Saturated excitation (SAX) microscopy; depth resolution
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Recently we have demonstrated the use of nonlinear fluorescence response emerging under saturation phenomena of fluorescence excitation for improving the spatial resolution in three-dimensions \cite{1,2}. To detect the nonlinear signals in the emitted fluorescence, the technique called saturated excitation (SAX) microscopy uses modulation of the excitation intensity at a single frequency and demodulation of the nonlinear fluorescence signals at the harmonic frequencies. In SAX microscopy, higher harmonic demodulation gives higher-order nonlinear fluorescence signals to realize further improvement of the spatial resolution.

Because SAX microscopy uses nonlinear fluorescence response appearing prominently in the center of the laser focus, it is expected to achieve significant suppression effects of out-of-focus signals in a similar manner as two-photon fluorescence microscopy, resulting in improvement of the depth-discrimination property. In this report, the depth-discrimination property of SAX microscopy was investigated theoretically and experimentally.

We calculated and measured the axial response of a thick fluorescent layer by scanning in the axial direction \cite{3}. Since the thick fluorescent layer gives large amount of out-of-focus signals under laser irradiation, it allows us to evaluate background rejection capability. We used Rhodamine 6G solution (10 \textmu M) as a sample. The dye was excited with CW laser ($\lambda = 532$ nm) and a water-immersion objective lens (NA: 1.2). The figure shows calculated and measured axial responses obtained in confocal and SAX microscopy using pinholes with different sizes. Because the axial resolution of SAX microscopy does not become worse with increasing pinhole size, we confirmed the improvement of the optical sectioning capability by SAX microscopy in our results.

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\begin{figure}
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\includegraphics[width=\textwidth]{axial_response.png}
\caption{Axial response of a thick fluorescent layer by confocal and SAX microscopy with finite pinholes\cite{3}}
\end{figure}