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Nonlinear optical microscopy in biology: Combining second-harmonic generation and two-photon fluorescence imaging KOEN CLAYS, University of Leuven, Belgium — Optical microscopy has been since long a truly enabling visualization technique in the biological and biomedical sciences. Linear optical microscopy relies on simple linear optical effects. Nonlinear optical microscopy relies on the nonlinear optical properties of endogenous or exogenous chromophores to produce a better image. Two-photon fluorescence (TPF), a third-order nonlinear optical effect and observed at the focal spot only due to the quadratic intensity dependence, results in inherently higher resolution than possible for one-photon fluorescence, observed over the complete Rayleigh range. Second-harmonic generation (SHG) is a second-order nonlinear optical effect only observed for non-centrosymmetric arrangements of non-centrosymmetric chromophores. While this does put a restriction on the chromophores that can be used, it also results in structural information about symmetry when used in combination with TPF. TPF, being a third-order nonlinear process, is not restricted by any symmetry consideration. We will review the molecular design criteria for exogenous probes for combined SHG and TPF nonlinear microscopy, provide examples of optimized chromophores and show microscopy images demonstrating the use of such chromophores in nonlinear microscopy.

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