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An Automatic Colonies Counting Based on Piecewise Circle Fitting

San-Ding Luo[1], Zheng Zou[1], Guang-Ya Tian[1]

[1]School of Information Science and Engineering
Central South University
No.932, Lushannan Road, Changsha, Hunan, 410005, China
sdluo@csu.edu.cn, zouzheng84@csu.edu.cn

Jeng-Shyang Pan[2,3] and Shi-Jian Liu[2,3]

[2]School of Information Science and Engineering
[3]The Key Laboratory of Big Data Mining and Applications of Fujian Province
Fujian University of Technology
No.3, Xueyuan Road, University Town, Minhou, Fuzhou, Fujian, 350118, China
jspan@cc.kuas.edu.tw, liusj2003@fjut.edu.cn

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Abstract. *and counting the total number of bacterial colonies on agar plates can offer essential indicator for microorganism survival rates. However, manual counting is time-consuming while for computer-aided colony counting, the adhesive colonies usually make the colonies counting challenging. To improve the accuracy and efficiency, we propose a fully automatic counting method. Firstly, a color identification process is used for distinguishing the colonies with different color. Secondly, according to the curvature characteristic along the colony boundary, a segments extraction strategy is used for finding the proper split points. Thirdly, piecewise fitting algorithm ranks candidate circles by confidence, and removes the redundant candidate circles to separate touching colonies. Once the circles are determined, all the colonies are marked by corresponding circles. Our method demonstrates a new way of using circle fitting for accurately separating the touching colonies. The experimental results demonstrated that our method is better than two other most cited methods in accuracy and consuming time.*
Keywords: Colonies counting, Petri dish, Clustering, Circle fitting, Colony segmentation

1. **Introduction.** In microbiological research, the accurate quantification of colony forming units formed on semisolid medium in a Petri dish is a crucial indicator for antibiotic screening [2], toxicology testing [1], genotoxicity measuring [3] and bacterial phase identification [4, 17]. The manual counting of colonies is a laborious process [5] and often prone to human errors due to phenotypic difference in genetically similar microorganisms and presence of background microflora [6, 7]. Moreover, there are uncertainty and large amounts of variations in manual colony counting [8].

Automatic colony counting is challenging and remains an open problem due to the complex appearances, variably intensity, low signal-to-noise ratio and touching colonies in colony images. Specifically, though a colony can be seem as a small circular region because of the surface tension of bacterial membranes, colonies within a Petri dish generally overlap with each other, which makes the appearance of colonies differently and the identification of individual colony difficultly by computer. Furthermore, the problems such

as uneven illumination, specular reflection introduced by poor light conditions and noises in the image make the colony counting more challenging. In additional, different kinds of colonies may exist and touch with each other, and the overlapping region may make partial information of colonies lost for detection. In this paper, we aim to automatically identify individual colonies from the Petri dish image and acquire the counting results with high accuracy.

2. **Related Work.** There are some counting systems developed to efficiently detect colonies and prevent inconsistencies of images. For example, the NICE [26], OpenCFU [27], Colony counter (CC) [28], Clono-Counter [29] and plugins of ImageJ [10]. However most of them either are not accurate enough or need human intervention to some extent. Due to inappropriateness for different imaging system or limitation in performance among many others, automatic colony detecting systems have not been widely accepted as a routine tool in microbiology. The previously developed automatic colonies segmentation method uses special culture medium containing fluorogenic substrates or a spectrograph [6] for acquiring reflectance image to enhance the image quality for segmentation. This method depending on the design of imaging is useful for detecting colonies, but the imaging system is costly and the imaging parameters needs adjusting for several time to get the proper value according to different bacteria.

Segmenting colonies from background and acquiring the correct individual colony is the difficulties of detecting colonies, which is critical for the results in accuracy. Methods based on gradient [9] such as watershed [20] are efficient for separating overlapping objects and can acquire the center of colonies, but if intensity variation of the touching colonies is not strong, the watershed based methods usually have the problem of over-segmentation or under-segmentation. Chiang et al. [16] incorporate distance transform into watershed to determine the colony with help of the skeleton obtained in distance mapping space. However, if there exists big proportion adhesion and severe overlap in a colony block which is formed by a single colony or several colonies as a visible cluster in Petri dish, the skeleton of part with relatively small size is obscure and difficult to acquire the extremum. Apart from the gradient information for segmentation, Bai et al. [17] analyze the concavity of the contour of the touching colonies to separate the touching colonies. But the contour has some false regions due to possible noise, which will affect the extraction of concavity. Some model based methods such as adaptive active physical deformable model [18] and graph-cut-based methods [19] also are proposed to solve the colony segmentation, but their result largely depend on the use of appropriate weighting parameters. Machine learning based methods such as support vector machine [24] and neural network [25] are also used in accurately detecting cells or colonies in connected domain. However due to the strong randomness in overlapping level and touching way of objects, the training is complex and time-consuming. Moreover, because the most common type [5] of bacterial colonise is quasi-circular, some ellipse based methods are proposed [17, 21, 22] to find every possible single colony in the touching colonies and achieve the purpose of dividing touching colonies. These methods are effective but offer incomplete segmentation because they pay more attention to the single pixel for fitting and neglect that colonies are continuous regions.

Aiming for accurate and robust segmentation for chromatic and achromatic colonies, we separate the touching colonies based on curve characteristics of colony boundary. The main contribution of our proposed method are as follows. Firstly, a principal color analysis is incorporated into preprocessing to better distinguish chromatic colonies, and some refinements including smoothing reduce the disturbance and make the method more robust. Secondly, a segment extraction strategy based on the curve characteristic finds the

correct split points for dividing the extracted boundary into segments, and the strategy that only the convex segments are used as the candidate can largely reduce the complexity of subsequent fitting. Thirdly, according to piecewise contour information offered by segments , the fitting algorithm with confidence ranking is used and adaptively classifies the candidate circles into some categories, then each category represents an individual colonies. Our method demonstrates a new way of using circle fitting based on the segments extraction strategy for efficiently separating the touching colonies and get the counting results.

The rest of this paper is organized as follows. Section 3 presents details of the proposed method. Section 4 gives some experimental results including the evaluation and comparison. Section 5 concludes the paper.

3. **The Proposed Method.**

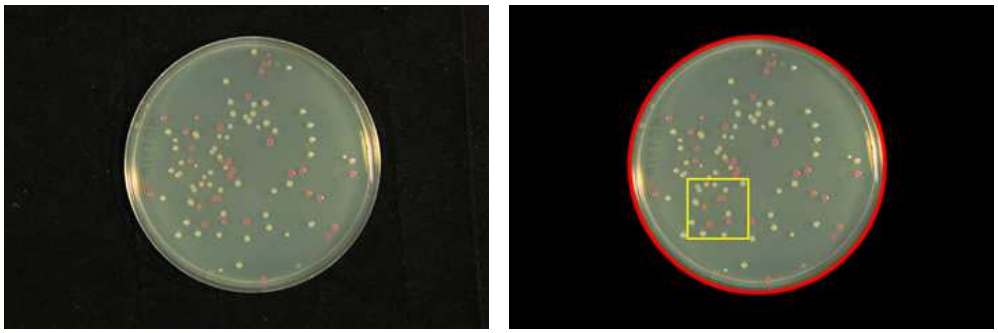
The proposed method takes Petri dish image as input and gives the colony counting result as output, it contains three major steps. The first step (see Section 3.1) focuses on problems such as Region of Interest (ROI) extraction, color classification and their boundary extraction, etc. In the second step (see Section 3.2), the extracted boundary of each colony block is divided into several boundary curves referred to as segments. The third step (see Section 3.3) fits circles to segments and clusters them, and the counting problem would be solved as long as the number of fitting circles are determined.

3.1. **Colony boundary extraction.**

Because the original images contains two parts : a Petri dish region and a big dark region around the dish. Locating the Petri dish region as our ROI can greatly reduce complexity and exclude disturbances from background. In order to successfully locate the Petri dish, Hough transform [11] is applied for our ROI extraction due to its quasi-circular shape. As demonstrated in Fig. 1, the red circle is a automatical detection result with the Hough transform of original image in Fig. 1(a), and the region inside of the circle is our ROI, while region outside is changed into black by masking operation.

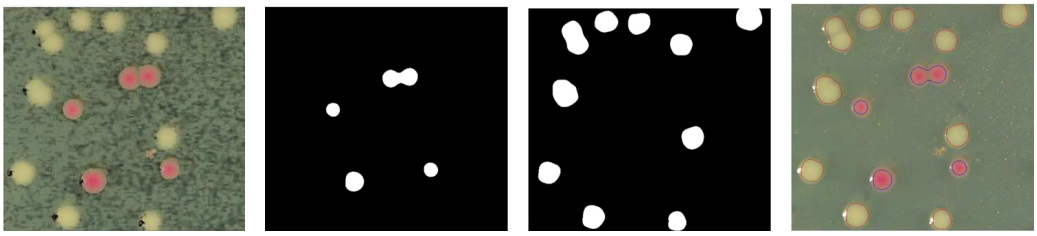
Due to uneven illumination, there may exists light spots on the bulged surface of the colony, which may results in fault boundaries points if the light spot locates in the neighbouring region close to the boundary, and we use the method proposed by Yang et al. [12] to remove the disturbance of highlight. Fig. 2(a) describes the highlight removal result of the yellow rectangular region of Fig. 1(b).

In Fig. 1(a), there are some touching colonies formed by red chromatic colonies and yellow achromatic colonies, the touching colonies can be preliminarily separated according to color information. In order to automatically classify the colonies with different color, the analysis of histograms in HSV (Hue, Saturation, and Value) color space [14] is used for the classification. The HSV color space has been proved to be better and more intuitive than other color models such as RGB, and it also can be transformed from the RGB by method such as the one proposed by Chen et al. [15]. We adopt a method similar to the one presented by Chen et al. [13]. In their method the three-dimensional parameters (i.e., H, S and V) are considered, but our method only analyze the hue channel of pixels, and the pixels with either a lower saturation or a lower value are neglected in advance. That is because pixels with lower saturation is supposed to close to gray, which is meaningless for color-based segmentation. Let B_S and B_V denote the upper bound of saturation and value respectively, pixels with $S < B_S/8$ or $V < B_V/8$ are treated as background in our method. The principle color in images can be distinguished according to several different peak values of distributions histograms in hue channel, regions in specific color can be



(a) (b)

FIGURE 1. The ROI extraction. (a) The original image; (b) The ROI region marked by red circle.



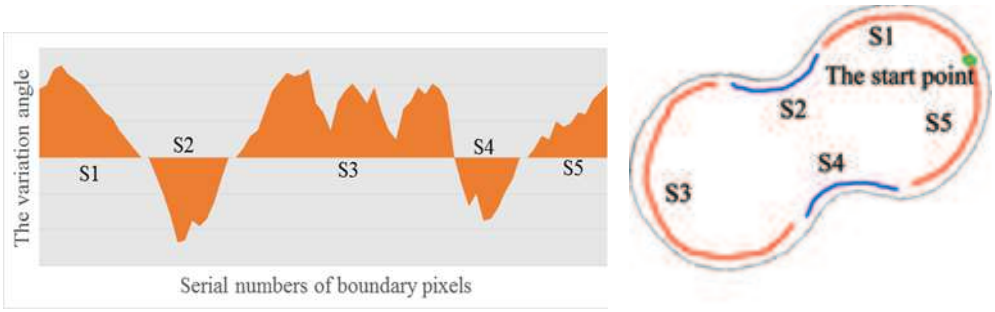
(a) (b) (c) (d)

FIGURE 2. The boundary extraction. (a) The image with highlight removal; (b) The chromatic colony region after color classification; (c) The common colony region after color classification; (d) Final boundaries of all colonies.

identified. The two types of the colony are separated into two parts as described in Fig. 2(b) and Fig. 2(c).

The boundaries of colony regions with different colors are extracted respectively, in our paper canny edge detection [30] is adopted to detect the boundary. There often exists serrate boundary resulting from the low resolution, and the serrate boundary will results in some fake inflection points and effect the subsequent fitting accuracy, morphological opening and closing operation is used to filter noisy part and smooth the boundary. The final boundary result as shown in Fig. 2(d), the yellow colony block are marked by red line, and the red colony block are marked by yellow line after classification. If the block boundary contains only an individual colony, a circle fitting process as proposed in Section 3.3 can be used to locate the colony. But more generally, there also exists the block which consists of multiple touching colones. Therefore, a separation strategy will be used before the circle fitting, which is one of the core contributions of this work (see Section 3.2).

3.2. **Boundary loop separation.** The entire boundary curve are mainly made up of three kinds of segments including the flat, concave and convex one, and the first step is finding the split points to decompose the entire boundary into segments according to different property of points. To analyze the property at points along the boundary, the variation angle at boundary points denoting the change in direction of the curvature is used. Specifically, let $\{P_i\}(1 \leq i \leq N)$ denote the points forming a block boundary(N is the number of boundary points), and $L(P_i)$ represents the line segment vector at each P_i, and the line starts from P_i to $P_i + 1$. Then the variation angle between all the pairs of



(a) (b)

FIGURE 3. An example of the segments extraction based on the variation angle. (a) The histogram of the variation angle along the contour of Fig.5(b) from the start point; (b) The corresponding segments result.

consecutive line segments are denoted as $A(P_i)$, where $A(P_i) \in [-\pi, \pi])$ is the clockwise angle from $L(P_i + 1)$ to $L(P_i)$. It can be obtained according to Eq. 1.

$$A(P_i) = \begin{cases} L(P_1) - L(P_i), & i = N \\ L(P_{i+1}) - L(P_i), & otherwise \end{cases} \tag{1}$$

The value of $A(P_i)$ is an indicator of the change in the curvature of the block contour. If any $A(P_i)$ is close to zero, which implies its change curvature is small or flat; if the absolute value of $A(P_i)$ is large, then the curvature at the point P_i is considered to be sharp. But only the variation angle is not enough to find split points. For example, in Fig. 3(b), there is a block containing two individual colonies. From a start point, we traverse all pixels of boundary curve in counterclockwise direction. According to the histogram of the variation angle of all pixels in Fig. 3(a), if the variation angle of some pixels with consecutive serial number are larger than zero or smaller than zero, then the segment which consists of those pixels should cover the same colony, for example in Fig. 3(b), all pixels along the segment $S1$ belongs to the same colony, while the segment $S1$ and the segment $S2$ belong to different colonies respectively. Therefore the zero crossing point is the pixel cover the connecting point between two neighboring colonies. Furthermore for the case that there exists no obvious concave curve due to severe overlapping colonies, the value of pixels in the same colony should float around the mean variation angle, then the pixels much less than the mean value is the turn point. Therefore, the possible split point P_s should satisfy the following rules: (1) $|A(P_{s-2}) + A(P_{s+2})| < |A(P_{s-2})| + |A(P_{s+2})|$ and $A(P_s) = 0$ or (2) $|A(P_s)| < 2\pi/N$.

According to the split point $\{P_{sj}\}(1 \le j \le M_1)$ (M_1 denotes the total number of split points), the entire boundary contour is divided into several segments $\{S_j\}$ from a start point as shown in Fig. 3. While in our extracting segment strategy only the convex segments are used as the final segments for the subsequent fitting. In order to pick the convex segments from the original segments set, the criterion we use for detecting the convex property of segment S_j is that the connecting line $\overline{P_{sj}P_{sj+1}}$ pass through the inside of colony. Then the final segments $\{S_j\}(1 \le j \le M_2)$ (M_2 denotes the total number of convex segments) can be obtained accordingly. As shown in Fig. 4, the convex segments marked by bold line are used for fitting and the others marked by thin line are removed. The strategy that removing the concave or flat segments greatly improves the efficiency of subsequent fitting.

3.3. **Circle fitting and counting.** Every image has multiple regions of touching colonies, and every boundary of the touching colonies may have multiple segments. The main aim

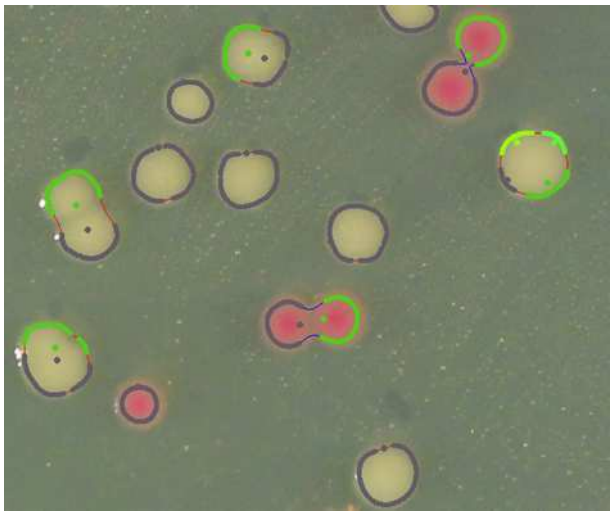


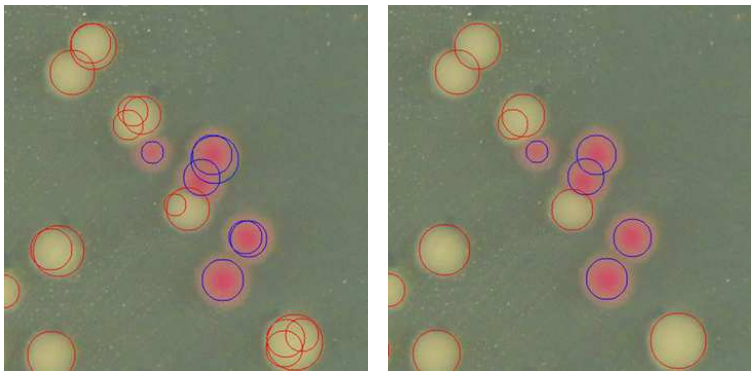
FIGURE 4. The candidate convex segments for fitting

for circle fitting processing is to separate the touching colonies into individual circles which denote individual colonies. Because in our extracting segment strategy only the convex segments are used for the circle fitting, which largely reduces the numbers of original fitting circle and reduce the probability of the case that the circle doesn't cover the colony due to the disturbance from concave segments. Firstly, as a effective method for fitting, the least square method is used in our paper to fit each segment the boundary to a circle. Secondly, the circles are further processed by our ranking rules which is incorporated into prior knowledge of the colony images, and some circles can be combined if they belong to the same colony and some circles can be kept if they belong to different colonies. Moreover we select pixels over the segment at a given distance interval for fitting to reduce the complexity of fitting. Among all the step for fitting above, the strategy of circle selection and combination is our focus, the details are given below.

After circle fitting, each segment has a fitting circle. To remove the redundant candidate circles. We rank the all candidate circles according to their confidence as described in Eq.2, where $Confidence$ denotes the proportion of overlapping region between circle region and original image to the circle area. The candidate circles with lower confidence are removed, and the selected circles are of high confidence and fit the contour segments well. The remaining circles are unsupervised clustered and automatically classified into several categories according to similarity in shape, and the measurement of similarity between the circle and the existing categories is computed as Eq.3. If the circle is similar to more than one category, then it is combined into the category with the highest similarity; if there is no similar category or no category, then according to overlapping region between the circle and original image, a new category is created. The clustering will not stop until all the candidate circles are compared. Once all categories, which represent individual colonies in the original image, are determined, the counting result including the total colony amount and the center or the radium of each colony is also obtained. The candidate result of the fitting and the final fitting result can be described in the Fig. 5

$$Confidence = \frac{Area(Circle \bigcap OriginalImage)}{Area(Circle)} \tag{2}$$

$$Similarity(a,b) = min\{ \frac{Area(Circle_a \bigcap Category_b)}{Area(Circle_a)}, \frac{Area(Circle_a \bigcap Category_b)}{Area(Category_b)} \} \tag{3}$$



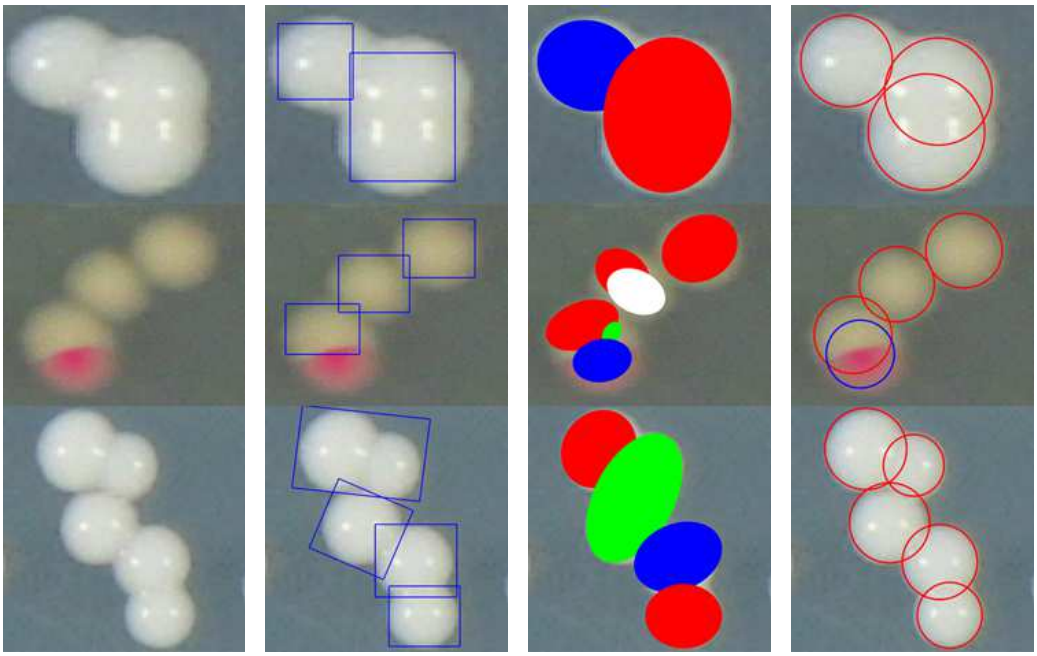
(a) (b)

FIGURE 5. The colony detection result with ranking confidence. (a) The candidate circles after fitting; (b) The detected circles after combing the candidate circles

4. **Experiments and Results.** In this study, Escherichia coli is selected for our experiments, the bacterial colonies is cultured in circular agar plate. The image captured by the CCD has 17 million effective pixels (5184(H) * 3456(V)) as shown in Fig. 1(a). In Fig. 1(a), there are two kinds of colonies with different color, and the red colonies results from effective gene splicing of red protein, while yellow ones does not successfully spliced by colored protein. These colonies in one plate are required to be detected and counted respectively according to different color. Our method is applied on 28 images and the range of the number of colonies was from 127 to 429. All the experiments are carried out on a computer with Intel Core CPU(2.2GHz), 8GB memory, programmed with C++ and OpenCV.

4.1. **Comparison results in detecting colonies.** To evaluate and demonstrate the performance of our method, two other algorithms are used for comparison in this paper. The system OpenCFU [28] as a effective open-source system for colony counting recently use a watershed transform based method [16] (WT) for dividing the touching colonies. Reference [28] proposed a ellipse fitting based method (EF) for splitting the touching one. We choose these two method for comparison, because they are two classic and latest method especially designed for splitting touching colonies, and OpenCFU has been used in commercial application. WT, EF and our method are applied on 28 images with 6256 colonies in total, and there are 339 touching colonies in the image set. Five measurements are used for the performance of these methods. They are the proportion of correctly separated colonies in total touching colonies (PCS), the precision for all the colonies detection (Precision)[16] , recall all the colonies detection (Recall)[16], F-measure for all the colonies detection (F-M)[16], and the consuming time of detection for all the images (Time). In comparison, the manual counting result, which are manually generated by experienced experts, is taken as the standard.

There are three original images with typical touching colonies for comparison of detection results as shown in Fig. 6. The first row, the second row and the third row of Fig. 6 are the high overlapping touching colonies, the chromatic touching colonies and the multiple touching colonies respectively. In detecting chromatic colonies, The EF based method and our method are better than the WT based method, which only use the global threshold for color classification obtained by user interactive. Because EF based method and WT based method are sensitive to fluctuations, and neither of them detect the right number of individual colony when the colonies are heavily touching (see Fig. 6(c)). In the



(a) (b) (c) (d)

FIGURE 6. The detection results with three methods. (a) The original image; (b) The result using WT based method marked by rectangle; (c) The result using EF based method marked by colored ellipse region; (d) The result of our method marked by circle.

TABLE 1. The statical results of the image set

	Precision (%)	Recall (%)	F-M (%)	PCS (%)	Time (ms)
Our method	98.97	96.29	97.43	96.68	6319
WT	93.29	90.24	92.57	81.98	8862
EF	98.38	94.21	95.16	95.99	10357

multiple touching colonies images, the redundant results of EF based method results from the fitting only according to local distance between the segment and candidate ellipse, and our method incorporate both the local characterises of segments and integrated shape information of a colony into fitting. These comparison results show that our method is more efficient for separating the touching colonies with different overlapping levels.

4.2. **Statistical results in performance.** The final statical results are shown in Table 1. Our method is better than the other two methods in Precision, Recall and F-M values. With regard to the accuracy in separating touching cell, The value of PCS for EF and our method are very close. However the ellipse fitting in EF need computer fitting similarity with the algebraic distance between the contour points and ellipse pixel by pixel, therefore the complexity of EF is higher, accordingly the consuming time of EF is highest among three method. Moreover, the WT based method requires the user to manually select the counting area or mark the color prior to the automated process, by contrast, no user intervention is required for our method.

5. **Conclusion and Future work.** This study proposes a fully automated colony segmentation method. The proposed method can adaptively locate the ROI and distinguish the chromatic color with shape priori knowledge of Petri dish and principal color analysis. Combined with the confidence ranking, our method use a circle fitting based on extracting

convex segments for effectively separating the touching colonies and largely reduce the complexity of automatical counting. Because the proposed method detects the colony with circle fitting, it can be also used for the detection of other quasi-circular objects. In the future, efforts will be made to provide a more robust method with the capability to count and analyze various strains of colonies in relevant media which vary in color and opacity. Moreover, the detecting the colonies in the rim of Petri dish may missing or error in counting results due to the rim turn and darker light, which makes a little influence for contour extraction of colony in the rim region of dish, a part of the future work is to find a more effective method for improve the colony detecting of the rim region. We will collect more colony image to expand our image set and valid the effectiveness of our method in detecting more complex image.

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REFERENCES

[1] T.Gura, Toxicity Testing Moves from the Legislature to the Petri Dishand Back, *Cell*, vol.134, no.4, pp. 557-559, 2008.

[2] C. J. Ingham, A. Sprenkels, and J. Bomer, The micro-Petri dish, a million-well growth chip for the culture and high-throughput screening of microorganisms, *Proceedings of the National Academy of Sciences*, vol.104, no.46, pp. 217-222, 2007.

[3] X. Wang , J. Guo , and T. Chen, Multi-walled carbon nanotubes induce apoptosis via, mitochondrial pathway and scavenger receptor, *Toxicology in Vitro An International Journal Published in Association with Bibra*, vol.26, no.6, pp. 799-806, 2012.

[4] C. Zhang and W. B. Chen, An effective and robust method for automatic bacterial colony enumeration, *International Conference on Semantic Computing*, pp. 581-588, 2007.

[5] H. Ates , O. N. Gerek, An image-processing based automated bacteria colony counter, *International Symposium on Computer and Information Sciences*, pp. 18-23, 2009.

[6] S. C. Yoon, K. C. Lawrence, and B.Park, Automatic counting and classification of bacterial colonies using hyperspectral imaging, *Food and Bioprocess Technology*, vol.8, no.10, pp. 2047-2065, 2015.

[7] G. E. Tillman, J. L. Wasilenko and M. Simmons, Isolation of shiga toxin-producing escherichia coli serogroups O26, O45, O103, O111, O121, and O145 from ground beef using modified rainbow agar and post-immunomagnetic separation acid treatment, *Journal of Food Protection*, vol.75, no.9, pp. 1548-1554, 2012.

[8] J. E. L. Corry, B.Jarvis and S. Passmore, A critical review of measurement uncertainty in the enumeration of food micro-organisms, *Food Microbiology*, vol.24, no.3, pp. 230-253, 2007.

[9] H. Men, Y. Wu and X. Li, Counting method of heterotrophic bacteria based on image processing, *IEEE Conference on Cybernetics and Intelligent Systems*, pp. 1238-1241, 2008.

[10] J. M. M. Perez and J. Pascau, Image processing with ImageJ, *Biophotonics International*, vol.11, no.5, pp. 36-42, 2003.

[11] J. Illingworth, J. Kittler, A survey of the Hough transform, *Computer Vision Graphics and Image Processing*, vol.43, no.2, pp. 765-768, 1988.

[12] Q. Yang, S. Wang and N. Ahuja, Real-Time specular highlight removal using bilateral filtering, *European Conference on Computer Vision*, 2010, pp. 87-100, 2010.

[13] T. W. Chen, Y. L. Chen and S. Y. Chien, Fast image segmentation based on K-Means clustering with histograms in HSV color space, *Multimedia Signal Processing*, pp.322-325, 2008.

[14] J. R. Smith, Color for image retrieval, *Image Databases: Search and Retrieval of Digital Imagery*, John Wiley and Sons, pp. 285-311, 2002.

[15] W. Chen, Y. Q. Shi and G. Xuan, Identifying computer graphics using HSV color model and statistical moments of characteristic functions, *IEEE International Conference on Multimedia and Expo*, pp. 1123-1126, 2007.

[16] P. J. Chiang, M. J. Tseng and Z. S. He, Automated counting of bacterial colonies by image analysis, *Journal of Microbiological Methods*, vol.108, pp. 74-82, 2015.
[17] X. Bai, C. Sun and F. Zhou, Splitting touching cells based on concave points and ellipse fitting, *Pattern Recognition*, vol.42, no.11, pp. 2434-2446, 2009.
[18] M. E. Plissiti and C. Nikou, Overlapping cell nuclei segmentation using a spatially adaptive active physical model, *IEEE Transactions on Image Processing A Publication of the IEEE Signal Processing Society*, vol.21, no.11, pp. 4568-1580, 2012.
[19] A. K. Yousef, L. Wiem and L. William, Improved automatic detection and segmentation of cell nuclei in histopathology images, *IEEE transactions on bio-medical engineering*, vol.57, no.4, pp. 841-852, 2009.
[20] C. Jaquet, E. And and G. Viggiani, Estimation of separating planes between touching 3D objects using power watershed, *Mathematical Morphology and Its Applications to Signal and Image Processing*, Springer Berlin Heidelberg, pp. 452-463, 2013.
[21] L. Zhang, H. Kong and C. T. Chin, Segmentation of cytoplasm and nuclei of abnormal cells in cervical cytology using global and local graph cuts, *Computerized Medical Imaging and Graphics the Official Journal of the Computerized Medical Imaging Society*, vol.38, no.5, pp. 369-380, 2014.
[22] D Yu, T D Pham and X Zhou, Segmentation, recognition and tracing analysis for high-content cell-cycle screening, *International Symposium on Computational Models of Life Sciences*, AIP Publishing, pp.66-75, 2007.
[23] Z Zhou, N Sang and X Hu, A parallel nonlinear adaptive enhancement algorithm for low- or high-intensity color images, *Journal on Advances in Signal Processing*, vol.1, pp. 1-14, 2014.
[24] G. Turra, N. Conti and A. Signoroni, Hyperspectral image acquisition and analysis of cultured bacteria for the discrimination of urinary tract infections, *Journal of the Brazilian Chemical Society*, vol.19, no.19, pp. 397-404, 2015.
[25] A. Ferrari, S. Lombardi, and A. Signoroni, Bacterial colony counting with convolutional neural networks in digital microbiology imaging, *Pattern Recognition*, vol.61, pp. 629-640, 2016.
[26] M. L. Clarke, R. L. Burton and A. N. Hill, Low-cost, high-throughput, automated counting of bacterial colonies, *Cytometry Part A*, vol.77, no.8, pp.790-797, 2010.
[27] Q.Geissmann, OpenCFU, a new free and open-source software to count cell colonies and other circular objects, *Plos One*, vol.8, no.2, pp. e54072, 2012.
[28] S. D. Brugger, C. Baumberger and M. Jost, Automated counting of bacterial colony forming units on agar plates, Plos One, vol.7, no.3, pp. e33695, 2012.
[29] M. Niyazi, I. Niyazi and C.Belka, Counting colonies of clonogenic assays by using densitometric software, *Radiation Oncology*, vol.2, no.1, pp. 848-858, 2007.
[30] S Sieuwerts, F A M D Bok and E Mols, A simple and fast method for determining colony forming units, *Letters in Applied Microbiology*, vol.47, no.4, pp. 275-278, 2008.