Counting of Bacterial Colonies of the Low Quality Image Using Perona-Malik Diffusion Filters and Image Morphology Operators

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Abstract. Microbiology has an important role in solving human problems that are complex in many fields, such as environment, medical, and biotechnology. One of the important researches in microbiology fields is the counting of the number of bacterial colonies. This research is very important to estimate the number of bacterial cells in each millilitre or gram sample. However, the manual counting of bacterial colonies is a time-consuming task and is also very tedious. To help microbiologists, an automatic counting method of bacterial colonies based on bacterial colonies images is needed to be developed. However, the bacterial colonies images taking by the phones camera has low-quality. They are noise presence and lack of contrast, so the pre-processing method is necessary to be developed. This paper proposes the pre-processing method by using modified Perona-Malik Diffusion (PMD) and Contrast Limited Adaptive Histogram Equalization (CLAHE). The modified PMD filter is used to reduce noise, while the CLAHE method is used to enhance the contrast. Moreover, the morphology operators are used for the petri dish extraction and bacterial colonies counting process. The experimental results show that the proposed method has better performance than the original PMD based on the average values of precision, recall, and F-measure.

1. Introduction
Microbiology is the study of microbes, such as bacteria, viruses, fungi, algae, archaea, and protozoa. One of the important researches in microbiology fields is the counting of the number of bacterial colonies. Several the counting methods of bacterial colonies have been proposed [1], [2], [3], [4]. This method is used to estimate the number of bacteria cells in each milliliter or gram sample. In particular, the counting method of bacterial colonies in the medical field has been applied for estimating the level of infection experienced by a patient [3]. The manual counting of bacterial colonies on a petri dish is a time-consuming task [3] and is also very tedious [5]. However, this task must be done by a microbiologist as the first step in a study.

Image processing is an alternative solution to solve the problem of counting bacterial colonies. There are a lot of researches about counting the number of colonies using image processing techniques. All the previous methods use high-quality image samples for counting
microorganisms and colonies. To take the high-quality image samples, they need a special camera. The special camera is very expensive. For this reason, this paper uses images taken by phone cameras, though the phone cameras some times results in the low-quality images. The counting method of bacterial colonies with low-quality images using image processing results in the low level of accuracy, since it dues to the presence of noise and the low contrast level of an image. Therefore, pre-processing techniques are needed to improve image quality so that the features of images can be extracted successfully [6].

To reduce the noise, the smoothing filters are necessary to be used. Some smoothing filters methods have been proposed, such as the mean filter, median filter, Perona-Malik Diffusion (PMD) filter, etc. The PMD filter has advantages compared with the mean and median filter. However, the diffusion coefficient on this method is very sensitive that is called contrast parameter or gradient threshold parameter [8]. furthermore, determining the suitable gradient threshold (k) parameter value for each image input is not easily determined manually [7]. For this reason, this paper proposed the PMD filter with Sobel mask method to overcome this problem. Sobel mask method is used to determine the gradient threshold parameter of an image.

To optimize the performance proposed method, the Contrast Limited Adaptive Histogram Equalization (CLAHE) method is applied to enhance the contrast of an image. The CLAHE is a modification of Adaptive Histogram Equalization (AHE) [9]. The advantage of this method is the contrast enhancement that carried out on sub-regions in an image. The regions in images with uneven lighting distribution are increased in each sub-region specifically until the lighting distribution is uniform [10].

After enhancing the contrast of an image, the bacterial colonies pixel has a brighter contrast compared with the pixel background. The top-hat filtering method can be used to extract the bright contrast features [11]. For this reason, the top-hat filtering method is used to extract the bacterial colonies.

Based on this background, this paper proposes the automatic counting method of bacterial colonies of the low-quality image by using the PMD Filters and the image morphology operators. To enhance the images, the PMD filter and CLAHE method are used. Moreover, the morphology operators are used for the petri dish extraction and bacterial colonies counting process. This proposed method will save time and help microbiologists. This method needs a low fund in term of counting bacterial colonies since the image samples use a mini studio and phones camera.

2. Proposed method
The proposed method in this study is developed by taking the advantages of several methods. The first method is PMD filter which is used to reduce noise by determining the gradient magnitude using Sobel mask. The next method is CLAHE to enhance the contrast of an image. The other methods are the Otsu’s method, filling holes, top-hat filtering, and bwlabel function on the labelling connected component operator. In several steps of the proposed method are petri dish extraction, pre-processing, bacterial colonies extraction and bacterial colonies counting. These steps are shown in figure 1.

2.1. Input image
Bacterial colonies samples used as input image in this study are acetic acid bacteria. Growth media used is colored and non-transparent. The bacterial colonies produce clear zones as shown in figure 2. The sampling uses a simple tool called the mini-studio and cellphone camera, where students generally use this tool because it is more efficient and practical. The mini-studio as shown in figure 3(a) has lighting from the LED lights as shown in figure 3(b) where the lights use energy from a power bank or USB charger adapter. The image extension, resolution, and bit depth of image samples are JPG, 400 dpi, and 24.
2.2. Petri dish extraction
Extraction on the Petri dish is carried out to separate the Petri dish from the background in order to avoid detection of objects that are not needed. The first step, the image of RGB figure 2 is converted into a grayscale image as shown in figure 4(a). The grayscale image is needed for the next step which is to convert the RGB image into a binary image using Otsu’s method as shown in figure 4(b). To avoid the results as shown in figure 4(b), the filling holes operation is carried out as shown in figure 4(c). The binary image (figure 4(c)) is used as a mask to extract the Petri dishes containing bacterial colonies, the results are shown in figure 4(d). After this process, the next step is to do the pre-processing technique.

2.3. Preprocessing
To increase the accuracy of the counting of bacterial colonies, the noise reduction method and the contrast enhancement method are needed to be used for increasing the quality of image.
The first step in this technique is to reduce noise where the method used is the Perona-Malik Diffusion (PMD) filter, then the next step is to increase the contrast of the sample image used using Contrast Limited Adaptive Histogram Equalization (CLAHE).

2.3.1. Perona-Malik Diffusion (PMD) Filter. Figure 5 is a figure 4(d) that is zoomed in. If the image is considered carefully, there are still spots that are known as noise. Therefore, PMD filters need to be applied. PMD filter is a reducing noise method in images that use the diffusion process. Diffusion on noise reduction is done by spreading the intensity of a pixel that has a
Figure 6. Sobel mask kernel

The diffusion equation has a form as follows:

\[
\begin{align*}
\frac{\partial I_t(x, y)}{\partial t} &= \text{div} \left\{ c_t(x, y) \cdot \nabla I_t(x, y) \right\} \\
I_{t=0} &= I_0
\end{align*}
\]

where \( I_t \) is an image on \( t \)-iteration, \( \text{div} \) is divergence operator, \( \nabla I_t(x, y) \) is an image gradient on \( t \)-iteration, and \( c_t(x, y) \) is the diffusion coefficient on \( t \)-iteration. Diffusion coefficient is defined as a function of image gradient \( \nabla I_t(x, y) \) by Perona and Malik so that equation (1) becomes a nonlinear diffusion equation also known as the anisotropic diffusion model. The coefficient is defined as follows:

\[
c_t(x, y) = g(||\nabla I_t(x, y)||)
\]

\[
g(||\nabla I||) = \frac{1}{1 + \left( \frac{||\nabla I||}{k} \right)^2}
\]

Different parameter values of \( k \) cause different diffusion effects on an image. The smoothing effect is less and the edges are preserved when \( ||\nabla I|| > k \), and the smoothing effect is greater and the diffusion coefficient is bigger when \( ||\nabla I|| < k \). in other words, \( k \) is a contrast parameter or gradient threshold [8].

2.3.2. Sobel mask. Sobel mask is proposed to get the suitable value of the parameter \( k \) automatically. The gradient threshold is approached based on the result of the \( G_x \) and \( G_y \) that are horizontal and vertical direction of image gradient. \( G_x \) and \( G_y \) are defined as follows:

\[
\begin{align*}
G_x &= f(x + 1, y) - f(x, y) \\
G_y &= f(x, y + 1) - f(x, y)
\end{align*}
\]

Gradient directions on equation (4) are applied on \( 3 \times 3 \) kernel (figure 6), then an approach from horizontal and vertical gradients is obtained as follows:

\[
\begin{align*}
G_x &= -f(x - 1, y - 1) + f(x + 1, y - 1) - 2f(x - 1, y) + 2f(x + 1, y) \\
    &\quad - f(x - 1, y + 1) + f(x + 1, y + 1) \\
G_y &= -f(x - 1, y - 1) + f(x - 1, y + 1) - 2f(x, y - 1) + 2f(x, y + 1) \\
    &\quad - f(x + 1, y - 1) + f(x + 1, y + 1)
\end{align*}
\]
The formula for obtaining the gradient threshold value \( k \) is shown as follows:

\[
k = \sqrt{F_x^2 + F_y^2},
\]

where,

\[
F_x = \max\{|G_x(i, j)|, \ i = 1, 2, 3, ..., M, \ j = 1, 2, 3, ..., N \}
\]

\[
F_y = \max\{|G_y(i, j)|, \ i = 1, 2, 3, ..., M, \ j = 1, 2, 3, ..., N \}
\]

Then to obtain the right gradient threshold value, the Sobel mask is applied to the center of the colony image as shown in figure 7(a). The result of noise reduction is shown in figure 7(b).

2.3.3. Contrast Limited Adaptive Histogram Equalization (CLAHE). If the figure 7(b) is zoomed in as shown as figure 8, the contrast of the image is still lacking so the image requires the CLAHE method to increase the contrast. CLAHE is proposed by S.M. Pizer where the histogram equalization is applied to a contextual region. The center of the contextual region contains each pixel of the original image. The difference of Histogram Equalization (HE) and CLAHE is that there is a maximum high limit value of a histogram called clip limit. The original histogram of an image is clipped based on the clip limit obtained and the clipped pixels are distributed to each gray-level so that CLAHE can limit the noise increasing. The application of this method is illustrated in the following figure:

The steps of the CLAHE method that is used to enhance the original image are shown as below:

Step 1: The original intensity image is divided into non-overlapping contextual regions. The total number of image tiles is the same as \( M \times N \), and a good value for maintaining image chromatic data of the total number of image tiles is \( 8 \times 8 \).

Step 2: The histogram of each contextual region is calculated based on the gray level in the array image.

Step 3: The contrast-limited histogram of the contextual region is calculated by the clip limit.
value as shown in the following equation:

\[ N_{\text{avg}} = \frac{N_{rX} \times N_{rY}}{N_{\text{gray}}}, \] (7)

where \( N_{\text{avg}} \) is the average number of pixels, \( N_{rX} \) and \( N_{rY} \) are the number of pixel in the x and y dimensions respectively, and \( N_{\text{gray}} \) is the number of gray levels from the contextual region.

The actual clip limit that is defined as \( N_{\text{CL}} \) can be written as:

\[ N_{\text{CL}} = N_{\text{clip}} \times N_{\text{avg}} \] (8)

where \( N_{\text{clip}} \) is the normalized clip limit in the range of \([0, 1]\). The pixels will be clipped if the number of pixels is greater than \( N_{\text{CL}} \). The total number of clipped pixels is defined as \( N_{\Sigma_{\text{clip}}} \), then the average of the remain pixels that will be distributed to each gray level uses the following formula:

\[ N_{\text{avggray}} = \frac{N_{\Sigma_{\text{clip}}}}{N_{\text{gray}}} \] (9)

The following statements are the rule of the histogram clipping

\[
\begin{align*}
\text{If } & H_{\text{reg}}(i) > N_{\text{CL}} \text{ then } \\
& H_{\text{reg,clip}}(i) = N_{\text{CL}} \\
\text{Else if } & (H_{\text{reg}}(i) + N_{\text{avggray}}) > N_{\text{CL}} \text{ then } \\
& H_{\text{reg,clip}}(i) = N_{\text{CL}} \\
\text{Else } & H_{\text{reg,clip}}(i) = H_{\text{reg}}(i) + N_{\text{CL}}
\end{align*}
\] (10)

where \( H_{\text{reg}}(i) \) and \( H_{\text{reg,clip}}(i) \) are the original histogram and the clipped histogram of each region at \( i \)-th gray level.

Step 4: the remaining pixels are redistributed until all the remaining pixels have been all distributed. the step of the redistributed pixels is given by:

\[ \text{Step} = \frac{N_{\text{gray}}}{N_R} \] (11)

where, \( \text{Step} \) is the positive integer at least 1 and \( N_R \) is the remaining number of clipped pixels.

The algorithm starts search from the minimum to the maximum of gray level with based the above step. If the number of pixels in the gray level is less than \( N_{\text{CL}} \), a pixel will be distributed to the gray level. If the pixels are not all distributed, the new step will be calculated according to the equation (11) and the new search is began until all remaining pixels distributed.
Step 5: Intensity values in each region is enhanced by Rayleigh transform. The clipped histogram is transformed to cumulative probability, $P_{\text{input}}(i)$, that is provided to create transfer function. The Rayleigh transform that is given in equation (12)

$$y(i) = y_{\text{min}} + \sqrt{2\alpha^2 \ln \left( \frac{1}{1 - P_{\text{input}}(i)} \right)}$$

where $y_{\text{min}}$ is the lower bound of the pixel value and $\alpha$ is a scaling parameter of Rayleigh distribution that is defined depending on each input image. The output probability density of each intensity value can be written as following:

$$p(y(i)) = \frac{(y(i) - y_{\text{min}})}{\alpha^2} \cdot \exp \left( -\frac{(y(i) - y_{\text{min}})^2}{2\alpha^2} \right) \quad y(i) \geq y_{\text{min}}$$

(13)

A higher $\alpha$ value will result in a more significant enhancement in contrast of the image, meanwhile increasing the saturation value and amplification of the noise level.

Step 6: The suddenly changing effect is reduced. The output from equation (13) is re-scaled using linear contrast stretch that can be expressed as:

$$y(i) = \frac{x(i) - x_{\text{min}}}{x_{\text{max}} - x_{\text{min}}}$$

(14)

where $x(i)$, $x_{\text{max}}$, and $x_{\text{min}}$ are denoted as the input value, maximum value and minimum value of the transfer function.

Step 7: The assignment of new gray levels of pixels in sub-matrix contextual region is calculated using bi-linear interpolation between four different mappings to eliminate artifacts [13].

The contrast enhancement of figure 7(b) that is shown in figure 10 using the CLAHE method shows that the light intensity between the growth media (background) and the bacterial colonies (objects) becomes very clear so that the counting accuracy of the proposed method becomes better.

2.4. Bacterial colonies extraction

Bacterial colonies extraction in figure 10 is carried out using the top-hat filtering method, where this method extracts bright objects. The formula of top-hat filtering is given in equation (15):

$$T_{\text{hat}}(f) = f - (f \circ B)$$

(15)

where $f$ is the grayscale image, $B$ is the structuring element, and the operation $\circ$ is the opening operation.
The result of the top-hat filtering method is shown in figure 11. The next step is to binarization figure 11 using Otsus method, the result is shown in figure 12(a). In the binary results, there are several objects that are not bacterial colonies, such as the edge of the dish. In addition, there are also a few small invisible objects. By utilizing the connected component labeling operation, the object can be detected as shown in figure 12(b) and 12(c). Unwanted objects are then removed from figure 12(a) so that figure 12(d) is obtained.

2.5. Bacterial Colonies Counting

Bacterial colonies counting that is done on figure 12(d) uses bwlabel that is one of the functions on labeling connected components. The result of bwlabel show total number of bacterial colonies on a Petri dish.

3. Result and discussion

The performance evaluation of the proposed method in the counting of bacterial colonies is done by counting the level of detection accuracy. The detection accuracy of bacterial colonies [2] is based on comparisons with observations made by microbiologists. The measure of accuracy used is the F-measure which is a combination of precision and recall. Precision is a measure of speed known as a positive predictive value, whereas recall is a measure of completeness, also known as sensitivity. Precision and recall are formulated as follows:

\[
\text{Precision} = \frac{\text{number of colonies taken}}{\text{total number of objects taken}} = \frac{\text{true positive}}{\text{true positive + false positive}}
\]
Recall = \frac{\text{number of colonies taken}}{\text{total number of existing colonies}} = \frac{\text{true positive}}{\text{true positive} + \text{false negative}} \tag{17}

where a colony is denoted as true and not a colony denoted as false. In this proposed method, objects identified as colonies are denoted as positive and objects identified as non-colonies are denoted as negative. Based on this, there are 4 possibilities that can occur, namely true positive (the correct result) is the number of objects whose colonies are identified as colonies, false positive (undesirable results) is the number of objects that are actually not colonies identified as colonies, false negative (wrong results) is the number of objects that are actually identified as non-colonies, and true negative (correct absence) is the number of non-colony objects identified as not colonies. Based on equation (16) and (17), so the formula of F-measure is given as follows:

\[ F - \text{measure} = 2 \cdot \frac{\text{precision} \cdot \text{recall}}{\text{precision} + \text{recall}} \tag{18} \]

**Table 1.** Accuracy of the modified PMD and original PMD method

|                  | The modified PMD | The original PMD |
|------------------|------------------|------------------|
|                  | Precision | Recall | F-Measure | Precision | Recall | F-Measure |
| Sample a         | 0.902     | 0.984   | 0.941     | 0.844     | 0.984   | 0.908     |
| Sample b         | 0.835     | 0.953   | 0.890     | 0.813     | 0.953   | 0.877     |
| Sample c         | 0.952     | 0.963   | 0.958     | 0.963     | 0.941   | 0.952     |
| Average          | 0.896     | 0.966   | 0.929     | 0.873     | 0.959   | 0.912     |

Accuracy detection of the proposed method uses 3 image samples, with the results of accuracy shown in Table 1. The average detection accuracy of the proposed method is almost close to the
maximum value of accuracy in equation (16), (17), and (18) that is 1.00. The results of precision and recall respectively are influenced by the number of false positives and false negatives. In this study, it is due to the LED light reflection from the mini-studio on the surface of the petri dish which caused the reflection of the light to be extracted as a colony. Moreover, there are colonies whose colors were similar to growth media are not extracted as colonies. The image samples and extraction results of each sample are shown in figure 13.

4. Conclusions
This study proposes a method of automatic bacterial colonies counting for low-quality image. In this proposed method, the PMD filter is modified, where the gradient threshold of each image is determined by using a Sobel mask so that the noise reduction on image samples that contain noise becomes maximal. From the experimental results, the proposed method has a good performance than the original PMD method. The accuracy of the proposed method based on the value of precision, recall and F-measure is better than the accuracy of the original PMD method.

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