Spectroscopy: Introduction

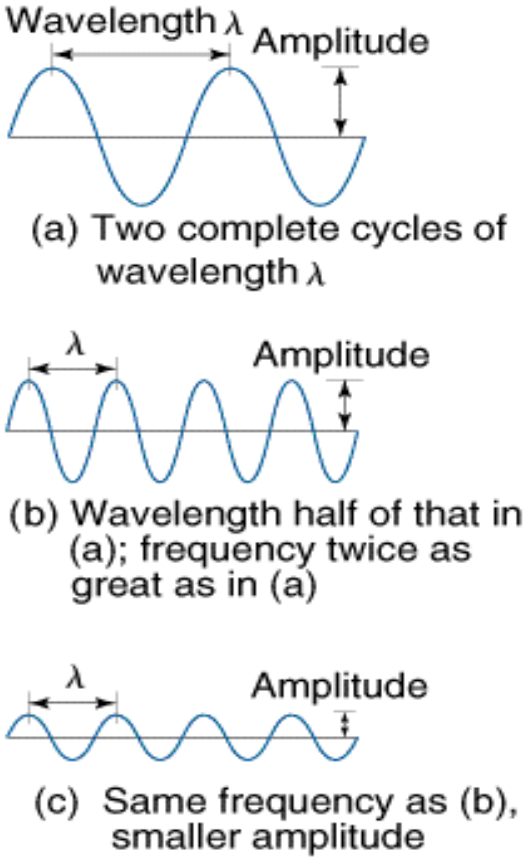
Required reading

Chapter 18 (pages 378-397)

Chapter 20 (pages 424-449)

Spectrophotometry is any procedure that uses light to measure chemical concentrations

Properties of Light



electromagnetic radiation – energy that moves by means of oscillating, perpendicular electric and magnetic fields. "Visible Light" is one small part of electromagnetic radiation.

Wavelength (λ) – distance between successive crests of a wave

Frequency (ν) – number of wave crests passing a point in space per unit time (second)

one **hertz** is one cycle per second (1/s)

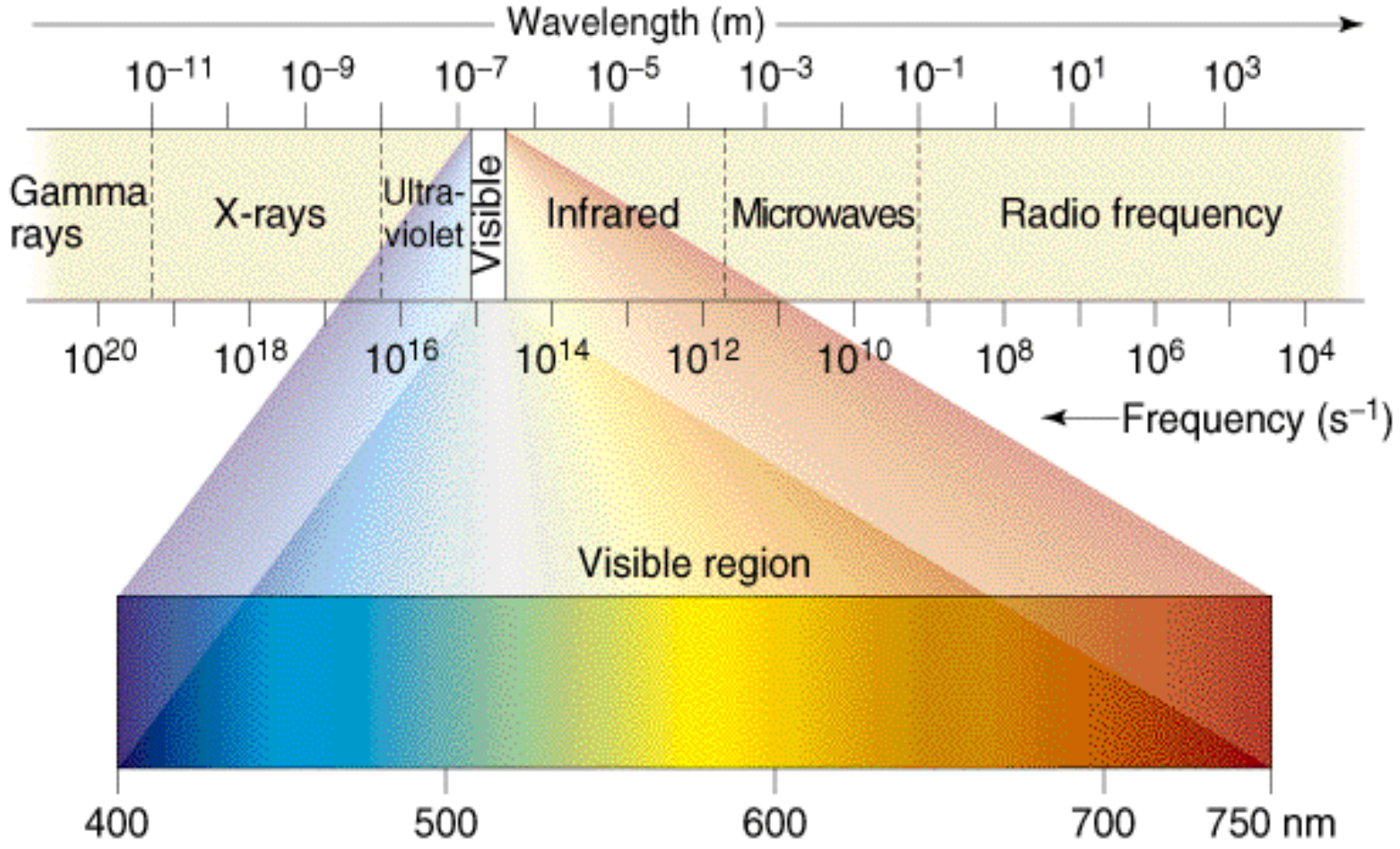
The wavelength and frequency of light are related:

$$\lambda \times \nu = c$$

(c = speed of light in vacuum = 2.998×10^8 m/s)

Regions of the Electromagnetic Spectrum

Frequency	Wavelength	Energy	Name	Uses
10^{20} to 10^{21}	10^{-12}	Nuclear	Gamma Rays	Cancer treatment
10^{17} to 10^{19}	10^{-10}	Electronic	X rays	imaging
10^{15} to 10^{16}	10^{-7}	Electronic	Ultraviolet	Black Lights
10^{13} to 10^{14}	10^{-6}	Electronic	Visible	Illumination
10^{12} to 10^{13}	10^{-4}	Vibrational	Infrared	Heating
10^{9} to 10^{11}	10^{-2}	Rotational	Microwave	Cooking
10^{5} to 10^{8}	10^{2}		Radio	Signal Transmission


Wavelength (m)
10^{-11}
10^{-9}
10^{-7}
10^{-5}
10^{-3}
10^{-1}
10^{1}
10^{3}
Gamma rays
X-rays
Ultra-violet
Visible
Infrared
Microwaves
Radio frequency
10^{20}
10^{18}
10^{16}
10^{14}
10^{12}
10^{10}
10^{8}
10^{6}
10^{4}
Frequency (s^{-1})
Visible region
400
500
600
700
750 nm

photon – individual bundles of energy; each photon has an energy that is directly proportional to the frequency:

$$E_{photon} = h\,\nu_{photon}$$ (h = 6.626 x 10^{-34} J s)

Often, we like to express the frequency in terms of inverse wavelength,

$$E = h\ \nu = h\ \mathrm{c}/\lambda = h\ \mathrm{c}\ \sigma$$

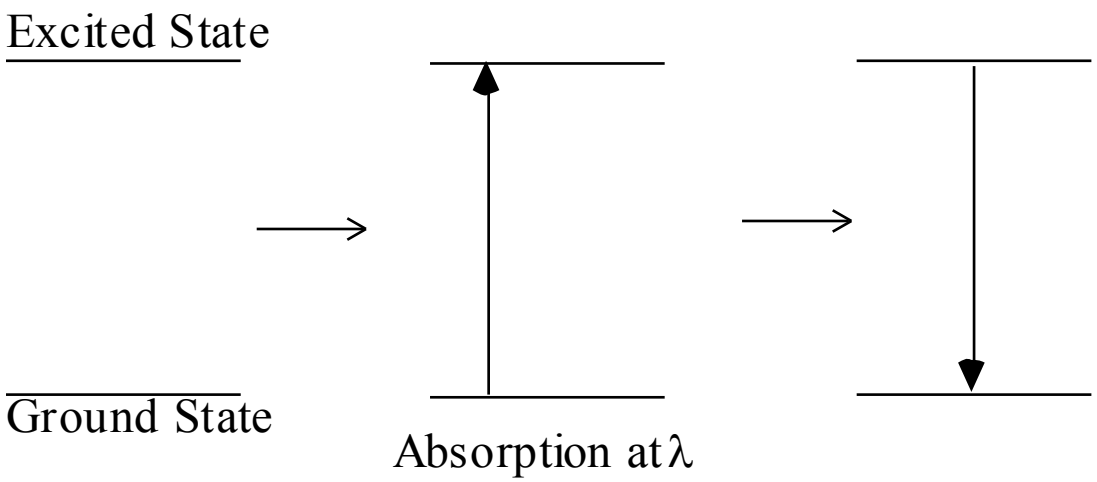
where **σ** is called the **wavenumber**. We will use this in infrared spectrometry.

Light of various *wavelengths* or *energies* does different things with matter:

The absorption of light by a *molecule* causes an increase in the energy of the molecule.

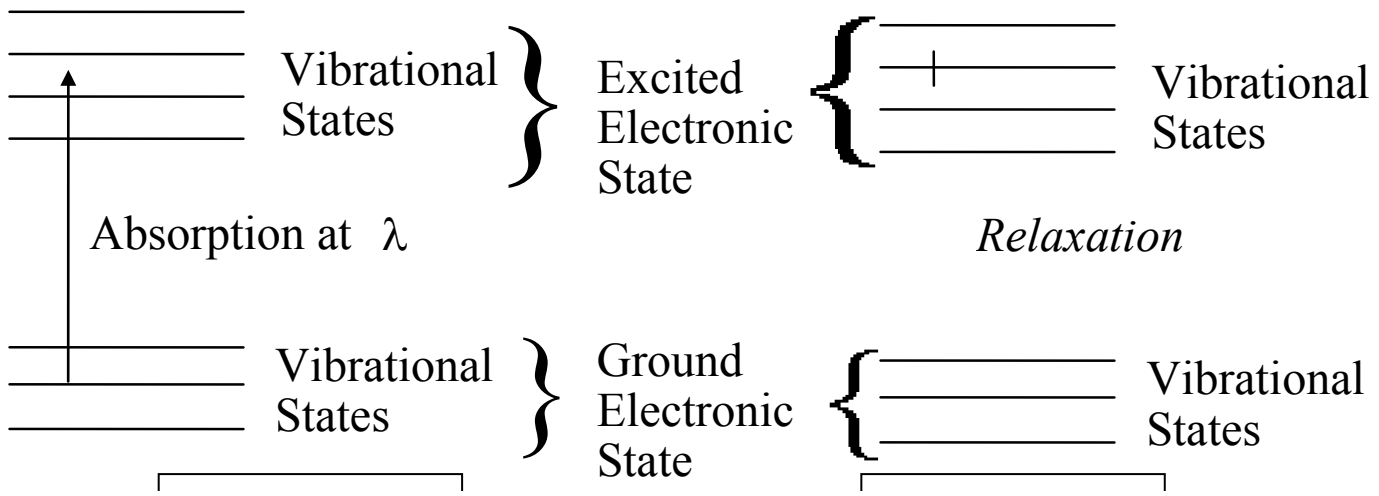
This may be in terms of an increase in vibrational, rotational, electronic or other types of molecular energy states.

Typically, in the *UV-Vis region*, we will disturb *electronic states* by the absorption of light in the range of 200-800 nm.

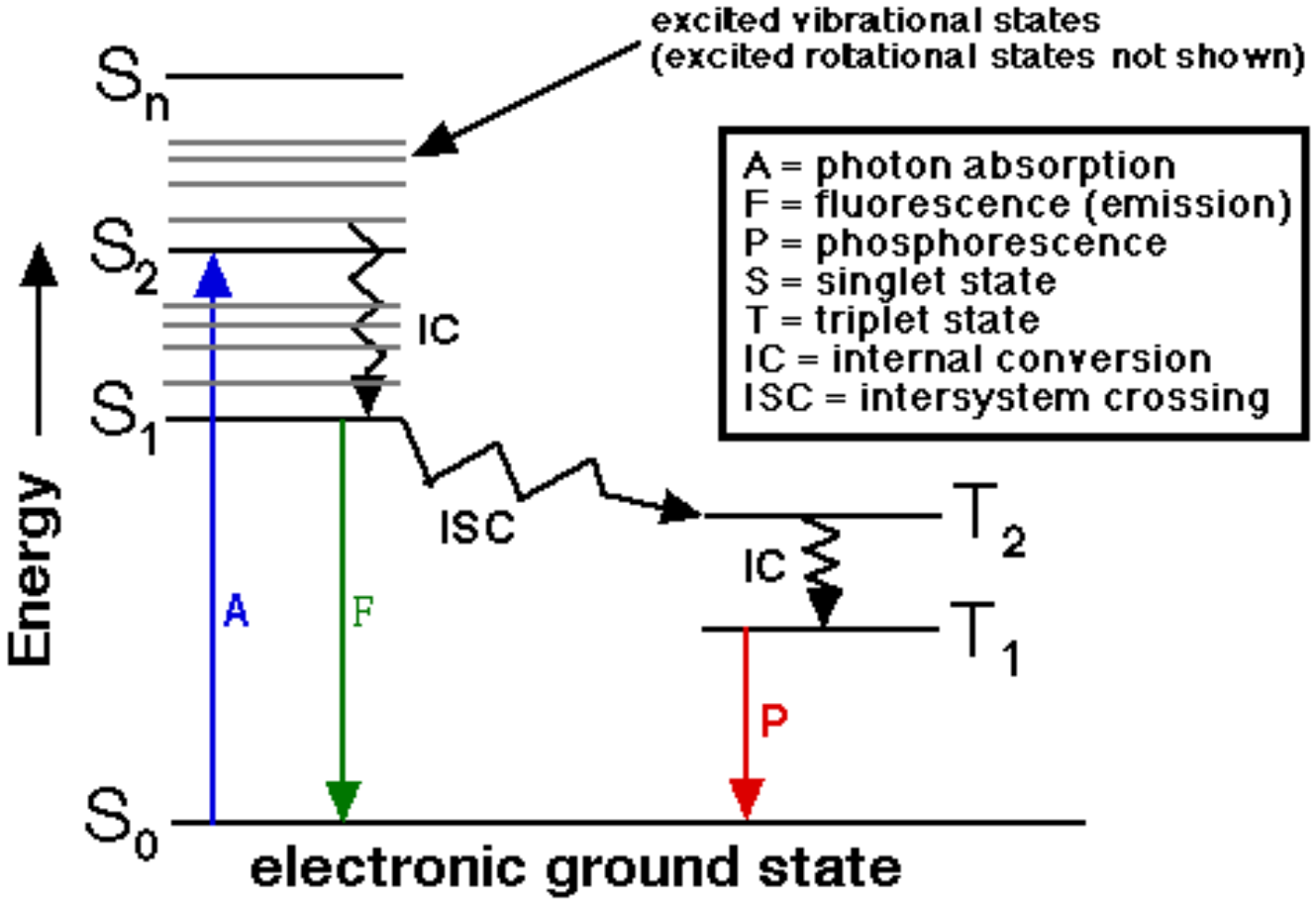


- If a molecule absorbs energy from a photon, then an electron can be promoted to a higher energy level in that molecule.
- That electron originally "resided" in a *molecular orbital* of the molecule which is at a relatively low energy, we call that the GROUND STATE.
- The new place of residence for the excited electron is a higher energy molecular orbital.
- The excited energy level of the molecule has not only an electronic energy level associated with it, but also *vibrational* and *rotational energy levels*.

- Basically, we have to dissipate the quantized energy in the different vibrational, rotational and electronic states of the molecule.



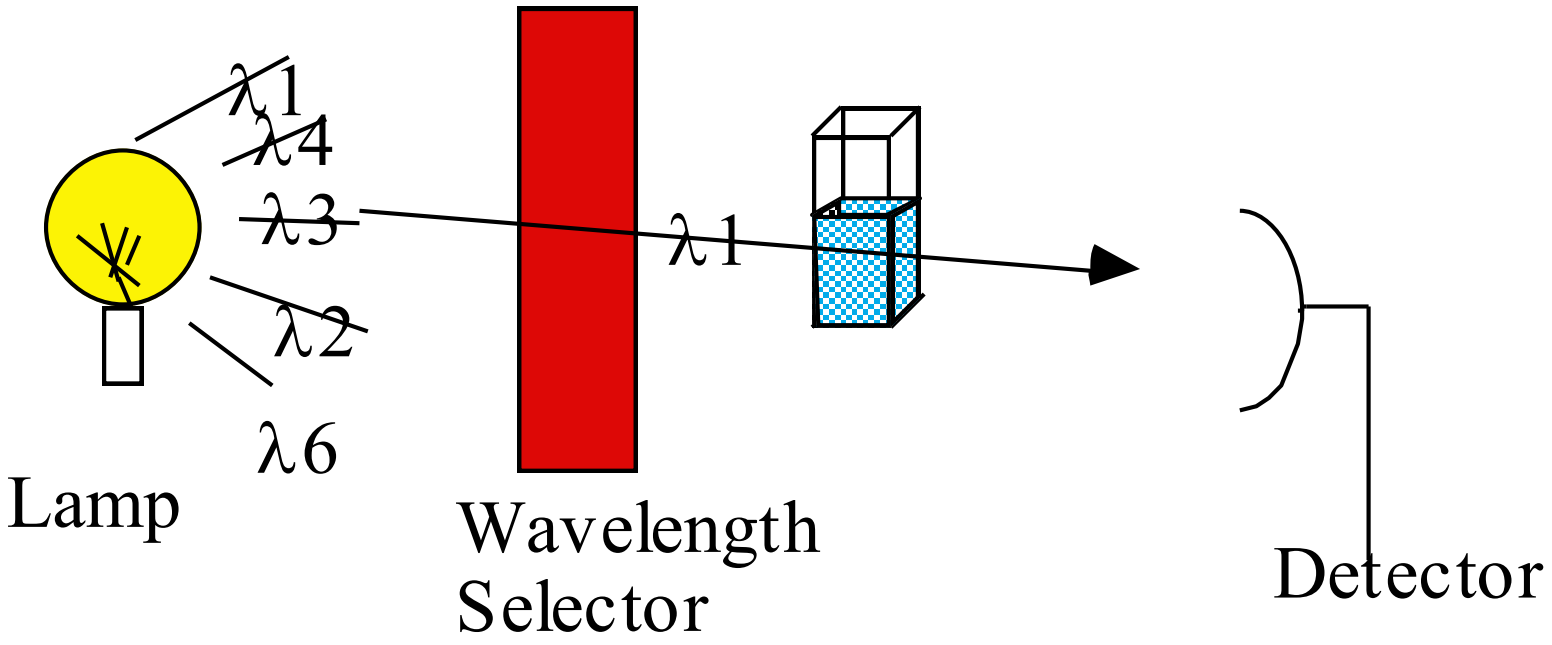
- So, the absorbed energy can be dissipated in a lot of different ways, as shown in the *JABLONSKI DIAGRAM* below:



- Fluorescence occurs on a timescale of about 10^{-6} sec.

- Phosphorescence is much slower, about 1 to 10^{-6} sec. This is because the electron has undergone spin inversion. Remember the Pauli Exclusion Principle.

- What if we have a sample holder known as a *cuvette* in the path of Electromagnetic radiation of some energy:



If the incident light has an incident power, P_o, and the exiting light has intensity, P, then the transmittance through the path length b (cm) of the sample is:

$$T = P/P_o \qquad \text{(Range of 0 to 1)}$$

The ***absorbance*** at that wavelength is:

$$A(\lambda) = -\log(P/Po) = -\log(T)$$

If we do some calculus, we come up with the *Beer-Lambert Law*:

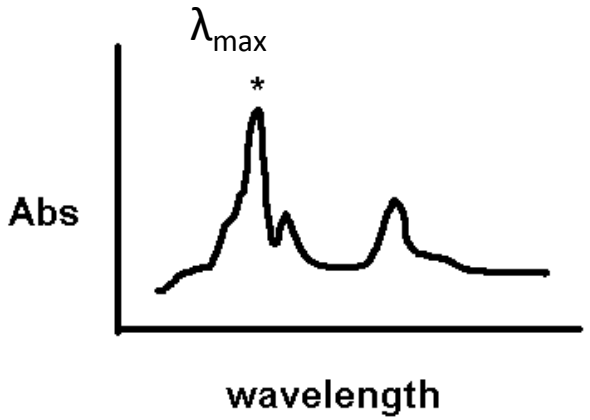
$$A(\lambda) = \varepsilon_\lambda \, b \, C \quad \text{(dimensionless)}$$

Beer's Law will deviate at high concentrations of analyte.

Solvent is chemically affected by high concentrations of analyte, thus impacting ε

The distribution of analyte components as a result of equilibria events is drastically affected by high analyte concentrations

An absorption spectrum is a graph showing how A varies with λ.



Absorption Spectrum

Note: Any substance that absorbs visible light will appear colored when white light is transmitted through it or reflected from it. The substance absorbs certain wavelengths of the white light, and our eyes detect the wavelengths that are not absorbed. The observed color is called the complement of the absorbed color.

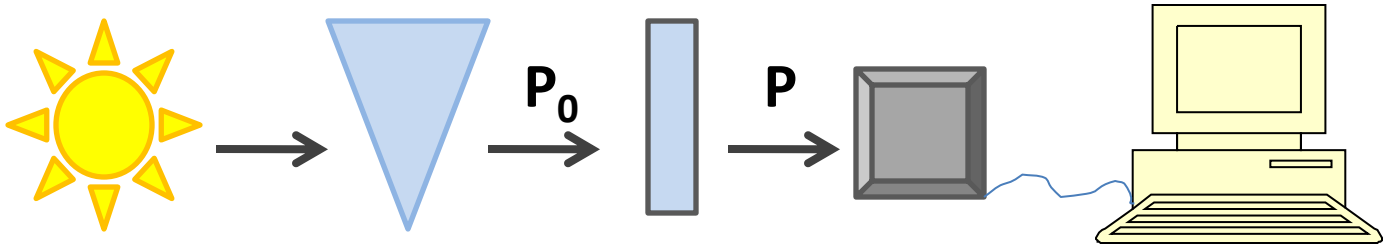
The Absorption Spectrophotometer

- Light from a continuous source is passed through a **monochromator**.
- This monochromatic light travels through a sample of pathlength *b*, and the radiant power of the emergent light is measured.
- Visible light generated from a quartz halogen lamp. UV light generated from a deuterium arc lamp.
- **cuvette** – sample cell that has flat quartz faces. Quartz transmits both UV and visible light – plastic or glass only transmits visible light.

Single-beam Spectrophotometer

First measure *Po* for a reference cuvette containing pure solvent. Then replace reference cuvette (containing the blank) with cuvette containing sample to measure *P*.

Block Diagram of Single-beam Spectrophotometer



Inconvenient, somewhat inaccurate, poorly suited to continuous measurements

Double-beam Spectrophotometer

Light passes alternately through the reference and sample cuvettes. A **chopper** is a mirror that rotates in and out of the light path diverting the light between the reference and sample cuvettes.

Routine procedure is to first record a baseline spectrum with two reference cuvettes. The absorbance of the reference is then subtracted from the absorbance of the sample to obtain the "true" absorbance at each wavelength.

Double-beam Spectrophotometer

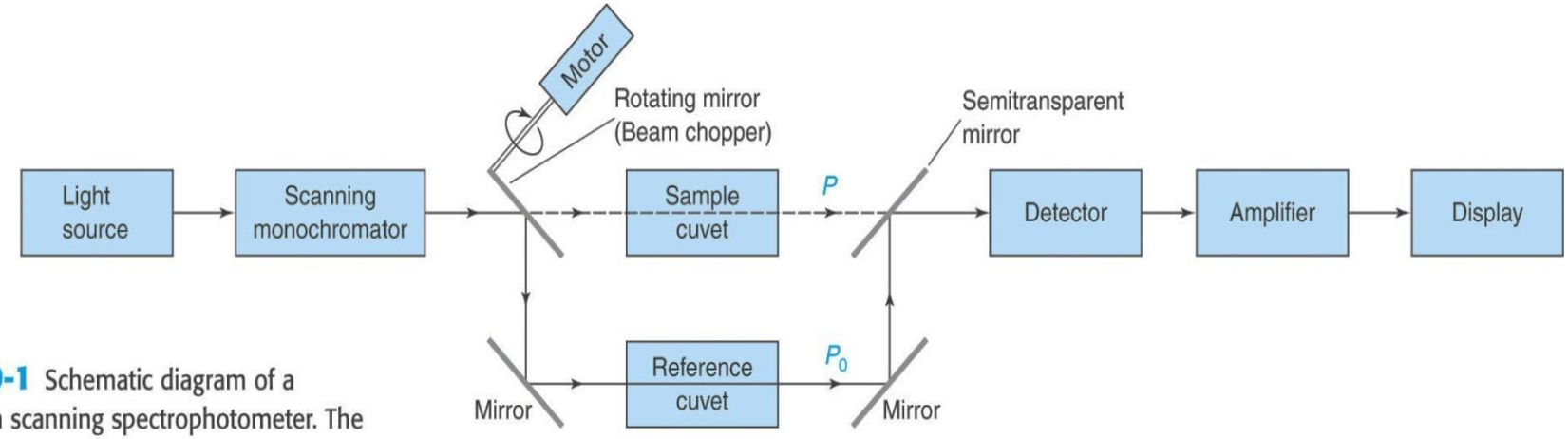


FIGURE 19-1 Schematic diagram of a double-beam scanning spectrophotometer. The incident beam is passed alternately through the sample and reference cuvets by the rotating beam chopper.

Precautions

- Most spectrophotometers operate best at values for $A \approx 0.4 - 0.9$
- If absorbance too high, intensity is hard to measure.
- If absorbance too low, hard to distinguish between reference and sample
- **Slight mismatches between reference and sample cuvettes leads to systematic error**
- Samples must be dust free. Cuvettes must be wiped clean before use.

Light Sources

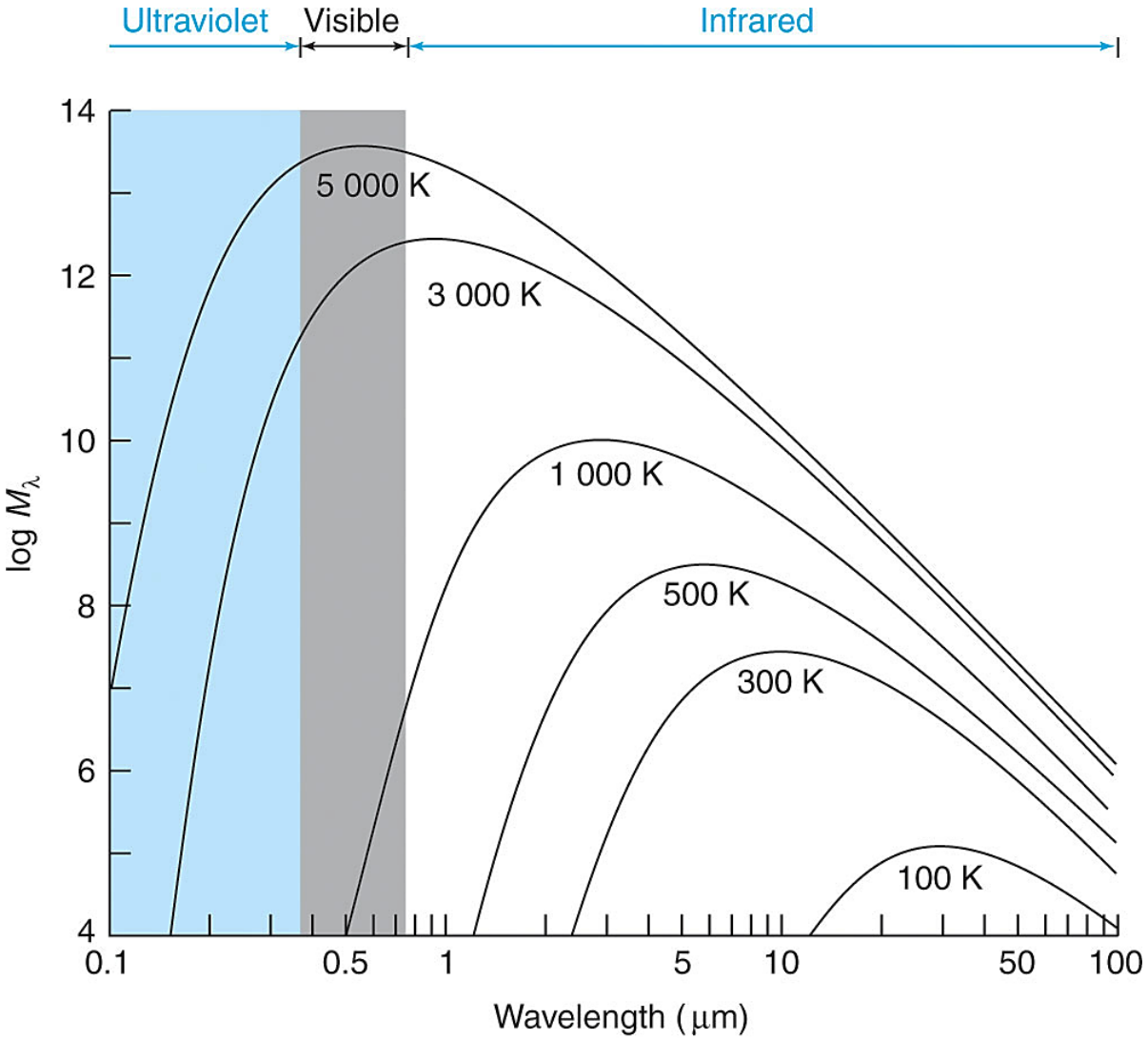
Any object that is heated emits **radiation**. Emission from real objects such as a tungsten filament light bulb emulate blackbody radiation (the emission is a continuous spectral distribution).

Visible and infrared lamps as light sources approach blackbody radiators. The radiation from an object's surface expressed as power per unit area is the exitance (emittance), *M.*

$$M = \sigma T^4$$

Where σ is the Stefan-Boltzmann constant ~ 5.7 X 10^{-8} W/(m^2K^4)
T = Temperature (K)

Spectral Distribution of Blackbody Radiation



Lamps for Absorption Spectrometers

Lamps are broad-band, polychromatic light sources. Typically they are inexpensive and stable.

i) Visible and Near Infrared: Tungsten Lamp

ii) Ultraviolet and Visible: Quartz Halogen Lamp

iii) Ultraviolet: Deuterium Arc Lamp

iv) Infrared: nichrome wire

Spectral Distribution of Different Light Sources

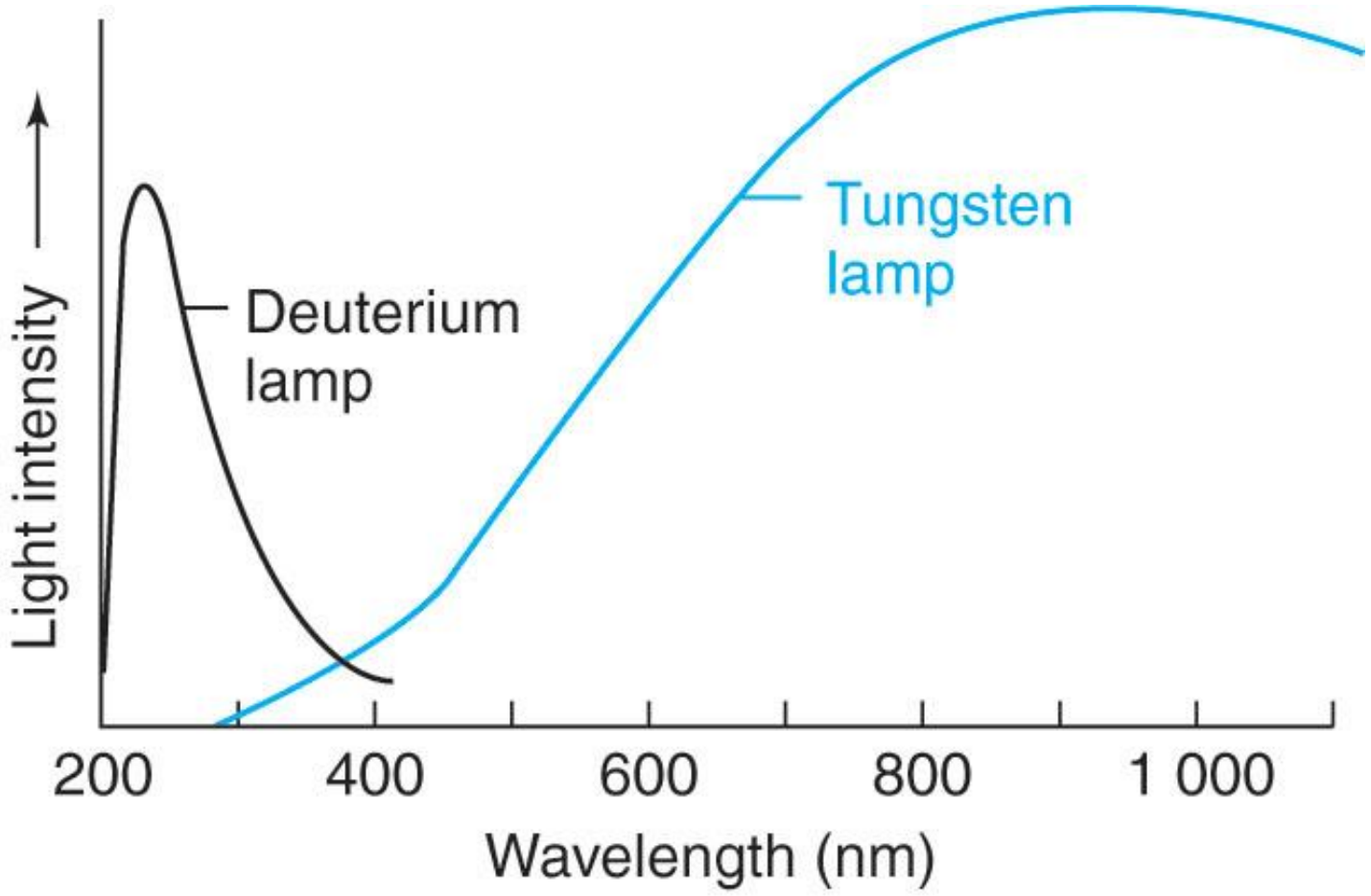


FIGURE 19-4 Intensities of a tungsten filament at 3 200 K and a deuterium lamp.

Lasers

Lasers provide ~ single λ

Very bright sources for spectroscopy

Properties of Lasers:

Monochromatic (only one wavelength)

Collimated (emit in one direction)

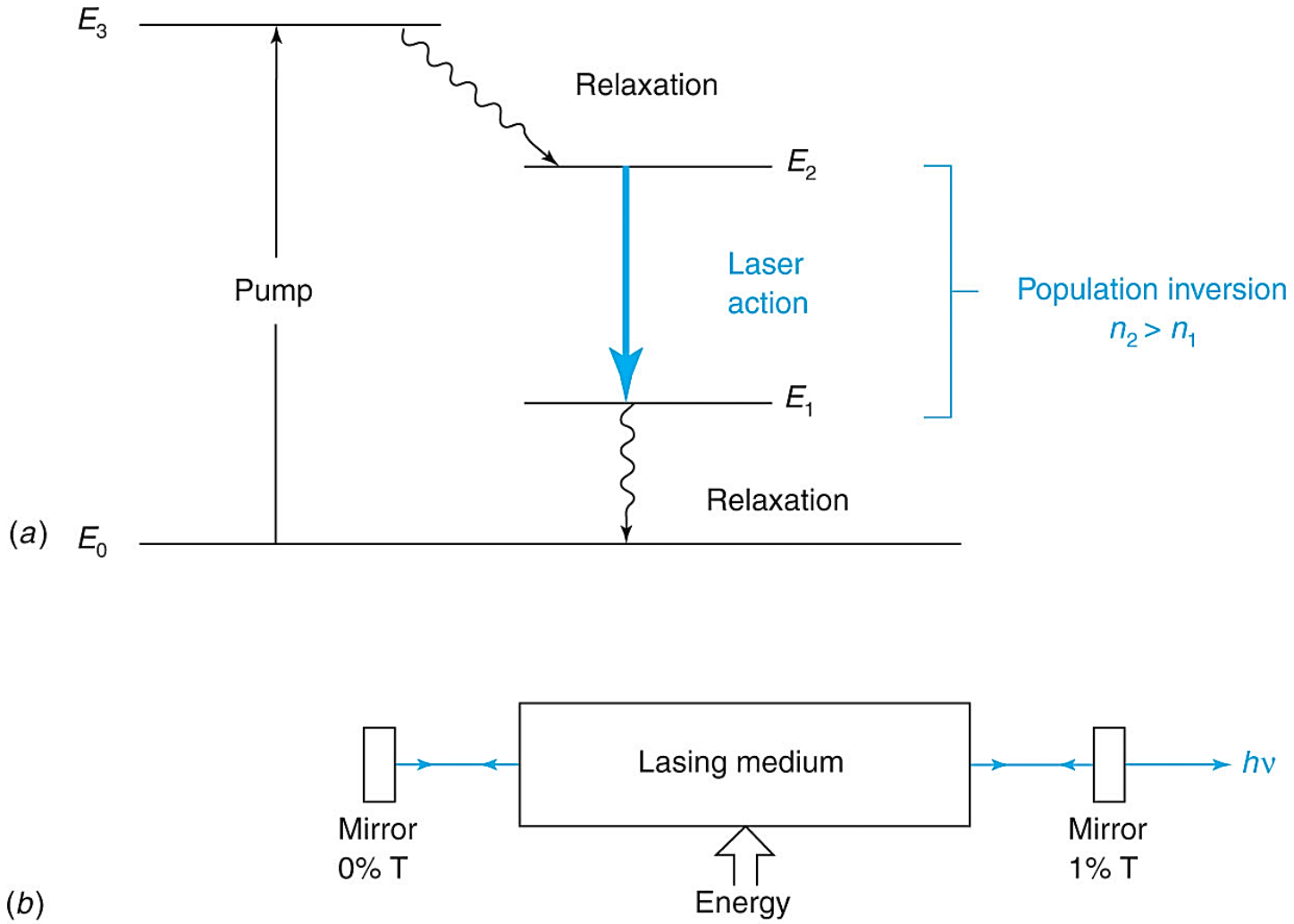
Polarized (only one electric field vector)

Coherent (electric/magnetic fields in phase)

expensive

high maintenance

Laser Operation Principles



Operation of a Laser

Population Inversion: A higher energy state has a greater population of electrons than a lower energy state

Lifetimes of electrons in various energy levels: E2 >> E3 or E1

Two popular lasers: helium-neon laser ~ red light

Laser diode ~ solid state, p-n junction ~ red, or near IR

Wavelength Selection Devices

- **Prisms** were used in older instruments, Quartz or salt crystals.
- **Monochromators** separate wavelengths of light; they consist of both entrance & exit slits, mirrors, and diffraction grating or refraction lens/prisms, and filters.

Grating–Monochromators

Polychromatic light is collimated (focused) into a beam of parallel rays by a concave mirror (monochromatic-one wavelength; polychromatic-many wavelengths).

Rays strike the reflection grating *(see next figure)* and different wavelengths are diffracted (separated) at different angles.

Diffracted light is focussed by a second concave mirror so that only one wavelength passes through the exit slit at a time.

Grating Equation:

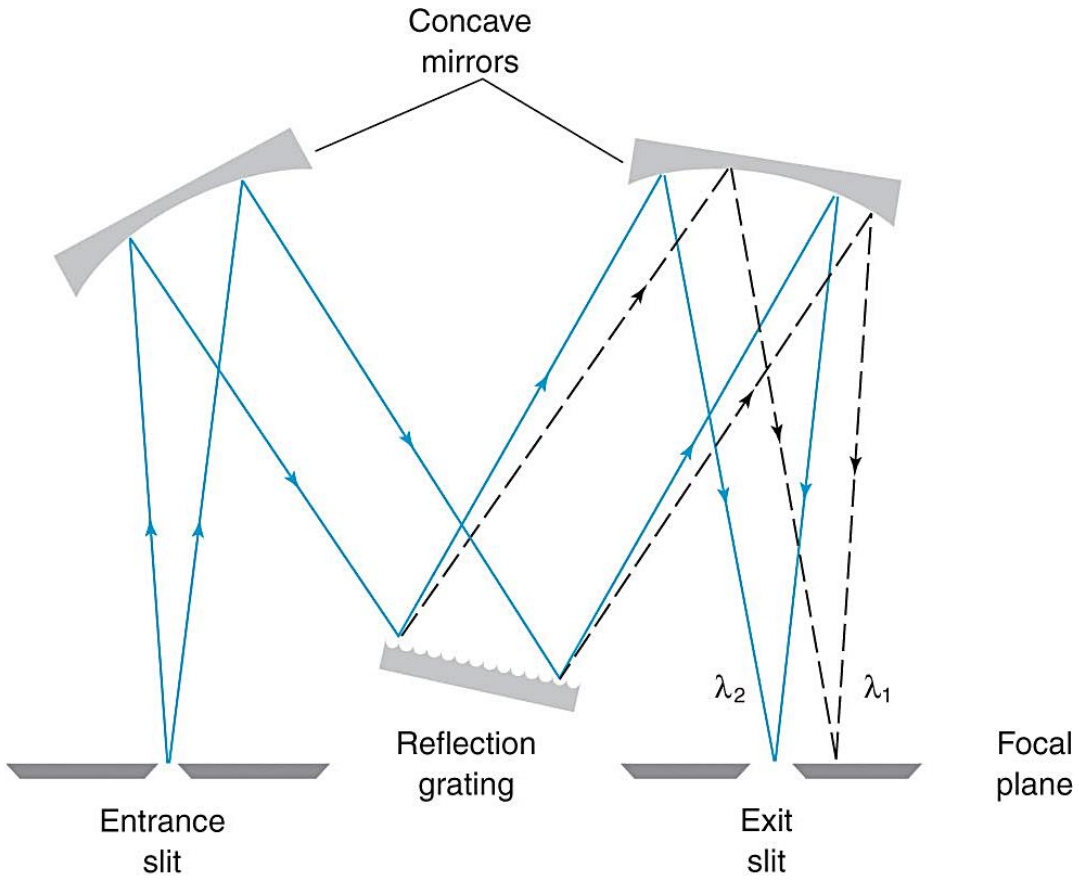
$n\lambda = d(\sin\theta - \sin\phi)$

n = diffraction order (1…n)

d = groove spacing

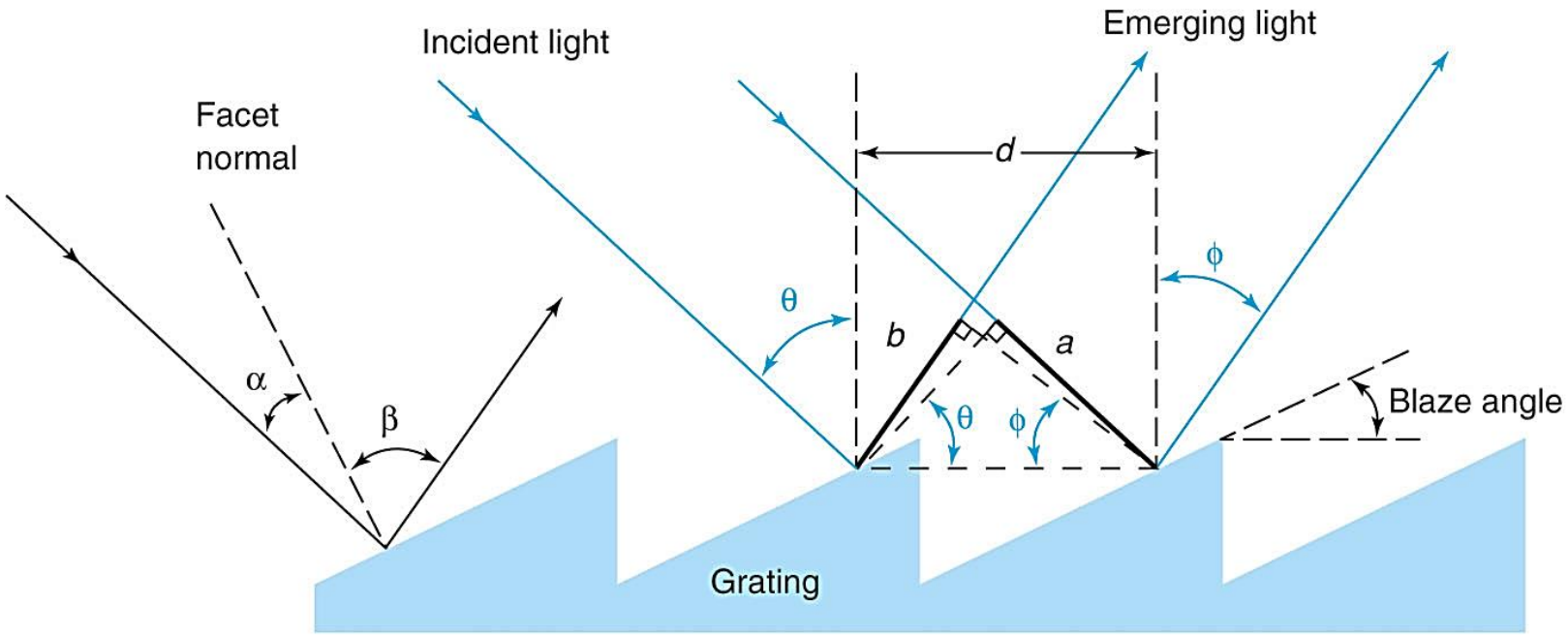
ϕ= angle of reflection

θ = angle of incidence



Components of a Grating Spectrophotometer

1) diffraction grating



A diffraction grating is ruled with a series of closely spaced parallel grooves separated by distance *d*.

These are often constructed from aluminum metal and coated with a non oxidative coating applied.

When light is reflected from the grating, each groove behaves as a source of light. When adjacent rays are in phase, they reinforce each other. When adjacent rays are out of phase, the partially or completely cancel each other. Thus can be aligned to allow only certain wavelengths to pass through.

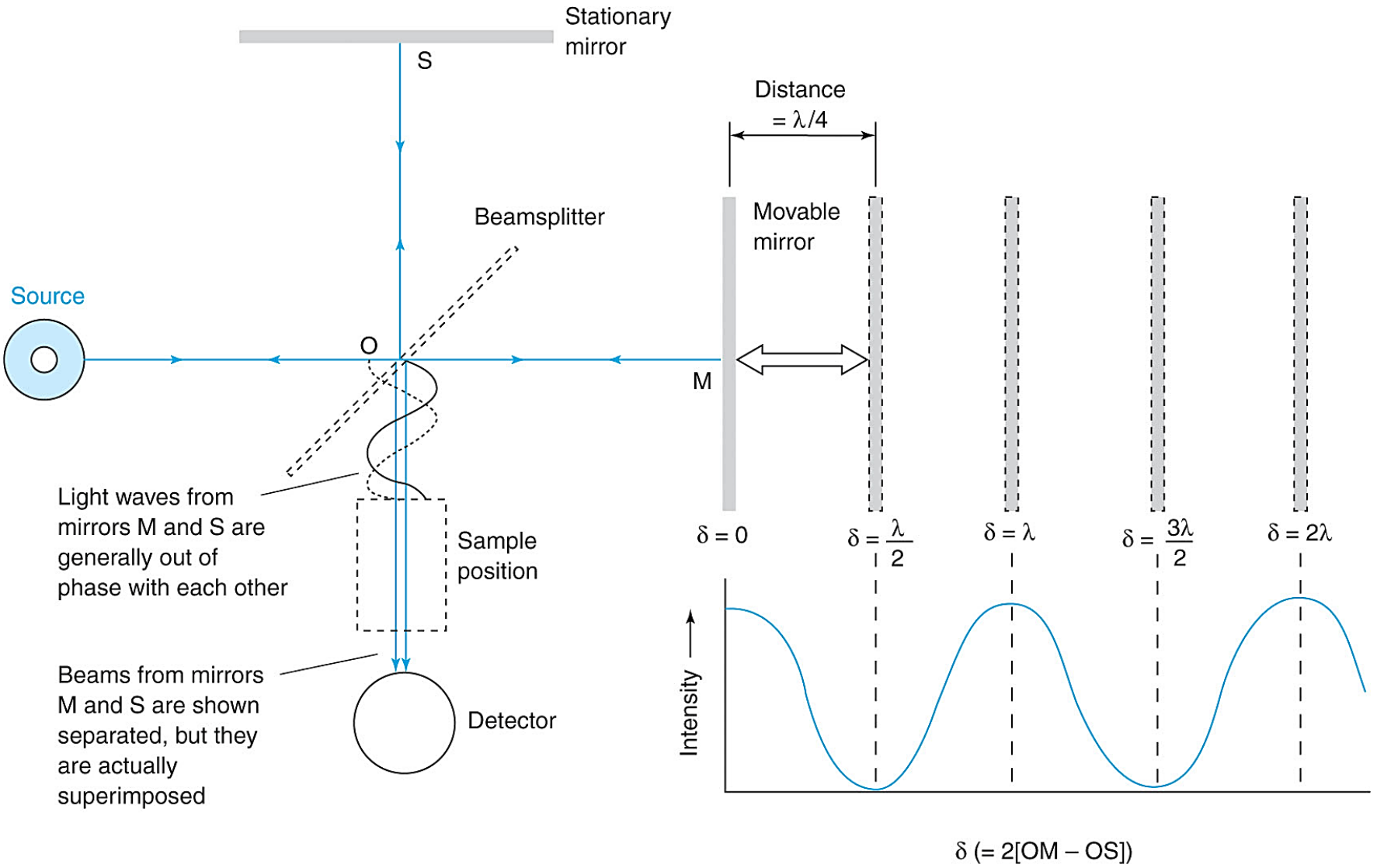
2) Slits

Slits are constructed by machining a sharp edge onto two metal pieces. These lie in a plane and the spacing between them, the slit width, can be adjusted. The smaller the slit width, the better the spectral resolution.

3) Filters:

Filters are used to pass on only desired wavelengths of light. A filter could be colored glass. Most likely they are also based on constructive or destructive interference of light waves.

4) **Interferometers -** alternative wavelength selection process

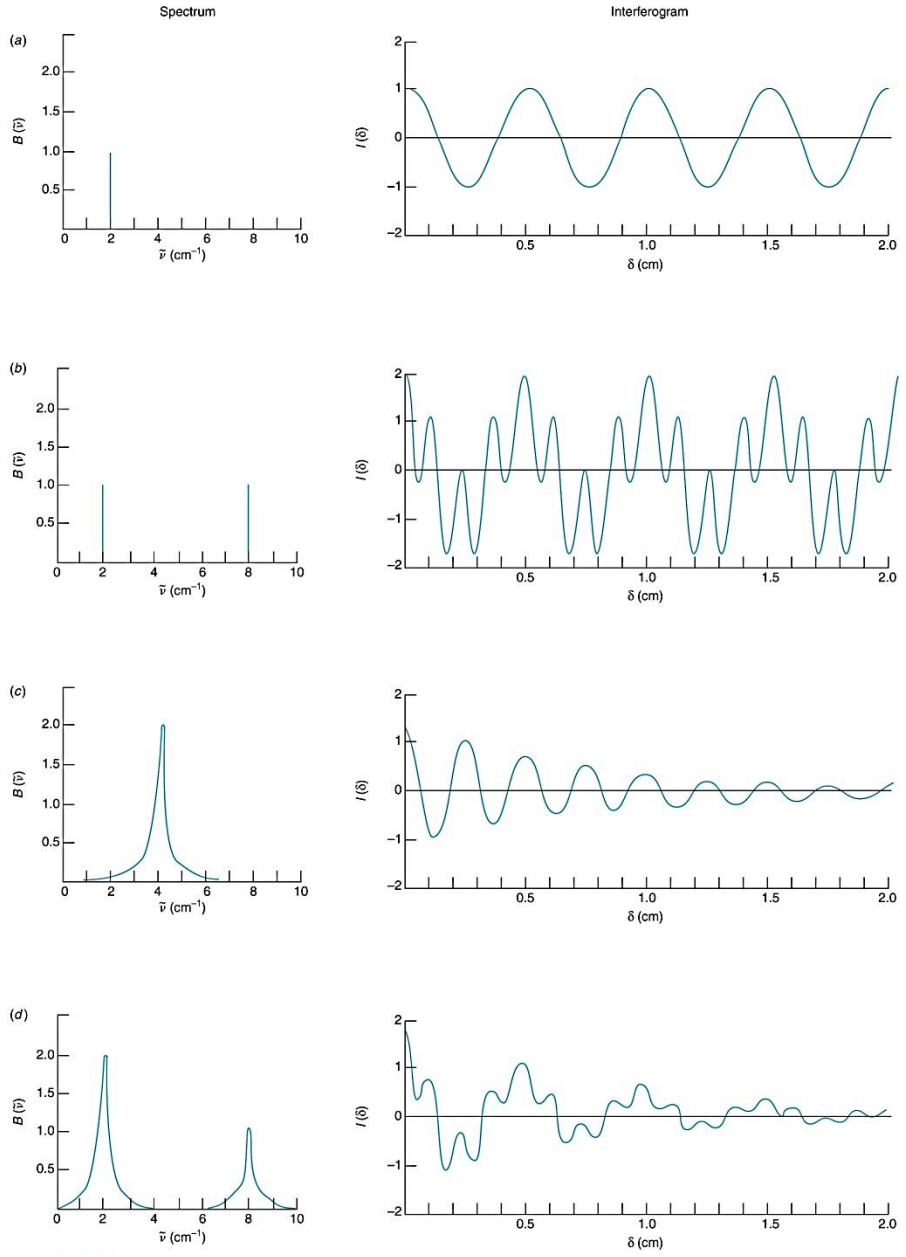


Interferometers

- allows all wavelengths to simultaneously reach the detector
- Radiation from source reaches beam splitter, where half of the radiation hits the moving mirror and half hits the fixed mirror.
- The beams reflect and re-combine, the emerging radiation for a wavelength exhibits constructive or destructive interference.

Interferometers

- With constant mirror velocity, the wavelength modulates in a regular sinusoidal manner.
- Both the sampling rate of radiation reaching the detector and the mirror velocity is modulated by a helium-neon laser.
- The resulting detector signal typically is stored as a time domain spectrum (interferogram).
- Converted to a spectrum in the frequency domain using the mathematical process of Fourier Transform.



Fourier Transform:

1) allows for signal averaging
2) allows all wavelengths to be monitored simultaneously
3) mathematical process that converts data obtained in the time domain to be converted into the frequency domain.

Fourier Transform

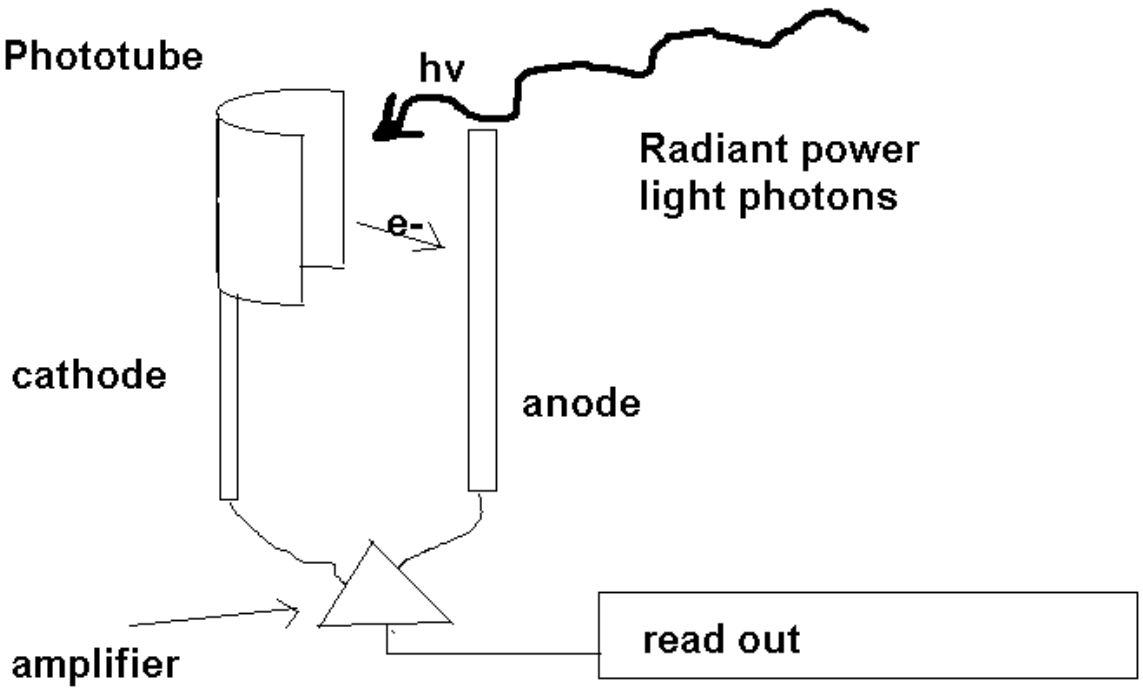
FT is used quite extensively in IR (infrared), mass spectroscopy and in NMR (nuclear magnetic resonance) applications.

In addition to IR spectroscopy, the use of Fourier transform has become quite common in certain spectroscopic methods due to the affordability of PCs.

Types of Detectors

A **detector** produces an electric signal when struck by photons.

Phototube detector



Phototube Detector

The phototube is used frequently as a detector in UV-Vis spectrometers.

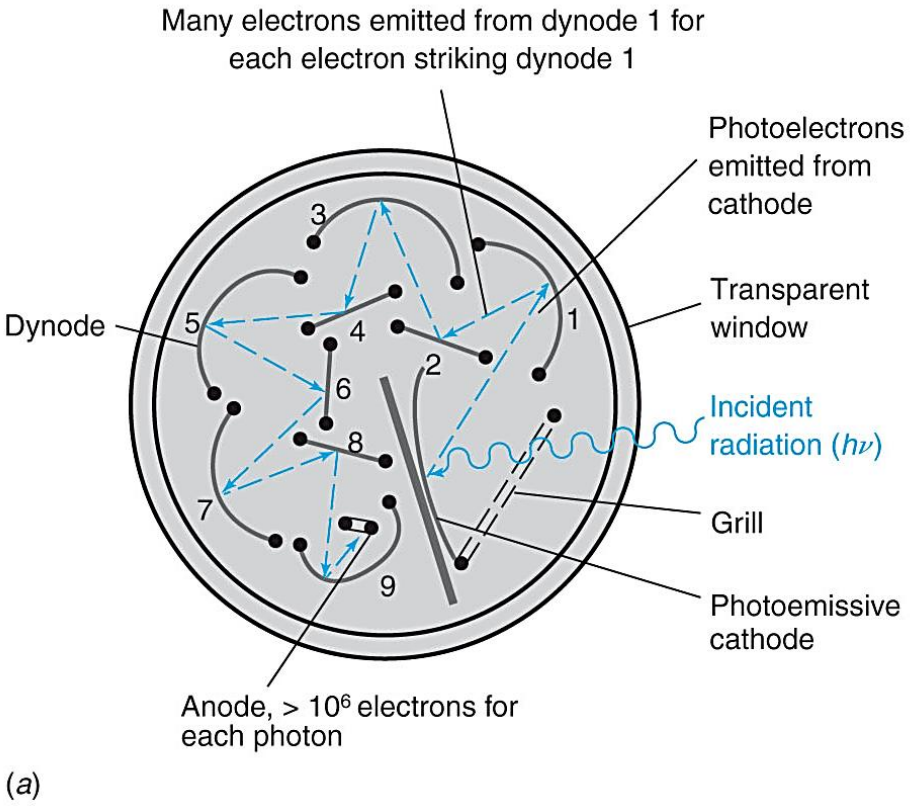
The cathode consists of a photo-emissive surface.

Electrons are ejected from the cathode proportional to the radiant power (photons) striking its surface.

The emitted electrons are attracted to the anode.

The accompanying voltage is fed to an amplifier and converted to a signal.

Photomultiplier Tubes

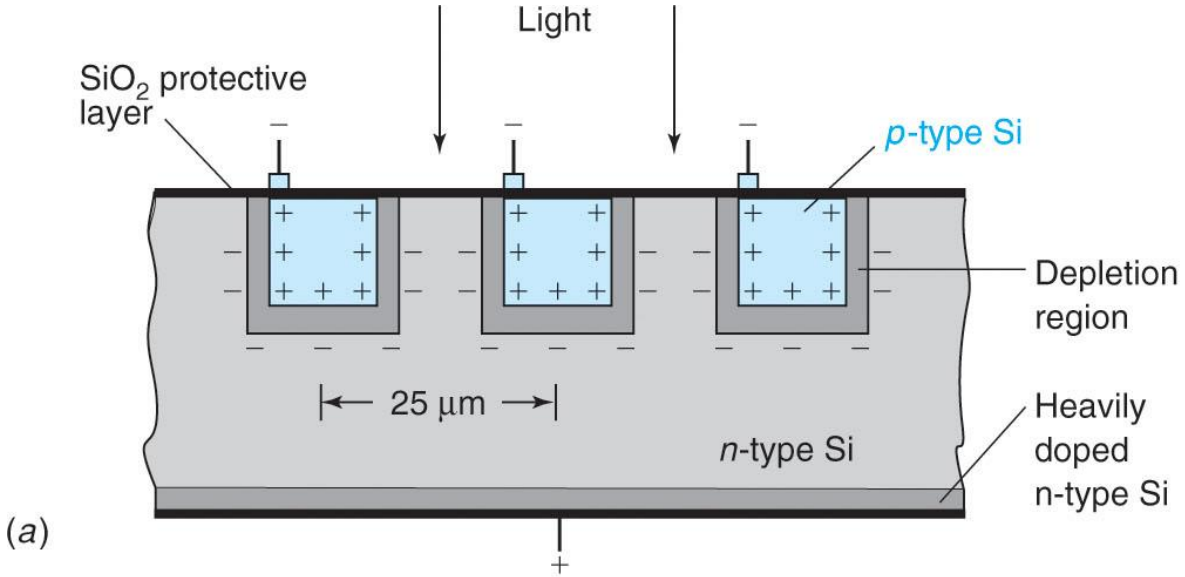


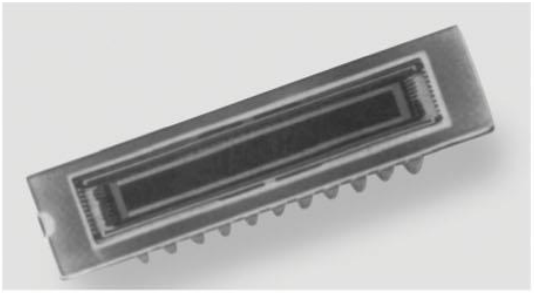
- The Photo Multiplier Tube, (PMT) is similar to the photo tube, but is a vast improvement.

Photomultiplier Tubes

- In addition to the cathode and anode, the PMT has dynodes, which produce a cascade effect on the electron emission production.
- Each photon causes a ~ 10^7 additional electrons to be produced.
- The PMT possesses high sensitivity, good S/N ratio, and excellent dynamic range.
- PMTs are highly sensitive to visible and UV excitations at extremely low power conditions, (very low concentrations of analyte).
- Intense light sources (such as daylight or stray light) can destroy and damage PMTs.

PHOTO DIODE ARRAY: *silicon diode*



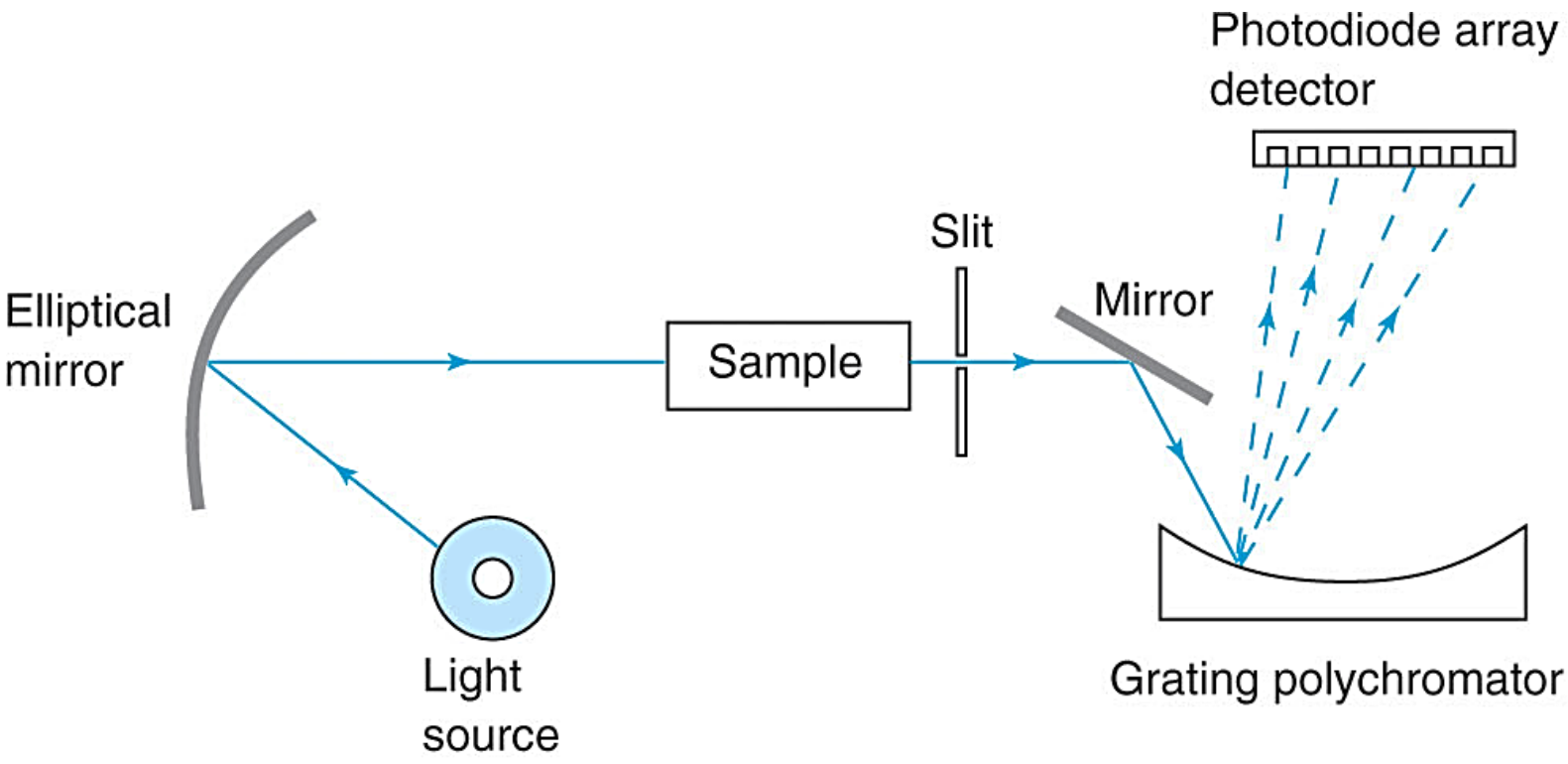


a) Schematic cross-sectional view of photodiode array
b) Picture of array with 1024 elements, each 25 μm wide and 2.5 mm high. The central black rectangle is the photosensitive area. The entire chip is 5 cm in length.

PHOTO DIODE ARRAY

- Silicon diode detectors, such as those used in a photo diode arrays (PDA), are composed of reverse-biased p-n junctions formed on a silicon chip.
- The doping of different elements in the silicon can produce electron rich and electron poor domains.
- As seen in (a) holes and electrons are randomly distributed.
- The addition of an outside power source (b) causes a depletion layer where conductance is zero.
- When a photon of light strikes the junction (c), holes and electrons form which provide the completion of the circuit.
- The current is proportional to the radiant power.

Photodiode Arrays



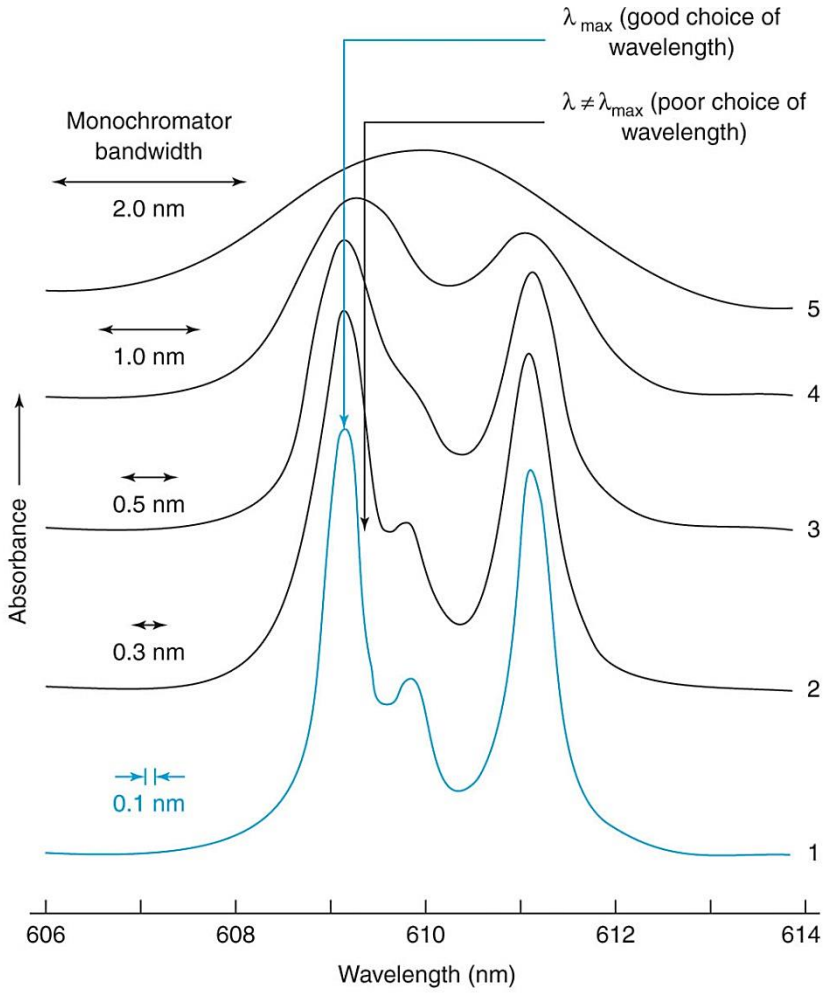
- PDAs are a series of silicon photo diodes, with each having a storage capacitor, and a switch that are combined in a integrated circuit on a silicon chip.
- The number of sensors (silicon photodiodes) in a PDA range from 64 to 4096.

Photodiode Arrays

- The slit width of the instrument allows the radiation to be dispersed over the entire array, allowing the spectral information to be accumulated simultaneously.
- PDAs are not as sensitive nor have the same S/N ratio as the PMT, but one gains the advantage of gathering multi-channel information (all of spectrum collected simultaneously).
- Advantage of the PDA is recording the entire spectrum in a fraction of the time required for a conventional scanning spectrometer to scan one wavelength at a time.
- An example of PDA use is in atomic emission spectroscopy (AES), UV-vis spectrophotometry, fluorescence spectrometry, Raman spectrometry.

Errors in Spectrophotometry

Choice of the Wavelength and Bandwidth



For Quantitative Analysis

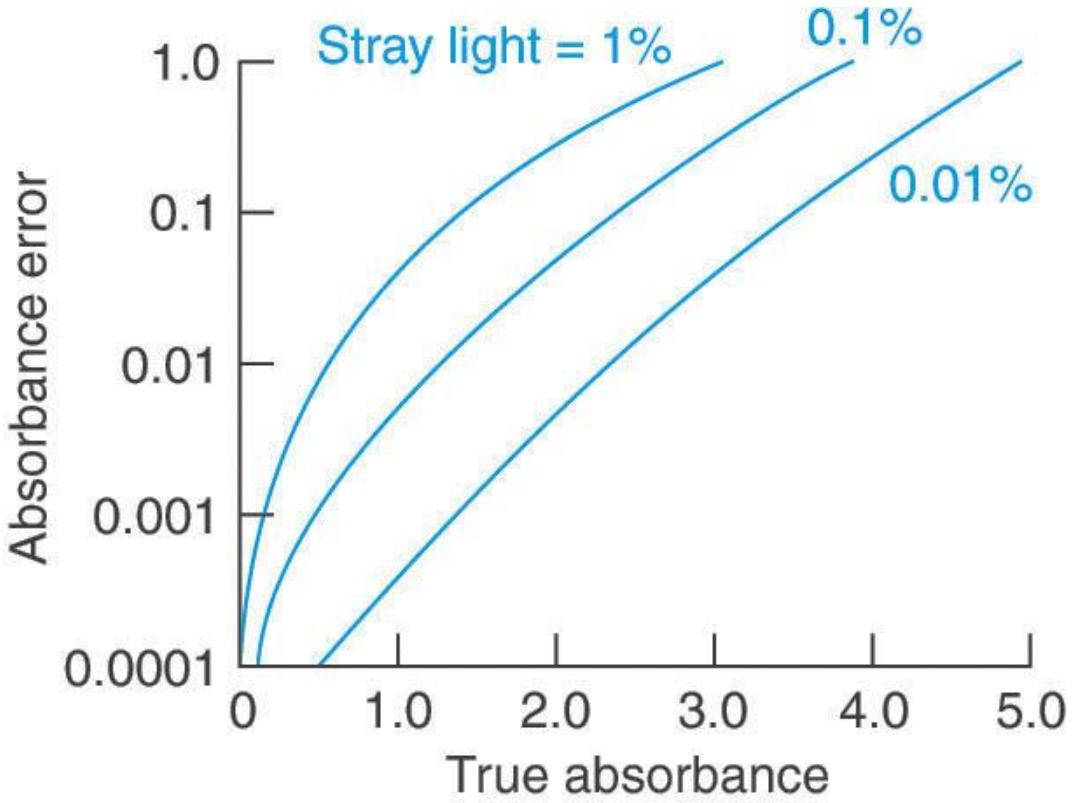
Absorbance should not be too large or small(~0.4-0.9 units)

Absorbance should be measured at the lambda max providing no interference from other species at this lambda.

Monochromator bandwidth should be as large as possible but small compared to the spectral bandwidth. Slit width to small allows less light to reach the analyte, smaller S/N ratio and precision. Too wide a monochromator band width (slit width) distorts the peak shapes of the spectrum.

- As shown in the previous figure; the instrumental setting with the band width at 0.1 nm and the choice of $\approx$ 609 nm for λ_{max} would be desirable for the spectra shown. At the 2.0 nm setting there is no fine structure shown in this spectrum.

Stray Light, Electrical Noise, Cell Positioning



Stray light arises from two major sources:

1) Misdirected rays coming from the monochromator.

2) Light coming from outside the instrument such as the sample compartment lid not closed properly.

Other concerns;

- the correct choice of the sample cell (does it require glass or quartz?)
- the alignment of the sample cell (and/or the sample cell holder)
- dust & fingerprints on the cell

Obtaining Quantitative Information from UV-vis Absorption Spectrophotometry

Beer's Law (aka Beer-Lambert Law)

$A = \varepsilon \times b \times C$

where *c* is the concentration (M), *b* is the path length (cm), and ε is the **molar absorptivity** (M–1 cm–1). ε is wavelength dependent.

Beer's Law works for dilute (~ 0.01 M) solutions in which the absorbing species is not participating in a concentration-dependent equilibrium.