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Characterization of a new MALDI-TOF axial benchtop instrument "neofleX™" for MALDI Imaging application

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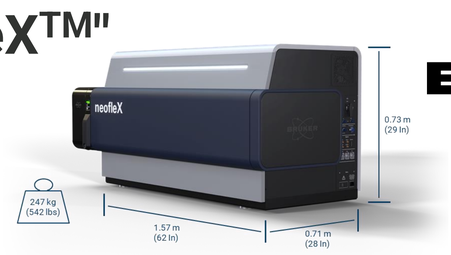

Introduction:

Expanding analytical utility from discovery-based research to translational utility requires tools that can meet the challenge. We characterized a new benchtop MALDI axial TOF mass spectrometer, the neofleX™ Imaging Profiler (Bruker Daltonics, Bremen, Germany), for broad application use cases and showcase the analytical applicability to perform MALDI Imaging measurements in clinically relevant research areas.

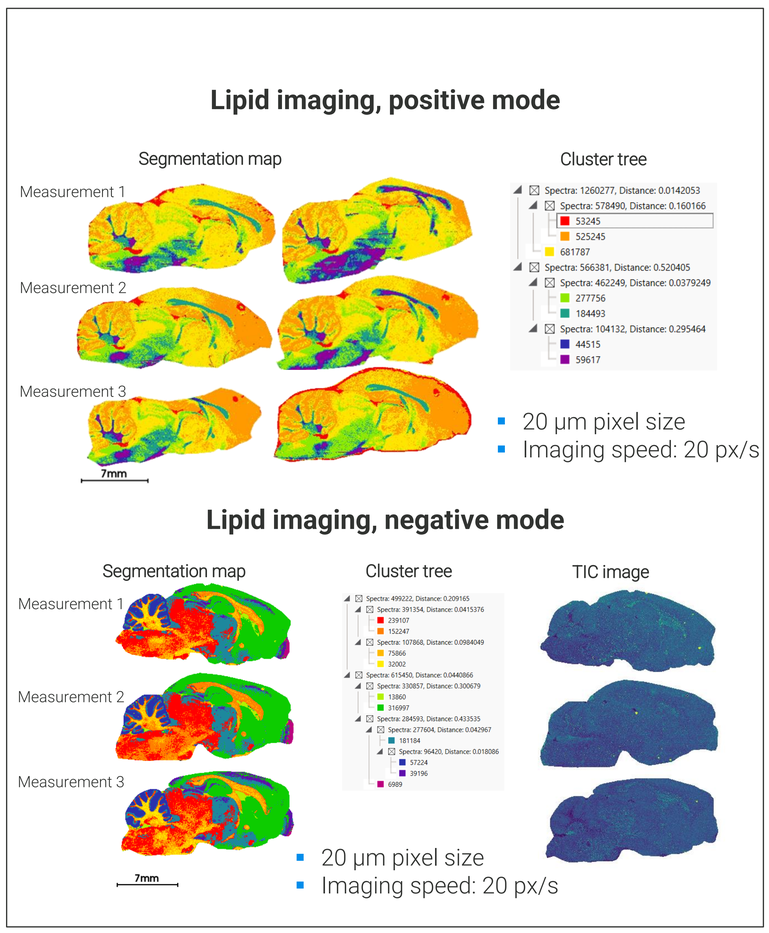

Figure 1. Measurement stability during lipid imaging runs in positive and negative ion mode on consecutive acquisitions of rat brain sections.

Methods:

Biological tissue sections were mounted on IntelliSlides® and prepared for MALDI Imaging acquisitions to map the distribution of lipids, targeted proteins, and glycans. The MALDI Imaging experiments were conducted on a neofleX Imaging Profiler in positive and negative ion mode at 20-30 µm pixel size. The instrument was equipped with a movable stage and an adjustable smartbeam 3D laser. New enhanced imaging detectors were installed which have stable gain even for many hours long imaging runs with high ion currents. The data was acquired with the FlexCompass 2025 package. Data visualization was performed with a SCiLS™ Lab 2024b or SCiLS Scope 1.

Results:

MALDI Imaging of lipids (Figure 1)

Three consecutive measurements were performed with two rat brain sections each sprayed with DHB matrix. An unsupervised clustering was conducted on data from all three measurements together. The segmentation analysis split in between tissue regions indicating high stability over a total of one million pixels.

Negative ion mode lipid imaging was performed with NEDC matrix on three rat brain sections yielding a similar result. The TIC image of all three datasets demonstrates that signals were stable over a total of thirteen measurement hours.

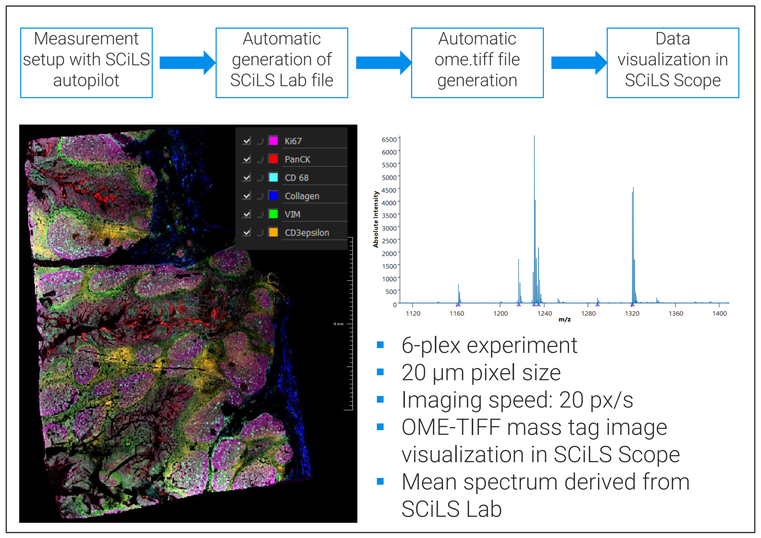

Figure 2. MALDI HiPLEX-IHC on a human tonsil sample.

MALDI HiPLEX-IHC of a human tonsil sample (Figure 2)

MALDI HiPLEX-IHC, a spatial biology workflow enabling multiplexed targeted protein expression analysis, was. performed on a pre-stained human tonsil sample which was purchased from AmberGen, Inc. (Billerica, MA, USA). After UV-cleavage, DHB matrix was sublimated and recrystallized. The measurement was setup with SCiLS autopilot using a default acquisition method. During setup, the automatic SCiLS Lab file and OME-TIFF file generation was enabled so that the antibody mass tag images could be readily visualized in the new SCiLS Scope software.

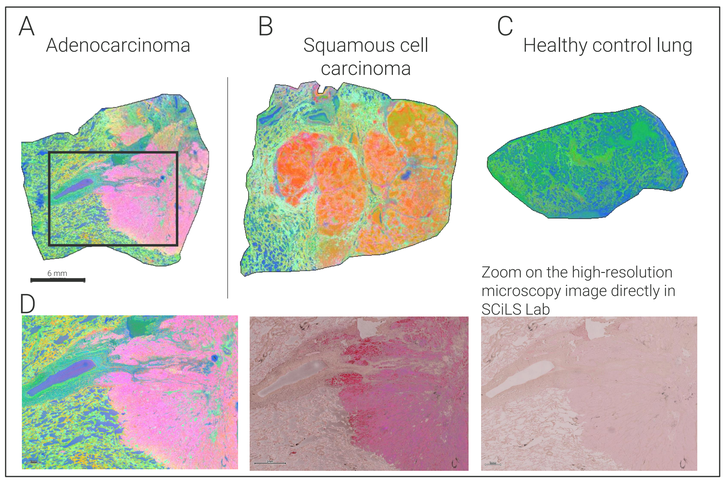

Figure 3. pLSA analysis of a 116-plex MALDI HiPLEX-IHC of lung (A) adeno- and (B) squamous cell carcinoma and (C) a control sample at 30 µm pixel size. Zoom on the adenocarcinoma tumor area (D, pink) superimposed on the H&E stain of the same section (40-fold magnification).

116-plex MALDI HiPLEX-IHC of lung cancer (Figure 3)

A pLSA analysis distinguished cancerous from healthy lung tissue and discriminated adenocarcinoma from squamous cell carcinoma in this pilot experiment (Figure 3A-C). Individual antibody profiles were characteristic for the adenocarcinoma tumor region (rose, pink components) or the squamous cell carcinoma tumor region (orange/ red components).

Importantly for interpretation, the microscopy image of the same section, as shown here for the adenocarcinoma sample (Figure 3D), was co-registered with the MALDI HiPLEX-IHC data. Zooming into the original image resolution is supported in SCiLS Lab by external storage of the image file in pyramidal format.

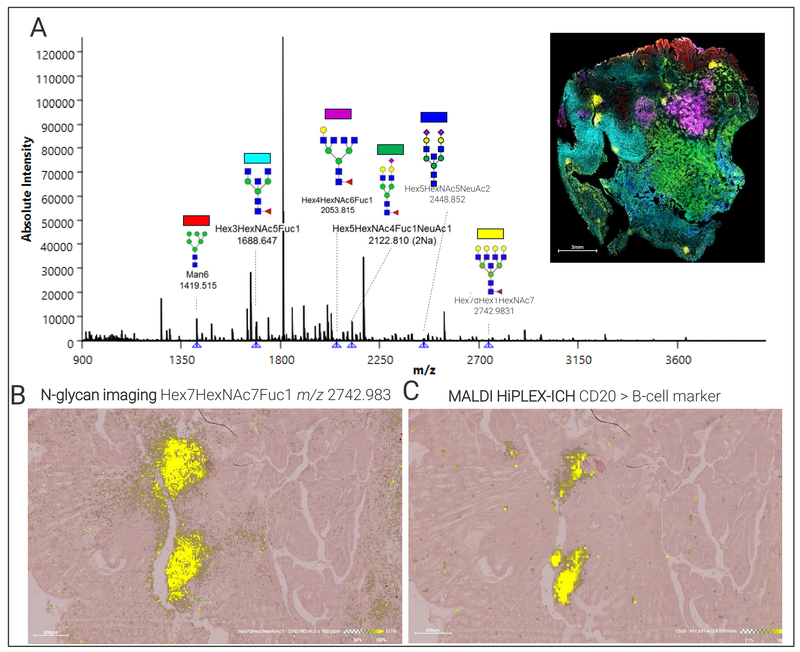

Figure 4. Multimodal imaging combining released N-glycans imaging and MALDI HiPLEX-IHC from a colorectal cancer sample overlayed with an H&E image of the same section.

MALDI Imaging of released N-glycans (Figure 4)

Distributions of released N-glycans from a colorectal cancer section were imaged and MALDI HiPLEX-IHC was performed on the same section post acquisition. The N-glycans with the putative structures indicated below show distinct distributions specific for different tissue phenotypes such as normal crypt (light blue), mucinous tumor (pink), tumor stroma (green, dark blue) (Figure 4A). A complex fucosylated *N*-glycan (*m/z* 2742.983, yellow, Figure 4B) was co-localized with a MALDI HiPLEX-IHC image of CD20, which is a marker for B-cells (yellow, Figure 4C).

Conclusion:

- The neofleX Imaging Profiler showed excellent instrument performance for various MALDI Imaging use cases:
- Robust measurements with over 1 millon pixels over three runs
- High speed acquisitions at 20 px/s
- MALDI HiPLEX-IHC provides complementarity to other spatial biology applications, and maximum protein information in one run
- Software solutions supporting automated workflows

Technology

COI Disclosure: C.L., M.E., J.O., S.B., C.N., A.H., J.K., T.B., U.H., A.S., K.S., J.H. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation and products, including mass spectrometers and software used in this study.

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