Different MALDI Mass Spectrometry Imaging Applications on a Prototype MALDI-Q-TOF Instrument

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Introduction

MALDI-MSI allows the detection of a wide variety of different molecular species in their histological context. As a result of this uniquely broad range of applications, MALDI-MSI has emerged as a powerful technique for fields where knowledge of molecular spatial distribution is essential, such as pharmaceutical and biomedical research. We present a range of different MALDI Imaging applications typically performed on ultra-high mass-resolving instruments, measured on timsTOF fleX, a MALDI-Q-TOF system consisting of a high spatial resolution MALDI source and stage mounted on a timsTOF Pro. This combination allows for the fast acquisition of different molecular species at high mass resolution.

Methods

Drug and Lipid Preparation, Measurement & Analysis: Fresh frozen kidney sections from rats dosed with substance Factor Xa antagonist were cut at 10 µm and mounted onto conductive glass slides (Bruker Daltonik GmbH, Bremen, Germany). For positive mode measurements, sections were sprayed with 15mg/ml DHB in 90% ACN/H2O using a TM sprayer (HTX Technologies, Chapel Hill, NC, USA); for negative mode measurements, sections were sprayed with 10mg/ml 9-aminoacridine (9-AA) in 70% ethanol/H_2O using the same device. Tissues were measured using the following parameters: *m/z* range: 200-1000, 500 shots, 10 kHz laser frequency, pitch: 100 µm. Mass spectra were imported into and visualized using SCiLS Lab MVS software (Bruker Daltonik).

N-glycan Preparation, Measurement & Analysis: FFPE human hepatocellular tumor tissues were cut at 5 µm, mounted on conductive glass slides (Bruker Daltonik), and prepared for N-glycan measurements using a well-established protocol [1]. In brief, slides underwent deparaffinization, rehydration and heat-induced antigen retrieval. N-glycans were released by spraying samples with 0.1 µg/µl PNGaseF Prime-LY solution (N-Zyme Scientifics) using a TM Sprayer, and digested under humid conditions for 2 hours. 7mg/ml alpha-cyano-4-hydroxycinnamic acid matrix in 50% ACN/H_2O + 0.1% TFA was deposited on the sample using the same spraying device, then measured using the following parameters: positive mode; *m/z* range: 500-3500, 1000 shots; 10 kHz laser frequency; pitch: 20 µm. Mass spectra were imported into SCiLS and the signals compared against a master list of 61 N-linked glycans that had been generated using a 7T solariX for peak detection [1]. Hierarchical cluster analysis which allows statistical grouping of similar spectra was also conducted. Spectra of a particular cluster were then assigned to a selected color and displayed as a spatial segmentation map.

Metabolite Preparation, Measurement & Analysis: FFPE human lung tumor tissues were cut at 5 µm, mounted onto conductive glass slides, and prepared for endogenous metabolite measurements using a protocol by Ly et al. with modifications [2]. Sections were deparaffinized and coated with 10mg/ml 9-AA in 70% ethanol/H_2O using a TM sprayer. The sample was measured using the following parameters: negative mode; *m/z* range: 200-1000, 400 shots; 10 kHz laser frequency; pitch: 30 µm.

Results

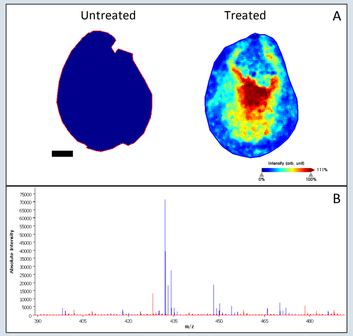


Figure 1. Factor Xa distribution measured on a timsTOF fleX. (A) The compound is detected in the renal medulla of treated animals; signal is absent in the non-treated samples. (B) Zoom of the range *m/z* 390-490 with treated in blue and untreated in red. Scale bar is 2mm; relative intensities indicated by false color coding using a jet color bar.

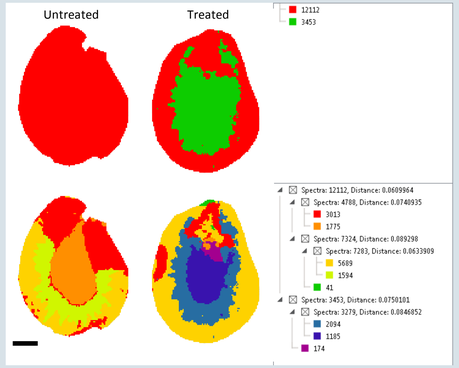


Figure 2. Segmentation map and cluster tree of untreated and Factor Xa-treated kidneys. Statistical analysis using the bisecting *k*-means algorithm with correlation distance clusters spectra from the medulla of treated animals separately from untreated animals. Scale bar indicates 2mm.

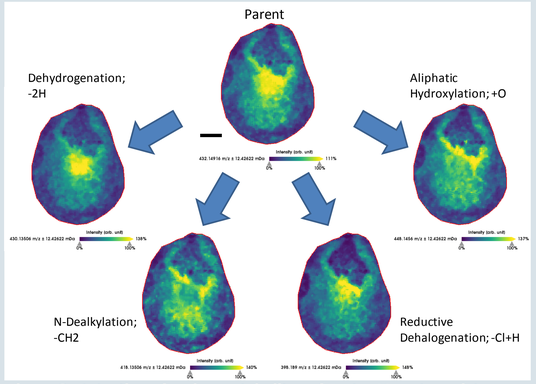


Figure 3. Detection of major metabolites of Factor Xa antagonist. Factor Xa antagonist metabolites are largely confined to where the parent is detected. Scale bar indicates 2mm; relative intensities indicated by false color coding using viridis color bar.

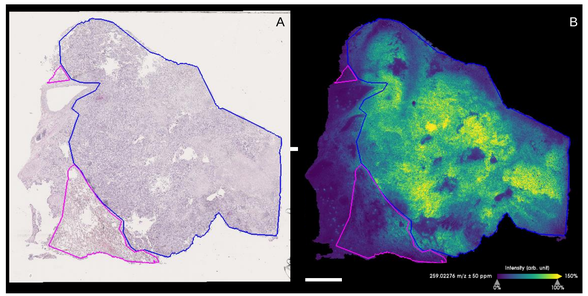


Fig. 4. Endogenous metabolites from FFPE human lung tumor sample. The detection of endogenous metabolites can be completed in FFPE tissues, but is often performed using extremely high mass resolving instruments rather than time-of-flight instruments due to high interference from isobaric matrix ions. (A) H&E-stained human lung tumor sample after MALDI acquisition. Independent assessment by a pathologist identified regions corresponding to tumor (blue) and lung parenchyma (pink). (B) Hexose-6-phosphate (*m/z* 259.023) is most intense in the tumor region. Scale bar indicates 3 mm; relative intensity indicated by false color coding using viridis color bar.

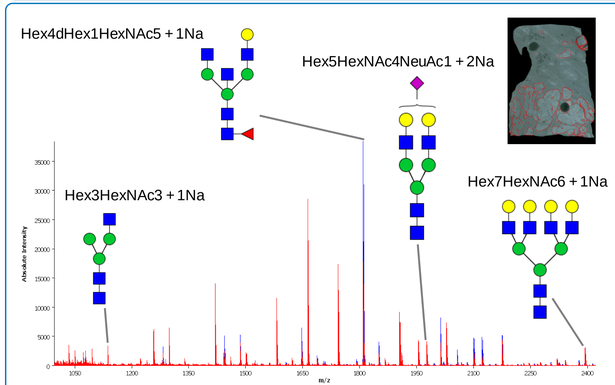
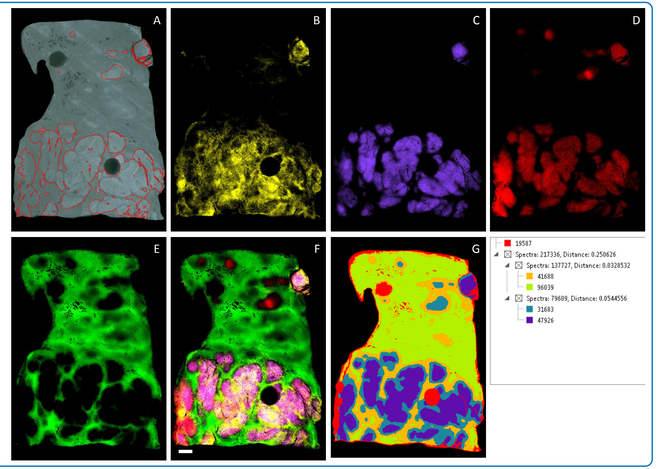


Figure 5. timsTOF fleX measurement of hepatocellular carcinoma *N*-glycans. Differences in N-Glycan spectra from non-tumor (blue) versus tumor regions (red) can clearly be discerned. The tumor regions in the section are outlined in red. (inset). Highlighted glycan structures are *m/z* 1136.3964, *m/z* 1850.6659, *m/z* 1976.666, and *m/z* 2393.845

Figure 6. Distribution of different *N*-glycans in a hepatocellular carcinoma section. (A) Pre-measurement scan of the hepatocellular carcinoma section indicates the tumor regions outlined in red. (B) Ion map of *m/z* 1976.666 (Hex5HexNAc4NeuAc1 + 2Na) indicates that it is largely detected in and between tumor regions. (C) *m/z* 2393.845 (Hex7HexNAc6 + 1Na) and (D) *m/z* 1136.3964 (Hex3HexNAc3 + 1Na) are localized to tumor. (E) In contrast, *m/z* 1850.6659 (Hex4dHex1HexNAc5 + 1Na) is largely confined to non-tumor regions. (F) Merge of the different images demonstrates the similarities and differences in *N*-glycan distribution. (G) Segmentation map and cluster tree calculated clearly divides the spectra into non-tumor (green), tumor (purple), tumor-edges (orange, blue), and non-tissue (red) regions. Scale bar indicate 1 mm.



Conclusions

- It is possible to conduct different MALDI imaging applications with high speed and high lateral resolution robustly on the timsTOF fleX
- These applications (detecting a dosed compound and compound metabolites in tissue, glycomics, endogenous metabolites) are typically performed on extreme mass resolving instruments.
- Different molecular species were highly correlated to distinct histological regions across all applications
- The timsTOF fleX generates high quality MALDI data can be used for SpatialOMx studies.

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References

1. West CA., J Proteome Res. 2018. 17: p.3454-3462.
2. Ly A., et al., Nat Protoc, 2016.11: p. 1428-43.

timsTOF fleX