Chapter 15 - Molecular Fluorescence Spectroscopy

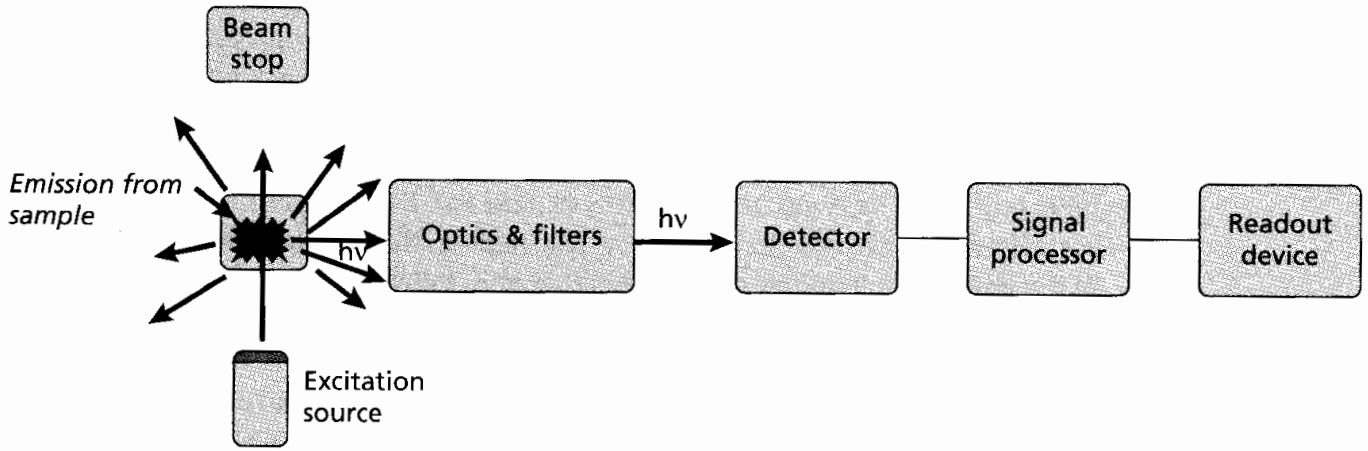
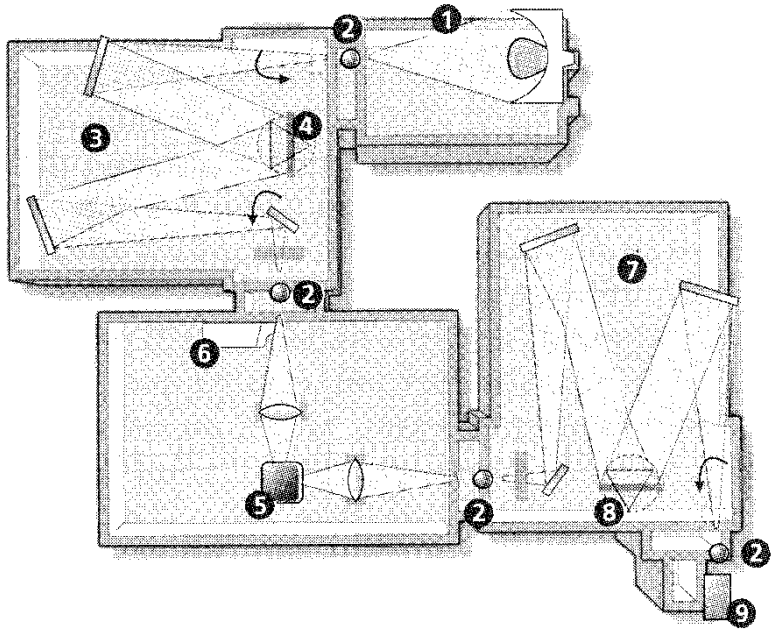


Figure 8.2 Basic setup for measuring luminescence.

Luminescence spectroscopy is an important technique used in a wide range of applications including food science, water quality, pharmaceuticals, nanotechnology and biochemistry.

The technique is extremely sensitive and femtomolar (fM) concentrations of gas, solid and liquid analytes have been measured.

Fluorescence Spectrometer



1. Xenon lamp
2. Adjustable slits
3. Excitation monochromator
4. Excitation grating
5. Sample compartment
6. Excitation correction
7. Emission monochromator
8. Emission grating
9. Detector

Notice off-axis detection of the luminesced light!

Figure 8.11 Commercial fluorescence and phosphorescence instrument (both time resolved and steady state), including optical layout.

Basic Design of a Simple Fluorometer

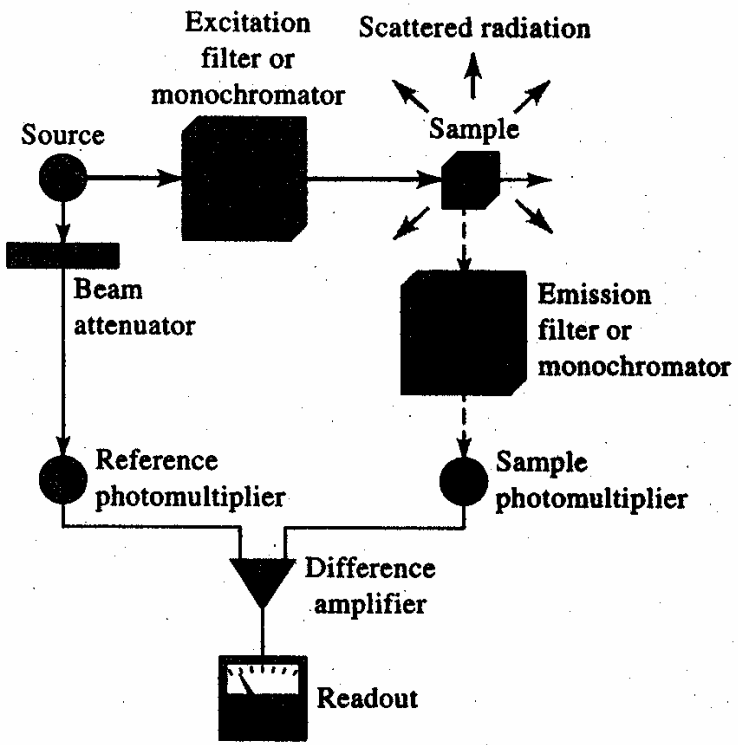


Figure 15-4 Components of a fluorometer or a spectrofluorometer.

Process of Excitation and Emission

- **Absorption of light** - 10^{-15} s and related to ε
- **Vibrational relaxation** – excess vibrational energy in solution immediately lost in solution due to collisional deactivation, 10^{-12} s.
- **Internal conversion** – intermolecular process by which a molecule passes to a lower energy electronic state *without* emission of light. Overlap of vibrational energy levels in two electronic energy levels.
- **External conversion** – deactivation of an excited electronic state by interaction and energy transfer between the excited molecule and solvent or other solutes.
- **Intersystem crossing** – process in which spin of an excited electron is reversed and change in multiplicity results. Most common when vibrational manifold overlap exists and when the molecule has a heavy atom substituent (e.g., Br, I).
- ***Fluorescence and Phosphorescence*** – relaxation of an excited state via light emission. Time scales range from 10^{-6} s to 100's s.

Quantitative Aspects of Fluorescence Measurements

$$F = 2.3\Phi\varepsilon bCP_o$$

- Φ = quantum efficiency = # molecules emitting/total # molecules excited
- ε (L/mol-cm) and b (cm) have their usual meanings
- P_o in incident radiant power density (watts/cm^2)

- Linear relationship, F = KC

- *Self-absorption* and *self-quenching* cause negative deviations from linearity (i.e., reduced fluorescence intensity).

- Φ increases with lower temperature, increased structural rigidity, $\pi \longrightarrow \pi^*$ transition, and can be affected by solvent type and pH.

- Electron donating groups (NH_2, OH) tend to enhance fluorescence while electron withdrawing groups (Cl, COOH) tend to inhibit it.

Wavelength Selector

Monochromator – disperses light into its component wavelengths

Diffraction = bending of light rays by a grating

Refraction = bending of light rays by a prism or lens

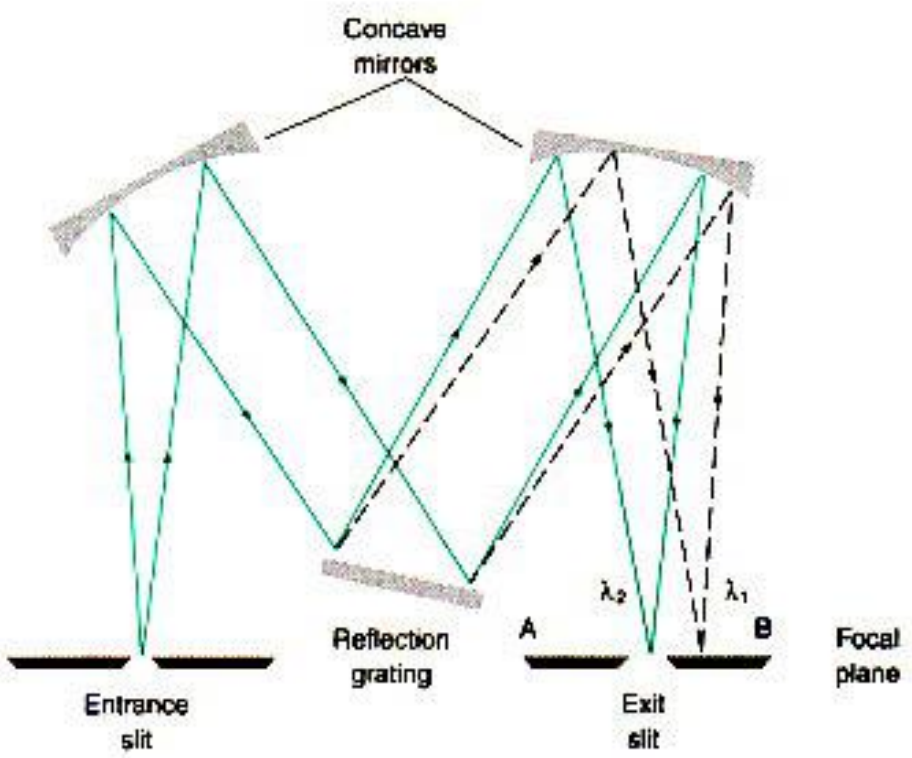


Figure 19-5 Czerny-Turner grating monochromator.

Dispersive instrument

Trade-off between resolution and signal: the narrower the exit slit, the greater the ability to resolve closely spaced at the expense of decreased signal-to-noise ratio.

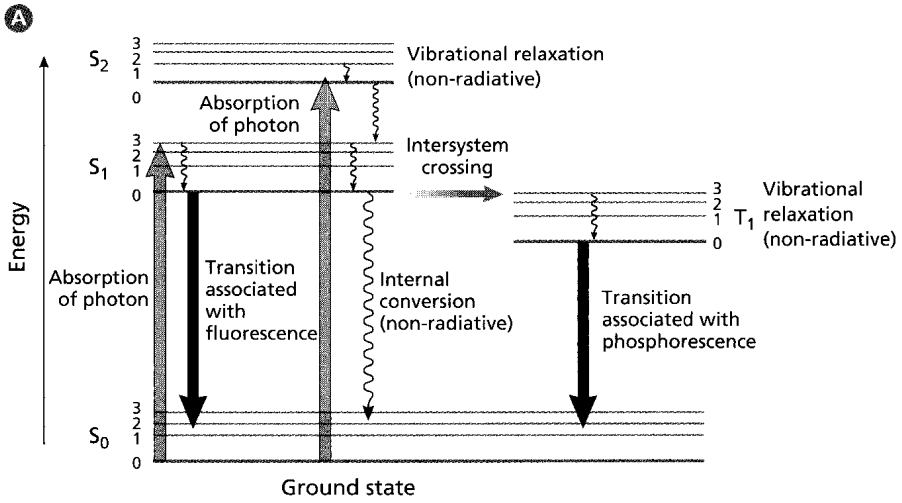
Performance Criteria of Monochromators

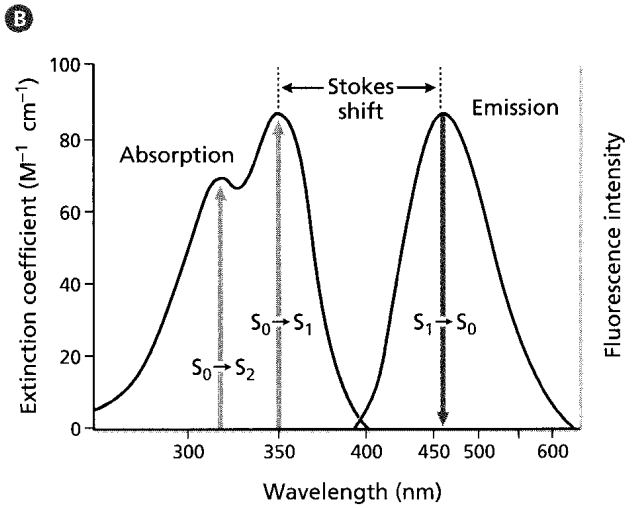
- Stray light rejection
- Resolving power $R = \frac{\lambda}{\Delta\lambda}$
- Light gathering power (1-10)
  Lower the number, the better the light gathering power

  $f = \frac{F}{d}$
- Linear dispersion $D = \frac{\partial y}{\partial \lambda}$

Jablonski Diagram

The few molecules that fluoresce do so from S1 (v=0) state.





Lifetime of excited vibrational states (10^{-15} s)

Lifetime of excited electronic states (10^{-9} s)

Potential Energy Diagram

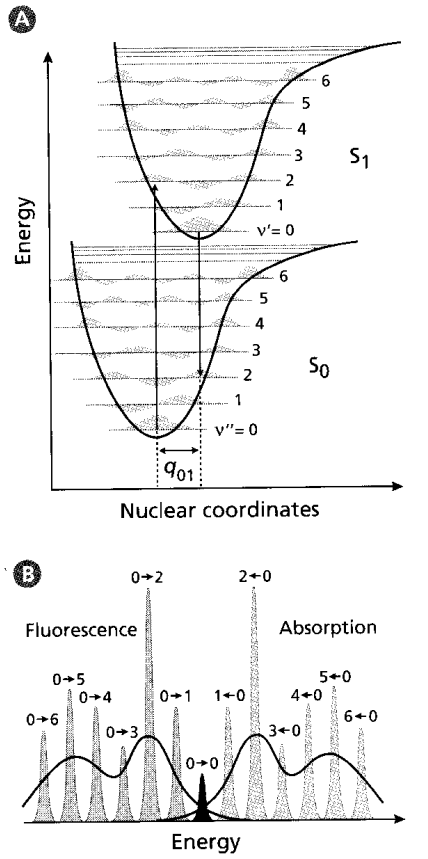


Figure 8.4 (A) Potential energy diagram showing two electronic states, ground state S_0 and excited state S_1, with vibrational levels (v) and (B) associated absorption and fluorescence spectra.

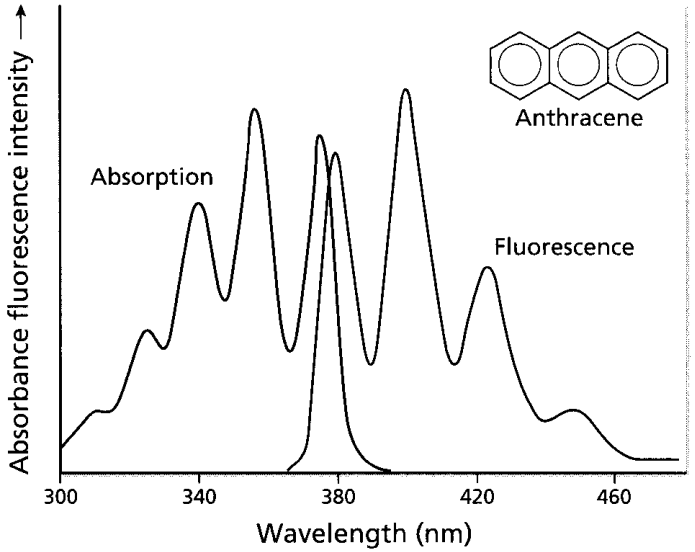


Figure 8.5 Fluorescence and absorption of anthracene.

***Mirror image rule*: for molecules in which excitation is into S1 (and not S2…), that the absorption spectrum is the mirror image of the emission spectrum.**

Quantum Yield

TABLE 8.1: Fluorophore Quantum Yields

FLUOROPHORE	QUANTUM YIELD, ϕ
Quinine sulfate	0.59
Rhodamine 6G	0.91
Fluorescein	0.89
Anthracene	0.28
Oxazine 1	0.15

From Wurth, C.; et al. Relative and Absolute Determination of Fluorescence Quantum Yields of Transparent Samples. *Nature Protocols*, 2013, 8, 1535–1550.

$$\phi = \frac{k_f}{k_f + k_{nr}}$$

k_r = rate constant for fluorescence

k_{nr} = sum of various non-radiative decay rate constants

$$\frac{number\ of\ molecules\ fluorescing}{number\ of\ molecules\ excited\ by\ photon\ absorption}$$

Fluorescence Measurement

Example 8.1

In the fluorescence spectrum of a given molecule, an emission peak at 490 nm is from $S_1(v_0)$ to $S_0(v_0)$. In the absorption spectrum, the peak is near 395 nm.

(a) Calculate the difference in energy between the S_1 and S_0 states associated with emission. Determine this energy in joules.

$$\Delta E = \frac{hc}{\lambda} = \frac{1.99\times10^{-25}\ \text{Jm}}{490\times10^{-9}\ \text{m}} = 4.06\times10^{-19}\ \text{J}$$

We can also express the energy of this one photon in terms of the energy of one mole of these same photons. Completing this conversion:

$$(4.06\times10^{-22}\ \frac{kJ}{photon})(6.02\times10^{23}\ \frac{photons}{mole}) = 244.4\ \frac{kJ}{mole}$$

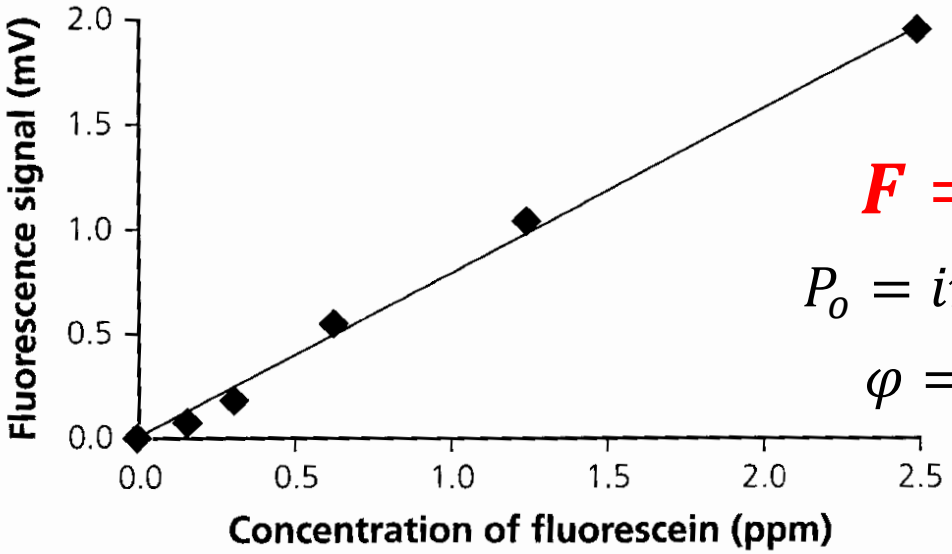
For a sense of scale, you may recall that bond energies or bond enthalpies (for gases) are on the order of tens to many hundreds of kJ/mole.

(b) Calculate the thermal energy imparted to the molecule as a result of the absorption to emission process. The thermal energy is the difference in energy between the absorbed photon at 395 nm and the emitted photon at 490 nm.

$$E_{abs} - E_{emission} = hc\left(\frac{1}{\lambda_{abs}} - \frac{1}{\lambda_{emission}}\right) = 1.99\times10^{-25}\ \text{Jm}\left(\frac{1}{395\times10^{-9}\ \text{m}} - \frac{1}{490\times10^{-9}\ \text{m}}\right)$$
$$= 9.77\times10^{-20}\ \text{J}$$

Note the subtle difference in how we have used the equation relating wavelength and energy. In part (a), we found the energy difference between two energy states from the wavelength associated with the transition. In part (b), we found the difference between two energies, each of which has an associated wavelength.

Fluorescence Intensity



$$F = 2.303\varepsilon bCP_0\varphi$$

$P_o = incident\ intensity\ (power)$

$\varphi = quantum\ efficiency$

$$I = I_{max}e^{\frac{-t}{\tau}} \qquad \tau = \frac{1}{k_f + k_{nr}}$$

Quenching

One way in which fluorescence can be a powerful tool to study the local chemical environment around a molecule is through *quenching.*

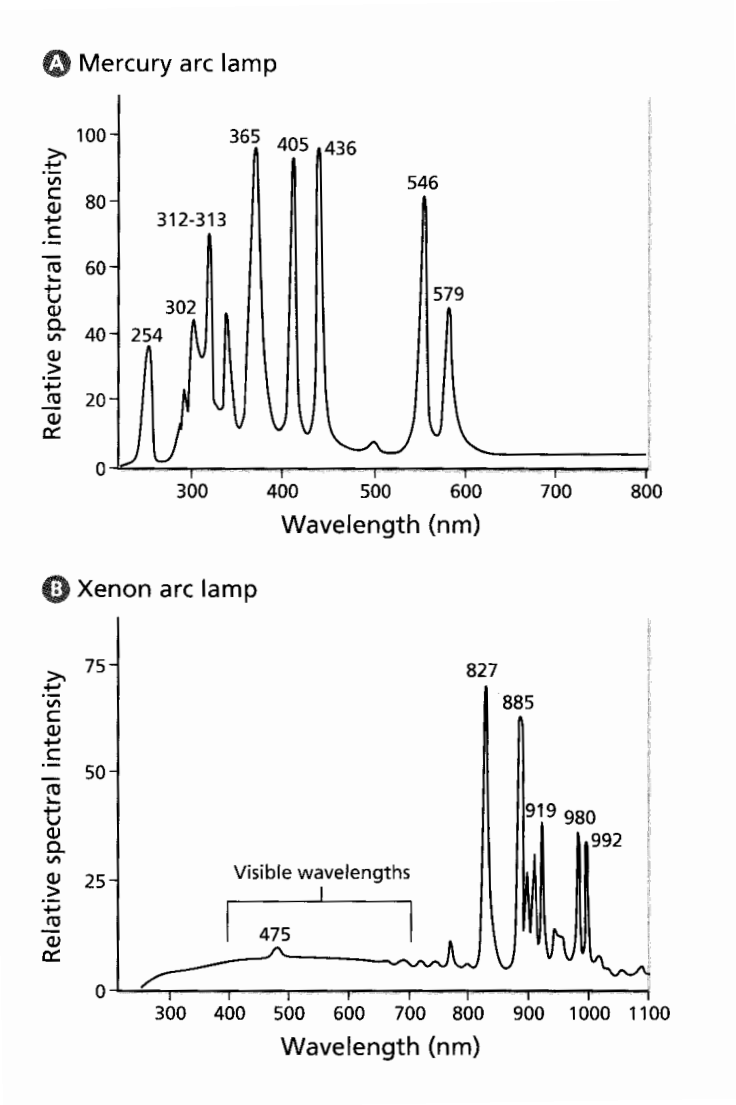
One fluorophore transfers energy to a different type of molecule (*quenching*) – reduced fluorescence. (robs energy from excited molecule) O_2 is an example of a quencher.

One fluorphore transfers energy to the same unexcited molecule (*self-quenching*)

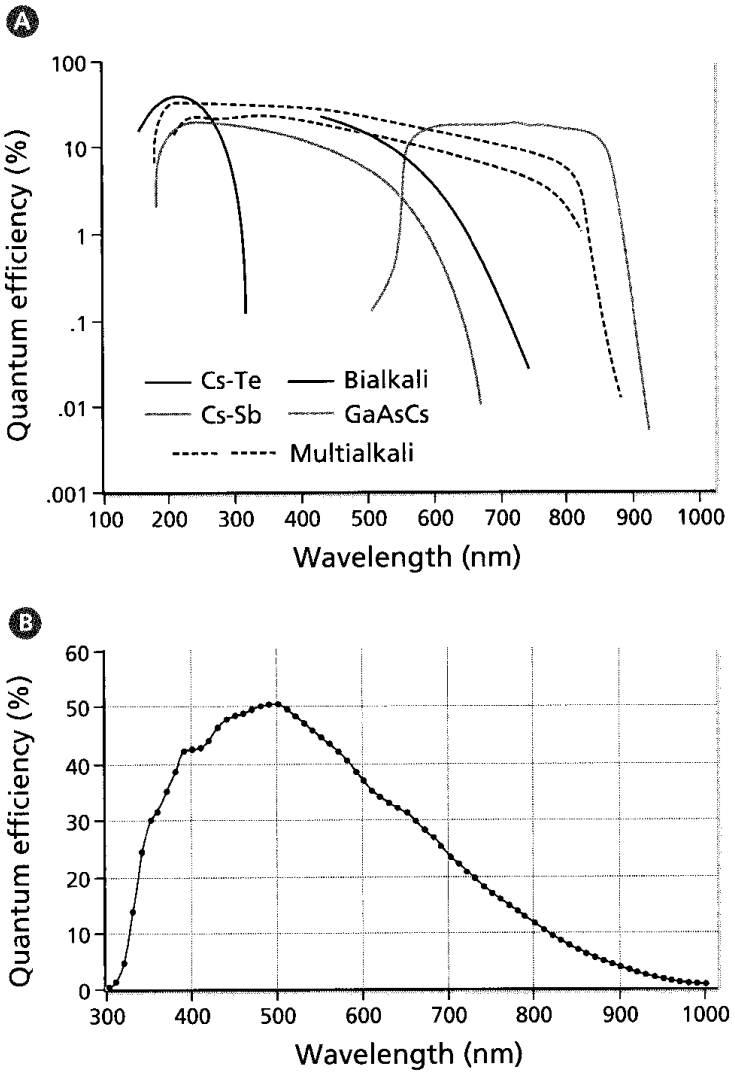
$$\frac{F_0}{F} = 1 + K_q[Q]$$

F_o = fluorescence intensity without quencher
F = fluorescence intensity with quencher
K_q = Stern-Volmer constant (quenching rate constant)
[Q] = quencher concentration

Broadband Light Sources



Photon Detectors

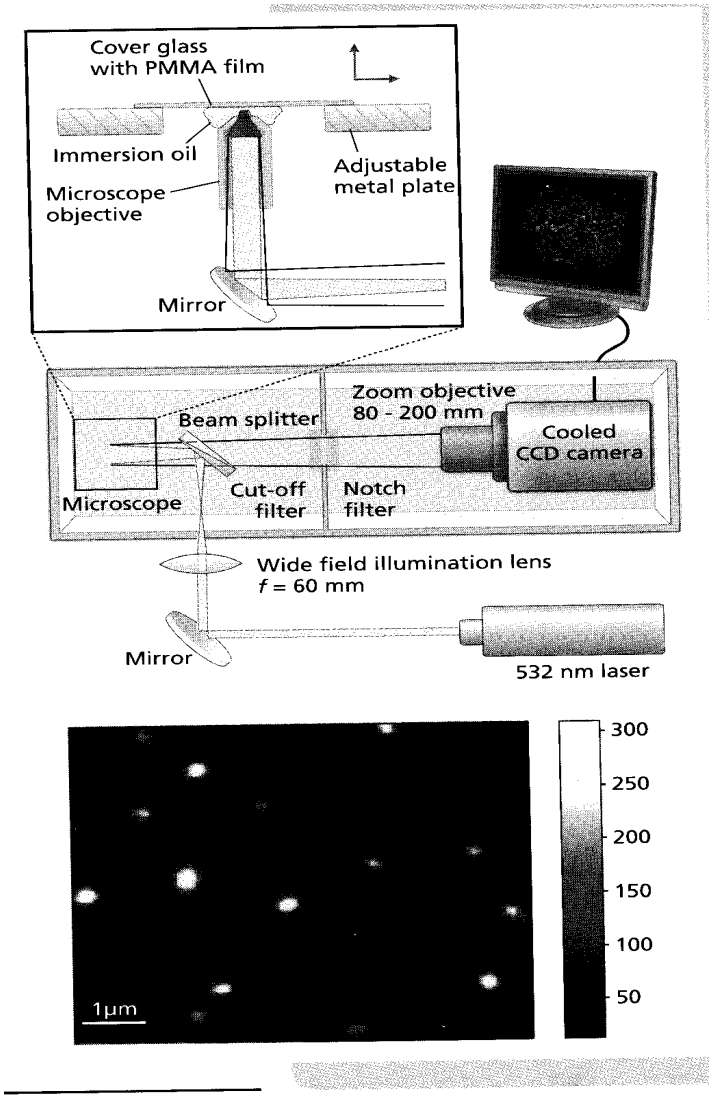


$$i_{photo} = kP + i_{dark}$$

Continuous quantum efficiency over range of wavelengths.

Figure 8.13 (A) Quantum efficiency as a function of wavelength for several different types of PMT photocathode materials and (B) a Si CCD.

Laser-Induced Fluorescence



Single molecule detection!

Laser light source

No monochromators