REDUCING BACKGROUND MODULATION IN TWO-PHOTON FOCAL MODULATION MICROSCOPY

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Optical imaging provides a high spatial resolution to resolve individual neurons and neuronal processes within scattering brain tissues [1].With the increase of penetration depth, the obtained images become blurred due to the scattering nature of the tissue. In order to enhance the penetration depth of optical imaging within scattering brain tissues, multiphoton microscopy (MPM) is developed to substantially extend the penetration depth of high-resolution optical imaging, which can enable functional imaging of populations of neurons in their native environment. However, the imaging depth for MPM in scattering biological tissue is limited by the signal-to-background ratio (SBR) [2]. Recently, focal modulation microscopy (FMM) has been developed and provide an alternative option to reject background scattering [3].

In this paper, combined with focal modulation techniques, two-photon focal modulation microscopy (TPFMM) is demonstrated using a novel spatiotemporal phase modulator. First, TPFMM in turbid media using a novel spatiotemporal phase modulator (STPM) is theoretically investigated using the vector diffraction theory. At the destructive stage during the excitation beam modulation, this STPM is equivalent to a strip-shaped pupil filter with a sinusoidal phase distribution. Compared to the binary filter patterns with sharp phase transitions, the contribution of out-of-focus ballistic excitation to the background is largely reduced using the continuous phase filters. In addition, this new STPM has been designed and integrated into TPFMM to achieve high-performance imaging of the biological tissues. It is found that TPFMM using this new STPM can significantly suppress scattered excitation and reduce out-of-focus ballistic excitation with acceptable modulation depth and resolution. Therefore, TPFMM with some new STPMs creates opportunities to further extend the penetration depth in imaging the scattering biological tissues, especially for the scattering brain tissue.

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