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Multi-photon near-infrared emission saturation nanoscopy using upconversion nanoparticles

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Multiphoton fluorescence microscopy (MPM), using near infrared excitation light, provides increased penetration depth, decreased detection background and reduced phototoxicity. Using stimulated mission depletion (STED) approach, MPM can bypass the diffraction limitation, but it requires both spatial alignment and temporal synchronization of high power (femtosecond) lasers, which is limited by the inefficiency of the probes. Here, we report that upconversion nanoparticles (UCNPs) can unlock a new mode of near-infrared emission saturation (NIRES) nanoscopy for deep tissue super-resolution imaging with excitation intensity several orders of magnitude lower than that required by conventional MPM dyes. Using a doughnut beam excitation from a 980 nm diode laser and detecting at 800 nm, we achieve a resolution of sub 50 nm, 1/20th of the excitation wavelength, in imaging of single UCNP through 93 µm thick liver tissue. This method offers a simple solution for deep tissue super resolution imaging and single molecule tracking.

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