Two image preprocessing methods for Confocal Laser Scanning Microscopy.

Jan Michálek[1], Martin Čapek[1,2] and Lucie Kubínová[1]

1. Department of Biomathematics, Institute of Physiology ASCR, Praha, Czech Republic
2. Czech Technical University, Faculty of Biomedical Engineering, Kladno, Czech Republic

michalek@biomed.cas.cz
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Series of stacks of fluorescent images captured by a confocal laser scanning microscope (CLSM) from different depths of a biological specimen are often used to produce 3D representation of the underlying biological volume, image mosaics covering an area larger than the field of view of the CLSM with a given magnification of the objective etc. Most of the 3D-reconstruction or image stitching algorithms make a tacit assumption that the brightness mapping between a certain type of tissue and the related grayscale-level is independent of the location of the tissue element in the specimen. However, this assumption is as a rule not true for raw captured images in a CLSM stack. Two types of brightness corruption are most prominent:

- *inter-frame brightness variability,* which manifests itself by the fact that CLSM images captured from deep layers of a specimen are often darker than images from the top layers
- *intra-frame brightness variability,* by which we mean that tissue regions having the same fluorescent dye density appear brighter in some locations of the CLSM image and darker in others.

If these two undesirable effects are left unattended, 3D-reconstruction or image mosaicking algorithms may produce visually unappealing results, or fail altogether.

To forestall algorithm performance deterioration caused by the above two image distortions, we propose two dedicated, fast, fully automatic methods:

Fast algorithm for matching of CLSM image pairs for constant brightness.

Image pairs of adjacent layers in a confocal image stack exhibit brightness attenuation due to absorption and scattering of both excitation and fluorescent light. In a previous work [1], we applied the dynamic histogram warping method to post-processing of confocal microscope image stacks (typically tens of images). The dynamic programming-based algorithm used in [1] takes about 3 sec to process a single 256 gray-level image on a Pentium 4, 2.8 GHz CPU.

To speed up processing of large series of confocal microscope images we propose an algorithm based on matching the *brightness distribution function*, rather than *histogram matching,* of darker images from deeper layers of the confocal stack to a reference brightness distribution. The speed up is based on the idea that distribution functions, being monotonical, are amenable to much faster matching algorithms than histograms, which may assume an arbitrary shape. Using a distibution function matching algorithm, the computation takes about 0.02 sec on a 1.6GHz laptop, with resulting image quality comparable to that of dynamic histogram warping. While our focus was on processing large numbers of CLSM images, the fast algorithm is also applicable to other tasks where the brightness of a pair of images needs

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to be matched, such as disparity map or depth evaluation in stereo vision, optical flow estimation in motion or image registration, or image mosaicking.

Fast algorithm for compensation of illumination inhomogeneities in confocal laser scanning microscope images.

Ideally, CLSM image regions corresponding to the same concentration of fluorescent dye in the specimen should be mapped to the same grayscale levels. In practice, unfortunately, even ideally homogeneous specimens like artificial calibration grids exhibit darkening of image edges, lightening of the centre etc.; the effects are yet more pronounced in images of real biological specimens. A host of reasons account for the effect: optical imperfections of the objective, surface of the specimen being non-perpendicular to the laser beam which makes the rays bend depending on the angle of incidence, locally curved surface of the specimen which concentrates or fans out the light, non-planar, curved, surface of focus, rather than a plane, which may miss a thin fluorescent layer, etc. Our solution to the problem relies on *estimating a spatially variable gain* which models the adverse effects of uneven illumination, and correcting images by inverting the estimated gain. The proposed approach exploits fast implementation of a morphological operation called the *upper Lipschitz cover.*

1. Capek M., Janacek J., Kubinova L.: Methods for Compensation of the Light Attenuation with Depth of Images Captured by a Confocal Microscope, Microscopy Research and Technique 69:624–635 (2006).
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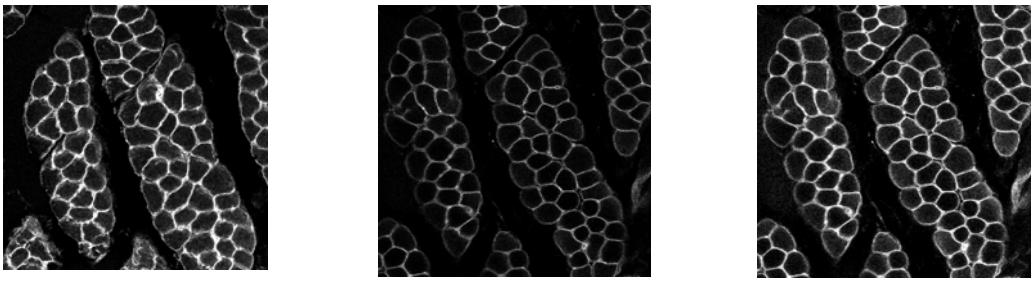
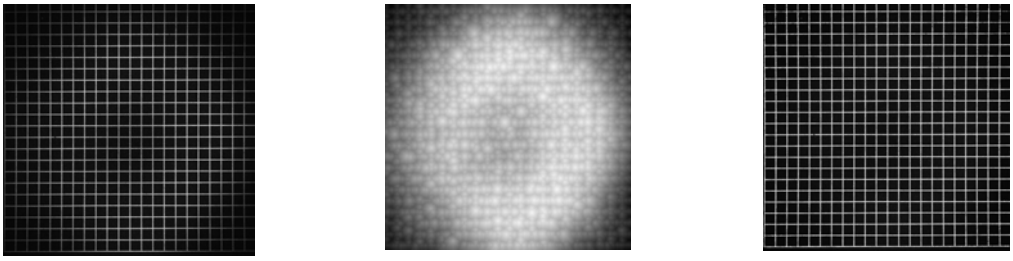


reference image

image #57 from the confocal stack **before** *brightness matching*

image #57 from the confocal stack **after** *brightness matching*

Figure 1. Brightness matching of a pair of images pair showing the reference, an original dark image, as well as the same, brightness matched, image.



recorded image

Lipschitz - cover estimated gain

corrected image

Figure 2. Correction of inhomogeneous illumination of the image of a calibration grid.