Title: Fluorescence Lifetime in our Lifetime: Discovery of Approaches to Measure Molecular Excited State Kinetics and Fluorescence Day by Flow Cytometry

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Summary:
The scientific objective this research is to apply new approaches in time-dependent flow cytometry for the high-throughput measurement of fluorescence decay kinetics from molecular species that are in or on single cells and small particles. Future work should enable quantification of intracellular phenomena at the essential throughput of cytometry and address widespread cytometry issues related to unwanted Rayleigh, Raman, and fluorescence light signal overlap.

In the field of flow cytometry, cells and particles reach elevated energy levels as they travel through fluidic chambers and traverse laser beams over microsecond transit times. The difficulties and complexities required for measuring the excited state kinetics has resulted in a lack of assays, applications, and instruments capable of high-throughput fluorescence decay measurements. In this research we study the fluorescence dynamics of intracellular molecules and proteins as well as the time-dependent characteristics of tagged microspheres using the capabilities of a new time resolved cytometry sorting and analysis system developed by our laboratory. The objectives are:

1. discover changes in the autofluorescence lifetime of intrinsic cellular molecules upon differences in cell cycle and cell viability, and understand when, how, and why autofluorescence dynamics are altered
2. explore fluorescent protein lifetime changes associated with intracellular protein transport, protein-protein interactions and protein complex formation detectable by Forster resonance energy transfer (FRET) and loss of FRET
3. identify new encoding approaches for multiplexed microsphere assays dependent on excitatory-state kinetics measurements.

Various frequency-domain cytometry systems are used on the three applications to (1) acquire multiple lifetimes from single cytometric events, (2) sort and separate single cells based on multiple fluorescence dynamics, (3) explore digital signal processing approaches that expand fluorescence lifetime resolution limits, (4) null cytometric signals based on the fluorescence lifetime, and (5) investigate time-dependent gradients across a

single cell at a high-throughput level. The cellular and bead-based systems were chosen because if successful they could lead to transformative cytometry protocols that remedy capricious autofluorescence signals. Also these approaches should enable the discovery of new intracellular fluorescence dynamics and demonstrate new time-resolved approaches for multiplex-based cytometric assays.