

Rapid spatial molecular insights into human brain tumors by axial MALDI TOF mass spectrometry imaging

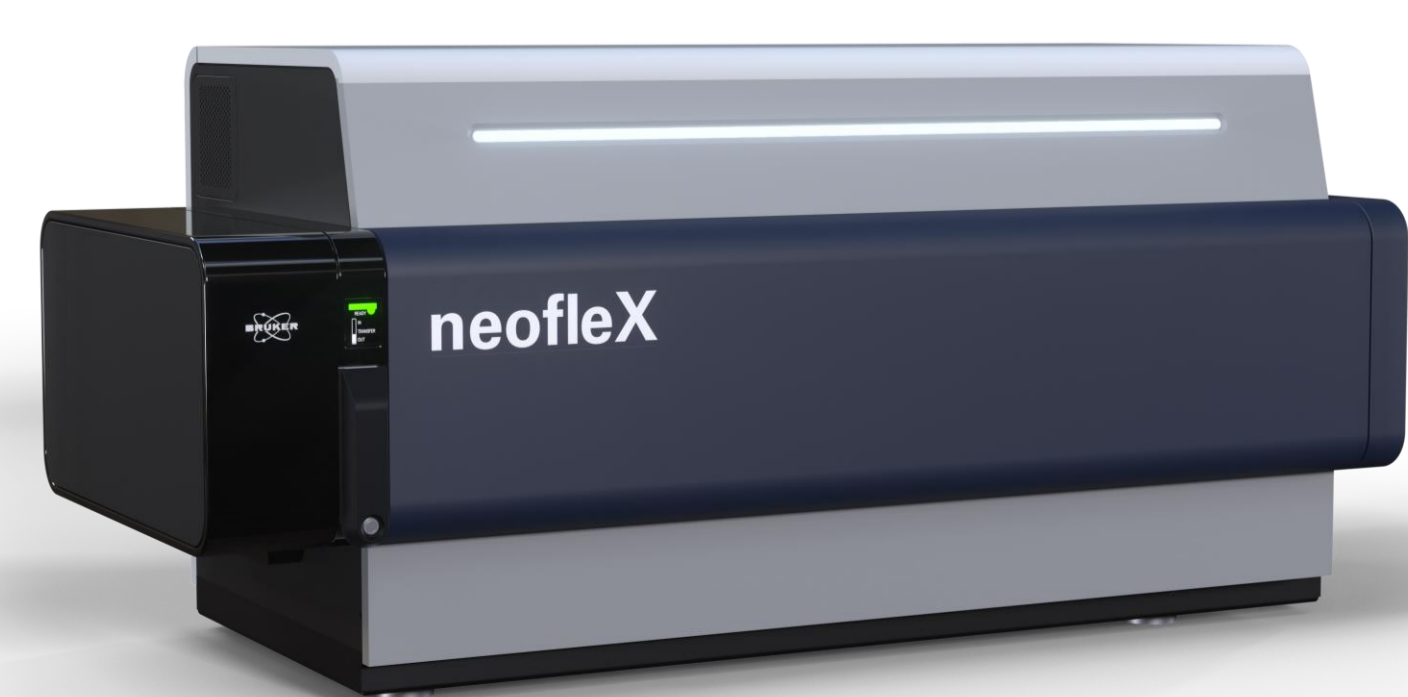
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Introduction

The diagnosis of human brain tumors currently relies primarily on traditional histopathological methods, such as hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC), combined with bulk tissue genomics. While this approach is iterative and time-consuming, it aligns with the current WHO classification. MALDI mass spectrometry imaging (MALDI Imaging) offers an untargeted, spatially resolved molecular method with significant potential to complement traditional approaches, aligning with the goals of precision diagnostics. Here, we utilized MALDI Imaging on the neoflex™ Imaging Profiler to distinguish key features of a disseminated medulloblastoma from normal neural tissues using unbiased lipid and glycan profiles. This highlights the potential of MALDI Imaging on a rapid axial MALDI TOF system to transform clinical research workflows and enhance our understanding of disease mechanisms.

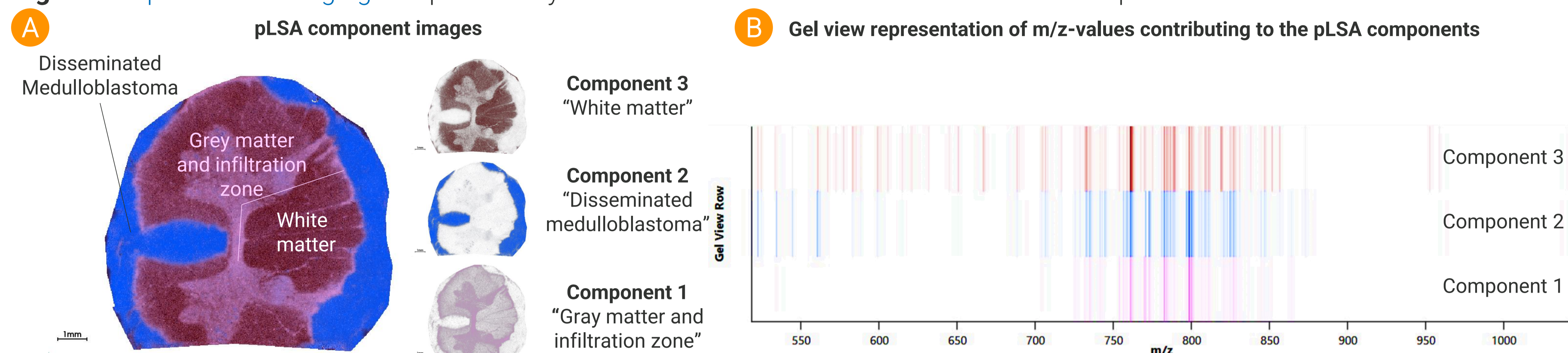
Methods

Fresh frozen and FFPE human medulloblastoma samples of the spinal cord were mounted on IntelliSlides® (Bruker). The fresh-frozen sections were spray-coated with DHB matrix using a M3+ sprayer (HTX Technologies). Spatial lipid profiles were acquired on a neoflex Imaging Profiler (Bruker) in the mass range 500-1100 m/z . The FFPE samples were processed for released N-glycan analysis by a PNGase F digestion according to a standard procedure (Bruker). CHCA matrix was sprayed using a M3+ sprayer, and data was acquired on the neoflex in the mass range 900-4000 m/z . Acquisitions were performed in positive ion mode with 20 μm pixel size. Samples were stained with H&E post-MALDI, and digital microscopy images were generated. All data were analyzed with SCLS Lab 2025b.



Results

Figure 1: Lipid MALDI Imaging and pLSA analysis of a Medulloblastoma metastasized to the spinal cord.



Lipid profiles obtained by MALDI Imaging of lipids from a fresh-frozen sample and subsequent Probabilistic Latent Semantic Analysis (pLSA) of the data distinguish the metastasized medulloblastoma region from white matter of the spinal cord as well as of the grey matter and infiltration zone (Figure 1A). The tissue specific mixture of m/z features contributing to the pLSA components are represented in Figure 1B.

Figure 2: N-glycan MALDI Imaging and pLSA analysis of a Medulloblastoma metastasized to the spinal cord.

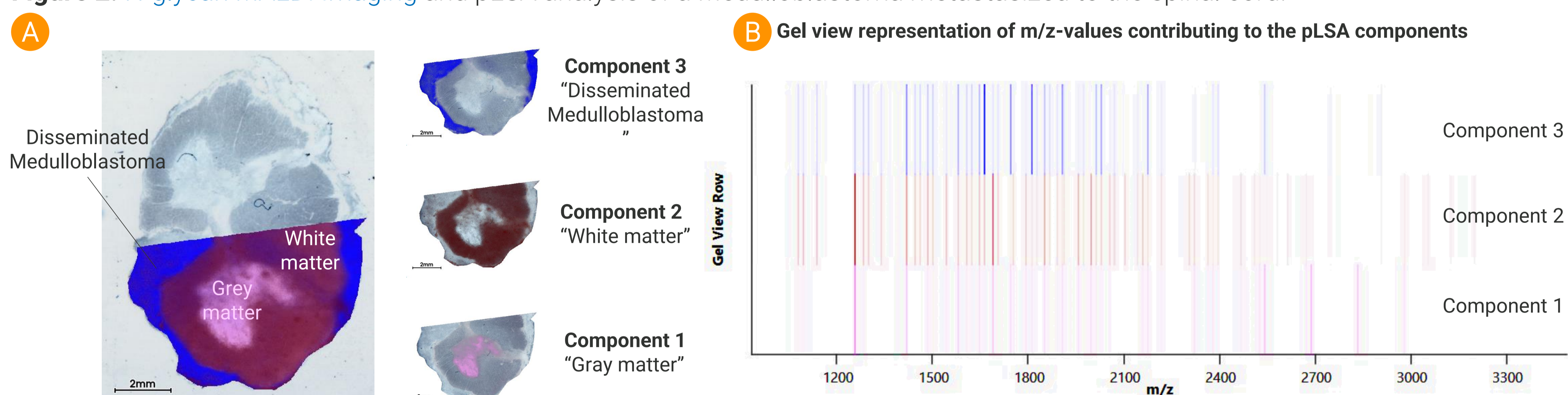


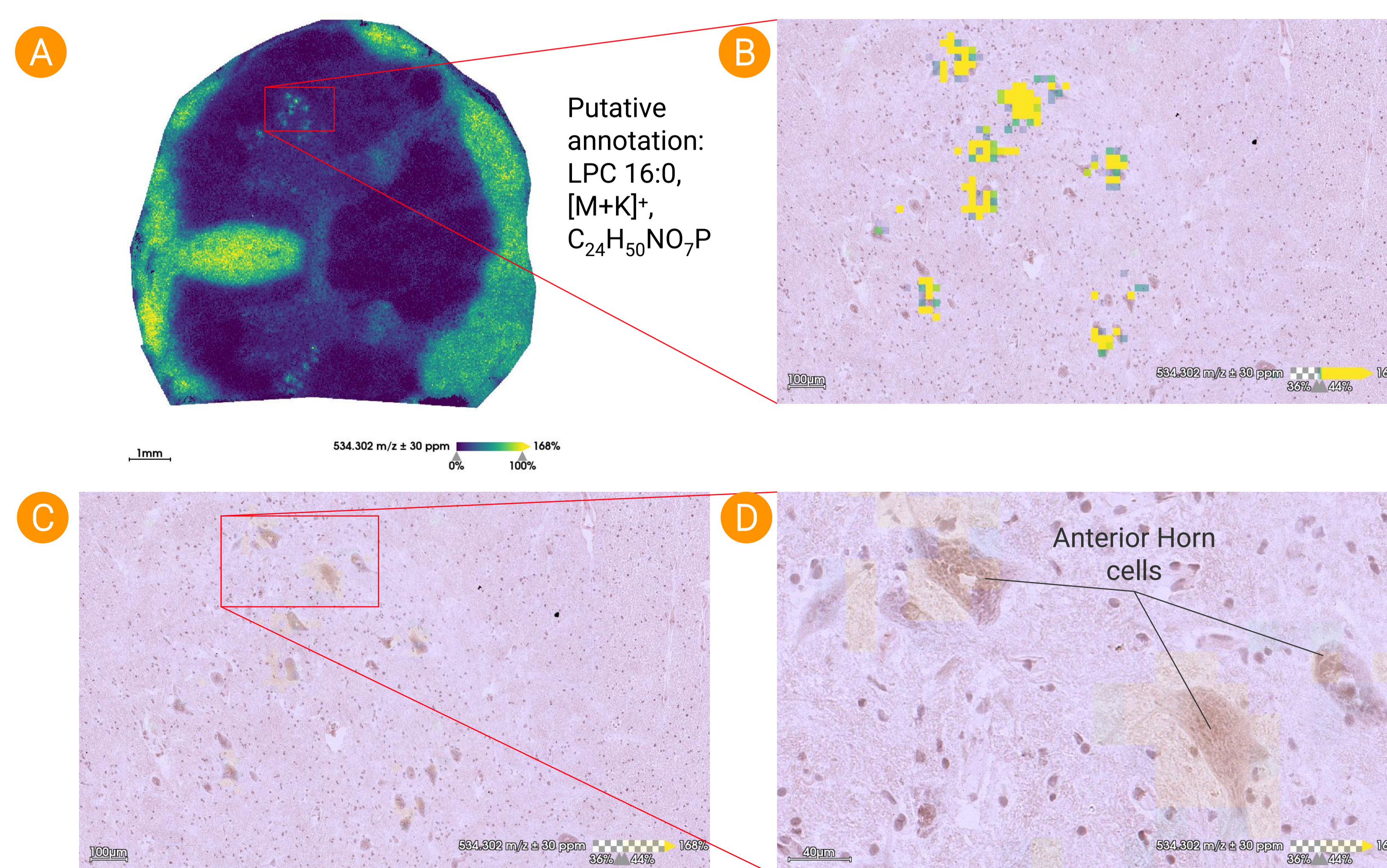
Figure 1. (A) Resulting image of the pLSA analysis with three components automatically clustering the disseminated medulloblastoma region (component 2) from the white matter region (component 3) and from the gray matter and infiltration zone (component 1). **(B)** Gel view representation of the m/z profiles contributing to the three pLSA components.

The lipid imaging data resulted in a total of 190,153 pixels with an imaging speed of 20 pixels per second. The total measurement time was approximately 2.6 hours for an area of 8.7x8.7 mm².

Figure 2. (A) pLSA analysis and corresponding component images superimposed on a low-resolution grey-scale scan of the section. Component 1 corresponds to the grey matter, component 2 to the white matter and component 3 to the medulloblastoma region **(B)** Gel view representation of the m/z profiles contributing to the three pLSA components. The broader the line, the bigger is the contribution to the separation.

Enzymatically released N-glycans from a FFPE section of a disseminated medulloblastoma were measured by MALDI Imaging on an axial MALDI TOF instrument. Like lipid profiles, also N-glycan profiles separate the medulloblastoma region from the spinal cord grey matter and white matter regions (Figure 2A). The tissue specific profiles with N-glycans contributing to the pLSA components are represented in Figure 2B.

Figure 3: Cell specific lipid distribution.



Rapid axial MALDI TOF Imaging data of lipids can reveal cell specific profiles as shown in Figure 3. A lysophosphatidylcholine (LPC)16:0 is abundantly present in Anterior Horn cells of the spinal cord. Anterior Horn cells are large, lower motor neurons signaling from the spinal cord to the muscles. These cells are degenerated in amyotrophic lateral sclerosis (ALS) making axial MALDI TOF imaging an interesting tool for clinical research and cell specific analysis.

- MALDI Imaging on an axial TOF system effectively distinguishes between tumor and healthy spinal cord tissue in a metastasized medulloblastoma.
- High-speed axial MALDI TOF Imaging provides a rapid methodology for molecular characterization of human brain pathology.
- Lipid imaging at 20 μm pixel size identifies Anterior Horn cell in the spinal cord, demonstrating the ability of this technology for cell specific analysis

Imaging MS

COI Disclosure. J.O., M.G., J.F. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study.