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A time-resolved fluorescence microscope.

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Abstract

We have constructed a time-resolved fluorescence microscope system that can collect images which show differences in fluorescence decay times. The microscope system has the abilities of the more common steady-state fluorescence imaging microscope and, additionally, can determine fluorescence intensity as a function of time. A modulated laser beam is the light source and a modulated microchannel plate coupled to a CCD camera serves as the detector. Data arrim at the detector at a chosen frequency (DC-150 MHz) and is heterodyned by the microchannel plate to a frequency slower than the camera's sampling rate (frame rate). Alternatively, the microchannel plate can be set to homodyne the incoming signal at which point the system becomes capable of real time analysis. The homodyning or heterodyning process maintains the spatial coherence of the images, which may have a resolution of 0.5 microns. The frequency domain images (DC intensity, AC intensity, phase shift and demodulation) can be interpreted directly or new combinations can be created for further analysis (e.g., lifetime images or phase-resolved images). Lifetimes as short as about 2 ns can be resolved with a resolution of about 50 pa. Using phase resolved techniques, fractional components of lifetimes at each pixel can be distinguished, potentially with only a single frequency data run. Supported by NIH RR03155.