Al-Rasheed University
College
Medical Instrumentation
Tech. Eng.

Clinical Chemistry Instrumentation and Technology

Lecture (4)

Spectrum Instruments and Uses

2nd stage – 2022/2023

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Light Structure

- Light is a set of photos propagate in the medium as waves. It is a part of electromagnetic spectrum.

$$f = \frac{c}{\lambda} = \frac{2.998\times10^{8}\ \textbf{(m/s)}}{\lambda\ \textbf{(m)}} = \textbf{Value (1/s) = Value (Hz)}$$

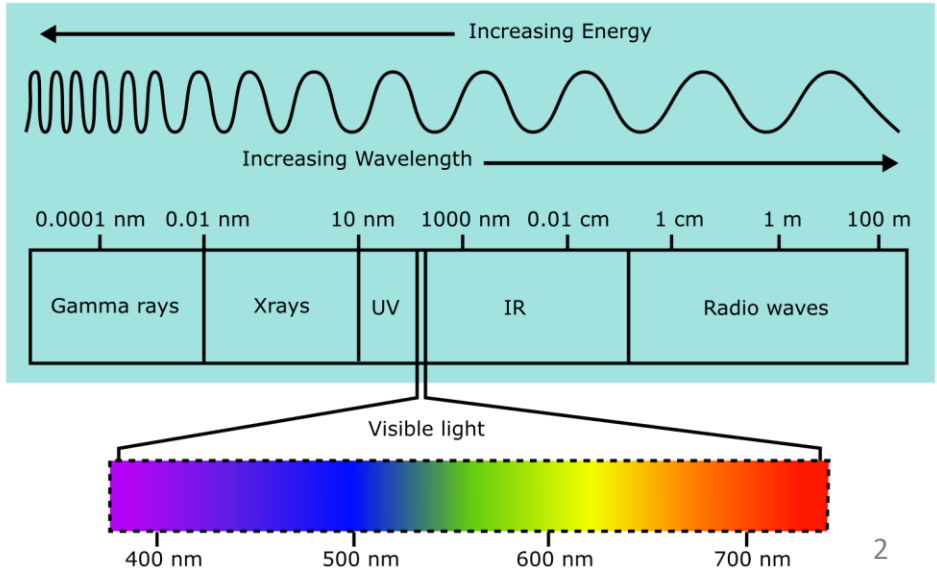
- The energy of photons is given by:

$E = hf$, h = **Planck Constant** = 6.63×10^{-34} **J.s**

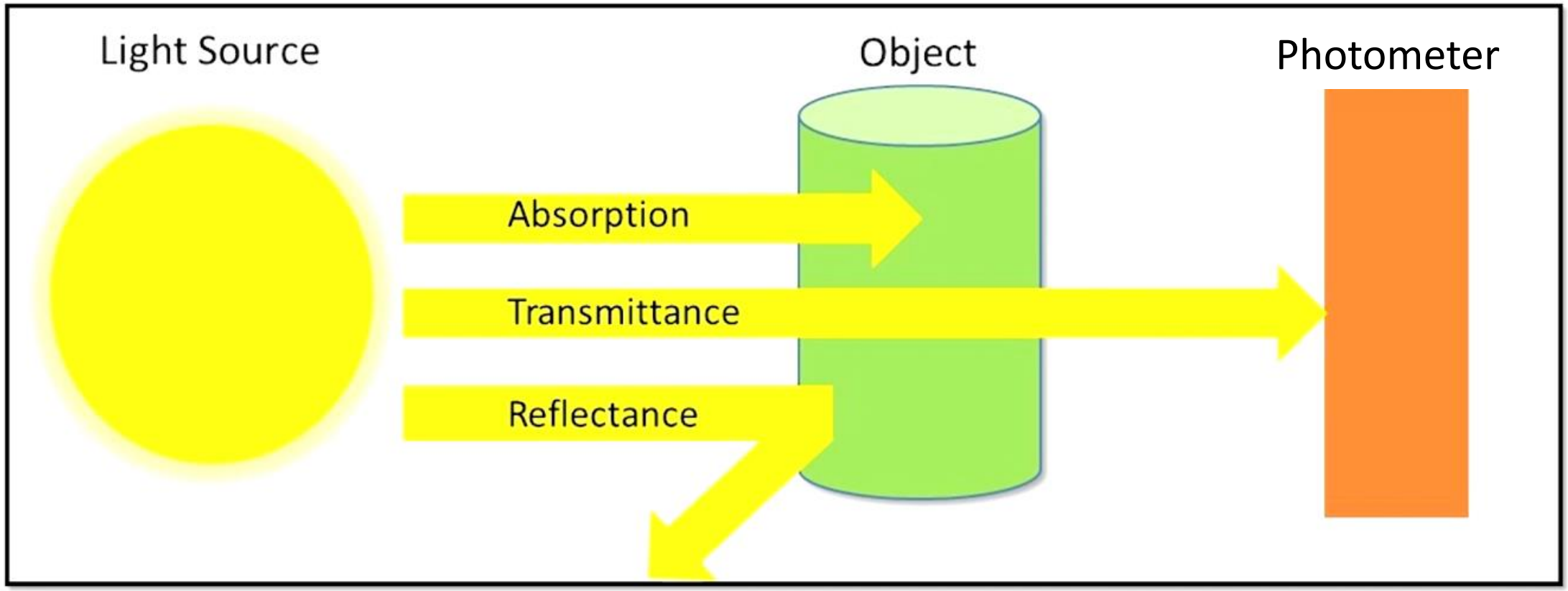
Example (1): What is the energy of a photon with a wavelength of 650 nm.

Key ans.:

$E = 3.056\times10^{-19}$ **J**



Interaction of EM radiation with materials
ثلاث عمليات ممكن أن تحصل عندما تعترض الموجة الكهرومغناطيسية الأجسام.



A spectrophotometer is made up of two instruments: a spectrometer and a photometer. The spectrometer ***is to produce light of unique wavelength***, while the photometer is ***to measure the intensity of light***.

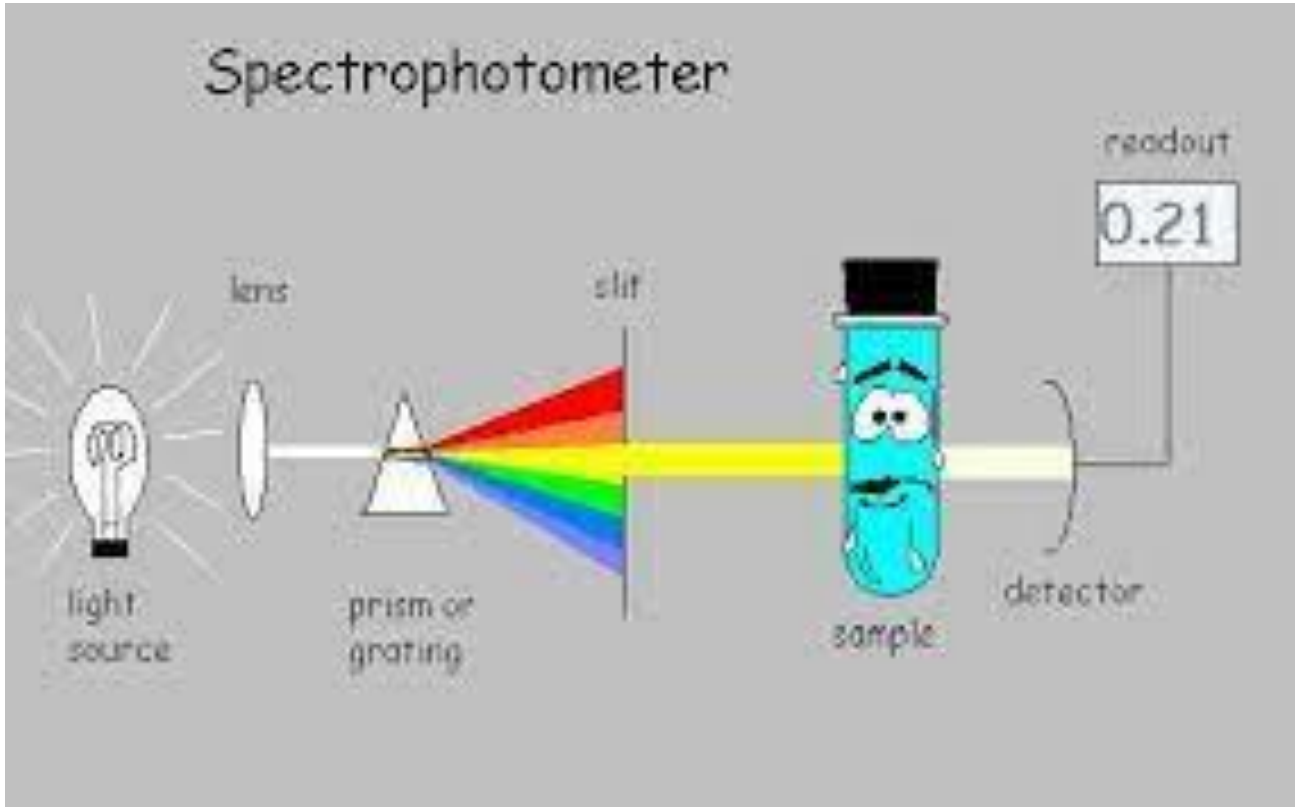
- The liquid or a sample is placed between spectrometer and photometer.
- The photometer measures the amount of light that passes through the sample and delivers a voltage signal to the display.
- **Colorimeter** is a device that can select and apply a single beam of light (with one wavelength, λ) to determine the concentration of specific components in the serum or liquid based on colour-light absorption.
- There are three main types of spectrum instrument (colorimeter, spectrophotometer, flame photometer).

The relationship between the wavelength and colour absorbed. العلاقة بين الأطوال الموجية والألوان الضوئية الممتصة من قبل المواد.

Wavelength, λ	Name	Color absorbed
180-320	Short UV	Not visible
320-380	Long UV	Not visible
380-440	Visible	Blue
500-580	Visible	Green
580-600	Visible	Yellow
600-620	Visible	Orange
620-750	Visible	Red
750-2000	Short infrared	Not visible

Working principle of spectrum instrument:

- The basic concept of spectrophotometer devices depends on Beer-Lambert law. Spectrophotometry is based on two principles: (1) substances absorb light at unique wavelengths, and (2) the amount of light absorbed is proportional to the amount of substance present.
- The solution to be measured must be coloured, and if it is not coloured, then it should be treated chemically to be coloured. The colour grading should represent the concentration of the solution. Therefore, the absorbed light intensity increases proportionally with solution concentration.
- يجب أن يكون المحلول المراد قياسه ملون، وإذا لم يكن كذلك يجب معاملته كيميائيا حتى يكون ملون، شريطة أن يكون غمق اللون يتناسب طرديا مع تركيز المادة المراد قياس نسبة تركيزها.
- Passing single light through the solution, then determines the intensity of the light passing through and exiting the solution (transmitted). The darker the colour solution, the more concentration and then more absorbance and less transmittance.



Solution concentration, light intensity, transmittance light quantity (penetrated).

شدة الضوء المرسل أو الضوء النافذ خلال العينة يتناسب عكسيا مع شدة تركيز المادة المقاسة.

Types of Photometers

(1) **Colorimeter** is a photometric device that uses an optical filter and works on the visible light range only. The most important parts of a colorimeter are:

1. **Light source** is usually an ordinary filament lamp (white light), such as **Tungsten lamps**.
2. **An aperture** (exit slit): set the direction of the light beam toward the sample tube or cuvette. Therefore, a smaller slit opening makes the colorimeter more accurate.
3. **Optical filters** to filter out unwanted wavelengths and keep the desired wavelength pass through.
4. **Cuvette** is a sample container made of quartz, plastic, or glass (Visible range only).
5. **Detector** (photocell) receives the transmitted light and converts its intensity to electrical energy.
6. **Output**: the output of the colorimeter may be shown in graphs or tables by an analogue or digital meter. The data may be printed on paper or stored on a computer.

(2) **Spectrophotometer:** is a photometric device that uses an **prisms** and **diffraction grating** instead of optical filters to generate **monochromatic** light beams, as well as it covers both UV and Visible light ranges. The common parts of the spectrophotometers include:

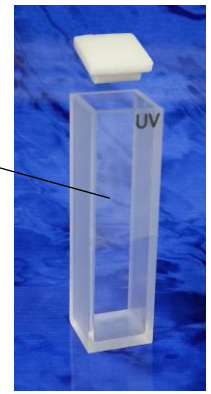
1. **The light source**, or the lamp, provides wavelengths of light in the visible or ultraviolet (UV) range. **Tungsten lamps** are used for ***visible wavelengths*** (approximately 380 to 750 nm). **Deuterium** or **mercury-arc** lamps generate ***UV light***. Some light sources such as **Xenon lamps** operate on ***both UV and visible light ranges***.

2. The **monochromator**, such as a prism, or diffraction grating, is used to eliminate unwanted wavelengths of light and allow the desired light (λ) to reach the sample.

3. A **cuvette.**

4. **Detector**.

5. **Display or printer to present or play results.**



Transparent
to EM energy

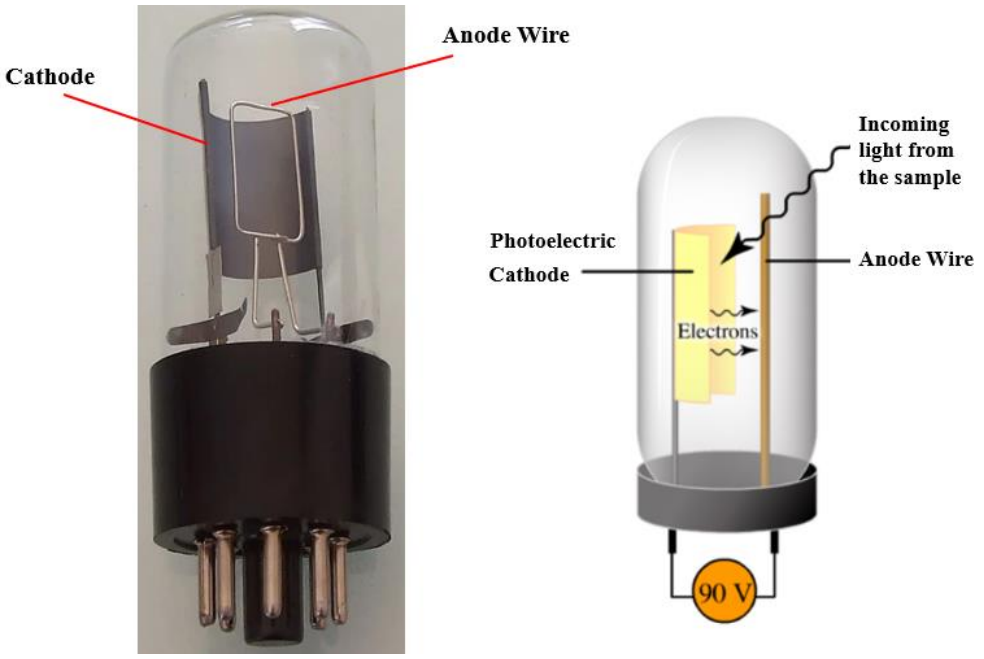
Non

Types of Photodetectors

1. **Photocell الخلية الضوئية** is also known as a photomultiplier tube, which consists of anode wire and photocell cathode.

✓ The photocell cathode is made of **photosensitive material**. When a beam of light falls on the cathode, photoelectrons are ejected out and are attracted by the anode generating an electric field. Thereby sending a current through the circuit measured by the galvanometer connected to the circuit.

2. Photosensitive semiconductor device.



The photomultiplier tube

Wavelength Selection

There are five basic types of filters that can be used in the spectrometer الفلاتر الأساسية في المطياف الضوئي:

1. **Gelatine filter**
2. **Glass filter**
3. **Interference filter**
4. **Diffraction grating**
5. **Prism**

Gelatine filter

- These are low-cost selection devices which produce or transmit a **wideband** of radiation, usually **20 nm**. (Not good, Narrow band is more accurate).

- كلما كان حزمة الضوء المفلتر أضيق كان دقة النتائج أعلى.

- The most common gelatine filter is constructed by sandwiching a thin layer of dyed gelatine of the desired colour between two thin glass plates.

- يصنع فلتر الجلاتين من خلال صب جلاتين مصبوغ باللون المرغوب به في قالب يتكون من لوحي زجاج.

- They absorb approximately 30% – 40% of all incident radiation, thereby reducing energy throughput to the detector. يمتص نسبة كبيرة من الطاقة المطبقة فبالتالي يقلل من شدة الطاقة النافذة الى الكاشف الضوئي.

Glass filter

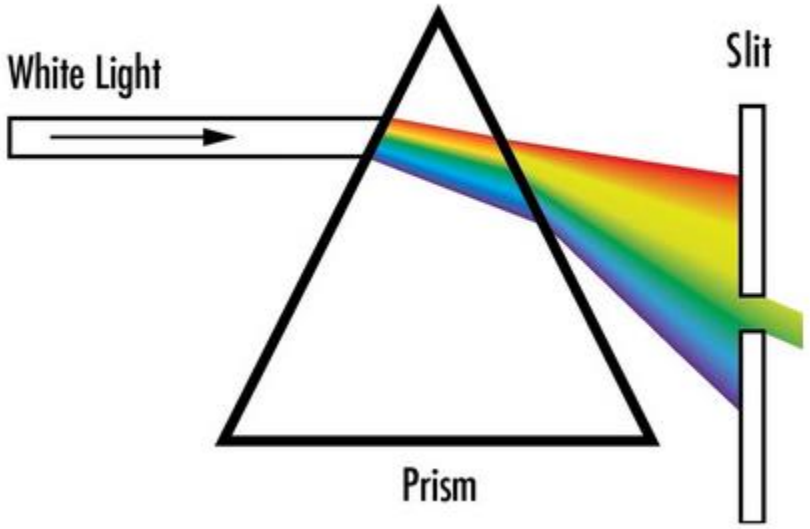
- Coloured class filters, each colour represents a wavelength. It is **less used**. Because it has a **wideband** often up to **150 nm**.

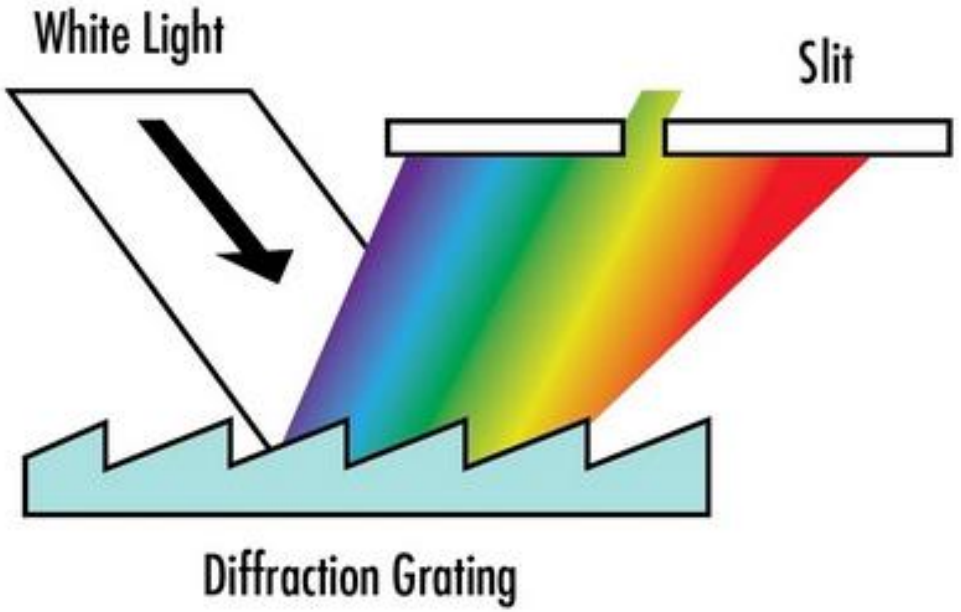
Interference filter

- These are used to select wavelengths **more accurately** by providing **a narrow bandpass** of around **10 nm**.
- The interference filter only absorbs approximately **10%** of the incident radiation over the whole spectrum, allowing **higher intensity light to reach the detector**.

Prism filter vs Diffraction gratings filter

- The **prism** is a type of an optical filter that separate wavelengths through **refraction** الإنكسار. While the **diffraction gratings** is a type of an optical filter that separate wavelengths through **diffraction** الحيود because of their surface structure.





Types of spectrophotometer

1. VIS spectrophotometer
2. UV-VIS spectrophotometer.
3. Infrared spectrophotometer.
4. Fluorescence spectrophotometer.
5. Atomic absorption spectrophotometer.

(1) VIS Spectrophotometer

- VIS spectrophotometer is an instrument used to measure absorbance and conduct quantitative analysis at the visible light (400 ~ 760nm), known as the visible spectrophotometer.
- **Bacterial cell density can be determined at 600 nm.**

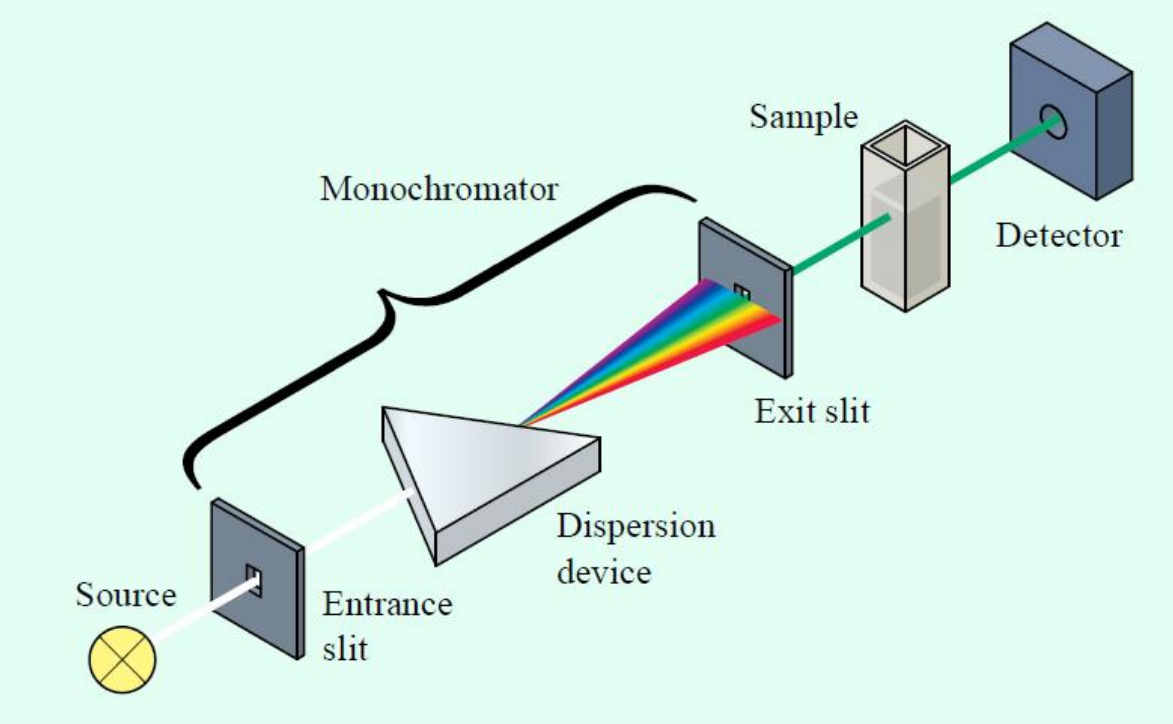
(2) UV-VIS Spectrophotometer

- UV-VIS spectrophotometer is used to measure the material of absorbance and quantitative analysis at the visible or ultraviolet light (200 ~ 760nm).
- It is applied to measure the **nucleic acid** and **protein concentrations**, as well as the **bacterial cell density**.

❑ Spectrophotometer can be divided into a ***single beam***, ***split beam***, and ***double beam*** for different applications.

A single beam is an analytical instrument in which all the light waves coming from the light source pass through the sample.

- These single beam spectrophotometers are compact, optically simpler, and unsuitable for highly demanding pharmaceutical and quality inspection industries.
- The **advantages** of a single beam configuration are that there are often **fewer moving parts**, which makes the instrument simpler and less likely to have parts get out of alignment.
- **The measurements taken from single beam spectrophotometers are <u>less reproducible</u>** because a single light beam is used at a two different times (not at the same time).

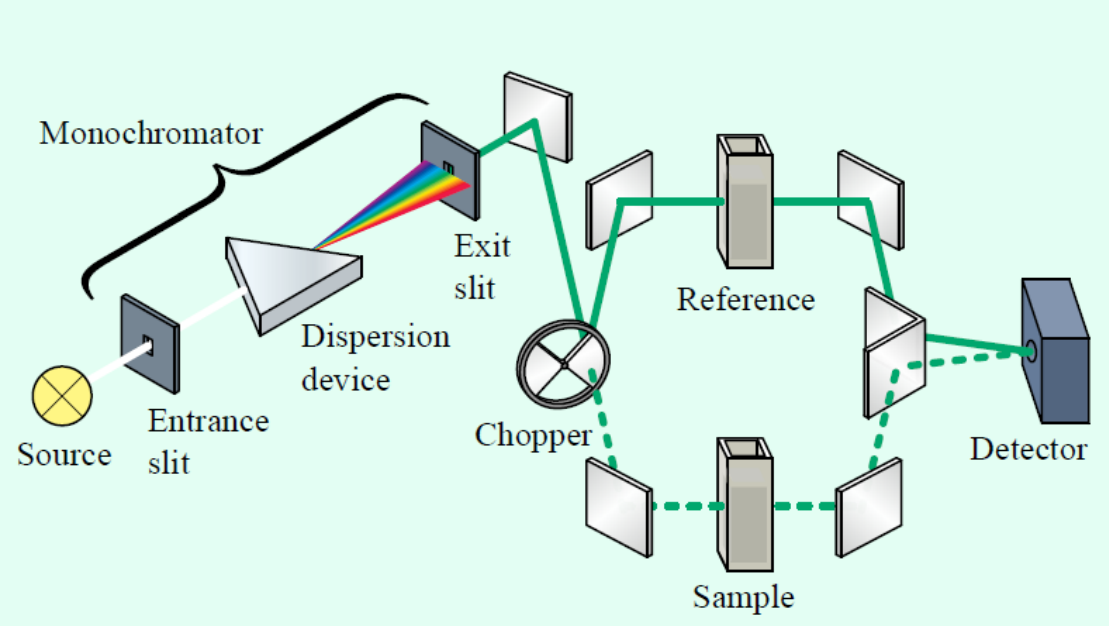


Schematic of a single beam spectrophotometer.

Note: In a conventional single-beam spectrophotometer, the blank and the sample are measured consecutively, with an interval of several seconds for a single wavelength measurement and up to several minutes for a full spectrum measurement with a single beam spectrometer. Lamp drift can result in significant errors over long time intervals. conventional instrument. Lamp drift can result in significant errors over long time intervals.

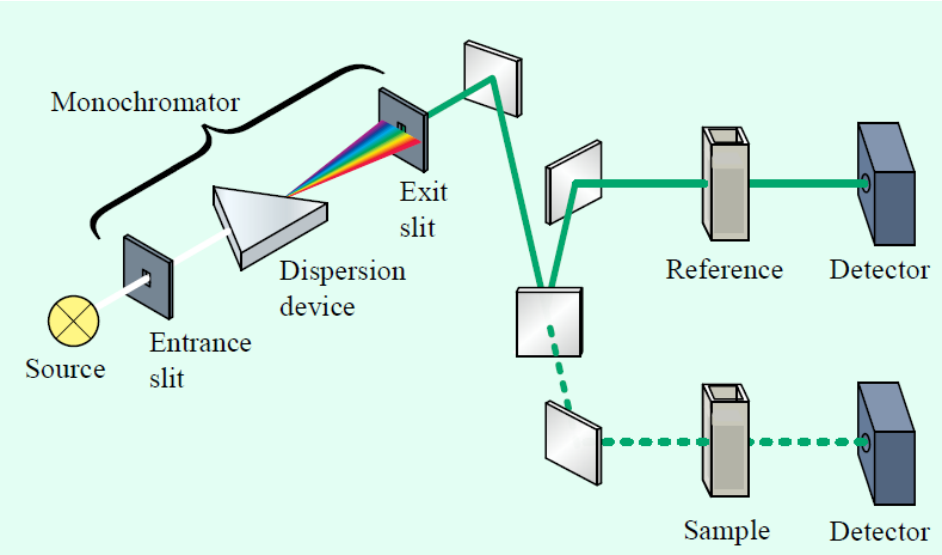
A double beam UV-visible spectrophotometer utilizes a splitting mirror and exiting slits in one piece. The desired beam is forwarded into two paths; one through a reference cell (the transmission is 100%) and another path through the sample to be tested.

- **The measurements taken from double beam spectrophotometers are highly reproducible** because electronic and mechanical effects on both sample and reference beams are equal (occur at the same time for both reference – distilled water – and sample).



Split beam spectrophotometer: The light emitted by the same monochromator is split into two beams, one of which reaches the detector directly, and the other passes through the sample and reaches the other detector.

- ❑ The split-beam design is mechanically simpler than the dual-beam instrument and requires fewer optical elements.



Note: This design provides high stability, although not as high as a dual-beam instrument since two detectors can drift independently, and high noise, compared to a single-beam instrument since the light is split so that less than 100 % passes through the sample.

Quiz Lecture 5

Please answer all questions and submit them in the next lecture.

Thank you ☺