Auto-focusing with Multi Focus Color Image Fusion Based on Curvelet Transform on Microscopic Imaging

Hülya DOĞAN[1], Selen AYAS[1], Murat EKİNCİ[1]

[1]Departmant of Computer Engineering, Karadeniz Technical University, Trabzon, Turkey
hulya@ktu.edu.tr, selenguven@ktu.edu.tr, ekinci@ktu.edu.tr

Abstract

The fundamental operation before analyzing bacteria on the microscopic system is optimal focusing. Laboratory technicians implement this process with eye-hand coordination. During auto-focus process avoiding the dependence on technicians, auto-focus functions giving a value about focusing of the images are used in literature. At the end of the auto-focusing based on auto-focus functions, some regions in the in-focus image can be blurred. In this paper auto-focusing based color image fusion is implemented to obtain all of region in-focus image. In this study, firstly an image sequence is captured with moving the microscope stage along *Z*-axis. The reference image with the highest focus value on the sequence is found. Reference image and several images around the reference image are fused with curvelet transform preferred to obtain curve and line information of image. Moreover, various evaluation criteria are utilized to analyze the performance of the proposed auto-focus approach on different color models.

1. Introduction

In order to begin scanning on the optical imaging systems for recognition of bacteria, microbiology laboratory technicians firstly find in-focus image. This process is implemented with eye-hand coordination performed by the technician. Auto-focus process for automatic optical imaging system is developed to avoid the dependence on technicians, and has very major role to obtain an image with most information and high resolution. The traditional auto-focus systems generally use auto-focus functions giving a focus value about degree of focusing for the images within the same scenes. In literature many auto-focus functions such as gradient, tenengrad, sobel were proposed, it is accepted that the image with maximum value was in-focus. In the traditional auto-focus system, because the auto-focus evaluation functions use all pixels in the images to calculate the auto-focus value, they can give a global value about the information and the details of the images. Therefore, some regions in the sample can be blurred or may not be seen clear at the in-focus image, as shown in "Fig.1". When the in-focus image based on the auto-focus function is used for the analysis of bacteria in order to diagnose a disease, a bacteria on blurred region of sample may not be seen, so misdiagnosed and treatment may be occurred. It is desirable to obtain one single microscopic image that contains all regions in-focus. This can be achieved through fusion of images with same scenes on the Z axis.

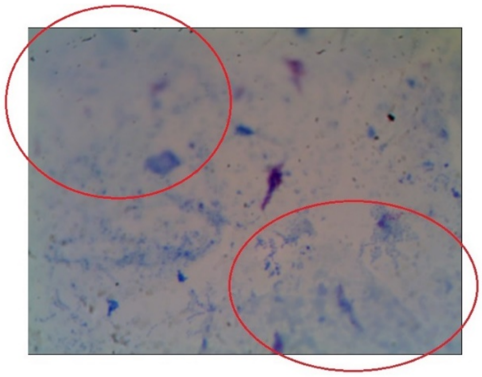

Fig. 1. In-focus image with maximum auto-focus value

Image fusion process obtains a single image with all information and details in-focus by integrating series of sequential images without distortion [1]. In the literature many image fusion algorithms are improved. Computing the average gray values of the pixels and selecting maximum gray level of source images are the most classical fusion algorithms. Moreover, Laplacian pyramid, Gradient pyramid, spatial and frequency domain, PCA, Discrete Wavelet Transform, Curvelet transform and Morphology pyramid are used as methods for fusion of images [2, 3]. The studies in literature show that fusion techniques based wavelet are generally more effective than other methods for optical imaging [4]. However, because wavelet transform can't capture curves and edges of images well, the images obtained with this transform have undesirable side effects. To minimize the limitation of the wavelet transform, Donoho proposed the curvelet transform [5, 6]. Tensens implemented image fusion techniques based curvelet transform and RGB color model for optical imaging systems [7]. Moreover, the studies in the literature indicate that RGB color model is not suitable for color image fusion, so different image color models are used [1-8].

The studies in literature show that the choice of color space and the method of fusion is important for image fusion process. Therefore, in this study multi-focus color image fusion methods based on different color models (YIQ, YCbCr, HSV) and transforms (Discrete Wavelet Transform and Curvelet Transform) are implemented. It is demonstrated that curvelet transform captures more the geometric information, solves the limitations of classical wavelet transform and the color models (YIQ, YCbCr, HSV) are more suitable than RGB color model for color image fusion.

2. Auto-Focusing on Optical Imaging System

The traditional auto-focusing methods based on auto-focus functions select a single in-focus image with maximum auto-focus value on the sequential images with same scenes. When this auto-focusing is implemented on optical imaging system, some regions of sample can be blurred or not seen on the in-focus image. Therefore, a novel auto-focusing system based image fusion method is improved in this study. In our approach as shown in "Fig. 2", multi-focus color image fusion methods based on different color models and transforms are implemented for auto-focusing on optical imaging system. The steps of our proposed system: (1) A series of sequential images (with the same of field) is captured by moving platform on the Z axis. (2) The focus value of each image on the series is calculated by using auto-focus function, and a probability density function (pdf) for the images in the series is estimated by using their focus values. (3) The image with maximum value of pdf is determined, and it is assigned to the reference image. (4) At last stage, an optimal auto-focusing in all region of the color image is achieved by applying image fusion approach on the color channels of reference image and images in which their pdf values are very close to the reference image.

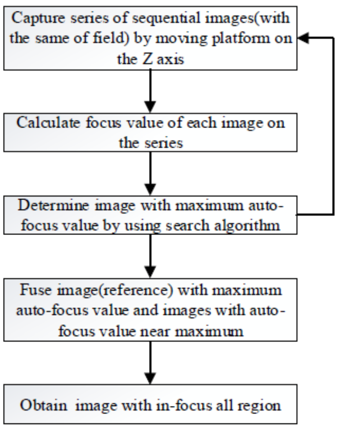

Fig. 2. General structure of proposed auto-focusing based image fusion

2.1. Auto Focus Functions

In literature, many types of auto-focus functions giving a significant value about information and details of the image exist. In this study, Entropy based on image histogram is used to calculate focus values of images.

- Entropy Function:

$$F = -\sum_{k} p_k \log_2 p_k \qquad (1)$$

2.2. Multi-Focus Color Image Fusion

Multi-focus color image fusion process purposes to produce a single image with all region in-focus by integrating a series of sequential images with same scene [2-3]. This process can be implemented in three levels: pixel level, feature level and decision level. In literature because of the increase of application fields, many types of image fusion methods are proposed. But some of these methods may cause the loss of curve and edge characteristic and undesirable contrast reduction. In addition, the results of wavelet based methods are generally better than the other methods [9]. However, DWT based methods cannot contain the geometric information of the image [10]. Novel geometric image transforms such as curvelet, ridgelet, wedgelet and contourlet are developed to obtain geometric information of images nowadays, and in this study curvelet based image fusion is proposed to decrease limitations of wavelet.

2.3. Curvelet Transform

Curvelet transform was proposed by Candes and Donoho in 2000 [6]. It was originally proposed as continues transform, then its digital implementations were improved with increase of application fields. Although wavelet transform decomposes the images into only multiscale components, curvelet transform decomposes the images into multiscale and multidirectional components. Therefore, in literature it is shown that curvelet transform is more preferred to obtain curve and line information of image than wavelet transform. The steps of the digital curvelet transform as presented in "Fig. 3": (1) Fourier coefficients are obtained by applying 2D Fourier transform. (2) A discrete localizing window is implemented to localize Fourier transform near the sheared wedges. (3) The data is indexed again by applying wrapping transformation. (4) Discrete curvelet transform coefficients are obtained by using inverse 2D Fourier transform.

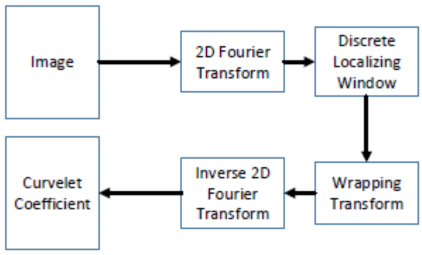

Fig. 3. The steps of the digital curvelet transform

2.4. Proposed Method

In our image fusion process reference image and images with auto-focus value near reference are fused to create a single color microscopic image with all of region in-focus, as shown in "Fig. 4".

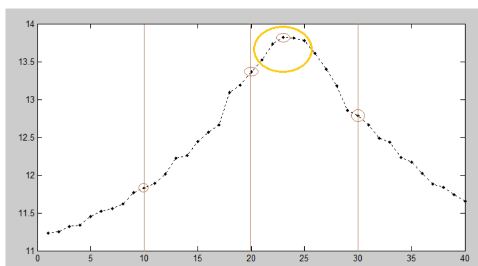

Fig. 4. The reference image and images with values near maximum

The steps in the proposed image fusion algorithms are given in "Fig. 5" and also as follows: (1) The RGB components of each image are converted to the other color models (HSV, YIQ, YCbCr). (2) Curvelet transform is performed on the intensity (I) components of the images. (3) Select maximum method is applied onto the curvelet coefficients for (I) components. Inverse curvelet transform of the selected curvelet coefficients is then taken to obtain the combined intensity (I) values, (4) Sharp areas of images are also chosen (X and Y) by using image fusion method (select maximum method). (5) Finally, X, Y and I components of the fused image are obtained and converted to RGB model.

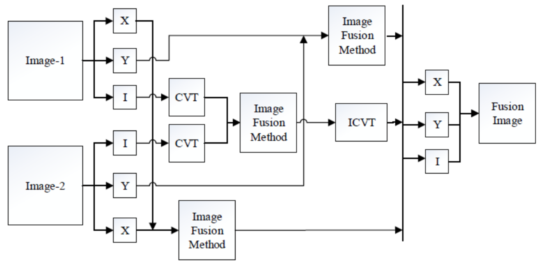

Fig. 5. The steps in the proposed image fusion algorithms

2. Experimental Results

2.1. Datasets

In this study, a database consisting of microscopic images is created to evaluate the performance of the proposed approaches. This images are obtained from ZN-sputum smear slides prepared in the Mycobacteriology Laboratory at Faculty of Medicine in Karadeniz Technical University. Image acquisition system consisting of a standard PC, a light microscopy and a digital camera was setup in our computer vision and pattern recognition laboratory [11]. A Nikon Eclipse 80i bright-light microscope with a 100x oil objective and 1.0 numerical aperture is used to scan sample slides. The images of database are captured with Digital microscope eyepiece with MA88-300 USB CMOS camera. The images with 640 × 480 resolution are stored in JPEG file format, with 24 bits per pixel, in color.

In literature, different auto-focus functions were improved to implement auto-focusing based auto-focus functions [8]. According to results of these studies, it is shown that different autofocus functions can't find same in-focus image. Moreover, some regions in the sample can be blurred and may not seen clear at the in-focus image. To solve this problems, in our study auto-focusing on microscopic imaging based on image fusion method is purposed instead of selecting a single in-focus image with maximum autofocus function value on the sequential images on the Z axis. In this process, firstly the image with maximum autofocus function value is found by implementing auto-focusing based on autofocus function (Entropy), and assigned to the reference image (Fig. 6.b). However, a blurred area in this image (Fig. 6.b right bottom) is still observed, while the same area is monitored as a nonblurred zone, as shown in "Fig. 6.a". The image in Fig. 6.a is the previous image in the sequence. In addition, the other part of the image (Fig. 6.a top left) is observed as a blur region, but the same part is viewed more clearly in "Fig. 6.b" (top left). The reference image and four images with maximum values, as shown in "Fig. 7", are fused with multi-focus color image fusion method based on CVT + HSV components to solve this problem and obtain all of region in-focus image as shown in "Fig. 8". In addition, DWT and the different color spaces (RGB, YCbCr, YIQ) are used to evaluate the performance of the fusion process.

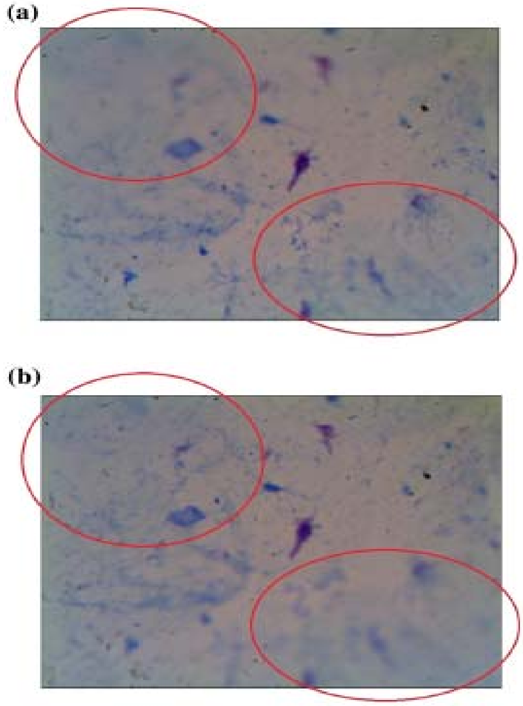

Fig. 6. (a) The image with value near maximum (b) the reference image

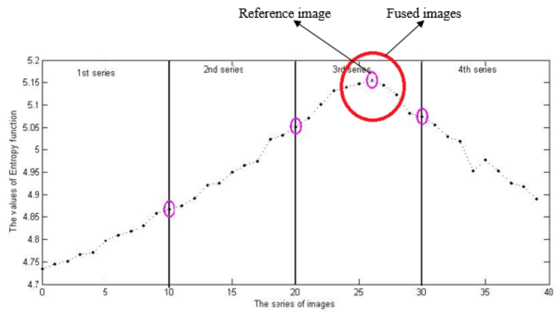

Fig. 7. The reference image and the other four images with values near maximum

2.2. Evaluation of Fused Image Quality

In this study, various criteria is used to decide which fusion method is better and which color model is suitable for microscopic image fusion. These methods:

1. Peak Signal Noise Rate (PSNR)

$$PSNR = 10\log \frac{255^2 MN}{\sum_{i=1}^{M}\sum_{j=1}^{N}(R(i,j)-T(i,j))^2} \qquad (2)$$

2. Root Mean Square Error (RMSE)

$$RMSE = \sqrt{\frac{\sum_{i=1}^{M}\sum_{j=1}^{N}(R(i,j)-T(i,j))^2}{MN}} \quad (3)$$

3. Mean Absolute Error (MAE)

$$MAE = \frac{1}{MN}\sum_{i=1}^{M}\sum_{j=1}^{N}|R(i,j)-T(i,j)| \quad (4)$$

Where, R – reference image, T – fused image, i – pixel row index, j – pixel column index, M and N – number of row and column.

The qualities of fused image results are compared and evaluated by using PSNR, RMSE and MAE. "Table 1" shows the statistical results of fusion methods based on different color space + DWT and CVT. We can see that CVT giving better results than DWT, is more suitable in transform domain to fuse microscopic images. In "Table 1", image fusion method that uses only RGB color gives lower results than transform domain. It is shown that multiscale geometric transforms such as DWT and CVT obtain more significant information from the image than spatial domain. Moreover, RGB color model is not suitable for color image fusion and HSV color model gives best results for microscopic image fusion.

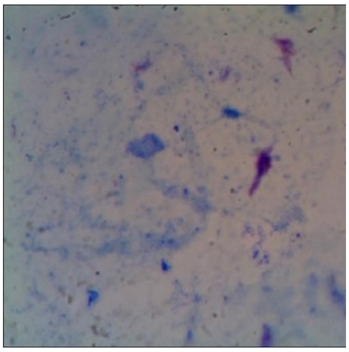

Fig. 8. The image obtained with multi-focus color image fusion method based on CVT + HSV components

Table 1. The statics results of fusion methods based on different image model + DWT and CVT

Fusion Method	PSNR	RMSE	MAE
RGB	37.4330	0.0053	0.1938
RGB + DWT	37.5758	0.0063	0.5752
RGB + CVT	38.7183	0.0062	0.9603
HSV + DWT	37.9139	0.0068	0.9093
HSV + CVT	40.0373	0.0071	1.8844
YCbCr + DWT	39.0230	0.0065	1.1989
YCbCr + CVT	38.6596	0.0062	1.1643
YIQ + DWT	37.8591	0.0058	0.2674
YIQ + CVT	38.6759	0.0062	1.2193

4. Conclusions

In the other studies auto-focusing based on selecting a single image with maximum auto-focus function value on the sequential images on the Z axis was implemented to obtain focus image. Therefore, some regions in the image can be blurred or may not be seen clear. In this study, to ensure detection of the image with all region in-focus auto-focusing on microscopic imaging based on image fusion method is proposed. At the end of studies it is shown that multiscale geometric transforms such as DWT and CVT are more effective than spatial domain to obtain significant information from the image. The results indicate that the in-focus images obtained with our proposed auto-focusing approach (based Curvelet transform) on different color components have higher focus values than the auto-focusing based on selection of a single image from the image series.

5. References

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