

# Simultaneous, high-throughput detection of RNA and protein biomarkers using the Orion™ imaging system and HCR™ Gold for comprehensive analysis of tumor samples in clinical trials

Jon Ladd<sup>1</sup>, Adam Maddox<sup>2</sup>, Nathan Schurman<sup>1</sup>, Daniel Tanoehusada<sup>1</sup>, Aneesh Acharya<sup>2</sup>, Arturo B Ramirez<sup>1</sup>

<sup>1</sup>RareCyte, Inc. Seattle, WA <sup>2</sup>Molecular Instruments, Inc. Los Angeles, CA

## BACKGROUND

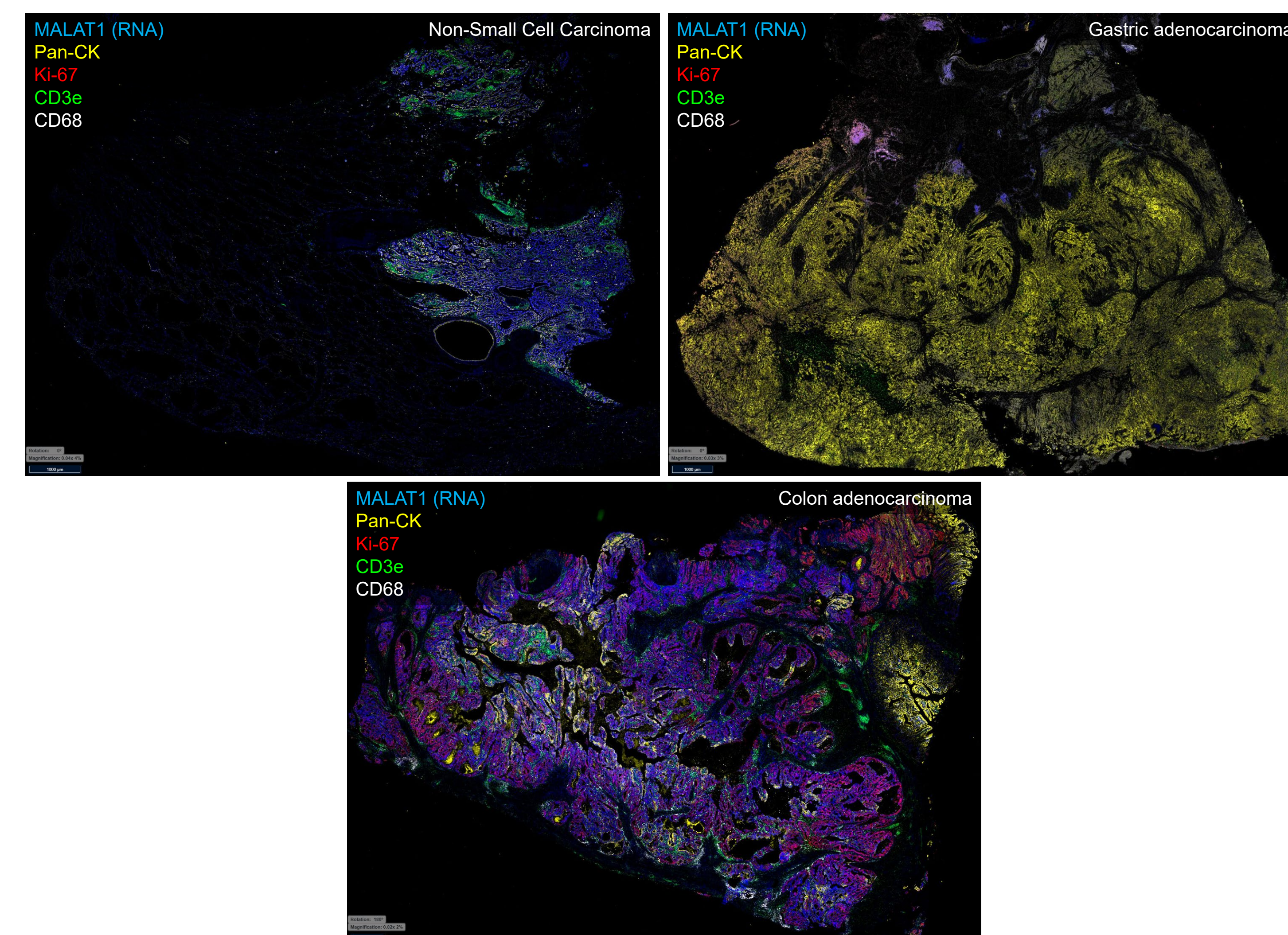
Understanding the tumor microenvironment (TME) during cancer progression and treatment has been aided by multiomic analysis of tissues collected at initial patient screening and after treatment. Cyclic staining methods have recently demonstrated multiomic capabilities on a single tissue, but with drawbacks that can include tissue degradation and processing times up to several days or weeks. The Orion™ imaging system overcomes these limitations through single round staining and scanning of whole tissue samples of up to 18 biomarkers simultaneously. When paired with Molecular Instruments' HCR™ Gold RNA-FISH, a novel product featuring a truly protease-free workflow with next-generation amplification technology, transcriptomic and proteomic multiplex immunofluorescence (mIF) analysis can be carried out in parallel on a single tissue section in a straightforward, high-throughput approach to provide comprehensive insight into tumor biology and immune response.

## METHODS

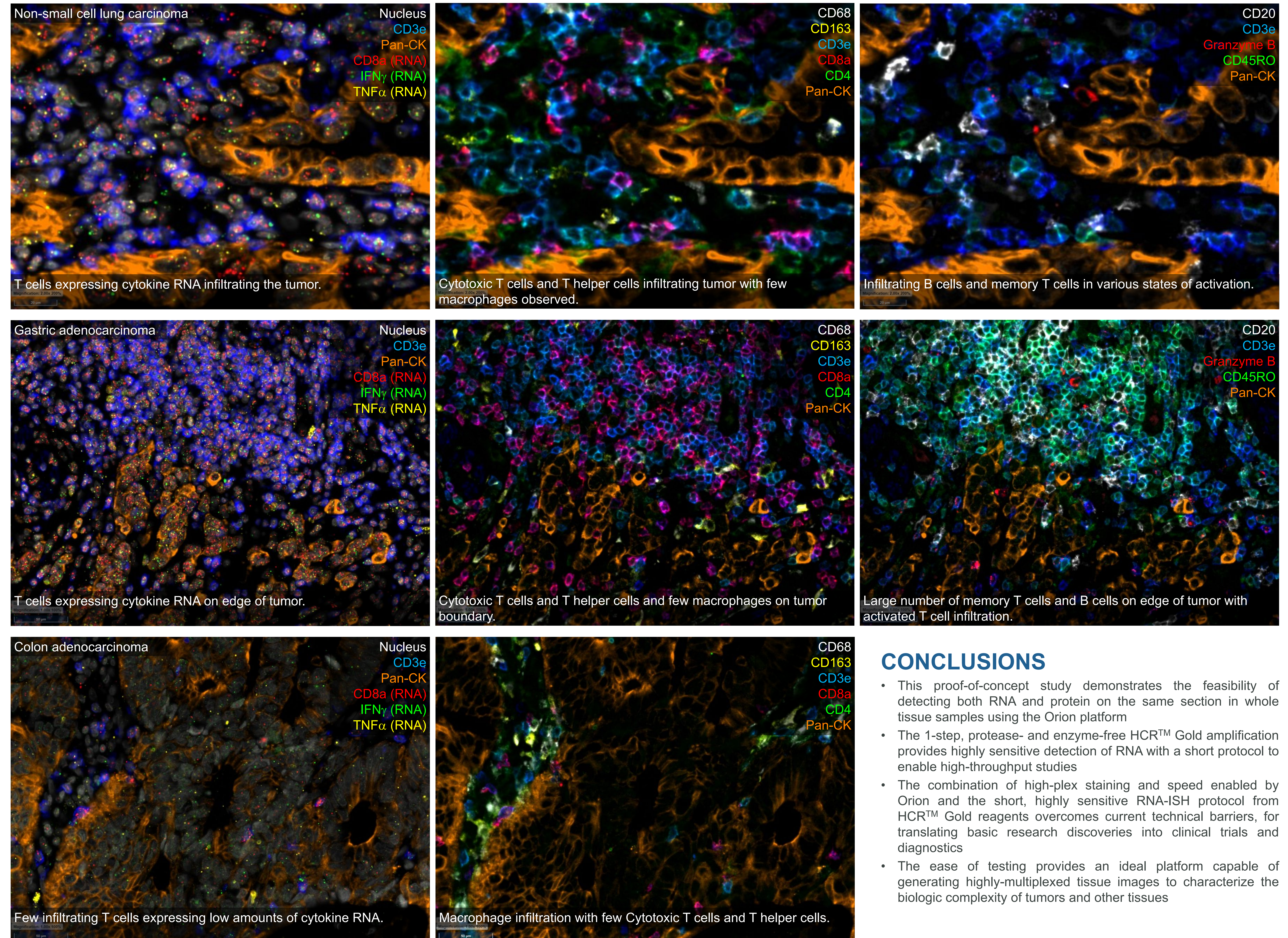
A 15-plex multiomic assay featuring 10 protein markers, 4 RNA markers, and a nuclear dye was developed to interrogate whole-slide formalin-fixed paraffin-embedded (FFPE) tissue sections. Following dewaxing and antigen retrieval, HCR™ HiFi Probes against RNA targets of interest were incubated on the tissue sample followed by fluorescently-labeled HCR™ Gold amplifiers for signal generation and scanned on the Orion instrument. Following an autofluorescence quench step, ArgoFluor™-conjugated antibodies directed to proteins of interest were incubated in a single cocktail on the same section, streamlining the staining procedure and decreasing downsides inherent to some amplification-based IF methods. Finally, whole tissues were again scanned on Orion. Images were processed and interrogated with a custom analysis pipeline.

RNA Targets	Protein Targets	
MALAT1	CD20	Granzyme B
CD8a	CD3e	CD68
TNF $\alpha$	CD8a	CD163
IFN $\gamma$	CD4	Ki-67
	CD45RO	Pan-CK

## WHOLE TISSUE IMAGES



## RESULTS



## CONCLUSIONS

- This proof-of-concept study demonstrates the feasibility of detecting both RNA and protein on the same section in whole tissue samples using the Orion platform
- The 1-step, protease- and enzyme-free HCR™ Gold amplification provides highly sensitive detection of RNA with a short protocol to enable high-throughput studies
- The combination of high-plex staining and speed enabled by Orion and the short, highly sensitive RNA-ISH protocol from HCR™ Gold reagents overcomes current technical barriers, for translating basic research discoveries into clinical trials and diagnostics
- The ease of testing provides an ideal platform capable of generating highly-multiplexed tissue images to characterize the biologic complexity of tumors and other tissues