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Automated segmentation of overlapped nuclei using concave point detection and segment grouping

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ABSTRACT

Nuclei assessment and segmentation are essential in many biological research applications, but it is a challenge to segment overlapped nuclei. In this paper, a new automatic method is proposed to segment overlapped nuclei robustly and efficiently. The proposed method mainly contains four steps: contour extraction, concave point detection, contour segment grouping and ellipse fitting. Blurry nuclei splitting and unobvious concave point detection are always difficult problems in nuclei segmentation. Contour extraction algorithm provides a smooth contour result and it is employed to estimate the blurriness degree of the image. The blurry level determines parameters in subsequent steps, which improves the accuracy of blurry nuclei splitting. Different methods to extract obvious and unobvious concave points from candidate points are proposed. In addition, grouping rules are proposed to assign segments divided by concave points into groups. Comparison study is performed and experimental results showed the effectiveness of the proposed method.

1. Introduction

Analyzing the morphology of nuclei is important to study nuclei polarization and arrangement on a micro-patterned substrate [1,2]. And it is an essential step to learn the contribution of the nucleus to the mechanical properties of cells [3]. However, the assessment of nuclei is a huge task for manual measurement. Manual segmentation is subjective and time consuming for large number of nuclei analysis. Thus, automatic nuclei segmentation is needed.

Many algorithms have been proposed to split overlapped cells or nuclei automatically [4–15]. Watershed method is one of the most popular methods to segment overlapped nuclei [4–6]. Method in [5] is based on watershed. Method in [6] and the first method in [4] are based on marker-controlled watershed. Watershed is not good at dealing with highly overlapped nuclei and is easy to cause over segmentation problem. Marker-controlled watershed reduces over segmentation, but the detection of markers is still not accurate in high overlapped nuclei. The second method proposed in [4] is pixel classification-based supervised segmentation. Another pixel classification-based nuclei segmentation method using Bayesian classification is proposed in [8]. One common defect of pixel classification-based method is its high computational complexity. Curvature detection is another method that is widely used in nuclei segmentation [9–15]. Different methods to detect concave points were employed. The accuracy of nuclei segmentation is highly dependent on the accuracy of concave point detection. These concave point detection methods are easily affected by noisy nucleus boundary. Unobvious concave point cannot be detected. There is either over segmentation or under segmentation problems in these methods. In [16], nucleus contours are approximated to line segments using a variation of Ramer-Douglas-Peucker algorithm [17]. Sharp turns and inflexion points are detected by evaluating angular change of the line segments. Then contours segmented by sharp turns and inflexion points are set into groups to fit to an ellipse if they satisfy certain criteria. As curvature changes slowly at unobvious concave point, its line segment approximation may miss the unobvious concave point and combine curves in two nuclei as one line segment. In addition, because some nuclei contour segments are short and noisy, it is difficult to find an accurate geometric center by this algorithm. Thus, the group of contours is affected.

To overcome the limitations mentioned above, a novel method is proposed in this paper. Concave points are detected and contour segments are set into groups to fit to ellipses. The contribution of our method can be summarized in three aspects:

(1) Level set method is proposed to extract contours and evaluate whether a nucleus is blurry. The three-phase level set method proposed in [18] is employed in the detection of nucleus contour for the first time. It's able to obtain a closed

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and smooth contour without any loss of corner or small turn information in contour. The detection result is used to judge whether the nucleus is well focused during image acquisition, which is helpful to set parameters automatically in concave point detection.

(2) A novel concave point detection algorithm is proposed. Instead of calculating angles or convexity on edge pixels, a candidate point detection method is proposed. Features are extracted on these candidate points. The robustness of concave point detection is improved. In addition, a candidate point is considered to be a concave point if it fulfills either obvious concave point extraction algorithm or unobvious concave point algorithm. Two different algorithms are proposed which focus on these two kinds of concave point respectively.

(3) A simple but efficient method to set contour segments into groups is proposed. The three criteria proposed in initial grouping and group verification are able to ensure that contour segments in the same group belong to one nucleus.

This paper is organized as follows. In Section 2, a three-phase level set method [18] is employed to detect the contour of nucleus. Concave points are detected and contour are split into segments by the concave points. In Section 3, the contour segments are assigned into groups. Each group of contour segments is fitted to an ellipse. Experimental results and conclusion are presented in Sections 4 and 5 respectively.

2. Contour extraction and concave point detection

It's a challenge to smooth nucleus contours without smoothing the turns of contours. Nucleus edge may be blurry and the slope of some turns may change slowly. It's easy to lose useful information during the process of smoothing. Three-phase level set is employed to extract nucleus contour as an initial segmentation. An image is segmented into three regions: nucleus, transition area and background.

Then a group of candidate points are detected. Concave points are further selected from candidate points.

2.1. Initial segmentation

Level set is widely used in image segmentation [19,20]. One advantage of level set method is its capability to represent contours with complex topology. There are mainly two classes of level set models: region-based models [21,22] and edge-based models [23]. Edge-based models utilize edge information to guide the motion of active contour while region-based models utilize region information. Edge-based models are usually sensitive to noise [24]. A region-based three-phase level set [18] method is employed to detect the contour of nucleus.

2.1.1. Three-phase level set formulation

Three-phase level set method segments an image into three regions: nucleus, background and transitional area. To deal with the intensity inhomogeneity during image acquisition, an observed image I can be modeled as:

$$I = bJ + n \tag{1}$$

where J is the original image, b stands for intensity inhomogeneity and n is additive noise. Let Ω be an image domain, and Ω_i be the disjoint regions segmented by contour. For each $y \in \Omega$, $\mathcal{O}_y \triangleq \{x : |x - y| < \rho\}$ represent a circular neighborhood with radius ρ. Under the assumptions that bias field b changes slowly in Ω and J takes a constant value c_i in Ω_i, we can define an function in $\mathcal{O}_y$:

$$\varepsilon_y = \sum_{i=1}^{N_y} \int_{\Omega_i} K(y - x)|I(x) - b(y)c_i|\, dx \tag{2}$$

where N_y denotes the number of Ω_i in $\mathcal{O}_y$. $K(y - x)$ is a kernel function, and $K(y - x) = 0$ if $x \notin \mathcal{O}_y$. The membership functions of three-phase level set are defined as $M_i(\Phi) \triangleq M_i(\phi_1(y), \phi_2(y))$. ϕ_1 and ϕ_2 are level set functions and $M_1(\phi_1, \phi_2) = H(\phi_1)H(\phi_2)$, $M_2(\phi_1, \phi_2) = H(\phi_1)(1 - H(\phi_2)$, $M_3(\phi_1, \phi_2) = (1 - H(\phi_1))H(\phi_2)$. $H(\phi)$ is Heaviside function. Then the final energy function is defined as:

$$F(\Phi, c, b) = \int \sum_{i=1}^{N} \int_{\Omega_i} K(y - x)|I(x) - b(y)c_i|\, M_i(\Phi) dx\, dy + \mathcal{R}_p(\Phi) \tag{3}$$

where $\Phi = (\phi_1, \phi_2, \phi_3)$. $\mathcal{R}_p(\Phi)$ is distance regularization term and it keeps a signed distance profile near the zero level set [25]. It is defined with a potential function such that the derived level set evolution has a unique forward-and-backward (FAB) diffusion effect. In this paper, $R_p(\Phi) \triangleq \int (1/2)(|\phi| - 1)^2 dx$. By minimizing the energy function, two level set contours are obtained. The energy function minimization problem is solved by iterative process. The initial contours are obtained by Otsu's method. For each iteration, c_i and b are calculated according to the result of last iteration. c_i is the mean value after last bias, that is:

$$c_i = \frac{\int (b * K) I u_i dy}{\int \left(b^2 * K\right) u_i dy} \tag{4}$$

where $u_i = M_i(\phi(y))$. Then b is calculated to bias the gray scale of each pixel in the region into c_i:

$$b = \frac{\left(I \sum_{i=1}^{N} c_i u_i\right) * K}{\sum_{i=1}^{N} {c_i}^2 u_i * K} \tag{5}$$

With fixed c_i and b, Φ is solved by calculus of variations [26]:

$$\frac{\partial \phi}{\partial t} = -\frac{\partial \mathcal{F}}{\partial \phi} = -\delta(\phi)(e_1 - e_2) + \upsilon \delta(\phi) div\left(\frac{\nabla \phi}{|\nabla \phi|}\right) + \mu div\left(\frac{p^{'}|\nabla \phi|}{|\nabla \phi|} \nabla \phi\right) \tag{6}$$

where ∇ is the gradient operator and $div(\,\cdot\,)$ is the divergence operator. After interaction, the level set contour is obtained. Each level set contour is fitted to a rectangle to evaluate the radius of ellipse. Most of the nuclei in the image is not overlapped. The highest peak of rectangle's length distribution is evaluated as $2\hat{R}_{Lm}$. And similarly the width is evaluated as $2\hat{R}_{Wm}$. $\hat{R}_{Lm}$ and $\hat{R}_{Wm}$ are the estimated major radius and minor radius.

2.1.2. Nuclei image blurry evaluation

Level set performs better than Otsu's method [27] in splitting light overlapped nuclei. Fig. 1 shows the contours detected by level set. In nuclei junctions indicated by using yellow arrows, level set method succeeded in splitting nuclei while Otsu's method didn't. Nuclei in the first row are well focused and distance between nucleus contour boundary (blue contour) and transitional area contour boundary (red contour) is short. While there is a relatively long distance between the two contours in blurry nuclei image shown in the second row. For each nucleus contour with N points, the distance is measured by the following equation:

$$D = \frac{1}{N_i} \sum_{i=1,\ D_i < Th_1}^{N} D_i \tag{7}$$

where D_i is the minimal distance between point i and transitional area contour. N_i is the number of points whose D_i are smaller than