Published in final edited form as:

Proteomics Clin Appl. 2013 December ; 7(0): 733–738. doi:10.1002/prca.201300055.

Imaging Mass Spectrometry: A New Tool for Pathology in a Molecular Age

Jeremy L. Norris and Richard M. Caprioli*

National Research Resource for Imaging Mass Spectrometry, Mass Spectrometry Research Center, and Department of Biochemistry, Vanderbilt University School of Medicine, Medical Research Building III, 465 21st Avenue South Nashville, TN 37232-8575

Abstract

Mass spectrometry provides unique advantages for the analysis of clinical specimens, and these capabilities have been critical to the advancement of diagnostic medicine. To date, liquid chromatography mass spectrometry (LC-MS) is the platform most commonly used for diagnostics; however, LC-MS based proteomics is very labor intensive and costly to implement for high volume assays. Furthermore, when analyzing tissue samples, additional laborious sample preparation steps must be employed (*e.g.*, extraction methods or laser microdissection). The direct analysis of cells and tissues by matrix assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) has developed significant momentum for applications that have diagnostic potential. MALDI IMS provides molecular information from specific cell types within tissue sections; however, this laser-based approach significantly reduces the analysis time for each location sampled. This Viewpoint discusses the technologies for direct analysis of tissues, the potential for diagnostic applications using MALDI IMS, and the challenges faced in the transfer of the technology to the clinical laboratory.

The histopathological examination of tissue and cells provides clinically important and necessary taxonomic insights that directly impact patient care [1]. Indeed, in the absence of information regarding the pathogenesis of a particular disease, examination and characterization of altered tissue morphology is a serviceable substitute that allows pathologists to stratify patient specimens and ultimately provides diagnostic indices that deliver a consistent standard of care. Classically, pathology relies heavily on visual inspection of stained tissue sections to elucidate the disease state of a patient sample. Inherently these procedures possess a degree of subjectivity due to the reliance on the experience of individual pathologists [2]. Rigorous training in specific pathology subspecialties has been instituted to clarify the standard of care across the field and to provide, where possible, measurable criterion and proficiency testing programs that can be applied to standardize interpretation and avoid mistakes [3]. In spite of these rigorous standards, there are situations in which objectivity cannot be guaranteed and where significant discordance exists even among elite specialists. For example, a study of 340 patients who received a second opinion after a biopsy diagnosis of breast cancer found that, in 80% of cases, some change in pathology or prognostic factors was made by the second pathologist. In 7.8% of cases, surgical therapy was altered, and additional prognostic factors were identified in 40% of the cases [4]. These data underscore the issue that in a significant number of cases, current best practices fall short of delivering high quality, reliable diagnoses. In these situations, a more objective approach is needed to provide clarity to the physician and patient.

Of equal importance to the quality of care is the recognition that present histopathology techniques can be inadequate to provide answers to many important clinical questions in modern medicine [5]. Today, increased understanding of the molecular basis of disease is revolutionizing the practice and delivery of health care. Analytical measurement of molecular markers has been a cornerstone of diagnostic medicine for decades and technologies for clinical measurements have significantly improved, adding increased throughput and sensitivity. These capabilities allow for the further development and validation of molecular markers for clinical use, and provide important insights into the mechanism of disease that augment observations of tissue morphology.

Among the many advanced technologies used for clinical measurements, mass spectrometry (MS) plays an important role due to the unique advantages of high sensitivity, wide dynamic range, molecular specificity, and the flexibility to address many varied analytes on a single platform. The impact of MS on diagnostic medicine is undeniably significant. Testing for biomarkers of endocrine function and drugs of abuse in body fluids (*e.g.*, serum, plasma, urine, etc.) are among those most commonly employed in high volume clinical laboratories. Further, analytical technologies have matured to a degree that enables this molecular revolution to be translated to anatomic pathology. Technologies that combine the analytical capabilities of mass spectrometry with the benefits of microscopy to elucidate and understand the molecular events that are happening in specific cell types within a tissue will transition anatomic pathology into the next generation. These capabilities will enable the diagnosis and prognosis of diseases, along with further assessment of treatment modalities that cannot be realized using current techniques.

Most modern clinical laboratories have adopted liquid chromatography-mass spectrometry (LC-MS) for routine analysis of low molecular weight compounds, and these assays are performed in many laboratories up to tens of thousands of times daily. LC-MS applied to targeted molecular species is the strategy most widely adopted by clinical laboratories for carrying out routine testing. Despite increased adoption and investment by instrument manufacturers in these technologies, LC-MS largely remains the domain of clinical chemists and has not been of substantial benefit to resolve clinical questions relating to the expression and distribution of proteins in tissues. The analytical platforms used to carry out LC-MS analyses have been designed to prepare and analyze samples in solution, making it difficult to adapt to the analysis of tissue sections. This lack of crossover is due to two significant technical problems that needed to be solved, lack of adequate methods to analyze formalin-fixed paraffin embedded (FFPE) tissues and a lack of cell type specific sampling techniques that permit targeting of specific cells within a tissue section for analysis. Multiple methodologies for the MS analysis of FFPE sample have been developed that maintain the important spatial information that is necessary to relate the molecular measurements to specific populations of cells in the tissue. Figure 1 shows the two alternative approaches most commonly used to measure proteins from FFPE tissue specimens while maintaining spatial registration with the tissue morphology, 1) laser microdissection (LM) followed by LC-MS analysis and 2) direct analysis of tissues using matrix assisted laser-desorption/ionization imaging mass spectrometry (MALDI IMS). These techniques are termed histology-directed experiments because the tissue pathology is used to guide the mass spectrometry analysis.

LM is a technique that can be used to specifically sample cell types from tissues [6, 7]. This approach uses focused laser energy to target cells for removal from the tissue and collection in a manner that allows for analytical measurements to be performed. Laser microdissection has been successfully paired with a number of molecular techniques for research and diagnostic purposes, including genomics, transcriptomics, and proteomics [8, 9]. Although initially thought to be an unlikely source of proteomic information, methodologies have

been developed that allow quality proteome analysis of laser microdissected samples from FFPE specimens on a routine basis [10, 11]. One noted application demonstrates the diagnostic utility of coupling LM with LC-MS proteomic analysis, the typing of amyloid deposits [12, 13] and represents the first example of this technology put into clinical practice. Amyloidosis is a disorder in which proteins form deposits in tissues that disrupts normal tissue function. The protein that comprises the amyloid deposit can vary; currently 28 types of amyloidosis are known [13]. Initial determination of amyloid deposits in the affected tissue is performed using Congo Red staining, which is a general indicator of the presence of amyloid deposit, but this stain lacks the molecular specificity to determine the specific type. Treatment of the disease can vary greatly depending upon the specific protein that comprises the deposit; therefore, accurate typing now relies upon a method whereby the deposit is dissected from the tissue by LM, peptides are liberated using enzymatic digestion, and the proteins in the deposit are identified using LC-MS/MS [12]. Although some specimens could be accurately typed by homogenizing the entire sample, sampling of targeted regions of the tissue provides the advantage over the analysis of low density amyloid deposits because the analysis is only targeted for tissue regions that are histologically aberrant. This reduces the time spent characterizing tissue subtypes that are not of interest and that may preclude the measurement of disease proteins of interest. Although this example shows how cell type specific, histology-directed sampling followed by MS analysis provides valuable molecular information that can be used to diagnose disease, there are disadvantages to the particular approach described. The primary disadvantage is the lack of throughput of the approach. Under the best conditions, amyloid typing of a single specimen using LM and LC-MS/MS takes several hours per sample. Both the sampling approach and the management of the analytical platform require significant expertise and are time consuming relative to other routine clinical assays. These issues limit the application of this particular combination of technologies mainly to diseases and assays where the sample volumes and required turnaround times are not demanding.

Laser-based MS analysis methods, *e.g.*, MALDI, provide a substantial throughput advantage over chromatography-based approaches. The analysis of samples using MALDI IMS employs a laser to desorb and ionize the sample, reducing the analysis time to seconds rather than the minutes to hours required for many chromatography-based approaches. Although not used for the typing of amyloid described above, direct analysis of human cells by MALDI MS has been demonstrated [14].

MALDI MS based approaches to the diagnosis of disease have been the subject of uncertainty early on as a result of the discrediting of some high profile studies carried out using SELDI-MS [15, 16]. Unfortunately, the zeal to commercialize the approach before the technology was mature enough to deliver on the promise of the study and before adequate validation had been performed to confirm the accuracy of the test, severely hindered commercial development of diagnostic applications of MALDI MS. Recent years have seen a reemergence of the use of MALDI MS and an increase in the use of this technology for clinical applications.

The first publication describing MALDI IMS for the imaging of biological tissues appeared more than 15 years ago [17, 18], and the first clinical application of the technology that demonstrated diagnostic and prognostic potential was reported soon thereafter [19, 20]. Since that time, MALDI IMS has been used in numerous clinically important studies, including the study of cancer, neuroscience, and drug distribution and metabolism [21]. One recent notable application to cancer research is the determination of human epidermal growth factor receptor 2 (HER2) status in cancer tissue [22, 23]. HER2 expression characterizes a patient population with a high likelihood to respond to treatment with trastuzumab (Herceptin). HER2 expression analysis has been recommended to all patients

diagnosed with invasive breast cancer since 2006 [24]. The two tests for HER2 status, immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH), rely on a highly subjective manual interpretation step. By employing MALDI IMS, Walch and colleagues constructed a model for the classification of breast tumor HER2 status based upon a curated set of fresh frozen human breast tumors that could be used to classify a validation set of tumor specimens with a sensitivity of 83% and specificity of 92% [22]. Using the same training set and classification algorithm, HER2 status was also predicted in gastric tumors as well as breast cancer demonstrating a general molecular signature for breast and non-breast cancer HER2 status with a sensitivity of 78% and a specificity of 88% [23].

Another recent example demonstrating the diagnostic potential of MALDI IMS is in the study of skin cancer. Spitzoid neoplasms describe a spectrum of disease states that range from benign melanocytic lesions called Spitz Nevi (SN) to Spitzoid Malignant Melanoma (SMM). These two diseases have quite different prognoses, and the approach to treatment is also radically different given the aggressive nature of the malignant disease. Thus, accurate diagnosis is critical to guide the physician to make the most appropriate treatment decision. Even though SN and SMM are vastly different diseases, these disorders display conflicting histopathologies. It is estimated that as many as 25% of cases cannot be distinguished by routine histopathology [25]. Using histology-directed MALDI IMS, the molecular differences between these two disorders were examined [26]. A set of 114 samples, including 56 SN and 58 SMM randomized into either training or testing cohorts, were analyzed in this study. Serially collected hematoxylin and eosin (H&E) stained sections were examined by a dermatopathologist who then annotated areas that were to be analyzed using MALDI IMS. FFPE specimens were analyzed in this study, so each area of interest was trypsin digested and MALDI matrix was applied to prepare the sample for analysis. Statistical analysis of the resulting peptide data from each specimen was employed to construct a classification model using the training data set which was validated using the testing cohort. The final classification model consisted of 5 peptides that were differentially expressed. The model predicted SN correctly with 97% sensitivity and 90% specificity.

Another example of a clinical application for MALDI MS is the MALDI Biotyper, introduced by Bruker Daltonics, for the characterization of bacterial cultures by collecting molecular profiles and searching a prepopulated database of profile spectra to identify the unknown microbe down to the species level [27]. This technology has received the CE Mark and FDA approval for the device is being pursued in the U.S. [28]. Currently, there are hundreds of platforms installed in laboratories worldwide. Other manufacturers also offer commercial solutions, further validating the approach. The success for this application of MALDI MS is due to the performance advantages of the mass spectrometer, and the final solution provides the data to the clinician faster, more cost effectively, and more accurately than the classical microbiological approaches [29]. Importantly, this example provides validation for the use of multiple molecular markers to provide accurate classification of biological samples. The use of MALDI MS to classify microbes is similar to the use of MALDI IMS in histology-directed experiments to classify patient samples. The primary difference is the addition of the spatial information that is used to relate the molecular information to the tissue morphology.

In addition to the two applications of MALDI IMS just described and the use of MALDI MS to identify microorganisms, numerous other examples in the literature show that MALDI analysis of cells and tissues can be used to generate molecular data that has diagnostic and prognostic value [21]. Due to the advantages mentioned for MALDI analysis including speed and ease-of-use along with the increased availability of MALDI technology, there continues to be rapid growth in the use of MALDI for diagnostically relevant clinical research. Figure 2 shows the growth of publications that report the use of all of the

approaches mentioned for clinical applications. Since 2009, the diagnostic applications of MALDI (IMS and microbiology) continue to grow, now exceeding more than 100 publications annually. During the same time period, only modest growth in the use of LM to prepare clinical samples for MS analysis is shown. These data suggest increased adoption of MALDI IMS in clinical research which is a prerequisite to adoption in clinical diagnostics. This trend represents the beginning of the work that must be done to complete the transition of the technology to the clinic.

As with any technology, there are inherent limitations with current instrumentation that represent a challenge as the technology moves forward. Perhaps the primary challenge is to increase the overall coverage of biomolecules in tissues. Currently, many hundreds to several thousand molecules can be measured but this falls short of the tens of thousands of biomolecules typically present in tissues. A second issue involves the ionization efficiency and ionization suppression phenomena of MALDI, i.e., although the comparison of relative analyte intensities in like tissue specimens is a reasonable indicator of change for that analyte, the intensity of a signal of an analyte is not a reliable indication of its relative abundance with respect to other analytes. In short, peak intensities are not a reliable indicator of actual tissue abundance. Thirdly, the use of different matrix compounds can result in different mass spectra, obviating the need for carefully controlled sample preparation procedures. Lastly, further development of the sample preparation and analysis procedures must be performed to minimize the time and labor costs required to obtain clinically useful results. For example, the development of matrix pre-coated slides can significantly enhance both sample throughput and reproducibility of analysis [30, 31]. Although there are clearly examples where the technology could be diagnostically applied using existing methodologies, limited sample preparation throughput currently prohibits MALDI IMS from being effective for high volume assays requiring rapid turnaround. Nevertheless, with these cautions in mind, high quality reproducible mass spectra can be generated from tissues and these data can be effectively used for assessing molecular pathologies.

We believe that in order to move this technology toward clinical applications a coordinated collaboration between the clinician, analytical scientist and instrument manufacturers must be established. Each group has unique roles to play in ensuring that these methods are used responsibly to make clinical decisions. Until recently, the sole responsibility has been in the hands of the analytical scientist to develop the tools and methods that can be used to reproducibly analyze clinical specimens. Significant effort has been put forth to develop methods for quality sample preparation, reproducible analysis, and data processing and analysis methods that can provide reliable results. With mature methodologies that can be transferred to trained clinical laboratory personnel, clinicians can now consider the application of MALDI IMS to solve clinical problems. The examples previously presented not only show the capability of MALDI IMS; these applications also address clinical needs that have tangible economic benefit if a functional diagnostic can be developed. As the technology transfer proceeds from the research lab to clinical lab, the community must carefully select clinical problems that are suitable to be addressed by state-of-the-art MALDI IMS methods. This approach will provide the basis for pathologists to critically evaluate the technology for clinical applications, a prerequisite for widespread adoption of the technology. Many proof-of-principle studies have been published with the assistance of clinical research partners to give confidence that the molecular information that is generated by such methods provides value. The key next step is the realization of a validated clinical test that makes an impact on patient care. Adoption of MALDI IMS for even a select number of clinical problems and the successful implementation of the technology to deliver commercial tests will provide much needed market validation that will further promote application of the technology to other clinical problems. Reaching this milestone will

encourage instrument manufacturers to further invest in the MALDI IMS platform. The technology exists to create a MALDI platform that functions as efficiently as a clinical analyzer; however, instrument manufacturers are reluctant to invest until the clinical market is tested. However, this investment will benefit the clinician in several ways, providing optimized workflows that save time, money, and provide the ease of use that makes the platform feasible for installation in a clinical laboratory. Additionally, market demand will encourage competition in the tools space as demonstrated for the MALDI typing of microbes, further fueling innovative instrument development.

In conclusion, it has been shown that histology-directed mass spectrometry measurements provide unique insight into clinical questions for which the tools available to pathologists today cannot currently provide adequate answers. Histology images routinely used by the pathologist today to diagnose disease are not underpinned with enough specific molecular information to provide the next generation of diagnostic tests that will extend and improve the quality of life. Although there may be a number of technologies that are ultimately used to deliver molecular information to pathology practice, MALDI IMS is poised to revolutionize the practice of anatomic pathology in the coming decade.

Acknowledgments

Preparation of this article was supported by grants from the National Center for Research Resources (5 P41 RR031461) and the National Institute of General Medical Sciences (8 P41 GM103391) from the National Institutes of Health.

References

1. Cotran, RS.; Kumar, V.; Collins, T. Robbins pathologic basis of disease. 6th ed.. Philadelphia: W.B. Saunders Co.; 1999.
2. Rizzardi AE, Johnson AT, Vogel RI, Pambuccian SE, et al. Quantitative comparison of immunohistochemical staining measured by digital image analysis versus pathologist visual scoring. *Diagn. Pathol.* 2012; 7:42. [PubMed: 22515559]
3. College of American Pathologists. Northfield, IL: 2013. <http://www.cap.org/>
4. Staradub VL, Messenger KA, Hao N, Wiley EL, Morrow M. Changes in breast cancer therapy because of pathology second opinions. *Ann. Surg. Oncol.* 2002; 9:982–987. [PubMed: 12464590]
5. Hillan KJ, Quirke P. Preface to genomic pathology—a new frontier. *J. Pathol.* 2001; 195:1–2. [PubMed: 11568885]
6. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, et al. Laser capture microdissection. *Science.* 1996; 274:998–1001. [PubMed: 8875945]
7. Espina V, Heiby M, Pierobon M, Liotta LA. Laser capture microdissection technology. *Expert Rev. Mol. Diagn.* 2007; 7:647–657. [PubMed: 17892370]
8. Cheng L, Zhang S, MacLennan GT, Williamson SR, et al. Laser-assisted microdissection in translational research: theory, technical considerations, and future applications. *Appl. Immunohistochem. Mol. Morphol.* 2013; 21:31–47. [PubMed: 22495368]
9. Cheng, L.; Zhang, S.; Davidson, DD.; Kuhar, M., et al. Molecular Genetic Pathology. Cheng, L.; Zhang, D.; Eble, J., editors. Berlin: Springer-Verlag; 2013. p. 466–482.
10. Hood BL, Darfler MM, Guiel TG, Furusato B, et al. Proteomic analysis of formalin-fixed prostate cancer tissue. *Mol. Cell. Proteomics.* 2005; 4:1741–1753. [PubMed: 16091476]
11. Hood BL, Conrads TP, Veenstra TD. Mass spectrometric analysis of formalin-fixed paraffin-embedded tissue: unlocking the proteome within. *Proteomics.* 2006; 6:4106–4114. [PubMed: 16800036]
12. Vrana JA, Gamez JD, Madden BJ, Theis JD, et al. Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. *Blood.* 2009; 114:4957–4959. [PubMed: 19797517]

13. Leung N, Nasr SH, Sethi S. How I treat amyloidosis: the importance of accurate diagnosis and amyloid typing. *Blood*. 2012; 120:3206–3213. [PubMed: 22948045]
14. Xu BJ, Caprioli RM, Sanders ME, Jensen RA. Direct analysis of laser capture microdissected cells by MALDI mass spectrometry. *J. Am. Soc. Mass Spectrom.* 2002; 13:1292–1297. [PubMed: 12443019]
15. Check E. Proteomics and cancer: running before we can walk? *Nature*. 2004; 429:496–497. [PubMed: 15175721]
16. Ransohoff DF. Lessons from controversy: ovarian cancer screening and serum proteomics. *J. Natl. Cancer Inst.* 2005; 97:315–319. [PubMed: 15713968]
17. Caprioli RM, Farmer TB, Gile J. Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS. *Anal. Chem.* 1997; 69:4751–4760. [PubMed: 9406525]
18. Stoeckli M, Chaurand P, Hallahan DE, Caprioli RM. Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues. *Nat. Med.* 2001; 7:493–496. [PubMed: 11283679]
19. Chaurand P, Schwartz SA, Caprioli RM. Assessing protein patterns in disease using imaging mass spectrometry. *J. Proteome Res.* 2004; 3:245–252. [PubMed: 15113100]
20. Schwartz SA, Weil RJ, Johnson MD, Toms SA, Caprioli RM. Protein profiling in brain tumors using mass spectrometry: feasibility of a new technique for the analysis of protein expression. *Clin. Cancer Res.* 2004; 10:981–987. [PubMed: 14871976]
21. Norris JL, Caprioli RM. Analysis of tissue specimens by matrix-assisted laser desorption/ionization imaging mass spectrometry in biological and clinical research. *Chem. Rev.* 2013; 113:2309–2342. [PubMed: 23394164]
22. Rauser S, Marquardt C, Balluff B, Deininger S-O, et al. Classification of HER2 Receptor Status in Breast Cancer Tissues by MALDI Imaging Mass Spectrometry. *J. Proteome Res.* 2010; 9:1854–1863. [PubMed: 20170166]
23. Balluff B, Elsner M, Kowarsch A, Rauser S, et al. Classification of HER2/neu Status in Gastric Cancer Using a Breast-Cancer Derived Proteome Classifier. *J. Proteome Res.* 2010; 9:6317–6322. [PubMed: 21058730]
24. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J. Clin. Oncol.* 2007; 25:118–145. [PubMed: 17159189]
25. Gerami P, Jewell SS, Morrison LE, Blondin B, et al. Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma. *Am. J. Surg. Pathol.* 2009; 33:1146–1156. [PubMed: 19561450]
26. Lazova R, Seeley EH, Keenan M, Gueorguieva R, Caprioli RM. Imaging mass spectrometry--a new and promising method to differentiate Spitz nevi from Spitzoid malignant melanomas. *Am. J. Dermatopathol.* 2012; 34:82–90. [PubMed: 22197864]
27. Bessede E, Angla-Gre M, Delagarde Y, Sep Hieng S, et al. Matrix-assisted laser-desorption/ionization biotyper: experience in the routine of a University hospital. *Clin. Microbiol. Infect.* 2011; 17:533–538. [PubMed: 20518792]
28. Check W. Infectious disease meets mass spectrometry. *CAP Today*. 2011; 25:1.
29. Dhiman N, Hall L, Wohlfiel SL, Buckwalter SP, Wengenack NL. Performance and cost analysis of matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine identification of yeast. *J. Clin. Microbiol.* 2011; 49:1614–1616. [PubMed: 21270234]
30. Yang J, Caprioli RM. Matrix precoated targets for direct lipid analysis and imaging of tissue. *Anal. Chem.* 2013; 85:2907–2912. [PubMed: 23418860]
31. Grove KJ, Frappier SL, Caprioli RM. Matrix pre-coated MALDI MS targets for small molecule imaging in tissues. *J. Am. Soc. Mass. Spectrom.* 2011; 22:192–195. [PubMed: 21472558]

Figure 1. Histology-directed analysis of tissue sections using mass spectrometry is primarily accomplished using one of two approaches, direct analysis of the tissue using MALDI IMS (top) and MS analysis of a laser microdissected sample using LC-MS (bottom).

Figure 2. Publications reporting the application of three different emerging mass spectrometry approaches to the diagnosis of disease. Data was collected from PubMed searches of the following key words: MALDI IMS (maldi AND imaging mass spectrometry AND diagnosis); MALDI Microbiology (maldi biotyper OR maldi AND microorganism identification); LM / MS (laser microdissection AND mass spectrometry AND diagnosis). The solid bar results for 2013 represent the first 6 months of 2013, with the dotted bar representing a linear projection for the remaining months.