Quantitative analysis of colorectal adenocarcinoma images obtained by one step 15-plex staining followed by imaging with the Orion™ spatial biology platform

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Keywords

Colon cancer, immune profile, CRC, TME, tumor microenvironment, quantitative spatial imaging, multiplex biomarker quantitation, high resolution imaging, whole slide quantitation, single cell quantitation, sub-cellular quantitation

Background

To understand the tumor microenvironment (TME), high resolution imaging of multiple biomarkers with whole slide context can be used as a basis for downstream biomarker quantitation and predicting patient outcomes[1]. Here we investigate a sample of invasive colorectal adenocarcinoma using whole slide, single-step high-plex staining and imaging at single-cell resolution followed by quantitative analysis.

Methods

Tissue staining and scanning protocol

- Mount sections on glass slides
- De-paraffinize and perform antigen retrieval
- Quench autofluorescence
- Stain slides with panel of ArgoFluor™ conjugated antibodies
- Coverslip with ArgoFluor Mounting Medium and cure overnight
- Image whole slides at 20X magnification using Orion instrument
- Process to ome.TIFF and analyze
- De-coverslip in aqueous solution
- Perform H&E staining and scanning on same section

Quantitative analysis

- Manually annotate regions of interest (ROI) on the tissue
- Segment whole slide images into cells with UnMICST and S3segmenter
- Import mask and clean data in QuPath
- Generate primary feature data table with QuPath
- Classify cells into populations in QuPath
- Quantify spatial biomarkers
- Compare cell populations and their spatial interactions in the different ROIs

Results

Data revealed a distinction between normal colonic epithelium, well-differentiated adenocarcinoma with immune cell collection, and an infiltrating border of the carcinoma. Differences in immune cell content and spatial organization were measured, and the infiltrating border found to contrast with other tumor regions by showing differences in E-cadherin and cytokeratin expression patterns, as well as an increased presence of all immune cells and an elevated presence of blood vessels. Additionally, cells within the superficial tumor were measured to have an elevated proliferative fraction (Ki-67, nuclear) compared to other regions within the tissue. These data highlight the importance of sufficient plex, resolution and whole slide context to derive reliable spatial biomarkers of potential prognostic value.

Further, hours vs. days speed for whole-section multiplexed biomarker quantitation, along with same-section conventional chromogenic analysis, makes this approach suited to multi-patient clinical studies.

[1] JR Lin et al., High-plex immunofluorescence imaging and traditional histology of the same tissue section for discovering image-based biomarker. *Nat Cancer*, 4, 1036-1052 (2023).

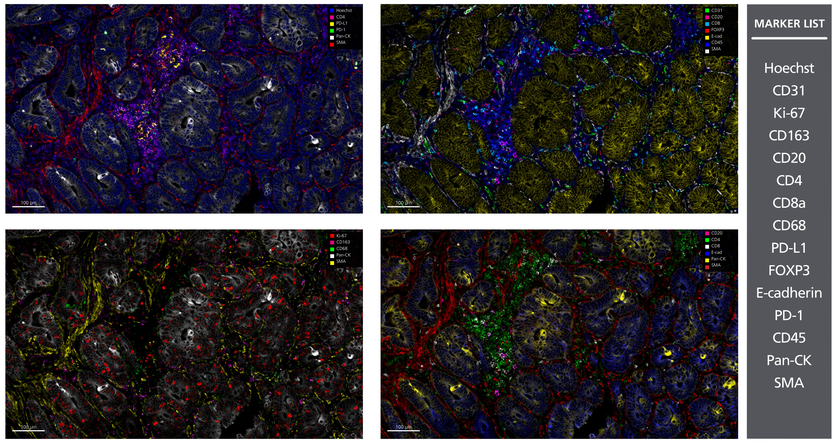


Fig 2. Region of the tissue showing expression of all biomarkers. T-cells, B-cells and macrophages are largely present between tumor clusters, with limited immune cells infiltrating into the clusters.

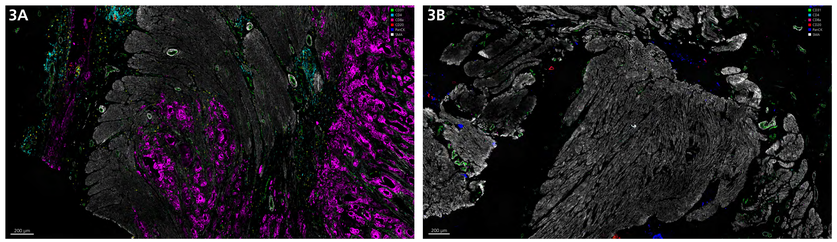


Fig 3. T- and B- lymphocytes, macrophages and endothelial cells are all found in and around the region where tumor has infiltrated into the submucosa, inner circular muscle layer and outer longitudinal muscle layer (A) while minimal immune and endothelial cell presence observed in the normal smooth muscle (B).

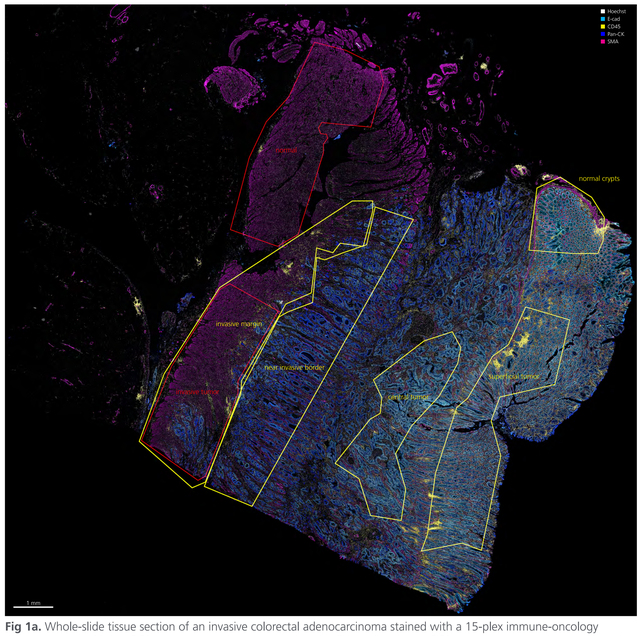


Fig 1a. Whole-slide tissue section of an invasive colorectal adenocarcinoma stained with a 15-plex immune-oncology biomarker panel. Yellow ROIs correspond to analyzed regions for Figure 4 while red ROIs correspond to regions analyzed for Figure 5.

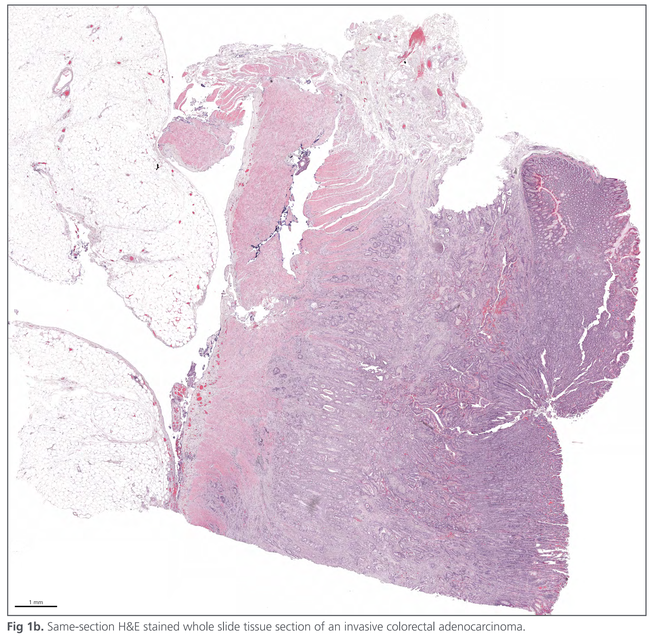
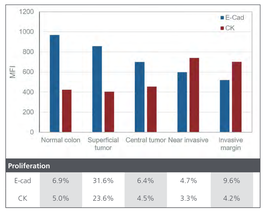


Fig 1b. Same-section H&E stained whole slide tissue section of an invasive colorectal adenocarcinoma.



Proliferation					
E-cad	6.9%	31.6%	6.4%	4.7%	9.6%
CK	5.0%	23.6%	4.5%	3.3%	4.2%

Fig 4. Quantification of MFI for E-cad (blue) and Pan-CK (red) among all cells that were positive for E-cad or Pan-CK. A reduction in E-cad MFI was measured from normal colon to the invasive margin, while CK was increased, matching the results observed in Figure 4. Inset table: Proliferation was highest in the superficial tumor, and consistently elevated in the E-cad positive cells compared to the CK positive cells.

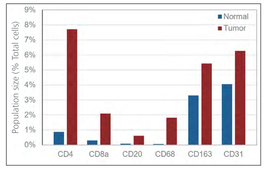


Fig 5. Proportion of T- and B-lymphocytes, macrophages and endothelial cells were elevated in the tumor region compared to the healthy muscle.

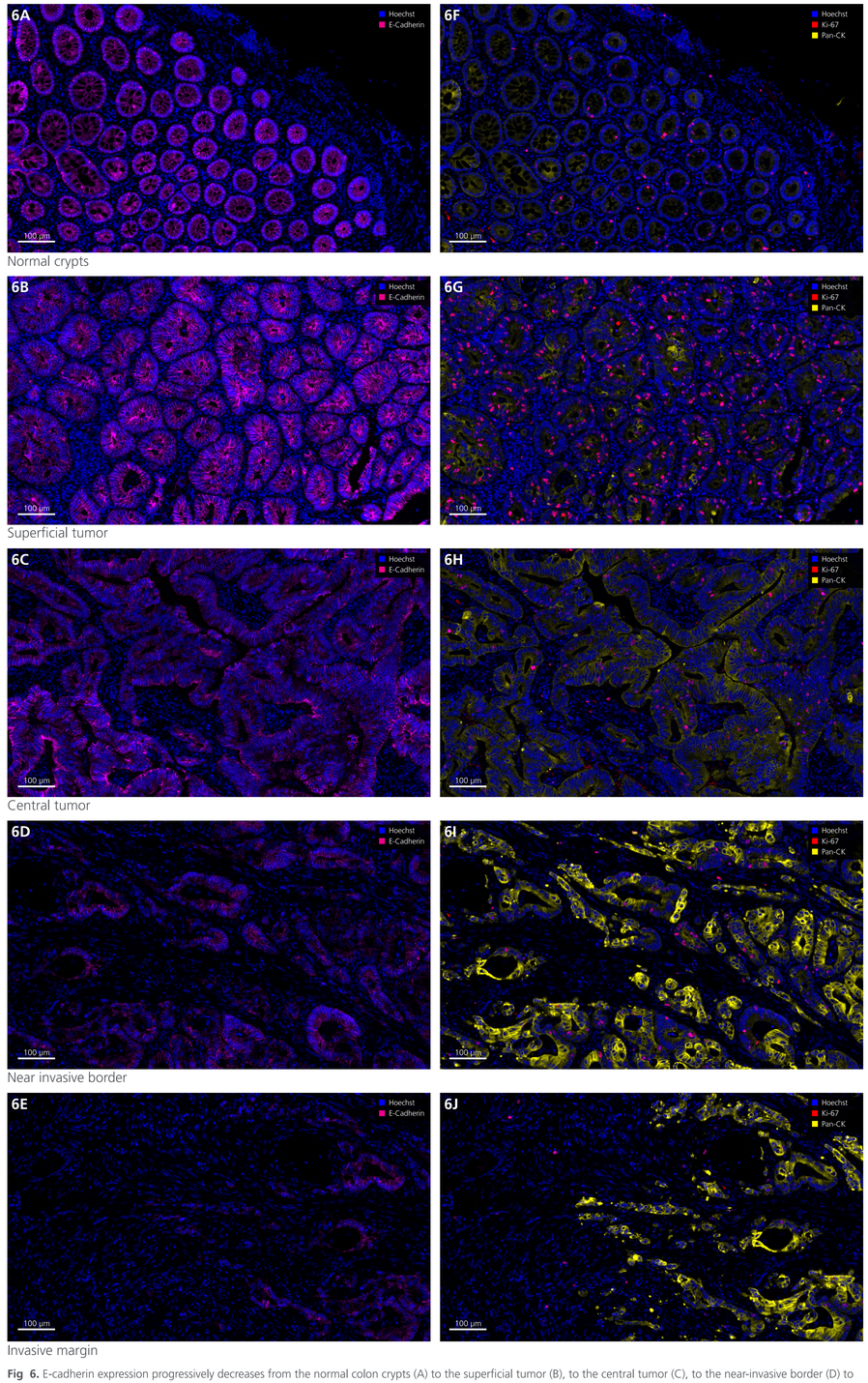


Fig 6. E-cadherin expression progressively decreases from the normal colon crypts (A) to the superficial tumor (B), to the central tumor (C), to the near-invasive border (D) to the invasive margin (E), while Pan-CK expression shows the opposite trend (F-J). Proliferation is elevated in the superficial tumor (G) compared to all other regions, as indicated by the increased Ki-67 (red). Representative images of different expression levels taken from each ROI shown in Fig 1.