Towards Single-Cell MALDI HiPLEX-IHC: Multiplexed, Multiomic and Multimodal Imaging of Targeted Intact Proteins and Untargeted Metabolites

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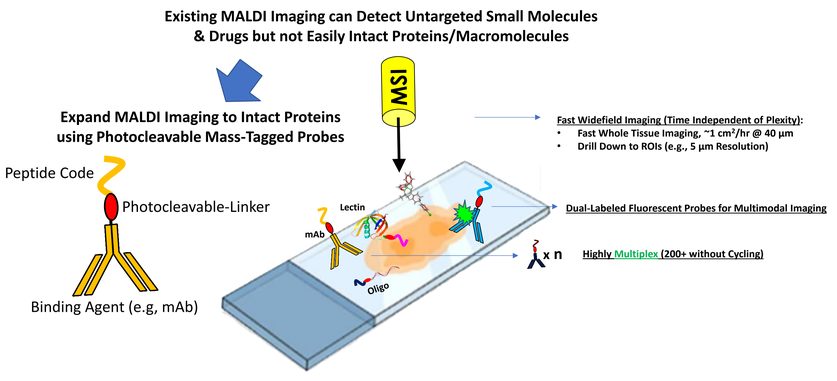
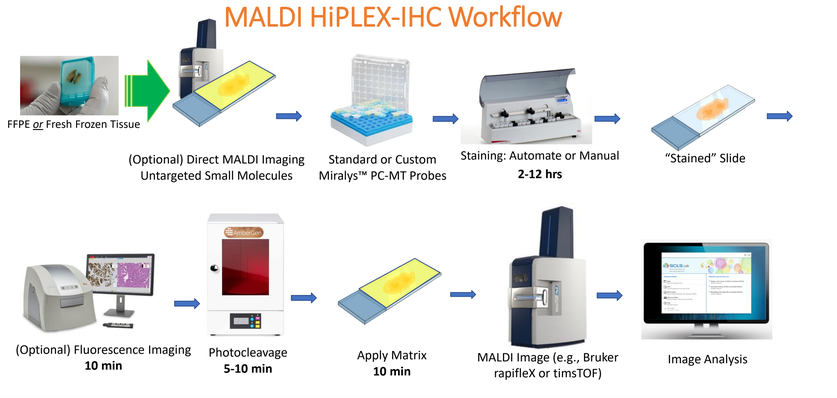
Introduction

An important goal in the biological sciences is to characterize the metabolomic, transcriptomic and proteomic profiles of intact biological specimens at the single cell level. Towards this goal, the emerging field of spatial multiomics is transforming our ability to understand human disease by providing a comprehensive picture of the immense complexity, heterogeneity, and interactions within biological systems. While significant progress has been made, existing imaging and spatial profiling methods are still not capable of combined metabolomic and proteomics measurements on the same sample, on a single instrument and using an integrated workflow. We describe here several examples of a new approach termed **MALDI HiPLEX-IHC**[1,2] based on the use of novel **Miralys™ Probes** developed by AmberGen. The examples illustrated here demonstrate the ability of MALDI HIPLEX-IHC to perform high-plex, multiomic and multimodal imaging of both small molecules, and targeted intact proteins in tissues. High resolution MALDI HiPLEX-IHC data at 5 µm was obtained using Bruker's **microGRID** which combines MALDI stage and smartbeam 3D laser beam positioning technology to facilitate high quality imaging at the single cell level.

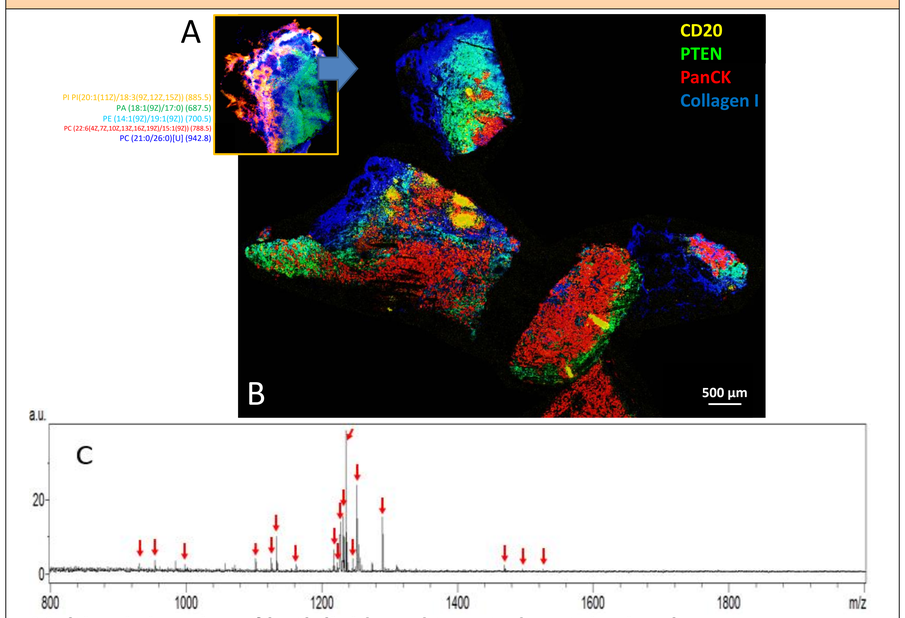
Key Features of MALDI HiPLEX-IHC



Methods

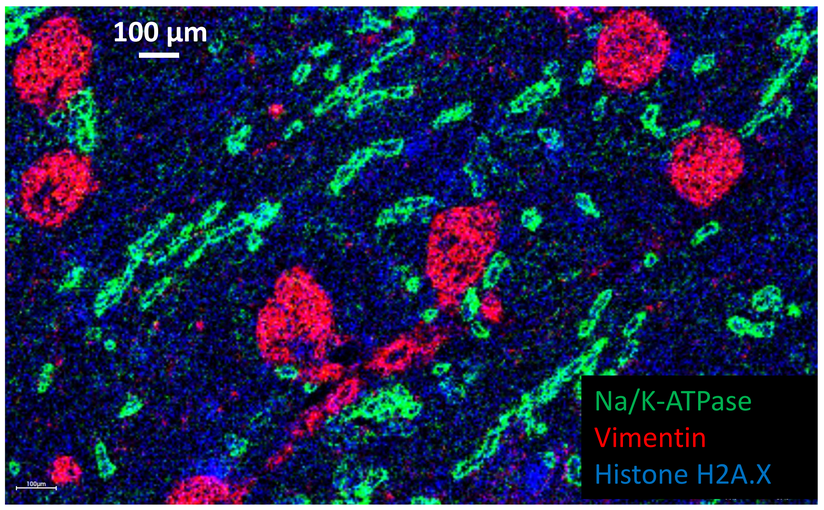


High-Plex & Multiomic Imaging of Small & Large Molecules (lipids & targeted intact proteins)



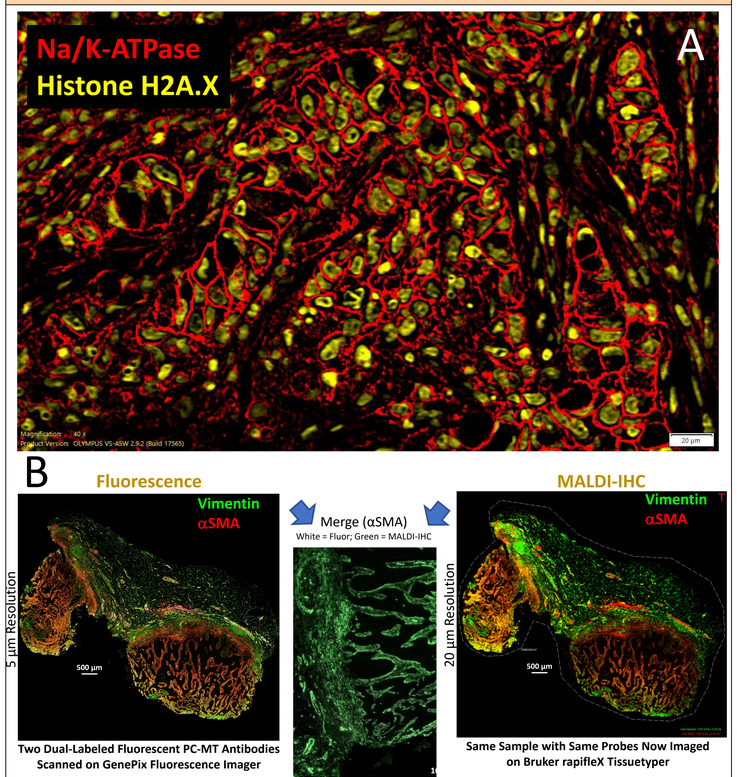
Multiomic imaging of both lipids and targeted proteins in a breast cancer tissue specimen. A) Mass spectrometry imaging (MSI) showing selected lipid molecules detected after a first MALDI-MSI run (see color key in figure for 5 example lipids). B) Following matrix removal, the tissue was stained and imaged using MALDI HiPLEX-IHC with 29 Miralys™ antibody Probes. Colors correspond to 5 example mass markers for CD20, PTEN, PanCK and Collagen (see color key in figure). The region corresponding to Figure A where initial lipid MSI was measured is indicated by the arrow. C) Average spectrum from Figure B with arrows showing the m/z values of the 18 detected mass-tags.

5 µm Resolution MALDI HiPLEX-IHC of Kidney Tissue



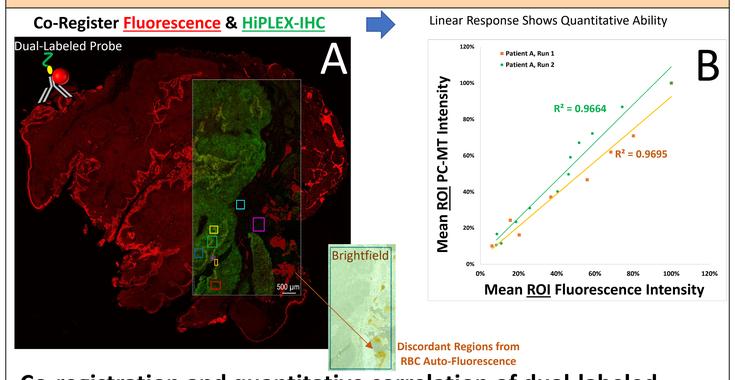
MALDI HiPLEX-IHC image of FFPE normal rat kidney tissue section. MALDI HiPLEX-IHC images were obtained on a Bruker timsTOF flex equipped with microGRID and recorded at 5 µm resolution. A simple 3-Plex panel of Miralys™ antibody Probes was used to stain tissue (see color key in figure). Structures identified include glomeruli (red: strong vimentin staining due to capillary beds), kidney tubules (green: Na/Ka-ATPase staining which according to ProteinAtlas.org is highly enriched in the kidney tubules), and individual cellular nuclei (blue: detected by the major Histone H2A.X).

High-Plex & Multimodal Imaging (Fluorescence & MALDI HiPLEX-IHC)



Multimodal imaging of an FFPE breast cancer tissue using dual-labeled Miralys™ Probes. A) High resolution immunofluorescence microscopy of dual-labeled fluorescent mass-tagged Probes for cell segmentation purposes (see color key in figure for Probes). (B) Fluorescence and MALDI HiPLEX-IHC images are both from the same tissue section and are readily merged to leverage the relative strengths of each. Ultimately, the high-resolution fluorescence will be used to effectively enhance the MALDI HiPLEX-IHC images.

Proof-of-Concept for Relative Quantification using MALDI HiPLEX-IHC



Co-registration and quantitative correlation of dual-labeled Miralys™ Probes on tonsil. A) A dual-labeled fluorescent (red) mass-tagged (green) Probe against Na/K-ATPase was used to stain tonsil and the images co-registered. (B) Intensity values were extracted from the same regions of interest from both images (ROIs; see boxes in A for examples) and plotted to show quantitative correlation.

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References: 1. Yagnik, G., Liu, Z., Rothschild, K. J., and Lim, M. J. (2021) Highly Multiplexed Immunohistochemical MALDI-MS Imaging of Biomarkers in Tissues. *J Am Soc Mass Spectrom* **32**, 977-988; 2. Claes, B. S. R., Krestensen, K. K., Yagnik, G., Grgic, A., Kuik, C., Lim, M. J., Rothschild, K. J., Vandenbosch, M., and Heeren, R. M. A. (2023) MALDI-IHC-Guided In-Depth Spatial Proteomics: Targeted and Untargeted MSI Combined. *Anal Chem* **95**, 2329-2338