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Direct Molecular Analysis of Whole-Body Animal Tissue Sections by MALDI Imaging Mass Spectrometry

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Abstract

The determination of the localization of various compounds in a whole animal is valuable for many applications, including pharmaceutical absorption, distribution, metabolism, and excretion (ADME) studies and biomarker discovery. Imaging mass spectrometry is a powerful tool for localizing compounds of biological interest with molecular specificity and relatively high resolution. Utilizing imaging mass spectrometry for whole-body animal sections offers considerable analytical advantages compared to traditional methods, such as whole-body autoradiography, but the experiment is not straightforward. This chapter addresses the advantages and unique challenges that the application of imaging mass spectrometry to whole-body animal sections entails, including discussions of sample preparation, matrix application, signal normalization, and image generation. Lipid and protein images obtained from whole-body tissue sections of mouse pups are presented along with detailed protocols for the experiments.

Keywords

Imaging mass spectrometry; whole-body sections; drugs; proteins; lipids

1. Introduction

MALDI imaging mass spectrometry (MS) is a powerful tool for acquiring molecularly specific profiles directly from tissue sections. Many analyte classes present in biological sections, including peptides, proteins, lipids, and endogenous metabolites, may be analyzed using this technology. Thus, one can extend this technology to the localization of very different compounds throughout a whole-body section. Whole-body imaging can therefore illuminate a broad picture of the biological state of an animal at a given point in time in both health and disease. One can identify what analytes co-localize together, where analytes are present or absent, and what analytes may be expressed at the same time but in different places. This type of information will be invaluable in expanding our understanding of basic biological processes, the origin and progression of disease as well as the response to drug treatments.

The pharmaceutical industry routinely uses quantitative whole-body autoradiography (QWBA) in order to map the distribution of drug compounds in animals with time. QWBA

requires the use of a radiolabeled drug compound, and indeed only the radiolabel is followed in the experiment. Nonetheless, the results give a global picture of the localization of the drug in the body and may alert investigators to unforeseen potential toxicological issues. Performing the QWBA experiment with imaging MS provides much more information because the compounds of interest are detected with high molecular specificity, with the dosed drug differentiated from metabolites that differ in mass. Protein targets of the drug may also be analyzed from the same or a serial section, providing additional information.

Imaging MS can be directly applied to larger, whole-body tissue specimens for small molecule, lipid, or protein analyses. The general theory, instrumentation, and advantages and limitations to the experiment are the same. The main differences will be discussed in the following section.

1.1. Unique Aspects of the Whole-Body MS Imaging Experiment

Due to the large size and multiple tissue types typically present in a whole-body section, there are specific considerations that must be taken into account when preparing the tissue for analysis, as well as for acquiring and processing the data.

1.1.1. Specimen Size and Sectioning—Acquiring sections from whole animals requires special considerations due to the increased size of the specimens. The bodies of adult rats can be up to approximately 30-cm long, while adult mice can approach 12-cm long, and obtaining sections from these whole animals requires a cryostat designed for larger specimens. As a result, the sample preparation for whole animals is more complex than the simple flash-freezing approach used for most single organs (1).

After sacrifice, the animal must be frozen, preferably after exsanguination to minimize blood pooling and artifacts that arise from compounds present in the blood. Freezing must occur while the animal is in a satisfactory position for obtaining sections in the desired plane (sagittal, coronal, or axial). This is typically accomplished by resting the animal in a foil or plastic boat, on their back or on their side, while soaking in a dry ice/hexane bath. Prior to sectioning, the frozen animal must be encased in a block of ice or other embedding media in order to stabilize the whole body and minimize tearing of the sections. As with smaller sections, care must be taken to ensure the embedding media (typically a small percentage of carboxymethylcellulose (CMC)) does not interfere with the MALDI signal. Pure water usually stabilizes the specimen adequately without adversely affecting the mass spectrum (2). This is illustrated in Fig. 17.1, which shows a 3-day-old mouse pup embedded in a block of ice (A) and a 1-day-old mouse pup mounted on a cryostat chuck with OCT embedding compound (B), after sagittal sections have been cut.

Due to the presence of multiple organs in a given section, additional stabilization is required to keep the organs together and maintain their spatial integrity once removed from the animal. Typically this is accomplished by acquiring the section onto a piece of tape. For whole-body autoradiography, this is acceptable, as it does not interfere with the subsequent radioactivity analysis. However, for MALDI, this is problematic for several reasons. First, the tapes used (acetate film tape) are non-conductive and may induce charging effects and mass shifts, depending on the instrument used. Second, they must be affixed to standard

MALDI sample plates for insertion into the instruments. This is accomplished using with conductive double-sided tape. Care must be taken in any event to ensure even transfer of the section to the MALDI plate (avoiding air bubbles, etc.). Third, certain compounds may preferentially interact with the tape and may not be easily extracted into the matrix. Alternatively, signals originating from the tape itself may cause ionization suppression or otherwise adversely affect the MALDI signal. Overall, this whole-body section mounting approach has been found useful for the imaging MS of drugs and their metabolites on MALDI QqTOF and ion trap systems. In these instruments, where the ionization source is decoupled from the mass analyzer, the nonconductive behavior of the sample is not a factor.

An alternative to tape collection is to collect whole-body sections on rice paper which is affixed to the acetate tape. When the sections are cut they adhere to the rice paper instead of the acetate tape. These are then are transferred (thaw-mounted) to the MALDI target plate. In this case, the sections are directly mounted on the target plate without the use of tape. This is beneficial because the adverse affects of the tape are avoided; however, this approach generally results in poor section quality because transfer efficiency is often unequal for different organs and across the section.

Another protocol may be used that minimizes the conductivity and ion suppression issues present with acetate tape. The tape transfer methodology (CryoJane tape transfer system, Instrumedics, Inc., St. Louis, MO, USA) (3) utilizes a proprietary tape instead of the acetate tape. Once the section has been obtained, it is placed on a glass slide pre-coated with a UV-sensitive adhesive. Irradiation of the section with a flash of UV light releases the tape and binds the tissue to the activated adhesive present on the slide. Whole-body sections obtained using the CryoJane tape transfer system have been found to be of much higher quality than those obtained using the rice paper approach. In order for the slide to be used as a MALDI sample plate, it must be in a shape and size that can fit in the instrument holder and must be conductive (ITO coated) when used in time-of-flight mass spectrometers. Conductive glass slides and plates are available in larger formats and have been successfully used for MALDI analysis (4) and in particular with this tape transfer system. For example, Fig. 17.1c shows a 20- μ m thick whole-body section cut from the ice block containing the 3-day-old mouse pup (shown in Fig. 17.1a) using a full-body cryostat and mounted on a conductive glass slide. Although the CryoJane transfer approach removes the tape from the sample that will be analyzed, it does introduce a new variable in the adhesive that is applied to the glass slide. The effects of the adhesive on the MALDI experiment still need to be fully examined.

Finally, due to the large size of some specimens, the entire section may be too big to fit onto a standard MALDI plate. For whole-body sections, the larger microtiter format MALDI sample plates can hold a whole-mouse section or approximately half of an adult rat section. The largest commercially available conductive glass slides (and matching target holder) are 50 $\times$ 75 mm and will hold at best a (small) whole-mouse section. Thus whole sections must be divided, often into several smaller sections, and each section affixed to its own MALDI plate. This can induce experimental variations, as data will be acquired from each sub-section independently.

Size may not be a limiting factor as some specimens are small enough in size to be cut using standard biomedical cryostats. This is demonstrated in Fig. 17.1d which shows a 12- μ m thick section cut from the 1-day-old mouse pup in Fig. 17.1b. This pup has a length of only ~3 cm; sections can then be directly thaw-mounted on MALDI target plates, in this case a conductive glass slide. When using rice paper, the CryoJane transfer approach or direct thaw-mounting, sample conductivity is not an issue since the sections are in direct contact with the MALDI target plates. These approaches are therefore preferred for imaging MS of lipids, peptides, and proteins using MALDI TOF mass spectrometers.

1.1.2. Matrix Application—Proper application of matrix is critical in obtaining a meaningful image in MALDI mass spectrometry (1). Application of matrix to whole-body tissue sections requires additional considerations. Choice of matrix to be used depends on the analyte of interest, as with single organs. Commonly, sinapinic acid is used for proteins, CHCA is used for peptides, and DHB is used for lipids and most pharmaceutical compounds. To date, matrix deposition for imaging MS of whole-body sections has been performed by manual spray deposition. The spray system usually consists of a small pneumatic nebulizer (typically used for thin layer chromatography) coupled to a high-pressure nitrogen gas outlet via a regulator. Matrix is prepared in the appropriate solvent system and manually sprayed on the sections. For whole-body sections mounted on tape, the solvent that the matrix is dissolved in must be compatible with the tape used. Dissolution of the tape, loss of adhesion of the sample to the plate, and/or poor MALDI signal may result from a matrix solution too high in organic composition. A homogeneous matrix coating is progressively built by alternating spraying and drying cycles. Matrix deposition by spray on large surfaces such as from whole-body sections is fast (typically ~1 h); however, the risk of delocalizing analytes during the spray process is high. (For more information on analyte extraction and migration, *see Chapter 8.*) Further, the quality of the mass spectra in terms of signal-to-noise and the overall number of detected mass peaks is significantly poorer compared to the spectral quality obtained from discrete matrix spots. This is particularly true for peptides and proteins (5).

Matrix may crystallize differently on different organs (2), and the signal response for a single compound may be affected by the unique microenvironments present in different organs (6). This is obviously an issue for whole-body analyses, where multiple organs are present in each section. Addition of an internal standard to the matrix solution may allow normalization to account for these differences, although this is not straightforward either. However, such a normalization approach is of value for imaging MS of single compounds such as administered pharmaceuticals. In this case, it is preferable to use a drug compound with a similar structure but a different molecular weight (ideally, a stable isotope-labeled analog). For lipid or protein imaging MS, it is difficult to find one internal standard that would be of use for all compounds of interest in the tissue. In these cases, normalization to the total ion current (TIC) may be a useful approach (see Section 1.1.5).

1.1.3. Imaging MS Data Acquisition—Imaging MS data acquisition from whole-body sections is performed in the same manner as from any other tissue section. (For more information on imaging of biological tissue sections, *see Chapter 15.*) Phospholipids,

peptides, and proteins are directly acquired in the MS mode generally using MALDI TOF instruments in the linear mode (proteins) or reflex mode (peptides and phospholipids). Other MALDI mass spectrometers may also be used. To minimize acquisition time per spot (or pixel), the minimal number of laser shots necessary to acquire quality data needs to be optimized. Although imaging MS of administered drugs (and their metabolites) can be acquired in the MS mode, MS/MS mode (or selected reaction monitoring, SRM) is often used to increase sensitivity and specificity by following a unique precursor/fragment transition (7, 8). In this case, MALDI QqTOF or ion trap mass spectrometers are generally used. (For more information on imaging MS of pharmaceuticals, see **Chapter 5**.)

1.1.4. Time—The increased sample size has effects on the time required for the experiment. As with all imaging experiments, the time required is a function of resolution (essentially the number of pixels) and acquisition time per pixel. These times are further influenced by instrumental parameters, such as laser frequency and time required for motor movements, as well as the duty cycle of the instrument used. The most obvious difference for whole-body images is an increase in the total number of pixels. Even for a moderate resolution image (~500 μm), the number of pixels it takes to image a whole-rat section (assume ~6×17 cm) is ~40,000. This assumes a rectangular shape; if the instrument can be programmed to only scan the tissue, this could reduce the total number by several thousand to ten thousand pixels. Nonetheless, assuming a relatively fast scan speed of 1 s/pixel, mass spectral acquisition would still require approximately 10–12 h.

1.1.5. Data Processing—The processing of imaging MS data files has proven to be useful in removing matrix deposition artifacts resulting in sharper recovered ion images (9). Processing imaging MS data typically consists of a series of pre-processing steps including background/noise reduction and smoothing and may include steps of signal realignments and data normalization. Signal realignment is typically based on a series of stable signals (~10) selected throughout the acquired mass range. For whole-body imaging MS data in particular, realignment can be performed only if enough mass signals are found to be stable throughout the section. Considering the huge variations in tissue types and molecular compositions encountered through whole-body sections, this is rarely the case. However, spectral realignment is only necessary if obvious mass shifts are observed during the course of data acquisition potentially due to sample plate charging or progressive source contamination from the ablated matrix. Starting with a clean ion source usually permits imaging MS data acquisition without observation of significant peak shifting.

Normalization of imaging MS data by total ion current (TIC) within a predetermined m/z range has proven to be useful in improving the quality of the recovered ion images (9). For whole-body imaging MS, normalization by TIC carries the risk of skewing the data resulting in incorrect ion signal images and thus needs to be carefully evaluated. MS data normalization by TIC assumes that the total ion signal for each pixel is somewhat constant or at least does not significantly deviate from an average value. Considering the very different tissue types encountered during the course of imaging MS of whole-body sections, this is rarely the case. For example, MS signals recovered from bone tissue typically produce a very low TIC in comparison with brain or liver tissues which produce very high TIC.

Further, some tissues contain in high abundance one or several proteins that will overwhelm the spectra and produce obvious ion suppression effects. This, for example, is the case for lung tissue from which intense hemoglobin signals are detected. This is again a situation where the validity of MS data normalization by TIC needs to be carefully evaluated. For whole-body imaging MS, other normalization alternatives still need to be explored.

1.1.6. Ongoing Improvements—As outlined above, challenges still exist in all of the aspects of whole-body imaging from tissue sectioning and handling, matrix application, and imaging MS data acquisition, visualization, and analysis. Because of the potentially large size of the whole-body tissue sections, significant improvements within the analytical workflow have the potential to reduce the overall time of analysis. As seen above, alternative tape transfer methodologies (3) to more efficiently transfer and stabilize whole-body sections onto (conductive) glass slides are emerging in the field of whole-body imaging MS. Background signals coming from binding polymer have, however, been observed and may interfere with the expression of tissue signals. Most manufacturers of MALDI mass spectrometers have adopted the Society for Biomolecular Screening microtiter sample plate format which allows the mounting of larger tissue sections. Special sample plate holders able to accommodate for larger dimension glass slides have also been designed by some manufacturers. This allows imaging MS of whole (mouse)-body sections in a single experiment avoiding analysis of multiple independent tissue pieces, from which the results have to be electronically reassembled.

Matrix deposition can also be a time-limiting step. Usually, matrix deposition for whole-body imaging is performed by manual spray coating. Although rapid to perform, significant section-to-section coating variability can be observed, even in experienced hands. Beyond analyte delocalization issues, in most instances, MALDI MS signal quality, especially for peptides and proteins, can be significantly degraded with respect to the quality recovered from individual matrix spots (5). Current matrix deposition alternatives involve automated matrix deposition using acoustic (see below) or Piezo-based matrix printing systems (10, 11). These offer very reproducible matrix deposition conditions without analyte delocalization as well as high-quality MALDI MS signals. Other alternatives include systems for automated spray deposition. These allow a precise control of the spray conditions including spray height, volume, temperature, and movement over the sections (9). For phospholipid imaging MS, matrix deposition via sublimation (12) or dry coating (13) is also a rapid and attractive alternative (see below).

The acquisition speed of mass spectrometers has also greatly improved within the past decade. Whereas early imaging MS experiments were performed with MALDI TOF instruments operating at laser repetition rates of a few hertz, modern systems routinely acquire MS data at repetition rates of 200–1000 Hz. The next generation of (TOF) mass spectrometers is expected to work at repetition rates of several kilohertz with continuous sample stage movement. This will further streamline data acquisition by reducing the overhead data writing time to the computer. Such improvements should significantly reduce imaging MS data acquisition times, for whole-body imaging in particular.

1.2. Examples from the Literature

To date, several reports have been published using MALDI imaging MS on whole-body (rat and mouse) tissue sections. Images of peptides, proteins, drugs, and metabolites have been presented. All of the published reports have used spray coating for application of matrix, but otherwise, they have differed in application and methodology. The earliest detailed report describes the acquisition of protein images as well as drug and metabolite images from rat whole-body sections (2). MS data were acquired on a MALDI TOF instrument for the protein analysis, while tandem mass spectra were acquired via a MALDI QqTOF instrument for drug and metabolite imaging. Interestingly, for the protein analysis, a mix of matrices (sinapinic acid and 2,5-dihydroxybenzoic acid (DHB), 4:1) was found to provide the best conditions in terms of tissue coverage and signal intensity, while DHB alone was utilized for the small molecule experiments. This report was the first to show imaging MS of a dosed drug (olanzapine, m/z 313) and two of its phase-one metabolites from one experiment as shown in Fig. 17.2. The MS/MS imaging results for olanzapine correlated quite well for location with autoradiographic results and quantity with LC–MS/MS results from individual organ extracts.

Another approach to imaging dosed compounds involves using MS scans alone (14, 15). This was demonstrated for both an injected β -peptide (15) and a small molecule with its metabolites (14) in whole-body rats and mice. The advantage to this approach is mostly speed of acquisition, as all signals obtained in the full mass range can be imaged in one experiment. However, it lacks the unambiguous identification of compounds that is typically obtained from an MS/MS experiment.

One recent application has incorporated ion mobility separation into the whole-body imaging experiment (16). In this case, the anticancer drug, vinblastine, was detected from whole-body rat sections. Comparisons of images obtained via conventional MS imaging to imaging with ion-mobility separation show that the latter significantly reduces chemical noise by eliminating interferences from isobaric ions, likely lipids. For example, the product ion of vinblastine at m/z 355 was shown overlaid onto a background of an endogenous lipid in the brain, with separation by ion mobility clearly showing vinblastine localized only to the ventricle.

Whole-body imaging MS has also started to be used to explore the proteome of other (non-vertebrate) animals. For example, some efforts have been focused on identifying protein signals associated with the nervous system of the medicinal leech (17, 18). Examples of peptide and protein whole-body imaging from zebrafish sections have also been reported by the Bruker Daltonics Corporation at the 2005 HUPO meeting.

2. Materials

2.1. Sample Preparation

1. Embedding polymer, such as optimal cutting temperature (OCT) polymer used to affix the specimen to the cryostat chuck (OCT, -50°C frozen section medium, Richard-Allan Scientific).

2. MALDI-compatible indium-tin oxide-coated glass slides (Delta Technologies Ltd., Stillwater, MN, USA).
3. Cryostat (CM 3050 S, Leica Microsystems, GmbH, Wetzlar, Germany).
4. 70 and 95% v/v isopropanol in water for section fixation and washing prior to protein imaging MS (HPLC grade).

2.2. Histological Staining

1. Microscope slides (ColorFrost microscope slides with clipped corners, Fisher Scientific).
2. Hematoxylin solution: 1 g hematoxylin, 25 g aluminum potassium sulfate, 400 ml water, 100 ml glycerol, 0.1 g sodium iodate.

Dissolve hematoxylin in glycerol. Dissolve aluminum potassium sulfate in 375 ml of water. Dissolve sodium iodate in 25 ml of water. Add aluminum potassium sulfate solution to hematoxylin solution, mix well. Add sodium iodate solution, mix well. Filter complete solution before use. The remaining solution should be wrapped in foil to protect from the light.

3. Eosin and phloxine solution: 57 ml 1% phloxine, 1 g phloxine B, 100 ml water (Milli-Q), 50 ml eosin.
4. Rinsing solutions: ethanol (200 proof), water (Milli-Q), xylene (ACS grade, Acros).
5. Cytoseal XYL mounting medium (Richard-Allen Scientific).
6. Slide coverslips (Fisher Scientific).

2.3. Matrix Application

1. For protein imaging: 20 mg/ml solution of sinapinic acid (Sigma, St. Louis, MO, USA) in 50:50 v/v acetonitrile:water with 0.2% trifluoroacetic acid.
2. Robotic spotter (Portrait 630, Labcyte, Sunnyvale, CA, USA).
3. For lipid imaging: 2,5-dihydroxybenzoic acid, finely ground (Sigma, St. Louis, MO, USA).

2.4. Data Acquisition and Processing

1. For protein imaging: Autoflex II MALDI TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA).
2. For lipid imaging: Ultraflex II MALDI TOF/TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA).
3. For image acquisition and visualization: FlexImaging 2.0 software (Bruker Daltonics, Billerica, MA, USA).

3. Methods

We present below two examples of protein and lipid whole-body imaging acquired by MALDI MS from sagittal sections obtained from a 1-day-old mouse pup.

3.1. Cryostat Sectioning

1. The pup was sacrificed by isoflurane gas.
2. Since the animal was small, about 1×3 cm, it was directly mounted on a cryostat chuck (Fig. 17.1b).
3. Thin 10-μm sections were cut at −21°C using a cryostat.
4. The sections to be imaged were thaw-mounted on MALDI compatible (indium-tin oxide-coated) glass slides (4).
5. Serial sections were also cut, thaw-mounted on regular microscope slides, and stained by hematoxylin and eosin (H&E). This allows alignment of the imaging MS data with mouse histology (Figs. 17.3a and 17.4a).

3.2. H&E Staining

1. Hematoxylin and eosin staining for thin tissue sections mounted on glass slides was performed in a truncated manner from that typically performed for high-resolution histological approaches. The quality of the stain is sufficient for alignment of the MS ion images to the histological image. The truncated H&E staining protocol used is as follows: Serially immerse the slide in 95% ethanol (30 s), 70% ethanol (30 s), water (30 s), hematoxylin solution (2 min), water (20 s), 70% ethanol (30 s), 95% ethanol (30 s), eosin solution (1 min), 95% ethanol (30 s), 100% ethanol (30 s), and xylene (2–2.5 min).
2. Remove excess xylene by gentle wiping.
3. Deposit a small drop of cyto seal solution on section and gently deposit the coverslip slide on top of the cyto seal drop. Make sure the coverslip covers the entire tissue section. For whole-body sections, several coverslip slides may be necessary.

3.3. Protein Image Protocols

1. The section to be analyzed for proteins was rinsed with 70 and 95% isopropanol solutions according to published protocols (19) to improve overall signal detection from all parts of the body.
2. Sample coating for imaging MS was achieved by printing arrays of small matrix droplets using a Portrait 630 reagent multispotter (10). The volume for each droplet was estimated to be ~170 pL. Sinapinic acid was printed in arrays with a center-to-center distance of 400 μm. By interlacing four print patterns, a final center-to-center spacing between spots of 200 μm was achieved.

3. Each print pattern was repeated for 30 cycles ejecting one drop of matrix per cycle. The final array consisted of a rectangular $48 \times 142 = 6,816$ spot pattern spaced by 200 μm center-to-center (Fig. 17.3b) (*see* Note 1).
4. The section was analyzed by MALDI TOF MS using an Autoflex II mass spectrometer operated in positive linear mode geometry under delayed extraction conditions time focused at $\sim m/z$ 15,000. In this case, signal resolutions (M/M measured at full width at half maximum) close to $\sim 1,000$ were attainable for ions at or around m/z 15,000, whereas acceptable resolutions in the range of 600 were obtainable for m/z values in the range of 5,000–25,000.
5. One mass spectrum was acquired per matrix spot. Each spot was analyzed in the same manner by averaging signals from 250 laser shots acquired in five series of 50 shots with each series acquired at a different location within the spot using a random walk pattern.
6. The collected mass spectra were baseline corrected before image assembly.
7. Imaging MS data was acquired, assembled, and visualized using FlexImaging 2.0 software. Data acquisition of the 5,325 matrix spots on tissue took 6 h 47 min generating 3.85 GB of data.
8. From each matrix spot (pixel), between 200 and 300 protein signals were typically detected with some of these being very organ or tissue specific. Figure 17.3c presents an overlay of 12 ion images from protein signals with some specificity for different tissues or organs. For example, the signal at m/z 4,062 was found to have a very strong expression in dental tissue whereas the signal at m/z 10,530 was found in higher abundance in the pancreas. Other signals such as m/z 5,679 specific for muscle and m/z 7,368 consistent with fat deposits were found throughout the section.

3.4. Lipid Image Protocols

1. The section to be analyzed by imaging MS for lipids was left unrinsed.
2. Finely ground 2,5-dihydroxybenzoic acid (DHB) used as matrix was homogeneously deposited onto the section using a dry-coating technique, in which solid matrix particles were filtered directly onto the tissue through a 20- μm stainless steel sieve as previously described (13) (*see* Note 2).
3. Imaging MS data acquisition was performed using an Ultraflex II TOF/TOF mass spectrometer operated in positive reflex mode geometry under optimized delayed extraction conditions time focused at $\sim m/z$ 800. In this case, signal resolutions (M/M) above 10,000 were routinely attainable for the phospholipid mass range from m/z 600–1,000.
4. Data acquisition was performed over the entire tissue section at a spatial resolution of 300 μm accumulating signals from 300 successive laser shots per position.
5. The collected mass spectra were baseline corrected before image assembly.

6. Imaging MS data was acquired, assembled, and visualized using FlexImaging 2.0 software. Data acquisition of the 5,444 spots on tissue took 5 h 23 min generating 1.0 GB of data.
7. From each position, intense phospholipid signals were observed with some of these being very organ or tissue specific. Figure 17.4c presents an overlay of six ion images from phospholipid signals with some specificity for different tissues or organs. For example, the signal at m/z 844.51 was found to have a very strong expression in liver, whereas the signal at m/z 810.60 was uniquely found in the adrenal gland. Other signals such as m/z 691.04 abundant in muscle and m/z 822.51 consistent with fat deposits were found throughout the section.

3.5. Conclusions

Clearly, imaging MS has tremendous analytical potential for interrogating whole-body tissue sections. Many analyte classes present in biological specimens – including lipids, peptides, proteins, endogenous metabolites, and administered drug compounds – can be analyzed and imaged via MALDI mass spectrometry. As with so many advanced technologies, “the devil is in the details”. Application of imaging MS to large, very heterogeneous whole-body sections requires special considerations in terms of experimental design, sample handling, data acquisition, data processing, and image generation in order to obtain meaningful data. While not trivial, these extra challenges can be overcome, and the tremendous stores of biological information present in a whole animal can begin to be understood. The methods and protocols presented in this chapter should help interested researchers successfully apply imaging MS to their own applications.

4. Notes

1. For this particular example, because of the relatively small dimensions of the section, matrix deposition using an automated printing approach was possible within a time frame of about 4 h. Matrix has also been successfully deposited on larger sections from rats and mice using the same approach but with larger center-to-center distances in order to keep printing times within a two working day period. Compared to manual matrix spray deposition, the spatial resolution with printed arrays is limited by the center-to-center distances between spots. Since imaging MS from whole-body sections is typically done at lower resolutions (200–500 μm) to keep acquisition times manageable, this is typically not an issue.
2. The resulting crystalline film of matrix is fairly homogeneous (Fig. 17.4b) and allows imaging by MALDI MS with spatial resolutions as small as 30 μm (13). Further, because of its simplicity, this matrix coating technique is easily applicable to larger whole-body tissue sections. Since matrix is applied dried on the section, this eliminates any risks of analyte delocalization.

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Fig. 17.1.

Generation of whole-body tissue sections from fresh frozen mouse pups. Photomicrographs of (a, c) a 3-day-old pup frozen in a block of ice from which 15- μ m thick sections were cut using a whole-body cryostat and mounted on conductive glass slides using the CryoJane system; (b, d) a 1-day-old pup held into position using OCT from which 12- μ m thick sections were cut in a biological specimen cryostat and directly thaw-mounted on conductive glass slides. See text for details.

Fig. 17.2.

Olanzapine (oral administration, 8 mg/kg) and metabolite distribution at 2 h post-dose in a whole-rat sagittal tissue section monitored by imaging MS. Organs are outlined in *red* on the photomicrograph of the section (a). MS/MS ion images of olanzapine (b) and its *N*-desmethyl (c) and 2-hydroxymethyl (d) metabolites are displayed. (Adapted from Ref. (2) with permission.)

Fig. 17.3.

Whole-body imaging MS of proteins from a 1-day-old mouse pup. (a) H&E stained section from which numerous organs are clearly visible. (b) Serial section used for imaging MS on which matrix (sinapinic acid) has been automatically deposited in an array manner with a final center-to-center spacing of 200 μm . (c) Overlay of 12 individual organ or tissue-specific protein images, each presented with a different color. See text for details.

Fig. 17.4.

Whole-body imaging MS of lipids from a 1-day-old mouse pup. (a) H&E stained section from which numerous organs are clearly visible. (b) Serial section used for imaging MS on which matrix (DHB) has been homogeneously applied using the dry deposition approach. (c) Overlay of six individual organ or tissue-specific lipid images, each presented with a different color. See text for details.