REVIEW

The use of MALDI MSI for the study of different tissues

Roman Guran[1,2*], Lucie Vanickova[3,4], Ondrej Zitka[1,2], Vojtech Adam[1,2] and Rene Kizek[1,2]

[1] Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic; E-Mails: zitkao@seznam.cz , vojtech.adam@mendelu.cz, kizek@sci.muni.cz

[2] Central European Institute of Technology, Brno University of Technology, Technicka 10, 616 00 Brno, Czech Republic

[3] Laboratory of Chemical Ecology, Institute of Chemistry and Biotechnology, Federal University of Alagoas, Av. Lourival de Melo Mota, s/n, Tabuleiro, 57072-970 Maceió, Brazil; E-Mail: luci.vanickova@gmail.com

[4] Institute of Organic Chemistry and Biochemistry, AS CR, Flemingovo nam. 2, 166 10 Prague, Czech Republic

* Author to whom correspondence should be addressed; E-Mail: r.guran@email.cz;
Tel.: +420 545 133 350; Fax: +420 545 212 044.

Received:2.7.2015 / Accepted:7.7.2015 / Published:1.10.2015

Matrix-assisted laser desorption/ionization mass spectrometry imaging technique (MALDI MSI) has mainly focused on imaging the spatial distribution of biomarkers, drugs and metabolites in different tissues. Due to ion suppression, MALDI MSI is usually used in the *m/z* range from 0 to 30 kDa. To detect a wide range of analyte concentrations, it is necessary to prepare tissue samples appropriately; cryosectioning has been found to be a successful method. There is also a need to process an enormous amount of formaldehyde-fixed tissue samples from histopatology/histochemistry. This article presents a short history and recent progress in MALDI MSI techniques for the imaging of diverse tissues.

Keywords: MALDI imaging; biomarker; tumor; skin; insect; plant

1. Introduction

The matrix assisted laser desorption/ionization (MALDI) technique was introduced by Karas et al. in 1985 [1]. Three years later, the same research group published a first study on the utilization of this ionization method for mass spectrometry of proteins [2]. Since its introduction, MALDI mass spectrometry was developed rapidly. Nowadays, it is routinely used for characterization of peptides, proteins and identification of bacteria. Because of its soft biomolecules ionization, MALDI was found to be useful for mass spectrometry imaging of a variety of samples where information regarding the spatial distribution of molecules is needed. At the turn of the third millennium, MALDI mass spectrometry imaging (MALDI MSI, MALDI imaging) was firstly applied for the determination of protein expression in mammalian tissues [3]. Usually, MALDI is combined with time-of-flight mass spectrometry (TOF MS), because it measures complete mass spectra over wide mass ranges at the same time [4]. There also exist other types of mass spectrometers connected with MALDI, such as Fourier transform ion cyclotron resonance mass spectrometers (FT-ICR MS) or linear ion trap with orbitrap mass spectrometers (LTQ Orbitrap MS) [5-7]. Currently, the MALDI MSI technique is the subject of comprehensive research to improve it in different ways – time of analysis [8,9], spatial resolution [10], and sensitivity and detection of different analytes [11,12]. Information gained from MALDI MSI can be correlated with immunohistochemical images [13] or with images from other techniques such

as magnetic resonance imaging (MRI) [14] or laser ablation-inductively coupled plasma mass spectrometry/atomic emission spectrometry (LA-ICP MS/AES) [15]. There exist several extensive reviews on recent progress in MALDI MSI and on the development of MALDI imaging techniques that are recommended to readers with interest in this field [16-18].

In the following paragraphs, a brief description of the current state of the use of MALDI MSI in research on different analytes in tissues will be given.

2. MALDI MSI in study of various tissues

In order for MALDI MSI analyses to generate reproducible data, proper sample preparation is crucial. Usually, various organs [19,20], plant tissues [21], bacterial colonies [22] or cells [23] are analyzed. There are different ways of preparing tissue sections depending on the state of tissue sample – whether it is fresh, frozen (-80 °C), conserved in ethanol or fixed in formaldehyde and embedded in paraffin [24,25]. The most frequently analyzed tissues are liver, kidneys, lungs, brain, heart and all types of tumors. The preferred preparation of tissue is quick and deep freezing of fresh samples – this minimalizes the degradation of analytes and fixes their spatial distribution. Rapid freezing of the entire tissue is crucial to prevent the sample from cracking and the formation of ice crystals. The tissue sample is firstly wrapped in thin aluminum foil and then is immersed repeated in the freezing liquid (nitrogen, ethanol, isopropanol). Afterwards, the tissue sample is stored at -40 or -80 °C, depending on the used freezing liquid. The optimal thickness of frozen tissue sections, made in cryotome is 5-20 µm. Fixation of frozen tissue sections on the conductive ITO (indium-tin oxide) glass slide is generally conducted by dehydration – usually a glass slide with tissue is briefly soaked in 70% ethanol and then soaked for a few minutes in 90% and 100% ethanol. For protein analysis, prior to the application of matrix, lipids and salts are washed away with ethanol and water or other organic solvents (xylene, chloroform). To desalt tissue samples prior to lipid analysis a solution of ammonium acetate or ammonium formate is recommended [23].

A general MALDI MSI workflow consists of cutting the tissue into slices, scanning the glass slide, applying a matrix solution, measuring the mass spectra, and analyzing the data using 2D mass maps (Fig. 1). For MALDI MSI of tissue sections that have been formalin-fixed and paraffin-embedded, sample preparation is more complicated. Paraffin can suppress ionization, and formaldehyde fixation causes dehydration, denaturation, crosslinking (methylene bridges), and the precipitation and agglutination of proteins, which prevents detection. Therefore, the deparaffination of tissue samples by incubation in xylene for a few minutes is needed. Then, rehydration is carried out by soaking the slides with the tissue samples in a series of ethanol solutions gradually decreasing in concentration. Antigens are recovered by breaking methylene bridges of cross-linked proteins at high temperatures and incubating in buffers differing in pH and ionic strength [13].

2.1 MALDI MSI of tumors

Tumors are the most frequently studied tissues by MALDI MSI. Oncology research plays an important role in the rapid development of MALDI MSI techniques.

The majority of researchers are searching for new cancer biomarkers or for distributions of drugs and their metabolites inside tumor tissue. In order to obtain the necessary data, sample preparation and matrix application must be optimized for each type of investigated analyte. Some analytes suffer from ion suppression, for example, and require different methods for their study.

Nearly all types of tumors have been investigated by MALDI MSI techniques. The most often applied MALDI MSI technique is MALDI-TOF MSI. For example, a time-of-flight detector was used to image metabolites in colorectal liver cancer metastases in a mouse model [26]. The applied matrix was N (1 naphthyl)ethylenediamine dihydrochloride (NEDC). This matrix was proven to be useful in the analysis of oligosaccharides and glycerophospholipids. In another study, MALDI-TOF MSI was com-

bined with MALDI-FT-ICR MSI to visualize and quantify the distribution of the anticancer drug, irinotecan, and its active metabolite, SN-38, in colon cancer (in a murine model) [27]. Rodrigo et al. reviewed that MALDI-TOF MSI was also used in studies of gastrointestinal cancer, cancer of the respiratory system, renal and bladder cancer, and prostate, breast and ovarian cancer, in order to find new cancer biomarkers or to study the spatial distribution of anticancer drugs in tumors [28].

MALDI-LTQ Orbitrap MSI is another technique that has been used for drug imaging in human lung tumor sections and rat xenograft tissue sections, where thin sections were exposed to pharmaceutical drugs (erlotinib, gefitinib, and tiotropium), and were characterized by microenvironment localization [31]. This method was also applied in a study, where a comparison between two different ways of carrying out pulmonary drug administration (inhalation of a nebulized aerosol of aqueous

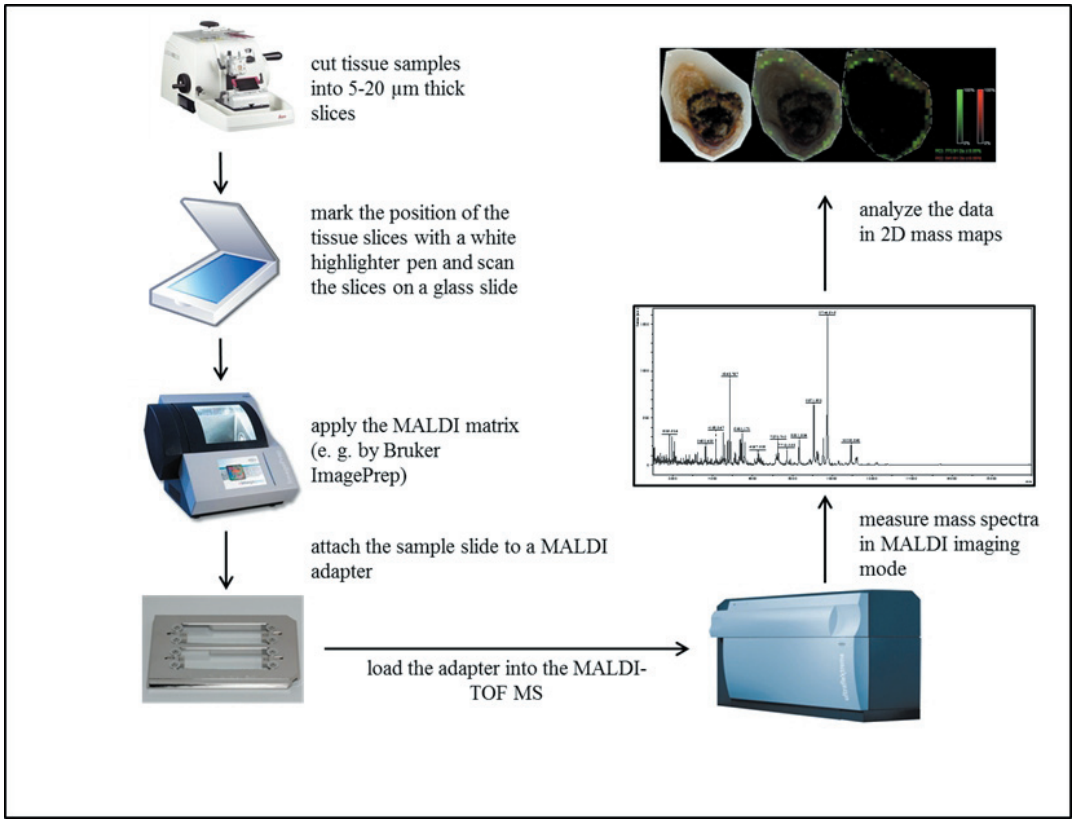

Figure 1: The scheme of typical MALDI MSI workflow.

MALDI-FT-ICR MSI was employed in in situ studies of lipids in head and neck tumors [29], where it served for gaining high resolution data sets for higher mass accuracy and better interpretation of lipidome, and in a study, where it helped to find and optimize an ex vivo model for better and faster optimization of sample preparation procedures in MALDI drug imaging studies [30].

drug solutions and intratracheal administration) was carried out in guinea pigs (model organism). Results indicated different distributions of the drug in connection with different method of administration [32].

2.2 MALDI MSI of other tissues

The (analytical) power of MALDI MSI has been demonstrated in many studies and with diverse types of tissues. For example, dermato-

logy/cosmetics use MALDI MSI to investigate the effect of age and peptide applications on the skin [33]. In other study from the fields of cosmetics and forensics, MALDI MSI was used to investigate reactions of hydrogen peroxide with cocaine in hair to provide information whether cocaine and his reaction products can be detected in hair after bleaching. It was found that all compounds of interest are in the hydrogen peroxide and wash solutions, and thus all evidence of cocaine use might be lost after a hair bleaching treatment [34]. An interesting study on MALDI MS/MS imaging of the drug tilidine in hair after external contamination revealed that for correct segmental hair analysis it is necessary to collect hair samples not only several weeks after intake but also within the first days after intake [35]. Another study on detection of blood in fingerprints revealed that MALDI MSI can support existing presumptive tests by detecting the molecules of haem and haemoglobin through their *m/z* ratios. Moreover, MALDI MSI is compatible with other methods employed for enhancing fingerprints contaminated by blood [36].

Mammalian retinas have also been investigated, and high resolution MALDI MSI was used to obtain information about the distribution of lipids on the retina [37]. Resolution at the level of a single cell was achieved.

Mesenchymal stem cells were investigated by MALDI MSI for the characterization of lipid markers of chondrogenic differentiation [38], and 20 different lipid species were identified.

Several studies focused on MALDI MSI of plant tissues. Soares et al. investigated hesperidin and rutin in Citrus sinensis grafted on Citrus limonia after infection by Xylella fastidiosa [39]. They suggested that hesperidin plays a role in the plant-pathogen interaction. In previous study, hypericin (a red anthraquinone-derivative with medicinal properties) and related phytochemicals were investigated in the leaves of Hypericum species by high resolution MALDI MSI [40]. Yet another study focused on using MALDI MSI to detect fungicide residue on wheat leaf surfaces, demonstrating the potential of MALDI MSI for monitoring the distribution of agrochemicals on leaves [41].

Insects have also been the subject of MALDI MSI. Klein et al. investigated plant-pest chemical interfaces inside leaves, specifically the interactions between soybean and aphids and rice and bacteria were studied [42]. Whole-body sections and various organs of the rove beetle, Paederus riparius, were investigated by atmospheric pressure high resolution scanning microprobe MALDI MSI to locate metabolites of the defensive compounds pederin, pseudopederin and pederon [43]. An interesting study from Brazil focused on queen bee signals in the stingless bee Friesella schrottkyi and illustrated the spatial distribution of active compounds on queen bees [44]. The spatial distribution of lipids in Drosophila melanogaster was also analyzed by MALDI MSI to provide more information about this model organism [45].

3. Conclusion

It is clear that MALDI MSI has potential in different research fields. Some significant improvements of MALDI imaging techniques have been made and surely these techniques will undergo important improvements and modernization to serve analyze tumors and other tissues. Further developments and the combination of MALDI MSI with other imaging techniques or quantitative methods will result in wider use of MALDI MSI, and clinical methods using MALDI MSI could be validated and applied in routine analytical processes in laboratories.

MALDI MSI can be used as supportive technique in clinical research, mainly in oncology. Finding new cancer biomarkers is so important that all suitable methods should be tested. The task for researchers is to find relevant analytical standards and make MALDI MSI a standard analytical method as soon as possible.

List of abbreviations

AES … atomic emission spectrometry
FT … Fourier transform
ICP … inductively coupled plasma
ICR … ion cyclotron resonance
ITO … indium-tin oxide
LA … laser ablation
LTQ … linear ion trap/linear trap quadrupole

MALDI … matrix assisted laser desorption/ ionization
MRI … magnetic resonance imaging
MS … mass spectrometry/spectrometer
MSI … mass spectrometry imaging
NEDC … N (1 naphthyl)ethylenediamine dihydrochloride
TOF … time-of-flight

Acknowledgments

The authors acknowledge Dr. Tara Massad for the language corrections and valuable comments on the early version of the manuscript. The financial support from "CEITEC–start_up" project (CZ.1.05/1.1.00/02.0068) and doctoral project "MALDI-IMAGE" (PGS27_2014) is greatly acknowledged.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Karas, M.; Bachmann, D.; Hillenkamp, F. Influence of the wavelength in high-irradiance ultraviolet-laser desorption mass-spectrometry of organic-molecules. Analytical Chemistry 1985, 57, 2935-2939.
2. Karas, M.; Hillenkamp, F. Laser desorption ionization of proteins with molecular masses exceeding 10000 Daltons. Analytical Chemistry 1988, 60, 2299-2301.
3. Stoeckli, M.; Chaurand, P.; Hallahan, D.E.; Caprioli, R.M. Imaging mass spectrometry: A new technology for the analysis of protein expression in mammalian tissues. Nature Medicine 2001, 7, 493-496.
4. Caprioli, R.M.; Farmer, T.B.; Gile, J. Molecular imaging of biological samples: Localization of peptides and proteins using MALDI-TOF MS. Analytical Chemistry 1997, 69, 4751-4760.
5. Solouki, T.; Marto, J.A.; White, F.M.; Guan, S.H.; Marshall, A.G. Attomole biomolecule mass analysis by matrix-assisted laser-desorption ionization fourier-transform ion-cyclotron resonance. Analytical Chemistry 1995, 67, 4139-4144.
6. Strupat, K.; Kovtoun, V.; Bui, H.; Viner, R.; Stafford, G.; Horning, S. MALDI produced ions inspected with a linear ion trap-orbitrap hybrid mass analyzer. Journal of the American Society for Mass Spectrometry 2009, 20, 1451-1463.
7. Chen, B.M.; Lietz, C.B.; Li, L.J. In situ characterization of proteins using laserspray ionization on a high-performance MALDI-LTQ-Orbitrap mass spectrometer. Journal of the American Society for Mass Spectrometry 2014, 25, 2177-2180.
8. Bednarik, A.; Kuba, P.; Moskovets, E.; Tomalova, I.; Krasensky, P.; Houska, P.; Preisler, J. Rapid matrix-assisted laser desorption/ionization time-of-flight mass spectrometry imaging with scanning desorption laser beam. Analytical Chemistry 2014, 86, 982-986.
9. Prentice, B.M.; Chumbley, C.W.; Caprioli, R.M. High-speed MALDI MS/MS imaging mass spectrometry using continuous raster sampling. Journal of Mass Spectrometry 2015, 50, 703-710.
10. Korte, A.R.; Yandeau-Nelson, M.D.; Nikolau, B.J.; Lee, Y.J. Subcellular-level resolution MALDI-MS imaging of maize leaf metabolites by MALDI-linear ion trap-Orbitrap mass spectrometer. Analytical and Bioanalytical Chemistry 2015, 407, 2301-2309.
11. Flinders, B.; Morrell, J.; Marshall, P.S.; Ranshaw, L.E.; Clench, M.R. The use of hydrazine-based derivatization reagents for improved sensitivity and detection of carbonyl containing compounds using MALDI-MSI. Analytical and Bioanalytical Chemistry 2015, 407, 2085-2094.
12. Wang, X.D.; Han, J.; Yang, J.C.; Pan, J.X.; Borchers, C.H. Matrix coating assisted by an electric field (MCAEF) for enhanced tissue imaging by MALDI-MS. Chemical Science 2015, 6, 729-738.
13. Caldwell, R.L.; Gonzalez, A.; Oppenheimer, S.R.; Schwartz, H.S.; Caprioli, R.M. Molecular assessment of the tumor protein microenvironment using imaging mass spectrometry. Cancer Genomics & Proteomics 2006, 3, 279-287.
14. Acquadro, E.; Cabella, C.; Ghiani, S.; Miragoli, L.; Bucci, E.M.; Corpillo, D. Matrix-assisted laser desorption ionization imaging mass spectrometry detection of a magnetic resonance imaging contrast agent in mouse liver. Analytical Chemistry 2009, 81, 2779-2784.
15. Bianga, J.; Bouslimani, A.; Bec, N.; Quenet, F.; Mounicou, S.; Szpunar, J.; Bouyssiere, B.; Lobinski, R.; Larroque, C. Complementarity of MALDI and LA ICP mass spectrometry for platinum anticancer imaging in human tumor. Metallomics 2014, 6, 1382-1386.
16. Dreisewerd, K. Recent methodological advances in MALDI mass spectrometry. Analytical and Bioanalytical Chemistry 2014, 406, 2261-2278.
17. Rompp, A.; Spengler, B. Mass spectrometry imaging with high resolution in mass and space. Histochemistry and Cell Biology 2013, 139, 759-783.
18. Svatos, A. Mass spectrometric imaging of small molecules. Trends in Biotechnology 2010, 28, 425-434.
19. Sun, N.; Ly, A.; Meding, S.; Witting, M.; Hauck, S.M.; Ueffing, M.; Schmitt-Kopplin, P.; Aichler, M.; Walch, A. High-resolution metabolite imaging of light and dark treated retina using MALDI- FTICR mass spectrometry. Proteomics 2014, 14, 913-923.
20. Wang, H.Y.J.; Wu, H.W.; Tsai, P.J.; Liu, C.B.; Zheng, Z.F. Matrix-assisted laser desorption/ionization mass spectrometry imaging of cardiolipins in rat organ sections. Analytical and Bioanalytical Chemistry 2014, 406, 565-575.
21. Shroff, R.; Schramm, K.; Jeschke, V.; Nemes, P.; Vertes, A.; Gershenzon, J.; Svatos, A. Quantification of plant surface metabolites by matrix-assisted laser desorption-ionization mass spectrometry imaging: Glucosinolates on Arabidopsis thaliana

leaves. Plant Journal 2015, 81, 961-972.
22. Louie, K.B.; Bowen, B.P.; Cheng, X.L.; Berleman, J.E.; Chakraborty, R.; Deutschbauer, A.; Arkin, A.; Northen, T.R. „Replica-extraction-transfer" nanostructure-initiator mass spectrometry imaging of acoustically printed bacteria. Analytical Chemistry 2013, 85, 10856-10862.
23. Nimesh, S.; Mohottalage, S.; Vincent, R.; Kumarathasan, P. Current status and future perspectives of mass spectrometry imaging. International Journal of Molecular Sciences 2013, 14, 11277-11301.
24. Kriegsmann, J.; Kriegsmann, M.; Casadonte, R. MALDI TOF imaging mass spectrometry in clinical pathology: A valuable tool for cancer diagnostics. International Journal of Oncology 2015, 46, 893-906.
25. Thomas, A.; Chaurand, P. Advances in tissue section preparation for MALDI imaging MS. Bioanalysis 2014, 6, 967-982.
26. Wang, J.N.; Qiu, S.L.; Chen, S.M.; Xiong, C.Q.; Liu, H.H.; Wang, J.Y.; Zhang, N.; Hou, J.; He, Q.; Nie, Z.X. MALDI-TOF MS imaging of metabolites with a N (1 naphthyl)ethylenediamine dihydrochloride matrix and its application to colorectal cancer liver metastasis. Analytical Chemistry 2015, 87, 422-430.
27. Buck, A.; Halbritter, S.; Spath, C.; Feuchtinger, A.; Aichler, M.; Zitzelsberger, H.; Janssen, K.P.; Walch, A. Distribution and quantification of irinotecan and its active metabolite SN-38 in colon cancer murine model systems using MALDI MSI. Analytical and Bioanalytical Chemistry 2015, 407, 2107-2116.
28. Rodrigo, M.A.M.; Zitka, O.; Krizkova, S.; Moulick, A.; Adam, V.; Kizek, R. MALDI-TOF MS as evolving cancer diagnostic tool: A review. Journal of Pharmaceutical and Biomedical Analysis 2014, 95, 245-255.
29. Krasny, L.; Hoffmann, F.; Ernst, G.; Trede, D.; Alexandrov, T.; Havlicek, V.; Guntinas-Lichius, O.; von Eggeling, F.; Crecelius, A.C. Spatial segmentation of MALDI FT-ICR MSI data: A powerful tool to explore the head and neck tumor in situ lipidome. Journal of the American Society for Mass Spectrometry 2015, 26, 36-43.
30. Huber, K.; Aichler, M.; Sun, N.; Buck, A.; Li, Z.; Fernandez, I.E.; Hauck, S.M.; Zitzelsberger, H.; Eickelberg, O.; Janssen, K.P., et al. A rapid ex vivo tissue model for optimising drug detection and ionisation in MALDI imaging studies. Histochemistry and Cell Biology 2014, 142, 361-371.
31. Vegvari, A.; Fehniger, T.E.; Rezeli, M.; Laurell, T.; Dome, B.; Jansson, B.; Welinder, C.; Marko-Varga, G. Experimental models to study drug distributions in tissue using MALDI mass spectrometry imaging. Journal of Proteome Research 2013, 12, 5626-5633.
32. Zecchi, R.; Trevisani, M.; Pittelli, M.; Pedretti, P.; Manni, M.E.; Pieraccini, G.; Pioselli, B.; Amadei, F.; Moneti, G.; Catinella, S. Impact of drug administration route on drug delivery and distribution into the lung: An imaging mass spectrometry approach. European Journal of Mass Spectrometry 2013, 19, 475-482.
33. Mondon, P.; Hillion, M.; Peschard, O.; Andre, N.; Marchand, T.; Doridot, E.; Feuilloley, M.G.J.; Pionneau, C.; Chardonnet, S. Evaluation of dermal extracellular matrix and epidermal-dermal junction modifications using matrix-assisted laser desorption/ionization mass spectrometric imaging, in vivo reflectance confocal microscopy, echography, and histology: Effect of age and peptide applications. Journal of Cosmetic Dermatology 2015, 14, 152-160.
34. Cuypers, E.; Flinders, B.; Bosman, I.J.; Lusthof, K.J.; Van Asten, A.C.; Tytgat, J.; Heeren, R.M.A. Hydrogen peroxide reactions on cocaine in hair using imaging mass spectrometry. Forensic Science International 2014, 242, 103-110.
35. Poetzsch, M.; Baumgartner, M.R.; Steuer, A.E.; Kraemer, T. Segmental hair analysis for differentiation of tilidine intake from external contamination using LC-ESI-MS/MS and MALDI-MS/MS imaging. Drug Testing and Analysis 2015, 7, 143-149.
36. Bradshaw, R.; Bleay, S.; Clench, M.R.; Francese, S. Direct detection of blood in fingermarks by MALDI MS profiling and imaging. Science & Justice 2014, 54, 110-117.
37. Ly, A.; Schone, C.; Becker, M.; Rattke, J.; Meding, S.; Aichler, M.; Suckau, D.; Walch, A.; Hauck, S.M.; Ueffing, M. High-resolution MALDI mass spectrometric imaging of lipids in the mammalian retina. Histochemistry and Cell Biology 2015, 143, 453-462.
38. Rocha, B.; Cillero-Pastor, B.; Eijkel, G.; Bruinen, A.L.; Ruiz-Romero, C.; Heeren, R.M.A.; Blanco, F.J. Characterization of lipidic markers of chondrogenic differentiation using mass spectrometry imaging. Proteomics 2015, 15, 702-713.
39. Soares, M.S.; da Silva, D.F.; Forim, M.R.; da Silva, M.F.d.G.F.; Fernandes, J.B.; Vieira, P.C.; Silva, D.B.; Lopes, N.P.; de Carvalho, S.A.; de Souza, A.A., et al. Quantification and localization of hesperidin and rutin in Citrus sinensis grafted on C. limonia after Xylella fastidiosa infection by HPLC-UV and MALDI imaging mass spectrometry. Phytochemistry 2015, 115, 161-170.
40. Kusari, S.; Sezgin, S.; Nigutova, K.; Cellarova, E.; Spiteller, M. Spatial chemo-profiling of hypericin and related phytochemicals in Hypericum species using MALDI-HRMS imaging. Analytical and Bioanalytical Chemistry 2015, 407, 4779-4791.
41. Annangudi, S.P.; Myung, K.; Adame, C.A.; Gilbert, J.R. MALDI-MS imaging analysis of fungicide residue distributions on wheat leaf surfaces. Environmental Science & Technology 2015, 49, 5579-5583.
42. Klein, A.T.; Yagnik, G.B.; Hohenstein, J.D.; Ji, Z.Y.; Zi, J.C.; Reichert, M.D.; MacIntosh, G.C.; Yang, B.; Peters, R.J.; Vela, J., et al. Investigation of the chemical interface in the soybean-aphid and rice-bacteria interactions using MALDI-mass spectrometry imaging. Analytical Chemistry 2015, 87, 5294-5301.
43. Bhandari, D.R.; Schott, M.; Rompp, A.; Vilcinskas, A.; Spengler, B. Metabolite localization by atmospheric pressure high-resolution scanning

microprobe matrix-assisted laser desorption/ ionization mass spectrometry imaging in whole-body sections and individual organs of the rove beetle Paederus riparius. Analytical and Bioanalytical Chemistry 2015, 407, 2189-2201.
44. Nunes, T.M.; Mateus, S.; Favaris, A.P.; Amaral, M.; von Zuben, L.G.; Clososki, G.C.; Bento, J.M.S.; Oldroyd, B.P.; Silva, R.; Zucchi, R., et al. Queen signals in a stingless bee: Suppression of worker ovary activation and spatial distribution of active compounds. Scientific Reports 2014, 4.
45. Niehoff, A.C.; Kettling, H.; Pirkl, A.; Chiang, Y.N.; Dreisewerd, K.; Yew, J.Y. Analysis of Drosophila lipids by matrix-assisted laser desorption/ ionization mass spectrometric imaging. Analytical Chemistry 2014, 86, 11086-11092.