Label-free molecular imaging of the kidney

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In this review, we will highlight technologies that enable scientists to study the molecular characteristics of tissues and/or cells without the need for antibodies or other labeling techniques. Specifically, we will focus on matrix-assisted laser desorption/ionization imaging mass spectrometry, infrared spectroscopy, and Raman spectroscopy.

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AN AGE OF MOLECULAR DISCOVERY

The past few decades have seen a rapid evolution of research conducted in the fields of molecular biology and molecular medicine. Increasingly, scientists and clinicians are concerned with the understanding of health and disease at the molecular level. This focus at the molecular and cellular levels enables research in curative therapies to target specific mechanisms of basic cellular biology. These in turn necessitate advances in research tools that provide higher levels of molecular specificity and cellular detail to investigators, which lead to new insights into the underlying biology.

The light microscope has been a favorite tool of the biologist since the 1600s and has produced detailed and magnified images of biological specimens. However, the current age of molecular discovery necessitates imaging modalities that go beyond brightfield microscopy to provide increased powers of molecular specificity and spatial resolution. Fluorescence-based approaches such as immunohistochemical (IHC) and genetic labeling strategies have seen widespread use because of their excellent resolution and sensitivity. However, standard histologic techniques cannot access the broad array of molecular species present in a tissue or cell population. For example, even the most specific antibodies struggle to differentiate between highly similar molecules (e.g., protein post-translational modifications or lipid molecules that differ by only one double bond or a few carbon atoms in a fatty acid chain length). Moreover, these studies can be time consuming and expensive. In addition, these approaches require *a priori* knowledge of the targeted molecule of interest, which severely limits their utility in discovering novel target species. Approaches that can specifically and simultaneously map the broad spectrum of molecular species present in a cell population are critical for our understanding of complex biological processes. In this review, we will highlight technologies that enable scientists to study the molecular characteristics of tissues and/or cells without the need for antibodies or other labeling techniques. Specifically, we will focus on matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS), infrared spectroscopy, and Raman spectroscopy.

I. MALDI IMAGING MASS SPECTROMETRY: A MOLECULAR MICROSCOPE FOR BIOLOGY AND MEDICINE

A technology that enables the untargeted, regiospecific measurement of a wide array of molecules present in tissue specimens is imaging mass spectrometry.[1–4] Using MS as an

imaging modality leverages the analytical advantages of high sensitivity and molecular specificity of modern mass spectrometers in producing images that are representatives of tissue biology on the basis of specific molecules (e.g., drugs, metabolites, lipids, peptides, and proteins). While there are several MS ionization methods that can be used to directly assess tissue specimens, MALDI has garnered the most attention since being described for IMS almost 20 years ago and will be the focus of the current discussion.[1] For convenience, in this article, MALDI IMS is simply abbreviated as IMS.

In a typical MALDI IMS experiment, a thin tissue section is mounted onto a flat substrate such as a microscope slide and then coated with a MALDI matrix (Figure 1). This matrix is typically a small organic acid with a strong absorbance at the wavelength of the incident MALDI laser. A raster of the tissue surface is performed with the laser, which desorbs and ionizes analytes mixed with the matrix molecules, generating a mass spectrum at each x, y coordinate (i.e., pixel). Maps of ion intensity can then be constructed as a function of x, y coordinates across the tissue surface. Ions of interest are identified using one or a combination of several techniques, including accurate mass measurements[5,6] and tandem MS (MS/MS).[7–13]

Recent advances in sample preparation, instrumentation, and bioinformatics approaches have greatly improved the power of IMS technology. Sample preparation protocols are typically designed to maximize sensitivity toward an analyte class of interest while still preserving the spatial integrities of those analytes of interest.[14,15] For example, on-tissue washes,[16,17] enzymatic digestion,[18–20] and chemical derivatization[21–24] strategies have been reported to improve protein, peptide, and small molecule sensitivities, respectively. The manner by which the MALDI matrix is applied to the tissue surface can also affect IMS sensitivity.[25] Matrix solution compositions[26,27] and/or vapor-assisted matrix recrystallization protocols[28] can be used during matrix application processes to aid in the extraction and cocrystallization of analytes into the matrix layer, thereby improving desorption/ionization. In addition, the identity of the MALDI matrix has been shown to greatly influence the sensitivity of IMS measurements with certain analyte classes. While organic molecules such as 4,6-tryhydroxyacetophenone (for drugs and metabolites),[29–30] 1,5-diaminonapthalene (for lipids),[31] 2,6-dihydroxyacetophenone (for lipids and proteins),[32–35] and α-cyano-4-hydroxycinnamic acid (for peptides and proteins)[36] are more common, alternative matrices such as colloidal silver nanoparticles for analyzing cholesterol[37–41] have also been reported. Recent reports have also described the use of basic matrices such as 9-aminoacridine (for small molecules),[42] the screening of rationally designed matrices such as 4-phenyl-α-cyanocinnamic acid amide (for lipids),[43] and matrices that preferentially generate multiply charged ions such as 2-nitrophloroglucinol (for proteins).[44]

The use of IMS in biological and clinical settings necessitates systems capable of high throughput and with high molecular specificity to rapidly and accurately analyze large sample cohorts.[45] In contrast to workflows that image a continuous region of a tissue, IMS profiling workflows only acquire the mass spectra from several discrete spots on the

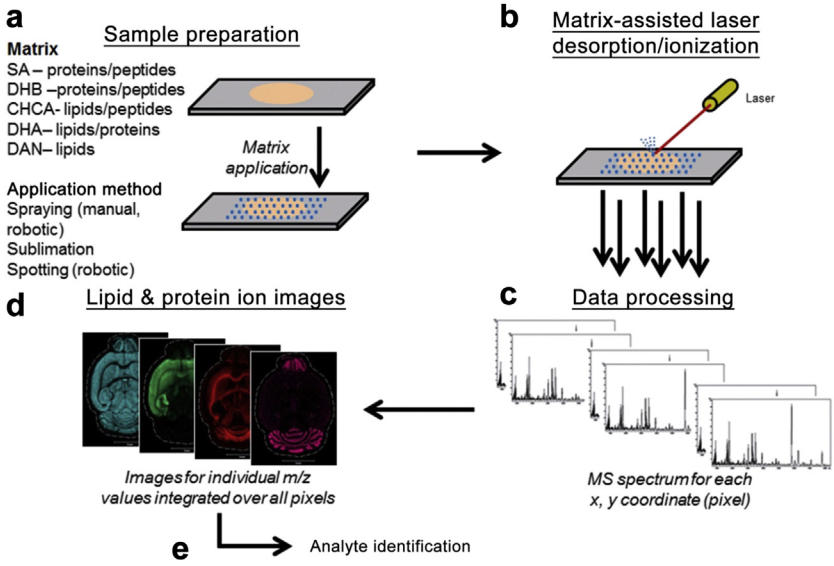

Figure 1 | Imaging mass spectrometry (IMS) workflow. (**a**) Specimens are prepared for analysis by mounting thinly sliced tissue sections onto slides. Then, matrix application is performed via any number of methods prior to (**b**) matrix-assisted laser desorption/ionization analysis. (**c**) Mass spectra generated at each x, y coordinate are then used to (**d**) construct intensity map images for any single ion of interest. (**e**) Analyte identification can be performed by 1 or a combination of several techniques.[45] Reprinted with permission from publisher from Prentice BM, Caprioli RM. The need for speed in MALDI imaging mass spectrometry. *J Postdoc Res.* 2016;4:3–13. Matrix abbreviations are as follows: CHCA, α-cyano-4-hydroxycinnamic acid; DAN, 1,5-diaminonapthalene; DHA, 2,5-dihydroxyacetphenone; DHB, 2,5-dihydroxybenzoic acid; SA, sinapinic acid. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.