FRET-FLIM and Phasor Analysis

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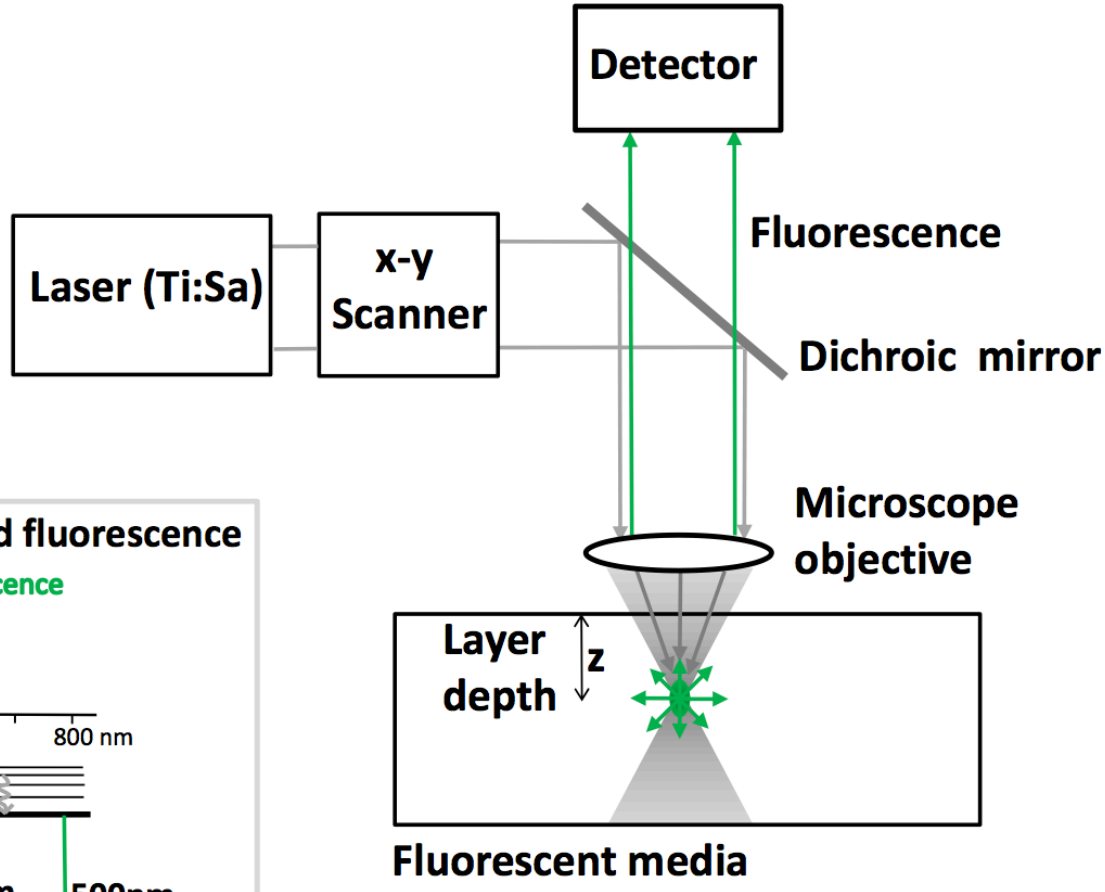
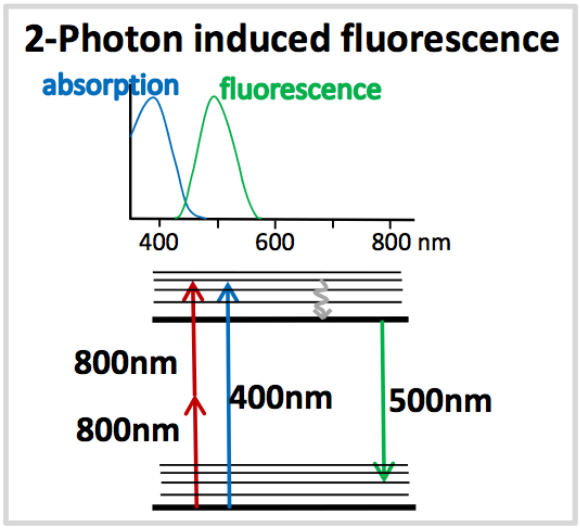
FLIM-FRET and Phasor Analysis

- FLIM: Fluorescence life time imaging microscopy
  - Measured with time domain and frequency domain
- FRET: Förster resonance energy transfer
  - Donor only
  - Change in lifetime of donor (CFP) represents energy transfer to acceptor (YFP)
- Phasor analysis
  - A way to graph and analyze the time domain and frequency domain detected with FLIM
  - Provides a global view of FLIM data for each pixel in the image

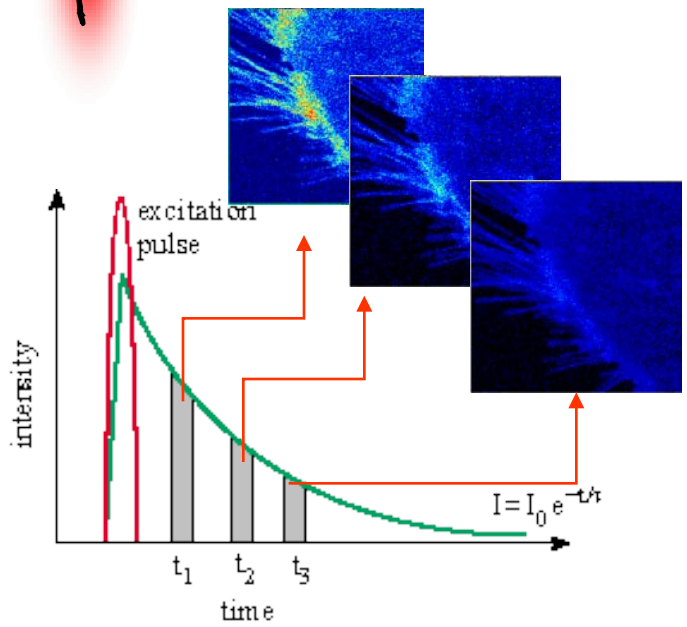
Two-photon fluorescence imaging





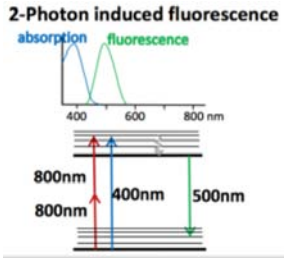
(Enrico Gratton and Alexander Dvornikov, LFD UCI)

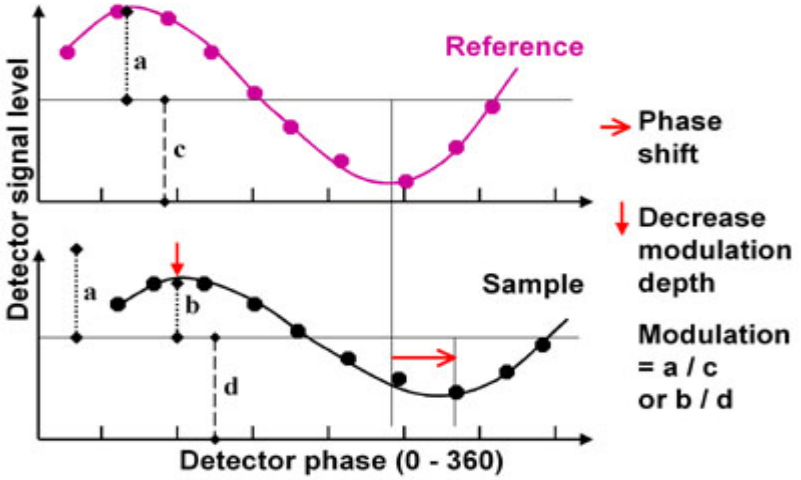
Florescence Life Time Measured with the Time Domain and Frequency Domain



A sample is flashed many times by a short duration laser source

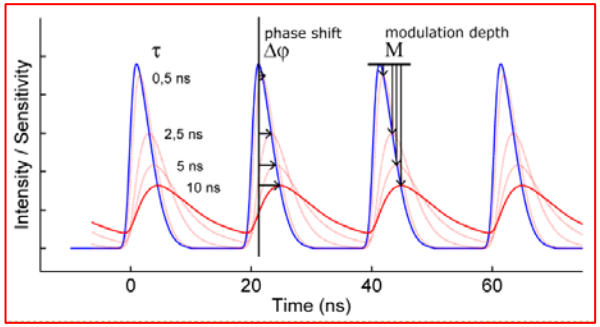
The histogram of the time intervals between the excitation flash, and 1st emitted photon is measured





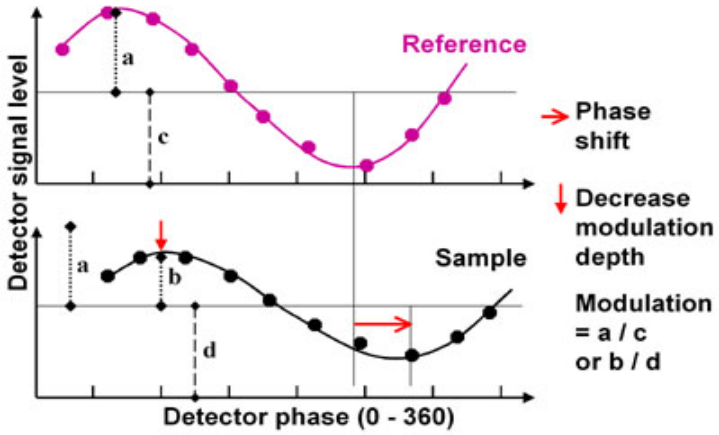
A sample is excited by a modulated light source

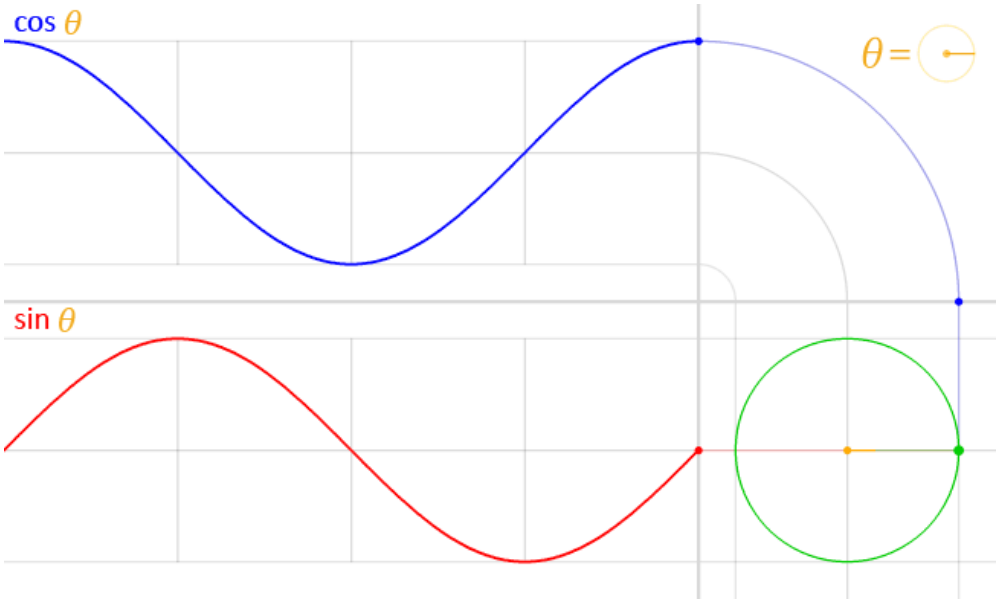
The fluorescence emission has the same frequency but is modulated and phase-shifted from the excitation source

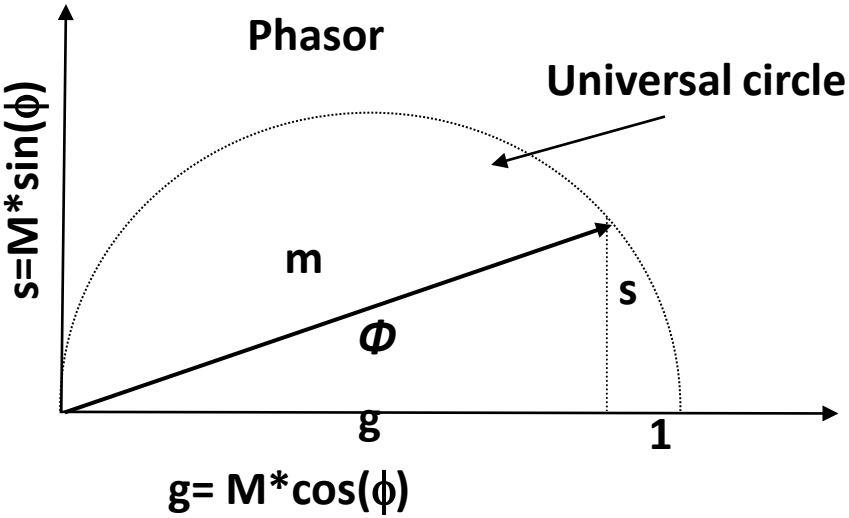


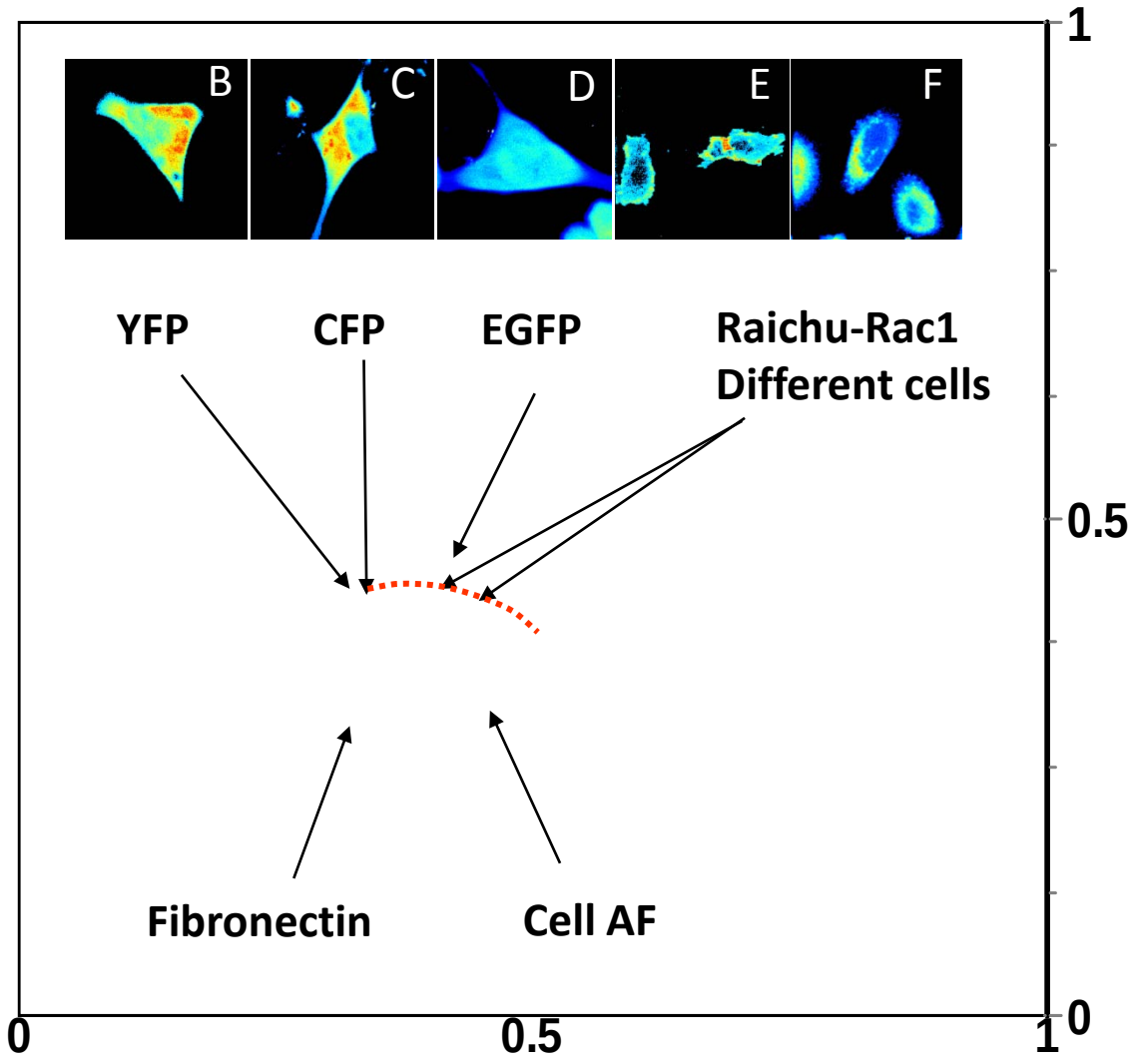
(Michelle Digman, LFD UCI)

The Phasor Plot (Universal circle)



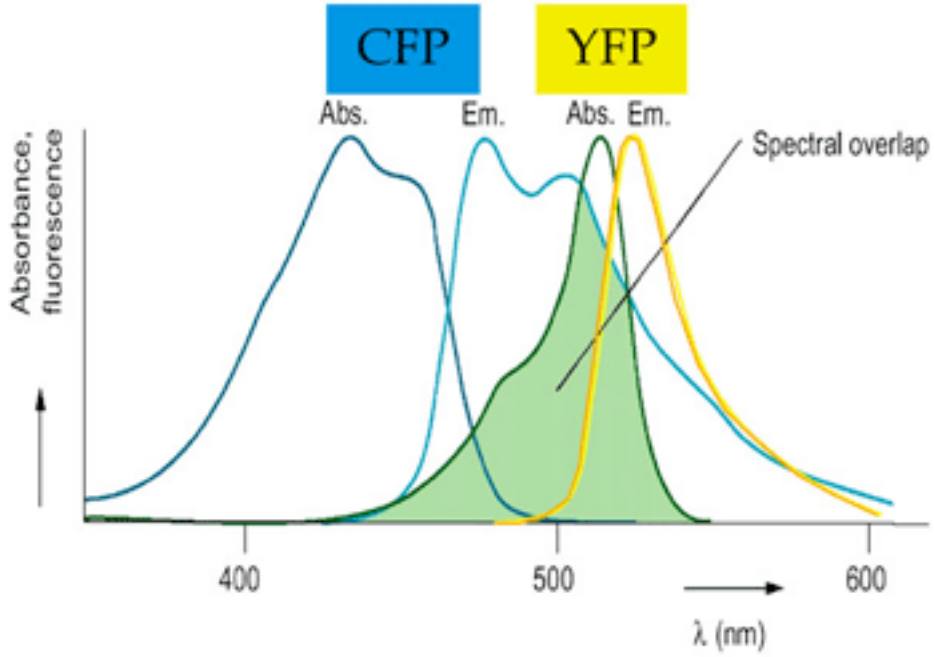




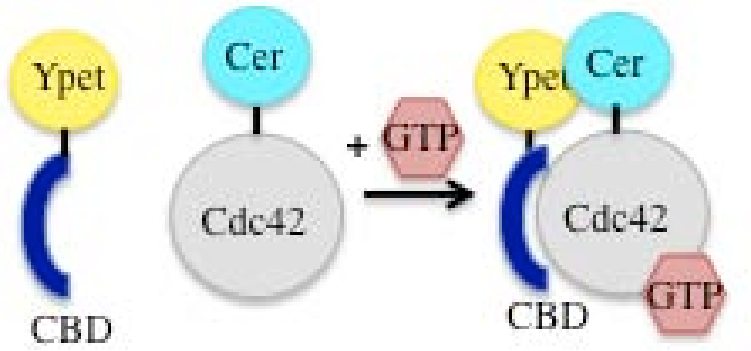


(Slide from Michelle Digman, LFD UCI)

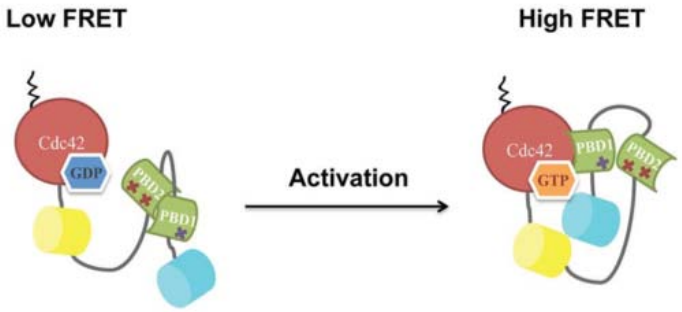
CFP-YFP FRET



Dual Chain

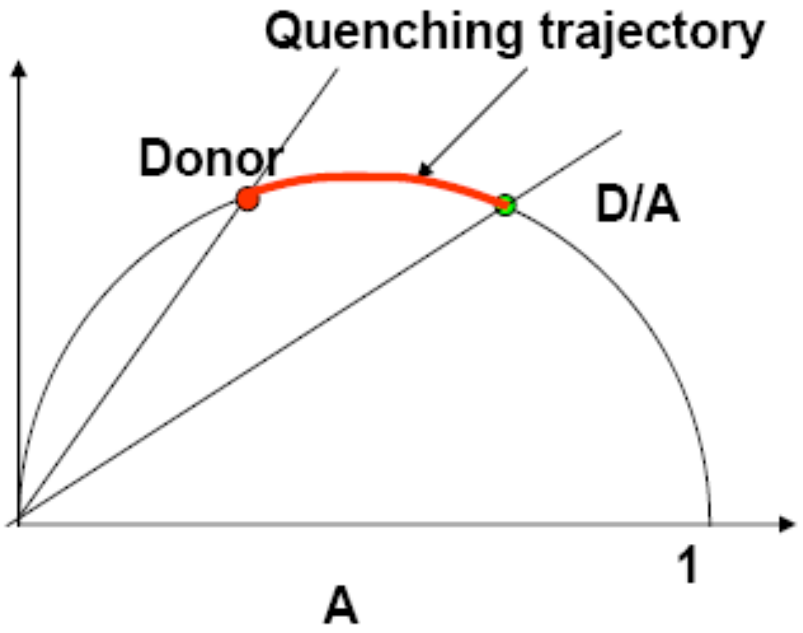


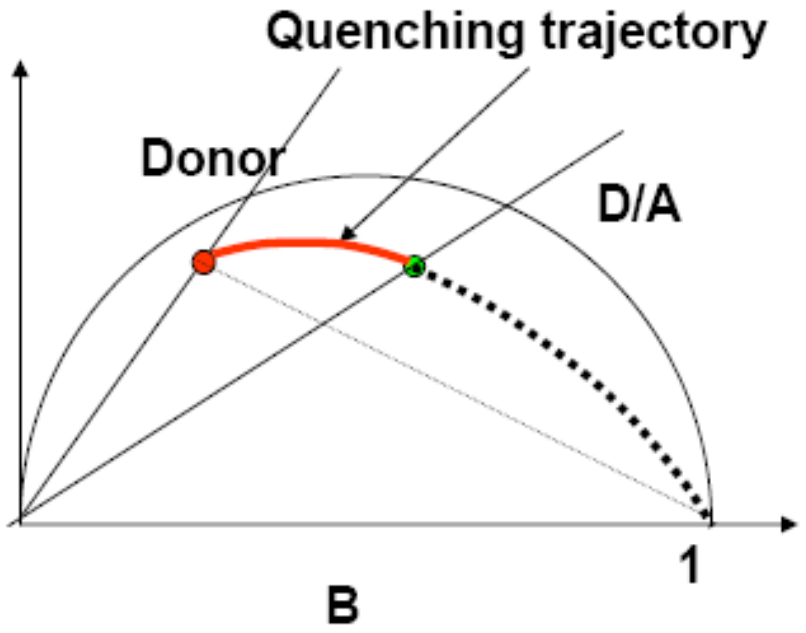
Single Chain



The FRET calculator

If we have a donor with a single exponential decay that is quenched by the presence of a acceptor. What should we expect?



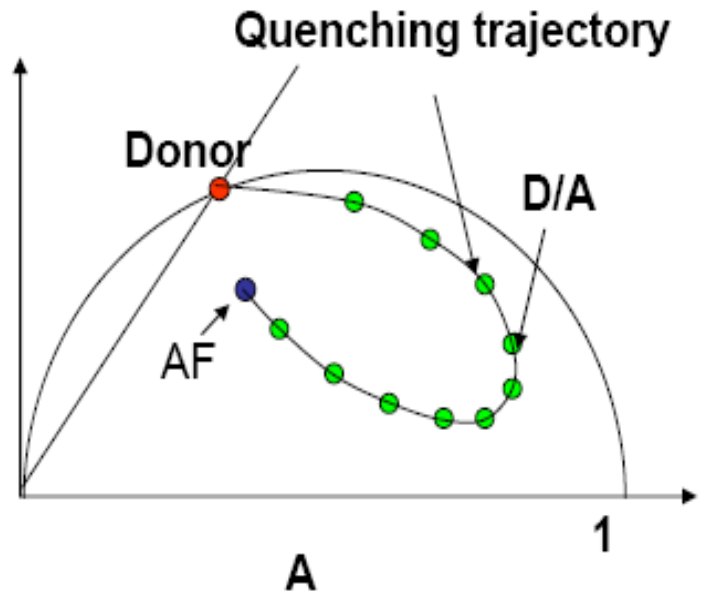


The lifetime of the donor is quenched
The FRET efficiency can be calculated by the ratio of the two lifetimes

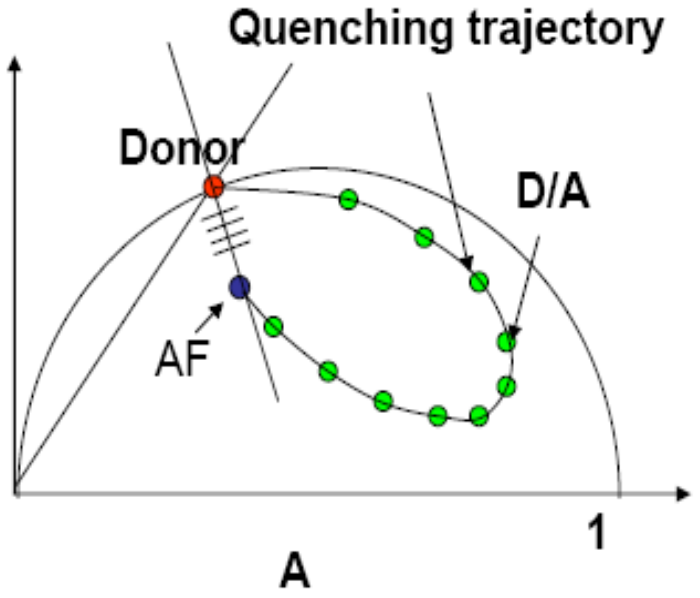
The lifetime of the donor is along a different "trajectory", Why is the trajectory an arc rather than a line to the (1,0) point?

The FRET Calculator

After all the Donor is quenched, what is left? The cell autofluorescence!!

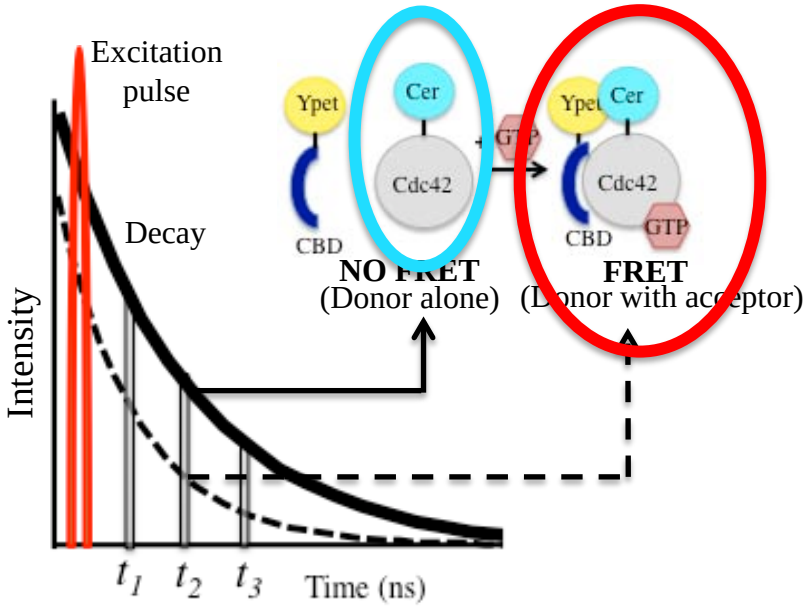


As the lifetime of the Donor is quenched, the phasor of the quenched Donor is added to the phasor of the autofluorescence

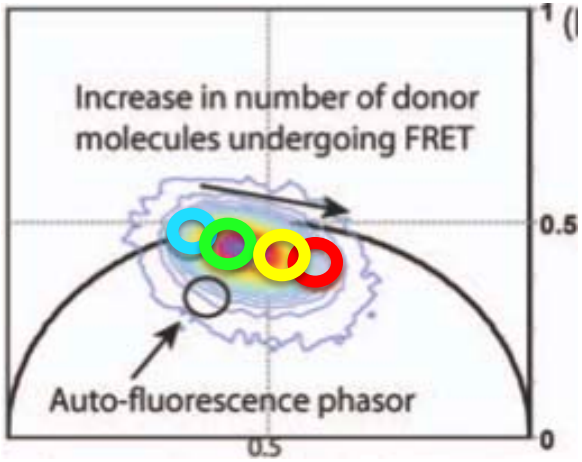


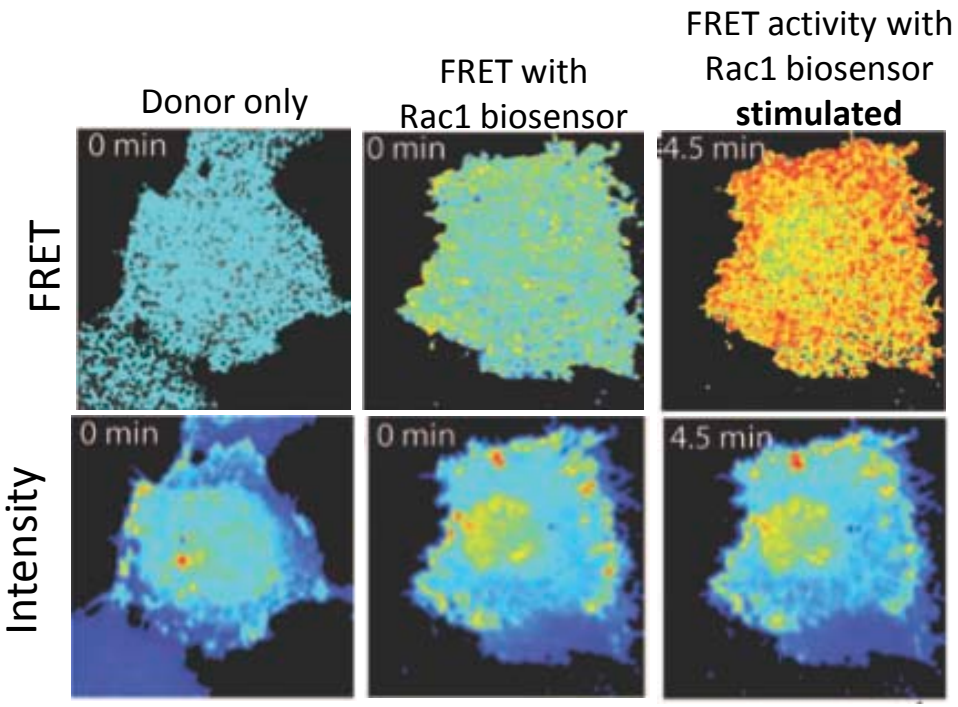
If there is a fraction of Donor that cannot be quenched, the final point will be along the line joining the Donor with the autofluorescence phasor

FRET signal is independent of intensity with phasor analysis (Measured with *donor only*)

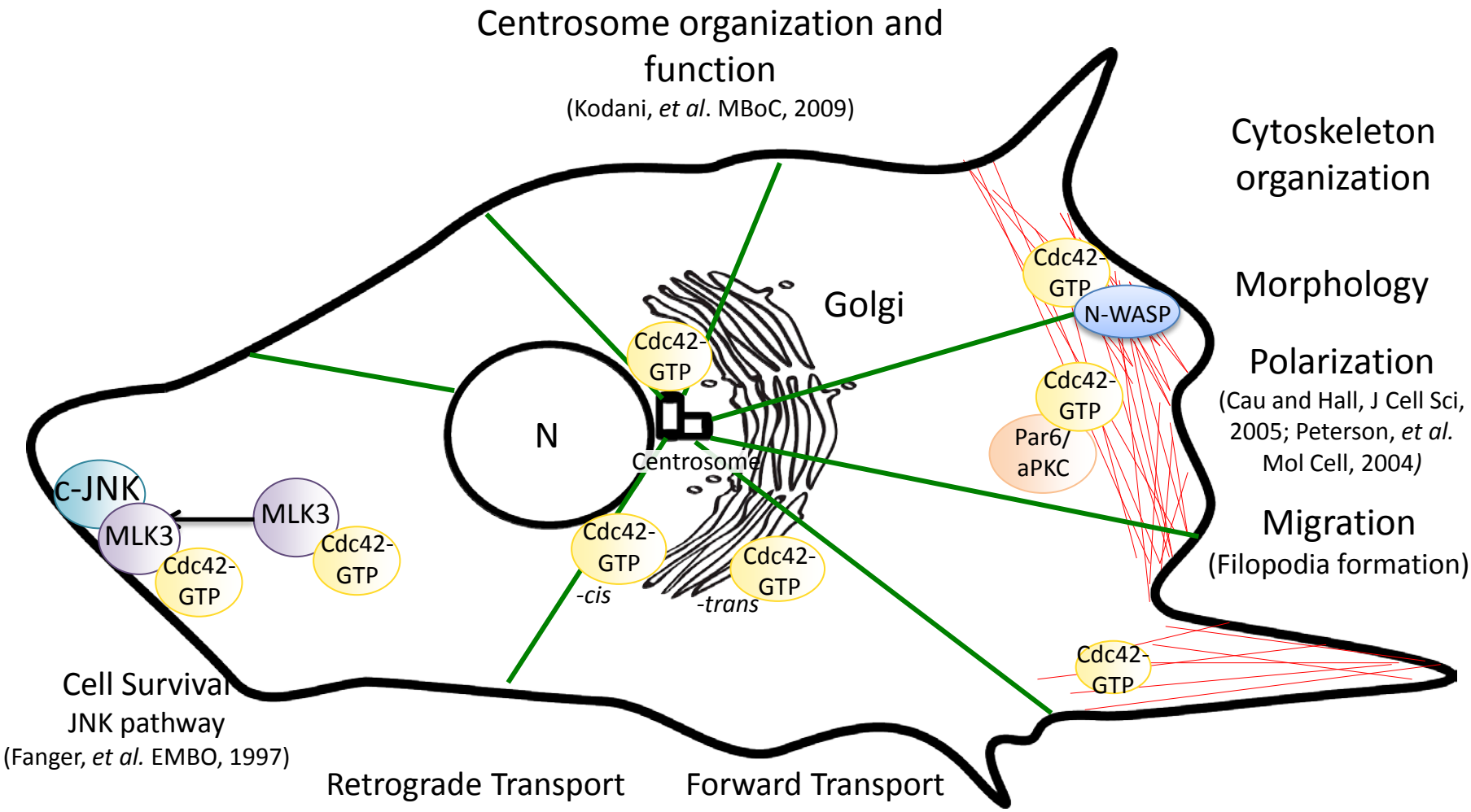


Donor (Cerulean) lifetime

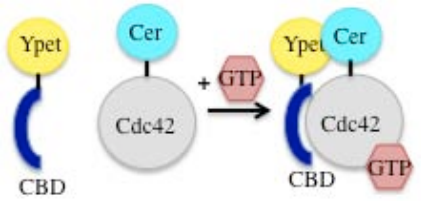


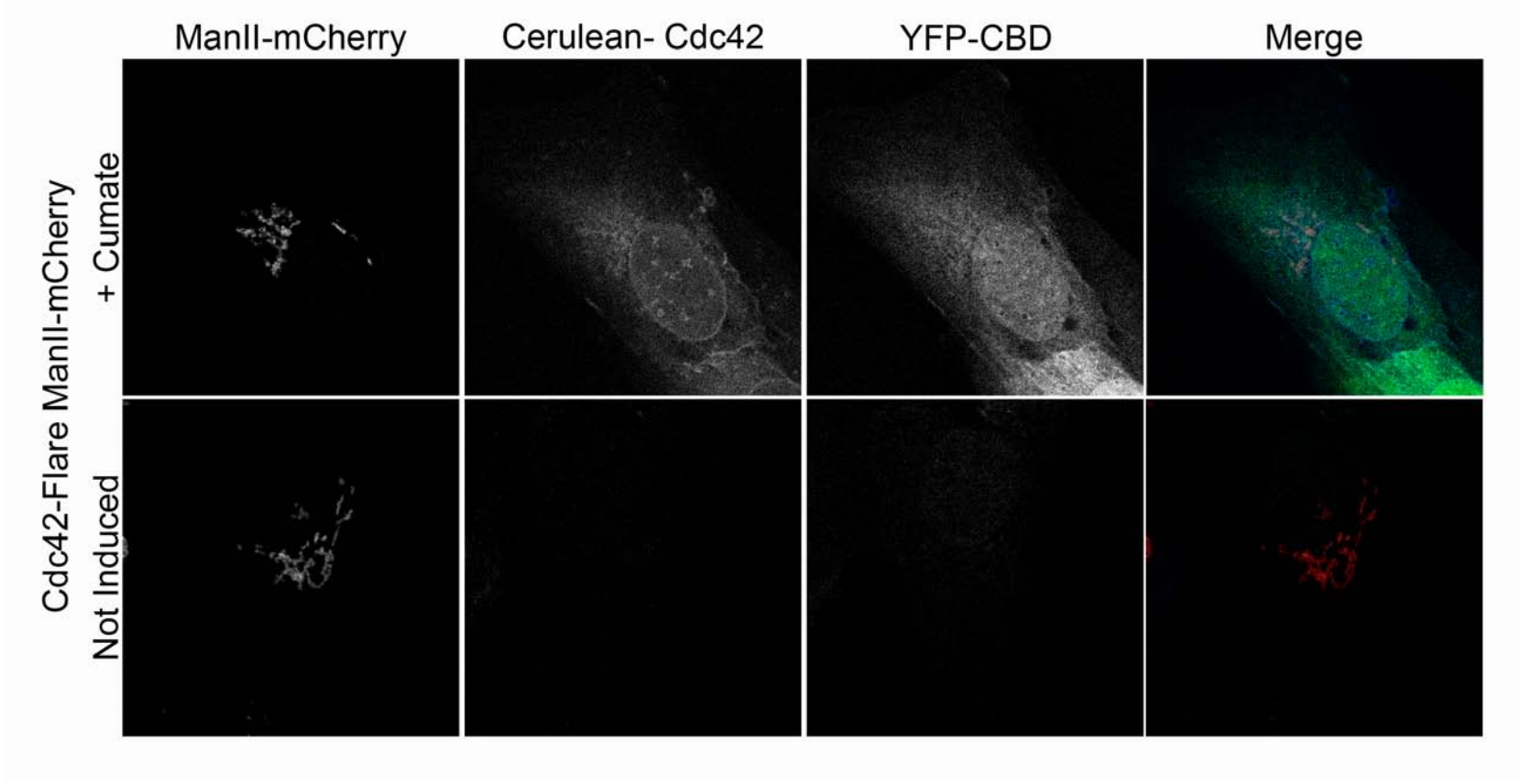


Cdc42 is a functional component of the Golgi



Cdc42-Flare ManII-mCherry U2OS cell line

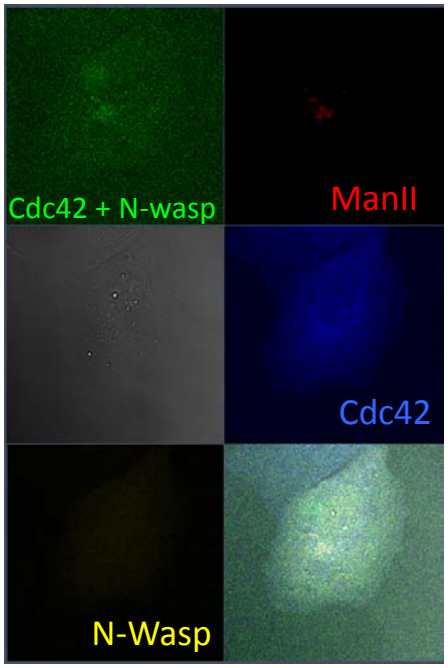




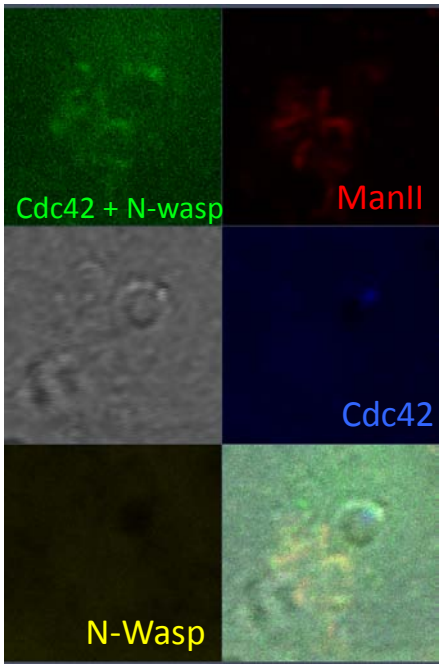
Higher resolution with Tuba depleted cells

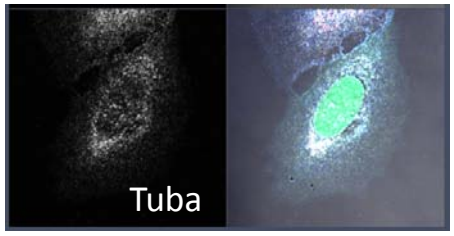
Scrambled

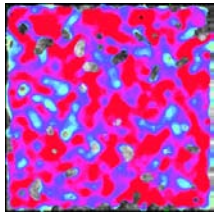
Whole Cell



Zoom in on Golgi

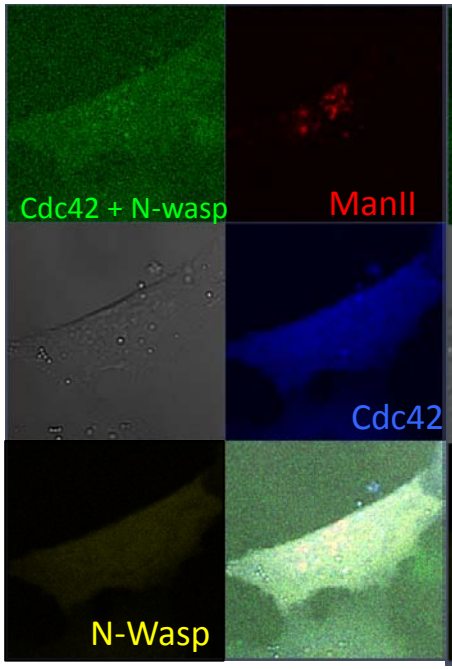




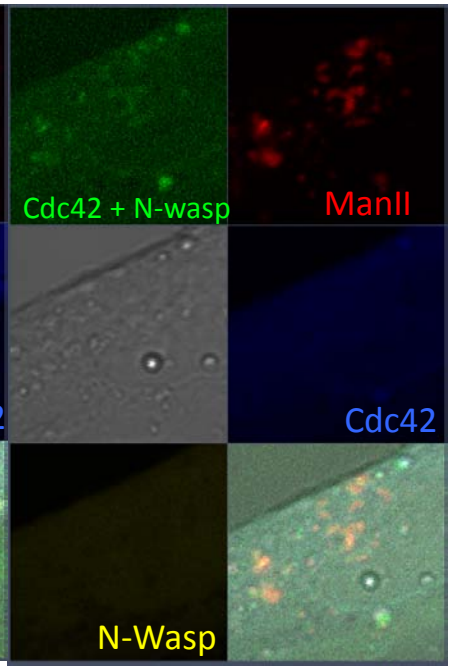


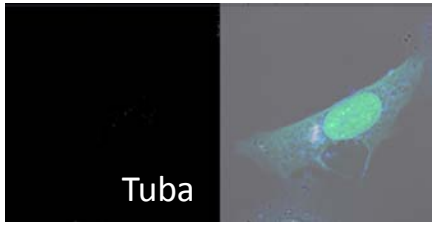
Tuba KD

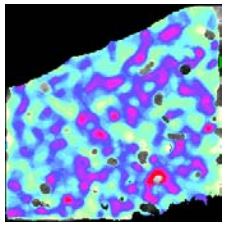
Whole Cell



Zoom in on Golgi







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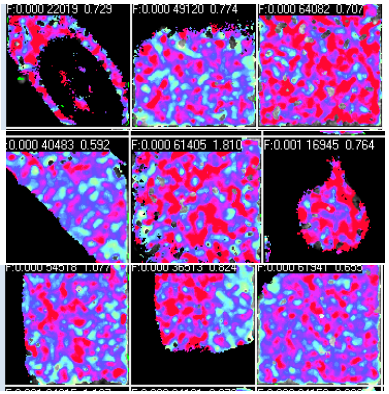

Tuba