Sequential same slide multiplex immunofluorescence and H&E staining for combined phenotypic and morphological characterization of formalin-fixed paraffin-embedded tissue sections

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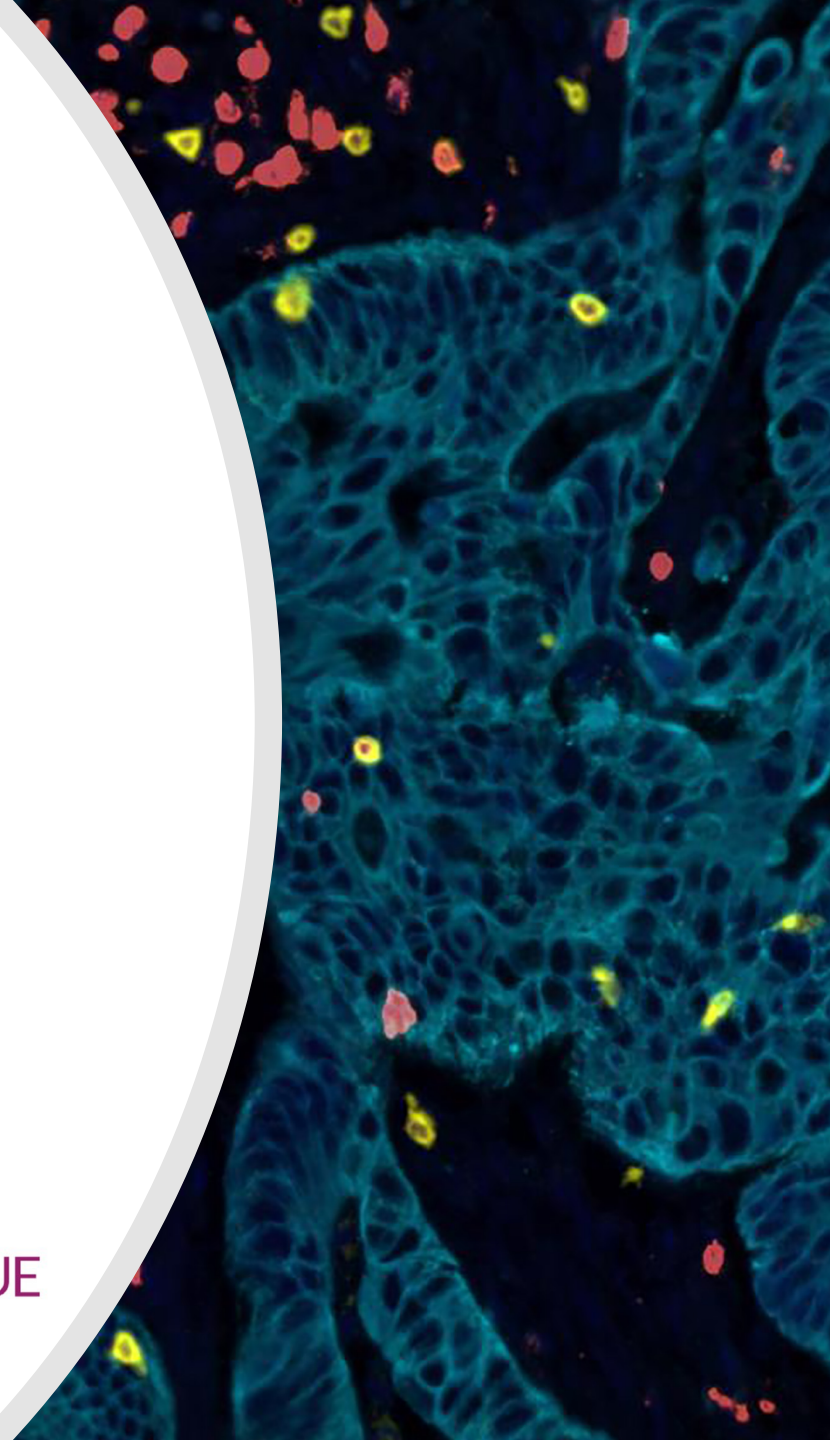
INTRODUCTION

The UltiMapper® I/O PD-L1 kit was used for multiplex immunofluorescence staining of CD8, CD68, PD-L1, and pan-Cytokeratin in formalin-fixed, paraffin-embedded (FFPE) samples from 3 serial sections of human tonsil and primary colon and lung tumor biopsies using the Leica® Biosystems BOND RX autostainer. Stained tissues were imaged in five spectrally distinct fluorescence channels (DAPI, FITC, TRITC, Cy5, Cy7) on the RareCyte CyteFinder® II HT Instrument. Slides were de-coverslipped and stained with H&E, then imaged with brightfield using the CyteFinder® II HT Instrument.



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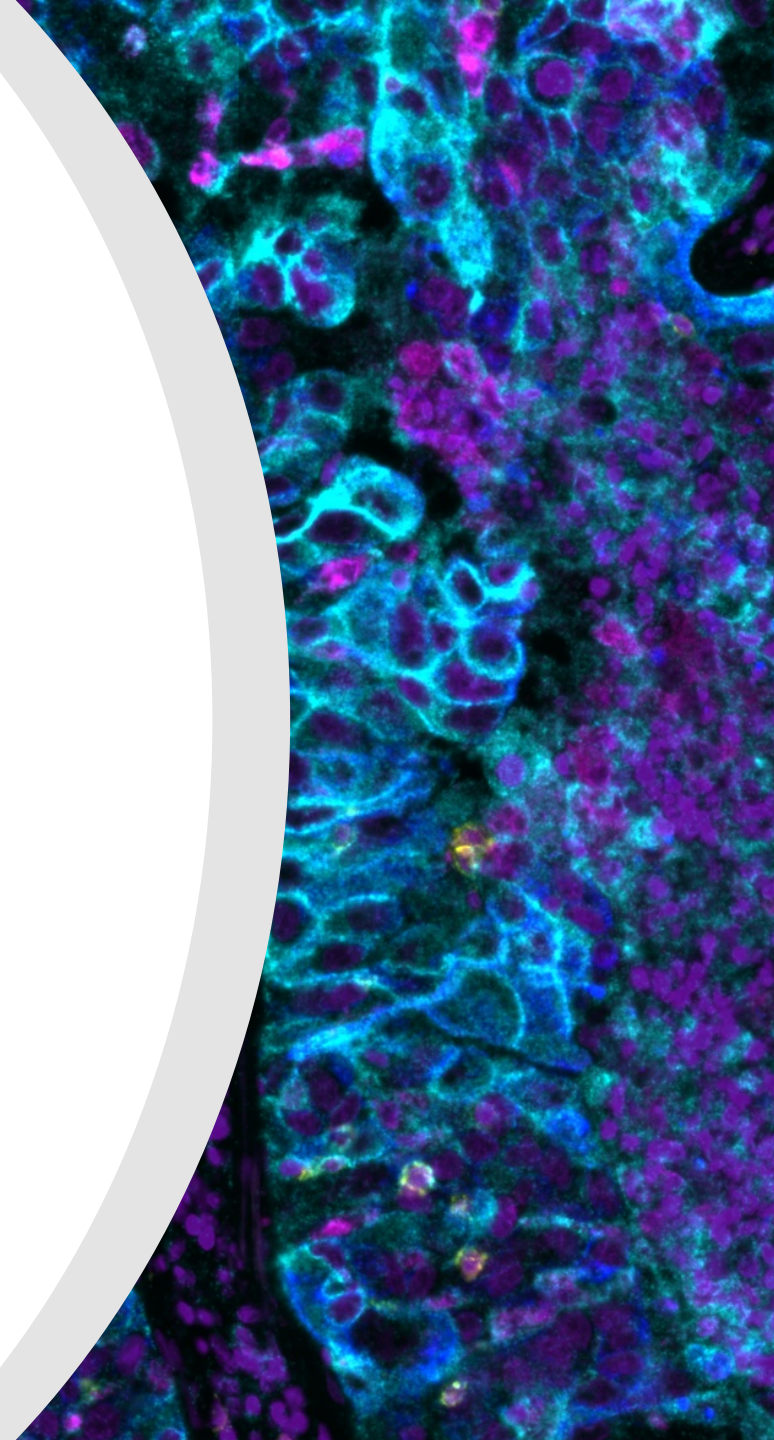
METHODS

The UltiMapper® I/O PD-L1 kit was used for multiplex immunofluorescence staining of CD8, CD68, PD-L1, and pan-Cytokeratin in formalin-fixed, paraffin-embedded (FFPE) samples from 3 serial sections of human tonsil and primary colon and lung tumor biopsies using the Leica® Biosystems BOND RX autostainer. Stained tissues were imaged in five spectrally distinct fluorescence channels (DAPI, FITC, TRITC, Cy5, Cy7) on the RareCyte CyteFinder® II HT Instrument. Slides were de-coverslipped and stained with H&E, then imaged with brightfield using the CyteFinder® II HT Instrument.



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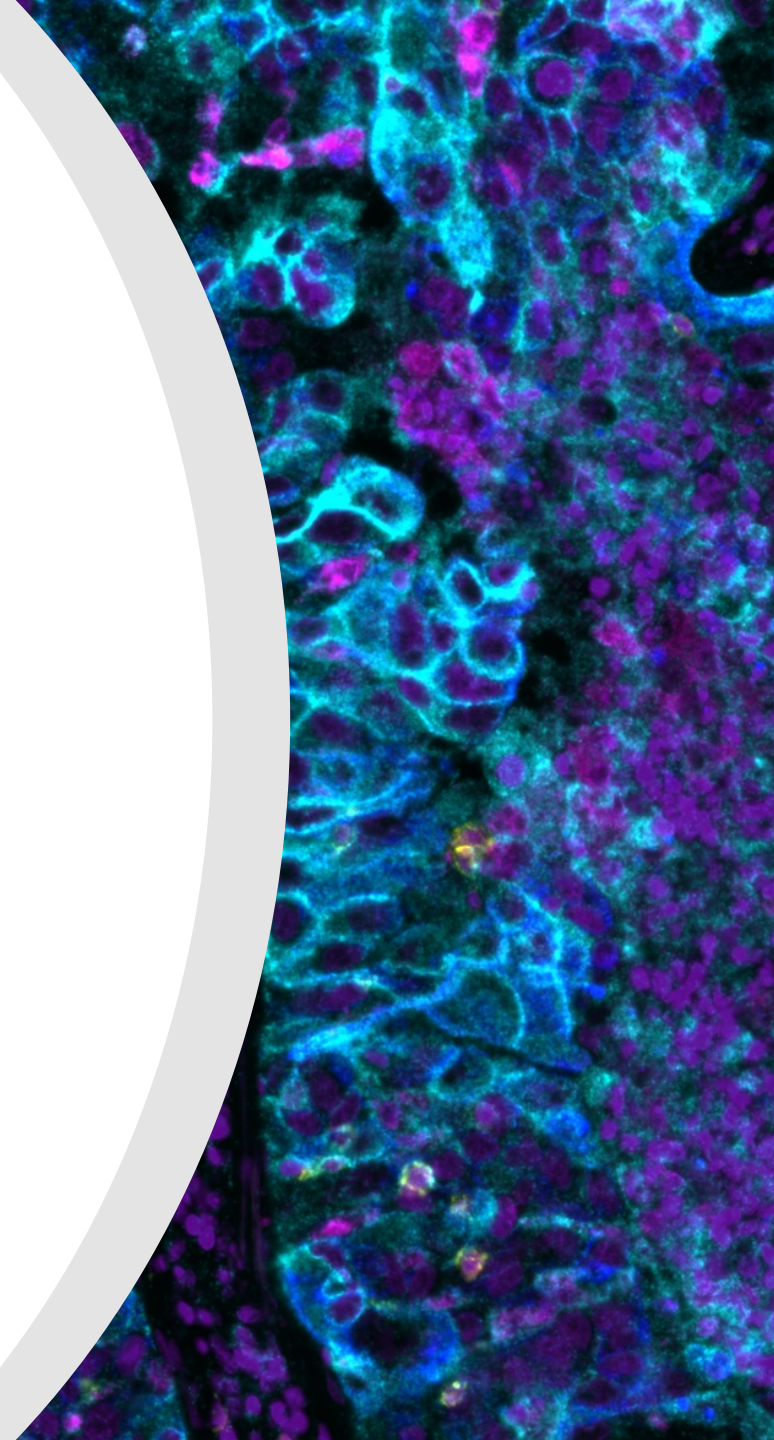
METHODS

To segment the tumor and stroma tissue regions, a HALO AI™ classifier was created for the lung and colon H&E images. Fluorescence images were analyzed using the HALO® Highplex FL module to identify CD8+ cytotoxic T-cells, CD68+ macrophages, CD68+/PD-L1+ immuno-suppressive macrophages, pan-CK+ tumor cells, and pan-CK+/PD-L1+ immune-evading tumor cells within the tumor and stromal regions identified by the H&E stain. As a comparison, a classifier was also trained on the fluorescent CK and DAPI signal.



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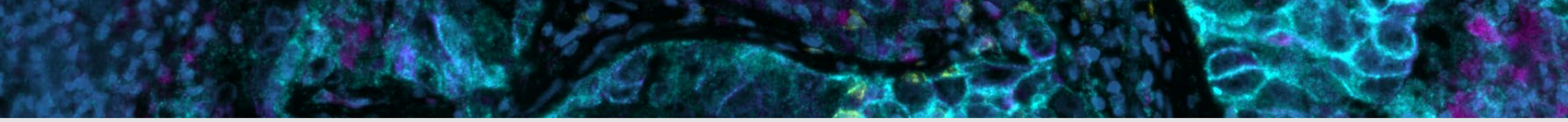
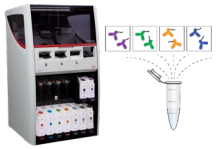


SEQUENTIAL STAINING AND ANALYSIS WORKFLOW

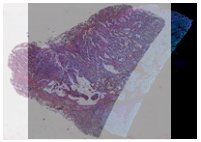


Stain slides with the UltiMapper® I/O PD-L1 kit on the Leica® BOND RX

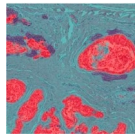
Image in fluorescence on the CyteFinder® II HT Instrument

Decoverslip and stain with H&E

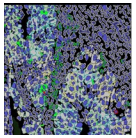
Image in brightfield on the CyteFinder® II HT Instrument



Align H&E image to fluorescence image using the Serial Stain Registration tool in HALO®



Classify tumor and stroma regions from H&E image using HALO AI™



Phenotype and quantify cell types within regions using HALO® Highplex FL

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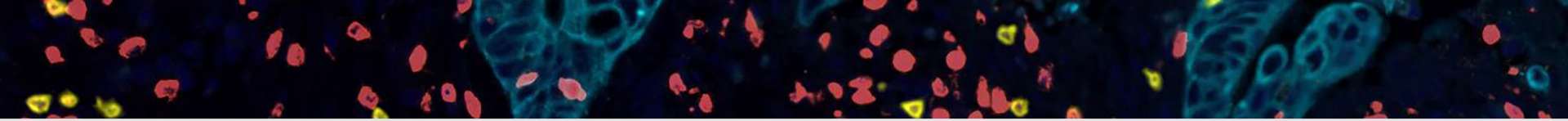
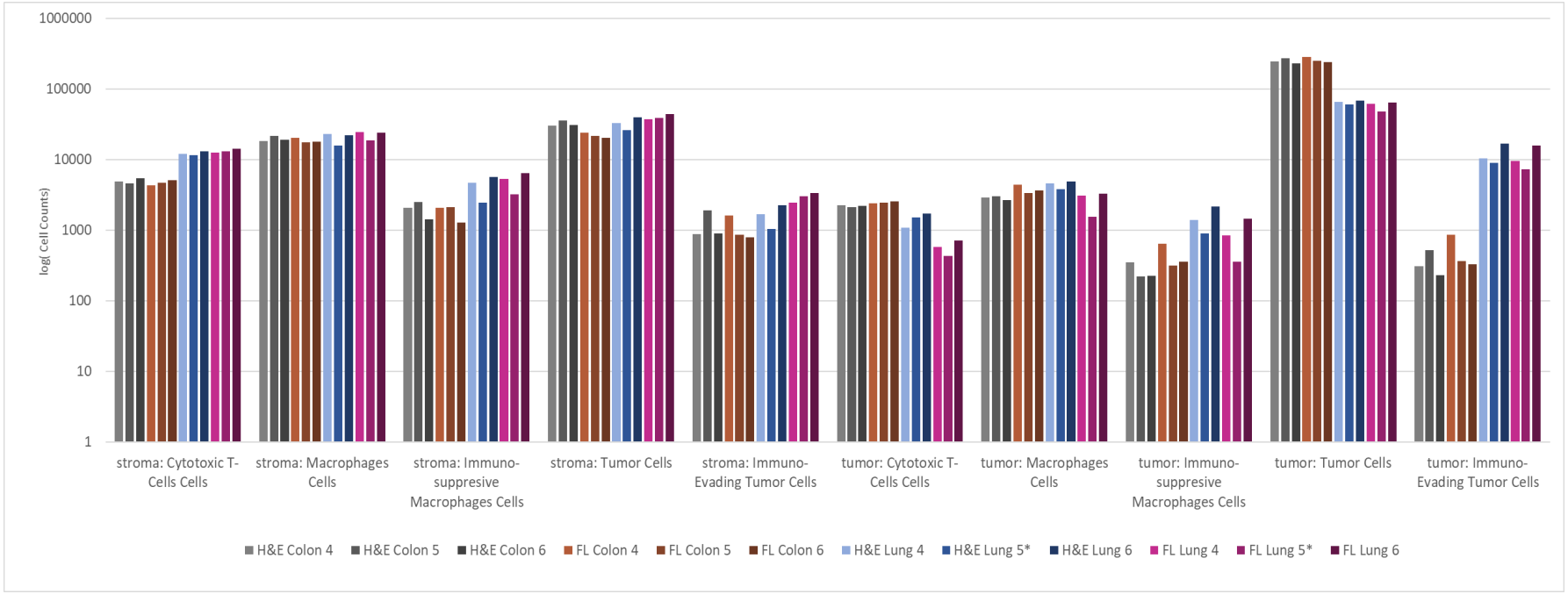


TUMOR MARKER IS NOT REQUIRED IN PANEL WHEN H&E USED FOR TISSUE ARCHITECTURE CLASSIFICATION



Cell counts/region comparing a classifier trained on the H&E image vs the fluorescence CK+DAPI signal are comparable. Both classifiers provide equivalent results, indicating that the tumor marker could be dropped from the fluorescence panel if using the H&E for tissue architecture classification.

*Lung 6 tumor/stroma segmentation was applied for Lung 5, since the tissue was damaged during decoverslipping.

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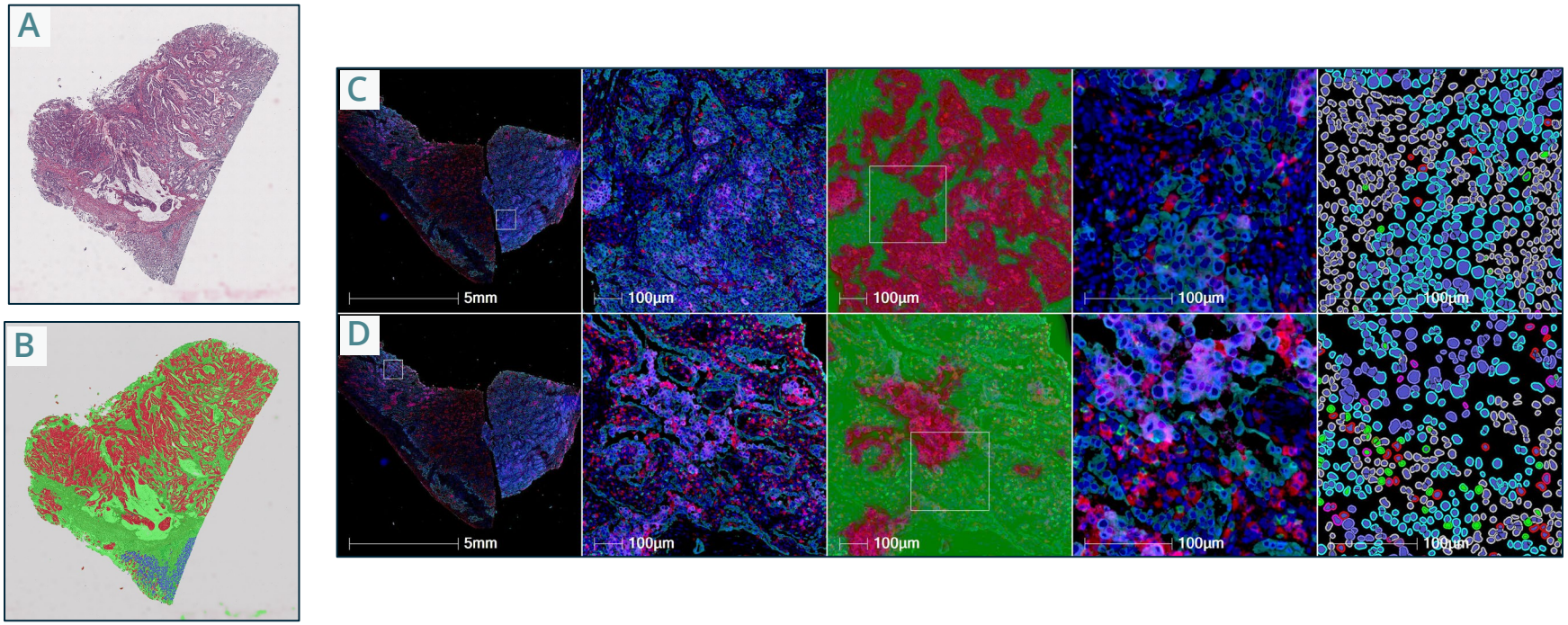
COLON TISSUE CLASSIFIED FROM H&E IMAGE



(A) H&E image of colon tissue (B) classified into tumor epithelium (red), stroma (green), and normal epithelium (blue) regions. (C) Tumor region and (D) normal region of the fluorescence image shown in closeup and segmented with color-coded phenotypes outlines: CK-positive (cyan), CD8-positive (green), CD68- positive (red), PD-L1-positive (pink), negative cytoplasm (grey).

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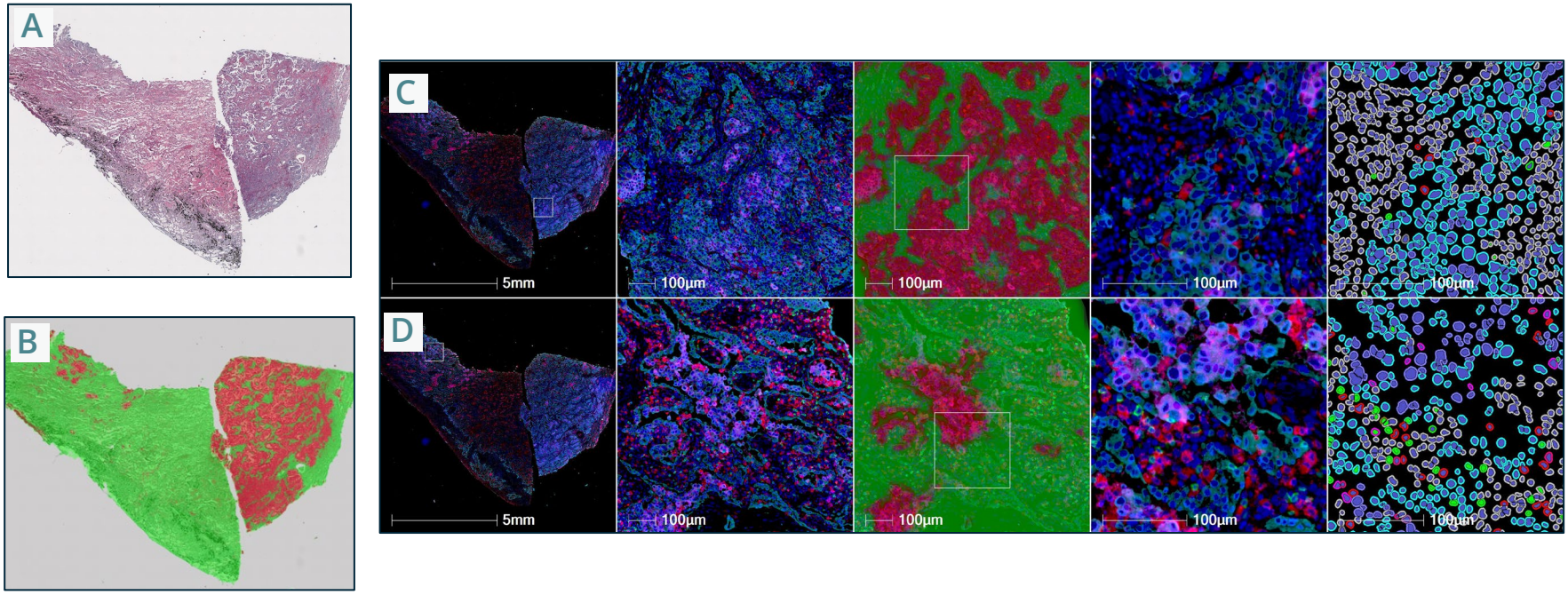
LUNG TISSUE CLASSIFIED FROM H&E IMAGE



(A) H&E image of lung tissue (B) classified into tumor epithelium (red), and stroma (green). (C) Tumor region and (D) stromal region of the fluorescence image shown in closeup and segmented with color-coded phenotypes outlines: CK-positive (cyan), CD8-positive (green), CD68-positive (red), PD-L1-positive (pink), negative cytoplasm (grey).

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CONCLUSIONS

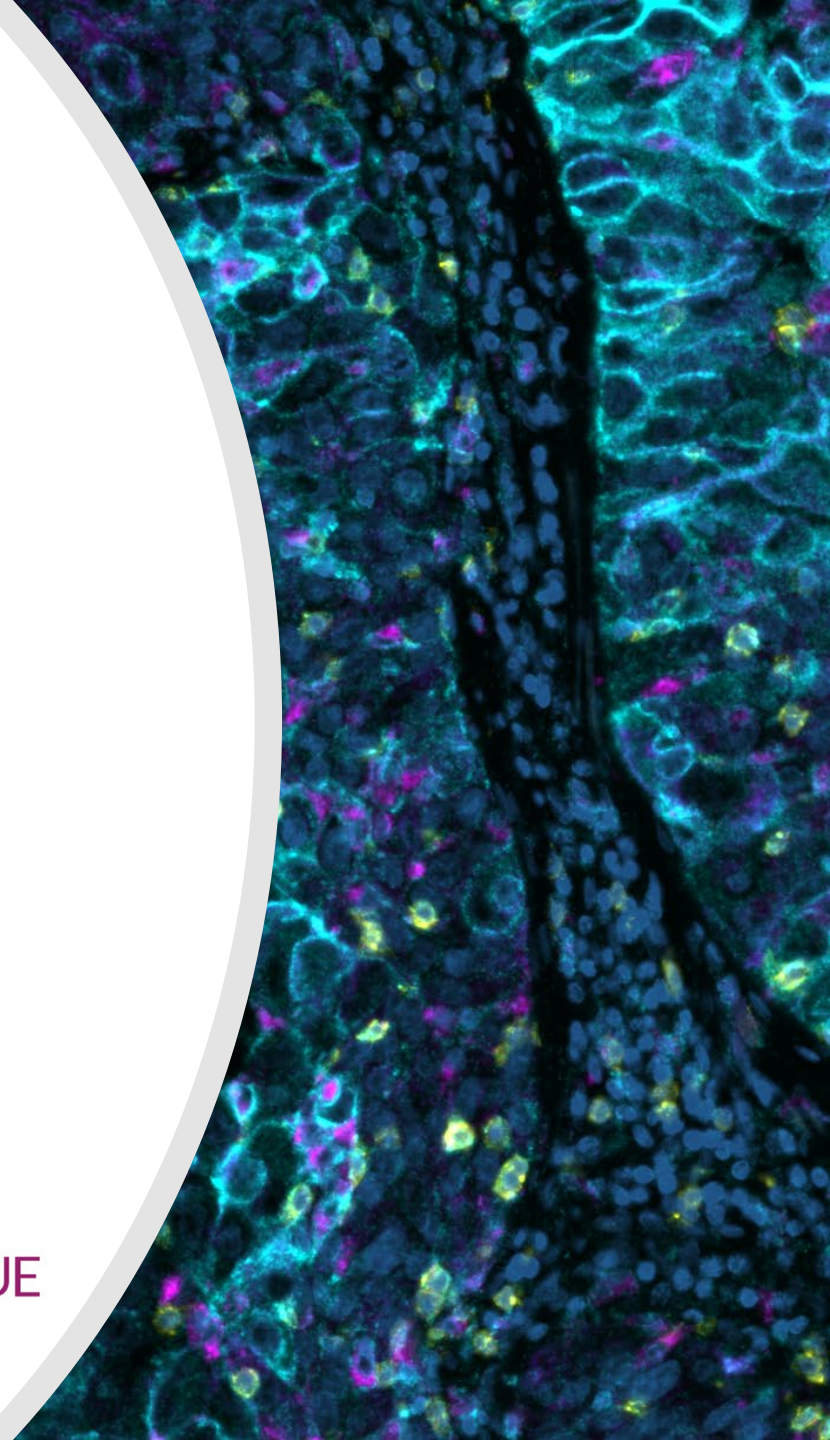
Here we demonstrate a tissue-preserving workflow to generate H&E images from a slide that is previous stained and imaged in fluorescence on the CyteFinder® II HT Instrument.

These H&E images can be used to delineate tissue architectural regions such as tumor and stroma, eliminating the need for a tumor-specific biomarker in the fluorescence panel.

H&E trained classifiers perform equivalently to classifiers trained on fluorescent signal from DAPI and pan-CK.

The UltiMapper® I/O PD-L1 kit produces reproducible staining results across multiple serial sections.

HALO® Highplex FL can be used to identify phenotypic cell populations and expression levels within tissue architectural regions.



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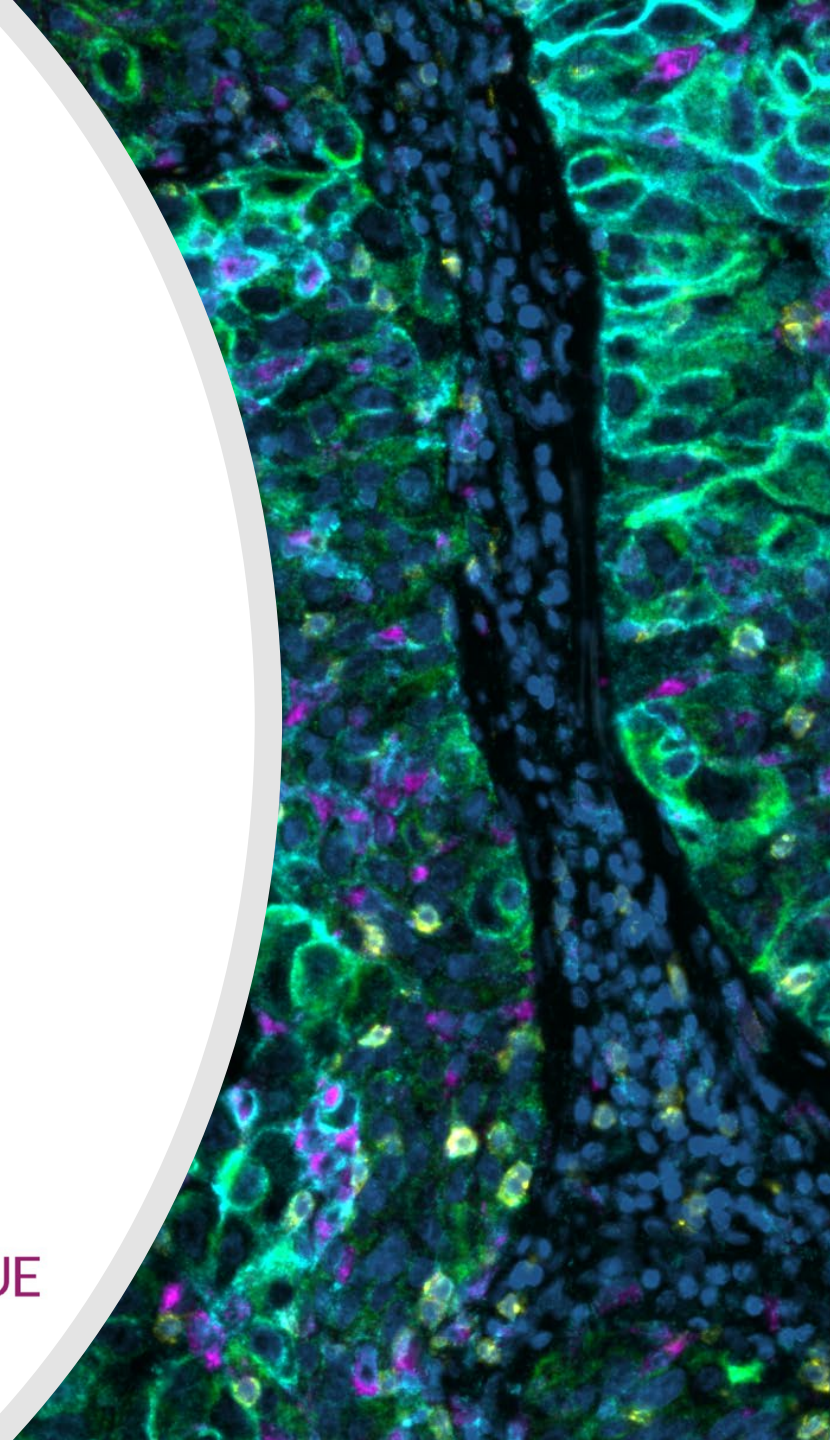
PRODUCT INFORMATION

CyteFinder® II HT Instrument

UltiMapper® I/O PD-L1 kit

HALO® image analysis platform

HALO® Highplex FL



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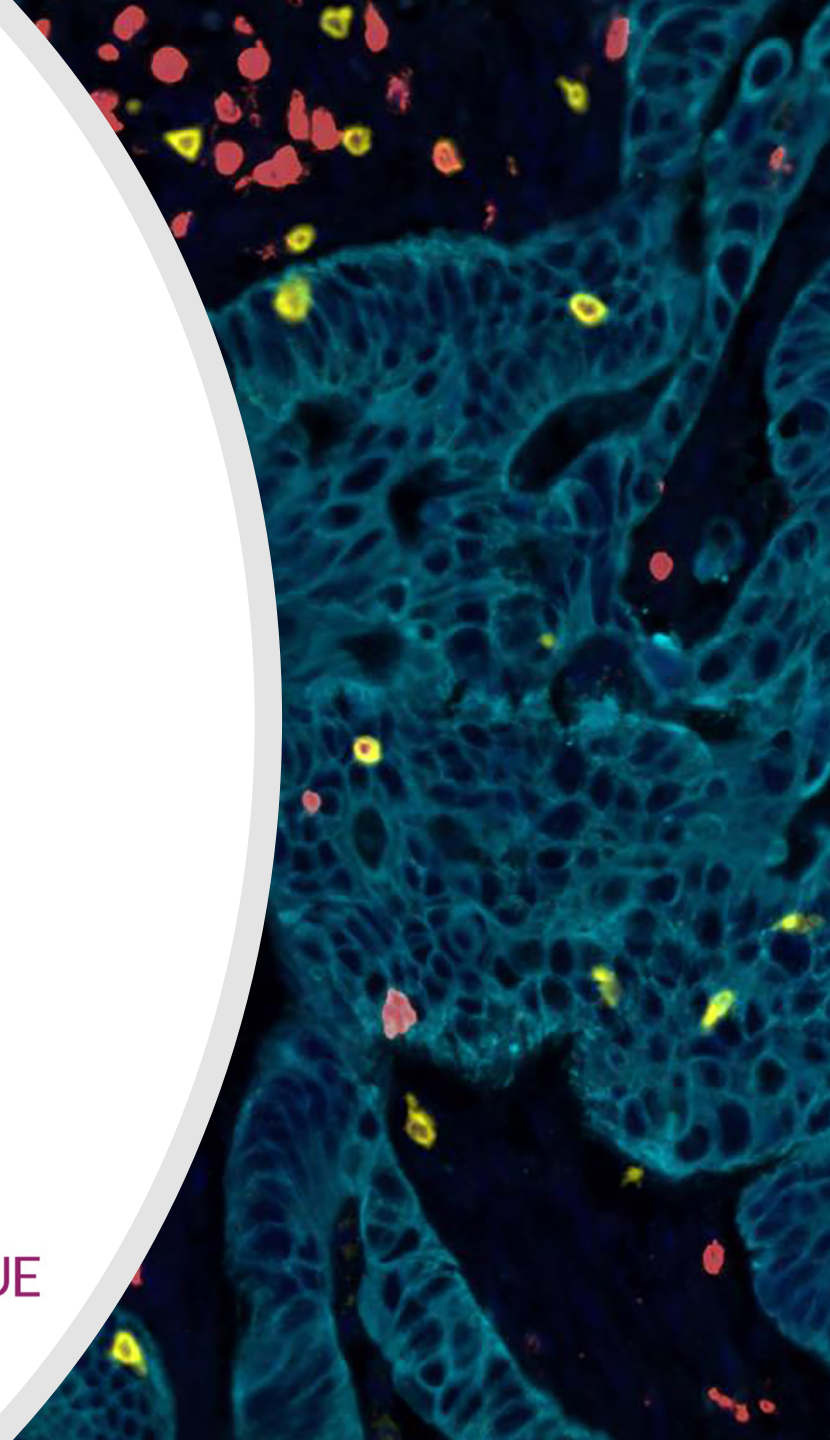
CONTACT INFORMATION

- To learn more about UltiMapper® I/O kits, email contact@ultivue.com
- To learn more about the CyteFinder® II HT Instrument, email info@rarecyte.com
- To learn more about HALO® image analysis workflows, email info@indicalab.com



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