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The Stimulated Emission Depletion Properties of Spiro-BTA

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In the field of optical imaging, diffraction limit is one of the most serious barriers that limit the spatial resolution to roughly half of wavelength. It was not until last decade had the diffraction limit been broken and from then on, super-resolution microscopy has generated strong impact to biological and material researches. Among these techniques, stimulated emission depletion (STED) microscopy is arguably the most successful one, which reaches super-resolution by turning fluorophores on and off with stimulated emission process. This technique needs two beams: a short-wavelength beam with solid shape to excite the fluorophore and a long-wavelength beam with doughnut shape to suppress spontaneous fluorescence via STED. As a result, spontaneous fluorescence is only allowed in the central region of the doughnut-shaped beam, and therefore resolution can be effectively improved. The higher the STED beam intensity, the better the resolution can be enhanced. However, the fluorophores need extraordinary photostability to sustain strong depletion beam as well as repeated excitation and depletion.

In this work, we demonstrated STED property from Spiro BTA, which is an ultra-stable organic fluorophore designed for organic light emitting diode applications.. Due to its exceptionally large cross section, we expect it to have good stimulated emission depletion property. Moreover, it is known that Spiro BTA has high photoluminescence and high bio-compatibility, which are both main considerations in biological application. We studied the suppression efficiency of fluorescence emitted from Spiro-BTA with different depletion intensity and obtained the effective saturation intensity of Spiro-BTA from the analysis of depletion curve. Moreover, in order to demonstrate our sample, Spiro-BTA's capability of reaching super-resolution, we wrap the dye in silica balls with diameter of 50nm. By measuring the point spread function of the silica balls, we confirm that the silica balls' point spread function is effectively narrowed, with FWHM only 100nm.

As a result, we confirmed that Spiro-BTA has great potential in application in super resolution imaging via STED microscopy.