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### Title

Fluorescence lifetime of single cells using a flow cytometer.

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Weiming Yu, Gary Durack, William W Mantulin, and Enrico Gratton.

**Fluorescence lifetime of single cells using a flow cytometer.**

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**Abstract**

We have developed a novel technique for flow cytometry capable of measuring fluorescence lifetime from a single cell at a rate of several hundred cells per second. Our instrument is based on an existing Coulter flow cytometer, the "EPICS Elite". A lifetime measurement mode is introduced using a fast frequency-domain heterodyning technique. For our current setup, a Pockels cell is used to sinusoidally modulate an Argon ion laser at the desired frequency. The fluorescence is collected through the standard optics of the Coulter flow cytometer and detected by using a Hamamatsu R928 photomultiplier tube which is gain modulated at a frequency 500 KHz to 1 MHz greater than that of the laser modulation. The waveform of this frequency difference, the cross-correlation frequency, is digitized and Fourier transformed to obtain the phase and the modulation of the signal. By using a second reference PMT for the laser, we obtained the phase shift and the demodulation due to sample fluorescence. A standard deviation of less than 0.5 degrees in phase was obtained by a fluorescein stream. We have tested our instrument on single CHO cells stained with different fluorochromes including: fluorescein diacetate, acridine orange, propidium iodide and 7aad, and proved to be useful for cell population study and cell sorting. Our lifetime flow cytometer has a resolution of about 1 ns. Supported by NIH RR03155 and UIUC CRI program.