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Review

A concise review of mass spectrometry imaging

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ABSTRACT

Mass spectrometric imaging allows the investigation of the spatial distribution of molecules at complex surfaces. The combination of molecular speciation with local analysis renders a chemical microscope that can be used for the direct biomolecular characterization of histological tissue surfaces. MS based imaging advantageously allows label-free detection and mapping of a wide-range of biological compounds whose presence or absence can be the direct result of disease pathology. Successful detection of the analytes of interest at the desired spatial resolution requires careful attention to several steps in the mass spectrometry imaging protocol. This review will describe and discuss a selected number of crucial developments in ionization, instrumentation, and application of this innovative technology. The focus of this review is on the latest developments in imaging MS. Selected biological applications are employed to illustrate some of the novel features discussed. Two commonly used MS imaging techniques, secondary ion mass spectrometric (SIMS) imaging and matrix-assisted laser desorption ionization (MALDI) mass spectrometric imaging, center this review. New instrumental developments are discussed that extend spatial resolution, mass resolving power, mass accuracy, tandem-MS capabilities, and offer new gas-phase separation capabilities for both imaging techniques. It will be shown how the success of MS imaging is crucially dependent on sample preparation protocols as they dictate the nature and mass range of detected biomolecules that can be imaged. Finally, developments in data analysis strategies for large imaging datasets will be briefly discussed.

Contents

1. Introduction

Mass spectrometry imaging, the combination of molecular mass analysis and spatial information, provides visualization of molecules on complex surfaces. The inception of MALDI-time-of-flight (MALDI-TOF) based MS imaging techniques in 1997 [1] has led to a surge in methodological and instrumental developments and subsequent applications of mass spectrometry imaging. In addition, the biological applications of time-of-flight SIMS (TOF-SIMS) have increased, due to the high spatial resolution offered by SIMS. Multi-modal MS imaging strategies, as well as the adaption of proteomics protocols, have made MS imaging a powerful tool for spatial localization and identification of elements, pharmaceuticals, metabolites, lipids, peptides and proteins in biological tissues. Fig. 1

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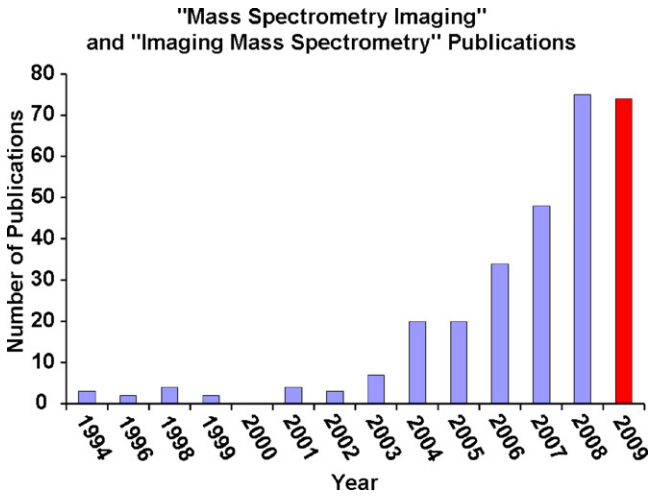

Fig. 1. Number of publications per year from an ISI Web of Science search of the topics "imaging mass spectrometry" and "mass spectrometry imaging".

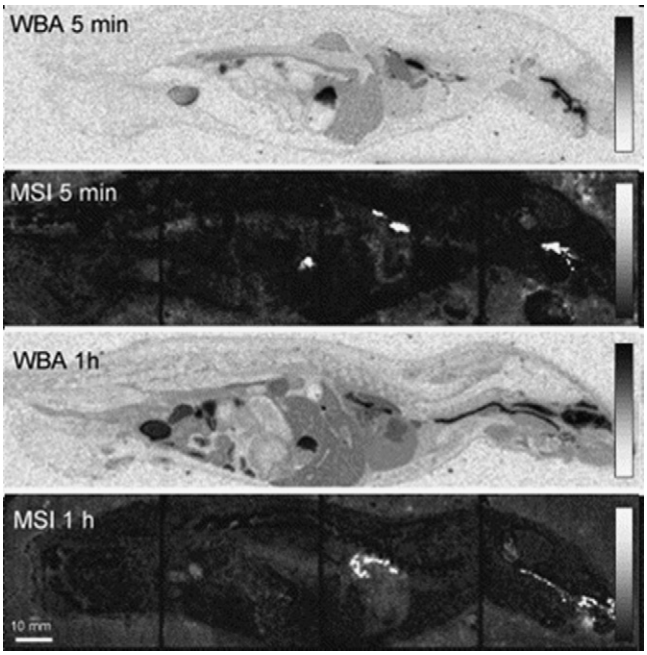

Fig. 2. Comparison of mass spectrometry imaging (MSI) with whole-body autoradioluminography (WBA) using whole-body sections after intra-tracheal administration of a compound (0.5 mg/kg) to rats. The two corresponding sections are from the same animal, but from different positions. The comparison of the methods shows remarkable similarity in the results: high levels are detected in the trachea, the lung and the stomach and lower levels in blood [30].

shows the number of publications per year from an ISI Web of Science search of the topics "imaging mass spectrometry" and "mass spectrometry imaging". Here, we will refer to "mass spectrometry imaging", MSI, or simply "MS imaging" to avoid the confusion of "imaging mass spectrometry", IMS, with "ion-mobility separation".

Mass spectrometry based "-omics" technologies have become routine for the analysis of biomolecules related to various diseases [2–5]. High-throughput methods combining mass spectrometry and separation techniques, such as liquid-chromatography and electrophoresis, have identified numerous biomarkers for diseases in bulk bodily fluids [6–8]. Biomolecules isolated from cell-lysates or tissue homogenates offer a more selective approach, also analyzed by MS based proteomics [4]. Laser capture microdissection (LMD) offers the highest regional selectivity and can be used for single cell analyses [9,10]. This approach is time consuming and requires a high number of cells in order to detect low abundance molecules. Voxelization and subsequent LC–MS of a mouse brain tissue section have been used for spatial mapping and quantitation of over 800 proteins, but at the cost of low spatial resolution ($1\,mm^3$) and extensive sample preparation [11].

Clinical imaging methods, such as magnetic resonance spectroscopy imaging (MRI), positron emission tomography (PET), immunostaining and fluorescent based techniques are used for the spatial localization of substances in biomedical studies [12–18]. Immunostaining is highly specific and allows the visualization of one analyte per histological section at high spatial resolution. Similarly, fluorescence tags allow highly specific visualization of proteins with high spatial resolution [18]. In both cases the spatial resolution is limited by classical diffraction theory (~200 nm). *In vivo* techniques, such as MRI and PET, target specific classes of molecules but with low specificity within the class. The spatial resolution of MRI varies from $1\,cm^3$ [19] to $1\,mm^3$ and new PET instrumentation designs been developed to archive less than $1\,mm^3$ isotropic volume resolution [20]. Mass spectrometry imaging allows for label-free discovery of multiple classes of biomolecules directly from a tissue surface, and can be combined with traditional imaging and proteomic methods.

MS imaging has been combined with MRI for complementary localization of proteins in mouse brain [21,22]. Biomedical applications range from neurodegenerative diseases [23] including spatial localization of amyloid beta peptides, related to Alzheimer's disease, on brain sections form transgenic mice [24] to protein detection on breast tumor xenograft and human formalin-fixed paraffin-embedded breast cancer [25] and cancer cell type discrimination [26]. The distribution of pharmaceuticals and their metabolites can be followed in whole-body sections [27], as well as in specific organs [28,29]. Fig. 2 shows whole-body sections comparing whole-body autoradioluminography (WBA) with mass spectrometry imaging (MSI) of a rat after compound administration. The results are comparable, while MS imaging precludes the need for compound labeling [30]. Also, TOF-SIMS has shown accumulation of several lipid classes as well as the depletion of vitamin E in nonalcoholic fatty liver [31] Desorption electrospray ionization (DESI) allows ambient sampling of tissue sections and has been used to map lipids in human liver adenocarcinoma, rat brain and several other tissue types [32]. These applications combined demonstrate the power of mass spectrometry imaging as a tool for drug discovery, proteomics and metabolomics research.

MS imaging studies began with laser microprobe mass spectrometry (LMMS) and laser microprobe mass analysis (LAMMA), commercially available in the late 1970s [33]. LMMS is used in the analysis of biological samples as well as inorganic samples. A focused UV laser pulse is used to desorb and ionize solid samples without matrix and different mass analyzers have been implemented (e.g. TOF and Fourier transform ion cyclotron resonance (FT-ICR)) [34–36]. In LAMMA, the laser beam is used to generate light pulses and ionizes the analytes which are accelerated into the mass spectrometer. This technique is used for the analysis of powdered samples, where the molecules are excited to an ionized state by a focused laser beam [37,38]. In addition, the development of static SIMS in the late 1960s, combined with the high-sensitivity of TOF instruments, has made TOF-SIMS a powerful tool for mass spectrometry imaging [39].

A TOF-MS is the mass analyzer of choice for both MALDI and SIMS mass spectrometry imaging. Speed, sensitivity and broad mass range detection (m/z ~1–100,000) make it attractive for imaging purposes. Recently, there have been a number of instrumental developments for MS imaging to increase throughput, mass resolving power, mass accuracy, MS/MS capabilities and spatial

resolution. Traveling-wave ion-mobility TOF adds a gas-phase separation step prior to analysis that aids analysis of complex samples to recover spatial information caused by unresolved peaks. In addition, the ion-mobility cell (or quadrupole in QTOF systems) can be used for improved MS/MS. The development of MALDI mass spectrometric imaging sources for high mass resolution and high mass accuracy mass analyzers, such as FT-ICR and FT-Orbitrap (FT-MS), allows for the separation of ions with the same nominal mass and confident assignment of elemental formulas for small molecules and lipids. Furthermore, various MS/MS techniques are available for FT-MS systems. The development of scanning microprobe MALDI for imaging can provide spatial resolution as low as 8 μm with the high-sensitivity ionization of biomolecules by MALDI [40].

Sample preparation is crucial for successful detection of desired molecules [41,42]. Recent advances in method development have increased both chemical and spatial information from imaging experiments. Washing protocols for tissue sections have proven useful for enhanced ionization of specific molecules in both SIMS and MALDI, likely due to the minimization of ion suppression due to the presence of salts and other undesired analytes [41,43]. Metal-enhanced and matrix-assisted SIMS allow for the detection of a broader range of analytes than unmodified surfaces [44]. In addition, the choice of MALDI matrix dictates the molecular weight range observed. Moreover, careful MALDI matrix deposition must insure adequate sample extraction without surface diffusion. The stretched-sample method [45] and dry matrix application [46] have proven useful for reducing surface diffusion of analytes.

In this concise review, we will discuss the current state and recent advances in mass spectrometry imaging instrumentation and method development. The focus will be on improvements for spatial and chemical resolving power, and increased sample throughput, with selected applications highlighting specific advances. Also, the advances in mass spectrometry imaging require new data treatment approaches for large imaging datasets, which will be briefly discussed. Finally, perspectives for mass spectrometry imaging will be discussed.

2. Ionization methodologies for imaging MS

2.1. Secondary ion mass spectrometry (SIMS)

SIMS uses high energy primary ions (e.g. Ar^+, Ga^+, In^+) to strike the sample surface. The primary ion penetrates the sample surface and induces a collision cascade with atoms and molecules in the surface region. Secondary ions are released from the surface when their kinetic energy is increased above the binding energy to the substrate. This typically occurs at a depth of 10 Å and is size independent. The detailed fundamentals on cascade theory and aspects of organic and inorganic SIMS are reviewed in detail elsewhere [39,47–49].

SIMS typically desorbs and ionizes elements and small molecules. The practical mass range is limited to ~m/z 1000 as a result of extensive surface fragmentation [39]. The use of a matrix in matrix-enhanced (ME)-SIMS can overcome this limit through the use of an organic MALDI matrix. This approach improves detection of higher mass species.

In SIMS, a distinction is made between the static mode or dynamic mode. Each technique has a different extent of surface damage and is used for different purposes. Static SIMS uses a primary dose lower than 10^{12} ions/cm^2, which minimizes the interaction of primary ions to the top monolayer of molecules. Each primary ion strikes an undisturbed region and the interaction between primary ion beam and atoms and molecules is limited to less than 1% [50–52].

Dynamic SIMS is more destructive, as a larger primary ion dose is used, which results in interactions with deeper sample surface layers. The differences between static and dynamic SIMS have a direct effect on their applications; static SIMS is largely used for qualitative imaging [53] while dynamic SIMS application consists primarily of quantitative elemental imaging [54–56]. The details of dynamic and static SIMS can be found elsewhere [39].

2.2. MALDI

The mechanism of ion formation in MALDI is a complex phenomenon. The desorption process and ion formation mechanisms have been intensively reviewed [57,58]. The addition of a matrix to sample surface serves several purposes: extraction of analytes from the sample surface and the formation of analyte-doped crystals; and absorption of laser energy for soft-ionization of sample molecules.

2.3. DESI

Desorption electrospray ionization (DESI) is performed by the interaction of electrospray generated charged droplets with the sample surface. The impact of the droplets with the surface produces a second generation of charged droplets with dissolved surface molecules. These secondary droplets proceed through an electrospray-type mechanism to form gaseous ions which are directed into an atmospheric inlet of the mass spectrometer [59,60]. The angle of the secondary electrospray plume and the MS inlet must be optimized to allow the maximum ion volume to enter the mass spectrometer. The ions measured directly from tissue surfaces by DESI are mainly singly charged lipids [28,32,59,61–64]. DESI allows ambient surface sampling without sample pretreatment, albeit with lower spatial resolution than SIMS and MALDI.

Table 1 summarizes important aspects of the common desorption/ionization methods used for mass spectrometry imaging.

3. Instrumentation

Recent instrumental developments aim to increase the throughput, mass resolving power, mass accuracy, tandem-MS capabilities and spatial resolution for mass spectrometry imaging. McDonnell et al. have constructed a sample handling system for automatic

Table 1
Desorption and ionization methods.

Source	Examples	Environment	Energy	Spot size (d)	Surface current	MW range (m/z)
Liquid metal Ion gun	Ga^+, In^+, Au^+ Au^{2+}, Au^{3+}	UHV	>25 eV	>1 μm	1–10 nA	0–3000
Solid-state gun	Cs^+	UHV	10 keV	2–3 μm	<10 nA	0–3000
C_{60}^+ cluster source	C_{60}^+	UHV	5 eV–40 keV	200 nm–200 μm		0–3000
MALDI	Nd:YAG, N_2 Nd:YLF	UHV, HV Ambient	100–200 μJ/pulse	5–300 μm	n/a	100–500,000
DESI	Solvent (e.g. H_2O, MeOH)	Ambient	n/a	>150 μm	0.5–50 nA [66]	100–66,000

introduction of sample slides from an environment controlled sample chamber to the mass spectrometer, for more time-efficient mass spectrometer use [65]. The Caprioli group has constructed two MALDI-TOF systems which operate in the continuous scanning mode with a 5 kHz laser, which show significant improvements in speed (>2×) over commercially available MALDI-TOF instruments [67].

While TOF-MS offers great throughput for MALDI MS imaging experiments, its lack of mass resolving power can result in loss of important chemical and spatial information. New technologies for MS imaging are under investigation and being implemented to overcome this limitation. Among these new technologies are ion-mobility based mass spectrometry imaging, MALDI FT-ICR MS and MALDI FT-Orbitrap. Ion-mobility separation offers a gas-phase separation dimension, based on the ions' collisional cross-section, which allows separation of ions at the same nominal mass, originating from the tissue surface or matrix clusters [68,69]. The combination of ion-mobility separation with MS imaging has been shown to improve the spatial mapping of the anti-cancer drug vinblastine, by the removal of an interfering endogenous lipid [27]. High performance mass spectrometry methods, such as FT-ICR MS and FT-Orbitrap, offer high mass resolving power and high mass accuracy, and FT-ICR MS been shown to resolve and identify ions at a single nominal mass that are commonly present in MALDI imaging mass spectra [64,70,71]. In addition, the high mass accuracy of FT-ICR has been used for high confidence identification of olanzapine and its metabolites from rat kidney (<1 ppm across the entire dataset) [22]. Fig. 3 shows MALDI FT-ICR MS spectra and mass selected images of hydroxymethyl olazapine and three ions at the same nominal mass from a dosed rat kidney. Further, the tandem-MS capabilities of FT-ICR MS have been shown for the identification of drugs and molecules directly from biological tissues [22,71,72].

Tandem-MS has traditionally not been available for static SIMS imaging instruments, due to the need to retain the spatial distribution of ions from the surface to the detector. However, the improvement of cluster-beam sources has improved the sensitivity, mass range and lateral resolution for biological SIMS [73].

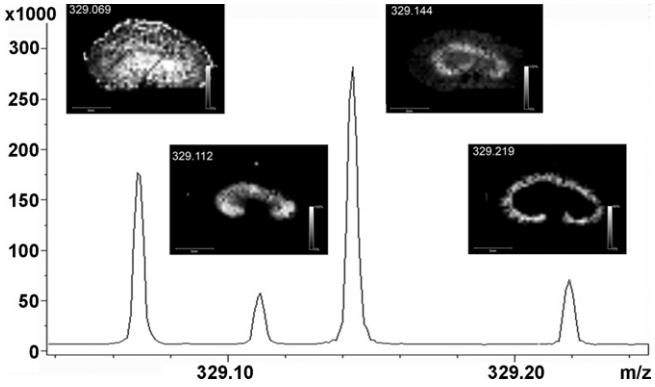

Fig. 3. FT-ICR MS images of hydroxymethyl OLZ and three other ions at m/z 329.2 [70].

The Winograd group has developed a C_{60} SIMS hybrid-quadrupole orthogonal-TOF-MS with tandem-MS capabilities [74]. This instrument has been employed to directly map gramicidin S (m/z 1141) under a copper grid at spatial resolution of 25–30 μm in both MS and MS/MS modes. Similarly, the Vickerman group has constructed a TOF-based instrument which employs a C_{60} ion source, operated in direct-current mode, with a secondary ion buncher, a collision-cell for MS/MS and a reflectron-TOF analyzer [75].

The increase of spatial resolution in MS imaging experiments is pursued through efforts to decrease the spot size of the laser or ion beam. A coaxial objective has been employed in MALDI decreasing the laser spot size to 1 micrometer or better [40]. However, fluence considerations limit the applicability of this approach. An alternative approach to increase the spatial resolution is the use of the microscope mode imaging technology [76]. This approach decouples the attainable spatial resolution from the desorption and ionization spot size. The spatial resolution is determined by the quality of the image forming ion optics and the quality of the position (X–Y) and time detector used. We have coupled a C_{60}

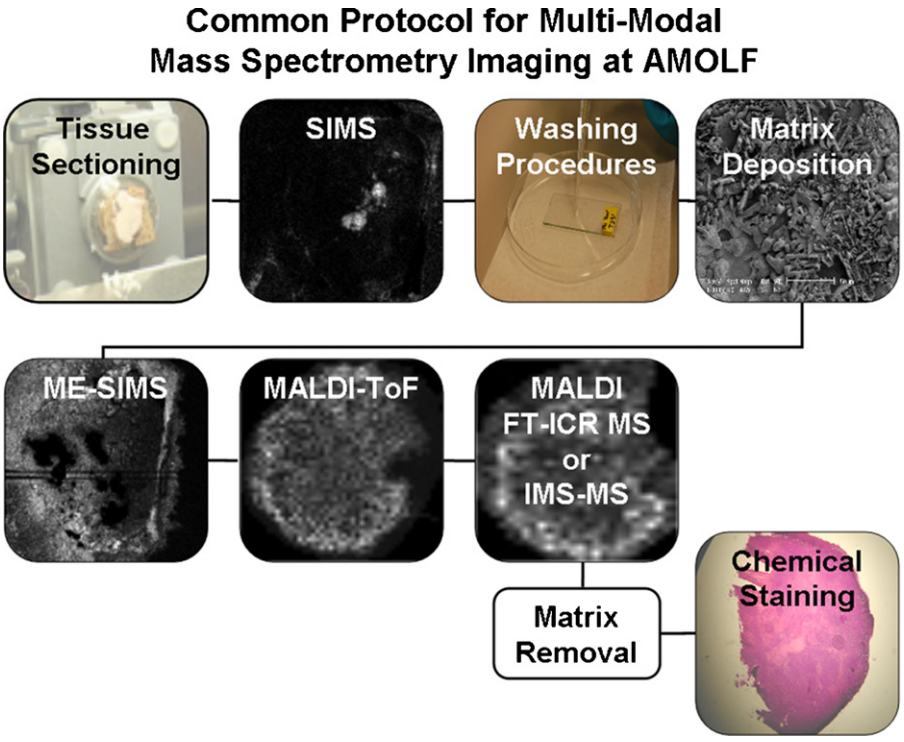

Fig. 4. A common multi-modal MS imaging protocol used at the FOM Institute AMOLF.

Table 2
Matrix selection for MALDI MS.

Matrix	Applications
2,5-Dihydroxybenzoic acid (DHB, gentisic acid) [77–79]	Lipids, small peptides, carbohydrates, nucleotides, glycopeptides, glycoproteins and small proteins
α-Cyano-4-hydroxycinnamic acid (HCCA) [79,80]	Peptides, glycopeptides, small proteins, glycoproteins, and oligonucleotides
3,5-Dimethoxy-4-hydroxycinnamic acid (SA, sinapinic acid) [79,81,82]	Proteins with MW > 10kDa, especially glycoproteins and hydrophobic proteins
4,6-Trihydroxyacetophenone (THAP) [79]	Oligonucleotides
3-Hydroxypicolinic acid (3-HPA) [79,83]	Oligonucleotides, peptides and glycoproteins
2,6-Dihydroxyacetophenone (DHA) [43,79,84]	Phospholipids
2,4-Dinitrophenylhydrazine (2,4-DNPH) [85]	Peptides, proteins (also for FFPE tissues)
HCCA/aniline [86]	Peptides
HCCA/N,N-dimethylaniline [86]	Peptides
HCCA/2-amino-4-methyl-5-nitropyridine [86]	Peptides

SIMS approach with a delay-line detector for microscope mode high spatial resolution (3–4 μm) TOF-SIMS imaging. The position-sensitive delay-line-detector allows the C_{60} source to be operated at full current and without the need for cluster-beam focusing [87].

4. Sample preparation

Several factors for MS imaging sample preparation must be considered, from sample collection to surface treatment prior to analysis. The proper and immediate treatment of tissue after surgical removal is essential to avoid degradation and spatial rearrangement of molecules [42]. A common protocol for multi-modal MS imaging at AMOLF is shown in Fig. 4.

Thin, flat surfaces are required for MS imaging analysis. Samples are typical prepared by cryo-sectioning on a microtome, where the temperature of sectioning depends on the type of tissue (e.g. fatty tissue requires lower temperatures than more water based tissues). The sectioned tissue slice can be directly applied on a conductive surface (e.g. metal MALDI target or indium tin oxide (ITO)-coated glass slide), for full tissue imaging. An alternate approach is the sample stretching method. Here, the tissue slice is deposited onto glass beads embedded in parafilm and stretched homogeneously in two dimensions and the parafilm is attached to a glass slide for stability. The stretching method allows increased spatial resolution and improved analyte extraction without diffusion concerns [45,88].

In both methods, the sample may require further sample preparation after the tissue section has been mounted onto a target. Washing steps, enzymatic digestion and matrix deposition can all be utilized depending on the mass analyzer, ionization method and desired molecule type. Several procedures and applications have been reviewed in past few years and frequently used protocols will be discussed here [41–44,89,90].

No washing steps are recommended if the molecules of interest are salts or lipids, as they are often removed with common washing solvents. One or more wash steps are recommended prior to matrix deposition for peptides or proteins [41]. The most commonly used washing procedure for the removal of surface lipids or salts is 70–80% cold ethanol (4 °C) [42,89,91]. The removal of salts, which may react with the matrix, allows homogeneous crystallization of the matrix, which increases the ionization efficiency of peptides and proteins. Salts and lipids are readily ionized, and may cause ion suppression and saturation of the detector. A more severe washing procedure with organic solvents such as chloroform, hexane or acetone can improve peptide and protein detection on older tissues sections [92]. Harsh washing procedures, however, may damage the sample and remove peptides of analytical interest. It is recommend to optimize matrix and washing procedures on adjacent slices, in order to evaluate the best sample preparation protocol to be used in each sample type. For DESI, there is typically no sample pretreatment.

On-tissue enzymatic digestion (e.g. tryptic digestion) can be performed to improve protein detection and identification capabilities. This method is always preceded by washing steps to prevent denaturation or deactivation of the proteolytic enzymes used. [11,25,85,90,93]. On-tissue digestion protocols are typically accompanied by analysis of an adjacent slide for intact protein identification.

4.1. Surface treatment for SIMS

The upper mass limit in SIMS can be partially overcome by dedicated surface treatments. Here, we will briefly describe two surface modification techniques that are commonly used in biomedical imaging studies; metal assisted SIMS (MetA-SIMS) and matrix-enhanced SIMS (ME-SIMS).

For MetA-SIMS, a thin layer (1–5 nm) of metal (e.g. gold) is coated on top of the sample. MetA-SIMS enhances the ionization of several species, including cholesterol and lipids [44]. The metal layer can be quickly and reproducibly applied using plasma sputter coaters.

ME-SIMS uses an organic MALDI matrix (e.g. α-cyanno-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid) deposited on the surface of the sample. ME-SIMS can increase the mass range to m/z ~2000 and peptides not detectable in normal SIMS preparations can be observed. Matrix deposition is the critical step for ME-SIMS, since the crystal size determines the spatial resolution for this method. Remarkably, ME-SIMS yields spectra similar to MALDI. Static mode ME-SIMS consumes a significantly lower amount of surface material than MALDI. Thus, the same tissue can subsequently be used for other desorption/ionization methods that require a matrix.

4.2. Matrix selection and deposition for MALDI and ME-SIMS

Matrix deposition for both MALDI and ME-SIMS is another crucial step in the sample preparation protocol for MS imaging. Matrix deposition must be homogeneous, reproducible, provide sufficient sensitivity and should be easy to use. In addition, the size of the matrix crystals determines the maximum spatial resolution attainable. Matrix deposition may be performed with or without previous wash steps for biological samples (e.g. tissue from biopsy) [41]. Below, we will discuss a number of matrix deposition considerations for MS imaging.

The successful detection of analyte molecules depends on the correct choice of the MALDI matrix. The matrix must not react with the analytes in the tissue section and should posses a low sublimation rate. The latter is important since most MS imaging studies are conducted under HV or UHV conditions, but not for ambient desorption methods that employ a matrix. In addition, the type and molecular weight of the analytes of interest must be considered when choosing a MALDI matrix. Table 2 lists common MALDI matrices and their applications for biological MS imaging.

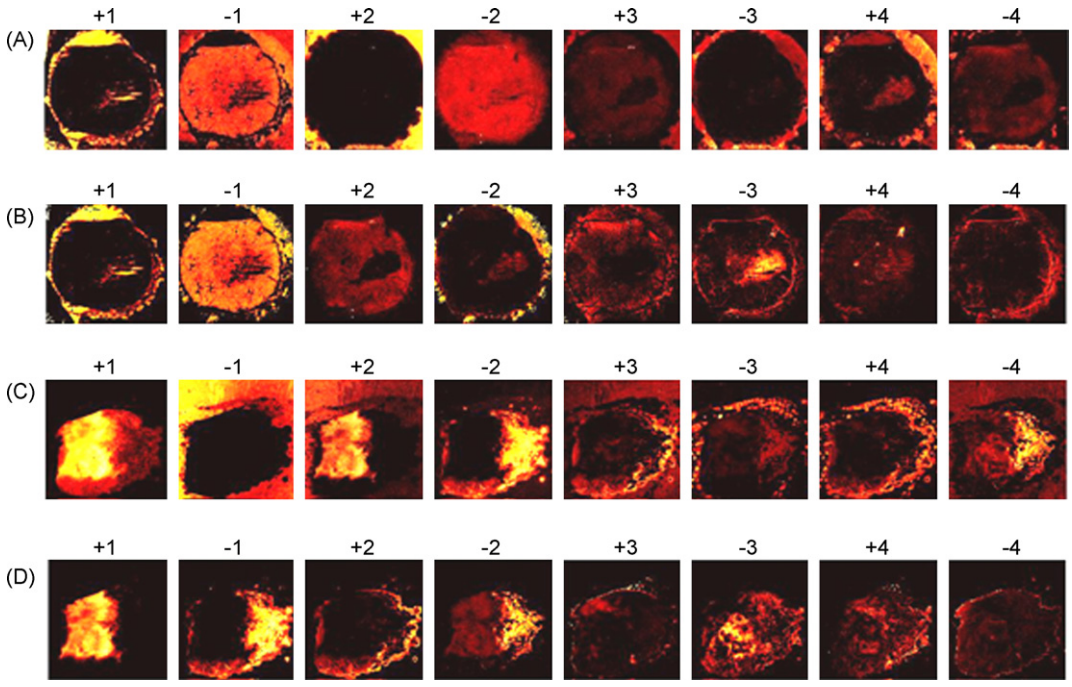

Fig. 5. PCA analysis of SIMS imaging dataset of (A) xenograft human breast tumor MDA-MB-231 and (B) the data from section A after removal of component +2 (background removal); (C) xenograft human breast tumor MCF-7 and (D) the data from section C after removal of component −1 (background removal) [53].

The matrix deposition method for MALDI and ME-SIMS MS imaging should also be carefully considered. The matrix layer should be as homogeneous as possible, in order to avoid local variations in desorption and ionization. The environmental conditions (e.g. humidity, presence of oxygen) during matrix deposition are also important, since they may result in poor interaction between matrix and analytes. For example, if the matrix reaches the surface already or almost dry there is little opportunity for extraction of surface analytes into the matrix. Conversely, a film of matrix solution can lead to surface diffusion [94,95]. The principal methods for matrix deposition methods for mass spectrometry imaging are briefly described below. A more in-depth review of this subject matter can be found elsewhere [41].

- *Dried-droplet method:*
  The matrix solution is applied on the sample manually with a pipette. This method is simple and fast and ideal for profiling, but it is not recommended for imaging purposes. Diffusion may occur inside the matrix spot, the size of the spot is typically large and there may be irregular distribution of matrix crystals.
- *Pneumatic nebulization:*
  The matrix solution is sprayed onto the sample with a hand-held thin layer chromatography sprayer or airbrush [42,44]. A gentle spray allows the formation of a homogeneous crystal layer on the sample surface. The spray has to be optimized previous to each application, thus reproducibility may be an issue. This method can also be automated in a controlled environment such as shown by Stoeckli et al. [96].
- *Chemical inkjet printer:*
  Small droplets of matrix solution are applied with a piezoelectric droplet printing device. This technique allows the deposition of small volumes per spot (100 pL) per cycle. Each single matrix spot has a diameter of 150 μm or smaller, dependent on the total amount of solution applied per cycle. Examples of devices are the chemical inkjet printer (ChIP) [97,98] of Shimadzu, the acoustic reagent multi-spotter of Labcyte [99] and the acoustic matrix deposition method developed by Aerni et al. [99]. The deposition is precise, highly reproducible and the droplets are uniform. Due to the droplet size, it is more suitable for lower spatial resolution MSI. Several home build device using inkjet printing technology have also been reported in literature [100].
- *Automated vibrational spray coater (ImagePrep by Bruker Daltonics):*
  This commercial device is dedicated for MALDI imaging MS and produces a homogeneous matrix layer with small crystals and operates in a closed system. Control of droplet size limits surface diffusion and the interaction time between analytes and matrix can be optimized for maximum co-crystallization [101].
- *Matrix sublimation:*
  Solid matrix is placed on the bottom section of a condenser and the sample is attached to the condenser with double sided thermally conductive tape facing the solid matrix. The sublimation occurs under low pressure and high temperature (approximately 125 °C) which allows the matrix to condense on the cold sample surface. This method provides enhanced purity of matrix applied to the sample, small crystal size, and uniformity of deposition [102].
- *Dry-coating:*
  Finely ground solid matrix is filtered directly onto the tissue surface through a 20 μm sieve. This method is useful for fast and reproducible matrix application for analysis of lipids. Similar to the sublimation method, surface diffusion is limited due to the absence of solvents [46].

The choice of the matrix deposition is governed by the type of application pursued. Automated methods are better for large scale samples and high-throughput studies. The manual methods are favored when testing new protocols. Some of the matrix deposition devices, such as the Chip-1000, the ImagePrep and the Labcyte spotter are also suited for the deposition of proteolytic enzymes for on-tissue digestion. Matrix deposition technologies are being researched heavily, as they may prove to be the limiting factor for future high-throughput biomedical studies, as rapid and reproducible matrix deposition is currently difficult.

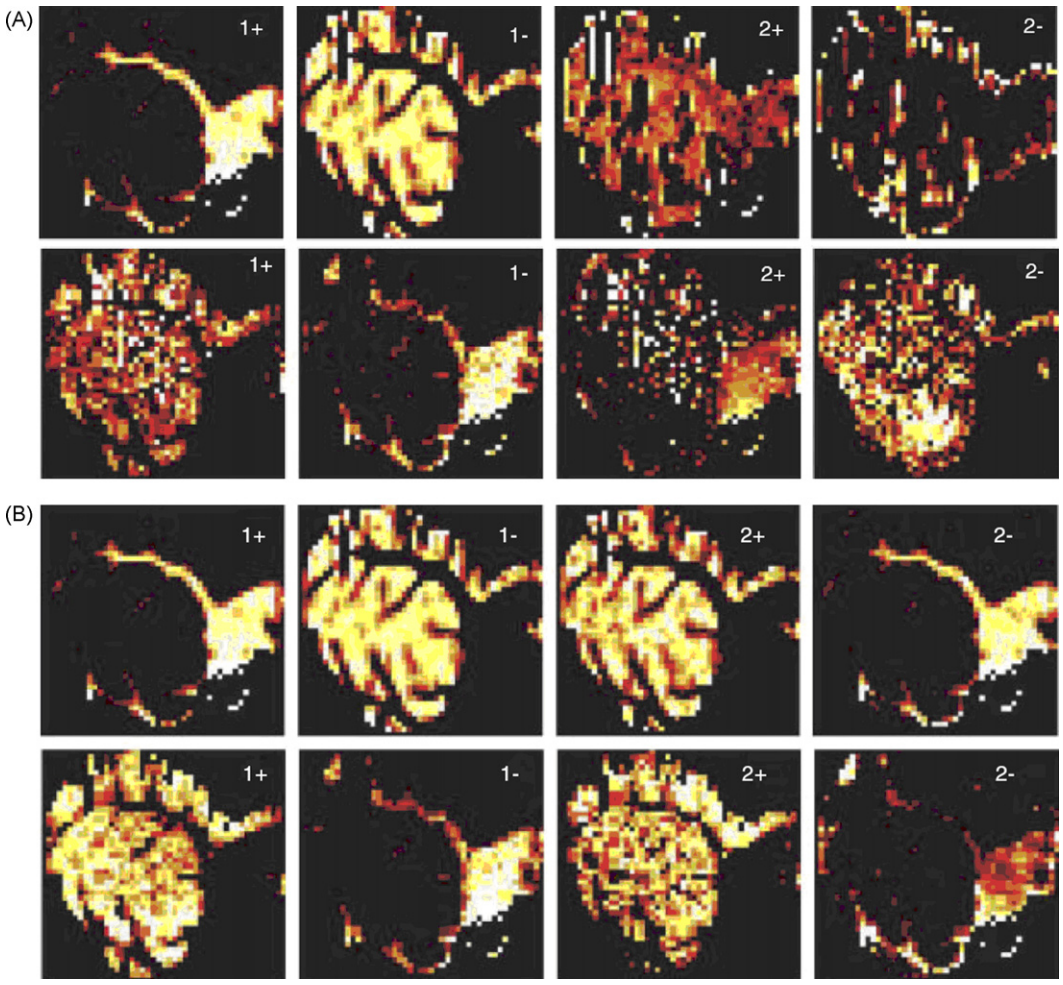

Fig. 6. (A) Principle component analysis score images for SIMS (top row) and MALDI (bottom row) data of human cerebellum tissue. (B) Canonical correlation analysis score images for SIMS (top row) and MALDI (bottom row) data of human cerebellum tissue [53].

5. Data analysis

A number of different software packages are available to generate images from position correlated mass spectra. These include Novartis' BioMap, Brukers' Flex software series and AMOLFs' "datacube" generator and viewer. The data generated by a MS imaging experiment can vary from >100 Mbytes in size up a few Gbytes per sample, and is dependent on the spatial resolution and mass analyzer used. Thus, manual interpretation of the data often becomes very difficult and time consuming. To these ends, a number of statistical software based tools have been developed to aid in MS imaging data interpretation.

Principal component analysis (PCA) [103,104] is a widely used multivariate data analysis method that is easily applied to MS imaging datasets. PCA is an unsupervised method that is used to identify groups of closely correlated variables; spatial coordinates and mass for MS imaging. Fig. 5 shows the use of principal component analysis with AMOLF's ChemomeTricks toolbox for MATLAB version 7.0 (The MathWorks, Natick, MA) [53]. Here, components that are associated with areas outside the tissue are subtracted and yield improved correlations of the remaining components.

Where PCA can have non-negative components, probabilistic latent semantic analysis (pLSA) results in non-negative components decomposition of imaging datasets that combine with noise reduction and automated tissue classification, also called digital staining [105,106]. PCA combined with canonical correlation analysis (CCA) can be used to correlate MS imaging data obtained from different techniques. This approach correlates the spatial and spectral components from each dataset, and leads to improved individual results. Fig. 6A shows PCA scores (top) for SIMS and MALDI MS imaging of human cerebellum, and correlation scores via CCA analysis are shown in Fig. 6B. Correlation of the results leads to marked improvement of component 2+/− for both SIMS and MALDI datasets. Quantification of correlations between mass spectrometry images has also been described [107].

6. Perspectives

Recent developments, from instrumentation to sample preparation, have improved the sensitivity, spatial resolution and identification capabilities for mass spectrometry imaging by SIMS and MALDI. These improvements are broadening the applications of MS imaging for lipid, peptide and protein biomarker identification, as well as drug and metabolite imaging. The combination of MS imaging, direct tissue MS/MS, and standard bioanalytical protocols (e.g. LC–MS/MS and electrophoresis) will allow confident identification of pathologically relevant compounds and their localization in cells and tissues. Efforts toward three-dimensional image construction will allow more global understanding of tumor environments and organ physiology. In addition, the integration of MS imaging with current clinical imaging techniques (e.g. MRI,

PET and CT) promises chemically specific complimentary spatial information.

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