A Deep-Learning Based Approach to Accelerate Groundtruth Generation for Biomarker Status Identification in Chromogenic Duplex Images

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Abstract

Immunohistochemistry based companion diagnosis relies on the examination of single biomarkers for patient stratification. However, recent years have seen an increasing need to characterize the interactions between biomarkers in the tumor microenvironment. To this end, chromogenic multiplexing immunohistochemistry (mIHC) serves as a promising solution, which enables simultaneous detection of multiple biomarkers in the same tissue sections. To automate whole-slide scoring for mIHC, a crucial analysis step involves the detection of cell localization along with the biomarker staining status, which we call biomarker status identification. However, developing algorithms for such analysis, especially deep-learning (DL) models, often requires manual labeling at the cell-level, which is time-consuming and resource-intensive. Here, we present a DL based method to accelerate groundtruth label generation for chromogenic duplex (tissue samples stained with two biomarkers) images. We first generate approximate cell labels and then develop a DL based interactive segmentation system to efficiently refine the cell labels. Our method did not require extensive manual labeling and reduced the time of label generation to 50%-25% of manual labeling, while achieving <5% error rate in pathologist review.

Methodology

Approximate Cell Levels -

We used HALO (Indica Labs HALO image analysis platform) for initial stain unmixing, followed by tissue segmentation and biomarker status identification. The tissue segmentation was performed for three classes, (i) tumor, (ii) stroma and (iii) other. The biomarker status identification was performed to classify four types of cells: (i) PDL1+CK7+ (ii) PDL1+CK7- (iii) PDL1-CK7+ (iv) PDL1-CK7-.

Interactive Tissue Segmentation –

We observed inadequate performance of HALO tissue segmentation and thus developed a DL-based interactive segmentation system, inspired by (Sofiiuk et al., 2022). We first trained a DL model that learnt to respond to user input and then developed a GUI to enable users to provide input (mouse clicks) to the model at test time. Unlike existing DL-based interactive segmentation models (Sofiiuk et al., 2022, 2020) segmenting one target class at a time, we designed a three-class model. A high level working mechanism of this GUI is shown in Figure 1.

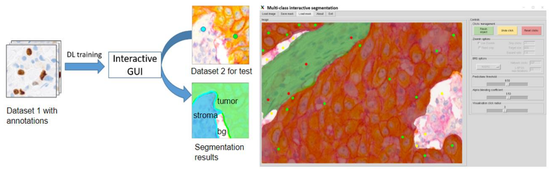

Figure 1 : High Level Working Mechanism of a Deep Learning Based Fast Interactive Segmentation GUI

Refining approximate cell labels with tissue masks - With the aforementioned segmentation, we identified the CK7+ cells in non-tumor regions as well as a large number of PDL1+ macrophages and non-tumor PDL1+ cells in necrotic and stroma regions, all of which we re-labeled as the fifth cell class, “Other”.

Visual Results

We observed erroneous segmentation of tumor regions with HALO as well as errors in the approximate cell detection results: (1) macrophages with moderate/strong membrane staining were detected as PDL1+; (2) benign epithelial cells showing Dabsyl staining were detected as CK7+; (3) Lots of cells in stroma region showing Tamra staining had been detected as PDL1+ (4) Multiple PDL1+ cells were incorrectly detected in the necrotic regions. Some examples are shown in Figure 2.

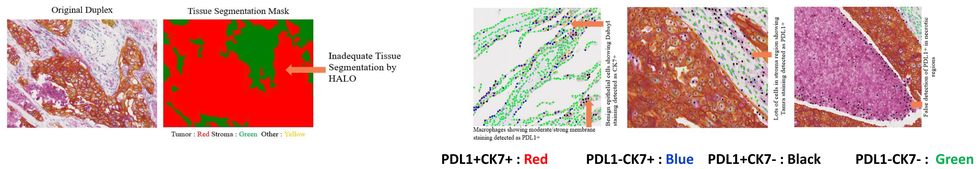

Figure 2 : Visual Representations of Errors in Segmentation and Approximate Cell False Detection Results by HALO

Visual Results

With the designed interactive segmentation system, we were able to generate accurate tissue segmentation masks (Figure 3(b)) with a few clicks per tissue class. We found that this system, while trained with natural scene images, was generalizable to mIHC images because it was trained to respond to arbitrary classes of regions guided by the simulated user clicks. With such tissue masks, incorrect cell labels for macrophages, CK7+ cells in non-tumor regions, PDL1+ cells in the stroma and necrotic regions were re-labeled as ‘Other’ cell types (Figure 3(c)).

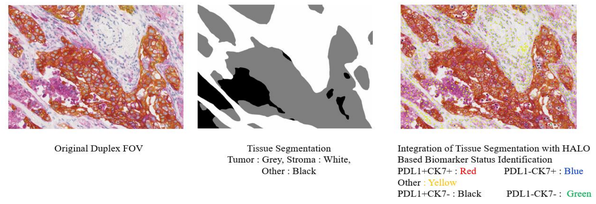

Figure 3: (a) An Example FOV (b) Tissue Segmentation Mask Refined Cell Labels

Quantitative Results

To ensure the validity of the cell labels, we first performed stain unmixing (Ruifrok and Johnston, 2001) of the duplex images to generate synthetic CK7 and PDL1 singleplex images respectively, followed by pathologist scoring within the tumor regions for each synthetic singleplex image. Three pathologists provided scores and their consensus scores (median of all three pathologists’ scores) were compared with the groundtruth scores from the corresponding cell labels, as shown in Figure 4, where vertical bars indicate range of pathologist scores. We observed that the scores of the generated labels aligned well with the pathologists’ scores, demonstrating the effectiveness of the proposed cell-level label generation method.

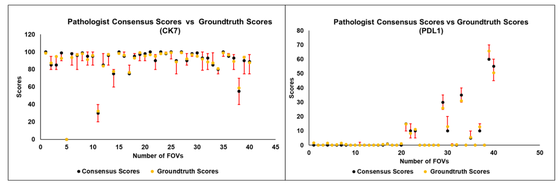

Figure 4: Comparison between Pathologist Scores and Groundtruth Scores

With this labeling approach, it only took around 15-20 minutes to label an FOV of 600x600 pixels in size, whereas manual annotation took around 45 minutes to 1 hour. With the generated labels, we were able to develop a UNet-based (Ronneberger et al., 2001) DL model to identify biomarker status at cell-level with >90% accuracy, which was confirmed by 3 pathologists.

Conclusion

In this project, we have developed a DL based method for accelerating cell-level labeling with minimal manual input. We first generate approximate cell labels and then develop a DL based interactive segmentation system to efficiently refine the cell labels. We demonstrated that our labeling approach is highly effective, fast and generalizable to various multiplex assays.

References

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