

# Rapid spatial molecular insights into human brain tumors by MALDI Imaging on a neofleX

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*AB and JO are employees of Bruker Corporation. Bruker manufactures and sells analytical instruments including mass spectrometers and software used in this study.*

Human brain tumor diagnosis traditionally relies on histopathology (H&E staining, IHC) and bulk tissue genomics, which are time-consuming and iterative. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI Imaging) offers an untargeted, spatially resolved molecular approach that can complement these methods and advance precision diagnostics. We applied MALDI Imaging using the neofleX Imaging Profiler to distinguish medulloblastoma from normal neural tissue based on unbiased lipid and glycan profiles. Our results highlight the potential of rapid axial MALDI TOF systems to streamline diagnostic workflows and provide deeper insights into tumor biology.

## Introduction

Human brain tumor diagnosis traditionally relies on histopathology (H&E staining, IHC) and bulk tissue genomics, which are time-consuming and iterative. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI Imaging) offers an untargeted, spatially resolved molecular approach that can complement these methods and advance precision diagnostics. We applied MALDI Imaging using the neofleX Imaging Profiler to distinguish medulloblastoma from normal neural tissue based on unbiased lipid and glycan profiles. Our results highlight the potential of rapid axial MALDI TOF systems to streamline diagnostic workflows and provide deeper insights into tumor biology.

## Methods

Frozen and FFPE medulloblastoma spinal cord samples were mounted on IntelliSlides (Bruker). Fresh-frozen sections were coated with DHB matrix using an M3+ sprayer (HTX Technologies), and lipid profiles were acquired on a neofleX Imaging Profiler (Bruker) in the 500–1100 m/z range. FFPE samples underwent PNGase F digestion (GlycoPath) for N-glycan analysis, followed by CHCA matrix coating and acquisition in the 900–4000 m/z range. Data were collected in positive ion mode at 20  $\mu\text{m}$  pixel size. Post-MALDI, samples were H&E stained, digitally imaged, and analyzed using SciLS Lab 2025b.

## Results

We performed MALDI Imaging on a medulloblastoma metastasis in the spinal cord to obtain spatial lipid and released N-glycan profiles. Unsupervised segmentation of both analyte classes clearly separated tumor tissue from normal spinal cord. Furthermore, the analysis distinguished grey matter from white matter within the spinal cord.

We identified specific m/z features that co-localized with tumor regions. Tumor-associated lipids

included m/z 560.32 and 796.53, tentatively annotated as LPE 18:1 [M+K]<sup>+</sup> and PC 34:2 [M+K]<sup>+</sup>, along with N-glycans at m/z 1305.82 and 1665.59. The infiltration zone showed enrichment of a lipid at m/z 753.58, corresponding putatively to SM 36:1;O<sub>2</sub> [M+Na]<sup>+</sup>. Distinct molecular profiles also characterized gray and white matter. N-glycans at m/z 1337.11 and 1033.09 were linked to white matter, whereas gray matter was enriched in features such as m/z 1257.42 and 2832.08.

## **Conclusion**

Lipid imaging of the spinal cord metastasis revealed that individual anterior horn cells are distinguishable by their lipid profiles using axial MALDI TOF Imaging. A putative LPC 16:0 [M+K]<sup>+</sup> was notably enriched in these cells. This capability highlights the technology's value for clinical research into molecular profiles and cellular architecture in neurological diseases. For example, profiling anterior horn cells could provide insights into motor neuron degeneration, supporting diagnosis and monitoring of conditions such as Amyotrophic Lateral Sclerosis (ALS).