

# Multimomics analysis for advanced tumor typing of lung cancer using 116plex MALDI HiPLEX-IHC and released N-glycans on the neoflex

Janina Oetjen<sup>1</sup>, Corinna Henkel<sup>1</sup>, Michael Easterling<sup>2</sup>, Nannan Tao<sup>2</sup>, Mark Lim<sup>3</sup>, Gargey Yagnik<sup>3</sup>, Andrew Yatsuhashi<sup>3</sup>, Kenneth Rothschild<sup>3,4</sup>, Mengze Stettler-Zhang<sup>5,6</sup>, Konrad Steinestel<sup>7</sup>, Bernd Bodenmiller<sup>5,6</sup>.

<sup>1</sup>Bruker Daltonics GmbH & Co. KG, Bremen, Germany; <sup>2</sup>Bruker Scientific, Billerica, MA, USA; <sup>3</sup>AmberGen Inc. Billerica, MA, USA; <sup>4</sup>Department of Physics and Photonics Center, Boston, MA, USA; <sup>5</sup>University of Zurich, Zurich, Switzerland; <sup>6</sup>ETH Zurich, Zurich, Switzerland; <sup>7</sup>Bundeswehrkrankenhaus Ulm, Ulm, Germany.

## Introduction

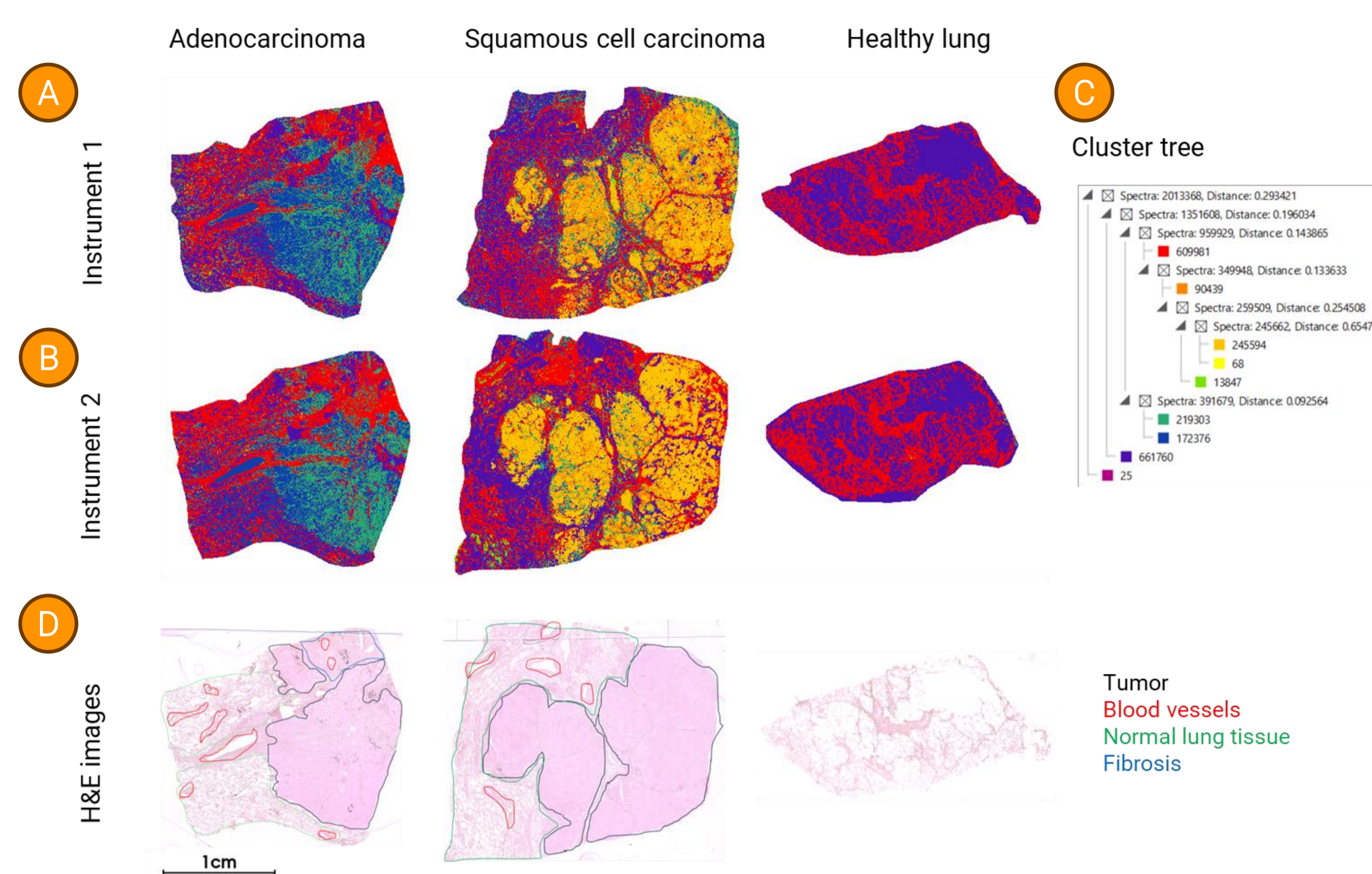
Spatially resolved molecular tumor typing is at the forefront of promising technologies in clinical research. MALDI HiPLEX-IHC is an antibody-based technique used to obtain spatial expression information from proteins in tissues. This proof-of-concept experiment uses a 116-plex of markers for an approach that removes the bias from a smaller panel, and enables a broader biological understanding of other potential protein targets that would otherwise not be considered. The antibody probes are tagged with photocleavable mass-tags (PC-MTs), which are detectable via MALDI mass spectrometry imaging (MALDI Imaging).

## Methods

The MALDI HiPLEX-IHC experiment with 116 PC-MT labeled probes (AmberGen Inc., Billerica, MA\*) was performed on lung adenocarcinoma (AD) and squamous cell carcinoma (SQ) compared against a healthy lung section using a previously published method [1]. The antibody probes were selected to target a variety of proteins including structural cell components, diagnostic markers, predictive markers, proteins involved in cell proliferation and cell state, as well as signaling pathways. The MALDI Imaging data were acquired on a neoflex™ Imaging Profiler with 30 μm pixel size in positive ion mode. After imaging, slides were further processed for N-linked glycan analysis with on-tissue PNGase digestion following protocols from GlycoPath (Charleston, SC). Lastly, the slide was stained with H&E for further detail on tissue morphology. All data were analyzed in SCiLS™ Lab version 2025a.

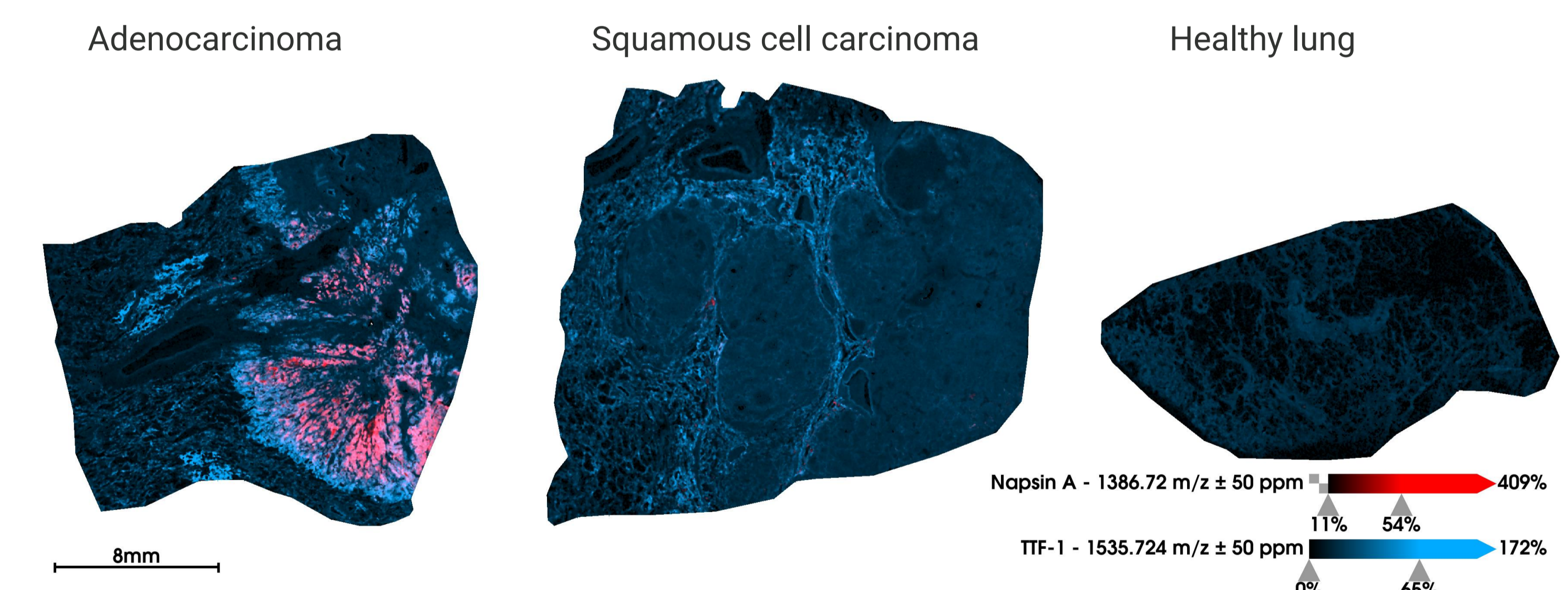
## Results

With simultaneous data collection of all 116-protein markers a broad spatial expression of the protein landscape was observed. Segmentation analysis of all 116-features was performed on two independent measurements to establish data reproducibility (Figure 1). The analysis separated normal lung tissue from tumor tissue. Furthermore, segmentation differentiated the adenocarcinoma tumor region from the squamous cell carcinoma region. Segmentation results were in accordance with pathologist annotation of the H&E stain. Lastly, segmentation demonstrated acquisition robustness given the reproducibility from measurements performed on two different instruments.



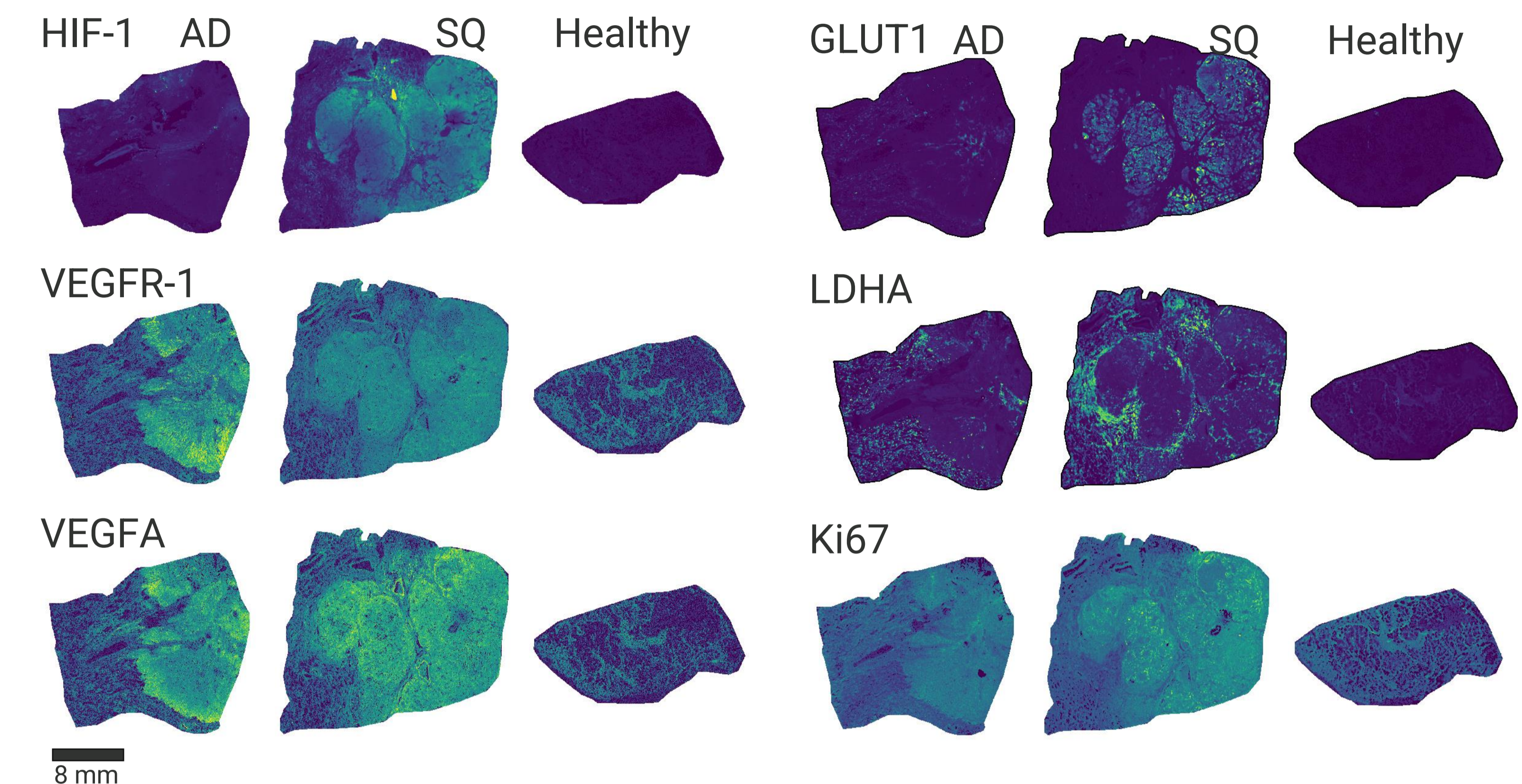
**Figure 1:** Unsupervised segmentation analysis of duplicates of the 116-plex MALDI HiPLEX-IHC data measured on two neoflex instruments (A and B) in comparison to pathological annotation of the H&E stain (D). The segmentation cluster tree is shown in C.

This 116-plex panel contained markers used in a clinical research setting to differentiate lung adenocarcinoma from squamous cell carcinoma (Figure 2). As expected for cancer biology, thyroid transcription factor 1 (TTF-1) showed higher abundance inside the tumor area of the adenocarcinoma and to a lesser extent in peritumoral healthy lung tissue of the squamous cell carcinoma and in the non-tumor control. Napsin A was specifically present in the adenocarcinoma tumor region, corroborating the finding that double staining with TTF-1 is more specific for distinguishing adenocarcinoma from squamous cell carcinoma tumors [2].



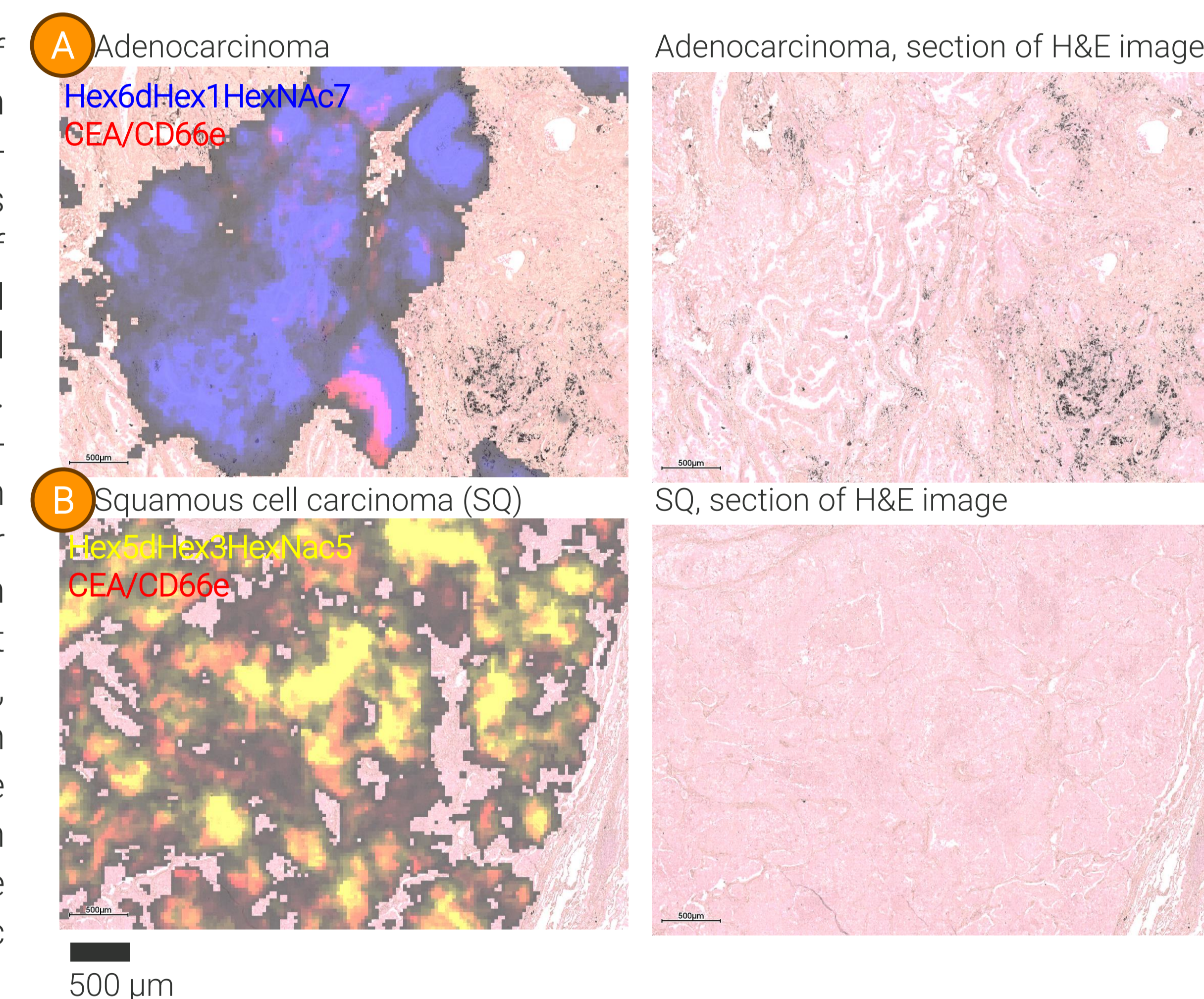
**Figure 2:** Ion images of two markers used in routine diagnostic settings to differentiate adenocarcinoma of the lung from squamous cell carcinoma, TTF-1, Thyroid Transcription Factor 1 and Napsin A.

Highly multiplexed targeted protein imaging provides the benefit of addressing various clinical research questions in one single experiment. Several examples are shown in Figure 3. Hypoxia-Inducible Factor 1-alpha (HIF-1) is a marker for the cell's oxygen status and often overexpressed in tumors due to higher oxygen demand of cancer cells. Expression of vascular endothelial growth factor A (VEGFA) and its receptor (VEGFR-1) is associated with tumor growth promotion by enhancing angiogenesis and vascularization. Glucose transporter 1 (GLUT-1) indicate metabolic adaptation to higher energy consumption. Lactate dehydrogenase (LDHA) is often overexpressed in cancer to meet the higher energy demands of rapidly dividing cells. High abundance in the tumor stroma area can be a sign of metabolic cooperation between stromal and cancer cells. Higher proliferation activity was indicated by expression of Ki67 in both tumor areas.



**Figure 3:** Example ion images of targeted proteins involved in proliferation and cell state.

Multimomics integration of MALDI HiPLEX-IHC data with enzymatically released N-glycan imaging data allows adding an additional layer of information for potential tumor markers and biological understanding (Figure 4). Interestingly, two different N-glycans were co-expressed in CEA/CD66e positive cells for both cancer types. Even though it is not clear what these differences mean, investigations have shown that alterations in the glycosylation of adhesion molecules like CEA/CD66e can influence the metastatic potential of cancer cells [3].



**Figure 4:** Multimomics integration of released N-glycans, with additional H&E overlay.

- MALDI HiPLEX-IHC enables simultaneous spatial expression analysis of more than 100 proteins.
- Leveraging multiplexing capabilities enables several clinical research questions to be addressed within one experimental setup.
- Integration of additional molecular classes, such as N-glycans, brings a multimomic view that can further inform on cellular characterization.

## Imaging: Spatially-Resolved Omics I

### References.

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- [2] Turner BM, Cagle PT, Sainz IM, Fukuoka J, Shen SS, Jagiradar J. (2012). *Arch. Pathol. Lab Med.* 136(2), 163-171.
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\*Miralys™ photocleavable mass tag imaging probes plus their high-plex and multimomic oriented workflows are patented by AmberGen Inc.



COI Disclosure: J.O., C.H., M.E., N.T. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study. M.L., G.Y., A.Y., K.R. are employees of AmberGen Inc.