1314 POLARITY MEASUREMENTS FROM MULTIPLEX IMAGING SUGGEST IMMUNE CELL ACTIVATION

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Background Multiplexed immunofluorescence (mIF) methods image dozens of molecules at subcellular resolution and whole-slide scale, allowing detailed characterization of tumor microenvironments. Although cells exhibit differential subcellular localization of proteins and other molecules depending on their functional states,[1–3] these morphological features are difficult to quantify. They are discarded in standard analysis workflows. In this work, we develop a metric defining surface protein polarity from mIF images and apply it to enhance immune cell phenotyping. We define six cell subtypes relating to morphology and measure their relation to patient outcomes.

Methods Our polarity quantification is described in figure 1. Briefly, we apply a polar transformation to cell-centered image patches, denoise, and take the area-under-the-signal-curve, resulting in a polarity score (range 0–1). The score is thresholded to produce a classification into polar and non-polar cells.

We train two models: a 3-layer multilayer perceptron (MLP) neural network that accepts the percent composition of cell types per sample and predicts binary 60-month survival; and a graph-based neural network[4] that accepts 3-hop cell neighborhoods and predicts sample-level survival status.

Results We verify that polarity events (figure 1) are correlated with positive patient outcomes, even after controlling for technical artifacts. Introducing polarity-aware cell subtypes improves model performance in predicting survival status across three patient cohorts (table 1). Given that polar expression can indicate antigen engagement during contact with tumor cells,[5] we confirm that polar cells are more likely than uniform cells to be adjacent to tumor cells; conversely, cells with tumor cells as direct neighbors are more likely to be polar. Tumor cells are significantly more likely to neighbor polar than non-polar immune cells (figure 2).

Finally, keeping the number of polar cells constant, we permute the spatial arrangement of polar cells. This significantly influences survival predictions (figure 3), suggesting that polar cell contacts, not simply their presence in a sample, are important.

Conclusions We describe a robust, interpretable subcellular morphology metric that reflects macrobiological states. Although labeling polarity events as immune synapses is premature, they represent biologically relevant signals in tumor microenvironments.

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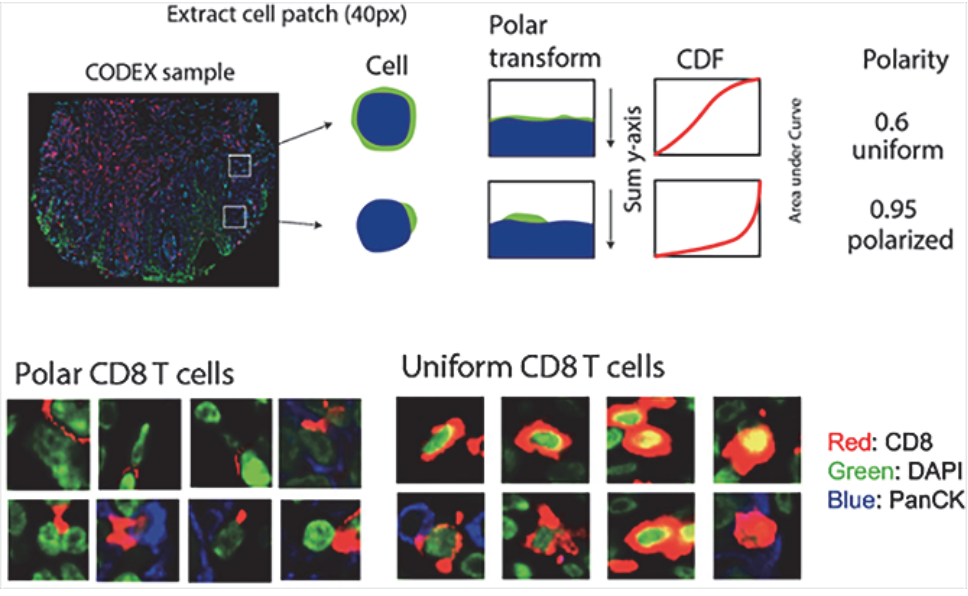
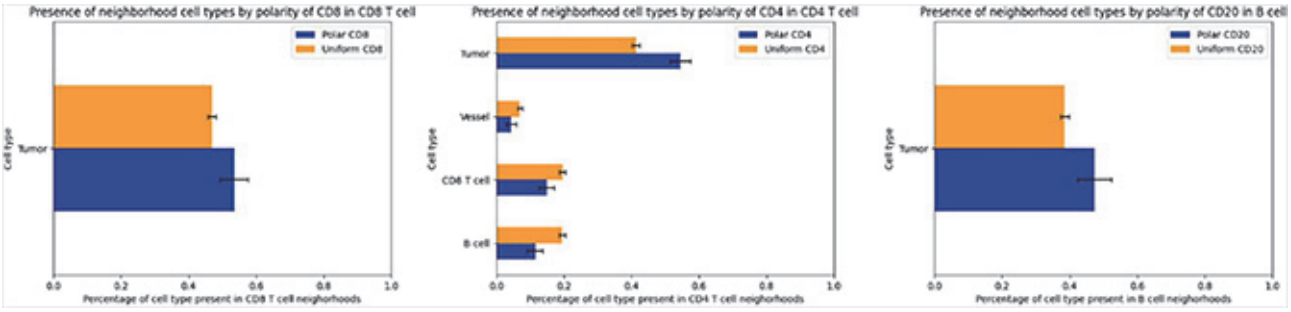
Abstract 1314 Table 1 Prediction performance on patient survival status increases when introducing the additional polarity-derived cell types. This effect is observed across multiple datasets and disease types

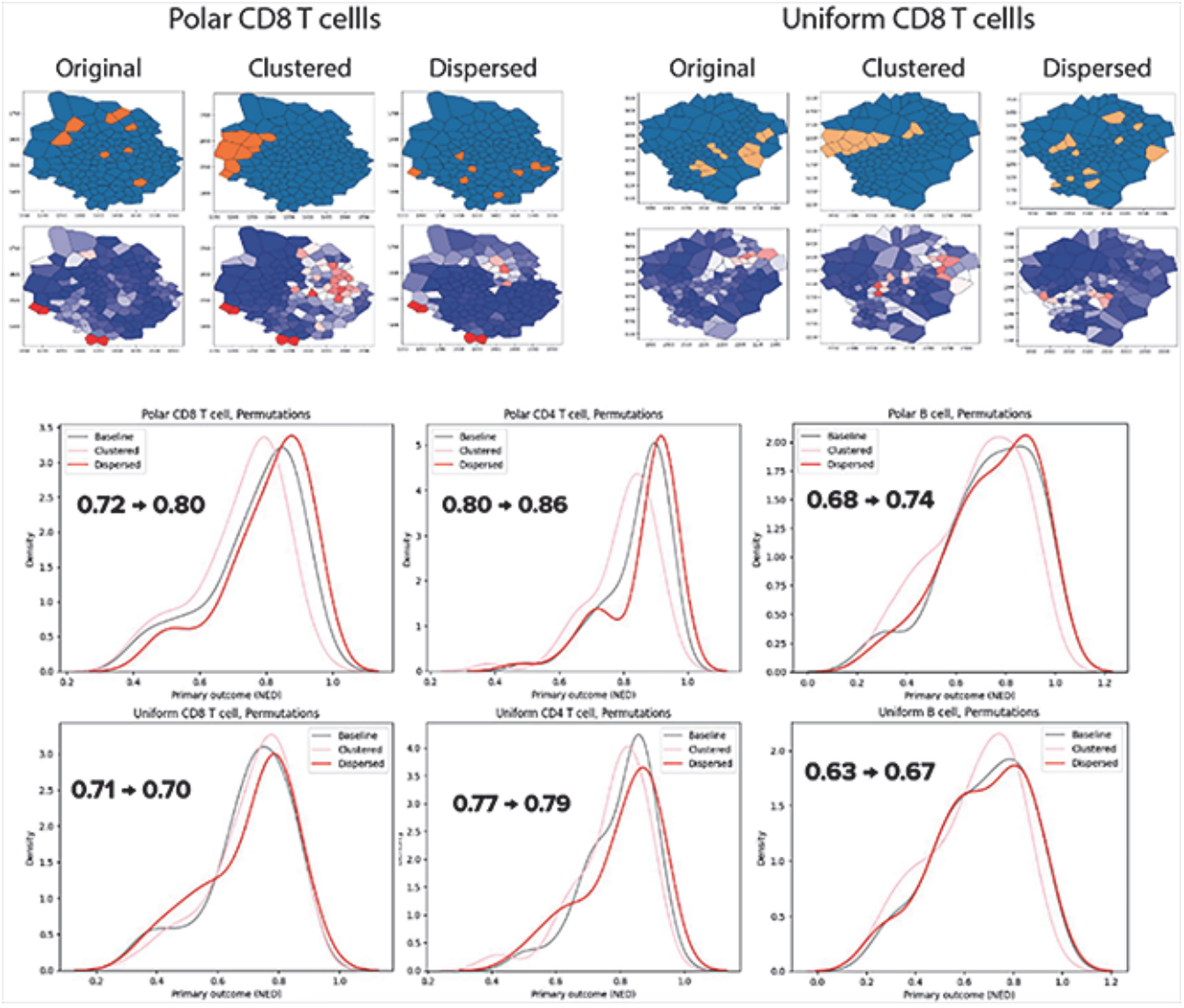
Survival status	UPMC-HNC	Stanford-CRC	DFCI-HNC (using UPMC-HNC model) generalization
3-layer MLP (full sample)			
- Baseline	0.767	0.481	0.630
- Adding polar and uniform CD8+ T cells, CD4+ T cells, B cells	0.805	0.502	0.652
GNN			
- Baseline	0.839	0.684	0.853
- 6 additional cell types	0.856	0.743	0.880



Abstract 1314 Figure 1 Workflow of computing polarity and Samples of polar and uniform cells



Abstract 1314 Figure 2 Neighborhood composition of polar vs uniform T and B cells. Tumors are significantly more present next to polar cells vs uniform cells. Only neighbor cell types with significant differences between polar and uniform are shown



Abstract 1314 Figure 3 Permutation experiments: Sample 7-hop neighborhood permutations for CD8 T cells. Left is for polar CD8+ T cells, and right is for uniform CD8+ T cells

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