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Special Section on New and Emerging Areas and Technologies in Drug Metabolism and Disposition, Part I—Minireview

Recent Advances in Mass Spectrometry-Based Spatially Resolved Molecular Imaging of Drug Disposition and Metabolomics

Yu Chen, Ying Liu, Ximei Li, Yan He, Weiwei Li, Ying Peng, and Jiang Zheng

State Key Laboratory of Functions and Applications of Medicinal Plants, Key Laboratory of Pharmaceutics of Guizhou Province, Guizhou Medical University, Guiyang, Guizhou, P.R. China (Y.C., Y.L., X.L., Y.H., W.L.); School of Basic Medicine, School of Pharmacy, Guizhou Medical University, Guiyang, Guizhou, P.R. China (Y.C., Y.L., X.L., Y.H., W.L.); Division of Pain Management, The Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, P.R. China (Y.C.); and Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang, Liaoning, P.R. China (Y.P., J.Z.)

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ABSTRACT

Mass spectrometric imaging is a nontargeted, tag-free, high-throughput, and highly responsive analytical approach. The highly accurate molecular visualization detection technology enables qualitative and quantitative analyses of biologic tissues or cells scanned by mass spectrometry in situ, extracting known and unknown multiple compounds, and simultaneously assessing relative contents of targeting molecules by monitoring their molecular ions and pinpointing the spatial locations of those molecules distributed. Five mass spectrometric imaging techniques and their characteristics are introduced in the review, including matrix-assisted laser desorption ionization mass spectrometry, secondary ion mass spectrometry, desorption electrospray ionization mass spectrometry, laser ablation electrospray ionization mass spectrometry, and laser ablation inductively coupled plasma mass spectrometry. The mass spectrometry-based techniques provide the possibility for spatial metabolomics with the capability of high throughput and precision detection. The approaches have been widely employed to spatially image not only metabolome of endogenous amino acids, peptides, proteins, neurotransmitters, and lipids but also the disposition of exogenous chemicals, such as pharmaceutical agents, environmental pollutants, toxicants, natural products, and heavy metals. The techniques also provide us with spatial distribution imaging of analytes in single cells, tissue microregions, organs, and whole animals.

SIGNIFICANCE STATEMENT

The review article includes an overview of five commonly used mass spectrometers for spatial imaging and describes the advantages and disadvantages of each. Examples of the technology applications cover drug disposition, diseases, and omics. Technical aspects of relative and absolute quantification by mass spectrometric imaging and challenges for future new applications are discussed as well. The reviewed knowledge may benefit the development of new drugs and provide a better understanding of biochemical processes related to physiology and diseases.

Introduction

The main imaging techniques traditionally used are autoradiography, fluorescent imaging, immunohistochemical staining, and magnetic resonance imaging. Autoradiography images whole bodies or organ systems of experimental animals. This method provides information about radioactivity localization in histologic preparations at the cellular level but without chemical structural identity. This technique, although sensitive, requires tedious and costly radiolabeling and organic synthesis (Solon et al., 2010; Solon, 2015). Additionally, operators performing experiments could face harmful effects due to exposure to radioactivity. Fluorescence often involves the detection of analytes labeled with fluorescent probes, but it is challenging to simultaneously provide high resolution, speed, large volume, and good bio-compatibility in a single imaging technique (Hobson and Aaron, 2022). Neither of the two can distinguish drugs from their metabolites, and they are expensive to use and have long experimental cycles. Immunohistochemical staining requires reagents specific to the targets and a low-throughput procedure (Schwamborn and Caprioli, 2010). Visualization of antibody-antigen pairs can be done by optical or

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ABBREVIATIONS: 2D, two-dimensional; 3D, three-dimensional; AFADESI, airflow-assisted desorption electrospray ionization; DESI, desorption electrospray ionization; LAESI, laser ablation electrospray ionization; LA-ICP, laser ablation inductively coupled plasma; m/z, mass-to-charge ratio; MALDI, matrix-assisted laser desorption ionization; MSI, mass spectrometric imaging; SIMS, secondary ion mass spectrometry; TOF, time-of-flight.
fluorescent imaging. Magnetic resonance imaging is an in vivo visualizing technique used for clinical diagnosis, mainly for soft tissue imaging, without ionizing radiation (Chabanova et al., 2014). Other imaging methods, such as positron emission tomography/computed tomography, provide little structural information using a wide range of molecular probes and metabolic parameters requiring a radiotracer (Gao et al., 2022b). These imaging tools lack spatial information or lack specificity. For example, further analysis after tissue homogenization using high-performance liquid chromatography or high-performance liquid chromatography tandem mass spectrometry can easily lead to an inability to distinguish the spatial distribution of parent drugs from their metabolites (Prideaux and Stoeckli, 2012). It is also necessary to know the target molecules of interest in advance and to label the molecules with probes, such as radioisotopes, fluorescence, chemiluminescence, and other tracers. These techniques often are time-consuming, which severely drags research progress.

MSI is a powerful label-free approach that simultaneously offers multiplexed images through acquiring hundreds of molecules and the capability to map those molecules in a sophisticated system with high flexibility and subcellular space analysis in technical terms (Buchberger et al., 2018; Unsihuay et al., 2021). MSI can provide a spatial distributational approach lacking in traditional liquid chromatography-tandem mass spectrometry methods and determine chemical specificity for endogenous and exogenous substances with good discrimination and selectivity for rapid screening and evaluating.

**Mass Spectrometric Imaging**

**Principles**

MSI was initially proposed by Caprioli and coworkers in 1997 (Caprioli et al., 1997) with the workflow as shown in Fig. 1. Samples need to be processed properly (e.g., tissue sections are embedded in paraffin after formalin treatment or frozen at optimal cutting temperature). The areas of the selection are simultaneously scanned by use of high-energy

![Schematic workflow of MSI. Created with BioRender.com.](image-url)
ion beams or lasers. Biologic specimens are rastered by label-free ionization using an ion detector to produce charged material for mass spectrometric analysis. A mass spectrometric map generates for each virtual raster pattern position. Mass analyzers can determine the mass-to-charge ratio \( m/z \) and measure intensity of ions on sample surfaces. Mass spectrometry software is used to perform the analysis, and a heat map of ion density can be generated for each \( m/z \) value detected to visualize its spatial localization and relative intensity. A two-dimensional (2D) spatial dispersion of multiple molecules or ions on the sample surface builds a map. In this model, a three-dimensional (3D) spatial distribution of analytes can be generated from the 2D distributed maps of the successive slices of continuously processed samples (Ye et al., 2012; Buchberger et al., 2018; Qin et al., 2018) enabling the establishment of qualitative and quantitative maps accompanied by localization functions (He et al., 2018).

**Ionization Techniques**

The selection of ionization method is closely related to MSI’s spatial resolution and signal intensity. Several ionization techniques are commonly used: matrix-assisted laser desorption ionization (MALDI) mass spectrometry, secondary ion mass spectrometry (SIMS), desorption electrospray ionization (DESI) mass spectrometry, laser ablation electrospray ionization (LAESI) mass spectrometry, and laser ablation inductively coupled plasma (LA-ICP) mass spectrometry.

**MALDI-MSI**

MALDI involves mixing a solution of analyte molecules with a resolution of matrix molecules that are then evaporated and dried on a MALDI target plate to form cocrystals of matrix molecules and analytes. When irradiating the crystal with laser light, the matrix crystals sublime as the matrix molecules absorb energy from the radiation and cause rapid heat production. The mass analysis measures ions’ \( m/z \) and ionic intensity at pixel points on the sample surface by expanding the matrix and analytes. In combination with the MSI software, the signal intensity and location of an ion on the surface of the sample can be determined, and the map is constructed by establishing the 2D distribution of complementary molecules or ions on the surface of the sample (Cornett et al., 2007; Flatley et al., 2014).

**SIMS-MSI**

SIMS uses a single beam of primaries injected onto the sample surface to perform measurements under a high vacuum condition, which causes the atoms on the surface to be electrostatic. The resulting secondary ions are sputtered and desorbed on the material's texture, followed by analysis using mass spectrometry and imaging by...
magnetic and electric fields to accurately measure the signals on the surface to be explored. The primary ion beam's energy is usually high so that the sputtering technique breaks covalent bonds by analyzing removed samples analytes. During the detection, many atomic and molecular debris obtained are characterized by an ion beam sputtering the surface components within each volumetric element, a small part of which is ionized as either positive or otherwise negative ions. SIMS is, therefore, generally suitable for the analysis of inorganic analytes as well as that of substances that are chemically bonded at the surface. The mass analyzer is also used for elemental and inorganic analysis as well as the detection of small molecules. SIMS has the advantages of high lateral resolutions in the micron or submicron range (Crecelius et al., 2014). The analyzers can be TOF-SIMS (Van Nuffel and Brunelle, 2022), Fourier-transform ion cyclotron resonance-SIMS, and nano secondary ion mass spectrometry (Mayali, 2020).

The procedure for tissue sectioning is the same as that for MALDI mass spectrometric analysis. Both require frozen sample sectioning, and tissue samples for SIMS can be analyzed directly without additional processing.

**DESI-MSI.** MALDI and SIMS mass spectrometry techniques generally require a vacuum environment and are inconvenient to use. For this reason, Cooks' group introduced DESI (Takáts et al., 2004) and developed it for atmospheric pressure mass spectrometric analysis. DESI is an environmental ionization technology that requires no substrates or specimen preparation. The basic principle includes a droplet-carrying mechanism that impinges an atomized solvent droplet on the surface of tissue sections to directly desorb and ionize analytes for qualitative and quantitative detection, followed by sputtering into the gas phase and desolvation of the charged droplet by nitrogen purging and forming gas-phase ions, which enter the mass spectrometric interface for analysis. The DESI technique is generally considered a combination of electrospray ionization and desorption. The approach is widely used for atmospheric pressure MSI. The analyzer produces mass spectra similar to electron spray ionization, mainly displaying the analyte's single or multicharged molecular ions (Cook et al., 2006; Beneito-Cambrá et al., 2020).

Solvents play a crucial role in the quality and efficiency of DESI mass spectrometry. This instrument can extract information about the distributions of different compounds on the sample surface optimized by changing the solvent systems (Eberlin et al., 2010). The distance and angle between the DESI nebulizer, the specimen surface, and the mass spectrometry entrance affects the resolution and signal intensity of imaging. In recent years, several novel techniques have been developed, such as DESI-MSI combined with ion flow separation for rapid qualification of analytes (Pierson et al., 2020). Ion mobility spectroscopy has been merged into MSI. The relatively new technique allows the separation of isomers with the same molecular weight, which has been employed for imaging particular isomers, e.g., lipids and lipopeptides (McCann et al., 2021).

Airflow assisted desorption electrospray mass spectrometry imaging (AFADESI-MSI) improves the sensitivity of DESI-MSI detection by introducing a high-rate airflow into the ion source to strengthen the in situ collection to increase the sampling of liquid droplets. The modified DESI-MSI system is equipped with tissue homogenization to optimize the spray solvent for analyte detection and a high-quality resolution orbital mass spectrometer with custom-developed highly discriminative imaging software MassImager. The system improves sensitivity, specificity, and feasibility and is suitable for identifying functional molecules and tissue architecture.

Sample preparation is similar to that for MALDI-MSI analysis. Again, DESI-MSI does not require a matrix coated on sample's surface, reducing the possibility of analytes leaving the domain before analysis (Kertesz et al., 2008). Following the collection and rapid tissue freezing, sections are placed on slides and stored in a dry environment pending tests.

**LAESI-MSI.** LAESI is an innovated technology with high-throughput ability and distinctive potential for nontargeted analysis and spatial location in intact plant samples without the need of extraction or extensive specimen preparation (Kulkarni et al., 2018). The technique uses a medium infrared laser beam to stimulate the water molecules in the samples, and the target area absorbs laser beam energy, resulting in the evaporation of the water molecules. When the energy density exceeds a critical value, a small fraction of the sample is sputtered, intercepted at right angles by the conical spray current, and ablated into gas-phase ions, which then enter the mass spectrum analyzer for group spectrometric analysis, enabling recording units of pixels at its origin. LAESI eschews the need for a remarkably flat surface, high-precision sample preparation, and matrix as a solvent. Due to these properties, it might be a good choice for spatially resolved food analysis (Nielen and van Beek, 2014). The practice of sample analysis by LAESI is similar to that of DESI. However, models do not need to be dried, and plant samples should not be sectioned before analysis.

**LA-ICP-MSI.** The LA-ICP technique is used for elemental analyses of solid materials and tissues. Liquid samples are first atomized in a sample introduction system to produce a fine aerosol and transfer it into the argon plasma applied. The high-heat plasma atomizes and electrically ionizes samples, producing ions pulled through the interfacial area and into a group of electrostatic mirrors called ion optical. The ion optomechanics focuses and guides the ion bunch into the four-pole mass spectrometer. The massive analyzer separates the ions depending on their $m/z$ and measures them on the detection unit (Wilschefski and Baxter, 2019). LA-ICP can detect and quantify proteins bound with metal-labeled antibodies (Sussulini et al., 2017). Electrophoretic protein segmentation (PAGE or SDS) allows analysis of metal-containing proteins to be visualized in 2D gels. LA-ICP with multiple element ability enables the accurate measurement and visualization of metals in tissues with high spatial resolution, flexibility, quantitative capability, and excellent repeatability (Weiskirchen et al., 2019). In the analysis of biologic samples, LA-ICP combined with TOF allows identification of proteins labeled with a trace of elements.

The five techniques have their own characteristics as listed in Table 1 and Fig. 3. With a high resolution, SIMS-MSI can offer 3D imaging without requiring matrixes. However, the method is only suitable for detecting small molecules with low sensitivity, making it more difficult to provide quantitative data. DESI-MSI requires neither complex sample pretreatment nor matrixes, facilitating the expansion of the analysis to liquid and gas samples. However, the technique has the weakness of relatively poor resolution and low signal susceptibility to external environmental interference. LAESI-MSI sample preparation is simple, compassionate, and matrix-free but requires aqueous and relatively stable samples and shows poor resolution. The MALDI-MSI technique provides high resolution and is suitable for all biologic tissue samples with no molecular weight limitation. However, sample presentation and matrices can disturb the detection of analytes (Porta Siegel et al., 2018). LA-ICP-MSI offers elemental imaging, trace metal detection, and quantification of metal-labeled antibodies, but isobaric interference lacks matrix-matched standards for quantification (Spruill et al., 2022).

**Spatially Resolved Imaging and Applications**

**Drug Disposition**

Various methods for measuring and visualizing medication uptake, distribution, and excretion have been explored for MSI. Nilsson and
coworkers studied propranolol, metoprolol, and atenolol absorption in small intestines of rats using a quantitative MALDI-MSI technique (Nilsson et al., 2017). They identified the sites for intestinal absorption of the three b-blockers. Chen and coworkers used MALDI-TOF-MSI to visualize detailed distribution of five central nervous system drugs in the brain of mice after intraperitoneal administration with these agents (Chen et al., 2020). Gruner and coworkers evaluated erlotinib, an inhibitor of epidermal growth factor receptor for the therapy of pancreatic ductal adenocarcinoma, and found that the agent was spatially distributed in healthy pancreas and unhealthy pancreas of mice using MALDI-MSI (Gruner et al., 2016). Cesca and coworkers employed MALDI-MSI to examine the potentiating effect of bevacizumab on paclitaxel-mediated anticancer activity and succeeded in establishing 3D distribution of the two antitumor agents in tumor sections (Cesca et al., 2016).

The information about fixation-related effects on MSI of metabolomics and drug disposition is limited. Dannhorn and coworkers assessed the changes in endogenous metabolome and xenobiotics in rat liver and kidney tissues using DESI- and MALDI-MSI. DESI does not require a complicated pretreatment process. The mass spectra of small molecules, such as amino acids, organic amines, lipids, and fatty acids, can be directly detected on freshly frozen sections. However, the technique does not work well for formalin-fixed paraffin-embedded samples, due to low sensitivity. The MALDI-based technique has the ability to analyze exogenous substances and metabolites as well as endogenous lipids and proteins with high sensitivity. Little difference in sensitivity of detection is obtained between the analysis of formalin-fixed samples and that of freshly frozen samples. No significant changes in the sensitivity to detect diphenhydramine, dexamethasone, and terfenadine distributed in liver and kidney were observed in formalin fixed and freshly frozen organs obtained from animals after the treatment of the antihistamine agents (Dannhorn et al., 2022). However, freshly frozen tissues are recommended for imaging the distribution of low abundant analytes, such as metabolites of losartan, terfenadine, and fexofenadine (Schnackenberg et al., 2022).

Seneviratne and coworkers successfully imaged the distribution of tenofovir and its active metabolites in colorectal biopsies in healthy volunteers, using MALDI-MSI (Seneviratne et al., 2018). Later, they examined the spatial distribution of emtricitabine, tenofovir, efavirenz, and rilpivirine, alone with some endogenous biomolecules, in the heart, liver, spleen, kidney, and brain in mice treated with the four anti-human immunodeficiency virus agents and succeeded in detecting these antiretroviral agents in the organs analyzed (Seneviratne et al., 2020). Ntshangase and coworkers used MALDI-MSI combined with liquid chromatography-tandem mass spectrometry to assess the pharmacokinetics and spatial distribution of efavirenz, tenofovir, and emtricitabine in the brain in rats after intraperitoneal injection of the three agents. Tenofovir was mainly located in the cortex; emtricitabine was mainly distributed in the thalamus, corpus callosum, and hypothalamus; and efavirenz was found in the brain (Ntshangase et al., 2019).

### Diseases

**Tumors.** The metabolic reprogramming of carcinoma cells, a new hallmark of cancer, occurs during tumor growth and progression. Cancer cells independently alter their throughput by various metabolic pathways to meet the elevated bioenergetic and biologic synthetic demands and to reduce the oxidative stress required for cancer cell multiplication and survival. Besides the known cytologic and molecular resistance mechanisms, other factors can influence the distribution and concentration of chemotherapeutic agents in tumors (Dey et al., 2021). The metabolism of the tumor-immune microm environment by off-tumor cells, including endothelium, fiber, and immune cells, should be considered in targeting cancer therapy (Martinez-Reyes and Chandel, 2021; Stine et al., 2022).

Guenther and coworkers found higher levels of fatty acids and phospholipids in patients with breast tumors using the DESI-MSI technique. The metabolomic work attempted to define the association of endogenous substances, such as estrogens, progesterones, fatty acids, and phospholipids, with the tumor's gradings (Guenther et al., 2015). Abliiz's team identified abnormal expressions of six enzymes, including pyroline-5-carboxylate reductase 2, glutaminase, uridine phosphorylase 1, and survival. Besides the known cytologic and molecular resistance mechanisms, other factors can influence the distribution and concentration of chemotherapeutic agents in tumors (Dey et al., 2021). The metabolism of the tumor-immune microm environment by off-tumor cells, including endothelium, fiber, and immune cells, should be considered in targeting cancer therapy (Martinez-Reyes and Chandel, 2021; Stine et al., 2022).

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### Table 1

Characteristics of different mass spectrometry images

<table>
<thead>
<tr>
<th>Ion source</th>
<th>Scanning beam</th>
<th>Pressure regimen</th>
<th>Mass range (Da)</th>
<th>Spatial resolution (μm)</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic SIMS</td>
<td>Primary charged particles</td>
<td>Vacuum/LP</td>
<td>1–300</td>
<td>0.03–0.5</td>
<td>Elements and small atomic clusters (Debois et al., 2008; Kertesz and Van Berkel, 2008; Becker et al., 2010a)</td>
<td></td>
</tr>
<tr>
<td>Static SIMS</td>
<td>Primary charged particles</td>
<td>Vacuum/LP</td>
<td>100–1,500</td>
<td>0.5–50</td>
<td>Low molecular mass compounds (&lt;1,000 Da) (Hölscher et al., 2009; Bartels and Svatoš, 2015; Dong et al., 2016)</td>
<td></td>
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<tr>
<td>LDI</td>
<td>UV laser</td>
<td>Vacuum, IP, or AP</td>
<td>100–5,000</td>
<td>1–500</td>
<td>Monitoring of molecular species (containing a compatible chromosome with the laser used) (Korte et al., 2015; Shariatgorji et al., 2015; Bai et al., 2016; Sturtevant et al., 2016; Zhu et al., 2022)</td>
<td></td>
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<tr>
<td>MALDI</td>
<td>UV or IR laser</td>
<td>Vacuum, IP, or AP</td>
<td>300–50,000</td>
<td>1–100</td>
<td>All kinds of biologic samples (Small molecules, lipids, peptides, and proteins) (Korte et al., 2015; Shariatgorji et al., 2015; Bai et al., 2016; Sturtevant et al., 2016; Zhu et al., 2022)</td>
<td></td>
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<tr>
<td>DESI</td>
<td>Charged spray-jet</td>
<td>AP</td>
<td>100–5,000</td>
<td>40–200</td>
<td>Small molecules, lipids, and proteins (&lt;2000 Da) (Tillner et al., 2017; Pierson et al., 2020)</td>
<td></td>
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<tr>
<td>Nano-DESI</td>
<td>Liquid bridge</td>
<td>AP</td>
<td>100–10,000</td>
<td>10–100</td>
<td>Same as DESI (Moore et al., 2014; Unsihuay et al., 2021)</td>
<td></td>
</tr>
<tr>
<td>LAESI</td>
<td>IR laser</td>
<td>AP</td>
<td>1–66,000</td>
<td>100–300</td>
<td>Small and large molecules (Bartels and Svatoš, 2015, 2022; Kalkarni et al., 2018)</td>
<td></td>
</tr>
<tr>
<td>LA-ICP</td>
<td>UV laser</td>
<td>AP</td>
<td>7–250</td>
<td>&lt;10</td>
<td>Elements, trace metal detection and quantification (Becker et al., 2010b; Wattrous and Dorrestein, 2011; Chen et al., 2022)</td>
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AP, atmosphere pressure; Da, Dalton; IP, intermediate pressure; LP, low pressure.
histidine decarboxylase, fatty acid synthase, and ornithine decarboxylase in 256 patients with esophageal squamous cell carcinoma by use of AFADESI-MSI (Fig. 4) (Sun et al., 2019). The spatial integration of distinguished enzymes and the corresponding downstream metabolites would facilitate the understanding of tumor metabolism and discovery of new metabolic pathways.

Recently, MSI technologies combined with MALDI, SIMS, and DESI have been quickly applied in animal and clinical studies of breast, kidney, ovarian, prostate, colon, brain, lung, thyroid, skin, pancreatic, and esophageal cancers. Similarly, rapid development has been achieved in metabolomic (e.g., lipids, fatty acids, amino acids, and glucose) imaging of various tumors (Cho et al., 2017; Inglese et al., 2017; Jirasko et al., 2017; Zhang et al., 2017; Bluestein et al., 2018; Paine et al., 2019; Zang et al., 2021). These techniques may be beneficial for the development of biomarkers and metabolic pathway discovery. High-resolution MSI is an invaluable tool for metabolomic analysis of cancer tissues, recording the differential production of biomolecules, and specifying the spatial distribution within the samples, which facilitates precise treatment of tumors.

**Other Diseases.** MSI has been used to identify disease-related pathologic features, diagnose diseases, and evaluate the effectiveness of intervention. The technique is employed in neurologic diseases, such as Alzheimer's disease (Cruz-Alonso et al., 2019), Parkinson's disease (Shariatgorji et al., 2014), Huntington's disease (Hunter et al., 2018), multiple sclerosis (Maccarrone et al., 2017), amyotrophic lateral sclerosis (Hanrieder et al., 2013), and frontotemporal dementia (Agrawal et al., 2022). Kaya and coworkers observed colocalized lipids such as ceramide, sulfatides, phosphatidylinositol, and lysophosphatidylcholine with plaque-associated Aβ subtypes in the hippocampal region of Alzheimer's disease transgenic mice using MALDI-MSI (Kaya et al., 2017). These findings suggested a definite link between amyloid accumulation and lipid metabolism alterations in response to oxidative stress, inflammation, demyelination, and the death of cells. The MSI technique is also used in the study of rheumatic immune diseases, and images of articular cartilage, synovium, and bone have been successfully constructed. This facilitates the better understanding of joint destruction and further characterizing and diagnosing of osteoarthritis, rheumatoid arthritis, and osteoporosis (Rocha et al., 2017) as well as developing predictive biomarkers for these rheumatic immune diseases. The technology is also employed in kidney and aging-related diseases, providing detailed diagnostic information by recording ion imaging of the spatial distribution of endogenous molecules and proteins as well as exogenous substances in specimens and by distinguishing the differential distribution of those small and large molecules in humans and experimental animals (Abbas et al., 2019; Rossiter et al., 2022).

**Omics**

**Metabolome.** Grove and coworkers investigated the mechanistic action of the hepatotoxicity of amodiaquine, a well-known antimalarial agent, using MALDI-MSI. Depletion of glutathione, along with the production of amodiaquine-derived glutathione conjugate, was visualized in the central lobular region of the liver in rats given amodiaquine. Additionally, accumulated parent amodiaquine was observed in the periportal area relative to the centrilobular area (Grove et al., 2019).
spatial imaging findings provided histologic details for the metabolic activation of amodiaquine responsible for the reported idiosyncratic hepatotoxicity. Bis-Choline tetra thiomybdate is a therapeutic agent for various cancers, Wilson’s disease, and multiple sclerosis. Foster and coworkers determined the distribution of the agent in different organs and tissues, such as suprarenal gland, liver, spleen, kidney, brain, and testis, using LA-ICP mass spectrometry, and they found the accumulation of molybdenum in these organs of animals receiving a high dose of the metal agent (Foster et al., 2022). The team also defined the correlation between the accumulation of molybdenum and its toxicities.

Resistance to clinic-associated bacterial specimens, including enterobacteriaceae, non-ferme strains, and other bacteria, has become an issue of concern. Florio and coworkers successfully developed several methods for rapid detection of metabolites of antibiotics associated with antimicrobial resistance using a combined MALDI-TOF mass spectrometry approach. In-depth studies of the most common methods include the understanding of the pharmacologic action of this particular candidate, the development of amodiaquine responsible for the reported idiosyncratic hepatotoxicity.

Metabolomics often refers to the collective study of endogenous small biomolecules within cells, biofluids, tissues, or organisms and of their interactions within a biologic system. It has been developed rapidly in recent decades and shown remarkable promise in multiple fields, such as chemistry, life sciences, and clinical medicine. In 2020, *Nature Methods* named “Tools for Metabolomics” as one of the methods to look forward to (Singh, 2020). Spatial multiomics, including spatial metabolomics, was named by *Nature* in 2022 as one of the top seven technologies of the year, with a greater emphasis on spatial distribution in the x, y, z axis. Integration of MSI technology with metabolomics is called spatially resolved metabolomics. The advantage is mainly to study the space distributional characteristics and spatiotemporal dynamics of biomolecules in tissue sections to visualize the overall metabolome in situ (Fox and Schroeder, 2020).

In 2015, He and coworkers combined AFADESI-MSI technology with metabolomics and proposed a new methodology for MSI with metabolomics (He et al., 2015). In the same year, Guenther and coworkers employed the DESI-MSI technique for the detection of endogenous substances to diagnose breast cancer (Guenther et al., 2015). These laid the groundwork for the application of spatially resolved metabolomics.

In 2019, Ahliz and coworkers established an entire body spatial resolution imaging approach for monitoring the distribution of YZG-331 and YZG-330, two isomeric sedative and hypnotic drug candidates, and their metabolites. The same technique was employed to image the changes in the allocation of neurotransmitters in the brain of rats administered individual epimers. They succeeded in defining the correlation between the distribution of each epimer in the brain versus stomach with their efficacy. Additionally, they found the changes in levels of glutamate and glutamine in the cerebral brain of rats (Fig. 5) (Luo et al., 2019) were consistent with the effectiveness of the two isomeric drug candidates. The team continued to mine the distribution of other drugs regarding spatial metabolomic dynamics and related pharmacologic properties (Zhang et al., 2020). Additionally, the team used AFADESI and MALDI to image the location of endogenous sugars, amino acids, nucleotides, fatty acids, lipids, vitamins, peptides, and metal ions associated with diabetic nephropathy across renal tissues in a rat model of diabetic nephropathy. Visualization of the improvement of metabolic disorders was observed in the animal model after 12-week administration of oral astragaloside IV (Wang et al., 2021).

**Fig. 4.** Six abnormally expressed metabolic enzymes in esophageal squamous cell carcinoma by AFADESI-MSI. (A–F) Quantification of immunohistochemistry signals of the six enzymes, including pyrroline-5-carboxylate reductase 2, glutaminase, uridine phosphorylase 1, histidine decarboxylase, ornithine decarboxylase, and fatty acid synthase in cancer, paired epithelium, and muscle tissues obtained from 256 esophageal squamous cell carcinoma patients. Reprinted with permission from Sun et al. (2019). Copyright © 2019 the author(s). Published by PNAS. (https://pubmed.ncbi.nlm.nih.gov/30559182).

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<th>PYCR2</th>
<th>GLS</th>
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Recently, He and coworkers determined the 3D distributional changes in metabolome resulting from the exposure to exogenous substances, such as drug candidates, toxicants, and herbal medicines. Those endogenous substances monitored included amino acids and their metabolites, lipids, neurotransmitters, and biomolecules associated with energy metabolism. Furthermore, the team succeeded in defining the correlation of those xenobiotics with multiple endogenous substances from the perspective of specific locations (Liu et al., 2020; Pang et al., 2021; Cong et al., 2022; Gao et al., 2022; Jiang et al., 2022; Liu et al., 2022). 3D-MSI has been used to visualize the distribution of pharmaceutical agents and enabled the establishment of the relationship between the structural heterogeneity of the microenvironment and the distribution of drugs within the tissues (Giordano et al., 2016). It is anticipated that more 3D studies will be performed with the development of sophisticated and advanced computational systems and bioinformatic methods.

Proteome. Spatial mapping of the distribution of proteins has become possible by using mass spectrometry. The method of mass spectrometry lies on incorporating ionizable metal attached to specific antibodies. Angelo’s team used a multiplexed ion beam imaging technique, a secondary ion mass spectrometry method for imaging antibodies labeled with isotopically pure elemental metals. They succeeded in spatially localizing estrogen receptor, progesterone receptor, c-calmodulin, Kif67 protein, vimentin, actin, keratin, and receptor tyrosine-protein kinase human epidermal growth factor receptor 2 stained with metal-coupled antibodies to analyze paraffin-embedded sections of human breast tumor tissues (Angelo et al., 2014). Giesen and coworkers employed imaging mass cytometry technology combined with a high-resolution laser ablation system and cytometry-TOF—a TOF LA-ICP-MS instrument mass cytometry—to visualize human mammary epithelial cells and human breast cancer samples by simultaneously imaging 32 proteins and their modifications at a cellular resolution of 1.0 μm (Giesen et al., 2014).

Rendeiro and coworkers analyzed 36 proteins expressed in cultured primary pneumocytes and lung tissues obtained from patients with COVID-19 and constructed spatial distribution of these proteins by mass spectrometric cytometry with high Query String imaging. The success provided comprehensive and spatial pictures of the human lung responses to the viral infection from macro to single-cell levels. Specifically, infiltrated monocytes with the expression of interleukin-1 beta were found in the lung of patients with early COVID-19 infection, while high degrees of inflammation, macrophage infiltration, complement activation, and fibrosis were observed in the lungs of individuals with COVID-19 late stages. The findings not only provided structural, immunologic, and clinical insights into lung pathology but also plotted a landscape of lung pathologic changes (Rendeiro et al., 2021).

Hamidi and coworkers used MALDI-TOF mass spectrometry to distinguish bacteria Brucella abortus from Brucella melitensis. Additionally, significant protein mass signals were successfully identified for ribosomal and structural proteins for each vaccine and virulent strain represented by the corresponding biomarker peaks (Hamidi et al., 2022). Mass spectrometry-based spatial proteomics can determine the nature of proteoform and subcellular localization, which enables a better understanding of the complexity of protein morphology, along with the significance of exploring unknown functions (Lundberg and Borner, 2019).

Single Cell. Single-cell analysis is a single-cell-based study of genomics, transcriptomics, proteomics, metabolomics, and cell–cell interactions (Wang and Bodovitz, 2010; Merouane et al., 2015). Single-cell spatial imaging may be used to detect the colocalization of the distribution of specific molecular species, such as particular lipids and proteins, and to define the association with morphologic characteristics of tissue sections, both of which are crucial for molecular pathology and cancer treatment (Rappez et al., 2021). Small-size cells require high resolution for imaging. Due to analyze delocalization and degradation, matrix crystal size, laser focusing limitation, and detector sensitivity are hurdles for spatial resolution (Ščupáková et al., 2020). The latest SpaceM, an open-source system for in situ single-cell metabolomics based on MALDI and light microscopy, can detect more than 100 molecules per hour in > 1,000 individual cells with fluorescent readouts and morpho spatial features. The developed technique is currently applied to identify the metabolic state within genetic cell lines, such as differentiated human hepatocytes (Rappez et al., 2021).
Yuan and coworkers developed a spatial single nuclear metabolomics approach, a multiscale spatial resolution platform combining wet experiments with computational algorithms, to characterize metabolic intra- or intercellular features. This technique allowed them to visualize the tissue architecture of cultured primary hepatic cells and to distinguish hepatocytes, Kupffer cells, and endothelial cells of mouse liver sections by monitoring various biomolecules responsible for those individual types of cells. Furthermore, the technique enabled them to image the elevated transmembrane transport of amino acids in human fibrotic liver tissue (Yuan et al., 2021).

Single-cell imaging is a growing technology in biomedical sciences in three aspects, including single-cell microscopy combined with microfluidics, MSI for subcellular compound localization, and imaging mass cytometry. The operations of the approach not only require centralized identification and maintenance of cell culture conditions while being able to receive high-resolution imaging for extended periods of time but also necessitate the development of new and advanced equipment (Skyllaki et al., 2016). The challenges of single-cell imaging include the complexity of data acquired and the requirement of more advanced computational analysis and bioinformatic methods for data processing, due to their multivariate nature (Pomerantz et al., 2019).

Conclusion and Perspectives

The MSI technique provides label-free and in situ imaging with high coverage and wide detection ranges. This relatively new analytical approach has been widely employed in biomedical research and clinical practice. The imaging with spatial information is achieved by mass spectrometry-based detection of various endogenous and exogenous substances. The endogenous substances include amino acids, peptides, proteins, neurotransmitters, lipids, and other biomolecules (Goodwin et al., 2008; Luan et al., 2019; Denti et al., 2020; Nachtigall et al., 2020). Varieties of exogenous chemicals, such as pharmaceutical agents (their metabolites), environmental pollutants, toxicants, natural products, heavy metals, and others, have been analyzed for molecular imaging. The most important and advantageous features of the technology is that the mass spectrometry-based molecular imaging allows us to establish spatial distribution and accumulation of the detected molecules in a variety of organisms. Those mass analyzers include MALDI, SIMS, DESI, LAESI, LA-ICP, and their combinations. These applications can certainly benefit life sciences in the future. However, these techniques are currently limited by the low spatial resolution of DESI and even MALDI. The instrument conditions, sample preparation, and data processing all need to be improved (Heeren et al., 2009). In particular, increases in detection sensitivity and resolution will be the focus of immediate efforts in the future (Hou et al., 2022).

Quantitation is an important issue for any analytical approach. Unfortunately, limited iteration of absolute quantitative MSI is available so far. During the sample processing phase, an authentic standard of known concentration is often deposited onto an adjacent untreated control sample that is being processed and analyzed simultaneously, allowing the estimation of absolute quantification. This method was applied to determine the abundance of tiotropium bromide in rat lung tissue sections (Goodwin et al., 2012). Relative quantification relies on internal standards or selected endogenous molecules in samples to obtain relative changes in the concentration of other molecules in different samples. This approach is commonly used for the nontargeted analysis of systematic phenotypic changes under different conditions (Unsihuay et al., 2021).

The rapid development of metabolomics provides room for the improvement of spatial metabolomics imaging. This would allow us to visualize the metabolome in situ to picture the spatial distribution of characteristic biomolecules and to construct a more accurate foundation for biochemical processes in the whole living species (Fox and Schroeder, 2020). Tumor heterogeneity and its microenvironment are biologically responsible for tumor staging, grading, and classification. Spatial metabolomics provides a novel approach not only to precisely diagnose tumors by identifying tissue metabolic heterogeneity and cellular microenvironment but to provide new strategies for targeting treatment of cancers. Molecular imaging also offers a powerful tool for the determination of the spatial distribution of pharmaceutical agents and their metabolites, dramatically facilitating new drug discovery (Miao et al., 2018; Vitale et al., 2021; Wang et al., 2022; Zhang et al., 2023).

The disposition of medicinal herbs is more complicated than that of a pure pharmaceutical agent. Little progress has been achieved in the application of MSI to spatial mapping distributed chemical components in animals after exposure to traditional Chinese medicines. The MSI technique is certainly able to provide spatial qualitative and quantitative identities of multiple chemical components distributed in tissues, organs, and whole body in animals given herbal medicines. Such a technique would facilitate the understanding of mechanisms of therapeutic and toxic actions of medicinal herbs with increasing consumption.

Single-cell imaging, 3D targeted imaging, and quantitative metabolomics will play an increasingly important role in future standardization and translation for clinical practice (Chen and Abliz, 2017; Blutke et al., 2020; Zhou et al., 2020), and the merge of multidimensional mass spectrometry, biochip, artificial intelligence, and other technologies will promote the development of life sciences and other related fields (Li et al., 2022).

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Wrote or contributed to the writing of the manuscript: Chen, Liu, X. Li, He, W. Li, Peng, Zheng.

References


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