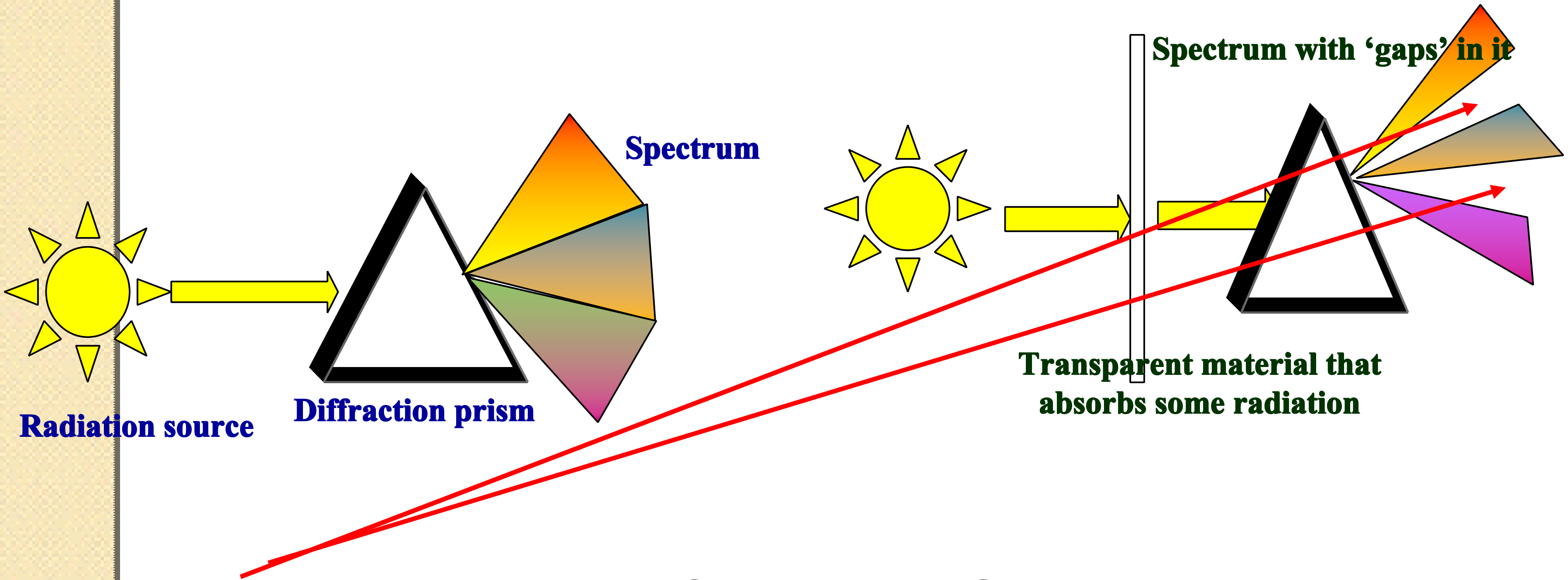
Ultraviolet (UV) Spectroscopy – Use and Analysis



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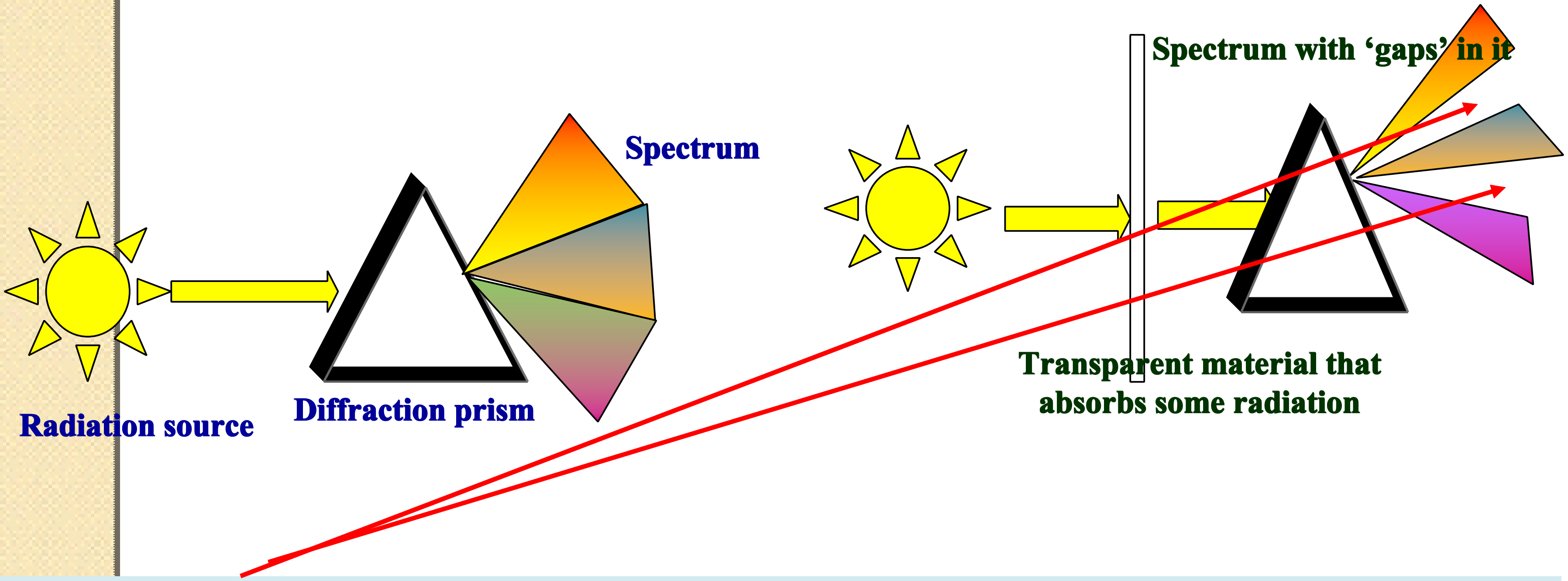
Ultraviolet (UV) Spectroscopy – Use and Analysis

When continuous wave radiation passes through a **transparent material (solid or liquid) some of the radiation might be absorbed by that material.**

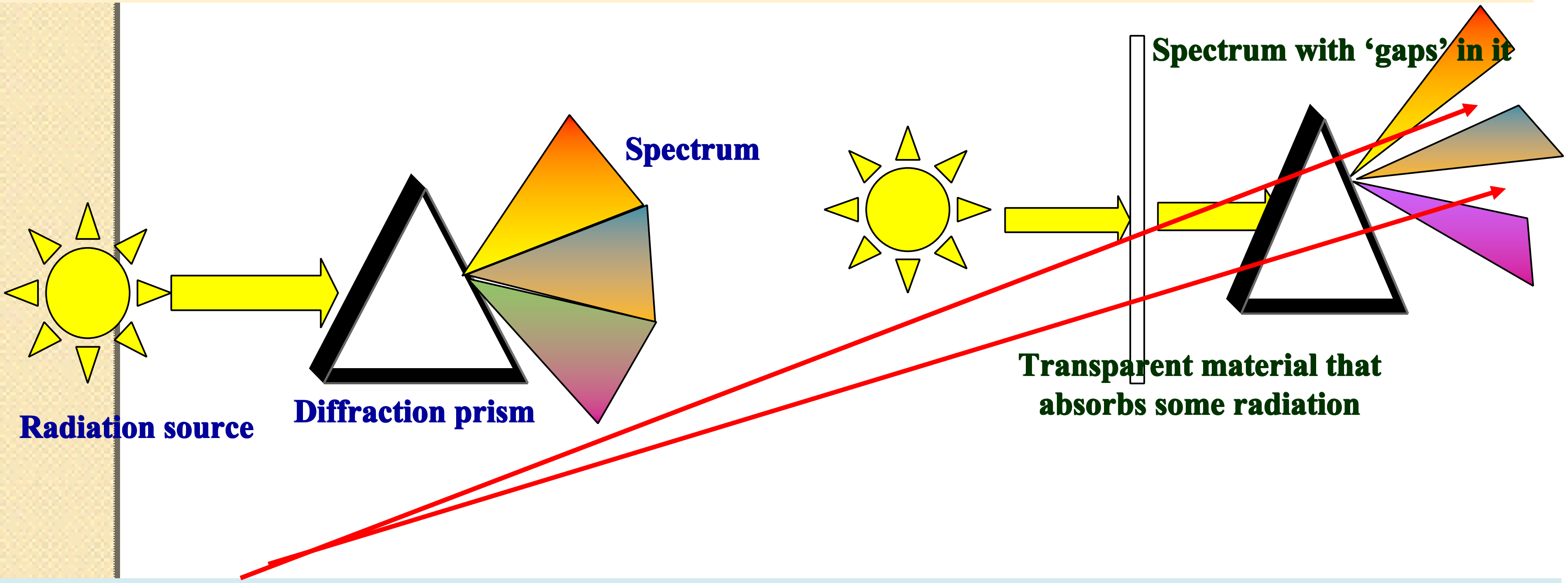


- The gaps in the light spectrum caused by the **absorption of radiation** by the transparent material through which is passed.

Ultraviolet (UV) Spectroscopy – Use and Analysis

When continuous wave radiation is **passed through a prism** a **diffraction pattern** is produced (**a spectrum**)

A spectrum is made up of **all the wavelengths** associated with the **incident radiation.**



- The gaps in the light spectrum caused by the **absorption of radiation** by the transparent material through which is passed.

Ultraviolet (UV) Spectroscopy

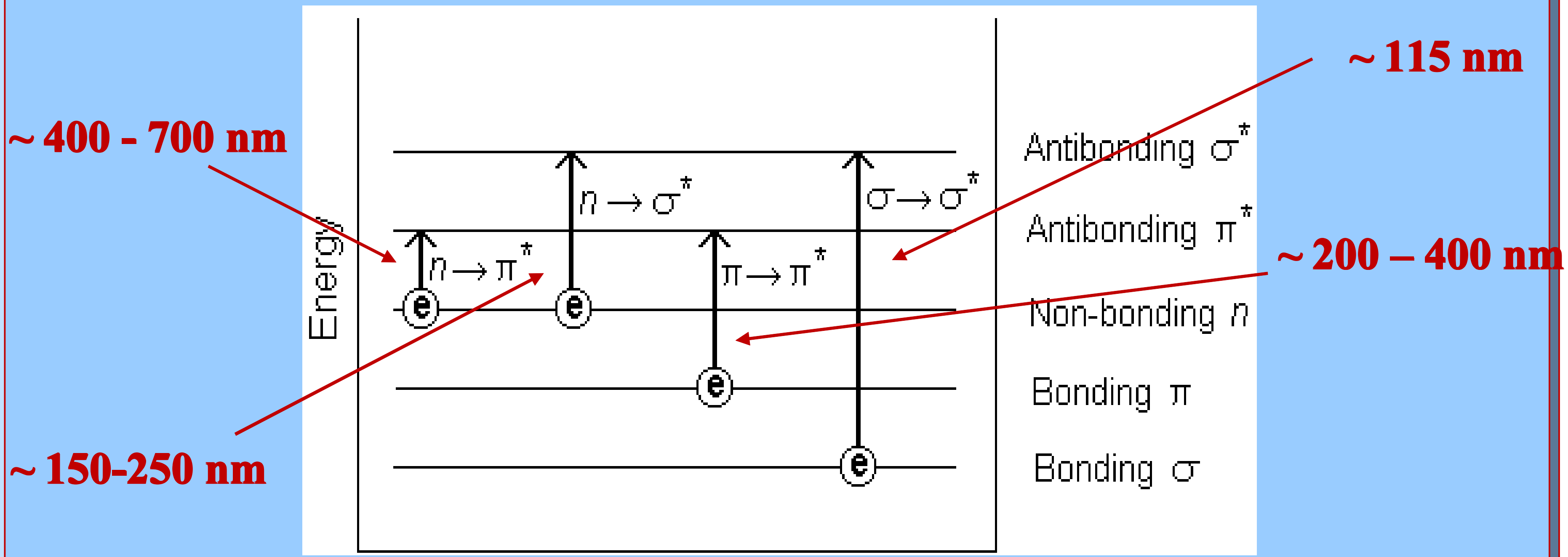
- **The absorption of UV radiation of energy causes transition of bonding electrons from a low energy orbital to a higher energy orbital**

missing' parts of the spectrum = **The energy difference between the orbitals involved in the transition.**

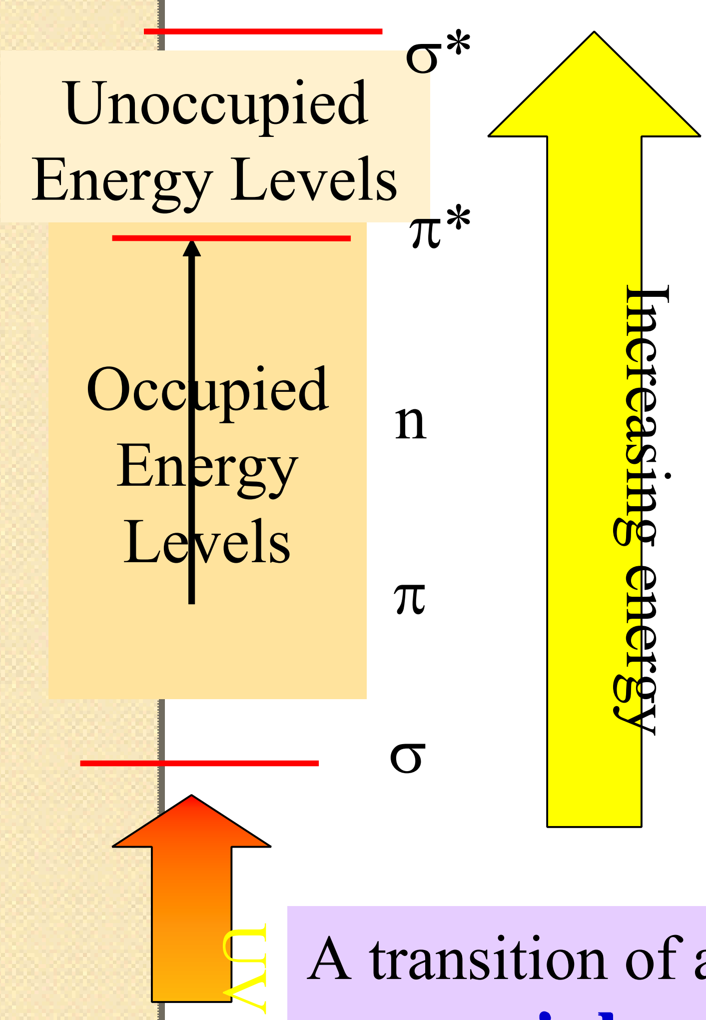
Absorption: Physical Basis

- Absorption occurs when the energy contained in a photon is **absorbed by an electron** resulting in a transition to an excited state
- **Since photon and electron energy levels are quantized, we can only get specific allowed transitions**

$E = h\nu$ ($h = 6.626 \times 10^{-34}$ J•s)



Ultraviolet (UV) Spectroscopy



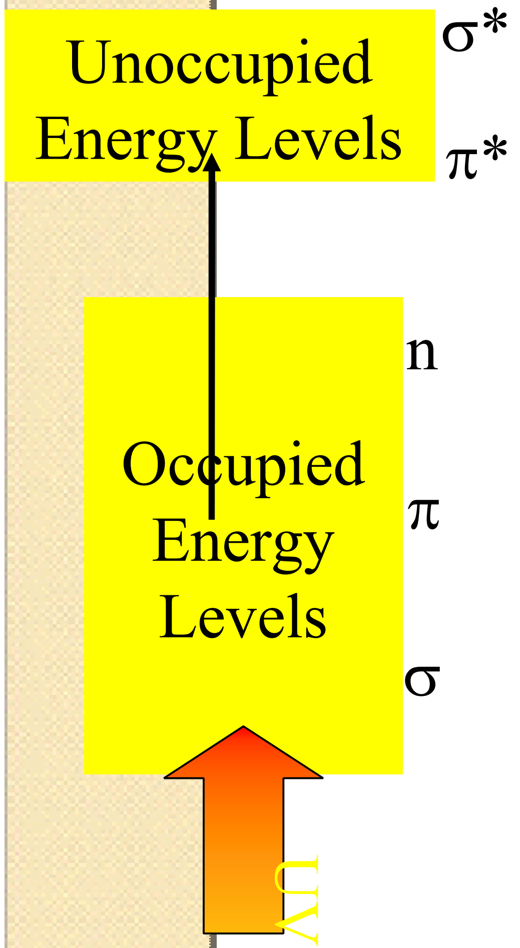
Antibonding orbitals are unoccupied in the ground state and can only be occupied by an electron in an excited state

- π* orbital
- σ* orbital

A transition of an electron **from occupied to an unoccupied energy level** can be caused by UV radiation.

π to π* implies that **UV is useful** with **compounds** containing **double bonds [200-400nm]**

Ultraviolet (UV) Spectroscopy



Types of orbitals might be occupied in the ground state

1- The σ-bonding orbitals

alkanes are low energy

C-C

2- The π-bonding Orbitals

all functional groups that contain double and triple bonds

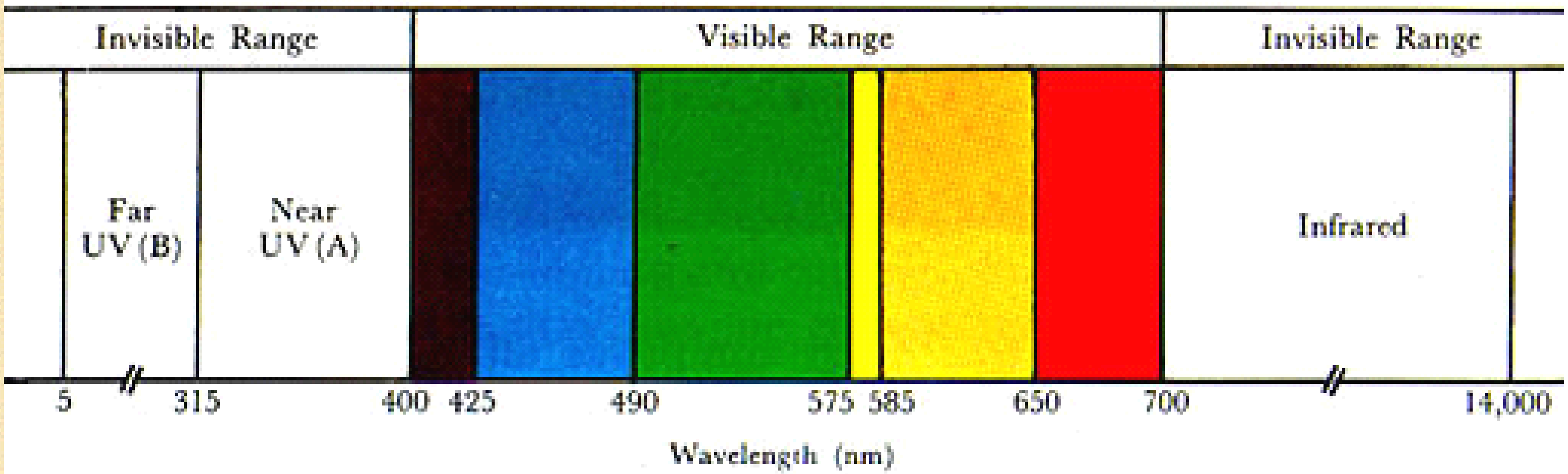
C=O, C=C

3- non-bonding orbitals

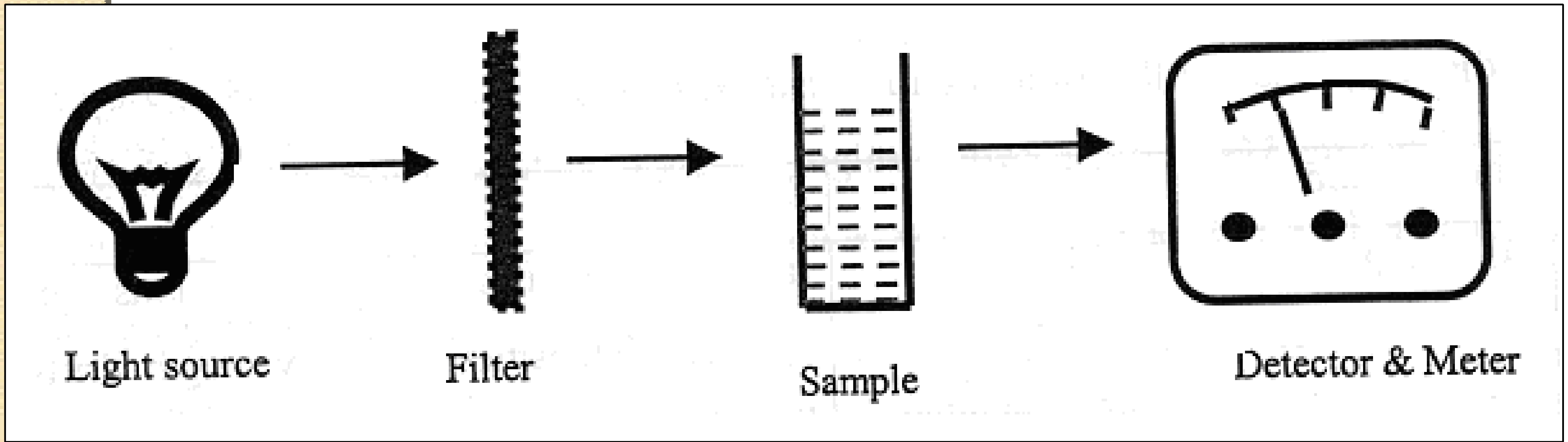
atoms that have **lone pair(s)** of electrons

O, N, S, Halogens

Radiation range	Visible range	UV range	UV – VIS range
Instrument	Colorimeter	UV spectrophotometer	UV-VIS spectrophotometer



Components of a colorimeter



Instrumentation

Optical path

1- Single beam

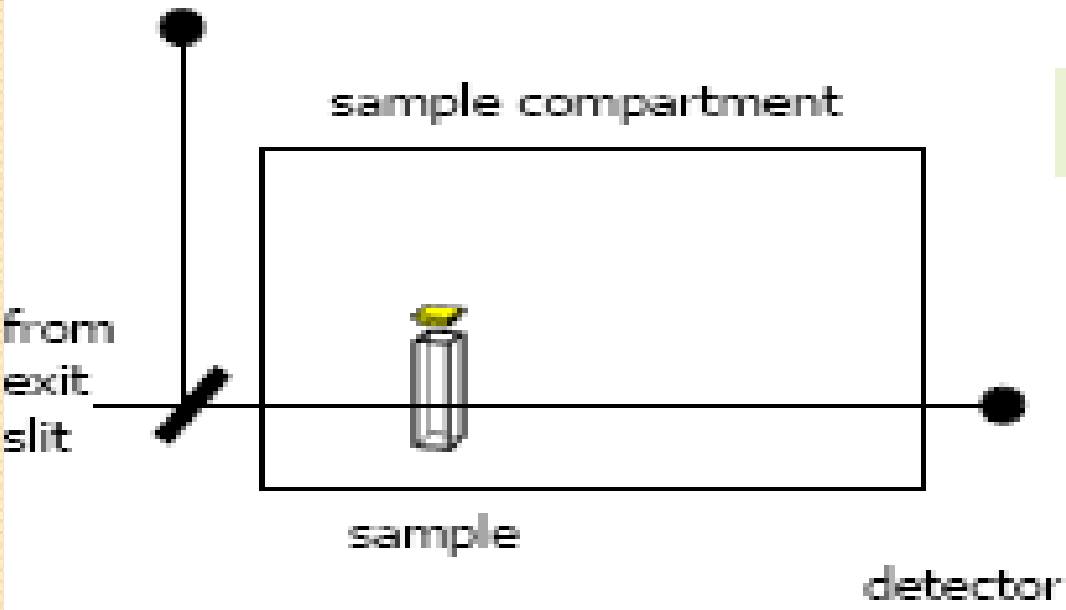
Light passes through sample

2- Double beam

Light passes through

- Sample — Sample beam
- Blank — Reference

Single beam



Advantages

a. Cheap

b. Useful when λ is known

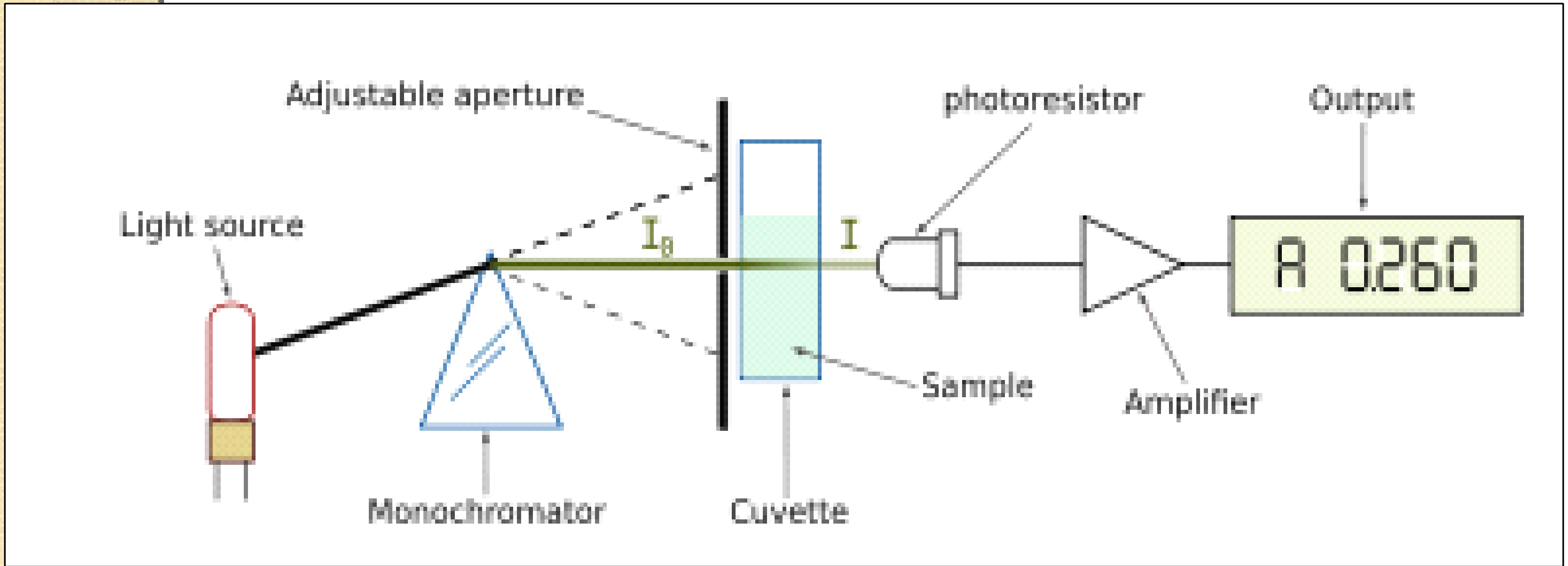
Disadvantages

a. Identification of λ_{max} is tedious

b. Time lag between blank & sample reading

1- Single beam

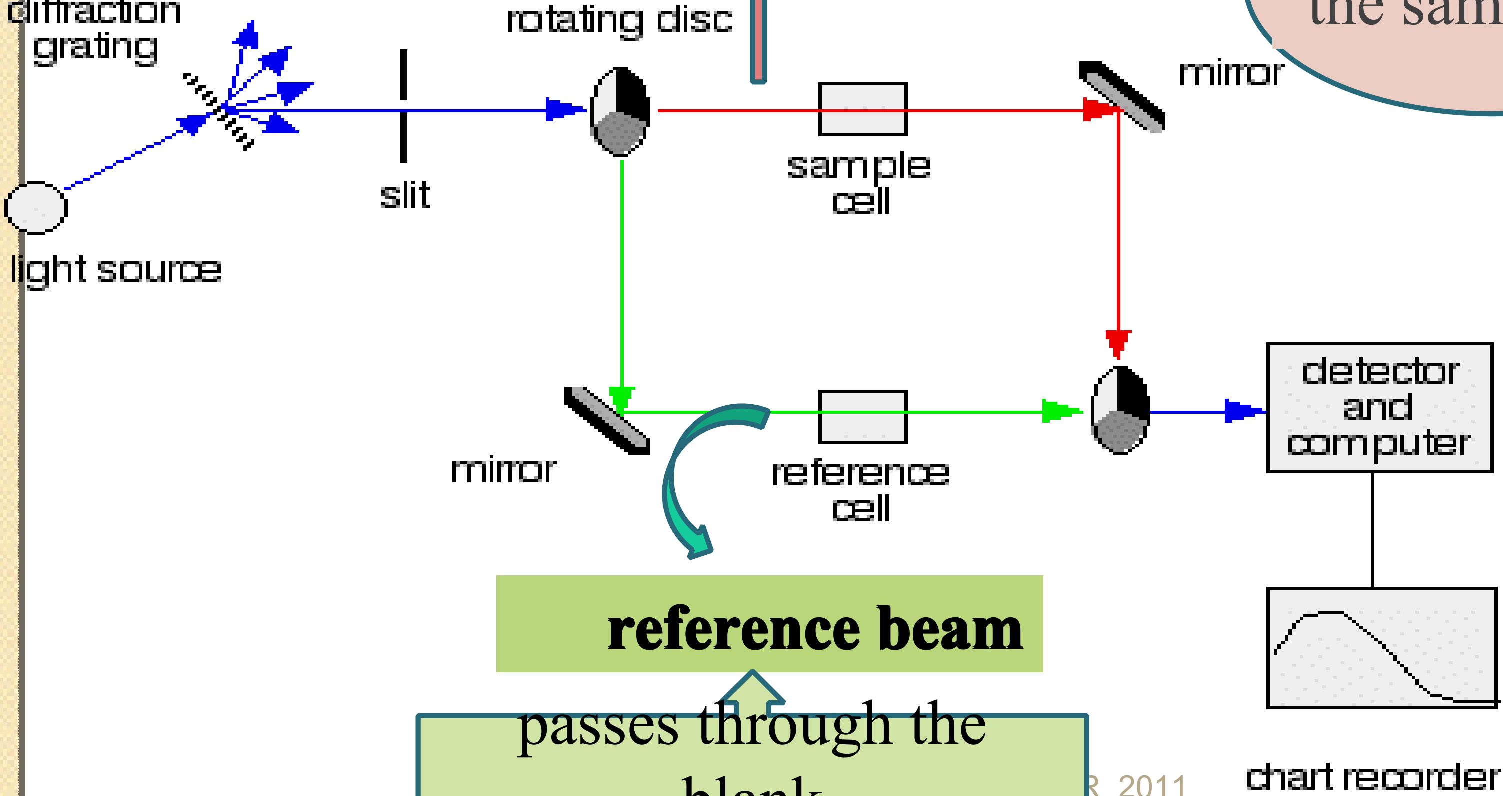
Light from monochromator passes only through sample solution before reaching the detector.



Double beam

Light from monochromator is split into two parallel beams:

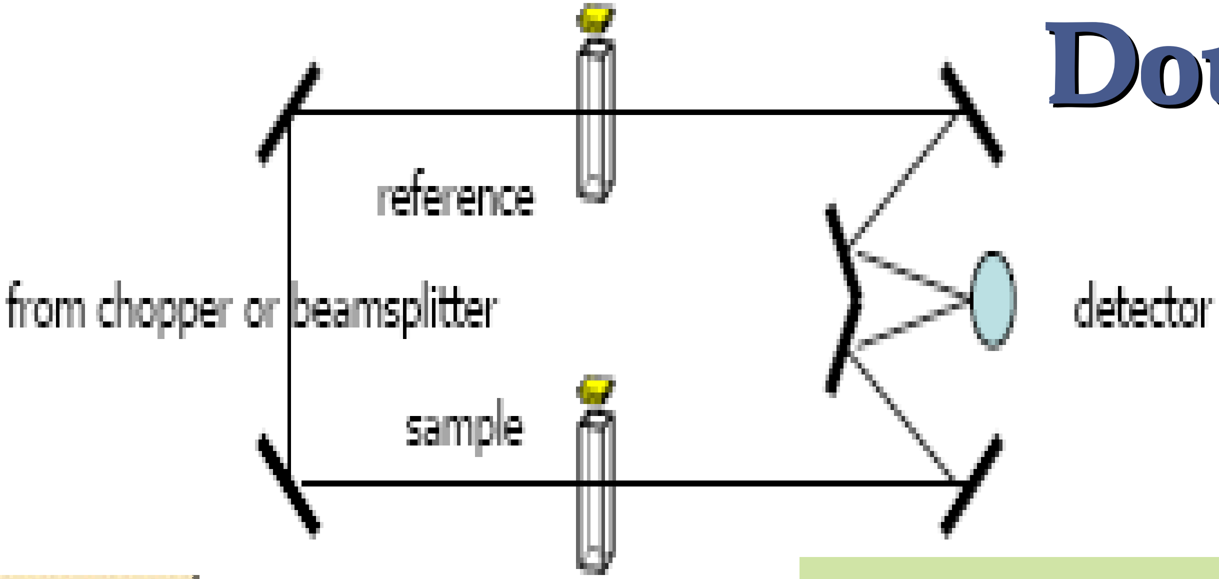
sample beam – passes through the sample



reference beam – passes through the blank

UV Visible spectrophotometer

Double beam



Advantages

a. Compensate for fluctuation in I & λ

b. Useful when λ_{max} is not known

c. λ_{max} easily obtained

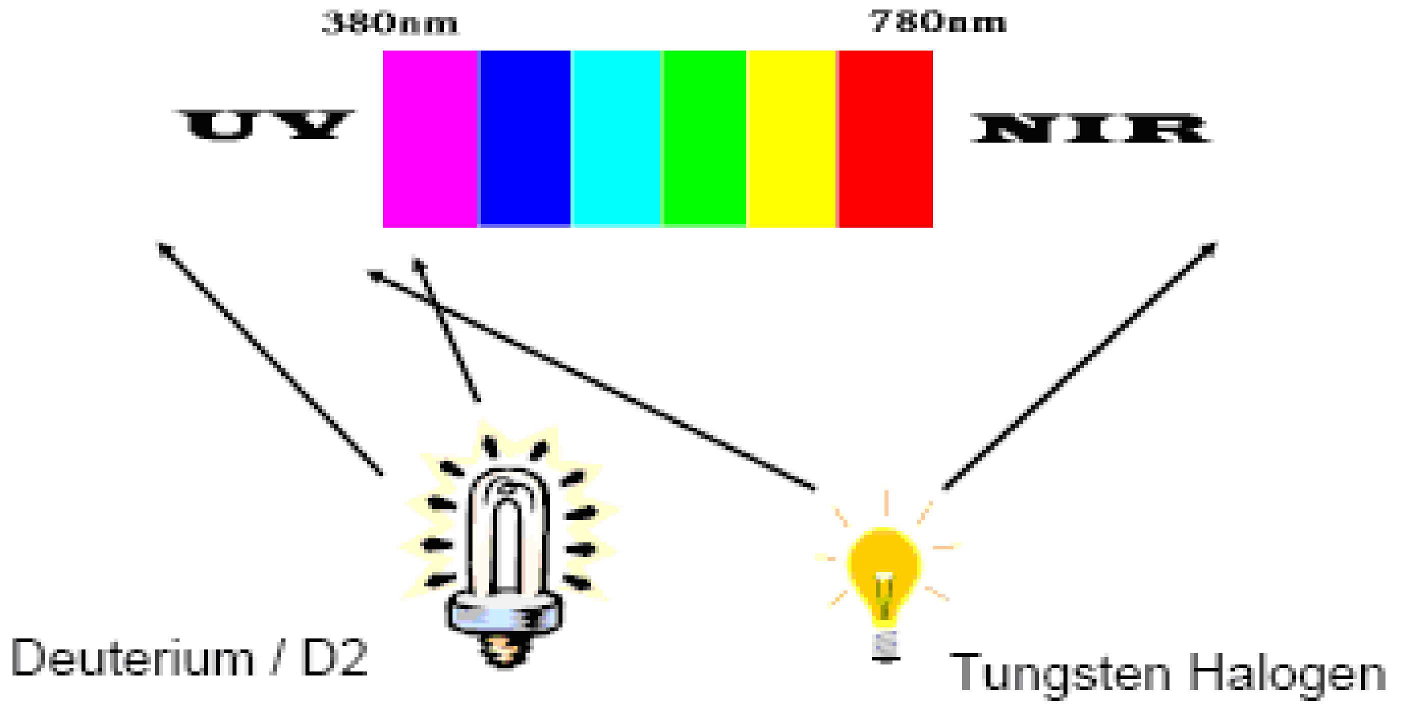
Disadvantages

a. Expensive

b. If reference beam does not pass through a blank then the I is not corrected.

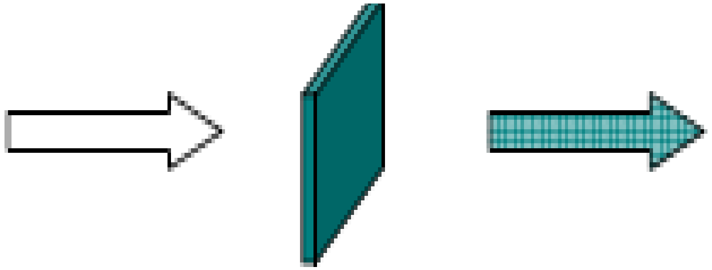
UV-VIS sources

- **Tungsten filament** — Visible & near IR
- **Deuterium lamp** — UV, 185 nm – 370 nm
- **Xenon Arc lamp** — UV-Vis

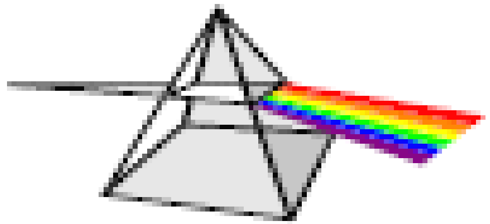


Monochromators

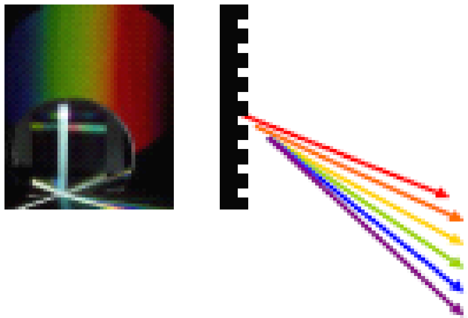
Disperse light into its component wavelengths and selects a narrow band of wavelengths to pass on to the sample or detector



Filter

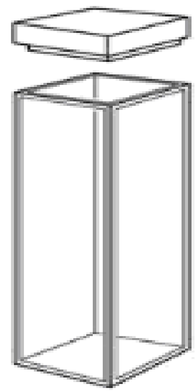


Prism



Grating

Sample compartment



Open-top
normal with lid

- **Cuvette:** **The cell which contain samples.**
- **Most common cuvettes have 1.00 cm pathlength**
    - **Made of fused silica, quartz, and glass**

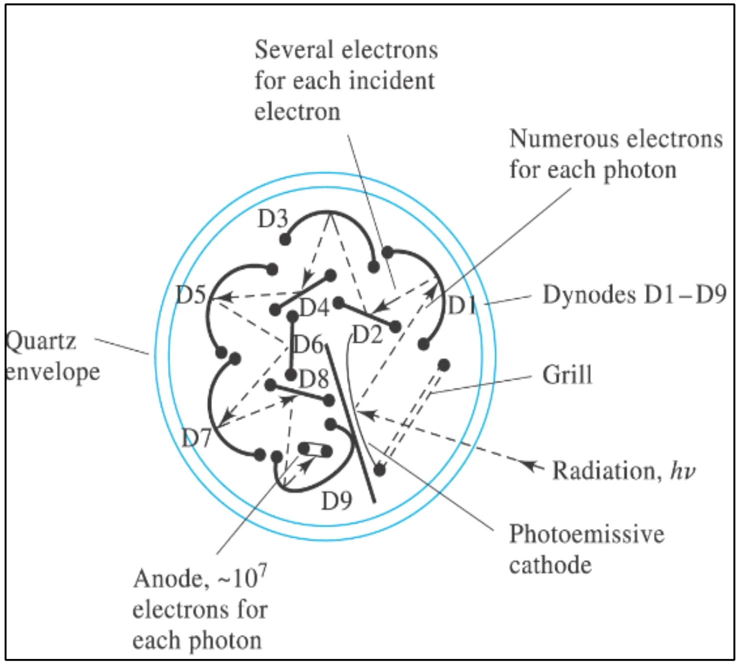
UV **Visible**

Glass absorbs ultraviolet radiation

Detector

converts radiation energy into an electrical signal for measurement

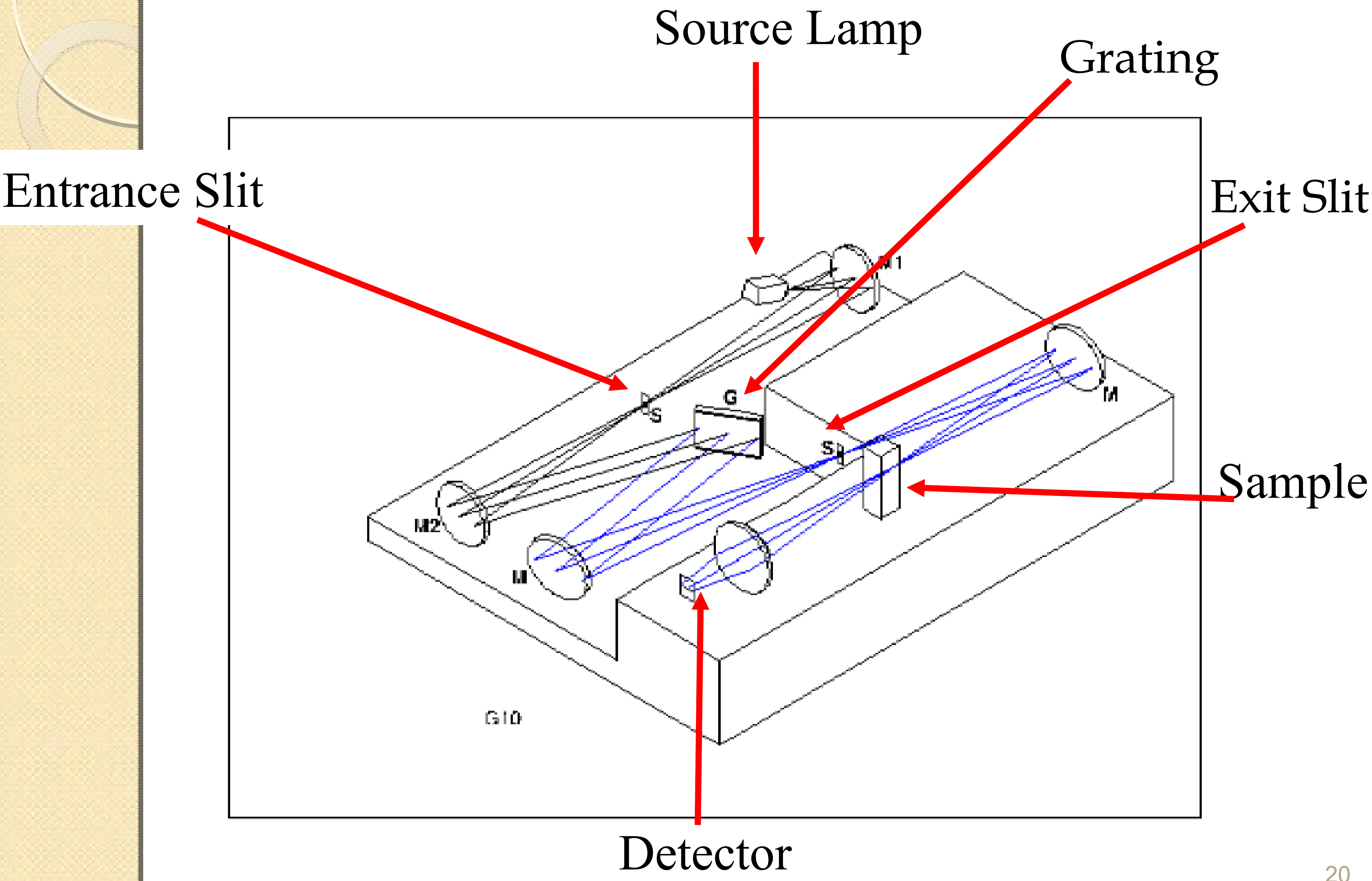
PMT Photo-multiplier tube



photodiodes

a semiconductor detector consisting of multiple individual diodes typically constructed from **silicon or germanium**

Overall instrument



Advantages of UV-VIS spectroscopy

1. **Applicable to organic, inorganic & biological samples.**

2. **High sensitivity ,**
   detection limit (10^{-5} M to 10^{-7} M)

3. **High selectivity**

4. **High accuracy, 95-99%**

5. **Easy automation**

Precautions & problems

1.Cuvette

- Use **plastic and glass** ones for measurements in the **visible region**
- Use **quartz cuvettes** for the **UV** measurements
- In **double-beam** instruments, **identical** cuvettes must be used for both blank and sample
- Avoid cuvettes with stains and/or scratches
- Wipe with tissue the **fingerprints** and liquids adhering to the outside walls.

Precautions & problems

1.Cuvette

2. Sample

- must be particle free in order to avoid scattering of radiation
- avoid contamination

3. Calibration of wavelength

- Use solution of known wavelength of maximum absorbance

Problems		Solutions
Interferences	a. If contaminant is not Known	liquid-liquid extraction/ solid phase extraction
	b. If contaminant is known	add it to sample and standards
Deviation from Beer-Lambart's law	A > I	Dilution of standards & unknown