Comparison of multiplex immunofluorescence and H&E-based approaches for characterization of the tumor microenvironment

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Background

- Predictive models applied to digital pathology images show promise for the rapid and objective analysis of patient samples to identify features of the tumor microenvironment (TME) predictive of treatment response.
- Multiplex immunofluorescence (mIF) and deep learning models applied to H&E-stained slides are two methods to interrogate the TME for tissue and cell identification.

Methods

- Adjacent sections from primary or metastatic tumors (n=91) from patients with colorectal, non-small cell lung, ovarian, pancreatic, and breast cancer were stained by mIF (Akoya PhenoImager) and H&E.
- mIF image analysis using QuPath was done for tumor-stroma segmentation and to identify necrotic tissue within the pathologist-annotated tumor bed. Cytotoxic T cells, immune cells, and fibroblasts were identified using CD8, CD45, and COL1A1 stain thresholding, respectively.
- AI-powered TME models developed by PathAI (Boston, MA; commercially available as PathExplore™) were deployed on the H&E slides for tissue classification (tumor epithelium, stroma, necrosis) and cell identification (cancer cells, lymphocytes, macrophages, plasma cells, fibroblasts).
- Tissue and cell features were compared between the approaches. Areas of tumor epithelium, stroma, and necrosis were assessed qualitatively with areas of disagreement undergoing independent pathologist review. The density of CD8+ cells from mIF was compared to lymphocytes from H&E, of CD45+ immune cells from mIF to lymphocytes, macrophages, and plasma cells from H&E, and of COL1A1+ cells from mIF to fibroblasts from H&E, recognizing that these cell populations do not overlap completely.

Conclusions

- Automated analysis of digital pathology images is a rapidly emerging field with broad potential to analyze pathology tissues accurately and reproducibly across tumor types.
- PathAI's TME models are a robust tool to distinguish tissue and cell features from H&E slides, comparable to mIF image analysis, but requiring less effort, time, and expense.
- Indication specific differences in cell classifications point to more accurate performance by H&E models than mIF.
- With additional refinement, these technologies could allow efficient evaluation of large pathology datasets for discovery of novel features to inform biology and patient care.

Results

- The mIF and H&E approaches showed good tissue segmentation performance, producing broadly similar annotations, with differences attributable to staining co-occurrence in mIF, lower performance of H&E models on metastatic samples, and disagreement at the tumor bed periphery.

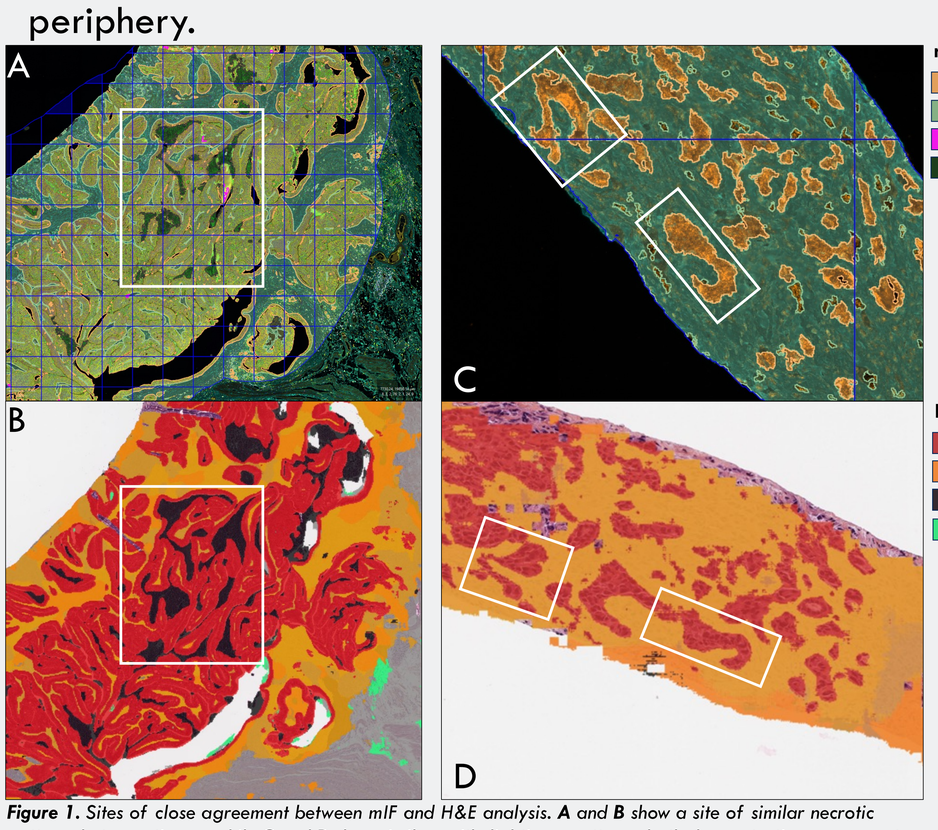

Figure 1. *Sites of close agreement between mIF and H&E analysis. **A** and **B** show a site of similar necrotic patterns between tissues, while **C** and **D** show similar epithelial tissue patterns, both demonstrating agreement between the two methods (see areas highlighted in white boxes).*

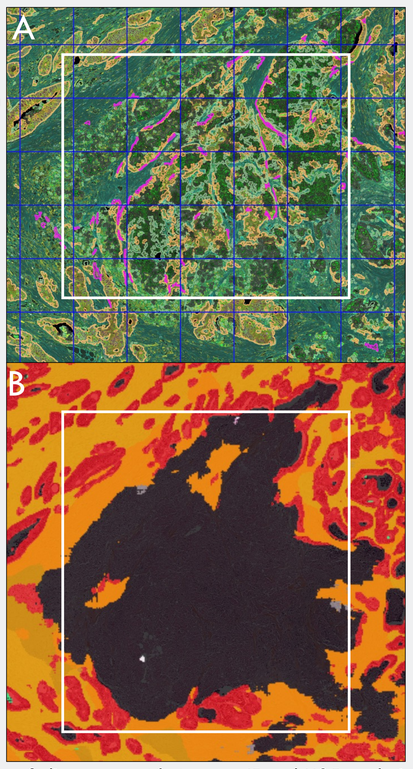

Figure 2. *Sites of disagreement between mIF and H&E analysis. Extensive collagen staining in a necrotic region (white box) confuses necrosis detection in mIF (**A**) but does not affect H&E (**B**)*

- Cell identification showed broad agreement between the density of CD8+ by mIF and lymphocytes by H&E (r=0.66, range 0.30-0.93 by indication), CD45+ cells by mIF with immune cells by H&E (r=0.60, range 0.23-0.87), and COL1A1+ cells by mIF with fibroblasts by H&E (r=0.51, range 0.08-0.56) (**Figure 3, Table 1**).

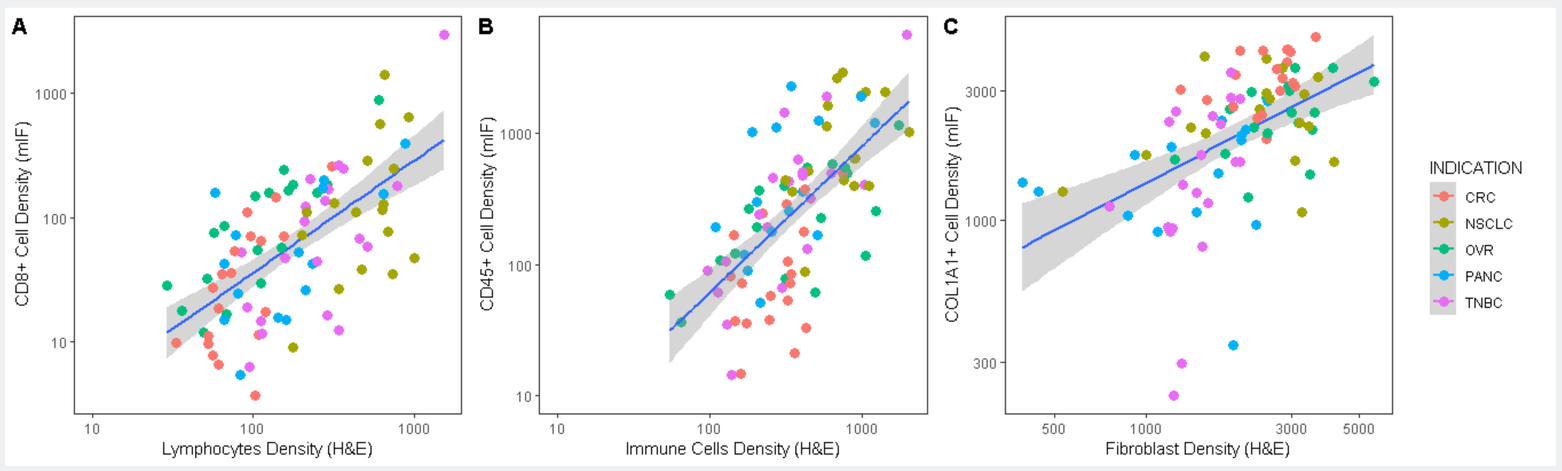

Figure 3. *Cell identification comparison between H&E models and mIF image analysis. A) Correlation of lymphocyte density by H&E with CD8+ cell density by mIF; B) Correlation of immune cell density by H&E with CD45+ cell density by mIF; C) Correlation of fibroblast density by H&E with COL1A1+ cell density by mIF. Colors by indication. The trend line is shown for all indications together.*

Indication	CD8+ Cells (mIF) to Lymphocyte Density (H&E)		CD45+ Cells (mIF) to Immune Cell Density (H&E)		COL1A1+ Cells (mIF) to Fibroblast Density (H&E)	
	R	SE	R	SE	R	SE
CRC	0.86	0.12	0.64	0.19	0.46	0.21
NSCLC	0.30	0.25	0.23	0.25	0.08	0.24
OVR	0.93	0.09	0.68	0.18	0.48	0.21
TNBC	0.88	0.11	0.87	0.12	0.56	0.19
PDAC	0.81	0.16	0.52	0.24	0.36	0.26
All Indications	0.66	0.08	0.60	0.09	0.51	0.09

Table 1. *Cell identification correlations between mIF and H&E-based approaches (low correlation in NSCLC attributable to highly variable staining in mIF samples, particularly COL1A1).*

CRC: Colorectal Cancer; NSCLC: Non-Small Cell Lung Cancer; OVR: Ovarian Cancer; PANC: Pancreatic Cancer; TNBC: Triple Negative Breast Cancer; SE: Standard Error

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