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SIMS and MALDI MS imaging of the spinal cord

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Abstract

The application of mass spectrometry to imaging, or MS imaging (MSI), allows for the direct investigation of tissue sections to identify biological compounds and determine their spatial distribution. We present an approach to MSI that combines secondary ion mass spectrometry (SIMS) and MALDI MS for the imaging and analysis of rat spinal cord sections, thereby enhancing the chemical coverage obtained from an MSI experiment. The spinal cord is organized into discrete, anatomically defined areas that include motor and sensory networks composed of chemically diverse cells. The MSI data presented here reveal the spatial distribution of multiple phospholipids, proteins, and neuropeptides obtained within single, 20- μ m sections of rat spinal cord. Analyte identities are initially determined by primary mass match and confirmed in follow-up experiments using LC MS/MS from extracts of adjacent spinal cord sections. Additionally, a regional analysis of differentially localized signals serves to rapidly screen compounds of varying intensities across multiple spinal regions. These MSI analyses reveal new insights into the chemical architecture of the spinal cord and set the stage for future imaging studies of the chemical changes induced by pain, anesthesia, and drug tolerance.

Keywords

Imaging; MALDI MS; Peptides; SIMS; Spinal cord

1 Introduction

Understanding the nature of complex biological processes occurring in a tissue or organism requires identifying the compounds involved and determining where they are located. Most biological and pathological processes in the body, including cell-to-cell signaling, disease, and therapeutic or illicit drug action, involve the dynamic spatial redistribution and interaction of a broad spectrum of compounds ranging from simple molecular or elemental ions to macromolecular complexes. Investigating the chemical complexity of biological tissues entails a vast array of analytical tools. One such discovery tool, mass spectrometry, has become increasingly integral to the study of complex biological systems. In a single MS experiment, one is able to examine complex samples, detect and identify a large number of known and unknown compounds, as well as characterize unknown compounds.

Adding an imaging modality to mass spectrometry serves to integrate the multiplexed detection capability of MS with a spatially resolved sampling process. The mass spectrometry imaging

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(MSI) approach can be used to detect and probe the molecular content of tissues in an anatomical context. MSI creates distribution maps of selected compounds in a similar manner to the more traditional biochemical techniques such as chemical staining, immunohistochemistry, and radiochemistry. However, unlike MSI, these traditional methods are often limited by the specificity of the applied labels, the number of compounds that may be studied simultaneously, and the requirement of *a priori* knowledge of the target compounds. These capabilities have made MSI particularly attractive as a discovery tool, which may be used to guide future, more in-depth studies of novel compounds or those that display interesting spatial localization.

Interest in MSI, particularly of biological tissues, has grown considerably in recent years, focusing on the development and application of secondary ion mass spectrometry (SIMS) and MALDI MS. Tissue sample preparation for MSI experiments are starting to become standardized [1,2], although methodological developments and abstractions continue [3–6]. The two ionization approaches are relatively complimentary in nature. SIMS is well-suited for higher spatial resolution imaging over a lower mass range (<1000 Da), whereas MALDI MS has a lower spatial resolution, but a much broader mass range (~500 Da to >100 kDa). Beyond imaging considerations, MALDI MS may also be used to identify detected compounds via MS/MS. As such, MALDI MS has become a workhorse for proteomic and peptidomic studies when linked with LC separations and fraction collections [7–9].

Traditionally a surface analysis technique, the high spatial resolution afforded by SIMS is particularly attractive for the imaging of tissue sections and single isolated cells, often at subcellular resolution [10–12]. As examples, SIMS has been used to study the subcellular distribution of vitamin E in the cellular membrane [12], lipid changes during *Tetrahymena* mating [13], and semiquantitate cholesterol in differentially treated macrophages [14]. Additional studies involving the analysis of diseased [15–18], adipose [19], kidney [20], aortic [21], and neuronal tissues [22–25] have provided valuable information regarding their involvement in multiple biological processes.

Having recently garnered significant interest, the use of MALDI MS for imaging is now applied to a broad range of scientific inquiry, with current attention focusing on various aspects of disease pathology [26] and pharmaceutical treatment [27], as well as examining fundamental biological questions. Native peptide and protein imaging in neurological systems is of great interest and has been used to study single neurons [28], crustacean [29] and cricket neuronal tissues [30], rat pituitary [31] and brain sections [3,5,26,32,33].

Here, we use SIMS imaging, MALDI imaging, and LC MALDI MS/MS to study the native distribution and identity of atomics, lipids, and peptides in rat spinal cord sections. The spinal cord transmits neural signals between the peripheral nervous system and the brain, and thus serves as a potent model system for the study of neuronal transmission, pain, anesthesia, and drug tolerance. The gross morphology of the spinal cord consists of white matter, the peripheral region containing sensory and motor neurons and myelinated axons, which surrounds the gray matter, composed primarily of neuronal cell bodies.

Both SIMS and MALDI MSI have been used in this work to increase the mass range of imaged compounds. In addition, LC-coupled MALDI MS/MS of extracts from serial sections of the spinal cord increases confidence in the identification of signals observed with the MALDI MSI. This combination of techniques can be implemented by collecting sections for LC-MALDI MS/MS that are nearly identical to the imaged tissue. Ion images for a large number of both identified and unknown compounds provide information on the native composition and distribution of biologically relevant compounds in the spinal cord.

2 Experimental

2.1 Chemicals

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

2.2 Tissue dissection

Protocols for animal care and procedures were approved by the UIUC LACAC and are in full compliance with federal guidelines for the humane care and treatment of animals. Spinal tissue sections were prepared from non-anesthetized adult Long-Evans rats (Harlan, Indianapolis, IN). Briefly, following rapid decapitation, the vertebral column was exposed immediately and transected at approximately L1. A 20-gauge syringe needle was inserted into the vertebral foramen at L1 and artificial cerebral spinal fluid (aCSF) (in mM 125 NaCl, 5 KCl, 1.25 NaH₂PO₄, 2 MgSO₄, 2 CaCl₂, 25 NaHCO₃, 10 glucose at pH 7.4) was pressure-injected by means of a 50-cc syringe. The positive pressure from aCSF injection typically extruded spinal tissue out of the opposite C1–3 vertebral column region previously transected by decapitation.

Collected spinal tissues were immediately wrapped in aluminum foil and snap-frozen in liquid nitrogen. Tissues were stored at -80°C until needed for further preparation (< 8 h). Spinal tissues were typically obtained and frozen within 2–3 min of decapitation to limit analyte redistribution and/or enzymatic degradation. The anatomical integrity of tissue cross-sections was visually assessed under 20 $\times$ magnification.

2.3 Tissue preparation for imaging

On the same day as the dissections, thin tissue sections (20 μm) were prepared with a cryomicrotome (Microm International, Walldorf, Germany) at -20°C . Segments of spinal cord from the upper thoracic region (~ 2 cm caudal to cervical decapitation) were affixed to the microtome sample stage with a small quantity of water to eliminate the need for an embedding medium (e.g., TissueTek), which can otherwise cause significant mass spectral interference or suppression of analyte signals acquired from biological samples [34]. Sections were then thaw-mounted to raw silicon shards (Montco Silicon Technologies, Spring City, PA) or indium-tin oxide coated glass slides (Delta Technologies, Stillwater, MN) for SIMS or MALDI MS experiments, respectively. Several sections immediately adjacent to the sections for imaging were collected into a vial for LC MALDI MS as outlined below. Visual observation was used to confirm the preservation of gross morphology during preparation. SIMS samples were analyzed without further treatment. Samples for MALDI MSI were then briefly rinsed in a dry ice-ethanol solution to remove excess salts and dried under nitrogen. Tissues were then manually coated with CHCA matrix (10 mg/mL) sprayed from a 1:1 acetone:water solution with 0.5% TFA via an artist's airbrush in several thin layers, which were allowed to nearly dry between each spray cycle.

2.4 SIMS

SIMS imaging experiments were performed on a TRIFT III TOF secondary ion mass spectrometer (Physical Electronics, Chanhassen, MN) equipped with a 22 keV gold liquid metal ion source. The primary ion beam (Au^{+}) was pulsed at 8 kHz with a 25 ns pulse width and scanned across the sample surface in a 256×256 raster pattern in a random manner. Positive and negative ion mass spectra were collected in the m/z 1–2000 range in consecutive acquisitions at a mass resolution of ~ 3000 ($m/\Delta m$ @ FWHM, m/z 384). The total primary ion dosage was kept well below the static limit ($< 10^{13}$ primary ions cm^{-1}). Although the ion beam was focused to a sub-micron spot size, the spatial resolution of the presented ion images is 2.3 $\mu\text{m}/\text{pixel}$ as limited by the spacing between positions in the raster, which is determined by the spatial dimensions of the area analyzed. No charge compensation was used. All data analyses

were performed with Wincadence software (Physical Electronics). Although many ions were of sufficient intensity to create images without manipulation, some images were “convolved” such that each pixel is effectively expanded to a 3×3 pixel size and overlapping intensities are then summed. This simplifies image visualization without greatly reducing the spatial resolution of the resultant image. All convolved images are noted as such in the figure captions.

2.5 MALDI MSI

MALDI mass spectra were acquired using an Ultraflex II TOF-TOF mass spectrometer (Bruker Daltonics, Billerica, MA) with a solid-state UV laser capable of a variable repetition rate. Positive-ion mass spectra were collected at 100 Hz in the reflectron mode with a delay time of 20 ns and an acceleration voltage of +25 kV. Mass spectra were acquired at 125- μ m intervals in a regular raster pattern. Each unsmoothed mass spectrum is the sum of 100 laser shots. All mass spectra were externally calibrated and data analyses were performed with the FlexAnalysis and FlexImaging software packages (Bruker Daltonics). Additionally, BioMap (Novartis, Basel, Switzerland) was used for region-of-interest analyses by importing the imaging file produced by the FlexImaging software.

2.6 Tissue Extracts

In order to increase the degree of confidence in the analyte identifications, adjacent sections not destined for the imaging experiments were collected, homogenized, and extracted in acidified acetonitrile (60:40:1 ACN:H₂O:TFA) overnight at 4 °C. Insoluble material was pelleted via centrifugation (5804R, Eppendorf, Hamburg, Germany) for 10 min at $15,000 \times g$. The supernatant was then dried (Savant SpeedVac, Thermo, Milford, MA) and resuspended in minimal 5% ACN prior to use of a 10 kDa molecular weight cut-off filter to remove large proteins from the sample (Millipore, Billerica, MA). The supernatant was then dried and resuspended in minimal 5% ACN prior to LC separation.

2.7 LC MALDI MS and MS/MS

Extracts were chromatographically separated with a capLC (Micromass, Manchester, UK) HPLC system coupled to a robotic fraction spotter (ProteineerFC, Bruker Daltonics). Portions (10 μ L) of the extract were injected using a manual injector (Valco Instruments Co, Inc., Houston, TX) and loaded onto a trap column (PepMap, C18, 5 μ m, 100 Å, Dionex, Sunnyvale, CA) and washed for 5 min. The trapped peptides were then eluted onto a reversed phase capillary column (LC Packings 300- μ m i.d. $\times$ 15 cm, C18 PepMap100, 100 Å, Dionex) at a 2 μ L/min flow rate. Fractions were collected at 2-min intervals directly onto a stainless steel MALDI target using the robotic spotter. Aliquots of 2,5-dihydroxybenzoic acid (1 μ L, 10 mg/mL, 50% ACN, 0.1% TFA) were then manually applied to the collected fractions on target. Mass spectra were collected using the same parameters as in the imaging experiment. These spectra were analyzed using both FlexAnalysis and WarpLC (Bruker Daltonics). Selected signals were fragmented using the MS/MS capabilities of the Ultraflex instrument. Tandem mass spectra were used for both partial *de novo* sequencing, comparisons to predicted fragmentation products from known neuropeptides, and Mascot searches to verify analyte identities with the BioTools software package (Bruker Daltonics).

3 Results and Discussion

3.1 SIMS imaging

To relate chemical composition to distinct anatomical regions within the tissue, a 20- μ m thick section of spinal cord has been imaged. By collecting both positive and negative ions in consecutive acquisitions, the distribution of an increased number of compounds may be studied in a single experiment. As shown in Fig. 1, the distribution of a wide variety of secondary ions

ranging from atomic ions to intact fatty acids has been imaged in the spinal cord. The selected ion images are expected to be highly specific as several peaks are often visible within a single m/z range and only a single peak is used to create the ion images. In addition, matrix effects should be minimal as ion images from non-specific organic fragments such as C_5H_9 (m/z 69⁺) appear homogenous across the imaged area. Many of the ion images show a particular localization in either the gray matter (dark central region in Fig. 1A), which contains the cellular bodies, or white matter (light peripheral region in Fig. 1A), which contains myelinated axons. This will be discussed in greater detail below.

SIMS has traditionally been used for atomic analyses and we observe a number of atomic species in our images. For example, a marked increase in the sodium (Fig. 1B) and potassium cation signals (Fig. 1C) are observed in the gray matter, although they are detected in both regions of the spinal cord. The ion signal relating to the $C_5H_9^+$ non-specific organic fragment (Fig. 1D), however, is relatively homogenous across the tissue, with some slight decrease in the white matter. Yet, when the intensity is normalized to total ion count, the regional difference is minimal. As is expected, the CH^- fragment ion was observed at high intensity across the tissue, with some increase in the white matter (Fig. 1J).

Phosphate ions (PO_3^-) are indicative of phospholipids (with some additional signal expected from genetic material) and were detected at elevated levels in the gray matter, matching the observed localization of various ions corresponding to choline (Fig. 1E) and phosphocholine (Fig. 1G), which are fragments of phosphatidylcholine (PC) and sphingomyelin lipids. Additionally, a signal at m/z 122⁻, which corresponds to the phosphatidylethanolamine (PE) lipid head group [35], was elevated in the gray matter (Fig. 1L). Cholesterol, however, was present in the white matter (Fig. 1H, I), with no apparent difference in distribution between the $[M-H]^+$ and the $[M-OH]^+$ ions of cholesterol. These distributions were expected on the basis of previously reported analyses of brain sections whereby lipid-related ions were present in the tissue region enriched in cellular bodies, while cholesterol was associated with the myelinated axons [22,24,25].

Several signals corresponding to fatty acids show some variation in their spatial distribution that correspond to the level of unsaturation. The images from the 16-carbon fatty acids, palmitoleic acid (C16:1) and palmitic acid (C16:0), vary in their distribution such that palmitoleic acid is more diffuse across the tissue (Fig. 1N) than palmitic acid (Fig. 1O), which is localized in the gray matter. A similar trend was observed for the C18 fatty acids including linoleic acid (C18:2) (Fig. 1P), oleic acid (C18:1) (Fig. 1Q), and stearic acid (C18:0) (Fig. 1R). These differences in localization based on the level of fatty acid unsaturation have been previously reported [36].

Several unknown compounds were also imaged including m/z 107⁺ (Fig. 1F) and m/z 237⁻ (Fig. 1M). Although these compounds remain unknown, with MSI we can examine the distribution of such compounds without prior knowledge of their identity. Identification of unknown ions can be driven by interesting or functionally relevant distributions. Additional ion images of both known and unknown compounds are presented in the Supporting Information (Fig. S1), as is a table of the imaged signals (Table S1). These ion distributions were confirmed by analyzing similar spinal tissue sections from other rats.

3.2 MALDI MSI

The analysis of peptide and protein distribution in tissues is complicated by their often low levels of expression and tendency to fragment under SIMS conditions. Therefore, we applied traditional MALDI MSI protocols to examine the distribution of peptides within a similar spinal cord section as was used for the SIMS experiments. During method optimization, we observed that the application of a dry-ice-cooled ethanol rinse greatly improved matrix crystallization

and signal intensities across the tissue. Under these conditions, we did not observe any indication of analyte redistribution during rinsing or spreading from the tissue to the surrounding glass substrate. Care was taken during matrix application to prevent analyte redistribution and none was observed; no biologically relevant signals were obtained from positions just immediately adjacent to the tissue sections.

Several selected ion images of peptides detected in the spinal cord section are presented in Fig. 2. The images selected here were chosen based on their signal identification by LC-MALDI MS/MS and interesting distribution patterns. Additional images of both identified and unidentified signals are presented in the Supporting Information (Figs. S2 and S3). As in the SIMS images, ion distributions were confirmed via analysis of similar sections collected from additional rats. For ease of comparison, an optical image (Fig. 2A) has been outlined to delineate the gray and white matter within the tissue section. As could be expected, several signals were detected from myelin basic protein (Fig. 2B). Similar distributions were observed for several other fragment products of myelin basic protein and are presented in the Supporting Information (Fig. S2).

A number peptides of particular interest were observed. For example, substance P (Fig. 2C) shows a unique localization in the dorsal horn of the spinal cord gray matter in agreement with previously published immunohistochemical localizations [37,38]. Multiple peptides from the proSAAS precursor, SAAS (Fig. 2D) and PEN (Fig. 2I), were also detected and similarly distributed. Although both compounds had increased levels in the gray matter, the images illustrate possible subtle shifts to their distribution in the spinal cord. Similarly, the endogenous opioid, proEnkephalin A (219–229) (Fig. 2G), is predominately expressed in the dorsal horn of the spinal cord, although it does appear, to a limited extent, within the white matter as well. Both substance P and the enkephalins have been shown to modulate nociception [39], again confirming the role of the dorsal horn as a major integrator of pain transmission pathways. Other peptides or protein fragments such as PEP-19 (Fig. 2E), complexin fragment 1–13 (Fig. 2F) and chromogranin B (Fig. 2H), although more homogeneously distributed within the gray matter, are also present in the white matter.

3.3 LC MALDI MS and MS/MS

Confident identification of signals arising directly from thin tissue sections is hindered when compounds are present at low abundances. Although success has been had via *in situ* enzymatic digestion and MS/MS directly from tissues [40], such experiments largely have focused on structural components or small molecules, which may be present at much higher levels than many of the neuropeptides detected here. All imaged signals presented in Fig. 2 were identified by MALDI MS/MS analysis of an extract from adjacent tissue sections following fractionation by LC. The compounds identified via MS/MS are presented in Table 1. The selection of these ions for MS/MS analysis was driven primarily by the detection of signals with matching masses directly from the tissue in the corresponding MALDI MSI experiment. These resulted in many strong signals not being characterized. Ion images of all identified compounds and labeled mass spectra from the LC separations are presented in the Supporting Information (Fig. S4).

Several interesting images and intense peaks were observed that do not match known signaling molecules or matrix adducts. Some of these images are presented in the Supporting Information. Further characterization of similar tissues will enhance the identification of such signals. Within the method optimization context presented here, we attempted to identify as many signals from the imaging experiments as feasible with MS/MS fragmentation.

The ability to identify unknown signals or verify primary mass matches is invaluable for imaging experiments. Often, several biologically relevant peptides have the same nominal mass; this may complicate analyses, particularly with regard to signal identification.

Nonetheless, when the imaged tissue and the tissue used for MS/MS are chemically similar, as with the serial sections used in this study, the presence of a single peak at a given m/z during an LC run, accompanied by high quality MS/MS spectra, can often unambiguously verify a signal's identity.

3.4 Regional analysis of tissue sections

Imaging experiments generate vast amounts of information that often create significant challenges during data analysis. Many signals detected in the summed mass spectrum produced from the collected spectra are of insufficient intensity to create meaningful ion images. In such cases, we have found that region-of-interest analyses highlight compounds that are spatially localized in a particular morphological feature, but may not be intense enough to create strong overall ion images. These region-of-interest examinations provide a broad comparison of the differential presence of a wide array of compounds in distinct anatomical regions. This rapid assay aids in the selection of those signals that are likely to produce meaningful ion images rather than creating larger numbers of arbitrary, less significant ion images.

Region-of-interest analyses are performed by summing and normalizing the mass spectra from selected regions to either total ion count or a ubiquitous ion. The normalized mass spectra are then subtracted to produce a difference plot, as shown in Fig. 3 for the SIMS experiments. Normalization is required to account for differences in the number of mass spectra present in each selected region. The total ion count has been used to normalize signals as the image of the total ions appeared homogenous across the tissue. Referring to Fig. 3, many signals beyond those presented in Fig. 1 show notable localization to either the gray or white matter. This includes several additional PC fragments (m/z 104⁺, 224⁺) [35,41], phosphatidylserine (PS) (m/z 208⁺) [35], phosphatidylinositol (PI) (m/z 223⁻) [41], 7-ketocholesterol (m/z 399⁻) [21], vitamin E (m/z 430⁺) [12], multiple signals of unknown identity, and several apparent lipid moieties in the m/z 600 to 900 region.

A similar analysis was performed for the MALDI MSI dataset (Fig. 4). The results were normalized to total ion count in a similar manner as the SIMS data. Many of the imaged, MS/MS verified signals are readily visible in the difference plot. Included are several myelin basic protein fragments, showing increased signals in the white matter, whereas neurokinin A, substance P, SAAS, complexin (1–13), proEnkephalin A (219–229), chromogranin B, and little SAAS are all elevated in the gray matter. Additionally, α -neoendorphin, hemoglobin, neurotensin, insulin β -chain, and β -thymosin were detected at elevated levels in the gray matter on the basis of a primary mass match; however, MS/MS confirmation was not possible due to a lack of detection in the extract or low signals in the LC-MALDI MS experiment. Many of the observed peaks in the regional analysis do not match known neuropeptide masses and suggest the need for further experiments to identify such signals.

4 Concluding remarks

MSI enables a new range of multi-analyte localization and discovery studies using tissue sections. Analytical challenges are still encountered when studying a broad mass range and/or imaging compounds that are present at levels low enough to preclude direct tissue analysis with MS/MS. Therefore, techniques capable of imaging the distribution of biological compounds directly from tissues facilitate efforts to address fundamental biological questions, advance applied medical diagnostic studies, and develop prognostic biomarkers. In the methodology presented here, similar tissue sections are analyzed by both SIMS and MALDI MS imaging. Furthermore, MS/MS analysis of tissue sections collected from areas adjacent to those used for MALDI MSI (and thus, chemically similar to the imaged tissues), increases the number and confidence of the peak assignments.

We imaged neuropeptides, lipids, small molecules, peptides, and protein fragments from the spinal cord of the rat. Several of these images correspond well to previously reported immunohistochemical localizations, while others are currently unreported and may guide future investigations of spinal cord function. We observe peptides from several larger proteins, including a hypothetical protein and synapsin, which are localized within the gray matter. These unexpected results will be examined in more detail in the future. Additionally, we present the co-localization of several neuropeptides within the spinal cord. This includes somatostatin-14 and substance P in the first few laminae of the dorsal horn. Upcoming experiments will utilize both imaging and separation strategies to study not only normal tissue but also how the spatial distribution and expression levels of analytes change due to inflammatory pain and drug tolerance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

aCSF	artificial cerebrospinal fluid
MSI	mass spectrometric imaging
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PS	phosphatidylserine
PCA	principal component analysis
SIMS	secondary ion mass spectrometry

6 References

1. Cornett DS, Reyzer ML, Chaurand P, Caprioli RM. MALDI imaging mass spectrometry: molecular snapshots of biochemical systems. *Nat Methods* 2007;4:828–833. [PubMed: 17901873]

2. McDonnell LA, Heeren RM. Imaging mass spectrometry. *Mass Spectrom Rev* 2007;26:606–643. [PubMed: 17471576]
3. Andersson M, Groseclose MR, Deutch AY, Caprioli RM. Imaging mass spectrometry of proteins and peptides: 3D volume reconstruction. *Nat Methods* 2008;5:101–108. [PubMed: 18165806]
4. Monroe EB, Jurchen JC, Koszczuk BA, Losh JL, et al. Massively parallel sample preparation for the MALDI MS analyses of tissues. *Anal Chem* 2006;78:6826–6832. [PubMed: 17007502]
5. Agar NY, Yang HW, Carroll RS, Black PM, Agar JN. Matrix solution fixation: histology-compatible tissue preparation for MALDI mass spectrometry imaging. *Anal Chem* 2007;79:7416–7423. [PubMed: 17822313]
6. Parry S, Winograd N. High-resolution TOF-SIMS imaging of eukaryotic cells preserved in a trehalose matrix. *Anal Chem* 2005;77:7950–7957. [PubMed: 16351142]
7. Mueller DR, Voshol H, Waldt A, Wiedmann B, Van Oostrum J. LC-MALDI MS and MS/MS—an efficient tool in proteome analysis. *Subcell Biochem* 2007;43:355–380. [PubMed: 17953403]
8. Li L, Garden RW, Sweedler JV. Single-cell MALDI: a new tool for direct peptide profiling. *Trends Biotechnol* 2000;18:151–160. [PubMed: 10740261]
9. Li L, Sweedler JV. Peptides in our brain: Mass spectrometric-based measurement approaches and challenges. *Annu Rev Anal Chem*. 2008;10.1146/annurev.anchem.1.031207.113053 in press
10. Colliver TL, Brummel CL, Pacholski ML, Swanek FD, et al. Atomic and molecular imaging at the single-cell level with TOF-SIMS. *Anal Chem* 1997;69:2225–2231. [PubMed: 9212701]
11. Sjoval P, Lausmaa J, Nygren H, Carlsson L, Malmberg P. Imaging of membrane lipids in single cells by imprint-imaging time-of-flight secondary ion mass spectrometry. *Anal Chem* 2003;75:3429–3434. [PubMed: 14570193]
12. Monroe EB, Jurchen JC, Lee J, Rubakhin SS, Sweedler JV. Vitamin E imaging and localization in the neuronal membrane. *J Am Chem Soc* 2005;127:12152–12153. [PubMed: 16131155]
13. Ostrowski SG, Van Bell CT, Winograd N, Ewing AG. Mass spectrometric imaging of highly curved membranes during *Tetrahymena* mating. *Science* 2004;305:71–73. [PubMed: 15232100]
14. Ostrowski SG, Kurczy ME, Roddy TP, Winograd N, Ewing AG. Secondary ion MS imaging to relatively quantify cholesterol in the membranes of individual cells from differentially treated populations. *Anal Chem* 2007;79:3554–3560. [PubMed: 17428032]
15. Quintana C, Bellefqih S, Laval JY, Guerquin-Kern JL, et al. Study of the localization of iron, ferritin, and hemosiderin in Alzheimer's disease hippocampus by analytical microscopy at the subcellular level. *J Struct Biol* 2006;153:42–54. [PubMed: 16364657]
16. Gazi E, Dwyer J, Lockyer N, Gardner P, et al. The combined application of FTIR microspectroscopy and ToF-SIMS imaging in the study of prostate cancer. *Faraday Discuss* 2004;126:41–59. [PubMed: 14992399]discussion 77–92
17. Roy S, Touboul D, Brunelle A, Germain DP, et al. Imaging mass spectrometry: a new tool for the analysis of skin biopsy. Application in Fabry's disease. *Ann Pharm Fr* 2006;64:328–334. [PubMed: 17095952]
18. Smith DR, Chandra S, Coderre JA, Morrison GH. Ion microscopy imaging of 10B from p-boronophenylalanine in a brain tumor model for boron neutron capture therapy. *Cancer Res* 1996;56:4302–4306. [PubMed: 8813111]
19. Malmberg P, Nygren H, Richter K, Chen Y, et al. Imaging of lipids in human adipose tissue by cluster ion TOF-SIMS. *Microsc Res Tech* 2007;70:828–835. [PubMed: 17576131]
20. Nygren H, Malmberg P, Kriegeskotte C, Arlinghaus HF. Bioimaging TOF-SIMS: localization of cholesterol in rat kidney sections. *FEBS Lett* 2004;566:291–293. [PubMed: 15147911]
21. Malmberg P, Borner K, Chen Y, Friberg P, et al. Localization of lipids in the aortic wall with imaging TOF-SIMS. *Biochim Biophys Acta* 2007;1771:185–195. [PubMed: 17240191]
22. Sjoval P, Lausmaa J, Johansson B. Mass spectrometric imaging of lipids in brain tissue. *Anal Chem* 2004;76:4271–4278. [PubMed: 15283560]
23. Todd PJ, McMahon JM, McCandlish CA Jr. Secondary ion images of the developing rat brain. *J Am Soc Mass Spectrom* 2004;15:1116–1122. [PubMed: 15234370]
24. McDonnell LA, Piersma SR, MaartenAltelaar AF, Mize TH, et al. Subcellular imaging mass spectrometry of brain tissue. *J Mass Spectrom* 2005;40:160–168. [PubMed: 15706616]

25. Nygren H, Borner K, Hagenhoff B, Malmberg P, Mansson JE. Localization of cholesterol, phosphocholine and galactosylceramide in rat cerebellar cortex with imaging TOF-SIMS equipped with a bismuth cluster ion source. *Biochim Biophys Acta* 2005;1737:102–110. [PubMed: 16300993]
26. Chaurand P, Schwartz SA, Caprioli RM. Assessing protein patterns in disease using imaging mass spectrometry. *J Proteome Res* 2004;3:245–252. [PubMed: 15113100]
27. Stoeckli M, Staab D, Schweitzer A. Compound and metabolite distribution measured by MALDI mass spectrometric imaging in whole-body tissue sections. *Int J Mass Spectrom* 2007;260:195–202.
28. Rubakhin SS, Greenough WT, Sweedler JV. Spatial profiling with MALDI MS: distribution of neuropeptides within single neurons. *Anal Chem* 2003;75:5374–5380. [PubMed: 14710814]
29. DeKeyser SS, Kutz-Naber KK, Schmidt JJ, Barrett-Wilt GA, Li L. Imaging mass spectrometry of neuropeptides in decapod crustacean neuronal tissues. *J Proteome Res* 2007;6:1782–1791. [PubMed: 17381149]
30. Verhaert PD, Conaway MCP, Pekar TM, Miller K. Neuropeptide imaging on an LTQ with vMALDI source: The complete 'all-in-one' peptidome analysis. *Int J Mass Spectrom* 2007;260:177–184.
31. Altaalar AFM, Taban IM, McDonnell LA, Verhaert P, et al. High-resolution MALDI imaging mass spectrometry allows localization of peptide distributions at cellular length scales in pituitary tissue sections. *Int J Mass Spectrom* 2007;260:203–211.
32. Reyzer ML, Hsieh Y, Ng K, Korfmacher WA, Caprioli RM. Direct analysis of drug candidates in tissue by matrix-assisted laser desorption/ionization mass spectrometry. *J Mass Spectrom* 2003;38:1081–1092. [PubMed: 14595858]
33. Taban IM, Altaalar AF, van der Burgt YE, McDonnell LA, et al. Imaging of peptides in the rat brain using MALDI-FTICR mass spectrometry. *J Am Soc Mass Spectrom* 2007;18:145–151. [PubMed: 17055739]
34. Kruse R, Sweedler JV. Spatial profiling invertebrate ganglia using MALDI MS. *J Am Soc Mass Spectrom* 2003;14:752–759. [PubMed: 12837597]
35. Ostrowski SG, Szakal C, Kozole J, Roddy TP, et al. Secondary ion MS imaging of lipids in picoliter vials with a buckminsterfullerene ion source. *Anal Chem* 2005;77:6190–6196. [PubMed: 16194078]
36. Amaya KR, Monroe EB, Sweedler JV, Clayton DF. Lipid imaging in the zebra finch brain with secondary ion mass spectrometry. *Int J Mass Spectrom* 2007;260:121–127.
37. Shults CW, Quirion R, Chronwall B, Chase TN, O'Donohue TL. A comparison of the anatomical distribution of substance P and substance P receptors in the rat central nervous system. *Peptides* 1984;5:1097–1128. [PubMed: 6085163]
38. Milhorat TH, Mu HT, LaMotte CC, Milhorat AT. Distribution of substance P in the spinal cord of patients with syringomyelia. *J Neurosurg* 1996;84:992–998. [PubMed: 8847594]
39. Otsuka M, Yanagisawa M. Pain and neurotransmitters. *Cell Mol Neurobiol* 1990;10:293–302. [PubMed: 1701358]
40. Lemaire R, Desmons A, Tabet JC, Day R, et al. Direct analysis and MALDI imaging of formalin-fixed, paraffin-embedded tissue sections. *J Proteome Res* 2007;6:1295–1305. [PubMed: 17291023]
41. Touboul D, Brunelle A, Halgand F, De La Porte S, Laprevote O. Lipid imaging by gold cluster time-of-flight secondary ion mass spectrometry: application to Duchenne muscular dystrophy. *J Lipid Res* 2005;46:1388–1395. [PubMed: 15834124]

Figure 1.

Selected SIMS ion images at 2.3- μm spatial resolution. (A) Optical image of spinal cord section with the imaged area outlined. Selected ion images of (B) Na^+ , m/z 23 $^+$, (C) K^+ , m/z 39 $^+$, (D) C_5H_9^+ , m/z 69 $^+$, (E) choline, m/z 86 $^+$, (F) unknown, m/z 107 $^+$, (G) phosphatidylcholine fragment, m/z 184 $^+$, (H) cholesterol $[\text{M}-\text{OH}]^+$, m/z 365 $^+$, (I) cholesterol $[\text{M}-\text{H}]^+$, m/z 384 $^+$, (J) CH^- , m/z 13 $^-$, (K) PO_3^- , m/z 79 $^-$, (L) phosphatidylethanolamine fragment, m/z 122 $^-$, (M) unknown, m/z 237 $^-$, (N) C16:1, m/z 253 $^-$, (O) C16:0, m/z 255 $^-$, (P) C18:2, m/z 279 $^-$, (Q) C18:1, m/z 281 $^-$, and (R) C18:0, m/z 283 $^-$. Scale bar in panel A = 1 mm. All ion image scale bars = 100 μm .

Figure 2.

Selected MALDI MSI images at 125- μ m spatial resolution. (A) Optical image with the gray and white matter outlined. (B) Myelin basic protein (2–12), m/z 1293.74, (C) substance P, m/z 1347.61, (D) SAAS, m/z 1367.72, (E) PEP-19, m/z 1392.78, (F) complexin (1–13), m/z 1424.57, (G) proEnkephalin A (219–229), m/z 1466.68, (H) chromogranin B (secretogranin I), m/z 1761.79, and (I) PEN, m/z 2301.31. All scale bars = 1 mm.

Figure 3.

Regional analysis of spinal cord imaged with SIMS. By normalizing and subtracting the mass spectra from the gray and white matter, the resulting difference plot highlights the compounds that are differentially expressed in each region. Labeled peaks are presented as ion images in Figure 1 and the Supporting Information. (a) Na^+ , (b) K^+ , (c) C_5H_9 , (d) choline, (e) PC fragment, (f) PC fragment, (g) PS fragment, (h) PC fragment, (i) cholesterol ($-\text{OH}$), (j) cholesterol, (k) vitamin E, (l) CH , (m) PO_3^- , (n) PE fragment, (o) PI fragment, (p) $\text{C}_{16:1}$, (q) $\text{C}_{16:0}$, (r) $\text{C}_{18:2}$, (s) $\text{C}_{18:1}$, (t) $\text{C}_{18:0}$, and (u) 7-ketocholesterol.

Figure 4.

Regional analysis of the spinal cord imaged with MALDI MSI. The labeled peaks are presented as ion images in Figure 2 or the Supporting Information. Identities have been verified by LC MALDI MS/MS except where noted. (a) α -neoendorphin (primary mass match), (b) myelin basic protein (119–128), (c) neurokinin A, (d) myelin basic protein (2–12), (e) hemoglobin (primary mass match), (f) substance P, (g) SAAS, (h) complexin 1–13, (i) proEnkephalin A (219–229), (j) neurotensin (primary mass match), (k) chromogranin B, (l) little SAAS, (m) myelin basic protein (2–17), (n) myelin basic protein (2–19), (o) insulin β -chain (primary mass match), and (p) β -thymosin (primary mass match).

Compounds identified from spinal cord via LC MALDI MS/MS. All signals are also presented as ion images in the Supporting Information.

Table 1

observed <i>m/z</i>		Name	Sequence	calculated [M+H] ⁺
LC MS	Imaging			
877.43	877.5	MetEnk - ArgPhe	YGGFMRF	877.40
900.32	900.6	MetEnk - ArgGlyLeu	YGGFMRGL	900.44
ND	1100.4	α-neoendorphin (mass match)	YGGFLRKYP	1100.59
1104.58	1104.7	Myelin Basic Protein S (119–128)	SRSGSPMARR	1104.57
1133.65	1133.8	Neurokinin A	HKTDSFVGLMa	1133.58
1236.67	1236.7	Myelin Basic Protein S (2–11)	AcASQKRPSQRH	1236.66
1293.74	1293.9	Myelin Basic Protein S (2–12)	AcASQKRPSQRHG	1293.68
1309.83	1310.0	Hypothetical protein RGD1304952 (261–272)	pQPKGAPAFQLNR	1309.70
ND	1324.5	Hemoglobin β (33–42) (mass match)	LVVYPWTQRY	1324.70
1347.64	1347.9	Substance P	RPKPQQFFGLMa	1347.74
1367.75	1367.8	SAAS	AVPRGEAAGA VQEL	1367.73
1392.81	1392.9	PEP-19 (51–62)	RKFQKKAGSQS	1392.81
1424.60	1424.7	Complexin fragment (1–13)	AcMEFV MKQALGGAT	1424.69
1432.74	1432.9	Myelin Basic Protein S (182–195)	GRDSRSGSPMARR	1432.72
1466.71	1466.8	ProEnk A 219–229	VGRPEWWM DYQ	1466.65
1508.80	1509.2	Myelin Basic Protein S (2–14)	AcASQKRPSQRHGSK	1508.80
1637.76	1637.8	Somatostatin-14	AGCKNFFWKTF TSC	1637.72
1671.78	1671.6	Myelin Basic Protein S (2–15)	AcASQKRPSQRHGSKY	1671.87
ND	1673.0	Neurotensin (mass match)	pQLYENKPRRPYIL	1672.94
1713.80	1713.9	Synapsin I fragment (1–13)	AcMNYLRRRLSDSNF	1713.85
1761.82	1761.8	Chromogranin B (secretogranin I)	pQYDDGVAELDQLLHY	1761.80
1785.01	1785.3	Little SAAS	SLSAASAPLAETSTPLRL	1784.98
1855.88	1856.0	Myelin Basic Protein S (2–17)	AcASQKRPSQRHGSKYLA	1855.99
2028.04	2028.2	Myelin Basic Protein S (2–19)	AcASQKRPSQRHGSKYLATA	2028.07
2301.34	2301.4	PEN	AVDQDLGPEVPPENVLGALLRV	2301.24
ND	3434.0*	Insulin-2 β-chain (mass match)	FVKQHLGSHLVEALYLVCGERGFFYTPMS	3434.04*
4964.58*	4963.4*	Thymosin β-4 (mass match)	AcSDKPDMAEIEKFDKSKLKKTTET QEKNP LPSKETIEQEKQAGES	4964.55*