DEGRADATION OF IMAGES ACQUIRED BY CONFOCAL MICROSCOPY
IN DIFFERENT DEPTHS OF BIOLOGICAL SPECIMENS

Oleksandr Chernyavskiy, Lucie Kubínová
Department of Biomathematics, Institute of Physiology
Academy of Sciences of the Czech Republic v.v.i.,
Videnska 1083, 14220 Prague 4, Czech Republic
E-mail: cernavsky@biomed.cas.cz

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Image acquisition by a confocal microscope provides true 3D imaging. However, it is often affected by a number of factors deteriorating the overall image quality. Postprocessing algorithms, such as deconvolution, applied to raw data can significantly improve the image quality, e.g. resolution and signal-to-noise ratio. On the other hand, when applying such algorithms, one should take care that the meaningful information is kept unimpaired, so that quantitative measurements of the processed 3D image data are not biased. For example, one can achieve improvement in volume estimation after deconvolution using either a theoretical point spread function (PSF), or experimentally measured PSF yielding better results [1]. The experimental PSF is usually acquired from confocal microscopic 3D images of microbeads having subresolution size, embedded in medium of the same refractive index (RI) as the used objective immersion. However, different biological tissues have different optical properties, such as RI and transparency. This can cause considerable degradation of the PSF, especially in deeper layers of the specimen.

The PSF changes in different depths of a biological specimen can be measured on PSF-like structures [2], or by direct measurements of fluorescent beads in highly scattered medium as well as in RI mismatch conditions [3]. In the present study we evaluated the depth dependence of experimental PSF in thick tissue sections of rat brain cortex and other biological tissues having different optical properties. We obtained experimental PSFs directly from microbeads located at different depths of the specimen. Such measurements provided more exact information for data quantification; they can be also used for testing different deconvolution algorithms.

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REFERENCES