Enhancing insights in low-plex multiplex immunofluorescence: leveraging the potential of same-slide H&E analysis

Becky Arbiv[1,4], Assaf Debby[2], Nethanel Asher[3], Guy Ben-Betzalel[3], Ron Elran[1], Pinchas Birnbaum[1], Shai Bookstein[1], Tal Dankovich[1], Lena Tsabari[1], Yuval Shachaf[1], Yoad Cohen[1], Amit Bart[1], Oscar Puig[1], Kenneth Bloom[1], Ronnie Shapira-Frommer[3], Iris Barshack[2], Ettai Markovits, MD[1]

[1]Nucleai, Tel Aviv, Israel; [2]Ella Lemelbaum Institute for Immuno-oncology, Sheba Medical Center, Tel Hashomer, Israel; [3]Institute of Pathology, Sheba Medical Center, Tel Hashomer, Israel; [4]Corresponding author: becky@nucleai.ai

#64

Introduction

- Multiplex immunofluorescence (mIF) is a powerful tool for characterizing the tumor-microenvironment (TME).
- However, the adoption of high-plex panels in the clinical setting is limited due to cost and technical complexity.
- Hematoxylin and eosin (H&E) staining provides morphologic information about tissue structure and cellular composition but despite its use in routine pathology, its potential for extracting meaningful data in combination with mIF has been largely overlooked.
- In this work, we leveraged same-slide H&E and mIF images to enrich our analysis, enabling identification of known biomarkers, unidentifiable from low-plex mIF alone.

Methods

- We stained consecutive slides from 57 melanoma biopsies using two 6-plex immunofluorescence antibody panels and same-slide H&E staining.
- The panels consisted of markers for immune and tumor cells, with the first panel including T-cell markers (CD4, CD8) and the second panel composed of B-cell markers (CD20, CD38).
- Using a deep learning-based multiplex imaging analysis pipeline[1], we generated predictions for 6 cell types for each mIF panel (Figure 1).
- H&E slides were processed with deep learning models to detect and classify cells. The mIF and H&E slides were registered, and mIF marker-negative cells were reclassified based on their registered H&E cell type to identify novel cell types.
- TLS detection was performed on the T-cell panel based on identification of non-T cell lymphocyte aggregates and was evaluated against TLS annotations based on the B-cell panel and corresponding H&E.

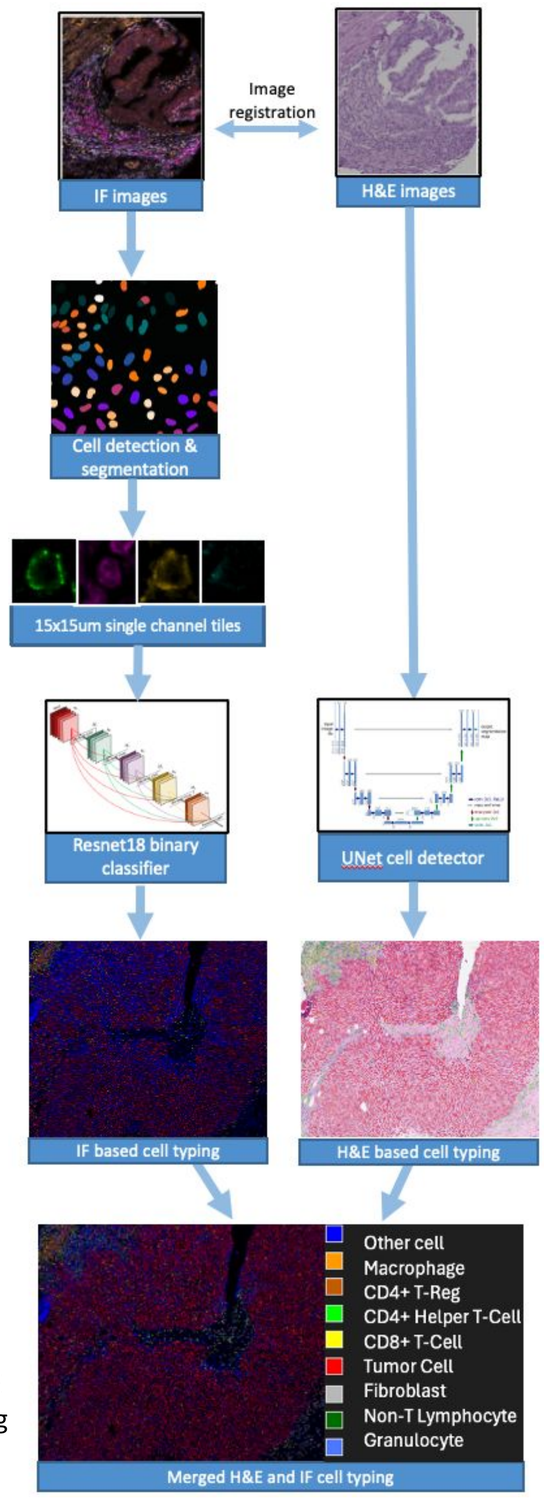

Figure 1: merged mIF and H&E deep learning-based cell typing workflow.

Results

- From the 20 million cells detected in the B-cell panel, 9 million were successfully classified as tumor-, dendritic-, plasma-, or B-cells. However, a significant number of cells were classified as marker-negative cells.
- Integrating H&E cell typing, we reclassified 6 million cells as SOX10-negative tumor cells, fibroblasts, granulocytes, and non-B-lymphocytes.
- The non-B-lymphocytes, derived from H&E images, showed strong correlation with T-cell counts identified by the T-cell mIF panel, enabling us to assess T-cell infiltration in the tumor invasive margin using the B-cell panel (Figure 2).

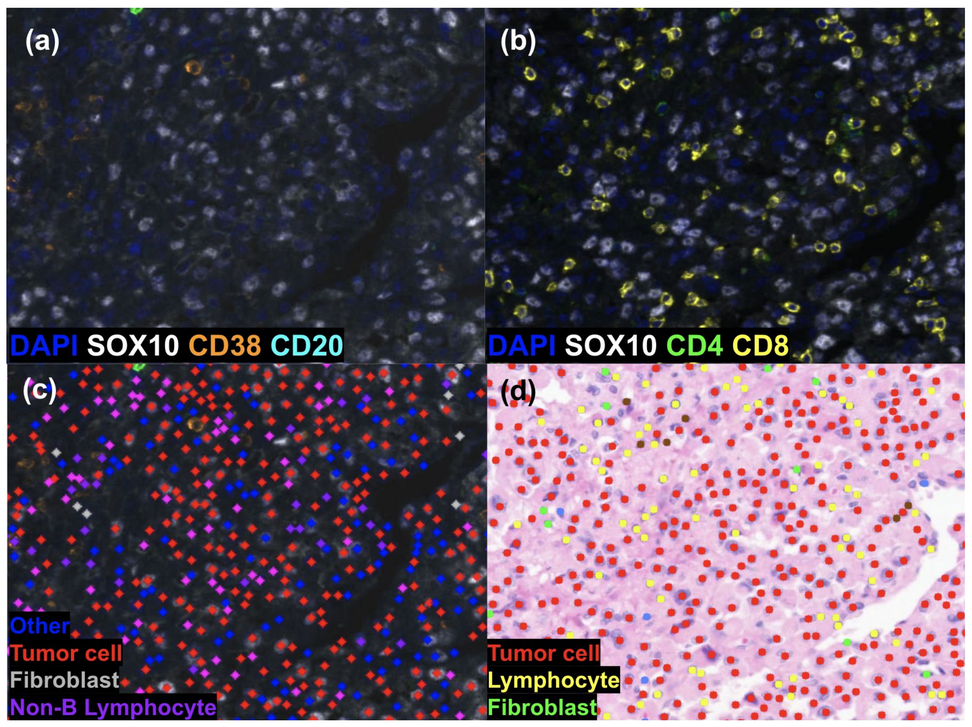

Figure 2. Tumor infiltrating lymphocytes identified from B-cell panel using H&E enhancement (a) B-cell panel, (b) T-cell panel, (c) mIF with H&E enhanced cell type predictions, (d) H&E cell type predictions

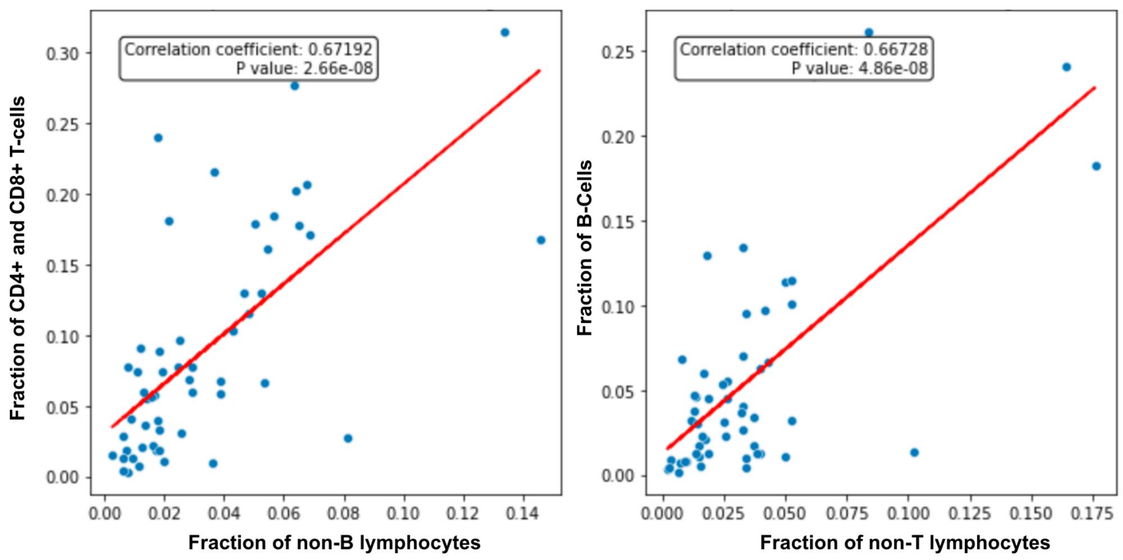

Figure 3: (a) Correlations between mIF detected T-cells from T-cell panel and non-B cell lymphocytes from the corresponding B-cell panel with H&E enhancement (b) Correlations between mIF detected B-cells from B-cell panel and non-T cell lymphocytes from the corresponding T-cell panel with H&E enhancement

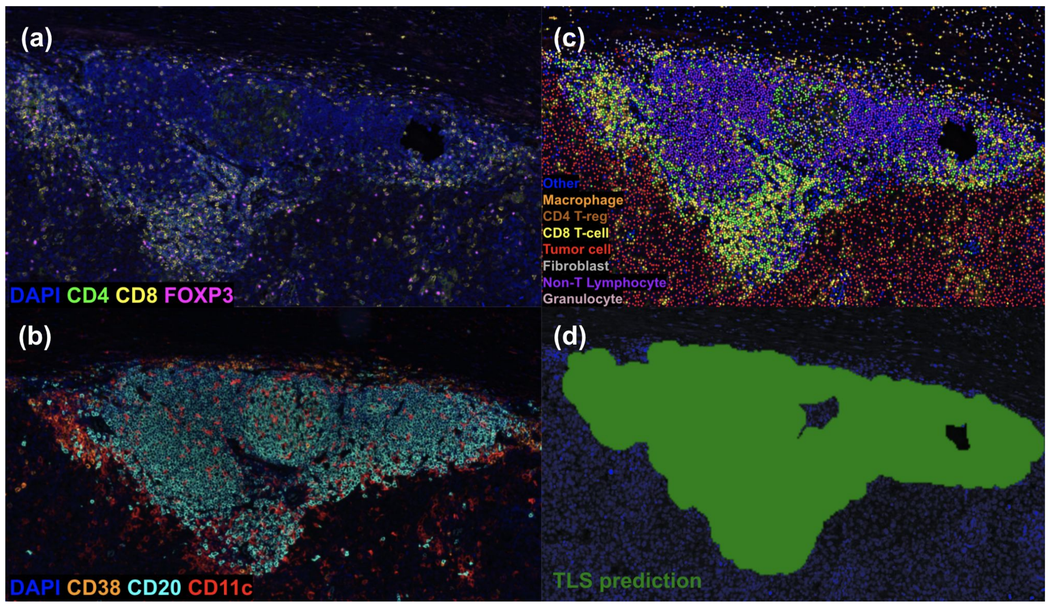

Figure 4: TLS identification from T-cell panel using H&E enhancement. (a) T-cell panel, (b) B-cell panel, (c) mIF with H&E enhanced cell type predictions, (d) TLS model prediction

- In the T-cell panel we reclassified 5.5 million cells, including non-T-lymphocytes, which showed high correlation with B-cell numbers in the B-cell panel (Figure 3).
- Leveraging these reclassified cells, we developed a TLS identification model (Figure 4).
- TLS were identified using the B-cell panel as dense aggregates of CD20+ displaying a germinal center
- Using the merged cell typing, we were able to identify TLS from only the T-cell panel and H&E with 88% accuracy (compared against mIF-based manual annotations)

Conclusions

- Our analysis demonstrates the value of integrating same-slide H&E analysis in low-plex mIF studies.
- By leveraging the morphologic information provided by H&E slides, we were able to identify additional cells and accurately quantify known biomarkers for immunotherapy response, such as TLS and T-cell tumor infiltration, which were not identifiable otherwise.

References

1. Markovits, E. et al. A novel deep learning pipeline for cell typing and phenotypic marker quantification in multiplex imaging. bioRxiv 2022.11.09.515776 (2022) doi:10.1101/2022.11.09.515776.