

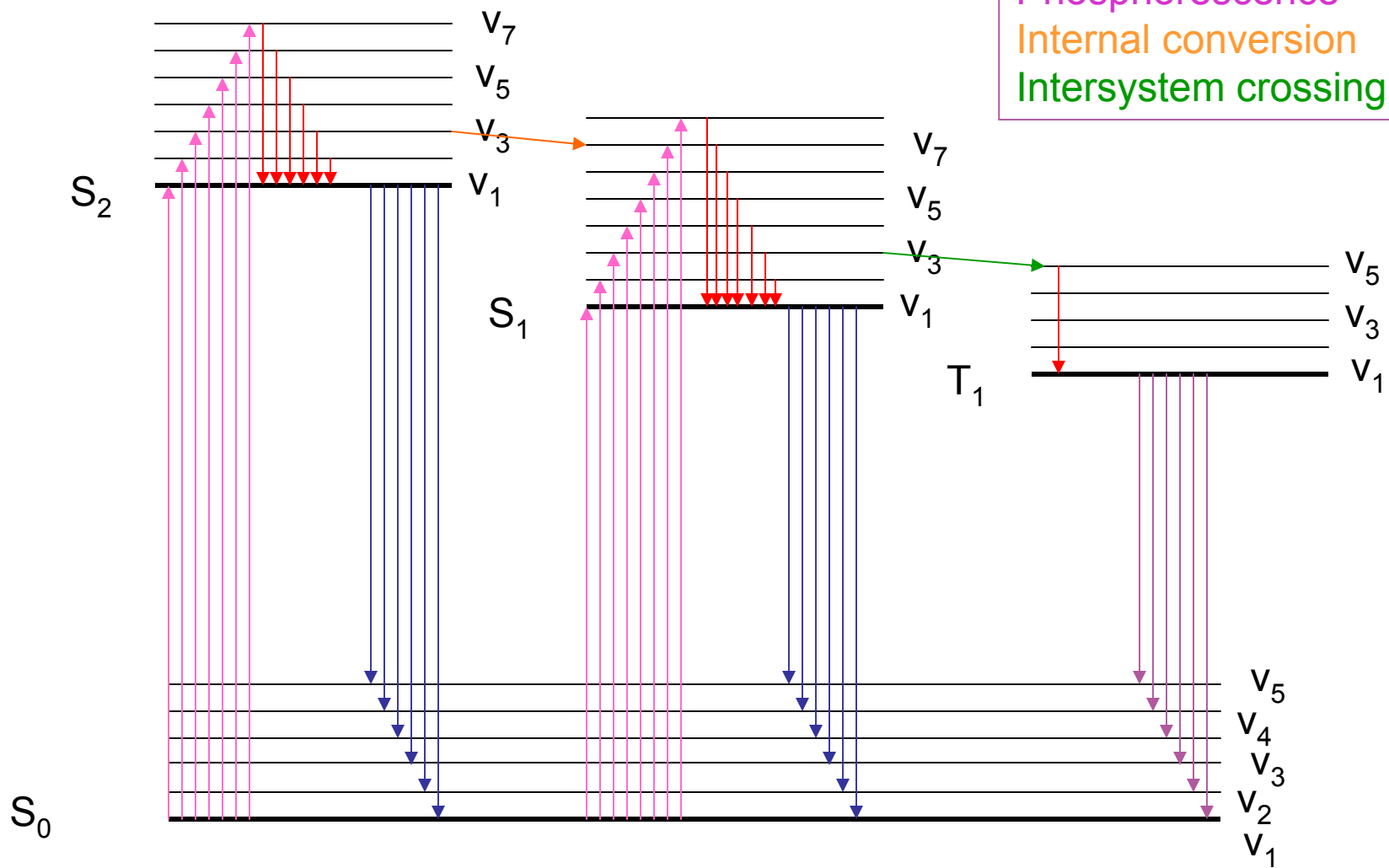
Fluorescence

Topics

- Definition
- Instrumentation
- Sensitivity (nM-pM)
 - Contrast UV-vis measurements
- Derivatization
- Laser-induced Fluorescence

Molecular Fluorescence

Absorption
Vibrational relaxation
Fluorescence
Phosphorescence
Internal conversion
Intersystem crossing



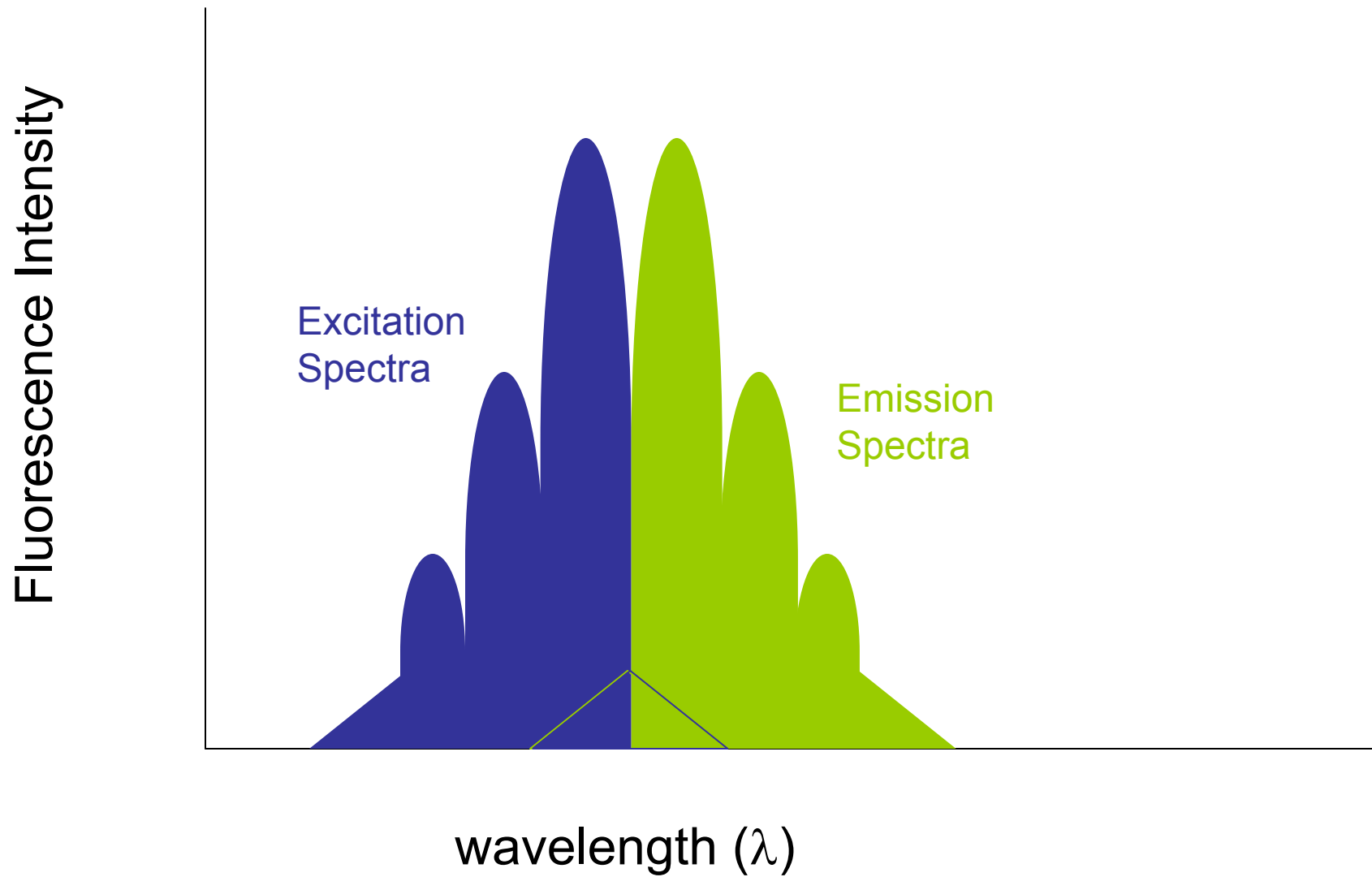
Competing Rates

- Absorption occurs on the femtosecond time scale (10^{-15} s)
- Relaxation from the ground state occurs through the fastest available process
- Most molecules do not fluoresce because the excited vibrational states of S_0 overlap with the S_1 state and relaxation can take place rapidly by vibrational relaxation
- Generally on the picosecond timescale

Fluorescence

- Photons emitted through molecular fluorescence are always less energetic than the photons that are absorbed
 - Look closely at energy diagram on slide 3
 - Photons are absorbed to higher electronic and higher vibrational states. Vibrational relaxation to ground vibrational state of excited electronic state occurs rapidly without emission of photons
 - Emission occurs from this state to ground electronic state at various vibrational states

Spectra



Molecules that fluoresce

- Fluorescence- relaxation through the emission of a photon
- Generally occurs on the ms-ns timescale.
- Molecules that fluoresce tend to be rigid aromatic compounds that possess limited vibrational freedom
- Phosphorescence is the emission of a photon from an excited triplet state
- Phosphorescence is an extremely rare property

Parameters

The intensity of the fluorescence is given by

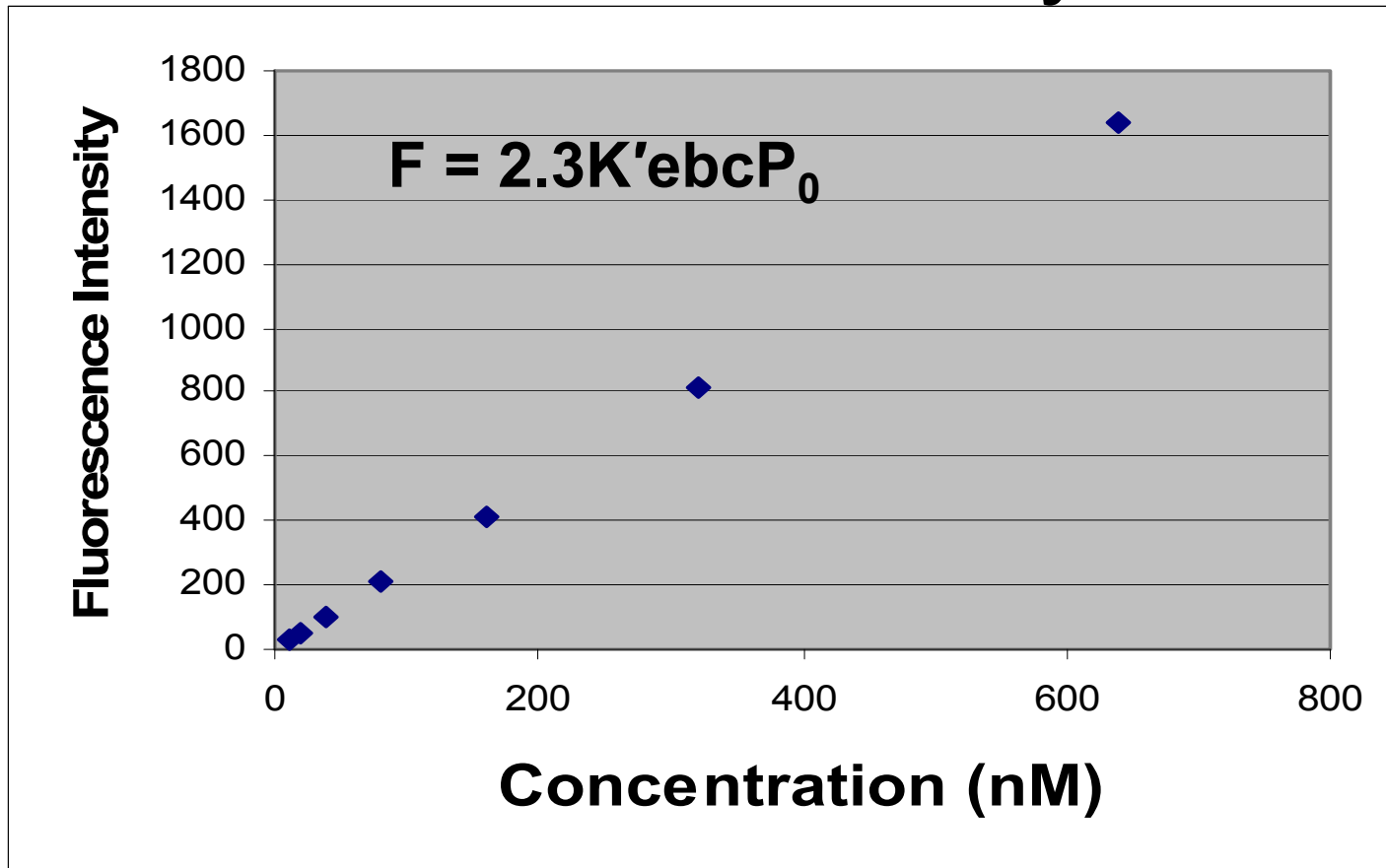
$$F = 2.3K'\epsilon bcP_0$$

K' is directly proportional to ϕ , the quantum efficiency

$$\Phi = k_f / (k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d),$$

competing rates

Typical standard curve for fluorescence analysis

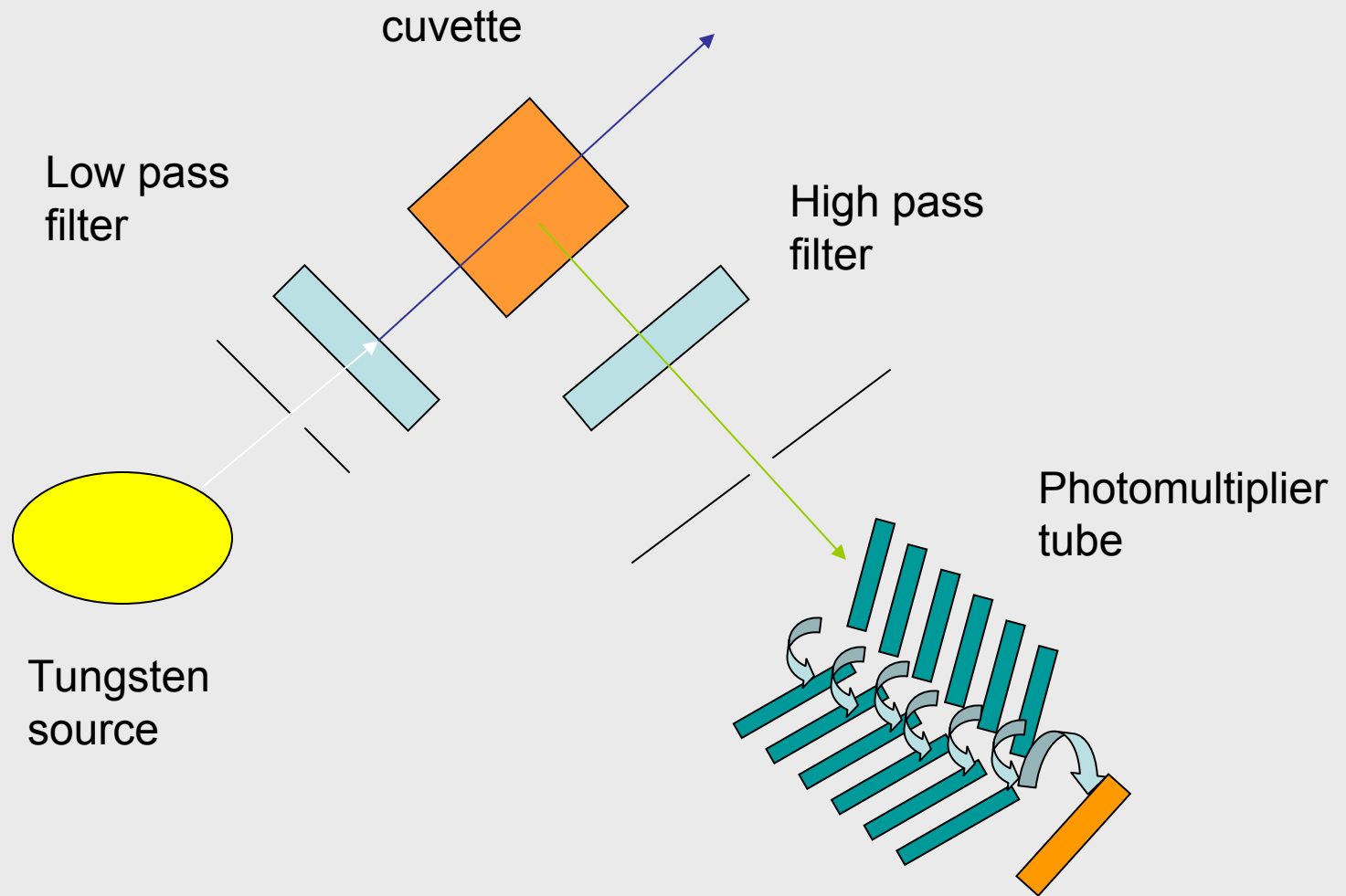


Very large dynamic range

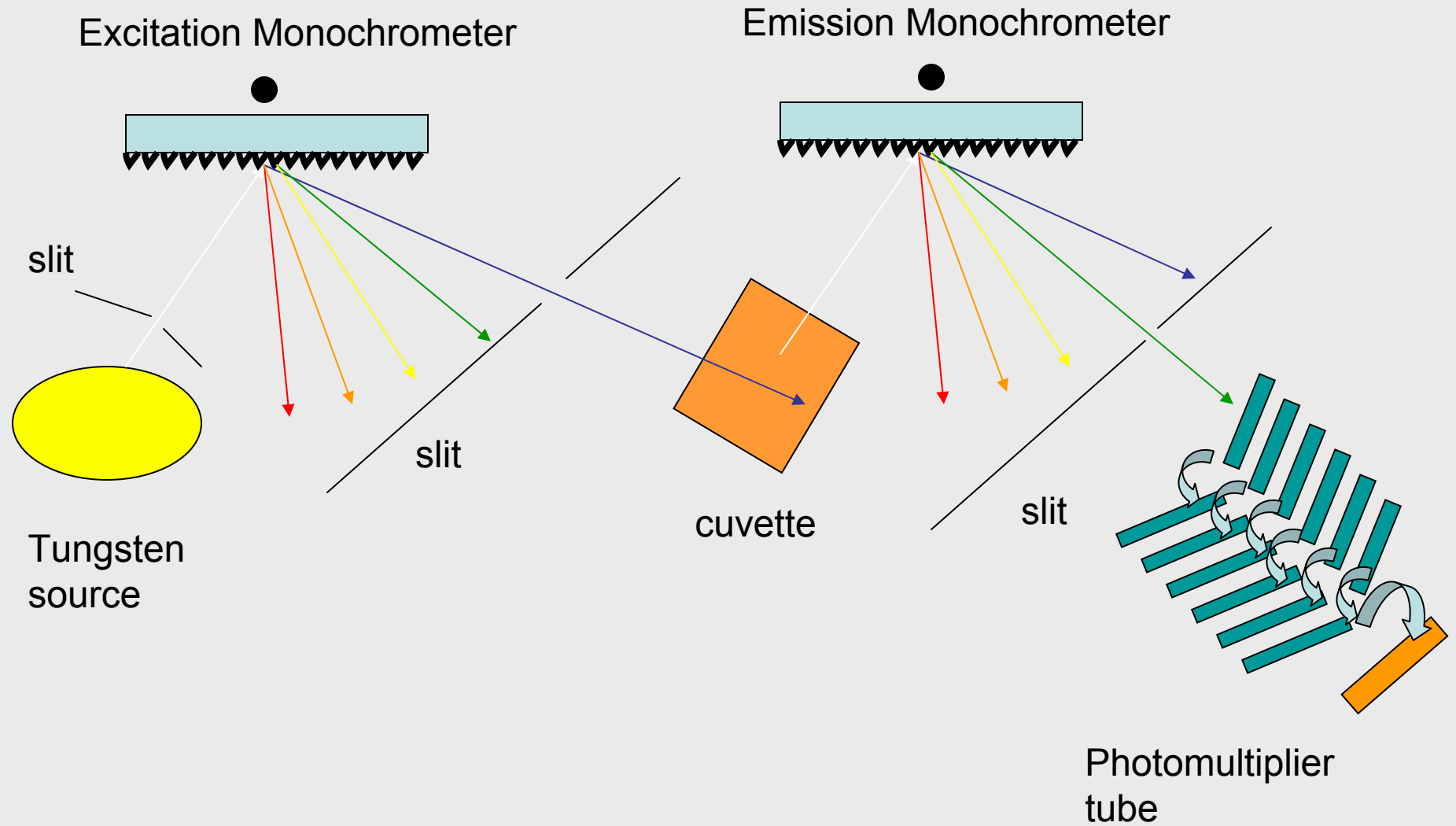
Instruments

- Fluorimeter
- Spectrophorimeter

Fluorimeter



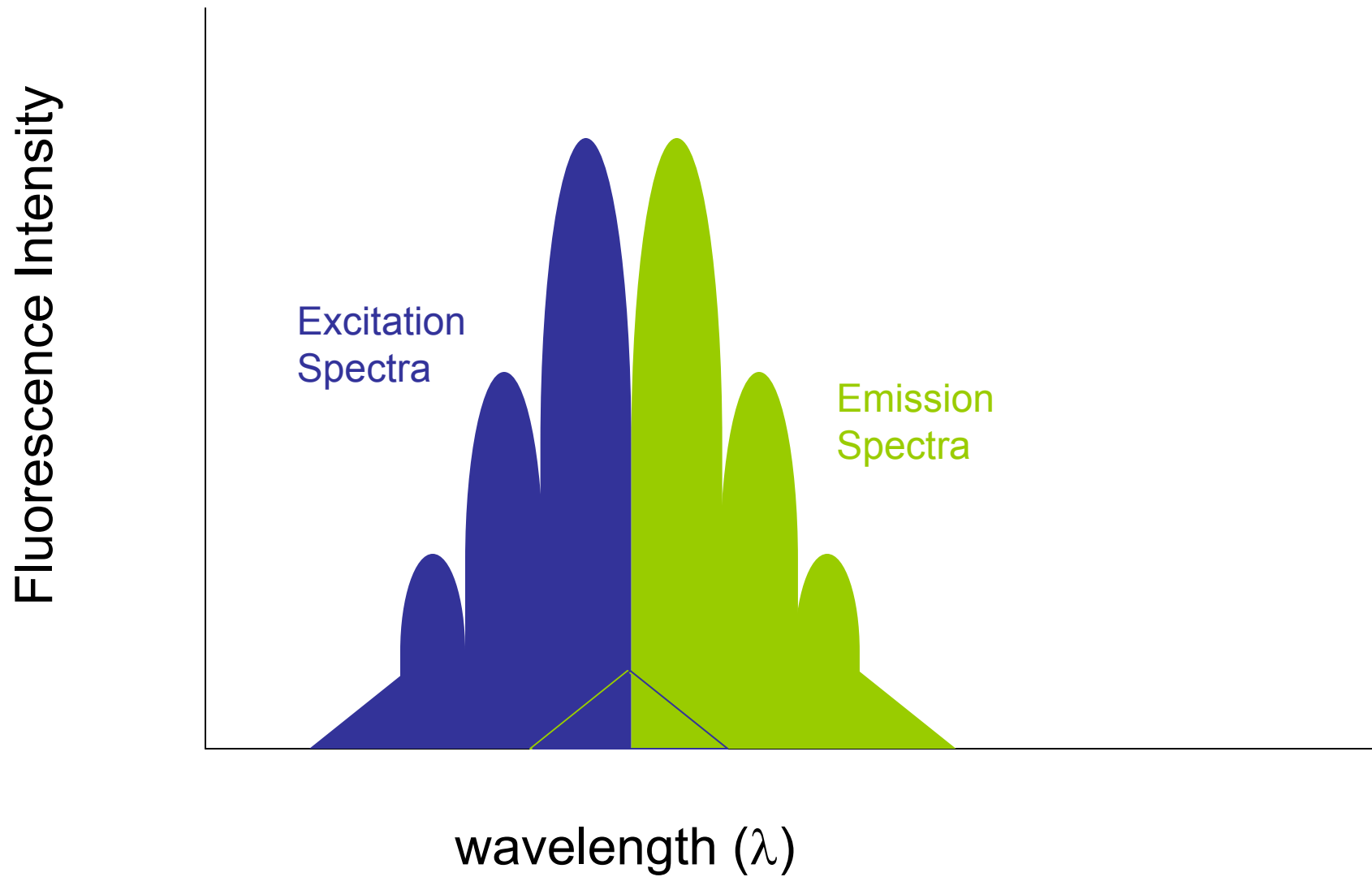
Spectrofluorimeter



Experiment with a spectrofluorimeter

- Select a trial excitation wavelength
- Collect the emission spectrum (scan emission wavelength)
- Select the wavelength of max emission
- Collect the excitation spectra (scan excitation wavelength)
- Select wavelength of max excitation
- Perform experiment with wavelength of max emission and max excitation

Spectra



Why fluorescence?

- More sensitive than UV/vis absorption by a factor of 10^3 - 10^5 .
- Measuring against a zero background
- Large dynamic range
- High specificity
- Biochemical tool; Fluorescent tagging
- Laser Induced fluorescence
 - F is proportional to intensity of source
 - Selectivity of monochromatic light source
 - Convenient with HPLC

Fluorescence tagging

- Green Fluorescence protein
 - Jelly fish
 - Excite 488 nm, emit @ 509 nm
- Genetic engineering to attach to protein of interests
 - Many applications;
 - Martin Chalfie, Osamu Shimomura and Roger Y. Tsien share the 2008 [Nobel Prize in Chemistry](#) for their discovery and development of the green fluorescent protein.
- fluorescence resonance energy transfer
 - Real time monitoring of the formation of protein complexes
 - Two chromophores on two interacting proteins
 - Emission changes when the two proteins are in close contact