143 A COMPREHENSIVE WORKFLOW FOR HIGHPLEX IMAGING, TISSUE SEGMENTATION, AND MULTIPLEX CELLULAR PHENOTYPING FOR TUMOR MICROENVIRONMENT ANALYSIS

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Background The growth in cancer immunotherapy agents requires an understanding of the immune contexture of the tumor microenvironment (TME). One way to understand immune contexture is to use multiplex staining, imaging, and analysis to obtain multi-marker phenotypes of specific cells and analyze their biodistribution in the TME. Imaging Mass Cytometry™ (IMC) is the method of choice for single-step staining and highplex imaging of FFPE tissues. FFPE tissue is autofluorescent, which limits the utility of immunofluorescence methods, particularly when done without amplification. Lung and colorectal tissue (and bone, skin, etc) are highly autofluorescence, and therefore are a good target for IMC imaging, which has no autofluorescence issues. However, developments in analysis software with a single-package workflow for highplex imagery have not kept pace. We present here a comprehensive workflow in the Oncotopix® Discovery platform designed specifically for highplex IMC image analysis, covering tissue segmentation, cell segmentation based on IMC DNA images, cellular phenotyping, and spatial analyses.

Methods Lung and colorectal tissue sections with a 40-marker panel comprised of structural, tumor, stroma, immune cell markers, and immunoregulatory proteins that are targets of immunotherapy, were imaged (Hyperion, Standard BioTools). Highplex image analysis was performed as a multi-step workflow in a single software package that includes: conversion of IMC images to pyramidal format; easy visualization methods for displaying different marker subsets; a paint-to-train algorithm for tissue segmentation (into tumor, stroma, necrosis, etc); deep-learning-based nuclear segmentation pre-trained specifically on IMC DNA channels; cellular phenotyping based on thresholds set based on visual assessment of positivity; spatial biodistribution metrics for cell populations; and a flexible set of outputs for further downstream analysis.

Results This study demonstrates that a simple workflow can be used to analyze highplex images of different tissue types with no programming knowledge and few changes between tissue types. Visualization templates for the marker subsets and the pre-trained IMC nuclear segmentation are reusable. A new tissue segmentation algorithm for each tissue type is required, as are new thresholds for biomarker positivity. Spatial biodistribution metrics and heatmaps were generated for each tissue type with a minimum of work required.

Conclusions IMC highplex imaging of lung and colorectal tumor samples is a simple and effective means of obtaining highplex images without interfering autofluorescence. Having a comprehensive workflow for the analysis of this complex data makes obtaining useful results from highplex images more accessible to biologists and immunologists by circumventing the requirement for expert programming for each specific application.

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