MULTIPHOTON LIGHT-SHEET IMAGING OF SHG NANOPROBES

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KEY WORDS: nonlinear microscopy, in vivo imaging, fast imaging, single-plane illumination microscopy, second harmonic generation, nanocrystals, nanoparticles, zebrafish.

Multiphoton microscopy has demonstrated unique advantages for *in vivo* imaging, including a large imaging depth or the ability to perform multimodal imaging by combining nonlinear fluorescence excitation with other nonlinear contrast mechanisms, such as second harmonic generation (SHG). However, the acquisition speed is often a critical limit for multiscale imaging or for investigating fast biological phenomena. Among recent strategies to improve the acquisition speed in multiphoton microscopy, light-sheet illumination exhibits unique advantages for fast live imaging [1-2] resulting from the orthogonal configuration of illumination and detection. Yet, this orthogonal geometry questions the ability to collect coherent signals such as SHG and perform multimodal imaging. Here, we present our recent investigation of the combination of two-photon excited fluorescence with second harmonic generation in a light-sheet microscope. We first investigate the orthogonal detection of coherent scattering from structures of different sizes and show point-like sources, such as SHG nanoprobes, can be efficiently imaged using light-sheet microscopy. SHG nanoprobes exhibit unique properties as contrast agents for microscopy metrology [3] and biological imaging [4]: indeed, they are biocompatible, achromatic, and the signal they produce is not limited by saturation, blinking or photo-bleaching. Based on numerical simulations and experimental analyses, we investigate the experimental parameters that govern the detection efficiency of SHG nanocrystals, such as the symmetries of their second-order susceptibility tensor, their rotational motion or the incident laser polarization. Finally, we demonstrate that fast (~200 fps) *in vivo* light-sheet imaging combining SHG and two-photon excited fluorescence can be achieved with SHG nanoprobes in live zebrafish embryos [5].

References:

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