

# QUASI-SPHERICAL FOCUS IN TWO-PHOTON SCANNING MICROSCOPY

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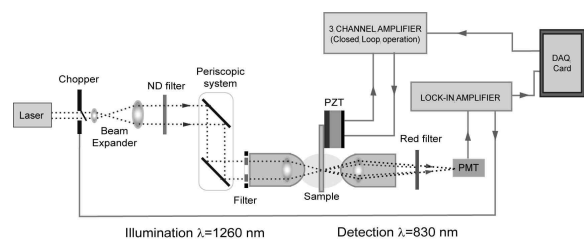
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The main feature of single-photon excitation confocal microscopes is their proverbial depth-discrimination capacity, which results from their ability for rejecting the light proceeding from parts of the object not on focus. A serious drawback of these systems is photobleaching, which appears since the entire sample is bleached when any single plane is imaged. Another drawback of this technique is its poor depth-penetration capacity. To solve these problems, two-photon excitation (TPE) scanning microscopy was proposed. This non-linear imaging technique relies on the simultaneous absorption of two photons, whereby a single fluorescence photon is emitted. Since the fluorescence intensity is, here, proportional to the square of the illumination intensity, TPE scanning microscopes inherently possess optical sectioning capacity despite the absence of pinholed detection. TPE generally uses near-infrared light that is less absorbed and scattered by biological or medical tissues, which allows deeper penetration of the excitation beam. Besides, since photobleaching depends here on the time-averaged square of the intensity distribution, it is restricted to the neighborhood of the imaged plane.

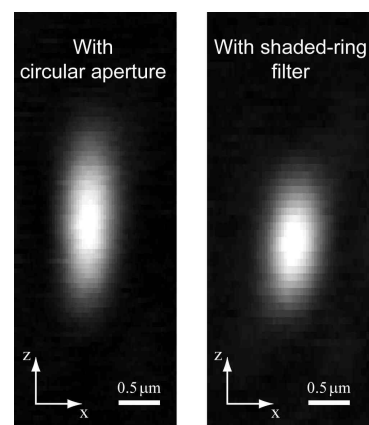
In the TPE mode the point spread function (PSF) is defined as the fluorescence distribution generated by focusing a collimated beam onto the sample. Such a distribution is proportional to the probability of simultaneous absorption of two photons, namely, to the square of the illumination intensity distribution. Therefore, even with the highest available NA, the PSF is much wider in the axial direction than in the lateral one, leading to anisotropic 3D imaging quality.

In this contribution we design a shaded-ring filter for optimum application in TPE scanning microscopy, and provide experimental evidence of the utility of such filters for obtaining a quasi-spherical 3D PSF. Specifically we prove that, under linearly polarized excitation, when the adequately designed filter is used as a pupil mask of a 1.2 NA water-immersion objective, the two-photon fluorescence PSF is axially shortened by 21% over its clear-pupil counterpart.

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Schematic geometry of the TPE microscope



Typical measurements for the 3D PSFs