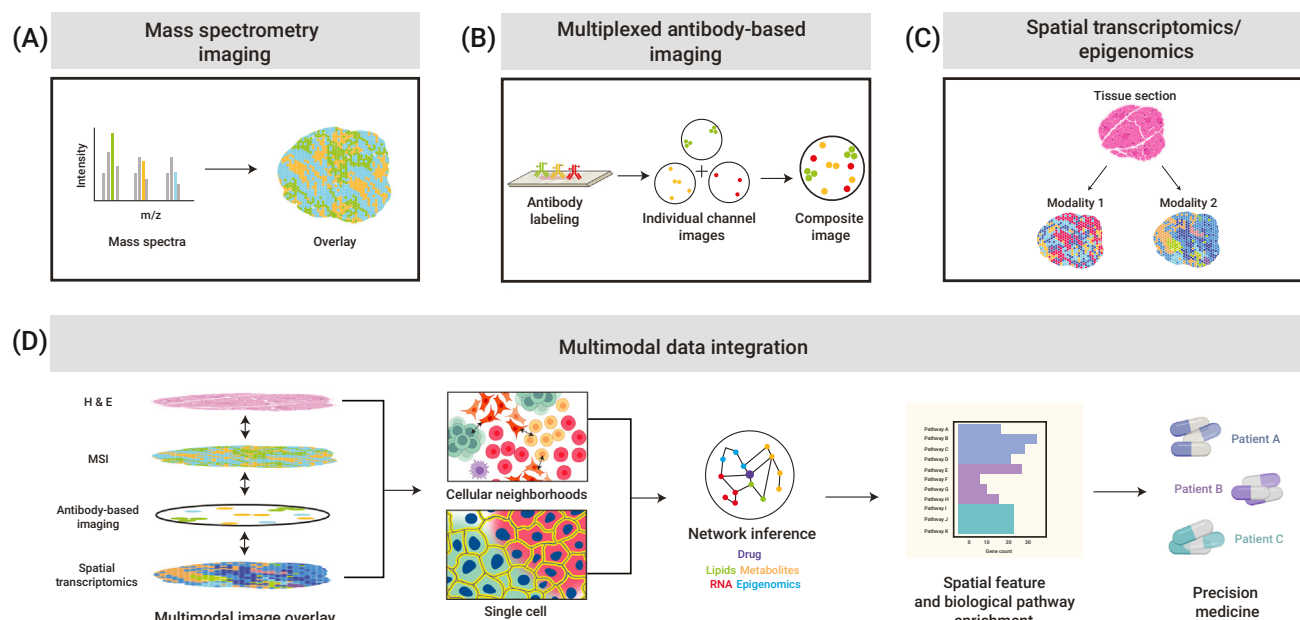
Where in the drug discovery process does MSI provide the most value?

What key modalities should be included in multimodal MSI for integrative systems biology?

Can MSI workflows be standardized to improve rigor and reproducibility?

When should MSI be used as a companion diagnostic or clinical diagnostic?

Can MSI become accessible to more companies and research laboratories?



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Figure 3. Multimodal mass spectrometry imaging (MSI) reveals new insights. Multimodal imaging using different technologies, such as (A) metabolomics using individual or integrative multimodal MSI, (B) proteomics using highly multiplexed antibody imaging for cellular phenotyping, and (C) transcriptomics and epigenomics using corresponding spatial technology. (D) Data processing includes multimodal image alignment and registration, followed by cellular or cellular neighborhood segmentation for multimodal feature extraction. Intra- and inter-modality molecular network analysis can be performed to infer spatial features and biological pathways impacting on precision medicine. Abbreviation: H & E, hematoxylin/eosin stain.

the diagnosis and prevention of disease, explaining response to treatment, and insights into drug pharmacology. Researchers have improved MSI instrumentation for sensitivity, spatial resolution, throughput, and chemical coverage, such that there is now an array of tools that perform well for complementary classes of molecules with impressive spatial resolution. ML and DL algorithms developed for image analysis have been translated to MSI to dissect and comprehend spatially and spectrally rich imaging data. Multimodal imaging has the potential to change the face of biomedicine.

Challenges that need to be addressed in the emerging field of spatial pharmacology include rigorous validation of data analysis workflows, data integration for multimodal imaging, and annotation of ions (see [Outstanding questions](#)). Clear guidelines for MSI methods for research and clinical studies are needed. Increased adoption of these technologies by diverse research laboratories will improve the learning process. The knowledge gained from application of these technologies and computational workflows will guide the next generation of instrumentation, as well as experimental and computational pipelines to improve rigor and reproducibility. Community efforts to provide resources and standardization, as recently demonstrated by the construction of antibody panel maps for different tissues [80], should be implemented in the MSI field. Consortium efforts such as the Human BioMolecular Atlas Program (HuBMAP) [81] and the Human Tumor Atlas Network (HTAN) [82], which aim to build spatial multiomic maps of healthy and diseased tissues, play an important role in bringing together researchers using different technologies, computational biologists, pathologists, and clinicians for interdisciplinary collaborations to decode human health and disease. Continued advances in technological developments, data analytics, cross-cohort validation studies, and community-building efforts will propel the field of MSI into mainstream biomedical and pharmaceutical research, transforming biology and medicine.

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Declaration of interests

B.R.S. is an inventor on patents and patent applications involving ferroptosis; co-founded and serves as a consultant to ProJenX, Inc. and Exarta Therapeutics; holds equity in Sonata Therapeutics; serves as a consultant to Weatherwax Biotechnologies Corporation and Akin Gump Strauss Hauer & Feld LLP. The remaining authors declare no competing interests.

References

1. Singh, N. *et al.* (2023) Drug discovery and development: introduction to the general public and patient groups. *Front. Drug Discov.* 3, 1201419
2. Spracklin, D.K. *et al.* (2020) Mini-review: comprehensive drug disposition knowledge generated in the modern human radiolabeled ADME Study. *CPT Pharmacometrics Syst. Pharmacol.* 9, 428–434
3. Granborg, J.R. *et al.* (2022) Mass spectrometry imaging in drug distribution and drug metabolism studies – principles, applications and perspectives. *TracTrend Anal. Chem.* 146, 116482
4. Bajinath, S. *et al.* (2022) Advances in spatial mass spectrometry enable in-depth neuropharmacodynamics. *Trends Pharmacol. Sci.* 43, 740–753
5. Alexandrov, T. (2020) Spatial metabolomics and imaging mass spectrometry in the age of artificial intelligence. *Annu. Rev. Biomed. Data Sci.* 3, 61–87
6. Scupakova, K. *et al.* (2020) Cellular resolution in clinical MALDI mass spectrometry imaging: the latest advancements and current challenges. *Clin. Chem. Lab. Med.* 58, 914–929
7. Morato, N.M. and Cooks, R.G. (2023) Desorption electrospray ionization mass spectrometry: 20 years. *Acc. Chem. Res.* 56, 2526–2536
8. Jia, F. *et al.* (2023) Advancements in ToF-SIMS imaging for life sciences. *Front. Chem.* 11, 1237408
9. Goodwin, R.J.A. *et al.* (2020) A critical and concise review of mass spectrometry applied to imaging in drug discovery. *SLAS Discov.* 25, 963–976
10. Meng, Y.F. *et al.* (2021) Single-cell mass spectrometry imaging of multiple drugs and nanomaterials at organelle level. *ACS Nano* 15, 13220–13229
11. Cooper-Shepherd, D.A. *et al.* (2023) Novel hybrid quadrupole-multireflecting time-of-flight mass spectrometry system. *J. Am. Soc. Mass Spectrom.* 34, 264–272
12. Rivera, E.S. *et al.* (2020) Integrating ion mobility and imaging mass spectrometry for comprehensive analysis of biological tissues: a brief review and perspective. *J. Mass Spectrom.* 55, e4614
13. Jiang, L.X. *et al.* (2023) High-throughput mass spectrometry imaging of biological systems: current approaches and future directions. *Trends Anal. Chem.* 163, 117055
14. Frenais, M. *et al.* (2022) Analytical performance evaluation of new DESI enhancements for targeted drug quantification in tissue sections. *Pharmaceuticals (Basel)* 15, 694
15. Wu, V. *et al.* (2022) High resolution ambient MS imaging of biological samples by desorption electro-flow focussing ionization. *Anal. Chem.* 94, 10035–10044
16. Mesa Sanchez, D. *et al.* (2022) Mass spectrometry imaging of diclofenac and its metabolites in tissues using nanospray

- desorption electrospray ionization. *Anal. Chim. Acta* 1233, 340490
17. Dilmetz, B.A. *et al.* (2021) Quantitative approach using matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry. *Methods Mol. Biol.* 2228, 159–166
 18. McMillen, J.C. *et al.* (2021) Enhancement of tryptic peptide signals from tissue sections using MALDI IMS postionization (MALDI-2). *J. Am. Soc. Mass Spectrom.* 32, 2583–2591
 19. Hejls, B. *et al.* (2020) MALDI-2 for the enhanced analysis of N-linked glycans by mass spectrometry imaging. *Anal. Chem.* 92, 13904–13911
 20. Barre, F.P.Y. *et al.* (2019) Enhanced sensitivity using MALDI imaging coupled with laser postionization (MALDI-2) for pharmaceutical research. *Anal. Chem.* 91, 10840–10848
 21. Niehaus, M. *et al.* (2019) Transmission-mode MALDI-2 mass spectrometry imaging of cells and tissues at subcellular resolution. *Nat. Methods* 16, 925–931
 22. Mokosch, A.S. *et al.* (2021) High-resolution AP-SMALDI MSI as a tool for drug imaging in *Schistosoma mansoni*. *Anal. Bioanal. Chem.* 413, 2755–2766
 23. Islam, A. *et al.* (2022) Application of AP-MALDI imaging mass microscope for the rapid mapping of imipramine, chloroquine, and their metabolites in the kidney and brain of wild-type mice. *Pharmaceuticals (Basel)* 15, 1314
 24. Hogan, K.A. *et al.* (2023) Using mass spectrometry imaging to visualize age-related subcellular disruption. *Front. Mol. Biosci.* 10, 906606
 25. Tian, H. *et al.* (2021) Multimions imaging using high-energy water gas cluster ion beam secondary ion mass spectrometry [(H₂O)_n-GCIB-SIMS] of frozen-hydrated cells and tissue. *Anal. Chem.* 93, 7808–7814
 26. Costa, C. *et al.* (2021) Imaging mass spectrometry: a new way to distinguish dermal contact from administration of cocaine, using a single fingerprint. *Analyst* 146, 4010–4021
 27. Zhang, J. *et al.* (2020) Cryo-OrbiSIMS for 3D molecular imaging of a bacterial biofilm in its native state. *Anal. Chem.* 92, 9008–9015
 28. Green, F.M. *et al.* (2023) Development of high throughput microscope mode secondary ion mass spectrometry imaging. *J. Am. Soc. Mass Spectrom.* 34, 1272–1282
 29. Thomen, A. *et al.* (2020) Subcellular mass spectrometry imaging and absolute quantitative analysis across organelles. *ACS Nano* 14, 4316–4325
 30. De Castro, O. *et al.* (2022) Magnetic sector secondary ion mass spectrometry on FIB-SEM instruments for nanoscale chemical imaging. *Anal. Chem.* 94, 10754–10763
 31. Dannhorn, A. *et al.* (2023) Targeted desorption electrospray ionization mass spectrometry imaging for drug distribution, toxicity, and tissue classification studies. *Metabolites* 13, 377
 32. Barry, J.A. *et al.* (2019) Quantification and assessment of detection capability in imaging mass spectrometry using a revised mimetic tissue model. *Bioanalysis* 11, 1099–1116
 33. Tobias, F. and Hummon, A.B. (2020) Considerations for MALDI-based quantitative mass spectrometry imaging studies. *J. Proteome Res.* 19, 3620–3630
 34. Barry, J.A. *et al.* (2019) Multicenter validation study of quantitative imaging mass spectrometry. *Anal. Chem.* 91, 6266–6274
 35. Castellino, S. *et al.* (2021) The emergence of imaging mass spectrometry in drug discovery and development: making a difference by driving decision making. *J. Mass Spectrom.* 56, e4717
 36. Bowman, A.P. *et al.* (2022) Evaluation of quantitative platforms for single target mass spectrometry imaging. *Pharmaceuticals (Basel)* 15, 1180
 37. Song, X. *et al.* (2022) Mass spectrometry imaging advances and application in pharmaceutical research. *Acta Materia Medica* 1, 507–533
 38. Gromova, M. *et al.* (2020) Biomarkers: opportunities and challenges for drug development in the current regulatory landscape. *Biomark. Insights* 15 1177271920974652
 39. Locke, D. and Hoyt, C.C. (2023) Companion diagnostic requirements for spatial biology using multiplex immunofluorescence and multispectral imaging. *Front. Mol. Biosci.* 10, 1051491
 40. Balluff, B. *et al.* (2021) Batch effects in MALDI mass spectrometry imaging. *J. Am. Soc. Mass Spectrom.* 32, 628–635
 41. Verbeeck, N. *et al.* (2020) Unsupervised machine learning for exploratory data analysis in imaging mass spectrometry. *Mass Spectrom. Rev.* 39, 245–291
 42. Hu, H. and Laskin, J. (2022) Emerging computational methods in mass spectrometry imaging. *Adv. Sci. (Weinh)* 9, e2203339
 43. Nikparvar B, T.J.-C. (2021) Machine learning of spatial data. *ISPRS Int. J. Geo-Inf.* 10, 600
 44. Abdelmoula, W.M. *et al.* (2021) Peak learning of mass spectrometry imaging data using artificial neural networks. *Nat. Commun.* 12, 5544
 45. Derwae, H. *et al.* (2023) Spatiochemical characterization of the pancreas using mass spectrometry imaging and topological data analysis. *Anal. Chem.* 95, 10550–10556
 46. Goncalves, J.P.L. *et al.* (2022) Mass spectrometry imaging spatial tissue analysis toward personalized medicine. *Life (Basel)* 12, 1037
 47. Bollwein, C. *et al.* (2022) MALDI mass spectrometry imaging for the distinction of adenocarcinomas of the pancreas and biliary tree. *Molecules* 27, 3464
 48. Zhang, J. *et al.* (2020) Mass spectrometry imaging enables discrimination of renal oncocytoma from renal cell cancer subtypes and normal kidney tissues. *Cancer Res.* 80, 689–698
 49. Gularyan, S.K. *et al.* (2020) Investigation of inter- and intratumoral heterogeneity of glioblastoma using TOF-SIMS. *Mol. Cell. Proteomics* 19, 960–970
 50. Tideman, L.E.M. *et al.* (2021) Automated biomarker candidate discovery in imaging mass spectrometry data through spatially localized Shapley additive explanations. *Anal. Chim. Acta* 1177, 338522
 51. Sun, C. *et al.* (2020) Mass spectrometry imaging-based metabolomics to visualize the spatially resolved reprogramming of carnitine metabolism in breast cancer. *Theranostics* 10, 7070–7082
 52. Banerjee, S. (2020) Empowering clinical diagnostics with mass spectrometry. *ACS Omega* 5, 2041–2048
 53. Keren, L. *et al.* (2018) A structured tumor-immune microenvironment in triple negative breast cancer revealed by multiplexed ion beam imaging. *Cell* 174, 1373–1387
 54. Tian, H. *et al.* (2021) Successive high-resolution (H₂O)_n-GCIB and C₆₀-SIMS imaging integrates multi-omics in different cell types in breast cancer tissue. *Anal. Chem.* 93, 8143–8151
 55. Verkhoturov, D.S. *et al.* (2021) Nanoprojectile secondary ion mass spectrometry for analysis of extracellular vesicles. *Anal. Chem.* 93, 7481–7490
 56. Yagnik, G. *et al.* (2021) Highly multiplexed immunohistochemical MALDI-MS imaging of biomarkers in tissues. *J. Am. Soc. Mass Spectrom.* 32, 977–988
 57. Song, X. *et al.* (2023) Immuno-desorption electrospray ionization mass spectrometry imaging identifies functional macromolecules by using microdroplet-cleavable mass tags. *Angew. Chem. Int. Ed. Eng.* 62, e202216969
 58. Bemis, K.A. *et al.* (2023) Cardinal v3 – a versatile open source software for mass spectrometry imaging analysis. *bioRxiv* Published online February 21, 2023. <https://doi.org/10.1101/2023.02.20.529280>
 59. Foll, M.C. *et al.* (2019) Accessible and reproducible mass spectrometry imaging data analysis in Galaxy. *Gigascience* 8 giz143
 60. Choi, M. *et al.* (2020) MassIVE.quant: a community resource of quantitative mass spectrometry-based proteomics datasets. *Nat. Methods* 17, 981–984
 61. Moore, J.L. *et al.* (2023) Prospective on imaging mass spectrometry in clinical diagnostics. *Mol. Cell. Proteomics* 22, 100576
 62. Baquer, G. *et al.* (2023) What are we imaging? Software tools and experimental strategies for annotation and identification of small molecules in mass spectrometry imaging. *Mass Spectrom. Rev.* 42, 1927–1964
 63. Tian, H. *et al.* (2022) Multi-modal mass spectrometry imaging reveals single-cell metabolic states in mammalian liver. *bioRxiv* Published online September 28, 2022. <https://doi.org/10.1101/2022.09.26.508878>
 64. Lamont, L. *et al.* (2018) Targeted drug and metabolite imaging: desorption electrospray ionization combined with triple quadrupole mass spectrometry. *Anal. Chem.* 90, 13229–13235
 65. Spraggins, J.M. *et al.* (2019) High-performance molecular imaging with MALDI trapped ion-mobility time-of-flight (timsTOF) mass spectrometry. *Anal. Chem.* 91, 14552–14560
 66. Ujma, J. *et al.* (2019) Cyclic ion mobility mass spectrometry distinguishes anomers and open-ring forms of pentasaccharides. *J. Am. Soc. Mass Spectrom.* 30, 1028–1037
 67. Richardson, L.T. *et al.* (2022) Referenced Kendrick mass defect annotation and class-based filtering of imaging MS lipidomics experiments. *Anal. Chem.* 94, 5504–5513

68. Agamah, F.E. *et al.* (2022) Computational approaches for network-based integrative multi-omics analysis. *Front. Mol. Biosci.* 9, 967205
69. Zhang, D. *et al.* (2023) Spatial epigenome-transcriptome co-profiling of mammalian tissues. *Nature* 616, 113–122
70. Vermeulen, I. *et al.* (2022) Multimodal molecular imaging in drug discovery and development. *Drug Discov. Today* 27, 2086–2099
71. Zemaitis, K.J. *et al.* (2021) Streamlined multimodal DESI and MALDI mass spectrometry imaging on a singular dual-source FT-ICR mass spectrometer. *Metabolites* 11, 253
72. Krijnen, K. *et al.* (2023) A multimodal SIMS/MALDI mass spectrometry imaging source with secondary electron imaging capabilities for use with timsTOF instruments. *J. Am. Soc. Mass Spectrom.* 34, 720–727
73. Goossens, P. *et al.* (2022) Integrating multiplex immunofluorescent and mass spectrometry imaging to map myeloid heterogeneity in its metabolic and cellular context. *Cell Metab.* 34, 1214–1225
74. Jacobsen, S.C. *et al.* (2021) Desorption electrospray ionization mass spectrometry imaging of Cimbi-36, a 5-HT_{2A} receptor agonist, with direct comparison to autoradiography and positron emission tomography. *Mol. Imaging Biol.* 23, 676–685
75. Honda, K. *et al.* (2021) On-tissue polysulfide visualization by surface-enhanced Raman spectroscopy benefits patients with ovarian cancer to predict post-operative chemosensitivity. *Redox Biol.* 41, 101926
76. Verbeek, N. *et al.* (2017) Connecting imaging mass spectrometry and magnetic resonance imaging-based anatomical atlases for automated anatomical interpretation and differential analysis. *Biochim. Biophys. Acta, Proteins Proteomics* 1865, 967–977
77. Balluff, B. *et al.* (2022) An overview of image registration for aligning mass spectrometry imaging with clinically relevant imaging modalities. *J. Mass Spectrom. Adv. Clin. Lab.* 23, 26–38
78. Krassowski, M. *et al.* (2020) State of the field in multi-omics research: from computational needs to data mining and sharing. *Front. Genet.* 11, 610798
79. Stahlschmidt, S.R. *et al.* (2022) Multimodal deep learning for biomedical data fusion: a review. *Brief. Bioinform.* 23, bbab569
80. Quardokus, E.M. *et al.* (2023) Organ mapping antibody panels: a community resource for standardized multiplexed tissue imaging. *Nat. Methods* 20, 1174–1178
81. Jain, S. *et al.* (2023) Advances and prospects for the Human Bio-Molecular Atlas Program (HuBMAP). *Nat. Cell Biol.* 25, 1089–1100
82. Rozenblatt-Rosen, O. *et al.* (2020) The Human Tumor Atlas Network: charting tumor transitions across space and time at single-cell resolution. *Cell* 181, 236–249
83. Barre, F. *et al.* (2019) Faster raster matrix-assisted laser desorption/ionization mass spectrometry imaging of lipids at high lateral resolution. *Int. J. Mass Spectrom.* 437, 38–48
84. Gyngard, F. and Steinhäuser, M.L. (2019) Biological explorations with nanoscale secondary ion mass spectrometry. *J. Anal. At. Spectrom.* 34, 1534–1545
85. Beng, H.M. *et al.* (2021) Quantitative DESI mass spectrometry imaging of lung distribution of inhaled drug. *J. Drug Deliv. Sci. Tec.* 66 ARTN 102794
86. Kallback, P. *et al.* (2020) Cross-validated matrix-assisted laser desorption/ionization mass spectrometry imaging quantitation protocol for a pharmaceutical drug and its drug-target effects in the brain using time-of-flight and fourier transform ion cyclotron resonance analyzers. *Anal. Chem.* 92, 14676–14684
87. Mamun, M.A. *et al.* (2023) Detection of distinct distributions of acetaminophen and acetaminophen-cysteine in kidneys up to 10 μm resolution and identification of a novel acetaminophen metabolite using an AP-MALDI imaging mass microscope. *J. Am. Soc. Mass Spectrom.* 34, 1491–1500
88. Traberg, A. *et al.* (2022) Quantitative mass spectrometry imaging of bleomycin in skin using a mimetic tissue model for calibration. *Pharmaceuticals (Basel)* 15, 1583
89. Rabasco, S. *et al.* (2021) Localization and absolute quantification of dopamine in discrete intravesicular compartments using NanoSIMS imaging. *Int. J. Mol. Sci.* 23, 160
90. Dexter, A. *et al.* (2019) Imaging drugs, metabolites and biomarkers in rodent lung: a DESI MS strategy for the evaluation of drug-induced lipodosis. *Anal. Bioanal. Chem.* 411, 8023–8032
91. Ntshangase, S. *et al.* (2019) Mass spectrometry imaging demonstrates the regional brain distribution patterns of three first-line antiretroviral drugs. *ACS Omega* 4, 21169–21177
92. Passarelli, M.K. *et al.* (2017) The 3D OrbiSIMS-label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power. *Nat. Methods* 14, 1175–1183
93. Groseclose, M.R. and Castellino, S. (2019) An investigation into retigabine (ezogabine) associated dyspigmentation in rat eyes by MALDI imaging mass spectrometry. *Chem. Res. Toxicol.* 32, 294–303
94. Xue, J. *et al.* (2018) Mass spectrometry imaging of the in situ drug release from nanocarriers. *Sci. Adv.* 4, eaat9039
95. Rovira-Clave, X. *et al.* (2021) Subcellular localization of biomolecules and drug distribution by high-definition ion beam imaging. *Nat. Commun.* 12, 4628
96. Wang, Z. *et al.* (2020) In situ metabolomics in nephrotoxicity of aristolochic acids based on air flow-assisted desorption electrospray ionization mass spectrometry imaging. *Acta Pharm. Sin. B* 10, 1083–1093
97. Pang, X. *et al.* (2021) Mapping metabolic networks in the brain by ambient mass spectrometry imaging and metabolomics. *Anal. Chem.* 93, 6746–6754
98. Zhu, T. *et al.* (2020) Anti-ischemia/reperfusion injury effects of notoginsenoside R1 on small molecule metabolism in rat brain after ischemic stroke as visualized by MALDI-MS imaging. *Biomed. Pharmacother.* 129, 110470
99. Vallianatou, T. *et al.* (2019) Molecular imaging identifies age-related attenuation of acetylcholine in retrosplenial cortex in response to acetylcholinesterase inhibition. *Neuropsychopharmacology* 44, 2091–2098