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Gradient-Based Computational Approach for Identification and Counting of Microorganisms in Light Microscopic Images

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Abstract: Microbial colony enumeration is an essential process to determine the concentration of microbes present in the sample. Colony counts are used to detect and count the microbes. There might exist hundreds or thousands of colonies and counting is often performed manually by well-trained technicians. There is some inherit human error and a lot of time involved in counting colonies manually. Therefore, it is necessary to develop an automated colony identification and counting methods. In this paper, Gradient-based computational approach for automatic identification and counting of microorganisms in Light Microscopic Images is proposed. Prior to the counting process, this proposed method removes noise present in these images, then the gradient edges are obtained. We apply region labelling algorithm for automatic counting. The colony count value obtained by this proposed method is compared with the manual count value and the count value of the existing method.

Key words: Colony counting · Microbial images · Gradient edge · Light microscopic image

INTRODUCTION

Microbiology is the scientific study of microorganisms. Microbes are single-celled organism, they exist in the free living or symbiotically associations with plants and animals as well as in the human body [1]. Fungi is a lower from of eukaryotic microorganism, exist in the form of single-celled or multi-celled and obtain their food by either decomposing dead organisms or by living as parasites on higher organism. Protozoa are another lower form of eukaryotic single-celled organism belonging to animal kingdom. They come in many different shapes and sizes ranging from an Amoeba which can change its shape to Paramecium with its fixed shape and complex structure. Algae are lower form of photosynthetic organism and exist as single celled or multi-celled. All algae reproduce asexually and are abundant in fresh water, salt water, soil and have symbiotic association with some plants. Viruses are smallest of the microbes, they are living or non-living organism and mostly they are pathogenic.Microbial image can be obtained from various imaging techniques, such as Light Microscopes Image (LMI), Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and Confocal Microscope (CM). The Light microscope image uses light and lenses to magnify the specimen. Light microscopy helps to identify different kind of bacteria [2]. The various type of light microscopy includes bright-field microscopy, dark-field microscopy, phase contrast microscopy and fluorescence microscopy. Each method has specific applications and advantages, but the most commonly used in classes and clinical laboratories is bright-field microscopy. Bright-field microscopy produces an image from light that is transmitted through a specimen. The specimen restricts light transmission and appears “shad owy” where light enters the microscope unimpeded. The dark-field microscopy has the contrast between internal components even without stain. A special condenser is used so that only light reflected off the specimen from a dark contrast field background and with the better resolution that of the bright field microscope. Phase contrast microscopy uses special optical components to exploit subtle difference in the refractive indices of water and cytoplasmic components to produce contrast.

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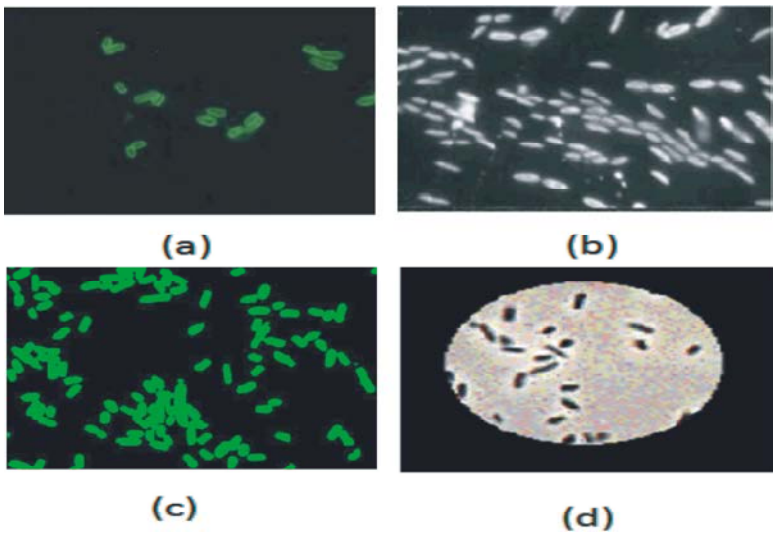

Fig. 1: Types of Light Microscopy images; (a) Bright filed (b) Dark field (c) Fluorescence (d) Phase contrast

As a result the specimen appears as various levels of darks against bright background. Fluorescence microscopy uses a fluorescent dye that emits fluorescence when illuminated with ultraviolet light. In Some cases, specimen posses naturally fluorescence chemicals and no dye is needed. Some example images of Light microscopy are shown in Figure 1.

Identification and manual counting of microbes in these microbial images are very difficult and error prone.Therefore an automated method is needed for identification and counting [3]. Some existing methods are available for automatic segmentation and counting, Zhang *et al* [4] proposed an automatic colony counting method which uses image processing techniques based on the RGB color theory to count white bacterial colonies in clear plate medium. This method produced accurate result even for the images having varying shape and size. A fully automatic and cost efficient method for counting microbes in color and clear medium is proposed in [5]. This method is a software center and the counting process includes detecting spirals regions, identifying colonies, separating aggregated colonies and reporting colony counts.An edge-based method to segment the bacterial images is proposed in [6]. This method uses canny edge detection on the bit-plane sliced images and combined the edge image of the high order bit plane images to produce final output.Automation of colony counting has been of increasing interest for many decades, and these methods has shown to be more consistent than manual counting.

It is also found that automated colony counts had significantly less variation when reanalyzing plates than those manually determined by individual or multiple observers [7]. The existing colony counting devices were then developed and commercialized in the market [8]. Some automatic counters [9] still require users to manually specify the plate/dish area and provide parameters prior to the actual enumeration process. Some may need operators to adjust the threshold values in order to handle dishes/plates/medium that differ from their default settings. In such cases, human operators are heavily involved in the operation, and thus it is not efficient for high throughput processing of plates/dishes in a batch.Greenspan *et al*. [10] proposed an automatic method based on statistical computation for identifying the bacteria. Bacteria cell classification using data mining techniques is employed for the classification of HE_p-2 cells in [11], which uses a simple set of shape features for the classification.

Thomas Posch *et al*. [12] developed a new image analysis tool to study biomass and morphotypes of three major bacterioplankton groups in an alpine lake based on geometric features, and the counting is performed on the edge image.Shen Wei-zheng and WU Ya-chun [13] developed a new automatic colony counting system, which makes use of image processing technology to feasibly count white bacterial colonies in clear plates according to the RGB color theory. It has been proved that the method greatly improves the accuracy and efficiency of the colony counting and the counting result is not affected by the shape or size of the colony.

Kalavathi and Naganandhini [14] proposed a hybrid approach for automatic counting of microorganism based on morphological operation, Chan-Vese (CV) active contour and labelling method. The colony enumeration

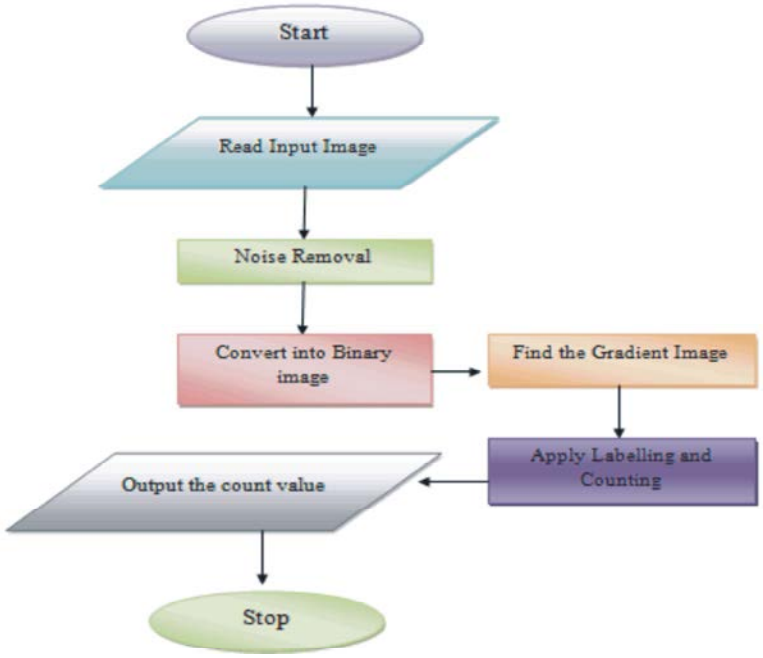

Fig. 2: Flowchart of the proposed method

device poses a significant challenge to many laboratories to perform huge amount of enumeration task, therefore a simplified method to automatically count bacterial colony counting unit is developed by Putman *et al*, [15].

Gurpreet karur *et al*. [16] proposed a simple and cost efficient methodology for automatically counting the bacterial colonies based on digital image processing techniques and it was tested with different types of filter images. It is observed that the results obtained with the proposed counter were not significantly different from the manual counting. A method proposed by Michael Putman *et al*. [17] proved that, it is not necessary to use costly hardware and imaging system to collect the images of bacterial colonies. With the development of cameras and document scanners, images of bacterial colonies can be obtained easily.In this paper, we proposed a method for automatic identification and counting of microorganisms in Light Microscopic images. This method uses Gradient edges to detect the microbes. The remaining part of the paper is organized as follows: In section 2, the methodological detail of the proposed method is given; the results and discussion are given in Section 3 and the conclusion is given in Section 4.

Methods: A Gradient-based automatic method to identify and count the microbes present in the Light Microscopic images is presented in this paper. The overall flowchart of this method is shown in Figure 2.

This method use noise-removal as a preprocessing step, since input image may have variations in brightness and contrast, this may affect the counting process. Because high levels of noises are always undesirable and hence noise removal has to be employed before applying further analysis [18-19]. A typical image noise is a Gaussian noise and it is independent of the signal intensity and it is also independent at each pixel. Some noise can also increase the appearance and sharpness of an image [20]. Moreover, the gradient-based method is sensitive to image noise. Therefore, in this method we removed the image noise using adaptive wiener filter [21]. Wiener filter is a type of linear filter based on local image variance. If the variance is large,it performs little smoothing, otherwise it performs more smoothing in the image. This approach often produces better result than the other linear filters. Once the noise is removed in the input image, we apply thersholding technique to obtain binary image. The thersholding process computes a threshold value and segment the image into background and foreground based on the computed threshold value. We use Otsu's [22] thersholding method for automatic computation of threshold value to obtain binary image.Edge detection refers to the identifying and locating sharp discontinuities in an image. Edge detection is one of the most frequently used techniques in digital image processing [23]. The gradient method detects the edge by looking for the maximum and minimum in the first

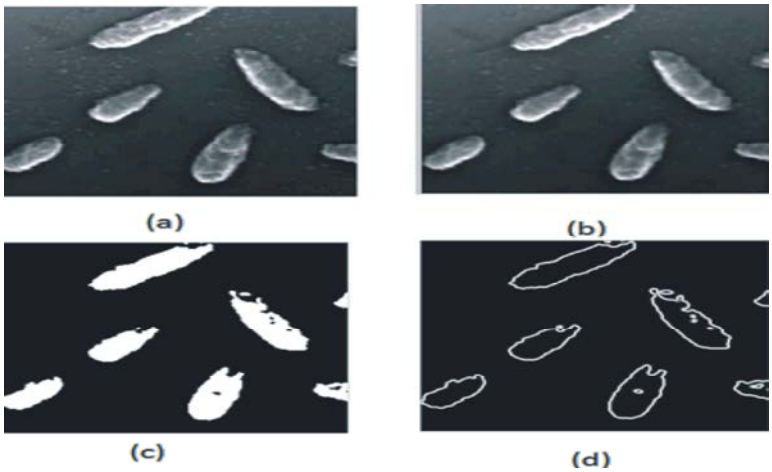

Fig. 3: Segmentation process by the proposed method; (a) Original image (b) Noise removed image (c) Obtained binary image (d) Gradient edge image

derivative of the image. The magnitude of the gradient is the most powerful technique that forms the basis of various approaches such as edge detection, image sharpening etc [24]. The gradient vector point in the direction of maximum rate of change for a function f(x,y), the magnitude of the gradient of f at the coordinate (x,y) is defined as:

$$\nabla f = \begin{bmatrix} G_x \\ G_y \end{bmatrix} = \begin{bmatrix} \partial f/\partial x \\ \partial f/\partial y \end{bmatrix} \quad (1)$$

In edge detection, an important quantity is the magnitude of this vector and is given by:

$$|\nabla f| = \sqrt{G_x^{\,2} + G_y^{\,2}} \quad (2)$$

The gradients takes it's maximum rate of increase of $f(x, y)$ per unit distance in the direction of ∇f. The gradient magnitude is commonly approximated by:

$$|\nabla f| = |G_x| + |G_y| \quad (3)$$

The direction of the gradient vector is also important and is given by:

$$\alpha(x, y) = \tan^{-1}\left[\frac{G_x}{G_y}\right] \quad (4)$$

where, $[G_x, G_y]$ returns the directions gradients, G_x and G_y is same size as the input image. When applying the gradient operator at the boundaries of the image, the values outside the boundary of the image are assumed to be equal to the nearest image border value.After identifying the edges, labelling process is performed to group the image pixels into regions based on 4-connected neighbourhood [25]. In 4-connected neighbourhood, the pixels are connected to the center pixel in its four neighbours left, right, top and bottom. After identification of the connected regions, we apply the region labelling algorithm [26] to assign a label number to each region for counting the number of individual microbes present in the microscopic images. The Figure 3 illustrates the process of segmenting the microscopic images by the proposed method.In this Figure, Image 3(a) is the original image. The noise removed image is given in Figure 3(b) and the binary image is given in Figure 3(c). The gradient edge image is shown in Figure 3(d). Summary of the steps involved in the proposed method is given in Algorithm 1.

Algorithm 1: Summary of the Steps involved in the proposed method

- Read input image.
- Remove the noise present in the input image.
- Obtain the binary image.
- Find the Gradient edge image.
- Apply labelling Process for counting regions.
- Output the final count value.

RESULTS AND DISCUSSION

To evaluate the performance of the proposed method, we have tested our method with the light microscopic images obtained from the internet. A sample images along with the results obtained by the proposed method are shown in Figure 4. The computed count value by the proposed, existing and manual methods is given in Table 1.

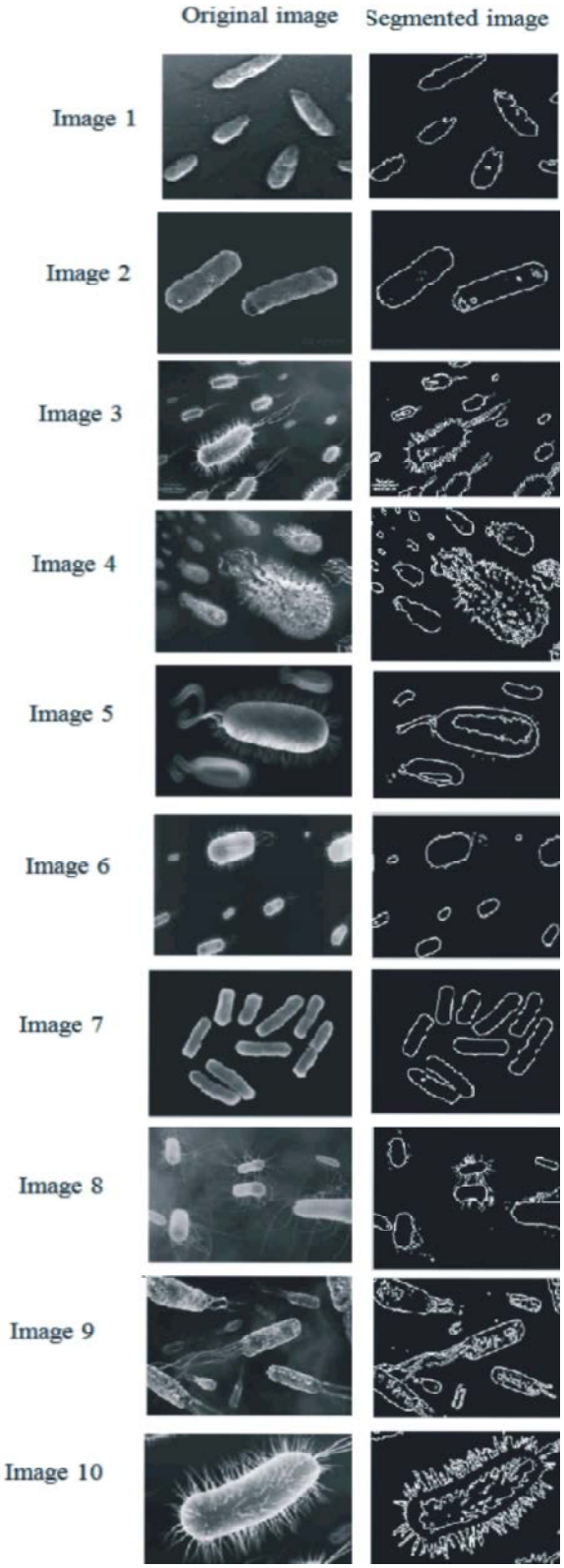

Fig. 4: Segmented image by the proposed method; (a) Original image (b) Segmented image

Table 1: Microbial count obtained by Manual Count, Existing Method and Proposed Method

	Manual count	Count value by the Existing method [14]	Proposed method
Image 1	7	8	7
Image 2	2	2	2
Image 3	14	10	13
Image 4	16	12	16
Image 5	3	4	5
Image 6	8	7	8
Image 7	9	8	8
Image 8	7	5	7
Image 9	8	10	8
Image 10	2	2	2

It is evident from Figure 3 and Table-1 that, this proposed method is one of the simple and efficient methods for automatic counting of microbes in light microscopic images. We have also given the manual count for the respective image for quantitative evaluation and comparison of the proposed method with the existing and manual count value. This proposed method produced accurate counting measures for Image1, Image 2, Image 4, Image 6, Image 8, Image 9 and Image 10 as shown in Figure 3. For other image, it fails to produce the accurate count value. Whereas,the existing method except for Image 2 and Image 10, for other images it has produced incorrect count value. Because the existing method is an active contour based method, active contour are always best suited for identifying the boundary of larger and disconnected object. But this proposed method finds gradient edge which could efficiently disconnect the overlapped microbes and thus produced correct count value for most the tested images.

CONCLUSIONS

The proposed approach is a simple and efficient method for automatic segmentation and counting of microorganism present in the microscopic images. The experimental results are compared with the manual count and also with the existing method. This proposed method has produced accurate result than the existing method on the entire tested image. However in this method we used only the light microscopy images, in future it maybe tested for other types of image modalities.

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