Ambient Mass Spectrometry Imaging and Its Applications

Subjects: Chemistry, Applied

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Ambient mass spectrometry imaging (AMSI) has attracted much attention in recent years. As a kind of unlabeled molecular imaging technique, AMSI can enable in situ visualization of a large number of compounds in biological tissue sections in ambient conditions. In this review, the developments of various AMSI techniques are discussed according to one-step and two-step ionization strategies. In addition, recent applications of AMSI for lipid and metabolite analysis (from 2016 to 2021) in disease diagnosis, animal model research, plant science, drug metabolism and toxicology research, etc., are summarized. Finally, further perspectives of AMSI in spatial resolution, sensitivity, quantitative ability, convenience and software development are proposed.

ambient mass spectrometry imaging (AMSI) lipid metabolite

1. Introduction

Mass spectrometry imaging (MSI) is a powerful analytical method, which is able to visualize the spatial distribution of a large number of compounds from the complex sample surface in a single experiment [1]. Generally in MSI experiments, the sample sections should be carefully prepared and then scanned and ionized by various desorption/ionization methods. The ion intensity of each individual compound at the target mass-to-charge ratio (m/z) are extracted from each pixel's mass spectrum and combined into a heat map revealing the relative distribution of that compound throughout the sample surface. Compared with conventional tagprobe labeling optical imaging methods, MSI enables the un-targeted imaging of multiple compounds without the need for labeling.

Ambient mass spectrometry refers to those ionization techniques operated in an atmospheric environment with little or no sample preparation [2][3]. It was firstly introduced by Cooks et al. in 2004 with the invention of desorption electrospray ionization (DESI) [4]. Due to its high sensitivity, high speed and easy operation at native conditions, ambient mass spectrometry was widely used in MSI, and ambient mass spectrometry imaging (AMSI) has been developed to be an important branch of MSI. In AMSI, compounds are desorbed from the sample surface at ambient conditions, ionized by charged microdroplets, photons or plasma, and then introduced into the mass spectrometer for further detection. Up to now, AMSI techniques based on different ionization methods have been proposed for the improvement of sensitivity and spatial resolution, and they have been widely applied in disease diagnosis, drug metabolism, toxicology research, forensic investigation and plant science [5][6][7][5–7]. Lipid and metabolite are the small-molecule entities that have key roles for the establishment of physiological function within the biological systems. The MSI of a global lipid and the metabolite profile from a biological tissue can help with an enhanced understanding of disease molecular mechanisms, the discovery of biomarkers and the elucidation the

mechanisms of drug action[8].

Several excellent reviews on different topics of AMSI have been reported. For example, Xue et al. summarized AMSI techniques from the aspects of ion source devices, ionization mechanism, resolution, sensitivity and applications in 2019 [9]. Xiao et al. introduced the important applications of the AMSI technique in pharmacology, drug metabolism, clinical diagnosis and toxicological evaluation in 2020 [10]. In this review, we will summarize the developments of AMSI technologies according to one-step/two-step ionization strategies and their application advances in lipid and metabolite from 2016 to 2021. In addition, the prospects of AMSI techniques and their applications for biological samples in the near future are discussed. Figure 1 shows the schemes of AMSI for lipid and metabolite analysis in this work.

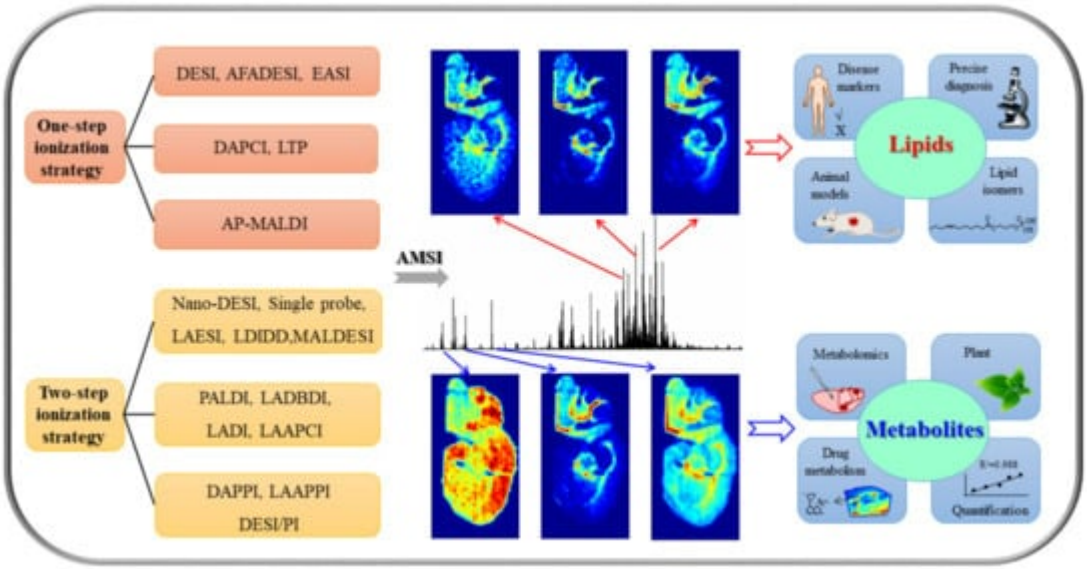

Figure 1. The schemes of ambient mass spectrometry imaging for lipid and metabolite analysis. DESI: desorption electrospray ionization; AFADESI: airflow-assisted desorption electrospray ion-ization; EASI: easy ambient sonic spray ionization; DAPCI: desorption atmospheric pressure chemical ionization; LTP: low-temperature plasma; AP-MALDI: atmospheric pressure matrix-assisted laser desorption/ionization; Nano-DESI: nanospray desorption electrospray ionization; LAESI: laser ablation electrospray ionization; LDIDD: laser desorption/ionization droplet delivery; MALDESI: matrix-assisted laser desorption electrospray ionization; PALDI: plasma-assisted laser ionization; LADBDI: laser ablation dielectric barrier discharge ionization; LADI: laser ablation direct analysis in real time; LAAPCI: laser ablation atmospheric pressure chemical ionization; DAPPI: desorption atmospheric pressure photoionization; LAAPPI: laser ablation atmospheric pressure photoionization; DESI/PI: desorption electrospray ionization/postphotoionization.

2. Development of AMSI Techniques

In AMSI, the process of target analytes on the sample surface being desorbed and simultaneously ionized is called the one-step ionization strategy, whereas when the desorbed analytes are post-ionized by another ionization source this is called the two-step ionization strategy.

2.1. One-Step Ionization Strategy

2.1.1. Desorption Electrospray Ionization (DESI)

2.1.2. Desorption Atmospheric Pressure Chemical Ionization (DAPCI) and Low-Temperature Plasma (LTP)

2.1.3. Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization (AP-MALDI)2.2. Two-Step Ionization Strategy

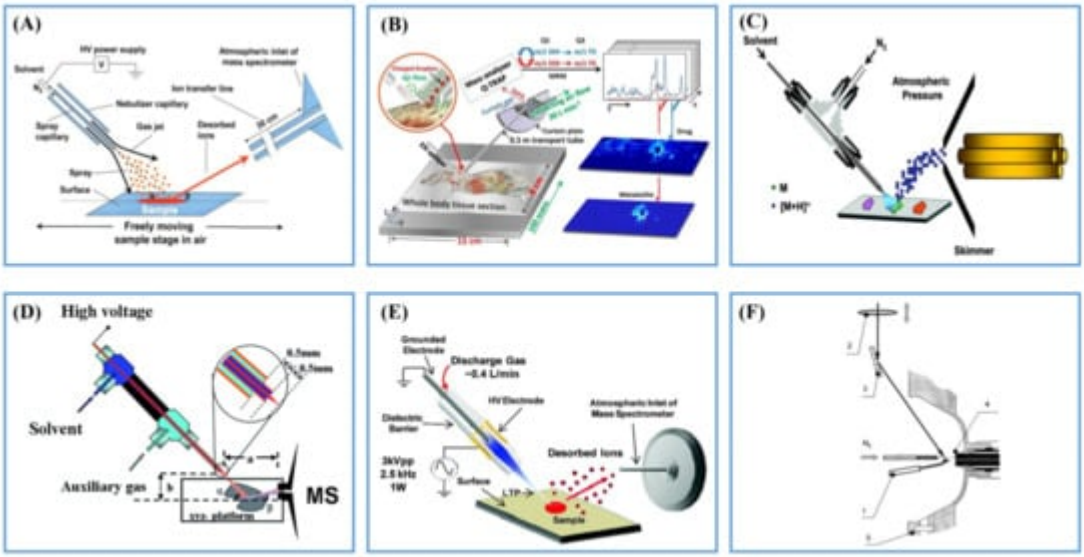

Figure 2. (**A**) Scheme of DESI ion source. Reprinted with permission from the authors of[4]. Copyright (2004) American Association for the Advancement of Science. (**B**) Scheme of AFADESI ion source. Reprinted with permission from the authors of [11]. Copyright (2013) American Chemical Society. (**C**) Scheme of EASI ion source. Reprinted with permission from the authors of [12]. Copyright (2008) American Chemical Society. (**D**) Scheme of DAPCI ion source. Reprinted with permission from the authors of [13]. Copyright (2017) Royal Society of Chemistry. (**E**) Scheme of LTP ion source. Reprinted with permission from the authors of [14]. Copyright (2008) American Chemical Society. (**F**) Scheme of AP-MALDI ion source. Reprinted with permission from the authors of [15]. Copyright (2000) American Chemical Society.

In the two-step ionization strategy, desorption and ionization of analytes are separated into two steps: (1) generating analyte-containing droplets/particles/gastification products from target samples; (2) post-ionizing the desorbed neutral species. The first step is normally fulfilled by thermal desorption, laser desorption and droplet pick-up, etc. As is well known to us, some compounds can be ionized during the initial laser desorption and microdroplets pick-up processes. However, due to the matrix effect in the microenvironment of biological tissues, most of the desorbed molecules are not ionized [16]. In the second step, the desorbed neutral species can be post-ionized by using charged microdroplets, plasma or photons in ambient conditions.

2.2.1. Post-Ionization by ESI

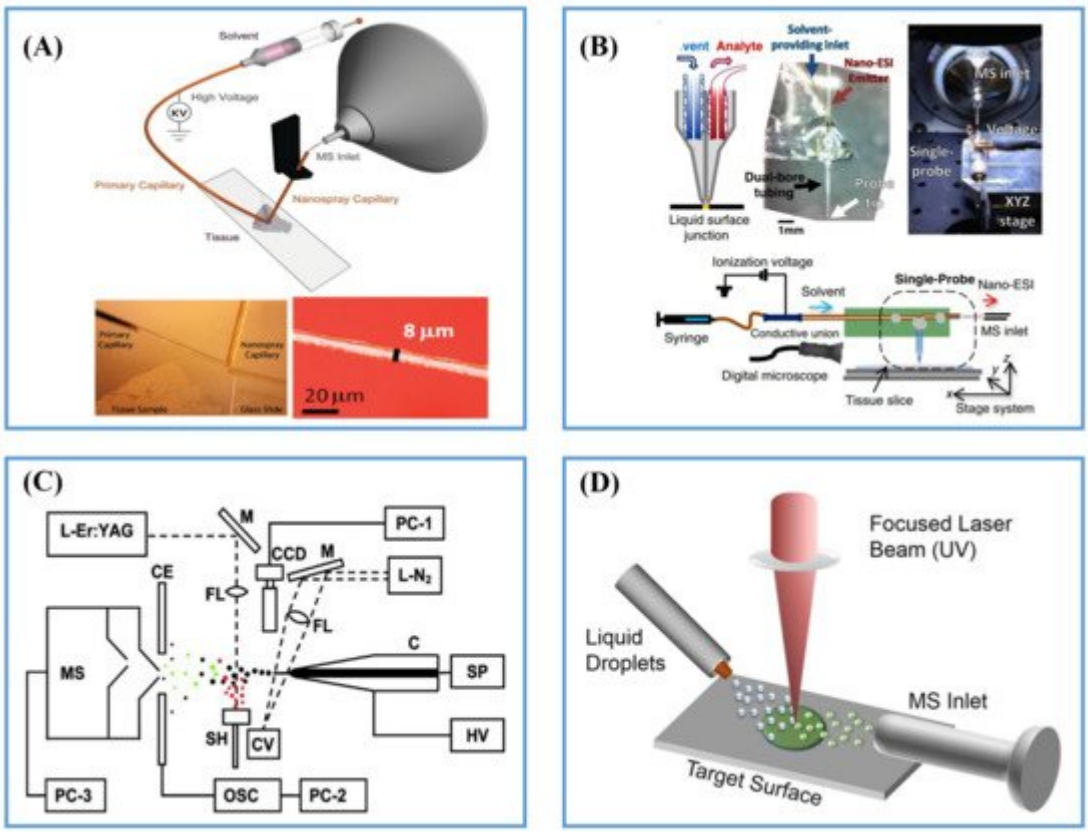

Figure 3. (**A**) Scheme of nano-DESI ion source. Reprinted with permission from the authors of [17] Copyright (2012) American Chemical Society. (**B**) Scheme of single probe ion source. Reprinted with permission from the authors of [18]. Copyright (2015) American Society for Mass Spectrometry. (**C**) Scheme of LAESI ion source. Reprinted with permission from the authors of [19]. Copyright (2007) American Chemical Society. (**D**) Scheme of LDIDD ion source. Reprinted with permission from the authors of [20]. Copyright (2016) American Chemical Society.

2.2.2. Post-Ionization by Plasma Ionization

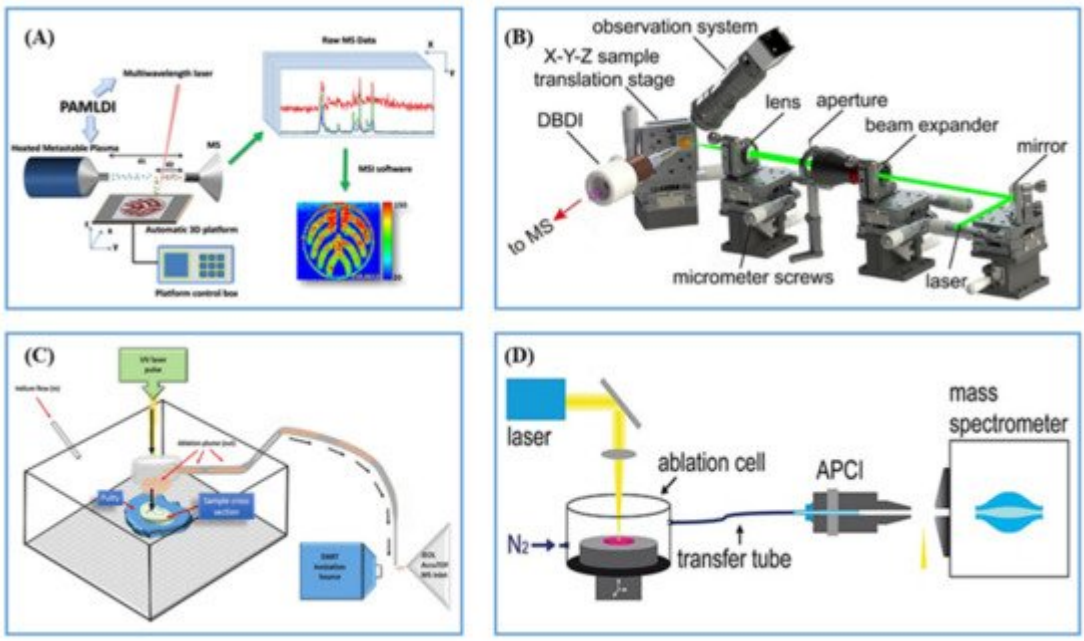

Figure 4. (**A**) Scheme of PALDI ion source. Reprinted with permission from the authors of [21]. Copyright (2014) American Chemical Society. (**B**) Scheme of LADBDI ion source. Reprinted with permission from the authors of [22]. Copyright (2021) American Chemical Society. (**C**) Scheme of LADI ion source. Reprinted with permission from the authors of [23] Copyright (2017) American Chemical Society. (**D**) Scheme of LAAPCI ion source. Reprinted with permission from the authors of [24]. Copyright (2013) John Wiley and Sons Ltd.

2.2.3. Post-Ionization by Photoionization (PI)

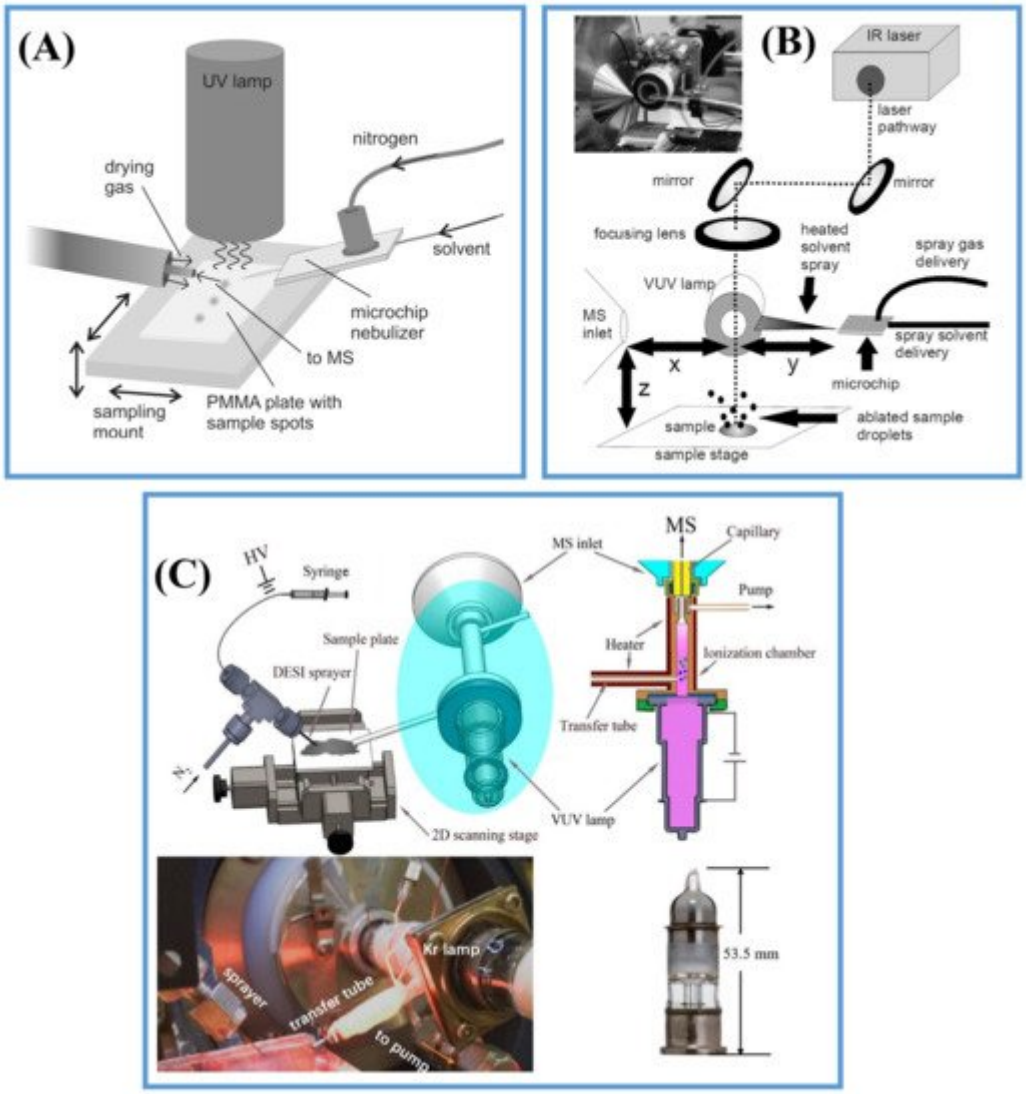

Figure 5. (**A**) Scheme of DAPPI ion source. Reprinted with permission from the authors of[25]. Copyright (2007) American Chemical Society. (**B**) Scheme of LAAPPI ion source. Reprinted with permission from the authors of [26]. Copyright (2012) American Chemical Society. (**C**) Scheme of DESI/PI ion source. Reprinted with permission from the authors of[27]. Copyright (2019) American Chemical Society.

3. Applications in Lipids and Metabolites

3.1. Lipids

Lipids are components of the cell membrane, and they play a vital role in cell membrane fluidity, neurotransmitter transmission and transport and energy supply[28][29] Lipid compositions can reflect histological type and cell growth state; hence, the alteration of lipid metabolism is linked to the occurrence of several human diseases[30], such as Alzheimer's disease, breast cancer [31] and basal cell carcinoma [32]. Lipidomic analysis can provide valuable information for understanding the molecular pathological mechanisms of many diseases, diagnosis and

differentiation of diseases and assessment of resection margins during clinical surgery, etc.[6][33][34][35]. It should be noted that the spatial distribution of proteins could also be visualized by the fluorescent labeling method, termed immunohistochemistry and immunofluorescence, whereas few other technologies can image lipids [36]. In past years, it has been demonstrated that AMSI techniques can be performed to visualize the spatial distribution of the sample surface compounds in the native state, and that they have a high sensitivity to lipids and other small molecules in diseased tissues, animal models and plants, etc.

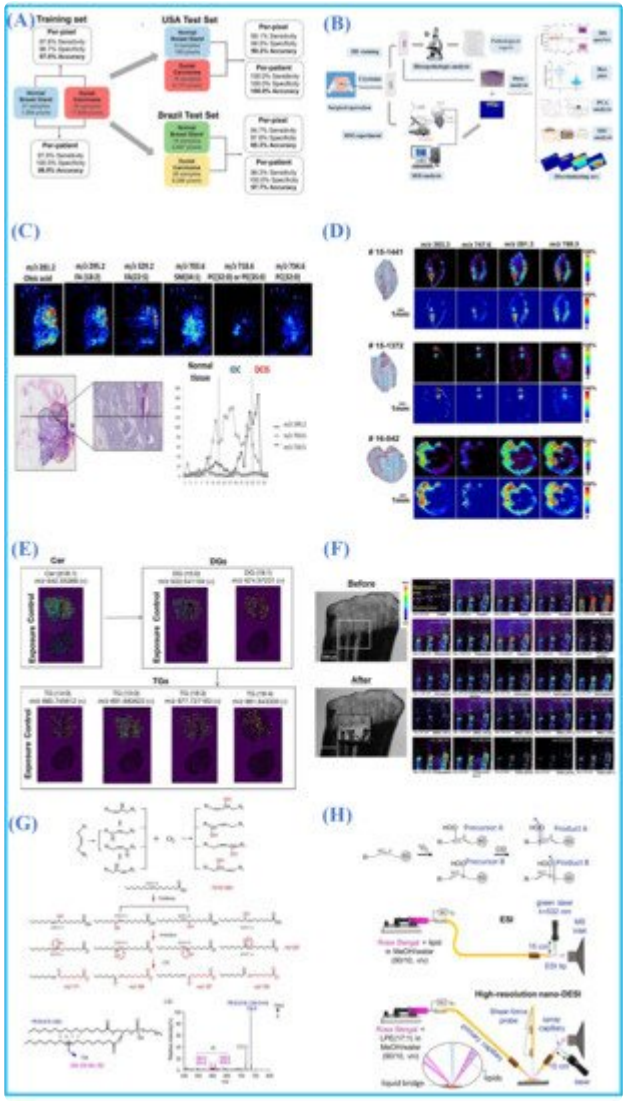

Figure 6. (**A**) Classification of per-pixel and per-patient prediction results from normal and IDC samples. Reprinted with permission from the authors of [37]. Copyright (2018) American Chemical Society. (**B**) The proposed workflow for diagnosis of melanocytic nevi. Reprinted with permission from the authors of [38]. Copyright (2021) Elsevier B.V. (**C**) Representative compounds MSI images and H&E staining of normal tissue, IDC and DCIS samples. Reprinted with permission from the authors of [39]. Copyright (2016) Springer Nature. (**D**) Selected 2D MSI ion images and H&E stained BCC and normal tissue in three specimens. Reprinted from [32] with the permission of (2018) National Academy of Sciences. (**E**) AP-MALDI MSI images of representative lipids from liver tissues of mice under cadmium exposure. Reprinted with permission from the authors of [40]. Copyright (2021) Elsevier B.V. (**F**) Representative MSI ion images and optical images of zebrafish caudal fin. Reprinted with permission from the authors of [41]. Copyright

(2018) American Chemical Society. (**G**) Mechanism of the oxidation of unsaturated lipids. Reprinted with permission from the authors of [42]. Copyright (2021) American Chemical Society. (**H**) Mechanism experiment setup of the online single oxygen reaction with lipids. Reprinted with permission from the authors of [43]. Copyright (2021) Wiley-VCH GmbH.

3.2. Metabolites

Molecular metabolites such as neurotransmitters, amino acids and vitamins play an important role in biosynthesis, energy production and supply, signal transduction and regulation and cognitive processes [44][45][46]. Changes in small molecules metabolites are often closely related to the nervous system and disease states, such as depression, Alzheimer's disease, movement disorders, being overweight, obesity and so on[47][48][49][50][51]. Therefore, a comprehensive and detailed understanding of the relative abundance and spatial distribution of small-molecule metabolites in organisms is an outstanding contribution to further understanding the metabolic reorganization of tumors, elucidating the metabolic mechanism in the process of disease development and searching for potential metabolic markers for disease diagnosis. As an unlabeled molecular imaging method, AMSI technique can obtain spatial distribution information of many small-molecule metabolites in a single experiment with little or without any pretreatment. For the imaging of labile metabolites in ambient conditions, the labile group could be protected via in situ chemical derivatization[52] .

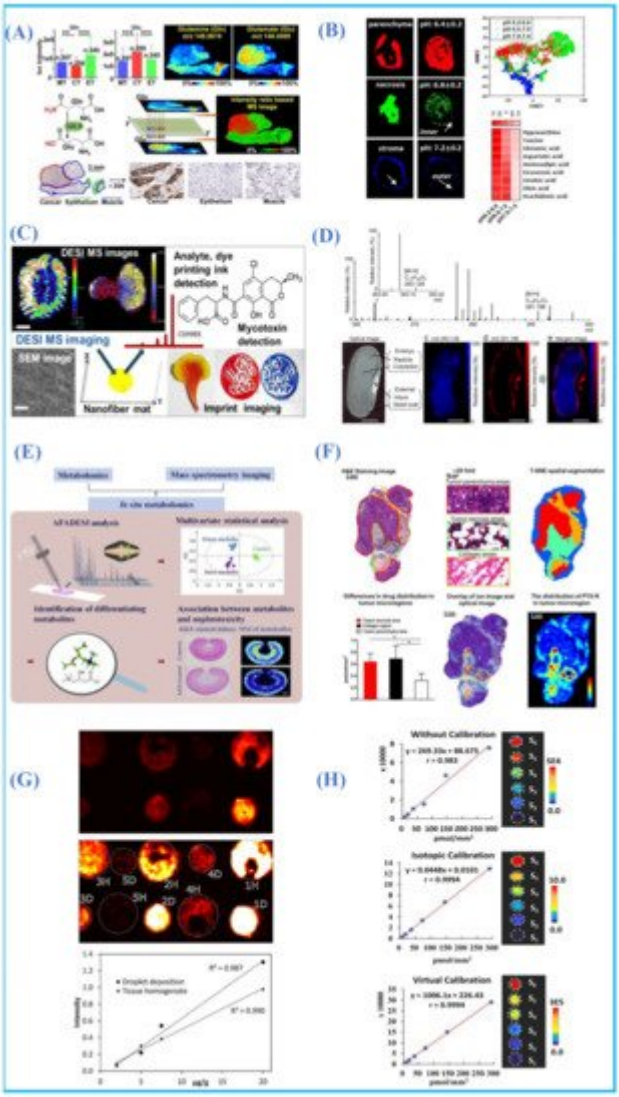

Figure 7. (**A**) In situ visualization of crucial metabolites and metabolic enzymes in the glutamine metabolism pathway. [53] Copyright (2019) National Academy of Sciences. (**B**) Images of the acidic TME and contributive acidic species. Reprinted with permission from the authors of [54]. Copyright (2021) American Chemical Society. (**C**) Electrospum nanofiber mats as “smart surfaces” for MSI and imprint imaging. Reprinted with permission from the authors of [55]. Copyright (2016) American Chemical Society. (**D**) MS spectrum, optical image and MSI imaging of immature phaseolus vulgaris L. seed. Reprinted with permission from the authors of [56]. Copyright (2017) Springer Nature. (**E**) In situ metabolomics in nephrotoxicity of aristolochic acids in rat kidneys. Reprinted with permission from the authors of [57]. Copyright (2020) Chinese Pharmaceutical Association and Inetitute of Materia Medica, Chinese Academy of Medical Science, production and hosting by Elsevier B.V. (**F**) Intratumoral distribution of PTX-R with heterogeneous characteristics. Reprinted with permission from the authors of [58]. Copyright (2020) the author(s) and published by Ivyspring International Publisher. (**G**) Calibration curves based on tissue homogenates and droplet deposition. Reprinted with permission from the authors of [59]. Copyright (2016) American Chemical Society. (**H**) Standard curves obtained with different calibration methods. Reprinted with permission from the authors of [60]. Copyright (2019) American Chemical Society.

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