AI-Based Detection and Scoring of TROP2 Expression in IHC-Stained NSCLC Specimens

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Abstract

Lung cancer is the leading cause of cancer-related deaths in the United States and non-small cell lung cancer (NSCLC) accounts for approximately 85 percent of all lung cancer cases[1,2]. Trophoblast cell-surface antigen 2 (TROP2), a transmembrane glycoprotein that normally serves as a Ca^{2+} signal transducer linked to cell growth, proliferation, and migration, is frequently observed in NSCLC and elevated expression levels are associated with increased metastatic risks and poor prognostic outcomes. Due to the adverse effects of TROP2 on NSCLC, as well as on many other common cancers, strategies such as antibody-drug conjugates (ADCs) and CAR-NK cells targeting TROP2-expressing tumors have emerged as areas of active investigation and therapeutic development. To serve these efforts, we present an end-to-end workflow for the quantification of TROP2 expression in NSCLC specimens that consists of an IHC staining assay optimized for the detection of TROP2 in FFPE tissue sections together with an AI-based image analysis routine for the automated detection and scoring of TROP2 tumor expression in whole-slide image (WSI) specimens. Our analysis algorithms were highly concordant with manual interpretation by pathologists when evaluated using Pearson's correlation coefficient, demonstrating both accurate tumor identification and TROP2 expression scores. By integrating our TROP2 IHC assay with algorithmic image analytics, we offer a comprehensive, scalable solution for discovery-based research efforts and clinical drug trials focused on TROP2 in NSCLC.

1) American Cancer Society. Facts & Figures 2025. American Cancer Society. Atlanta, GA. 2025
2) Siegel, R.L., Kratzer, T.B. Giaquinto, A.N, Sung, H., & Jemal, A. (2025). Cancer statistics, 2025. CA: A Cancer Journal for Clinicians, 75(1). Https://doi.org/10.3322/caac.21871

TROP2 IHC Assay

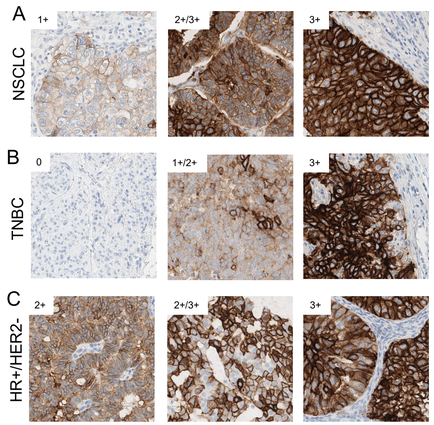


Figure 1: TROP2 IHC Assay in Lung and Breast Specimens. A IHC staining assay was developed, optimized, and LDT validated for the detection of TROP2 in **A)** lung NSCLC, **B)** breast TNBC, and **C)** breast HR+/HER-. **A-C)** Examples of TROP2 staining in the specified indications: 0 (negative), 1+ (weak), 2+ (moderate), 3+ (strong).

H&E Tumor Segmentation

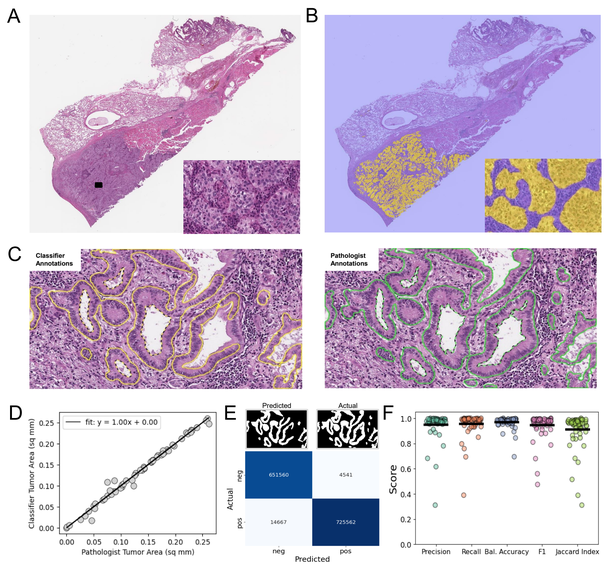


Figure 2: NSCLC H&E Tumor Segmentation and Classifier Validation. A custom AI-based NSCLC H&E tumor model was developed using our proprietary pipeline, converted to .onnx format, and imported into Indica Halo v4.2 for deployment. All NSCLC H&E images were analyzed in Halo. **A)** A representative WSI and **B)** associated overlay displaying the area classified as tumor in yellow following segmentation using our custom model; insets show magnified areas indicated by the black box in A. **C)** 20x field of views (FOVs, 0.36 mm^2) demonstrating classifier-derived annotations (yellow) in comparison to pathologist drawn ground truth annotations (green). **D)** Scatter plot illustrates the total tumor area per FOV determined by the classifier compared to the pathologist; n = 60 FOVs from 30 WSIs. Black line shows the regression fit of the data; Pearson's correlation coefficient (r) = 0.99 **E)** Confusion matrix demonstrating the pixel-level comparison between the classifier-derived (Predicted) and the pathologist-derived (Actual) binary masks of the images depicted in panel C showing tumor (white) and outside tumor (black). **F)** Strip plots summarize the tumor classifier's performance for key metrics on a per FOV basis; black bars illustrate the means.

H&E Tumor Annotations Transferred to IHC

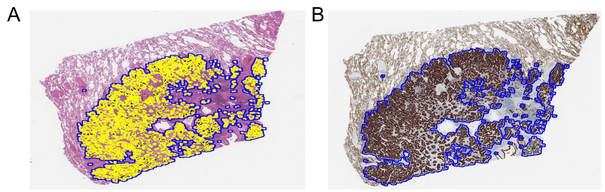


Figure 3: Dilation and Transfer of H&E tumor annotations to Registered IHC image. **A)** Representative H&E showing annotations generated by the H&E tumor classifier (yellow) and dilated annotations (blue) generated using a custom code to enlarge and merge the individual tumor annotations. B) Dilated annotations transferred to the registered IHC image.

IHC Tumor Segmentation

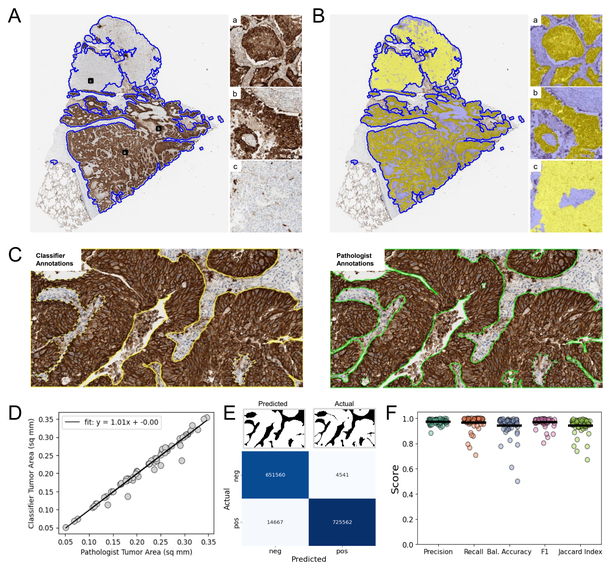


Figure 4: NSCLC TROP2 IHC Tumor Segmentation and Classifier Validation. A custom TROP2 IHC tumor classifier was developed with Halo AI and used to analyze all TROP2 IHC images in Halo v4.2. **A-F)** Panel layout and descriptions match those in Figure 2.

Detection of TROP2 Membrane Staining

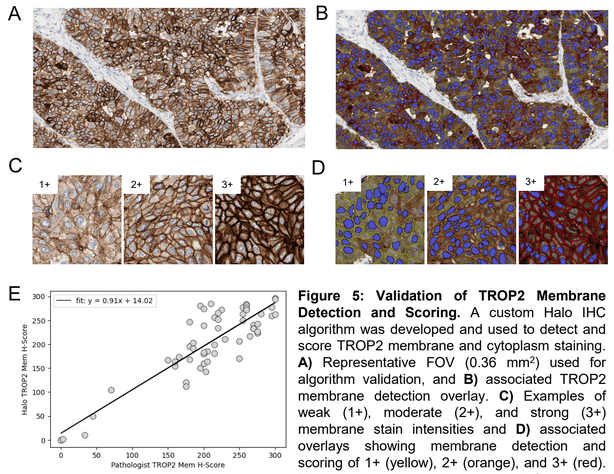


Figure 5: Validation of TROP2 Membrane Detection and Scoring. A custom Halo IHC algorithm was developed and used to detect and score TROP2 membrane and cytoplasm staining. **A)** Representative FOV (0.36 mm^2) used for algorithm validation, and **B)** associated TROP2 membrane detection overlay. **C)** Examples of weak (1+), moderate (2+), and strong (3+) membrane stain intensities and **D)** associated overlays showing membrane detection and scoring of 1+ (yellow), 2+ (orange), and 3+ (red). **E)** Halo vs. pathologist membrane H-Scores with regression fit; n = 58 FOVs from 29 WSIs, r = 0.88.

Detection of TROP2 Cytoplasm Staining

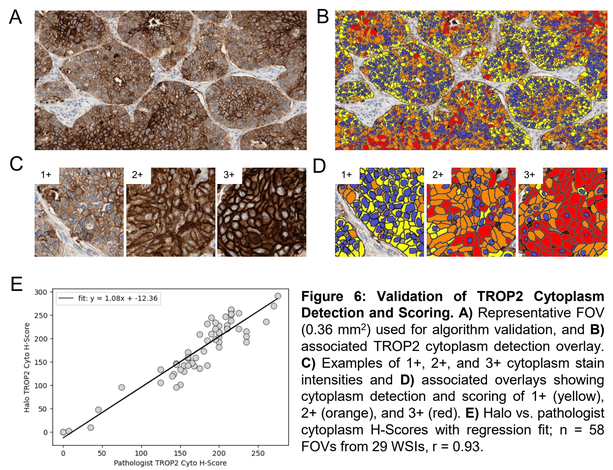
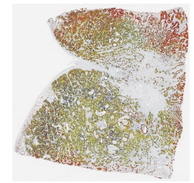


Figure 6: Validation of TROP2 Cytoplasm Detection and Scoring. A) Representative FOV (0.36 mm^2) used for algorithm validation, and **B)** associated TROP2 cytoplasm detection overlay. **C)** Examples of 1+, 2+, and 3+ cytoplasm stain intensities and **D)** associated overlays showing cytoplasm detection and scoring of 1+ (yellow), 2+ (orange), and 3+ (red). **E)** Halo vs. pathologist cytoplasm H-Scores with regression fit; n = 58 FOVs from 29 WSIs, r = 0.93.

TROP2 IHC Reportables



Slide Level
Membrane H-Score
Cytoplasm H-Score
Cell Level
Membrane Optical Density (OD)
Cytoplasm OD
Normalized Membrane Ratio* *[Mem OD/(Mem OD + Cyto OD)]

Summary

- A TROP2 IHC assay was developed, optimized, and LDT validated in lung NSCLC specimens, and breast TNBC and HR+/HER- specimens.
- Custom NSCLC H&E and IHC tumor classifiers closely matched manual tumor segmentation by a pathologist.
- TROP2 IHC detection algorithm was highly concordant with pathologist scores of membrane and cytoplasm stain intensity.
- Integrating the TROP2 IHC assay with AI-driven image analytics in a GxP validated environment offers a comprehensive, scalable solution for discovery-based research efforts and clinical drug trials focused on TROP2.
- Quantification of compartmental TROP2 staining enables insights into cellular expression profiles that could serve to improve patient stratification and therapeutic decisions.

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