Chapter 5
An Overview of Matrix-Assisted Laser Desorption/Ionization (MALDI) Mass Spectrometry and Some of Its Applications

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Abstract Matrix-assisted laser desorption/ionization time-of-flight (TOF) mass spectrometry (MS) is a powerful tool for both the qualitative and quantitative analysis of wide array of analytes. MALDI-TOF MS is simple, both in design and practice, and offers the advantages that analysis times are very short, the mass range is virtually unlimited, and sensitivity is high. This chapter describes the theory and practice of MALDI and illustrates the utility of approach by reviewing its common applications.

Keywords MALDI • Mass spectrometry • Microbiology • Clinical applications • Imaging

5.1 Introduction

5.1.1 History

The term matrix-assisted laser desorption ionization (MALDI) was coined in 1985 by Franz Hillenkamp and Michael Karas [1, 2]. These investigators found that the amino acid alanine could be ionized more easily if it was mixed with the amino acid

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tryptophan and irradiated with a pulsed 266 nm laser. The availability of small and relatively inexpensive nitrogen lasers operating at a wavelength of 337 nm, and the introduction of the first commercial instruments in the early 1990s, lead to widespread adoption of MALDI by researchers.

5.1.2 Definitions

MALDI is the commonly used abbreviation for matrix assisted laser desorption/ionization. MALDI is frequently coupled with a time-of-flight mass analyzer (TOF) and the abbreviation therefore becomes MALDI-TOF. Other types of analyzers can be used and are commercially available, but these alternative configurations constitute a small portion of the market because, in practice, the marriage of MALDI ionization with a TOF analyzer is close to ideal.

5.1.3 An Overview of MALDI

MALDI is a widely used and powerful "soft" ionization method suitable for almost all analytes that can accept (positive ion mode) or lose (negative ion mode) a proton. Because of its versatility, MALDI has found wide application in the analysis of large and/or labile molecules, lipids, peptides, proteins, oligonucleotides, and synthetic polymers. Special applications of increasing importance include: protein characterization, clinical chemistry, clinical microbiology, single cell analysis, metabolomics and imaging. (These specific applications are discussed in greater detail later.)

For MALDI, the sample is intimately mixed with matrix and dried on a target (or MALDI plate). The target is then loaded into the mass spectrometer where the sample/matrix mixture is irradiated by the laser. This process transfers both energy and charge to the sample molecules: *i.e.*, in positive ion mode the analyte molecules are transferred to the gas phase and protonated. Ions are accelerated out of the source region by an electric field (20–30 kV) and fly down the flight tube which is typically about 1 m long. At the end of the flight tube a detector records both the intensity of the ion current and the time-of-flight.

5.1.4 The Ionization Process

In positive ion MALDI the matrix absorbs the laser energy and transfers a proton to the analyte molecule. Together, the analyte and proton form a quasimolecular ion: *e.g.,* $[M+H]^+$. Other possibilities include $[M+Na]^+$ in the case of an added sodium ion, or $[M-H]^-$ in the case of a removed proton. The ions generated are all even-electron species although radical cations (photoionized molecules) can be observed, e.g., in case of matrix molecules or some organic molecules.

5.1.5 The MALDI Mass Spectrum of a Pure Component

In a typical MALDI mass spectrum of a pure compound the major peak corresponds to $[M+H]^{1+}$. In some instances, however, molecules adduct cations rather than a proton. For example, acidic peptides can bind sodium and potassium ions, arising from glass vessels as well as buffers, and these acidic peptides show up at 22 and 38 mass units higher than expected (*i.e.,* higher than the anticipated M+H ion.) In addition, MALDI spectra are also frequently complicated by both multiply charged ions (*i.e.,* $[M+nH]^{n+}$) and singly charged, multimeric forms (*i.e.,* $[nM+H]^{+}$). The propensity to form these more complex species is a function of the matrix, the laser intensity and the analyte (most especially its mass). Molecule-molecule and molecule-matrix adducts are also evident in some spectra.

5.1.6 The Laser

First-generation instruments employed UV lasers such as nitrogen lasers (337 nm), but frequency-tripled or quadrupled Nd:YAG lasers (355 nm and 266 nm respectively) are now more commonly employed. Infrared lasers have sometimes been used because they give a softer mode of ionization, less low-mass interferences, and are compatible with other matrix-free laser desorption mass spectrometry methods.

5.1.7 Time-of-Flight Analyzers

The operating principle of a TOF analyzer is that a population of ions having a distribution of masses and moving in the same direction with (more-or-less) constant kinetic energy will have a distribution of velocities inversely proportional to their mass. Their arrival times at a target plane (parallel to the plane of origin) will be distributed according to the square root of m/z. That is to say, for a given energy (E) and distance (d), the mass is proportional to the square of the flight time of the ion: *i.e.,* $m = (2E/d^2)t^2$.

5.1.8 The Matrix and Its Properties

The matrix is central to the ionization process and it is typically a low mass conjugated aromatic molecule. The matrix must be: (a) able to isolate and entrap analyte molecules (*e.g.,* by co-crystallization), (b) able to transfer or accept protons from nonvolatile analytes, (c) chemically inert (*i.e.,* no reactivity with analytes), (d) stable under vacuum in the MALDI source and (e) both soluble in solvents and compatible with analytes and samples. Commonly used matrices and their properties are included in Table 5.1.

Table 5.1 Commonly used MALDI matrices and their features

Matrix	Wavelength	Typical applications
2,5-Dihydroxybenzoic acid (2,5-DHB)	337/355 nm	Proteins and oligosaccharides
Sinapinic acid (SA)	337/355 nm	Proteins
α-Cyano-4-hydroxycinnamic acid (HCCA)	337/355 nm	Peptides and proteins
3-Hydroxycinnamic acid (3-HPA)	337/355 nm	Oligonucleic acids
Picolinic acid (PA)	266 nm	Nucleic acids
2,4,6-Trihydroxyacetophenone (2,4,6-THAP)	337/355 nm	Oligonucleic acids and acidic oligosaccharides
6-Aza-2-thiothymine	266, 337, 355 nm	Oligonucleic acids and acidic oligosaccharides
2-(4′-Hydroxybenzeneazo) benzoic acid (HABA)	337/355 nm	Proteins and carbohydrates
2,6-Dihydroxyacetophenone (2,6-DHAP)	337/355 nm	Oligonucleic acids
3-Aminoquinoline	337 nm	Oligosaccharides
3-Hydroxy picolinic acid	337 nm	Nucleic acids
Nicotinic acids	266 nm	Proteins, peptides and adduct formation
Thiourea	266 nm	Large protein

5.1.9 Sampling from the Target

Typically the laser is fired multiple times at a fixed location on the target and spectra are averaged or summed with the objective of maximizing the observable signal-to-noise ratio. If the amount of analyte is low, it may be necessary to irradiate additional regions on the target and sum/average many of these to yield an acceptable spectrum (Fig. 5.1). For example, when a sample is irradiated 10, 100, 1,000 & 11,000 times and the signal is averaged, there is a marked increase in signal-to-noise in the observed mass spectrum.

5.1.10 Characteristics of the MALDI Spectrum of a Mixture

Ideally, each component in a complex mixture would yield a single peak in the mass spectrum and relative peak heights would reflect relative amounts of each component of the mixture. The reality, however, is that the spectrum of a mixture is (very) complex. Each component often gives rise to multiple peaks and because components differ in how readily they ionize (*i.e.,* their ionization efficiencies), relative peak heights do not reflect relative amounts. Interpretation of the MALDI mass spectrum of a mixture is therefore far from trivial.

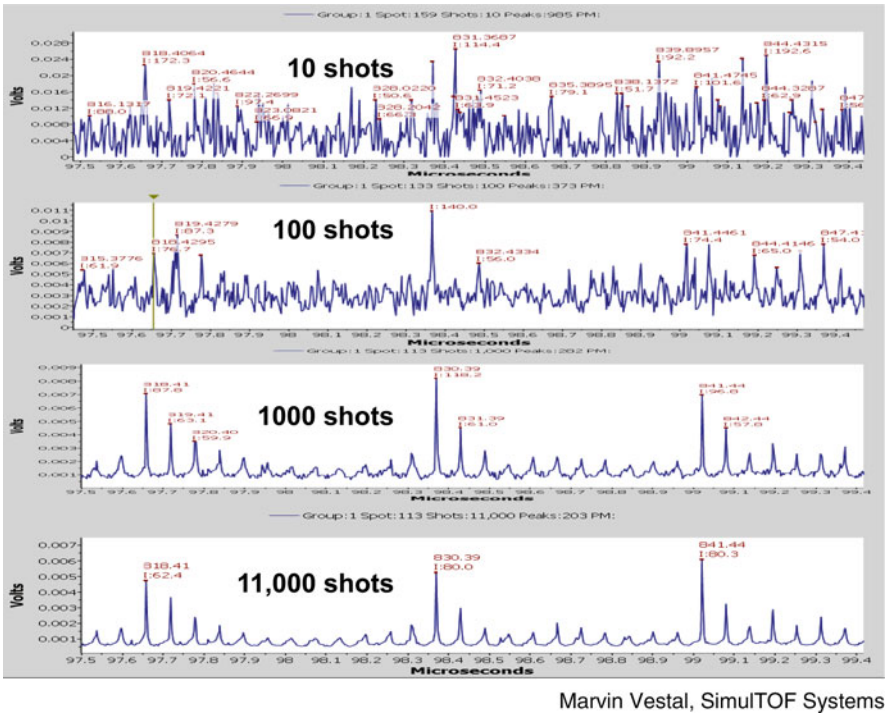

Fig. 5.1 The impact of increasing the number of laser shots on the signal-to-noise ratio

5.1.11 Instrument Refinements

There have been several refinements to the basic MALDI-TOF configuration aimed at improving both the resolution and information content of spectra. The most important of these are outlined below.

Delayed extraction: In a conventional continuous ion extraction MALDI-TOF mass spectrometer, the achievable resolution is limited by the spread in the initial velocities of ions of identical *m*/*z*. In delayed extraction, a short delay is introduced between the laser pulse and the application of the accelerating voltage to compensate for this. Delayed extraction improves mass resolution [3].

The Reflectron: A reflectron can be located at the end of the flight tube and is comprised of a series of electrodes that form a linear field opposite in direction to the initial accelerating voltage. The reflectron is used to reverse the direction of travel of the ions. It significantly improves mass resolution by ensuring that ions of the same *m*/*z*, but different translational energies, arrive at the detector at the same time [4].

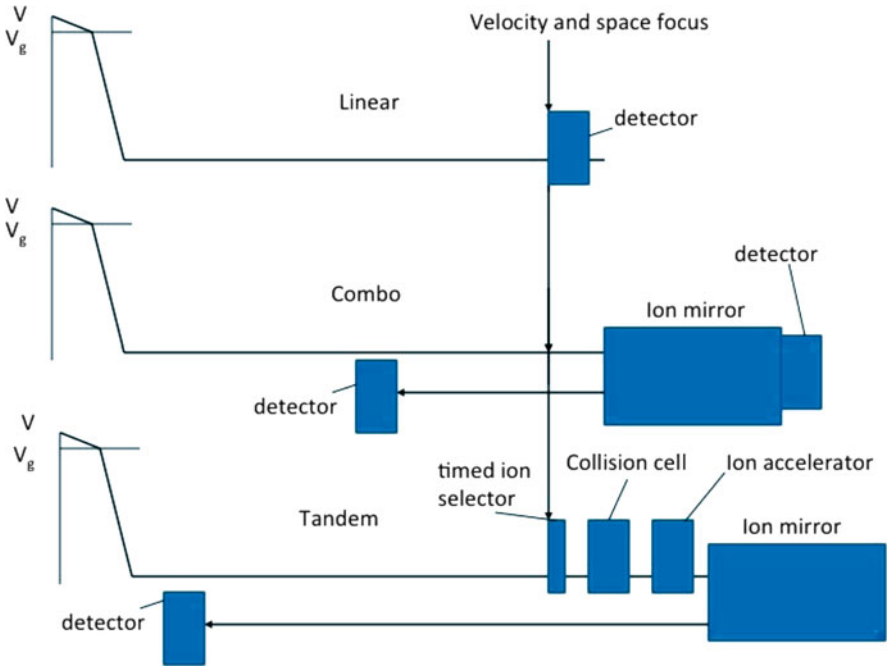

Fig. 5.2 Commonly adopted instrument configurations for MALDI-TOF instruments. From top to bottom, this figure shows the configuration of a simple linear MALDI-TOF system incorporating delayed extraction. The center panels show the incorporation of a reflectron (*i.e.,* ion mirror) to enhance resolution. In the third panel the TOF/TOF design incorporates a collision cell and a second stage of (product) ion separation to give fragmentation and structural information not otherwise accessible by MALDI

Post Source Decay (PSD) and TOF/TOF: There is little to no fragmentation in a typical MALDI mass spectrum. Several approaches have therefore been developed to overcome this limitation and to allow investigators to generate product ion spectra. The first of these was PSD [5]. Although powerful, the approach is limited because it is not possible to select a specific precursor and fragment it. More recently, a high energy collision cell has been introduced between two time-of-flight analyzers to allow selection of a precursor ion of interest, collisional dissociation to induce fragmentation, and then generation of a product ion spectrum [6] (Fig. 5.2).

5.1.12 Major Applications of MALDI (MALDI-TOF)

Some of the most common areas of application of MALDI-TOF mass spectrometry are outlined in the sections that follow.

5.1.12.1 Applications in Organic Chemistry: Characterization of Synthetic Materials and Natural Products

MALDI can be used to aid in the characterization of natural products and synthetic materials. Contrary to common views, MALDI can be applied to very low mass analytes and provides accurate MW assignments with errors in the very low ppm range. This makes it possible to assign elemental compositions [7]. Synthetic polymer characterization is a challenging exercise, but MALDI offers a powerful solution and its applications have been comprehensively reviewed [8].

5.1.12.2 Applications to Proteomics: Peptide Mass Fingerprints (PMF) and MS/MS

It is possible to identify a purified protein by an approach known as peptide mass fingerprints. This is a powerful strategy that is simple and fast to perform that gives valuable information on a "purified" protein and its contaminants. The process involves four steps: (1) protein isolation (PMF requires that a single protein or just a few proteins are present); (2) protein digestion; (3) MALDI-TOF analysis to generate MWs of proteolytic peptides and (4) application of database search algorithms to determine the best match. The algorithm performs a theoretical digest of each entry in the protein database and calculates the mass of all peptides derived from each; the algorithm then compares observed (*i.e.,* experimental) peptide masses with those calculated *in silico* from the database entries (*i.e.,* theoretical). The best matches are determined and scores assigned to each that can be used to assess the certainty of the match. The process generally works well, but factors including the number of masses observed, the mass tolerance/accuracy (set/measured; ppm or Da), protein modifications, and the attributes of the relevant database(s) can all influence the outcome.

Protein identification is feasible by PMF because instead of searching all conceivable sequences, the search space is restricted to consider only the small subset of known proteins that are represented in the database. However, depending on the specific objectives, it may prudent to generate additional data in support of a protein assignment made by PMF. This could include generating one or more peptide MS/MS spectra (*e.g.,* generated by MALDI TOF/TOF) and matching these against the theoretical MS/MS spectra of putative sequence for the peptide.

Notably, these approaches can only be used to assign an ID to proteins that have already been identified and where their sequence resides in, or can be derived from, an accessible database. In some instances, however, matches to closely homologous entries can provide important clues to protein identity, even if the target is not included in the database(s), but for obvious reasons, the exact splice variant, mutant protein and structure homologue may be difficult, if not impossible, to identify. Similarly, complications can arise if proteolysis is incomplete, there are non-specific cleavages or if multiple proteins are present in the sample (*i.e.,* because it becomes computationally intensive to account for all possibilities).

Numerous reviews highlight other applications of MALDI-TOF to peptide and protein characterization [9–14].

Applications in Microbiology

The identification of microbial pathogens by MALDI-TOF mass spectrometry is an important and rapidly growing application. MALDI-TOF is particularly attractive in the field of clinical microbiology because it provides accurate results at markedly reduced costs relative to the more conventional approaches that have been adopted for decades. Further, the rugged, reproducible nature of the technique means fewer replicates are required to confirm pathogen identifications. In addition, training in the MALDI technique typically takes a week; contrastingly, training in the more conventional approaches may take years.

With MALDI-based identification one basic strategy can be used to confidently identify a range of microbial pathogens including bacteria, yeast, molds, fungi and mycobacteria [15]. The approach involves taking a small aliquot of a clinical sample (*e.g.,* urine or blood) and incubating this on a Petri dish with standard agar growth media to amplify the number of pathogens and to generate "pure" colonies. Culture time remains the main rate-limiting step, even with MALDI, because it is necessary to have adequate biomass (*i.e.,* about 10^5 cells/ml) to generate mass spectra of sufficient quality for confident pathogen typing. (In the case of *Mycobacterium tuberculosis*, it can take up to 6–8 weeks to culture the sample.)

After culturing, a representative colony is sampled from the Petri dish with an inoculation loop, smeared onto the MALDI target, matrix is added (*i.e.,* sinapinic acid) and the sample is air-dried. A MALDI mass spectrum of the intact peptides and proteins is then generated. Custom software is used to match the spectral peaks derived from the unknown pathogen peptides and proteins against those in a microbe spectral library. The software assigns a score based on the quality of the match between the sample and the best match in the library. The pathogen peptides/proteins are not identified as a part of this process, but for the most part they are ribosomal proteins. Their sequences, and therefore their masses, are sufficiently constant and unique to allow robust characterization [16]. The approach is reported reproducible from lab-to-lab and is largely independent of culture conditions (*e.g.,* time and temperature) and culture medium [17].

There are, however, several limitations to the technique. For example, atypical organisms or complex samples comprised of multiple pathogens may still require nucleic acid sequencing. Although MALDI approaches to antibiotic susceptibility testing are under development, they are not yet commercially available. As anticipated, species identification is highly dependent on the spectral library, how complete it is, how carefully it is curated, and how well the software matches/discriminates between mass fingerprints.

Despite current limitations, MALDI-based approaches are now routinely adopted, and they allow the reliable characterization of pathogens that would not have been identified by traditional workflows just a few years ago. For example,

MALDI bacterial typing can identify life-threatening infections such as multi-drug resistant bacteria many hours faster than more conventional diagnostic strategies [18]. This is because blood borne bacteria can be detected in culture media after only 6–8 h incubation whereas more conventional approaches require overnight culturing on selective media. Streamlining the process translates directly to better outcomes because early and appropriate therapy is the most important factor in preventing mortality from potentially fatal infections such as septicemia [19].

Rapid change in this area continues. Commercial spectral libraries are regularly updated with new species, workflows are being optimized to incorporate new developments and research into new applications is ongoing. For example, MALDI-based approaches to identify viral pathogens and bacterial endotoxins have also been reported [20–22]. Also, methods have been reported that allow for the direct identification of urine and blood infections without prior culturing [23]. However, when multiple pathogens infect the sample, identification can be problematic because the matching algorithms can fail if the MALDI mass spectrum is a composite derived from several distinct organisms. The problem is compounded because without isolation and enrichment through culturing, high abundance components – not all necessarily derived from the same pathogen – can contribute a strong signal that overwhelms and masks others. Under these conditions it can be difficult to get a high quality match, and less abundant pathogens may be overlooked completely.

Applications of MALDI to Clinical Diagnostics

The development of diagnostics centered round MALDI-TOF mass spectrometry is an area of very active research. A wide variety of endogenous analytes including nucleic acids, glycans, lipids, exogenous small molecules and drug metabolites have been measured in these studies, but peptides and proteins have been the focus of the majority of clinical biomarker studies. Typically these studies involve some approach to profiling (or fingerprinting) multiple components simultaneously, either on the crude sample, or following affinity enrichment, depletion or pre-fractionation strategies. A wide variety of tissue and fluids have been explored including biopsy samples, blood products (*e.g.,* serum and plasma), urine, tears, saliva, and cerebral spinal fluid (CSF). MALDI-TOF has been employed across all phases of biomarker development – from discovery, through verification and validation, to routine testing. In recent years several clinically validated and FDA approved assays have been developed on a MALDI platform and these are now becoming commercially available. In this section some of the strategies adopted, and recent developments from research and commercial sectors are highlighted.

There are many instances of where MALDI has been applied to biomarker discovery. For example, changes in the glycosylation pattern/profile of cellular and circulating proteins have been explored in a variety of cancers and inflammatory disorders. In a case-control study aimed at identifying new glycan biomarkers of epithelial ovarian cancer (EOC), N-glycans were enzymatically cleaved from affinity enriched serum glycoproteins, permethylated to stabilize them and characterized

by MALDI [24]. A diagnostic algorithm was then constructed from the peak areas of mannose and fucose containing glycan motifs. This approach reportedly delivered 97 % sensitivity and 98.4 % specificity in the diagnosis of EOC. (This compares with a 97 % sensitivity and 88.9 % specificity for the common tumor antigen CA-125.) In a similar manner, van Swelm et al. [25] describe a MALDI-TOF case-control study of urine collected from psoriatic patients at risk of hepatic fibrosis, an adverse reaction associated with long-term immunosuppression with methotrexate (MTX) therapy. They identified candidate urinary protein biomarkers that may be useful in monitoring MTX-induced hepatic fibrosis. Tear analysis has also been explored as a minimally invasive approach to the diagnosis of breast cancer. Bohm et al. employed MALDI-TOF-TOF to identify over 20 proteins that appeared to be differentially abundant in 25 patients with primary invasive breast carcinoma relative to 25 age-matched healthy controls [26].

Numerous companies now offer bespoke assays, affinity reagents, standards and software solutions based on the MALDI-TOF platform. Examples include the MASStermind® cardiac assay (Pronata, www.pronota.be), Veristrat® lung cancer drug response assay (Biodesix Inc., www.biodesix.com), SISCAPA reagents, affinity depletion frets for MALDI mass spectrometric immunoassay (MSIA®; Thermoscientific, www.thermoscientific.com), MassARRAY® nucleic acid analysis (Sequenom, www.sequenom.com) and MALDI spectral analysis software products from Premier Biosoft (www.premierbiosoft.com) and Bioinformatic Solutions (www.bioinfor.com). The Veristrat® and MassARRAY® tests are prescient examples that showcase the technical capability and clinical potential of MALDI based products.

Sequenom's MassARRAY® system combines primer extension chemistry with MALDI-TOF mass spectrometry for quantification of nucleic acids and detection of genetic variability. The MassARRAY® system provides multiple nucleic acid testing options including genotyping, somatic mutation and methylation profiling, copy number variation and quantitative gene expression. Single nucleotide polymorphism (SNP) genotyping is commonly used for fine mapping of gene variance associated with specific clinical phenotypes (GWAS studies). Sequenom SNP genotyping combines two core elements: first the generation of an allele-specific product from template cDNA and second, extension primers are designed to anneal directly adjacent to every SNP position to be assayed [27]. During subsequent thermocycling, a thermosequence enzyme adds a nucleotide that naturally differs in mass from the other three available. Samples are then cleaned up by ion exchange to remove potential interferents. For these applications 3-hydroxy picolinic acid is used as a matrix to ionize and dissociate the primer-template duplex. The allele specific SNP products can then be discriminated by m/z differences as low as 16 within the mass range m/z 4,000–9,000. The technology was commercialized around the development of a dedicated MALDI-TOF analyzer and specific reagents and has been cited in over 2,000 publications to date.

Veristrat® is a clinically validated protein diagnostic test, released in 2009, that aims to personalize drug treatments for non-small cell lung cancer (NSCLC) patients. Instead of SNP genotyping, Veristrat uses MALDI-TOF to examine a

panel of eight spectral features (or specific *m*/*z* values) in the patient blood sample. A bespoke algorithm uses these features to differentiate between those subjects that are likely to respond to a specific drug type (*i.e.,* a protein kinase inhibitor) and those who likely will not. Biodesix does not define the specific proteins that underpin the Veristrat® signature, but in a subsequent study by Milan et al. [28], serum amyloid A protein 1 (SAA1), together with its truncated forms, are reported to be over-expressed in the plasma of patients classified as Veristrat-poor. These authors report that SAA1 and its variants contribute four out of the eight classifiers composing the Veristrat® signature. To date, Veristrat® has been used in over 5,000 patients and over 80 clinical trials. Ackerly et al. report that 90 % of post-test treatment recommendations positively correlated with Veristrat® test results [29]. The test also has the potential to serve as a breast and colorectal cancer outcome prediction tool.

Applications of MALDI to Imaging

In imaging mass spectrometry a sample of tissue is immobilized on the MALDI target and coated with matrix. Imaging mass spectrometry retains the spatial fidelity of the sample with the objective of locating specific molecules within the sample. Generating an image using MALDI MS is accomplished by rastering the laser across the tissue in order to collect molecular information from a regularly spaced array of positions (or pixels). The molecular information encoded at each location is extracted and plotted to create ion images that can be directly correlated with the location of specific biological molecules.

Typically in MALDI imaging a fresh frozen tissue sample is cut and mounted on a conductive target. Matrix is then applied in an ordered array across the tissue section and mass spectra (or MS/MS spectra) are generated at each x, y coordinate. The distribution of one or more components is visualized within the tissue sample by using a color scale to represent the relative intensity of the components.

5.2 Conclusions

MALDI ionization, especially when combined with time-of-flight ion separation, is a cost-effective, rapid, sensitive, reproducible and exceedingly versatile approach to both qualitative and quantitative analysis. MALDI efficiently ionizes a wide range of compounds including high mass biopolymers and thermally labile compounds and it is a valuable complement to electrospray ionization. Because of these attributes, MALDI is increasingly being applied to issues relating to bioterrorism including the rapid and sensitive identification of potent toxins [30–32] and dangerous pathogens [33–35] that might be intentionally introduced into the environment.

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References

1. Karas M, Bachmann D, Hillenkamp F (1985) Influence of the wavelength in high-irradiance ultraviolet laser desorption mass spectrometry of organic molecules. Anal Chem 57(14):2935–2939
2. Karas M et al (1987) Matrix-assisted ultraviolet laser desorption of non-volatile compounds. Int J Mass Spectrom Ion Process 78:53–68
3. Vestal ML, Campbell JM (2005) Tandem time-of-flight mass spectrometry. Methods Enzymol 402:79–108
4. Mamyrin BA et al (1973) The mass-reflectron, a new nonmagnetic time-of-flight mass spectrometer with high resolution. Sov Phys JETP 37:45
5. Kaufmann R et al (1996) Post-source decay and delayed extraction in matrix-assisted laser desorption/ionization-reflectron time-of-flight mass spectrometry. Are there trade-offs? Rapid Commun Mass Spectrom 10(10):1199–1208
6. Medzihradszky KF et al (2000) The characteristics of peptide collision-induced dissociation using a high-performance MALDI-TOF/TOF tandem mass spectrometer. Anal Chem 72(3):552–558
7. Bucknall M, Fung KYC, Duncan MW (2002) Practical quantitative biomedical applications of MALDI-TOF mass spectrometry. J Am Soc Mass Spectrom 13(9):1015–1027
8. Weidner SM, Trimpin S (2010) Mass spectrometry of synthetic polymers. Anal Chem 82(12):4811–4829
9. Yang H, Liu N, Liu S (2013) Determination of peptide and protein disulfide linkages by MALDI mass spectrometry. Top Curr Chem 331:79–116
10. Stuhler K, Meyer HE (2004) MALDI: more than peptide mass fingerprints. Curr Opin Mol Ther 6(3):239–248
11. Hamdan M, Galvani M, Righetti PG (2001) Monitoring 2-D gel-induced modifications of proteins by MALDI-TOF mass spectrometry. Mass Spectrom Rev 20(3):121–141
12. Bonk T, Humeny A (2001) MALDI-TOF-MS analysis of protein and DNA. Neuroscientist 7(1):6–12
13. Roepstorff P (2000) MALDI-TOF mass spectrometry in protein chemistry. EXS 88:81–97
14. Chaurand P, Luetzenkirchen F, Spengler B (1999) Peptide and protein identification by matrix-assisted laser desorption ionization (MALDI) and MALDI-post-source decay time-of-flight mass spectrometry. J Am Soc Mass Spectrom 10(2):91–103
15. Croxatto A, Prod'hom G, Greub G (2012) Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev 36(2):380–407
16. Intelicato-Young J, Fox A (2013) Mass spectrometry and tandem mass spectrometry characterization of protein patterns, protein markers and whole proteomes for pathogenic bacteria. J Microbiol Methods 92(3):381–386
17. Welker M (2011) Proteomics for routine identification of microorganisms. Proteomics 11(15):3143–3153
18. Berrazeg M et al (2013) Biotyping of multidrug-resistant Klebsiella pneumoniae clinical isolates from France and Algeria using MALDI-TOF MS. PLoS One 8(4):e61428
19. Schneiderhan W et al (2013) Work flow analysis of around-the-clock processing of blood culture samples and integrated MALDI-TOF mass spectrometry analysis for the diagnosis of bloodstream Infections. Clin Chem 59(11):1649–1656
20. Gao X et al (2013) Screening of influenza mutations using base-specific cleavage and MALDI mass spectrometry. Clin Chim Acta 420:89–93

21. Peng J et al (2013) Sensitive and rapid detection of viruses associated with hand foot and mouth disease using multiplexed MALDI-TOF analysis. J Clin Virol 56(2):170–174
22. Kilar A, Dornyei A, Kocsis B (2013) Structural characterization of bacterial lipopolysaccharides with mass spectrometry and on- and off-line separation techniques. Mass Spectrom Rev 32(2):90–117
23. Ferreira L et al (2011) Rapid method for direct identification of bacteria in urine and blood culture samples by matrix-assisted laser desorption ionization time-of-flight mass spectrometry: intact cell vs. extraction method. Clin Microbiol Infect 17(7):1007–1012
24. Biskup K et al (2013) Serum glycome profiling: a biomarker for diagnosis of ovarian cancer. J Proteome Res 12(9):4056–4063
25. van Swelm RP et al (2013) Biomarkers for methotrexate-induced liver injury: urinary protein profiling of psoriasis patients. Toxicol Lett 221(3):219–224
26. Bohm D et al (2012) Comparison of tear protein levels in breast cancer patients and healthy controls using a de novo proteomic approach. Oncol Rep 28(2):429–438
27. Tost J, Gut IG (2005) Genotyping single nucleotide polymorphisms by MALDI mass spectrometry in clinical applications. Clin Biochem 38(4):335–350
28. Milan E et al (2012) SAA1 is over-expressed in plasma of non small cell lung cancer patients with poor outcome after treatment with epidermal growth factor receptor tyrosine-kinase inhibitors. J Proteomics 76:91–101
29. Akerley WL et al (2013) The impact of a serum based proteomic mass spectrometry test on treatment recommendations in advanced non-small-cell lung cancer. Curr Med Res Opin 29(5):517–525
30. Ramos Catharino R et al (2005) Aflatoxin screening by MALDI-TOF mass spectrometry. Anal Chem 77(24):8155–8157
31. McGrath SC et al (2011) Detection and quantification of ricin in beverages using isotope dilution tandem mass spectrometry. Anal Chem 83(8):2897–2905
32. Alam SI, Kumar B, Kamboj DV (2012) Multiplex detection of protein toxins using MALDI-TOF-TOF tandem mass spectrometry: application in unambiguous toxin detection from bioaerosol. Anal Chem 84(23):10500–10507
33. Pierce CY et al (2007) Strain and phase identification of the U.S. category B agent Coxiella burnetii by matrix assisted laser desorption/ionization time-of-flight mass spectrometry and multivariate pattern recognition. Anal Chim Acta 583(1):23–31
34. Jiang J et al (2007) An immunoaffinity tandem mass spectrometry (iMALDI) assay for detection of Francisella tularensis. Anal Chim Acta 605(1):70–79
35. Ayyadurai S et al (2010) Rapid identification and typing of Yersinia pestis and other Yersinia species by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. BMC Microbiol 10:285