Cyclic MALDI-IHC for Successive High-plex and Multimodal Imaging of Tissues and Tissue Microarrays

AmberGen

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Introduction

MALDI-IHC is a recently introduced top-down mass spectrometry imaging (MSI) approach based on novel photocleavable mass-tags (PC-MTs) conjugated to antibody probes. It provides a basis for achieving high-plex, multiomic, MALDI-based workflows to image both small molecules and intact proteins on the same tissue sample and even on individual cells. Moreover, multimodal dual-labeled antibody probes enable same-slide mass spectrometry and fluorescent imaging of targeted intact proteins. Here, we demonstrate a novel workflow to perform a second cycle of MALDI-IHC and fluorescent imaging on the same tissue section or tissue microarray (TMA). This approach, termed cyclic MALDI-IHC (cMALDI-IHC), enables successive imaging of archived samples with the same or different antibody panels as well as reimaging of selected Regions of Interest (ROIs) at higher spatial resolution.

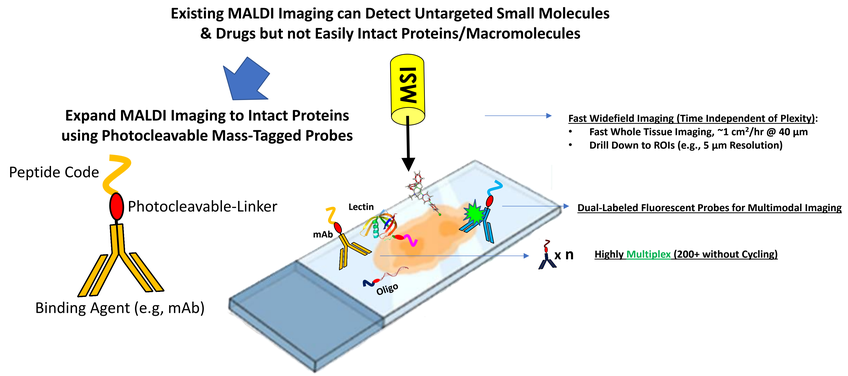
Methods

TMAs containing 24 normal organ tissue FFPE cores from TissueArray.com (#MC245c) or Alzheimer's disease (AD) transgenic mouse brain FFPE tissue sections (strain hAbetaSAA from JAX) were successively stained and imaged in two cycles using a novel workflow with the same or different antibody panels. Each cycle consisted of MALDI-IHC staining/antigen retrieval and incubation, fluorescent imaging (Olympus VS200 fluorescence scanner), MSI (Bruker timsTOF fleX) and a final repeat fluorescence scan. Fluorescent imaging and MSI were conducted at ~1.6 µm (4x objective) and 20 µm spatial resolution, respectively, unless mentioned otherwise in figure legends. After each cycle, matrix removal was performed. We assessed conservation of staining morphology by non-elastically aligning images from both cycles using FIJI/ImageJ and calculating the Pearson's correlation coefficient using in-house developed Python scripts. We also evaluated variation of signal intensity between cycles on a pixel-by-pixel basis using in-house developed Python scripts.

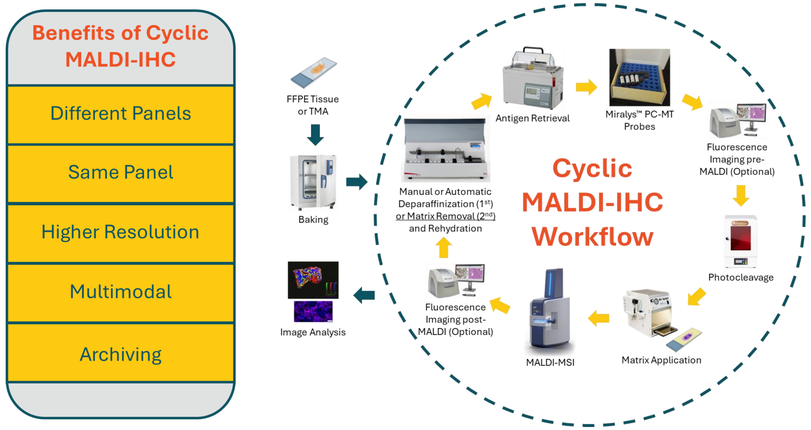
Novel Aspects

cMALDI-IHC and associated workflows enable successive high-plex and multimodal imaging on the same large tissue section or multi-core TMA.

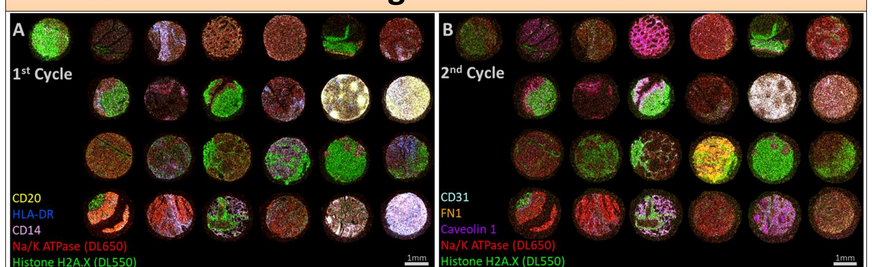
Key Features of MALDI-IHC



Benefits of Cyclic MALDI-IHC and Workflow

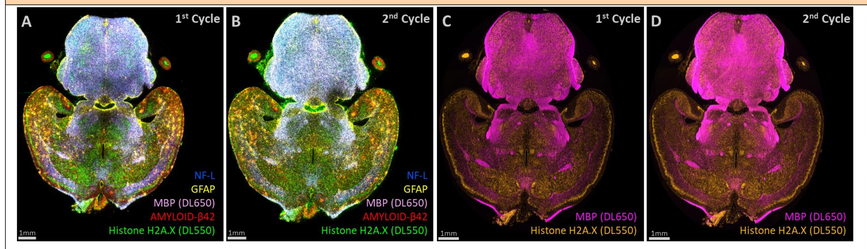


cMALDI-IHC on Multi-Organ TMA with Different Panels



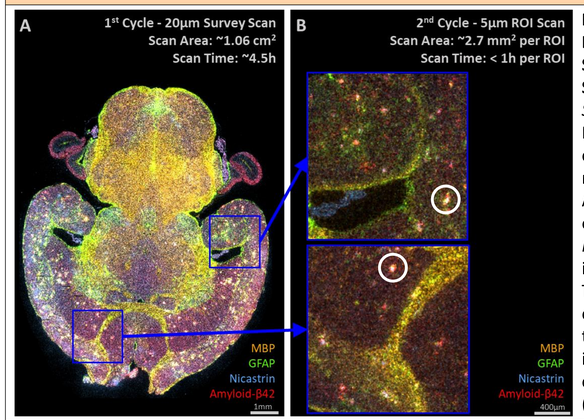
Multiplex, Cyclic MALDI-IHC on FFPE Multi-Organ Tissue Microarray (TMA) Using Different Pannels of Miralys™ Probes. (A) 26-plex MALDI-IHC image of FFPE Multi-Organ TMA corresponding to the 1st cycle of cMALDI-IHC. (B) 14-plex MALDI-IHC image of the same TMA corresponding to the 2nd cycle of cMALDI-IHC. Note almost identical staining pattern for two common probes shown in both cycles (Histone H2A.X and Na/K ATPase).

Multimodal cMALDI-IHC on Transgenic Mouse Brain Results in Nearly Identical Images Across Cycles when Using Same Panel



Multimodal, Multiplex, Cyclic MALDI-IHC on FFPE Transgenic Mouse Brain Tissue Specimens. (A) 21-plex MALDI-IHC image of FFPE Transgenic Mouse Brain Tissue Specimen corresponding to the 1st cycle of cMALDI-IHC. (B) 21-plex MALDI-IHC image of the same tissue specimen corresponding to the 2nd cycle of cMALDI-IHC. (C) 2-plex Fluorescent Microscopy image of the same tissue specimen corresponding to the 1st cycle of cMALDI-IHC using dual-labeled Miralys™ probes (Fluorophore and Mass-tag). (D) 2-plex Fluorescent Microscopy image of the same tissue specimen corresponding to the 2nd cycle of cMALDI-IHC using dual-labeled Miralys™ probes. (E) Data analysis pipeline starting with data processing and exporting '.tiff' images using software (Bruker SCiLS™ Lab and OlyVIA for MALDI and Microscopy, respectively), followed by co-registration of images from 1st and 2nd cycles using FIJI, and staining pattern (Pearson Coefficient) and signal intensity (Best Fit Line's Slope) conservation analyses using in-house developed Python scripts. Analysis of 4 independent FFPE Transgenic Mouse Brain Tissue Specimens using the aforementioned 21-plex panel results in nearly identical staining pattern across runs (Average Pearson Coefficient = 0.87 ± 0.07) and similar signal intensity (Average Best Fit Line's Slope = 0.94 ± 0.1) when comparing images on a box-by-box basis (2 by 2 pixels boxes).

Rapid MALDI-IHC Survey Imaging of Large Transgenic Mouse Brain Specimen Followed by High Resolution Imaging of Selected ROIs



Rapid, High-Plex MALDI-IHC Survey Imaging of Large FFPE Transgenic Mouse Brain Tissue Specimen Followed by High Resolution Imaging of Selected Regions of Interest. (A) *First Rapid Survey Scan*: 21-plex MALDI-IHC image of FFPE Transgenic Mouse Brain Tissue Specimen (~1.06 cm²), corresponding to the 1st cycle of cMALDI-IHC measured at 20 µm spatial resolution in ~4.5h. Alternatively, a 40 µm spatial resolution Survey Scan of the same area can be done in ~1.5h. (B) *Second Rapid Region of Interest Scans*: 21-plex MALDI-IHC image of the same FFPE Transgenic Mouse Brain Tissue Specimen corresponding to the 2nd cycle of cMALDI-IHC conducted at 5 µm spatial resolution taking < 1h per ~2.7 mm² ROI. Circled regions show, in white, example Alzheimer's Disease plaques with colocalizing proteins: Amyloid-β42 (red), Nicastrin (blue) and GFAP (green).

Conclusions

These results demonstrate that cyclic MALDI-IHC is a robust workflow enabling more information to be obtained from the same tissue sections or tissue microarrays. Key features include:

- **Successive MALDI-IHC Imaging:** Same or different panels of Miralys™ Antibody Probes can be used for successive MALDI-IHC cycles
- **Archive Specimens:** After the initial MALDI-IHC scan, a specimen can be stored and then rescanned with the same or different Miralys™ Antibody Probes
- **Identify and Image Regions of Interest:** After a fast MALDI-IHC image scan, a second cycle can be performed at higher resolution on a region of interest
- **Multimodal:** Fluorescence and MS images on same tissue specimen with Dual-Labeled Miralys™ Probes

References:

1. Yagnik et al. (2021) J Am Soc Mass Spectrom 32(4): 977-988 https://doi.org/10.1021/jasms.0c00473
2. Lim, Yagnik et al. (2023) Front. Chem 11 https://doi.org/10.3389/fchem.2023.1182404

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Conflict of Interest: Authors LGD, GY, PC, ML and KR are employed by the company AmberGen, Inc.