Spatial Proteomic Profiling of ADC and Cancer Immunotherapy-Related Biomarkers Directly on Routine H&E Sections Using Imaging Mass Cytometry

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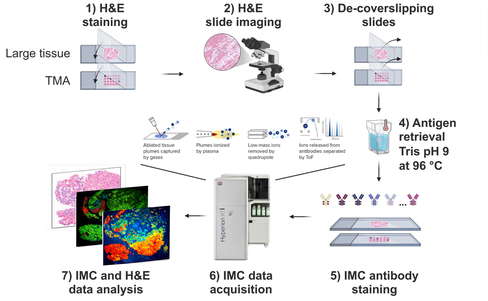
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INTRODUCTION

Hematoxylin and eosin (H&E) staining remains the cornerstone of histopathology, providing essential morphological context for tissue architecture and disease characterization. However, morphology alone cannot capture the spatial molecular heterogeneity that underlies therapeutic response and resistance, including in antibody-drug conjugate (ADC) development. Imaging Mass Cytometry™ (IMC™) technology enables highly multiplexed (40-plus markers) quantitative protein detection at subcellular resolution using metal-tagged antibodies and a wide linear dynamic range. Importantly, routine H&E-stained slides are compatible with IMC workflows, enabling the same tissue section to be reviewed by standard brightfield histology and then profiled by IMC technology, with computational alignment to overlay multiplexed biomarker maps directly onto familiar H&E features. This integrated approach preserves histologic interpretability while adding spatially resolved molecular phenotyping to support translational biomarker discovery and ADC target evaluation.

Methods and materials

FFPE tissue sections were prepared using standard histology workflows and stained with H&E. H&E whole slide images were acquired by brightfield microscopy. Following H&E imaging, the same tissue section was processed for IMC technology using metal-tagged antibody panels designed to profile ADC target biomarkers (for example, HER2, TROP2, EGFR) alongside tumor, stromal and immune lineage markers. Single-cell segmentation was performed using nuclear and/or membrane markers, followed by cell phenotyping and quantification of marker expression. Spatial analyses were used to map ADC target-positive cells and characterize their distribution relative to histologic structures and immune contexture. MCD™ SmartViewer v1.2 was used for image analysis.



ADC and clinical biomarkers investigated
HER2ECD
HER2ICD
ER
PR
TROP2
Nectin-4
CA9
CEACAM5
MSLN

Figure 1. Integrated H&E and IMC workflow for spatial tissue analysis. FFPE tissue sections were first processed by standard H&E staining (1) and high-resolution whole slide imaging (2) to capture tissue morphology and histopathologic context. Following digital acquisition, coverslips were removed (3) and the same tissue sections were reprocessed, including antigen retrieval (4), to enable downstream multiplexed protein analysis. Slides were stained with a panel of metal-conjugated antibodies (5) for IMC workflows. The panels consisted of clinically actionable targets – ADCs – and other relevant phenotyping markers. IMC data was acquired at subcellular resolution (6) and computationally integrated with corresponding H&E images to enable joint analysis of tissue architecture, cellular phenotypes and spatial marker expression (7). The table shows nine ADC and clinical markers that were investigated.

CONCLUSIONS

Integrating IMC workflows with routine H&E histology enables spatially resolved, quantitative mapping of ADC-relevant biomarkers, improving interpretation of target expression and microenvironmental context for translational decision-making.

- Demonstrated compatibility of IMC technology with freshly stained and archived H&E slides
- Revealed spatial heterogeneity in biomarker distribution and microenvironmental features not apparent from morphology alone
- Quantitatively identified and enumerated ADC target-positive cells (for example, HER2, TROP2, EGFR) within specific histopathologic contexts

Results

Post-H&E IMC uncovers important spatial features of breast cancer TME

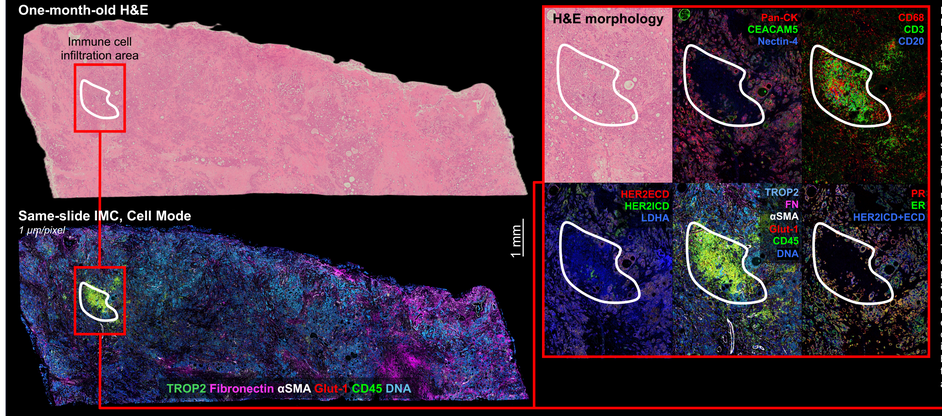


Figure 2. Linking conventional histopathology of breast cancer with high-parameter spatial proteomics with IMC technology. Side-by-side whole slide images show conventional H&E histopathology alongside the corresponding multiplexed IMC data acquired from the same tumor slide using Cell Mode (1 µm/pixel). The IMC panel includes epithelial, stromal, metabolic and immune markers enabling simultaneous visualization of tumor cells, stromal compartments, metabolic states and immune infiltration at single-cell resolution across intact tumor tissue. Inset images highlight a tertiary lymphoid structure (TLS), revealing localized expression of clinically relevant ADC targets together with immune markers and markers associated with breast cancer diagnosis, such as HER2, ER and PR. Scale bar – 1 mm

This integrated H&E and IMC approach demonstrates how IMC technology provides a powerful bridge between routine histopathology and high-dimensional proteomic profiling, enabling direct spatial linkage of tissue morphology with clinically actionable protein expression patterns. By preserving whole slide context while delivering multiplexed single-cell information, IMC technology uniquely supports biologically and clinically meaningful insights into tumor heterogeneity, immune microenvironments and therapeutic target expression.

IMC with simulated IHC enables pathologist-friendly workflows

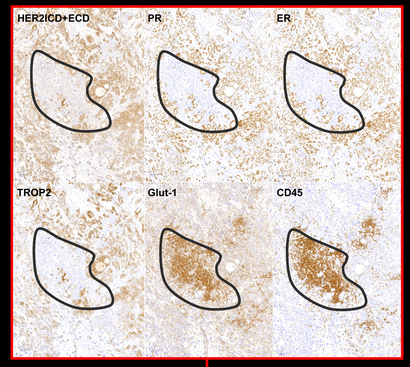


Figure 3. IMC technology-derived simulated IHC visualization of clinically relevant protein markers. Individual protein markers acquired by IMC workflows are rendered as simulated immunohistochemistry (IHC)-like images to facilitate intuitive interpretation by pathologists and translational researchers. IMC signal for HER2, TROP2, estrogen receptor (ER), progesterone receptor (PR), Glut-1 and CD45 was pseudocolored to resemble conventional DAB-based IHC staining, while preserving the quantitative multiplexed nature of IMC data. This representation enables direct visual comparison with standard diagnostic assays while simultaneously supporting high-dimensional spatial analysis from a single tissue section.

IMC technology-derived simulated IHC images provide a familiar histology-inspired visual framework for research interpretation while overcoming the limitations of singleplex IHC, allowing multiple biologically relevant and therapeutically researched immune and tumor markers to be assessed concurrently with preserved spatial context. This approach bridges established pathology workflows with multiplexed proteomic profiling, supporting biomarker research, exploratory cohort stratification and therapeutic target assessment in research studies

IMC provides a high-throughput spatial proteomic workflow to generate quantifiable metrics of TME cellular composition and activity

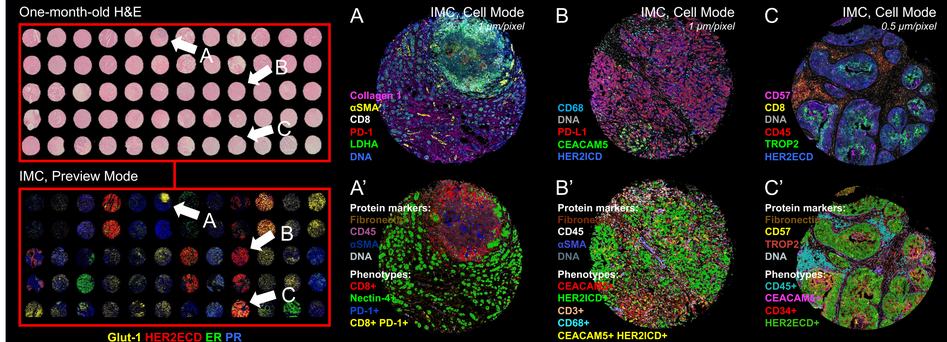


Figure 4. High-throughput spatial and single-cell analysis of breast cancer tissue microarrays using integrated H&E and IMC technology. Breast cancer TMAs were processed using the same integrated workflow, with H&E staining and imaging followed by IMC imaging on the same tissue section. Preview Mode was used to rapidly screen and identify TMA cores exhibiting focal accumulation of biologically and clinically relevant markers. Three representative cores are shown, illustrating multiplexed Cell Mode images. Single-cell segmentation and supervised phenotypic clustering analysis using QuPath revealed quantifiable distribution of relevant cell populations. This analysis shows the presence of PD-1-expressing cells in immune aggregates (A'), CEACAM5 and HER2 double positive tumor cells (B') and stromal immune cells (CD45+) surrounding HER2-expressing tumor cells (C'). Image analysis was performed in MCD SmartViewer v1.2.

Extending the integrated H&E and IMC workflow to TMAs demonstrates the scalability of IMC technology for high-throughput tissue profiling, enabling efficient screening, prioritization and deep spatial characterization of clinically relevant regions across large sample cohorts. This approach supports robust biomarker discovery, comparative analysis across patients, and translational insights relevant to cancer biology and therapeutic target evaluation.

IMC on archived H&E-stained slides visualizes cell populations relevant to breast and lung cancer

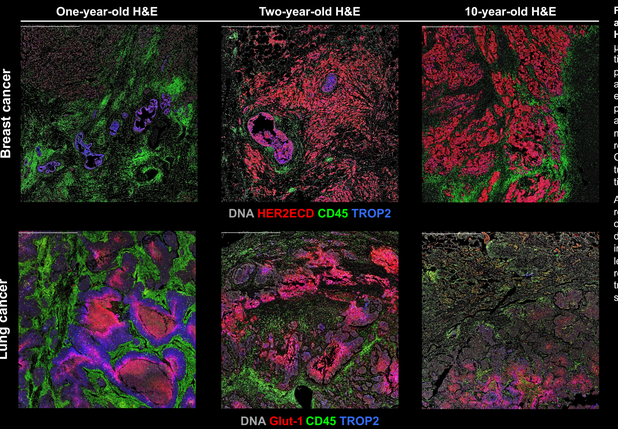


Figure 5. High-dimensional spatial proteomic analysis enabled on long-stored archival H&E-stained slides. Representative Cell Mode (1 µm/pixel) images are shown from breast cancer tissue sections that had been H&E stained, permanently processed, and stored for approximately one, two and 10 years. Despite extended storage and prior histopathologic processing, IMC technology was successfully applied to these archival slides, enabling robust multiplexed detection of clinically and biologically relevant markers, including HER2, TROP2, Glut-1, CD45 and DNA. IMC images reveal preserved tumor, immune and metabolic features within aged tissue sections. Scale bar – 1 mm

Archived H&E slides can be successfully repurposed for IMC workflows, allowing extraction of meaningful high-dimensional spatial proteomic data. This capability unlocks previously inaccessible biological and clinical insights from legacy pathology collections, enabling retrospective studies, biomarker discovery and translational research without the need for newly sectioned tissue.

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