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Advances in mass spectrometry imaging coupled to ion mobility spectrometry for enhanced imaging of biological tissues

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Tissues present complex biochemical and morphological composition associated with their various cell types and physiological functions. Mass spectrometry (MS) imaging technologies are powerful tools to investigate the molecular information from biological tissue samples and visualize their complex spatial distributions. Coupling of gas-phase ion mobility spectrometry (IMS) technologies to MS imaging has been increasingly explored to improve performance for biological tissue imaging. This approach allows improved detection of low abundance ions and separation of isobaric molecular species, thus resulting in more accurate determination of the spatial distribution of molecular ions. In this review, we highlight recent advances in the field focusing on promising applications of these technologies for metabolite, lipid and protein tissue imaging.

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Introduction

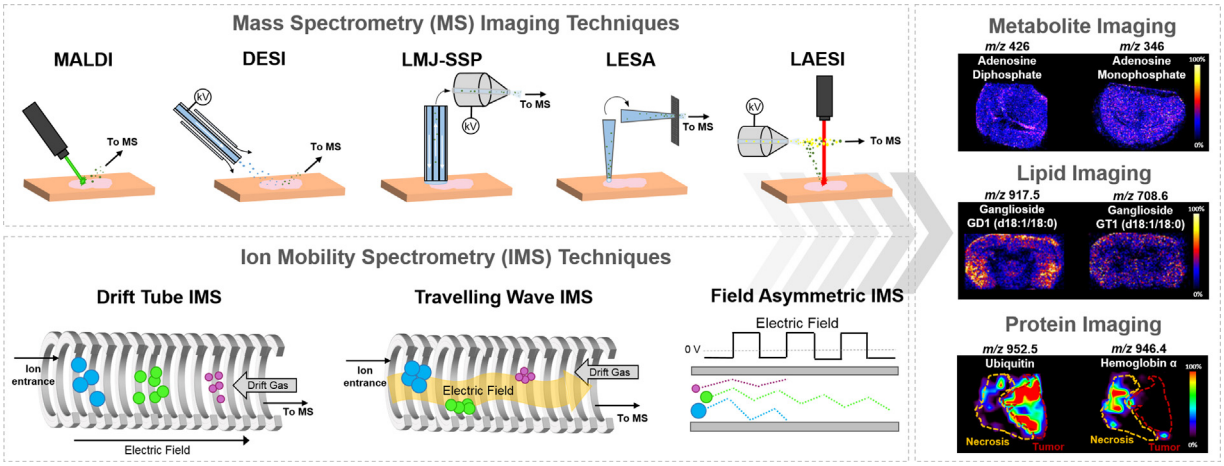
Imaging technologies provide transformative capabilities to investigate and visualize the complexity and cellular heterogeneity of biological systems [1]. Spatial and molecular complexity of biological systems are intrinsically associated to many physiological processes related to normal organ development as well as aberrant disease progression [2–4]. Cancer initiation and promotion, for example, involve multistep cellular and genetic changes that result in complex tumorigenic state and tissue features [5]. Molecular imaging technologies including MS imaging are key approaches for investigating complex biological tissues, allowing spatial characterization of molecular components associated to heterotypic interactions exhibited by different cell types and their diverse microenvironment [6,7,8•].

MS imaging allows untargeted analysis of hundreds of molecular species directly from a tissue sample, providing direct spatial correlation between their abundances and histological features. Molecular species are chemically identified based on high mass accuracy measurements of their mass-to-charge ratios (m/z), isotopic distributions, and tandem MS fragmentation patterns [9,10]. The speed, sensitivity, and specificity of MS imaging techniques have been widely explored for in depth investigation of the cellular environment of healthy and diseased thin tissue sections [11–13]. Despite its powerful analytical capabilities, direct tissue analysis by MS imaging presents challenges including matrix interferences and chemical noise, which can hinder detection and identification of less abundant molecular species. Moreover, the inability of MS imaging techniques to separate isomeric molecular ions prior to mass analysis can lead to inaccurate assessment of their spatial distribution within the tissue samples. In an attempt to address these challenges, gas-phase ion mobility spectrometry (IMS) technologies have been integrated into MS imaging workflows, allowing rapid separation of molecular ions post ionization and prior to mass analysis. In this review, we provide a brief overview of MS imaging and IMS techniques, focusing on recent advances coupling these approaches for enhanced imaging of metabolites, lipids and proteins (Figure 1). Promising applications to tissue imaging are highlighted to demonstrate the value of these methods in biomedical research.

Mass spectrometry imaging techniques

MS imaging techniques provide a range of analytical and imaging capabilities for biological tissue imaging. Secondary ion MS (SIMS) was the first ionization technique introduced for surface imaging [14]. However, the molecular fragmentation processes inherent to SIMS analysis at that time hindered biomolecule analysis for tissue imaging [15,16]. Following SIMS, matrix assisted laser desorption/ionization (MALDI) was developed for surface analysis, enabling softer ionization of molecular ions and imaging of intact biomolecules with molecular weight up to thousands of Daltons. MALDI–MS typically utilizes a UV laser beam to transfer energy to a matrix-embedded analyte surface, desorbing molecules into the gas phase and promoting molecular ionization [17].

Figure 1



Schematic representation of the MS imaging and IMS techniques that have been integrated for enhanced imaging of metabolites, lipids and proteins from biological tissues. MS imaging techniques highlighted include MALDI, DESI, LMJ-SSP, LESA and LAESI. IMS techniques include DTIMS, TWIMS and FAIMS. 2D ion images of metabolites (adenosine diphosphate and adenosine monophosphate), lipids (Gangliosides GD1 (d18:1/18:0) and GT1 (d18:1/18:0)), and proteins (ubiquitin and hemoglobin α) obtained using MS imaging and IMS are shown [26•,28••,56••].

MALDI has been extensively used for biological tissue imaging of metabolites, lipids and proteins with spatial resolution ranging from ~5 to 200 μm [18–24], and has been integrated with IMS for biological applications [25••,26•,27,28••,29–33,34•,35••,36]. The development of cluster SIMS allowed softer ionization of molecular ions and thus its application for imaging of intact metabolites and lipids from biological samples [16,37,38], with sub-micrometer spatial resolution [39]. Nevertheless, no approaches to couple SIMS imaging with IMS have been reported.

Ambient ionization MS techniques were introduced in 2004 with the development of desorption electrospray ionization (DESI) [40]. Ambient ionization MS enables real-time and in *situ* analysis of samples at atmospheric pressure conditions with minimal to no-sample preparation, and was thus rapidly adapted for imaging applications [41]. DESI utilizes a spray of charged solvent droplets to desorb and ionize analyte molecules from a sample surface, allowing efficient analysis of metabolites and lipids from biological samples [40]. The demonstration of DESI-MS for tissue imaging in 2006 drove its use in biomedical research [42], and it is now broadly used for tissue imaging with a typical spatial resolution of 150 μm [43••,44,45–48]. The increased application of DESI led to the development of tens of other ambient ionization MS techniques, many of which have been successfully adapted for tissue imaging [49]. Among these, liquid extraction based techniques, such as liquid extraction surface analysis (LESA) [50], liquid microjunction surface sampling (LMJ-SS) [51], and nanoDESI [52], employ a solvent 'liquid microjunction' that interacts with a sample surface to extract molecules in a pulsed or continuous approach [53•]. Extracted molecules are re-aspirated, ionized and introduced into the mass spectrometer by electrospray ionization. LESA, LMJ-SSP and nanoDESI have been used for biological tissue imaging providing moderate to high spatial resolution (500–1000 μm, 630 μm and 15 μm, respectively) [54••,55,56••]. Other ambient ionization MS techniques have employed hybrid methods for desorption and ionization. Laser desorption electrospray ionization (LAESI), for example, combines a laser pulse to ablate material from a tissue surface and ESI to intercept the resulting plume for ionization [57].

Ion mobility spectrometry

IMS technologies are well suited for coupling with MS imaging techniques as they provide high-throughput separation capabilities (milliseconds), without significant increase in typical imaging analysis time (<1 s/pixel), which is infeasible with other separation techniques such as liquid-chromatography (LC) approaches (minutes to hours). IMS are used for gas phase separation of molecular ions based on their size, shape, and charge within an electric field, also defined as their 'mobility' (K) [58]. The most established form of IMS, named drift tube ion mobility spectrometry (DTIMS) employs a constant low electric field used to propel ions through a drift tube cell, while a high pressure buffer gas (typically helium or nitrogen) flows in the opposite direction of the ions, slowing their acceleration [59•]. The number of collisions an ion experiences, dictated by its collisional cross section (CCS), influences its velocity through the drift cell, thus allowing separation from other ions. Besides DTIMS, traveling wave ion mobility spectrometry (TWIMS) is a widespread IMS technology that also employs an electric field to drive ions through a traveling wave ion guide.