**Background**
Understanding the complex interactions between tumor, immune, and stromal cells in the tumor microenvironment (TME) is essential for understanding how patients respond to treatments as well as for the development of next-generation immunotherapies. Here, we demonstrate the multimomic capability of the RNAscope™ technology by combining it with protein detection to interrogate the TME of breast cancer and lung cancer tissue.

**Methods**
A section of a tissue microarray containing breast and lung tissue was stained using RNAscope™ Multiplex V2 assay with target probes for PanCK, PD1, and CTLA4 and was stained using immunofluorescence (IF) for the PD-L1 checkpoint biomarker using the Integrated Co-Detection workflow from ACD. A second section was stained using RNasecope probes for TNFA, CCR5, and IFNG and was stained for CD4 using IF. Slides were scanned on Vectra Polaris scanner and were imported into the HALO® platform as QPTIFF images for image analysis. HALO AI was used to create an artifact classifier that removed highly autofluorescent red blood cells and blood vessels from the analysis. HALO AI was additionally used to create a tumor/stroma classifier to classify breast and lung tissue. The FISH IF module of HALO® was used to quantify RNA probes and IF signal in the tumor and the stroma compartments. Infiltration analysis using the HALO® Spatial Analysis module was performed on phenotypes of interest.

**Results**
Phenotypes of interest, such as PD-L1+ CTLA4+ (immune cells), PD-L1+ PanCK+ (tumor cells), PanCK+ (tumor cells), CD4+ TNFA+ IFNG+ (Th1 cells), and CD4+ CCR5+ (T cells) were analyzed across the breast and lung tumor and stromal compartments. In both tissue types, the percentage of CD4+ IFNG+ TNFA+ Th1 cells as well as the percentage of CD4+ CCR5+ T cells was greater in the stroma compared to the tumor compartment. FISH scores for each probe were calculated as well as a H-score for each probe in the four tissue compartments. An infiltration analysis of Th1 cells revealed distinct profiles in the stroma of lung and breast tumor tissue.

**Conclusions**
We demonstrate a powerful workflow to enable characterization and quantification of RNA and proteins expression in a single assay. This combined staining technique conserves tissue samples, provides flexibility to examine RNA and protein expression in the same sample, and enables detection of immune cell populations using antibodies and activation signatures using RNA probes against cytokines and chemokines.

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