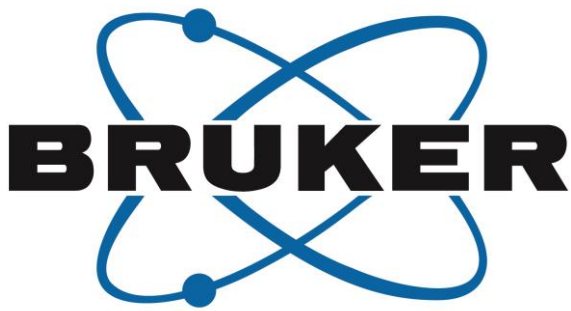


A Multiplex Immunofluorescence Panel Reproduces Routine Colorectal Adenocarcinoma Antibody Stains with Spatial Context on a Single Section on the CellScape® Precise Spatial Proteomics Platform



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Introduction

Advances in colorectal adenocarcinoma research are rapidly expanding the demand for immunohistochemistry test utilization to aid in diagnosis, prognosis and identification of therapeutic targets for treatment interventions. Each IHC requires the cutting of additional sections and personnel time associated with staining procedures. Multiplex immunofluorescence has recently emerged as a compelling evolution in the traditional workflow that uses a single tissue section and maintains true spatial relationships between cell populations that may be lost when reviewing serial sections. In this poster, we present a study on the Bruker Spatial Biology CellScape® platform where 34 markers associated with the development of colorectal adenocarcinoma were stained on a single slide containing 24 paired tumor/normal cases and a normal placenta positive control. After visual inspection, cells were segmented, classified and phenotyped. Cells were analyzed for clinically relevant expression patterns such as mismatch repair status, up- or down-regulation, immune cell infiltration and presence of tertiary lymphoid structures. Although not a clinical validation, this study demonstrates the power of multiplex immunofluorescence to replicate a clinical immunohistochemistry workflow using minimal tissue and a fully automated staining/imaging workflow that can power the evolution of spatial signature-based studies in the anatomic pathology laboratory.

Methods

A panel of 35 markers representing a broad range of functional categories in the diagnostic, prognostic and therapeutic journey for colorectal adenocarcinoma. Where available, antibodies with direct fluorescent conjugates were selected from commercial vendors. For 3 antibodies, a direct conjugate clone does not exist so unconjugated primary antibodies were labeled with Bruker Spatial Biology VistaFlex™ Antibody Labeling Kits using fluorophores indicated in the panel design figure. A commercial colorectal adenocarcinoma tissue microarray (Biochain T8235790D) was sectioned onto a charged slide and subjected to standard deparaffinization and rehydration followed by antigen retrieval with a Tris-EDTA pH 9.0 buffer (BioLegend 422704) in a TintoRetriever pressure cooker (Bio SB 7087). A Bruker Spatial Biology Whole Slide Imaging Chamber (WSIC) was adhered to the slide and placed on the CellScape® instrument for staining. Antibodies diluted in CellScape® Storage Buffer were then cycled on the CellScape® instrument with 60-minute room temperature incubations followed by a wash cycle and fluorescent imaging using the 10X objective. At the end of each cycle, the fluorescent signal was removed through a photochemical bleaching step with EpicIF buffer. This gentle treatment does not affect epitope stability or tissue integrity and allows for high-plex staining without loss of staining efficiency. Images were exported as standard OME.TIFF files that were imported into QuPath software for initial analysis. Additional python scripts were developed for deeper data investigation.

Multiplex Immunofluorescence Process

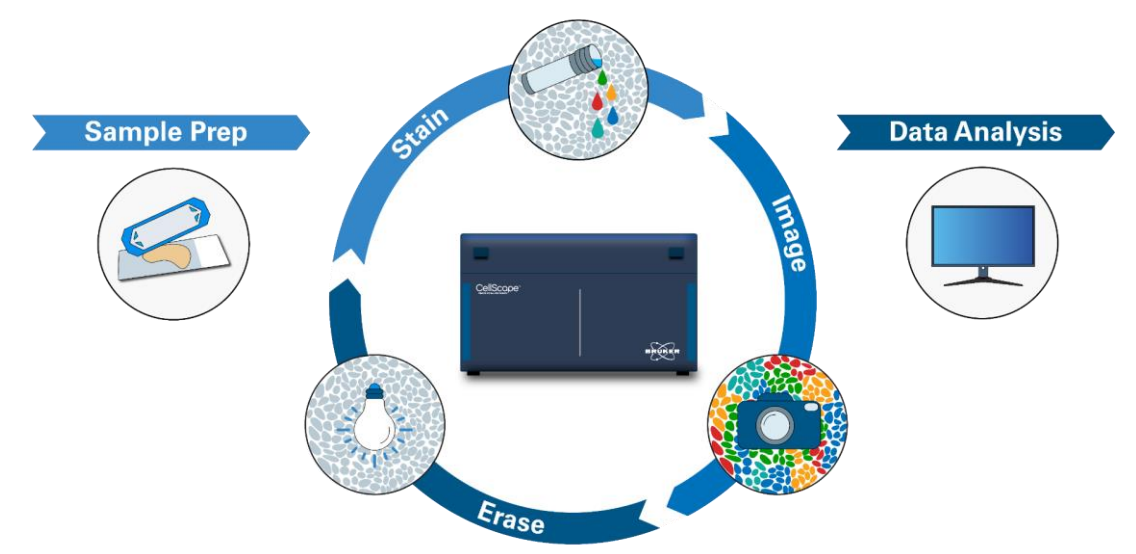


Figure 1. The CellScape®/EpicIF workflow. Cycles of staining, imaging, and gentle signal removal enable spatial biomarker detection at single-cell resolution.

Panel Composition

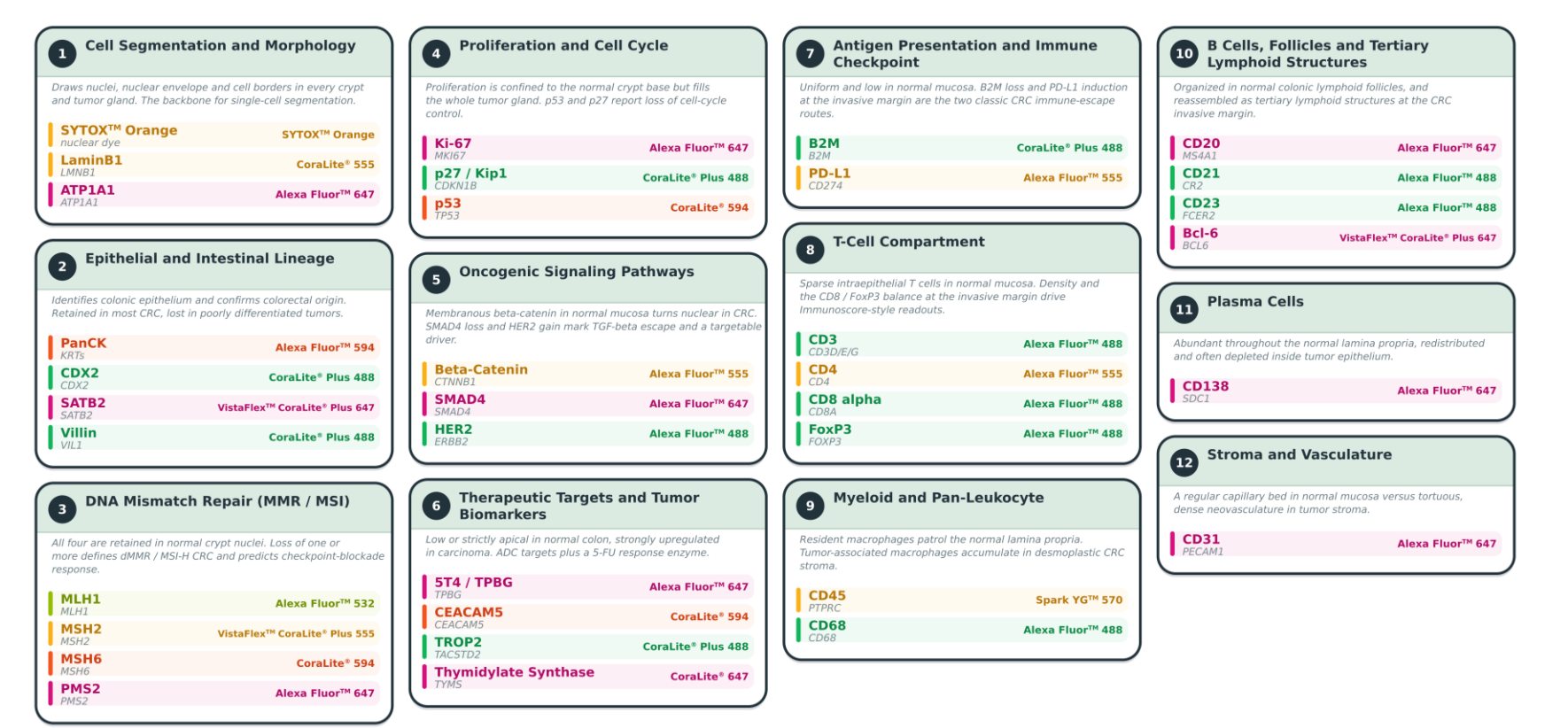


Figure 2. Panel Composition. The above panel lists all markers, fluorophores and nuclear stain used in the development of this colorectal adenocarcinoma panel.

Sample Reinterrogation

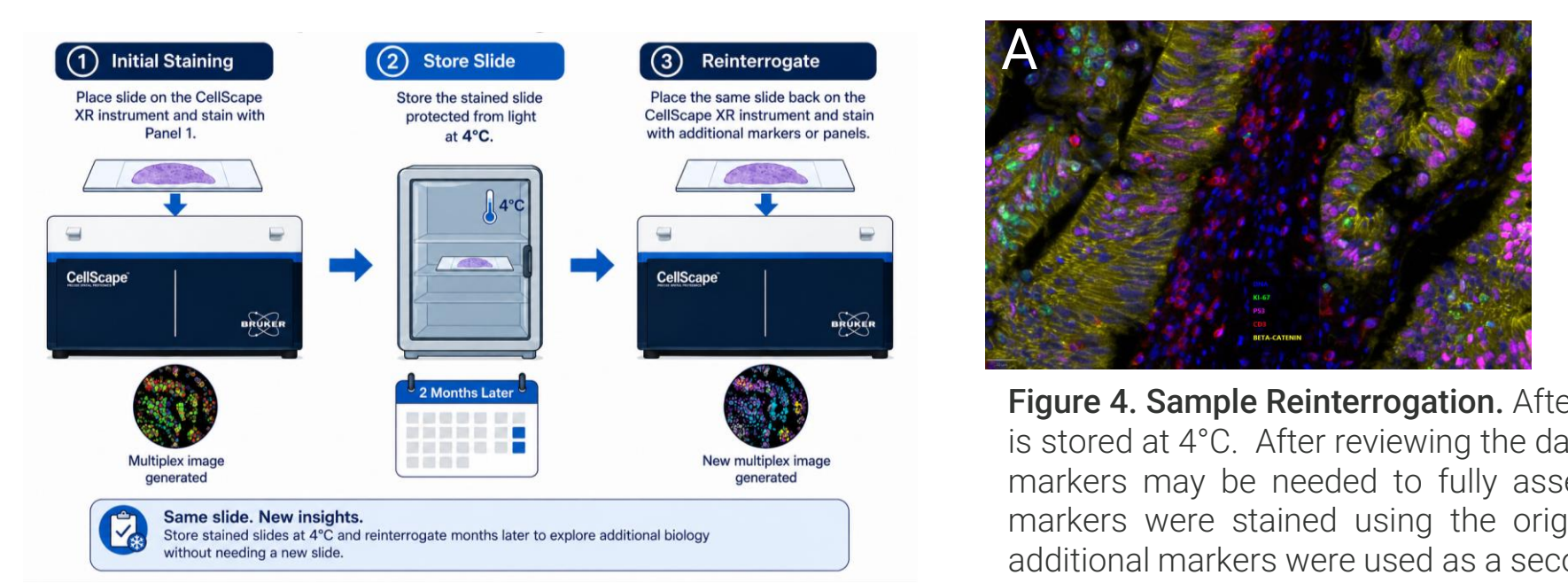
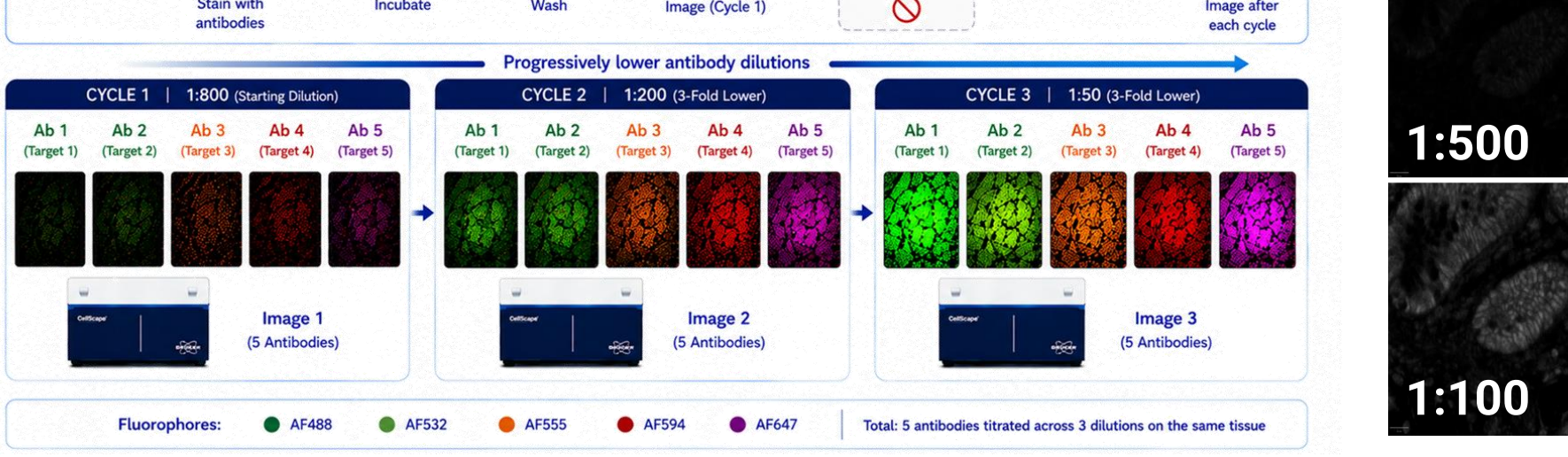


Figure 4. Sample Reinterrogation. After an experiment is completed, the slide is stored at 4°C. After reviewing the data or in light of new findings, additional markers may be needed to fully assess the sample. In this example, 28 markers were stained using the original panel (A). Two months later, 7 additional markers were used as a secondary panel to stain this TMA (B).

Single Slide Antibody Titration



Advanced Analysis

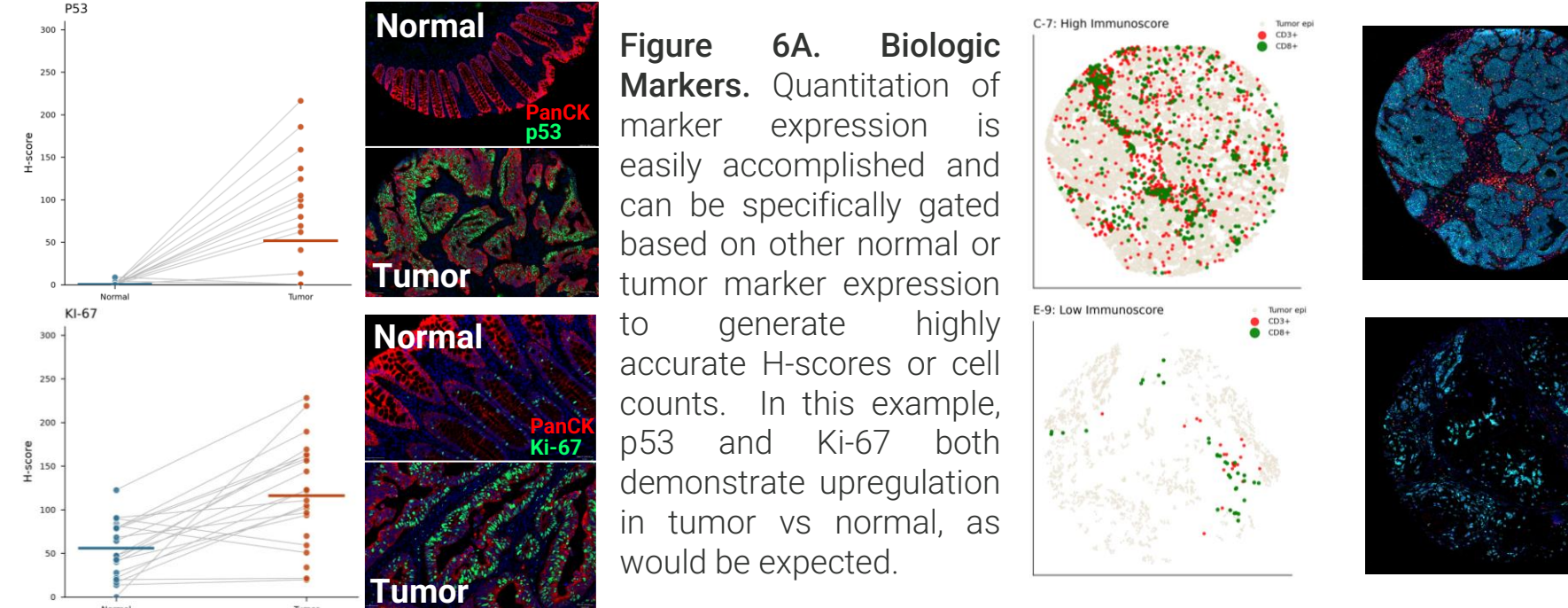


Figure 6A. Biologic Markers. Quantitation of marker expression is easily accomplished and can be specifically gated based on other normal or tumor marker expression to generate highly accurate H-scores or cell counts. In this example, p53 and Ki-67 both demonstrate upregulation in tumor vs normal, as would be expected.

Mismatch Repair Status

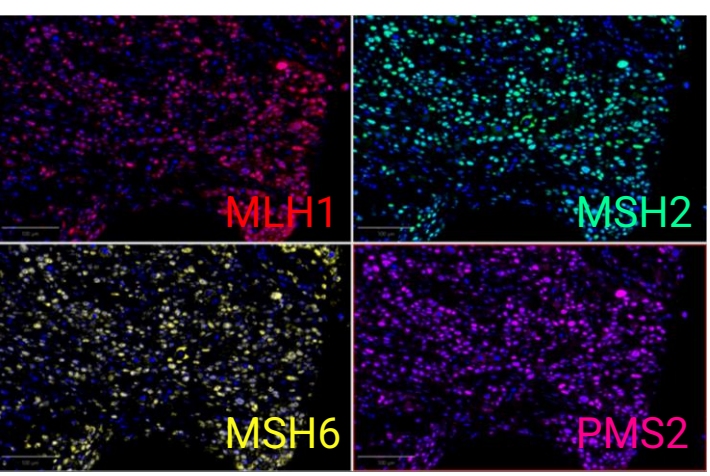


Figure 3. Mismatch Repair Status. Only 8/22 could be reliably assessed for MMR status due to poor PMS2 and MSH2 staining when comparing tumor epithelium to stromal/immune compartment staining which serves as an internal control. Of the 8 cores interpretable, none showed loss of MMR function. Representative staining is shown in the figure above.

Example Staining

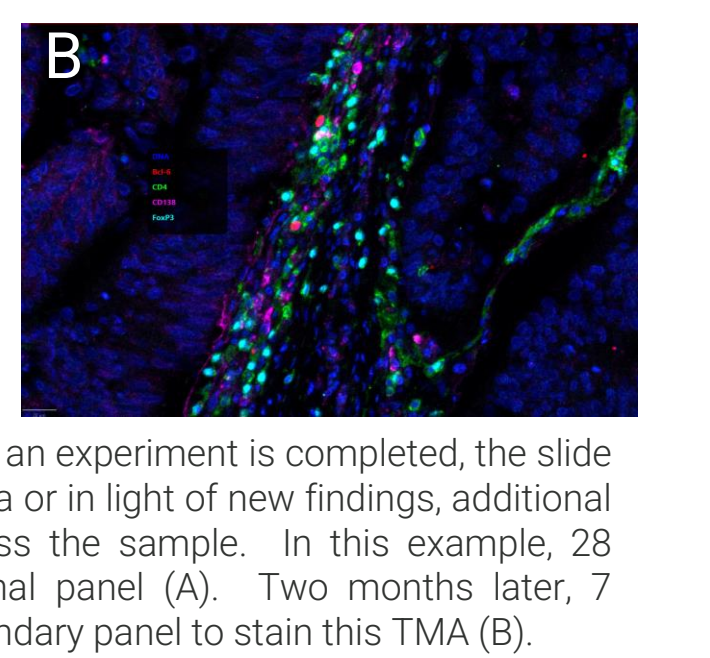


Figure 7. Example Staining. Individual fluorescent channels are shown in greyscale in panel A. An overview of the entire TMA is shown in B. Multimarker images are shown in C (normal colon) and D (adenocarcinoma). Note the bright B and T-cell staining observed in the normal mucosa-associated lymphoid tissue (C).

Conclusion

- 35 markers used routinely in the diagnosis, prognosis and therapeutic characterization of colorectal adenocarcinoma were successfully used to stain a single TMA section
- Advanced analyses were able to demonstrate quantitative and spatially resolved marker changes affecting patient management and prognosis
- The CellScape® Precise Spatial Proteomics Platform is uniquely positioned to meet the walkaway automation and imaging resolution requirements of clinical research workflows

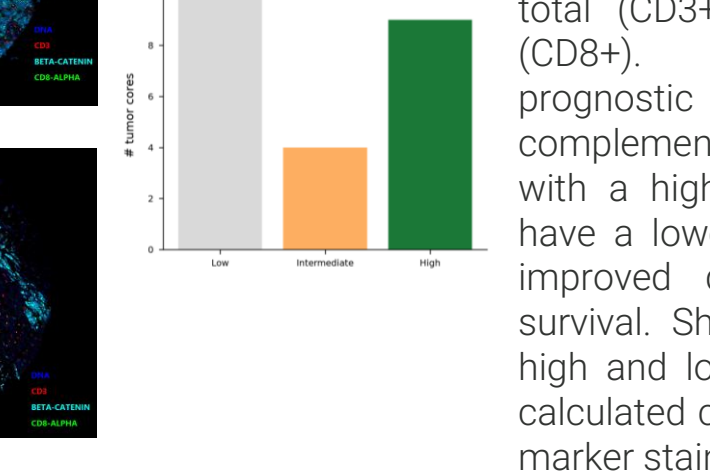


Figure 6B. Immunoscore. The Immunoscore is a validated immune biomarker score that measures the density of tumor infiltrating T cells in total (CD3+) and cytotoxic T cells (CD8+). This score provides prognostic information that complements TNM staging. Patients with a high Immunoscore generally have a lower risk of recurrence and improved disease-free and overall survival. Shown are examples of both high and low scores represented by calculated cell phenotypes and actual marker staining shown adjacent.