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Two-photon excitation point spread function measurements and modeling.

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Abstract

Two-photon techniques are very important for quantitative microscopy due to the intrinsic three dimensional localization of the excitation point spread function (PSF). Quantitative two-photon measurements strongly depend on the exact shape of the PSF. The shape of the two-photon PSF depends on several factors including: excitation beam profile, aberrations in the excitation optical path, and aberrations caused by sample inhomogeneities. We are performing a series of experiments and models to explore the relationship between these factors and their effects on quantitative measurements. Specifically, the PSF is measured and modeled in the presence and absence of various spatial filters. The photon counting histogram (PCH) is used to correlate a brightness parameter for various beam profiles. Parameters recovered from fluorescence correlation spectroscopy (FCS) are compared with the measured PSF and modeled parameters. In addition we are modeling the effect of different apodizations of the objective's back aperture on the PSF. We will discuss the relevance of these studies to the design of actual experiments. Supported by the National Institutes of Health, PHS 5 P41 RRO3155.