

# ANTI-STOKES FLUORESCENCE MICROSCOPY WITH ALEXA FLUOR 568

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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, and Alexa Fluor 568 [3].

Due to the nature of the excitation possible applications for the hot-band active anti-Stokes emitting dyes were proposed [3]: signal-to-noise ratio increase via autofluorescence reduction, two colour measurements using a single excitation wavelength, drift correction.

## References:

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