Crossdisciplinary Applications of Microscopy Techniques, e.g. Physic-Life Science Interfaces

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Enhancing effective depth-of-field using spectra-specific wavelet based multi-focus image fusion for digital pathology applications

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Optical microscopy is inherently characterised by diffraction-limited wavelength-specific depth-of- focus causing objects beyond this limit to appear defocused in the image. We present a wavelet-based multi-focus image fusion algorithm aimed at enhancing the effective depth of field of bright-field optical microscopy. The objective of the proposed algorithm is to generate an "All-in-Focus" image within the microscope's field of view by selectively fusing multiple partially focussed images acquired as a focus-merging z-stack. All-in-Focus image has higher extended depth of focus and can be subsequently used by human and machine vision platforms for image analysis [1].

The proposed method for image fusion utilizes a pixel-by-pixel non-linear image fusion scheme i.e. fusion of the images at the highest resolution level. In the present application, images are acquired using Lieca DM750 Bright-field (white light illumination) Compound Microscope under 40x A-plan objective magnification (NA – 0.65), manually maintaining an inter slice-distance of 2 µm in Z-direction on either side of a user-defined plane (central focus). The effective depth of field at this objective magnification is 1µm at an image depth of 12.8 mm. The number of slices in the z-stack acquisition is selected to have the complete depth of the sample covered. In order to reduce blurring due to accumulated averaging and relax the computational burden on the fusion routines, images corresponding to spectra-specific local maximas of focus measures are incorporated into the fusion pool for further processing. These spectra-specific focus graphs are generated using focus measure calculated as the ratio of Daubechies 'Db6' wavelet coefficients [2]. The focus profiles for a selected sample dataset are presented in Figure 1. Further, the selected images are fused pair-wise using the proposed wavelet-based multi-focus fusion algorithm as described below. The results of fusion using this framework on a sample dataset are presented in Figure 1.

Stage 1: Coefficient Extraction: The source images are decomposed using Discrete Wavelet Transform using the selected wavelet basis with a decomposition level of 2, to obtain the approximate and detail wavelet coefficients.

Stage 2: Approximate Component Blending: A weighted-average based approach is used to blend the approximate components of the source images. The weights are determined proportional to the gradient based focus measures derived using Gaussian Steerable Filters as proposed in [3]. This composite approximate coefficient image is used for reconstruction of the fused image.

Stage 3: Detail Component Blending: The coefficient-specific activity of the detail components is determined by the absolute value of the corresponding coefficients. For the composite coefficient selection, a Choose-Max strategy is implemented, followed by a 5×5 window-based majority filter consistency verification step to remove spurious selections [4].

Stage 4: Fused image reconstruction: The final fused image is generated by applying Inverse Wavelet Transform to the reconstructed composite wavelet coefficients obtained after Step 2 and 3.

The above fusion procedure is applied pair-wise on each of the selected images in the fusion pool. At each stage of fusion, the improvement in the cumulative focus measure is evaluated. For the present application, to choose an optimal wavelet family for optimal fusion, the relative improvement in the focus measure measured as ratio between the focus measure of the fused image and the most focussed source image, is used as an evaluation metric. An exhaustive evaluation on the sample dataset presented here using wavelet families like Daubechies, Biorthogonal, Morlet, Coiflets and Symlets, it was observed that Daubechies 'Db4' wavelet basis gave the most optimal improvement in the focus measure of 4.783. The experiments on the image z-stacks obtained for the current application established the applicability of the proposed method to generate images with better information and higher focus measure than any of the source images. To conclude, the presented strategy is envisaged to solve the inherent problem of short depth of focus of optical microscopes and generate sharp in-focus images of the imaged sample.

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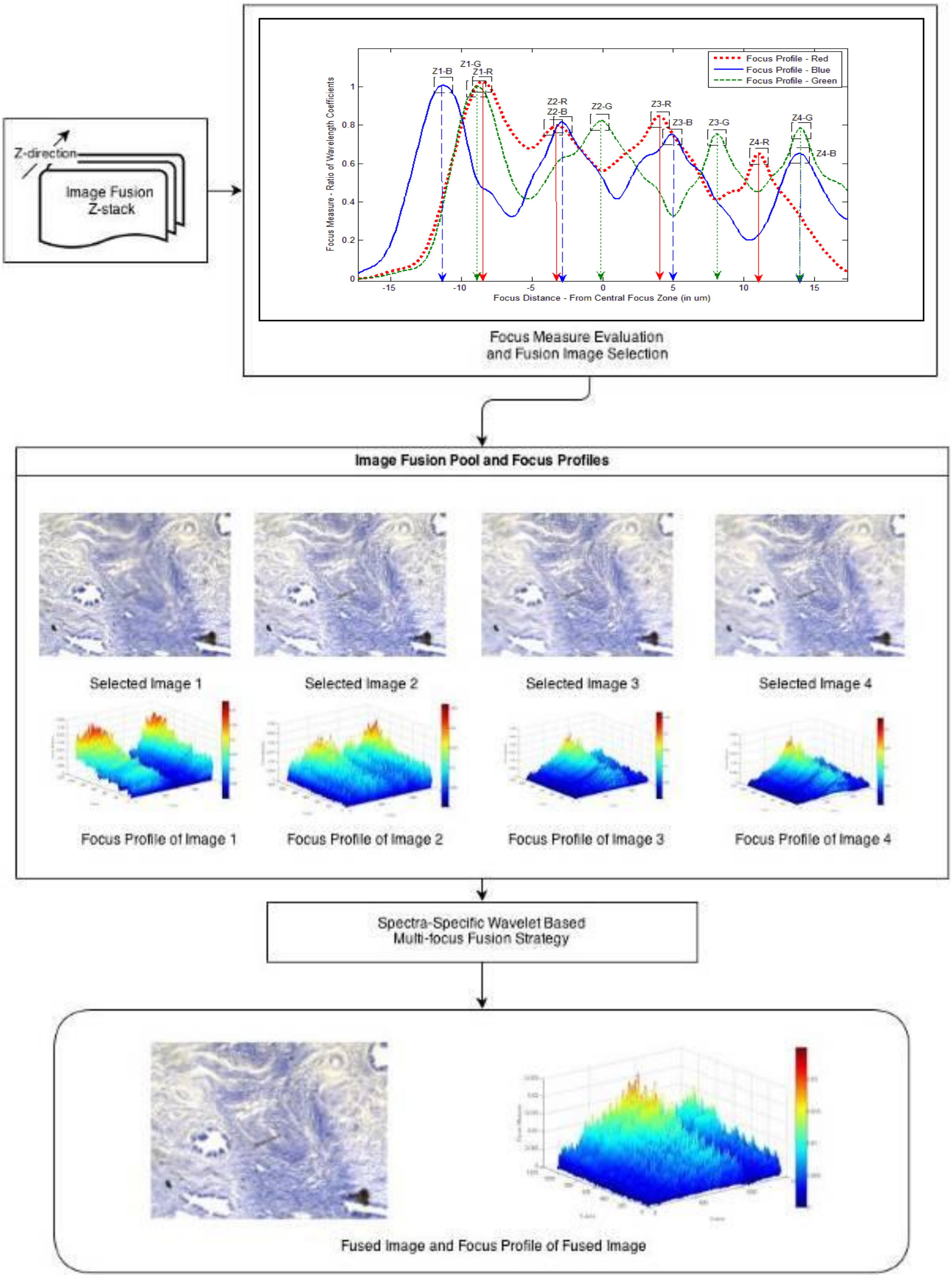

Figure 1. Schematic drawing of the framework for Spectra-Specific Multi-focus Fusion Scheme for extension of Depth of Focus. The images to be fused are selected from the image z-stack based on the Focus measure evaluation and the selected images are fused using the proposed spectra specific wavelet based multi-focus strategy to generate an "All-in-focus" Image.