

# MALDI-TOF Mass Spectrometry in Microbiology

Subjects: [Microbiology](#) | [Infectious Diseases](#)

Contributor: eva Torres Sangiao

Mass spectrometry (MS) is an analytical technique that has emerged as a powerful tool in clinical microbiology for identifying peptides and proteins, which makes it a promising tool for microbial identification. Matrix assisted laser desorption ionization–time of flight MS (MALDI-TOF MS) offers a cost- and time-effective alternative to conventional methods, such as bacterial culture and even 16S rRNA gene sequencing, for identifying viruses, bacteria and fungi and detecting virulence factors and mechanisms of resistance.

MALDI-TOF MS

microbial identification

proteomics

resistome

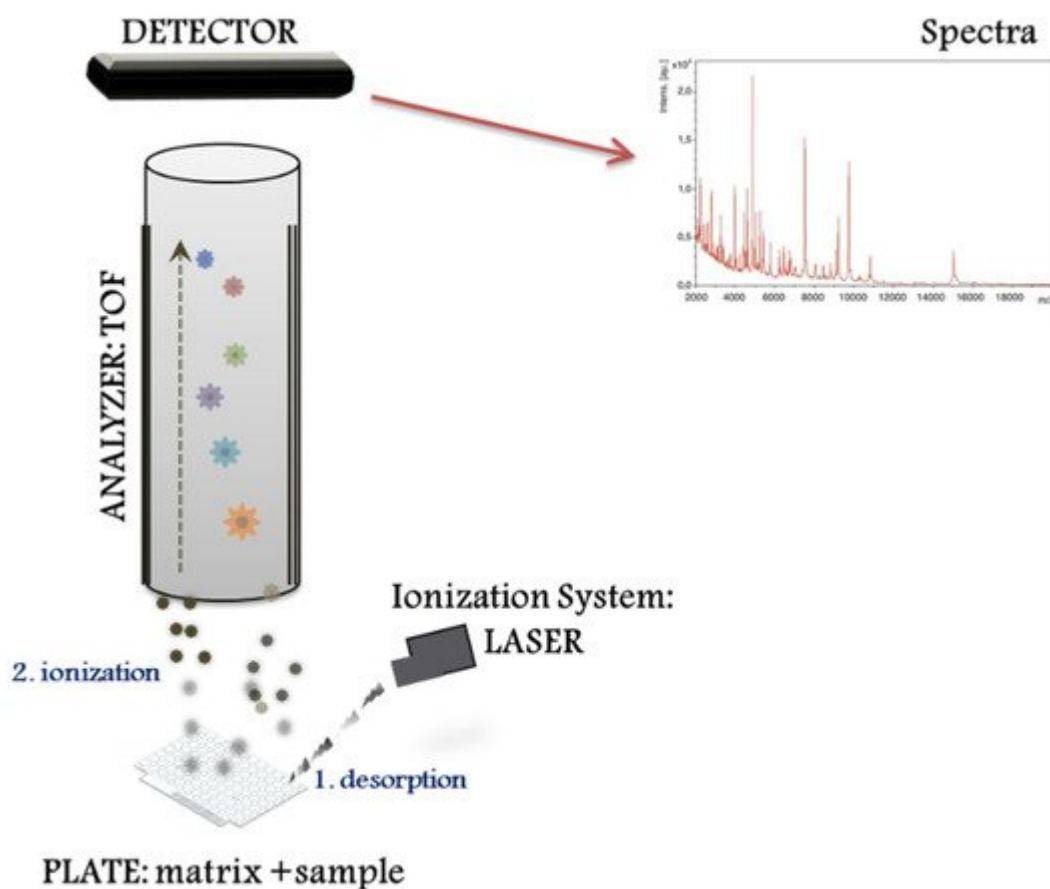
disease biomarkers

## 1. Background

Mass spectrometry (MS) was originally developed at the end of the 19th century to measure the masses of atoms, and one of its first contributions to science was demonstrating the existence of isotopes, at the beginning of the 20th century <sup>[1]</sup>. MS is an analytical approach that measures the mass-to-charge ratio ( $m/z$ ) of chemical compounds and calculates its exact molecular weight. Laser desorption/ionization (LDI), matrix-assisted laser desorption ionization (MALDI) or surface-enhanced laser desorption/ionization (SELDI) as well as electrospray ionization (ESI) are currently the most widely used ionization techniques for analyzing chemical structures in biological systems <sup>[2]</sup>. In the late 1980s, with the introduction of soft ionization, protein analysis developed rapidly, revolutionizing MS. In the late 1990s, the pioneering application of MS in microbiology <sup>[1]</sup> demonstrated that intact bacterial cells could be distinguished using MALDI coupled to a time of flight (TOF) analyser <sup>[3]</sup>. These achievements stimulated the fast development of MALDI-TOF MS system approaches as promising tools for the microbial characterization of bacteria <sup>[4]</sup>, fungi <sup>[5]</sup>, viruses <sup>[6]</sup>, and even nematodes <sup>[7]</sup>.

The MALDI-TOF MS system performs different proteomic strategies using intact or digested proteins. The “top down strategy” is used for direct analysis of intact proteins, proteoforms and post-translational protein modifications <sup>[8]</sup>, whereas the “bottom up” is used for mixtures of peptides derived from protein digestion (i.e., peptide sequencing). MALDI-TOF MS systems represent a basic configuration workflow in a linear mode. **Figure 1** presents a schematic overview divided into three compartments: (1) the ionization source system (laser), (2) the mass analyser (TOF), and (3) the ion detector. First, the sample for analysis is prepared by mixing with a matrix, an energy-absorbent, organic compound solution. Then, after the mixture (matrix and sample) crystallize upon drying, the sample is ionized using a laser beam. In the process of desorption and ionization using the ionization source system, the molecules are converted to gas-phase ions and individually charged  $[M+H]^+$ , so that they can be manipulated by external electric and magnetic fields. MALDI is based on a soft ionization method that preserves

the integrity of the sample without massive fragmentation [9]. Soft ionization allows the analysis of proteins and peptides and large organic molecules (i.e., polymers, dendrimers) [10], which tend to become brittle and fragmented in other ionization methods.



**Figure 1.** Schema showing the linear mode workflow in a MALDI-TOF MS system.

## 2. Direct Microbial Identification from Human Samples

MALDI-TOF MS has recently been used in laboratories for the rapid identification of microorganisms in emergency and inpatients, resulting in shorter hospital stays, particularly in intensive care units [11]. Sepsis is a life-threatening organ dysfunction caused by an unregulated host response to infection [12] and is the major cause of mortality from infectious disease, according to World Health Organization (<https://www.who.int/news-room/fact-sheets/detail/sepsis>, accessed on 26 August 2020).

Commercial protocols remain the reference procedures for the extraction of bacterial proteins from direct samples [10][13]. However, more and more modified and in-house methods have emerged to address several fundamental issues related to precision and speed of identification. As discussed in the previous section, sample preparation and an adequate method of protein extraction are key steps that may influence sensitivity, resolution, and reproducibility. Poor sample preparation will lead to lower peak resolution with a consequently lower sensitivity and reproducibility, since ion generation by MALDI-TOF depends on an optimal ratio of matrix substance and analyte

[10]. Therefore, it is of the utmost importance to pretreat a sample properly considering that human fluids contain proteins other than bacterial or fungal. These preliminary steps must drain and separate blood and other human cells (i.e., haemoglobin), and selectively recover bacterial proteins [14]; otherwise, a wrong or unknown identification will result.

### 3. Microbial Identification Using Reference Databases and Open Free Libraries

Microorganism databases are the key component for the identification in MALDI-TOF MS. Proprietary databases (i.e., Bruker) are continuously updated and increasing in size as new microbial species are discovered and new annotations are made. In recent years, open database platforms, spectrum libraries and computer tools have emerged and been made available to the scientific community. Most of them are used to handling long sequences of peptide data [15]. Although, the conventional clinical microbiologist would find working with proprietary programs already in the operating system to be more practical, comfortable and familiar, open-source software platforms, in combination with proprietary ones such as OpenMS [16], allows for additional MS-based workflows and user interfaces that must be considered for clinical diagnoses. The standardization of protocols, the increase in the resources of spectrum libraries and databases, as well as the development of more intuitive computer programs, is allowing the MALDI-TOF system to consolidate itself as the method of choice for microbiological diagnosis [15] (Table 1).

There are two main platforms for commercial use: (1) MALDI BioTyper and (2) VITEK<sup>®</sup> MS. Both provide a microbial diagnosis based on the detection and identification of low-molecular-weight spectra ranging from 2 to 20 KDa, typically represented by ribosomal proteins and a few housekeeping proteins [17]. They also show high levels of identification at the species level (S ~85%) [18][19] with MALDI BioTyper being better suited for bacteria and yeast [19] and VITEK<sup>®</sup> MS (v3.0) being better suited for mycobacteria, actinomycetes [19][20][21] and filamentous fungi [22]. The MALDI BioTyper and VITEK<sup>®</sup> MS platforms include software programs to explore and analyze the acquired mass spectra data. BioTyper and VITEK<sup>®</sup> platforms are continuously updating their databases with discoveries of new specimens and annotations. These computer programs allow comparison, analysis of spectra clusters for strain classification [23], dendrogram performance, multidimensional analysis [24] and resistance determination using hydrolysis ratios [25][26]. The main difference and limitation between the platforms is in access to other networks or databases, and the resources of their respective computer programs. Despite having private access, BioTyper allows mass spectra and new identifications to be shared among all users.

### 4. MS Big Data and Machine Learning Applied to Clinical Diagnosis

The increasing amounts of publicly available MS platforms in open access databases, mass spectrum libraries or analytical methods for visualization, standardization and validation [27][28] (Table 1), together with automated colony picking in the laboratory [14], are improving the typing or characterization of strains and identification of microbes.

**Table 1.** Overview of platforms for the analysis of big MS data of acquired mass spectra from MALDI-TOF MS. MDS: Multi-dimensional Scaling; PCA: principal component analysis; QA/QC: Quality Assurance/Quality Control; SOM: self-organization Map.

Platform	Statistical Analysis	Spectra Analysis	Accepted Formats	URL	Reference
BioNumerics	ANOVA, MANOVA, PCA, MDS, SOM and other statistical, parametric and non-parametric tests. Dendrograms, cluster analysis, biocustering, generation of phylogenetic trees. QA/QC.	Creation, identification and classification (spectrum libraries). Pre-processing: optimization, normalization, alignment, subtraction, smoothing. Peak detection, identification and quantification.	mzML, *.btmsp, *.txt, RAW	<a href="https://www.applied-maths.com/applications/maldi-tof-bacterial-identification">https://www.applied-maths.com/applications/maldi-tof-bacterial-identification</a> (accessed on 19 July 2021)	Bionumerics™ software (Applied Maths BVBA, Sint-Martens-Lantern, Belgium).
MaldiQUANT	Computational framework in R language: statistical analysis, dendrograms, clustering, probability distributions, quality control, etc.	Pre-processing: optimization, normalization, alignment, subtraction, smoothing. Peak detection, identification, and quantification.	mzML, mzXLM, imzML *.csv, *.fid, *.tab	<a href="http://strimmerlab.org/software/maldiquant/">http://strimmerlab.org/software/maldiquant/</a> (accessed on 19 July 2021)	Gibb S and Strimmer [29]
Mass-Up	PCA, classification analysis, biomarker discovery, clustering and biocustering. QA/QC.	Preprocessing: intensity transformation, optimization, alignment, subtraction, smoothing and peak analysis. Peak detection and identification.	mzML, mzXLM, *.csv, *.muc	<a href="http://www.sing-group.org/mass-up/">http://www.sing-group.org/mass-up/</a> (accessed on 19 July 2021)	López-Fernandez et al. [30]

Platform	Statistical Analysis	Spectra Analysis	Accepted Formats	URL	Reference
MATLAB	Regression, ANOVA, PCA, multivariate analysis, probability distributions, cluster analysis.	Pre-processing: optimization, smoothing, alignment, signal statistics, peak analysis, envelope extraction. Spectral analysis.	*.txt, *.xls, *.xlsx	<a href="http://es.mathworks.com/products/matlab-online/">http://es.mathworks.com/products/matlab-online/</a> (accessed on 19 July 2021)	MATLAB® software (MathWorks Inc., Natick, MA, USA)
PEAKS	Algorithms and support for analysis.	Pre-processing: optimization, normalization, alignment, subtraction, smoothing, peak analysis. Peak detection, identification and quantification. Sequence editor.	mzML, mzXLM, mzDATA, MGF, ASCII	<a href="http://www.bioinfor.com/">http://www.bioinfor.com/</a> (accessed on 19 July 2021)	Peaks® software (Bioinformatics Solutions Inc., Waterloo, ON, Canada)

RCM 2002, 16, 1352–1357.

6. Colquhoun, D.R.; Schwab, K.J.; Cole, R.N.; Halden, R.U. Detection of norovirus capsid protein in authentic standards and in stool extracts by matrix-assisted laser desorption ionization and nanospray mass spectrometry. *Appl. Environ. Microbiol.* 2006, 72, 2749–2755.

Other approaches are multidimensional analysis (MDS) or principal component analysis (PCA) (Table 1). MDS and PCA are mathematical approaches that use proximity measures such as the correlation coefficient or Euclidean distance to generate a spatial configuration of points in multidimensional space where distances between points reflect the similarity among isolates. MDS and PCA analyses have been extensively used to discover discriminative peaks [31] and identify potential sets of biomarkers [32,33] in a statistically reliable way [34], as well as distinguish among different strain isolates [33] from a large data set of selected genes or proteins [34][35].

9. Altun, O.; Botero-Kleiven, S.; Carlsson, S.; Ullberg, M.; Ozenci, V. Rapid identification of bacteria from positive blood culture bottles by MALDI-TOF MS following short-term incubation on solid media. *J. Med. Microbiol.* 2015, 64, 1346–1352.

5. The State-of-the-Art Combining Approaches

Figure 2 presents a standard MS workflow with the exact identification of proteins and their relative quantification that provides detailed knowledge of protein expression, microorganisms and parasites, and their final integration and interaction in the human body.

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

11. Ge, M.-C.; Kuo, A.-J.; Liu, K.-L.; Wen, Y.-H.; Chia, J.-H.; Chang, P.-Y.; Lee, M.-H.; Wu, T.-L.; Chang, S.-C.; Lu, J.-J. Routine identification of microorganisms by matrix-assisted laser

- desorption ionization time-of-flight mass spectrometry. Success rate, economic analysis, and clinical outcome. *J. Microbiol. Immunol. Infect.* 2017, 50, 662–668.
12. Singer, M.; Deutschman, C.S.; Seymour, C.W.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G.R.; Chiche, J.-D.; Coopersmith, C.M.; et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016, 315, 801–810.
13. Nomura, F.; Tsuchida, S.; Murata, S.; Satoh, M.; Matsushita, K. Mass spectrometry-based microbiological testing for blood stream infection. *Clin. Proteom.* 2020, 17, 14.
14. Tsuchida, S.; Umemura, H.; Nakayama, T. Current Status of Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in Clinical Diagnostic Microbiology. *Molecules* 2020, 25, 4775.
15. Singhal, N.; Kumar, M.; Kanauiya, P.K.; Viridi, J.S. MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. *Front. Microbiol.* 2015, 6, 791.
16. Röst, H.; Sachdev, T.; Aiche, S.; Bielow, C.; Weisser, H.; Aichele, F.; Andreotti, S.; Ehrlich, H.C.; Gutenbrunner, P.; Keny, E. et al. OpenMS: A flexible open source software platform for mass spectrometry data analysis. *Nat. Methods* 2016, 13, 741–748.
17. Croxatto, A.; Prod'homme, G.; Greub, G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiol. Rev.* 2012, 36, 380–407.
18. Jamal, W.Y.; Ahmad, S.K.; Khair, U.; Rotimi, V.O. Comparative evaluation of two matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems for the identification of clinically significant yeasts. *Int. J. Infect. Dis.* 2014, 26, 167–170.
19. Levesque, S.; Dufresne, P.J.; Soualhine, H.; Domingo, M.C.; Bekal, S.; Lefebvre, B.; Tremblay, C. A Side by Side Comparison of Bruker Biotyper and VITEK MS: Utility of MALDI-TOF MS Technology for Microorganism Identification in a Public Health Reference Laboratory. *PLoS ONE* 2015, 10, e0144878.
20. Wilson, C.B.; McMullen, A.R.; Burnham, G.A. Comparison of Sample Preparation Methods for MALDI-TOF MS. *J. Clin. Microbiol.* 2015, 53, 2308–2315.
21. Brown-Elliott, B.A.; Fritsche, T.R.; Olson, B.J.; Vasireddy, S.; Vasireddy, R.; Iakhiaeva, E.; Alame, D.; Wallace, R.J.; Branda, J.A. Comparison of Two Commercial Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) Systems for Identification of Nontuberculous Mycobacteria. *Am. J. Clin. Pathol.* 2019, 152, 527–536.
22. Sun, Y.; Guo, J.; Chen, R.; Hu, L.; Xia, Q.; Wu, W.; Wang, J.; Hu, F. Multicenter evaluation of three different MALDI-TOF MS systems for identification of clinically relevant filamentous fungi. *Med. Mycol.* 2020, 59, 81–86.

23. Garbino, C.; Sierra, A.; Dominguez, M.; Ferrer, N.; Navarro, M.; Vilagut, R.; et al. Automated control of categorization of methicillin-resistant *Staphylococcus aureus* clinical isolates into different clonal complexes by MALDI-TOF mass spectrometry. *Clin. Microbiol. Infect.* 2015, 22, 161.e1–161.e7.
24. Deol, P.; Girard, V.; Hyman, J.; Miller, E.; Dussoulier, R.; Mailler, S.; Schrenzel, J.; Beni, A.M.; Ninet-Bescher, B.; Walsh, J.; et al. Identification of Mycobacteria by VITEK® MS Matrix-Assisted Laser Desorption Ionization—Time of Flight Mass Spectrometry; BioMerieux, Ed.; BioMerieux: Marcy-l'Étoile, France, 2013; Volume 1, p. 32. Available online: <http://www.biomerieux-diagnostics.com/vitek-ms> (accessed on 19 July 2021).
25. Mirande, C.; Canard, I.; Perrot, N.; Welker, M.; Van Belkum, A.; Chatellier, S. Matrix-Assisted Laser Desorption Ionization—Time of Flight Mass Spectrometry for Rapid Antibiotic Resistance Detection; BioMerieux, Ed.; BioMerieux: Marcy-l'Étoile, France, 2013; Volume 1, p. 32. Available online: <http://www.biomerieux-diagnostics.com/vitek-ms> (accessed on 19 July 2021).
26. Angeletti, S.; Dicuonzo, G.; Lo Presti, A.; Cella, E.; Crea, F.; Avola, A.; Vitali, M.A.; Fagioni, M.; De Florio, L. MALDI-TOF mass spectrometry and *bla*KPC gene phylogenetic analysis of an outbreak of carbapenem-resistant *K. pneumoniae* strains. *New Microbiol.* 2015, 38, 541–550.
27. Perez-Riverol, Y.; Xu, Q.W.; Wang, R.; Uszkoreit, J.; Griss, J.; Sanchez, A.; Reisinger, F.; Csordas, A.; Ternent, T.; Del-Toro, N.; et al. PRIDE Inspector Toolsuite: Moving Toward a Universal Visualization Tool for Proteomics Data Standard Formats and Quality Assessment of ProteomeXchange Datasets. *Mol. Cell. Proteom. MCP* 2016, 15, 305–317.
28. Uszkoreit, J.; Plohnke, N.; Rexroth, S.; Marcus, K.; Eisenacher, M. The bacterial proteogenomic pipeline. *BMC Genom.* 2014, 15 (Suppl. 9), S19.
29. Gibb, S.; Strimmer, K. MALDIquant: A versatile R package for the analysis of mass spectrometry data. *Bioinformatics* 2012, 28, 2270–2271.
30. Lopez-Fernandez, H.; Santos, H.M.; Capelo, J.L.; Fdez-Riverola, F.; Glez-Pena, D.; Reboiro-Jato, M. Mass-Up: An all-in-one open software application for MALDI-TOF mass spectrometry knowledge discovery. *BMC Bioinform.* 2015, 16, 318.
31. Worley, B.; Powers, R. Multivariate Analysis in Metabolomics. *Curr. Metab.* 2013, 1, 92–107.
32. Mantini, D.; Petrucci, F.; Pieragostino, D.; Del Boccio, P.; Sacchetta, P.; Candiano, G.; Ghiggeri, G.M.; Lugaresi, A.; Federici, G.; Di Ilio, C.; et al. A computational platform for MALDI-TOF mass spectrometry data: Application to serum and plasma samples. *J. Proteom.* 2010, 73, 562–570.
33. Santos, T.; Capelo, J.L.; Santos, H.M.; Oliveira, I.; Marinho, C.; Goncalves, A.; Araujo, J.E.; Poeta, P.; Igrejas, G. Use of MALDI-TOF mass spectrometry fingerprinting to characterize *Enterococcus* spp. and *Escherichia coli* isolates. *J. Proteom.* 2015, 127, 321–331.
34. Landgrebe, J.; Wurst, W.; Welzl, G. Permutation-validated principal components analysis of microarray data. *Genome Biol.* 2002, 3, research0019.1.

35. Werth, M.T.; Halouska, S.; Shortridge, M.D.; Zhang, B.; Powers, R. Analysis of metabolomic PCA data using tree diagrams. *Anal. Biochem.* 2010, 399, 58–63.
36. Nachtigall, F.M.; Pereira, A.; Trofymchuk, O.S.; Santos, L.S. Detection of SARS-CoV-2 in nasal swabs using MALDI-MS. *Nat. Biotechnol.* 2020, 38, 1168–1173.
37. Montoro Bustos, A.R.; Ruiz Encinar, J.; Sanz-Medel, A. Mass spectrometry for the characterisation of nanoparticles. *Anal. Bioanal. Chem.* 2013, 405, 5637–5643.
38. Torres-Sangiao, E.; Holban, A.M.; Gestal, M.C. Advanced Nanobiomaterials: Vaccines, Diagnosis and Treatment of Infectious Diseases. *Molecules* 2016, 21, 867.
39. Torres Sangiao, E.; Holban, A.M.; Gestal, M.C. Applications of Nanodiamonds in the Detection and Therapy of Infectious Diseases. *Materials* 2019, 12, 1639.
40. Yang, Y.; Long, C.L.; Li, H.P.; Wang, Q.; Yang, Z.G. Analysis of silver and gold nanoparticles in environmental water using single particle-inductively coupled plasma-mass spectrometry. *Sci. Total Environ.* 2016, 563, 996–1007.
41. Baker, M.J.; Trevisan, J.; Bassan, P.; Bhargava, R.; Butler, H.J.; Dorling, K.M.; Fielden, P.R.; Fogarty, S.W.; Fullwood, N.J.; Heys, K.A.; et al. Using Fourier transform IR spectroscopy to analyze biological materials. *Nat. Protoc.* 2014, 9, 1771–1791.
42. Vatanashenassan, M.; Boekhout, T.; Mauder, N.; Robert, V.; Maier, T.; Meis, J.F.; Berman, J.; Then, E.; Kostrzewa, M.; Hagen, F. Evaluation of Microsatellite Typing, ITS Sequencing, AFLP Fingerprinting, MALDI-TOF MS, and Fourier-Transform Infrared Spectroscopy Analysis of *Candida auris*. *J. Fungi* 2020, 6, 146.
43. Naumann, D.; Helm, D.; Labischinski, H. Microbiological characterizations by FT-IR spectroscopy. *Nature* 1991, 351, 81–82.
44. Martak, D.; Valot, B.; Sauget, M.; Cholley, P.; Thouverez, M.; Bertrand, X.; Hocquet, D. Fourier-Transform InfraRed Spectroscopy Can Quickly Type Gram-Negative Bacilli Responsible for Hospital Outbreaks. *Front. Microbiol.* 2019, 10, 1440.
45. Burckhardt, I.; Sebastian, K.; Mauder, N.; Kostrzewa, M.; Burckhardt, F.; Zimmermann, S. Analysis of *Streptococcus pneumoniae* using Fourier-transformed infrared spectroscopy allows prediction of capsular serotype. *Eur. J. Clin. Microbiol. Infect. Dis.* 2019, 38, 1883–1890.
46. Cordovana, M.; Mauder, N.; Kostrzewa, M.; Wille, A.; Rojak, S.; Hagen, R.M.; Ambretti, S.; Pongolini, S.; Soliani, L.; Justesen, U.S.; et al. Classification of *Salmonella enterica* of the (Para-)Typhoid Fever Group by Fourier-Transform Infrared (FTIR) Spectroscopy. *Microorganisms* 2021, 9, 853.
47. Alexandrov, T. MALDI imaging mass spectrometry: Statistical data analysis and current computational challenges. *BMC Bioinform.* 2012, 13 (Suppl. 16), S11.

48. Caprioli, R.M.; Farmer, T.B.; Gile, J. Molecular imaging of biological samples: Localization of peptides and proteins using MALDI-TOF MS. *Anal. Chem.* 1997, 69, 4751–4760.
49. Attia, A.S.; Schroeder, K.A.; Seeley, E.H.; Wilson, K.J.; Hammer, N.D.; Colvin, D.C.; Manier, M.L.; Nicklay, J.J.; Rose, K.L.; Gore, J.C.; et al. Monitoring the inflammatory response to infection through the integration of MALDI IMS and MRI. *Cell Host Microbe* 2012, 11, 664–673.
50. Watrous, J.D.; Dorrestein, P.C. Imaging mass spectrometry in microbiology. *Nat. Rev. Microbiol.* 2011, 9, 683–694.
51. Chaurand, P.; Cornett, D.S.; Angel, P.M.; Caprioli, R.M. From whole-body sections down to cellular level, multiscale imaging of phospholipids by MALDI mass spectrometry. *Mol. Cell. Proteom. MCP* 2011, 10, S1–S11.

---

Retrieved from <https://encyclopedia.pub/entry/history/show/35204>