Histogram Equalization with Filtering Techniques for Enhancement of Low Quality Microscopic Blood Smear Images

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***Abstract*—This paper presents image enhancement and filtering techniques for microscope blood smear image, in order to improve low image quality that have characteristics: blurred, the diminished true color of objects which are cells , unclear boundary and low contrast between the cells and background. Therefore in this paper proposed histogram equalization (HE) technique followed with filtering techniques such as median filter. HE utilizing to adjust the contrast which based on intensity pixels values, hence able to measure image quality through image histogram as shown in results, while removing noise from the images using filtering and gamma correction parameter in order to distinguish between background and foreground (cells) to get clear borders also. These techniques have been implemented on 46 blood samples. The proposed method successfully improve the readability of the cells in the low quality of blood smear images this mean that contain more information with a good effectiveness which lead for the correct sickness detection and data analysis.**

***Index Terms*—Blood Samples; Histogram Equalization Image Filtering; Image Enhancement.**

I. INTRODUCTION

Image enhancement is the process of improving the appearance of a subsection or an image for enhanced image contrast or imagining of certain structures to facilitate a more precise image analysis. With image improvement, the distinguishability of elected features in an image can be improved, but the ingrained information content cannot be increased [1]. The design of a good image precision algorithm should consider the detailed features of interest in the microscopic image and the imaging process itself [2]. The purpose of this study is to get good image quality, sharpen objects included removing the noise (filtering) and smoothing of the pixel or voxel data by performing some sort of local averaging function [4].

For microscopy applications, numerous image enhancement procedures have been established and used [5]. These procedures are divided into two classes: transform domain and spatial domain. Spatial domain procedures contain processes carried out on the entire image or on a confined area chosen based on the image data. Methods that are appropriate for this category comprises of histogram equalization, image averaging, sharpening of vital characteristics like contours or edges and nonlinear filtering. Transform domain enhancement approaches tamper image information in transform domains, such as Fourier and wavelet transform. Frequently, image information of interest cannot be divided in the spatial domain but can be isolated in the transform domain [6]. For example, some coefficients can be amplified in Fourier domain and the image recovered in the spatial domain to show the image content of interest [7]. The enhancement of blood smear image include the enhancement of features which are the cells; white blood cells (WBC), red blood cells (RBC) and platelets. The enhancement process make the features clear and sharpen which make the cell analysis easier and more accurate.

Several studies have attempted to use green channel of the RGB color or other color spaces. For example authors in [8] suggest to use L*a*b* color model for reduced color feature. In addition, in study [9] used combining B from RGB and Y component from CMYK color spaces to have more contrast. In other hand many efforts have been devoted to reduce the noise including wavelet which is more practical and widely used but the biggest challenge in wavelet is finding a suitable threshold value [10].In term of blood cell detection in study [11] median filter was used to de-noise blood microscope images but its effect the sharp of the cells. The technique proposed in this paper combined HE with other filters which can conserve proper cell edge. Image intensities have measured to check image quality.

II. MATERIAL AND METHODS

A. Preparing of Blood Smear Samples

The first step in our research is preparing blood smear sample. The sample was prepared in BIOMEMS lab equipped with (stain A, stain B, blood cell drop, slide), preparing of the sample started with putting one drop of blood in the slide then spread it on the slide. After the drop was dried it was dipped in the stain. The same process was repeated with purple stain. Once the slide was dried, it was subjected to microscope for analysis. The difference among our samples is related with the blood that is used it to prepare the sample. Figure 1 shows respectively (a) microscopic oil used on the samples and image taken under 100 times (100X), (b) the stain chemical used for coloring the cells and (c) the stain which enables us to see the cells with coloring and without background.

Figure 1: Microscopic oil and chemical stains used to prepare blood sample

B. Image Acquisition

The process for displaying the RBC image will include digitization of optical image with 40 times (40X) objective which equal to approximately 400 times magnification, or optical image with 100 times objective (100X) which requires microscopic oil on the RBC sample.

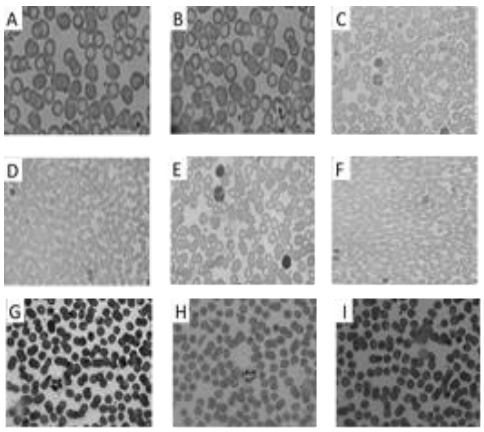

Figure 2: Blood smear images under microscope

C. Pre-processing of Blood Images

The pre- processing is preparing clear features of RBC images to start correct process that ensure fast and right processing. Brightness and contrast Adjustment including histogram equalization are important. After that the image can be converted. Defining the image in binary as 1 and 0 is the easiest way to distinguish the background from the object. Improve of image quality requires image enhancement which is the process of enhancing the appearance of an image or a subset of the image for better contrast or visualization of certain features and to facilitate subsequently more accurate image analysis. For the enhancement, some of the techniques that could be used are: point processing, histogram based techniques or mask processing in spatial domain, or (high, low) filter in frequency domain [9]. In this paper, some spatial domain techniques were implemented. Spatial domain techniques implemented in this research. Spatial domain methods are helpful because it includes operations carried out on a whole image or on local region selected on the basis of image statistics such as histogram equalization, image averaging sharpening of important features such as contours or edges and nonlinear filtering (median filter).

D. Histogram Equalization

The gray-level histogram of an image is the probability of occurrence of each gray level in the image. The purpose of histogram equalization is to remap the image gray level so as to obtain a uniform (flat) histogram if no prior information is available about gray level distribution, it is often useful to distribute the intensity information uniformly over the available intensity levels [12]. Also it is easier to compare two images taken under different conditions if their histogram match. Mathematically, the normalized histogram $h(r_i)$ can be expressed as$h(r_i) = {}^{n_i}/_{n}$, where r_i is the gray level an image having a total of L values, n_i is the number of occurrences of gray level r_i in the image, and n is the total number of pixels in the image. It is possible to use the transformation T(r) to map the original gray levels r_i of the input image into new gray levels s_i such that, for the output image [13].

$$s_i = T(r_i) = \sum_{j=0}^{i} h(r_i) = \sum_{j=0}^{i} \frac{n_i}{n}, \quad i = 0,1 \dots, l-1 \qquad (1)$$

where the transformation T is the rising distribution function of the image gray levels, which is always monotonically increasing. The resulting image will have a histogram that is ‘‘flat’’ in a local sense, since there is only a finite number of gray levels available [13].

Local histogram equalization is a variant of the histogram equalization process described earlier. It applies histogram equalization to small, overlapping areas of the image that contain local features. This nonlinear operation can significantly increase the visibility of subtle features in the image. However, because histogram equalization is carried out in local areas, it is computationally intensive, and the complexity increases with the size of the local area used in the operation. There is also a number of other variations in image histogram transformations that take into account local image characteristics, such as the local standard deviation [14].

E. Median Filtering

The median filter is a type of standing filter because it is based on the statistics derived from rank –ordering the elements of a set, this filter is often salutary because it can reduce noise with preserve sleek edges. The median filter is a commonly used nonlinear operator that replaces the original gray level of a pixel by the median of the gray levels of the pixels in a specified neighborhood. The din curtailment effect of the median filter depends on two factors which are the spatial extent of its neighborhood and the number of pixels involved in the median calculation [15].

When more than one image of stationary object is available, averaging over$N$ image is a simple way to improve the signal to noise ratio by $\sqrt{N}$. In microscopic imaging, multiple images are often obtained. These multiple images if duly registered can then be average to reduce the noise [16].

III. Results and Discussions

This part presents the results for images taken under microscope and enhancement techniques results (histogram equalization, contrast adjustment and median filter have been used). Certain images have been taken under light microscope with 40 times (40x) and 100 times (100X) the order of images from A to I respectively, Images were saved as tiff format of class unit 8.Tiff format was used because it is a very flexible format that is used in many applications.

In this images manifested that facing issue prevent to distinguish the objects form background or separate between cells this is due to weak brightness and contrast that is interpreted defocused image, with this issue the boundary between cells cannot be defined also for nucleus and cytoplasm region. The transition of intensity levels is unclear (image F and image I). Moreover this issue was observed in all images from (A to I). The small non-cells Objects in the background can affected cell counting, which is a serious problem [10]. Histogram equalization was used to adjust the illumination and contrast and to enhance the image quality. The Figure 3 shows histogram equalization for the images chosen as pattern, while table shows measuring the cell intensities of one of images as example.

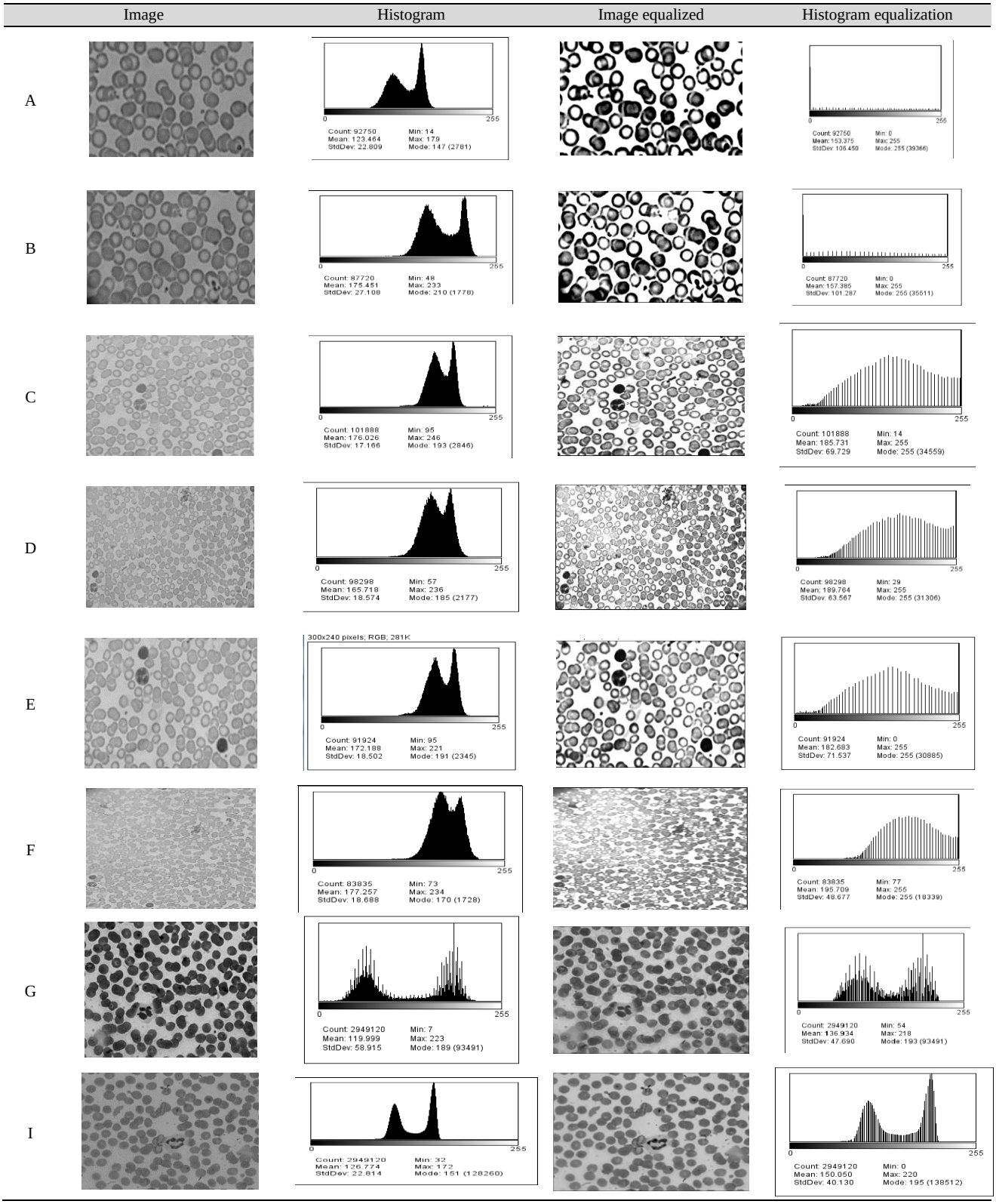

Figure 3: Normal images and equalized with their histogram

Figure 3 shows images in the first column attaching with their histogram in the second column including histogram parameters (pixel count, mean parameter, stdDev: stander deviation and minimum pixel histogram start to the max histogram). From the minimum value to the maximum value is histogram intensity average. Moreover the third column include image equalized results using histogram equalization techniques. In terms of needed enhancement the most important features of this images are dark and have low dynamic range. This can be seen in the histograms in figure 4, in which the dark nature of the image is expected because the histogram is biased toward the dark end of the gray scale. The low dynamic range is obviously from the fact that the width of the histogram is narrow with respect to the entire gray scale.

The images from A to I in Figure 4 show the histogram equalized result. The enhancement in average intensity and contrast are quite evident. The image features also are evident

in the histogram of images. The increase in overall intensity image is due to the fact that the average intensity level in the histogram of the equalized image is higher (lighter) than the original. The histogram equalization method has the desire characteristic of being able to increase the dynamic range for intensity level in the image so far it's helpful to produce a flat histogram. In case of image (D, F, and G) Histogram equalization do not give good results the intensity levels have been shifted to the upper one half of gray scale, thus giving the image a washed-out appearance.

It is able observe some small dots in images specially images (F, G, H, I) that is carrying out that after enhance image intensity and fixe the low dynamic range needs to applying some filters to remove out the noise from images were used. Figure 4 shows the results of applying median filter, gamma correction filter or average filter depends on the noise nature of each image.

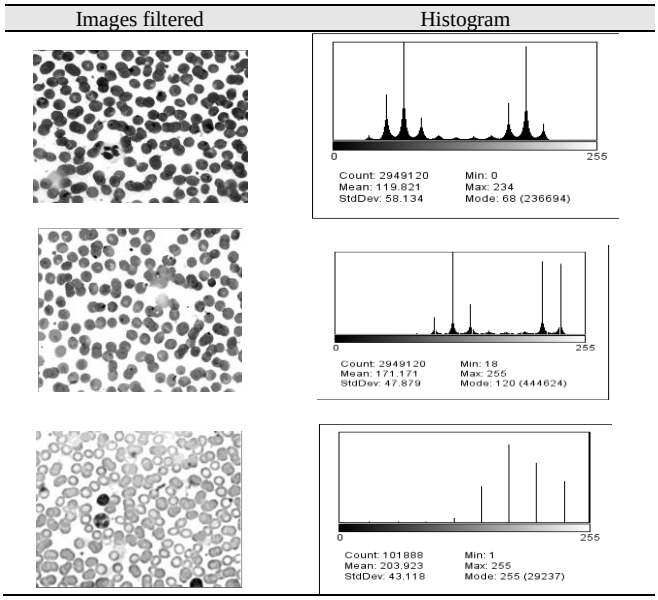

Figure 4: Filtered images using median filter

Can use the filters which mentioned before with assist of gamma correction what we found it is helpful to reduce small noise, Gamma correction is the parameter that specifies the shape of curve that maps the intensity value in the image, we have use it in low bright, if gamma is greater than 1 the tamping is weighted toward lower (darker) and if it's less than 1 so the mapping is weighted toward higher (brighter). Image I, H, C were chosen after applying median filter. The problem related with median filter is blurring the objects (cell) borders so it is no suitable for all images samples we have which need furthermore process like morphological operations.

The statistical measuring of cell intensities shows on table 1 helps to count percent-positives which can detect intensity average for the cells in image so could confirm image quality to start the right process.

Based on the histograms in Figure 4 can observed that the histogram cemetery to two parts that makes possible to distinguish the foreground from background flat histogram which was desired. After removing unlike objects with other operations and methods. For other images the applying of median filter blurring the edge and make it not smooth. If do comparison of filtered images with normal images or equalized able to see the difference that the features or objects are clearer and less noise in background.

Table 1
Measure Cell Intensities for Image I

Image	Object	Feature	Mean	Median	STD
Image I	cell	IntegratedIntensity	8.049	6.259	8.095
Image I	cell	MeanIntensity	0.054	0.04	0.06
Image I	cell	StdIntensity	0.016	0.003	0.054
Image I	cell	MinIntensity	0.034	0.035	0.004
Image I	cell	MaxIntensity	0.093	0.047	0.185
Image I	cell	IntegratedIntensityEdge	2.208	2.125	0.489
Image I	cell	MeanIntensityEdge	0.041	0.04	0.009
Image I	cell	StdIntensityEdge	0.004	0.003	0.006
Image I	cell	MinIntensityEdge	0.036	0.035	0.004
Image I	cell	MaxIntensityEdge	0.054	0.047	0.033
Image I	cell	MassDisplacement	0.04	0.021	0.062
Image I	cell	LowerQuartileIntensity	0.041	0.039	0.009
Image I	cell	MedianIntensity	0.048	0.039	0.038
Image I	cell	MADIntensity	0.009	0.001	0.033
Image I	cell	UpperQuartileIntensity	0.069	0.043	0.112
Image I	cell	CenterMassIntensity_X	104.509	104.164	44.001
Image I	cell	CenterMassIntensity_Y	81.113	82.885	47.26
Image I	cell	MaxIntensity_X	105.763	106.5	44.306
Image I	cell	MaxIntensity_Y	81.579	84.0	46.861

IV. CONCLUSION

Image enhancement indeed related with the quality of a digital image, which depends on the size of the pixels, relative to the size of the image and the number of available values of gray tone that are accessible. Image data represented by the histogram. Desired flat histogram form this technique and can provide wide intensity average and good contrast. When Histogram equalization don't give a good results. The intensity levels have been shifted to the upper one half of gray scale, thus giving the image a washed out appearance. To enhance blood cell images features, spatial filter domain were used. Like median filter to enhance image quality, and find out that the image equalization technique gives very good results if not much noise in the image and for more image improvement median filter is suitable for red blood cell images to remove the noise but does not keep smooth borders, perhaps need to implement morphological operation or local thresholding to support histogram equalization technique.

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