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Phenoplex™ spatial analysis of whole-slide colon adenocarcinoma imaged with Orion™ 13-plex one-round staining and imaging

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Abstract

Background

The ability to image tissue microenvironments (TME) at high-plex over an entire slide without requiring multiple staining steps allows for unprecedented insights into tissue architecture and the molecular mechanisms of immune and disease processes. Here we investigate a whole slide tissue section of high-grade colon adenocarcinoma, a hot tumor that contains prominent clusters of PD-L1 expression scattered throughout, using single-step high-plex staining and imaging at single-cell resolution followed by the analysis of single-cell phenotypes, tissue segmentation and spatial proximity and nearest neighbor analysis.

Methods

A standard pathology slide was stained in one round with a 13-plex immune-oncology panel then a 21.6 by 18.5 mm area was imaged in one round with the Orion™ spatial biology platform (RareCyte). Analysis was performed on the resulting OME.TIFF file using Phenoplex™ (Visiopharm) with the following pipeline: tissue Segmentation into epithelial, necrosis and stromal regions of interest; cell segmentation using the Visiopharm Blueprint APP for DAPI nuclear detection; and phenotyping and spatial biodistribution analysis using the Guided Highplex Workflow.

Results

Data revealed intricate architecture and cellular transitions as tumor cells express varying levels of Pan-CK, with a subset proliferating, and PD-L1 clusters within the lamina propria. Lymphocyte clusters are seen interacting with PD-L1+ macrophages and, at higher zoom level, the proximity can be measured of infiltrating T and B cells near the tumor cells. A variety of spatial distance-metric analyses were performed. As an example, neighbor analysis within the stromal ROI showed that PD-L1+ macrophages had a different neighborhood of immune cells within their vicinity compared to PD-L1- macrophages.

Conclusions

These data highlight the importance of sufficient plex, resolution and whole slide context to derive reliable spatial biomarkers of potential prognostic value. The ability to generate such rich data in just hours across a whole section with multiplexed biomarker quantitation makes this approach suited to multi-patient clinical studies. Deep-learning based image analysis enables deeper insights into the TME of cancer samples and enables researchers to robustly and accurately quantify and classify tissue samples to understand biology of disease.

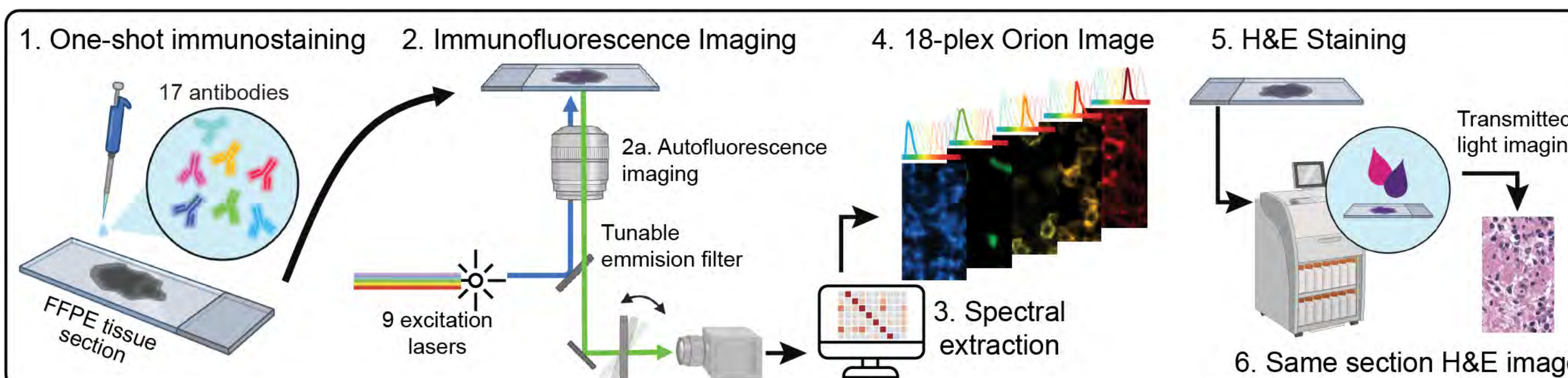

Figure 1 Orion workflow. Orion staining is completed in one-shot, with all antibodies (up to 17) applied simultaneously to whole slide FFPE or fresh frozen tissue. All channels, including two dedicated to autofluorescence, are also collected simultaneously. Individual channels are then spectrally extracted to isolate biomarker staining. After immunofluorescence staining, H&E can be performed on the same section and the brightfield and fluorescence images can be aligned.

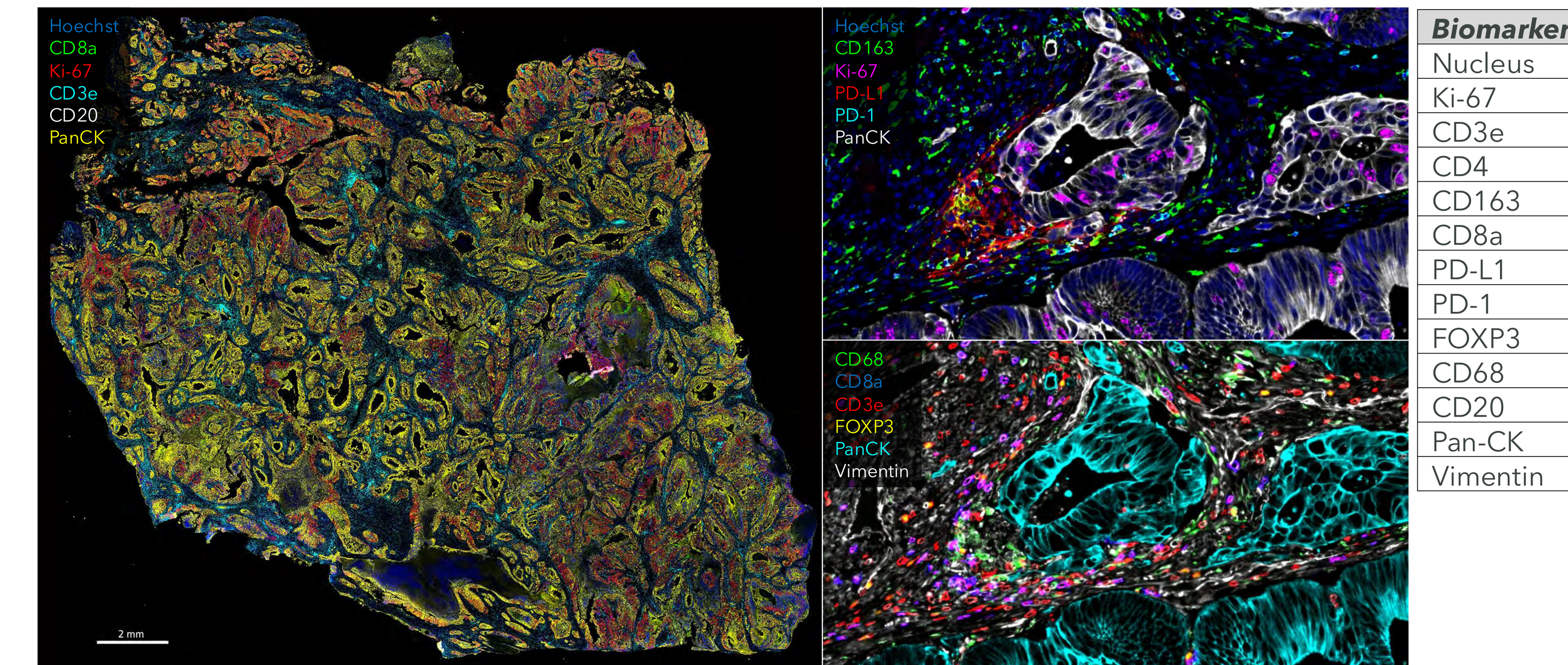

Figure 2 Whole slide imaging. Whole-slide tissue section (left) of an invasive colorectal adenocarcinoma stained with a 13-plex immune-oncology biomarker panel. Magnified image showing the interaction of tumor with adjacent immune cells (middle), including T cells (some of which express PD-1) and macrophages (some of which express PD-L1). Full panel listed on the right.

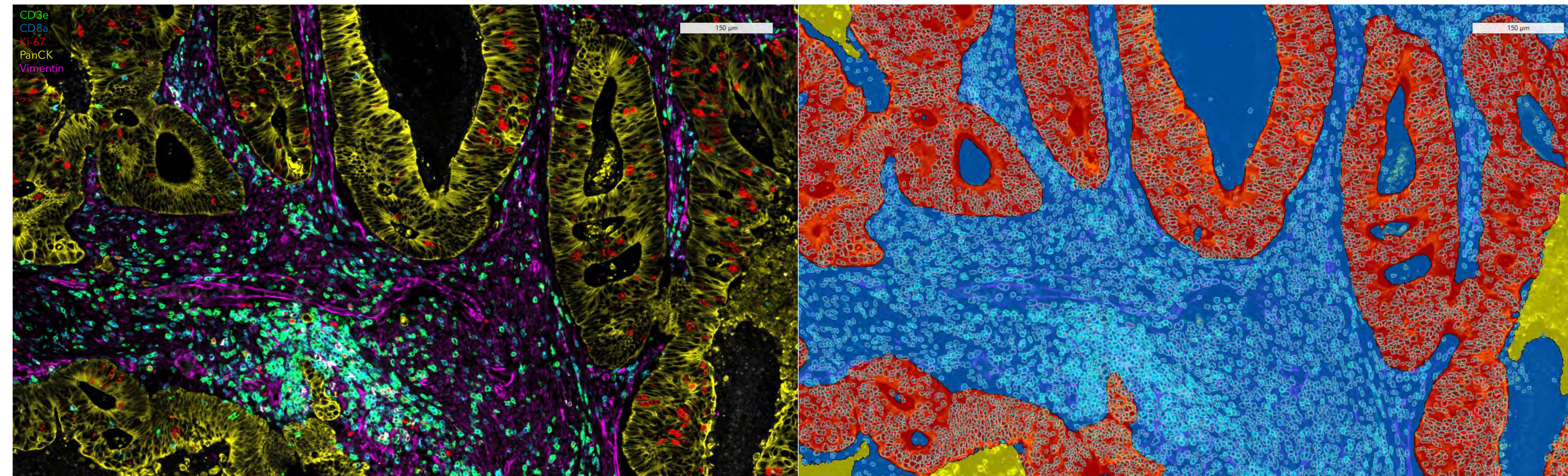

Figure 3 Tumor and lymphocytes. Higher magnification IF image (left) highlights the tumor organization as well as the surrounding stroma, including many lymphocytes (cyan CD3E and green CD8A). Corresponding tissue mapping (right) includes shading to indicate necrosis (yellow), tumor (red) and stroma (blue), in addition to the single cell segmentation mask used to calculate marker intensities. Tissue and cell level masks are then used for downstream compartmental cell density and cell neighborhood analyses.

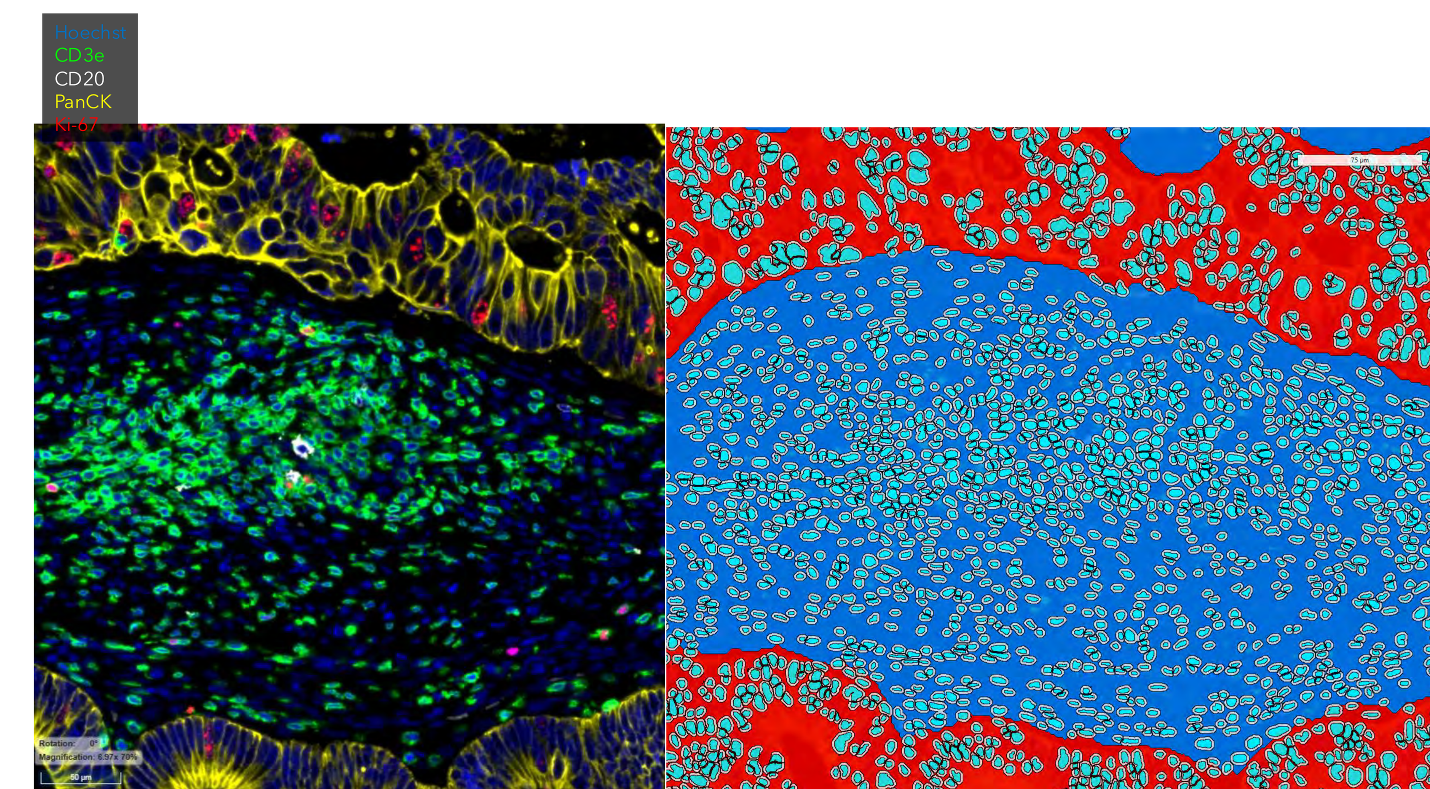

Figure 4 Magnified segmentation. The IF image (left) can be segmented into both tissue compartments and individual cells (right). The Phenoplex mIF DNA pretrained Knowledge APP segments the cells in the stroma and epithelial area (pan-CK, yellow) well.

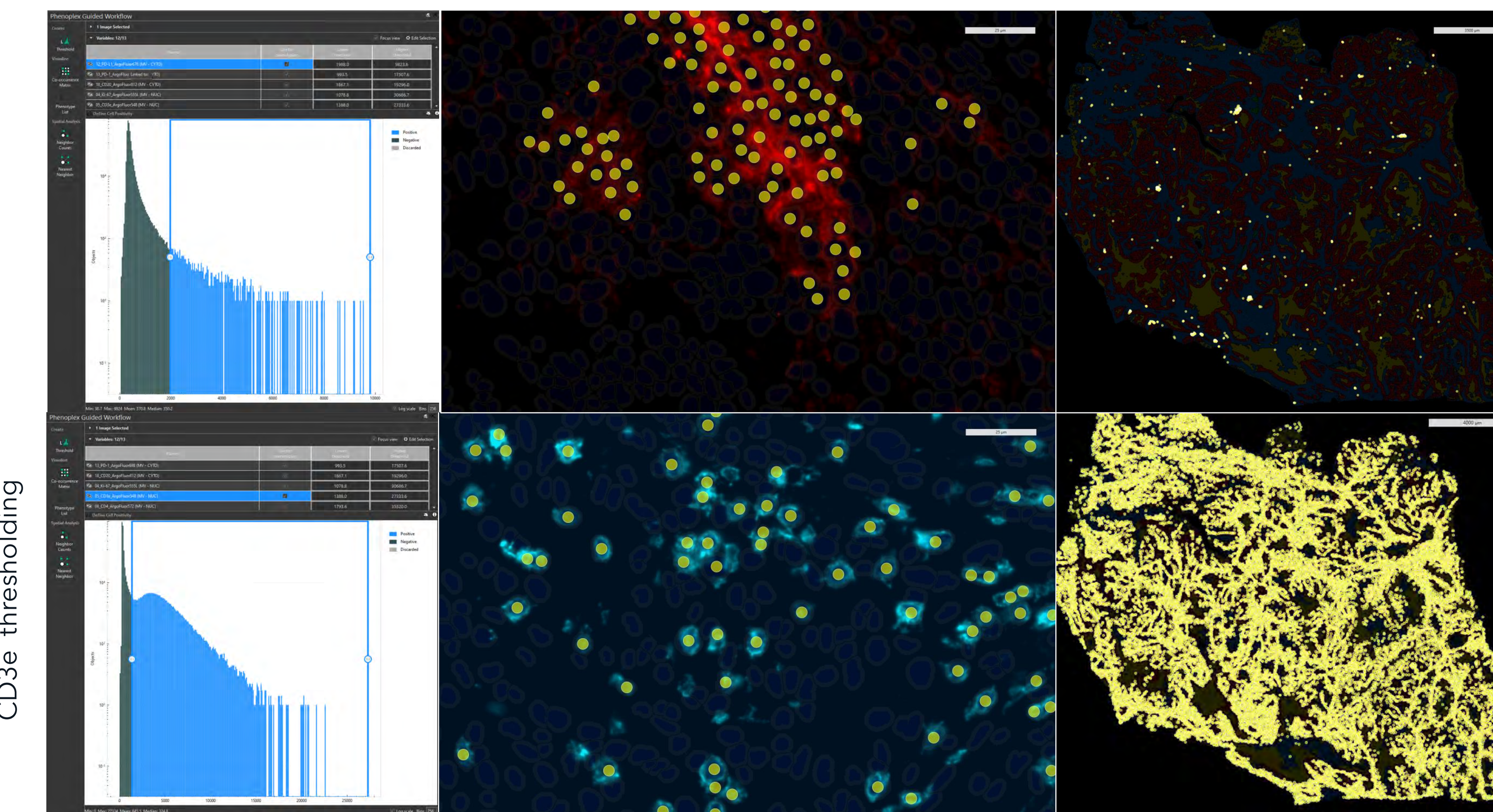

Figure 5 Positivity Thresholding. The Phenoplex Guided Workflow provides fast and intuitive gating for biomarker positivity on the whole slide level and confirmation on higher magnification. Users select the cellular compartment in which to gate, here PD-L1 in the cytoplasm, and CD3e in the membrane compartment. Histograms show the intensity distributions, all blue bin bars are set as positive objects. Yellow overlay dots on cells indicate which cell objects are positive, allowing for a fast QC of results and are updated in real time.

Color scale: 15.21 – 0

Neighbor Counts within 30µm around Target cells		Neighbor												
		CD3+CD8a+	CD3+CD8a+ PD-1+	CD3+CD4+	CD3+CD4+ FOXP3+	CD3+Ki67+	CD3+CD8a+ Ki67+	CD163+PD-L1+	CD68+PD-L!+	CD68+CD163+	CD68+	CD20+	pan-CK+	CD3+PD-1+
Target	CD163+PD-L1+	15.21	2.98	10.68	2.11	1.48	0.89	10.05	3.41	4	5.85	1.56	0.77	7.87
	PD-L1+	11.73	2.25	10.02	2.2	1.16	0.54	6.17	3.49	3.52	6.9	1.41	1.51	6.68
	CD3+CD8a+	3.75	0.34	5.41	1.63	0.4	0.17	0.35	0.15	0.88	1.81	0.17	0.47	2.01
	CD3+CD4+	2.4	0.19	5.52	1.79	0.33	0.1	0.11	0.06	0.72	1.59	0.11	0.47	1.85
	CD3+Ki67+	3.16	0.27	5.85	1.9	0.54	0.19	0.27	0.12	0.97	1.96	0.21	0.78	2.13
	CD8a+Ki67+	4.4	0.41	5.7	1.72	0.63	0.32	0.53	0.18	1.09	2.05	0.27	0.76	2.2
	CD3+CD4+ FOXP3+	2.32	0.16	5.77	2.04	0.35	0.1	0.07	0.04	0.72	1.51	0.1	0.4	1.9
	CD20+	4.87	0.71	7.11	2.1	0.82	0.3	1	0.5	1.4	2.79	1.76	4.5	3.28

Figure 6 Neighbor counts. In the stroma, PD-L1 expressing macrophages are clustering with T cells , M2 macrophages. Target are the center cells for measurements. Maximum radius distance for counting of neighbor was set to 30µm.

Color scale: 16.3 µm – 0 µm

Nearest neighbor matrix in µm (maximum radius for measurements 30µm)		Neigbor												
		CD3+CD8a+	CD3+CD8a+ PD-1+	CD3+CD4+	CD3+CD4+ FOXP3+	CD3+Ki67+	CD3+CD8a+ Ki67+	CD163+PD-L1+	CD68+PD-L1+	CD68+CD163+	CD68+	CD20+	pan-CK+	CD3+PD-1+
Target	CD163+PD-L1+	3.1	8.2	3.1	11.2	11.4	12.5	2.5	6.9	6.3	5	10.7	14.7	4.9
	PD-L1+	3.8	9	3.6	10.5	11.4	11.7	4.9	6.2	6	2.8	10	12.7	5
	CD3+CD8a+	7.5	12.7	6.9	12.1	14.1	13.8	9.9	12.3	13.3	11.6	14.3	15.5	11.3
	CD3+CD4+	10.8	14.2	5.6	10.9	14.6	15.7	11.7	12.7	13.4	11.7	14.9	15.5	10.8
	CD3+Ki67+	9.1	13.1	5.7	10.7	8.8	10.7	11.9	13.2	12.8	11	14.1	14.3	10.4
	CD8a+Ki67+	6.3	11.8	6.7	11.9	9	7.7	9.2	10.6	12.7	10.9	13	14.5	11.1
	CD3+CD4+ FOXP3+	11	14.8	5.5	9.3	14.3	15.7	12.8	13.4	13.5	11.9	14.9	16.3	11.1
	CD20+	7.8	11.8	5.6	10.9	12.9	14	8.4	8.5	11.6	9.9	3.7	15.8	9.6

Figure 7 Nearest neighbor distances. In the stroma, PD-L1 expressing macrophages are close to other macrophages, CD3+CD8+ and CD3+CD4+ cells, and further away from proliferative T cells and Treg cells. Target are the center cells for measurements. Maximum distance for measurements was set to 30µm.

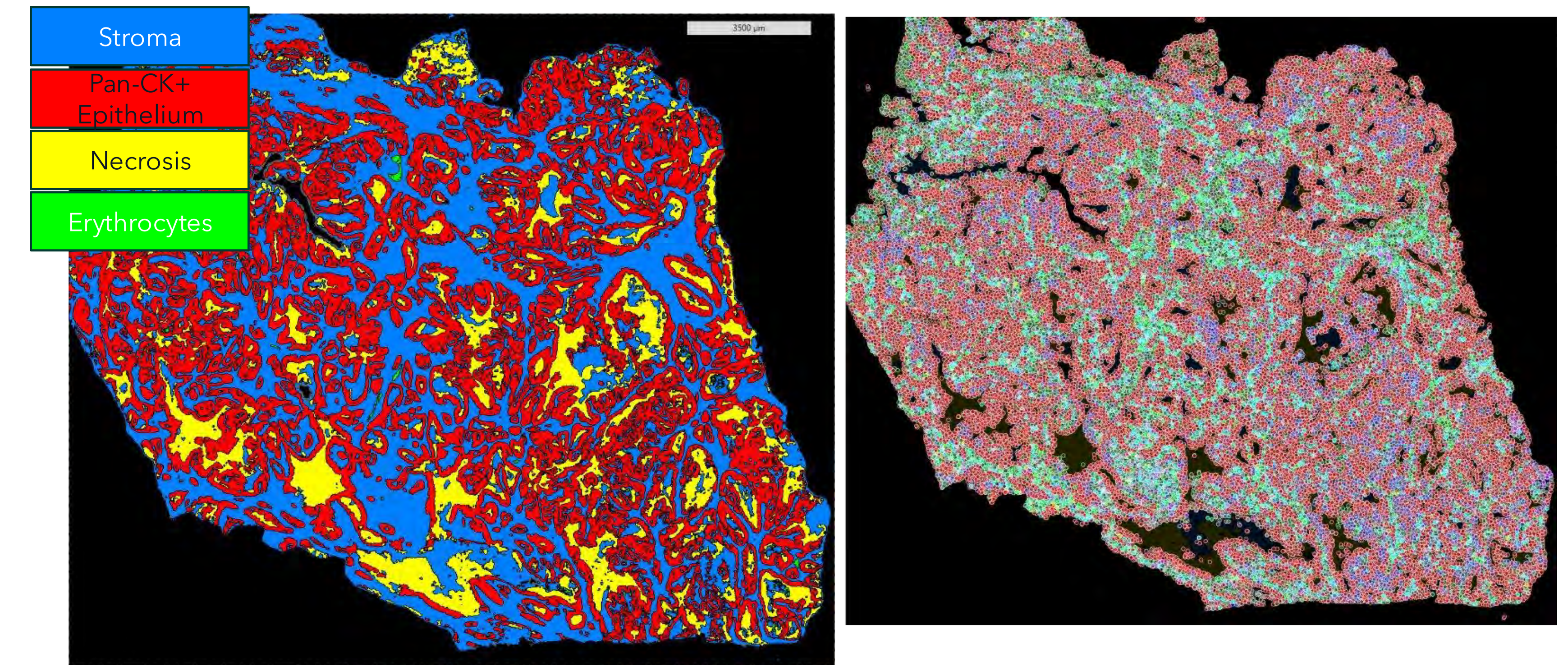

Figure 8 Tissue Mapping and phenotyping. Whole tissue rendering (left), using a trained AI network to segment tissue into different stroma, epithelium, necrosis and erythocytes. Phenotype review (right) showed localization of pan-CK+, panCK+Ki67+ cells mainly within the epithelial region and the T cells, CD3+CD4+, CD3+CD8+, and macrophages found in the stromal areas. (note: overlays of phenotypes are shown subsampled to allow visualization at 1x overview).

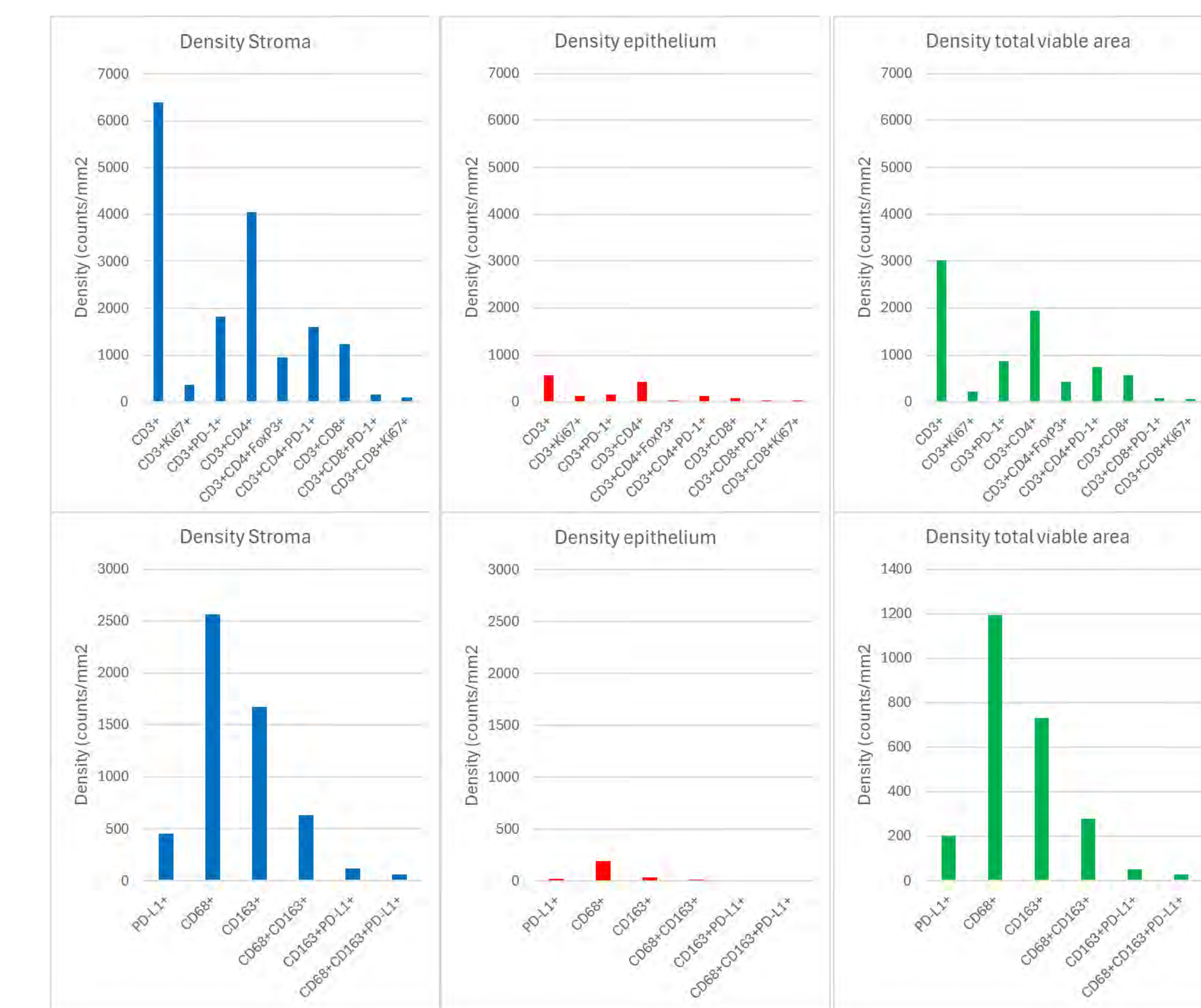

Figure 9 Cell densities. Density plots for T cell phenotypes and macrophage phenotypes in the stromal, epithelial and total viable areas. Data show that this case has many T cells in the stroma but few are infiltrating the epithelial area.

Summary

- RareCyte Orion allows for fast whole slide, whole resection tissue imaging of samples
- Phenoplex can display and analyze millions of cells from Orion images
- Phenoplex offers a flexible and powerful image analysis tool box that provides intuitive phenotyping of large data sets and allows for easy setup of spatial calculations

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