Published in final edited form as:

Biochim Biophys Acta. 2011 November ; 1811(11): 970–975. doi:10.1016/j.bbalip.2011.04.012.

MALDI Imaging of Lipids after Matrix Sublimation/Deposition

Robert C. Murphy, Joseph A. Hankin, Robert M. Barkley, and Karin A. Zemski Berry

Department of Pharmacology, University of Colorado Denver, Mail Stop 8303, 12801 E. 17th Avenue, Aurora, CO 80045

Abstract

Mass spectrometric techniques have been developed to record mass spectra of biomolecules including lipids as they naturally exist within tissues and thereby permit the generation of images displaying the distribution of specific lipids in tissues, organs, and intact animals. These techniques are based on matrix-assisted laser desorption/ionization (MALDI) that requires matrix application onto the tissue surface prior to analysis. One technique of application that has recently shown significant advantages for lipid analysis is sublimation of matrix followed by vapor deposition directly onto the tissue. Explanations for enhanced sensitivity realized by sublimation/deposition related to sample temperature after a laser pulse and matrix crystal size are presented. Specific examples of sublimation/deposition in lipid imaging of various organs including brain, ocular tissue, and kidney are presented.

Keywords

imaging mass spectrometry; sublimation/deposition; MALDI; phosphatidylcholine; sphingolipids; brain; kidney; retina; spinal cord; human lens; glycerophospholipids

1. Introduction

Recent advances in mass spectrometry have enabled this technique to be used as an imaging platform to determine the localization of biomolecules in tissues. When these biomolecules are lipids, it is often possible to define their structures when a tandem mass spectrometer is used as the imaging instrument. The advantage of generating images of defined molecules is that a unique body of information can be generated relevant to biochemical events that happen at precise locations in a tissue. This information can be quite useful for studies of lipid biochemistry in tissues and organs. While most of the early work in this area used ion beams to generate images (secondary ion mass spectrometry, SIMS [1]), recent work has used matrix assisted laser desorption/ionization (MALDI) (2) to generate images of the distribution of molecular ions. A major reason for using MALDI has been the observation that more abundant intact molecular ions are obtained due to the favorable characteristics of this ionization process relative to SIMS. SIMS is highly energetic and leads to extensive bond cleavage of biomolecules, including lipids, to produce secondary ions with a variable degree of information that can be traced only partially to the molecule of origin and the

© 2011 Elsevier B.V. All rights reserved.

Correspondence to: Robert C. Murphy, Ph.D., Department of Pharmacology, University of Colorado Denver, Mail Stop 8303, 12801 E. 17th Ave, Aurora, CO 80045, Tel: 303-724-3352 | Fax: 303-724-3357 | robert.murphy@ucdenver.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

intact lipid is often not detected. Specific examples would be SIMS imaging of lipids in tissues by recording m/z 184 (3), a highly stable ion derived from phosphatidylcholines and sphingomyelins or m/z 369, the highly stable cholesteryl cation [4] derived from cholesterol or cholesteryl esters. MALDI imaging can generate positive and negative ions that generally correspond to $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, or $[M-H]^-$ phospholipids of reasonable abundance in a tissue. While one of the advantages of MALDI over SIMS is the detection of intact lipid molecular species, one of the disadvantages of MALDI compared to SIMS imaging is that the latter technique has a substantially higher lateral resolution [1].

Matrix deposition is a factor that governs the MALDI process and the quality of a MALDI image in terms of mass resolution, detection sensitivity, spatial resolution, and reproducibility. The effectiveness of the matrix is determined by the size, density, analyte extraction, and homogeneity of the crystals that form on the tissue surface and the final goal is to get a complete and homogeneous coverage of the tissue surface with limited analyte delocalization. The methods that have been used to apply matrix to tissue sections for MALDI imaging are quite extensive.

Most of these methods use solutions of matrix dissolved in a water/organic solvent mixture in the deposition of the matrix, such as the dry droplet approach [5], electrospray deposition [6], spray coating using an airbrush or TLC reagent sprayer [7], and inkjet printing [8]. However, the chance of analyte delocalization is significant due to the water/organic mixture used in these matrix application methods. Recognition of this possibility has led to adapted applications that reduce liquid levels as the matrix contacts the tissue surface. An oscillating capillary nebulizer has been successfully used to spray small droplets of matrix aerosol onto the tissue surface with good matrix homogeneity and spatial resolution for visualization of several types of lipids including sulfatides, gangliosides, and glycerophospholipids [9]. Additionally two solvent free techniques, dry-coating [10] and sublimation [11], have been reported as a method of deposition of matrix on tissue sections for lipid analysis with, theoretically, no analyte delocalization. The dry-coating technique deposits finely ground matrix onto tissue slices by the direct filtering of solid matrix particles through a 20 micron stainless steel sieve. Vapor-phase deposition of matrix through sublimation of matrix produces a homogeneous coating of matrix across the tissue section. What was observed with the initial sublimation/deposition experiments was a significantly enhanced signal for lipids, reduction in laser spot-to-spot variation of secondary ion yield, as well as reduction in alkali metal contamination.

This review will discuss the sublimation process, provide lipid images rendered from such tissues after sublimation/deposition of matrix, and discuss the advantages and disadvantages of this technique applied to MALDI IMS.

1.1 Sublimation/deposition

Sublimation is the process of a solid passing directly into a gas without intermediate formation of a liquid phase. Most organic molecules with a significant vapor pressure either under vacuum or atmospheric pressure can undergo this endothermic process at specific temperatures and pressures, if thermal decomposition does not interfere. The opposite of sublimation is deposition, where a gas becomes a solid on a surface in an exothermic phase change process without intermediate formation of a liquid phase. In 2007, we reported matrix application using DHB (2,5-dihydroxybenzoic acid) by sublimation/deposition onto the tissue surface specifically for the generation of molecular images of lipid distributions by MALDI imaging [5]. The rationale for initiating this approach was a potential minimization in lateral movement of tissue lipids compared to other matrix application methods, such as electrospray, dry droplet, airbrush, or inkjet printing application, where organic solvents are added to a tissue surface. In such cases, lipids could become solubilized in the solvent

droplet and diffused laterally. What was observed with the initial sublimation/deposition experiments was a significant enhanced lipid signal, reduction in laser spot-to-spot variation of secondary ion yield, as well as reduction in alkali metal contamination. This latter observation was likely due to the purification properties of the sublimation approach. In practical terms, this technique was very fast and simple to implement and yielded a very even and thin coating of matrix crystals on the tissue. Most importantly, the observed crystals that were deposited on tissue surface were exceedingly small implying the potential for better lateral resolution. Subsequent studies in our and other laboratories have confirmed these observations and fundamental studies of the MALDI desorption process has been examined with respect to matrix applied either by sublimation/deposition or the dry droplet method [12]. It should be stated that sublimation of matrix and lipid molecules from tissues have been reported as adverse properties [13]. While not discussed further in this review, the ambient vacuum conditions employed during the imaging process can have a major effect on in-source matrix sublimation during the rather lengthy imaging experiments. Most imaging experiments using DHB matrix have been carried out where the tissue/matrix is exposed to only moderate vacuum and certainly not high vacuum conditions.

Several models of ionization and desorption mechanisms have emerged to explain the MALDI desorption process [14, 15]. Dreisenwerd [16] proposed a thermal desorption mechanism [17] that stressed the importance of higher matrix temperature in secondary ion yield. Direct measurements of the temperature that samples (matrix) achieve during the MALDI process whether applied to the dry droplet or sublimation/deposition revealed a significantly higher effective temperature, up to 1200 K, when matrices were vapor deposited [12]. It has been reasoned that very small crystals have difficulty in the dissipation of laser energy because of limited number of molecules in the crystal lattice and poor energy transfer between adjacent crystals. Larger crystals have more molecules within each lattice structure and larger area over which energy can disperse. This inability to dissipate energy would become manifest as local heating of the small crystals compared to large crystals after each laser pulse. The theoretical desorption mechanism of phase explosion and ejection of large clusters from the crystal lattice [18] would also be consistent with the increased ion abundances observed from the sublimed/deposited matrix due to these higher temperatures achieved in smaller crystals. Also, a factor of two increase in ion velocity of analytes was directly measured in sublimed/deposited matrix when compared to the ion velocity from the same target molecules to which matrix had been applied in the dry droplet technique [12]. With these increased ion velocities there would be a corresponding lower internal energy (and lower fragmentation pathways) due to expansion cooling of the ejected molecules during the phase explosion process [17]. The sublimed/deposited matrix was also found to be more completely removed by the MALDI process as compared to the dry droplet approach which would further account for the increased sensitivity observed in the sublimed/deposited matrix method. Not all matrices show these favorable characteristics due to multiple factors, including enthalpy of sublimation (DHB has $-\Delta H_{\text{sub}}$ of $109 \pm 3 \text{ kJ} \cdot \text{mol}^{-1}$) [19] and UV absorption properties of the solid powder [16] relative to the laser wavelength.

The first use of sublimation/deposition for direct MALDI analysis was reported by Kim [20]. The sublimation process was used to apply both sample and matrix in an even fashion to the sample plate and reported as an excellent method to apply matrix and sample when they were not soluble with each other. After our demonstration of the value of matrix sublimation/deposition for imaging, this method of matrix application was implemented for both direct sample analysis by MALDI as well imaging applications. In a study of direct MALDI analysis, sample plates were vapor deposited with α -cyano-4-hydroxycinnamic acid matrix prior to application of samples of a membrane protein digest. With this approach a significant increase in signal derived from peptides was reported [21]. In another study,

DHB was deposited by sublimation directly onto intact cells that had been treated with two different HIV protease inhibitors, nelfinavir (m/z 568.3) and saquinavir (m/z 671.4). These drugs were then followed by high resolution MALDI-FTICR analysis and the two signals for these inhibitors were obtained in reasonably high yield from the cell surface [22]. Additionally, images of neutral lipids such as wax esters on insect wings have also been reported [23]. In this study, sublimation/deposition of DHB was used to generate images of lipid location on the wings as alkali attachment adducts (K^+ and Na^+) of wax esters [23]. Also, when sublimed/deposited matrix was used to image peptides and/or proteins in cells placed on a MALDI plate, enhanced lateral resolution as well as sensitivity was observed when a hydration step was used after deposition of the matrix in order to facilitate a re-crystallization [24]. This approach followed from the suggestion that re-crystallization of MALDI matrix applied by dry droplet or spray techniques on physiological samples that had high salt concentrations such as those found *in situ* in tissues greatly improved the yield of MALDI ions [25]. This re-crystallization could be effected by storing the matrix coated samples in a chamber at high relative humidity.

Confirmation of the advantages that have been reported for matrix sublimation/deposition has now appeared that validate the value of this method to improve imaging by MALDI mass spectrometry. The generation of a thin, microcrystalline matrix layer on a membrane surface increased the intensity of ions that can be observed in the MALDI experiment likely due to an increase temperature achieved within each of the microcrystals after a laser pulse as well as complete ablation of the matrix after only a few laser pulses. The ease of preparation of these vapor deposited matrices on tissue slices, the purification of matrix during sublimation, the absence of a need for expensive matrix application devices, and simplicity of the apparatus needed to make high quality matrix coatings continues to make this an attractive matrix application technique.

1.2 Brain

Rodent brain has been a widely studied tissue in the development of MALDI IMS because it is readily available, rich in lipids, and easy to slice. Perhaps most importantly, the brain has well defined anatomical regions that can be readily visualized using histological tools, with detailed annotated atlases available to help interpret experimental MALDI images [26].

MALDI IMS using various matrix application methods, including sublimation/deposition have been employed to examine the distribution of lipids in brain slices.

Glycerophosphocholine lipids and sphingomyelin molecules as mixed protonated, sodiated and potassiated forms dominate MALDI spectra of brain tissue in positive ion mode [27], while sulfatide and glycerophosphatidylinositol molecules are dominant ionizing species in negative ion mode [28]. MALDI images reveal remarkable definition of anatomical regions of the brain for common cell membrane glycerophosphocholine lipids components (PC(16:0/18:1), Figure 1A) as well as a unique distribution of other specific molecular species of phospholipids such as PC(18:0/22:6) observed in the grey matter region of the cerebellum (Figure 1B). These findings are consistent across different matrix application methods, different mass spectrometers, and between different species (mouse and rat).

Recently, regional ion intensity derived by MALDI IMS was correlated to the relative regional amount of that ion for glycerophosphocholine lipid-derived ions in rat brain tissue coated with matrix by sublimation [29]. In this study, all of the ionizable forms ($[M+H]^+$, $[M+Na]^+$ $[M+K]^+$) of the PC molecules were combined for comparisons. Another recent MALDI IMS study revealed regional differences in alkali metal adducts of PC molecules experimentally related to traumatic brain injury (TBI) in rats. There was a localized injury-related increase in sodium glycerophosphocholine lipids adducts with depletion of corresponding potassium adducts attributed to loss of the Na^+/K^+ -ATPase activity localized to the site of the injury (Figure 1C) [30]. The use of sublimation for application of matrix on

tissue in a solvent free manner allowed the unexpected detection of differences in alkali metal attachment ions related to a change in tissue condition. Similar MALDI IMS regional differences of sodiated and potassiated adducts of wax esters were found on fly wings when DHB matrix was applied by sublimation [23].

1.3 Kidney

MALDI imaging of kidney tissue obtained with sublimation of DHB matrix has revealed unique distributions of specific lipids in the cortex, medulla, and pelvic regions of murine kidney tissue as well as perinephric tissue [31]. Cholesterol was found to be distributed evenly throughout the kidney tissue based upon a rather uniform image of the kidney at m/z 369.3 and molecules derived from triacylglycerols were found to be localized in the perirenal fat tissue. The image at m/z 796.6 derived from $PC(16:0/18:2)+K^+$ in mouse kidney appeared to have a high abundance in the cortex region (Figure 2A), while the ion at m/z 798.6 corresponding to $PC(16:0/18:1)+K^+$ was most abundant in the cortex and pelvic regions of the kidney (Figure 2B). Interestingly many abundant ions observed in this tissue were potassiated species. Many phospholipid and sphingomyelin ions have been observed in the kidney with one interesting aspect of a high abundance arachidonic acid containing PC present in the medulla region of this organ, suggesting that arachidonic acid containing PC might be a source of arachidonic acid that is converted into biologically active eicosanoids that play an important role in kidney function [32].

1.4 Ocular tissue

MALDI imaging has been used effectively to study lipids present in specific regions of the eye such as tissue slices of the lens as well as the retina. A study of the effect of age on the distribution of sphingomyelin lipids in the lens from human subjects employed MALDI imaging after DHB matrix was applied by sublimation [33]. The MALDI images indicate that the distribution of dihydrosphingomyelin (SM (d18:0/16:0)) is relatively homogeneous in a younger lens and that there is an annular distribution of this lipid in older lenses (Figure 3A). The size of the dihydrosphingomyelin annular ring was consistent with the barrier of diffusion, which becomes evident in human lenses at middle age and is a characteristic of age-related nuclear cataracts. This study also revealed that dihydroceramide, Cer (d18:0/16:0), was most abundant in the nuclear region of older lenses while being absent in the outer regions of the lens (Figure 3B). Such changes in lipid composition have a significant impact on the physical properties of the fiber cell membranes and may be associated with the formation of a barrier.

The salamander retina is another tissue that has been examined using MALDI imaging techniques after applying DHB by sublimation [34]. The images showed a different distribution of phosphatidylcholine molecular species in the different layers of the retina. For example, phosphatidylcholine lipids containing saturated and monounsaturated fatty acids, $PC(16:0/16:0)$, $PC(16:0/16:1)$, and $PC(16:0/18:1)$, were detected in the outer and inner plexiform layers (OPL and IPL) of the retina (Figure 4). In contrast, the phosphatidylcholine lipids containing polyunsaturated fatty acids, $PC(16:0/20:4)$, $PC(16:0/22:6)$, and $PC(18:0/22:6)$, were found in the inner segment (IS), the outer segment (OS), and the retinal pigment epithelium (RPE) (Figure 4). The presence of PCs containing polyunsaturated fatty acids in the OS layer implied that these phospholipids form flexible lipid bilayers, which facilitate phototransduction process occurring in the rhodopsin-rich OS layer. Such anatomical detail, in terms of the location of specific phospholipid molecular species, would be quite difficult to obtain by any other technique.

2. Conclusions

Imaging of lipids by mass spectrometry has emerged as a powerful technique to follow unique biochemical events within tissues as well as the whole organism. Information about specific molecular species and their distributions relative to anatomical features provides a unique insight into events taking place *in vivo* that would not be possible to observe using alternative approaches such as dissection and analysis using lipidomic protocols. Significant advances are being rapidly made in both instruments that can carry out these experiments as well as means to apply matrix. The sublimation/deposition of matrix has greatly facilitated the imaging of lipids in tissue slices because of the unique features of enhanced sensitivity, reduction of the potential for lateral diffusion of lipids and purification of matrix during sublimation. The sublimation/deposition method is easy to implement and it generates a very even and uniformly thin layer of MALDI matrix.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported, in part, by the LIPID MAPS large scale collaborative grant from the Institute of General Medical Sciences (GM069338) of the National Institutes of Health.

Abbreviations

DHB	2,5-dihydroxybenzoic acid
SIMS	secondary ion mass spectrometry
MALDI	matrix-assisted laser desorption/ionization
IMS	imaging mass spectrometry
PC	glycerophosphocholine lipids

References

1. Amstalden van Hove ER, Smith DF, Heeren RM. A concise review of mass spectrometry imaging. *J Chromatogr A*. 2010; 1217:3946–3954. [PubMed: 20223463]
2. 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]
3. Roddy TP, Cannon DM Jr, Ostrowski SG, Ewing AG, Winograd N. Proton transfer in time-of-flight secondary ion mass spectrometry studies of frozen-hydrated dipalmitoylphosphatidylcholine. *Anal Chem*. 2003; 75:4087–4094. [PubMed: 14632121]
4. Nygren H, Böner K, Hagenhoff B, Malmberg P, Månsson 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*. 2005:102–110. [PubMed: 16300993]
5. Juhasz P, Costello CE, Biemann K. Matrix-assisted laser desorption ionization mass-spectrometry with 2-(4-hydroxyphenylazo)benzoic acid matrix. *J Am Soc Mass Spectrom*. 1993; 4:399–409.
6. Axelsson J, Hoberg AM, Waterson C, Myatt P, Shield GL, Varney J, Haddleton DM, Derrick PJ. Improved reproducibility and increased signal intensity in matrix-assisted laser desorption/ionization as a result of electrospray sample preparation. *Rapid Commun Mass Spectrom*. 1997; 11:209–213.
7. Wang HY, Jackson SN, Post J, Woods AS. A minimalist approach to MALDI imaging of glycerophospholipids and sphingolipids in rat brain sections. *Int J Mass Spectrom*. 2008; 278:143–149. [PubMed: 19956342]

8. Baluya DL, Garrett TJ, Yost RA. Automated MALDI matrix deposition method with inkjet printing for imaging mass spectrometry. *Anal Chem.* 2007; 79:6862–6867. [PubMed: 17658766]
9. Chen Y, Allegood J, Liu Y, Wang E, Cachón-Gonzalez B, Cox TM, Merrill AH Jr, Sullards MC. Imaging MALDI mass spectrometry using an oscillating capillary nebulizer matrix coating system and its application to analysis of lipids in brain from a mouse model of Tay-Sachs/Sandhoff disease. *Anal Chem.* 2008; 80:2780–2788. [PubMed: 18314967]
10. Puolitaival SM, Burnum KE, Cornett DS, Caprioli RM. Solvent-free matrix dry-coating for MALDI imaging of phospholipids. *J Am Soc Mass Spectrom.* 2008; 19:882–886. [PubMed: 18378160]
11. Hankin JA, Barkley RM, Murphy RC. Sublimation as a method of matrix application for mass spectrometric imaging. *J Am Soc Mass Spectrom.* 2007; 18:1646–1652. [PubMed: 17659880]
12. Jaskolla TW, Karas M, Roth U, Steinert K, Menzel C, Reihls K. Comparison between vacuum sublimed matrices and conventional dried droplet preparation in MALDI-TOF mass spectrometry. *J Am Soc Mass Spectrom.* 2009; 20:1104–1114. [PubMed: 19299165]
13. Piehowski PD, Carado AJ, Kurczy ME, Ostrowski SG, Heien XL, Winograd N, Ewing EG. MS/MS methodology to improve subcellular mapping of cholesterol using TOF-SIMS. *Anal Chem.* 2008; 80:8662–8667. [PubMed: 18925746]
14. Karas M, Gluckmann M, Schafer J. Ionization in matrix-assisted laser desorption/ionization: Singly charged molecular ions are the lucky survivors. *J Mass Spectrom.* 2000; 35:1–12. [PubMed: 10633229]
15. Knochenmuss R. A quantitative model of ultraviolet matrix-assisted laser desorption/ionization. *J Mass Spectrom.* 2002; 37:867–877. [PubMed: 12203681]
16. Dreisewerd K, Schürenberg M, Karas M, Hillenkamp F. Influence of the laser intensity and spot size on the desorption of molecules and ions in matrix-assisted laser desorption/ionization with a uniform beam profile. *Int J Mass Spectrom Ion Process.* 1995; 141:127–148.
17. Zhigilei LV, Leveugle E, Garrison BJ, Yingling YG, Zeifman MI. Computer simulations of laser ablation of molecular substrates. *Chem Rev.* 2003; 103:321–348. [PubMed: 12580634]
18. Garrison BJ, Postawa Z. Computational view of surface based organic mass spectrometry. *Mass Spectrom Rev.* 2008; 27:289–315. [PubMed: 18421766]
19. Price DM, Bashir S, Derrick PR. Sublimation properties of x,y-dihydroxybenzoic acid isomers as model matrix assisted laser desorption ionisation (MALDI) matrices. *Thermochim Acta.* 1999:167–171.
20. Kim SH, Shin CM, Yoo JE. First application of thermal vapor deposition method to matrix-assisted laser desorption ionization mass spectrometry: determination of molecular mass of bis(p-methyl benzylidene) sorbitol. *Rapid Commun Mass Spectrom.* 1998; 12:701–704.
21. Poetsch A, Schlüsener D, Florizone C, Eltis L, Menzel C, Rögner M, Steinert K, Roth U. Improved identification of membrane proteins by MALDI-TOF MS/MS using vacuum sublimated matrix spots on an ultraphobic chip surface. *J Biomol Tech.* 2008; 19:129–138. [PubMed: 19137096]
22. Dekker LJ, van Kampen JJ, Reedijk ML, Burgers PC, Gruters RA, Osterhaus AD, Luider TM. A mass spectrometry based imaging method developed for the intracellular detection of HIV protease inhibitors. *Rapid Commun Mass Spectrom.* 2009; 23:1183–1188. [PubMed: 19283784]
23. Vrkslav V, Muck A, Cvacka J, Svatos A. MALDI imaging of neutral cuticular lipids in insects and plants. *J Am Soc Mass Spectrom.* 2010; 21:220–231. [PubMed: 19910210]
24. Bouschen W, Schulz O, Eikel D, Spengler B. Matrix vapor deposition/recrystallization and dedicated spray preparation for high-resolution scanning microprobe matrix-assisted laser desorption/ionization imaging mass spectrometry (SMALDI-MS) of tissue and single cells. *Rapid Commun Mass Spectrom.* 2010; 24:355–364. [PubMed: 20049881]
25. Monroe EB, Koszczuk BA, Losh JL, Jurchen JC, Sweedler JV. Measuring salty samples without adducts with MALDI MS. *Int J Mass Spectrom.* 2007; 260:237–242.
26. Hochheiser H, Yanowitz J. If I only had a brain: Exploring mouse brain images in the Allen Brain Atlas. *Biol Cell.* 2007; 99:403–409. [PubMed: 17567264]
27. Jackson SN, Wang HY, Woods AS. *In situ* structural characterization of phosphatidylcholines in brain tissue using MALDI-MS/MS. *J Am Soc Mass Spectrom.* 2005; 16:2052–2056. [PubMed: 16253515]

28. Jackson SN, Wang HY, Woods AS. *In situ* structural characterization of glycerophospholipids and sulfatides in brain tissue using MALDI-MS/MS. *J Am Soc Mass Spectrom.* 2005; 18:17–26. [PubMed: 17005416]
29. Hankin JA, Murphy RC. Relationship between MALDI-MSI intensity and measured quantity of selected phospholipids in rat brain sections. *Anal Chem.* 2010; 82:8476–8484. [PubMed: 20853893]
30. Hankin JA, Farias S, Barkley RM, Heidenreich K, Frey LC, Hamazaki K, Kim H-Y, Murphy RC. MALDI Mass Spectrometric Imaging of Lipids in Rat Brain Injury Models. *J Amer Soc Mass Spectrom.* In press.
31. Murphy RC, Hankin JA, Barkley RM. Imaging of lipid species by MALDI mass spectrometry. *J Lipid Res.* 2009; 50:S317–S322. [PubMed: 19050313]
32. Medhora M, Dhanasekaran A, Gruenloh SK, Dunn LK, Gabrilovich M, Falck JR, Harder DR, Jacobs ER, Pratt PF. Emerging mechanisms for growth and protection of the vasculature by cytochrome P450-derived products of arachidonic acid and other eicosanoids. *Prostaglandins Other Lipid Mediat.* 2007; 82:19–29. [PubMed: 17164129]
33. Deeley JM, Hankin JA, Friedrich MG, Murphy RC, Truscott RJ, Mitchell TW, Blanksby SJ. Sphingolipid distribution changes with age in the human lens. *J Lipid Res.* 2010; 51:2753–2760. [PubMed: 20547889]
34. Roy MC, Nakanishi H, Takahashi K, Nakanishi S, Kajihara S, Hayasaka T, Setou M, Ogawa K, Taguchi R, Naito T. Salamander retina phospholipids and their localization by MALDI imaging mass spectrometry at cellular size resolution. *J Lipid Res.* 2011; 52:463–470. [PubMed: 21149645]

Research Highlights

- Sublimation/deposition of MALDI matrix has several distinct advantages in purification of matrix, increase signal, minimize lateral diffusion of lipids
- Small matrix crystal size leads to increase temperature of analyte during desorption process that increases lipid signal strength
- Sublimation/deposition particularly good for imaging lipids in tissue slices.
- Specific examples of images obtained by matrix sublimation/deposition of the brain, lens, retina, and liver are provided.

Figure 1.

(A) Positive ion MALDI image showing the distribution of m/z 760.6 ($[M+H]^+$ PC16:0/18:1) mouse brain, sagittal section, matrix (DHB) applied by sublimation. With permission of Elsevier [11]. (B) Positive ion MALDI image showing the distribution of m/z 834.6 ($[M+H]^+$ PC(18:0/22:6) mouse brain, sagittal section, matrix (DHB) applied by sublimation. With permission of Elsevier [11]. (C) Positive ion MALDI images of rat brain coronal sections showing the different distribution of m/z 782.6 ($[M+Na]^+$ PC(16:0/18:1)) and of m/z 798.6 ($[M+K]^+$ PC(16:0/18:1)) related to traumatic brain injury (TBI). Matrix (DHB) applied by sublimation. This research was originally published in the Journal of Lipid Research as reference [30]© the American Society for Biochemistry and Molecular Biology.

Figure 2.

(A) Positive ion MALDI image showing the distribution of m/z 796.6 (potassiumated PC(16:0/18:2)) in a mouse kidney. Matrix (DHB) applied by sublimation. This research was originally published in the Journal of Lipid Research as reference [31]© the American Society for Biochemistry and Molecular Biology. (B) Positive ion MALDI image from the same MALDI IMS data acquisition showing the distribution of m/z 798.6 (potassiumated PC(16:0/18:1)) in this mouse kidney.

Figure 3.

(A) Positive ion MALDI image showing the distribution of m/z 705.6 (SM(d18:0/16:0)) of a 23, 64, and 70 year old human lens. (B) Positive ion MALDI image showing the distribution of m/z 540.6 (Cer(d18:0/16:0)) of a 23, 64, and 70 year old human lens. Matrix (DHB) applied by sublimation. This research was originally published in the Journal of Lipid Research as reference [33]© the American Society for Biochemistry and Molecular Biology

Figure 4.

Distribution of phosphatidylcholine lipids in a salamander retina. (A) Optical image of a salamander retinal section. Positive ion MALDI images of (B) PC(16:0/16:0), (C) PC(16:0/16:1), and (D) PC(16:0/18:1) which are present in the outer plexiform layer (OPL) and inner plexiform layer (IPL). Positive ion MALDI images of (E) PC(16:0/20:4), (F) PC(16:0/22:6), and (G) PC(18:0/22:6) that are found in the inner segment (IS), outer segment (OS), and retinal pigment epithelium (RPE). Matrix (DHB) applied by sublimation. This research was originally published in the Journal of Lipid Research as reference [34]© the American Society for Biochemistry and Molecular Biology