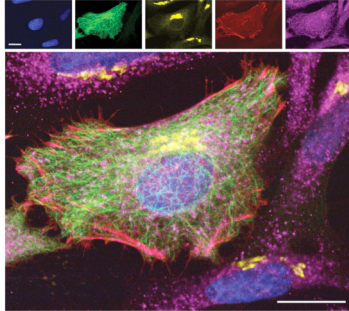


Fig. 3. Parallel application of targeting methods and fluorophores

Excitation (nm; 2 photon)	488	532	568	637
Emission (nm)	410-490	500-530	555-565	580-620
Fluorophore	Hoechst	GFP	QD595	RbAxi1
Targeting	direct affinity	genetic	immuno	genetic
Target	DNA	α -tubulin	gaudin	β -actin
Structure	nuclei	microtubules	grg1	stress fibers



B. N. G. Giepmans et al., Science 312, 217-224 (2006)

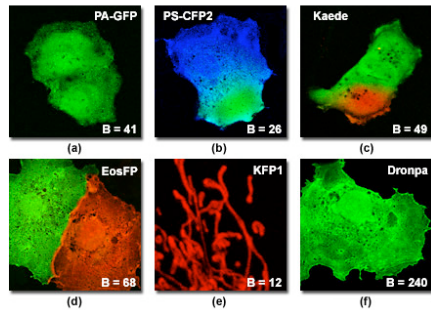


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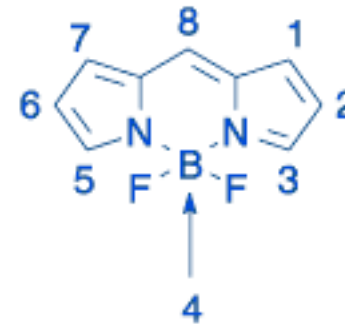
Types of fluorochromes

1. organic molecules (polyphenolic)
 - natural & synthetic
2. metal chelates (lanthanides)
3. semiconductor crystals (Q-dots)
4. fluorescent proteins
 - natural & engineered
5. expressible affinity reagents

Brightness Levels and Images of Optical Highlighter Proteins

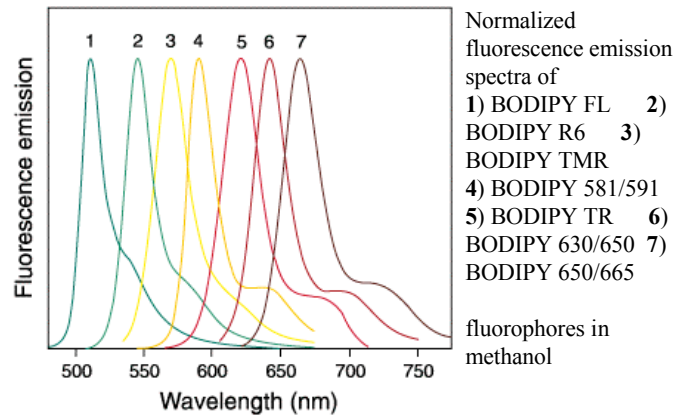


BODIPY fluorophores

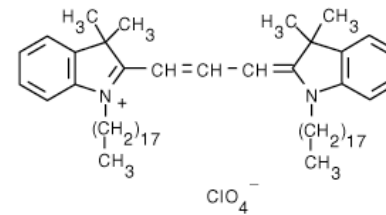


4,4-difluoro-4-bora-3a,4a-diaza-s-indacene

BODIPY fluorophores



DiI Family

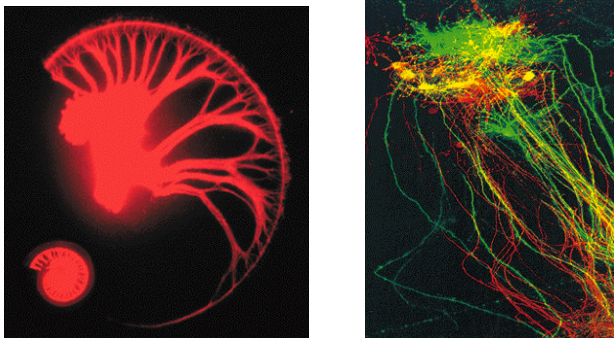


1,1'-diiodo-3,3',3'-tetramethylindocarbocyanine perchlorate ('DiI'; DiI₁₈(3))

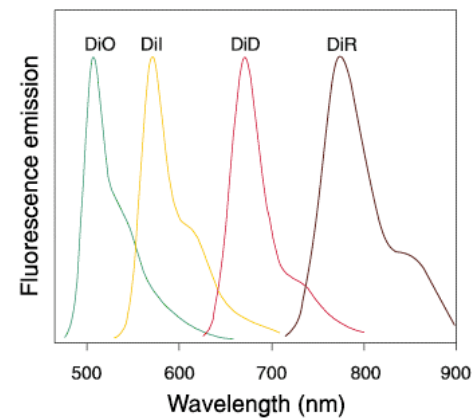
Tract tracing in fixed tissue

Remarkably stable – up to 2 years in vitro

DiI images

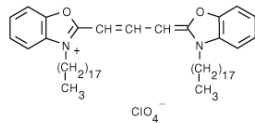


Fluorescence Spectra for DiXs



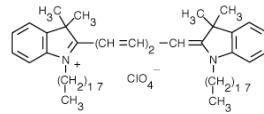
DiI Family

DiO



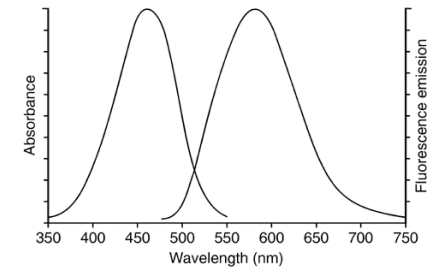
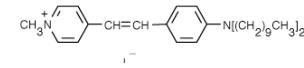
3,3'-dioctadecyloxycarbocyanine perchlorate ('DiO'; DiOC₁₈(3))

DiD



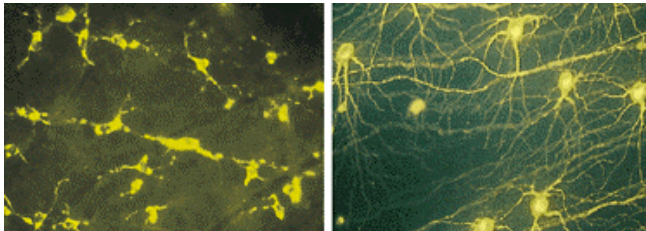
1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate ('DiD' oil; DiIC₁₈(5) oil)

DiA



4-(4-(dihexadecylamino)styryl)-N-methylpyridinium iodide (DiA; 4-Di-16-ASP)

DiA in mouse retina



3 days after optic nerve transection

10 days after optic nerve transection

Dextrans

Substituted dextran polymers

Poly-(α -D-1,6-glucose) linkages, which render them resistant to cleavage by most endogenous cellular glycosidases

Wide molecular weight range (3000 – 300K)

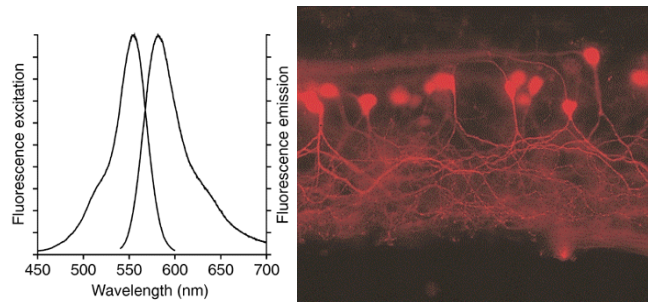
Multiple fluorophores

Biotin

Lysine

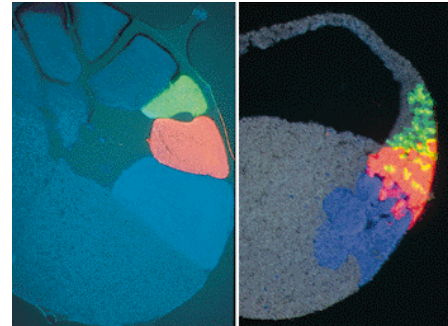
Fluoro-Ruby

Fluoro-Ruby



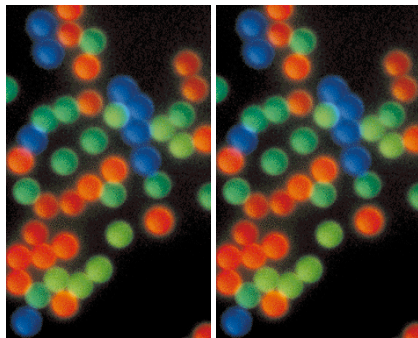
Fish spinal cord

Cell lineage with dextrans



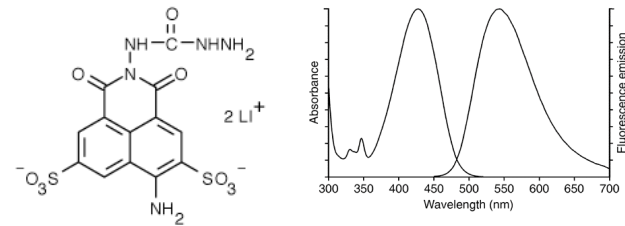
The image on the left shows a 13 μm-thick section of a stage 6 (32-cell) *Xenopus* embryo fixed right after injection; this section exhibits significant autofluorescence due to the presence of residual yolk. The image on the right is a stage 10 (early gastrula) embryo

Fluorescent Microspheres

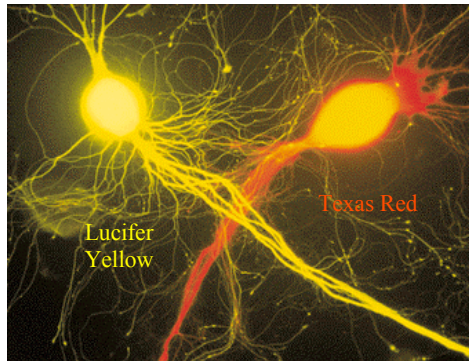


polystyrene microspheres 0.02 – 15 μm diameter

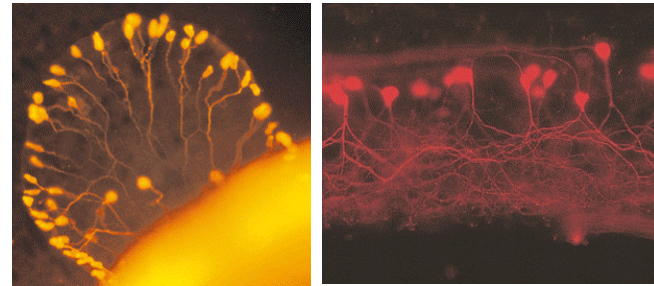
Lucifer Yellow



Lucifer Yellow

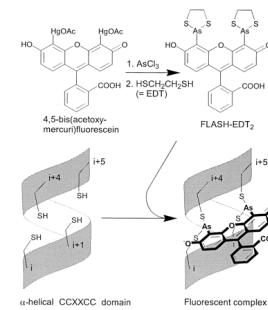


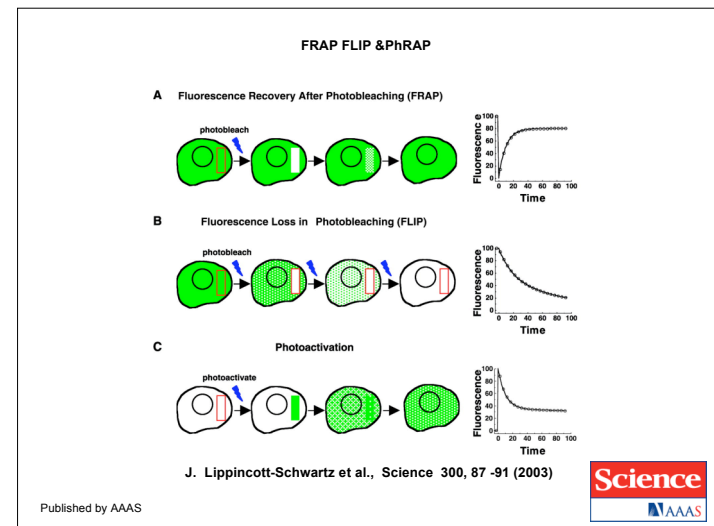
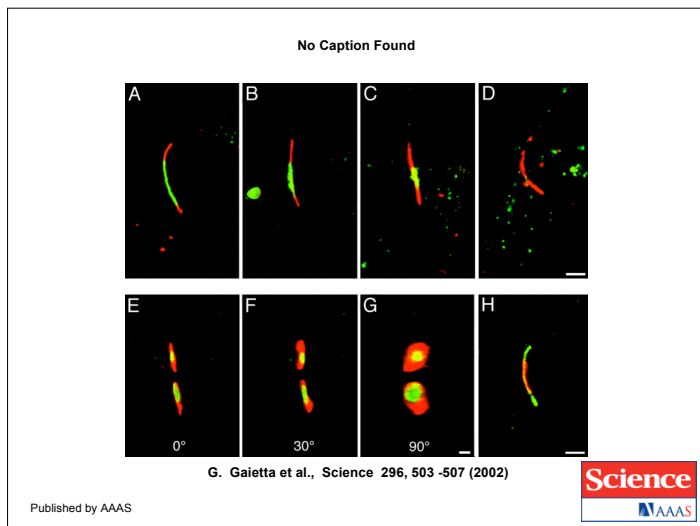
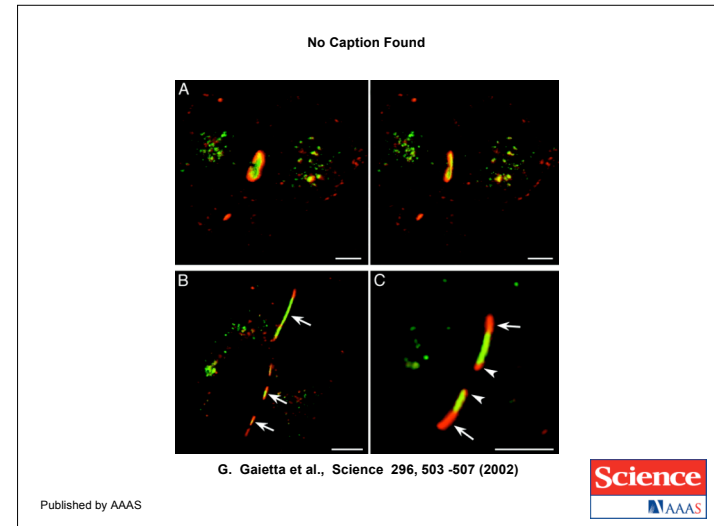
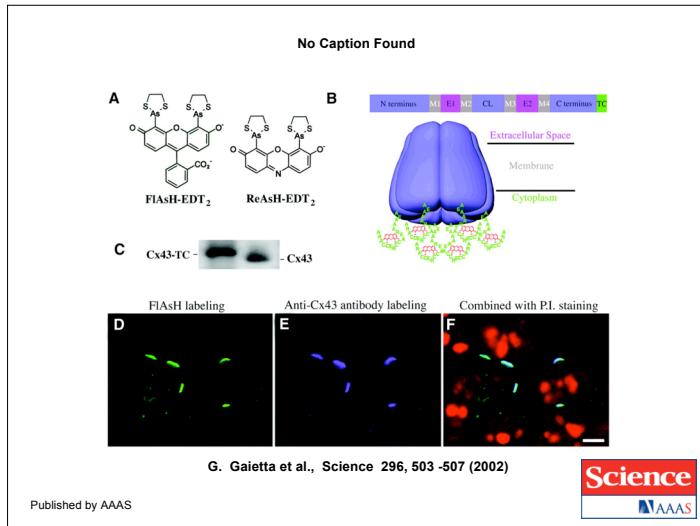
End of tract tracers



Types of fluorochromes

1. organic molecules (polyphenolic)
natural & synthetic
2. metal chelates (lanthanides)
3. semiconductor crystals (Q-dots)
4. fluorescent proteins
natural & engineered
5. expressible affinity reagents





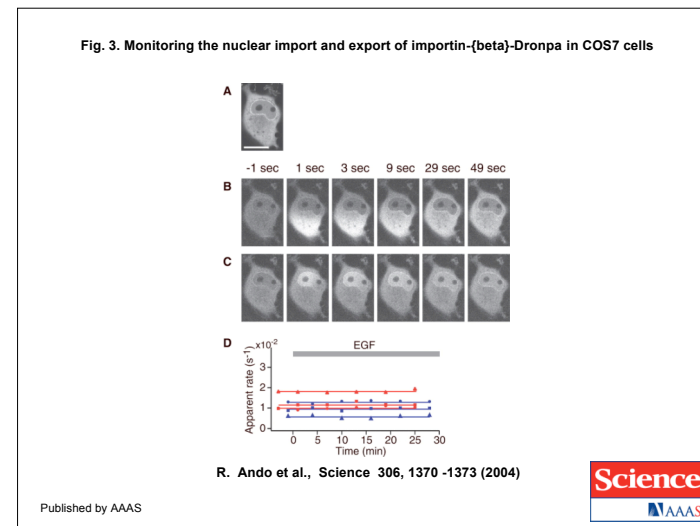
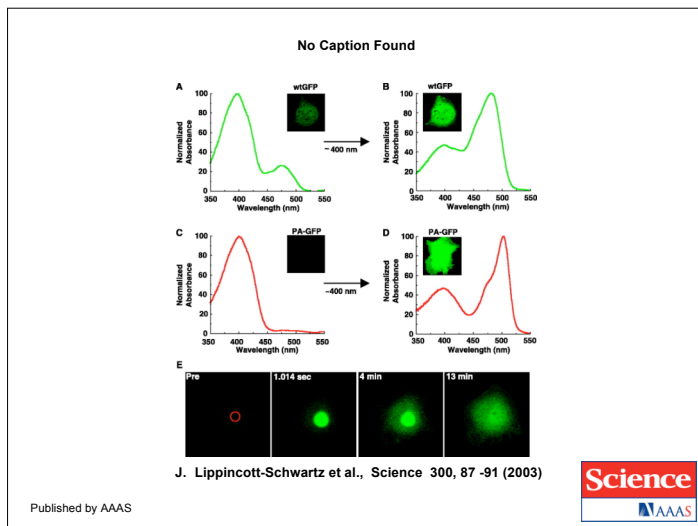
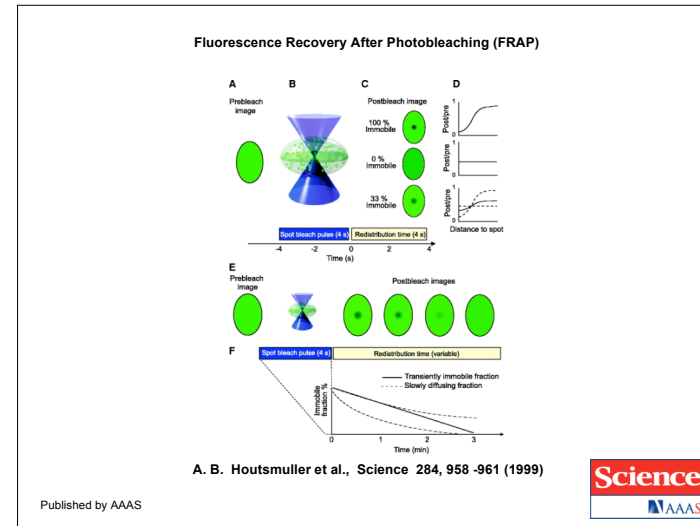
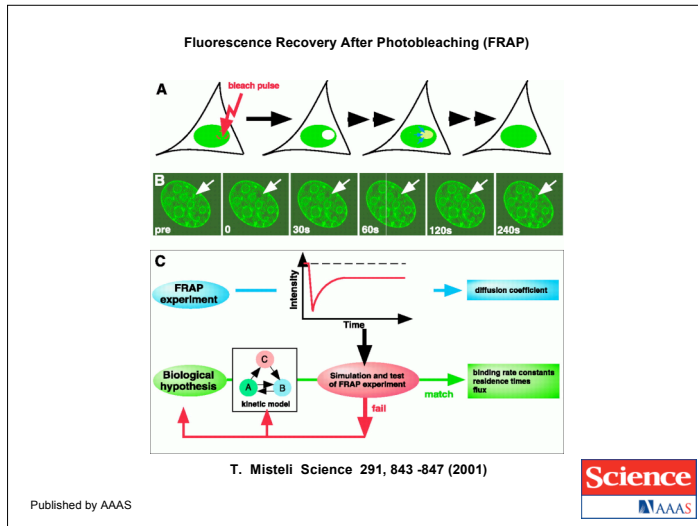
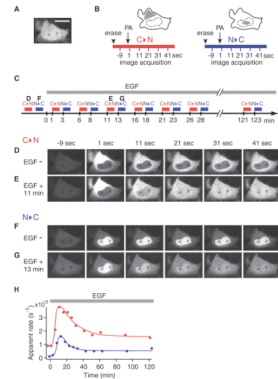


Fig. 2. Monitoring the nuclear import and export of ERK1-Dronpa in COS7 cells during stimulation with 100 ng/ml EGF

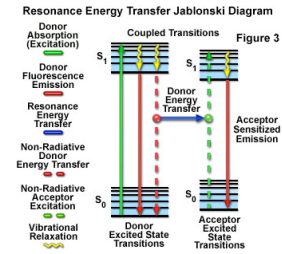


R. Ando et al., *Science* 306, 1370 -1373 (2004)



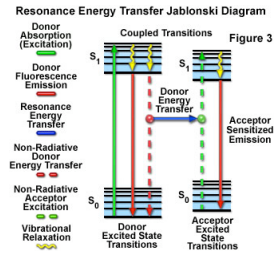
Published by AAAS

Foerster Resonance Energy Transfer



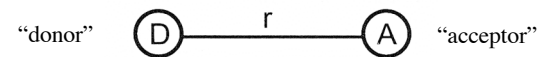
dipole - dipole interaction

Foerster Resonance Energy Transfer



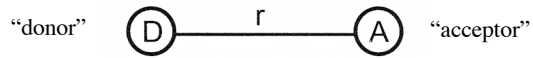
1. Distance between fluors
2. Spectral overlap
3. Orientation of dipoles

Foerster Resonance Energy Transfer



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

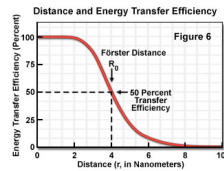
Foerster Resonance Energy Transfer



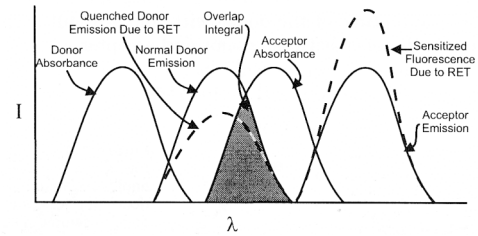
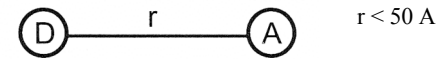
$$E = \frac{1}{1 + (r/R_0)^6}$$

efficiency

Foerster radius



Foerster Resonance Energy Transfer



Foerster Resonance Energy Transfer (FRET)

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

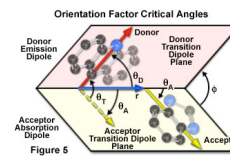
$$R_0^6 = 8.8 \times 10^{-28} \kappa^2 n^{-4} Q_0 J$$

Foerster Resonance Energy Transfer (FRET)

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

$$R_0^6 = 8.8 \times 10^{-28} \kappa^2 n^{-4} Q_0 J$$

dipole orientation factor



$$\kappa^2 \sim 2/3$$

Foerster Resonance Energy Transfer (FRET)

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

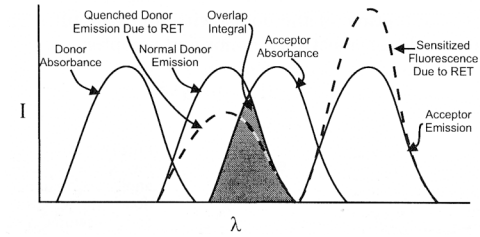
$$R_0^6 = 8.8 \times 10^{-28} \kappa^2 n^{-4} Q_0 J$$

refractive index

quantum yield of donor

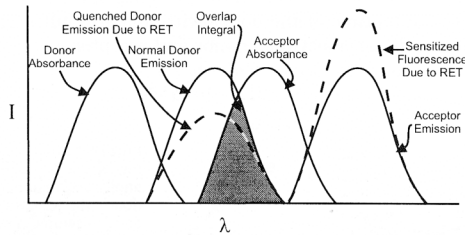
spectral overlap integral

Foerster Resonance Energy Transfer (FRET)



$$J = \int f_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda$$

Foerster Resonance Energy Transfer (FRET)



$$J = \int f_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda$$

normalized donor spectrum

molar extinction coef of acceptor

wavelength

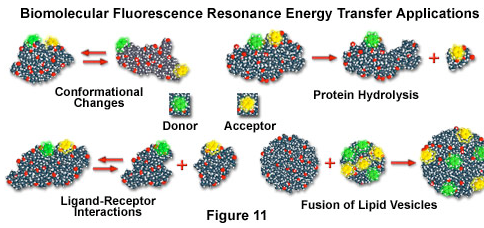
Foerster Resonance Energy Transfer (FRET)

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

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$$J = \int f_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda$$

Foerster Resonance Energy Transfer



Foerster Resonance Energy Transfer

FRET Detection of *in vivo* Protein-Protein Interactions

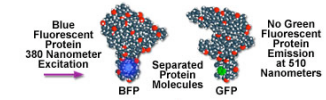
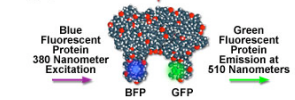


Figure 2 Intermolecular Association



Mitochondrial Protein-Protein Association with FRET

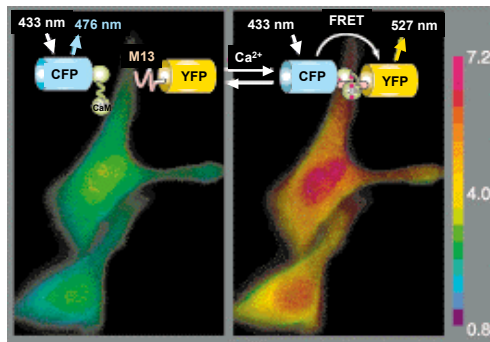
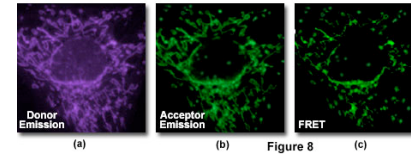
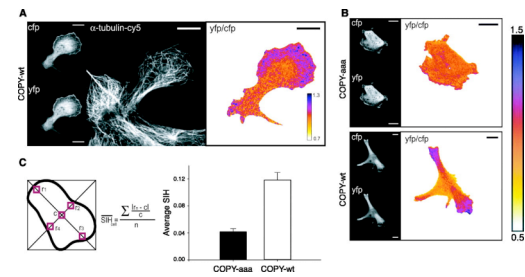


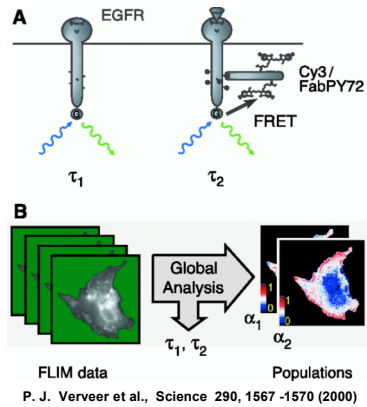
Fig. 2. Phosphorylation-dependent patterns of stathmintubulin interaction in *Xenopus* A6 cells



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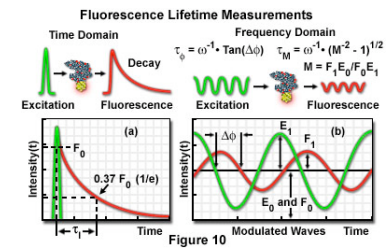
Fluorescence Lifetime Imaging



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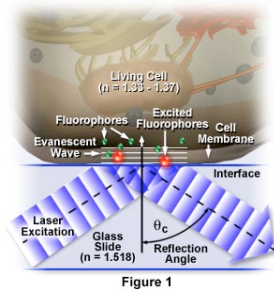


Foerster Resonance Energy Transfer

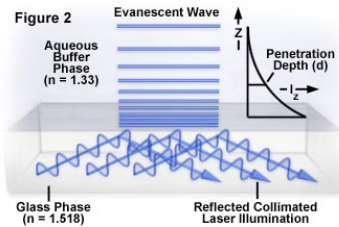


Total Internal Reflection Microscopy

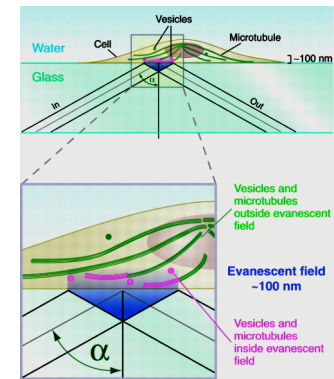
Total Internal Reflection Fluorescence



Evanescent Wave Exponential Intensity Decay



Total Internal Reflection Microscopy



D. J. Stephens et al., *Science* 300, 82-86 (2003)

Published by AAAS



