Correction of Non-Uniform illumination for Biological Images Using Morphological Operation Assessing with Statistical Features Quality.

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Received in :23/March/2016,Accepted in :17/May/2016

Abstract

Non Uniform Illumination biological image often leads to diminish structures and inhomogeneous intensities of the image. Algorithm has been proposed using Morphological Operations different types of structuring elements including (dick, line, square and ball) with the same parameters of (15).To correct the non-uniform illumination and enhancement biological images, the non-uniform background illumination have been removed from image, using (contrast adjustment, histogram equalization and adaptive histogram equalization)**.** The used basic approach to extract the statistical features values from gray level of co-occurrence matrices (GLCM) can show the typical values for features content of biological images that can be in form of shape or specific features. In this research, the application of gray level co-occurrence matrix (GLCM) statistical features correlation, contras, energy and Homogeneity have presented these features which have high accuracy and efficiently. The color biological images had been used taken which is from microbiology laboratory at the Biological Department College of Science Al-MustansiriyhUniversity. The algorithms have been applied on ten different biological color images, in this work only two images have been displayed.

Keywords: Non uniform illumination, morphological operations, histogram Equalization, (GLCM) statistical features

Introduction

Images play an increasingly important role in many fields of science and its countless applications. Biology is one of the best examples of fields that have come to depend heavily upon images for progress. As a consequence of the ever increasing resolving power and efficiency of microscopic image acquisition hardware and the rapidly decreasing cost of mass storage and communication media, biological image data sets grow exponentially in size and carry more and more information. Extracting this information by visual inspection and manual measurement is labor intensive, and the results are potentially inaccurate and poorly reproducible. Hence there is a growing need for computerized image processing and analysis, not only to cope with the rising rate at which images are acquired, but also to reach a higher level of sensitivity, accuracy, and objectivity than can be attained by human observers. The future biologists will increasingly resort to automated image processing and analysis technology in exploiting their precious data. In order to benefit from this technology, it is importance to have at least a basic understanding of its underlying principles: biologically highly relevant information and improper use of image processing and analysis tools [1, 2 and].The image acquires phase which converts the differences in coloring and shading in the picture into binary values that a computer can process. The enhancement phase includes image enhancement 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 of 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 [4].

Non-uniform illumination

Background problems due to 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. The observed image is given as the following equation [5].

$$f(x; y) = [s(x; y) I(x; y)] + n(x; y) \quad (1)$$

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 processing image registration based on correlation metrics. 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 biological images. Morphological operations is a technique that helps to compute the details of the components present in the image, their shape, size (area) and other characteristics of the particles or objects present in an image [6,7].

Morphlogical Operations

Morphology is a technique of image processing based on shapes. The 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, you can construct morphological operation that is sensitive to specific shapes in the input image [8].Morphological operations such as erosion, dilation, opening, and closing. Often combinations of these operations are used to perform morphological image analysis. There are many useful operators defined in mathematical morphology. Morphological operations apply structuring elements to an input image, creating an output image of the same size. Irrespective of the size of the structuring element, the origin is located at its center [9].

Opening and Clouding

The two basic operations, dilation and erosion, can be combined into more complex sequences. The most useful of these for morphological filtering are called opening and closing [10]. Opening consists of an erosion followed by a dilation and can be used to eliminate all pixels in regions that are too small to contain the structuring element. In this case the structuring element is often called a probe, because it is probing the image looking for small objects to filter out of the image. The Opening process is as equation below:

$$AoB = (A\Theta B) \oplus B \quad (2)$$

Opening generally smoothed the contour of an image, breaks narrow gaps.

Structuring Elements

Morphological techniques probe an image with a small shape or template called a structuring element. The structuring element is positioned at all possible locations in the image and it is compared with the corresponding neighborhood of pixels. Some operations test whether the element "fits" within the neighborhood, while others test whether it "hits" or intersects the neighborhood. A structuring element is simply a binary image that allows us to define arbitrary neighborhood structures. The structuring element is a small binary image, i.e. a small matrix of pixels, each with a value of zero or one. The matrix dimensions specify the size of the structuring element. The pattern of ones and zeros specifies the shape of the structuring element. [10].Two dimensional or flat, structuring elements consist of a matrix of 0's and 1's, typically much smaller than the image being processed. The center pixel of the structuring element, called the origin, identifies the pixel of interest—the pixel being processed. The pixels in the structuring element containing 1's define the neighborhood of the structuring element. These pixels are also considered in the dilation or erosion processing. Three dimensional or non-flat, structuring elements use 0's and 1'sto define the extent of the structuring element in the *x*- and *y*-plane and add height values to define the third dimension [4].Table (1): include kind of structure element and using in this paper and its description**.**

Contrast Enhancement

Analysis of a biological image involves examining every particle of the image and removes any of the problems such as non-uniform illumination, less brightness etc. contrast enhancement techniques are used to enhance the contrast of the biological image, it spreads the intensity values over full range. The enhancement techniques are used to improve biological image.

Contrast Adjustment

Contrast Adjustment

Intensity adjustment is a technique for mapping image's intensity values to a new range. In addition to increasing or decreasing contrast, you can perform a wide variety of other image enhancements Under Contrast adjustment, overall lightness or darkness of the image is changed. [12].

Histogram equalization

Histogram equalization is one of the well-known image enhancements for contrast enhancement this method is simple and effective. In the latter case, preserving the input brightness of the image is required to avoid the generation of non-existing artifacts in the output image. Although these methods preserve the input brightness on the output image with a significant contrast enhancement, they may produce images which do not look as natural as the input ones. The basic idea of histogram equalization method is tore-map the gray levels of an image. Histogram equalization tends to introduce some annoying artifacts and unnatural enhancement. This technique is widely used to improve images with poor lighting. The histogram equalization technique consists in reordering the grey level intensities within the image to obtain a uniformly distributed histogram [13][14].

Adaptive Histogram Equalization

Histogram equalization. The term adaptive implies that different regions of the image are processed differently depending on local properties. This is generally effective in increasing local contrast, but 'block art effects' can occur as a result of processing each such region in isolation and art effects at the boundaries between the inner windows can tend to produce the impression of an image consisting of a number of slightly incongruous blocks. The art effects can generally be reduced by increasing the size of the outer window relative to the inner window [15].

Extraction of Features Of Biologcal image

Gray Level Co-Occurrence Matrix (GLCM) has been proved to be a popular statistical method of extracting feature from images. According to co-occurrence matrix, the features measured from the probability matrix to extract the characteristics of statistics of images [16, 17]. In this paper four important features, angular second moment, contras, correlation, energy and homogeneity. Table (2) shows the formulation of statistical features and discription.

Methodology

The propsed methodology can be devided into six steps.

Step 1:-Rread color biological image

Step 2:- Convert color biological image to gray scale image

Step 3:- Applying opening morphlogical operation on gray scale biological image using different kind of structure elemants (disck, squer,line and ball), with the same of (15) parameter to estimate illumination function.

Step 4:- Subtract original from background to separate interest objects of biological image from their background .

Step 5:- Applying conteract enhancement techniques to biological image obtain from step(4)using different kind of enhancement techniques include (conterast adjustment, histogram equalization and adaptive histogram equalization).

Step 6:- Calculating Gray Level Co-Occurrence Matrix (GLCM) and the statistical features are contras, correlation, energy and homogeneity for enhancement biological images to assess morphological operation quality.

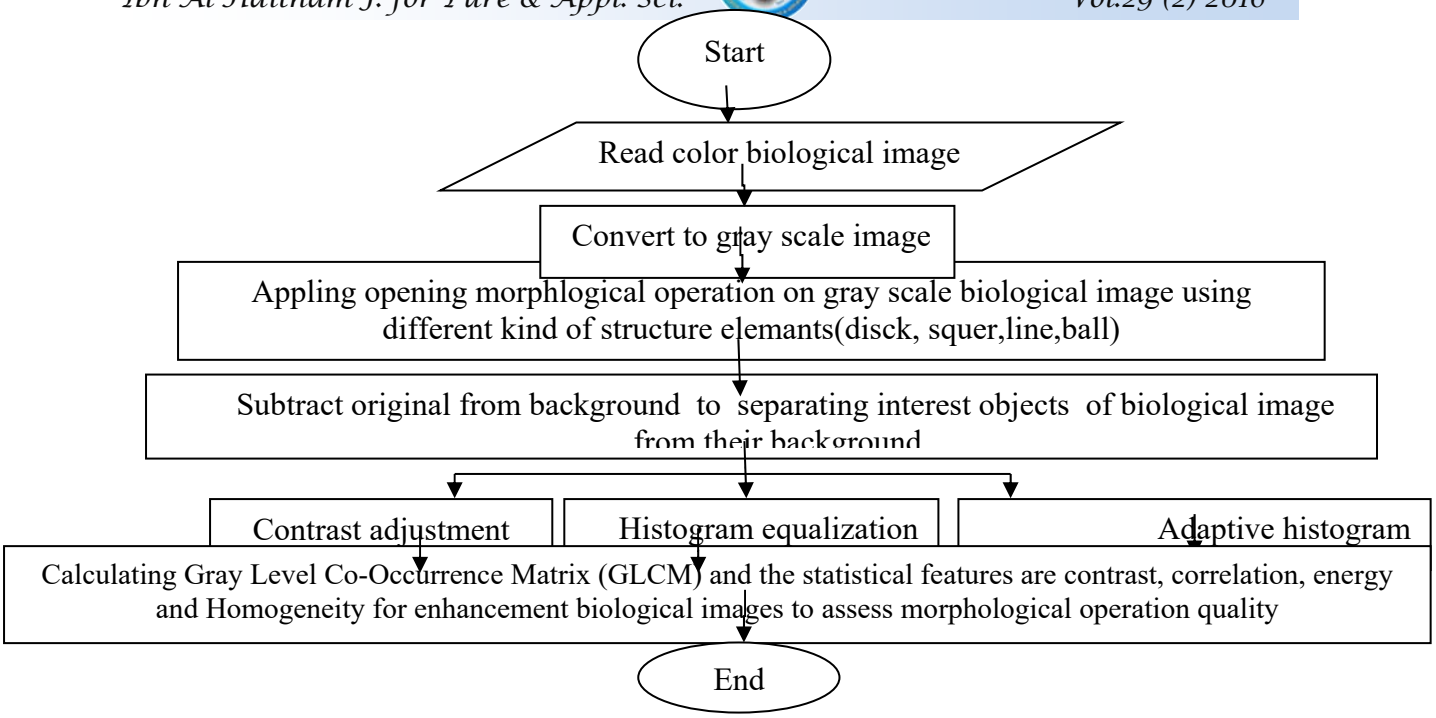

Figure (1) Flow-chart of the proposed algorithm

Results and Discussion

The following results describe the proposed algorithm for two real biological images:

1. Figures (2) and (3) show the result of applying the proposed algorithm on two real biological images. Morphological were introduced to correct the non-uniform illumination of biological images operations using different structure element SE include (dick, square, line and ball). After removing the non uniform illumination, the resulting image has the problem of less brightness than the original image due to morphological operation and the particles appear to be slightly less bright than their original view. In order to remove these problems, image enhancement techniques can be performed at the output of the image including (contrast adjustment, histogram equalization and adaptive histogram equalization).
2. Table (3) shows the comparison between the values of Gray Level Co-Occurrence matrixes (GLCM), the statistical features for contrast, correlation, energy and homogeneity were calculated from original biological images and enhancement biological images using (contrast adjustment, histogram equalization and adaptive histogram equalization) after appling opening morphlogical operations with different kind of structure elemants (disck, squer,line and ball) with same parameter 15.

Conclusions

The analysis of the results showed that

1. The best result was obtained when using the (disk) structure element for correction of non-uniform illumination of biological image. However, the worst is the (line) structure element.
2. Adaptive histogram equalization and contrast adjustment are better than histogram equalization to enhancement biological image after non-uniform illumination removal.
3. The results that have been obtained by applying contrast and correlation are a good indicator image quality because they are easy to generate and seemingly unbiased

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Table (1): Kind of structure element and its description

Kind of structure element	Description
disk	Creates flat ,disk-shaped structuring element with radius R.
Line	Creates flat ,linear structuring element
square	Creates square structuring element
ball	Structuring element decompositions used for the 'disk' and 'ball' shapes Are approximations [11].

Table(2): Gray Level Co-Occurrence Matrix (GLCM)the statistical features are contras, correlation, energy and homogeneity of gray level pixels defined as [18].

(GLCM)or Statistical Feature	Discription
$\text{Conteras} = \sum_i \sum_j (i-j)^2 p(i,j)$	Measure of the intensity contrast between a pixel and its neighbor over the whole image.
$\text{Correlation} = \sum_i \sum_j \frac{(i-\mu_{i)(j-\mu_j)}}{\sigma_i \sigma_j} p(i,j)$	Measure of how correlated apixel is to its neighbor over the whole image and it measures the joint probability of occurrence of the specified pixel pairs
$\text{Energy} = \sum_i \sum_j p(i,j)^2$	Measures the overall probability of having distinctive gray –scale patterns in image
$\text{Homogneity}=\sum_i \sum_j \frac{p(i,j)}{1+\lvert i+j \rvert}$ where i and j are two different gray levels of the image .p is the number ofco-appearance of gray levels i and j	Measures the value that closeness of the distribution of elements in the GLCM to the GLCM diagonal [11].

Table (3) The values of statistical image quality by different of morphological structuring elements and Contrast Enhancement Techniques (CET).

Structure Morphological element	Statistical Image Quality							
	Image1				Image2			
	Contrast	Correlation	Energy	Homogeneity	Contrast	Correlation	Energy	Homogeneity
Original	1.4093e+04	-0.0145	1.4158e-05	0.0321	7.9540e+04	4.1291e-06	3.1682e-06	0.0160
	contrast adjustment							
disk	**1.3842e+04**	**-0.0151**	**1.5294e-05**	**0.0322**	**8.1158e+04**	**6.6424e-04**	**4.5635e-06**	**0.0159**
square	1.3541e+04	-0.0133	1.6889e-05	0.0323	7.5186e+04	-1.1169e-04	5.7358e-06	0.0162
line	1.2995e+04	-0.0022	2.6104e-05	0.0320	6.8616e+04	0.0025	9.5459e-06	0.0165
ball	1.3817e+04	-0.0159	1.5381e-05	0.0322	8.0316e+04	-2.4689e-04	4.7202e-06	0.0159
	Histogram equalization							
disk	1.4884e+04	-0.0104	1.2928e-05	0.0316	7.9295e+04	1.6602e-04	4.1563e-06	0.0159
square	1.4740e+04	-0.0073	1.2692e-05	0.0316	7.5512e+04	-1.2526e-04	4.1031e-06	0.0161
line	1.5213e+04	-0.0023	1.1917e-05	0.0314	7.3792e+04	3.5081e-04	3.6191e-06	0.0162
ball	1.5181e+04	-0.0084	1.2249e-05	0.0314	7.8573e+04	-4.1196e-04	4.1449e-06	0.0159
	Adaptive Histogram equalization							
disk	1.3988e+04	-0.0153	1.5633e-05	0.0322	7.9437e+04	3.5082e-04	4.2274e-06	0.0160
square	1.3676e+04	-0.0111	1.6672e-05	0.0322	7.5442e+04	-1.1513e-04	4.8152e-06	0.0161
line	1.3435e+04	-0.0033	2.0738e-05	0.0322	7.0314e+04	0.0021	6.2499e-06	0.0163
ball	1.3947e+04	-0.0167	1.5804e-05	0.0322	7.9767e+04	-5.2512e-04	4.3465e-06	0.0160

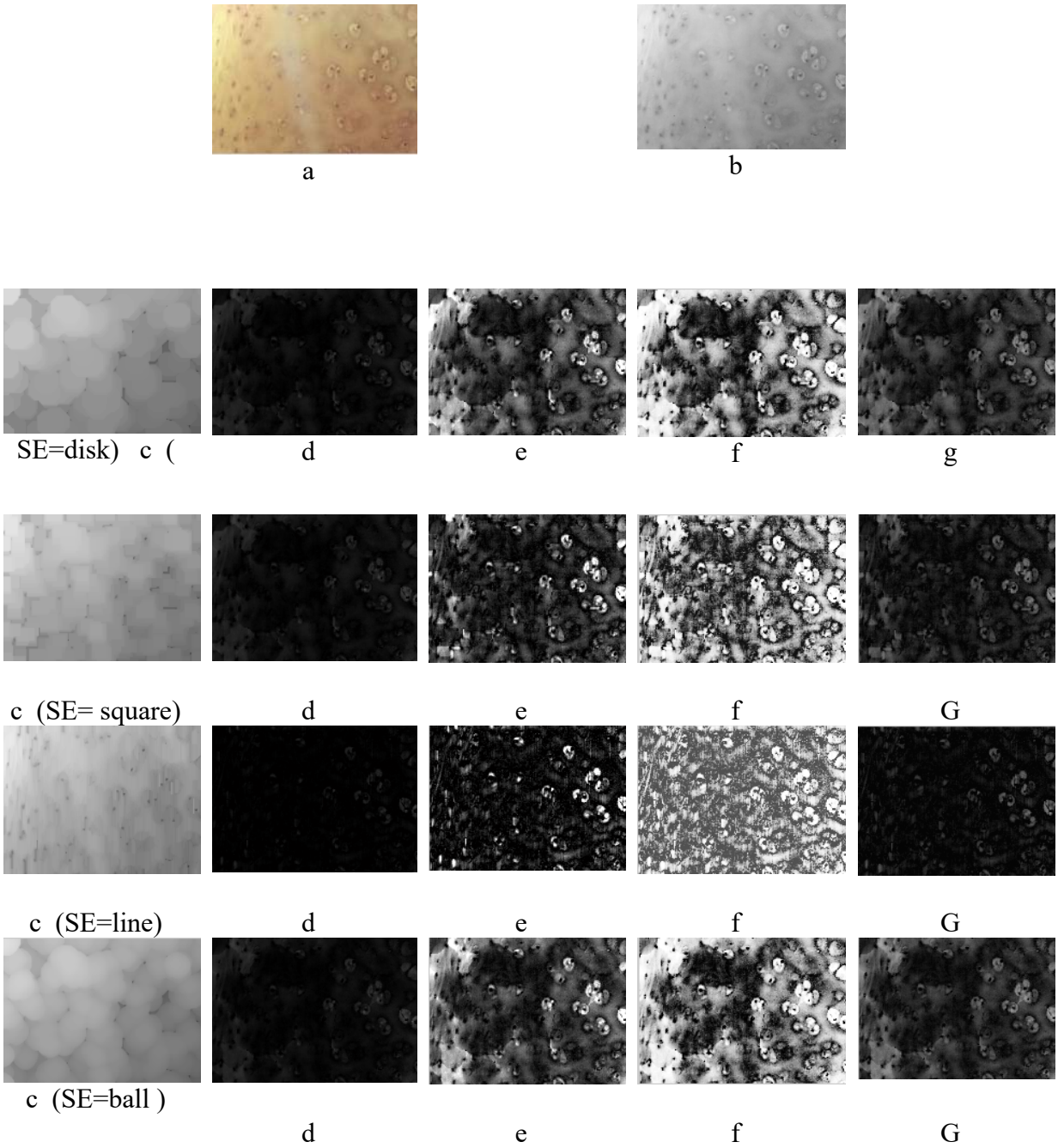

Figure (2):- Correction of non-uniform illumination morphological opening for color biological image1. First row (a) original image; (b) grayscale image **From the second to four row** (c) estimate of illumination through function by morphological opening of grayscale image using different types of structuring element (disk, square, line& ball) (d) with illumination subtracted; contrast-enhanced version of image using (e) contrast adjustment (f) histogram equalization (g) adaptive histogram equalization of biological images.

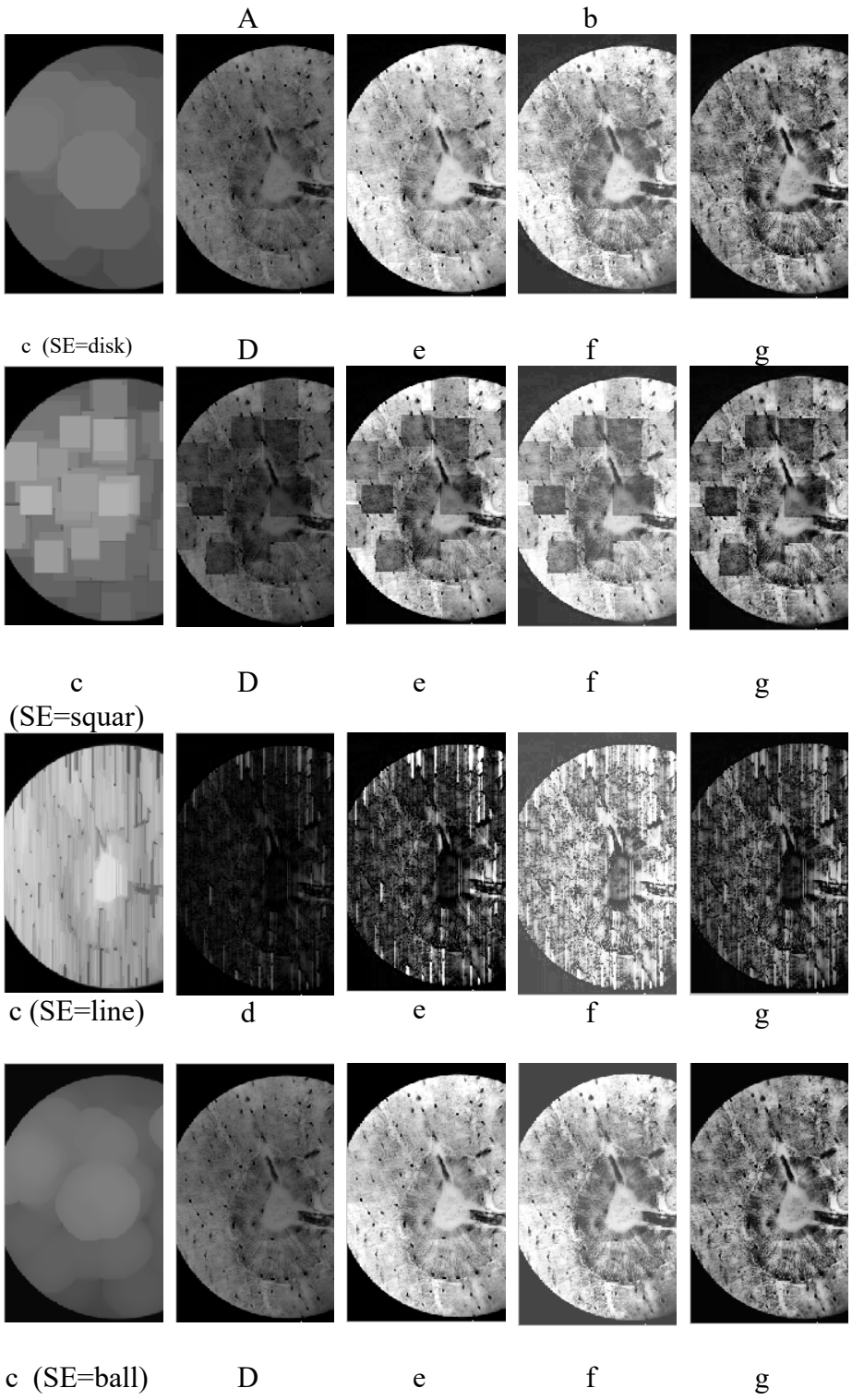

Figure (3):- Correction of non-uniform illumination morphological opening for color biological image2. First row (a) original image; (b) grayscale image **From the second to four row** (c) estimate of illumination through function by morphological opening of grayscale image using different types of structuring element (disk, square, line& ball) (d) with illumination subtracted; contrast-enhanced version of image using (e) contrast adjustment (f) histogram equalization (g) adaptive histogram equalization of biological images.

تصحيح الاضاءة غيرالمنتظمة للصور البيولوجية باستعمال عملية التشكيل الرياضية والتقييم بالخصائص الاحصائية للجودة

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استلم البحث في :23/اذار/2016 ، قبل في 17/مايس/2016

الخلاصة

عدم الانتظام في اضاءة الصور البيولوجية غالبا مايؤدي لتقليص الهياكل والشدة غير المتجانسة في الصوة.استعملت الخوارزمية المقترحة العمليات التشكلية وانواع مختلفة من العناصر المورفولوجية شملت (قرص،خط,مربع,كرة)وللمعامل نفسه (15).لتصحيح الاضاءة غير المنتظمة للصورة يتم ازالة الاضاءة الخلفية غير المتجانسة من الصور ثم تحسين الصور البيولوجية باستعمال (تعديل التباين,تسوية المخطط التكراري(المحلي والمحور).يمكن ان يظهر النهج الأساسي المستعمل لقيم الخصائص الاحصائية المستعملة من المستوى الرمادي لمصفوفاتGLCM القيم النموذجية لمحتوى خصائص الصور البيولوجية التي يمكن ان يكون في هيئة شكل او خصائص معينة .

قدم هذا البحث تطبيق مصفوفات المستوى الرمادي GLCM (الترابط,التغاير,الطاقة,التجانس) اذ اظهرات نتائج الميزات الاحصائية لها دقة عالية وكفاءة في التميز.في هذا البحث استعملت الصور البيولوجية الملونة الماخوذة من مختبر علم الاحياء المجهرية في كلية العلوم قسم علوم الحياة الجامعة المستنصرية .قد تم تطبق الخوارزمية لعشر صور بيولوجية ملونة تم في هذا العمل عرض اثنين فقط.

الكلمات المفتاحية: الاضاءة غير المنتظمة, عمليات التشكيل الرياضية, تسوية المخطط التكراري, مصفوفات المستوى الرمادي (GLCM)