MALDI-ICC and SpaceM on PBMCs Spiked with Cancer Cells for Highly Multiplexed, Multiomic and Multimodal Single-Cell Profiling

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Introduction

Immunocytochemistry (ICC) and multiparameter flow cytometry (MFC) are used extensively to profile protein expression in single cells. However, these techniques are limited by the number of biomarkers (multiplexity) and/or the types of biomarkers (multiomic) which can be detected. These capabilities are critical for profiling diverse types and states of cells isolated from tissues and present in liquid biopsies. We evaluated a new approach, termed MALDI-ICC, based on novel photocleavable mass-tags (PCMTs) conjugated to antibodies which are imaged by MALDI mass spectrometry (MSI) after photocleavage (1,2). When combined with SpaceM, a method for spatial single-cell metabolomics, this approach has the potential to rapidly profile millions of cells at very high multiplexity (>100) and to detect both metabolite and protein biomarkers.

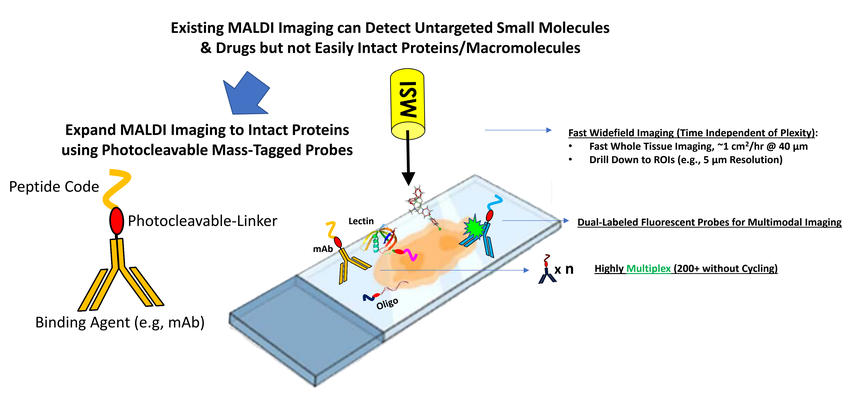
Methods

Various methods were used to prepare and image cells by MALDI-ICC. In one case, peripheral blood mononuclear cell (PBMC) suspensions spiked with HeLa and MCF7 cancer cells were formalin-fixed and paraffin-embedded (FFPE) and 3 µm sections subsequently immunostained with a high-plex panel of PCMT-antibodies targeting various cell specific biomarkers. A fluorophore-labeled histone-targeted antibody was also included. Staining methods were similar to those previously reported for MALDI-IHC tissue imaging. Cells were first imaged on an Olympus VS200 fluorescence microscope using a 20x objective, photocleaved and then coated with matrix. The cells were imaged using a Bruker timsTOF fleX at 10-20 µm resolution. In a similar workflow, PBMCs were processed by depositing and desiccating them on a glass slide followed by a modified immunostaining protocol. Custom image analysis was based on FIJI/ImageJ, Python scripts, and SpaceM.

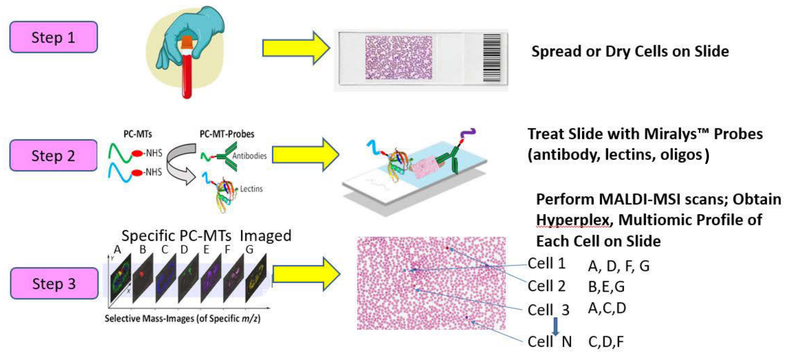
Novel Aspects

MALDI-ICC based on photocleavable mass-tags combined with SpaceM provides rapid multiplex and multiomic molecular profiling of millions of cells.

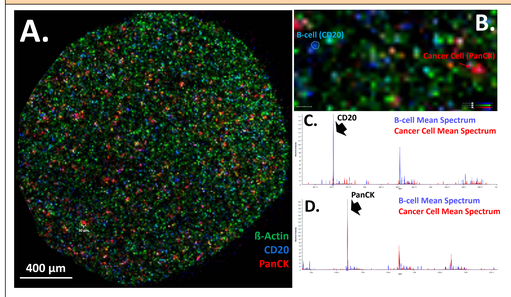
Features of MALDI-ICC



Basic Workflow for MALDI-ICC

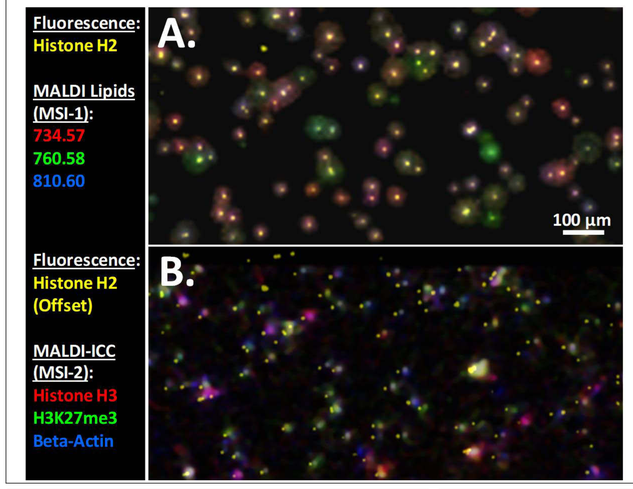


MALDI-ICC on High-Density PBMC/Cancer Cell Pellet



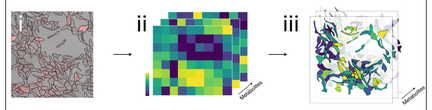
High-density PBMC cell pellets spiked with HeLa and MCF7 cancer cells (~10% each) were prepared as FFPE blocks and thin-sectioned onto conductive slides for MALDI-ICC using a 21-plex panel of PCMT antibody probes. Cell density was ~625,000/cm² and MALDI-MSI scanning resolution was 10 µm. (**A.**) Overlaid images of the PCMTs for ß-actin (all cells), PanCK (cancer cells), and CD20 (B-cells) are shown as an example. (**B.**) Magnified view with Regions of Interest (ROIs) drawn around a representative B-cell and cancer cell, as indicated. (**C.**) Overlaid mean spectra of the ROIs for the B-cell (blue) and cancer cell (red) in the mass range for the CD20 PCMT. (**D.**) Overlaid mean spectra of the ROIs for the B-cell (blue) and cancer cell (red) in the mass range for the PanCK PCMT. PCMTs indicated with arrows.

Multiomic/Multimodal MALDI-ICC Imaging



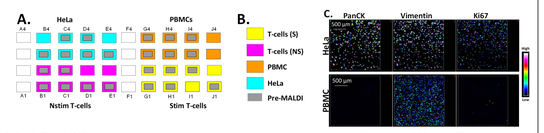
Multiomic "Double-MALDI": Untargeted small molecule imaging followed by MALDI-ICC of targeted proteins. Positive-ion mode MALDI-MSI of untargeted label-free metabolites/lipids was first performed (MSI-1) on unfixed PBMCs deposited on slides, followed by MALDI matrix removal, cell fixation, and 12-plex MALDI-ICC (MSI-2) on the same sample. (**A.**) Single-cell lipid imaging (MSI-1) with 3 example lipids colorized according to the key (tentative assignments: 734.57, PC(32:0); 760.58 (PC(34:1); 810.60 PC(38:4)). (**B.**) MALDI-ICC (MSI-2) with 3 example biomarkers colorized according to the key. (**A. and B.**) Both images were co-registered with the Histone H2A.X fluorescence microscopy (yellow; intentionally offset in the case of Panel B).

SpaceM Workflow for MALDI-ICC



SpaceM workflow): i) Microscopic imaging at high resolution of cells dispersed on surface along with cell segmentation to capture morphology and with fluorescence information; ii) Subsequent normalized image of specific metabolites captured by lower resolution MALDI with squares showing heat map for individual pixels and layers showing individual metabolites; iii) Superposition of the information captured in steps i and ii, where the laser-ablation marks in the matrix coating are used to co-registered both images (3).

MALDI-ICC of Multiple Samples on Single Slide



MALDI-ICC of various single-cell types dispersed on a 40-well array formed on a conductive Bruker Intellislide. (**A**.) Configuration of a single-cell MALDI-ICC run using a 40-well array (designation of each well by rows [1-4] and columns [A-J]; each well can be used for separate samples or experimental conditions). (**B**.) Color code for different cell types and if well has been subjected to pre-MALDI to measure metabolites. (**C**.) Differential pattern of PanCK, Vimentin and Ki67 shown for HeLa and PBMC cells for example. All 40 wells were stained in a single workflow and imaged in a single MALDI-MSI run following the SpaceM workflow at 20 µm spatial resolution on a Bruker rapifleX.

Conclusions: MALDI-ICC for Profiling Single Cells

These initial results demonstrate the potential for MALDI-ICC to rapidly profile millions of cells in liquid biosamples and for the first time has been combined with SpaceM based single-cell metabolomics. Key features include:

- **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:** Fluorescence and MALDI-MS images on same tissue sample uisng Dual-Labeled Probes

References:

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