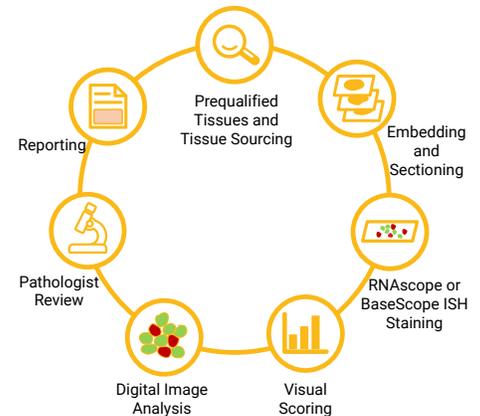


Pharma Assay Services Data Analysis Offerings

To accurately interpret gene expression in tissues, scientists are in need of a quantitative assay that provides spatial information at the molecular level. The RNAscope® and BaseScope™ ISH technologies enable single molecule detection of RNAs *in situ* within the tissue context. ACD's Pharma Assay Services (PAS) provides RNAscope and BaseScope ISH assay services to support clinical and pre-clinical studies for global pharma and biotech partners. Tissue sectioning, ISH staining, high resolution brightfield and fluorescence scanning, scoring, and image analysis are all performed by a dedicated team of highly trained specialists, scientists, and board-certified pathologists.



Digital Image Analysis

- Quantitative cell-by-cell gene expression data
- Identify tissue types or regions of interest
- Optional heat maps provide full-tissue spatial expression

Image analysis by our PAS scientists is performed using HALO® (Indica Labs), the powerful digital pathology tool for quantitative gene expression analysis. HALO reports morphological and multiplexed gene expression data on a cell-by-cell basis across entire tissue sections, providing image analysis capabilities for BaseScope assays and RNAscope singleplex, duplex, and multiplex fluorescence assays.

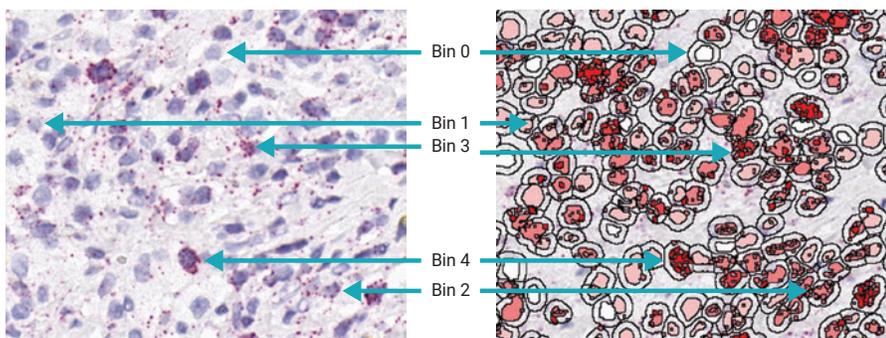


FIGURE 1. Heterozygous expression of EGFR in human breast cancer tissue. RNAscope staining (left) and HALO image analysis markup (right), showing cells binned according to level of gene expression based on dots per cell

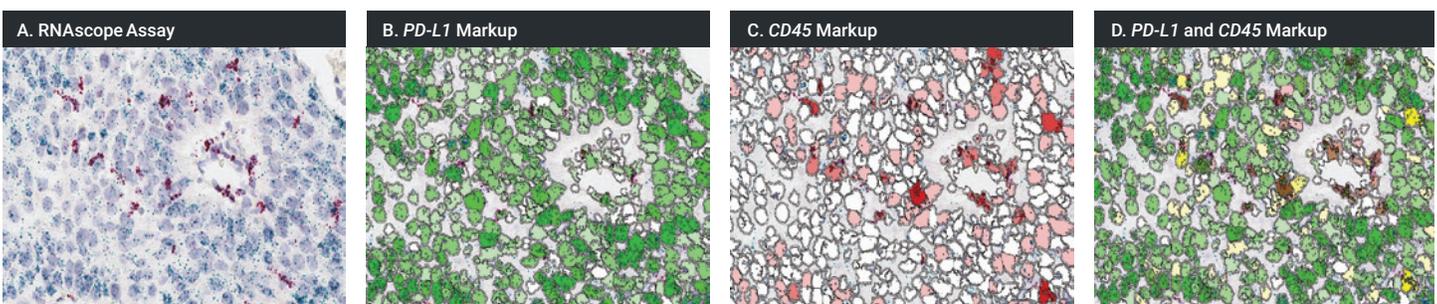


FIGURE 2. RNAscope duplex staining (A) on NSCLC tissue for PD-L1 (green chromogen) and CD45 (red chromogen). Associated HALO mark-up images (B-D) show cell segmentation and identification of cells positive for PD-L1 (green), CD45 (red) or dual (yellow)

Regions of Interest

Using the HALO Tissue Classifier Module, a machine-learning algorithm, to identify different cell types or tissue types based on color, texture, and contextual features. The user establishes settings to train the software to classify cell populations or regions of interest (ROI), for example categorizing a tissue into tumor cells versus stromal cells.

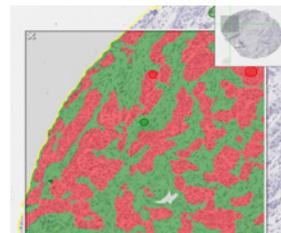


FIGURE 3. Tumor (red) and stromal (green) cells defined using the Tissue Classifier Module

Digital image analysis data output includes:
Total cell count in region of interest
Average probe copies per cell
Percentage of cells in each bin for each probe
H-score for each probe

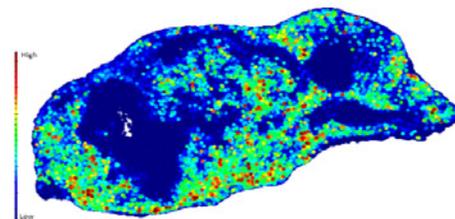


FIGURE 4. Heat map generated using HALO software showing regions of high versus low expression

Pathologist Review

- In-house pathologists for image annotations
- Histopathology notes include tumor load and visual H-scoring
- Teleconference with pathologists for data presentation and review

Your pathologist's workload can be reduced by utilizing the PAS team's in-house and contract pathologists who will provide additional notations or review for any PAS study. Annotations can be performed manually either to identify regions of inclusion or regions of exclusion, for instance to exclude regions of necrosis or poor positive control staining. Visual H-scoring can also be performed by a pathologist to provide more quantitative results.

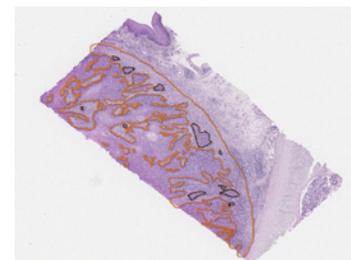


FIGURE 5. Manual annotation of tumor-associated stromal regions

Visual Scoring

- Semi-quantitative scoring performed by highly trained experts
- Rapid scoring shortens turnaround time and speeds delivery of results
- Provides expression level and percentage of cells positive

For gene expression studies where understanding relative signal intensity is sufficient, visual semi-quantitative scoring can be used to provide a single score based on the expression level observed. RNAscope and BaseScope ISH staining generates punctate chromogenic or fluorescent signals. Scoring is based on counting the number of dots per cell, with each dot corresponding to a single RNA molecule. Visual scoring (0–4) is performed based on the predominant staining pattern seen throughout the entire sample or within a defined region of interest. Heterogeneity or non-uniformity of expression will be noted. Percentage of cells positive is scored visually based on number of cells with ≥ 1 dot/cell and binned into categories (i.e. 0%, 1–25%, 26–50%, 51–75%, 76–99%, 100%).

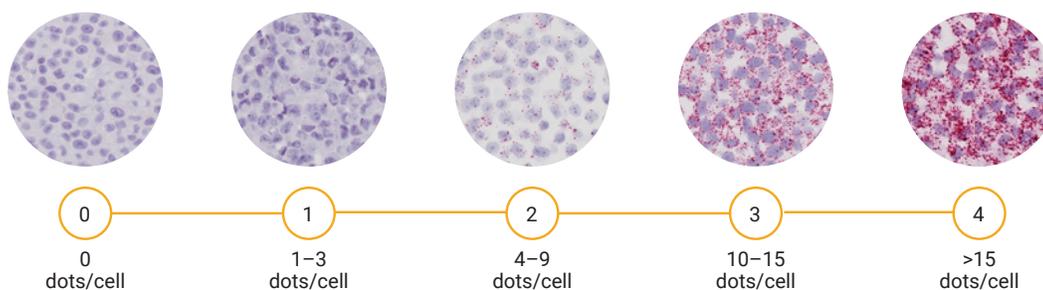


FIGURE 6. Scores (0–4) are assigned to a tissue sample based on the number of dots/cell

Learn more about ACD's PAS Data Analysis services by contacting acd_pas@bio-techne.com