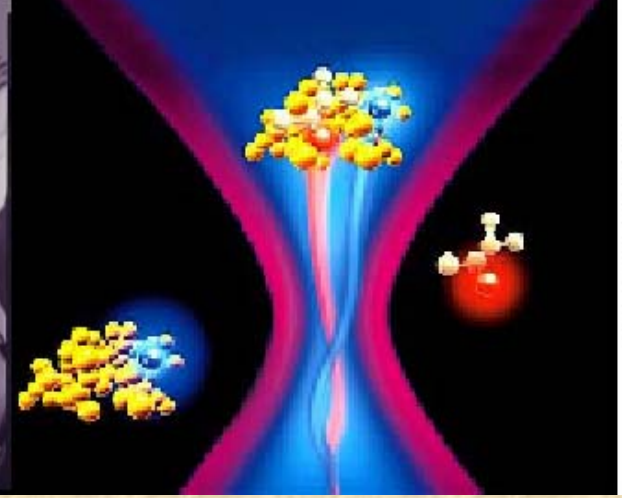
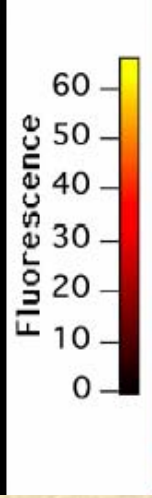
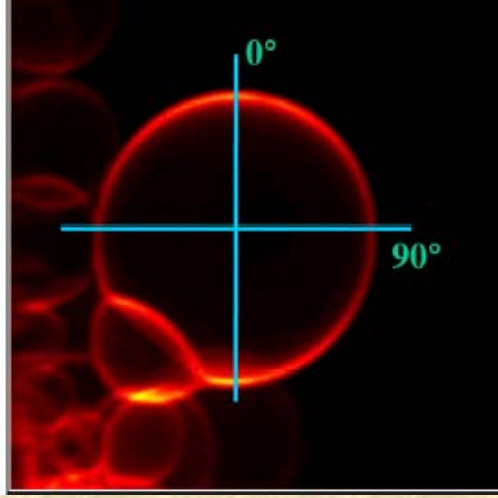






Fluorescence Workshop
UMN Physics
June 8-10, 2006

Quantum Yield and Polarization (1)
Joachim Mueller

Quantum yield, polarized light, dipole moment, photo-selection, dipole radiation, polarization and anisotropy

Virtually all fluorescence data required for any research project will fall into one of the following categories.

1. The fluorescence emission spectrum
2. The excitation spectrum of the fluorescence
3. The quantum yield
4. The polarization (anisotropy) of the emission
5. The fluorescence lifetime

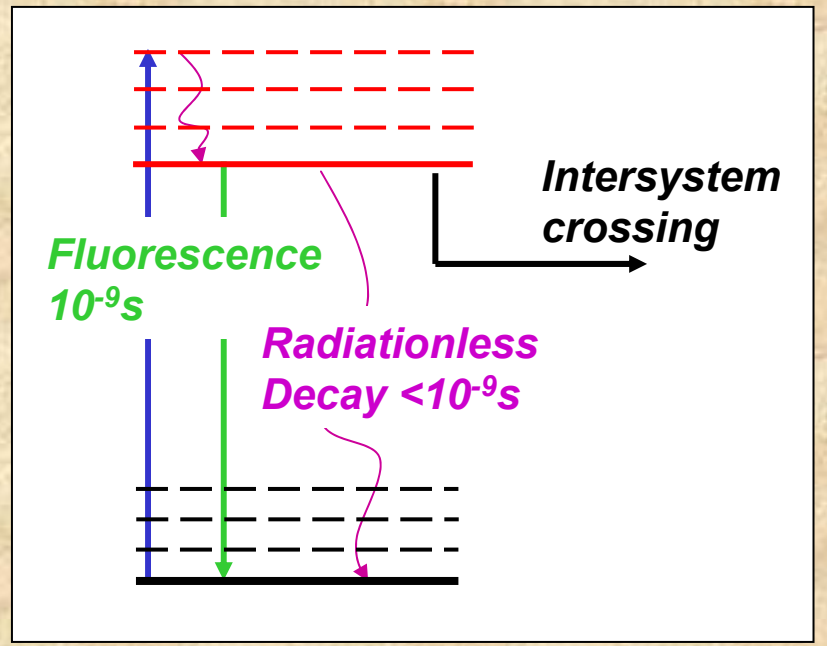
In these lectures, we examine each of these categories and briefly discuss historical developments, underlying concepts and practical considerations

Quantum Yield Φ

quantum yield:
The number of fluorescence emissions which occur per *photon* absorbed by the fluorophore.

Φ = (number of emitted photons)/(number of photons absorbed)

Example:
A quantum yield of 100% (Φ=1) means that every absorbed photon leads to an emitted photon (fluorescence),
while a quantum yield of 0% (Φ=0) means that the molecule is non-fluorescent.



There is a competition between the fluorescence process and all other process that leave the excited state S_1 .

Each process has a rate coefficient *k* (which is the inverse of its lifetime τ): $k = \frac{1}{\tau}$

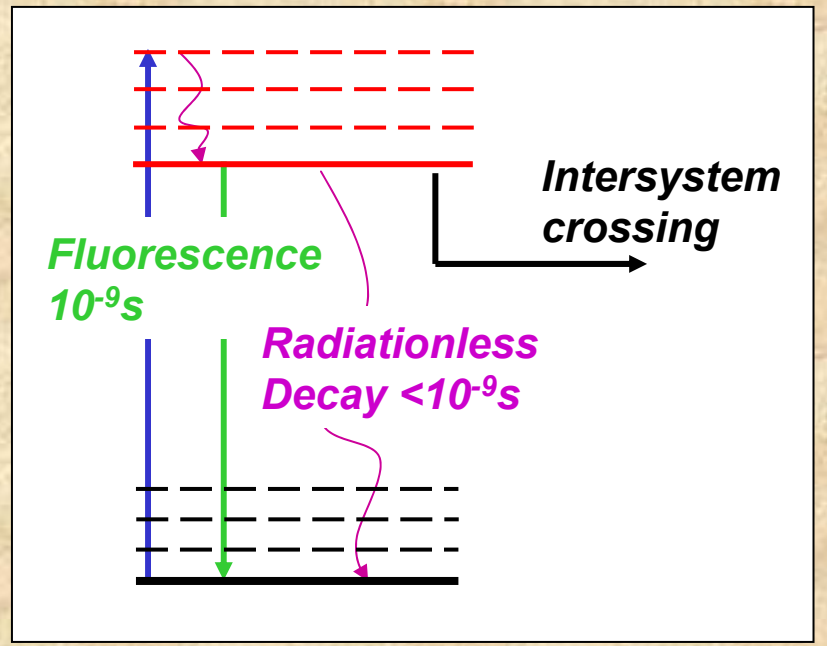
A lifetime of 1 ns corresponds to a rate of $10^9\ s^{-1}$

Quantum Yield Φ

The quantum yield of fluorescence (Φ) is dependent on the ***rate of the emission process*** divided by the sum of the ***rates of all deactivation processes:***

$$\Phi = \frac{k_f}{k_f + k_i + k_x}$$

k_f is the rate of fluorescence
k_i is the rate of radiationless decay
k_x is the rate of intersystem crossing



If the rates of the deactivation processes are slow compared to k_f then the Φ *is high*

However, if the rates of these other processes are fast compared to k_f then Φ is low

List of quantum yields from “Molecular Fluorescence” by Bernard Valeur

Tab. 6.1. Standards for the determination of fluorescence quantum yields

Range	*Compound*	*Temp.* (°C)	*Solvent*	Φ_F	*Ref.*
270–300 nm	Benzene	20	Cyclohexane	0.05 ± 0.02	1
300–380 nm	Tryptophan	25	H_2O (pH 7.2)	0.14 ± 0.02	2
300–400 nm	Naphthalene	20	Cyclohexane	0.23 ± 0.02	3
315–480 nm	2-Aminopyridine	20	0.1 mol L^{-1} H_2SO_4	0.60 ± 0.05	4
360–480 nm	Anthracene	20	Ethanol	0.27 ± 0.03	1, 5
400–500 nm	9,10-diphenylanthracene	20	Cyclohexane	0.90 ± 0.02	6, 7
400–600 nm	Quinine sulfate dihydrate	20	0.5 mol L^{-1} H_2SO_4	0.546	5, 7
600–650 nm	Rhodamine 101	20	Ethanol	1.0 ± 0.02	8
				0.92 ± 0.02	9
600–650 nm	Cresyl violet	20	Methanol	0.54 ± 0.03	10

1) Dawson W. R. and Windsor M. W. (1968) *J. Phys. Chem.* **72**, 3251.
2) Kirby E. P. and Steiner R. F. (1970) *J. Phys. Chem.* **74**, 4480.
3) Berlman I. B. (1965) *Handbook of Fluorescence Spectra of Aromatic Molecules*, Academic Press, London.
4) Rusakowicz R. and Testa A. C. (1968) *J. Phys. Chem.* **72**, 2680.
5) Melhuish W. H. (1961) *J. Phys. Chem.* **65**, 229.
6) Hamai S. and Hirayama F. (1983) *J. Phys. Chem.* **87**, 83.
7) Meech S. R. and Phillips D. (1983) *J. Photochem.* **23**, 193.
8) Karstens T. and Kobs K. (1980) *J. Phys. Chem.* **84**, 1871.
9) Arden-Jacob J., Marx N. J. and Drexhage K. H. (1997) *J. Fluorescence* **7(Suppl.)**, 91S.
10) Magde D., Brannon J. H., Cramers T. L. and Olmsted J. III (1979) *J. Phys. Chem.* **83**, 696.

Quenchers

Molecules which decrease a fluorophore's quantum yield by direct interaction are often termed "quenchers."

Fluorescence quenching can result from interaction of the quenching molecule with the excited fluorophore – a process known as collisional (or dynamic) quenching – or by formation of a ground state complex between the quencher and the fluorophore (static quenching).

Both of these quenching processes are usually reversible, as opposed to photobleaching of the excited fluorophore, which is usually (but not always) irreversible.

more later …

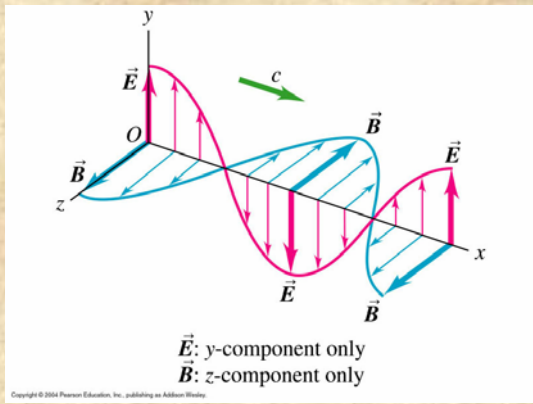
Virtually all fluorescence data required for any research project will fall into one of the following categories.

1. The fluorescence emission spectrum
2. The excitation spectrum of the fluorescence
3. The quantum yield
4. The polarization (anisotropy) of the emission
5. The fluorescence lifetime

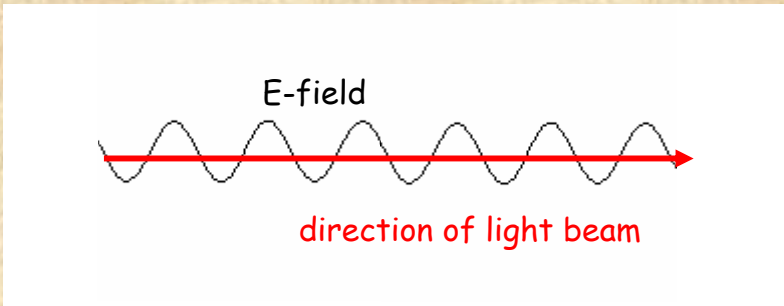
In these lectures, we examine each of these categories and briefly discuss historical developments, underlying concepts and practical considerations

Polarization Basics (1)

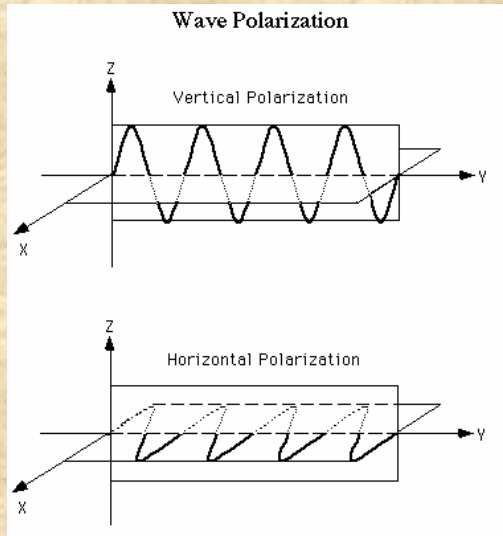
As stated earlier, light can be considered as oscillations of an electromagnetic field - characterized by electric (E) and magnetic (B) components - perpendicular to the direction of light propagation.



Let's forget about the magnetic field (it is not important for what we want to do) and only consider the E-field:

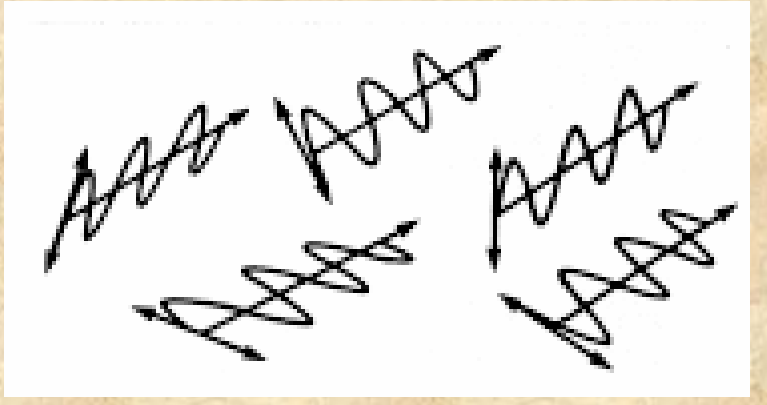


The electric field (E) is always perpendicular to the direction of travel, but its orientation may differ. Here we show two possible configurations of the electric field for light traveling to the right. The direction of the electric field defines the polarization of the wave. The picture illustrates vertically and horizontally polarized light.



Polarization Basics (2)

Most light sources are unpolarized which means that the electric field vector can assume any direction of oscillation perpendicular to the propagation direction of the light.

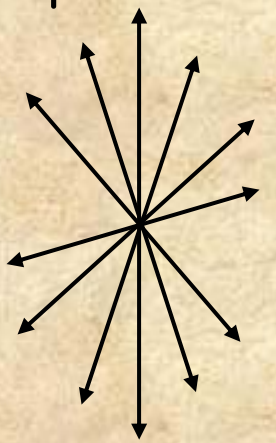


To simplify drawings it is custom to indicate the direction of the E-field oscillations by a double arrow

Note, the direction of propagation is perpendicular to the E-field vector

Thus, we can represent unpolarized light by arrows pointing in all directions:

Unpolarized (natural) light

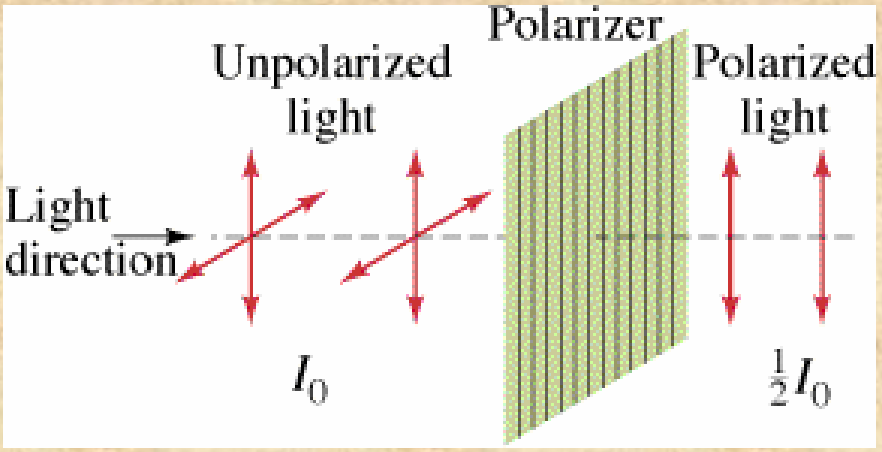


Light Propagation Direction

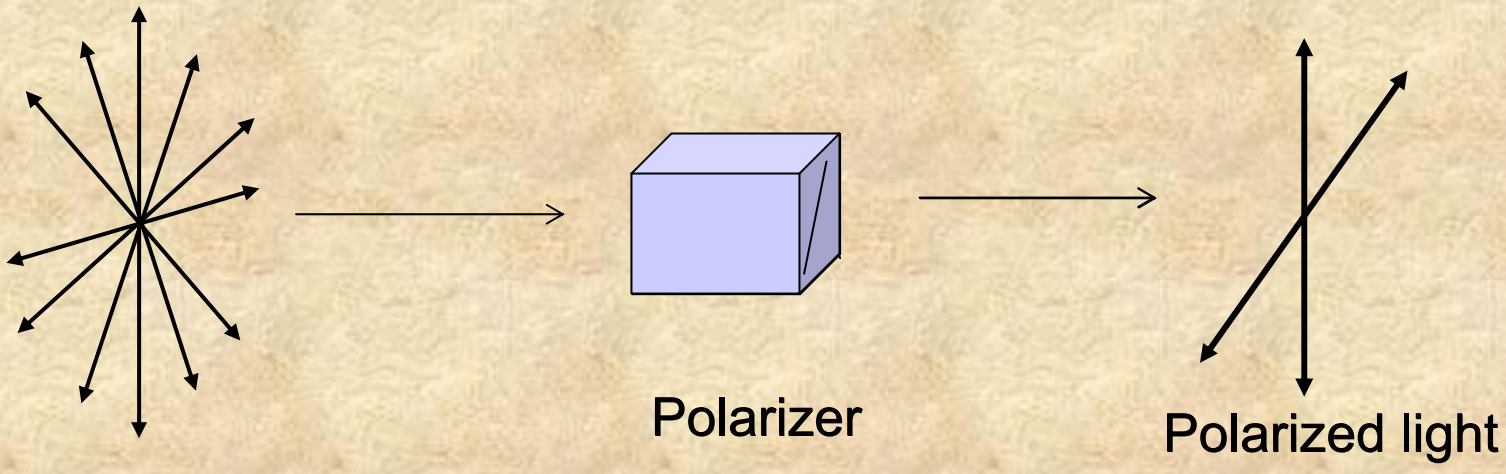
Note, some light sources are polarized or partially polarized.
Examples: most lasers and light reflected from surfaces

Polarizer (1)

Polarizers are optically active devices that can isolate one direction of the electric vector.



Because the polarization axis of the polarizer is vertical only the vertical component of the impinging electric field can pass. Note that we lost half of the original light intensity.



Unpolarized (natural) light

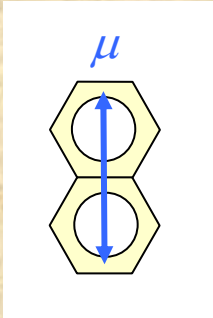
Polarizer (2)

The most common polarizers used today are (1) dichroic devices, which operate by effectively absorbing one plane of polarization (e.g., Polaroid type-H sheets based on stretched polyvinyl alcohol impregnated with iodine) and (2) double refracting calcite ($CaCO_3$) crystal polarizers - which differentially disperse the two planes of polarization (examples of this class of polarizers are Nicol polarizers, Wollaston prisms and Glan-type polarizers such as the Glan-Foucault, Glan-Thompson and Glan-Taylor polarizers)

Polarizers have, in fact, been in use for a very long time - the Vikings used a "sunstone" (now thought to have been composed of the mineral cordierite, a natural polarizing material) to observe the location of the sun on foggy or overcast days. Since scattered sunlight is highly polarized compared to light coming along the direction to the sun, the distribution of the sky's brightness could be observed through the sunstone and hence the sun's position could be localized and, if the time of day were known, the compass directions.

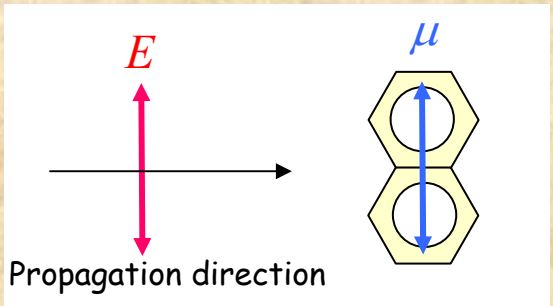
Electric Dipole Moment (1)

The electric field of light interacts with the electrons of a molecule. The electrons are bound to the molecule and thus restricted in their movement. Typically there is a direction within the molecule where the electrons can move more easily. Let's call this axis the electric dipole moment μ of the molecule.

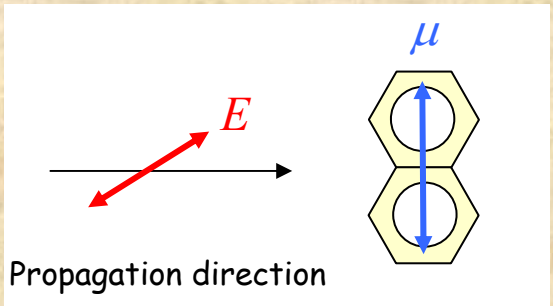


Light is absorbed by the molecule if the electric field (E) of the incoming light is properly oriented relative to the electric dipole moment (μ) of the molecule.

Maximum absorption occurs, if E and μ are parallel to each other

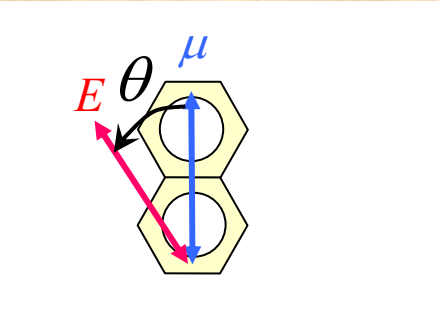


No absorption occurs, if E and μ are normal to each other



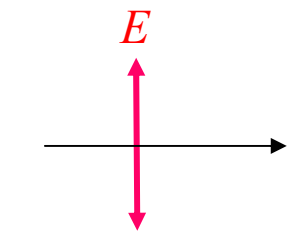
Electric Dipole Moment (2)

The probability of the absorption is proportional to the cosine squared of the angle θ between the direction of the electric field of the exciting light and the electric dipole moment.

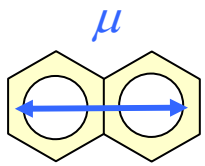


Absorption probability $\propto \cos\theta^2$

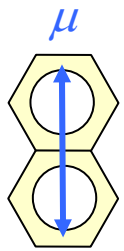
Thus, if light of a particular electric vector orientation (plane polarized light) impinges on a sample, only those molecules which are properly oriented relative to this electric vector can absorb the light.



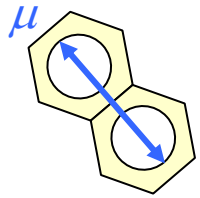
Incoming vertically polarized light



No absorption



Maximum absorption

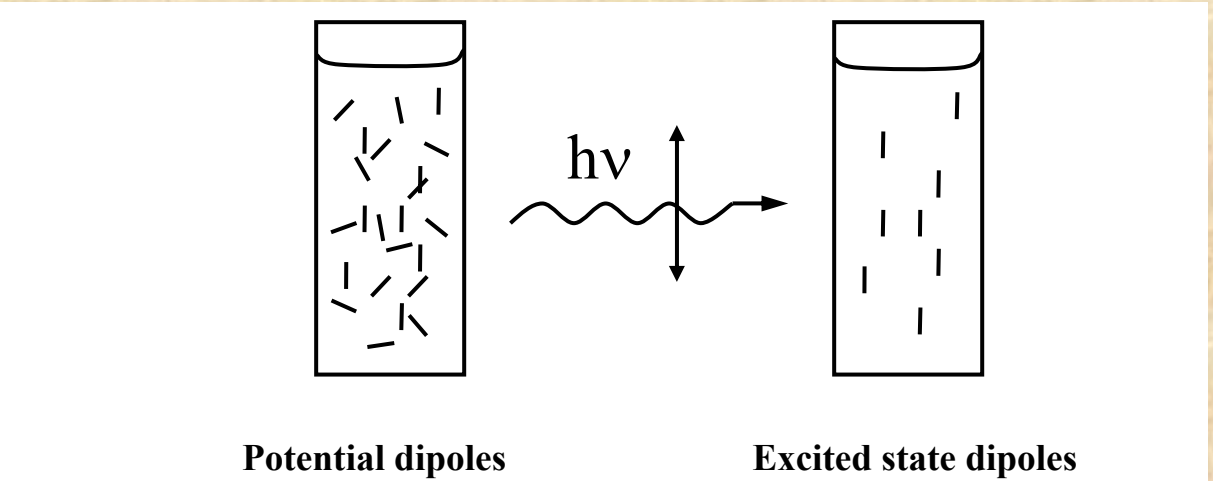


Some of the molecules with this orientation will absorb light.

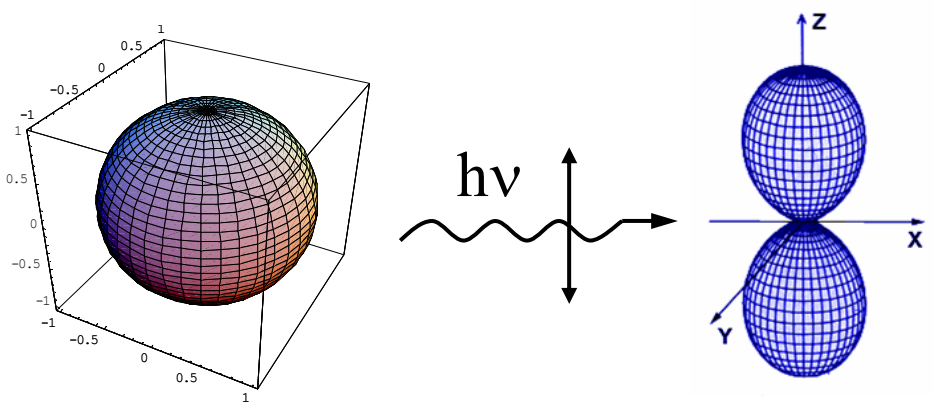
Photoselection

When we excite an ensemble of randomly oriented fluorophores with plane-polarized light we are performing a *photoselection* process, creating a population of excited molecules which nominally have their excited dipoles lines up with the polarization direction of the excitation. This process is illustrated below:

Schematic of photoselection process

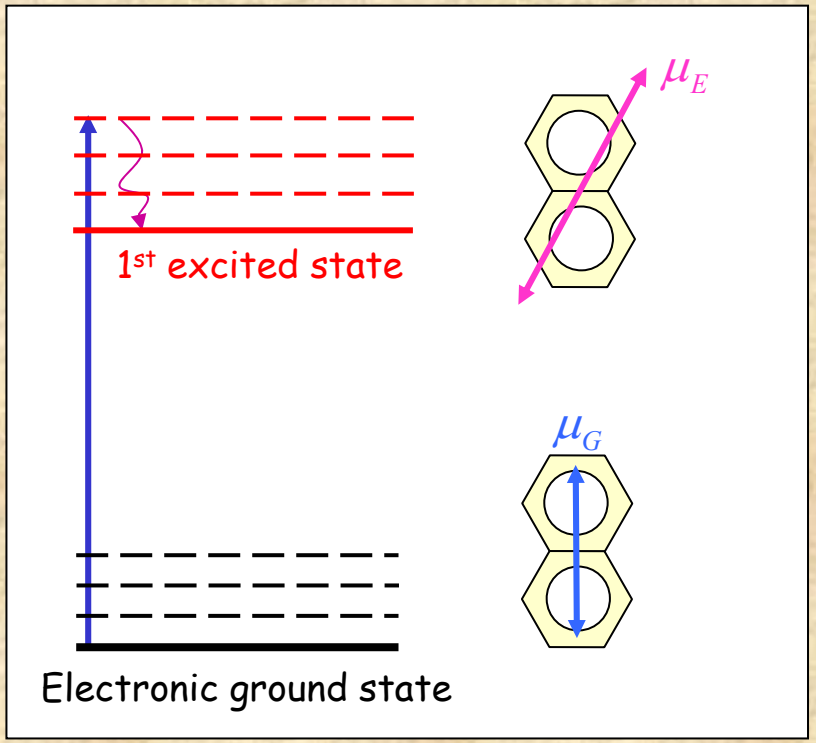


Polar plot of dipole populations

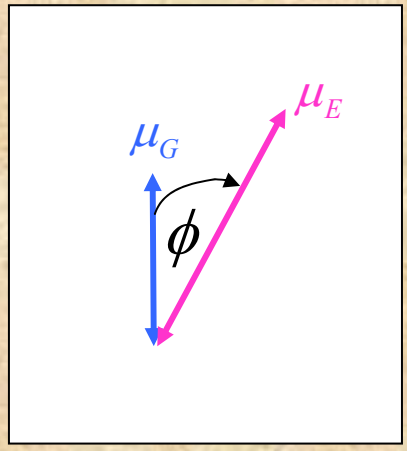


Absorption and Excited State Dipole

Upon absorption of a photon the electrons of the excited molecule redistribute. This leads to an excited dipole moment μ_E in the fluorophore (usually of different magnitude and direction from the ground state dipole μ_G). The orientation of this dipole moment relative to the nuclear framework, and its magnitude, will be determined by the nature of the substituents on the molecule. This excited state dipole moment is also known as the transition dipole or transition moment.



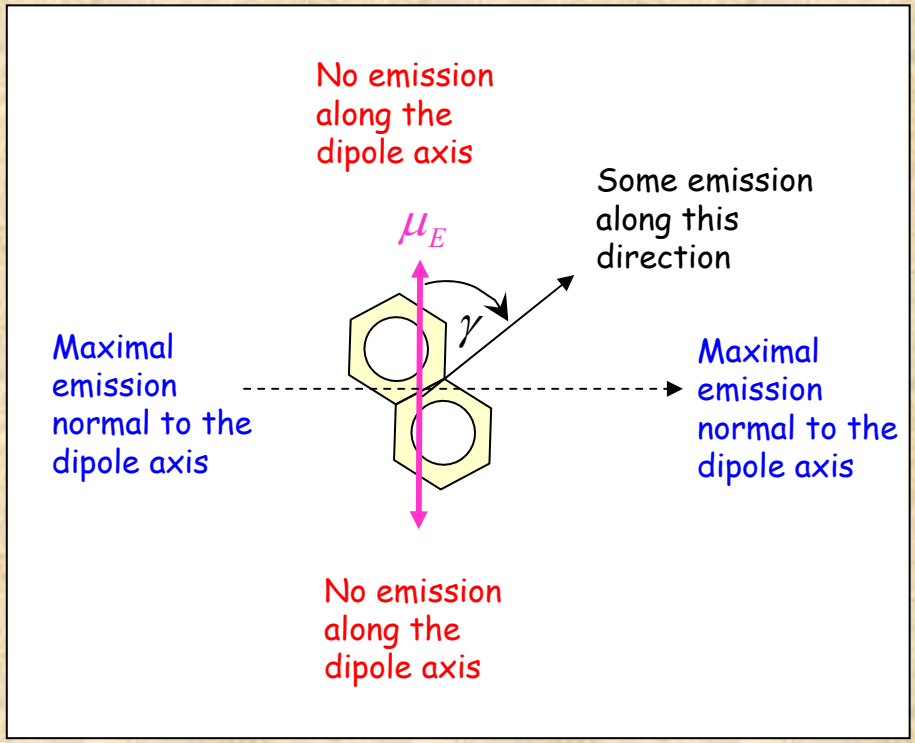
ϕ is the angle between absorption and emission dipoles (we will use it later)



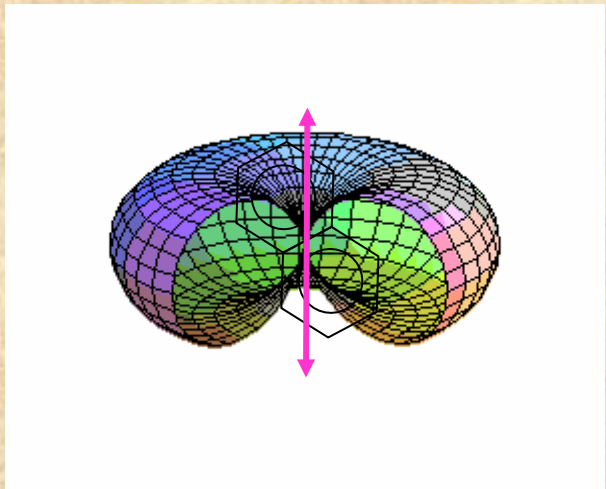
Fluorescence Emission from an Excited Molecule

Consider an excited molecule with a transition dipole μ_E that returns to the ground state via emission of a photon (fluorescence). The direction of emission is not random, but follows a sine squared law of electric dipole radiation.

Emission probability $\propto \sin \gamma^2$



Dipole radiation pattern



Polarization of Fluorescence Emission

In 1920, F. Weigert discovered that the fluorescence from solutions of dyes was polarized. Specifically, he looked at solutions of fluorescein, eosin, rhodamine and other dyes and noted the effect of temperature and viscosity on the observed polarization. Wiegert discovered that polarization increased with the size of the dye molecule and the viscosity of the solvent, yet decreased as the temperature increased. He recognized that all of these considerations meant that fluorescence polarization increased as the mobility of the emitting species decreased.

We developed in the last few slides the fundamental tools to understand polarization of fluorescence:

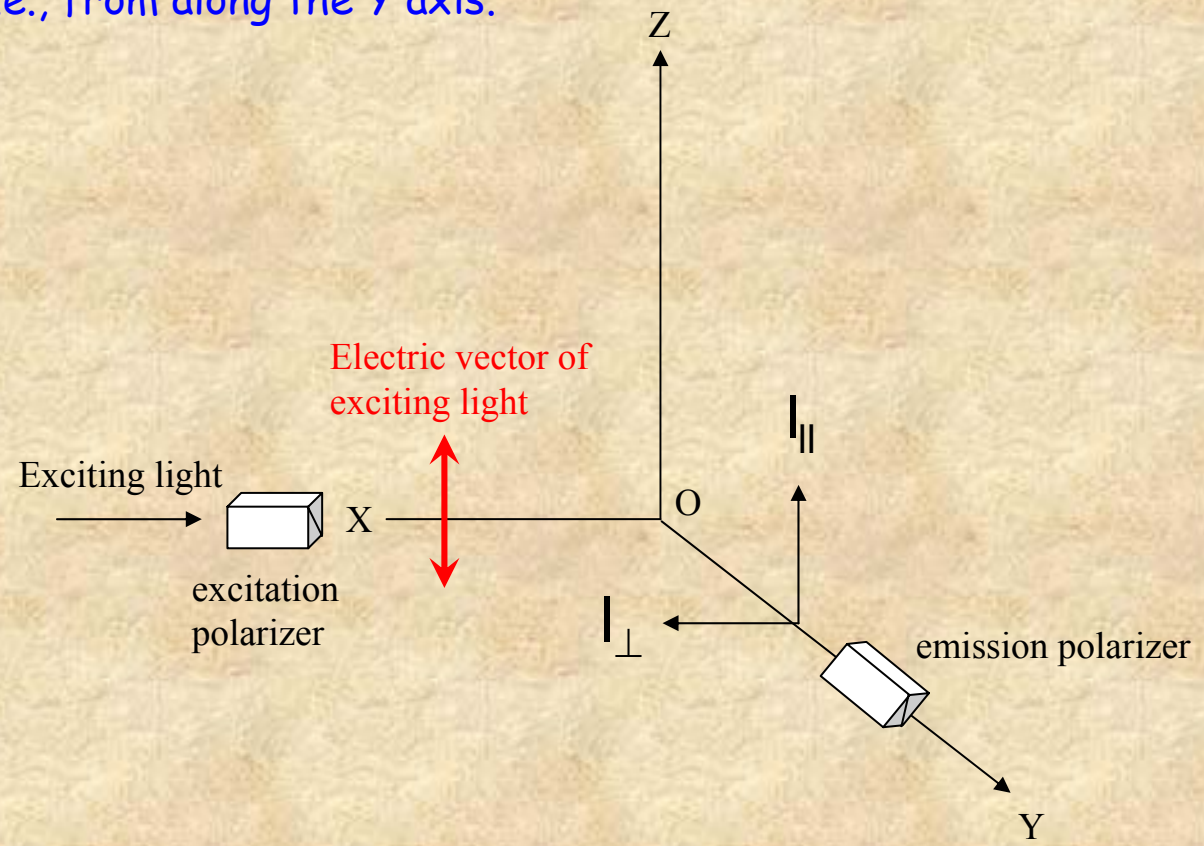
- Polarization of light
- Electric dipole moment of a molecule
- Photoselection
- Radiative emission of an excited dipole

In the following we will illustrate how these effects determine the polarization of fluorescent light. For now we assume that the molecules are fixed and cannot rotate. We will treat the effect of rotation later.

Fluorescence Polarization Experiment

Consider an XYZ coordinate framework with a fluorescent solution placed at the origin, as shown below, where XZ is in the plane of the page.

In this system, the exciting light is traveling along the X direction and is polarized along the Z direction. The polarized exciting light will be absorbed by the fluorophore at the origin and give rise to fluorescence **which is typically** observed at 90° to the excitation direction, i.e., from along the Y axis.



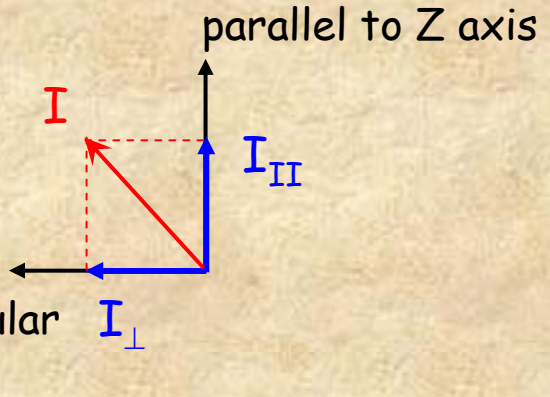
By inserting a polarizer into the emission path we can measure the intensity of the fluorescence with an electric vector along the axis of the polarizer. By rotating the polarizer one can isolate different electric vector orientations of the fluorescent light.

Polarization P

We initially consider that this fluorescence can have any direction of polarization. The actual direction of the electric vector of the emission can be determined by viewing the emission through a polarizer which can be oriented alternatively in the parallel or perpendicular direction relative to the Z axis or laboratory vertical direction. Polarization is then defined as a function of the observed parallel (I_{II}) and perpendicular intensities ($I_{\perp}$) :

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

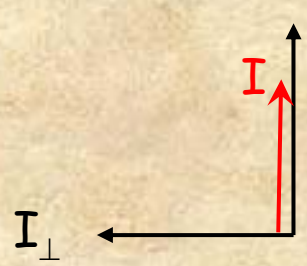
Example: Assume the fluorescence light with intensity I is polarized as shown. Aligning the emission polarizer along the Z axis determines the intensity along the Z axis (I_{II}), while orienting the polarizer perpendicular to the Z axis determines the perpendicular intensity component ($I_{\perp}$)



Limits of Polarization

- If the fluorescence emission is completely polarized in the parallel direction, i.e., the electric vector of the exciting light is totally maintained, then:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} = \frac{I - 0}{I + 0} = 1$$



$I_{\parallel} = I$

$I_{\perp} = 0$

- If the emitted light is totally polarized in the perpendicular direction then:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} = \frac{0 - I}{0 + I} = -1$$

$I_{\parallel} = 0$

$I_{\perp} = I$

The limits of polarization are thus +1 to -1

Anisotropy r

Another term frequently used in the context of polarized emission is anisotropy (usually designated as either A or r) which is defined as:

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

By analogy to polarization, the limits of anisotropy are +1 to -0.5.

A comment about the relationship between polarization and anisotropy:

Given the definition of polarization and anisotropy, one can show that:

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

For example:

P	r
0.50	0.40
0.30	0.22
0.10	0.069

Clearly, the information content in the polarization function and the anisotropy function is identical and the use of one term or the other is dictated by practical considerations as will be discussed later.

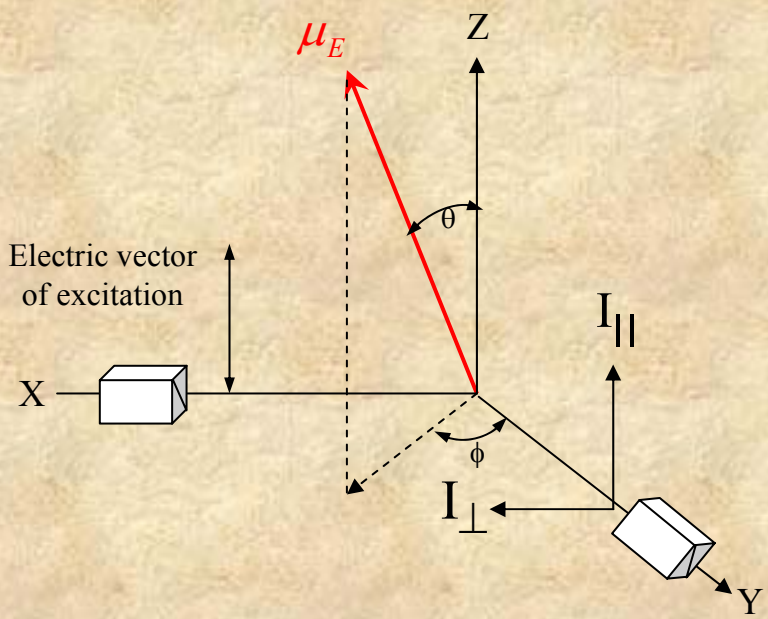
Fluorescence Polarization in Solution

In a solution the limiting polarization values (e.g., +/-1) cannot be realized, because of

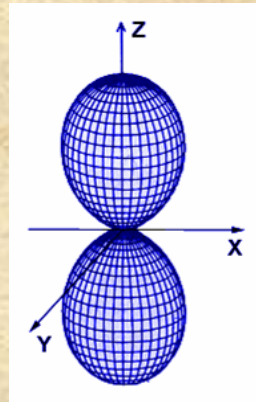
(1) photoselection

(2) the different orientation between ground and excited state dipoles

Consider, as shown to the right, a single fluorophore at the origin of our coordinate system with an excited state dipole μ_E. Calculate the emitted intensities I_{II} and $I_{\perp}$ for electric dipole radiation.



Now we take photoselection into account and repeat the above calculation for a population of excited dipoles that are distributed according to the $\cos^2 \theta$ photoselection rule.

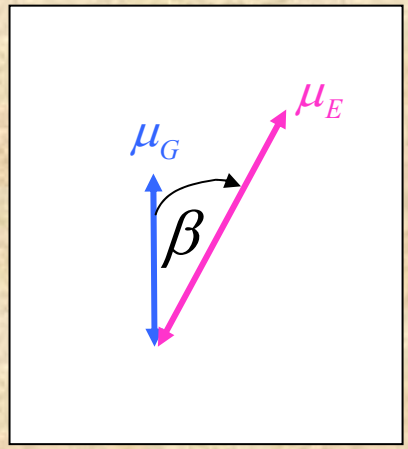


Once this is all done we arrive at the following expression …

Limiting Polarization in Solution

$$r_o = \frac{2}{5}\left(\frac{3\cos^2\beta - 1}{2}\right) \qquad P_o = \frac{1+3\cos(2\beta)}{7+\cos(2\beta)}$$

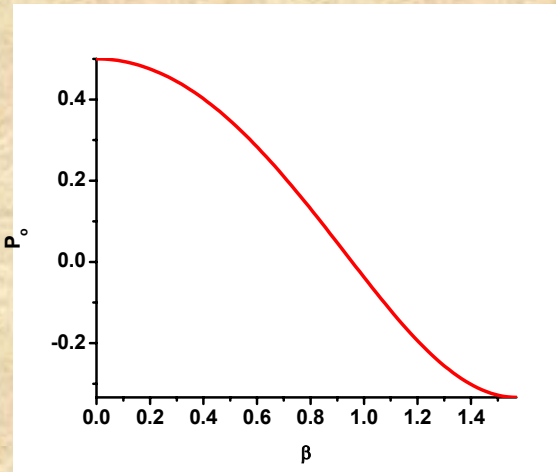
where β is the angle between absorption and emission dipoles.



Note that we ignored rotations of the dipole in our model (we will discuss the effect of rotational diffusion later). The polarization values, in the absence of rotation (and other depolarization effects such as energy transfer), are termed limiting or intrinsic polarizations and are denoted as P_o. Similarly, the limiting anisotropy is denoted r_o.

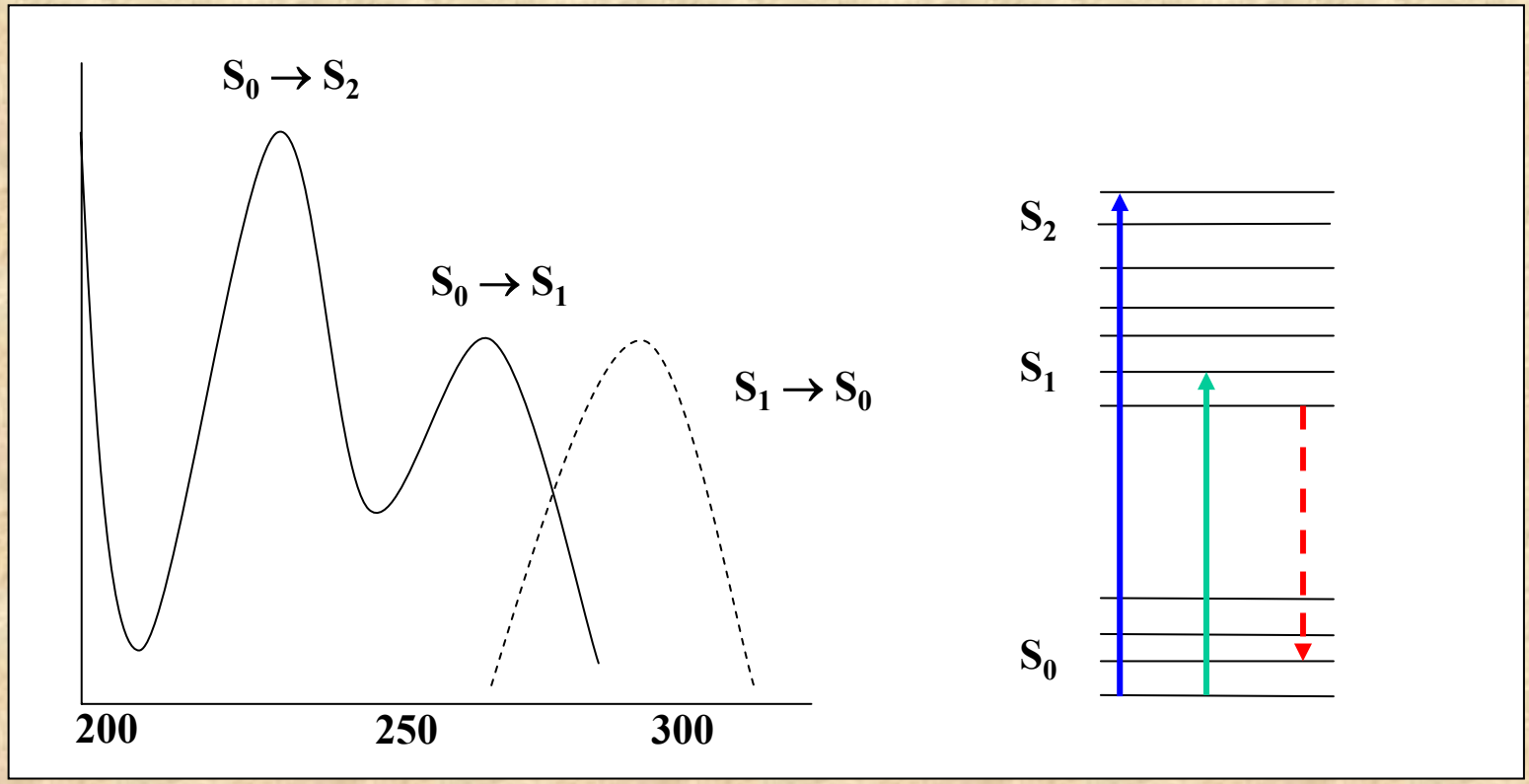
Plot of the intrinsic polarization as a function of the angle between the ground and excited state dipole of the molecule.

Note that the intrinsic polarization P_o has a maximum of $\frac{1}{2}$ (ground and excited state dipoles are parallel) and a minimum of – 1/3 (ground and excited state dipoles are normal to each other).



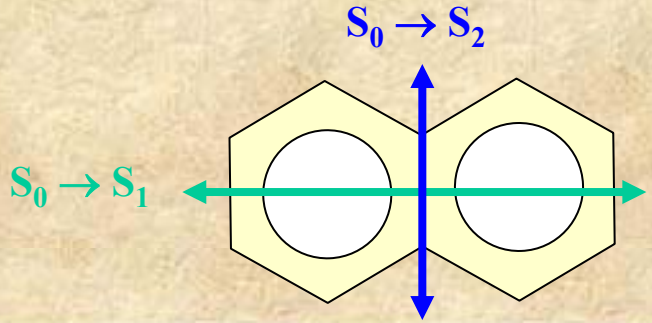
Example

Consider a molecule with the following attributes:



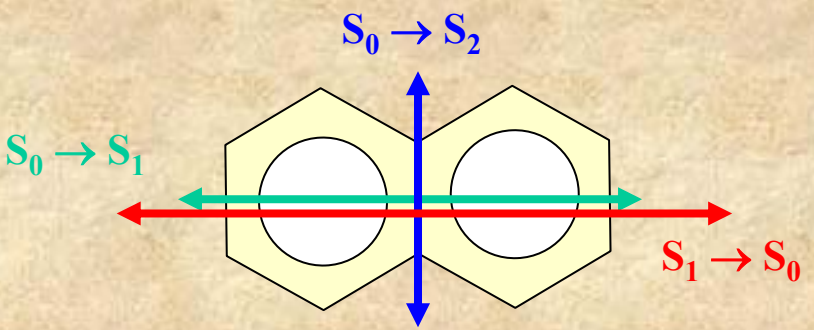
Here are depicted two principle absorption bands for a compound along with the emission band. The energy level diagram corresponding to this system is also depicted.

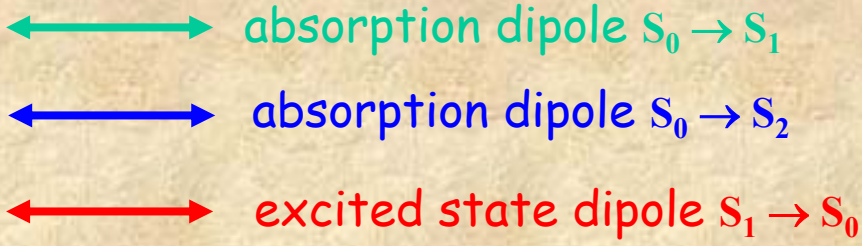
The directions of the absorption dipoles – relative to the nuclear framework – may differ greatly for the two transitions as illustrated on the right.



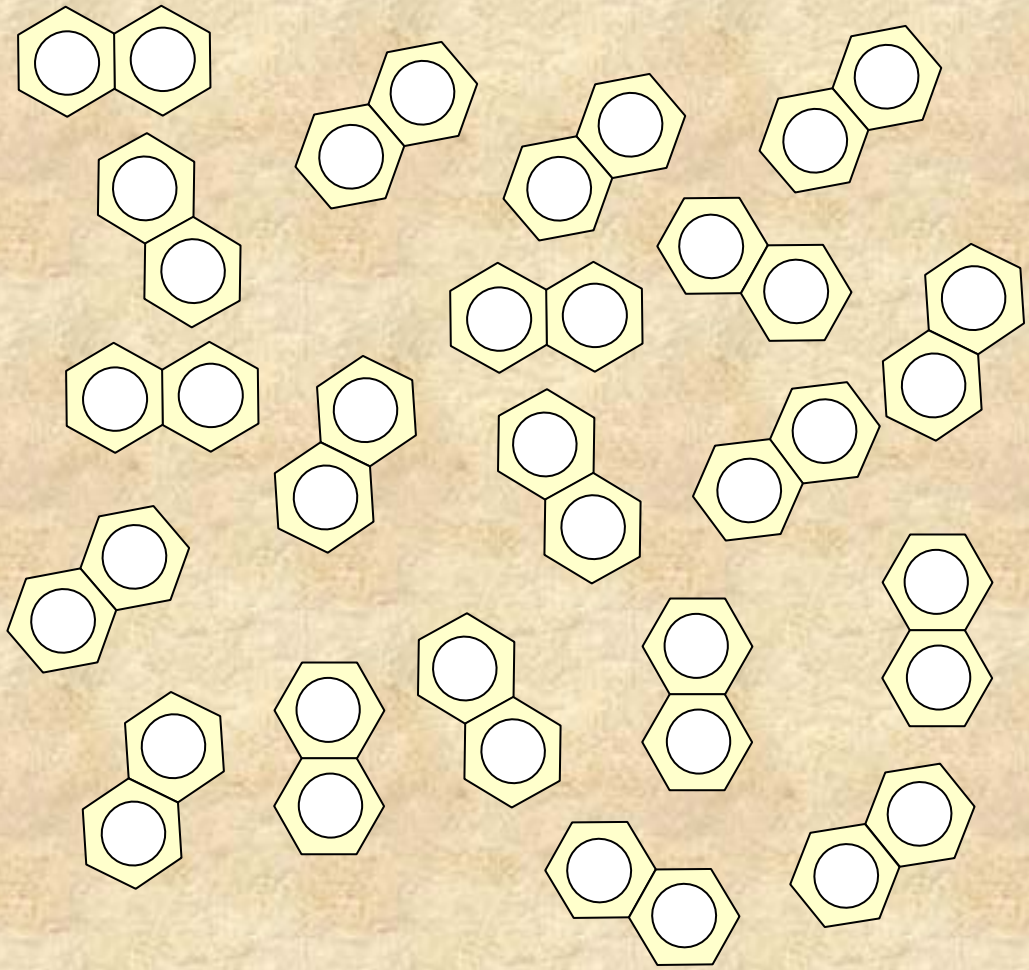
The two excited dipoles corresponding to the $S_0 \rightarrow S_1$ and the $S_0 \rightarrow S_2$ transitions may be oriented at an arbitrary angle. After the excitation process, however, regardless of whether the absorption process corresponded to the $S_0 \rightarrow S_1$ or the $S_0 \rightarrow S_2$ transition, rapid thermalization leaves the excited fluorophore in the S_1 level.

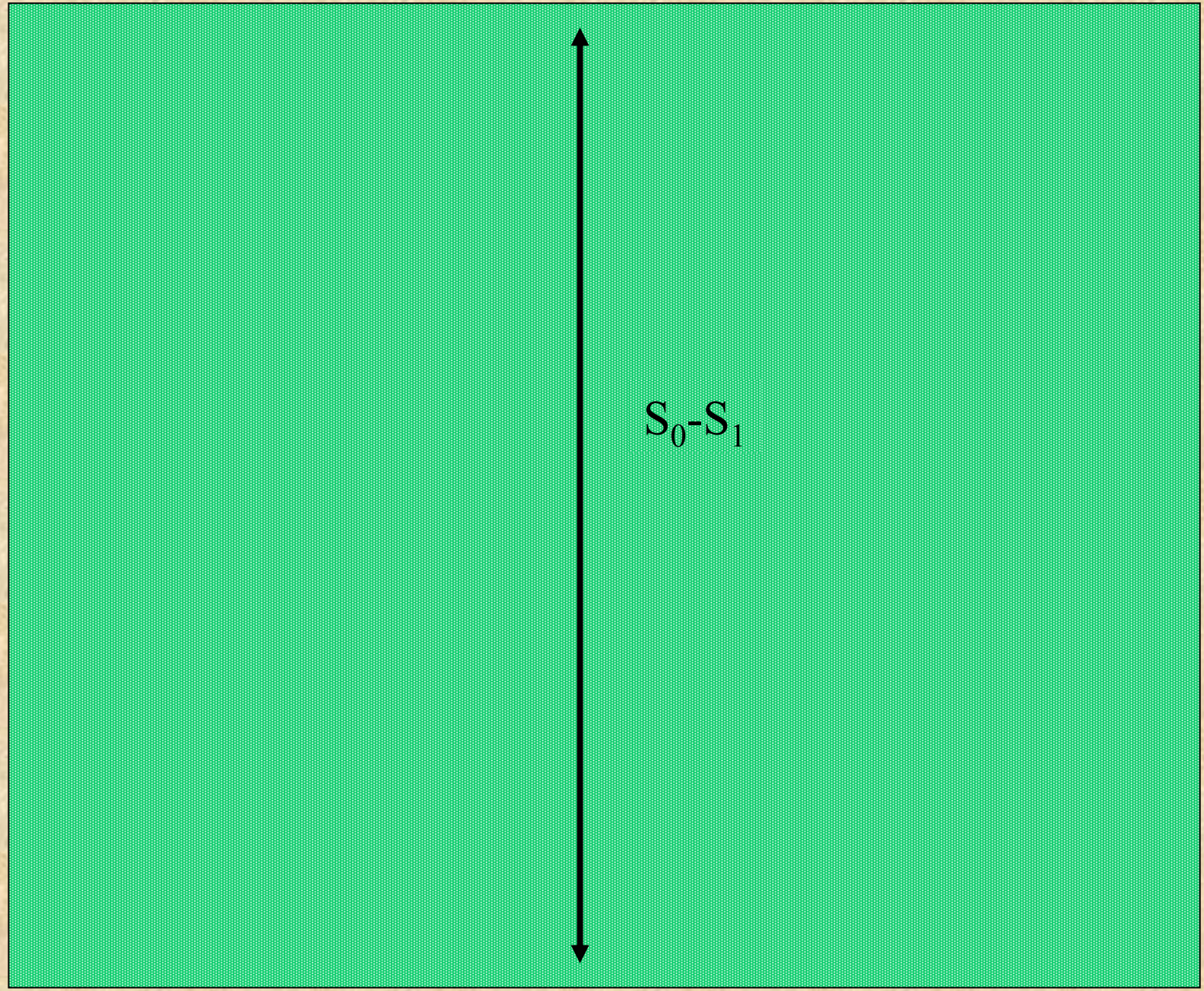
Thus the important dipole moments are the absorption dipoles for the $S_0 \rightarrow S_1$ and the $S_0 \rightarrow S_2$ transitions and the excited state dipole for the $S_1 \rightarrow S_0$ transition. Consider the following situation:

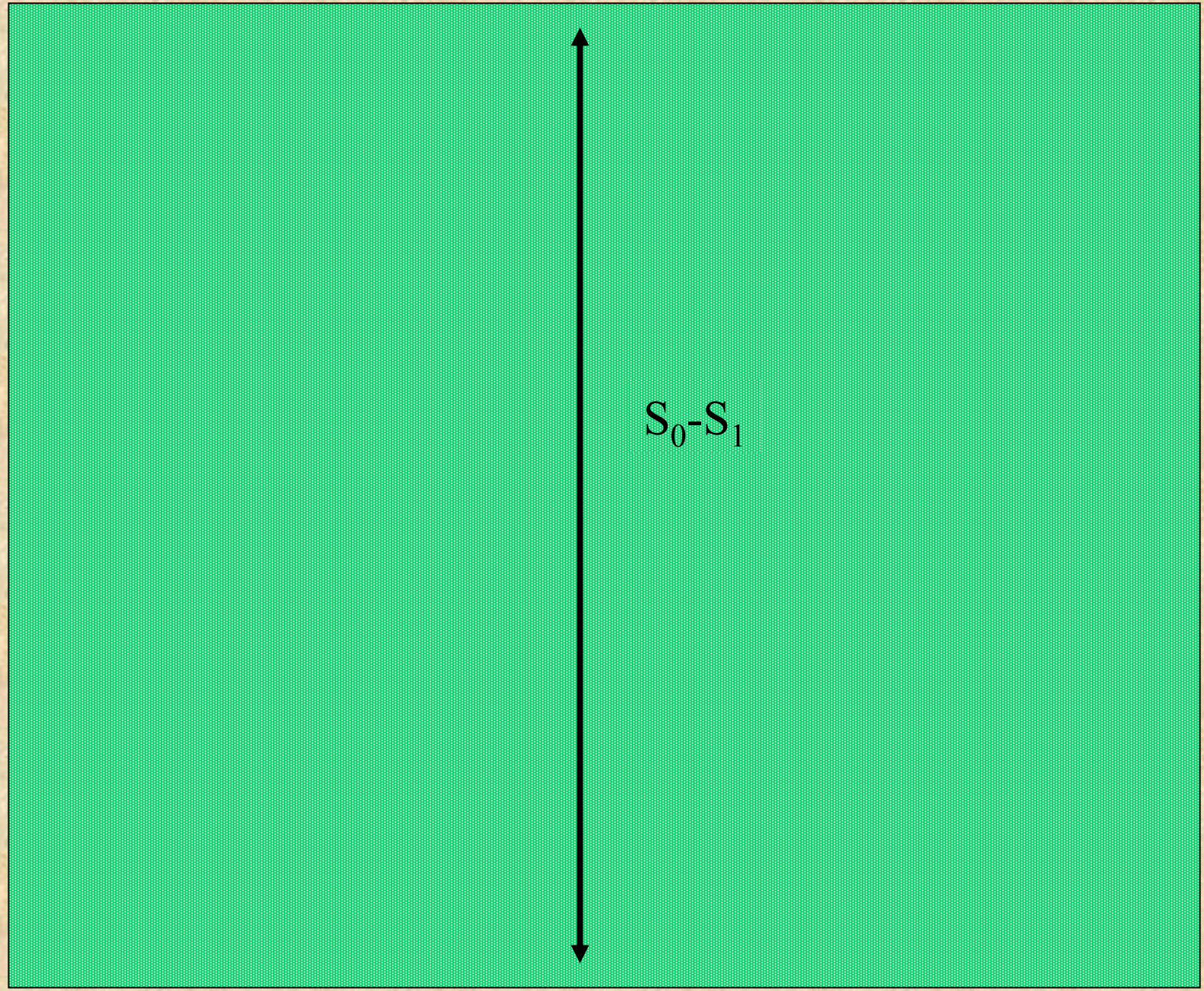


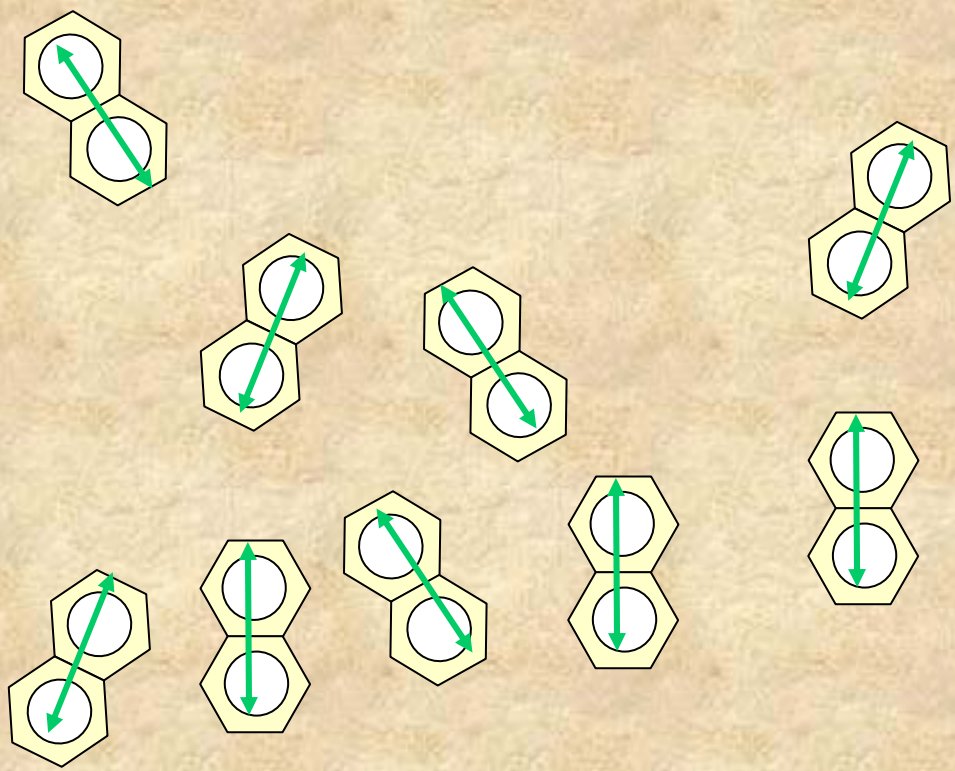


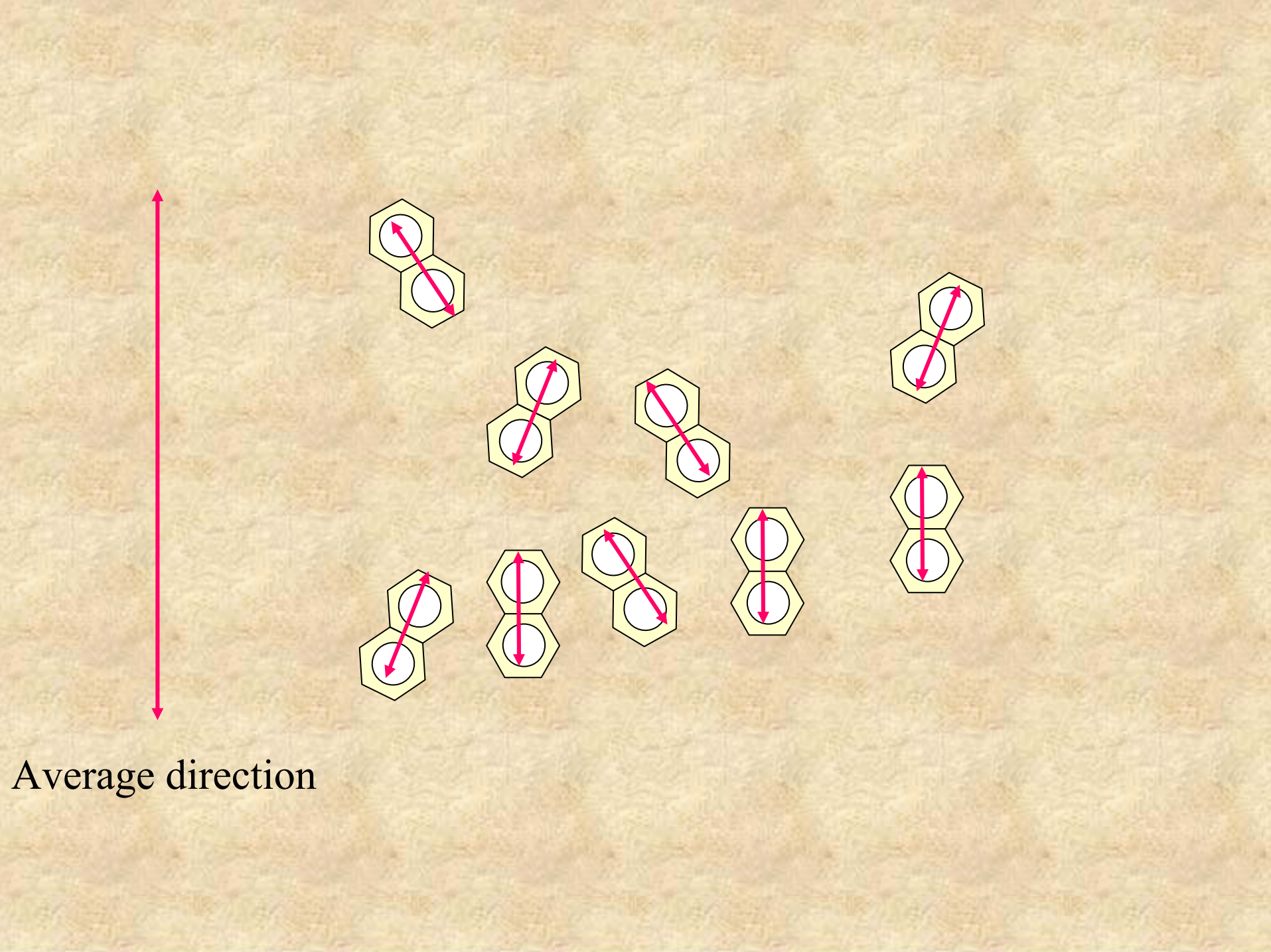
Thus the two absorption dipoles originally photoselected by the exciting light will lead to excited dipoles which differ in their average orientation as illustrated in the next few slides …

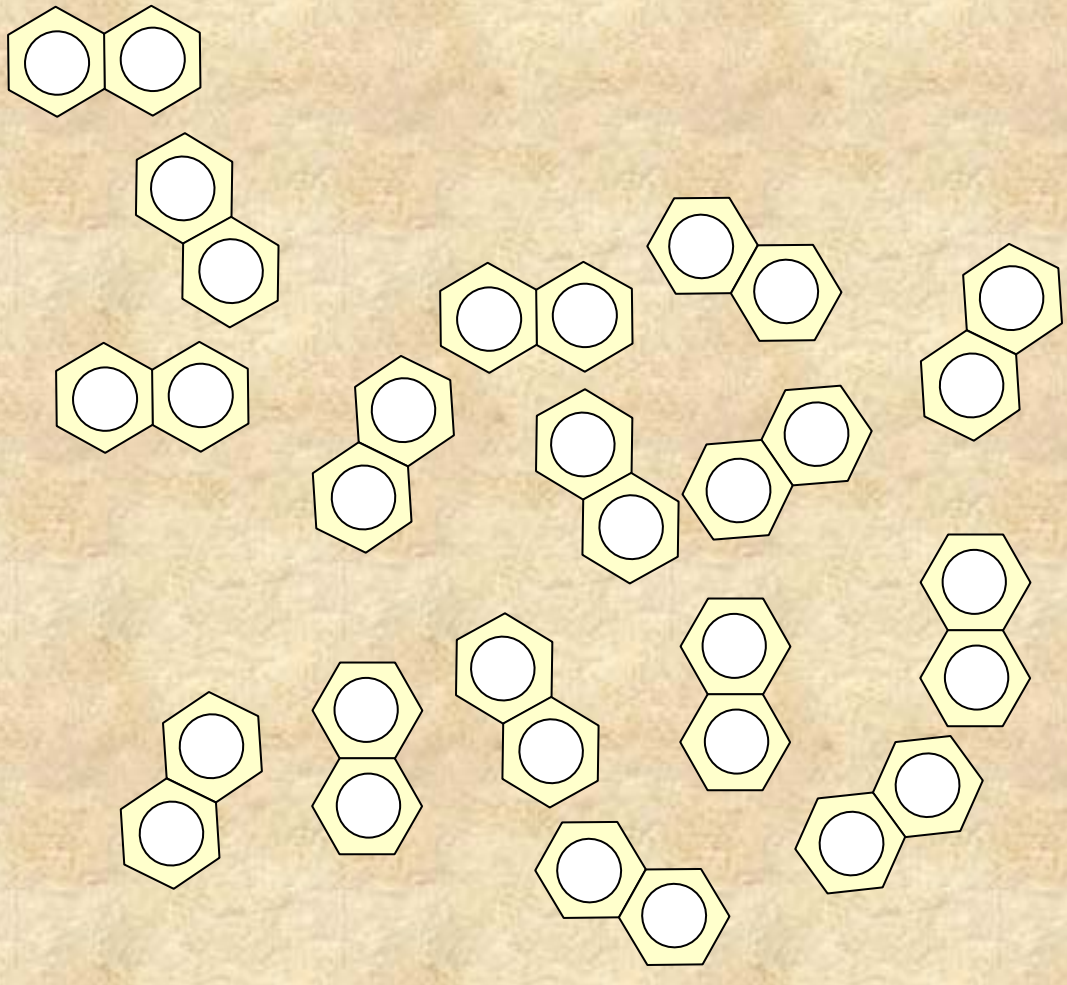


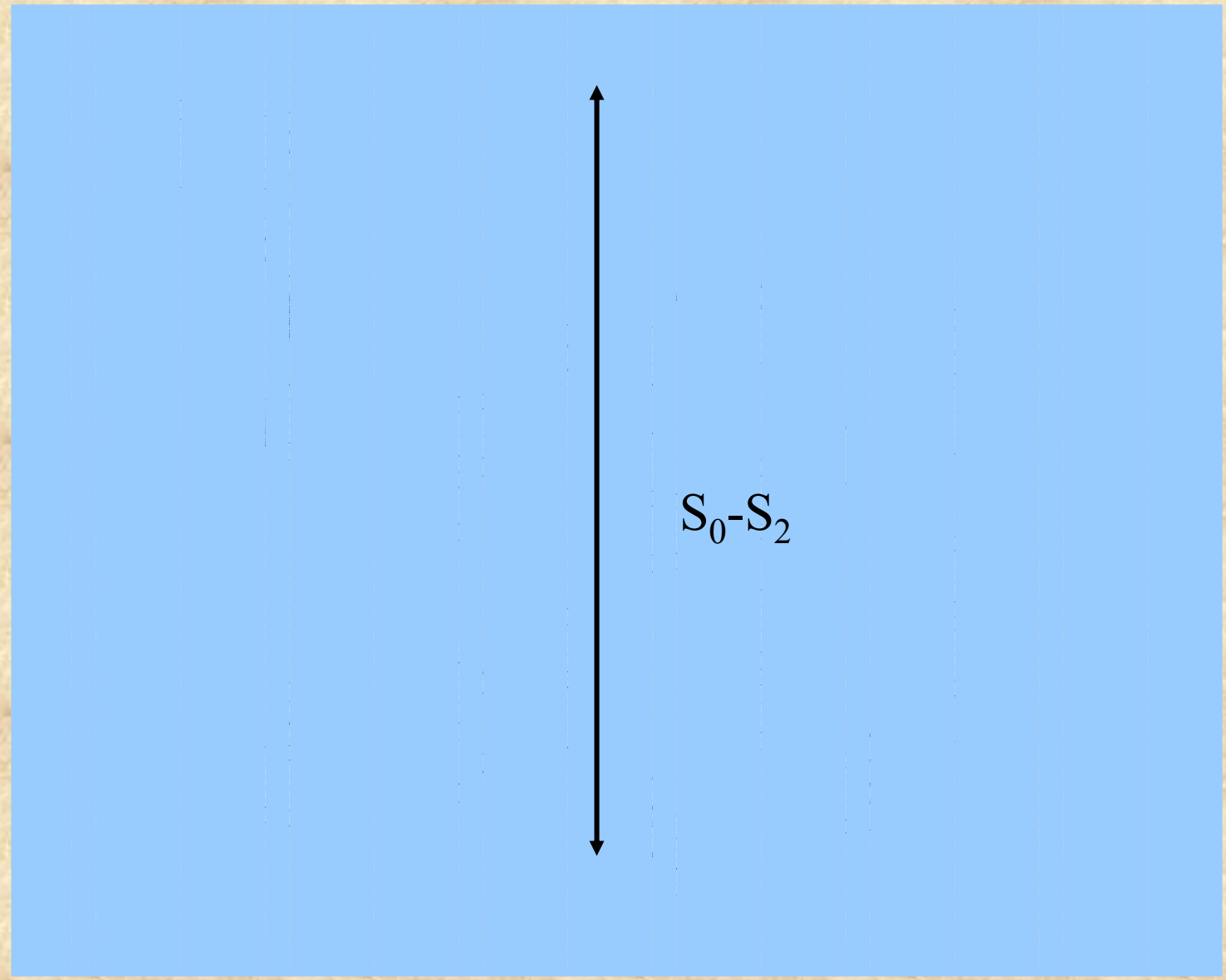

S_0-S_1

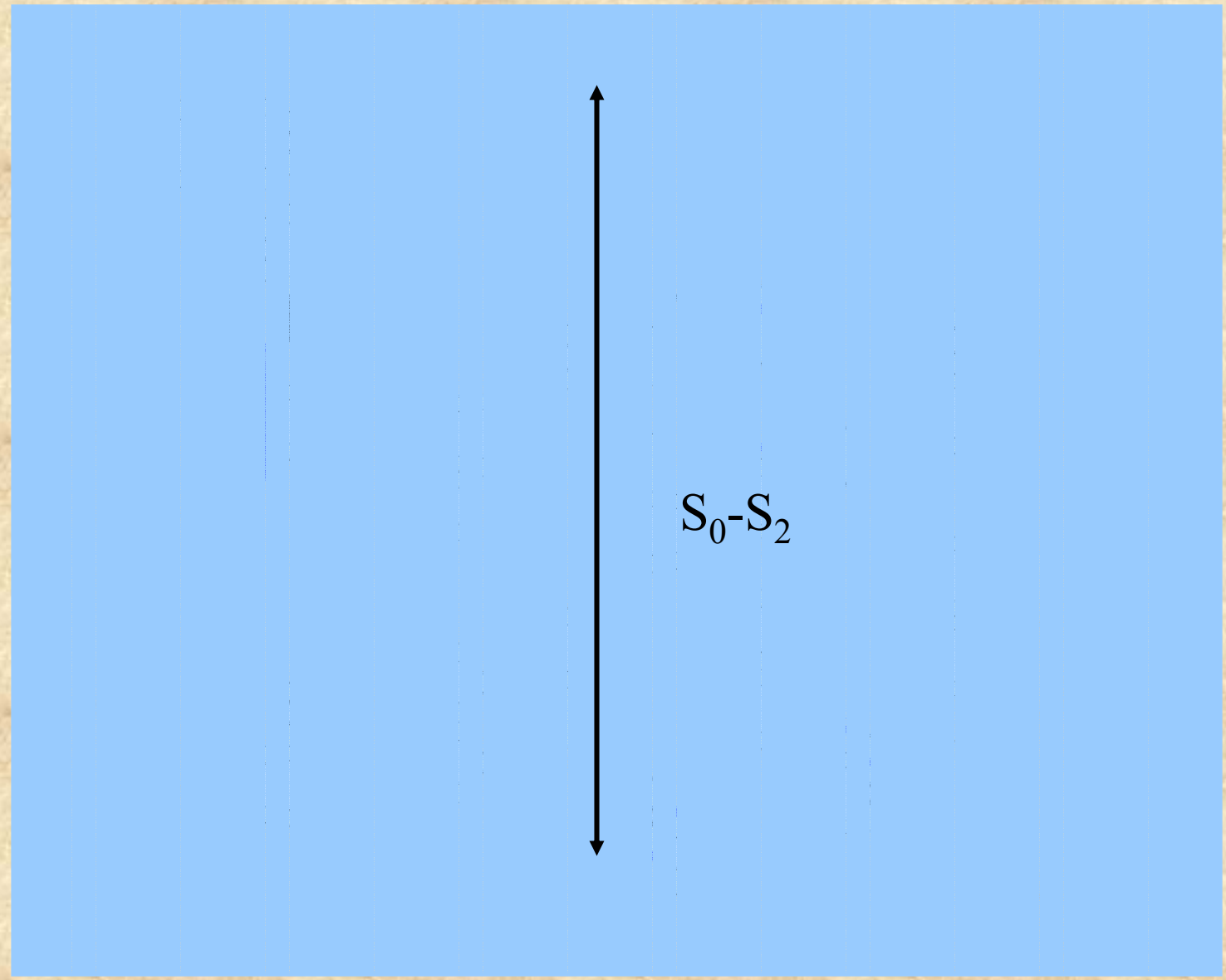

S_0-S_1

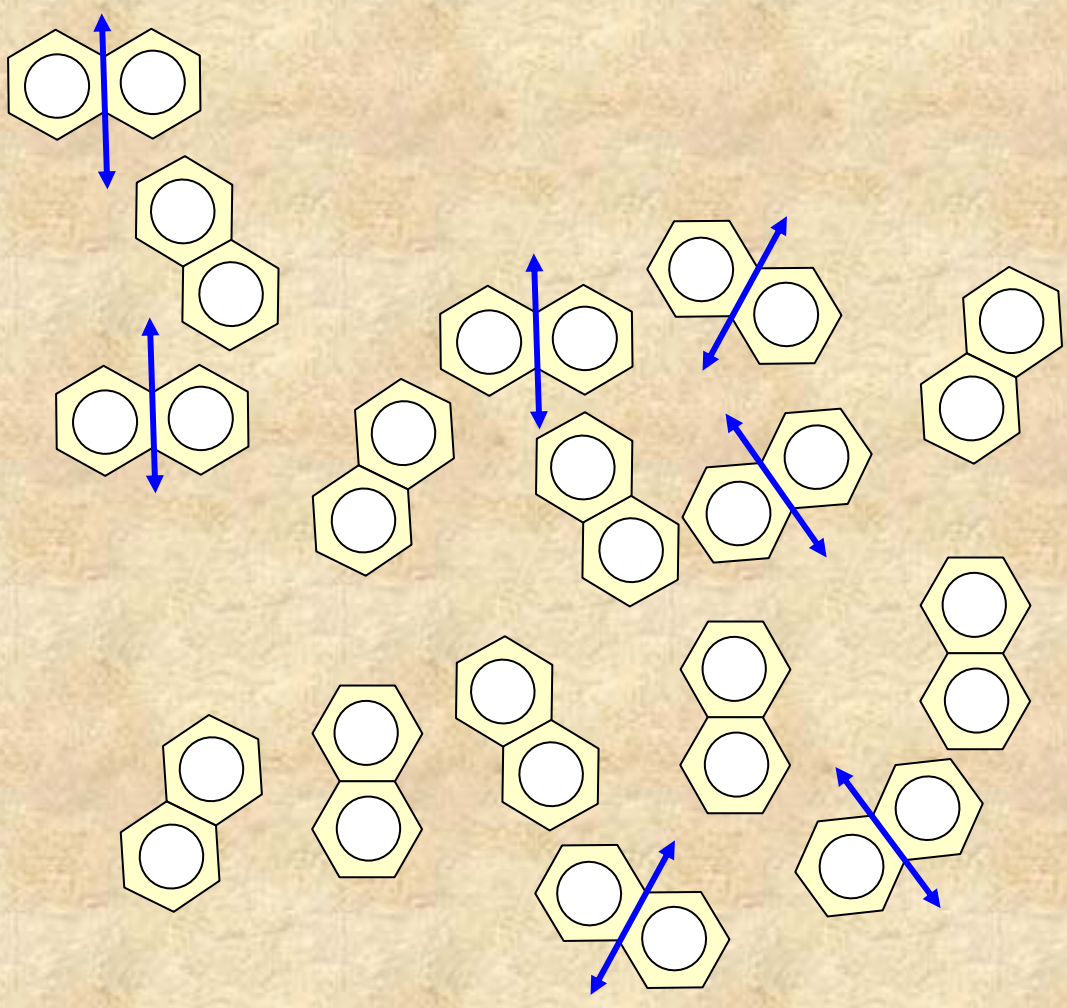


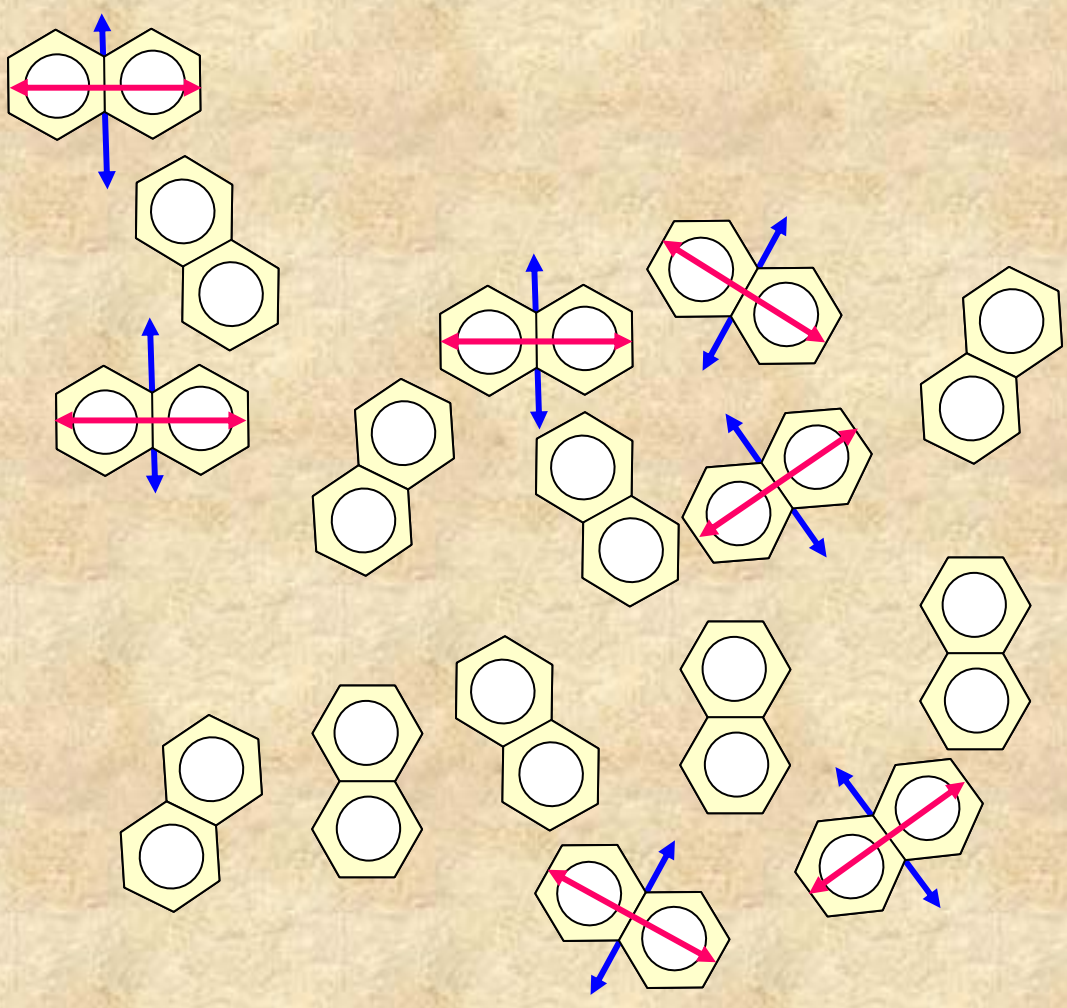

Average direction

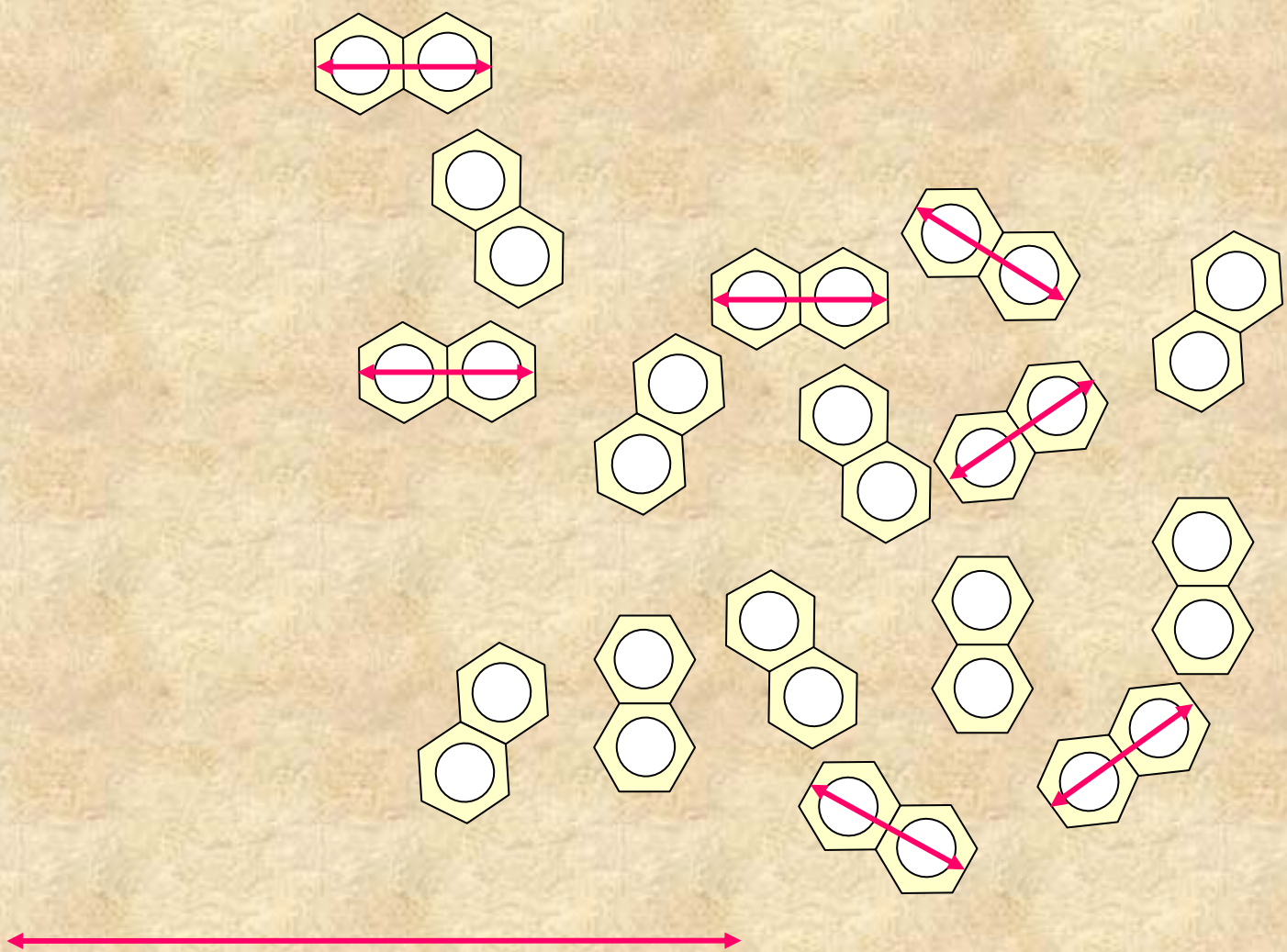



S_0-S_2


S_0-S_2

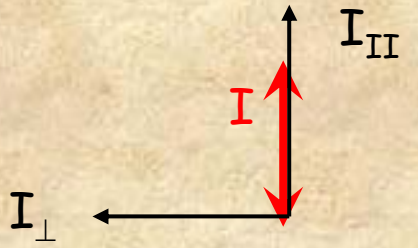





Average direction

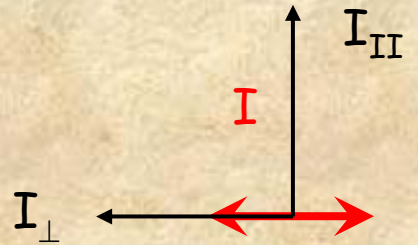
This example illustrates how the limiting polarization of a fluorophore can depend upon the excitation wavelength.

for $S_0 \rightarrow S_1$ absorption we observed predominantly vertically polarized emission. Thus the polarization value is positive



$$P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp} > 0$$

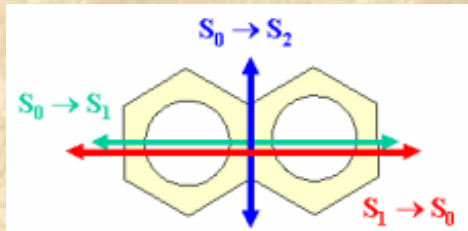
for $S_0 \rightarrow S_2$ absorption we observed predominantly horizontally polarized emission. Thus the polarization value is negative



$$P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp} < 0$$

This can also be seen by the equation describing the limiting polarization (or anisotropy):

$$P_o = \frac{1 + 3\cos(2\beta)}{7 + \cos(2\beta)} \qquad r_o = \frac{2}{5}\left(\frac{3\cos^2\beta - 1}{2}\right)$$



with $\beta = 0^0$ for $S_0 \rightarrow S_1$ and $\beta = 90^0$ for $S_0 \rightarrow S_2$

Consider the excitation polarization spectrum for phenol (in glycerol at - 70 C).

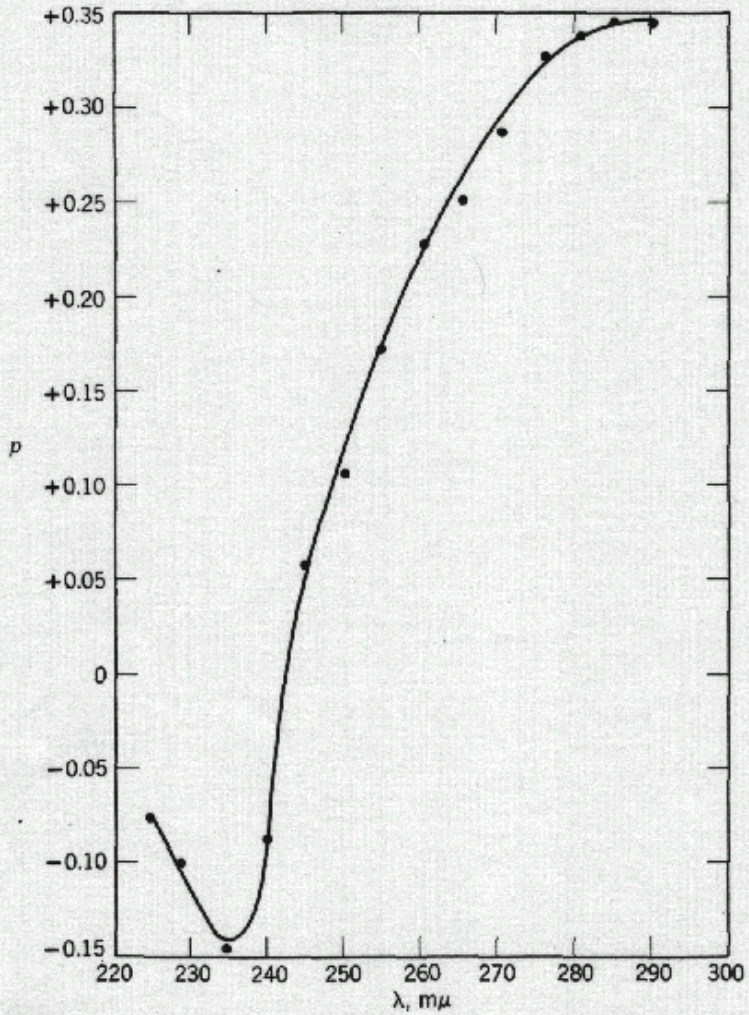


Fig. 8-5. Fluorescence polarization spectrum of phenol at −70°C in propylene glycol. Ordinate = polarization, p; abscissa, exciting wavelength in mμ. Redrawn from Weber (18).

In cases where there are multiple overlapping absorption bands at various angles, the excitation polarization spectrum can be somewhat complex as shown below for indole.

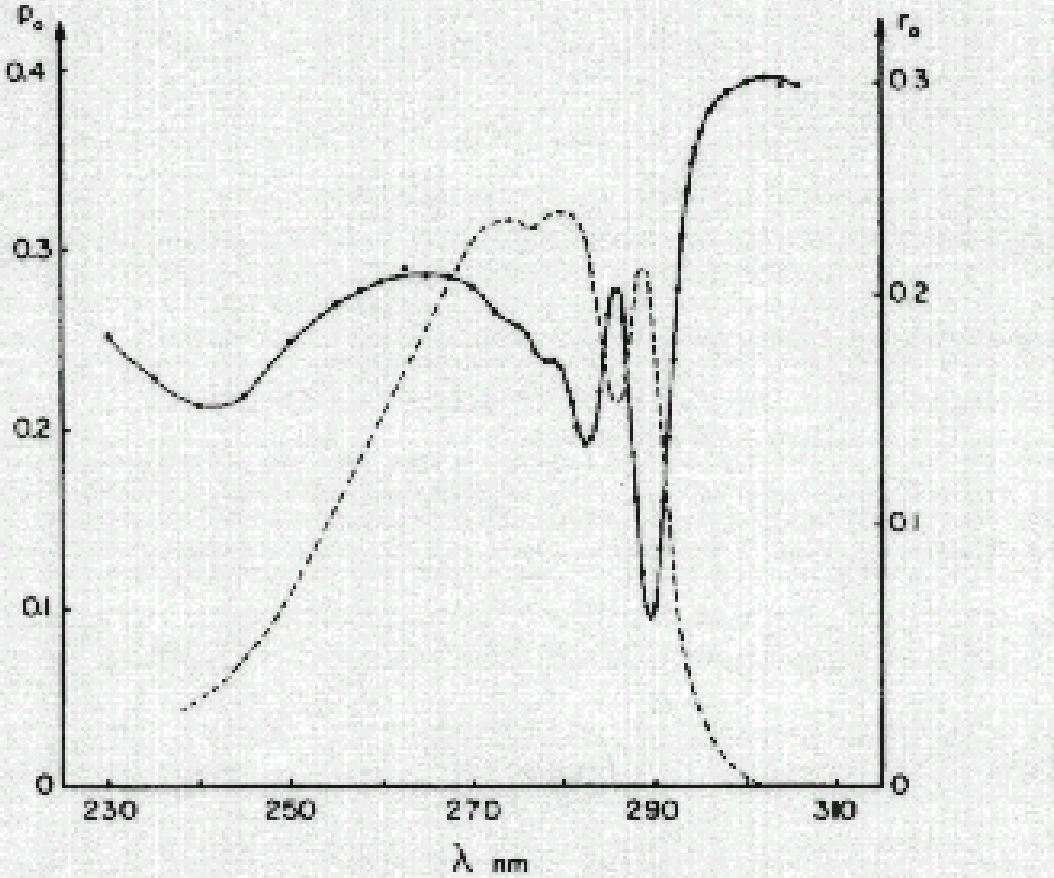


Figure 2. Corrected excitation spectrum (broken line) and excitation polarization spectrum of indole in propylene glycol at −58 . The fluorescence is observed through a Corning 7-39 filter.

Excitation polarization spectra of rhodamine B embedded in a Lucite matrix at room temperature. Emission was viewed through a cut-on filter passing wavelengths longer than 560nm; slits were ~4nm.