Foreground Extraction in Histo-Pathological Image by Combining Mathematical Morphology Operations and U-Net

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Abstract: In recent years, computational pathology is rapidly developing. This resulted in various artificial intelligence approaches that have been proposed and applied to images common to the pathology practice, i.e. Whole Slide Images. It is very important to pre-process these images for a deep learning classifier because they are simply too large to feed into such a network. In order to get useful information from these images, we propose a new background removal method for the extracted Regions Of Interest in these images. We combine traditional morphology image operators and a U-Net framework. Firstly, we pre-process the images by using Contrast Limited Adaptive Histogram Equalization and thresholding. Then we predict the mask by using pre-trained U-Net weights. Finally, we use morphological opening and propagation operators on the predicted mask to refine the masks. The experiments based on different types of staining (H&E, PAS, and JONES silver) show the effectiveness of our method compared to 3 state-of-the-art models.

1 INTRODUCTION

Pathological examination of biopsies is an important method for clinical diagnosis and plays a crucial role in the diagnostic process. Over the past decade, digital pathology has become one of the main directions for development in pathology (Li et al., 2022). The introduction and use of digital slide scanner systems provide high-resolution whole slide images (WSIs) which are obtained from the traditional pathological slides resulting in image sizes in gigabyte order. Digitization of pathological slides contributes a lot to the preservation, sharing, and analysis of pathological information. On the basis of WSIs, an automated or computer-assisted diagnosis comes into reach. This requires that dedicated pattern recognition systems need to be developed and, consequently, the WSIs need to be prepared for these pattern recognition procedures. At present, pattern recognition methods are based on, so-called, deep learning systems. So, WSIs can be used in computational approaches to recognize certain pathologies in these images (Neuner et al., 2021). Pattern recognition can assure efficient and accurate pathological assessment of diseases. Although the computer-aided diagnosis of histo-pathological images gains critical acclaim for its accuracy, stability and efficiency, still, the quality of the histo-pathological images has posed various challenges to those proposed techniques. We will discuss some of these limitations in terms of slide preparation and computational preparation. In this paper, we employ histo-pathological images of the kidney, in particular by investigating biopsies taken from patients with a kidney transplant.

There are several staining methods for tissues commonly used in kidney pathology; haematoxylin and eosin (H&E) is the most frequently used staining technique. For kidney transplants, also the Periodic Acid Schiff (PAS) and the JONES silver staining are used. Examples of these three different staining methods are depicted in Figure 1. These images are typical examples for kidney biopsies.

As mentioned, the WSIs are large and have a high resolution, this complicates the use of the image as a whole for pattern recognition; i.e., computer memory is still limited. Therefore, it is important to first find where slide of the biopsy and thus on the WSI the

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relevant information is.. Once the locations of information are found, just the tissue information on those locations needs to be extracted. Only this part of the WSI is considered relevant for further processing.

The relevant information on the biopsy WSI is referred to as the foreground, and this part is used to find the patterns. Most often there are a number biopsy sections mounted on one slide. The first step is to identify the regions where the sections are. The empty background, i.e. the part of the slide where no sections are mounted, is often indicated with one color, this needs to be set to zero first; an example of this can be seen in Figure 2a.

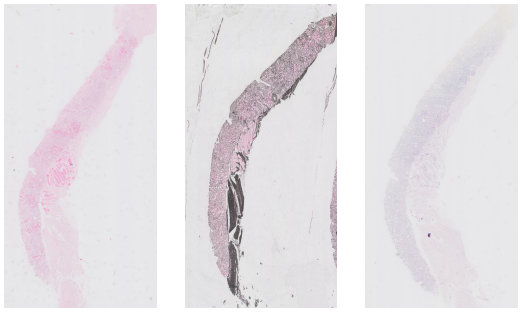
Existing methods for foreground extraction for WSIs are mostly focused on H&E staining (Riasatian et al., 2020). In kidney transplant biopsies, however, also the PAS staining is important for histo-pathological analysis. Foreground extraction of PAS stained WSI can be complicated as the staining fades over time resulting in a low contrast image. In general, the stained tissue in a WSI should be evaluated for fading, artifact, dirt, and low contrast. In Figure 2b and 2c some typical examples are depicted.

In order to analyse the information in the tissue, all tissue areas need to be identified. The analysis consists of building a classifier for the pathological state of the tissue. Therefore it is important to only use relevant, i.e. tissue, information in the classification. Therefore, the WSI needs to be pre-processed to that this information can be submitted to the classification system. We focus on getting this information from the WSI.

Once the tissue regions in the WSI are identified, the tissues themselves need to be identified as foreground. To this end, a segmentation procedure is applied. Therefore, the next step of histo-pathological image analysis is tissue segmentation (Khened et al., 2021).

The classification of the foreground parts is accomplished using a deep neural network. The computational task is facilitated trough the use of graphic processing units (GPUs). However, the memory of GPUs has limitations. Consequently, the common approach is to divide the image into smaller patches. These patches are then used to train the deep neural network.

So, the preparation of the WSIs for the training of a classifier requires constructing “patches” for the relevant areas with tissue. Once, these areas are established and “clean”, the patching is the last step for the preparation. This needs to be done in such a manner that the patches contain useful information for the training of a classification. Therefore our work aims to pre-process the WSI in such a manner that first the regions are established where sections are on the slide, and subsequently process the tissue area in each of the regions such that a binary mask is obtained that can be used for the construction of patches that contain relevant tissue information.



(a) H&E (b) JONES (c) PAS

Figure 1: Different types of staining.

(a) Empty area (b) fade staining (c) dirty staining

Figure 2: Some challenging examples for tissue segmentation.

In our approach, the empty area, cf. Figure 2a, of the WSI is set to zero through a simple thresholding operation. Next, the tissue part of the WSI needs to be assessed. In order to deal with low contrast in the image, image enhancement is used. This is typically the case for staining that is fading over time. We assess the contrast distribution in the image and enhance the contrast through a redistribution of the intensity values. For our approach we use Contrast Limited Adaptive Histogram Equalization (CLAHE) (Pizer et al., 1990) for low-contrast images. This enhancement method operates on a local assessment of the intensity distributions and combines well with the subdivision of patches later in the process. Artifacts and dirt on the slides are often seen as tissue by the segmentation procedure. In order to remove these artifacts we use mathematical morphology operators. The combination of these procedures will result in areas that are suitable for consistent and robust patching.

Further, based on results from the literature, we propose a new method that combines mathematical morphology operations with a U-Net deep learning structure. We first assess the contrast distribution, then we use thresholding to remove the empty parts

from the analysis. Next, we use the MobileNet neural network (Howard et al., 2017) as the U-Net encoder backbone to predict the tissue foreground areas. This neural network was pre-trained on The Cancer Genome Atlas (TCGA) datasets [1]. Next, we post-process the initial masking area through mathematical morphology operations. The resulting mask is then used to create the patches for the classifier.

The main contributions of this paper are: (1). A better solution for the extraction of the relevant foreground from the WSI. (2). Development of a new generic procedure for the pre-processing of kidney biopsy images. (3) Comparison with three state-of-the-art methods (Otsu, MobileNet, and EfficientNet-B3.) on 7 typical images with two binary evaluation indexes.

The remainder of this paper is organized as follows: in section 2, several existing approaches related to our algorithm are presented. Then, in section 3, we will introduce our method. Section 4 provides the experiment results. Finally, we present our conclusions in section 5.

2 RELATED WORK

For the processing of WSIs There are two categories related to our work: background removal and whole slide image processing.

2.1 Background Removal

In tissue segmentation tasks, background removal can refer to different approaches. One is to correct for uneven illumination in the background. And elaborate methods are available for this (Cai and Verbeek, 2015). For WSIs background correction refers to the removal of non-object parts in the image. This often uses a segmentation method and the crux is to find the right threshold value(s). Here we see two categories, one based on traditional machine learning algorithms and the other based on a deep learning method. The traditional approaches entail region growing, the watershed-based method, and Otsu thresholding (Otsu, 1979). The main idea of the threshold-based algorithm is to compute an optimum. In a deep learning approach, patching over the image is applied to find local optima.

By dividing the section parts of the WSI, aka the regions of interest (ROI), into small patches, a deep neural network for the segmentation can be used. This works by predicting a label for each of the pixels in a patch. The label denotes whether it is foreground or not. Next, the segmented patches are stitched back to the overall images. There are several neural network models for image segmentation (Sultana et al., 2020), such as FCN (Fully Convolutional Network) (Long et al., 2015), U-Net (Ronneberger et al., 2015), and Mask R-CNN (He et al., 2017). Riasatian et al. compared different U-Net topologies for background removal in histo-pathological images (Riasatian et al., 2020). By training on different backbones in their experiments, they have shown that MobileNet and EfficientNet-B3 (Tan and Le, 2019) perform better than the others.

2.2 Whole Slide Image Pre-Processing

Chen et al. proposed a tissue localization pipeline to process WSIs (Chen and Yang, 2019). They use thresholding on grayscale images followed by filling the holes, which works well on H&E staining. Neuner et al. developed an open-source library to process WSIs, which helps the training and evaluation task for classification (Neuner et al., 2021). The general procedure of their software consists of several steps: ROI definition, tile filtering, tile extraction, and tile collection. After this procedure, we can get a batch of tiles, which can be directly used for downstream tasks. They employed several kinds of filters to segment the background and foreground. Clustering-constrained Attention Multiple Instance Learning (CLAM) is another deep-learning-based method that uses attention-based learning to classify the WSIs (Lu et al., 2021). In CLAM, thresholding is used on the saturation channel after blurring the image with a median filter. In addition, morphological operators are used to fill the small holes.

3 METHOD

Our method consists of three main steps: image pre-processing by contrast assessment and thresholding, MobileNet mask prediction using pretrained weights, and mask post-processing using propagation and morphological opening. The workflow of our method is shown in Figure 3.

3.1 Image Pre-Processing

Since there is often some debris on the slide as well as color coding of non-section areas in the WSIs, we use thresholding to remove the very dark and very light

[1]Pretrained model available at: https://kimialab.uwaterloo.ca/kimia/index.php/data-and-code/

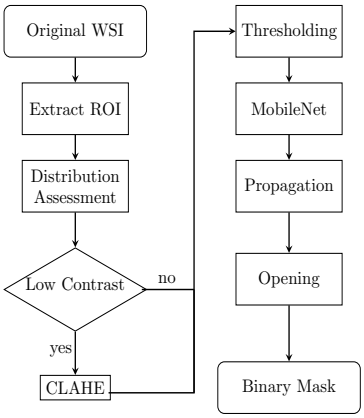


Figure 3: Flow chart of our method.

areas. First, we convert the image to a grayscale image and then assess the contrast, i.e. low or sufficient. The grayscale image is calculated using this conversion:

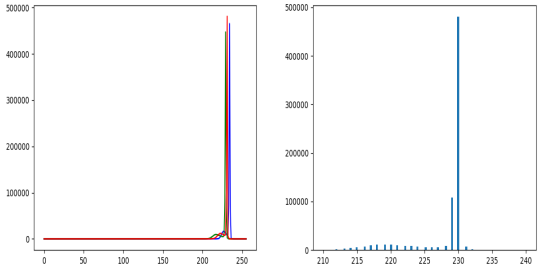
$$Y = 0.299 * R + 0.587 * G + 0.114 * B \quad (1)$$

Where R, G, B is the intensity value of red, green, and blue channels. From the resulting intensity image, we calculate the contrast by using:

$$C_M = \frac{I_{max} - I_{min}}{I_{full}} \quad (2)$$

where I_{max} and I_{min} is the maximum and minimum intensity value in the image respectively. I_{full} represents the dynamic range for the given image type; typical, for an 8-bit image, this is $[0, 255]$. If the image contrast is lower than a given value, it is considered a low-contrast image (see Figure 4 for a histogram example from the source image in Figure 2(b)). For this image, the max gray value is 241, and the min gray value is 80. As we can see, the value above 231 and below 214 are no more than 1%. In this case, the $I_{max}$is 231, and I_{min} is 214. I_{full} is 256. So the contrast value would be 0.066; meaning that only 6.6% of the dynamic range is used. We consider this image to be of low contrast and we employ CLAHE as an image enhancement method on this image. If it is not the low-contrast image, we directly proceed to thresholding.

In the thresholding step, we set the pixels whose intensity values are below 70 and above 230 to be 255 (from empirical assessments). By doing this, the pixels with values beyond this interval are viewed as background by the neural network. Through image enhancement and thresholding, some of the noise is removed. After thresholding, we will get an image that keeps almost all of the tissues and contains less background.



(a) (b)

Figure 4: Histogram of low contrast image (Figure 2(b)) (a) RGB histogram of the image. (b) Zoom in to the highest frequency gray values.

3.2 U-Net Architecture

Image segmentation tasks can be accomplished in many ways. In order to further classify the image content, we need to create masks over the regions where the tissues are found. Following recent accomplishments in WSI segmentation, we invoke a deep learning strategy for the segmentation. As a widely used convolutional neural network model, U-Net works well for image segmentation tasks, especially for biomedical microscopy images (Ronneberger et al., 2015). There are several different backbones that we can choose for the tissue segmentation task. According to experiments in previous work (Riasatian et al., 2020), the MobileNet works better than the others. The main idea of MobileNet is the depth-wise separable convolution, with which the number of parameters can be reduced. Therefore, we choose the MobileNet as the backbone of U-Net, and we use the default settings (the patch size is 400) to produce the (initial) mask.

3.3 Post-Processing

After the initial prediction of U-Net, we get the mask with small holes both in the tissue are and on the border of the tissue area. To fill the holes in the tissue, we process the mask using image propagation. Image propagation is to fill the holes in the overall mask. As for small concavities on the border of the tissue, we use an opening with a rectangular structuring element, size 20x20, to fill in the small concavities. Finally, in this manner. we obtain a binary mask for each of the ROIs.

Given the generated mask, we could keep only tissues by combining it with the original image using logic AND operation. Subsequently, we generate the image patches for the neural network classifier from the masked area. A simple patch example is shown in Figure 5. The green line represents the overall mask outline. We generate 256*256 sized patches. As an

Table 1: Similarity measure between the masks from our method and the others.

Image	binary correlation			binary overlap		
	EfficientNet	MobileNet	Otsu	EfficientNet	MobileNet	Otsu
I_1	0.9864	0.9817	0.8592	0.9919	0.9890	0.9055
I_2	0.7178	0.7967	0.4940	0.7476	0.8258	0.5733
I_3	0.9060	0.6395	0.8753	0.9184	0.6215	0.8915
I_4	0.9217	0.3483	0.1447	0.9269	0.3363	0.0387
I_5	0.9661	0.9782	0.9108	0.9703	0.9810	0.9199
I_6	0.9881	0.9923	0.0335	0.9900	0.9935	0.0230
I_7	0.9902	0.9860	0.6770	0.9919	0.9885	0.7365

extra heuristic, we establish if there is sufficient information in the patch for the training. This is to prevent the classifier to train on the background. In a patch, the tissue should have at least an area (256*256)/2 pixels, i.e. 50%, for it to be relevant and kept to feed it into the classifier (cf. shown in red square in Figure 5).

Figure 5: A simple example of the resulting patches.

4 EXPERIMENT

4.1 Dataset Preparation

We have selected 7 WSIs from kidney transplants on different types of staining, which are difficult for the neural network to predict nice masks. The WSIs we used are scanned with a Philips DP v1.0. The Automated Slide Analysis Platform (ASAP) is used to annotate the best quality ROIs. From the annotation generated by ASAP (XML file), we extract the information on the ROIs. A WSI is acquired at different resolutions, aka levels. These levels are ranged from 0 to 9. On average 1 ROI per WSI, which leads in total to 500 useful patches for training. To speed up the prediction of neural networks and include as much information as possible, we extract the level 5 ROIs corresponds with a magnification of 1.25×. The original images are shown in Figure 6 (a). The images from top to down are denoted as I_1 to I_7. I_1 is the H&E staining. I_3 is the PAS staining. The others are all JONES staining. I_1, I_2, and I_3 are from the same kidney but with different types of staining.

4.2 Experimental Settings

We have implemented our algorithms in Python and use the OpenCV and Diplib library [2] to process the image. The experiments were run on a Windows 11 system with Intel 3.4GHz Processor and 16GB memory. We compare our method with Otsu threshold segmentation in OpenCV, MobileNet, and EfficientNet-B3 [3]. The mask results are shown in Figure 6.

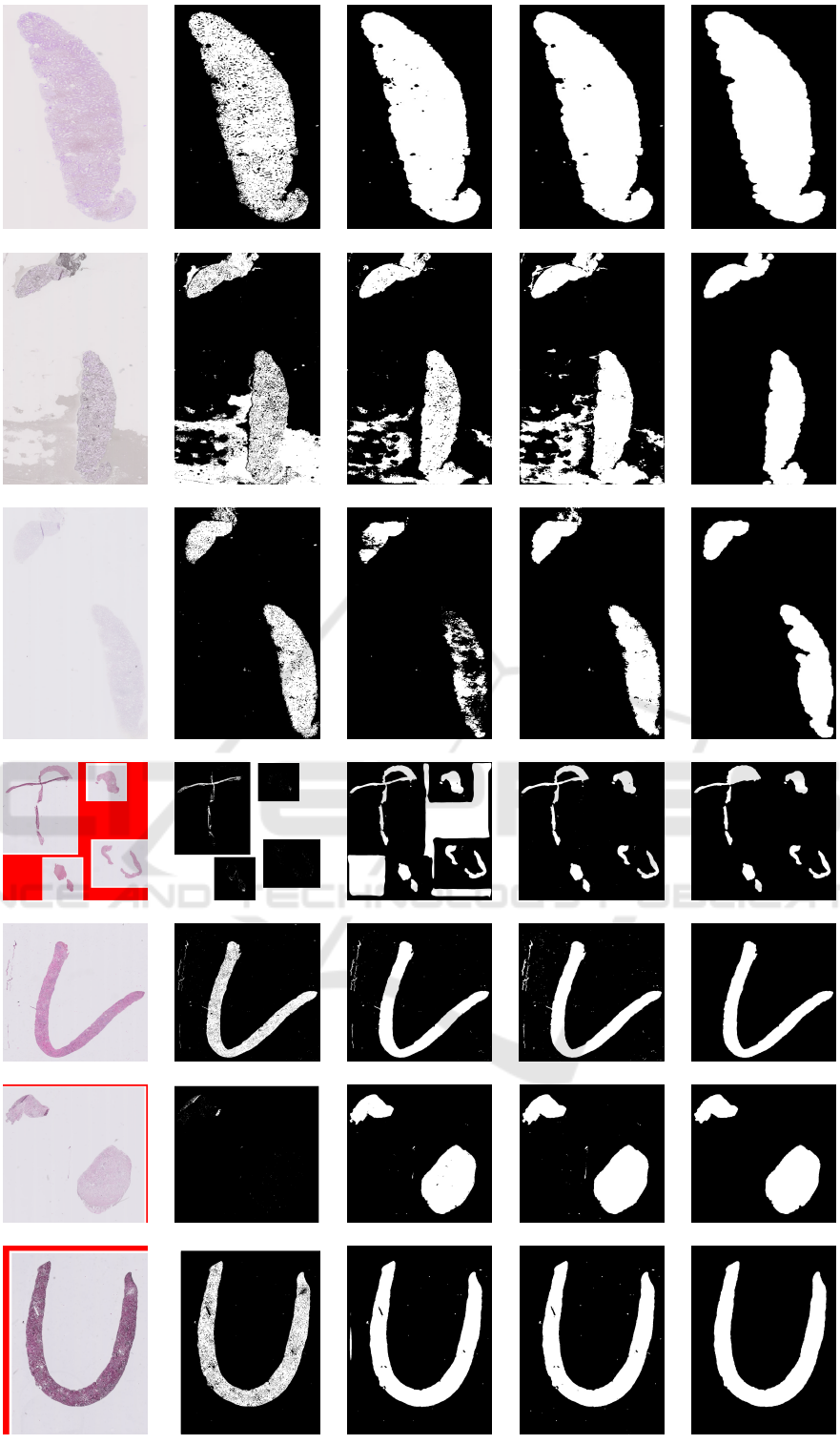
4.3 Performance Evaluation

To be able to compare the masks generated by our method with other approaches, we employ binary correlation and binary overlap to measure the correlation between two binary images (Verbeek, 1995). In this calculation, the two images should be binary images of the same size. The total pixels in the image are denoted as N_{tot}. The binary correlation could be calculated as:

$$bc(I_1,I_2) = \frac{|N_{I_1 \cap I_2} * N_{tot} - N_{I_1} * N_{I_2}|}{\left[(N_{I_1} * N_{tot} - N_{I_1}^2) * (N_{I_2} * N_{tot} - N_{I_2}^2)\right]^{1/2}} \quad (3)$$

[2]https://diplib.org/.

[3]Code and pretrained weights available at: https://kimialab.uwaterloo.ca/kimia/index.php/ijcnn-2020-u-net-based-background-removal-in-histopathology/.



(a) Original (b) Otsu (c) MobileNet (d) EfficientNet (e) Our method

Figure 6: The results of the different methods.

where I_1 denotes the mask generated by our method, and I_2 denotes the mask generated by another method. N_{I_1} is the number of object pixels in I_1. N_{I_2} is the number of object pixels in I_2. $N_{I_1 \cap I_2}$ denotes the number

of the patterns that result from the logical AND operation of I_1 and I_2. And the binary overlap could be calculated as:

$$bo(I_1, I_2) = \frac{2 * N_{I_1 \cap I_2}}{N_{I_1} + N_{I_2}} \quad (4)$$

4.4 Results Analysis

The binary correlation and binary overlap between the masks from our method and the others are shown in Table 1. The measures indicate the discrepancies between our methods compared to the other approaches. The results show that all the methods work well for H&E staining. Our method can, however, remove all the background in the image resulting from a dirty staining I_2. For images resulting from a weak staining I_3, MobileNet predicts fewer tissues and EfficientNet could find more tissues. Otsu and MobileNet view the empty area in I_4 as foreground, EfficientNet and our method can recognize the empty area and only consider the tissue as foreground. Due to the empty area and the white part inside the empty area, the Otsu missed most tissues in I_4 and I_6. Our method removes the small holes predicted by MobileNet in I_5, I_6, and I_7.

5 CONCLUSIONS

In this paper, we have proposed a solution for the construction of a tissue mask as a pre-processing step for tissue classification. The masking is based on a tissue segmentation task, which uses a combination of mathematical morphology processing on results from the U-Net architecture. Several experiments of our method on different types of staining show the method performs well and leads to better results for the patching. For the PAS staining, there are still a few parts of tissue missing. So, here we need to do further filter and parameter optimization to be as complete as possible in identifying the tissue parts. Furthermore, we aim to automatically extract the parameters from the images. This will require further analysis of a larger number of images.

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