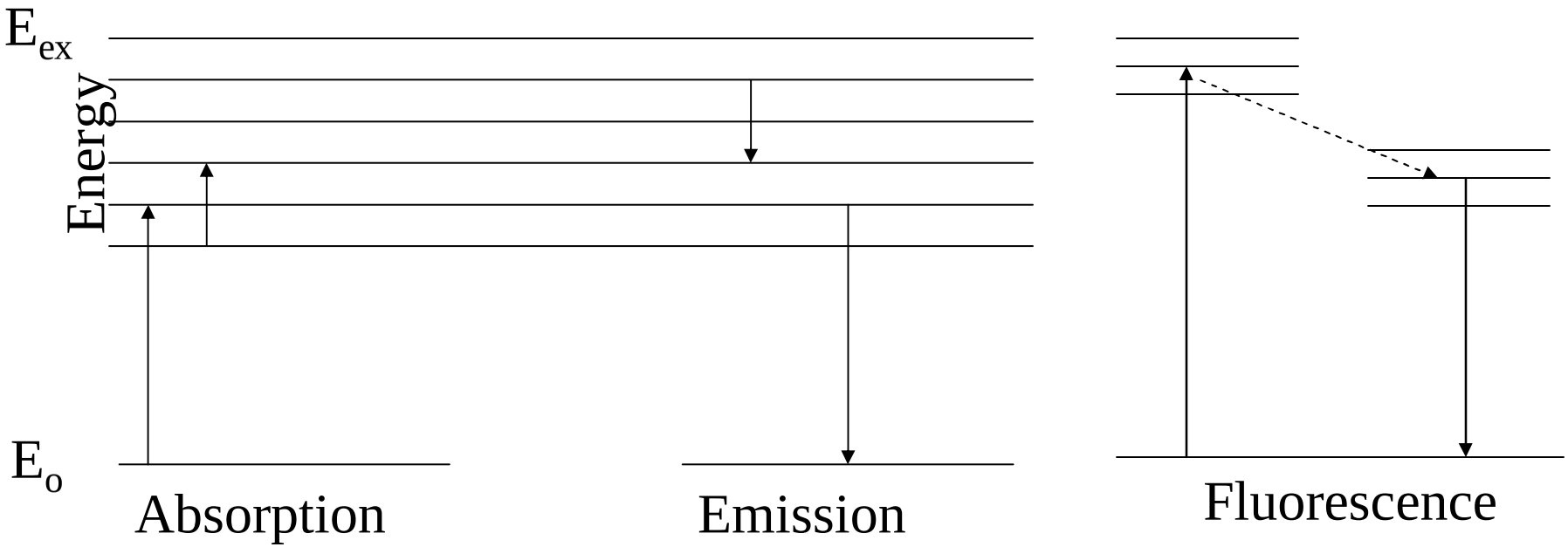
Chapters 18 – 20 Spectrophotometry or Optical Spectroscopy

Review

Nine properties of electromagnetic radiation

- **Diffraction**
- **Refraction**
- **Dispersion**
- **Reflection**
- **Scattering**
- **Polarization**
- **Transmission**
- **Absorption**
- **Emission**

- **Absorption: atom or molecule "absorbs" a photon of specific energy, goes to a higher energy state**
- **Emission: atom or molecule releases a photon of specific energy, goes to a lower energy state; nonradiative excitation**
- **Fluorescence: absorption followed by re-emission**



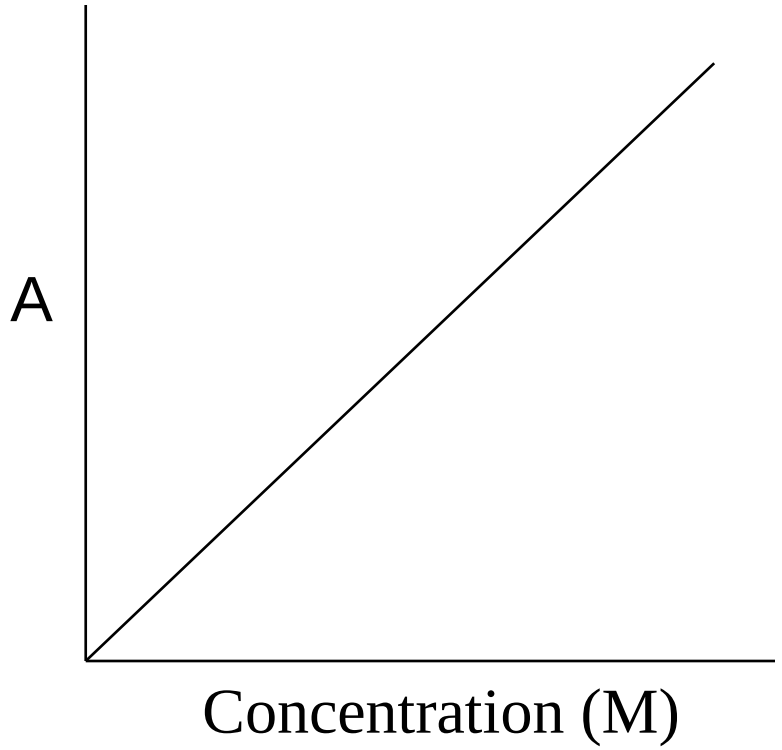
Absorption Methods, Beer's Law

$$A = \varepsilon bc$$

b => path length (cm)

c => concentration (M)

ε => molar absorptivity ($M^{-1}cm^{-1}$)



Question 1.
Which of the following relationships between absorbance and %Transmittance is **correct** ?

a) $A = -\log_{10} T$
b) $A = 2 - \log_{10} T$
c) $A = \log_{10} T$

Question 2.
In the equation, $A = \varepsilon bc$, what quantity is represented by "**ε**" ?

a) Absorbtivity
b) Molar absorbtivity
c) Path length

Question 3.
Why is it generally preferable to use absorbance as a measure of absorption rather than % Transmittance?

a) Because %T cannot be measured as accurately as absorbance
b) Because %T is dependant on the power of the incident radiation
c) Because absorbance is proportional to the concentration of the analyte, whereas %T is not.

Question 4.
Does a compound with high molar absorbtivity have a higher or lower limit of detection than a compound with low molar absorbtivity?

Beer's law problem

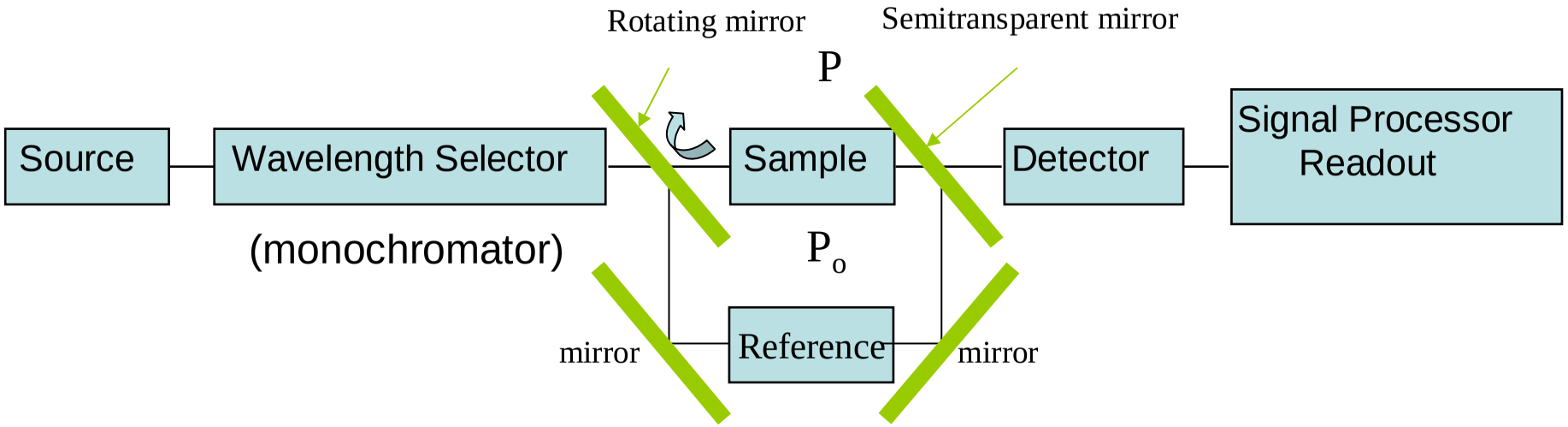
A 3.96×10^{-4} M solution of compound A has an absorbance of 0.624 at 238 nm in a 1.0 cm cuvet. A blank solution containing only solvent had an absorbance of 0.029 at the same wavelength.

a) What is the molar absorptivity of compound A?

b) The absorbance of an unknown solution of compound A in the same solvent and cuvet was 0.375 at 238 nm. Find the concentration of A in the unknown solution.

c) A concentrated solution of compound A in the same solvent was diluted from an initial volume of 2.0 mL to a final volume of 25.0 mL. The diluted solution had an absorbance of 0.733. What is the concentration of A in the concentrated solution?

Components of Optical Instruments



Noise

- Unwanted component of the measured signal that affects and limits measurements of the chemically relevant signal component
- The absolute magnitude of noise may not be important; however, the **signal-to-noise ratio (S/N)** is critical!
- Improving the S/N is critical in some measurements

S/N = (magnitude of signal) / (magnitude of noise)

The **S/N** gets lower as one approaches the detection limit. Some methods of determining the LOD are based upon detection limit, e.g. **S/N** = 2 at the detection limit.

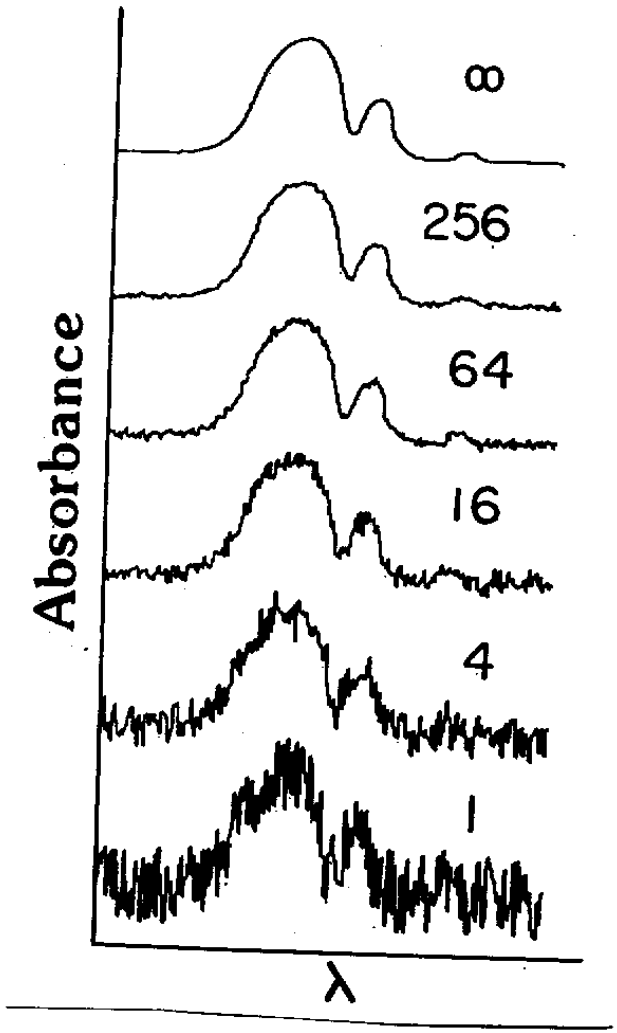
How to improve the S/N:

- Improve background (sample matrix, electronics, detector, etc.)
- Signal average multiple measurements

S/N improves as $\sqrt{n}$

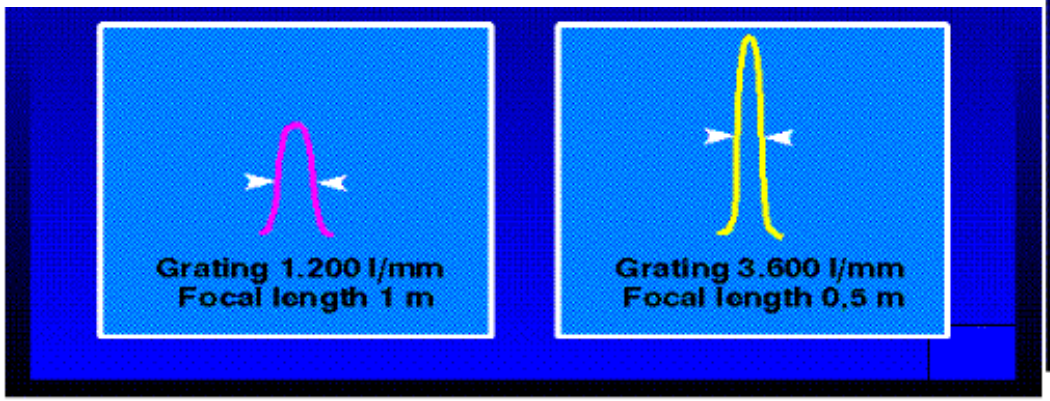
(where n is the number of measurements)

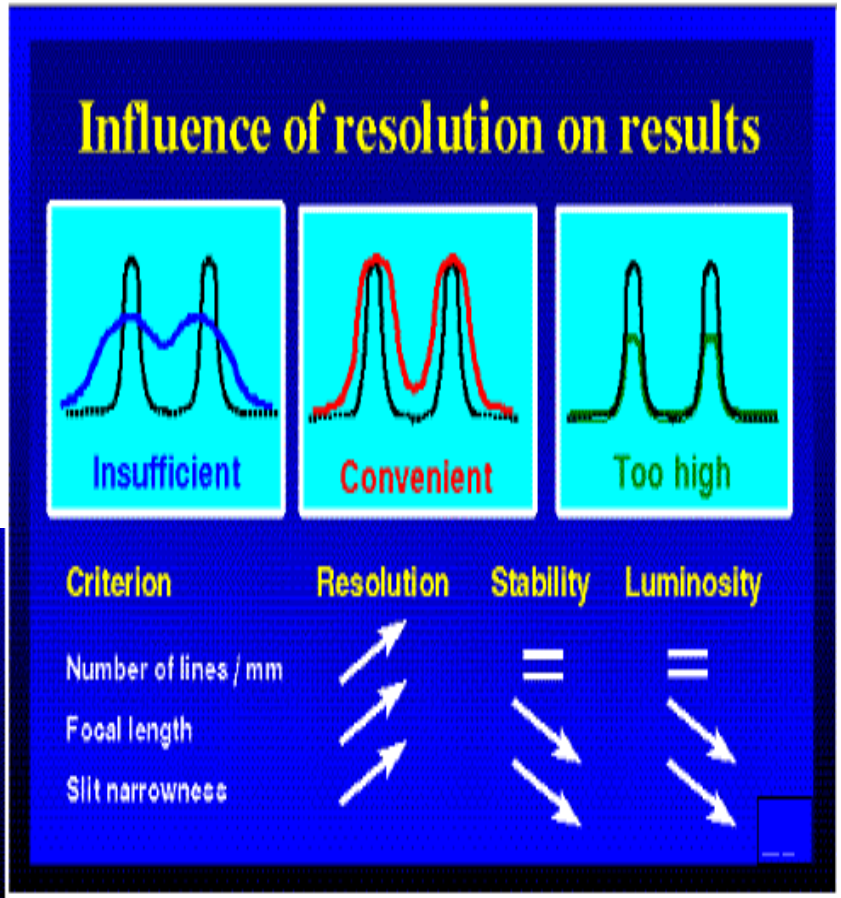
Example of S/N improvement by signal averaging



Resolution of the spectrum
Resolving power of the spectrometer

- **Resolving power** = $\lambda / \Delta\lambda$
- Resolving power is influenced by design - number of grooves/mm, and focal length





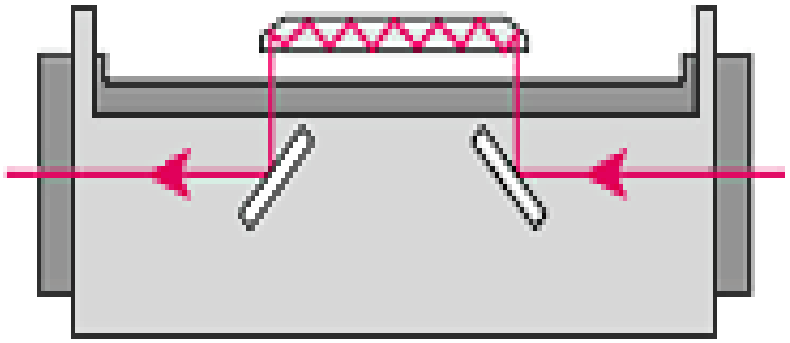
Oximetry – measurement of O_2 content

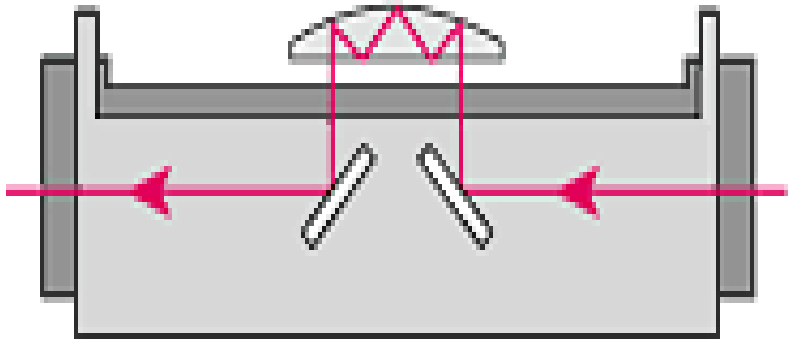
Small instrument based on the color difference of oxygenated blood (bright red) and deoxygenated blood (dark red, almost purple). Absorption spectroscopy used to quantitatively determine O_2 in blood quickly.

http://www.avweb.com/articles/oximeter.html

Attenuated total reflectance IR Spectroscopy

- The beam is confined to an “ATR” crystal (ZnSe is common for IR).
- Sample is coated on the surface
- If the sample absorbs light, the transmitted light beam is attenuated
- Diamond ATR crystal allows for analysis of very “hard” samples





http://www.nicolet.com/