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Digital heterodyne method for fluorescence lifetime measurement

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#### Nicholas P Barry, John S Eid, and Enrico Gratton. **Digital heterodyne method for fluorescence lifetime measurement.** 47th Annual Meeting of the Biophysical Society, San Antonio, Texas, 2003. *Biophys J.* 2003; 84(2), 2325-Pos/B701. Abstract

Digital circuitry has reached clock speeds (GHz) applicable to measurement of fluorescence lifetime. In direct digital methods, a detected photon is converted into a logic pulse using a high speed discriminator. Lifetime information is extracted from the pulse train without use of intervening analogue circuitry such as a TAC. We present measurements using a digital heterodyne technique similar to the frequency domain method for lifetime measurement. The primary advantage of a digital implementation of the frequency domain lifetime method is the signal to noise improvement afforded by the use of a discriminator compared to analogue operation of a PMT. The mixing/heterodyning step takes place in a simple Flip-Flop circuit. A reference pulse train with a fixed frequency offset from the excitation frequency beats with the detected photon pulses in this circuit. As with the analogue heterodyne method, the circuitry downstream of the mixer does not have to work with nanosecond precision. Standard pulse counting data aquisition cards may therefore be used. Using the same analysis algorithms as in the analogue frequency domain lifetime, the fluorescence lifetime is calculated from the phase delay of the modulated output compared to the excitation light.