



Accurate, high-throughput spatial profiling of whole slide samples with the Palettra™ multiplexed image analysis pipeline

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Abstract: Multiplexed immunofluorescence (mIF) enables spatially resolved single-cell phenotyping within the tumor microenvironment (TME), which is being increasingly recognized as an important predictor of patient response to immunotherapies. Palettra™ is a mIF full service provided by NeoGenomics Laboratories, Inc capable of staining and analyzing up to 60 proteins on a single formalin-fixed paraffin-embedded tissue section, with an image-able area of nearly 10 square centimeters. This capability enables profiling of millions of individual cells in a single slide, providing unbiased spatial phenotyping at scale.

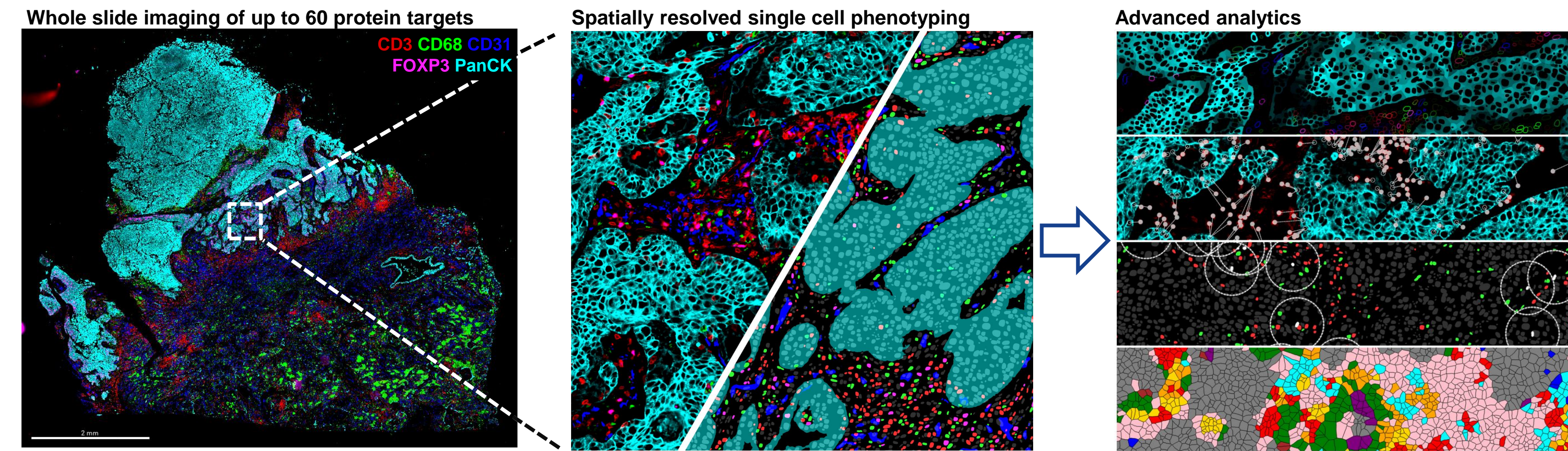
Here we present the Palettra image analysis pipeline that has been optimized for whole-slide analysis. We demonstrate the pipeline's capabilities using a 16-marker TME panel to spatially characterize a set of 20 non-small cell lung cancer (NSCLC) samples. Accuracy is benchmarked against clinically validated immunohistochemistry (IHC) for a subset of representative immune phenotypes. Cell density and intensity data generated by the image analysis pipeline demonstrated strong concordance with IHC assays for all markers evaluated.

To achieve high throughput whole slide processing while maintaining high accuracy, we use an automated pipeline backed by 100% human quality control (QC). Automation begins with image acquisition, where QC algorithms monitor image collection in real-time to detect imaging failures and trigger corrective action. In post-processing, tissue and stain QC algorithms then identify regions of tissue artifacts such as tissue loss, tissue folding, and necrotic regions to be excluded from downstream analysis.

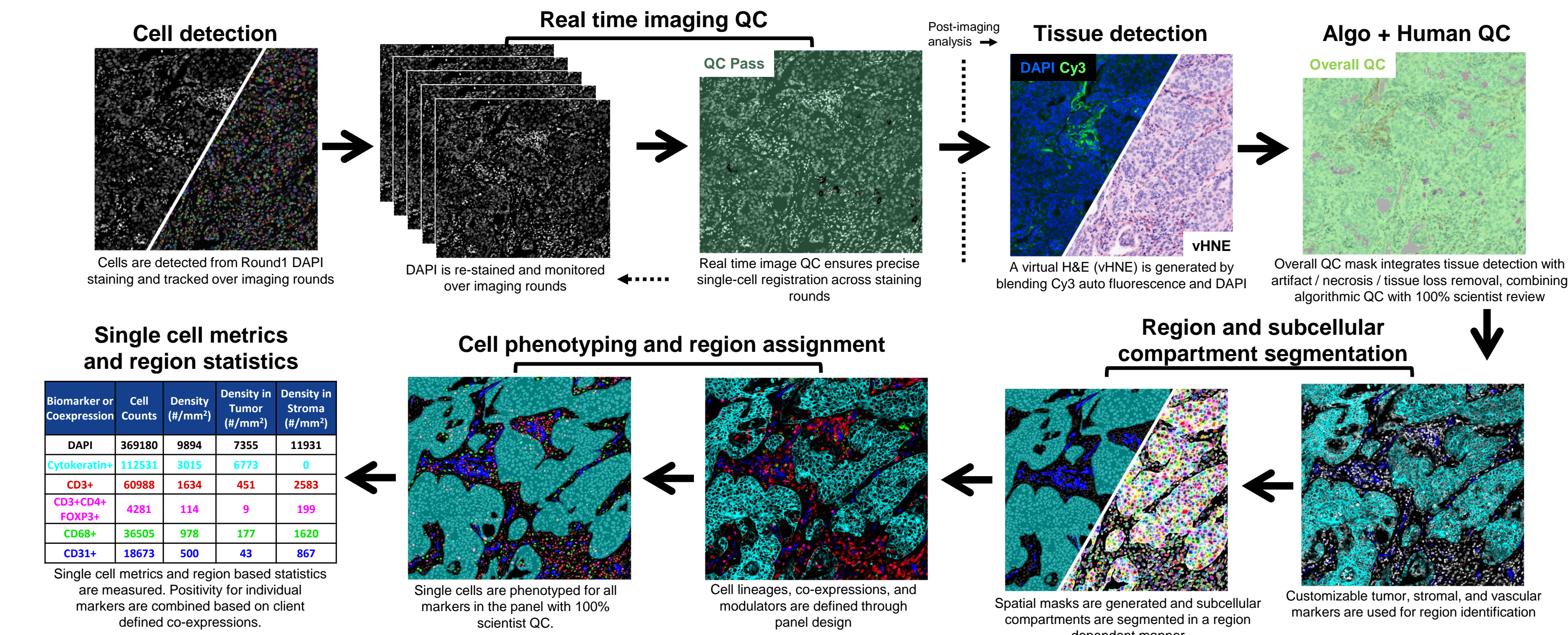
We next transform our mIF images into a single-cell map of the TME by segmenting the tissue into spatial regions and phenotyping on a single cell level. Region segmentation delineates the tissue from surrounding glass, and then further segments the tissue into different regions such as intratumoral, stromal, vasculature, and leading-edge regions. Single cells and subcellular compartments are segmented, which enables accurate intensity quantification over the relevant expression patterns of each marker. Cells are phenotyped using an in-house library of AI algorithms trained on millions of annotated cells – the models integrate morphological and intensity features to accurately distinguish target populations from artifacts and identify phenotypes across diverse tissue types and qualities.

Finally, we demonstrate our comprehensive spatial profiling capabilities on the cohort of 20 NSCLC samples. Cell distributions are quantified through a combination of unsupervised neighborhood clustering, nearest neighbor distances, and tissue region profiling. By mapping critical cell populations—such as tumor-infiltrating lymphocytes, cancer-associated fibroblasts, and other cellular signatures—across distinct tissue regions, we extract salient spatial information that provides biological insight into the TME.

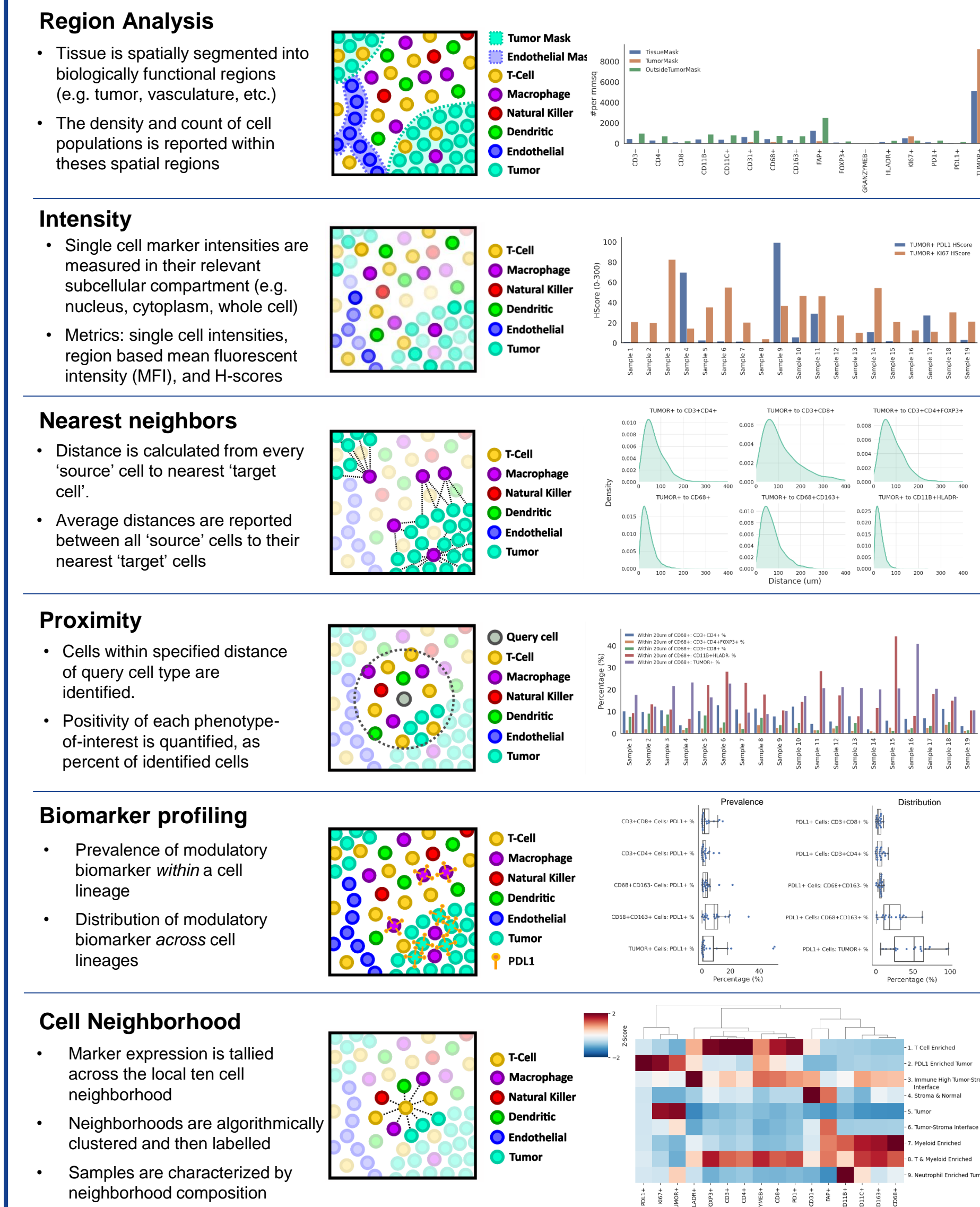
Palettra transforms tumor samples into single cell maps of the TME



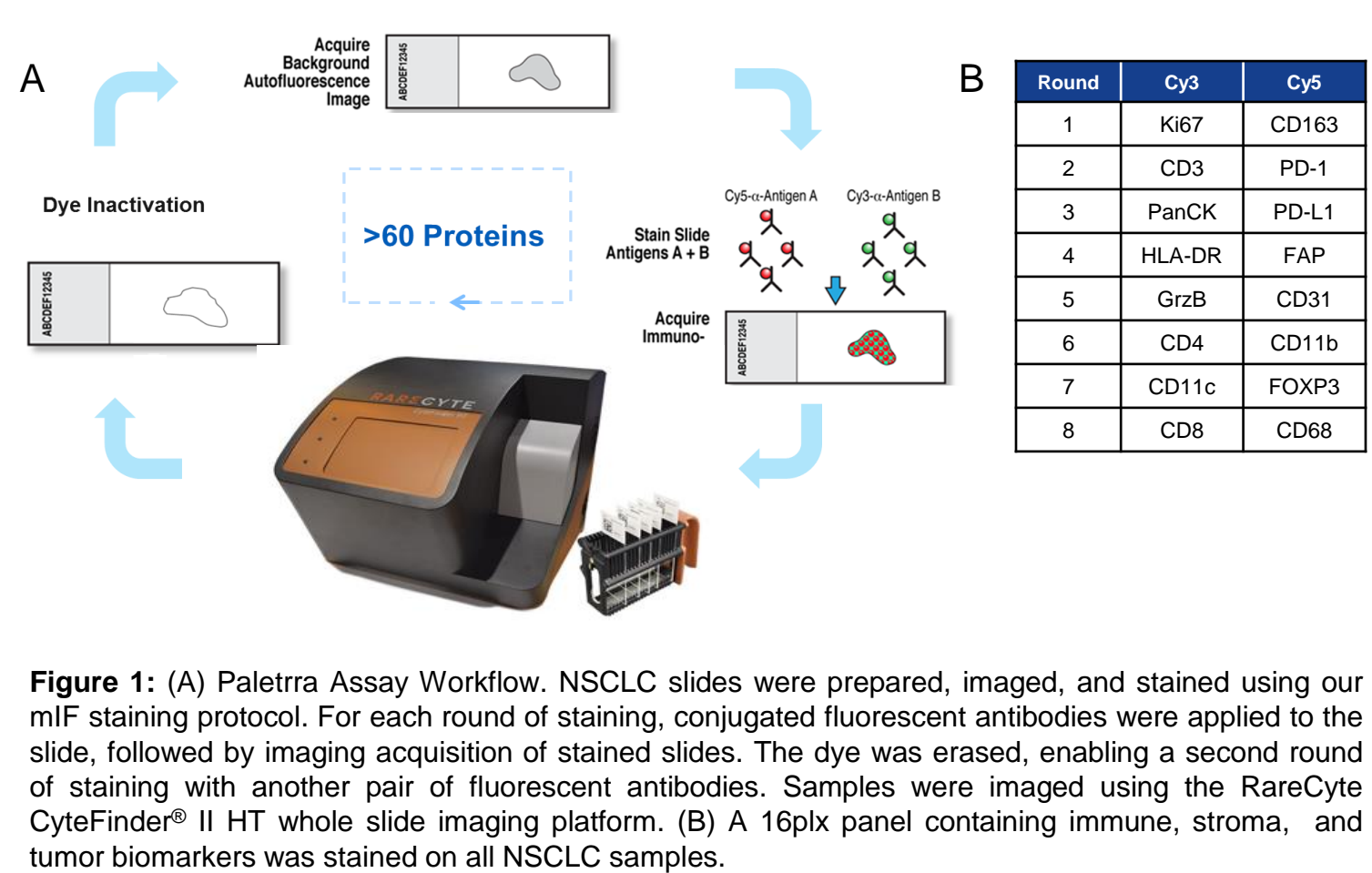
Palettra AI-enabled image analysis



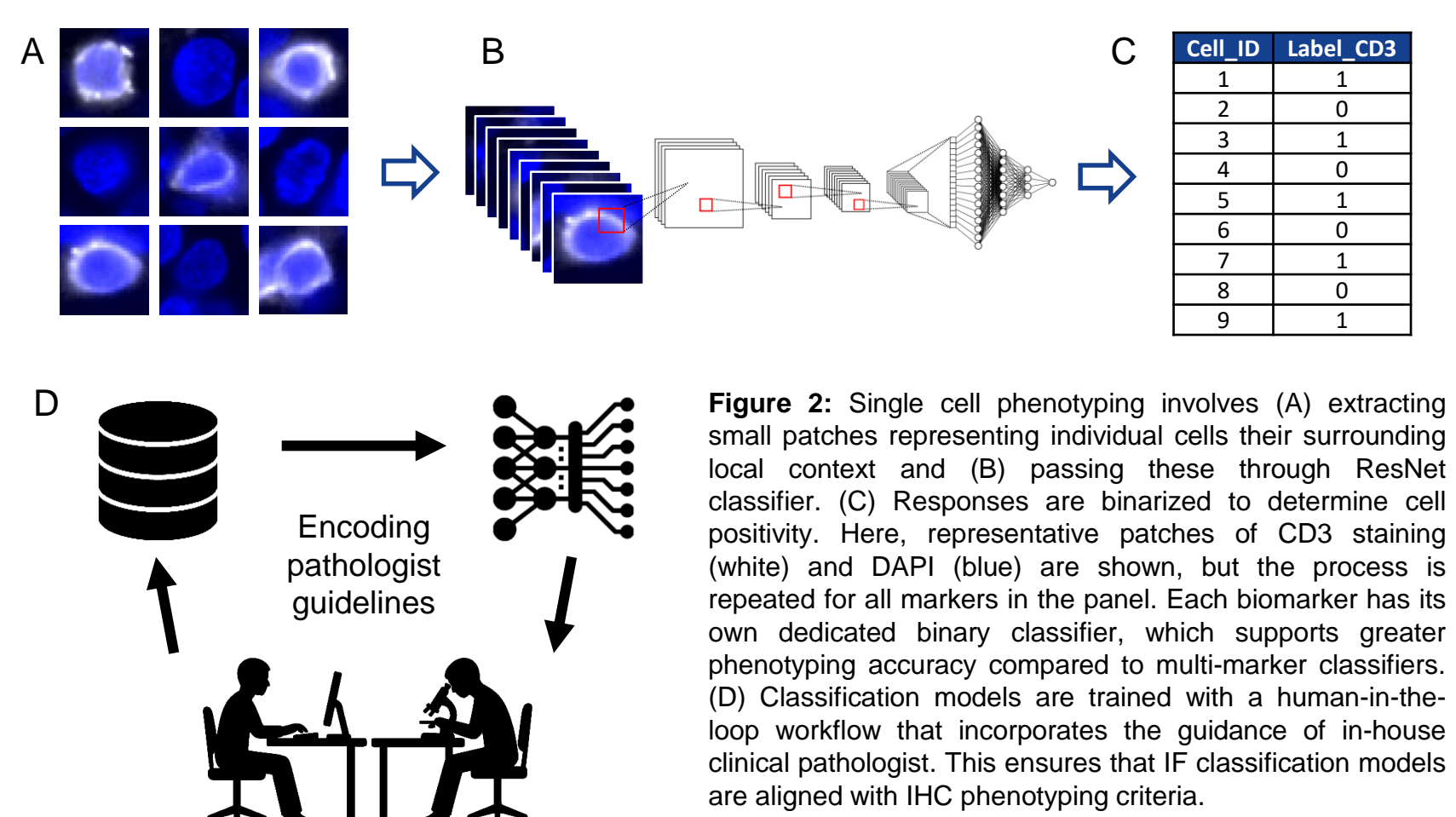
Advanced analytics



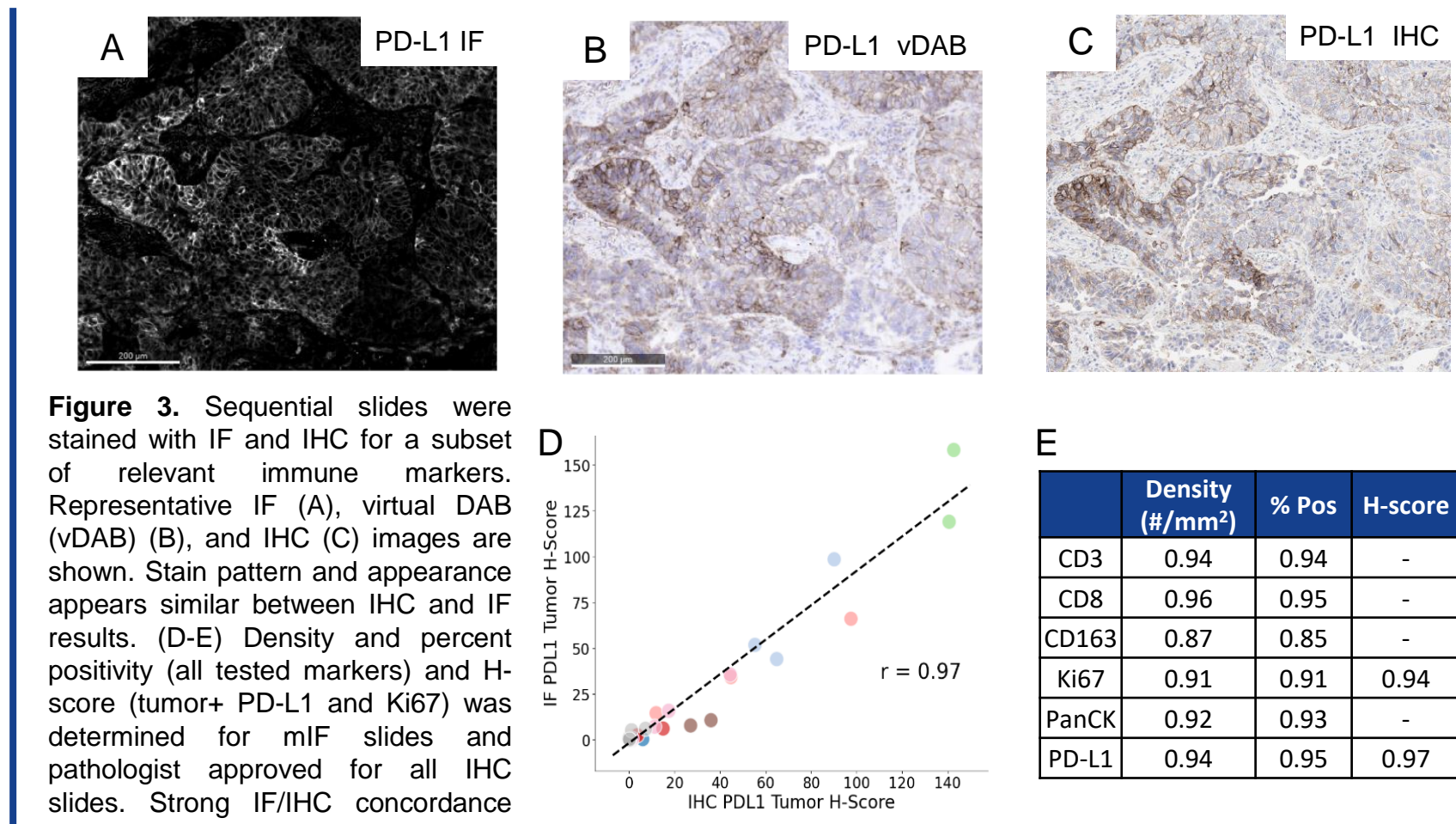
Palettra staining and imaging workflow



Accurate single cell phenotyping



IHC concordant density and intensity outputs



Tissue scale features

