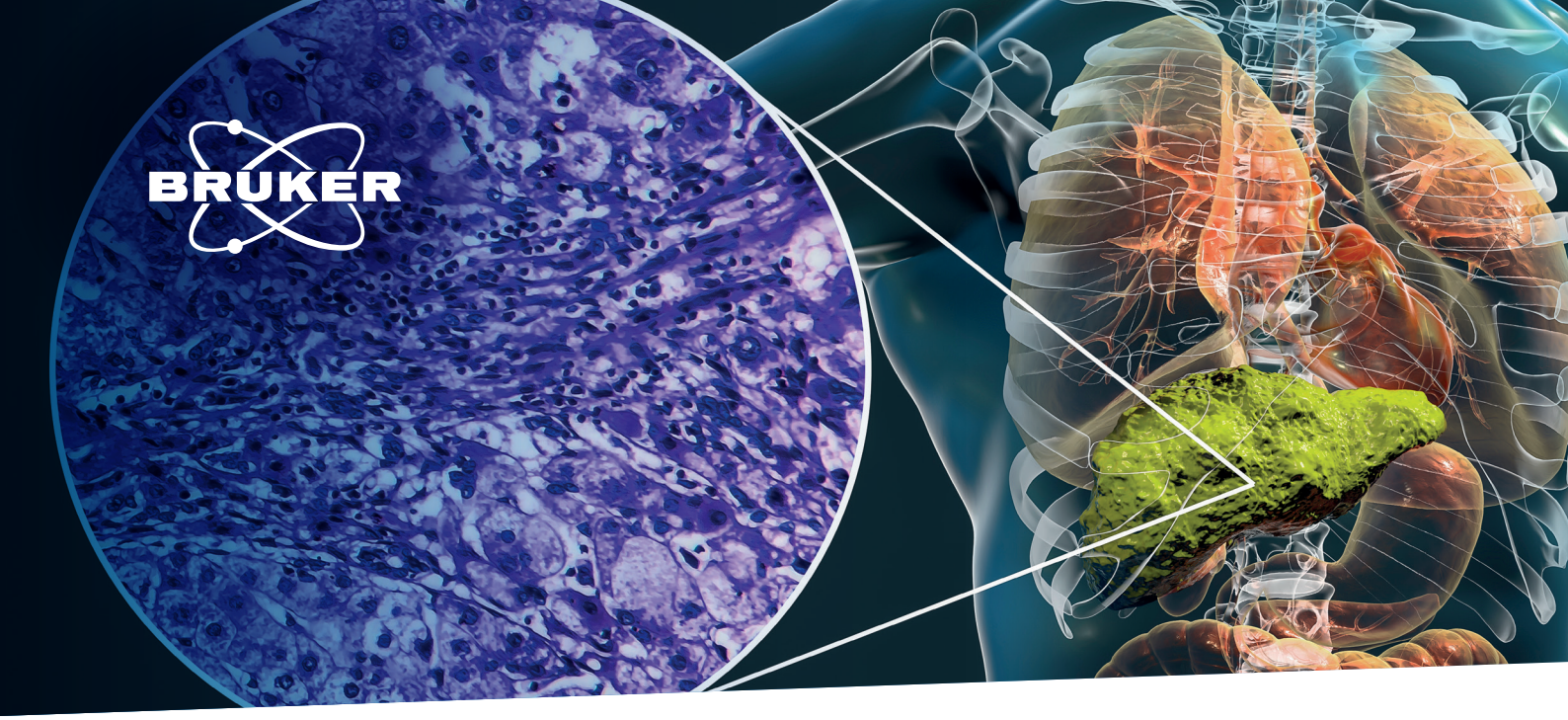

MALDI Imaging of glycans made simple

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been shown to be an important tool for high-throughput glycan profiling and here we show how to analyse such data.

Objectives

It is described in detail how to prepare a tissue sample to measure glycans and how to analyse the data with Bruker's software solutions afterwards. In this technical note you will learn about optimized methods to use for glycan analysis, and you can practice on your own using an example dataset that is downloadable from Bruker's website. The investigated tissue was liver and breast tumor treated with PNGase F and measured on a timsTOF fleX as described below.

Keywords:
timsTOF fleX,
MALDI imaging, MSI,
PNGase F, glycans,
oligosaccharides

Introduction

Just as genomics and proteomics have revolutionized translational research, glycomics brings the same promise, particularly in the fields of immunology and pathology. *N*-glycosylation facilitates protein folding and protein trafficking as well as signal transduction. However, N-glycans have been shown to be altered in disease states like cancer, immune disorders, and neurodegenerative disorders. For this reason, they present as noteworthy biomarkers that can help inform clinicians and accelerate diagnoses. In this example, we show that MALDI Imaging is capable of uncovering glycan profiles that are unique to pathologies within and across tissue types. Glycans with particular structural motifs are more abundant in liver tumors, liver stroma, breast tumors or breast stroma, whereas other glycans are shared between tumors, presenting a more generic marker for the cancer microenvironment. This is just one example showing the wealth of biological information waiting to be characterized and uncovered in the burgeoning field of glycomics.

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Methods

The detailed description of tissue preparation is described in Drake et al. (1). First, dewaxing of FFPE tissue sections, mounted on IntelliSlides®, was performed through 1 h incubation at 60°C followed by sequential soaking in fresh xylenes (3 min, 2x), 100% ethanol (1 min), Carnoy's solution (3 min, 2x), 95% ethanol (1 min), 70% ethanol (1 min) and HPLC water (3 min, 2x). After drying, tissues were subjected to pressurized heat (95°C) for 20 minutes in citraconic buffer (pH=3) for antigen retrieval. An HTX M5 TM-Sprayer (25 µL/min, 15 passes, 1200 mm/min, 45°C, 10 psi, 3 mm track spacing; 40 mm distance from tip of the nozzle to slide surface) was used to spray 0.1 mg/mL PNGase F (PNGase F Prime Ly, N-zyme Scientifics). After PNGase F application, the tissues were transferred to a humidity chamber and incubated at 37.5°C for two hours to optimize enzyme activity. After digestion, an automated pump coupled with a M5 TM-Sprayer (100 µL/min, 10 passes, 79°C, 10 psi, 2.5 mm track spacing; 40 mm distance from tip of the nozzle to slide surface) was used to apply 7 mg/mL α-cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile and 0.1% trifluoroacetic acid. MALDI Imaging was performed using a timsTOF fleX in positive ion mode with *m/z* range of 700-4000, transfer time of 130 µs and pre pulse storage of 30 µs. Data was collected using a spatial resolution of 150 µm with 300 laser shots per pixel. Data evaluation was performed in SCiLS™ Lab 2023b and MetaboScape® 2023.

Results and discussion

The investigation of liver and breast tumor tissue after treatment with PNGase F shows the power of MALDI Imaging to unravel glycan profiles. The overall mass spectra from the two imaging runs can be displayed in SCiLS Lab and represent good quality data and clear signal-to-noise ratios, which allows precise peak picking (Figure 1).

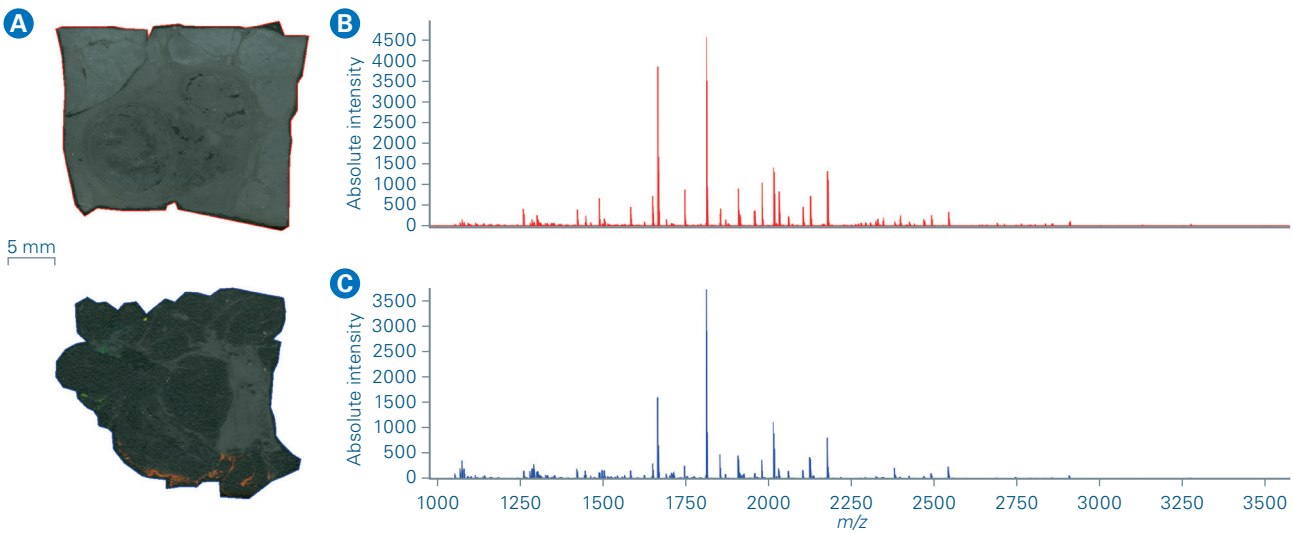

Figure 1

Optical images of tissue sections from liver tumor (Ⓐ, top) and breast tumor (Ⓐ, bottom) as well as corresponding sum average spectra collected from the MALDI imaging experiments (Ⓑ, red, liver tissue section and Ⓒ, blue, breast tumor section).

SCiLS Lab can be used to perform statistical analysis in order to provide context to the data. Here a segmentation analysis was used to perform an unsupervised statistical data analysis uncovering the different areas of the tissues. The tool was started from the "tools"-menu following the guide and using a TIC normalization, no denoising, the bisecting k-Means method and Correlation Distance metric. In the segmentation analysis pixel containing similar mass spectra are grouped together in labels which are then represented by different colors in the map. After calculation by the software is finished, the result is stored in the Labels tab. By double clicking on specific labels, the map can be interactively adjusted by expanding or collapsing classes. One example of a segmentation analysis is shown in Figure 2. Matching this result to histological annotations made by a pathologist, reveal that the liver tumor shows up

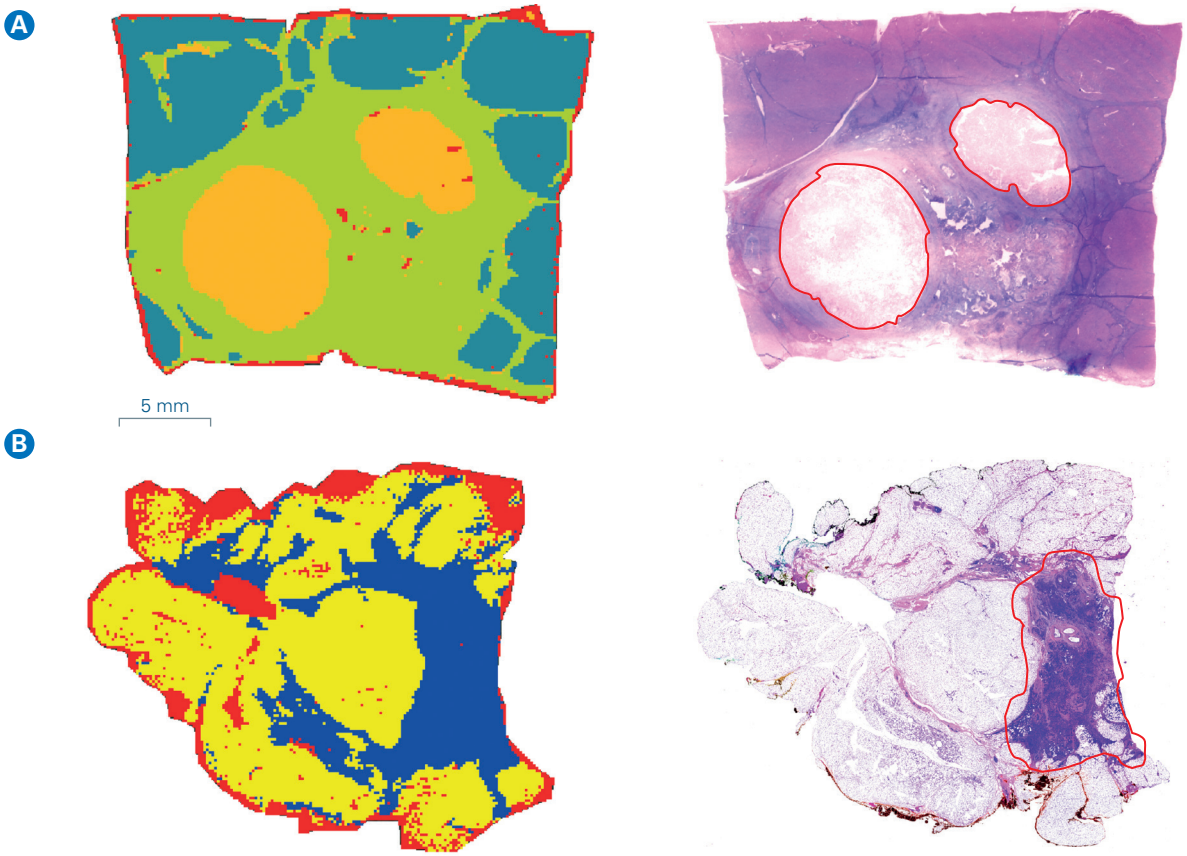

Figure 2
Result of a segmentation (bisecting k-means) analysis using SCiLS lab software for statistical analysis of hepatocellular carcinoma and surrounding liver tissue (Ⓐ, left) and breast cancer and surrounding breast tissue (Ⓑ, left). Corresponding H&E stained tissue with the tumor regions marked by a pathologist on the right side.

in orange (Figure 2A) whereas the breast cancer in the other dataset is located in the region marked in blue (Figure 2B). These two classes were saved as regions in order to find *m/z* features that are located there.

With the unsupervised segmentation result it is possible to find discriminative *m/z* features (glycans) which are overrepresented e.g., in the tumor regions and serve as biomarkers. From the tools menu the option "find values colocalized to region" was chosen to use the ion images of the overall "Region" and correlate with the orange (liver tissue) or blue (breast tissue) region. The next step included matching these data against the glycan database, which has been developed for MetaboScape together with Rick Drake and his team. Therefore, the data were exported from SCiLS Lab through the objects tab using the function "export for MetaboScape". Then this file was used to import the MALDI Imaging dataset into MetaboScape. Following the wizard using all pre-defined standard parameters a Feature List is created. The features from this list were then matched against the Target List *N-Glycan human* (which can be downloaded from Bruker's webpage) delivering annotations for the *m/z* features. MetaboScape identified a number of different glycans, of which some relevant examples are listed in Table 1. The annotated features were then exported from MetaboScape by generating a .mca file which can be reimported to SCiLS Lab for visualization of the spatial distributions in the two investigated tumor tissues.

Table 1
Examples of identified glycans

m/z	Assigned glycan	Mass error
1298.448		Δ 0.924 ppm
1419.475		Δ 0.410 ppm
1485.535		Δ 0.942 ppm
1581.533		Δ 2.97 ppm
1647.587		Δ 0.303 ppm
1663.585		Δ 2.16 ppm
1743.581		Δ 0.057 ppm
1809.642		Δ 1.49 ppm
2012.725		Δ 3.13 ppm
2174.774		Δ 1.15 ppm

Data evaluation of the annotated features revealed that specific glycans are unique to pathologies within and across tissue types. It can be shown that some glycans are more abundant in liver tumors (Figure 3A), normal hepatocytes (Figure 3B), breast tumors (Figure 3C) or breast stroma (Figure 3D). There are also other glycans identified as more generic markers for cancer that show localization in both tumors (Figure 3E).

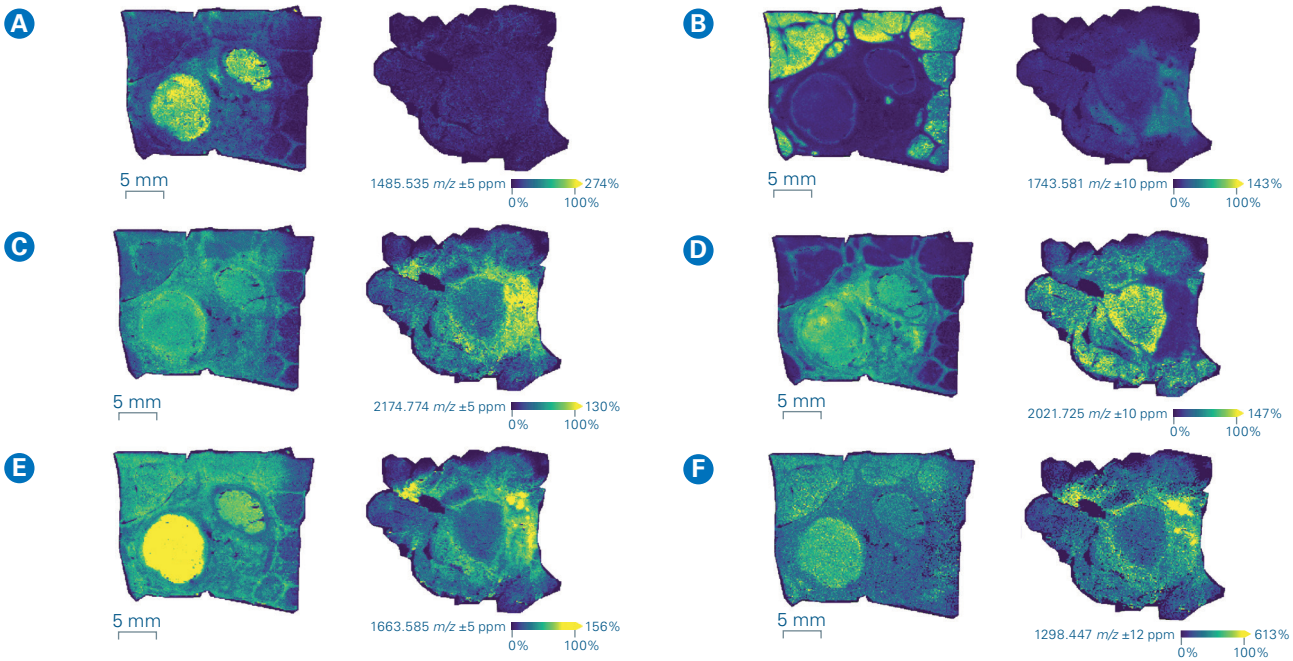

Figure 3

Extracted ion images showing the distribution of different glycans in liver tumor tissue (left part) and breast tumor tissue (right part). (A) ion image of *m/z* 1485.535 which is overrepresented in the liver tumor, (B) ion image of *m/z* 1743.581 localized in normal hepatocytes, (C) ion image of *m/z* 2174.774 localized in the breast tumor region and (D) ion image of *m/z* 2012.725 representing the breast adipocytes adjacent to tumor. The glycans corresponding to *m/z* 1663.585 and 1298.447 are present in both tumor types ((E),(F)).

Conclusion

MALDI Imaging can detect glycans directly from tissue after releasing with PNGase F. Unsupervised statistical analysis enabled distinguishing between the localization of tumor and surrounding regions of liver and breast tissue and confirmed the histological annotation a pathologist is able to make. Visualization of distinct glycans in SCiLS Lab software, which were annotated in MetaboScape, allow for identification of biomarkers that are specific for the two tumor types that were investigated in this proof of principle study.

Resources to try your own dataset

- SCiLS Lab trial
- Glycan dataset
- Contact your local sales rep to set up a software demo

References

[1] Drake RR, Powers TW, Norris-Caneda K, Mehta AS, Angel PM (2018). *In Situ Imaging of N-Glycans by MALDI Imaging Mass Spectrometry of Fresh or Formalin-Fixed Paraffin-Embedded Tissue.* Curr Protoc Protein Sci. **94**(1):e68. doi: 10.1002/cpps.68. Epub 2018 Aug 3. PMID: 30074304.

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