MALDI MS Imaging of Cereals

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Overview

MALDI MS Imaging is applied to common cereals, such as oat, barley, rye or wheat flakes. These (phospho)lipid and carbohydrate-containing samples are used to demonstrate the following:

I. Workflow solution for MALDI MS Imaging of tissue samples
II. Analysis by High Resolution / Accurate Mass (HRAM) scanning across cereal flakes to establish distinct features of flakes from various sources, such as flakes from barley, oat, rye and wheat
III. Need for mass resolution better than 50,000 in order to distinguish isobaric lipids that occur at *m/z* 719 each displaying a different spatial distribution
IV. Carbohydrate occurrence in flakes visualized by MALDI MSI in wheat flakes

Introduction

The usage and the benefits of an Orbitrap™ mass analyzer-based instrumentation for the detection of MALDI-produced ions were successfully demonstrated earlier using an intermediate pressure interface (75 mtorr): High mass resolution and accurate mass (HRAM) for MALDI ions were proven to be accessible with a collisional cooling interface followed by Orbitrap detection providing high dynamic range in single FTMS scans with few laser shots.[1] Here, we apply the intermediate pressure MALDI interface and HRAM detection of Orbitrap technology to imaging of various cereal flakes. The goal is to characterize each grain's composition and compound distribution by MALDI MS Imaging (MALDI MSI). Also, studies follow the aim to distinguish the different cereal grains by MALDI MS Imaging.

Methods

Sample Preparation: Flakes from commercially available cereals - herein kindly provided as flakes separated by their origin grains (oat, rye, barley, wheat) - deriving from conventional farming are attached with double-sided adhesive tape (PLANO, Wetzlar, Germany) on MALDI sample plate holders, typically used to place metal slides.
CHCA matrix is provided in methanol / water (7/3) with a concentration of 5 g/L and sprayed using a SunCollect MALDI Sprayer (SunChrom™, Friedrichsdorf, Germany). Five to 10 layers of matrix are deposited using a syringe flow rate of 1.5 uL/min.

Preparation and Pre-Processing of Samples for MALDI MSI: Sample plates with matrix-sprayed flakes are optically scanned with a commercial scanner. Regions of interest for MALDI MSI analyses – such as the entire flake area - are selected along with laser raster step size (e.g. 50 or 100 µm) and saved as a position file. These position files are treated similar to autosampler positions in LCMS experiments.

Analyses such as a.) an FTMS full scan and b.) a data-dependent or targeted MSMS scan from the same location are designed and interrogated in a sequence setup. Raw file acquisition for MALDI Imaging applications monitor the corresponding x-y position for every scan.

Thickness of flakes is between some hundreds micrometer up to 1 mm; the given MALDI instrumentation does not require the covering of the samples with a conductive grid or the use of conductive glue to hold the relative thick flakes in place: The de-coupling between MALDI ion production and ion detection results in high quality high resolution / accurate mass data even from thick and insulating samples.

The imaging workflow consists of tissue sectioning, matrix spraying on tissue sections, mass analysis and data processing in x-y resolved extracted ion chromatograms (XICs). This is displayed in **Figure 1**. The software solution for the creation of position files in Tune 2.5.5 (Instrument Control software) and its interrogation in the Thermo Scientific Xcalibur software sequence setup is explained in detail in **Figure 2**.

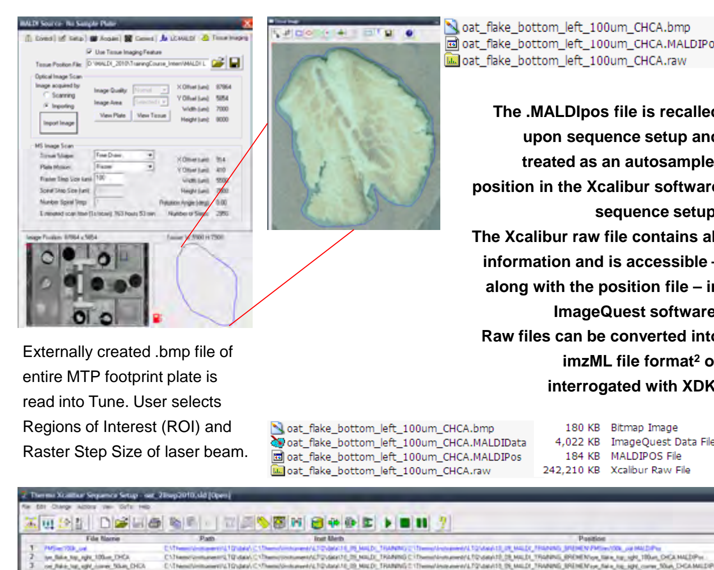
Mass Spectrometry: All experiments are performed on a Thermo Scientific MALDI LTQ Orbitrap XL Mass Spectrometer described elsewhere[1]. Briefly, ions are desorbed / ionized into a collisional cooling interface helt at 75 mtorr nitrogen pressure.

Data Processing of MALDI MS Imaging Raw Files: With Thermo Scientific ImageQuest software, the mass spectral raw data – along with the position file information – is displayed in a spatially resolved manner. For every x-y coordinate, FTMS and MSMS data can be read, and color-coded spatially resolved extracted ion chromatograms are generated. In this study, Extracted Ion Chromatograms (XICs) are normalized relative to Total Ion Current (TIC), and logarithmic scaling is used for displaying the images. Under the rainbow color scheme of ImageQuest™ software, relative intensities are displayed as red > green > yellow> blue.

Workflow

FIGURE 1. Workflow for MALDI MS Imaging involves sectioning tissue, matrix spraying, mass spectrometric imaging acquisition, and processing raw files for the creation of color-coded images.

FIGURE 2. Workflow using a Tune and Instrument method (Tune 2.5.5 and Xcalibur™ software 2.0.7 or 2.1). A bitmap file of the entire plate is acquired on an optical scanner. Bitmap files of the Region Of Interest (ROI) are created in the MS instrument and allow for optical visualization of ROI for MS Imaging. The thereby created .MALDI position file knows start and end positions and the raster step size, e.g. 100 µm. The .MALDIposition file is recalled upon a sequence started and treated like an autosampler position. The raw file is then opened with the visualization tool ImageQuest software (.MALDIdata). Typical file sizes (area of cereal flakes) of corresponding .bmp, .MALDIpos, .raw, and .MALDIdata are given.



Results

Figure 3 and **Figure 4** display results obtained from **rye flakes**. Color-coded Extracted Ion Chromatograms (XICs) are done with ImageQuest software and show three different *m/z* ratios or **(phospho)lipids being located in three different regions**, **Figure 3b**. Here, images are displayed with overlay functionality with up to 3 colors (red, green, blue). If a position or area shows two or three selected *m/z* ratios, the pixel or area is coded in yellow color, (no data shown here).

FIGURE 3. a.) Optical and b.), c.), d.) and e.) images of a rye flake scanned with 50 µm laser step size (FTMS full scan data). b.) displays the overlay of the XICs made in c.) – e.) *m/z* ratios and corresponding color coding is given on the bottom of each XIC image. The *m/z* ratios are made in agreement to HRAM performance of the used Orbitrap detector-based instrumentation and the 50 µm raster step size[3]. The fact that XIC images can be created upon full usage of HRAM performance using ImageQuest software is the key differentiator to other software solutions and enables for the extraction of clear images. Lower mass resolution and/or less accurate mass data do not allow for such high quality images.

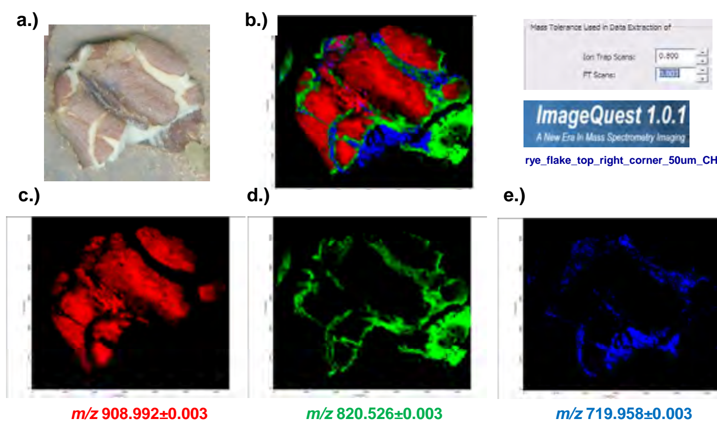
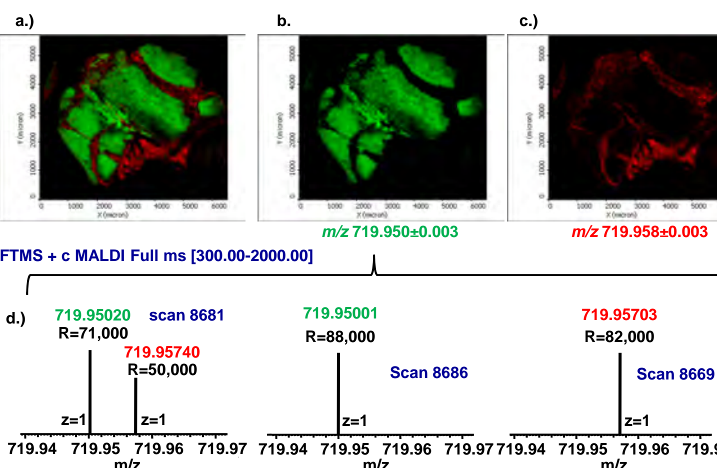


FIGURE 4. Necessity for both High Resolution AND Accurate Mass for MALDI MS imaging data. Displayed by images and XICs of *m/z* 719.950 and *m/z* 719.958 (FTMS full scan data) shown in a.), b.) and c.) Spectra are acquired with a resolving power of 90,000 at the given mass (no co-migration); this lowers to app. 60,000 at the few x-y locations where both compounds are observed. This is illustrated by showing insets into single full scan FTMS spectra from locations where the isobaric species appear singly or together. d). Clearly, XIC (*m/z* 719.950) and XIC (*m/z* 719.958) can be distinguished by *m/z* ratios, and both *m/z* ratios derive from different locations in the rye flake.



Figures 5, 6 and 7 display results obtained from **wheat flakes**. Color-coded extracted ion chromatograms (XICs) are done with ImageQuest software and demonstrate the **carbohydrate distribution** (Figures 5, 6) and **distribution of four specific phospholipids** (Figure 7) in the flake. **Table 1** shows proposed chemical formulas for selected carbohydrate ions from **Figures 5 and 6**. Many more polymeric carbohydrates were detected than are shown here.

FIGURE 5. Selected spatially resolved XICs of FTMS full scan data upon scanning across a wheat flake with 50 µm raster step size. The *m/z* ratios selected for the five XICs are between *m/z* 543 and *m/z* 2325. These *m/z* ratios display subsequent increase in multiples of 162.0528233 u, indicative of the mass increment of a Hexose, $C_6H_{10}O_5$. The corresponding optical image is shown on the right hand side along with the scanned area (red box). Refer to Table 1 for data interpretation of the assigned *m/z* ratios.

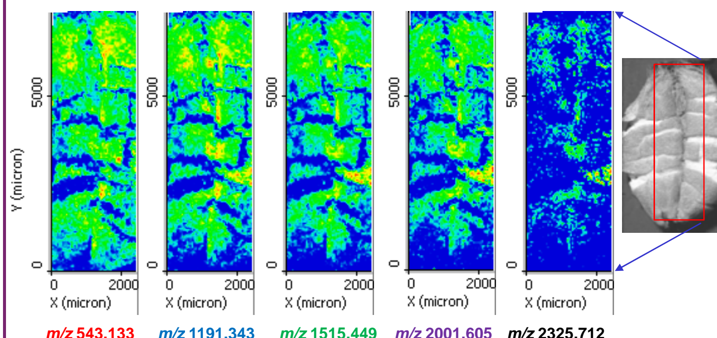


Table 1: Data interpretation for carbohydrates observed from wheat flakes, see Figure 5.

m/z 543.1330	$C_{18}H_{32}O_{16}K$ equals to $(H_20 + C_6H_{10}O_5)_3 + K^+)$, RDBe 2.5
m/z 1191.343	543.133 plus additional 4 glucose units, equals to $C_{42}H_{72}O_{36}K^+$ or $(H_20 + C_6H_{10}O_5)_7 + K^+)$, RDBe 6.5
m/z 1515.449	1191.343 plus another 2 glucose units, equals to $C_{54}H_{92}O_{46}K^+$ or $(H_20 + C_6H_{10}O_5)_9 + K^+)$, RDBe 8.5
m/z 2001.605	1515.449 plus another 3 glucose units, equals to $C_{72}H_{112}O_{61}K^+$ or $(H_20 + C_6H_{10}O_5)_{12} + K^+)$, RDBe 11.5
m/z 2325.712	2001.605 plus another 2 glucose units, equals to $C_{84}H_{132}O_{71}K^+$ or $(H_20 + C_6H_{10}O_5)_7 + K^+)$, RDBe 13.5

All values are measured m/z ratios. Mass accuracy is less than 2 ppm. $C_6H_{10}O_5$ = mass increment of **glucose = 162.05282 u**. Straight forward sum formula assignement benefits from HRAM performance

Figure 6: Mass spectrum is an average of 4 scans from subsequent positions (50 µm spacing, area of 4 subsequent positions, less than 100 laser shots in total) and demonstrates the carbohydrate distribution with regard to *m/z* range.

Peaks differ by hexose units, $C_6H_{10}O_5$. m/z values and mass resolution are given.

FIGURE 7. Four different phospholipids displayed as spatially resolved XICs of FTMS full scan data using ImageQuest software. The mass difference of 2.01565 mass units between subsequent lipids chosen below (*m/z* 818, *m/z* 820, *m/z* 822, *m/z* 824) indicates differences in saturation of the alkyl fatty acid chain (see Reference 4). Data is acquired upon scanning across a wheat flake with 50 µm raster step size. Displayed images are extracted from the identical raw file as that from Figure 5. Note that significantly less phospholipids are observed in the upper part of this flake where more carbohydrates are found.

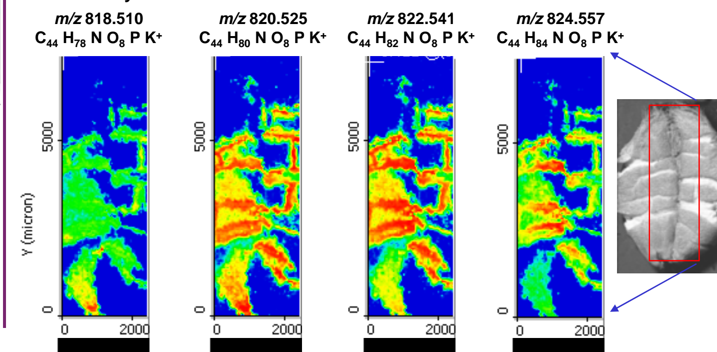
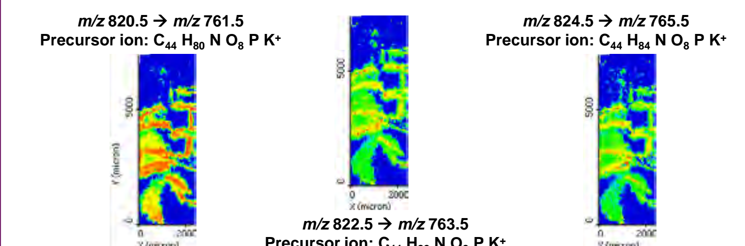


Figure 8 displays results obtained from **wheat flakes**. The instrument method was designed so that one FTMS full scan and three subsequent MS/MS scans derive from the identical location. Images from FTMS and ITMS/MS can be extracted and compared by selecting the appropriate scan filters in ImageQuest software. Features of high mass resolution (FTMS) and selectivity upon fragmentation (MS/MS) are effectively combined. ITMS/MS data are performed in a targeted manner on *m/z* 820.5, *m/z* 822.5, and *m/z* 824.5, three of the phospholipids displayed in **Figure 7**. The neutral loss of 59, trimethylamine, confirms their classification as phosphatidylcholine or sphingomyelin.[5]

FIGURE 8. Selected spatially resolved XICs of ITMS/MS data upon scanning across a wheat flake with 50 µm raster step size. Images of fragment ions are displayed. Three phospholipids from the FTMS full scan in Figure 7 are chosen as precursor ions; precursor ions can be assigned as $(M+K)^+$ quasimolecular ions. Selected are three different phospholipids, investigated with regard to FTMS full scan information in Figure 7. Here, the answer to their MSMS channels is shown.



Conclusion

- Despite of the thickness of (insulating) cereal flakes, MALDI MS Imaging can be performed straightforward to obtain high quality spectra in the given instrumentation.
- MALDI MS imaging data of FTMS can be obtained from all different flakes; herein data from rye and wheat flakes are shown. Data from barley and oat show very similar tendencies with regard to carbohydrate distribution and phospholipid content.
- HRAM capability provided by the given instrumentation is essential for the unambiguous assignment of isobaric peaks that can then be extracted and displayed as images showing different localization. This is demonstrated with two compounds differing by 8 mmu.
- The key differentiator of ImageQuest software is its ability to extract ion images and take full advantage of the high resolution, accurate mass Orbitrap data.
- Various carbohydrates, as well as lipids differing in fatty acid chain length and degree of saturation, are distinguished by high resolution and accurate mass capabilities. Future work will concentrate on identifying the structures by MS^n (Reference 5) and further understanding the differences between cereals using MALDI imaging.

References

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Acknowledgements

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