Two-Photon Absorption Properties of Far-Red Fluorescent Probes

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Two-photon excitation (TPE) microscopy relies on fluorescent probes that are easily excitable through two-photon absorption. Unfortunately, the two-photon absorption properties of a given fluorescent probe cannot be predicted from its single-photon excitation (SPE) spectra but require careful quantification. Especially in the 1990s, the two-photon action cross-sections of a large range of organic dyes and fluorescent proteins were measured (see for example [1, 2]) and the data quickly became an essential reference for the successful application of TPE microscopy. The recent introduction of highly photostable dyes in the red emission range (ATTO647N, STAR635P and others) offer intriguing new opportunities for TPE microscopy. The new silicon-rhodamine (SiR) dye [3] is of particular interest as it is a membrane permeable fluorophore that can be coupled to genetically encoded protein tags for in vivo labelling. The red-shifted emission wavelengths of these new dyes allow for deeper penetration depths and better biocompatibility. Moreover, the dyes themselves are likely more photostable in TPE than fluorescent proteins, which promises better image quality or longer time-lapse sequences. A particularly appealing application is the combination of TPE with stimulated emission depletion (STED) nanoscopy [4], where many of these new fluorophores have already been successfully used with SPE. However, their two-photon absorption characteristics have not yet been quantified and as such, they have been of limited use in TPE-STED nanoscopy and TPE microscopy in general.

Here we present the wavelength-dependent TPE action cross-sections of some of the most promising far-red STED-compatible organic dyes: ATTO647N, STAR635P, STAR520SXP and SiR. For TPE, we used a mode-locked Ti:sapphire laser emitting ~100 fs pulses at ~80 MHz over a wavelength range from 690 nm to 1040 nm. For action cross-section measurements, we used a protocol adapted from [2]. Our data demonstrates quantitatively the two-photon excitability of the listed STED compatible dyes and moreover, it supports the possibility of using the SiR dye as an alternative fluorescent marker for TPE-STED neuroimaging applications in live animals.

References