

**ORION™: 17-PLEX SINGLE-STEP STAIN AND IMAGING OF COLORECTAL ADENOCARCINOMA**Selena Larkin\*, Eric Kaldjian, Tad George. *RarCyte, Inc., Seattle, WA, USA*

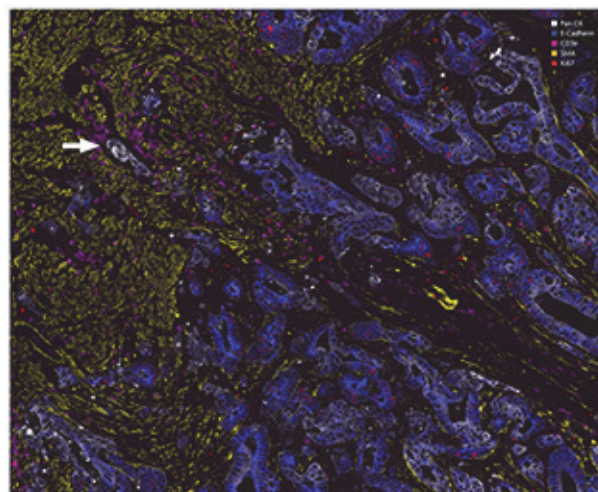
**Background** In order to understand the subcellular nature of the tumor microenvironment (TME), high quality imaging at single-cell resolution is needed as a basis for downstream biomarker quantitation and predicting patient outcomes. Here we investigate a sample of invasive colorectal adenocarcinoma using whole slide, single-step stain and imaging at single-cell resolution.

**Methods** These images profiled a whole slide tissue section of an invasive colorectal adenocarcinoma, stained with a 17-plex immuno-oncology biomarker panel. In this profile, we designed a high-plex panel of 17 biomarkers where the tissue autofluorescence was imaged and isolated as an additional fluorescence channel. Whole slide spatial staining and imaging was conducted on the Orion spatial biology platform, and H&E staining was performed after immunofluorescence (IF) imaging on the same section and imaged by brightfield microscopy. The full protocol is fairly quick and simple, using standard histology tools:

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

**Results** Data revealed a distinction between normal colonic epithelium, well-differentiated adenocarcinoma with immune cell collection, and an infiltrating border of the carcinoma. Multiplexed imaging uncovered the invasive border of the tumor, where the infiltrating border contrasts with other tumor regions by showing a lower proliferative fraction (Ki-67, nuclear), and the staining of E-cadherin is reduced/presence of cytokeratin's is more pronounced (figure 1). This is consistent with a down-regulation of E-cadherin in the invasive cells that is part of epithelial to mesenchymal transition, leading to more aggressive tumor behavior.

**Conclusions** There is a need to show biology of the TME in a high quality, subcellular fashion as it provides a more in-depth understanding into such biology and thus, can lead to a greater spatial investigation for human disease. Here multiplexed imaging identified distinct immune cell collections and revealed the invasive border of the tumor, confirming the biological differences in tissue composition and cellular interactions between these tissues and provides crucial information for future spatial quantitation.



**Abstract 101 Figure 1** Multiplexed imaging reveals the invasive border of the tumor where clusters of malignant cells (white arrow) appear to have broken off from the glandular structures.

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