CELL BIOLOGY

Dr. Treefa F. Ismail **4th stage**

Lecture 1: Introduction into cell biology **4/10/2021**

Cell biology (also **cellular biology** or **cytology**) is a branch of biology studying the structure and function of the cell (the basic unit of life), regarding the physiological properties which include the study of cell metabolism, cell communication, the organelles they contain; interactions with their environment; and their life cycle, division, and death.

Cell biology research extends to both the great diversities of single-celled organisms like bacteria and the complex specialized cells in multicellular organisms like humans.

History (The discovery of Cells):

*Cells were first seen in Europe in **1665** with the invention of the compound microscope.

*In **1667**, Robert Hooke termed the building block of all living organisms as "cells" after looking at a piece of cork and observing a cell-like structure, however, the cells were dead and gave no indication to the actual overall components of a cell.

*In **1674**, Anton Van Leeuwenhoek was the first to analyze live cells in his examination of algae.

All of this preceded the **cell theory** which states that:

(1) all living things are made up of cells

(2) cells are the functional and structural unit of organisms. This was ultimately concluded by plant scientist, Matthias Schleiden and animal scientist, Theodor Schwann in 1839, who viewed live cells in plant and animal tissue, respectively.

19 years later, Rudolf Virchow further contributed to the cell theory, adding that **(3) all cells come from the division of pre-existing cells.**

Although widely accepted, there have been many studies that question the validity of the cell theory. Viruses, for example, lack common characteristics of a living cell, such as membranes, cell organelles, and the ability to reproduce by themselves. Scientists have struggled to decide whether viruses are alive or not and whether they are in agreement with the cell theory.

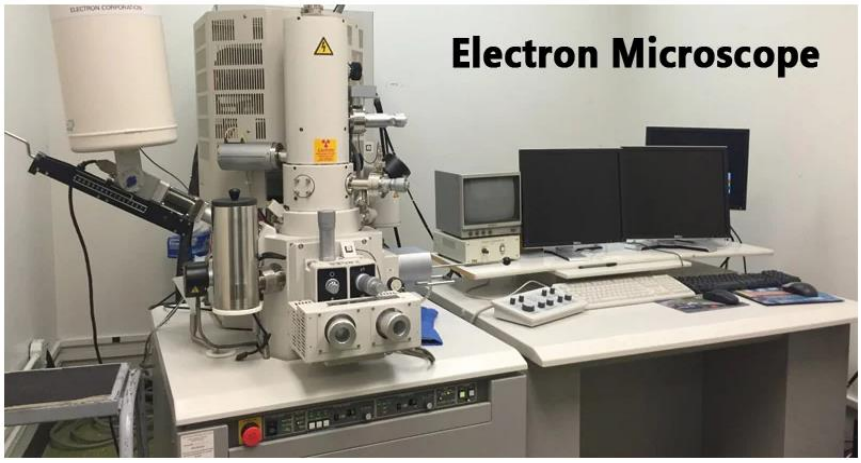
Techniques commonly used to study cells:

Most **cells** are so small that they cannot be viewed with the naked eye. Therefore, scientists must use microscopes to **study cells**. A microscope is an instrument that magnifies an object.
Modern-day cell biology research looks at different ways to culture and manipulate cells outside of a living body to further research in human anatomy and physiology, and to derive medications. Research in cell biology is interconnected to other fields such as genetics, molecular genetics, biochemistry, molecular biology, medical microbiology, immunology, and cytochemistry.

When Hooke first looked at cells using a handmade microscope with a candle as a light source, he used a light microscope.
A **light microscope** is a microscope that uses light passing through optical lenses to magnify objects. For many years this was the only type of microscope used, and it allowed many advancements to be made.

Electron Microscopes

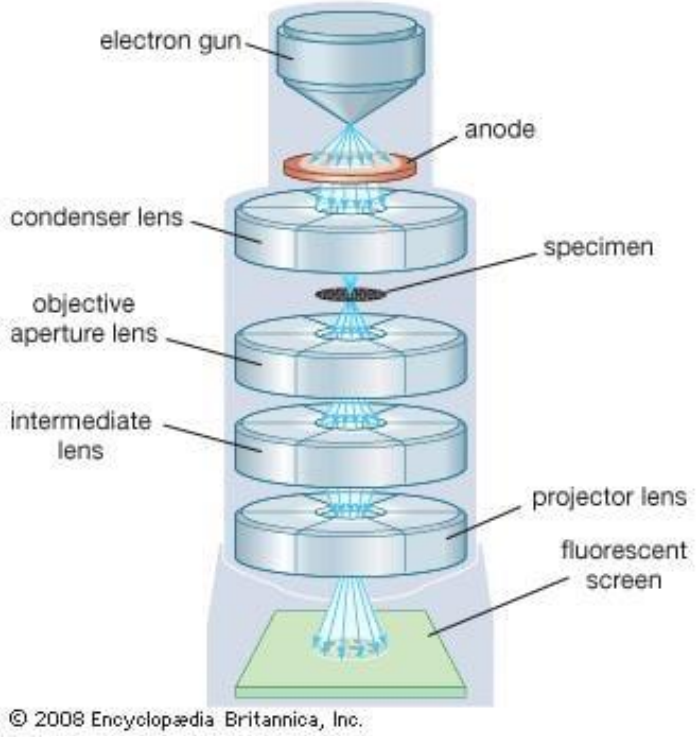
- An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination.
- It is a special type of microscope having a high resolution of images, able to magnify objects in nanometres, which are formed by controlled use of electrons in vacuum captured on a phosphorescent screen.
- Ernst Ruska (1906-1988), a German engineer and academic professor, built the first Electron Microscope in 1931, and the same principles behind his prototype still govern modern EMs.
- Preparation of a specimen for viewing under an electron microscope will kill it; therefore, live cells cannot be viewed using this type of microscopy.

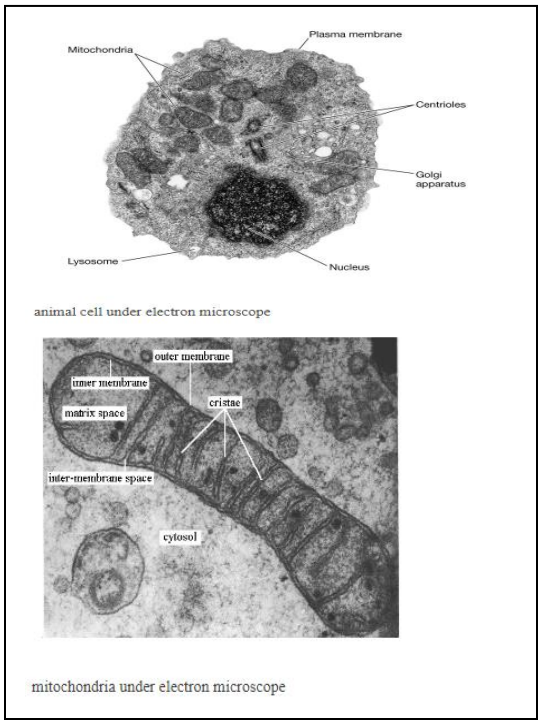


Types of Electron microscope

There are two types of electron microscopes, with different operating styles:

1. **The transmission electron microscope (TEM)**





- The transmission electron microscope is used to view thin specimens through which electrons can pass generating a projection image.
- The TEM is analogous in many ways to the conventional (compound) light microscope.
- TEM is used, among other things, to image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture).

Main steps in preparing samples for TEM:

1) Fixation:The tissues are fixed in 2.5%-6% glutaraldehyde prepared in buffer (cacodylate buffer) Ph 7.2-7.4,

2) Postfixation: postfixed in 1% osmium tetroxide for one hour, OsO_4 is a fixative and electron dense stain, but it is dangerous so you must be careful in handling because when it touches the skin it will oxidize and produces a black precipitate in which it will be dangerous to eyes. It has a low penetration rate

because it contain osmium with high molecular weight, that is why it is used after primary fixation

3) Dehydrated in ascending concentrations of ethanol or acetone 50%, 70%, 90% and 100%

4) Cleared in propylene oxide because ethanol is immiscible with the plastic embedding medium

5) Infiltration by a mixture of resin (plastic medium) + propylene oxide

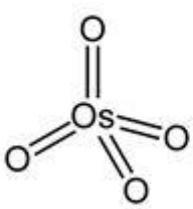
6) Embedded in resin

7) Sectioning using ultramicrotome (glass or diamond knives) into thick and semi-thin sections (0.5-1micrometer) or ultrathin sections (600-900A).

8) Mounting: transferring the ultrathin sections to the electron microscope grids which are either copper, nickel or gold, copper is used because it is cheap.

9) Staining: The thick and semithin sections on the grids are stained by 1% toluidine blue, while the ultrathin sections are stained by heavy metals such as uranyl acetate and lead citrate.

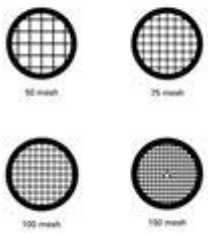
10) Examining by electron microscopy.



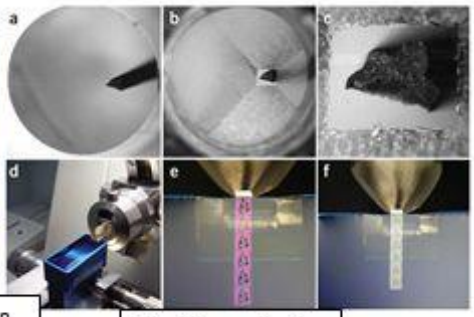
Osmium tetroxide

Moulds for

resin blocks



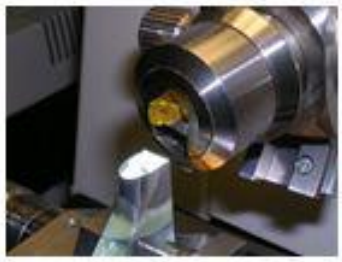
EM grids



Grid tweezer block and grid rack

Staining ultrathin section

Ultrathin sectioning

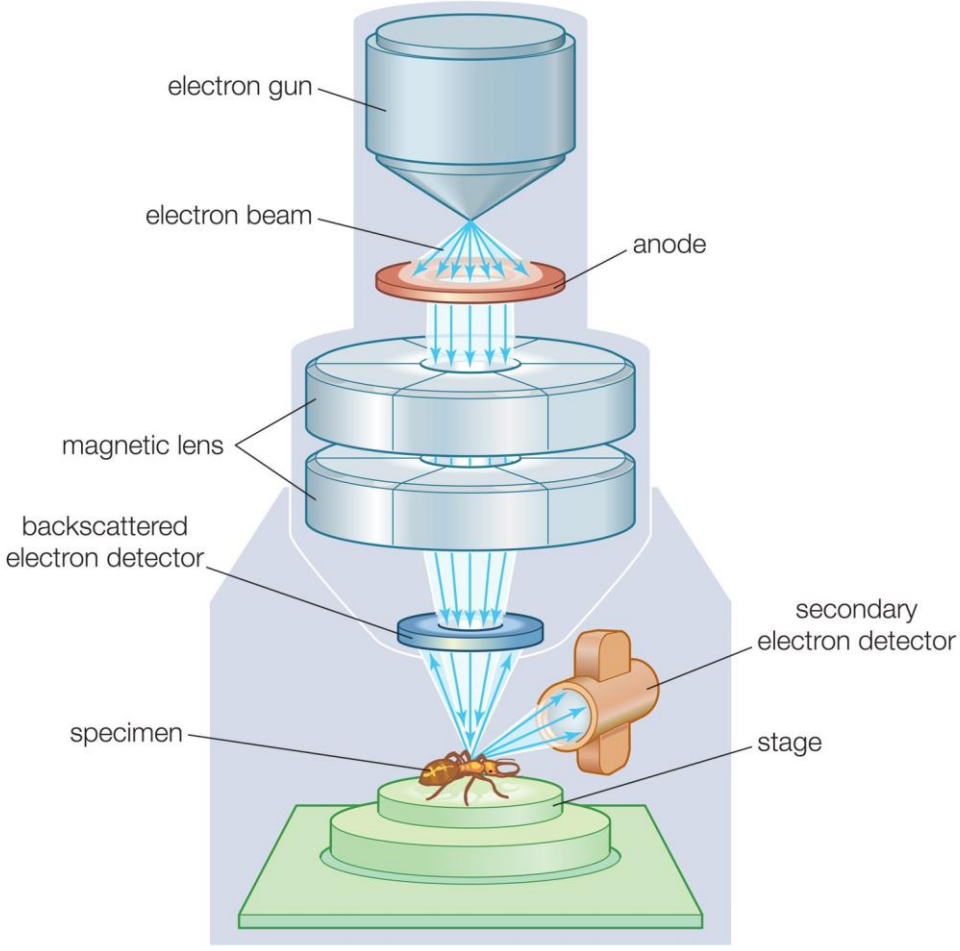


ultramicrotome

Diamond knife

Glass knife

2. The scanning electron microscope (SEM)



- Conventional scanning electron microscopy depends on the emission of secondary electrons from the surface of a specimen.
- Because of its great depth of focus, a scanning electron microscope is the EM analog of a stereo light microscope.

- It provides detailed images of the surfaces of cells and whole organisms that are not possible by TEM. It can also be used for particle counting and size determination, and for process control.
- It is termed a scanning electron microscope because the image is formed by scanning a focused electron beam onto the surface of the specimen in a raster pattern.
- Typically, a biological specimen is chemically fixed, dehydrated through an acetone or ethanol series and then dried at the critical point - a method used to minimize specimen distortion due to drying tensions. SEM can also be used to investigate smooth surfaces of industrial samples. The samples are mounted on a stub of metal with adhesive, coated with 40 - 60 nm of metal such as Gold/Palladium and then observed in the microscope.
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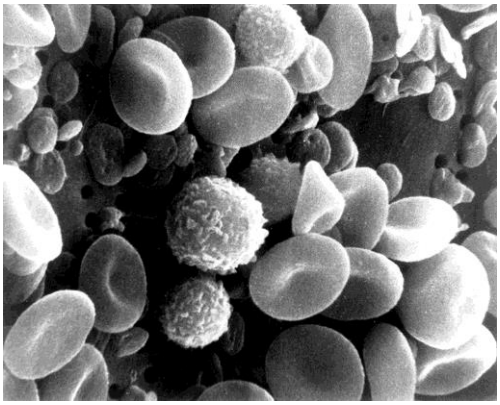
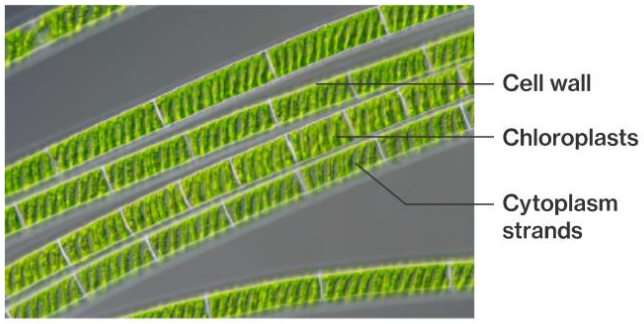


-A transmission electron microscope passes electrons through a specimen, while a scanning electron microscope reflects electrons off the surface of a specimen.
-The magnification and resolution of an SEM are slightly lower than those of a TEM; however, the SEM requires less power to operate because the electrons do not have to pass through the sample.
-The techniques used by the TEM and SEM can be combined, the result being the scanning transmission electron microscope (STEM), which gives the greatest resolution.

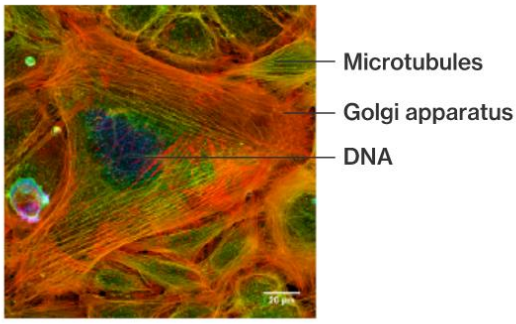
Fluorescence microscopy:

Most cellular components are colorless and cannot be clearly distinguished under a microscope. The basic premise of fluorescence microscopy is to stain the components with dyes.
Fluorescent dyes, also known as fluorophores or fluorochromes, are non-protein molecules that absorb excitation light at a given wavelength (generally UV), and

after a short delay emit light at a longer wavelength. The delay between absorption and emission is negligible, generally on the order of nanoseconds.
The emission light can then be filtered from the excitation light to reveal the location of the fluorophores.



Spirogyra



HeLa Cells

Immunohistochemistry:

Immunohistochemistry (IHC) is a powerful microscopy-based technique for visualizing cellular components, for instance proteins or other macromolecules in tissue samples.
Immunohistochemistry (IHC), or immunohistochemical staining, is a technique which employs antibodies to detect antigens in cells within a tissue section. This application is used to locate specific antigens in tissue sections with labeled antibodies based on antigen-antibody interactions
Immunohistochemistry is commonly used by clinicians to detect and diagnose abnormal cells found in disease states such as cancer. Such biomarkers are specific to the disease state and are characteristic of particular events such as cell death, apoptosis or proliferation, which give rise to the abnormality.

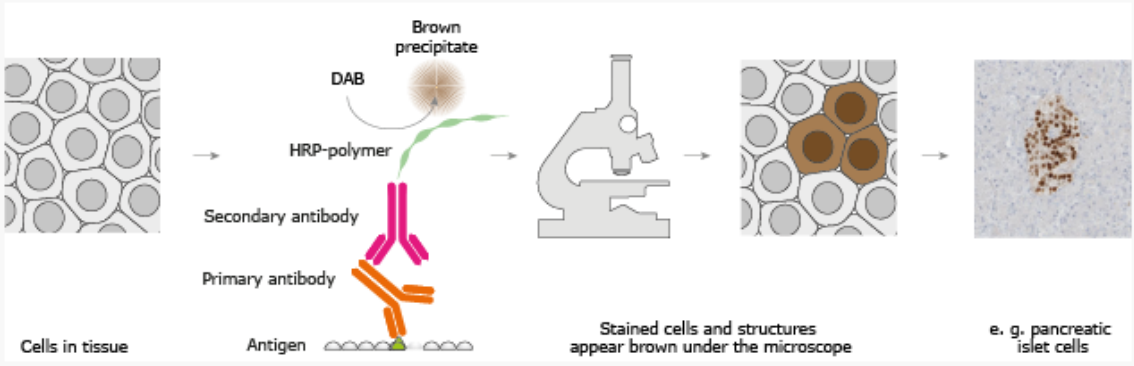


Figure 1. The basic principle of immunohistochemistry.

Tissue Culture

Is the basic technique of growing cells in a laboratory independent of an organism (in vivo), but in culture (in vitro), Utilizes rapidly growing cells on artificial media which allows for a large amount of a specific cell type and an efficient way to study cells.

When the cultured cells are derived from a single cell, it is known as a **cell line**. Because of this derivation, all the cells are considered to be genetically identical.

Many kinds of cells are maintained in cultures, but due to the fact that a great deal of research is concerned with humans, human-derived tissue culture lines are of the highest importance. The most common and thus most widely used human cell culture line is called HeLa. These cells were obtained from the cervical tumor of Henrietta Lacks in 1951. Ms. Lacks was a young mother who visited the Johns Hopkins Hospital when she noted vaginal bleeding, and was subsequently diagnosed with cervical cancer. The cells taken from Ms. Lacks were given her name—Henrietta (He) and Lacks (La), hence the name HeLa cell.

To grow the cells, researchers place a single cell in a nutrient-rich growth medium and place that medium in a glass flask incubated at 37°C, human body temperature. The cells divide and divide again, continuing until the flask is filled. At that point some cells can be removed and placed in a new flask, and the process can be repeated. In this way, HeLa cells have been maintained continuously since 1951.