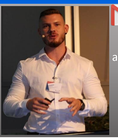
Sequential MALDI-MS imaging of lipids, N-Glycans, and tryptic peptides on a single FFPE tissue section



andrew.smith@unimib.it
LinkedIn andrew-smith-5a453a38
Andrew_Smith102

Vanna Denti[1], Isabella Piga[1], Francesca Clerici[1], Lisa Pagani[1], Lucrezia Criscuolo[1], Clizia Chinello[1], Katherine A. Stumpo[2,3], Dale S. Cornett[3], Giuseppe Paglia[1], Fulvio Magni[1], and Andrew Smith[1]

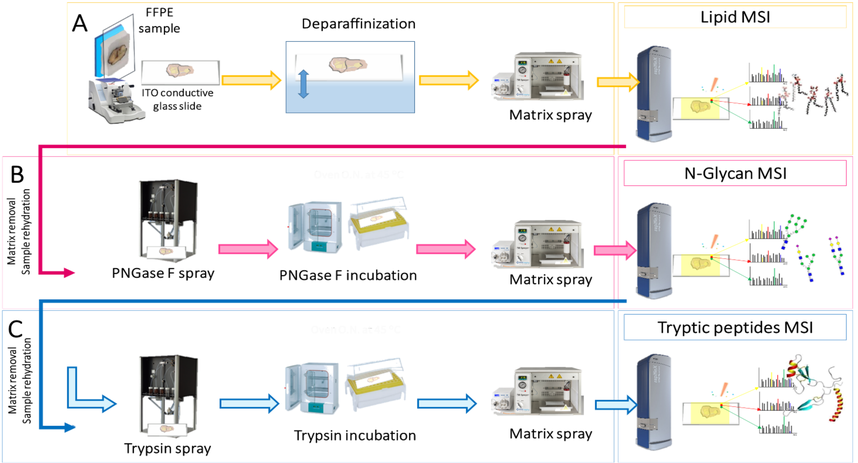
[1]*University of Milano-Bicocca, Department of Medicine and Surgery, Proteomics and Metabolomics Unit, Vedano al Lambro, Italy*
[2]*Bruker Scientific LLC, Billerica, MA 01821*
[3]*The Johns Hopkins University School of Medicine, Russell H. Morgan Department of Radiology and Radiological Science, Division of Cancer Imaging Research, Baltimore, MD 21205*

Scan Me

RATIONALE

Mass spectrometry imaging (MSI) is an emerging technology that is capable of mapping various biomolecules within their native spatial context and performing spatial multi-omics on formalin-fixed paraffin-embedded (FFPE) tissues may further increase the molecular characterisation of pathological states. However, it is not uncommon for the amount of clinical tissue available to be limited, as well as the cellular distribution differing even between serial sections. Thus, we present a novel workflow which enables the sequential MSI of lipids, N-Glycans, and tryptic peptides on a single FFPE tissue section and highlight the enhanced molecular characterisation that is offered by combining the multiple spatial omics datasets.

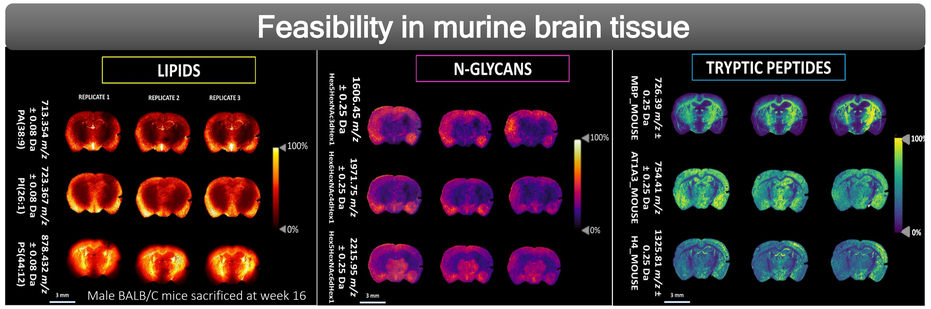
WORKFLOW



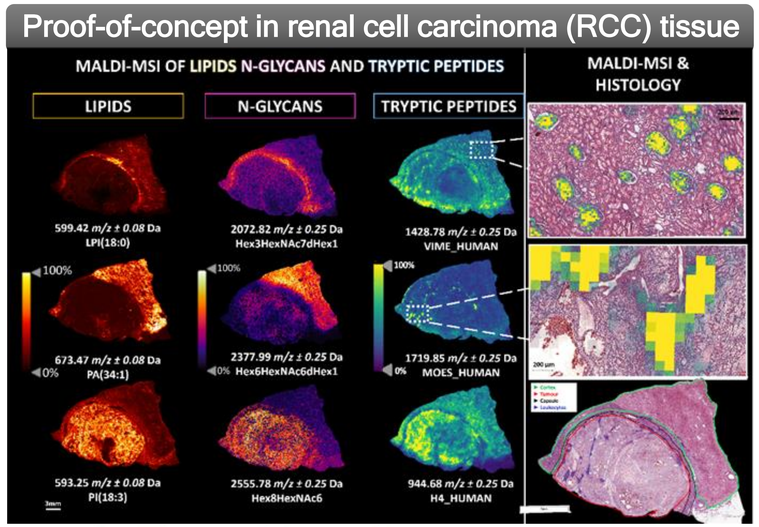
For the imaging of lipids, FFPE tissue sections were deparaffinised prior to the deposition of 10 mg/mL 9-Aminoacridine (9-AA) using a HTX TM-Sprayer. Following MSI analysis, 9-AA was washed from the tissue. For the imaging of N-Glycans, antigen retrieval was performed prior to the deposition of PNGase F using an iMatrixSpray automated spraying system and left to digest overnight. Upon completion, 5 mg/mL α-cyano-4hydroxycinnamic acid (CHCA) was deposited. Following MSI analysis, CHCA was also washed from the tissue. For the imaging of tryptic peptides, trypsin was deposited using an iMatrixSpray and left to digest overnight. Finally, 10 mg/mL CHCA was deposited. All imaging analyses were performed using a rapifleX MALDI Tissuetyper™, employing a beam scan setting of 46 µm and a raster of 50 µm in both *x and y* directions..

RESULTS

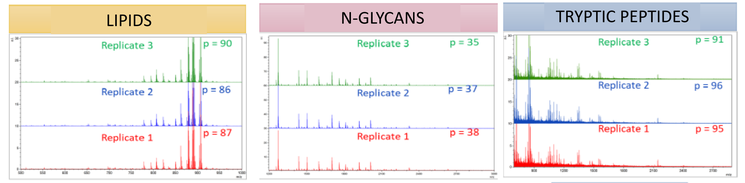
Feasibility in murine brain tissue



Proof-of-concept in renal cell carcinoma (RCC) tissue

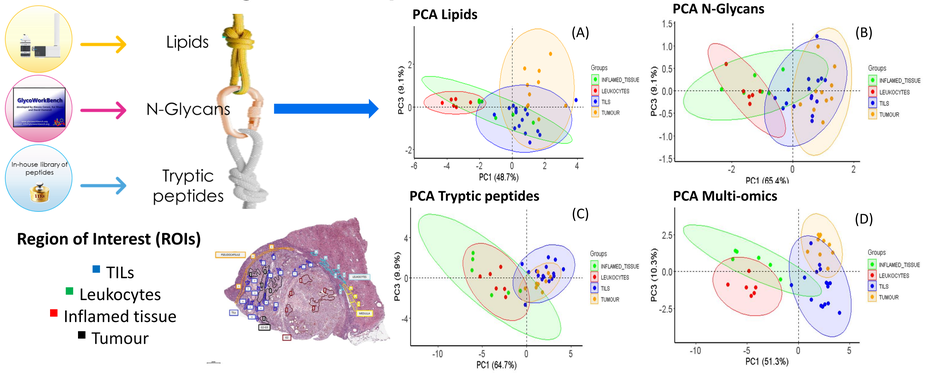


Coefficient of variance <20% for each molecular class



(Left) MALDI-MS images of lipids, N-Glycans, and tryptic peptides in three technical replicates of murine brain tissue. The tissue distribution of the selected *m/z* features (rows) are highlighted in each of the technical replicates (columns), with a different colour scheme for each omics class. Scale and intensity bars are provided. **(Right)** Average lipid, N-Glycan, and tryptic peptide profiles obtained from the three technical replicates of murine brain tissue. The number of detected peaks, along with those common among all three technical replicates, are reported.

Integration of spatial multi-omics dataset



(Left) Sequential MALDI-MSI of lipids, N-Glycans, and tryptic peptides on the same RCC tissue section. The molecular annotation for each *m/z* feature are provided along with scale and intensity bars. The insets present the tissue distribution of vimentin (*m/z* 1428.72) and moesin (*m/z* 1719.47), co-localised to the glomeruli and tumour infiltrating leukocytes (TILs). **(Right)** Principal Component Analysis (PCA) score charts (PC1 (*x* axis) vs PC3 (*y* axis)) generated from the lipid (A), N-Glycan (B), and tryptic peptide (C) MSI analysis as well as the integrated spatial multi-omics dataset, which increased the ability to distinguish among the four major histopathological groups. 95% confidence intervals are highlighted by the respective background colour.

TAKE HOME

With the presented workflow, it is possible to perform sequential MSI of lipids, N-Glycans, and tryptic peptides on a single FFPE tissue section and in a reproducible manner. Moreover, each omics class may provide complementary information that, when integrated, may facilitate more comprehensive mapping of the molecular complexity present within pathological tissue.

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