Immunohistochemistry-informed AI systems for improved characterization of tumor-microenvironment in clinical non-small cell lung cancer H&E samples

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CONCLUSION

This study demonstrates the value of combining histomorphological and immunohistochemistry (IHC) data for improved cell annotation and model training

We provide a quantitative benchmark of the limitations of human cell-level annotations on hematoxylin and eosin (H&E) alone and demonstrate the improvement in inter-rater agreement when a co-registered IHC stain is provided

We also utilize registered same-section H&E and IHC stains to simultaneously automate the extraction of H&E training data and capture the increase in annotation quality achieved when humans had access to IHC

17% increased agreement between pathologists measured in cell count correlation and **62%** increased agreement considering location of individual cells, when registered IHC images are provided, compared to annotating on H&E alone

IHC-informed automatically extracted labels can be used to train H&E-based machine learning models that perform on par (carcinoma, plasma cells) and outperform (lymphocytes) pathologists

INTRODUCTION

- Automated cell-level characterization of the tumor microenvironment (TME) at scale is key to data-driven immuno-oncology.[1] Artificial intelligence (AI)-powered analysis of hematoxylin and eosin (H&E) images provides a scalable solution and has recently been translated into diagnostics[2]
- Typically, deep learning models for cell classification are trained using manual pathologist annotations, which are both expensive and time consuming to generate.[3] Additionally, manual pathologist annotations are most frequently made using the H&E stain alone. Given that H&E staining is inherently non-specific to different cell types, this results in disagreement between pathologists on the identity of individual cells
- Accurately co-registered immunohistochemistry stains provide cell-specific labels, which have the potential to improve the accuracy of manual annotation and can facilitate automated label extraction

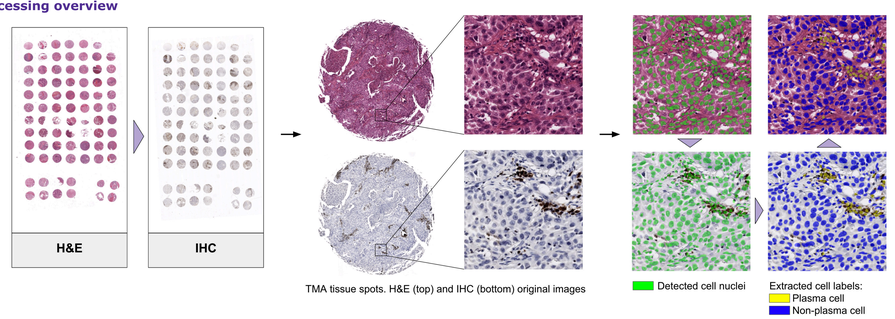
OBJECTIVES

1. Investigate and quantify error introduced by pathologists in defining carcinoma, lymphocyte, and plasma cells from H&E scans
2. Establish a novel image analysis workflow to enable training of improved AI-systems for morphological assessment of H&E scans based on (a) high-precision image registration and (b) incorporation of IHC information
3. Assess the performance of AI-models trained with this novel approach regarding tumor and TME characterization from H&E scans

METHODS

- The work was carried out on 239 clinical non-small cell lung cancer (NSCLC) cases
- The workflow is based on co-registration of same-section H&E and IHC stained images with micrometer precision
- CK-KL1, CD3+CD20, and Mum1 were used for defining carcinoma, lymphocyte and plasma cells, respectively
- Cells were detected in H&E and labelled using rule-based algorithms that incorporated IHC information
- This H&E data was used to train neural networks (NN)
- For evaluation, representative regions were annotated by three trained pathologists

Figure 1. Data processing overview



RESULTS

Figure 2. The inter-rater agreement of pathologists annotating on H&E is increased when information from registered IHC images is provided

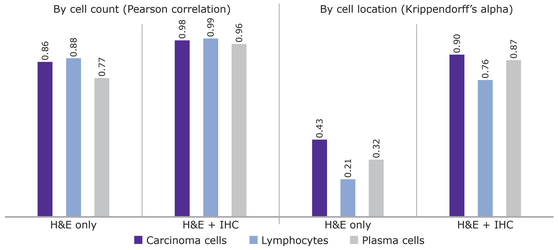
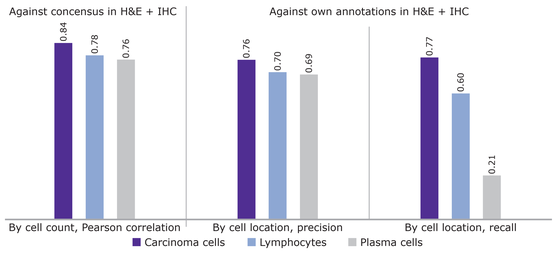


Figure 3. The performance of pathologists annotating on H&E only compared to H&E + IHC shows that pathologists miss or misclassify cells with a certain error



H&E, hematoxylin and eosin; **IHC**, immunohistochemistry

- Providing pathologists access to the co-registered IHC-stain improved the cell count inter-rater agreement for all cell types (carcinoma: +0.12, lymphocytes: +0.11, plasma: +0.19, as measured by Pearson correlation). The location-based inter-rater agreement was also consistently increased when IHC was available (carcinoma cells: +0.47, lymphocytes: +0.55, plasma cells: +0.55, as measured by Krippendorff's alpha) (**Figure 2**)
- Considering the IHC-informed pathologist annotations as 'ground truth' allows direct evaluation of pathologists annotating in H&E alone. When compared against their own IHC-informed annotations, annotators had an average precision of 0.76 in carcinoma cells, 0.70 in lymphocytes and 0.69 in plasma cells. Recall was notably low in plasma cells (0.21) (**Figure 3**)
- Neural networks trained on high quality IHC-informed automatically extracted labels show impressive performance on all three cell types (**Table 1**)

Table 1. NNs trained with IHC-based labels achieve similar performance for cell type classification on H&E as pathologists annotating on H&E

Cell type	By cell count (Pearson correlation)
Carcinoma cells	0.84
Lymphocytes	0.92
Plasma cells	0.75

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