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Lateral Brightness Correction In Confocal Microscopy Images Using Mathematical Morphology Filters

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In images acquired by confocal laser scanning microscopy (CLSM), regions corresponding to the same concentration of fluorophores in the specimen should be mapped to the same grayscale levels. In practice, due to multiple distortion effects, CLSM images of even homogeneous specimen regions exhibit irregular intensity variations, e.g. darkening of image edges and lightening of the centre (Fig.1a). A spatially varying grayscale map complicates image postprocessing e.g. in image stitching of neighbouring fields of view or in registration of images of successive sections for three-dimensional (3D) reconstruction. We present a fast correction method based on estimating a spatially variable gain which models the lateral brightness distortion, and multiplying acquired CLSM images by the inverse of the estimated gain. The estimation is done from the CLSM image itself, not from some kind of a reference image. The proposed approach exploits two types of mathematical morphology operators: the median filter and the upper Lipschitz cover [5].

Related problems have been adressed in different fields of biomedical imaging. Mangin [2] presented an entropy minimization-based method for automatic correction of intensity nonuniformity in MR images. For CLSM images, Mangin's assumptions are not granted. Besides, the processing time (~minutes/image) is too long to allow correction of large stacks stacks of CLSM images.

Lee and Bajcsy [3] propose mean-weight filtering for lateral intensity correction of CLSM images. It is not clear how well mean-weight filtering performs in practice, since the authors present only one result for a real CLSM image. Feasibility of the method for *automatic* processing of large CLSM image stacks is questionable, since the size and shape of the filtering kernel has to be found interactively by successive optimization.

Hovhannisyan et al. [4] present a method of image heterogeneity correction in 3D multiphoton microscopy based on mutiplication of the acquired image by a lateral correction factor, which, unlike in [2] and [3], needs to be estimated from a reference image of a uniform fluorescent sample, not from the image being corrected itself. The necessity to use a reference image restricts the flexibility of the method of [4].

To overcome the drawbacks of the quoted methods, we propose a method for lateral brightness correction of *large stacks of CLSM images* which is *fast*, *fully automatic*, and does not rely on uniform fluorescent samples. For the intensity of the emitted light in CLSM, Heintzmann [1], presents the formula:

$$I_{em}(x,y) = I_{ex}(x,y) \cdot Obj(x,y) \qquad (1)$$

with $I_{ex}(x,y)$ the excitation intensity, and $Obj(x,y)$ the concentration of the fluorescent dye. If excitation intensity is constant across the specimen, recorded light intensity for object regions having the same dye concentration should *ideally* be the same. In *real* CLSM images as in Fig.1a, image brightness obviously varies depending on the pixel location within the frame. To model this dependence, we suppose that the emitted light at different pixel positions is amplified or attenuated by a single function called $gain(x,y)$ that together with Eq.(1) yields the relationship between the dye concentration and the light intensity:

$$I_{rec}(x,y) = gain(x,y)\cdot I_{em}(x,y) = gain(x,y)\cdot Obj(x,y)\cdot I_{ex} \qquad (2)$$

In a recorded image, we separate the gain from the object based on the fact that the gain changes slowly, is continuous and has only a small number of minima or maxima, while the concentration of the fluorescent dye may change abruptly on tissue boundaries, is discontinuous and may have arbitrarily many minima and maxima. To estimate the *gain*(x,y) , we assume that local maxima in the CLSM image correspond to specimen regions with the highest fluorescent dye concentration, and span a slowly varying function with limited rate of change over the maxima using a morphological filter called the *upper Lipschitz cover* [5]. In noisy images, noise is first reduced using a fast median filter.

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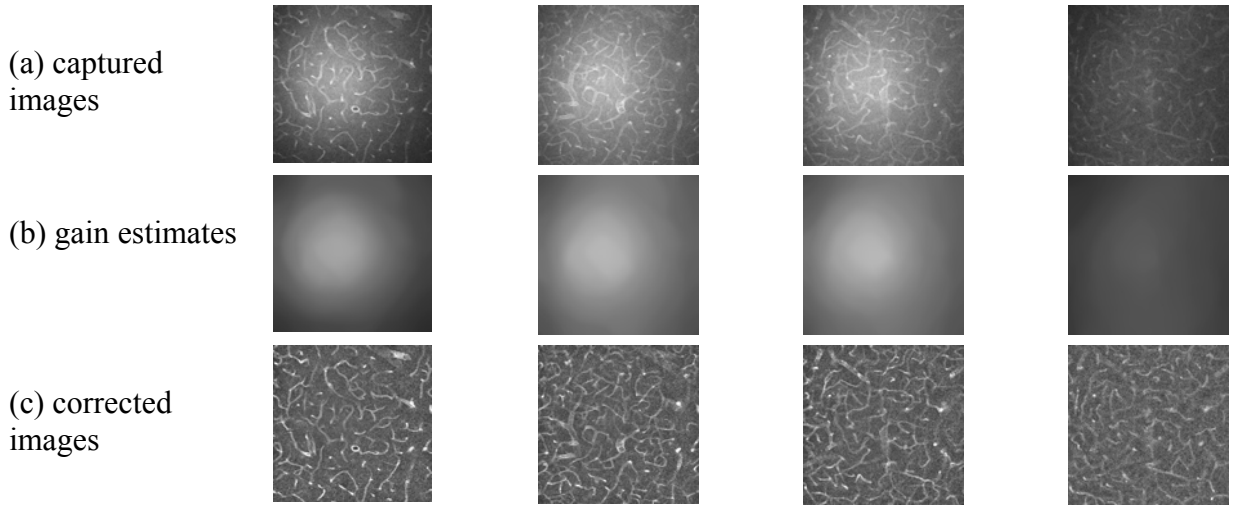

FIG. 1. Brightness correction of a confocal stack of a cross section of a rat brain cortex with stained capillaries captured by a Leica SPE CLSM using a HC PL FLUOTAR 20x objective, NA=0.15.

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