NONLINEAR IMAGING WITH EXTENDED DEPTH-OF-FIELD USING SHAPED EXCITATION

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Multiphoton microscopy can be used to probe the nonlinear optical (NLO) properties of thick biological tissue samples, and to perform optical biopsies. Different contrast mechanisms such as two-photon excited fluorescence, second- and third-harmonic generation (SHG, THG) can be used in combination to provide complementary information, which are together potentially useful to derive physiological scores (e.g. [1]). However the NLO characterization of an extended volume is time consuming, because multiphoton imaging typically relies on point-scanning and the acquisition rate is usually limited by signal level.

We study theoretically [2] and experimentally the use of shaped beams providing extended depth-of-field, such as Bessel beams, to rapidly probe the nonlinear optical properties of a thick sample. Shaped probe beams with programmable lateral and axial extensions can be produced in a multiphoton microscope using a liquid crystal-based spatial light modulator. We analyze the differences between the cases of fluorescence and harmonic extended-depth imaging. We show that signal level and emission directionality exhibit different behaviors as a function of sample size and beam shape depending on the imaging modality. Based on this analysis, we show experimental conditions where shaped beams can probe NLO properties more rapidly and more quantitatively than the usual Gaussian 3D scanning scheme