

การประมวลผลภาพและประยุกต์วิธี SVM ในการนับจำนวนและ วัดขนาด Staphylococci จากกล้องจุลทรรศน์

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บทคัดย่อ

การนับจำนวนแบคทีเรียและวัดขนาดด้วยกล้องจุลทรรศน์มีความจำเป็นในงานตรวจสอบและการวิจัยทางจุลชีววิทยา แบคทีเรียเรียสแตฟีโลคอคโคเป็นกลุ่มแบคทีเรียที่มีความสำคัญทั้งในอุตสาหกรรมอาหารและโรงพยาบาล แต่ในการนับจำนวนแบคทีเรียต้องอาศัยผู้เชี่ยวชาญในการแยกแยะระหว่างโคโลนีของแบคทีเรียที่ต้องการตรวจสอบและสิ่งอื่นๆ และต้องนับไม่ต่ำกว่า 10 บริเวณที่ส่องกล้อง ซึ่งต้องใช้เวลาและส่งผลต่อสายตาของผู้นับอย่างมาก งานวิจัยนี้จึงนำเสนอซอฟต์แวร์ MicroStaph ที่พัฒนาขึ้นเพื่อช่วยในการนับจำนวนและวัดขนาดแบคทีเรียที่มีรูปร่างกลมขนาดเล็กนี้ โดยใช้เทคนิคต่างๆ มาสกัดลักษณะเด่นหลายประการของโคโลนีจากภาพเพื่อช่วยในการประมวลผลภาพ จากนั้นนำลักษณะเด่นเหล่านั้นมาใช้ในการแบ่งแยกโคโลนีออกจากสัญญาณรบกวนต่างๆ ด้วย Support Vector Machine (SVM) ทำให้ง่ายต่อการนับจำนวนที่แท้จริง ส่วนการวัดขนาดของเซลล์ทำโดยการเปรียบเทียบจำนวนของพิกเซลจากภาพถ่ายที่กำลังขยาย

เดียวกันของสเตรจไมโครมิเตอร์กับพื้นที่ที่เล็กที่สุดของกล้องครอบภาพหลังการหมุนแกน ผลการทดลองแสดงให้เห็นว่า ลักษณะเด่นที่ใช้ในการแบ่งแยกแยะระหว่างโคโลนีและสัญญาณรบกวนนั้นสามารถใช้แบ่งแยกได้อย่างมีประสิทธิภาพ และผลการนับที่ได้มีความใกล้เคียงกับผลการนับจากผู้เชี่ยวชาญสูง เนื่องจากจำนวนที่นับได้โดยซอฟต์แวร์และผู้เชี่ยวชาญมีความสัมพันธ์เป็นเส้นตรงค่าสหสัมพันธ์ (r^2) เป็น 0.99 สำหรับภาพกำลังขยาย 400 เท่า และ 0.98 สำหรับภาพกำลังขยาย 1000 เท่า นอกจากนี้การนับจำนวนแบคทีเรียสแตฟีโลคอคโคด้วย MicroStaph ที่นำเสนอนี้สามารถลดเวลาในการนับได้มากกว่าครึ่งหนึ่งของการนับโดยผู้เชี่ยวชาญ ขนาดของเซลล์ที่วัดได้โดยซอฟต์แวร์มีเส้นผ่านศูนย์กลางอยู่ในช่วง 0.5 – 0.9 ไมครอน ซึ่งสัมพันธ์กับขนาดจริงของแบคทีเรีย

คำสำคัญ: แบคทีเรีย สแตฟีโลคอคโค การนับจำนวนโคโลนี การนับด้วยกล้องจุลทรรศน์ การประมวลผลภาพ วิธีหาขอบวัตถุ Support Vector Machine

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Image Processing and SVM Application for Microscopic Counting and Size Measuring of Staphylococci

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Abstract

Microscopic count and size measurement of bacteria are necessary for microbiological work. Staphylococci are one of the most frequently found bacteria in food industry and hospitals. However, an expert is needed for such count. He needs to perform at least 10 visual counts, which cause eye strain and are time consuming. This research proposes the developed software, namely Micros-Staph, to aid in the microscopic count of these bacteria as well as the size measurement of the individual cell using extracted features from image processing techniques. These features are used to train Support Vector Machine (SVM) to differentiate the bacterial colony from noises in the images. Therefore, the number of colony can be easily counted. The cell size is obtained by comparing the number of pixels on the stage micrometer with the cell bounding box at the smallest

area found after angle rotation. The experimental results show that the extracted features can be used to count the number of colony effectively. The number of colony counted by SVM provides a linear relationship with those counted by the experts at the correlation value (r^2) of 0.99 and 0.98 for the images captured with 400 and 1,000 times of magnification, respectively. Additionally, the time of microscopic count spent by the Micros-Staph software is reduced by more than half. The cell size measured by the software is 0.5 – 0.9 μm in diameter which is absolutely correlated to the actual cell size of staphylococci.

Keywords: *Staphylococcus*, Bacteria, Colony Count, Microscopic Count, Image Processing, Canny Edge Detection, Support Vector Machine

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1. Introduction

Counting of bacterial cells under microscope considers as an important technique that often perform in microbiological laboratory as well as food safety. Staphylococci are group of bacteria appear round in shape and have cluster arrangement. Cells sizes are 0.5-1.5 micrometer (μm) in diameter [1]. The widely known species are *Staphylococcus aureus* and *S. epidermidis*. Both are skin flora of warm-blooded animals, but the former species is well recognized as foodborne pathogen which produces heat stable enterotoxins [2].

Microscopic count is one of the tools to provide the number of bacteria per gram or per ml of sample [3]. The results can be obtained more rapid than the viable plate count method. However this technique needs an expert to perform which is time consuming and eyes exhausting. Although there is some commercial software available, e.g. Image-Pro Plus, etc. The prices are costly and the user must buy it as a package with famous-make microscope. Furthermore the changes in operating systems such as Windows® affect to old software in such a way that it is unable to operate with the new version of Windows®. These are the limitation of some small laboratories, therefore this research aimed to develop the software that can aid the microscopic count and cell sizing of staphylococci in any computer.

2. Background and Related Work

An example of bacterial image of 1024×1280 pixel obtained under the light microscope at 400 times of magnification is shown in Figure 1. However the micrograph image contains both bacteria (see arrow 1 and 4) and non-bacteria or noises such as spot of culture media (see arrow 2) and the shadow of a dust inside the microscopic lens (see arrow 3). These noises

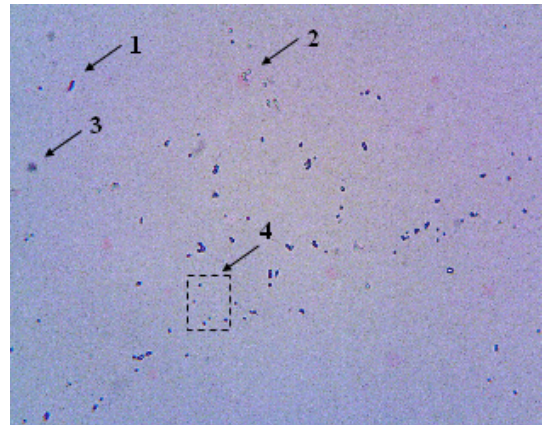


Figure 1 *Staphylococcus*.

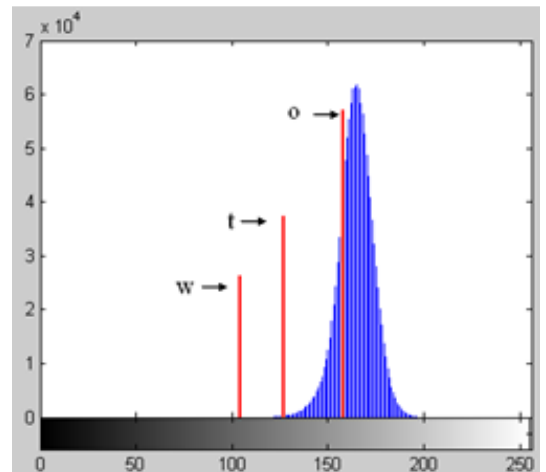


Figure 2 Histogram of micrograph image in Fig. 1.

have similar size to the bacterial cells which are the obstacle of microscopic examination both by man and by computer program.

It is obviously seen from Figure 1 that this bacteria has a very small size. The micrograph image can be segmented into the foreground which consists of bacteria and noises, and background or the spaces. However, the foreground cover only 1% of the micrograph image and the histogram of this micrograph is shown in Figure 2. It is noticeable that the histogram is uni-modal

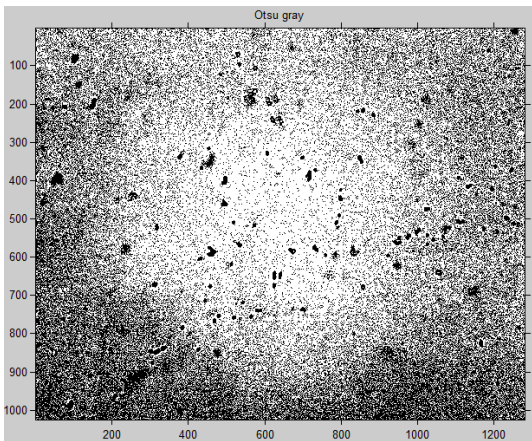


Figure 3 Otsu's method thresholding.

meaning that bacteria and noises cannot be clearly separated.

Many of image thresholding methods based on bi-modal or multi-modal gray level histograms such as Otsu's method [4], [5] cannot be applied to separate bacteria out of background. From Figure 2, four famous image thresholding methods are used to find threshold value to separate bacteria and background. The arrow 'o' in Figure 2 showed a threshold value obtained from Otsu's method. The arrow 't' showed a threshold value obtained from Rosin's method (Triangle) which is designed especially for uni-modal histogram. However, these threshold values are unable to distinguish bacteria from the background. Figure 3 showed an example of using threshold value obtained from Otsu's method to separate bacteria and background.

Although a freeware named "CellC" which is purposely designed for counting bacteria in an image taken from Fluorescence microscope or Light microscope [6], this freeware does not provide acceptable accuracy when counting bacteria in staphylococcal images. Moreover, "ImageJ", a free and well known image processing application software [7], [8], with

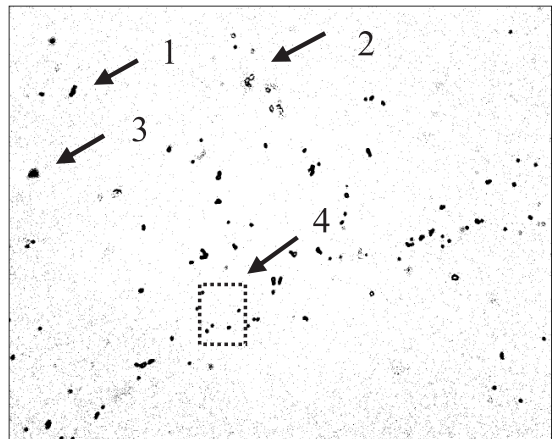


Figure 4 The image thresholding result using ImageJ with Adaptive Threshold.

its adaptive threshold plugin [9] is also used to count the bacteria. Although the results are better than that of "CellC", many noises still exist, as shown in Figure 4 and Table 1. Thus this results in inaccuracy of counting performance of the software.

Table 1 The example of objects from the resulting thresholding image using ImageJ

Arrow No.	Color Image	Black/White Image	Type
1			Colony
2			Noise
3			Noise
4			Colony

The aforementioned characteristics of microscopic images and the size of staphylococcal bacteria possess high difficulties to image thresholding of microscopic image. Neither local nor global image thresholding methods can be easily done. Moreover, some application software ranging from commercial software to well-known freeware cannot be used to determine a threshold value efficiently. In 2010, Zhang et al. [10] proposed to apply Support Vector Machine (SVM) to recognize and count of *Escherichia coli*, but it did not cover *Staphylococcus* bacteria.

This research proposed the simple but effective method to recognize and distinguish the cell/ colony of staphylococci from several types of noises appeared in the image. The analyses of global and local threshold level are incorporated with edge detection method to extract features of cocci bacteria from the images. These features will be used to train SVM to recognize cell/ colony of staphylococci. This work has been extended from our previous work [11] in order to enhance the efficiency of the software to facilitate the microscopic counting as well as cell size measurement of images taken from the light microscope at 400 and 1000 times of magnification.

3. Proposed Methods

This section provides concept and details of methods we used in our proposed work.

3.1 Grey-scale Weighted Threshold Method (GW)

Hong Men et al. [12] proposed an iterative weighting method to determine a threshold value. The foreground and background group of pixels is given different weights by which the summation of the two weights is equal to 1. Let ω is a weight given to

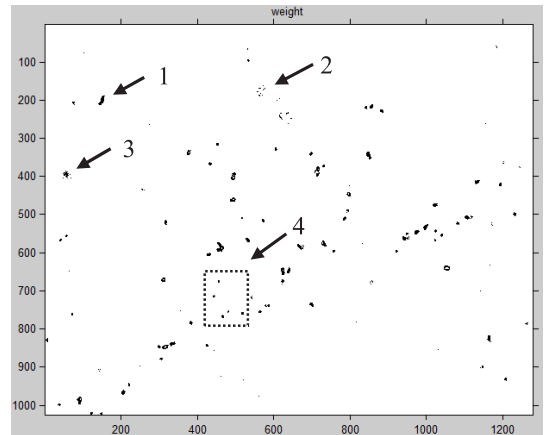


Figure 5 The resultant grayscale image from Grey-scale weight method.

foreground then the weight given to background is $1 - \omega$. The threshold value can be calculated as follow:

$$t_{i+1} = \omega \cdot \mu_1(t_i) + (1 - \omega) \cdot \mu_2(t_i) \quad (1)$$

when t_1 and t_{i+1} are threshold value of current and next iteration, respectively. Whereas $\mu_1(t_i)$ and $\mu_2(t_i)$ are mean of foreground and background group of pixels at current iteration, respectively. The threshold value will be iteratively computed until there is no change or it reaches predefined number of maximum iteration. This method is robust to illumination noises which is good for microscopic images. In this paper, we set the weight ω to 0.65 as suggested in the work of Hong Men et al. [12]. The example of image thresholding result using the threshold value found by this method (see arrow 'w' in Figure 2) is shown in Figure 5.

From Figure 5, the segmented objects can either be colonies of bacteria or noises. It is quite difficult to distinguish them since the bacteria are so small, hence the differences between colonies and noises are hardly noticeable. Moreover, some colonies are smaller

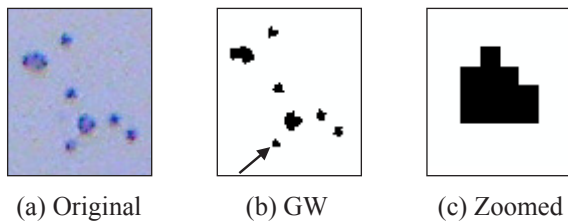


Figure 6 Small size colony (12 pixels).

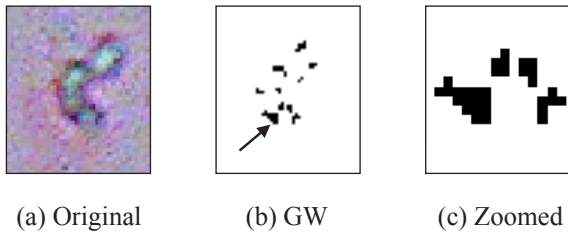


Figure 7 noise (16 pixels).

than noises so the size of the object cannot be used to differentiate them. The example of such colony is shown in Figure 6 (a), which is corresponding to example 4 in Table 1. The arrow in Figure 6 (b) points to an object which is considered as a colony of bacteria. This object is as small as the object of 12 pixels in the image whereas the object pointed by an arrow in Figure 7(b) (see arrow 2 in Table 1) is an object of 16 pixels which is larger than 12 pixels and is considered as noises. Thus such examples are the difficulty in analyzing the image of *Staphylococcus* bacteria.

However the threshold found by this method is a global threshold, so it alone is not sufficient to segment bacterial colonies and noises in staphylococcal image. The analysis of local neighboring pixels is required to solve this problem. Therefore, a method to detect edge of the object is applied to obtain the boundary information of the object. In this work, we select Canny edge detection method. The overview of this method and rationale for selecting this method is given in next subsection.

3.2 Canny Edge Detection

John Canny [13] proposed an algorithm to find edges of the objects in the images based on mathematical analysis of difference in order to get better results than previous methods such as Sobel and Prewit. In this paper, Canny edge detection was employed to detect the edges of the objects in the original greyscale image since it gave a thin edge which was suitable for finding edge on small objects. Moreover, the area covered by the edges of one object obtained from this method is approximately the same as the area covered by that object obtained from GW method.

Canny edge detection method starts from smoothing the greyscale image with Gaussian filter to reduce noises. Then, the gradient of magnitude and orientation are computed. The non-maximum value of pixels along the orientation of the gradient are suppressed to thinning the edges. Finally, the two threshold values, a pair of high and low value, are used to select and connect the edges. This is called Hysteresis thresholding.

The two threshold values, t_1 and t_2 , used in this paper are $t_1 = (0.4)T$ and $t_2 = T$ where T is a threshold value obtained by GW. Moreover, the value of sigma for Gaussian filter is set to 2 in order to reduce salt and pepper noise as suggested in reference [14]. The results are illustrated in Figure 8. The advantage of Canny edge detection method is that the very small intensity gradients, which normally corresponds to edges of noises, will be discarded. Therefore, some noises can be excluded from further analysis.

Moreover, the colonies usually have Canny edges lines form a closed contour even a very small one as shown in Figure 9. The arrow in Figure 9 (b) points to the resulting Canny edges of a very small colony in

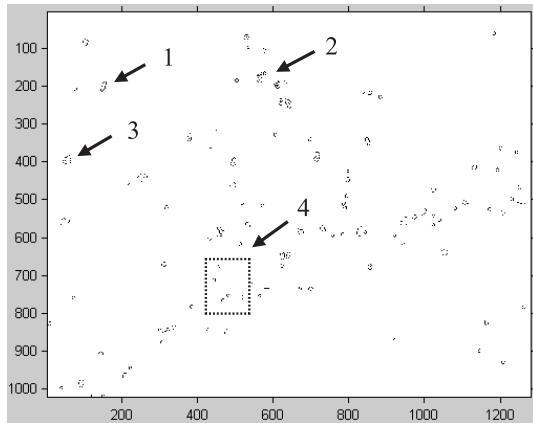


Figure 8 The Canny edges for the bacteria in Fig. 1.

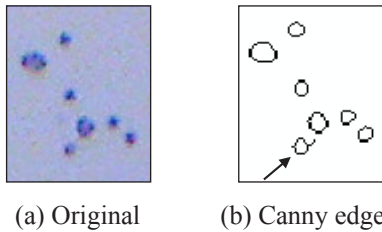
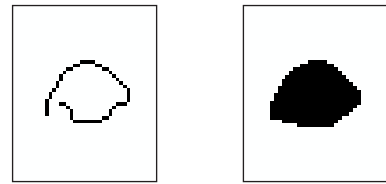


Figure 9 The resulting Canny edges of Fig. 6.

Figure 6 (b). Apparently, there is a little tail, so-called spur, pointed out from the closed contour of the Canny edges. This spur has an effect to the closed contour of Canny edges, but it does not affect to the size measurement of the colony. The effect of this spur will be further discussed.

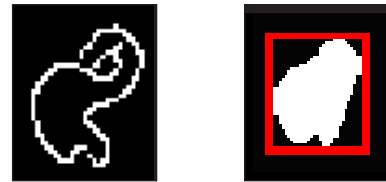
3.3 Mask

The area of the object obtained from Canny edge detection is called Mask in this paper. The Mask is applied to make an insight analysis of the object in order to differentiate between colony and noise. However, the Canny edges of the objects can be connected and form a curve shape. This Canny curve may not be a closed contour. To create Mask, the closed contour of the object has to be found. Thus the convex hull is used



(a) Canny curve (b) convex hull area

Figure 10 Mask of the Canny curve.



(a) Two Canny curves (b) The Mask

Figure 11 Mask of two Canny curves.



Figure 12 The length of the major and minor axis of the eclipse.

to find the area of Canny curve. Figure 10 (a) illustrated the Canny curve of the Image 3 in Table 1, whereas its convex hull area or Mask is shown in Figure 10 (b).

If there are two or more Canny curves that are closed together, their convex hull area might be overlapped. In this case, their convex hull areas will be union to create the Mask. The noise as displayed in Image 2 of Table 1, for instance, has two Canny curves as shown in Figure 11 (a) and its Mask is shown in Figure 11 (b).

The eclipse that has the value of the second central moment equals to that of each Mask will be found. After that, the length of Major and Minor axis of that eclipse can be obtained as shown in Figure 12.

These two lengths of the eclipse can be used as feature to distinguish between bacterial colony and noise. While the other attributes of the object such as angle of the axis are not significant attribute since they cannot be used to differentiate between colony and noise efficiently. Hence, the other attributes of the eclipse are omitted. Unfortunately, the features extracted from the eclipse are not enough to classify the object. Then the other features should be extracted. The additional feature used to classify the object in this work is described in the following subsection.

3.4 Number of Objects in Mask

The colony of bacteria obviously consists of one or more bacteria. Though there is more than one bacterium in a colony, the edge lines of the colony should be connected to form only one contour shape. Consider the Mask which is created from the union of Canny curves of the object, the Mask and the contour of the same object should fit together, in other word they should cover the same area. Therefore, number of contour shapes (N) in the Mask can be used to classify whether the object is a colony or not. The number of contour shapes in the Mask can be determined from the intersection between the Mask and the contour shapes of the object obtained from GW method at the same location of that particular Mask. For example, the intersection of the Mask in Figure 9 (b) and the contour shapes in Figure 6 (b) show that these objects are all colony since there is only one contour in each Mask (N=1) as can be seen in Figure 13.

Moreover, the intersection between the Mask in Figure 11 (b) and the contour shapes in Fig 7 (b) display that none of these objects can be considered as colony as demonstrates in Figure 14 since there is more than

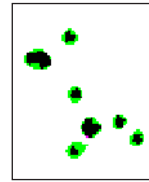
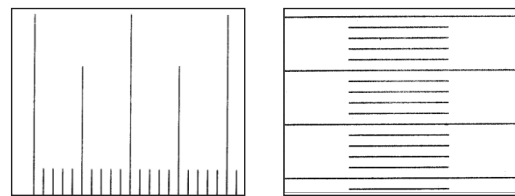


Figure 13 These objects can be classified as colony.



Figure 14 These objects cannot be classified as colony.



(a) horizontal axis (b) vertical axis

Figure 15 Stage Micrometer at 400X.

one object in one Mask (N=10).

The number of contour shapes (N) can therefore be used as a feature to distinguish between the colony and the noise.

3.5 Scaling with Stage Micrometer

The micrograph images of stage micrometer took at 400 and 1000 times of magnification (400X and 1000X), both in vertical and horizontal, were used to scale the bacterial size. Each smallest gap between the bar (Figure 15) is equal to 10 μm which is then converted into number of pixels. This conversion has been done to ensure that the calibration unit conversion from pixels to micron can be done correctly.

In order to measure the gap between each bar of stage micrometer, the projection of the image was carried out to get the average distance converting into total number of pixels in both Y and X axes. The method is displayed in Figure 16, the gap between each bar of stage micrometer has an average of 52.9250 pixels. In other word, the length of 1 pixel on the x-axis means 0.1889 micron on the average for the images taken at 400X.

Likewise, on the y-axis, 1 pixel equal to 0.16895 micron. The conversion at 1000X is also applied with the same procedure.

3.6 Colony's Size Measurement

To measure the size of staphylococcal cell/ colony, the distances of the bounding box placed over a cell image in x and y axes are defined. If the colony laying inclined from the axis with the $|\theta|$ angle of more than 1 degree (as shown in Figure 17), the size would be derived from comparison of the pixels obtaining from the bounding box before and after axes rotation. The lowest area of bounding box found is selected to convert into size (see Table 2).

Table 2 The size measure of colonies at 400X

	Image	Size (in pixels)		θ	Size (in pixels)	
		Original	Rotated		X	Y
1		x = 3 y = 5		90	0.57 (3)	0.84 (5)*
2		x = 15 y = 26	x = 30 y = 12	68.7	5.66 (30)	2.02 (12)
3		x = 19 y = 26	x = 28 y = 21	81.5	3.59 (19)	4.39 (26)

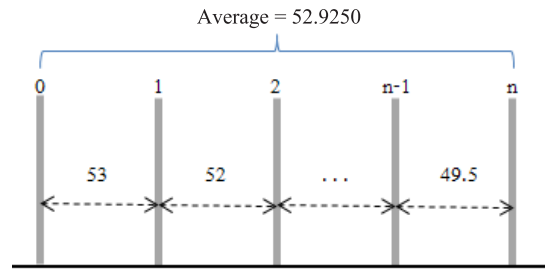


Figure 16 The average number of pixels in 1 μm .

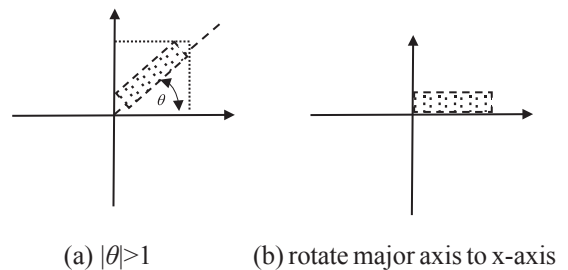


Figure 17 The rotation of major axis of the colony.

However, the size of the colony is equal to the smallest area of the bounding boxes. Table 2 shows the examples of the size measurement.

It is noted that on Image 1 in Table 2, the values of x and y are not rotated because θ is on the axis. They can be directly used in the measurement. Whereas in Image 2, the values of x and y used in the measurement are rotated by θ since it is not on the axis. The values of x and y in Image 3 are not rotated because the original bounding box is smaller than the rotated one.

4. Experimental Settings

4.1 Preparing Microscopic Images

Staphylococcal bacteria were grown overnight on Nutrient agar at 37°C. The colonies were then diluted in normal saline solution (0.85% NaCl). The suspended cells were smeared on a glass slide, heat-fixed and Gram's stained [3], [14]. The slide was examined under the light microscope which connected to the digital

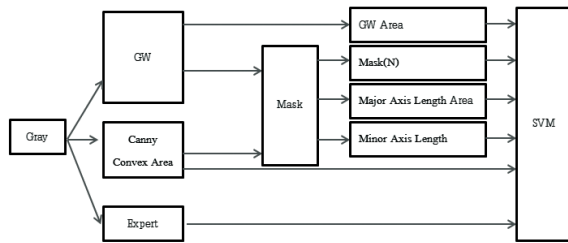


Figure 18 The features used in SVM Training.

camera. At least 10 microscopic fields were observed and the images of 400 and 1,000 times of magnification were saved into jpeg files.

4.2 Recognition of Bacterial Colonies

The microscopic images of *Staphylococcus* bacteria of size of 1024×1280 pixels were used in this present work. The images are taken at 400X and 1000X. There are 10 images of each magnification used in this work.

Each original image was transformed from Color image to grayscale image by using Grey-scale weighted threshold method (GW). Edges of each object were then obtained by using Canny edge detection. After the edges were detected, the features of the bacterial colonies can be extracted. These features are the convex area of GW, convex area of Canny, length of Major axis, length of Minor axis, and number of convex area of GW under the same Mask (N). They were used to train SVM. Additionally, there was more information in order to train SVM. It is the decision from the experts whether which object is colony or noises. Therefore, each set of features was classified as colony or noises based on the corresponding decision from the experts. The recognition framework for the SVM training part of this software is shown in Figure 18.

3	38	8.279	5.933	1	0
3	8	3.651	3.055	1	0
6	12	5.422	3.169	1	0
11	16	5.416	4.041	1	1
24	16	5.416	4.041	1	1
24	32	7.006	5.953	1	1
8	8	3.651	3.055	1	1

Figure 19 Example of sets of features with corresponding decisions from the experts used in SVM training.

The example of sets of features with corresponding decisions from the experts used to train SVM in this paper is shown in Figure 19. Each row consists of convex area of GW, convex area of Canny, length of Major axis, length of Minor axis, and number of convex area of GW under the same Mask (N) respectively.

4.4 Software Evaluations

The microscopic images of both 400X and 1,000X were manually counted with 3 experts. Those counts were compared with the count processed by the software developed from this work (Micros-Staph) and the other 2 freewares, i.e. CellC and ImageJ on the PC with Pentium® CPU Dual T2390 1.86 GHz at 2 MB RAM using Windows XP operating system. Correlation between manual and software counting was implemented, r^2 more than 0.95 was required in order to accept the accuracy of software. Percentage of difference was calculated from the subtracted value of the highest and lowest count of the same image divided by the highest number and multiplied by 100.

5. Results and Discussion

The results in Table 3 show the percentage of differences among 3 experts and between the expert and each software, when all images were enumerated.

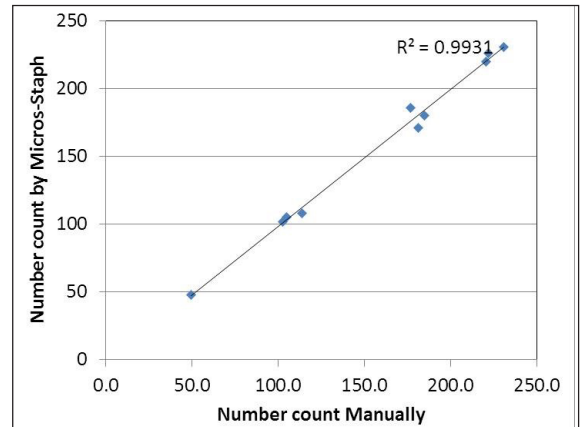
It was found that the differences among 3 experts were much higher than those obtained between the expert and Micros-Staph. Whereas the results of counting by CellC gave inaccurate numbers particularly at 1000X (99% difference). For ImageJ the percentage of difference was comparable to the differences among 3 experts. It was noticed that the different percentages were higher at higher magnification (1000X) than at lower magnification (400X) except that exhibited in Micros-Staph.

Table 3 Comparison of the differences in counting by experts and software expressed in percentage of average of 10 images

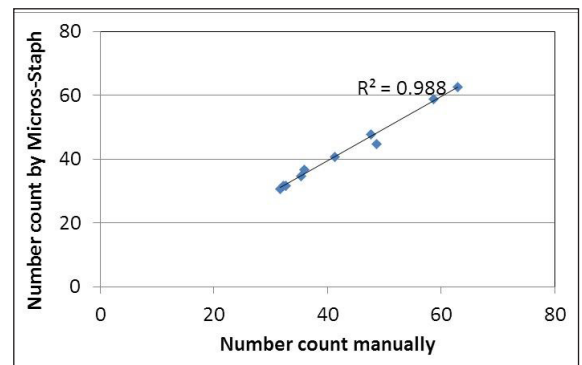
Comparison between	Percentage of difference of counting at	
	400 X	1000 X
3 Experts	7.0	10.3
Expert and Micros-Staph	2.4	1.8
Expert and CellC	19.2	99.6
Expert and ImageJ	5.8	11.2

The linear correlation of the average numbers count by 3 persons and the number count by Micros-Staph at 400X and 1000X display in Figure 20, the r^2 values were 0.99 and 0.98, respectively. This indicated that the enumerated numbers obtained by Micro-Staph was equal to those attained manually.

When consider the times required to operate the count of 10 images, the Micros-Staph software performed nearly half of the time needed by manual count (Table 4). In addition the lower the number of colonies in an image, the less of the time required in counting by Micro-Staph (100-240 colonies at 400X and 30-70 colonies at 1000X). In contrast the processing times of CellC and ImageJ softwares were not subject to the number of the colonies in each



(a)



(b)

Figure 20 The number of staphylococcal count by Micros-Staph software compared with the average number from manual counting from 3 experts at 400X (a) and 1000X (b).

image, since the process times were similar at both magnifications.

Table 4 Time used in counting process of 10 images by men and three softwares

	Processing time (sec.) used in counting at	
	400 X	1000 X
Average of 3 experts	574	124
Micros-Staph	230	72
CellC	211	210
ImageJ	223	216

Size measurement can also be obtained by Micros-Staph, the software provided the diameter of coccal cells by interpreting an average of the pixels of a cell from two axis. The results of the cell sizes show in Table 5 related to the size-range of Staphylococcus which was 0.5-1.5 μm in diameter [1].

Table 5 Size measurement of single cell staphylococci performed by Micros-Staph

400 X	Pixel obtained	Cell size (μm) in diameter
Image 1	x = 0.57, y = 0.84	0.70
Image 2	x = 0.56, y = 0.50	0.53
Image 3	x = 0.75, y = 0.67	0.71
Image 4	x = 0.94, y = 0.84	0.89
Image 5	x = 0.94, y = 0.84	0.89
1000 X		
Image 1	x = 0.40, y = 0.81	0.60
Image 2	x = 0.50, y = 1.01	0.75
Image 3	x = 0.61, y = 0.81	0.71
Image 4	x = 0.50, y = 1.01	0.75
Image 5	x = 0.71, y = 1.01	0.86

When compare the functions of the softwares used in this work, Micros-Staph had two distinct advantages over the other two softwares as summarized in Table 6. These are the efficiency of reducing noise and accuracy in counting of microscopic images of staphylococci.

Table 6 Comparison of softwares upon their functions

	Software		
	Micros-Staph	CellC	ImageJ
Can obtain microscopic count of staphylococci	Yes	No	Yes
Can measure the cell size	Yes	No	Yes
Number of steps require to process counting	Few	Few	Many
Display results in grey scale, so the correctness of count can be followed	Yes	Yes	Yes
Capable of reducing noise	High	Low	Must be adjusted manually
Counts are accurate	Very	No	Fairly

6. Conclusions

The Micro-Staph software has been developed to aid microscopic counting of staphylococcal bacteria as well as estimating the size of the cell. Although “CellC” and “ImageJ” are two well-known freeware for aiding microscopic counting, their performances on staphylococcal bacteria, which has very small size, are unacceptable. Regardless of the aforementioned software, our software was designed to incorporate global and local analysis to classify whether the object is a colony of bacteria or noise. The grey-scale weight threshold method was used to determine a global threshold to separate foreground and background. The convex area of each foreground object was found. As a local analysis, Canny edge detection was used to find the edges of the objects which later be union into a convex hull area called Mask. The lengths of major and minor axis of Mask were determined. Moreover, the convex hull area of each foreground object must be intersected with the Mask at the same location to determine the number of objects in this area (N). Finally, these findings were used to train SVM to classify that which foreground object is colony of bacteria. The evaluation results confirmed that the propose software, Micros-Staph, is applicable as it required half of time in processing the count and gave accurate number comparable to the human counting.

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