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Capabilities of high-sensitivity spectral fluorescence-lifetime imaging for resolving spectroscopically overlapping species JUSTIN CRAWFORD, University of Tennessee Space Institute - Center for Laser Applications, LLOYD DAVIS, BRIAN CANFIELD, University of Tennessee Space Institute — The ability to separate the contributions from spectroscopically overlapping fluorophores has enabled significant breakthroughs in cellular imaging. However, commercial microscopes for this purpose generally use analog light detection with least-squares curve-fitting analysis. Improvements in sensitivity are possible and will lead to new applications. To this end, we have constructed a microscope with a high-throughput Brewster-prism spectrometer and four high-quantum efficiency single-photon detectors, coupled with time-correlated single photon counting electronics to provide added temporal resolution. We have demonstrated the use of maximum-likelihood (ML) methods for analyzing small numbers of photons to find the contributions from fluorescent species with differences in excitation and emission spectra. However, it is difficult to resolve fluorophores with different temporal decay profiles because the single-photon counting modules exhibit a count-rate-dependent time-walk. We discuss extension of the ML-analysis to account for a varying time-walk and results from Monte Carlo simulations to ascertain the minimum number of photons needed to reliably resolve specific fluorophores.

Justin Crawford
University of Tennessee Space Institute - Center for Laser Applications

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