# EXTENDED DEPTH-OF-FIELD FOR COLOR IMAGES IN LIGHT MICROSCOPY: IMAGE FUSION AND 3D VISUALIZATION

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**INTRODUCTION:** Bright-field microscopy suffers from a relatively small depth-of-field. Typically, the specimen's profile covers a range larger than the depth-of-field, and parts of the specimen that lie outside the object plane appear blurred. The specimen can be 'scanned' by moving the object along the optical axis, and different images will contain different areas that are sharp. The purpose of image fusion is to combine those images into one single image with an extended depth-of-field. One promising method is based on the wavelet transform. We show how the wavelet-based image fusion technique can be successfully applied to obtain a sharp composite color image. The selection of the proper type of wavelets can improve the results. We also introduce a way to apply this technique for color images without introducing false colors. Finally, we show how height information on the specimen, which we obtain as a side product from our algorithm, can be used for 3D visualization.

**METHODS:** To extend the depth-of-field, we have to define an in-focus criterion. Typically, an image that is in-focus has a maximal number of visible details, whereas defocused images are blurred by the point-spread-function of the microscope. Therefore, we assume that the areas of an image that are focused contain more high frequency components than the out-of-focus areas. Classical frequency analysis, using the Fourier transform, does not provide any spatial localization. The discrete wavelet transform DWT, by contrast, seems to be the ideal high saliency detection tool, since it allows a local analysis of the image's frequency content. The wavelet approach computes the DWT of the image slices at various focal distances, and constitutes the wavelets coefficients of the composite image by a maximum-absolute-value selection rule. The final composite image is obtained after computing the inverse DWT [3]. The image fusion algorithm proposed here has several refinements compared to the classical approach. First, we perform a vector-to-scalar conversion in a preprocessing step: Multi-channel (or color) data is converted to a single channel using principle component analysis. Then the algorithm is applies only once, and not on every channel separately. Second, we propose the use of complex-valued wavelets instead of hitherto used real ones. The complex wavelets introduce phase information, which yields stability for consistency checks applied in the fusion step. Moreover, they preserve details during the denoising step. Third, as post-processing step we propose a scalar-to-vector conversion that reassigns information from the original image stack to obtain multi-channel data. Fourth, we use the height map obtained as a byproduct from the reassignment for a 3D reconstruction of the specimen.

**RESULTS:**

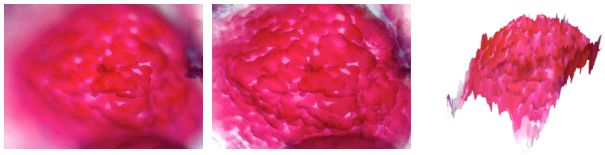

*Fig. 1. From left to right: Image from the original stack (Courtesy of ISREC), resulting fused image and 3D reconstruction).*

**DISCUSSION & CONCLUSIONS:** By measurements on simulated data and by examples on real images, we showed before [1,2] that our choice of a complex wavelet transform out-performs real wavelets and the variance method. We showed that a careful multi-channel con-version with subsequent reassignment avoids the introduction of false colors, and suppresses arti-facts. Our algorithm is freely available at http://bigwww.epfl.ch/demo/edf/ as a plug-in for ImageJ and is used in practice by biologists at the ISREC cancer research facility in Lausanne. The algorithm can be applied to visualize and present light microscopy images.

**REFERENCES:** [1]B. Forster, D. Van De Ville, et al. (2004) *Extended depth-of-focus fur multi-channel microscopy images: a complex wavelet approach*. Second 2004 IEEE Int. Symp. on Biomedical Imaging, Arlington, USA. [2]B. Forster, D. Van De Ville et al. (2004) *Complex Wavelets for Extended Depth-of-Field: A New Method for Fusion of Multi-Channel Microscopy Images*. Submitted to Microscopy Research and Technique. [3]A. G. Valdecasas et al. (2001) On the extended depth of focus algorithms for bright field microscopy. Micron **32**, 559-569.

**ACKNOWLEDGEMENTS:** The first author acknowledges the financial support from the German Academy of Natural Scientists Leopoldina (BMBF-LPD 9901/8-64) and from the European Union's Human Potential Program (HPRN-CT-2002-00285).