

4PI-MICROSCOPY WITH 1-PHOTON EXCITATION

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Over the last years two-photon excitation 4Pi-microscopy with an axial resolution of ~100 nm has been demonstrated in multiple biological applications [1-3]. The artefact-free presentation of the recorded structures has been possible because of the contiguous optical transfer function (OTF), which allows reliable image deconvolution. The use of two-photon excitation (2PE) has been critical to suppress the artefact-producing side-lobes of the 4Pi-point spread function.

Unfortunately, the dependence of 4Pi microscopy on 2PE also brings about disadvantages such as the need for expensive laser systems. Moreover, the main advantage of 2PE, that is the capability of penetrating deeply into scattering tissue, cannot be exploited because 4Pi-microscopy is restricted to sample thicknesses of several tens of microns.

Therefore, we have investigated conditions for which 1-photon-excitation (1PE) still gives low enough interference sidelobes, i.e. conditions for which the minima in the 1PE 4Pi-OTF are sufficiently high above the noise level. It turns out that 2PE can partly be replaced by choosing the optimum detection pinhole size and by utilizing the combination of 4Pi-excitation and 4Pi-detection in conjunction with a large Stokes-shift.

We show the potential of 1PE 4Pi-microscopy in comparison to its 2PE counterpart depending on different imaging parameters. Experimental results achieved with the Leica TCS 4PI underscore the potential in practical imaging.

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