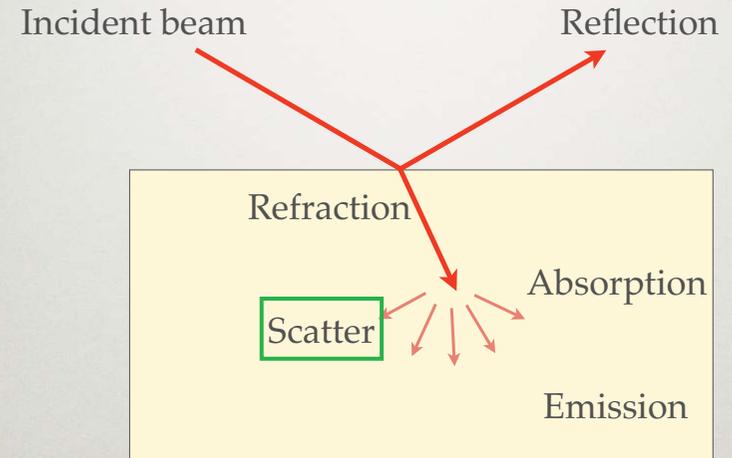


# INTERACTION OF LIGHT WITH MATTER:

## SCATTER, ABSORPTION, EMISSION, LUMINESCENCE

MIKLÓS KELLERMAYER

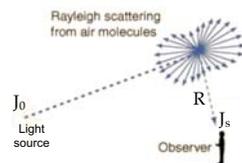
# INTERACTION OF LIGHT WITH MATTER



# LIGHT SCATTERING



Lord Rayleigh (1842-1919)



- Elastic collision: photon energy (wavelength) does not change
- Emission by resonating dipoles

$$J_s = J_0 \frac{8\pi^4 N \alpha^2}{\lambda^4 R^2} (1 + \cos^2 \Theta)$$

$J_s$  = intensity of scattered light  
 $J_0$  = intensity of incident light  
 $N$  = number of scattering particles  
 $\alpha$  = polarizability (dipole moment per electric field)  
 $\lambda$  = wavelength of light  
 $R$  = distance between scatterer and observer  
 $\Theta$  = angle of scattered light

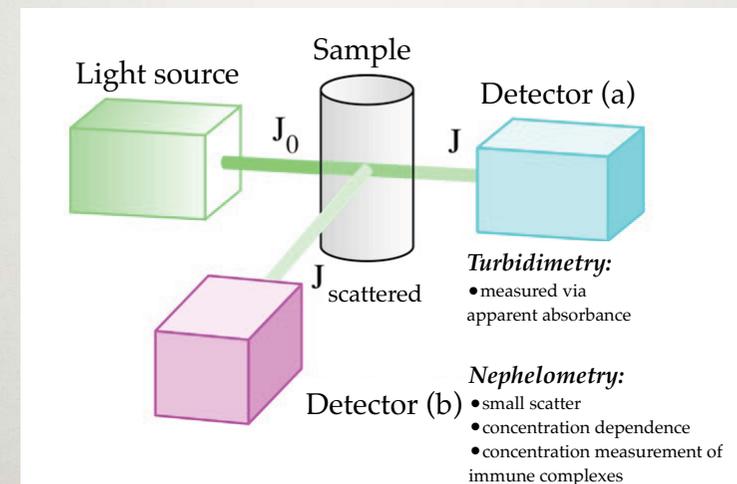


Strong wavelength dependence  $\rightarrow$  enhancement of short wavelengths  $\rightarrow$  blue sky

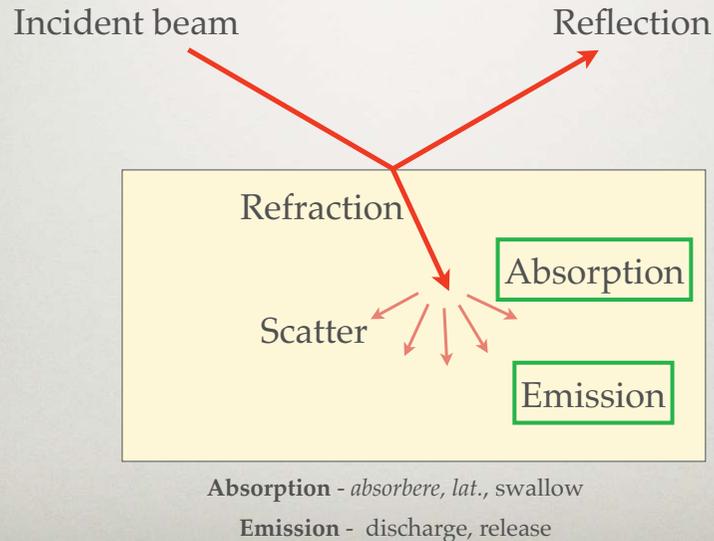


If scatterers are interacting particles the the overall size of which is comparable to the wavelength  $\rightarrow$  interference, cancellation  $\rightarrow$  gray clouds

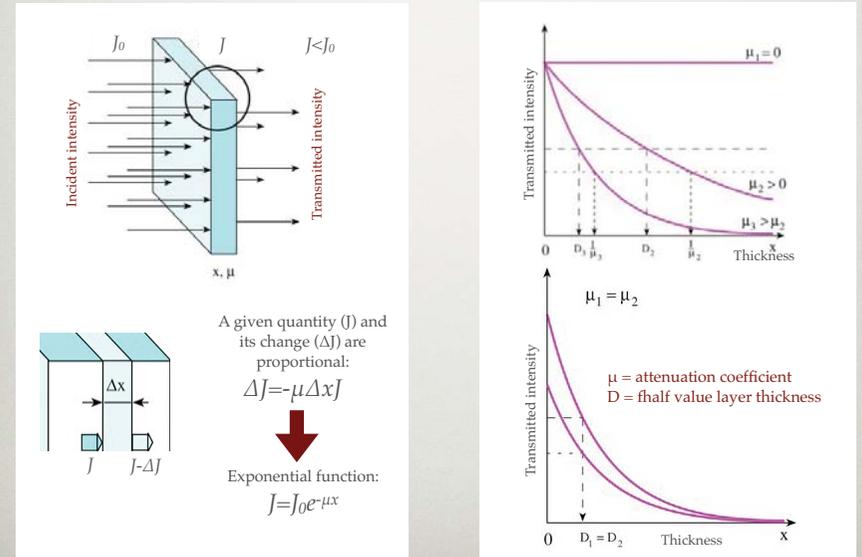
# BIOMEDICAL APPLICATIONS OF LIGHT SCATTERING



# INTERACTION OF LIGHT WITH MATTER



# GENERAL ABSORPTION (ATTENUATION) LAW

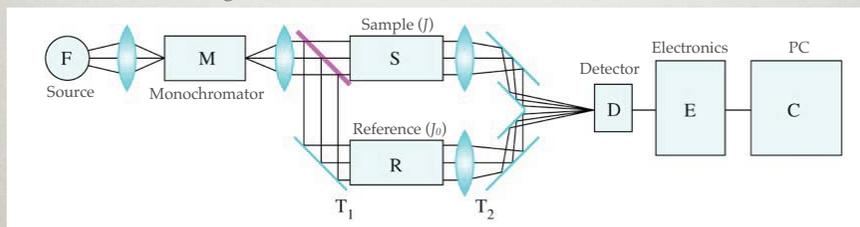


# PARAMETERS AND MEASUREMENT OF ABSORPTION

Absorbance (A):  $A = \lg \frac{J_0}{J} = \lg e \cdot \mu \cdot x$  Dimensionless number  
Synonyms: extinction, optical density (OD)

Transmittance (T):  $T = \frac{J}{J_0} \cdot 100$  Expressed in percent (%)  
Synonym: transmission coefficient

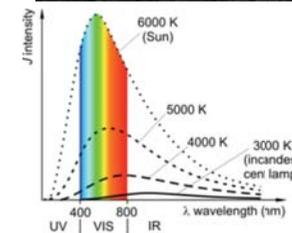
Photometry ("measurement of light"):



# SOURCE OF EMISSION

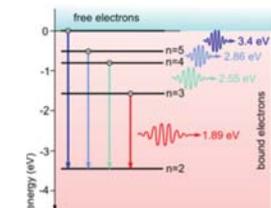
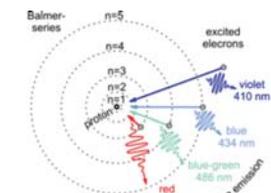
1. Thermal (black body) radiation

Mechanism: thermal motion of atoms, molecules

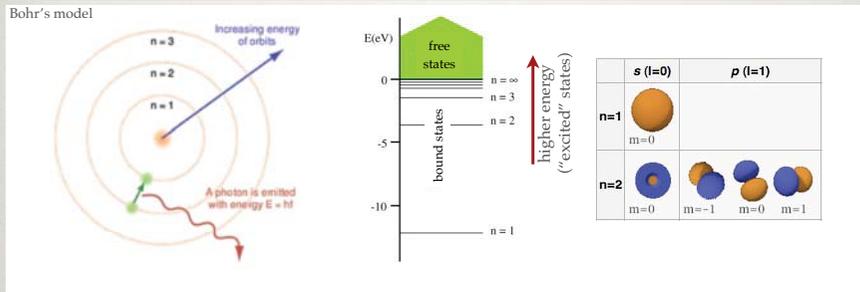


2. Luminescence

Mechanism: emission of excited-state energy



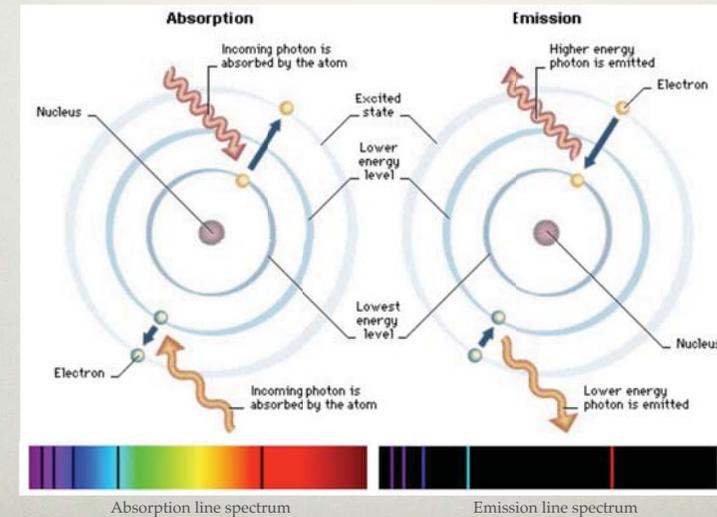
# ABSORPTION AND EMISSION DEPEND ON THE ATOMIC STRUCTURE



name	symbol	orbital meaning	range of values	value example
principal quantum number	$n$	shell	$1 \leq n$	$n = 1, 2, 3, \dots$
azimuthal quantum number (angular momentum)	$\ell$	subshell	$(0 \leq \ell \leq n - 1)$	for $n = 3$ : $\ell = 0, 1, 2$ (s, p, d)
magnetic quantum number, (projection of angular momentum)	$m_\ell$	energy shift	$-\ell \leq m_\ell \leq \ell$	for $\ell = 2$ : $m_\ell = -2, -1, 0, 1, 2$
spin projection quantum number	$m_s$	spin	$-\frac{1}{2}, \frac{1}{2}$	for an electron, either: $-\frac{1}{2}, \frac{1}{2}$

- **Principal quantum number:** distance of electron from nucleus (energy)
- **Azimuthal (angular or orbital) quantum number:** shape of atomic orbital
- **Magnetic quantum number:** orientation of orbital in magnetic field
- **Spin quantum number:** intrinsic angular momentum of the electron

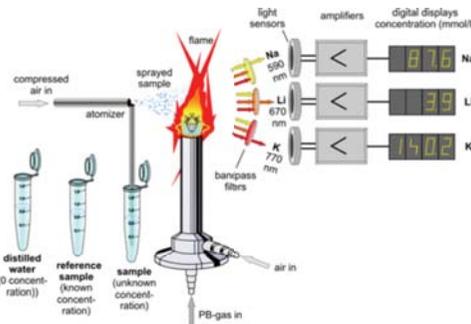
# ABSORPTION AND EMISSION BY AN ATOM



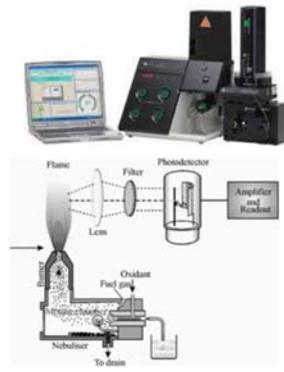
# APPLICATION OF EMISSION SPECTROSCOPY

## Flame photometry

Qualitative and quantitative analysis of alkali metals

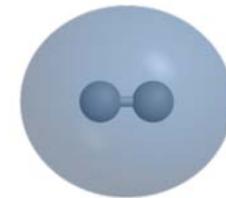


Clinical practice: determination of serum ions ( $\text{Na}^+$ ,  $\text{K}^+$ )



# STATE OF A MOLECULE IS AFFECTED BY ITS MOTIONAL MODES

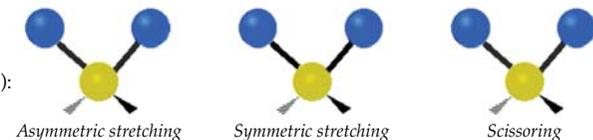
Molecule: atoms connected by chemical bonds  
Simplest case: diatomic molecule (e.g., hydrogen molecule)



Molecules *vibrate* and *rotate*!

**Vibration:** periodic motion *along* the axis of the covalent bond  
**Rotation:** periodic motion *around* the axis of the covalent bond

Examples of vibrational motion in the triatomic methylene group ( $-\text{CH}_2-$ ):



# ENERGY OF A MOLECULE



Max Born  
(1882-1970)



J. Robert Oppenheimer  
(1904-1967)

Born-Oppenheimer approximation:

$$E_{total} = E_e + E_v + E_r$$

### Important notions:

- Types of energy states are independent (not coupled)
- Energy states are non-continuous, but discrete
- Transition between states involves packets (quanta) of energy
- Scales of transition energies between different states are different:

$$E_e \sim^{100x} E_v \sim^{100x} E_r$$

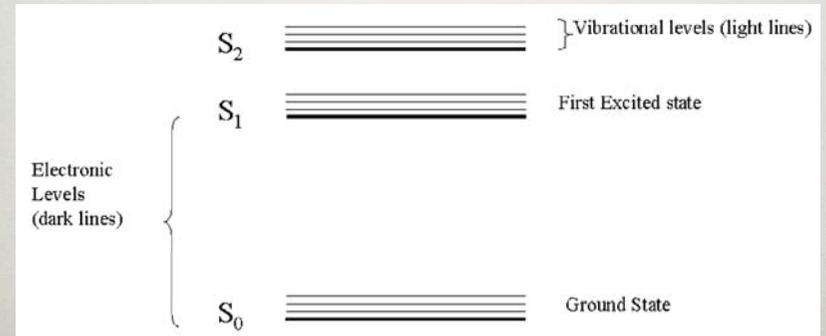
$$\sim 3 \times 10^{-19} \text{ J } (\sim 2 \text{ eV}) > \sim 3 \times 10^{-21} \text{ J} > \sim 3 \times 10^{-23} \text{ J}$$

# REPRESENTATION OF ENERGY STATES

Jabłoński diagram:  
illustrates the electronic states of a molecule and the transitions between them (with arrows)



Alexander Jabłoński  
(1898-1980)



# SPIN STATES

Wolfgang Pauli  
(1900-1958)



### Pauli's exclusion principle:

- Each quantum state can be occupied by a single electron.
- Within an atom there cannot be two electrons for which all four quantum numbers are identical.



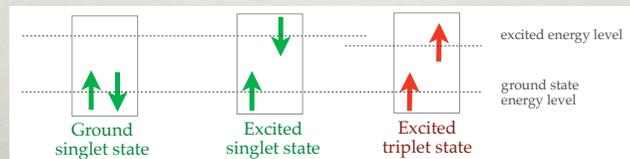
fully occupied subshell: spin pairing  
(opposite-spin electrons pair)

### Singlet and triplet states:

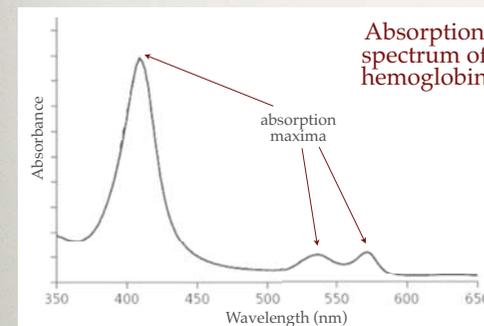
number of orientations of magnetic moment associated with net spin state (in magnetic field) =  $2S+1 = 1$  (singlet) or  $3$  (triplet). ( $S$  = net spin, e.g., in fully occupied subshell  $(+1/2)+(-1/2) = 0$ )

**S: singlet state:** paired electrons with opposite spins, net spin ( $S$ ) =  $0$ , number of orientations ( $2S+1$ ) =  $1$ .

**T: triplet state:** there are identical spin-state electrons in the molecule, net spin =  $1$  (e.g.,  $(+1/2)+(+1/2) = 1$ ), number of orientations ( $2S+1 = 2+1$ ) =  $3$ .



# MOLECULES HAVE BAND ABSORPTION SPECTRA



General attenuation law:

$$A = \lg \frac{J_0}{J} = \lg e \cdot \mu \cdot x$$

For dilute solutions - Lambert-Beer law:

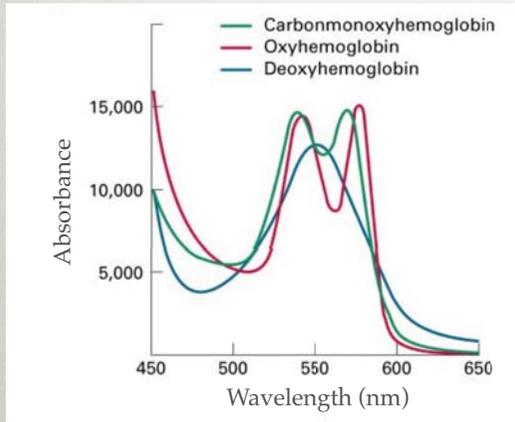
$$A_\lambda = \lg \frac{J_0}{J} = \epsilon_\lambda \cdot c \cdot x$$

$\epsilon_\lambda$  = molar extinction coefficient  
 $c$  = concentration

- SI unit of molar extinction coefficient ( $\epsilon_\lambda$ ):  $\text{m}^2 \cdot \text{mol}^{-1}$
- Method ideal for concentration measurement
- Based on the wavelength (at maximum) the transition energy may be calculated:

$$E_2 - E_1 = E_{\text{photon}} = h \cdot f = h \cdot \frac{c}{\lambda}$$

# ABSORPTION SPECTROSCOPY



- **Spectrum:** intensity (or its derived units, e.g., OD) as a function of photon energy (or its derived units, e.g., frequency, wavelength).
- **Spectroscopy:** qualitative analysis of the spectrum.
- **Spectrometry, spectrophotometry:** quantitative analysis of the spectrum.
- **Applications:** analysis of chemical structure, concentration measurement, etc.

# EMISSION BY LUMINESCENCE: EVERYWHERE



Photoluminescence

# LUMINESCENCE EVERYWHERE



Display lights

Aurora borealis

Radioluminescence

# LUMINESCENCE EVERYWHERE



Bioluminescence

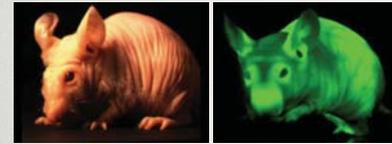
# LUMINESCENCE EVERYWHERE



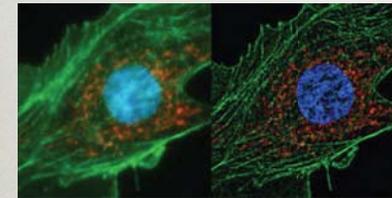
Bioluminescence  
Firefly



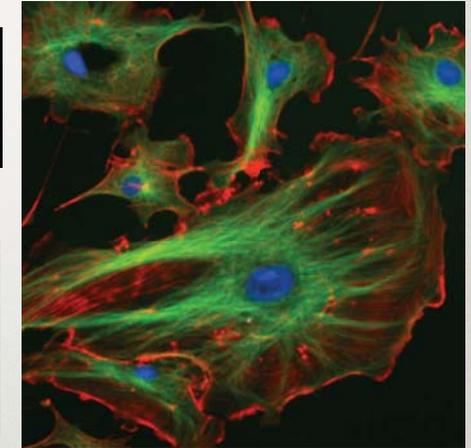
# LUMINESCENCE EVERYWHERE



GFP-mouse (green nude mouse)



Superresolution microscopy (Nobel-prize 2014)



Epifluorescence microscopy (cytoskeletal system)

Fluorescence

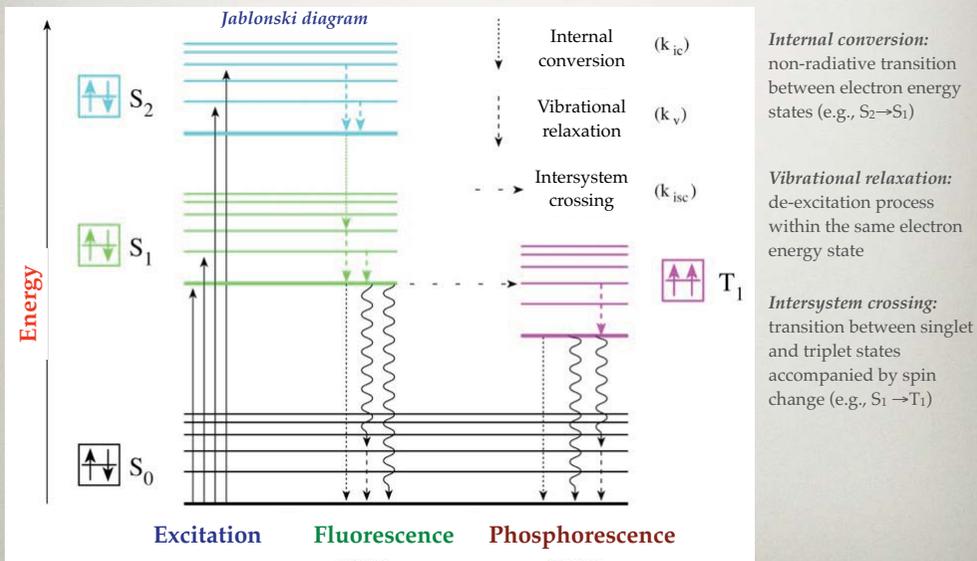
# DEFINITIONS OF LUMINESCENCE

- Relaxation from excited state followed by light emission
- Radiation emitted by matter in excess of thermal emission
- "Cold light"
- Processes of fluorescence and phosphorescence

# TYPES OF LUMINESCENCE

(a) Excitation Mode	Luminescence Type
absorption of radiation (UV/VIS)	photoluminescence
chemical reaction	chemiluminescence, bioluminescence
thermally activated ion recombination	thermoluminescence
injection of charge	electroluminescence
high energy particles or radiation	radioluminescence
friction	triboluminescence
sound waves	sonoluminescence
(b) Excited State (Assuming Singlet State)	Luminescence Type
first excited singlet state	fluorescence, delayed fluorescence
lowest triplet state	phosphorescence

# PROCESSES OF LUMINESCENCE



**Internal conversion:**  
non-radiative transition between electron energy states (e.g.,  $S_2 \rightarrow S_1$ )

**Vibrational relaxation:**  
de-excitation process within the same electron energy state

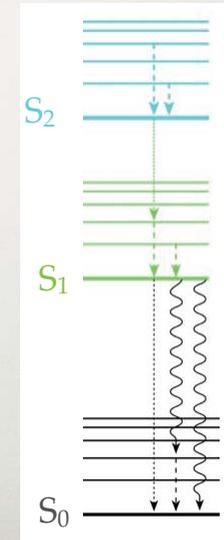
**Intersystem crossing:**  
transition between singlet and triplet states accompanied by spin change (e.g.,  $S_1 \rightarrow T_1$ )

# KASHA'S RULE

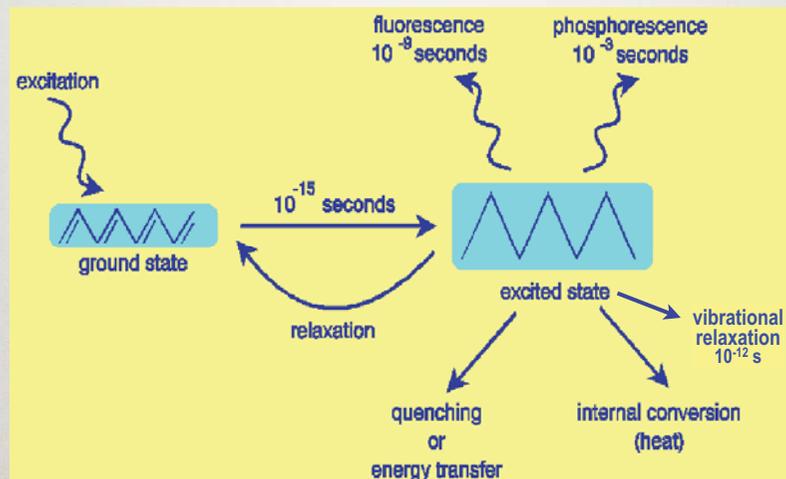
**Photon emission** (fluorescence or phosphorescence) occurs only from the lowest-energy excited electronic state (i.e.,  $S_1$  or  $T_1$ ) of a molecule.



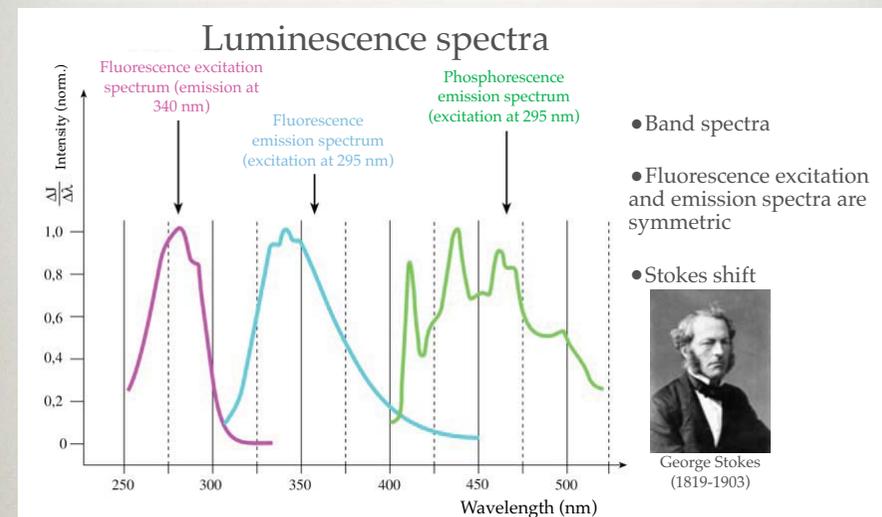
Michael Kasha (1920-)  
American physicist



# TIME SCALE OF TRANSITIONS



# CHARACTERIZATION OF LUMINESCENCE I.



Fluorescent dyes: "fluorophores"  
By the specific attachment fluorophores, non-fluorescent molecules may also be studied (fluorescent labeling)

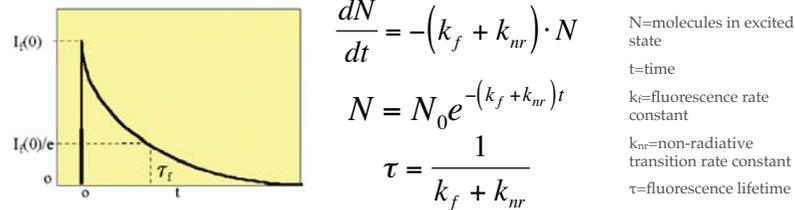
# CHARACTERIZATION OF LUMINESCENCE II.

## Quantum yield

$$\Phi = \frac{\text{number of emitted photons}}{\text{number of absorbed photons}} \leq 1 \quad \Phi = \frac{k_f}{k_f + k_{ic} + k_{isc} + k_Q}$$

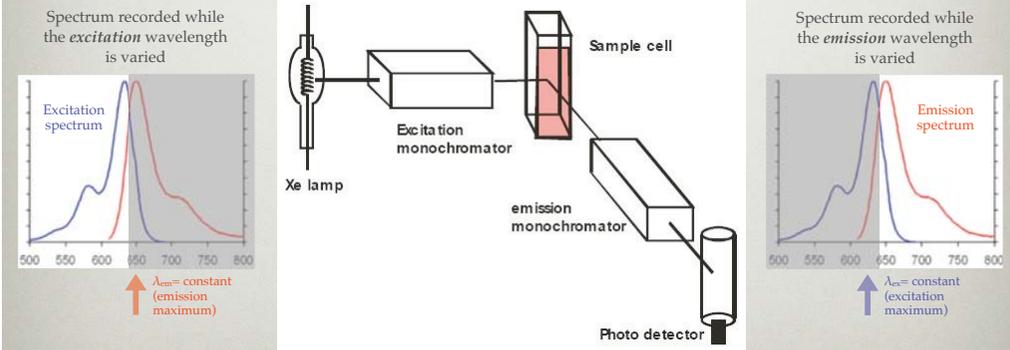
$k_{nr}$ =non-radiative transition rate constants

## Excited-state lifetime



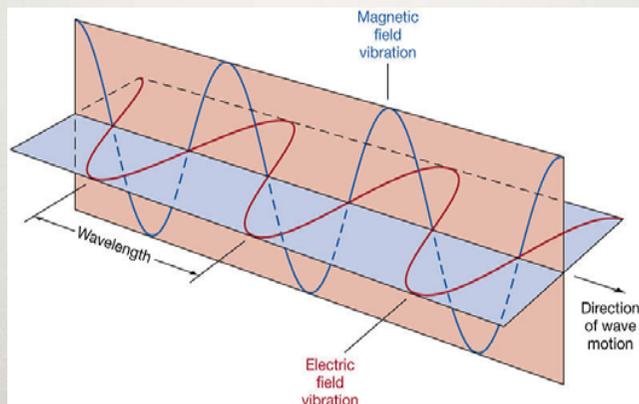
# MEASUREMENT OF FLUORESCENCE

## Fluorescence spectrometer ("Steady-state" spectrofluorometer)

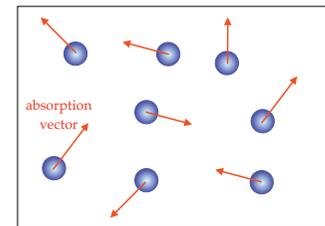


# LIGHT IS ELECTROMAGNETIC RADIATION

- Propagating electromagnetic disturbance.
- Transverse wave.
- Therefore, it can be polarized.



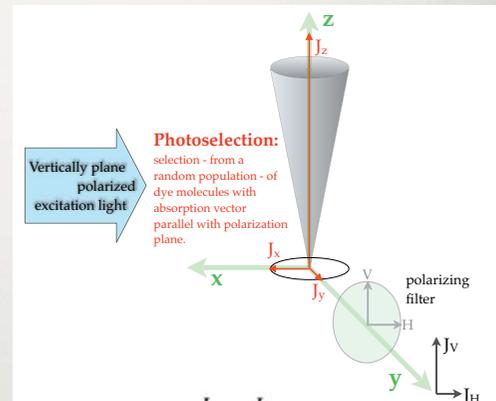
# EXCITATION WITH POLARIZED LIGHT



**Absorption and emission vectors** of fluorophores: determine the probabilities of photon absorption and emission.

Absorption is maximal if absorption vector and electrical polarization axis of light are parallel.

Absorption depends on  $\cos^2\alpha$  ( $\alpha$  is the angle between absorption axis and electrical polarization vector).



$$\text{Polarization: } p = \frac{J_{VV} - J_{VH}}{J_{VV} + J_{VH}}$$

$$\text{Anisotropy: } r = \frac{J_{VV} - J_{VH}}{J_{VV} + 2J_{VH}}$$

• additive quantity  
 • In the denominator: total excitation intensity ( $J_{VH} = J_{HV}$ )