

COMPENSATION OF THE BRIGHTNESS AND CONTRAST ATTENUATION WITH DEPTH IN CONFOCAL MICROSCOPY

Martin Capek* **, Lucie Kubinova*

* Institute of Physiology, Academy of Sciences of the Czech Republic
Videnská 1083, 142 20 Prague 4, Czech Republic

** Institute for Biomedical Engineering, Czech Technical University
Zikova 4, 166 36 Prague 6, Czech Republic
E-mail: capek|kubinova@biomed.cas.cz

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Intensities of fluorescent images captured by a confocal laser scanning microscope (CLSM) from deep layers of a specimen are often darker than images from the top most layers due to absorption and scattering of both excitation and fluorescent light. These effects cause problems in subsequent analysis of biological objects. Methods [e.g. 1, 2] used for solution of this problem are generally based on the post-processing of data. We have chosen, first, an approach, which lies in software optimization of the microscope hardware during scanning and, second, a post-processing method based on a simple optimization of image intensities in the image stack.

In our experiments we have used a Leica SP2 AOBS CLSM. It is controlled by a personal computer equipped by the Leica Confocal Software which contains a Macro Developer giving a user a possibility to develop macros in Visual Basic language to tune the CLSM hardware.

We have developed a macro called SCOM (Series Capture Optimization Macro) in the above described environment. In the beginning the macro tests the specimen according to level of intensities in the predefined number of evenly spaced layers of the specimen. As a measure of the intensity level we have chosen an average value of pixel intensity values of a layer (AVPI). To get the same value of AVPI in all tested layers, the value of PMT Gain is optimized by “n-step search”. This optimization strategy uses decreasing steps to achieve the global minimum of difference of AVPI of two layers. Having values of PMT Gain of tested layers, the values of PMT Gain of all layers are piecewise linearly interpolated and the resulting stack of images is captured. The method preserves the information content of images within the whole specimen, but the level of background noise grows, especially in deep layers.

Therefore, we have developed a post-processing method too. The method optimizes AVPI of all images in the stack directly by adding or subtracting a value to image pixels. At the end of the procedure the whole stack is intensity equalized. The method has been implemented within a modular Ellipse software system (ViDiTo Company, Slovakia; <http://www.ellipse.sk>) for the visualization and processing of 2D/3D biological data sets. This post-processing method is computationally fast, simple, and at the same time yielding good visualization results.

[1] Margadant, T. Leemann, P. Niederer: A precise light attenuation correction for confocal scanning microscopy with $O(n^4/3)$ computing time and $O(n)$ memory requirements for n voxels. *J. Microsc.*, **182**, 121-132 (1996).

[2] C. Kervrann, D. Legland, L. Pardini: Robust incremental compensation of the light attenuation with depth in 3D fluorescence microscopy. *J. Microsc.*, **215**, 297-314 (2004).

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