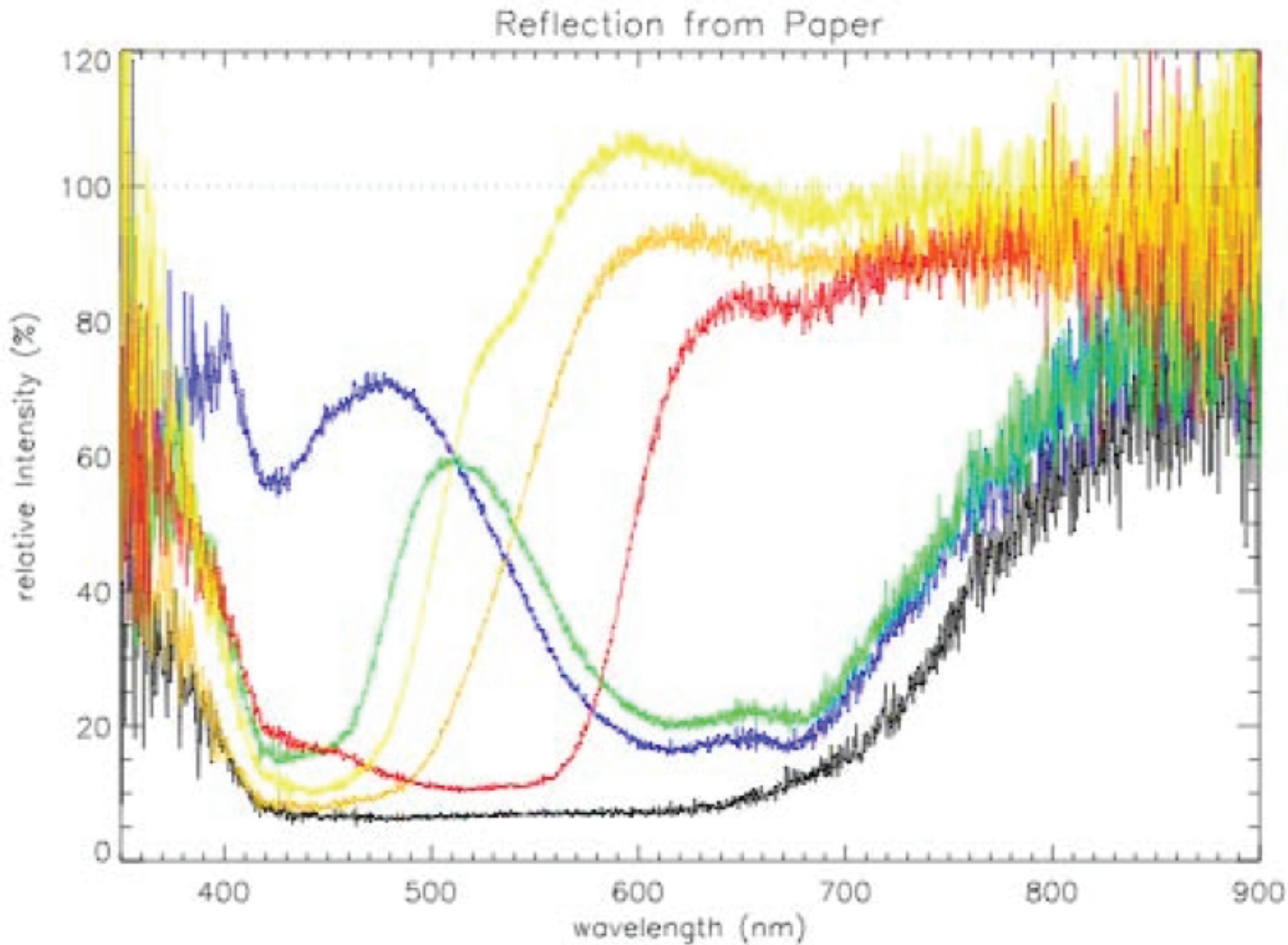


Reflectance spectra from colored paper



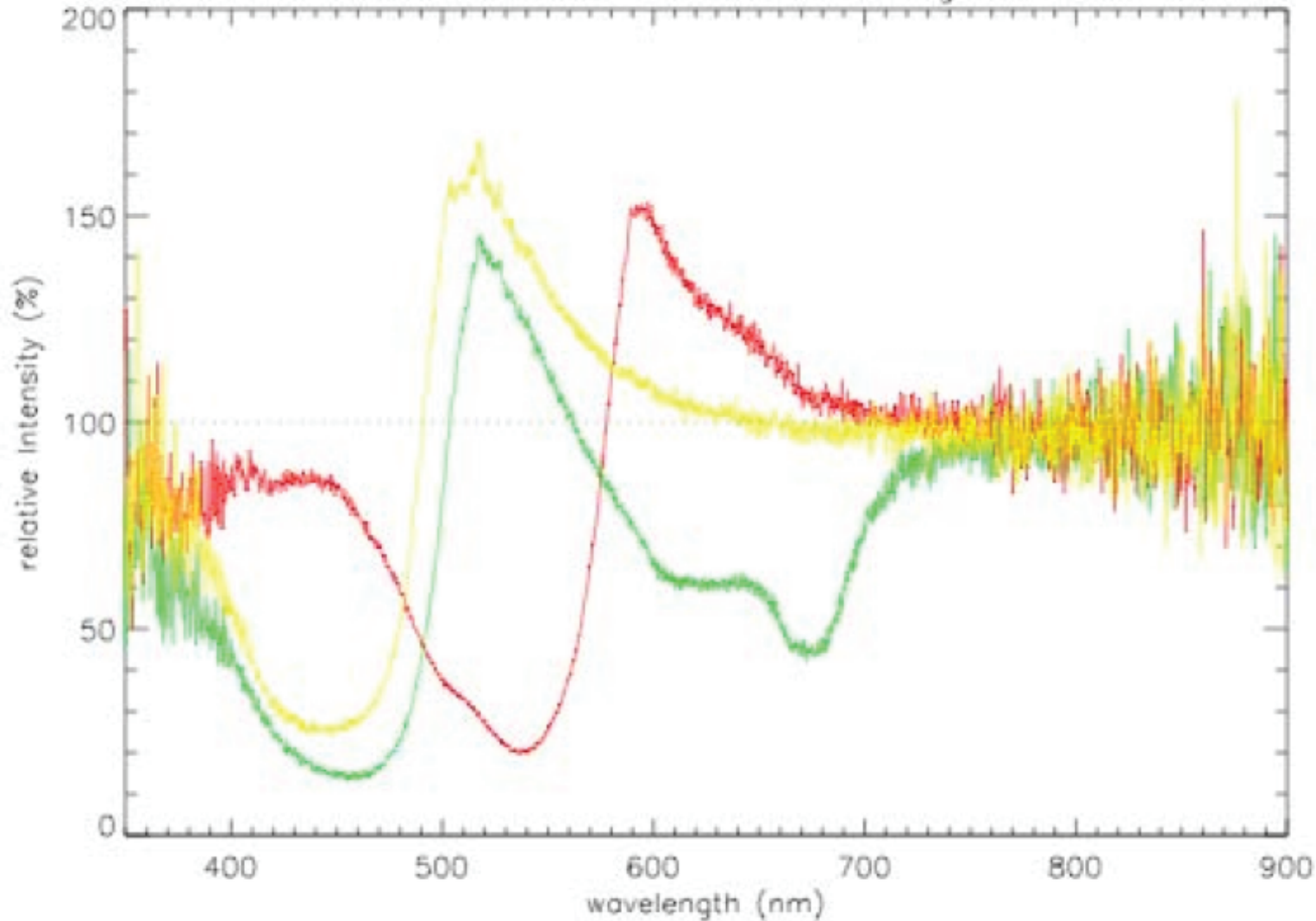
Spectra of sunlight reflecting off of color papers appearing :

blue
green
yellow
orange
red
black

aside from slight fluorescence in the yellow paper, colors operating by reflectance only never peak above 100%

Emission spectra for fluorescent markers (highlighter pens)

Reflection from Fluorescent Highlighters

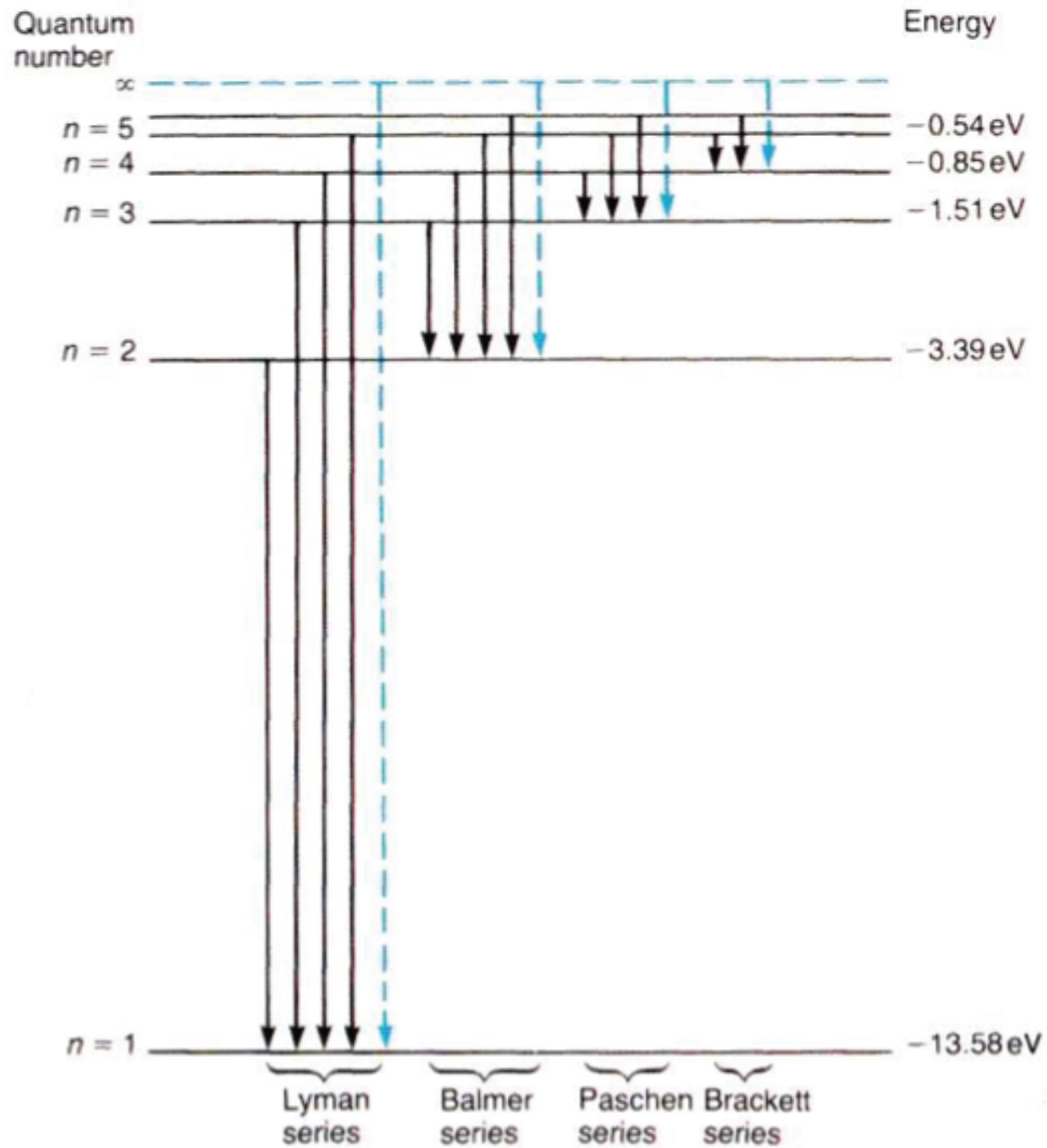


Fluorescent markers convert light from lower wavelengths to higher wavelengths.

green highlighter
yellow highlighter
pink highlighter

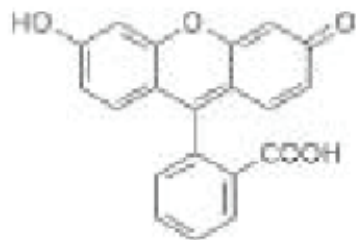
All three highlighters have emissions that exceed the 100% that would be possible from reflection alone.

Energy levels for atomic spectra of hydrogen

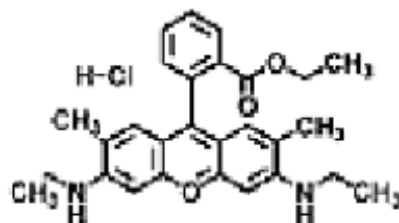


Absorption & emission spectra of fluorescent organic dyes

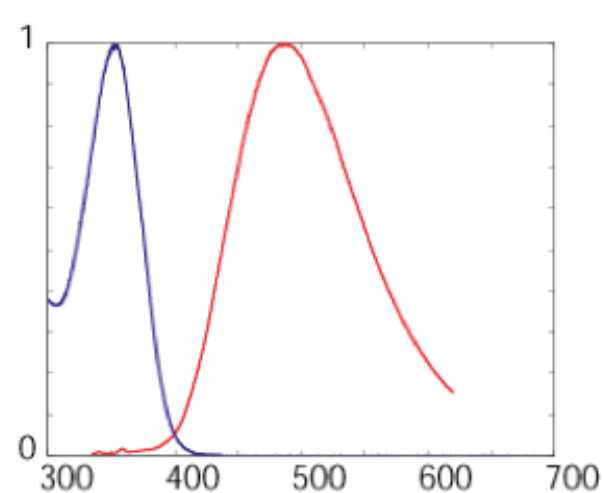
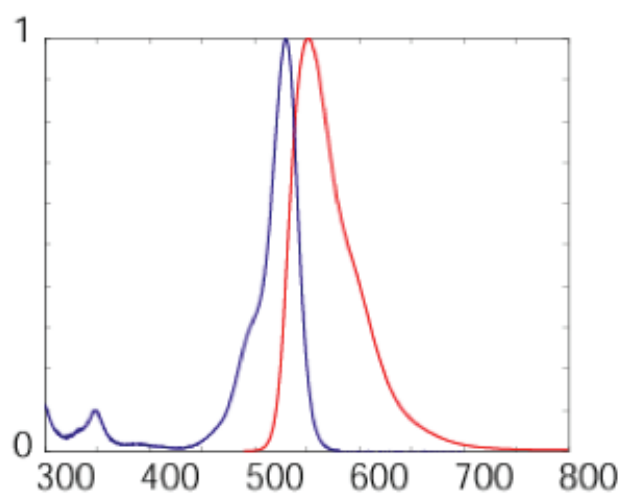
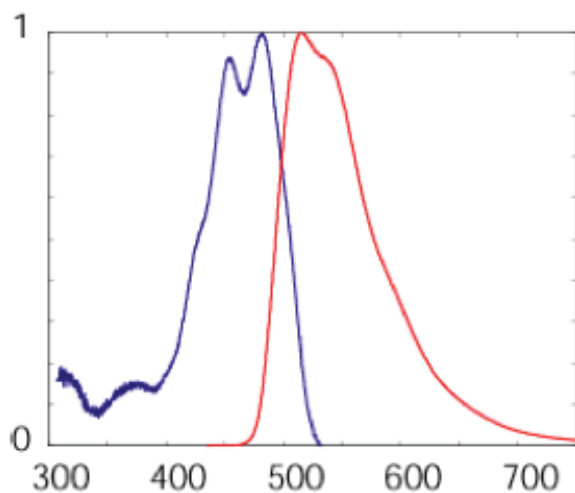
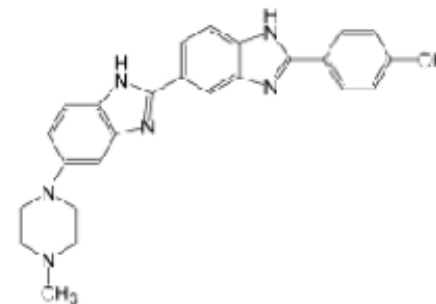
Fluorescein



Rhodamine 6G



Hoechst 33258

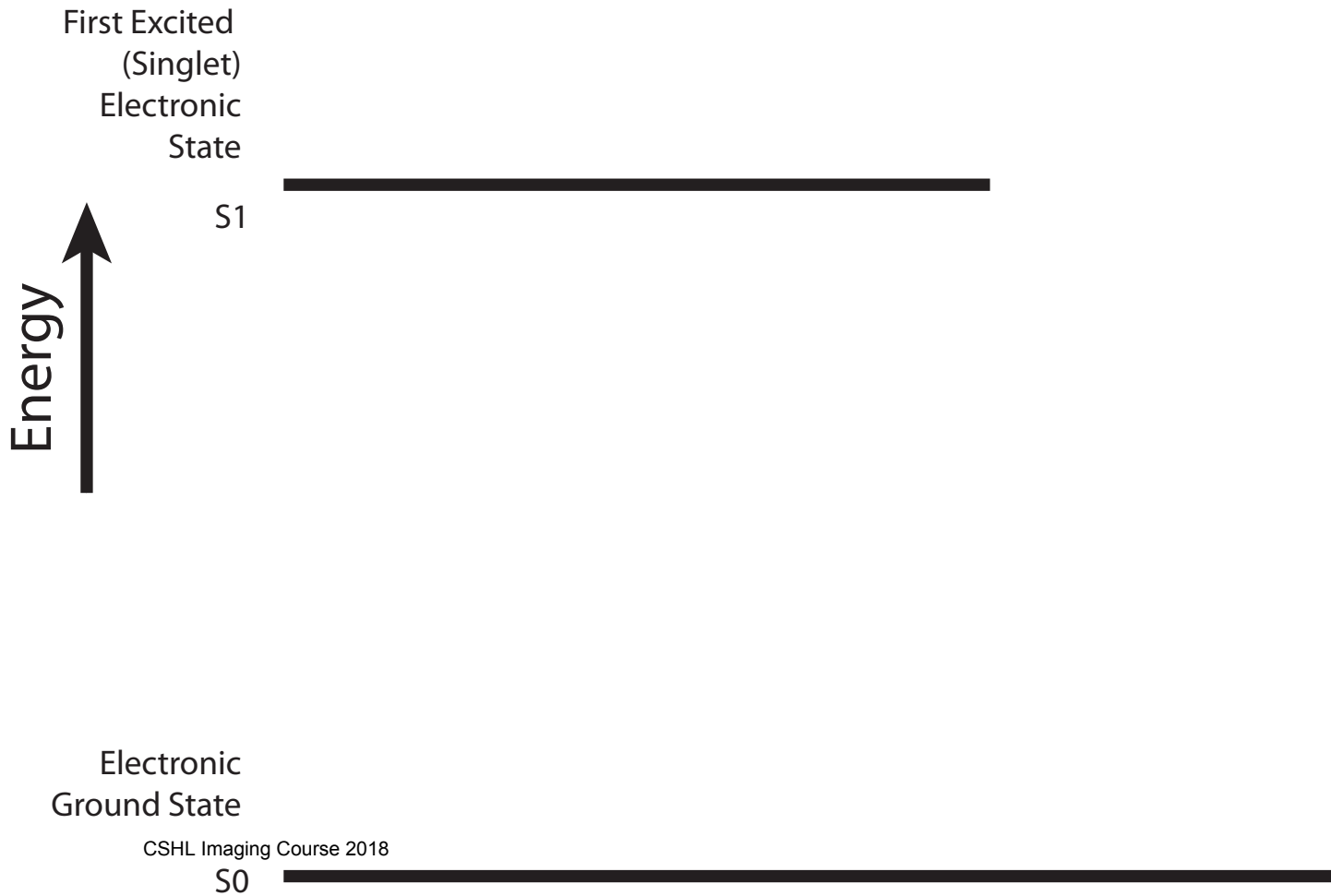


Wavelength (nm)

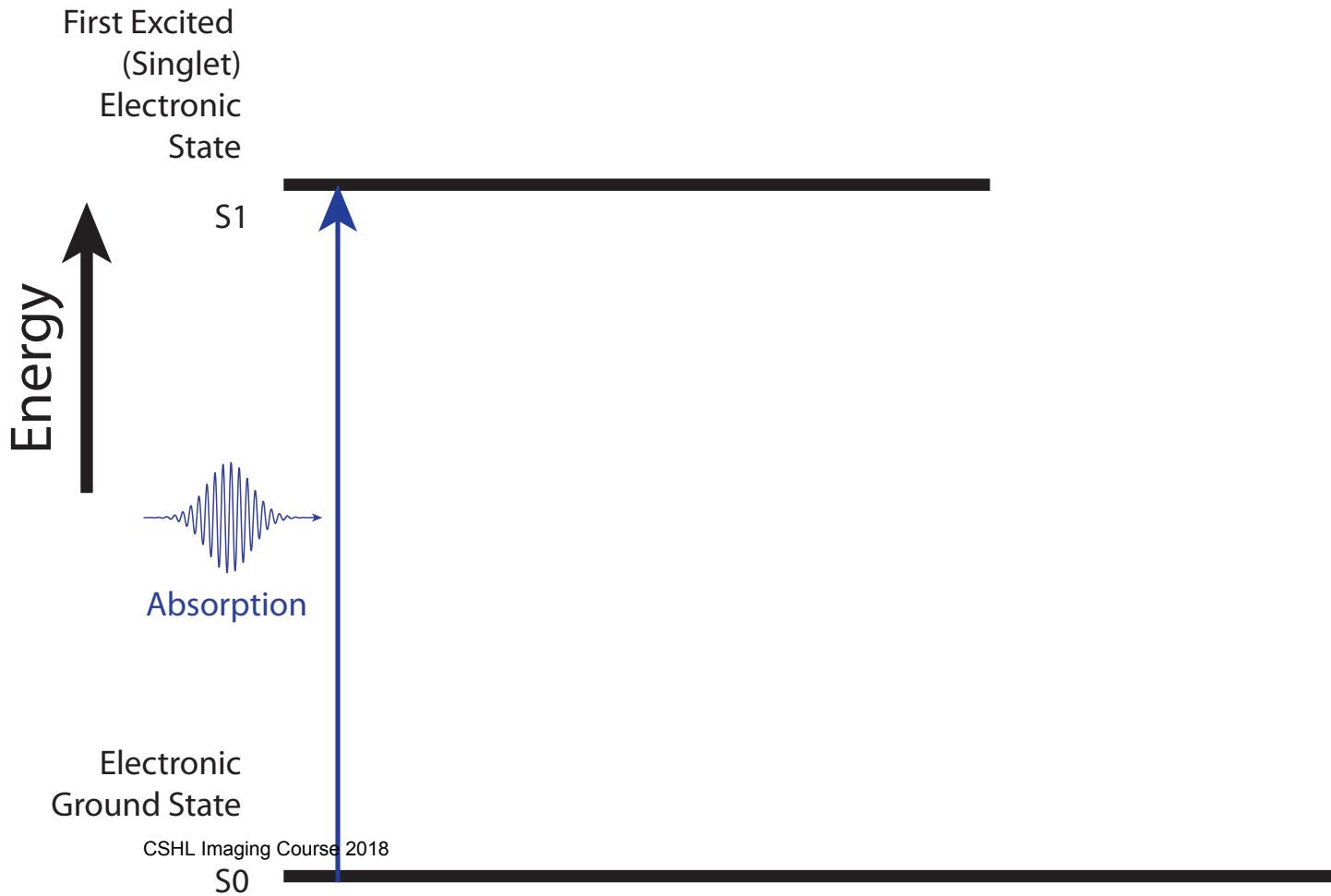
— Normalized Absorption Spectrum

— Normalized Fluorescence Emission Spectrum

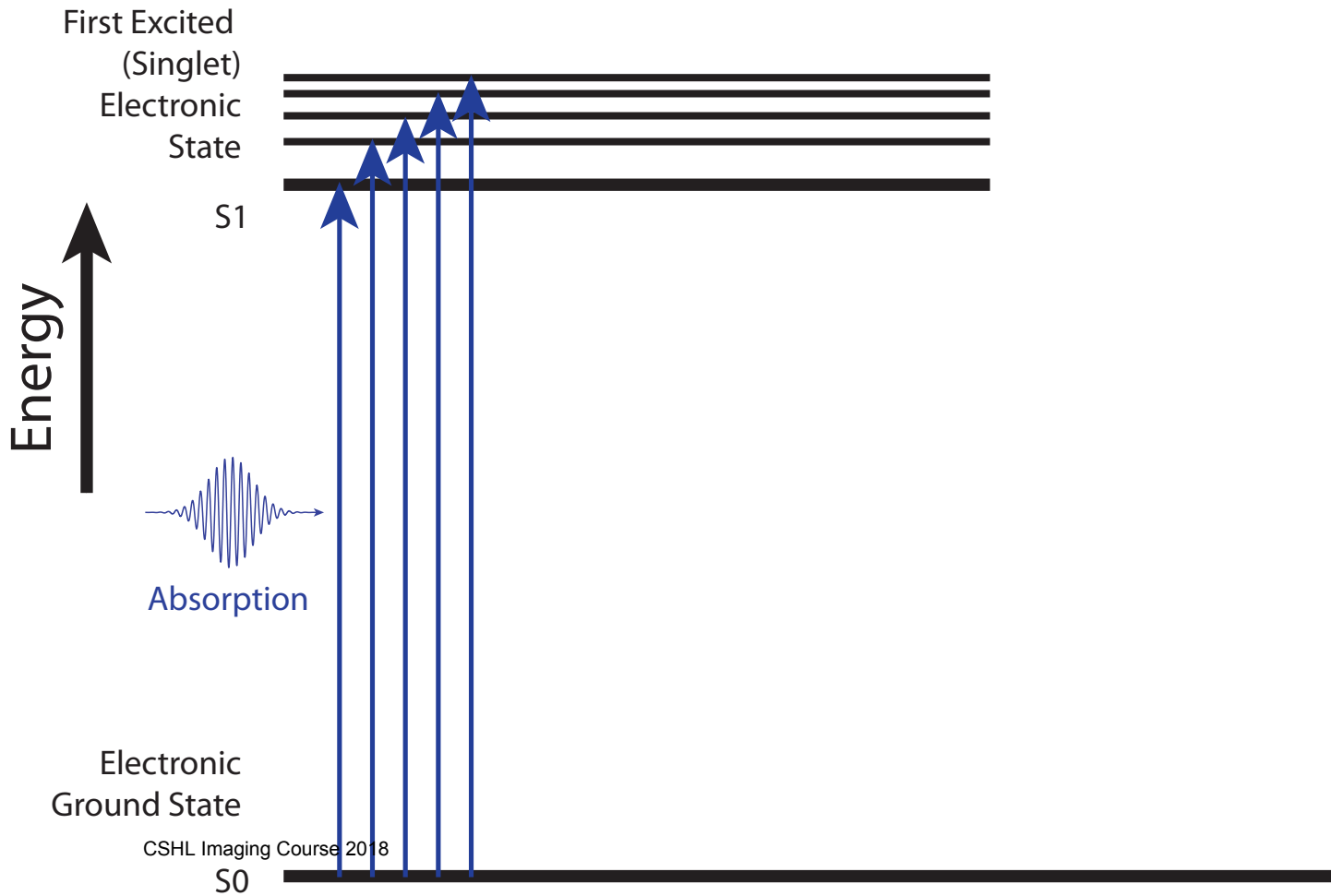
Jablonski Energy Diagram



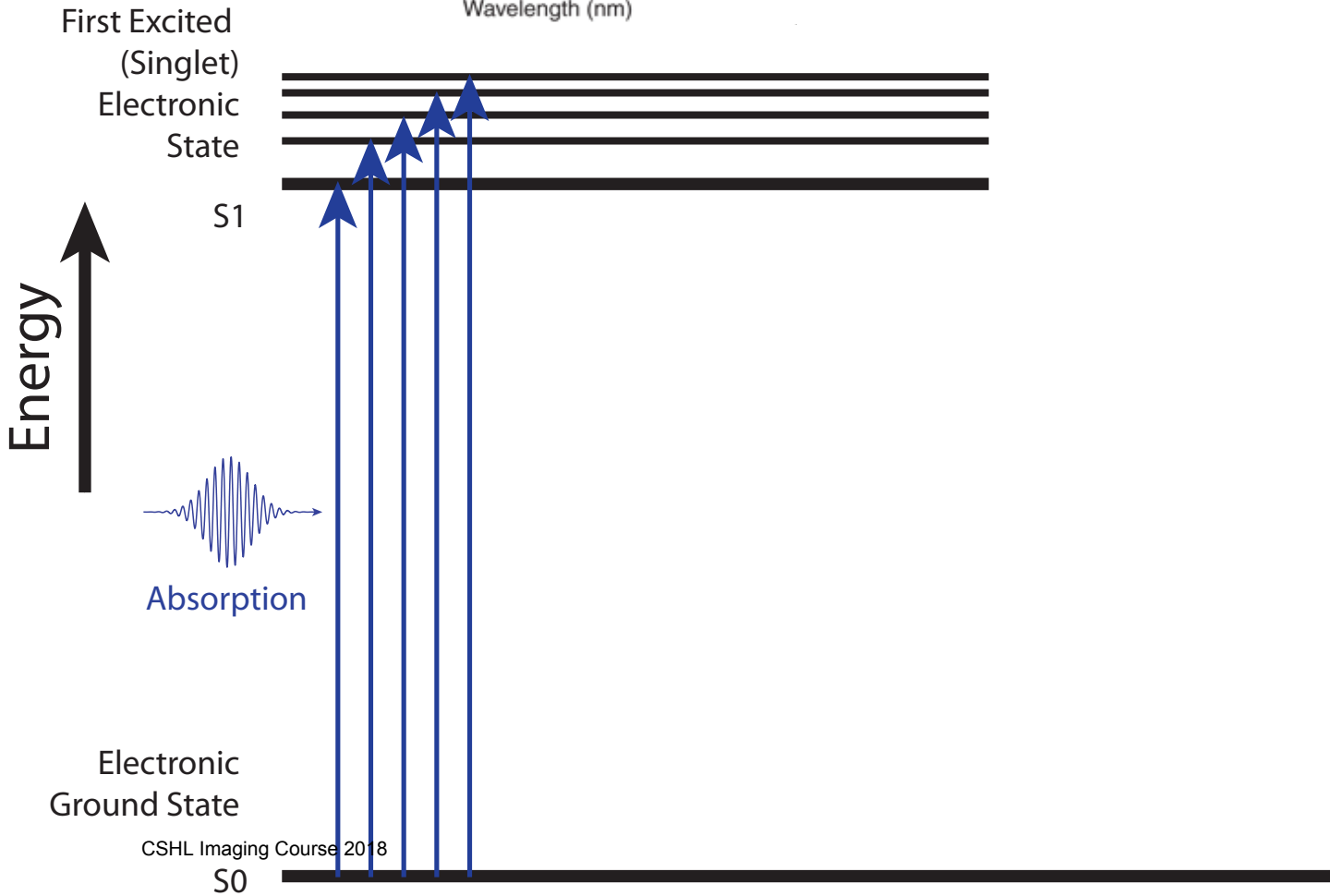
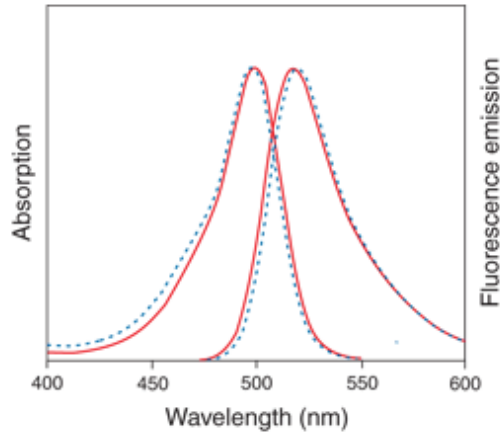
Jablonski Energy Diagram



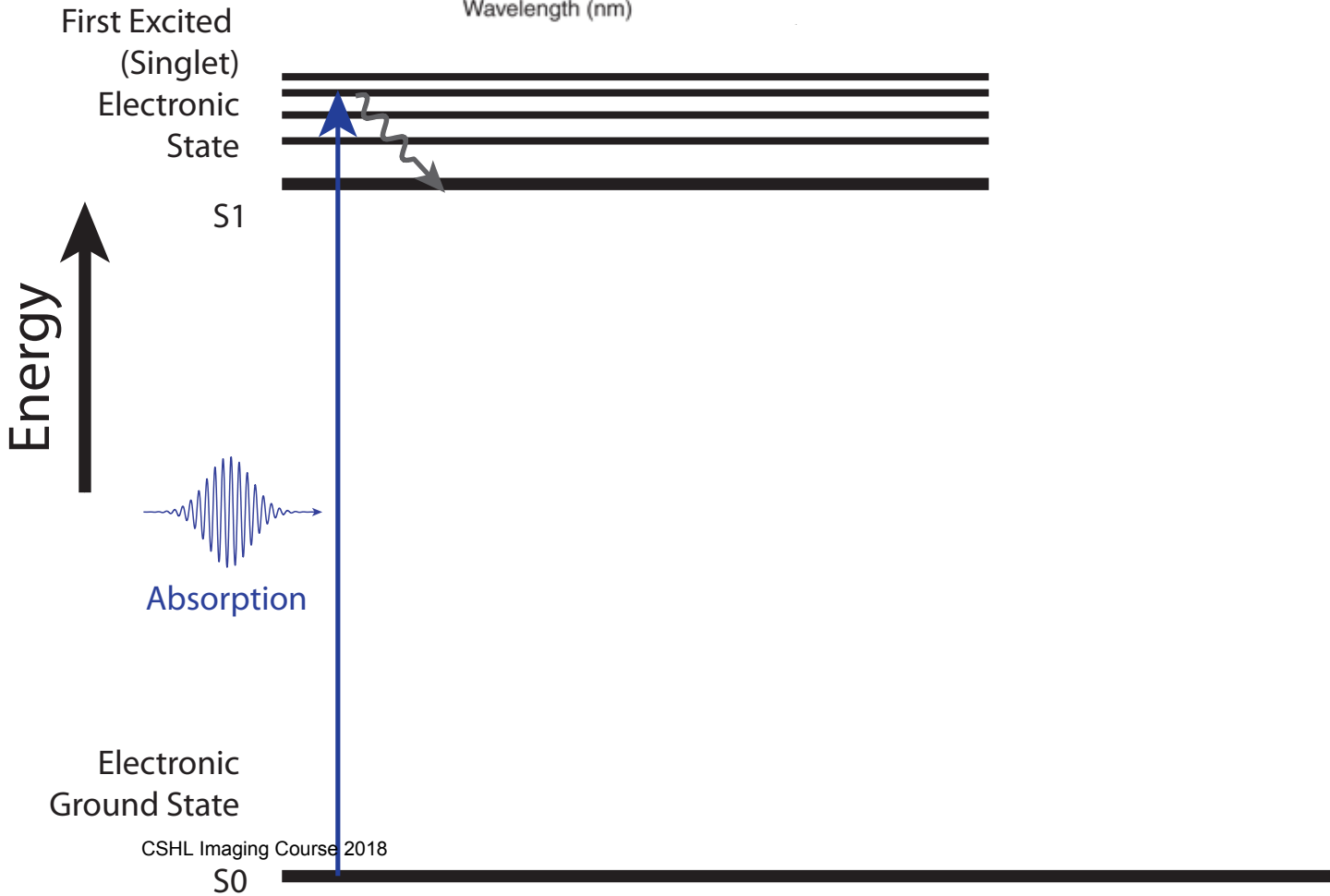
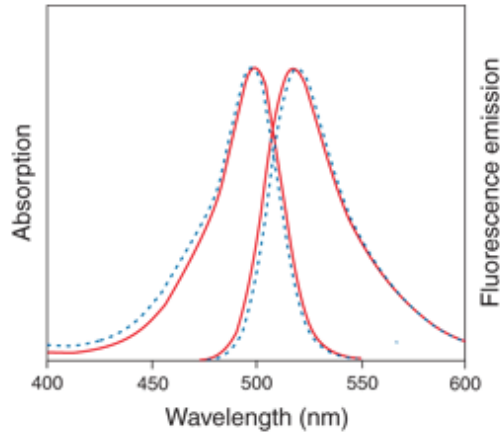
Jablonski Energy Diagram



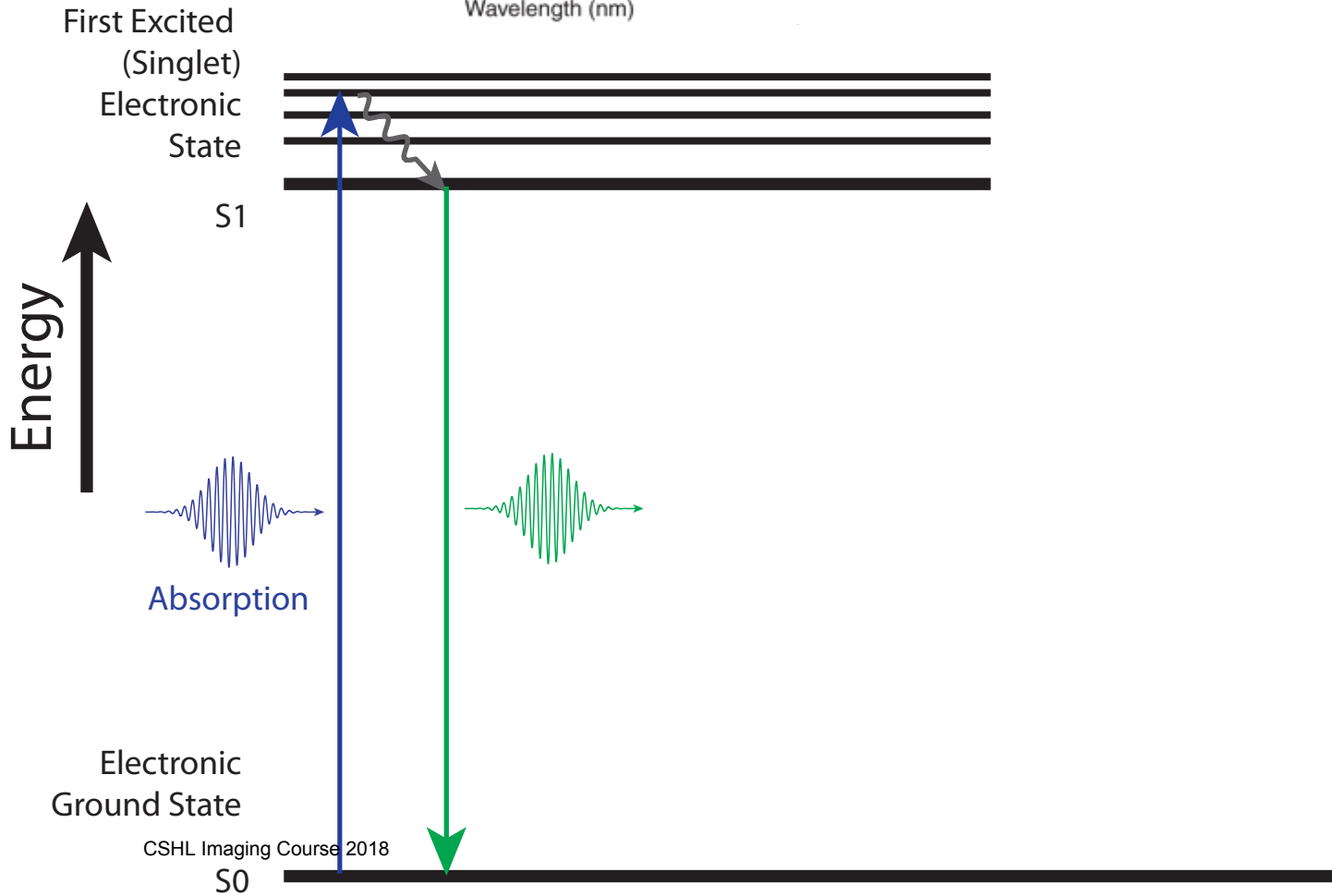
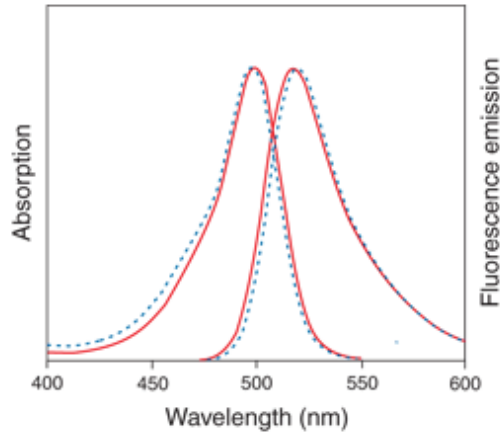
Jablonski Energy Diagram



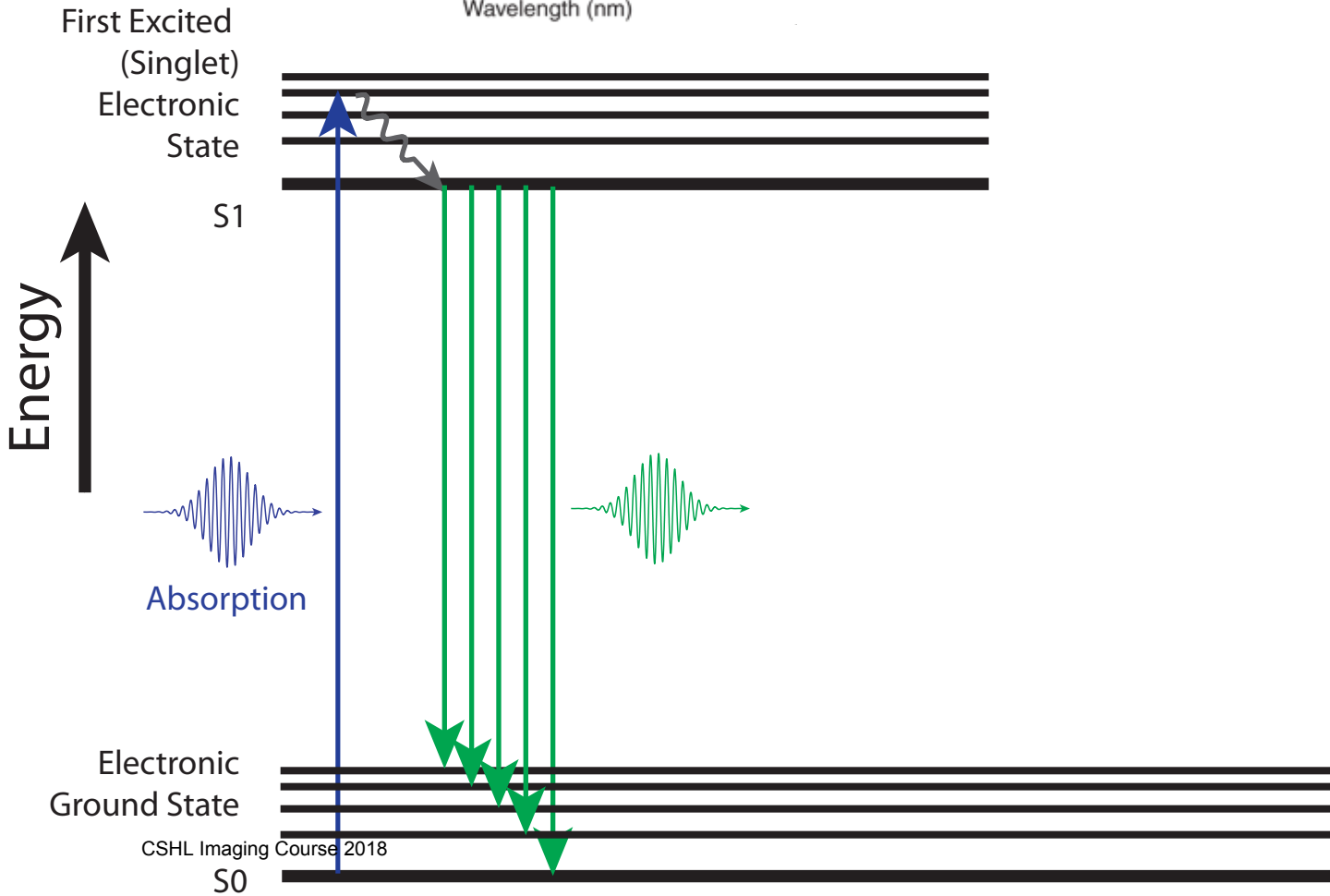
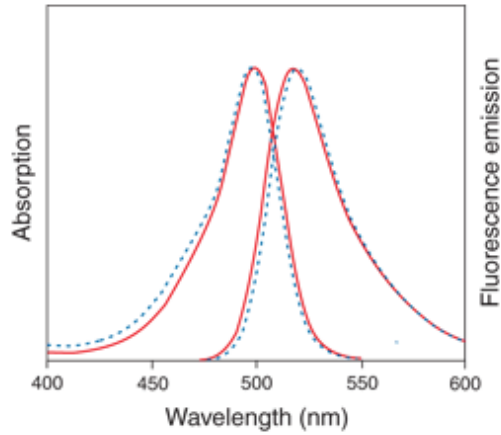
Jablonski Energy Diagram



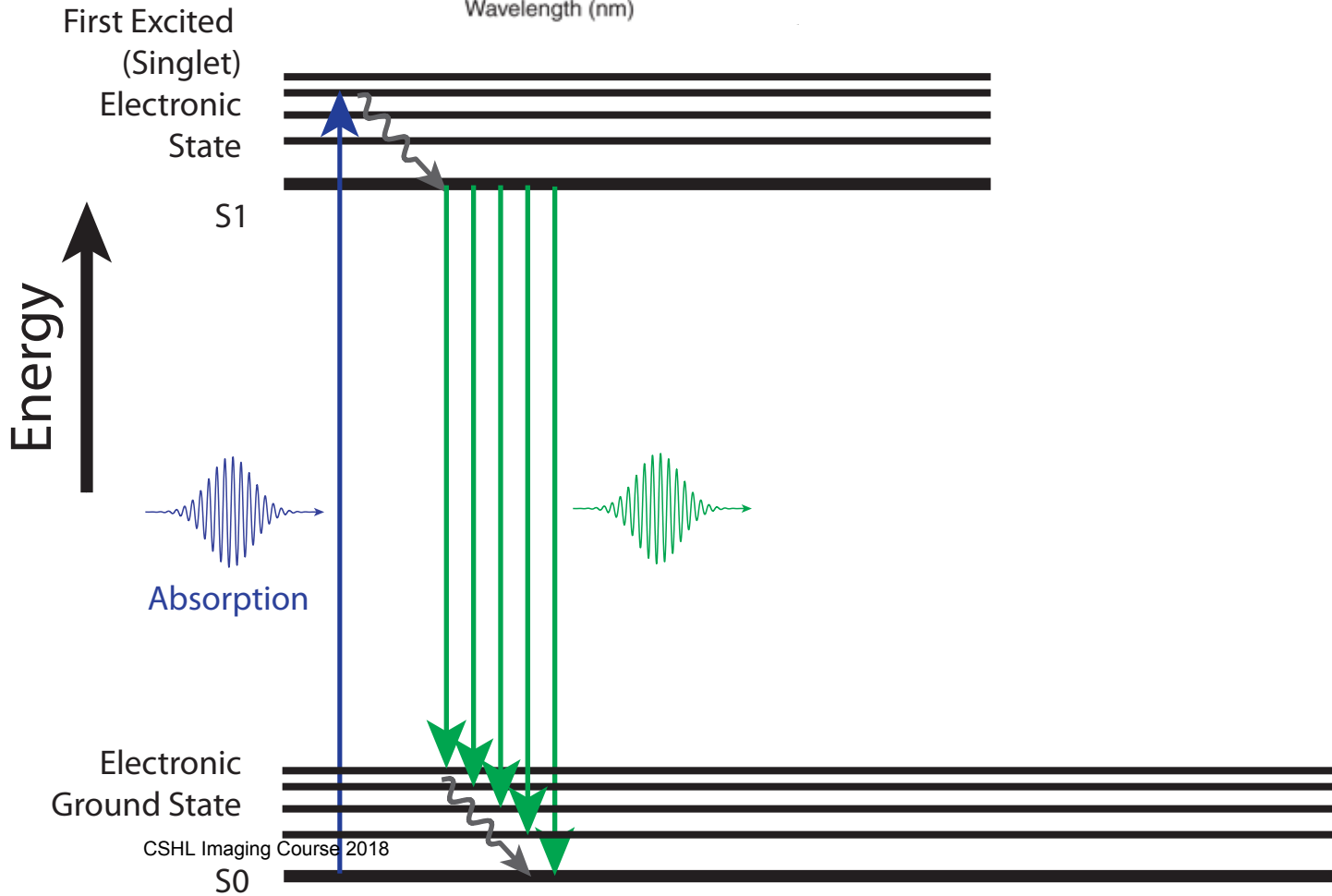
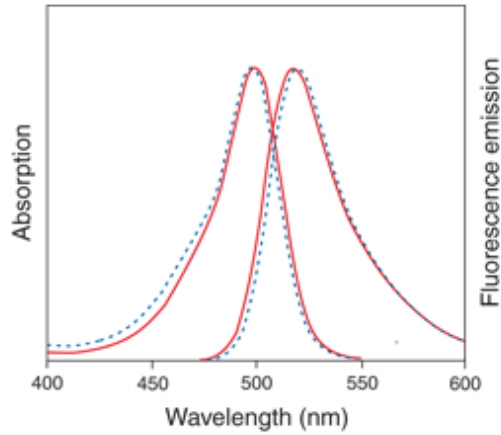
Jablonski Energy Diagram



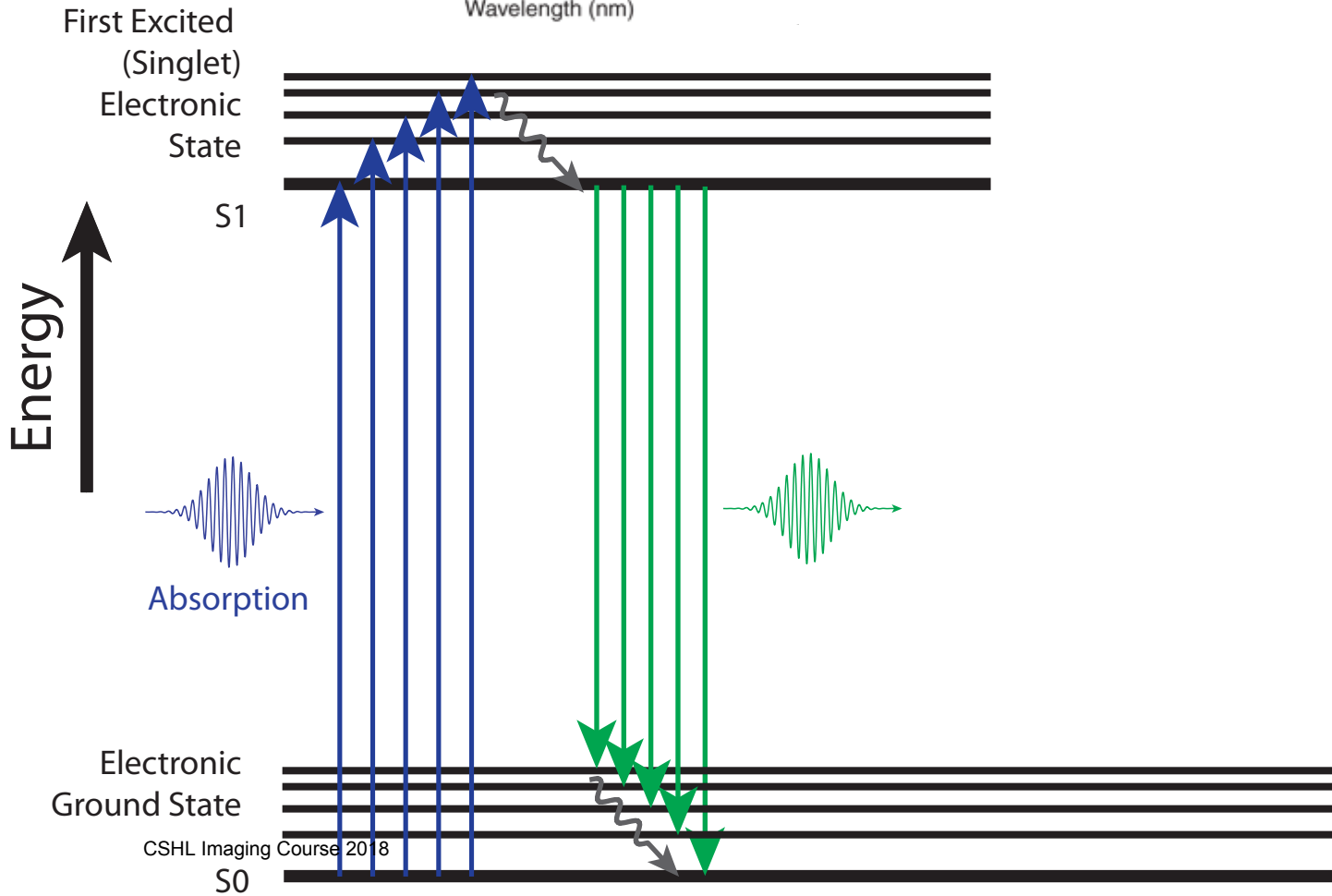
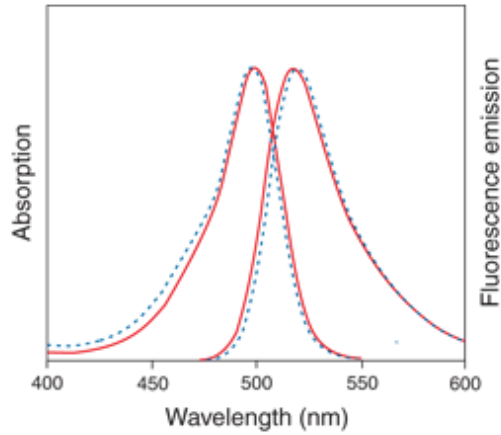
Jablonski Energy Diagram



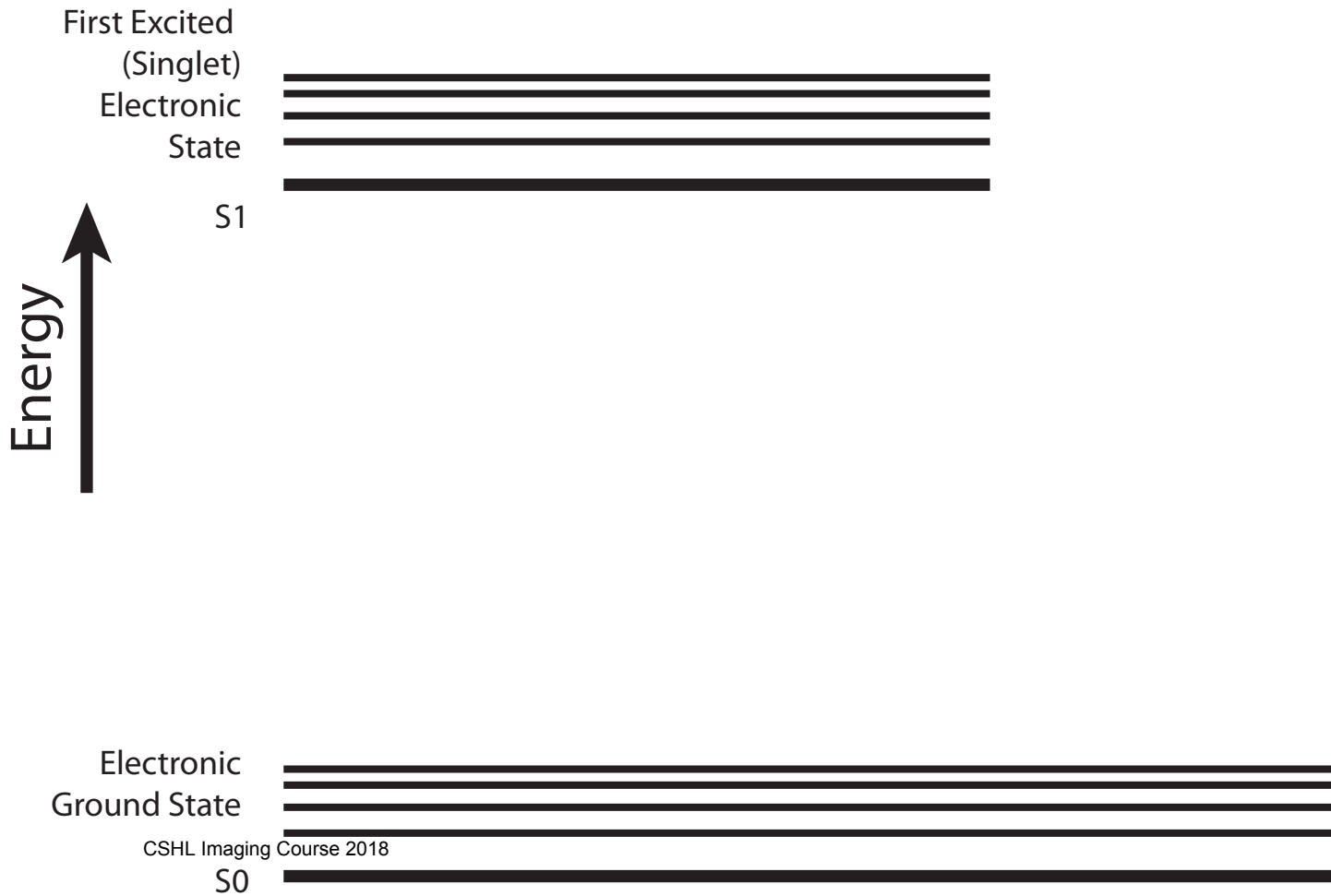
Jablonski Energy Diagram



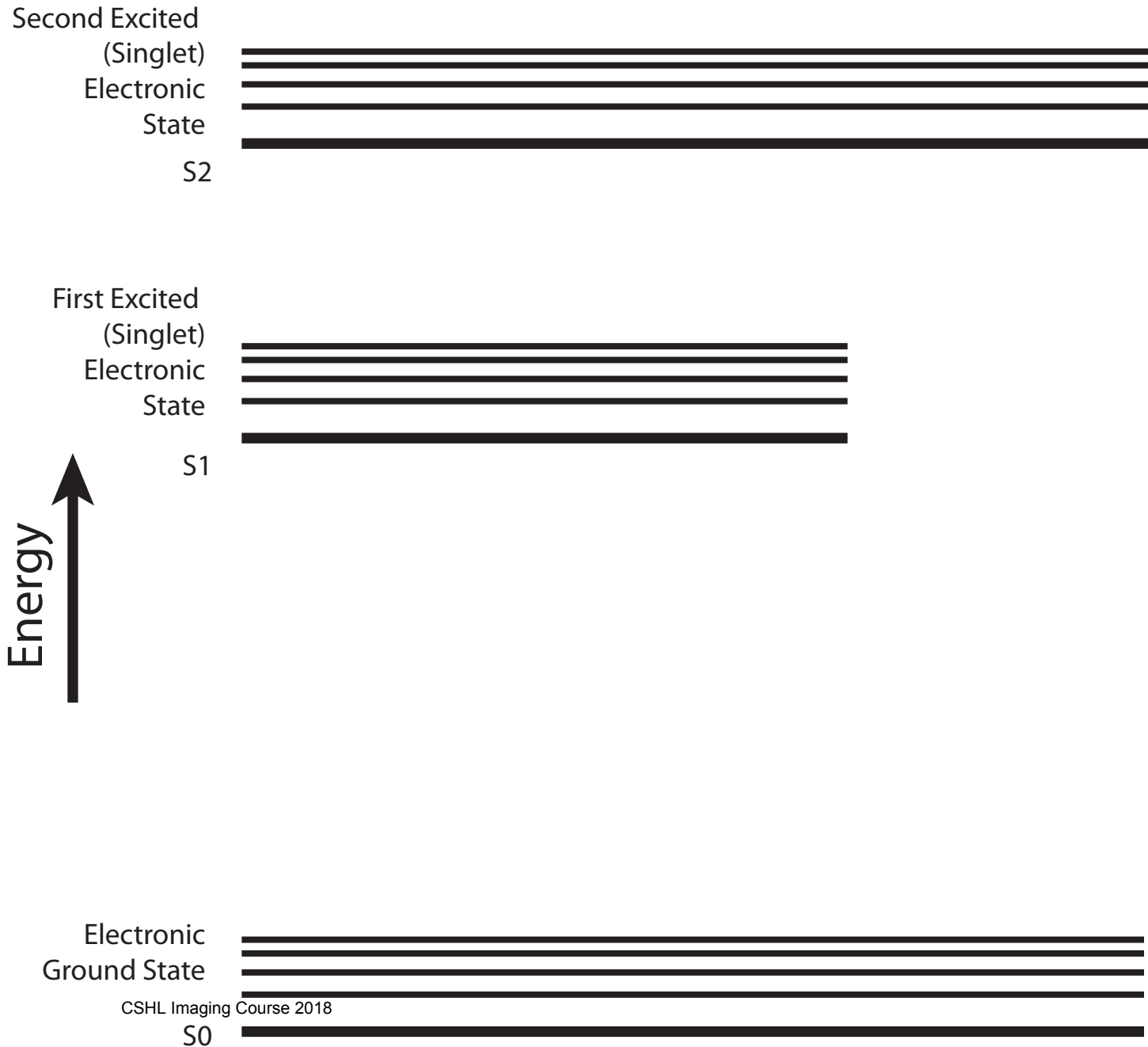
Jablonski Energy Diagram



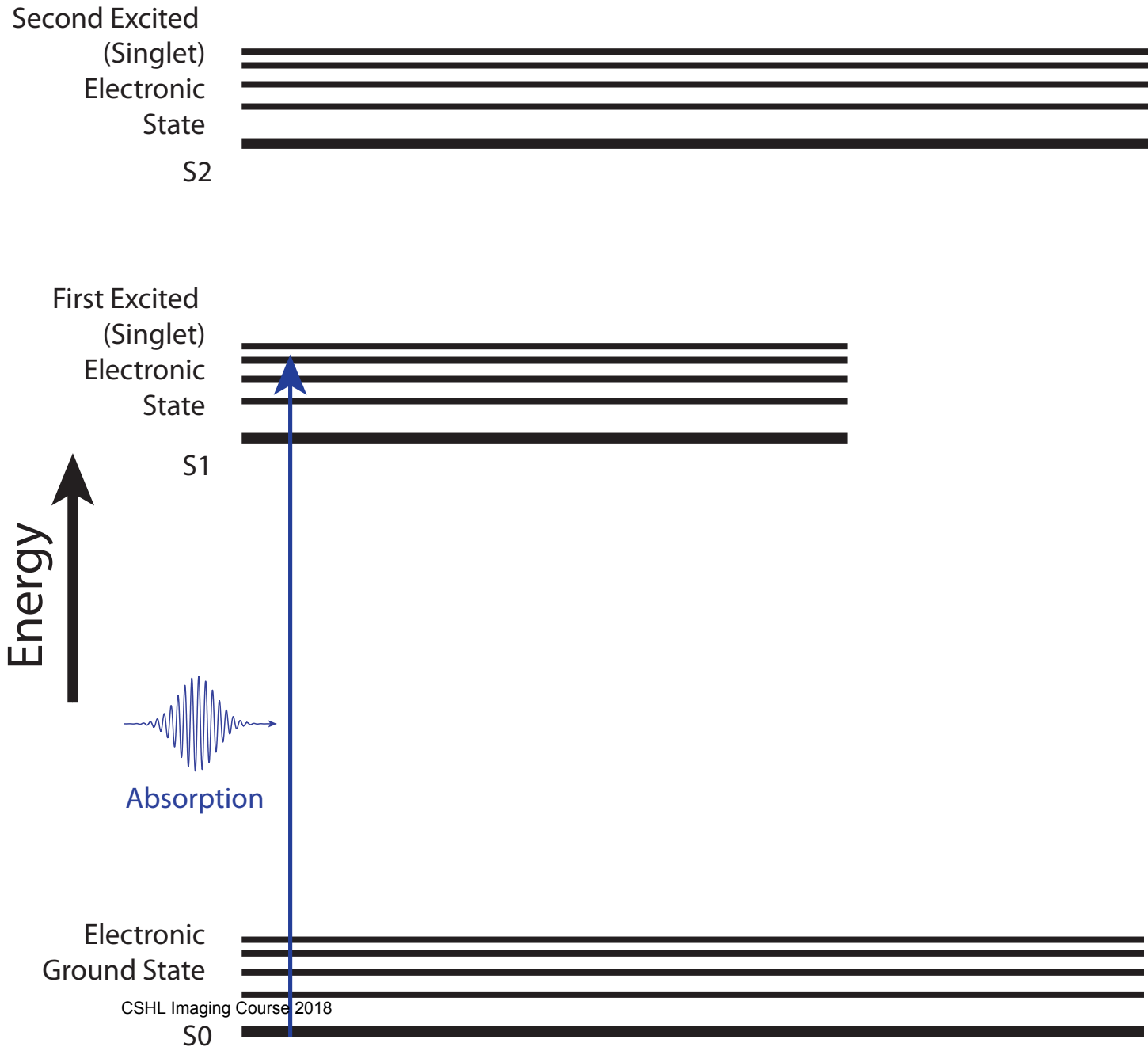
Jablonski Energy Diagram



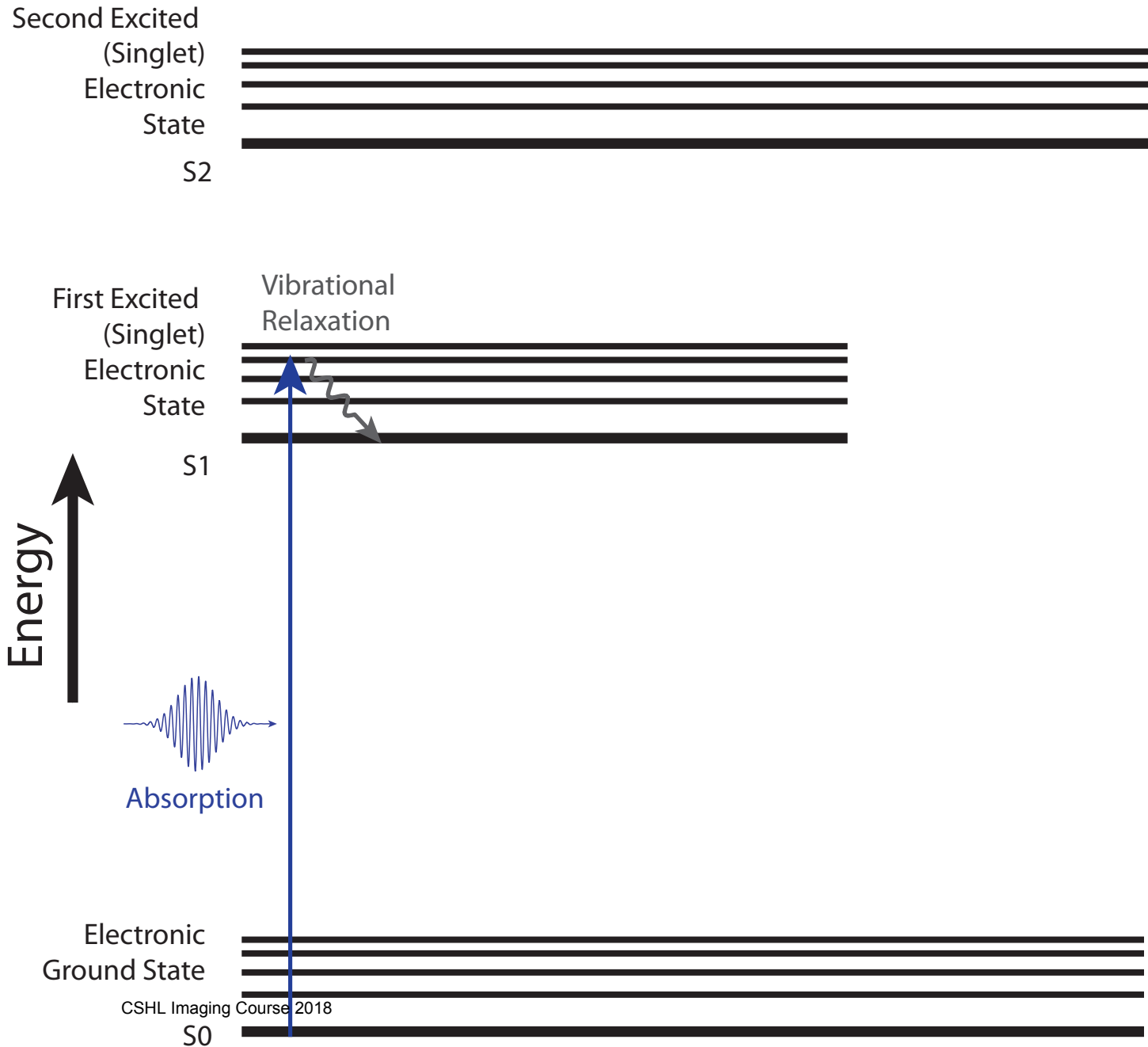
Jablonski Energy Diagram



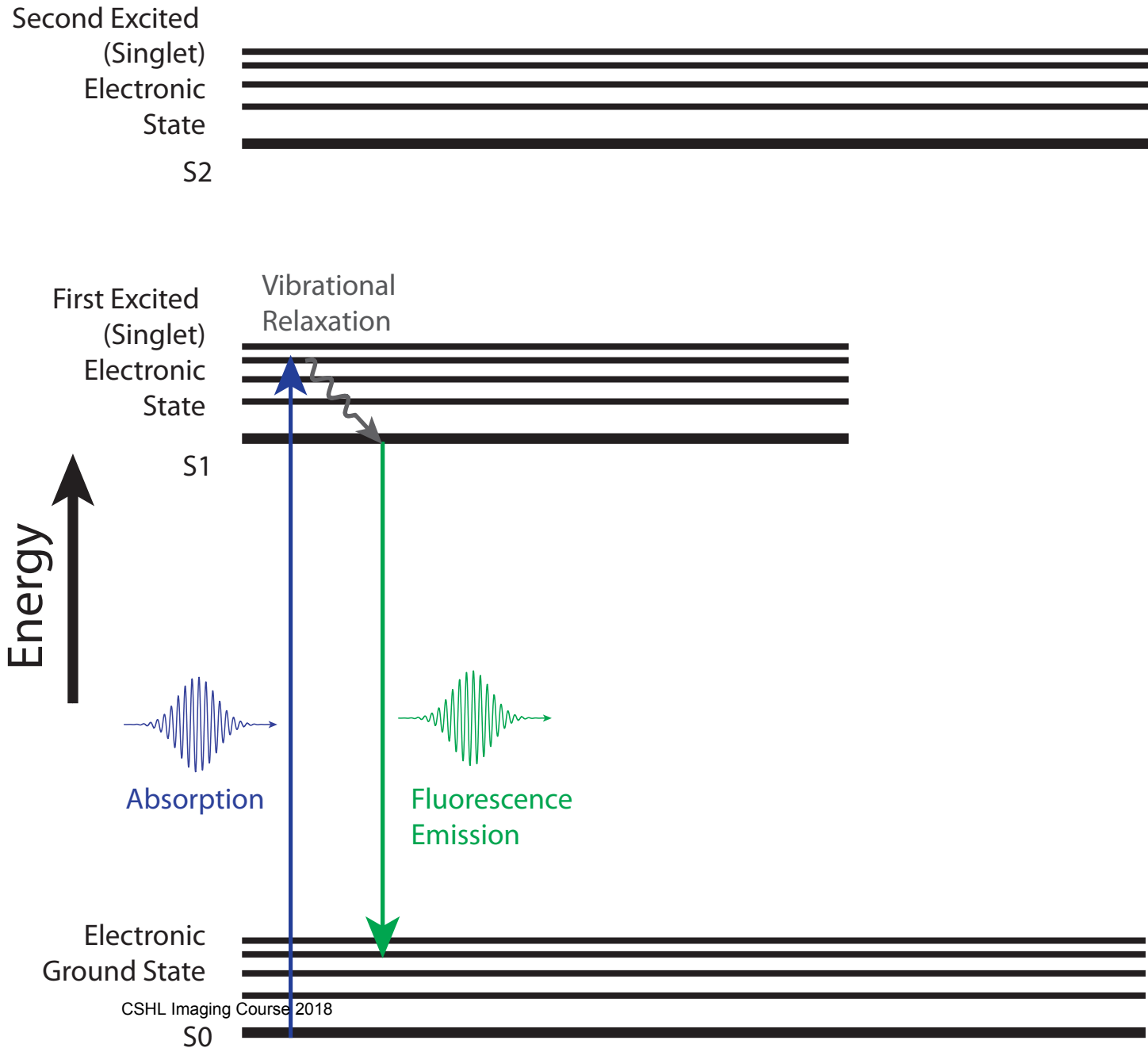
Jablonski Energy Diagram



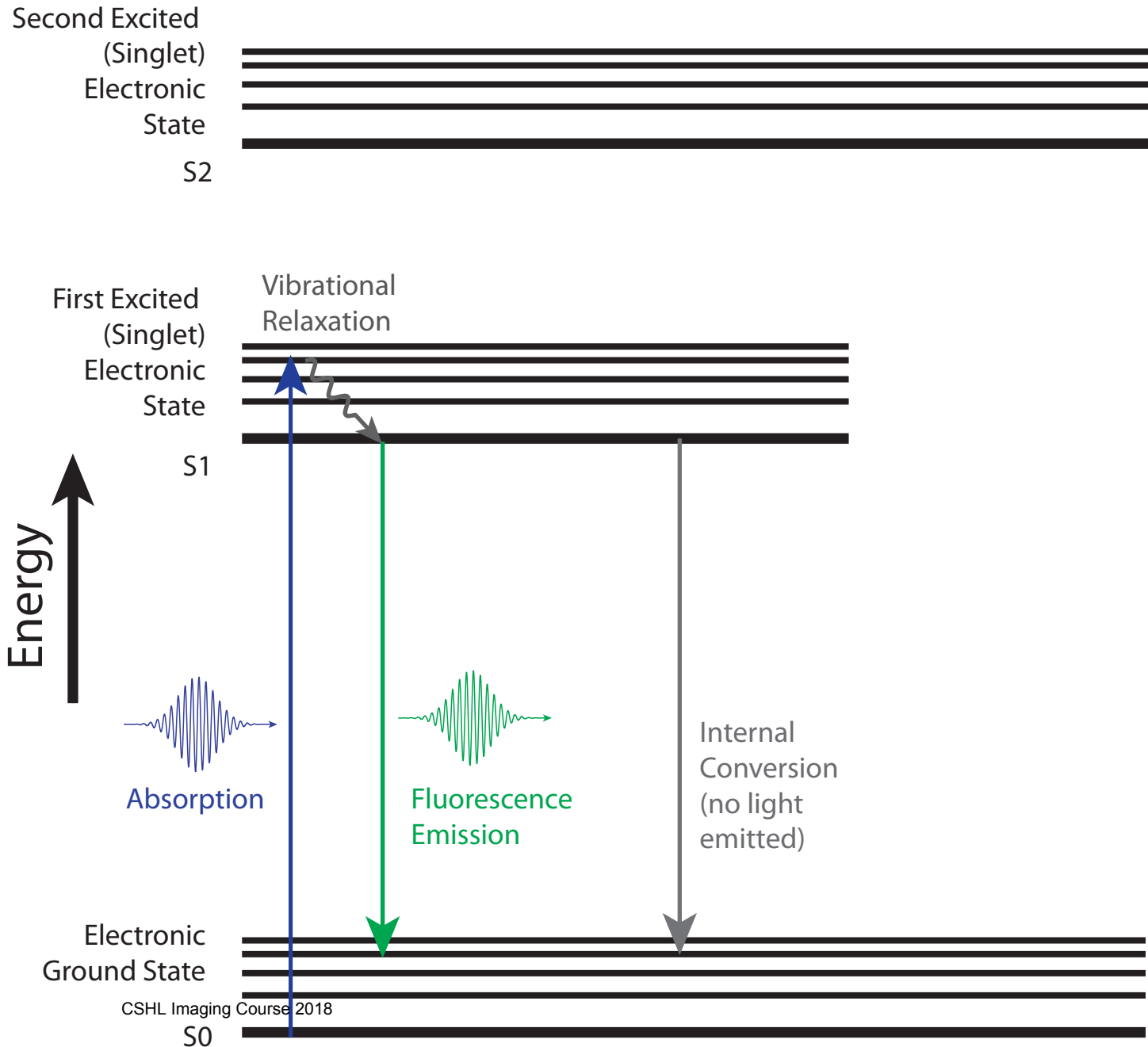
Jablonski Energy Diagram



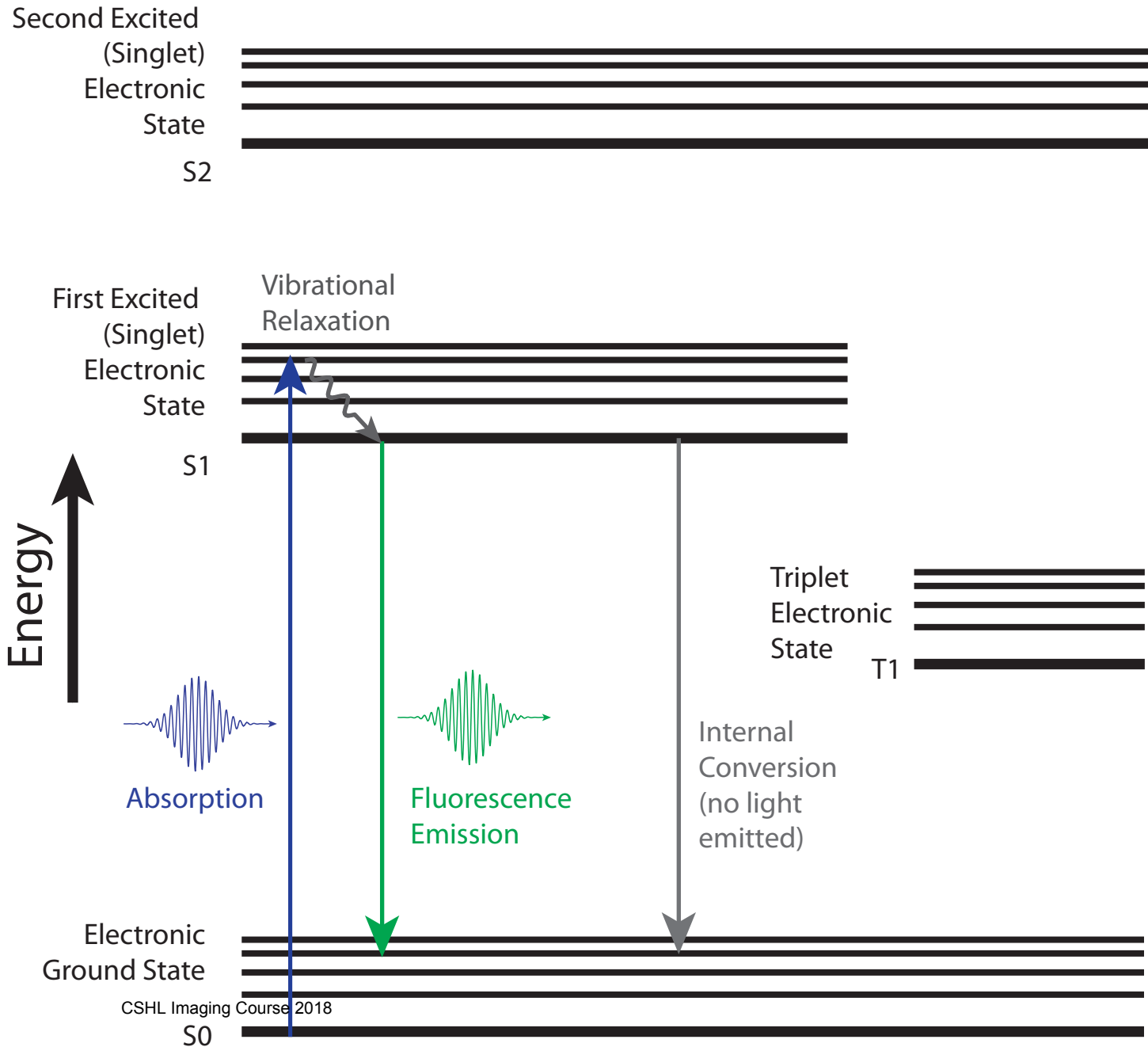
Jablonski Energy Diagram



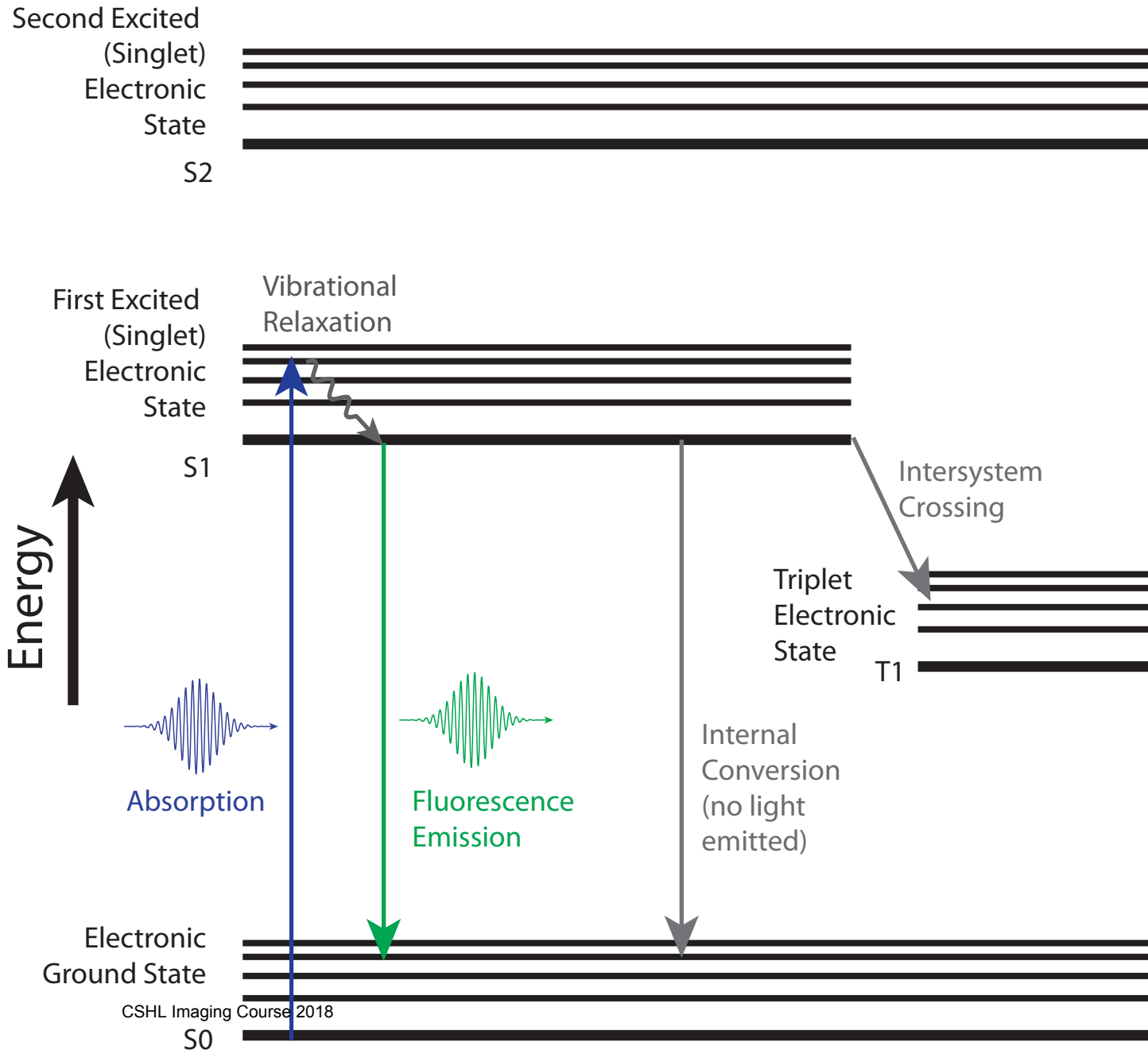
Jablonski Energy Diagram



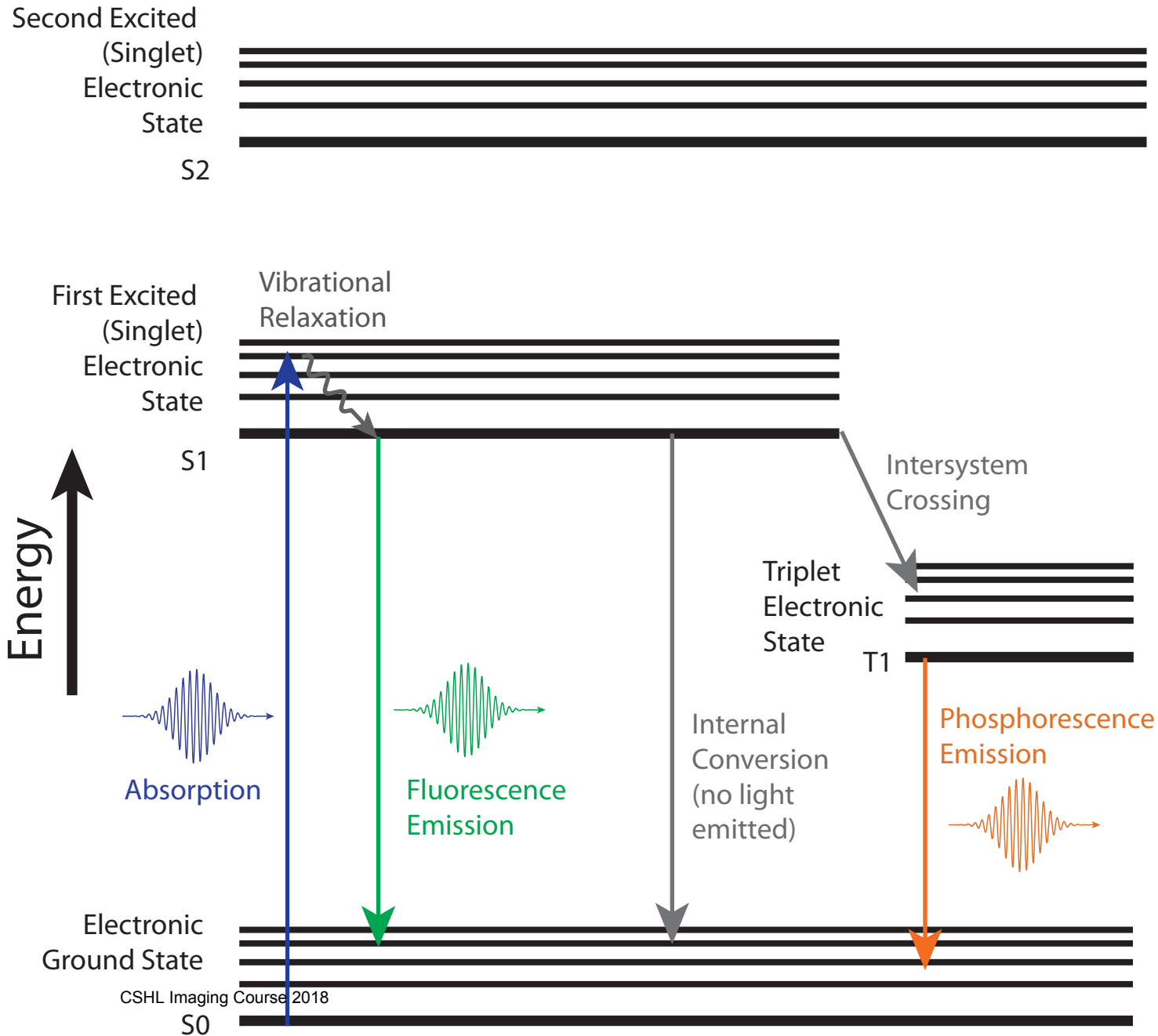
Jablonski Energy Diagram



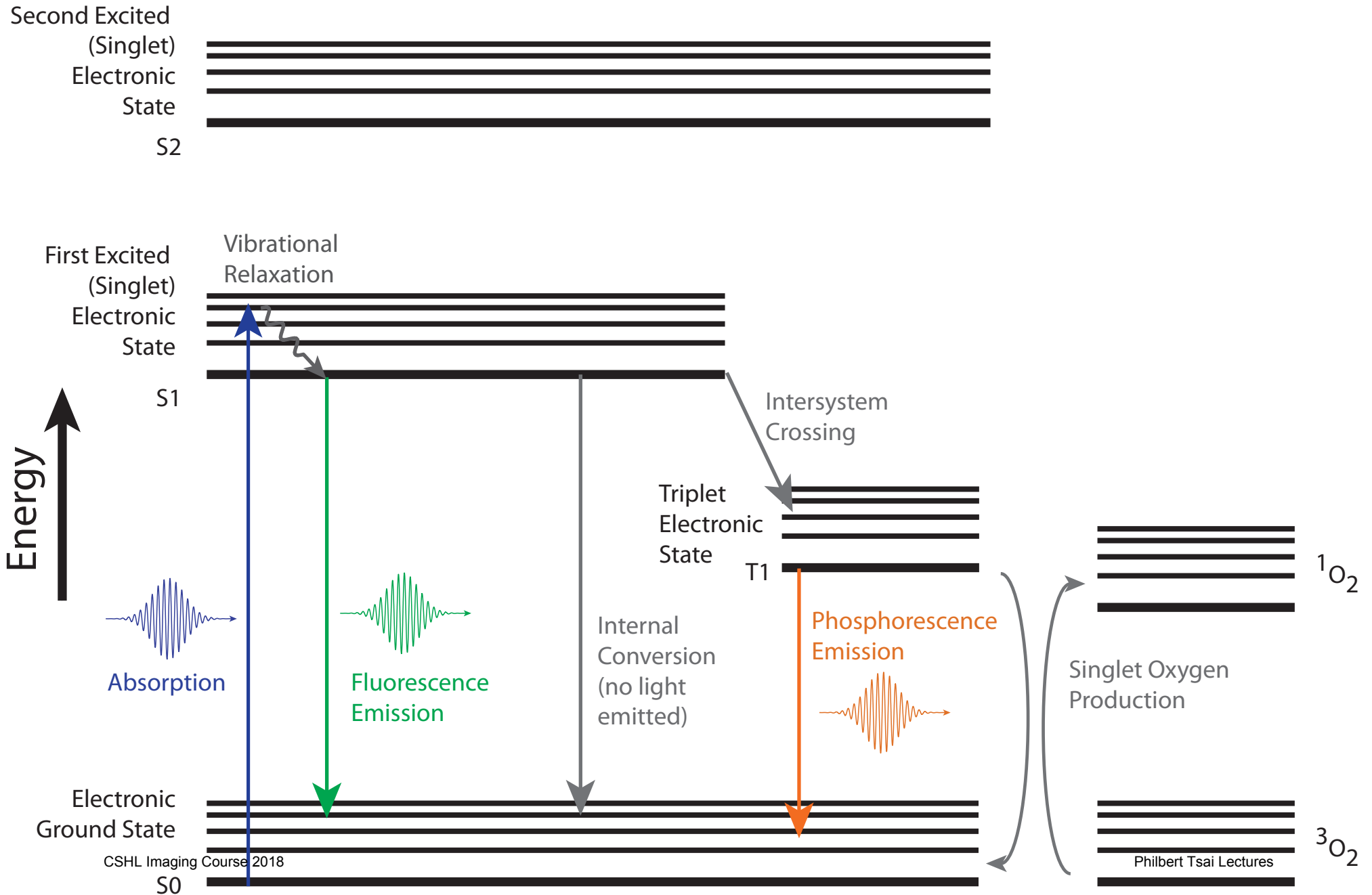
Jablonski Energy Diagram



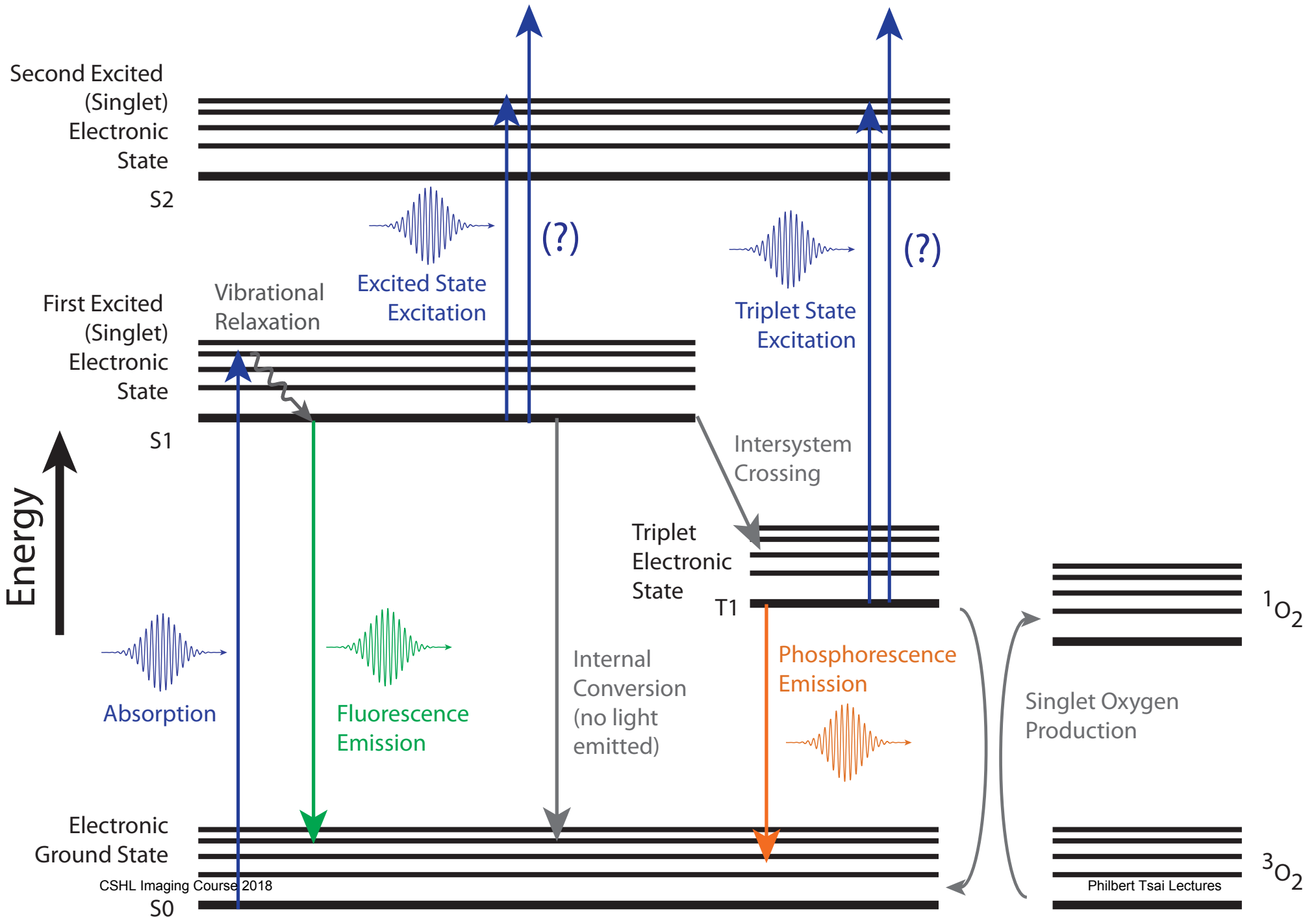
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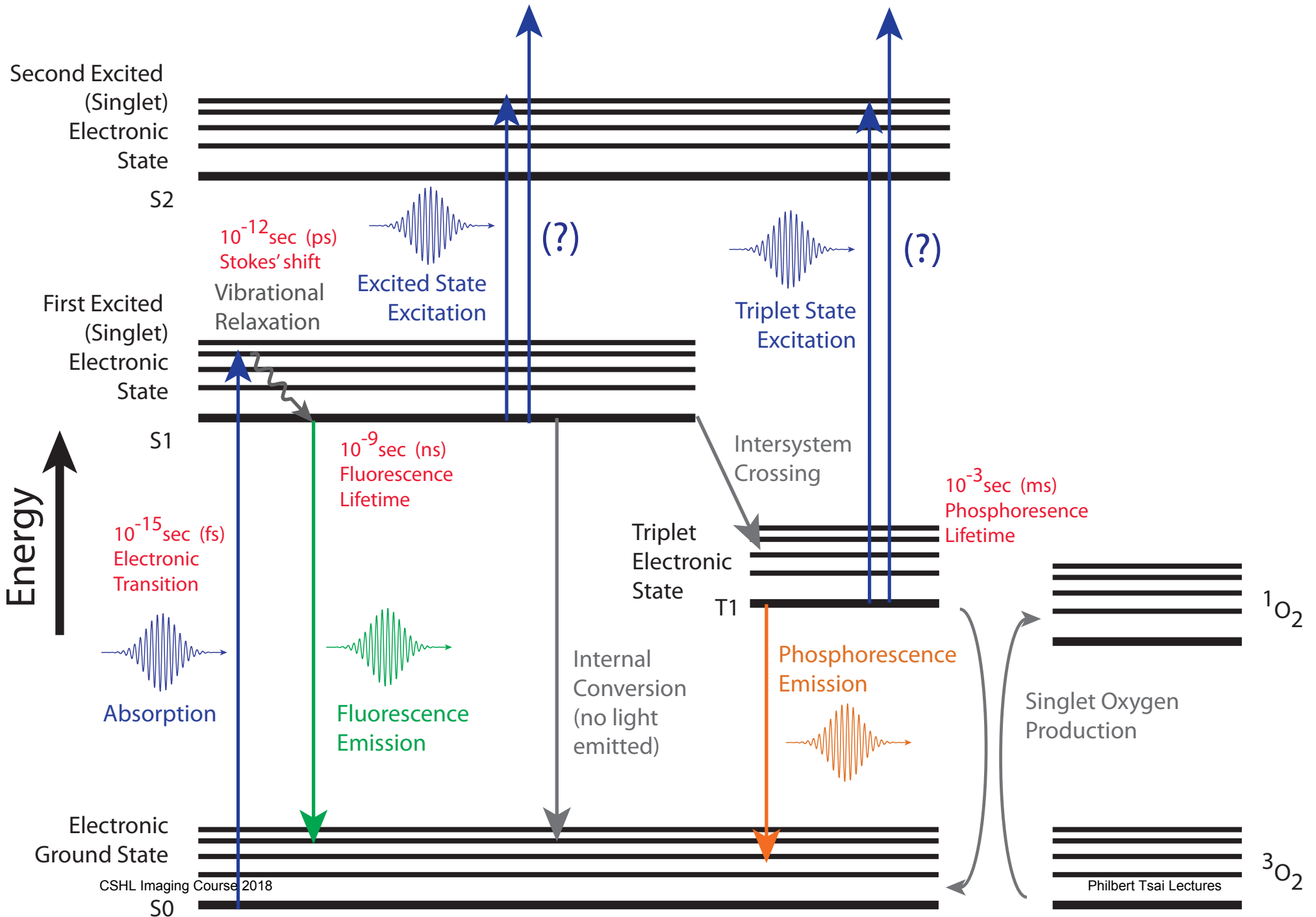
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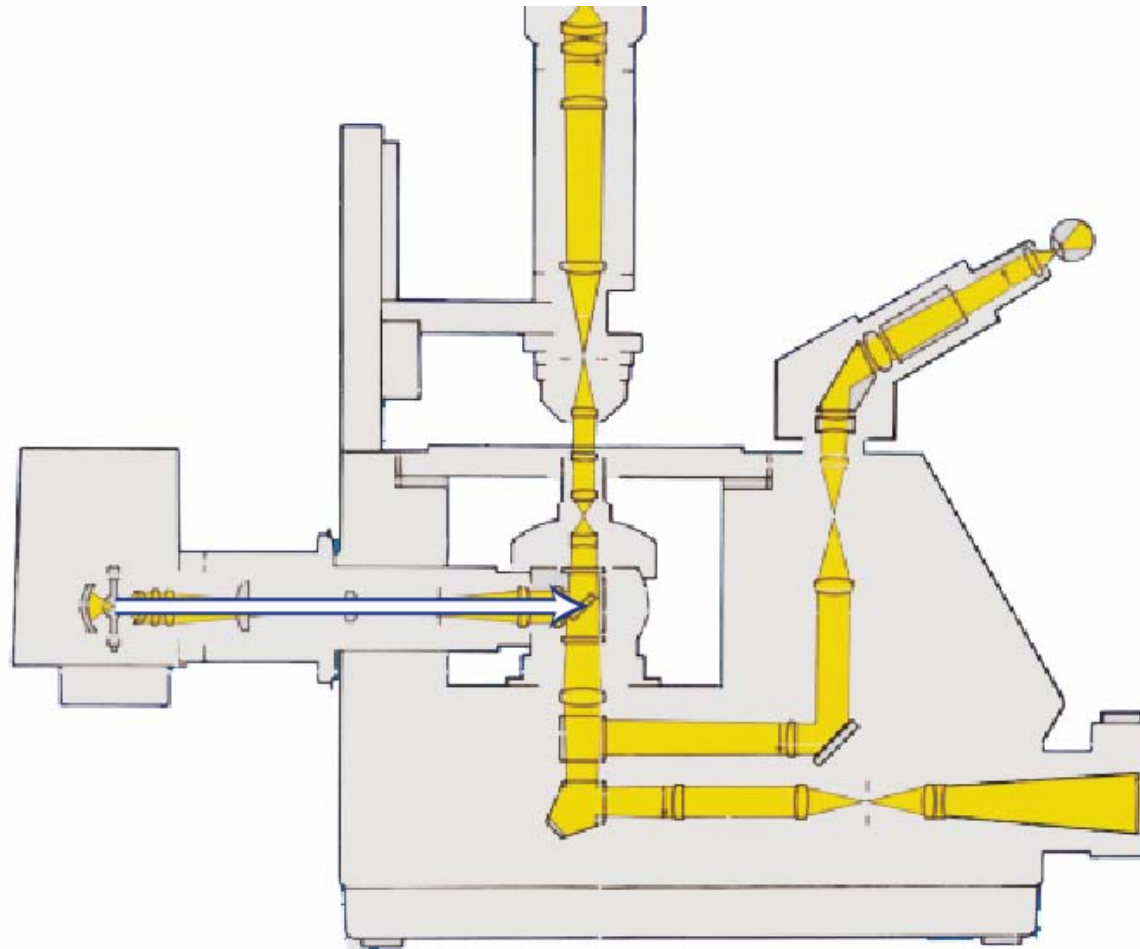
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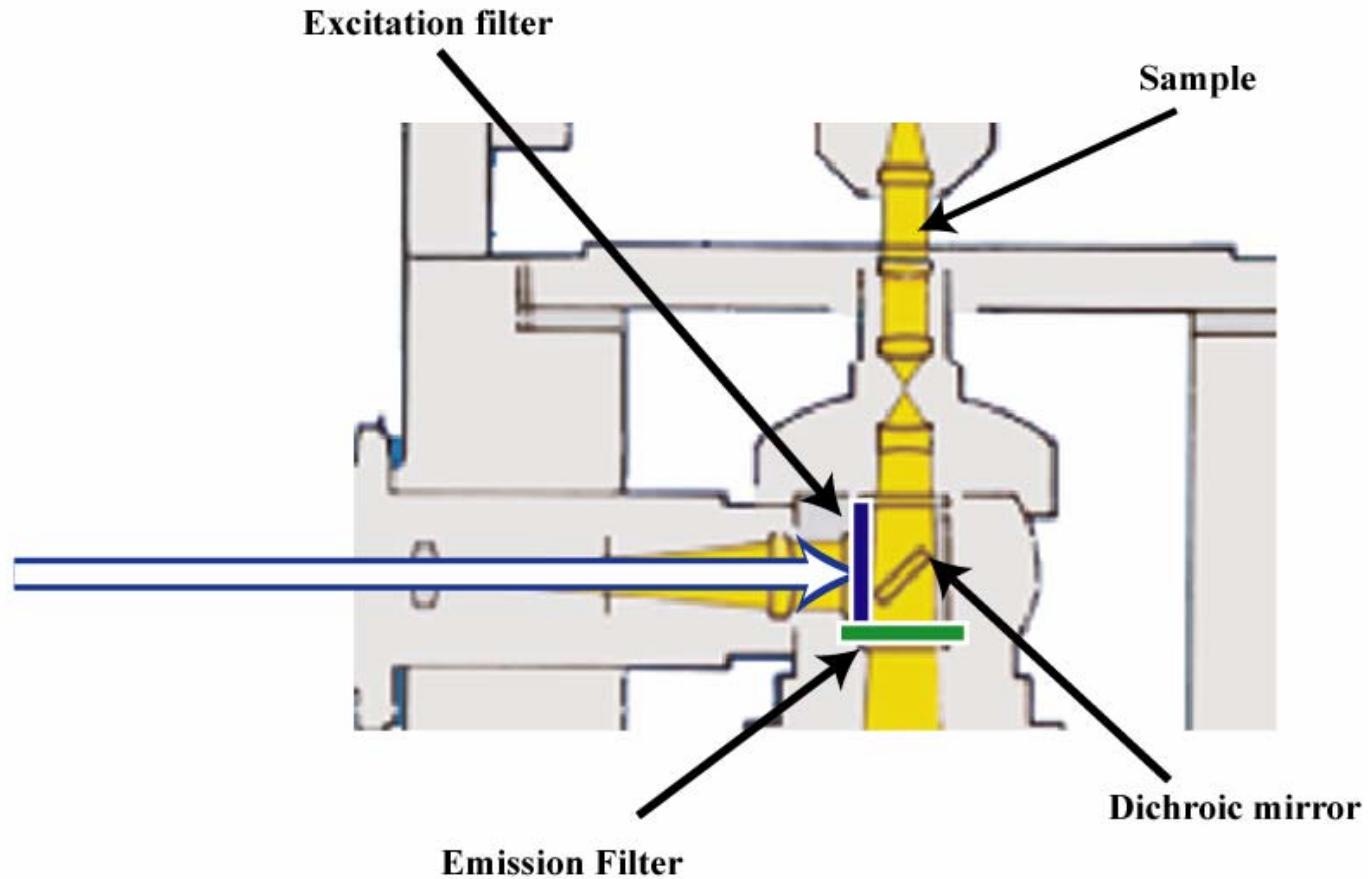


Optical schematic of an inverted epifluorescence microscope



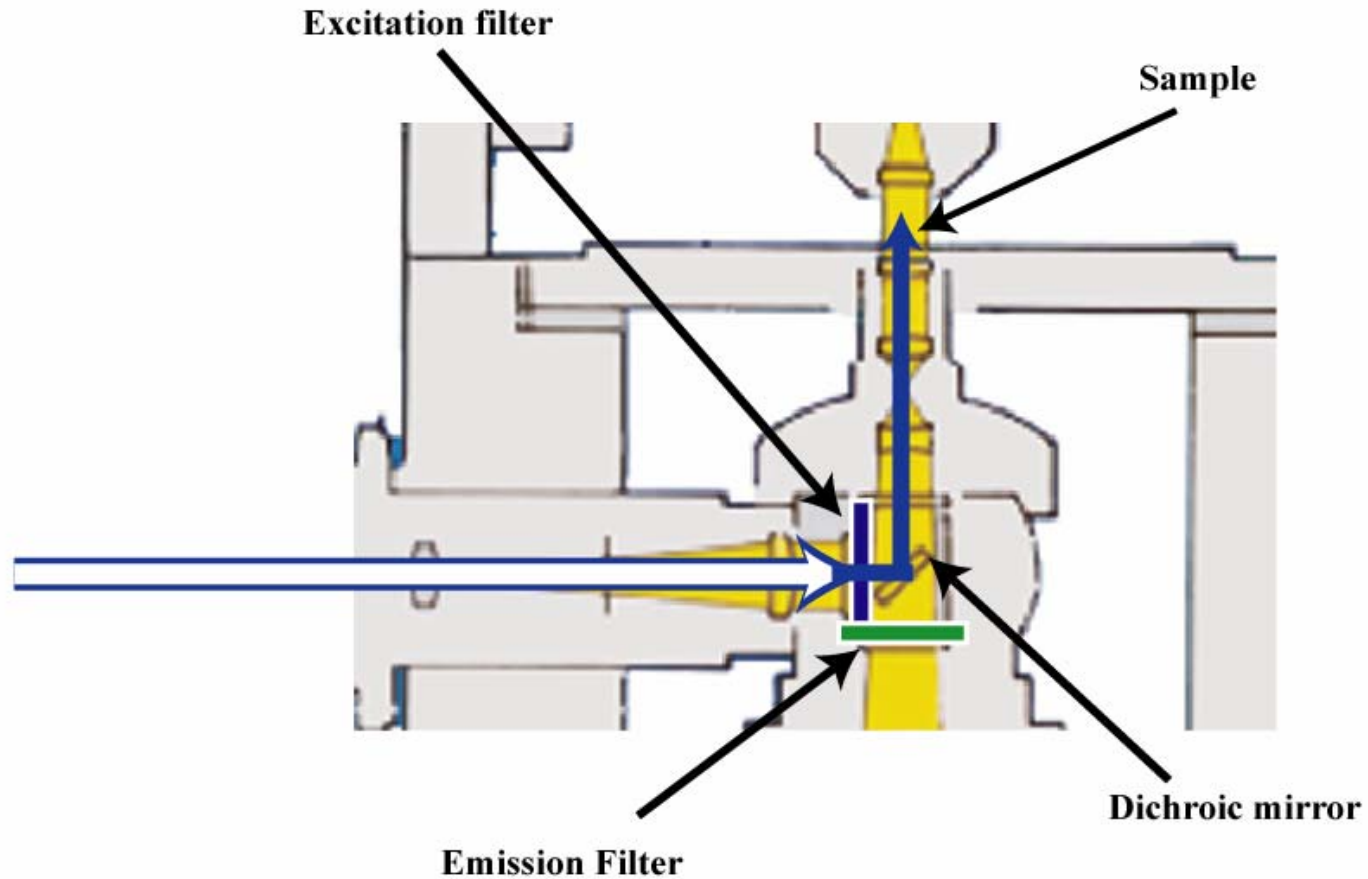
Philbert S. Tsai, July 28, 2010

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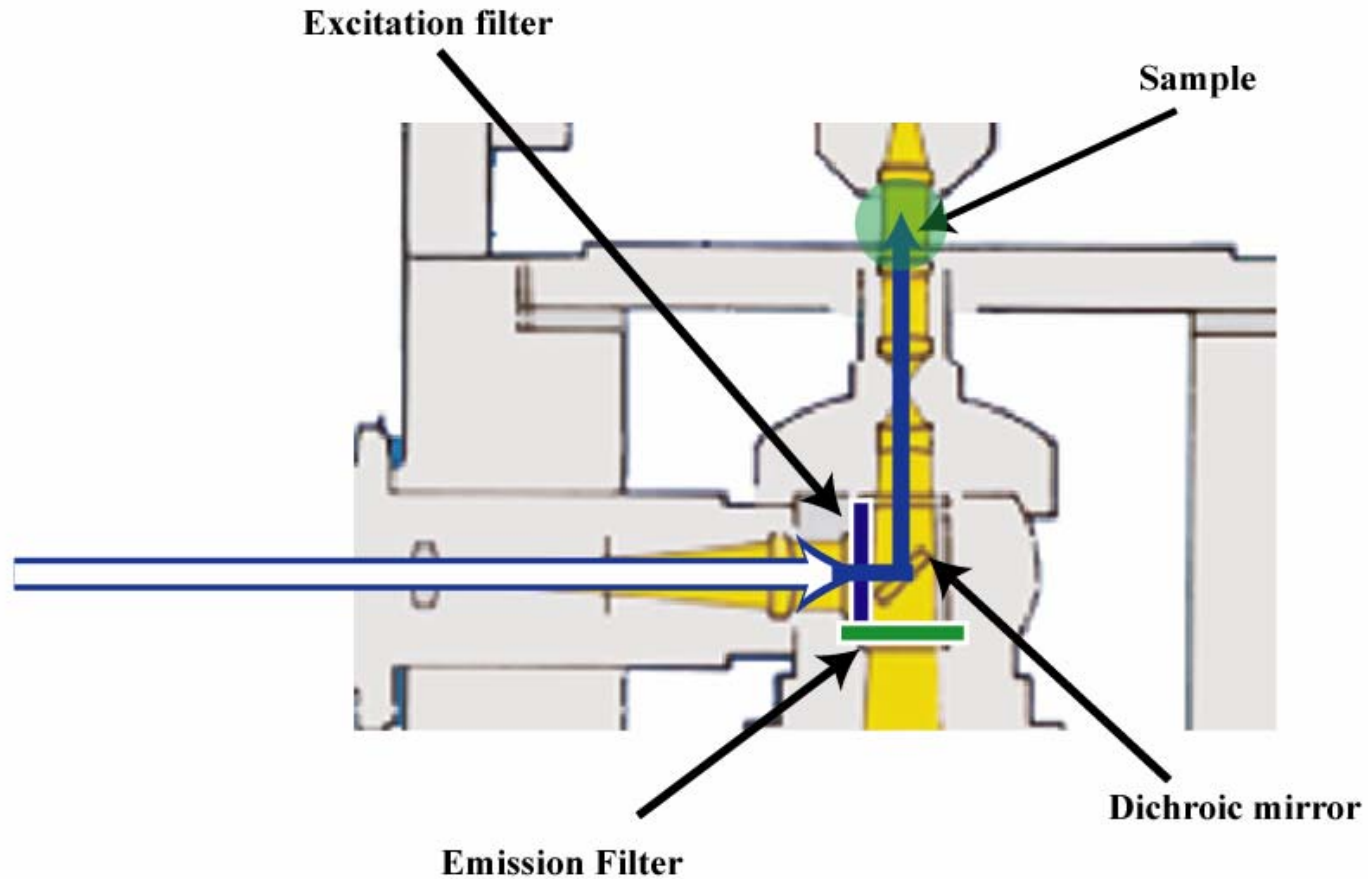
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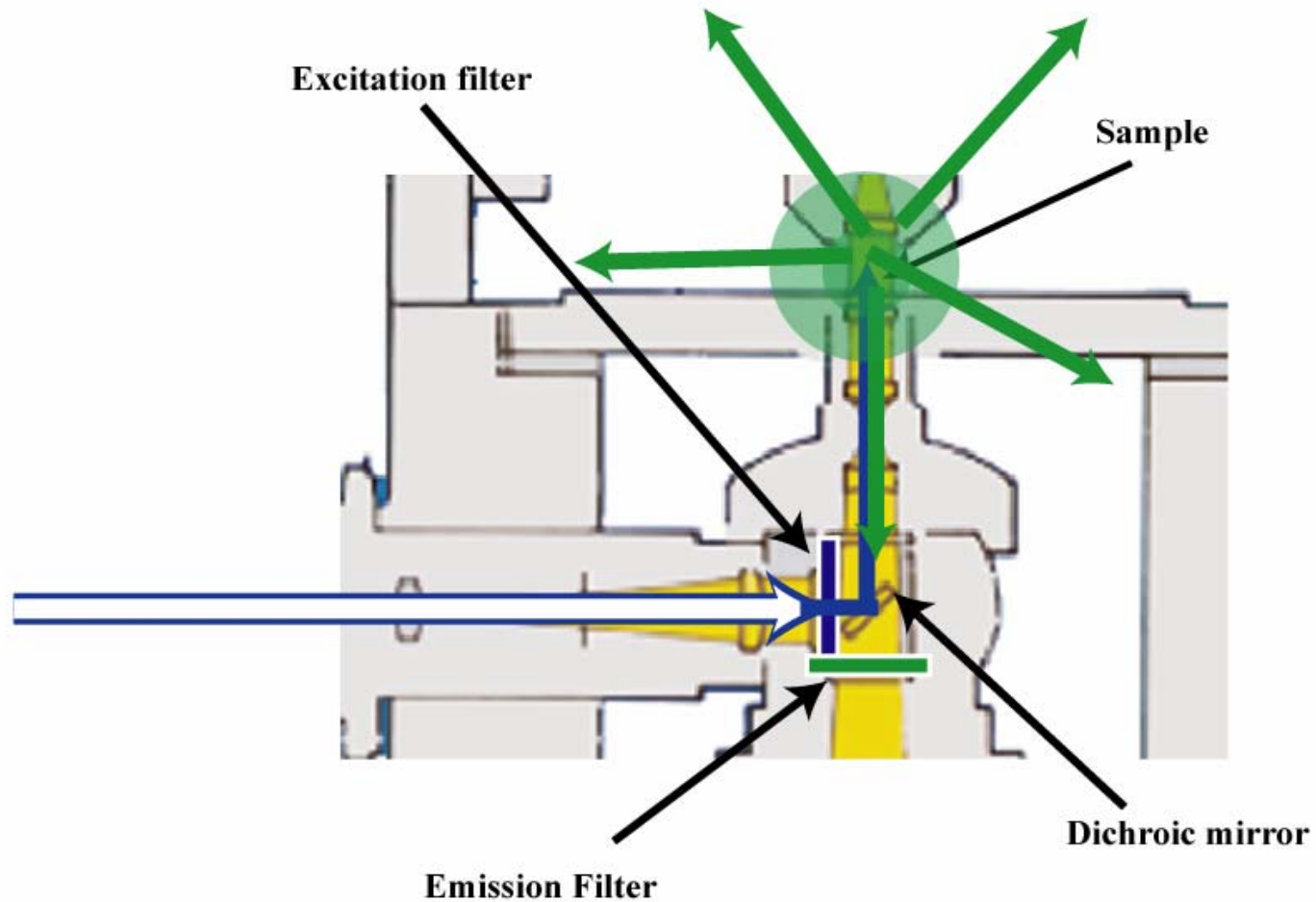
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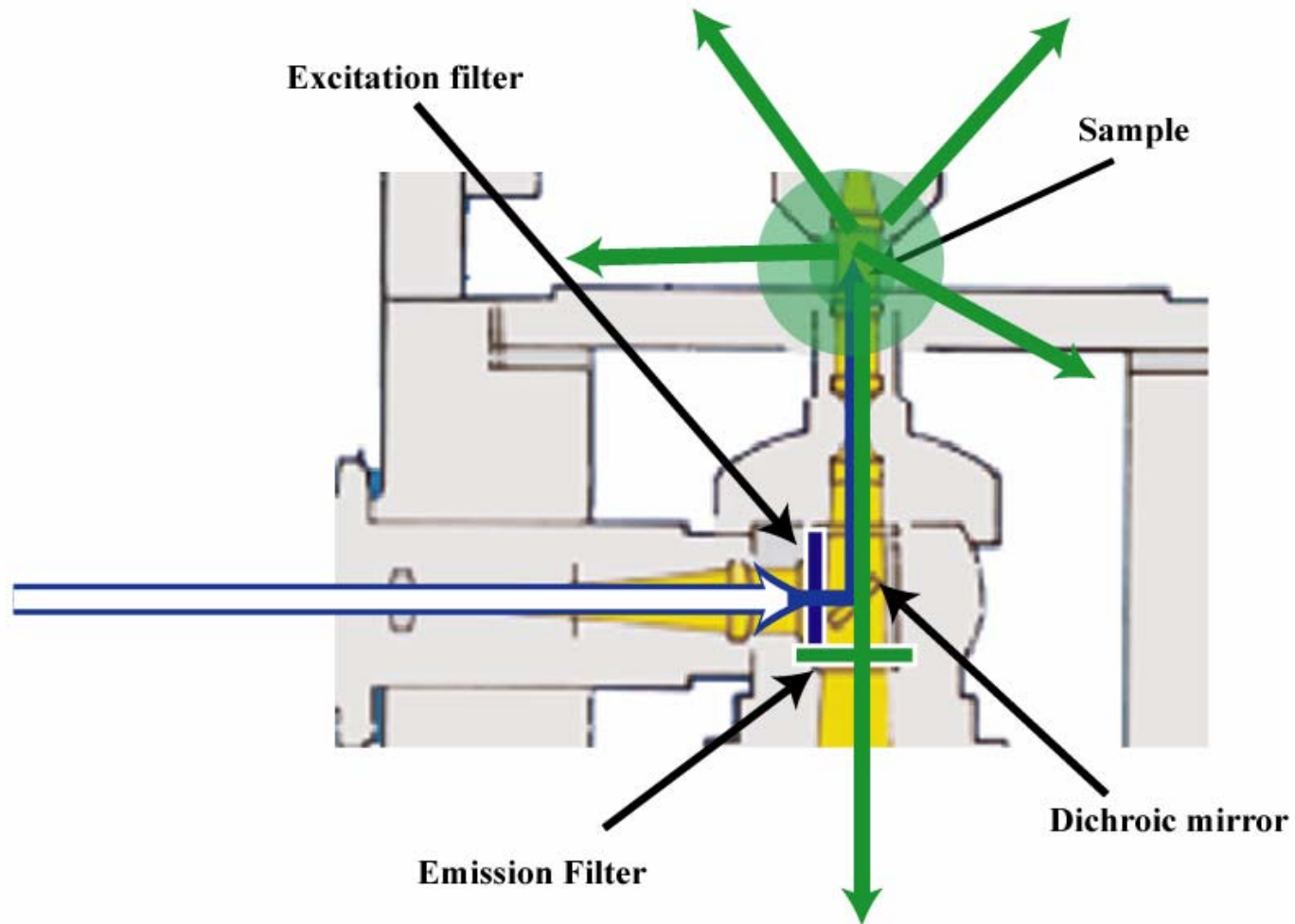
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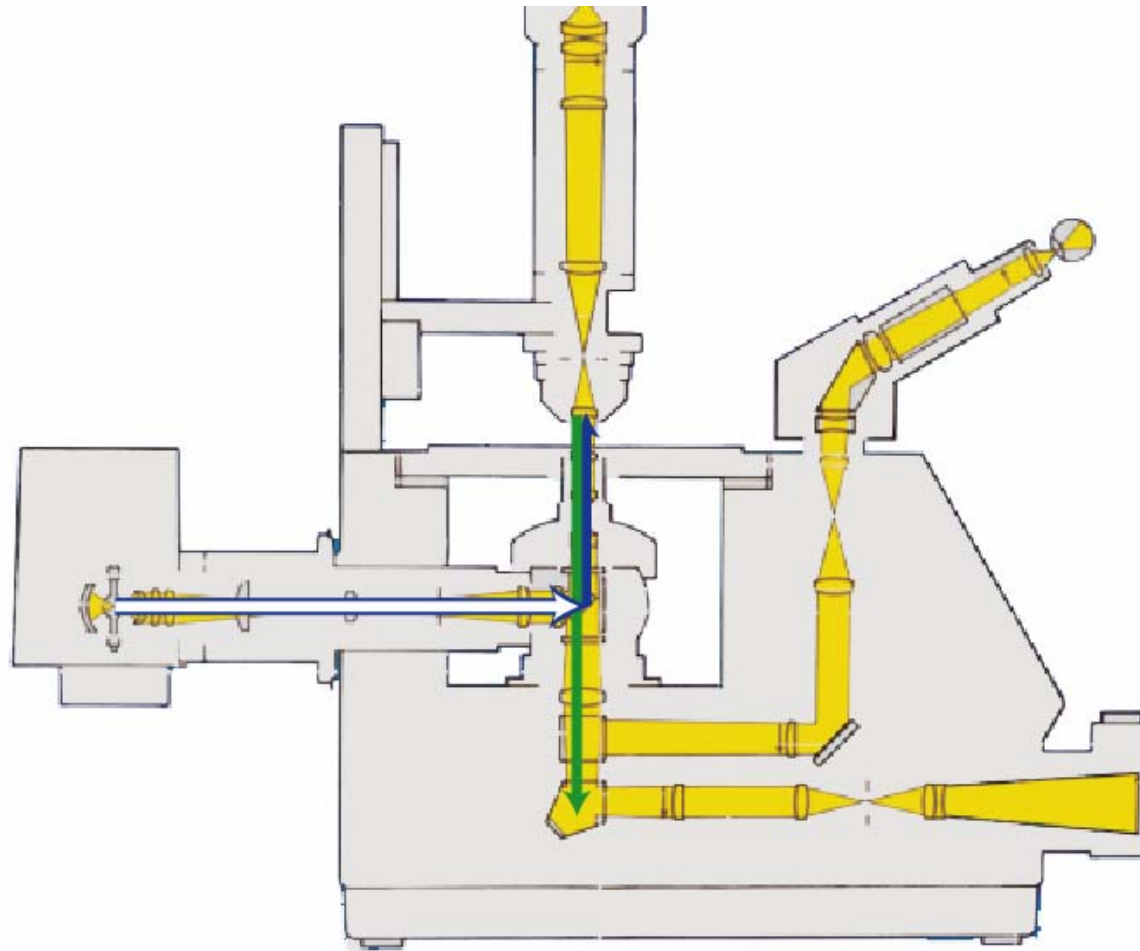
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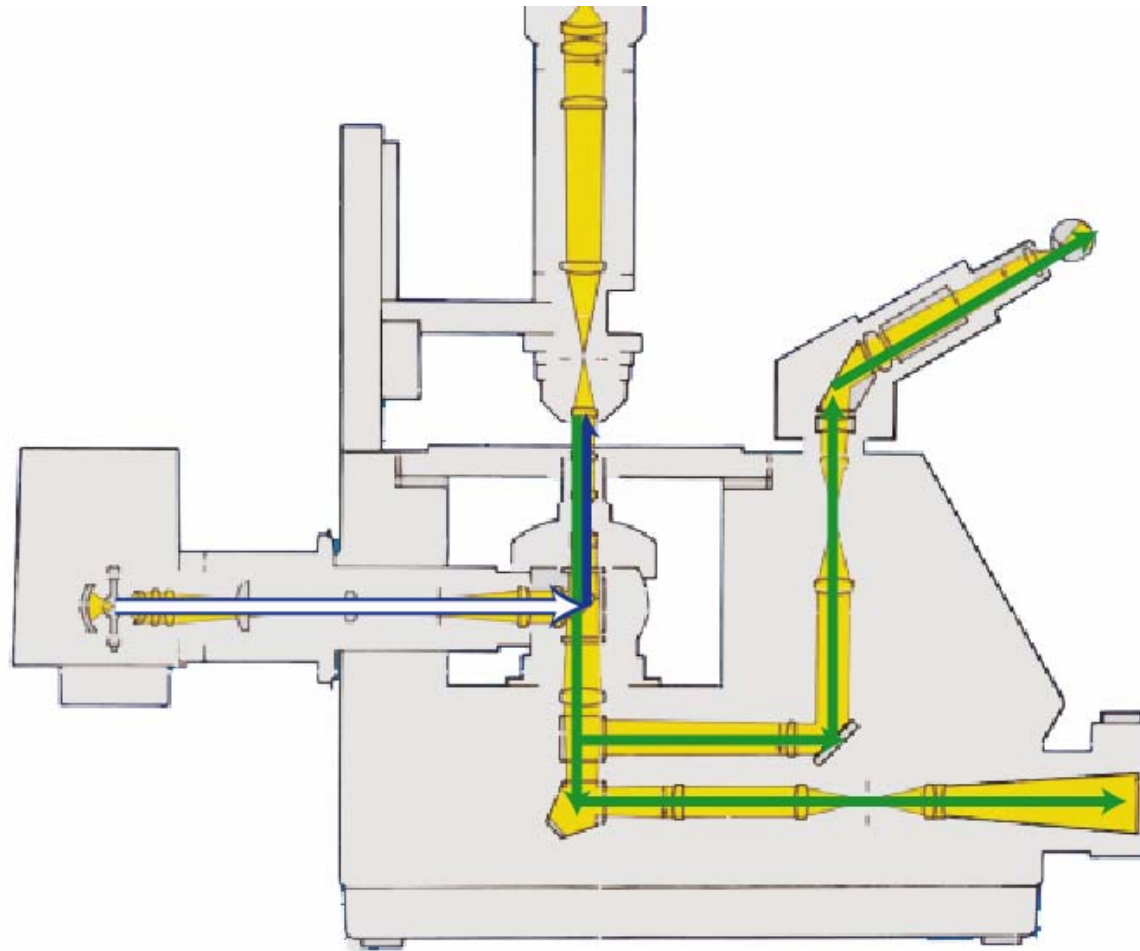
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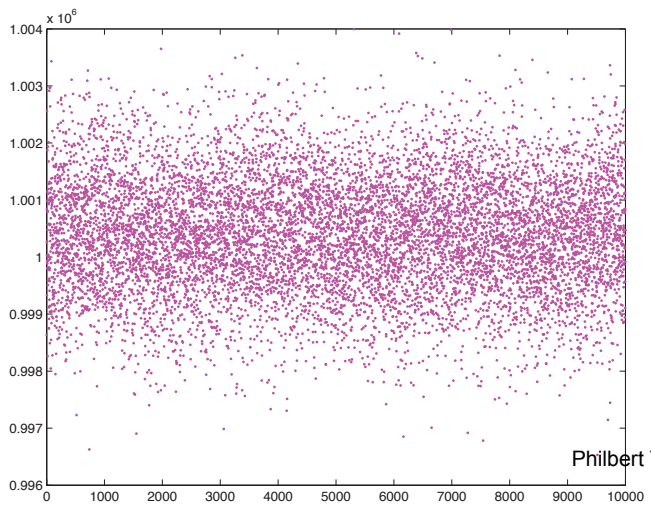
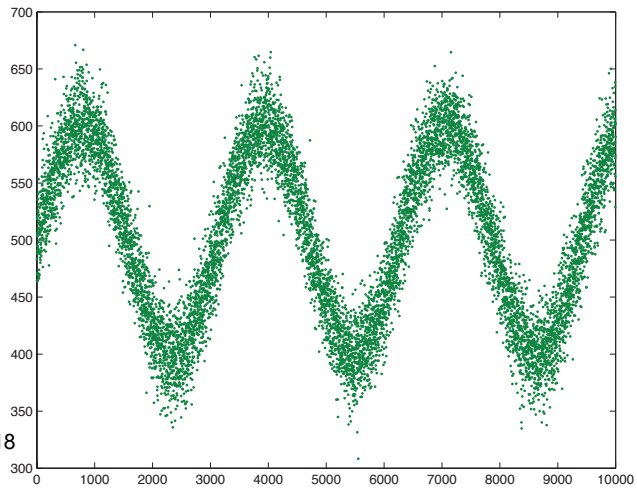
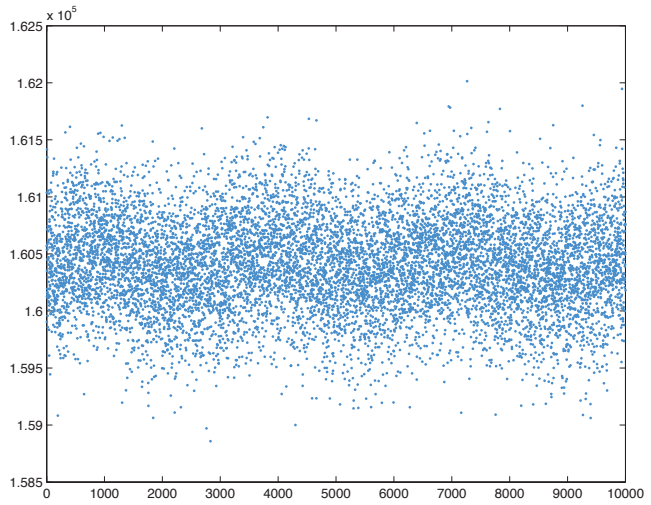
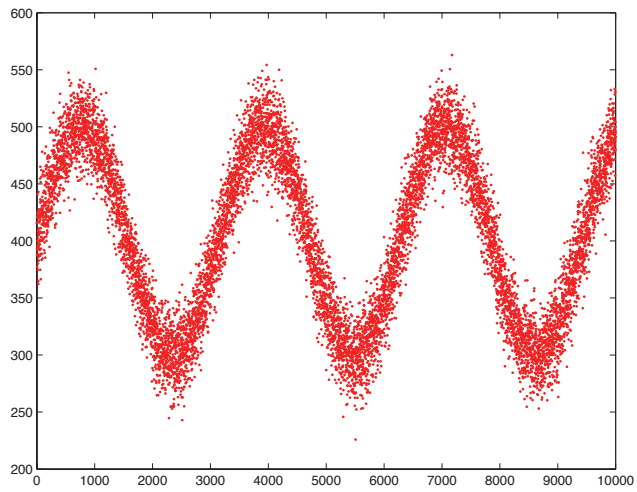
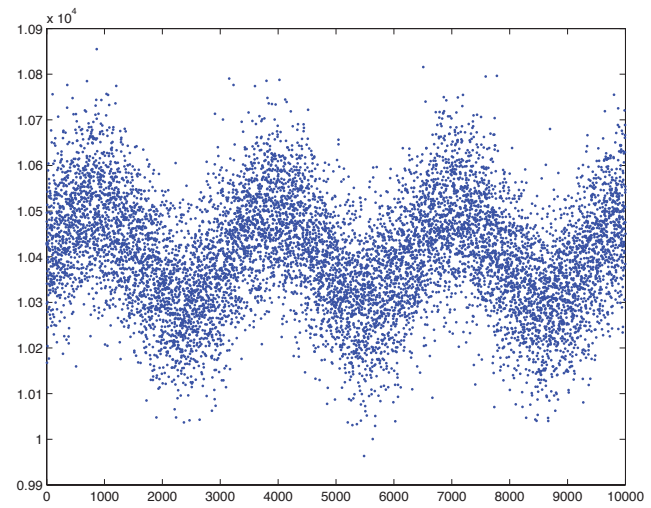
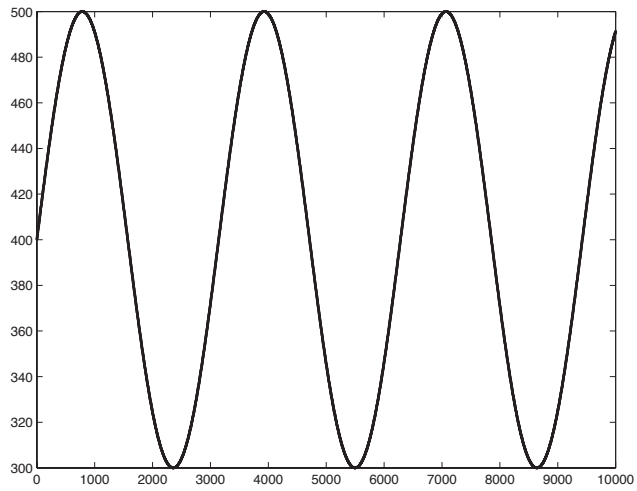


Philbert S. Tsai, July 28, 2010

Optical schematic of an inverted epifluorescence microscope



Philbert S. Tsai, July 28, 2010



Some Relevant Parameters

Extinction Coefficient

$\epsilon = 5,000 - 200,000 \text{ M}^{-1} \text{ cm}^{-1}$ for most fluorophores
refers to the absorption at a single wavelength (typically the maximum)

Quantum Yield

$Q = 0.05 - 1$ for most fluorophores
refers to the integrated photon emission over the entire emission spectrum

Below saturation, total fluorescence intensity $\sim Q * \epsilon$

Lifetime

$\tau = 1 - 10 \text{ ns}$ for most fluorophores

A quick & dirty calculation of saturation

Absorption cross-section, σ

$$\varepsilon = 80,000 \text{ M}^{-1} \text{ cm}^{-1}$$

$$\sigma = \varepsilon \cdot \ln(10) / 6.023 \cdot 10^{23}$$

$$\sigma(\text{fluorescein}) = 3 \cdot 10^{-16} \text{ cm}^2 \cdot \text{molecule}^{-1} \cdot \text{photon}^{-1}$$

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Assume 1mW of green light (around 488 nm) at the sample:

$$1 \text{ mW} = 1 \cdot 10^{-3} \text{ J/s}$$

$$1 \text{ photon (488nm)} \leftrightarrow 2.5 \text{ eV} = 4 \cdot 10^{-19} \text{ J}$$

$$1 \text{ mW (488nm)} = 2.5 \cdot 10^{15} \text{ photons/s}$$

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Epifluorescence : Illuminate 500 x 500 um area $\sim 2 \cdot 10^{-3} \text{ cm}^{-2}$

$$\text{Intensity} = \text{Power} / \text{Area} \sim 1.25 \cdot 10^{18} \cdot \text{photons} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$$

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Point Scanning : Illuminate 0.5 x 0.5 um area $\sim 2 \cdot 10^{-9} \text{ cm}^{-2}$

$$\text{Intensity} = \text{Power} / \text{Area} \sim 1.25 \cdot 10^{24} \cdot \text{photons} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$$

A quick & dirty calculation of saturation

Absorption and Fluorescence Rates

$$k_a = \sigma \cdot I$$

$$k_f = 1/\tau$$

Let x = fraction of fluorophores in the excited state

$$k_a \cdot (1-x) = k_f \cdot x \quad \leftarrow \text{in steady state}$$

$$\mathbf{x = k_a / (k_a + k_f)}$$

A quick & dirty calculation of saturation

Epifluorescence :

$$I = \text{Power} / \text{Area} \sim 1.25 \cdot 10^{18} \cdot \text{photons} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$$

$$\sigma(\text{fluorescein}) = 3 \cdot 10^{-16} \text{ cm}^2 \cdot \text{molecule}^{-1} \cdot \text{photon}^{-1}$$

$$k_f = 1/\tau = 1 / 4.5 \text{ ns} = 2.2 \cdot 10^8 \text{ s}^{-1}$$

$$k_a = \sigma \cdot I = 375 \text{ s}^{-1}$$

$$x = k_a / (k_a + k_f) = 1.7 \cdot 10^{-6}$$

Only ~ 2 out of every billion fluorophores is in the excited state

A quick & dirty calculation of saturation

Point Scanning :

$$I = \text{Power} / \text{Area} \sim 1.25 \cdot 10^{24} \cdot \text{photons} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$$

$$\sigma(\text{fluorescein}) = 3 \cdot 10^{-16} \text{ cm}^2 \cdot \text{molecule}^{-1} \cdot \text{photon}^{-1}$$

$$k_f = 1/\tau = 1 / 4.5 \text{ ns} = 2.2 \cdot 10^8 \text{ s}^{-1}$$

$$k_a = \sigma \cdot I = 3.75 \cdot 10^8 \text{ s}^{-1}$$

$$x = k_a / (k_a + k_f) = 0.63$$

63% of the fluorophores is in the excited state!