Image Enhancement and Analysis of Microscopic Images using various Image Processing Techniques

***Ms. Shivani Gupta **Mr. Satyajit Sen Purkayastha**
(M.Tech student Deptt. of Electronics and Comm., IET Bhaddal)
(Asstt.Professor, Deptt. of Electronics and Comm., IET Bhaddal)

ABSTRACT-Image Enhancement is one of the basic requirements for making an image useful for various statistical analysis and particle identification that form the basis of various tests and measurements in Digital Photography, Biomedical Tests and Measurements, Microscopic Image Enhancement and many more Digital Imaging Applications. Various Techniques have been studied and tested on different images for their results and effects in border extraction, edges detection, pixel value matrix computation and controlling and contrast of images. MATLAB based image processing tools have been utilized for the complete enhancement and analytical operations.
KEYWORDS: **Image Enhancement, Histogram Equalization, Linear Filtering, Opening and Closing, Digital Image Processing.**

1. INTRODUCTION

Digital image processing is an ever expanding and dynamic area with applications into our everyday life such as medicine, space exploration, authentication, automated industry inspection and many more areas. Image Processing basically includes analysis, manipulations, storage and display of graphical images from sources such as photographs, drawings and so on. Image Enhancement basically includes noise reduction from the image. This paper gives the analysis of the image with the help of histogram equalization,

edges detection or border extraction and morphological operations.

2. IMAGE ENHANCEMENT AND ANALYSIS TECHNIQUES

Particle Analysis of a biological image involves examining every particle of the image and remove any of the problems such as non-uniform illumination, less brightness etc. Various techniques such as Histogram equalization and edge detection have been studied by editing the input picture of microscopic bacteria as in fig 1.

2.1 HISTOGRAM EQUALIZATION AND CONTRAST ENHANCEMENT

Histogram equalization is used to enhance the contrast of the image, it spreads the intensity values over full range. Under Contrast adjustment, overall lightness or darkness of the image is changed.

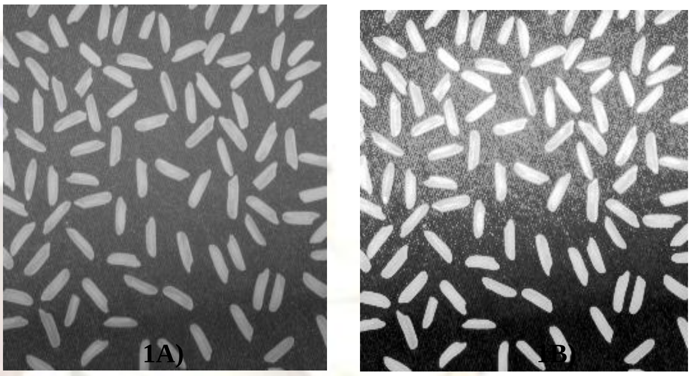


Fig 1 A): Original Image of cluster of particles with non uniform illumination at backround (MATLAB IMAQ toolbox)
Fig 1 B): Resulting Image after Histogram equalization for contrast enhancement on the input image shown above

Histogram equalization technique can’t be used for images suffering from non-uniform illumination in their backgrounds as this process only adds extra pixels to the light regions of the image and removes extra pixels from dark regions of the image resulting in a high dynamic range in the output image.

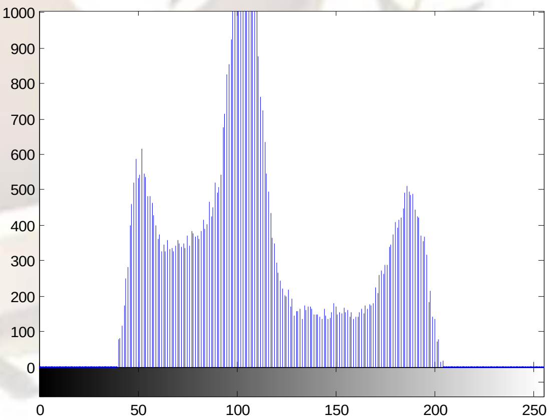


fig 2A): Histogram of the image I as shown in fig 10 (less dynamic range and high frequency of variation

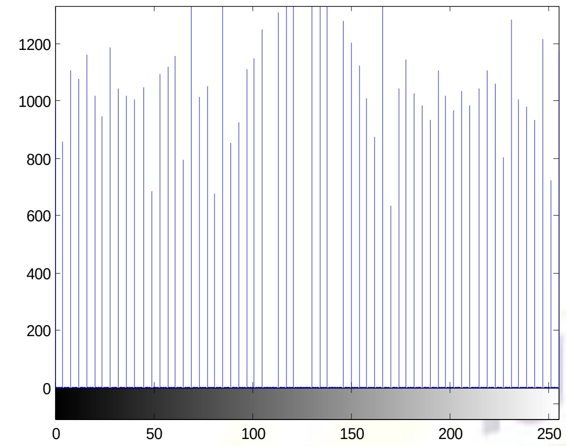


Fig 2B): Histogram of the image I as shown in fig 10 (high dynamic range and increased signal amplitudes)

Histograms indicate that the dynamic range is improved but the amplitudes for various pixels near the center of the image with light backrounds have been amplified resulting in excessive brightness near the particles present in specified locations.

Fig 3: Approximated non-uniform background image extraction using histogram equalization

Now by operating the surf command over the image background attained from histogram equalization, we get its background approximation.

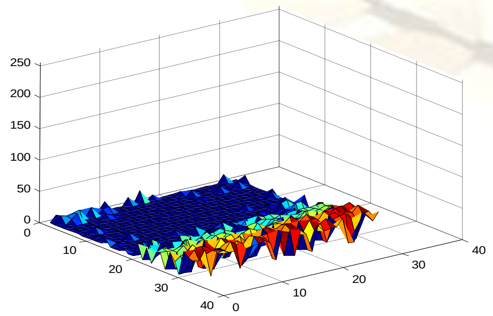


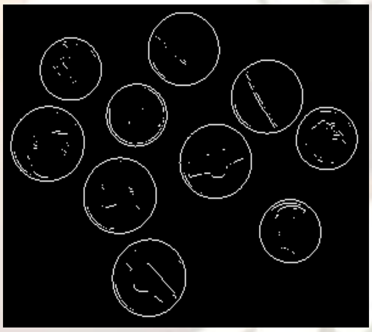
Fig 4): Surface approximation of the backround attained using histogram equalization

3.2 EDGE DETECTION AND BOUNDARY EXTRACTION

To find edges, this function looks for places in the image where the intensity changes rapidly, using one of these two criteria: Places where the first derivative of the intensity is larger in magnitude than some threshold. Or places where the second derivative of the intensity has a zero crossing. Edge provides a number of derivative estimators and each one these derivatives implement on any one of the criteria mentioned above. Consider the images of coins in an image that need to be extracted using boundary extraction techniques. This image is having a uniform background.

Fig 5A): Image of coins to be used for boundary extraction purposes (courtesy of NASA/ MATLAB Imaq toolbox/ imdemos)

Now, applying the sobel and canny edge detectors to the above image for boundary extraction.



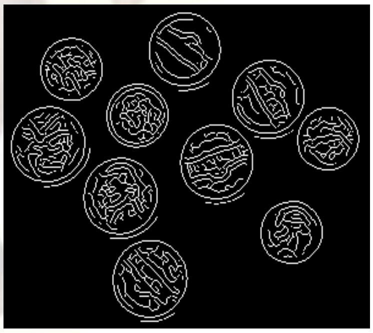
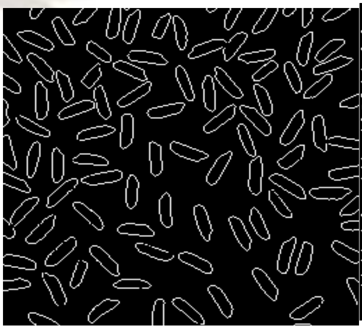
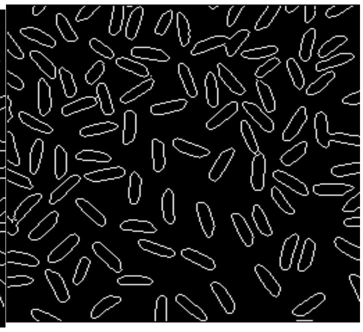


Fig 5B): Edge Detection through canny filter

Fig 5C): Edge Detection through Sobel filter

Now applying the same technique to the image shown in figure 1, results as shown.





a) **b)**

Figure 6: a) Sobel Edge detection b) Canny Edge Detection

The sobel filter has many edges missing in the particle and canny filter based edge detection has complete boundaries but the some of the particles present in close proximity have been merged together. This technique could not be implemented to enhance the image in terms of clear visualization of the particles.

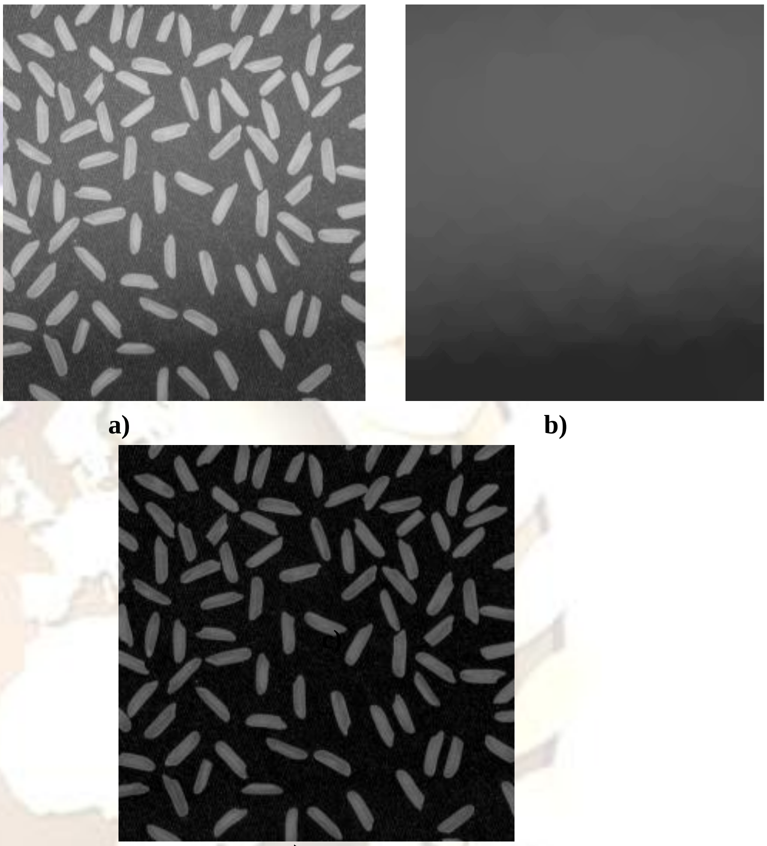
3 MORPHOLOGY

Mathematical morphology (MM) is a theory and technique for the analysis and processing of geometrical structures, based on set theory, lattice theory, topology, and random functions. MM is most commonly applied to digital images. Most of the techniques alone fail to accurately determine the objects real boundaries due to the problem of non-uniform illumination in the background . So, advanced image processing tools have to be used.

4. PROPOSED FLOW-CHART TO COMPARE VARIOUS IMAGE ENHANCEMENT TECHNIQUES

5. RESULTS

The modification performed uses the image enhancement after the removal of backround illumination for the lateral stages whereas in older algorithms, these enhancement methods were used in starting stages but also multiplied the effect of noise and non-uniform background. Results in uniform background that is suitable for particle analysis as shown



a) b)

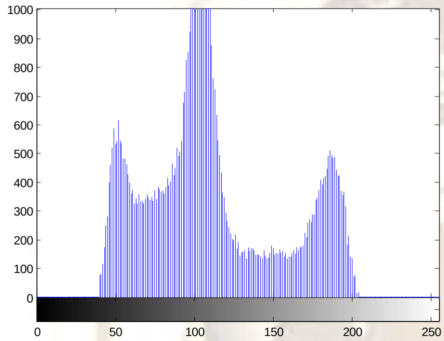
c)

Figure 7 a) Original Image with Non-Uniform Background7b) Non-uniform Background extraction from original image using morphological opening and successive erosion and dilation and structuring element approach. 7c) Iout = I – B, where Iout is the image obtained after the removal of non-uniform backround (B) from original image (I) uniform background throughout the image

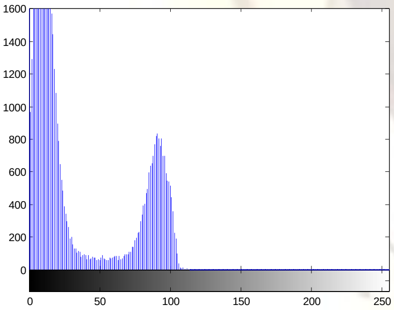
After removal of non-uniform illumination, we observe that the resulting image have the problem of less brightness than the original image due to morphological opening and particles appear to be slightly less bright than the original . In order to remove these problems, we performed image enhancement techniques at the output of the image including the contrast and brightness adjustment and finally thresholding was done

.
Figure 8: Final Image obtained for Particle Analysis application with full accuracy and non-uniform illumination removal and contrast enhancement obtained with new algorithm

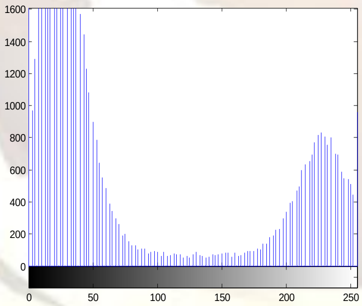
Histogram plot for the successive stages in fig 7 a), c) and fig 8 images have been compared and results are



9.x)



9.y)



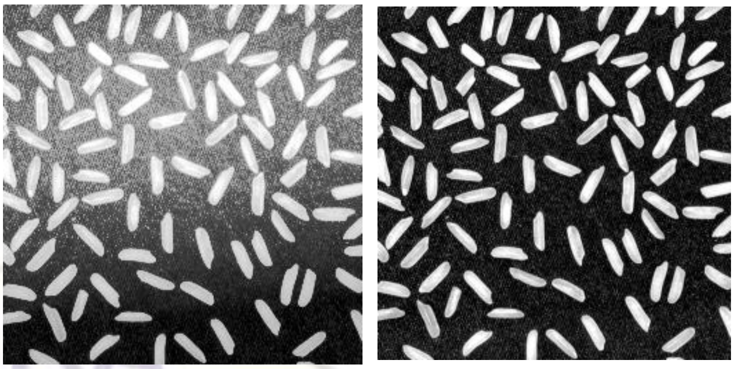
9.z)

Figure 9. x) **Histogram plot of original image**

Figure 9. y) Histogram plot after the removal of non-uniform backround indicating uniform variation corresponding the equal distributions of probability at the output.

Figure 9. z) Histogram plot of the final image indicating uniform distribution of graph (uniform backround) and Wide Dynamic Range for effective brightness of the image

Hence the desired output graph was obtained indicating removal of non-uniform backround illumination and image enhancement . Comparing the outputs from various techniques studied, following images were obtained.



a) b)

Figure 10: Comparision of the images obtained from old and new algorithm

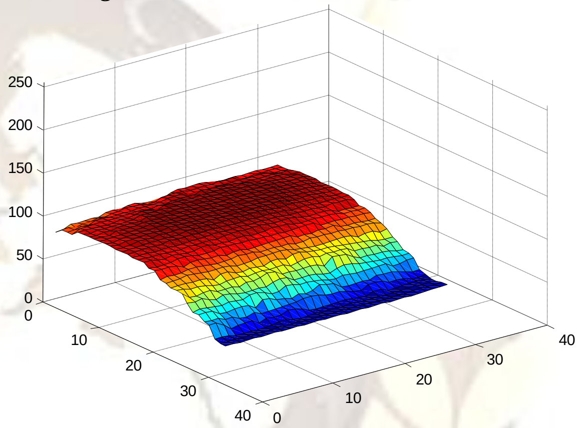


Figure 11) Surface approximation of background obtained by new algorithm based on morphological operations

6. CONCLUSIONS

The work indicates that histogram equalization technique can't be used for images suffering from non-uniform illumination in their backgrounds specifically for particle analysis purposes as this process only adds extra pixels to the light regions of the image and removes extra pixels from dark regions of the image resulting in a high dynamic range in the output image. The methodology used for solving the problem is estimating accurate background approximation as a surface to extract the non-uniform background from the image and then constructing the new image by subtracting this estimated background from the original image and then use the enhanced image to identify discrete objects/particles present in the image.

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