

Segmentation of Microscopic Images: A Survey

Bhupendra S. Deshmukh
Dept. of Electronics and Communication
Government Polytechnic Sakoli
bsdeshmukhp@gmail.com

Vijay H. Mankar
Dept. of Electronics and Communication
Government Polytechnic Nagpur
vhmankar@gmail.com

Abstract—Screening of microscopic slides is a manual process which involves its subjectivity. A semi-automated computer based system can contribute to the detection of screening error by the way of greater reliability. The objective of segmentation in microscopic image is to extract cellular, nuclear and tissue components. This problem is challenging because accurate segmentation of each cell is a difficult task and there is large variations in feature of each component. In this paper an attempt is made to overview various segmentation methods for microscopic images. The existing methods are grouped by their application in one of the following pathologist field: Cytology and Histology. This analysis is helpful for the better use of existing method and for improving their performance as well as designing new one.

Index Terms— Segmentation, Microscopic Image, Cytology Image, Histology image.

INTRODUCTION

Image segmentation is the key procedure in automating any computer aided diagnostic system. Accurate segmentation of image plays a crucial role because it can ultimately determine the success or failure of computerized analysis procedure [1]. Image processing methods are widely used in the last decade in the medical imaging and microscopic field received consistent efforts from research [2]. Considering the importance of pathologist results for human health and application difficulties, many computer aided image analysis system have been proposed.

Cell segmentation is a fundamental subject of quantitative analysis of cytological and histological images. Among various types of cell images, the one obtained from biopsy are more difficult to segment, because of the diversity of structure contained in the images, the intense variation of background caused by the uneven staining, and overlapped cell cluster. As most of the malignant characteristics of cell contained in the cell nucleus, the isolation of cell nucleus is an important part of segmentation for this kind of cell images [3].

The task of segmentation in the microscopic images refers to the process of finding the boundaries of cells, cells nuclei and histological structure with adequate accuracy of images of stained tissue with different makers. These papers study different image types from breast, thyroid, cervical, liver with different makers, e.g. hematoxyline and eosin, papnicolaous stain, immunidetection of lamin A/C.

This is not a comprehensive study of the methods, but rather an overview and classification of most used image

processing methods in this particular domain. We group this existing technique by their pathological field, i.e. cytology and histology and extract from each technique the most important image processing methods used, i.e. watershed, threshold etc.. This analysis is helpful for better use of existing methods and for improving their performance as well as for designing new one.

DIFFERENT METHODS

The *watershed segmentation* algorithm is inspired by natural observation, such as rainy day in the mountains [4]. A given image can be defined as a terrain on which nuclei correspond to valleys. The terrain is flooded by rainwater and arising puddles start to turn into basins. When the water from one basin begins to pour away to another, a separating watershed is created. The flooding operation has to be stopped when the water level reaches a given threshold θ . The threshold should preferably be placed somewhere in the middle between the background and a nucleus localization point [4].

Watershed algorithm when used directly leads to over segmentation problems due to noise and other irregularities, such as gradient. Xuqing Wu and Shishir Shah applied unsupervised clustering method to locate the “seed” for each region by using regional minima/maxima extraction to preserve the intensity variance information in the spatial domain [1]. An adaptive nonlinear diffusion algorithm for the reduction of noise whose characteristic is to preserves the edge information as well before applying the watershed algorithm [5] gives proper segmentation. The hybrid method of watershed algorithm with k-means clustering could separate cytoplasm and nucleus by converting RGB image into HSV [6].

Many real images are made up of various smooth and textured based regions, all of which need to be reliably identified in the segmentation algorithm. In this case existing methods fails to produce a meaningful segmentation. Therefore it would clearly require some prior knowledge of feature selection before segmentation. In this way, highly textured regions can be segmented using spatial frequency based features, while the smooth region can be segmented using local grey level statistic such as mean and variance. In this correspondence, a scheme that automatically selects the optimal feature for each pixel using “*wavelet*” analysis is proposed [7].

In the cytology images chromatin distribution is the indication of early stages in the development of future

abnormalities. Discrete wavelet transforms (DWT) based textural feature are used to classify the cancerous cells. DWT is inefficient for providing the phase information. Since, phase information plays an important role in signal and image analysis. Hence, Issac Niwas S *et al.* suggested to use some different complex wavelet transform such as the dual tree complex wavelet transform (DTCWT), the projection based complex wavelet transform, the steerable pyramid complex wavelet transform. It is described that after processing original image is represented by the sub-images at several scales. Every detailed sub image contains information of specific scale and orientation. Spatial information retained within the sub-images. There are two broad classes of filters that permit such a decomposition orthogonal and Biorthogonal. Real orthogonal filter are energy preserving, but lack linear phase. Biorthogonal filter are linear phase and permits use of continuous symmetric boundary extensions, but do not conserve energy in the transform domain [8].

The fast wavelet transform (FWT) is a computationally efficient form of discrete wavelet transform. It is a multiresolution analysis method that provides frequency decomposition of images using scaling and functions [9].

Thresholding is very simple and efficient method for the segmentation of microscopic images. The problems associated with the thresholding method in the cytology and histology images is the non-uniform distribution of staining and as per the colour density of images thresholding value differs with different images. Neerad Phansalkar *et al.* proposed a new local thresholding segmentation method to solve the problem of non-uniform staining using different colour spaces [10].

Segmentation of cell nuclei in the microscopic images using *snake* algorithm is one of the active contour method. The basic idea of snake is to search for a curve in the images where the weighted sum of internal energy and potential energy is minimum. The internal energy is defined by the curve itself to keep the model smooth during deformation. The potential energy is computed from the image data to move the curve towards an object boundary or other desired feature within an image. The implementation of this algorithm is based on greedy algorithm [11].

Automatic segmentation of microscopic images is a challenging task because of high variability of cells and their structure, limited resolution of acquired images, low contrast of boundaries of structural cell elements, loss of data in passing from 3D object to its 2D image. Hence, considering these problems O. L. Konvesky and Yu. V. Stepanets proposed an improved *Seeded Region Growing* (SRG) algorithm. The basic idea of this method consists in assigning image pixel to coherent group (cluster) according to the predetermine uniformity criterion. The growth of cluster start from their seed, which are selected automatically or manually as a result of certain image features. The cluster spread on by subsequently joining pixel adjacent to them and not yet assign to any other cluster. The growth continues until all pixels are included in the clusters [12].

A nucleus of the cell is the place where a cancer malignancy can be observed. It is observed that the cells have elliptical

shape. Unfortunately, the detection of ellipses using *Hough Transform* which are describe by two parameter a and b ($x = a \cos \alpha, y = b \sin \alpha$) and which can be additionally rotated, is computationally expensive. Thus detection of circle is much simpler, in the sense of required computation, because the required parameter is radius R only. Hence, Maciej *et al.* proposed a hybrid Hough transforms algorithm for the segmentation of cell nuclei of microscopic images [13]. It is observed that applying only Hough transform results in either under segmented image or missed some nucleus detection. Therefore, Hough transform [13] in conjunction with active contour methods is proposed which gives good segmentation results.

The method of microscopic image segmentation using *Fuzzy clustering* technique provides a mean of classifying pixel values with the great extent of accuracy. In real application where there is usually no sharp or crisp boundary between clusters, fuzzy clustering is often suited for the classification of data in decision oriented application like tissue classification, tumour detection etc. One of the most difficult tasks in image analysis and computer vision application is to classify correctly the pixel values as there are no crisp boundaries between objects in an image. In order to address this difficult task, fuzzy clustering techniques [14] are proving to be fruitful research area.

S. Schupp *et al.* presented a system of automatic microscopic image segmentation combining fuzzy clustering and active contour model. An automatic initialization algorithm based on fuzzy clustering is used to robustly identify and classify all possible seed region in the image. This seed are propagated outward simultaneously to localize the final contour of all objects [15].

Eric Dahai Cheng *et al.* proposed a fusion algorithm, where two different algorithms used to detect cell boundary information. It is observed that each algorithm has its strength and weakness, and no one can detect this cell perfectly, but they can compensate each other. The basic idea is to combine these two results into one, in which the probability of detection is increased [16].

Convolution network have been applied with great success for high level computer vision task such as object recognition. Recent study shows that they can also be used as a general method for low level image processing problems, such as denoising and restoration. A convolution network is an alternative sequence linear filtering and non-linear transformation operation. The architecture consist of input layer and output layers, each of includes one or more images, and various intermediate layers with "hidden" images called feature maps that are the internal representation and computation of algorithm. Each layer receives images or feature maps as input from only the previous layer.

Baochuan Pang *et al.* proposed convolution network for the segmentation of cell nucleus in the colour histopathological images and shows two important properties of the convolution network as a segmentation method. First, as a machine learning approach, the convolution network encodes enough high-level domain specific knowledge into the final segmentation strategy

by learning the training data. Second, the convolution network can use appropriate amount of context information in segmenting by optimizing the weights of the filters in the networks through the learning process [17].

In order to detect the cluster in the microscopic image a *cell graph* method is used. Graph theory based methods have an impressive record of modelling complex relationship in numerous contexts and have the ability to analyse the patterns of interaction in systems. In the cell graph method nucleus of cell is consider as a node (vertex) which is connected to the nearby nuclei of cells with the edges and analysed with graph theoretical methods. In a cell graph $G = (V, E)$ is an undirected and weighted graph without loops, with V and E being nodes (vertices) and edges of the cell graph respectively. Graph based representation of image in a slide can obtained by defining node set V as the set of centroid of nuclei and then probabilistically assigning a link between a pair of nodes in V . The coordinates of the centroids of these nuclei are calculated to identify the coordinates of the node set for cell graph generation.

Pournami S. Chandran *et al.* proposed a cell graph method for the cluster detection in cervical cancer cytological images. To avoid the major issue in the simple cell graph method like if euclidean distance is the only criteria for establishing links between the nodes, then nodes in a cluster need not be connected, rather neighbouring cells which are not the part of cluster may be connected, *adjacency matrix*, *centroid matrix* and *spectral analysis* introduced. The graph plotted, using the centroid matrix and the adjacency matrix. The adjacency matrix of a graph is $n \times n$ matrix in which n is number of nodes in the graphs [18].

CONCLUSION

Segmentation is an important step in advance image analysis and computer vision and therefore is an ongoing research area although a dense literature is available.

The techniques [1-3 & 5-18] reviewed in this survey are applicable to analysis of microscopic images in one of the pathologist field i.e. Cytology and Histology and in future can be applied to MRI and CT for better analysis. Furthermore in future a hybrid method based on segmentation algorithm and classifiers like Neural Network etc. can be combined to work on input data set for better results and previously designed method can be modified to work for color image segmentation.

REFERENCES

- [1] Xuqing Wu and Shishir Shah, "Comparative Analysis of Cell Segmentation using Absorption and Color Images in Fine Needle Aspiration Cytology", *IEEE International Conference on Systems, Man and Cybernetics SMC*, 2008.
- [2] Maciej Hrebien, Piotr Stec, Tomasz Nieczkowski, Andrzej Obuchowicz, "Segmentation of Breast Cancer Fine Needle Biopsy Cytological Images", *International Journal Appl. Math. Computer Science*, Vol. 18, No. 2, 159–170 2008.
- [3] Min Hu, Xijian Ping, Yihong Ding, "Automated Cell Nucleus Segmentation Using Improved Snake", *International Conference on Image Processing (ICIP)* 2004.

- [4] Rafael C Gonzalez, Richard E Woods "Digital Image Processing" Third Edition
- [5] M. Park, J.S. Jin, Y. Peng, P. Summons, D. Yu, Y. Cui, S. Luo, F. Wang, L. Santos and M. Xu, "Automatic cell segmentation in microscopic colour images using ellipse fitting and watershed" *The IEEE/ICME International Conference on Complex Medical Engineering, Gold Coast, Australia* July 13-15, 2010.
- [6] Jingqi Ao, Sunanda Mitra, Rodney Long, Brian Nutter and Sameer Antani, "A hybrid watershed method for cell image Segmentation", *978-1-4673-1830-3/12IEEE SSIAI* 2012.
- [7] Robert Porter and Nishan Canagarajah, "a robust clustering scheme for image segmentation wavelets", *IEEE transactions of image processing*, vol. 5, No.4 April, 1996.
- [8] S. Issac Niwas, P.Palanisamy and K. Sujathan, "Complex wavelet as nucleus descriptors for Automated Cancer cytology classifier system using ANN", *978-1-4244-5967-4/10 IEEE* 2010.
- [9] Pelin Görgel, Ahmet Sertbas, and Osman N. Ucan, "A Fuzzy Inference System Combined with Wavelet Transform for Breast Mass Classification", *978-1-4673-1118-2/12 IEEE* 2012.
- [10] Neerad Phansalkart, Sumit More, Ashish Sabale Madhuri Joshi "Adaptive Local Thresholding for Detection of Nuclei in Diversity Stained Cytology Images" *978-1-4244-9799-7/11 IEEE* 2011.
- [11] Min Hu, Xijian Ping, Yihong Ding, "Automated Cell Nucleus Segmentation Using Improved Snake", *International Conference on Image Processing (ICIP)*, 0-7803-8554-3/04/2004 *IEEE* 2004.
- [12] O. L. Kovnsky and Yu. V. Stepanets, "An Algorithm for Segmenting Cytological Images" *Proceedings of 7th International Conference on Pattern Recognition and Image Analysis: New Information Technologies St. Petersburg, Russian Federation, October 18–23, 2004*.
- [13] Maciej Hrebien, Piotr Stec, "The Hough Transform and Active Contours In Segmentation of Cytological Images", *Xi Conference Medical Informatics & Technologies* 2006.
- [14] Samina Naz, Hammad Majeed, Humayun Irshad "Image Segmentation Using Fuzzy Clustering", *6th International Conference on Emerging Technologies (ICET)*, *978-1-4244-8058-6/10/ IEEE* 2010.
- [15] S.Schupp, A.Elmoataz, J.Fadili, P.Herlin, D.Bloyet, "Image segmentation via multiple active contour models and fuzzy clustering with biomedical applications" *0-7695-0750-6/00 IEEE*, 2000.
- [16] Eric Dahai Cheng, Subhash Challa, Rajib Chakravorty, John Markham, "Microscopic Cell Segmentation by Parallel Detection and Fusion Algorithm", *978-1-4244-2295-1/08/IEEE* 2008.
- [17] Baochuan Pang, Yi Zhang, Qianqing Chen, Zhifan Gao, Qinmu Peng, Xinge You, "Cell Nucleus Segmentation in Color Histopathological Imagery Using Convolutional Networks", *978-1-4244-7210-9/10/ IEEE*, 2010.
- [18] Pournami S Chandran, Byju N B, Deepak R U, Rajesh Kumar R, Sudhamony S, Patrik Maim, Ewert Bengtsson, "Cluster Detection in Cytology Images Using the Cellgraph Method", *International Symposium on Information Technology in Medicine and Education*, *978-1-4673-2108-2/12/ IEEE* 2012.