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A novel approach for detection and delineation of cell nuclei using feature similarity index measure

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ABSTRACT

Accurate image segmentation of cells and tissues is a challenging research area due to its vast applications in medical diagnosis. Seed detection is the basic and most essential step for the automated segmentation of microscopic images. This paper presents a robust, accurate and novel method for detecting cell nuclei which can be efficiently used for cell segmentation. We propose a template matching method using a feature similarity index measure (FSIM) for detecting nuclei positions in the image which can be further used as seeds for segmentation tasks. Initially, a Fuzzy C-Means clustering algorithm is applied on the image for separating the foreground region containing the individual and clustered nuclei regions. FSIM based template matching approach is then used for nuclei detection. FSIM makes use of low level texture features for comparisons and hence gives good results. The performance of the proposed method is evaluated on the gold standard dataset containing 36 images (~8000 nuclei) of tissue samples and also in vitro cultured cell images of Stromal Fibroblasts (5 images) and Human Macrophage cell line (4 images) using the statistical measures of Precision and Recall. The results are analyzed and compared with other state-of-the-art methods in the literature and software tools to prove its efficiency. Precision is found to be comparable and the Recall rate is found to exceed 92% for the gold standard dataset which shows considerable performance improvement over existing methods.

1. Introduction

Image processing techniques are widely being used in different biomedical applications for accurate and efficient analysis [1,2]. Image analysis of cells and tissues is an active area of research as novel and accurate approaches are required for the scientific and quantitative analysis of cell and tissue specimens. The analysis of these cells and tissues involves cell nuclei counting; detecting abnormal cell nuclei; analyzing the presence of antigens within target cells, etc. These analyses help in diagnosing a wide spectrum of pathologies. In the early

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stages, manual methods were used by pathologists for analyses which were time consuming and yielded inaccurate results due to inconsistencies and human errors. With the advent of image processing techniques large numbers of algorithms are being developed for automating the process of cell and nuclei segmentation and analysis. Most of the existing methods rely on prior knowledge or require initial training on datasets for detection and segmentation of cell or nuclei. Active contour models are powerful tools for cell segmentation introduced in [3]. A functional formulation to optimize the segmentation is formulated by Mumford and Shah in [4]. The popular level set segmentation method was proposed in [5] and enhanced for region based segmentation in [6]. Automated segmentation of fluorescently labeled cell nuclei in 3D confocal microscope images is presented in [7]. Texture and contour based method for segmentation has been proposed in [8,9]. A nuclei segmentation method based on adaptive threshold is presented in [10]. There are many software tools which are used for microscopic image analysis. Most of these existing tools do not yield accurate segmentation.

Generally, the cellular images are obtained using multichannel fluorescent microscopes. In a two channel microscopy, the cells and nucleus will be separately highlighted by fluorescence. These detected nuclei are used as seeds for further segmentation as in [11]. A texture and watershed based method is presented in [12] for segmenting bright field and fluorescent microscopic images. An efficient method for segmenting moving cells in bright field and epi-fluorescent microscopic image sequences is presented in [13]. The segmentation of microscopic images captured by single channel microscopy is more challenging due to the inefficiency of detecting the seed points or nuclei regions accurately. So, other methods like detection of local maxima points using h-maxima transform [14,15] were used in such cases. Some of the existing methods for analyzing single channel microscopic images rely on the shape of nuclei as in [16,17], where circular nuclei are detected using Hough transform [18]. This method depends on the shape of the cell which is expected to be circular or elliptical. The shape based detection fails to give accurate results in most cases because nuclei may exhibit varying shapes and sizes in pathological samples.

Another challenging issue in the segmentation of cells is the delineation of overlapping nuclei. In [19] geometric active contours are used with a coupling constraint which helps in the delineation of overlapping nuclei. Training based methods adopts a physical model for analyzing the shape of the nuclei, which is then used for the detection of nuclei. Such a deformable active shape model is used in [20] for overlapping nuclei segmentation. Training based detection methods requires additional overhead of the training phase. The method in [21] combines shape, texture and intensity features for cell nuclei extraction. But this method does not address the detection of clustered nuclei. An algorithm for separating touching cells in hematoxylin-stained breast tissue microarray specimens is presented in [22]. The algorithm claims to separate touching cells in hematoxylin-stained breast TMA specimens using an active contour based level set segmentation method based on an interactive model. There are several template based nuclei detection techniques in literature. In [23] the round shape of cell is chosen as template for detecting the nucleus within the image. In [24] a template matching algorithm with shape models is used to identify glands and nuclei from the low-level likelihood scenes. Several other techniques of template matching also exist with prior knowledge of shape, number of nuclei or using cross correlation techniques as in [25] where a nucleon template is selected from several manually selected nuclei and is matched against the image using normalized cross correlation techniques.

The most important aspect of nuclear segmentation is the process of detecting set of points indicating the nuclei centers that are referred to as ''markers'' or ''seeds''. These seeds are then used by segmentation algorithms for delineating cell nuclei. The accuracy of the segmentation technique is of utmost importance which is highly dependent on the accuracy and reliability of initial seed point detection. Several approaches have been developed for seed point detection. The Euclidean distance map along with watershed algorithm was used for seed point detection in [26,27] but it has a major drawback of over segmentation.

In this paper, we propose a novel technique for automated seed point detection in single channel microscopic images using a template matching technique. The method identifies the locations of the nuclei within the image using a template matching technique based on feature similarity index measure (FSIM) [28]. The proposed method has been compared with [29,30], two popular methods of nuclei detection in literature. In [29], Wienert et al. presents an efficient contour based minimum model approach for detecting the nuclei points while in [30], seed points are detected by combining multiscale Laplacian-of-Gaussian filtering constrained by distance-map-based adaptive scale selection. The recall rate obtained using our method is better for most of the images in the data set compared to the methods in [29,30]. Another highlight of our method is the clustered nuclei detection. The template matching using the feature similarity index measure makes use of low level texture features in the image and hence gives good results and helps in delineating clustered nuclei. This method does not require any training phase but requires only a priori estimate of size of one nucleus which is selected as template and so only minimal user interaction is needed. The proposed method is called DCN_FSIM method (Detection of Cell Nuclei based on FSIM) here onwards.

2. Materials and methods

The DCN_FSIM method uses an initial clustering technique to separate the nucleus, cytoplasm and background. Due to the differences in staining several gradient variations can occur in the input image and traditional hard clustering methods like k-means algorithm fails in such cases. The Fuzzy C-Means (FCM) [31] clustering algorithm is hence chosen for clustering since it provides a membership degree for each pixel which denotes the degree to which each pixel belong to a particular cluster. The pixels having maximum membership to belong to the nuclei region are selected as nuclei regions. Once the image is clustered, the nuclei pixels in the image are found to have clustered around the minimum cluster centroid (darker shades) and hence they are separated to form a binary image containing all probable nuclei regions as foreground.