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Analysis of Point Spread Function Degradation in Thick Tissues

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Confocal fluorescence microscopy with one-photon excitation (1PE) or two-photon excitation (2PE) is becoming a routine tool for everyday use in biomedical research. Its optical sectioning capabilities can be exploited for measurements and geometrical characterization of biological structures and their 3D reconstructions, providing true 3D information about spatial tissue organization and morphology.

Although image acquisition by a confocal microscope provides true 3D imaging, 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 the either theoretical point spread function (PSF), or experimentally measured PSF (Fig. 1) while the latter yields better results [1]. The experimental PSF is usually measured 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, including their refractive indices and transparency. This can cause considerable degradation of the PSF, especially in deeper layers of the specimen.

PSF changes in different depths of a biological specimen were measured based 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 measured PSF directly from microbeads located in different depths of the specimen. Such measurements provided more exact information not only for data quantification, but also can be used for testing different deconvolution algorithms [4].

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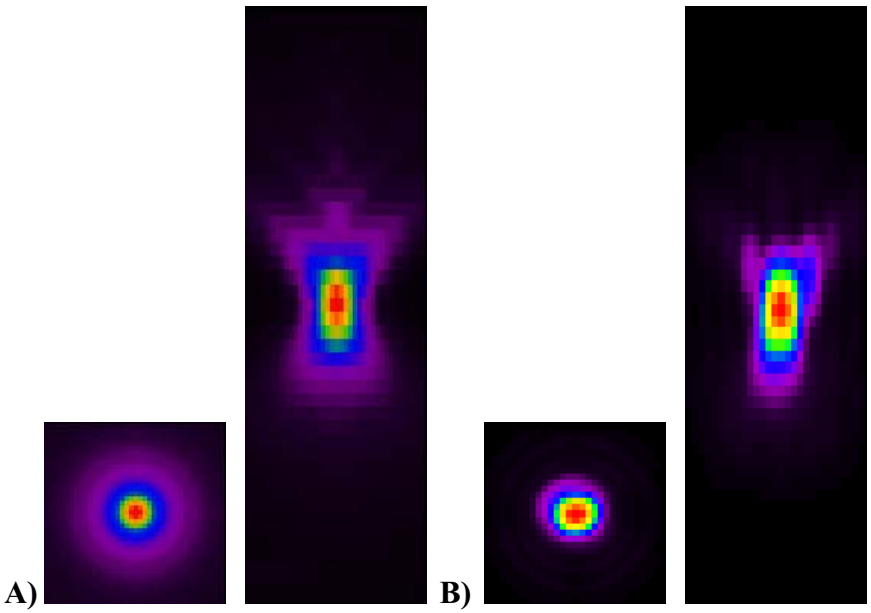

FIG. 1. Point spread function: A) theoretical PSF versus B) experimental PSF (Obj. 100x, oil, 1.4 NA, pinhole 1 Airy)

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