Generating Virtual Multiplex Images from Sequential Immunohistochemistry (IHC) Slides using Deep Learning

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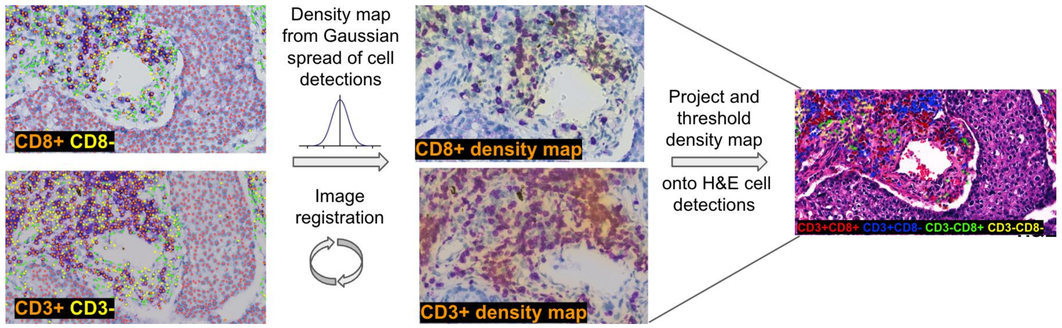
INTRODUCTION

- IHC staining is an important tool to assess antigen expression in tissue, however, it is limited when performed as a single-plex assay since it does not allow assessment of co-expression and other antigen interactions.
- We employed deep learning (DL) to generate a virtual multiplex (VM) image from H&E and four sequential IHC slides to calculate and visualize these features.
- This tool can overcome current challenges in integrating different H&E and IHC stains to a single image, including tissue artifacts, staining variability and slice differences.

METHODS

- Sequential slides stained for H&E, CD3, CD8, CD163, and PD-L1 IHC were collected from 120 non-small-cell lung cancer (NSCLC) patients. Cohort was split into training (n=43), validation (n=48) and test (n=29) sets.
- DL models based on Unet were trained on each stain separately to classify tumor cells, lymphocytes, granulocytes and fibroblasts, and tumor versus stromal areas. Model performance was evaluated by comparing DL detections to manually annotated test slides, and pathologist's review.
- An image alignment model that uses cell and area morphology was run pairwise between each IHC slide and the H&E slide to create a registration map between the two slides. IHC cell detections were then projected onto the corresponding H&E slide.
- Density maps for positive and negative cells were generated by spreading cell detection in a Gaussian distribution to overcome image alignment inaccuracies and tissue differences.
- To identify co-expression of IHC markers, the positive and negative density maps of each stain were interpolated at each H&E cell detection to determine its positivity or negativity for each marker. (CD163+)

METHODS



Workflow of the VM model generation

- In order to assess VM performance, we evaluated sensitivity by calculating the fraction of CD3+CD8+ (true positive) lymphocytes from CD3+CD8+ and CD3-CD8+ (false negative) lymphocytes, as all CD8+ lymphocytes should co-express CD3. Specificity was calculated as the proportion of CD8- non-lymphocytes (true negative) from CD8- and CD8+ (false positive) non-lymphocytes, since CD8 is expressed exclusively on lymphocytes.
- The VM can be visualized using a web-based platform, allowing users to analyze the spatial arrangement of immune cell subclasses on H&E slides.

RESULTS

- The H&E cell detection and area segmentation models reached an accuracy of **81% and 86%** on the test slides, respectively. IHC cell detection models reached an average accuracy of **81%** across different cell types and IHC stains (range: 77%-86%)

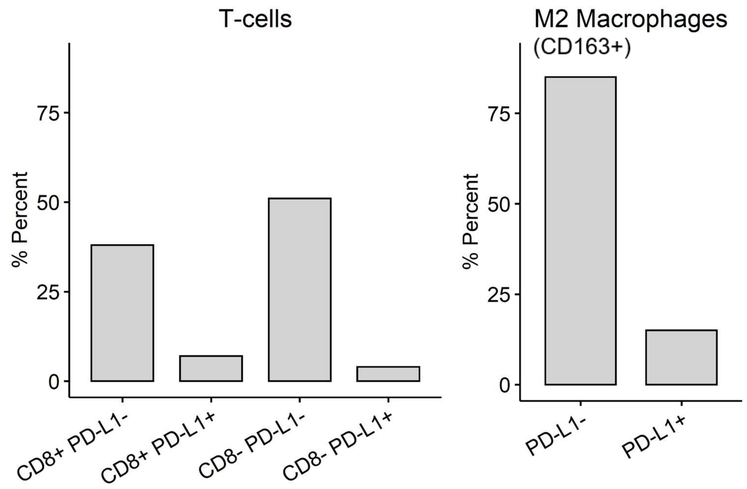
Reference \ Predicted	Tumor cells	Fibroblasts	Lymphocytes	Granulocytes
Tumor cells	94%	3%	3%	0%
Fibroblasts	15%	80%	4%	1%
Lymphocytes	3%	4%	91%	2%
Granulocytes	1%	2%	12%	85%

Accuracy of cell detection model

- Sensitivity of the VM was **70% and specificity was 90%**.

RESULTS

- 2,200,000 lymphocytes and 150,000 macrophages were detected in the cohort and the VM further classified them based on expression of phenotypic markers.



Cell classification based on VM results

CONCLUSIONS

- DL can utilize single IHC stained slides of sequential cuts to analyze combinations of markers to better understand the spatial arrangement of immune cell subclasses.
- This technique can be adapted to any number of markers and enable researchers to quantify co-expression between multiple proteins on the single cell level without the overhead of developing a multiplex antibody assay.
- Our unique image alignment model along with the high accuracy of IHC cell detection allow a reliable analysis and fast turnaround time on an easily accessible platform.

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