Chapter 17
MALDI Imaging with Ion-Mobility MS: Waters Corporation

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Abstract For reasons of the unique detection principle based on mass spectrometry, MALDI-IMS provides an attractive proposition for molecular imaging, as a large range of different compounds can be detected simultaneously with simple protocol and fast data acquisition; also, it does not require any labeling of compounds of interest. In addition, the combination of ion-mobility separation with MALDI TOF-MS provides a unique separation dimension to further enhance IMS; ion-mobility spectroscopy can be used to produce images without interference from background ions of similar mass, which can remove ambiguity from imaging experiments and lead to more precise localization of the compound of interest, such as drugs and metabolites. Thus, ion-mobility TOF-MS has the potential to reduce complexity and improve confidence in imaging experiments.

17.1   Introduction

The past few years have seen a rapid increase in the interest in imaging mass spectrometry (IMS). The aim of this type of experiment is to obtain information on the spatial distribution of compounds in biological samples. Mass spectrometry (MS) is an attractive proposition for imaging, as a large range of different compounds can be detected simultaneously with simple protocol and fast data acquisition, and it does not require any labeling of compounds of interest.

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In earlier studies, IMS was developed as a tool for protein imaging [1–3], and in fact most of the reports concerning matrix-assisted laser desorption/ionization (MALDI)-IMS thus far describe the direct detection and imaging of proteins or peptides. On the other hand, research regarding the detection and imaging of small organic molecules has been increasing recently. The emergence of IMS as a tool for metabolite imaging has great impact because we do not have established technology for metabolite imaging whereas well-established methods have been widely applied for imaging of transcripts and proteins [4]. In this context, metabolic imaging by IMS is critical for interpretations of various aspects of life science.

Despite the promising capability of IMS, especially in the imaging of small metabolites, this emerging technique has several remaining problems. Below, we discuss point-to-point approaches to address these by using the MALDI-SYNAPT high definition MS (HDMS) system (Fig. 17.1).
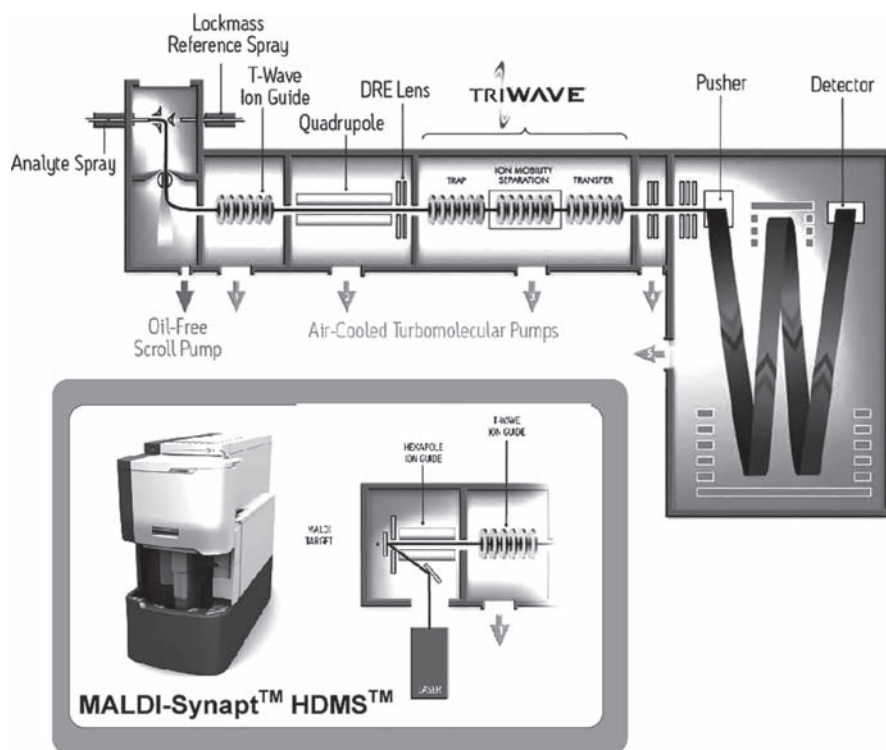

Fig. 17.1 Overview of MALDI-SYNAPT high definition (HD) MS: *Triwave* enables access to the unique benefits of ion-mobility spectroscopy, providing another separation capability; *oa-TOF* achieves high acquisition rates that complement ion-mobility separation time frames

17.1.1 Physical Properties of Tissue Samples

Tissue sections exhibit different physical properties from conventional MALDI samples: i.e., variation in electrical conductivity and sample morphology are observed [5].

In axial MALDI mass spectrometers, such as TOF/TOF systems, the source region, including the sample holder and sample, form part of the time-of-flight (TOF) system. Therefore, the properties of a tissue sample may result in a reduction of performance of the mass analyzer, leading to a decrease in mass accuracy and mass spectral resolution. This uncertainty carries the risk of incorrectly assigning masses. In this regard, the orthogonal acceleration mass analyzers used by MALDI-SYNAPT HDMS do not suffer from this limitation, as the source region does not form part of the mass analyzer and instrument performance is independent of the sample analyzed (see Fig. 17.1).

17.1.2 Sample Complexity

To produce unambiguous ion images, it is important to be able to separate and distinguish the compounds of interest from MALDI matrix background. In particular, the low-m/z region (m/z <1000) of a MALDI spectrum contains a large population of ions from endogenous metabolites as well as matrix-related adduct clusters and fragments [6, 7]. Such a high density of ions means that multiple compounds often share the same nominal mass.

With the introduction of the MALDI-SYNAPT HDMS system, it is possible to separate ions using ion-mobility separation before mass analysis. Ion-mobility separation allows for the separation of ions according to their size, shape, and charge state. Using this technique it is possible to separate different compound classes, giving additional confidence that the true distribution of an ion of interest is observed, as discussed next.

17.1.3 Abundance of Compound of Interest

Compounds of interest may be difficult to detect when they are present at low concentration. This problem can be compounded by the sample complexity described previously.

Targeted MS/MS analysis is an effective solution; a substantial improvement in the signal-to-noise ratio can be achieved when monitoring specific fragment ions from compounds of interest in the MS/MS mode of analysis. A further advantage of this technique is that by monitoring more than one fragmentation, it is possible to validate the distribution of a compound of interest.

Below, we introduce an application example of MALDI-SYNAPT HDMS for imaging of small metabolites in a mouse spinal cord section. The example clearly demonstrates the usefulness of ion-mobility separation for small *m/z* compounds in MALDI-IMS.

17.2   Methods

- 2,5-Dihydroxy benzoic acid (DHB) matrix was used at a concentration of 50 mg/ml in 70% MetOH, 0.1% trifluoroacetic acid.
- The frozen spinal cord sections were thaw-mounted on indium tin oxide-coated glass slides.
- The sections were coated with matrix using an airbrush (Procon Boy FWA Platinum; Mr. Hobby, Tokyo, Japan).
- The sample was analyzed using a MALDI-SYNAPT HDMS system.
- The instrument was operated in V-mode over a *m/z* range of 50–1,000 in positive ion detection mode.
- Spatial resolution at 200 µm was selected, and 200 raster shots were acquired per pixel at a laser repetition rate of 200 Hz.
- The area to be imaged was selected using MALDI Imaging Pattern Creator (Waters Corporation, Manchester, UK).
- After acquisition, HDMS data evaluation was performed using DriftScope (Waters Corporation, Manchester, UK).
- Data were converted into Anatyze file format using MALDI-Imaging Converter (Waters Corporation, Manchester, UK) for image analysis using BioMap (Novartis, Basel CH).

Supported by a number of software tools, the MALDI imaging workflow on the MALDI-SYNAPT HDMS system has been optimized to make the production of high definition imaging data as easy as possible. The steps involved in this workflow are outlined in Fig. 17.2.

17.2.1   Selection of Area for Mass Analysis

It is important to limit the area to be analyzed to the tissue of interest, as this saves a great deal of instrument time, especially for high-resolution images. The Creator imaging software simplifies the process, using a digital image of the prepared sample to guide selection of the areas of interest. Area selections are made using a drawing tool, allowing rectangular, elliptical, and free-drawn areas to be selected. Multiple areas can be selected for one experiment. The laser coordinates corresponding to the selected areas are automatically determined at a user-definable spatial resolution and then used during data acquisition.
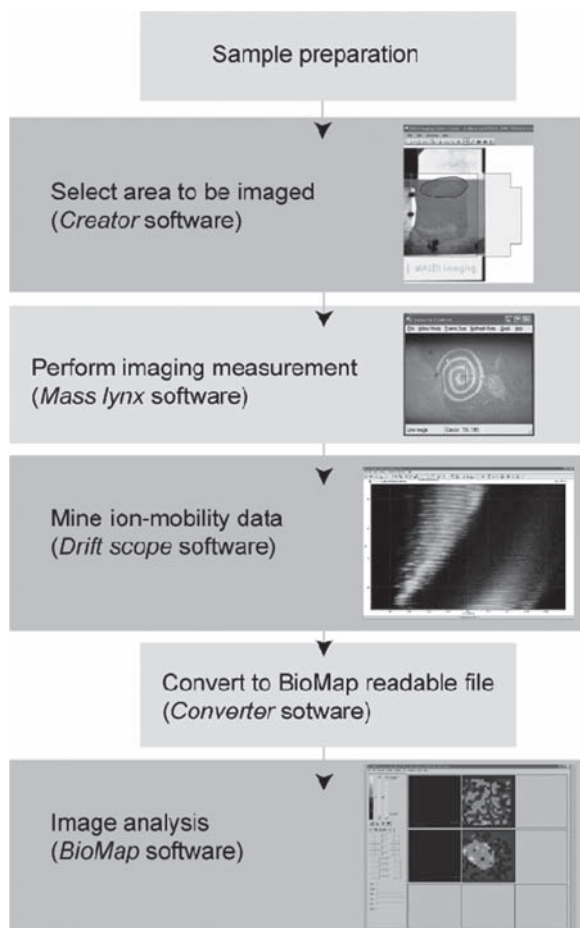
Fig. 17.2 Workflow of MALDI ion-mobility IMS with SYNAPT HDMS

## 17.2.2   Automated HDMS or MS Imaging Experiments

Imaging data acquisition is managed from the MassLynx software sample list. Researchers can choose between MS, MS/MS, HDMS, and HDMS/MS imaging experiments. Details of these experiments are shown in Table 17.1.

17.2.3   Ion-Mobility Data Conversion to Imaging Format

After data acquisition in HDMS or HDMS/MS mode, the mobility data can be viewed and mined using DriftScope software (see Fig. 17.3). A specific *m/z* and drift-time range can be selected and converted into ion intensity maps. These intensity

Table 17.1 MALDI-IMS experiments available on the MALDI-SYNAPT HDMS system

Type of imaging experiment	Ion-mobility separation	Type of mass spectral data
MS	No	MS
MS/MS	No	MS/MS on one or more precursor ions
HDMS	Yes	MS
HDMS/MS	Yes	Trap fragmentation
		Transfer fragmentation
		TAP fragmentation
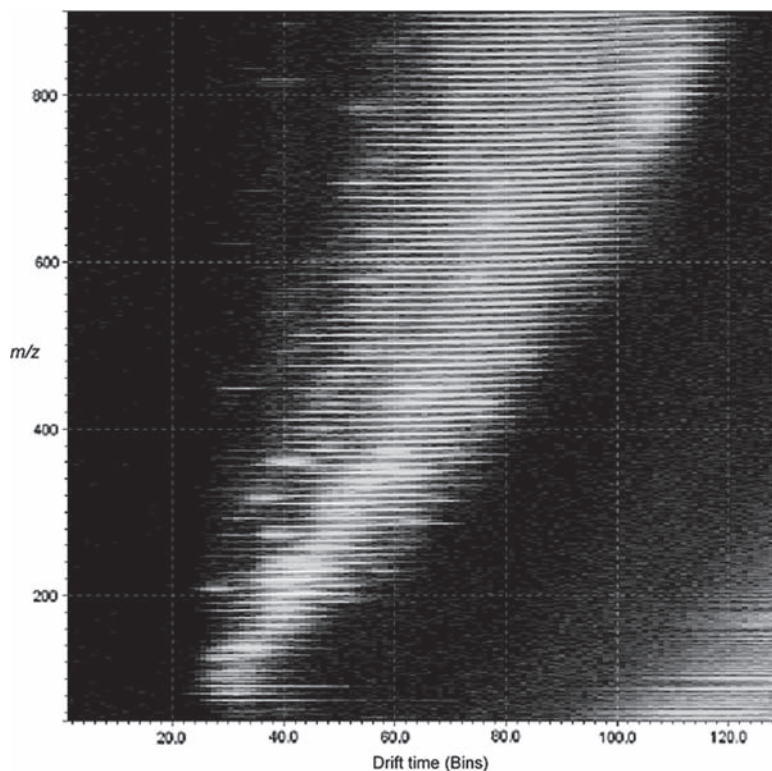
Fig. 17.3 MALDI ion-mobility 2D plot (*m/z* vs. drift time) of a mouse spinal cord tissue section with 2,5-dihydroxy benzoic acid (DHB) matrix in positive ion mode

maps can be imported into BioMap (Novartis, CH) using the MALDI imaging converter software tool.

17.3 Results and Discussion

Owing to the complexity of the tissue samples under investigation, a major limitation of MALDI-IMS is a risk of isobaric ions distorting the ion distribution and thus invalidating results, especially when applying single-stage MS imaging measurement.

In this regard, the MALDI-SYNAPT HDMS system achieves this by gas-phase separation of ions based on their size, shape, and charge using high-efficiency ion mobility. In the imaging mode, a full three-dimensional data set is acquired at every spatial position consisting of *m/z*, drift time, and ion intensity, ensuring maximum flexibility for mining the data.

Figure 17.3 shows a typical MALDI ion-mobility TOF spectrum (plot of *m/z*, *y*-axis vs. drift time, *x*-axis), obtained from the mouse spinal cord section. In a previous study using standard compounds, it was demonstrated that different classes of biomolecules such as lipids, oligonucleotides, and peptides form distinct familial trend lines on a two-dimensional (2D) plot of mobility drift times versus *m/z* [8]. Therefore, we can mine a highly specific imaging dataset for molecular species of interest by ion-mobility separation [9].

In the drift-time spectrum, an independent peak was observed around 110 bins (arrowhead in Fig. 17.4a). It has been demonstrated that lipid ion species were consistently slower than isobaric peptides and DHB matrix clusters [8, 9]. Based on previous studies [9, 10], we assumed the drift-time peak as a lipid-derived signal. Then, we extracted mass spectra from a distinct lipid trend area that is separated
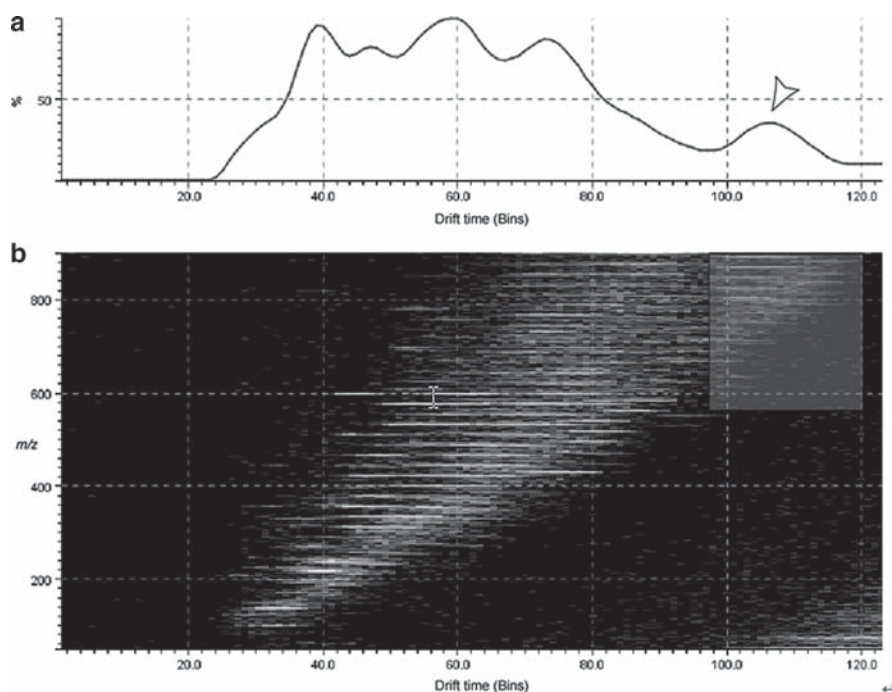

Fig. 17.4 Selection and separation of lipid-related signals by difference of drift time. (**a**) Overall drift time spectrum. *Arrowhead* represents a peak corresponding to lipid species [9]. (**b**) MALDI ion-mobility 2D plot (*m/z* vs. drift time) of a mouse spinal cord tissue section with DHB matrix in positive ion mode. A *square region* was selected (98–230 bins and 580–900 *m/z*), and using the 2D range of mobility time and mass, mass spectra were reconstructed for each spatial point
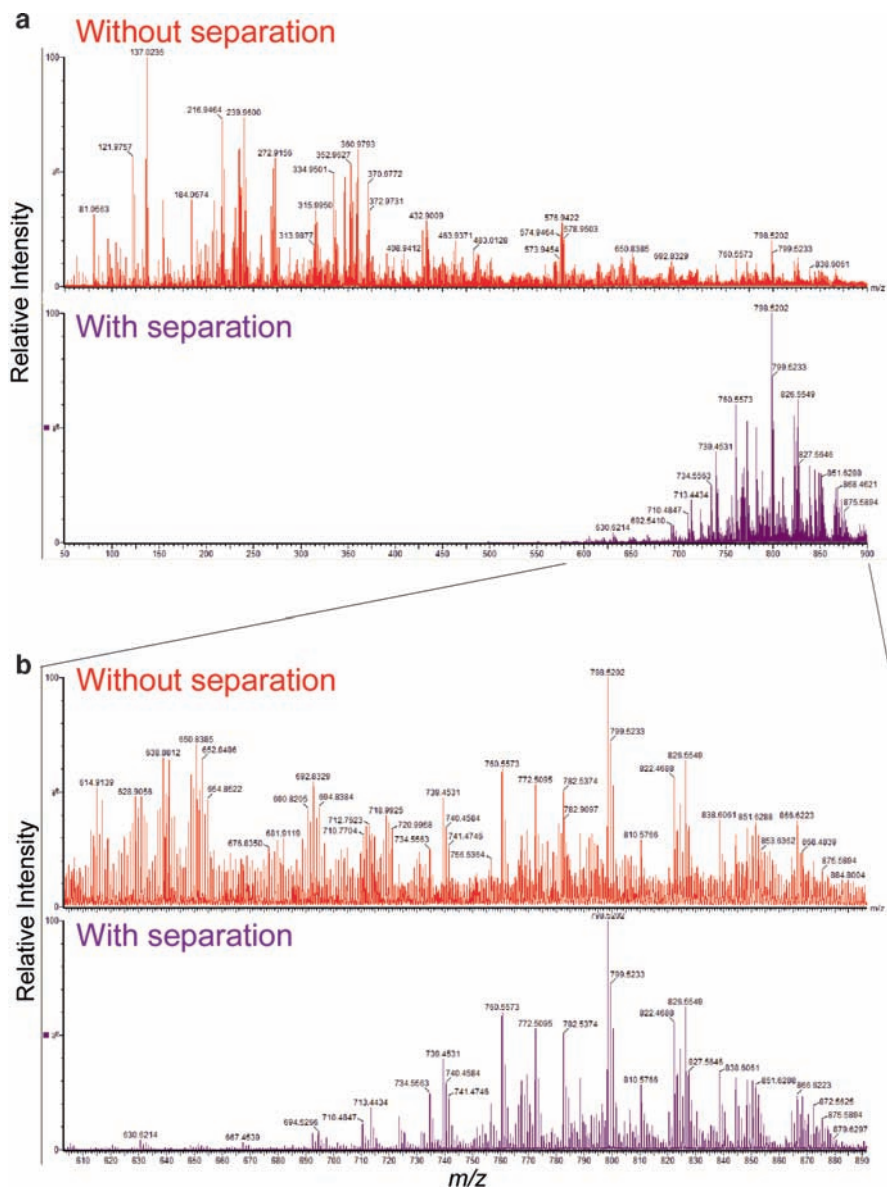
Fig. 17.5 Ion-mobility separation results in specific MS profiles for lipid-related signals

from other biomolecules (square region in Fig. 17.4b). It is this 2D range of mobility time and mass that was used to acquire the lipid images in the following study.

In Fig. 17.5, which shows mass spectra with/without ion-mobility separation, it can clearly be seen that ions of lipid species were separated by the ion-mobility
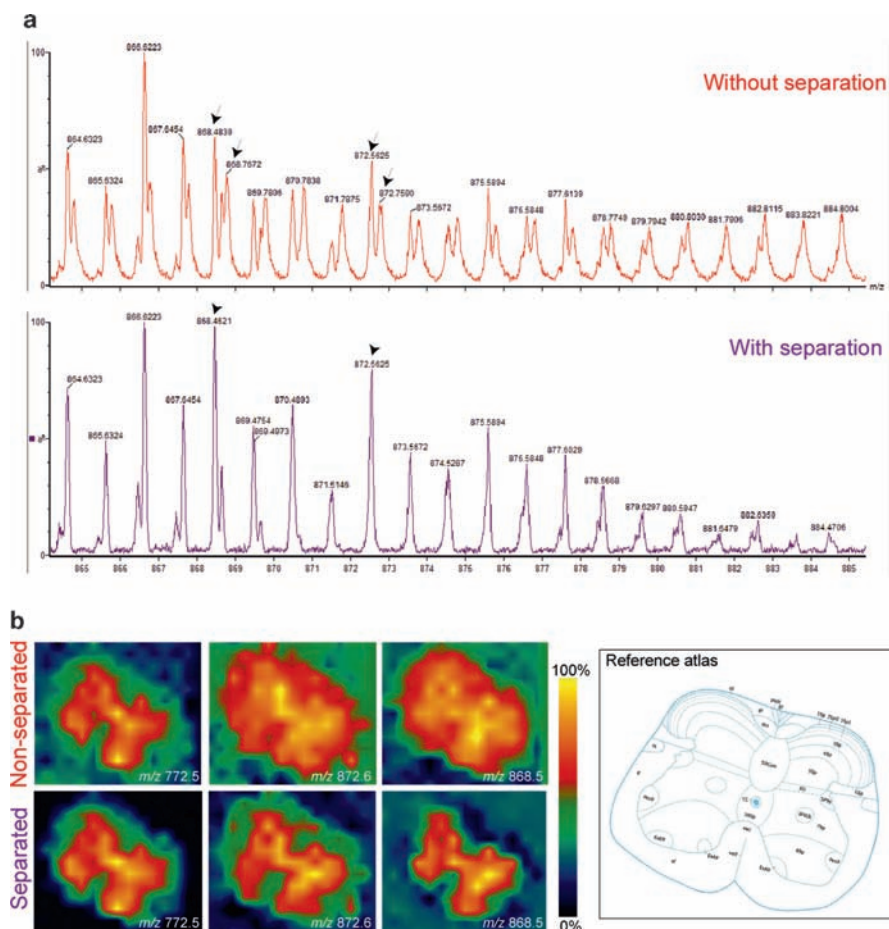
Fig. 17.6 Ion-mobility separation helps to provide the true spatial tissue distribution of the endogenous metabolite by removing any contribution of the interfering matrix ions. (**a**) Mass spectrum with/without ion-mobility separation. Signals presumably derived from matrix clusters were removed by the separation (*arrows*), and signal-to-noise ratio of lipid signals was enhanced (*arrowheads*). (**b**) Ion distribution images of phosphatidylcholines reconstructed from mass spectra with or without ion-mobility separation

dimension from the complex ion mixture containing other trends of ion molecules, such as DHB clusters.

Figure 17.6 demonstrates that ion-mobility separation provides a more specific ion distribution image of compounds by removing matrix-related signals. Mass peaks presumably derived from matrix clusters were removed by the separation process (arrows in Fig. 17.6a). On the other hand, the signal-to-noise ratio of lipid signals was improved (arrowheads).

Such improvement on signal selectivity clearly helps to provide the true spatial tissue distribution of the endogenous metabolite by removing any contribution of the interfering matrix ions. Fig. 17.6b shows ion images for the ion of phosphatidylcholines with or without ion-mobility separation [11]. When ion-mobility separation is applied, the ion images for phosphatidylcholines have a significantly lower background noise level, as was especially true for the lipids at low concentration (*m/z* 872.6 and 868.5).

17.4 Conclusions

- The combination of ion-mobility separation with MALDI provides a unique separation dimension to further enhance IMS.
- Ion-mobility spectroscopy can be used to produce images without interference from background ions of similar mass; this can remove ambiguity from imaging experiments and lead to more precise localization of the compound of interest, such as drugs and metabolites.
- HDMS has the potential to reduce the complexity and improve confidence in imaging experiments.
- MALDI imaging offers a complementary approach to established imaging techniques, such as whole-body autoradiography. It has the advantage that no labeling of the compounds of interest is required and that multiple compounds can be monitored simultaneously, e.g., drugs and metabolites. As a result, the experiments can take place rapidly and the technique is relatively inexpensive.

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