Optical Spectroscopy--Molecular and Atomic Part II. Con't of Molecular Spect.

Analytical Spectroscopy: method to examine or measure the amount of species present based on a selective and characteristic interaction of the analyte with electromagnetic radiation

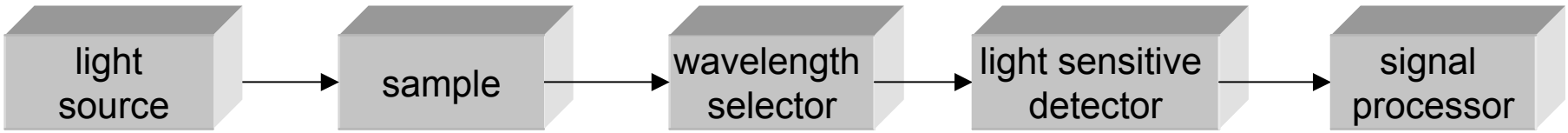
UV-Visible Absorption--more details!

Fluorescence (Luminescence) Spectroscopy

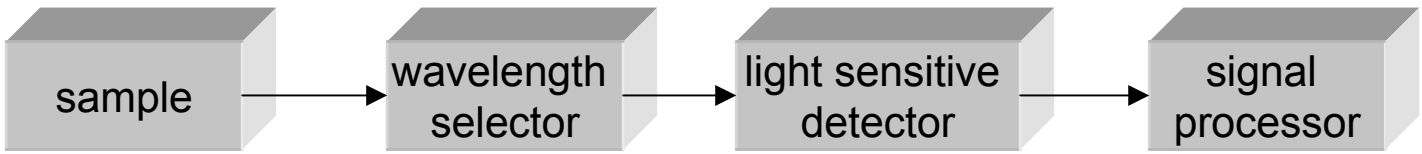
Introduction to Basic instrumentation

Basic Types of Spectroscopy

Absorption Spectrometry

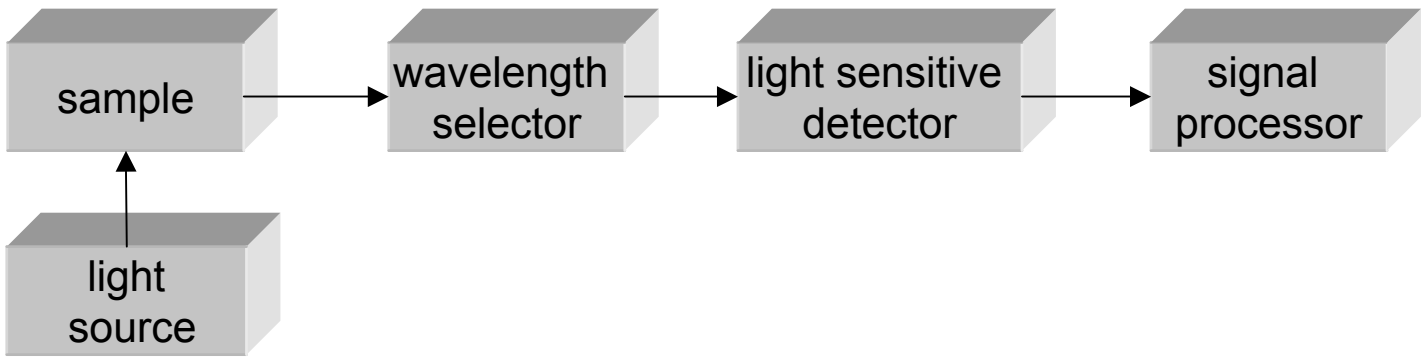


Emission Spectroscopy



Fluorescence Spectroscopy

(& Scattering Methods)



Absorbance

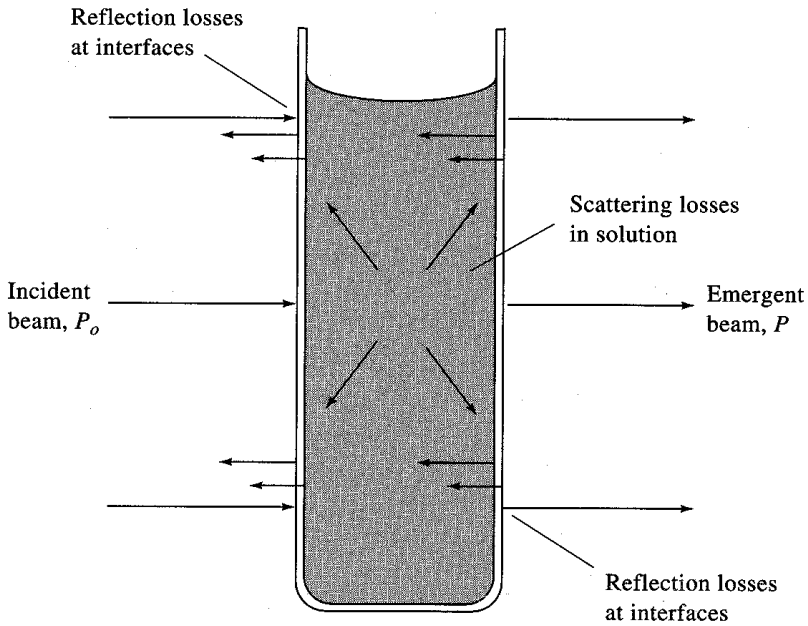
Transmittance $T = \frac{P}{P_0} \approx \frac{P_{sample}}{P_{blank}}$

Absorbance $A = -\log T$

Power $P = \frac{energy}{area \cdot \sec}$

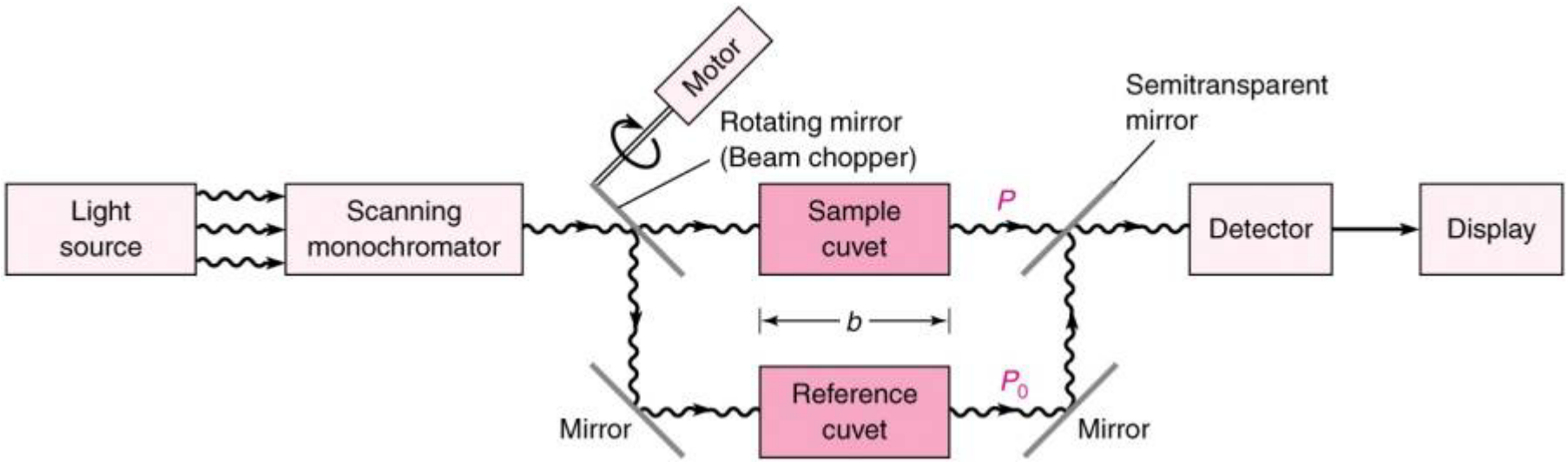
Beer's Law $A = abc = \varepsilon l[C]$

molar absorptivity $\varepsilon = \frac{L}{cm \cdot mol}$



- cuvette is quartz for UV; glass for visible
  1 cm is standard path length
- surface must be highly polished and clean to minimize scattering and reflection

Simple Spectrophotometer Dual-Beam

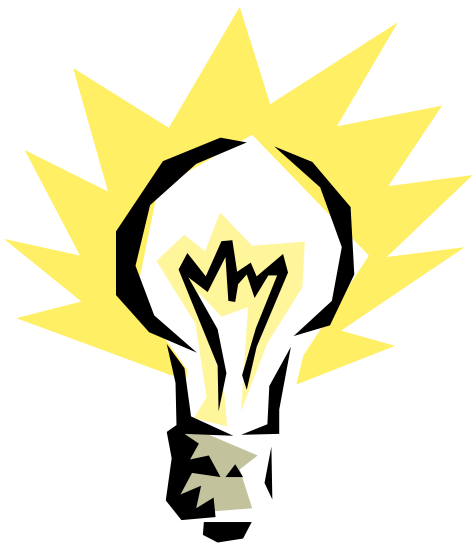


Transmittance $$T = \frac{P}{P_0} \approx \frac{P_{sample}}{P_{blank}}$$

Absorbance $$A = -\log T$$

Light Sources

- Tungsten (incandescent) lamp
  - for visible and near IR spectra
  - 350 to 900 nm
  - basically a "white" hot wire



- D_2 or H_2 arc lamp
- for UV spectra

$$D_2 \xrightarrow{E_e} D_2^* \longrightarrow D' + D'' + h\nu$$

$$E_e = E_{D_2^*} = E_{D'} + E_{D''} + h\upsilon$$

kinetic energy of the photons

continuum spectrum

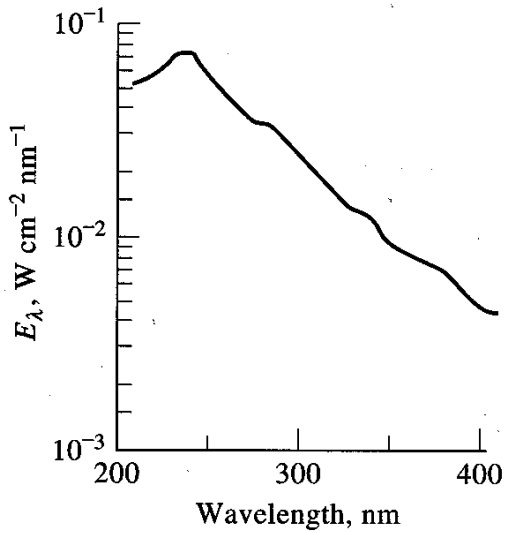
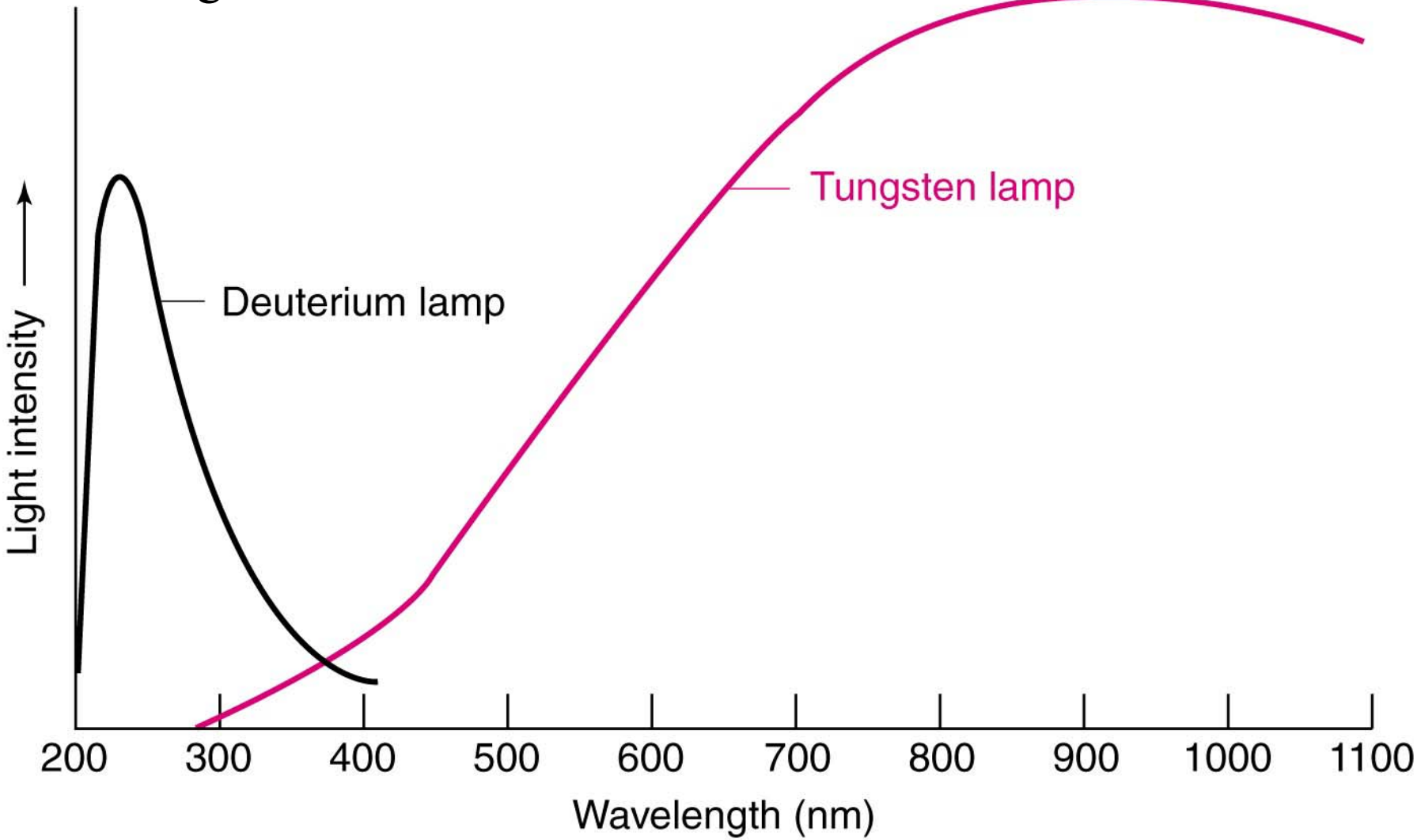


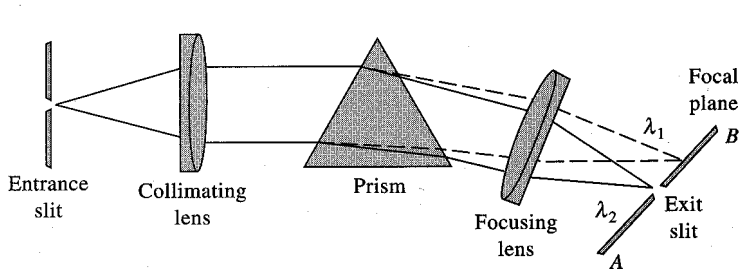
Figure 13-11 Output from a deuterium lamp.

<u>Continuum Sources</u>----yield light over a wide range of adjacent wavelengths---but intensities are not same at different wavelengths!



Wavelength Selection

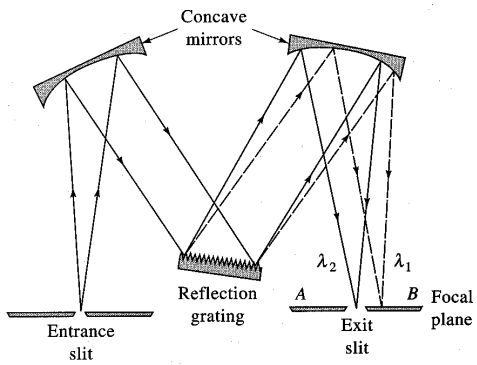
Prism Monochromator



(b)

(Bunsen type)

Grating Monochromator



(a)

(Czerney-Turner type)

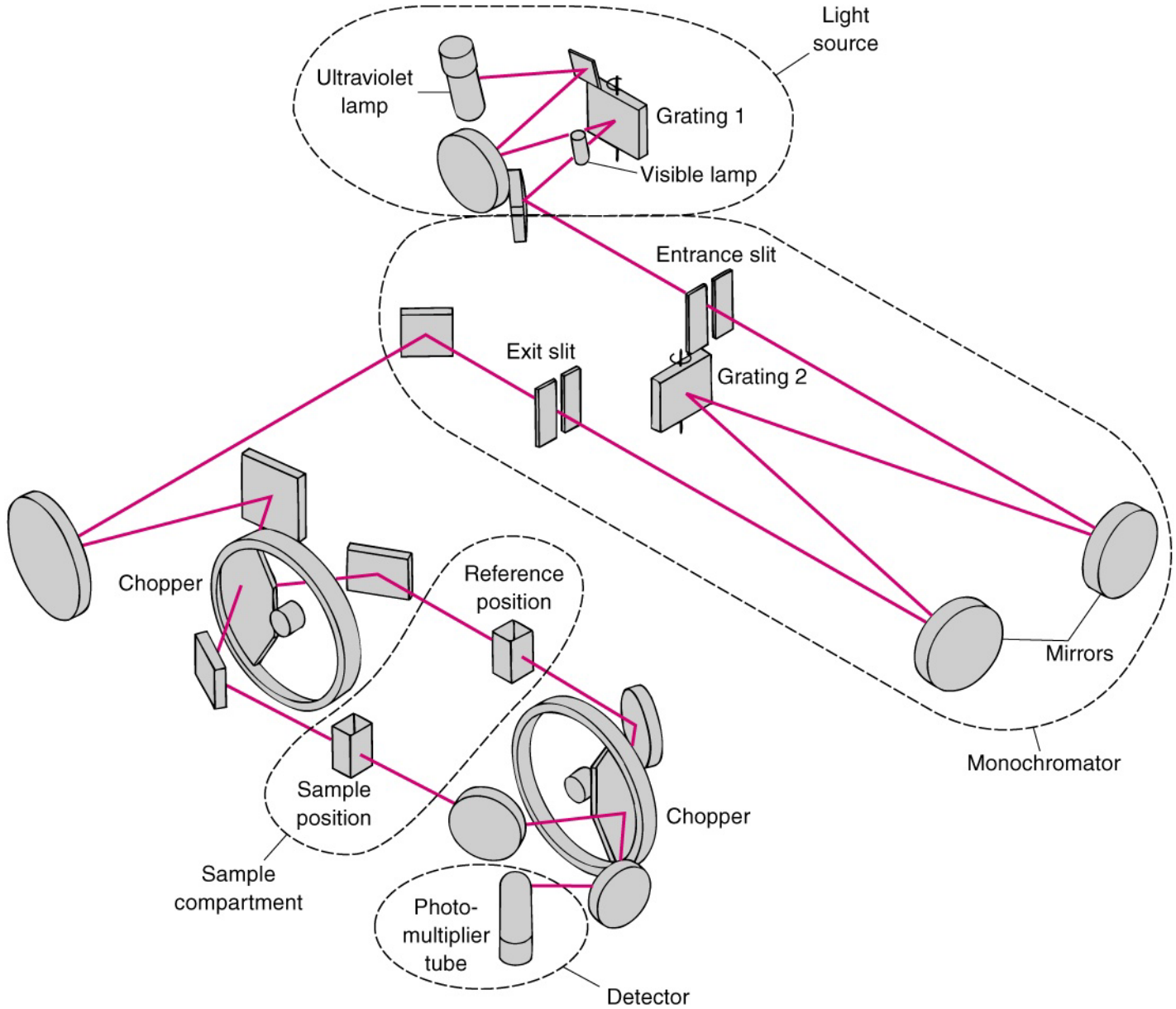
Monochromator: optical device that selects one wavelength (or band of similar wavelengths)

Refraction (bends light):

- Light slows down in dense materials like quartz (SiO_2)
- Angle that light bends depends on the wavelength

λ	η
1,060	1.4497
545	1.4601
365	1.4744
215	1.5343

Inside of high quality UV-vis spectrophotometer quite complex



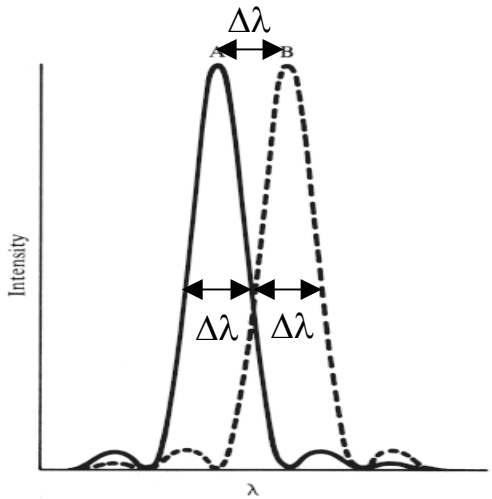
(*b*)

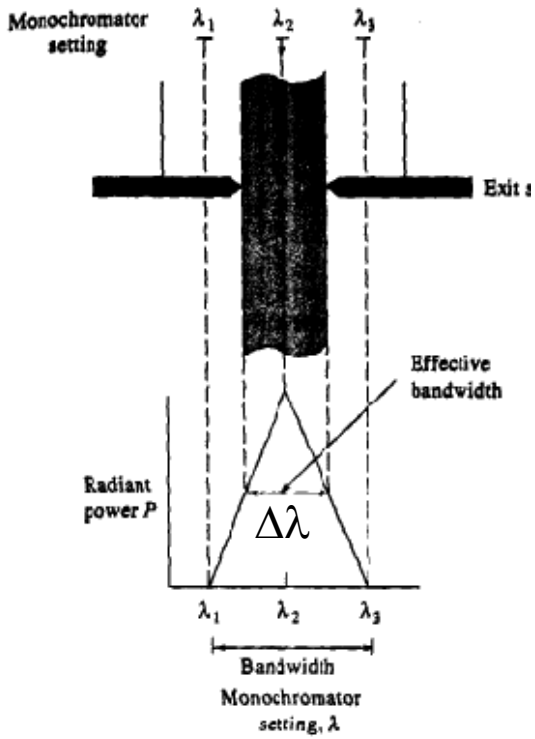
Resolution in Spectroscopy

Controlled by quality of monochromator (prism not as good as grating!)
Also ---by slit width of monochromator;

Resolution is the ability to distinguish between unique spectral features

- for example, different analyte species
- $\Delta\lambda$, numerical figure for resolution

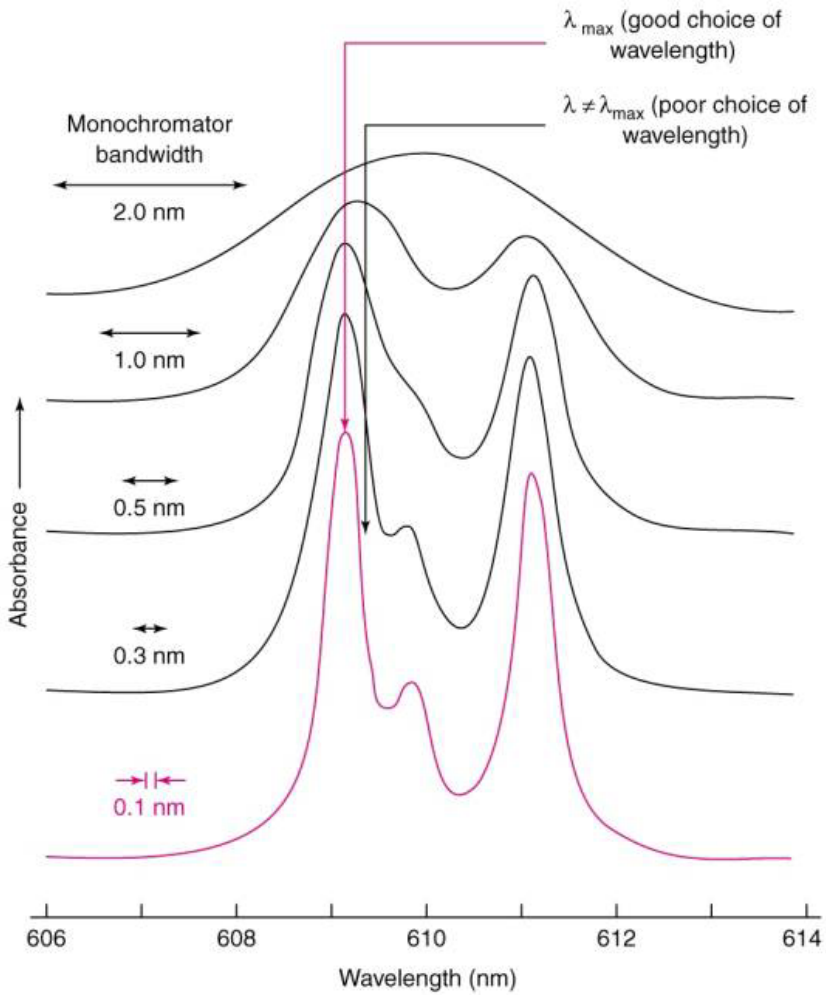




Resolving Power is a property of the monochromator and slit widths

- the higher the resolving power the better the resolution (possible)
- $R = \lambda / d\lambda = (nN)$
- actual power throughput is Gaussian (normal distribution)

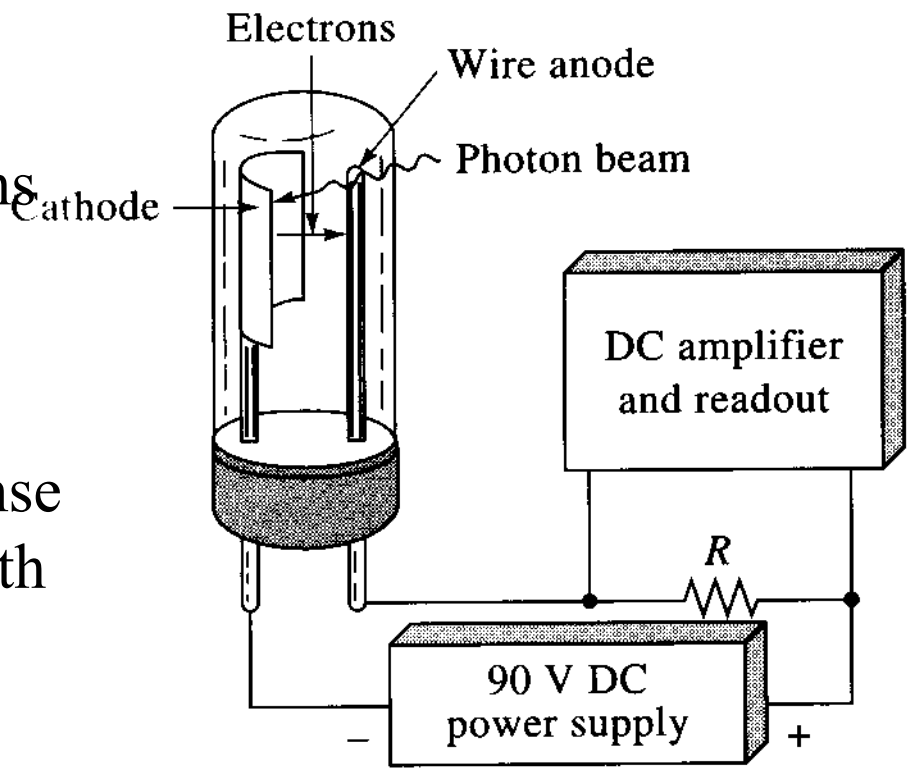
Wavelength Selection, Scanning Monochromator



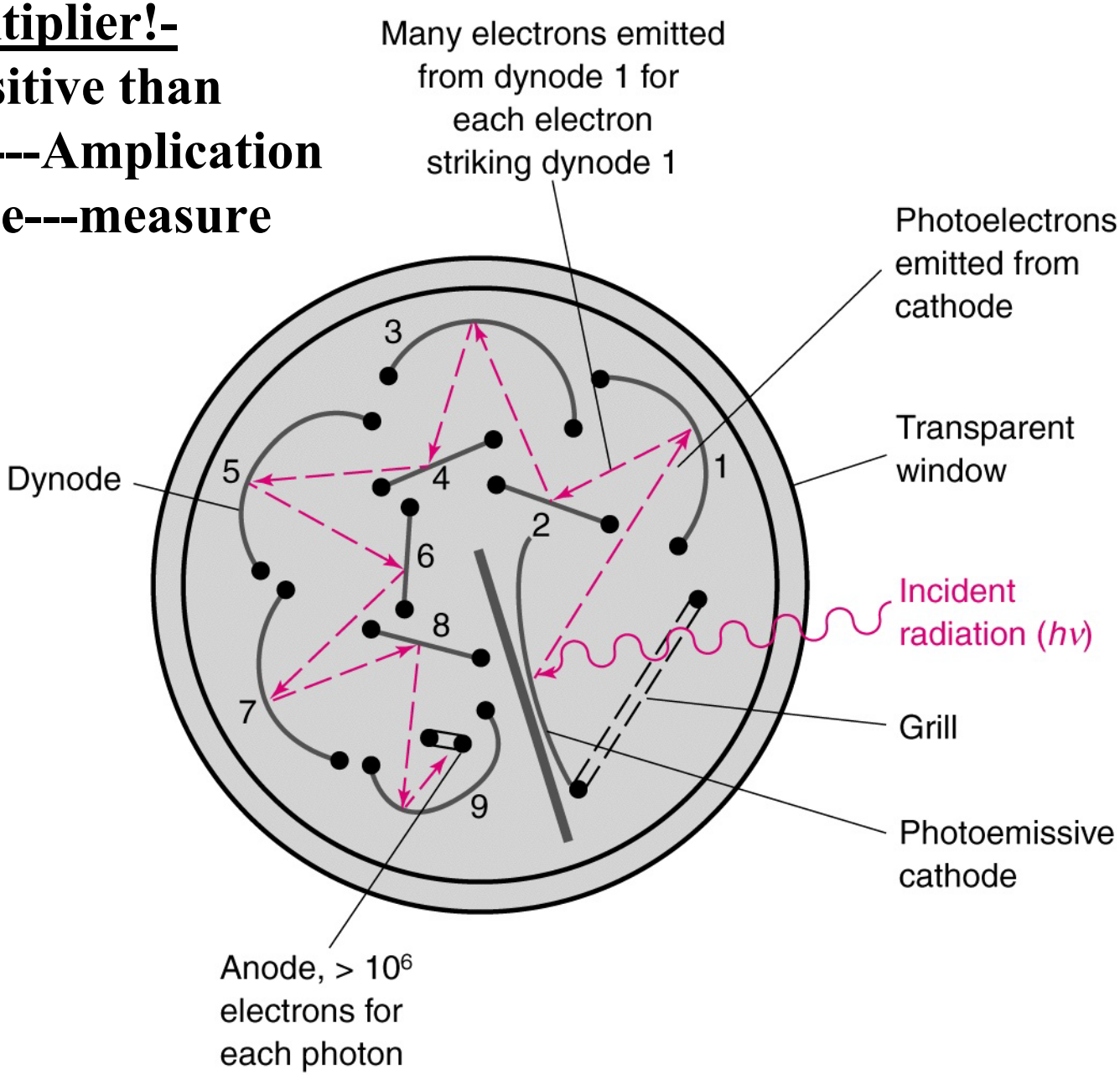
Photosensitive Detectors

Photocathode

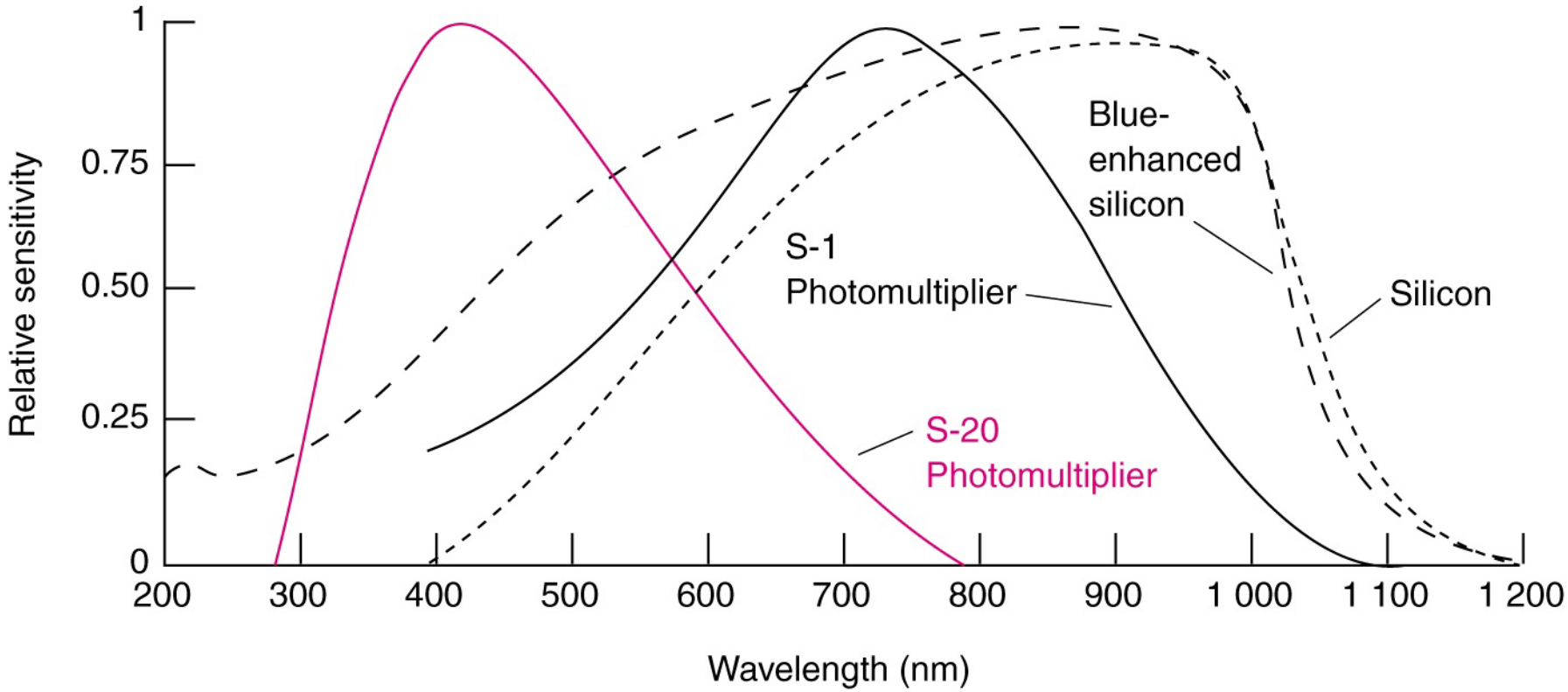
- Cathode surface emits electrons when excited by a photon
- Surface is a special alloy:
  - mixed alkali is common
  - Ga/As gives flattest response over the widest spectral width
- Single stage transducer
  - (one cathode - one anode)
  - relatively low gain



Photomultiplier!-
more sensitive than photocell---Amplication by cascade---measure current!

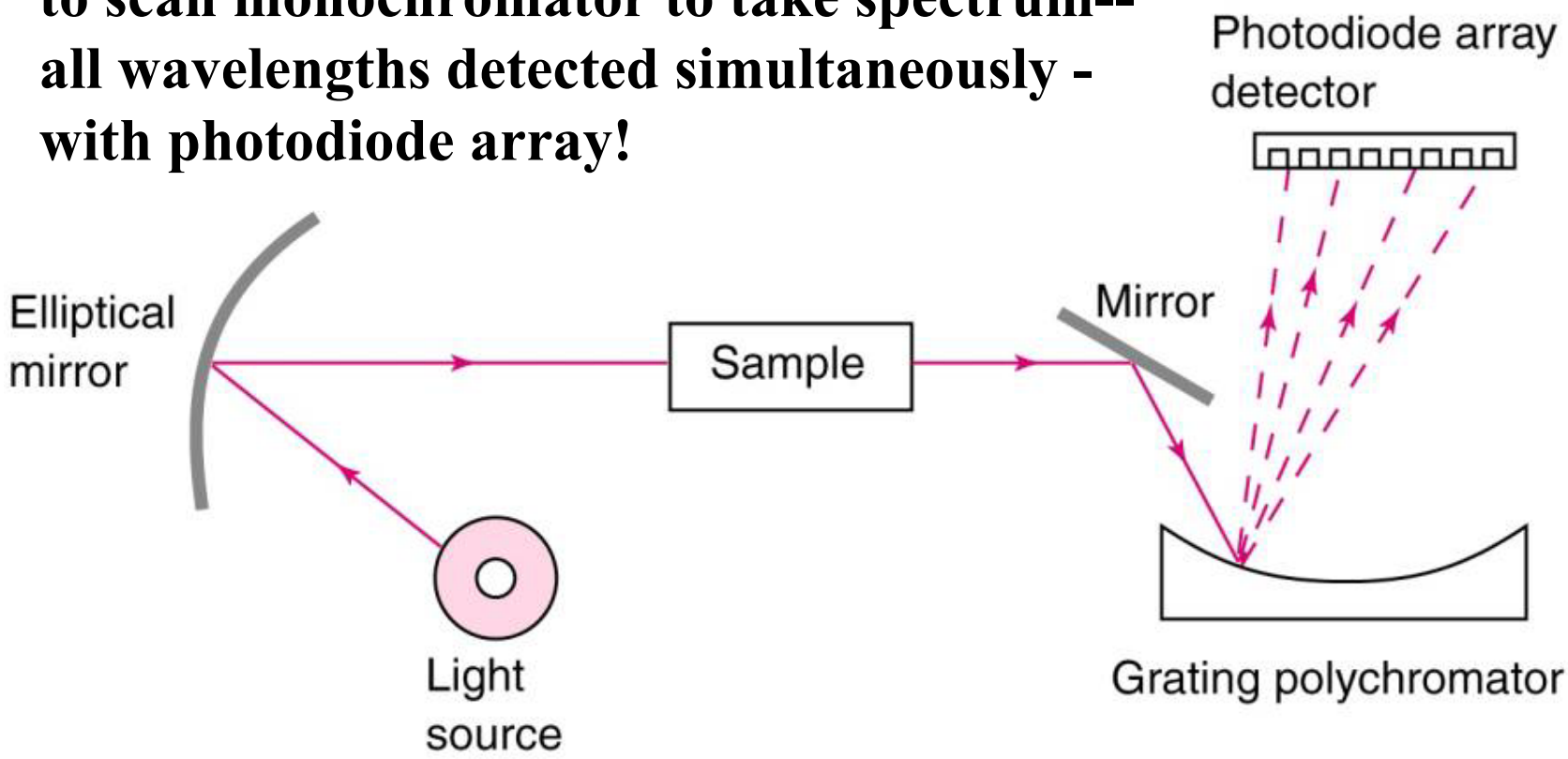


Detectors with different surfaces have different sensitivities in over the wavelength range of interest!



Simple Mutliplex Spectrometer

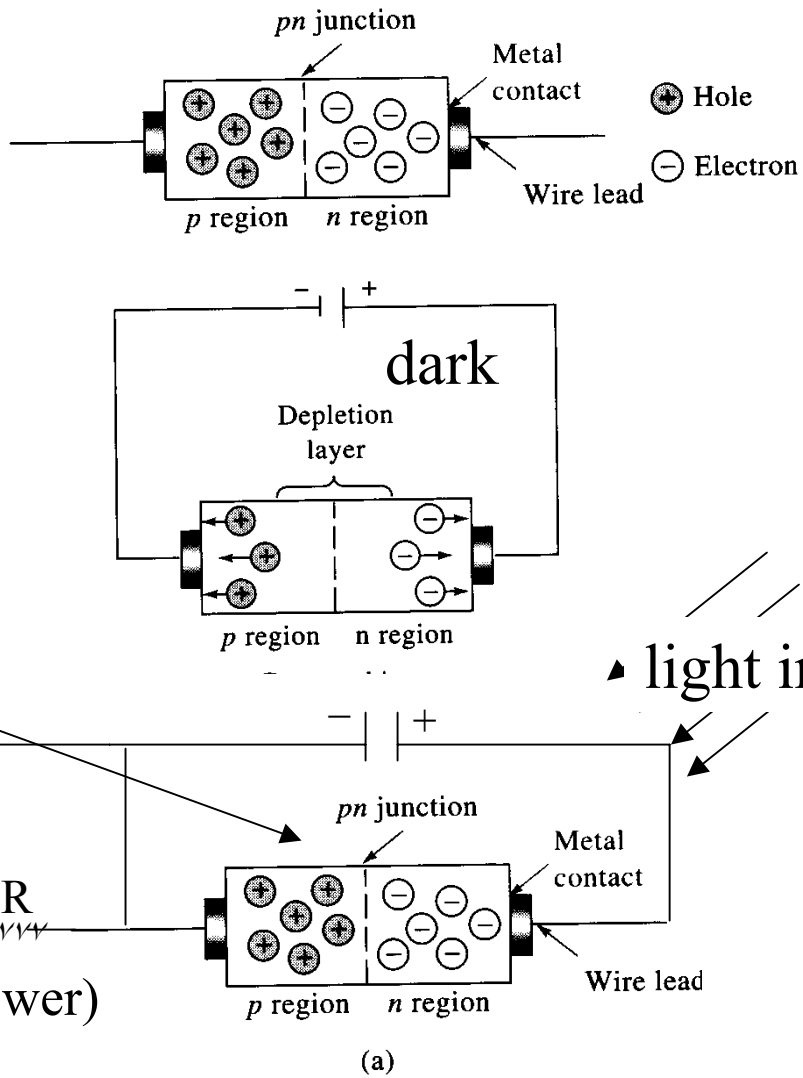
diode array spectrophotometer---don't need to scan monochromator to take spectrum--all wavelengths detected simultaneously - with photodiode array!



Photodiode

- Semiconductor diode: transducer that acts like a one way switch

- Diode under reverse bias
- typical condition steady-state, or at rest

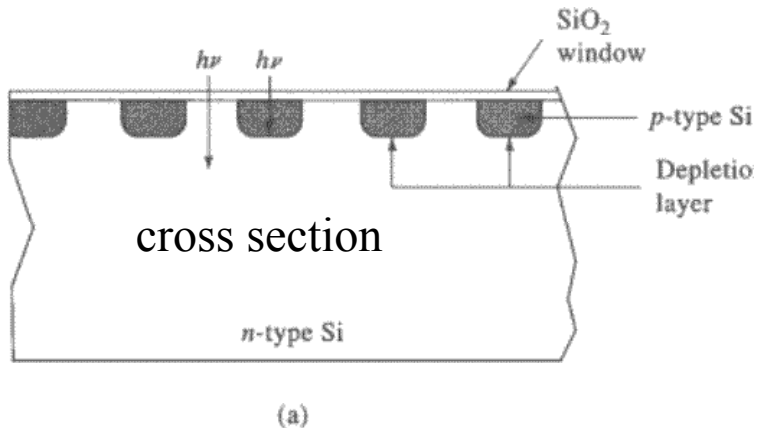


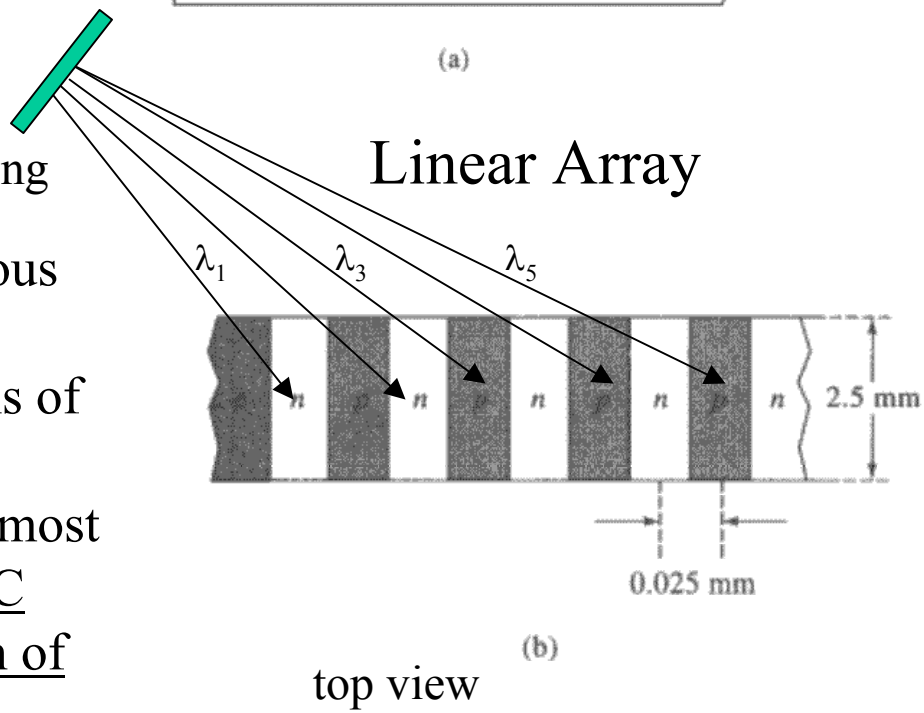
I (current)=γ (response factor)•P_i (radiant power)

Diode Arrays

Advantages: tiny and rugged -large bandwidth -good dynamic range -lower power required

Disadvantage: -devices tend to be noisier than the PMT





- Linear diode array allows simultaneous detection of all wavelengths!
  - this is a huge advantage in terms of speed of analysis
- The full spectrum can be acquired almost instantaneously (1-2 ms)--<u>good for LC detectors---can obtain entire spectrum of eluting analyte!!</u>

Molecular Photon Emission

- Not all molecules fluoresce, even though they absorb.
- Non-radiative process can dominate
- phosphoresence also occurs for select syster

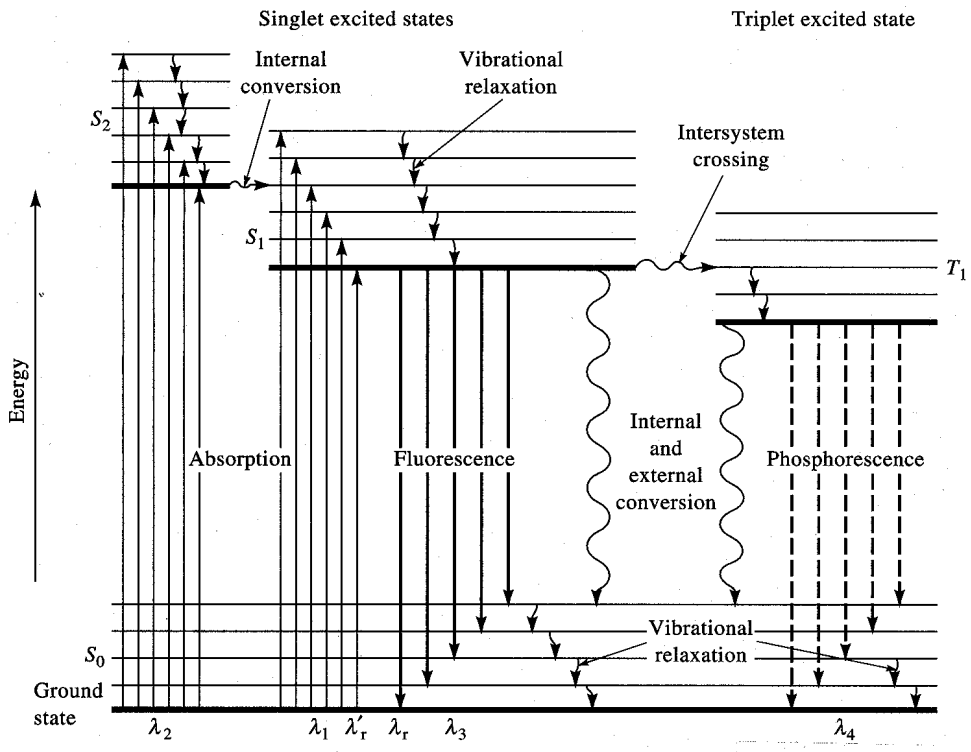
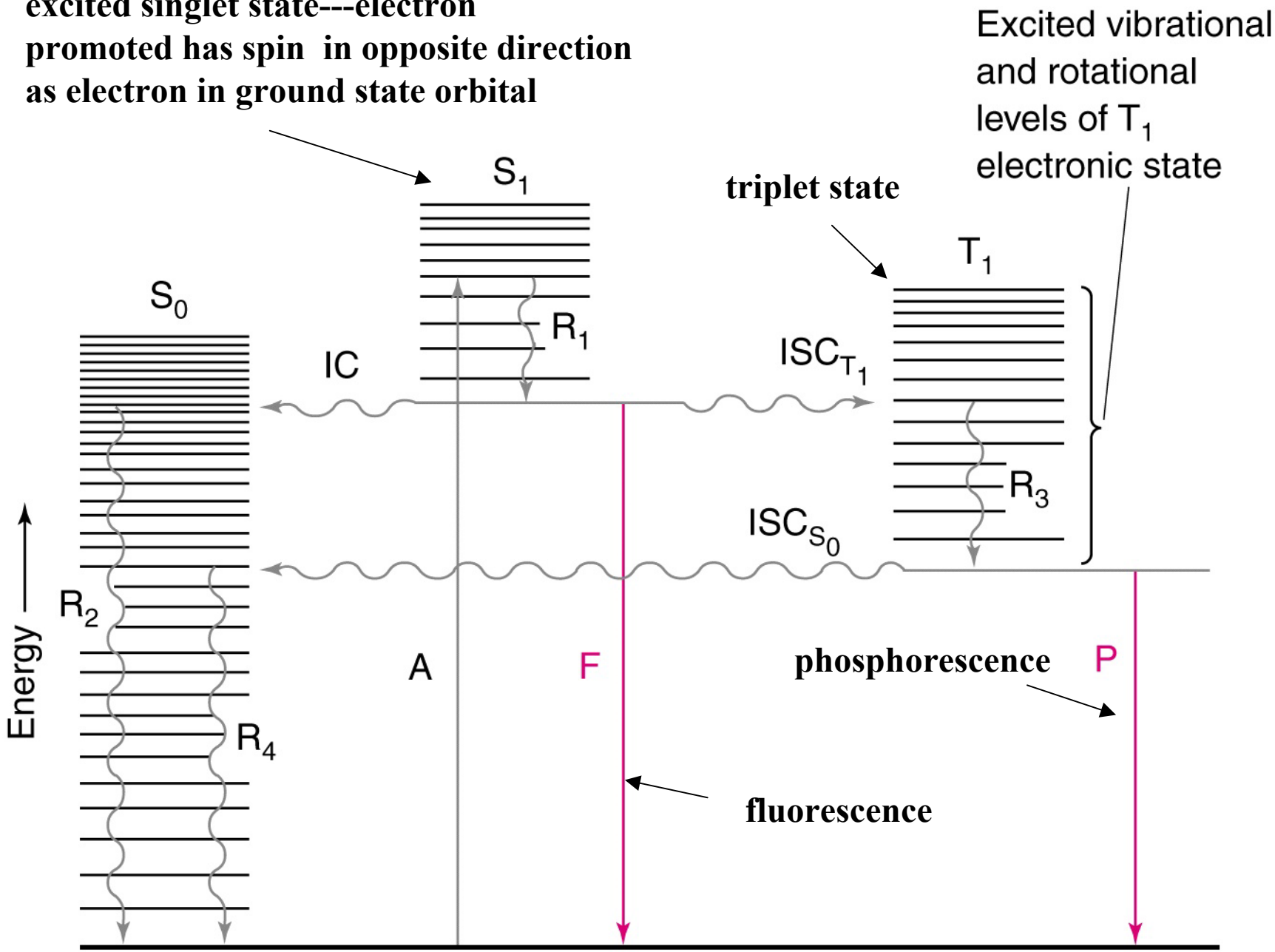
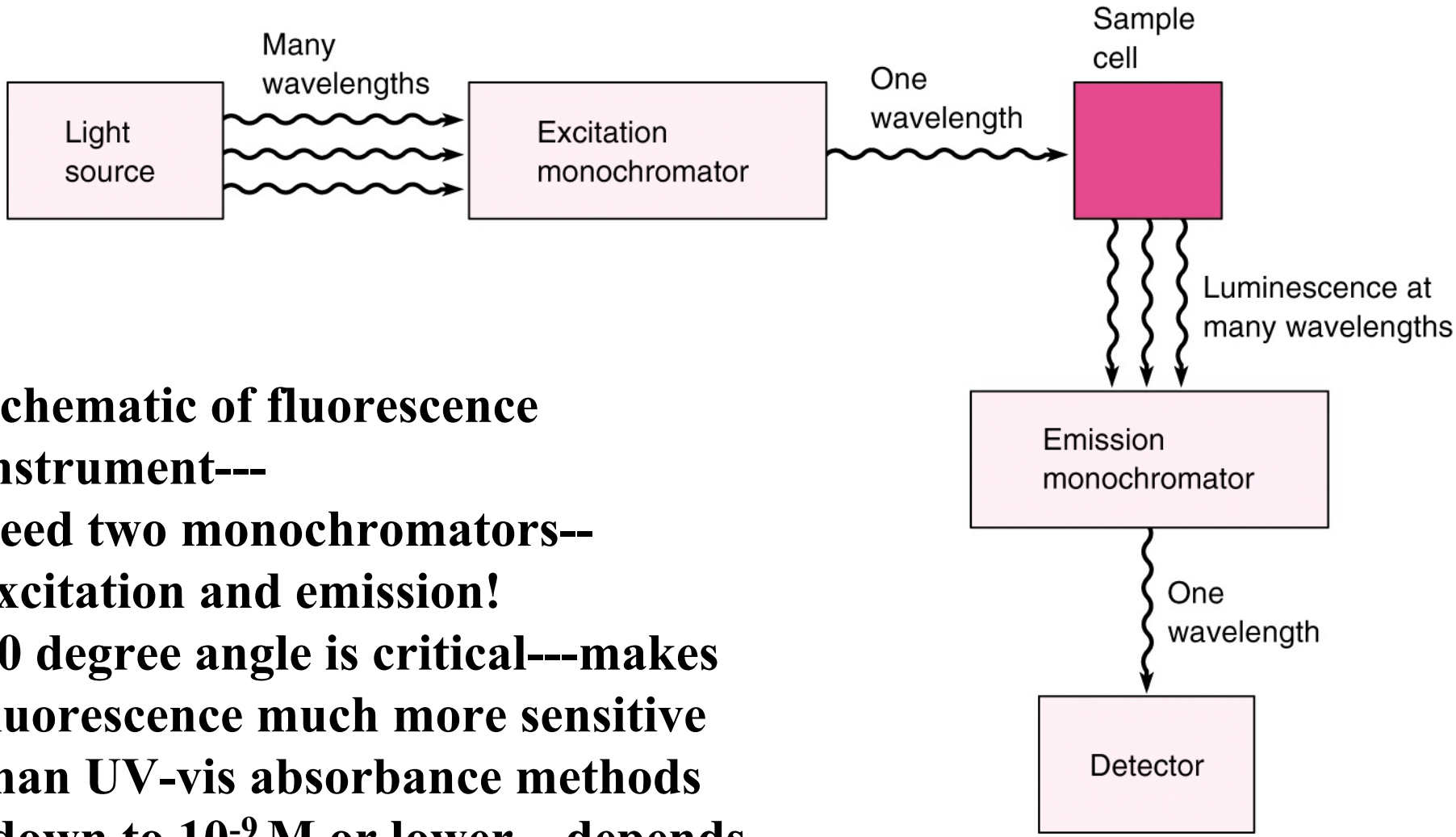


Figure 15-1 Partial energy diagram for a photoluminescent system.

<u>Quantum Yield</u>---# photons emitted as fluorescence--divided by # of photons absorbed--
the most fluorescing species have Q near 1.


excited singlet state---electron promoted has spin in opposite direction as electron in ground state orbital
Excited vibrational and rotational levels of T_1 electronic state
triplet state
S_1
T_1
S_0
R_1
IC
ISC_{T_1}
R_3
ISC_{S_0}
R_2
Energy
A
F
phosphorescence
P
R_4
fluorescence



Schematic of fluorescence instrument---
need two monochromators--excitation and emission!
90 degree angle is critical---makes fluorescence much more sensitive than UV-vis absorbance methods (down to 10^{-9} M or lower---depends on intensity of source--and quantum yield of molecule (analyte)
(measuring signal on top of nearly zero background--not like UV-vis)

Fluorescence Red-Shift

- Vibrational and non-radiative energy losses reduce the energy of the photons released from the molecule.
- The red-shift of fluorescence can be very useful.
- No background from electrically scattered photons (source)
- finger-print the molecule, since few molecules are likely to have the same λ_{max} absorption and λ_{max} fluorescence

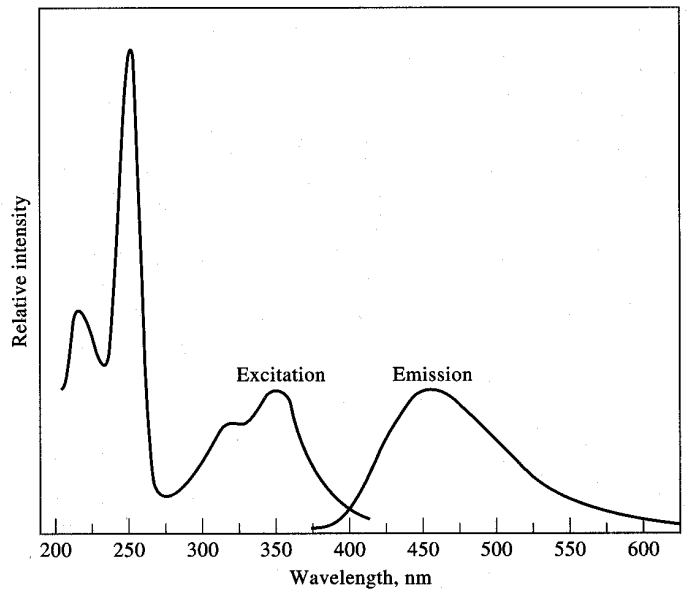
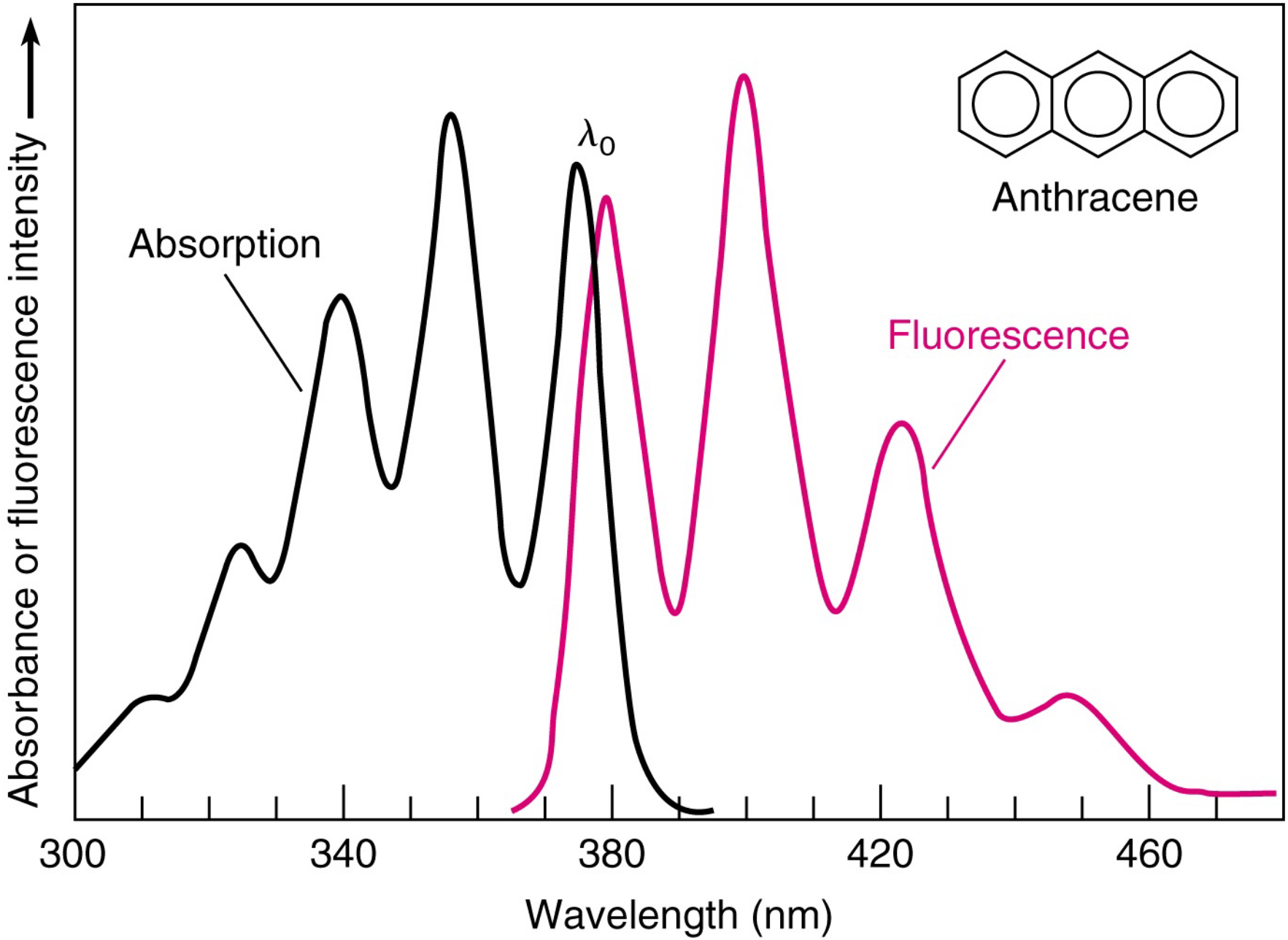
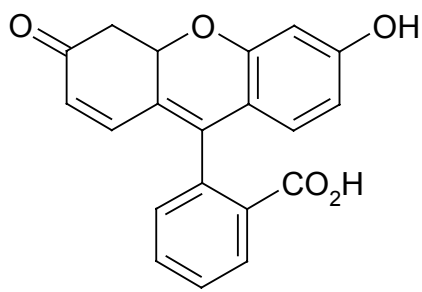


Figure 15-2 Fluorescence excitation and emission spectra for a solution of quinine.

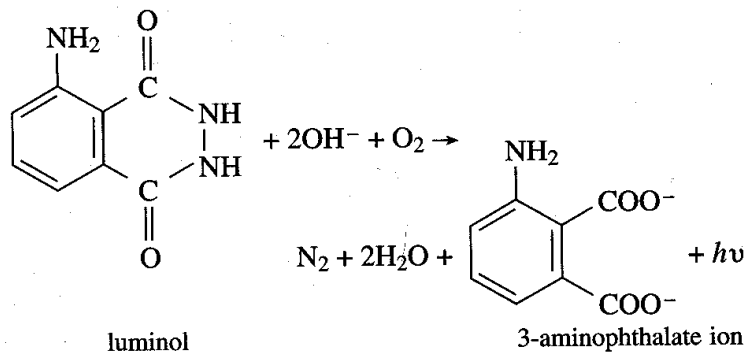

Absorbance or fluorescence intensity
Absorption
λ_0
Fluorescence
Anthracene
300
340
380
420
460
Wavelength (nm)

Applications of Fluorescence-- and also other luminescence methods

- Most applications are quantitative
- most exploit the verylow detection limits
- fluorescence quenching
  - powerful indirect method
- <u>chemluminescence:</u> light emitted as a side-product of a chemical reaction
  - does not require a light source
  - great potential for in vivo, or remote applications



fluorescein a common indicator



luminol

3-aminophthalate ion

$NO + O_3$ ----> $NO_2^* + O_2$

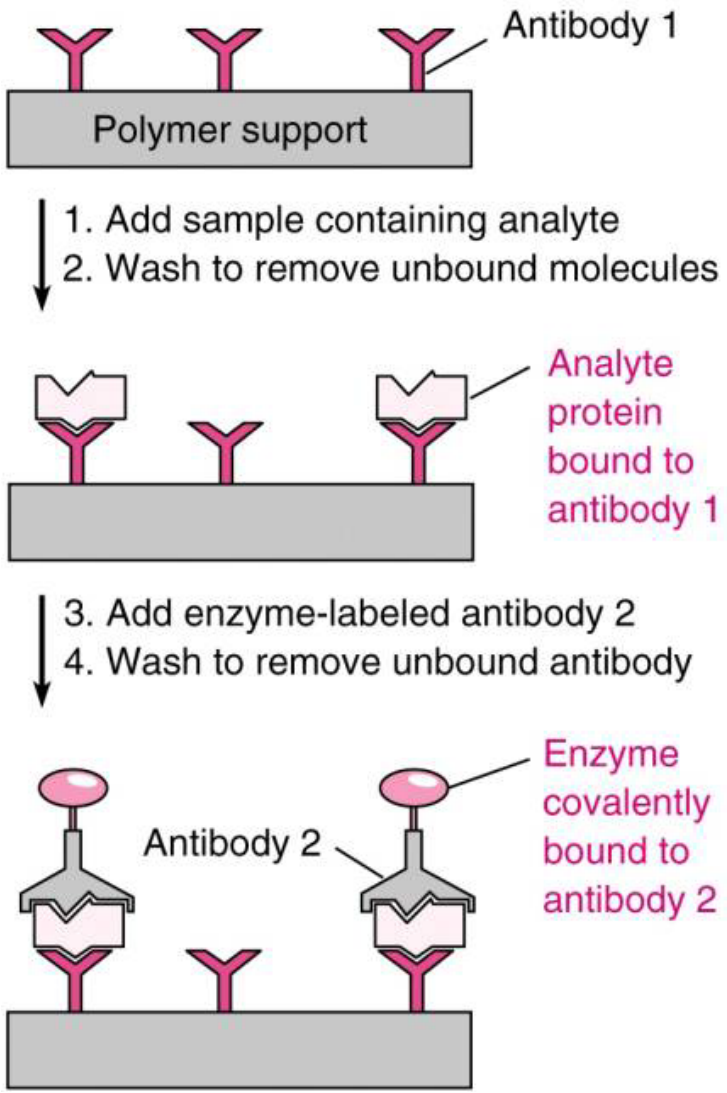
excited state species

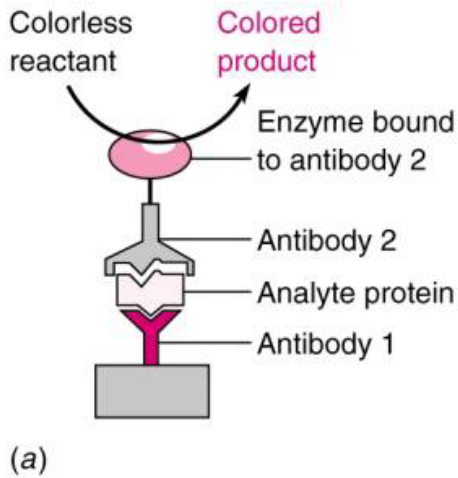
$NO_2 + h\nu$

Fluorescence Probes for Single Cell or Single Molecule Applications

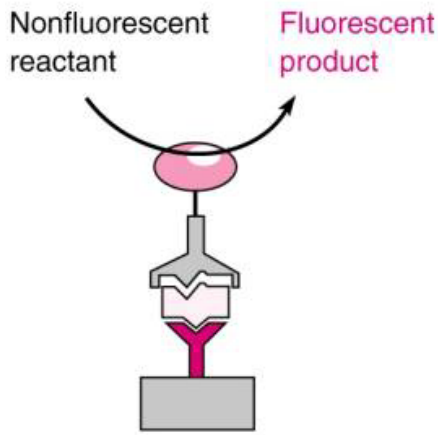
- Rare earth, transition metal chelate complexes
  - Advantages: – indestructible – high ε. – long excited state
  - Disadvantages: – long excited state – can be toxic
    - fewer excitation/emission cycles/sec
- Polymer bead encapsulated metal chelates
  PEBBLES (Kopelman, UM)
- Quantum dots - submicron semiconductor particles: Cd/Se
  (Alivosatos, UC Berkeley; Nie, Indiana)
  - Advantages: – indestructible – high density (sensitivity) – low toxicity
  - Disadvantages: not applicable to single molecule applications

Immunoassays use Fluorescence





(*a*)



(*b*)