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RESEARCH ARTICLE

Estimation of the Microscope Point Spread Function by Using an Adaptive Image Deblurring Method

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Abstract

As one of the most widespread techniques in biological investigation and dynamic process, light compound microscopy has used to analyze the optical properties of biological images. The optical microscope is often used to study and analyze the structures of biological cells and tissues. Microscope images are characterized by a number of specific parameters. Blurring is a major cause of image degradation in optical microscopes. We present an adaptive deconvolution based on the Richardson–Lucy (RL) algorithm to restore micrographs corrupted by out-of-focus blur with parametric estimation of the point spread function (PSF) of the acquisition system by using the blind deconvolution algorithm. This technique improves the quality of digital micrographs with defocus blur effectively. The evaluation algorithm is implemented to improve image quality by removing out-of-focus blur. The algorithm proceeds by randomly generating the PSF at each generation by using non-reference image quality. The PSF is used to estimate the actual image by applying the adaptive RL algorithm. Experimental results show that the proposed blind deconvolution method can estimate the core of the PSF, make good-quality restorted images with a slight ringing effect even, when the image is hardly blurred, and select input images for the non-reference image quality method for deconvolution.

Keywords: *Image Processing***,** *Image restoration, Point Spread Function, Deblurring, blind deconvolution.*

Introduction

Light microscopy is a vital technique in different fields because of its intuitiveness and cost effectiveness compared with other types of microscopy [1,4].The optical microscope produces blurred images (the Accuracy along the optical axis is restricted) due to the diffraction of light, dim of signals, and out of focus light. Since all the pattern is lighted for all image, light is often recorded from in focus and, from out of focus planes [5]. Out of focus share in a large amount of the distortion distinction the point spread function that is the impulse response of the optical imaging system [6, 7].

The Images formation of any microscope can be qualified by the mathematical form of convolution, where the original signal is convolved with distortion effect (which in this paper is out of focus effect) from the microscope. Image restoration is an active image deconvolution technique to enhance image quality.

It is a method to invert the blur due to convolution. Deconvolution takes in consideration optical properties and noise. This paper proposes a method to recover the PSF from blurred images to enhance micrographs in the range of 40×10 magnification by using the blind deconvolution method. Deconvolution mitigates the distortion created by the microscope [6]. It is an importance image deconvolution technique to enhance micro-graph quality [8, 9]. The accuracy of any restoration algorithm relies on the form of the used PSF.

The PSF has been estimated from the measurements of microspheres or computed from the optical properties of a microscope system. Other options also exists, at the PSF can be evaluated from the recorded images with each other with the observed objects (blind restoration). Blind deconvolution or

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restoration methods are used when the blur function cannot be accurately modeled or measured. It is a convolution of the original image with a PSF [7, 10, 11].

Related Work

Image restoration is a senior topic with applications in the image processing reconstruction and deblurring of astronomic images. Many methods have been proposed to recover the sharp latent image and the PSF. One of the first deblurring algorithms for optical microscopy is the Van Cittert used algorithm [12], which a popular iterative algorithm in the field of image is deblurring. Image restoration problems are often solved by the Richardson Lucy (RL) restoration method [13], which iteratively arrives at a solution by alternately optimizing the latent image and the PSF estimates. Diaz et al [14].

Suggested a simple algorithm which deblurs microscope images, giving an effective 3D-like rendering and a clear restoration of the inner texture .Levin et al [15].Used a sparse derivative to avert the ringing artifacts in restoration .Hom et al. [16] presented an adaptive image deconvolution algorithm, namely, the blind deconvolution framework for 2D and 3D data.

The PSF on the imaging system is almost known and provided in the form of the Optical Transfer Function (OTF). Shan et al. [17] used a probability model of the latent image, PSF, and noise. Their results are particularly useful when applied to motion-blurred images. Pan et al. [18] suggested a method to estimate the blur mask from a blurred image by regularizing the sparsity speciality of natural images. In this work, a regularization algorithm of deconvolution kernel were presented, that is based on randomly generating the PSF using non-reference algorithm depending on adaptive RL algorithm, the results gives high-quality deblurred images even when the image is severely blurred.

Image Degradation: Causes of Blurring

Image degradation is the act of loss of quality of image, due to different reasons. In event of Image degradation, the image gets blurry and loses its quality to much extent. Image degradation is caused by four independent factors: scatter, noise, glare and blur [19, 20, 11]. Scattering is a common physical operation where light is forced to drift from a straight path by one or more paths due to localized non-uniformities in the medium through that light pass. While noise is a random disarrangement of detail in the image. The random distribution of noise is unknown. In optical microscope images, the source is either the signal itself so called "photon noise", which is characterized by a Poisson distribution, or the digital imaging system [19, 21]. Glare is result from a significant ratio of lightness between the task and the glare source. Factors such as, the angle between the glare source and task, and the eye adaptation have great effects on the test of glare.

Noise is a random disarrangement of detail in the image. The random distribution of noise is unknown, but In optical microscope images, the source of noise is either the digital imaging system or the signal itself (so called "photon noise") [19, 21]. Blur is a nonrandom spreading of light caused by the passing of light through the imaging system and lenses. The three main causes of degradation by blurring are object motion, where blurring of image is due to movement of the subject or imaging system, out of focus (defocus aberration) and Gaussian blur that is a result of blurring on the images by a Gaussian function.[8, 20, 9]. Image is denoted as matrix inside computer.

Each image contains a lot of features like edge, contrast etc. In image processing features have to be extracted from the image for further study of image. To restore the image, we need a mathematical expression of how it was blurred; we commonly start with a shift-invariant model this meaning that all points in the original signal move out the same way in forming the blurry signal. We model this with convolution.

The purpose of image restoration or deconvolution is to reconstruct the original image from a degraded image. This restoration process is difficult in the image processing applications. In spite of the fact that, classical linear image restoration has been thoroughly studied, the more hard problem of blind images restoration has numerous research possibilities [1, 2]. The theoretical model of blur, which is important in deconvolution, is introduced in detail in the following section.

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Degradation Model and Mathematical Formalism

The degradation process given in equation (1) can be modeled or described as (PSF), blur function or convolution of the of the system (H) and the reference (original image O) to produce a distortion, that, Which, along with noise, works on input image I. Image deconvolution is a mathematic process that calculate an object approximation, presented the gained image I, H represent the degradation and N is the noise.

Generally, knowing of N is restricted to the knowing of its statistical nature. In the image capture form microscopy, mostly degradation is result from the optical blur which is created by out of focus aids of the object in the focal plane and noise. The noise undergo a Poisson distribution that is one of the distribution noise in the optical microscope [5, 9, 20], it can be closed by a Gaussian distribution for simplification purposes [22, 23].

$$I = noise\,(O \otimes H) \quad (1)$$

Point Spread Function (PSF)

Objective and tube lens of the microscope do not image a point in the specimen as a high light disk with defined edges but as a little blurred spot that is surrounded by diffraction rings called airy disk (Figure 1(a)). The image for a point source shown in the form of a disk containing (84%) of the total energy, the disk is surrounded by a number of fewer bright rings where the remaining energy is distributed [31].

Airy is the first who calculated the intensity of the image of a point object formed by the diffraction therefor the central disk is called the Airy disk .The representations of the diffraction pattern near the intermediate image plane are known as the PSF (Figure 1(b)). The PSF is a 3D representation of the airy disk [8, 20, 11, 24], as shown in Figure 1(c) [22].

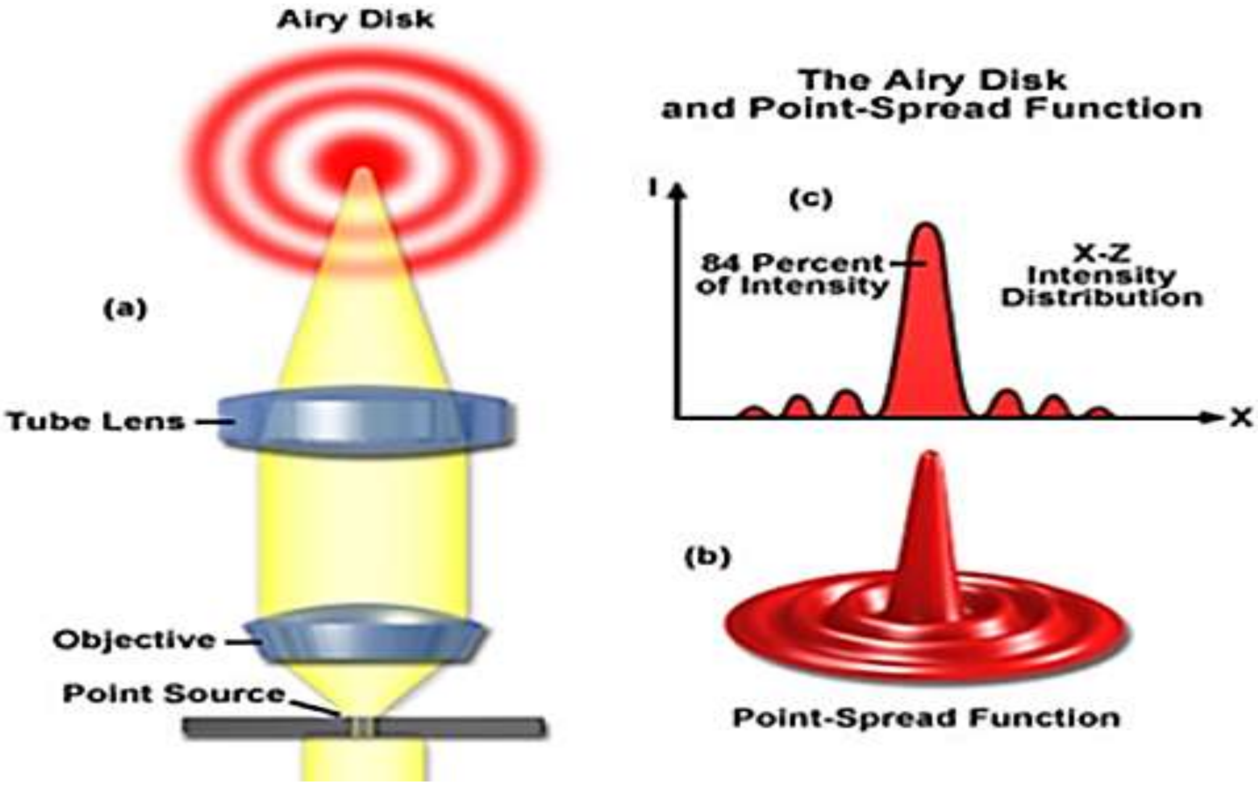

Figure 1: The Airy disk [22]

The blurred model that has included in optics is depended on the function of a PSF [19]. The diffraction pattern of an isolated point, source of light is the PSF. Any type of the blur is described by the point spread function. The light propagated from a point source travels through the optical system (optics of the microscope, including the objective lens), and it is diffracted (Figure 2).

The qualities of the optical system device rely on the spread or blurred point object. Thus, the operation of the convolution is characterizes the implementation of the PSF at every point in the object (i.e., light that emitted from all point in the object or point source is wrap with the PSF to make the image).

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Therefore, blurring is described as the convolution of the original image with a PSF (Figure 3).

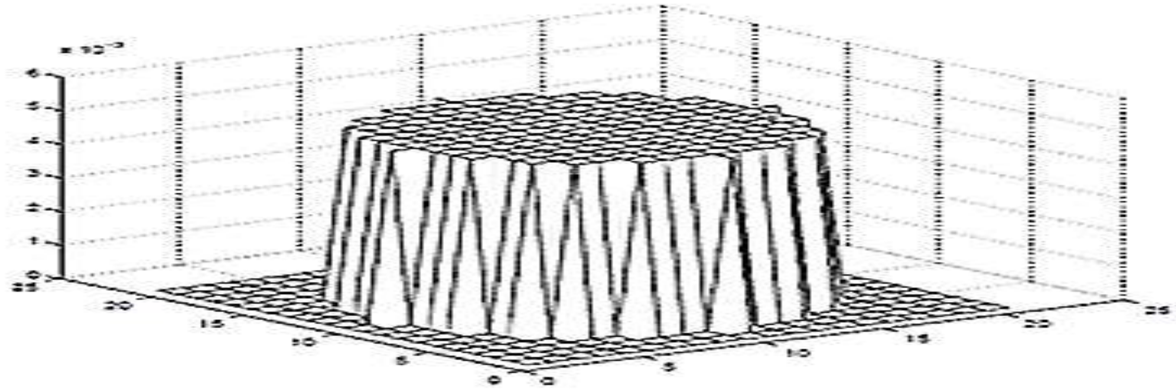

Figure 2: Out of focus PSF [20]

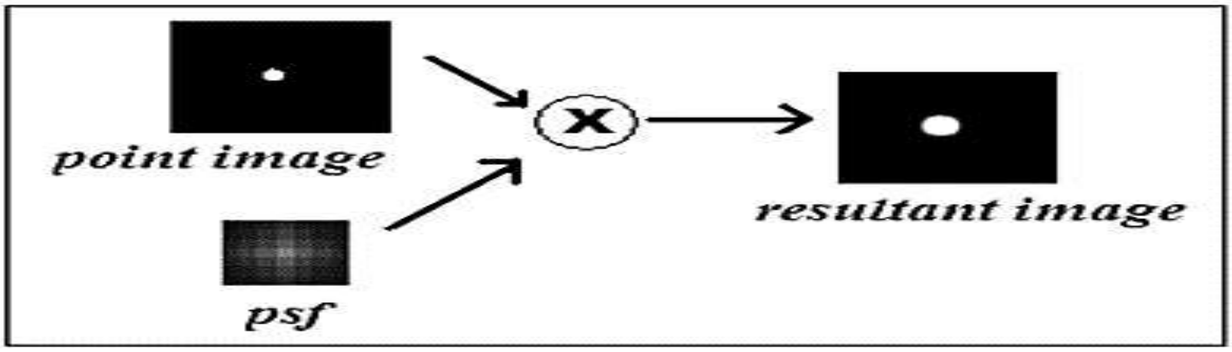

Figure 3: Image formation with PSF [11]

The captured (blurred) image g(x, y) can be described as a convolution of the original image f(x, y) and a blur function or PSF [11, 24, 25] as:

$$g(x,y) = f(x,y) \otimes PSF + n(x,y) \quad (2)$$

Where n(x, y) represents noise image and $\otimes$being the convolution operator .If the PSF can be measured or accurately modeled, then the deconvolution process can be applied to $g(x,y)$ to estimate (x,y) .When an image has been captured by any recording optical device, the lightness of pixels is directly proportional to the intensity of the corresponding section of the sight captured. Virtually, the lightness of pixels is influenced by the blur or noise.

Imaging Theory of Defocus

The term of PSF, in this study is out-of-focus PSF. This blurring is produced by a defocus optical system, as shown in Figure (2) [20]. The degree of defocus relies on the lens diameter and the long between the object and the sensor [1].

The defocus PSF is given by [11, 20, 8, 19]

$$PSF = \begin{cases} \frac{1}{\pi r^2} & \text{if } (x^2 + y^2) \leq r^2 \\ 0 & \text{otherwise} \end{cases} \quad (3)$$

r Being the radius of the circle of confusion, and (x, y) is the center of the out-of-focusPSF.

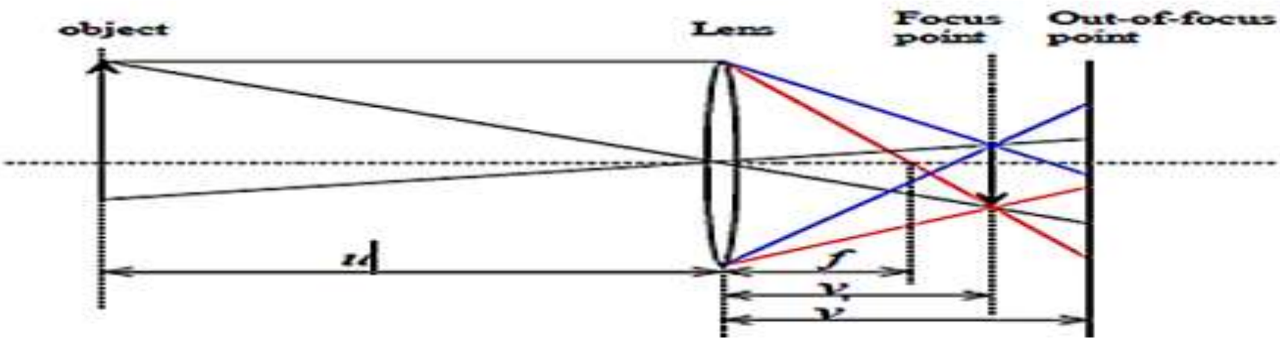

Figure 4: Theory of image blurs [1]

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Proposed Method

Blind deconvolutions are used in cases where the blur function cannot be measured or actually modeled. In this paper an attempt to extract the unknown PSF from a blurred image using the RL by estimating the PSF (RLEPSF) algorithm are done, which is effective for different blur types, such as motion and defocus blur.

The aim of No-Reference Image Quality Assessment (NR-IQA) is to suggest some mathematic models, can accurately and automatically find the visual quality of distorted images without prior knowledge of the original reference images. generally, the image quality assessment algorithms is to estimate the perceptual quality of the digital images without access to reference images and the types of distortions present using an objective degree, that should be highly correlation with the human subjective degree [30,31,32].

No-reference Image Equality Algorithm

The proposed method restores the image from distortion images without PSF, depending on the estimated PSF by using the no-reference image quality scale called No-reference Quality Metrics of the Blur (NQMB), where the NQMB measures the PSF for the different sizes and then calculates the quality metrics at maximum value. And then best PSF had been estimated. The stepwise description of the proposed method is as follows:

No-reference Quality Metrics of the Blur (NQMB

Input: Image blurs (g)

Output: NQMB

- Input Image g(x, y).
- Calculate Sobel mask for g(x, y) to find edges, to obtain ge (xe, ye).
- Find original image values in the edge coordinates g(xe, ye).
- No-reference Quality Metrics of the Blur for g (xe, ye) by finding the mean value: NQMB = mean (g (xe, ye)).

Estimation PSF by RL method (RLEPSF) algorithm:

This algorithm computed the resorted image (deblurrung image) using RLEPSF algorithm depending on the best restored PSF .best estimation of PSF depends on the best computed radius from blurring metric BM. The general steps of the proposed system are as follows:

Algorithm2: (RLEPSF) method

Input: Image blur (g)

Output: restored image (gr)

- Input g(x,y) at position x,y .
- Restore image by the RL method for many radius at ri where (r= 1:14).
- Specify different window sizes for PSF, where hi = $\frac{1}{\pi r^2}$.
- To obtain gdbi, which is the image deblur at the different radius.
- Find No-reference Quality Metrics of the Blur for gdbi to obtain NQMB i .
- Calculate the maximum value of NQMBi to obtain NQMB_max that corresponds to the PSF_max.
- Calculate PSF_max in Step 2 to obtain the restored image $\hat{f}$.

Experimental Results

This section presents the experimental results to demonstrate the effectiveness of the proposed method when defocus blur is presented. The optical system used in these studies is an optical microscope. The light source is a lamp that transmits incoherent light to the microscope. Images of human tissue are acquired using a CCD camera.

The captured images are saved at 24 bit. The distorted images are divided into three datasets: two sets for 100× magnification images and one set for 400× magnification images. Each set contains 30 images of geometrically in-focus, small miss-focus, and large miss-focus imaging, as shown in Figures 5a, 5b, and 5c for 100×, 400×, and 100×, respectively.

Quality Image Analysis (NRIQA)

In this study, we evaluate the visual quality of the specimen images without access to the reference image.

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The evaluation result of our algorithm for NRIQA is shown in Figures 6, 7, and 8, which present the three best images of sets a, b, and c, respectively. The improvement of our BM for estimated quality results is clearly visible in Figure 9. The plot (in which each data point represents one test image) shows that the estimated quality QM is low for out-of-focus images, and the largest value of QM is obtained at the origin (low or no blur).

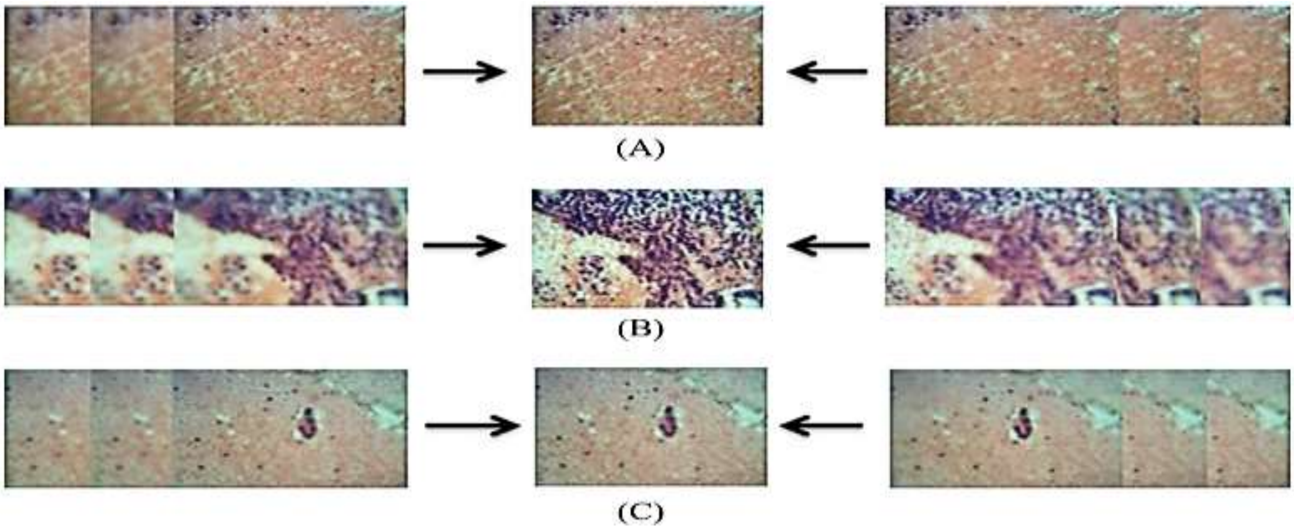

Figure 5: The samples (A, B and C) of the captured images

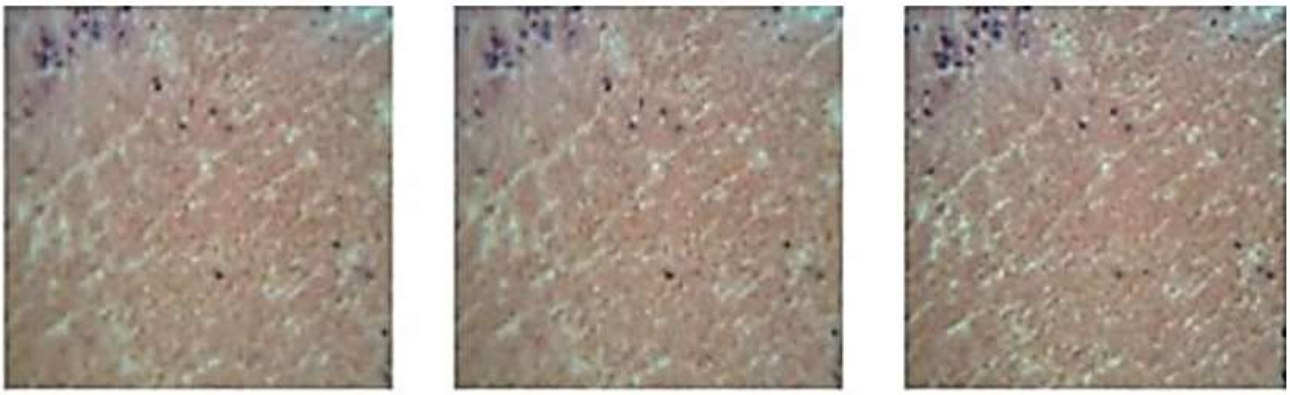

Figure 6: The best images from BM for the sample (A)

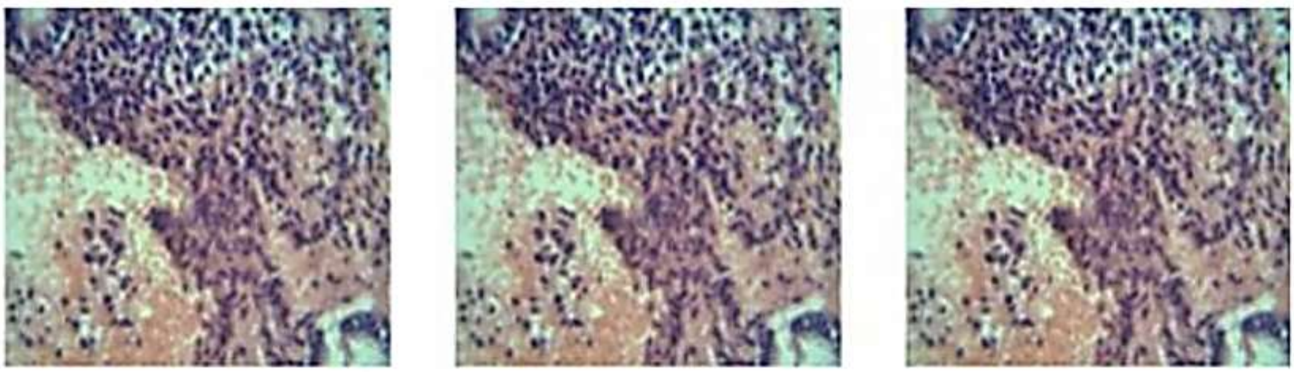

Figure 7: The best images from BM for the sample (B)

Figure 8: The best images from BM for the sample (C)

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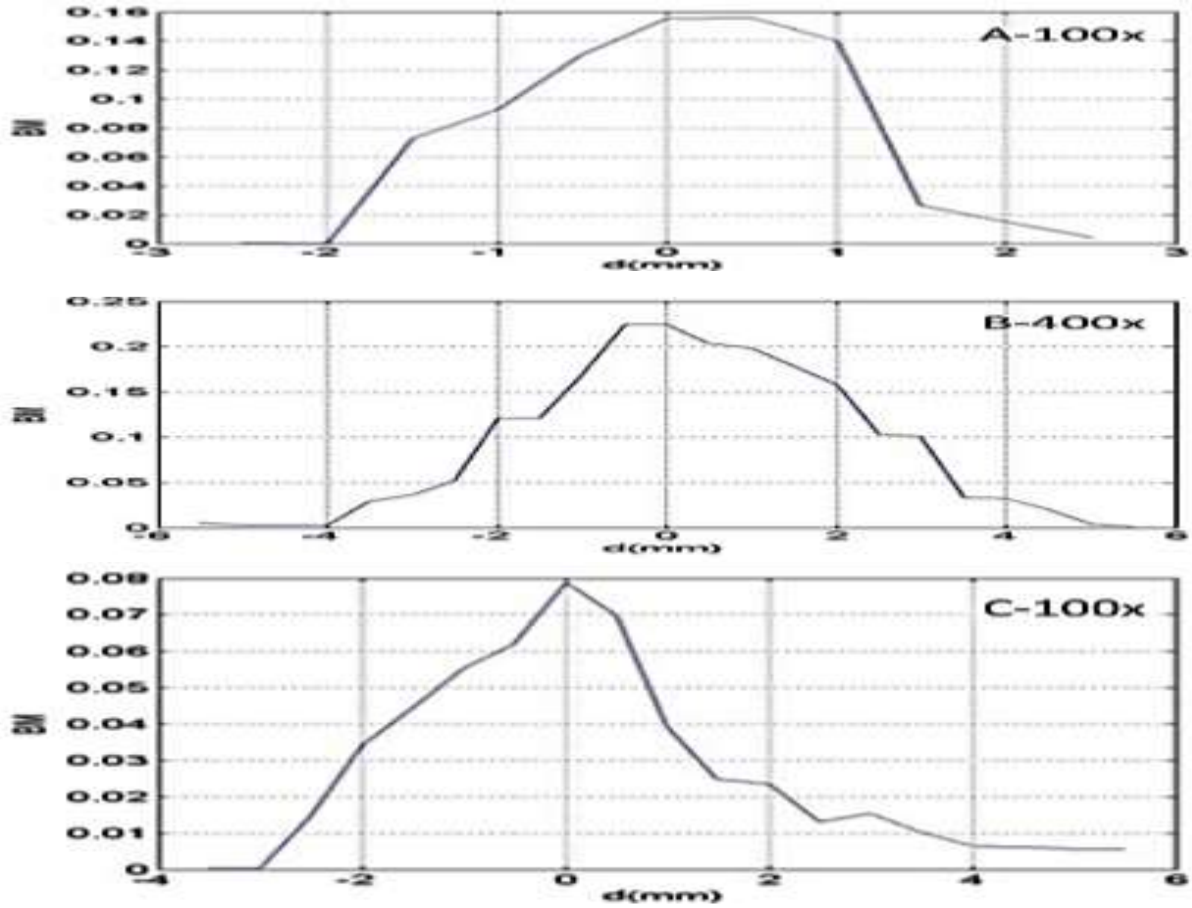

Figure 9: The QM as a function of the distance from focus

PSF Estimation

For blind restoration, the PSF is calculated from the blur images; permit the deconvolution to be implemented. After the sharp images are predicted from the NQMB metric, the PSF has been estimated as the kernel that, when convolution with the sharp image, produces the blurred image. The quality of the deblurred image is mainly determined by the knowledge of the PSF. It shows an interesting structure, which results from a combination of diffraction and blur. Figure 10 show that the obtained PSF is highly localized at the center of the discrete grid.

Evaluating the Blind Deblurring Algorithm

The deblurred images shown in Figures 11-15 are obtained from the proposed adaptive RL algorithm. The blur is reduced effectively in both magnification images. The visual quality of the images using the proposed method can better preserve image details. The distribution of intensity values (histogram) provides a global impression of the quality of an image. The graphical results of histogram are shown in Figures 12, 14, and 16 for the blurred and deblurred images. The enhancement of the contrast in the deblurred images indicates that the width of the distribution is a combined measure of the strength of artifacts and blurred images.

Conclusion

This paper presents an image restoration method based on blind deblurring by using the adaptive RL algorithm with approximate PSF estimation for optical micrographs. The goal of the image enhancement algorithm is to enhance a quality or characteristic of a digital image, in order to, the resulting image is better than the distorted image. One of the most common methods, degradations in medical microscope images are their less contrast quality like blur and noise Experimental results show that the blur problem is effectively suppressed and the restored quality is improved.

The blur scale that quantifies the blur problem on a blurry image enables the system to identify the appropriative parameters that can bias deblurring, so the proposed method is beter solution to gives autofocusing system to find the best focus of the microscope.

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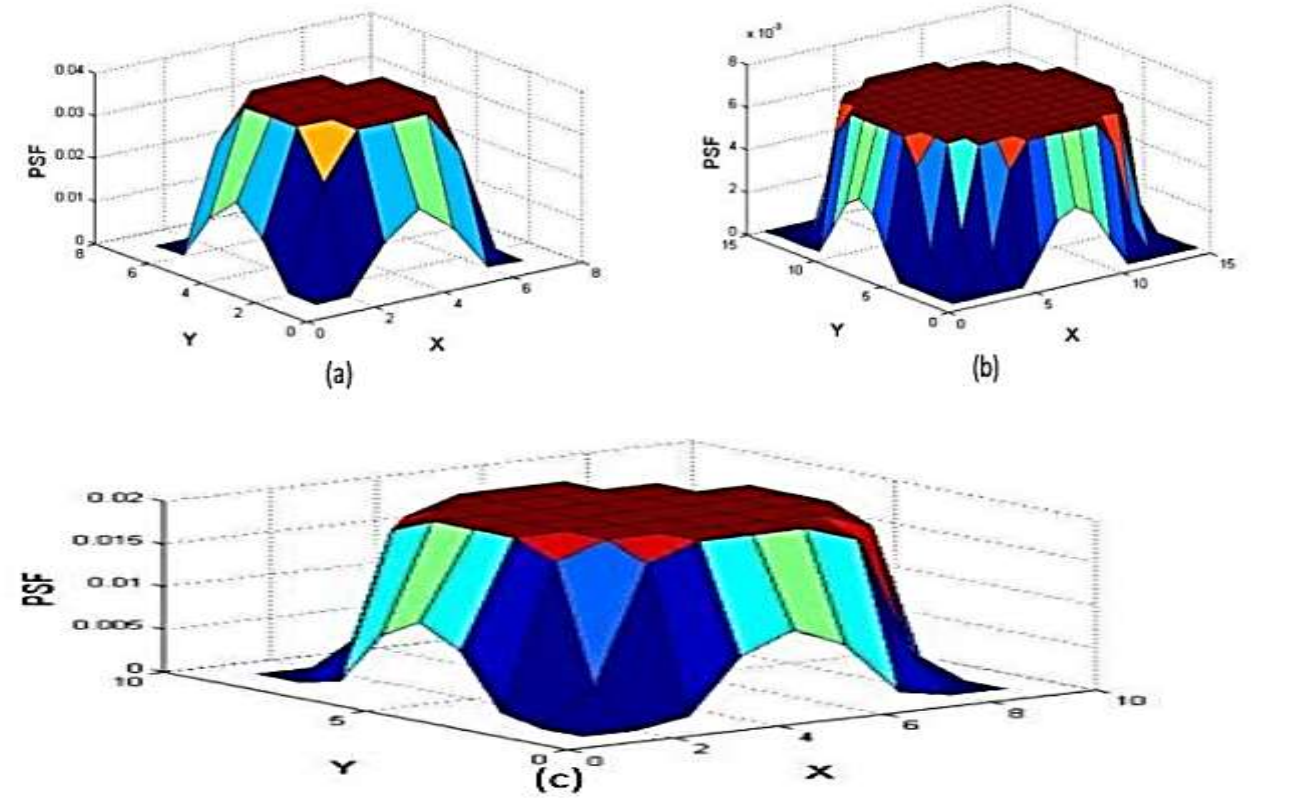

Figure 10: Estimated PSF for the best images: (a) for sample (A). (b) For sample (B). (c) For sample (C)

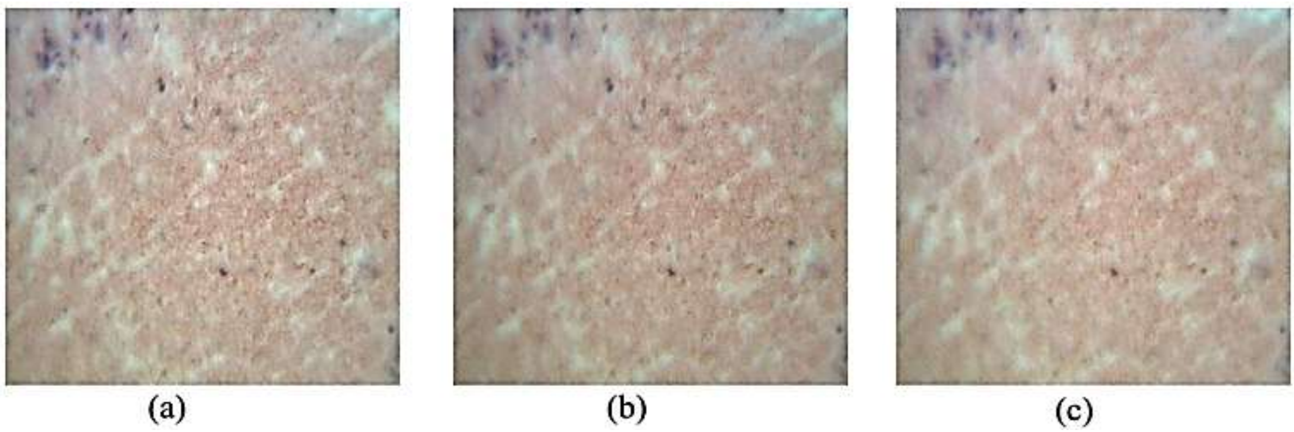

Figure 11: Blind deblurring of the best images in the sample (A)

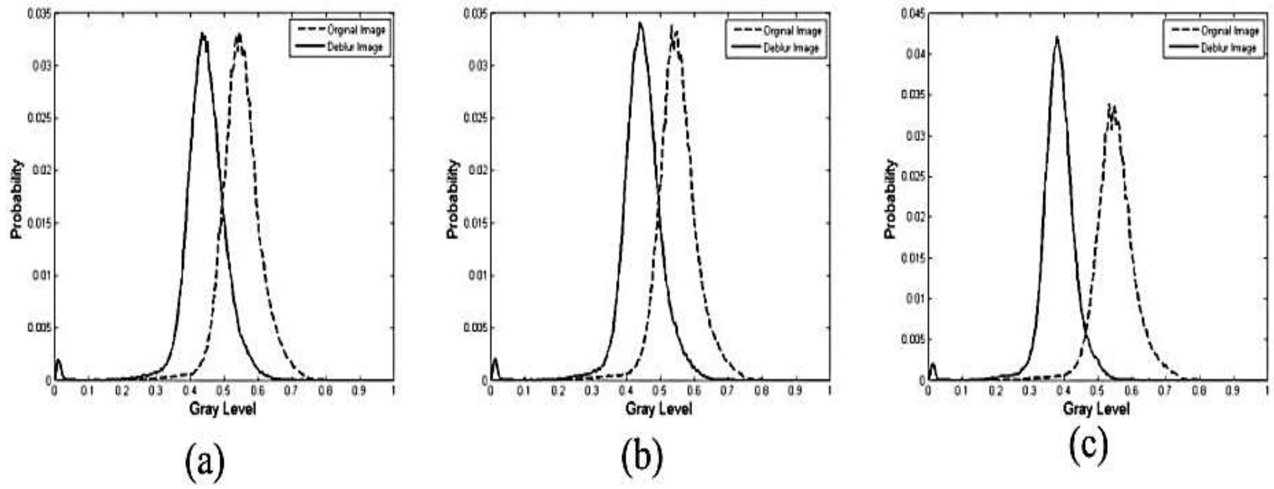

Figure 12: The histogram for a best images and it's deblur in the sample (A)

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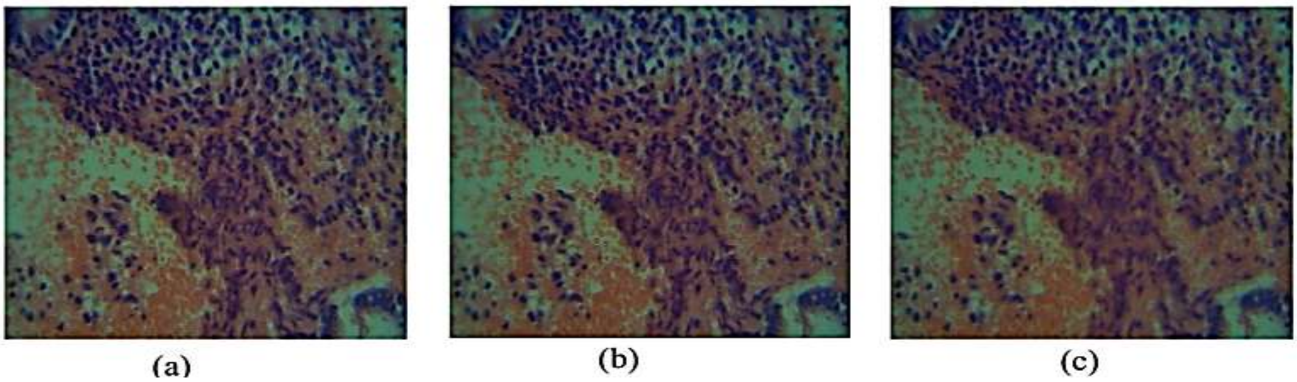

Figure 13: Blind deblurring of the best images in the sample (B)

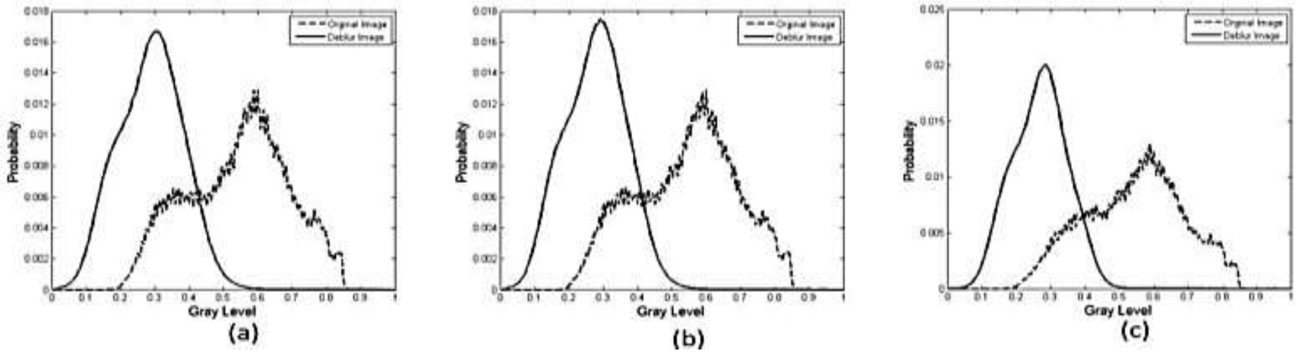

Figure 14: The histogram for the best images and its deblur in the sample (b)

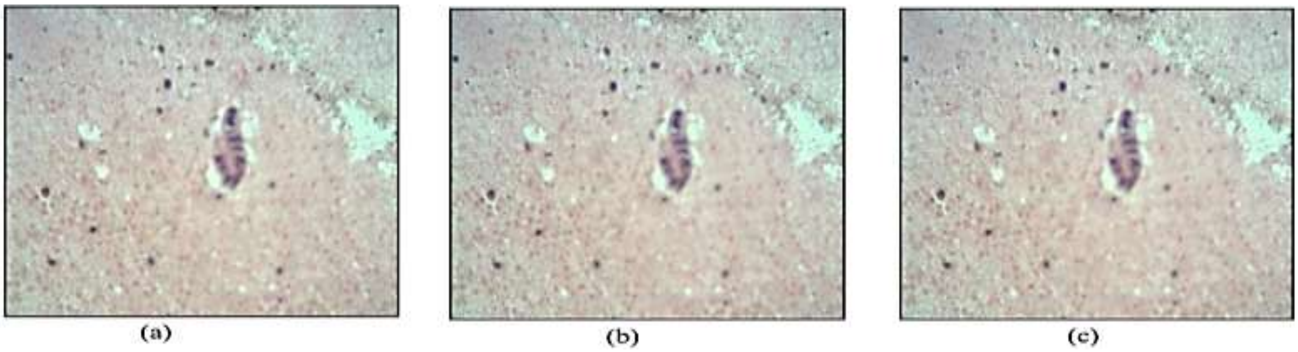

Figure 15: Blind deblurring of the best images in the sample (c)

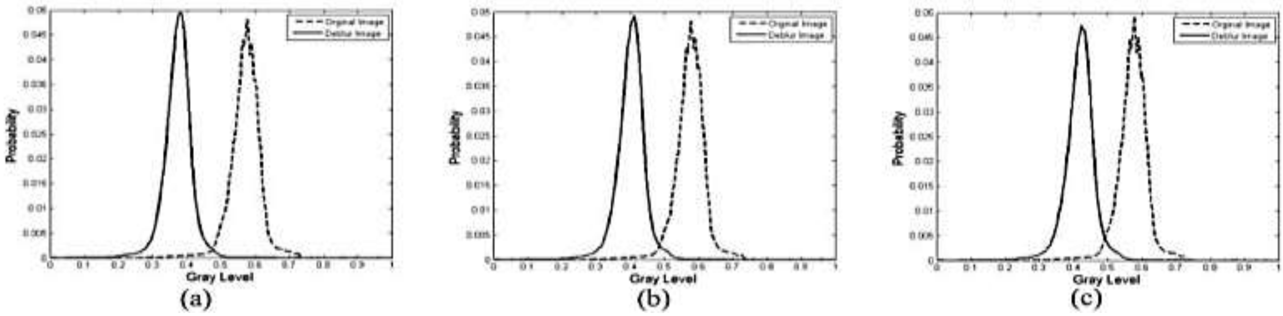

Figure 16: The histogram for the best images and its deblur in the sample (C)

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