Counting of Micro-Organisms for Medical Diagnosis using Image Processing Method

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Abstract

Recognizing and counting the total number of microorganisms in a particular sample is one of the main task that is performed in biological research and diagnosis field. Usually the procedure for counting the total number of microorganisms is done manually which makes this task more tedious, lengthy as well as error prone. To overcome these drawbacks various algorithms have been presented in this paper that aims to automatically count the total number of microorganisms present in a sample using image processing methods. To achieve this goal object recognition method is used which recognize the microorganisms by their shape. Thresholding technique is also applied to the image containing microorganisms for counting. In last thresholding using morphological operations with object recognition method has been applied which overcome all the drawbacks and count total number of microorganisms automatically.

Keywords: Object Recognition Method, Circular Hough Transform, Thresholding Method, Moore Neighbour Tracing Algorithm, Morphological Operations, Dilation, Erosion, Opening

I. INTRODUCTION

Microorganisms are small microscopic organisms which cannot not be observed through the naked eyes, they can be a small cell, bacteria, virus etc. Recognizing and counting the number of bacteria is an important task in the field of biology for research and diagnosis purpose. Counting of bacteria or virus in a blood sample, helps us in determining the level of threat it pose on humans. So it becomes a very crucial task to correctly recognize and count the total number of microorganisms in a particular sample. Counting microorganisms manually is a lengthy as well as tedious and the probability of mistake in the result is quite high. For this reason a generic techniques have been explored for automatic counting of microorganisms using various image processing tools.

To determine the total number of microorganisms in a particular sample we need to consider two important points i.e. first recognizing the microorganisms in the image sample and then counting the total recognised microorganisms. While counting microbes, various issues are to be considered for exact counting of microbes in the sample without any error. Some of the issues are overlapping of microbes, contrast between the microbes and the background, degree of microbes clustering, shape variation etc.

Various algorithms have been proposed in this paper. The first algorithm proposed is object recognition method which works on analysing the shape of the desired microbe and then counting them. To overcome the drawback of previous algorithm thresholding method is used. But this also fails when the microbes are overlapping and densely clustered. So to overcome that, morphological tools are applied to thrasholding method. And in last thresholding using morphological operations with object recognition method is used to achieve the best results by overcoming all the drawbacks.

II. OBJECT RECOGNITION METHOD

Object recognition method is the first and very basic algorithm that is analysed in this paper. In this particular algorithm shape of the microorganisms is considered to find out the total number of microbes in the image sample [1]. The figure shown below is the block diagram representation of object recognition method.

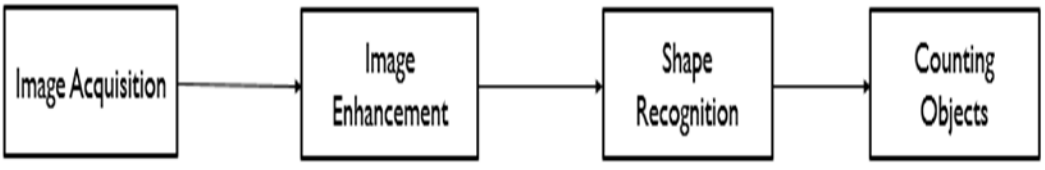

Fig. 1 Basic Model for Object Recognition Method

Now the first block shown in fig.1 is image acquisition [1]. In this particular phase the digital image of the sample containing the microorganisms is obtained using cameras attached to the microscope. The image so acquired is fed into the next block i.e. image enhancement. This particular block plays a crucial role in identification of the desired microbes in the image sample. Operations like Histogram Equalisation, Histogram Specification etc. are be applied in this block. The main task of this block is contrast enhancement. In this paper Histogram Equalisation operation has been applied to the acquired image. Histogram Equalisation is an image processing method in which contrast adjustment is done for improving the image quality. In this method the contrast of the degraded image whose quality is to be improved is spread uniformly throughout the histogram. In other words we can state it as the technique that allows the low contrast area of the image to gain high contrast. The main reason for contrast enhancement is to clearly differentiate the microbes with the background region in the image. The output of the image enhancement block is given to shape recognition block. This block is the heart of object recognition method. It is to be noted that circular shaped microorganisms are considered for analysis in this paper. To recognise the shape of the microbes in the image sample, Circular Hough Transformation technique is used [5]. This is a very unique technique used to determine the circular objects in the image. To search for the circular objects or microbes in in the image, four axis are considered i.e. one horizontal, one vertical and two diagonal. Now the centre point where all the axis meet is origin. This origin is overlapped on each of the pixel in the image traversing from left to right and top to bottom of the sample image. Now whenever this origin lies on a particular pixel of the image, the variation or the chance in the intensity of the pixel is tested across all the axes form the centre of the axis i.e. origin. The object is considered to be having a circular shape when it satisfies two conditions i.e.

1) There is change in the intensity at all axis with respect to the origin pixel
2) The distance between the origin and the pixel where the intensity is changed should be same for all the axis.

If the above mentioned conditions get satisfied, the microbe is considered to be in circular shape. Now the last section is to count the total number of circular objects detected using Circular Hough Transformation [5]. The figure shown below shows the simulation results of the above mentioned algorithm:

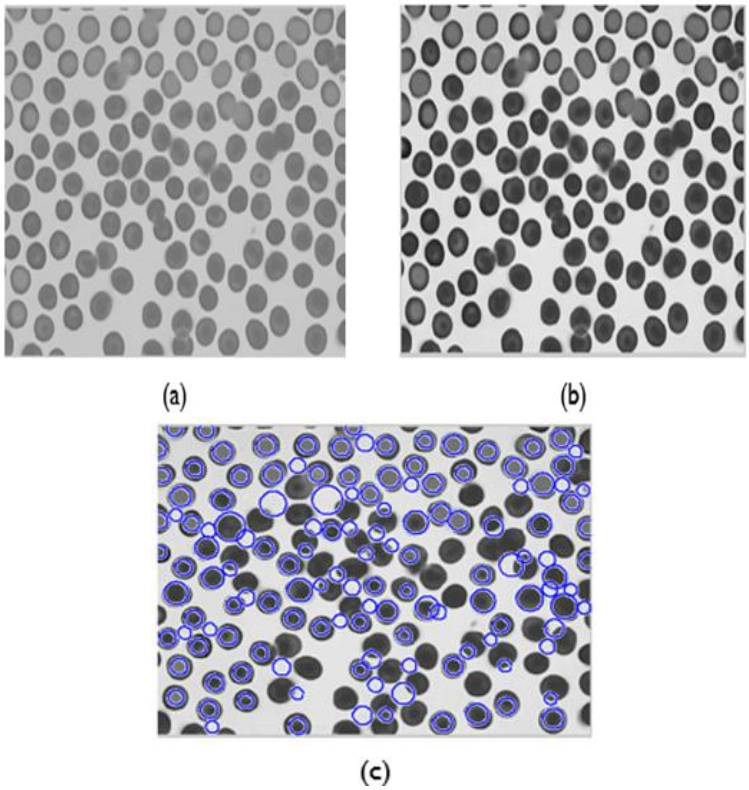

Fig. 2: Simulation Results for Object Recognition Method (A) The Original Image Containing Microbes (B) Image After Enhancement (C) Image with Total Recognised Circular Objects

From the simulation results we can conclude that there are many microbes in the image that have not been recognised by the algorithm. It can also been seen that there are certain recognised circles which are encircling regions other than microbes, which is because of the change in the intensity level in the background region which lead to wrong prediction of circle. Other drawbacks of this algorithm are that, if the microbes are overlapping, this algorithm fails to consider this fact. If there is slight variation in the shape of the microbe form circle then in that case also the microbe will not be recognised. Total number of microbes calculated by this method is 215 whereas the actual count is 146 [4]. This variation is due to the drawback discussed above.

III. THRESHOLDING METHOD

Thresholding Method is a technique in which we convert our image in black and white image and search for the bounded objects in the image [2]. This method helps in tackling few of the drawbacks which were seen in the previous algorithm. The figure shown below is the block diagram representation of the Thresholding Method.

Fig. 3: Block Diagram Representation of Thresholding Method

The first block shown in fig.3 is image acquisition in which we get the digital image of the sample containing microbes. The image so obtained is given to RGB to Grayscale converter, where the coloured image is converted in to a grayscale image with the pixel values in between 0 to 255. Now the quality of this grayscale image is enhanced using contrast enhancement technique in the next block. In this analysis, histogram equalisation technique is applied for contrast enhancement [3]. The next block i.e. thresholding is the main part of this algorithm. In this block the grayscale image is converted into black and white image. The main purpose of converting the grayscale image to black and white image is to find the bounded objects in the image, which in our analysis is microbes. After conversion of image to black and white a unique technique called Moore-Neighbour Tracing algorithm is applied [6]. This is a technique, which is used to determine the bounded objects in the image i.e. those objects which have closed boundaries. This algorithm works by traversing through all the pixels in the image from bottom left to upward and from left to right direction. As this algorithm is applied in black and white image, whenever any pixel which represents black is encountered, it represent it as a start pixel. Now whenever a black pixel is found, this will backtrack i.e. it will go back to the white pixel on which it was previously standing upon. Then the final step is to move in clockwise direction around the black pixel until it hits the next black pixel. By repeating this algorithm again and again whenever a black pixel is obtained, we can recognise the bonded objects in the image. After doing this we will count the total number of bounded objects in the sample image, which will be equal to the total number of microbes in the image. The images shown below are the simulation results of the thresholding method:

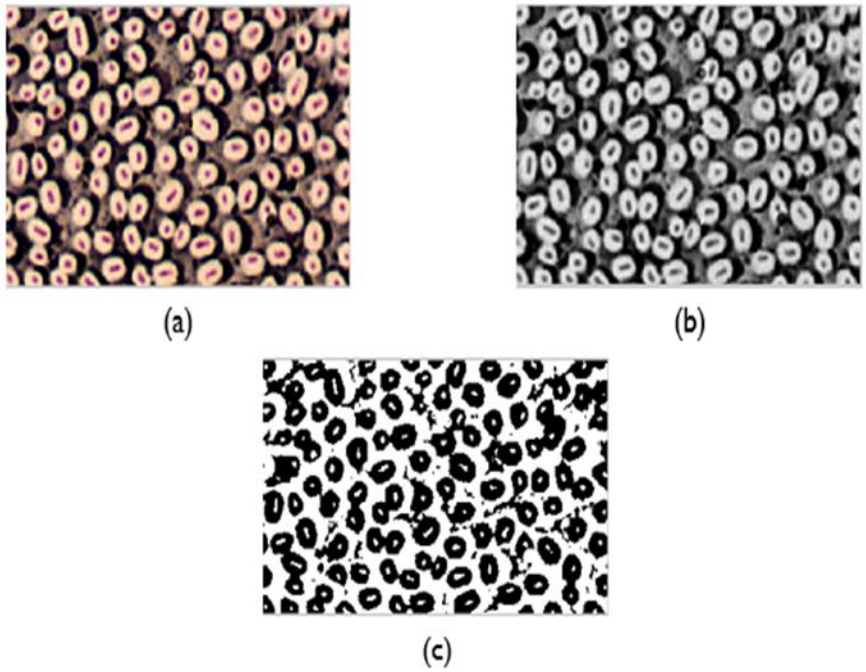

Fig. 4: Simulation Results for Thresholding Method (A) the Original Image Containing Microbes (B) Image after Grayscale Conversion and Enhancement (C) Black & White Image after Thresholding Operation

The total number of microorganisms in the image sample that is obtained from thresholding algorithm is 249. But the actual number of microbes in the image sample are 152 [4]. From this algorithm we are able to overcome few drawbacks which we there in the previous algorithm. Some of them are that, this algorithm does not depend on the shape of the microorganisms, so if there is slight variation in the shape of the microbe, then also this algorithm will count that object. The circles which were being

considered in the image because of the intensity variation in the background region of image is overcome by this algorithm. But still there are certain issues like overlapping, undesired objects being recognized which are dealt in the next algorithms.

IV. THRESHOLDING METHOD WITH MORPHOLOGICAL OPERATIONS

This method is an up gradation to the method discussed in the previous section. In this method we will be using morphological tools to overcome the problems like overlapping of the microbes in image sample, removal of undesired objects in the image other than microbes. Morphological Image Processing is the technique for analysis and processing of geometric structure using set theory [8]. It analyze the image with the help of Structural element. So structural element is the basic unit used in morphological operation. The structural element act like a window which is traversed throughout the image form left to right and top to bottom. It is to be noted that the shape and the size of the structural element plays an important role in refining the shape of the microbes. The shape of the structural element in this analysis is circular disc, as the microbes in the image are in circular shape. Now there are two conditions which are to be considered i.e. fit (it is the case when all the 1's of structural elements overlap on 1's of the image pixel), hit (when at least one of the 1's of the structural element overlaps on the 1's of the image pixel), if neither of the case is followed in that case it is neither fit nor hit. Each structural element contain its origin. Now this structural element is traversed through the sample image and the cases i.e. fit or hit are analyzed and accordingly the value of the image pixel which is overlapped by the origin of the structural element is decided. Following are the different types of morphological operation with their simulation results applied to the image fig.4 (a).

1) Erosion: Erosion of the binary image by the structural element is given by $f \ominus g$, where f is the original image and g is the structural element [8]. In this the pixel of the original image which correspond to origin of the structural element is set to one, when the fit condition is satisfied. Following are the simulation results of erosion from which it can be seen that the unnecessary objects are reduced to great extant and the boundary of the microbes has also been reduced.

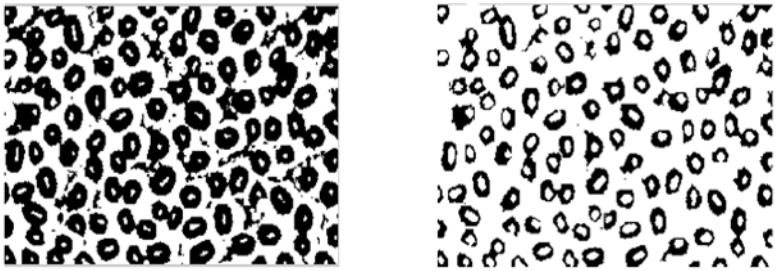

Fig. 5 Simulation Results for Erosion (A) Image after Thresholding (B) Image after Applying Erosion

2) Dilation: Dilation of the binary image by the structural element is given by $f \oplus g$, where f is the original image and g is the structural element [8]. In this the pixel of the original image which corresponds to origin of the structural element is set to one, when the hit condition is satisfied. Following are the simulation results of dilation from which we can conclude that the boundary of the objects has become much thicker.

Fig. 6: Simulation Results for Dilation (A) Image after Thresholding (B) Image after Applying Dilation

3) Opening: Opening is the technique in which erosion is applied followed by dilation [8]. The significance of this particular technique is that by applying erosion first, all the unnecessary objects are removed. The overlapped boundaries of the microbes also detach from each other as shrinking of objects takes place because of erosion. After this dilation is applied to the image which will convert the shrink microbes into their actual size. So if we conclude the overall result, the size of the microbes in the image sample remains same and all the unnecessary objects are removed from the image. Following are the simulation result of opening:

Fig. 7 Simulation Results for Opening (A) Image after Thresholding (B) Image after Applying Opening

So the total count of microbes which is determined from the image obtained after applying opening technique is 112 whereas the actual count is 152 [4]. It is to be noted that the counting of microbes is done by considering the bounded objects in the image, obtained from opening technique. So the result obtained through this algorithm is quite close to the actual count.

V. THRESHOLDING USING MORPHOLOGICAL OPERATIONS WITH OBJECT RECOGNITION METHOD

Thresholding using morphological operations with object recognition is the perfect technique with the minimum percentage of error in the output result among all the algorithms discussed till yet. In this particular technique initially thresholding is applied to the image which converts the grayscale image to black and white image [7]. After applying thresholding, morphological operation i.e. opening is applied to the image. The significance of opening is that it will remove all the unnecessary bounded objects and will help in removing the overlapping between the boundaries of the microbes. It is to be noted that because of the application of opening technique to the sample image, the shape of microbes in the image is redefined to much more circular orientation. Now the final step is to apply object recognition method to the output obtained from the opening technique. As the microbes are circular in shape, circular hough transform is used to recognize the circular microbes. Following are the simulation results of thresholding using morphological operations with object recognition method:

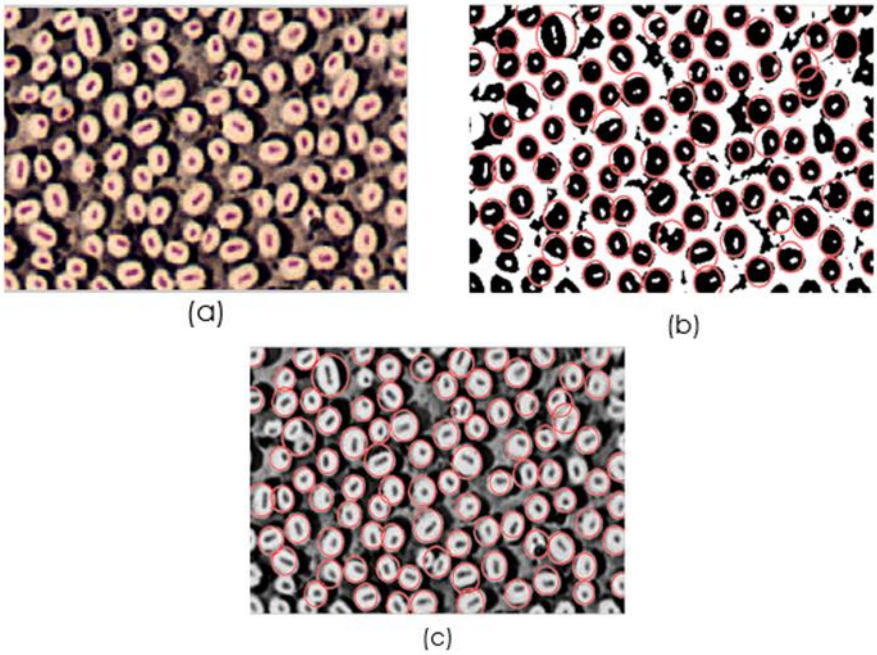

Fig. 8: Simulation Results for Thresholding using Morphological Operations with Object Recognition Method (A) Original Image Containing Microbes (B) Image after Applying Thresholding and Opening Technique (C) Grayscale Image with Perfectly Identified Microbes

So the simulation results obtained, clearly shows that all the microbes present in the sample image are clearly recognised by the algorithm. The total number of microbes counted by this algorithm is 141, whereas the actual count is 152 [4]. This variation is because of the microbes which are present at the border of the image. So this algorithm shows the satisfactory results in determining the total number of microbes in the image.

VI. CONCLUSION

In this paper, the framework for counting the total number of microorganisms in a sample image is presented. We first examined a technique called Object Recognition Method, which counts the number of objects by recognizing the shape of the microorganisms in image. The error percentage in the results obtained through this method is approximately about 32%. Then we discussed a more generic technique i.e. is Thresholding method, which counts the microorganisms depending on the number of bounded objects in the black and white image of the image sample. The percentage error or the percentage deviation of the result from the actual count is 38%. Then to overcome the drawbacks in thresholding method, morphological techniques are employed. So the percentage deviation is reduced to 25%. In last the most promising algorithm has been discussed in which thresholding followed by morphological operation and in last object recognition method is applied. The percentage deviation of the result obtained from the algorithm in comparison to the actual count is 7%. Which indicated the least error in result obtained through thresholding using morphological operations with object recognition method?

REFERENCES

[1] J. G. A. Barbedo, ”An Algorithm for Counting Microorganisms in Digital Images” , IEEE LATIN AMERICA TRANSACTIONS, VOL. 11, NO. 6, DECEMBER 2013.
[2] Ates, H. , Gerek, O.N, “An image-processing based automated bacteria colony counter” , Computer and Information Sciences, 2009. ISCIS 2009. 24th International Symposium, 14-16 Sept. 2009.
[3] D. P. Mukherjee, A. Pal, S. E. Sarma, and D. D. Majumder, “Bacterial colony counting using distance transform,” International Journal of Bio-Medical Computing, vol. 38, pp. 131–140, 1995.
[4] http://www.microbeworld.org, “Types of microorganisms database”.
[5] Khairosfaizal, W.M.K.W.M,” Circle detection in images using Circular Hough Transform” Signal Processing & Its Applications, 2009. CSPA 2009. 5th International Colloquium , vol. 21, pp. 132–136, 2013.
[6] http://en.wikipedia.org/wiki/Moore_neighborhood.
[7] F. Schönholzer, D. Hahn, B. Zarda, and J. Zeyer, “Automated image analysis and in situ hybridization as tools to study bacterial populations in food resources, gut and cast of Lumbricus terrestris L.” Journal of Microbiological Methods, vol. 48, pp. 53–68, 2002
[8] Beham, M.P. , Gurulakshmi, A.B., “Morphological image processing approach on the detection of tumor and cancer cells”, Devices, Circuits and Systems (ICDCS), 2012 International Conference, 15-16 March 2012.
[9] J. Osowsky and H. R. Gamba, “Sistema automático para contagem de colônias em placas de Petri,” Revista Brasileira de Engenharia Biomédica, vol. 17, pp. 131–139, 2001.
[10] J. Marotz, C. Lübbert, and W. Eisenbeiß, “Effective object recognition for automated counting of colonies in Petri dishes (automated colony counting),” Computer Methods and Programs in Biomedicine, vol. 66, pp. 183–198, 2001.
[11] Ates, H. , Gerek, O.N, “An image-processing based automated bacteria colony counter”, Computer and Information Sciences, 2009. ISCIS 2009. 24th International Symposium, 14-16 Sept. 2009.