Chapter 12
Technologies and Principles of Mass Spectral Imaging

Kevin A. Douglass, Demian R. Ifa, and Andre R. Venter

Abstract The field of imaging is rapidly becoming more of an analytical chemistry tool. Included in this is the capability of mass spectrometry (MS) to give anatomical and metabolic information. Mass spectrometry has also entered the imaging world as an analytical chemistry tool. This chapter will attempt to cover a variety of techniques using mass spectral approaches for metabolic imaging such as MALDI, SIMS, DESI, LAESI, AP-fs-LDI, LA-FAPA, PESI, and LMJ-SSP. Each of these will be covered with their respective physics as well as the mechanics of how to employ these in the drug and biologics development setting as alone or as alongside of other inclusive imaging modalities. The reader should also investigate Chap. 6 (Autoradiography; Solon and Moyer) in this volume for some of the classical approaches to metabolic imaging.

12.1 Introduction

Determining the distribution of drugs and drug metabolites in tissues is a necessity for drug discovery and development. Many kinds of studies rely on this information, including absorption, distribution, metabolism, and excretion (ADME), pharmacokinetic (PK), and toxicology studies. Traditionally, this information has been obtained using radiographic imaging methods such as whole-body autoradiography (WBA) and positron-emission tomography (PET). In these radiographic methods, the drug compound is given a radioactive label which is detected within the imaging

K.A. Douglass • A.R. Venter (✉)
Department of Chemistry, Western Michigan University, 1903 W Michigan Avenue, Kalamazoo, MI 49008-5413, USA
e-mail: andre.venter@wmich.edu

D.R. Ifa
Department of Chemistry, York University, 4700 Keele Street, Toronto, ON, M3J 1P3, Canada

B.R. Moyer et al. (eds.), *Pharmaco-Imaging in Drug and Biologics Development*, AAPS Advances in the Pharmaceutical Sciences Series 8, DOI 10.1007/978-1-4614-8247-5_12,

experiment. Recently, mass spectral imaging (MSI) has emerged as an attractive alternative.

MSI offers several advantages over WBA and PET. MSI does not require the use of compounds labeled with radioactive isotopes, which can be expensive, time-consuming to prepare, and have a short window of use. Since WBA and PET detect the decay of the radioactive isotope instead of molecules, they are unable to distinguish between the parent compound and its metabolites. MSI, on the other hand, can be used to determine the localization of the parent drug and its metabolites as well as other biologically relevant compounds present in the tissue sample, all in one imaging experiment. In addition, many MSI instruments are capable of high-accuracy mass measurements and/or MS/MS experiments, offering confident identification of drugs and their metabolites simultaneously with the imaging experiment without requiring subsequent LC-MS experiments.

12.1.1 What Is Mass Spectral Imaging?

MSI is a surface analysis technique in which the distribution of chemicals on a surface are determined to create a chemical image for one or more dimensions. Initially, the area to be imaged is divided into several smaller areas, or pixels. This process is illustrated in Fig. 12.1 where a sample image (a) is divided into a 10×10 grid of 100 pixels (b). Each individual pixel is then analyzed, and one mass spectrum becomes associated with it (c). After the entire area has been interrogated, an average mass spectrum of all the pixels can be created that represents all of the ions that were present on the total surface area of all pixels. Using imaging software, peaks in the mass spectrum can be selected to generate chemical distribution maps or chemical images based on their distribution on the surface. For instance, the simple image in Fig. 12.1a is comprised of three molecules, each of which is localized in a separate area of the image. By selecting to display an individual ion peak, a representative chemical image for the corresponding compound is created (d). These individual images can be overlaid to generate a single image of the chemicals on the surface (e).

12.1.2 Important Terms for Mass Spectral Imaging

12.1.2.1 Lateral (Spatial) Resolution

Lateral, or spatial, resolution is the minimum distance in which two distinct objects can be resolved on the surface, similar to optical resolution. A typical strategy to measure resolution is to compare features of known dimensions present on a sample, determined using microscopic methods, with the chemical image obtained by MSI. A sample is sometimes prepared solely for this purpose, with typical choices being a printed grid (Kertesz and Van Berkel 2008; Shelley et al. 2008;

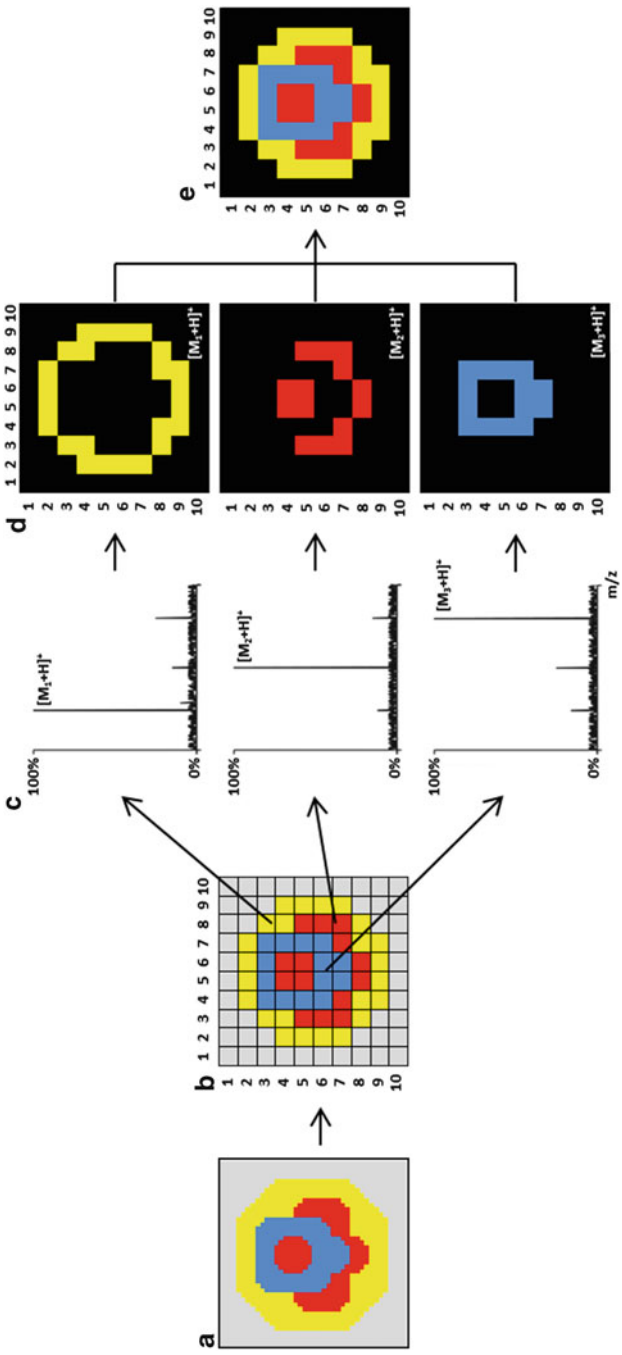

Fig. 12.1 An outline of the IMS experiment. An image (**a**) is initially divided into the desired number of pixels (**b**) and a mass spectrum is then obtained for each pixel (**c**). A chemical image can be created by plotting the intensity of a selected peak within each pixel (**d**), and these images can then be combined into a single image (**e**)

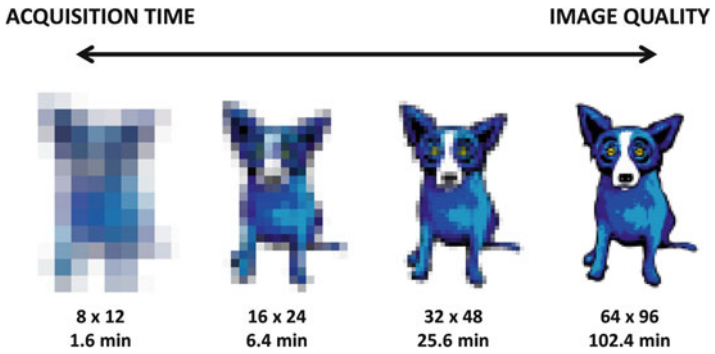

Fig. 12.2 Image quality is inversely related to acquisition time. It is best to choose the resolution that has the shortest acquisition time while still able to adequately answer the question at hand. The Blue Dog Series is a group of original paintings by George Rodrigue (Reproduced with permission of the copyright owner Rodrigue Studios, LLC)

Ovchinnikova et al. 2010; Laskin et al. 2012) or a set of lines with increasing width along one dimension (Ifa et al. 2007; Liu et al. 2010). In most cases, spatial resolution is limited by the probe dimensions, although a theoretical limit exists for the pixel size where the amount of material present in a pixel is below the detectable limit, in which case the pixel size is limited by the sensitivity of the mass spectrometer.

One factor to consider when increasing the pixel count by increasing the resolution is that acquisition time (AT) increases quadratically with pixel count as shown in Eq. (12.1), where ST is the scan time and X and Y are the vertical and horizontal dimensions:

$$\mathrm{AT} = \mathrm{ST}\frac{X * Y}{(\text{pixel size})^2} \tag{12.1}$$

Therefore, MSI analysis is typically a balance between image quality and acquisition time. For example, Fig. 12.2 shows several images of a dog at different resolutions. The image farthest on the left is 8×12 pixels. If the scan time is 1 s, the image would take 96 s (or 1.6 min) to acquire. However, the image resolution is poor and may not be adequate to answer the question the image was supposed to answer. On the other hand, the image farthest on the right is composed of 64×96 pixels and would take 102 min to acquire with the same scan time of 1 s. The level of detail is now very good, but the acquisition time has increased dramatically. In practice, one should choose a resolution that is adequate to answer the question at hand while minimizing acquisition time. One approach is to use a constant number of pixels per inch, or pixel density. The acquisition time scales with area for images with identical pixel densities, so good resolution can be obtained for a small area of interest while keeping the acquisition time short.

Table 12.1 The resolving powers of several mass analyzers

Mass analyzer	Resolving power (FWHM)
FT-ICR	1,000,000
FT-Orbitrap	100,000–300,000
TOF	10,000–40,000
Quadrupole/iontrap in ultrazoom mode	10,000
Quadrupole/iontrap	1,000

12.1.2.2 Depth Resolution

Depth resolution describes the thickness of the layer of material that is removed from the sample with each analysis. For some MSI techniques, such as secondary ion mass spectrometry (SIMS) or laser ablation electrospray ionization (LAESI), it is possible to continue resampling the sample spot after an initial desorption event to obtain information about successive layers of the sample. In this way, layer by layer can be removed and analyzed to create a three-dimensional chemical image. For other techniques, such as matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI), it is possible to obtain a three-dimensional image by dividing the sample into thin slices and imaging each slice (Eberlin et al. 2010a). Several factors affect the depth resolution, such as sample preparation, the energy and destructiveness of the chosen ionization source, the required signal intensity, the scan time, and the time for analysis.

12.1.2.3 Mass Resolution

Mass resolution refers to the ability of a mass analyzer to separate two adjacent mass spectral peaks. Different types of mass analyzers have different resolving powers r (Table 12.1), where m designates a mass value and Δm is the peak width necessary for separation at m:

$$r = m / \Delta m$$

Figure 12.3 shows two spectra for the short peptide bradykinin at two different resolving powers. At a resolving power of 1,000, there is no differentiation of the isotopic peaks, but at a resolving power of 5,000, the individual isotope peaks are distinguished. Mass resolution is an important performance parameter that determines the ability to obtain information about chemical species whose mass spectral peaks have very similar m/z values. For example, Fig. 12.4 shows the distribution of phospholipids in the sagittal sections of a rat brain (Manicke et al. 2010). Ions used to form the images in the left panel (A,C,E,G) have m/z values very similar to the corresponding ions used to form the images on the right panel (B,D,F,H). Without adequate mass resolution, the two species would have appeared as a single peak, and the resulting image would be a combination of the two separate chemical images.

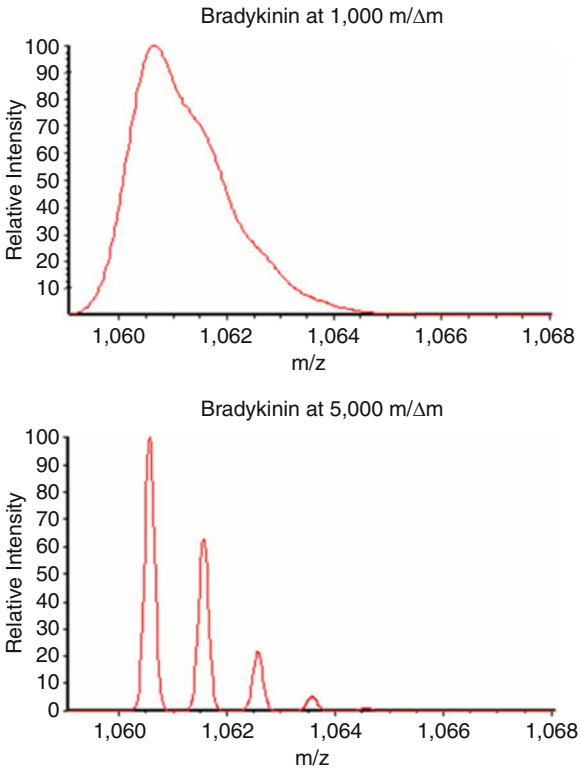

Fig. 12.3 Bradykinin at two resolving powers

12.1.3 General Equipment Needed to Perform Mass Spectral Imaging

12.1.3.1 Sample Preparation

For MSI, the typical ADME workflow begins with the dosing of an animal with the compound of interest. The animal is then sacrificed at a designated time interval, and slides are prepared. If performing whole-body analysis, which gives information about the distribution of the compound within the entire animal, the whole body is sectioned. This may require the use of an embedding medium to rigidify the animal, the most popular choice being carboxymethylcellulose (Ullburg et al. 1977). Alternatively, individual organs or other tissue samples may be removed and sectioned. Transfer of the sections to slides requires the use of adhesive tape or paper (Prideaux et al. 2010). Adhesive tape is not amenable to MSI techniques which require a conductive substrate, such as MALDI, unless a conductive matrix is applied to the sample, but paper suffers the disadvantages of incomplete transfer and sample distortion. A washing step is sometimes applied to the sample prior to analysis in order to remove interfering salts or other suppressants (Shariatgorji et al. 2012). Care must be taken to minimize the solubility of the target compound in the

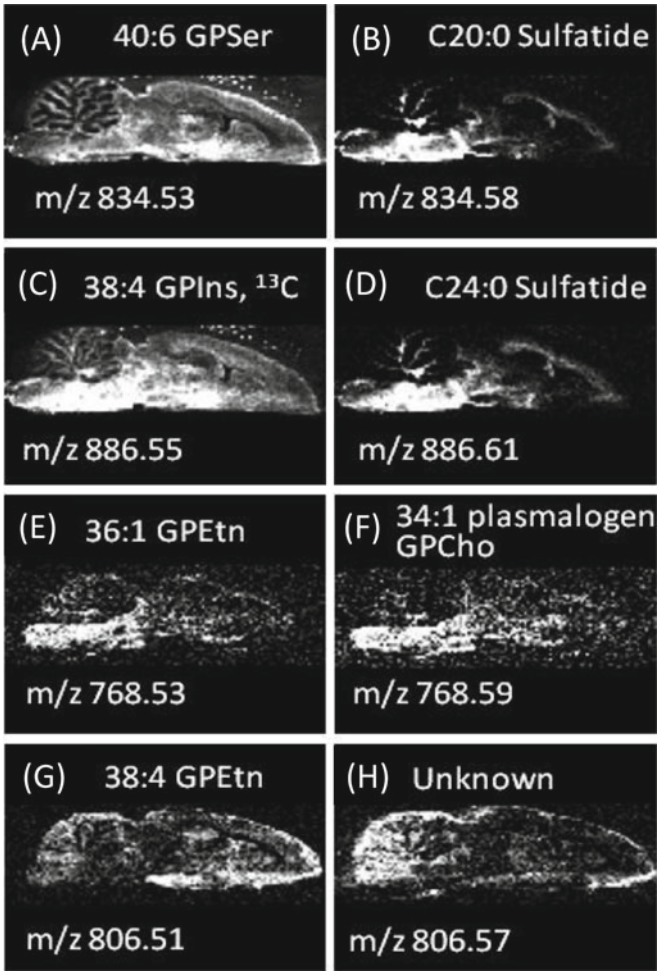

Fig. 12.4 The distribution of phospholipids in the sagittal sections of a rat brain. The panels on the left (A,C,E,G) and the adjacent panels on the right (B,D,F,H) have highly similar m/z values, requiring a high resolution mass analyzer for differentiation

wash solution to minimize removal and delocalization. Once prepared, samples should be analyzed immediately to minimize sample degradation in the open laboratory environment (Goodwin et al. 2012).

The sample may or may not require additional sample preparation depending on the nature of the sample and the MSI technique that will be used. Ambient mass spectrometry ionization methods such as DESI require little to no sample preparation, while methods such as MALDI require considerable sample preparation. In general, the imaged surface should be flat, and the sample must meet the analytical requirements of the specific technique that is used. For instance, some techniques require that the sample be placed in a vacuum chamber for analysis, while some require that the analyte of interest be soluble in a particular solvent system.

12.1.3.2 Sample Positioning Stage

In an imaging experiment, the desorption/ionization source and mass spectrometer are typically held stationary while the sample is moved relative to them. To accurately raster a sample, a moving stage is required. The moving stage is an important part of the experimental setup to consider as the quality of the image depends on the robustness and repeatability of the sampling. Typical moving stages use computer-controlled stepping motors in the x and y dimensions. For samples analyzed under ambient conditions, a height adjustment is also required for optimizing the sample position relative to the ionization source and mass spectrometer inlet. The minimum step size determines the minimum lateral resolution, while the minimum and maximum velocities impact the time required for analysis. In the case of spray desorption methods such as DESI (discussed below), a smooth scanning motion is required, as these techniques typically acquire data as line scans. For techniques which use a laser or a probe device for desorption, data is acquired one pixel at a time, and a fast movement to the following pixel is desired. The reproducibility of sample placement is also important for indexing of pixels.

12.1.3.3 Desorption/Ionization Source and Mass Analyzer

The desorption/ionization source is important because it desorbs the sample, generates the gas-phase ions that are ultimately detected, and sets limits on the lateral and depth resolutions. There are many kinds of desorption and ionization agents available, such as lasers, ion beams, plasmas, liquid microjunctions, solid probes, and solvent sprays. Many sources are a combination of two or more of these agents (Huang et al. 2011).

There are many kinds of mass analyzers available as well, such as the triple quadrupole, Q-trap, orbitrap, or time of flight (TOF). Different mass analyzers have different mass ranges and mass resolutions. Analyzers with high mass resolution can be used to determine the elemental composition of the detected compounds, which enables unambiguous identification simultaneous with the imaging experiment. Similarly, analyzers with MS/MS capabilities can be used to fragment a peak of interest for identification purposes. In some cases, fragments can be selected and fragmented again, offering structural information about the parent compound. Most sources can be paired with any mass analyzer, but some common configurations exist, such as MALDI-TOF.

12.1.3.4 Post-processing

Computer software is used to process the imaging data after it has been collected. The software allows the visualization of the chemical image and includes features for data manipulation. Various commercial programs, as well as many programs developed in-house, exist for processing MSI data. A popular software option is BioMAP

(http://www.maldi-msi.org/), which can be downloaded for free from the Internet. Depending on the resolution of the mass analyzer and the pixel count, imaging files can become quite large. Therefore, the processing speed and storage capabilities of the computer used for data acquisition and processing must be considered before performing an MSI experiment. The processing of MSI data typically includes (1) concatenating individual mass spectra into a single file, (2) building a table of all the ions detected over the entire image, (3) extracting the ions of interest, and (4) displaying the distribution of these particular ions across one or more images.

12.2 MSI Methods

The lateral, depth, and mass resolutions, as well as sample preparation, can vary significantly between the different MSI methods, so the choice of which method to use depends on the application. Established techniques for the MSI of drug distributions in tissues include secondary ion mass spectrometry (SIMS) and matrix-assisted laser desorption/ionization (MALDI). These techniques offer the advantages of high sensitivity and high spatial resolution, but typically require a substantial amount of sample preparation. The introduction of desorption electrospray ionization (DESI) (Takats et al. 2004) and direct analysis in real time (DART) (Cody et al. 2005) instigated a rapid emergence of the so-called ambient ionization techniques. Ambient methods such as DESI and laser ablation electrospray ionization (LAESI) are becoming increasingly popular choices for MSI. These techniques require little-to-no sample preparation, allowing high-throughput analysis to be done in the open environment. Ambient ionization techniques are able to analyze samples directly because they include both desorption and ionization capabilities. While these two events can be accomplished with a single agent in some cases, most ambient methods use separate agents for desorption and ionization. Many permutations of the desorption and ionization agents have been achieved (Huang et al. 2011; Venter et al. 2008). The desorption processes of most ambient ionization methods are localized, so these methods have the ability to be used for MSI (Wu et al. 2013).

A figure containing schematics for several MSI techniques discussed below is presented at the end of the chapter.

12.2.1 Common MSI Techniques Used for Drug Discovery

12.2.1.1 Secondary Ion Mass Spectrometry (SIMS)

In SIMS, an ion beam is used to bombard a sample in vacuum. Ions are liberated from the surface and subsequently detected. Many ion beam choices are available, including mono- and polyatomic clusters. Polyatomic clusters and molecular ions

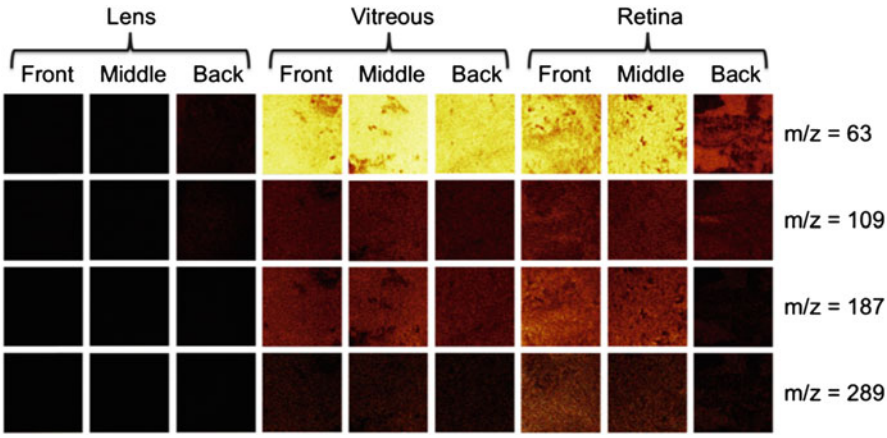

Fig. 12.5 The distribution of dexamethasone sodium phosphate drug fragments in various sections of the lens, vitreous humor, and retina of perfused ovine ocular tissue determined using Bi3+ cluster TOF-SIMS. (From Mains et al. 2011; with permission)

such as C_{60} reduce the amount of fragmentation that is common during SIMS. Both the spatial and depth resolutions are very good for SIMS, as low as 100 nm (Pacholski and Winograd 1999) and 15 nm (Cheng and Winograd 2005), respectively, making it an ideal choice if high resolution is required. SIMS can be operated in one of two modes by adjusting the density of ions bombarding the surface. Static SIMS, achieved with a low density of ions, is minimally destructive. Dynamic SIMS, which is more destructive, occurs with a high density of ions. Dynamic SIMS can be used to remove layers of the sample to construct a 3D chemical image of the sample (Seeley and Caprioli 2012). SIMS is typically used for low molecular weight molecules, although interference from fragmentation can be quite severe in the low mass range of the spectrum, making the interpretation of the data difficult.

Figure 12.5 shows an example of using Bi_3^+ cluster TOF-SIMS to map the distribution of dexamethasone sodium phosphate drug fragments in various sections of the lens, vitreous humor, and retina of perfused ovine ocular tissue (Mains et al. 2011). By comparing the distributions with those obtained from non-perfused tissue, the authors were able to show that the movement of dexamethasone through the eye is not the result of diffusion alone. The images are 500 × 500 μm, demonstrating the extremely high resolution of this technique.

12.2.1.2 Matrix-Assisted Laser Desorption/Ionization (MALDI)

MALDI is another vacuum method with high spatial resolution. Resolutions as low as 30 μm (Burnum et al. 2008) have been attained, although resolutions around 200 μm are more typical. MALDI is usually paired with TOF mass analyzers and as such offers a large mass range, 600 Da to 1 MDa. The ionization agent is a laser pulse. As the name implies, MALDI requires the application of a matrix compound

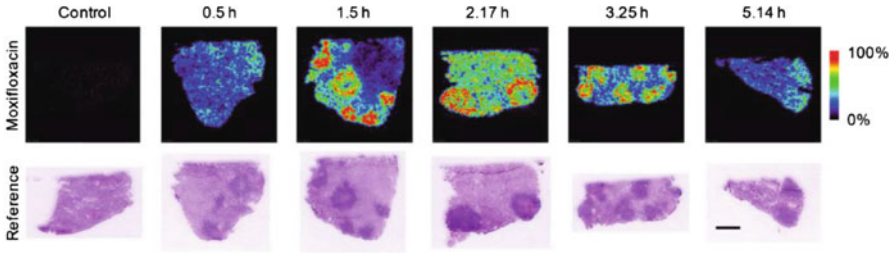

Fig. 12.6 The distribution of moxifloxacin in tuberculosis-infected rabbit lung biopsies determined using MALDI-TOF. The scale bar in the reference image at 5.14 h represents 5 mm. (From Prideaux et al. 2011; with permission)

prior to analysis to absorb the laser energy and aid with ionization, which usually occurs through protonation or deprotonation reactions. There are many matrix compounds used in MSI MALDI, and the choice of matrix depends on the application (Kaletaş et al. 2009). For instance, 2,5-dihydroxybenzoic acid (DHB) is usually used for sugars or peptides, while sinapinic acid is used for proteins or lipids. There are several methods for applying the matrix, including droplet deposition, dry coating, sublimation, and electrospray (Kaletaş et al. 2009). Choosing the correct matrix compound and applying it are critical to the success of MALDI. When the matrix dries, crystals are typically formed and the size and homogeneous distribution of these crystals are important for obtaining good spatial resolution when attempting MSI by MALDI. One drawback of MALDI is the interference in the low mass range from the formation of matrix clusters, making the interpretation of data for lower molecular mass compounds difficult. However, higher molecular weight compounds can ionize easily in MALDI, making it ideal for the study of biopolymers such as proteins, DNA, or RNA.

Figure 12.6 shows the distribution of the tuberculosis drug moxifloxacin in infected rabbit lung biopsies (Prideaux et al. 2011). The drug was shown to accumulate in the granulomatous lesions over time, preferentially in the outer regions known to contain active microphages as opposed to the central necrotic caseum, where lower levels were detected. Since extracellular bacteria populations are thought to reside in this central area, this information may be clinically relevant. The scale bar for these images is 5 mm, so the pixel size is much larger than what can be attained by SIMS, but the resolution is adequate to show the various distributions of the target compound.

Many modifications to the traditional MALDI setup have been used for MSI. The laser pulse is typically applied from above the plane of the sample, but by using a transparent MALDI substrate, it is possible to apply the laser pulse from behind. This enables the sample to be placed much closer to the mass spectrometer inlet to improve ion transmission (Vertes et al. 1990). Mass spectral imaging has been demonstrated in the transmission geometry (Richards et al. 2011). Laser desorption ionization (LDI) is a method operationally similar to MALDI (McMahon 1985), with the exception that it is not necessary to apply a matrix to the sample prior to

analysis. LDI has been used to image metabolites in plant tissues (Cha et al. 2009), lipid distributions in mouse brain tissues (Goodwin et al. 2011), and antibiotic compounds on the surface of beewolf larval cocoons (Kroiss et al. 2010). Finally, intermediate and atmospheric pressure MALDI (AP-MALDI) sources have been used for the MSI of lower molecular weight compounds (Guenther et al. 2011; Garrett et al. 2007).

12.2.1.3 Desorption Electrospray Ionization (DESI)

DESI, one of the pioneering ambient techniques, (Takats et al. 2004) is performed by directing an electrosprayed solvent at the sample section. Initial droplets create a thin liquid film into which material is dissolved. Subsequent droplets impact this film and produce secondary droplets which fly towards the mass spectrometer inlet (Costa and Graham 2008). Ionization from these secondary droplets occurs in the same way as traditional electrospray ionization (Takáts et al. 2005). For compounds not easily analyzed by DESI, reagents can be added to the electrospray to react with these molecules and form product molecules that are more easily ionized and detected. This method is called reactive DESI (Cotte-Rodriguez et al. 2005). The typical resolution for a DESI MSI experiment is around 200 μm, although a resolution as high as 40 μm has been reported (Kertesz and Van Berkel 2008).

Of all the ambient methods, DESI has been developed the most as a MSI technique, especially for the analysis of lipids in tissue samples (Eberlin et al. 2011). The three-dimensional analysis of lipids has been demonstrated for the lipids in rat brain tissue (Eberlin et al. 2010a) as well as the mapping of lipids (Paglia et al. 2010) after two-dimensional high-performance thin-layer chromatography (2D-HPTLC) separation and peptides (Pasilis et al. 2008) after one-dimensional HPTLC. DESI MSI analysis has also included the mapping of drug distributions in tissues (Kertesz et al. 2008), natural products imaging (Lane et al. 2009; Li et al. 2011; Muller et al. 2011; Thunig et al. 2011; Ifa et al. 2011), and cancerous tissue discrimination (Dill et al. 2009; Eberlin et al. 2010b). In addition, reactive DESI has been used to image cholesterol in adrenal gland tissue (Wu et al. 2010), a compound not amenable to standard DESI analysis.

A comparison between the whole-body imaging of a mouse sagittal section by DESI-MS/MS and WBA (Kertesz et al. 2008) is shown in Fig. 12.7. The specimen was intravenously dosed with 7.5 mg/kg of propranolol and euthanized 60 min after dosing. Each pixel in the DESI-MS/MS image corresponds to an area of approximately 140 μm by 200 μm in size. Comparison of panels B and D shows that DESI-MS/MS was able to detect propranolol in the brain, lung, and stomach regions. The autoradioluminograph obtained by WBA showed accumulation in the liver, kidney, and salivary gland as well, which was not picked up by DESI-MS/MS, but this signal may have resulted from the detection of propranolol metabolites, which WBA cannot distinguish from the parent drug.

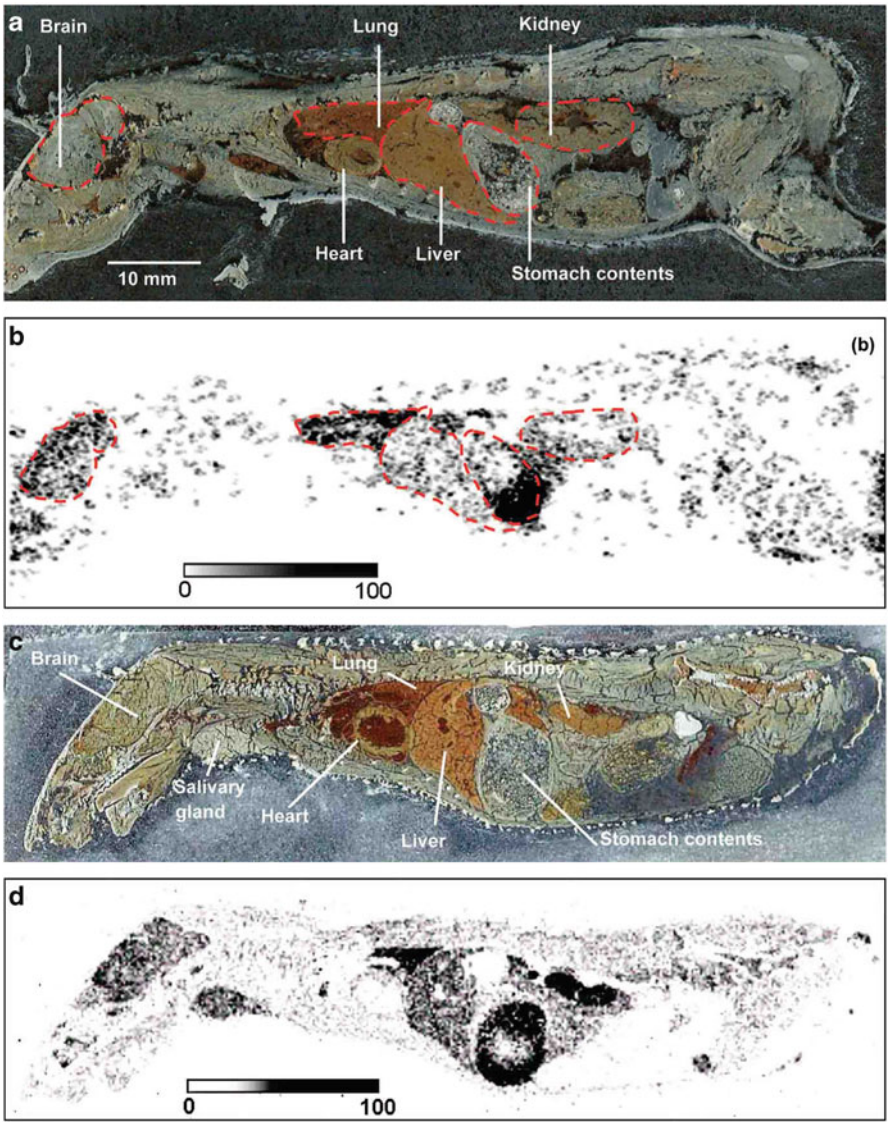

Fig. 12.7 (**a**) and (**c**) are optical images of whole-body tissue sections of a mouse dosed with 7.5 mg/kg propranolol and euthanized 20 min after dosing. (**b**) Distribution of propranolol determined using DESI-MS/MS. (**d**) Distribution of propranolol determined using WBA. (From Kertesz et al. 2008; with permission)

12.2.2 Other Techniques

Besides the MSI techniques commonly used for drug discovery, many other desorption/ionization techniques have been developed that have high potential for MSI. Many of these techniques are part of the boom of ambient techniques that began

with DESI and DART and share the advantages common to all ambient techniques, such as operation at atmospheric pressure and limited sample preparation. While ambient ionization is a relatively young field compared to SIMS and MALDI, it is currently receiving a lot of attention and is developing rapidly. Besides DESI, some methods have already become commercially available, such as LAESI and LMJ-SSP. The following section contains a brief introduction for various MSI techniques which have shown the potential to be used for drug discovery and development. They are divided according to the desorption mechanism of their ionization source, the parameter which typically has the largest impact on lateral resolution.

12.2.2.1 Laser Desorption Techniques

In each of the following ambient techniques, a laser is directed towards the sample and material is ablated with the firing of the laser, similar to MALDI or LDI. After ablation, ionization is achieved by one of several methods, depending on the technique.

Laser Ablation Electrospray Ionization (LAESI)

During LAESI analysis (Nemes and Vertes 2007), material is ablated from the sample using a mid-infrared laser at 2,940 nm. The ablated material then coalesces with the droplets of an electrospray that is directed towards the mass spectrometer inlet. Ionization occurs by the typical electrospray ionization mechanisms (Nemes and Vertes 2007). The amount of material that is ablated can be controlled by adjusting the number of laser pulses applied to each sample position. This makes it possible to perform 3D imaging experiments using this method (Nemes et al. 2009).

The bio-applications demonstrated by LAESI have included the ability to map both the 2D (Nemes et al. 2008) and 3D (Nemes et al. 2009) distributions of plant metabolites present in *Aphelandra squarrosa* leaves (Fig. 12.8), the mapping of small metabolites and lipids in rat brain tissue sections (Nemes et al. 2010), and the in vitro analysis of metabolites in the electric organ of the fish *Torpedo californica* (Sripadi et al. 2009). In addition, the in situ analysis of single cells (Shrestha and Vertes 2009; Shrestha et al. 2011) has also been demonstrated by LAESI.

Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS)

LA-ICPMS (Durrant 1999) begins by using a laser to ablate material on the sample. This material is then transferred via a flow of gas to a plasma source for ionization, where the high temperature of the plasma breaks the material into individual atoms, some of which are ionized. A lateral resolution of 4 μm has been reported (Durrant 1999). Because it uses a plasma ionization source, this technique is very good for elemental analysis. For example, Fig. 12.9 shows the distribution of Cu, Fe, Zn, and Mn in a rat brain at 2 h, 7 days, and 28 days after the administration

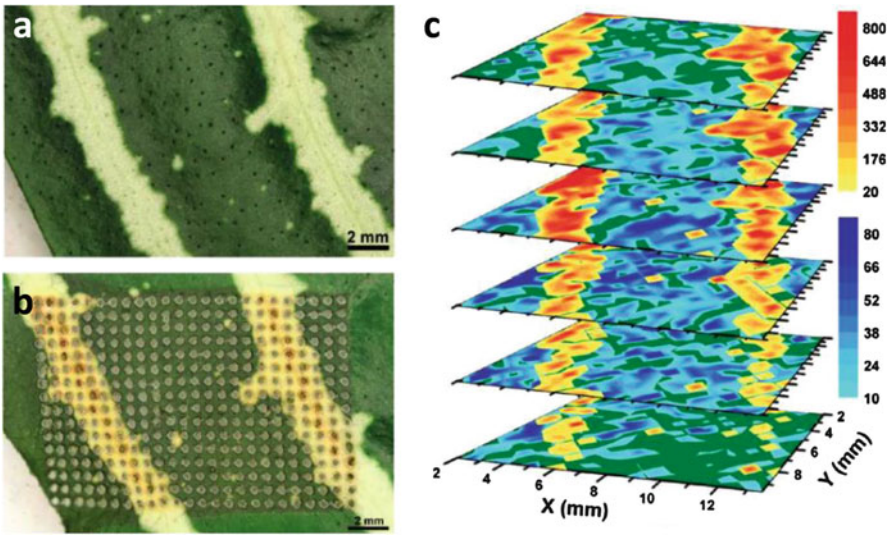

Fig. 12.8 Optical image of an *Aphelandra squarrosa* leaf before (**a**) and after (**b**) LAESI analysis. The 3D distribution of kaempferol/luteolin (**c**) followed the variegation pattern of the optical images. (From Nemes et al. 2009; with permission)

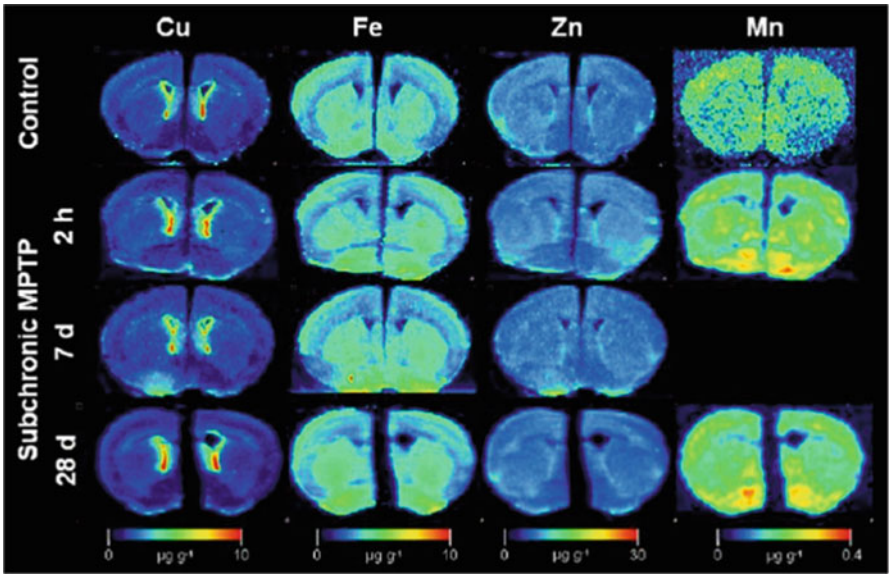

Fig. 12.9 Cu, Fe, Zn, and Mn distributions in rat brain sections at various time points following administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP). (From Matusch et al. 2010)

of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP), a drug used to model Parkinson's disease (Matusch et al. 2010). It was possible to observe an increase of Fe over time in the interpeduncular nucleus as well as a significant decrease of Cu in the periventricular zone and fascia dentate at 2 h and 7 days followed by over-compensation at 28 days.

Infrared Laser Ablation Metastable-Induced Chemical Ionization (IR-LAMICI)

Similar to LAESI, a mid-infrared laser at 2,940 nm is used to ablate material during IR-LAMICI (Galhena et al. 2010). However, unlike LAESI, after ablation the material is introduced to a metastable plume directed towards the mass spectrometer inlet, where ionization occurs by gas-phase chemical ionization. IR-LAMICI has been used for natural product imaging on biological surfaces (Galhena et al. 2010) as well as the small molecule imaging of drug tablets (Galhena et al. 2010).

Atmospheric Pressure Femtosecond Laser Desorption Ionization (AP fs-LDI)

AP fs-LDI uses near-infrared laser pulses with high peak power densities to desorb and ionize the sample (Coello et al. 2010). Because the sample absorbs the laser energy directly, sample fragmentation can occur (Lozovoy et al. 2008). Similar to LAESI, AP fs-LDI can be combined with an electrospray, a technique referred to as laser electrospray mass spectrometry in the literature (LEMS) (Judge et al. 2010). The mass range for AP fs-LDI is limited at around *m*/*z* 400, but it has the best reported spatial resolution of the laser-based ambient techniques at around 20 μm (Coello et al. 2010). The ability to analyze biological tissues was demonstrated for AP fs-LDI by imaging a monolayer of onion epidermis, which allowed the visualization of individual cells (Fig. 12.10) (Coello et al. 2010).

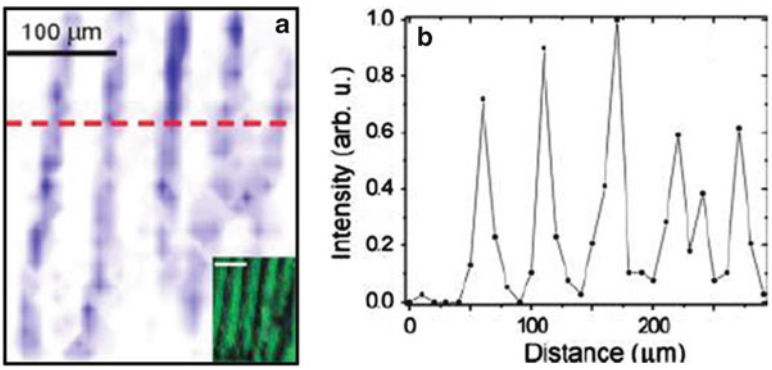

Fig. 12.10 Individual onion epidermis cells visualized using AP fs-LDI. Panel (**a**) shows the MSI chemical image and corresponding optical image (inset). Panel (**b**) shows the intensity distribution for scan represented by the red dashed line in panel (**a**). (From Coello et al. 2010)

Laser Ablation Coupled to Flowing Atmospheric-Pressure Afterglow (LA-FAPA)

The last of the laser desorption methods is LA-FAPA (Shelley et al. 2008). LA-FAPA uses an ultraviolet laser to ablate material. This material is subsequently carried by a nitrogen stream to the afterglow of a helium atmospheric pressure glow discharge ionization source where analyte molecules are mixed with the plasma and ionized. LA-FAPA can be used to analyze molecules up to 1 kDa with a spatial resolution as low as 20 μm, depending on the size of the laser spot. LA-FAPA has been used for drug tablet analysis as well as the imaging of tissue loaded with lidocaine (Shelley et al. 2008).

12.2.2.2 Plasma and Vapor Desorption Methods

Low-Temperature Plasma Source (LTP)

The low-temperature plasma source (LTP) (Harper et al. 2008) uses plasma for both desorption and ionization. In the LTP source, an alternating voltage is applied between two electrodes separated by a dielectric layer, generating a low-temperature plasma which is then applied to the surface to desorb and ionize the sample at a spatial resolution as low as 250 μm (Liu et al. 2010).

Desorption Atmospheric Pressure Photoionization (DAPPI)

DAPPI (Haapala et al. 2007) uses a heated jet containing solvent vapor to desorb material from the sample, which is then ionized by photoionization using a photoionization lamp. The heated jet increases the temperature of the sample where it is applied, a parameter important for efficient desorption (Luosujärvi et al. 2008). For this reason, substrates with low thermal conductivity are best. Pharmaceutical and illicit drugs have been detected using DAPPI (Haapala et al. 2007; Luosujärvi et al. 2009), and MSI analysis of phytocompounds from plant leaves, as well as the selective ionization of a sterol lipid from a brain tissue sample, has also been demonstrated with a spatial resolution of 1 mm (Pól et al. 2009).

12.2.2.3 Probe Electrospray Ionization (PESI)

During PESI (Hiraoka et al. 2007), a solid needle is used to penetrate the surface of the sample, some of which adheres to the needle on extraction. The needle is then moved to a position in front of an spray emitter, where droplets from the spray wet the needle tip containing the desorbed material. A high voltage applied to the needle then initiates an electrospray at the needle tip, which ionizes the sample and transfers it to the mass spectrometer. Once the ionization event is finished, the needle is moved to the next sample position, and the process is repeated.

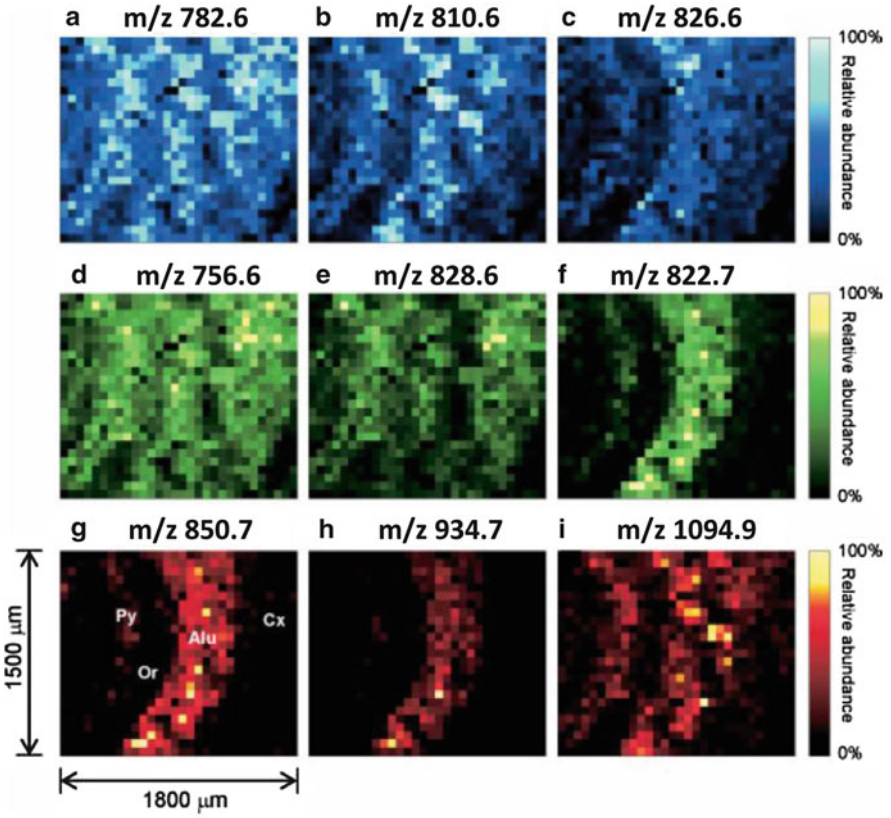

Fig. 12.11 Chemical images of phospholipids (**a**–**e**), galactosylceramides (**f**–**h**), and an ion at *m*/*z* 1,094.9 present in a mouse brain section determined using PESI (**i**). (From Chen et al. 2009; with permission)

The lateral resolution is limited by the size of the probe, which is typically around 100 µm or less. Depth profiling is also possible using PESI, although carryover resulting from increasing hole size during subsequent punctures makes quantitative measurement difficult (Chen et al. 2009). PESI has been used to analyze phospholipids and galactosylceramides from mouse brain sections (Fig. 12.11) with a lateral resolution of 60 µm (Chen et al. 2009).

12.2.2.4 Liquid Microjunction Methods

Several closely related ambient methods have been developed where a liquid junction is formed between the sample and a probe, including the liquid microjunction surface-sampling probe (LMJ-SSP), the nanospray desorption electrospray ionization (nano-DESI) source, and the liquid extraction surface analysis (LESA) source. Solvent is delivered directly to the sample, and material from the sample is

dissolved into the junction. The solvent containing the sample is then transferred to a position where it is ionized by electrospray ionization.

Liquid Microjunction Surface-Sampling Probe (LMJ-SSP)

The LMJ-SSP (Van Berkel et al. 2008) uses a set of coaxial capillaries where solvent from the outer capillary forms the junction with the sample while the inner capillary transfers solvent sampled from the junction to a pneumatically assisted electrospray. A noncontact method of LMJ-SSP called proximal probe thermal desorption/secondary ionization has been developed where the continuous flow probe is positioned immediately above the surface, catching material from a transmission mode laser ablation event (Ovchinnikova et al. 2011). Traditional LMJ-SSP has demonstrated tissue imaging capabilities (Van Berkel et al. 2008), though the lateral resolution is larger than many other ambient techniques at 670 μm. Proximal probe thermal desorption/secondary ionization has a lateral resolution around 50 μM (Ovchinnikova et al. 2010), similar to other methods using laser desorption.

Nanospray Desorption Electrospray Ionization (Nano-DESI)

The nano-DESI source (Roach et al. 2010) forms a liquid microjunction between the sample and two closely positioned capillaries, one that delivers solvent and another with a self-aspirating nanospray at the other end, which is positioned in front of the mass spectrometer inlet. The analysis of human carcinoma and rat brain tissues (Laskin et al. 2012) has demonstrated nano-DESI's ability to achieve a lateral resolution as low as 12 μm (Fig. 12.12).

Liquid Extraction Surface Analysis (LESA)

During LESA (Eikel et al. 2011), the probe is first positioned close to the surface. When in position, the probe extrudes a droplet to form a liquid microjunction with the sample. The droplet is then retracted back into the probe and transferred to a position from where it is subsequently electrosprayed. A lateral resolution of 1 mm was demonstrated for the imaging of the pharmaceutical product terfenadine and its metabolite fexofenadine in whole-body mouse sections (Eikel et al. 2011).

12.2.3 Summary Table

A summary of the MSI methods is presented in Table 12.2. The table summarizes reported lateral and depth resolutions. It also illustrates applications of the various MSI methods. Figure 12.13 is also provided as a simplified depiction of several of

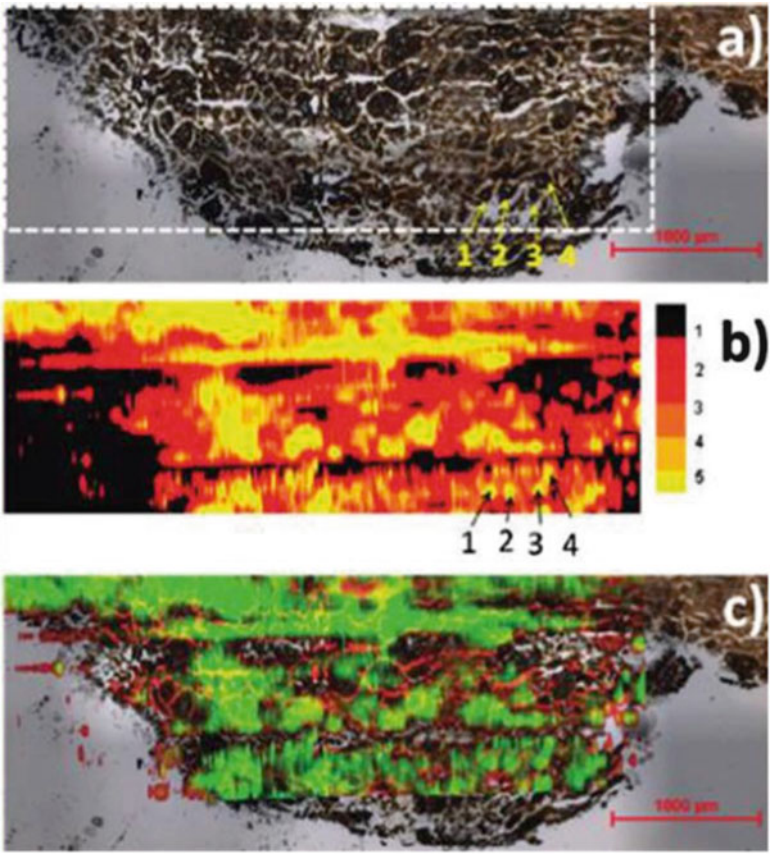

Fig. 12.12 (**a**) Optical image of a papillary renal cell carcinoma human tissue sample. (**b**) Ion image of the cholesteryl ester at *m*/*z* 687.5 obtained using nano-DESI. (**c**) Overlay of the optical image and chemical image. The scale bar represents 1 mm. (From Laskin et al. 2012, with permission)

the IMS methods presented in this chapter. Many methods have been used for biomedical applications, and others listed are likely to be used for such applications soon. While no other method has yet to approach the lateral resolution capable by SIMS, most have reported lateral resolutions of 200 μm or less, adequate for many imaging applications at tissue level.

12.3 Concluding Remarks

MSI has become a valuable tool which can provide complementary data to radiographic and optical studies of drug distributions. As the field of mass spectrometry continues to expand, the already extensive collection of available ionization sources and mass

Table 12.2 Summary of mass spectrometry methods that have been used for imaging experiments

Technique	Desorption agent	Ionization agent[a]	Spatial resolution[b]	Depth resolution[b]	Applications
SIMS	Ion beam	Ion beam	100 nm (Pacholski and Winograd 1999)	15–20 nm (Cheng and Winograd 2005)	Pharmaceuticals, various bio-applications. Several recent reviews (Seeley and Caprioli 2012; Barnes et al. 2011; Lee et al. 2012)
MALDI	Laser	*	30 µm (Burnum et al. 2008)	–	Tissue analysis, plant proteins, drug development, etc. Several recent reviews (Seeley and Caprioli 2011, 2012; Lagarrigue et al. 2011; Goto-Inoue et al. 2011; Grassl et al. 2011; Balluff et al. 2011; Bonnel et al. 2011; Fernández et al. 2011; Kaspar et al. 2011)
LA-ICPMS	Laser	Plasma	4 µm (Durrant 1999)	–	Bioaccumulation of metals in tissues (Matusch et al. 2010)
LAESI	Laser	Electrospray	200 µm (Nemes et al. 2010)	30 µm (Nemes et al. 2009)	Plant products (Nemes et al. 2008, 2009), tissue analysis (Sripadi et al. 2009)
IR-LAMICI	IR-laser	Metastable plume	300 µm (Galhena et al. 2010)	–	Natural products (Galhena et al. 2010), pharmaceuticals (Galhena et al. 2010)
AP fs-LDI	Laser	*	20 µm (Coello et al. 2010)	–	Tissue imaging (Coello et al. 2010)
LA-FAPA	UV-laser	Metastable plume	20 µm (Shelley et al. 2008)	36 µm (Shelley et al. 2008)	Pharmaceuticals (Shelley et al. 2008), tissue imaging (Shelley et al. 2008)
LTP	Plasma	*	250 µm (Liu et al. 2010)	–	

(continued)

Table 12.2 (continued)

Technique	Desorption agent	Ionization agent[a]	Spatial resolution[b]	Depth resolution[b]	Applications
DAPPI	Solvent vapor	Photochemical ionization	1 mm (Pól et al. 2009)	–	Pharmaceuticals (Haapala et al. 2007), drugs (Luosujärvi et al. 2009), lipids (Pól et al. 2009), plant products (Pól et al. 2009)
PESI	Solid probe	Electrospray	60 µm (Chen et al. 2009)	–	Tissue imaging (Chen et al. 2009)
LMJ-SSP	Liquid microjunction[c] laser[d]	Electrospray	670 µm[c] (Van et al. 2008) 50 µm[d] (Ovchinnikova et al. 2010)	–	Tissue imaging (Van et al. 2008)
Nano-DESI	Liquid microjunction	Nanospray	12 µm (Laskin et al. 2012)	–	Tissue imaging (Laskin et al. 2012)
LESA	Liquid microjunction	Electrospray	1 mm (Eikel et al. 2011)	–	Tissue imaging (Eikel et al. 2011)
DESI	Electrospray	*	40 µm (Kertesz and Van Berkel 2008)	–	Pharmaceuticals (Kertesz et al. 2008), lipids (Eberlin et al. 2011), peptides (Pasilis et al. 2008), natural products (Lane et al. 2009; Li et al. 2011; Muller et al. 2011; Thunig et al. 2011; Ifa et al. 2011)

[a]An "*" indicates desorption/ionization occur together using the desorption agent
[b]Lowest reported value found in the literature. An "–" indicates no available data
[c]Contact mode LMJ-SSP
[d]Proximal probe LMJ-SSP

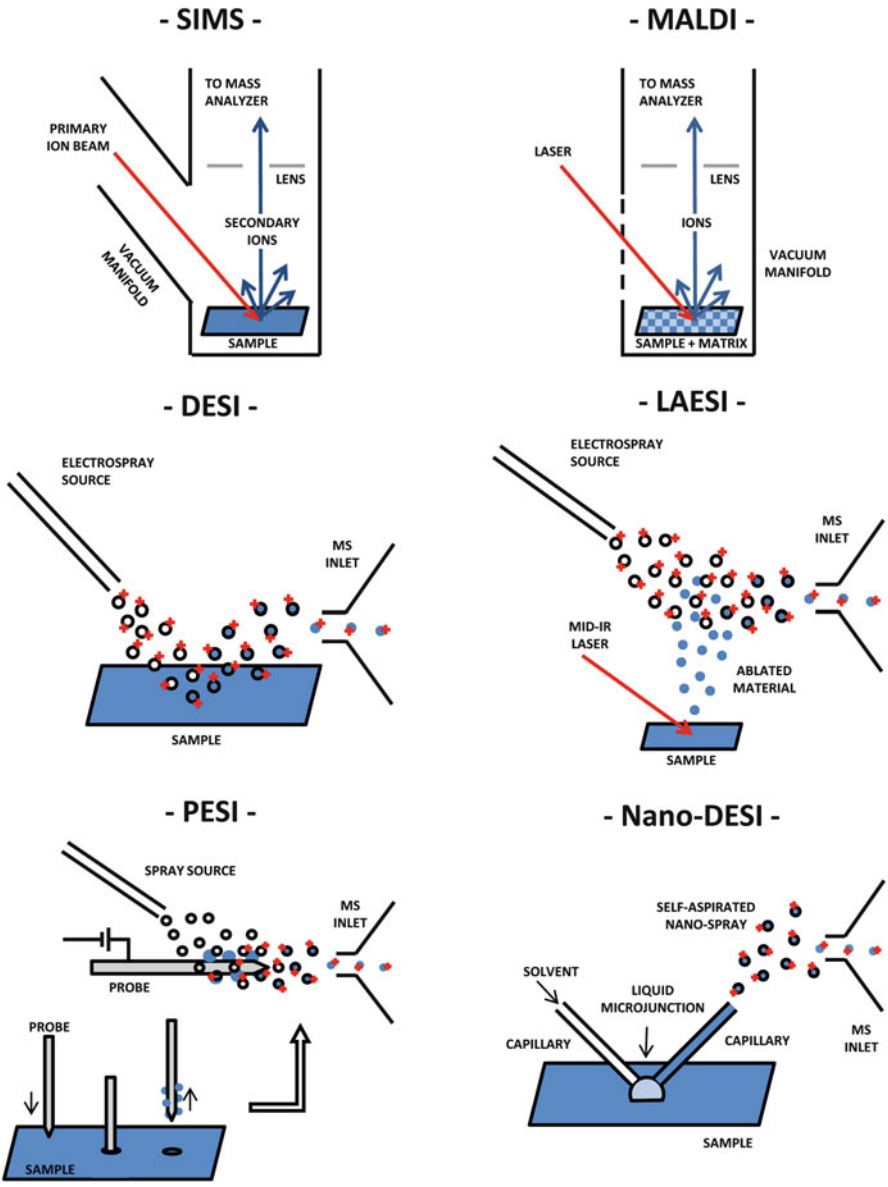

Fig. 12.13 Schematics of various ionization/desorption methods used for MSI

analyzers, the number of possible techniques with which to perform MSI, will continue to grow as well. As we have shown, many recently introduced ambient ionization sources have already found utility in MSI for drug discovery. With continued development, they will increasingly present an attractive alternative to established techniques such as MALDI and SIMS. The ambient methods offer several advantages over SIMS

as well as MALDI. For many applications, the ambient methods offer satisfactory resolution while requiring much less sample preparation, improving the time of analysis.

Wu et al. have identified several target areas in which progress would aid the adoption of the ambient MSI techniques into the field of drug discovery and development (Wu et al. 2013). These areas include:

1. A better understanding of ion suppression and matrix effects which are common in ambient mass spectrometry
2. Increased robustness in ambient MSI source design and experimental setup to increase reproducibility
3. Increased use of high-resolution mass analyzers and tandem mass spectrometry for molecular identification
4. The development of imaging software and other tools for handling increasingly large sets of imaging data
5. Improved spatial resolution of the ambient methods
6. The development of quantitative methods, such as the inclusion of internal standards

References

Burnum KE, Frappier SL, Caprioli RM (2008) Matrix-assisted laser desorption/ionization imaging mass spectrometry for the investigation of proteins and peptides. Annu Rev Anal Chem 1(1):689–705

Balluff B et al (2011) MALDI imaging mass spectrometry for direct tissue analysis: technological advancements and recent applications. Histochem Cell Biol 136(3):227–244

Bonnel D et al (2011) MALDI imaging techniques dedicated to drug-distribution studies. Bioanalysis 3:1399–1406

Barnes TJ, Kempson IM, Prestidge CA (2011) Surface analysis for compositional, chemical and structural imaging in pharmaceutics with mass spectrometry: a ToF-SIMS perspective. Int J Pharm 417(1–2):61–69

Coello Y et al (2010) Atmospheric pressure femtosecond laser imaging mass spectrometry. Anal Chem 82(7):2753–2758

Cha S et al (2009) Direct profiling and imaging of epicuticular waxes on arabidopsis thaliana by laser desorption/ionization mass spectrometry using silver colloid as a matrix. Anal Chem 81: 2991–3000

Costa AB, Cooks RG (2008) Simulated splashes: elucidating the mechanism of desorption electrospray ionization mass spectrometry. Chem Phys Lett 464:1–8

Cotte-Rodriguez I et al (2005) Desorption electrospray ionization of explosives on surfaces: sensitivity and selectivity enhancement by reactive desorption electrospray ionization. Anal Chem 77(21):6755–6764

Chen LC et al (2009) Ambient imaging mass spectrometry by electrospray ionization using solid needle as sampling probe. J Mass Spectrom 44:1469–1477

Cheng J, Winograd N (2005) Depth profiling of peptide films with TOF-SIMS and a C60 probe. Anal Chem 77(11):3651–3659

Cody RB, Laramée JA, Durst HD (2005) Versatile new ion source for the analysis of materials in open air under ambient conditions. Anal Chem 77(8):2297–2302

Durrant SF (1999) Laser ablation inductively coupled plasma mass spectrometry: achievements, problems, prospects. J Anal At Spectrom 14(9):1385–1403

Dill AL et al (2009) Lipid profiles of canine invasive transitional cell carcinoma of the urinary bladder and adjacent normal tissue by desorption electrospray ionization imaging mass spectrometry. Anal Chem 81(21):8758–8764
Eberlin LS et al (2010a) Three-dimensional vizualization of mouse brain by lipid analysis using ambient ionization mass spectrometry. Angew Chem Int Ed 49(5):873–876
Eberlin LS et al (2011) Desorption electrospray ionization mass spectrometry for lipid characterization and biological tissue imaging. Biochim Biophys Acta Mol Cell Biol Lipids 1811(11): 946–960
Eberlin LS et al (2010b) Cholesterol sulfate imaging in human prostate cancer tissue by desorption electrospray ionization mass spectrometry. Anal Chem 82(9):3430–3434
Eikel D et al (2011) Liquid extraction surface analysis mass spectrometry (LESA-MS) as a novel profiling tool for drug distribution and metabolism analysis: the terfenadine example. Rapid Commun Mass Spectrom 25(23):3587–3596
Fernández J et al (2011) Matrix-assisted laser desorption ionization imaging mass spectrometry in lipidomics. Anal Bioanal Chem 401(1):29–51
Goodwin RJA, Iverson SL, Andren PE (2012) The significance of ambient-temperature on pharmaceutical and endogenous compound abundance and distribution in tissues sections when analyzed by matrix-assisted laser desorption/ionization mass spectrometry imaging. Rapid Commun Mass Spectrom 26:494–498
Galhena AS et al (2010) Small molecule ambient mass spectrometry imaging by infrared laser ablation metastable-induced chemical ionization. Anal Chem 82(6):2178–2181
Guenther S et al (2011) AP-MALDI imaging of neuropeptides in mouse pituitary gland with 5 μm spatial resolution and high mass accuracy. Int J Mass Spectrom 305:228–237
Goodwin RJA et al (2011) Matrix-free mass spectrometric imaging using laser desorption ionization Fourier transform ion cyclotron resonance mass spectrometry. Rapid Commun Mass Spectrom 25:969–972
Grassl J, Taylor N, Millar AH (2011) Matrix-assisted laser desorption/ionisation mass spectrometry imaging and its development for plant protein imaging. Plant Meth 7(1):21
Goto-Inoue N et al (2011) Imaging mass spectrometry for lipidomics. Biochim Biophys Acta (BBA) Mol Cell Biol Lipids 1811(11):961–969
Garrett TJ et al (2007) Imaging of small molecules in tissue sections with a new intermediate-pressure MALDI linear ion trap mass spectrometer. Int J Mass Spectrom 260:166–176
Huang MZ et al (2011) Ambient ionization mass spectrometry: a tutorial. Anal Chim Acta 702(1):1–15
Harper JD et al (2008) Low-temperature plasma probe for ambient desorption ionization. Anal Chem 80(23):9097–9104
Haapala M et al (2007) Desorption atmospheric pressure photoionization. Anal Chem 79(20): 7867–7872
Hiraoka K et al (2007) Development of probe electrospray using a solid needle. Rapid Commun Mass Spectrom 21(18):3139–3144
Ifa DR et al (2007) Development of capabilities for imaging mass spectrometry under ambient conditions with desorption electrospray ionization (DESI). Int J Mass Spectrom 259(1–3):8–15
Ifa DR et al (2011) Tissue imprint imaging by desorption electrospray ionization mass spectrometry. Anal Meth 3(8):1910–1912
Judge EJ et al (2010) Analysis of pharmaceutical compounds from glass, fabric, steel, and wood surfaces at atmospheric pressure using spatially resolved, nonresonant femtosecond laser vaporization electrospray mass spectrometry. Anal Chem 82(8):3231–3238
Kroiss J et al (2010) Symbiotic streptomycetes provide antibiotic combination prophylaxis for wasp offspring. Nat Chem Biol 6:261–263
Kaspar S et al (2011) MALDI-imaging mass spectrometry—an emerging technique in plant biology. Proteomics 11(9):1840–1850

Kertesz V et al (2008) Comparison of drug distribution images from whole-body thin tissue sections obtained using desorption electrospray ionization tandem mass spectrometry and autoradiography. Anal Chem 80(13):5168–5177
Kaletaş BK et al (2009) Sample preparation issues for tissue imaging by imaging MS. Proteomics 9(10):2622–2633
Kertesz V, Van Berkel GJ (2008) Improved imaging resolution in desorption electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 22(17):2639–2644
Laskin J et al (2012) Tissue imaging using nanospray desorption electrospray ionization mass spectrometry. Anal Chem 84(1):141–148
Liu Y et al (2010) Imaging mass spectrometry with a low-temperature plasma probe for the analysis of works of art. Angew Chem Int Ed 49(26):4435–4437
Lozovoy VV et al (2008) Control of molecular fragmentation using shaped femtosecond pulses. J Phys Chem A 112(17):3789–3812
Luosujärvi L et al (2008) Desorption and ionization mechanisms in desorption atmospheric pressure photoionization. Anal Chem 80(19):7460–7466
Luosujärvi L et al (2009) Analysis of street market confiscated drugs by desorption atmospheric pressure photoionization and desorption electrospray ionization coupled with mass spectrometry. Rapid Commun Mass Spectrom 23(9):1401–1404
Lee YJ et al (2012) Use of mass spectrometry for imaging metabolites in plants. Plant J 70(1):81–95
Lagarrigue M et al (2011) Matrix-assisted laser desorption/ionization imaging mass spectrometry: a promising technique for reproductive research. Biol Reprod 86(3):74
Lane AL et al (2009) Desorption electrospray ionization mass spectrometry reveals surface-mediated antifungal chemical defense of a tropical seaweed. Proc Natl Acad Sci 106(18): 7314–7319
Li B et al (2011) Characterization of barley leaf tissue using direct and indirect desorption electrospray ionization imaging mass spectrometry. J Mass Spectrom 46:1241–1246
Muller T et al (2011) Direct plant tissue analysis and imprint imaging by desorption electrospray ionization mass spectrometry. Anal Chem 83(14):5754–5761
Manicke NE et al (2010) High-resolution tissue imaging on an orbitrap mass spectrometer by desorption electrospray ionization mass spectrometry. J Mass Spectrom 45(2):223–226
Mains J, Wilson CG, Urquhart A (2011) ToF-SIMS analysis of dexamethasone distribution in the isolated perfused eye. Invest Ophthalmol Visual Sci 52:8413–8419
Matusch A et al (2010) Cerebral bioimaging of Cu, Fe, Zn, and Mn in the MPTP mouse model of Parkinson's disease using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). J Am Soc Mass Spectrom 21(1):161–171
McMahon JM (1985) The analyses of six common vitamins by laser desorption mass spectroscopy. Anal Biochem 147(2):535–545
Nemes P, Vertes A (2007) Laser ablation electrospray ionization for atmospheric pressure, in vivo, and imaging mass spectrometry. Anal Chem 79(21):8098–8106
Nemes P et al (2008) Ambient molecular imaging and depth profiling of live tissue by infrared laser ablation electrospray ionization mass spectrometry. Anal Chem 80(12):4575–4582
Nemes P, Woods AS, Vertes A (2010) Simultaneous imaging of small metabolites and lipids in rat brain tissues at atmospheric pressure by laser ablation electrospray ionization mass spectrometry. Anal Chem 82(3):982–988
Nemes P, Barton AA, Vertes A (2009) Three-dimensional imaging of metabolites in tissues under ambient conditions by laser ablation electrospray ionization mass spectrometry. Anal Chem 81(16):6668–6675
Ovchinnikova OS, Kertesz V, Van Berkel GJ (2010) Molecular surface sampling and chemical imaging using proximal probe thermal desorption/secondary ionization mass spectrometry. Anal Chem 83(2):598–603

Ovchinnikova OS, Kertesz V, Van Berkel GJ (2011) Combining transmission geometry laser ablation and a non-contact continuous flow surface sampling probe/electrospray emitter for mass spectrometry based chemical imaging. Rapid Commun Mass Spectrom 25:3735–3740

Prideaux B, Staab D, Stoeckli M (2010) Applications of MALDI-MSI to pharmaceutical research. Meth Mol Biol 656:405–413

Pasilis S et al (2008) Using HPTLC/DESI-MS for peptide identification in 1D separations of tryptic protein digests. Anal Bioanal Chem 391(1):317–324

Paglia G et al (2010) Desorption electrospray ionization mass spectrometry analysis of lipids after two-dimensional high-performance thin-layer chromatography partial separation. Anal Chem 82:1744–1750

Pacholski ML, Winograd N (1999) Imaging with mass spectrometry. Chem Rev 99(10): 2977–3006

Pól J et al (2009) Automated ambient desorption–ionization platform for surface imaging integrated with a commercial Fourier transform ion cyclotron resonance mass spectrometer. Anal Chem 81(20):8479–8487

Prideaux B et al (2011) High-sensitivity MALDI-MRM-MS imaging of moxifloxacin distribution in tuberculosis-infected rabbit lungs and granulomatous lesions. Anal Chem 83:2112–2118

Richards AL et al (2011) Imaging mass spectrometry in transmission geometry. Rapid Commun Mass Spectrom 25:815–820

Roach PJ, Laskin J, Laskin A (2010) Nanospray desorption electrospray ionization: an ambient method for liquid-extraction surface sampling in mass spectrometry. Analyst 135(9):2233–2236

Shariatgorji M et al (2012) Controlled-pH tissue cleanup protocol for signal enhancement of small molecule drugs analyzed by MALDI-MS imaging. Anal Chem 84:4603–4607

Seeley EH, Caprioli RM (2012) 3D imaging by mass spectrometry: a new frontier. Anal Chem 84(5):2105–2110

Sripadi P et al (2009) In vitro analysis of metabolites from the untreated tissue of *Torpedo californica* electric organ by mid-infrared laser ablation electrospray ionization mass spectrometry. Metabolomics 5(2):263–276

Shrestha B, Vertes A (2009) In situ metabolic profiling of single cells by laser ablation electrospray ionization mass spectrometry. Anal Chem 81(20):8265–8271

Shrestha B, Patt JM, Vertes A (2011) In situ cell-by-cell imaging and analysis of small cell populations by mass spectrometry. Anal Chem 83(8):2947–2955

Shelley JT, Ray SJ, Hieftje GM (2008) Laser ablation coupled to a flowing atmospheric pressure afterglow for ambient mass spectral imaging. Anal Chem 80(21):8308–8313

Seeley EH, Caprioli RM (2011) MALDI imaging mass spectrometry of human tissue: method challenges and clinical perspectives. Trends Biotechnol 29(3):136–143

Takats Z et al (2004) Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science 306(5695):471–473

Takáts Z, Wiseman JM, Cooks RG (2005) Ambient mass spectrometry using desorption electrospray ionization (DESI): instrumentation, mechanisms and applications in forensics, chemistry, and biology. J Mass Spectrom 40(10):1261–1275

Thunig J, Hansen SH, Janfelt C (2011) Analysis of secondary plant metabolites by indirect desorption electrospray ionization imaging mass spectrometry. Anal Chem 83(9):3256–3259

Ullburg S (1977) The technique of whole-body autoradiography: cryosectioning of large specimens, in special issue on whole-body autoradiography. Instr J Sci Tools. O. Elvefeldt (ed) LDK-Producter AB, Bromma, Sweden, pp 2–29

Vertes A, Balazs L, Gijbels R (1990) Matrix-assisted laser desorption of peptides in transmission geometry. Rapid Commun Mass Spectrom 4(7):263–266

Venter A, Nefliu M, Cooks RG (2008) Ambient desorption ionization mass spectrometry. TrAC Trends Anal Chem 27(4):284–290

Van Berkel GJ et al (2008) Liquid microjunction surface sampling probe electrospray mass spectrometry for detection of drugs and metabolites in thin tissue sections. J Mass Spectrom 43(4): 500–508