

# 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.

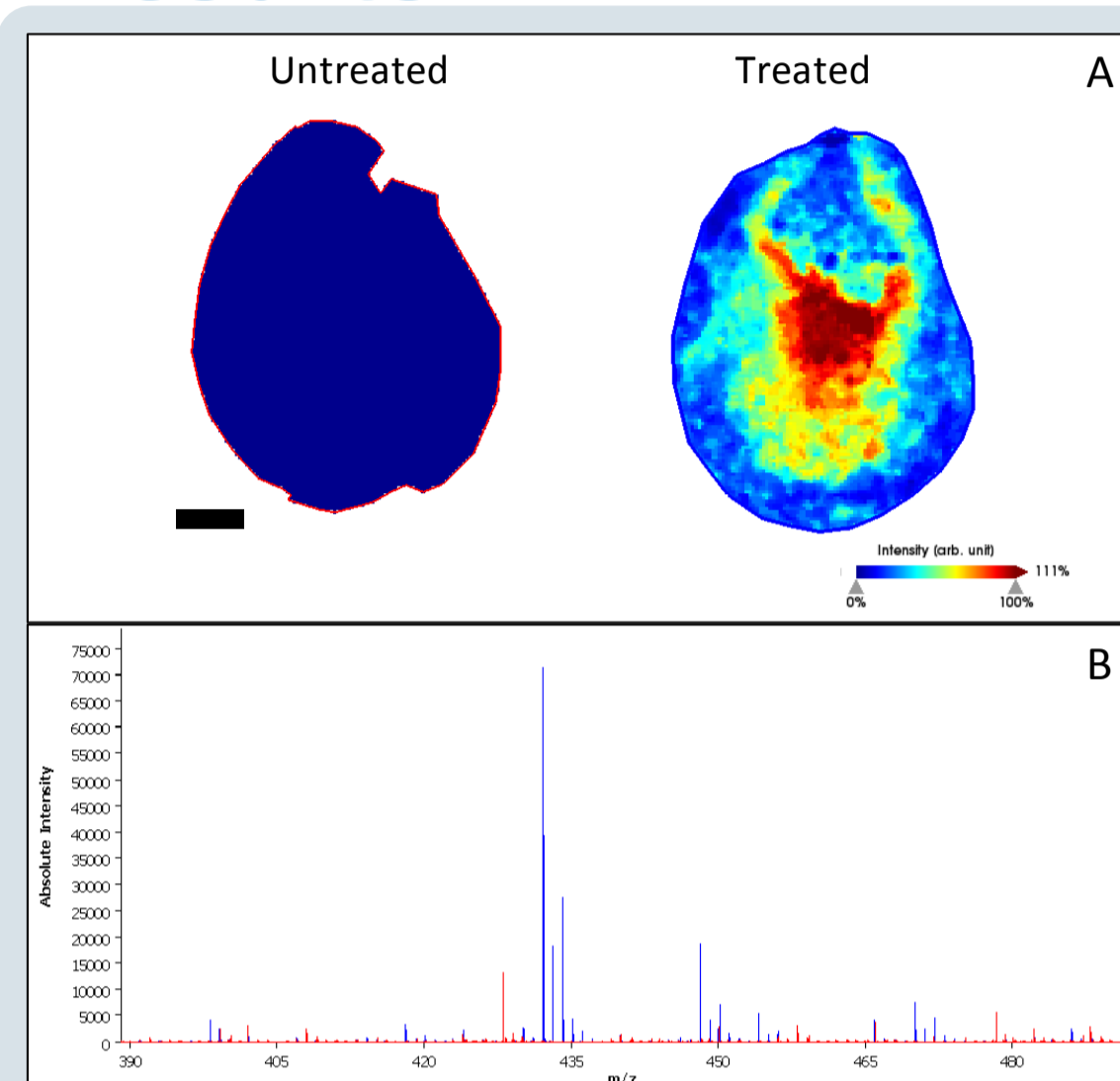
## Methods

**Drug and Lipid Preparation, Measurement & Analysis:** Fresh frozen kidney sections from rats dosed with substance Factor Xa antagonist were cut at 10  $\mu$ m 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/H<sub>2</sub>O 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<sub>2</sub>O using the same device. Tissues were measured using the following parameters:  $m/z$  range: 200-1000, 500 shots, 10 kHz laser frequency, pitch: 100  $\mu$ m. Mass spectra were imported into and visualized using SCI.LS Lab MVS software (Bruker Daltonik).

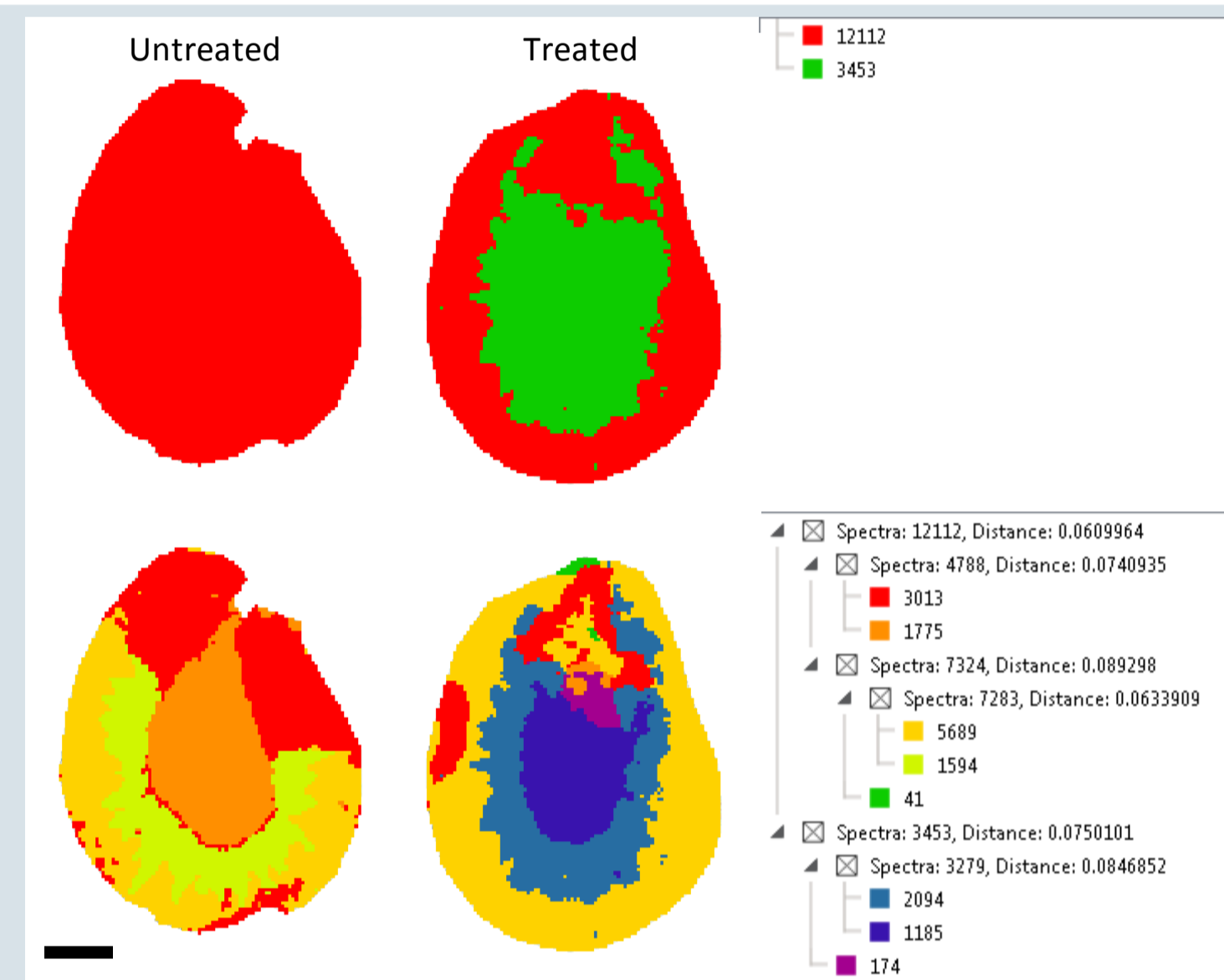
**N-glycan Preparation, Measurement & Analysis:** FFPE human hepatocellular tumor tissues were cut at 5  $\mu$ m, mounted on conductive glass slides (Bruker Daltonik), and prepared for N-glycan measurements using a well-established protocol [1]. In brief, slides underwent deparaffinization, rehydration and heat-induced antigen retrieval. N-glycans were released by spraying samples with 0.1  $\mu$ g/ $\mu$ l PNGaseF Prime-LY solution (N-Zyme Scientific) using a TM Sprayer, and digested under humid conditions for 2 hours. 7mg/ml alpha-cyano-4-hydroxycinnamic acid matrix in 50% ACN/H<sub>2</sub>O + 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  $\mu$ m. Mass spectra were imported into SCI.LS 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  $\mu$ m, mounted on 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<sub>2</sub>O 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  $\mu$ m.

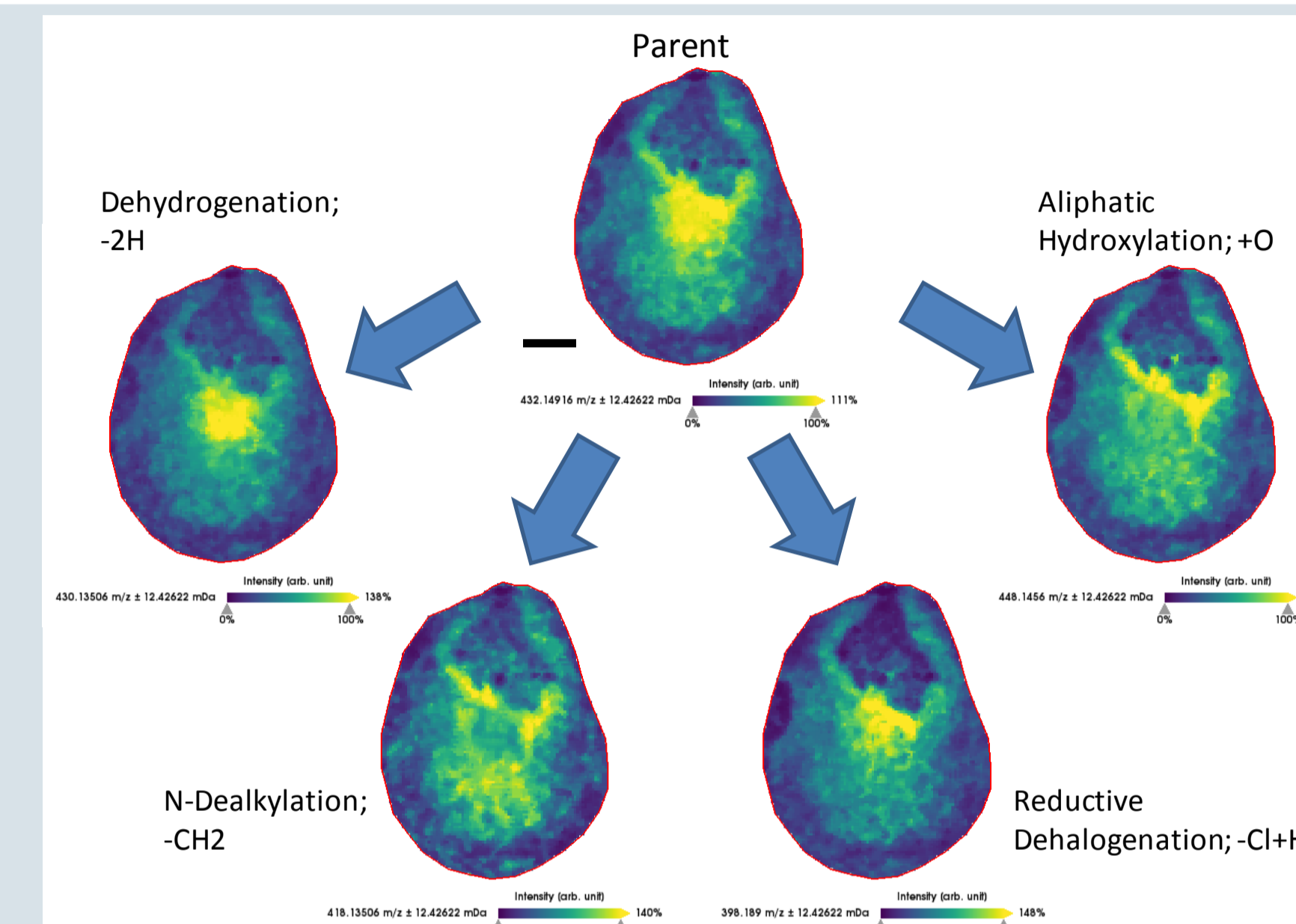
## Results



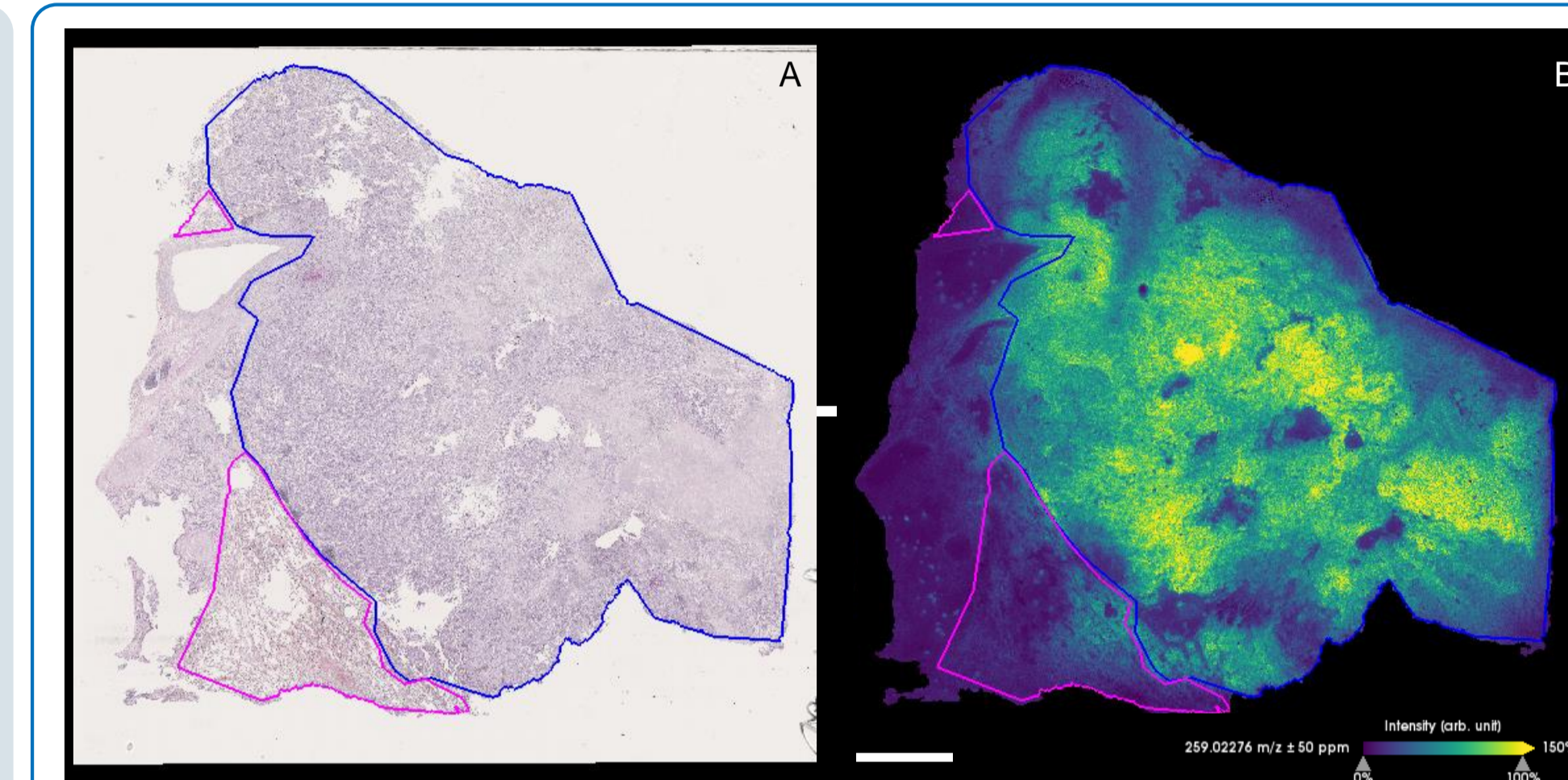
**Figure 1. Factor Xa distribution measured on a timsTOF fleX.** (A) The compound is detected in the renal medulla of treated animals; signal is absent in the non-treated samples. (B) Zoom of the range  $m/z$  390-490 with treated in blue and untreated in red. Scale bar is 2mm; relative intensities indicated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



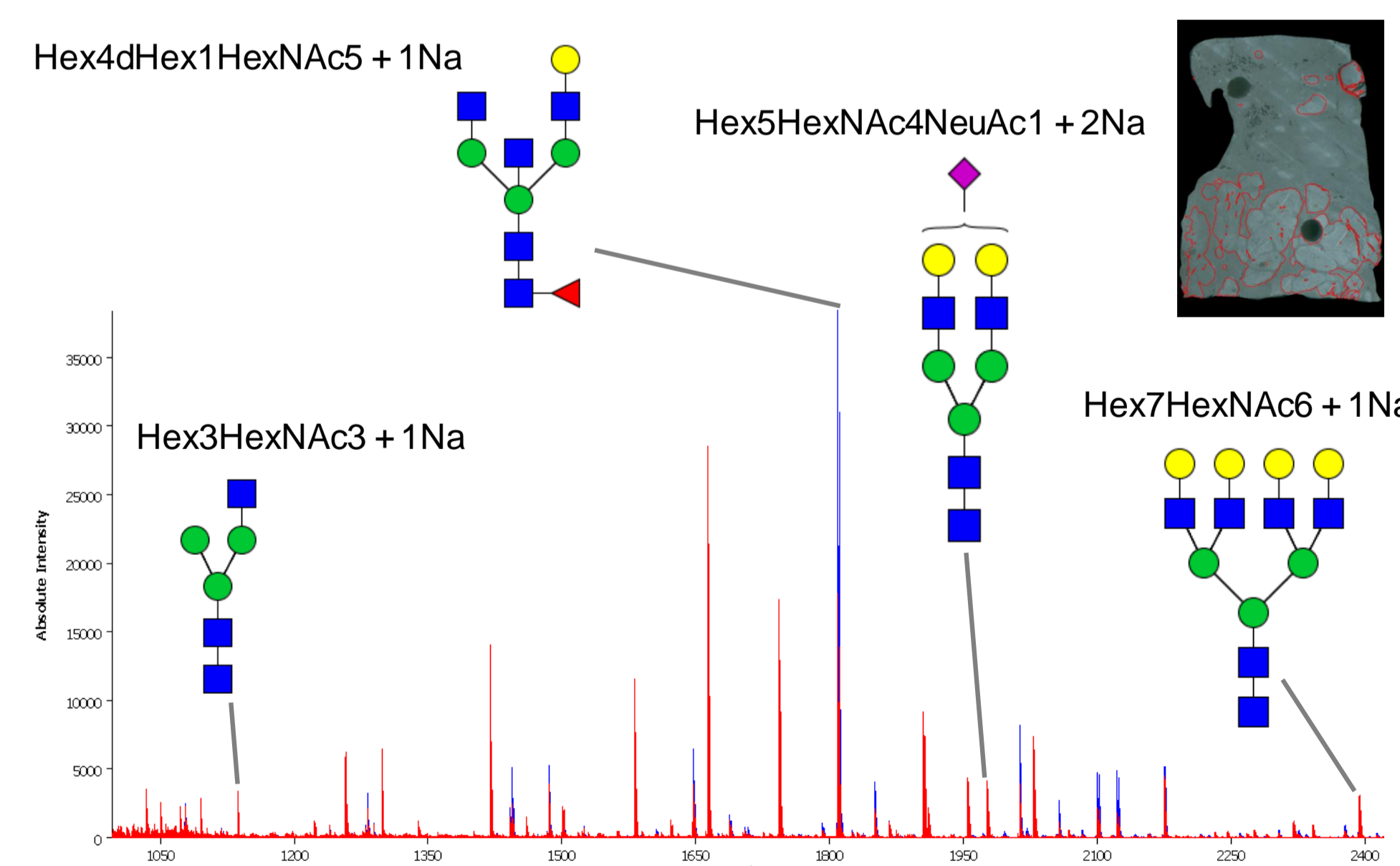
**Figure 2. Segmentation map and cluster tree of untreated and Factor Xa-treated kidneys.** Statistical analysis using the bisecting  $k$ -means algorithm with correlation distance clusters spectra from the medulla of treated animals separately from untreated animals. Scale bar indicates 2mm.



**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.



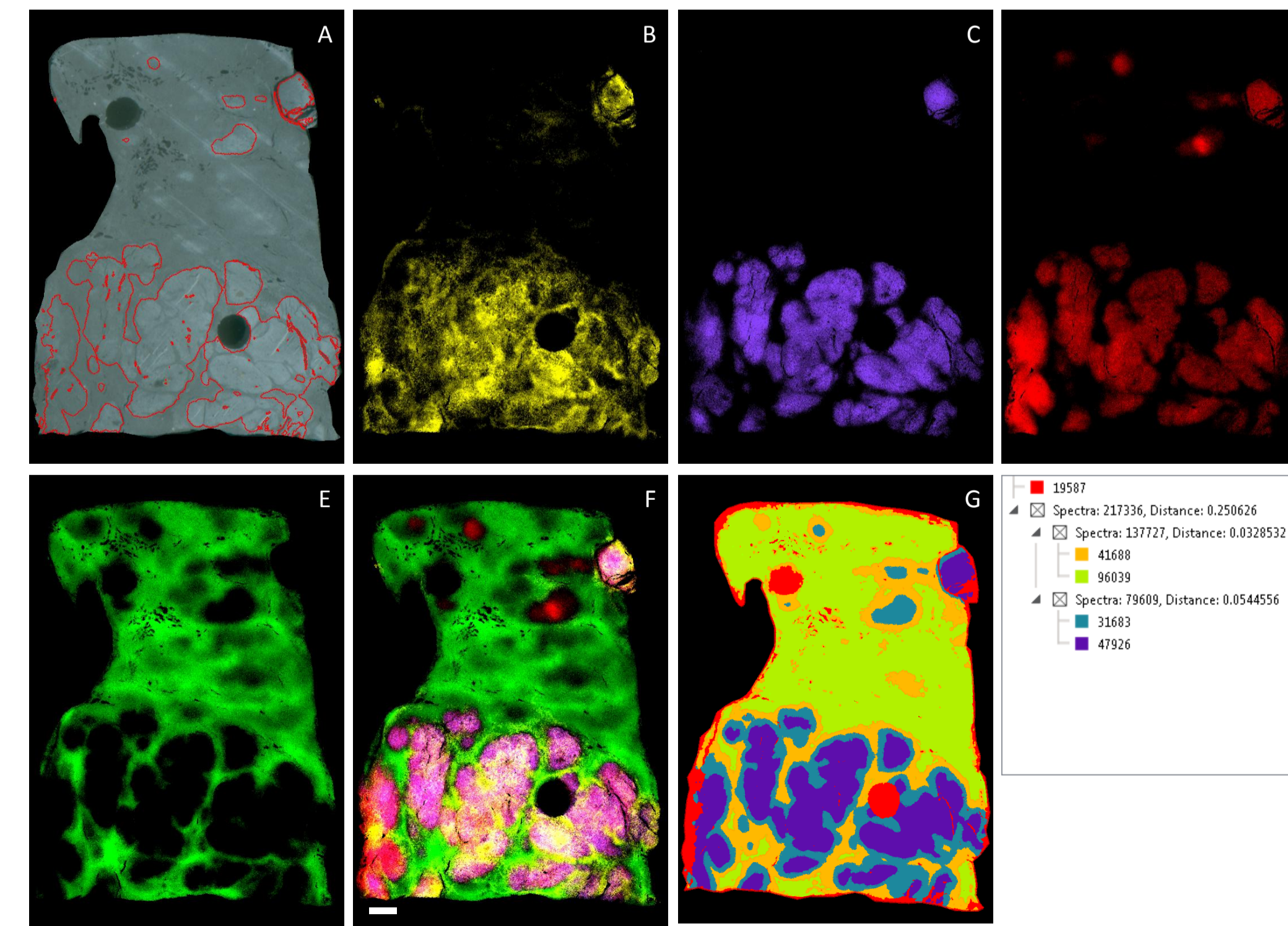
**Fig. 4. Endogenous metabolites from FFPE human lung tumor sample.** The detection of endogenous metabolites can be completed in FFPE tissues, but is often performed using extremely high mass resolving instruments rather than time-of-flight instruments due to high interference from isobaric matrix ions. (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.

## Acknowledgements

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## References

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timsTOF fleX