BIO & PHARMA ANALYTICAL TECHNIQUES

Chapter 9 Electron Microscopy

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Chapter Description

- Aims
  - Discuss theory, principles and application of analytical techniques used in material characterisation, pre-formulation development, manufacturing process and storage stability.

- Expected Outcomes
  - Explain general facts of electron microscopy including application in other field
  - Illustrate theory and principle of scanning electron microscopy (SEM)
  - Discuss on the application of SEM in pharmaceutical

- References

  - Gunzler H. & Williams A. (2002). Handbook of Analytical Techniques. Wiley-VCH, Weinheim, Germany.
  - Mullertz, A., Perrie, Y. and Rades, T. (2016) Analytical Techniques in the Pharmaceutical Sciences (Advances in Delivery Science and Technology). Springer, United States.

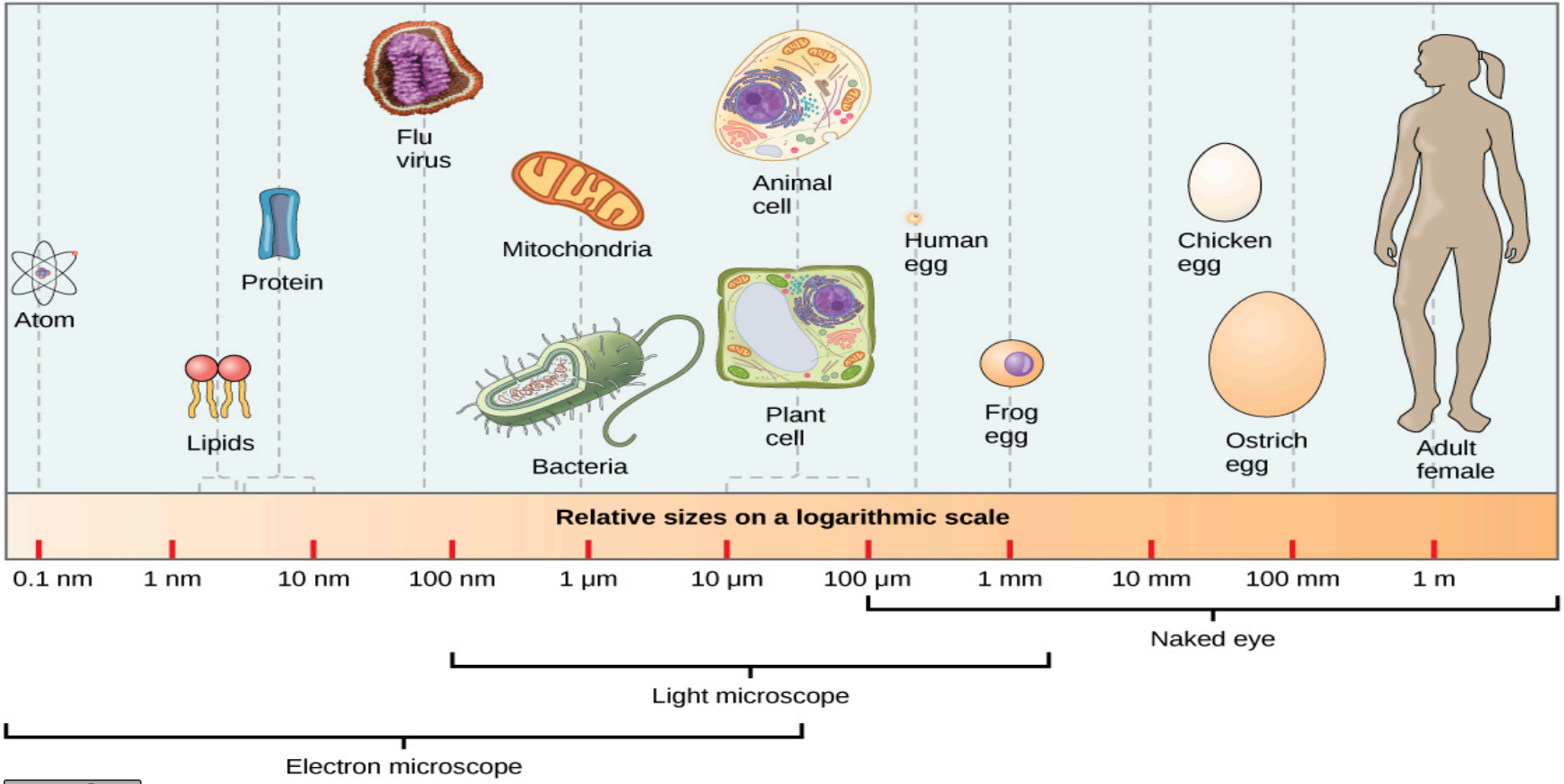
MICROSCOPY

- Microscopy word came from the Greek :

  mikros,-means small and skoopein- means to look or see
- It is an instrument use to see objects which is too small for the naked eye.
- The science of investigating small objects using such an instrument is called **microscopy**

Microscope Resolution

is defined as the capacity to distinguish small gaps between two separate points which humans cannot distinguish.



THE LIGHT MICROSCOPE *vs* THE ELECTRON MICROSCOPE

FEATURE	LIGHT MICROSCOPE	ELECTRON MICROSCOPE
Sources	Visible light 760nm (red) – 390nm Colours visible	Electrons app. 4nm Monochrome
Maximum resolving power	app. 200nm	0.2nm Fine detail
Maximum magnification	x1000 – x1500	x200 000
Radiation source	Quartz halogen lamp	High voltage (50kV) tungsten lamp
Focussing screen	Human eye (retina), photographic film	fluorescent (TV) screen, photographic film

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THE LIGHT MICROSCOPE v THE ELECTRON MICROSCOPE

FEATURE	LIGHT MICROSCOPE	ELECTRON MICROSCOPE
Preparation of specimens	Temporary mounts living or dead	Tissues must be dehydrated = dead
Fixation	Alcohol	Glutaraldehyde or OsO_4
Embedding	Wax	Resin
Lenses	Glass	Magnets
Sectioning	Hand or microtome slices Š20 000nm Whole cells visible	Microtome only. Slices Š 50nm Parts of cells visible

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Electron Microscopy (EM)

The electron microscope is a type of microscope that **uses a beam of electrons to create an image of the specimen.**

It is capable of much **higher magnifications** and has a **greater resolving power** than a light microscope, allowing it to see much smaller objects in finer detail.

They focus the electron beam using electromagnetic coils instead of glass lenses (as a light microscope does) because electrons can't pass through glass.

TYPES OF ELECTRON MICROSCOPE

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TRANSMISSION ELECTRON MICROSCOPY

- Microscopy technique in which **a beam of electrons is transmitted through an ultra- thin specimen**, interacting with the specimen as it passes through it.

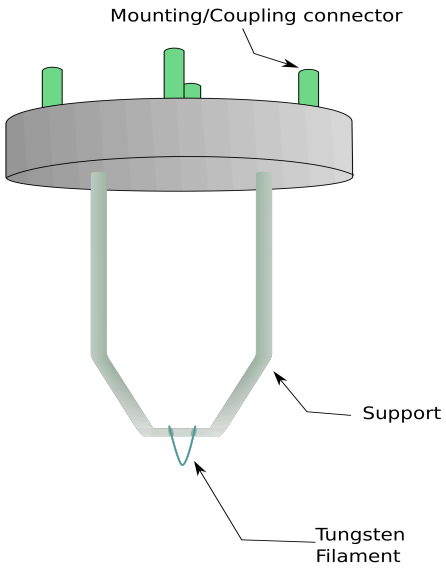
SCANNING ELECTRON MICROSCOPY

- The scanning electron microscope (SEM) uses a focused beam of **high-energy electrons** to generate a **variety of signals** at the surface of solid specimens.

SEM CORE TECHNOLOGY: Electron Gun

How is the **electron beam created**?

- Method 1: Heating a tungsten filament
- Method 2: Cold Field Emission Gun (Cold FEG)



SEM CORE TECHNOLOGY: Vaccuum

1. Electron microscopes use a vacuum to make electrons behave like light.
2. Ensures that the electron beam interacts with the sample rather than the air.
3. Samples to be viewed with the SEM must be able to withstand a vacuum and need to be conductive.
4. Samples that are not conductive can be coated with a thin layer of conductive material by a process called sputter coating.

How are the electrons accelerated?

- With an accelerating voltage!
- The anode is a positively charged plate that attracts the electrons from the tip.
- Accelerating voltages range from 500 v to 30 kV.
- The higher the voltage, the faster the electrons

SEM CORE TECHNOLOGY: Magnetic Lenses

How are the electrons focused?

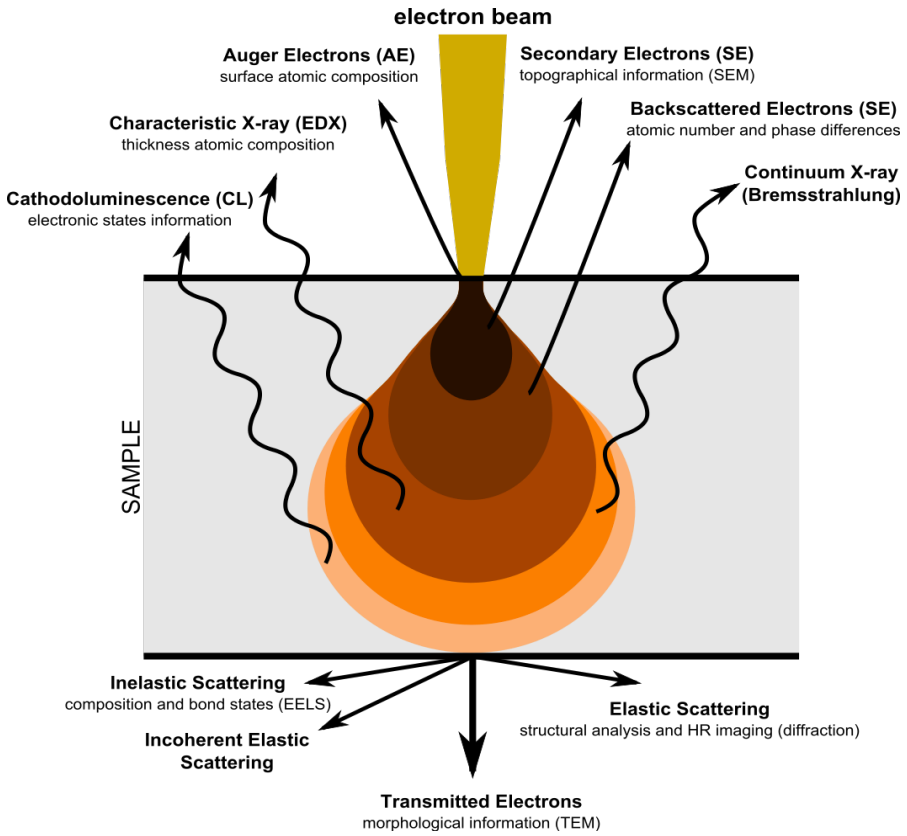
- Using a “magnetic lens”

- This magnetic field “bends” the path of an electron.

How is the image created?

- Magnetic lenses focus the electron beam to a point.
- As the electrons "smash" into the surface, there are three types of "interactions"

1. Secondary electrons
2. Backscattered electrons
3. X-rays



Source: https://en.wikipedia.org

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PRINCIPLES

Electrons are generated at the top of the microscope by a metallic filament (electron gun)

↓

The emitted electrons are then formed into a beam and accelerated down the column toward the specimen

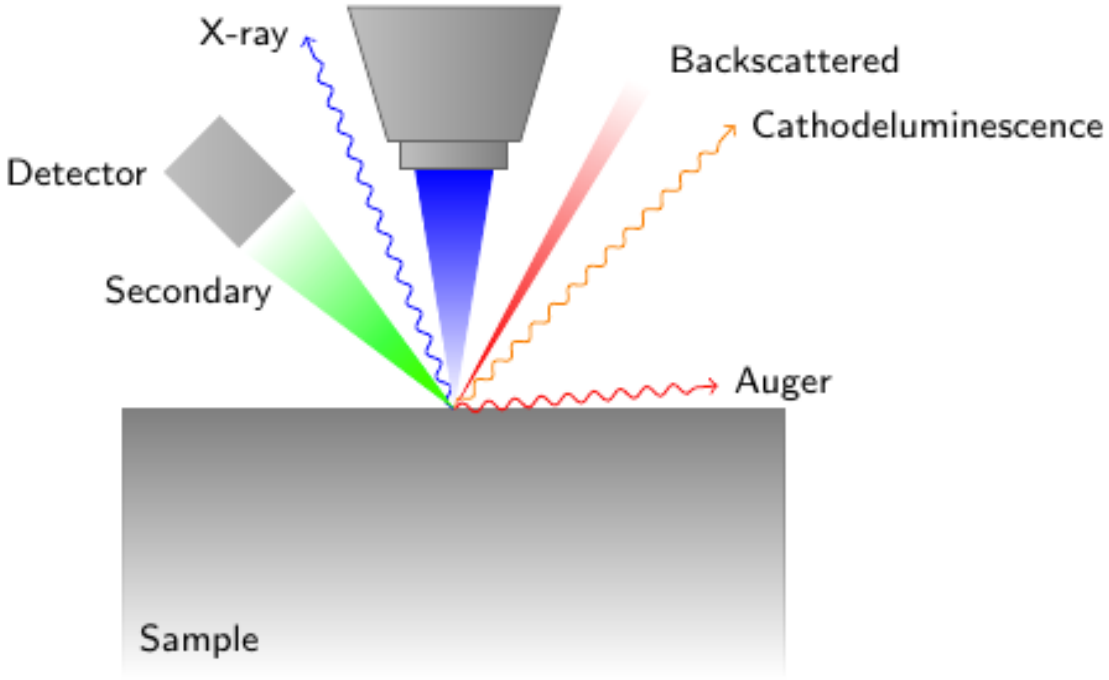
↓

The beam is further focused and directed by electromagnetic lenses as it moves down the column

↓

When the beam reaches the specimen, electrons are knocked loose from the surface of the specimen (secondary electrons)

These electrons are “seen” by a detector that amplifies the signal and sends it to a monitor



Source: https://www.texample.net/

1. Secondary Electrons

Electron beam collides with a sample atom **electron**

↓

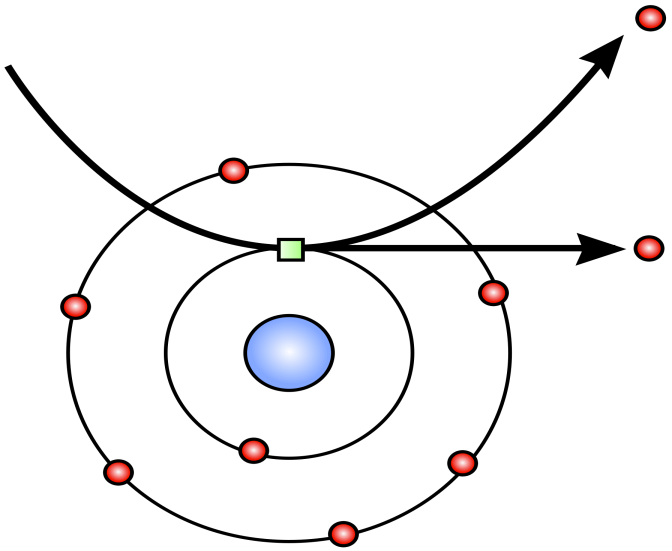
Sample atom electron knocked out of its shell (secondary electron)

↓

Collect by electron detector

↓

Send to CRT for display



2. Backscattered electron (BSE)

Electron beam collides with a sample atom **nucleus**

↓

The electron will **bounce back** out of the sample (BSE)

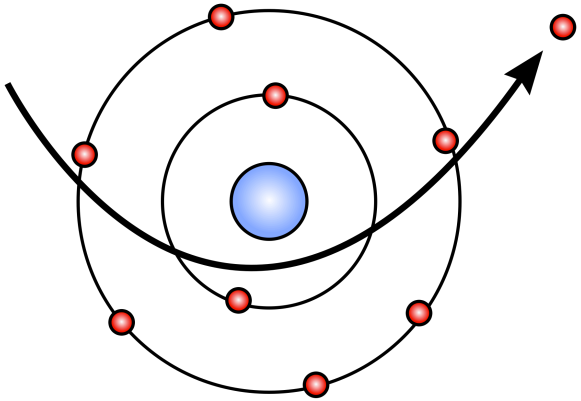
↓

High energy electron. More density

Sample → more electrons

↓

Send to BSE detector for image display



X-ray Emission

Electron beam interact with atoms in sample

Causing shell transition

X-ray emitted from the sample (elemental characteristic of the sample)

Detected by Energy Dispersive Spectroscopy (EDS) → X-ray line profile / spectrum

APPLICATIONS (Pharmaceutical)

- Today, all pharmaceutical and biopharmaceutical companies follow **testing requirements** which set standards for inspection of **visible particulates**, and also for examining the **size and quantity** of sub-visible particulates in **final products**.

- These **quality control** mechanisms are typically employed at the end of the production cycle, and are typically done via **optical inspection.**

- However, pharmaceutical developers and manufacturers are finding that they need the high resolution provided by a scanning electron microscope (SEM) to **characterize, control, and elementally quantify the size and shape of these particles**.

PARTICLE CONTAMINATION

- Foreign particles or particulate matter in drug powder products is an area of extreme concern.
- Unwanted particles should be controlled and their sources identified.
- Contamination due to an excessive amount of particulate matter in pharmaceutical products might lead to quality and safety problems
- Particle contamination in manufacturing sites might even result in the **suspension** of production

- But, even the cleanest rooms can produce particulate matter **shed by gowns, gloves, skin, sample preparation equipment, and glassware**.
- Containers and closures, specifically **rubber** closures, contribute particulate matter due to leaching chemical reactions, friction, and changes in physical properties.
- Some of the most common materials identified in pharmaceutical environments are **stainless steel, silica, aluminum, salts, minerals, organic fluorinated compounds, and carbonaceous materials** in varying sizes and shapes.

API IN PHARMA TABLETS

Pharmaceutical tablets are composed of a number of different materials, each of which is designed to improve performance.

- API
- Excipients, act as fillers, bulking agents, tablet disintegrants, and tablet coatings (to protect the core and to mask taste).

EDS maps and BSE **(Back Scattered Electron)** images of **tablet cross-sections** are two related means of directly examining excipient and API distribution within a tablet.

QUALITY CONTROL

For example: measuring thickness of the tablet's coating.

Tablet coating from an **Ibuprofen** sample using a **Back Scattered Electron** (BSE) Image.

PARTICLE SIZE

- Being able to examine each particle individually has led to microscopy being considered as an absolute measurement of particle size.

- Can distinguish aggregates from single particles

➢ LIMITATIONS

- Vacuum compatibility typically required
- SEM may spoil sample for subsequent analyses.
- Size restrictions may require cutting the sample.

Comparing SEM and TEM

	TEM	SEM
Electron Beam	Broad, static beams	Beam focused to fine point; sample is scanned line by line
Voltages Needed	TEM voltage ranges from 60-300,000 volts	Accelerating voltage much lower; not necessary to penetrate the specimen
Interaction of the beam electrons	Specimen must be very thin	Wide range of specimens allowed; simplifies sample preparation
Imaging	Electrons must pass through and be transmitted by the specimen	Information needed is collected near the surface of the specimen
Image Rendering	Transmitted electrons are collectively focused by the objective lens and magnified to create a real image	Beam is scanned along the surface of the sample to build up the image

Conclusion of the Chapter

- The SEM uses a beam of electrons scanned across the surface of a sample.
  - Collecting **secondary electrons** produces a **3-D image** of the surface.
  - Collecting **x-rays** allows an **elemental analysis** of the surface.
  - Collecting **back scattered electrons (BSE)** allow an **primary mapping of the surface**.

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Any Question?

Please refer to:

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