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MORPHOLOGY BASED NON UNIFORM BACKGROUND REMOVAL FOR PARTICLE ANALYSIS: A COMPARATIVE STUDY

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ABSTRACT- Non Uniform Illumination in an image often leads to diminished structures and inhomogeneous intensities due to different texture of the object surface and shadows cast from different light source directions. It is one of the most important factors affecting the appearance of an image, more adverse in case of biological images. Various techniques such as segmentation, edge detection and contrast enhancement using Histogram Equalization could not differentiate between some of the particles and their background. For applications, including particles/objects to be studied or analysed, these techniques give faulty results due to changes in actual shapes and sizes of the particles in the resulting image. 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 such as visualizing and estimation of growth of a 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 Opening. 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. Results reveal the various particles present in the image with exact boundaries along with the removal of non-uniform illumination at the background. Histograms are plotted of the resulting images and compared at the end. Surface approximation plots for background of images from two techniques are compared to estimate the exact non-uniform background from image by both the steps.

KEYWORDS: Morphology, Histogram Equalization, Thresholding, Structuring Element.

1.INTRODUCTION

Image processing is used to modify pictures to improve them (enhancement, restoration), extract information (analysis, recognition), and change their structure (composition, image editing). Images can be processed by optical, photographic, and electronic means, but image processing using digital computers is the most common method because digital methods are fast, flexible, and precise. Image processing technology is used by planetary scientists to enhance images of Mars, Venus, or other planets. Doctors use this technology to manipulate CT scans and MRI images. Image Enhancement improves the quality (clarity) of images for human viewing. Removing blurring and noise, increasing contrast, and revealing details are examples of enhancement operations. Image Processing basically includes analysis, manipulations, storage and display of graphical images from sources such as photographs, drawings and so on. Image processing spans a sequence of 3 phases, which are the image acquire, processing and display phase. The image acquires phase converts the differences in colouring and shading in the picture into binary values that a computer can process. The enhancement phase can include image enhancement and data compression. The last phase consists of display or printing of the processed image. The term **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 analyse 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

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value of each pixel in the output image is based on a comparison of the corresponding pixel in the input image with its neighbours. By choosing the size and shape of the neighbourhood, 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 have many sources: aging filaments, faulty reference voltages, contaminated apertures, or non-uniform support film fabrication. 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 modelled as a multiplicative effect. The observed image is given as

$f(x; y) = s(x; y)\, I(x; y) + n(x; y);$ (1.1) , 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. For example, an image might be taken of an endothelial cell, which might be of low contrast and somewhat blurred. Reducing the noise and blurring and increasing the contrast range could enhance the image. The original image might have areas of very high and very low intensity, which mask details. The image could have been taken in a non-uniform illumination environment which might make the details of the image less visible, our problem is to solve the issues of background illumination and enhance the image with the help of morphological operations for applications of particle analysis in microscopic images. Particle Analysis is a technique that helps to compute the details of the components present in the image, their shape, size (area) and number and other characteristics of the

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particles or objects present in an image. This research paper presents the work to be done on images having distorted or uneven background and filtering the images to compute statistics of the objects present in the image. This problem is severe in case of microscopic images captured for the purpose of bio-medical research where it is difficult to find out the exact shape, size and number of microscopic particles due to non-uniform illumination and sensitivity to even small fluctuations in light.

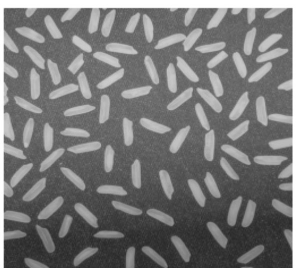

Figure 1: Grey-Scale Image showing a cluster of bacteria present in a fluid having non- uniform texture, brighter on the top and center portions and darker at the bottom.

So, a particular defined area of a photographic plate is taken and exposed by the particles the characteristics of which are to be computed. The technique used would be to make an algorithm to finally 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 on the microscopic image shown in figure 1. 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 alone fail to accurately determine the objects real boundaries due to the problem of 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 using 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 or neighbouring pixels and boundaries and shapes of the resulting object changes. Even when the particles

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

Particle Analysis of a biological image involves examining 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 common techniques such as Histogram equalization and edge detection have been studied by editing the input picture of microscopic bacteria as shown in figure 1. Related problems with these existing technologies are studied on 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 below.

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. 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 as an example given below.

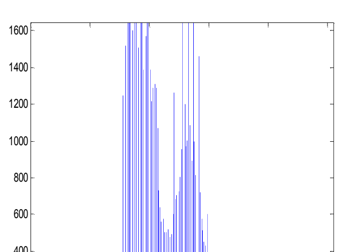

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Fig 2: Grey Scale Image with low contrast **Fig 3: Histogram plot of the Input Image**

Performing histogram equalization on the above image to spread the intensity values over the full range of the image improving the contrast and brightness of the overall image as shown below in figure 4 and its histogram in figure 5.

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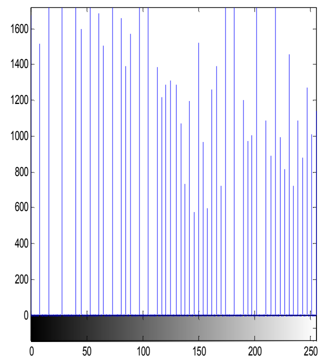
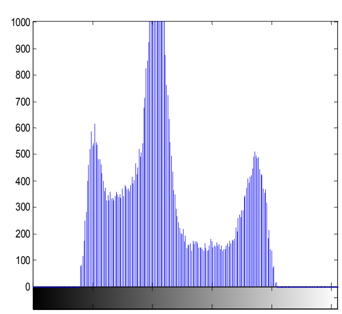


Fig 4: Grey scale image with histogram equalization

Fig 5: Histogram of output image after global histogram (contrast enhancement) equalization(dynamic range)

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. Histogram equalization is applicable to the gray scale images where the main target is to enhance the image in order to see the details in the image clearly for future processing and this is achieved by increasing the dynamic range of the entire histogram.The Following is the flow-diagram of algorithms followed in **previous approaches** to solve this desired problem:



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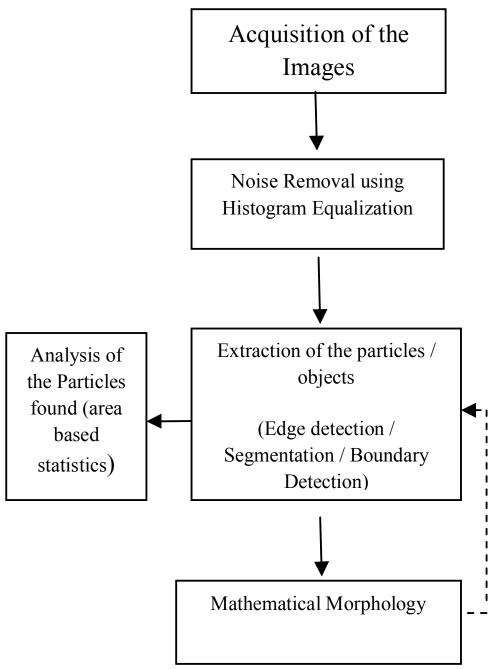

Fig.6 Image processing algorithm previously used.

Considering this approach, histogram equalization technique was studied over the required image with non-uniform background as shown in figure 1. Following the histogram equalization, resultant image is shown in figure 8.

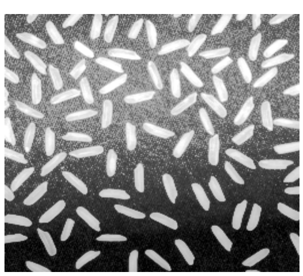

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Fig 7: Resulting Image after Histogram equalization for contrast enhancement on the input image shown above

As indicated in figure 8, it is clear 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 with the histograms of both the images obtained shown below in figure 8 and 9.

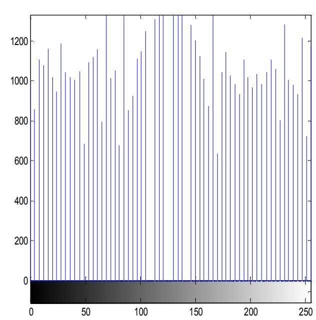

Fig 8: Histogram of the image as shown in fig 1 (less dynamic range and high frequency variation

Fig 9: 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.Approximated background image

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estimated by the technique of histogram equalization is shown in fig. 10 and its surface approximation in fig.11

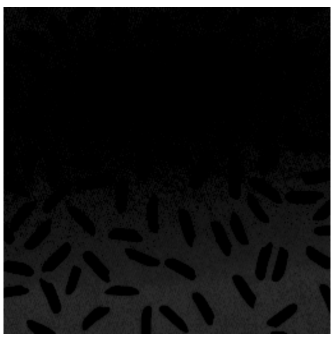

Fig 10: Approximated non-uniform background image extraction using histogram equalization

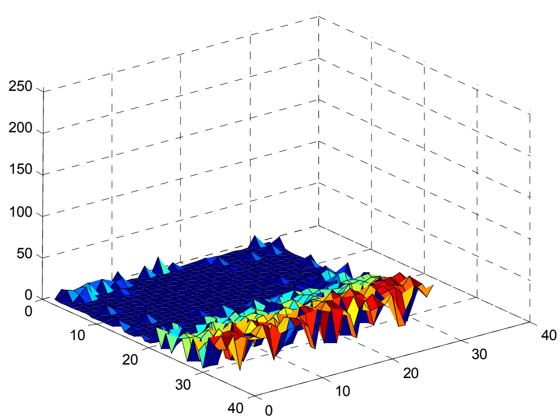

Fig 11: Surface approximation of the background attained using histogram equalization

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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. 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. Other techniques for particle identification under the condition of non-uniform background studied here are edge detection or boundary extraction techniques and their results shown in the next step.

3.2 EDGE DETECTION AND BOUNDARY EXTRACTION

[7] and [4] have studied the particle analysis based upon boundary tracings and edge detection techniques. 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. Operation sensitive area could also be defined in edge detection such that it includes places like detection across horizontal edges, vertical edges, or both. This technique when used returns a binary image of the input, i.e. 1’s and 0’s. Consider the images of coins in an image that need to be extracted using boundary extraction techniques. Following is an example of image of coins having a uniform background.

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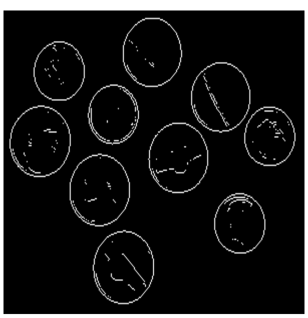
Fig 12: Image of coins to be used for boundary extraction purposes

Now, applying the sobel and canny edge detectors to the above image, resulting image obtained is shown in figure 13 and figure 14.



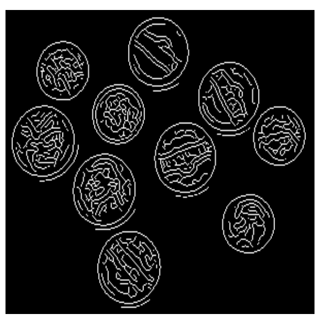
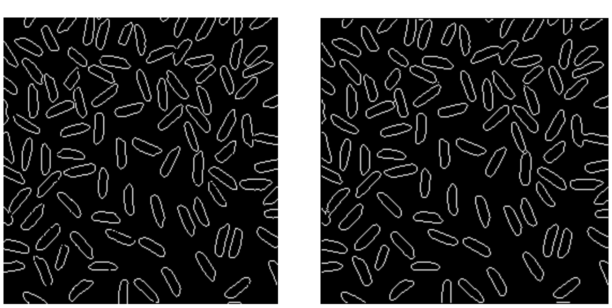


Fig 13: Edge Detection through Sobel filter

Fig 14: Edge Detection through canny filter

Above figures indicate effective boundary extraction for the images of coins using linear filtering approach. Now applying the same technique for particle extraction purposes in the image shown in figure 1, having non-uniform backgrounds results in the new images as shown in figure 15.



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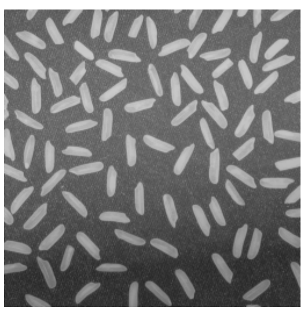
a) **b)**



c)

Figure 15:a) Sobel Edge detection b) Canny Edge Detection c)Original Image

Considering the figure 15, it could be seen that image obtained (a) after sobel filter has many edges missing in the particle analysis and canny filter based edge detection has complete boundaries but the some of the particles present in close proximity have been merged together giving the faulty readings at the final stages for particle based study and area based statistics plotting. Also, another problem faced with the edge detection technique is that it could be used only for rough particle extraction from an

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image and returns it in the form of binary image, i.e. in terms of 0's and 1's. This technique could not be implemented to enhance the image in terms of clear visualization of the particles present and give grey scale image at the output. Considering the problems faced due to non-uniform illumination at the background of the image in particle analysis, we propose a new algorithm for image analysis and non-uniform background elimination from the microscopic image shown in figure 1 based on morphological openings and structuring element design followed by histogram equalization and contrast enhancement.

3.3 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, but it can be employed as well on graphs, surface meshes, solids and many other spatial structures, Topological and Geometrical space concepts such as size, shape, convexity, and geodesic distance, can be characterized by MM on both continuous and discrete spaces. MM is also foundation of morphological image processing, which consists of a set of operators that transform images according to the above characterizations.

Denoting an image by *f(x)* and the structuring function by *b(x)*, the grayscale dilation of *f* by *b* is given by:

$$(f \oplus b)(x) = \sup_{y \in E}[f(y) + b(x-y)] \quad -1$$

where "sup" denotes the supremum. Similarly, the erosion of *f* by *b* is given by

$$(f \ominus b)(x) = \inf_{y \in E}[f(y) - b(y-x)] \quad -2$$

MM was originally developed for binary images, and was later extended to grayscale functions and images. The subsequent generalization to complete lattices is widely accepted today as MM's theoretical foundation. In binary morphology, an image is viewed as a subset of a Euclidean space Rd or the integer grid Zd, for some dimension *d*. In the morphological dilation and erosion

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operations, the state of any given pixel in the output image is determined by applying a rule to the corresponding pixel and its neighbors in the input image. The rule used to process the pixels defines the operation as dilation or erosion. Dilating a binary image, we create an output image such that the value of the output pixel is the maximum value of all the pixels in the input pixel's neighborhood. In a binary image, if any of the pixels is set to the value 1, the output pixel is set to 1 as shown in figure below.

structuring element

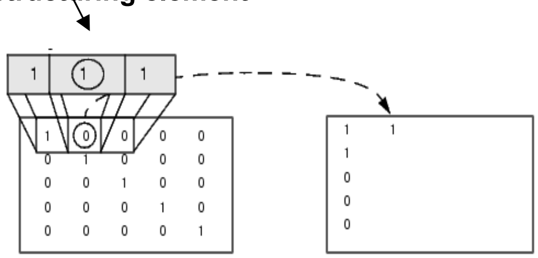

Figure 16: Dilating a binary image with a structuring element

Note how the structuring element defines the neighborhood of the pixel of interest, which is circled. The dilation function applies the appropriate rule to the pixels in the neighborhood and assigns a value to the corresponding pixel in the output image. In the figure, the morphological dilation function sets the value of the output pixel to 1 because one of the elements in the neighborhood defined by the structuring element is on. Considering this morphological dilation on a grey scale image,

structuring element

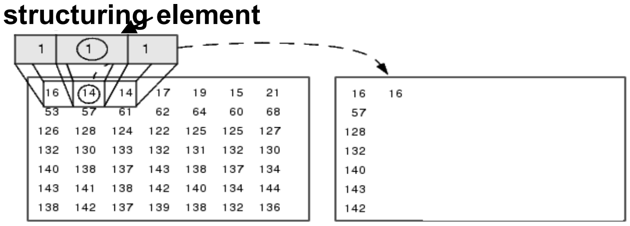

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Figure 17: Dilating a grey scale image with a structuring element

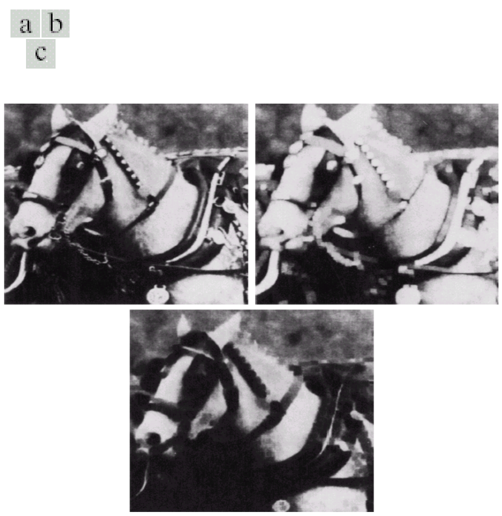

Fig.18(a) Original Image (b) Result of Dialation (c) Result of Erosion.

Erosion on the other hand removes the extra pixels from the specified areas. Above picture of a horse shows the basic concept of dilation and erosion of the image. But these operations alone could not be utilized for the extraction and analysis of particles of an image having non-uniform background illumination. Some of the basic elements of binary morphology includes Structuring Element, Basic Operators: Euclidean Distance and Shift Invariant, Erosion and Dilation and Opening and Closing of binary Images. The basic idea in binary morphology is to probe an image with a simple, pre-defined shape, drawing conclusions on how this shape fits or misses the shapes in the image. This simple "probe" is called structuring element, and is itself a binary image. Dilation adds pixels to the boundaries of objects in an image, while erosion removes pixels on object boundaries.Grayscale structuring elements are also functions of the same format, called "structuring functions". Various techniques and common approaches to solve the problem of particle identification are Image Filtering, Boundary detection, Edge Detection, Linear Filtering, Segmentation, Morphological operations: Dilation and

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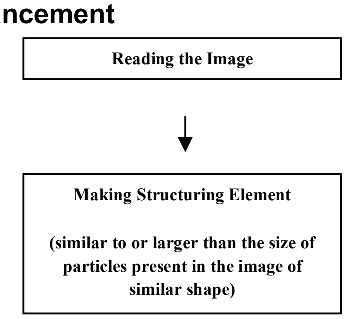
Erosion etc. But most of these techniques alone fail to accurately determine the objects real boundaries due to the problem of non-uniform illumination in the background of the image due to which most of the particles appear to be either dark or light in an image and using techniques such as histogram equalization, edge detection and general image processing algorithms based on 'region of interest' could not differentiate between some of the particles and their background or neighboring pixels as proved in the above steps. 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.

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 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. 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.

Algorithm Designed for non-uniform background removal for particle analysis and image enhancement



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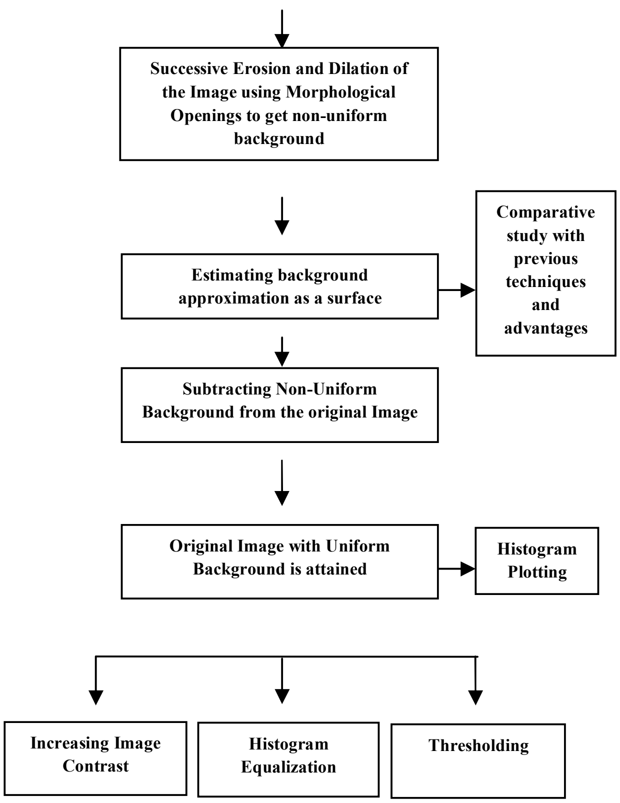

Fig.19

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 20.

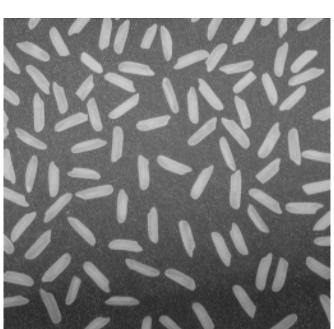

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Fig 20.a) Original Microscopic Image with non-uniform background in which particle analysis is to be performed (bright in center, slightly bright in top and dark at the bottom)

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 20.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

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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. 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); (1.1)

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.

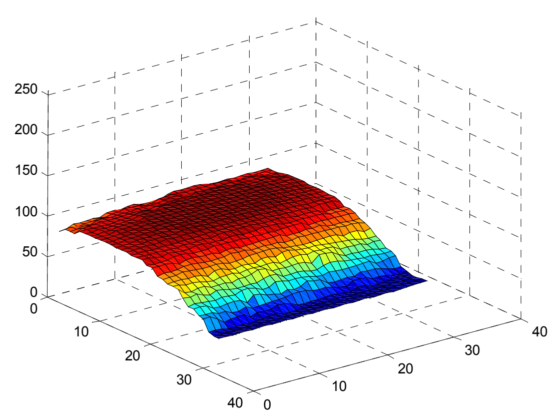

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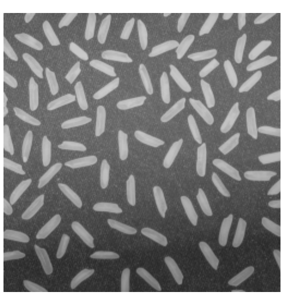
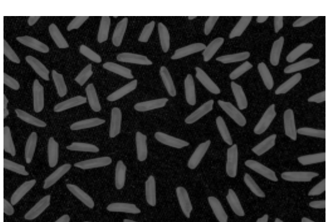
Fig.21. Background approximation as a surface in 3-d view

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 22.



a) **b)**



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c)

Figure 22

a) Original Image with Non-Uniform Background.

b) Non-uniform Background extraction from original image using morphological opening and successive erosion and dilation techniques and structuring element approach

b) 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 23 that is computed to be suitable for applications in particle analysis.

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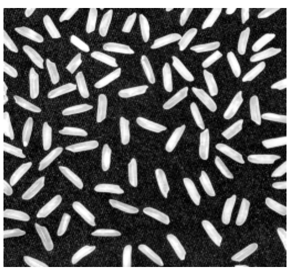

Figure 23: Final Image obtained for Particle Analysis application with full accuracy and non-uniform illumination removal and contrast enhancement obtained with new algorithm

Histogram plot for the successive stages in fig 22 a), c) and fig 23 images have been compared and results have justified in following figure x, y, z of figure 24 correspondingly.

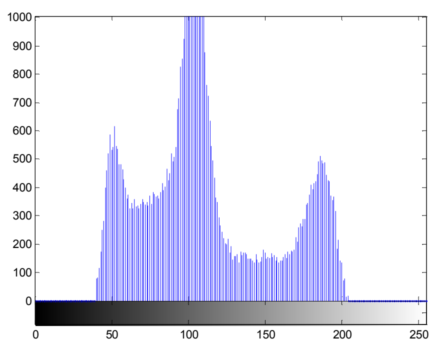

Figure 24. x) Histogram plot of original image in 24.a)

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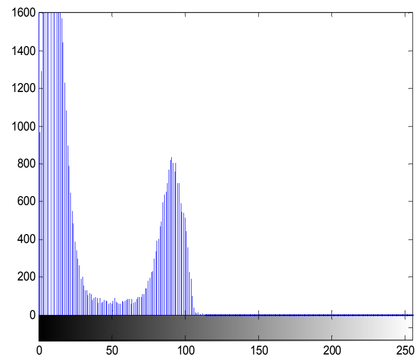

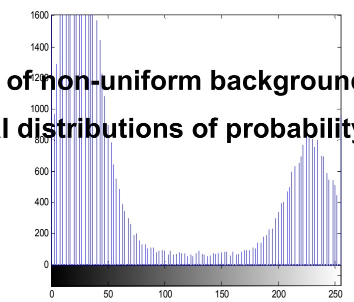

Figure 24. y) Histogram plot after the removal of non-uniform background indicating uniform variation in the image corresponding the equal distributions of probability at the output.

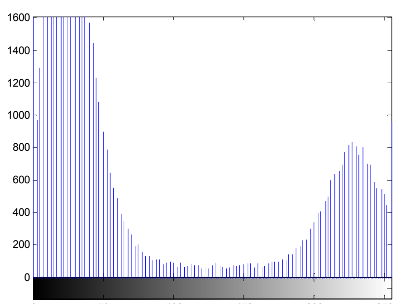

Figure 24.(z) Histogram plot of the final image indicating uniform distribution of graph (uniform background) and Wide Dynamic Range for effective rightness of the image

After obtaining the histogram plot on the successive images, the desired output graph was obtained with the new algorithm designed indicating both, the removal of non-uniform background illumination and image enhancement and clear visualization shown on the histogram plot in the image 24.(z).

7.COMPARISION OF RESULTS

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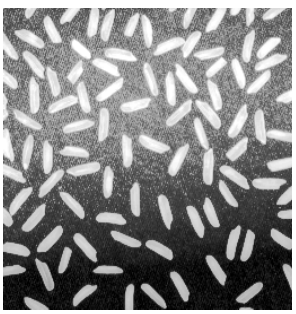
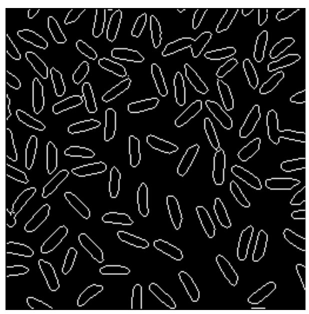
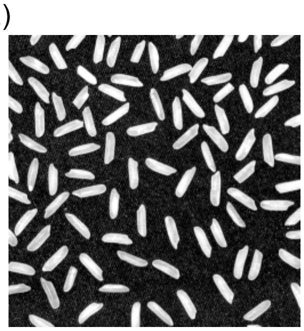
Comparing the outputs from various techniques studied, following images were obtained from the input image.





a) b)



c)

Figure 25: Comparision of the images obtained from old and new algorithm

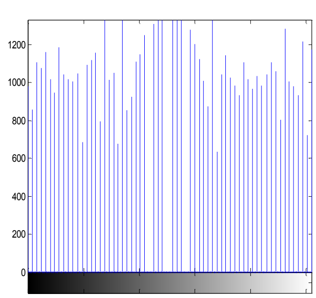


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Figure 26: a) b)

a) Histogram plot from new algorithm

b) Histogram plot from old algorithm

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, whereas, the histogram plot obtained from the old algorithm using conventional image processing operations such as histogram equalization and segmentation based on edge detection is shown in figure b where that indicates inhomogeneous intensities at the output displaying lack of removal of non-uniform background. Thus it is clear 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 histogram for the two old 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 for biological and microscopic image processing. it could be seen that image obtained (a) after sobel filter has many edges missing in the particle analysis and canny filter based edge detection has complete boundaries but the some of the particles present in close proximity have been merged together giving the faulty readings at the final stages for particle based study and area based statistics

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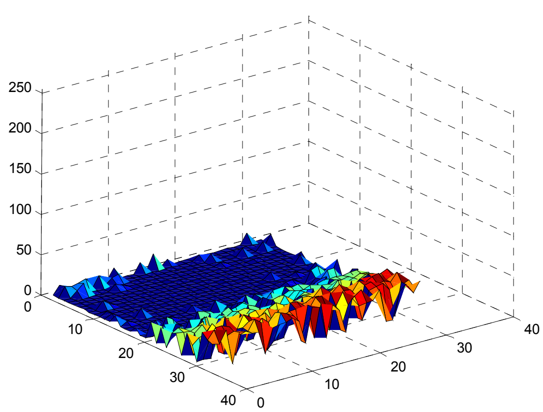
plotting. Also, another problem faced with the edge detection technique is that it could be used only for rough particle extraction from an image and returns it in the form of binary image, i.e. in terms of 0's and 1's. This technique could not be implemented to enhance the image in terms of clear visualization of the particles present and give grey scale image at the output. The approach used to solve the problems of non-uniform illumination suggested in the paper however results in the image figure 26, c) indicating accurate particle identification and image enhancement resulting in the removal of non-uniform background and clear image visualization of particles present in the 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. 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. Following are the background approximations obtained by the two algorithms.



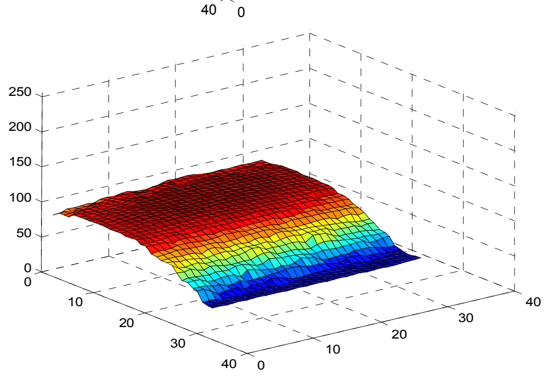


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Figure 27. a) Surface approximation of background obtained by old algorithm

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

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.

4. CONCLUSIONS AND FUTURE WORK

It has been concluded that 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 neighbouring pixels. For applications, including particles/objects to be studied or analysed, 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

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contrast enhancement and wide dynamic range indicating clear visibility of the image along with effective non-uniform background removal, whereas, the histogram plot obtained from the old algorithm using conventional image processing operations such as histogram equalization and segmentation based on edge detection indicates inhomogeneous intensities at the output displaying lack of removal of non-uniform background. So it is clear 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. It could be seen that image obtained after sobel filter has many edges missing in the particle analysis and canny filter based edge detection has complete boundaries but the some of the particles present in close proximity have been merged together giving the faulty readings at the final stages for particle based study and area based statistics plotting. Also, another problem faced with the edge detection technique is that it could be used only for rough particle extraction from an image and returns it in the form of binary image, i.e. in terms of 0's and 1's. This technique could not be implemented to enhance the image in terms of clear visualization of the particles present and give grey scale image at the output. The approach used to solve the problems of non-uniform illumination suggested in the paper. 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.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.

REFERENCES

[1] Emerson Carlos Pedrino,et al."A Genetic Programming Approach to Reconfigure a Morphological Image Processing Architecture", pp1-10,2011.

ISSN (Online) 2249 - 054 X

International Journal of Computing and Corporate Research

Specialized and Refereed Journal for Research Scholars, Academicians, Engineers and Scientists

http://www.ijccr.com

VOLUME 1 ISSUE 3 MANUSCRIPT 14 NOVEMBER 2011

[2] YanFeng Sun,et al.“A Multi-scale TVQ-based Illumination Normalization Model” ,vol.I pp1-6, 2011.

[3]Yadong Wu,et al.”An Image Illumination Correction Algorithm based on Tone Mapping”, IEEE pp245-248, 2010.

[4] M Rama Bai,“A New Approach For Border Extraction Using Morphological Methods”, pp3832-3837, 2010.

[5] Kevin Loquin,et al. “Convolution Filtering And Mathematical Morphology On An Image: A Unified View”, pp1-4, 2010.

[6] Przemysław Kupidur,”Semi-automatic method for a built-up area intensity survey using morphological granulometry”,pp271-277, 2010.

[7] Abhishek Acharya,et al. “FPGA Based Non Uniform Illumination Correction in Image Processing Applications Vol 2”, pp349-358, 2009.

[8] Komal Vij,et al. “Enhancement of Images Using Histogram Processing Techniques Vol 2” , pp309-313, 2009.

[9] Jean-Michel Morel,et al. “Fast Implementation of Color Constancy Algorithms Vol.19”, pp2825-2837, 2009.

[10] Yan Wan,et al.”A Dual Threshold Calculating Method for Fiber’s Edge Extraction”, IEEE pp 247-254,2009.

[11] M. Kowalczyk,et al.“Application of mathematical morphology operations for simplification and improvement of correlation of images in close-range photogrammetry”,pp153-158, 2008.

[12] David Menotti,“Contrast Enhancement in Digital Imaging using Histogram Equalization” ,pp1-85, 2008.

[13]Ley,et al.“GPU-Based Background Illumination Correction for Blue Screen Matting” , pp1-102, 2007.

[14]Joanna Sekulska,et al.” Digital image processing methods in biological structure recognition a short review”,pp24-27, 2006.

[15] M. Ranzato,et al.“Automatic Recognition of Biological Particles in Microscopic Images”,pp1-19, 2006.