

# HIGH-RESOLUTION IMAGING OF FLUORESCENT NANODIAMONDS BY SATURATED EXCITATION (SAX) MICROSCOPY

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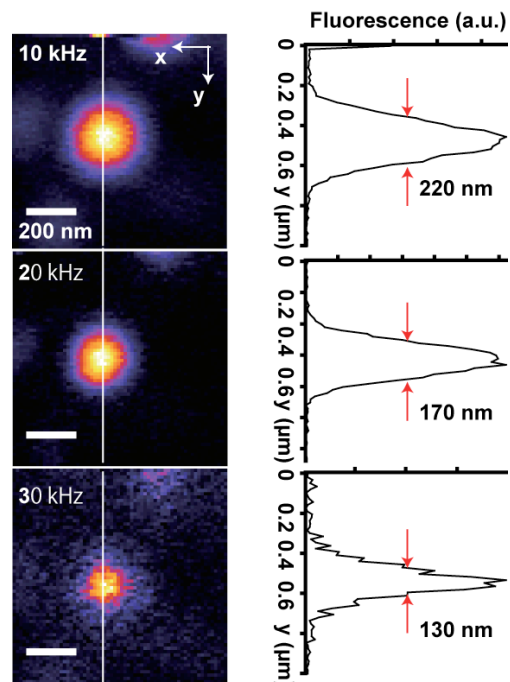
Diamonds containing nitrogen-vacancy defects are recently considered as a promising fluorescent probe because they emit bright fluorescence without photobleaching [1]. Here, we demonstrate high-resolution fluorescence imaging of the nano-sized diamonds by saturated excitation (SAX) microscopy[2, 3].

SAX microscopy exploits nonlinear fluorescence responses under saturation phenomena in single-photon excitation of fluorescent molecules to break the diffraction limit. Since the nonlinear fluorescence responses induced by the saturation appear notably in the area smaller than the laser focus, we can achieve the spatial resolution beyond the diffraction limit by detecting the nonlinear fluorescence signals. In SAX microscopy, we modulated the excitation intensity temporally at a fundamental frequency ( $\omega$ ) and demodulated the fluorescence intensity at higher-order harmonic frequencies ( $2\omega, 3\omega, \dots$ ) to extract the nonlinear fluorescence signals.

We observed fluorescence nanodiamonds fixed on a cover glass by SAX microscopy. The right figure shows fluorescence images of the diamonds and their intensity profiles spanning the dotted lines in the images. In these observations, we modulated the excitation intensity at 10kHz and demodulated the fluorescence intensity at the fundamental, the 2nd (20 kHz), and the 3rd (30 kHz) harmonic frequencies. We used a CW solid-state laser (wavelength 532nm) and an oil-immersion objective lens (NA 1.45, x60) for the observaton. The samples were excited with an excitation intensity of 4.4, 11, and 22 kW/cm<sup>2</sup> for figure a) to c), respectively. From these results, we confirmed that the spatial resolution was improved and fluorescence signals demodulated with higher order harmonic frequencies gives higher spatial resolution.

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**References:** [1] Chang et al., Nat. Nanotech., **3**, 284-288 (2008). [2] Yamanaka et al., J. Biomed. Opt., **13**, 050507 (2008). [3] Fujita et al., Phys. Rev. Lett, **99**, 228105 (2007).



**Figure** Images of fluorescence nanodiamonds by demodulation at 10 kHz, 20 kHz, and 30 kHz.