Experiment 3: Fluorescence Spectroscopy
I: Introduction to steady state spectra

Prelab Lecture 30jan19
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WHAT IS FLUORESCENCE?

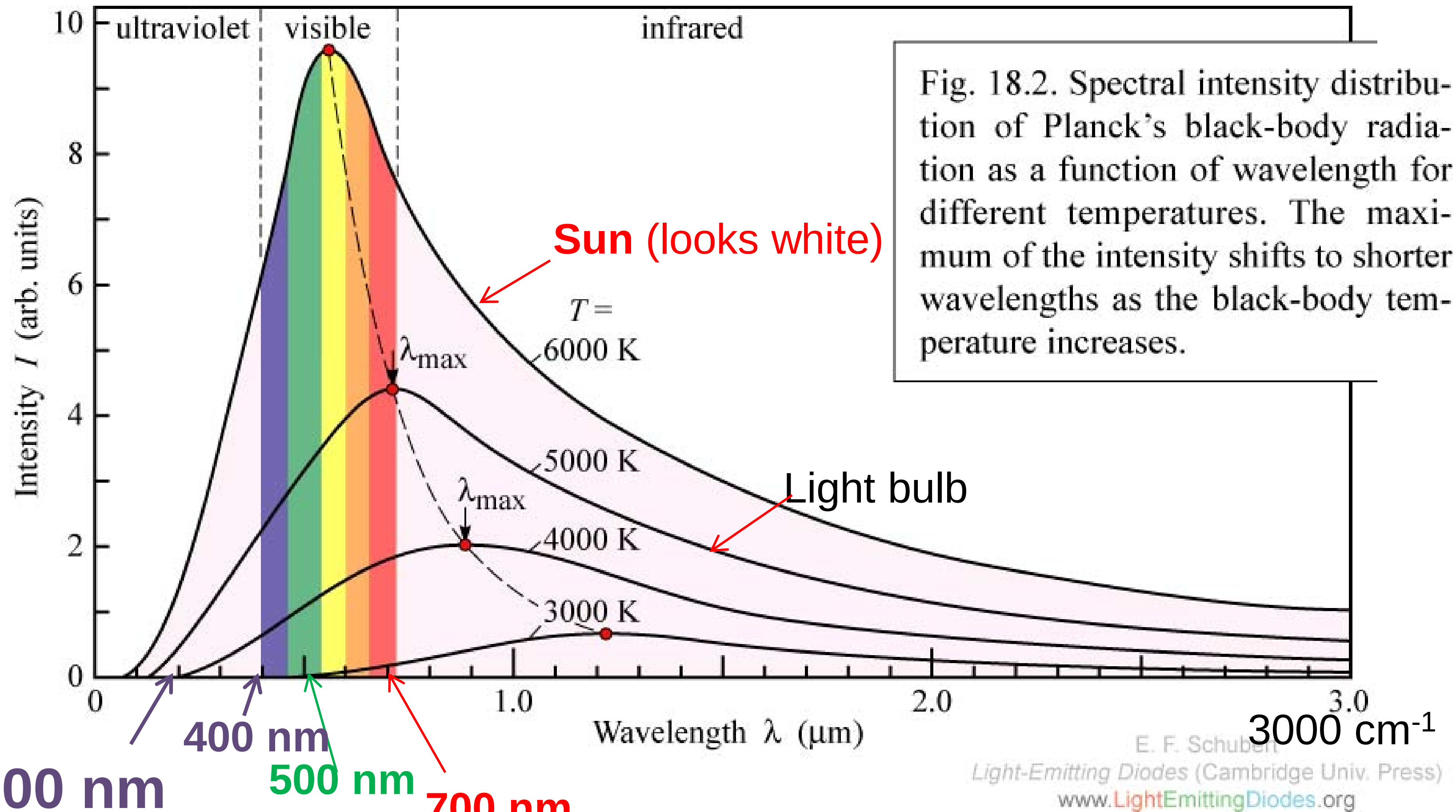
ALL light comes from fluorescence, e.g., the sun, light bulbs, fire, cell phone screens, some chemical reactions, etc.

Fluorescence *usually* refers to a form of spontaneous emission in the Visible or UV wavelengths coming promptly from an electronically excited state (decay time ps to μs i.e., 10^{-12} to 10^{-6} s).
The most-used fluorescence probes have nanosecond decay times.

Spontaneous emission happens whenever a system is in an excited state. WHY?

ANSWER: caused by strong fluctuations in the zero-point electromagnetic field, present even in dark vacuum!

Electromagnetic radiation is emitted by all objects not at 0 Kelvin.



200 nm

Where N2 and O2 start to absorb

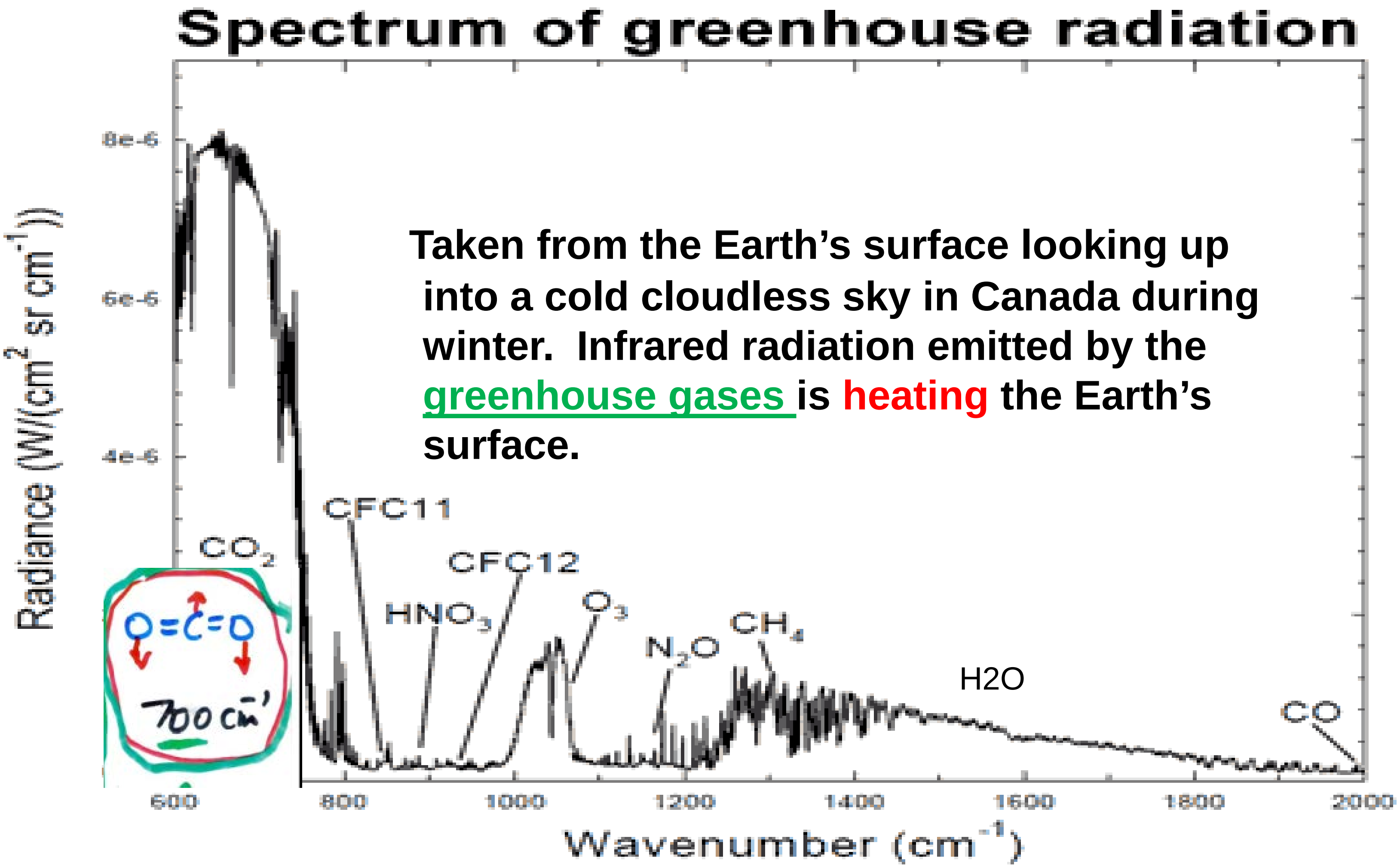
Temperature dependence is from the **Boltzmann** ratio for probability to be in an excited state:

$$N_{excited}/N_{ground}=\exp(\Delta E/k_BT),$$

where $\Delta E = h\nu = hc/\lambda$

IR fluorescence from the atmosphere.

Evidence: looking skyward with an IR spectrometer:



Taken from the Earth's surface looking up into a cold cloudless sky in Canada during winter. Infrared radiation emitted by the greenhouse gases is heating the Earth's surface.

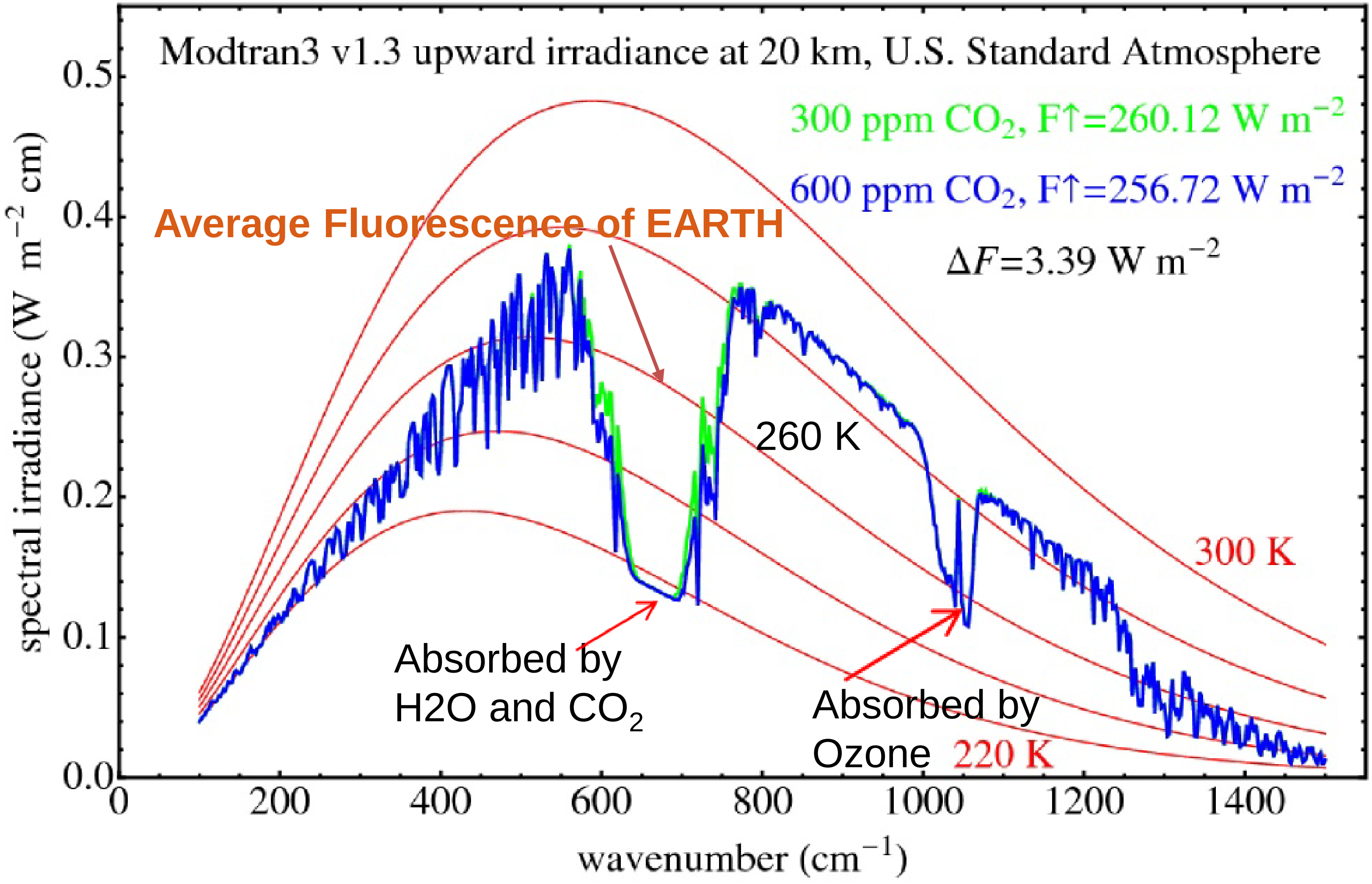
Why is CO_2 so strong compared to water??

There is much more water in air.

2 J

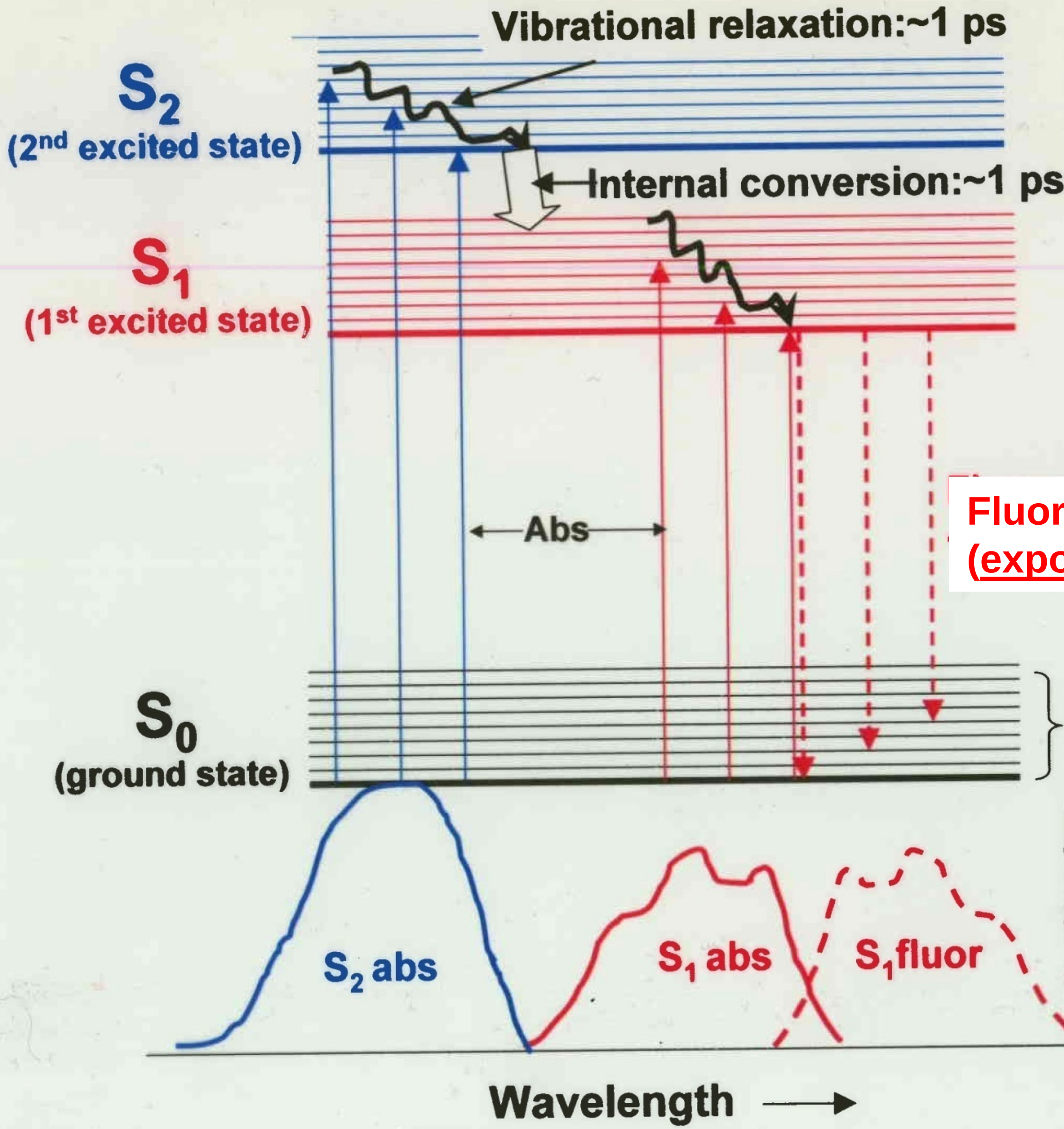
(Evans 2006).

Fluorescence of EARTH at different temperatures seen from space.



ABSORPTION & FLUORESCENCE

Jablonski Diagram



Fluorescence lifetime ~5 ns (<u>exponential</u> decay)

<u>Kasha's Rule:</u>
Fluorescence is 99.9% from S_1 ***<u>independent</u>*** of excitation wavelength; ~***<u>mirror image</u>*** of S_1 abs.

Beer-Lambert Law

photon + M ---> M* (electronically excited)

$$\frac{d[\text{photon}]}{dt} = -k[M][\text{photon}] = -k\,[M]\,I$$

$$[\text{photon}] = \text{light intensity} = I$$

$$\frac{dI}{dt} = -k[M]I\text{, where } k[M] \text{ is a pseudo first-order rate constant}$$

for the disappearance of photons

Intensity of beam falls off exponentially

$$f = \frac{I}{I_0} = e^{-k[M]t} = e^{-k[M]x/c} = 10^{\frac{-k[M]x}{2.303c}} = 10^{-\varepsilon[M]x} = 10^{-A}$$

In this context **f = fraction of photons remaining after travelling distance x**

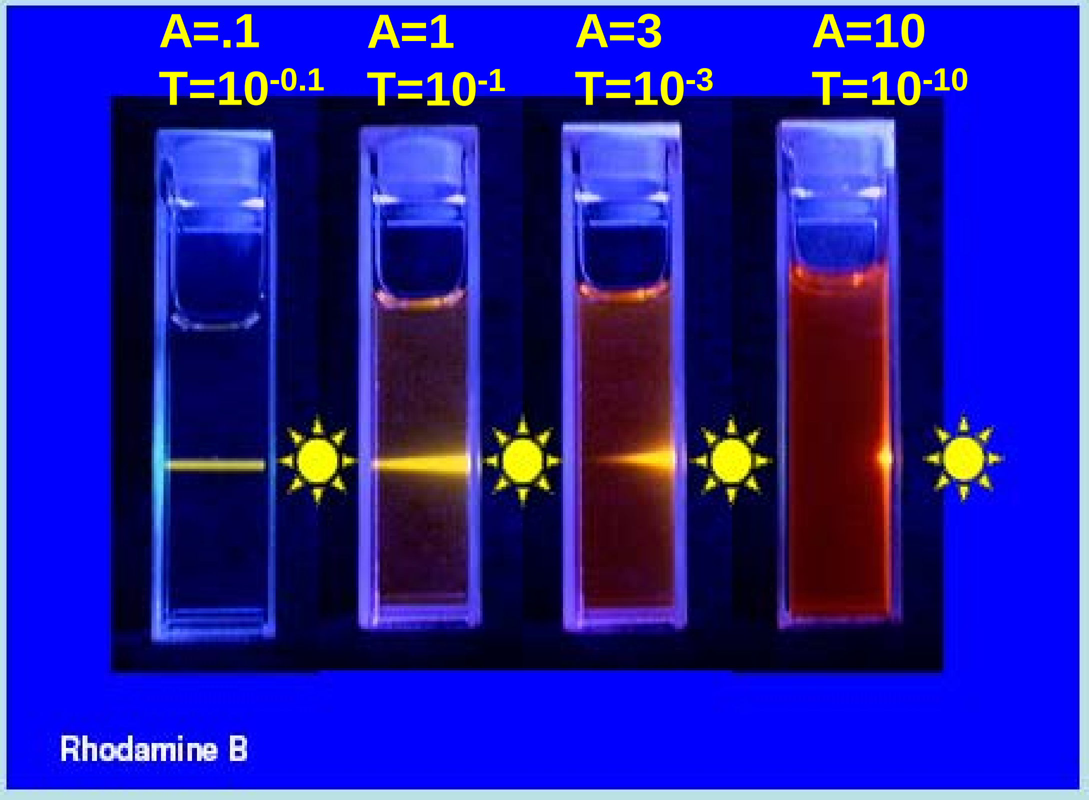
i.e., **f= Transmittance = T = $10^{-\varepsilon cx}$ = 10^{-A}**

10^{-A} is just telling you that **A = εcx = –log T**

Attenuation of the Excitation Light through Absorbance

Sample concentration & the *inner filter effect*

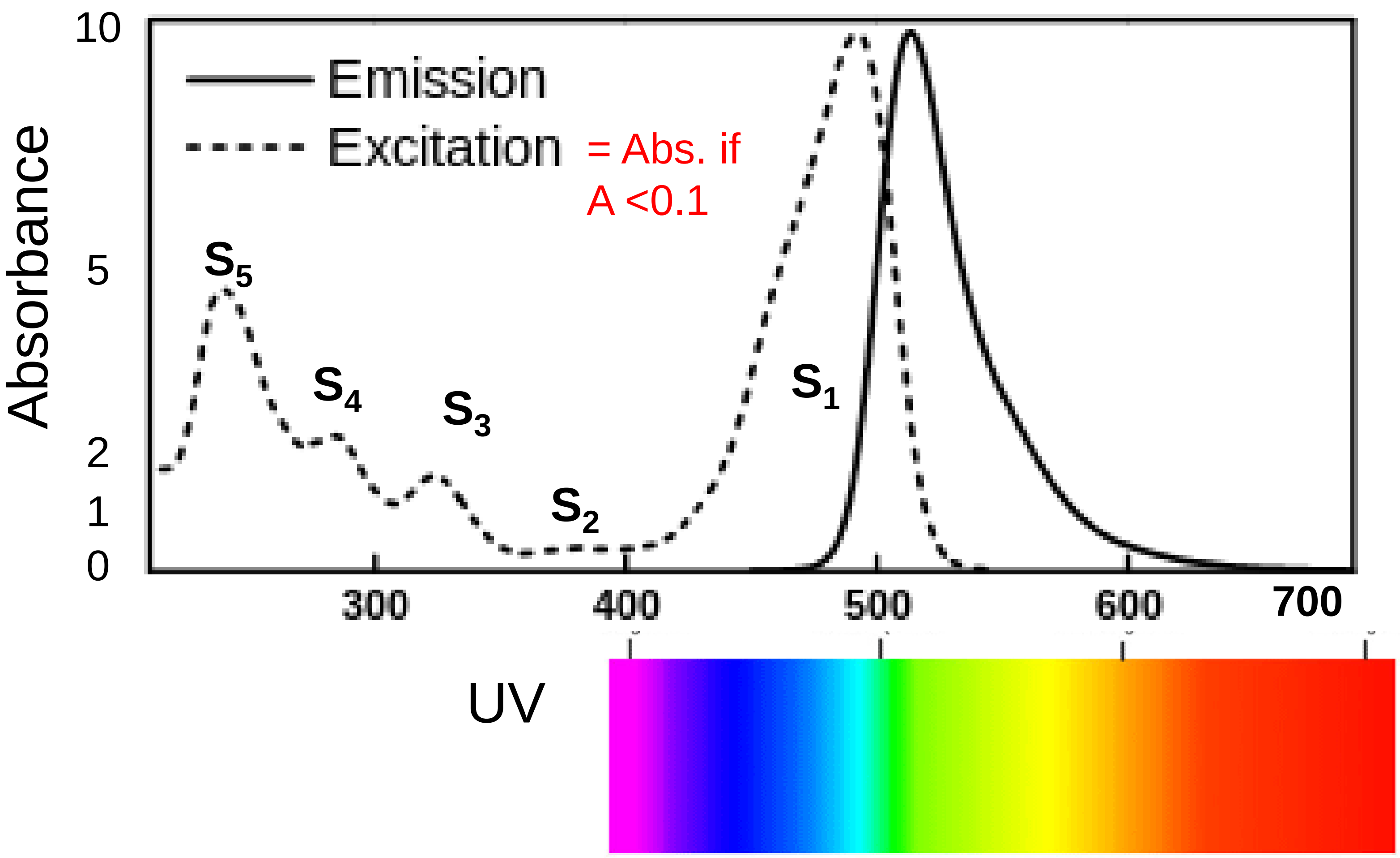
Guess the Absorbance



David Jameson http://www.fluorescence-foundation.org/2007Lectures/Lecture12007.pdf

from Jameson et. al., Methods in Enzymology (2002), 360:1 https://en.wikipedia.org/wiki/Fluorescein

Fluorescein



file:///J:/374-14/Fluorescence-I/Application%20Notes%20-%20HORIBA.htm

Fluorescence from single molecucles under a microscope

http://www.youtube.com/watch?v=CDald68tTz0

Fig. 13.34 Tinoco et al.

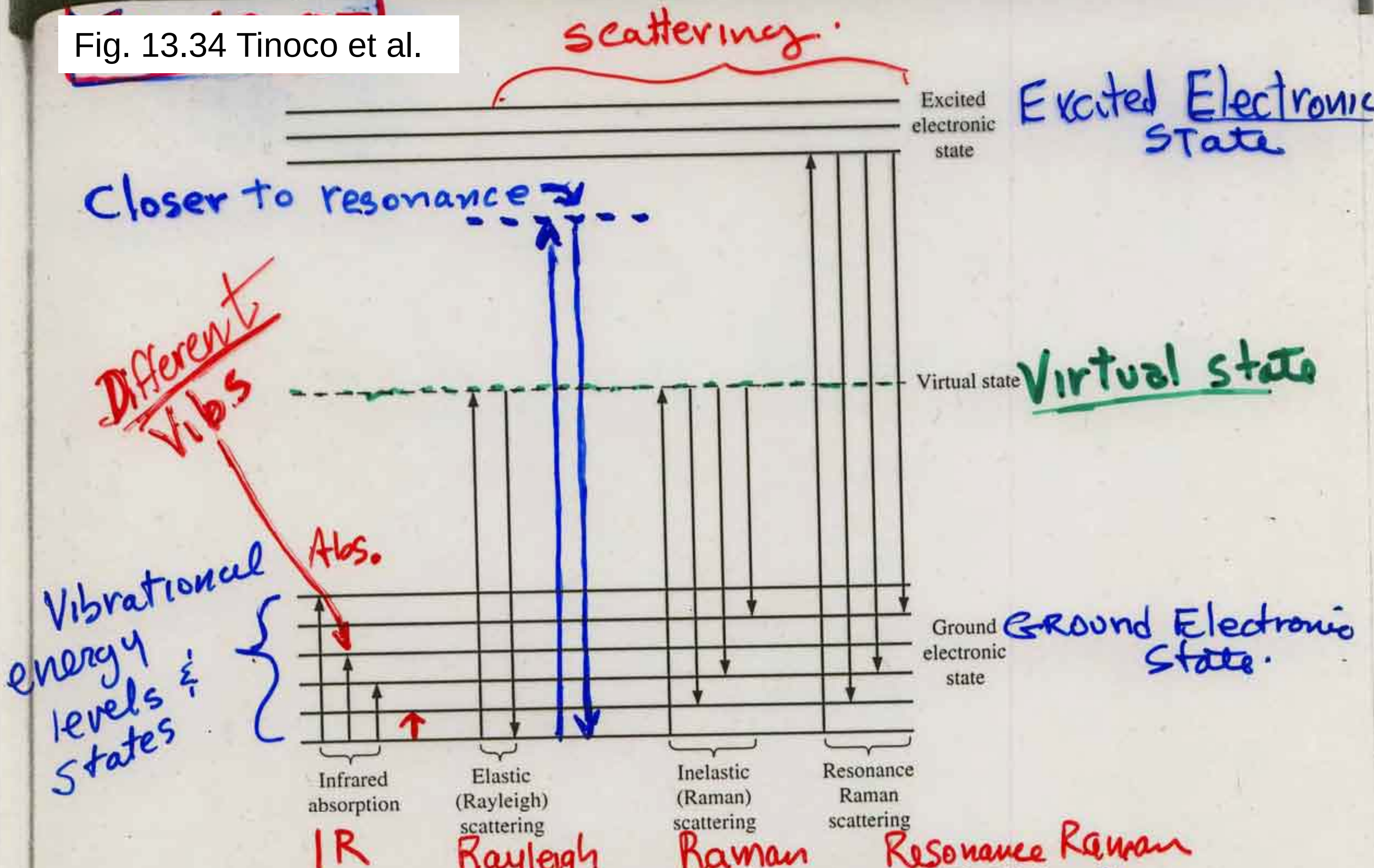
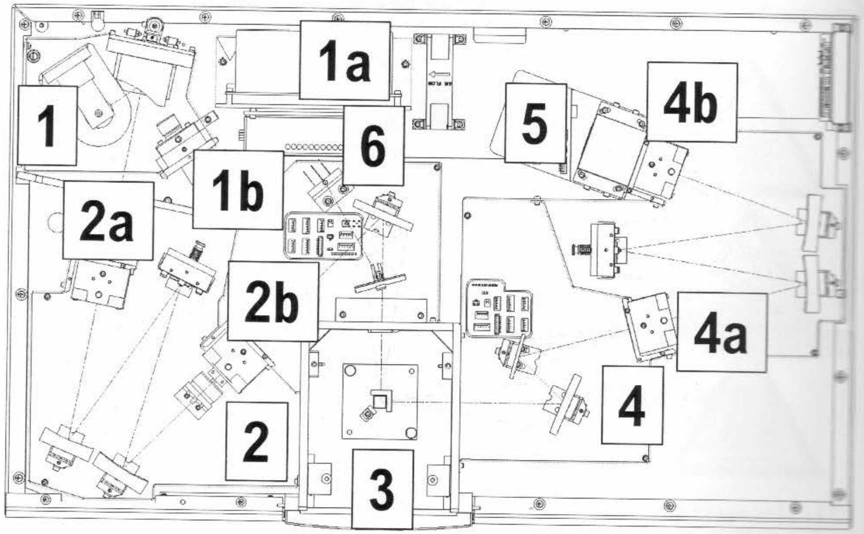


Fig. 10.41 Energy-level diagram for infrared absorption, elastic (Rayleigh) scattering, and inelastic (Raman) scattering. Transitions between vibrational energy levels of the ground electronic state absorb infrared radiation; not all transitions are allowed. For elastic scattering the frequency of the incident light is equal to the frequency of the scattered light. For inelastic scattering the frequency of the scattered light is different from that of the incident light. The virtual state shown can have any energy; the scattering of light occurs in any region of the spectrum.

How do we measure fluorescence and scattering?

Horiba Fluorimeter

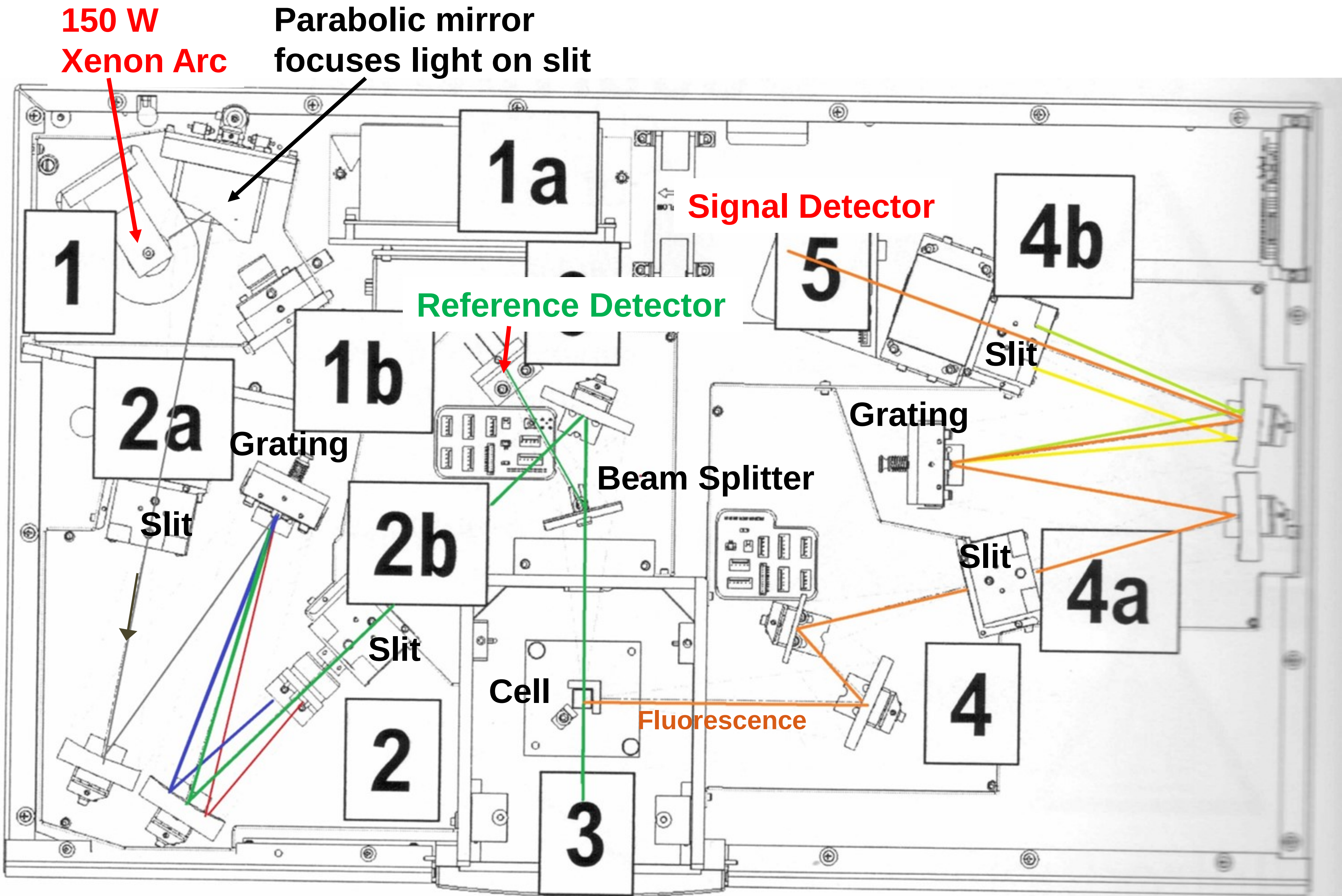
Optical layout



1 Xenon arc-lamp and lamp housing
1a Xenon-lamp power supply
1b Xenon flash lamp (FluoroMax®-4P only)
2 Excitation monochromator
2a & 2b Slits
3 Sample compartment
4 Emission monochromator
4a & 4b Slits
5 Signal detector (photomultiplier tube and housing)
6 Reference detector (photodiode and current-acquisition module)
Host computer (not on diagram)

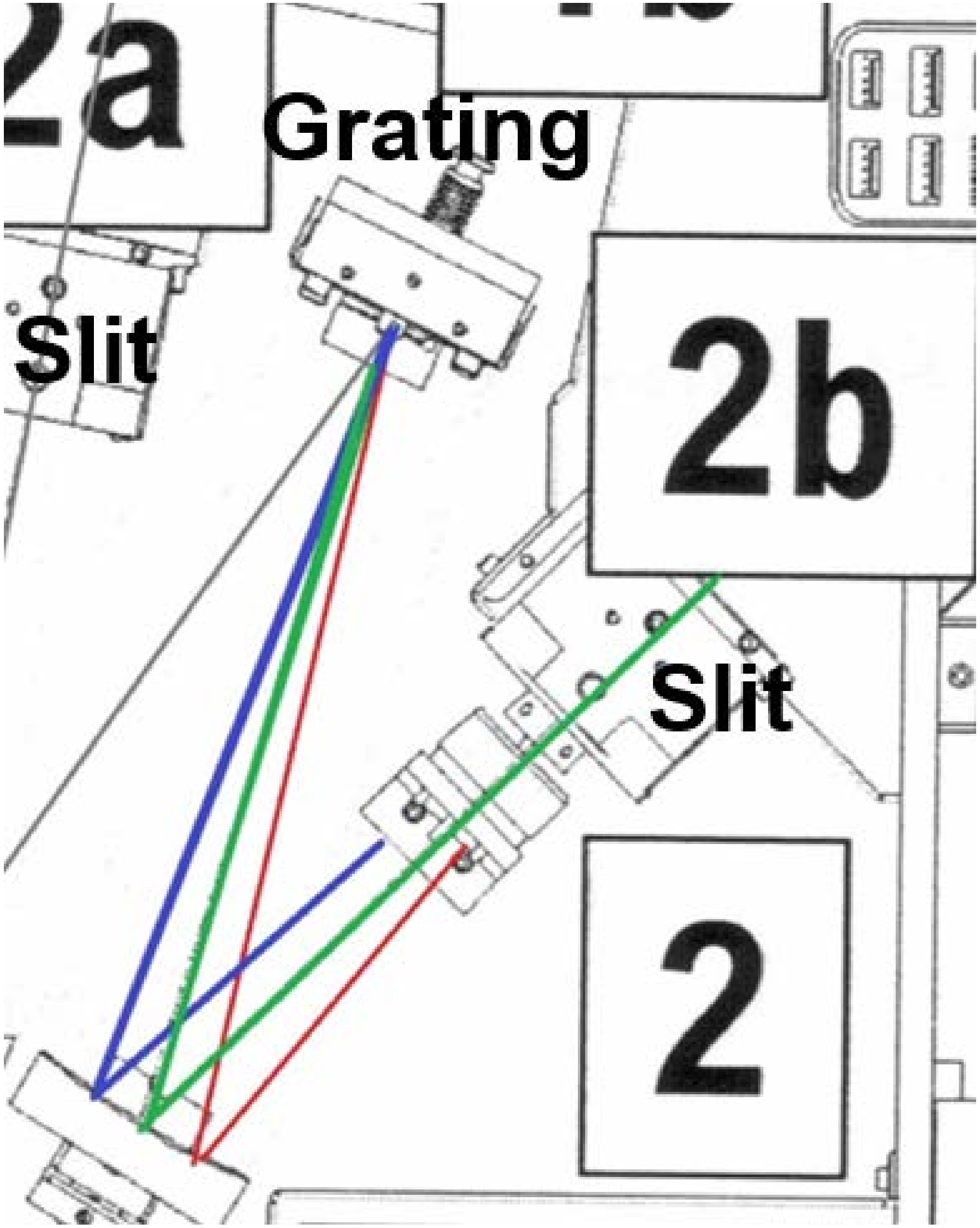
Illuminator (xenon arc-lamp, 1)

The continuous light source is a 150-W ozone-free xenon arc-lamp. Light from the lamp is collected by a diamond-turned elliptical mirror, and then focused on the entrance slit of the excitation monochromator. The lamp housing is separated from the excitation monochromator by a quartz window. This vents heat out of the instrument, and protects against the unlikely occurrence of lamp failure.

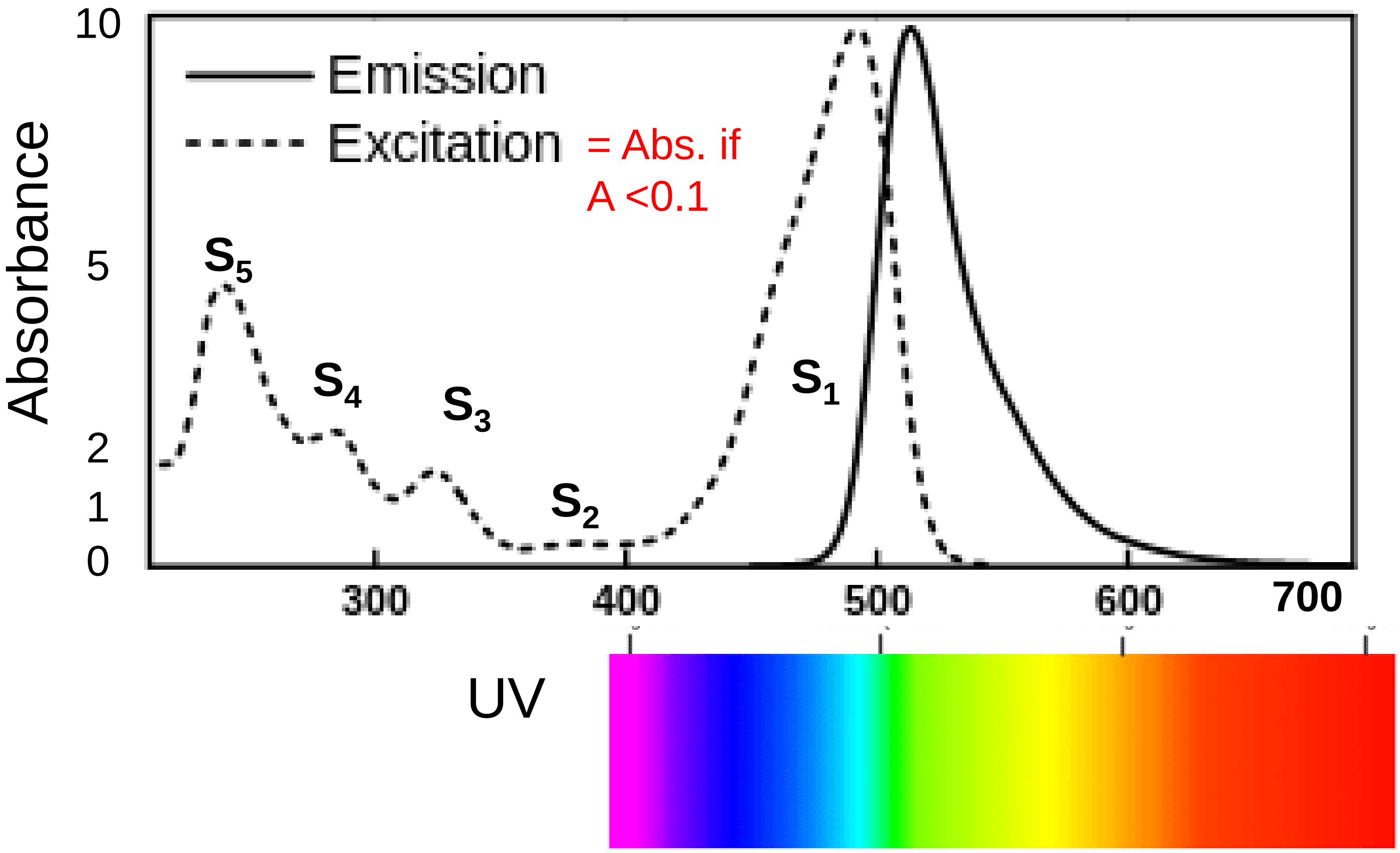


Excitation Monochromator

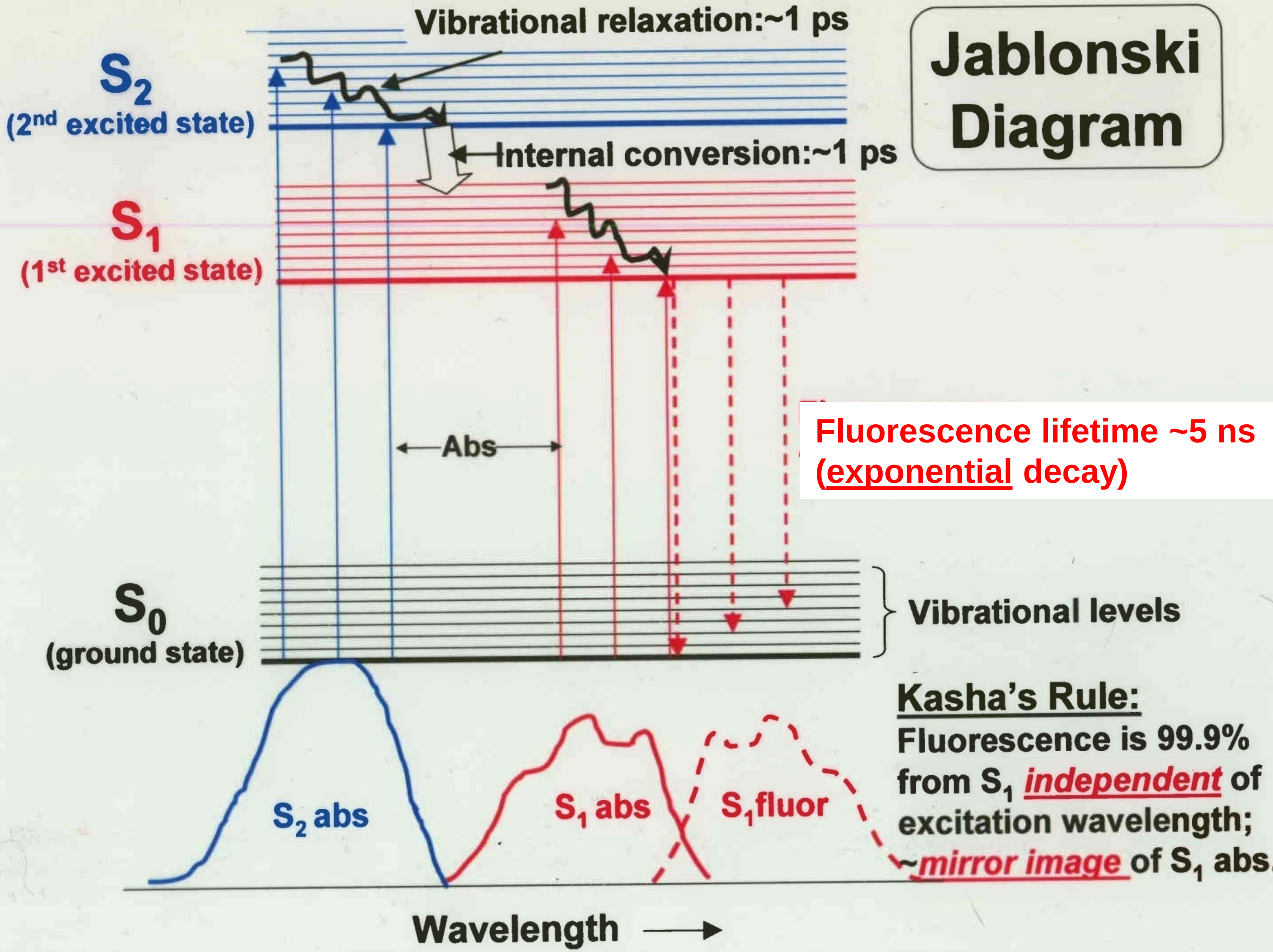
Emission Monochromator


2a
Grating
Slit
2b
Slit
2

https://en.wikipedia.org/wiki/Fluorescein



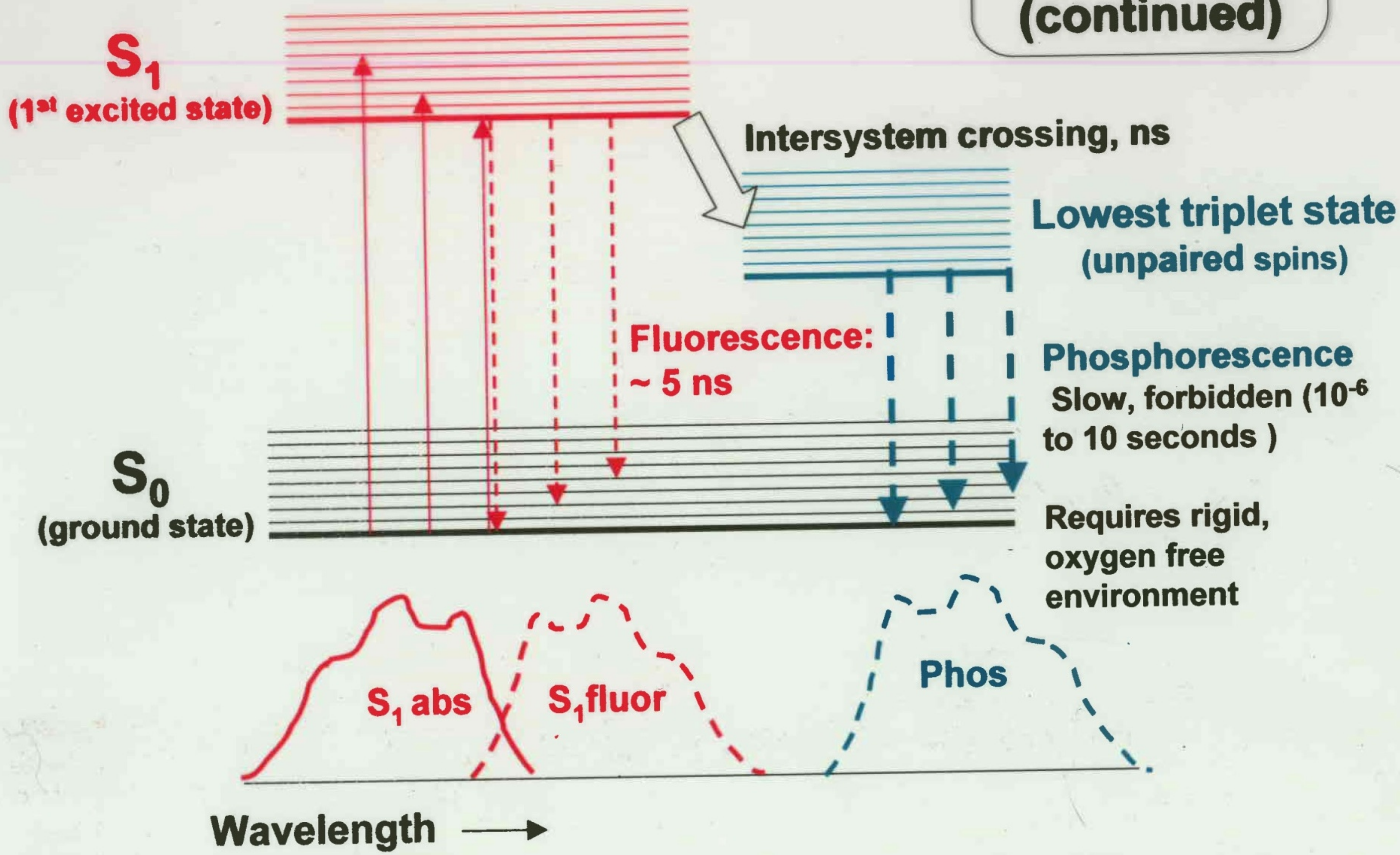
ABSORPTION & FLUORESCENCE



Kasha's Rule:
Fluorescence is 99.9% from S_1 ***independent*** of excitation wavelength; ~***mirror image*** of S_1 abs.

Phosphorescence

Jablonski Diagram (continued)



Fluorescence Quantum Yield

4.2.3 Fluorescence Quenching by Iodide

1. Calculate he quantum yield for each concentration of the iodide, [Q], using numbers from the Theory document and :

Quantum Yield = $$\Phi_f = \frac{k_{rad}}{k_{rad} + k_{ic} + k_{isc} + k_q[Q]}$$

Fluorescence Lifetime = 1/(sum of rate constants)

$$= \mathbf{1/(k_{rad} + k_{ic} + k_{isc} + k_q[Q]) = \tau_f}$$

What is fluorescence lifetime?

d[excited molecules]/dt = -k [excited molecules]

d(Intensity)/dt = -k (Intensity) **1st order reaction**

Solution to this differential equation?

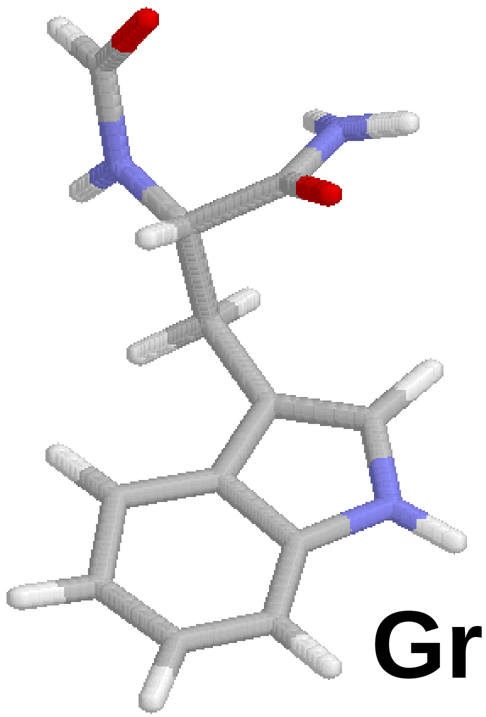
Fluor. intensity at time t = (Fluor. Intensity at time 0) x e^{-kt}

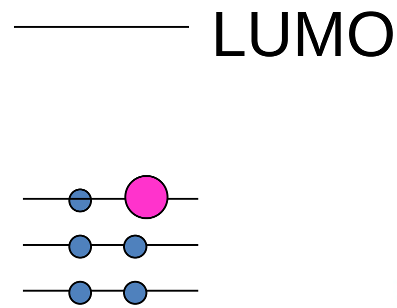
or = (Fluor. Intensity at time 0 x) $e^{-t/\tau}$

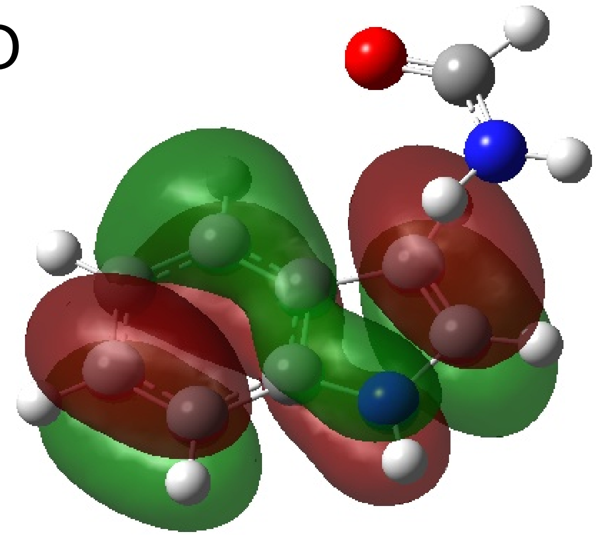
τ = "lifetime" = 1/k

τ = inverse of 1st order rate constant

“Quenching”

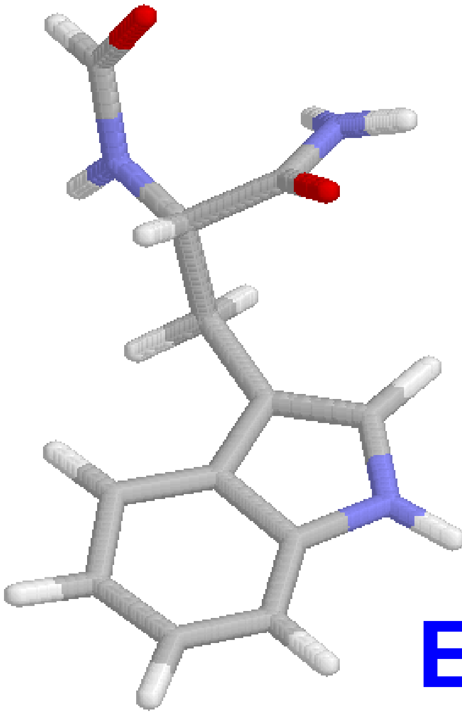


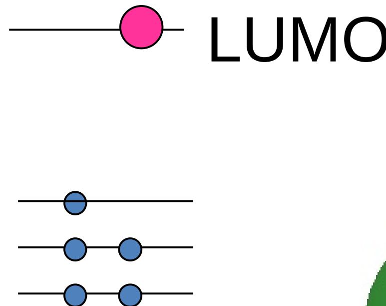


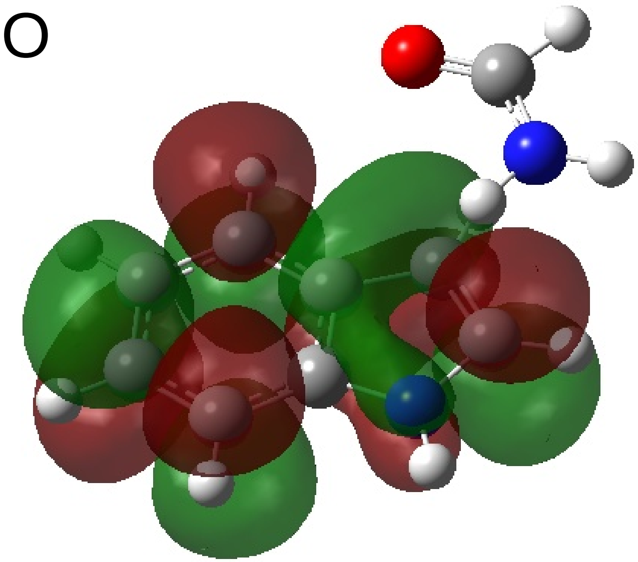


Ground State

Highest Occupied Molecular Orbital



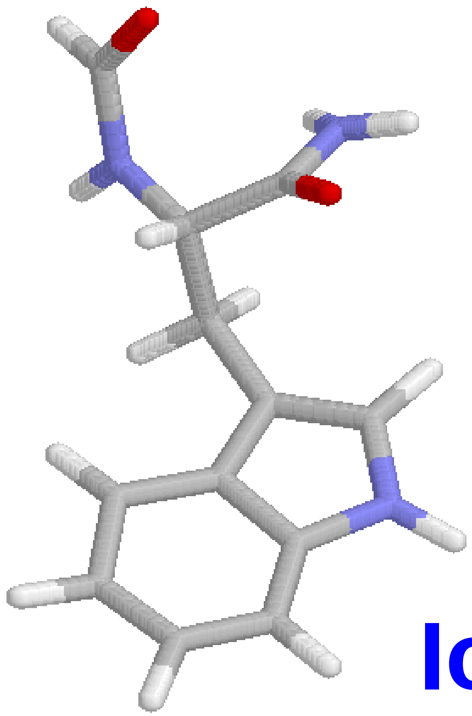


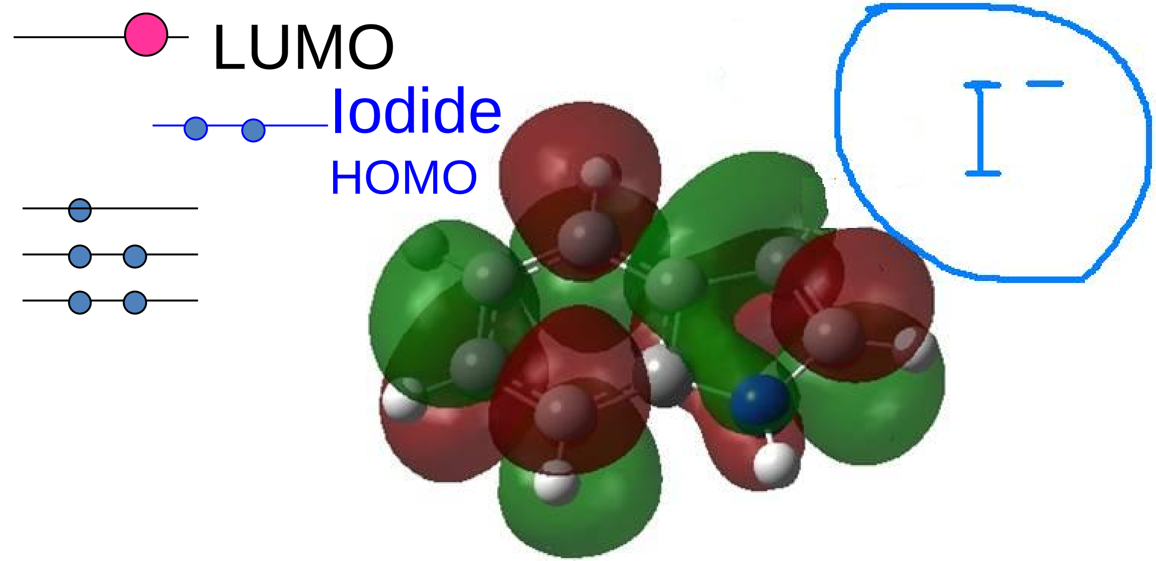


Excited State (fluorescing state)

Lowest Unoccupied Molecular Orbital (electron excited)

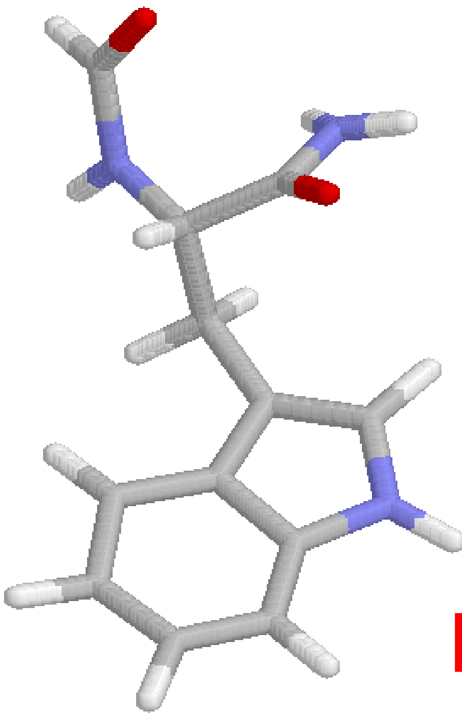
Quenching by iodide ion

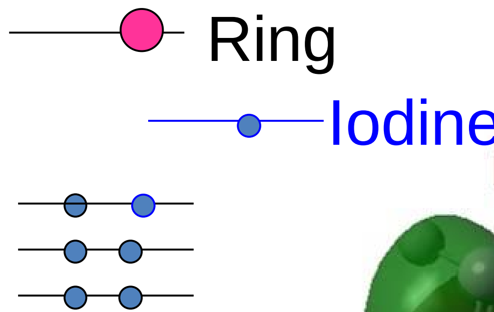


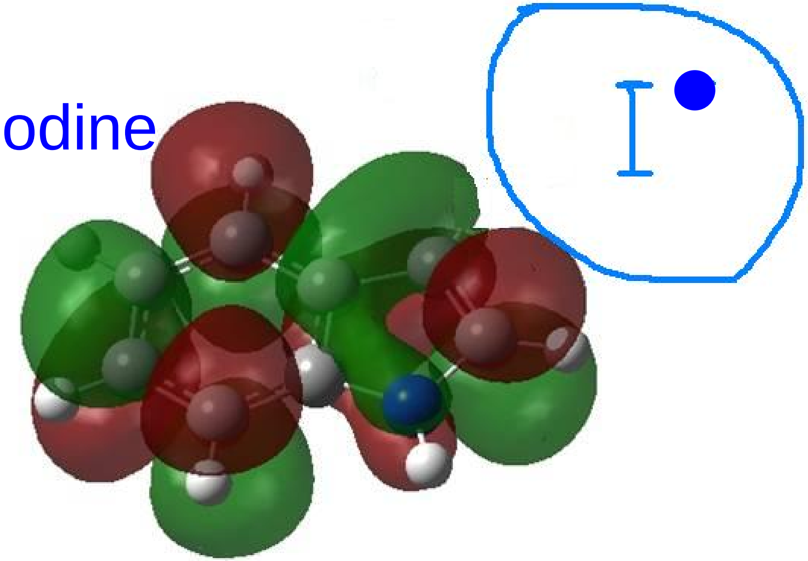


Iodide ion collides;
(has higher HOMO)
will quench fluorescence

Electron transfer from I^- to indole makes a radical pair that cannot fluoresce.
(would violate Pauli exclusion)







Radical Pair (can't fluoresce)

Electron transferred from iodide to vacancy in HOMO of ring i.e., QUENCHING