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MINIREVIEW

MALDI mass spectrometric imaging meets "omics": Recent advances in the fruitful marriage

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A. C. Crecelius,[ab *] U. S. Schubert[ab] and F. von Eggeling[bcde]

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Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI MSI) is a method that allows the investigation of the molecular content of surfaces, in particular tissues, within its morphological context. The applications of MALDI MSI in the field of large-scale mass spectrometric studies, which are typically denoted by the suffix "omics", is steadily increasing. This is because, on one hand, technical advances regarding sample collection and preparation, matrix application, instrumentation, and data processing have enhanced molecular specificity and sensitivity of MALDI MSI; and, on the other hand, the focus of the "omics" community has moved from establishing an inventory of certain compound classes to exploring their spatial distribution to gain novel insights. Thus, the aim of this mini-review is twofold, to display the state-of-art in terms of technical aspects in MALDI MSI and to highlight selected applications within the last two years, which either have significantly advanced a certain "omics" field or have introduced a new one through pioneering efforts.

[a]Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Humboldtstraße 10, 07743 Jena, Germany.
[b]Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena, Philosophenweg 7, 07743 Jena, Germany, E-mail: anna.crecelius@uni-jena.de.
[c]Institute of Physical Chemistry, Friedrich Schiller University Jena, Helmholtzweg 4, 07743 Jena, Germany.
[d]Department of Otorhinolaryngology, Jena University Hospital, Lessingstraße 2, 07743, Jena, Germany.
[e]Leibnitz Institute of Photonic Technology (IPHT), Albert-Einstein-Straße 9, 07745 Jena, Germany.

Anna C. Crecelius obtained her Ph.D. in 2002 under the supervision of Prof. Malcolm R. Clench at the Sheffield Hallam University (U.K.). She has conducted post-doctoral research stays at universities including Vanderbilt University (USA) and Ludwig Maximillians University Munich. Currently, she is a senior research scientist in the group of Prof. Ulrich S. Schubert at the Friedrich Schiller University Jena. Her main research area is the development and application of mass spectrometric imaging techniques.

Ulrich S. Schubert studied chemistry in Frankfurt and Bayreuth and the Virginia Commonwealth University (USA). His Ph.D. studies were performed at the Universities of Bayreuth and Tampa (USA). After postdoctoral training with Jean-Marie Lehn at the University in Strasbourg (France), he moved to the TU

München and obtained his Habilitation in 1999. From 1999 to 2000 he was Professor at the Center for NanoScience, University of Munich, and from 2000 to 2007 Full-Professor at TU Eindhoven (The Netherlands). Currently he holds a chair at the Friedrich Schiller University Jena with research interest in nanoparticle systems as sensor and drug delivery devices, supramolecular chemistry, inkjet printing of polymers, polymers for energy applications, and self-healing materials.

Ferdinand von Eggeling studied Biology and Chemistry in Erlangen and finished his Ph.D (1995) and his Habilitation (2003) at the Friedrich Schiller University Jena in Genetics. Since 2001 he was head of the Core Unit Chip Application (CUCA) at the Institute of Human Genetics. In 2009 he received the apl. Professor for Genetics at the Friedrich Schiller University Jena. Currently he has an affiliation at the Institute of Physical Chemistry and the ENT-Department of the University Hospital in Jena. His main interest is in mass spectrometric imaging of different tumour entities and the biological characterisation of functional tissue components.

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Introduction

One of the simplest instruments to generate images is a pinhole camera, which astronomers have been used in the 13th century to observe sunspots. In the 16th century the microscope was developed which allowed detecting small objects like mammalian eggs, sperms or bacteria. The next level of imaging was reached by the development of X-rays (1895) which enabled physicians to look into a body. Since 1973 magnetic resonance imaging is also used for the examination of organs or the whole body in 3D. Another medical imaging technique that produces also a three-dimensional image from functional processes in the body is positron emission tomography. With this imaging technique molecular processes could be made visible to a certain extent, however radioactive labels are required. An emerging label free technique is mass spectrometric imaging (MSI), which was developed at the end of the 20th century. Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI MSI) was one of the first soft ionization techniques applied to it.[1, 2] One key aspect of a successful MALDI MSI experiment is the procedure for the matrix application,[3] and beforehand the right choice of matrix.[4] Due to the increasing applications of ultra violet (UV) MALDI MSI in various "omics" field, the information, which matrix is suitable for which family of compound classes and mass range can be easily found in the literature. In particular in metabolomics, considerable efforts have been carried out in the last two years to discover new matrices, such as the versatile matrix 9-aminoacridine.[5-7] Hence, Table 1 provides a snapshot of the current state-of-art of common and new UV MALDI MSI matrices, subdivided into the corresponding "omics" fields. We listed only one recent reference, published within the last two years, in Table 1 for each presented MALDI MSI matrix candidate based on the selected matrix application procedure. Additional information regarding suitable UV MALDI MSI matrices for animal tissue analysis can be found in the recent review by Thomas and Chaurand.[8]

In order to circumvent the disadvantages, such as lateral analyte migration induced through matrix deposition,[9] the possibility of using heterocyclic based dyes,[10] nanostructured indium tin oxide coated glass slides,[11] or nanostructure-assisted laser desorption/ionization[12] are promising alternatives, which can be employed for the analysis of small molecules, e.g. in the fields of peptidomics, metabolomics, glycomics, or plantomics.

Even though, UV MALDI MSI is more robust than infrared (IR) MALDI MSI,[13] its few applications should be presented in this mini-review. The advantage of IR MALDI MSI over UV MALDI MSI is that no sample preparation is required, since the physiological cell water of the biological tissue can be used as matrix.[14] During measurements, the evaporation of the physiological cell water can be prevented by employing an atmospheric pressure (AP) ion source with a cooling stage.[15, 16] For improving the ionization yield, AP IR MALDI MSI can be combined with an electrospray postionization step, such as accomplished in matrix-assisted laser desorption electrospray ionization (MALDESI).[17-19]

Despite the right choice of matrix, it should be kept in mind that the complete sample preparation protocol determines e.g. spectrum quality, spatial resolution, as well as reproducibility of the MALDI MSI experiments.[8, 20, 21] Key aspects of sample handling and preparation include tissue processing (fresh frozen *vs.* embedded tissue),[22] washing steps,[23, 24] on-tissue chemistry (e.g. enzymatic digestion,[25, 26] derivatization,[27] transfer to functionalized surfaces),[28] and instrumentation for the application of the matrix.[3, 29-31] A variety of embedding media has been tested, ranking from gelatin,[32] carboxymethylcellulose,[33] poly[*N*-(2-hydroxypropyl) methacryl amide],[22] to eye yolk.[34] Hence, the optimization of all these parameters based on the type of sample, ranking from human, animal, plant, food tissue, cells, cosmetics, to polymeric materials, is essential for the outcome of the MALDI MSI experiment. No wonder that especially during the last year more and more efforts have been devoted to methodology optimization,[3, 35-37] validation,[27, 38] and standardization,[39] which is essential for translating e.g. biomedical applications of MALDI MSI into the clinics.[40, 41]

One advancement in MALDI MSI has certainly contributed to the translation into the clinic research, the analysis of formalin-fixed paraffin-embedded (FFPE) tissue.[42] In comparison to fresh frozen tissue, for which MALDI MSI was originally developed in the field of proteomics,[1] FFPE tissue enables the storage at room temperature for a long period of time. However, several additional sample preparation steps (deparaffination, rehydration, antigen retrieval, and enzymatic digestion) are necessary in comparison to fresh frozen tissue.[43] Hence, a great number of publications were published recently to tackle this issue.[14] For tissue preservation not only FFPE tissue is suitable, also heat treatment is efficient.[44]

Another feature, which is necessary for clinical applications, is the increased sample throughput.[45] One possibility is to use pre-coated slides, bypassing the matrix application after sectioning.[46] Finally, the correlation of the extracted ion images with the underlying histology represents an important aspect in the clinical setting towards personalized medicine.[47] This can be achieved in different ways: (1) staining of the tissue before MALDI MSI analysis,[48] (2) removal of the matrix after MALDI MSI analysis and staining of the tissue[49] or (3) staining of a serial section.[50] All three options have their advantages and disadvantage, e.g. option one is only applicable to certain stains, which are compatible with MALDI MSI, option two can lead to disrupter of the tissue integrity caused by the matrix removal, and option three can influence the co-registration step of ion and optical image. Hence, the right choice varies from case to case, e.g. head and neck tissue tears during matrix removal,[50] therefore option two is not applicable, and hematoxylin-eosin (H&E) staining is not compatible with MALDI MSI, therefore only option three is suitable.

Typically, the majority of studies belonging to one "omics" field are focusing on a single principal of mass analysis in order to fulfil the requirements for the specific compound class, as illustrated in Table 1. For example in proteomics, in which molecules of high molar mass are of interest, the time-of-flight

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(TOF) or TOF/TOF analyser, is generally employed due to the highest practical mass range.[51] However, the TOF/TOF instruments have a limited precursor-ion selectivity for MS/MS experiments, which makes the on-tissue structural elucidation of the recorded ions in the performed MALDI MSI experiments challenging. For the analysis of small molecules, by means of metabolomics, plantomics, etc. an analyzer with high mass resolving power is so beneficial, that a loss in dynamic range is taken into account. Hence, Fourier-transform ion cyclotron resonance (FT-ICR) or Orbitrap analyzer are commonly used. However, the trend to achieve high, even towards cellular spatial resolution is creating, especially with these kind of instruments, new challenges. The question, which nowadays often arises is "how do I get fast meaningful results from these big datasets?", since the aim is to process and analyse these datasets in an efficient, fast and standardized manner. The MALDI MSI community is currently responding to this issue by suggesting various computational strategies.[50, 52-58] Finally, in drug imaging,[59, 60] besides ion traps (FT-ICR and Orbitrap), triple quadropole analyser is additionally in favour, due to its selected reaction monitoring capabilities.[61, 62]

Since more and more research groups are employing MALDI MSI nowadays, new software has been developed to visualize (MIRION),[63] process (MSiReader,[64] OpenMSI[65]), and to store[66] the generated datasets.

The mini-review will subsequently focus on selected applications of MALDI MSI. Publications within the last two years, starting from 2013 up to the first quarter of 2015 are considered, which either substituted a common "omics" field or introduced a new one owing to its potential.

Selected "omics" applications

Mass spectrometry has become an indispensable tool in the "omics" analysis due to its vast expansion. Often not only the identification and quantification of a certain compound class is of interest, but also its localization within the biological system, which cannot be determined by classical MS based approaches alone without any additional fractionation, extraction steps. As a consequence, MALDI MSI is gaining growing significance, in particularly in biological and clinical studies for improved diagnostic and therapeutic approaches.[45] Due to the rapidly growing number of publications in this field within the last two years, only significant applications of MALDI MSI in the different "omics" based areas will be discussed. For further biological applications of MALDI MSI, the review by Spengler is highly recommended, who covers the last three years.[14]

Table 1. Overview of widely used and new MALDI MSI matrices, classified according to the "omics" field. (Time-of-flight, TOF; quadrupole time-of-flight, QTOF; fourier-transform ion cyclotron resonance, FT-ICR; ion mobility, IM)

"Omics" field	Compound class	Matrix	Matrix application procedure	Spatial resolution [µm]	Specimen	MS analyzer (ion mode)	Ref.
Proteomics	Proteins	Sinapinic acid	Automatic vibrational sprayer	80	Mouse brain	TOF/TOF (positive linear)	67
			Sublimation device followed by rehydration	50, 100	Human ulcers	TOF (positive linear)	68
			Pre-coated gold covered microscopic slides	10	Mouse brain and rat kidney	TOF (positive linear)	46
		2,5-Dihydroxyacetophenone	Sublimation device followed by recrystallization Automatic nozzle/air sprayer system	5	Mouse and rat brain	TOF and TOF/TOF (positive linear)	69
			Automatic nozzle sprayer	1	Mouse brain	TOF (transmission geometry)	70
	Peptides	α-Cyano-4-hydoxycinnamic acid	Automatic vibrational sprayer	150	Human knee synovial and cartilage tissue	IM (positive-V-mode)	71
				80	Rat muscles	TOF/TOF (positive reflector)	72
			Automatic pneumatic sprayer	100	Mouse brains	TOF/TOF (positive reflector)	73
Peptidomics	Peptides	2′,4′,6′-Trihydroxyacetophenone	Automatic vibrational sprayer	50-200	Rat intestine	TOF (positive linear)	74
	Neuropeptides	2,5-Dihydroxybenzoic acid	Airbrush	25	Stromatogastric nervous tissue of crabs	TOF/TOF (positive reflector) FT-ICR (positive)	75
				50		TOF/TOF (positive reflector)	76
		α-Cyano-4-hydoxycinnamic acid	Chemical Printer	50	Honeybees brains	TOF/TOF (positive reflector)	77
Metabolomics	Lipids	2,5-Dihydroxybenzoic acid	Sublimation device	10	Mammalian retina	TOF or TOF/TOF (positive reflector)	35
		Alkylated 2,5-dihydroxybenzoic acid	Capillary spraying device	50	Rat brain	TOF/TOF (positive, negative reflector)	78
		α-Cyano-4-hydoxycinnamic acid	Automatic vibrational sprayer	150	Mouse kidney (mouse model for fabry disease)	FT-ICR (positive)	79
			Automatic nozzle sprayer	70	Human colorectal cancer tissue	IM (positive-V-mode)	80
		Binary matrix of 2,5-dihydroxybenzoic acid and α-cyano-4-hydoxycinnamic acid	Automatic vibrational sprayer	150	Mouse liver (mouse model for HBV infection)	TOF (positive reflector)	81
		2,6-Dihydroxyacetophenone	Sublimation device	10	Mouse eyes (mouse model for stargardt disease)	FT-ICR (positive)	82

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		9-Aminoacridine	Automatic vibrational sprayer	50	Mouse kidney	TOF/TOF (negative reflector)	5
		1,5-Diaminonapthalene	Sublimation device	80, 100, 255	Pig and mouse brain homogenates	FT-ICR (positive)	83
				10, 100	Mouse brain, *xiphosphorus maculatus* fish	TOF/TOF (positive and negative reflector)	84
		1,8-Bis(dimethyl-amino)naphthalene	Sublimation device followed by recrystallization	20, 1000	Human cancer tissue	FT-ICR (negative)	85
		Quercitin	Automatic vibrational sprayer	200	Porcine adrenal glands	FT-ICR (positive, negative)	86
		Curcumin	Airgun, Automatic pneumatic sprayer, Acoustic droplet ejection system	50, 100, 150	Human fingermarks, rat lung, living skin equivalent	Q-TOF (positive) IM (positive)	87
		Dithranol	Airbrush	10	Mouse brain	IM (negative)	88
		Ag nanoparticles	Implanter	50	Rat heart	Orbitrap (positive, negative)	89
		1,5-Napthalenediamine hydrochloride	Automatic vibrational sprayer	50, 100, 200	Mouse and rat brains (MCAO rat model)	TOF/TOF (negative reflector)	90
		4‘-Phenyl-α-cyanocinnamic acid amide	Automatic pneumatic sprayer	100	Rat brain	TOF (positive, negative reflector)	91
	Nucleotides	9-Aminoacridine	Automatic pneumatic sprayer	50	Porcine eyes	FT-ICR (negative reflector)	6
	Nucleotides, Lipids	*N*-(1-Naphthyl) ethylenediamine dihydrochloride	a) Electrospray deposition device b) Automatic vibrational sprayer	a) 10 b) 25, 50, 100	Mouse brain, kidney, liver (colorectal liver metastasis model)	TOF/TOF (negative reflector)	92
	Fatty acids	Silver	Sputter coater	5, 50, 150	Mouse kidney, testis, liver, brain	TOF/TOF (positive reflector)	93
Glycomics	N-linked glycans	2,5-Dihydroxybenzoic acid	Automatic vibrational sprayer	25	Mouse brain, human kidney	FT-ICR (positive)	94
			Automatic nozzle sprayer	100	Murine kidney	TOF/TOF (positive reflector)	95
Plantomics	Metabolites	9-Aminoacridine	Sublimation device	50	*Arabidopsis thaliana* leaves	TOF (negative reflector)	7
		1,8–Bis (dimethyl-amino)naphthalene	Airbrush	50	*Medicago truncatula* roots and root nodules	TOF/TOF (negative reflector)	96
		α-Cyano-4-hydoxycinnamic acid	Automatic vibrational sprayer	50	Lupine roots	Orbitrap (positive)	97
		2,5-Dihydroxybenzoic acid	Pneumatic sprayer	20	Grapes	Orbitrap (positive)	98
		Binary matrix of 2,5-dihydroxybenzoic acid and α-cyano-4-hydoxycinnamic acid	Automatic vibrational sprayer	35	*Citrus sinensis* grafted on *citrus limonia* petioles and leaves	TOF/TOF (positive reflector)	99
		1,5-Diaminonapthalene	Sublimation device	25	Corn leafs	Orbitrap (negative)	32
		Silica TLC powder	Dry coating technique	50	*Eucalyptus* stem	Orbitrap (negative)	100
Foodomics	Fungal secondary metabolites	2,5-Dihydroxybenzoic acid	Pneumatic sprayer	15	Mushrooms	Orbitrap (positive)	33
	Proteins	α-Hydroxycinnamic acid and aniline	Automatic vibrational sprayer	250	Tomatoes	TOF/TOF (positive linear)	101
	Flavonoids	2′,4′,6′-Trihydroxyacetophenone	Automatic vibrational sprayer	70	Strawberries	TOF/TOF (negative reflector)	102
Cosmetomics	Lipids	α-Cyano-4-hydoxycinnamic acid	Airbrush	100	Cosmetic products	Orbitrap (positive, negative)	103
Materiomics	Lipids	Binary matrix of 2,5-dihydroxybenzoic acid and α-cyano-4-hydoxycinnamic acid	Chemical printer	10 to 150	Ultra-high molecular weight polyethylene joint implants	TOF/TOF (positive reflector)	104
	Polymers	2,5-Dihydroxybenzoic acid	Pneumatic sprayer	20 to 100	Polymeric dialyzer membranes	Orbitrap (positive)	105

Proteomics

Even though, MALDI MSI has been extensively utilized in the field of proteomics since its discovery,[1, 2] the analysis of large intact proteins (above 25 kDa) is still challenging.[106] Hence, more and more researchers are employing various on-tissue digestions techniques, developed for the specific tissue type they are working on[25, 73, 107, 108] Additionally in 2013, a multi-center study has been set up to improve and validate the current protocols under the roof of the COST action BM1104. The idea to image tryptic peptides opened the door for analysing FFPE tissue samples, which are commonly archived in clinics with the corresponding meta-data (survival and treatment response). Therefore, it is obvious that the move of MALDI MSI towards clinical biomarker discovery is currently on its way,[40, 42] substituted by robust methods,[43] and reference databases.[109] The clinical applications rank from various cancer types, such as clear cell renal cell,[110] heptatocellular,[111] thyroid,[112] prostate,[113] pancreatic,[114] salivary gland,[115] breast and gastric carcinoma[116] to other diseases, such as osteoarthritis,[71] trauma,[72] wounds,[68] cartilage,[117] ectopic calcification,[107] in order to differentiate and classify pathological regions on the basis of known and unknown proteins. The findings are subsequently further validated by traditional histology.[47]

MALDI MSI has been proven in the past as a useful tool in molecular neuroscience,[118, 119] exploring the biomolecular mechanism of neurodegenerative disorders, such as amyotrophic lateral sclerosis,[120, 121] parkinson’s disease,[122] and childhood absence epilepsy.[123]

A recent example of MALDI MSI,[107] which helped in understanding the underlying biology of calcification is presented in Figure 1. The MALDI MSI data (Figure 1 B) were integrated with the histological observations (Figure 1 A) to

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reveal molecular markers for morphological defined tissue areas, such as calgizzarin (*m/z* 11651) for epidermis, or thymosin beta-4 (TYB-4, *m/z* 4965) for papillary dermis or whole dermis in the case of diseased and healthy skin tissue, respectively. The subsequent assignment of the protein identities to the peaks was accomplished by bottom-up strategies using *in situ* digestion *via* hydrogel discs.[124]

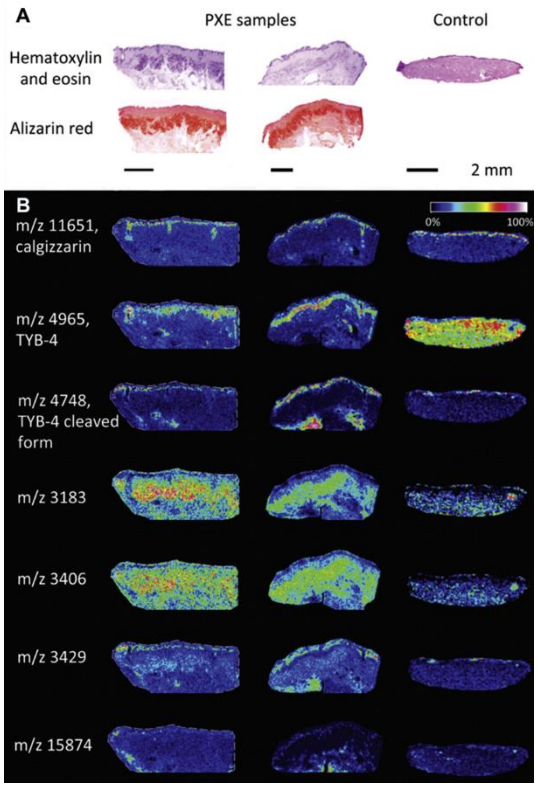

Fig. 1. MALDI MSI of a patient sample affected by pseudoxanthoma elasticum (PXE) in comparison to a control one. (A) Optical images of tissue sections after hematoxylin-eosin or alizarin red staining; (B) Seven extracted ion images of known and unknown proteins associated with ectopic calcification. Reprinted in a modified version with permission from ref.[107], D. Taverna, *et al.*, *Bone*, 2015, **74**, 83-94. Copyright 2015, Elsevier Inc.

The second approach commonly used in proteomics for protein identification, which is called top-down, has also been employed in MALDI MSI studies,[67] even the combination of both.[125] In top-down a mass spectrometer is used to fragment the proteins without any digestion step, e.g. in-source decay can be used, which is a metastable decay of molecules during the desorption/ionization process.[67] Although, all three approaches (bottom-up, top-down, combination of both) resulted in confident protein identifications, and consequently in the establishment of a public database,[126] it certainly remains an important issue to be solved in the future to push this technique towards a valuable tool for clinicians (grading, margins, prognosis, prediction of tumours) in the framework of personalized medicine.

Peptidomics

The MS-based "omics" discipline that focuses on the comprehensive analysis of peptides involved in cell-to-cell communications is often referred to as peptidomics. There are not many MALDI MSI studies in this field presumably due to the low abundance of peptides, and especially neuropeptides, which are hampering the analysis. However, MALDI MSI with its high specificity is certainly a powerful method for localizing neuropeptides to identify neuronal signalling pathways[118, 127] or exploring neurodegenerative diseases, such as parkinson's,[128-130] alzheimer's,[131, 132] and huntington disease.[133] The resulting molecular knowledge is important to understand for example how the human nervous system enables us to interact with our environment. A common used model system in this respect is the stromatogastric nervous system of the Johns crab *cancer borealis*, which consists of a set of distinct but interacting motor circuits. A recent MALDI MSI study[76] identified 57 neuropeptides originating from eleven different neuropeptide families in stomatogastric ganglion tissue. The spatial localization of e.g. NFDEIDRSGFGFA from the orcokinin neuropeptide family (*m/z* 1474.6), and HIGSLYRa from the YRamide neuropeptide family (*m/z* 844.5) were determined as predominantly localized in the central region of the ganglion, where the neutrophil is formed, as shown in Figure 2 B and C, respectively.

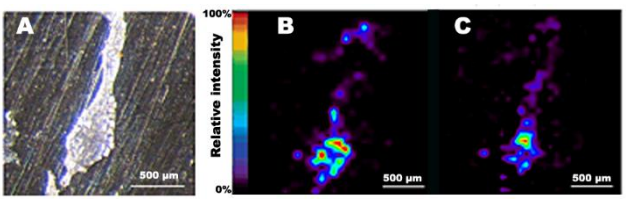

Fig. 2. MALDI MSI analysis of neuropeptides in stomatogastric ganglion tissue, extracted from a John crab *cancer borealis*. (A) Optical image prior matrix coating; (B) and (C) two selected ion images: NFDEIDRSGFGFA from the orcokinin neuropeptide family (*m/z* 1474.6), and HIGSLYRa from the YRamide neuropeptide family (*m/z* 844.5), respectively. Reprinted in a modified version with permission from ref.[76], R. Chen *et al.*, *Rapid Commun. Mass Spectrom.*, 2014, **28**, 2437-2444. Copyright 2014, John Wiley & Sons, Ltd.

Current limitations associated with identification of MALDI MSI measured peaks might be tackled by using the approach presented by Minerva *et al.*[134] in an obese mouse model. The strategy was based on high-resolution MALDI MSI, MS/MS and LC-MS, facilitating the identification of 46 peptides out of 136 detected signals by MALDI MSI.

Furthermore, to address the drawbacks originating from the sample preparation, as discussed in the introduction section, pre-spotted targets can be used, as shown by the group of Salzet.[135] They successfully analysed endogenous peptides on targets pre-spotted with ionic matrices. Another possibility is to use nanostructure-initiator chips, although, a recent study has proven that MALDI MSI is superior for analyzing neuropeptides leading to twelve identified neuropeptide distributions compared to none in using nanostructure-initiator chips.[136]

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Metabolomics

Metabolomics is considered as one of the later "omics" technologies that provides additional functional information for understanding biological systems. The metabolome consists of small molecular building blocks (e.g., amino acids, nucleotides), metabolic intermediates (e.g., fatty acids), and structural and signalling elements (e.g., lipids). Since these small molecules are easily detectable *via* MALDI MSI, the last two years were characterized by a strong interest in this "omics" field,[137-139] with special emphasis in the tumour-specific metabolism.[140] Since lipids, which are classified as a subset of metabolomics, are playing many important roles in cancer progressing,[141] their spatial localization has been investigated in colorectal,[80] clear cell renal cell,[110], thyroid, gastric, lung, esophageal, breast,[85] head and neck cancer,[50, 142] as well as xenografts.[143, 144] Compared to the list of proteomic MALDI MSI studies in cancer research, as presented two sections above, this list is nearly as long. Moreover, the subsequent combination of both "omics" fields has already been started, e.g. by exploring protein and lipid markers, which differentiate tumour from non-tumour regions within one MALDI MSI study.[110] In particular, in cancer research, the correlation of the lipidomic organization revealed by MALDI MSI with the underlying histology is very important to gain further information of histological structures to improve personalized medicine. In the case of head and neck cancer, the spatial molecular characterization is important, since half of the patients develop a locoregional recurrence although radical therapy was prescribed. Figure 3 shows exemplary a correlation of molecular and histological information.[50]

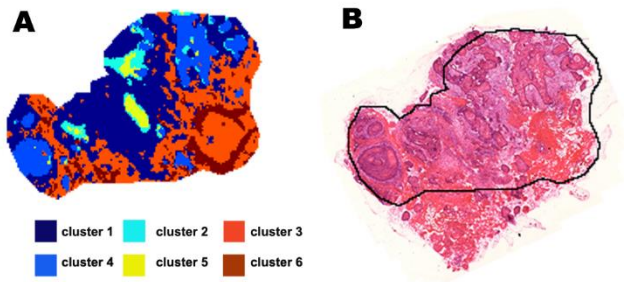

Fig. 3. MALDI FT-ICR MSI analysis of a human head and neck tumour tissue. Comparison of (A) metabolic segmentation map generated by the MSI data, and (B) histopathology (hematoxylin-eosin stain). Reprinted in a modified version with permission from ref.[50], L. Krasny *et al.*, *J. Am. Soc. Mass Spectrom.*, 2015, **26**, 36-43. Copyright 2015, Springer.

With the help of computational algorithms, the head and neck tissue sample was subdivided into six clusters based on the spectral similarities (Figure 3 A). The thereby detected heterogeneity of the functional lipidome was reflected in the morphology, as observed in the H&E stain (Figure 3 B). To enable such a comparison of spatially defined molecular and histological signatures a registration step of the MALDI MSI dataset to the histological stain is essential, which was performed in this case manually on three defined landmarks. Alternatively, an automated registration procedure can be performed,[145] which ultimately facilitates the correlation to anatomical atlases.[146] And beyond this, the image fusion of extracted ion images with H&E stains generated by MALDI MSI and microscopy, respectively, can assist in predicting ion distributions in tissue areas that were not analyzed by MALDI MSI.[147]

Besides, cancer applications, various other diseases, such as infections of hepatitis B virus,[81] atherosclerosis,[148] fabry,[79] peridontal,[149] alzheimer's,[150-152] epilepsia,[153] stroke,[154] schizophrenia,[155] and stargardt disease[82] have been investigated to shade light into the underlying disease mechanism. Within the spatial mapping of lipids, certain classes, such as phospholipids,[156] which can degrade in time,[36] sphingolipids,[157] and ceramides[158, 159] have been extensively investigated. Even the possibility to introduce a wavelength-tuneable postionization laser to increase the ion yields of lipids.[160]

As discussed for the two earlier "omics" fields, in metabolomics is the identification of the detected species directly on the analysed tissue as well crucial. Generally, two approaches are possible: 1) MS identification based on the high mass accuracy obtained from an orbitrap or an FT-ICR MS instrument,[161] and 2) MS/MS fragmentation patterns enabling the differentiation between isobaric species.[162]

Nowadays, ion mobility (IM) instruments are often used in MALDI MSI,[163] owing to several advantages, such as an improved signal-to-noise ratio caused by the physical separation of the noise, a larger peak capacity, and the possibility to separate isomers on the basis of shape differences. The latter feature beyond doubt can facilitate the identification procedure.

Besides the identification, the number of detected metabolites is important, which can be increased by carrying out the MALDI MSI analysis in both, positive and negative ionisation mode, using certain matrices (1,5-diaminonaphthalene,[84] quercetin,[86] dithranol,[88] silver nanoparticles[164] or the combination of two (α-cyano-4-hydoxycinnamic acid and 9-aminoacrdine).[165]

To go beyond the *in situ* visualization of metabolic changes, the utilization of additional chemical imaging technique,[166] has been shown as a promising tool, e.g. for analysing bacterial biofilms,[167] three-dimensional cell cultures,[168] mouse brain,[169] or rat spinal cord sections.[170] Novel designs of mass spectrometers even allow the acquisition of two imaging modi in one instrument,[170, 171] promoting the idea of multimodal imaging to understand complex biological systems.

Within the reported lipidomic MALDI MSI studies, insects are the smallest animal bodies investigated, ranking from complete insect bodies, such as *drosophila melanogaster* flies to *paderus riparius* beetles.[172-174] The gained knowledge can possibly help in understanding mechanistic and physiological principals, explaining the recent interest in this class of invertebrates.

At the end of this section, the developments towards quantitative MALDI MSI should be addressed. Certainly, there is still space for improvements; however, useful strategies in metabolomics as well as pharmacogenomics have already established and the reader is revered to the references listed.[5, 83, 175, 176] Among these methods, one strategy is to use a calibration curve generated from the deuterated analogue to quantify the absolute concentration of a target compound. In the case of acetylcholine (ACh), a neurotransmitter, which can be used to identify cholinergic

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neurons in the central nervous system, MALDI MSI was performed in parallel on a sagittal mouse brain section of a control and a treated animal (10 mg/kg of the cholinesterase inhibitor tarcine), as shown in Figure 4.[177] The relative concentration of ACh is visualized in the extracted ion images, (Figure 4 A), and the absolute concentrations in the different brain areas is calculated using the deuterated standard spotted on control sections (Figure 4 B). From this, it could be demonstrated that the administration of tracine caused overall a seven-times increase of ACh in the mouse brain. Typically, the concentration of ACh is determined by quimioluminiscent, electro-chemical, fluorescent, and high-performance liquid chromatography coupled to electrospray mass spectrometry.[178] However, none of these techniques enabled the spatial quantification of ACh in such a way, as possible by MALDI MSI.

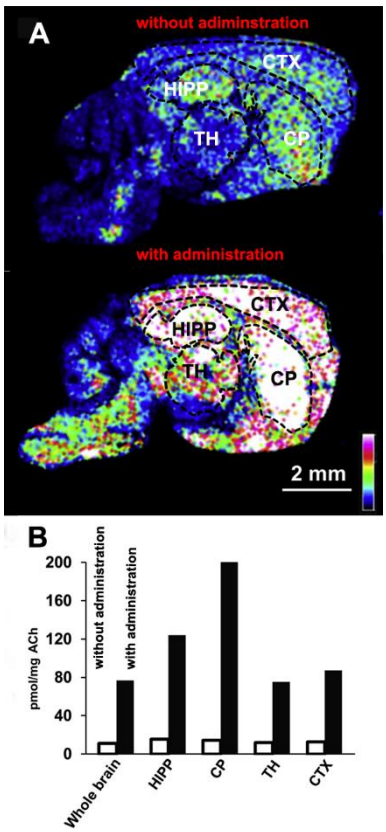

Fig. 4. MALDI MSI analysis of a sagittal mouse brain section to quantify (A) the relative and (B) the absolute concentration change of the neurotransmitter ACh in different brain areas (hippocampus, HIPP; caudate putamen, CP; cerebral cortex, CTX; thalamus, TH) induced through the administration of 10 mg/kg tracine. Reprinted in a modified version with permission from ref.[177], M. Shariatgorji *et al.*, *Neuron*, 2014, **84**, 697-707. Copyright 2014, Elsevier Inc.

The considered study[177] is two-fold remarkable, firstly due to the link of the two "omics" fields metabolomics and pharmacogenomics, and secondly due to the aspect of absolute quantification.

Other neurotransmitter, besides ACh, such as e.g. γ-amino butyric acid are only detectable through a derivatization step,[177] which is nowadays often applied in MALDI MSI studies to allow the successful analysis of e.g. amino acids,[27, 179] or pharmaceuticals.[180] MALDI MSI has already been employed as a tool to close the gap between metabolome changes in response to drug administration and certainly will be continued in the future in clinical settings.[62]

Besides the impressive applications of MALDI MSI in medicine, its potential in forensic science has already been proven, e.g. to gain chemical information on latent fingermarks to help in convicting suspects, when the typical matching of the patterns fails.[181] Further developments in this domain are focused on replacing the use of organic matrices to overcome disadvantages, which are linked with its use, such as the difficulties to get reproducible signal intensities, or the circumstance of masking analyte signals.[182, 183]

Glycomics

Glycomics is focusing on the isolation and characterization of glycans, which nowadays are routinely analysed by MS based methods, providing both, qualitative and quantitative information employing top-down techniques.[184] In order to understand the roles of N-glycans in the physiology and molecular pathology of diseases, MALDI MSI of N-linked glycans was developed within the last two years, emerging from profiling[94] to MALDI MSI of FFPE tissues.[95, 185, 186]

Plantomics

Plantomics describes the application of "omics" technologies in plant science to which the analysis of plant metabolites by MALDI MSI certainly counts. Often there exists an overlap with foodomics, as e.g. recognizable in the next section in this mini-review.

The crucial step in plantomics is certainly the sample preparation. Two simple questions are arising in this contest: "how do I get intact sections from my sample, and which matrix can I use?" Commonly, animal and human tissue is cut in a cryomicrotome at 10 to 20 µm tissue thickness, however plant tissue is more demanding, especially if fragile leaves or petals are cut. Hence, a typical thickness can vary from 20 up to 100 µm. In the case of even uneven leaves, the imprinting onto porous PTFE prior matrix application is recommended to circumvent a broadening of the peak shape due to variations of the recorded time-of-flight of the ions when originating from different tissue heights.[187] Plant tissue compared to animal and human tissue is special, since it consists of many different cell types; therefore, the right choice of matrix is crucial. As presented in Table 1, a variety of matrices have already been investigated in plantomics, ranking from 9-aminoacridine[7] and diaminonapthalene[32] for the negative ion mode to common MALDI MSI matrices for the positive ion mode, such as α-cyano-4-hydroxycinnamic acid,[97] 2,5-dihydroxybenzoic acid,[98] or the combination of both.[99] Even the use of silica TLC powder[100] according to the protocol described by Puolitaival *et al.*[188] was successfully employed. Another possibility is to use no matrix, however only applicable if the plant metabolites have chromophores, which absorb the incident laser light.[189] Since only recent studies have been discussed here, the review by

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Bjarnholt *et al.*[190] is recommended for further reading, who covers publications up to the end of 2013.

Foodomics

Foodomics is the comprehensive, high-throughput approach for the exploitation of food science in the light of an improvement of human nutrients.[191] The main objective of this new approach is to improve human health and well-being. A variety of tissue types have been investigated by MALDI MSI ranking from barley,[192] blueberry,[193] apple,[194] tomatoes,[101] to mushrooms[33] explore the spatial distribution of mainly small organic molecules, such as lipids, flavonoids, fungal secondary metabolites, or amino acids.[195] An example of the MALDI surface analysis of fruits is presented in Figure 5.

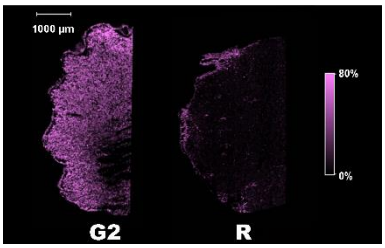

Fig. 5. Localization of citric acid $C_6H_7O_7$ ($[M-H]^-$ *m/z* 191) in strawberries (*Fragaria × ananassa*) of two development stages (G2: green fruit; R: full-ripe red fruit).[102]

Strawberries are fruits, which are widely appreciated for their aroma, red color, juicy texture and sweetness. The acidity comes mainly from citric acid which comprises about 88 % of the acid content, along with malic acid and ellagic acid. When strawberries ripen, the acidity decreases. To visualize this change in concentration and to figure out its localization we performed MALDI MSI on garden strawberries (Fragaria × ananassa) and could show that citric acid is homogenously distributed in green fruits (Fig. 5 G2), and only at the skin in the full-ripe red fruit (Fig. 5 R).[102] This example shows very nicely the potential of food imaging, and since the current spatial resolution of MALDI MSI is sufficient for analysing food, it can be expected that this technique will find its way as an analytical tool for the quality control of food products, besides novel ambient ionization techniques, which do not require any sample preparation.

Cosmetomics

Cosmetic products are gaining more and more importance in our daily life to improve e.g. the skin care, or odor of our body due to the rapid increase of our population. Hence, analytical methods are required to control their quality. Due to the complexity of cosmetics advanced characterization techniques, such as high-performance liquid chromatography coupled to mass spectrometry, gas chromatography mass spectrometry, and inductively coupled plasma mass spectrometry are routinely employed.[196] The application of MALDI MSI as a quality control tool of cosmetic products was presented by de Oliveira *et al.*[103] Upon the lipid profiles it was possible to test if a lipstick or eyeliner was expired or not. The imaging capabilities of MALDI MSI in the area of cosmetomics have certainly to be investigated further to fulfil the requirements as a robust quality control technique in the cosmetic industry. However, in cosmetomics not only the quality of the cosmetic products is of interest, but also e.g. the penetration rate of cosmetics into the skin. The potential of MALDI MSI in the spatial analysis of skin has already been demonstrated,[197-199] and hence now it is the right time to determine e.g. the penetration depth of certain sun-cream ingredients into the skin.

Materiomics

Materiomics is defined as the comprehensive study of material systems, including polymer systems, also known as polymeromics,[200] connecting physiochemical material properties with material characteristics and functions. Besides, polymeric surfaces, materials, such as ceramic or metal are investigated in this "omics" discipline for their later application, which often comprises of biological environments. The characterization of polymer surfaces by MALDI MSI is certainly possible, when the right sample preparation technique is figured out.[201] For example, in the case of a negative photoresist layer,[202] which is generally used to manufacture printed circuit boards, the main component novolac, the active component benzophenone, and the solvent tetrahydrofuran were mixed with the matrix dithranol and the salt additive LiTFA, and spin-coated onto an ITO-conductive glass slide. In another application the best way to analyse ultra-high molecular weight poly(ethylene) (UHMW-PE) joint implants was to slice them at 14 to 16 µm in a cryomicrotome and to apply a binary matrix consisting of 2,5-dihydroxybenzoic acid and α-cyano-4-hydoxycinnamic acid in e.g. potassium chloride saturated methanol using a chemical printer.[104] The interesting point in this study was to visualize the material failure and to connect it to the biological implication.

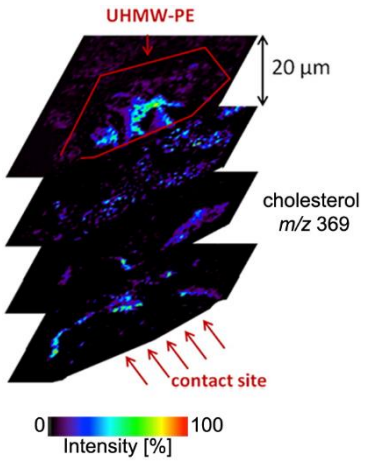

Fig. 6. MALDI MSI analysis of a ultra-high molecular weight poly(ethylene) (UHMW-PE) hip joint explant. Reprinted in a modified version with permission from ref.[104], S. M. Froehlich *et al.*, *Anal. Chem*., 2014, **86**, 9723-9732. Copyright 2014, American Chemical Society.

For this, a piece of a hip joint explant was sliced and serial sections were analysed by MALDI-MSI for the spatial lipid distributions inside the material. Figure 6 shows the 3D reconstruction of cholesterol $[M-H_2O+H]^+$ at *m/z* 369 on four

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consecutive UHMW-PE slices. It is clearly visible that cholesterol diffuses into the material possibly caused by mechanical stress during implantation in the patient. Subsequent follow up studies by e.g. nanoindentation or fourier-transform infrared spectroscopy (FT-IR) can might reveal further insides in this material wearout.

The "omics" field materiomics is still in it's infancy, however the possibility to gain spatial molecular information on materials by MALDI MSI and to correlate these findings with common used techniques in material science, such as e.g. indentation or FT-IR seems to be a promising way to proceed.

Conclusions

In total, over 150 publications have been covered in this mini-review, published between 2013 and 2015. Figure 7 summarizes the cited publications according to the different "omics" fields per year. From this it can be clearly seen that within the last two years most efforts were undertaken in the field of metabolomics with strong emphasis on methodology improvements.

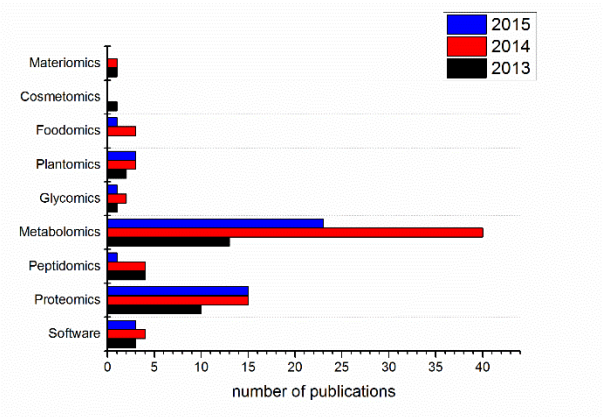

Fig. 7. Overview of covered MALDI MSI publications in this mini-review.

In the end the present mini-review represents an attempt to provide a flavor of possible future directions of MALDI MSI in a variety of different "omics" fields to enable the readership to place this emerging technique into perspectives. Since steadily new "omics" areas are emerging, the type of samples as well as compound classes analysed by MALDI MSI will increase. This will consequently lead to the development of new sample preparation techniques, processing steps, as well as new mass spectrometers for MALDI MSI to fulfil the future needs to move this technique from the academic side to industry, e.g. as a potential quality control tool by employing standardized protocols.[39] The recent possibility of sharing, exchanging MALDI MSI datasets on a public repository is certainly one-step towards this direction leading to multicentre studies.[66, 203]

Acknowledgements

The authors regret that space constrains prevented to cite all recent publications related to MALDI MSI. Financial support of the DFG (grand no. EG 102/4-1, EG 102/5-1, and SCHU 1229/15-1), the Thüringer Kultusministerium (grand no. B515-07008), as well as the Ernst-Abbe foundation are acknowledged.

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