928 STANDARDIZING THE ANALYSIS OF SPATIAL IMAGING FEATURES IN TUMOR SAMPLES

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Background The analysis of cellular imaging data produces annotations of image regions, derived features, and cellular annotations that are the basis for quantitative reports. This data can come from different instruments and annotations applied to regions of interest (ROI); annotations of this data require can arrive in many different formats, and these formats require standardization so they can be analyzed using a common syntax. Our software package, Pythologist (https://github.com/dfci/pythologist), provides the functionality for extracting spatial and cellular features from analyzed images.[1,2] Here we demonstrate how this software can be used to standardize the process of investigating and generating reports from annotated tumor samples.

Methods Multiplex-Immunofluorescence images from FFPE were analyzed by the DFCI Center for Immuno-Oncology as part of the Human Tumor Atlas Network (HTAN) to study the tumor immune microenvironment of cancers. We analyze samples stained by two panels: 1) a checkpoint specific panel measuring Tumor (SOX10 or Cytokeratin), CD8, PD-1, PD-L1, and FOXP3, and 2) a macrophage specific panel includes CD3, CD68, PD-L1, CD163, and Ki-67 in addition to the Tumor marker. Cellular features were annotated by InForm software, and region annotations were manually drawn, these include: cell segmentation, processed image regions, drawn tumor regions, and cellular annotations produced in InForm. A subset of cases were analyzed for cell density and percent cellularity measures in the context of the full-ROI but also within the tumor and invasive margin compartments. Furthermore, spatial features including cell-proximity, cell-cell-contacts, and nearest-neighbor distances are extracted and reported through the same tool.

Results As a vignette to demonstrate how this software package can be used to generate reports, two colorectal samples where the manually-annotated invasive margin annotations were available are analyzed. Within these two samples, treating all regions of interest as a single image, a greater density of CD8+ T cells (535 and 506 cells/mm2) are adjacent to the tumor region (within ~40um from the outside of the tumor region) compared to those CD8+ T cells within the tumor region (101 and 370 cells/mm2).

Conclusions Spatially informed annotations, enable us to examine cell populations in these different contexts. By standardizing imaging data annotation formats our tool provides a common platform for generating reports from the analyzed data. These capabilities provide a straight-forward approach to analyzing and visualizing tumor samples and provide insights into meaningful spatial contexts by performing hypothesis driven analyses of the tumor-immune microenvironment.

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Ethics Approval The HTAN study is approved by the DFCI Institutional Review Board as protocol 18-452.

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