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A novel extended depth of field process based on nonsubsampled shearlet transform by estimating optimal range in microscopic systems

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ABSTRACT

Increasing the depth of field (DOF) and maintain a high resolution have been the classical challenges for acquiring a single 2D image sample investigated with the microscope to enhance a complete in-focus. The extended depth-of-field microscope is implemented to overcome these problems. Various studies have proposed the wavefront coding and image fusion as the remedies for the blurred content of the in-focus image. In this study, a classical extended depth of field (EDOF) process based on the image fusion is implemented by moving a microscope platform along the fixed range, a distance between initial and final positions, to determine a random Z axis. During the movement of the platform in this range, a certain number of multi-focus images are acquired at infinite steps (Δd). However, it is seen that the magnification objective affects the range and number of the multi-focus images. Instead of determining the range randomly, the optimal range is selected to extract a significant information from the multi-focus images. In this study, a novel EDOF process based on the multi-scale representations is improved, estimating the optimal range in the microscopic systems. Our proposed EDOF process is performed in two main stages: pre-process and image fusion. In the pre-processing stage, various ranges with different initial and final positions are extracted to scan the whole structure of the sample on the Z axis. In the second stage, a novel image fusion approach based on the Nonsubsampled Shearlet transform (NSST) is implemented into all ranges to obtain the optimal fused image. To evaluate the performances of the proposed image fusion approach and to show the effects of other color spaces on the image fusion approaches based on the multi-scale representations, fused images created with the different fusion approaches including Maximum Absolute Selection, Variance, Tenengrad, Discrete Complex Valued Wavelet Transform, Discrete Curvelet Transform, and other color spaces (HSV, YIQ and YCbCr) are tested in terms of their transferred focus information, outliers and blurring. From the obtained experimental results, the fused image created with our proposed approach contains more detailed information and fewer outliers and artifacts. Furthermore, the YCbCr and HSV color models provide the highest performances that capture the critical information in terms of focusing.

1. Introduction

The depth of field (depth of focus) is the distance where objects on the scene appear clearly in-focus. The microscopy depth of field is defined as an axial depth at which a sample is moved without loss of focusing while the imaging device is stationary. When the sample size is examined under a microscope, the sample is thicker than the depth of field as it is not possible to acquire a single 2D image with the whole scene completely in-focus. The sample areas outside the depth of field appear less sharp. In literature, the basic mathematical formulation to analyze the depth of field for thin lenses under the microscope is represented by Eq. (1).

$$d = \frac{\lambda}{n(\sin\alpha)^2} \tag{1}$$

Eq. (1) describes the relationship between the depth of field (d), the wavelength of illumination (λ), the reflective index of the medium (oil or air) between the objective lens and the sample (n) and the angular semi-aperture (α) of objective [1–3]. Moreover, the numerical aperture (N) and magnification of objective (M) are inversely related to the depth of field according to Eqs. (2) and (3) [4]. As the ratio of magnification

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objective increases, it is not possible to project the sample 3D structure, investigated in the microscope, to the 2D image plane, to make the whole area in-focus completely.

$$NA = n \sin \alpha \quad (2)$$

$$M = const * NA \quad (3)$$

This restriction is addressed by extending the depth of field in the microscopic system, and various techniques, such as the wavefront coding and image fusion have been proposed to solve this problem [5].]. The wavefront coding is a technique firstly proposed by Dowski and Cathey [6]. In this technique, the depth of field is extended by placing phase-only elements in the back focal plane of a microscope's objective [7,8]. The image fusion is a digital approach that obtains a single image with all the information and details in-focus, integrating a series of the multi-focus images. The image fusion algorithm can be implemented into three different levels: low (pixel) level, middle (feature) level and high (decision) level [9,10]. Low-level algorithms are applied directly to the pixels of the images in the spatial or frequency domain. In the middle-level algorithms, focus features are extracted for the fusion. In the high-level algorithms, the probabilistic information of the images assists the integration of the images.

The image fusion approaches for the extended depth of field are be classified as follows [11]:

1. Multi-scale representations based approaches: The basic principle of these approaches is that the most in-focus region contains higher frequency components than the others. Therefore, the multi-scale representations of the input images are used as focus features in the image fusion approaches. Laplacian pyramids [12], gradient pyramids [13], Fourier transform [14], discrete cosine transform (DCT) [15,16], discrete wavelet transform (DWT) [17–21] curvelet transform [22,23], contourlet transform [24,25] and shearlet transform [26] are some of the preferred methods to obtain the frequency coefficients of the input images.
2. Sparse representations based approaches: To keep the saliency information on the fused image, the sparse representations of the input images are utilized as the focus features [27]. These approaches are implemented using the following steps [11]: (1) Partitioning of the multi-focus images into overlapped patches. (2) Decomposition of the patches to obtain sparse representations. (3) Selection of the coefficients with the fusion rule. (4) Reconstruction of the fused coefficients to obtain a fused image.
3. Neighborhood-based approaches: To select the in-focus pixels from the input images, the focus value of each pixel is extracted using the focus operators that use the neighboring pixels to calculate the focus value. In the literature, several focus operators are proposed: (1) Gradient-based operators that use the first derivative of the image: Thresholded Absolute Gradient [28], Tenengrad [29], Squared Gradient [28] and Brenner [30,31]. (2) Laplacian-based operators that utilize the second derivative of the image: Energy of Laplacian [31] and Modified Laplacian [32]. (3) Statistics based operators that use the statistical knowledge, such as the histogram and the texture on the image to calculate the focus value of the pixel: Variance [28,32], Normalized Variance [32] and Entropy [31].
4. Neural Computing based approaches: To integrate shift-dependent in the fused images, these approaches decompose the input images into blocks [33]. These blocks are trained in the neural network to determine clearer regions, and the clearer regions are fused into a single image. Instances of neural networks are the artificial neural networks (ANN) [34], pulse coupled neural network (PCNN) [35–37], and deep learning [38].
5. Hybrid approaches: To minimize the shortcomings of the previous approaches, hybrid approaches, based on the combination of different transforms, are proposed. Some examples of the hybrid approaches are IHS and wavelet [39], wavelet and contourlet [40], PCA and wavelet [41], IHS and PCA [42], wavelet, and sparse representation [43].

The classical EDOF process based on the image fusion of the microscopy system consists of three steps: (1) the creation of a series of multi-focus images. This step is implemented by moving the microscopy platform along the fixed range, a distance between the initial and final positions, to determine the randomly Z axis. To obtain a fused image, a certain number of multi-focus images between these positions acquire the infinite steps (Δd). During the image acquisition process, this number is not changed according to the types of the sample and microscope objective. However, it is seen that the range between the initial and final positions and the number of multi-focus images on the Z-axis are effective in extracting a significant information from the focus of the sample. During the EDOF process, scanning the whole sample 3D structure on the Z axis is guaranteed to create a 2D fused image with a precise information and details. To demonstrate the efficiencies of a total number of multi-focus images, the range and by not scanning the whole 3D structure of the sample of EDOF process, a series of 1000 multi-focus images are captured by moving the platform on the Z axis in the step size of 0.0125 μm. In the acquisition process of this image, the initial and final positions are determined randomly. The focus values calculated with the Variance function are shown in Fig. 1a. Based on the information presented in Fig. 1a, the spread of the images with more comprehensive focusing information is approximately 450 and 550 indices. The EDOF process that uses all the images in Fig. 1a gives a fused image as shown in Fig. 1b. When the range between the initial and the final positions is long, a large number of images that do not affect this process is used unnecessarily, leading to disorders and noises on the fused image as shown in Fig. 1b. However, this process requires a huge amount of computation time and power. On the other hand, two other fused images extracted, using different initial and the final positions, are determined randomly on this series. For the fused image shown in Fig. 1c, a series of images are taken from the initial position (200) to the final position (450).

Also, 350 images from the initial position (550) to the final position (900) used for the fused image is shown in Fig. 1d. Reducing the number of the images affects the noisy and erroneous of the fused images as shown in Figs. 1b–1d. It is thought that this blurring is caused by not scanning the whole sample on the Z axis. In contrast to the previous approaches [4,22,44], a pre-process stage to ensure the whole sample is scanned on the Z axis is implemented in this study. The total number of the multi-focus images is optimized regardless of the types of the sample and the microscope objective, which is a critical factor affecting the EDOF process on the microscopic parameter.

Moreover, the optimal range between the initial and final positions is estimated by formulating the number of the multi-focus images with a specific mathematical model. (2) The extraction of the focus features: It is aimed at this step to obtain the critical features from the multi-focus images for the EDOF process. In recent years, many multi-scale images transform, such as wavelet, curvelet, contourlet and shearlet, have been proposed. To extend the depth of field in microscopy, Forster proposed the complex-valued wavelet, which outperformed the traditional real-valued wavelet [4]. However, the wavelet families have some limitations in the representation of curves and edges on the images. Tessens developed a curvelet-based image fusion approach to extend the depth of field in microscopy to overcome the limitations identified in wavelets [22]. In our study, non-subsampled shearlet transform, that has the better sparse representation ability, which demonstrates a faster computational speed than other multiscale image transforms, is proposed for the extended depth of field in microscopy. (3) The fusion of the focus features: In this step, the fusion rules combine the focus features into a single image. Maximum absolute selection and averaging are the examples of the classical fusion rules. Due to the extraction of