A Deep-Learning Approach to Guide Acquisition Region Selection for Imaging Mass Cytometry

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Introduction

The growth in cancer immunotherapy agents requires an understanding of the immune contexture of the tumor microenvironment (TME). This can be aided by high-plex imaging and analysis to obtain phenotypes of specific cells and study their biodistribution and interactions. Imaging Mass Cytometry™ (IMC™) technology is the method of choice for single-step staining and high-plex imaging of tissues, avoiding the complications of autofluorescence and cyclic imaging.

IMC technology has three new imaging modes: Preview Mode (PM), Cell Mode (CM) and Tissue Mode (TM). PM rapidly scans a stained tissue to provide a comprehensive overview, mapping out the distribution of over 40 markers and revealing tissue heterogeneity. This enables researchers to make informed decisions about which areas warrant closer examination. Following PM, regions of interest (ROIs) are selected for high-resolution imaging. This is a critical step that is informed by biomarker expression using automated AI algorithms. CM offers high-resolution imaging for detailed analysis of the ROIs identified during PM, all using the same slide. TM provides fast acquisition of the entire tissue at 5-micron resolution, optimal for quantitative pixel-based analysis. These modes support automated, continuous imaging of more than 40 large tissue samples (400 mm²) weekly.

Methods and Materials

Tissue sections of colon adenocarcinoma were stained with a 30-marker IMC panel of structural, tumor, stromal, immune cell and immune activation markers. Images were acquired on the Hyperion XTi™ Imaging System (Standard BioTools™), first in PM and then in CM with automatic selection of ROIs using Phenoplex™ software (Visiopharm®). ROIs were automatically selected based on two criteria: 1) actively proliferating and non-proliferating tumor regions; 2) cold and hot tumor regions as identified by immune hotspots within stromal or epithelial tumor regions. An adjacent serial section was acquired in TM.

Single-cell analysis of the images obtained in CM was performed using Phenoplex. Tissue segmentation divided the tissue into tumor epithelial and stromal regions; cell segmentation was based on iridium DNA channels; and phenotyping was performed using the guided workflow. This data was used to compare the immune contexture through a series of t-SNE plots partitioned by spatial region and clinical variables.

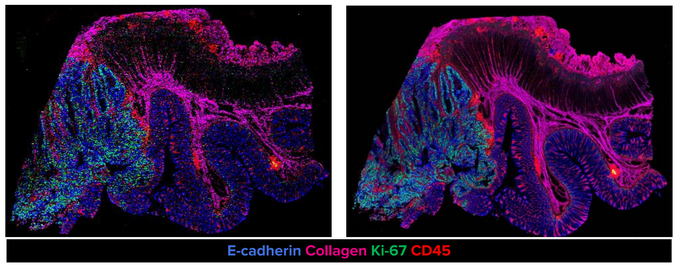
Results

Whole slide imaging with IMC

Here we show whole slide imaging of colon adenocarcinoma tissue using PM and TM. Heat maps generated by Phenoplex from PM were used to guide ROI selection for acquisition in CM. A high degree of immune infiltration was observed in the tumor, with significant levels of infiltrating myeloid cells. Tissue phenotypic signatures of the TME were uncovered through the determination of immune cell types found in the vicinity of cancerous cells, using Phenoplex.

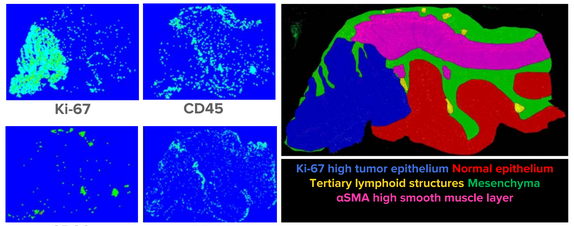
A Preview Mode IMC image

Tissue Mode IMC image



A) PM (1 µm resolution, 25 µm spacing) generates a whole slide preview of the tissue sample in minutes by using subsampling to visualize all stained markers. It is used to provide guidance on where to place ROIs and ablate the same section using CM at 1 µm resolution. TM is used to visualize the whole tissue sample at 5 µm resolution within a few hours. The data can then be analyzed using pixel-based analyses.

B Preview Mode annotations to guide ROI selection

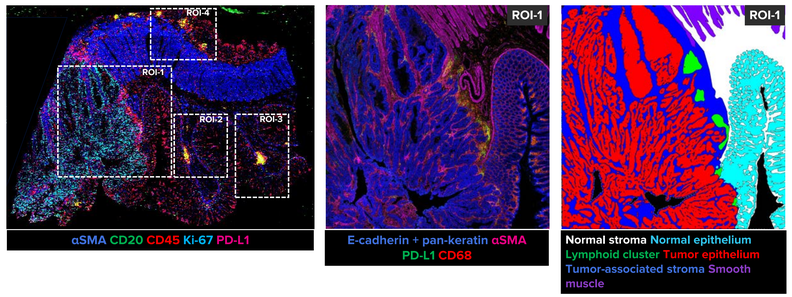


B) Heat maps of selected individual biomarkers were generated by Phenoplex using the PM image of colon adenocarcinoma tissue shown in (A). These heat maps can be used to guide the ROI selection for high-resolution acquisition in CM. Heat maps for proliferation (Ki-67) and immune phenotypic signatures (CD45, CD20, PD-L1) of the TME were generated to guide ROI placement. Biomarker-based regional annotations were created to depict tissue regions for easy selection of ROIs.

C Guided ROI selection in Preview Mode

D Cell Mode image

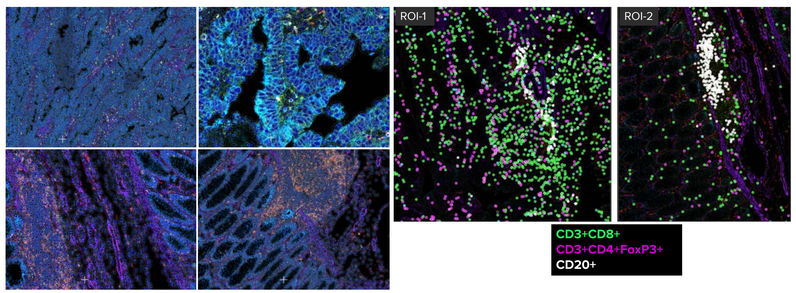
E AI-based tissue segmentation



C) Whole slide imaging of colon adenocarcinoma in PM with overlaid ROIs (1–4) guided by biomarker heat maps as generated in (B) using Phenoplex. **D)** ROI-1 acquired on the same section in CM at 1 µm resolution is depicted here. **E)** AI-based tissue segmentation of ROI-1 for differentiation of stroma and epithelial areas, both tumor and normal, was trained using E-cadherin, DNA-1 and collagen channels as input for the deep-learning network. Smooth muscle segmentation was guided by αSMA positivity, and lymphoid clusters were added as additional segmentations for more detailed analysis options.

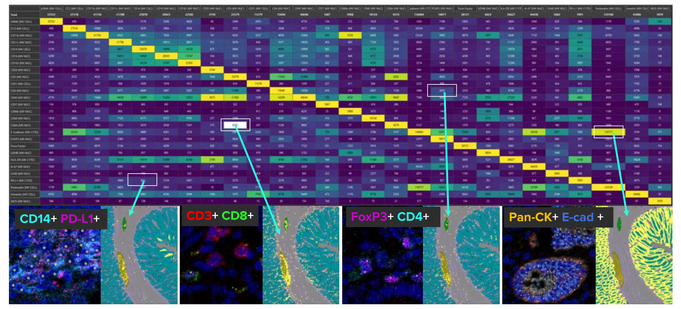
F Cell segmentation

G Phenotyping



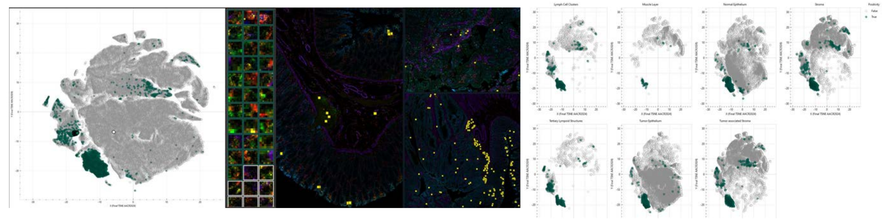
F) Visiopharm's pretrained deep-learning-based cell segmentation algorithm for IMC technology, trained on DNA1 and DNA2 as input channels, was applied to all CM images. A postprocessing step added a cytoplasmic compartment to the nuclei. This generates three distinct outputs for phenotyping, providing users the option to select nuclear, cytoplasmic/membrane and whole-cell object-based outputs for determination of object positivity (for example, phenotype). **G)** Examples of generated phenotypes are shown as overlays on ROI-1 and ROI-2: CD3+CD8+ (green), CD3+CD4+FoxP3+ (magenta) and CD20+ (white). Phenotypes can be visualized directly from the Phenoplex workflow, allowing users to investigate the distribution of cell populations across multiple samples simultaneously.

H Cellular phenotypes in the interactive co-occurrence matrix



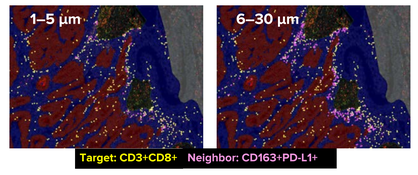
H) The co-occurrence matrix provides users with an overview of paired biomarker combinations. Each biomarker is represented as a column and a row. Co-occurrences are normalized to each column's total count of positive objects. Users can interactively investigate the co-localization of the gated biomarker pairs by selecting the relevant table cells. The double-positive cell objects are highlighted on all images and can be investigated by users. Here we show examples for immune populations and epithelial cells in the adjacent normal ROI-2. Yellow dots represent double-positive objects on the images.

I Interactive data exploration using t-SNE plots and cell object gallery



I) Generated cell objects were plotted as a t-SNE using the built-in data-plotting capabilities of Phenoplex. All data visualization plots are fully interactive and allow users to color objects (green = positive objects for CD3+CD8+), review selected objects in an object gallery and display selected objects across all open images. Additional options allow users to split (facet) generated plots by any variable or metadata; here data was split by ROI names.

J Spatial analysis tools



J) Phenoplex neighbor count analysis enables users to define specific targets as center points and neighbors based on the positivity gates of the objects and quantify the number of neighbors around targets. Here we show the analysis of targets CD3+CD8+ (yellow dots) and neighbor counts for immune-suppressive macrophages CD163+PD-L1+ double-positive cell objects (magenta dots) specifically within the TME (blue and red regions). Analysis was performed at two distinct distance bands (left: 1–5 µm and right: 6–30 µm distance from target cell objects).

Here we show region-specific analysis and visualization options of the neighbor count analysis for FoxP3+CD4+CD3+ (Tregs, magenta dots) in the vicinity of CD8+CD3+ (cytotoxic T cells, yellow dots) within the TME (left image) and the adjacent normal tissue (right image). The data table provides counts of the number of adjacent neighbor cell objects as mean with standard deviation.

Conclusions

- The multimodal features of the Hyperion™ XTi Imaging System can greatly accelerate the ability of IMC users to gain useful insights from complex biological samples
- Phenoplex enables a comprehensive workflow for the analysis of this complex data, providing automated ROI selection, phenotyping and spatial analyses of high-resolution IMC images for biological assessment

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