Ryan A Colyer, Claudia Y Lee, and Enrico Gratton.
Time-resolved frequency-domain fluorescence lifetime imaging microscopy in the photon-counting regime.
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Abstract
Fluorescence lifetime imaging microscopy (FLIM) is growing in usefulness as a means of distinguishing molecular species, observing environmental regions which affect the lifetime of a fluorophore, and observing fluorescence resonance energy transfer (FRET). We present a convenient technique for performing FLIM in a laser scanning microscope using the photon counting regime of the photomultiplier detector in conjunction with time-resolved frequency-domain data acquisition hardware.

In our technique we synchronize the internal clock of a field programmable gate array (FPGA) to a sampling frequency which is fractionally offset from the laser repetition frequency, and use heterodyning to evenly sample the entire repetition time of the laser. The sampling clock period is divided into time-resolved measurement windows which sample the arrival of photons in different portions of the lifetime curve with a 100% duty cycle. The heterodyning frequency domain approach allows us to evenly sample the lifetime curve with each time-resolved measurement window. The data is processed automatically by software which accumulates a phase histogram of photon counts across the cross-correlation period, and then automatically calculates the phase and modulation of the emission. From the phase and modulation, the lifetime values can be calculated, or the results can be better analyzed using the phasor approach.

The advantages of this system are the very low cost, the 100% duty cycle of the method, compatibility with normal microscope acquisition, relatively fast frame rates, and the constant high sensitivity of the detector. This technique provides cost-effective and statistically rigorous FLIM capability which could be easily added to many ... [truncated at 250 words]