Abstract Submitted for the TSF15 Meeting of The American Physical Society

Multiple-Pulse Pumping for Enhanced Fluorescence Detection and Molecular Imaging in Tissue RYAN RICH, Texas Wesleyan University, IGNACY GRYCZYNSKI, RAFAL FUDALA, JULIAN BOREJDO, DOROTA STANKOWSKA, RAGHU KRISHNAMOORTHY, University of North Texas Health Science Center, SANGRAM RAUT, Texas Christian University, BADRI MALIWAL, University of North Texas Health Science Center, DMYTRO SHU-MILOV, HUNG DOAN, ZYGMUNT GRYCZYNSKI, Texas Christian University, CENTER FOR FLUORESCENCE TECHNOLOGY AND NANOMEDICINE COLLABORATION — Fluorescence based imaging techniques in cellular and tissue environments are severely limited by the background fluorescence of endogenous components of cells, tissue, and the fixatives used in sample processing. We report here a multi-pulse excitation approach to confocal fluorescence microscopy that allows for a many-fold increase in the relative intensity of a moderately long-lived probe above the background. By using separate, closely spaced excitation pulses in repeated bursts, we can increase the number of probe molecules in the excited state. At the end of this multi-pulse burst, the probe molecules are allowed to spontaneously return to the ground state. The shorter-lived background, on the other hand, will completely decay to the ground state in between each excitation pulse, and will therefore will not experience an additive effect on its fluorescence signal. Using a confocal microscopy system equipped with a pulsed laser diode and time correlated single photon counting (TCSPC) detection, we are able to enhance the signal of a long-lived Ruthenium (Ru)-based probe by nearly an order of magnitude while the background is unaffected.

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Date submitted: 08 Oct 2015

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