

# Chapter 7

# Fluorescence

Bibliography:

*Molecular Fluorescence: Principles and Applications.* Bernard Valeur

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The world of fluorescence  
is a world of beautiful  
color. In the darkness all  
the ordinary colors of our  
daylight world disappear.  
Only the intensely  
glowing hues of  
fluorescent substances  
touched by the ultraviolet  
beam shine out with  
striking clarity.

Sterling Gleason, 1960

show

**Fluorescence** is the emission of light by a substance that has absorbed light or other EM radiation. It is a form of [luminescence](#). In most cases, the emitted light has a longer wavelength , and therefore lower energy, than the absorbed radiation. The most striking example of fluorescence occurs when the absorbed radiation is in the UV region of the spectrum, and thus invisible to the human eye, while the emitted light is in the visible region, which gives the fluorescent substance a distinct color that can be seen only when exposed to UV light. Fluorescent materials cease to glow nearly immediately when the radiation source stops, unlike [phosphorescent](#) materials, which continue to emit light for some time after.

*Fluorescence* and *phosphorescence* are particular cases of luminescence. The mode of excitation is absorption of a photon, which brings the absorbing species into an electronic excited state. The emission of photons accompanying de-excitation is then called *photoluminescence* (fluorescence, phosphorescence or delayed fluorescence), which is one of the possible physical effects resulting from interaction of light with matter, as shown in Figure 1.1.

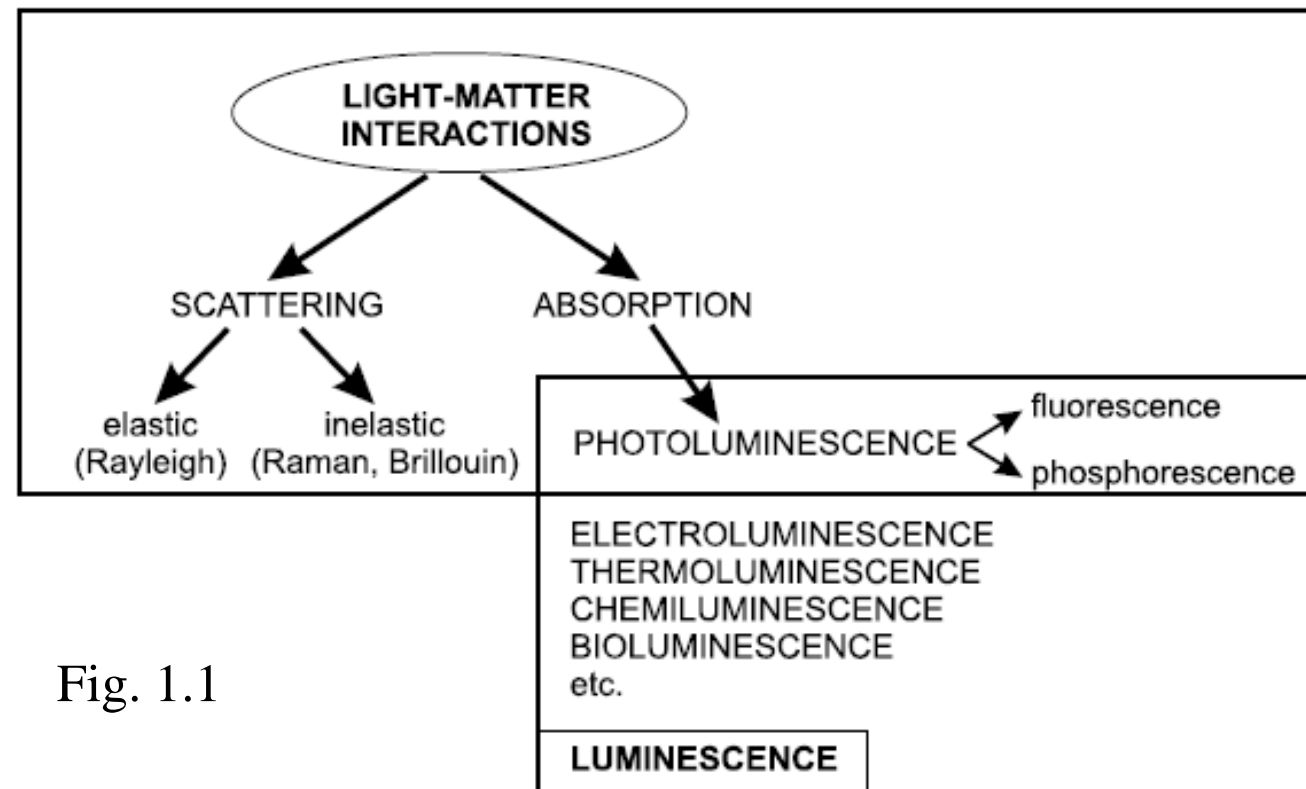


Fig. 1.1

When one of the two electrons of opposite spins (belonging to a molecular orbital of a molecule in the ground state) is promoted to a molecular orbital of higher energy, its spin is in principle unchanged so that the total spin quantum number ( $S = \sum s_i$ , with  $s_i = +\frac{1}{2}$  or  $-\frac{1}{2}$ ) remains equal to zero. Because the multiplicities of both the ground and excited states ( $M = 2S + 1$ ) is equal to 1, both are called *singlet state* (usually denoted  $S_0$  for the ground state, and  $S_1, S_2, \dots$  for the excited states) (Figure 2.2)<sup>1)</sup>. The corresponding transition is called a singlet-singlet transition.

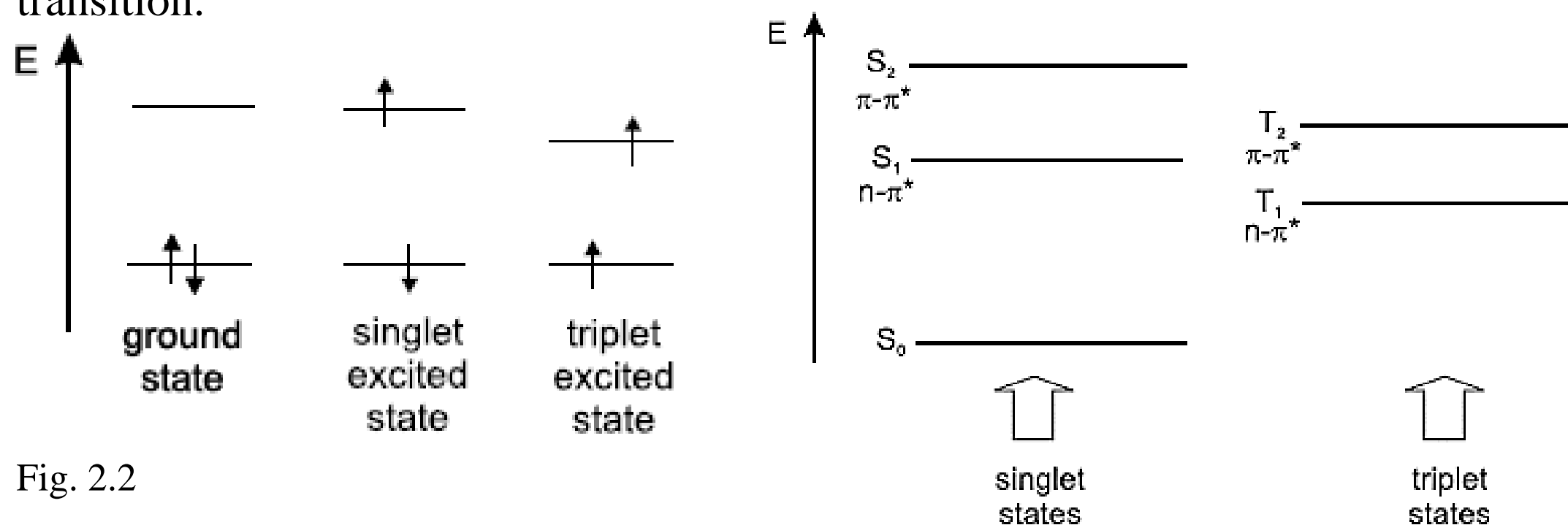
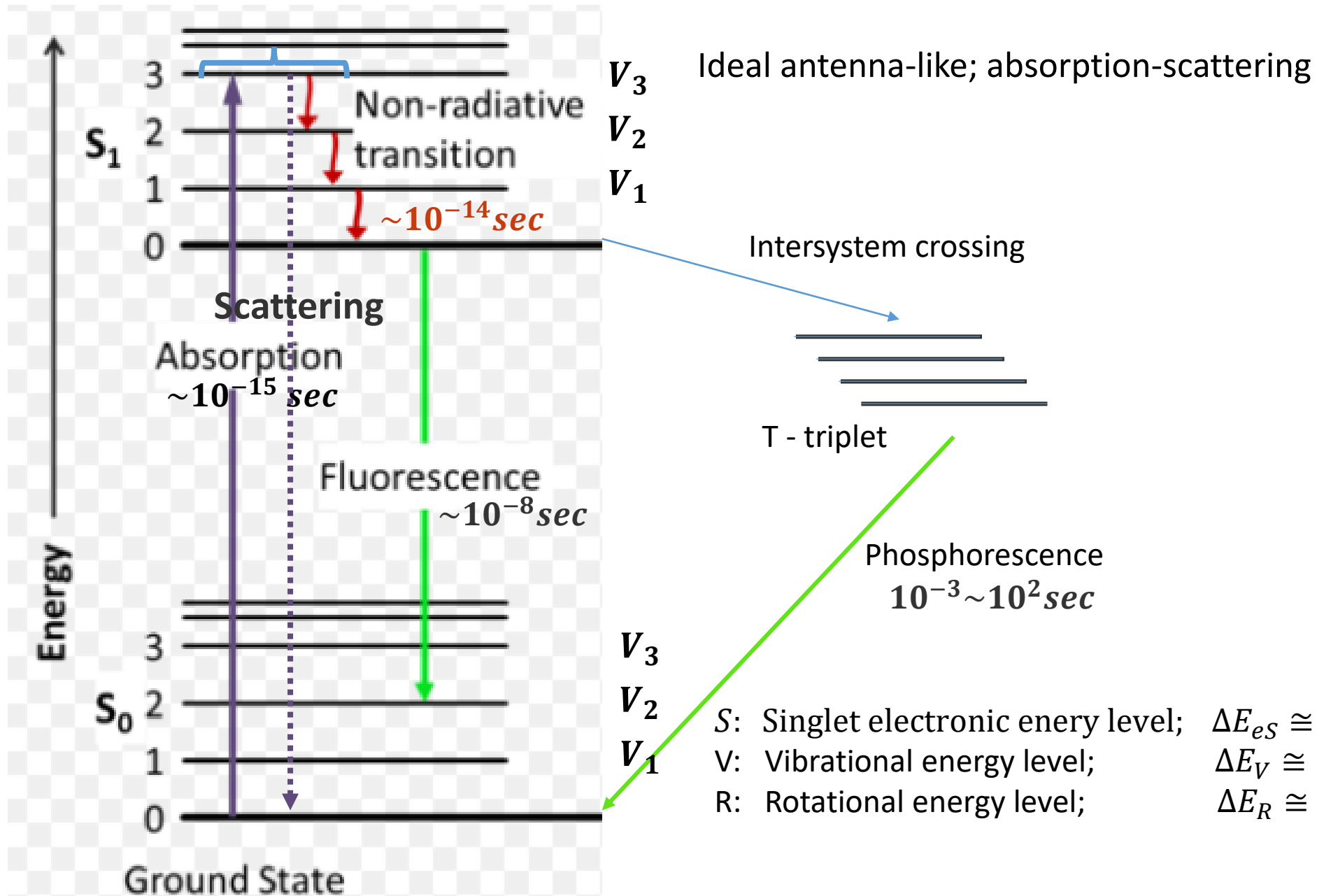


Fig. 2.2



Sir George Stokes 1819-1903

Jablonski diagram including vibrational levels for absorbance, non-radiative decay, and fluorescence



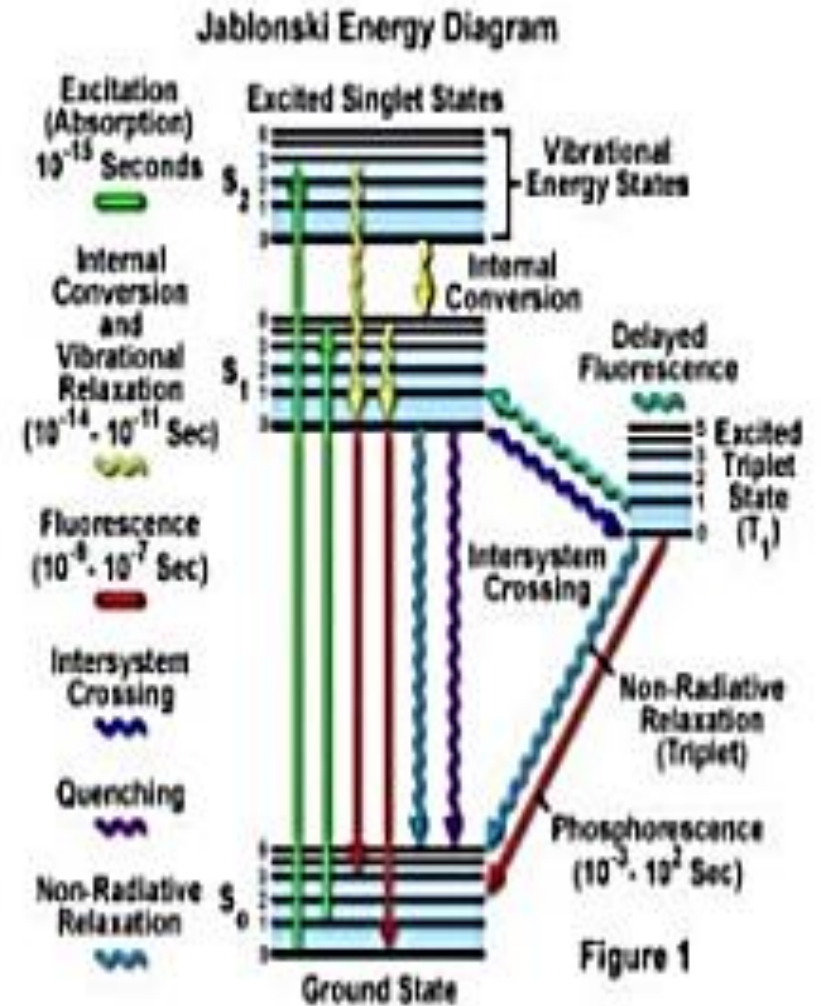
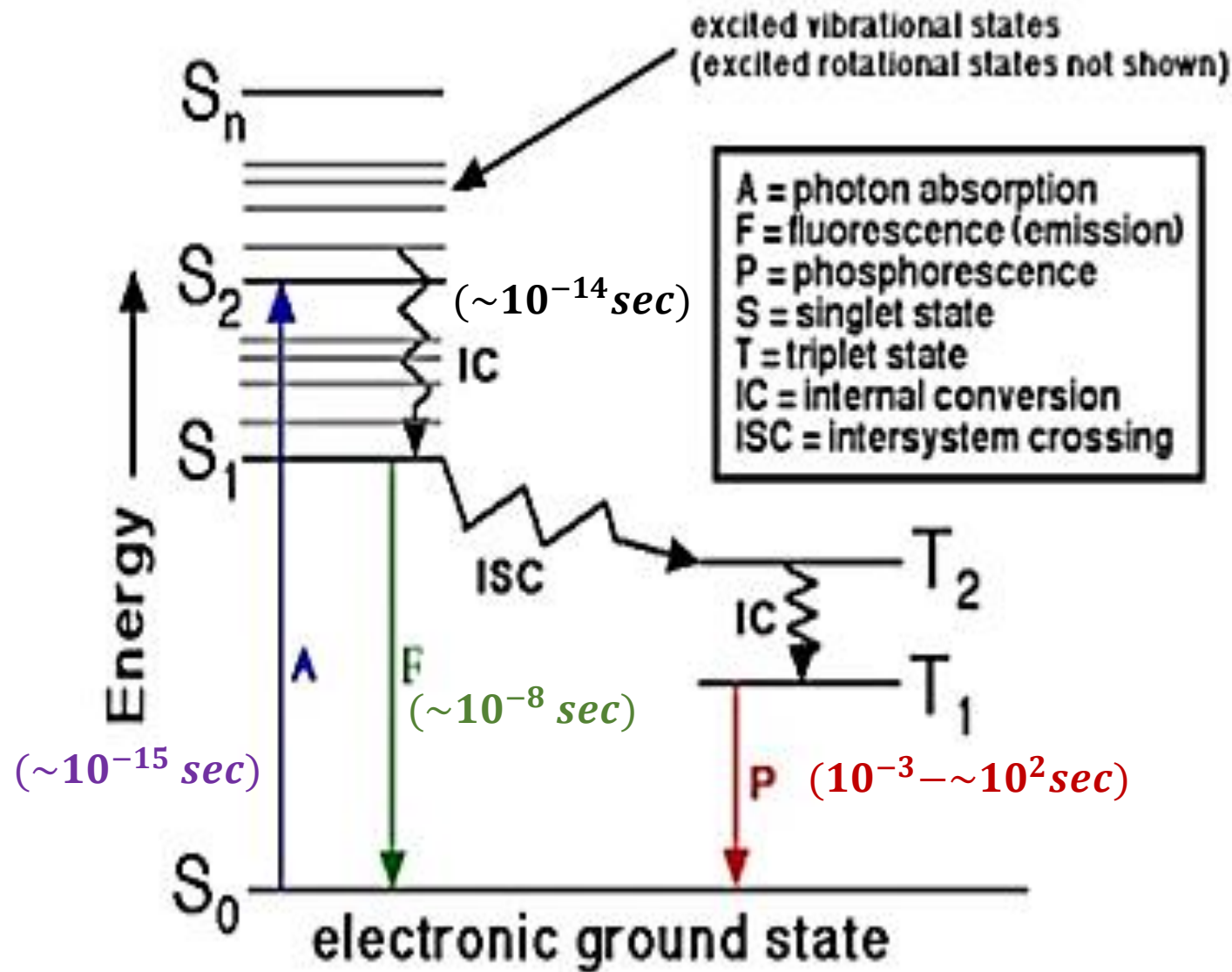
S: Singlet electronic energy level;  
V: Vibrational energy level;  
R: Rotational energy level;

$$\Delta E_{eS} \cong 2.5 \text{ eV} = 20000 \text{ cm}^{-1}$$

$$\Delta E_V \cong 0.12 \text{ eV} = 1000 \text{ cm}^{-1}$$

$$\Delta E_R \cong 0.0012 \text{ eV} = 10 \text{ cm}^{-1}$$

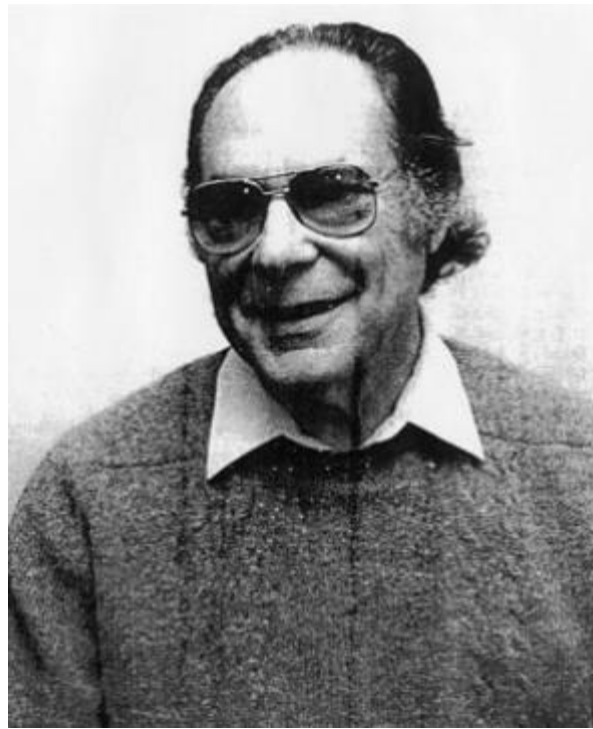
# Energy level diagram (Jablonski diagram)







Francis Perrin 1901 – 1992

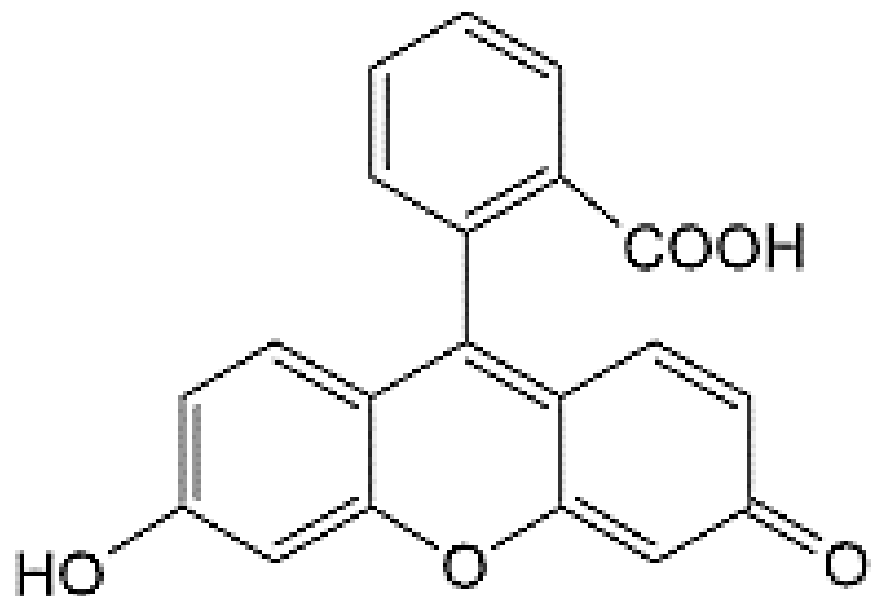


Gregorio Weber 1916-1997

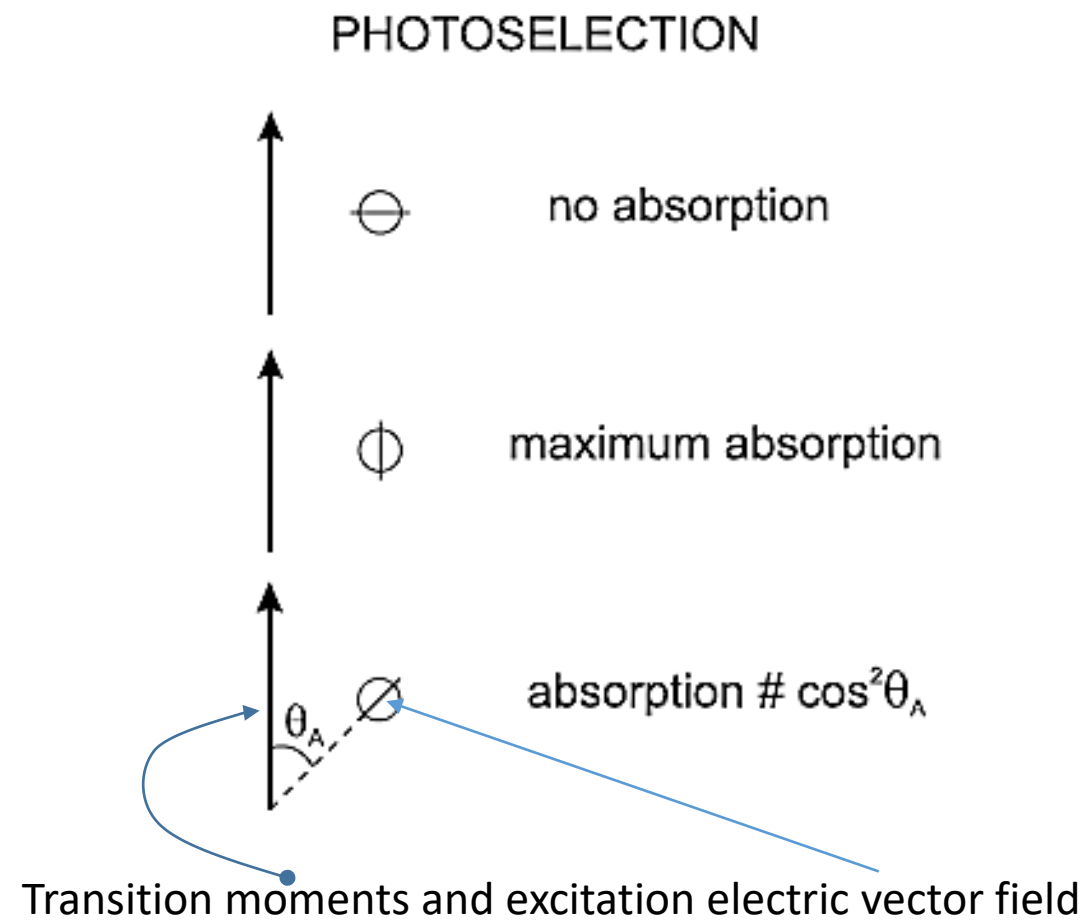


Theodor Förster 1910 – 1974)

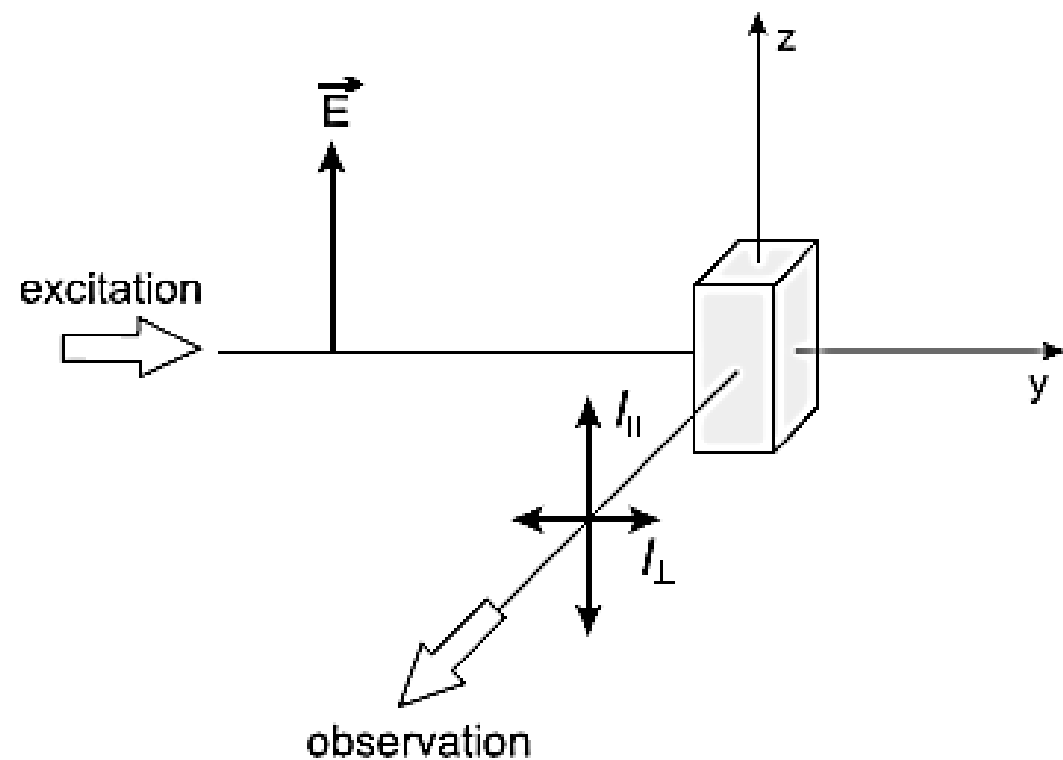
## Fluorescence polarization



fluorescein



Right hand configuration for measuring fluorescence polarization.



polarization ratio

$$p = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

emission anisotropy

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

$$I_{\text{tot}} = I_{\parallel} + 2I_{\perp}$$

$$r = \frac{2p}{3 - p}$$

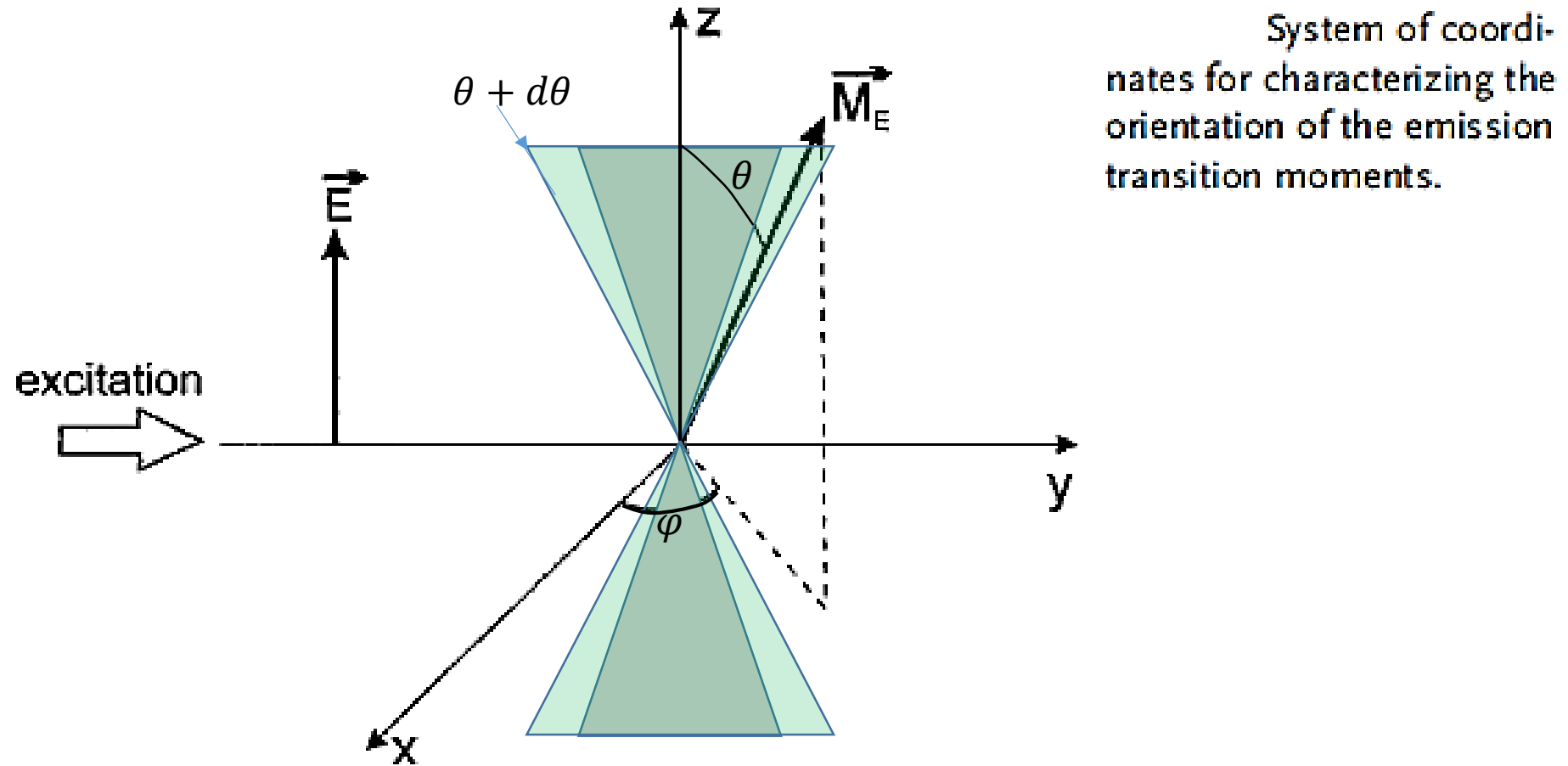


Fig 5.6

Before excitation, the number of molecules whose transition moment is oriented within angles  $\theta$  and  $\theta + d\theta$ , and  $\phi$  and  $\phi + d\phi$  is proportional to an elementary surface on a sphere whose radius is unity, i.e.  $2\pi \sin \theta d\theta d\phi$  (Figure 5.6).

Taking into account the excitation probability, i.e.  $\cos^2 \theta$ , the number of excited molecules whose transition moment is oriented within angles  $\theta$  and  $\theta + d\theta$ , and  $\phi$  and  $\phi + d\phi$ , is proportional to  $\cos^2 \theta \sin \theta d\theta d\phi$ . The fraction of molecules oriented in this direction is

$$W(\theta, \phi) d\theta d\phi = \frac{\cos^2 \theta \sin \theta d\theta d\phi}{\int_0^{2\pi} d\phi \int_0^\pi \cos^2 \theta \sin \theta d\theta} \quad (5.21)$$

The denominator, which is proportional to the total number of excited molecules, can be calculated by setting  $x = \cos \theta$ , hence  $dx = -\sin \theta d\theta$ , and its value is  $4\pi/3$ . Equation (5.21) then becomes

$$W(\theta, \phi) d\theta d\phi = \frac{3}{4\pi} \cos^2 \theta \sin \theta d\theta d\phi \quad (5.22)$$

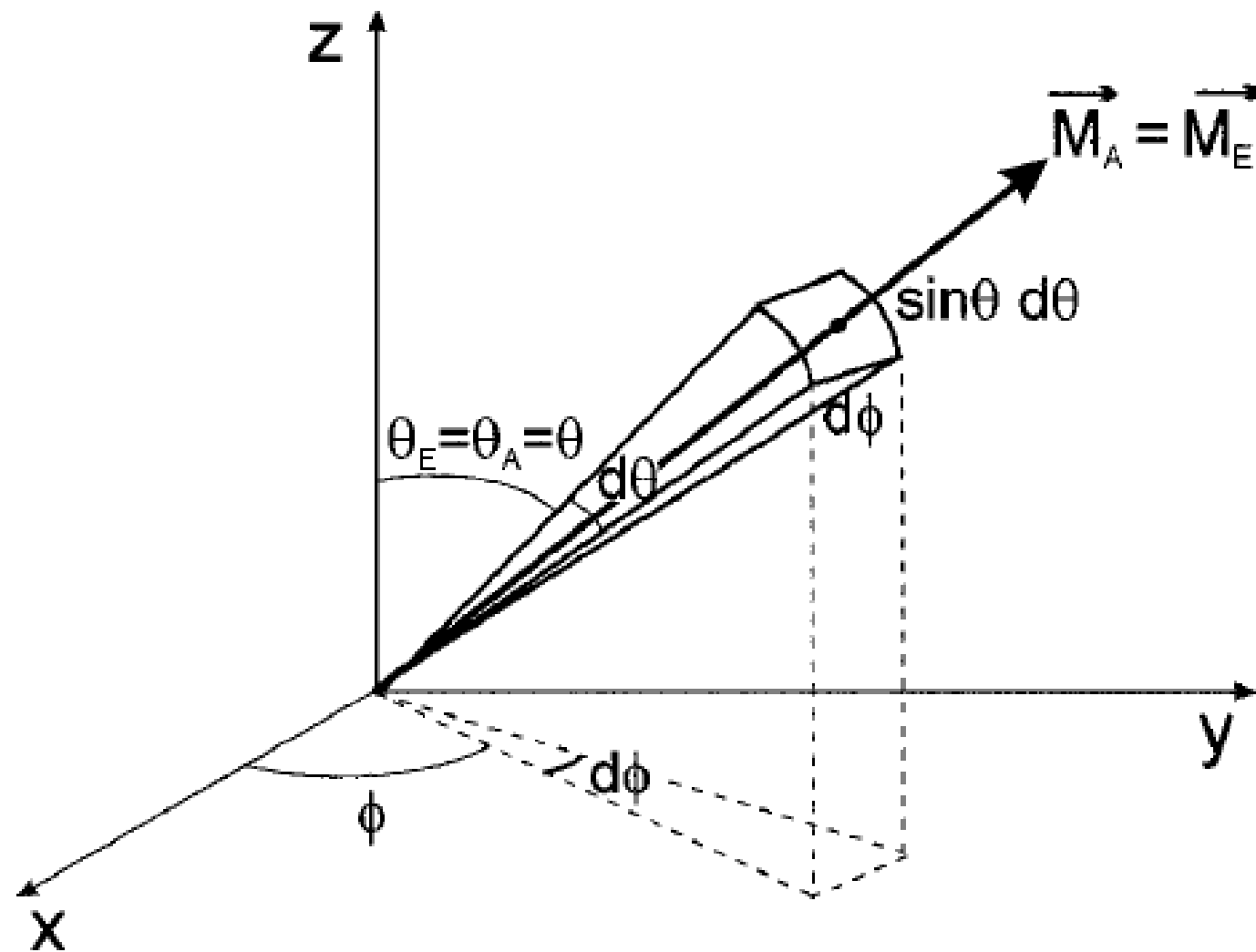


Fig. 5.6. The fraction of molecules whose absorption and emission transition moments are parallel and oriented in a direction within the elementary solid angle. This direction is defined by angles  $\theta$  and  $\phi$ .

It is then possible to calculate the average of  $\cos^2 \theta$  over all excited molecules

$$\begin{aligned}\overline{\cos^2 \theta} &= \int_0^{2\pi} d\phi \int_0^\pi \cos^2 \theta W(\theta, \phi) d\theta \\ &= \frac{3}{4\pi} \int_0^{2\pi} d\phi \int_0^\pi \cos^4 \theta \sin \theta d\theta \\ &= 3/5\end{aligned}\tag{5.23}$$

$$r_0 = \frac{3 \overline{\cos^2 \theta} - 1}{2} = \frac{2}{5} = 0.4$$

$$p_0 = 0.5\tag{5.24}$$

A nanoparticle (yellow dot in the figure) suspended in solution will undergo a random walk due to the summation of these underlying collisions. The rotational correlation time ( $\phi_r$ ), the time it takes for the molecule to rotate 1 radian, is dependent on the viscosity ( $\eta$ ), temperature (T), Boltzmann constant ( $k_B$ ) and volume ( $V$ ) of the nanoparticle:[5]

$$\phi_r = \frac{\eta V}{k_B T}$$

Perrin equation 
$$\left( \frac{1}{p} - \frac{1}{3} \right) = \left( \frac{1}{p_0} - \frac{1}{3} \right) \left( 1 + \frac{\tau}{\tau_c} \right)$$

$$\tau_c = V\eta/RT$$

