Detailed cellular profiling and unbiased molecular exploration of brain cancer using MALDI Imaging

Rapid single cell spatial mapping with the neofleX Imaging Profiler for comprehensive molecular histology.

Key Learning Objectives

- Provide more spatial molecular context to traditional histopathological techniques for disease understanding and subtyping
- Incorporate the innovative approach of MALDI Imaging for unbiased molecular information

Background and Introduction

Keywords:
MALDI Imaging, Medulloblastoma, Pathology, Lipidomics, Glycomics, Multiomics, Histology

Histopathology remains the cornerstone of tissue diagnostics, with methods such as hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) enabling visualization of tissue morphology and identification of specific biomarkers. In cancer research, this approach is often combined with bulk-tissue genomic analyses to arrive at an 'integrated' histomolecular diagnosis. While effective for confirming diagnoses according to the current WHO Classification, this approach is iterative and usually targets predefined biomarkers that may miss more specific molecular information that can affect accurate tissue subtyping. In the era of precision diagnostics and rapidly evolving bespoke clinical trials for these rare tumors, the current approach misses a wealth of spatially resolved biological data relevant for drug target discovery, patient stratification, and trial outcome analyses. To improve confirmation and spatial context to existing diagnostics, a fundamental change can be achieved by adding spatially resolved unbiased multiomics. This shift to improve phenotypic subtyping incorporates the well-established technique of matrix-assisted laser desorption/ionization (MALDI) Imaging mass spectrometry [1].

Jasmine Reese[1], Janina Oetjen[2], Katherine Stumpo[3], Olaf Ansorge[1]; [1]Nuffield Department of Clinical Neurosciences, University of Oxford; [2]Bruker Daltonics GmbH & Co. KG, Bremen, Germany; [3]Bruker Scientific LLC, Billerica, MA.

MALDI Imaging offers an innovative approach to address this limitation by delivering unbiased actionable clinical research information on one tissue section. This proof-of-concept study to evaluate core features of a medulloblastoma was performed on the neofleX™, a benchtop instrument with easy-to-use features and automated set-up.

Here, we focus on glycan and lipid MALDI Imaging patterns of a medulloblastoma with intrathecal (spinal) dissemination. This malignant brain tumor develops in childhood, with extremely poor outcomes. Given the rarity of these tumors, many patients are recruited into clinical trials, adding the possibility of longitudinal studies that monitor changes in molecular expression or drug-target effects. This example demonstrates the capabilities of the neofleX on standard histology blocks to reveal spatially resolved multiomic information across macroscopic and microscopic scales, down to single cell resolution.

By integrating this technology into current workflows, pathologists can potentially achieve dual objectives in the future: precise diagnosis according to current criteria and methods, and simultaneous exploration of the spatial molecular biology of disease down to the single cell level using mass spectrometry.

Methods

Tissue preparation

All human tissues were analysed with UK National Research Ethics Committee (REC) Approval: Reference: 23/SC/0241. Frozen and FFPE human tumor samples were sectioned on a cryostat and microtome, respectively, at 10 µm thickness and mounted on UV-irradiated (235 nm, 30 min) IntelliSlides® (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). The FFPE tissue slide was baked at 37°C overnight and transported at room temperature. The frozen tissue slide was dried under vacuum overnight and vacuum-sealed for transport. A grayscale reference scan was generated on an Epson Perfection V850 Pro flatbed scanner.

Matrix application

For frozen tissue, Dihydroxybenzoic acid (DHB) matrix (15 mg/mL DHB in 90% ACN, 0.1% TFA) was applied using Bruker's standard protocol on the M3+ sprayer (HTX Technologies, Chapel Hill, NC, USA). For FFPE tissue, PNGase F was sprayed to release N-glycans using the M3+ sprayer. The slide was transferred to a humidity chamber and incubated at 37°C for two hours. After digestion, α-cyano-4-hydroxycinnamic acid (HCCA) matrix (7 mg/mL HCCA in 50% ACN and 0.1% TFA) was applied using Bruker's standard protocol on the M3+ sprayer. Red phosphorus was spotted next to the sections for external calibration purposes.

Data acquisition

MALDI Imaging data was acquired on a neofleX Imaging Profiler instrument in positive ion mode for the mass ranges of 500-1100 *m/z* for lipid imaging, and 900-4000 *m/z* for N-glycan imaging at 20 µm pixel size. 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 8.7×8.7 mm^2.

Histological staining and image pairing

After MALDI Imaging, the same section was used for hematoxylin and eosin (H&E) staining. First, the matrix was removed by washing for 2 minutes in 70% ethanol. The slide was then stained with H&E using a standard protocol. Slides were digitized using a 20x and 40x objective on a Hamamatsu NanoZoomer. Regions and cells of interest were confirmed in the MALDI images via visual inspection of the histological image and were confirmed by a neuropathologist.

MALDI Imaging data analysis

All data were processed, analyzed, and visualized using SCiLS™ Lab version 2025b. MALDI Imaging data was registered to the H&E Image and imported. Total ion count (TIC) normalization was performed. An unsupervised component analysis using Probabilistic Latent Semantic Analysis (pLSA) was performed with random initialization.

Results

Unbiased lipid and glycan analysis of a metastasized medulloblastoma

Unbiased lipid MALDI Imaging in a medulloblastoma metastasized to the spinal cord distinguished tumor compared to the spinal cord, as well as grey and white matter of the spinal cord with unbiased pLSA analysis (Figure 1A). The anatomy can be visualized without a priori biological information, with the medulloblastoma presented in the pLSA image of component 2 (blue), the white matter of the spinal cord in component image 3 (brown), the tumor infiltration zone and grey matter in component image 1 (pink). Each region has specific spectral *m/z* profiles that are characteristic (Figure 1B). The spectral profiles represent a combination of individual *m/z* features, in this case lipids, that contribute to the separation of the components (i.e., in phenotypically distinct regions). The lines in the gel-view representation of the *m/z* profiles correspond to detected lipids. The darker the line, the higher is the contribution to the respective pLSA component. Importantly, it is the combination of several lipids that is specific for the phenotype which might in the future help characterizing disease state. Biomarker discovery and the association with key tissue architecture provides context for additional phenotypic regions.

Similar distinctions can be made with unbiased N-glycan MALDI Imaging, which again distinguished tumor compared to the spinal cord, as well as grey and white matter of the spinal cord in a pLSA analysis (Figure 2A). Medulloblastoma is presented in component 3 (blue) from the pLSA analysis, the white matter of the spinal cord in brown as component 2, and the grey matter in pink as component 1. Specific *m/z* profiles corresponding to a combination of N-glycans were enriched in the three tissue regions (Figure 2C). Individual features can be deduced from the N-glycan profiles (Figure 2B). Features such as *m/z* 1663.58 and *m/z* 1809.64 corresponding to N-glycans with the putative composition Hex5HexNAc4 and Hex5dHex1HexNAc4, respectively, were abundant in the tumor area. The grey matter of the spinal cord is enriched in features such as *m/z* 2832.02 (putative composition Hex7dHex3HexNAc6) and *m/z* 2685.96 (putative composition Hex7dHex2HexNAc6). Note that the grey matter region is more accurately reflected in the component image compared to the individual ion images showcasing the power of the pLSA analysis to separate the phenotypic distinct areas. The white matter has enriched features such as *m/z* 1257.42 (putative composition Hex5HexNAc2) and *m/z* 1688.61 (putative composition Hex3dHex1HexNAc5).

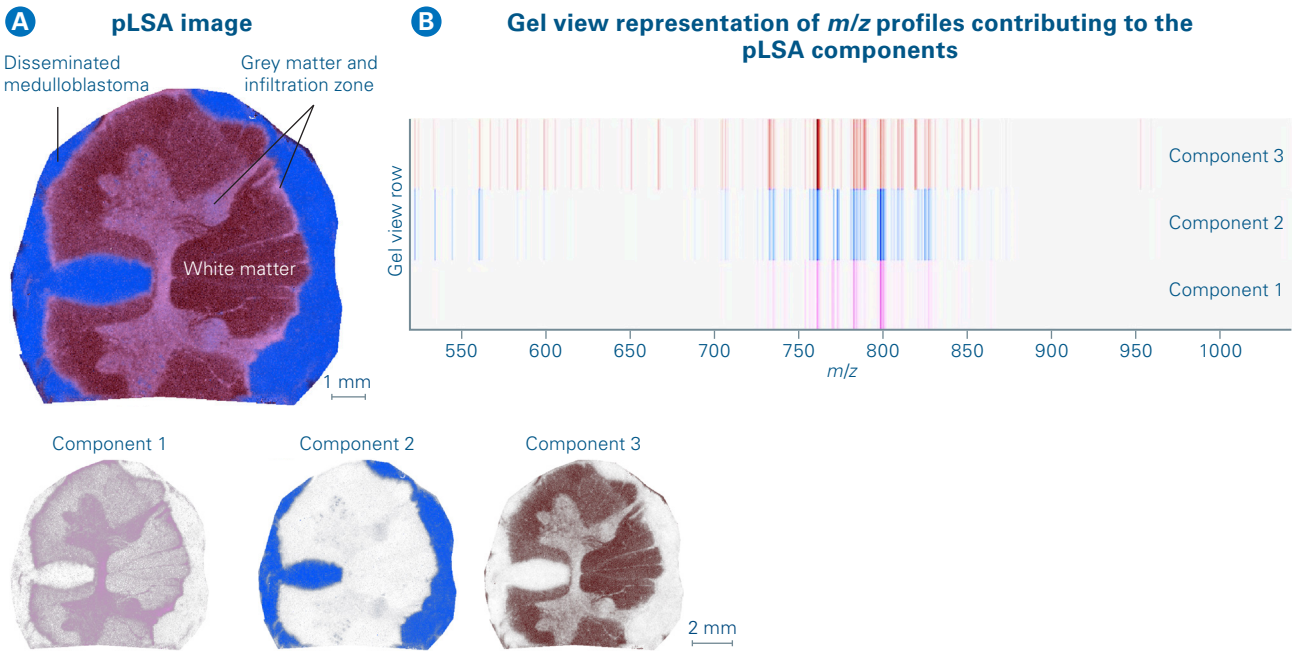

Figure 1. Lipid MALDI Imaging of a medulloblastoma metastasized to the spinal cord.
(A) Resulting image of the pLSA analysis with three components automatically clustering the disseminated medulloblastoma region (component 2) from the white matter region (component 3) and from the grey matter and infiltration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

Figures 1 and 2 demonstrate that MALDI Imaging with the neofleX is capable of providing unbiased spatial clustering of tumor regions with both lipid and glycan molecular profiling, providing clearcut borders between tumor and healthy brain for resections, as well as providing rich molecular insights into the molecular profiles of healthy versus tumor regions and the infiltration zones in a clinical sample.

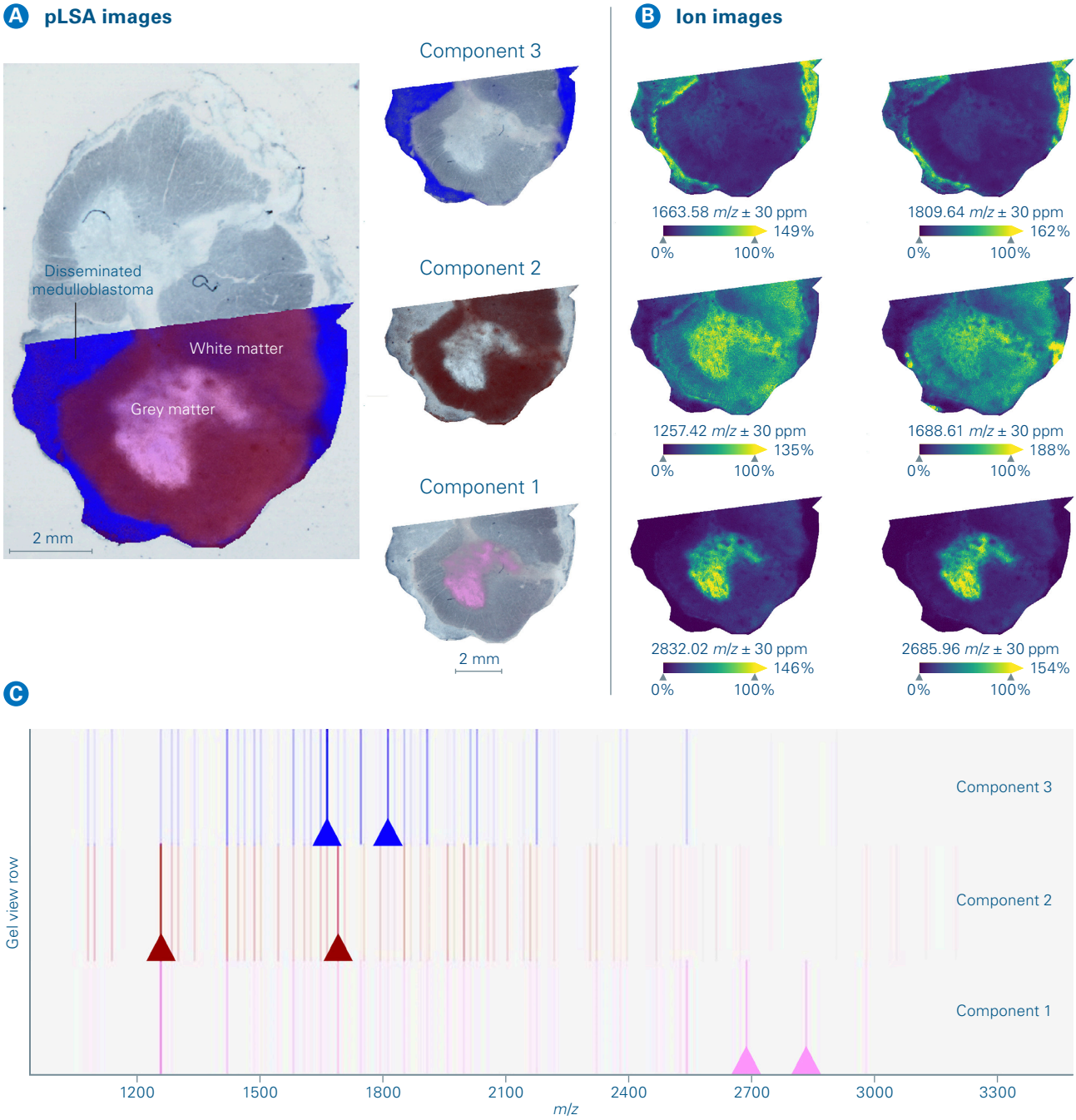

Figure 2. **N-glycan MALDI Imaging of a medulloblastoma metastasized to the spinal cord.**
Ⓐ pLSA analysis and corresponding component images superimposed on a low-resolution greyscale scan of the section. Component 1 corresponds to the grey matter, component 2 to the white matter and component 3 to the medulloblastoma region Ⓑ Ion images of individual *m/z* values contributing largely to the components. Ⓒ Gel view representation of the *m/z* profiles contributing to the three pLSA components. The *m/z* values used to produce the ion images in Ⓑ are marked by a triangle.

The neofleX is capable of distinguishing single cells in their spatial context

In addition to the molecular information that is gained from the disseminated medulloblastoma, the lipid imaging of the spinal cord revealed that single Anterior Horn cells can be visualized based on lipid distributions with the neofleX (Figure 3). These capabilities place the neofleX in an ideal position to contribute to clinical research into the molecular profiles of both the regional as well as the finer cell structural architecture of neurological diseases. For example, the ability to visualize molecular profiles of Anterior Horn cells could also provide insights into motor neuron degeneration, in diseases such as Amyotrophic Lateral Sclerosis (ALS).

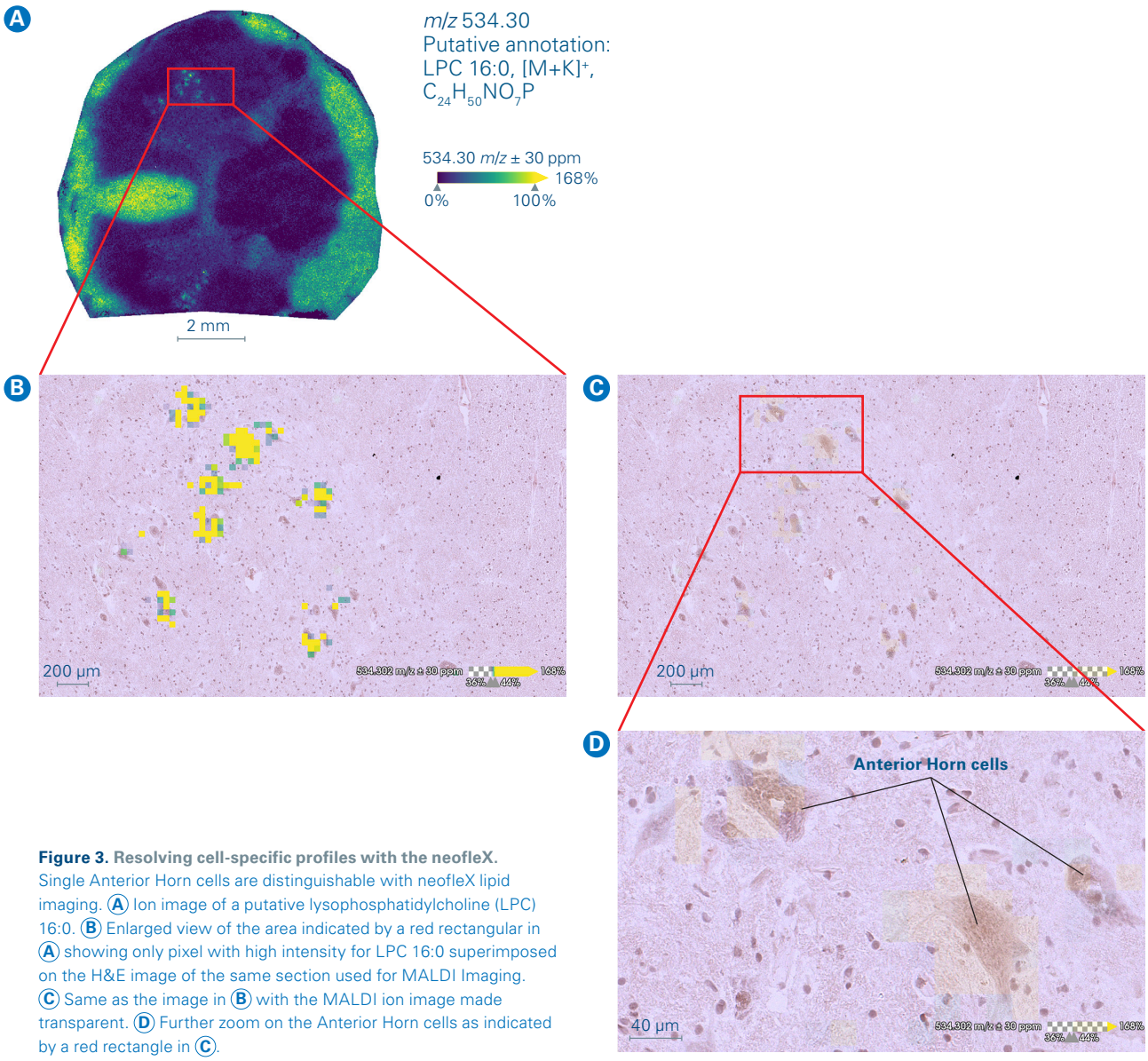

Figure 3. Resolving cell-specific profiles with the neofleX. Single Anterior Horn cells are distinguishable with neofleX lipid imaging. (A) Ion image of a putative lysophosphatidylcholine (LPC) 16:0. (B) Enlarged view of the area indicated by a red rectangular in (A) showing only pixel with high intensity for LPC 16:0 superimposed on the H&E image of the same section used for MALDI Imaging. (C) Same as the image in (B) with the MALDI ion image made transparent. (D) Further zoom on the Anterior Horn cells as indicated by a red rectangle in (C).

Conclusion

- The benchtop neofleX demonstrates significant promise as a clinical research tool for pathology departments, offering dual functionality for neuropathological precision diagnostics while simultaneously providing unbiased molecular profiling of spatially-resolved disease biology that cannot be captured with genomic technologies.
- neofleX provides a fast solution for high-throughput molecular imaging that holds potential to be embedded into legacy clinical research pathways. It achieves a total acquisition speed of approximately 2.8 hours for 200,000 pixels at 20 µm resolution for an 8.7 mm^2 tissue section.
- Lipid and N-glycan imaging using the neofleX effectively distinguished between tumor zones, infiltration regions, and healthy brain tissue in a metastasized medulloblastoma.
- Lipid imaging at 20 µm pixel size successfully identified individual Anterior Horn cells in the spinal cord, demonstrating the ability for single cell analysis, showcasing the capability of the neofleX for high-resolution, cell-specific analysis.

References

[1] Aichler M, Walch A (2015). *MALDI Imaging mass spectrometry: current frontiers and perspectives in pathology research and practice*. Lab Invest **95**, 422–431. https://doi.org/10.1038/labinvest.2014.156

For Research Use Only. Not for use in clinical diagnostic procedures.

Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com

Learn more at **www.bruker.com/neoflex**