

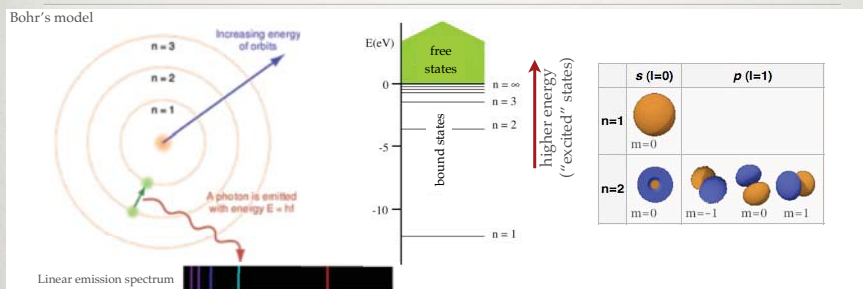
LUMINESCENCE

FUNDAMENTALS, PROPERTIES

LUMINESCENCE

- Energy of a molecule
- Spin states
- Types of luminescence
- Transitions of luminescence
- Parameters of luminescence
- Measurement of luminescence
- Polarization, anisotropy
- Applications, specialties

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) | ℓ | 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_ℓ | 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

MOLECULAR STRUCTURE

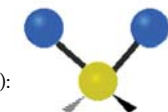
Molecule: atoms connected by chemical bonds
Simplest case: diatomic molecule (e.g., hydrogen molecule)



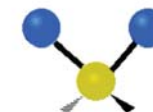
Molecules *vibrate* and *rotate*!

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

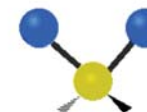
Examples of vibrational motion in the triatomic methylene group ($-\text{CH}_2-$):



Asymmetric stretching



Symmetric stretching



Scissoring

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:

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

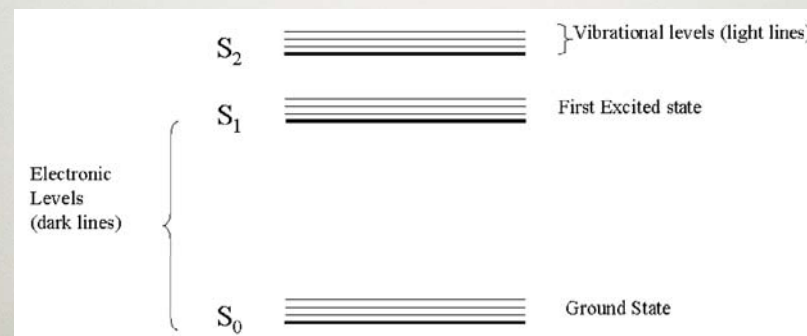
$$\sim 3 \times 10^{-19} \text{ J } (\sim 2 \text{ eV}) > \sim 3 \times 10^{-21} \text{ J} > \sim 3 \times 10^{-23} \text{ J}$$

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.



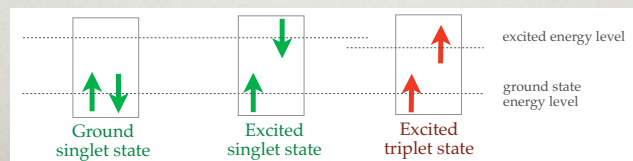
fully occupied subshell: spin pairing
(opposite-spin electrons pair)

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.



DEFINITION OF LUMINESCENCE

• Relaxation from excited state followed by light emission

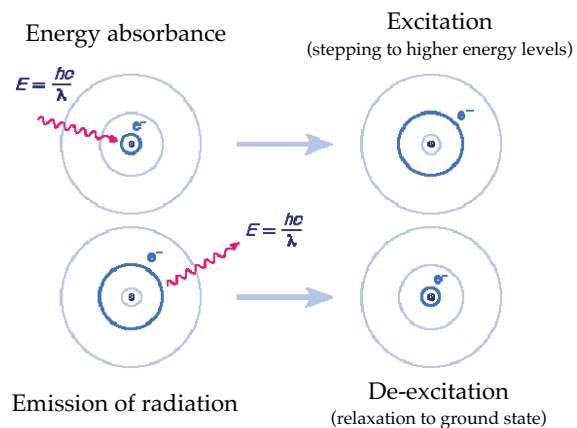
• Radiation emitted by matter in excess of thermal emission

• “Cold light”

• Processes of fluorescence and phosphorescence

SIMPLIFIED STEPS OF LUMINESCENCE

(Atomic system!)



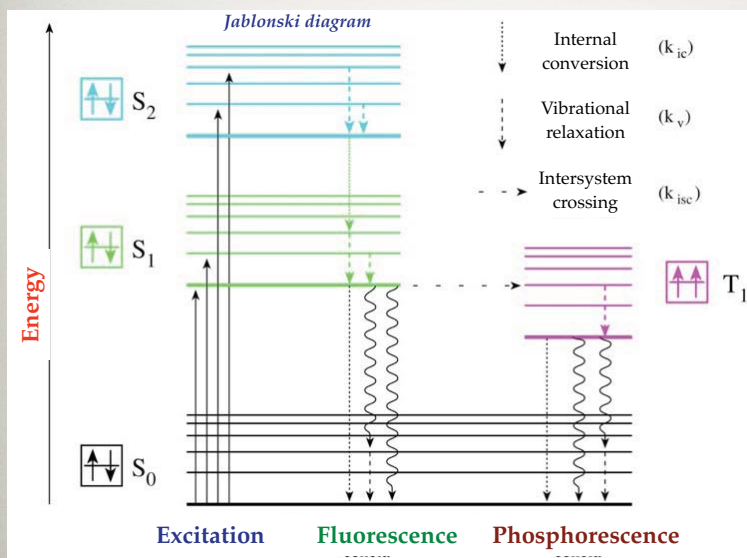
TYPES OF LUMINESCENCE

| (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 |

Bioluminescence



PROCESSES OF LUMINESCENCE



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

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

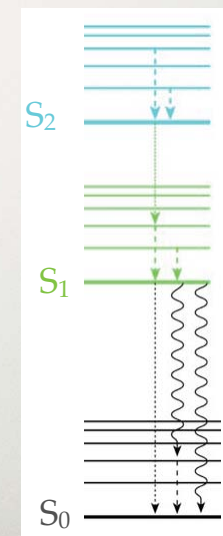
Intersystem crossing:
transition between singlet and triplet states accompanied by spin change (e.g., $S_1 \rightarrow T_1$)

KASHA'S RULE

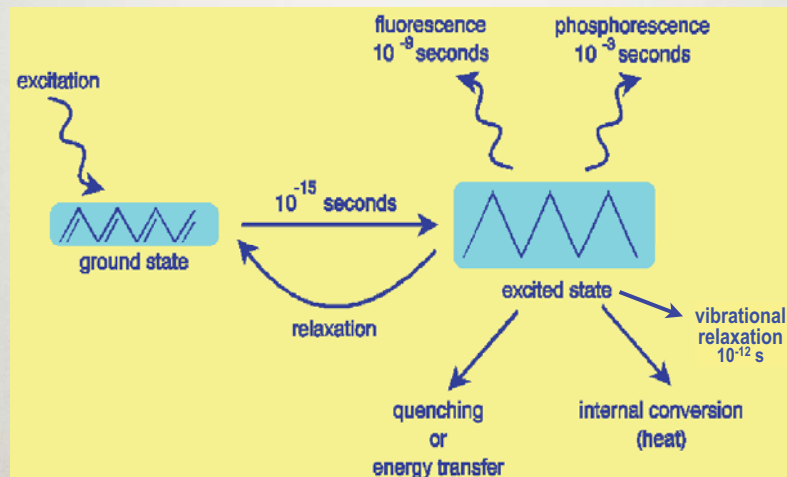
Photon emission (fluorescence or phosphorescence) occurs only from the lowest-energy excited electronic state (i.e., S_1 or T_1) of a molecule.



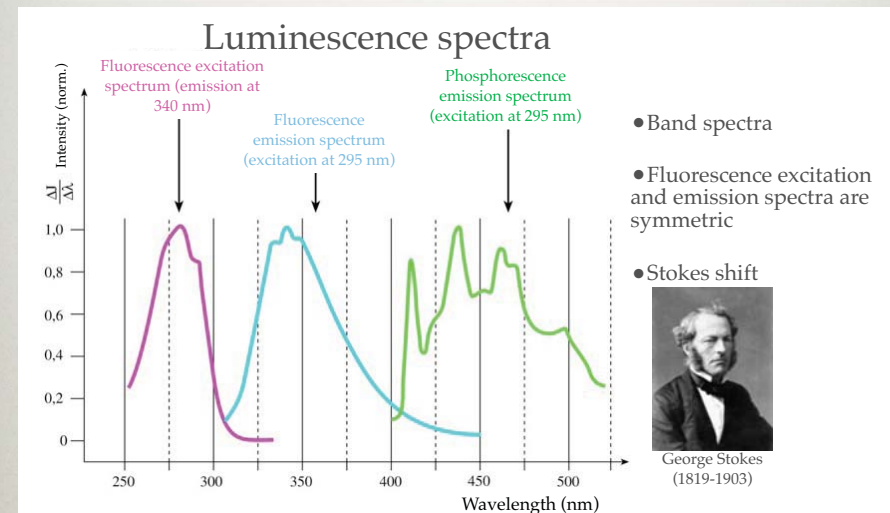
Michael Kasha (1920-)
American physicist



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)

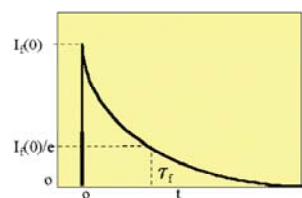
CHARACTERIZATION OF LUMINESCENCE II.

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



$$\frac{dN}{dt} = -(k_f + k_{nr}) \cdot N$$

$$N = N_0 e^{-(k_f + k_{nr})t}$$

$$\tau = \frac{1}{k_f + k_{nr}}$$

N =molecules in excited state

t =time

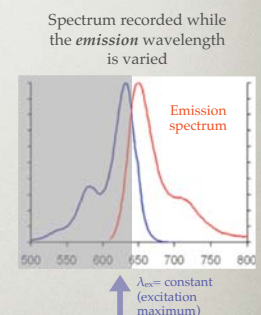
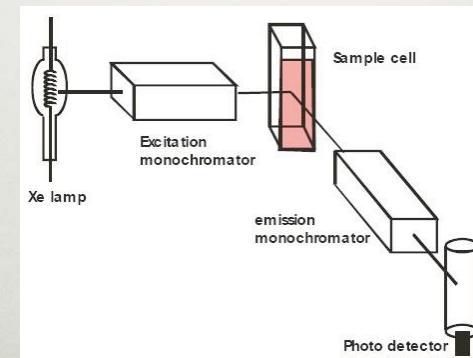
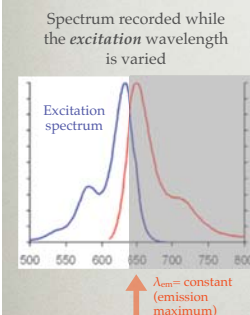
k_f =fluorescence rate constant

k_{nr} =non-radiative transition rate constant

τ =fluorescence lifetime

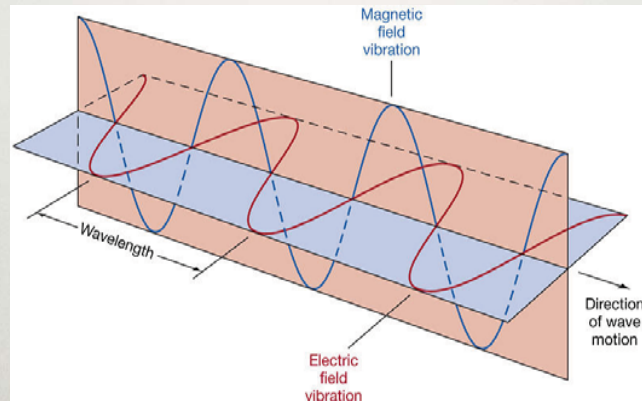
MEASUREMENT OF FLUORESCENCE

Fluorescence spectrometer ("Steady-state" spectrofluorometer)

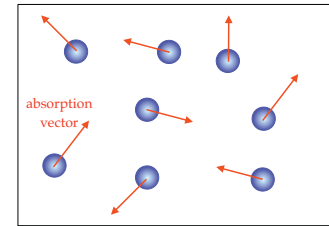


LIGHT IS ELECTROMAGNETIC RADIATION

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



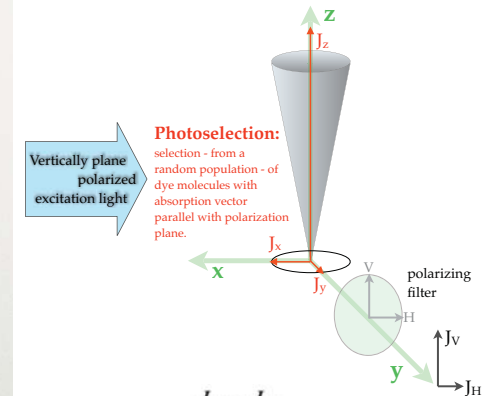
POLARIZATION, ANISOTROPY



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



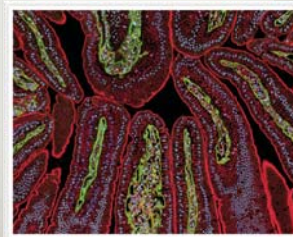
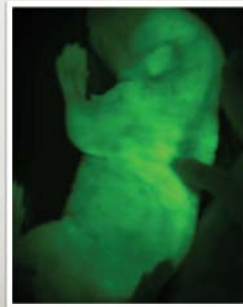
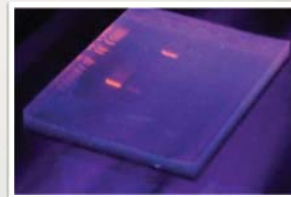
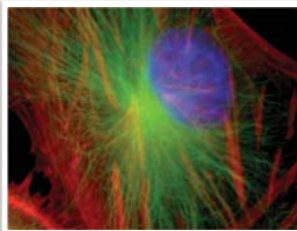
$$\text{Polarization: } p = \frac{J_{VV} - J_{VH}}{J_{VV} + J_{VH}}$$

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

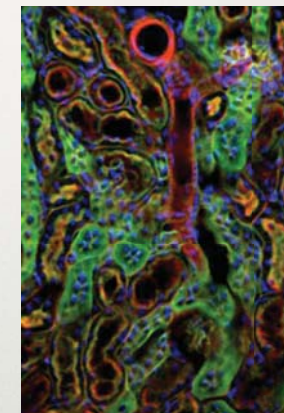
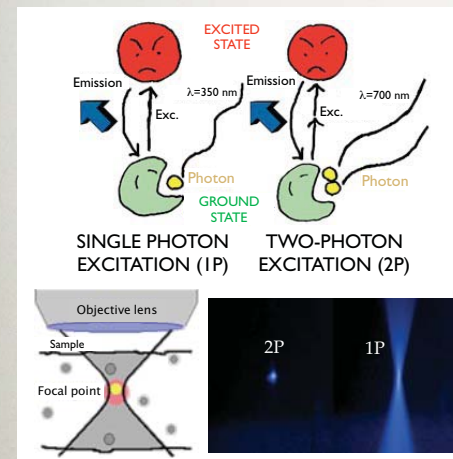
• additive quantity
• In the denominator: total excitation intensity ($J_{VH} = J_{VH}$)

BIOMEDICAL APPLICATIONS OF FLUORESCENCE

- Fluorescence microscopy
- DNA sequencing (chain termination method)
- DNA detection (EtBr)
- DNA microarray
- Immunofluorescence
- Fluorescence-activated cell sorting (FACS)
- Förster resonance energy transfer (FRET)
- Fluorescence recovery after photobleaching (FRAP)
- Fluorescent protein conjugation technologies
- Quantum dots



TWO-PHOTON FLUORESCENCE



Kidney cortex - tubular system (red: blood vessel)

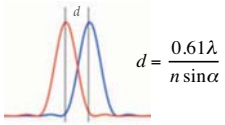


Cortical pyramidal cells

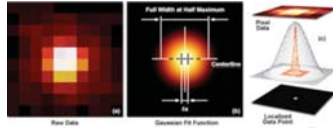
SUPER-RESOLUTION MICROSCOPY

Resolution problem is converted into position-determination problem

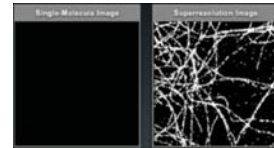
Resolution problem (Abbé)



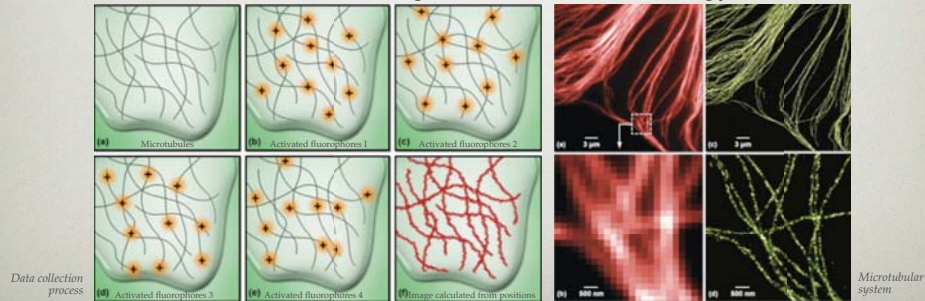
Position determination problem
(precision depends on photon count)



"Stochastic" data collection, single fluorophores



STORM: "stochastic optical reconstruction microscopy"



SUMMARY

- Molecular structure and energy states are important in luminescence.
- Luminescence is molecular de-excitation followed by radiation.
- Fluorescence spectra are characterized by Stokes shift.
- Quantum yield and lifetime are important luminescence parameters.