

ANTI-STOKES FLUORESCENCE PROPERTIES OF ALEXA FLUOR 568

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KEYWORDS: spectroscopy, upconversion, hot-band absorption, anti-Stokes fluorescence

The emission spectra of fluorescent dyes can be explained via radiative and non-radiative transitions between the electronic states and internal conversions. When a molecule absorbs a photon, it is then excited to a higher electronic state (S_1) from the ground state (S_0). Because of the energy loss due to internal conversion, when the molecule relaxes the emitted photon has a longer wavelength. This effect is called the Stokes-shift.

However, during the anti-Stokes fluorescence, the emitted photon has higher energy (lower wavelength) than the excitation photon. This excitation process requires some additional energy, which can come from the absorption of multiple photons or from heat-populated vibrational states. Hot-band absorption has been observed with several organic dyes: Rhodamine 101 [1], 6G, 640, B, Oxazine 1. Although Alexa Fluor 568 is a widely used fluorescence dye in confocal and super-resolution microscopy, to our knowledge, its anti-Stokes emission properties have not been studied.

To distinguish between the possible excitation models, the relationships between excitation power, excitation wavelength, emission intensity, lifetime and temperature were measured for Alexa Fluor 568 and for Rhodamine 101. Due to its nature possible applications for the hot-band active anti-Stokes emitting dyes effect were proposed [3]: signal-to-noise ratio increase via autofluorescence reduction, fast ROI selection during two colour measurements, drift correction.

References:

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