Fluorescence Theory

Fluorescence Basics

- Fluorescence definition
- The fluorescence process
- Molar extinction coefficients
- Quantum yield
- Brightness
- Fluorescence lifetime
- FRET

Fluorescence

Spontaneous emission of radiation (luminescence) from an excited molecular entity with the formation of a molecular entity of the same spin multiplicity*

***From International Union of Pure and Applied Chemistry Glossary of Terms used in Photochemistry (http://www.unibas.ch/epa/glossary/glossary/htm)**

Fluorescence Definition

- Fluorescence can be more simply defined as

  "the molecular absorption of light energy (photon) at one wavelength and its re-emission at another, usually longer, wavelength"

- Molecules which are able to absorb light are known as chromophores

- Molecules which are able to absorb and emit light are known as fluorochromes or fluorophores

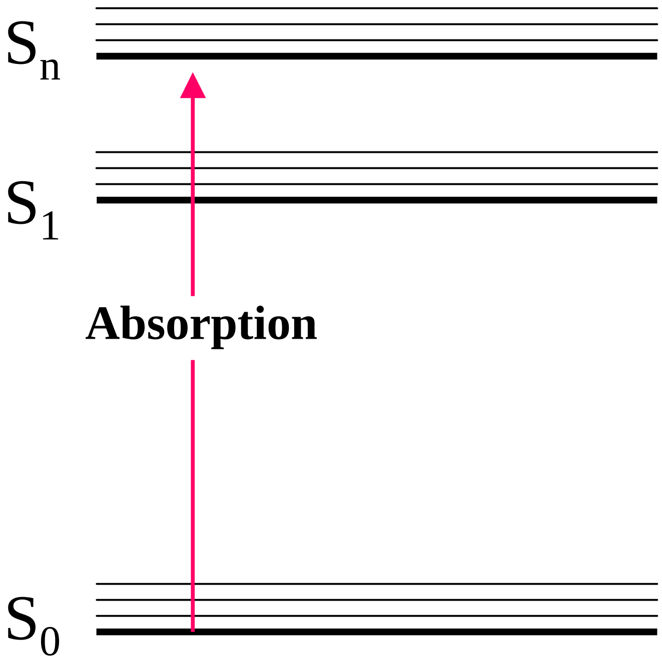
The Fluorescence Process

- The fluorescence process can be broken down into three phases

1. **Excitation** - absorption of light of an appropriate wavelength by fluorophore
2. **Excited state** - fluorophore undergoes vibrational and conformational changes
3. **Emission** - photon of light is emitted

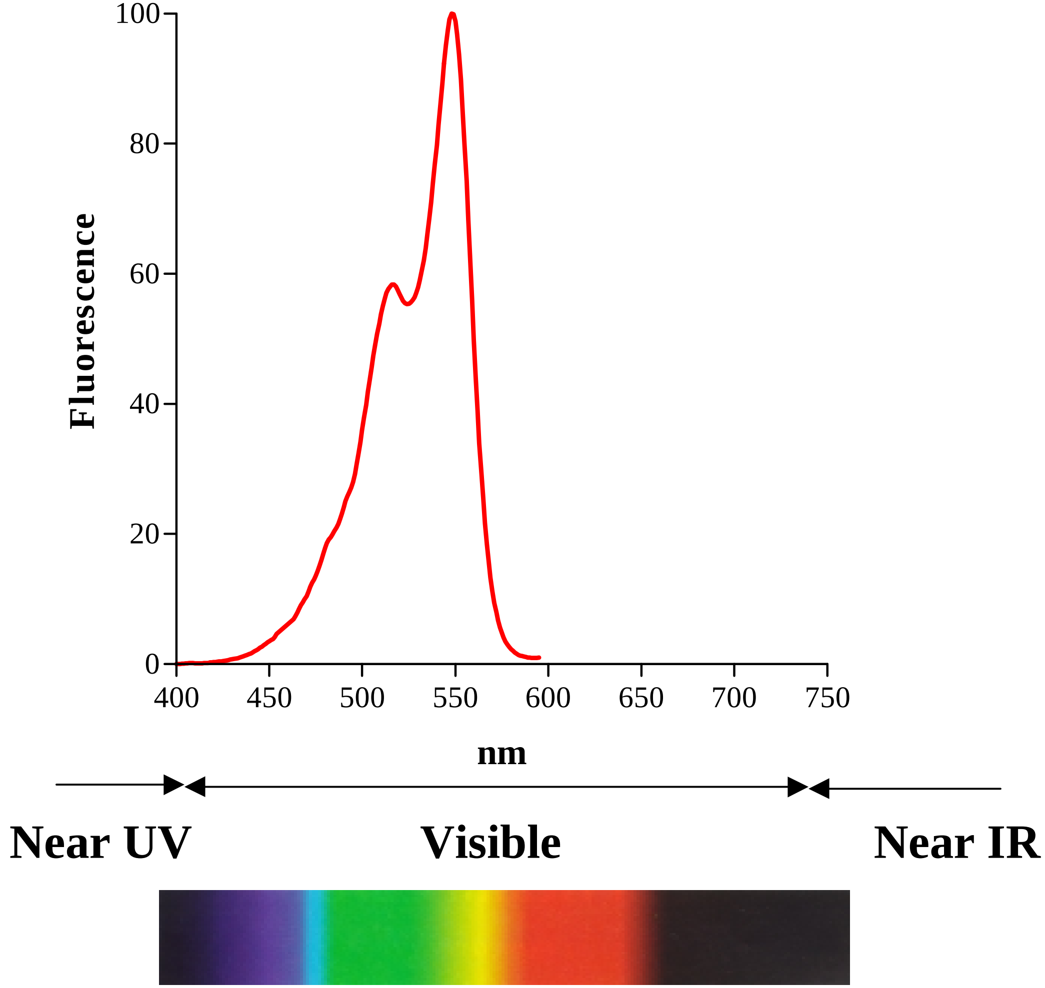
- The fluorescence process is cyclical therefore a fluorophore can be excited repeatedly

Excitation



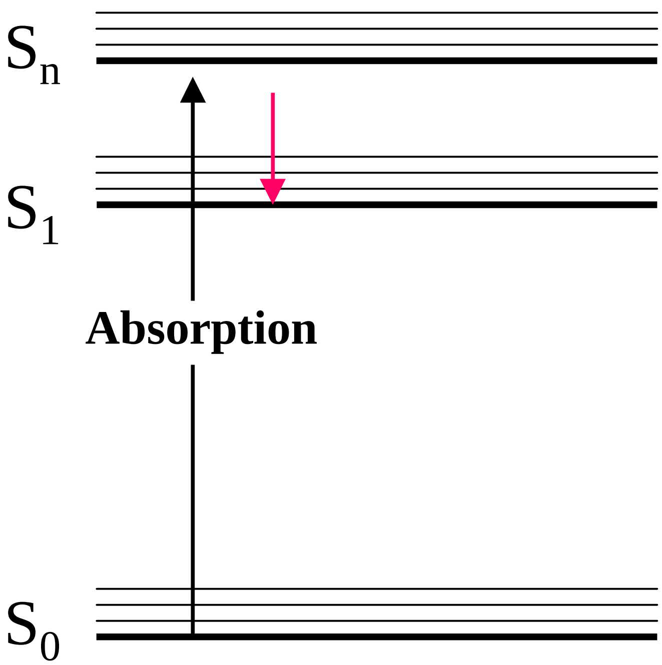
Absorption of a photon and thus excitation to S_1 or S_n respectively

Cy3 Excitation Spectrum



R&D

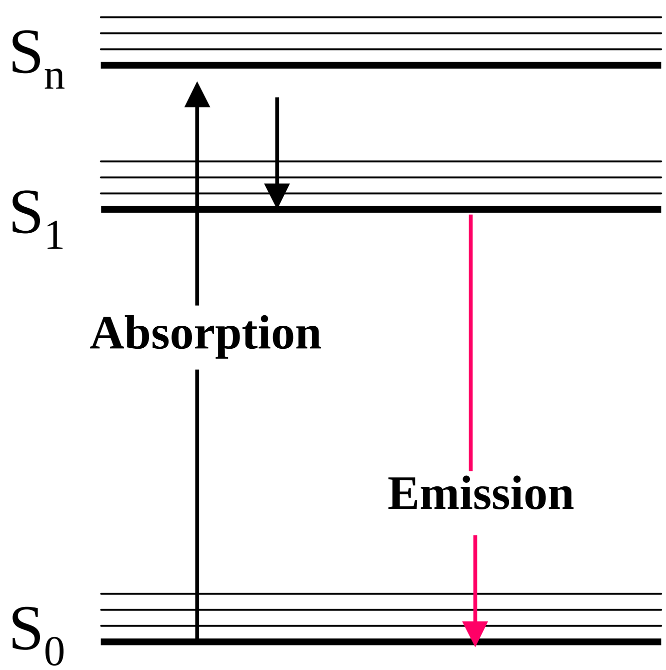
Excited State



Absorption of a photon and thus excitation to S_1 or S_n respectively

Radiationless energy loss to return to S_1V_1

Fluorescence Emission

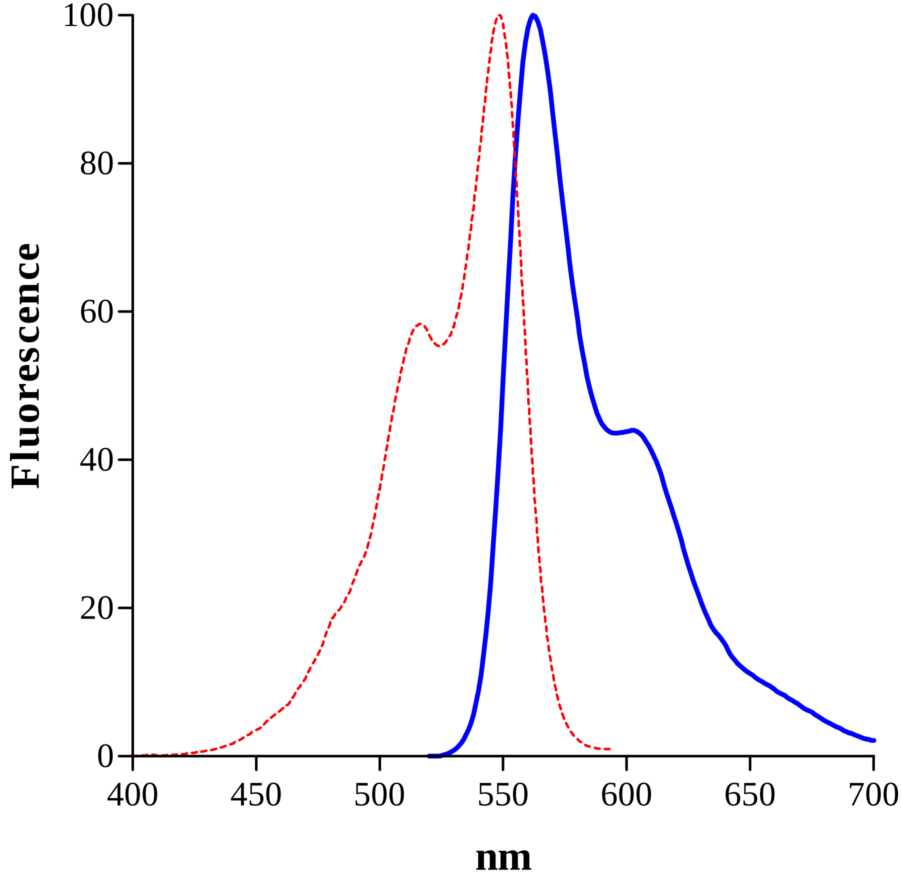


Absorption of a photon and thus excitation to S_1 or S_n respectively

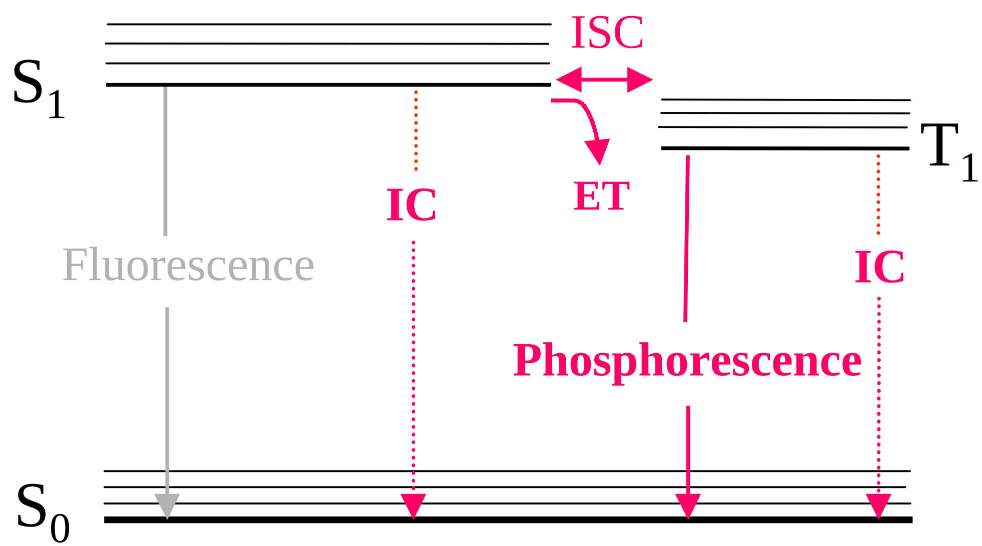
Radiationless energy loss to return to S_1

Reconversion to S_0 from S_1 with emission of radiation - fluorescence

Cy3 Emission Spectrum



Competing Processes



ET Energy transfer to a nearby chromophore

IC Radiationless internal conversion to the ground state

ISC Intersystem crossing to triplet state
energy dissipated via radiative (phosphorescence) or non-radiative pathways

Extinction Coefficient (ε)

- The molar extinction coefficient is a measure of the light absorbing capacity of a dye - dyes with large molar extinction coefficients are efficient absorbers

- The molar extinction coefficient is required when determining the concentration of a dilute solution of fluorophore using the Beer-Lambert law

$$A = \varepsilon c l$$

Where A is absorbance

ε is molar extinction coefficient

c is the concentration of the absorbing species

l is the absorption path length

Quantum Yield (ϕ)

- The fluorescence quantum yield is the ratio between the number of fluorescence photons emitted and the number of photons absorbed:

$$\phi = \frac{\text{number of photons emitted}}{\text{number of photons absorbed}}$$

Brightness

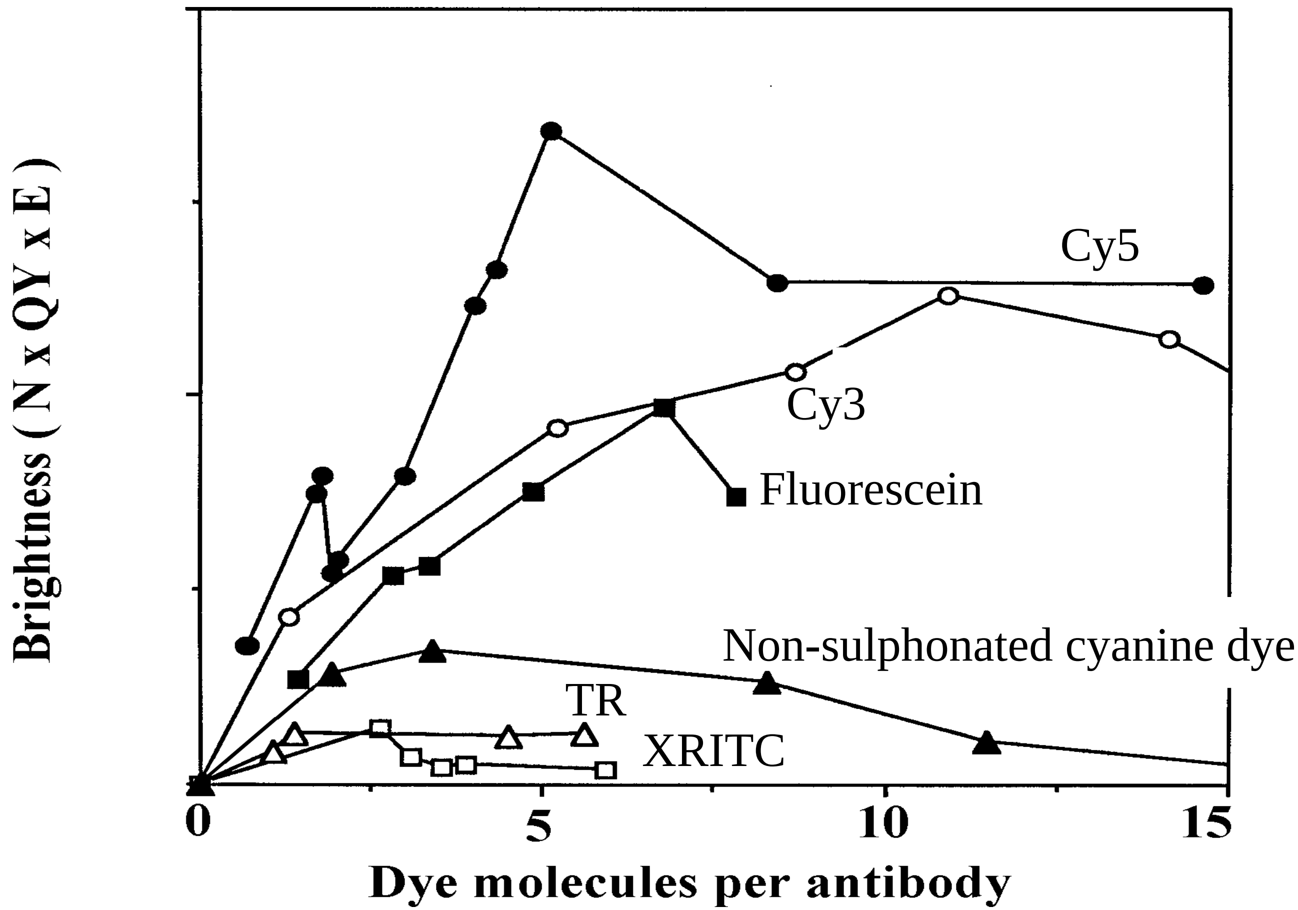
- Brightness is proportional to the product of the extinction coefficient and quantum yield

Brightness } εϕ

- The brightness of a fluorophore labelled molecule is proportional to the extinction coefficient, quantum yield and number of dyes per molecule

Brightness } nεϕ

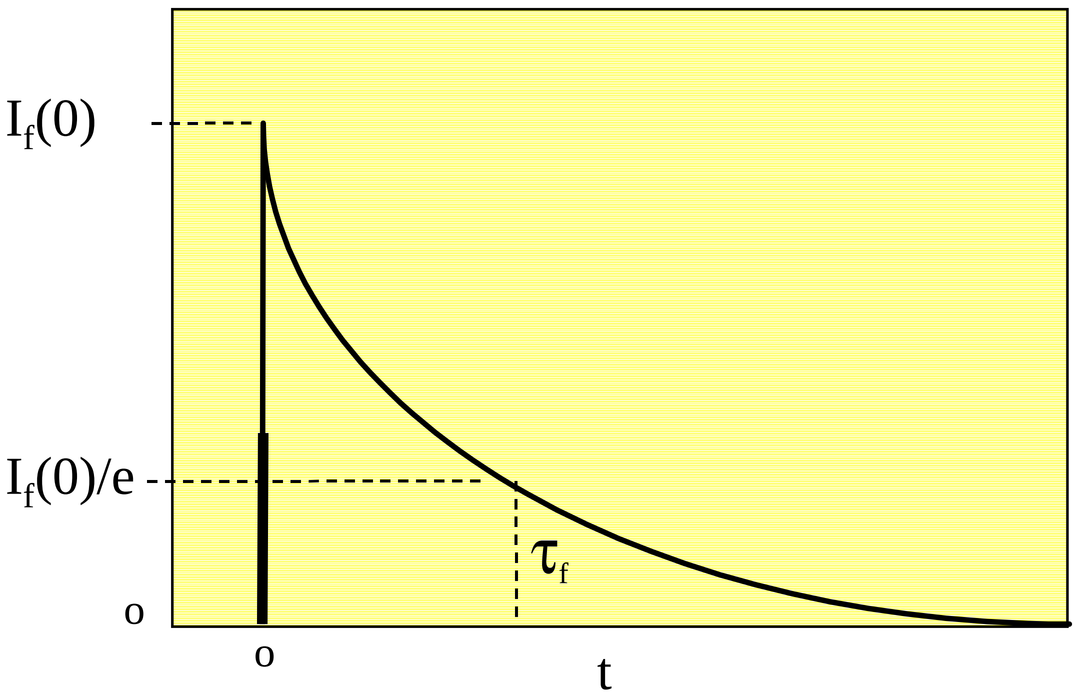
Relative Brightness



Fluorescence Lifetime (τ)

- The fluorescence lifetime is the mean time spent in the excited state
- Natural or intrinsic fluorescence lifetime (τ_f)
  - Theoretical
- The excited state fluorescence lifetime (τ_{ex})
  - Measured value
- Excited state lifetimes can change with changes in fluorophore environment

Fluorescence Lifetime (τ)

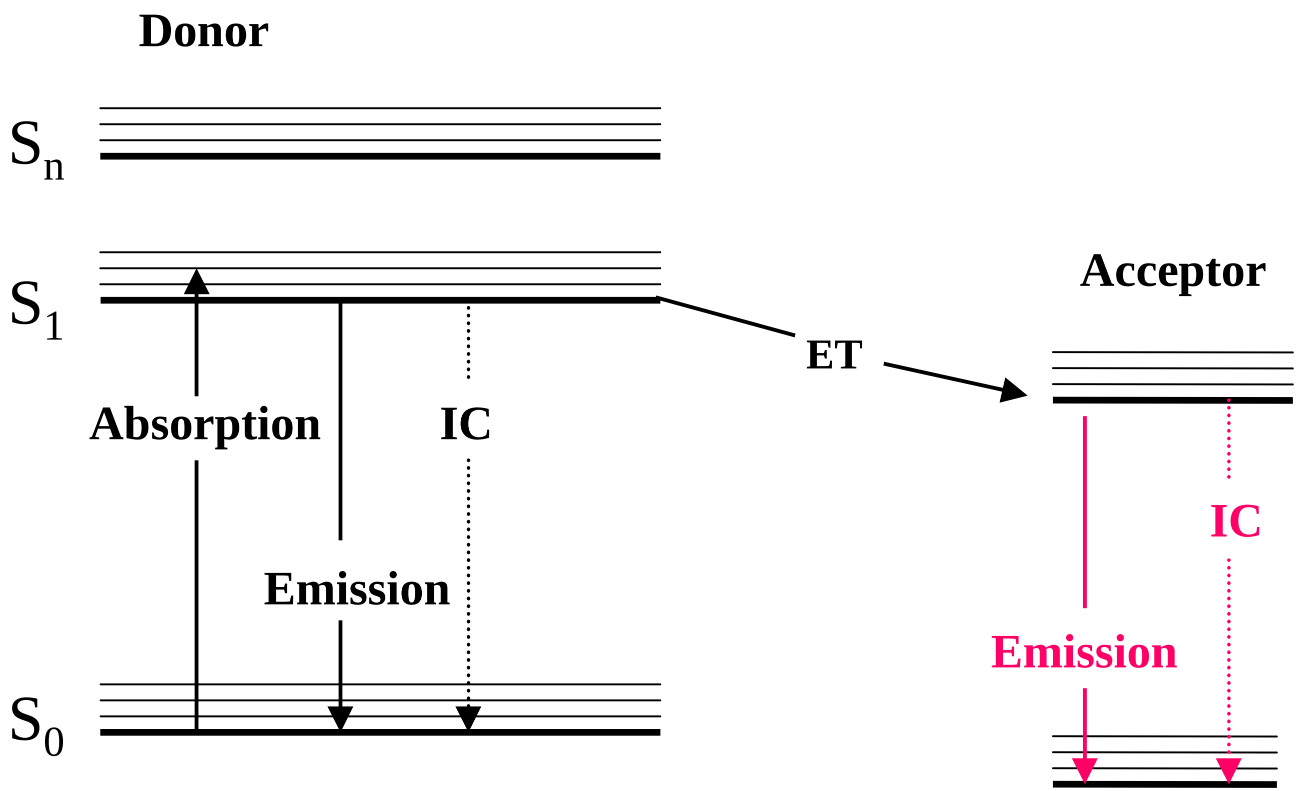


FLUORESCENCE
DECAY CURVE

FRET

- Is a through space transfer of excitation energy from a donor fluorophore to an acceptor
- Can occur over distances of 10 - 100Å (1 - 10nm)
- The donor and acceptor must be spectrally related
- There is no emission of light by the donor
- The acceptor may or may not be fluorescent

Fluorescence Resonance Energy Transfer



Energy Transfer Efficiency (*E*)

$$E = \left[\frac{R_0^6}{R_0^6 + r^6} \right]$$

R_0 = Distance at which energy transfer efficiency is 50%

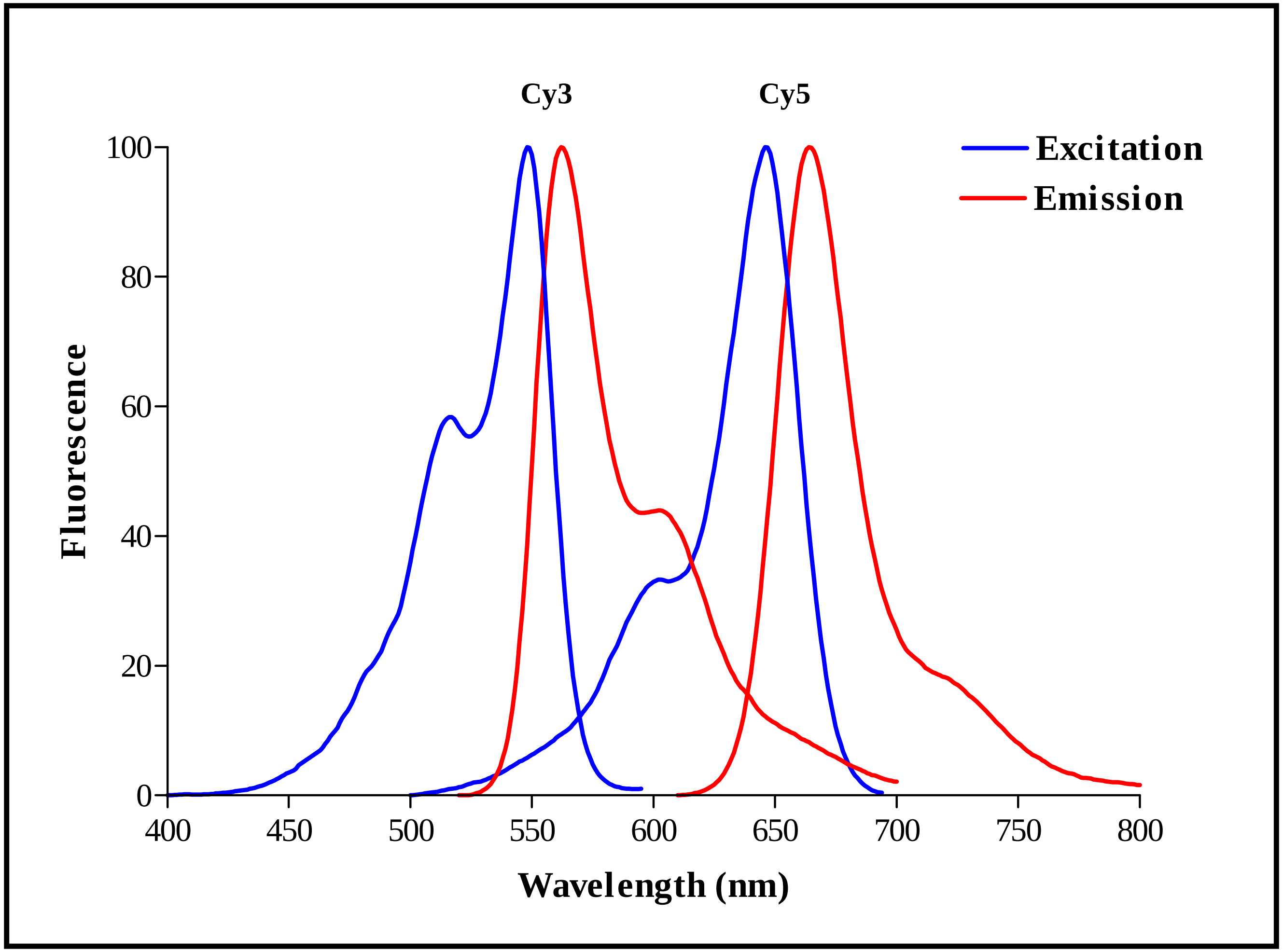
r = Distance between donor-acceptor (Å)

R_0 Value

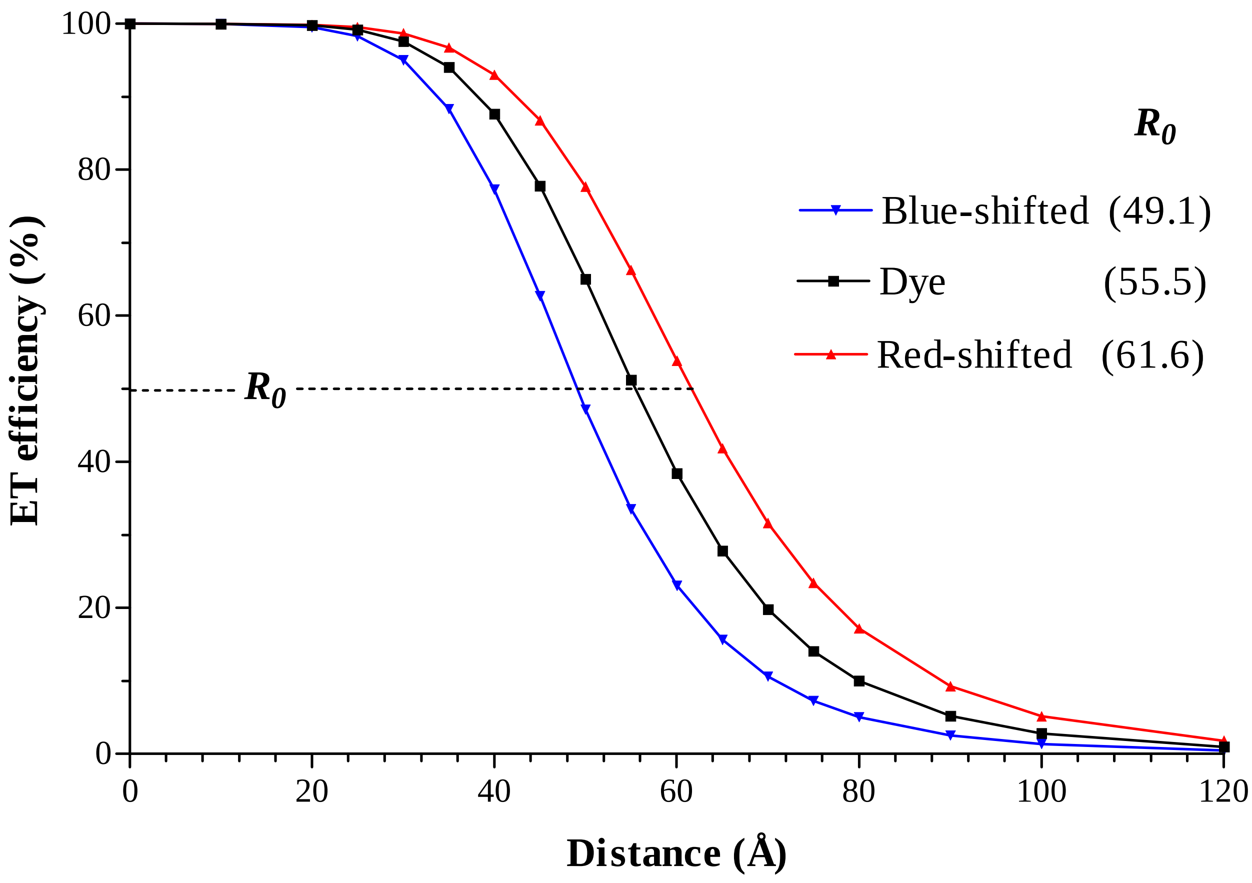
$$R_0 = 9.8 \times 10^3 \left(J \kappa^2 \phi_d n^{-4} \right)^{1/6}$$

R_0	= Distance at which energy transfer efficiency is 50%
J	= Spectral overlap integral (extinction coefficient of acceptor buried within J)
κ^2	= Orientation factor
ϕ_d	= Donor quantum yield (in absence of acceptor)
n	= Solvent refractive index

Cy3 and Cy5 Spectra



Dependence on Spectral Overlap (J)



R&D

Summary

- Define fluorescence
- The fluorescence process
- Molar extinction coefficients
- Quantum yield
- Brightness
- Fluorescence lifetime
- FRET

Fluorescence Modules

- Fluorescence Basics
- CyDye™ Chemistry
- CyDye Applications
- CyDyes and Fluorescence Polarisation

Further Information

Can be found at Amersham Biosciences "Drug Screening Application Zone"

http://www.amershambiosciences.com

SPA (password prompt) → SPA

- **Fluorescence theory:** **Fluorescence Overview**
- **FRET:** **Energy Transfer**
- **CyDye reagents:** **CyDye Fluor**