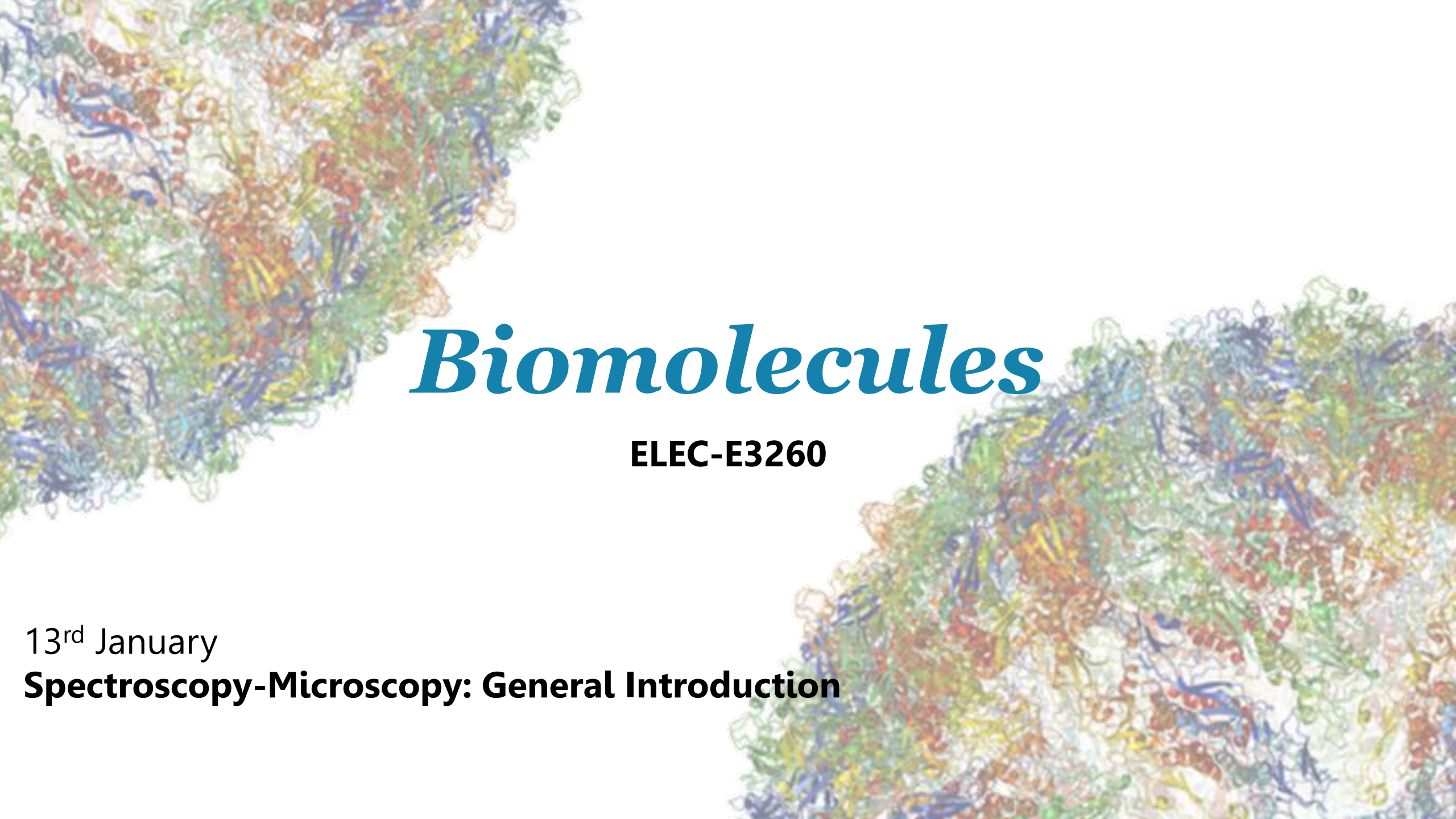


Biomolecules

ELEC-E3260

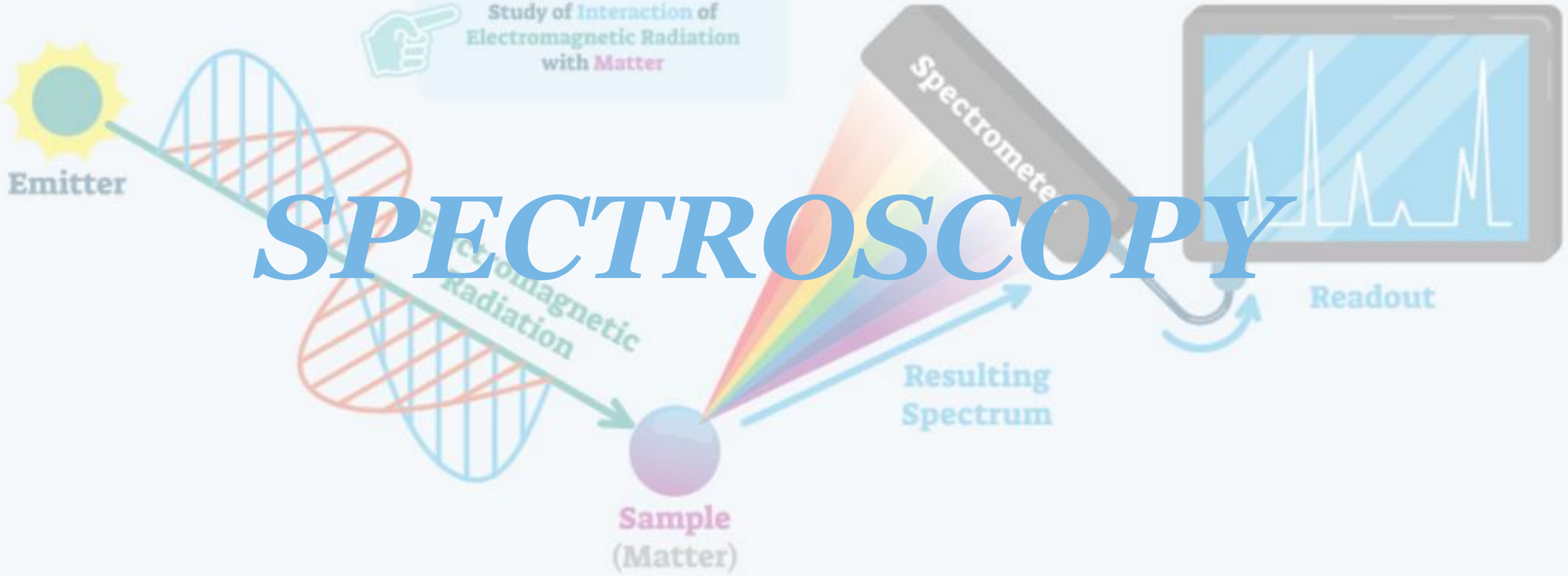
13rd January

Spectroscopy-Microscopy: General Introduction

Summary

- Spectroscopy
- Microscopy
- Difference between Spectroscopy and Microscopy
- In-class activity

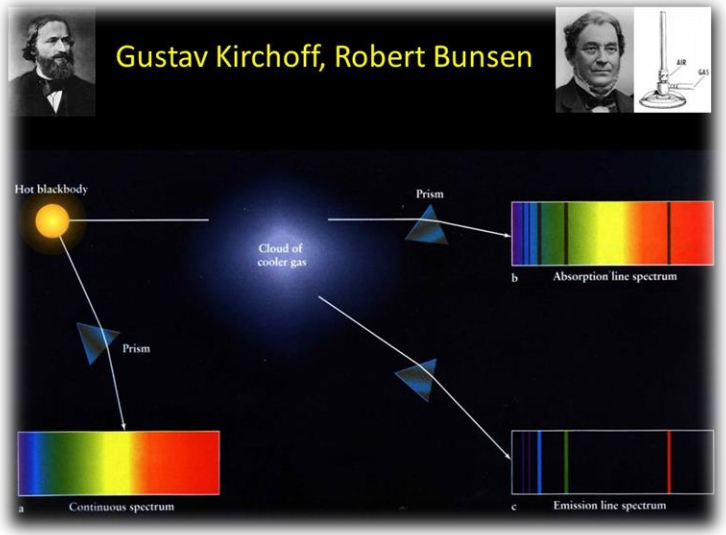
SPECTROSCOPY



Brief History

- **Isaac Newton :** Founder of the spectroscopy (1666).

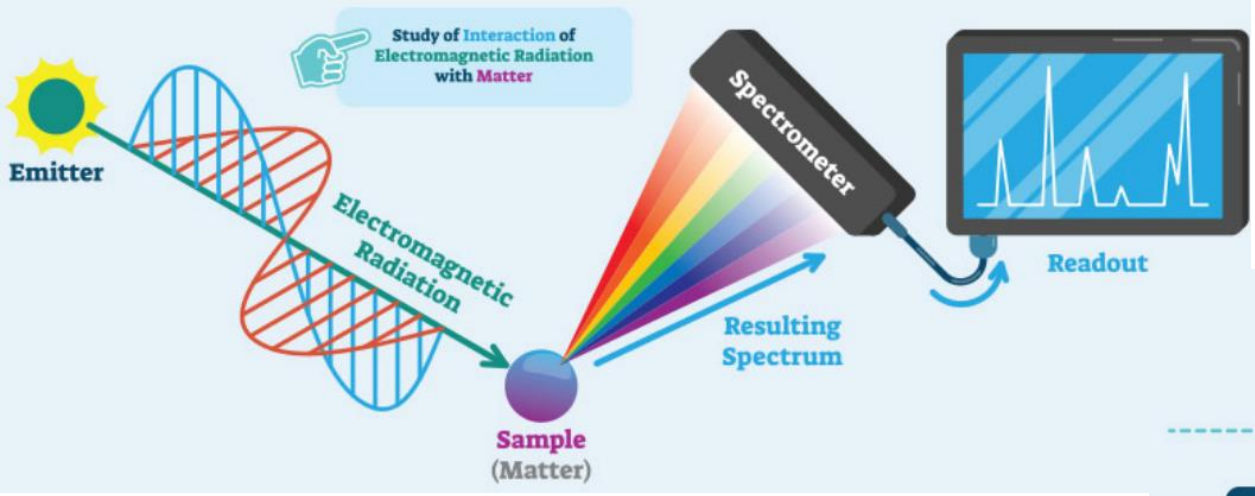
- **Gustav Robert Kirchhoff and Robert Wilhelm Bunsen :** Spectroscopy as an analyze technique is born (1859).

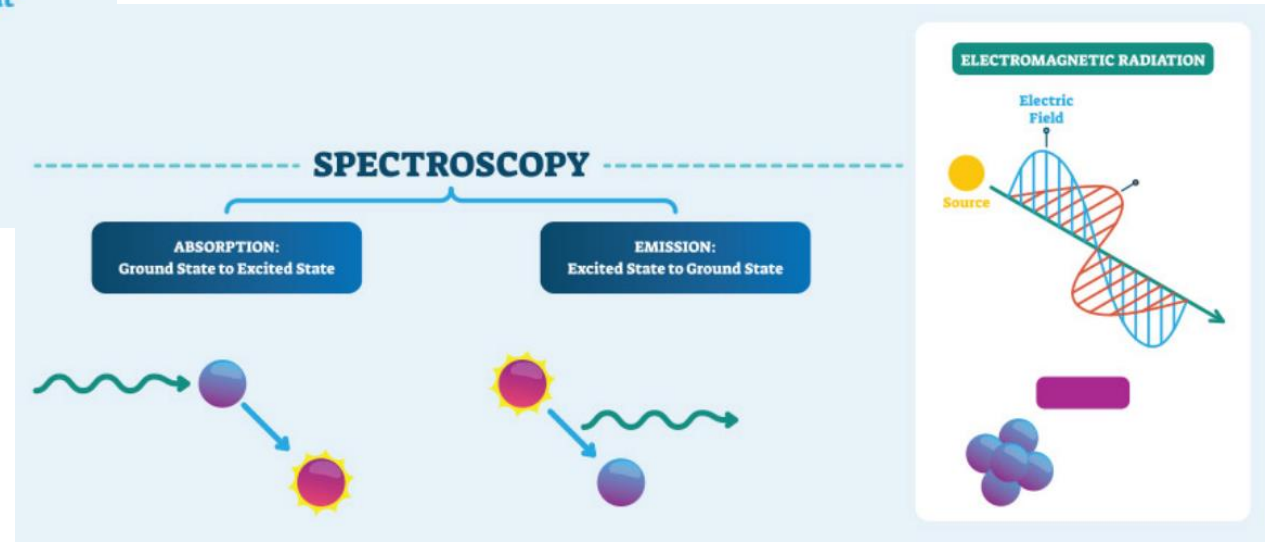


- **Sir Normal Lockyer :** Determined the exact wavelength of the Helium (5876Å) with spectroscopy technique (1868).

What is spectroscopy ?

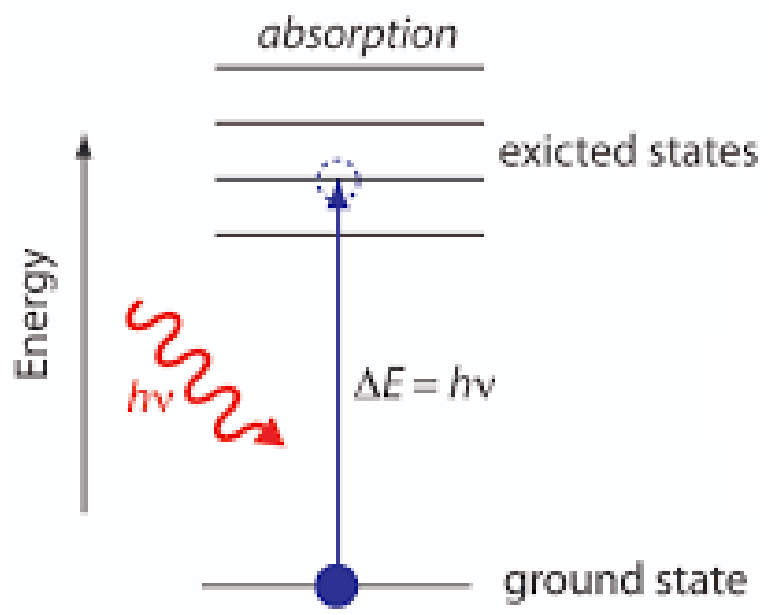
- **Spectroscopy** is the investigation and measurement of spectra produced by matter interacting with or emitting electromagnetic radiation.

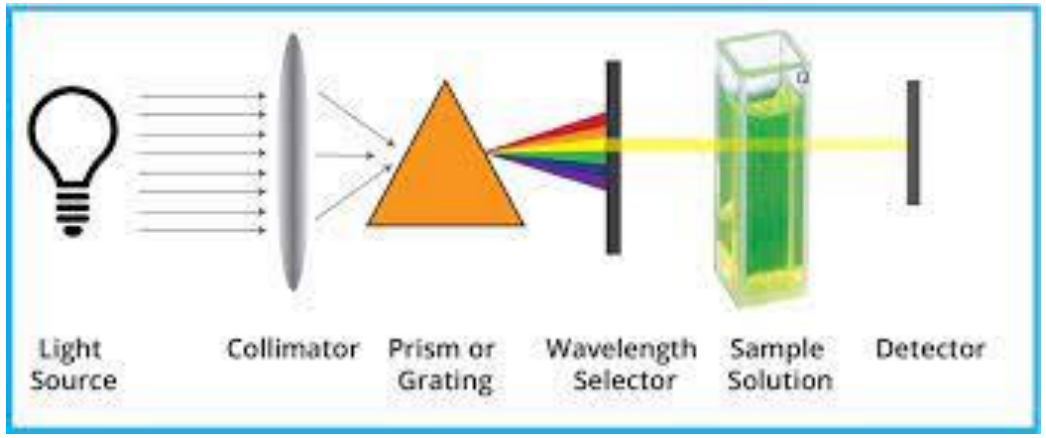


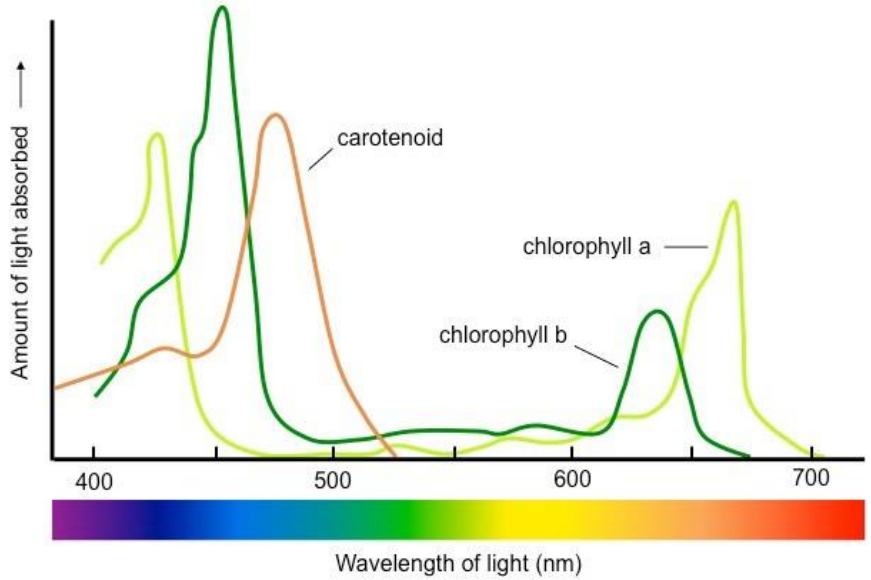


What is spectroscopy ?

- **Absorption :** Measures how much light is absorbed by a sample over a range of wavelengths defined by the electromagnetic spectra

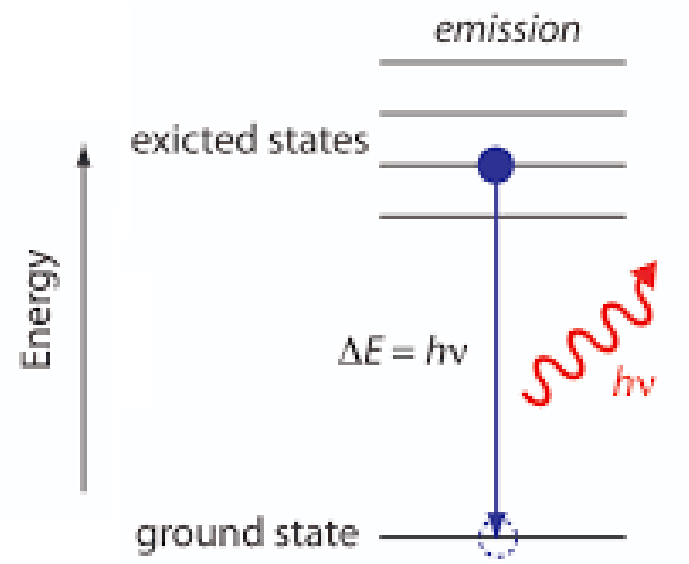


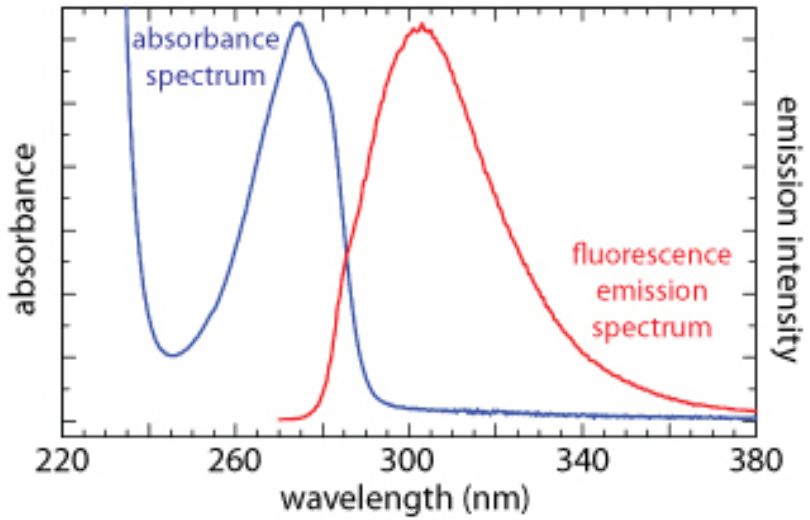




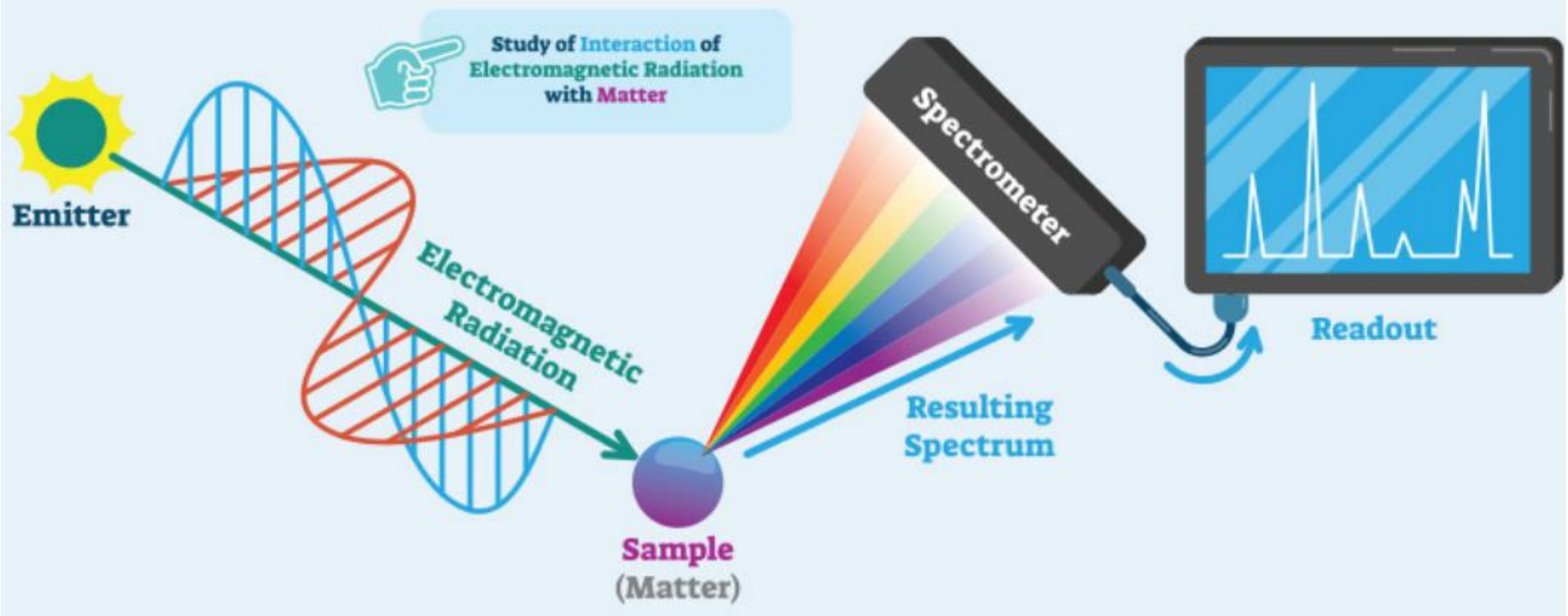
What is spectroscopy ?

- **Emission :** Measures how much light is emitted by a sample over a range of wavelengths defined by the electromagnetic spectra. It is created when the atoms of an element releases energy.



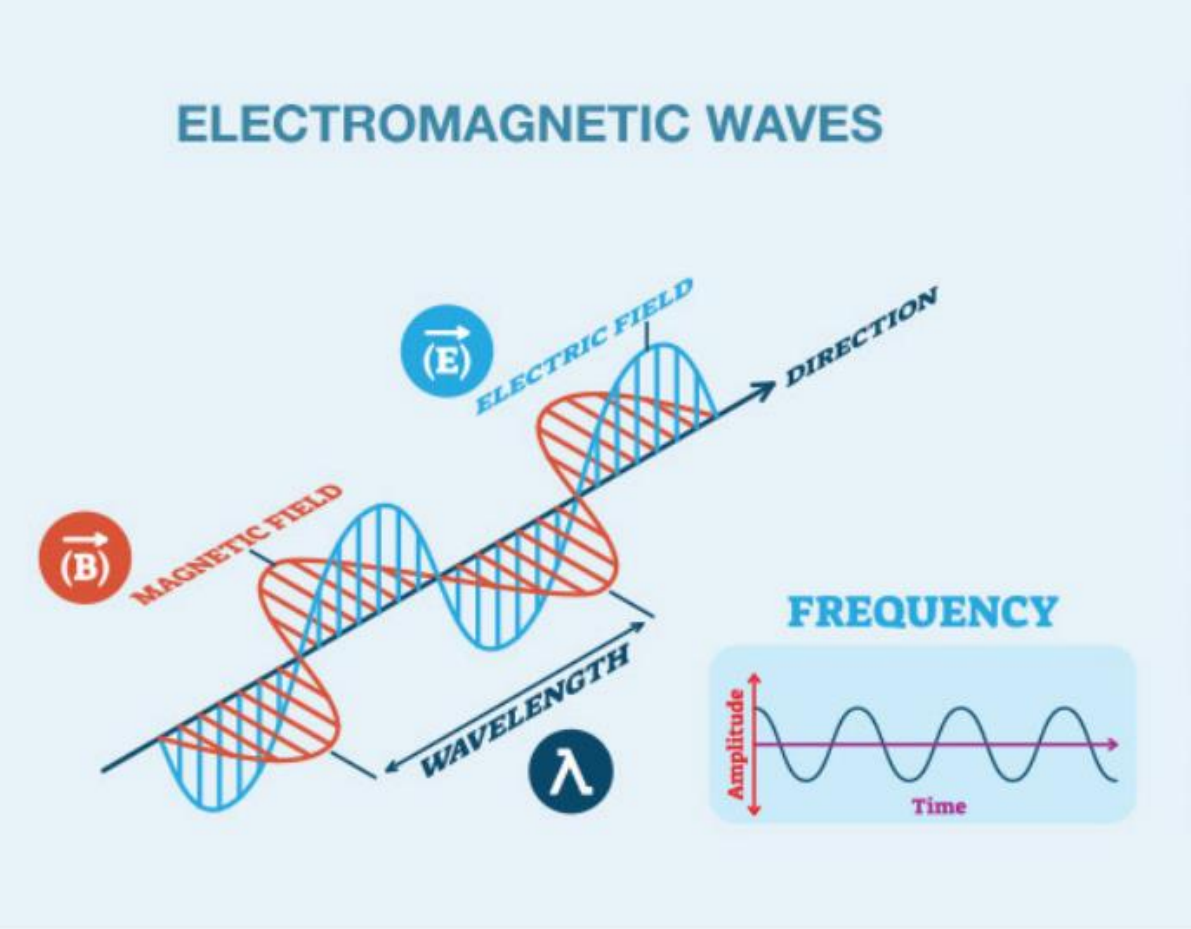


More Concrete



- Absorption : System is perturbed
- Emission : System is going back to his equilibrium

Electromagnetic field

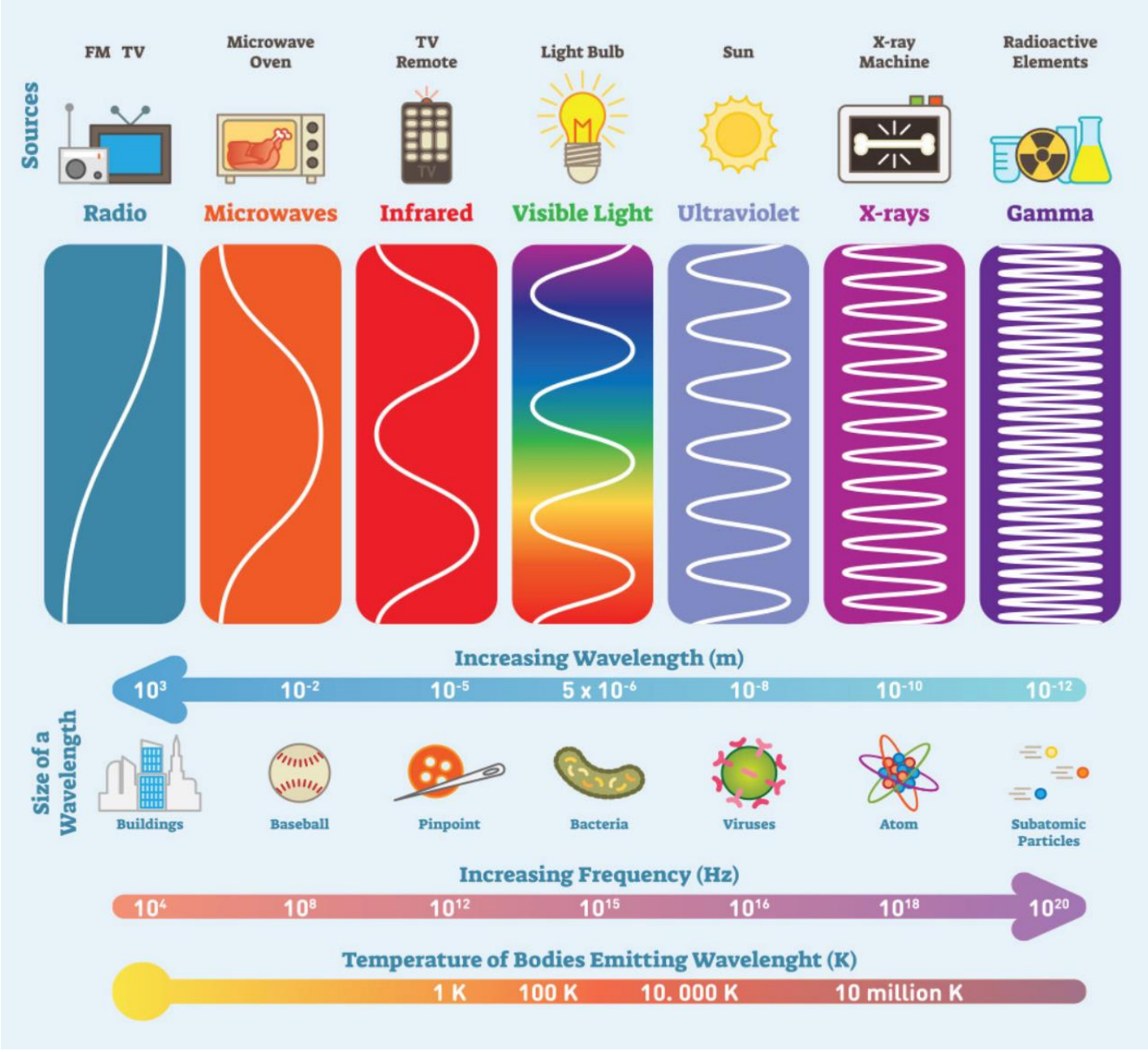


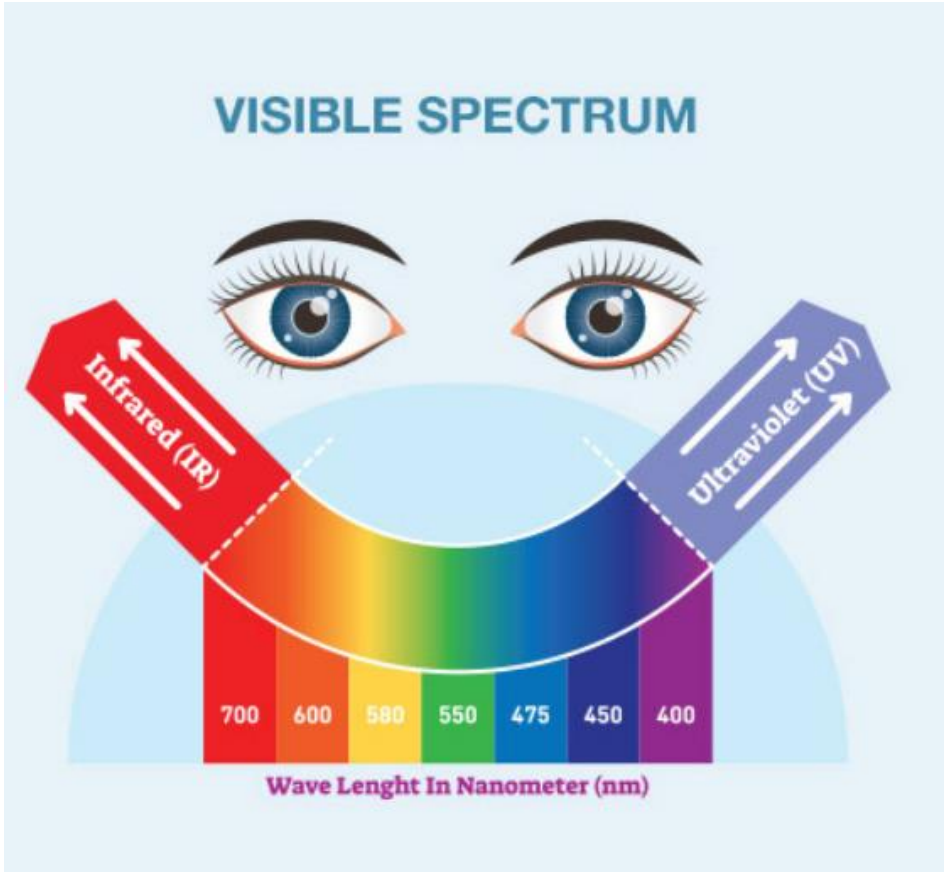
$$\nu = \frac{1}{T} \qquad \lambda = cT = \frac{c}{\nu} \qquad \overline{\nu} = \frac{1}{\lambda}$$

$$E = h\nu$$

ν frequency (Hz); T period (s); λ wavelength (m); c celerity of the light (3.10^8 m/s); $\overline{\nu}$ wavenumber (cm^{-1}); E energy of the photon (J) and h Plank constant ($6{,}624.10^{-34}$ J.s).

Electromagnetic spectrum





Quantification of the energy levels

- **Born-Oppenheimer Approximation:**

- $m_{nuclei}=2000*m_{electrons}$
- Nuclei are fixed and unmoving

- $E_{tot}= E_{el} + E_{nucleide}$
- $E_{nucleide} = E_{vib} + E_{rot} + E_{tr}$

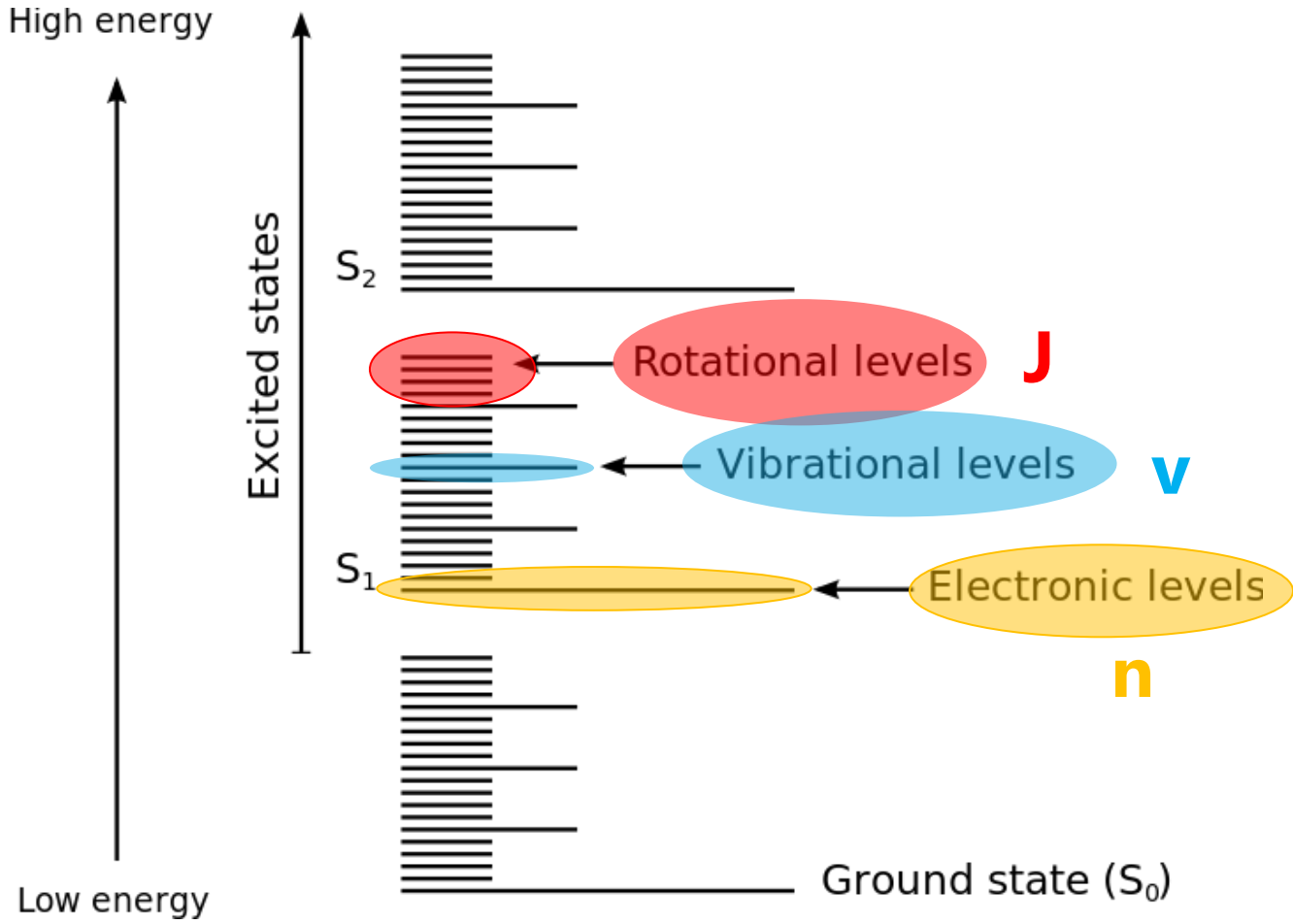
$=> E_{tot}= E_{el} + E_{vib} + E_{rot}$

Quantification of the energy levels

Order of magnitude :

- $\Delta E_{el} >> \Delta E_{vib} >> \Delta E_{rot}$

- UV-vis: Investigate about Electronic Transition
- MIR and NIR : Investigate about Vibration Transition
- MW : Investigate about Rotation Transition
- FIR : Investigate about Rotation and Vibration Transition according to the molecule.



Selection rules

- Selection rules determine if a transition is allowed or not.

- Light-matter interaction is possible if :
  - The frequency of the light corresponds to the difference of energy **ΔE** between two energy levels concerned.
  - Creation of the dipolar moment μ variation of the system at the same frequency.

- If μ is *electric* : Dipolar Electric Transition
- If μ is *magnetic* : Dipolar Magnetic Transition

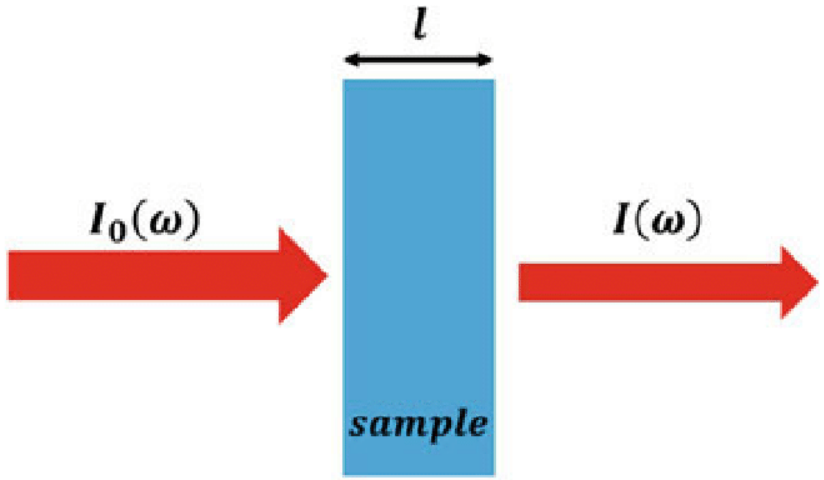
Some important formula

- Maxwell-Boltzmann law :

$$N_J = N_0 . e^{\Delta E_{i \to 0} / K_B T}$$

- Beer-Lambert law : (For liquid at low concentration)

- $A = log\left(\frac{I_0}{I}\right) = \varepsilon l C$
- $T = \frac{I}{I_0}$; $log\left(\frac{1}{T}\right) = A$



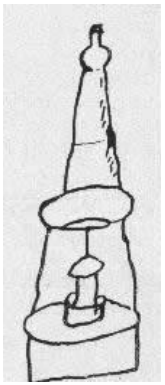
What is spectroscopy used for ?

- Determining the atomic structure of a sample
- Determining the metabolic structure of a muscle
- Monitoring dissolved oxygen content in freshwater and marine ecosystems
- Studying spectral emission lines of distant galaxies
- Altering the structure of drugs to improve effectiveness
- Characterization of proteins
- Space exploration
- Respiratory gas analysis in hospitals

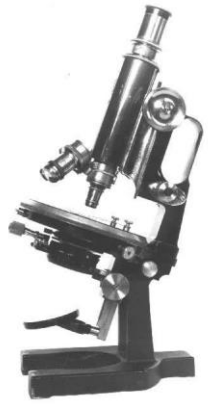
Microscopy

Brief History

- First microscope was made in the late 1300's
- 1609 **Galileo Galilei** perfected the first device known as a microscope.
- In the late 16th century, **Anton van Leeuwenhoek** the founder of the study of microscopy and an played a vital role in the development of cell theory.
- The first **electron microscope** was developed in the 1930's by **Max Knoll** and **Ernst Ruska**.
- **Scanning tunneling microscope** invented in 1981 by **Gerd Bennig** and **Heinrich Rohrer**.
- Later **Bennig** and his colleagues, in 1986, went on to invent the **atomic force microscope.**

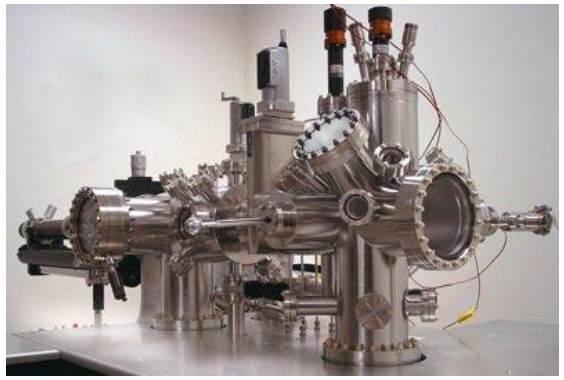


First drawing of a microscope (1625)



Zeiss Microscope (1912)

Electron Microscope

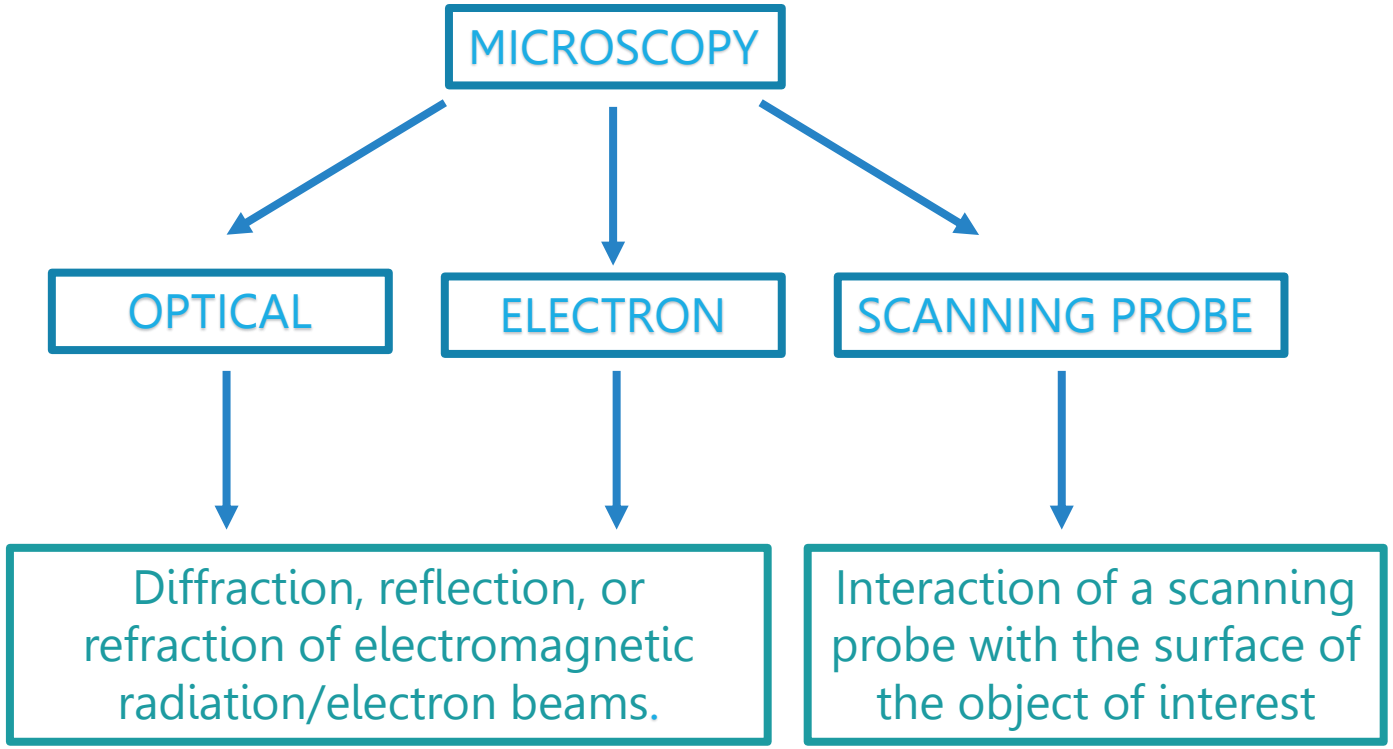


Scanning Tunneling Microscope

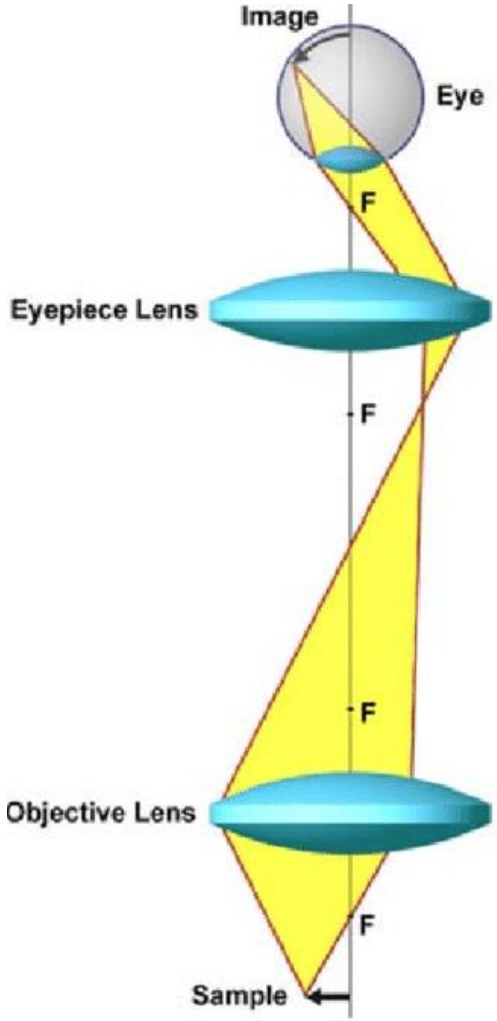
Atomic Force Microscope

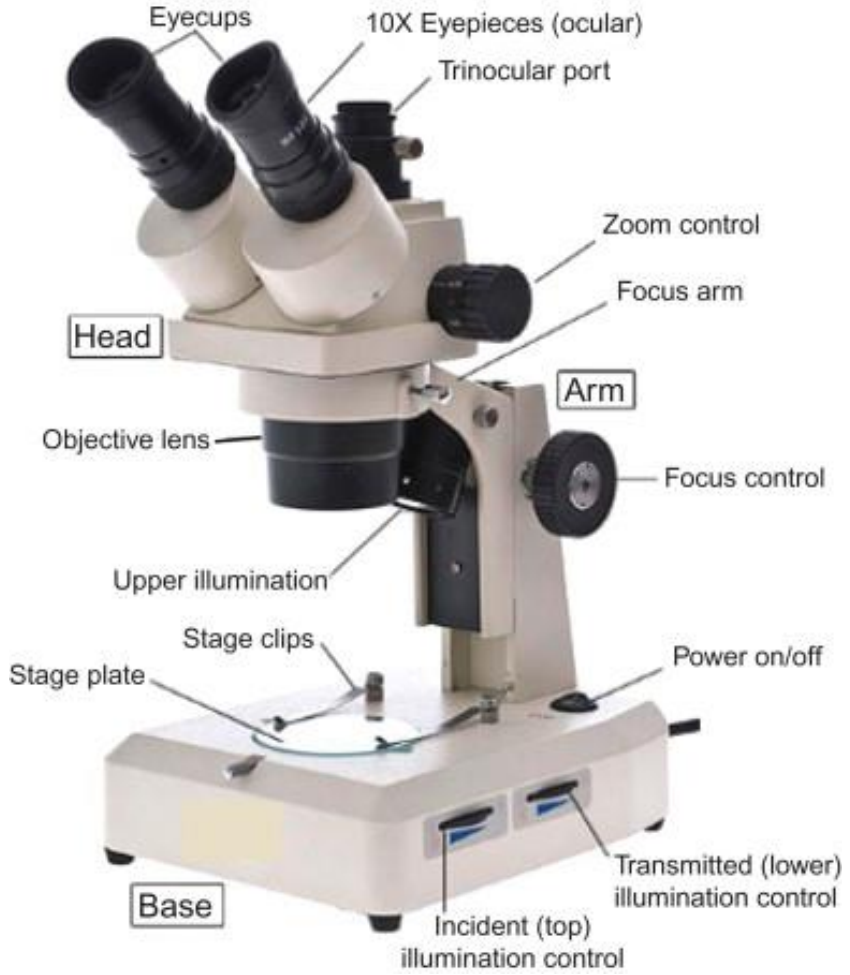
What is Microscopy ?

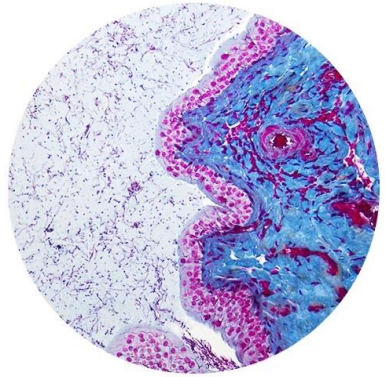
- **Microscopy** to view objects (cannot be seen with naked eye)

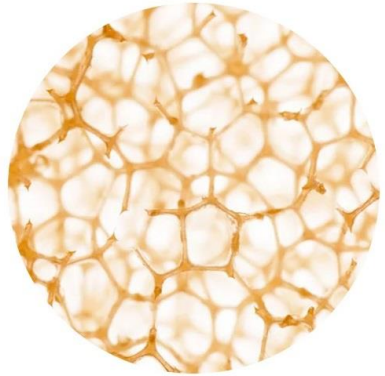


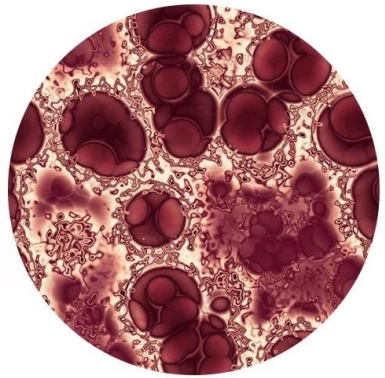
Optical Microscopy











Types of optical microscopes

Simple Microscope

Compound Microscope

Digital Microscope

Stereo Microscope

Comparison Microscope

Inverted Microscope

Optical Microscopy: Applications and limitations

Applications:

- Microbiology
- Microelectronics
- Nanophysics
- Biotechnology
- Pharmaceutical
- Biological samples for medical diagnoses

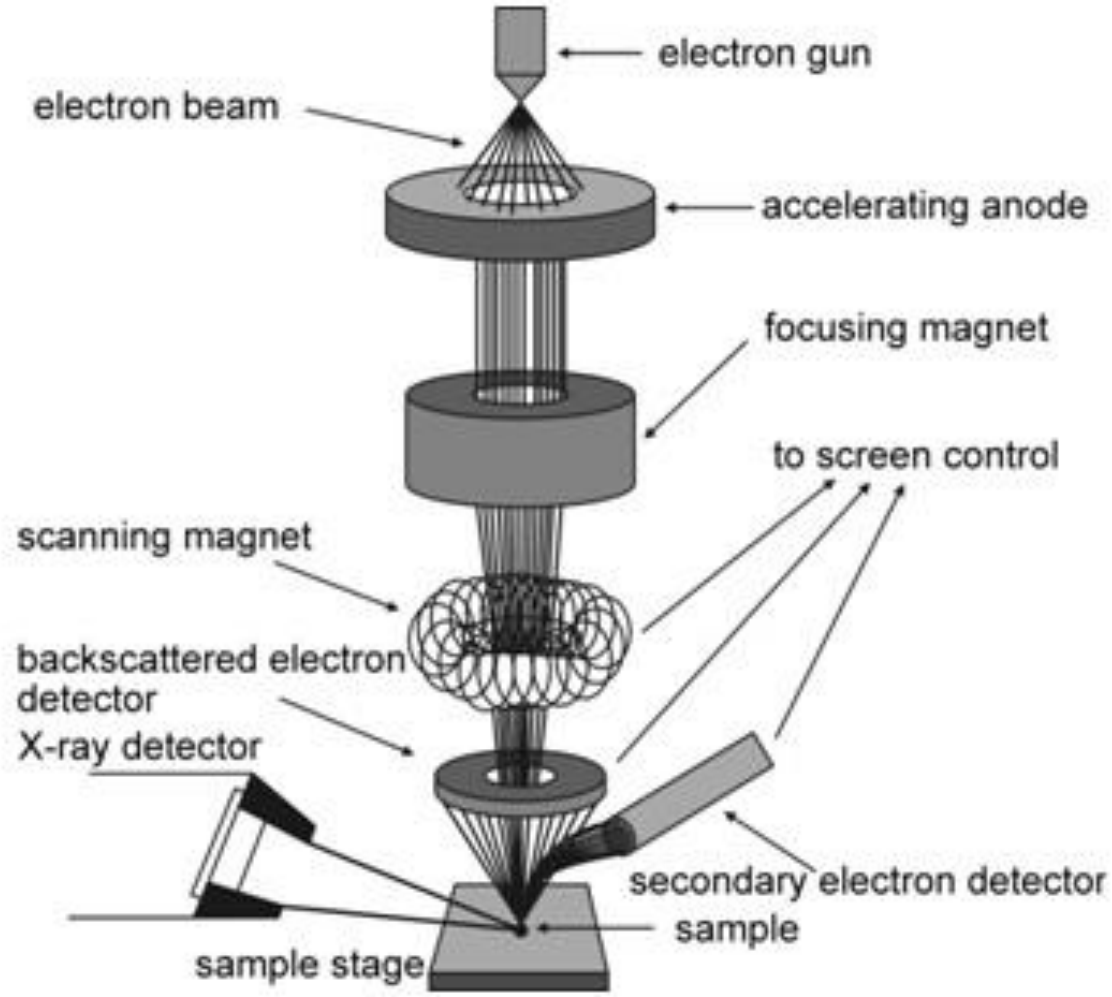
Limitations

- Airy Disks
- Diffraction limit

$$d = \frac{\lambda}{2nsin(\alpha)}$$

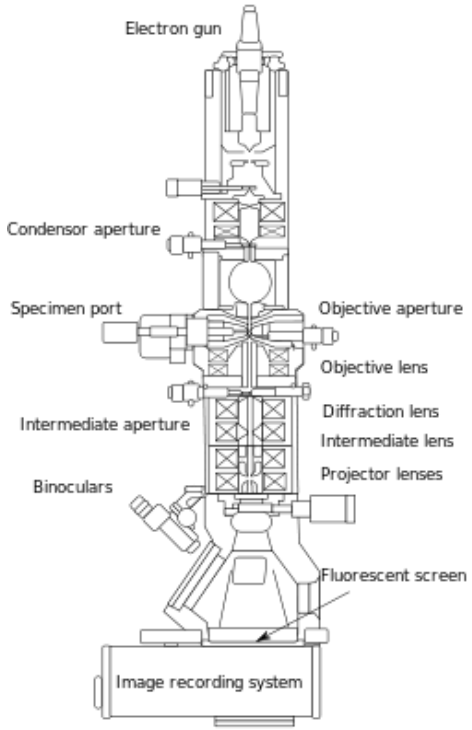
Electron Microscopy

- The electron microscope uses a beam of electrons and their wave-like characteristics to magnify an object's image.
- Can resolve features that are more than 1 million times smaller than optical microscope.
- Gain information as to sample's structure and composition.

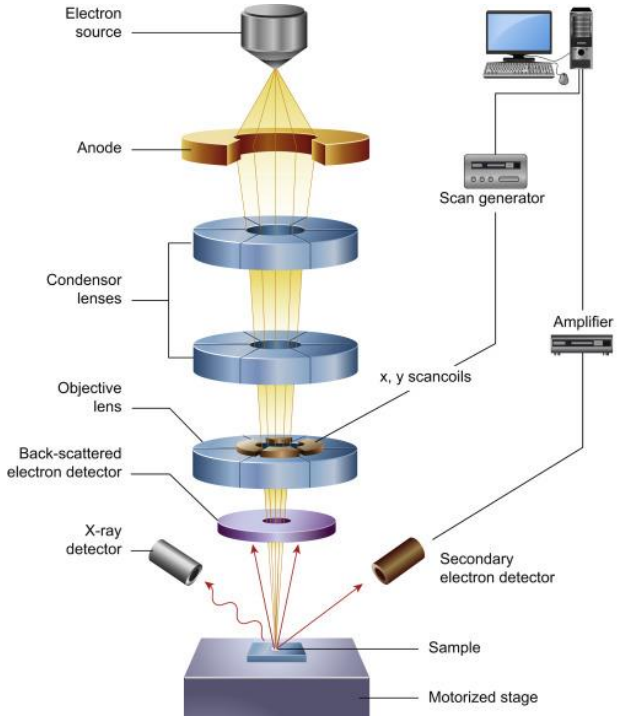


Types of electron Microscopes

Transmission Electron Microscope



Scanning Electron Microscope



Electron Microscopy: Applications and limitations

Applications

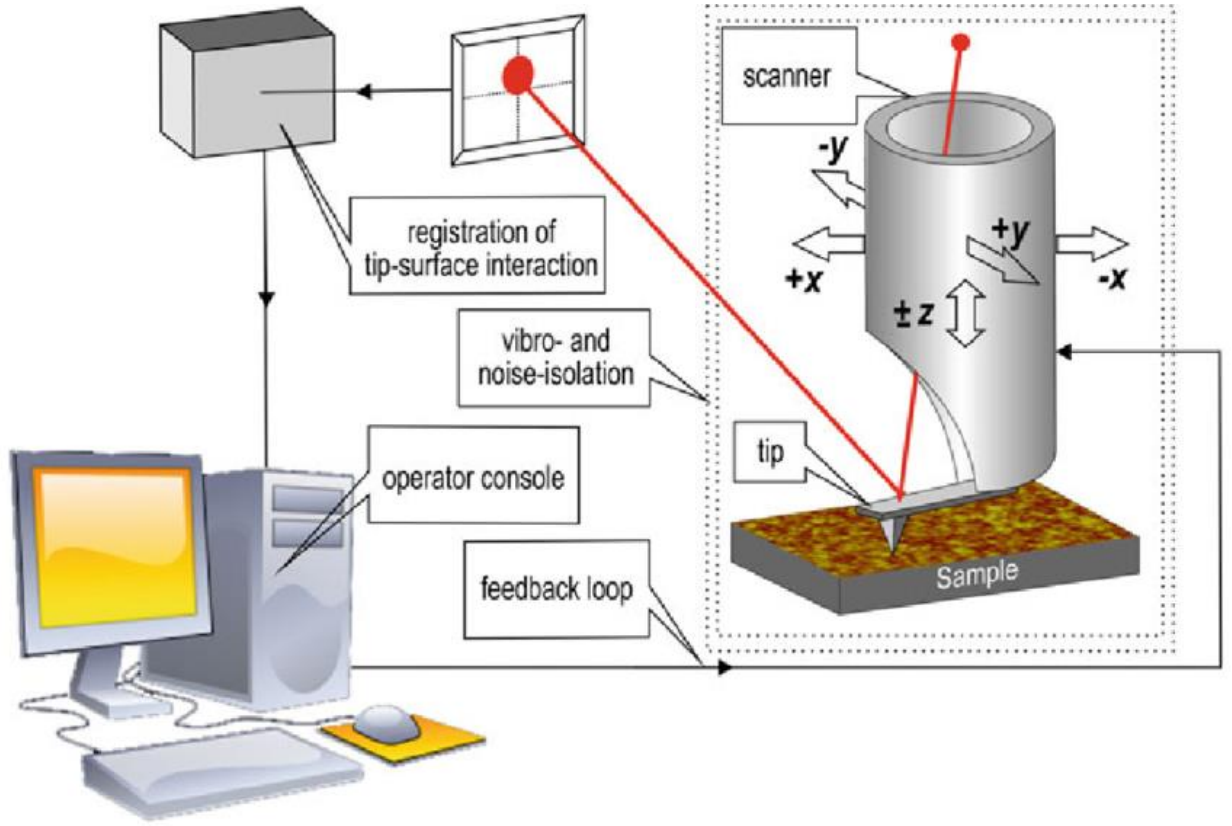
- **Investigate:**
  - Microorganisms
    - Cells
  - Large molecules
  - Biopsy samples
    - Metals
    - Crystals
- **Quality control and failure analysis.**

Limitations

- Cost
- Size
- Maintenance
- researcher training
- Image artifacts

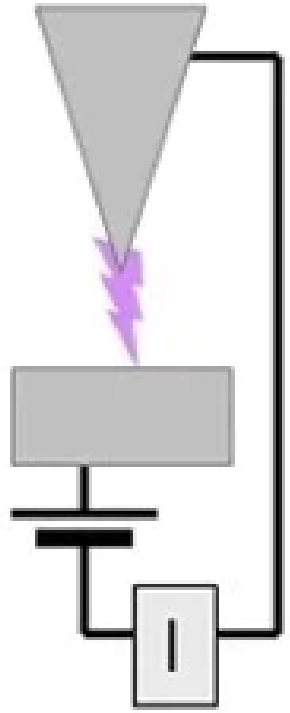
Scanning Probe Microscopy

- Scanning the sample surface with a tip and tracking the strength of the interaction between the surface and tip.
- At a distance of a few nanometers or angstroms.
- Simple
- User-friendly

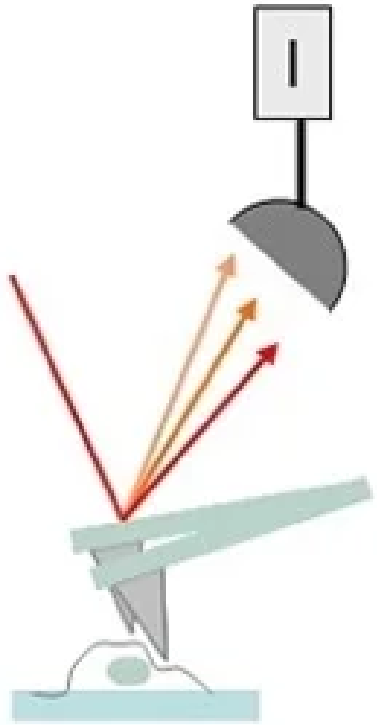


Types of Scanning Probe Microscopes

Scanning Tunneling Microscopy (STM)



Atomic Force Microscopy (AFM)



Scanning Near-field Optical Microscopy (SNOM)

Scanning Probe Microscopy: Applications and limitations

Applications

- Study surfaces and modify them
  - Determination of the effect of atomic interactions and quantum effects on surface friction
  - Industries such as automotive, aerospace and electronics
- Examine peptide structures and determine their stiffness and structure
  - Map cell surfaces
- Examine the cell membrane's stiffness

Limitations

- Images are produced in black and white or grayscale which can exaggerate a specimen's actual shape or size.

Differences between Spectroscopy and Microscopy

Microscopy

- **Improve the visibility of a structure that is too small for the naked eye to observe**
- **Uses specific lenses to improve the visibility of very fine particles**

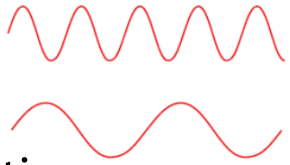
Spectroscopy

- **Determine how electrons respond to light energy, determine the spectral lines and/or energy of a sample**
- **Uses electromagnetic radiation at specific wavelengths to investigate a sample's absorbance or transmittance**

Any Questions ?

In-Class Activity

- 1. Calculate percent transmittance from the following values of absorbance:
  a) 4.0
  b) 0.23
  c) 1.6
- 2. Convert the following from percent transmittance to absorbance.
  a) 0.10%
  b) 23%
  c) 84%
- 3. In a 5.0 M solution, a solute absorbs 90% of a visible light as the beam passes through an 80 mm cell. Calculate the molar absorptivity of this solute.
- 4. When molecules are exposed to radiation with frequency, v, such that $\Delta E = h\nu$, do they travel through a transition from a higher to lower state, or lower to higher state?
- 5. a) Which wave has the higher frequency?
  b) If one wave represents visible light and the other represents infrared radiation, which wave is which?



- 6. The wavelength of the red line in the Hydrogen spectrum is 656 nm. What is the wavenumber and frequency of it?
- 7. The yellow light given off by a sodium vapor lamp used for public lighting has a wavelength of 589 nm.
  a) What is the frequency of this radiation?
  b) Calculate the energy of one photon of this yellow light