Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Review

Mass spectrometry in pathology – Vision for a future workflow

Jörg Kriegsmann[a,b], Rita Casadonte[b], Katharina Kriegsmann[c], Rémi Longuespée[d], Mark Kriegsmann[d,*]

[a] MVZ for Histology, Cytology and Molecular Diagnostics Trier, Trier, Germany
[b] Proteopath GmbH, Trier, Germany
[c] Department of Hematology, Oncology and Rheumatology, University Hospital Heidelberg, Heidelberg, Germany
[d] Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany

ARTICLE INFO

Keywords:
MALDI Imaging
Mass Spectrometry
Molecular Pathology
Pathology Workflow

ABSTRACT

Mass spectrometric (MS) techniques are applied in various areas of medical diagnostics. For the detection of microbiological germs and genetic mutations, MS is a method used in routine. Since MS also allows the analysis of proteins and peptides, it seems an ideal candidate to supplement histopatholological diagnostics. Matrix-assisted laser desorption/ionization time-of-flight Imaging MS links molecular analysis of numerous analytes with morphological information about their spatial distribution in cells or tissues. Herein, we review principle MS techniques as well as potential applications in pathology and discuss our vision for a future workflow.

1. Introduction

Mass spectrometric (MS) techniques are applied in various areas of medical diagnostics.

In microbiological germ detection, MS is a routine diagnostic method since the last years [17,58]. Similarly, this technique has been introduced into toxicology [51], as well as in forensic medicine [6,28]. For the analysis of DNA, this technique allows the detection of several hunrdred different mutations and is therefore applied in molecular pathology already today [41,45]. However, as the identification of mutations by MS is based on prior PCR amplification, the method is most suitable for the detection of hotspot mutations and there are limitations when it comes to the identification of complex mutations or genetic aberrations.

As MS also allows the analysis of proteins [68] and peptides [40,48], it seems an ideal candidate for histopatholological diagnostics which strongly relies on the detection of proteins and peptides by immunohistochemical (IHC) methods [71]. An advantage of MS as compared to IHC, is the detection of numerous proteins or peptides without the need for target-specific antibodies [56]. On the other hand, the identification of the detected ion peaks might be a challenge. MS can be applied in the profiling mode where a region of a tissue section has been chosen for application of the laser which ionizes the proteins or peptides or in the imaging mode, where numerous proteins or peptides can be localized in a given tissue section [2].

Furthermore, other chemical classes can be detected in tissue such as lipids or phospholopids [14,31,39,70], carbohydrates and glycoconjugates [37], exogenous or endogenous small molecules, especially molecules playing a role in drug metabolism [34,50,65] and nucleic acids [38,57].

Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) Imaging MS (MALDI-IMS), first described by Caprioli et al. in 1997 [9], links molecular analysis of numerous analytes with morphological information about their spatial distribution in cells or tissues [7,12,22,63] and provides unbiased visualization of the arrangement of biomolecules [29].

The information contained in tissues cannot be replaced by investigation of serum or blood [67]. Therefore, pathology is not only a large field of medical research but also a basis for diagnostics of various diseases and treatment decisions.

2. General principle of mass spectrometry

MS is a wide field with various different specialized methods applied. However, all are based on the fact that molecules from a target *e.g.* a tissue section are ionized and subsequently measured. MALDI is the most common method applied for ionization. In MALDI experiments, the time-of-flight of the respective analyte is measured by a

Abbreviations: DNA, desoxyribonuleic acid; EGFR, epidermal growth factor receptor; H&E, hematoxylin and eosin; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; LC, liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; MALDI-IMS, matrix-assisted laser desorption/ionization imaging mass spectrometry; MS, mass spectrometry; NRAS, neuroblastoma RAS viral oncogene homolog; PCR, polymerase chain reaction; TOF, time-of-flight

* Corresponding author at: Institute of Pathology, University Hospital Heidelberg, Im Neuenheimer Feld 224, Germany.
E-mail address: mark.kriegsmann@med.uni-heidelberg.de (M. Kriegsmann).

https://doi.org/10.1016/j.prp.2018.05.009
Received 8 October 2017; Received in revised form 23 April 2018; Accepted 11 May 2018
0344-0338/

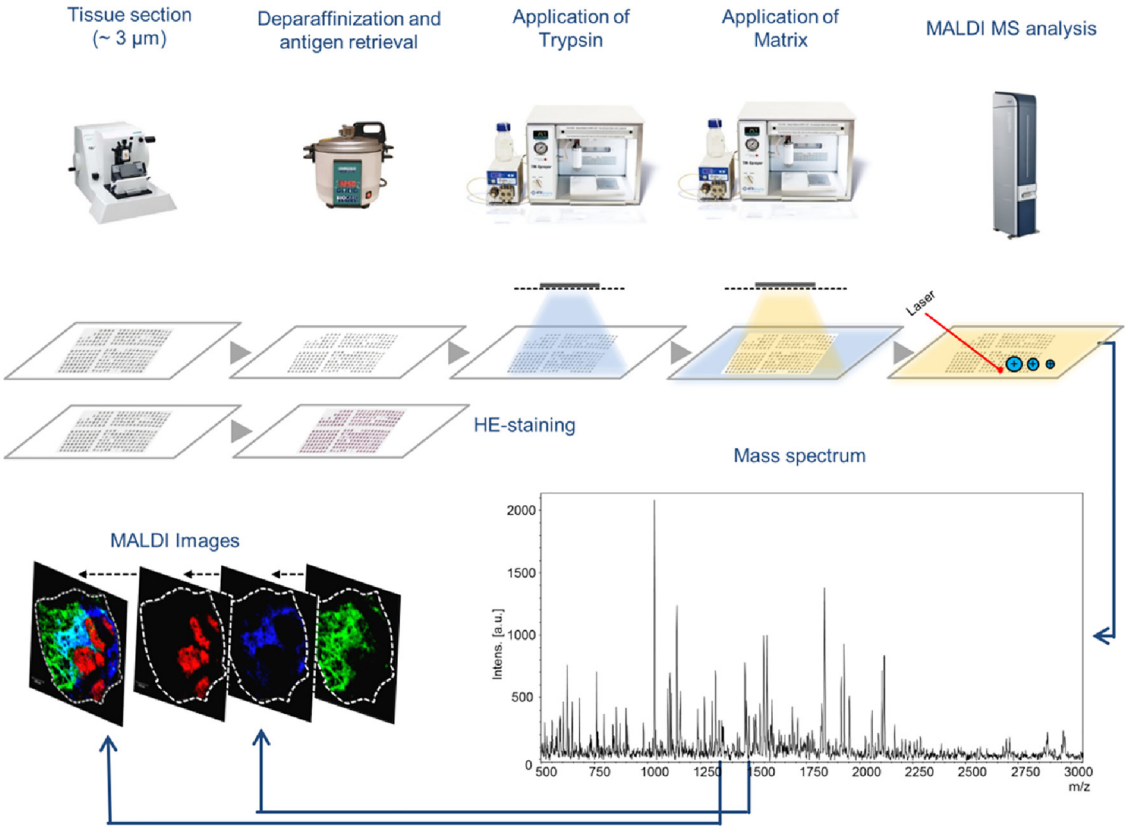

Fig. 1. Workflow of a MALDI-IMS experiment.
A typical workflow of a MALDI-IMS experiment is illustrated. Tissue sections are mounted on an indium-tin-oxide covered glass slide, the sample is prepared for the MALDI analysis and mass spectra are acquired in a rastered fashion at a resolution of 10–50 µm. For each peak, the distribution throughout the tissue can be visualized.

detector and the ions are categorized based on their mass-to-charge ratio (m/z value). Matrix is applied to a sample in order to prepare the sample for analysis and a laser subsequently irradiates the solid preparation. This leads to the ionization and desorption of the molecules (Fig. 1). Various substances such as proteins, peptides, carbohydrates or lipids can be detected by MALDI. IMS allows to localize specific molecules throughout a tissue section. Other common ionization methods applied in IMS are summarized in Table 1. An example of a pancreatic cancer specimen analyzed by MALDI-IMS is shown in Fig. 2. The detection of genetic mutations is based on DNA isolation followed by PCR-amplification of the respective DNA sequence. If a genetic mutation is present, a mass peak of the wild-type allel and a second mass of the mutated allel are detected at specific locations in the mass spectrum.

3. Detection of different molecules

The following paragraph illustrates examples for the detection of various molecules. With regard to proteins, Meding et al. analyzed 171 fresh frozen samples from adenocarcinomas of esophagus, breast, colon, liver stromach and thyroid. A primary set was used for training and a secondary set was utilized for confirmation. In the confirmation set, an accuracy of > 80% for the classification of the six different tumor origins was achieved [53]. Concerning peptides, most studies have analyzed formalin-fixed paraffin-embedded tissue samples from routine diagnostic archives [44,72,74]. In a recent study, we show that adenocarcinoma and squamous cell carcinoma of the lung could be reliably differentiated by MALDI-IMS. Out of 118 samples, 117 could be correctly classified, which surpasses the accuracy of a single IHC marker [42]. Among the 339 m/z values that were used for classification in this study, some were subsequently identified by tandem MS and validated by IHC. Interestingly, we found known immunohistochemical discriminative markers such as cytoceratin 5, but also potential new markers such as cytokeratin 15 or Heat-shock-protein-beta 1. Although these results should be validated in a larger dataset, they highlight the potential of MALDI-IMS on routine paraffin material.

Also carbohydrates and glycoconjugates may be dectected for classification purposes [20] and it is well documented that alterations and changes in cell surface glycosylation occur during tumorigenesis [16]. Thus, it is not surprising that tumor and stroma show distinct N-glycan distributions, which has recently been demonstrated in high-grade serous ovarian cancer and hepatocellular carcinoma [25,62].

Lipids are another molecule class that can be detected by MS. In a prove of concept study including 36 gliomas, a correct classification (subtype and grade) on an independent test set could be made in 79%, based purely on lipids [24]. Naturally, pathologists do have a sound understanding of proteins and peptides, as the application of histochemistry and IHC targets these molecules. However, other molecule classes might very well contribute to a more detailed understanding and ultimately to a better classification of diseases.

Besides proteins, carbohydrates, glycoconjugates and lipids, nucleic acids can be detected. This application of MS is already well established in many laboratories; either for the detection of *e.g.* viruses such as human papilloma virus, bacteria including their resistances to antibiotics and parasites [13,45,55,76,78], or for the detection of genetic mutations in various genes including *KRAS*, *NRAS*, *EGFR* and others [30,41,49,75].

4. Perspective workflow in pathology including mass spectrometry

The regular workflow in pathology includes as a first step diagnosis on hematoxylin and eosin (H&E)-stained tissue sections [33]. Adjunct

Table 1
Ionization methods employed in mass spectrometry imaging.

Ionization method	Description	Spatial Resolution	Advantages	Limitations
DESI	Chared primary droplets generated by electrospray ionization impact the sample surface to desorb the sample in secondary droplets	100–500 µm	no sample preparation required minimal sample fragmentation	Sensitive to surface welting, low spatial resolution Mostly for the detection of lipids and small molecules
LA-ICP LA-APC	Molecules are extracted from the surface by laser ablation. The aerosol is transported by a carrier gas in a plasma source where atomization and ionization occur.	2 µm	Low matrix effects High sensitivity	Mostly for the detection of metal- and heteroatomcontaining compounds
MALDI	Molecules are extracted from the tissue surface by laser ablation. A matrix absorbs laser energy leading to desoprtion and ionization of the analytes	10–200 µm	wide range of molecules can be analyzed	spatial resolution limited by matrix droplet size and diameter of the laser beam, Matrix-derived ions interfere with the analysis of small molecules
NIMS	Molecules are ionized through a laser-induced rearrangement of the tissue surface structure	< 1 µm	no sample preparation required minimal fragmentation high sensitivity	Laborious chip fabricateon Mostly for the detection of metabolites
LAESI	Molecules are extracted from the surface by laser ablation followed by electrospray ionization	< 50 µm	no sample preparation required minimal sample fragmentation a wide range of molecules can be analyzed	water condensation affects imaging quality
LESA	Molecules are extracted from the surface by liquid microjunction and ionized by electrospeay ionization	> 200 µm	no sample preparation required minimal sample fragmentation a wide range of molecules can be analyzed	sample clean-up required to avoid ionization of intact protein complexes
SIMS	Molecules are extracted from the surface using a focused primary ion beam	< 1 µm	High spatial resolution	Extensive sample preparation Lack of sensitivity Fragmentation of large molecules Mostly for the detection of lipids, metabolites and chemical elements

DESI, Desorption electrospray ionization, MALDI, Matrix-assisted laser desorption/ionization, LA-ICP, laser ablation inductively coupled plasma MS, LA-APCI, laser ablation atmospheric pressure chemical ionization mass spectrometry, LAESI, Laser ablation electrospray ionization, LESA, liquid extraction surface analysis, MALDI, matrix-assisted laser desorption/ionization, NIMS, nanostructure-initiator mass spectrometry, SIMS, secondary ion mass spectrometry. Table adopted from [64].

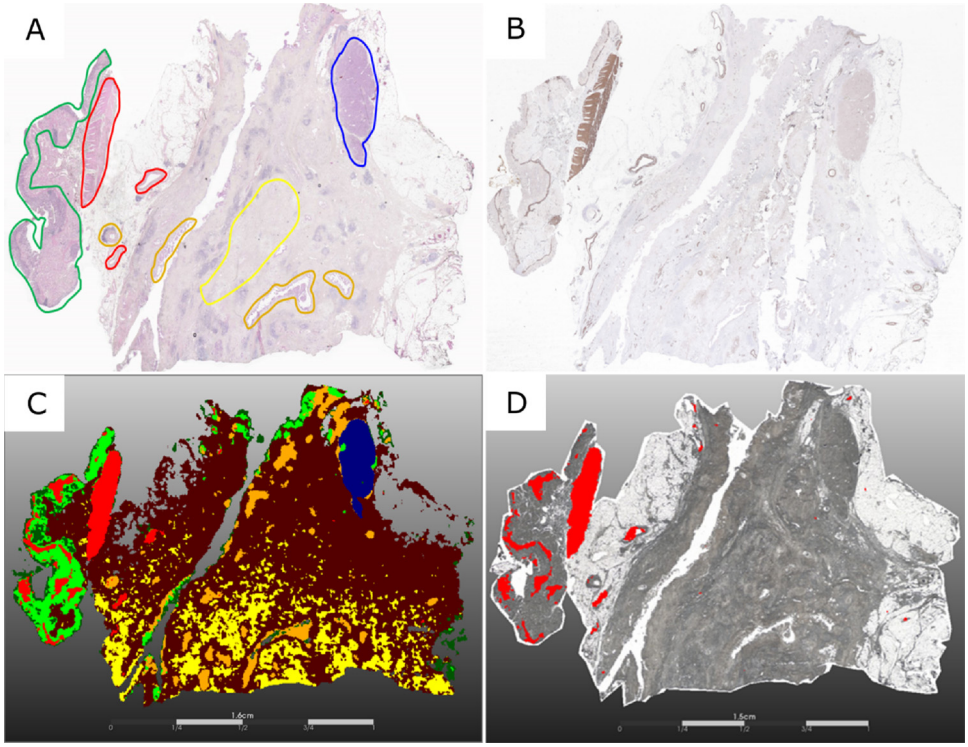

Fig. 2. Example of a pancreatic adenocarcinoma MALDI Image.
A typical example of a pancreatic adenocarcinoma is shown. Illustrated are (A): the duodenal mucosa (green), regions with high smooth muscle content (duodenal wall and vessels), pancreatic parenchyma (blue), pancreatic ducts (orange) and tumor (yellow) (HE, 2x). Immunohistochemistry with antibodies against actin highlights smooth muscles (B). Actin positive areas show brown immunreactivity. The MALDI Image with a predictive peptide of actin demonstrates an *m/z* signal in the same areas that are positive by immunohistology (D). A color-encoded overlay of six different peaks highlights specific morphological structures (C).

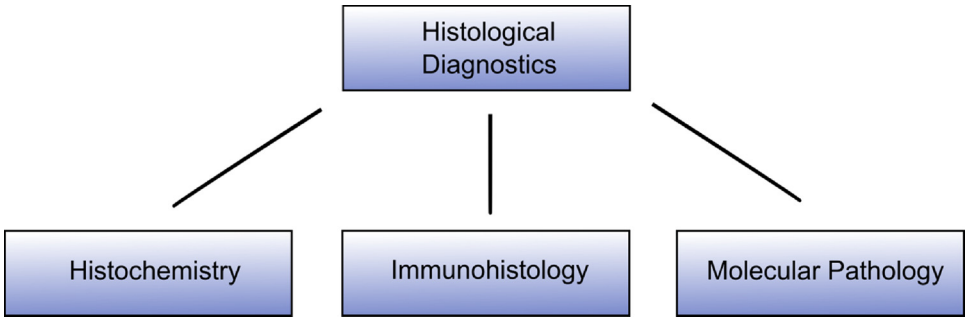

Fig. 3. Current panel of methods in pathology. The current workflow on the basis of a conventional H&E stained tissue section is depicted. Adjunct methods to secure diagnoses and/or to provide prognostic and/or predictive information include histochemistry, immunohistochemistry and molecular pathological methods such as sequencing techniques and in-situ hybridization assays among others.

methods are histochemistry and IHC as well as molecular pathology to classify various diseases and tumors [27] (Fig. 3).

MS has the potential to replace histochemistry since histochemical methods allow detecting mainly various peptides, proteins or sugars [43]. We have shown, that for example hemosiderin can be identified by MS, visualized by the Prussian blue reaction, by measuring *m/z* values of ferritin light or heavy chains [43]. The *m/z* values of these molecules were localized in the same tissue regions as the reaction product of the Prussian-blue reaction. Tumor typing has been shown to be possible by various groups [8,11,42,44]. Numerous antibodies are often needed for the classification of tumors [46]. Since classification of a tumor when applying MALDI-IMS requires only one tissue section, this technique is superior to IHC especilly if only very small tissue samples are available because of minimal invasive methods introduced in the past [42] (Fig. 4). For this reason, MALDI-IMS saves tissue for further molecular classification of the tumor or screening for mutations e.g. in lung tumors [61]. The combination of MALDI-IMS for the classification of the tumor with typing and grading and subsequent application of MALDI-TOF-techniques for screening of mutations could be a future sensitive and cost-effective workflow and would reduce the bias of a subjective evaluation of a pathologist. Moreover, the tournaround time for sample preparation and MS analyses (both for the analysis of peptides and genes) does not exceed one working day and therefore seems suited for routine applications. In this regard, published results indicate that MALDI-IMS has a great potential to develop into a valuable diagnostic technique that can complement established methods. Especially, as the biomarkers or classifiers can be robustly reproduced in different biomedical centers [18]. Meding et al. reported in 2006 that six common tumor entities could be reliably distinguished by MS [53]. Other groups have shown subtyping of non-small cell lung cancers [35,42], renal cell carcinoma [73] and gastric carcinoma [54].

In the future, MALDI-IMS could strongly assist histopathological diagnosis of pathologist by giving objective measures of various conditions. In routine histopathological diagnostics, gastric biopsies play a major role in daily work of pathologists allover the world. Classification of gastritis into A- B- and C- gastritis is not challenging for experienced histopathologists, but time consuming. A future workflow could include the following procedure: MALDI-IMS might be integrated in the histopathological workflow. Special proteomic patterns would be able to discern the three main types of gastritis and could make a proposal for a diagnosis of A-, B-, or C-gastritis. At the same time, slides could be scanned for digital microscopy. As soon as both, result of MS and scanned images merge at the workplace of a pathologist, on one screen histopathological diagnosis produced by MS would be provided, while on the other screen H&E images would be visible for pathological review. If both would be in good concordance, the pathologist could finalize the report by sealing the diagnosis and signing out the report, which was prepared based on the MS result (Fig. 5). Only in case of any

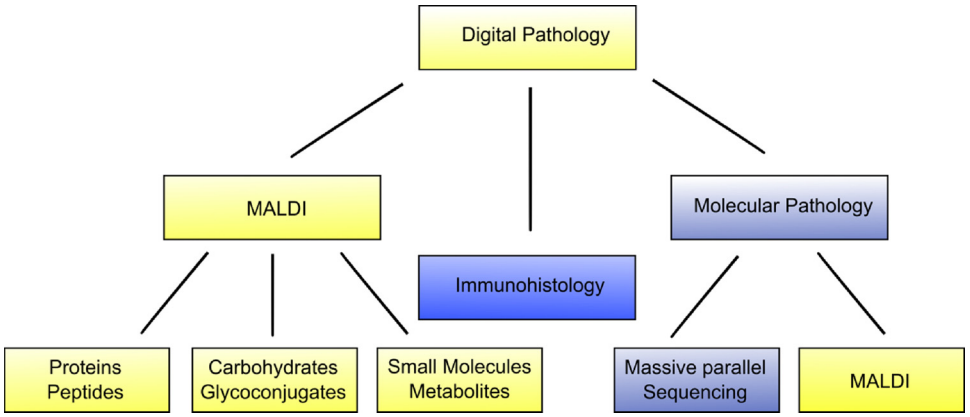

Fig. 4. Perspective panel of methods in pathology.
A perspective panel of methods in pathology will certainly integrate digital software solutions, but will still be based on H&E-stained histpathological slides. Single immunohistochemical stains will probably be replaced by techniques that integrate multi-dimensional data. In our point of view, MALDI techniques have the potential to supersede histochemical methods and possibly also immunohistochemistry in the future. Together with massive parallel sequencing, MS-based techniques allow already today a sensitive and robust detection of DNA. In blue, techniques that are implemented in the pathology workflow today, in yellow novel techniques that may enter the pathology workflow.

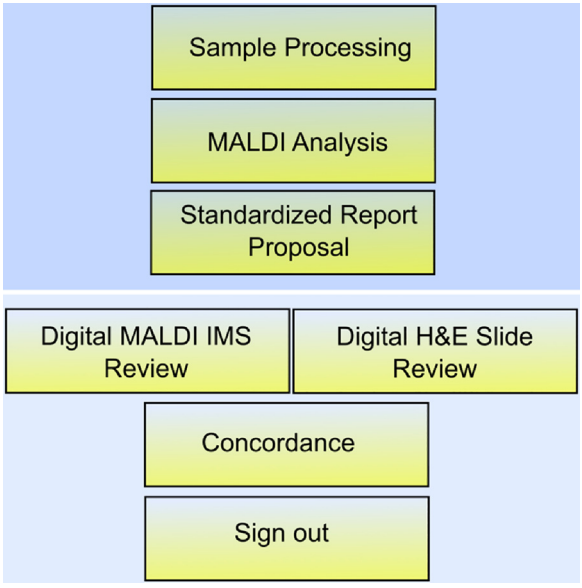

Fig. 5. Possible future workflow in pathology – a vision.
Many laboratory steps can be automated and also reporting may be based on standardized texts. In a possible future workflow the degree of standardization may allow the pathologist to focus on the review of the automatically regenerated results. Only, in case of any doubt, based on the digital review of the available methods, microscopical assessment of the glass slides is necessary.

doubt, the pathologist would have to go deeper into the image of the digital scan or request the slide to clarify the diagnosis. Furthermore, especially in gastric biopsies a few signet ring cells can be "missed" by the pathologist. If mass spectra of various tumors including signet ring cell carcinoma would be in a MS-library, they would unlikely be missed, applying this diagnotic procedure. In this regard, precision of diagnoses and prevention of failures would be one of the advantages using this perspective workflow.

5. Applications of mass spectrometry

In the past, MS has been used to analyse tumor- and non-tumor tissue. In the literature, MS has been applied for tumor typing, grading, to determine molecular margins, and to identify prognostic biomarkers. An objective technique for tumor classification would be highly desirable and some examples for tumor typing have already been mentioned above (for a comprehensive review see also [40]). The tumor grading is an important pathological variable to determine prognosis. In this regard, MS revealed signals specific to poorly differentiated gastric cancer [54] and high-grade myxoid sarcomas could be separated from low grade tumors [77]. A potential advantage of MS for the evaluation of tumor margins is the ability to identify molecular changes that are not yet detectable from histopathological evaluation alone [5]. Furthermore, the detection of new prognostic biomarkers by multidimensional methods such as MALDI-IMS is certainly beneficial and proteins related to prognosis have been identified [15]. Also, hypoxia related proteins that are present in necrotic tumors and clinically associated with increased potential for metastases have been detected by MS [19]. Additionally, MALDI-IMS seems to be ideal to study intratumoral heterogeneidity [1,4]. Finally, the distribution of drugs and metabolites can be examined, which might explain why some patients respond well to chemotherapeutic agents and others do not [7,26].

MS cannot be applied only to tumor samples, but is also able to support classification of other diseases. A good example for this, is typing of amyloid in amyloidosis. A group from the Mayo Clinic have implemented a liquid-chromatography (LC) tandem mass spectrometry (MS/MS) method for amyloid subtyping and were able to determine the amyloid subtype in > 97% of cases in a large investigation including 474 patients [66,69]. For the purpose of amyloid subtyping, MS became the method of choise, especially as some types of amyloidosis are difficult to be diagnosed by IHC assays, e.g. Leukocyte chemotactic factor 2 amyloidosis [10,59].

Furthermore, the classification of meniscal lesions in acute or chronic, low-grade and high-grade may be objectived by MS [60]. The diagnosis of periprosthetic joint infections relies on multiple parameters including clinical investigation, laboratory tests, radiological methods and tissue sampling for histological investigation. The latter is mainly based on the amount of neutrophils identified. However, the threshold of neutrophils per defined area of tissue among various studies is very inconsistent. Thus, the detection of neutrophilic peptides in periprosthetic tissues might well be a surrogate for counting neutrophils and ultimately may assist in the diagnosis of periprosthetic joint infection [32].

6. Methods in mass spectrometry

Various different methods of MS including, but not limited to the following exist: MALDI profiling, MALDI-IMS, MALDI-IMS in profiling mode, MALDI MS/MS directly from tissue, LC–MS/MS and laser microdissection based LC–MS/MS [52]. In MALDI profiling, a specific region of interest is targeted and only few mass spectra are acquired. The major advantage of this approach is the speed of data acquisition. In MALDI-IMS, the whole tissue section is usually analyzed. Using this method, a comprehensive analysis of the spatial distribution of molecules is possible, but much data is acquired that might not all be relevant to the scientific question. In MALDI-IMS in profiling mode, whole tissue sections are analyzed, as by MALDI-IMS, but only specific regions of interest are selected for data analysis. This approach

increases the speed of data acquisition and reduces the amount of data for analysis. For classification purposes, this method is usually recommended. Spectra acquired by MALDI methods are composed of peaks representing *m/z* values. In order to identify these *m/z* species and to assign peptides to these values, subsequent analyses have to be performed. In brief, either direct identification on the respective tissue section (MALDI MS/MS), or analysis by liquid based MS (LC–MS/MS) can be done. Laser microdissection followed by LC–MS/MS is an advanced technique with the ability to detect > 1000 proteins from samples containing < 3000 cells. However, using this technique, information on spatial distribution of molecules is reduced. Based on the scientific or diagnostic question, the appropriate method has to be selected. For a more detailed description of the methologies, various reviews are available in the literature [3,12,21,23,36,47,48].

Funding

This manuscript was supported by the Eurostars-2 Programme, the German Aerospace Center (DLR) and the Federal Ministry of Education and Research (BMBF).

References

[1] D. Alberts, C. Pottier, N. Smargiasso, D. Baiwir, G. Mazzucchelli, P. Delvenne, M. Kriegsmann, D. Kazdal, A. Warth, E. De Pauw, R. Longuespee, MALDI imaging-Guided microproteomic analyses of heterogeneous Breast tumors-A pilot study, Proteomics Clin. Appl. 12 (1) (2018), http://dx.doi.org/10.1002/prca.201700062 Epub 2017 Sep 15..

[2] M. Andersson, P. Andren, R.M. Caprioli, MALDI imaging and profiling mass spectrometry in neuroproteomics, in: O. Alzate (Ed.), Neuroproteomics, 2010 Boca Raton (FL).

[3] T.C. Baker, J. Han, C.H. Borchers, Recent advancements in matrix-assisted laser desorption/ionization mass spectrometry imaging, Curr. Opin. Biotechnol. 43 (2017) 62–69.

[4] B. Balluff, C.K. Frese, S.K. Maier, C. Schone, B. Kuster, M. Schmitt, M. Aubele, H. Hofler, A.M. Deelder, A. HeckJr, P.C. Hogendoorn, J. Morreau, A.F. Maarten Altelaar, A. Walch, L.A. McDonnell, De novo discovery of phenotypic intratumour heterogeneity using imaging mass spectrometry, J. Pathol. 235 (2015) 3–13.

[5] K.J. Boggio, E. Obasuyi, K. Sugino, S.B. Nelson, N.Y. Agar, J.N. Agar, Recent advances in single-cell MALDI mass spectrometry imaging and potential clinical impact, Expert Rev. Proteomic. 8 (2011) 591–604.

[6] N.W. Brown, Toxicology in clinical laboratories: challenging times, Br. J. Biomed. Sci. 74 (2017) 110–120.

[7] A. Buck, M. Aichler, K. Huber, A. Walch, In situ metabolomics in cancer by mass spectrometry imaging, Adv. Cancer Res. 134 (2017) 117–132.

[8] D. Calligaris, D.R. Feldman, I. Norton, P.K. Brastianos, I.F. Dunn, S. Santagata, N.Y. Agar, Molecular typing of meningiomas by desorption electrospray ionization mass spectrometry imaging for surgical decision-making, Int. J. Mass Spectrom. 377 (2015) 690–698.

[9] R.M. Caprioli, T.B. Farmer, J. Gile, Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS, Anal. Chem. 69 (1997) 4751–4760.

[10] R. Casadonte, M. Kriegsmann, S.O. Deininger, K. Amann, R. Paape, E. Belau, D. Suckau, J. Fuchser, J. Beckmann, M. Becker, J. Kriegsmann, Imaging mass spectrometry analysis of renal amyloidosis biopsies reveals protein co-localization with amyloid deposits, Anal. Bioanal. Chem. 407 (2015) 5323–5331.

[11] R. Casadonte, M. Kriegsmann, F. Zweynert, K. Friedrich, G. Baretton, M. Otto, S.O. Deininger, R. Paape, E. Belau, D. Suckau, D. Aust, C. Pilarsky, J. Kriegsmann, Imaging mass spectrometry to discriminate breast from pancreatic cancer metastasis in formalin-fixed paraffin-embedded tissues, Proteomics 14 (2014) 956–964.

[12] R. Casadonte, R. Longuespee, J. Kriegsmann, M. Kriegsmann, MALDI IMS and cancerancer tissue microarrays, Adv. Cancer Res. 134 (2017) 173–200.

[13] Y. Charretier, J. Schrenzel, Mass spectrometry methods for predicting antibiotic resistance, Proteomics Clin. Appl. 10 (2016) 964–981.

[14] P. Chaurand, D.S. Cornett, P.M. Angel, R.M. Caprioli, From whole-body sections down to cellular level, multiscale imaging of phospholipids by MALDI mass spectrometry, Mol. Cell. Proteomics: MCP 10 (2011) 004259 (O110).

[15] J.H. Choi, N.R. Shin, H.J. Moon, C.H. Kwon, G.H. Kim, G.A. Song, T.Y. Jeon, D.H. Kim, D.H. Kim, D.Y. Park, Identification of S100A8 and S100A9 as negative regulators for lymph node metastasis of gastric adenocarcinoma, Histol. Histopathol. 27 (2012) 1439–1448.

[16] M.N. Christiansen, J. Chik, L. Lee, M. Anugraham, J.L. Abrahams, N.H. Packer, Cell surface protein glycosylation in cancer, Proteomics 14 (2014) 525–546.

[17] A.E. Clark, E.J. Kaleta, A. Arora, D.M. Wolk, Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology, Clin. Microbiol. Rev. 26 (2013) 547–603.

[18] T.J. Dekker, B.D. Balluff, E.A. Jones, C.D. Schone, M. Schmitt, M. Aubele, J.R. Kroep, V.T. Smit, R.A. Tollenaar, W.E. Mesker, A. Walch, L.A. McDonnell, Multicenter matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) identifies proteomic differences in breast-cancer-associated stroma, J. Proteome Res. 13 (2014) 4730–4738.

[19] M.C. Djidja, J. Chang, A. Hadjiprocopis, F. Schmich, J. Sinclair, M. Mrsnik, E.M. Schoof, H.E. Barker, R. Linding, C. Jorgensen, J.T. Erler, Identification of hypoxia-regulated proteins using MALDI-mass spectrometry imaging combined with quantitative proteomics, J. Proteome Res. 13 (2014) 2297–2313.

[20] R.R. Drake, T.W. Powers, E.E. Jones, E. Bruner, A.S. Mehta, P.M. Angel, MALDI mass spectrometry imaging of N-Linked glycans in cancer tissues, Adv. Cancer Res. 134 (2017) 85–116.

[21] K. Dreisewerd, Recent methodological advances in MALDI mass spectrometry, Anal. Bioanal. Chem. 406 (2014) 2261–2278.

[22] M. Dufresne, N.H. Patterson, N. Lauzon, P. Chaurand, Assessing the potential of metal-assisted imaging mass spectrometry in cancer research, Adv. Cancer Res. 134 (2017) 67–84.

[23] M.W. Duncan, D. Nedelkov, R. Walsh, S.J. Hattan, Applications of MALDI mass spectrometry in clinical chemistry, Clin. Chem. 62 (2016) 134–143.

[24] L.S. Eberlin, I. Norton, A.L. Dill, A.J. Golby, K.L. Ligon, S. Santagata, R.G. Cooks, N.Y. Agar, Classifying human brain tumors by lipid imaging with mass spectrometry, Cancer Res. 72 (2012) 645–654.

[25] A.V. Everest-Dass, M.T. Briggs, G. Kaur, M.K. Oehler, P. Hoffmann, N.H. Packer, N-glycan MALDI imaging mass spectrometry on formalin-fixed paraffin-embedded tissue enables the delineation of ovarian cancer tissues, Mol. Cell. Proteomics: MCP 15 (2016) 3003–3016.

[26] A. Feuchtinger, A. Walch, M. Dobosz, Deep tissue imaging: a review from a preclinical cancer research perspective, Histochem. Cell Biol. 146 (2016) 781–806.

[27] M. Fiorentino, M. Scarpelli, A. Lopez-Beltran, L. Cheng, R. Montironi, Considerations for standardizing predictive molecular pathology for cancer prognosis, Expert Rev. Mol. Diagn. 17 (2017) 47–55.

[28] S. Francese, R. Bradshaw, N. Denison, An update on MALDI mass spectrometry based technology for the analysis of fingermarks − stepping into operational deployment, Analyst 142 (2017) 2518–2546.

[29] M.M. Gessel, J.L. Norris, R.M. Caprioli, MALDI imaging mass spectrometry: spatial molecular analysis to enable a new age of discovery, J. Proteomics 107 (2014) 71–82.

[30] R. Giannini, C. Lupi, E. Sensi, G. Ali, A. Proietti, L. Boldrini, A. Servadio, M. Giordano, E. Macerola, R. Bruno, N. Borrelli, A. Chella, F. Melfi, M. Lucchi, A. Ribechini, E. Vasile, F. Cappuzzo, A. Mussi, G. Fontanini, EGFR and KRAS mutational analysis in a large series of Italian non-small cell lung cancer patients: 2,387 cases from a single center, Oncol. Rep. 36 (2016) 1166–1172.

[31] D. Gode, D.A. Volmer, Lipid imaging by mass spectrometry − a review, Analyst 138 (2013) 1289–1315.

[32] S. Gravius, T.M. Randau, R. Casadonte, M. Kriegsmann, M.J. Friedrich, J. Kriegsmann, Investigation of neutrophilic peptides in periprosthetic tissue by matrix-assisted laser desorption ionisation time-of-flight imaging mass spectrometry, Int. Orthop. 39 (2015) 559–567.

[33] J. Griffin, D. Treanor, Digital pathology in clinical use: where are we now and what is holding us back, Histopathology 70 (2017) 134–145.

[34] M.R. Groseclose, S. Castellino, A mimetic tissue model for the quantification of drug distributions by MALDI imaging mass spectrometry, Anal. Chem. 85 (2013) 10099–10106.

[35] M.R. Groseclose, P.P. Massion, P. Chaurand, R.M. Caprioli, High-throughput proteomic analysis of formalin-fixed paraffin-embedded tissue microarrays using MALDI imaging mass spectrometry, Proteomics 8 (2008) 3715–3724.

[36] J. Hajduk, J. Matysiak, Z.J. Kokot, Challenges in biomarker discovery with MALDI-TOF MS, Clin. Chim. Acta 458 (2016) 84–98.

[37] D.J. Harvey, Analysis of carbohydrates and glycoconjugates by matrix-assisted laser desorption/ionization mass spectrometry: an update for 2009–2010, Mass Spectrom. Rev. 34 (2015) 268–422.

[38] C. Honisch, Y. Chen, C. Mortimer, C. Arnold, O. Schmidt, D. van den Boom, C.R. Cantor, H.N. Shah, S.E. Gharbia, Automated comparative sequence analysis by base-specific cleavage and mass spectrometry for nucleic acid-based microbial typing, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 10649–10654.

[39] P.J. Horn, K.D. Chapman, Lipidomics in situ: insights into plant lipid metabolism from high resolution spatial maps of metabolites, Prog. Lipid Res. 54 (2014) 32–52.

[40] J. Kriegsmann, M. Kriegsmann, R. Casadonte, MALDI TOF imaging mass spectrometry in clinical pathology: a valuable tool for cancer diagnostics (review), Int. J. Oncol. 46 (2015) 893–906.

[41] M. Kriegsmann, N. Arens, V. Endris, W. Weichert, J. Kriegsmann, Detection of KRAS NRAS and BRAF by mass spectrometry − a sensitive, reliable, fast and cost-effective technique, Diagn. Pathol. 10 (2015) 132.

[42] M. Kriegsmann, R. Casadonte, J. Kriegsmann, H. Dienemann, P. Schirmacher, J. Hendrik Kobarg, K. Schwamborn, A. Stenzinger, A. Warth, W. Weichert, Reliable entity subtyping in non-small cell lung cancer by matrix-assisted laser desorption/ionization imaging mass spectrometry on formalin-fixed paraffin-embedded tissue specimens, Mol. Cell. Proteomics: MCP 15 (2016) 3081–3089.

[43] M. Kriegsmann, R. Casadonte, T. Randau, S. Gravius, P. Pennekamp, A. Strauss, J. Oldenburg, K. Wieczorek, S.O. Deininger, M. Otto, J. Kriegsmann, MALDI imaging of predictive ferritin: fibrinogen and proteases in haemophilic arthropathy, Haemophilia 20 (2014) 446–453.

[44] M. Kriegsmann, R. Longuespee, P. Wandernoth, C. Mohanu, K. Lisenko, W. Weichert, A. Warth, H. Dienemann, E. De Pauw, T. Katzenberger, D. Aust, G. Baretton, J. Kriegsmann, R. Casadonte, Typing of colon and lung adenocarcinoma by high throughput imaging mass spectrometry, Biochim. Biophys. Acta 1865 (2017) 858–864.

[45] M. Kriegsmann, P. Wandernoth, K. Lisenko, R. Casadonte, R. Longuespee, N. Arens, J. Kriegsmann, Detection of HPV subtypes by mass spectrometry in FFPE tissue

specimens: a reliable tool for routine diagnostics, J. Clin. Pathol. 70 (2017) 417–423.
[46] H. Loffler, N. Pfarr, M. Kriegsmann, V. Endris, T. Hielscher, P. Lohneis, G. Folprecht, A. Stenzinger, M. Dietel, W. Weichert, A. Kramer, Molecular driver alterations and their clinical relevance in cancer of unknown primary site, Oncotarget 7 (2016) 44322–44329.
[47] R. Longuespee, D. Alberts, C. Pottier, N. Smargiasso, G. Mazzucchelli, D. Baiwir, M. Kriegsmann, M. Herfs, J. Kriegsmann, P. Delvenne, E. De Pauw, A laser microdissection-based workflow for FFPE tissue microproteomics: important considerations for small sample processing, Methods 104 (2016) 154–162.
[48] R. Longuespee, R. Casadonte, M. Kriegsmann, C. Pottier, G. Picard de Muller, P. Delvenne, J. Kriegsmann, E. De Pauw, MALDI mass spectrometry imaging: a cutting-edge tool for fundamental and clinical histopathology, Proteomics Clin. Appl. 10 (2016) 701–719.
[49] G. Magliacane, G. Grassini, P. Bartocci, I. Francaviglia, E. Dal Cin, G. Barbieri, G. Arrigoni, L. Pecciarini, C. Doglioni, M.G. Cangi, Rapid targeted somatic mutation analysis of solid tumors in routine clinical diagnostics, Oncotarget 6 (2015) 30592–30603.
[50] M.L. Manier, J.M. Spraggins, M.L. Reyzer, J.L. Norris, R.M. Caprioli, A derivatization and validation strategy for determining the spatial localization of endogenous amine metabolites in tissues using MALDI imaging mass spectrometry, J. Mass Spectrom. JMS 49 (2014) 665–673.
[51] M.M. Mbughuni, P.J. Jannetto, L.J. Langman, Mass spectrometry applications for toxicology, EJIFCC 27 (2016) 272–287.
[52] L.A. McDonnell, P.M. Angel, S. Lou, R.R. Drake, Mass spectrometry imaging in cancer research: future perspectives, Adv. Cancer Res. 134 (2017) 283–290.
[53] S. Meding, U. Nitsche, B. Balluff, M. Elsner, S. Rauser, C. Schone, M. Nipp, M. Maak, M. Feith, M.P. Ebert, H. Friess, R. Langer, H. Hofler, H. Zitzelsberger, R. Rosenberg, A. Walch, Tumor classification of six common cancer types based on proteomic profiling by MALDI imaging, J. Proteome Res. 11 (2012) 1996–2003.
[54] Y. Morita, K. Ikegami, N. Goto-Inoue, T. Hayasaka, N. Zaima, H. Tanaka, T. Uehara, T. Setoguchi, T. Sakaguchi, H. Igarashi, H. Sugimura, M. Setou, H. Konno, Imaging mass spectrometry of gastric carcinoma in formalin-fixed paraffin-embedded tissue microarray, Cancer Sci. 101 (2010) 267–273.
[55] J. Murugaiyan, U. Roesler, MALDI-TOF MS profiling-advances in species identification of pests parasites, and vectors, Front. Cell. Infect. Microbiol. 7 (2017) 184.
[56] J.L. Norris, R.M. Caprioli, Analysis of tissue specimens by matrix-assisted laser desorption/ionization imaging mass spectrometry in biological and clinical research, Chem. Rev. 113 (2013) 2309–2342.
[57] I. Okamoto, K. Sakai, S. Morita, H. Yoshioka, H. Kaneda, K. Takeda, T. Hirashima, Y. Kogure, T. Kimura, T. Takahashi, S. Atagi, T. Seto, T. Sawa, M. Yamamoto, M. Satouchi, M. Okuno, S. Nagase, K. Takayama, K. Tomii, T. Maeda, S. Oizumi, S. Fujii, Y. Akashi, K. Nishino, N. Ebi, K. Nakagawa, Y. Nakanishi, K. Nishio, Multiplex genomic profiling of non-small cell lung cancers from the LETS phase III trial of first-line S-1/carboplatin versus paclitaxel/carboplatin: results of a West Japan Oncology Group study, Oncotarget 5 (2014) 2293–2304.
[58] R. Patel, MALDI-TOF mass spectrometry: transformative proteomics for clinical microbiology, Clin. Chem. 59 (2013) 340–342.
[59] P. Paueksakon, A.B. Fogo, S. Sethi, Leukocyte chemotactic factor 2 amyloidosis cannot be reliably diagnosed by immunohistochemical staining, Hum. Pathol. 45 (2014) 1445–1450.
[60] J. Petzold, R. Casadonte, M. Otto, M. Kriegsmann, M. Granrath, A. Baltzer, J. Vogel, P. Drees, S. Deininger, M. Becker, J. Kriegsmann, MALDI mass spectrometry of the meniscus Objectification of morphological findings, Zeitschrift fur Rheumatologie 74 (2015) 438–446.
[61] N. Pfarr, A. Stenzinger, R. Penzel, A. Warth, H. Dienemann, P. Schirmacher, W. Weichert, V. Endris, High-throughput diagnostic profiling of clinically actionable gene fusions in lung cancer, Genes. Chromosomes Cancer 55 (2016) 30–44.
[62] T.W. Powers, S. Holst, M. Wuhrer, A.S. Mehta, R.R. Drake, Two-dimensional N-Glycan distribution mapping of hepatocellular carcinoma tissues by MALDI-Imaging mass spectrometry, Biomolecules 5 (2015) 2554–2572.
[63] J. Quanico, J. Franck, M. Wisztorski, M. Salzet, I. Fournier, Progress and potential of imaging mass spectrometry applied to biomarker discovery, Methods Mol. Biol. 2017 (1598) 21–43.
[64] B. Rocha, C. Ruiz-Romero, F.J. Blanco, Mass spectrometry imaging: a novel technology in rheumatology, Na. Rev. Rheumatol. 13 (2017) 52–63.
[65] A. Rompp, S. Guenther, Z. Takats, B. Spengler, Mass spectrometry imaging with high resolution in mass and space (HR(2) MSI) for reliable investigation of drug compound distributions on the cellular level, Anal. Bioanal. Chem. 401 (2011) 65–73.
[66] S.M. Said, S. Sethi, A.M. Valeri, N. Leung, L.D. Cornell, M.E. Fidler, L. Herrera Hernandez, J.A. Vrana, J.D. Theis, P.S. Quint, A. Dogan, S.H. Nasr, Renal amyloidosis: origin and clinicopathologic correlations of 474 recent cases, Clin. Jo. Am. Soc. Nephrol.: CJASN 8 (2013) 1515–1523.
[67] C. Schone, H. Hofler, A. Walch, MALDI imaging mass spectrometry in cancer research: combining proteomic profiling and histological evaluation, Clin. Biochem. 46 (2013) 539–545.
[68] K. Schwamborn, M. Kriegsmann, W. Weichert, MALDI imaging mass spectrometry − From bench to bedside, Biochim. Biophys. Acta 1865 (2017) 776–783.
[69] S. Sethi, J.A. Vrana, J.D. Theis, A. Dogan, Mass spectrometry based proteomics in the diagnosis of kidney disease, Curr. Opin. Nephrol. Hypertens. 22 (2013) 273–280.
[70] L.J. Sparvero, A.A. Amoscato, C.E. Dixon, J.B. Long, P.M. Kochanek, B.R. Pitt, H. Bayir, V.E. Kagan, Mapping of phospholipids by MALDI imaging (MALDI-MSI): realities and expectations, Chem. Phys. Lipids 165 (2012) 545–562.
[71] A. Stenzinger, M. Kriegsmann, W. Weichert, The role of pathology in the diagnostics of CUP syndrome, Der Radiologe 54 (2014) 124–133.
[72] S. Steurer, C. Borkowski, S. Odinga, M. Buchholz, C. Koop, H. Huland, M. Becker, M. Witt, D. Trede, M. Omidi, O. Kraus, A.S. Bahar, A.S. Seddiqi, J.M. Singer, M. Kwiatkowski, M. Trusch, R. Simon, M. Wurlitzer, S. Minner, T. Schlomm, G. Sauter, H. Schluter, MALDI mass spectrometric imaging based identification of clinically relevant signals in prostate cancer using large-scale tissue microarrays, Int. J. Cancer 133 (2013) 920–928.
[73] S. Steurer, A.S. Seddiqi, J.M. Singer, A.S. Bahar, C. Eichelberg, M. Rink, R. Dahlem, H. Huland, G. Sauter, R. Simon, S. Minner, E. Burandt, P.R. Stahl, T. Schlomm, M. Wurlitzer, H. Schluter, MALDI imaging on tissue microarrays identifies molecular features associated with renal cell cancer phenotype, Anticancer Res. 34 (2014) 2255–2261.
[74] S. Steurer, J.M. Singer, M. Rink, F. Chun, R. Dahlem, R. Simon, E. Burandt, P. Stahl, L. Terracciano, T. Schlomm, W. Wagner, W. Hoppner, M. Omidi, O. Kraus, M. Kwiatkowski, O. Doh, M. Fisch, A. Soave, G. Sauter, M. Wurlitzer, H. Schluter, S. Minner, MALDI imaging-based identification of prognostically relevant signals in bladder cancer using large-scale tissue microarrays, Urol. Oncol. 32 (2014) 1225–1233.
[75] H.X. Tian, X.C. Zhang, Z. Wang, J.G. Chen, S.L. Chen, W.B. Guo, Y.L. Wu, Establishment and application of a multiplex genetic mutation-detection method of lung cancer based on MassARRAY platform, Cancer Biol. Med. 13 (2016) 68–76.
[76] A. van Belkum, S. Chatellier, V. Girard, D. Pincus, P. Deol, W.M. Dunne Jr., Progress in proteomics for clinical microbiology: MALDI-TOF MS for microbial species identification and more, Expert Rev. Proteomic 12 (2015) 595–605.
[77] S.M. Willems, A. van Remoortere, R. van Zeijl, A.M. Deelder, L.A. McDonnell, P.C. Hogendoorn, Imaging mass spectrometry of myxoid sarcomas identifies proteins and lipids specific to tumour type and grade, and reveals biochemical intratumour heterogeneity, J. Pathol. 222 (2010) 400–409.
[78] L. Xiu, C. Zhang, Z. Wu, J. Peng, Establishment and application of a universal Coronavirus screening method using MALDI-TOF mass spectrometry, Front. Microbiol. 8 (2017) 1510.