PROFESSIONAL ASSAY SERVICES
IMAGE ANALYSIS OFFERINGS

To accurately interrogate gene expression in tissues, scientists are in need of a quantitative assay that provides spatial information at the molecular level. The RNAscope™, BaseScope™, and miRNAscope™ ISH technologies enable single molecule detection of RNAs *in situ* within the tissue context. Professional Assay Services operates under Good Clinical Laboratory Practices (GCLP) compliance and provides custom assay services using all three platforms to support clinical and preclinical studies for global academic and biopharma customers. Tissue sectioning, ISH staining, high resolution brightfield and fluorescence whole-slide scanning, visual scoring, and digital image analysis are all performed by a dedicated team of highly trained specialists, scientists, and board-certified pathologists. A decade of experience across thousands of RNAscope studies gives our scientists unparalleled expertise.

DIGITAL IMAGE ANALYSIS

- Quantitative cell-by-cell gene expression data

- Define cell populations or regions of interest

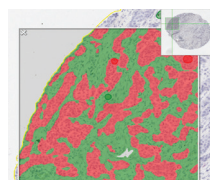- Heat maps provide full-tissue spatial mapping

FIGURE 1. Tumor (red) and stromal (green) cells defined using the Tissue Classifier Module
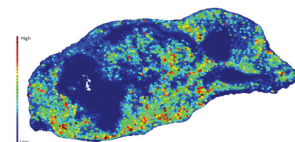

FIGURE 2. Heat map generated using HALO software showing regions of high versus low expression

Image analysis is performed using HALO® (Indica Labs), the powerful digital pathology tool for quantitative gene expression analysis. HALO reports gene expression data on a cell-by-cell basis across entire tissue sections, and is designed for RNAscope, BaseScope, and miRNAscope singleplex, duplex, and multiplex fluorescence assays. Sorting and filtering capabilities allow the user to mine millions of cells while visually assessing the corresponding cells *in situ*. The software can be trained to classify cell populations or regions of interest (ROI), for example categorizing a tissue into tumor cells versus stromal cells. The readouts are defined based on the scientific objectives of the study, and each project is supported by consultations with the scientific team.
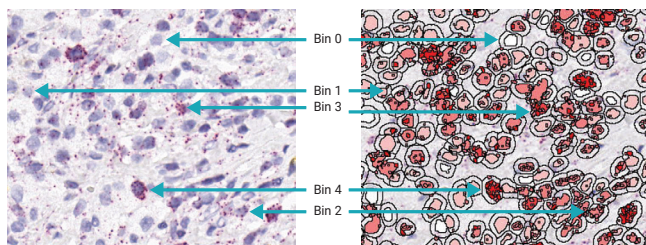

Bin 0
Bin 1
Bin 3
Bin 4
Bin 2

FIGURE 3. Heterozygous expression of EGFR in human breast cancer tissue. RNAscope staining (left) and HALO image analysis mask (right), showing cells binned according to level of gene expression based on dots per cell.

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
Co-expression data for multiplex assays

A. RNAscope Assay **B. PD-L1 Markup** **C. CD45 Markup** **D. PD-L1 and CD45 Markup**
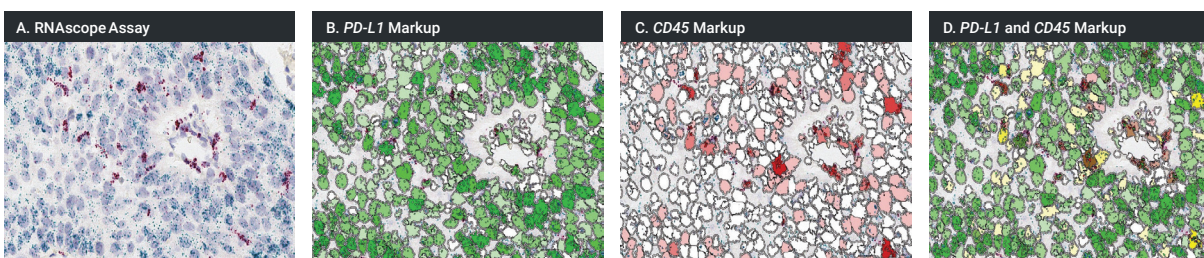
FIGURE 4. RNAscope duplex staining (**A**) on NSCLC tissue for *PD-L1* (green chromogen) and *CD45* (red chromogen). Associated HALO mark-up images (**B-D**) show cell segmentation and identification of cells positive for *PD-L1* (green), *CD45* (red) or dual (yellow)

PATHOLOGIST REVIEW

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

Your pathologist's workload can be reduced by utilizing our team's in-house and contract pathologists who will provide additional notations or review for any 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.
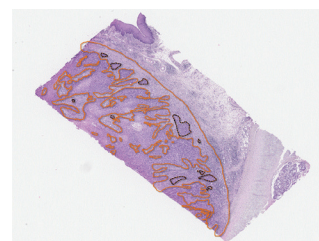

FIGURE 5. Manual annotation of tumor-associated stromal regions

VISUAL H-SCORING

To provide a more quantitative evaluation, and to account for heterogeneity in expression, H-Scoring can be performed visually, typically by a pathologist, to assess the predominant staining pattern across the entire sample. Cells are grouped into 5 bins based on the number of dots per cell, and percentage of cells in each bin is scored. The H-score is calculated by totaling the percentage of cells in each bin according to the weighted formula shown. H-scores are provided on a scale of 0-400.

H-Score= 0*(% of cells in bin 0) + 1*(% of cells in bin 1) + 2*(% of cells in bin 2) + 3*(% of cells in bin 3) + 4*(% of cells in bin 4)

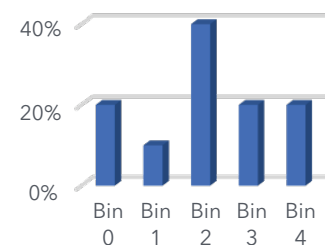|	% OF CELLS	WEIGHTED FORMULA
Bin 0 (0 dots/cell)	20%	0 * 20
Bin 1 (1-3 dots/cell)	10%	+ 1 * 10
Bin 2 (4-9 dots/cell)	40%	+ 2 * 40
Bin 3 (10-15 dots/cell)	20%	+ 3 * 20
Bin 4 (>15 dots/cell)	20%	+ 4 * 20
H-SCORE = 230		

FIGURE 6. Example for Visual H-Scoring and binning of gene expression (left). Binned expression histogram (right).

SEMI-QUANTITATIVE VISUAL SCORING

- Semi-quantitative scoring performed by highly trained specialists
- 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 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%).
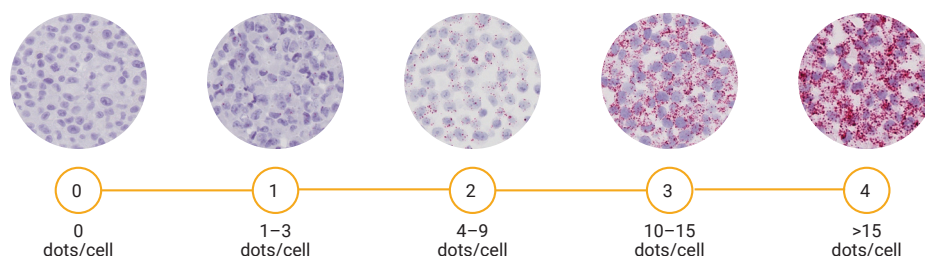

FIGURE 7. Scores (0–4) are assigned to a tissue sample based on the number of dots/cell

0	1	2	3	4
0 dots/cell	1–3 dots/cell	4–9 dots/cell	10–15 dots/cell	>15 dots/cell

Learn more about our image analysis offerings at:
acdbio.com/services/quantitative-analysis

Contact Professional Assays Services at:
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