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Segmentation of Rotavirus-A Particles in Microscopic Images Based on Feature Fusion in Active Contour Model

Manjunatha Hiremath[1] , P. S. Hiremath[2]

[1]Department of M.C.A., School of Computer Science
Central University of Karnataka, Aland Road, Kadaganchi, KALABURGI, KA

[2]Department of Computer Applications (MCA)
KLE Technological University, BVBCET Campus, Hubli -580 031, Karnataka

Abstract

The segmentation is the prominent stage in image processing, where a significant commitment is made during automated analysis by delineating structures of interest and discriminating them from background. This separation, which is generally effortless and swift for the human visual system, can become a considerable challenge in algorithm development. In many cases, the segmentation approach dictates the outcome of the entire analysis, since measurements and other processing steps are based on segmented regions. The objective of the present study is to develop an automatic tool to identify and classify the Rotavirus-A particles in digital microscopic images based on fusion of gray level co-occurrence matrix (GLCM) and Gabor features in active contour model. The Geometric features are used to identify and classify the Rotavirus-A particle.

Keywords: Active Contour Model (ACM), Gray level co-occurrence matrix (GLCM), Gabor filters, feature fusion, segmentation;.

1. INTRODUCTION

Image segmentation is one of the most extensively studied problems in computer vision tasks, which is crucial to image analysis, understanding and interpretation. Texture image segmentation has been an important topic of pattern recognition in image processing field for a long time, which aims at segmenting a texture image into several regions with different texture features. There is no known method that is able to consistently and accurately segment all kinds of texture images. Generally, the overall quality of texture segmentation is determined by both the performance of texture features and the segmentation approach. There are numerous methods focusing on image segmentation [1-9], such as region-growing, split-and-merge, Bayesian, Neural networks (NN) and active contour model (ACM), etc. The digital image processing techniques have been successfully used in microbiology for bacterial classification.

A virus is an infectious agent too small to be seen directly with a light microscope. They are not made of cells and can only replicate inside the cells of another organism (the viruses' host). Viruses infect all types of organisms, from animals and plants to bacteria and archaea. Viruses are found in almost every ecosystem on Earth and these minute structures are the most abundant type of biological entity. Viruses consist of two or three parts: all viruses have genes made from either DNA or RNA, long molecules that carry genetic information; all have a protein coat that protects these genes; and some have an envelope of fat that surrounds them when they are outside a cell. Viruses vary from simple helical and icosahedral shapes, to more complex structures. Most viruses are about one hundred times smaller than an average bacterium. Viruses spread in many ways ; plant viruses are often transmitted from plant to plant by insects that feed on sap, such as aphids, while animal viruses can be carried by blood-sucking insects. Influenza viruses are spread by coughing and sneezing.

The Rotavirus is a genus of double-stranded RNA virus in the family Reoviridae. It is the leading single cause of severe diarrhoea among infants and young children, and is one of several viruses that cause infections commonly known as stomach flu, despite having no relation to influenza. By the age of five, nearly every child in the world has been infected with rotavirus at least once. However, with each infection, immunity develops, subsequent infections are less severe, and adults are rarely affected. There are seven species of this virus, referred to as A, B, C, D, E, F and G. Humans are primarily infected by species A,B and C, most commonly by species “A”. All seven species cause disease in other animals [11-14].

Automated image analysis and segmentation of rotavirus particles will play an important role to identify rotavirus particles by using digital image processing techniques. Previously, the segmentation and statistical analysis of individual rotavirus particles is done by Venkataraman, et al. [15]. Active contours without edges based on Mumford-Shah segmentation techniques and the level set method has been done by Tony F. Chan [21-25].

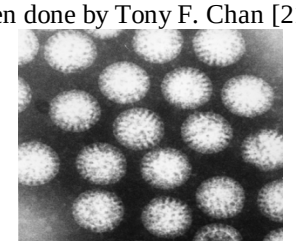

Fig. 1: Rotavirus-A TEM image from the faeces of an infected child [17].

The object identification by using snakes, shapes and gradient vector flow was done by Chenyang Xu and Jerry[20]. Multigrid geometric active contour models for segmenting and tracking object in image analysis and computer vision has been done by George Papandreou and Petros Maragos[23]. Automated identification and classification of Rotavirus-A particles using marker controlled watershed algorithm is investigated by Hiremath et al.[16,18,28]. In this paper, the objective is to propose a method to segment and identify the Rotavirus-A particles in digital microscopic images using active contour model. Geometric features are used to identify the Rotavirus-A particles. Recently, ACM has been one of the most successful methods for image segmentation. Compared with other methods, ACMs have many advantages compared to other state of the art segmentation methods.

The algorithm introduced in this paper deals with the problem of rotavirus-A particle segmentation and classification. The remainder of this paper is organized as follows. The Section 2 describes the background of the proposed method. Experimental results are presented in the Section 3 followed by discussion. The conclusion and future directions are presented in the Section 4.

2. PROPOSED METHOD

The proposed algorithm segments the Rotavirus-A particle images by minimizing the proposed energy function GMFT on the combination of the PCA optimized 24-D GLCM texture feature and 5-D Gabor texture feature vectors. The flowchart of the proposed algorithm is shown in the Fig. 2 . First, the 24-D GLCM based texture feature maps and the 5-D Gabor based texture feature maps of the input texture image are extracted and optimized by PCA separately. Second, PCA optimized GLCM features and Gabor features are fused together. Finally, the texture features with stronger discrimination is selected from the fused texture features by maximum difference scheme (MDS). Then the contour is initialized by the level set function ϕ_0. The level set function ϕ_n of the convex energy function GMFT is updated by the fast dual formulation until the iteration error $\| \phi_{n+1} - \phi_n \|$ is smaller than a given threshold ε [27].

Gray Level Co-occurrence Matrix Feature :

The Gray level co-occurrence matrix (GLCM) method is a texture feature extraction method. The calculation of GLCM contains two steps. The first one is to compute a co-occurring probability matrix. The elements of the matrix are the conditional joint probabilities of all pair wise combinations of gray levels (i, j) in a given spatial window (of size N). In the GLCM matrix computation process two parameters need to be determined : interpixel orientation (θ) and distance (δ).

$$P(i, j) = \Pr(i, j | \delta, \theta, G, N)$$

Usually, a variety of orientations and inter-pixel distances are selected. Besides, the quantization of gray level G and the window size N should also be determined. Coarser quantization G can significantly accelerate calculations and reduce noise overcoming the high computational complexity of GLCM. The window size parameter N affects the ability of GLCM to capture texture features. Small windows can lead to poor local estimates while large windows increase the risk of misleading classification for the multiple texture features appearing in the window.

On the basis of co-occurring probability matrix, many texture statistics are defined. The second step is to apply the predefined statistics to extract corresponding texture features. A texture statistic can identify some structural aspect of the co-occurring probabilities which in turn reflect some qualitative characteristic of the local image texture, e.g., smoothness or roughness. Each window generates a feature vector which is associated with the center pixel of the window. As a result , all pixels in the image have a feature vector associated with it [27].

Gabor Feature :

The Gabor filtering is a frequency transform method and has the ability to model the frequency and orientation sensitivity characteristic of the human visual system. It has been applied in various image processing tasks, such as texture feature extraction, face recognition and so on. A Gabor function is a Gaussian modulated complex sinusoid function in spatial domain. The two-dimensional Gaussian function has an aspect ratio of δ_x / δ_y. The complex exponential has a spatial frequency of F and an orientation θ. The mathematical tractability of Gabor filter in the spatial-frequency domain is appealing since it can be simplified as a Gaussian function centered on the frequency of interest, e.g.,

$$H(u, v) = \exp(-2\pi^2((u - F)^2\sigma_x^2 + v\sigma_y^2))$$

Typically, a filter configuration is created by allowing for the complete coverage of spatial –frequency plane. The filters are set up in a pseudo wavelet format to match the filter's frequency with its spatial extent. Each pixel will have a response to each filter, so each pixel is represented by a feature vector dimensioned to the number of filters. Although there exist many techniques to extract features from Gabor filter outputs, there is experimental evidence to support using the magnitude of Gabor filter n response [27].

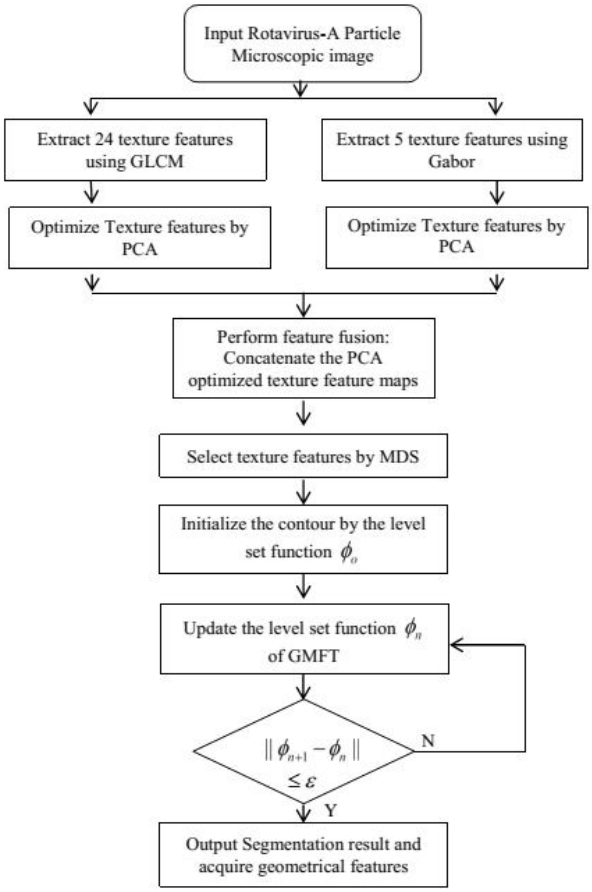

Fig. 2 : Typical block diagram of Proposed Method

3. EXPERIMENTAL RESULTS AND DISCUSSION

For the purpose of experimentation, 50 digital images of rotavirus-A particles (non-overlapping) with x82,000 magnification are considered which are taken from transmission electron microscopy. The implementation is done on a Intel core 2 duo processor @ 2.83GHz machine using MATLAB 7.9. The input of rotavirus-A particle image is converted into gray scale image. The resulting image is segmented using proposed method to obtain binary image. The segmented image is labelled and for each segmented region (known particles), the geometric features are extracted. The Table I presents the geometric feature values computed for the segmented rotavirus-A particle regions of the image in the Fig. 2. The minimum and maximum values of geometric features are stored in the knowledge base of the rotavirus-A particle and is presented in the Table II. Some sample training images of rotavirus-A are shown in the Fig.3.

The algorithms for the training and testing phases of the proposed method for the segmentation and identification of rotavirus-A particles are given below:

Algorithm 1: Training phase

1. Input the Rotavirus colour image.
2. Convert the colour image into gray scale image and adjust the image intensity values.
3. Extract 24 GLCM and 5 Gabor features.
4. Optimize the acquired features by applying PCA. Concatenate the PCA optimized texture feature maps; Initialize the contour by the level set function;
5. Perform geometric active contour model upto N iterations to obtain segmented image, where N is the number of iterations required for the snake curve to reach the object boundary and does not move thereafter[19-23].
6. Binarize the segmented image of Step 5.
7. Remove the border touching cells obtained in binary image and then perform labeling the segmented binary image.
8. For each labeled segment, compute geometric shape features (Area, Eccentricity, Perimeter, Circularity, Tortuosity, Length/Width ratio, Compactness) and store them.
9. Repeat steps 1 to 6 for all the training images.
10. Compute minimum and maximum of feature values of rotavirus-A particle and store them as knowledge base.

Algorithm 2: Testing phase

1. Input the Rotavirus colour image.
2. Convert the colour image into gray scale image and adjust the image intensity values.
3. Extract 24 GLCM and 5 Gabor features.
4. Optimize the acquired features by applying PCA. Concatenate the PCA optimized texture feature maps; Initialize the contour by the level set function;
5. Perform multigrid geometric active contour model upto N iterations to obtain segmented image, where N is the number of iterations required for the snake curve to reach the object boundary and does not move thereafter.
6. Binarize the segmented image of Step 5.
7. Remove the border touching cells obtained in binary image and then perform labeling the segmented binary image.
8. For each labeled segment, compute geometric shape features (Area, Eccentricity, Perimeter, Circularity, Tortuosity, Length/Width ratio, Compactness) and store them.
9. Apply rule for identification of the rotavirus-A particles: A segmented region is of Rotavirus-A, if its feature values lie in their corresponding min-max range.

Table I. The geometric feature values of the Rotavirus-A particles of the image in Fig. 2(d).

Rotavirus-A Features	Area	Eccentricity	Perimeter	Circularity	Tortuosity	Length/ Width ratio	Compactness
Particle 1	702	0.26	141	0.41	0.21	1.08	2.36
Particle 2	878	0.22	151	0.47	0.22	1.02	2.14
Particle 3	756	0.44	159	0.40	0.22	1.18	2.43
Particle 4	810	0.26	140	0.49	0.23	1.03	2.06
Particle 5	730	0.49	178	0.37	0.19	1.19	3.14
Particle 6	801	0.48	159	0.42	0.23	1.11	2.47
Particle 7	808	0.42	163	0.48	0.22	1.19	2.40
Particle 8	901	0.26	167	0.41	0.21	1.03	2.26
Particle 9	1025	0.19	143	0.64	0.24	1.22	1.65
Particle 10	920	0.39	179	0.42	0.21	1.17	2.50
Particle 11	1034	0.35	142	0.66	0.25	1.06	1.62

Table II The knowledge base for rotavirus-A particles containing minimum and maximum values of features

Features	Area	Eccentricity	Perimeter	Circularity	Tortuosity	Length/ Width ratio	Compactness
Minimum Value	646	0.13	109	0.289	0.181	1.005	1.129
Maximum Value	1289	0.57	198	0.881	0.319	1.159	3.498

The value of N, the number of iterations, is empirically determined and depends upon the image set in general. For the experimental data set considered in the present study, N is found to be 220 iterations.

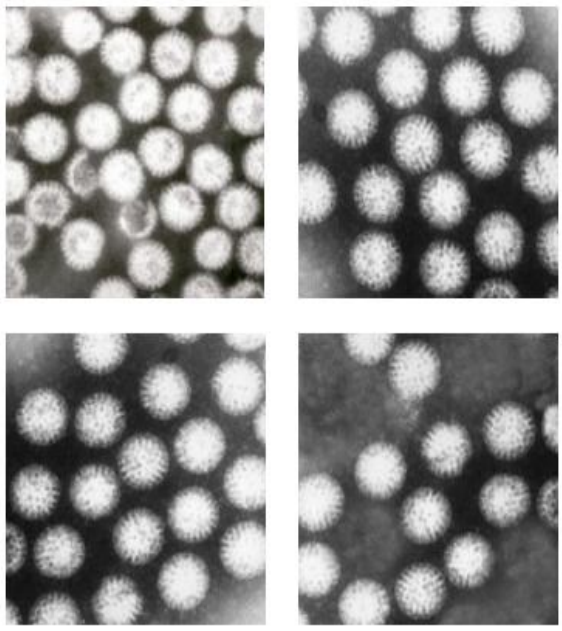

Fig. 3 Sample training images of Rotavirus-A particles.

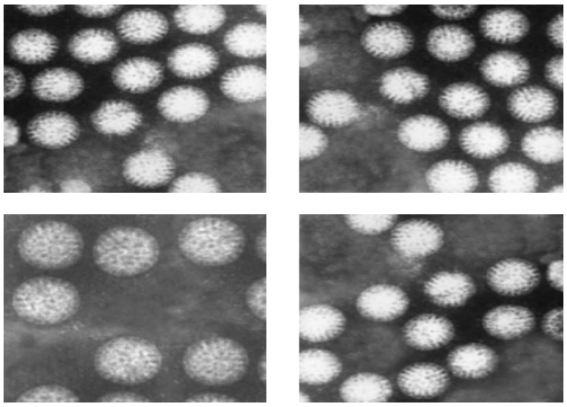

Fig. 4 Sample test images used for identification of Rotavirus-A particles.

4.CONCLUSION

In this paper, we have proposed a method for segmentation and identification of rotavirus-A particles in transmission electron microscope (TEM) images using fusion of gray level co-occurrence matrix and Gabor features in active contour model. The experimental results are validated by visual inspection conducted by microbiological experts. The proposed method is more reliable and computationally less expensive. It yields an identification rate of 99.10% for rotavirus-A particles, which is better than our earlier method [28] which yielded 96%. It could be improved further by better pre-

processing methods, feature sets and classifiers. The classification of other rotavirus particles also will beconsidered in future work.

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AUTHOR

Manjunatha Hiremath was born in July 1984, and has obtained M.Phil (2010) in Computer Science and M.Sc. (2008) in Computer Science from Gulbarga University, Gulbarga. Presently, he is working as Assistant Professor (on Contract) in Department of M.C.A, School of Computer Science, Central University of karnataka. His area of research interest is Image Processing and Pattern Recognition. He has published 17 research papers in peer reviewed International Journals and Proceedings of International Conferences.

Dr. P. S. Hiremath was born in May 1952, and has obtained Ph.D. (1978) in Applied Mathematics and M.Sc. (1973) in Applied Mathematics, from Karnataka University, Dharwad, Karnataka, India. He had been in the Faculty of Mathematics and Computer Science of various institutions in India, namely, National Institute of Technology, Surathkal (1977-79), Coimbatore Institute of Technology, Coimbatore (1979-80), National Institute of Technology, Tiruchirapalli (1980-86), Karnataka University, Dharwad (1986-1993), Gulbarga University, Gulbarga (1993-2014) and has been presently working as Professor of Computer Science in KLE Technological University, BVBCET Campus Hubli (2015-16). His research areas of interest are Computational Fluid Dynamics, Optimization Techniques, Image Processing and Pattern Recognition, and Computer Networks. He has published 156 research