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Nota de aplicación

Data Independent MALDI Imaging HDMS^E for Visualization and Identification of Lipids Directly from a Single Tissue Section

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Abstract

MALDI Imaging High Definition MS^E (HDMS^E) enables detection and identification of lipid species in a single analytical run. This unique methodology provides MS and MS/MS information from detectable ion species within the same experiment, without the need for precursor selection.

Benefits

- Identification of lipids based on high mass accuracy, fragment ion information, and spatial correlation – all obtained from a single experiment.
- Parallel fragmentation approach for a MALDI imaging experiment allowing structural identification of all detectable lipid species within the tissue section sample.
- Information obtained from valuable tissue sections is maximized.
- Simple and generic acquisition methodology reduces the need for designing targeted MS/MS experiments.

- Untargeted data sets provide an information-rich, digital archive of the sample to enable detection and identification of new species in the future, that is, acquire data first and ask (biological) relevant questions regarding the data later.

Introduction

Recent advances in mass spectrometry (MS) have enabled the simultaneous analysis of a wide range of chemically similar lipids as well as structurally diverse lipid classes, contributing to an increased interest in lipidomics research. However, the spatial localization of lipids within tissue micro-structures is often lost during the process of lipid extraction, as applied in more traditional analysis approaches, resulting in the loss of valuable information pertaining to origin and biological function.

Mass spectrometry imaging (MSI) visualizes the location of lipid species in entire tissue sections. The first step is typically an untargeted MS analysis experiment that enables large numbers of species to be detected and localized simultaneously. Structural identification of the detected lipid species is the next step; however, this can be time-consuming since it consists of manually conducting a series of MS/MS acquisitions on selected components, using either single or consecutive tissue sections.

A data independent MALDI imaging acquisition method called MALDI Imaging High Definition MS^E ($HDMS^E$), presented here, enables detection and identification of lipid species in a single analytical run. This unique methodology provides MS and MS/MS information from detectable ion species within the same experiment, without the need for precursor selection. Post acquisition, precursors and fragments are correlated on the basis of ion mobility (drift time) and spatial distribution to provide highly informative results for every detectable molecular component.

Experimental

Sample Description

A 30-µm-thick rat whole-body sagittal tissue section was mounted on invisible mending tape that was cut using

a scalpel to fit a Waters MALDI target with double sided tape. A solution of α-cyano-4-hydroxycinnamic acid (CHCA) matrix at 5 mg/mL was applied evenly to the sample in several coats using a SunCollect (SunChrom GmbH) nebulising spray device.

MS Conditions

Mass Spectrometer:	MALDI SYNAPT G2 HDMS
Mode:	Positive
Mass range:	100 to 1000 Da
Transfer collision voltage:	Low energy function: 4 eV Elevated energy function: 50 eV
Laser:	1 KHz solid state Nd: YAG laser (λ = 355 nm)
Spatial resolution:	200 µm (lateral)

Data Management

The raw data obtained were subsequently processed using High Definition Imaging (HDI) MALDI Software, whereby the low energy and elevated functions were processed and combined in a .txt output file. Only a limited drift time range, specific for lipids, from 100 to 160 mobility bins was considered.

Identification based on mass accuracy and fragmentation information was carried out using SimLipid 3 (PremierBioSoft, US) Software and LipidMaps MS tools (http://www.lipidmaps.org/tools/index.html).

Results and Discussion

Ions are generated in the source of the mass spectrometer and passed through the quadrupole (with no

precursor selection) in the Triwave region, as shown in Figure 1. The ions are rapidly separated (in 20 to 50 msec) based on their size, shape, and charge (i.e. ion mobility, or IM) to better enable detection of isobaric and isomeric components.[1] Following IM separation, ions pass through the TRANSFER T-Wave collision cell, where, in the first low energy function precursor ion spectra are recorded (intact lipid information), and in the second elevated energy function energy product ion spectra are recorded (lipid fragment information). The two functions can be acquired on the same pixel (to maximize spatial resolution) or consecutive pixels (to maximize sensitivity). The low energy precursors can be associated with the relevant elevated energy fragments since they share similar drift time values from the IM separation, as shown in Figure 1. The datasets are subsequently processed, using the High Definition Imaging (HDI) MALDI Software, where the data from both the low and elevated energy functions are peak detected, aligned, and a two-step correlation based on drift time and position between precursor and fragment ions is performed.

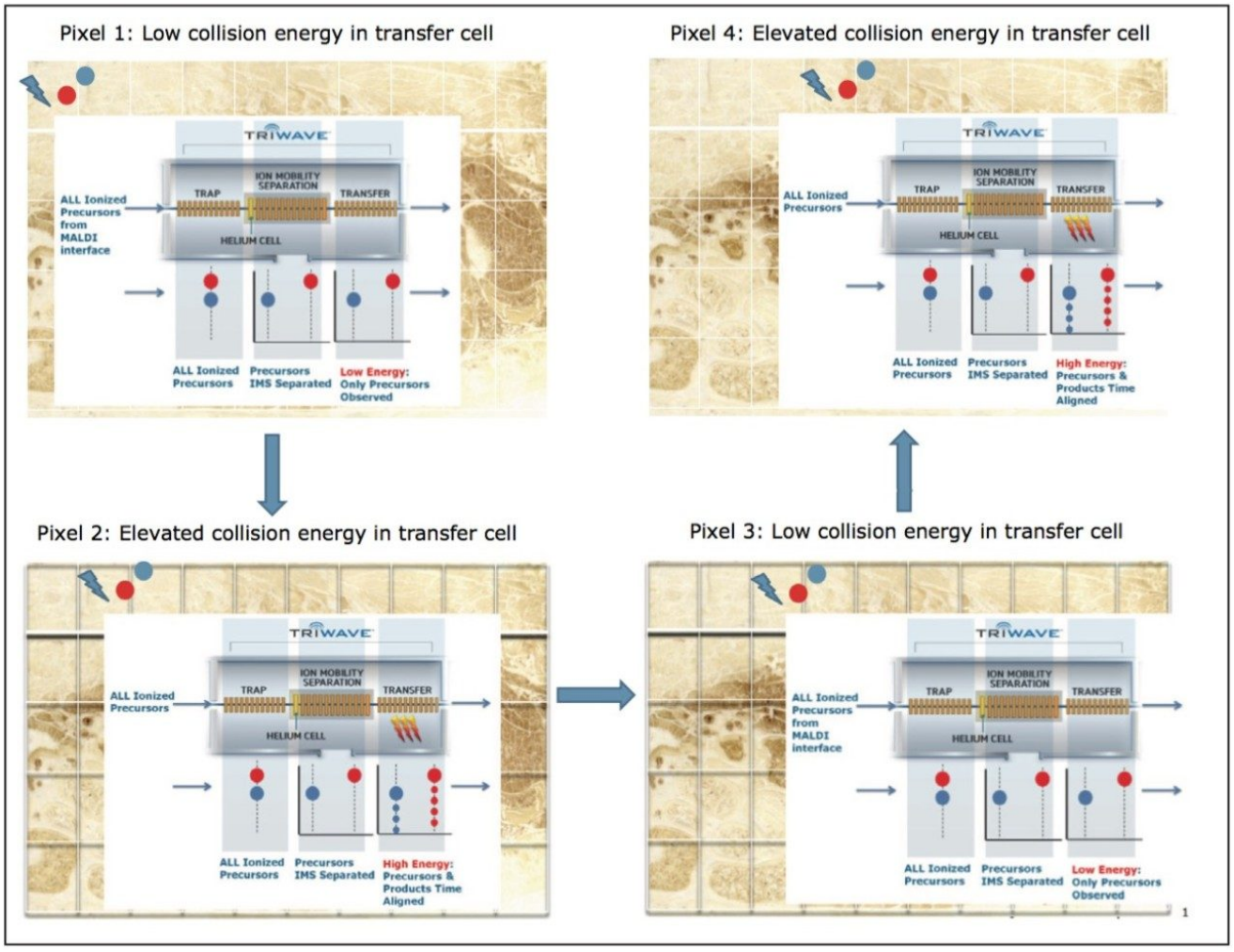

Figure 1. Schematic of a MALDI Imaging HDMS^E experiment.

The user interface of HDI Software and the display of the processed data are shown in Figure 2. In this view, the peak lists, mass spectra, and ion images from the two functions are integrated in an interactive manner. A two-dimensional plot of drift time versus *m/z* plot is also included, to enhance visualization and peak selection (blue dots represent the low energy information and green dots the elevated peak detected ions).

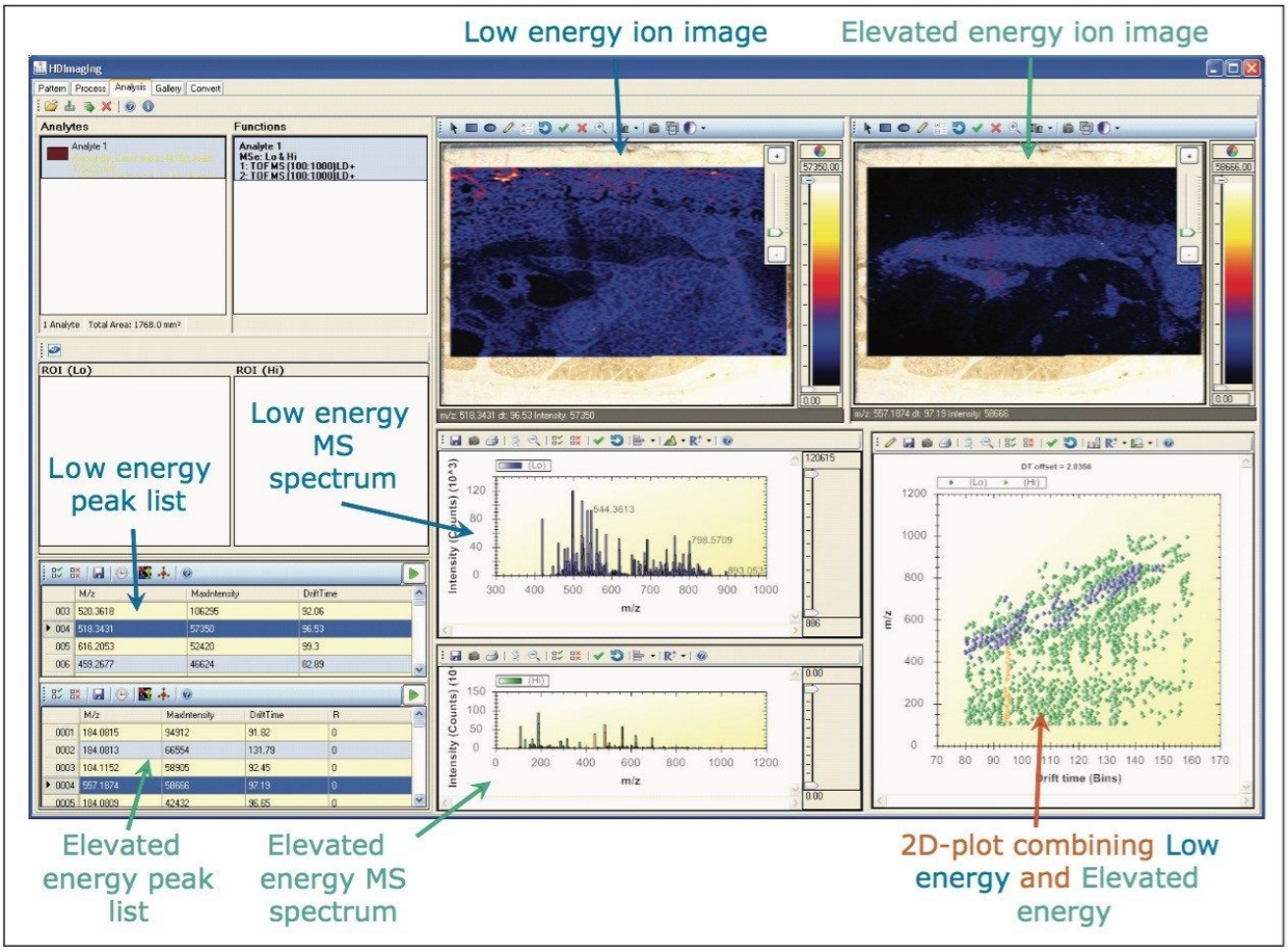

Figure 2. MALDI Imaging HDMS^E view of the processed data with High Definition Imaging HDI Software.

The main, distinct advantage of MALDI imaging HDMS^E is its ability to generate precursor ion and fragment ion information for every detectable molecular ion. Initial correlation is achieved on drift time similarity, which is realized within the IM cell. However, one particular challenge with lipid samples is the high number of species within a limited mass and drift range. Fragments that do not belong to the correct precursor can sometimes be assigned incorrectly, but this situation is strongly improved using a second correlation step based on spatial distribution similarity of fragment ions and their precursors.

The workflow of the two-step correlation process for fine association of fragment ions to their originating precursors is illustrated in Figure 3. For example, precursor lipid ion *m/z* 760.6 was selected in the top HDI window. In the *m/z* versus dt plot, the drift time associated fragment ions are displayed as orange dots. After

accepting the drift time correlation results, 108 potential fragments were drift time associated to this particular lipid precursor ion, as displayed in the middle window. The next step was the spatial correlation. When a correlation factor from 0.3 to 1.0 was applied, the number of potential fragment ions was reduced to 39, as can be seen in the bottom window.

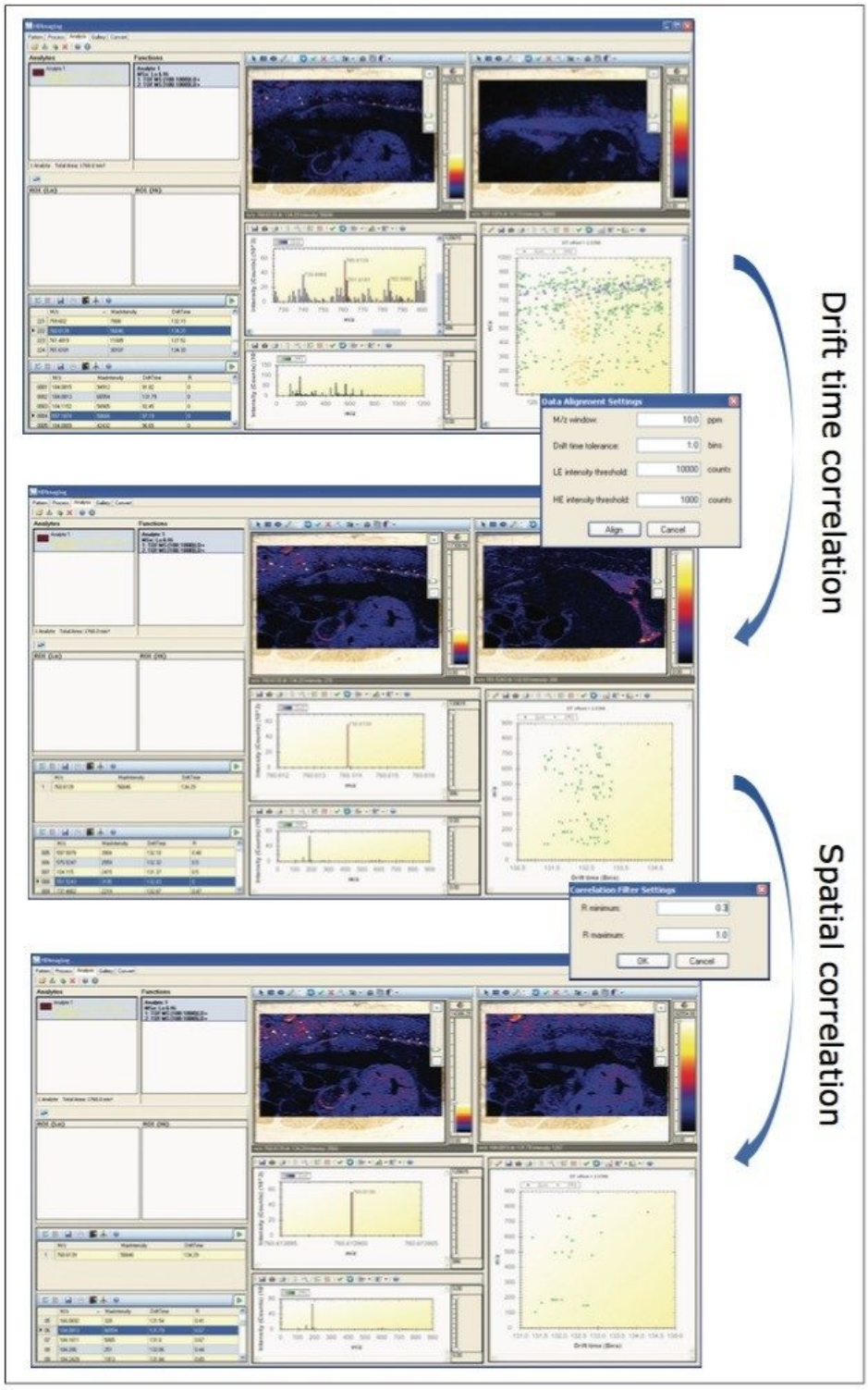

Figure 3. Workflow of the two-step correlation. The top image shows all processed data that were drift time

associated with precursor ion m/z 760.0. The middle image shows only fragment ions that were drift time associated to precursor ion m/z 760.6. In the bottom image, spatial correlated fragment ions are displayed (correlation factor 0.5 to 1.0).

The results from the two-step correlation process were imported into SimLipid 3 for lipid identification. Here, parent *m/z* values were internally lockmass-corrected after identification of lipid *m/z* 798.5. In this instance, lipid *m/z* 760.5859 was identified as either PC (16:0/18:1) H^+ or PC (18:1/16:0)H^+ with fragment ions *m/z* 478.3301 (M-18:1-H_2O) and 496.3404 (M-18:1). T he annotated MS/MS spectrum is shown in Figure 4.

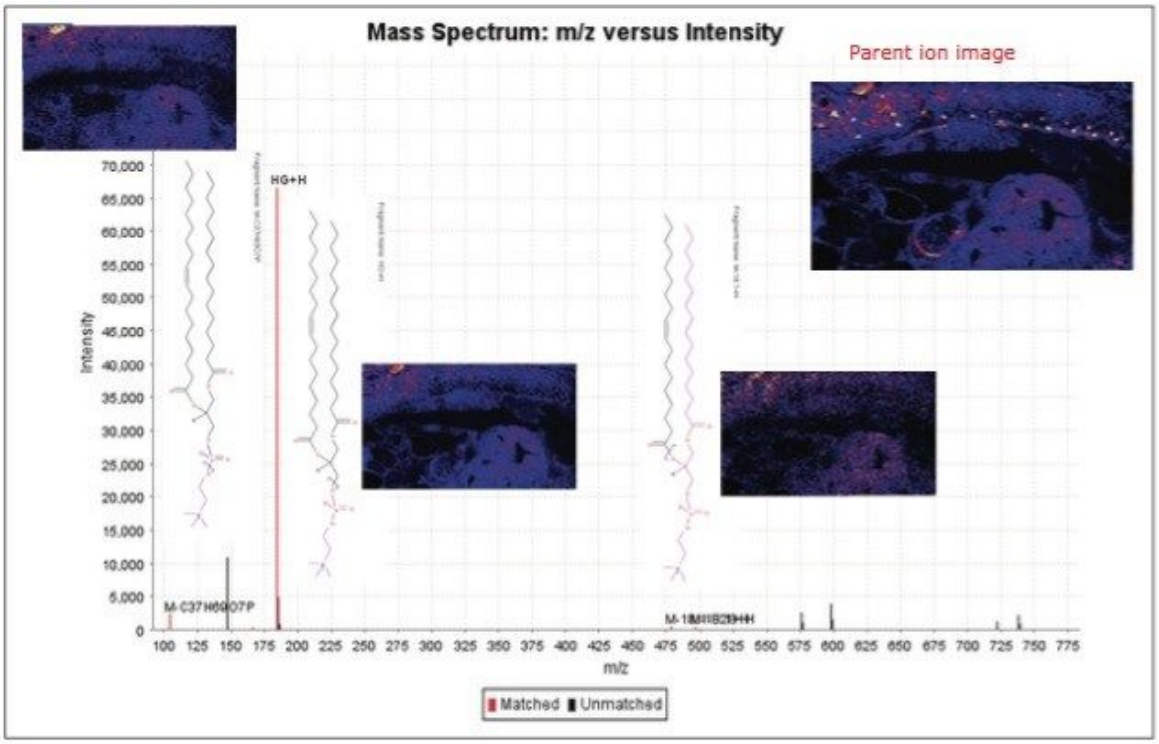

Figure 4. MS/MS spectrum generated from SimLipid 3 with lipid fragment structures and ion images displayed.

Using the information from the two-step correlation and mass accuracy, over 20 lipid species were identified from the single MALDI imaging $HDMS^E$ experiment and are summarized in Table 1.

Experimental *m/z*	Theoretical *m/z*	Systematic name		ppm
713.4543	713.4524	PA (14:0/20:1) or PA(20:1/14:0)	K+	2.7
721.4794	721.4803	PA (20:4/18:2) or PA(18:2/20:4)	H+	-1.2
721.4794	721.4803	PA (18:2/18:1) or PA(18:1/18:2)	Na+	-1.2
723.4948	723.4941	PA (18:1/18:1)	Na+	1.0
725.5581	725.5573	SM (d34:1)	Na+	1.1
734.571	734.57	PC (13:0/19:0) or PC (19:0/13:0)	H+	1.4
737.4536	737.4524	PA (14:1/22:2) or PA (22:2/14:1)	K+	1.6
739.4694	739.4674	PA (36:2)	K+	2.7
741.483	741.4831	PA (36:1)	K+	-0.1
741.5323	741.5313	SM (d18:1/16:0)	K+	1.3
745.4788	745.4786	PG (P-16:0/16:0)	K+	0.3
756.4964	756.4946	PE (12:0/22:1) or PE (22:1/12:0)	K+	2.4
756.4964	756.4946	PC (19:1/12:0) or PC (12:0/19:1)	K+	2.4
758.5706	758.57	PC 34:2	H+	0.8
760.5859	760.5856	PC (16:0/18:1) or PC (18:1/16:0)	H+	0.4
780.5527	780.5543	PC (16:0/20:5) or PC (20:5/16:0)	H+	-2.0
782.5695	782.5694	PE (19:0/20:4) or PE (20:4/19:0)	H+	0.1
796.5285	796.5259	PE-Nme (18:1/18:1)	K+	3.3
798.5415	798.5415	PC (14:0/20:1) or PC (20:1/14:0)	K+	0.0
820.528	820.5259	PE (17:2/22:2) or PE (22:2/17:2)	K+	2.6
824.5598	824.5572	PC:36:2	K+	3.2
826.573	826.5727	PE (P-20:0/22:6)	Na+	0.4
832.5842	832.5832	PE (20:4/21:0) or PE (21:0/20:4)	Na+	1.2
835.6686	835.6669	SM (d18:1/24:1) or SM (d24:1/18:1)	Na+	2.0

Table 1. Lipid identification summary from the MALDI Imaging HDMS^E experiment following the two-step correlation workflow.

Conclusion

- A novel, untargeted MALDI imaging experiment called MALDI Imaging HDMS^E is described, which allows precursor and fragment ion information to be collected from a single tissue section MALDI imaging experiment.
- Association of product ions with its precursor is confidently achieved with a high level of specificity by the described two-step correlation based on drift time and spatial distribution.
- Lipid species were identified directly from a single, untargeted MALDI imaging experiment while structural identification was made possible from the untargeted dataset using the high mass accuracy of the spectral data and the two-step (drift time and spatial location) correlation process.

References

1. Triwave – More Complete Characterization of Mixtures and Molecules. Waters Corporation. 2012; 720004176en.

Featured Products

MALDI SYNAPT G2-Si High Definition Mass Spectrometry <https://www.waters.com/134740682>

High Definition Imaging (HDI) Software <https://www.waters.com/134833914>

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