

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.

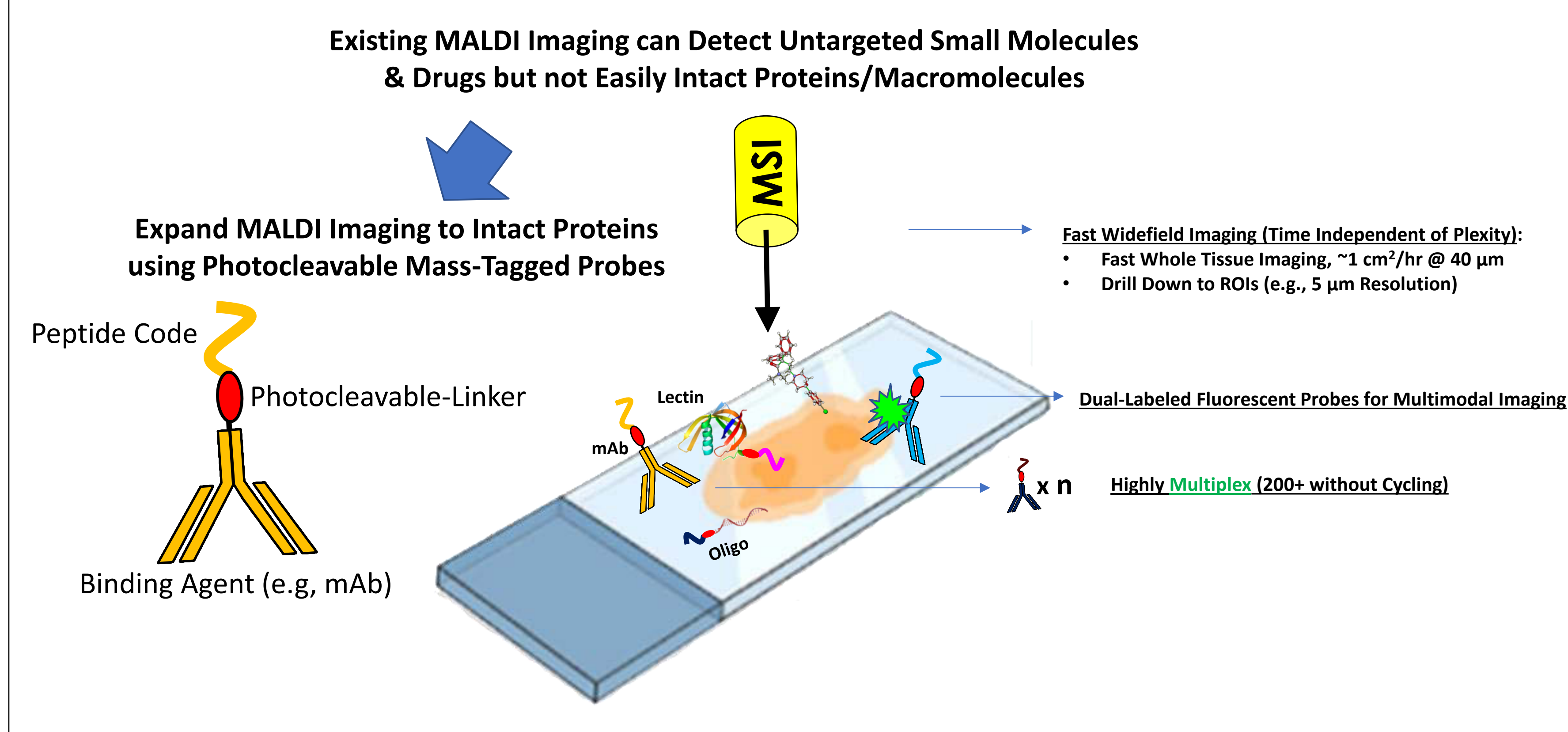
Methods

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.

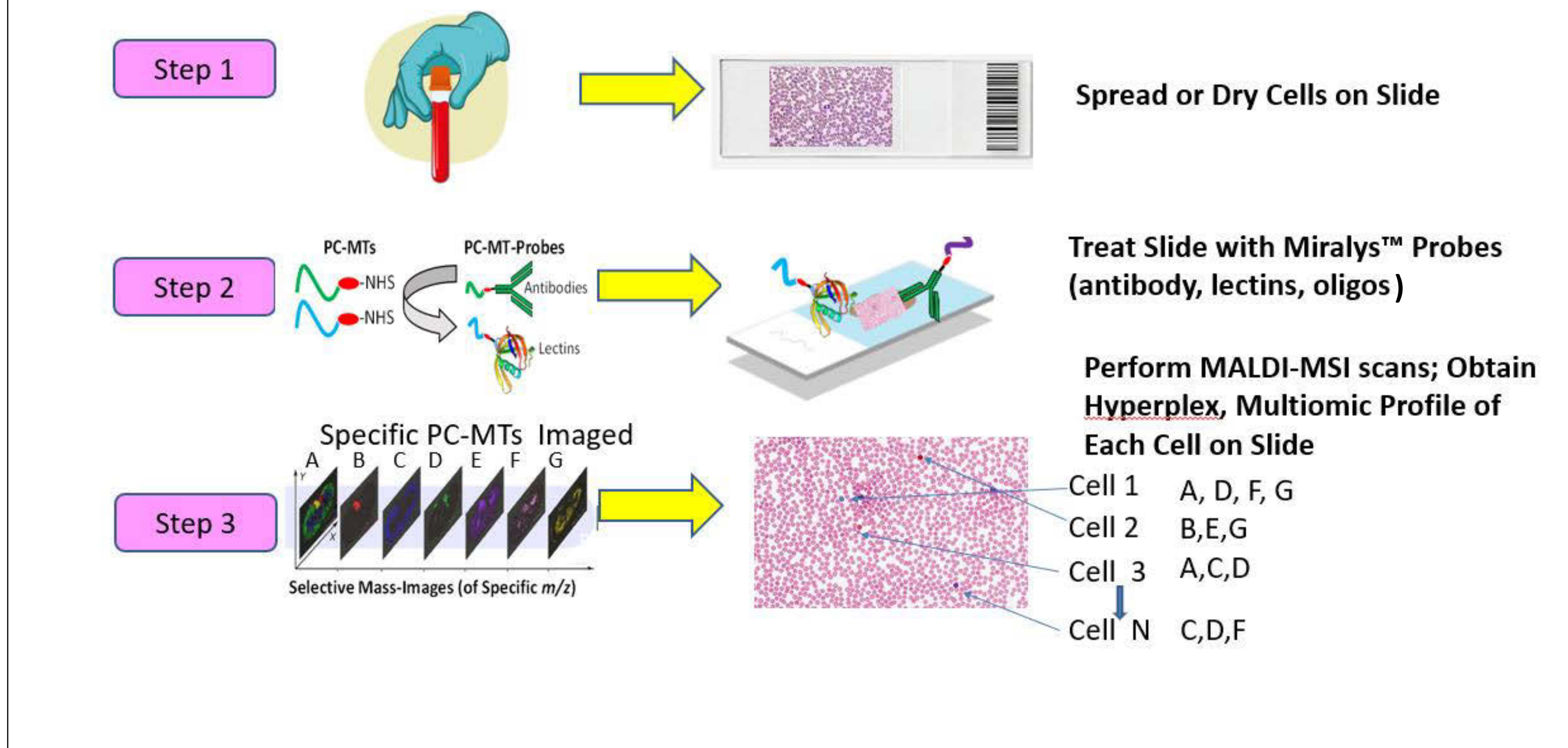
Novel Aspects

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

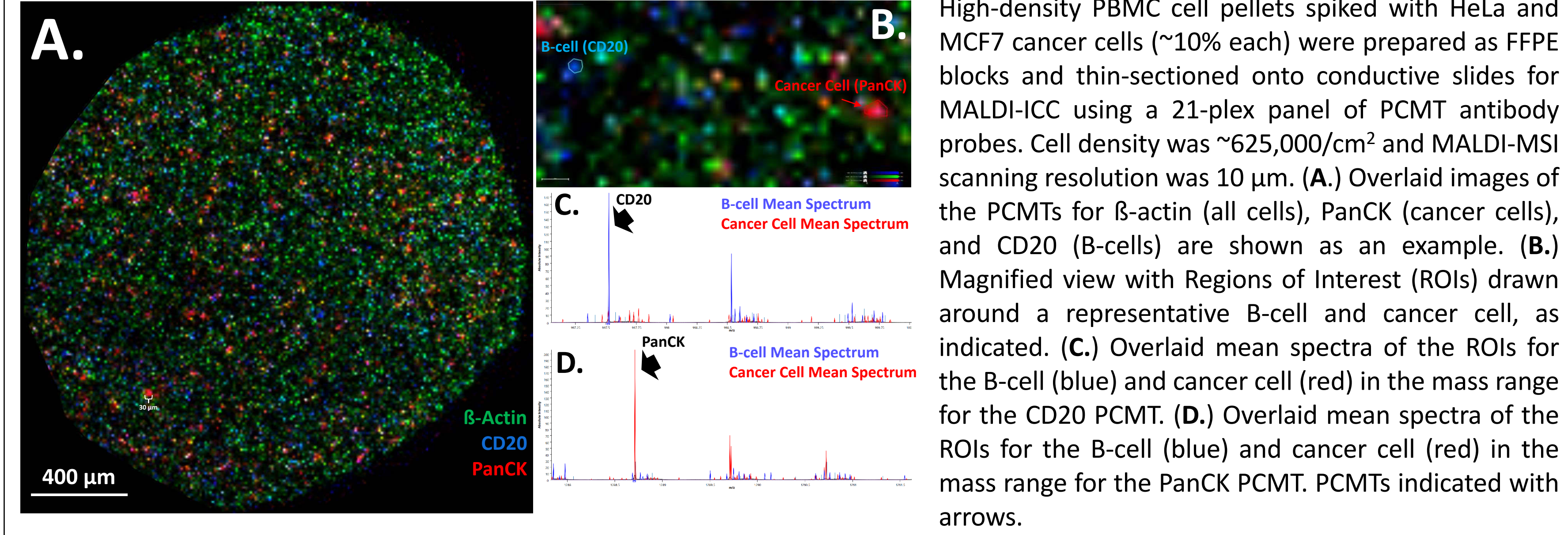
Features of MALDI-ICC



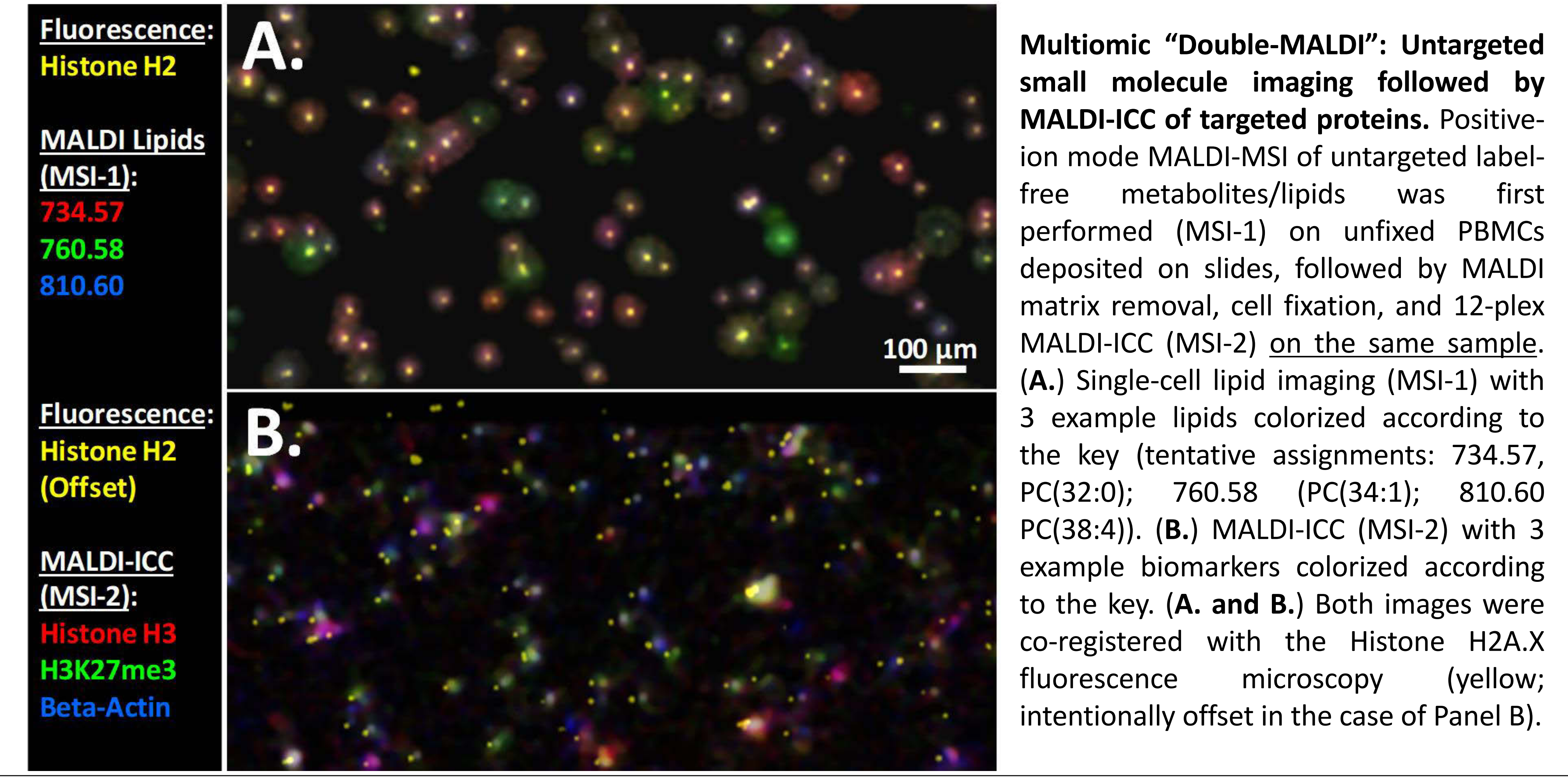
Basic Workflow for MALDI-ICC



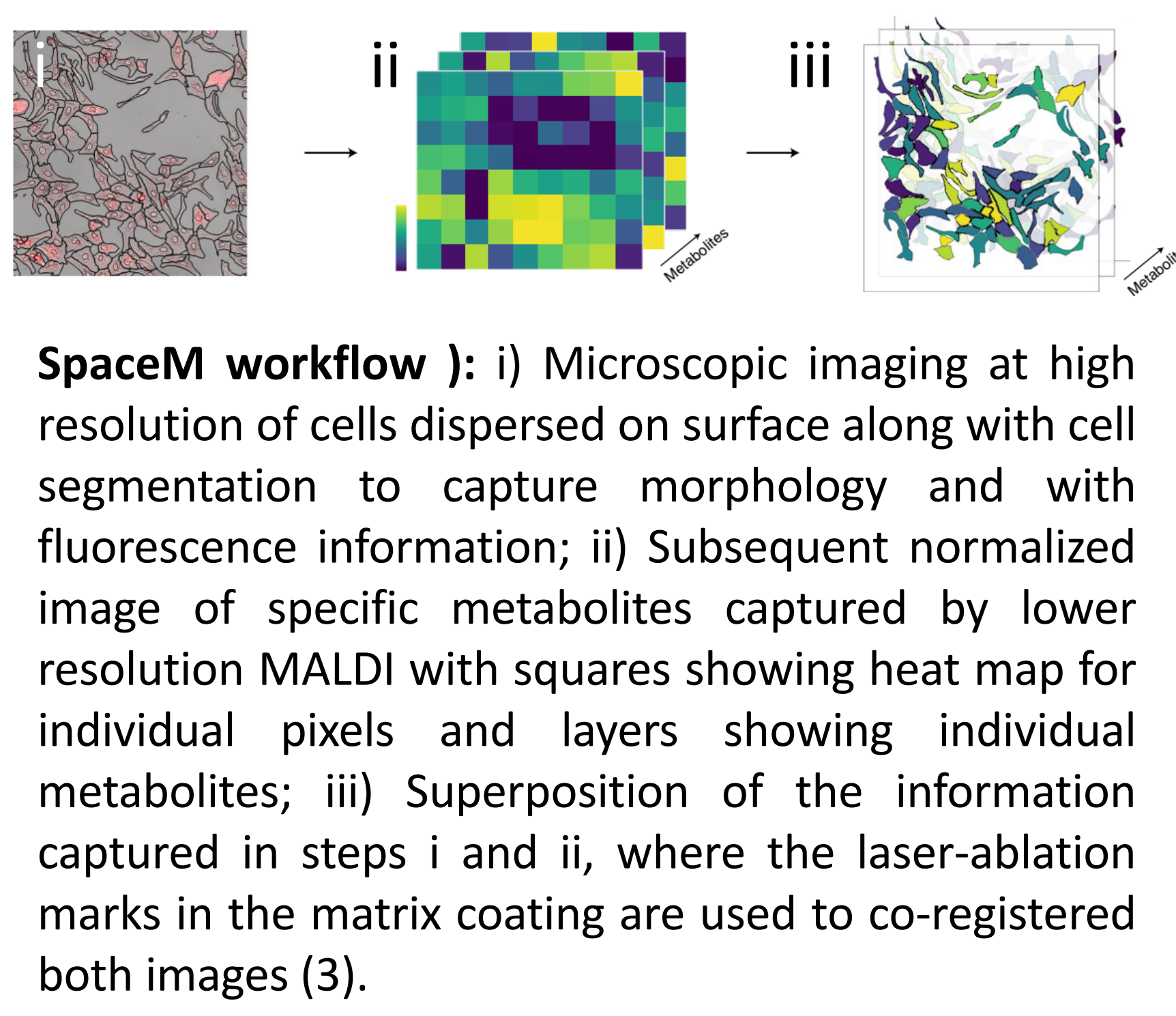
MALDI-ICC on High-Density PBMC/Cancer Cell Pellet



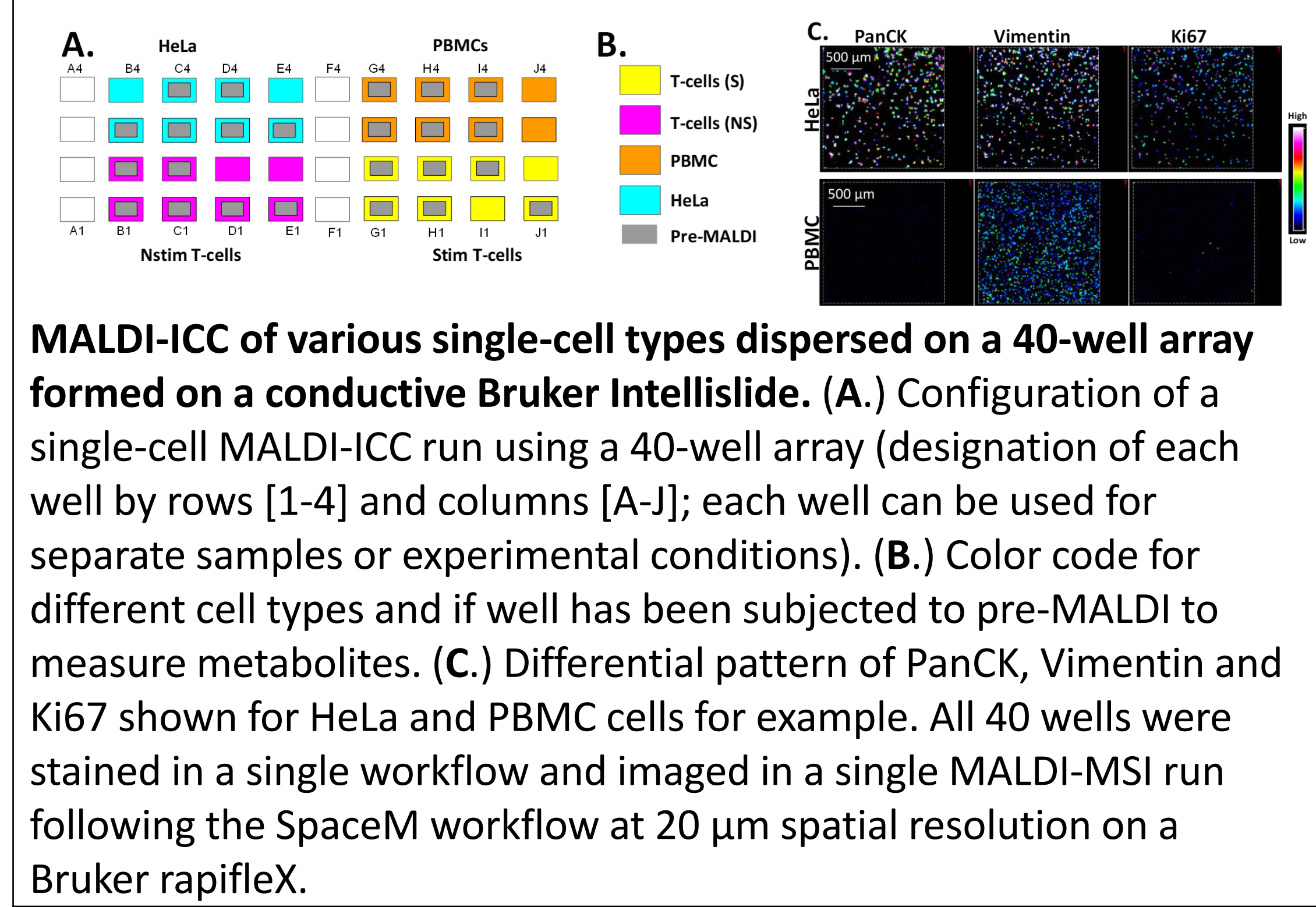
Multiomic/Multimodal MALDI-ICC Imaging



SpaceM Workflow for MALDI-ICC



MALDI-ICC of Multiple Samples on Single Slide



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 using Dual-Labeled Probes

References:

- Yagnik, Liu et al. (2021) *J Am Soc Mass Spectrom* 32(4): 977-988. pubs.acs.org/doi/10.1021/jasms.0c00473
- Lim, Yagnik et al. (2023) *Front. Chem* 11 <https://doi.org/10.3389/fchem.2023.1182404>
- Rappez, Stadler, et al. *Nat Methods* 18, 799–805 (2021). <https://doi.org/10.1038/s41592-021-01198-0>
- Rappez, L., M. Stadler, et al. (2021). "SpaceM reveals metabolic states of single cells." *Nat Methods* 18(7): 799-805.

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