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Accurate Single Molecule FRET Efficiency Determination for Surface Immobilized DNA Using Maximum Likelihood Calculated Lifetimes

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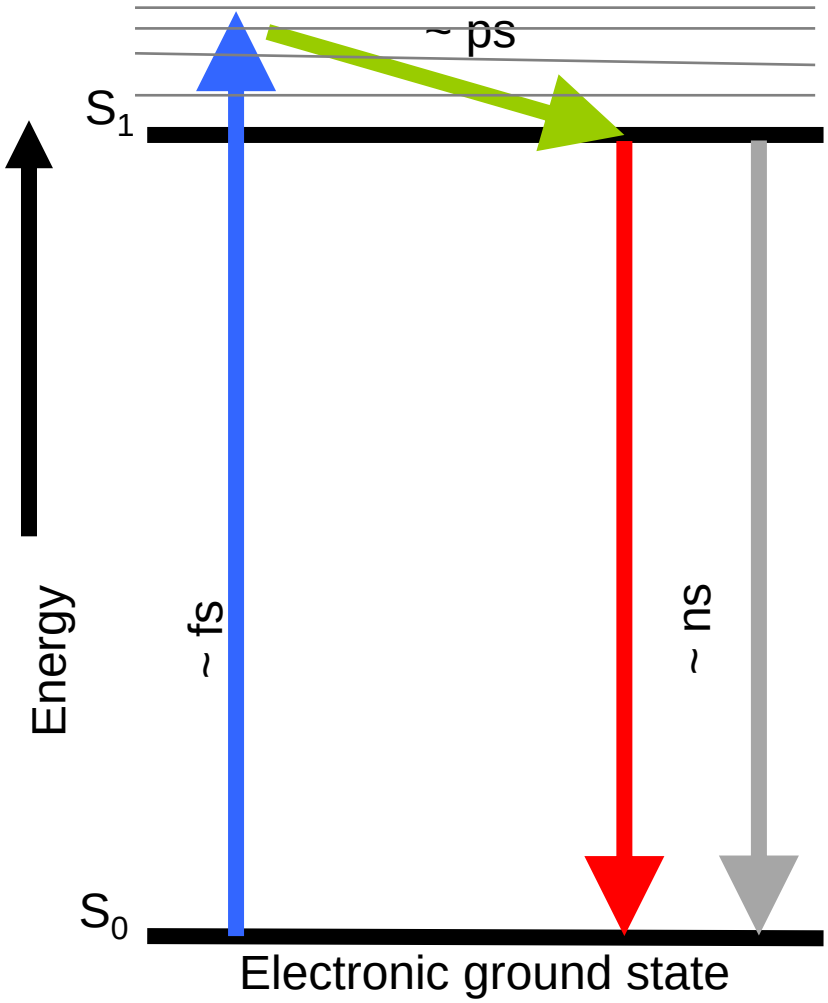
Single molecule fluorescent lifetime trajectories of surface immobilized double-stranded DNA coupled with a tetramethylrhodmaine and Cy5 FRET pair were directly measured using time-tagged single-photon counting and scanning confocal microscopy. A modified maximum likelihood estimator (MLE) was developed to compensate for localized background fluorescence and instrument response. With this algorithm, we were able to robustly extract fluorescent lifetimes from their respective decays with as few as 20 photons. Fluorescent lifetimes extracted using an MLE were found to be highly dependent on background fluorescence. We show that appropriate factors are required to extract true lifetime trajectories from single fluorophores.

Contents

Fluorescence lifetime

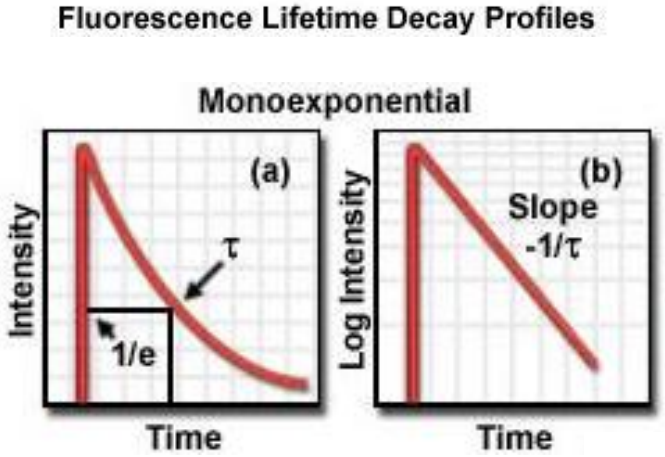


$$F(t) = F_0 e^{-t/\tau}$$

F : concentration of excited state molecules at time

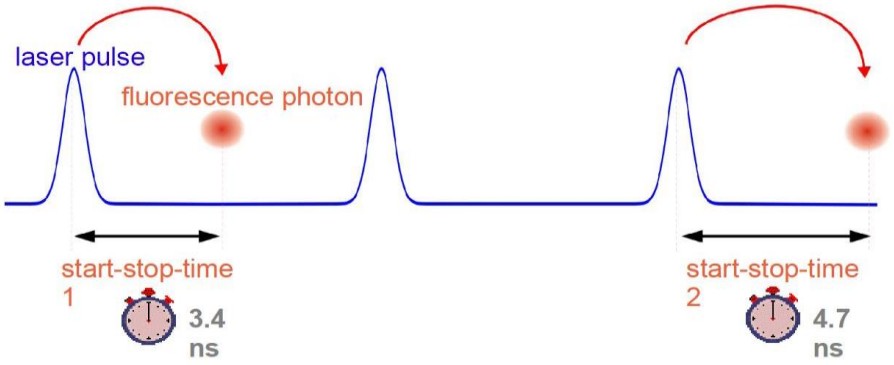
F_0 : initial concentration

Γ : decay rate or the inverse of the fluorescence lifetime

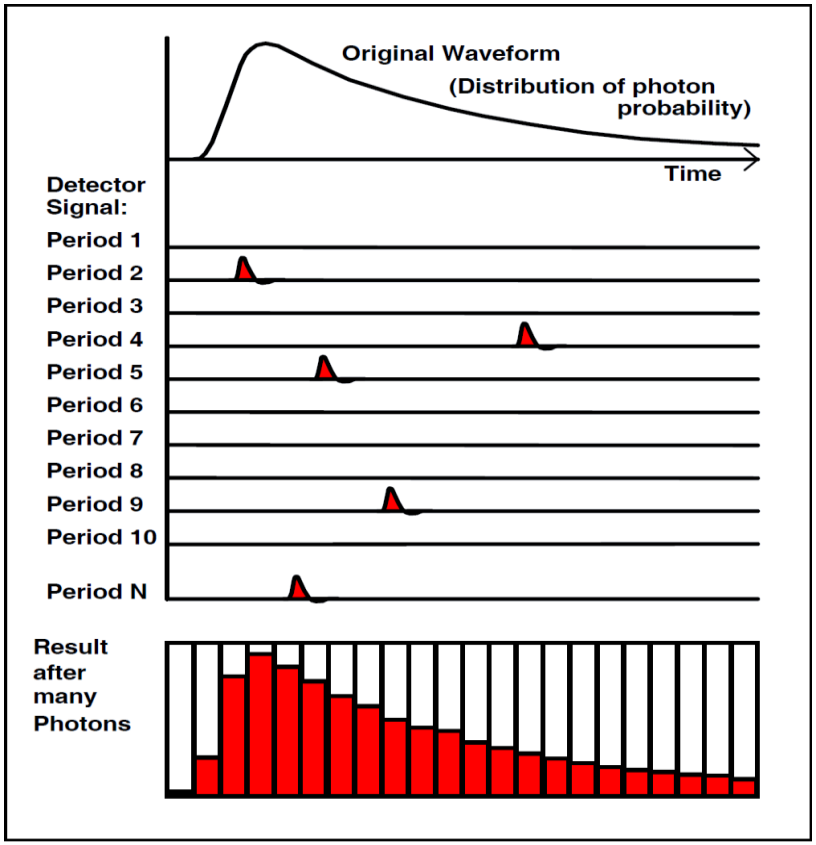


http://www.olympusmicro.com/primer/techniques/fluorescence/fluorescenceintro.html

TCSPC (Time-correlated single-photon counting)



picoHarp300 user's manual, PicoQuant



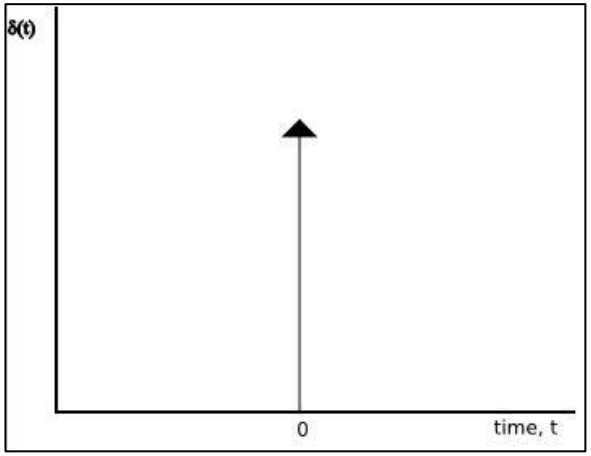
The fundamental signal in the TCSPC experiment is the time delay between the excitation laser pulse and a single photon emitted by the fluorophore

TCSPC

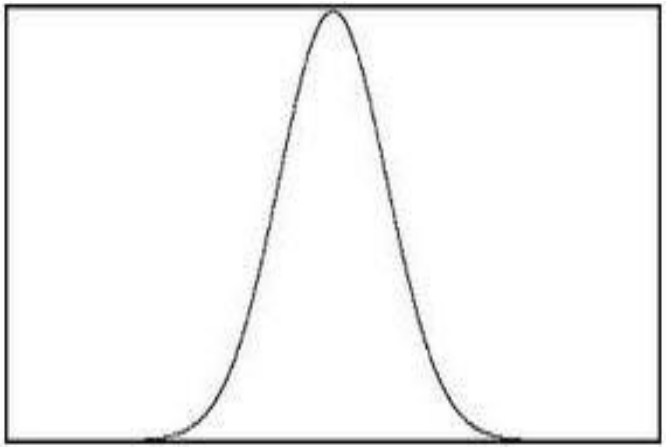
This signal contains a few artifacts.

1. IRF (Instrument response function)

Ideal detector IRF

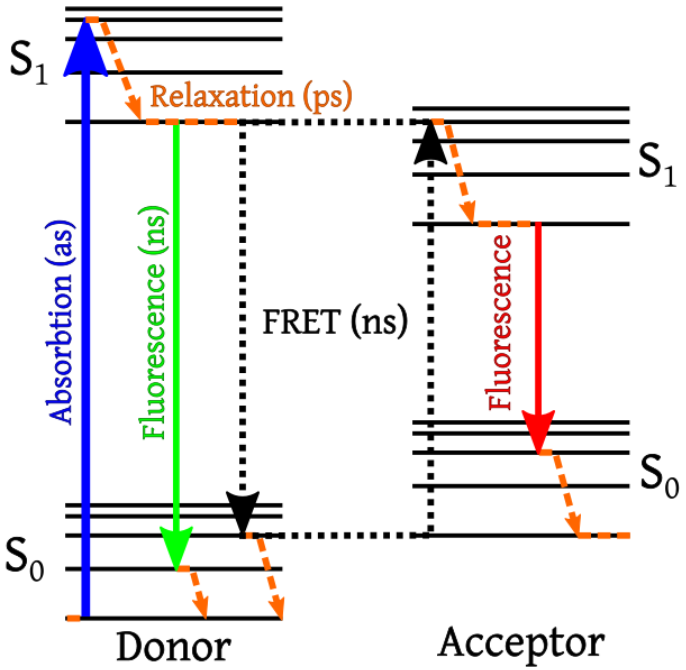


detector IRF

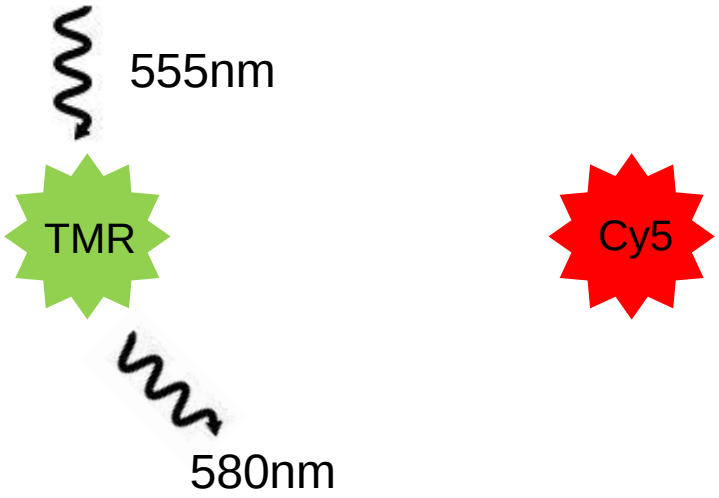


2. background fluorescence as well as scattering

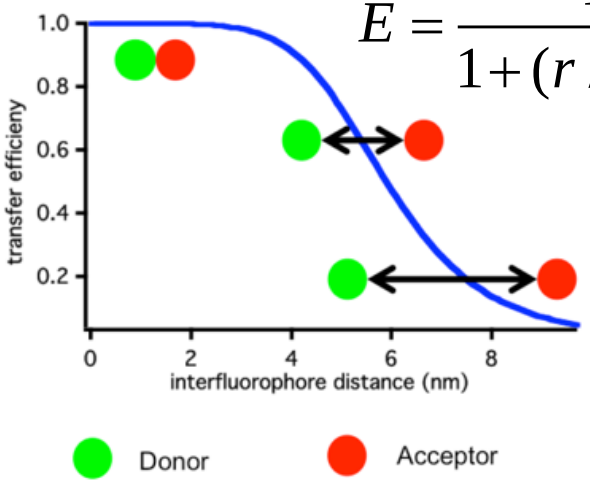
FRET (Förster resonance energy transfer)



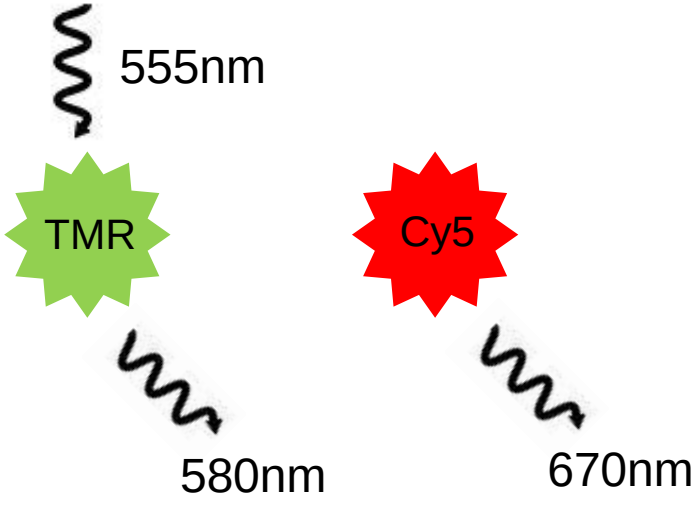
No FRET signal



$$E = \frac{1}{1+(r/R_0)^6}$$



FRET signal



Set up

Microscope : Zeiss Axiovert 200 microscope, 63 (N.A.1.45) oil immersion

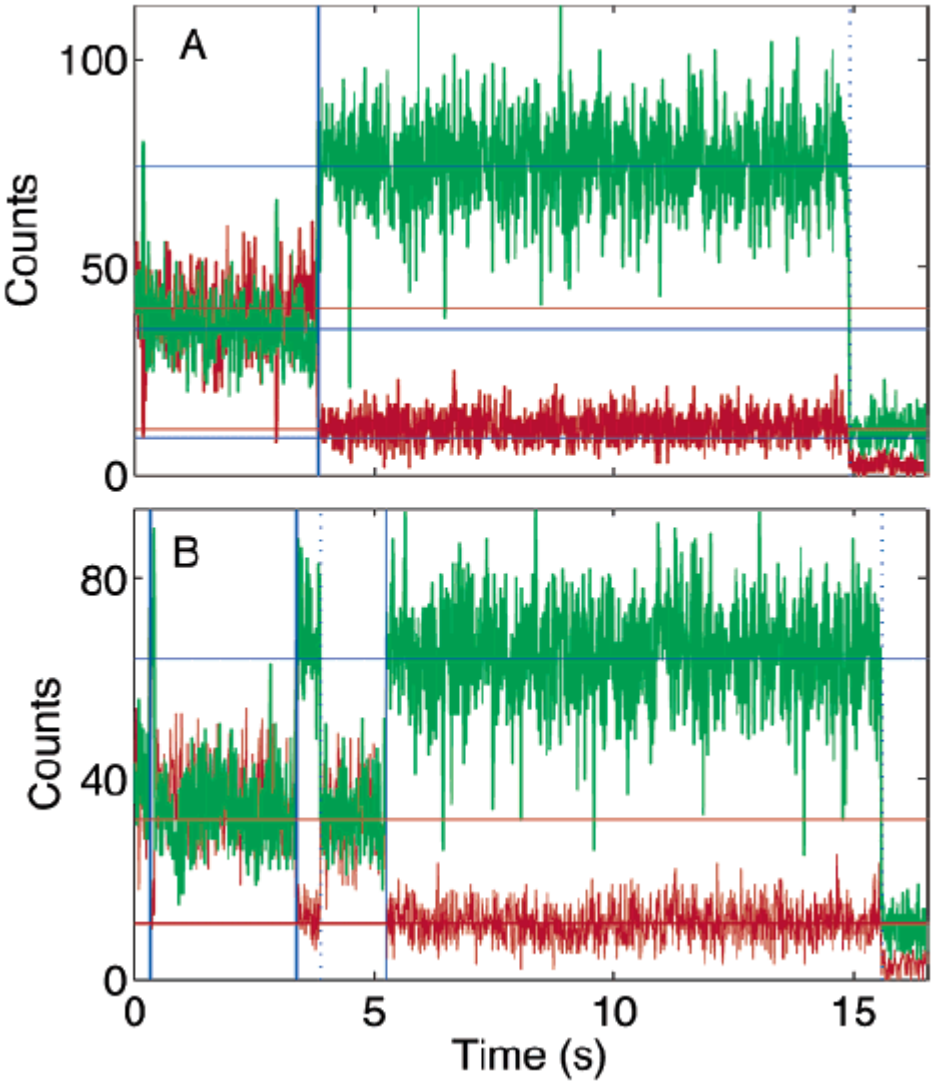
Piezo stage : piezo-driven nanopositioner (Physik Instrumente)

LASER : 80 MHz femtosecond Ti:Sapphire laser (Tsunami, Spectra Physics) operating at 1000 nm was frequency doubled using a lithium triborate crystal, with the resultant excitation wavelength at 500 nm.

Silicon avalanche photodiode detectors (Perkin-Elmer AQR14) were used to collect fluorescence in the red channel (650-750 nm) and green channel (505-635 nm)

Data & Result

Typical time traces of individual DNA molecules labeled with TMR and Cy5



Bin time resolution : 10ms

The fluorophores are separated by

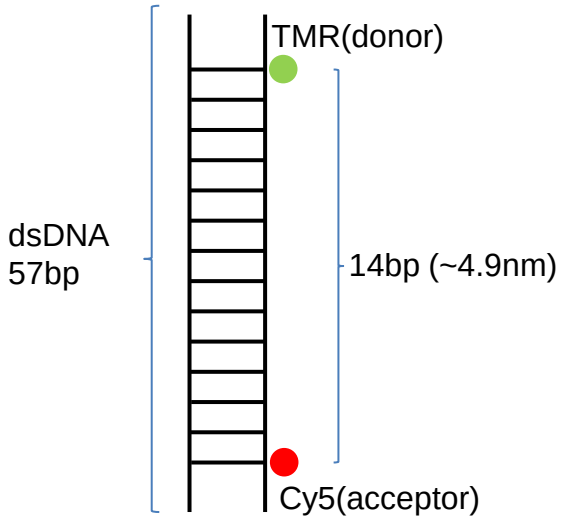
14 nucleotides (~4.9nm)

Cy5 : acceptor (red)

TMR : donor (green)

the TMR-Cy5 FRET pair undergoes energy transfer from 0-3.8 s

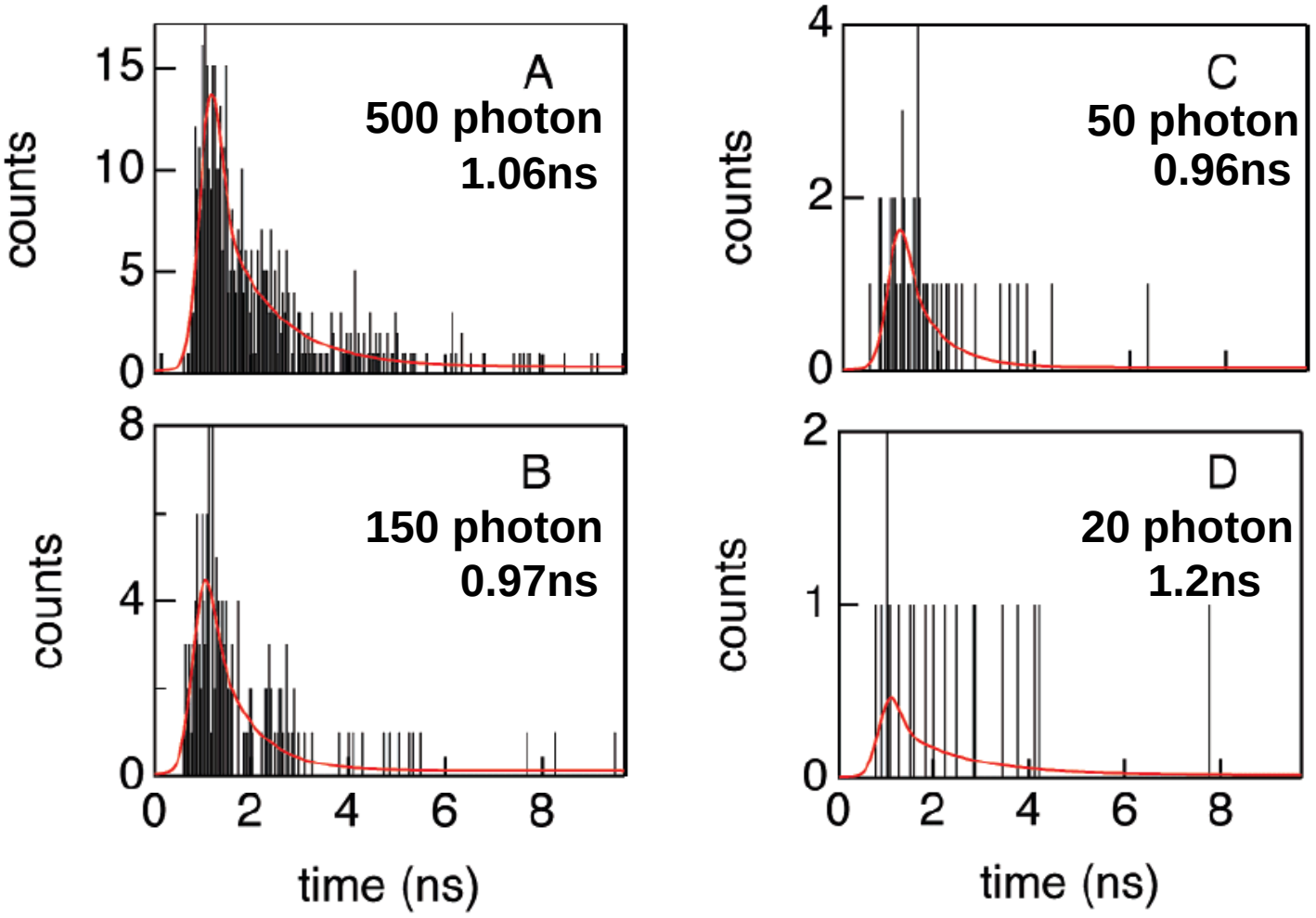
after which the acceptor photobleaches resulting in an increased count rate of the donor



Data & Result

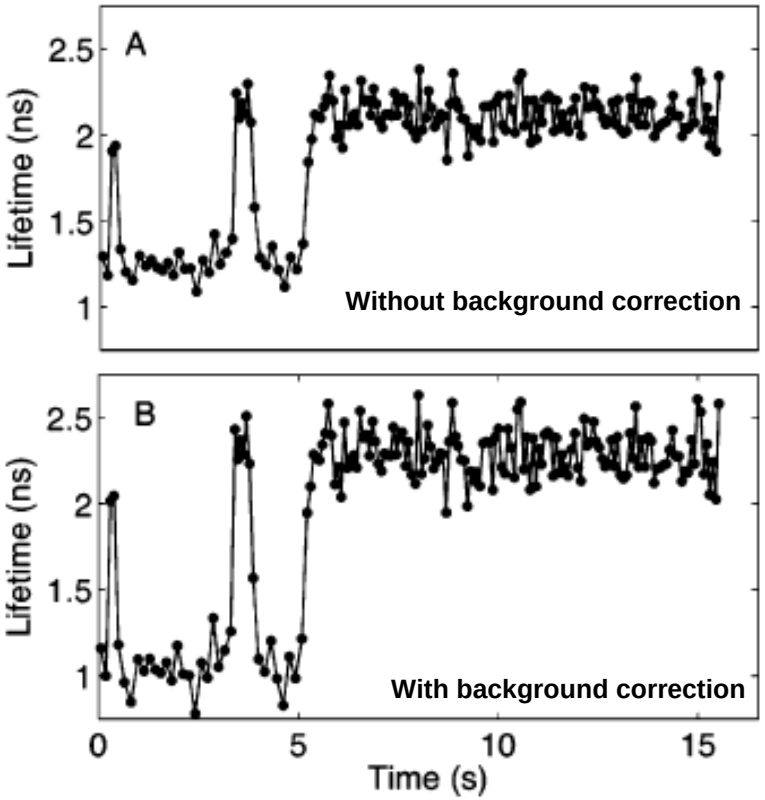
Fluorescent lifetime decays (TMR)

Using a maximum likelihood estimator fitting algorithm with background and scattering subtraction

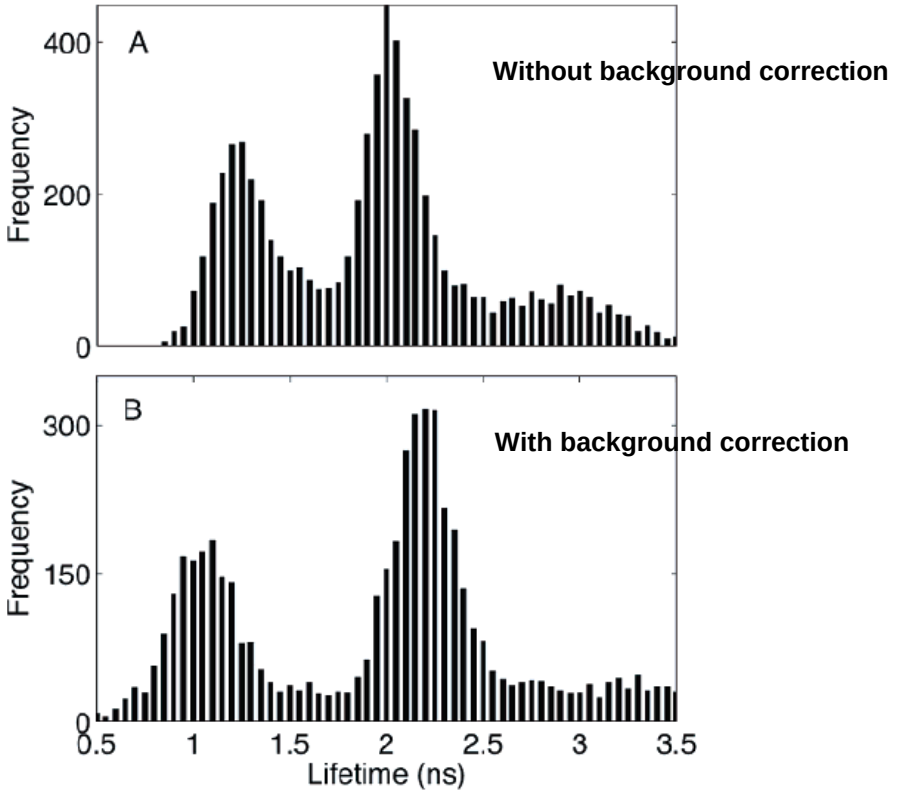


Data & Result

Fluorescent lifetime trajectories and histogram of the TMR



each lifetime was determined using 500 photons



Histogram from the accumulation of 61 independent single molecule lifetime trajectories for TMR using 500 photons per lifetime

Bulk lifetime of TMR (50nM)

FRET lifetime : 1.0ns
Non-FRET lifetime : 2.4ns

Figure A

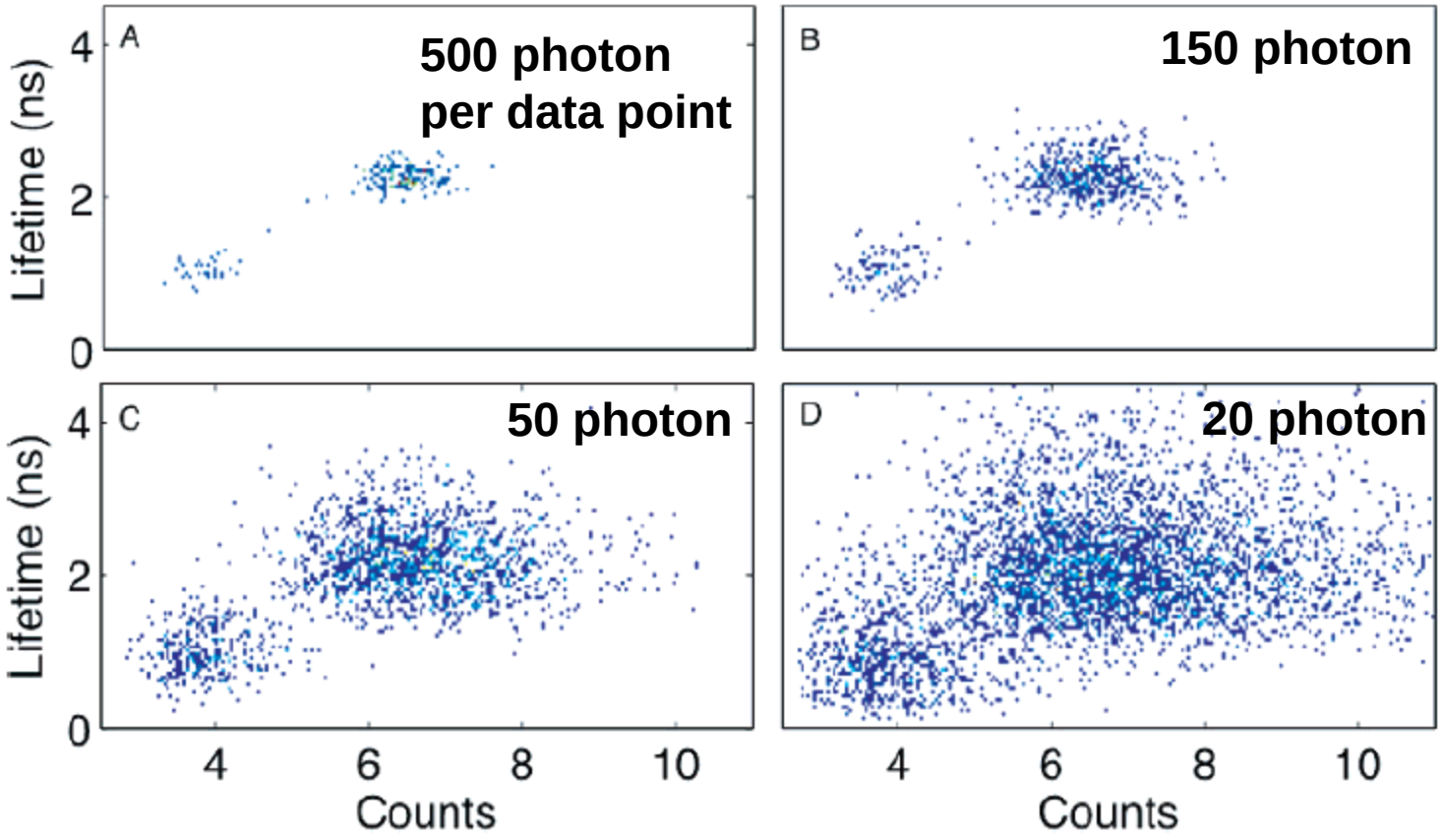
FRET lifetime : 1.25ns
Non-FRET lifetime : 2.0ns

Figure B

FRET lifetime : 1.0ns
Non-FRET lifetime : 2.3ns

Data & Result

Scatter plots of lifetime versus counts for TMR



even at 20 photons, the 2 lifetime populations of TMR are clearly distinguishable