Characterizing the tumor microenvironment of a colorectal adenocarcinoma sample with the Orion spatial biology platform and HALO quantitative image analysis

RARECYTE

Anne Hellebust[1], Amber Ortiz[1], Tad George[2], Edward Lo[2]
[1]Indica Labs, Inc., Albuquerque, NM [2]RareCyte Inc., Seattle, WA

indica labs

INTRODUCTION

Understanding the tumor microenvironment (TME) can help drive targeted treatments. High-grade colorectal adenocarcinoma (CRC) is poorly differentiated and spreads more quickly than others. Thus, with poor patient survival prognostic markers can help improve treatment. Multiplex immunofluorescence and digital pathology can provide a spatial understanding of cellular relationships within the TME. With the ability to not only identify but also quantify many biomarkers, this can provide insight into the interactions between tumorigenic and inflammatory cells. Advances in Artificial intelligence (AI) will allow for separation of tumor and stroma and thus identify these biological interactions between cells and immunoprofiling of the tumor or TME itself. Here we identify the inflammatory state of the tumor biopsy and explore the potential for targeted biomarker treatments such as the role of PD-L1/anti-PD1 therapy for CRC.

METHODS

Tissue Preparation & Orion™ Imaging

A formalin fixed paraffin embedded (FFPE) colorectal tissue section was stained using a multiplex immunofluorescence (IF) panel in one staining round, followed by whole-slide imaging with the Orion™ instrument (shown below). All the markers were captured in one imaging round, along with tissue autofluorescence, which was isolated as an additional channel to provide additional tissue morphology information. The panel consisted of Hoechst/nucleus, autofluorescence, Ki-67, CD3e, CD4, CD163, CD8a, PD-L1, PD-1, FOXP3, CD68, CD20, Pan-CK , and Vimentin. The digital slide confirmed the tissue was a high-grade colon adenocarcinoma hot tumor with prominent clusters of PD-L1 expression. All biomarkers were present and show expected staining patterns.

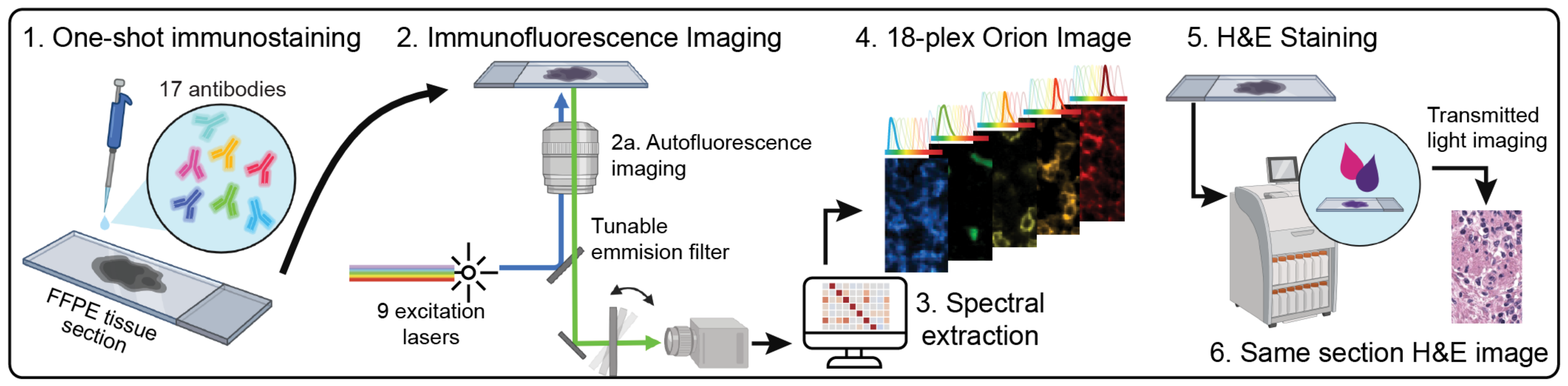


Figure 1: Immunostaining and imaging workflow.

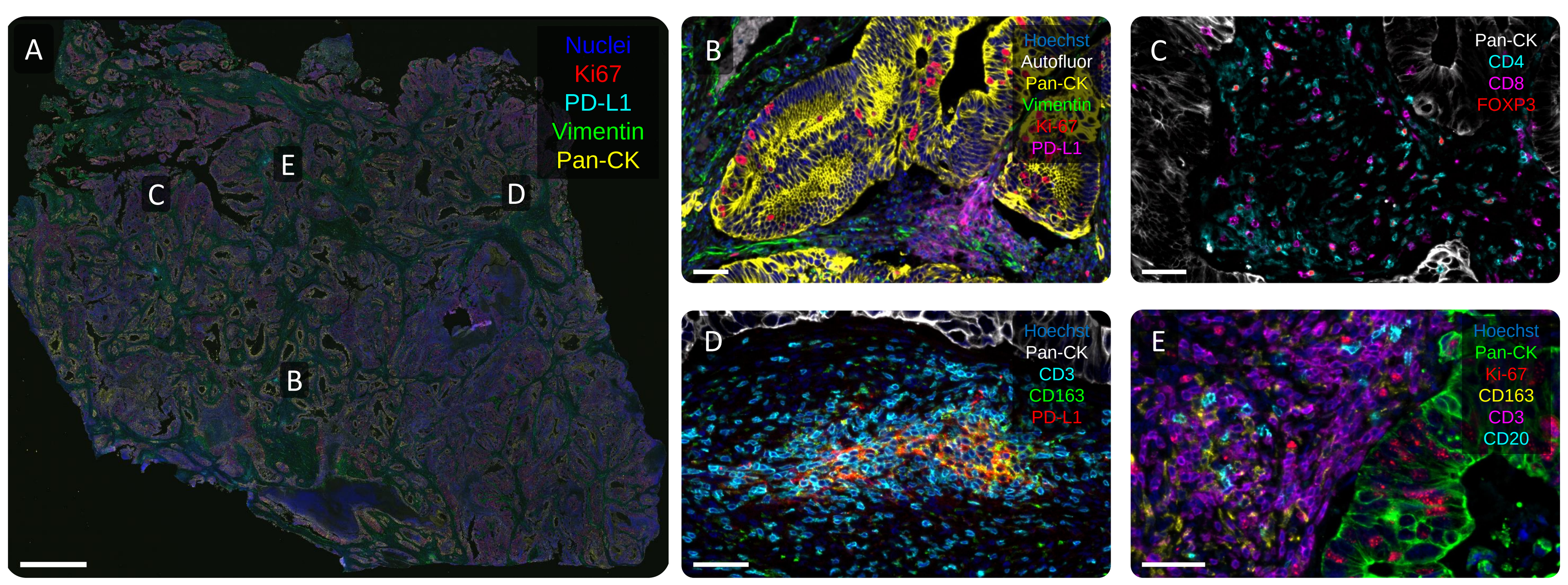
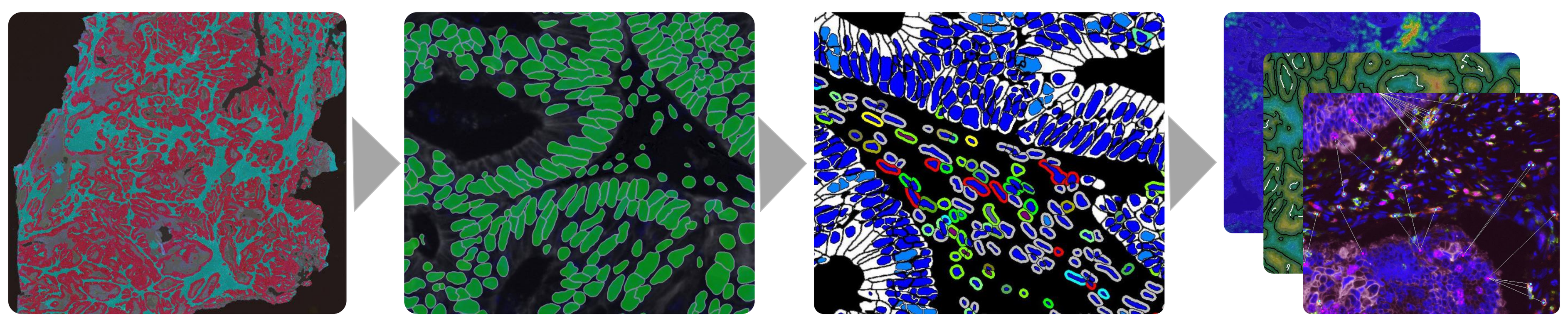


Figure 2: Whole section of colon adenocarcinoma (A). Scale bar is 2mm. Four regions of interest are shown to highlight staining patterns. A subset of channels are shown per panel for visibility. (B) PD-L1 cluster within the lamina propria. Vimentin identifies the stroma and cells within the lamina propria, including endothelial cells. (C) T-cell subsets including cytotoxic T-cells, T-helper cells, and T-regs are identified. (D) T-cells interacting with a cluster of PD-L1+ macrophages. (E) Leukocyte cluster containing B cells near proliferating tumor cells. Scale bars are 50µm for (B-E).

HALO Image Analysis Workflow

The resulting digital slide was analyzed using the HALO® image analysis platform with the Highplex FL analysis module from Indica Labs to better understand the TME. HALO AI networks were leveraged to segment tumor and stromal tissue compartments as well as utilized to improve nuclear and membrane segmentation across the whole slide image (WSI). Once tissue and cells were segmented cell phenotyping, proximity analysis, and density heat mapping was performed.



AI pattern-based tissue classification of the tumor, stroma, necrosis, and glass compartments

Custom HALO AI network for nuclear segmentation and pre-trained HALO AI membrane segmentation

Biomarker quantification of individual cells in the tumor and stroma regions

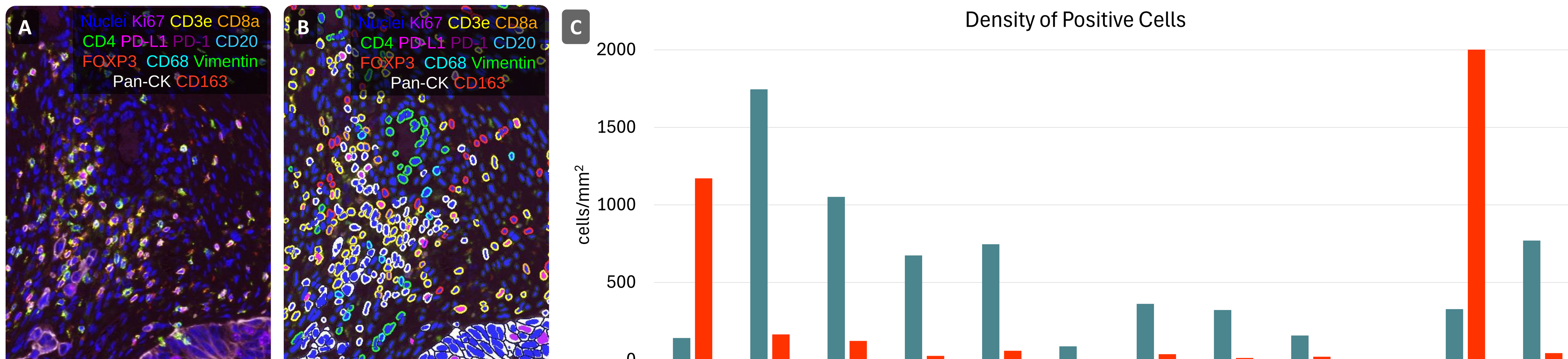
Assorted spatial analysis completed after segmentation and protein quantification steps

Figure 3: HALO image analysis pipeline.

RESULTS

HALO® Image Analysis using HALO AI

Nearly 2 million cells were quantified on this high-grade colon adenocarcinoma tumor specimen. A HALO AI DenseNet tissue classifier successfully segmented tumor, stroma, necrosis, and glass areas across the WSI. Allowing cell segmentation analysis to be restricted to areas of viable tissue. A custom AI nuclear segmentation network was trained to accurately segment nuclei stained with Hoechst. The pre-trained HALO AI membrane segmentation network was employed to segment cell boundaries based off the pan-CK staining pattern. These networks ran embedded in the Highplex FL HALO analysis module to quantify biomarker expression and positivity based off defined thresholds.

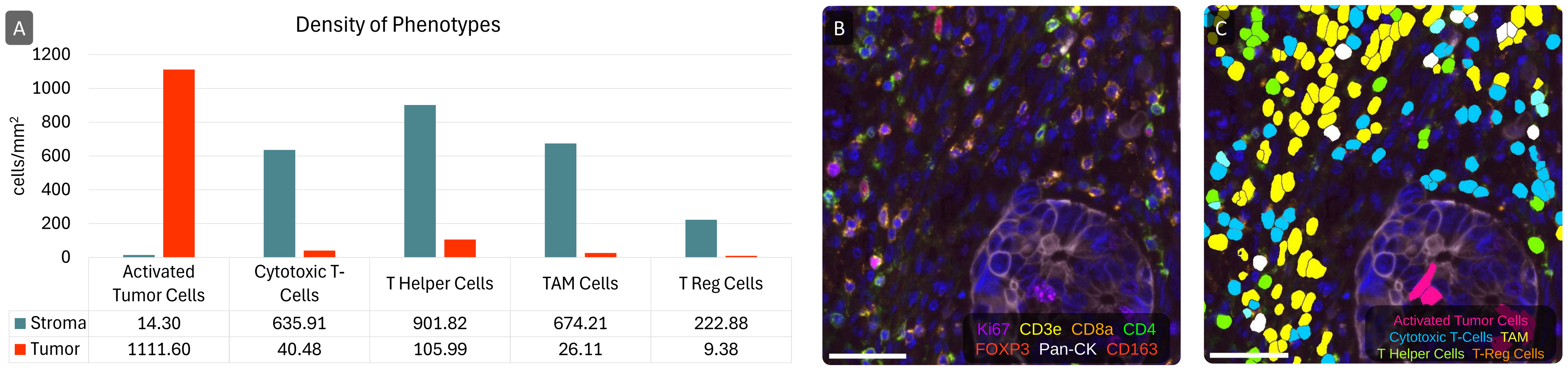


	Ki67	CD3e	CD4	CD163	CD8a	PD-L1	PD-1	FOXP3	CD68	CD20	Pan-CK	Vimentin
Stroma	141.39	1745.58	1051.78	674.21	746.42	87.60	362.12	322.15	157.63	0.38	328.15	769.93
Tumor	1171.57	164.94	122.81	26.11	59.20	4.50	37.13	12.87	21.07	0.22	9626.82	44.32

Figure 4: Quantification of protein biomarkers using the Highplex FL module of HALO with AI nuclear and membrane segmentation. Example input (A) and markup (B) of a region of interest from the CRC tissue sample with many of the 13 channels present. Scale bars are 100µm. Expression of these biomarkers was quantified and stratified across tumor and stroma tissue regions identified as in Figure 1A (C).

Defined Cell Phenotyping

Phenotypes of interest were specified based off protein biomarker expression positivity. Defined phenotypes for Activated Tumor Cells (Ki67⁺Pan-CK⁺), Cytotoxic T-Cells (CD8a⁺CD3e⁺), T Helper Cells (CD4⁺CD3e⁺), Tumor Associated Macrophages (CD163⁺), and T-Reg Cells (CD4⁺CD3e⁺FOXP3⁺) were used for this analysis. Defining these phenotypes enables not only tissue immunophenotyping, but also a more streamlined spatial analysis in the next step.



	Activated Tumor Cells	Cytotoxic T-Cells	T Helper Cells	TAM Cells	T Reg Cells
Stroma	14.30	635.91	901.82	674.21	222.88
Tumor	1111.60	40.48	105.99	26.11	9.38

Figure 5: Expression of cell phenotypes was quantified across the CRC tissue and further stratified across tumor and stroma tissue regions previously identified using HALO AI. The density of these phenotypes in each tissue compartment is shown in panel A. Example input (B) and analysis markup (C) of a region of interest from the CRC tissue sample, displaying the five defined phenotypes. Scale bars are 50µm.

Spatial Analysis

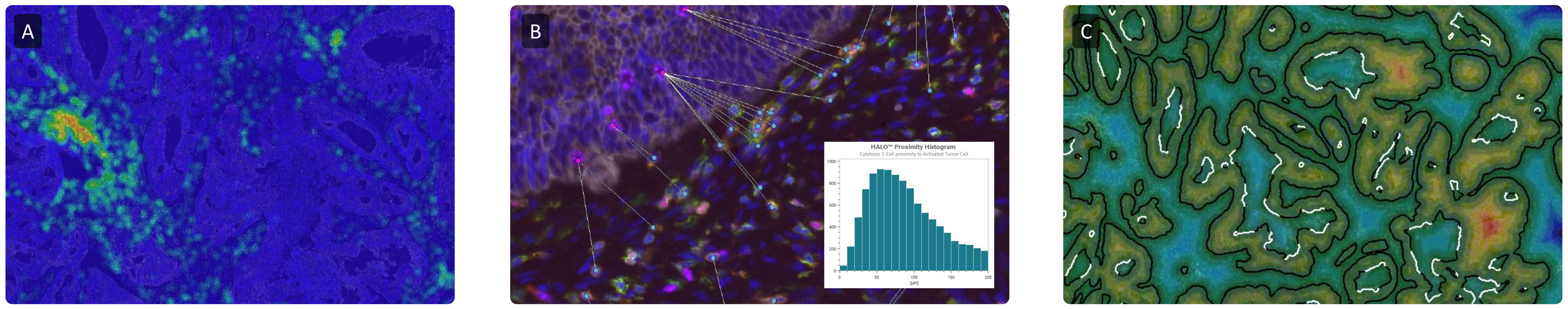


Figure 6: HALO supports four different types of spatial analysis. Three of those types are depicted in this panel. Density heatmap example showing cytotoxic t-cell distribution across an area of the CRC tissue (A) making it easy to quickly identify areas of increased expression across the tissue. Proximity analysis quantifies the spatial relationship between two distinct cell populations. The relationship between cytotoxic T-cells were an average distance of 99µm from activated tumor cells (B). Infiltration analysis measures the distance of a distinct cell population to a boundary (C). In this example, the distance of cytotoxic T-cells to the tumor boundary (generated by HALO AI) is shown.

CONCLUSIONS

A combined workflow using the Orion platform for multiplex IF staining and imaging with HALO quantitative image analysis delivers a comprehensive solution for exploring the immune landscape of the TME.

info@indicalab.com