

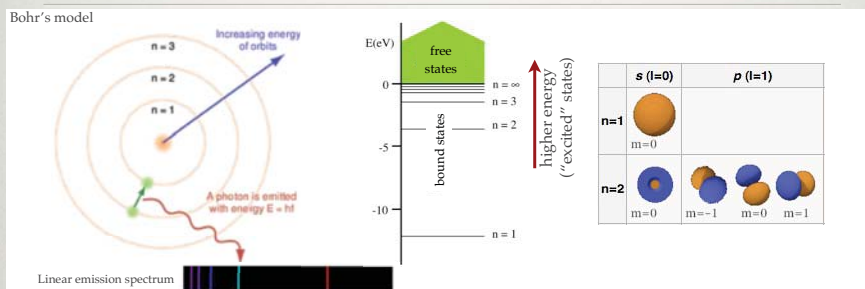
LUMINESCENCE

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

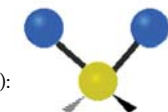
Molecule: atoms connected by chemical bonds
Simplest case: diatomic molecule (e.g., hydrogen molecule)



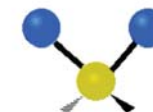
Molecules *vibrate* and *rotate*!

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

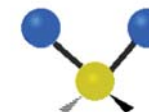
Examples of vibrational motion in the triatomic methylene group ($-\text{CH}_2-$):



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:

$$E_e \overset{\sim 100\times}{>} E_v \overset{\sim 100\times}{>} E_r$$

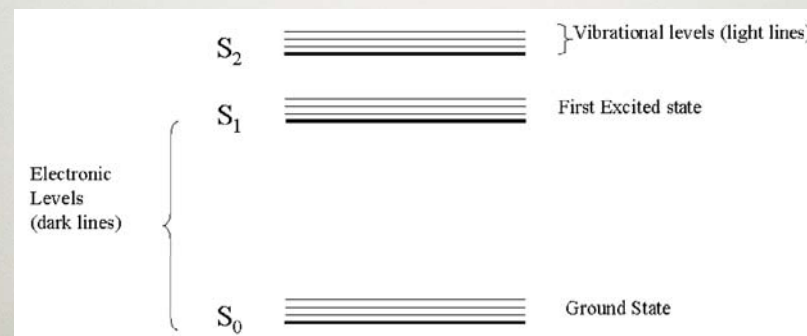
$$\sim 3 \times 10^{-19} \text{ J } (\sim 2 \text{ eV}) > \sim 3 \times 10^{-21} \text{ J} > \sim 3 \times 10^{-23} \text{ J}$$

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.



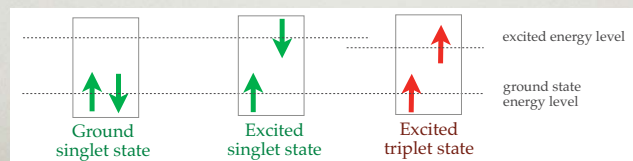
fully occupied subshell: spin pairing
(opposite-spin electrons pair)

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.



DEFINITIONS OF LUMINESCENCE

• Relaxation from excited state followed by light emission

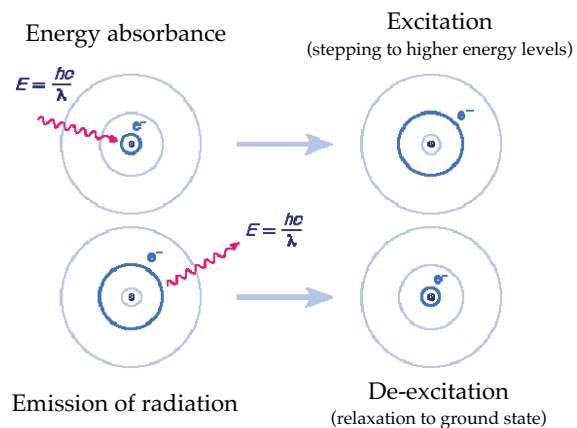
• Radiation emitted by matter in excess of thermal emission

• "Cold light"

• Processes of fluorescence and phosphorescence

SIMPLIFIED STEPS OF LUMINESCENCE

(Atomic system!)



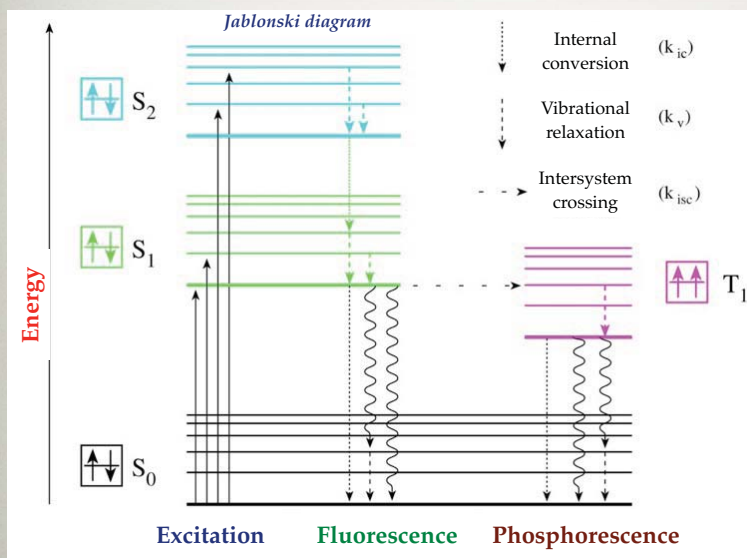
TYPES OF LUMINESCENCE

(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 (e.g., $S_2 \rightarrow S_1$)

Vibrational relaxation:
de-excitation process
within the same electronic
state

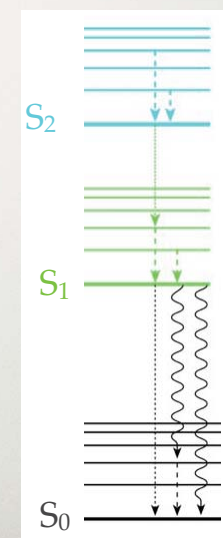
Intersystem crossing:
transition between singlet
and triplet states
accompanied by spin
change (e.g., $S_1 \rightarrow T_1$)

KASHA'S RULE

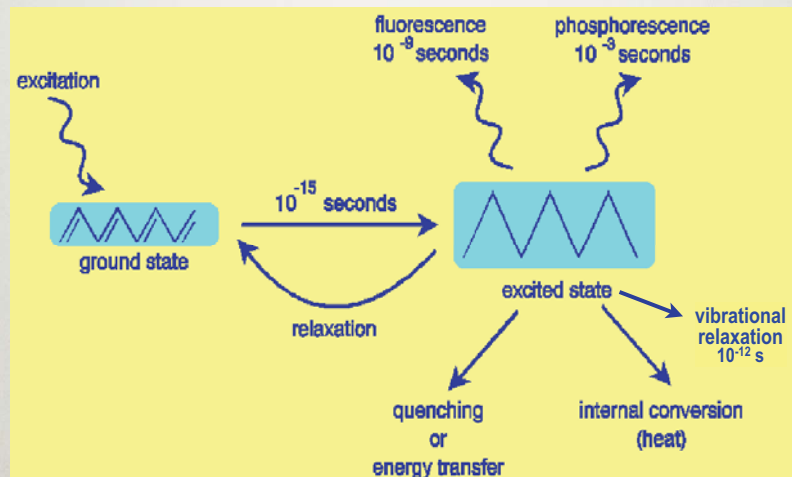
Photon emission (fluorescence or phosphorescence) occurs only from the lowest-energy excited electronic state (i.e., S_1 or T_1) of a molecule.



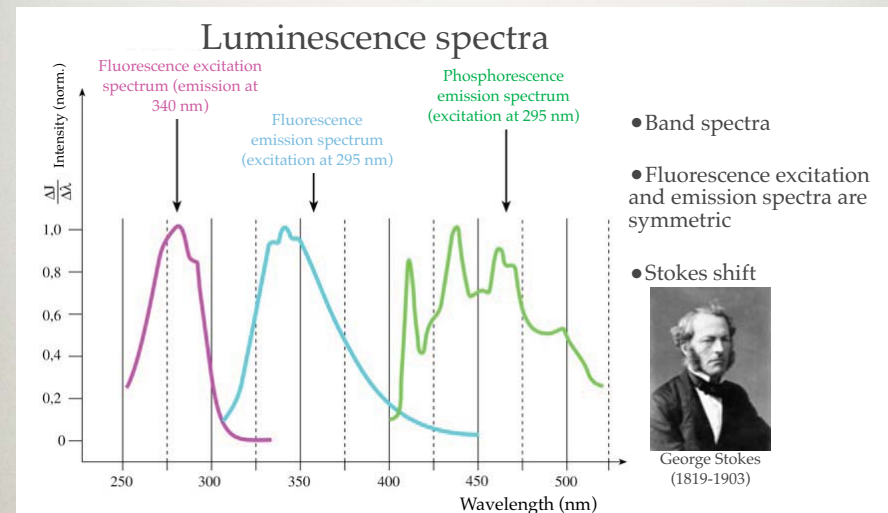
Michael Kasha (1920-)
American physicist



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)

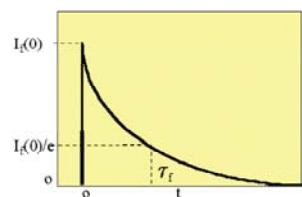
CHARACTERIZATION OF LUMINESCENCE II.

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

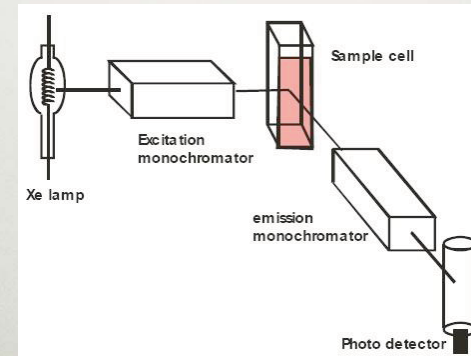
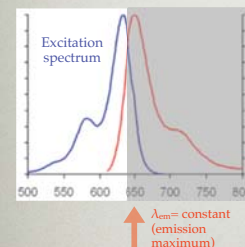
k_{nr} =non-radiative transition rate constant

τ =fluorescence lifetime

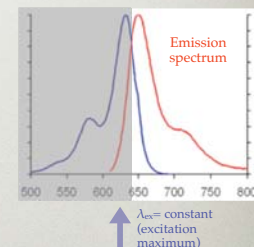
MEASUREMENT OF FLUORESCENCE

Fluorescence spectrometer ("Steady-state" spectrofluorometer)

Spectrum recorded while the excitation wavelength is varied

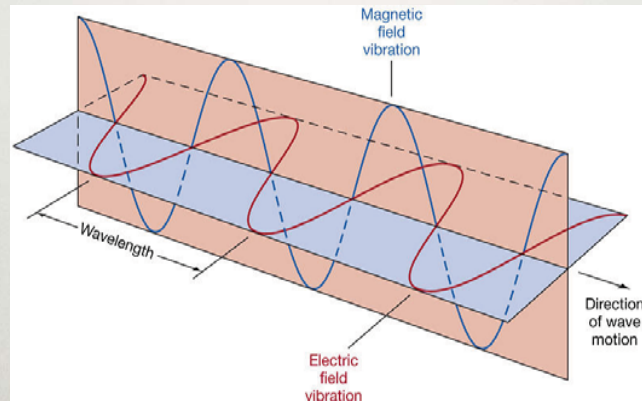


Spectrum recorded while the emission wavelength is varied

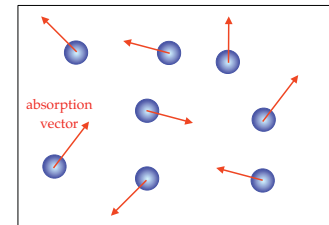


LIGHT IS ELECTROMAGNETIC RADIATION

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



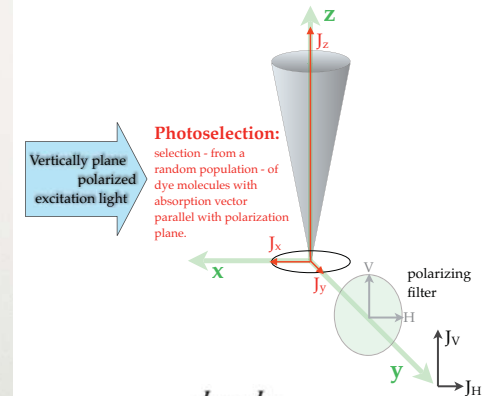
POLARIZATION, ANISOTROPY



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).



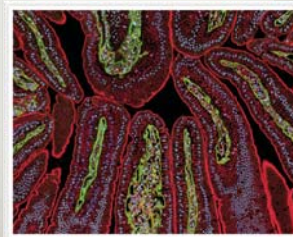
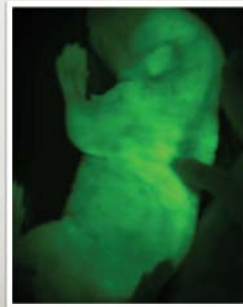
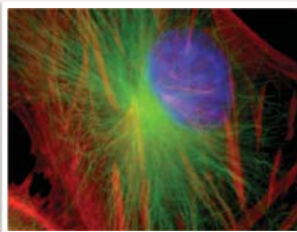
$$\text{Polarization: } p = \frac{J_{VV} - J_{VH}}{J_{VV} + J_{VH}}$$

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

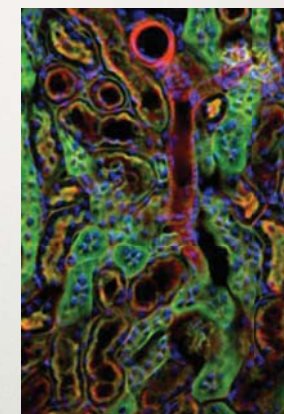
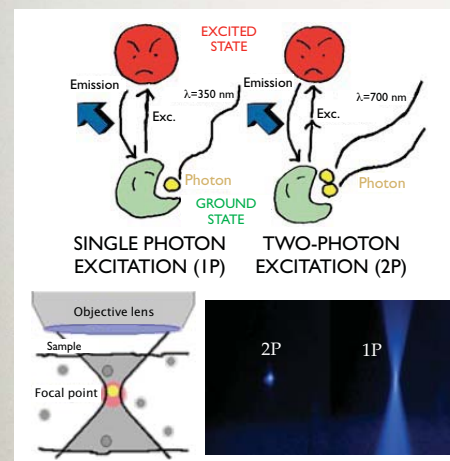
• additive quantity
• In the denominator: total excitation intensity ($J_{VH} = J_{VH_1} + J_{VH_2}$)

BIOMEDICAL APPLICATIONS OF FLUORESCENCE

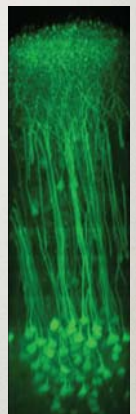
- Fluorescence microscopy
- DNA sequencing (chain termination method)
- DNA detection (EtBr)
- DNA microarray
- Immunofluorescence
- Fluorescence-activated cell sorting (FACS)
- Förster resonance energy transfer (FRET)
- Fluorescence recovery after photobleaching (FRAP)
- Fluorescent protein conjugation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



TWO-PHOTON FLUORESCENCE



Kidney cortex - tubular system (red: blood vessel)

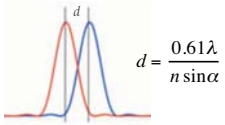


Cortical pyramid cells

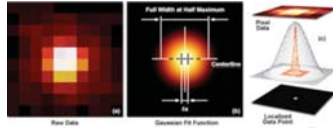
SUPER-RESOLUTION MICROSCOPY

Resolution problem is converted into position-determination problem

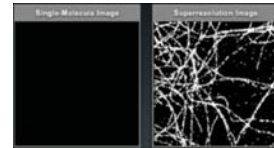
Resolution problem (Abbé)



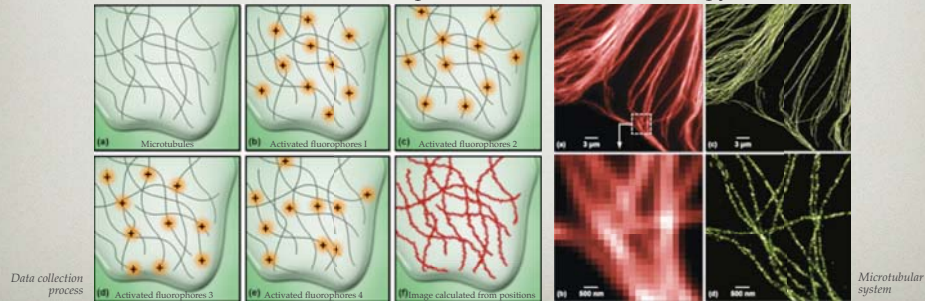
Position determination problem
(precision depends on photon count)



"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.