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Adhesion Segmentation Algorithm and Fluorescence Intensity Extraction for Human Umbilical Vein Endothelial Cells ⋆

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Abstract

Due to the complexity of adhesion and irregularity of human umbilical vein endothelial cells, we propose a novel cell image segmentation technique by combining cell image with corresponding nucleus image based on label-controlled watershed algorithm instead of segmenting the cell image directly. Moreover, nucleus segmentation and location in stained cells are introduced. The most fascinating advantage of the combined method is its accurate positioning, which ensures that the center of each cell could be positioned accurately and is conducive to the adhesion and irregular cells segmentation. To determine the activation time of nuclear factor kappa B and its signaling pathway blocking mechanism, relevant fluorescence parameters extraction approach is also discussed. Performance and result analysis demonstrate that this algorithm is valid, precise, and very helpful to further cell analysis and medical diagnosis.

Keywords: Human Umbilical Vein Endothelial Cells; Adhesion Segmentation; Nucleus Localization; Label-controlled Watershed Algorithm

1 Introduction

Human Umbilical Vein Endothelial Cell (Huvec) is one of commonly used vascular endothelial cell in vitro studies [1]. Numerous particles in endothelial cell, for example proinflammatory cytokines, are related to inflammation and hemostasis. Nuclear factor kappa B (NF-κB) is commonly found in variety of cells, whose signaling pathway regulates lots of adhesion molecules expression. In certain inflammatory cytokine stimulation, it can be phosphorylated and activated, and the activated NF-κB releases the protein and runs into the nucleus, which regulates many genes involved in transcription regulation. Therefore, activation of NF-κB is conducive to cellular immunology research [2]. With fluorescence analysis of the immunofluorescence staining human umbilical vein endothelial cells induced by toxins, we can obtain the fluorescence ratio of nuclear and cytoplasm,

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and according to the ratio, the activation time of NF-κB could be determined, which helps us to further study NF-κB signaling pathway blocking mechanism. The accurate segmentation of cells (especially the adhesion and irregular cells) and relevant fluorescence parameters extraction are crucial for the research.

Over the past decades, researchers have put forward various overlapping cell segmentation methods, including the active contour method [3–9], the watershed algorithm [10–14], the edge detection or gradient method [15], morphological erosion [16], the sliding band filter approach [17]. Watershed approach is an effective skill to separate adhesion cells. However, due to large numbers of local minima/maxima in the image or its gradient, directly applying the watershed algorithm to the image or its gradient may lead to severe over-segmentation. Therefore, many improvements on watershed algorithm [11, 18] have been proposed. The main ideas of improved watershed algorithm could be classified into three aspects, such as enhancing the image quality by smoothing before watershed transformation [19], adding constraints during the watershed operation process and merging the regions after watershed transform using fusion technology. Though there exist many adhesion cell image segmentation algorithms [3, 18, 20], the segmentation of human umbilical vein endothelial cells has not been discussed. Therefore, the study on segmenting human umbilical vein endothelial cells in this paper is an innovation. Unlike blood cells [19], human umbilical vein endothelial cells not only have different size, but also present various shapes such as round-like and fusiform. Moreover, they usually overlap each other with different degree. These features make the segmentation of human umbilical vein endothelial cell become more difficult than blood cells. To solve the problem, we propose a new improved watershed algorithm to segment the irregular and low intensity human umbilical vein endothelial cells image. The experimental results shows the proposed approach is precise and effective.

2 Principles and Method

Since the nucleus of human umbilical vein endothelial cells with round/oval features, we combine the nucleus image and cell image to segment cell image. Firstly, do distance transform for the binary nuclear image, extract "seed point" in the distance map, delete redundant seed points using neighborhood optimization method, get a new "seed point" and ensure one seed point corresponding to one nucleus; Secondly, binarize the cell image (target white, black background), obtain the "seed point" of each cell integrating the seed point map of nucleus acquired before, reconstruct the distance map of cell image based on the seed point map of cell found before; Thirdly, segment cells using label-controlled watershed algorithm. For obtaining the fluorescence intensity of each nucleus, the adhesion nuclei are firstly segmented using the watershed algorithm based on optimized seed point map of nucleus and then located in the cell image. After adhesion nuclei/cells segmentation and nuclei location, mark the segmented cells and nuclei and regulate the initial cell mark to ensure that each nucleus mark is consistent with each cell mark. Finally, extract fluorescence intensity of cell/nucleus/cytoplasm, and obtain the ratio of nucleus and cytoplasm fluorescence intensity. These obtained data make it possible for quantitative analysis of subsequent NF-κB activation results.

2.1 Image Analysis

From Fig. 1, we can see that cell fluorescence image quality is poor, which contains fuzzy edges