بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

HISTOLOGY

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

MID | Lecture #

Introduction to histology pt.2

Written by : Yaman Khalil
Yamen Aljarrah

Reviewed by : Emad Aljammal

MICROSCOPES

Types of microscope

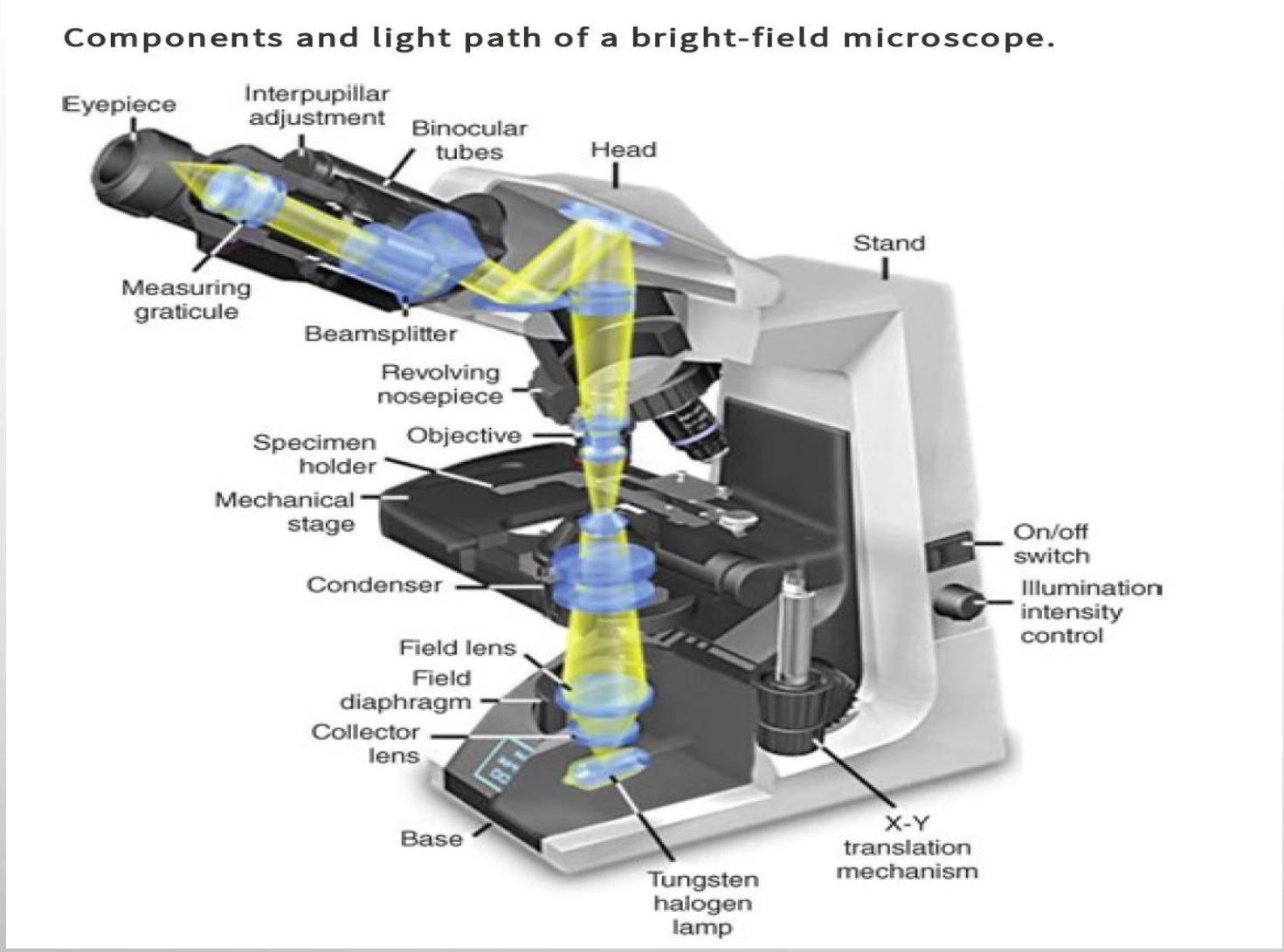
- **Light microscope.**

1. Bright-field microscopy
2. Fluorescence microscopy
3. Phase-contrast microscopy
4. Confocal microscopy
5. Polarizing microscopy

- **Electron microscope**

1. Transmission electron microscopy
2. Scanning electron microscopy

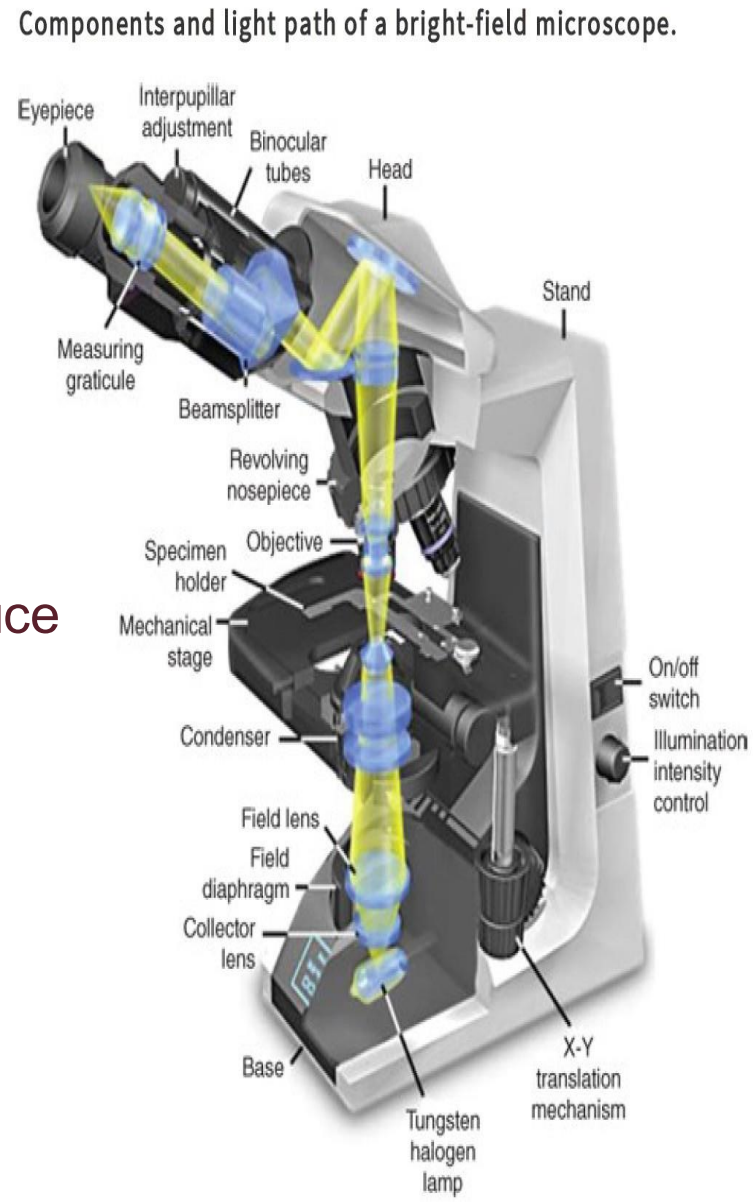
Light Microscope (Bright-field)



Bright-field Light Microscope

The most commonly used microscope

- Stained tissue is examined with ordinary light passing through the preparation. Visible light
- Includes an optical system and mechanisms to move and focus the specimen.
- The **condenser** collects and focuses a cone of light that illuminates the tissue slide on the stage. It is used to narrow the beam of light to reduce the scattering of the light .
- **Objective** lenses enlarge and project the illuminated image of the object toward the eyepiece.
- The two **eyepieces** or oculars magnify this image another 10X and project it to the viewer, yielding a total magnification of 40X, 100X, or 400X. The final (total) magnification = eyepiece magnification x objective lens magnification



1. The light beam is emitted from the tungsten halogen lamp (the source of light) when switching on the bright-field microscope.

2. The diaphragm is used to control the light beam.

3. The glass slide sits on the mechanical stage.

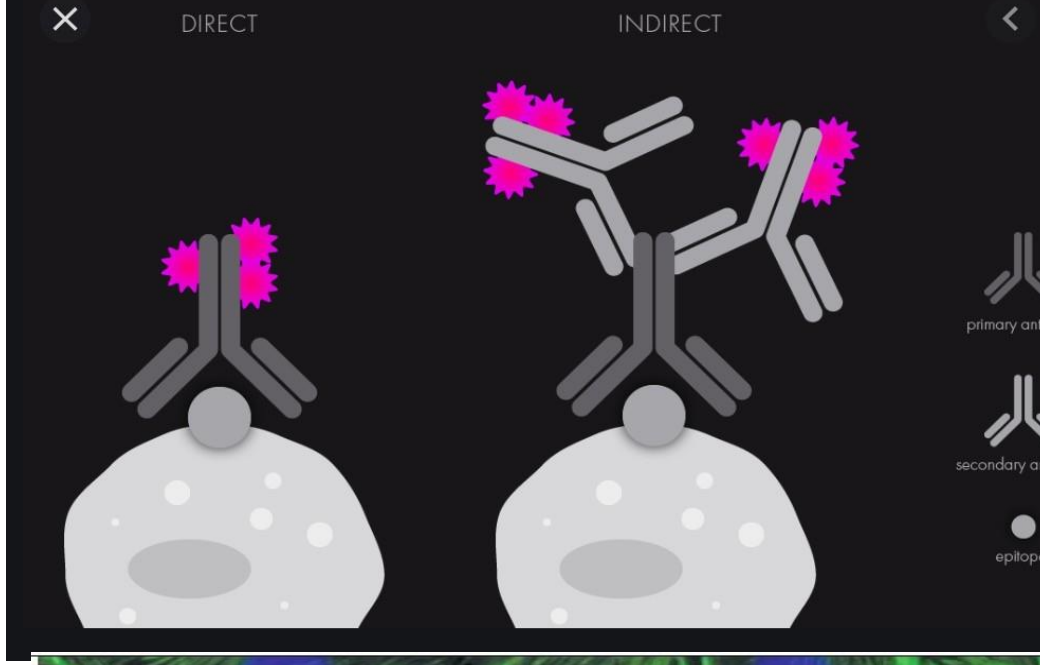
4. Each objective lens has a unique magnification.

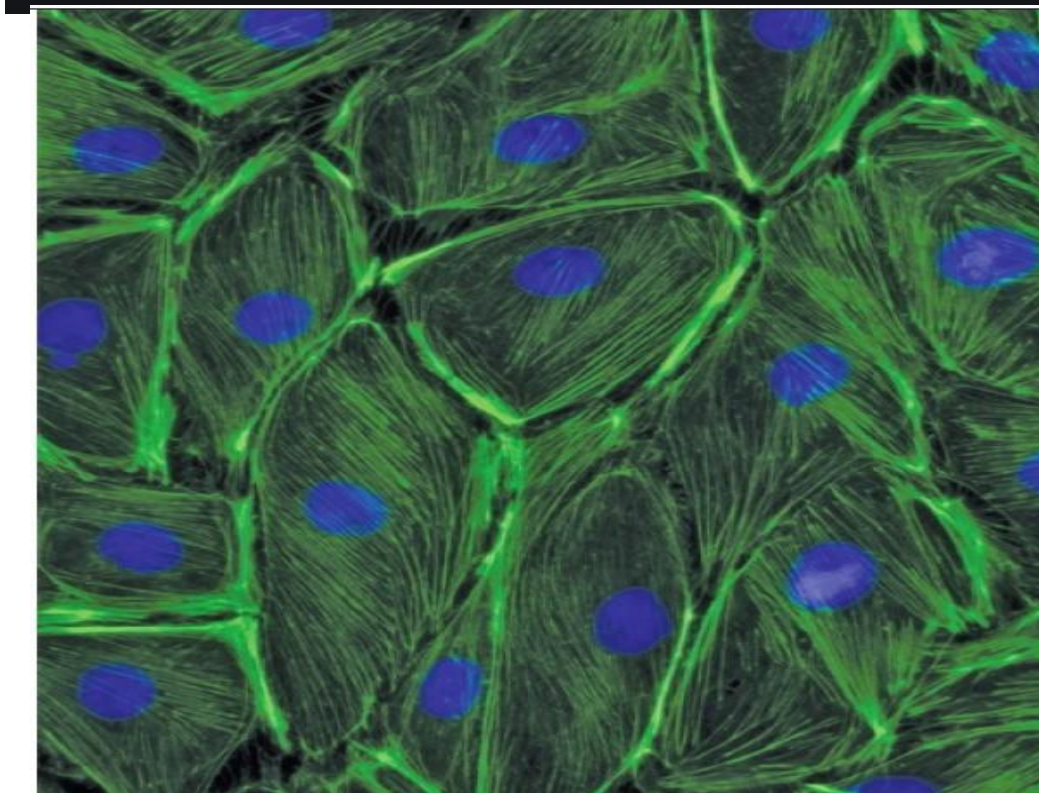
5. In final (total) magnification questions we consider the eyepiece lens as a x10 magnification lens unless the question states otherwise

Fluorescence Microscopy

(dark field)

- **Fluorescence**: when certain cellular substances are irradiated by light of a proper wavelength, as a result they emit light with a longer wavelength.
- In fluorescence microscopy, tissue sections are irradiated with
- Ultraviolet (UV) light and the emission is in the visible portion of the spectrum.
  1. The emission here refers to the light that is emitted by the tissue after it is exposed to the UV
  2. This microscope usually has a UV lamp unlike the bright-field.
- The fluorescent substances appear bright on a dark background.
- For fluorescent microscopy the instrument has a source of UV or other light and filters that select rays of different wavelengths emitted by the substances to be visualized.





· The fluorescence microscope uses immunostaining to target a specific protein.

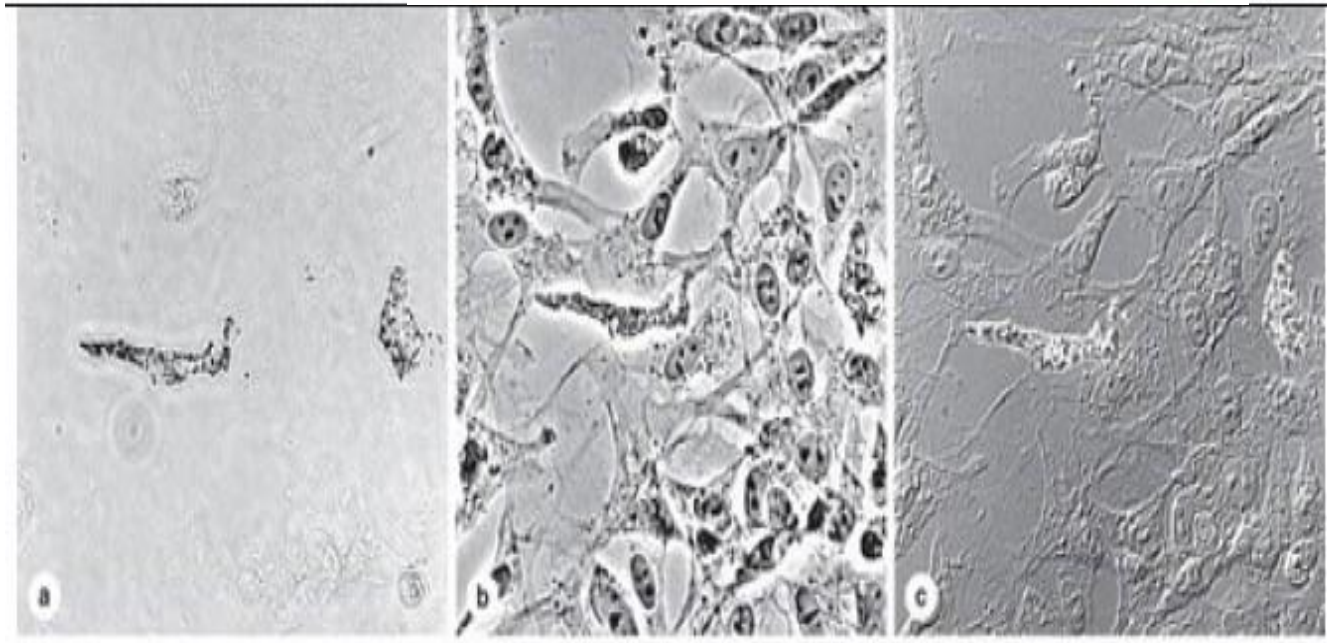
·The way that immunostaining work is that the protein that we are looking for acts as an antigen that binds to specific antibodies. Fluorophores (fluorescent materials) are bound to these specific antibodies in order to emit certain colors if the antibodies bind to the protein (in other words, if the protein exists in this tissue) allowing visualization of the protein.

·Antibodies that bind to the antigen directly are called primary antibodies
·Antibodies that bind to the primary antibody are called secondary antibodies

·The purpose of using secondary antibodies is to amplify the signal by increasing the number of fluorophores, making the protein easier to visualize.

Phase-contrast Microscopy

- Study unstained cells and tissue sections (colorless; similar optical densities.
- Uses a lens system that produces visible images from transparent objects and can be used with living, cultured cells.



- Is based on the principle that light changes its speed when passing through cellular and extracellular structures with different refractive indices--- appear lighter or darker in relation to each other.

- Studying unstained cells cannot be done with Bright-field microscope because the images are colorless and give no information.

- We use the Phase-contrast microscope in order to study unstained cells.

- In order to study alive cells, we cannot stain them because the staining process kills them, so we use the Phase-contrast microscope in order to study them.

- Cells in cultures tend to be social, that's why in the previous slide microscope images, there are some processes between cells which keep the cells alive and connected together.

Electron Microscope

- Interaction of tissue with a beam of electrons.

TEM

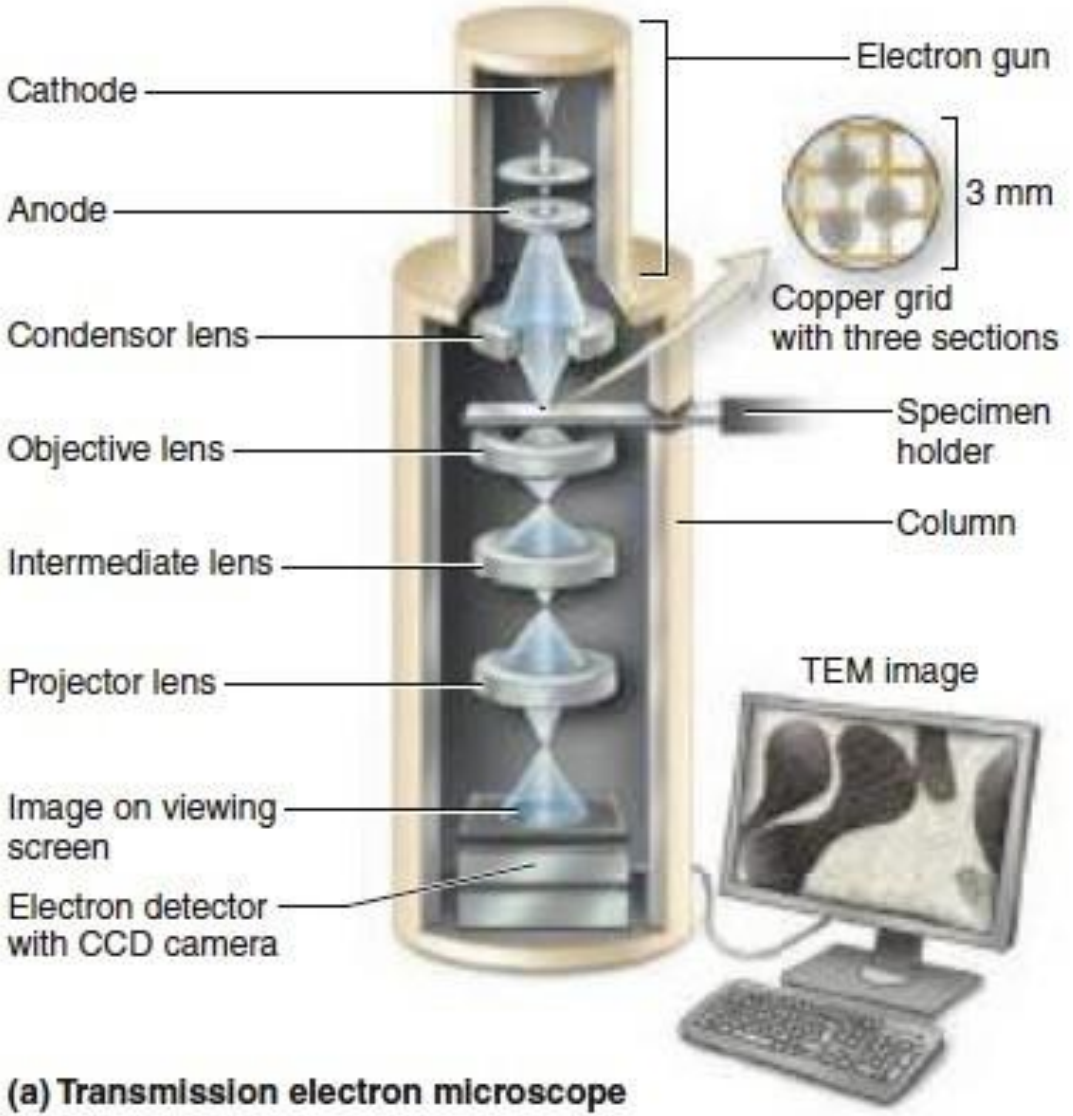
- The electron beam passes the tissue.
- Very high magnification
- Very thin sections, 40-90 nm.
- Electron beam interact with tissue(might be reflected , absorbed or it might pass actually through the tissue) producing 2D black, white and shades of gray images.

SEM.

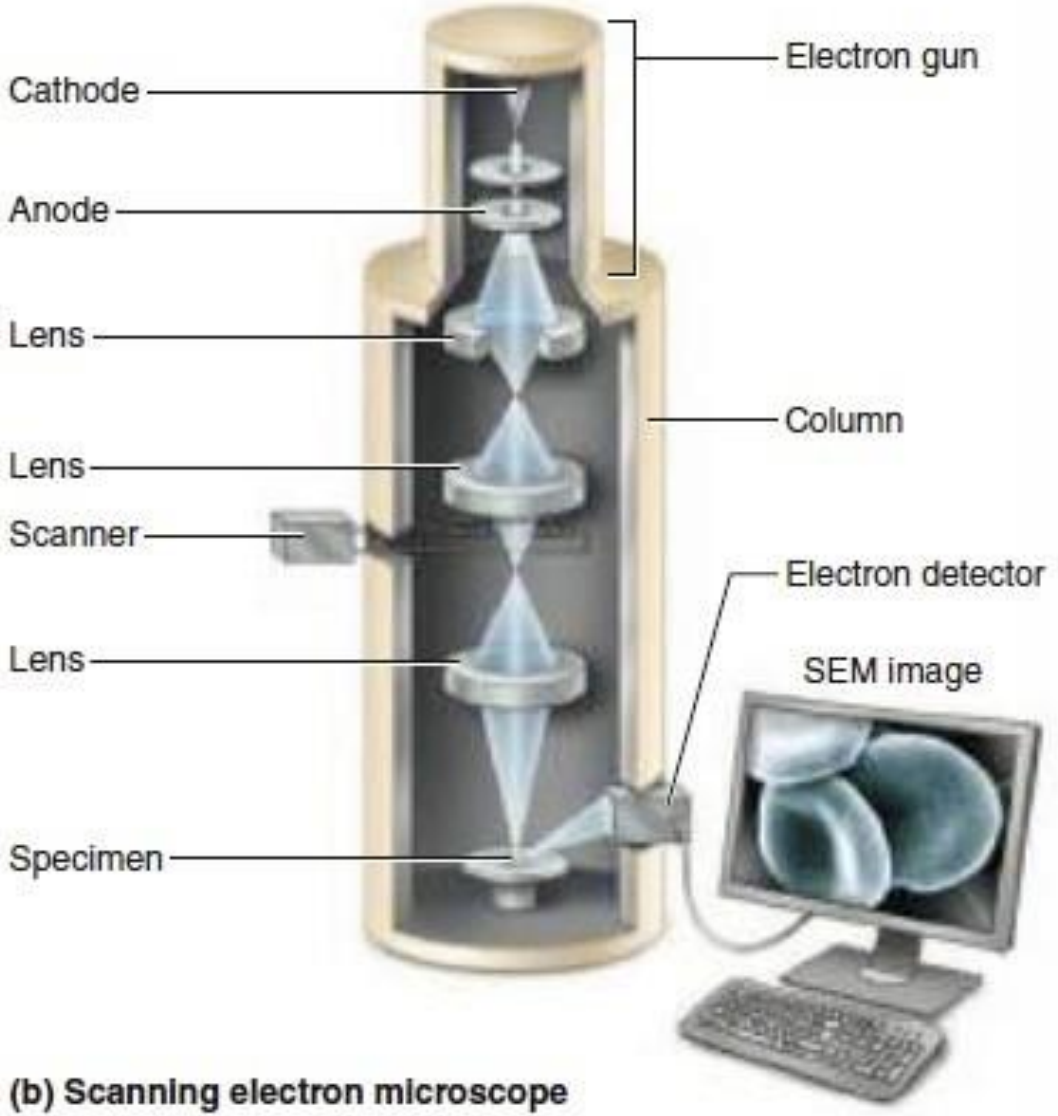
- The electron beam does not pass the tissue.
- The surface of cells and tissue is coated with heavy metals (gold)---which reflect the electrons---producing 3D images which is a recording of the specimen topography.

SEM images are also naturally black and white , any colors seen are added digitaly for more illustration

Electron Microscope

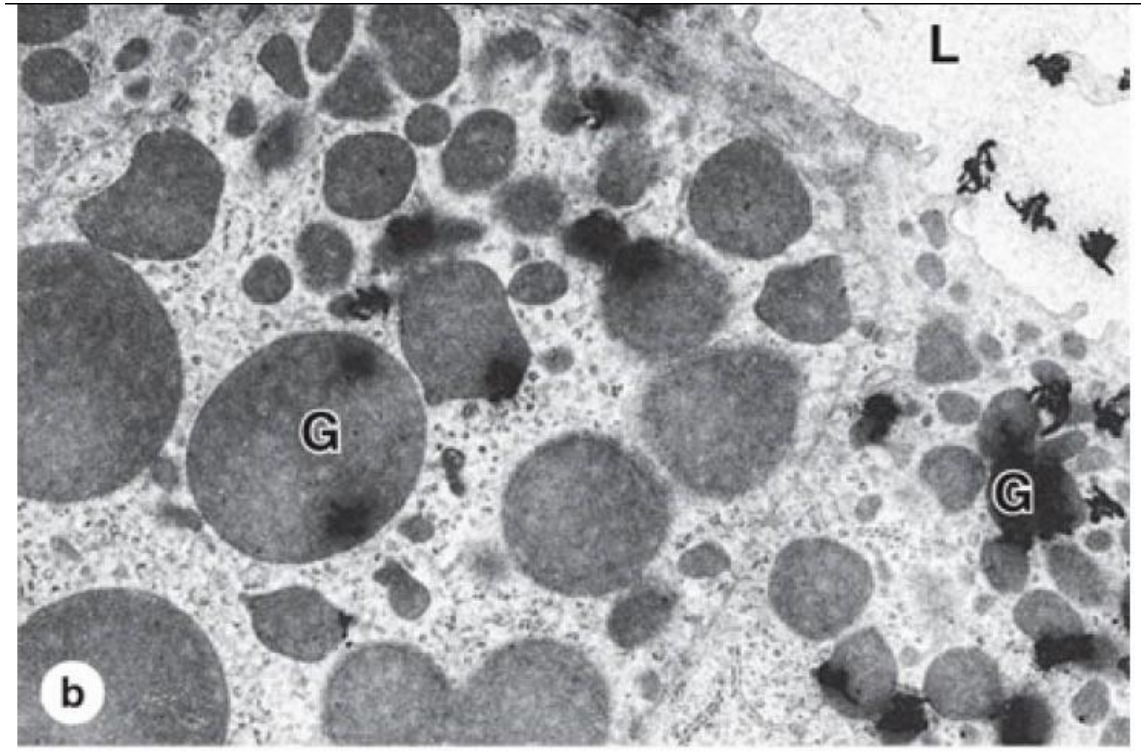


(a) Transmission electron microscope



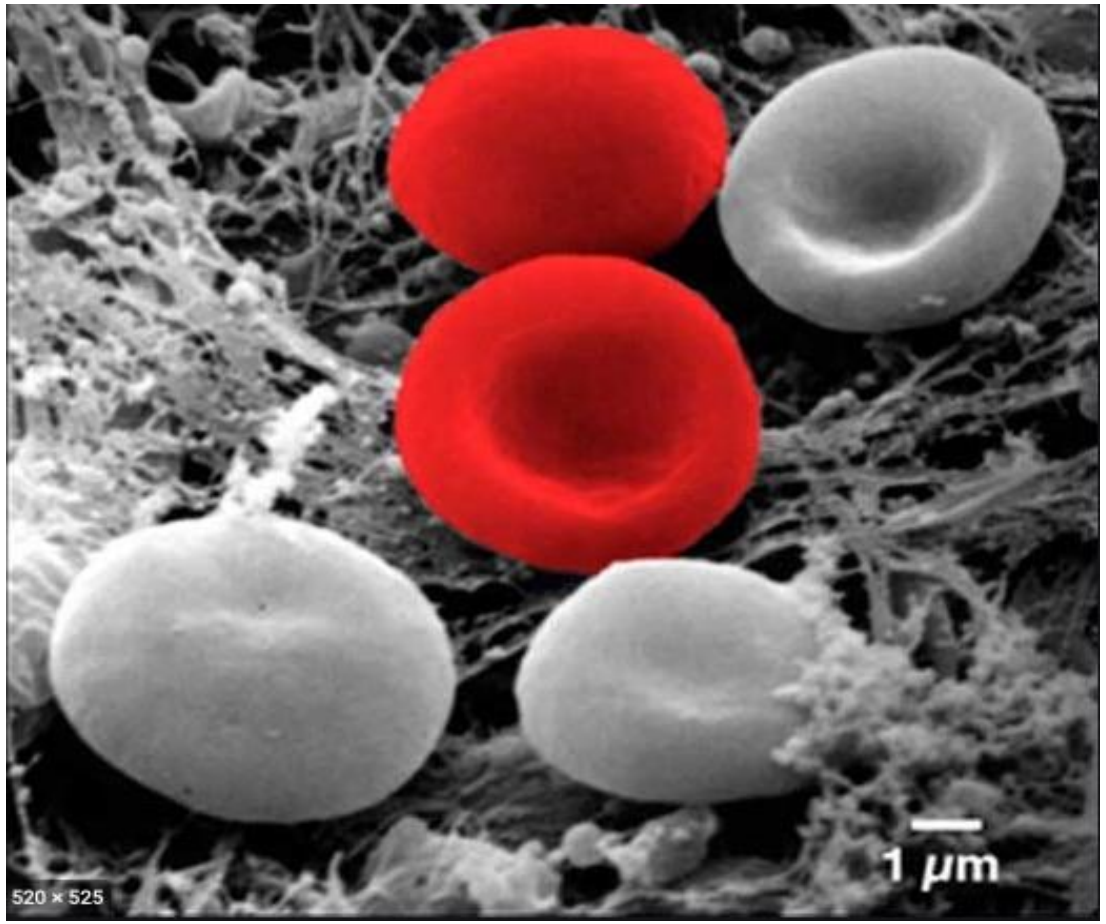
(b) Scanning electron microscope

TEM



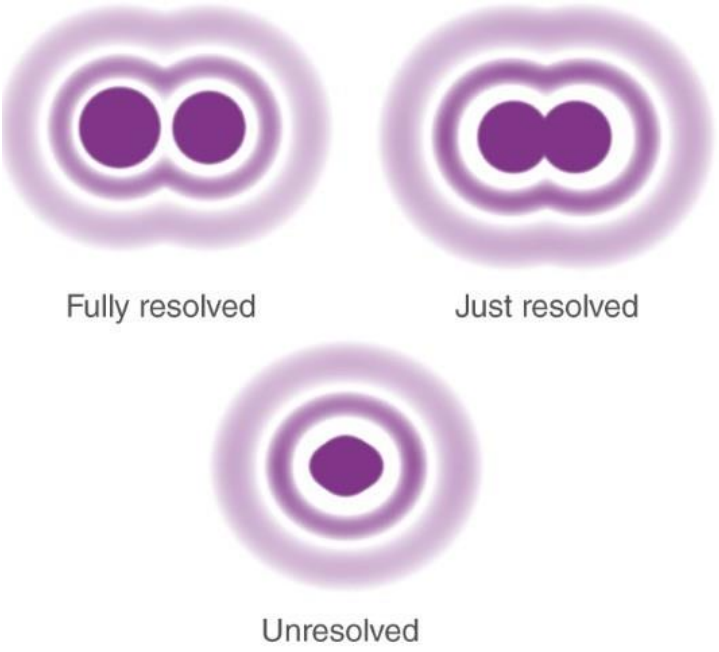
Source: Anthony L. Mescher: Junqueira's Basic Histology: Text and Atlas, 15th Edition.

SEM



Resolution

- **Resolving power**: the smallest distance between two structures at which they can be seen as separate objects.
- The maximal resolving power of the light microscope is approximately 0.2 µm--- can permit clear images magnified 1000- 1500 times.



- Objects smaller or thinner than 0.2 µm (such as a single ribosome or cytoplasmic microfilament) cannot be distinguished. (light microscopes)
- The microscope's resolving power determines the quality of the image, its clarity and richness of detail, and depends mainly on the quality of its objective lens.
- Magnification is of value only when accompanied by high resolution.
- Resolving of TEM is 3 nm (electron wavelength is shorter than that of light).

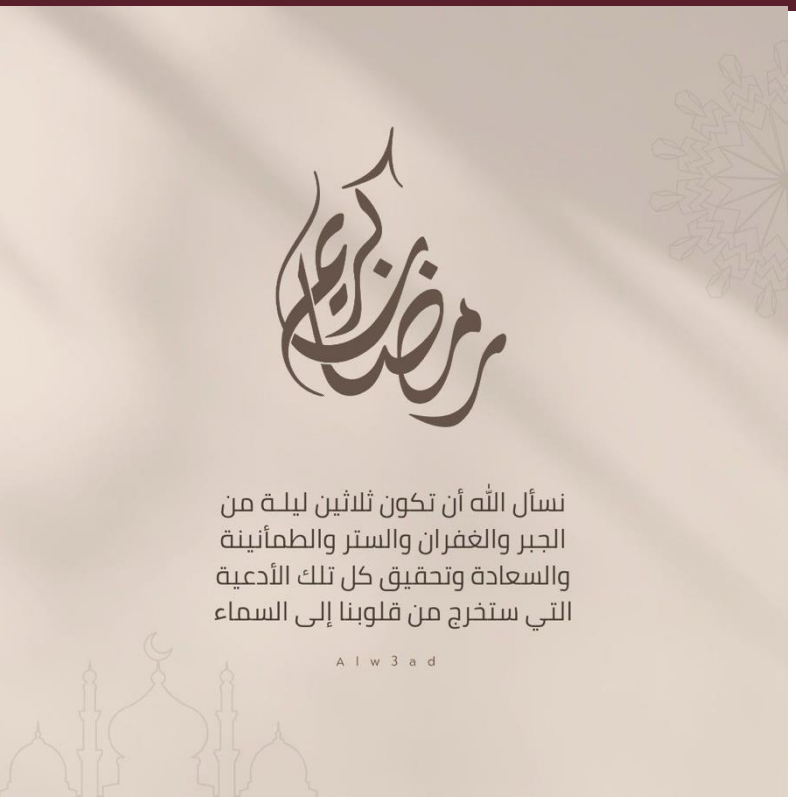
For any feedback, scan the code or click on it

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:



إذا بلغك الله عز وجل رمضان، فيا هنيئاً ثم يا هنيئاً ثم ياهنيئاً
قد فُتحت لك أبواب الرحمن
قد فتحت لك أبواب الجنة
قد فُتحت لك أبواب الرضوان

عن أبي هريرة رضي الله عنه قال:
كان رسول الله –صلى الله عليه وسلم– يُبَشِّر أصحابه بقدوم رمضان فيقول:
قد جاءكم شهر رمضان شهرٌ مبارك، كتب الله عليكم صيامه، فيه تُفتح أبواب الجنة وتغلق فيه أبواب الجحيم، فيه ليلةٌ خيرٌ من ألف شهر، من حُرِمَ خيرها فقد حُرِم.