Aperture Optimization to Improve Modulation Depth in Focal Modulation Microscopy

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Abstract

Focal Modulation Microscopy (FMM) was recently developed as a novel method for in-vivo imaging of thick biological tissues [1, 2]. FMM introduces a spatial phase modulator into the illumination beam path to modulate part of the beam, which results in temporal oscillation of the interference pattern in the focal volume; whereas no oscillatory emission occurs in the out-of-focus region because of spatial separation between the modulated and unmodulated beams. Only the modulated emission light from the focal volume is retrieved to form FMM images, so FMM can achieve improved imaging depth compared to conventional confocal microscopy [1, 2].

Modulation depth is an essential parameter in designing FMM because it determines the signal-to-noise ratio and the efficiency of FMM signal generation. Large modulation depth not only improves penetration depth, it also reduces the threshold of excitation power, which is important for avoiding photobleaching. In a previous work, only equal-area aperture pattern with uniform illumination was considered [3]. However, equal-area aperture pattern deteriorates modulation depth for high numerical aperture- objective lens and is not practical for non-uniform illumination as in the case of Gaussian beam illumination, for example. In fact, equal-area aperture pattern is not even the optimal design for uniform illumination.

Here, we propose a new aperture optimization method with the benefit of pinhole selectivity and field superimposition. Our results showed that the optimized apertures have much better improved modulation depth than equal-area apertures. Furthermore, this aperture optimization method can also be applied to the cases of high numerical aperture imaging using vector diffraction theory, and non-uniform illumination. Thus, we further investigated modulation depth for both linearly polarized incident beam and radially polarized incident beam. Our results show significant improvement in modulation depth along with enhanced resolution with radially polarized incident beam. Several optimized apertures with simple configurations and large modulation depth are proposed and demonstrated.