An Integrated Cell Region Reconstruction Method Based upon Mask R-CNN Model and Improved Voronoi Algorithm

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Abstract. Cell region segmentation in IHC staining images is the basis for quantitative analysis, which is very challenging due to the incomplete membrane staining in IHC images. In this paper, we propose a novel integrated cell region reconstruction method based upon Mask R-CNN model and improved Voronoi algorithm to reconstruct cell region in incomplete IHC staining images, in which Mask R-CNN model is introduced to perform nucleus segmentation, and then the improved Voronoi algorithm with the prior knowledge of nucleus-cytoplasm ratio is used to reconstruct the whole cell region. We also develop a framework for the integrated method to achieve automatic end-to-end cell region segmentation. Our method could better solve the problem of cell segmentation under incomplete staining than only deep-learning method or standard Voronoi algorithm, and the reconstructed cell region could better fit the real cell boundary.

1. Introduction

Precision medicine is a personalized diagnosis and treatment approach based on patient's pathological features or the genetic understanding of their disease. The quantitative analysis of programmed death-ligand 1 (PD-L1) immunohistochemistry (IHC) plays an increasing role in the precise treatment of tumors [1,2] in recent years, especially in the clinical immunotherapy in patients with non-small cell lung cancer, squamous cell carcinoma and adenocarcinoma. The expression level of PD-L1 is a basic metric for estimation of the effectiveness of drugs, which could be quantitated by tumor proportion score (TPS). TPS is defined by the percentage of viable tumor cells showing partial or complete membrane staining. In traditional clinical treatment, TPS is generally semi-quantitatively calculated by pathologists based on experience. Manual analysis not only takes a lot of time and effort, but is also extremely error prone. Therefore, designing an automatic quantitative analysis is in urgent need.

The TPS calculation should determine the number of positive tumor cells on the PD-L1 stained image first, of which the prerequisite is the automatic and accurate segmentation and classification of tumor cells. On IHC staining images, only some cells may respond to the target receptor, so the cell membrane with negative response is completely invisible; and the membrane staining of cells may also be incomplete, which could not visualize the complete cell membrane; furthermore, the superposition and diffusion of different stains on the sample will also introduce interference noise during slice staining. In view of the above reasons, it is very challenging to segment the cell membrane structure based on IHC staining images. We need to make full use of staining information as well as cell structure features to reconstruct or estimate intact cell structure, and then classify cells with the reconstructed cell structure.
At present, the research on cell membrane segmentation of IHC images is still rare, and most of the work can only deal with the automatic segmentation of the nucleus rather than the membrane segmentation [3, 4, 5]. The segmentation of the nucleus is relatively effective with current deep learning methods, such as U-Net [6], R-CNN [7], and Master R-CNN [8], however, it is still impracticable to obtain an accurate classification of cells based on only nucleus, because the staining reaction only exists on the cell membrane. [9, 10] uses elliptical approximation to treat cell membrane regions, which cannot accurately describe the morphological diversity of cells. [11, 12] achieves semi-automatic segmentation by artificially adding control points around the boundary of the target cell membrane, but still requires human intervention in the segmentation process. The active contour method [13, 14] can deal with the automatic segmentation of cell membrane, but this method is very sensitive to the staining quality and initialization process, and the segmentation result is also unstable. [15] can automatically segment the membrane according to the membrane staining color and the spatial position between the cells, but this method requires high quality of the staining and image pre-processing.

In order to address above problems, we proposes an integrated cell region reconstruction method based upon Mask R-CNN model and improved Voronoi algorithm to automatically segment cells in PD-L1 stained images. At the data level, pathologists and developers first accurately label the nucleus regions based on PD-L1 staining images to generate corresponding training sets. At the model level, we segment the nucleus regions based on the efficient, flexible and universal instance segmentation framework Mask R-CNN. The cell region is then reconstructed based on the improved Voronoi method and cytoplasmic ratio information. The proposed method can achieve cell region reconstruction under incomplete staining, and lay a foundation for more accurate cell classification based on the reconstructed cell regions.

2. Main Idea and Implementation

2.1. Main Idea
The integrated cell region reconstruction method based upon Mask R-CNN model and improved Voronoi algorithm mainly includes two key aspects of nucleus segmentation and cell membrane reconstruction. The main ideas are as follows: First, we train the Mask R-CNN model on the PD-L1 image dataset annotated with the nucleus region to automatically detect nucleus on real IHC image, as the annotation and detection of nucleus regions on PD-L1 images is relatively intuitive. Then, based on the identified nucleus region, the cell membrane area will be automatically reconstructed through the improved Voronoi algorithm and cellular cytoplasmic ratio parameters. The framework and modules of the integrated method is as shown in figure 1.

![Figure 1](image_url)

Figure 1. The framework and modules for the implementation of the integrated cell region reconstruction method based upon Mask R-CNN model and improved Voronoi algorithm.
The framework consists of an interactive UI, an image processing engine, an image segmentation agent, and a deep learning framework on the underlying layer. The image processing engine is the interface between the user and the developer, and is mainly used for the pre-processing and post-processing. In the pre-processing process, the image processing engine cuts the whole slide PD-L1 image (WSI) into smaller sub-images for the convenience of further annotation and detection. The post-processing module maps the detection result of the sub-images obtained by the image segmentation agent onto the original WSI, then the interactive UI could present these information to users. The interactive UI is the interface between the framework and users, mainly used for the data annotation and the presentation of the detection result. The image segmentation agent is the core module of the framework, which calls the underlying deep learning framework to perform automatic segmentation of the nucleus and automatic reconstruction of the cell membrane. The platform's modules are seamlessly integrated through pre-defined JSON files.

2.2. Implementation

2.2.1. Image annotation and dataset construction. The nucleus segmentation process of the cell region reconstruction method is implemented with supervised deep learning model, which could be trained on the annotated PD-L1 stained IHC image dataset. The scale and quality of the annotated dataset can greatly affect the prediction effect of the model. First, we annotate the nucleus area in the IHC staining image through the interactive UI, and the annotation of a single image will be saved as an individual JSON file. As shown in figure 2, the red polygonal area is a mask of the nucleus. Since the color features in nucleus area is relatively intuitive, the nucleus annotation does not require the participation of the clinical pathologist. The current dataset size is about $10^3$ scale, and the sub-image resolution is 1024*1024. As the dataset is continuously expanded and validated, the size and quality of the data set will continue to be improved.

![Figure 2. Part (a) is the original IHC stained image, where the blue region is the cell nucleus. Part (b) presents the manually annotated nucleus contours.](image)

2.2.2. Nucleus segmentation based upon Mask R-CNN Model. Based on the annotated PD-L1 dataset, we trained the Mask R-CNN [8] model for nucleus segmentation. The backbone network of Mask R-CNN model is ResNet101, which could automatically extract image features and perform multi-scale feature fusion to detect ROI regions. In order to decrease the error caused by the overlapping of neighboring nucleus, we introduce NMS (non-maximum suppression) algorithm in post-processing to improve the detection accuracy by eliminating the detection nucleus with high overlapping area or low classification confidence. The input images are normalized under Resnet rules, and augmented by means of rotation, inversion, cropping, Gaussian blur, and contrast adjustment to improve the training accuracy of Mask R-CNN model. The training process is scheduled with stochastic gradient descent method with a learning rate of 0.0001. The backbone network is initialized with the pre-trained Resnet weights. Initially, 50 epochs are used for initial training of the network header, and then 500 epochs
are used to refine the network weights. Finally the model weight at the 375 training epoch with best validation loss is finally selected as the best model for further detection. Using the trained network, we could obtain a detection result on test dataset. Our model could achieve a precision of 99% with the manually image annotation as ground truth. The detection results of two images in our test dataset are shown in figure 3, the yellow contour is the nucleus area automatically identified by the model.

![Detection Results](image)

Figure 3. Part (a) and (b) are the detection results of two IHC images in our test dataset with Mask R-CNN model.

2.2.3. Cell membrane reconstruction with improved Voronoi Algorithm. IHC-based precision medicine requires accurate segmentation, counting, or classification of stained regions or stained cells on IHC images. Cell counting and classification relies on complete cell region information, including information on the nucleus, cytoplasm, and membrane regions, therefore the cell region segmentation is the basis for further quantitative analyses. On IHC staining images with tumor, only some cells may respond to the target receptor, and the cell membrane with the negative response is completely invisible, and the membrane staining of the stained cells may also be incomplete, therefore the segmentation of cell region based on incomplete staining is very challenging. Cell region segmentation could be incomplete with only nucleus staining or partly cell membrane staining, therefore additional methods or additional information must be introduced in cell segmentation process in order to address above problems.

The Voronoi algorithm can be used for spatial segmentation. As shown in figure 4, the standard Voronoi algorithm divides the space based on a set of discrete reference points distributed in space, and other point is assigned to the adjacent region of the closest reference point accordingly. Voronoi algorithm will finally obtain a series of polygonal regions with the reference points as their centers. As shown in figure 4, part (a) is the result of standard Voronoi partition with the barycenter of the nucleus as the reference point. As can be seen, the boundary of the segmentation is straight lines, which is significantly different from the natural cell boundary. Furthermore, for some loosely distributed cells, the segmented cell region will greatly exceed the actual cell size, therefore, the segmentation quality is clearly far from satisfactory.

Essentially, there is a certain intrinsic correlation between nucleus size and cell size, which we can be defined by nucleus-cytoplasmin ratio (N:C ratio). N:C ratio is determined by cell type and cell maturity, and as the cell grows, N:C ratio will stabilize finally. For example, for mature erythrocytes, leukocytes, and megakaryocytes, N:C ratio will drop to about 2:1, while lymphocytes will stabilize at around 3:1. Therefore, we can estimate the extracellular boundary based on prior knowledge of N:C ratio and improve the segmentation result of Voronoi algorithm.
The problem of cell segmentation under incomplete staining is actually the problem of the allocation of free space between cells. In order to address these problems, we designed a novel integrated segmentation algorithm to reconstruct the cell region. The algorithm is improved based on the standard Voronoi algorithm, and N:C ratio is introduced to improve the segmentation effect as prior knowledge. The main flow of the algorithm is as follows:

**Algorithm 1**: an integrated cell segmentation algorithm based upon Voronoi algorithm and prior-knowledge

**Input**: nucleus contours obtained by Mask R-CNN model, nucleus-cytoplasm ratio

**Output**: cell contours

1. Set the nucleus regions in nucleus contours as the initial cell regions
2. Expand nucleus regions based upon nucleus-cytoplasm ratio as new cell regions
3. Calculate overlapping area between neighboring cell regions
4. Reallocate points in overlapping area to the cell region of which the point is nearest to the nucleus contour
5. Reconstruct cell contours from the obtained cell regions

In Section 2.2.2, the accurate outer contour of the nucleus can be automatically obtained with Mask R-CNN model. In Step 1 of Algorithm 1, we use the automatically detected nucleus region as the initial cell region, and subsequent steps will expand and refine this region. In Step 2, the algorithm estimates the position of the outer contour of the cell based on the input nucleus-cytoplasm ratio, namely expanding the nucleus contour with given nucleus-cytoplasm ratio as shown by the dotted contour in part (b) of Figure 4. Step 3 will calculate the overlapping areas of the adjacent expanded cell regions, and mark these areas as that need to be reallocated further. In Step 4, the algorithm will traverse points in these overlapping areas, calculate the distance from the point in the overlapping region to the outer boundary of the adjacent cell nucleus boundary, and then assign the point to the cell region closest to the point. As shown in part (b) of Figure 4, point P is a point in the overlapping area of three adjacent cells, and the distance from P to the cell nucleus boundary is d1, d2, and d3 respectively, where d3 is the minimum value, so P will be assigned to the cell region 3.

Herein, the distance calculation method is different from the standard Voronoi algorithm. The standard algorithm calculates the distance from P to the barycenter of the nucleus, while the improved algorithm calculates the distance from the point to the outer contour of the nucleus, therefore cell segmentation could be more close to the real contour as shape and structure information of cells are considered. After above steps, all the points in the space will be assigned to different cell regions, while the regions far away from any cells will be kept as empty areas after the segmentation. Finally, step 5 will reconstruct the outer contour of the cell based on the final cell region.
Figure 5. Part (a) is the original IHC staining image. The red contour in the Part (b) is the nucleus boundary detected by Mask R-CNN model. Part (c) is the reconstructed cell region with improved Voronoi algorithm.

As shown in figure 5, the algorithm can achieve automatic reconstruction of the cell region based on the stained image, and the reconstructed membrane region can well reflect the shape characteristics of cells (part (b) of figure 5). In areas where the cell space is loosely distributed and only nucleus staining is available, cell boundaries could also be estimated based on a given N:C ratio. As can be seen, this method can better solve the problem of cell segmentation under incomplete staining.

3. Conclusions and Future Work

Cell region segmentation in IHC staining images is the basis for quantitative analysis, which is very challenging due to the incompleteness of staining in IHC images. In this paper, we propose a novel integrated cell region reconstruction method based upon Mask R-CNN model and improved Voronoi algorithm to achieve automation end-to-end cell region segmentation in incomplete IHC staining images, design and develop an IHC image quantitative detection and analysis platform to achieve end-to-end processing of IHC images, which could lay a solid foundation for further quantitative detection and classification. In the future, we will further improve the related algorithm and implementation, increase the size of the annotated dataset, and improve the quality of the image annotation. Meanwhile, we will introduce color features in the membrane reconstruction process to further enhance the segmentation effect.

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