PSF ENGINEERING IN 3D SCANNING MICROSCOPY

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Far-field microscopy has experienced spectacular progresses in the implementation of novel types of 3D imaging techniques like confocal detection or multiphoton excitation. Such advances are of great importance in the inspection of thick biological tissues. Although these techniques are meanly featured by their unique optical sectioning capacity, biological imaging still demands the improvement of this capability. Progress in this direction can be achieved by use of 3D point spread function (PSF) engineering techniques.

Along the last years, our “3D Diffraction and Imaging Group” has improved the optical sectioning capabilities of scanning microscopes by designing pupil masks that properly shape their PSFs [1]. Our PSF engineering techniques are designed to work in the nonparaxial vectorial regime and are applied both to single-photon fluorescence confocal microscopy and also to non-linear scanning microscopy. Our proposals include both phase and amplitude masks applied to the 3D stretching of the PSF —i.e., increasing in 3D resolution— of different types of scanning microscopes such as conventional confocal microscopes, 4Pi-confocal systems or multiphoton scanning devices.

Additionally, we have designed optical masks that dramatically reduce the effect of sample-induced aberrations on the PSF of the system. These aberrations are variable throughout the scanning of the specimens and cannot be compensated statically. Our proposal extends the spatial invariance of the 3D PSF of the microscope, providing a sharp 3D recovery of thick samples.

In this work we present a brief review of our major results in this field, stressing the potentiality of our techniques to deal with different present and future challenges in 3D microscopy.

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