Digital Frequency-Domain FLIM

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Abstract: We present a mathematical model and physical implementation for a Digital Frequency Domain FLIM system which provides lifetime resolution comparable to TCSPC methods. We present data on cells and on molecules diffusing in solution.

Fluorescence lifetime imaging (FLIM) is a powerful microscopy technique for providing contrast of biological and other systems by differences in molecular species or their environments. However, the cost of equipment and the complexity of data analysis have limited the application of FLIM. We present a mathematical model and physical implementation for a low cost Digital Frequency Domain FLIM (DFD-FLIM) system which can provide lifetime resolution with quality comparable to time-correlated single photon counting methods. Our implementation provides data natively in the form of phasors. Based on the mathematical model, we present an error analysis which shows the precise parameters for maximizing the quality of lifetime acquisition, as well as data to support this conclusion. The hardware and software of the proposed DFD-FLIM method simplifies the process of data acquisition for FLIM, presents a new interface for data display and interpretation, and optimizes the accuracy of lifetime determination.