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A novel overlapped nuclei splitting algorithm for histopathological images

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ABSTRACT

Background and objective: Nuclei segmentation is a common process for quantitative analysis of histopathological images. However, this process generally results in overlapping of nuclei due to the nature of images, the sample preparation and staining, and image acquisition processes as well as insufficiency of 2D histopathological images to represent 3D characteristics of tissues. We present a novel algorithm to split overlapped nuclei.
Methods: The histopathological images are initially segmented by K-Means segmentation algorithm. Then, nuclei cluster are converted to binary image. The overlapping is detected by applying threshold area value to nuclei in the binary image. The splitting algorithm is applied to the overlapped nuclei. In first stage of splitting, circles are drawn on overlapped nuclei. The radius of the circles is calculated by using circle area formula, and each pixel's coordinates of overlapped nuclei are selected as center coordinates for each circle. The pixels in the circle that contains maximum number of intersected pixels in both the circle and the overlapped nuclei are removed from the overlapped nuclei, and the filled circle labeled as a nucleus.
Results: The algorithm has been tested on histopathological images of healthy and damaged kidney tissues and compared with the results provided by an expert and three related studies. The results demonstrated that the proposed splitting algorithm can segment the overlapping nuclei with accuracy of 84%.
Conclusions: The study presents a novel algorithm splitting the overlapped nuclei in histopathological images and provides more accurate cell counting in histopathological analysis. Furthermore, the proposed splitting algorithm has the potential to be used in different fields to split any overlapped circular patterns.

1. Introduction

Histopathological analysis is a common procedure in diagnosis, grading and treatment of diseases. This procedure is performed by examining a biopsy, a surgical specimen or a histopathological image. A specimen is obtained by a sequence of technical histological procedures, which are fixation, dehydration, clearing, infiltration, embedding, sectioning and staining respectively [1,2]. A histopathological image can be acquired by various imaging techniques depending on the using purpose, and the images are examined by an expert or a CAD system [2,3]. In practice, an expert visually examines tissue sections through a microscope. However, this approach is irreproducible [4], slow, and error-prone due to expert's personal take and experience. In addition, the agreement between experts is between 61% and 73% [5–7].

Computer Assisted Diagnosis (CAD) systems have been used in the field of histopathology as well as other medical fields to help experts in assessment process by providing remarkable quantitative values [8–20]. These values may also provide more confidence in decision-making process during a diagnosis. In addition, CAD systems can relieve the workload and expedite the diagnosis and treatment process.

Nuclei counting is crucial in diagnostic decision when nuclei play a central role in analysis of a tissue for diseases. The first step of nuclei counting is to segment nuclei from other tissue structures in histopathological image. Well-known general or specific image segmentation algorithms can segment the nuclei. General image segmentation algorithms such as K-Means [19,21,22], Watershed Segmentation [21,23], Fuzzy C-Means [24,25], Thresholding Segmentation [26] have been used in nuclei segmentation. In addition, specific nuclei segmentation algorithms have been proposed for increasing the quality and efficiency of segmentation [27–32]. However, segmentation can be problematic due to the nature of

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Table 1
The abbreviations used in the paper.

Abbreviation	Explanation
CC	Connected component
CCP	Circularity center of pattern
CP	Circular pattern
DBI	Digital binary image
PON	The proposed algorithm
c_i	ith circle
O_i	The pixel coordinate set of the CC intersect with the pixels of ith circle drawn on the CC
Θ	The origin of circle having maximum count of pixels in intersection of CC
δ_i	The number of pixels in O_i
φ	The set of pixels in circle with origin (x_0,y_0) and radius r
SR	The number of nuclei segmented by K-Means (Segmentation Result)
AS	The number of nuclei marked after splitting (After Splitting)
E	The number of nuclei marked by the expert
nTP	The number of nuclei marked by both of the expert and the proposed algorithm
nFP	The number of nuclei marked by the proposed algorithm, but not marked by the expert
nFN	The number of nuclei marked by the expert, but not marked by the proposed algorithm
T	The number of all nuclei, nTP + nFP + nFN
A	Accuracy
P	Precision
R	Recall
F_1	F_1 measure

images and the variability in the sample preparation, staining and image acquisition process.

The main challenge in nuclei segmentation is overlapping or superposing of nuclei. 2D histopathological images are orthographic projections of 3D tissues. Overlapping may occur due to insufficiency of 2D to represent 3D characteristics for the tissue and nuclei. Recently, the splitting schemes of overlapped nuclei such as improved touching-cell splitting [27], contour based cell detection and segmentation [29], parallel seed detection and repulsive level set [30], marker-controlled watershed algorithm [33], Gaussian-based hierarchical voting and repulsive balloon model [32], multiple clustered instance learning [31], and integrated framework for touching-cell splitting [34] have been adopted for segmentation and splitting of nuclei.

In this paper, we present a novel algorithm to split the overlapped nuclei. The histopathological images are initially segmented by K-Means segmentation algorithm. Then, the nuclei cluster are converted to binary image, and the background is eliminated. The overlapped nuclei are detected by applying a threshold area value to each nuclei in the binary image. The proposed nuclei splitting algorithm is applied to these overlapped nuclei, and split them. In the first stage of splitting, circles are drawn on the overlapped nuclei. The radius of the circles is calculated by using circle area formula. Each pixel's coordinates of the overlapped nuclei are selected as center coordinates of a circle. The number of circle drawn on the overlapped nuclei equals to the number of pixels in the overlapped nuclei. After the circles are drawn, the number of intersected pixels in both the circle and the overlapped nuclei are counted. The pixels in the circle that contains maximum number of intersected pixels are removed from the overlapped nuclei, and the filled circle labeled as a nucleus. The remaining parts of the overlapped nuclei are reprocessed. The splitting process is terminated when there are no overlapped nuclei.

The algorithm has been tested on histopathological images of healthy, and damaged kidney tissues. The results are evaluated by accepting expert evaluation as ground truth, and compared with the results provided by an expert and three related studies.

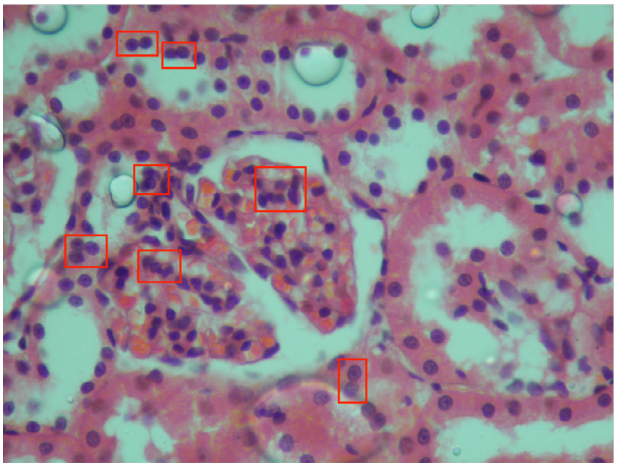

Fig. 1. A histopathological kidney image containing overlapped nuclei.

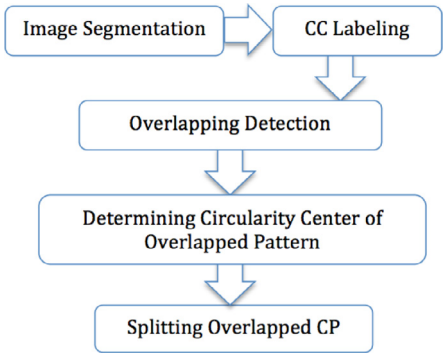

Fig. 2. The overall schematic of the proposed algorithm.

Experimental results and the evaluation demonstrated that the nuclei in an image containing overlapping can be segmented with accuracy of 84% by applying the proposed splitting algorithm.

The rest of this paper is organized as follows: the proposed algorithm is presented in Section 2; Section 3 describes experimental results; Section 4 presents discussion; Limitation and drawbacks are defined in Section 5. Section 6 explains the conclusions. The future works are mentioned in section 7. The abbreviations used in the paper are shown in Table 1.

2. Methods

Histopathological tissues are 3D objects. However, the images of histopathological tissues are 2D images. Thus, nuclei blocking the partial appearance of each other cause overlapping in the images. H&E stained histopathological image of damaged kidney tissue containing overlapped nuclei is shown in Fig. 1 as an example. The dark components represent nuclei or overlapped nuclei, some of which are shown in colored rectangles. The proposed algorithm aims to split the overlapped nuclei for accurate nuclei segmentation and counting. The overall schematic of the proposed algorithm to deal with the issue is shown in Fig. 2.

2.1. Image segmentation

Segmentation is important first step of computer-assisted histopathological image analysis systems. The success of the systems largely depends on the quality of segmentation. Segmentation algorithms aim to distinguish interested tissue structures or regions from others. For example; in [35,36], connecting tissues, nuclei, epithelial cells and lumen are clustered, and features