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来聴歓迎

Nonlinear Laser Scanning Microscopy (NLSM)

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要旨: Nonlinear laser scanning microscopy (NLSM) is advanced imaging technique particularly suitable for deep tissue and in vivo imaging. It utilizes ultra-short laser pulses (femto second pulses) in near infrared spectral region and due to nonlinearity the interaction with specimen material occurs only in the tiny focal volume which enables deep penetration. There are three modalities of NLSM: Two Photon Excitation Fluorescence (TPEF), Second Harmonic Generation (SHG) and Third Harmonic Generation (THG) imaging. Each of them provide complementary and valuable information and is used for various types of imaging. TPEF is the most similar to the single photon excitation fluorescence in confocal microscopy, but it has much higher penetration depth and axial resolution. TPEF relays very often on auto fluorescence, that originates from NADH and FAD mostly, when vertebrate specimens are imaged or chitin in arthropods. SHG imaging reveals ordered structures such as collagen (type I), myosin and starch with no need for labeling and with extremely high contrast. Also, it is the only optical technique that is used for quantification of degree of collagen molecules organization. THG is also used for unstained samples imaging providing information on steep changes of refractive index inside the specimen. With some limitations it is mostly used for in vivo imaging and real time tracking of some physiological processes, e.g. C. Elegance embryogenesis, physiological processes in zebra fish etc.

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