Non Uniform Background Removal using Morphology based Structuring Element for Particle Analysis

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ABSTRACT
Non Uniform Illumination in an image often leads to diminished structures and inhomogeneous intensities of the image due to different texture of the object surface and shadows cast from different light source directions. This effect is adverse in case of biological images. Techniques such as segmentation, edge detection and contrast or brightness enhancement using Histogram Equalization could not differentiate between some of the particles and their background or neighboring pixels. This paper is aimed to remove these problems in microscopic image processing by removing the problem of non-uniform background illumination from the image using Morphological Opening, Adaptive Histogram Equalization and Edge detection techniques for particle analysis .A comparative study have been shown and a new algorithm is proposed for removing the problem of non-uniform background illumination in biological images for visualizing and estimation of growth of fungus in a particular sample to transform the input image to its indexed form with maximum accuracy involving morphological openings and structuring element design using Morphological Processing.

Keywords
Morphological opening,skeletonization, Histogram Equalization, Thresholding, Structuring Element.

1. INTRODUCTION
Image processing technique is generally used to modify pictures to improve them, extract information (recognition), and change their structure (composition). Images can be processed by various means like optical, photographic, and electronic means, but using digital computers is the widely used method because of its speed, flexibility, and precise results. Image processing technology is used by scientists to view enhanced images of various planets. Doctors use the same to manipulate CT scans and MRI images. Image Enhancement improves the quality of images by removing blurring and noise, increasing contrast, and revealing details. The technique basically includes analyzing, manipulating, storing and displaying the graphical images from sources such as photographs, drawings and so on. Technique spans a sequence of 3 phases i.e acquisition, processing and displaying. [3]The image acquire phase converts the differences in coloring and shading in the picture into binary values that a computer can process. The enhancement phase includes image enhancement and data compression. The last phase consists of printing the processed image. Morphology means form and structure of an object. Sometimes it refers to the arrangements and inter-relationships between the parts of an object. Morphology is related to the shapes and digital morphology is a way to describe and analyze the shape of a digital object. In biology, morphology relates more directly to shape of an organism such as bacteria. Morphological opening is a name specific technology that creates an output image such that value of each pixel in the output image is based on a comparison of the corresponding pixel in the input image with its neighbors. By choosing the size and shape of the neighborhood, one can construct a morphological operation that is sensitive to specific shapes in the input image. Morphological functions could be used to perform common image processing tasks such as contrast enhancement, noise removal, thinning, skeletonization, filling and segmentation.

2. NON-UNIFORM BACKGROUND ILLUMINATION AND EFFECTS
Non-uniform illumination can be due to many reasons like aging filaments, faulty reference voltages, contaminated apertures or non-uniform support film fabrication. [1] Subtle electron illumination asymmetries are more evident at moderate-to-low magnifications and are often inadvertently enhanced by digital contrast adjustment. This Effect is similar to the intensity inhomogeneity problem observed in MRI. The MRI Intensity inhomogeneity problem is manifested as a slowly varying multiplicative effect in the acquired images. Similarly, the non-uniform illumination can be modeled as a multiplicative effect. The observed image is given as $f(x; y) = s(x; y)\, I(x; y) + n(x; y)$; where s is the true signal, I is the non-uniform illumination field and n is additive noise. The I-field varies slowly over the image; in other words, it does not have any high frequency content. Removal of non-uniform illumination effects is important for later processing stages such as image registration based on correlation metrics and segmentation based on intensity thresholding. The technique Particle Analysis helps to compute the details of the components present in the image, their shape, size, number and other characteristics of the particles present in an image. This problem is severe in case of microscopic images captured for bio-medical research where it is hard calculate exact size and number of microscopic particles due to non-uniform illumination. So, a particular defined area of a photographic plate is taken and exposed by the particles the characteristics of which are to be computed. So, the technique used would be to examine every particle of the image, to see clearly every object in the image, and remove any of the problems such as non-uniform illumination, less brightness etc.

that make it difficult to differentiate between the particles. Various techniques and common approaches to solve the problem of particle identification are Histogram Equalization, Image Filtering, Boundary detection, Edge Detection, Linear Filtering, Segmentation, Morphological operations: Dilation and Erosion etc. But most of these techniques fails to accurately determine the objects real boundaries due to non-uniform illumination in the background of the image due to which most of the particles appear to be either dark or light in an image and techniques such as histogram equalization, segmentation, edge detection and general image processing algorithms based on 'region of interest' could not differentiate between some of the particles and their background and thus shapes of the resulting object changes. Even when the particles are extracted, there are changes to their shape and size which leads to faulty readings in the computations of area of such particles. So, advanced image processing and image enhancement tools have to be used for maximum accuracy of the results and to identify the particles accurately from the image without even missing a single object.

3. TECHNIQUES FOR NON-UNIFORM BACKGROUND REDUCTION IN PARTICLE ANALYSIS AND RESULTS

Histogram equalization have been studied by editing the picture of microscopic bacteria. Related problems with these existing technologies are studied in the presence of non-uniform illumination field in the background of the image and an algorithm based on morphological opening and structuring element design have been studied in order to remove the problems of non-uniform background by background approximation techniques. Available techniques are listed here.

3.1 HISTOGRAM EQUALIZATION AND CONTRAST ENHANCEMENT

Histogram of an image represents the relative frequency of occurrence of grey levels within an image. Histogram modelling techniques modify an image so that its histogram has a desired shape. [2] Histogram equalization is used to enhance the contrast of the image such that it spreads the intensity values over full range. Under Contrast adjustment using histogram equalization, overall lightness or darkness of the image is changed, i.e. in this technique, pixel values below specified values are mapped to black and pixel values above a specified value are mapped to white. The result is linear mapping of a subset of pixel values to entire range of display intensities.

Fig 1: Grey Scale Image with low Image contrast

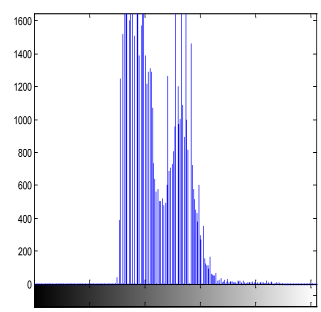


Fig 2: Histogram plot of the Input Image

Performing histogram equalization on the above image the results are shown below in figure 3 and its histogram in figure 4.

Fig 3: Grey scale image with histogram equalization

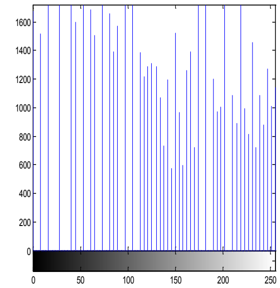


Fig 4: Histogram of output image after global histogram equalization

So, histogram equalization technique basically compares every pixel in the input image with a predefines pixel value that sets all the pixel values above the threshold values to be 1 i.e. white in colour and others below this value to be 0, or black. Considering this approach, histogram equalization technique was studied over the required image with non-uniform background as shown in figure 5.The resultant image is shown in figure 6.

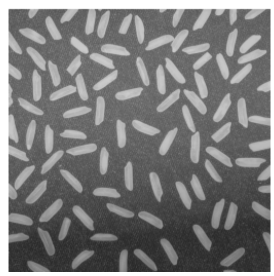


Figure 5: Grey-Scale Image showing a cluster of bacteria present in a fluid having non- uniform texture

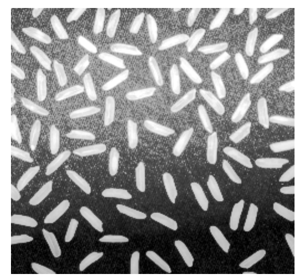


Figure 6: Resulting Image after Histogram equalization for contrast enhancement on the input image

As indicated in figure 6, it is clear that histogram equalization technique can't be used for images suffering from non-uniform illumination in their backgrounds. The histograms of both the images obtained shown below in figure 7 and 8.

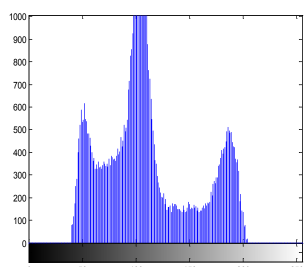


Fig 7: Histogram of input image (less dynamic range and high frequency variation)

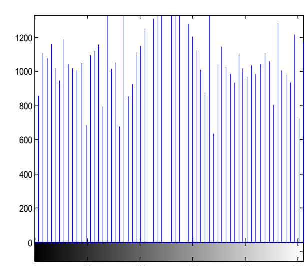


Fig 8: Histogram of the image as shown in fig 7 (high dynamic range and increased amplitudes)

Above histograms for the two techniques indicate that the dynamic range for the entire image is though improved but the amplitudes for various pixels near the center of the image with light backgrounds have been amplified resulting in excessive brightness near the particles present in specified locations making the resulting image unsuitable for Particle identifications and analysis.

Fig 9: Approximated non-uniform background image extraction using histogram equalization

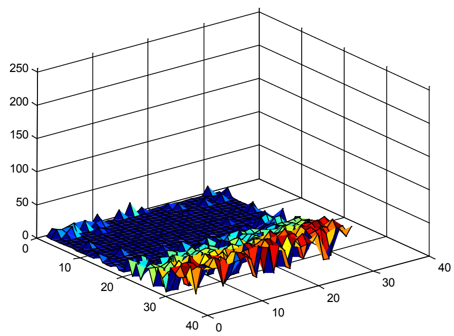


Fig 10: Surface approximation of the background attained using histogram equalization

Approximated background image estimated by the technique of histogram equalization is shown in fig. 10 and its surface approximation in fig.10.The surface display [0, 0] represents the origin, or upper left corner of the image. Surface approximation indicates the highest pixel values as a curvier portion and lower pixel values as a flat region. But the above region depicts a lot of irregularities in the background attained due to a lot of dark portion attained at the top of the image in the background. It is clear from the above graph plotting that histogram equalization alone could not be able to create an image of uniform background from non-uniform background due to the addition of large amplitude values to the lighter regions around the objects in case of particle analysis and hence results in faulty calculations at the end to determine each particle individually.

4. PROPOSED ALGORITHM AND WORK FOR NON-UNIFORM ILLUMINATION REMOVAL FOR PARTICLE ANALYSIS USING MORPHOLOGY

We have proposed an algorithm with morphological opening at first to first estimate the background of the image and then remove the non- uniform background illumination.

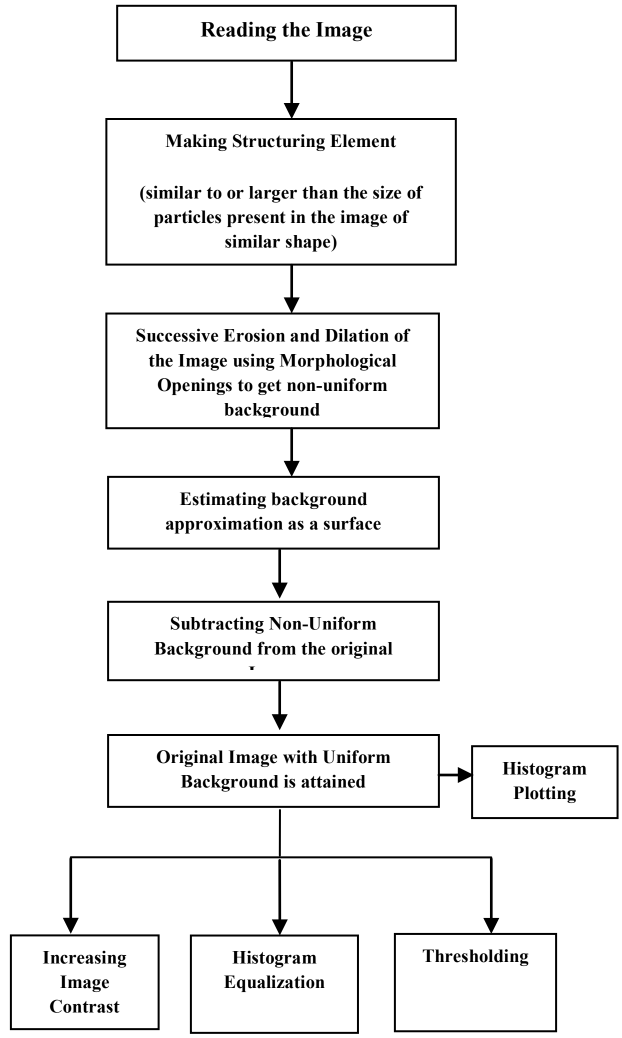


Fig.11: Algorithm Designed for non-uniform background removal for particle analysis and image enhancement

This is done by creating a structuring element of the size and shape similar to the particles present in the image (in this case, disk shaped: according to shape of bacteria) and morphological opening of the image with this structuring element.[5] Background approximation have been taken as the criteria to determine the close proximity to the non-uniform background extraction using various techniques such as Histogram Equalization, Linear Filtering and our new technique based on morphological processes and successive dilation and erosion followed by contrast enhancement for the accurate particle extraction for lateral image processing.

5. RESULTS

Image commands of Image Processing toolbox is used for computing the background of the image and enhancing the contrast, thresholding and computing the object statistics present in the image. Stepwise Results from the above operations are shown in figure 11.

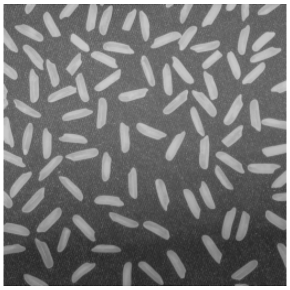


Fig 11.a) Original Microscopic Image with non-uniform background in which particle analysis is to be performed (bright in center, slightly bright in top and dark at the bottom)

Designing of a disk type structuring element was done and it was used in the successive dilation and erosion of the above image in order to perform morphological opening and non-uniform background field was estimated.

Fig 11.b) Non-Uniform background extraction by morphological operations in the above image

6. BACKGROUND APPROXIMATION AS A SURFACE

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. In the surface display [0, 0] represents the origin, or upper left corner of the image. Surface approximation indicates the highest pixel values as a curvier portion and lower pixel values as a flat region. [4] The accuracy of non-uniform illumination reduction and performance of the image enhancement for further particle analysis depends upon the factor s(x,y) to be extracted from the Image , I(x,y) where f(x; y) = s(x; y) I(x; y) + n(x; y);where f is the observed image, s is the true signal, I is the non-uniform illumination field and n is additive noise. Here, the function s is non-uniform illumination field that is variable and depends upon the many factors. N(x, y) is the external noise, an additive quantity and can be removed easily with the help of histogram equalization and brightness control techniques. But S(x, y) is a multiplicative quantity with the image and is difficult to remove as it is variable. With the help of background approximation from the original image, non-uniform field could be easily calculated and removed from the original image. S function is determined with the help of background approximation technique and with the help of our new methodology, accurate background has been estimated as compared to previous results from histogram equalization and linear filtering techniques.

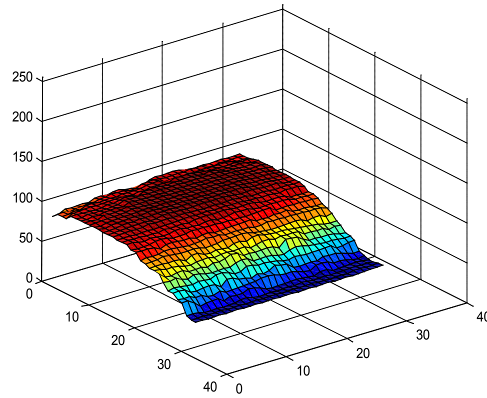
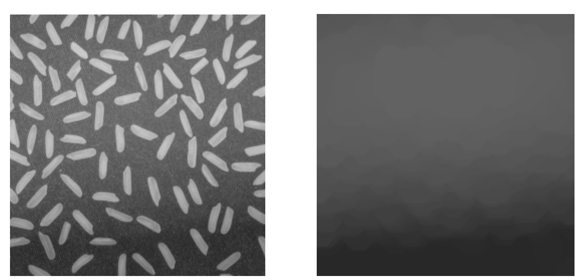
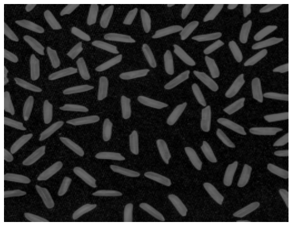


Fig.12 Background approximation as a surface in 3-d view

In the surface display, [0, 0] represents the origin, or upper left corner of the image. The highest part of the curve indicates that the highest pixel values of background (and consequently input image in figure 1) occur near the middle rows of the image. The lowest pixel values occur at the bottom of the image and are represented in the surface plot by the lowest part of the curve. This background approximation obtained is exactly similar to the original non-uniform background field and is a uniform 3-D graph with no-sudden changes in the surface plot as it was in case of other techniques such as histogram equalization. Resulting Image obtained after morphological opening is the difference of this background approximation from the original image with removal of non-uniform background problems. Further after the final image is obtained, there is still a remaining problem of noise in the image that is extracted by histogram equalization and contrast adjustment techniques. The modification performed uses the image enhancement after the removal of background illumination for the lateral stages whereas in older algorithms, these enhancement methods were used in staring stages but also multiplied the effect of noise and non-uniform background. Image obtained after subtracting the non-uniform field from the original image results in the required image with uniform background that is suitable for particle analysis as shown in figure 13.



a) **b)**



c)

Figure.13
a)Original Image with Non-Uniform Background. b)Non-uniform Background extraction from original image. c)Iout = I – B, where Iout is the image obtained after the removal of non-uniform background (B) from original image (I) uniform background throughout the image

After non-uniform illumination have been removed, we observe that the resulting image have the problem of less brightness than the original image due to morphological opening and the particles appear to be slightly less bright than their original view. 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. Image obtained after the image enhancement step results in the final image as shown in figure 14.

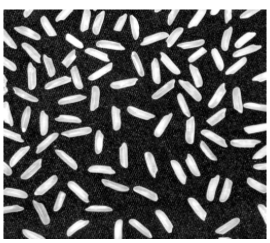


Figure 14: Final Image obtained for Particle Analysis application with new algorithm

Histogram plot for the successive stages in fig 13 a), 13 c) and fig 14 images have been compared and results have justified in following figure x, y, z of figure 15 correspondingly.

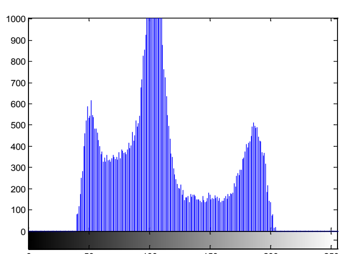


Figure 15. x) Histogram plot of original image in 24.a)

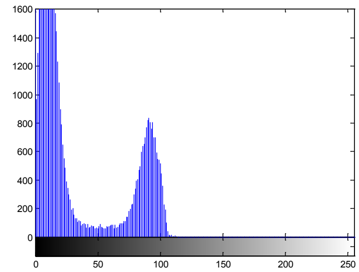


Figure 15. y) Histogram plot after the removal of non-uniform background indicating uniform variation in the image corresponding the equal distributions of probability at the output.

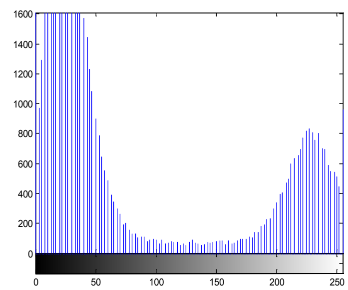


Figure 15.(z) Histogram plot of the final image indicating uniform background and Wide Dynamic Range for effective brightness of the image

7. CONCLUSIONS AND FUTURE WORK

It has been concluded that techniques such as segmentation, edge detection and contrast enhancement using Histogram Equalization could not differentiate between some of the particles and their background or neighboring pixels. For applications, involving particles/objects to be studied or analyzed, these techniques give faulty results due to changes in actual shapes and sizes of the particles in the resulting image. However the proposed technique produce optimum results. Histogram plots obtained from new algorithm indicates uniform distribution of intensities in the image along with contrast enhancement and wide dynamic range indicating clear visibility of the image along with effective non-uniform background removal. After non-uniform illumination removal the resulting image have the problem of less brightness than the original image due to morphological opening and the particles appear to be slightly less bright than their original view. 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. In future the proposal is to find characteristics of each particle, compute its area and to show results in area based statistics and histogram equalization.

7. REFERENCES

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