

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

Prelab Lecture 2feb17
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WHAT IS FLUORESCENCE?

ALL light comes from fluorescence, e.g., the **sun**, **light bulbs**, **fire**, **cell phone screens**, 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 (not in true ground state). **WHY?**

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

Light is emitted by almost **all objects not at 0 Kelvin.**

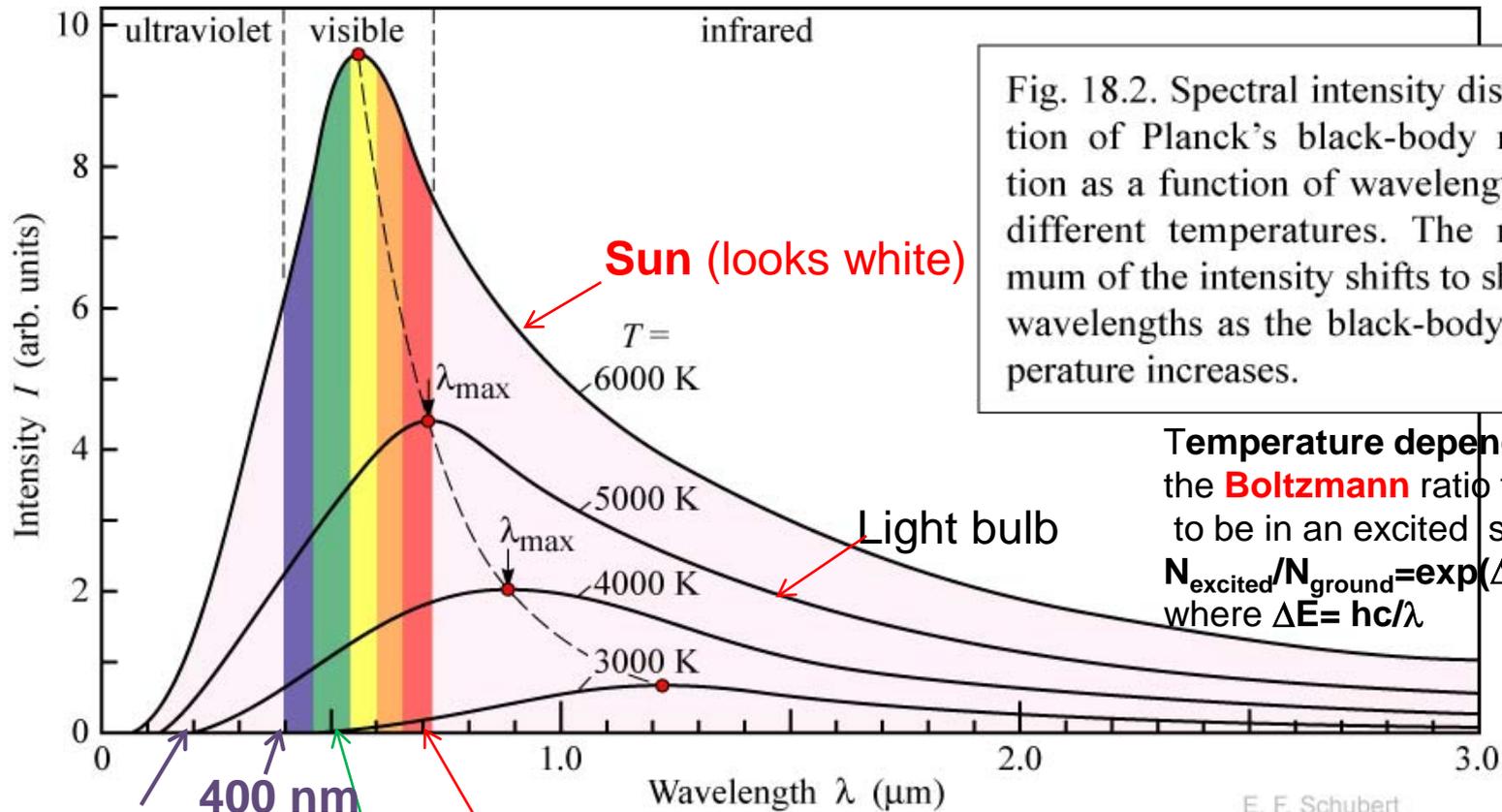


Fig. 18.2. Spectral intensity distribution of Planck's black-body radiation as a function of wavelength for different temperatures. The maximum of the intensity shifts to shorter wavelengths as the black-body temperature increases.

Temperature dependence is from the **Boltzmann** ratio for probability to be in an excited state:
 $N_{\text{excited}}/N_{\text{ground}} = \exp(\Delta E/k_B T)$,
 where $\Delta E = hc/\lambda$

200 nm
 Where N₂ and O₂
 start to absorb

400 nm
 500 nm
 700 nm

Sun (looks white)

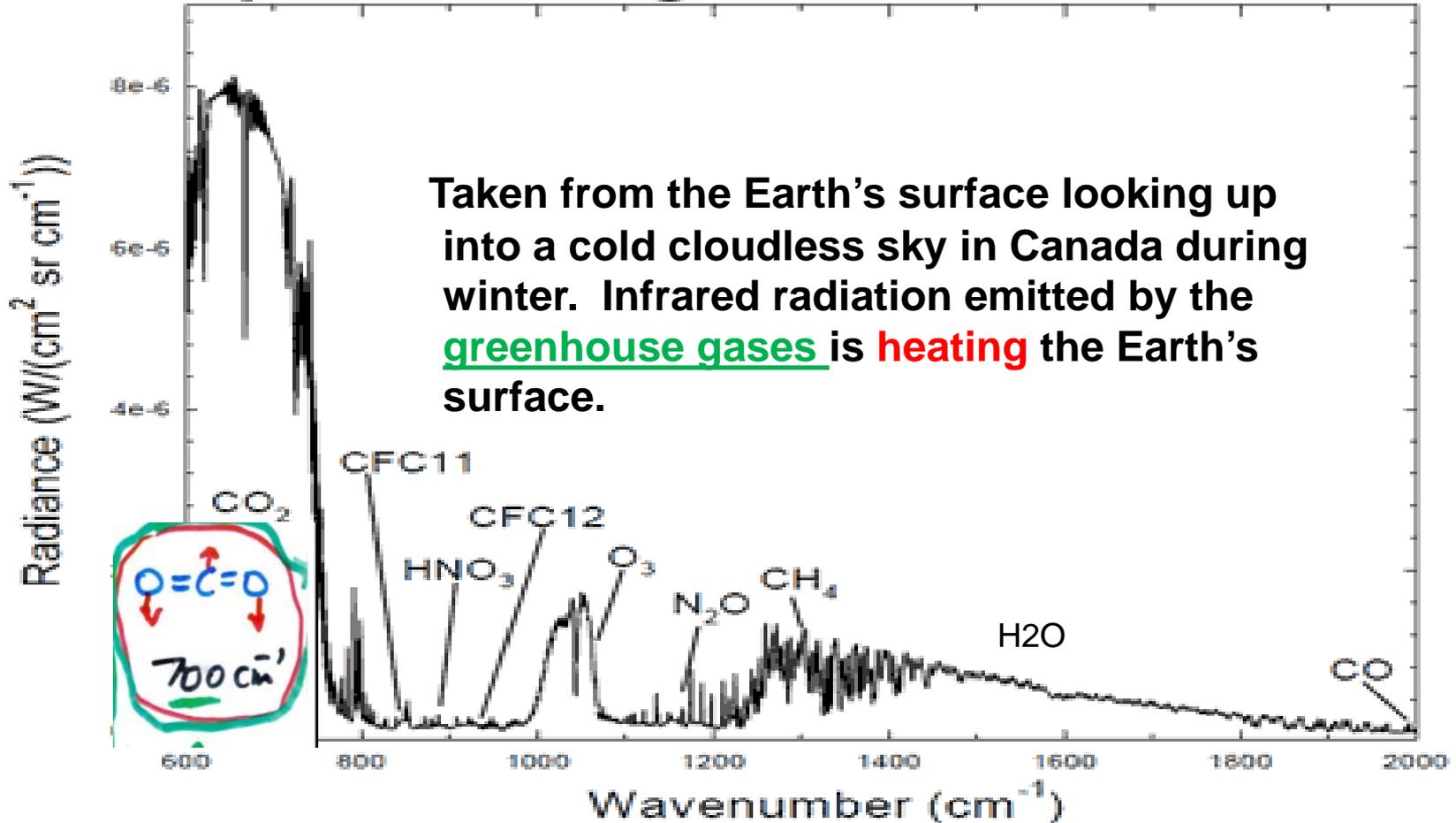
Light bulb

E. F. Schubert
 Light-Emitting Diodes (Cambridge Univ. Press)
 www.LightEmittingDiodes.org

IR fluorescence from the atmosphere.

Evidence: looking skyward with an IR spectrometer:

Spectrum of greenhouse radiation

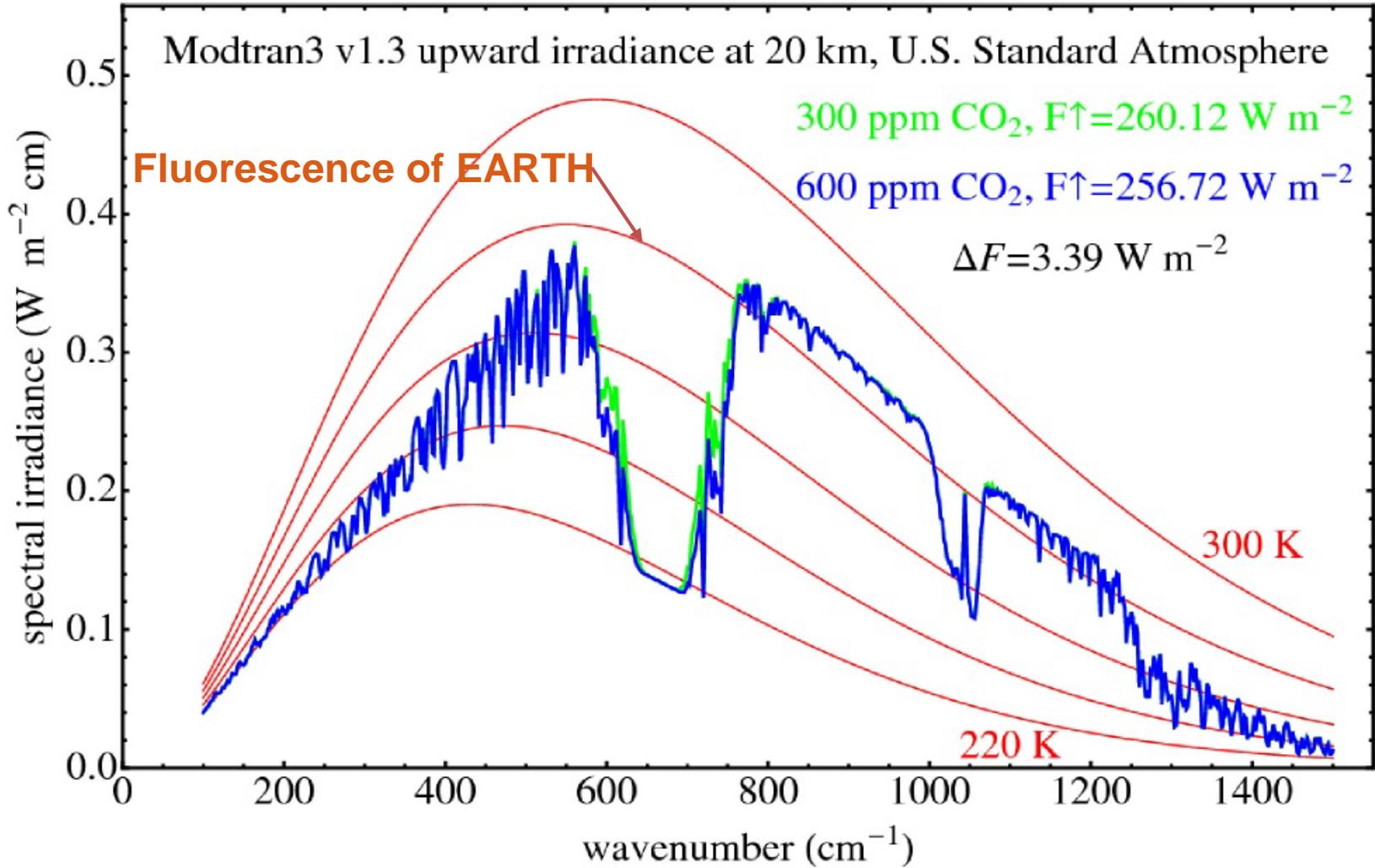


Why is CO₂ so strong compared to water??

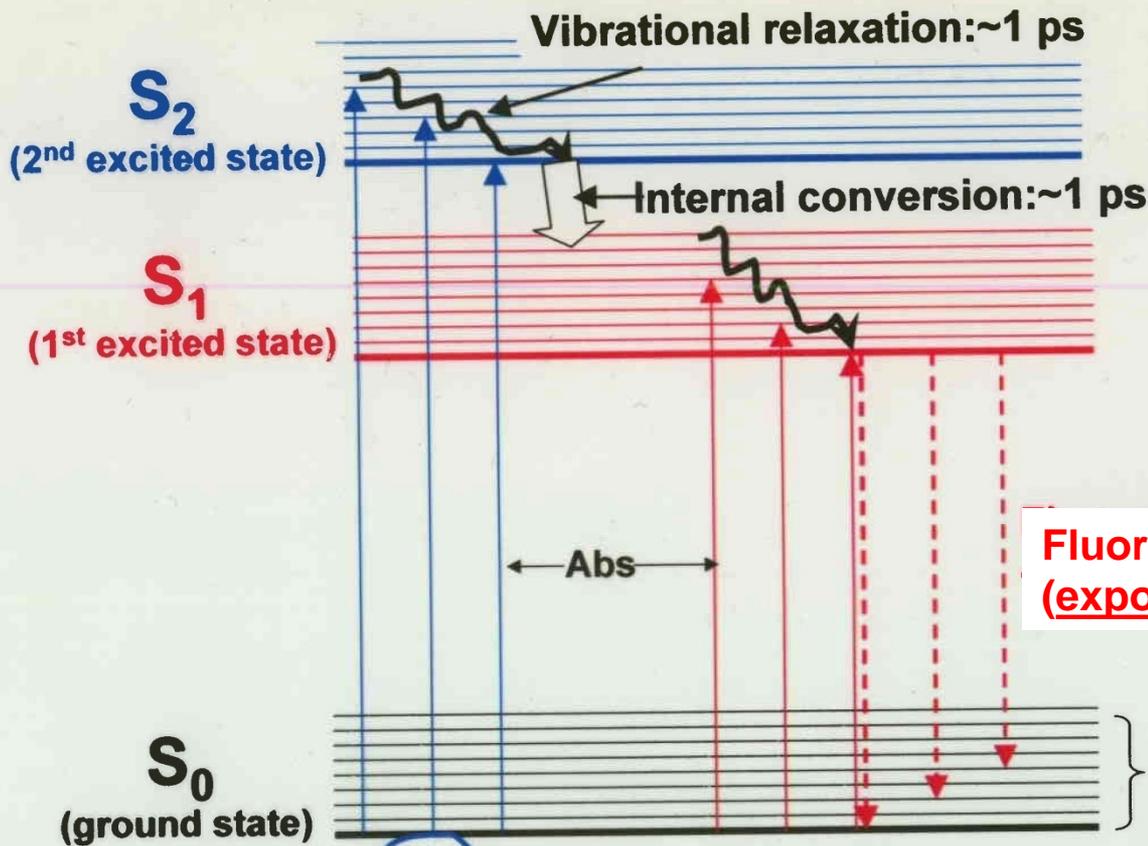
There is much more water in air.

([Evans 2006](#)).

CO₂



ABSORPTION & FLUORESCENCE



**Jablonski
Diagram**

Fluorescence lifetime ~5 ns
(exponential decay)

Vibrational levels

Kasha's Rule:

Fluorescence is 99.9% from S_1 independent of excitation wavelength; ~mirror image of S_1 abs.

S_2 abs

S_1 abs

S_1 fluor

Wavelength →

Beer-Lambert Law



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

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

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

for the disappearance of photons

Intensity of beam
falls off exponentially

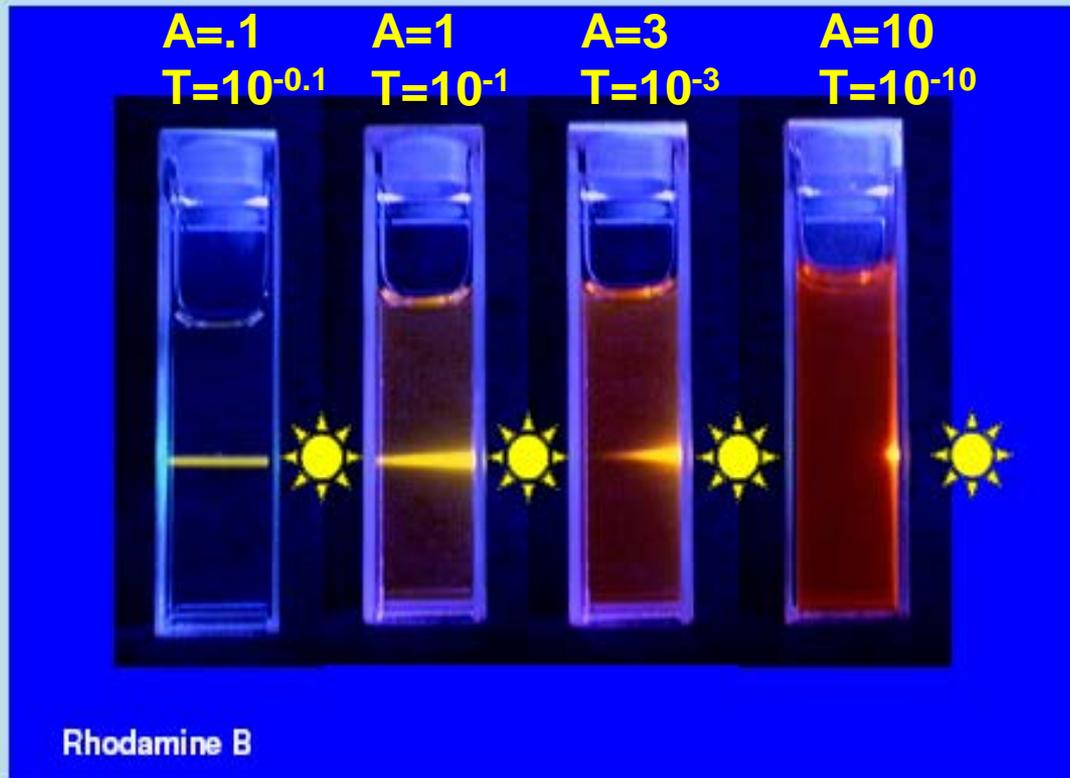
$$f = \frac{I}{I_0} = e^{-k[M]t} = e^{-k[M]x/c} = 10^{\frac{-k[M]x}{2.303c}} = 10^{-\epsilon[M]x} = 10^{-A}$$

In this context **f = fraction of photons remaining after travelling distance x**
i.e., **f = Transmittance = T = 10^{-εcx} = 10^{-A}**

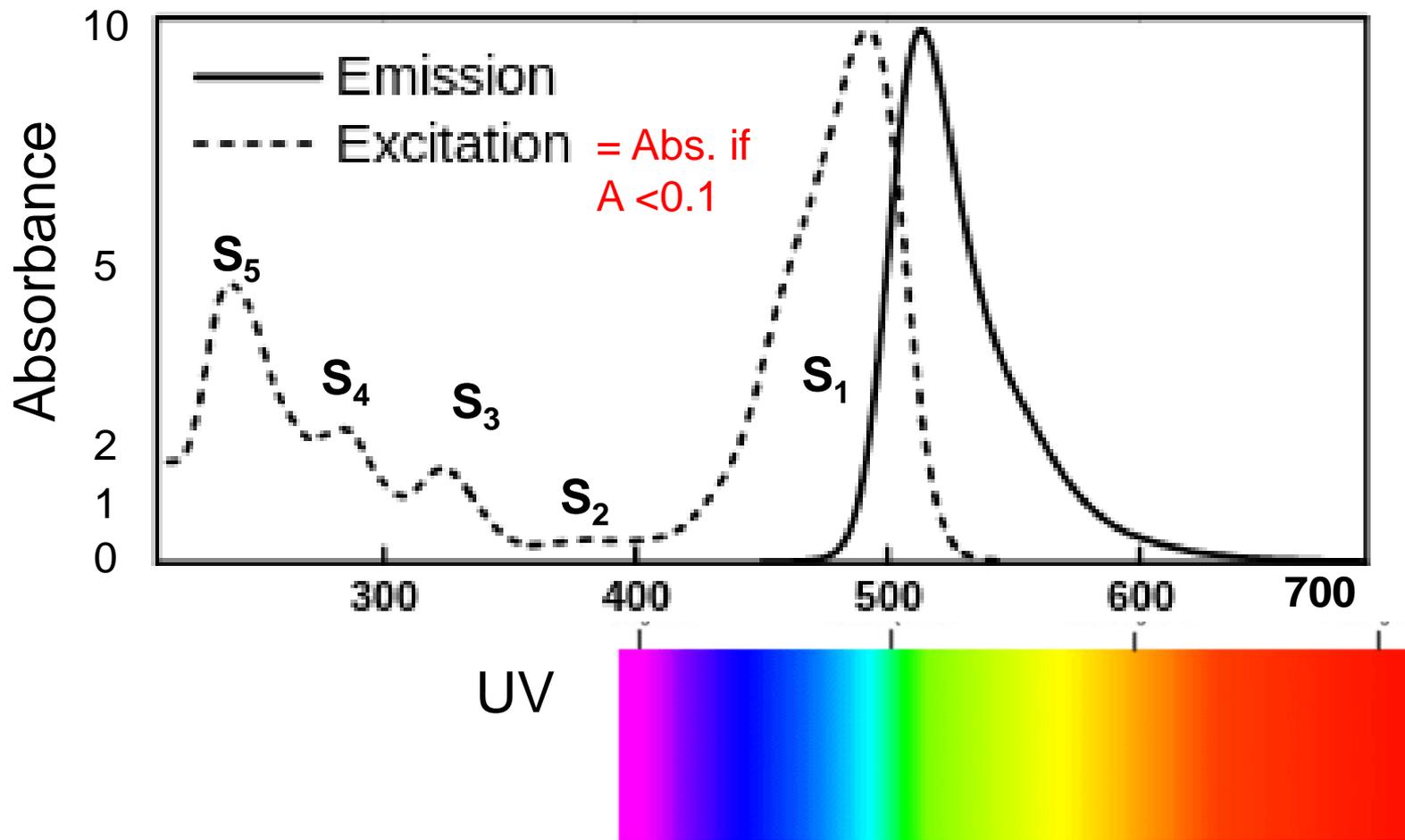
10^{-A} is just telling you that **A = εcx = -log T**

Attenuation of the Excitation Light through Absorbance

Sample concentration
& the *inner filter effect*



Fluorescein



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

Fluorescence from single molecules under a microscope

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

Fig. 13.34 Tinoco et al.

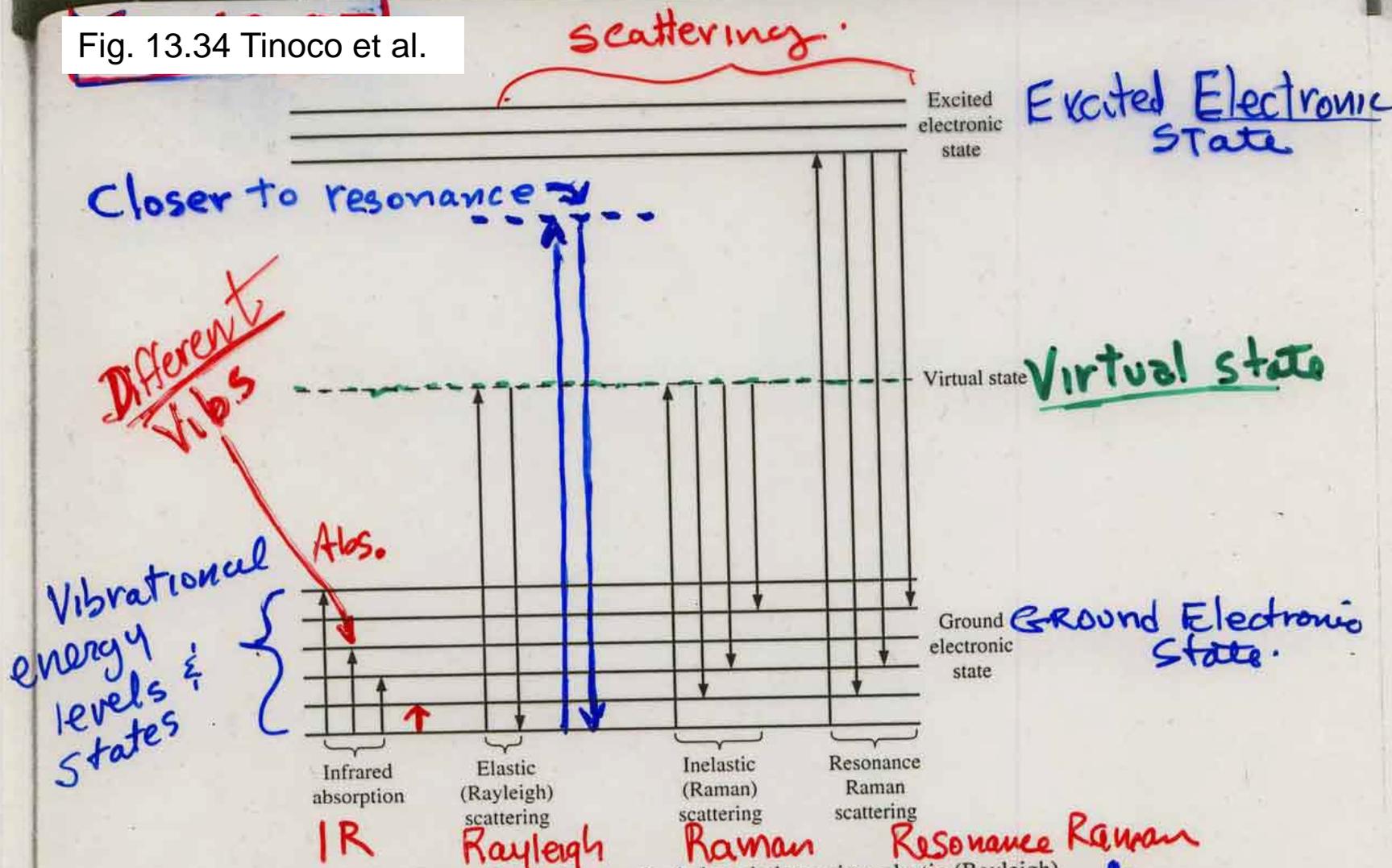


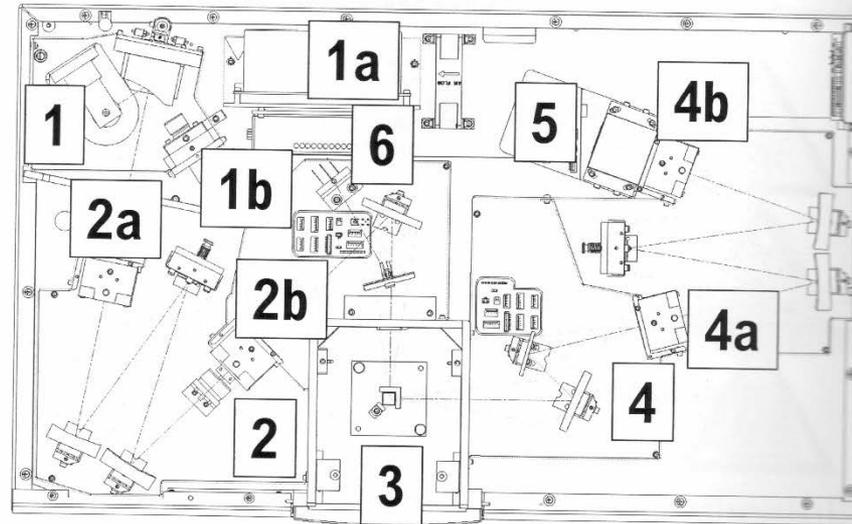
Fig. 10.41 Energy-level diagram for infrared absorption, elastic (Rayleigh) scattering, and inelastic (Raman) scattering. Transitions between vibrational energy levels of the ground electronic state absorb infrared radiation; not all transitions are allowed. For elastic scattering the frequency of the 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.

~ 1000 times enhanced.

How do we measure fluorescence and scattering?

Optical layout

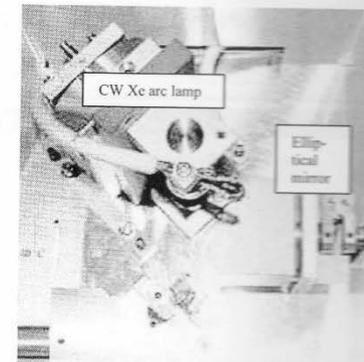
Horiba Fluorimeter



- 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)

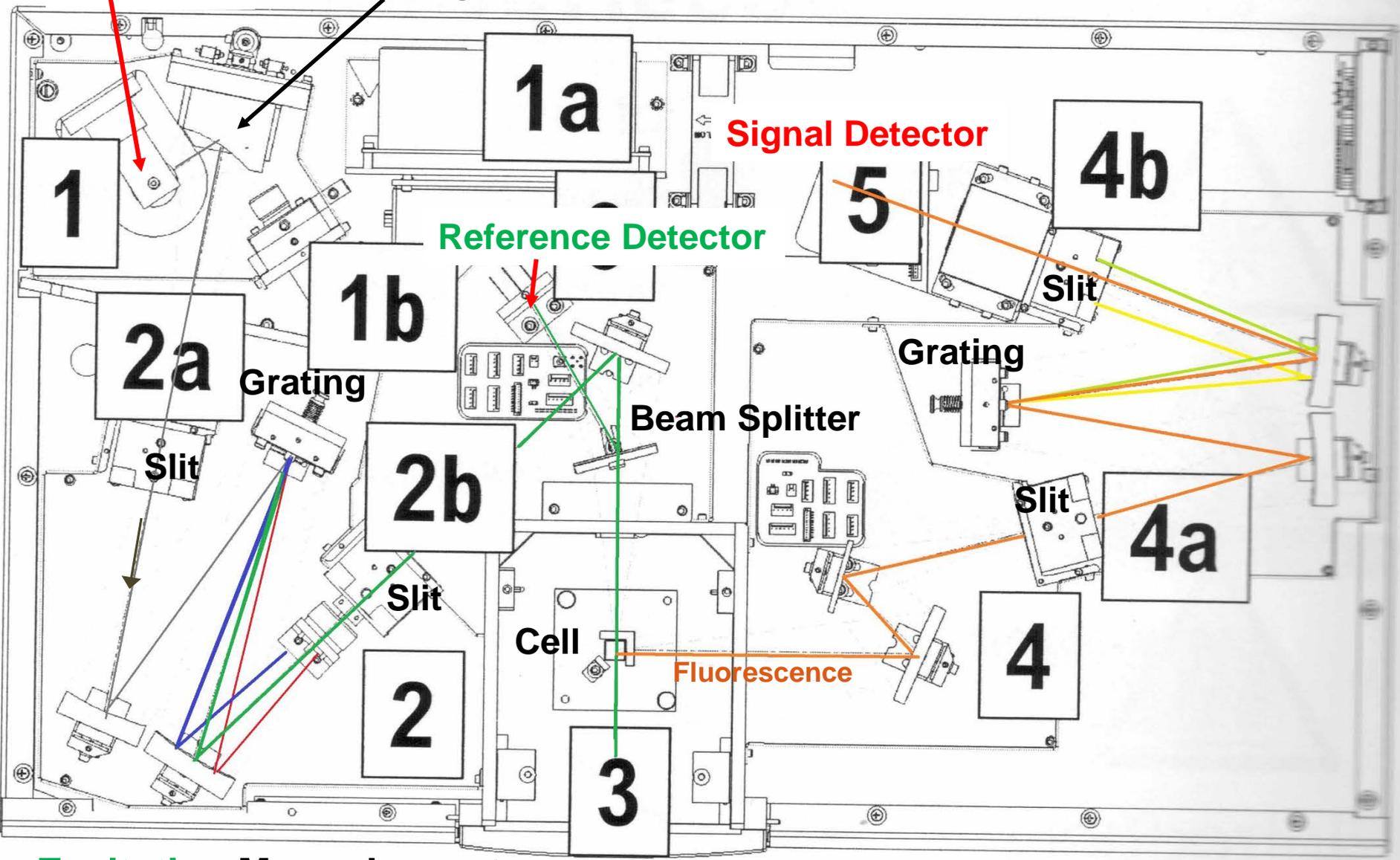
Illuminator (xenon arc-lamp, 1)

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.



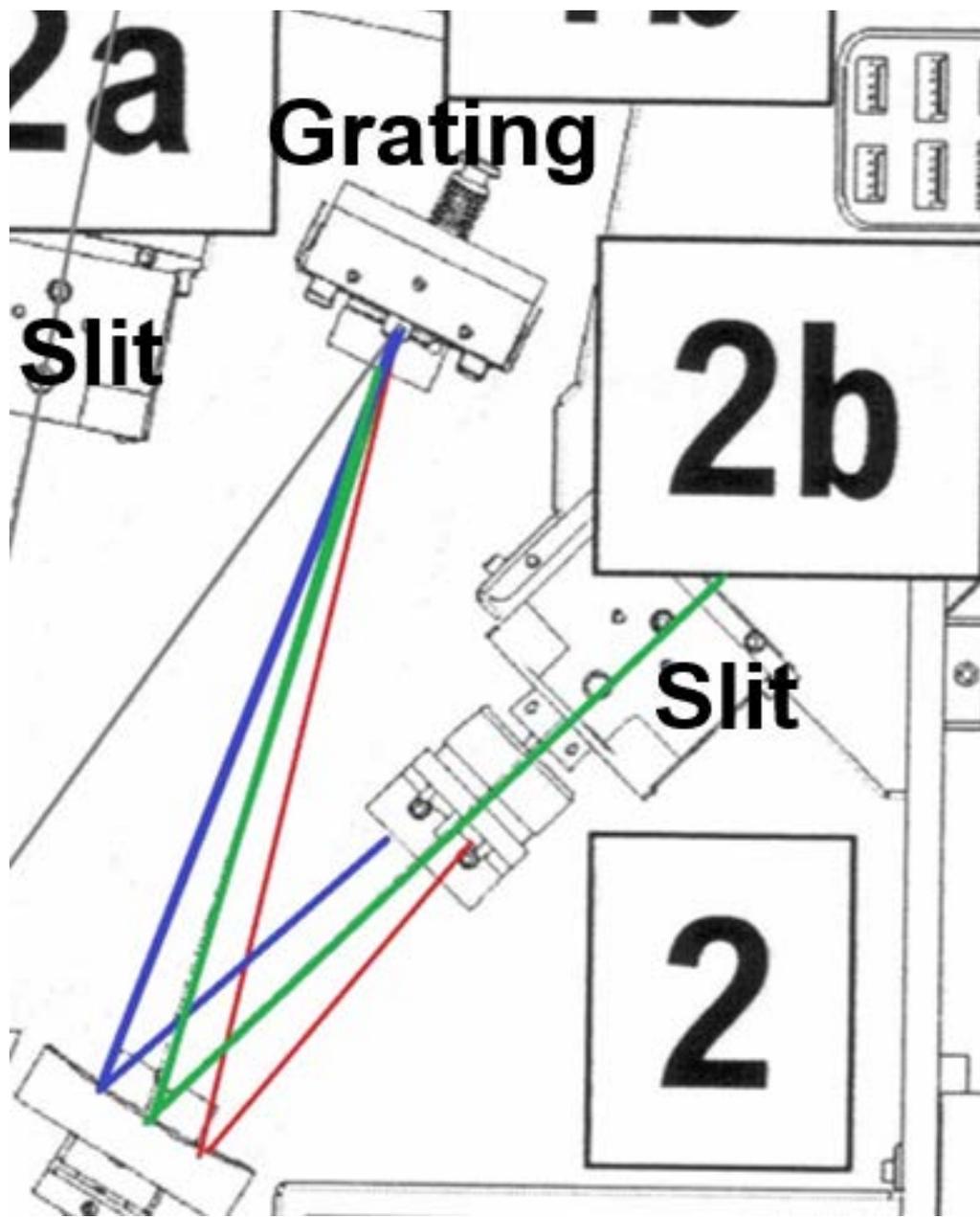
150 W Xenon Arc

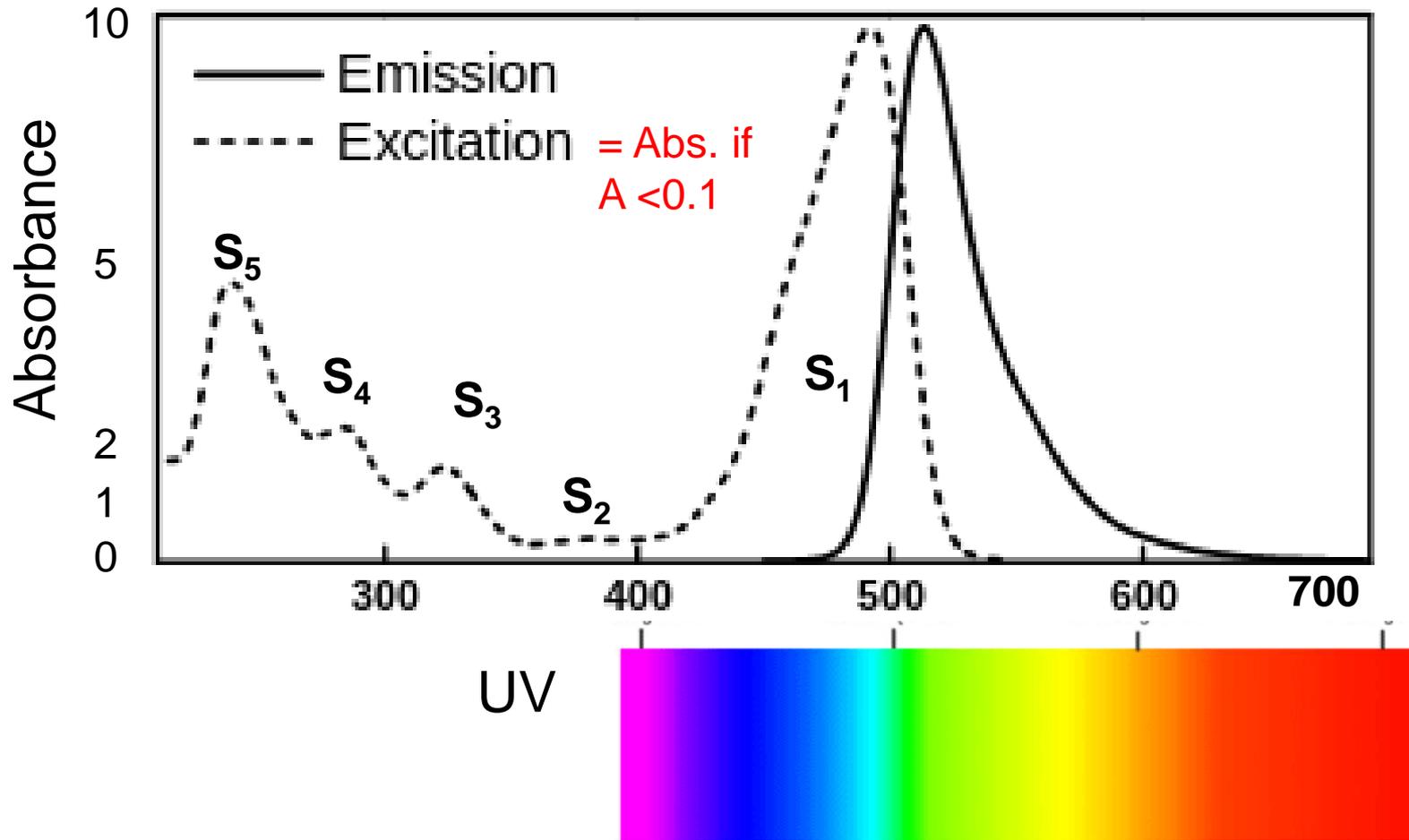
Parabolic mirror focuses light on slit



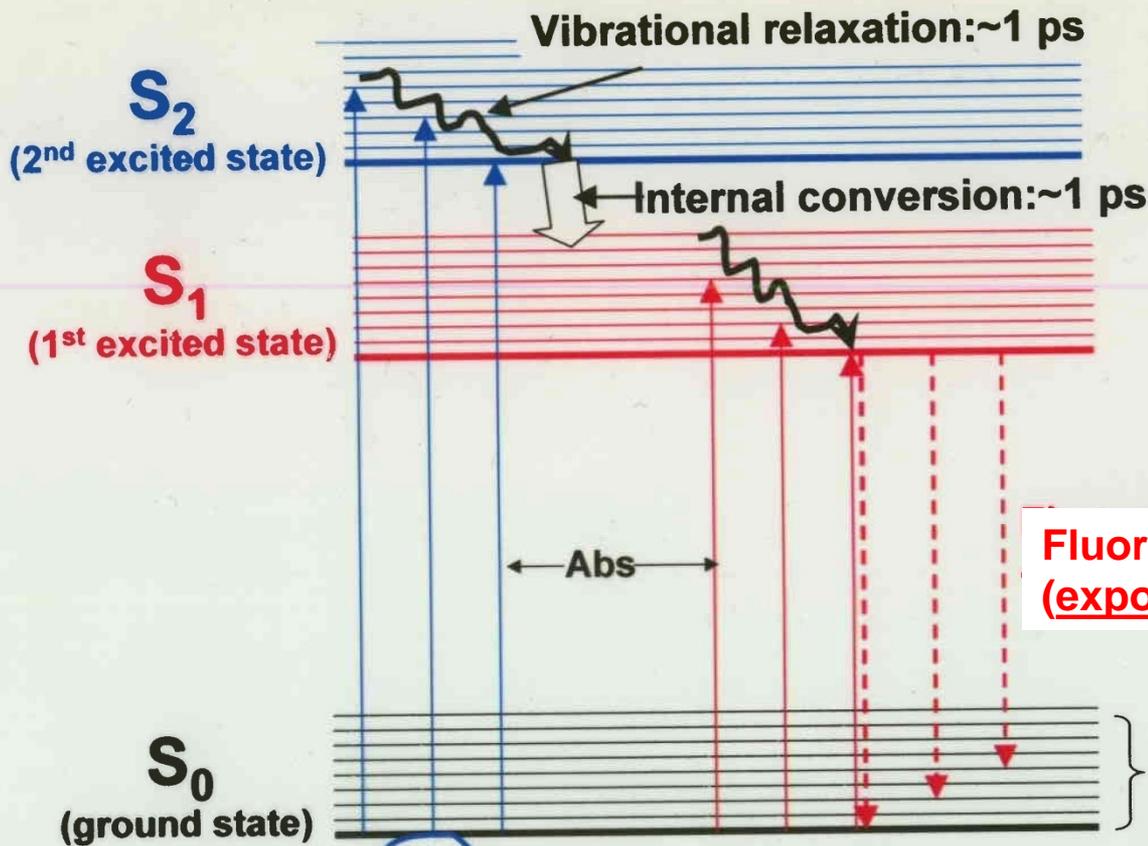
Excitation Monochromator

Emission Monochromator





ABSORPTION & FLUORESCENCE



Jablonski Diagram

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S_2 abs

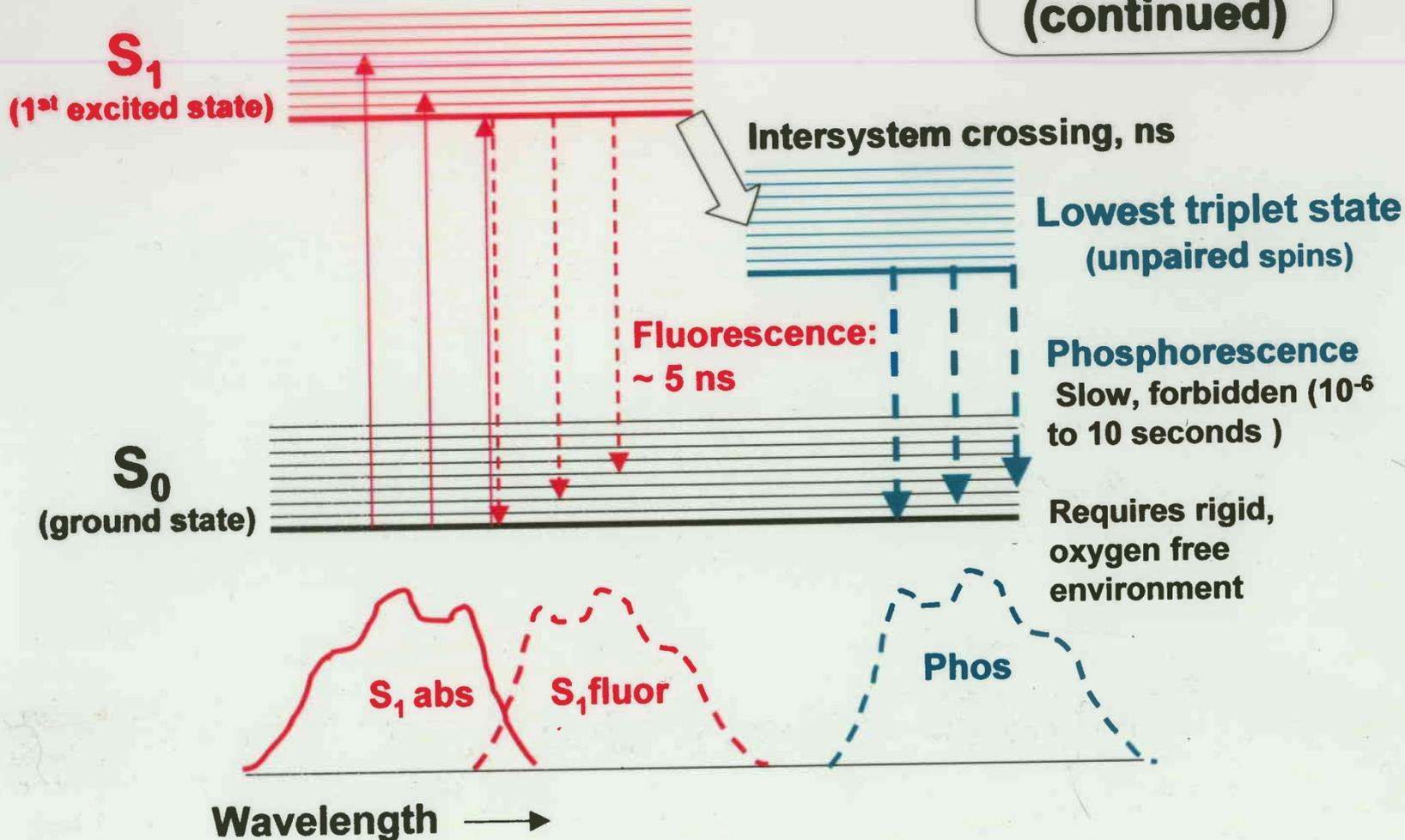
S_1 abs

S_1 fluor

Wavelength →

Phosphorescence

Jablonski Diagram (continued)



Fluorescence Quantum Yield

4.2.3 Fluorescence Quenching by Iodide

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

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

Fluorescence Lifetime = 1/(sum of rate constants)

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

What is fluorescence lifetime?

$$d[\text{excited molecules}]/dt = -k [\text{excited molecules}]$$

$$d(\text{Intensity})/dt = -k (\text{Intensity}) \quad \mathbf{1^{\text{st}} \text{ order reaction}}$$

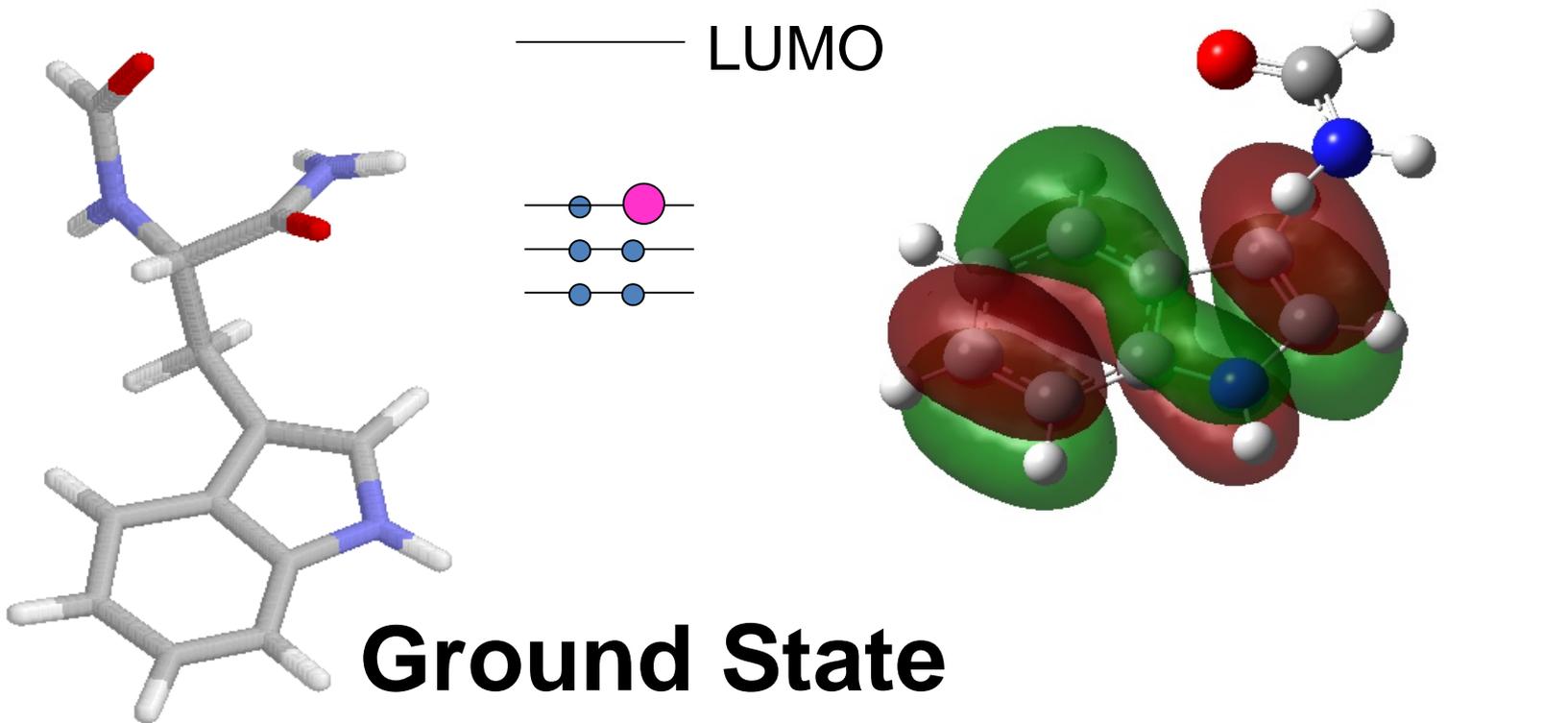
Solution to this differential equation?

$$\begin{aligned} \text{Fluor. intensity at time } t &= (\text{Fluor. Intensity at time } 0) \times \mathbf{e^{-kt}} \\ \text{or} &= (\text{Fluor. Intensity at time } 0) \times \mathbf{e^{-t/\tau}} \end{aligned}$$

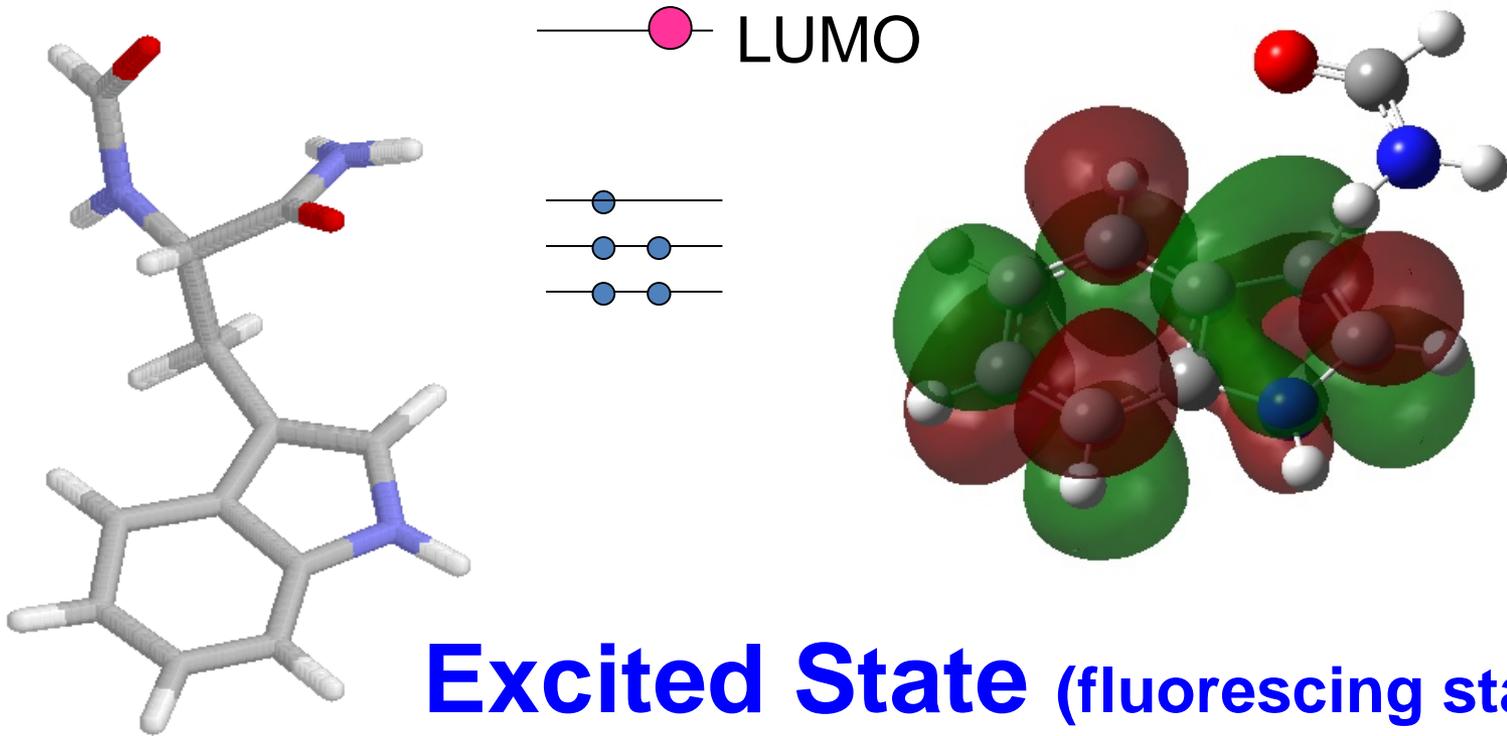
$$\mathbf{\tau = \text{“lifetime”} = 1/k}$$

$$\mathbf{\tau = \text{inverse of } 1^{\text{st}} \text{ order rate constant}}$$

“Quenching”



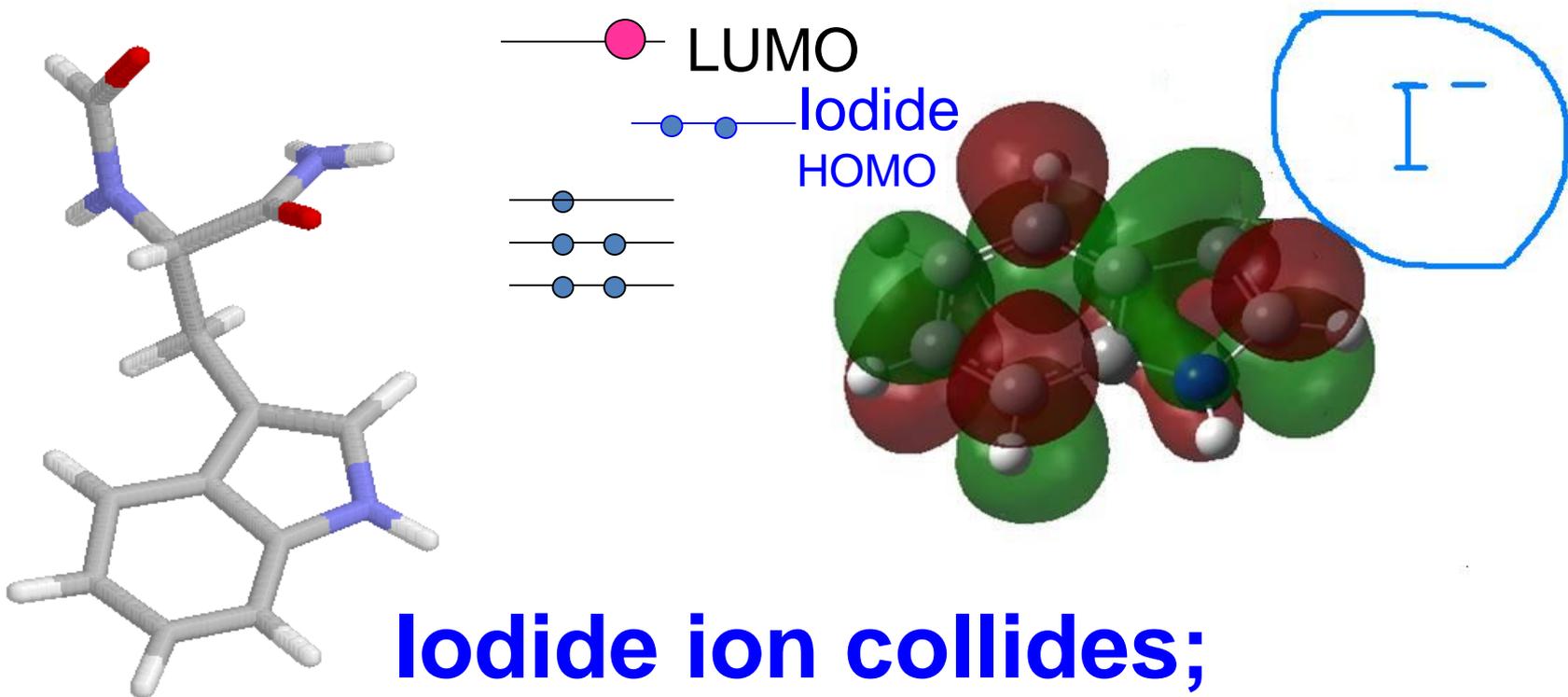
LUMO



Excited State (fluorescing state)

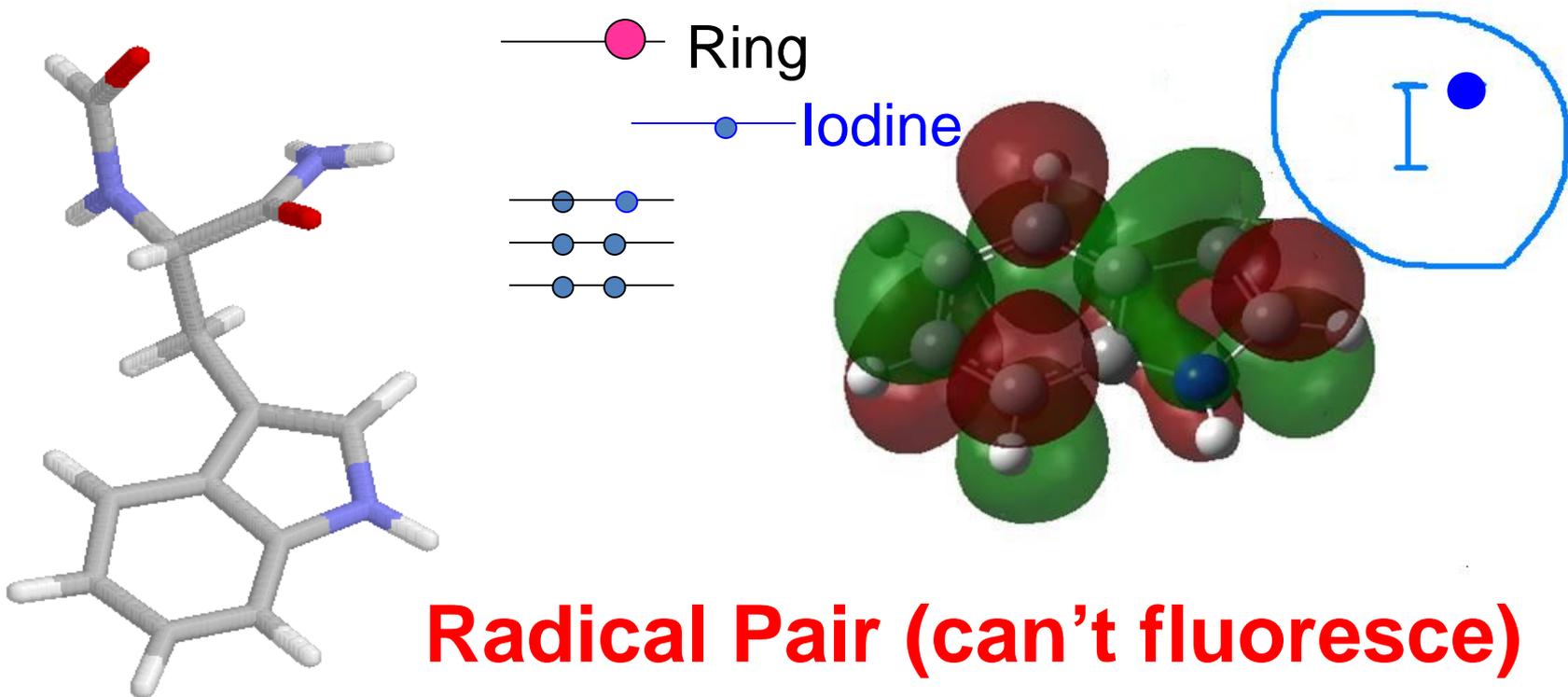
**Lowest Unoccupied Molecular
Orbital (electron excited)**

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)



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