Spatial Profiling of Exhausted T Cells Using High-Plex Imaging Mass Cytometry

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Introduction

The spatial organization and cellular composition of the tumor microenvironment (TME) has the potential to inform clinical and translational researchers about mechanisms of disease progression and therapeutic success. Of particular interest are immune cells, especially T cells, which can become exhausted due to chronic stimulation. This poses a significant challenge in cancer therapy as exhausted T cells have reduced effector functions and sustained expression of inhibitory receptors such as PD-1 and CTLA-4, resulting in failure to effectively eliminate tumor cells.

Imaging Mass Cytometry™ (IMC™) technology is a high-plex imaging technique that enables deep characterization of the heterogeneity and complexity of the TME. The Hyperion™ XTi Imaging System utilizes IMC technology to provide signal intensities over a wide dynamic range and entails one-step detection of 40-plus markers without issues of tissue autofluorescence, making it ideally suited for spatial biology applications. Whole slide imaging modes and an automated slide loader function enable a streamlined, versatile, scalable workflow for high-throughput analysis.

Methods and materials

The 41-marker panel used in this study was created by adding commercially available expansion panels and single antibodies to the Human Immuno-Oncology IMC Panel, 31 Antibodies. This expands our ability to conduct comprehensive high-plex tumor and immune cell profiling. Whole tumor tissue sections were stained with this comprehensive antibody panel. Tissue Mode imaging of whole slide tumor sections, combined with pixel-clustering analysis, provided a spatially resolved quantitative assessment of specific tumor and immune components within the TME. This approach was further enhanced by a quick tissue scan using Preview Mode, which was used to guide single-cell analysis of selected regions of interest (ROIs) in serial tissue sections that were acquired at single-cell resolution using Cell Mode. Together, these methods successfully delivered quantitative spatial biology analyses.

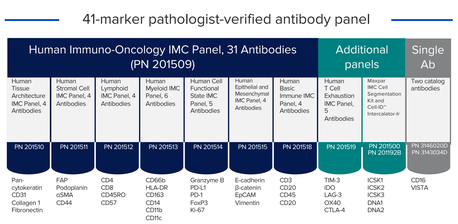


Figure 1. Pathologist-verified 41-marker antibody panel. The Human Immuno-Oncology IMC Panel is designed to explore immuno-oncological processes in human tumors. It includes 31 pathologist-verified antibodies in the base panel and is optimized for FFPE tissues. The panel's modular structure allows for customization, making it suitable for various translational and clinical samples. When combined with the Human T Cell Exhaustion IMC Panel and the Maxpar™ IMC Cell Segmentation Kit, it enables the detection of immune cell subtypes, tumor characteristics and microenvironment components, and the presence of cancer-associated fibroblasts (CAFs). This comprehensive approach enhances understanding of the TME in immuno-oncology research.

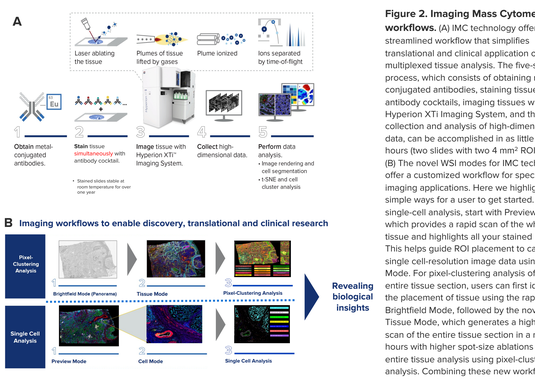


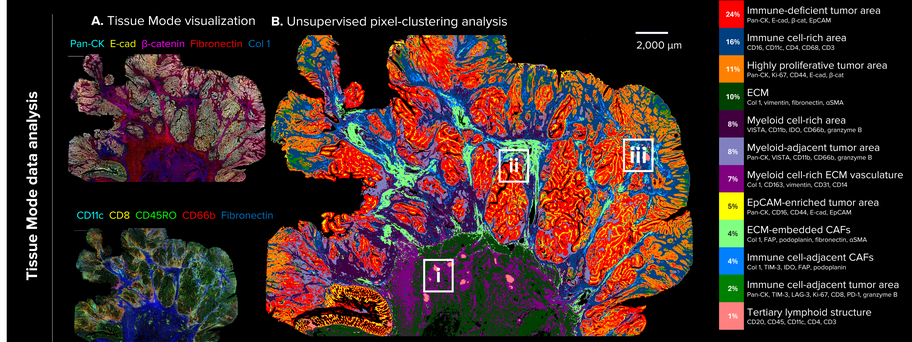
Figure 2. Imaging Mass Cytometry workflows. (A) IMC technology offers a streamlined workflow that simplifies translational and clinical application of multiplexed tissue analysis. The five-step process, which consists of obtaining metal-conjugated antibodies, staining tissues with antibody cocktails, 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 mm² ROI each). (B) The novel WSI modes for IMC technology 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 section 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 for the Hyperion XTi Imaging System streamlines IMC application and makes it a useful resource for high-throughput clinical and translational studies.

Conclusions

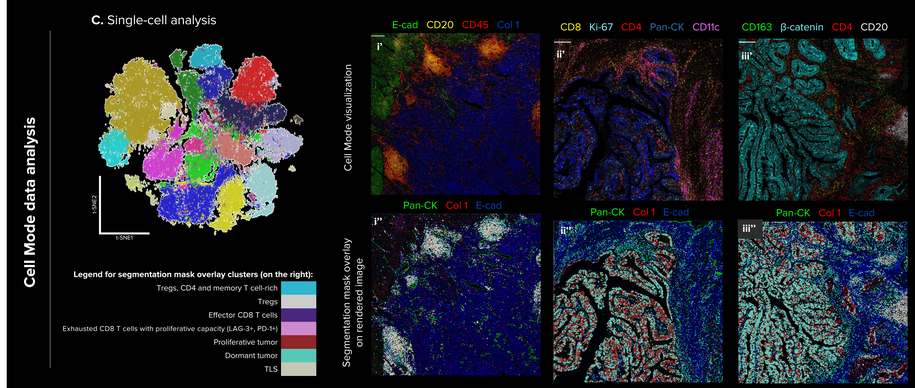
This work characterizing T cell exhaustion markers in multiple cancers showcases the capabilities of IMC technology and establishes it as a reliable high-plex, high-throughput spatial biology imaging platform. IMC technology is ideally suited for developing future translational and clinical applications and has the potential to help guide personalized therapeutic strategies for cancer treatment.

Results: IMC analysis revealed striking heterogeneity with distinct tumor and immune-rich niches in the TME of the cancer tissues.

Human colon adenocarcinoma



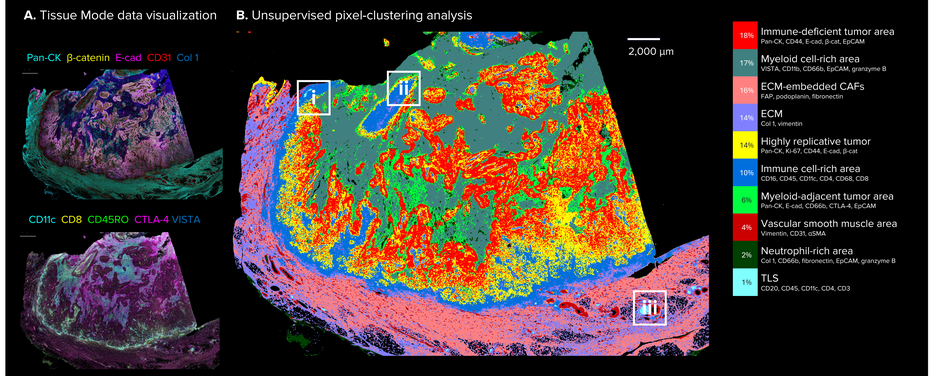
- Detected functional effector T cells and multiple tertiary lymphoid structures (TLSs)
  - (i) Formation of TLS adjacent to the tumor region; (ii) region with multiple TLSs; and (iii) region of tumor-immune infiltration
- CAFs are found in the areas in between the specialized tumor structures across the tissue
- Unsupervised pixel clustering and analyses of Tissue Mode data further delineated distinct tumor areas based on the presence of infiltrating immune cells, tumor replicative activity and spatial proximity to stromal components



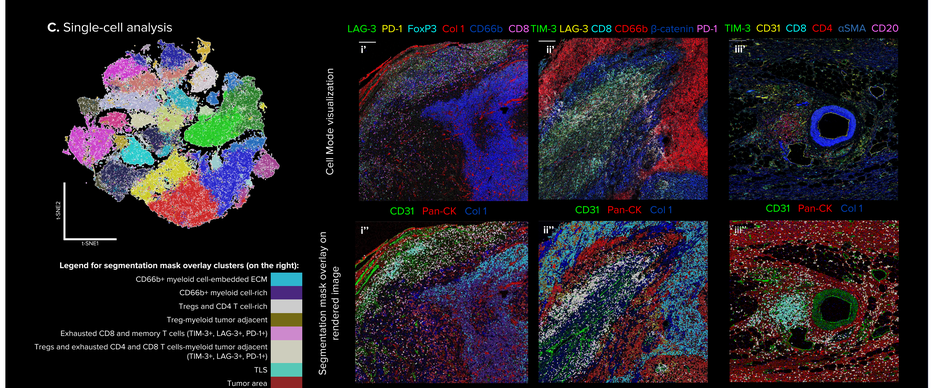
- Cell Mode confirmed presence of functional effector T cells and TLSs
- t-SNE and PhenoGraph clustering analysis of Cell Mode data segregated and spatially resolved the T cells into regulatory, effector and exhausted (LAG-3+/PD-1+) T cell subpopulations, which were mapped back to the segmented cell masks
- 23 clusters were identified in all ROIs including the tumor and various immune cell subpopulations, including exhausted T cells

Figure 3. Functional effector T cells and multiple TLSs were detected in human colon adenocarcinoma tissue.
(A) The regions of tumor (top) and the immune cell infiltration (bottom) in the Tissue Mode data are visualized using MCD™ Viewer. Scale bar is 2,000 µm.
(B) The tissue is further divided into 12 clusters following unsupervised pixel-clustering analysis along with hierarchical clustering using the MCD SmartViewer analysis pipeline. Three ROIs were investigated further in (C) and (D) using a serial section of the same tissue block (i, ii and iii).
(C) The three ROIs identified in (B) were acquired using Cell Mode and visualized using MCD Viewer (i', ii' and iii'). Scale bar is 200 µm.

Human urothelial carcinoma



- Identified hot tumor regions with immune cell infiltrates (immune cell-adjacent tumor area) and cold tumor regions (immune-deficient tumor area)
- T cells expressing PD-1 and TIM-3 at the tumor periphery suggest that they are in advanced exhaustion stage
- Unsupervised pixel clustering and analyses of Tissue Mode data further delineated distinct tumor areas based on the presence of infiltrating immune cells, tumor replicative activity and spatial proximity to stromal components



- The CD8 T cells are found to be in an exhausted state due to their co-expression of inhibitory receptors like TIM-3, LAG-3 and PD-1
- t-SNE and PhenoGraph clustering analysis segregated and spatially resolved the T cells into various subpopulations including Tregs, CD4, CD8 and memory T cells, which could then be mapped back to the segmented cell mask for each ROI (i'', ii'' and iii'')
- 28 clusters were identified in all ROIs including the tumor and various immune cell subpopulations

Figure 4. Exhausted T cells surround the tumor areas in human urothelial carcinoma.
(A) The regions of tumor (top) and the immune cell infiltration (bottom) in the Tissue Mode data are visualized using MCD Viewer. Scale bar is 2,000 µm.
(B) The tissue is further divided into 10 clusters following unsupervised pixel-clustering analysis along with hierarchical clustering using the MCD SmartViewer analysis pipeline. Three ROIs were investigated further in (C) and (D) using a serial section of the same tissue block (i, ii and iii).
(C) The three ROIs identified in (B) were acquired using Cell Mode and visualized using MCD Viewer (i', ii' and iii'). Scale bar is 200 µm.

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