

A Multi-Method Fusion Approach for Precise Nuclei Segmentation in Prostate Histopathological Images for Digital Pathology-Assisted Diagnosis

Linkai Sun

School of Health Science and Engineering, University of Shanghai for Science and Technology, Shanghai, 200093, China

ABSTRACT

This study designs a processing method for nuclear segmentation to provide a reliable basis for Gleason classification and quantitative analysis in digital pathology-assisted diagnosis. Aiming at the complex tissue structure, obvious nuclear overlap and adhesion, and difficult boundary identification in prostate histopathological images, an unsupervised segmentation framework based on multi-method fusion is constructed. First, fuzzy c-means clustering was used for preliminary region division to alleviate the influence of blurred nuclear boundaries. Then, Lab color space K-means and adaptive thresholding were introduced to further separate dark-stained areas and improve nuclear overlap and adhesion. Finally, Canny edge detection and morphological operations refined the segmentation results and located the centroid. Experiments on the public SICAPv2 dataset show this method can stably extract the nuclear region in different Gleason patterns. The positive predictive value was 89.94%, the intersection over union (IoU) was 75.13%, and the Dice similarity coefficient was 81.02%. The proposed method maintains high segmentation accuracy while controlling computational complexity and annotation cost, serving as a feasible solution for a basic image analysis module in digital pathology systems.

KEYWORDS

Prostate cancer; Digital pathology; Nuclei segmentation; Multi-method fusion; Computer-aided diagnosis

1 Introduction

Prostate cancer is one of the most common male malignancies in the world, and its incidence and mortality remain high. This phenomenon also has long placed considerable pressure on public health systems. In the clinical diagnosis and treatment stage, doctors make accurate judgment on the prognosis of patients, which is directly related to the rationality of individualized treatment plan^[1-2]. At present, clinical evaluation is still based on pathological tissue sections, and Gleason scoring system is used as the main evaluation method to judge the malignant degree of prostate cancer. However, the traditional manual slide reading highly relies on the experience of pathologists. The scoring process is cumbersome and time-consuming, and it is easy to produce significant differences between different observers^[3]. In recent years, with the continuous development of digital pathology technology, researchers have gradually introduced computer-aided diagnosis methods to objectively analyze the whole-slide images and describe the tissue characteristics in a quantitative way. Such methods have gradually become an important path to improve the diagnosis efficiency^[4-5]. Research on key steps in pathological image analysis has gradually shown its practical value in this process, and provides support for the application of related systems.

The existing methods for nuclear segmentation of pathological images can be divided into two categories, one focusing on traditional image processing technology, and the other relying on deep learning models. Among the traditional methods, Otsu method, watershed algorithm and active contour model are common. These methods usually have high computational efficiency in images with clear background and simple structure. However, when the image contrast is reduced and obvious nuclear adhesion occurs, the algorithm is prone to under-segmentation or over segmentation, and the overall stability is limited^[6-11]. In recent years, convolutional neural networks have gradually become the focus of research, in which a class of models represented by U-Net are outstanding in the task of medical image segmentation. By learning high-dimensional semantic features, such methods can complete relatively fine region division^[12-13]. However, deep learning models usually rely on a large number of pixel-level annotated data as the basis for training, so the data acquisition process is more difficult, and the cost of manual annotation also continues to increase^[14]. On the other hand, most of these models belong to the black box structure, and the internal feature extraction process is difficult to form a direct correspondence with the pathological morphological features. Therefore, in the clinical auxiliary diagnosis scenarios, the interpretation ability and result traceability are still insufficient, which also affects its practical application scope to a certain extent^[15].

2 Materials and Methods

2.1 Materials

In this study, the public prostate pathological image dataset SICAPv2 was selected as the experimental material for

performance evaluation of the segmentation algorithm^[16]. The dataset included whole-slide images of prostate tissue treated with standard staining. In order to support pixel-level computational pathology research, researchers have uniformly resampled the original slides and cut them into 512×512 pixel standardized image blocks. Around the nuclear segmentation task, the dataset further provides the nuclear binary mask of the corresponding image block, and the annotations were completed by professional pathologists, which also provides a reliable reference for the verification of segmentation accuracy.

These image samples cover normal glands and a variety of pathological states corresponding to different levels of Gleason pattern. The images contain not only well-defined nuclei, but also more challenging morphological scenes such as dense adhesion and spatial distribution disorder. The dataset retains substantial variability in the pathological microenvironment and is equipped with accurate mask information, so it can provide a solid evaluation basis for the multi-method fusion segmentation model in this paper. Subsequent experiments also carried out a quantitative analysis on the validity and stability of the model, and provided reference for the application evaluation of the method in the digital pathological image analysis task.

2.2 Methods

2.2.1 Preprocessing and Initial Segmentation

Pathological tissue sections are often affected by staining differences and background noise in the process of preparation and digital scanning. These factors will interfere with the subsequent feature extraction. Therefore, this study first preprocesses the input standardized pathological image blocks. The algorithm first uses bicubic interpolation to enlarge the resolution of the original image, and improves the local pixel density while maintaining the relatively smooth edge of the tissue. Subsequently, histogram equalization was introduced to stretch the overall contrast of the image to expand the gray difference between the dark-stained nuclei and the light colored cytoplasm and stromal background. On this basis, the algorithm further uses median filtering based on RGB three channel independent calculation to remove high-frequency point noise in the image. Specifically, the kernel size of the median filter was set to 3×3 . Such standardized preprocessing methods can improve the signal-to-noise ratio of pathological images and provide more stable input conditions for the subsequent segmentation process^[17-18].

After preprocessing, the algorithm introduces fuzzy c-means clustering to complete the preliminary segmentation according to the characteristics of unclear nuclear boundary in prostate pathological image. The conventional hard clustering method is prone to misjudgment when dealing with the transition region, while fuzzy c-means clustering calculates the soft membership of each pixel in the space belonging to the nucleus region or background region by minimizing the objective function, so it is more suitable for dealing with the boundary fuzzy problem. Previous studies have shown that this method can better deal with the fuzziness and uncertainty of medical image tissue boundaries^[19].

2.2.2 Multi-feature Channel Algorithm Fusion

After obtaining the preliminary segmentation mask, the algorithm further constructs a feature extraction and mask fusion model including three parallel channels for the common problem of nuclear overlap and adhesion and local uneven staining in prostate pathological images. This design is not only used to improve the segmentation accuracy, but also can enhance the stability of the algorithm in the face of complex pathological image input.

The first channel uses the ability of the Lab color space to separate brightness and color information, converts the preprocessed image to the Lab color space, and performs K-means clustering on this basis. Previous studies have shown that extracting dark-stained areas with independent color components is an effective way to process H&E-stained sections^[20]. By minimizing the sum of squares of errors within the cluster, the algorithm can separate the nuclear region from the background region

In the second channel, the adaptive local threshold processing method based on Gaussian window is adopted, with the specific window size set to 15×15 . The algorithm can dynamically adjust the threshold according to the contrast change of the local area, and identify the main body of the nucleus under the background of uneven illumination distribution. The third channel successively implements morphological closing and opening operations on the probability mask output in the preliminary segmentation stage, which can not only respond to local illumination distortion, but also filter out isolated noise^[21-23].

3 Experiments and Results

3.1 Experimental Configuration and Evaluation Metrics

All experiments were completed on a graphics workstation. The workstation is equipped with Intel Core i7-12700KF processor, NVIDIA RTX 3090 GPU and 16 GB memory. The software environment is built based on Python 3.9. The unsupervised segmentation algorithm in this paper mainly relies on open source libraries such as OpenCV and scikit-image. For the deep learning model U-Net as the comparison baseline, the research uses PyTorch 1.8 framework to complete the construction^[24], and divides the SICAPv2 training set and test set according to the ratio of 8:2. Prior to feeding the images into the U-Net model, the pixel values of all pathological images were normalized to the range of [0, 1] to ensure stable gradient descent. In the training phase, the stochastic gradient descent optimizer is used, the learning rate is set to 0.001, the batch size is set to 9, and the number of iterations is set to 100. At the same time, BCE-Dice loss is used to optimize the network parameters. Compared with the deep learning model that needs training process, the method in this paper mainly relies on deterministic image processing steps in the inference stage, so it has less dependence on the data scale, and also retains some simplicity of implementation.

In the quantitative evaluation stage, this paper selects three core indicators^[25] commonly used in the field of medical image segmentation, namely positive predictive value (PPV), intersection over union (IoU) and Dice similarity coefficient (DSC).

3.2 Results

3.2.1 Nucleus Segmentation Results

In order to quantify the performance of the algorithm, this paper compares the multi-method fusion model with traditional image processing methods and a basic deep learning model on the SICAPv2 test set. Baseline methods involved in the comparison include Otsu method^[26], watershed algorithm^[27] and U-Net model^[12]. Each method runs on the same test sample, and the statistical results of relevant evaluation indexes are shown in Table 1. This group of experiments is used to test the actual performance of this method as the basic module of pathological image segmentation.

The experimental results showed that Otsu method depended on the global threshold, and it was difficult to work stably in the face of the local illumination variations and uneven staining in prostate pathological sections. The lightly stained nuclei were easy to be ignored, and its Dice similarity coefficient was 65.34%. Watershed algorithm can separate the adhesion region to a certain extent, but it is sensitive to the high-frequency noise in the background and prone to over segmentation. At the same time, the number of false positive increases, and its positive predictive value is 68.55%. The U-Net model has strong adaptability in complex tissue environments by learning characteristics in a data-driven way, and its intersection over union reaches 72.45%.

Compared with the above methods, the multi-method fusion model does not rely on the training of large-scale pixel-level annotated data, and each index performs better. The experimental results show that the positive predictive value of this method is 89.94%, the intersection over union is 75.13%, and the Dice similarity coefficient is 81.02%. These results show that this method can maintain a stable segmentation effect in complex pathological images, and can also provide a reliable basis for feature extraction in subsequent digital pathology analysis.

Table 1 Quantitative comparison of nucleus segmentation performance among different models

Segmentation Models	PPV (%)	IoU (%)	Dice (%)
Otsu	71.26	58.42	65.34
Watershed	68.55	61.18	69.87
U-Net baseline	86.12	72.45	78.69
Proposed method	89.94	75.13	81.02

3.2.2 Robustness Evaluation Across Different Gleason Patterns

See Table 2 for the specific results. Through this set of analysis, the study can further determine the scope of application of this method in different digital pathology scenarios. The experimental results show that under the Gleason 3 pattern with relatively simple pathological structure, the model can maintain high segmentation accuracy, the Dice similarity coefficient is 83.45%, and the positive predictive value is 92.18%. With the gradual increase of pathological grade, the nuclear atypia increased, and the spatial overlap phenomenon became more obvious, so the segmentation indexes decreased to some extent. Nevertheless, the model relies on Lab color space features, adaptive local threshold and multi-channel cross constraints, and still maintains 78.62% Dice similarity coefficient and 87.41% positive predictive value in the face of the most complex Gleason 5 pattern. The performance fluctuation between different grades is always

controlled within a reasonable range, and there is no obvious decline on the whole. This set of fine-grained evaluation results show that the multi-method fusion framework still has good stability and robustness in the complex pathological microenvironment.

Table 2 Segmentation performance of the proposed model across different Gleason patterns

Gleason Pattern	PPV (%)	IoU (%)	Dice (%)
GP3	92.18	78.24	83.45
GP4	89.15	75.02	80.81
GP5	87.41	72.16	78.62
Overall	89.94	75.13	81.02

3.2.3 Ablation Experiments on the Segmentation Model

The experimental results show that the model can roughly locate the nuclear distribution area when only relying on fuzzy c-means clustering for preliminary segmentation, but it is still prone to misjudgment in severe adhesion tissues, with the intersection over union (IoU) of 61.20% and the Dice similarity coefficient of 73.15%. After adding the K-means clustering feature of Lab color space, the recognition ability of the model for hyperchromatic nuclei was enhanced, and the overall positive predictive value increased to 83.20%. If the adaptive local threshold processing is added on the basis of the preliminary segmentation, the adaptability of the algorithm to the uneven illumination region is improved, and the Dice similarity coefficient is increased to 77.85%. When the three feature channels participate in and complete the logical mask fusion at the same time, the algorithm can separate the adhesion region more effectively, and the overall segmentation performance is also improved. After continuing to stack the boundary refinement module, the complete model achieved the optimal results. Compared with the basic clustering model, each index was significantly improved, including the intersection over union (IoU) increased by 13.93%, and the Dice similarity coefficient increased by 7.87%. These ablation results show that the multi-channel cross-validation mechanism and boundary constraint mechanism are necessary for nuclear segmentation in complex pathological images.

Table 3 Quantitative evaluation of ablation experiments for the proposed segmentation model

Model variants	PPV (%)	IoU (%)	Dice (%)
Baseline (FCM only)	78.50	61.20	73.15
FCM + K-means	83.20	66.85	76.40
FCM + Adaptive Threshold	84.15	68.30	77.85
FCM + K-means + Adaptive Threshold	87.62	72.90	79.44
Proposed Full Model	89.94	75.13	81.02

4 Discussion

Once the prostate cancer tissue enters the high-grade pathological stage, the microscopic morphology will change significantly. The most prominent manifestation is that the gland structure is damaged, and the nuclei are highly dense and overlapping. In the face of such complex slides, the traditional single segmentation algorithm is often difficult to take into account the local information and the overall structure at the same time. The key reason why the multi-method fusion strategy can maintain good performance in the severely adherent regions is that the algorithm establishes a multi-dimensional feature cross-validation mechanism. Fuzzy c-means clustering firstly calculates the membership of pixels at the global scale, which is used to alleviate the common problem of tissue boundary ambiguity in medical images. Then, Lab color space clustering and adaptive local threshold extract deep staining features from color space and local gray gradient respectively. After strict bitwise logical filtering, the model only retains the pixel area recognized as the foreground nucleus by multiple channels at the same time. With the help of this multi-dimensional consensus mechanism, the algorithm can not only filter out the background artifacts caused by local uneven staining, but also more effectively separate the weak adhesion bridge formed by physical extrusion between nuclei. Combined with the subsequent edge contour constraint and Euclidean distance transformation, the model finally completes the fine separation and individual localization of dense nuclear groups, which also provides a more reliable basis for the subsequent quantitative analysis of pathological images.

5 Conclusion

This paper presents an unsupervised nuclear segmentation algorithm based on multi-method fusion to solve the problems of nuclear overlap and adhesion, as well as blurred boundaries in prostate pathological images. The algorithm

integrates fuzzy c-means clustering, color space feature extraction and adaptive local threshold processing to achieve individual nucleus separation and precise localization in a complex tissue microenvironment. Quantitative experimental results show that the model can maintain stable segmentation performance in multiple Gleason patterns, and does not rely on massive pixel-level annotated data, nor does it need high-cost graphical computing support. On this premise, it still achieves better segmentation accuracy than the conventional deep learning baseline model. The white box segmentation framework constructed in this paper has clear physical meaning, and can provide a reliable technical basis for the objective quantitative analysis of pathological images, auxiliary grading of prostate cancer and digital pathology related applications.

References

- [1] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2024; 74(3): 229-263.
- [2] Sekhoacha M, Riet K, Motloung P, Gumenku L, Adegoke A, Mashele S. Prostate Cancer Review: Genetics, Diagnosis, Treatment Options, and Alternative Approaches. *Molecules.* 2022; 27(17): 5730.
- [3] Bulten W, Kartasalo K, Chen PHC, et al. Artificial intelligence for diagnosis and Gleason grading of prostate cancer: the PANDA challenge. *Nat Med.* 2022; 28(1): 154-163.
- [4] Hanna MG, Reuter VE, Hameed MR, et al. Toward a new normal: integration of digital pathology into clinical practice. *Mod Pathol.* 2022; 35(2): 152-164.
- [5] Chen RJ, Ding T, Lu MY, Williamson DFK, et al. Towards a general-purpose foundation model for computational pathology. *Nat Med.* 2024; 30(3): 850-862.
- [6] Ahmedt-Aristizabal D, Armin MA, Denman S, Fookes C, Petersson L. A survey on graph-based deep learning for computational histopathology. *Comput Med Imaging Graph.* 2022; 95: 102027.
- [7] Guo Z, Liu H, Ni H, et al. Densely Convolutional Spatial Attention Network for nuclei segmentation of histological images for computational pathology. *Front Oncol.* 2023; 13: 1009681.
- [8] Hasan MR, Hossain MM, Islam MS, et al. Cluster Analysis of Cell Nuclei in H&E-Stained Histological Sections of Prostate Cancer and Classification Based on Traditional and Modern Artificial Intelligence Techniques. *Diagnostics.* 2022; 12(1): 15.
- [9] Madusanka N, Jayalath P, Fernando D, Yasakethu L, Lee BI. Impact of H&E Stain Normalization on Deep Learning Models in Cancer Image Classification: Performance, Complexity, and Trade-Offs. *Cancers.* 2023; 15(16): 4144.
- [10] Majanga V, Mnkandla E, Wang Z, Koulla Moulla D. Active Contours Connected Component Analysis Segmentation Method of Cancerous Lesions in Unsupervised Breast Histology Images. *Bioengineering.* 2025; 12(6): 642.
- [11] Chen Y, Huang Q, Geng M, Wang Z, Han Y. A systematic review on cell nucleus instance segmentation. *IET Image Process.* 2025; 19(4): 1020-1035.
- [12] Ronneberger O, Fischer P, Brox T. U-Net: Convolutional networks for biomedical image segmentation. *Med Image Comput Comput Assist Interv.* 2015; 9351: 234-241.
- [13] Azad R, Giaquinto M, Bozorgpour A, et al. Medical image segmentation review: The success of U-Net. *IEEE Trans Pattern Anal Mach Intell.* 2024; 46(8): 5522-5544.
- [14] Zhang H, He Y, Wu X, et al. PathNarratives: Data annotation for pathological human-AI collaborative diagnosis. *Front Med.* 2023; 9: 1070072.
- [15] Singh Y, Hathaway QA, Keishing V, et al. Beyond post hoc explanations: A comprehensive framework for accountable AI in medical imaging through transparency, interpretability, and explainability. *Bioengineering.* 2025; 12(8): 879.
- [16] Silva-Rodríguez J, Colomer A, Sales MA, Molina R, Naranjo V. Going deeper through the Gleason scoring scale: An automatic end-to-end system for histology prostate grading and cribriform pattern detection. *Comput Methods Programs Biomed.* 2020; 195: 105637.
- [17] Bankhead P, Loughrey MB, Fernández JA, et al. QuPath: Open source software for digital pathology image analysis. *Sci Rep.* 2017; 7(1): 16878.
- [18] Dimitriou N, Arandjelović O, Harrison DJ. Principles of machine learning in pathology. *J Pathol.* 2022; 256(4): 389-402.
- [19] Singh C, Ranade SK, Kaur D, Bala A. An Intuitionistic Fuzzy C-Means and Local Information-Based DCT Filtering for Fast Brain MRI Segmentation. *J Imaging Inform Med.* 2024; 37(5): 2287-2310.
- [20] Saxena P, Goyal A, Bivi MA, Singh SK, Rashid M. Segmentation of Nucleus and Cytoplasm from H&E-Stained Follicular Lymphoma. *Electronics.* 2023; 12(3): 651.
- [21] Xing F, Yang L. Robust Nucleus/Cell Detection and Segmentation in Digital Pathology and Microscopy Images: A Comprehensive Review. *IEEE Rev Biomed Eng.* 2016; 9: 234-263.
- [22] Canny J. A computational approach to edge detection. *IEEE Trans Pattern Anal Mach Intell.* 1986; 8(6): 679-698.
- [23] Naylor P, Laé M, Reyat F, Walter T. Segmentation of nuclei in histopathology images by deep regression of the distance map. *IEEE Trans Med Imaging.* 2019; 38(2): 448-459.
- [24] Paszke A, Gross S, Massa F, et al. PyTorch: An imperative style, high-performance deep learning library. *Adv Neural Inf Process Syst.* 2019; 32: 8024-8035.
- [25] Maier-Hein L, Reinke A, Godau P, et al. Metrics for evaluating 3D medical image segmentation: analysis, selection, and tool. *Nat Commun.* 2024; 15(1): 822.
- [26] Otsu N. A threshold selection method from gray-level histograms. *IEEE Trans Syst Man Cybern.* 1979; 9(1): 62-66.
- [27] Vincent L, Soille P. Watersheds in digital spaces: an efficient algorithm based on immersion simulations. *IEEE Trans Pattern Anal Mach Intell.* 1991; 13(6): 583-598.