MULTIPHOTON LIGHT-SHEET MICROSCOPY AT LOW LASER REPETITION RATE: MORE SIGNAL AND LESS PHOTODAMAGE

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Among strategies developed to increase the acquisition speed in multiphoton microscopy, light-sheet illumination exhibits unique advantages for fast live imaging with low photodamage [1-3] resulting from the orthogonal configuration of illumination and detection. Compared to the standard collinear arrangement, light-sheet microscopy uses specific illumination parameters, which drastically impact the photoperturbation processes involved in nonlinear imaging using pulsed lasers. Indeed, the illumination parallelization and a good axial resolution are obtained using very low illumination focusing of a single beam. It has several critical consequences, such as a high pulse energy limit for fluorescence saturation, relatively low laser peak intensity and mean power, as well as long pixel dwell times. Hence it suggests a very different photodamage regime compared to collinear techniques. However, the laser parameters governing linear and nonlinear photodamage in multiphoton light-sheet microscopy remain poorly investigated. Here, we investigate the nature of induced photodamage in multiphoton light-sheet microscopy and the influence of laser parameters on the signal-to-photodamage ratio, by using zebrafish embryonic heart beat rate as a sensitive reporter of photoperturbations. We experimentally characterize the linear and nonlinear effects involved during live imaging depending on laser parameters such as mean laser power, pulse frequency or wavelength. Such systematic analyses demonstrate femtosecond laser sources commonly used in multiphoton microscopy at 80 MHz repetition rate are not optimized to take full advantage of light-sheet illumination. We find an optimal pulse frequency of ~10 MHz for imaging mCherry labeled beating hearts at 1030 nm excitation wavelength [4]. We reach an order-of-magnitude enhancement in signal-to-photodamage ratio by optimizing the laser pulse frequency while maintaining low both laser average power and peak irradiance. High-speed multiphoton imaging is achieved (~0.5 kHz frame rate) without inducing additional heating or reaching nonlinear photodamage compared to previous implementation. More generally, using low laser repetition rate (10 MHz range) in multiphoton light-sheet microscopy result in a drastic improvement in fluorescence signal level without compromising live sample, which opens new opportunities for fast *in vivo* imaging.

References:
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