Next Generation of Spatial Biology: High-Throughput Multiplexed Imaging Mass Cytometry with Whole Slide Modes

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Introduction

Gaining spatial insights into the cellular composition of tumor tissue has tremendous potential to inform clinical and translational researchers about mechanisms behind spatial predictors of immunotherapy success, disease etiology and progression. Imaging Mass Cytometry™ (IMC™) technology is a high-plex spatial biology imaging technique that enables deep characterization of the diversity and complexity of the tumor microenvironment (TME). IMC platforms support detailed assessment of cell phenotype and function using 40-plus metal-tagged antibodies simultaneously on a single slide without issues associated with fluorescence-based spectral overlap, tissue autofluorescence or implementation of multiple washing and acquisition cycles.

Currently, IMC technology enables user-defined regions of interest (ROIs) in tissues to evaluate cellular and structural composition. To enhance the IMC user experience, we developed two new whole slide imaging (WSI) modes, which enable streamlined workflows using ultrafast Preview Mode (PM) and high-throughput Tissue Mode (TM). PM samples the entire tissue at predefined spacing to rapidly capture a low-resolution image of all expressed markers in the antibody panel. PM generates an image in minutes to enable informed ROI placements while leaving the stained tissue intact for higher-resolution imaging. TM rapidly acquires images of the whole tissue in hours at lower resolution at a quality that can be used for quantitative analysis of the tissue spatial biology. Specifically designed for high-throughput applications, TM in combination with a newly available 40-slide loader for the Hyperion XTi™ Imaging System permits automated and continuous imaging of more than 40 large tissue samples (400 mm^2 per tissue) per week.

Methods and materials

We showcase the application of WSI modes using the newly developed Human Immuno-Oncology IMC Panel, 31 Antibodies. The 31-marker panel was combined with expansion panels to create a 40-plus-marker panel that expands the ability to conduct comprehensive high-plex tumor and immune cell profiling. Tumor tissue microarrays (TMA) and whole tumor tissue sections were stained with the expanded panel. Single-cell analysis of selected ROIs, on whole tumor sections and TMAs, guided by PM data successfully provided quantitative analyses of spatial biology at single-cell resolution. In parallel, TM on whole tumor sections followed by pixel-clustering analysis provided a spatially resolved quantitative assessment of specific tumor and immune components of the TME.

Conclusions

Whole slide imaging modes highlight the power of IMC as a **high-plex spatial biology imaging platform** with **high-throughput capabilities** ideally suited for **translational** and clinical applications.

41-marker pathologist-verified antibody panel

Human Immuno-Oncology IMC Panel, 31 Antibodies (PN 201509)							Expansion panel	
Human Tissue Architecture IMC Panel, 4 Antibodies	Human Stromal Cell IMC Panel, 4 Antibodies	Human Basic Immune IMC Panel, 4 Antibodies	Human Lymphoid IMC Panel, 4 Antibodies	Human Myeloid IMC Panel, 6 Antibodies	Human Cell Functional State IMC Panel, 5 Antibodies	Human Epithelial and Mesenchymal IMC Panel, 4 Antibodies	Human Immune Cell Expansion IMC Panel, 7 Antibodies	Maxpar® IMC Cell Segmentation Kit
PN 201510	PN 201511	PN 201518	PN 201512	PN 201513	PN 201514	PN 201515	PN 201516	PN 201500
CD31 Collagen 1 Fibronectin PanCK	FAP Podoplanin αSMA CD44	CD3 CD20 CD45 CD68	CD4 CD8 CD45RO CD57	CD66b HLA-DR CD163 CD14 CD11b CD11c	Granzyme B PD-L1 PD-1 FoxP3 Ki-67	E-cadherin β-catenin EpCAM Vimentin	CD7 CD206 CD15 INOS CD16 MPO CD38	ICSK1 ICSK2 ICSK3

Figure 1. Human Immuno-Oncology IMC Panel. The new base 31-plus-marker panel is improved relative to the previous 17-marker panel and offers a more comprehensive set of pathologist-verified antibodies, which are highly relevant for accessing immuno-oncological processes in human tumors. The off-the-shelf modular structure of the panel offers excellent flexibility to customize IMC panels for application on any tumor samples. In combination with the Human Immune Cell Expansion IMC Panel and the Maxpar™ IMC Cell Segmentation Kit (ICSK), the fully loaded 41-marker panel permits detection of lymphoid and myeloid immune cell subtypes and their activation states, immunosuppressive, metastatic and growth state of tumors, tumor stemness, tertiary lymphoid structures, tumor vascularization, the presence of cancer-associated fibroblasts (CAFs) and extracellular matrix composition.

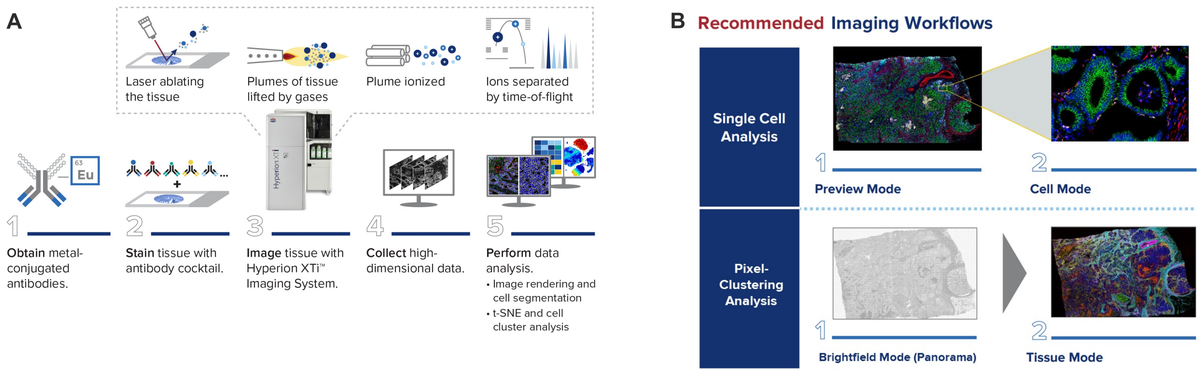

Figure 2. Imaging Mass Cytometry workflows. (A) IMC offers a streamlined workflow that simplifies translational and clinical application of multiplexed tissue analysis. The five-step process, which consists of obtaining metal-conjugated antibodies, staining tissues with antibody cocktails, imaging tissues with the Hyperion™ XTi Imaging System, and the collection and analysis of high-dimensional data, can be accomplished in as little as 72 hours (two slides with two 4 mm^2 ROI each). (B) The novel WSI modes for IMC platforms offer a customized workflow for specific imaging applications. Here we highlight two simple ways for a user to get started. For single-cell analysis, start with Preview Mode, which provides a rapid scan of the whole tissue and highlights all your stained markers. This helps guide ROI placement to capture single-cell resolution image data using Cell Mode. For pixel-clustering analysis of an entire tissue section, users can first identify the placement of tissue using the rapid Brightfield Mode, followed by the novel Tissue Mode, which generates a high-quality scan of the entire tissue sections in a matter of hours with higher spot-size ablations enabling entire tissue analysis using pixel-clustering analysis. Combining these new workflows with the newly available slide loader the Hyperion XTi Imaging System streamlines IMC application and makes it a useful resource for high-throughput clinical and translational studies.

Results

Preview Mode and Cell Mode imaging-mediated workflow enables rapid data-driven ROI selection for single-cell analysis of human tumors using a single slide.

Single-cell analysis of selected human multi-cancer TMA cores highlights immuno-oncological features of tumor microenvironment.

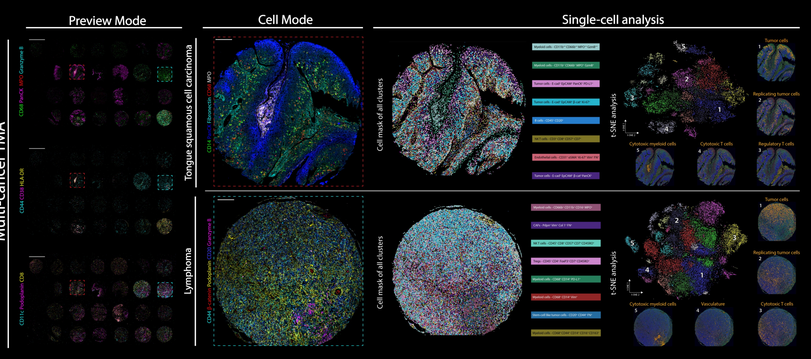

Figure 3. Rapid visualization of tissue cellular composition of human multi-cancer TMA cores using the Human Immuno-Oncology IMC Panel. Preview Mode scan of a single slide (three rendered images) rapidly identified tongue squamous cell carcinoma (TSCC) and lymphoma that contained immune-related structures. These cores were selected for subsequent Cell Mode acquisitions. In TSCC, high immune cell infiltration in the stromal tissue compartment and MPO-expressing myeloid cell accumulation was observed. In lymphoma tissue, granzyme B-expressing immune cells and podoplanin-expressing stromal cells were detected. Single-cell analysis through Cellpose and histoCAT enabled detection of tumor and immune cell populations on both tissue samples, as shown in the segmented cell mask. t-SNE and PhenoGraph clustering analysis successfully resolved specific subsets of tumor and immune cell populations, which could then be mapped back to the segmented cell mask for each tissue. Detection of these cell populations is critical for guiding immune-related therapies for cancer patients in the clinic. Scale bar in Preview Mode is 1.5 mm and Cell Mode is 200 µm.

Preview Mode and Cell Mode application on breast cancer tissue delineates cellular composition of immune and stromal infiltrates.

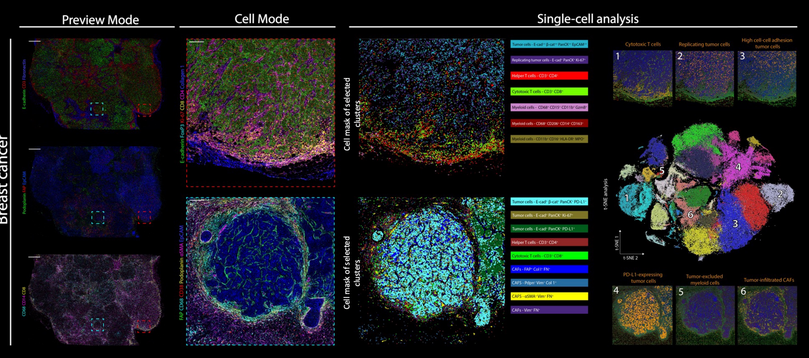

Figure 4. Streamlined single-cell analysis of breast cancer spatial biology using the Human Immuno-Oncology IMC Panel. Preview Mode scan rapidly demonstrated the heterogeneous landscape of the entire breast cancer (invasive ductal carcinoma) tissue, which assisted in guiding the placement of several ROIs at relevant positions. Multiple types of lymphoid and myeloid immune cells were detected at the tumor margin (red inset). A tumor structure with low immune penetration was detected surrounding the necrotic tissue (cyan inset). Subtypes of CAFs and immune cells can be observed localized at the margin of the PD-L1-expressing tumor structure. Single-cell analysis quantitatively delineated cell phenotype of tumor and immune cell populations in all ROIs. t-SNE and PhenoGraph clustering analysis successfully segregated and spatially resolved subsets of activated and dormant tumor cells and immune cell populations, which could then be mapped back to the segmented cell mask for each tissue. The detection of subpopulations of tumor and immune cells defines the highly heterogeneous nature of the breast cancer sample. Scale bar in Preview Mode is 2 mm and Cell Mode is 200 µm.

Preview Mode and Cell Mode facilitate detection of tertiary lymphoid structures and PD-L1-expressing tumor cells in the colon.

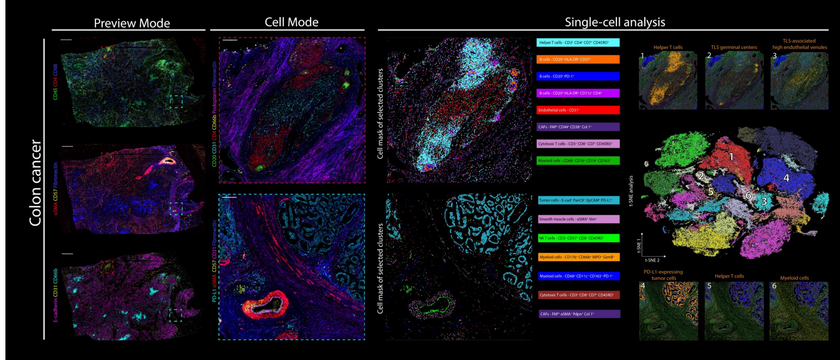

Figure 5. Efficient mapping of clinically relevant features of colon adenocarcinoma tissue using the Human Immuno-Oncology IMC Panel. Preview Mode scan highlighted several relevant tumor-specific structures such as tertiary lymphoid structures (TLSs) and high vascular and immune cell density landmarks on the tissue, which were further evaluated using Cell Mode. Detailed spatial layout of the TLS highlighted the presence of B cell-containing germinal center surrounded by T helper cells (red inset). The presence of TLS-specific high endothelial venules was detected as well. In the stromal compartment, a large vessel containing extravasating NK cells was detected along with PD-L1 (clone 73-10)-expressing tumor and immune cell populations. Single-cell analysis quantitatively detected the exact number of cells in the TLS and specialized tumor cells in the tissue. t-SNE and PhenoGraph clustering analysis successfully demonstrated immune-evasive tumor cell populations, CAFs and myeloid and lymphoid immune cell types, which could then be mapped back to the segmented cell mask for each tissue. Scale bar in Preview Mode is 2 mm and Cell Mode is 200 µm.

Tissue Mode and pixel-clustering analysis maps the spatial biology of heterogeneous tissue compartments in human tumors.

Pixel-clustering analysis using Tissue Mode reveals complex compartmentalization and heterogeneity of breast cancer tissue.

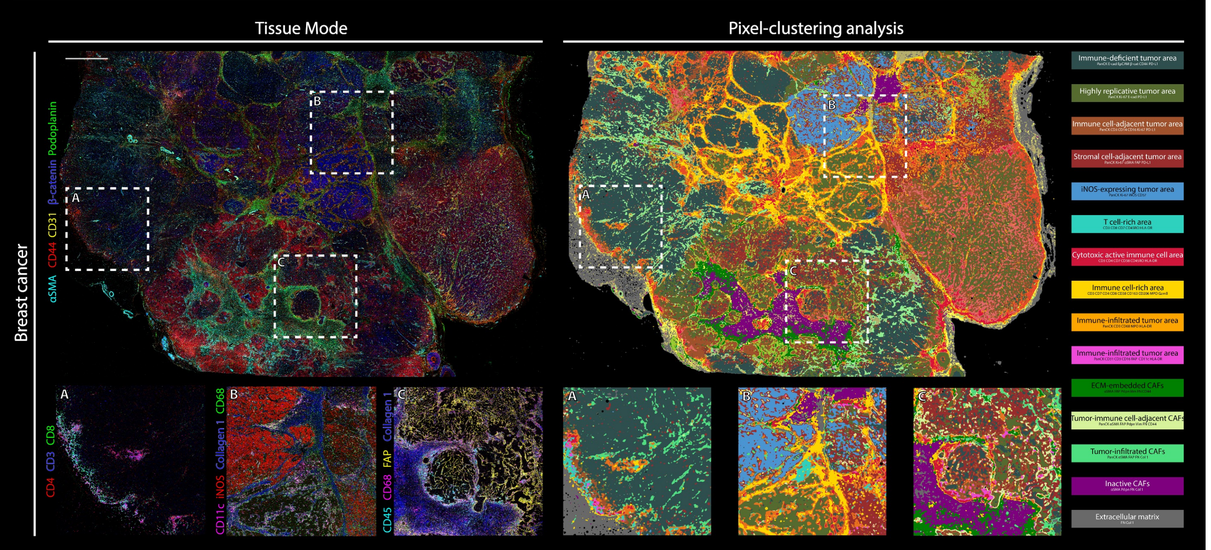

Figure 6. Rapid assessment of whole tissue spatial biology of human breast cancer tissue using the Human Immuno-Oncology IMC Panel. Tissue Mode imaging successfully demonstrates the expression pattern of Human Immuno-Oncology IMC Panel markers in breast cancer tissue. Striking heterogeneity of the tissue can be detected with specialized tumor structures across the tissue. Lymphocyte localization is observed at the tumor margins (A, inset). The presence of specialized tumor cells expressing iNOS surrounded by collagen 1-rich extracellular matrix is detected (B, inset). Tissue compartments containing interactive niches of CAFs and immune cells are detected as well (C, inset). Unsupervised pixel-clustering analysis along with hierarchical clustering using the MCD™ SmartViewer analysis pipeline highlighted several tumor areas such as immune-deficient, highly replicative, immune cell-adjacent, stromal cell-adjacent and tumor cells with high iNOS-expressing tumor regions. Areas with high immune cell infiltrations containing cytotoxic T cells, T helper cells and myeloid cells were detected. Interactive niches of several subtypes of CAFs are visualized and their spatial localization pattern can be readily observed. The detection of these tissue compartments provides potential key biological insights necessary for the prognostic evaluation of patients and determining the sequence of clinical intervention. Scale bar is 2 mm.

Pixel-clustering analysis using Tissue Mode identifies highly specialized tumor, immune and stromal tissue compartments in colon adenocarcinoma.

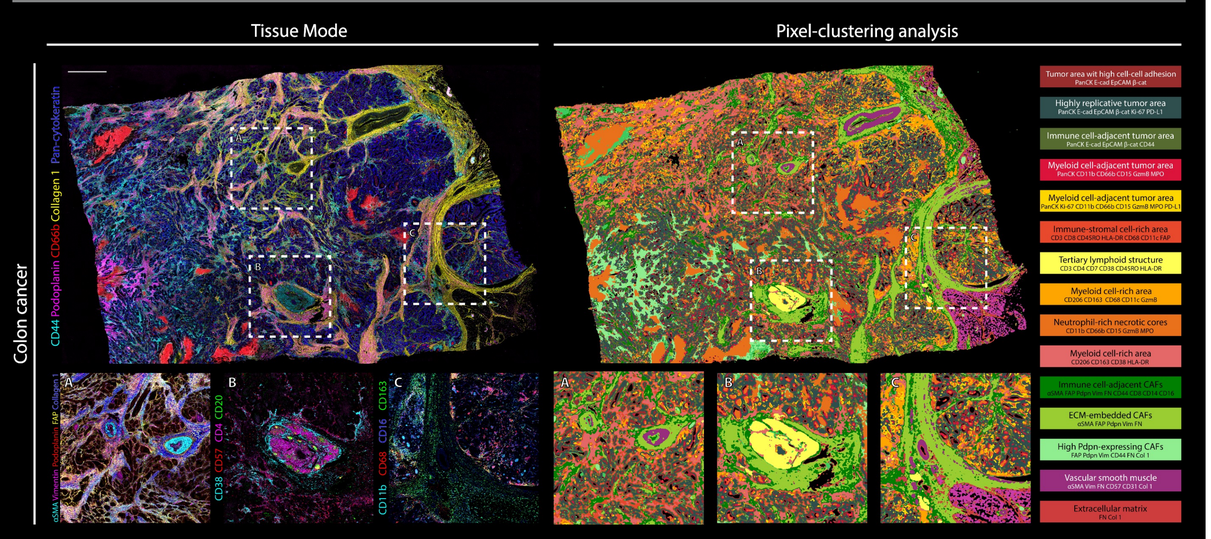

Figure 7. Rapid assessment of whole tissue spatial biology of human colon adenocarcinoma tissue using the Human Immuno-Oncology IMC Panel. Tissue Mode imaging successfully demonstrates the expression pattern of Human Immuno-Oncology IMC Panel markers in colon adenocarcinoma tissue. Tumor compartments containing collagen 1-rich ECM tracks, necrotic cores and stroma can be detected. Striking heterogeneity of CAFs expressing various combinations of CAF-specific markers from the Human Stromal Cell IMC Panel are detected (A, inset). A large TLS is detected by combining markers from the Human Lymphoid IMC Panel (B, inset). A wide variety of myeloid cells are observed present within the tumor cell-rich area as well as in the stromal compartment (C, inset). Unsupervised pixel-clustering analysis along with hierarchical clustering using the MCD SmartViewer analysis pipeline identified multiple tumor areas with high expression of cell-cell adhesion markers, replication markers and immune cell-adjacent areas. Immune cell-rich compartments containing immune-stromal interactive niches, myeloid cell enrichment, neutrophil-rich necrotic cores as well as TLSs can be visualized. Various subtypes of CAFs expressing high or low podoplanin are detected. Additionally, CAF-rich compartments are detected at specific locations in the tissue, which are embedded in the ECM or adjacent to other immune cells. Detection of these clinically relevant tumor parameters, which are made possible by whole slide imaging with Tissue Mode and pixel-clustering analysis, are important for determining the best course of clinical intervention for colon cancer patients. Scale bar is 2 mm.

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