Segmentation and Splitting of Touching Vaginal Bacteria Based on Superpixel and Effective Distance

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Abstract—In this paper, a new method for segmentation and splitting of touching vaginal bacteria based on superpixel method is proposed. Feature fusion is integrated with kernel-based support vector machine (SVM) for bacteria segmentation. After segmentation by superpixel, the touching bacteria regions are further separated according to the defined effective distance. Finally, the separated bacteria are counted finally for the performance evaluation. Our experimental results show that the proposed method has achieved promising segmentation result. Moreover, compared to the state-of-the-arts method, better segmentation results have also been achieved.

Keywords—segmentation; vaginal bacteria; effective distance; superpixel; splitting.

I. INTRODUCTION

Bacterial vaginosis(BV) is one of the most common vaginal infection when the normal bacteria balance is replaced by overgrowth of certain bacteria. BV is associated with pelvic inflammatory disease and increases ectopic pregnancy, infertility, and HIV infection risk, which could lead to high mortality rate [1, 2]. According to World Health Organization's statistics, BV rates are between 22%and 50% [2]. In 2009, NIH also launched the Human Genome Project [3] for the vaginal flora study. Therefore, BV study for disease pathogenesis and vaginitis treatment is of vital significance. Segmentation, touching bacteria splitting and counting is even more significant for the vaginitis disease treatment since total bacteria numbers are more helpful in the clinical application. The traditional method for BV diagnosis is mainly based on visual observation of microscope image, which is time-consuming, labor-intensive and highly subjective. Moreover, the psychological fatigue caused by the manual observation leads to misdiagnosis and missed diagnosis. To resolve this problem, automatic vaginal bacteria segmentation and identification techniques for the vaginal disorder, inflammation, and cancer diagnosis have attracted more and more interest and become a hot topic [2-4].

In the recent year, automatic-assisting approaches such as cell segmentation and detection have achieved great success [5]. The widely applied cell segmentation algorithms include *k*-means, mean shift, graph cuts [6],normalized cuts, edge detection [7], thresholding and active contours [8, 9]. However,

the existing methods are designed for nucleus segmentation rather than vaginal bacteria segmentation and splitting. Developing an automatic-assisting tool for the vaginitis diagnosis by vaginal bacteria segmentation is very desirable. It is proved that the superpixel algorithm with simple linear iterative clustering (SLIC) is an effective method for cell segmentation with distinct bacteria boundaries, reduced memory, fast speed and low complexity [10].SLIC has an advantage of providing the compactness and flexible number of superpixels. Another advantage is that it can group pixel into perceptually meaningful atomic region. In view of this, SLIC is investigated to partition H&E-stained vaginal bacteria cell into a set of superpixel regions. To the best of our knowledge, there is no prior work applying SLIC to segment the vaginal bacteria.

Vaginal bacteria can be broadly divided into two types: bacilli and cocci. Different types of bacteria have high color complexity with similar appearances. Though color, shape, texture, and edge intensity features in each superpixel boundary are very promising for bacteria detection, there are too many touching bacteria, segmenting touching bacteria especially the bacilli bacteria type in rod shape is even more challenging. Besides, vaginal bacteria are very small and have no distinct definition of the boundary. Most of the previous work focused mainly on the non-touching cell segmentation or round shape cell segmentation, which can be applied in the BV directly [11]. There is no theoretical definition and benchmark tool for the vaginal touching cell segmentation. So far, there is no previous work on the bacteria segmentation, touching vaginal bacteria segmentation and counting for BV treatment and diagnosis. In view of this, the main goal of this paper is to develop a high performance, low-cost, and labor-saving vaginal screening system to benefit female person especially those in the under-privileged country. The touching bacteria segmentation is solved by effective distance to find the marker. Both segmentation and touching bacteria splitting have achieved good performance. Moreover, the kernel-based SVM has a very high discriminative power to detect the bacteria desirably.
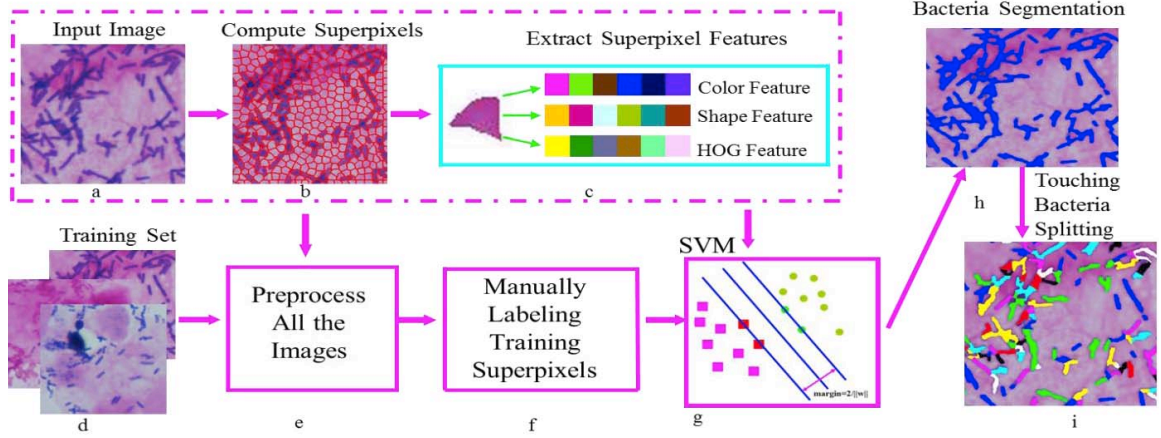
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Figure 1. System overview.

II. METHODLOGY

A. Syetem Overview

The framework of the vaginal bacteria segmentation is shown in Figure 1.The input image is first converted to grayscale image, and then the median filtering is utilized to remove the noises. The real-world collected images are often noised-disrupted, bad-stained and background interfered, preprocessing is conducted to remove the noises. Superpixel method is also exploited to divide the input image into small regions. Each region is represented by the color, shape, histogram of gradient (HOG) and combined features. Based on the feature vectors and the manually labeled training pixels, SVM is employed to detect vaginal bacteria. The vaginal microscope image has a lot of touching bacteria especially in the rod shape, the touching bacteria is further segmented based on the defined effective distance.

B. Superpixel

To efficiently compute superpixel, the graph-based SLIC method in [10] is selected due to the smoothed boundaries in the regular shape. SLIC takes advantage of the color similarity and the spatial information in the pixel to cluster the image. By adopting penalty rule, image gradient between the pixels is inversely proportional to other boundaries, which builds regularized boundaries well aligned with intensity edges. Compared to patch-based method, superpixel based method is more desirable with the controllable size to constrain the overall errors. Specifically, assuming image with N pixels, the regions of superpixelis K, and the centroid distance is approximated as: $S = \sqrt{(N/K)}$. To avoid the interference from the sample point in the edge position, the distance between each pixel and the centroid point is calculated as:

$$d_c = \sqrt{(l_j - l_i)^2 + (a_j - a_i)^2 + (b_j - b_i)^2}$$

(1)

$$d_s = \sqrt{(x_j - x_i)^2 + (y_j - y_i)^2}$$

(2)

$$D = \sqrt{d_c^2 + (\frac{d_s}{S})^2 w^2}$$

(3)

where l, a, b channel is the CIE LAB color space, x, y is the coordinate point, w is the weighting parameter to adjust the region size. Meanwhile, in the practical application of k-means clustering, centroid point is only searched within $2S \times 2S$ regions to improve the speed. The search will be stopped when the distance between the centroid is small enough. To ensure the connectivity of the regions, the small region area will be merged to the region with the nearest distance. There are two parameters to adjust in the SLIC algorithm, one is region area and another is weighting parameter w. As shown in Figure 2, small area can better capture the boundary, whereas large w will affect the connectivity. To tradeoff them, in our experiment, the parameters are set as: $N/K=20$, $w=0.05$.

C. Feature Representation

To detect the bacteria effectively, the features composed of color, morphological, texture and HOG feature are combined together to represent each superpixel region. Since the bacteria and non-bacteria regions are quite distinct, the maximum (Max), minimum (Min), mean and standard deviation (Std) of the R, G, B channel are selected. In most cases, the color differences in the same regions are very small, and hence the Max, Min, mean and Std of the H, S channel are also selected. Taking the morphological differences into account, 10 morphological features are incorporated. HOG feature with the connected region size of 6×6 with 8 directions are computed due the scale and rotation invariance. Meanwhile, considering the spatial correlation of every region in the superpixel, two background regions out of the superpixel regions are defined, one has a radius equal to 5 pixels, another has a radius equal to10 pixels. Max, Min, mean and Std values of the two background regions are included. The final feature
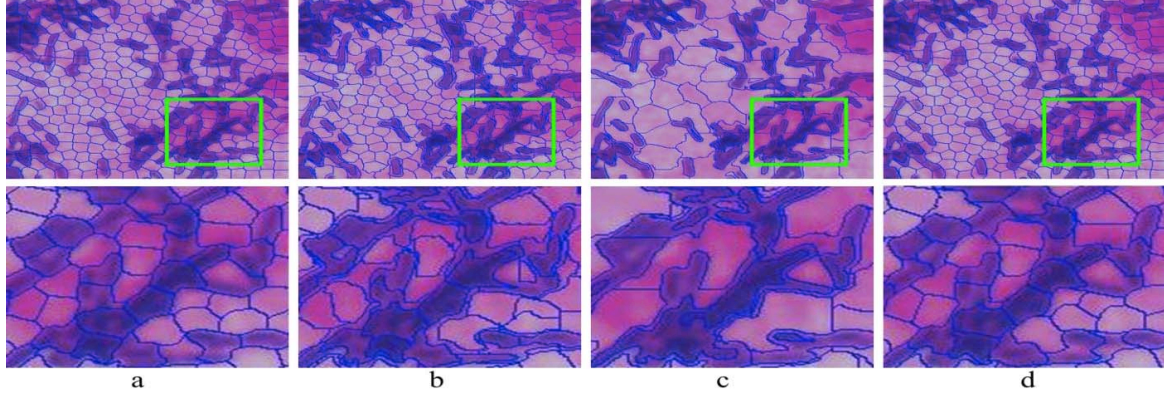
Figure 2. Illustration of different SLIC parameter. The upper row is the segmentation results by superpixel, the bottom rows are the green frame in the upper row: a. N/K=20, w=0.1, b. N/K=20, w=0.01, c. N/K=40, w=0.01, d. N/K=20, w=0.05.

representation is normalized within [0, 1] to avoid the bursting effect of some large numbers.

D. Kernel-Based Feature Mapping

SVM is one of the most popular machine learning methods since it gains an edge to solve the over-fitting problem. Kernel-based feature mapping is integrated with SVM to boost the segmentation performance. As discussed in [12], good performance can be obtained by mapping the linear space to non-linear one with an explicit feature map. The main motivation of feature mapping is to approximate a non-linear kernel as a linear one. Accordingly, the inner product in the linear SVM is operated in a non-linear way by feature mapping [12]. The linear method can be applied in this feature space by feature mapping, which is very beneficial to the distance based algorithms. The widely used kernels such as intersection kernel (IK), chi-square (Chi2) kernel, and Jensen-Shannon (JS) kernel are applied and investigated.

E. Touching Bacteria Splitting

In this paper, the bacteria are regarded as touching mainly based on the following criteria:

1) The region area >150;
2) The length/width ratio is less than 1.2 and the round ratio>0.9;
3) Convex rate>0.8;
4) Short axis is above 15.

In the vaginal smear bacteria image, the touching bacteria and region is mainly in the rod shape and different from the color, which is shown in Figure 3a. The original image is converted to HSV color space, and S channel is extracted. The pixel value in S channel is convoluted with Gaussian kernel $G(x,y;\sigma)$ to smooth the signal, and σ is determined by the bacteria size. The splitting of touching bacteria is based on the Hessian matrix, which is defined as follows:

$$H(x,y) = \begin{bmatrix} S_{xx} & S_{xy} \\ S_{xy} & S_{yy} \end{bmatrix} \tag{4}$$

The color change of the touching area is calculated by Hessian matrix's eigenvalue λ_1 and λ_2 as below:

$$\lambda_{1,2} = \frac{S_{xx} + S_{yy} \pm \sqrt{(S_{xx} - S_{yy})^2 + 4S_{xy}^2}}{2} \tag{5}$$

$$\lambda = \lambda_1 - \lambda_2 \tag{6}$$

Based on the obtained λ value, the superpixel region is enlarged into the range of [0 255], as shown in Figure 3b. In the following, an adaptive threshold is calculated as:

$$Th = \alpha(G_{max} - G_{med}) + \beta G_{med} \tag{7}$$

where G_{max} and G_{med} are the Max and medium values of the 5×5 regions, α and β are the weighting parameters, $\alpha = 0.3$ and $\beta = 1$ are empirically set in our experiment to optimize the performance. Morphological operation is then performed to find the touching marker (i.e. the green area), as illustrated in Figure3c. Different from the watershed method, an effective distance between the pixel values and the marker is defined first, and then the minimum distance is employed for the touching bacteria splitting as below:

$$D_g(T_i, M_j) = \min_{x \in M_j} d_i(x) \tag{8}$$

$$D_c = \left\| M_{M_j} - M_{T_i} \right\| \tag{9}$$

$$D = (1-\gamma)\frac{D_c^2}{D_g} + \gamma\frac{D_g^2}{D_c} \tag{10}$$

where $d_i(x)$ is the Euclidean distance between T_i and x, M_{M_j} is the gray pixel mean value of j-th marker M_j in the S channel, γ is an adjusting parameter and empirically set to 0.3 in our experiment. Since the bacteria area should be larger than a certain value, which is pre-specified to 150 based on the doctor's experience and distribution of our dataset. The superpixel area smaller than 150 is merged to the neighbouring area. Finally, the touching bacteria are split according to the defined minimum distance, which is described in Figure 3d.
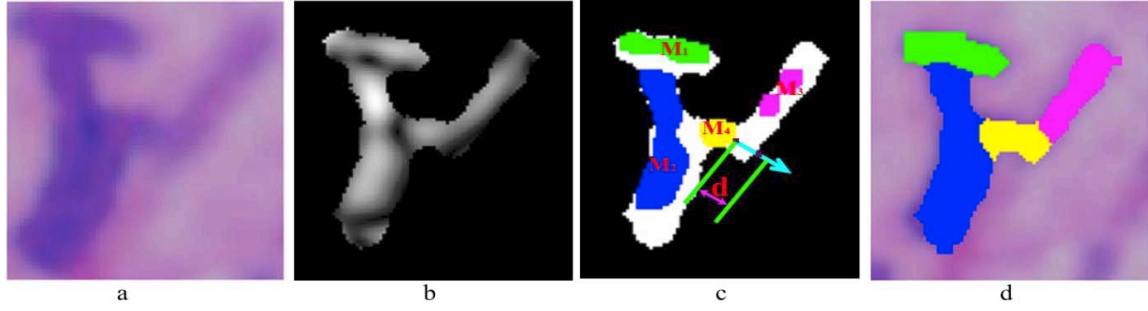
Figure 3. Illustration of touching bacteia splitting procedures: a. Superpixel region of bacteria,b. Gray image of Fig.3a, c. Detected markers of Figure3a (solid line is distance), d.Touching bacteria splitting after ultimate erosion (color represents the associate results).

TABLE I.   Segmentation Result.

Method	Morphological feature	Color feature	HOG	Feature fusion
BPNN	63.34%	80.21%	84.64%	89.76%
Linear SVM	73.06%	84.41%	89.87%	90.24%
IK-SVM	73.62%	87.96%	89.67%	93.33%
Chi2-SVM	**73.96%**	**88.67%**	89.84%	89.27%
JS-SVM	71.52%	85.48%	**89.97%**	**94.38%**

III. EXPERIMENTAL RESULTS

A. Materials and Dataset

Our experiments are based on 105 women subjects aged 18-50 from Shenzhen Sixth People's Hospital. By using oil immersion, a total of 105 bacteria slides are collected by the Olympus BX43 microscope at a magnification rate of 100 and a numerical aperture of 1.25. 44 out of them are diagnosed as BV, 15 of them are fungal infected, and the rest are normal. All images are labeled by the physicist with more than 15 years of residency. 319 images of size 1360×1024 in JPEG format containing the bacteria are utilized for actual diagnosis by doctors. 41×41 superpixel regions are extracted from the collected images based on the centroid of the superpixel. The total training samples from the superpixel region include 3435 bacteria, 3382 background, 1478 Leukocyte, 5792 nucleus, and 10096 cyptoplasm are selected as the training samples. All the reported results are based on 10-fold cross-validation. All slides were prepared using manual liquid-based cytology (MLBC) technique stained with H&E, and confirmed by biopsy. It should be noted that all materials used in this experiment were approved by the Ethics Committee of the university institution.

B. Segmentation Result

To evaluate the segmentation performance, the Zijdenbos similarity index (ZSI) is utilized as evaluation metric. To compare the performance with different methods and effectiveness of feature mapping, the unsupervised backward propagation neural network (BPNN) is compared with supervised SVM method based on different kernels. BPNN is an unsupervised method, which adjusts weights of each layer by minimizing the objective function using a gradient method. Table I summarizes the performance of different methods using the representative features. Based on the quantitative results, JS kernel based SVM is found to be the most effective and outperforms the other selected method. The ZSI results demonstrate that kernel based SVM is very effective for the vaginal bacteria segmentation. The performance of BPNN is still not quite satisfactory due to its limited network layer. The quantitative results of the segmentation performance in terms of different features demonstrate that the feature fusion method outperforms the single feature only since higher discriminative information is preserved in the feature fusion method. The segmentation results are high enough to detect the bacteria boundary.

The comparisons of the manual and automatic segmentation results based on 3 different bacteria images are illustrated in Figure 4. It is observed that the automatic segmentation is very desirable compared to the ground truth based on doctors' experience. The proposed segmentation method also outperforms the related methods in [3] and [4]. Obviously, the detected contours of the proposed method outperform the selected methods. The boundaries of each superpixel have a close match of the true image boundaries. It is noted that superpixels are very effective for segmentation by well-reserved image boundaries.

C. Touching Bacteria Splitting Result

After segmenting, the touching bacteria are further splitted to be more effctive for BVdiagnonsis based on the defined adaptive and effective distance. In the BV study, the touching bacteria evaluation is based on the following criteria:1) over-spliting, the original non-touching bacteria is regarded as the touching bacteria; 2) under-spliting, the touching bacteria is regarded as the non-touching bacteria; 3) erosion mistakes, the touching area does not have clear boudaries.10 images from our database are selected for the the performance

evaluaton and comparison. The experienced doctors with 15 years are asked to give the evaluation of the touching bacteria splitting. Table 2 shows the bacteria counting results after bacteria splitting. It is shown that the bacteria counting result are very useful and remarkable for the BV diagnosis. Besides, Figure 5 shows the touching bacteria splitting results. It can be seen that the splitting performance is very promising even under very complexed contrast and highly touched cases. Based on the splitting performance, it can be seen that the proposed method has achieved remarkable perfomance in the touching bacteria seperation and can be widely applied in the BV diaganosis.
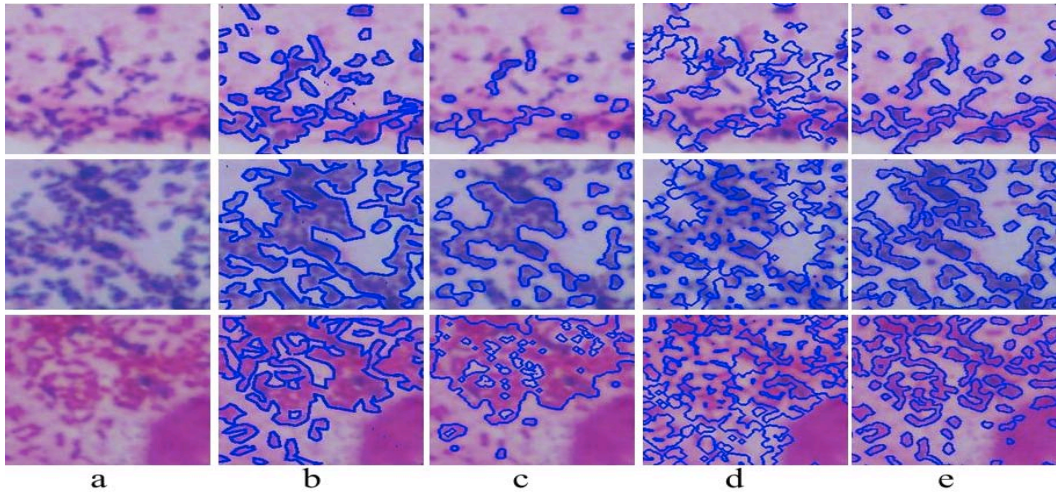

Figure4. Typical vaginal image samples and the segmentation results of the vaginal bacteria: a. Original image, b. Ground truth, c. Segmentation results in method [4], d. Segmentation results in method [3], e. Segmentation results in our proposed method.

TABLE II.   Bacteria Counting Results.

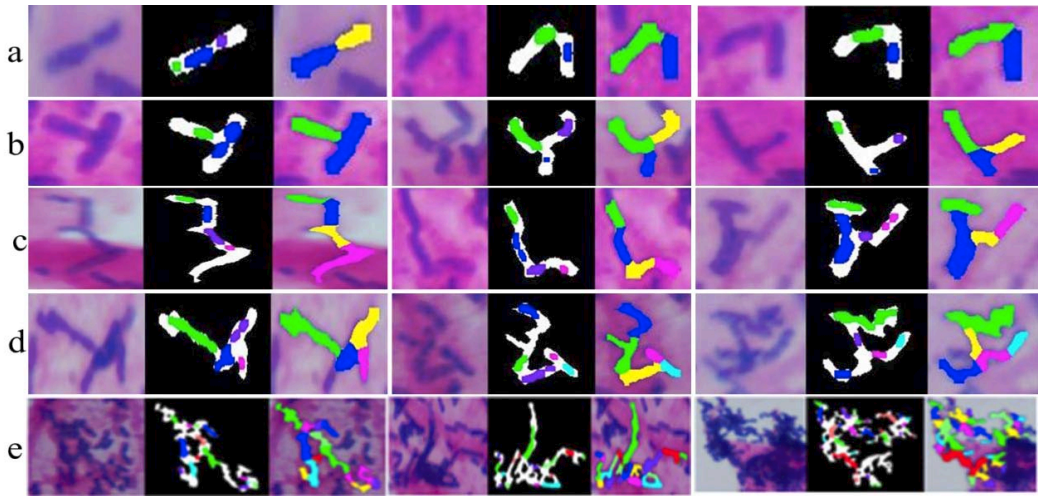| Image ID	1	2	3	4	5	6	7	8	9	10	Ave. numbers
Number of bacteria	41	51	63	108	124	152	194	342	247	214	153.6
Correct splitting	35	39	50	90	102	123	152	267	199	172	122.9
Under-splitting	2	3	8	7	3	9	9	16	14	14	8.5
Over-splitting	1	3	2	2	8	2	5	5	1	2	3.1
Erosion errors	1	1	0	2	5	3	5	24	19	9	6.9

Figure 5. Splitting results of touching bacteria. Splitting results of a. Two touching bacteria, b. Three touching bacteria, c. Four touching bacteria, d. Five touching bacteria, e. More than five touching bacteria.

IV. CONCLUSIONS

In this paper, a novel method for touching bacteria segmentation and splitting is proposed based on the superpixel and defined effective distance. Superpixel techniques are investigated to segment and identify the vaginal bacteria region. The adaptively defined distance is investigated to split the touching bacteria. Our experimental results indicate that

superpixel partitioning is highly effective for vaginal bacteria segmentation. The achieved remarkable segmentation and identification demonstrates that the practical bacterial vaginitis by the automatic diagnosis techniques.

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