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Increasing Fluorescence Signals by Pulsing the Excitation Laser CHERYL NIELSON, EYAL SHAFRAN, BEN MANGUM, CHUN MU, JORDAN GERTON, University of Utah — After photoexcitation, fluorescent molecules (fluorophores) can decay radiatively from the singlet excited state back to the ground state, or non-radiatively via a metastable triplet state. The triplet state has been associated with photobleaching whereby an irreversible photo-induced chemical change in the molecule suppresses further fluorescence. Photobleaching ultimately limits the number of fluorescence photons that can be emitted by a single fluorophore, and thus reduces the sensitivity of fluorescence microscopy. Recently it has been shown that if the triplet state is allowed to relax in the dark, the photobleaching rate can be reduced by up to a factor of  $\sim 20$ . This was demonstrated using a picosecond pulsed laser with a variable repetition rate. We are investigating whether such a dark-state relaxation scheme can be implemented with a CW laser that is chopped on and off with microsecond-scale pulses using an acousto-optic modulator. This scheme is much simpler since it does not require the use of a pulsed laser. Preliminary data show that our dark-state relaxation scheme does lead to reduced photobleaching, and thus an increase in total fluorescence signal.

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