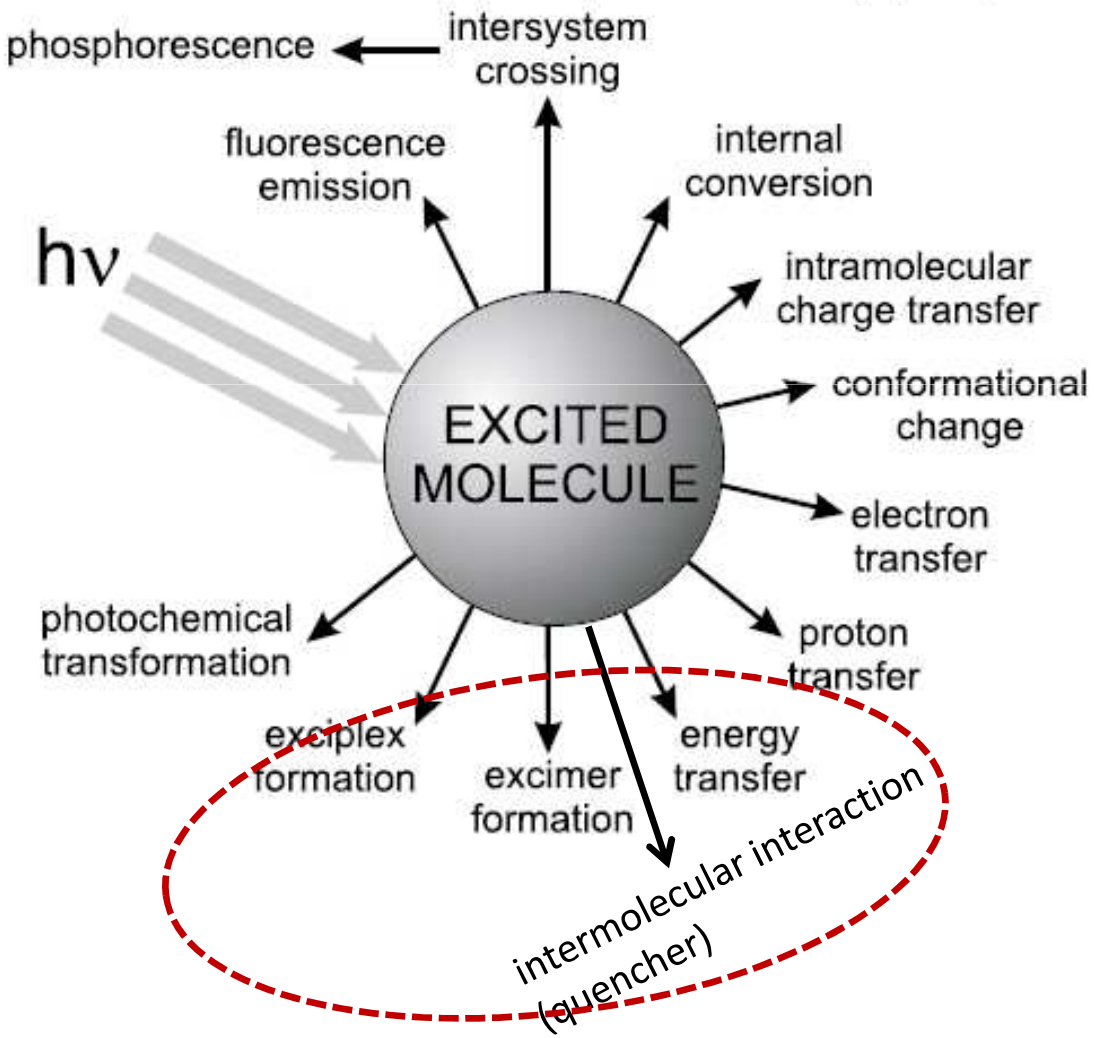
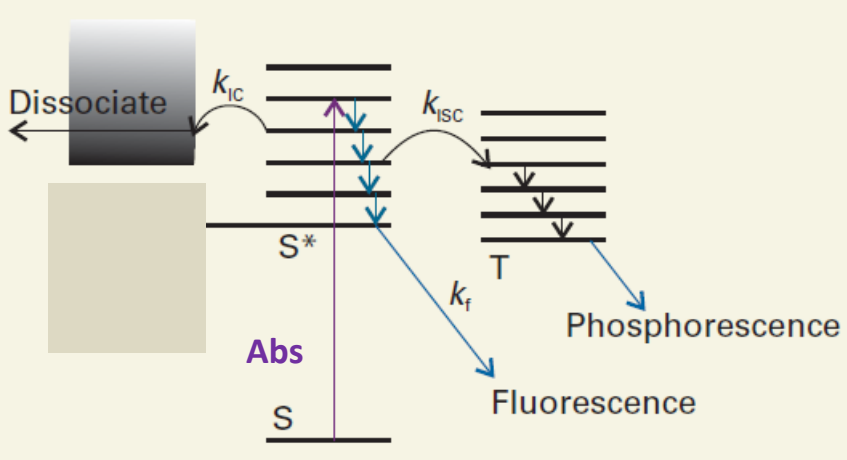
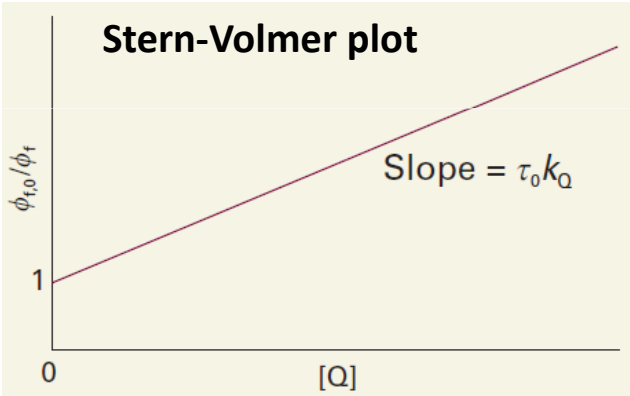
Pathways of decay of excited singlet states



Quenching





$$\text{Quenching:} \qquad S^* + Q \rightarrow S + Q \tag{11}$$

$$\frac{d[S^*]}{dt} = I_{abs} - (k_f + k_{ISC} + k_{IC} + k_Q[Q])[S^*] = 0 \tag{12}$$

The fluorescence quantum yield:

$$\phi_f = \frac{k_f}{k_f + k_{ISC} + k_{IC} + k_Q[Q]} \tag{13}$$

The quantum yield when [Q] = 0 is:

$$\phi_{f,0} = \frac{k_f}{k_f + k_{ISC} + k_{IC}} \tag{14}$$

$$\frac{\phi_{f,0}}{\phi_f} = \left(\frac{k_f}{k_f + k_{ISC} + k_{IC}}\right) \times \left(\frac{k_f + k_{ISC} + k_{IC} + k_Q[Q]}{k_f}\right) = 1 + \frac{k_Q}{k_f + k_{ISC} + k_{IC}}[Q]$$

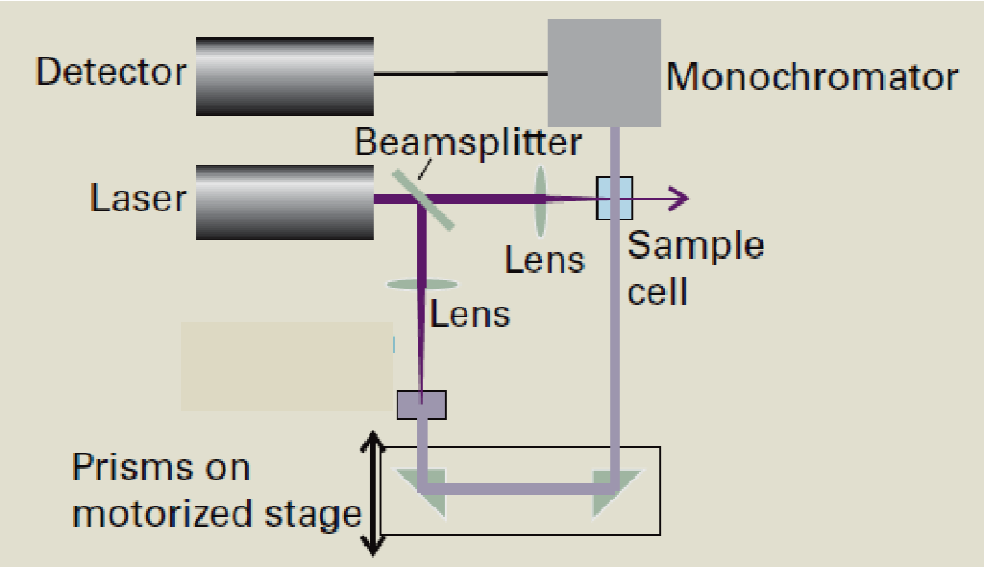
lifetime of excited singlet state $\tau_0 = \dfrac{1}{k_f + k_{ISC} + k_{IC}}$

The Stern–Volmer equation: $\dfrac{\phi_{f,0}}{\phi_f} = 1 + \tau_0 k_Q [Q]$ (15)

Determination of the lifetime of excited state (τ_0)

$$A + h\nu \rightarrow A^* \quad \text{(absorption)}$$

$$A^* \rightarrow A \quad \text{(emission)}$$



O_2: well-known quencher of fluorescence

Ground state: $^3\Sigma$

Excited singlet states: $^1\Delta$ and $^1\Sigma$

$$^1A^* + {}^3O_2(^3\Sigma) \rightarrow {}^1A + {}^1O_2(^1\Delta) \quad \text{or}$$

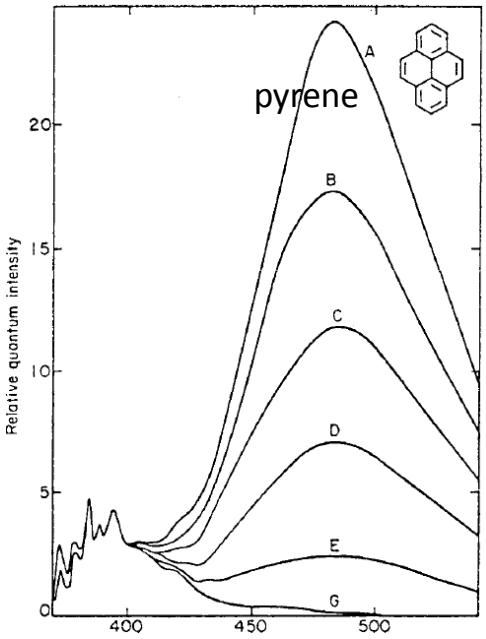
$$^3A^* + {}^3O_2(^3\Sigma) \rightarrow {}^3A + {}^1O_2(^1\Delta)$$

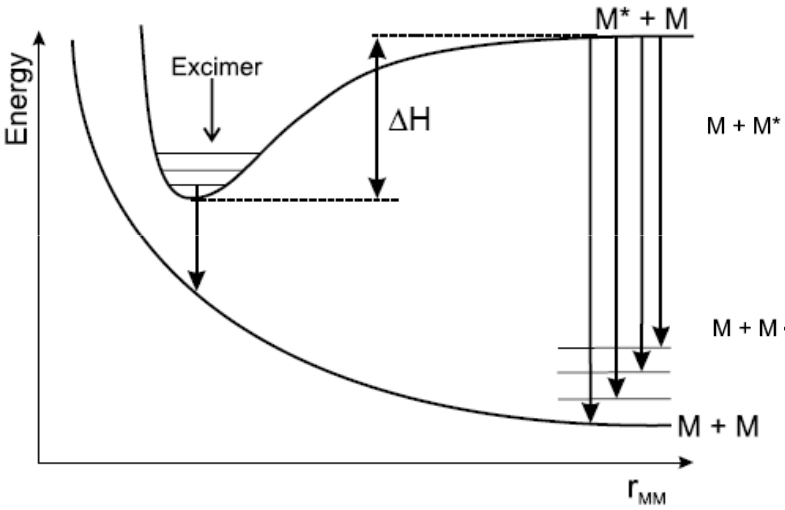
Excimer or Exciplex formation

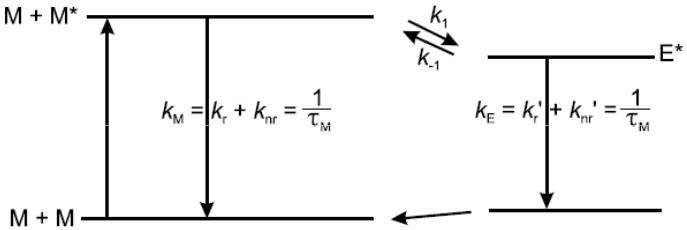
Excimers :'**exci**ted di**mer**'

Formation by collision: $^1M^* + {}^1M \rightleftarrows {}^1(MM)^*$

excitation energy is delocalized







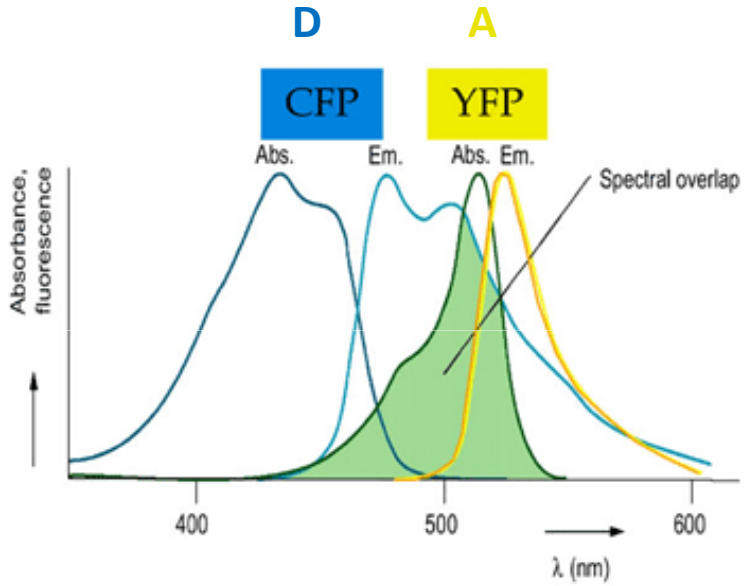
Exciplexes :'**exci**ted com**plex**'
D = donor
A = acceptor

$^1D^* + A \rightleftarrows {}^1(DA)^*$

$^1A^* + D \rightleftarrows {}^1(DA)^*$

Excitation energy transfer

$$D^* + A \rightarrow D + A^*$$



Radiative energy transfer:

- distances (D, A) larger than λ
- does not require any interaction between D and A
- depends on the spectral overlap and on the concentration

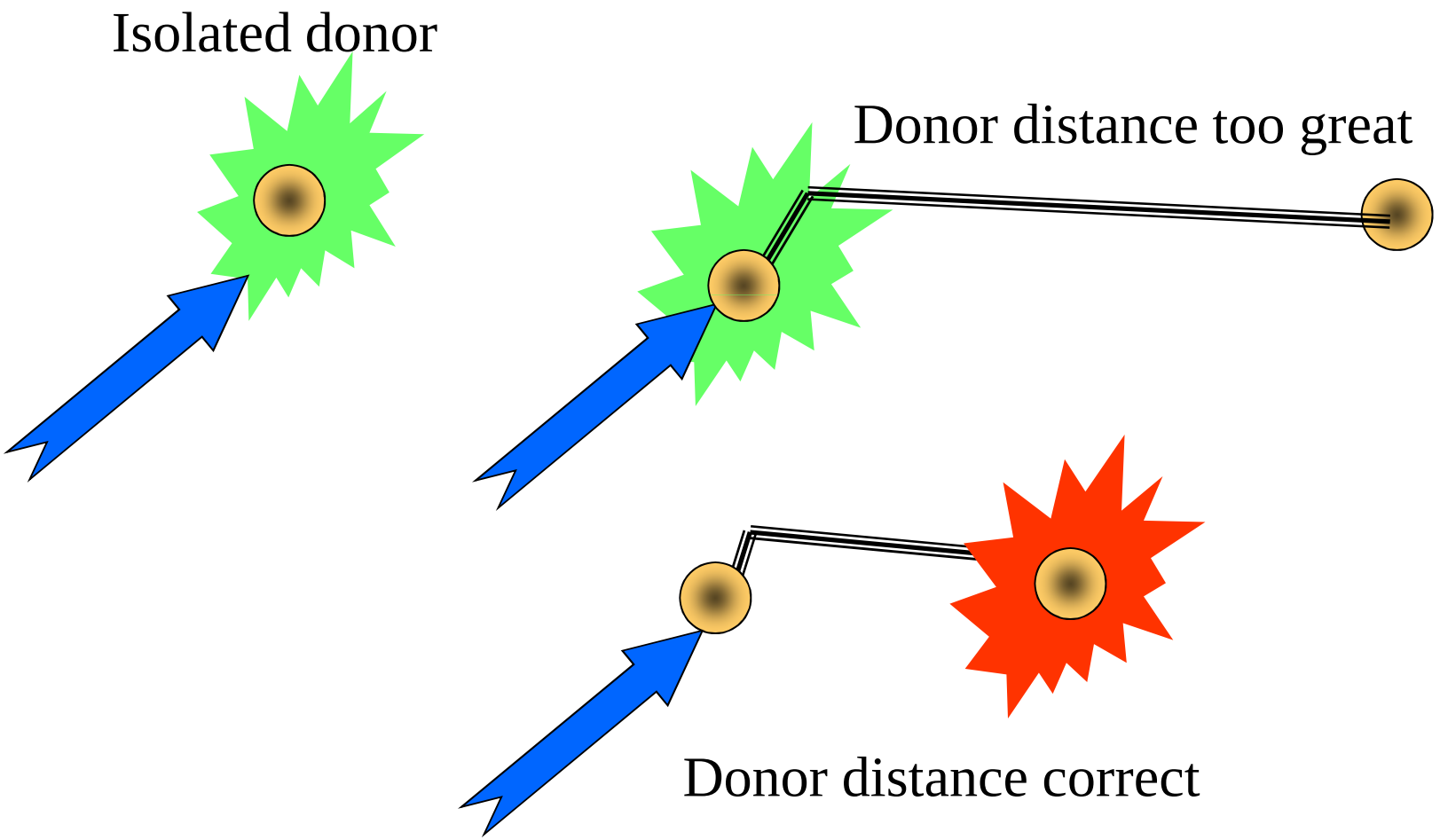
$$D^* \rightarrow D + h\nu$$

$$h\nu + A \rightarrow A^*$$

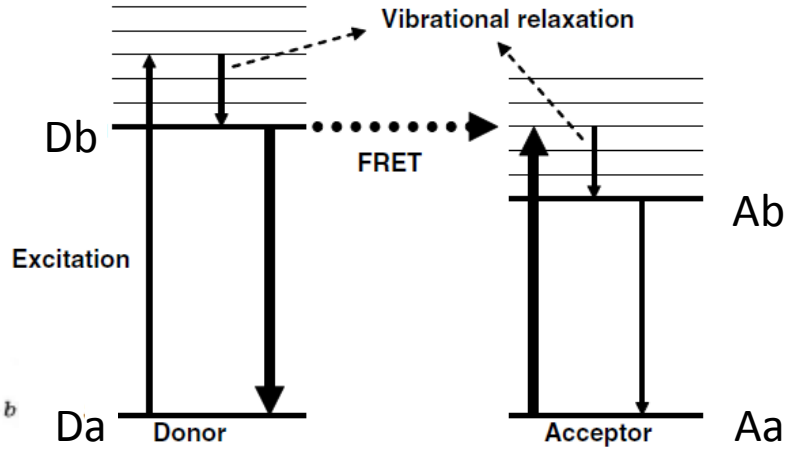
Non-radiative energy transfer:

- distances (80-100 Å) less than the wavelength
- requires interactions between D and A (dipole-dipole)
- photosynthesis

•Resonance energy transfer can occur when the donor and acceptor molecules are less than 100 Å of one another (preferable 20-50 Å)



Fluorescence resonance energy transfer (FRET)



Db+Aa $\rightleftarrows$ Da +Ab

Calculate the rate at which the state of D,A changes from

$$\Psi_{D_b}\Psi_{A_a} \text{ to } \Psi_{D_a}\Psi_{A_b}$$

The rate of energy transfer $k_T(v) \propto |\langle \Psi_{D_a}\Psi_{A_b}|\underset{\sim}{V}|\Psi_{D_b}\Psi_{A_a}\rangle|^2$ (16)

$$\underset{\sim}{V} = (\underset{\sim}{\mu}_D \cdot \underset{\sim}{\mu}_A)/R^3 - 3(\underset{\sim}{\mu}_D \cdot \mathbf{R})(\mathbf{R} \cdot \underset{\sim}{\mu}_A)/R^5$$

$$k_T(v) \propto |(\kappa/R^3)\langle \Psi_{D_a}\Psi_{A_b}|\underset{\sim}{\mu}_D||\underset{\sim}{\mu}_A|\Psi_{D_b}\Psi_{A_a}\rangle|^2$$

$$\underset{\sim}{V} = \kappa|\underset{\sim}{\mu}_D||\underset{\sim}{\mu}_A|/R^3 \quad (17)$$

κ geometry parameter

With (17) in (16): $k_T(v) \propto |(\kappa/R^3)\langle \Psi_{D_a}\Psi_{A_b}|\underset{\sim}{\mu}_D||\underset{\sim}{\mu}_A|\Psi_{D_b}\Psi_{A_a}\rangle|^2$

$$k_T(v) \propto (\kappa^2/R^6)|\langle \Psi_{D_a}|\underset{\sim}{\mu}_D|\Psi_{D_b}\rangle|^2|\langle \Psi_{A_b}|\underset{\sim}{\mu}_A|\Psi_{A_a}\rangle|^2$$

Absorption takes place at a single frequency ν: $|\langle \Psi_{A_b}|\underset{\sim}{\mu}_A|\Psi_{A_a}\rangle|^2 \propto \varepsilon_A v^{-1}$

$$D_{ab} \propto v^{-3}A_{ba} = v^{-3}\tau_S^{-1}$$

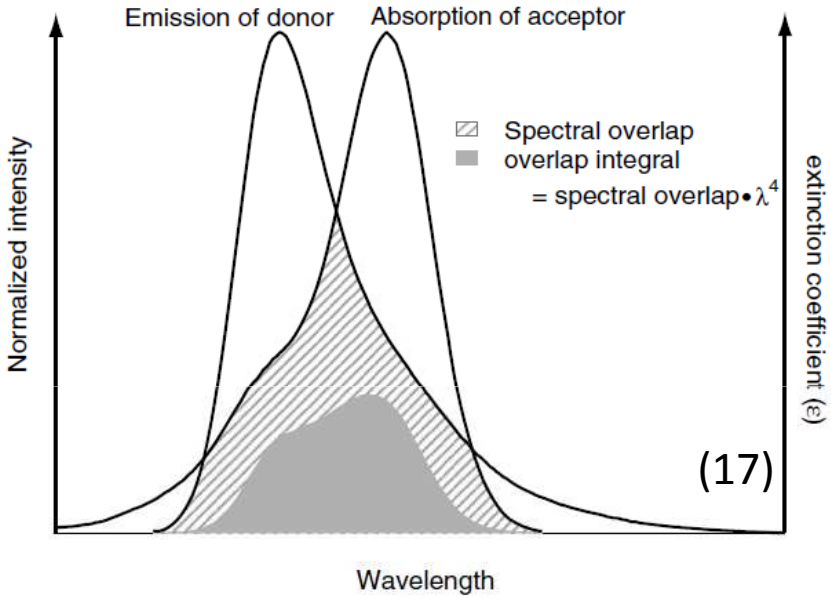
$\tau_S^{-1} = \phi_D/\tau_D$ $\Rightarrow$ $|\langle \Psi_{D_a}|\underset{\sim}{\mu}_D|\Psi_{D_b}\rangle|^2 \propto v^{-3}\phi_D/\tau_D$

FRET

The energy transfer rate: $k_T(v) \propto (\kappa^2/R^6)(\phi_D/\tau_D)\varepsilon_A v^{-4}$

Fluorescence of D and absorption of A occur over a range of frequencies:

$$k_T \propto (\kappa^2/R^6)(\phi_D/\tau_D)\int \varepsilon_A(v) f_D(v) v^{-4} dv = (\kappa^2 \phi_D/R^6 \tau_D) J$$



(17)

In a fluid medium, the true: interaction potential is V/n^2
n is the refractive index

Förster radius $R_0^6 = 8.79 \times 10^{-5} \cdot \frac{\Phi_D \cdot \kappa^2 \cdot J}{n^4}$ cm^6

R_0: The distance at which 50% energy transfer takes place

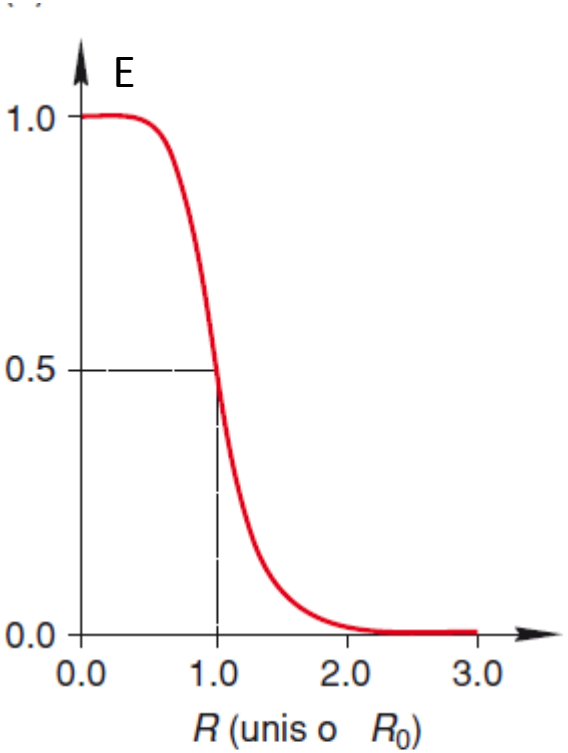
The energy transfer rate: $k_T = (R_0/R)^6 \tau_D^{-1}$ (18)

FRET efficiency E: $E = k_T / (k_T + \tau_D^{-1})$ (19)

$$E = \frac{R_0^6}{R_0^6 + R^6} \quad (20)$$

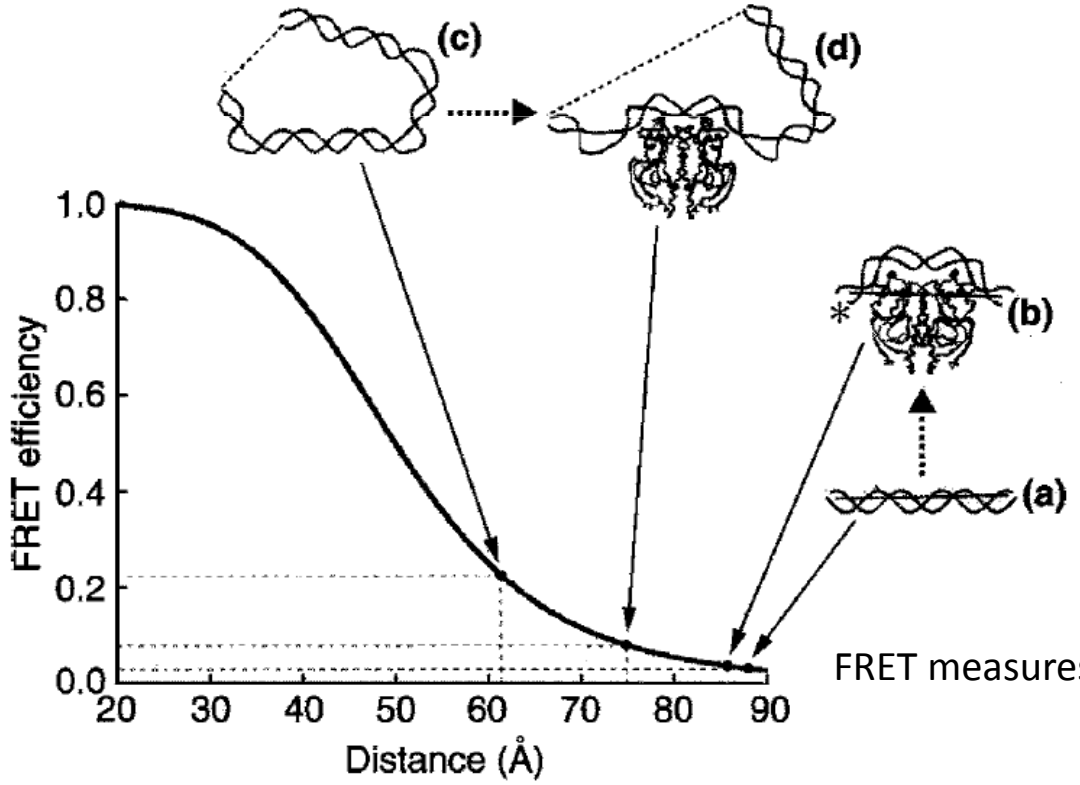
$$E = \frac{R_0^6}{R_0^6 + R^6} \qquad (20)$$

spectroscopic ruler
in the range 10-100 Å



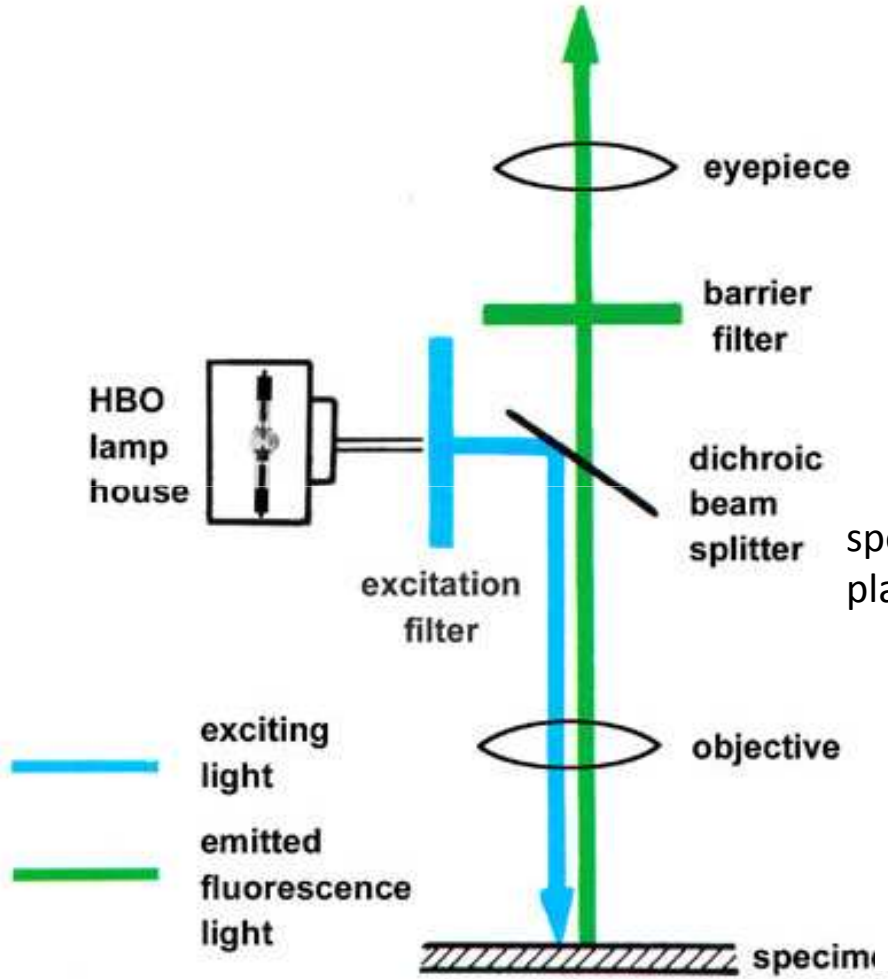
$0.5R_0 < R < 1.5R_0$

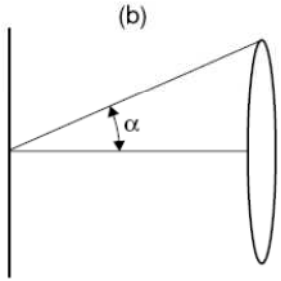
k^2 : relative orientation of the transition dipoles of D and A.
= 2/3 isotropic dynamic average



FRET measures distances in proteins

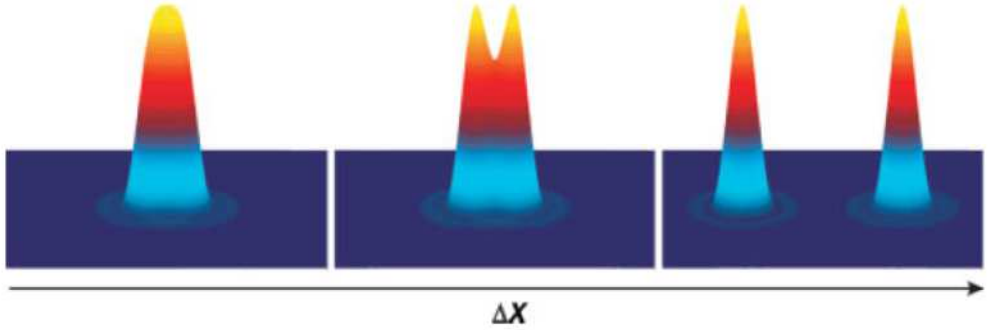
Fluorescence microscopy





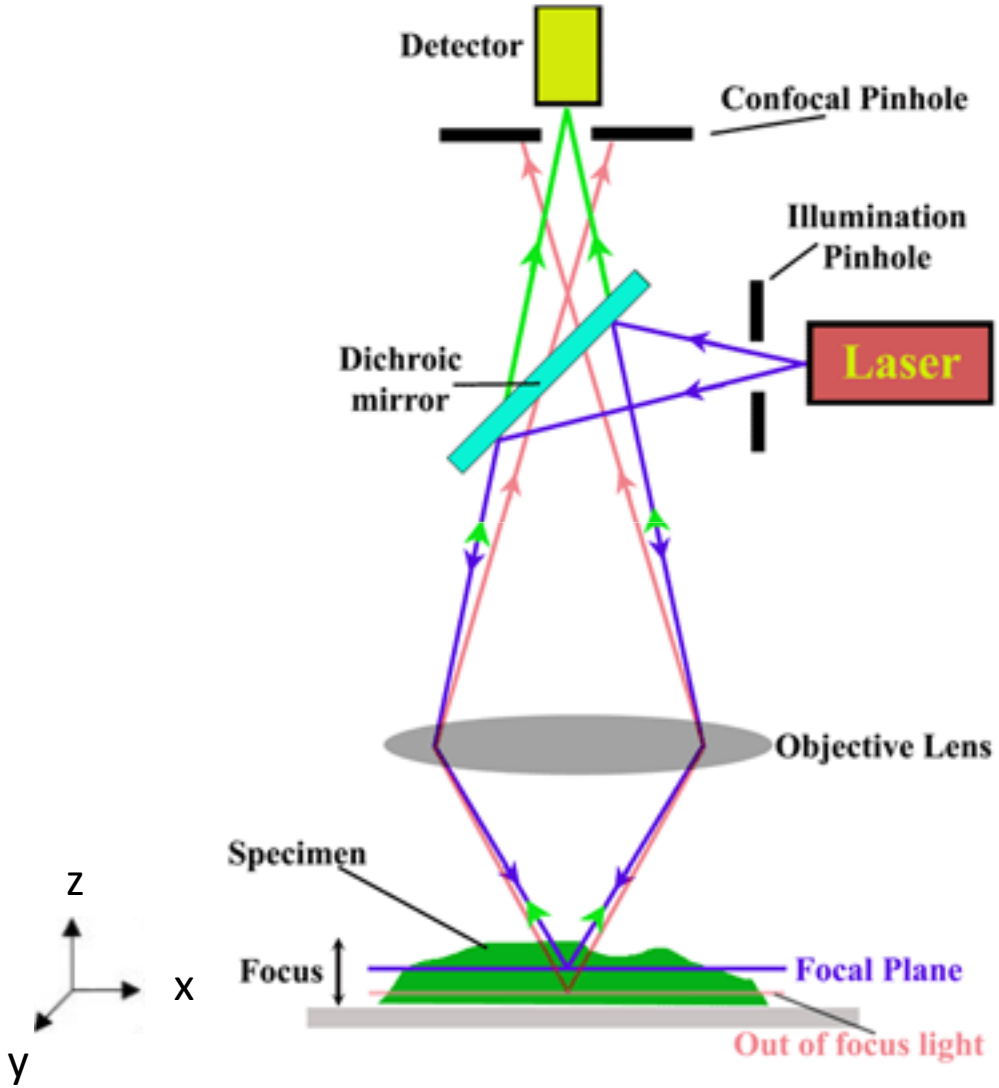
$$r = \frac{0.6\lambda}{n \sin\alpha}$$

lateral resolution (Rayleigh):
200 - 300 nm for visible radiation



axial resolution: 700 nm

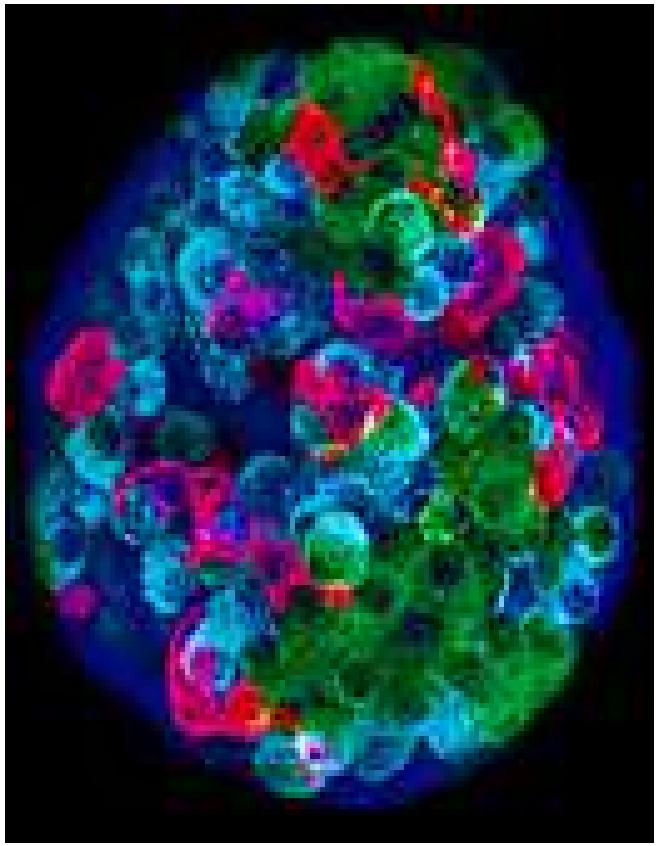
Fluorescence confocal microscopy: 3D images



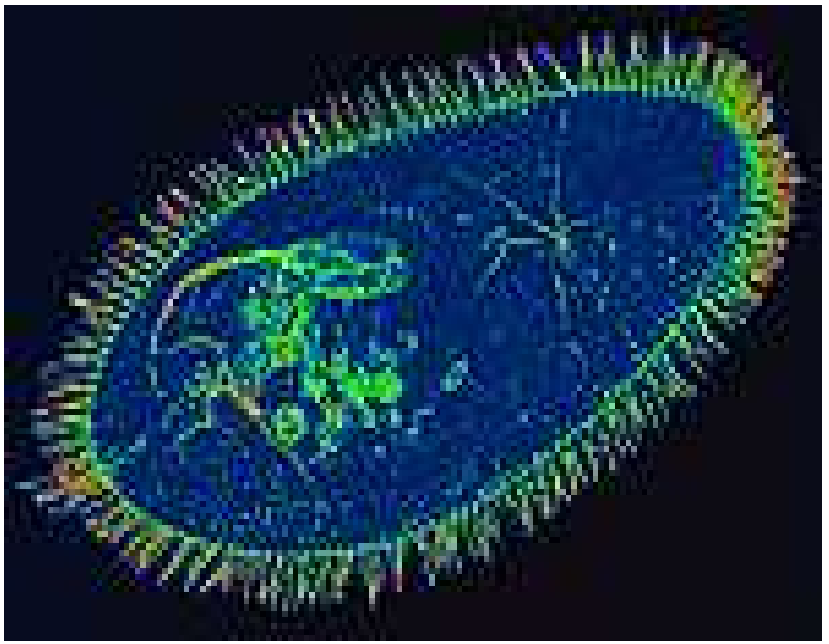
Advantage of confocal microscopy:
optical sectioning: 3D images of specimens

Disadvantage:
higher amount of photobleaching

Applications

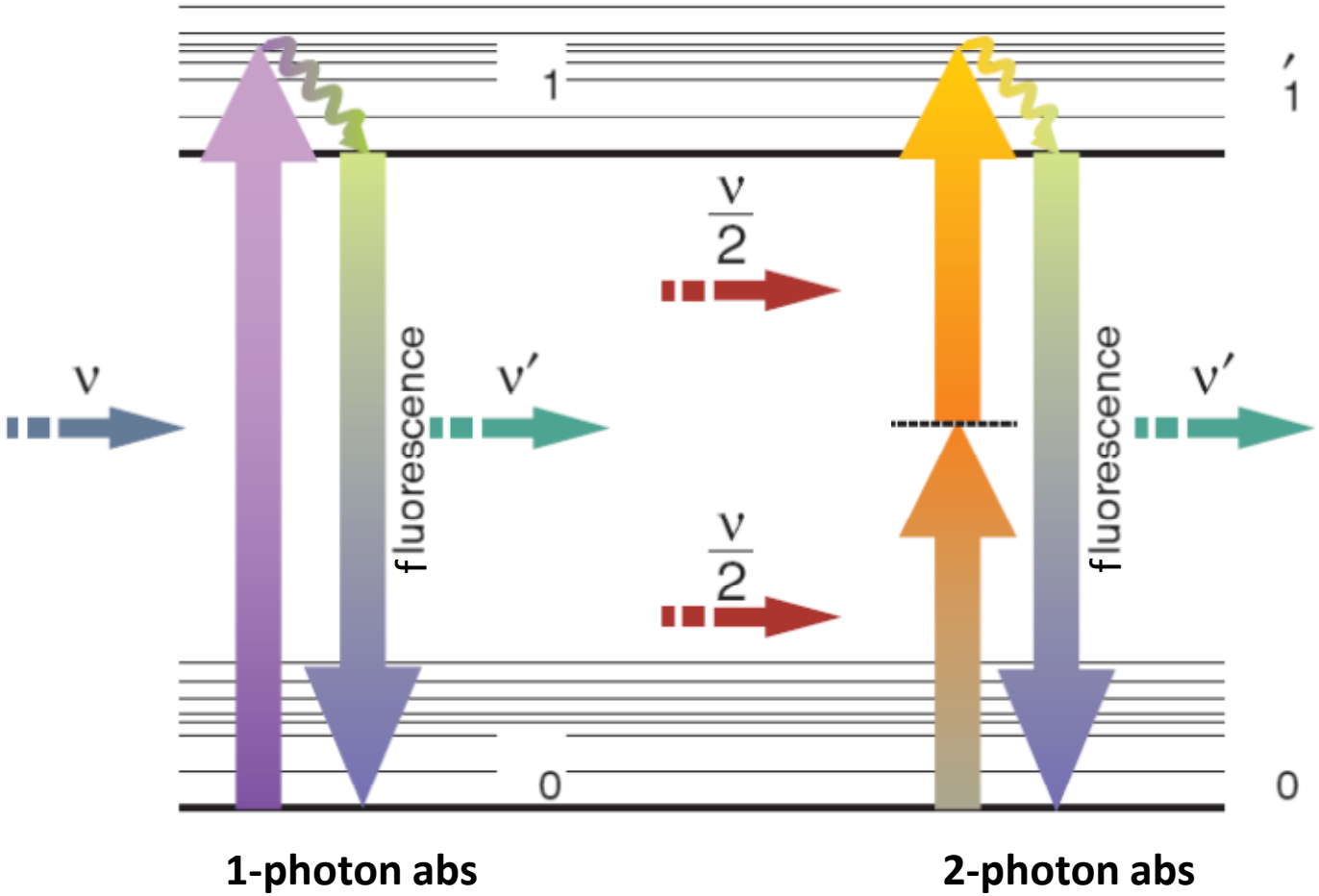


Projection of 25 optical sections
of a triple-labeled rat islet of Langerhans,
acquired with a krypton/argon laser.



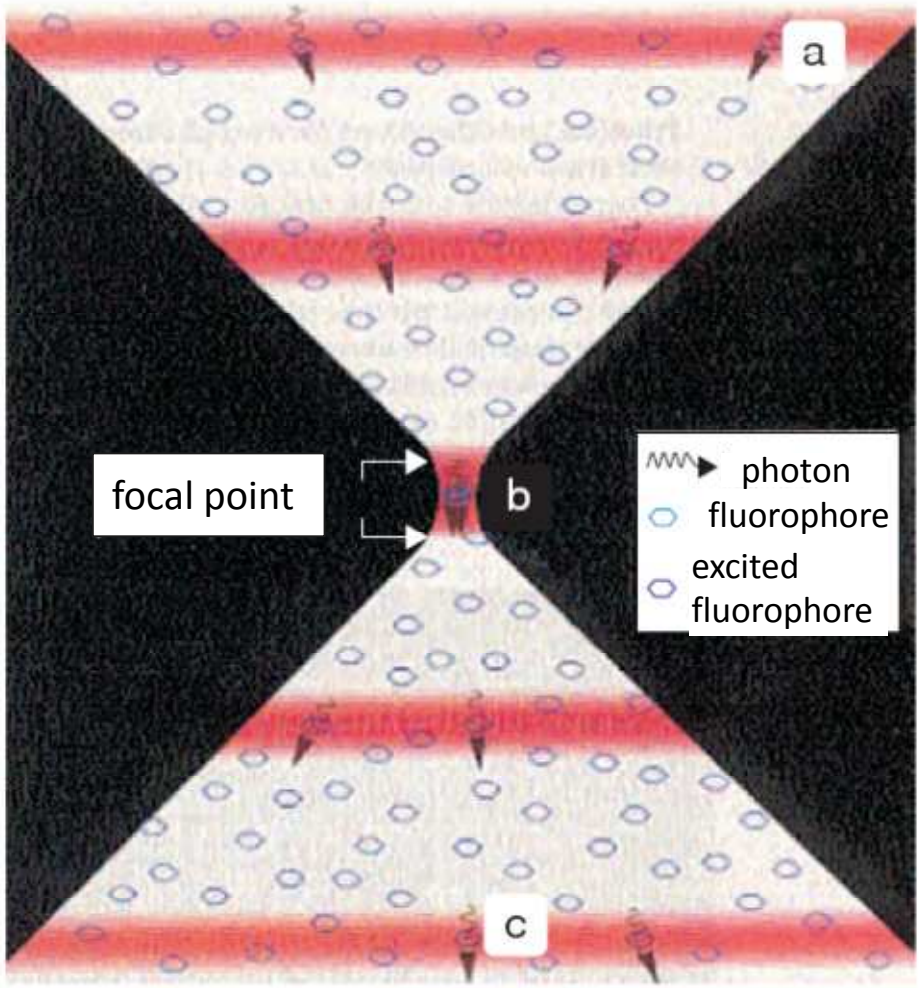
Paramecium:
thousands of cilia and internal microtubular structures.

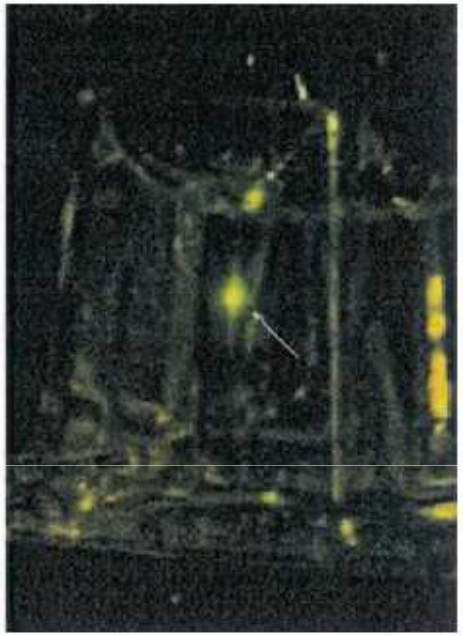
Two-photon excitation fluorescence microscopy: 3D images



The cross-sections for two-photon absorption: 10^{-50} cm^4 s $photon^{-1}$ $molecule^{-1}$ (rhodamine B).
Used lasers:*Titan:Sapphire laser (TiSa)*

Two-photon excitation fluorescence microscopy: 3D images





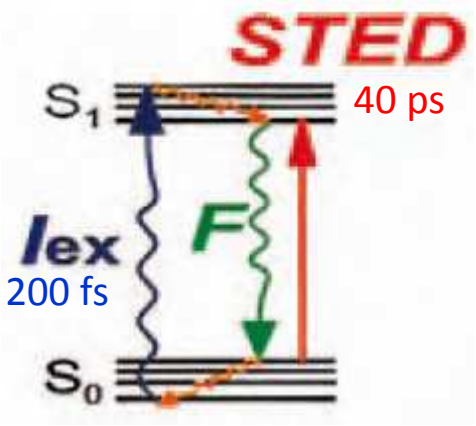
Advantages:

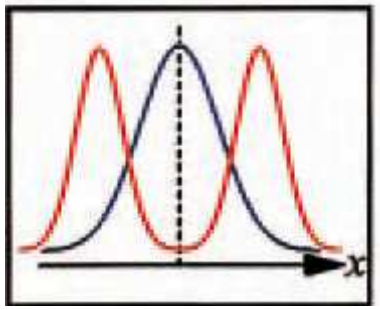
- no out-of-focus photobleaching
- excitation beam is not attenuated by out-of-focus absorption,(increased penetration depth)

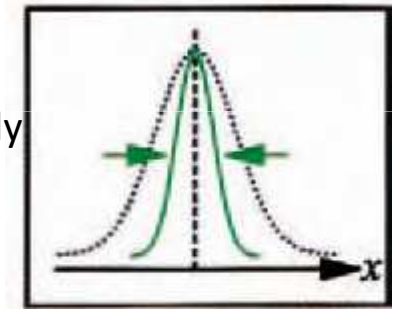
Disadvantage:

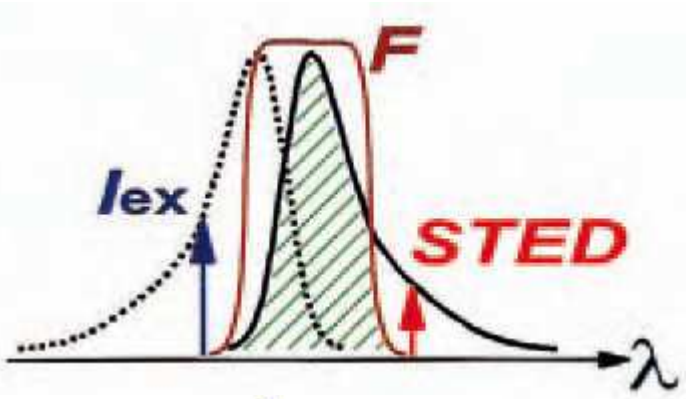
- lower spatial resolution then in confocal imaging (longer wavelength!)

Stimulated emission depletition (STED) microscopy: resolution beyond the Rayleigh limit







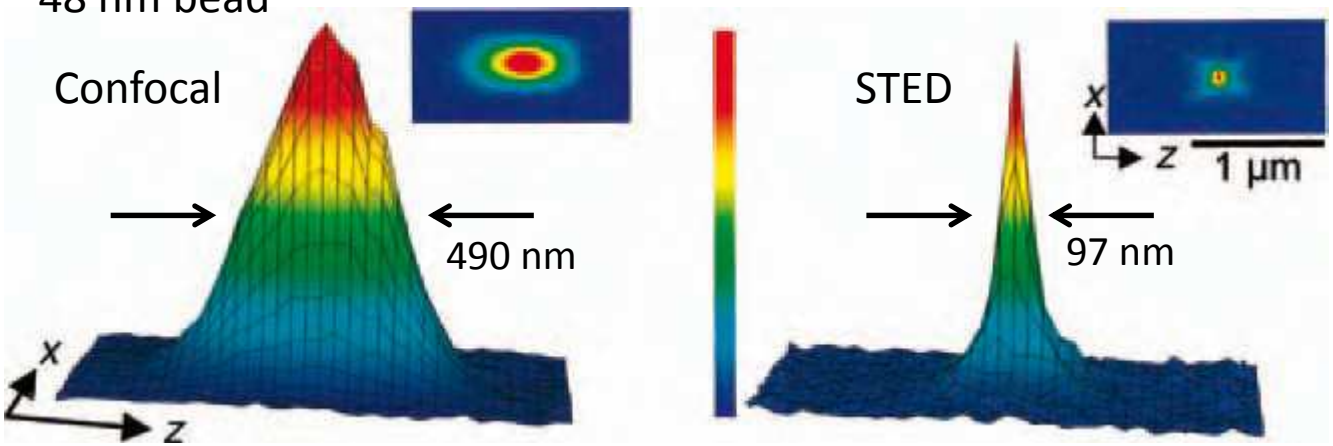


$$\Delta x \approx \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_{sat}}}$$

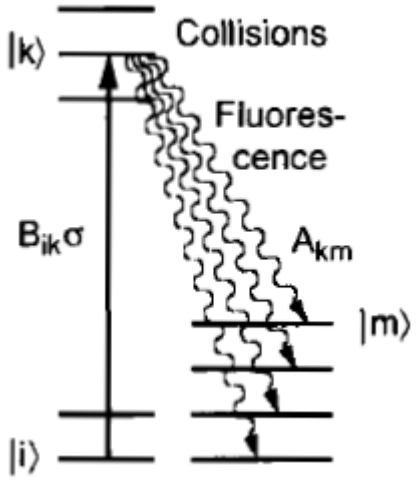
The fluorescence is confined spatially to sub-diffraction dimensions

I_{sat} :
intensity at which the fractional population of the excited state is depleted to 1/e
30 MW cm^{-2} in the visible range

48 nm bead



Laser induced fluorescence



The number of photons absorbed per second along the path length Δx : $n_a = N_i n_L \sigma_{ik} \Delta x$

n_L : *number of incident laser photons per second*
σ_{ik}: *absorption* cross section per molecule
Ni : density of molecules in the absorbing state *i*

The number of fluorescence photons emitted per second from the excited level:

$$n_{Fl} = N_k A_k = n_a \eta_k$$

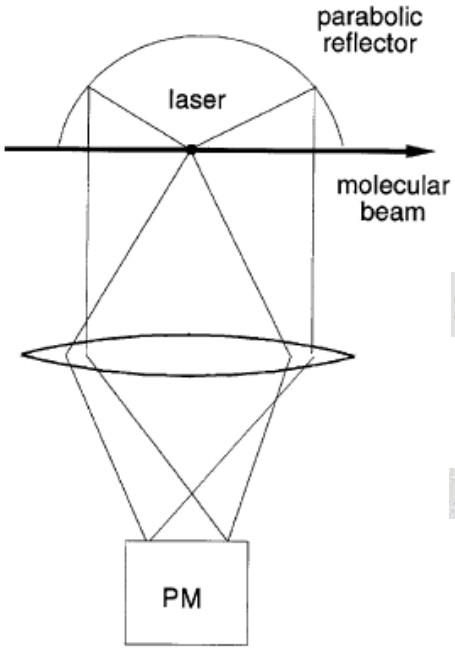
η_k quantum efficiency of the excited state

The number n_{pe} *of photoelectrons counted per second:*

$$n_{pe} = n_a \eta_k \eta_{ph} \delta = (N_i \sigma_{ik} n_L \Delta x) \eta_k \eta_{ph} \delta$$

$$\delta = d\Omega / 4\pi$$

η_{ph} quantum efficiency of the photocathode (0.2)



Ex:

$\eta_{ph} = 0.2$ $\delta = 0.1$ $d\Omega = 0.4\,\pi$ $n_{pe} = 100$ counts/s $\eta_k = 1$

$$n_a = 5 \times 10^3 \text{ /s}$$

laser power of 1 W at the wavelength $\lambda = 500$ nm $n_L = 3 \times 10^{18}$ /s

$$(I_0 - I_{trans}) / I_0 = 10^{-15}$$

Single molecule detection

spontaneous lifetime τ: 10^{-8} *s*
travel time T through the laser beam: 10^{-5} *s*
excitation-fluorescence cycles $n = T/\tau$: *500* photons / molec

→ **single molecule detection**