Journal of Artificial Intelligence and Capsule Networks (ISSN: 2582-2012)
www.irojournals.com/aicn/

Segmentation of Microscopy images using Multi-Scale Retinex with Chromacity Preservation and Otsu Thresholding

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Abstract

Bacteria play a significant role in our environment by being helpful or harmful; hence, it is crucial to identify the various bacterial species. The microscopic image captured by camera with microscope is not reliable due to the poor quality of image, making bacterial counting a difficult and time-consuming task. This paper proposes improved and enhanced Multi-Scale Retinex with Chromacity Preservation and Otsu Thresholding techniques for increasing the quality of images of bacterial cells for segmentation and contrast enhancement. A combinative procedure of image enhancement and segmentation is illustrated in this paper. The parameters for Image Quality Assessment (IQA) used are Enhancement Measure Estimation and Standard Deviation of the upgraded images. The proposed approach gives better segmentation results, as proven by the incremental changes in the IQA parameters.

Keywords: Microscopy images, Otsu Thresholding, Enhancement Measure Estimation, Multi-Scale Retinex with Chromacity Preservation, Standard Deviation, Image Quality Assessment

1. Introduction

The bacteria are an important part of our environment; they have a huge importance in farming, biochemistry, science, medicine, and food industry hence it becomes important to identify different bacterial species [3]. While some bacteria have positive impact on the environment, some have negative impact and if not controlled can cause disasters; thus, studying and identifying different bacteria is essential. It is not possible to see these

DOI: https://doi.org/10.36548/jaicn.2023.1.002
Received: 22.01.2023, received in revised form: 22.02.2023, accepted: 09.03.2023, published: 20.03.2023

microorganisms with bare eyes. The device used for the recognition is microscope. With the help of a microscope images are captured and these are termed as microscopic images [5].

Samples taken from different humans are placed on slides and the images of these samples are captured. These images are then used by pathologists for identification and counting of bacteria. These images do not have a clear contrast, thereby making it a difficult task for even a professional to identify the species of bacteria. The images have a bad contrast, visibility, and noise, and hence are not able to produce good results after segmentation. Therefore, it is necessary to enhance these images after segmentation for better results.Segmentation is a method to procure only the relevant section of the images. Pre-processing is used to achieve better results. In pre-processing, the colored images are converted to grey scale as the colored images are difficult to distinguish and they occupy large memory spaces. The contrast enhancement is achieved by Multi-Scale Retinex (MSR) [12]. Image color constancy and dynamic range compression are increased by the MSR method. The image enhancement and noise filtering are achieved by GIF [6]. Contrast enhancement is achieved by Single-Scale Retinex (SSR) [7]. Edge enhancement of the bacterial image can be achieved by unsharp masking [8]. Chromacity preservation and MSR, when used on bacterial images, give better results in the counting, segmentation, and morphological operations of WBCs and RBCs using Otsu Thresholding (OT) [2]. This paper uses OT and MSR to attain finer outcome. MSR used in this paper provide a dependable outcome which further provides better results on segmentation.

The subsequent sections of this work are as follows. Section 3 describes an overview of MSR and Multi-Scale Retinex with Chromacity Preservation (MSRCP). Section 4 elaborates the OT and Segmentation techniques. The IQ, results, and performance outcomes are summarized in section 5, and the conclusion is briefed in section

2. Literature Review

Paper [1] utilized a MSR model which is proficient in improvement by utilizing shading in the pictures, and following it the Cuckoo Search (CS) calculation is executed for the enhancement which is utilized to choose the determination of the weighted boundaries for various Gaussian channels. For all intents and purposes, CS calculation is a streamlining strategy which is bio-propelled and is applied broadly to take care of different enhancement issues. For all intents and purposes, the quantity of boundaries required are tiny when contrasted with GA, PSO and ABC. Subsequently, it's reasonableness is exceptionally high in the enormous number of advancement issues. A fruitful use of CS calculation was done to improve the decision of weighted boundaries. The proposed CS-MSR gave astounding exhibitions by the improvement of weight of Gaussian channels with no adjustment of the scale boundaries in a few conditions. The visual portrayal of the aftereffects of upgrade shows that the proposed method can work on the general brilliance and difference of tiny picture.

Article [2] utilized multi-scale portrayal into division calculation to eliminate the impact of clamor, powerless edges or surfaces on division results. Nonetheless, edges are incredibly critical to division, subsequently a superior picture multiscale portrayal ought to be edge-mindful. As of recently, many picture multi-scale portrayal calculations have been imagined. Laplacian pyramid is one of old style calculations. By the by, the Laplacian pyramid is faulted for inadequacy of addressing edges well and mismatched applications in edge-mindful activities, for example, edge-safeguarding smoothing and tone planning, since it is built with Gaussian pieces which are spatially invariant. As edges are of extraordinary importance to picture division, Laplacian pyramid does not hold for multiscale picture disintegration in picture division calculation. In the calculation, edges are initially grouped into two lists with a limit, huge scope edges and limited scope subtleties. Then, at that point a bunch of picture channels are intended to accomplish edge-safeguarding smoothing, detail improvement, and so on.

The pre-processing can also be done by the particle filtering which is a progressive Monte Carlo framework in which the crucial indication is to characterize the essential subsequent density function by a group of random samples with related loads (weights) as proposed in [4]. The feature extraction of the image can be obtained by a Hessian Matrix, which is obtained by converting the image to Hue Saturation Value color space, followed by gaussian 4 automated bacteria colony counter, diagnosis using microscopic images, and smoothening by Gaussian kernel via a convolution operation. After Gaussian smoothing, the Hessian matrix is obtained in each pixel via the second order derivations of S [10].

In the work of A. R. Yadav et al. [11], though several multi-resolution approaches are available, Gaussian Image Pyramid (GIP) was chosen as it requires less computational efforts. Image pyramid is useful for illustrating images at several resolutions and has been used for texture analysis due to local averages. In GIP, moving from bottom to top of the pyramid produces images of reduced size and resolution. Base image of the pyramid has high resolution while the top (apex) of the pyramid has low resolution. The original image is convolved with

Gaussian kernel function (low pass filter), and sub-sampled to generate next level of pyramid image.

3. Contrast Enhancement of Bacterial Images using MSRCP

3.1 Multi-Scale Retinex

The implementation of MSR is achieved by Single Scale Retinex which is an enhanced form or Retinex approach. The colour constancy and Dynamic Range Compression (DRC) are enhanced with the help of this coloured image enhancing algorithm. The limitation of SSR are DRC and colour rendition [12]. To avoid these limitations, MSR is used.

Eq.1 provides n^{th} spectral component.

$$MSR_n = \sum_{i=1}^{q} W_i \, R_i \tag{1}$$

Here, number of scales is given by q, w_i gives the weights corresponding to each scale and R_i is shown in Eq.2.

$$R_i(a,b) = \log I_n(a,b) - \log(C(a,b) * I_n(a,b)) \tag{2}$$

Here, pixel positions are defined by a, b and Gaussian function is defined by c(a,b), I(a,b) defines the original image, and * is convolution operator. MSR requires optimal amount of colour in the images, otherwise it produces distorted image outcomes. MSR outcome can be contort, if average common value of RGB doesn’t lead to a common grey value.

3.2 Multi-Scale Retinex with Chromacity Preservation

MSRCP Algorithm:

MSRCP Algorithm

BEGIN
Step 1:Input i= test images$(colored)$
Step2:Input a_1,a_2,a_3**= scales**
Step3:Input s_1,s_2**= saturation levels**
Step 4: $t = (i_R + i_G + i_B)/3$
Step 5: for each s_i
Step 6: d=log(t) – log (t*gauss)
Step 7: end for
Step 8: MSR=$\sum_i {}^1/_3\, d$
Step 9: $t_i = ColorBalance(MSR, S_1, S_2)$
Step 10: for each i
Step 11: $p = \max\,(i_R[i] + i_G[i] + i_B[i])$
Step 12: $l = min\left(\frac{255}{p}, \frac{t_i[i]}{t[i]}\right)$
Step 13: $MSRCP_R[i] = l * i_R[i]$
Step 13: $MSRCP_G[i] = l * G[i]$
Step 13: $MSRCP_B[i] = l * iB[i]$
Step 14: end for
Step 15: Output MSRCP
END

a1, a2, and a3 are the three parameters that are used in MSRCP [4]. At the start, the image that is given as input is converted to a grayscale image. With each parameter as described above, the grayscale image undergoes three convolutions simultaneously. The value of the result of the MSR is calculated. By taking the arithmetic weight of all RGB parts of bacterial image, the difference between the greyscale bacterial image and the convolution results are obtained. The value after the subtraction along with the saturation levels are used in the colour balance function. The value of l is calculated as shown in the above algorithm. The MSRCP value or output for all levels of colour (Red, Green, and Blue) is calculated by using the value of l. Finally, the output of MSRCP is calculated by taking the arithmetic mean of the values of all the levels of RGB colours and the resultant image. All the parts of bacterial image is enhanced significantly, as this method enhances the image quality. In the convolution function of the algorithm, Multiplication after Fast Fourier Transformation is performed, and then Inverse Fast Fourier Transformation is performed to extract the image within the time domain. To get the final value of MSRCP, multiplication is done multiple times, in iterations.

4. Segmentation using Otsu Thresholding

Segmentation is described as the grouping of similar items together. Segmentation provides pixel-level partitioning, which is further used in image processing and classification of bacteria species. It removes unwanted background from the images and focuses on pixel-level details. The partitioning into pixel-level and multiple segments makes it easier to analyze minute details [8]. After pre-processing, the OT technique is used for segmentation to extract the important pixel-level details in this work. The bacterial cells in these images have a distinct boundary and they are turned into white through binarization process. The unwanted background is removed and turned into black. It is also clear from the image that the overlapping bacterial cells are also removed from each other and they are clearly distinguished from each other. Binarization of the grayscale image is done after pre-processing. The mathematical equation of OT is given as:

$$p(x,y) = \begin{cases} 255 & q(x,y) \geq t \\ 0 & q(x,y) \leq t \end{cases} \quad (3)$$

Where, (x, y) is defined as pixel value at (x, y), (x, y) is defined as input image pixel value, and optimum threshold value is given as t.

Threshold has a significant task in OT as it is a region based segmentation algorithm. The working and accuracy of segmentation in OT largely depends on threshold, hence it requires a notable attention [13]. In this work, numerous values of threshold were taken but at t=0.8, the OT algorithm without any misconstrue produced best results. The methods described in this paper, when applied combatively, produce significantly good results. MSRCP gives very reliable results for bacterial image enhancement; further, this enhanced image undergoes segmentation by the method of OT.

5. Results and Discussion

5.1 Image Quality Assessment

As described above, MSR and MSRCP are involved in the contrast enhancement of the images. The assessment of the result is the verification of output at each step. Enhancement Measure Estimation (EME) parameter measures the level of enhancement in the microscopy images. It is an absolute valued parameter and used for comparison of the images for enhancement. EME for both input and output image are calculated. As the difference in these values is higher, the enhancement is better [8]. Entropy is the information that can be extracted from images [9]. Standard Deviation (SD) is the variability of the information obtained from images [10]. These are some absolute values that are responsible for the calculation of the degree of contrast enhancement done by MSR and MSRCP.

A good result is produced with the higher value of EME (entropy) and SD. Segmentation is done by OT. SD is also used for the assessment of quality after segmentation, as SD is an absolute value. A good result produces a higher value of SD.

5.2 Experimental Results

Digital Images of Bacteria Species dataset (DIBaS) [19] which contains microscopic images of 33 bacterial species with 20 images for each of them, has been used. The method described above are applied upon the bacterial images in the DiBAS dataset. Firstly, DiBAS dataset is divided into test images which contain 20% images of dataset and training images which contains 80% images of dataset. The contrast enhancement is done by MSR followed by MSRCP, as discussed in Section 2. The output from the above method are given below in figures 1 and 2. Each figure shows the input images and the various output produced by MSR and MSRCP. Following it, OT for segmentation is applied on the input images and the output images are shown in figures 1d and 2d.

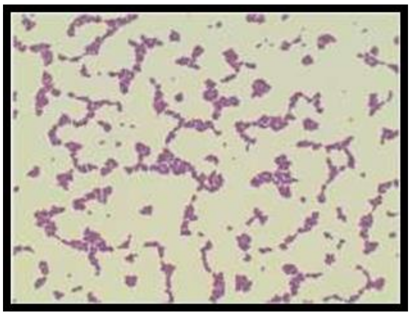

Figure 1a. Original Image 1

EME=1.623, SD= 0.193

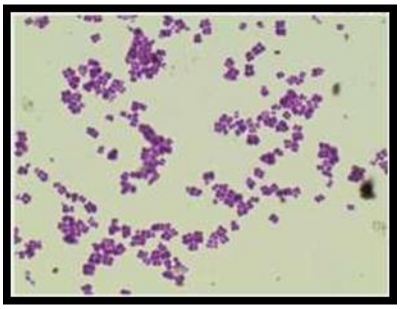

Figure 2a. Original Image 2

EME=1.61, SD=0.85

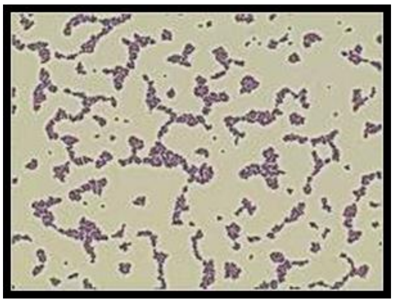

Figure 1b. Image 1 -MSR output

EME= 5.26. SD= 1.43

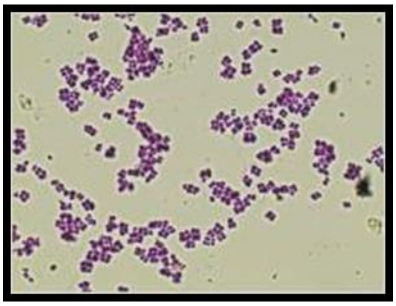

Figure 2b. Image 2 -MSR output

EME= 4.26, SD= 1.87

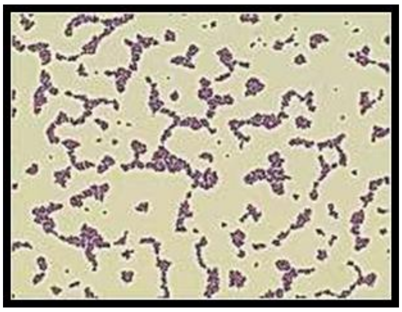

Figure 1c. Image 1 -MSRCP output

EME=7.93, SD=2.43

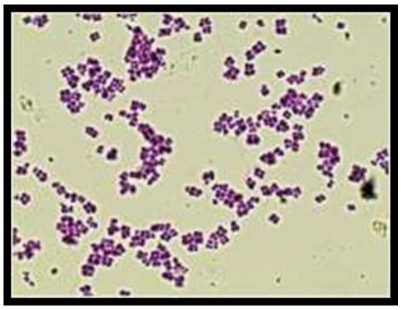

Figure 2c. Image 2 -MSRCP output

EME=8.45, SD=3.93

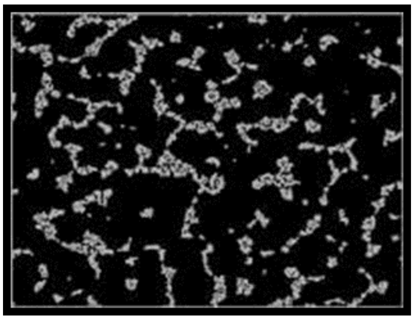

Figure 1d. Image 1 -Segmentation using OT

SD= 3.21

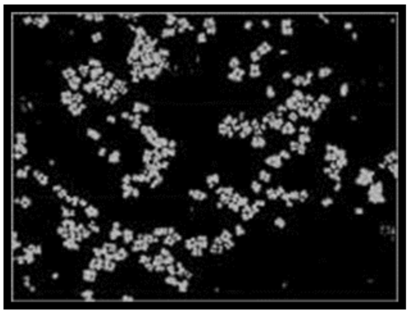

Figure 2d. Image 2 -Segmentation using OT

SD= 4.18

The images are evaluated on IQA parameters, and the results are analysed in the table given below.

Table 1. IQA values after Processing

Test Cases	**Original Images**		**MSR Output**		**MSRCP Output**		**OT Output**
	EME	SD	EME	SD	EME	SD	SD
Test Case 1	1.629	0.193	5.26	1.43	7.93	2.43	3.21
Test Case 2	1.61	0.85	4.26	1.87	8.45	3.93	4.18

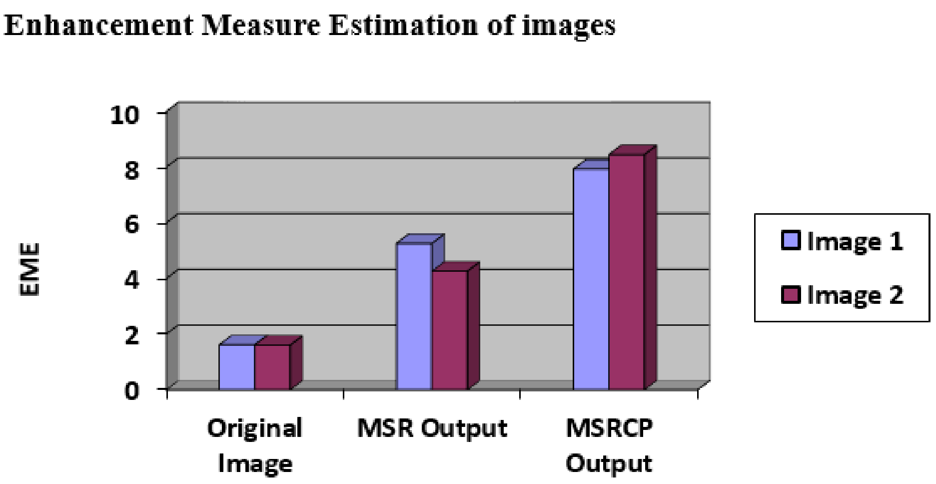

Figure 3. Graph depicting Enhancement Measure Estimation of images

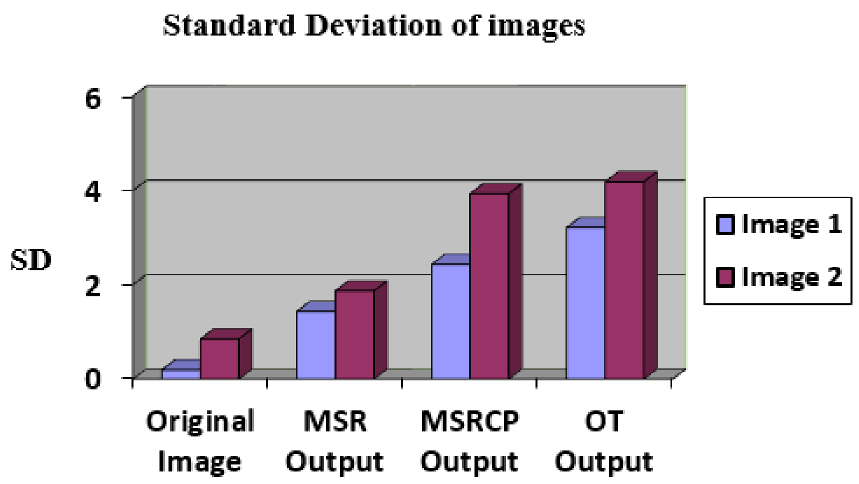

Figure 4. Graph depicting Standard Deviation of images

6. Discussion

The original bacterial images in figures 1 and 2 are hazy, making the segmentation very difficult, unreliable and some information loss. The MSR and MSRCP produce clear bacterial images that are distinguished from each other. Table 1 demonstrates the enhancement evaluated by the IQA parameters. The MSR produces clear images but the colours of the images are not good. This can be achieved by MSRCP which distinguishes the bacterial images from its background. Further segmentation is done to extract the required information.

7. Conclusion

In this paper, pre-processing is done using an improved method of contrast enhancement i.e., MSR and MSRCP which not only improves the image quality but also enhances the colour of the images, which are evaluated on the IQA parameters. The value of EME is improved by 7 times and the SD is improved by 5 times. Further, OT algorithm is used for segmentation. This OT method produces good results, which can be used for classification and feature extraction of bacterial species that helps in the diagnosis of diseases. Thus, this method provides reliable and remarkable results. In the future, optimization algorithms like Particle Swarm Optimization or Ant Colony Optimization can be used with MSRP for further improving the efficiency.

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