Automatic Classification of Bacterial Cells in Digital Microscopic Images

Article *in* Proceedings of SPIE - The International Society for Optical Engineering · February 2010

DOI: 10.1117/12.853303

CITATIONS
18

READS
457

2 authors:

Prakash Hiremath
Gulbarga University
179 PUBLICATIONS **2,021** CITATIONS
SEE PROFILE

Parashuram Bannigidad
Rani Channamma University Belgavi
32 PUBLICATIONS **147** CITATIONS
SEE PROFILE

International Journal of Engineering and Technology

Volume 2, Number 4, December 2009

Automatic Classification of Bacterial Cells in Digital Microscopic Images

P.S. Hiremath
Department of Computer Science, Gulbarga University, Gulbarga, Karnataka, India
hiremathps@hotmail.com

Parashuram Bannigidad
Department of Computer Science, Gulbarga University, Gulbarga, Karnataka, India
parashurambannigidad@gmail.com

Abstract

The objective of the present study is to develop an automatic tool to identify and classify the bacterial cells in digital microscopic cell images. Geometric features are used to identify the different types of bacterial cells, namely, bacilli, cocci and spiral. The current methods rely on the subjective reading of profiles by a human expert based on the various manual staining methods. In this paper, we propose a method for bacterial classification by segmenting digital bacterial cell images and extracting geometric features for cell classification. The experimental results are compared with the manual results obtained by the microbiology expert and demonstrate the efficacy of the proposed method.

Keywords Cell classification, segmentation, bacterial image analysis, bacilli, cocci, spiral.

1. Introduction

Bacteria are unicellular microscopic organisms which can only be seen through microscope. Bacteria exist in different sizes and shapes and they measure in micro-meter (which is a millionth part of a meter). Bacteria are found everywhere and in all types of environments. There are numerous types of bacteria in the world. Bacteria are mainly classified based on their shapes, biochemistry and staining methods [6]. Recently, along with the morphology, other profiles such as their metabolic activities, conditions required for their growth, biochemical reactions, antigenic properties, and other characteristics are also helpful in classifying the bacteria. However, each type of bacteria has its own characteristics. Most of the bacteria are characterized by three main shapes: rod (rod shaped bacteria are called bacilli), sphere (sphere shaped bacteria are called cocci) and spiral (spiral shaped bacteria are called spirilla or spiral). Some bacteria posses different shapes, which are more complex than the above mentioned shapes.

The statistical imaging method for automatic identification of bacterial types is proposed by Trattner and Greenspan[1], The artificial neural network approach for bacterial classification has been investigated by Nicolas Blackburn, et al.[2]. The data mining techniques are employed for the classification of HEp-2 cells by Petra Perner [3], in which a simple set of shape features are used for classification of bacterial cells. Hiremath and Parashuram[8] have investigated the automatic Gram-staining characterization of digital microscopic bacterial cell images.

In this paper, the objective is to propose a method for automatic classification of bacterial cells in digital microscopic images using geometric features that characterize the shapes of bacterial cells. The experimental results are compared with the manual results obtained by microbiology expert and demonstrate the efficacy of the proposed method.

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 scoopful 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 pickup the colony and go further identification by using staining techniques. A smear of mixed culture bacteria is deposited on a glass slide and thoroughly air-dried. It is stained for 1 min in Crystal Violet solution, 1 min in iodine solution, washed for 20s in ethanol and finally, counterstained with safranin for 1 min. The glass slide is examined under oil immersion at 1000x – 2500x magnification with direct illumination in a Dialux 20 microscope equipped with a 3 CCD Sony color camera and connected to a PC[6,9]. We have considered 100 color images for present study and these are converted into gray scale images [10].

3. Proposed method

The purpose of the automated image analysis of digital bacterial cell image is to identify the type of bacteria whether it is bacilli or cocci or spiral based on their geometric features.
Out of many geometric features used by various authors in the literature [4,7], it is observed that there are five geometric features, namely, circularity, compactness, eccentricity, tortuosity and length-width ratio, which provide better classification results. Hence, we have used these five features, which are defined as given below:

Circularity (x_1):	4π(Area)/perimeter^2
Compactness(x_2):	A measure of compactness (Perimeter^2/4π*Area)
Eccentricity(x_3):	It is the ratio of the length of the highest chord of the shape to the longest chord perpendicular to it. Eccentricity=$\text{Length}_{\text{major\_axis}}/\text{Length}_{\text{minor\_axis}}$
Tortuosity(x_4):	Major axis/perimeter
Length-width ratio(x_5):	Major axis/minor axis

The bacterial cell images generally contain noise, small debris and artifacts depending on the different staining methods. To remove this debris, we have preprocessed the image by applying morphological operations, namely, erosion, reconstruction and dilation. This stage is of high

importance in achieving good results in segmentation and further process. The gray scale image of cells is segmented using the adaptive global thresholding, which yields binary image. After labeling the segmented image, the geometric features x_i, i=1,2,..,5, are extracted for each labeled segment. These features are used as a basis for the cell classification. Using the training set of images (with known cell classification), for each feature $x_i^k, i = 1,2,..5$, of k[th] cell type, we compute the mean $\bar{x}_i^k$ and standard deviation σ_i^k of the sampling distribution of the feature values and store them as knowledge base (Table 1). In the testing phase, for a given test image, feature values $x_i^{(test)}$ of the segmented regions (cells) are computed and then cell classification is done using the 3□ rule, namely:

For a segmented region in the test image, if the feature values $x_i^{(test)}$ lie in the interval $\bar{x}_i^k \pm 3\sigma_i^k$, i=1,2,.., 5 , then the region is a cell of type k. The k=1,2,3 correspond to bacilli, cocci and spiral, respectively.

The proposed method for the classification of bacterial cells based on their geometric features is given below:

Training phase:

Algorithm 1: Extraction of features for knowledge base

Step 1: Input bacterial cell image (RGB color training image)
Step 2: Convert the RGB image into gray scale image
Step 3: Perform preprocessing method and segment the resulting binary image
Step 4: After removing border touching cells, perform labeling the segmented image
Step 5: For each labeled segment, compute geometric shape features x_i^k, i=1,2,..,5, (i.e. eccentricity, compactness, circularity, tortuosity and length-width ratio) for each cell type k. The k=1,2,3 correspond to bacilli, cocci and spiral, respectively.
Step 6: Repeat steps 1 to 5 for all the training images
Step 7: Compute mean $\bar{x}_i^k$ and standard deviation σ_i^k of the sampling distribution of the feature values for each cell type k and store them as knowledge base.

Classification phase:

Algorithm 2: Classification of bacterial cells.

Step 1: Input bacterial cell image (RGB color test image)
Step 2: Convert the RGB image into gray scale image
Step 3 : Perform preprocessing method and segment the resulting binary image
Step 4: After removing border touching cells, perform labeling the segmented image
Step 5: For each labeled segment, compute geometric shape features x_i , i=1,2,..,5, (i.e. eccentricity, compactness, circularity, tortuosity and length-width ratio) and store these features as $x_i^{(test)}$.

Step 6: Apply 3□ rule for classification of the bacterial cells: A segmented region is of cell type k, if its features $x_i^{(test)}$ lie in the interval $\bar{x}_i^k \pm 3\sigma_i^k$, i=1,2,..,5. The k=1,2,3 correspond to bacilli, cocci and spiral, respectively.

Step 7: Repeat the steps 5 and 6 for all labeled segments and output the classification of identified cells.

4. Experimental results and discussions

For the purpose of experimentation, 100 color digital bacterial cell images containing different types of bacterial cells (non-overlapping) namely, bacilli, cocci and spiral are considered (as described in section 2). The implementation is done on a Pentium P-IV 1.0 GHz machine using MATLAB 7.0. In the training phase, each input color image of bacterial cell (Fig. 1(a)) is converted into gray scale image (Fig.1(b)), and the morphological operations such as erosion, reconstruction and dilation are applied. The resulting image is thresholded to obtain segmented binary image (Fig. 1(c)). The segmented image is labeled and for each segmented region (known cells), the geometric features are computed. The Table 1 presents the geometric feature values computed for the segmented cell regions of the image in Fig. 1(d)-(f).

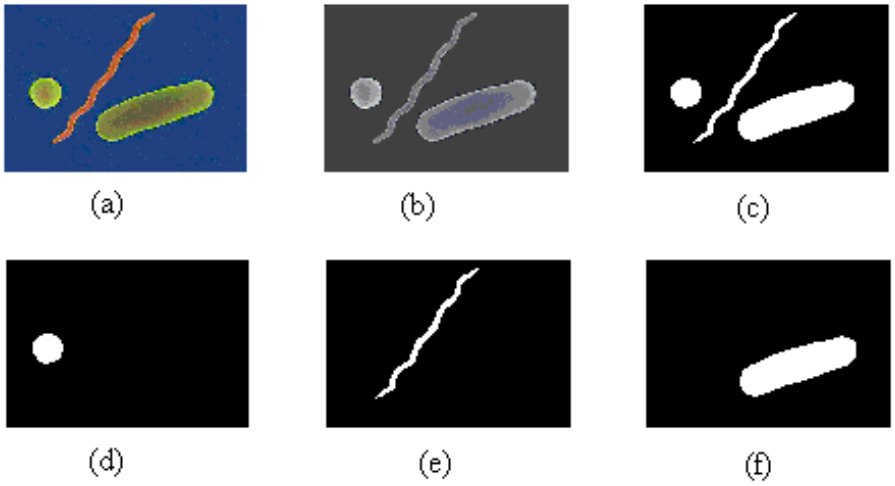

Fig. 1. (a) Original color image, (b) gray image of color image in (a), (c) segmented image, (d) the segmented region known as Cocci, (e) the segmented region known as Spiral, (f) the segmented region known as Bacili

The mean and standard deviation of the sampling distribution of these features obtained from the training images are stored in the knowledge base of the cells: bacilli, cocci and spiral, as shown in Table 2. Some sample training images are shown in Fig. 2.

Table 1. The geometric feature values of the cell regions of the image in Fig. 1(d)-(f)

Cell features	Bacilli	Cocci	Spiral
Circularity (x_1)	0.3695	0.06595	0.0469
Compactness x_2)	2.7060	1.5163	21.3002
Eccentricity(x_3)	0.9640	0.2849	0.9988
Tortuosity (x_4)	0.3786	0.2643	0.3847
LW ratio (x_5)	3.7630	1.0432	20.2628

Table 2. Mean and standard deviation of geometric features of bacterial cells of types: Bacili, Cocci and Spiral

Cell features	Bacili		Cocci		Spiral	
	Mean($\bar{x}_i^{-1}$)	Sd (σ_i^1)	Mean($\bar{x}_i^{-2}$)	Sd (σ_i^2)	Mean($\bar{x}_i^{-3}$)	Sd (σ_i^3)
Circularity (x_1)	0.4425	0.2203	0.6427	0.0236	0.0881	0.0239
Compactness x_2)	2.5441	0.7594	1.558	0.0581	12.3827	3.9769
Eccentricity(x_3)	0.9425	0.0373	0.481	0.1407	0.9846	0.0086
Tortuosity (x_4)	0.122	0.0433	0.2369	0.0144	0.0628	0.0211
LW ratio (x_5)	3.4023	0.974	1.1761	0.1397	7.7203	5.0824

Fig. 2. Sample training images of bacterial cells

In the testing phase, for a test image, the feature extraction algorithm is applied and the test feature values $x_i^{(test)}$ for each segmented region are used for classification using 3□ rule. The classification results are given in the Table 3. The Fig. 3 shows some sample test images used for classification of bacterial cells.

Table 3. Classification rates for the different bacterial cells

Bacterial cells	No. of cells in test images	Classification rate (%)
Bacilli	100	96
Cocci	100	94
Spiral	100	95

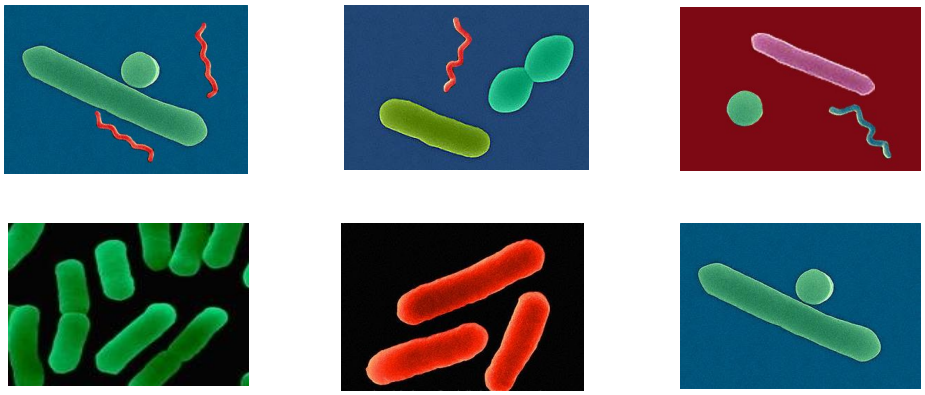

Fig. 3. Sample test images used for classification of bacterial cells

Although the comparison of classification performance of the various state-of-the art methods in the literature is difficult because of the different cell image data sets used for experimentation, it may be observed that, in [4] data mining approach has yielded 86.67% classification rate, in [3] neural network approach has yielded above 90% classification rate and in [2] the statistical methodology has yielded classification rates in the range 89% to 98% for different categorization methods. The proposed method is computationally less expensive and yet yields comparable classification rates in the range 94% to 96% for different cell types.

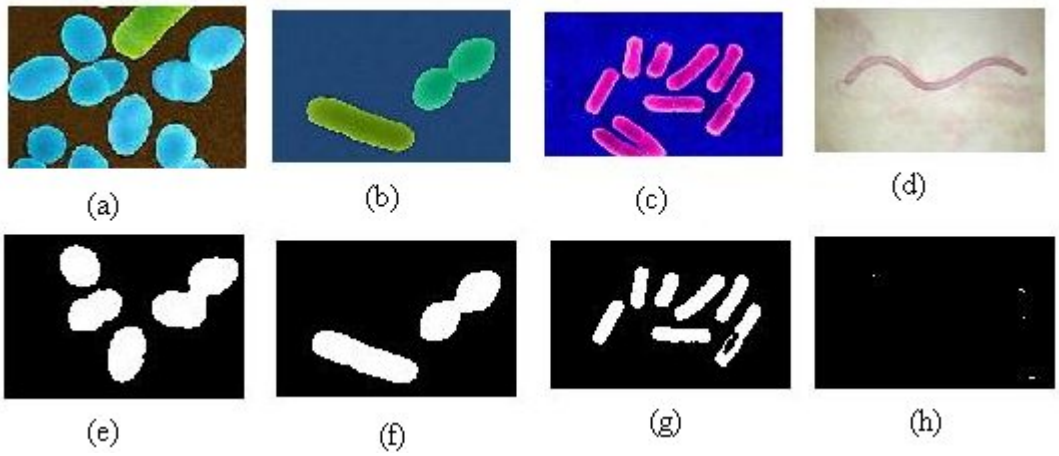

Fig. 4. Some sample images corresponding to misclassification results. (a)-(d) original cell images, (e)-(f) segmented images corresponding to cell images in (a)-(d).

The Fig. 4 shows some sample cell images corresponding to misclassification results. In Fig. 3(a) and (e), the cocci is classified as bacilli. Also, in Fig. 3(b) and (f), the cocci is classified as bacilli. In Fig. 3(c) and (g), a bacilli is not classified (i.e. unknown) due to over segmentation. Also, in Fig. 3(d) and (h), a spiral is not classified (unknown) due to over segmentation. These problems can be overcome by employing better segmentation methods. Further, the classification results can be improved by using better classification techniques. These aspects will be considered in our future work.

5. Conclusions

In this paper, we have proposed an automated bacterial cell classification by segmenting digital microscopic bacterial cell images and extracting geometric features of cells. The experimental results are compared with the manual results obtained by expert. The proposed method is computationally less expensive and yet yields comparable classification rates in the range 94% to 96% for different cell types. It could be improved further by better preprocessing methods and feature sets, which will be taken up in our future work.

6. Acknowledgements

The authors are grateful to Dr. A. Dayanand, Professor of Microbiology, Gulbarga University, Gulbarga and Dr. Ramakrishna, Department of Microbiology, Government Degree College, Gulbarga, for providing bacterial cell images and manual results of the cell images by visual inspection.

7. References

[1]. Sigal Trattner and Greenspan (2004), Automatic Identification of Bacterial Types Using Statistical Imaging methods, IEEE Transactions on Medical Imaging, Vol.23, No.7, pp.807-820.

[2]. Nicholas Blackburn, et al. (1998), Rapid Determination of Bacterial Abundance, Biovolume, Morphology, and Growth by Neural Network-Based Image analysis, Applied and Environmental Microbiology, Vol. 64, No.9, pp.3246-3255.

[3]. Petra Perner (2001), Classification of HEp-2 Cells using Fluorescent Image Analysis and Data Mining, Medical Data Analysis, Springer Verlag, INCS 2199, pp.219-224.

[4]. Carolina Wahlby, et al.(2002), Algorithms for cytoplasm segmentation of fluorescence labeled cells, Analytical Cellular Pathology, 24, pp.101-111.

[5]. Rafael C. Gonzalez and Richard E. Woods (2002), Digital Image Processing, Pearson Education Asia.

[6]. Madigon M.T. et al. (1999), Biology of Microorganism, 8th Ed. McGraw Hill Inc., Newyork.

[7]. S. Venkataraman, et al. (2006), Automated image analysis of atomic microscopy images of rotavirus particles, Ultramicroscopy, Elsevier, Vol.106, pp.829-837.

[8]. P.S. Hiremath and Parashuram Bannigidad, (2009) Automated Gram-staining Characterization of Digital Bacterial Cell Images, Proc. IEEE Int'l. Conf. on Signal and Image Processing ICSIP 2009, 12-14, August 2009, Mysore. (2009) pp. 209-211.

[9]. K.R. Aneja, (1996) Experiments in Microbiology Plant Pathology Tissue Culture and Mushroom Culture, Newage International Publications, New Delhi, India.

[10]. Dennis Kunkel Microscopy, Inc, Science Stock Photography, http://denniskunkel.com/DK/Bacteria/