Indian Journal of Engineering

ANALYSIS

ISSN 2319 – 7757 EISSN 2319 –7765

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Publication History
Received: 26 July 2016
Accepted: 20 August 2016
Published: October-December 2016

Citation
Vinothini A, Prasad B. H-Maxima Transformation Based Image Segmentation of Clump Nuclei in Phase Contrast Image. *Indian Journal of Engineering*, 2016, 13(34), 655-660

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H-Maxima Transformation Based Image Segmentation of Clump Nuclei in Phase Contrast Image

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ABSTRACT

The nucleus segmentation is the most important and tedious process in medical image analysis. The proposed method has three stages: K-means clustering, marker controlled watershed segmentation and texture analysis. First, the preprocessing stage uses top-hat filter to increase the contrast of nuclei and reduce the non-uniform illumination, and K-means clustering is used for rough segmentation of the cell image. By using K-meansalgorihm, image pixel is dividing into three parts: nuclei, cytoplasm and background. In second stage, the segmentation of nuclei consists of a distance transformation, h-maxima transformation and watershed segmentation. The markers are used to obtain segments of the nuclei in the h-TMC watershed segmentation. To detect the single marker in nucleus, we usethese transformations. Due to imaging artifacts, prolonged cell cytoplasm in the contrast image, nuclei may falsely be segmented and it leads to an inaccurate analysis of the cell image. To identify and remove the non-nuclei segments. The third stage of texture analysis is followed. The texture with adaboost algorithm is used for non-nucleus identification.

Keywords

Adaboostalgorithm, h-TMC watershed segmentation, K-means clustering,Nuclei segmentation, Phase contrast image.

1. INTRODUCTION

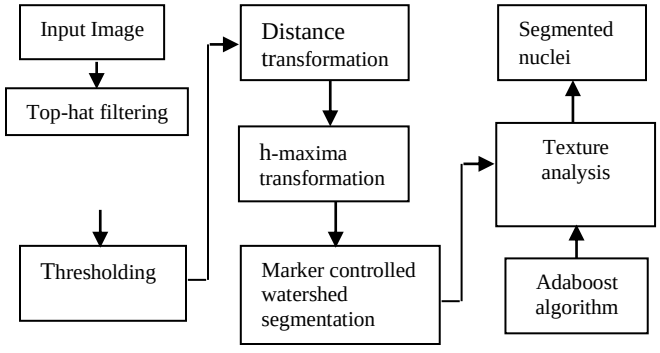
Automated phase contrast imaging systems are becoming more important for molecular cell biology research, because it is very essential for finding various diseases such as cancer, lack of fertility, and inflammation. Image segmentation is a fundamental process that partitioning the images into various regions or object. The effective segmentation of nuclei is that involves separating each nucleus from its background. This is the first step of these system includes cell/nucleus segmentation for developing various tools for automatic cell image analyses such as counting cell, quantifying molecular markers (antigens) or quantifying aspects associated with normal/diseased tissue architecture [1]. The performance of manual method is time consuming and prone to human error. These factors are developed by using automatic cell analysis tools to identifying the nucleus exactly and extract relevant characteristics of cell.

Phase contrast microscopy is particularly used in live cell microscopy, and how they proliferate through cell division. It is a widely used technology; it results in poor image quality due to uneven lighting conditions and short exposure times used to minimize the cell death.The illumination variation and imaging artifact changes also increase the complexity of automatic segmentation. The problem is more acute in nuclei overlap and touching nuclei one another in a cluster [2].

To overcome the above problems, the proposed framework depends on the following process regarding the characteristics of nucleus. First, a nucleus in a phase contrast images appears as a dark region surrounded by a bright halo artifact [3],[4] and segmentation operation on nucleus carried with the intensity information. There are many algorithms that were used in segmentation process namely top-hat filter [5], distance transformation [6], h-maxima transformation and marker controlled watershed segmentation [7],[8]. Second, the texture analysis is carried on the cell image with dust (or) imaging artifacts of nucleus. With these texture analysis, six measures are carried based on the Gray Scale Co-occurrence Matrix (GLCM) [9] with Adaboost algorithm [10].

2. METHODOLOGY

Nuclei segmentation is a common and complicated task in image analysis. Depending on the image characteristics the segmentation performed in different ways. An automated nucleus segmentation is significantly improves the performance of the irregularity of cell- shape, cell-to-cell variability and noise in image. Fig.1 shows the block diagram of the proposed method, it consists of three level: preprocessing, h-maxima transformation based marker controlled watershed segmentation and texture.



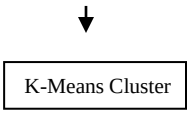


Fig.1. Block diagram for proposed method

1.1 Preprocessing

In a phase contrast image, due to non- uniform illumination, the image appears as noisy peaks during the image acquisition system. Then this stage consist two operations1) top-hat filter and 2) thresholding. The contrast of the images are increased in the first stage and reduces the noise content in the image. To improve this and to separate the nuclei from the background the top-hat filter is performed.

$$A_1 = \text{TopHat}(A'_0, B) = A'_0 - (A'_0 \circ B)$$

Where the "o" refers to the morphological opening, A_0 is an input, $A'_0$is the compliment image of A_0, A_1 is the filtered image and B is a non-flat disk shaped structuring element of radius 'r'. The shape of the structuring element B is defined as

$$B(r) = \{(x,y) \in Z^2 : \sqrt{x^2 + y^2} \leq (r + 0.5)\}$$

In the preprocessing stage, the top-hat filtering method is used to invert the input image and then applies morphological opening operation is performed. After performing of opening operation. Thenon-uniform illumination in the input image is corrected by subtracting the morphological processed image with unprocessed image. The performance of top-hat filter also increases the intensity between the nuclei and surrounding regions.

The thresholding operation is followed after the top-hat filtering on A_1. The otsu'sthresholdingapproximates the background and gray level distribution in the object. It is more approximated than by a normal distribution. These type of a thresholding is used to reduction of gray level image into a binary image. The selection of thresholding is to minimize the combined rangeand separate into two classes.The Otsu's thresholding is computed as follows:

$$\sigma_\omega^2(t) = \omega_1(t)\,\sigma_1^2(t) + \omega_2(t)\,\sigma_2^2(t)$$

The above equation shows the weight ωi is the probabilitiesof the two classes separated by a threshold, t and σ_i^2variances of these classes and it is the normalized histogram of the intensity of nuclei and non-nuclei regions.

1.1.1 K-means Cluster

K-means clustering aim for obtaining K-observation from n-observation in which each clustered observation is same with nearest mean. The K-means cluster divides the image pixel as follows:

1) Nuclei
2) Cytoplasm
3) Background

This clustering algorithm splits the nucleus image by filtering and merged the two pixels of same class that has the median gray levels. With this method the nucleus are corrected and intricate the rough divisions of thecell.

1.2 h-maxima based marker controlled watershed segmentation

1.2.1 Marker extraction

The nuclei from the background are separated by using thresholding method and there may exist touching nuclei in clusters. To separate the nuclei, it uses intensity information for identifying a marker in the nuclei of the cell image and it is followed bythe watershed segmentation. Generally the nuclei are elliptical shape. The center of the nuclei is taken as highest distance value from the outer edge of cell to propagate the distance into center of the nuclei. high accuracy of the nuclei is obtained through the h-maxima transformation.

In a distance transformation, Chamfer 5/7 distance is applied on thresholded image to compute the distance image. In this method, zero value is assigned initially for the boundary pixels and with infinity values for non-boundary pixels. These can be performed in two passes as forward and backward. Distance from left to right is modified by forward passes in 'D' as shown,

$$D[i,j] = min(D[i-1,j-1] + 7, D[i-1,j] + 5, D[i-1,j+1] + 7, D[i,j-1] + 5, D[i,j])$$

Distance from top to bottom is modified by backward passes in D as shown,

$$D[i,j] = min(D[i,j], D[i,j+1] + 5, D[i+1,j-1] + 7, D[i+1,j] + 5, D[i+1,j+1] + 7)$$

The value of D is normalized by dividing them by 5. D [i,j] is the distance of the nearest boundary pixel from the position (i,j). After performing distance transform the normalization of D and A_1 a shown,

$$norm(A_2) = \{\omega * norm(D)\} + \{(1-\omega)^* norm(A_1)\}$$

Where A_1 is the image that is corrected illumination parameters, A_2 is the transformed image andweighting parameter is ω, where it is set to 0.3.then the value of ω is set to 0.3. The false markers can be detected in less number after the h-maxima transformation, than this transformation detects local maxima as seed point being higher sometimes to texture.The purpose of marker detection is used to detectthe height of all the peaks in the regions, in relative with the surrounding 'h' values lesser. The normalization function h as follows:

$$h = \frac{\beta}{2} \times \frac{\{\overline{A_n} - \overline{A_b}\}^2}{\overline{A_n} - \overline{A_b}}$$

1.2.2 Marker controlled watershed segmentation

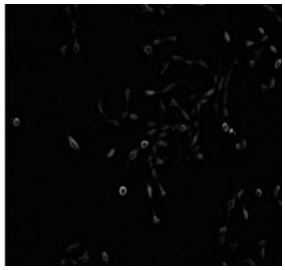
The nuclei segmentation uses the machine learning algorithm with the classical segmentation to deal the challenges faced in nuclei clustered and overlapping. This problem can be addressed by the watershed transform by collecting a group of catchment basins in the observed domain. Then this algorithm is applied to the radiant of input image and clustered nuclei. The flooding simulation is the process for the classical form of transform (watershed) and it start from the local minima. The marker controlled watershed algorithm removes these minima by markers. Never flooding simulation will starts from the local minima instead it the detected markers instead originator from detected markers in the cell.

1.3 Texture analysis

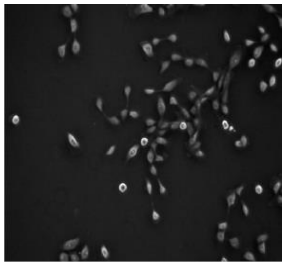
The texture measures tend to extract the typical nucleus in which the non-nucleus segments are shown in a phase contrast image. To identify the non-nucleus segments from nucleus, the Adaboost algorithm is used. The Adaboostalgorithm gives efficient for texture analysis. The feature value of the nucleus segments are stable compared with the non-nucleus segments. For these purpose the adaboost algorithm is used to separate both the nucleus and non-nucleus from the observed cell for texture analysis.

2. RESULT AND DISCUSSION

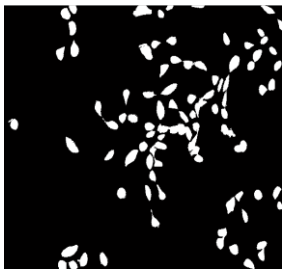
These algorithms are used to identify the non-nucleus segments of phase contrast image. Fig.2 (a) shows input image. Input image is a phase contrast image with les contrast image. Fig.2 (b) shows the illumination corrected image. The non-uniform illumination is eliminated and the contrast is improved with the top-hat filter processing.Fig.2 (c) shows the thresholded image. Then the nuclei are segmented by using Otsu's thresholding. Fig.3 (d) shows the K-means Segmentation and it also explained in section II.



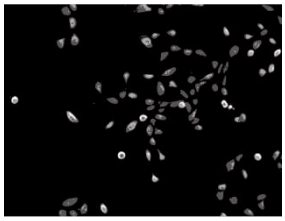
(a)



(b)



(c)



(d)

Fig.2 (a) Input Image (b) Illumination Corrected Image and (c) Thresolded Image (d) K-means Image

After preprocessing h-maxima transformation is obtained from distance transformation image bydetecting all regional peaks value of the individual nuclei is calculated to locate the nucleus. Fig.3 shows detection of marker using h-maxima transformation.

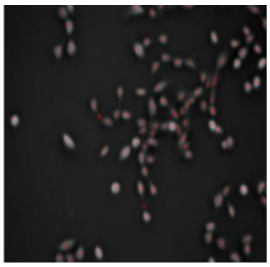


Fig.3 Detection of marker using h-maxima transformation

h-TMC based watershed segmentation is performed on the marker detected image. It separate the overlapped nuclei or touching nuclei in cluster. Fig.4 shows the h-TMC based watershed segmentation of nuclei.

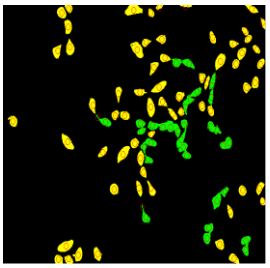


Fig.4 h-TMC based watershed segmentation

Fig.5 (a) shows the segmented nuclei in initial contour, where the watershed ridged lines are marked. Fig.5 (b) shows the segmented nuclei after texture analysis. Then the texture analysis is removes the false segmentation in the cell nuclei.

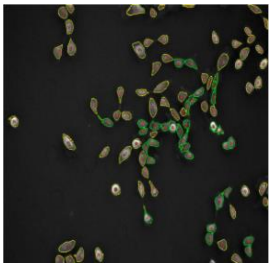
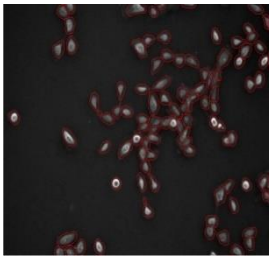


Fig.5 (a) segmented nuclei in initial contour

Effectiveness of the proposed method can be analyzed in term of accuracy detection for the segmentation process. The analysis of various algorithms is shown in Table I. False segmentation and touching nuclei are reduced compared with other method.



(b)

Fig.5(b) segmented nuclei after texture analysis

3. EVALUATION AND PARAMETER SELECTION

The parameters that are used to measure the detection accuracy are precision (p), recall (r), and F1-measure (f1). Various parameters are used effectively to calculate the segment of individual nuclei. Some of parameters calculated are cell nuclei area, cell radius, cell center.

TABLE I
ACCURACY COMPARISION WITH THE SOME OTHER EXISTING METHODS

Methods	Total number of frames	Nuclei count	P	R	F_1
Proposed method	220	4850	0.938	0.922	0.946
Graph cut	220	4850	0.912	0.845	0.878
Hybrid merging	220	4850	0.898	0.873	0.885
Compactness	220	4850	0.877	0.832	0.854
Watershed	220	4850	0.824	0.779	0.802

These parameters are calculated for each individual nucleus to segment efficiently. The accuracy detection can be improved at the texture analysis stage by identification and removal of non-nuclei segments.

4. CONCLUSION

An h-TMC based watershed segmentation method is used in nucleus segmentation. There are various methods to resolve the non-uniform illumination, low contrast and imaging artifacts from images, the prolonged cell cytoplasm produces noise in the image. To eradicate the above issues the proposed method yields better result in three levels. Firststage,follows the improvement of contrast and removal of non-uniform illumination and Rough division are segmented by K-means clustering.In the second stage, the angle marker in each nuclei from the intensity information are detected by h-maxima and distance transformation. The nucleus can be segmented efficiently by using the h-maxima transformation based marker controlled watershed with marker as minima. By using these transformation methodthe over and under segmentation of the nuclei can be reduced. The nucleus is processed efficiently in the second stage of the segmentation, still some imaging artifacts tends to false segmentation of the nuclei, due to the noise caused by prolonged cell cytoplasm. Thiscan be resolved by texture analysis method. Where the texture shows the nuclei from the non-nuclei based on the typical characteristics processed by them.Therefore the non-nuclei can be distinguished effectively by the texture analysis. Outputs obtained from the proposed method shows that the false nucleus segmentation is identified much well than the other nucleus identification techniques.

5. ACKNOWLEDGEMENT

The authors wish to thank the reviewers for their valuable feedback that resulted in an improved paper and we would also like to thank our guide for assisting with code development.

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