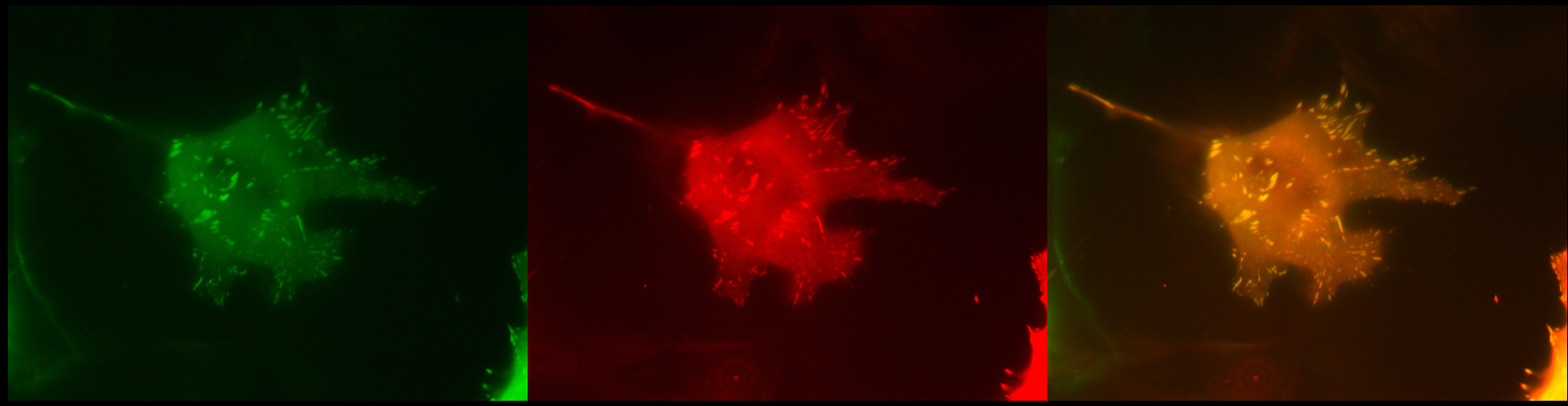
Advanced Fluorescence Microscopy



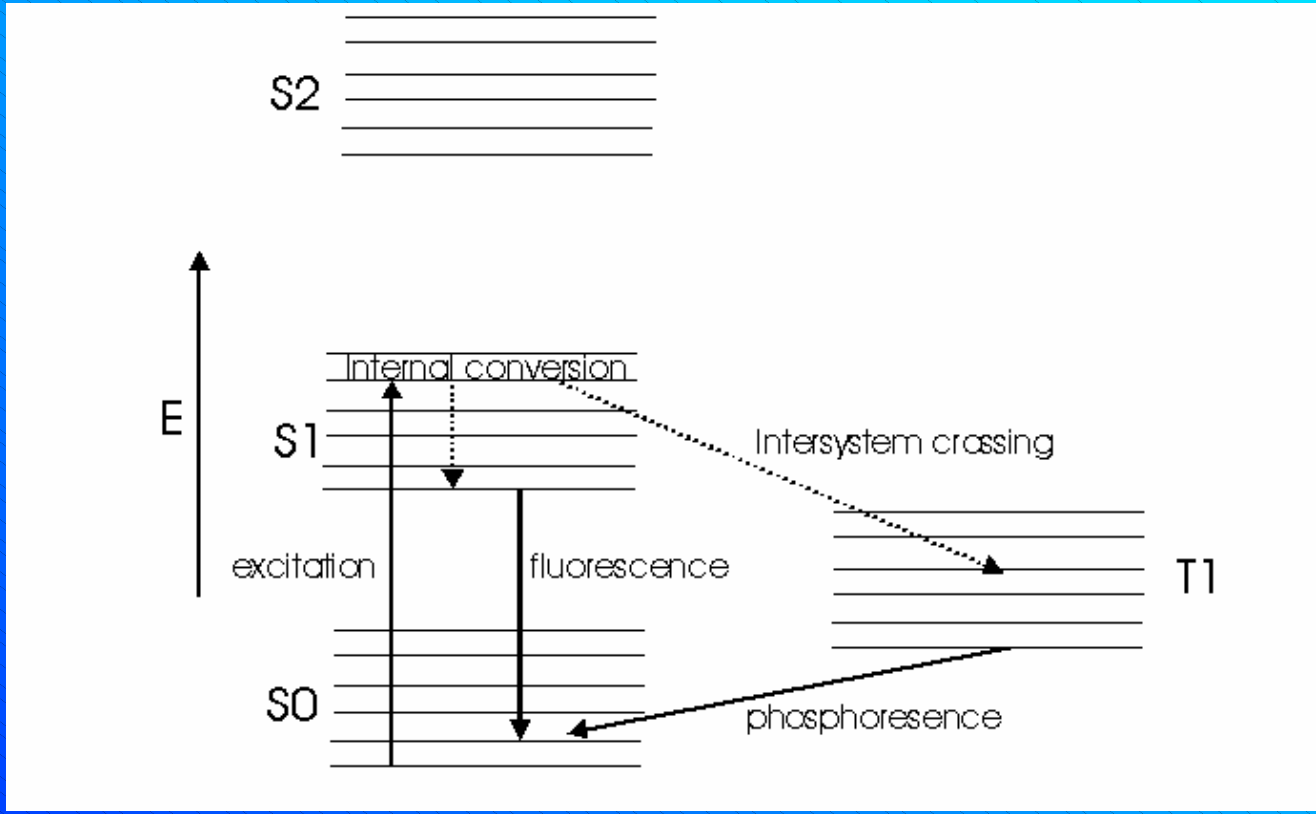
Spectral Resolved Microscopy

Lifetime Resolved Microscopy

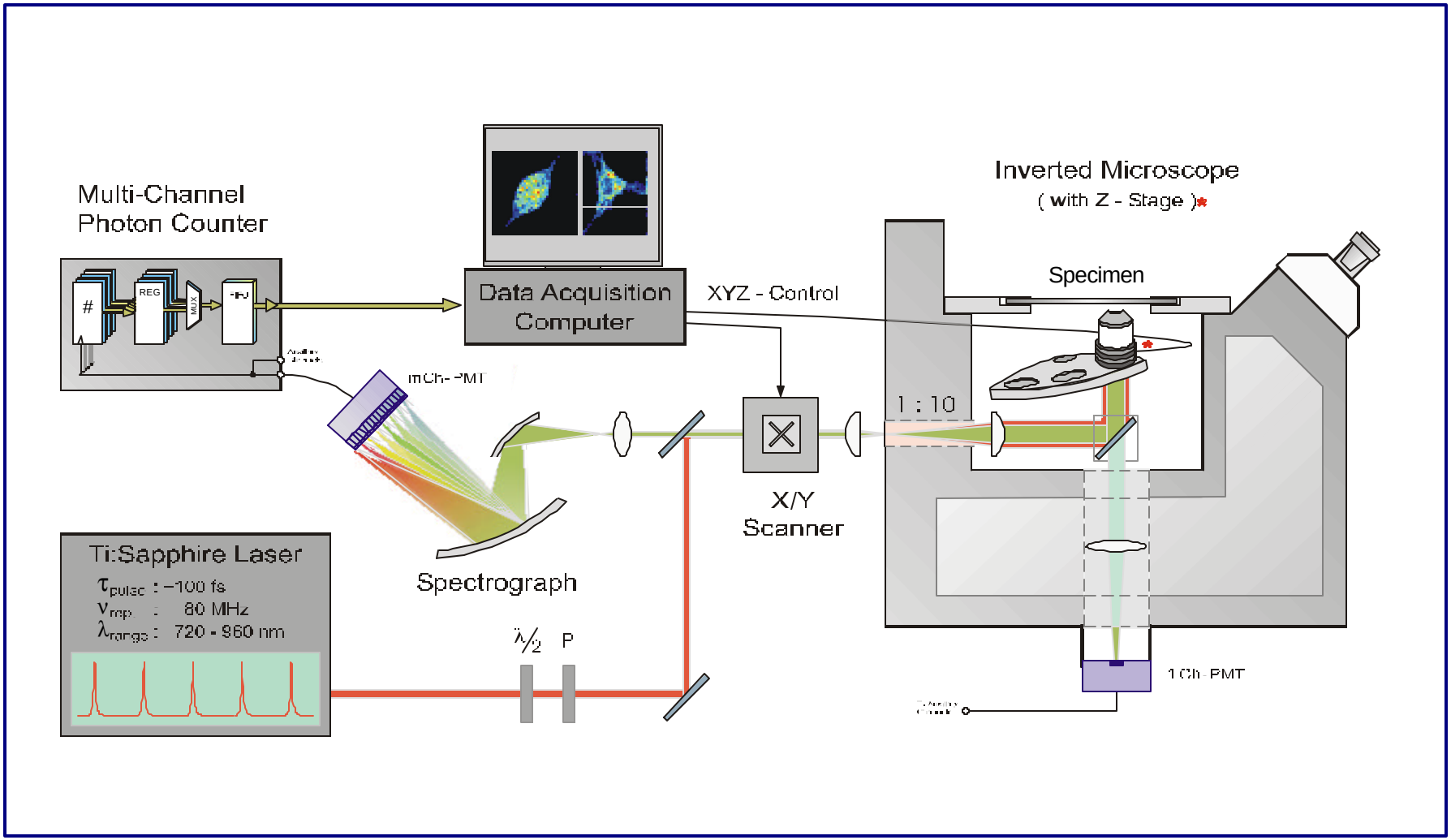
Foster Resonance Energy Transfer (FRET) Microscopy

Fluorescence

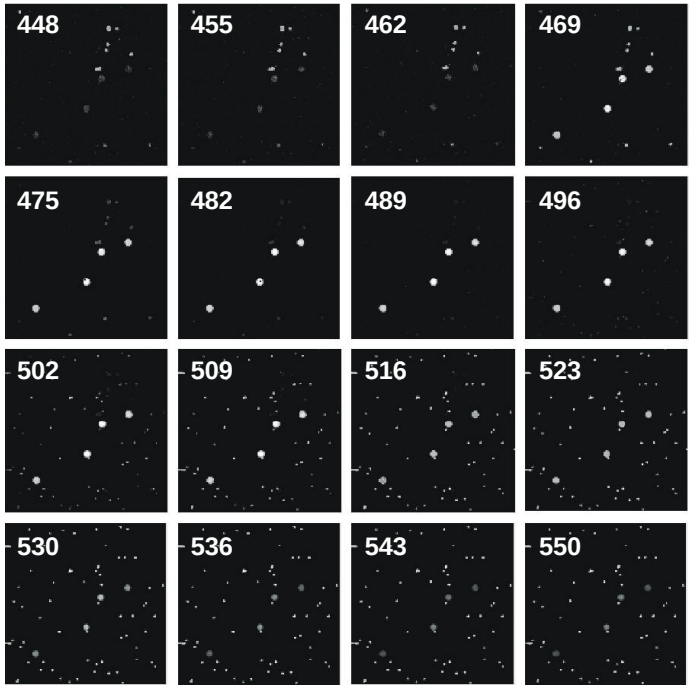
Jablonski Diagram



Spectral Resolved Fluorescence Microscopy



In collaboration with Dr. C. Buehler, PSI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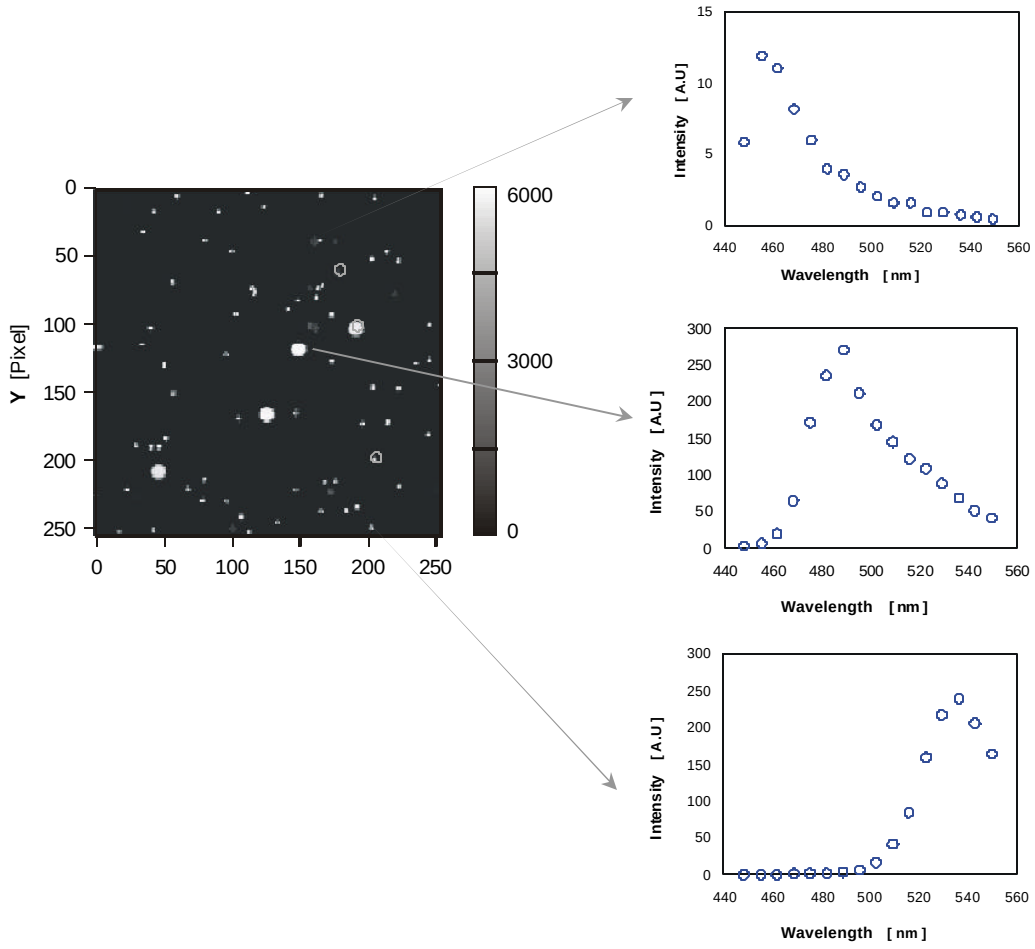
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50
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Intensity [A.U]
Wavelength [nm]
440
460
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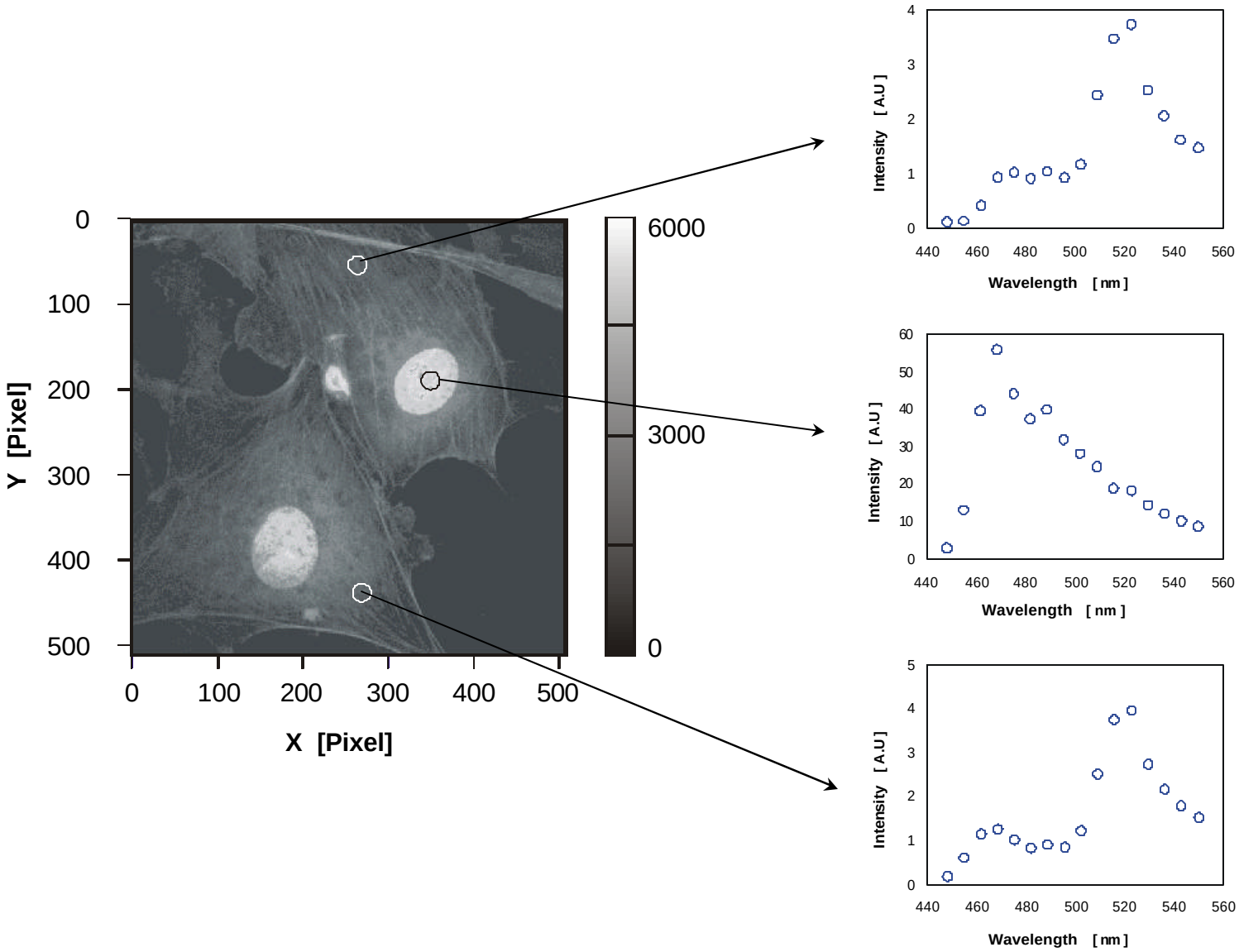
520
540
560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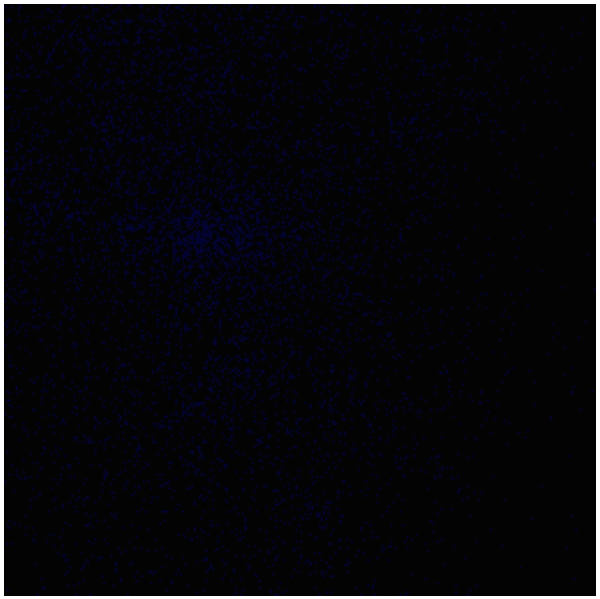
400
500
6000
3000
0
Intensity [A.U]
Wavelength [nm]
440
460
480
500
520
540
560

Spectrally Resolved 2-P 3-D Imaging

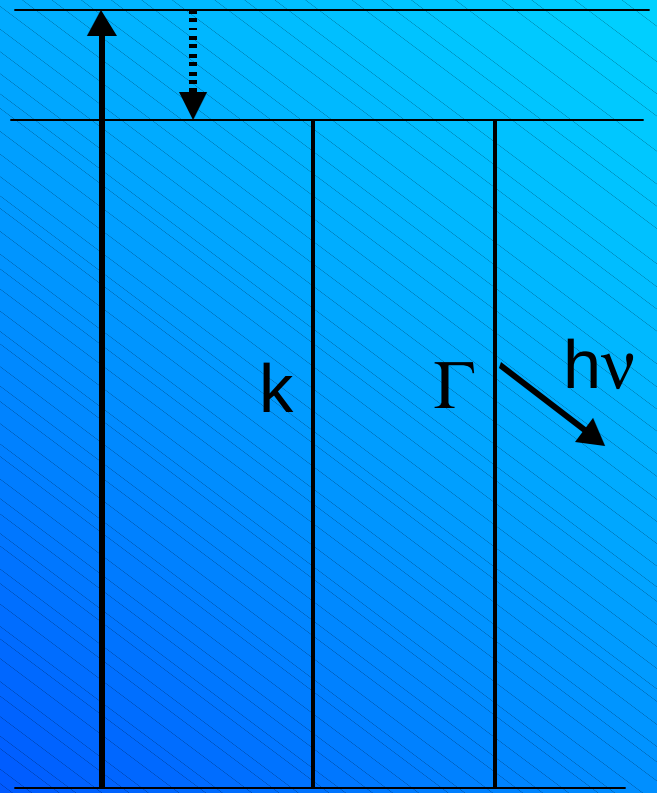
3 Color Latex Beads
(10 Hz frame rate)



Ex vivo human dermis
(0.2 Hz frame rate)

Fluorescence

Radiative & Non-Radiative Decay I



Fluorescence

Radiative & Non-Radiative Decay II

Fluorescence lifetime

$$t = \frac{1}{\Gamma + k}$$

Fluorescence Natural lifetime

$$t_0 = \frac{1}{\Gamma}$$

Fluorescence

Intensity Decay

Single Exponential Decay

$$\frac{dN_e}{dt} = -(k+\Gamma)N_e$$

$$F = F_0 e^{-(k+\Gamma)t} = F_0 e^{-t/t}$$

Fluorescence

Intensity Decay II

Multiple Exponential Decay

$$\frac{dN_e}{dt} = -\left(\frac{1}{t_1} + \frac{1}{t_2} + \ldots.\right) N_e$$

$$F = F_0 e^{-\Sigma t / t_i}$$

Fluorescence

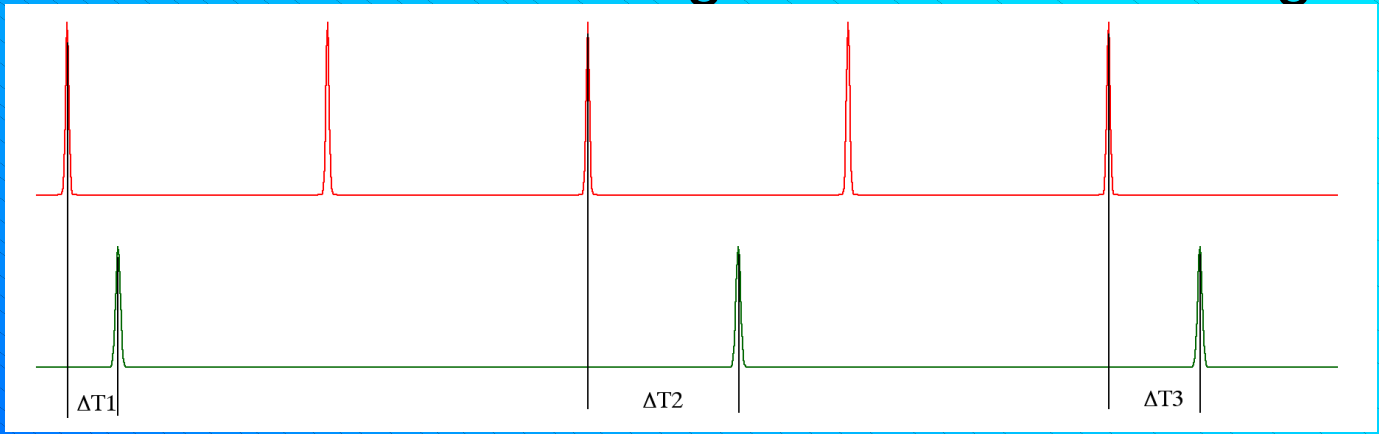
Lifetime of Fluorophores

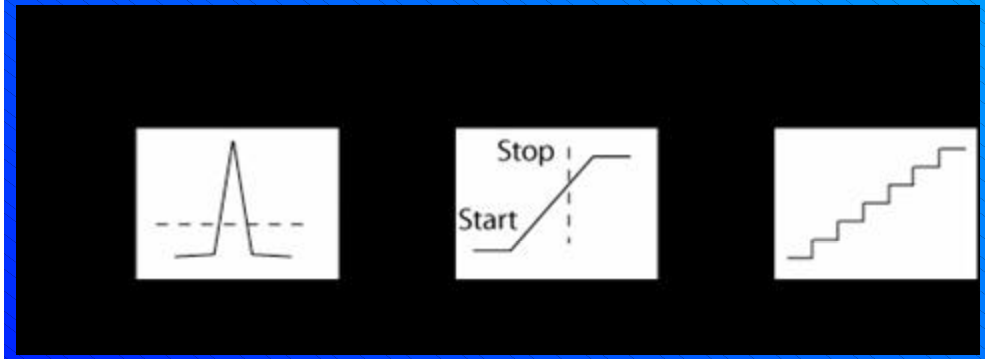
POPOP: 1.3 ns
Fluorescein: 4 ns
GFP: 2.5 ns
CdSe/ZnS Q-dot: 25-50 ns
Terbium, Rubidium compounds: >500ns

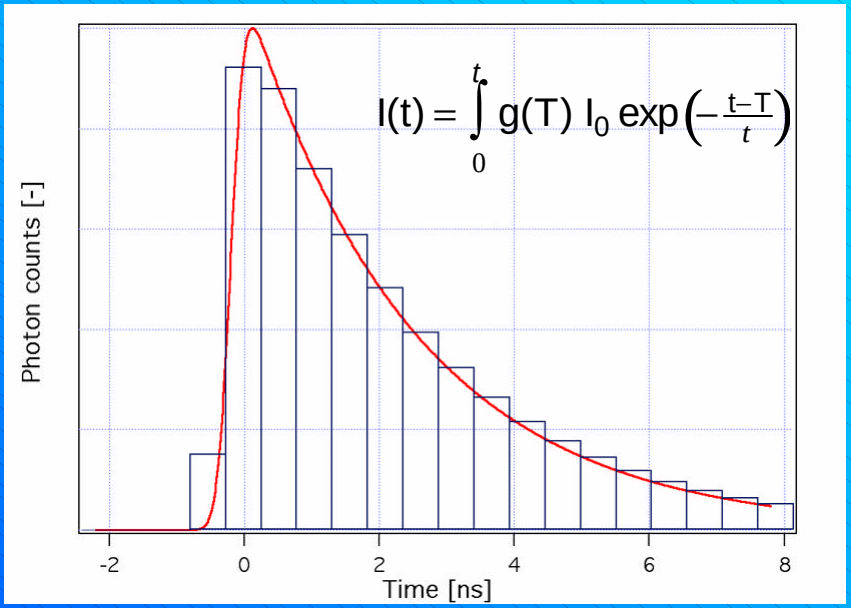
EtBr (free): 1.8 ns
EtBr (bound): 23 ns

Time domain

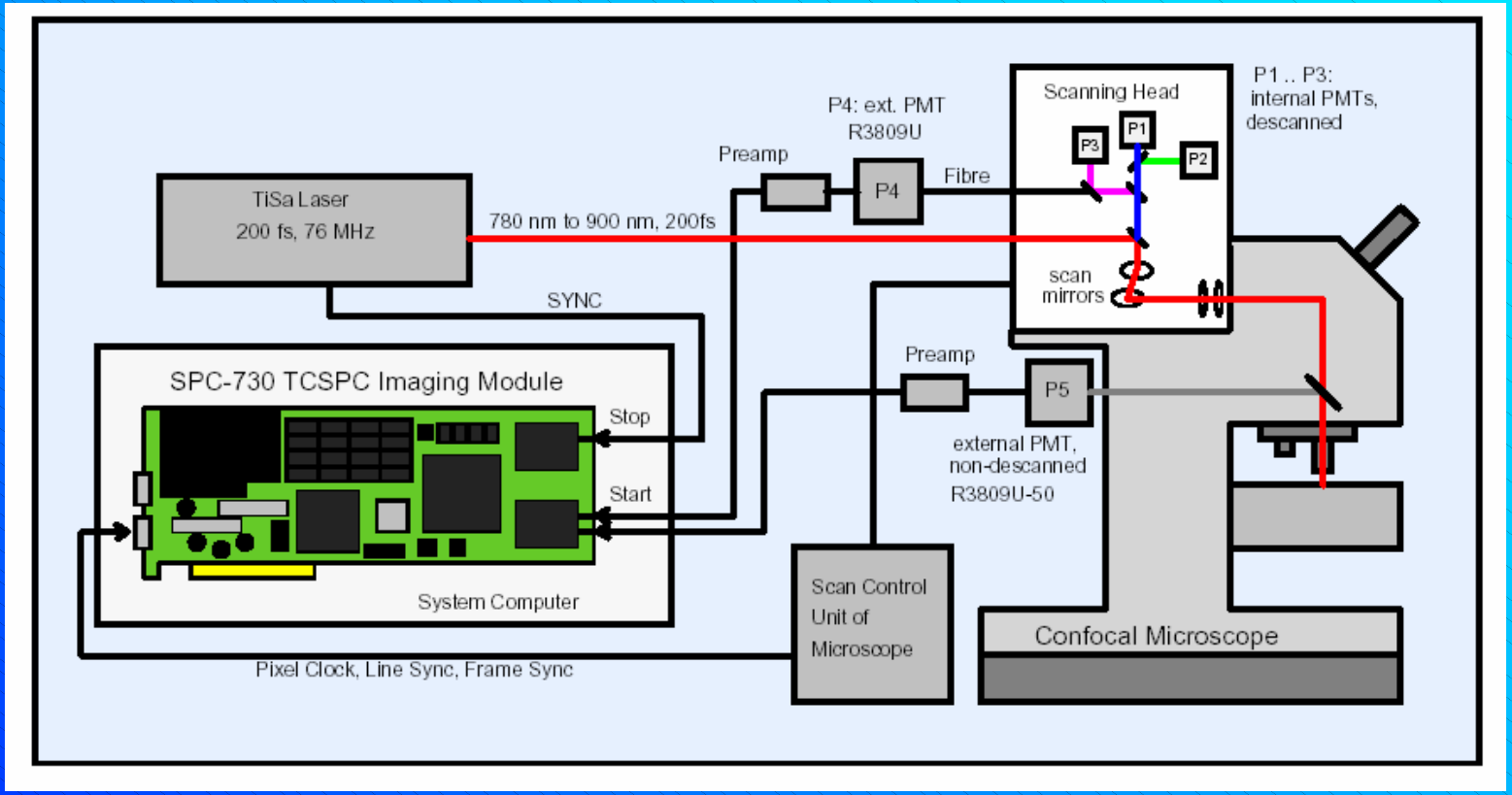
Time Correlated Single Photon Counting







Time-Correlated Single Photon Counting

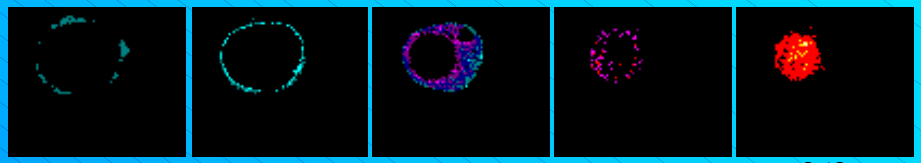


Becker et al, ECBO 2002

Lifetime imaging and biological functions

(1) Distinguish cellular organelles by multiple lifetime imaging

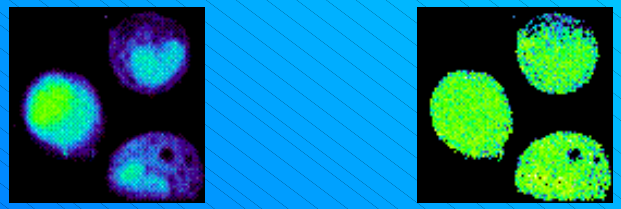
Lifetime Sections



1.6-2.4 2.4-4 4-8 8-9 9-19

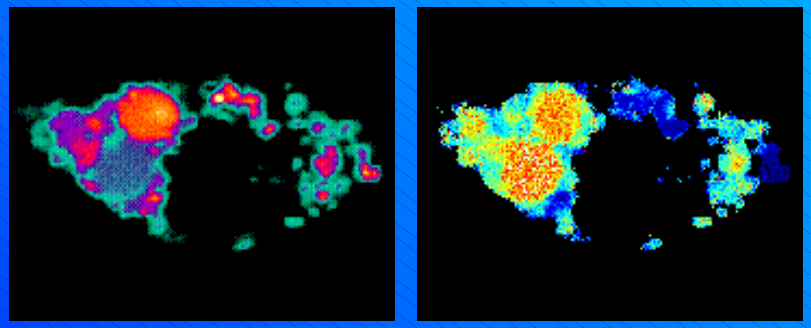
(2) Monitor metabolite concentration (Ca, pH etc)

Intensity Lifetime



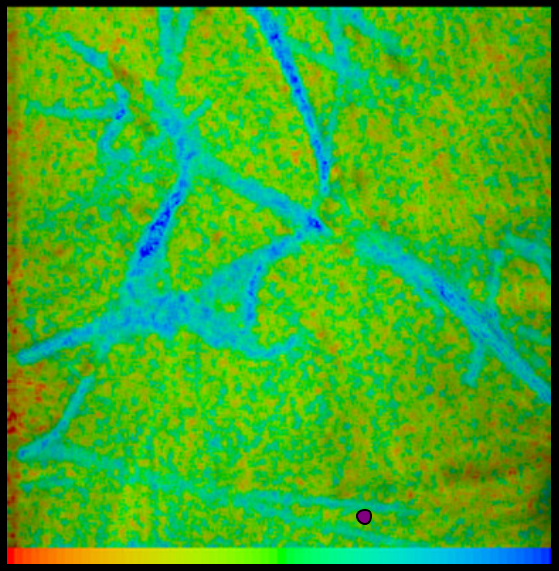
(3) Monitor cellular processes such as proteolytic activity

Intensity Lifetime



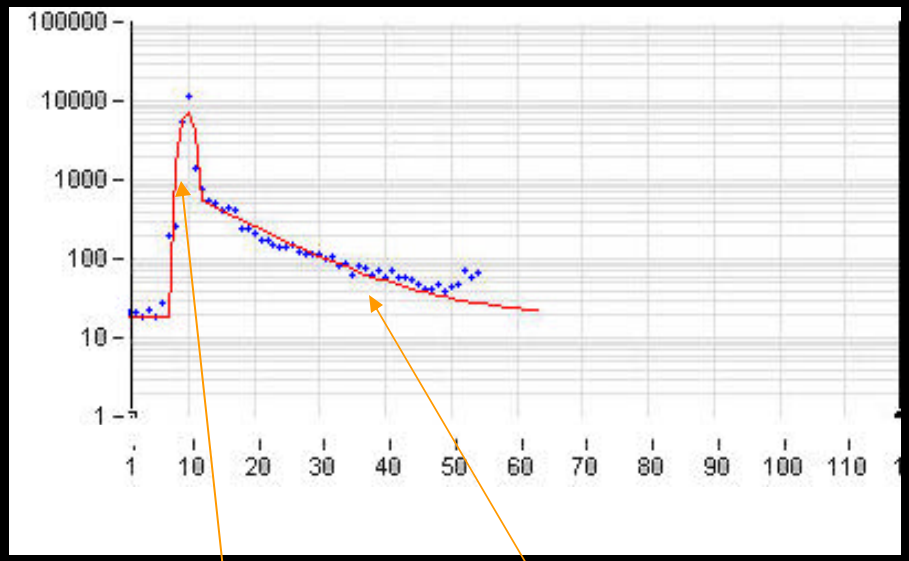
Lifetime Image of ECM in Dermis

Mean lifetime Image



2.0e3 3.5e3 ps

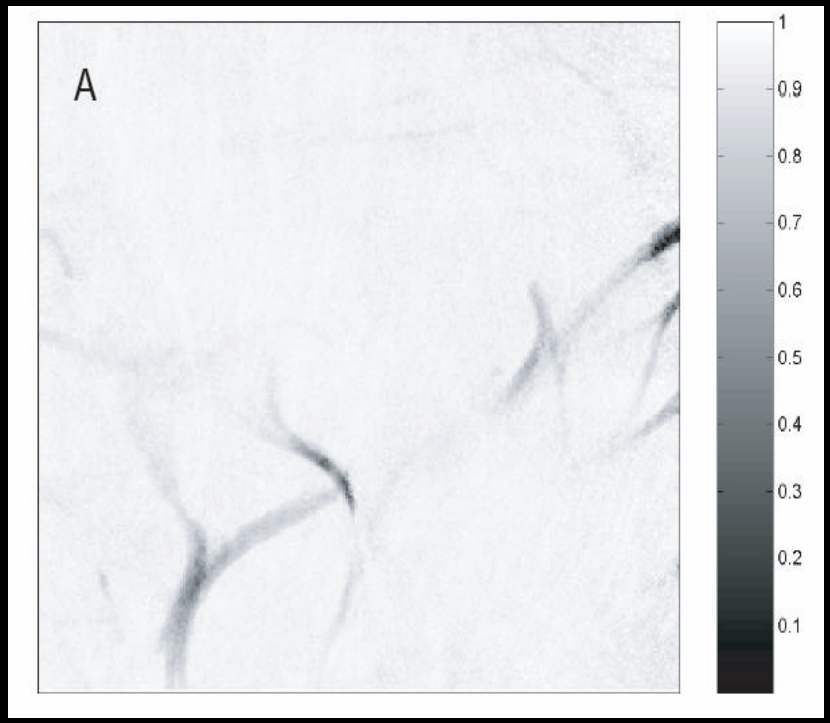
Lifetime decay at a selected point



Zero lifetime component: Collagen 2nd harmonic emission

ns lifetime component: Elastin fluorescence

Resolving Collagen & Elastin Fraction in the Dermis

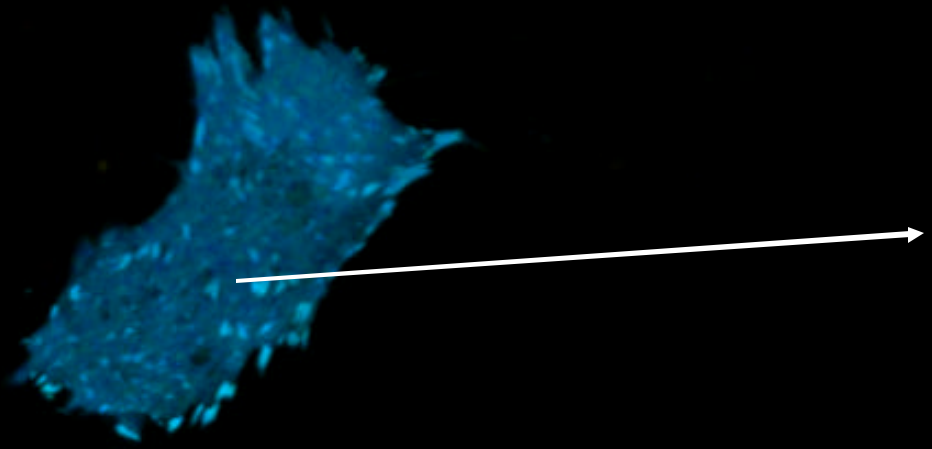


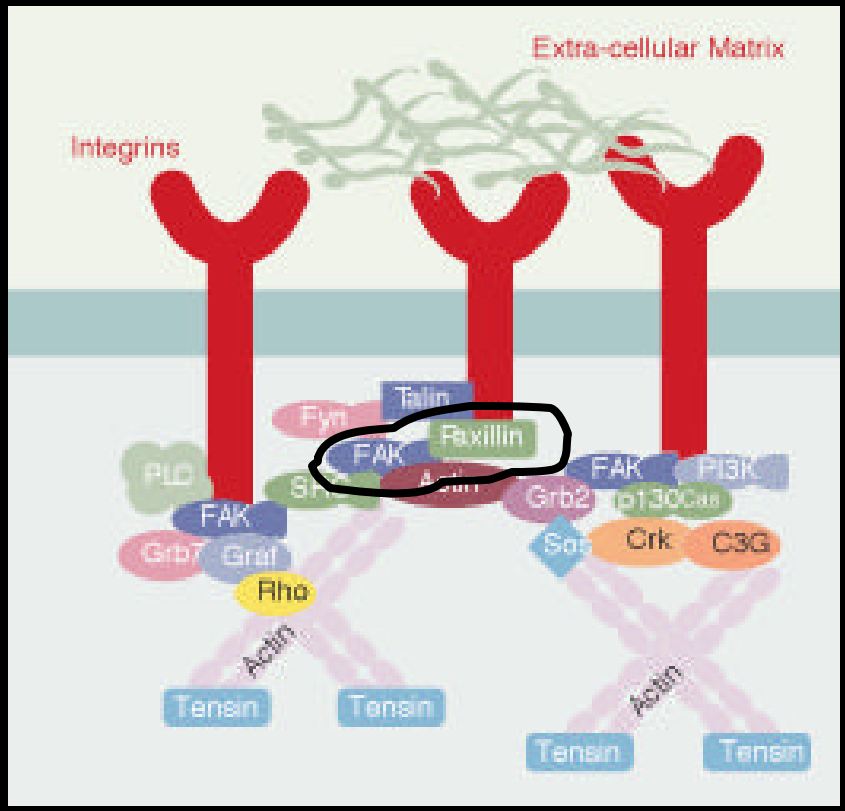
Fraction of SHG (Collagen)

Foster (Fluorescence) Resonance Energy Transfer

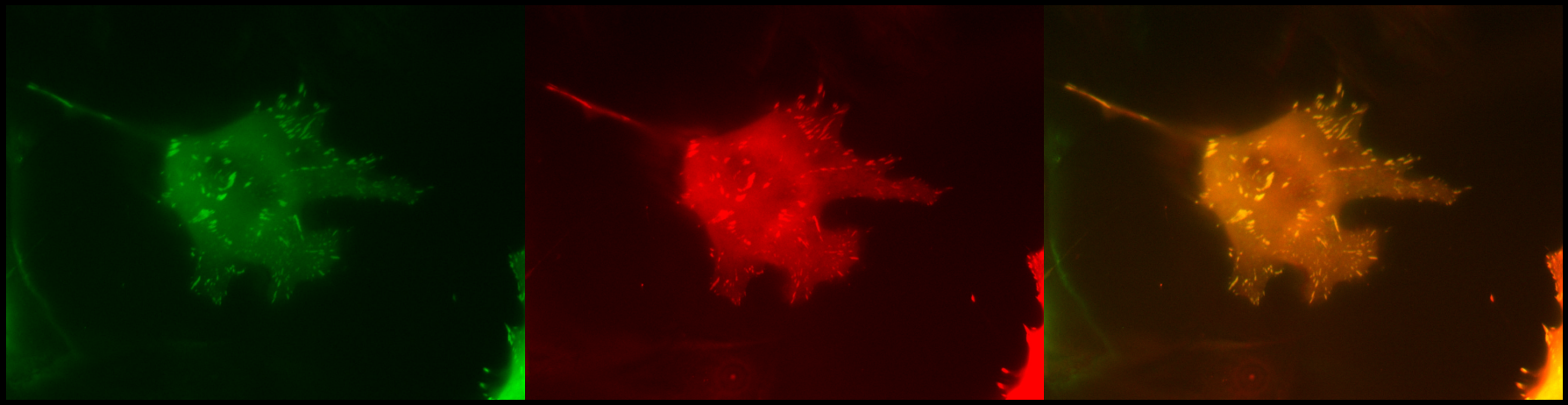
Focal Adhesion Complex – Paxillin-Focal Adhesion Kinase Interaction

*Double negative mutants of FAK is fatal. Tumor cells over expresses FAK
*FAK controls cell spreading, migration, and survival
*Focal adhesion localization results in auto-phosphorylation of FAK
*FAK phosphorylation is linked to cytoskeletal remodeling (Src, Rac 1)

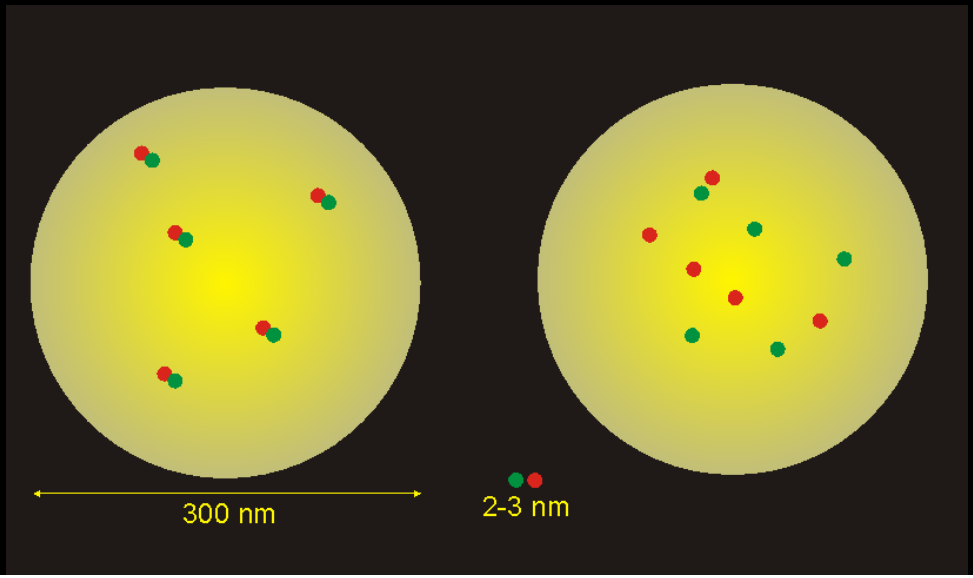




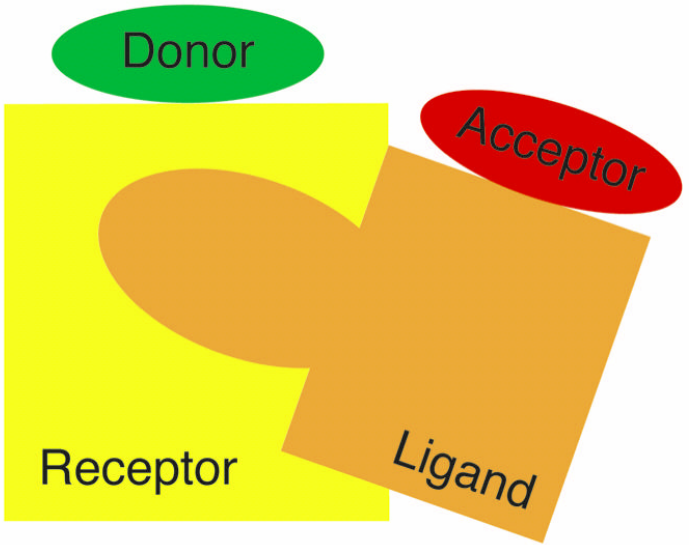
(1) Paxillin-FAT in endothelial cells



GFP-Paxillin **FAT-mCherry** **Spectral overlap**



Foster (Fluorescence) Resonance Energy Transfer



Dipole - dipole interaction

r^6 dependence

Efficiency

50% energy transfer

Förster distance

R_0 = 40 to 70 Å

Decrease excited-state lifetime

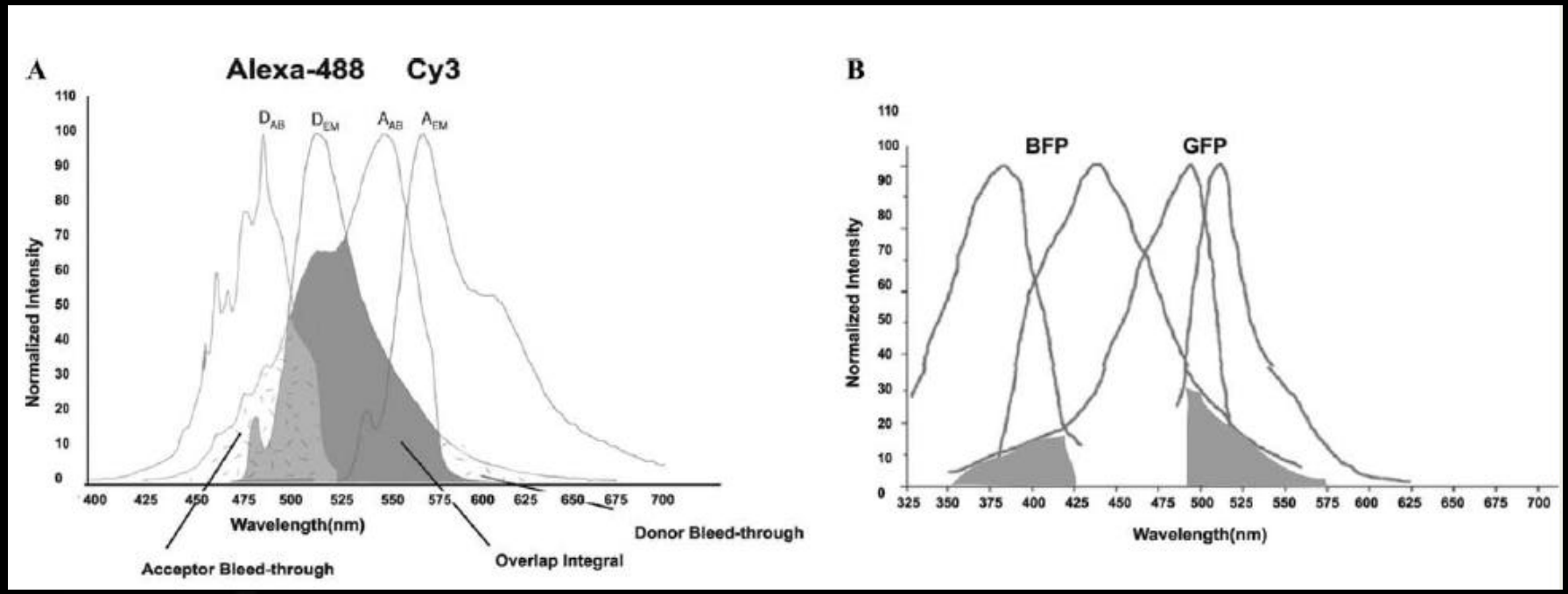
Fixed geometry yields constant FRET parameters

$$E = \frac{R_0^6}{R_0^6 + r^6} = 1 - \frac{t_{DA}}{t_D}$$

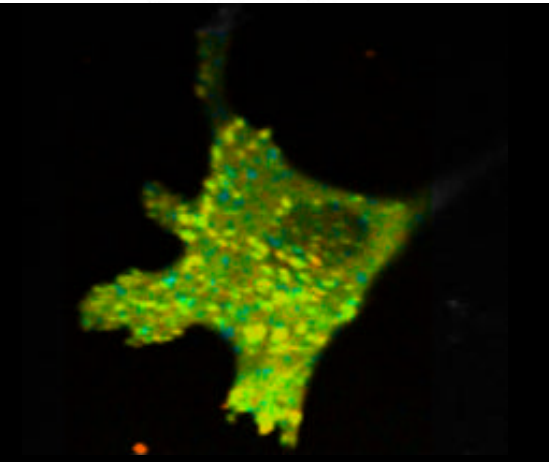
$$R_0^6 = \frac{9000 \ln(10)\, k^2\, f_D}{128\, p^5\, N_A\, n^4}\, J$$

where, $$J = \frac{\int F_D(l)\, e_A(l)\, l^4\, dl}{\int F_D(l)}$$

FRET & Spectral Bleedthrough

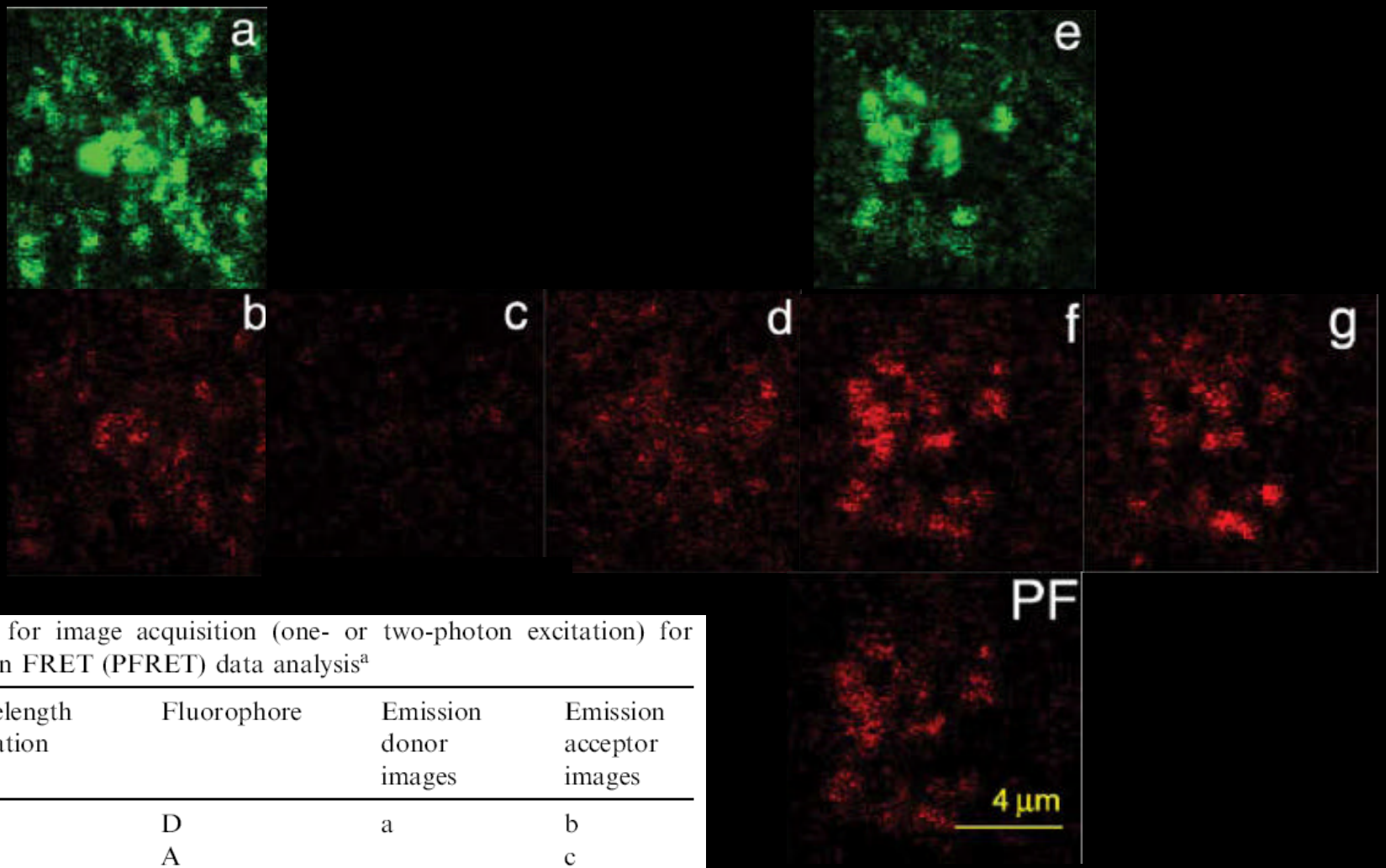


Elangovan, Methods, 2002



FRET Image: Are we ok now if we see acceptor fluorescence?

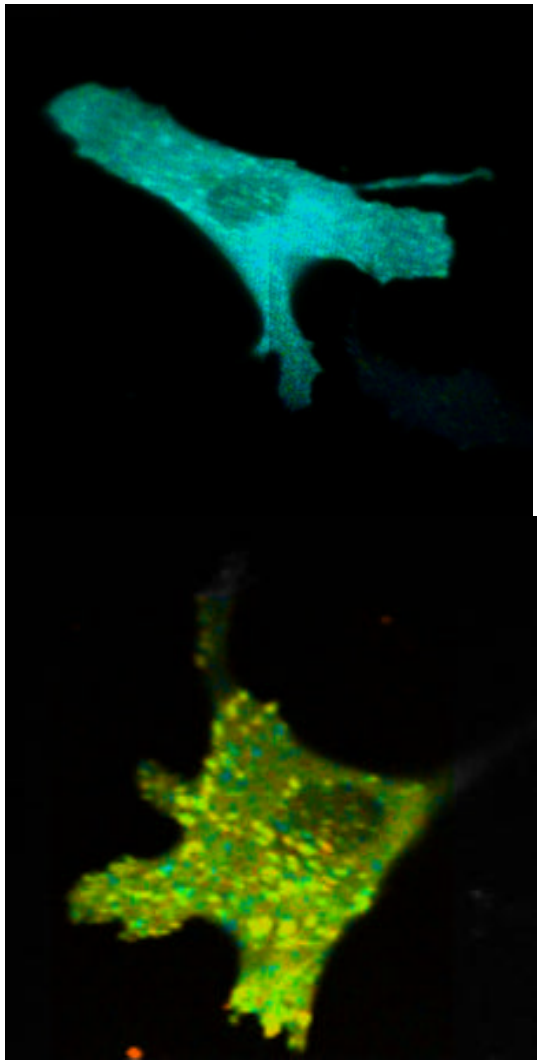
Spectral Resolved FRET



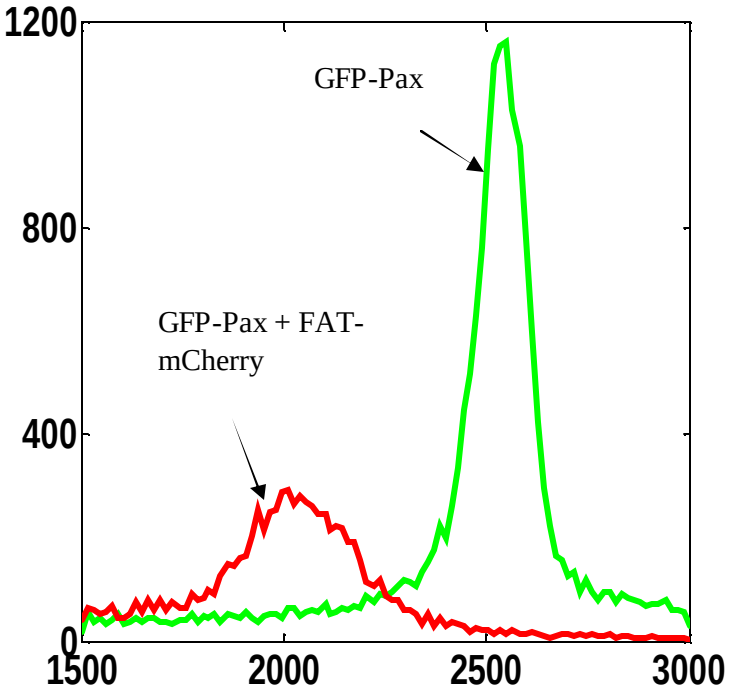
Layout for image acquisition (one- or two-photon excitation) for precision FRET (PFRET) data analysis[a]

Wavelength excitation	Fluorophore	Emission donor images	Emission acceptor images
λ_D	D	a	b
	A		c
	D + A	e	f
λ_A	A		d
	D + A		g

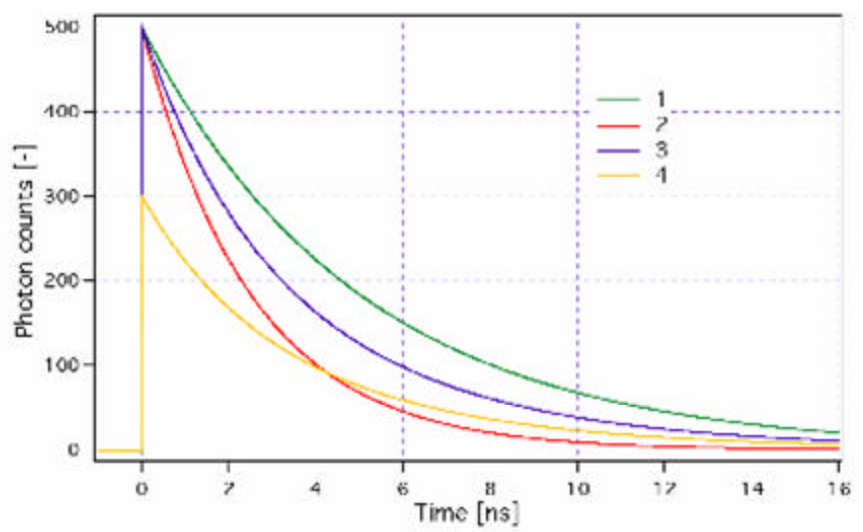
(1) Lifetime Resloved FRET



Lifetime (ns)

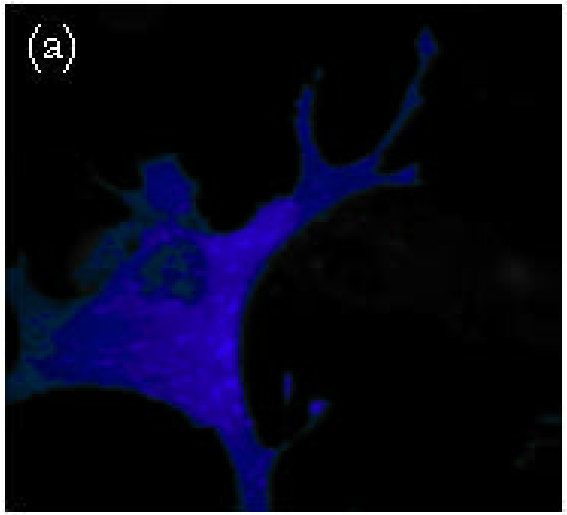


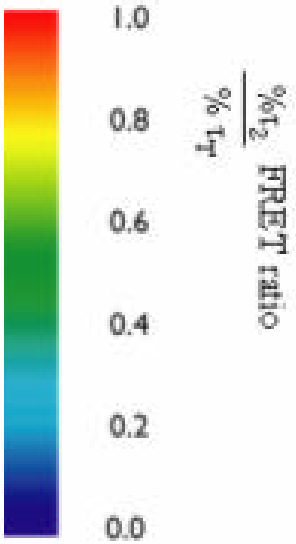
“Quantify” Signaling Pathway Using t-FRET

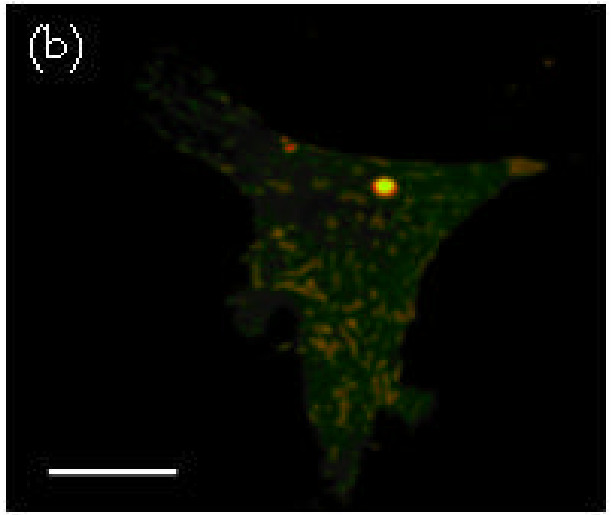


$$I_{\mathrm{i}}^{\mathrm{model}}(t) = \int_0^t G(t-T) \times c_{2\mathrm{i}+1} \left(c_{2\mathrm{i}+2} \exp\left(-\frac{T}{c_1}\right) + (1 - c_{2\mathrm{i}+2}) \exp\left(-\frac{T}{c_2}\right) \right) \mathrm{d}T.$$

(1) Resolving bound paxillin fractions In PAC and cytosol







FRET ratio

0.17

0.06

0.003

0

0

FRET ratio

0.54 ← cytoplasm

0.84 ← majority FA

1

0.65