An Introduction to Ultraviolet/Visible Molecular Absorption Spectrometry

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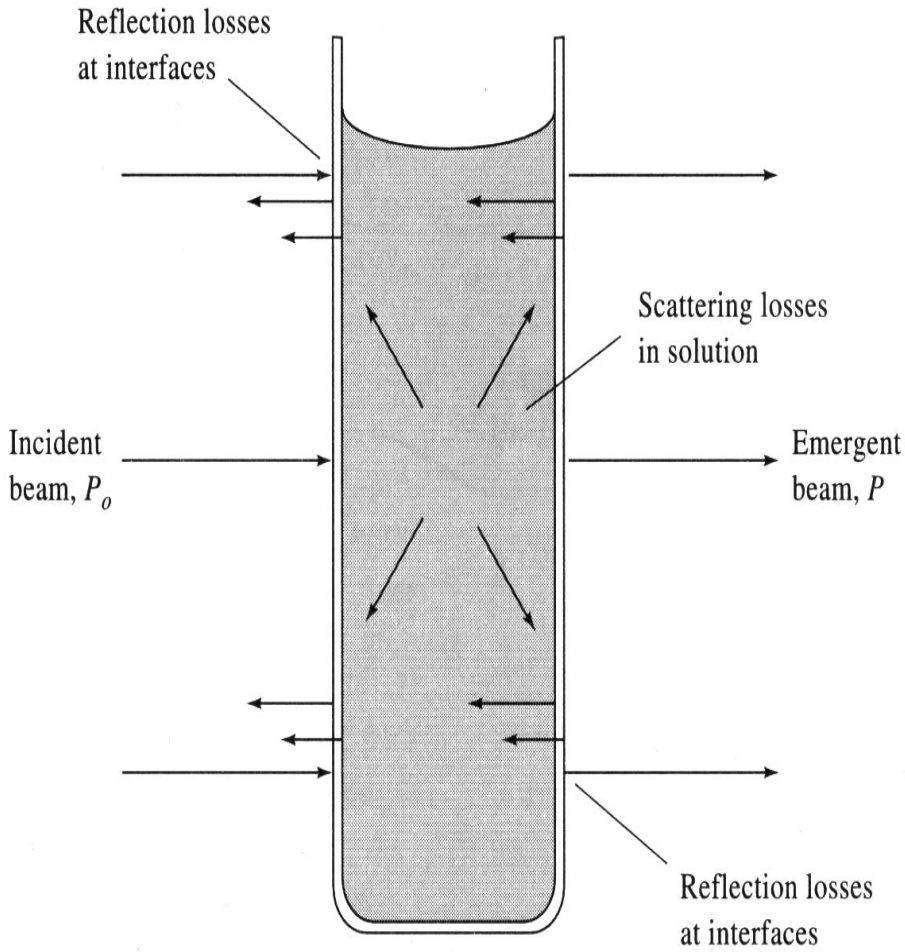
Measurement of Transmittance and Absorbance

- Transmittance (T)

$$T = \frac{P}{P_0} \quad \textit{and} \quad \%T = T \times 100$$

Where: P_0 = Power of incident beam,

P= Power of emergent beam



Measurement of Transmittance and Absorbance

- In reality, we do not measure the true P_0. We measure $P_{Solvent}$, the power of the beam passing through a cell containing solvent only.

$$T = \frac{P_{Solution}}{P_{Solvent}}$$

- Absorbance (A)

$$A = -\log T = -\log \frac{P_{Solution}}{P_{Solvent}} = \log \frac{P_{Solvent}}{P_{Solution}} = \log \frac{1}{T}$$

Beer's Law

- The proportion of radiation absorbed is proportional to the thickness of the absorbing layer (*b*), the molecular concentration (*c*) of the absorbing species, and the *molecular absorbing coefficient* (*a*) of the species, which is characteristic of the species at a given wavelength.

$$A = abc$$

Beer's Law

- When concentration (*c*) is expressed in molarity (mol/L) and thickness or path length (*b*) is expressed in centimeters, the *molecular absorbing coefficient* (*a*) is called *molar extinction coefficient or molar absorptivity* (*e*) and has units of liters per moles per centimeter (1/M•cm).

$$A = \varepsilon bc$$

- For mixtures, Beer's Law is additive.

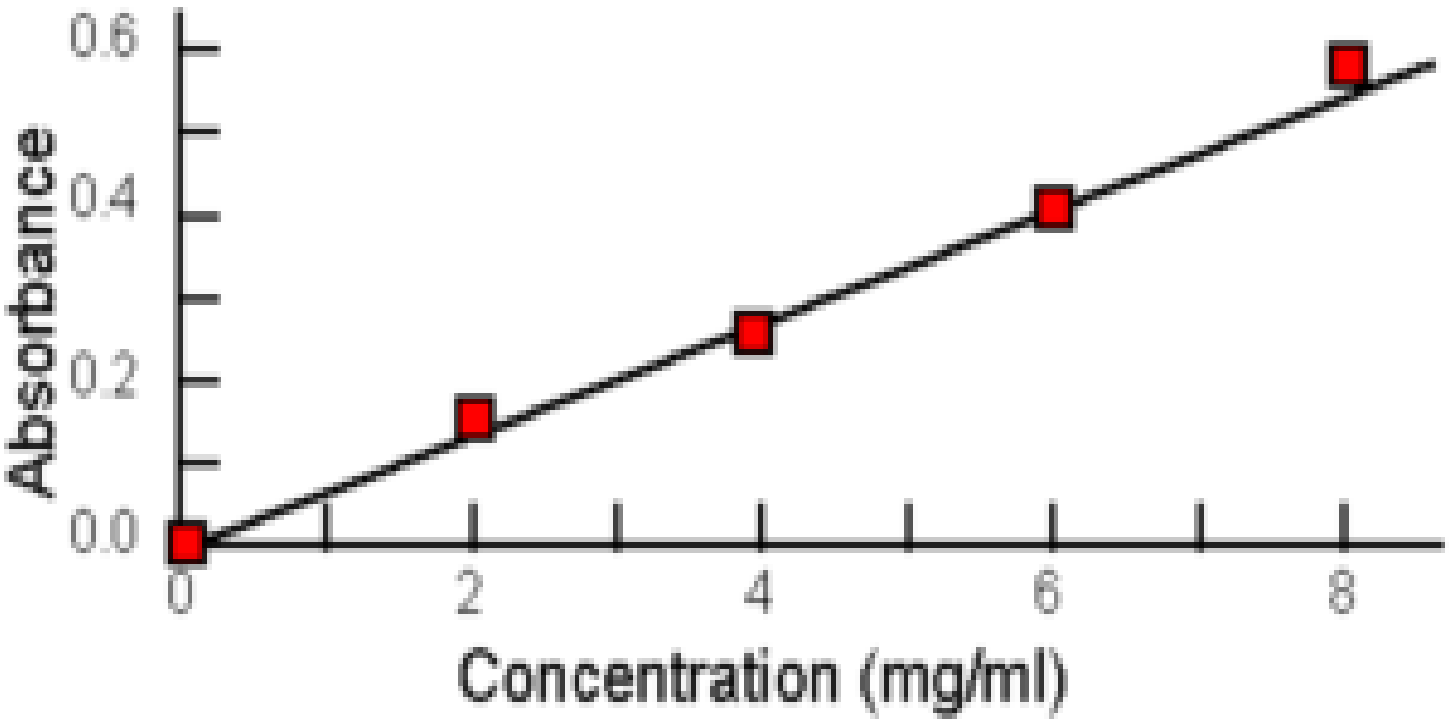
$$A_{Total} = A_1 + A_2 + A_3 ..... + A_n$$

or

$$A_{Total} = \varepsilon_1 b_1 c_1 + \varepsilon_2 b_2 c_2 + \varepsilon_3 b_3 c_3 ..... + \varepsilon_n b_n c_n$$

Beer's Law

- Working curve is produced by plotting the absorbance vs. the concentration.
- The curve is a linear regression.
- It also called a Beer's Law plot or a calibration curve.



Beer's Law

- According to Beer's Law absorbance (*A*) is linear with respect to path length (*b*) and concentration (*c*), yet there are some real limitations to this relationship.
  - A has no limitation with respect to b.
    - Use short path lengths for samples of high concentration (absorbance).
    - Use long path lengths for samples of low concentration (absorbance).

  Example: If $A = 0.410$ for a 1.0 cm cuvette ($b = 1.0$ cm)

  Then if: $b = 2.0$ cm, $A = 0.820$

  $b = 0.1$ cm, $A = 0.041$

- b becomes a constant for an analysis where the same cuvette is used for all samples.

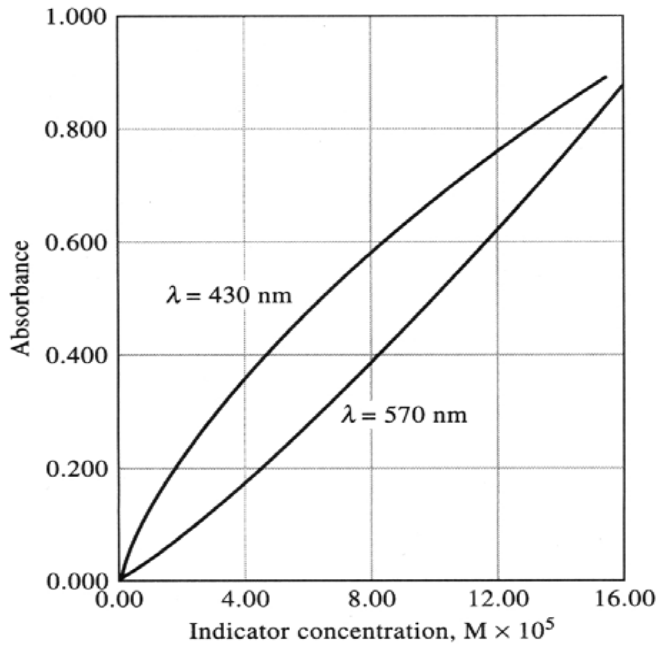
Beer's Law

- **Chemical Deviations**
  - *Absorbance* usually becomes nonlinear with concentration when *c* is greater than 0.10 M.
    - Above concentrations of 0.10 M, the distance between analyte molecules decreases to the extent that they change each others charge distribution, effectively changing the way they absorb radiation (i.e. ε changes).

Beer's Law

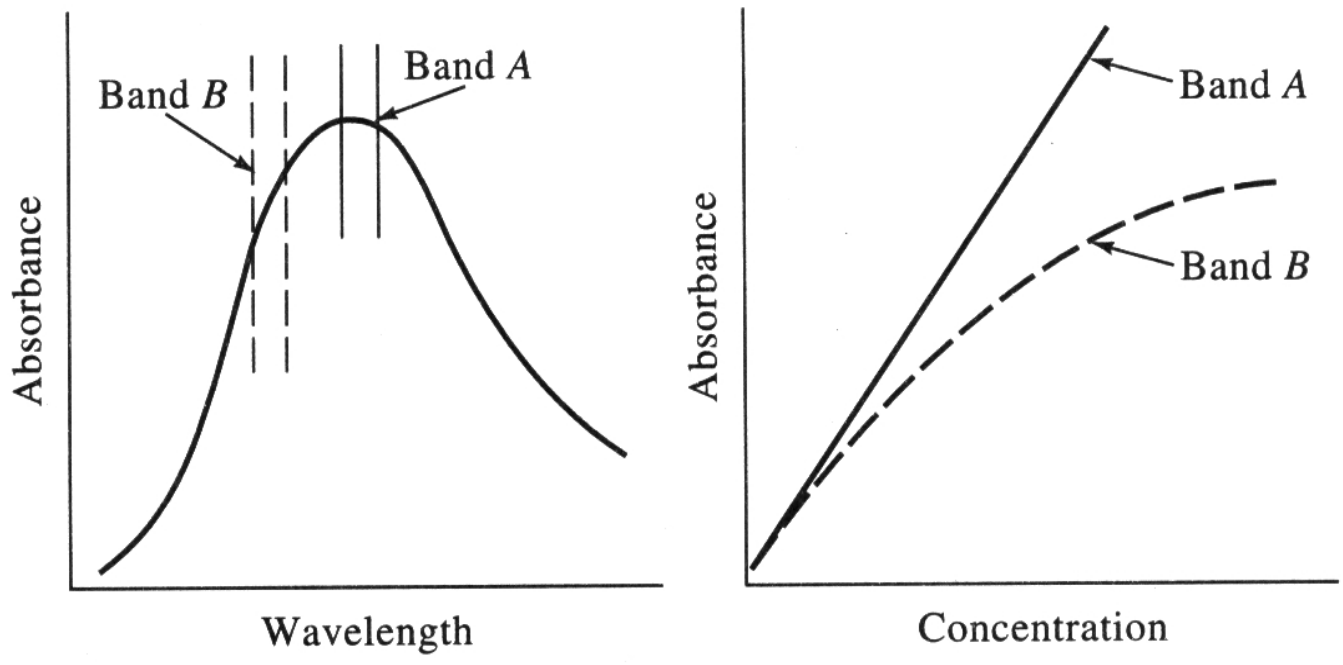
- *Absorbance* becomes nonlinear when chemical reactions occur.
  - If the associates, dissociates, or reacts with the solvent or other components in the solution deviations from Beer's Law will occur.

Example: $\underset{\textit{Color 1}}{\boldsymbol{HIn}} \leftrightarrow H^+ + \underset{\textit{Color 2}}{\boldsymbol{In}^-}$



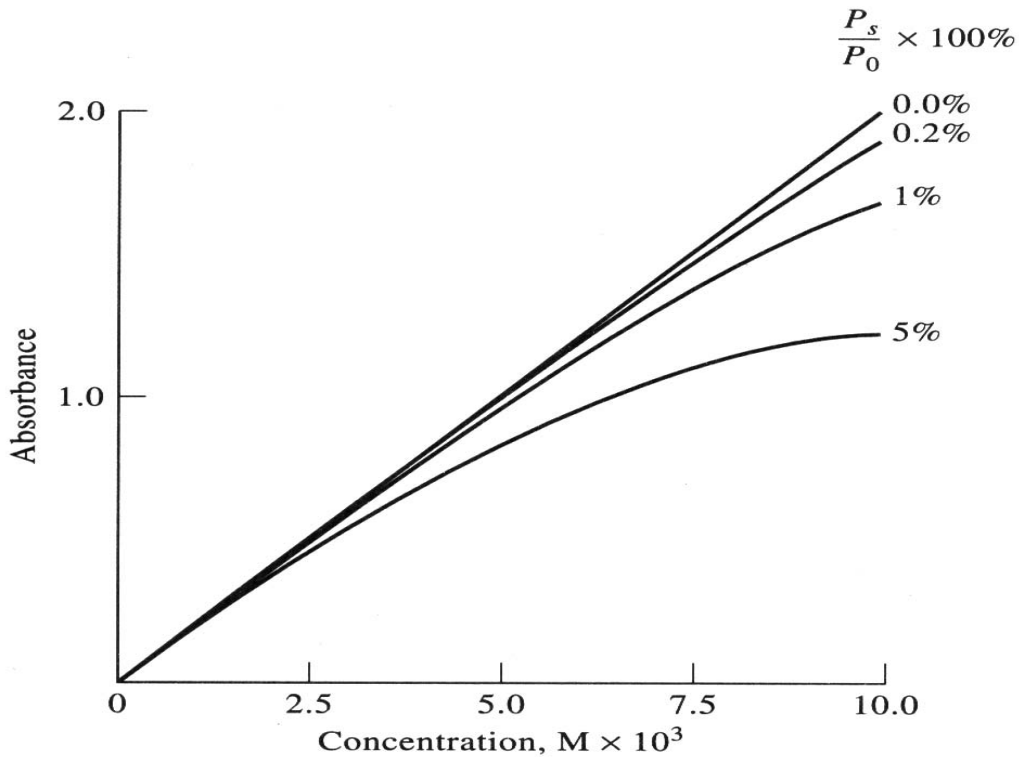
Beer's Law

- Instrument Deviations
  - Effect of polychromatic radiation
  - Ideally, a monochromator will pass radiation of a single wavelength, but in reality the monochromator passes a band of radiation. The bandwidth of the spectrometer will affect the linearity of Beer's Law.



Beer's Law

- Scattered light that reaches the detector will increase $P_{Solution}$.



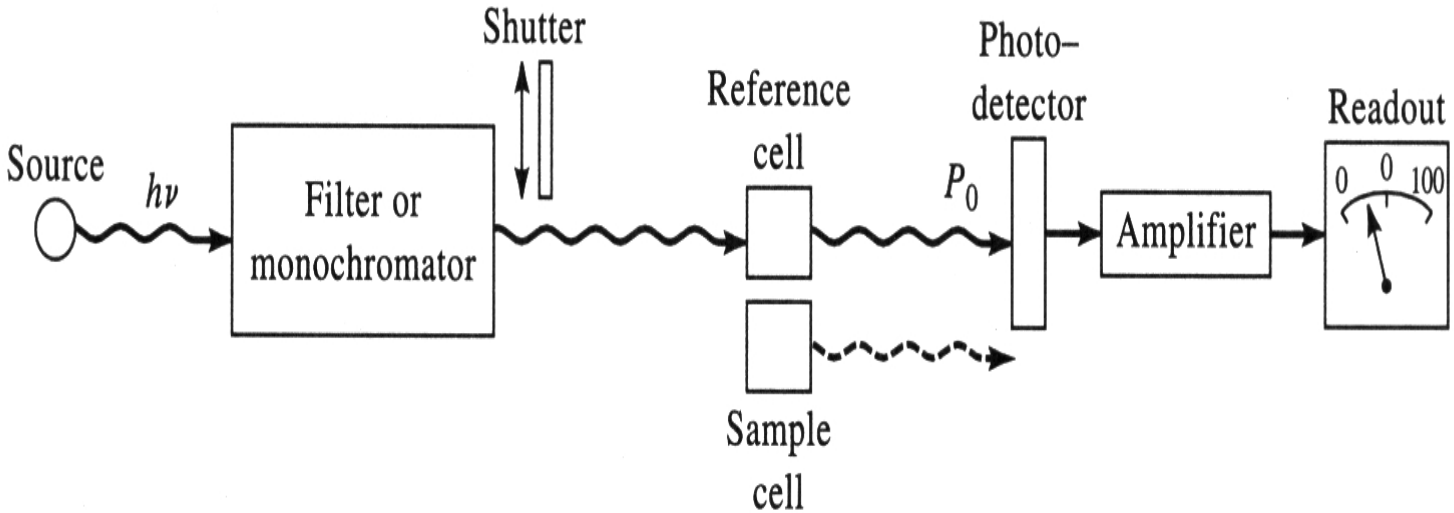
- Long path length cells cause more scattered light, which causes deviations from linearity in the relationship between *A* and *b*.
- Note that all instrument deviations lead to negative absorbance errors.

Instrumentation

- Four basic types of instruments used for UV/Vis Spectroscopy
  - Single-beam.
  - Double-beam in space.
  - Double-beam in time.
  - Multichannel.

Instrumentation

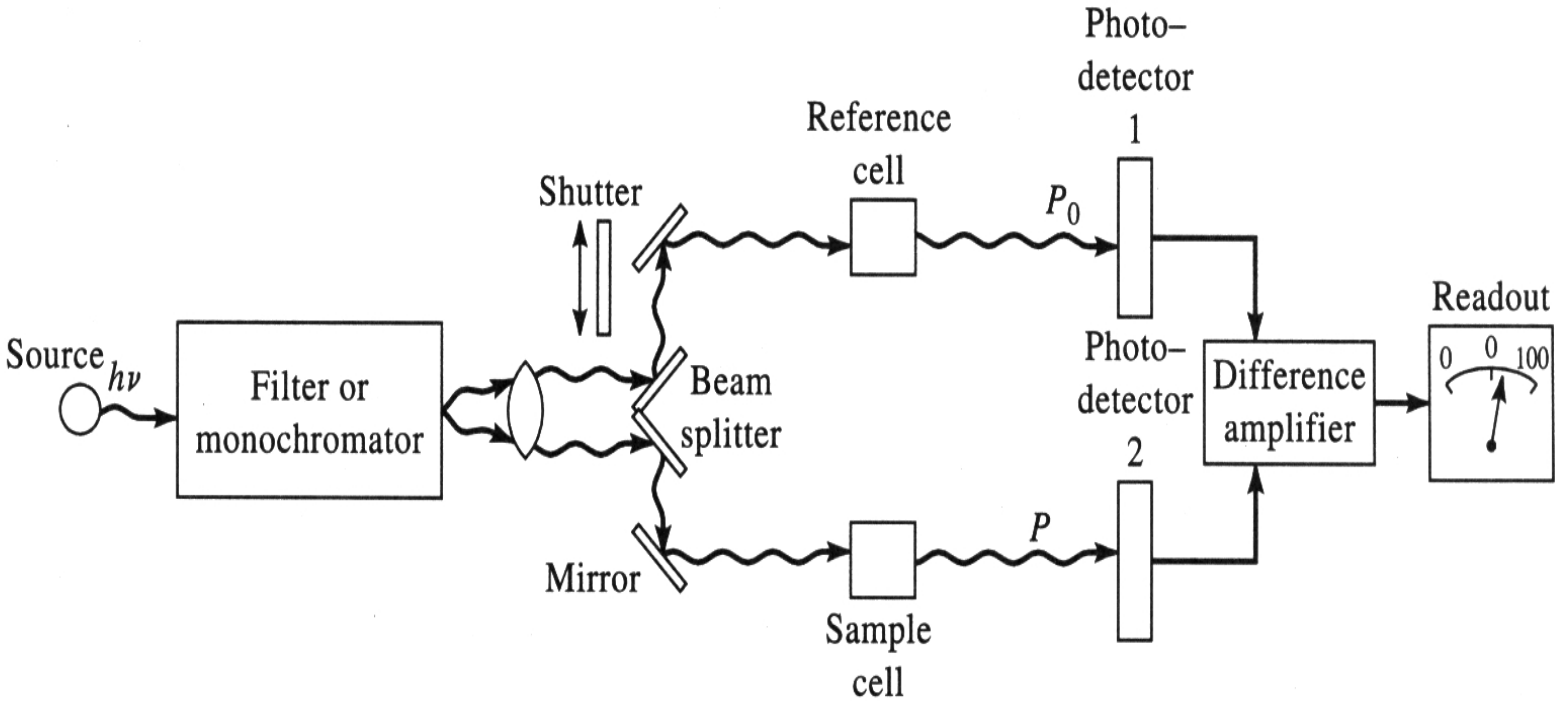
- Single-beam instrument
  - These can be either single channel or scanning instruments.



- 0% T is set with shutter in the beam path.
- 100% T is set with a reference in the beam path.
- Measurement is then made with the sample in the beam path.

Instrumentation

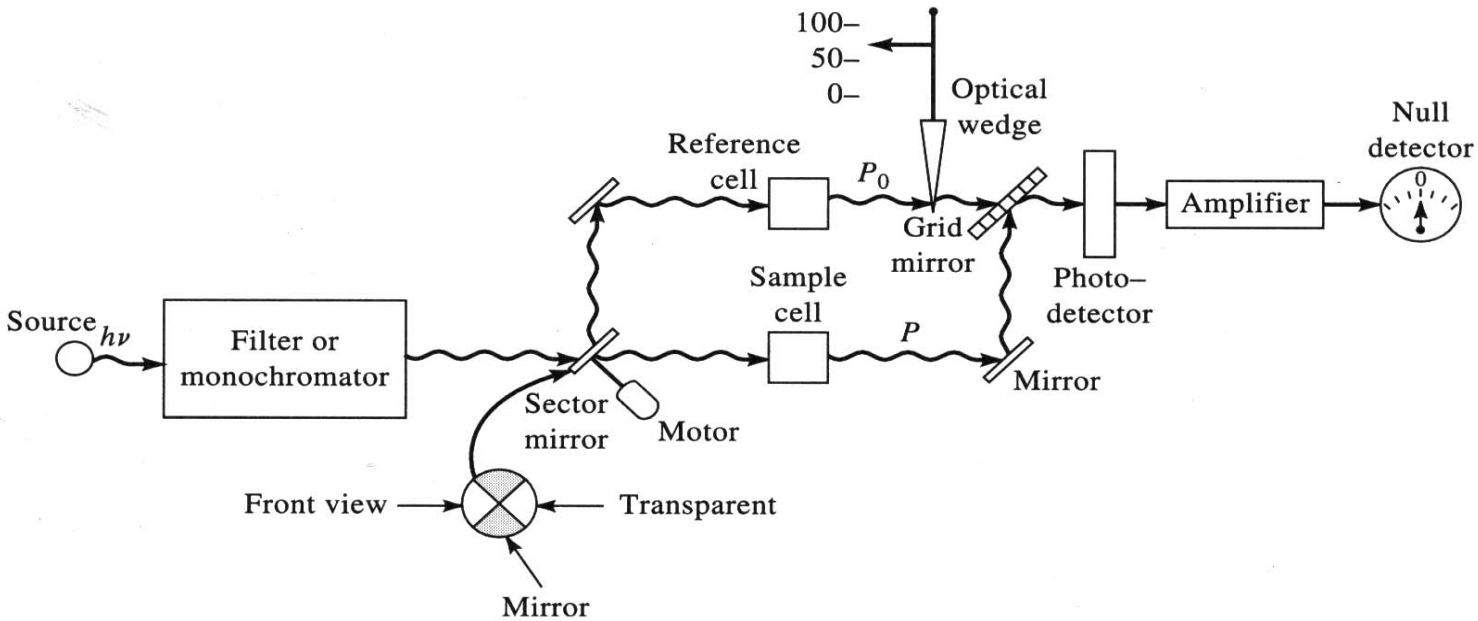
- Double-beam in space instrument.



- Sample and reference are measured simultaneously and the signal from the reference is subtracted from the sample signal.
- A major drawback of this type of instrument is the requirement of two detectors, which makes the instrument more expensive.

Instrumentation

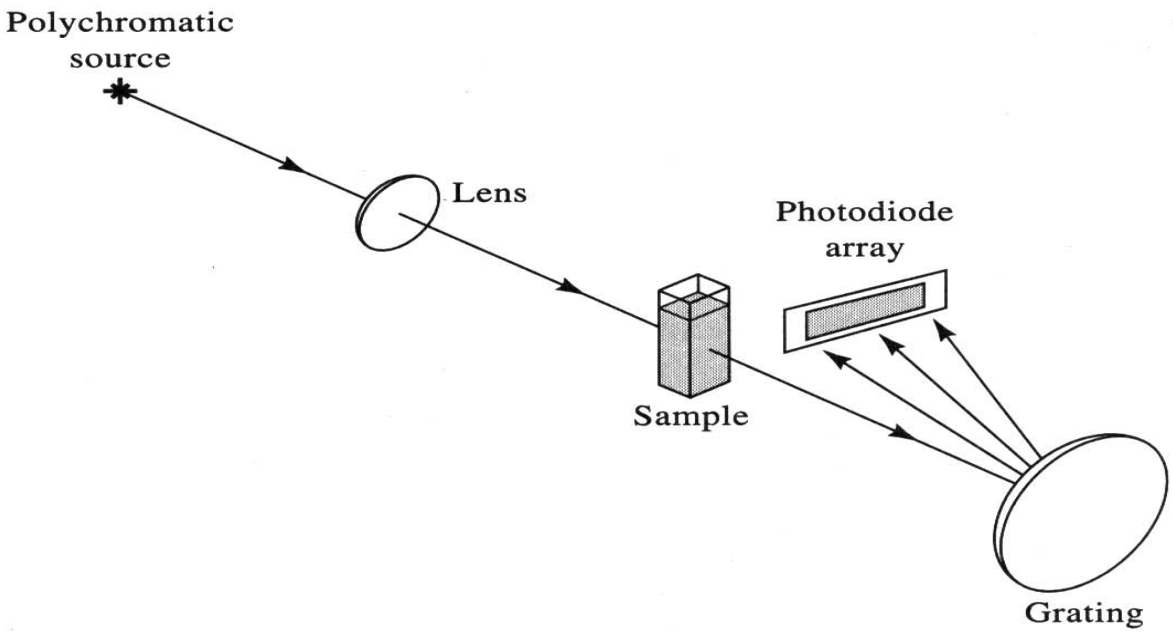
- Double-beam in time instrument



- Most common type of double-beam instrument commercially available.
- Advantages of a double-beam over a single-beam instrument:
  - **Compensate for variations in the source intensity.**
  - **Compensate for drift in the detector and amplifier.**
  - **Compensate for variation in intensity as a function of wavelength.**

Instrumentation

- Multichannel instruments



- Able to “scan” (collect) an entire spectrum in ~ 0.1 sec.
- Uses signal averaging over a period of 1 sec or more to enhance signal-to noise ratio.
- Have high throughput of radiant energy due to the minimal optics.
- Typically use a deuterium lamp source for a spectral range of 200 nm → 820 nm and have a spectral bandwidth (resolution) of 2 nm.

Components of UV/Vis Spectrometers

- Source
  - Tungsten lamp
    - Used for the visible region of the spectrum (350 – 800 nm).
    - Usually constructed of a quartz or glass bulb containing a tungsten filament.
  - H_2 or D_2 lamp
    - Used for the ultraviolet region of the spectrum (160 – 350 nm).
    - The excited D_2 will dissociate to give a continuous band of radiation.
    - Constructed of a quartz bulb filled with deuterium and a pair of electrodes.

Light Source

Deuterium lamps (UV)

Light Source

- Xe lamp
  - Used when a high intensity lamp is required and emits a continuous band of radiation from 200 → 1000 nm.
  - Not very common because they are expensive and have a short lifetime.

Sample Containers

- Sample containers are usually cuvettes.
  - Constructed of either quartz or glass.
  - Glass is used for the visible region because it is transparent from 350 → 2000 nm.
  - Quartz (silica) is used for the UV region because it is transparent below 350 nm.
  - Cuvettes are commercially available in path lengths of 0.1 → 10 cm.

Sample Cells

- You must use quartz cuvettes in the UV region.
  - These are also transparent in the visible.
  - They are expensive (about $100 a piece).

Sample Cells

- Silicate glass cuvettes can be used in the visible only.
  - Much cheaper.

Sample Cells

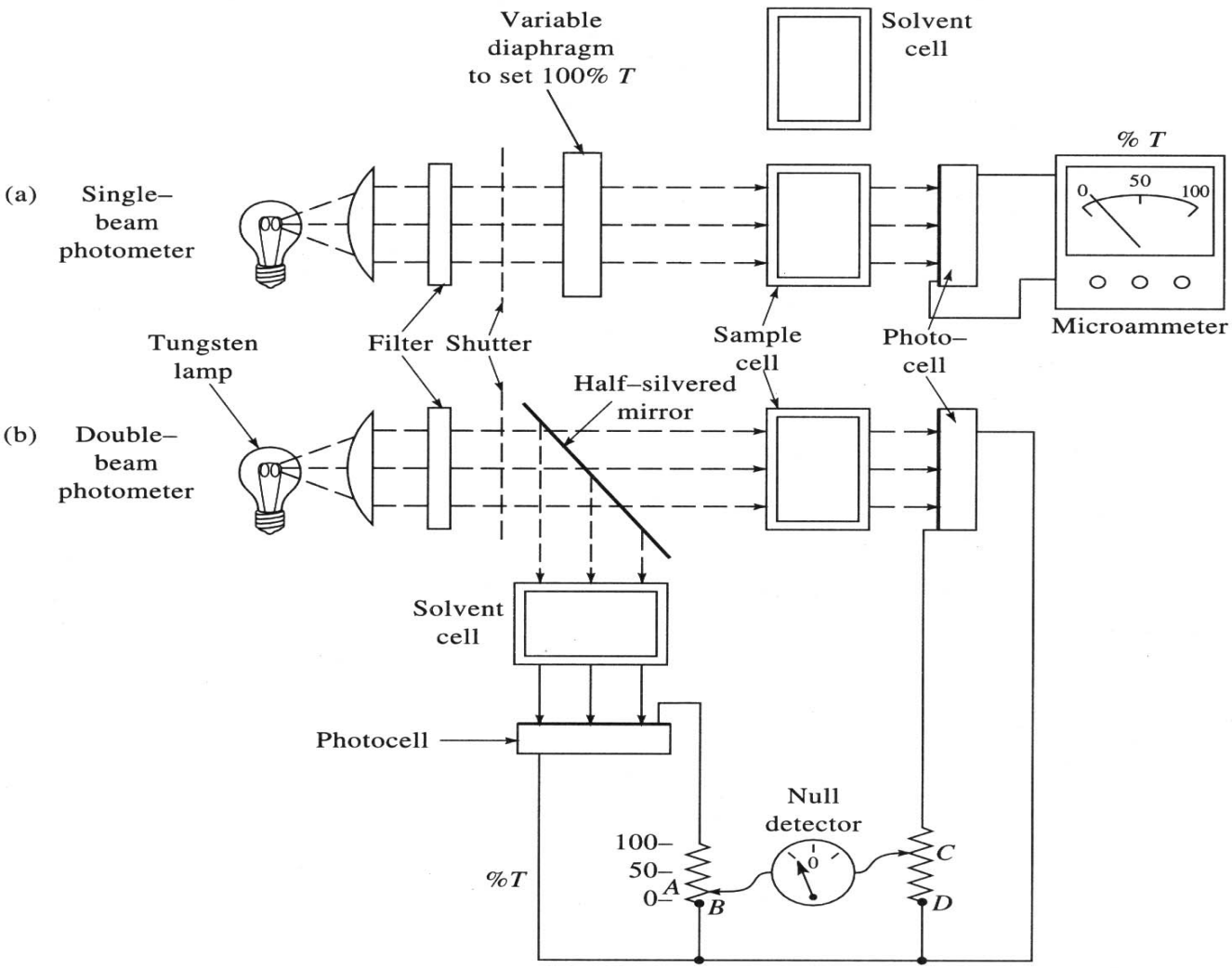
- Plastic (disposable) cuvettes can also be used in the visible region and transmit light from 350-900 nm
- You must make sure the solvent won't dissolve the cuvette !

Detectors and some type instruments

- Detectors
  - Use either a photomultiplier tube or a diode array.
- Some typical instruments
  - Two major classifications of instruments:
    - Photometers: Simple instruments that use *filters* to select wavelength. They can only detect a single wavelength at a time, have a high throughput energy due to the simple optics (good S/N), and are inexpensive.
    - Spectrophotometers: Instruments that contain a *monochromator* or *dispersive element* that allow them to scan various wavelengths. More expensive than photometers and usually have a lower S/N due to the more complex optics.

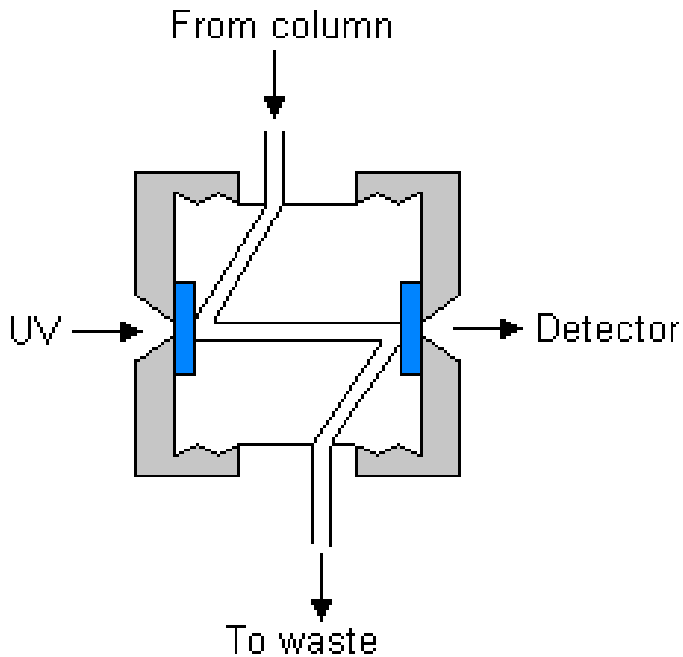
Photometers

- Visible photometers



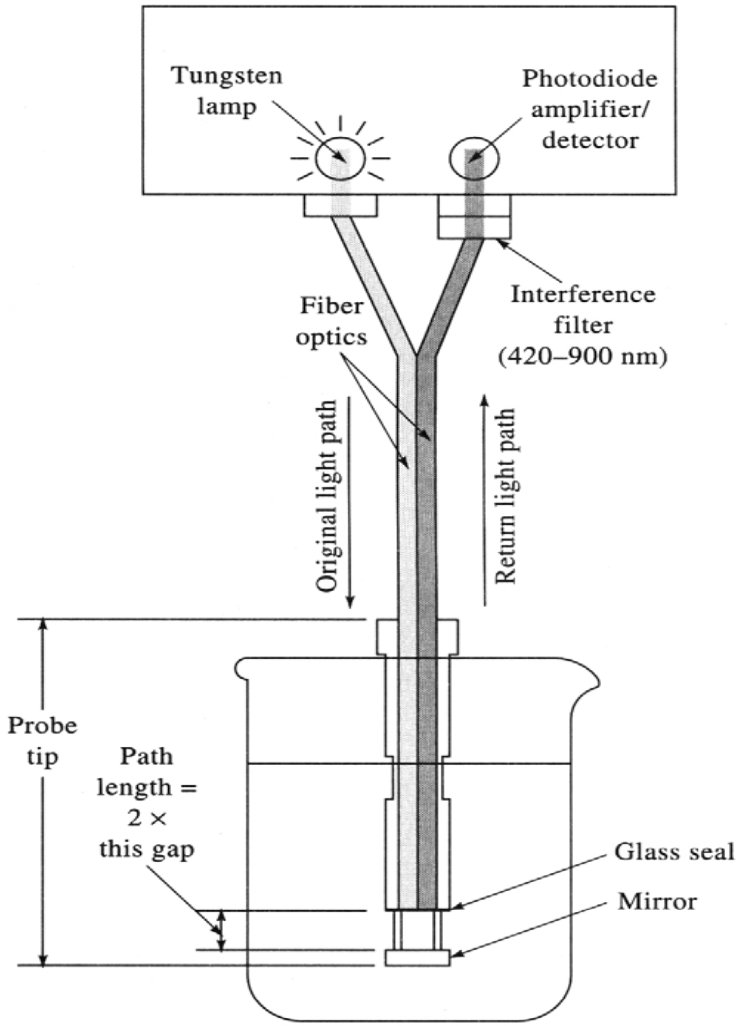
UV photometers

- Common detector used for high performance liquid chromatography.
- Usually contain a Hg lamp as a source and the emission line at 254 nm is isolated by a filter.
- Good for the detection of organic molecules, which absorb at 254 nm.



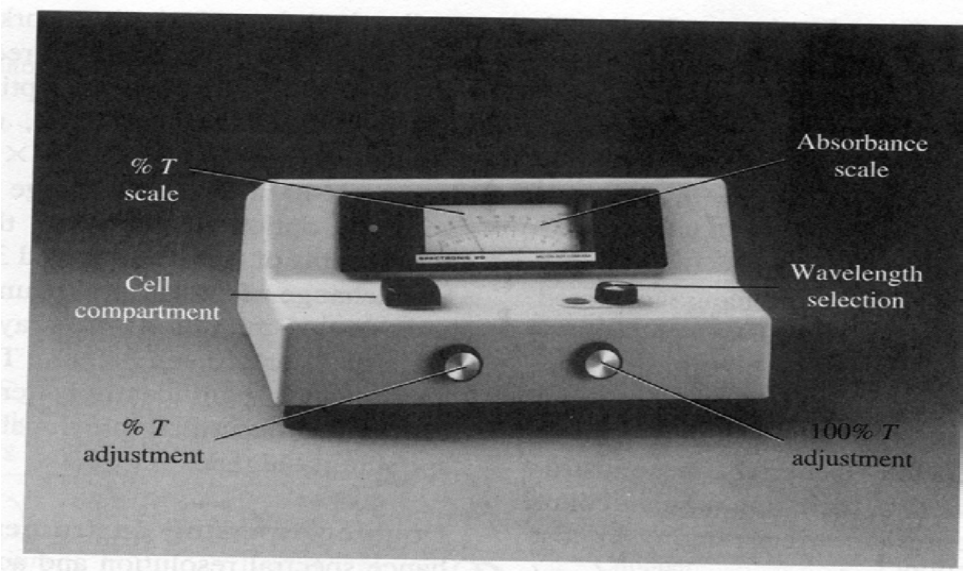
Probe type photometers

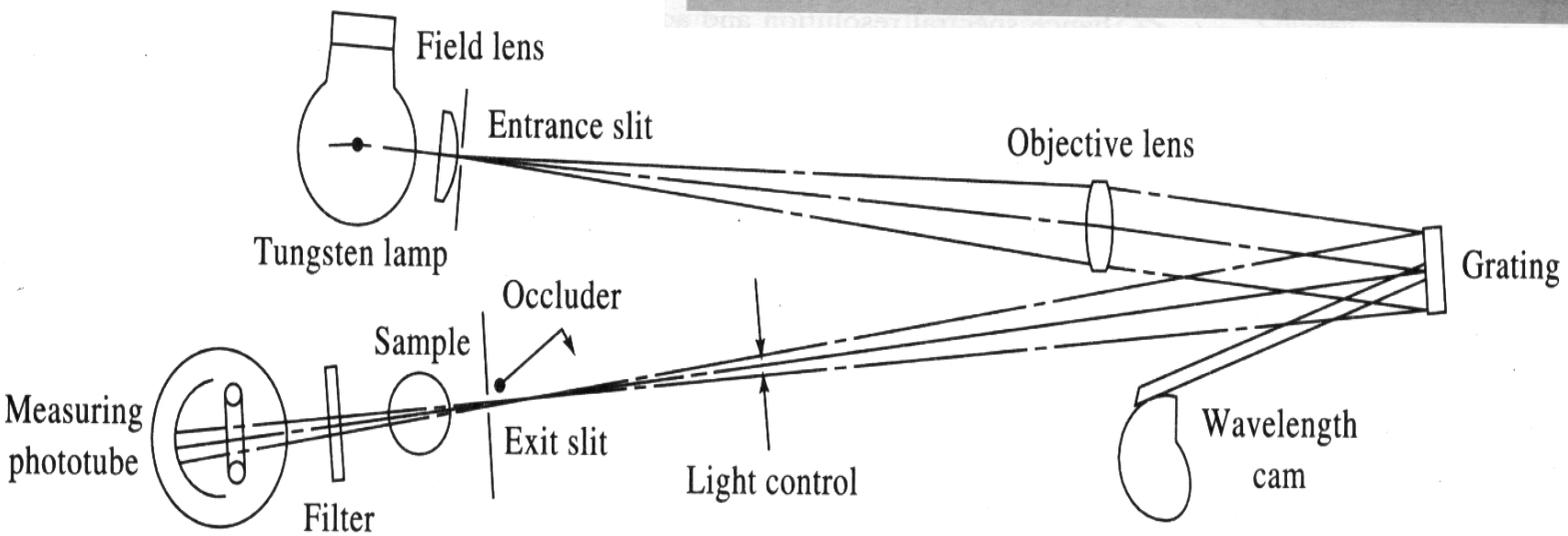
- Contain fiber optics and a mirror to project the source beam through the sample.
- Commonly found in field instruments and eliminate the need for a sample cell.



Spectrophotometers

- Visible instruments
  - Inexpensive ($500 to $3000).
  - Range: 380 – 800 nm.
  - Resolution: 8 –20 nm.



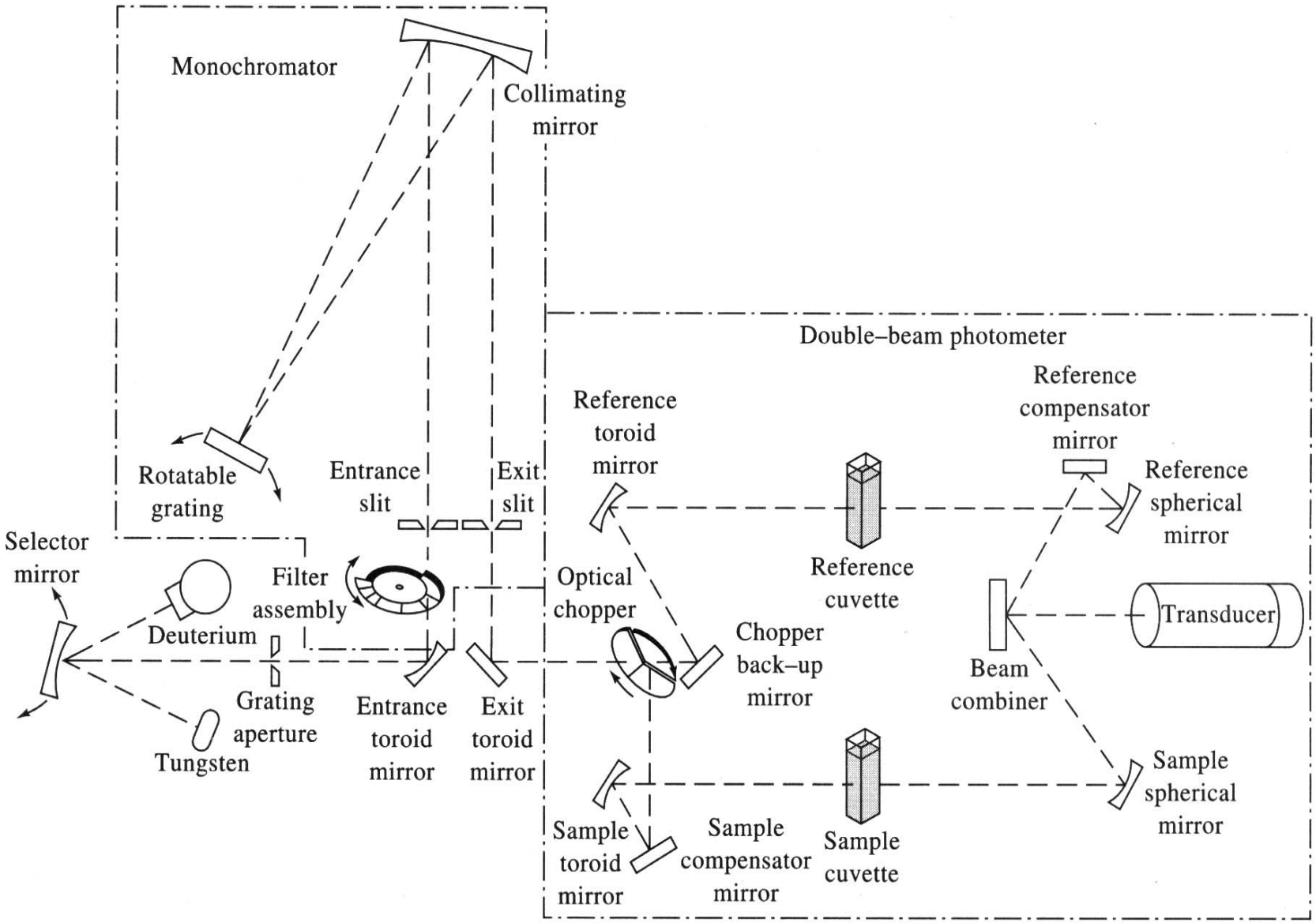


(b)

Single- and double-beam UV/Vis spectrophotometers

- Use exchangeable tungsten and D_2 lamps
- Range: 200 – 900 nm
- Cost: $3000 - $8000 for single-beam UV/Vis, $4000 - $15,000 for double-beam UV/Vis spectrophotometers.
- Resolution: 0.5 to 8 nm for single-beam UV/Vis, 0.1 to 3 nm for double-beam UV/Vis.
- Accuracy: ±0.005 Å for single-beam UV/Vis, ±0.003 Å for double-beam UV/Vis.

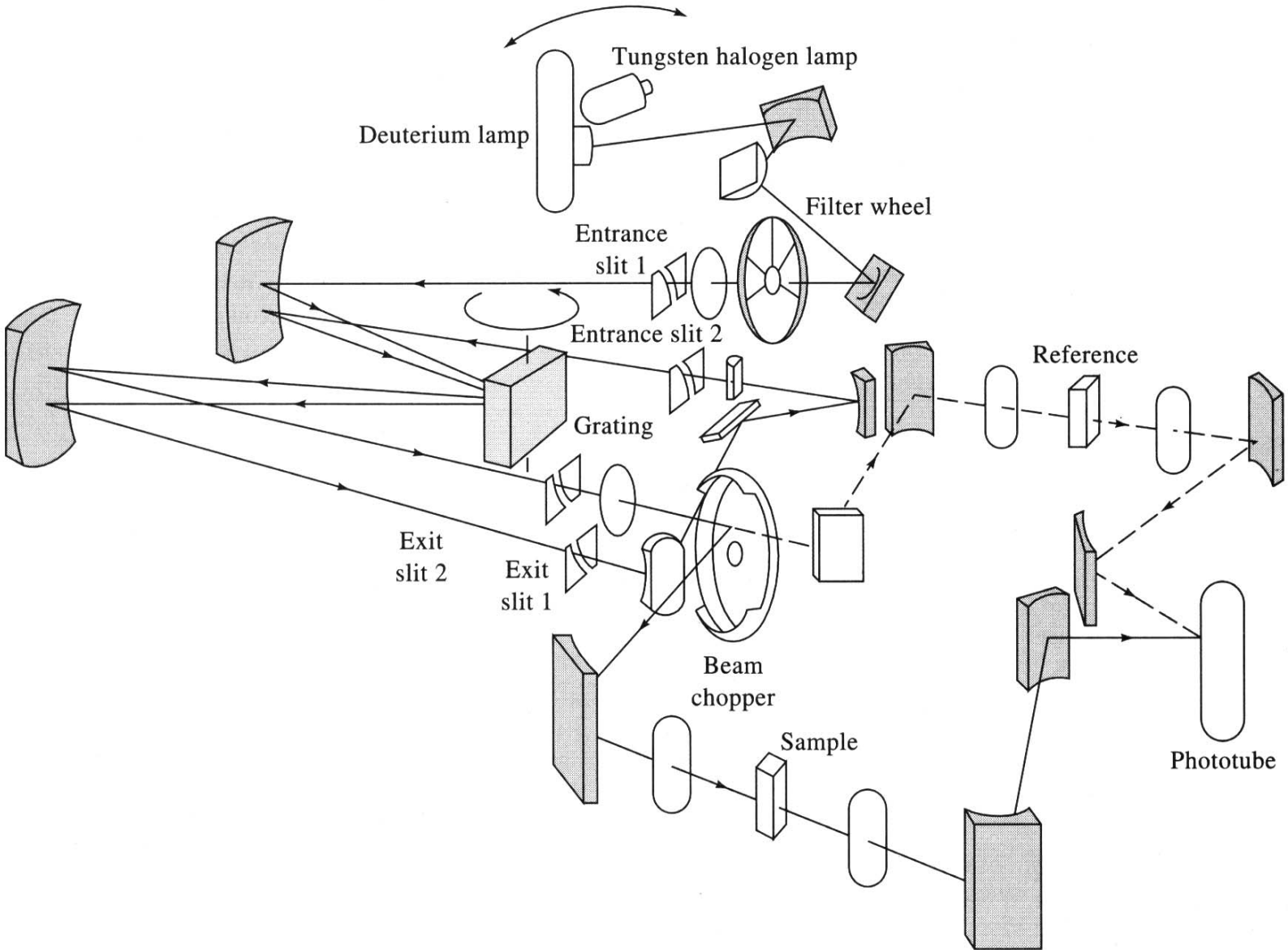
Double-beam UV/Vis Spectrophotometers



Double-dispersing Instrument

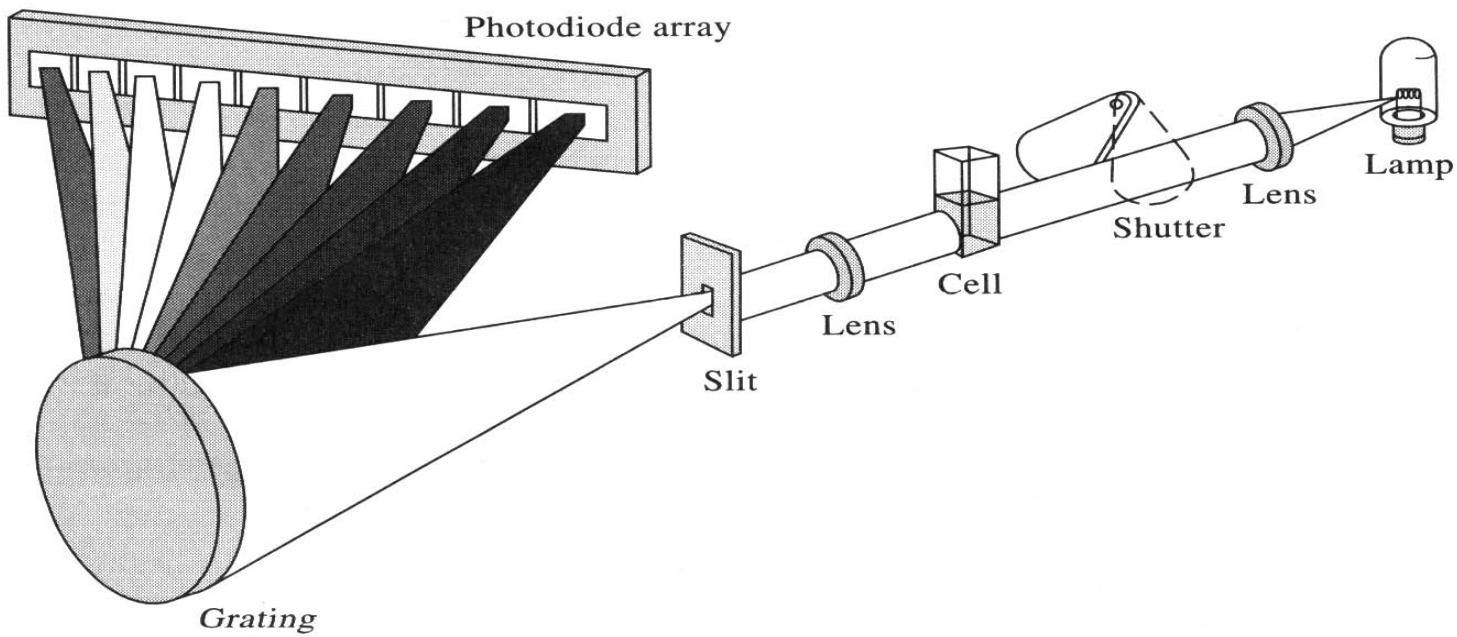
- Light passes through the monochromator twice resulting in very high resolution.
- Expensive: > $10,000
- Bandwidth: 0.07 nm
- Stray light: 0.0008%

Double-dispersing Instrument



Diode Array Instruments

- Can be very compact in size.
- Fast scanning (~ 0.1 s).
- Range: 200 → 820 nm.
- Bandwidth: 2 nm.
- Cost: $3000 → $10.000.



Diode Array Instruments