

Imaging mass spectrometry-based assessment of ER, PR and HER2 protein expression in breast cancer

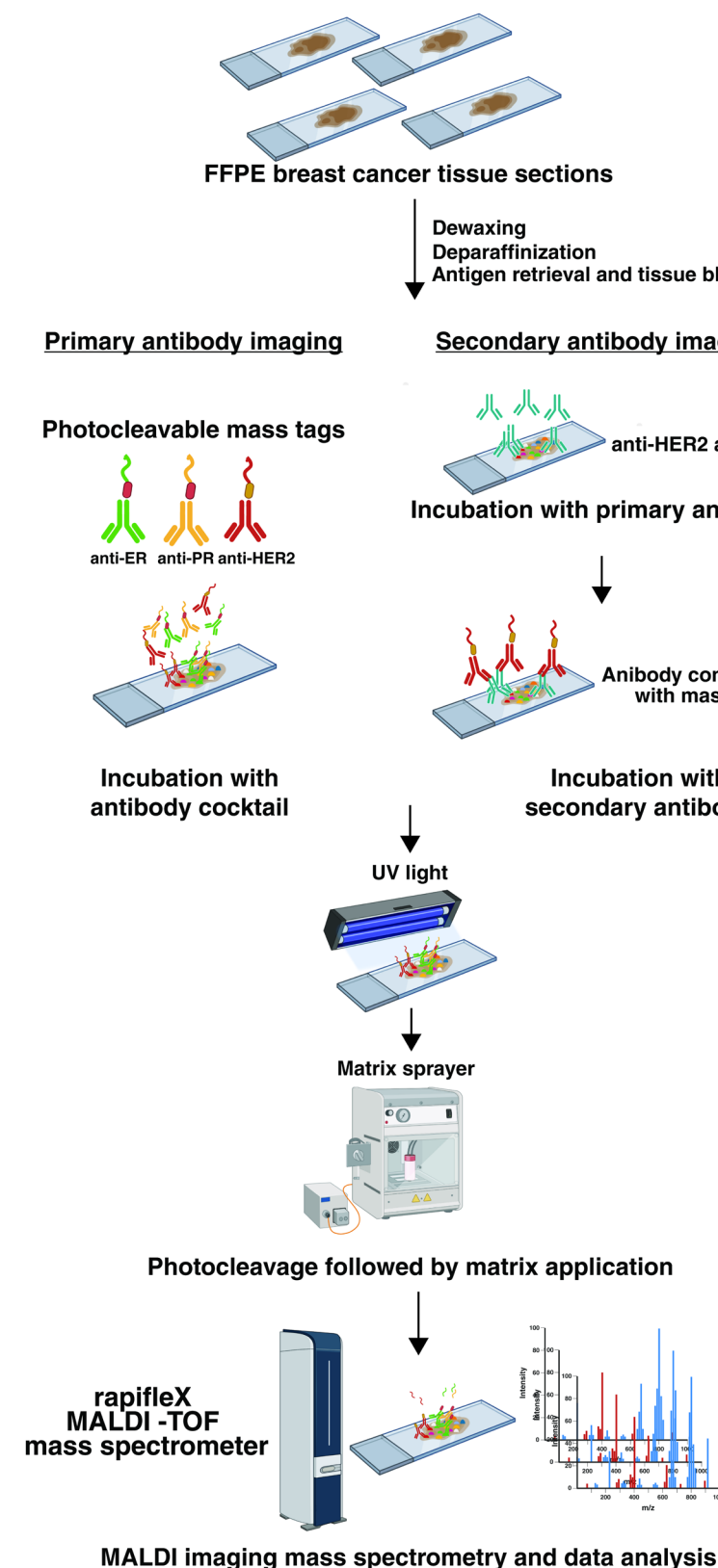
Kiran K. Mangalparthi¹, Daigo Gunji¹, Olajide E. Olaleye¹, Amy J. French¹, Gunveen Sachdeva¹, Shilpa Venkataraman¹, Neha Joshi¹, Tianqi Gao¹, Sumankalai Ramachandran³, Kenneth J. Rothschild⁴, Mark J. Lim⁴, Gargey Yagnik⁴, Saba Yasir¹, Michael Keeney¹, Akhilesh Pandey^{1,2}
¹ Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, ² Center for Individualized Medicine, Mayo Clinic, Rochester, MN, ³ Bruker Scientific LLC, MA, ⁴ AmberGen Inc., Billerica, MA

INTRODUCTION

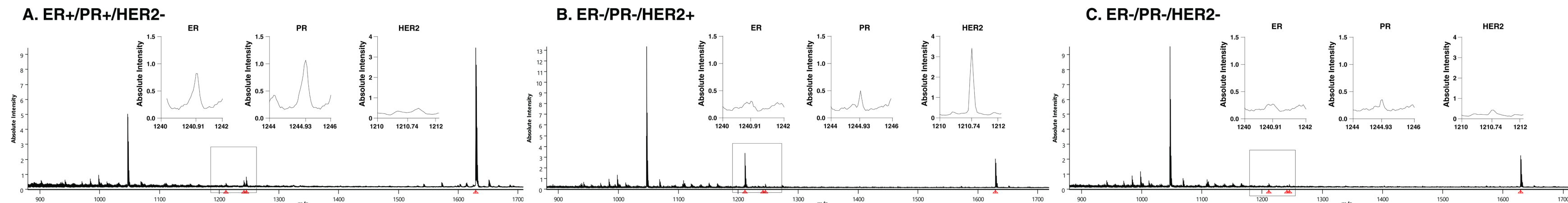
- Immunohistochemistry (IHC) is the gold standard diagnostic method used by pathologists to study the spatial localization of proteins in clinical tissue sections
- Traditional IHC is limited to detecting a single protein per slide and multiplexing approaches are constrained by labor-intensive cyclic staining procedures
- Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) enables spatial mapping of analytes such as lipids, metabolites, glycans with high multiplexing capability
- The integration of MALDI-MSI with IHC, termed as MALDI-IHC, is a novel workflow that combined photocleavable mass tags (PC-MTs) conjugated to antibodies and mass spectrometry detection enabling spatial visualization of target proteins using the high-resolution capabilities of the mass spectrometry
- In this study, we applied MALDI-IHC to a clinical cohort of 12 breast cancer patients to assess the expression of ER, PR, and HER2 markers in FFPE tumor tissue sections

METHODS

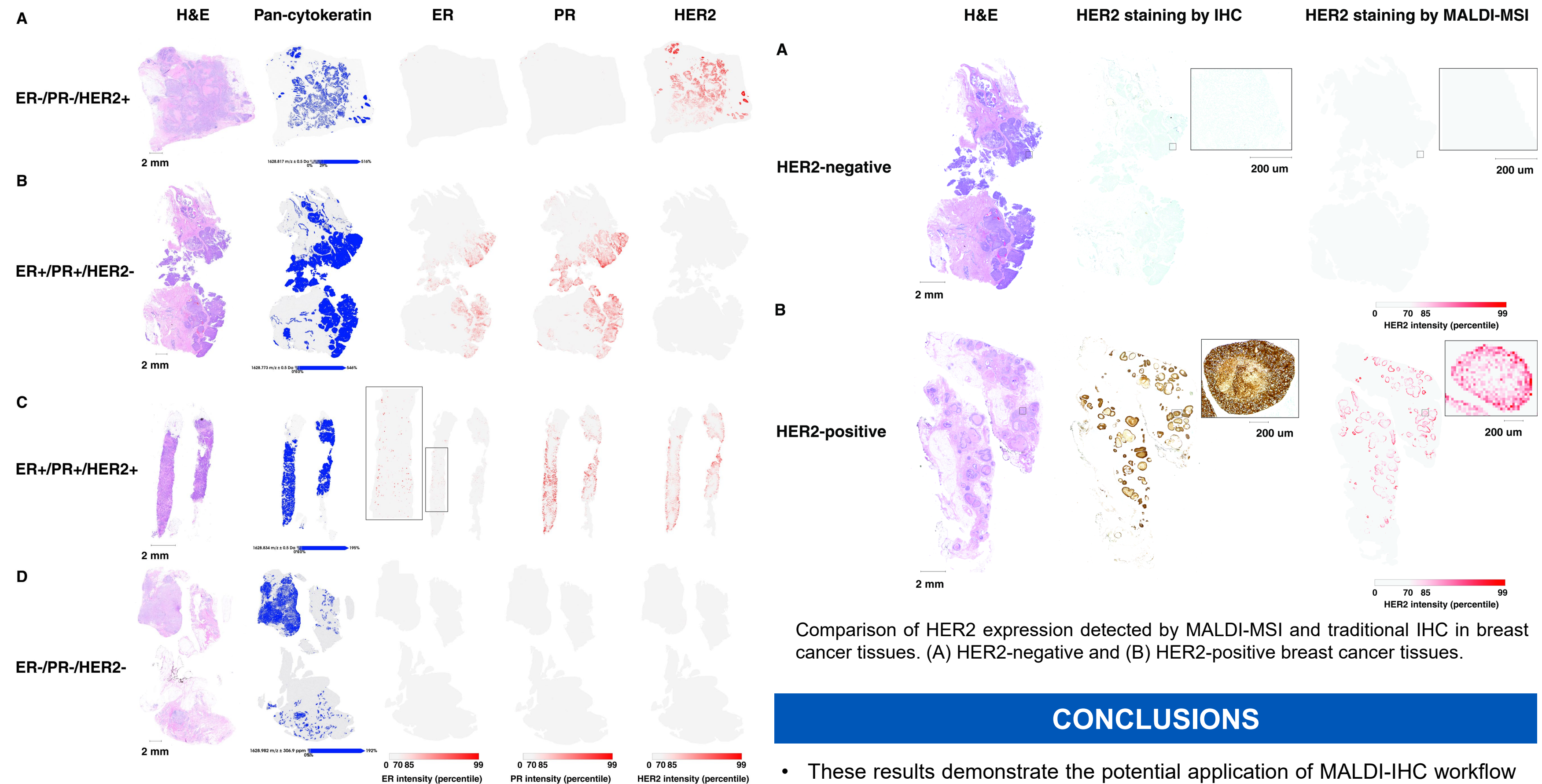
- Patient samples:** Twelve breast cancer cases were selected including triple-negative, ER/PR/HER2-positive, ER/PR-positive/HER2-negative and ER/PR-negative/HER2-positive cases. 5 μm thick FFPE sections were mounted on glass slides for H&E and IHC, while 10 μm thick sections on ITO-coated slides were used for MALDI-IHC
- PC-MT conjugated antibodies:** ER (clone SP1), PR (clone D8Q2J), HER2 (clone D8F12) and PanCK (clone C11) were used for staining (AmberGen, Inc.).
- Sample preparation and mass spectrometry analysis:** FFPE tissue sections were subjected to deparaffinization followed by antigen retrieval and incubated with PCMT antibody mix. PC-MTs were then cleaved by exposing the sections to UV light followed by matrix application, and mass spectrometry analysis using rapifleX MALDI-TOF mass spectrometer at 20 μm resolution
- Secondary antibody imaging for HER2:** Slides were incubated with a clinically used anti-HER2 antibody (clone 4B5, Roche), followed by an anti-Rabbit IgG secondary antibody crosslinked to PC-MT (AmberGen Inc.) for staining
- Data processing:** Raw mass spectrometry data were processed and visualized using SCiLS Lab Pro software. Standardized visualization was achieved using Python scripts with percentile-based color mapping for ER, PR, HER2 and PanCK



RESULTS



Average MALDI-MSI spectra of breast cancer subtypes—(A) ER+/PR+/HER2-, (B) ER-/PR-/HER2+, (C) ER-/PR-/HER2- —with red arrows indicating m/z values of photocleavable mass tags for ER (1240.91), PR (1244.93), HER2 (1210.74), and pan-cytokeratin (1628.79), demonstrate good concordance with the expected biomarker status.



Comparison of HER2 expression detected by MALDI-MSI and traditional IHC in breast cancer tissues. (A) HER2-negative and (B) HER2-positive breast cancer tissues.

CONCLUSIONS

- These results demonstrate the potential application of MALDI-IHC workflow to spatially profile clinically relevant biomarkers in cancer tissues.
- Optimal performance of the workflow requires careful selection, validation, and optimization of antibodies including concentration and target specificity.