Lab instruments ...Lecture-4

The Spectrophotometer-2

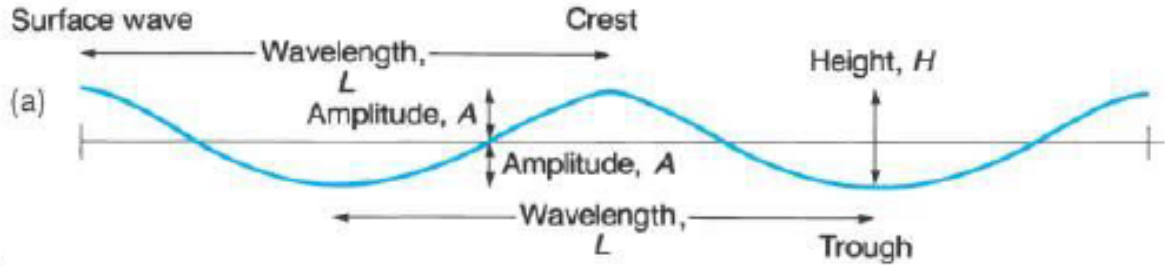
جامعة المعقل
الكلية التقنية الصحية
قسم تقنيات التحليلات المرضية

Out line :

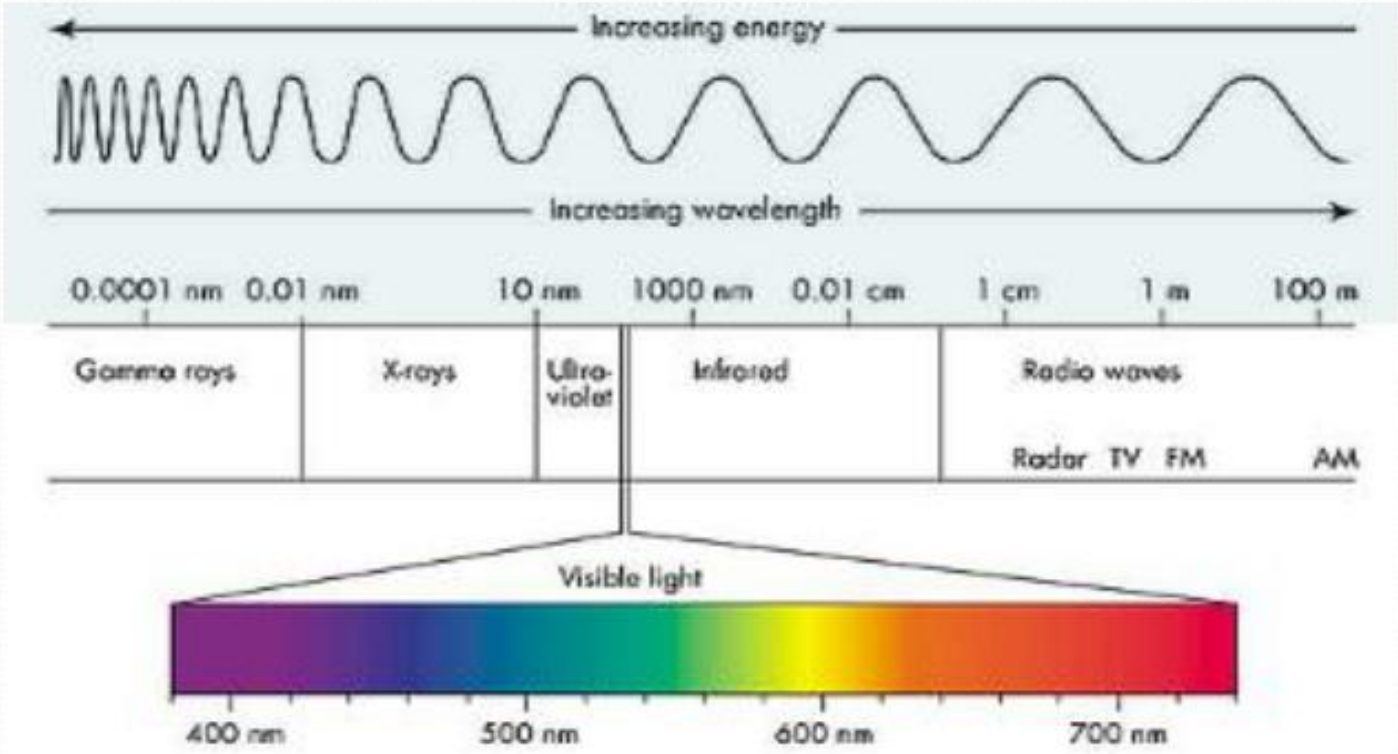
- Introduction to Spectrophotometry
- Principles of Spectrophotometer
- Instruments of Measurement
- Instrumentation (Spectrophotometers)
- Definitions & Symbols
- Beer - Lambert law
- Applications of a spectrophotometer

Introduction to Spectrophotometry

- **Properties of Light:**
- *Electromagnetic radiation* moves in waves
- **Light** (called **electromagnetic radiation**) moves in waves.
- Wavelength = different types of light have different wavelengths. Some are longer than others. For instance, in the visible light spectrum, red light waves are longer than blue light waves.
- Wavelengths are commonly given in ????



Electromagnetic spectrum



Colors & Wavelengths

Only those substances appear coloured that absorb VIS radiation
The colour is then determined by the reflected light (the colour of the substance is *complementary* to that one which has been absorbed):

Visible Light

Color absorbed	Color observed	Absorbed radiation(nm)
Violet	Yellow-green	400-435
Blue	Yellow	435-480
Green-blue	Orange	480-490
Blue-green	Red	490-500
Green	Purple	500-560
Yellow-green	Violet	560-580
Yellow	Blue	580-595
Orange	Green-blue	595-605
Red	Blue-green	605-750

What are Spectroscopy and Spectrophotometry??

- Light can either be ***transmitted*** **or** ***absorbed*** by dissolved substances.
- Presence & concentration of dissolved substances is analyzed by passing light through the sample.
- **Spectroscopes** measure electromagnetic ***emission***
- **Spectrophotometers** measure electromagnetic ***absorption***

Principles of Spectrophotometer

- A spectrophotometer consists of two instruments:
- **Spectrometer:** for producing light of any selected color (wavelength),
- **Photometer:** for measuring the intensity of light.

Instruments of Measurement

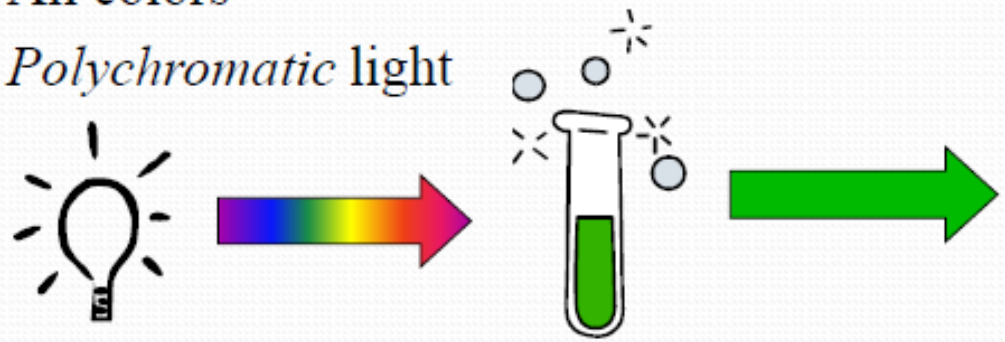
- **What do spectrophotometers measure?**

- The absorption of light indicates **the presence of the substance**. This is a qualitative measurement.

- The amount of light absorbed measures the **concentration of the dissolved substance.** This is a quantitative measurement.

Absorption of Light

- **White light**
  - All colors
  - *Polychromatic* light



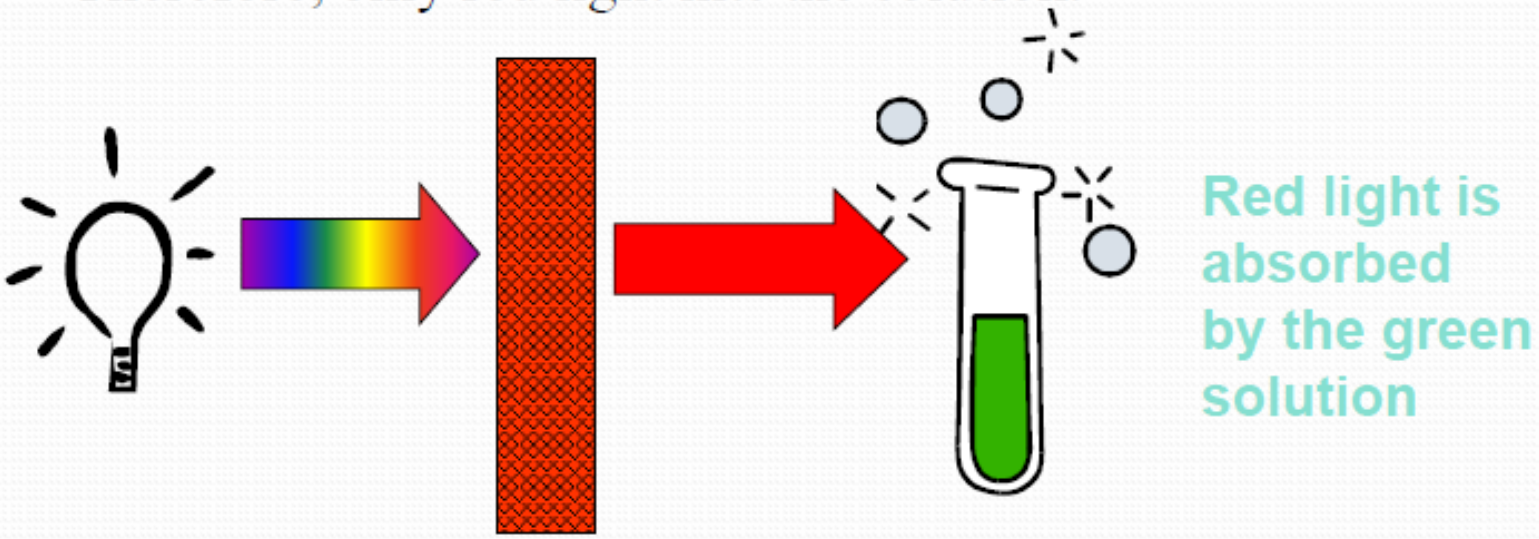
-When white (polychromatic) light passes through a coloured solution some of the light is absorbed by the substances in the solution, and the rest passes through.

-For Example: Green solution absorbs light other than green.

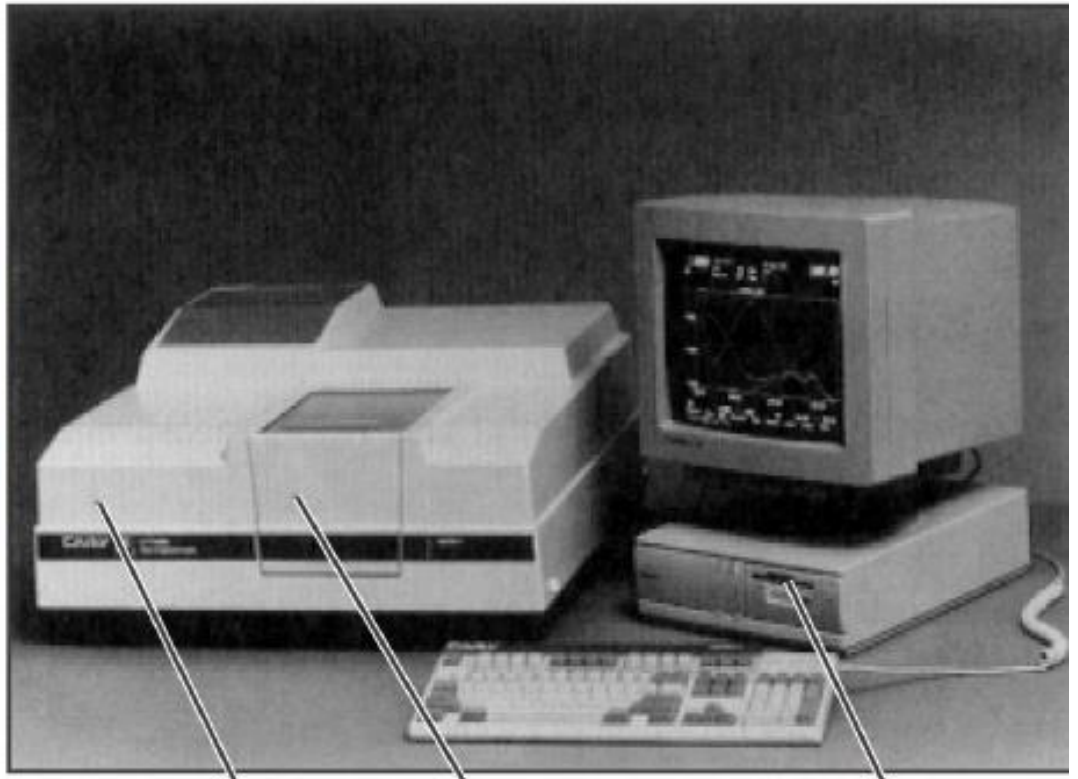
Absorption of Light

- **Monochromatic light**
  - Light of one color

- For example: If white light is made to pass through a red filter, all light except red is filtered out and absorbed. Therefore, only red light hits the solution.

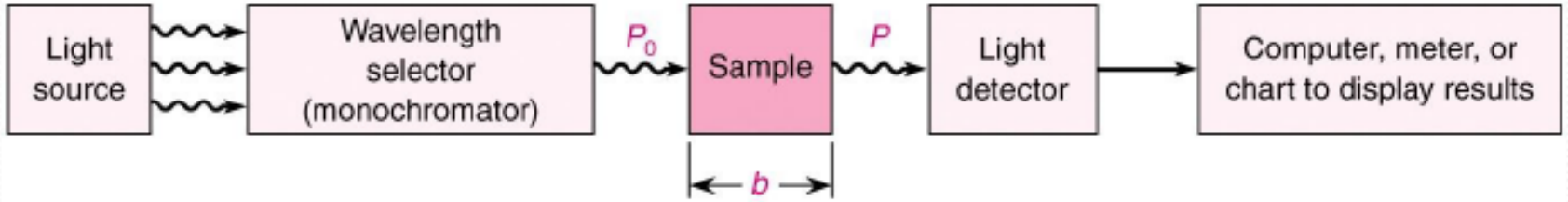


The Spectrophotometer

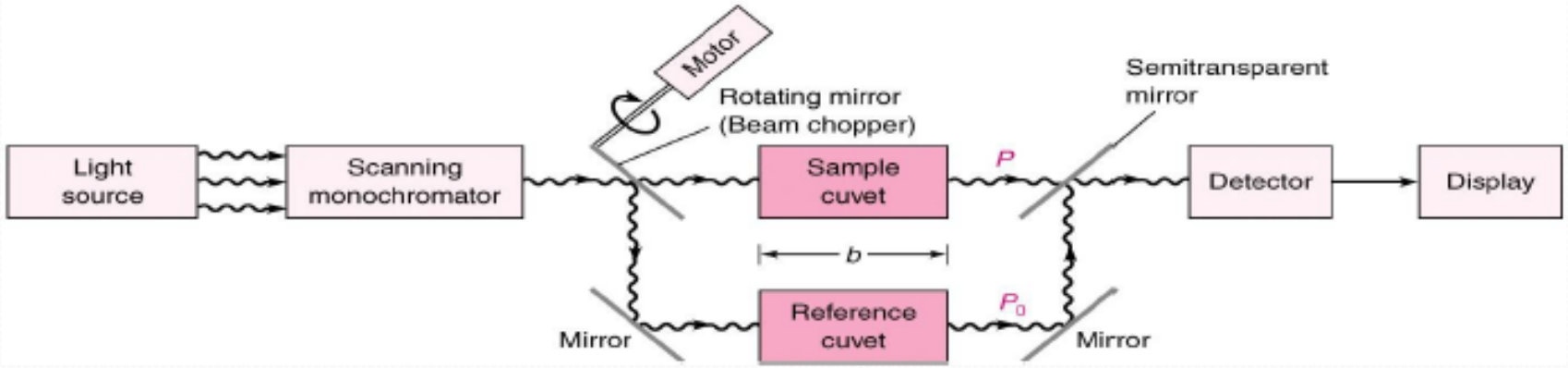


The Spectrophotometer

- a) Single-beam



- b) Double-beam



Instrumentation (Spectrophotometers)

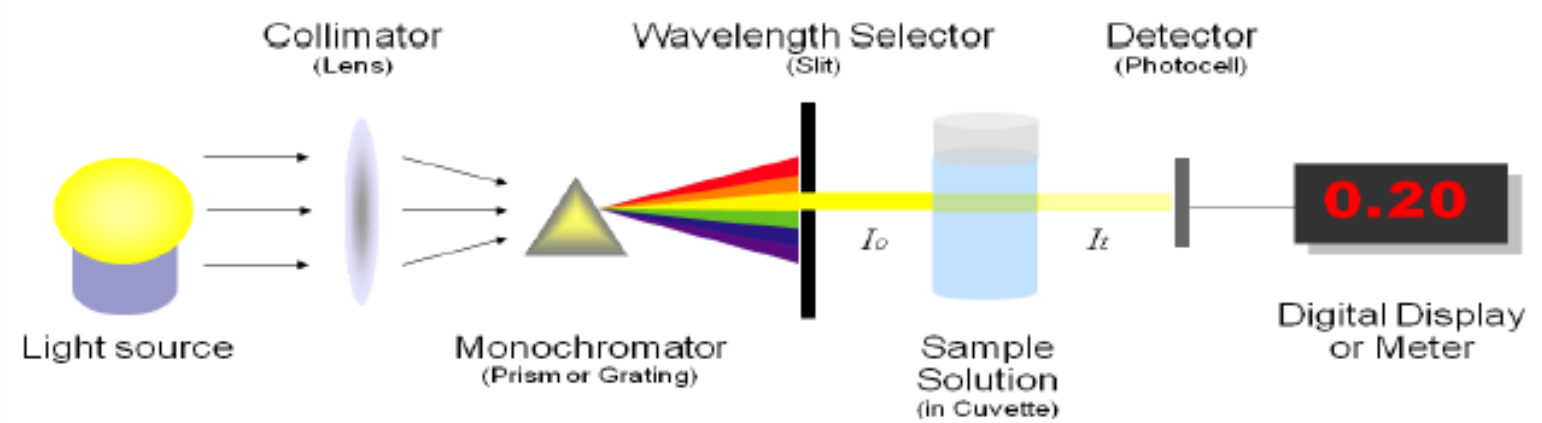
1- Sources of light

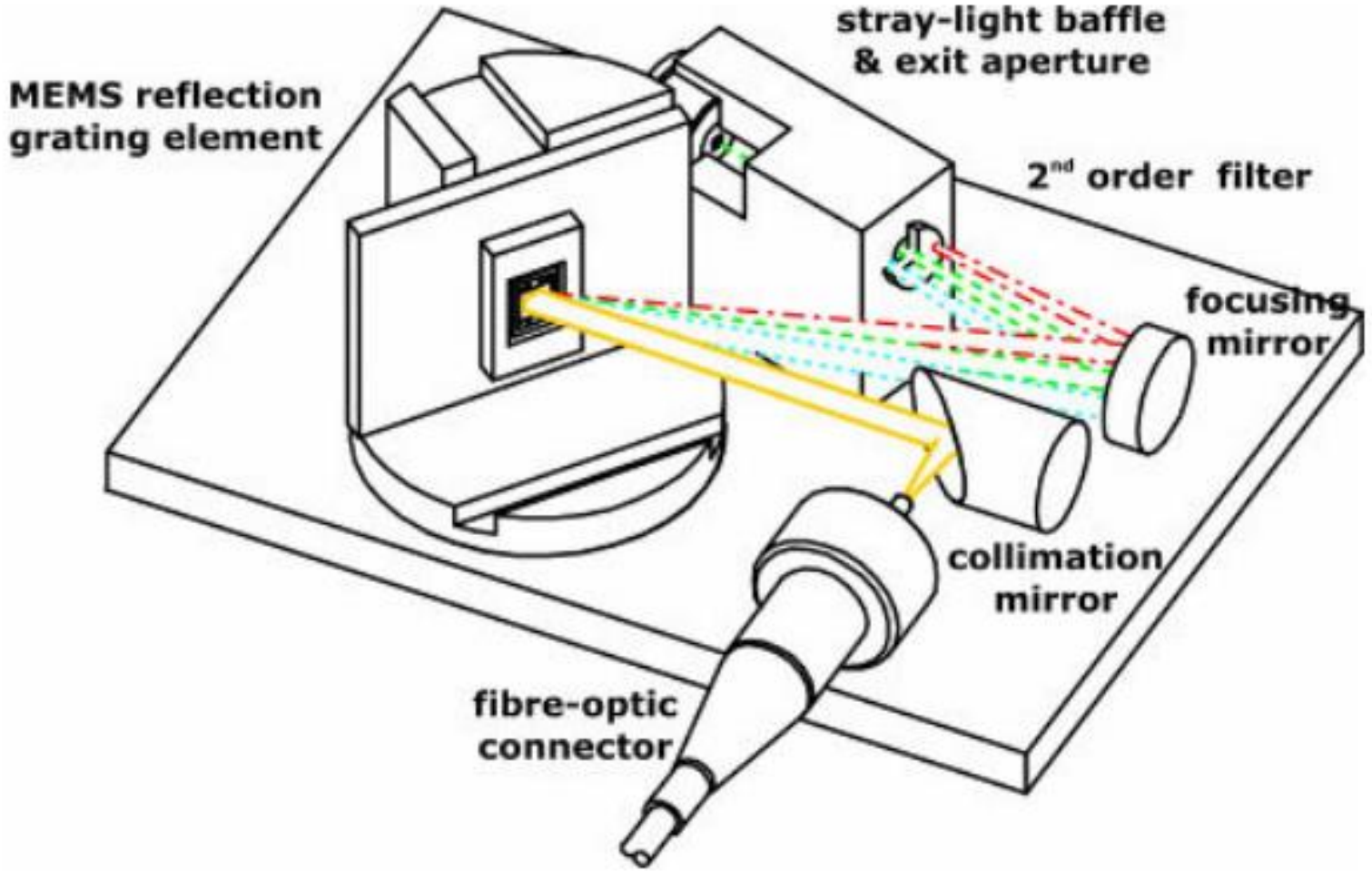
2- Wavelength Selectors

3- Sample Containers

4- Detectors

5- signal processor and readout

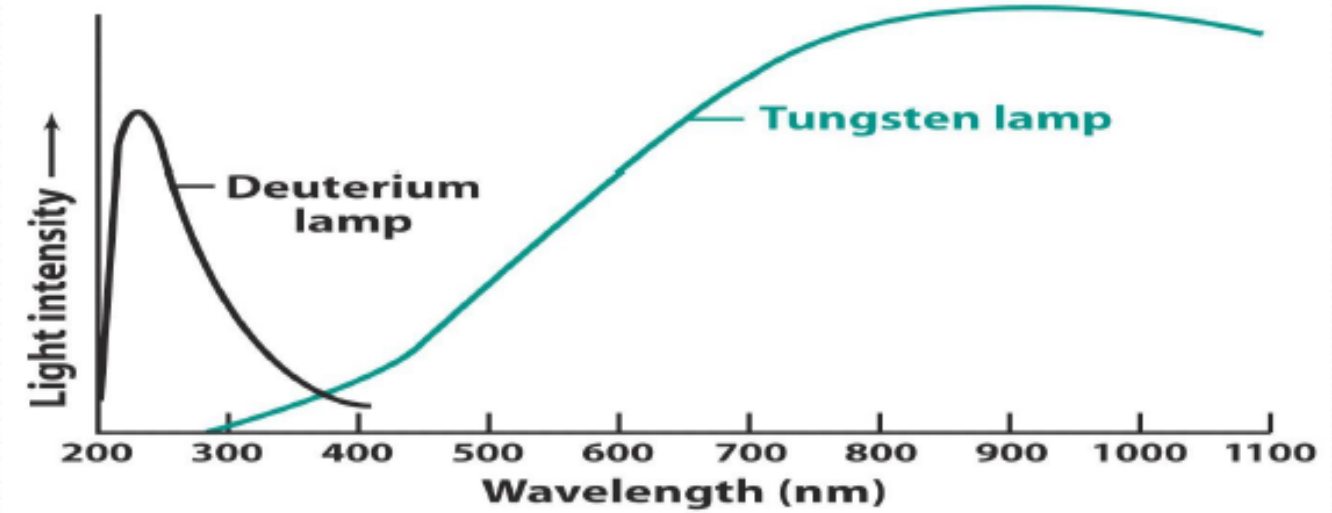



stray-light baffle
& exit aperture
MEMS reflection
grating element
2nd order filter
focusing
mirror
collimation
mirror
fibre-optic
connector

The Spectrophotometer

1-Source of Light

- **UV light from 200 to below 380 nm =** deuterium or hydrogen lamp.
- **Visible region from 380 nm to 780 nm** = tungsten or tungsten-halogen.



2- Wavelength Selectors

- To limit light to a certain wavelength
- Monochromator (prism or gratting) can isolate a specific wavelength of white light and allow it to pass through the solution being analyzed.

3- Sample Containers

- **Cell or cuvettes:**
- Visible range = glass cuvette and plastic cuvette
- UV range = quartz cuvette

4- Detectors

Detector: Convert radiant energy (photons) into an electrical signal.

- **Photocell:** To detect transmitted light,
- **Or Photomultiplier tube:** very sensitive detector

5- signal processor and readout

Out put:

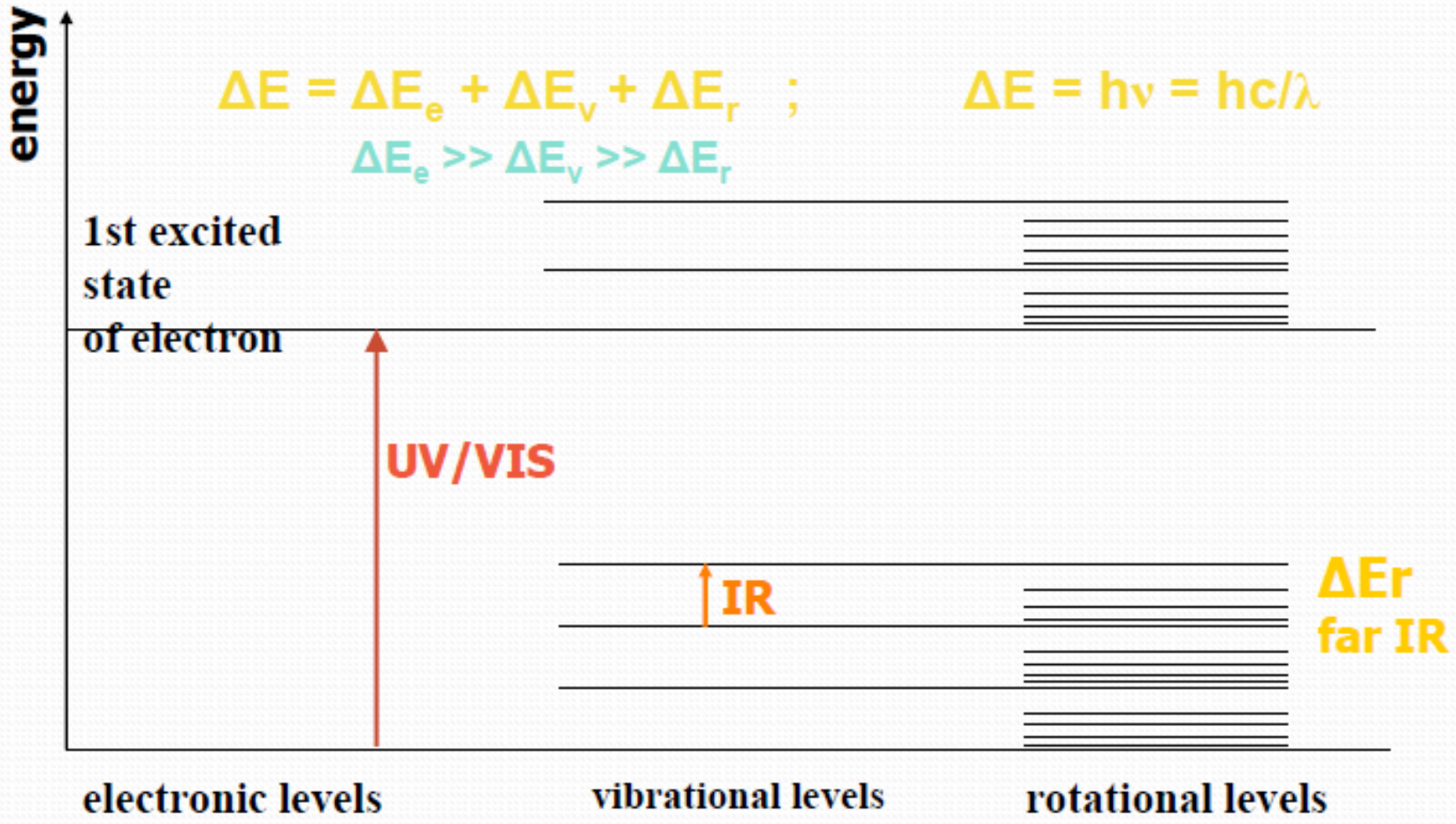
- The final instrument component, the output transducer, converts the modified electrical signal into information in a form useful to the analyst.
- Meter
- Digital

- Chart paper; or recorder
- Computerizes system

Absorption of radiation

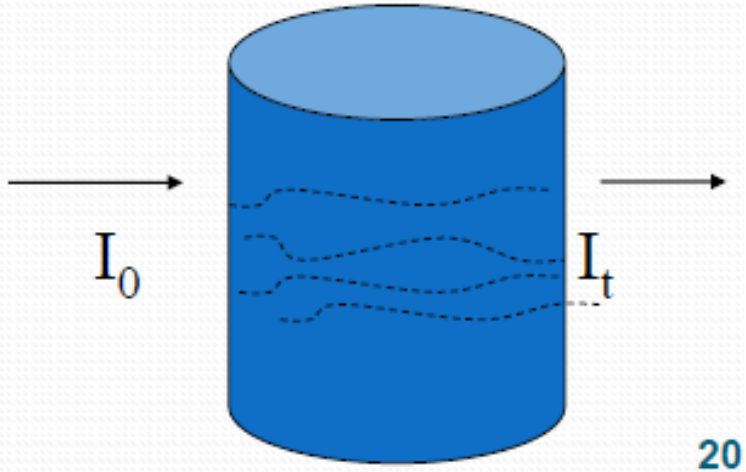
- Molecules of the sample absorb the photons of a suitable wavelength (λ) and change their energy level (state):
  - 1) in the microwave and far infrared region, the photons have such a low energy that, if absorbed, can cause only the changes of the rotational energy states
  - 2) absorption of photons of the infrared radiation can bring about the changes of the vibrational energy states
  - 3) energy of photons of UV and visible light (VIS) is sufficient to cause the transition of electron to a higher electronic energy level

Energy levels of a molecule



Definitions & Symbols:

- Radiation Intensity (I)
- $\mathbf{I_t}$: is the radiation transmitted by the solution.
- $\mathbf{I_o}$: is the radiation transmitted by the pure solvent (blank).
- **Transmittance (T)**
  - It's also referred to as %T or T x 100
  - $\mathbf{\%T = \frac{I_t}{I_o} \times 100}$



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Definitions & Symbols:

- **ABSORBANCE (A)**
- **$A = \log(1/T) = -\log(T)$**
- **$A = \log \frac{I_o}{I_t} = \log I_o - \log I_t$**
- Absorbance is what is generally recorded from a spectrophotometer.

Beer - Lambert law

More dissolved substance = more absorption and less transmittance.

Beer-Lambert's Law is:

$$A = \varepsilon b C$$

$$\text{Log}\left(\frac{I_o}{I_t}\right) = \varepsilon b C$$

A= Absorbance (no units)

ε = molar extinction coefficient, **molar absorptivity**

c = concentration of the absorbing species (mol/L)

b = path length of the light-absorbing sample (cm)

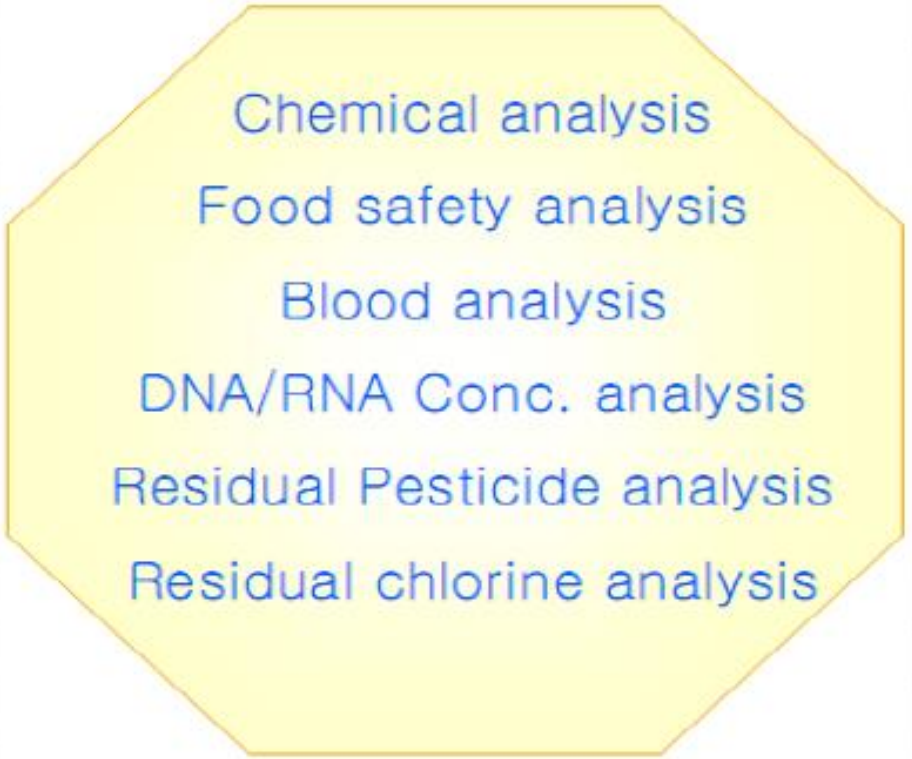
Sample Problem

- **Cytosine has a molar extinction coefficient of 6 x 10^3 mol^{-1} L cm^{-1} at 270 nm at pH 7. Calculate absorbance of 1 x 10^{-3} M cytosine solution in 1mm cell at 270 nm.**
- **Solution:**
- **A** = Log $\left(\frac{I_o}{I_t}\right)$ = εb C
- ε = 6 x 10^3 mol^{-1} L.cm^{-1}
- b = 1mm = 0.1 cm
- **C** = 1 x 10^{-3} M
- **A = ε b c** = (6 x 10^3)x (0.1) x (1 x 10^{-3})

  = 6 x 10^{-1}

  = 0.6

Applications of Spectrophotometry



The important characteristics of Spectrophotometric methods

1. Wide applicability to both organic and inorganic systems
2. High sensitivity of **10^{-6}-10^{-4}** M
3. Moderate to **high selectivity.**
4. **Good accuracy** the relative error encountered in concentration lie in the range from **1% to 3%**
5. **Ease and convenience** of data acquisition

Home work

- **Q1.//** Which one is more sensitive? Explain why?
  Photo tube or photomultiplier tube as detector.

- Q2.//Why can't we use glass or plastic cell in UV spectroscopy with wave length from 200-400nm?

Resources and references:

- Textbook: Principles of instrumental analysis, Skoog et al., 5th edition, chapter 7, 13.
- Quantitative chemical analysis, Daniel C. Harris, 6th edition , chapter 20.
- Lecture slides partially adopted from Dr. Raafat Aly slides.
- Useful links

- http://www.youtube.com/watch?v=pxC6F7bK8CU&feature=player_detailpage
- http://bio-animations.blogspot.com/2008/04/double-beam-uvvis-spectrophotometer.html