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Paletrra™ AI: Automated phenotyping of multiplex immunofluorescence datasets via information maximizing self-training

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Abstract:

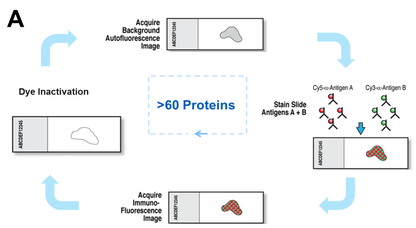
Multiplex immunofluorescence (mIF) is a powerful tool for profiling dozens of biomarkers from a single tissue section. Customized mIF panels enable oncology researchers to phenotype cells within the tumor microenvironment, interrogate activation status of immune cells, quantify expression levels of biomarker targets, and explore the spatial organization of cells. Yet, analysis of large mIF datasets remains a bottleneck, largely due to the difficulty of accurately phenotyping single cells across large tissues.

The incorporation of deep-learning algorithms into mIF analysis pipelines has helped overcome some limitations of traditional intensity gating by using stain morphology to add robustness to intensity variation, spatial spillover, and tissue artifacts. However, these deep-learning algorithms are costly to develop from a data volume and/or annotation perspective, often requiring fine-tuning on manual labels from target datasets to achieve acceptable performance. Therefore, there is demand for algorithms that efficiently generalize across batches, mIF panels, and tissue types.

Here, we present a weakly-supervised framework for adapting a pretrained single-channel feature extractor into a mIF whole panel classifier capable of zero-shot cell phenotyping. Our source model is a single-channel feature extractor trained on 20 million annotations spanning over 40 biomarker classes, with text conditioning to encode marker-specific interpretations. We adapt this model into a whole panel classifier by introducing both single-marker binary heads and a multi-marker phenotyping head, jointly trained through self-distillation to enforce per-channel consistency while promoting information maximization across the multiplexed panel. This strategy preserves interpretable single-marker classification while leveraging cross-channel context for accurate and scalable phenotyping of mIF datasets.

We applied this framework to 27 FFPE samples stained with a 17-marker mIF panel and analyzed them using Paletrra™ AI (NeoGenomics Laboratories, Inc). Notably, we showed that our framework achieved data-efficient cell phenotyping performance and reduced sensitivity to common mIF issues such as batch effects and spatial spillover, as compared to other mIF image analysis techniques. Overall, our method provides a lightweight, automated method for adapting pretrained mIF algorithms to new panels with strong generalization performance to novel biomarkers.

Staining and imaging workflow

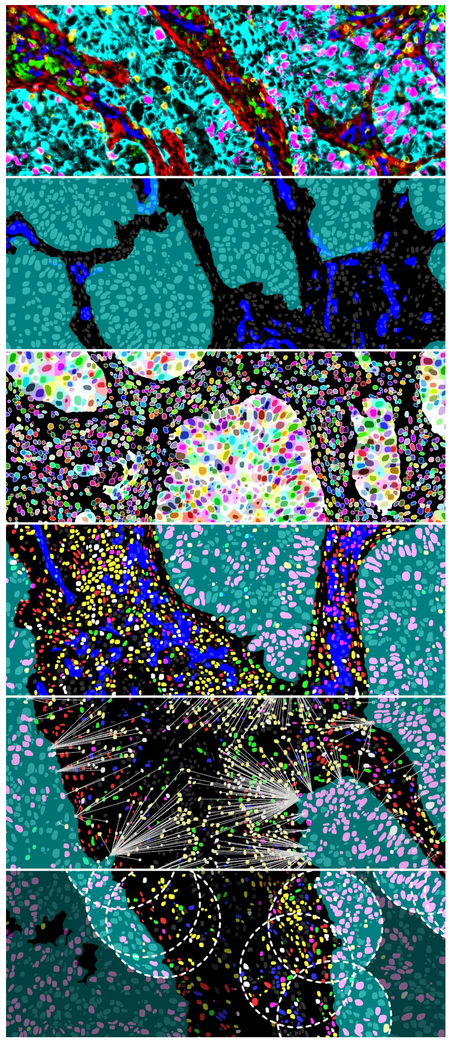


B

Biomarkers	
CD11B	CD163
CD3	CD31
CD4	CD68
CD8	CTLA4
FOXP3	GZMB
HLADR	LAG3
PD1	PD-L1
SOX10	TBET
TIM3	

Figure 1: (A) Assay Workflow. Melanoma slides were prepared, imaged, and stained using our mIF staining protocol. For each round of staining, conjugated fluorescent antibodies were applied to the slide, followed by imaging acquisition of stained slides. The dye was erased, enabling a second round of staining with another pair of fluorescent antibodies. (B) A 17plx panel containing immune, stroma, and tumor biomarkers was stained on all melanoma samples.

Paletrra AI-enabled image analysis



Biomarker Acquisition
- Biomarkers are imaged at subcellular resolution to enable precise biomarker interpretation and cell phenotyping

Tissue Segmentation
- Tissue is segmented into functional regions (e.g. tumor, vasculature)
- Artifacts & necrosis are automatically detected and removed

Cell Segmentation
- Cells are identified and subcellular compartments are segmented
- Single cell marker intensities are measured in the relevant staining compartment

Cell Phenotyping
- AI-enabled biomarker classification based on stain intensity and pattern
- Cells are phenotyped based on marker coexpressions

Spatial Analytics
- Spatial relationships between key cell phenotypes
- Nearest neighbor distances between two cell types (upper panel)
- Cell composition within a specified proximity of a target cell type or tissue structure (lower panel)
- Neighborhood analysis to identify cellular niches within the tumor microenvironment
- Intensity metrics to investigate biomarker expression heterogeneity

Figure 2: Paletrra AI workflow. Biomarker acquisition is followed by tissue segmentation, cell segmentation, cell phenotyping, and spatial analytics. Spatial analytics are configurable for each multiplexing panel and can be tailored to answer specific research questions.

Semi-automated single-cell phenotyping

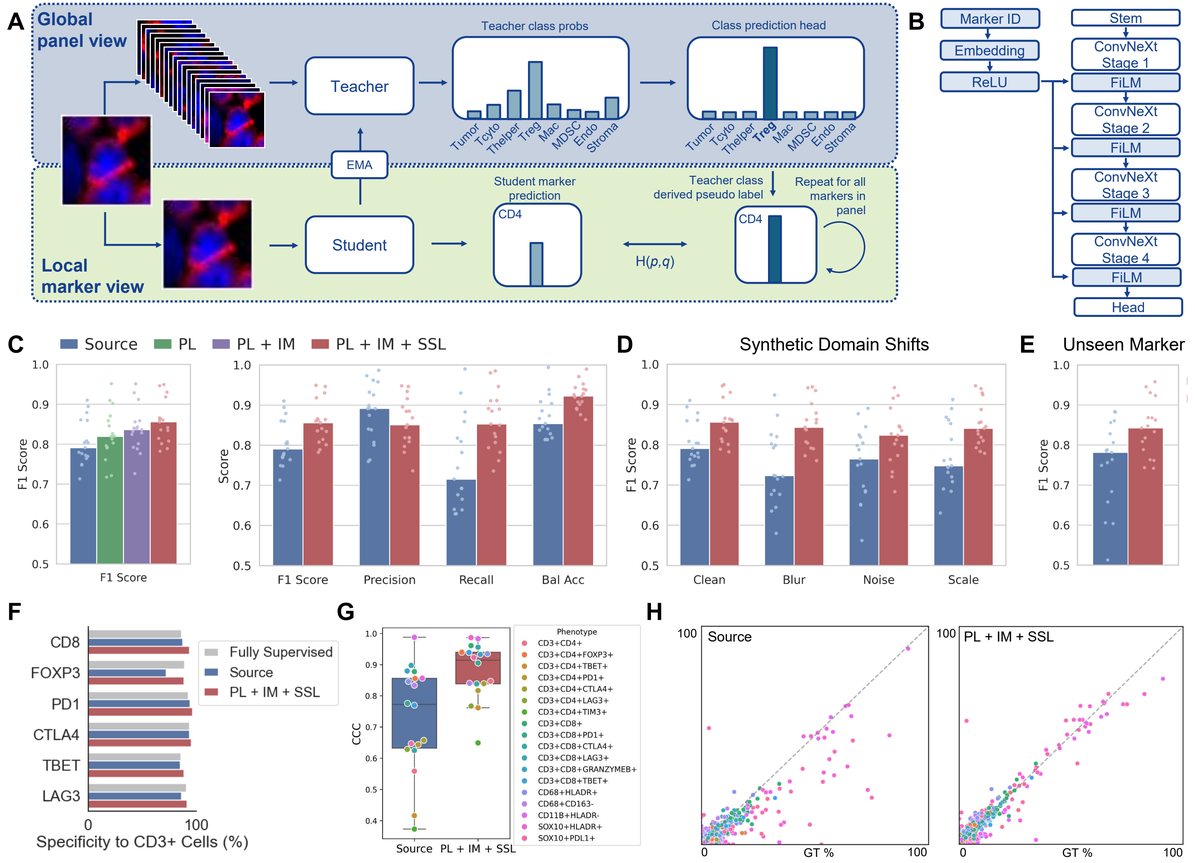


Figure 3. **A.** Schematic of weakly supervised mean teacher framework. The teacher network analyzes global, multi-marker cell views to predict phenotypes, while a student model uses marker-specific classifiers operating on single-marker inputs. Teacher phenotype predictions are mapped to marker-level pseudo-labels (PL) to supervise the student via a consistency objective. A class prediction head is attached to the teacher to maximize mutual information across the entire panel (IM) and align pseudo labels with phenotype patterns. Small amounts of manually labeled data can be spiked in to improve alignment with ground truth (SSL: 50 cells, or 0.16% of total training data). Adaptation procedure runs on a single A30 GPU in <20min. **B.** Schematic of the source model, a ConvNeXt encoder conditioned on marker identity via FiLM, where marker embeddings generate stage-wise feature modulations throughout the network. **C.** Performance metrics of the source model (blue) and three adaptation variants: PL only (green), PL + IM (purple), and PL + IM + SSL (red). Evaluation is against data labeled by a fully-supervised model trained on the melanoma dataset (GT, silver standard). **D.** The adapted model outperforms the source model across domain shifts: blur (Gaussian, σ 0.5μm), noise (multiplicative Gaussian, σ 0.25), and intensity scaling (0.5×). **E.** Performance on unknown markers evaluated by masking a single marker identity prior to adaptation. **F.** Specificity of T-cell markers to CD3+ cells, demonstrating robustness to spillover and artifacts relative to source. **G.** Concordance correlation coefficient for coexpressions, measured against ground truth positivity in a set of 100μm image crops. **H.** Scatterplot depicting the coexpression positivity used in G, with each dot representing a single coexpression and image crop.

Conclusions

- Paletrra AI-enabled image analysis pairs cell phenotyping with configurable spatial analytics for a comprehensive mIF image analysis solution.
- A semi automated cell phenotyping algorithm was developed for efficient single-cell quantification across biomarkers and panel configurations.
- Generalization to a specific mIF panel is achieved without panel-specific pretraining by adapting single-channel classifiers to phenotype-consistent expression patterns in the target dataset.
- The methodology is robust to domain shifts due to weak supervision based on expected co-expression patterns that guide consistent marker–phenotype relationships across datasets.