

MODULE-3

SPECTROPHOTOMETRIC TECHNIQUES

Electromagnetic spectrum

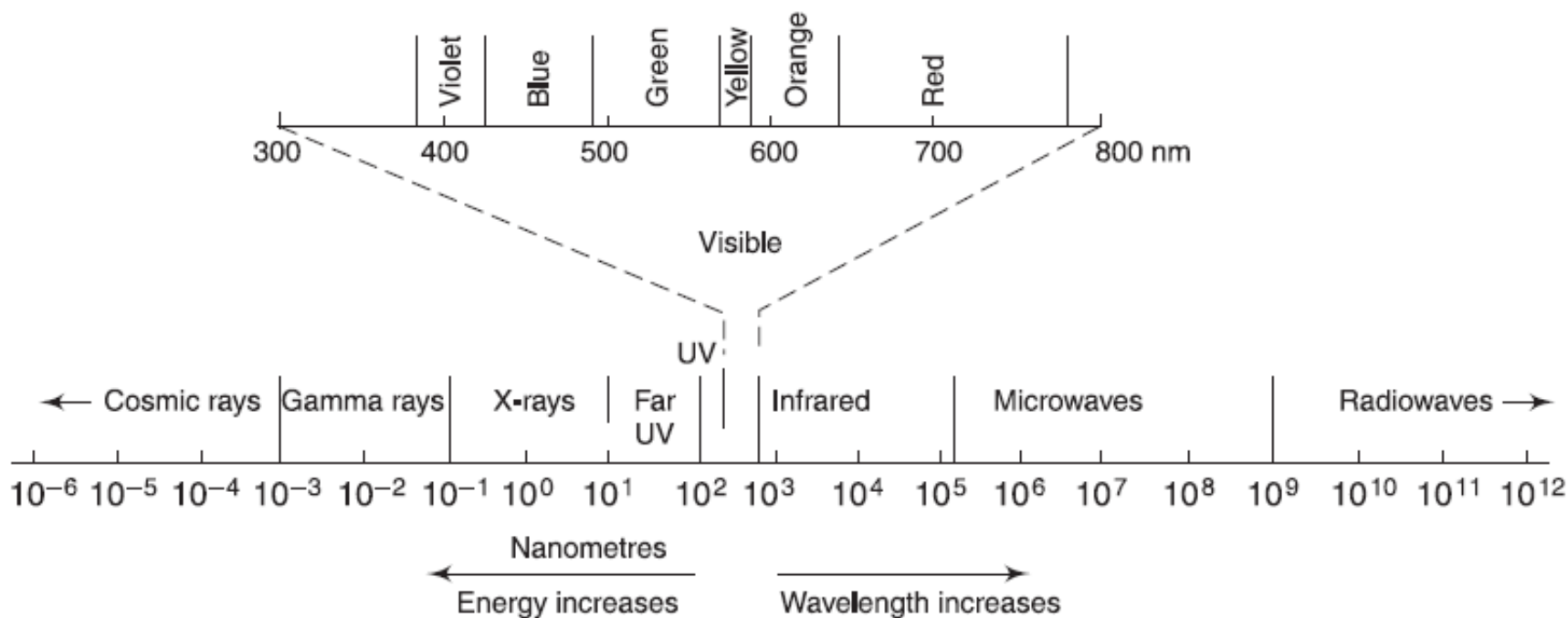
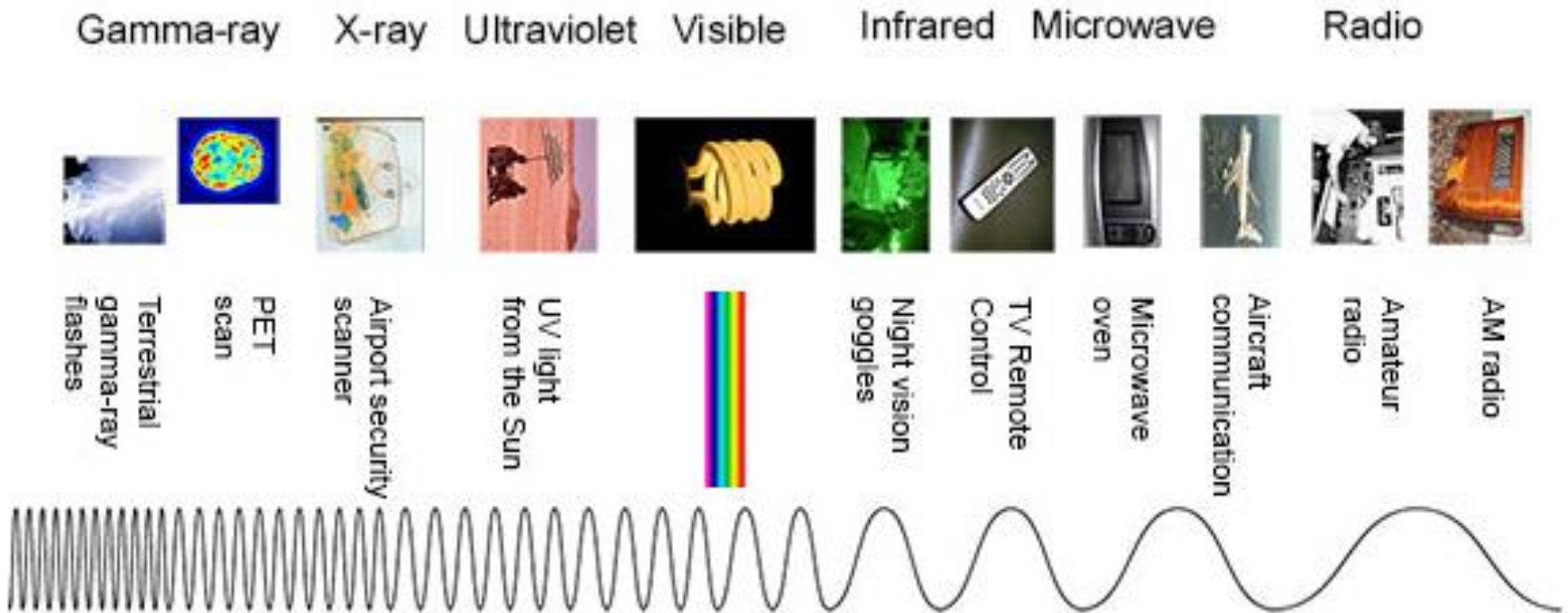


Figure: Various regions in the electromagnetic spectrum which are normally used in spectroscopic work

Electromagnetic spectrum



As wavelength increases frequency decreases.

Courtesy: <https://imagine.gsfc.nasa.gov/science/toolbox/emspectrum1.html>

Classification of spectroscopic techniques

The different types of spectroscopy include:

- a. Based on the level of study
- b. Based on the property of either absorption or emission.
- c. Based on the level of study i.e. electronic or magnetic levels.

a) Based on the level of study- Here the classification is done based on the study made at atomic or molecular level. When light is allowed to pass through the substance, changes in atomic level or molecular level is observed.

1) Atomic spectroscopy: Here energy changes takes place at atomic levels. The measurement is done to study the atoms and their quantity. There are two types: atomic absorption spectroscopy and atomic emission spectroscopy.

2) Molecular spectroscopy: Here the energy changes occurring at the level of molecule are studied. The characters like molecular absorption, emission and vibration are studied. The examples include, colorimetry, UV-spectroscopy, infrared, FTIR, fluorimetry etc.

B) Based on the property of either absorption or emission.

Here the principle of absorption or emission of electromagnetic radiation is taken into consideration.

1) Absorption spectroscopy: As the name suggests, here there is absorption of light by the sample. The extent of absorption and the wavelength of the absorbed light is considered. The wavelength of light absorbed tells the nature of the compound while the intensity of absorbed light tells the concentration

- The examples of the spectroscopic methods are colorimetry, UV-spectroscopy, infrared spectroscopy, NMR spectroscopy, atomic absorption spectroscopy.

2) Emission spectroscopy: Here the emitted light is measured. Once the light impinge on the sample, some of it is absorbed.

- This absorption of light leads to transition of electrons from ground state to excited state.
- These excited electrons return back to ground state by release of electromagnetic radiation (light) of specific wavelength.
- The intensity gives the concentration while the wavelength tells the nature of the compound.
- Examples of this method are fluorescence spectroscopy, flame photometry.

C) Based on the level of study i.e. electronic or magnetic levels.

Here the study is done based on electronic or magnetic properties of the compound. Light is an electromagnetic radiation. That is it has both electronic and magnetic properties.

- **Electronic spectroscopy:** So when a compound is estimated without the magnetic field we call it electronic spectroscopy. In this method the substance under test is exposed to light without the influence of magnetic field.
- Examples of this method are colorimetry, UV visible spectroscopy, IR, fluorimetry etc.
- **Magnetic spectroscopy:** Here the substance is exposed to electromagnetic radiation in presence of external magnetic field.
- Examples include Nuclear magnetic resonance spectroscopy (NMR), Electron spin resonance spectroscopy (ESR).

Laws relating to absorption of radiation

- When a beam of light is allowed to pass through a transparent medium the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of light.
- Laws relating to absorption of radiation:
 - Lambert's Law
 - Beer's Law
 - The Beer-Lambert Law

Lambert's Law

- Lambert's Law states that each layer of equal thickness of an absorbing medium absorbs an equal fraction of the radiant energy that traverses it.
- Let us suppose, I_0 is the incident radiant energy and I is the energy which is transmitted.
- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample is known as the transmittance.

- Lambert's Law is expressed as:

$$\text{Transmittance } T = I / I_0$$

- It is usually expressed as a percentage

$$\% \text{ Transmittance} = I / I_0 \times 100$$

- The logarithm of the reciprocal of the transmittance is known as absorbance.
- Absorbance = $\log_{10} (1/T) = \log_{10} (I_0/I)$

Beer's Law

- This law states that the absorption of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium in the light path.

The Beer-Lambert Law

- A combination of the two laws, known jointly as the Beer-Lambert Law defines the relationship between absorbance (A) and transmittance (T).
- It states that the concentration of a substance in solution is directly proportional to the 'absorbance', A, of the solution.

$$\text{Absorbance} = A = c\epsilon b$$

where: A = absorbance

c = concentration

ϵ = molar absorptivity (how much light is absorbed by 1 mole of a molecule)

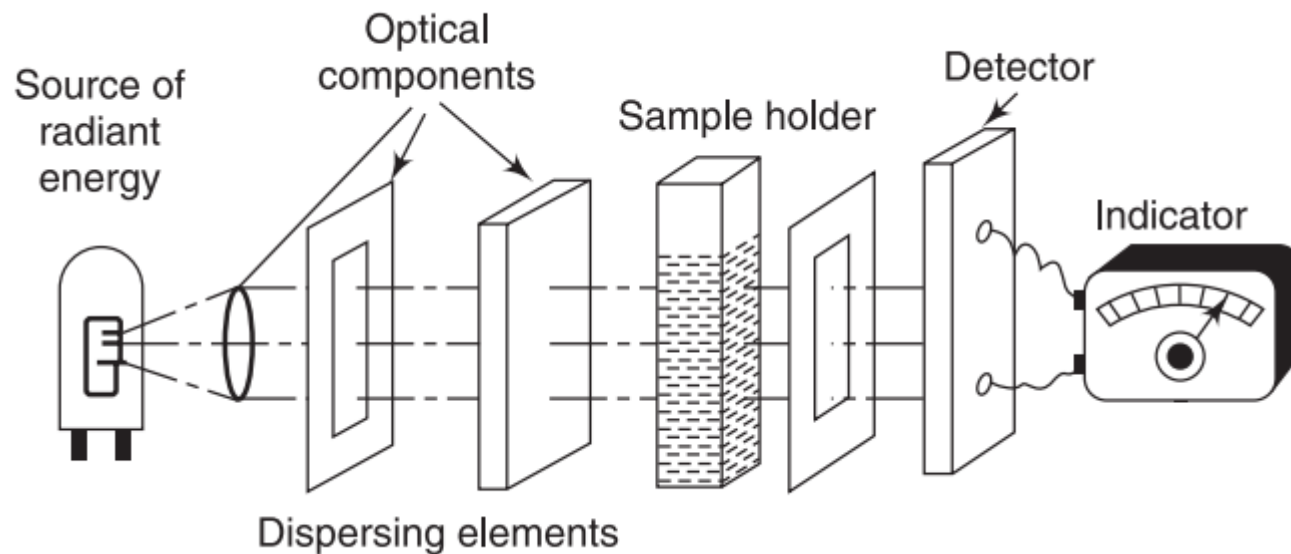
b = length of the path that the light travels

- ϵ is a function of wavelength. So, the Beer-Lambert Law is true only for light of a single wavelength or monochromatic light.

- Absorptivity is constant, depending upon the wavelength of the radiation and nature of the absorbing material.
- Absorbance is related to percentage transmittance T by the expression:

$$A = \log_{10} (I_0/I) = \log_{10} (100/T) = \epsilon bc$$

Various components of absorption instrument



The essential components are:

- A source of radiant energy, which may be a tungsten lamp, xenon-mercury arc, hydrogen or deuterium-discharge lamp, etc
- Filtering arrangement for selection of a narrow band of radiant energy. It could be a single wavelength absorption filter, interference filter, a prism or a diffraction grating.
- An optical system for producing a parallel beam of filtered light for passage through an absorption cell (cuvette). The system may include lenses, mirrors, slits, diaphragm, etc.
- A detecting system for measurement of unabsorbed radiant energy, which could be human eye, barrier-layer cell, phototube or photomultiplier tube.
- A readout system or display, which may be an indicating metre or numerical display.

Radiation Sources

- The function of the radiation source is to provide sufficient intensity of light suitable for making a measurement.
- The most common and convenient source of light is the tungsten lamp.
- Lamps convert electrical energy into radiation.
- Different designs and materials are needed to produce light in different parts of the electromagnetic spectrum.

Blackbody sources:

- A hot material, such as an electrically heated filament in a light bulb, emits a band of spectrum of light.
- The spectrum is approximated by Planck's radiation law for blackbody radiators:

$$B = \left\{ 2h \frac{\nu^3}{c^2} \right\} \left\{ \frac{1}{\exp \frac{h\nu}{kT} - 1} \right\}$$

Where h is Planck's constant, ν is frequency, c is the speed of light, k is the Boltzmann constant, and T is temperature in K.

- The most common incandescent lamps and their wavelength ranges are:
 - Tungsten filament lamps : 350 nm–2.5 mm
 - Glowbar : 1–40 mm
 - Nernst glower : 400 nm–20 mm



Figure: A tungsten lamp

- Tungsten lamps are used in visible and near infrared (NIR) absorption spectroscopy, glowbar, and Nernst glower are used for infrared spectroscopy.
- A major portion of the energy emitted by a tungsten lamp is in the visible region and only about 15–20% is in the infrared region.
- For work in the UV region, a hydrogen or deuterium-discharge lamp is used.

- The radiation from the discharge lamps is concentrated into narrow wavelength regions of emission lines.
- Practically, there is no emission beyond 400 nm in these lamps.
- Nernst filaments are operated at lower temperatures and radiates sufficient energy.
- The curves in figure show the energy distribution for a tungsten filament at three different temperatures.
- Such radiation is known as 'black body radiation'.

- In the figure, we can observe that how the emitted energy increases with temperature and how the wavelength of maximum energy shifts to shorter wavelengths.

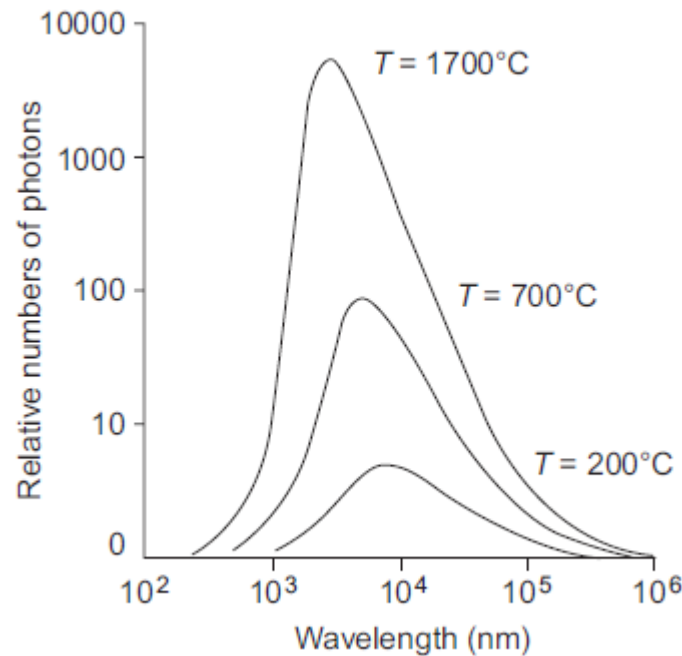


Figure: Tungsten filament radiation characteristics

The tungsten-halogen lamp:

- The introduction of the tungsten-halogen light source has a higher intensity output than the normal tungsten lamp in the region of 320–380nm and are used in colorimetry, spectrophotometry.
- The tungsten filament evaporates by releasing particles.
- The evaporated tungsten combines with halogen gas within the glass envelope to create tungsten – halogen molecule.

- The tungsten – halogen molecule then migrates back to the filament, eliminating blackening of the glass envelope.
- The tungsten is re-deposited and recycled onto the filament, strengthening the filament, extending the life of the lamp and the halogen gas is then free to start the cycle again.

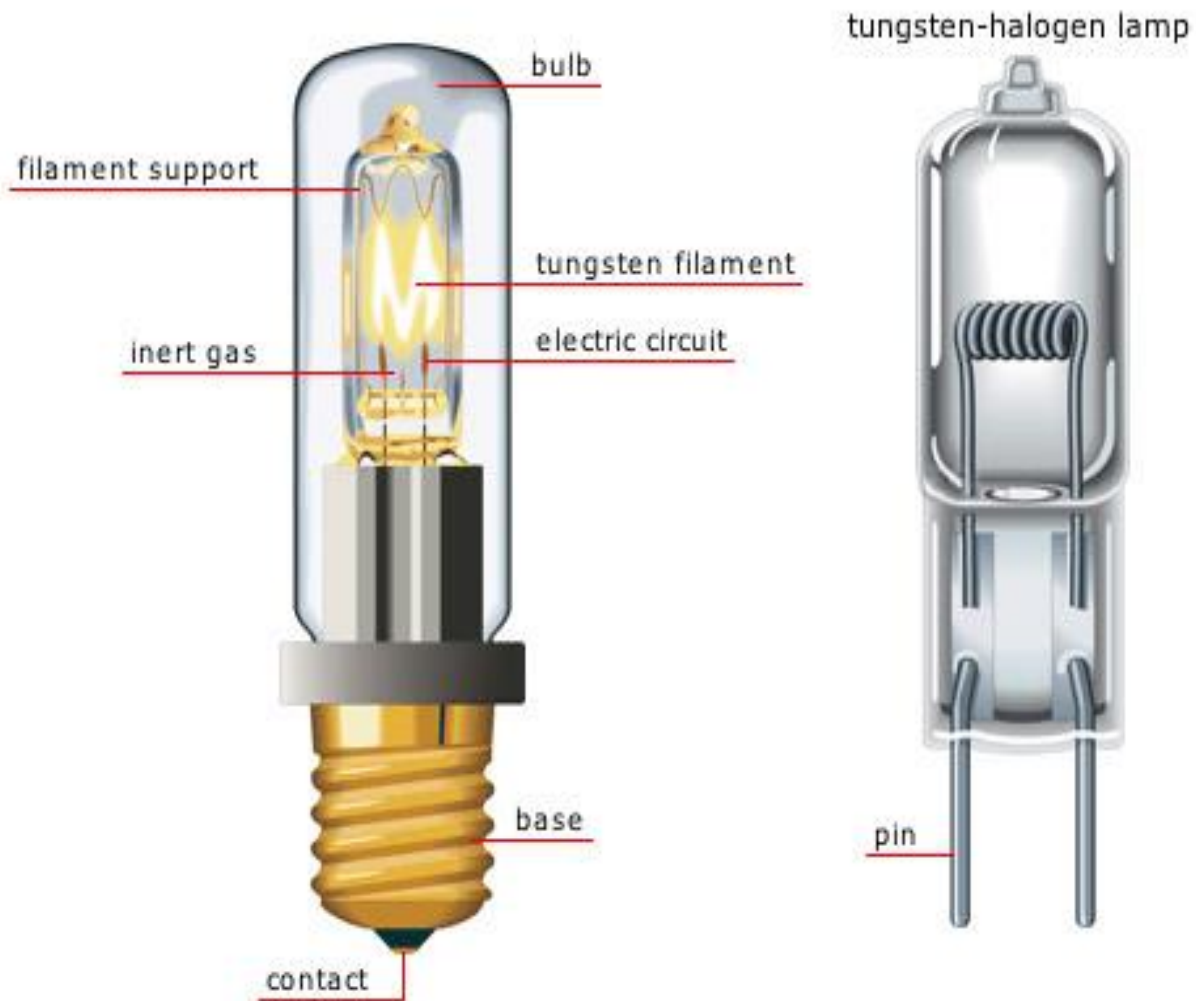


Figure: The tungsten-halogen lamp

Deuterium tungsten-halogen light source:

- A combination of deuterium tungsten-halogen light source combines the continuous spectrum of a deuterium UV light source and a tungsten-halogen VIS/Shortwave NIR light source in a single optical path.
- The combined light source produces a powerful, stable output from ~ 200 – $1,100$ nm.

Discharge lamp

- Discharge lamps, such as neon, pass an electric current through a rare gas or metal vapour to produce light.
- The electrons collide with gas atoms, exciting them to higher energy levels which then decay to lower levels by emitting light.
- Common discharge lamps and their wavelength ranges are given below:
 - Hydrogen or deuterium: 160–360 nm
 - Mercury: 253.7 nm, and weaker lines in the near UV and visible
 - Ne, Ar, Kr, Xe discharge lamps: many sharp lines throughout the near UV to near-IR
 - Xenon arc: 300 – 13 nm

- Deuterium lamps are the UV source in UV-Vis absorption spectrophotometers.
- For fluorescent work, an intense beam of UV light is required. This requirement is met by a Xenon arc or a mercury vapour lamp.
- Mercury lamps are usually run direct from the AC power line via a series ballast choke (fast warm up to the lamp to obtain operating temperature is).

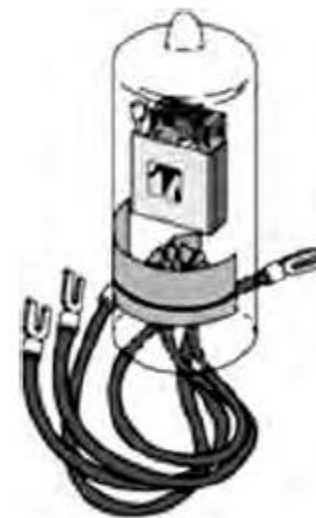


Figure: A deuterium lamp of the type used in spectrophotometers

- A deuterium arc lamp provides emission of high intensity and adequate continuity in the range of 190–380 nm.
- A quartz or silica envelope is necessary not only to provide heat shield, but also to transmit the shorter wavelengths of the UV radiation.
- Figure shows the energy output as a function of wavelength in case of deuterium arc lamp and tungsten-halogen lamp.

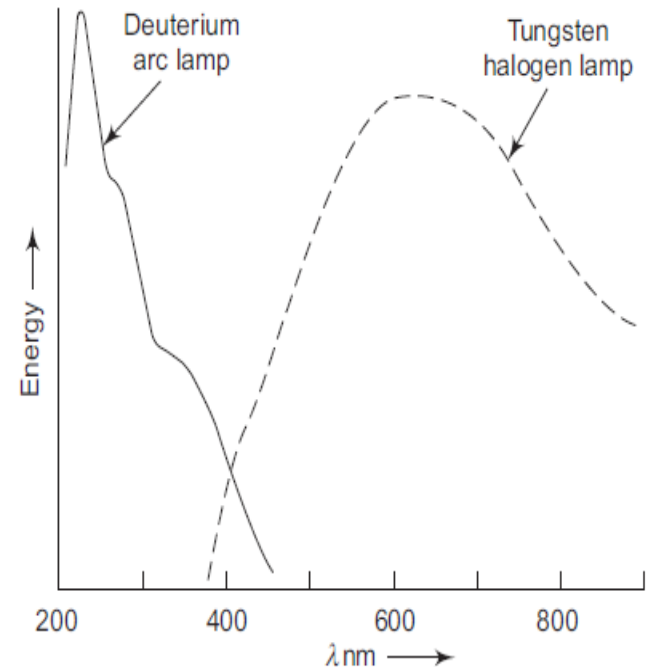


Figure: Energy output as a function of wavelength for deuterium arc lamp and tungsten halogen lamp

Lasers

- The term laser has been coined by taking the first letters of the expression 'Light amplification by simulated emission of radiation'.
- The laser beam has spatial and temporal coherence, and is monochromatic (pure wavelength).
- The beam is highly directional and exhibits high density energy which can be finely focused.
- The advantages include higher intensity, monochromaticity, low beam divergence, the availability of short and ultrashort pulses for studies of transient phenomena, and coherence (well-defined phase).
- Lasers have been used in many spectroscopic applications such as Raman Spectroscopy, UV/visible spectrophotometry.

Light Emitting Diodes (LEDs)

- The development of LEDs has resulted in the appearance of a new optical light source in analytical instrumentation.
- The first LED was developed in 1962 based on GaAsP layers, which emitted red light.
- Significant advances in III-V nitride manufacturing processes have resulted in high power commercially available LEDs in the region of 247–1,550 nm, covering UV, visible and near infrared regions.
- The advantages include increased lifetime, low cost, reduced power consumption, higher brightness, rugged construction, flexible configuration, enhanced spectral purity, small size, and breadth of spectral range.
- The most popular type of tri-colour LED has a red and a green LED combined in one package with three leads.
- When both the red and green LEDs are turned on, the LED appears to be yellow.

- The centre lead (K) is the common cathode for both LEDs, the outer leads (A1 and A2) are the anodes to the LEDs allowing each one to give separately, or both together to give the third colour.
- The use of bi-/tri- colour LEDs can provide a compact rugged multi-wavelength photometer source that can facilitate multi-component analysis.

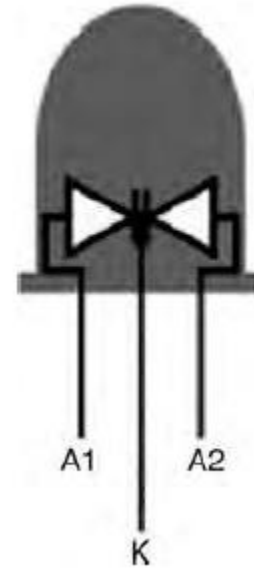


Figure: Schematic of Tri-colour LED

Optical Filters

- A filter may be considered as any transparent medium which by its structure, composition or colour enables isolation of radiation of a particular wavelength.
- For this purpose, ideal filters should be monochromatic (i.e. they must isolate radiation of only one wavelength).
- A filter must meet the following two requirements:
 - (a) high transmittance at the desired wavelength and
 - (b) low transmittance at other wavelengths

- Filters can be broadly classified as absorption filters and interference filters.

Absorption filters:

- The absorption type optical filter usually consists of colour media: colour glasses, coloured films (gelatine, etc.), and solutions of the coloured substances. This type of filter has wide spectral bandwidth, which may be 40–50 nm in width at one-half the maximum transmittance. Their efficiency of transmission is very poor and is of the order of 5–25%.

- Interference filters:
- Interference filters usually consist of two semitransparent layers of silver, deposited on glass by evaporation in vacuum and separated by a layer of dielectric (ZnS or MgF₂). In this arrangement, the semi-transparent layers are held very close. The spacer layer is made of a substance which is of low refractive index. The thickness of the dielectric layer determines the wavelength transmitted.

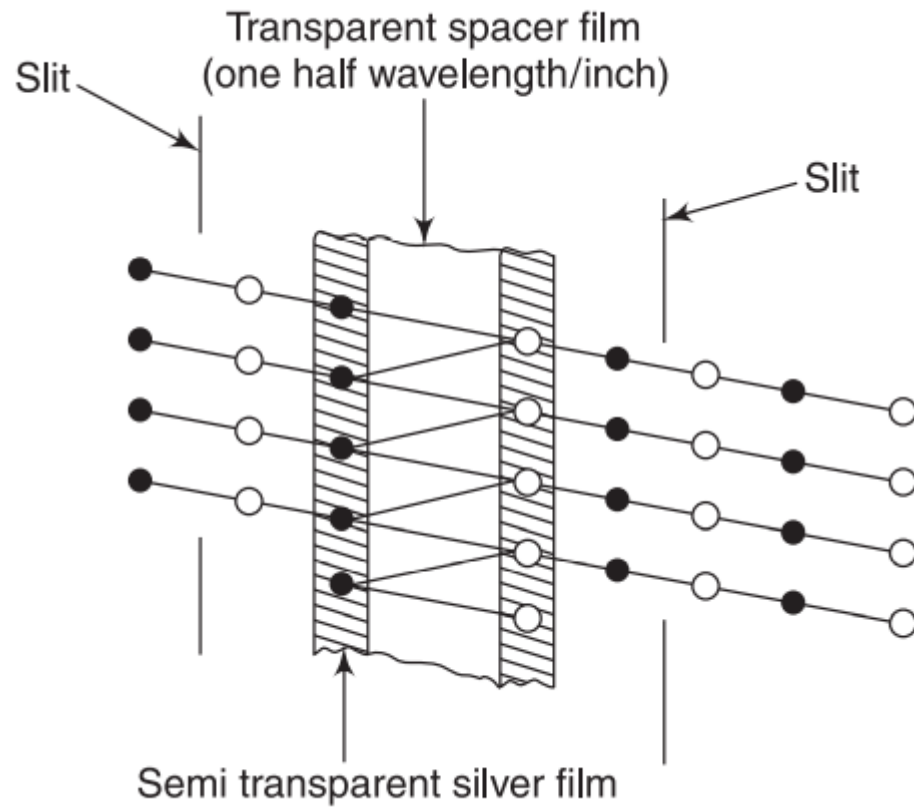


Fig: Path of light rays through an interference filter

- Some part of light that is transmitted by the first film is reflected by the second film and again reflected on the inner face of the first film, as the thickness of the intermediate layer is one-half a wavelength of a desired peak wavelength. Only light which is reflected twice will be in phase and come out of the filter, other wavelengths with phase differences would cause destructive interference. Constructive interference between different pairs in superposed light rays occurs only when the path difference is exactly one wavelength or some multiple thereof. The relationship expressing a maximum for the transmission of a spectral band is given by

$$m \lambda = 2d(n) \sin \theta$$

when light is incident normally, $\sin \theta = 1$

$$m\lambda = 2d(n)$$

Detectors

- Photosensitive Detectors
 - Photovoltaic or barrier layer cells
 - Photo-emissive cells
 - . High vacuum photo-emissive cells
 - . Gas-filled photo-emissive cells
 - . Photomultiplier tubes
- Silicon diode detectors
- Photodiode arrays (PDA)

Photosensitive Detectors

- After isolation of radiation of a particular wavelength in a filter or a monochromator, it is essential to have a quantitative measure of their intensities.
- This is done by causing the radiation to fall on a photosensitive element, in which the light energy is converted into electrical energy.
- The electric current produced by this element can be measured with a sensitive galvanometer directly or after suitable amplification.
- There are two types of photoelectric cells: photovoltaic cells and photo-emissive cells.

Photovoltaic or barrier layer cells

- Photovoltaic or barrier-layer cells usually consist of a semiconducting substance, which is generally selenium deposited on a metal base which may be iron and which acts as one of the electrodes.
- The semiconducting substance is covered with a thin layer of silver or gold deposited by cathodic deposition in vacuum. This layer acts as a collecting electrode.
- Figure shows the construction of the barrier-layer cell.
- When radiant energy falls upon the semiconductor surface, it excites the electrons at the silver-selenium interface.
- The electrons are thus released and collected at the collector electrode.

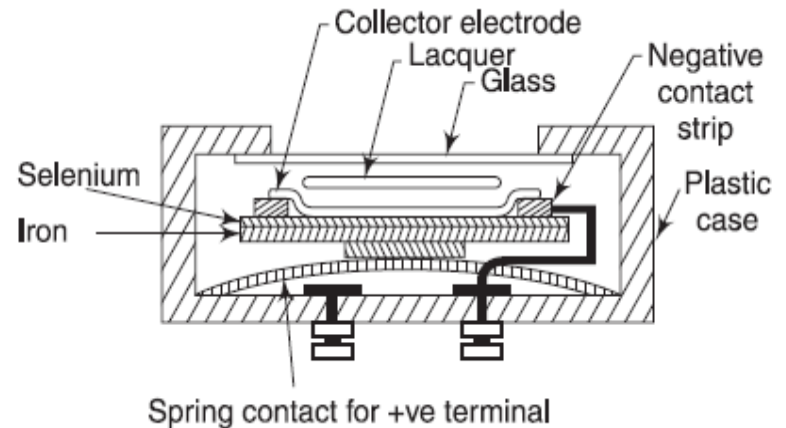


Figure: Construction of a barrier layer cell

- Photovoltaic cells are very robust in construction and no need of external electrical supply to work.
- The typical photocurrents produced by these cells are as high as 120 mA/lumen.
- At constant temperature, the current set up in the cell usually shows a linear relationship with the incident light intensity.
- Selenium cells are sensitive to almost the entire range of wavelengths of the spectrum

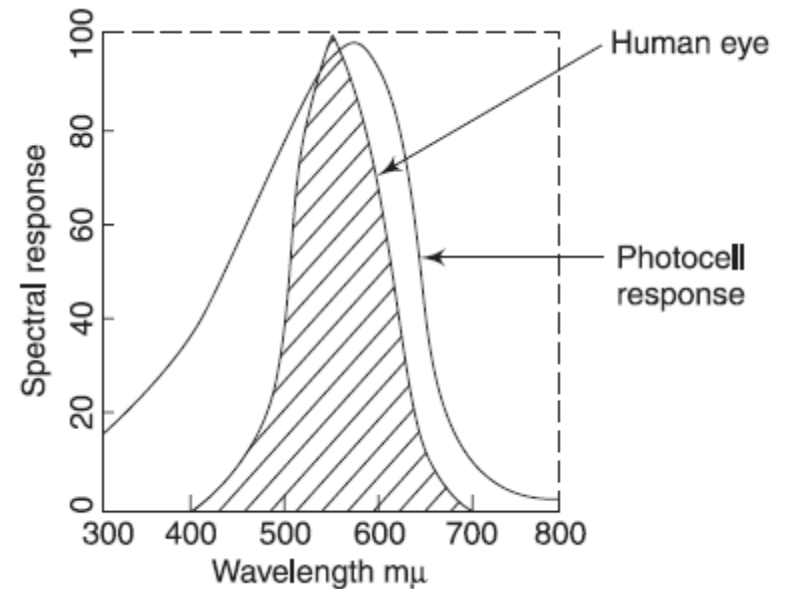


Figure: Spectral response of a selenium photocell and the human eye

Photo-emissive cells

- Photo-emissive cells are of three types:
 - (a) high vacuum photocells,
 - (b) gas-filled photocells and
 - (c) photomultiplier tubes.
- All of these types differ from selenium cells, in that they require an external power supply to provide a sufficient potential difference between the electrodes to facilitate the flow of electrons generated at the photosensitive cathode surface.
- Also, amplifier circuits are invariably employed for the amplification of this current.

High vacuum photo-emissive cells

- The vacuum photocell consists of two electrodes, cathode having a photosensitive layer of metallic caesium deposited on a base of silver oxide and the anode is either an axially centered wire or a rectangular wire that frames the cathode.
- The construction of the anode is such that no shadow falls on the cathode.
- The two electrodes are sealed within an evacuated glass envelope.

- When a beam of light falls on the surface of the cathode, electrons are released from it, which are drawn towards the anode which is maintained at a certain positive potential.
- This gives rise to photocurrent, which can be measured in the external circuit.
- The spectral response of a photo-emissive tube depends on the nature of the substance coating the cathode.

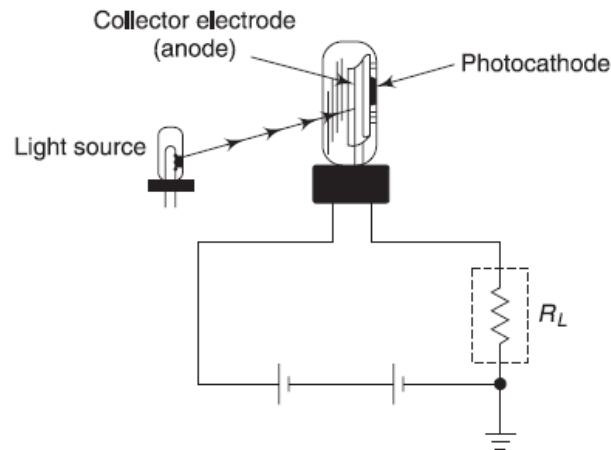


Figure: Typical circuit configuration employed with photo-emissive tubes

- Figure shows current-voltage characteristics of vacuum photo-emissive tube at different levels of light flux.
- They show that as the voltage is increased, the point is reached where all the photoelectrons are swept to the anode as soon as they are released and result in saturation photocurrent.

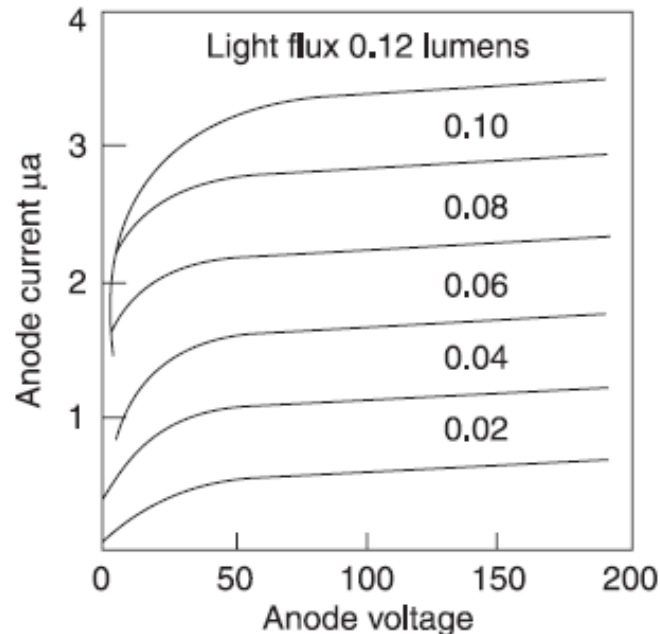


Figure: Current-voltage characteristics of vacuum

Gas-filled photo-emissive cells

- This type of cell contains small quantities of inert gas like argon, whose molecules can be ionised when the electrons present in the cell possess sufficient energy.
- The presence of small quantities of this gas prevents the phenomenon of saturation current, when higher potential differences are applied between the cathode and anode.
- Due to repeated collisions of electrons in the gas-filled tubes, the photoelectric current produced is greater even at low potentials.

Photomultiplier tubes

- Photomultiplier tubes (PMTs) convert photons to an electrical signal.
- They have a high internal gain and are sensitive detectors for low-intensity applications such as fluorescence spectroscopy.
- Photomultiplier tubes are used as detectors when it is required to detect very weak light intensities.
- The tube consists of a photosensitive cathode and has multiple cascade stages of electron amplification; in order to achieve a large amplification of primary photocurrent within the envelope of the phototube itself.
- When a photon of sufficient energy strikes the photocathode, it ejects a photoelectron due to the photoelectric effect.
- The photocathode material is usually a mixture of alkali metals, which make the PMT sensitive to photons throughout the visible region of the electromagnetic spectrum.

- The photocathode is at a high negative voltage, typically -500 to $-1,500$ V.
- The photoelectron is accelerated towards a series of additional electrodes called dynodes.
- These electrodes are each maintained at successively less negative potentials.
- Additional electrons are generated at each dynode. There may be 9–16 dynodes.
- This cascading effect creates 10^5 to 10^7 electrons for each photoelectron that is ejected from the photocathode.
- The amplification depends on the number of dynodes and the accelerating voltage.
- This amplified electrical signal is collected at an anode at ground potential, which can be measured.

- The sensitivity of the PMT can be varied by regulating the voltage of the first amplifying stage.
- Because of the relatively small potential difference between the two electrodes, the response is linear.
- The output of the PMT is limited to an anode current of a few milli amperes.

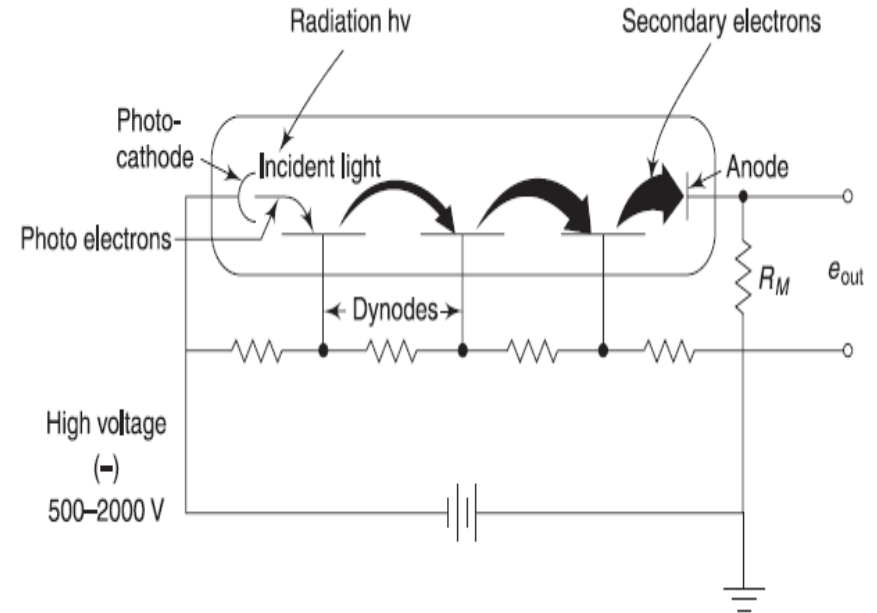


Figure: Schematic of a photomultiplier tube

- A direct current (DC) power supply is required to operate a photomultiplier, the stability of which must be at least one order of magnitude better than the desired precision of measurement.
- The devices are sensitive to electromagnetic interference, and they are also more costly than other photoelectric sensors.
- Photomultipliers are not uniformly sensitive over the whole spectrum.
- In the case of spectrophotometers, the photomultipliers normally supplied cover the range of 185–650 nm.
- For measurements at longer wave lengths, special red-sensitive tubes are offered.

Silicon diode detectors

- The photomultiplier which is large and expensive and requires a source of stabilised high voltage, can be replaced by a photodiode.
- This diode is useable within a spectral range of 400–1,050 nm, in a number of instruments (spectrophotometers, flame photometers).
- When a photon strikes a semiconductor, it can promote an electron from the valence band (filled orbital) to the conduction band (unfilled orbital) creating an electron (–) – hole (+) pair.
- The concentration of these electron-hole pairs is dependent on the amount of light striking the semiconductor, making the semiconductor suitable as an optical detector.

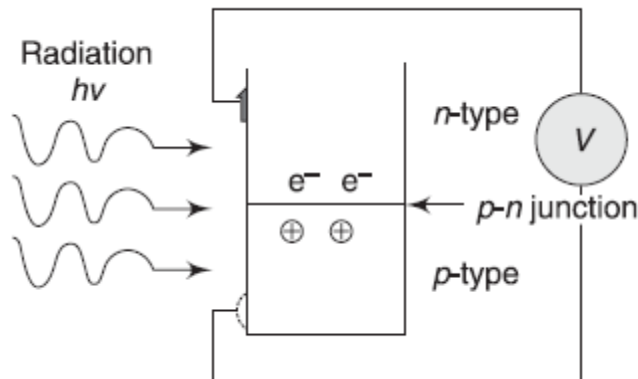


Figure: Schematic of a semiconductor detector

- There are two ways to monitor the concentration of electron-hole pairs.
- In photodiodes, a voltage bias is present and the concentration of light-induced electron-hole pairs determines the current through semiconductor.
- Photovoltaic detectors contain a p-n junction that causes the electron-hole pairs to separate to produce a voltage that can be measured.
- They cover a spectral range from 185 to 850 nm.

S.No.	Detector type	$\lambda(\mu)$
1.	Si	0.2–1.1
2.	Ge	0.4-1.8
3.	InAs	1.0-3.8
4.	InSb	1.0–7.0
5.	InSb (77K)	1.0–5.6
6.	HgCdTe (77K)	1.0–25.0

Table: The wavelength range for different solid-state detectors

- The figure shows spectral response of silicon diode detectors.
- The devices being solid state are mechanically robust and consume much less power.

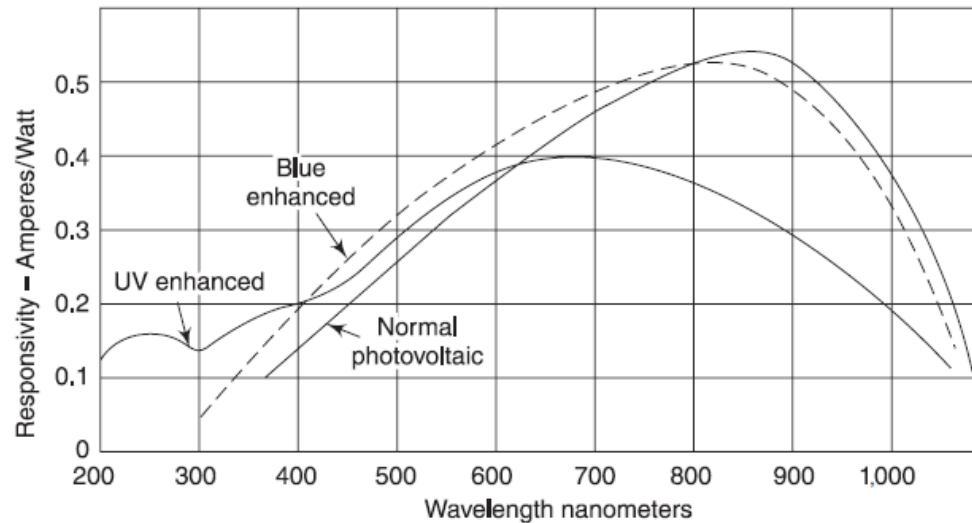


Figure: Spectral response of silicon diode detectors

Photodiode arrays (PDA)

- Diode arrays are assemblies of individual detector elements in linear or matrix form, which in a spectrophotometer can be mounted so that the complete spectrum is focused on to an array of appropriate size.
- The arrangement does not require any wavelength selection mechanism and the output is instantaneously available.
- However, resolution in diode arrays is limited by the physical size of individual detector elements.

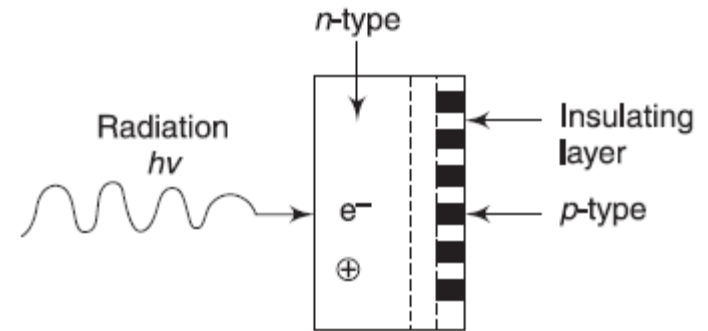


Figure: Schematic of a photodiode array

- A photodiode array consisting of discrete photodiodes is available on an integrated circuit (IC) chip.
- Array detectors are especially useful for recording the full uv-vis absorption spectra of samples that are rapidly passing through a sample flow cell, such as in HPLC detectors.
- PDAs are available with 512, 1,024, or 2,048 elements with typical dimensions of 25 mm wide and 1–2 mm high.
- Light creates electron-hole pairs and the electrons migrate to the nearest PIN junction.
- After a fixed integration time the charge at each element is read with solid-state circuitry to generate the detector response as a function of linear distance along the array.
- The detector consists of two silicon integrated circuits, each containing 211 photosensitive diodes and 211 storage capacitors.

UV – VIS spectrophotometer

Introduction

- Ultraviolet-visible (UV-Vis) spectrophotometry is a technique used to measure light absorbance across the ultraviolet and visible ranges of the electromagnetic spectrum.
- When incident light strikes matter it can either be absorbed, reflected, or transmitted.
- The absorbance of radiation in the UV-Vis range causes atomic excitation, which refers to the transition of molecules from a low-energy ground state to an excited state.
- Before an atom can change excitation states, it must absorb sufficient levels of radiation for electrons to move into higher molecular orbits.

- A UV-VIS spectrophotometer can use this principle to quantify the analytes in a sample based on their absorption characteristics.
- A UV-VIS spectrophotometer measures the intensity of light transmitted through a sample compared to a reference measurement of the incident light source.
- According to Beer-Lambert Law, the amount of light absorbed is directly proportional to the concentration of the sample and the distance the light travels through the sample; the path length.
- UV-VIS spectrophotometers are able to determine the concentration of specific analytes in a microvolume by controlling the analysis wavelengths and the path length.

- Since the UV-VIS range spans the range of human visual acuity of approximately 400–750 nm, UV-Vis spectroscopy is useful to characterise the absorption, transmission, and reflectivity of a variety of technologically important materials, such as pigments, coatings, windows, and filters.

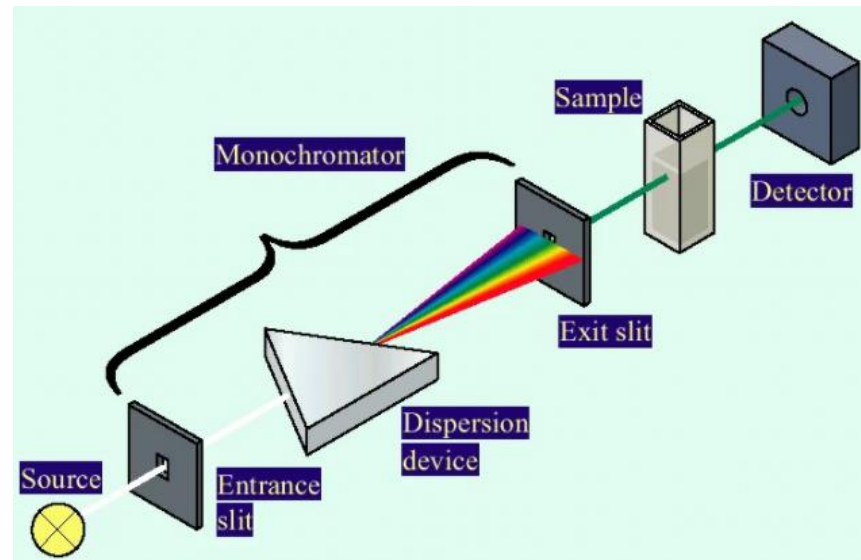


Figure: Single beam UV-VIS spectrophotometer

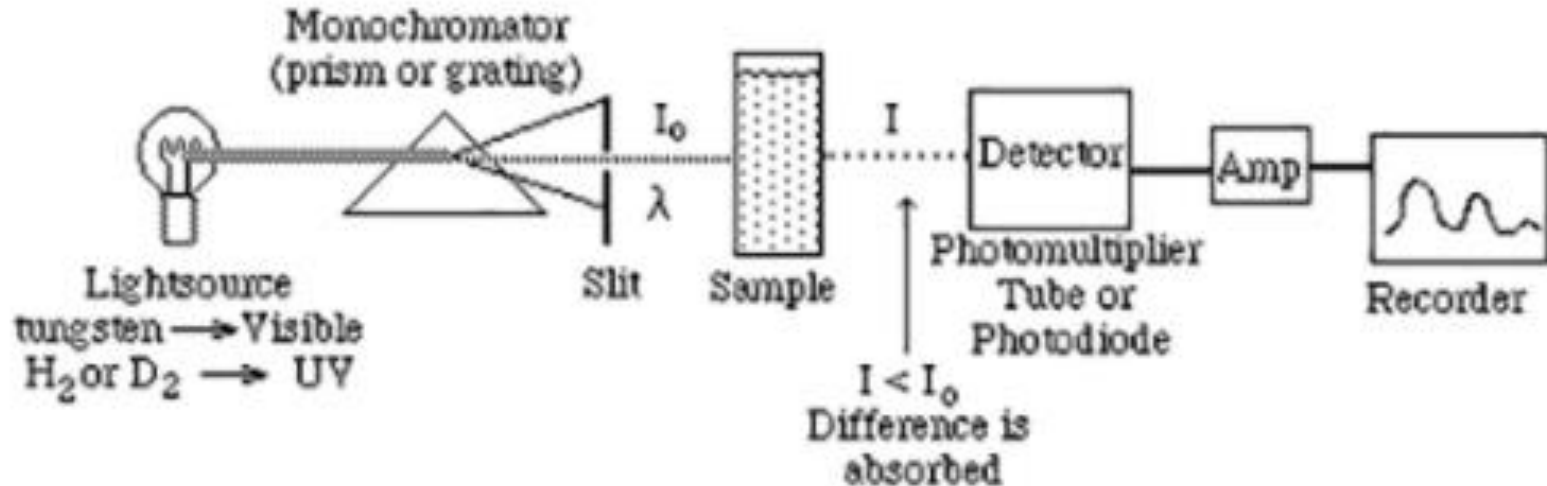
- The light source is usually a Tungsten-Halogen Lamp or deuterium-discharge lamp for UV measurements and a tungsten halogen lamp for visible and NIR measurements.
- The instruments automatically swap lamps when scanning between the UV and visible regions.
- The wavelengths of these continuous light sources are typically dispersed by a holographic grating in a single or double monochromator or spectrograph.
- The spectral bandpass is then determined by the monochromator slit width or by the array-element width in array-detector spectrometers.
- The detector in single-detector instruments is a photodiode, phototube or PMT.

- UV-Vis-NIR spectrometers utilise a combination of a PMT and a Peltier-cooled PbS IR detector.
- The light beam is redirected automatically to the appropriate detector when scanning between the visible and NIR regions.
- The diffraction grating and instrument parameters such as slit width can also be change.
- The UV-Vis spectral range is approximately 190–900 nm.
- The short-wavelength limit for simple UV-Vis spectrometers is the absorption of ultraviolet wavelengths less than 180 nm by atmospheric gases.
- High-end commercial UV-Vis spectrophotometers extend the measurable spectral range into the NIR region as far as 3,300 nm.

Types of UV-Vis spectrophotometer

- Single beam and
- Double beam

Single beam spectrophotometer

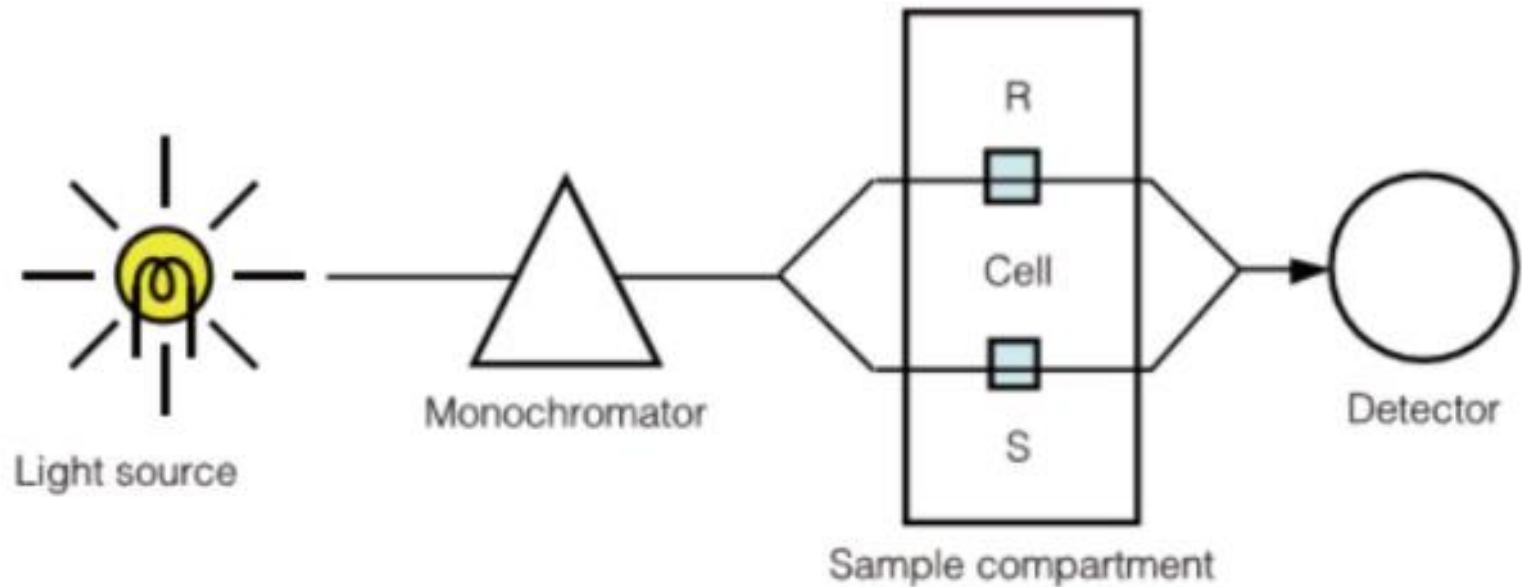


Working of Single beam spectrophotometer

- A spectrophotometer measures the light that passes through the sample, then strike the detector, where it is measured.
- Let's call the amount of light at wavelength λ incident on the sample cuvette I_0 and the amount of light exiting the sample to hit the detector I .
- If we now calculate the percent of the ratio these two values $(I/I_0)*100$ we get a percent transmission (%T) value.
- If the sample transmits all the light at a given wavelength then $\%T = 100$; however, if the sample absorbs light then we will have the case where $I < I_0$ and %T will be a number less than 100.
- If the beam is totally blocked or absorbed by the sample, then $\%T = 0$.

- When a spectrophotometer, the instrument has not been calibrated for the values of 100 %T or 0 %T.
- This 100 %T calibration is acquired by a process called background correction for a spectrum. it is also sometimes called auto-zero for a single wavelength measurement.
- A background correction is performed by removing the sample from the instrument and measuring the amount of light that strikes the detector in the scan range of all wavelengths in the spectrum.
- These 100 %T values (called a background scan) are then stored in memory for use in calculating accurate sample %T values for a sample.

Double beam spectrophotometer



- These instruments are configured to allow the automatic and simultaneous (or near simultaneous) measurement of the sample and reference beam intensities.
- In a double-beam system, the monochromatic light from the monochromator is split into a sample beam (S) and reference beam (R) before entering the sample compartment and hitting the detector.
- This can be accomplished using a beam splitter to divide the beam into the reference beam and sample beam.
- In the double-beam-in-space configuration, the intensities of the split beams are measured simultaneously after passing through the sample and reference cells.
- Alternately, in a double-beam-in-time spectrophotometer, the beams pass through a modulator which allows the detector to see either the reference beam or the sample beam.
- Since the source beam is directed through a reference cell part of the time and through the sample cell the rest of the time, the term 'double-beam-in-time' is used.

- The main disadvantage of single-beam spectrophotometer is that the instrument settings have to be adjusted to give a 100% reading with the reference in the beam, before the sample is examined.
- This drawback is overcome by using a double-beam instrument, wherein the arrangement is such that the radiation beam is shifted automatically to pass alternately through the sample and reference cuvettes.
- The cuvettes themselves are not shifted and remain in their fixed position.

Infrared Spectrophotometer

Introduction

- Infrared (IR) spectrophotometer is ideally suited for carrying out qualitative and quantitative analysis, particularly of organic compounds.
- IR region extends from 0.8 to 200 μm in the electromagnetic spectrum. But most of the commercial instruments are available in the region from 0.8 to 50 μm .
- The position of absorption bands in the IR spectrum is expressed both in wavelength as well as in wave numbers.
- IR spectrophotometers using prisms produce spectra which are spread linearly with wavelength, whereas instruments fitted with gratings generally deliver spectra spread linearly with wave number.

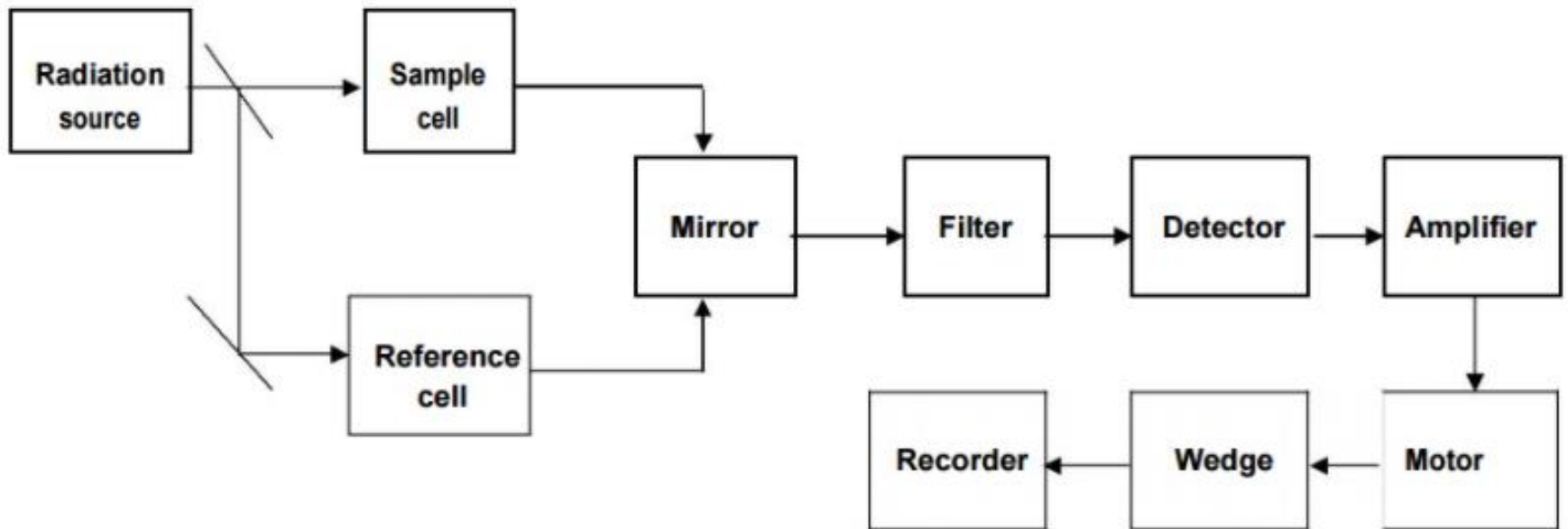
Various regions of the IR range of the spectrum

Region	Wavelength range (λ m)	Wavenumber range (cm^{-1})
Near	0.78–2.5	12800–4000
Middle	2.5–50	4000–200
Far	50–1000	200–10

Basic Components of IR Spectrophotometers

- Spectrophotometers for the IR range are composed of the same basic elements, as instruments in the visible and ultraviolet range, namely:
 - a source of radiation
 - a monochromator for dispersing the radiation
 - a detector which registers the residual intensity after selective absorption by the sample

Block diagram of IR Spectrophotometers



Radiation Sources

- The source of radiation in IR spectrophotometers is ideally a black body radiator.
- The energy emitted by a black body radiator varies with wavelength and with temperature.
- The optimum IR source is an inert solid heated electrically to temperatures between 1500 and 2000 K.
- The maximum radiant intensity at these temperatures occurs at 1.7–2 μm (5000–6000 cm^{-1}).

Types of IR sources

- There are three common practical IR sources: the Globar rod, the Nernst filament and the Nichrome wire.

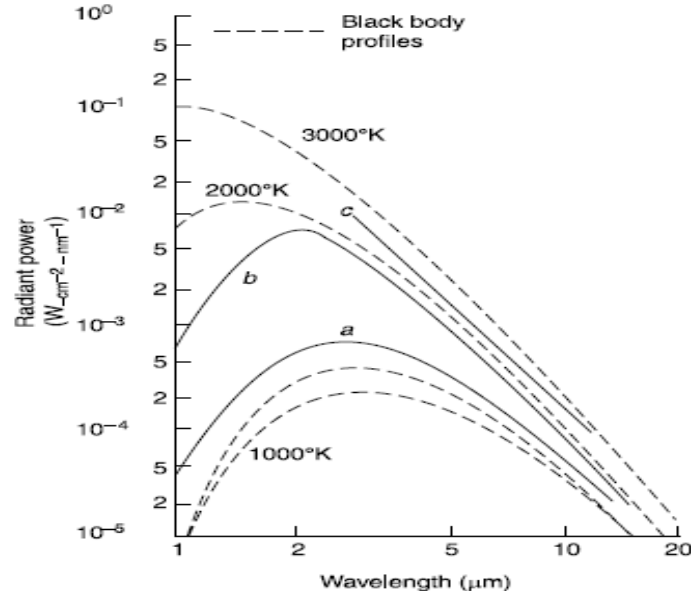


Figure: Typical spectral characteristics of some IR sources
(a) Globar, (b) Nernst glower, (c) Tungsten glower

Globar

- The Globar is a silicon carbide rod, which has a positive temperature coefficient of resistance.
- The rod is about 5 cm in length and 0.5 cm in diameter. It is electrically heated and run at a current to produce a temperature of about 1300 K.
- The heat dissipation at the ends of the rod is high, as they are the coolest parts.
- Therefore, water cooling of electrical parts is required to prevent arcing.
- The Globar finds applications for work at wavelengths longer than 15 μm (650 cm^{-1}), because its radiant energy output decreases less rapidly.



Nernst filament

- The Nernst filament is a small rod composed of fused rare earth oxides of zirconium and yttrium.
- The filament is of cylindrical shape having a diameter of about 1–2 mm and a length of 20–30 mm.
- Platinum leads are sealed to the ends of the cylinder to allow passage of current through it.
- The filament is heated to temperatures between 1500 and 2000°C and produces maximum radiation at about 7100 cm^{-1} .
- The device has a negative temperature coefficient of electrical resistance and is, therefore, operated in series with ballast resistance in constant voltage circuit.



Nichrome strip

- The nichrome strip, though, gives less energy than the Globar or Nernst, and is extremely simple and reliable in operation.
- In construction, it could be a tightly wound spiral of nichrome wire, heated by passage of current.
- The temperature is about 800–900°C. Some IR spectrophotometers employ a ceramic rod source, in which is embedded a platinum-rhodium filament producing temperature up to 1200°C.



NiCR Filament
Pulsable IR Sources

Monochromators

- Light from the entrance slit is rendered parallel after reflection from a collimating mirror and falls on the dispersing element.
- The dispersed light is focused on to the exit slit of the monochromator and passes into the detector section.
- The sample is usually placed at the focus of the beam, just before the entrance slit to the monochromator.
- IR monochromators generally employ several mirrors for reflecting and focusing the beam of radiation in preference over lenses to avoid problems with chromatic deviations.

- Both prisms and gratings are used for dispersing IR radiation.
- As a general rule, instruments operating below 25–40 μm have prism monochromators, whereas reflection gratings are utilised above 40 μm , because transparent materials are not easily available in that range.

- Materials for prism construction, which are found most suitable in the IR region.

Material	Optimum range
Glass (SiO₂)	300 mm to 2 m (5000 cm⁻¹)
Quartz	800 mm to 3 m (3300 cm⁻¹)
Lithium fluoride	600 mm to 6.0 m (1670 cm⁻¹)
Calcium fluoride	200 mm to 9.0m (1100 cm⁻¹)
Sodium chloride	200 mm to 14.5 m (625 cm⁻¹)
Potassium bromide	10 to 25 m (400 cm⁻¹)
Caesium iodide	10 to 38 m (260 cm⁻¹)

- The use of gratings as dispersing elements in the monochromators offers a number of advantages for the IR region.
- Better resolution is possible, because there is less loss of radiant energy than in a prism system.
- This facilitates employing of narrower slits. Also, gratings offer nearly linear dispersion.
- With these advantages, gratings have almost replaced prisms.
- The standard grating used in IR spectrophotometry is an echelon reflection grating.
- They are usually constructed from glass or plastic that is coated with aluminium.
- Grating instruments incorporate a sine-bar mechanism to drive the grating mount, when a wavelength readout is desired and a cosecant-bar drive when wave numbers are desired.
- One disadvantage of using gratings is that, they disperse radiant energy into more than one order.

Entrance and Exit Slits

- Most of the monochromators use a specially shaped cam to control the slit width, as the wavelength is scanned.
- Besides this, a servo system can also be employed to achieve the same purpose of the slit servo system, which maintains the output of an optical monochromator at a constant level, as the wavelength is scanned.
- The slit width control has the same importance as it has in ultraviolet and visible spectrophotometers, and a compromise of different factors is to be made while selecting the slit width to be used.
- Narrow slits produce smaller bandwidth which consequently results in better spectra definition, whereas wider slits permit larger amount of radiant energy reaching the detector and consequently greater photometric accuracy

Mirrors

- As the materials that are used for lenses are not transparent to IR radiation over the entire wavelength range, lenses are generally not preferred.
- Front surfaced mirrors are usually used in the IR instruments, of which plane, spherical, parabolic and toroidal types are the most common.
- Although highly reflecting aluminium-coated mirrors with a protective coating are usually employed

Detectors

- Detectors used in IR spectrometers usually convert the thermal radiant energy into electrical energy, which can subsequently be plotted on a chart recorder.
- Selection of a specific detector depends on the waveband of interest, the sensitivity required and the cost constraints.
- The detectors used may be divided into thermal and quantum types.
- The quantum detectors are useful in the near IR region, whereas thermal detectors can be used very much beyond this range.
- The main features of thermal-type detectors include responsiveness with little dependence on wavelength and operation at room temperature.
- However, the response speed and detectivity are lower than the quantum type. The commonly used thermal detectors are thermopiles, bolometers, pneumatic detectors and pyroelectric detectors.

Quantum-type detectors

- Quantum-type detectors feature high detectivity and fast response speed.
- Responsitivity is wavelength dependent and except for detectors in the near IR range, cooling is normally used with these detectors.
- Quantum-type detectors are classified into intrinsic types and extrinsic types.
- The wavelength limits of intrinsic-type detectors are determined by their inherent energy gap, and responsitivity drops drastically when the wavelength limit is exceeded.
- Typical examples of this type of detectors are photoconductive and photovoltaic detectors such as Hg CdTe or PbSnTe.
- Extrinsic-types of detectors are photoconductive detectors whose wavelength limits are determined by the level of impurities doped in high concentrations to the Ge or Si semiconductors.
- The major difference between intrinsic-type detectors and extrinsic-type detectors is the operating temperature.
- Extrinsic-type detectors must be cooled down to the temperature of liquid helium.

- Quantum-type detector

- Photoconductive cells
- Solid-State Photo detectors

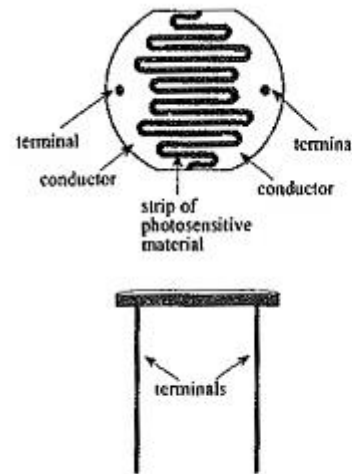
- Thermal detectors

These detectors depend on their response on the heating effect of the radiation.

- Thermocouples, bolometers, pneumatic and pyroelectric detectors are the three commonly used thermal detectors

Photoconductive Cell

- Light striking the surface of a material can provide sufficient energy to cause electrons within the material to break away from their atoms. Thus, free electrons and holes (charge carriers) are created within the material, and consequently its resistance is reduced. This is known as the Photoconductive effect.



(a) Photoconductive cell construction



(b) Circuit symbol

- Light-sensitive material is arranged in the form of a long strip zigzagged across a disc-shaped base.
- The connecting terminals are fitted to the conducting material on each side of the strip.
- Cadmium sulfide (CdS) and cadmium selenide (CdSe) are the two materials normally used in photoconductive cell manufacture.
- Both respond rather slowly to changes in light intensity.
- For cadmium selenide, the response time is around 10 ms, while for cadmium sulfide it may be as long as 100 ms.
- Temperature sensitivity is another important difference between the two materials.
- There is a large change in the resistance of a cadmium selenide cell with changes in ambient temperature, but the resistance of cadmium sulfide remains relatively stable.

Solid-State Photo detectors

- Photodiodes
- Phototransistors
- PIN diodes
- Avalanche photodiodes
- Silicon photo multipliers

Thermocouples

- **Thermocouples** are the most widely used detectors employed in IR spectrophotometers.
- In these detectors, the signal originates from a potential difference caused by heating a junction of unlike metals.
- They are made by welding together two wires of metals in such a manner that they form two junctions.
- One junction between the two metals is heated by the IR beam and the other junction is kept at constant temperature.
- Due to difference in work functions of the metals with temperature, a small voltage develops across the thermocouple.
- The receiver element is generally blackened gold or platinum foil, to which are welded the fine wires comprising the thermoelectric junction.
- The other junction is shielded from the incident radiation. Changes in temperature of the order of 10^{-6} °C can be detected.

Bolometers

- **Bolometers** give an electrical signal as a result of the variation in resistance of a conductor with temperature.
- It consists of a thin platinum strip in an evacuated glass vessel, with a window transparent in the IR range.
- Irradiation by the IR beam produces an increase in resistance of the metal strip, which is measured with a Wheatstone bridge.
- Usually, two identical elements are used in the opposite arms of the bridge.
- One of the elements is placed in the path of the IR beam and the other is used to compensate for the changes in the ambient temperature.
- Alternatively, the platinum strips may be replaced with thermistors, which show a negative thermal coefficient of electrical resistance.
- Bolometer arrays have become the focus of most uncooled detector development

Pneumatic detector

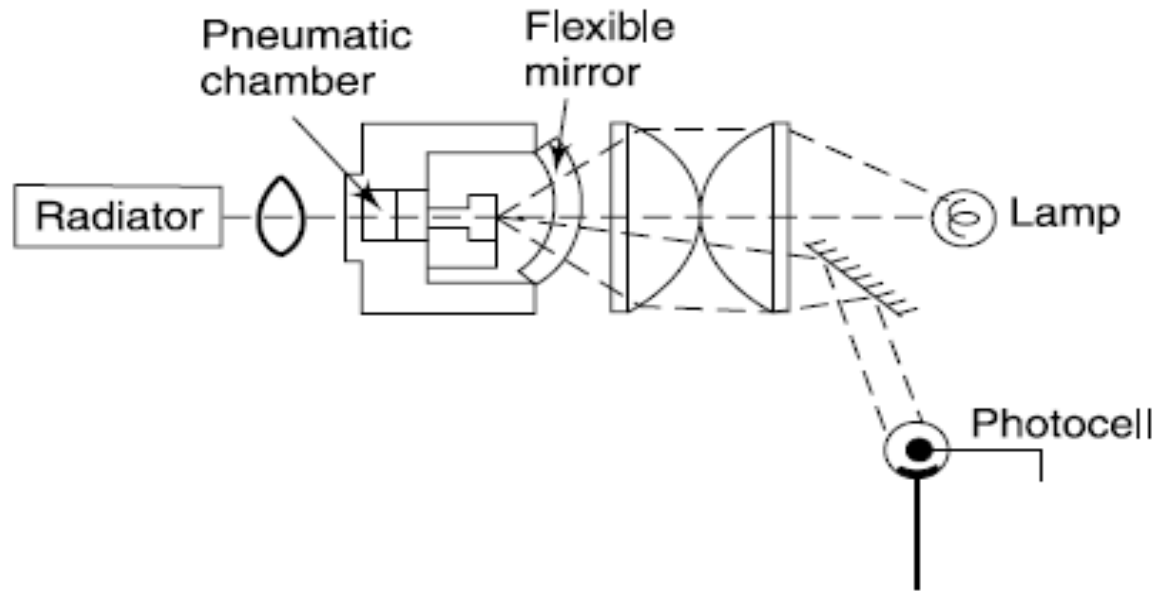


Figure: Golay pneumatic cell

Construction and Working Principle

- The pneumatic detector described by Golay is used to measure the intensity of IR radiation by following the expansion of a gas upon heating.
- The Golay cell comprises a chamber containing xenon, a gas of low thermal conductivity.
- It is sealed at its front end by a blackened receiver.
- The rear wall is a flexible membrane with a mirrored surface on its rear side.
- IR energy falling on the receiver warms up the gas in the chamber.
- A rise in temperature of the gas in the chamber produces a corresponding rise in pressure and therefore, a distortion of the mirror diaphragm.
- Light from a lamp inside the detector housing can be focused on the diaphragm, which reflects the light on to a photocell.
- Movements of the diaphragm corresponding to the amount of incident IR energy changes the incident light energy on the photocell surface and causes a change in the photocell output.
- By periodically interrupting the incident radiation with a chopper, an AC signal is produced by the photocell which can be amplified.

- Another form of pneumatic detector is capacitor microphone type in which the varying expansion of the gas affects the capacitor film, which in turn produces the variation in the electrostatic capacity.
- Pneumatic detectors function at all wavelengths throughout the IR region (as far as 400 μ m using a diamond window).
- They have a low response time.
- The whole receiver is sensitive to the radiations, thereby eliminating the need for optical alignment.

Pyroelectric detectors

- Pyroelectric detectors certain types of crystals (ferroelectric) get polarised in a well defined direction, known as the polar axis.
- Because the degree of polarisation is temperature dependent, heating or cooling a slice of such a crystal will create an accumulation of charge (on the faces normal to the polar axis), that is, proportional to the variation in polarisation caused by the temperature change.
- This is the pyroelectric effect.
- Temperature fluctuations produce a charge change on the surface of pyroelectric crystals, which produces a corresponding electrical signal. This temperature gradient can be created by the absorption of light.
- A pair of electrodes normal to the polar axis of the crystal may be used to measure the voltage generated within the crystal due to temperature changes.
- Pyroelectric detectors are mostly made from single crystal triglycine sulphate (TGS).

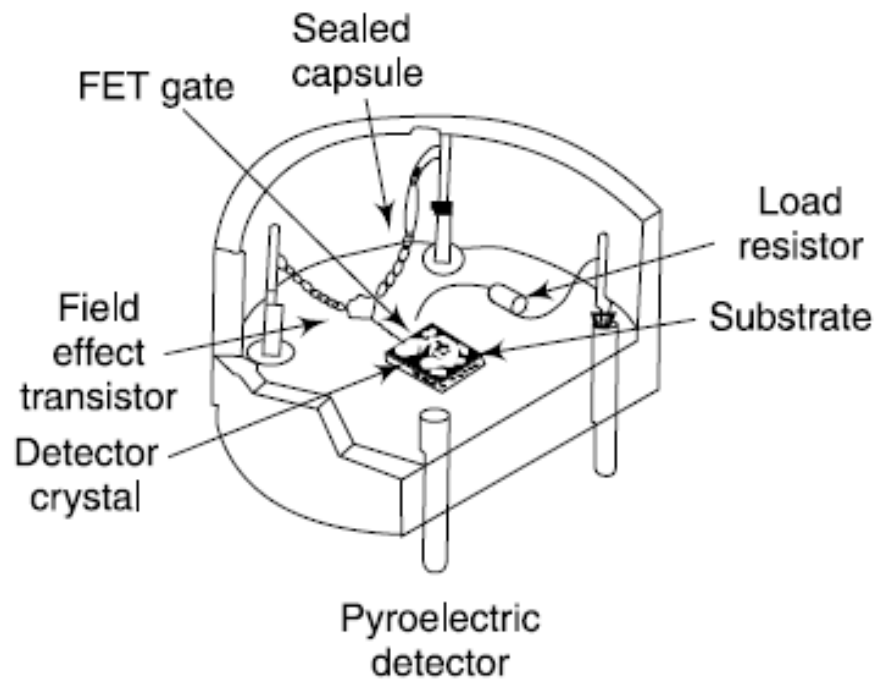


Figure: Pyroelectric detector

- A pyroelectric detector contains a piece of ferroelectric crystal material with electrodes on two sides – essentially a capacitor.
- One of those electrodes has a black coating (or a processed absorbing metal surface), which is exposed to the incident radiation.
- The incident light is absorbed on the coating and thus also causes some heating of the crystal, because the heat is conducted through the electrode into the crystal.
- As a result, the crystal produces some pyroelectric voltage.
- The voltage generated is usually applied to a field effect transistor, which is an integral part of the detector package.
- Pyroelectric detectors are current sources with an output that is proportional to the rate of change of their temperatures.
- Pyroelectric materials absorb quite strongly in the FIR and have essentially flat wavelength response from the near IR through the FIR.
- Though the pyroelectric detectors have low sensitivity as compared to Golay detector, they are preferred in the FIR range due to faster response.

Types of IR Spectrophotometers

- Optical Null Method
- Ratio recording

- The IR radiation is passed simultaneously through two separate channels, one containing the sample, the other the reference.
- The two beams are re-combined into a common axis and are alternately focused on the detector.
- If the intensities of the sample and reference beams are exactly equal, then no alternating intensity radiation goes through the slit.
- If the sample absorbs some radiation, an alternating intensity radiation is observed by the detector, which produces an AC signal.
- This AC signal can be selectively amplified in a tuned amplifier.
- As the amplifier is tuned to the chopping frequency of one light beam, signals of frequency different from the chopping frequency are not amplified.

- The alternating signal from the detector is used to drive a servomotor, which is mechanically coupled to an optical wedge or a fine-toothed comb attenuator.
- Movement of the comb occurs when a difference in power of the two beams, is sensed by the detector.
- The motor will stop when the reference and the sample beam intensities are exactly equal.
- The movement of the motor is synchronised with the recorder pen, so that its position gives a measure of the relative power of the two beams and thus the transmittance of the sample.
- The record obtained, therefore, is of the sample absorption as a function of spectral frequency.
- The teeth of the attenuator comb are accurately cut, so that a linear relationship exists between the lateral movement of the comb and the decrease in power of the beam.

Recording-type double-beam optical-null principle IR spectrophotometer

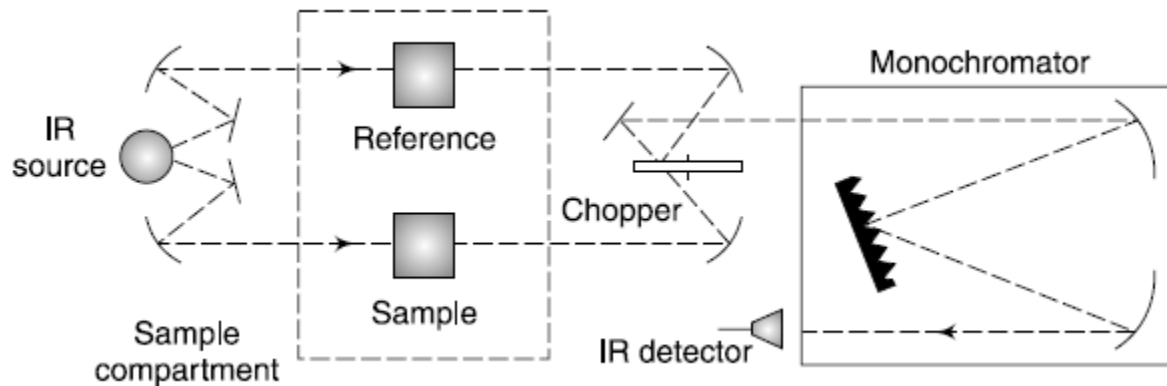


Figure: Block diagram of a double-beam IR spectrophotometer

Working Principle

- The two light beams from light source pass through the sample and reference cells.
- With a switching frequency of 12.5 Hz, the rotary sector mirror alternately conducts the radiation of the sample and reference channels into the monochromator.
- Here, the light of the respective wave number is reflected and passed on to the thermocouple, which delivers a 12.5 Hz AC voltage signal, when the intensities of the two optical paths are not equal.
- After amplification in the pre-amplifier and main amplifier and after phase-sensitive rectification in the demodulator, this AC voltage signal controls the servomotor, which adjusts the attenuator diaphragm in the reference channel until the intensities of both beams are equal and the signal disappears.
- The servomotor also drives the recording pen, which records the sample's transmittance corresponding to the position of the attenuator diaphragm.

Ratio Recording Method

- In the direct ratio recording system, radiation from the source is passed alternately through two separate channels, one passing through the sample cell and the other through the reference cell.
- The two signals from the detector are amplified, rectified and recorded separately.
- The sample signal is displayed as a proportion of the reference signal.
- In this method, attempts are not made to have a physical equalisation of the sample and reference beam intensities.
- The reference signal is used to drive the slit width control, so that the energy level reaching the detector is constant.
- This system is, however, not favoured in IR instruments, as it requires a very stable amplifier system and the chopping system.