MALDI HiPLEX-IHC: Highly Multiplex & Multiomic Tissue Imaging with Photocleavable Mass-Tagged Probes

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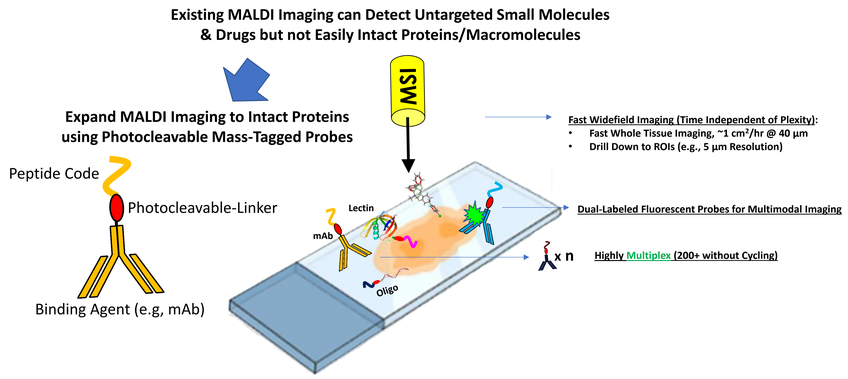
Introduction

Multiomics approaches stand to transform our ability to understand human disease by providing a comprehensive picture of the immense complexity, heterogeneity and interplay within biological systems. However, "omic" data must not forfeit spatial information through homogenization of the biospecimen, but rather, must be put into a spatial context at the multi-cellular and tissue levels. This is best achieved by tissue imaging approaches, yet current methods lack the multiplexity and flexibility to obtain multiomic scale information. For example, immunohistochemistry (IHC) provides an important and widely used tool for researchers and pathologists to image multiple protein biomarkers in tissue specimens. However, standard fluorescence based IHC is generally limited to 3-5 different biomarkers (hyperspectral/multispectral methods <10). IHC also cannot image small molecules in tissue specimens such as metabolites and drugs. While mass spectrometry (MS) is a proven proteomic/metabolomic tool and the advent of mass spectrometric imaging (MSI) has extended MS to the spatial dimension, it is generally limited to untargeted analysis of small molecules and peptides. To overcome this barrier, MALDI HiPLEX-IHC (MALDI-IHC) uses probes such as antibodies, lectins and oligonucleotides conjugated to photocleavable mass-tags (PC-MTs) for MSI of targeted proteins and other macromolecules in cells and tissues. MALDI HiPLEX-IHC significantly exceeds the multiplexity of both fluorescence and previous cleavable mass-tag based methods, without the need for iterative cycling procedures. Moreover, we have combined on the same tissue section untargeted MSI of endogenous small molecules with this targeted protein MSI approach for a truly unique multiomic capability. Novel dual-labeled fluorescent MALDI HiPLEX-IHC probes extend the utility of this new approach to allow co-registration of low-plex but high spatial resolution fluorescence images with MALDI HiPLEX-IHC images of the same tissue section, such as for cell segmentation purposes. Overall, this approach stands to transform the fields of "omics" based research and discovery, tissue pathology, tissue diagnostics, drug development, therapeutics and precision medicine.

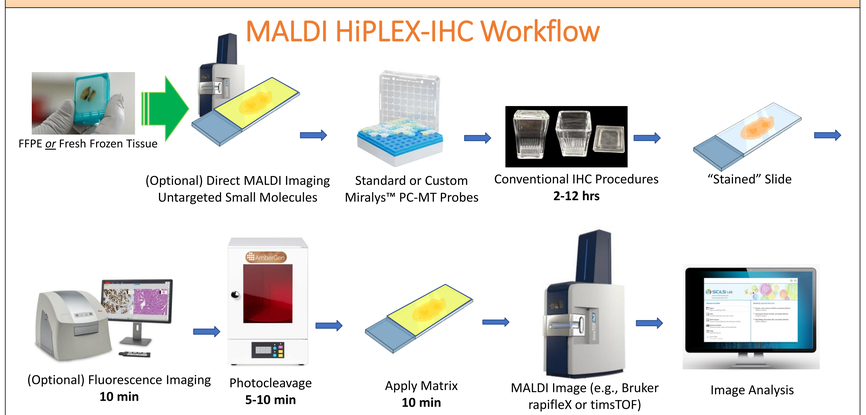
Instrumentation

Bruker rapifleX & timsTOF

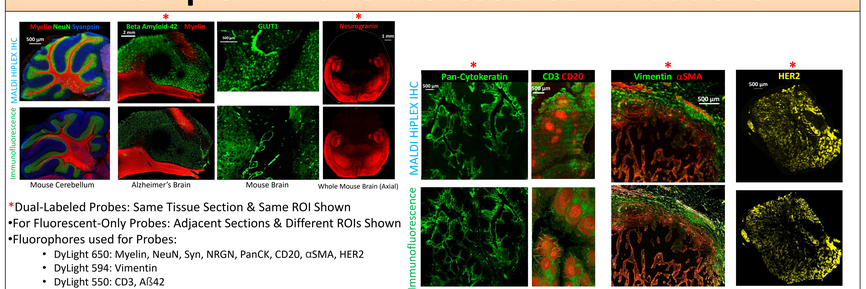
Features of MALDI HiPLEX-IHC



Methods

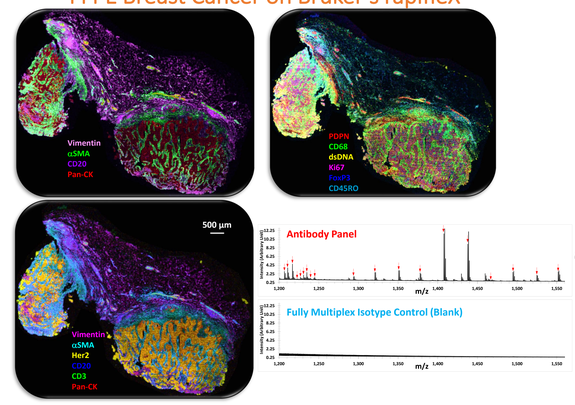


Example Immunofluorescence Validations

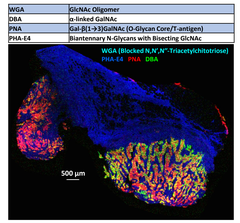


23-Plex Imaging with Antibody & Lectin Probes

1. Pan-CK
2. CD3
3. CD4
4. CD8
5. CD20
6. CD45RO
7. FoxP3
8. ER
9. PR
10. Her2
11. dsDNA
12. CD68
13. Ki67
14. ACTA2
15. VIM
16. FAP
17. PDPN
18. PDGFRB
19. PTEN



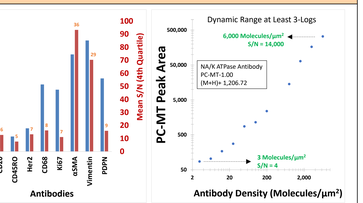
19 Antibody Probes



4 Lectin Probes

rapifleX Settings
- Positive Ion, Reflector
- 500 Shots/Pixel
- 10 kHz Laser Rate
- 20 µm Resolution

Dynamic Range, Sensitivity Estimate & Quantification

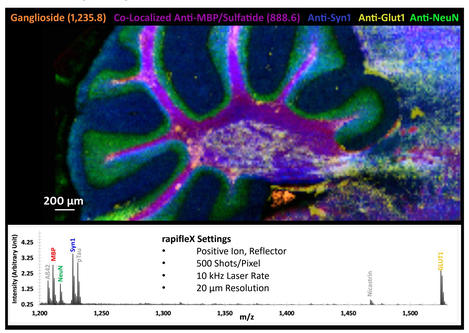


Script Based Total Tissue Analysis (98,827 Pixels). flexImaging script-based pixel-by-pixel analysis was used to call photocleavable mass-tag (PC-MT) positive and negative pixels using the SNAP2 algorithm with a S/N threshold of 3. Peak S/N for the PC-MT-positive pixels was then averaged over the entire breast cancer tissue section shown in the panel above. Example biomarkers are shown (red bars are mean of the top 25% of pixels while orange numbers indicate the mean of all PC-MT-positive pixels).

Sensitivity Estimate & Dynamic Range. PC-MT antibody was photocleaved and then subjected to a 2-fold serial dilution in saturated CHCA matrix in TA30 Solvent (30% acetonitrile and 0.1% TFA in mass spectrometry grade water). 2 µL was then spotted to fill each well of a standard 384-well MALDI-MS steel target and allowed to dry/crystallize. The spots were then subjected standard non-imaging MALDI-MS analysis on a Bruker rapifleX Tissuetyper.

Multiomic "Double-MALDI"

Untargeted Endogenous Small Molecule Imaging → Targeted Protein Imaging (MALDI HiPLEX-IHC)
Merged Image from same Mouse Brain Cerebellum Tissue Section



Conclusions

- **Multiplex:** High-Plex (up to 200) Probe-Based MALDI Imaging of Intact Proteins
- **Multiomic:** MALDI Imaging of label free small molecules and intact proteins on same tissue sample
- **Multimodal:** Fluoresence and MS images on same tissue sample with Dual-Labeled Probes

[Yagnik, Liu et al. (2021) J Am Soc Mass Spectrom 32(4): 977-988 pubs.acs.org/doi/10.1021/jasms.0c00473]
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