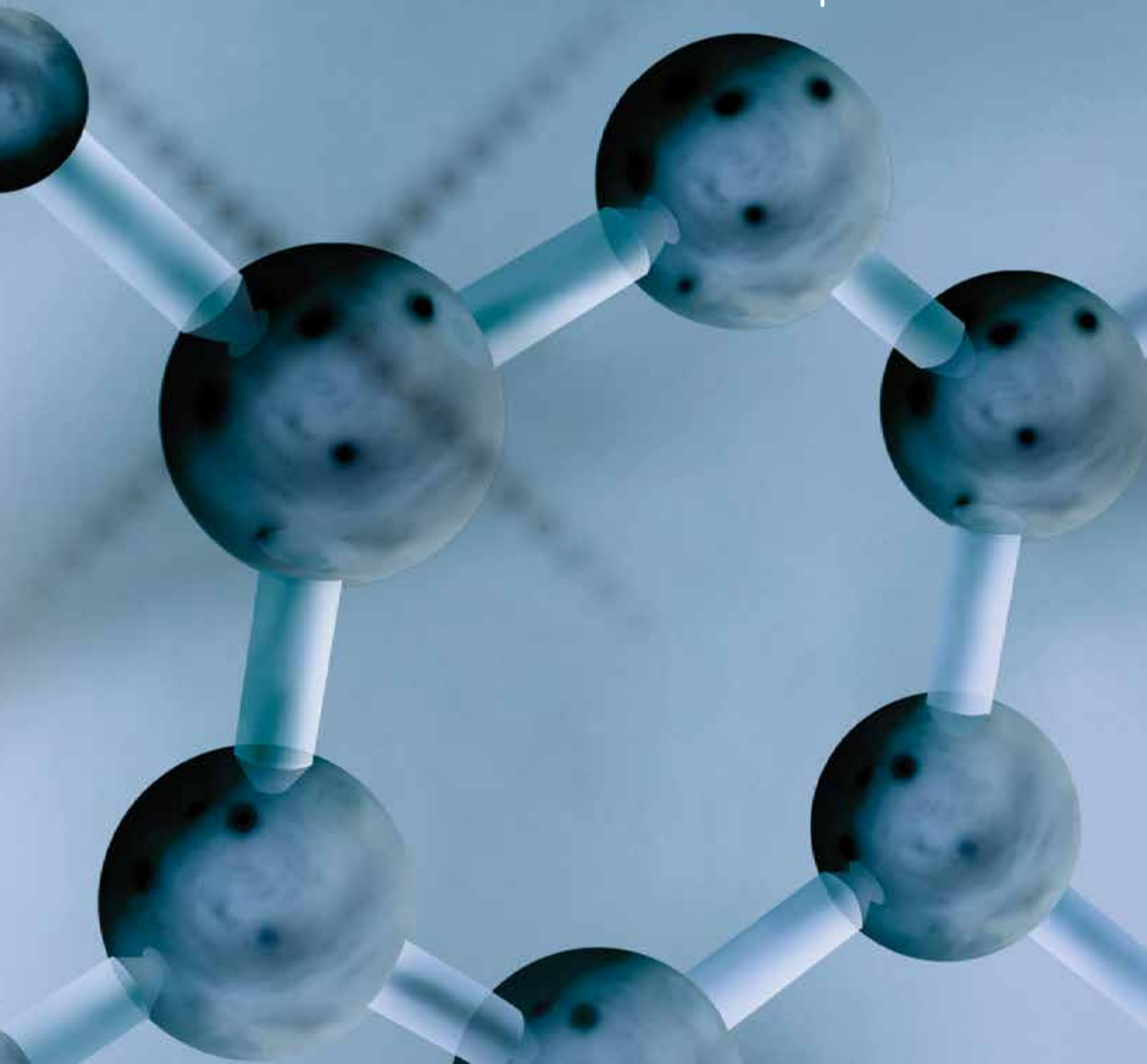
MS Imaging and Ambient Ionization-MS for Metabolomics and Lipidomics



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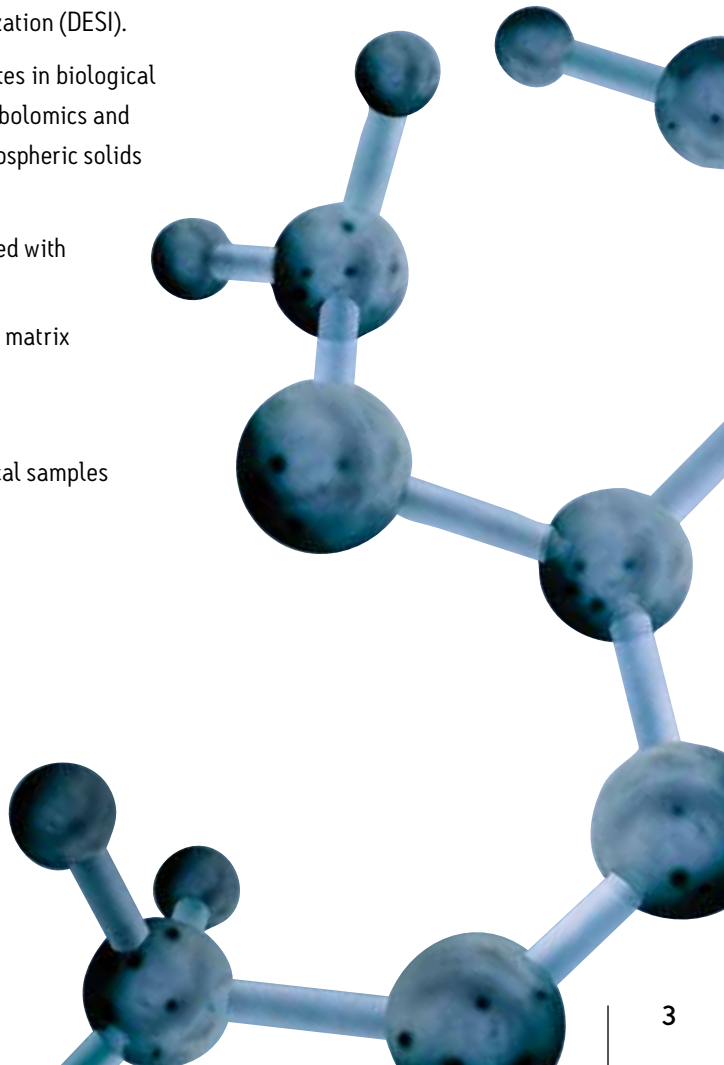
MS IMAGING AND AMBIENT IONIZATION-MS FOR METABOLOMICS AND LIPIDOMICS

There is a growing interest in applying surface-based desorption ionization (DI) techniques for metabolomics and lipidomics. In DI, the ionization process begins by irradiating a defined spot on the solid-state sample using a focused, excitatory beam such as a laser or charged solvent droplets. Upon impact, the sample's surface releases a vapor of ionized molecules that can be directed into a mass spectrometer. Alternatively, acoustic or thermal desorption could initiate the ionization process. The combined use of ion mobility after DI offers the unique opportunity to separate most of the mass interferences deriving from matrix, peptides and isobaric species, thus improving the overall sensitivity, signal-to-noise, and specificity of analysis. The two main DI-MS approaches for metabolomics and lipidomics are MS imaging and ambient ionization MS.

MS Imaging can provide information about the spatial distribution of metabolites and lipids on a sample (e.g., animal and plant tissues, entire insect body, etc.), generating topographic maps of the molecular composition. This level of information is often missed during traditional sample-preparation and extraction protocols for metabolomics and lipidomics. Various DI technologies, combined with ion mobility-MS, have been used for MS imaging in metabolomics and lipidomics, including matrix-assisted laser DI (MALDI) and desorption electrospray ionization (DESI).

Ambient ionization MS allows for real-time, rapid, in situ screening of analytes in biological samples. The main DI technologies that can be applied to real-time-MS metabolomics and lipidomics are rapid evaporative ionization mass spectrometry (REIMS), atmospheric solids analysis probe (ASAP), and direct-analysis in real time (DART).

- Spatial localization of metabolites and lipids with ion mobility-MS coupled with MALDI or DESI
- Unique capabilities for post-ionization separation of isobaric species and matrix interferences using ion mobility-MS (HDMS)
- High Performance, accurate Mass MALDI MS-MS
- Real-time, rapid, in situ screening of metabolites and lipids from biological samples



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Biomarker Discovery Directly from Tissue Xenograph Using High Definition Imaging MALDI Combined with Multivariate Analysis

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APPLICATION BENEFITS

New features have been implemented into Waters High Definition Imaging (HDI) 1.2 Software to allow multivariate data analysis, such as principal component analysis (PCA) and partial least squares discriminant (PLS-DA). These capabilities, integrated to the workflow, reduce the dimensionality of data and ease the comparison of multiple datasets. In this work, we demonstrate their use with MALDI imaging in studying the changes over time in the proteome on xenograph tissues sections after administration of an anti-cancer drug.

WATERS SOLUTIONS

MALDI SYNAPT® G2 HDMS™

High Definition Imaging (HDI) 1.2 Software

MassLynx® Software

KEY WORDS

Proteomics, multivariate analysis, principal component analysis (PCA), partial least squares discriminant (PLS-DA), MALDI imaging

INTRODUCTION

In biomedical research, proteomics has become an indispensable tool for the discovery of candidate biomarkers and drugs. Moreover, mass spectrometry imaging (MSI) enables researchers to determine the spatial distribution of proteins and peptides directly from a tissue section, without radioisotope labeling or tagging.

However, a MALDI imaging experiment can readily generate a vast amount of data, depending on tissue size and acquisition mass range. Both of these factors relate to the number of ion detections, number of pixels recorded, and the possible addition of ion mobility separation to improve the specificity of the analysis. Moreover, in the case of comparing multiple samples, such as different states of a disease or drug time-course experiment, data review complexity is multiplied, amounting in tedious and time-consuming data review to identify molecular species changes.

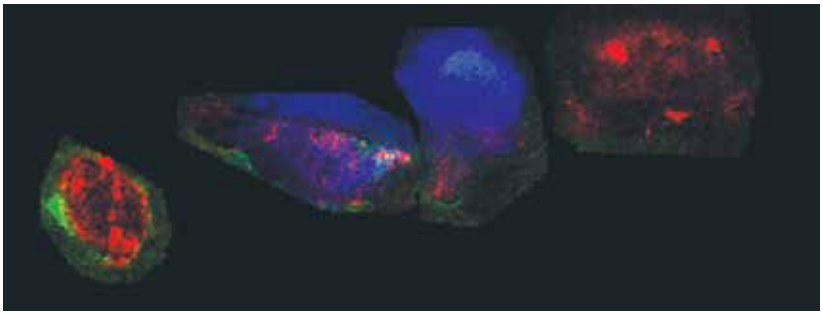

Figure 1. Overlay of three tryptic peptide ion images from xenograph tissue sections.

EXPERIMENTAL

Conditions

Data acquisition

System:	MALDI SYNAPT G2 HDMS, positive mode
Mass range:	600-3,000 Da
Laser:	1 KHz solid state Nd:YAG laser (λ = 355 nm)
Spatial resolution:	150 µm (lateral)

Data management

The obtained data were processed and visualised using HDI MALDI 1.2 Software. Regions of interest (ROIs) information were analysed using EZInfo (Umetrics Software). MassLynx Software was also utilized.

Sample description

Four rats were injected subcutaneously with a cancer cell line to allow the growth of tumor mass, called a xenograph. The animals were administrated with the anti-cancer drug Dasanitib at a concentration of 30 mg/kg and scarified at different time points (control, 1, 3, and 6 hours). After tumour excision, frozen tissue sections from each animal were thaw-mounted onto a single glass slide. Tissue samples were washed with different baths of cold ethanol and chloroform. *In situ* trypsin digestion was performed by spraying a solution of trypsin directly onto the tissue and incubating overnight in a humidity chamber at 37 °C.

Post-digestion, several layers of matrix, α-cyano-4-hydroxicinnamic acid (CHCA) containing aniline in acetonitrile/water/TFA (1:1:0,1), were also sprayed directly onto the tissue samples. A single jpeg image of the four tissue sections mounted on the glass slide was taken using a flatbed scanner. Using High Definition Imaging (HDI) MALDI software, the visual image was co-registered, and areas to be imaged by MSI were defined. This allowed the overlay of the MS information and the visual image later in the analysis (Figure 2A).

RESULTS AND DISCUSSION

Figure 2A represents the processed data loaded in the Analysis tab of HDI Software. It is possible to define one or several regions of interest (ROIs) on the four tissue sections as shown in Figure 2B. The ROIs can be drawn using the free draw tool, eclipse (or round), and rectangle (square) options.

By clicking on the Umetrics icon as shown in Figure 2B, the intensities for each entry (*m/z*, dt) present in the processed data are averaged and TIC normalized across the pixels present within each defined ROI. The output is reported in a csv file (Figure 2C). EZInfo can be launched directly from MassLynx Software by clicking on the Extended Statistics icon (Figure 2D). The output txt file is loaded in EZinfo where the different groups can be specified, as seen in Figure 2D).

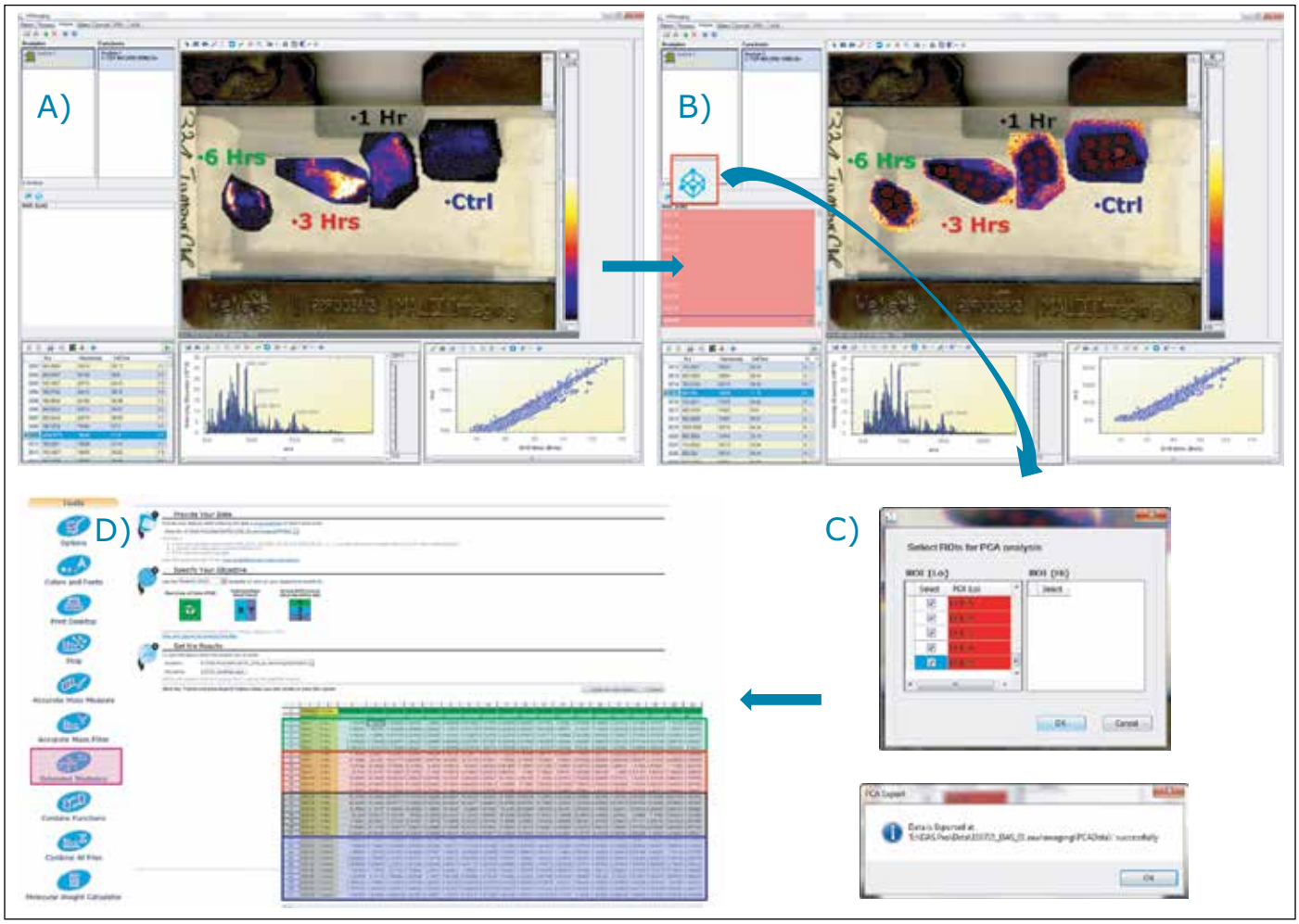

Figure 2. Workflow with A) MALDI imaging processed data loaded in HDI; B) Multiple ROIs drawn for each tissue section; C) Average normalized intensities calculated; D) View of the csv matrix where intensities are reported for each entry (m/z, dt) and ROI - EZinfo is launched directly from MassLynx Software.

Principal component analysis (PCA) of the MALDI imaging data

Following on from the data matrix loaded in EZInfo, as shown in Figure 2D, PCA is performed and the result scores plot displayed in Figure 3A. Each dot represents an ROI observation, which has been color coded to one of the four groups (*i.e.*, one of the tissue section). Scores values for each group clustered well together.

A second data processing step was performed within EZInfo to obtain the loadings data for the analysis as demonstrated in Figure 3B. Each loading dot, representing a (*m/z*, dt) entry, can be selected and a list can be generated comprising peak IDs of each of the selected variables with its associated PCA coordinates (Figure 3C). This list can be imported back into the HDI Software following the route shown in Figure 3D. The loadings plot is recreated in HDI Software (Figure 3E) and the dataset queried to highlight ion images associated with variables from the loadings distribution.

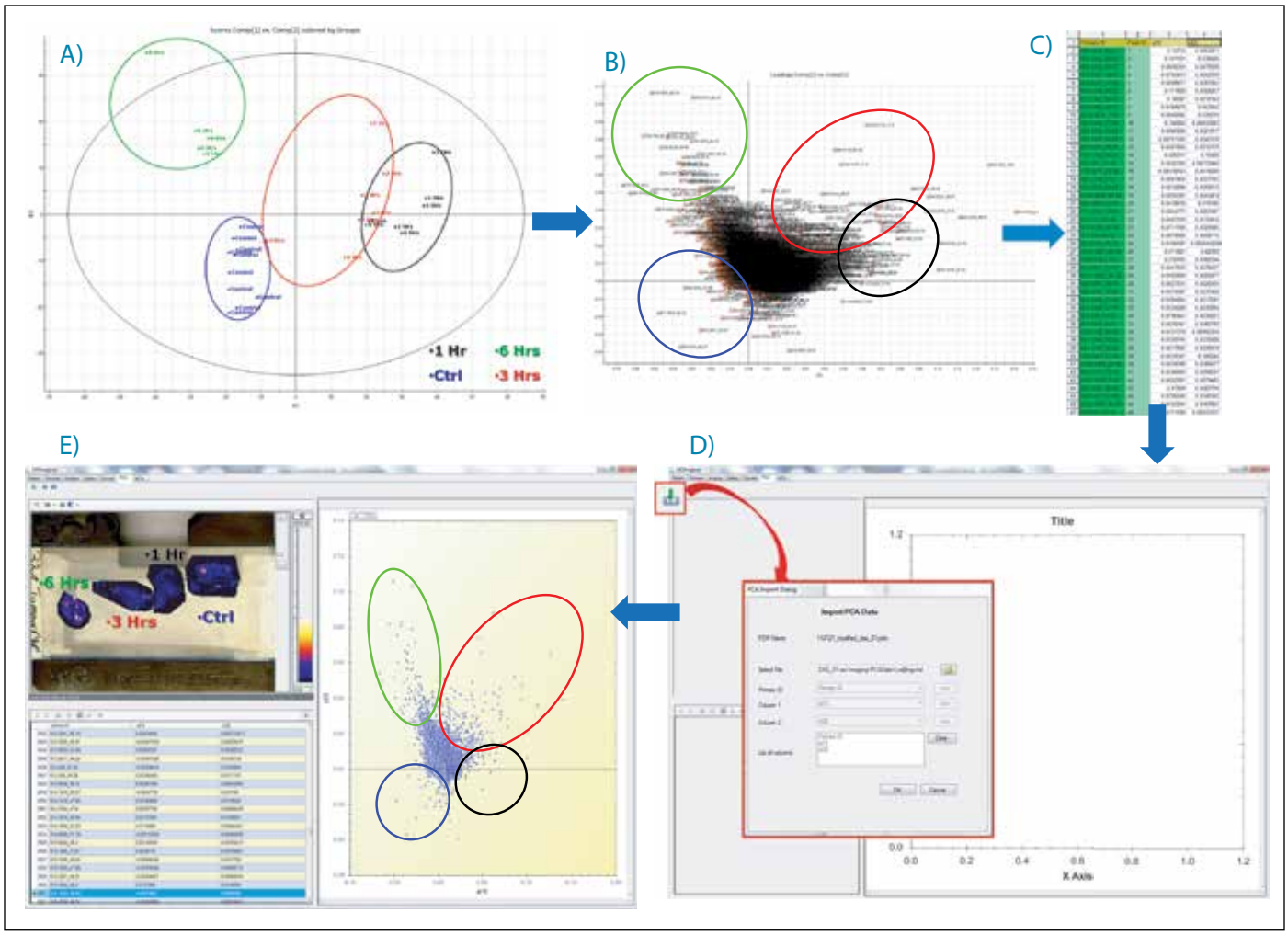

Figure 3. Workflow where A) PCA scores distribution is shown, and B) the resultant loadings distribution is displayed and the all variables are selected to generate the table in C). D) Route where the table is imported back in HDI Software. E) Loadings plot loaded in HDI Software with corresponded ion images displayed.

The latter is illustrated in Figure 4, where ion images of specific tryptic peptides are likely to distinguish the different conditions from the PCA experiments. Figure 4A shows the selection in the loadings distribution of variable (*m/z*_dt) 871.5_62, which is dominant in the control condition compared to the other tissue section images. Figure 4B shows the selection of (*m/z*_dt) 1144.6_74 that is more abundant in the 1 hour condition. Figure 4C shows the selection of (*m/z*_dt) 1033.6_72 that is more abundant in the 3 hours condition and Figure 4D shows the selection of (*m/z*_dt) 1027.54_70 that is more abundant in the 6 hours condition.

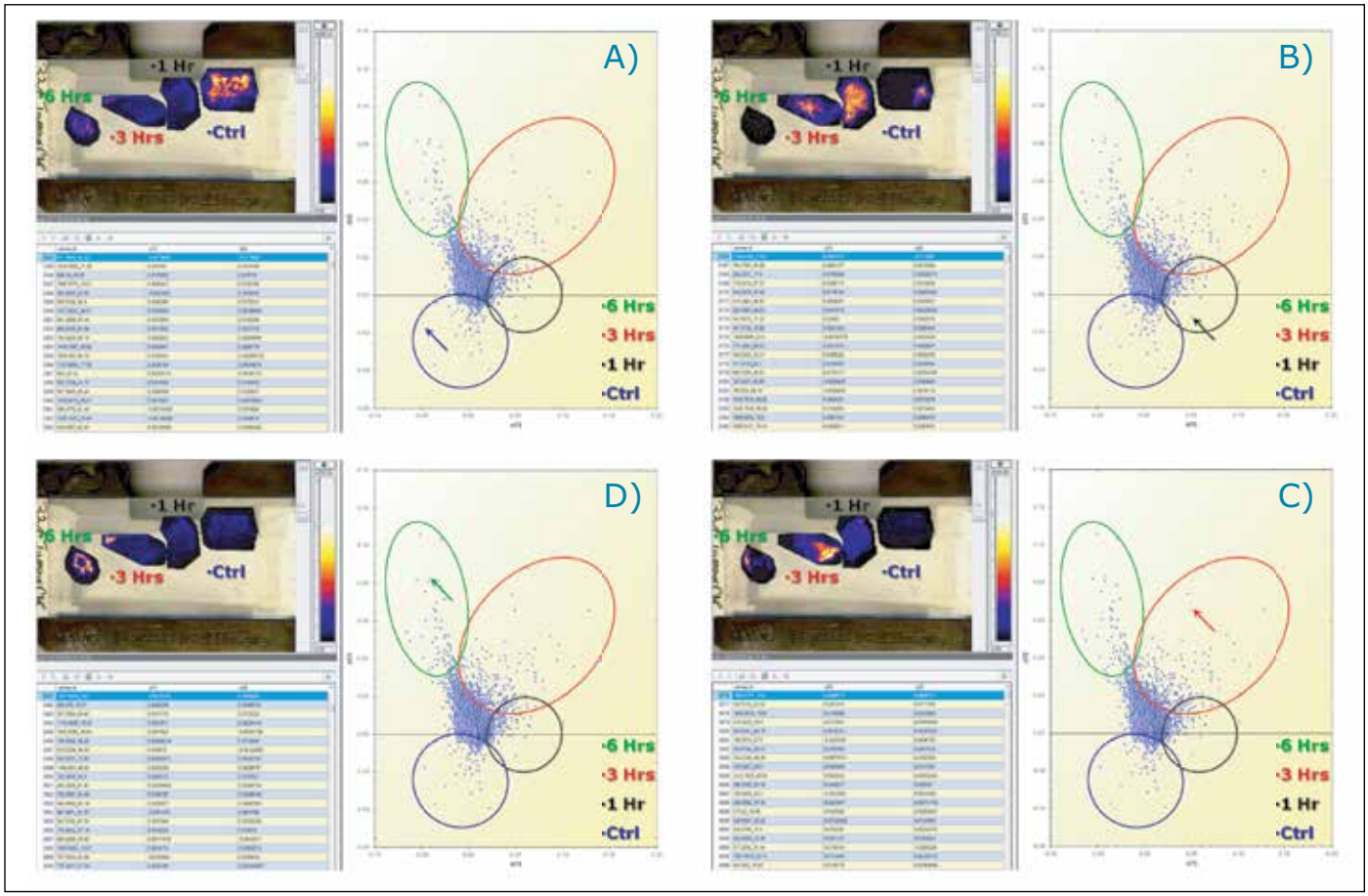

Figure 4. Querying of the PCA loadings distributions to display ion images of the analytes that differentiate the groups. Selection of tryptic peptides A) (m/z_dt) 871.5_62 for the control condition; B) (m/z_dt) 1144.6_74 for the 1 hr condition; C) (m/z_dt) 1033.6_72 for the 3 hrs; and D) (m/z_dt) 1027.54_70 for the 6 hrs condition.

OPLS/OPLS-DA of the MALDI imaging data

From the data matrix of (*m/z*_dt) analyte values, intensities, and group definitions shown in Figure 2D, it is possible to select only two groups, such as group 1 and 3 hours (Figure 5A), which were the two groups that were the least clearly differentiated in the unsupervised analysis.

An OPLS/OPLS-DA distribution was generated (Figure 5B) with its S-plot (Figure 5C) that illustrates confidence of change on the Y-axis and magnitude of change on the X-axis. As for the unsupervised analysis, the loadings data can be imported back into the HDI Software where analyte/ion images associations can be viewed (Figure 5D) and queried (Figure 6).

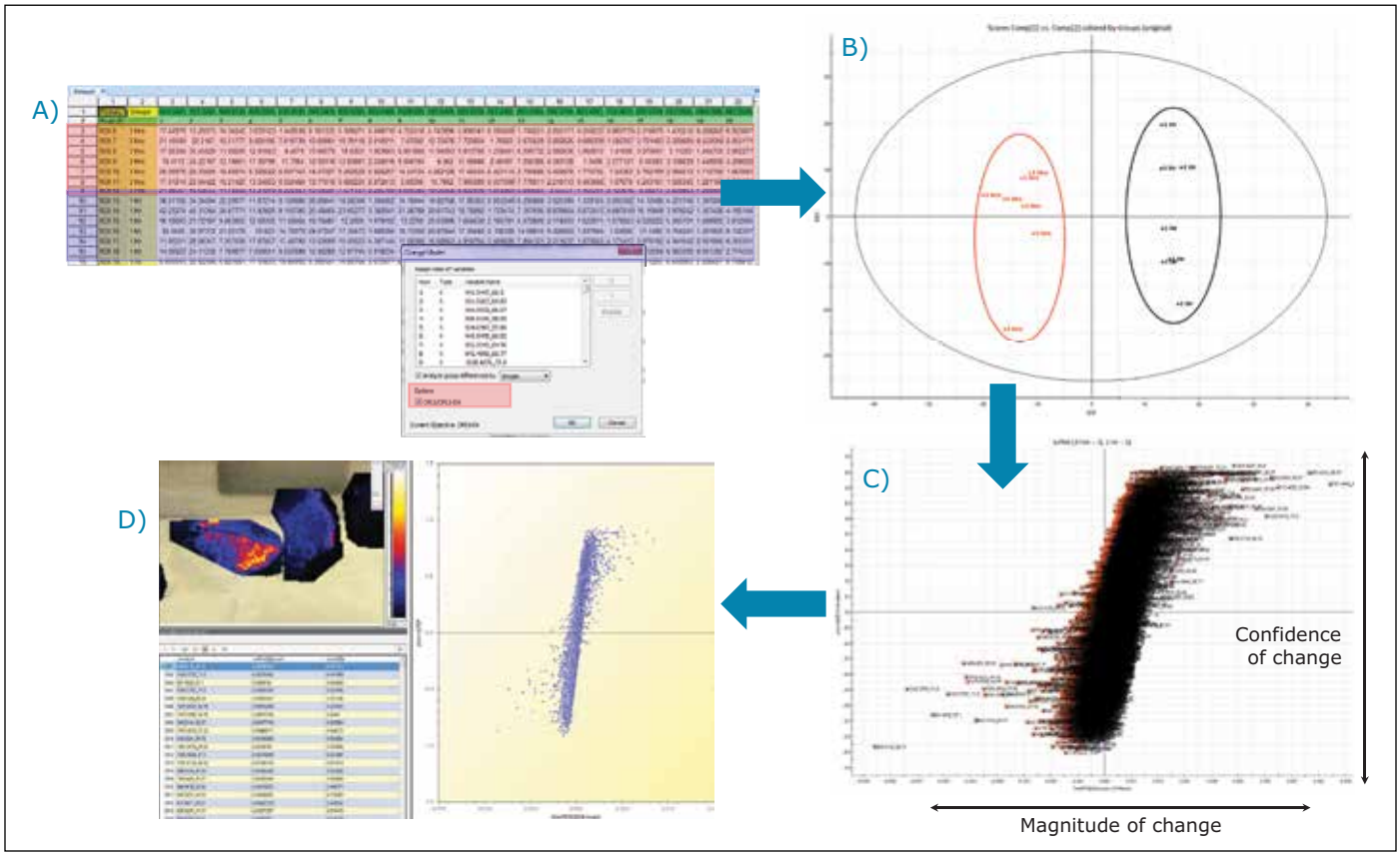

Figure 5. Workflow where A) Amended data matrix with two groups defined; B) OPLS/OPLS-DA scores plot is shown; C) the resultant S-plot is displayed and the all variables are selected to generated the table; D) Loadings plot loaded in HDI Software with corresponded ion images displayed.

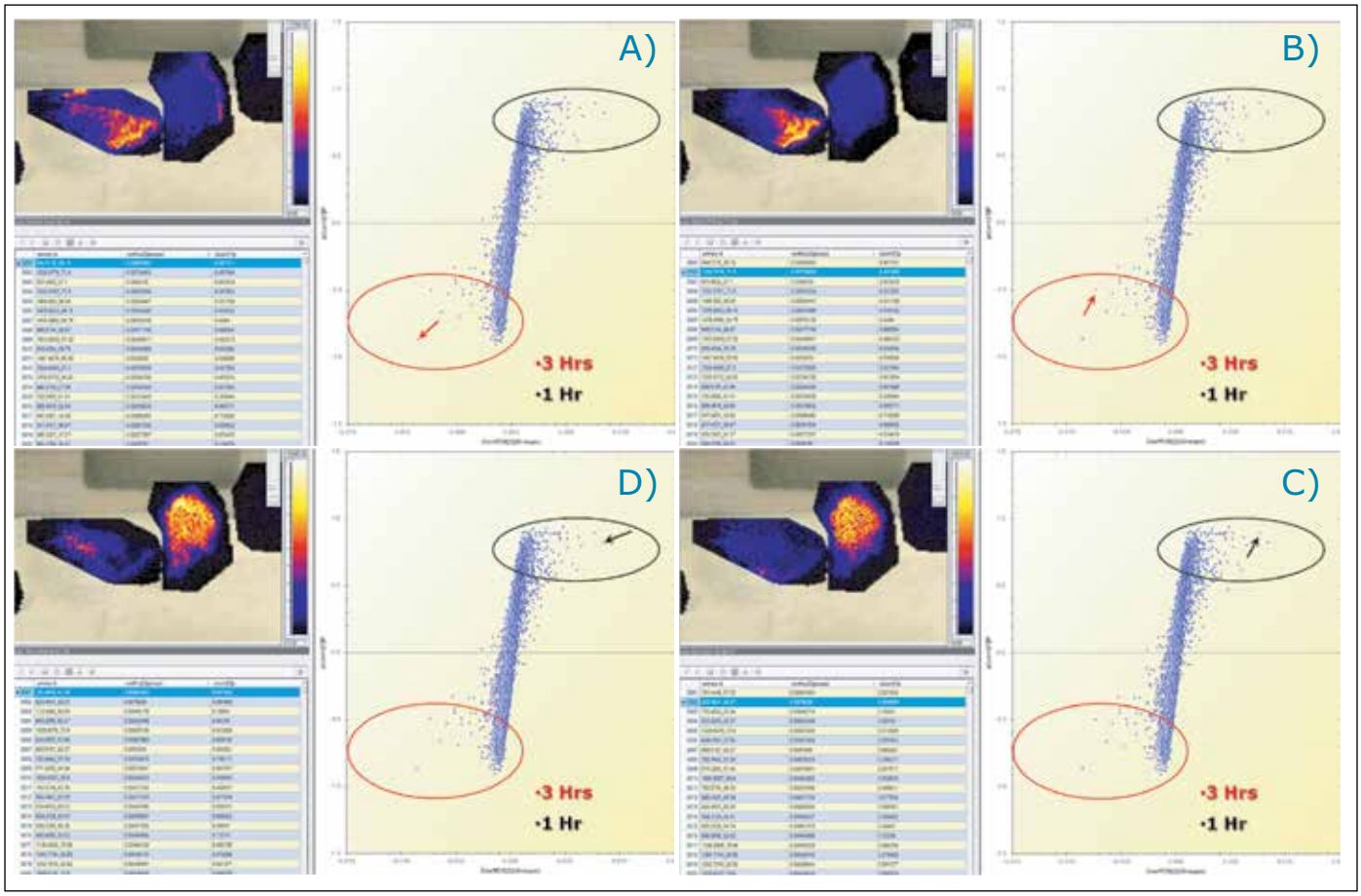

Figure 6. Querying of the OPLS/OPLS-DA S-plot to display ion images of the analytes that differentiate the groups. Selection of tryptic peptides more expressed in the 3 hr tissue section A) (m/z_dt) 944.51_68 and B) (m/z_dt) 1032.58_72. Selection of tryptic peptides more expressed in the 1 hr tissue section C) (m/z_dt) 823.45_60 and D) (m/z_dt) 781.45_57.

CONCLUSIONS

- High Definition Imaging (HDI) Software allows supervised and unsupervised multivarariant analysis on complex multidimensional imaging datasets.
- Multiple statistical analyses can be carried out on the same dataset, including PCA, PLS-DA, and OPLS/OPLS-DA.
- Full integration of the multivarariant analysis results in the HDI Software linked to ions images.
- The interrogation of the data is therefore greatly speeded up and simplified compared to manual mining of the data.

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Improved MALDI Imaging Quality and Speed Using the MALDI SYNAPT G2-S*i* HDMS

SUMMARY

A new laser with an improved laser focus profile has been incorporated into the MALDI SYNAPT® G2-S*i* HDMS™ System, resulting in enhanced ion imaging quality and higher sensitivity for imaging with sub-50-micron pixel sizes. The instrument control has also been optimized so that, combined with the higher laser repetition rates, data is acquired faster.

BACKGROUND

The use of solid-state lasers such as diode-pumped ND:YAG lasers has been critical in the development of MALDI imaging applications, providing higher repetition rates than could be achieved with nitrogen lasers. The MALDI SYNAPT G2-S*i* laser provides higher repetition rates, up to 2.5 kHz, increasing analytical speed. It also has a longer laser life-span, of typically several billion shots.

For high spatial resolution imaging, the main limitation is defined by the diameter of the laser focus, although oversampling can partially overcome this constraint. The improved beam profile of the MALDI SYNAPT G2-S*i* laser allows a tight focus to be produced.

With a new laser, the MALDI SYNAPT G2-S*i* HDMS offers improved MALDI imaging capabilities, with faster analysis of tissue sections and higher data quality for high spatial resolution experiments.

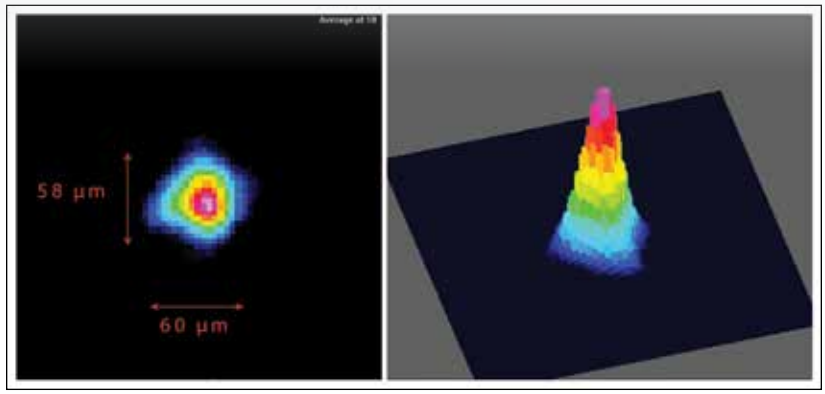

Figure 1. The focused laser beam profile of the new MALDI SYNAPT G2-Si laser.

THE SOLUTION

The MALDI SYNAPT G2-S*i* HDMS Mass Spectrometer introduces a new laser system that offers several enhancements, including:

- Faster maximum laser repetition rate, maximizing analytical speed
- Variable laser repetition rate control, from 100 Hz to 2.5 kHz
- Sharper and rounder beam profile with low eccentricity
- Increase in data quality for high spatially resolved MALDI imaging experiments
- Improved synchronization between the laser firing and the stage movement results in significantly shorter acquisition times

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Figure 1 illustrates the focus profile of the laser included with the MALDI SYNAPT G2-S*i* HDMS. It is circle-shaped with a minimal degree of eccentricity. The profile has an approximate diameter of 30 microns at FWHM, which is significantly smaller than the previous laser. The laser fluence, which also affects the ion desorption area, can be controlled using the built-in variable neutral density filter.

MALDI imaging experiments were carried out using a rat whole body tissue section comparing the quality of the data obtained using the previous laser system and the new laser system (same sample, sample preparation, and mass spectrometer).

The sample target plate was programmed to move in 15, 20, and 50 micron pixel sizes (pitches) while maintaining the minimum laser focus diameter possible for both lasers. The total image summed spectra for each experiment are displayed in Figure 2. In all three cases, the background noise is clearly less abundant, while the lipid peak intensities are higher with the MALDI SYNAPT G2-S*i* laser, especially for the 15 and 20 micron pixel sizes.

Figure 3 shows the overlay of three lipid species that are distributed in very specific anatomical regions of the cerebellum rat brain. The red ion image represents a lipid that is more concentrated in the grey matter of the cerebellum, whereas the blue ion image shows localization of the sample lipid, as an example, in the white matter. The green ion image represents a lipid more specific to the pia matter. It is noticeable that the white matter layer and the pia matter layer are distinguished although their cross sections are less than 100-200 microns.

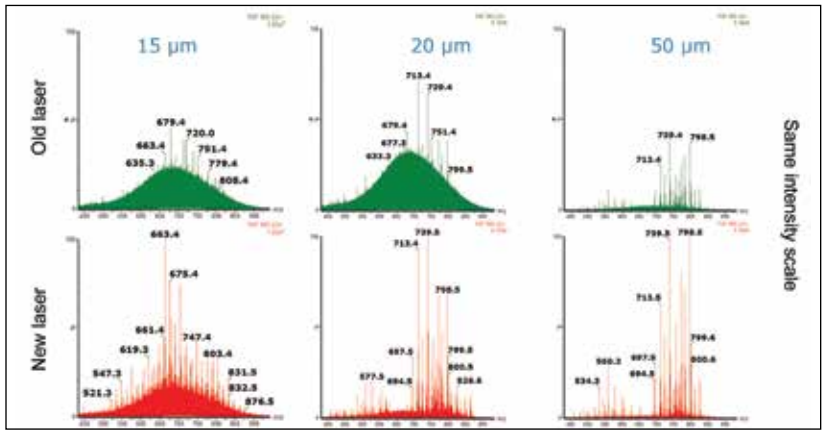

Figure 2. Comparing the summed spectra quality obtained for MALDI imaging experiments carried out with pixel sizes of 15, 20, and 50 microns. Overall, the background noise is lower, and the intensity of lipid species peaks higher with the MALDI SYNAPT G2-Si laser, particularly at spatial resolution sub-50-microns.

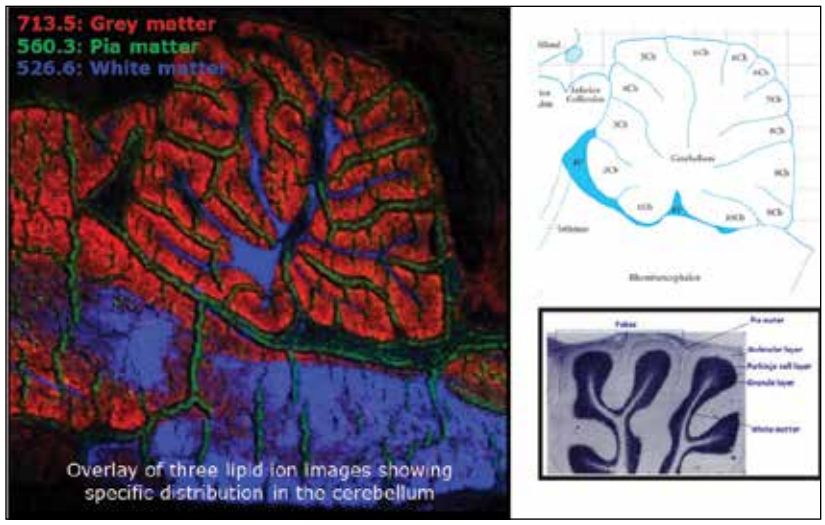

Figure 3. Overlay of three lipid ion images that are distributed to very explicit parts of rat cerebellum. Lipid m/z 713.5 is specific to the grey matter, lipid m/z 560.3 to the pia and lipid m/z 526.6 to the white matter.

SUMMARY

For high spatial resolution MALDI imaging experiments, the MALDI SYNAPT G2-S*i* HDMS can acquire both faster and with greater signal to noise. The enhancements are directly attributed to the improved laser focusing and pulse synchronization.

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

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Waters Corporation, Manchester, UK

APPLICATION 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.

WATERS SOLUTIONS

MALDI Imaging HDMS^E

MALDI SYNAPT™ G2-S HDMS™

KEY WORDS

MALDI imaging, high definition imaging, HDI, identification, lipids, HDMS^E

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.

Method conditions

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.

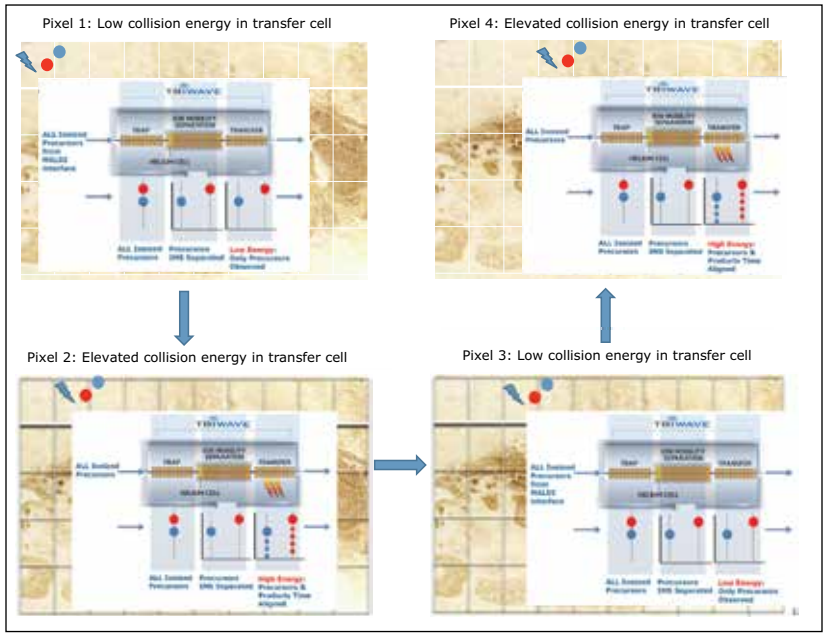

Figure 1. Schematic of a MALDI Imaging HDMS^E experiment.

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).

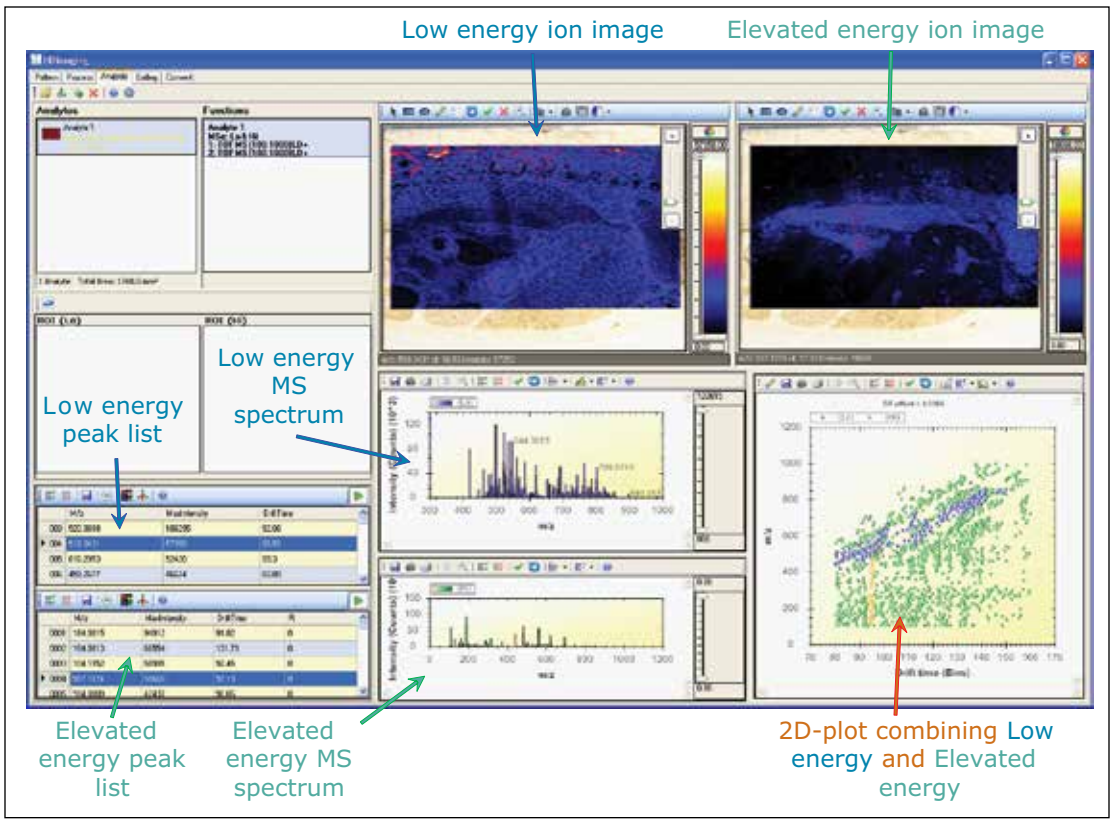

Figure 2. MALDI Imaging HDMS^E view of the processed data with High Definition Imaging DHI Software.

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.

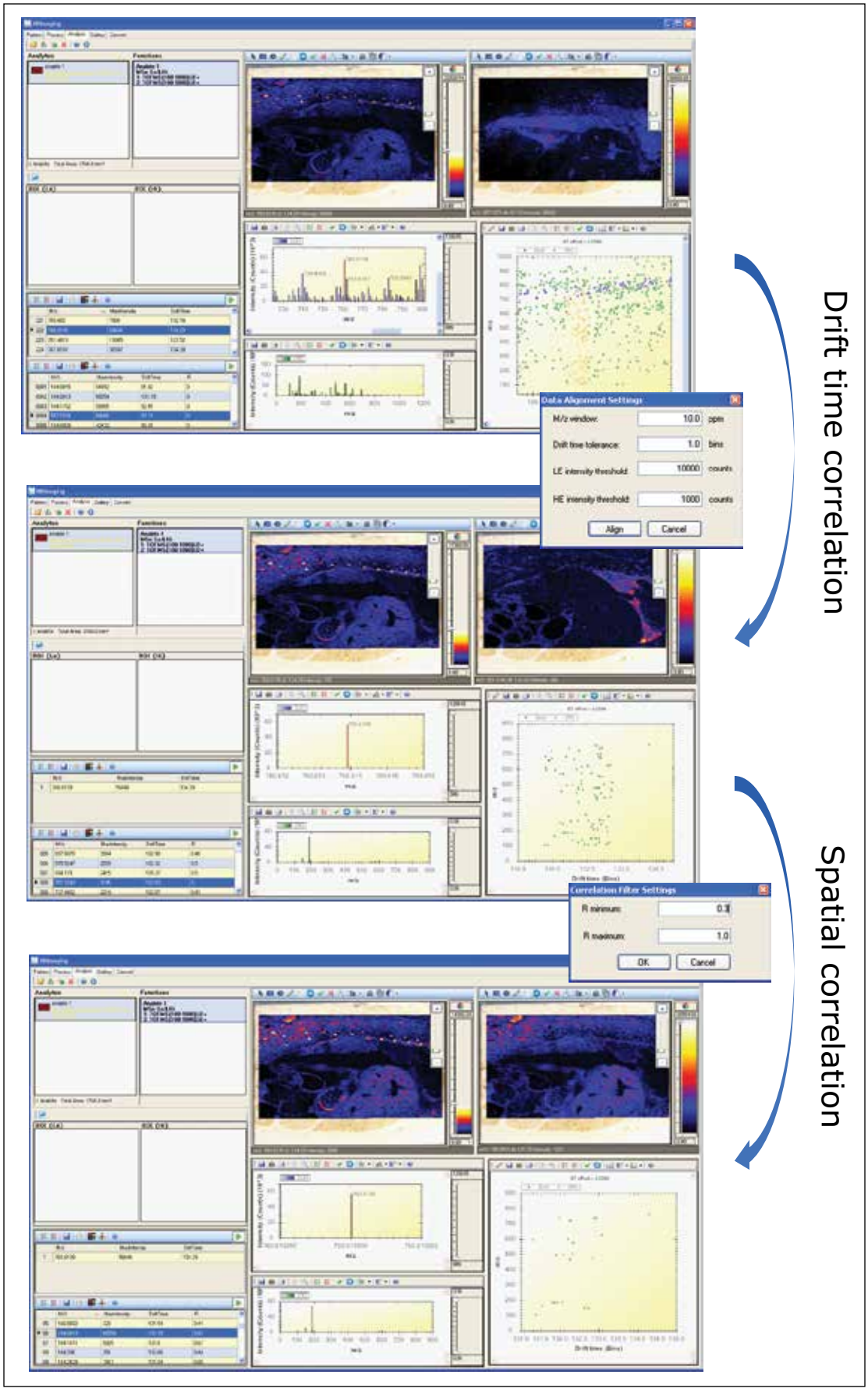

Figure 3. Workflow of the two-step correlation. The top image shows all processed data that were drift time associated with precursor ion m/z 760.0. The middle image shows only fragment ions that were drift time associated to precursor ion m/z 760.6. In the bottom image, spatial correlated fragment ions 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). The annotated MS/MS spectrum is shown in Figure 4.

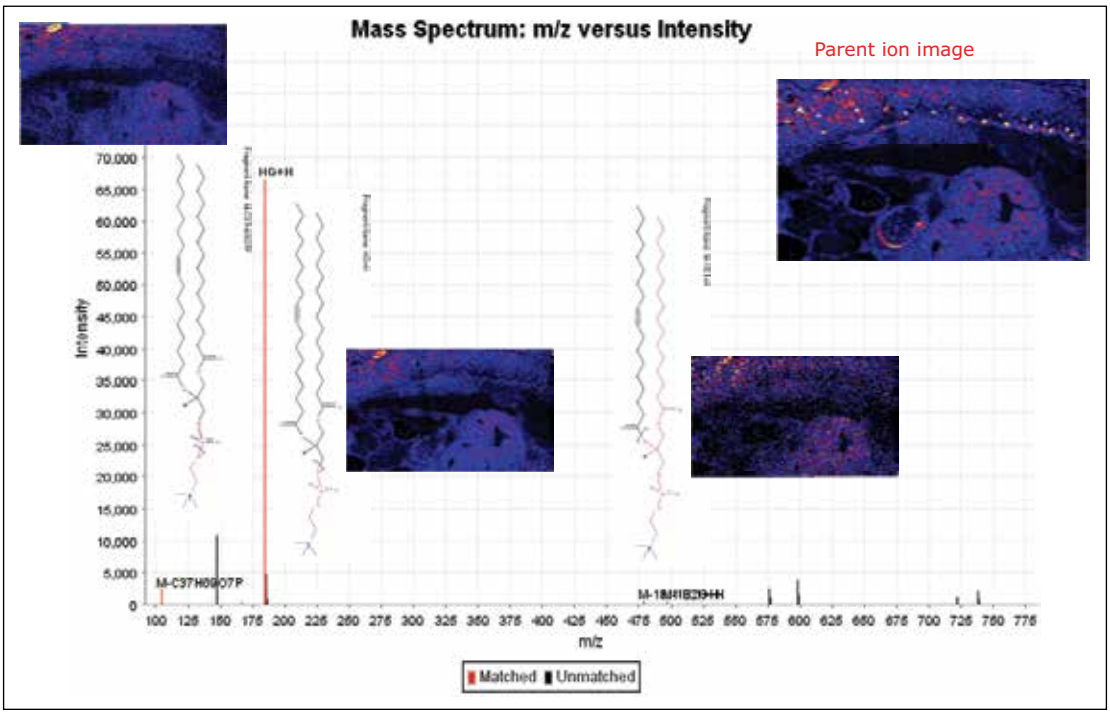

Figure 4. MS/MS spectrum generated from SimLipid 3 with lipid fragment structures and ion images displayed.

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.

CONCLUSIONS

- 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.

Reference

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

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October 2012 720004471EN AG-PDF

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Distribution of Biomarkers of Interest in Rat Brain Tissues Using High Definition MALDI Imaging

GOAL

To illustrate a complete MALDI imaging biomarker discovery workflow through visualization of the effects of various diets on the distribution of Biomarkers of Interest (BOIs) in the rat brain.

BACKGROUND

MALDI is one of several ionization methods that enables mass spectral analysis directly from the sample surface of fresh, unfixed tissues that are difficult to access. The use of MALDI to image tissue sections is gaining popularity, which promises to deliver a complete and accurate structural picture of the tissue for putative biomarker characterization and drug development. Not only does this technique allow determination of BOIs, but it also shows their localization with no anatomical distortion. With very little tissue manipulation and disruption, MALDI provides an important advantages for drug development and for the understanding of (metabolic) diseases. Undoubtedly, this technique will become very useful for the demonstration of central nervous system effects of diet, cognition, obesity, gut-brain interactions; metabolic diseases such as diabetes, hypertension, Alzheimer's; and inflammatory conditions.

Most metabolomics or lipidomics biomarker discovery studies use biofluids (mainly blood and urine) in order to evaluate the biological process that occurs in tissues and organs that are not easily accessible. In this technology brief, we demonstrate how MALDI imaging allows direct analysis of BOIs in tissues; the workflow is shown in Figure 1. This novel approach could lead to a better understanding of the physiological processes and the pathophysiology of diseases because it allows both discovery and localization of biomarkers.

Gain a better understanding of physiological processes and pathophysiology of diseases with High Definition MALDI Imaging.

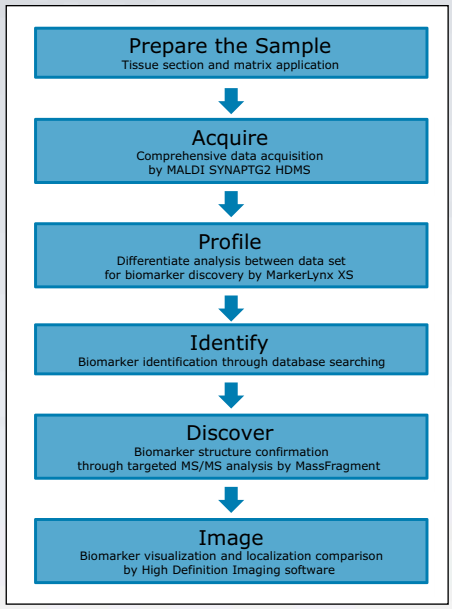

Figure 1. MALDI imaging bioanalysis workflow.

THE SOLUTION

The left brain hemispheres of two rats kept on different diets were sliced in 10 µm sections using a cryostat. Tissue sections were mounted on microscope slides, frozen, and stored at -80 °C until use. 2,5 dihydroxybenzoic acid (DHB) was used as the matrix and a TM Sprayer was used for the matrix application. MALDI imaging analysis was performed on the Waters® MALDI SYNAPT® G2 HDMS.™ Positive HDMS full scan data were acquired for the mass range of *m/z* 100 to 1000. The Nd:YAG laser was operated at a firing rate of 200 Hz with a spatial resolution of 75 µm. Waters new High Definition Imaging Software (HDI) was used for the visualization of MALDI imaging data.

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After data acquisition, the complex data set was processed by HDI software and analyzed using MarkerLynx™ XS Software. The discovered BOIs were identified by database searching using exact mass molecular ion. The identity of the BOIs was confirmed and validated by performing targeted MS/MS experiments, combined with MassFragment Software to propose assignments for the precursor and fragmentation peaks, and matching them between the data generated from the standards and directly from the tissue sample.

Visualization was performed and compared in HDI using both exact mass and drift time information. Figure 2 shows the MS/MS spectrum of an indentified BIOs, a lipid phosphatidylcholine (PC) (18:1 (9Z)/16:0) or PC (16:0/18:1 (9Z)) observed at *m/z*=760.5859. The structure of the lipid was confirmed by matching the diagnostic fragmentation pattern of the spectrum with the standard one. The average mass error for the six diagnostic ions in the MS/MS spectrum was 0.48 mDa. Figure 3 shows the MALDI image comparison for the same lipid from the brain tissues of the two rats fed by different diets. Very different distributions of the lipid are observed in the two images, indicating that the found lipid biomarker may be of importance.[1] Using this technology, it was possible to highlight several BOIs reflecting the impact of the different diets.

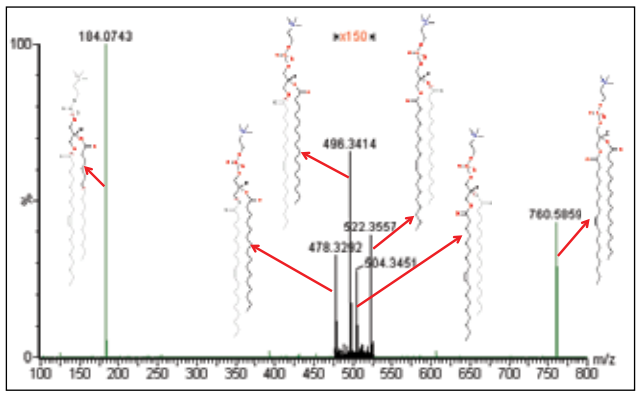

Figure 2. Biomarker structure confirmation through targeted MS/MS analysis. Fragment ions assigned by MassFragment.

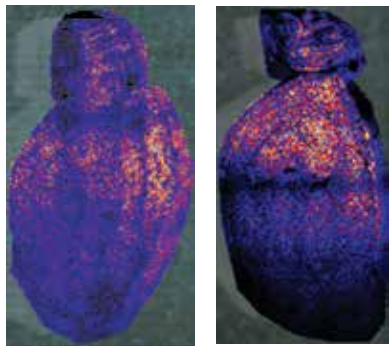

Figure 3. Lipid biomarker MALDI image localization and distribution comparison using High Definition Imaging (HDI) Software.

SUMMARY

This technology brief demonstrates a complete MALDI imaging biomarker discovery workflow for use in rat brain tissues and its modification by dietary components.

Waters High Definition Imaging (HDI) Software integrates pattern generation, HDMS data processing, and visualization in a single interface.

In high definition MALDI imaging, the combination of MarkerLynx XS and MassFragment software enable discovery, identification, and confirmation of BOIs within tissue samples.

Compared to traditional biomarker discovery analysis (mainly LC-ESI-MS approach), this workflow has the potential to lead to a better understanding of physiological processes, as well as the pathophysiology of diseases, with added information about their spatial distribution in heterogenous, not easily accessible tissue sample.

This approach may be applicable to biomarker discovery and distribution analysis in areas including metabolomics, lipidomics, and proteomics in various application fields such as: metabolic diseases, aging, gut-brain interactions, cognition, and inflammatory diseases associated to the health benefits linked to adequate nutritional interventions.

1. Koizumi S, *et. al.* Imaging Mass Spectrometry Revealed the Production of Lysophosphatidylcholine in the Injured Ischemic Rat Brain, Neuroscience 168 (1):219-25(2010).

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Tissue Imaging of Pharmaceuticals by Ion Mobility Mass Spectrometry

Stacey R. Oppenheimer[1], Emmanuelle Claude[2], and Tasneem Bahrainwala[3]
[1]Groton, USA; [2]Waters Corporation, Manchester, UK; [3]Waters Corporation, Beverly, MA, USA

Cyclosporin (CsA) is a drug commonly used as an immunosuppressant that functions as a signal transduction kinase inhibitor; however, CsA has been shown to induce kidney injuries in humans[1]. The objective of this study was to examine the distribution of CsA within renal tissues at varying known doses to induce a certain degree of toxicity.

The traditional approach for MALDI imaging of small molecules, e.g. drug compounds in tissue, utilizes a targeted MS/MS approach followed by mass analysis. This selective strategy provides confirmation of the identity of the drug and enables the molecules to be differentiated from endogenous signals of the same molecular weight. However, some small molecules do not produce satisfactory fragmentation and must therefore be monitored by their intact mass in the MS mode.

Cyclosporin (Figure 1), does not produce intense fragment ions in MS/MS mode and conventional MALDI-TOF MS alone was unable to provide the selectivity required for the analysis.

In this application note, High Definition Mass Spectrometry™ (HDMS™) was used as an alternative approach for imaging CsA distribution. HDMS is based on travelling wave (T-Wave™) technology[2] incorporated into the mass spectrometer. Triwave™ consists of three T-Wave devices, as shown in Figure 2. The first T-Wave (Trap) is used to trap ions during the period when an ion mobility separation (IMS) is being performed in the second T-Wave, thus greatly enhancing the efficiency of the IMS process. The final T-Wave (Transfer) transports the separated ions to the TOF analyzer.

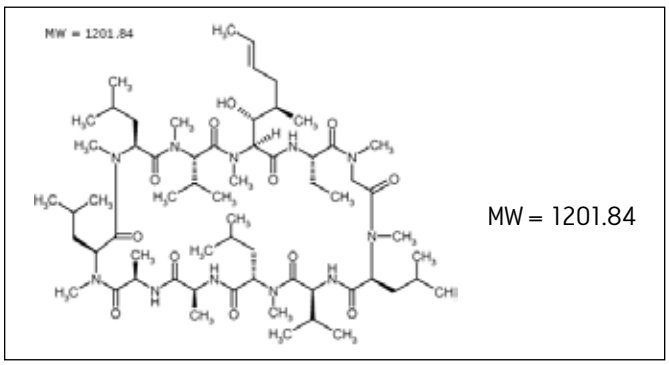

Figure 1. Chemical structure of Cyclosporin (CsA).

EXPERIMENTAL

Mouse kidneys (control, 20 mg/kg, and 80 mg/kg, frozen subcutaneously for seven days) were sectioned at 20 µm thickness and thaw-mounted onto MALDI target plates. Subsequent sections were acquired for histology staining and anatomical visualization. Matrix [30 mg/mL of 2,5-dihydroxybenzoic acid (DHB) in 50.0/50.0/0.1 (v/v/v) water/methanol/trifluoroacetic acid] was deposited with a nebulizing spray device (manual nebulizer or ImagePrep (Bruker Daltonics, Bremen, Germany).

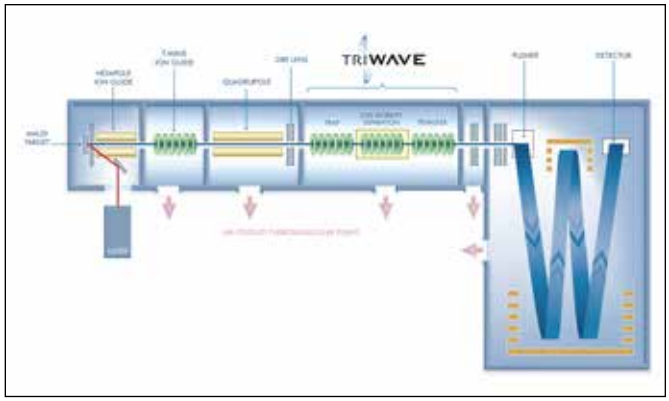

Figure 2. Schematic of the MALDI SYNAPT HDMS.

The image area was selected using MALDI Imaging Pattern Creator (Waters Corporation, Manchester, UK). Data were acquired using Waters® MALDI SYNAPT™ HDMS System in HDMS positive ion mode over the *m/z* range 100 to 1,500 at an image resolution of 150 x 150 µm and a laser speed of 200 Hz; Figure 2 shows a schematic view. Post acquisition, the ion mobility dimension of the data was evaluated using DriftScope™ Software. Image reconstruction was performed using BioMap (Novartis, Basel, Switzerland).

RESULTS

In the mass range of the drug compound, the background ions from the tissue and matrix were intense. Here, the most abundant ion species was the [M+K]+ signal at *m/z* 1240.84; without the selectivity of the ion mobility separation, it was difficult to distinguish drug-related ions.

Figure 3 shows a comparison of the DriftScope 2D plots obtained from the control kidney and the 80-mg/kg-dosed kidney, zoomed around the [M+K]+ ion. The red circle indicates the position of the ion species from CsA in the dosed kidney data. In addition, the drift time of the ion was different from the drift time of the interfering background ions to enable specific selection of CsA. Therefore, it is possible to extract very specifically the CsA [M+K]+ ion species from the DriftScope 2D plot and recreate the ion-image.

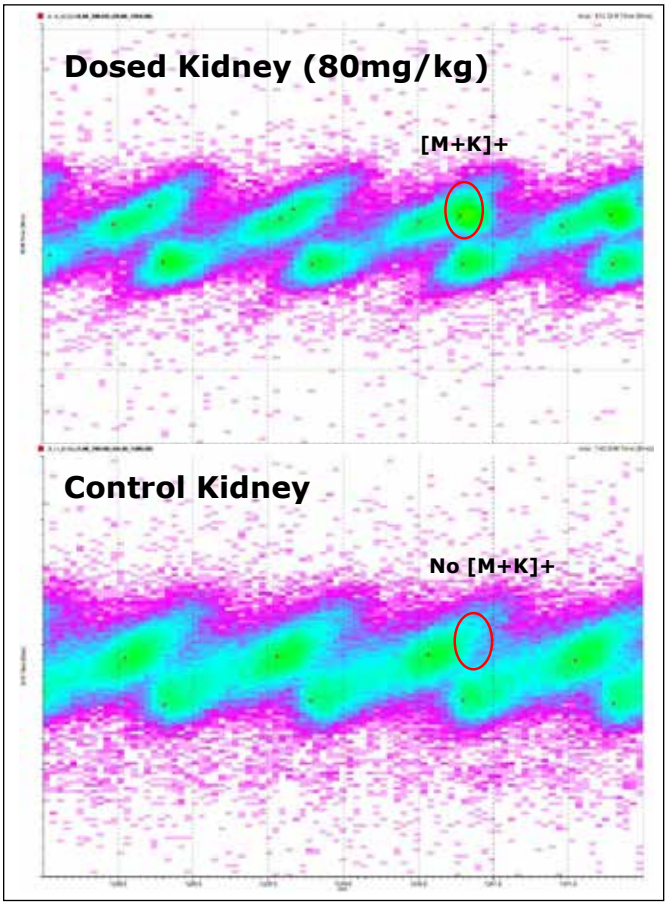

Figure 3. DriftScope 2D plots of the control and 80-mg/kg-dosed tissue sections.

Figure 4 shows the mobilogram (drift time versus intensity plot) of ion *m/z* 1240.8 in the dosed tissue, again showing the presence of two species at the same *m/z* value, each with different mobility.

The mass spectrum for each species can be extracted and the mass spectrum on the left-hand side represents the interference species, whereas the mass spectrum on the right-hand side corresponds to the Cyclosporin drug.

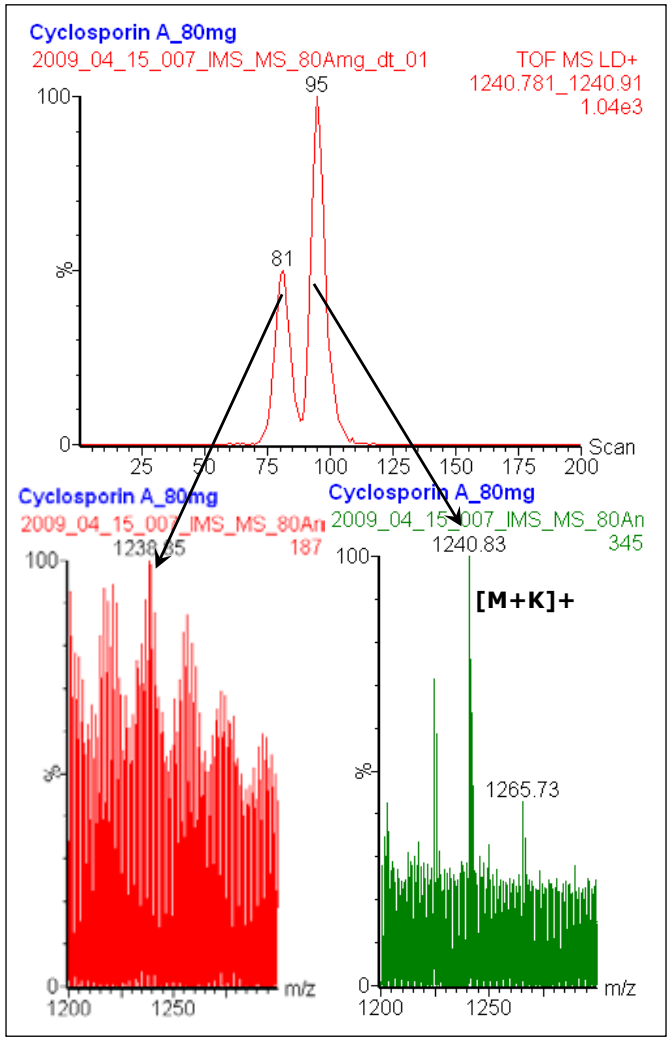

Figure 4. Top: Mobilogram of m/z 1240.8. Bottom: Extracted MS spectra with specific drift time from each species.

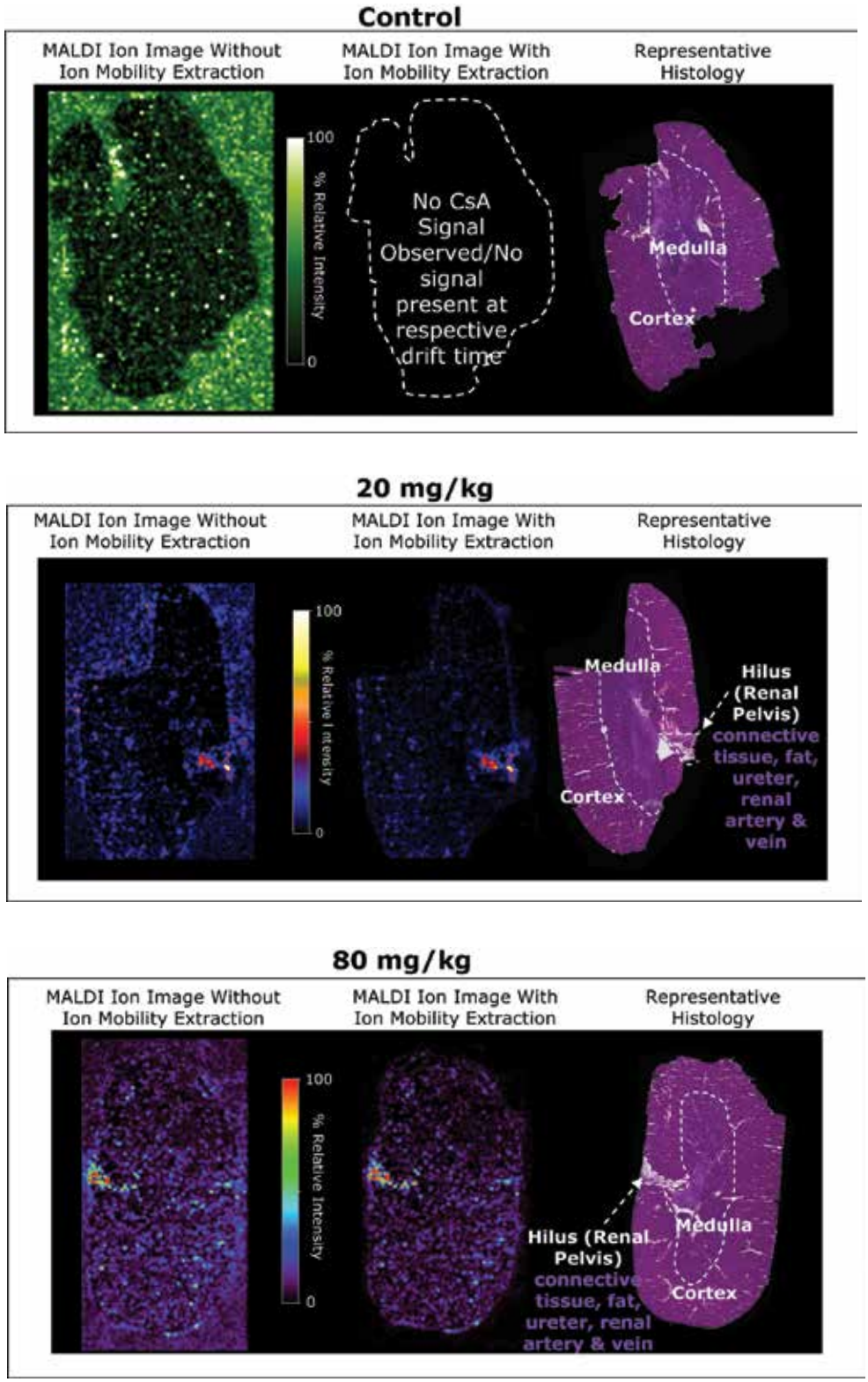

Figure 5. CsA ion reconstituted images of control, 20 mg/kg, and 80 mg/kg-dosed kidney, with and without ion mobility separation, compared to the histology image.

Figure 5 illustrates the effect of ion mobility on the MALDI ion images. Images of control, low, and high CsA-dosed renal tissues are shown. Each panel contains the ion image prior to and after drift time selective extraction of the CsA signal from the DriftScope data, together with the corresponding histology image. For each reconstructed image, the same *m/z* range was selected. The matrix ion was used for normalization purposes.

The ion mobility 2D plot from the control sample confirms that no signal corresponding to CsA is present endogenously in renal tissues, but the image reconstructed from the *m/z* value (without incorporating drift data) corresponding to CsA reveals the distribution of the background ions present at that *m/z* value. The ion images shown from dosed tissue, before and after the use of DriftScope to isolate the analyte, demonstrate the added selectivity provided by ion-mobility separation. The 20-mg/kg dose was near the lower limit of detection for CsA.

BIOLOGICAL DISCUSSION

Images from the 80-mg/kg and the 20-mg/kg CsA-dosed samples illustrate CsA's distribution to the renal medulla, cortex, papilla, and hilus. Less drug is present in the 20-mg/kg sample, but shows a similar distribution pattern to the 80-mg/kg sample.

The drug was more highly concentrated in the hilus region than in the cortex or the medulla. The hilus region contains the renal pelvis where concentrated urine, containing the drug to be excreted, accumulates prior to its passage to the bladder. The renal artery within the hilus region may also contribute to the higher drug concentrations. The renal artery carries blood into the kidney where it is filtered and then exits through the renal vein.

CONCLUSIONS

- The advantage of applying IMS as a first-dimension separation of ions prior to time-of-flight (TOF) mass analysis for imaging pharmaceuticals in tissues was demonstrated in this study.
- In the case of pharmaceutical compounds that do not give satisfactory MS/MS fragmentation, the selectivity of the traditional approach, where only mass analysis is taken into account, can be poor. In the case of CsA, the drug was confounded by unresolved background ions. The control image highlights the amount of interfering signal at the same m/z as CsA.
- Incorporation of ion mobility separation prior to TOF mass analysis in an imaging experiment enabled the true visualization of CsA distribution in the renal tissues, without interfering signal obstruction.

References

1. Kahah BD. Transplantation Proceedings, 2009; 41: 1423-1437.
2. Giles K, Pringle S, Worthington K, Little D, Wildgoose J, Bateman R. Rapid Commun. Mass Spectrom., 2004; 18: 2401-2414.

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A Novel High Definition Imaging (HDI) Informatics Platform

GOAL

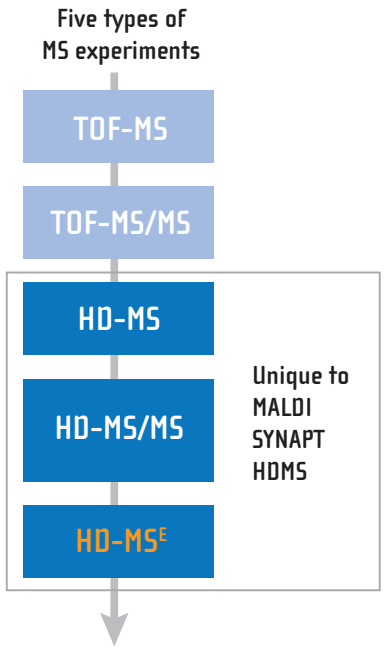
A brief overview of the new Waters® High Definition Imaging (HDI™) Software solution that allows the maximum information to be obtained from imaging experiments that combine ion mobility with mass spectrometry.

BACKGROUND

Imaging using mass spectrometry is a rapidly expanding area that has extensively used MALDI ionization. Waters has pioneered the use of ion mobility spectrometry with MALDI imaging experiments. Ion mobility allows ions to be separated in the gas phase by their size and shape prior to MS, allowing differentiation of isobaric species. This is directly integrated in all High Definition Mass Spectrometry (HDMS™) systems fitted with a MALDI source, and has been extensively used during the analysis of molecules directly ionized from tissue samples.

To access the detailed spatial information contained within the data, dedicated software is required. Waters MALDI SYNAPT® G1 and G2 raw data have previously been converted into an ANALYZE 7.5 format to be visualized using BioMAP (Novartis, Switzerland). However, this software approach is not integrated; therefore, it is not designed for incorporating the ion mobility dimension.

Waters has recently developed a proprietary software solution, designed for MS imaging, that provides a seamless workflow and makes full use of the ion mobility MS data.



HDI Imaging Software enables users to easily acquire and visualize MALDI HDMS^E data for fully incorporated ion mobility information.

Figure 1. Experiments supported using Waters HDI Imaging Software.

THE SOLUTION

Waters' new HDI Imaging Software is designed to simplify and streamline the imaging workflow allowing the user to fully integrate all of the steps in an MS imaging experiment for MALDI SYNAPT Mass Spectrometers using a single intuitive interface. An outline of the workflow is shown in Figure 1, which details the different experiments that are supported.

HDMS^E is a patented method of data acquisition that records the exact mass precursor and fragment ion information for every detectable component in a sample. HDMS^E rapidly alternates between two functions: the first acquires low-energy precursor ion spectra and the second acquires elevated-energy (CID) fragment ion data. Precursor and fragment ions are deconvoluted and reconstructed by alignment of their ion mobility drift-times. This drift-time aligned data can subsequently be visualized in Waters HDI Software.

Workflow of the HDI Software

The initial step is to use the pattern definition tool to assign reference points to the photographic image, in order to select the area of interest for HD imaging.

The processing experiment file is created directly from the HDI Software and loaded into a MassLynx™ Software sample list. Processing of the data using the algorithm Apex 3D to create a peak list with *m/z* and drift time information occurs automatically after acquisition.

The resulting raw data are processed in the Analysis Section of the software, where all types of experiments described in Figure 1 are supported. Analysis of the acquired imaging data sets fully incorporates the ion mobility information, which is integrated into the data processing and visualization, as shown in Figure 2. This allows the distribution of molecules such as drugs, lipids, or peptides to be determined without the interference of background ions or isobaric species.

Smooth interactions between the available visualizations – including the peak list table, mass spectrum, drift time versus *m/z* plot – and the ion images allow scientists to analyze their data in a powerful, user-friendly fashion.

Following fully automated HDI data acquisition and processing, the results can be exported as raw data for statistical treatment using MarkerLynx™ XS Application Manager, or directly into other MassLynx applications for further processing, such as the elemental composition tool, or MassFragment.™

The user-defined grid gallery allows the comparison of a series of ion images of interest by using the Red/Green/Blue (RGB) overlay capability.

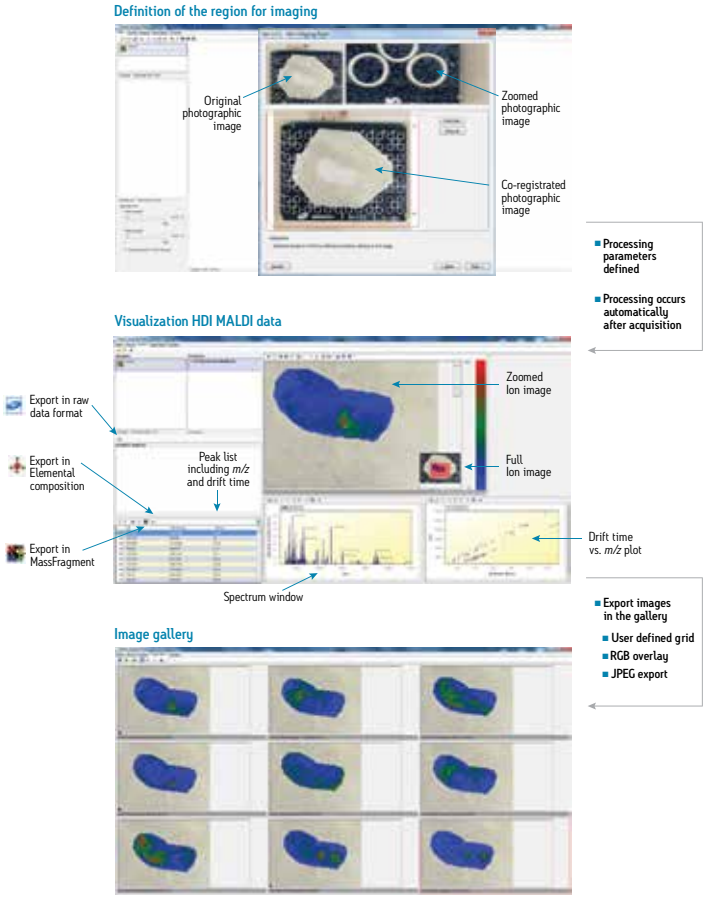


Figure 2. Acquired imaging data sets fully incorporate the ion mobility information that is integrated into the data processing and visualization tool.

SUMMARY

- Waters' High Definition Imaging Software is a new, fully integrated software suite for MALDI imaging experiments.
- Integration of HDI data acquisition processing and visualization is performed in a single interface.
- For the first time, MALDI imaging ion mobility information is fully incorporated and used within the imaging software.
- MALDI HDMS^E data can be acquired and easily visualized.
- Flexible export options are available for calculating elemental composition, statistical analysis using MarkerLynx XS Application Manager, or analysis with MassFragment.

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Collision Cross-Section Determination of Lipids on the MALDI SYNAPT G2 HDMS System

GOAL

To determine the collision cross-sections of singly charged lipid ions analyzed by MALDI mass spectrometry.

BACKGROUND

Lipidomics is a rapidly expanding field of research, where mass spectrometry plays a key role. Moreover, visualizing the localization of lipids within a tissue section is challenging since there are no antibodies specific to lipids. However, imaging by MALDI mass spectrometry allows the location of different classes of lipids directly from tissue sections to be visualized, thereby enhancing lipid studies.

The use of ion mobility to evaluate the size and shape of ions in the gas phase is a technique which is rapidly gaining recognition. Initial studies were carried out on proteins; however, it has now been demonstrated that it is possible to use ion mobility to measure the collision cross-section of other types of molecules, like lipids.

The MALDI SYNAPT® G2 HDMS™ System (schematic shown in Figure 1) provides an ideal platform to conduct these imaging studies, and in addition, allows the collision cross-sections to be calculated during the same experiment. Furthermore, data is acquired at high resolution, enabling exact mass measurements to be made on both precursor and fragment ions, coupled to the ability to separate target analytes from isobaric background interferences using gas-phase ion mobility separations.

The MALDI SYNAPT® G2 HDMS™ System provides an ideal platform to conduct lipid imaging studies, and in addition, allows the collision cross-sections to be calculated during the same experiment.

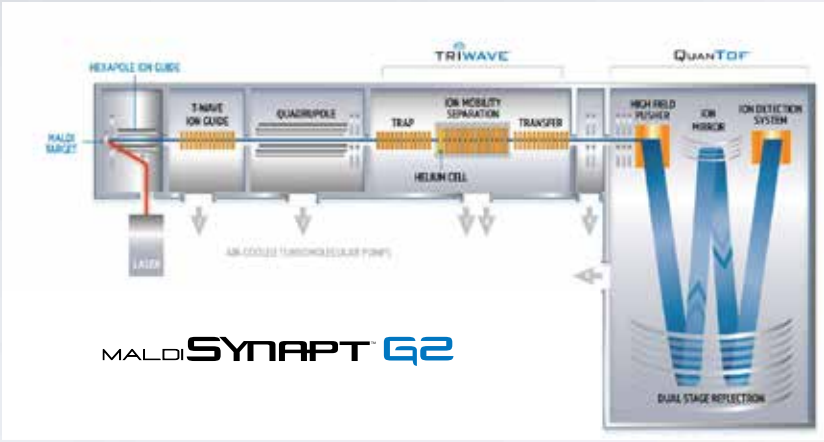

Figure 1. Schematic of the MALDI SYNAPT G2 HDMS System.

THE SOLUTION

First, we calibrated the IMS T-Wave®[1] using polyalanine, with previously determined collision cross-section values (from http://www.indiana.edu/~clemmer). The calibration curve was generated with DriftScope™ Software using a power trend line where R=0.9995.

To calculate the collision cross-sections of each lipid standard, PC (16:0/16:0), PS (18:1/18:1), and PG (16:0/18:1) (Avanti Polar Lipids) were mixed individually with α-cyano-4-hydroxycinnamic acid (CHCA) matrix, spotted onto a MALDI stainless steel target, and analyzed under identical conditions to those used for the polyalanine standard. The analysis was performed using a 1 kHz Nd:YAG laser system on a MALDI SYNAPT G2 operated in HDMS mode. CCS calculations were also determined for the sodiated and potassiated species, when present.

Figure 2 shows that the collision cross-section, which was calculated to be 214.9 Å^2 for MH$^+$ of PC (16:0/16:0). This is in accordance with previously published results[2] by Ridenour et. al., where their CCS calculation of these lipids on a MALDI SYNAPT (G1) HDMS instrument were found to be 215.3 Å2 +/- 3.6.

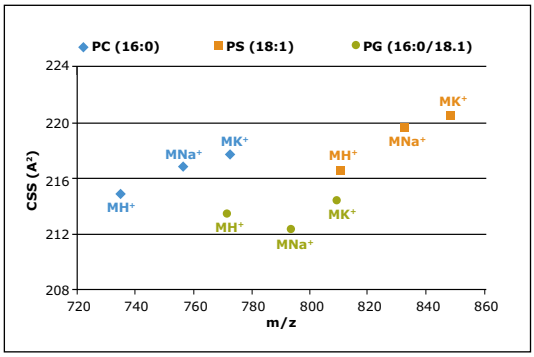

Figure 2. Collision cross-section calculation for lipid standards.

The collision cross-section of the Na$^+$ and K$^+$ species of the PC and PS standards indicate a more open configuration in the gas phase relative to the MH$^+$ species when compared to the PG lipid standard. Here the Na$^+$ and K$^+$ of the PG ion appear to be more compact in the gas phase versus their MH+, which could indicate that the lipid may be folded around the salt.

Further analysis was carried out on a rat tissue section, under the same IMS conditions and the CCS of lipids calculated directly from tissue section, as shown in Figure 3. As lipids tend to have similar mass defects for each class, the data was colored following the first decimal of the m/z. Here, the trend lines can be observed in the data following the mass defect.

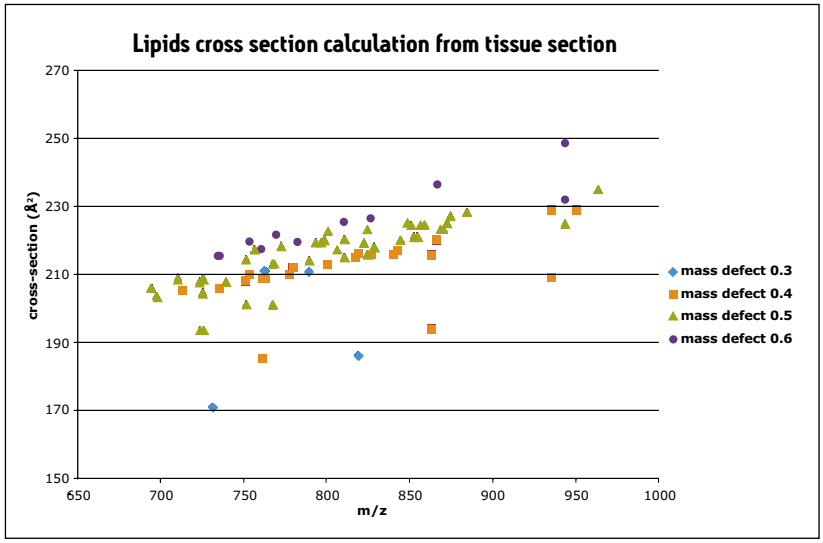

Figure 3. Collision cross-section calculation for lipids analyzed directly from a tissue section. Data is colored by mass defect of the lipids.

SUMMARY

The MALDI SYNAPT G2 HDMS System enables the calculation of collision cross-sections of lipid standards. Here we have observed that the sodiated PG lipid standard had a more compact configuration in the gas phase compared to its counterpart MH+.

This type of analysis can also be carried out for endogenous species analyzed directly from tissue sections. In a single experiment, it is possible to calculate collision cross-sections for endogenous species and carry out a MALDI imaging experiment to observe their location throughout the tissue.

References

1. The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).
2. Structural characterization of phospholipids and peptides directly from tissue sections by MALDI traveling-wave ion mobility-mass spectrometry. Ridenour WB, Kliman M, McLean JA, Caprioli RM. Anal Chem. 2010 Mar 1;82(5):1881-9.

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Utility of Desorption Electrospray Ionization (DESI) for Mass Spectrometry Imaging

Emmanuelle Claude and Emrys Jones

GOAL

To describe the DESI imaging technique as applied to mass spectrometry imaging using time-of-flight (TOF) mass spectrometers, such as the SYNAPT® G2-S*i* or the Xevo® G2-XS.

BACKGROUND

In the past few years, mass spectrometry imaging (MSI) has seen a rapid increase in interest and utilization in areas such as proteomics, biomarker discovery and validation, drug distribution, and clinical research. MSI was originally developed using a matrix assisted laser desorption ionization (MALDI) mass spectrometer, where the sample is prepared by first coating it with an ionizable matrix. Then, the sample is placed under vacuum and a rastering laser is used to ioninze molecules in the sample for analysis by a TOF mass spectrometer.

More recently, an ambient ionization technique called desorption electrospray ionization (DESI) was introduced and applied to MSI to allow for the direct analysis of surfaces at atmospheric pressure. DESI imaging uses a charged jet of solvent to deposit micro-droplets onto a surface where analytes are extracted and desorbed into the gas phase at ambient pressure and temperature. Subsequently, they are drawn into the MS inlet where they can be analyzed using a TOF-MS. This technique is compatible with Waters® SYNAPT G2-S*i* or Xevo G2-XS Mass Spectrometers.

DESI imaging provides effective and meaningful molecular spatial localization within a variety of samples with minimum sample preparation.

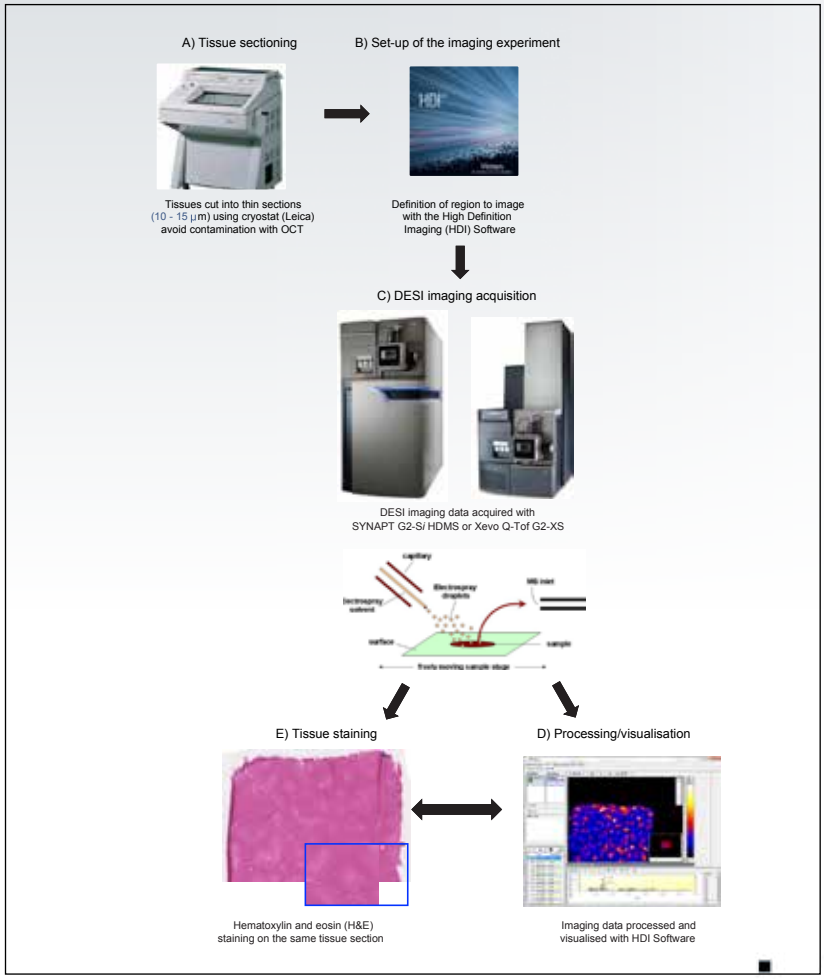

Figure 1. Workflow of a DESI imaging experiment.

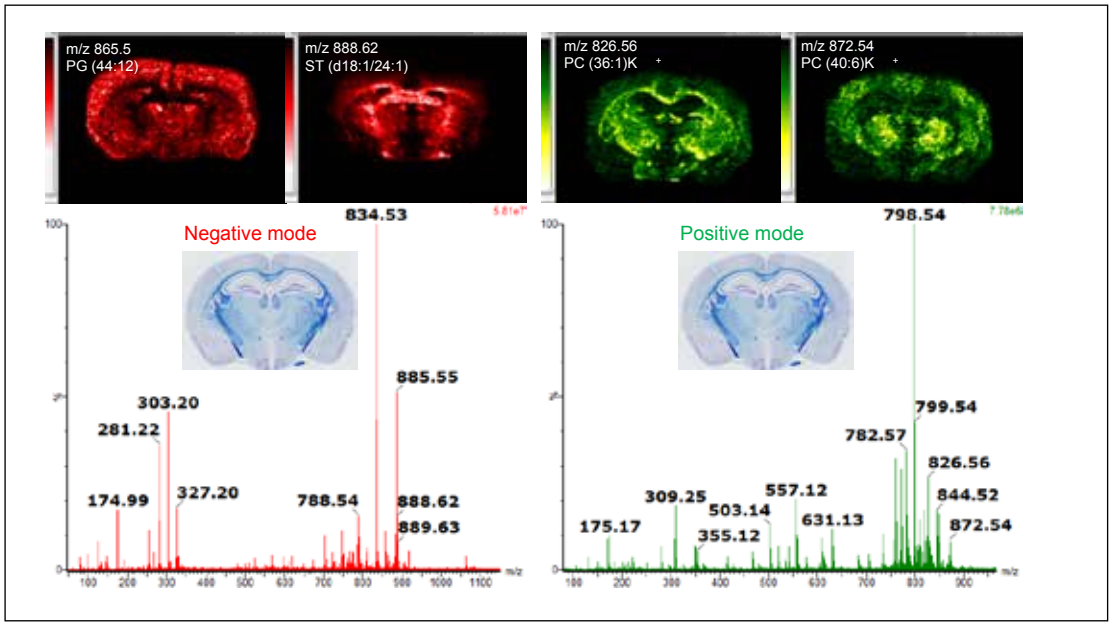

Figure 2. Multimodal imaging from a single sample. DESI imaging experiments on mouse brain tissue sections[1] with the full combined MS spectra in positive (green) and negative (red) ion modes.

THE SOLUTION

To perform a DESI imaging experiment, a fresh frozen thin tissue section is mounted onto a glass slide directly from the cryostat or freezer. The slide is placed onto the 2D linear moving stage of the DESI source without any other pre-treatment. An optical image is taken and co-registered with the High Definition Imaging (HDI®) Software (Figure 1B). This optical image is then used to define the rectangular area to be imaged. The surface of the tissue section is rastered line-by-line using the DESI sprayer with a mass spectrum collected at predefined X,Y coordinates. The pixel size in the X-direction is defined by the speed of the stage movement and acquisition rate of mass spectra. The pixel size in Y-direction is defined by the distance between two lines of acquisition. Typically, DESI imaging experiments are acquired with pixel sizes of 50 µm or more.

Raw imaging data is subsequently processed and visualized within the HDI Software (Figure 1D). When using optimized DESI imaging conditions, the sample is preserved to such an extent that the tissue section can be haemotoxylin and eosin (H&E) stained directly after MS acquisition (Figure 1E). This allows the H&E stained optical image to be overlaid with the DESI molecular images from the same tissue section.

Figure 2 displays ion images of lipids species from consecutive mouse brain sections;[1] one acquired in positive (green) ionization mode and one acquired in negative (red) ionization mode, using a solvent of 90:10 methanol: water, at a pixel size of 100 µm. Both polarities provide DESI MS spectra very rich in small molecules and phospholipid species that localize into specific features within the mouse brain.

DESI Imaging Versatility

DESI imaging, being a surface analysis technique, can be applied to numerous types of samples, varying from animal and human tissue samples, to plant material, pharmaceutical tablets, and even isolated bacterial colonies on agar. Figure 3 shows a selection of ion images from a variety of samples acquired using a range of pixel sizes from 100 to 200 µm, measured either in positive or negative ionization mode. Figure 3A is an ion image of oleic acid in porcine liver, highly concentrated in the center of the liver lobules co-localized with the central vein. Figure 3B shows the potential to apply DESI imaging to forensic trace evidence

analysis by capturing molecular images from a fingerprint. Figure 3C displays the overlay of two small molecules, differentially distributed within a bacterial colony grown on agar.[1] Figure 3D illustrates the analysis of a human tissue sample[2] that contains both normal tissue and a secondary tumor tissue. The distribution of two lipids *m/z* 698.51 (PE (O-34:3)) and *m/z* 773.53 (PG (36:2)) specifically correlates to the identity of the tissue type. In this example PE (O-34:3) is specifically localized within the tumor region.

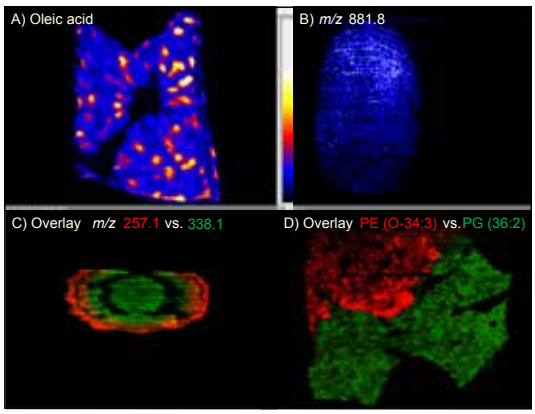

Figure 3. Application flexibility with DESI imaging. A) ion image of oleic acid in porcine liver, B) m/z 881.8 ion image of a human fingerprint, C) overlay of m/z 257.1 (red) and 338.1 (green) from a bacterial colony,[1] and D) overlay of ion images m/z 698.51 (PE (O-34:3) (red) and m/z 773.53 (PG (36:2)) (green) from human liver sample.[2]

SUMMARY

DESI imaging represents a significant enhancement in the capabilities of mass spectrometers to analyze and determine spatial localization and molecular distribution of target molecules within a variety of samples. Use of DESI imaging has the advantages of requiring minimal sample preparation to collect a wealth of molecular information. When optimized, the technique allows for either multiple analyses of a single sample (with different MS polarities if desired) or enables additional visualization techniques (i.e. staining) to be performed on the sample after DESI imaging is complete. As seen is these examples, DESI imaging has been shown to be very effective in analyzing small molecules such as lipids or other small molecule cellular metabolites.

The advantages of DESI imaging include:

- Accommodation of multimodal imaging analyses on a single sample (i.e., DESI and MALDI; positive and negative ion mode analysis)
- Minimum sample preparation
- Non destructive, multimodal image analysis to maximize information from precious samples
- Compatibility with additional analysis techniques after DESI imaging (i.e., staining)
- Excellent sensitivity for a variety of small molecule analytes (i.e., lipids and small molecules)
- Flexibility to analyze a wide variety of sample types and analytes

Acknowledgements

1. We thank Professor Ron M.A Heeren and Karolina Skraskova from Maastricht University, Maastricht, The Netherlands, for providing the mouse brain sample and Dr. Jacob Malone and Dr. Gerhard Saalbach from the John Innes Center, Norwich, UK, for providing the bacterial colony sample.
2. This study was carried out in conjunction with Imperial College London, UK. For the analysis of human samples, ethical approval was obtained from the National Research Ethics Service (NRES) Committee London – South East (Study ID 1/LO/0686).

   This work was supported by European Research Council under Starting Grant Scheme (Grant Agreement No: 210356) and the European Commission FP7 Intelligent Surgical Device project (contract no. 3054940).

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Generation of Multiple Images from a Single Tissue Section with Dual Polarity Desorption Electrospray Ionization Mass Spectrometry

Emmanuelle Claude and Emrys Jones
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GOAL

To demonstrate the flexibility of desorption electrospray ionization (DESI) imaging, showing that the same tissue can repeatedly be imaged at the molecular level in positive and negative ion mode, to maximize information about molecular distribution in tissue.

BACKGROUND

DESI imaging, a surface analysis technique incorporating an electrospray probe, can be utilized as an imaging technique by rastering a surface under an ionizing solvent sprayer using a high precision X,Y stage. As the droplets impact upon the surface, chemical constituents are desorbed and carried towards the atmospheric inlet of the mass spectrometer. Ionization of the desorbed molecules occurs via the charge imparted onto the droplets. In contrast to other ionization techniques often used for tissue imaging (i.e., MALDI), no special sample preparation, such as coating of the tissue section with a specialized solvent/ionizable matrix mixture, is required. In this study, gas and solvent flow rates as well as ionization voltages are optimized to allow DESI imaging experiments that preserve the tissue sample being analyzed. These less destructive DESI ionization conditions provide an opportunity for a single tissue section to be analyzed multiple times with the same or different experimental conditions or techniques.

Sequential analysis of a single tissue section for comprehensive molecular profiling is made possible with dual-polarity DESI imaging.

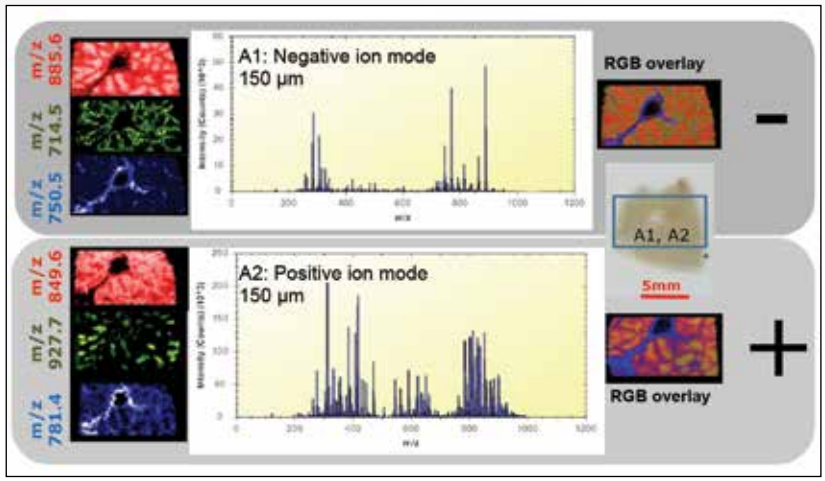


Figure 1. Two successive DESI imaging analyses of the same region of a tissue section from porcine liver. A1) First analysis, 150 µm spatial resolution negative ion mode, three color overlay and average spectrum. A2) Second analysis, same raster conditions– 150 µm spatial resolution positive ion mode, three color overlay and average spectrum.

Fresh frozen tissues of porcine and human liver were sectioned on a cryo-microtome to 15 µm thickness and thaw-mounted onto conventional glass slides. When required, the samples were stored at -80 °C. Immediately prior to analysis, the samples were brought to room temperature and placed directly onto the stage of the DESI source. No further sample preparation was required.

A 2D-DESI source was mounted onto a SYNAPT G2-S*i* HDMS Mass Spectrometer. Spray conditions were set as follows: flow rate of 1.5 µL/min, with a 90:10 MeOH:water mixture at 100psi N_2 gas pressure, and a voltage of 5kV for both polarities. To conduct the imaging experiment, a raster pattern was defined over the tissue region of interest and the scan speed and line spacing were selected appropriately for the target pixel dimensions. For 150 µm resolution images, the stage was scanned at 0.15 mm per second on the X-axis; and stepped 0.15 mm in the Y-axis between each DESI line scan. In all instances, the MS scan time was 1 second.

As the flow rates used are sufficiently low and the desorption was considered a soft event, the same tissue section can be analyzed more than once without modification or exhaustion of the surface molecules- allowing dual polarity analysis on the exact same section for increased information depth.

Initially, imaging experiments on porcine liver were performed with the MS operating in negative mode, subsequently followed by imaging the same tissue section in positive mode (Figure 1). In both modes of ionization, plentiful lipids and endogenous metabolites were detected, giving intense peaks for analysis by the mass spectrometer.

A second experiment was designed to evaluate whether the repeated imaging of the same tissue sample alters the chemical information obtained. Figure 2 compares the spectrum from a single DESI imaging experiment in positive mode (top), with the positive mode spectrum (bottom) generated from a consecutive tissue section, after first analyzing it in negative mode. Identical peaks were observed in both spectra with very similar relative intensities.

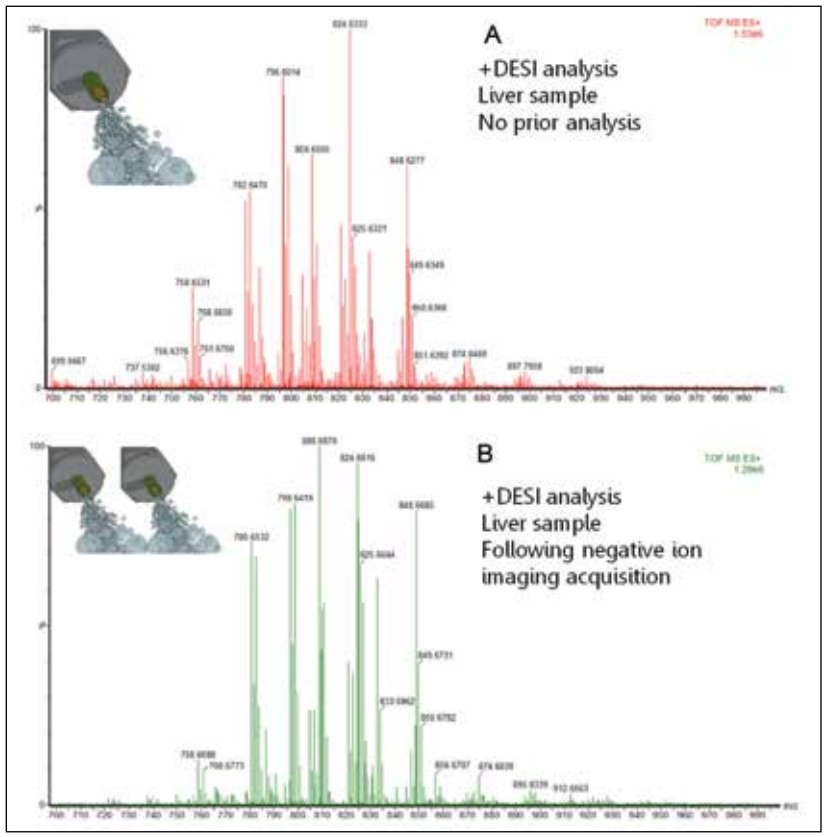
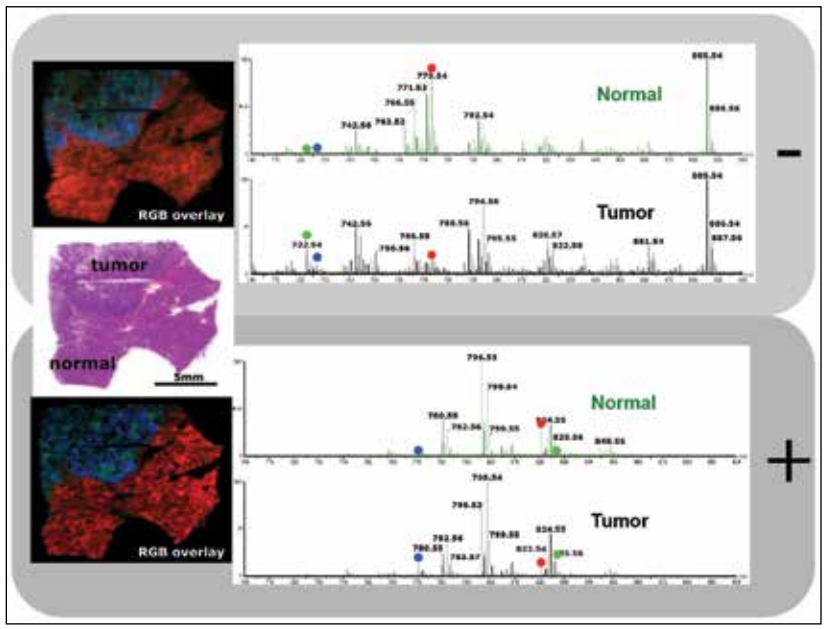


Figure 2. Combined positive mode mass spectra from similar regions of serial porcine liver sections: A) on a pristine surface B) on an altered surface where a full negative ion DESI imaging experiment was previously carried out.



The ability to revisit the same section to increase the amount of information could be of great importance when samples are precious, for example with a human liver sample. Figure 3 shows one such example where the same section was analyzed by MS imaging in both polarities. Lipid species specific to tumor tissue and healthy tissue were identified in positive and negative ion mode. This would seem to indicate that the tissue has not been significantly affected by the DESI imaging technique and is still relatively intact. By not perturbing the tissue with DESI imaging, subsequent additional surface analysis or staining techniques (i.e., H&E staining) could be utilized on the same tissue sample for further, more comprehensive characterization.

SUMMARY

DESI imaging provides metabolite and lipid molecular information directly from a tissue section with no tissue sample pre-treatment. With low flow rates, the tissue section can be analyzed multiple times without significant degradation of signal or modification of the chemical signature obtained from the tissue. Using the same analytical MS instrument setup, information rich spectra in both positive and negative ion mode can be generated without the need to change solvent or analysis conditions. Therefore, positive and negative ion mode DESI data can be obtained from the same tissue and combined for extended chemical coverage and sample differentiation.

Here we demonstrate that by optimizing DESI imaging conditions, a wealth of molecular information can be accessed from a single tissue section. Multiple images containing unique information can be collected from a tissue sample without the need for the analysis of a number of serial sections.

The advantages of DESI imaging include:

- Optimization of DESI conditions allows multiple imaging experiments to be performed on the same tissue sample without altering the architecture or composition of the tissue
- Both positive and negative ion mode DESI imaging data can be collected from a single tissue section
- Using optimized DESI conditions enables subsequent analysis of tissue sections by other visualization techniques (i.e. H&E staining)

Acknowledgments

1. This study was carried out in conjunction with Imperial College London, UK.
2. For the analysis of human samples, ethical approval was obtained from the National Research Ethics Service (NRES) Committee London – South East (Study ID 11/LO/0686).
3. This work was supported by European Research Council under Starting Grant Scheme (Grant Agreement No: 210356) and the European Commission FP7 Intelligent Surgical Device project (contract no. 3054940).

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Multiple, Sequential DESI Images from a Single Tissue Section at Different Spatial Resolution

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GOAL

To demonstrate that desorption electrospray ionization (DESI) imaging can be utilized as a non-destructive imaging technique, which allows multiple analyses in rapid succession on the same tissue section at different spatial resolution.

BACKGROUND

DESI, a surface analysis technique incorporating an electrospray probe, can be utilized as an imaging technique for a broad range of samples. Imaging of a sample is accomplished by rastering a surface under a spray of ionized solvent using a high precision X,Y stage. As the electrospray droplets impact the sample surface, chemical constituents are desorbed and carried towards the atmospheric inlet of a mass spectrometer for analysis. Ionization of various analytes is provided by the charge imparted onto the droplets. Unlike other mass spectrometry based imaging techniques, such as matrix assisted laser desorption ionization (MALDI), no sample preparation (i.e., matrix addition) is required for imaging a sample.

When collecting images from a sample using DESI imaging, the characteristics of the ionized solvent spray used to desorb analyte molecules from the sample affects the spatial resolution of the imaging experiment. The spatial resolution of the image collected can be manipulated to allow for higher or lower levels of spatial resolution as desired by the researcher.

DESI imaging allows multiple analyses of a single tissue section at different spatial resolutions.

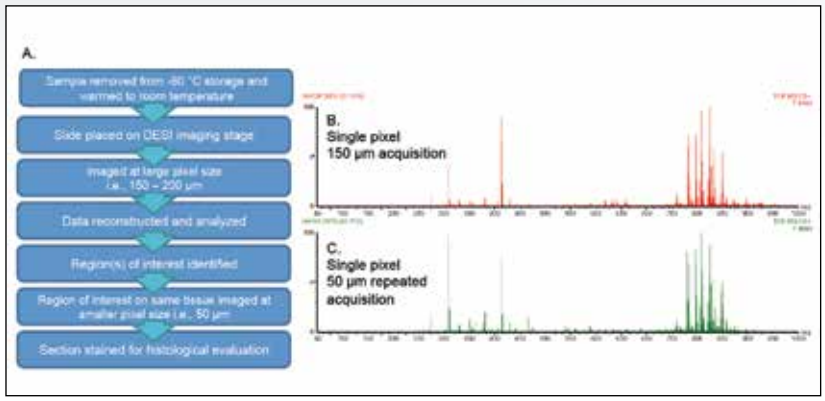

Figure 1. A: Workflow for acquiring multiple DESI images with different spatial resolutions. B: MS spectrum from a single pixel of 150 µm resolution on a pristine porcine liver section. C: MS spectrum from a single pixel of 50 µm resolution on a previously DESI imaged porcine liver section.

Moreover, by modifying the conditions used with the DESI technique, the amount of sample surface disruption can be tightly controlled such that the sample is not destroyed when obtaining an image.

This ability to control and manipulate many of the parameters utilized for DESI imaging allows a single sample to be analyzed multiple times with different experimental conditions or techniques (i.e., one experiment at low spatial resolution, followed with a higher spatially resolved experiment to further characterize a region of interest). This experimental flexibility also allows a DESI imaging study to be followed with hematoxylin and eosin (H&E) or the use of another staining or imaging technique on the same sample.

THE SOLUTION

In this study, a SYNAPT® G2-S*i* Mass Spectrometer equipped with an enhanced DESI imaging source was used to analyze a number of tissue samples. Data collection and image analysis were performed using MassLynx® and HDI® v1.3 Software.

Snap frozen tissues of porcine and human liver were sectioned on a cryo-microtome to 15 µm thickness and thaw mounted onto conventional glass slides. The samples were stored at -80 °C prior to analysis if needed. Immediately prior to analysis, the samples were brought to room temperature and placed onto the stage, without any further sample preparation. The enhanced DESI source was mounted onto a SYNAPT G2-S*i* HDMS. DESI spray conditions were set at 1.5 µL/min, 90:10 MeOH:water at 100psi N_2 gas pressure and a voltage of 5 kV for both polarities. The pixel size was determined in the X-direction by the speed of the stage movement and acquisition rate of mass spectra. The Y-direction was defined by the distance between two lines of acquisition.

In the first DESI imaging experiment, a raster pattern was defined over the whole tissue sections, with a pixel size of 150 µm for the porcine liver, and 200 µm for the human liver sample. The second experiment was carried out using a specific region of the same tissues, both at 50 µm. The workflow for these experiments is described in Figure 1A.

Figure 1B and 1C display mass spectra with plentiful lipid and endogenous metabolite signals observed from the DESI analysis of the tissue sections. Each spectrum was obtained from a single pixel acquired on the porcine tissue section at different spatial resolution (150 µm followed by 50 µm) from the same tissue. The relative intensities of the lipid signals are comparable at either spatial resolution.

Examples of images produced from the tissue samples can be seen in Figure 2. Figures 2A and 2B show the ion images of a phosphotidyl glycercol (PG) containing lipid at *m/z* 927.7 with A) being a pixelated ion image at a 150 µm spatial resolution from a pristine surface, and B) an image of the same tissue section, measured at 50 µm spatial resolution immediately after the 150 µm imaging experiment.

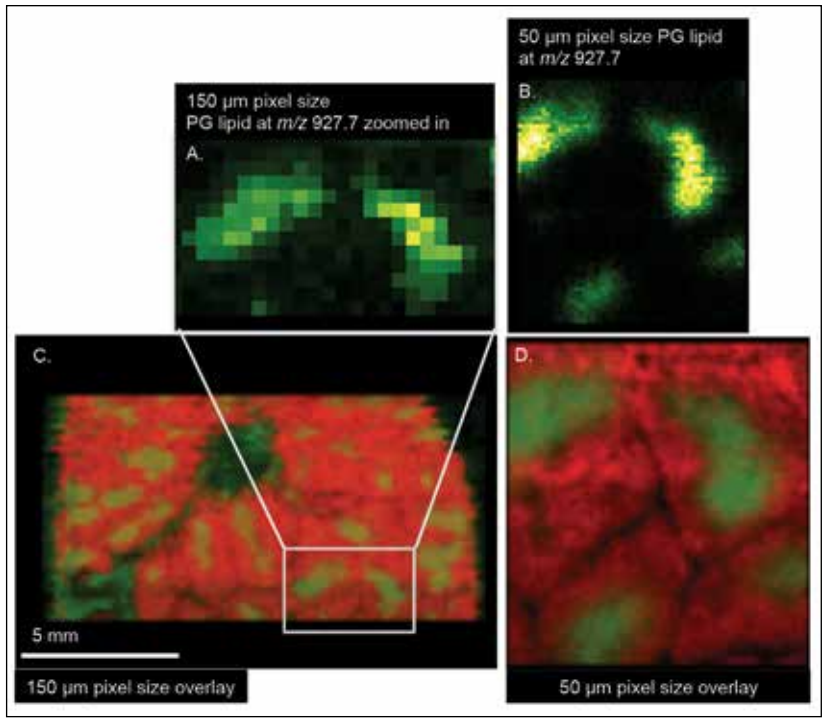

Figure 2. DESI imaging analyses of the porcine liver in positive ion mode A and B: ion images of m/z 927.7 with A: at 150 µm spatial resolution on pristine surface and B: at 50 µm spatial resolution on previously imaged surface. C and D: RG overlay of m/z 848.55 (PC (38:4) K^+) (red) and m/z 927.7 (green) in interpolate mode with C: at 150 µm spatial resolution on pristine surface and D: at 50 µm spatial resolution on previously imaged surface.

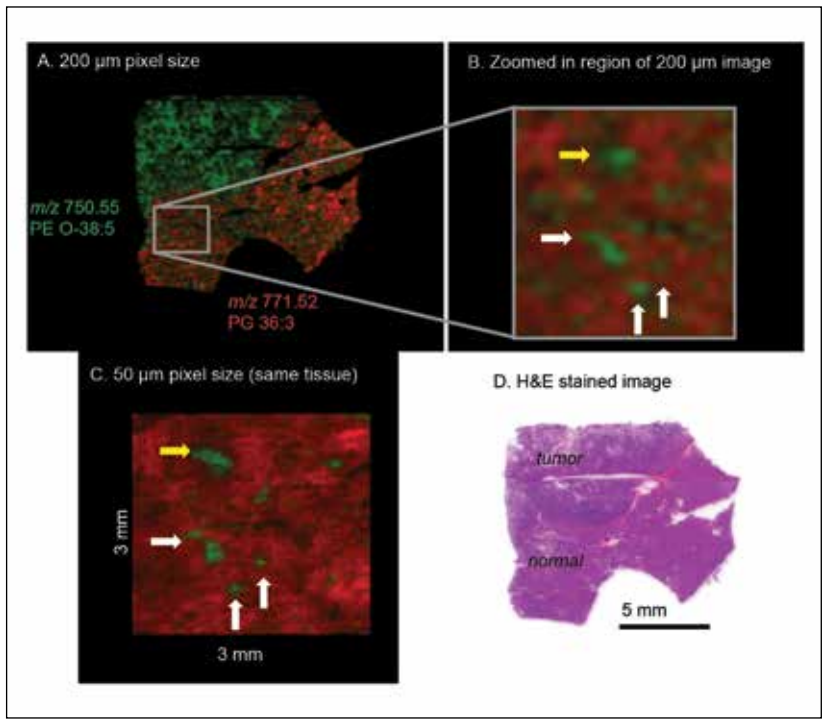

Figure 3. DESI imaging analyses of human liver sample in negative ion mode A: RG overlay of m/z 771.52 (PG (36:3)- (red ion image)) and m/z 750.55 (PE (O-38:5)- (green ion image)) at 200 µm resolution from a pristine surface. B: zoomed-in view of the "normal" tissue section. C: RG overlay of the same ion species from the 50 µm resolution previously imaged tissue section. D: H&E staining of the tissue section after the two DESI experiments.

As expected, the image quality noticeably improves at higher spatial resolution. But it is also noteworthy to see that there is no noticeable delocalization of ions from one imaging experiment to the next on the same tissue section. Delocalization can be a problem with other imaging techniques requiring the application of solvents or matrix to a sample for imaging (i.e., MALDI).

Figures 2C and 2D are a Red/Green (RG) overlay of ion images from a *m/z* 848.55 phosphotidyl choline (PC) containing lipid (38:4)K^+ (red ion image) with a PG containing lipid at *m/z* 927.7 (green ion image). This figure also shows the ion images of the same lipid species acquired during the second sequential imaging experiment acquired at 50 µm resolution.

A similar imaging study was carried out on a human liver biopsy tissue sample that contains both healthy cells and a secondary tumor (Figure 3). The entire tissue section was first imaged at 200 µm resolution. In this experiment, PG containing lipids of *m/z* 771.52 (36:3)- (red ion image) and phospotidyl ethanolamine (PE) containing lipids of *m/z* 750.55 (0-38:5)- (green ion image) were found to be specifically localized to either healthy or tumor tissue, and could be utilized to distinguish tissue type in the sample section (Figure 3A). A second imaging experiment on the same human liver tissue section focused on the region of the section that was identified as healthy. This sequential imaging experiment was performed at 50 µm spatial resolution and concentrated on the margin between healthy and cancerous tissue (Figure 3B and 3C). Looking closely at the images obtained at this level of resolution indicates that some tumor cells have begun invasively migrating through the intercellular spaces of the healthy liver tissue.

Finally, once DESI imaging experiments were completed, the tissue section was subsequently H&E stained for accurate correlation of DESI imaging observations with cell and tissue morphology (Figure 3D).

SUMMARY

DESI imaging has been shown to provide important information about tissue samples especially regarding the distribution of lipids and small molecules throughout a variety of tissues. Here, we have shown that the potential of using DESI imaging to gather information from samples can be enhanced by optimizing the conditions on various tissue sections. Specifically, controlling the gas and solvent flow rates, as well as the voltages applied to the instrument allows for effective DESI imaging at different spatial resolution (i.e., 50 and 200 µm).

This capability of DESI imaging allows for a relatively fast initial scan of a tissue sample, followed by a more high resolution, detailed imaging study of regions of interest identified by the initial experiment. Additionally, after DESI imaging is complete, the tissue section can be directly H&E stained for further morphological analysis.

The advantages of DESI imaging include:

- Sequential image analysis of a single tissue section at one or more levels of spatial resolution.
- Fast scan imaging and subsequent imaging of selected regions on the same tissue sample.
- Identification of tissue discriminating or tissue identifying marker compounds.
- Performance of imaging studies with little sample preparation.
- Capability to combine DESI image analysis of a tissue sample followed by subsequent H&E staining for morphological analysis.
- No potential for analyte relocalization during imaging.

Acknowledgments

1. This study was carried out in conjunction with Imperial College London, UK. For the analysis of human samples, ethical approval was obtained from the National Research Ethics Service (NRES) Committee London – South East (Study ID 11/LO/0686). This work was supported by European Research Council under Starting Grant Scheme (Grant Agreement No: 210356) and the European Commission FP7 Intelligent Surgical Device project (contract no. 3054940).

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Real Time Lipidomic Profiling Using Desorption Ionization with Ion Mobility MS

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[1] IonSense, Saugus, MA, USA
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APPLICATION BENEFITS

The combination of real time desorption ionization and ion mobility MS offers a convenient solution for phenotypic identification and comparative lipidomic analysis.

WATERS SOLUTIONS

SYNAPT® G2 HDMS™ System

HDMS Compare Software

KEY WORDS

Lipid, metabolomics, lipidomics, DART, ion mobility, T-Wave™

INTRODUCTION

Lipids are major constituents of food and biological tissues. Among lipid key properties are those to determine the caloric content, texture, and taste of food. Besides their importance in food and nutrition, lipid composition affects the physiology of living cells. Alterations in lipid profiles have been implicated in a wide range of pathologies in many types of organisms including plants and humans. Therefore, assessing lipid profiles and ratios between various lipid species can be indicative of the quality of food or health status of living organisms, as shown in Figure 1.

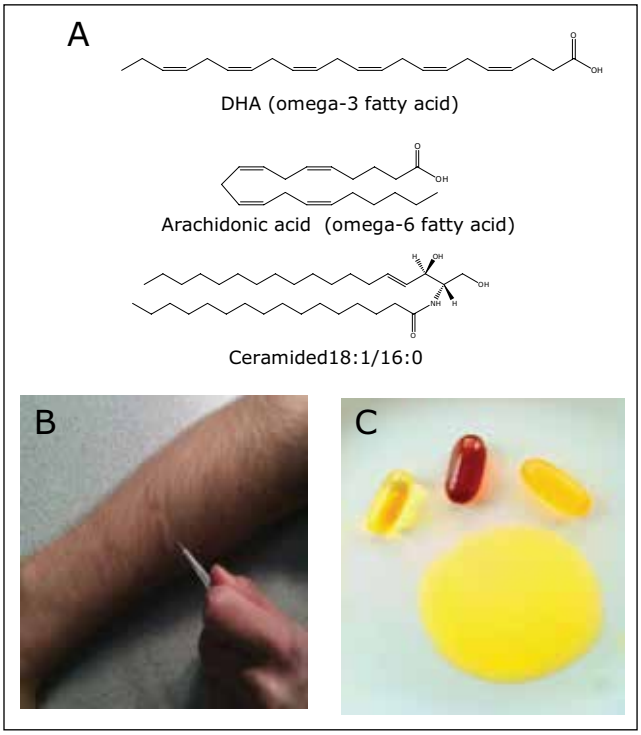

Figure 1. Representative lipid structures analyzed in the study (panel A). Lipids contained in human sebum from skin (panel B) and edible oils (panel C) have been used as representative samples for the DART-IMS-MS analyses.

EXPERIMENTAL

Sample description

No sample preparation is required. Samples were swiped on glass capillaries, which were held in the in metastable gas beam between the Direct Analysis in Real Time (DART, IonSense, MA, USA) ion source and SYNAPT G2 HDMS.

Lipid standards and extracts were purchased from Avanti Polar Lipids (AL, USA). Edible oils were purchased at the local grocery store and blindly analyzed.

MS conditions

Chromatographic separation is not required. Analyses were conducted using a DART source coupled with a Waters® SYNAPT G2 HDMS instrument. DART sources are designed to fit the Waters Xevo® MS family of instruments. Acquisition time was 5 to 10 seconds.

Mass spectrometer:	SYNAPT G2 HDMS
Ionization:	DART +ve and –ve
Cone voltage:	20 V
Source temp.:	120 °C
DART temp.:	50 to 450 °C
Cone gas:	30 L/h
Desolvation gas:	800 L/h (Nitrogen)
IMS gas:	90 mL/min (Nitrogen)
IMS T-Wave velocity:	833 m/s
IMS T-Wave height:	40 V
Acquisition range:	50 to 1200

The analysis of lipid composition often requires very laborious and time-consuming procedures. Furthermore, the detailed spatial distribution of lipid species on a surface is often missed using traditional sample preparation and lipid extraction protocols for large-scale lipid analysis (lipidomic analysis).

The use of desorption ionization (DI) techniques in lipidomics could provide a new level of description beyond the pure measure of lipid concentration. DI-MS techniques are useful for real-time, rapid, in-situ screening of various materials including food, plant, and animal tissue.[1] In particular, DI-MS spectra of biological samples feature ions corresponding mainly to lipids. By molar quantities, the most abundant ionic molecular species in biological tissue, lipids ionize well under DI conditions.

The *in-situ* generation of a particular profile of lipid ions has been proposed for real-time molecular fingerprinting and diagnosis. Here, a rapid (few seconds), real-time method using DI in combination with post-ionization ion mobility separation to analyze lipidomic profiles in food and biological samples is presented.

RESULTS AND DISCUSSION

For a rapid lipidomic analysis, we combined two emerging technologies: DART and ion mobility separation[2] to analyze lipids extracted from biological samples.

Belonging to the DI techniques, DART is an atmospheric pressure ion source that instantaneously ionizes samples in open air under ambient conditions. DART employs an electrical discharge to create a plasma that produces helium metastables, which react with ambient water, oxygen, or other atmospheric components to produce charged water clusters. Protons are then transferred to the analytes.

Samples were swiped on glass capillaries, held in the in metastable gas beam between the DART ion source and SYNAPT G2 HDMS. Without the need for chromatographic separation, lipids were ionized by DART and guided into the mass spectrometer, where they traveled to the Ion Mobility Separation (IMS) cell. A T-Wave mobility separator used a repeating train of DC pulses to propel lipid ions through a nitrogen-filled IMS cell in a mobility dependent manner. Lipids migrated with characteristic mobility times (drift times) according to their size and shape before TOF detection, as shown in Figure 2.

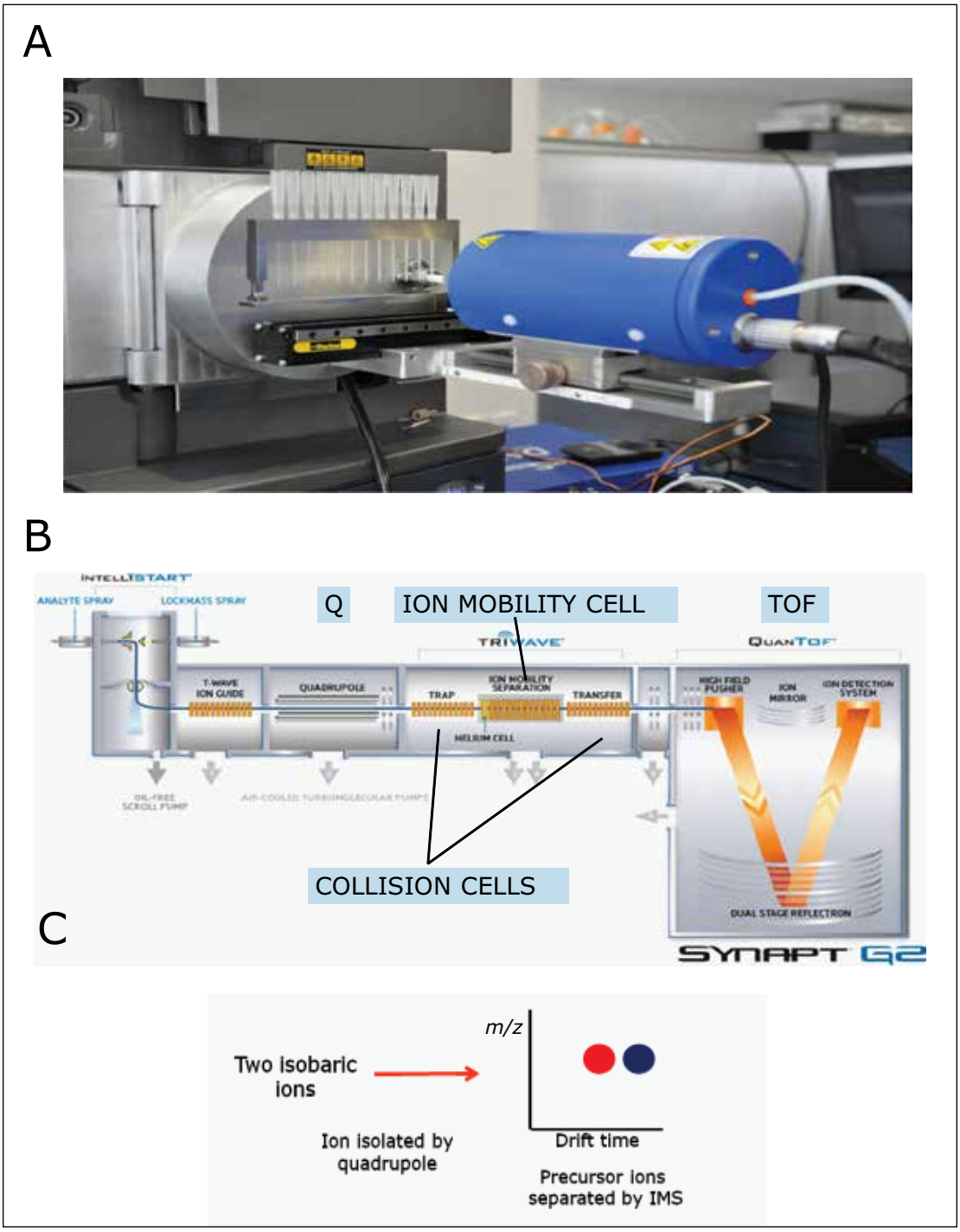

Figure 2. The DART ion source can be installed on Waters instruments (panel A). The ability to couple DART with a SYNAPT HDMS instrument (schematic in panel B) eliminates sample preparation and chromatographic steps because of the post-ionization separation by ion mobility (C).

As an example of the power of such an approach, lipid profiles of edible oils (fish oil and olive oil), and lipids extracted from biological samples and human sebum, which is the oily matter that lubricates and waterproofs human skin, were analyzed, as shown in Figure 1. Lipid molecules with different acyl chain length or number of double bonds resulted in characteristic drift times. This enabled the separation and detection of key lipids, such as fatty acids and ceramides, on the millisecond time-scale without the need for prior derivatization or chromatography, as shown in Figures 1, 3, and 4. Ion mobility enabled the separation of the entire lipid profile of a sample on the millisecond time-scale, and a complete DART-IMS-TOF analysis required just a few seconds (0.1 min), as shown in Figures 3 and 4.

Data processing allowed the generation of 3D molecular maps based on drift time, exact mass, and intensity of the signal relative to the various analytes present in the oils. Such representation highlighted the capacity of ion mobility to separate isobaric lipid species (species with the same mass) without the need for prior chromatography, as shown in Figure 3.

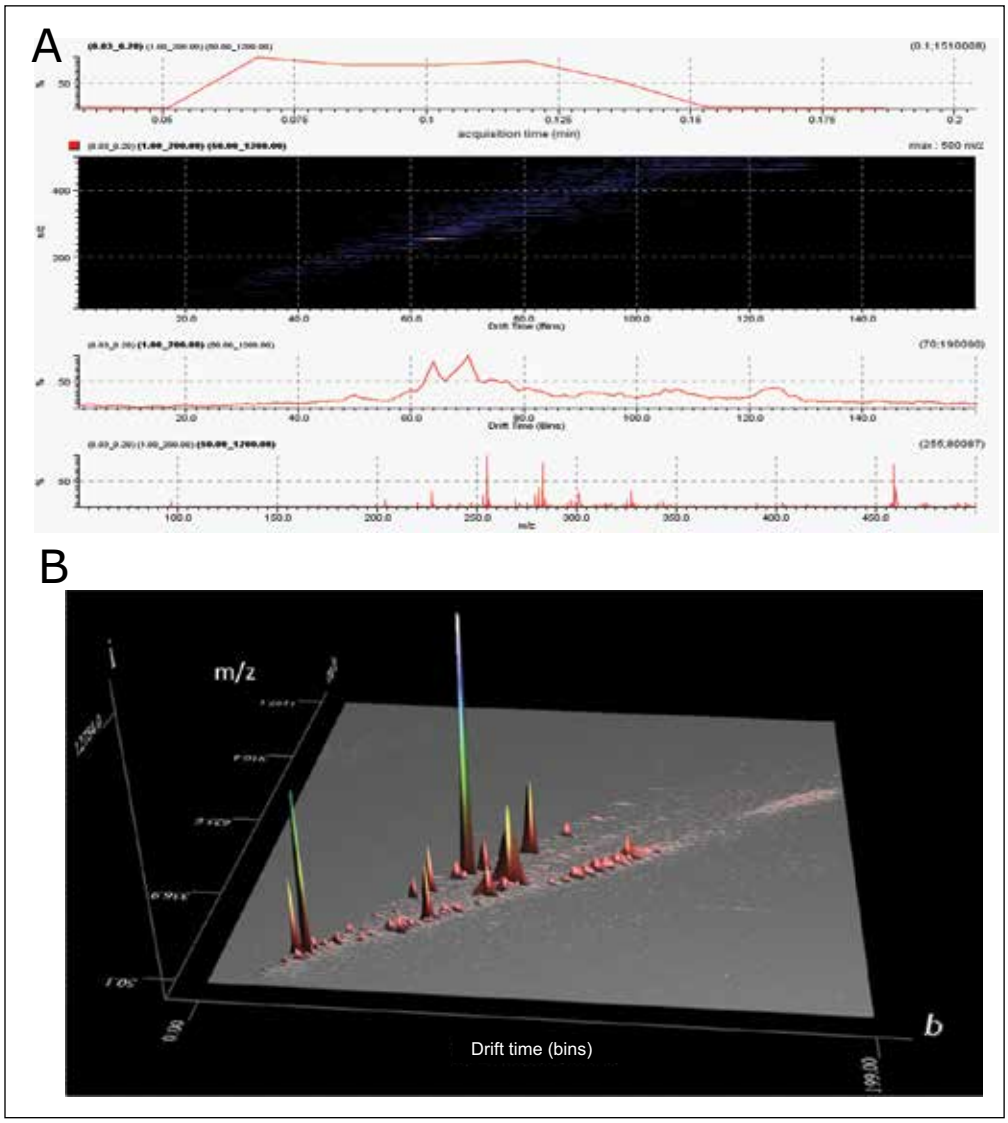

Figure 3. The entire DART-IMS-TOF analysis requires just a few seconds (0.1 min). Lipids are separated by ion mobility on the millisecond time-scale (panel A). Software processing of the data allows the generation of 3D molecular maps based on drift time, exact mass, and intensity of the signal relative to the various analytes present in the oils. Isobaric species are separated by ion mobility.

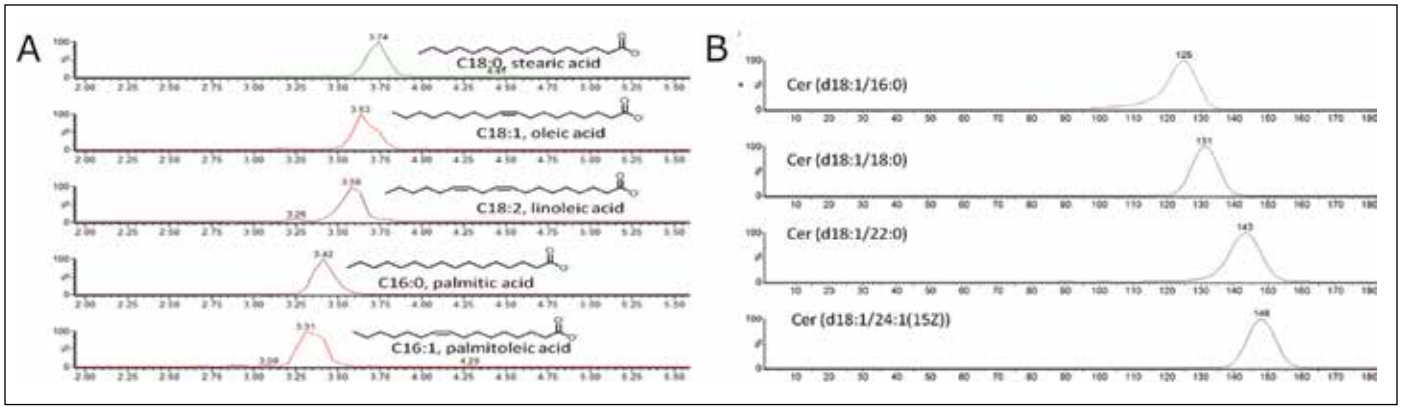

Figure 4. Ion mobility separation of fatty acids from olive oil (panel A), and ceramides from human sebum (panel B) after rapid DART ionization in negative and positive ion mode, respectively. Differences in the acyl chain length or number of double bonds affect the shape and size of lipid molecules, resulting in characteristic drift times.

A comparison of lipid profiles of human sebum, shown in Figure 5, and edible oils shown in Figure 6 was done based on the separation capabilities of IMS-TOF/MS. HDMS Compare Software was used for a rapid binary comparison of different driftograms (masses versus drift time matrices). The drift time and spectral information associated with the components responsible for the differentiation can be extracted from the dataset and analyzed to better understand the underlying reasons for the observed differences.

Figure 5. Comparison of sebum skin oils from two human subjects. Overlaying individual molecular maps clearly show areas where the samples are significantly different. Ion mobility data analysis and processing was done using HDMS Compare Software. Key areas of significant differences between two samples were clearly visualized and identified with two different colors. Regions of interest were easily selected and expanded in Zoomed Map view for further interrogation of important sample differences.

HDMS Compare Software also allows importing a list of target ions (mass and drift time) and reporting changes in the levels of these targets, as shown in Figure 6.

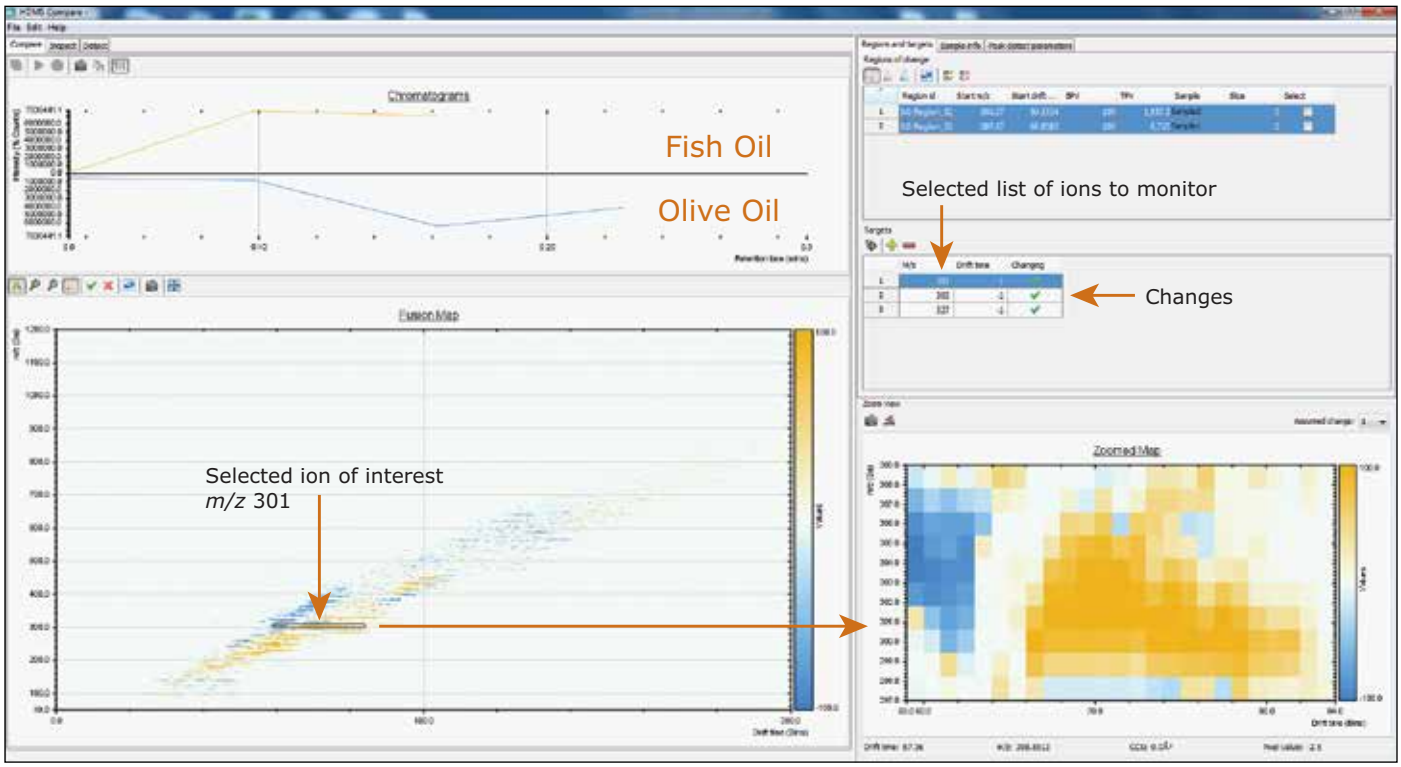

Figure 6. Comparison of edible oils. HDMS Compare Software was used to determine molecular difference in fish oil versus olive oil. The software automatically identified significant differences between the two oils in the levels of a selected list of ions, including m/z 301 (eicosapentanoic acid; EPA), 303 (arachidonic acid), and 327(docosahexaenoic acid; DHA).

CONCLUSIONS

- The combination of a desorption ionization technique such as DART with ion mobility-TOF offers a convenient solution for lipidomic profiling.
- Post-ionization separation by ion mobility allows resolution of complex mixtures of lipids.
- Software solutions provide overlay driftograms (plots of masses versus drift time) to compare different samples.
- More generally, these results suggest that the combination of desorption ionization techniques and the ion mobility approach is suitable for the rapid screening of bioactive lipids, including fatty acids and ceramides.
- Potential applications include phenotypic fingerprinting and comparative lipidomics in the areas of personalized medicine, disease diagnostics, food analysis, and traditional medicines.

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