UV Spectrophotometry (Technique)

UV Spectrophotometer (Instrument)

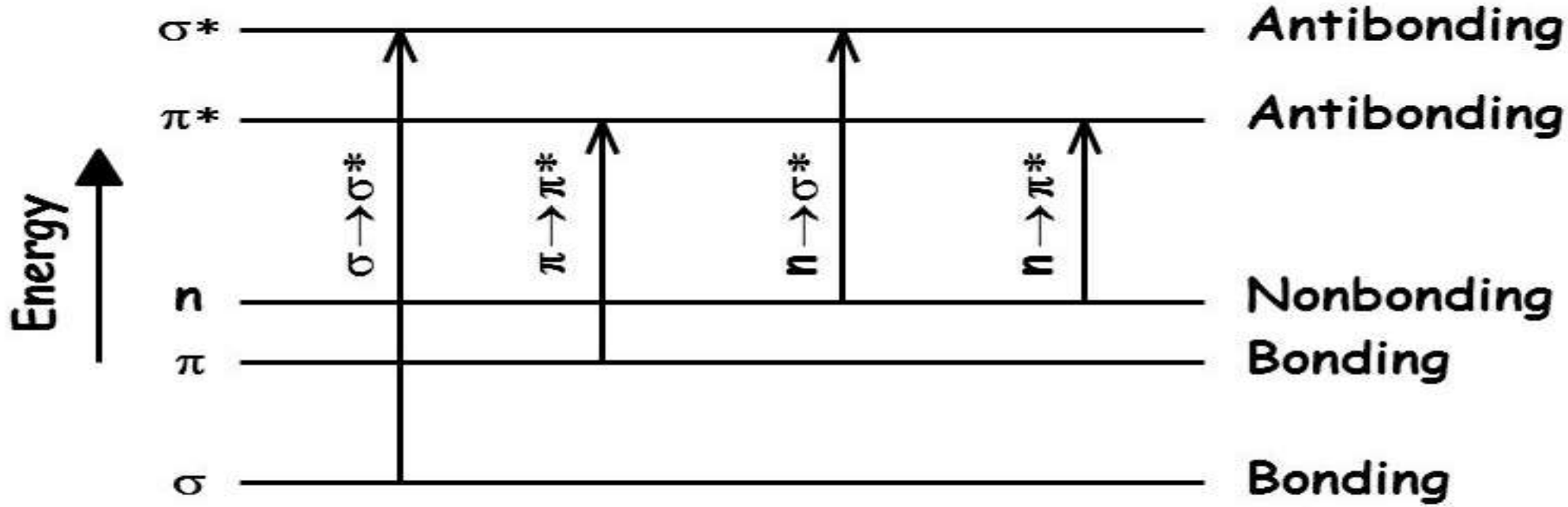
Introduction

- As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules
- Spectrophotometry is the measurement and interpretation of electromagnetic radiation absorbed when the molecules or atoms or ions of a sample moves from one energy state to another energy state
- UV spectrophotometry is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm) is absorbed by the molecule which results in the excitation of the electrons from the ground state to higher energy state

Principle

1. It is related to the interaction of light with matter
2. When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state
3. The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum which aids in the identification of the compound
4. Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals
5. There are four possible types of transitions (π–π*, n–π*, σ–σ*, and n–σ*), and they can be ordered as follows: σ–σ* > n–σ* > π–π* > n–π*

Bonding electrons appear in σ and π molecular orbitals
nonbonding in n



Electronic transitions can occur between various states.
The energy of the transitions increases in the following order:

$(n \rightarrow \pi^*) < (\pi \rightarrow \pi^*) < (n \rightarrow \sigma^*) < (\sigma \rightarrow \sigma^*)$

400-700 nm 200-400nm 150-250 nm 115 nm

Instrumentation

1. Light Source

- Tungsten filament lamps are rich in red radiations and emit the radiations of 375 nm
- Hydrogen-Deuterium lamps falls below 375 nm
- Xenon lamps for complete UV range

2. Monochromator

- Monochromators generally is composed of rotating prisms and slits
- The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelength to pass through the slits for recording purpose

3. Sample and reference cells

- Two cell, sample and reference solution
- One of the two divided beams is passed through the sample solution and second beam is passé through the reference solution
- These cells are made of either silica or quartz
- Glass can’t be used for the cells as it also absorbs light in the UV region

4. Detector

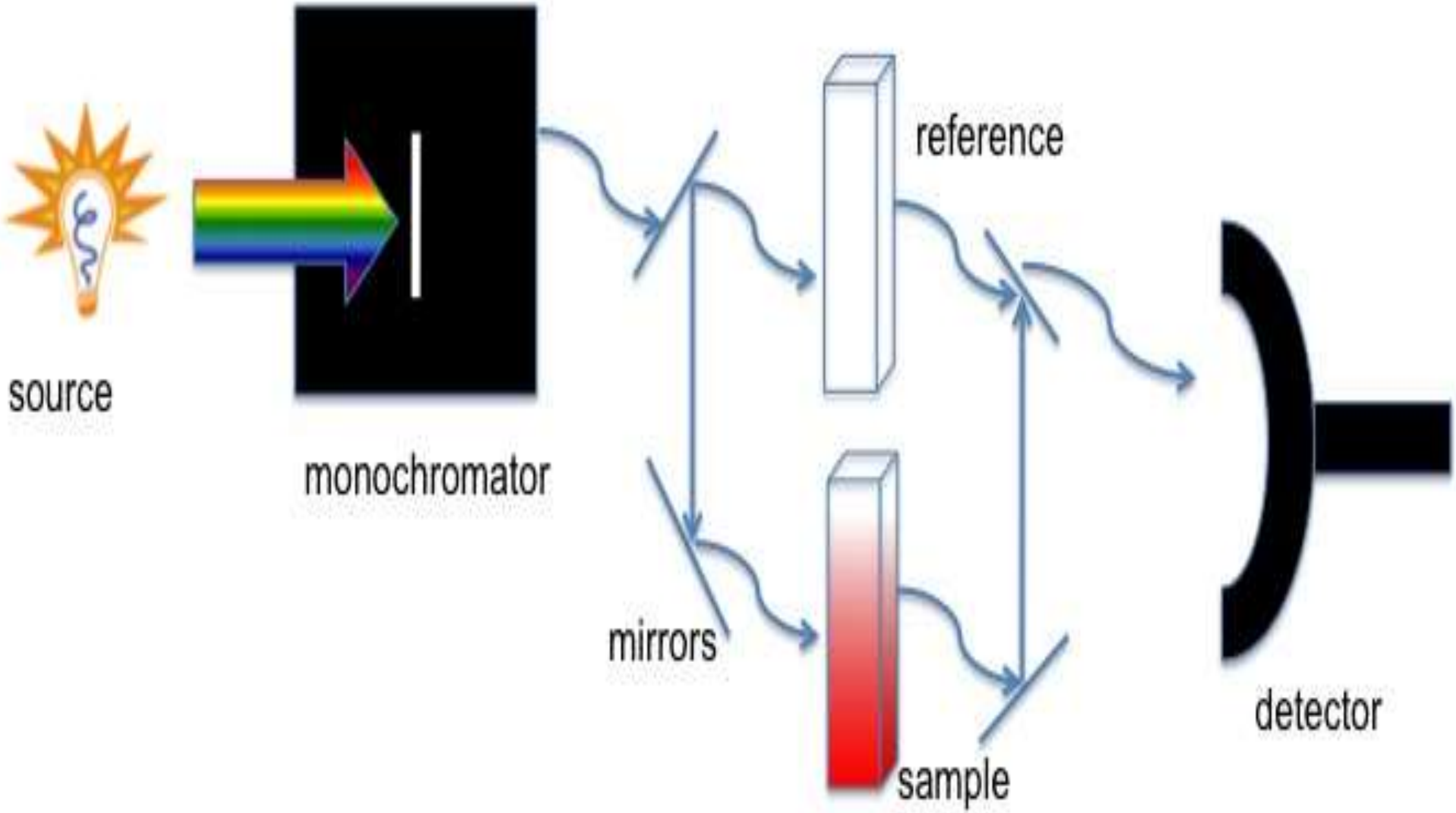
- Generally two photocells serve the purpose of detector
- One of the photocell receives the beam from sample cell and second detector receives the beam from the reference
- The intensity of the radiation from the reference cell is stronger than the beam of sample cell.
- This results in the generation of pulsating or alternating currents in the photocells

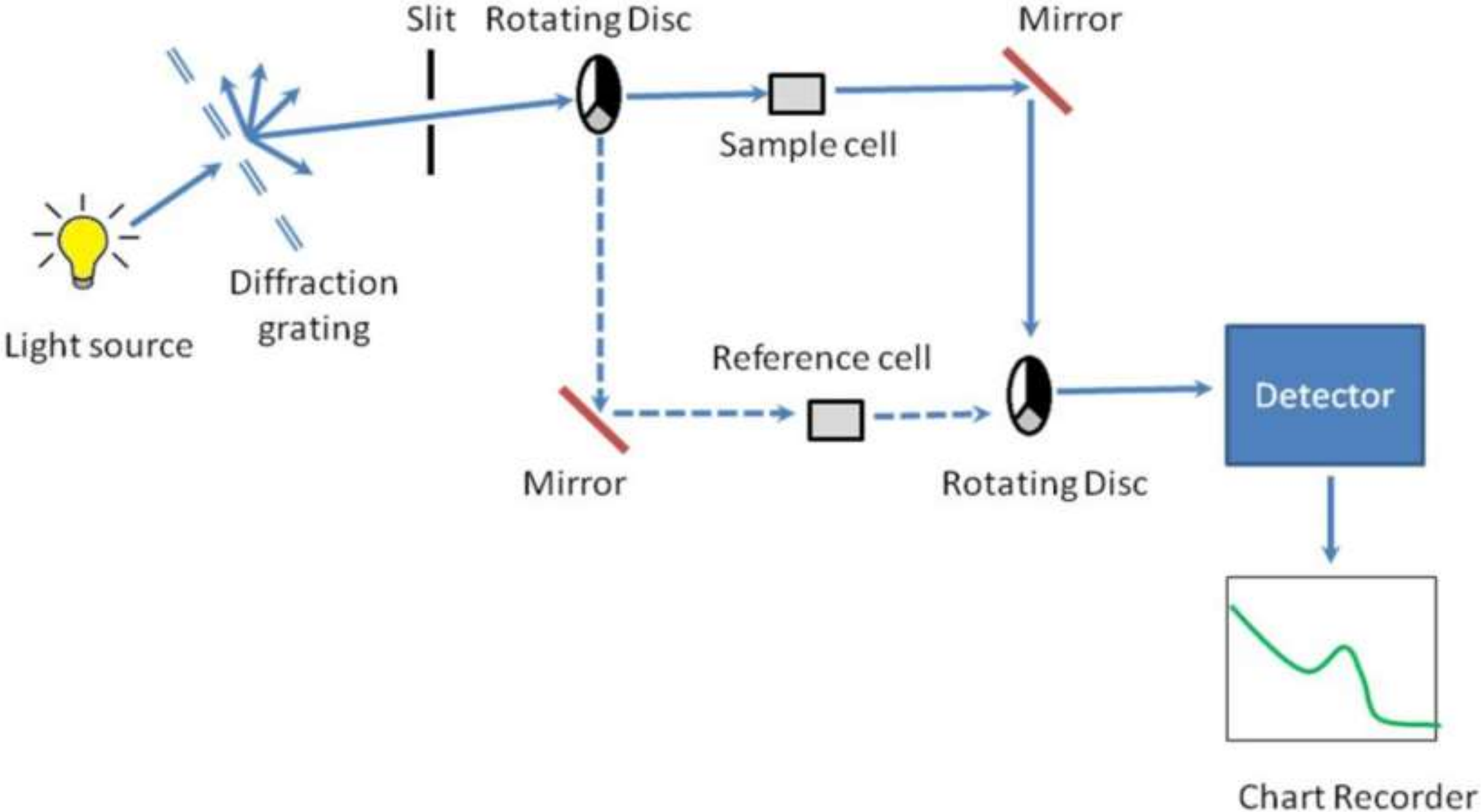
5. Amplifier

- Generally current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear and recordable signals.
- The amplifier is coupled to a small servo meter

6. Recording devices

- Most of the time amplifier is coupled to a pen recorder which is connected to the computer
- Computer stores all the data generated and produces the spectrum of the desired compound


source
monochromator
reference
mirrors
sample
detector


Slit
Rotating Disc
Mirror
Sample cell
Light source
Diffraction grating
Reference cell
Detector
Mirror
Rotating Disc
Chart Recorder

Applications

1. Detection of Impurities

- Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material
- By also measuring the absorbance at specific wavelength, the impurities can be detected

2. Structure elucidation of organic compounds

- It is useful in the structure elucidation of organic molecules, such as in detecting the presence or absence of unsaturation, the presence of hetero atoms.

3. Quantitative determination of compounds

4. Characterization of compounds which absorbs UV radiation

- Identification is done by comparing the absorption spectrum with the spectra of known compounds

5. Detection of presence or absence of functional group in the compound

- Absence of a band at particular wavelength regarded as an evidence for absence of particular group