MALDI imaging mass spectrometry: molecular snapshots of biochemical systems

Dale S Cornett, Michelle L Reyzer, Pierre Chaurand & Richard M Caprioli

Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) is emerging as a powerful tool for investigating the distribution of molecules within biological systems through the direct analysis of thin tissue sections. Unique among imaging methods, MALDI-IMS can determine the distribution of hundreds of unknown compounds in a single measurement. We discuss the current state of the art of MALDI-IMS along with some recent applications and technological developments that illustrate not only its current capabilities but also the future potential of the technique to provide a better understanding of the underlying molecular mechanisms of biological processes.

Understanding the complex biochemical processes that occur within living organisms requires not only the elucidation of the molecular entities involved in these processes, but also their spatial distribution within the organism at any point in its developmental cycle. It follows then that analysis tools should provide as much information as possible about a sample, including the identity of all species present and how they are distributed. The complexity of this task is immense, but it is made possible by innovative molecular analytical technologies of high sensitivity that allow for the identification of the biological molecules and their distribution in the organism. Chemical stains, immunohistochemical tags and radiolabels are common methods for visualizing and identifying molecular targets. However, there are limits to the specificity of these methods and to the number of target compounds which can be monitored simultaneously. Moreover, use of specific molecular tags requires a priori knowledge of the target species, thereby limiting their utility for molecular discovery.

Imaging mass spectrometry (IMS) is an emerging technology that combines the multichannel (m/z) measurement capability of mass spectrometers with a surface sampling process that allows one to rapidly probe and map the proteomic and small molecule content of tissues. Each ion measured is thus registered to a specific location in the sample, which facilitates rendering of the data into spatial distribution maps, or images, for the many hundreds of ions measured in the mass spectra. The images are, therefore, independent measures of the distribution of the respective compounds. The concept of using mass spectrometers to produce molecular images goes back several decades. However, a broad review of this field is beyond the scope of the current article (for a brief description of alternative technologies for IMS, see **Box 1**).

We focus here on the use of MALDI mass spectrometry for imaging, a technology that has broad applicability to biological research. A unique feature of this technology is its ability to analyze complex mixtures ranging from small drug compounds to very large proteins. Early on, molecular analysis using MALDI mass spectrometry was employed in studies of intact cells[1–3] to produce characteristic molecular fingerprints. Later, the first reports of MALDI-IMS showed that spatial distributions of molecules could be rendered from a two-dimensional array of MALDI mass spectra acquired directly from thin sections of tissue[4,5]. By plotting ion intensity as a function of x and y coordinates on the section, unique distribution maps can be produced for each of the many hundreds of ions produced from the section. Key advantages of this technology are that it does not require molecule-specific tags or chemical modifiers to facilitate detection and does not rely on any prior knowledge of the tissue proteome. This allows the discovery of protein markers that are either highly localized within specific tissue structures or expressed for only a brief time during the stages of development.

Interest in MALDI-IMS has grown rapidly, enhanced by the recent introduction of commercial devices for sample preparation and data acquisition and analysis. With MALDI-IMS instrumentation more readily available, studies are being published at an increasing rate. Much of the interest in MALDI-IMS has been for exploring disease pathology[6–8] and for pharmaceutical

The authors are at the Mass Spectrometry Research Center, 465 21st Avenue, Vanderbilt University Medical Center, Nashville, Tennessee 37232, USA.
email: r.caprioli@vanderbilt.edu

PUBLISHED ONLINE 27 SEPTEMBER 2007; DOI:10.1038/NMETH1094

BOX 1 IMAGING BY MASS SPECTROMETRY

Instruments used for imaging mass spectrometry can be classified according to how ions are generated from the sample: either by irradiation by pulsed laser or bombardment by energetic particles. Laser based systems include MALDI and laser desorption ionization (LDI) instruments. Secondary ion mass spectrometry (SIMS) systems use particle bombardment with a continuous beam of highly-focused, energetic ions such as Cs^+, Au_3^+ and C_{60}^+. Each approach has advantages and disadvantages depending on the particular application. Major analytical differences: SIMS primary beams can yield images with a maximum spatial resolution on the order of 100 nm, but the energetic particles, which have kinetic energies on the order of several keV, cause fragmentation of most molecular species larger than ~1,000 Da. Laser-based systems are capable of achieving routine resolution of about 20 μm, but using MALDI, the measurable *m/z* range can be well over 100,000. Several published articles discuss and compare MALDI and SIMS imaging[46,47].

applications[9–12]. The technique continues to advance these fields, but new applications are expanding the technology into fields such as developmental and systems biology. We discuss methodological developments and applications of MALDI-IMS that have emerged during the past two years and provide a perspective for coming years.

METHODOLOGY

MALDI-IMS uses the basic matrix-assisted laser desorption/ionization process for the analysis of spatially discrete areas of a sample. Tissue samples are typically thin sections, 5–20 μm thick, that have been spray-coated or microspotted with MALDI matrix. Once the sample has been placed inside the mass spectrometer, spectra are acquired at discrete locations about the sample surface according to a predefined rectangular *x*,*y* grid. The resulting dataset therefore contains an array of spectra in which each spectrum is an independent molecular profile of the area irradiated by the laser. Individual grid-cells can be thought of as 'pixels', each composed of a spectrum of thousands of signals of measured mass and intensity. The intensity of any given signal, or combinations of signals, can then be plotted on the original coordinate system, generating an image of that signal over the sample surface. Thus, in a relatively short time, a MALDI-IMS analysis yields hundreds of such relative distribution maps. The overall process is illustrated in **Figure 1**.

Sample handling and preparation of sections for image analysis are critical to the spatial integrity of measured molecular distributions. Obviously, any molecular degradation that occurs in the time between sample collection and analysis can adversely affect the results. A typical study may involve samples collected over a lengthy period of time, and standardized procedures are therefore required to minimize experimental variability over the time course of the study. Our experience shows the need for good communication among all personnel involved with collecting, storing and analyzing samples. Ideally, samples are frozen immediately after collection and stored until sections for MALDI-IMS analysis are cut on a cryomicrotome just before analysis. Once sections are cut, they are transferred to the MALDI plate and affixed through thaw-mounting or other mechanical means such as double-sided tape. Sucrose and polymer-based embedding media have been found to inhibit ion production if these are transferred to the surface of the section during the cutting process, and their use is discouraged[8]. Chemical fixation procedures such as formalin fixation that act by crosslinking proteins can inhibit molecular analysis. However, the many hundreds of thousands of chemically fixed samples banked in various institutions around the world constitute a potentially rich cohort for study, and early efforts to develop methods for analyzing these have shown promise[13].

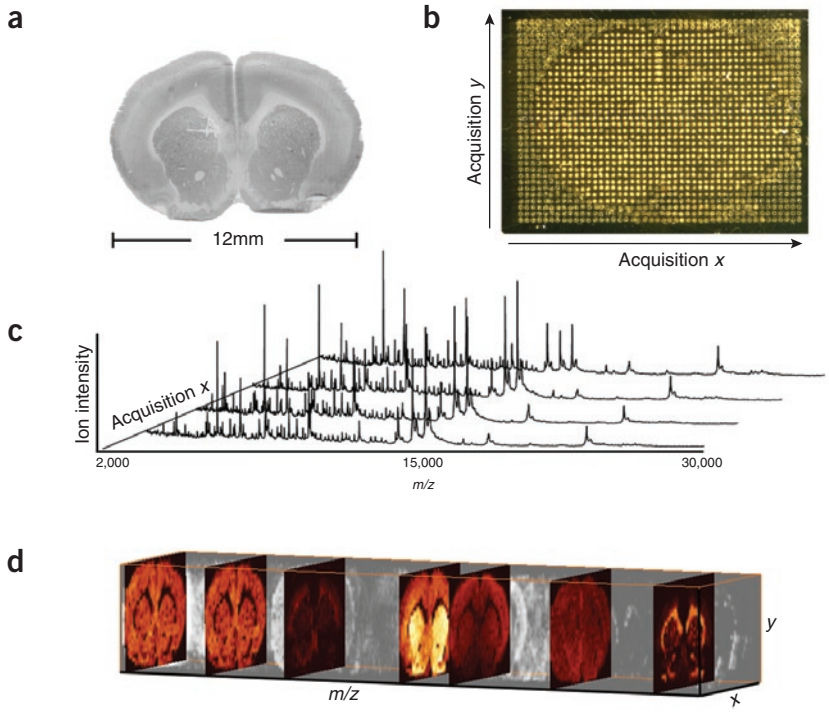

Figure 1 | General overview of MALDI-IMS. (**a**) Fresh section cut from sample tissue (shown here, mouse brain). (**b**) Mounted section after matrix application using a robotic picoliter volume spotter. (**c**) Partial series of mass spectra collected along one row of coordinates (*x* axis). (**d**) Three-dimensional volumetric plot of complete dataset with selected *m/z* 'slices' or ion images. Principal axes are *x*, *y* and *m/z*. Color of each voxel is determined by ion intensity.

The application of MALDI matrix to mounted sections is a critical determinant of spatial resolution as well as the number of unique ions detected. The most common and least expensive devices available for applying matrix are hand-held aerosol sprayers or airbrushes. The chief advantage of these devices is that, with careful application, a dispersion of very small droplets of matrix solution can be delivered onto the sample surface to form a homogeneous

layer of crystals that are typically no larger than the diameter of the focused laser of the mass spectrometer (usually 50–100 μm). As a result, the diameter of the laser beam determines the smallest surface area or pixel size that is represented by a single mass spectrum. However, overspraying the matrix increases the likelihood of analyte migrating away from the location from which it was extracted, whereas underspraying delivers to the surface a limited amount of solvent for extracting analyte. Automated sprayers minimize much of the operator variability, and several such devices are now available commercially. Early results from these devices are promising, but application of matrix to areas larger than the diameter of the aerosol requires repeated overlapping passes, which in turn may cause some artificial variations in ion intensity. These can, however, be partially corrected through data processing[14].

An alternative to aerosols is the delivery of matrix solution to the sample in the form of very small droplets from precision ejectors. Two different types of ejectors have been incorporated into such devices: inkjet-style piezo nozzles[15] and focused acoustic dispensers[16]. Both designs operate by drop-on-demand, producing highly reproducible drops of approximately 100 pl that dry to form crystalline matrix spots of ~150 μm diameter. Aerosol-generated droplets can be smaller in size, but spotting devices offer advantages of droplet uniformity and precision of placement, both of which translate into greater control over protein extraction. However, because spotted matrix is generally larger than the focused laser spot, it is the size of the droplet, or more accurately the surface area wetted by the droplet, that determines the maximum achievable spatial resolution. The typical operating mode is to 'print' an array of discrete microspots over the area to be imaged. The pitch of the array of matrix spots, which is typically 150–300 μm, is approximately 2–5 times lower than the maximum image resolution achieved with aerosols. This is offset somewhat by the fact that any lateral diffusion of analyte molecules is confined to the area covered by the droplet. In addition to controlling analyte extraction, the ability to deposit precisely onto the same location multiple times makes it possible to explore the products of chemical or enzymatic reactions *in situ* simply by changing the solution being spotted.

Almost any modern MALDI mass spectrometer can be configured for IMS analyses. Owing to their widespread availability, high sensitivity and unlimited *m*/*z* range, time-of-flight (TOF) mass analyzers are commonly used. MALDI-TOF instruments incorporating fast (>200 Hz) lasers permit data acquisition rates on the order of 1–2 spectra per second with the sensitivity to detect hundreds of proteins from an area containing only a few cells. However, for image analysis of small molecules there can be considerable interference from abundant matrix-related ions, particularly when both the target species and the interfering compound have the same integral molecular weight. These cases require an alternative approach such as the use of tandem mass spectrometry (MS/MS) and selected reaction monitoring (SRM), in which the molecular ion of the analyte is fragmented, and images are generated from one or more analyte-specific fragment ions. This approach has been extensively used for the analysis of drugs and their metabolites[9,12,17–20], as well as for endogenous lipid species[21,22]. Tandem mass spectrometry eliminates much of the chemical background that is present in the low molecular weight range.

A number of laboratories are pursuing the development of new instrumentation, seeking to improve the spatial resolution, molecular sensitivity and speed of the analysis. Scanning laser microprobe instruments with advanced laser optics offer high-resolution image analysis of tissue samples with pixel dimensions on the order of 10 μm (ref. 23,24). A microscope-mode instrument has been reported that maintains the spatial relationship of ions desorbed from within the area irradiated by the laser. This instrument is capable of resolving spatial features within the area irradiated by a laser beam, focused to a relatively large diameter of ~200 μm, to produce an effective spatial resolution of 4 μm (ref. 25). The high mass-resolving power of Fourier transform mass spectrometers allows imaging of isobaric ions[26,27]. Instruments like these offer some unique advantages, but they are still early in development. Presently, the widespread availability of TOF, quadrupole-TOF and ion trap systems make these the instruments of choice.

APPLICATIONS

MALDI-IMS applications are typically classified into two broad categories according to the analyte: proteins and small molecules.

Proteins

A majority of MALDI-IMS studies have been dedicated to the study of proteins contained in animal tissue sections. Applications in the field of pathology hold particular interest for many because of the

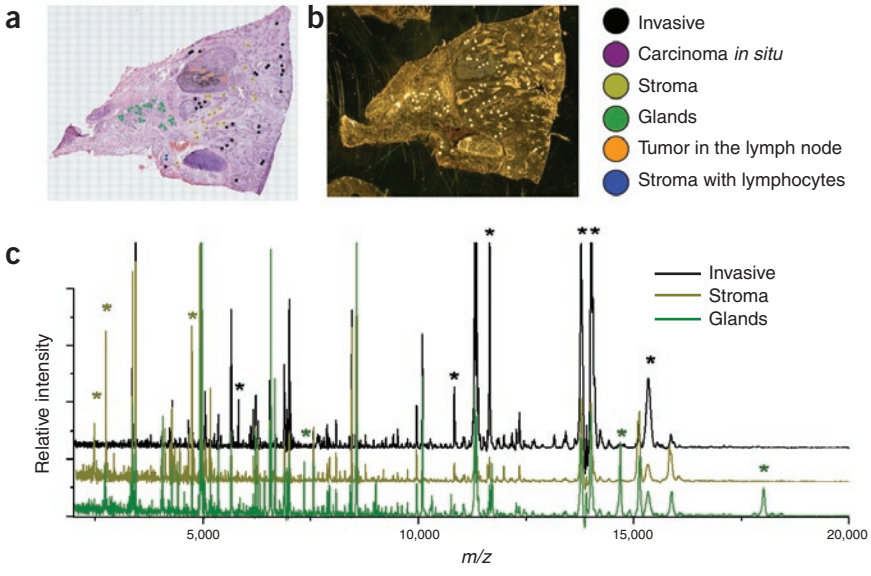

Figure 2 | Workflow for histology-directed profiling. Two adjacent sections of the sample tissue are required, one for staining and one for MALDI analysis. (**a**) A pathologist selects cells of interest using the histology image. (As shown here, the cells are colored according to type.) (**b**) The locations of the selected cells are registered to the matrix spotter that holds the section for MALDI analysis. After spotting with matrix, the coordinates of the matrix spots are registered to the mass spectrometer and spectra are automatically acquired from each location. (**c**) Spectra demonstrating the cell-specific differences (peaks marked with asterisks) that can be detected.

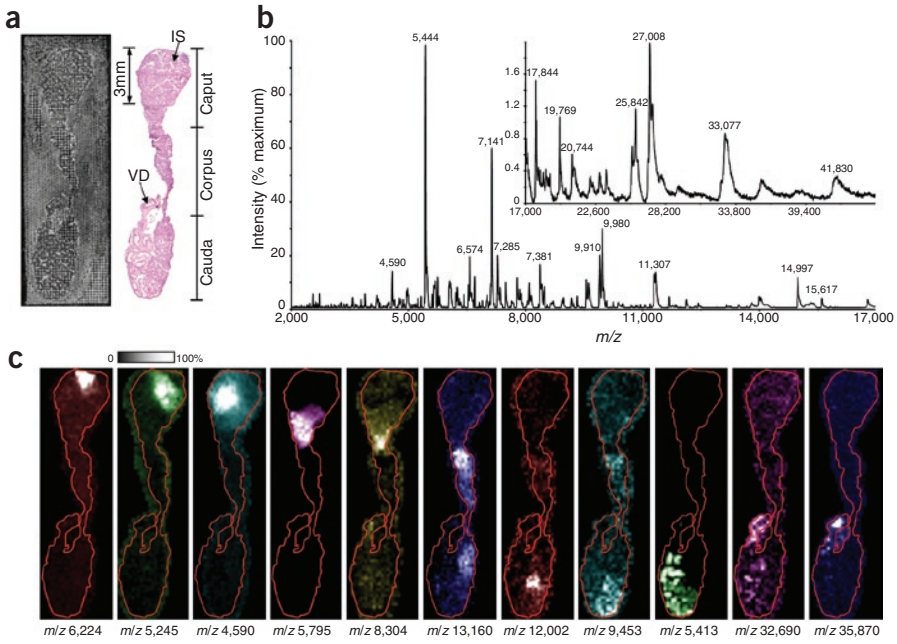

Figure 3 | MALDI-IMS analysis of 12-µm section of mouse epididymis. (**a**) Photomicrographs of the matrix-coated section and hematoxylin and eosin stained section after matrix removal. IS, initial segment; VD, vas deferens. (**b**) Average mass spectrum computed from all pixels. (**c**) False-color ion images of selected protein signals localized to distinct regions along the length of the organ. Ion images are shown with different colors according to *m/z*, but all are plotted with a common luminance.

potential benefit for clinical diagnoses and treatment. Other studies have focused on proteomic events occurring in normally developing tissues; some recent examples include the direct imaging of secretory peptides from frog skin[28] and bovine ocular lens proteins[29], as well as neuropeptides from rat pituitary[25], decapod crustacean neuronal tissue[26] and house cricket[30]. Below we describe several examples of applications of MALDI-IMS.

Pathology. Determining molecular changes associated with disease progression continues to be a main focus of MALDI-IMS studies[7,9,10,31–33]. The general approach taken by these studies is comparative proteomic analyses whereby mass spectral features (*m/z* peaks) are correlated with a variety of patient information, such as therapeutic regimen and overall outcome. Images can be generated from individual features or a combination of features that represent a signature for a particular aspect of the study.

In some cases, the goal of the experiment is to identify specific molecular changes associated with progression of disease. For these studies it is not necessary to acquire MALDI-IMS spectra from the entire section of tissue. Rather, spectra are acquired at discrete locations within the section that contain histological features of interest. The molecular signatures are then analyzed for feature differences specific to the types of cell being analyzed. For example, molecular signatures from glioma biopsies can be used to distinguish tumor grade and were found to be correlated with patient survival[32].

Identification of specific cellular changes within heterogeneous tissue can be particularly challenging from the perspective of matrix spot size and placement. As the mass spectrum contains ions from proteins extracted from all cells wetted by the droplet of matrix solution, it is essential that matrix be placed as precisely as possible onto only those cells of interest. Histopathology results can be used to direct the automated deposition of matrix[31]. The workflow, illustrated in **Figure 2**, is demonstrated for a lung tumor biopsy.

Developmental biology. Proteomic studies using MALDI-IMS can yield insight into some extremely complex biological processes. One example is the maturation of spermatozoa as they move through the epididymis (**Fig. 3**), interacting with protein-rich epididymal fluid to complete differentiation begun in the testis[34]. The epididymis is a long tubular organ composed of four anatomical regions: initial segment, caput, corpus and cauda. As spermatozoon DNA is transcriptionally inactive, the development of the capability for fertilization depends on sequential interactions between spermatozoa and proteins encountered during transit through the organ. MALDI-IMS analyses of epididymal sections have been used to determine the localized composition of the epididymal fluid from caput to cauda[35], confirming that different protein compositions are found along the length of the epididymis (**Fig. 3**).

In another study MALDI-IMS was used to probe proteomic changes in mouse uterine tissue surrounding a blastocyst from the time of implantation through early development (K.E. Burnum and R.M.C., unpublished data). One particularly challenging aspect of this study was the relatively small size (<3 mm diameter) of the uterine cross-section, with the developing embryo being approximately 300 µm long. The tissue is extremely delicate, and image analysis required high spatial resolution and precise positioning of the matrix.

***In situ* protein chemistry.** Sample pretreatment protocols using various enzymes and molecular tags can increase the amount of protein information obtained from MALDI-IMS analysis. Proteins can be hydrolyzed *in situ*, with the resulting peptides, rather than the proteins, analyzed by MALDI-IMS. Several reports have demonstrated image analysis of tissue sections treated with the enzyme trypsin[15,36]. MS/MS analysis of the tryptic fragments followed by database searching was used to identify many proteins. One of the potential benefits of such an approach is the ability to localize proteins that are not readily detected by a direct MALDI-MS analysis, such as high-molecular-weight or membrane-bound or membrane-associated proteins. Photocleavable molecular tags to enhance the specificity of MALDI-IMS analyses have also been designed[37,38]. In this strategy the sample section is incubated with the tagging reagent and image analysis is performed on the low-molecular-weight tag compound instead of the target molecule.

Small-molecule imaging

Many compounds that are biologically or pharmacologically relevant are less than 1 kDa in size and thus fall into the broad category of small molecules. These include both exogenous and endogenous

molecules, such as pharmaceutical compounds and their metabolites, drugs of abuse, environmental toxins, endogenous metabolites and lipids. Below we summarize the application of MALDI-IMS for the study of drug metabolism and phospholipid distribution.

Drug metabolism. Initial applications of small-molecule MALDI-IMS focused on pharmaceutically active compounds and their metabolites[12,19], and considerable effort continues in this field[17]. For example, one study reported the localization of the drug olanzapine and two first-pass metabolites in whole-rat sections[12]. The results of this study correlated well with both autoradiography and LC-MS/MS quantitative results, illustrating the power of MALDI-IMS for metabolite profiling. More recently, there has been considerable interest in adding quantitative capabilities to the MALDI-IMS experiment[39,40]. The technology also opens up the exciting possibility of correlating drug distribution with concomitant protein changes[41].

Phospholipids. Phospholipids are involved in many essential biological functions, including cell signaling, energy storage, and membrane structure and function, and defects in lipid metabolism have a role in many diseases. Analysis of lipids is challenging because of the wide molecular diversity of this class of compounds and their relative insolubility in aqueous systems. Using multiple stages of mass spectrometry[21,42], ion species may be unambiguously classified depending on how the lipid ion initially fragments. The use of ion mobility has also been reported in conjunction with MALDI MS as a means to fractionate lipids[43–45]. In this technique, ions are first separated by ion mobility and then analyzed by TOF mass spectrometry. The desorbed lipid ions fall on a trend line (a function of charge and collision cross-section) that is separate from those of oligonucleosides, peptides, proteins, and drugs and metabolites having the same nominal mass. This allows ions originating from lipids to be distinguished from other small molecules.

OUTLOOK

The correlation of molecular information to anatomical structures in tissues offers tremendous potential for a better understanding of biological processes. Using current technology the distribution of hundreds of proteins can be determined to spatial dimensions on the order of 50 μm. Molecular sensitivity depends upon the class and size of a particular compound but is typically on the order of hundreds of amol/μm^2 to low fmol/μm^2. Other limiting factors include aspects of both sample preparation and instrumentation. For example, the intensity and number of discrete ions observed is a function of protein solubility, dynamic range and ion suppression effects inherent to the MALDI ionization process, as well as the limited mass resolving power of mass spectrometers. Thus, of the tens of thousands of proteins expressed in the cells of a tissue, MALDI-IMS generally produces images for only about 1,000 discrete ions. Continued growth in the number of MALDI-IMS studies will undoubtedly drive future developments for improving sensitivity.

MALDI-IMS analysis of individual cells remains a difficult task. Lateral image resolution (that is, the surface area mapped by a single pixel) is determined by the largest of the following quantities: the diameter of the focused MALDI laser beam, the pitch of the microspotted matrix array, or the size of the matrix crystals on a sprayed or spotted sample. Achieving cellular resolution will require these quantities to be reduced to subcellular dimensions. Current instrumentation is capable of producing submicron laser spots, but improvements will be needed in ejector and aerosol designs in order to produce submicron matrix crystals with 100% coverage of the sample yet without delocalizing the analyte. Further, achieving high sensitivity is important; the amount of analyte decreases markedly at smaller spot sizes. Finally, the data load increases significantly: doubling the spatial resolution increases the number of acquired mass spectra by fourfold, with concomitant increases in acquisition time and data file size.

Ion images and the mass spectra from which they are derived are based solely on *m/z* information. Identifying the molecular entity producing a given signal requires additional extraction, purification and either enzymatic or chemical procedures that traditionally have been wet-chemistry based. However, with the liquid spotting technology now available, it should be possible to develop protocols for chemical procedures carried out directly on the tissue section. Such procedures have already been demonstrated to facilitate the *in situ* identification of proteins, but there is significant potential for developing procedures for optimizing other factors such as protein extraction and ionization. Ultimately this should lead to vastly improved specificity and sensitivity for the molecular images.

The outlook for MALDI-IMS is extremely positive with great opportunity for continued growth. As instrumental capabilities become more readily available and performance improves, more investigators are exploring the technology and using it for new applications. Visualizing the distribution of the target compound along with hundreds of other molecular species simultaneously without need for target-specific reagents such as antibodies is unique to MALDI-IMS and provides investigators with another powerful imaging tool for the study of biochemical processes.

ACKNOWLEDMENTS

The authors thank E. Seeley, N. Caprioli, M.-C. Orgebin-Crist and P. Massion for assistance with the figures and have received financial support from the US National Institutes of Health, 5R01-GM058008-08, and Department of Defense, W81XWH-05-1-0179.

Published online at http://www.nature.com/naturemethods/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions

1. Claydon, M.A., Davey, S.N., Edwards-Jones, V. & Gordon, D.B. The rapid identification of intact microorganisms using mass spectrometry. *Nat. Biotechnol.* **14**, 1584–1586 (1996).
2. Holland, R.D. *et al.* Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **10**, 1227–1232 (1996).
3. Krishnamurthy, T., Ross, P.L. & Rajamani, U. Detection of pathogenic and non-pathogenic bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **10**, 883–888 (1996).
4. Caprioli, R.M., Farmer, T.B. & Gile, J. Molecular imaging of biological samples: Localization of peptides and proteins using MALDI-TOF MS. *Anal. Chem.* **69**, 4751–4760 (1997).
5. Stoeckli, M., Chaurand, P., Hallahan, D.E. & Caprioli, R.M. Imaging mass spectrometry: A new technology for the analysis of protein expression in mammalian tissues. *Nat. Med.* **7**, 493–496 (2001).
6. Stauber, J. *et al.* New developments in MALDI imaging mass spectrometry for pathological proteomic studies; introduction to a novel concept, the specific MALDI imaging. *Mol. Cell. Proteomics* **5**, S247–S247 (2006).
7. Skold, K. *et al.* Decreased striatal levels of PEP-19 following MPTP lesion in the mouse. *J. Proteome Res.* **5**, 262–269 (2006).
8. Chaurand, P., Norris, J.L., Cornett, D.S., Mobley, J.A. & Caprioli, R.M. New developments in profiling and imaging of proteins from tissue sections by MALDI mass spectrometry. *J. Proteome Res.* **5**, 2889–2900 (2006).
9. Stoeckli, M., Staab, D. & Schweitzer, A. Compound and metabolite distribution measured by MALDI mass spectrometric imaging in whole-body tissue sections. *Int. J. Mass Spectrom.* **260**, 195–202 (2007).

10. Meistermann, H. *et al.* Biomarker discovery by imaging mass spectrometry — Transthyretin is a biomarker for gentamicin-induced nephrotoxicity in rat. *Mol. Cell. Proteomics* **5**, 1876–1886 (2006).
11. Kulkarni, M.J., Vinod, V.P., Umasankar, P.K., Patole, M.S. & Rao, M. Intact cell matrix-assisted laser desorption/ionization mass spectrometry as a tool to screen drugs in vivo for regulation of protein expression. *Rapid Commun. Mass Spectrom.* **20**, 2769–2772 (2006).
12. Khatib-Shahidi, S., Andersson, M., Herman, J.L., Gillespie, T.A. & Caprioli, R.M. Direct molecular analysis of whole-body animal tissue sections by imaging MALDI mass spectrometry. *Anal. Chem.* **78**, 6448–6456 (2006).
13. Lemaire, R. *et al.* Direct analysis and MALDI imaging of formalin-fixed, paraffin-embedded tissue sections. *J. Proteome Res.* **6**, 1295–1305 (2007).
14. Norris, J.L. *et al.* Processing MALDI mass spectra to improve mass spectral direct tissue analysis. *Int. J. Mass Spectrom.* **260**, 212–221 (2007).
15. Shimma, S., Furuta, M., Ichimura, K., Yoshida, Y. & Setou, M. Direct MS/MS analysis in mammalian tissue sections using MALDI-QIT-TOFMS and chemical inkjet technology. *Surf. Interface Anal.* **38**, 1712–1714 (2006).
16. Aerni, H.R., Cornett, D.S. & Caprioli, R.M. Automated acoustic matrix deposition for MALDI sample preparation. *Anal. Chem.* **78**, 827–834 (2006).
17. Reyzer, M.L. & Caprioli, R.M. MALDI-MS-based imaging of small molecules and proteins in tissues. *Curr. Opin. Chem. Biol.* **11**, 29–35 (2007).
18. Pevsner, P.H. *et al.* Direct identification of proteins from T47D cells and murine brain tissue by matrix-assisted laser desorption/ionization post-source decay/collision-induced dissociation. *Rapid Commun. Mass Spectrom.* **21**, 429–436 (2007).
19. Hsieh, Y. *et al.* Matrix-assisted laser desorption/ionization imaging mass spectrometry for direct measurement of clozapine in rat brain tissue. *Rapid Commun. Mass Spectrom.* **20**, 965–972 (2006).
20. Atkinson, S.J., Loadman, P.M., Sutton, C., Patterson, L.H. & Clench, M.R. Examination of the distribution of the bioreductive drug AQ4N and its active metabolite AQ4 in solid tumours by imaging matrix-assisted laser desorption/ionisation mass spectrometry. *Rapid Commun. Mass Spectrom.* **21**, 1271–1276 (2007).
21. Garrett, T.J. & Yost, R.A. Analysis of intact tissue by intermediate-pressure MALDI on a linear ion trap mass spectrometer. *Anal. Chem.* **78**, 2465–2469 (2006).
22. Woods, A.S. & Jackson, S.N. Brain tissue lipidomics: direct probing using matrix-assisted laser desorption/ionization mass spectrometry. *AAPS J.* **8**, E391–E395 (2006).
23. Spengler, B. & Hubert, M. Scanning microprobe matrix-assisted laser desorption ionization (SMALDI) mass spectrometry: instrumentation for sub-micrometer resolved LDI and MALDI surface analysis. *J. Am. Soc. Mass Spectrom.* **13**, 735–748 (2002).
24. Chaurand, P., Schriver, K.E. & Caprioli, R.M. Instrument design and characterization for high resolution MALDI-MS imaging of tissue sections. *J. Mass Spectrom.* **42**, 476–489 (2007).
25. Altelaar, A.F.M. *et al.* High-resolution MALDI imaging mass spectrometry allows localization of peptide distributions at cellular length scales in pituitary tissue sections. *Int. J. Mass Spectrom.* **260**, 203–211 (2007).
26. DeKeyser, S.S., Kutz-Naber, K.K., Schmidt, J.J., Barrett-Wilt, G.A. & Li, L.J. Imaging mass spectrometry of neuropeptides in decapod crustacean neuronal tissues. *J. Proteome Res.* **6**, 1782–1791 (2007).
27. Taban, I.M. *et al.* Imaging of peptides in the rat brain using MALDI-FTICR mass spectrometry. *J. Am. Soc. Mass Spectrom.* **18**, 145–151 (2007).
28. Brand, G.D. *et al.* Bradykinin-related peptides from *Phyllomedusa hypochondrialis*. *Peptides* **27**, 2137–2146 (2006).
29. Han, J. & Schey, K.L. MALDI tissue imaging of ocular lens alpha-crystallin. *Invest. Ophthalmol. Vis. Sci.* **47**, 2990–2996 (2006).
30. Verhaert, P.D., Conaway, M.C.P., Pekar, T.M. & Miller, K. Neuropeptide imaging on an LTQ with vMALDI source: The complete 'all-in-one' peptidome analysis. *Int. J. Mass Spectrom.* **260**, 177–184 (2007).
31. Cornett, D.S. *et al.* A novel histology-directed strategy for MALDI-MS tissue profiling that improves throughput and cellular specificity in human breast cancer. *Mol. Cell. Proteomics* **5**, 1975–1983 (2006).
32. Schwartz, S.A. *et al.* Proteomic-based prognosis of brain tumor patients using direct-tissue matrix-assisted laser desorption ionization mass spectrometry. *Cancer Res.* **65**, 7674–7681 (2005).
33. Caldwell, R.L., Gonzalez, A., Oppenheimer, S.R., Schwartz, H.S. & Caprioli, R.M. Molecular assessment of the tumor protein microenvironment using imaging mass spectrometry. *Cancer Genomics Proteomics* **3**, 279–288 (2006).
34. Suzuki, K. *et al.* Epididymis-specific promoter-driven gene targeting: a transcription factor which regulates epididymis-specific gene expression. *Mol. Cell. Endocrinol.* **250**, 184–189 (2006).
35. Chaurand, P. *et al.* Profiling and imaging proteins in the mouse epididymis by imaging mass spectrometry. *Proteomics* **3**, 2221–2239 (2003).
36. Groseclose, M.R., Andersson, M., Hardesty, W.M. & Caprioli, R.M. Identification of proteins directly from tissue: *in situ* tryptic digestions coupled with imaging mass spectrometry. *J. Mass Spectrom.* **42**, 254–262 (2007).
37. Thiery, G. *et al.* Multiplex target protein imaging in tissue sections by mass spectrometry—TAMSIM. *Rapid Commun. Mass Spectrom.* **21**, 823–829 (2007).
38. Lemaire, R. *et al.* Tag-mass: specific molecular imaging of transcriptome and proteome by mass spectrometry based on photocleavable tag. *J. Proteome Res.* **6**, 2057–2067 (2007).
39. Signor, L. *et al.* Analysis of erlotinib and its metabolites in rat tissue sections by MALDI quadrupole time-of-flight mass spectrometry. *J. Mass Spectrom.* **42**, 900–909 (2007).
40. Corr, J.J. *et al.* Design considerations for high speed quantitative mass spectrometry with MALDI ionization. *J. Am. Soc. Mass Spectrom.* **17**, 1129–1141 (2006).
41. Reyzer, M.L. *et al.* Early changes in protein expression detected by mass spectrometry predict tumor response to molecular therapeutics. *Cancer Res.* **64**, 9093–9100 (2004).
42. Cha, S.W. & Yeung, E.S. Colloidal graphite-assisted laser desorption/ionization mass spectrometry and MS^n of small molecules. 1. Imaging of cerebrosides directly from rat brain tissue. *Anal. Chem.* **79**, 2373–2385 (2007).
43. Woods, A.S. *et al.* IR-MALDI-LDI combined with ion mobility orthogonal time-of-flight mass spectrometry. *J. Proteome Res.* **5**, 1484–1487 (2006).
44. Jackson, S.N., Wang, H.Y.J. & Woods, A.S. Direct tissue analysis of phospholipids in rat brain using MALDI-TOFMS and MALDI-ion mobility-TOFMS. *J. Am. Soc. Mass Spectrom.* **16**, 133–138 (2005).
45. McLean, J.A., Ridenour, W.B. & Caprioli, R.M. Profiling and imaging of tissues by imaging ion mobility-mass spectrometry. *J. Mass Spectrom.* **42**, 1099–1105 (2007).
46. McDonnell, L.A. & Heeren, R.M.A. Imaging mass spectrometry. *Mass Spectrom. Rev.* **26**, 606–643 (2007).
47. Heeren, R.M.A. *et al.* Why don't biologists use SIMS? A critical evaluation of imaging MS. *Appl. Surf. Sci.* **252**, 6827–6835 (2006).