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Correlation analysis applied to fluorescence lifetime measurement

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Abstract

Typical fluorescence lifetimes lie in the range of 1 to 20 nanoseconds. The frequency domain technique with heterodyne detection has found widespread use in this application, primarily because, apart from the detector itself, fast (nanosecond) signal acquisition electronics are not required. The system still requires precise timing control between the excitation source and detector, usually achieved by phase locking. In the case of low signal levels or poor excitation source stability, averaging is used to improve error bars. This generally involves folding the acquired waveform or averaging the result of a FFT routine. An alternative method is to cross-correlate the signals from the reference detector and sample detector. This yields a waveform with the appropriate beat frequency, phase shift and demodulation characteristic of the fluorescence lifetime, without prior specification of its period. The cross-correlation operation is quadratic, compared to averaging (a linear operation). The manner in which phase noise propagates through the measurement algorithm is therefore different. We apply this technique to fluorescence lifetime measurement in both a cuvette instrument and two-photon scanning microscope and show that it is possible to measure lifetime without phase locking the excitation source to the detection electronics. Research supported by NIH, PHS 5 P41 RRO3155.