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STATISTICAL PARTICLE ANALYSIS IN MICROSCOPIC IMAGES USING MORPHOLOGY

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Abstract: An accurate and simple algorithm has been proposed to identify the microscopic particles present in an Image and compute Area Based Statistics of each and every particle in the Image accurately using Morphological Operations and Image Enhancement features with efficient removal of various negative factors including background noise and non-uniform illumination due to non alignment of the photographic camera with the microscope used to capture such images using an Image of cluster of bacteria obtained in a sample having non-uniform background illumination. Based on this algorithm and structuring element approach, various statistics of particles in an image have been computed including area, Centroid and bounding box of every particle in the image and area based statistics have been formulated.

Keywords: Pixel Region Matrix, Morphology, Image Enhancement.

1. INTRODUCTION

Due to non-uniform background illumination, most of the particles appear to be either dark or light in an image and using techniques such as 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. 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. Work is proposed in this paper to remove these problems in microscopic image processing by firstly removing the problem of non-uniform background illumination from the image using Morphological Opening in Image Processing Toolbox of MATLAB by estimation of the backround of image, adaptive Histogram Equalization and Image Enhancement to transform the input image to its indexed form with maximum accuracy involving thresholding and contrast adjustment techniques and finally employing the connected component strategy to find out number of particles present in the image, creating pseudo colored index image to compute the characteristics of every particle clearly and computing the characteristics of every particle and finally plotting area based statistics and histogram of the final image. Morphology is related to the shapes and digital morphology is a way to describe and analyse the shape of a digital object. 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 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. LITERATURE SURVEY

Various particle analysis techniques that have been proposed in research papers include edge detection and histogram modelling techniques. There are many research papers that propose Histogram modelling techniques to modify an image so that its histogram has a desired shape. This is useful in stretching the low-contrast levels of an image with a narrow histogram, thereby achieving contrast enhancement. This is driven by the lack of removal of non-uniform illumination, as a result of which presence of extra light pixels at some positions in the image and extra dark pixels around other particles in the image is variable, so this contrast enhancement at the starting of the image processing does not create the accurate boundaries of the objects to be detected.

3. PROBLEM FORMULATION

This research 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

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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.

4. RESEARCH PROBLEM

To propose and evaluate an accurate and simple algorithm to identify the microscopic particles present in an Image and compute Area Based Statistics of each and every particle in the Image accurately using Morphological Operations and Image Enhancement features with efficient removal of various negative factors including background noise and non-uniform illumination due to non alignment of the photographic camera with the microscope used to capture such images using an Image of cluster of bacteria obtained in a sample having non-uniform background illumination. Our problem is to solve the issues of background illumination and enhance the image with its contrast, histrogram equalization, and calculate the details of the components present in the image, their shape, size (area) and number and finally plot the area based statistics and histogram of the area. The idea behind these thesis is to enhance and modify the images taken under very dark environment and correcting one of the most common problems of non-uniform background illumination using efficient image processing tools under MATLAB software. 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. This research work to be done in this thesis is basically based upon images taken for biological studies such as images consisting of cluster of cells, bacteria, or other particles where it is important to find out the concentration of the particles. So, 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. Finally, when all the visualization and analyzing problems are removed, the characteristics of each particle, its area has to be computed and results would be shown in area based statistics and histogram equalization

The characteristics of the individual object/bacteria are to be found using MATLAB *.m* files and then computing statistics for all the similar particles in the image.

Figure 1: Image (Gray Scale Image) Showing a Cluster of Bacteria Present in a Fluid Having Non- uniform Texture

5. RESEARCH METHODOLOGY

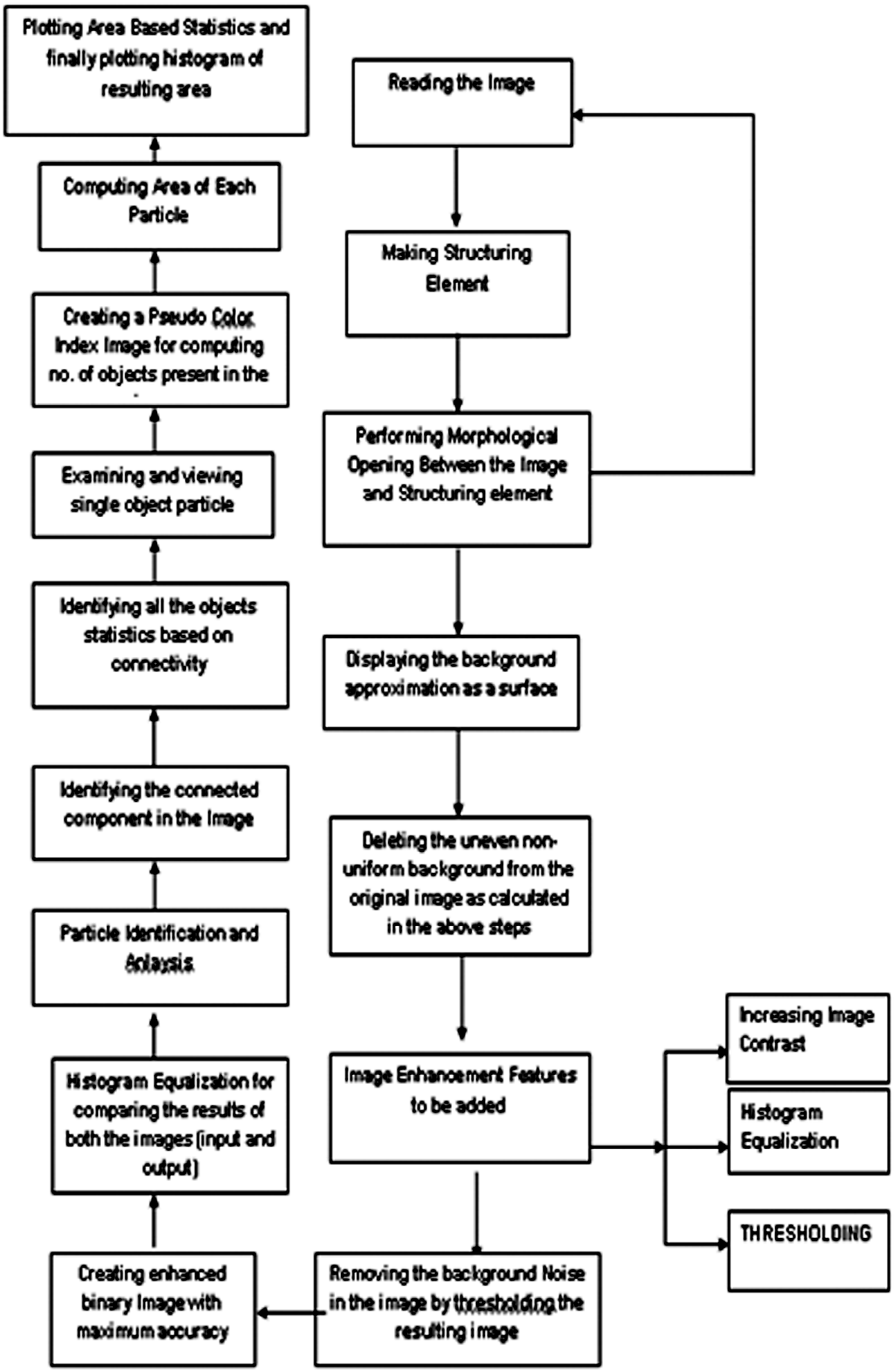
The approach here used is morphological opening to estimate the background of the image and adaptive histogram equalization. Also, other enhancements such as enhancing contrast of the image by controlling the threshold of the texture, identifying the objects present in the image individually, computing area of each object (in terms of pixels) and finally plotting Area-based statistics and histograms.

Imaq 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. Finally, histogram equalization technique would be used to compute the area based statistics.

6. TECHNIQUES USED

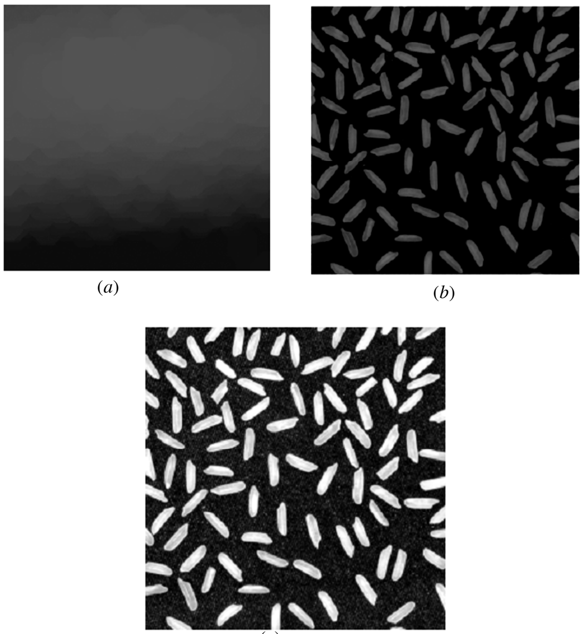
Various techniques and common approaches to solve the problem of particle identification are Image Filtering, Boundary detection, Edge Detection, Linear Filtering, Segmentation, Morphological operations: Dilation and Erosion etc. But most of these techniques alone fail to accurately determine the objects 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 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. So, advanced image processing and image enhancement tools have to be used for maximum accuracy of the results without even missing a single object.

7. ALGORITHM FOR NON UNIFORM ILLUMINATION REDUCTION AND PARTICLE ANALYSIS



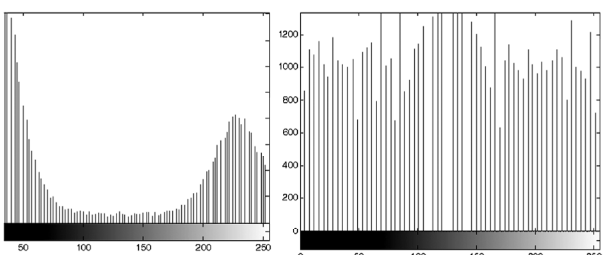
8. RESULTS

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



(*a*) (*b*) (*c*)

Figure 2: (*a*) Extraction of Non-Uniform Background by Morphological Operations. (*b*) Image Obtained with Uniform Background (*c*) Binary Image After Thresholding

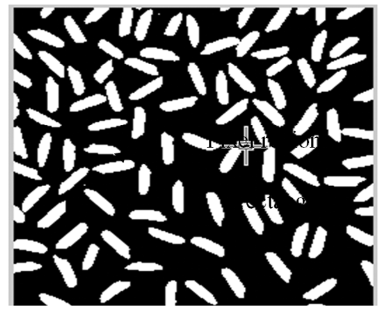


(*a*) (*b*)

Figure 3: (*a*) Histogram Plot of Final Image with Uniform Background (*b*) Histogram Plot of Original Image

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.

Viewing the Pixel Values using Labelled Matrices



(*a*)

63	63	63	0	0	0	0	0
63	63	63	63	0	0	0	0
63	63	63	0	0	0	0	0
63	63	63	0	0	0	0	0
63	63	63	0	0	0	0	0
63	63	0	0	0	0	0	0

(*b*)

Figure 4: (*a*) Pixel Region Rectangle (*b*) Pixel Region Matrix Inside the Rectangle

A pixel reigon rectangle is then created as shown above on the basis of 4-connected approach or connected-connected component strategy. The bwlabel function labels all the components in the binary image bw and returns the number of components it finds in the image in the output value, numObjects. [labeled,numObjects] = bwlabel(bw,4);

Result:

numObjects

ans = 101; indicating total number of objects computed to be 101 using pixel region matrix. Next step is to measure object or region properties in an image and return them in a structure array. When applied to an image with labelled components, it creates one structure element for each component.

Computing Area, Centroid And Bounding Box Of Every Particle In Image

The regionprops function returns three commonly used measurements: area, centroid (or center of mass), and bounding box. The bounding box represents the smallest rectangle that can contain a region.

particledata(51).Area

particledata(51).BoundingBox

particledata(51).Centroid

Results:

Particledata = 101×1 struct array with fields:

Area

Centroid

BoundingBox

ans = 140 (Area of Particle 51)

ans = 107.5000 4.5000 13.0000 20.0000 (Centroid of Particle 51)

ans = 114.5000 15.4500 (Bounding Box of Particle 51)

Similarly, we can find out values of Area, Centroid and Bounding Box of every Particle in the image using following commands:

particledata(*n*).Area

particledata(*n*).BoundingBox

particledata(*n*).Centroid

where, *n* is the number of particle in labelled matrix.

Maximum sized particle statistics computation

max([particledata.Area])

Results:

returns the area of maximum sized particle

ans =(Area of largest Particle)

Finding Particle Number with Largest Area

LargestParticle = find([particledata.Area]==404)

Results:

returns the number of the largest particle from labelled matrix

LargestParticle = 59

Finding Mean of all Particle Sizes to give an Average Area

mean([particledata.Area])

Results: ans = 175.0396

AVERAGE AREA BASED STATISTICS

Making a histogram containing 20 bins that show the distribution of particle sizes. The histogram shows that the most common sizes for Particles in this image are in the range of 150 to 250 pixels.

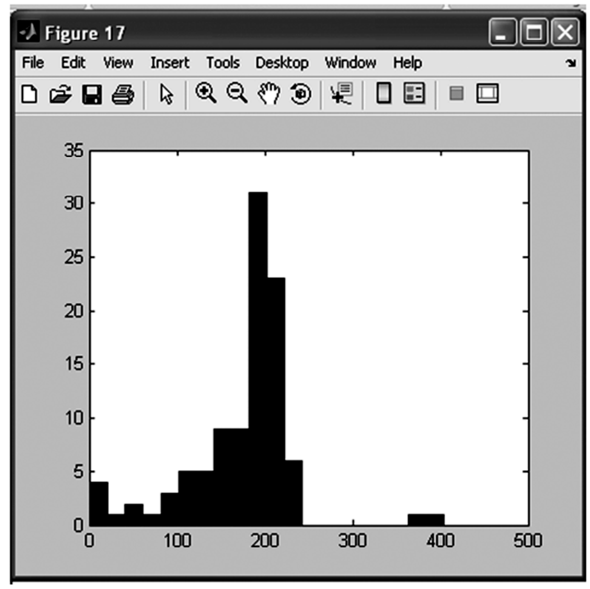


Figure 5: Area Based Statistics Computation

9. CONCLUSION AND FUTURE SCOPE

It has been concluded that due to non-uniform background illumination, most of the particles appear to be either dark or light in an image and using techniques such as 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. In future, it is plan to perform the simulations and analysis of an image by using this technique to correct for non uniform illumination and then use the enhanced image to identify discrete objects/ particles present in the image. The work to be done is based upon images taken for biological studies such as images consisting of cluster of cells, bacteria, or other particles where it is important to find out the concentration of the particles. Finally, when all the visualization and analyzing problems are removed, the characteristics of each particle, its area has been computed and results are be shown in area based statistics and histogram equalization. Also, the area based statistics computations indicate that the most common sizes for Particles in this image are in the range of 150 to 250 pixels.

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