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SHORT COMMUNICATION

Bacilli bacterial cell image analysis using active contour segmentation with SVM classifier

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ABSTRACT

The main aim of the present study is to develop an automatic method to identify and classify the different cell types of bacilli bacterial cells in digital microscopic cell images using active contour method. Snakes, or active contours, are used widely in computer vision and machine learning applications, particularly to locate object boundaries. GLCM, HOG and LBP features are used to identify the arrangement of bacilli bacterial cells, namely, bacillus, cocobacilli, diplobacilli, palisades and streptobacilli using SVM classifier. The current methods rely on the subjective reading of profiles by a human expert based on the various manual staining methods. In this paper, it is proposed a method for bacilli bacterial cell classification by segmenting digital bacterial cell images using active contour model and extracting GLCM, HOG and LBP features. The experimental results proves that, the SVM classifier has yielded an overall accuracy of 97.2% with GLCM features, HOG features has yielded an accuracy of 74.8% and LBP features yielded 91.2% accuracy. The GLCM features with SVM classifier has got good classification results compared to HOG and LBP feature sets for bacilli bacteria cell types.

***Keywords*:** Cell classification, Segmentation, Bacterial cell image analysis, Bacillus, SVM, Cocobacilli, Diplobacilli, Palisades, Streptobacilli, Active contour method, *Bacillus subtilis*

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1. INTRODUCTION

Numerous types of bacteria can be seen in the world and they are categorized based on their shapes, biochemistry and different staining methods. However, each bacteria has its own characteristics and they can be characterized by three main shapes: rod or bacilli, sphere or round and spiral. *Bacillus subtilis* is one of the prokaryotes types of bacteria and relatively large size have provided the powerful tools required to investigate a bacterium from all possible aspects.

1. 1. Arrangement of Bacilli bacterial cell groups

Normally, Bacilli bacteria exists alone, if they do group together, they arrange themselves with different pattern based on the shape. The single bacterial cell is belonging to Bacillus; Diplobacilli bacteria are arranged side by side with each other, Cocobacilli is in oval or circular shape, Palisades are picket fence and angular patterns, while bacteria in the Streptobacilli genus are arranged in chains. The arrangement of bacilli bacterial cell groups is shown in the Fig. 1 [6].

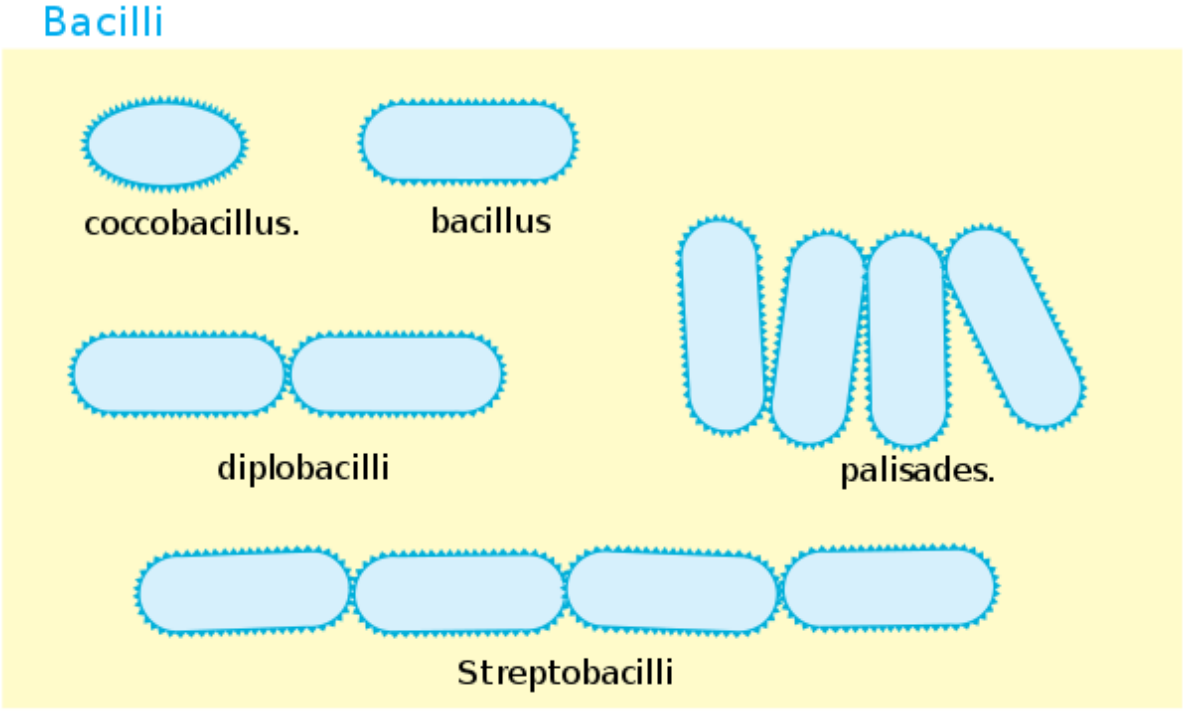

Figure 1. Arrangement of Bacilli bacterial cell groups.

Automatic image analysis of bacterial can be divided into four stages: (i) image acquisition, digitization, and segmentation to locate cells; (ii) automatic measurement to extract features of interest; (iii) classification of different cell types; and (iv) statistical analysis, computations, and interpretation of the data. Automatic identification of bacterial types using statistical approach was proposed by Trattner and Greenspan [1]. The artificial neural network technique for bacterial classification has been investigated by Nicolas Blackburn et al. [2].

The data mining approaches are employed for the classification of HEp-2 cells by Petra Perner [3]. Hiremath and Parashuram [11, 12] have investigated the automatic classification of bacterial cells using digital microscopic images using geometric shape features. Thomas Posch et al. [7] have proposed a new image analysis technique using geometric features. Carolina Wählby et al. [4], have investigated algorithms for cytoplasm segmentation of fluorescence labelled cells using statistical analysis tools with shape descriptive features.

In this paper, the objective is to propose a method for automatic identification and classification of bacilli bacterial cell types in digital microscopic images using active contour model. GLCM, HOG and LBP features are used to identify the arrangement of bacilli bacterial cells, namely, bacillus, cocobacilli, diplobacilli, palisades and Streptobacilli using SVM classifier.

2. MATERIALS AND METHODS

The spread plate technique is used for the separation of a dilute mixed population of micro-organisms, so that individual colonies can be isolated. Aseptically transfer the loopful of mixed culture on the Nutrient Agar medium. Spread uniformly with the help of L-shaped spreader on the surface of medium plates. After spreading, incubate at 37 ºC for 24-48 hours. After incubation, single colonies will appear on the Nutrient Agar media plates. Then pick up the colony and go further identification by using staining techniques. A smear of mixed culture of bacteria is deposited on a glass slide and thoroughly air-dried. It is stained for 1 min in Crystal Violet solution and wash with distilled water then add gram's iodine for 1 min, decolourised for 20s in ethanol and finally, counterstained with safranin for 1 min in distilled water. The glass slide is examined under oil immersion with direct illumination in a Dialux 20 microscope equipped with a 3 CCD Sony color camera and connected to a PC. However, we have considered 350 color images of size 512 × 512 and 15000x magnifications, acquired by SEM equipment, for present study and these are converted into gray scale images.

3. PROPOSED METHOD

Snakes or active contours are widely used in many applications, including edge detection, shape modelling, segmentation, and motion tracking [8-10]. The objective of the automated image analysis of digital bacilli bacterial cell image is to identify the type of bacteria whether it is bacillus, cocobacilli, diplobacilli, palisades and streptobacilli based on the features of GLCM, HOG and LBP using SVM classifier. The proposed method for the classification of bacilli bacterial cells is given below:

3. 1. Training phase

Algorithm 1: Extraction of features for knowledge base

Step 1: Input bacilli bacterial cell RGB colour image

Step 2: Convert the RGB image into gray scale image and adjust the image intensity values.

Step 3: Perform active contour without edges upto 1800 iterations to obtain segmented image.

Step 4: Binarize the segmented image of step 3.

Step 5: After removing border touching cells, perform labeling the segmented image.

Step 6: For each labeled segment, compute GLCM, LBP and HOG features, for each cell type i.e., bacillus, cocobacilli, diplobacilli, palisades and streptobacilli.

Step 7: Repeat steps 1 to 6 for all the training images and store them as knowledge base.

3. 2. Classification phase

Algorithm 2: Classification of bacterial cells.

Step 1: Input bacilli bacterial cell image (RGB color test image)

Step 2: Convert the RGB image into gray scale image and adjust the image intensity values.

Step 3: Perform active contour without edges upto 1800 iterations to obtain segmented image.

Step 4: Binarize the segmented image of step 3.

Step 5: After removing border touching cells, perform labeling the segmented image.

Step 6: For each labeled segment, compute GLCM, LBP and HOG features, for each cell type i.e., bacillus, cocobacilli, diplobacilli, palisades and streptobacilli.

Step 7: Repeat steps 1 to 6 for all the testing images

Step 8: Apply SVM rule for classification of the bacterial cells

Step 9: Repeat the steps 7 and 8 for all labeled segments and output the classification of identified cells.

4. EXPERIMENTAL RESULTS AND DISCUSSIONS

The experimentation is done on350 color digital bacterial cell images containing different types of bacilli bacterial cells (non-overlapping) namely, bacillus, cocobacilli, diplobacilli, palisades and streptobacilli with 15,000x magnification and above are considered (as described in section 2). The implementation is done on a Pentium Core 2 Duo @ 2.83 GHz machine using MATLAB 7.9. In the training phase, each input color image of bacilli bacterial cell (Fig. 2(a)) is converted into gray scale image and adjust the image intensity values using MATLAB function.

The resultant image is initiated by a fixed rectangular mask (Fig. 2(b)), and then, segmented using active contour method (Fig. 2(c)) to obtain binary image (Fig. 2(d)). Then, the segmented image is labeled and for each segmented region (known cells), the GLCM, HOG and LBP features are computed and stored them as knowledge base.

In the testing phase, for a test image, the feature extraction algorithm is applied and the test feature values GLCM, HOG and LBP for each segmented region are used separately for classification using SVM classifier. The classification results based on GLCM, HOG and LBP features extracted from Bacilli bacterial cell types with SVM classifier is given in the Table 1.

The confusion matrix results of Bacilli bacterial cell types with GLCM, HOG and LBP features using SVM classifier are given in the Table 2, 3 and 4, respectively.

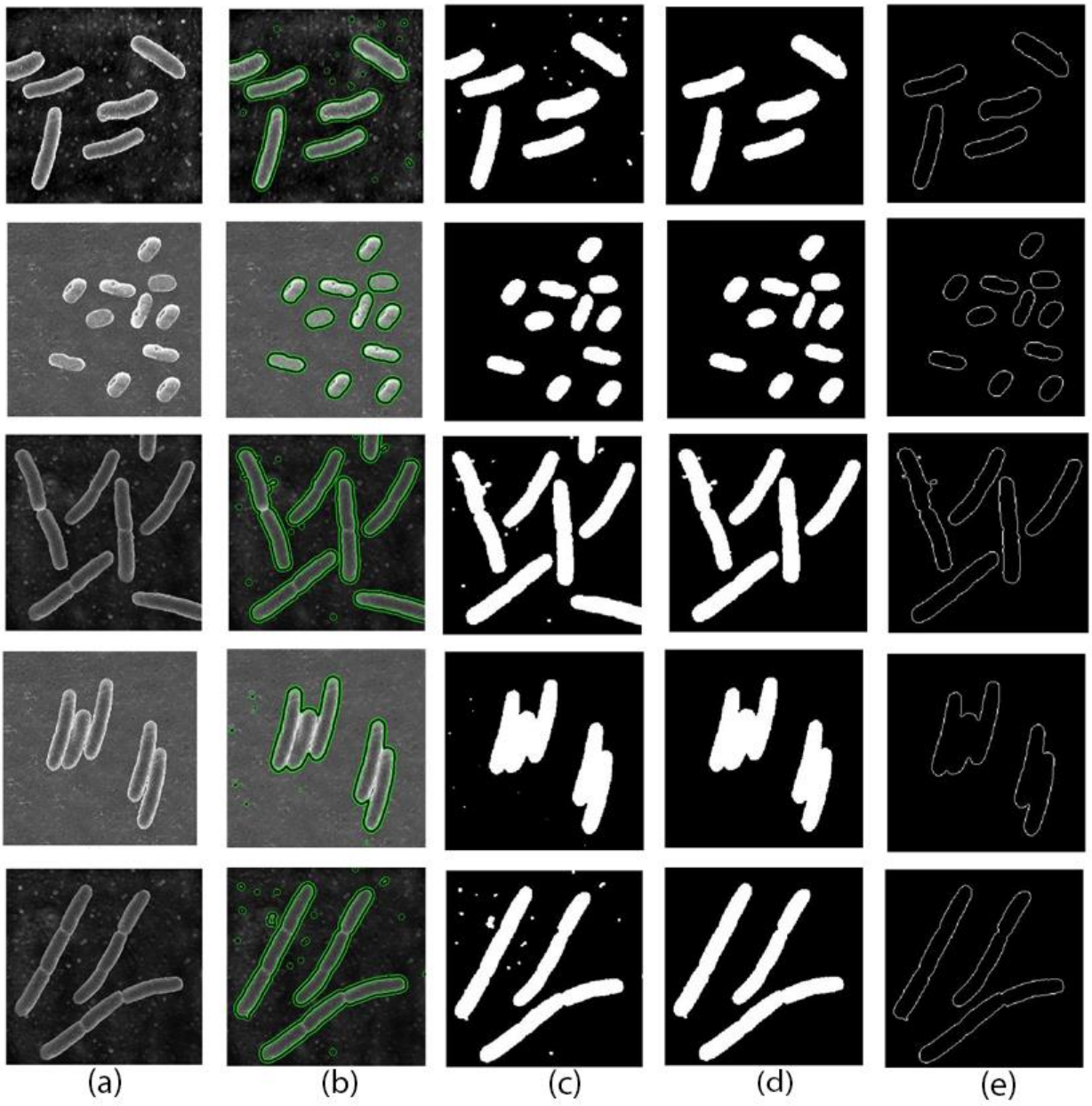

Figure. 2. (a) Original Bacilli color image, (b) Initializing fixed rectangular grid image of color image in (a), (c) image after 1800 iterations, (d) Binarized image after segmentation by active contour method, (e) perimeter of the image in (d).

The Table 1 summarizes the average classification rates obtained by different features sets, GLCM, HOG and LBP. For testing images, The SVM classifier is used to classify the bacilli bacterial cell groups and has yielded an overall accuracy of 97.2% with GLCM features, HOG features have yielded an accuracy of 74.8% and LBP features are yielded 91.2% accuracy. The GLCM features with SVM classifier has got good classification results compared to HOG and LBP feature sets for bacilli bacteria cell types.

Table 1. The classification results based on GLCM, HOG and LBP features extracted from Bacilli bacterial cell types with SVM classifier.

Bacilli cell groups	Accuracy based on GLCM, HOG and LBP features extracted from Bacilli bacterial cell types with SVM classifier		
	GLCM	HOG	LBP
Bacilli	100	90	97
Cocobacilli	100	62	93
Diplobacilli	95	73	89
Palisades	90	0	30
Streptobacilli	79	42	79
Overall percentage	97.2%	74.8%	91.2%

Table 2. The confusion matrix results of Bacilli bacterial cell types with GLCM features using SVM classifier.

Bacilli cell groups	Total Bacterial Cells	Bacilli	Cocobacilli	Diplobacilli	Palisades	Streptobacilli	Recognition Rate (%)
Bacilli	153	153	-	-	-	-	100
Cocobacilli	61	-	61	-	-	-	100
Diplobacilli	74	-	-	70	-	4	95
Palisades	10	-	-	1	9	-	90
Streptobacilli	19	-	-	4	-	15	79

Table 3. The confusion matrix results of Bacilli bacterial cell types with HOG features using SVM classifier.

Bacilli cell groups	Total Bacterial Cells	Bacilli	Cocobacilli	Diplobacilli	Palisades	Streptobacilli	Recognition Rate (%)
Bacilli	153	137	8	6	2	-	90
Cocobacilli	61	21	38	2	-	-	62

Diplobacilli	74	15	4	54	-	1	73
Palisades	10	8	-	1	-	1	0
Streptobacilli	19	3	-	7	1	8	42

Table 4. The confusion matrix results of Bacilli bacterial cell types with LBP features using SVM classifier.

Bacilli cell groups	Total Bacterial Cells	Bacilli	Cocobacilli	Diplobacilli	Palisades	Streptobacilli	Recognition Rate (%)
Bacilli	153	148	2	3	-	-	97
Cocobacilli	61	4	57	-	-	-	93
Diplobacilli	74	6	-	66	2	-	89
Palisades	10	3	-	3	3	1	30
Streptobacilli	19	-	-	3	1	15	79

5. CONCLUSIONS

In this paper, an automated method is proposed for bacterial cell classification by segmenting digital microscopic bacterial cell images using active contour model and extracting GLCM, HOG and LBP features of cells. The SVM classifier is used to classify the bacilli bacterial cell groups and has yielded an overall accuracy of 97.2% with GLCM features, HOG feature set has yielded an accuracy of 74.8% and LBP feature set yielded 91.2% accuracy. Hence, the GLCM features with SVM classifier has got good classification results compared to HOG and LBP feature sets for bacilli bacteria cell types. The experimental results are compared with the manual results obtained by biological experts. The results could be improved further by better preprocessing techniques, segmentation methods and feature sets, which will be taken up in our future work.

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