



Integrating Hematoxylin and Eosin Histology with Multiplexed Imaging Mass Cytometry for Spatial Proteomic Profiling of Antibody-Drug Conjugate Targeted Biomarkers

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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.

Materials and methods

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.

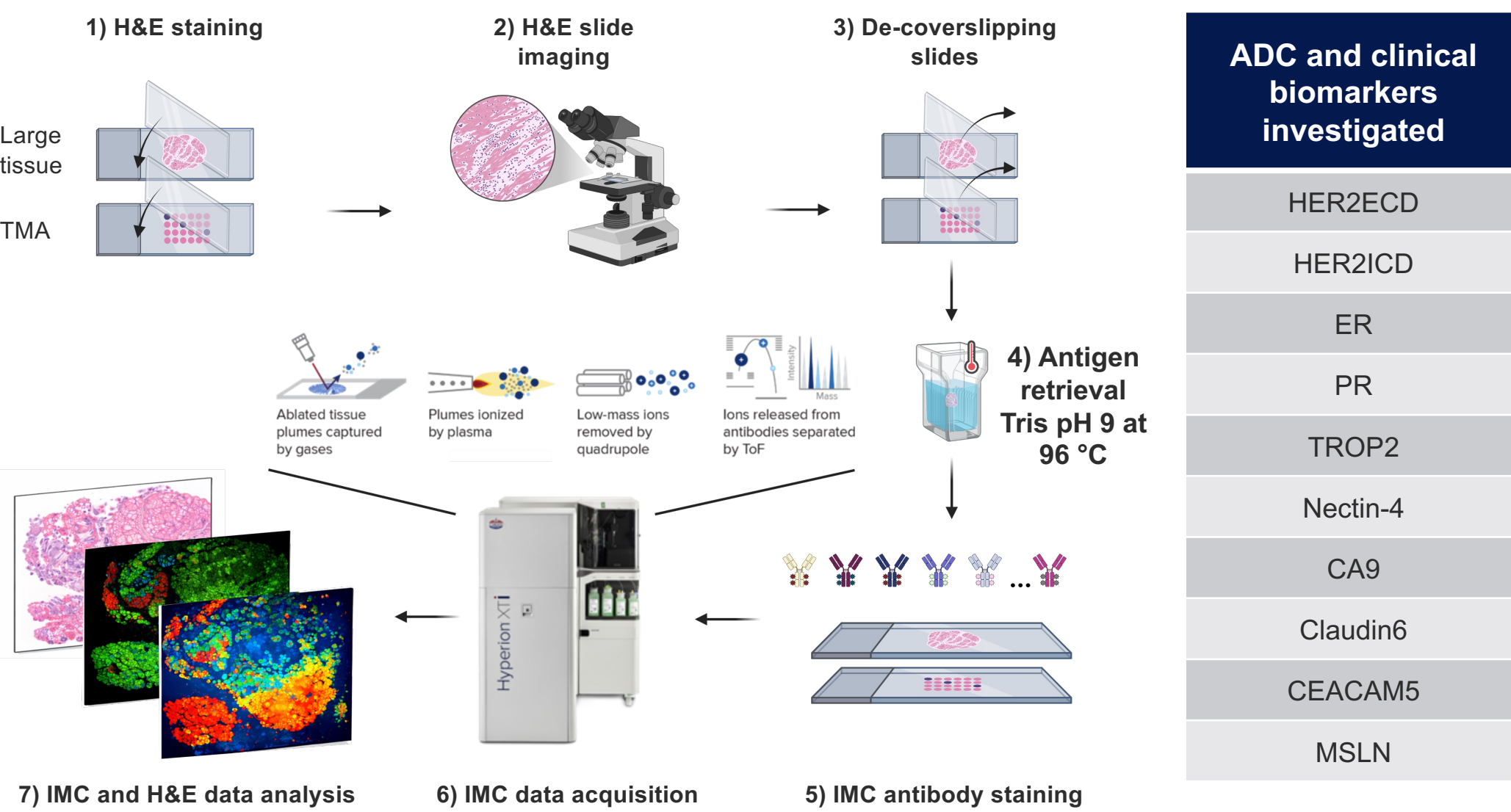


Figure 1. Integrated H&E-IMC workflow for spatial tissue analysis. FFPE tissue sections were first processed by standard H&E staining (1) and high-resolution whole slide imaging (2) to capture tissue morphology and histopathologic context. Following digital acquisition, coverslips were removed (3) and 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 10 ADC and clinical markers that were investigated.

CONCLUSION

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

