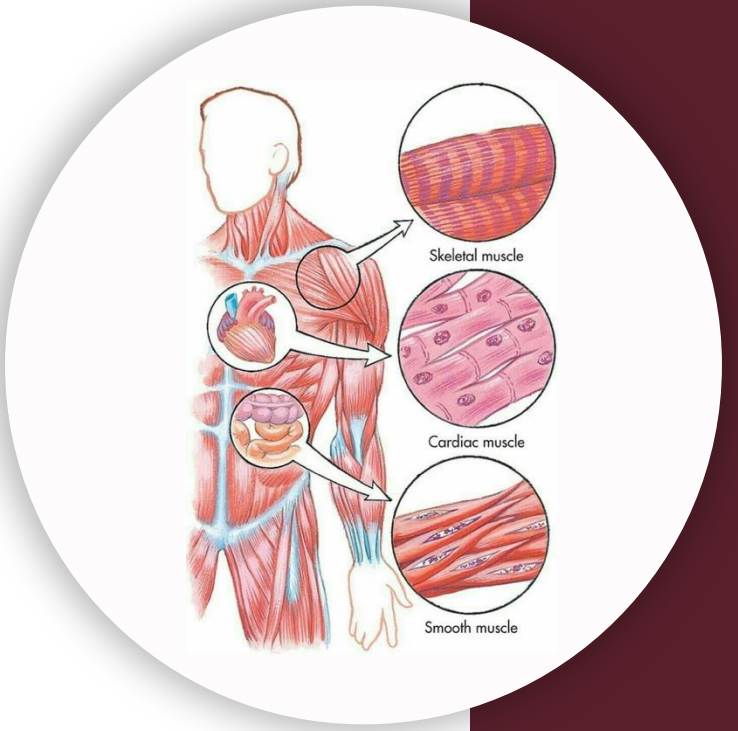
بسم الله الرحمن الرحيم

Histology - Lecture 2

Staining & Microscopy



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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

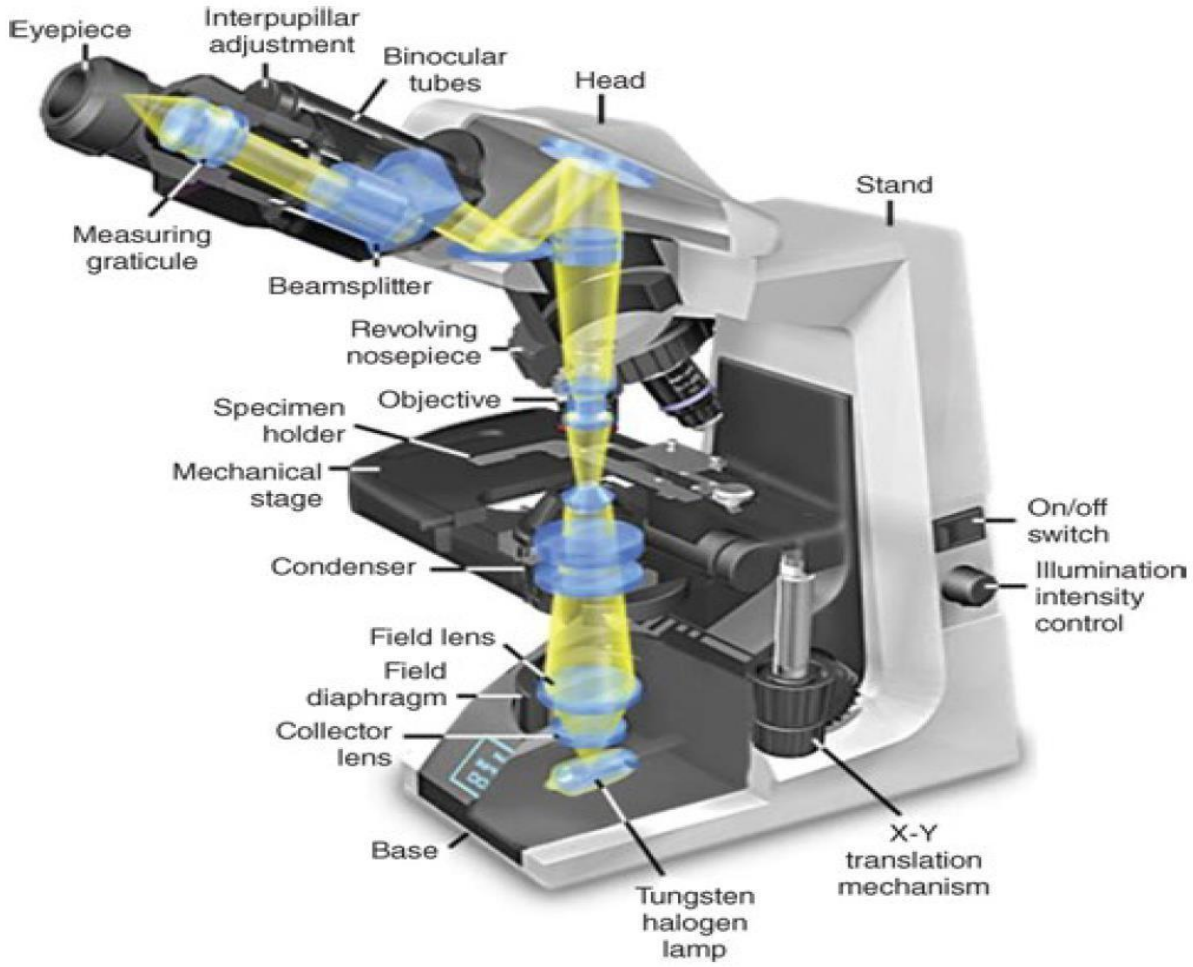
What are the differences between light microscopes and electron microscopes in tissue examination?
Light microscopes use visible light and optical lenses to magnify tissues, making them ideal for routine histology. Electron microscopes, however, use electron beams and offer much higher resolution, allowing us to observe fine details such as cell membranes and organelles.

How do microscopy techniques, such as fluorescence or confocal microscopy, improve tissue visualization?
Fluorescence microscopy uses fluorescent dyes to highlight specific structures, making it useful for studying proteins and DNA. Confocal microscopy provides high-resolution, three-dimensional images of tissues by eliminating out-of-focus light, improving clarity and detail

Why is the combination of staining and microscopy essential for accurate medical and scientific observations?
Staining and microscopy together allow for a detailed analysis of tissues, helping scientists and doctors identify structural abnormalities, diagnose diseases, and conduct medical research. Without proper staining, even advanced microscopes would fail to provide meaningful results.

Light Microscope (Bright-field)

Components and light path of a bright-field microscope.



Condenser narrow the beam of the light to reduce the scattering of the light .

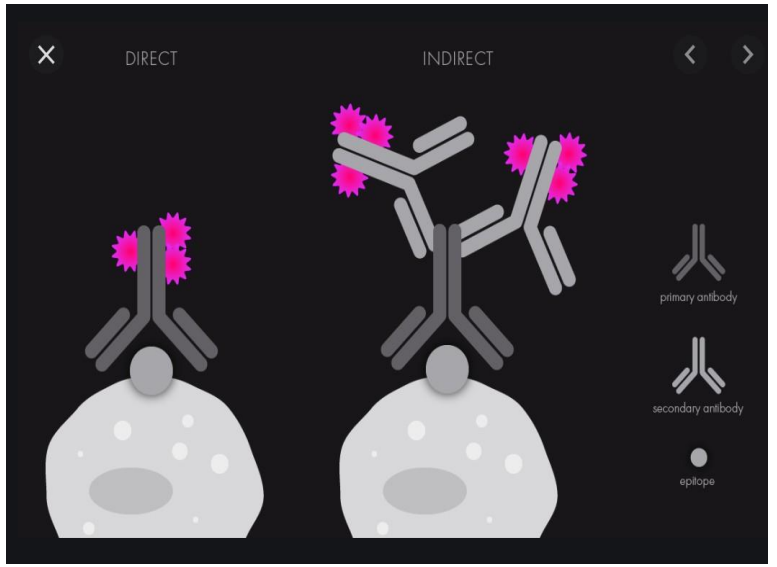
Diaphragm control and linnet the electricty .

1. **Eyepiece** – The lens through which the user looks.
2. **Interpupillary Adjustment** – Adjusts the distance between the two eyepieces.
3. **Binocular Tubes** – Holds the eyepieces and allows for proper alignment.
4. **Head** – Connects the eyepieces to the rest of the microscope.
5. **Beamsplitter** – Splits the light for different optical paths.
6. **Measuring Graticule** – A scale inside the eyepiece for measurements.
7. **Revolving Nosepiece** – Holds multiple objective lenses and allows for rotation.
8. **Objective Lenses** – Magnify the specimen at different levels.
9. **Specimen Holder** – Secures the slide in place.
10. **Mechanical Stage** – Moves the slide for precise viewing.
11. **Condenser** – Focuses light onto the specimen.
12. **Field Lens & Diaphragm** – Helps control and direct light.
13. **Collector Lens** – Collects and focuses light from the lamp.
14. **Tungsten Halogen Lamp** – The light source.
15. **Base** – The foundation of the microscope.
16. **Stand** – Supports the microscope structure.
17. **On/Off Switch & Illumination Intensity Control** – Adjusts brightness.
18. **X-Y Translation Mechanism** – Allows precise movement of the stage.

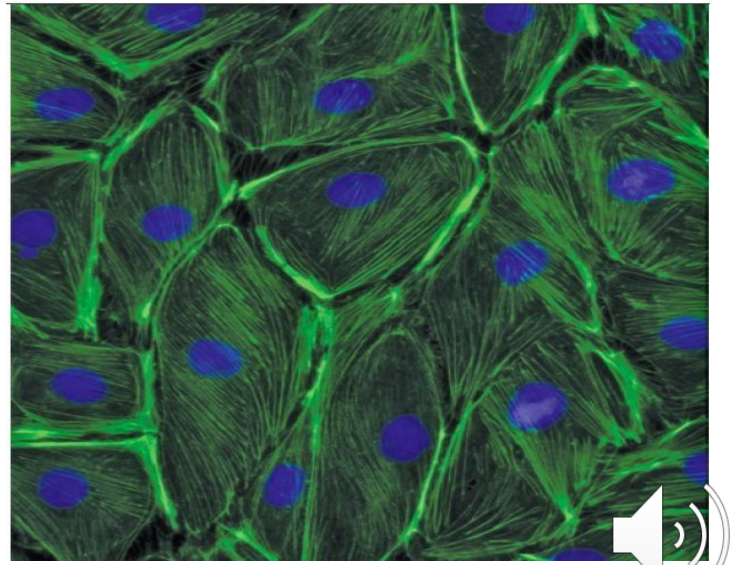
Bright-field Light Microscope

- **The most commonly used and the cheapest light microscopy.**
- Stained tissue is examined with ordinary light passing through the preparation.
- 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.
- **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.

Fluorescence Microscopy



- **Fluorescence**: when certain cellular substances are irradiated by light of a proper wavelength, 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.
- 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.



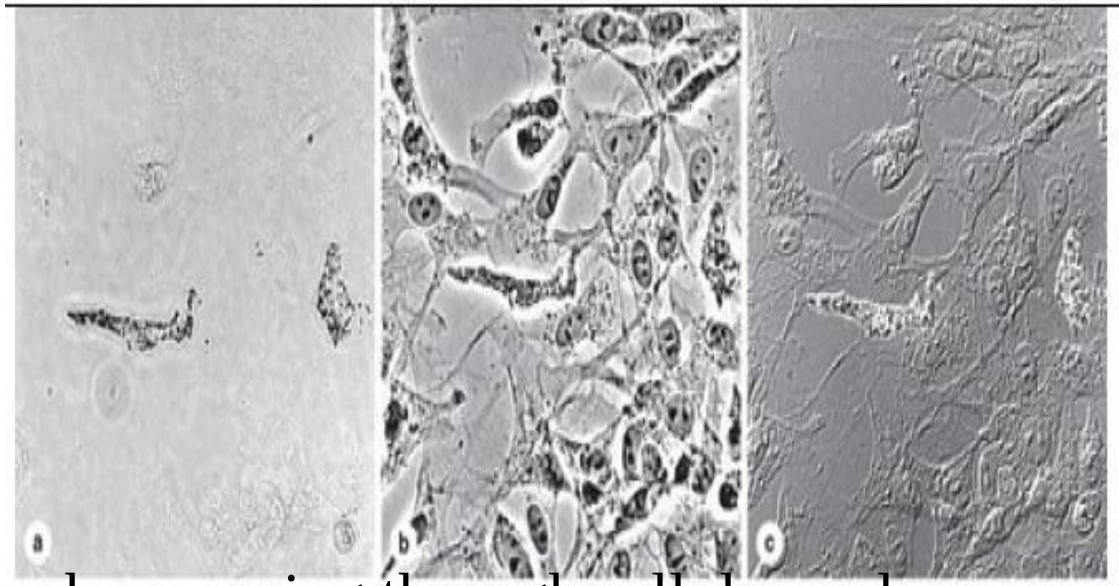
Why do I have to have secondary and primary antibody?

- Because the primary have limited fluorophore less than the force of secondary antibody , secondary load more fluorophore coming from the protein , so I am amplifying the signal so I can visualize the protein easier because the signal is stronger .
- The second picture show a cultured cells (cells grow in dishes (in Incubactor)) we use the florescence to visualize immunostained sections in this image. We have targeted filamentous , protein, concentrated under the plasma lamina .

- Cells in cultures their life depends on cells tend to be social and their communicate with each other .

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.

Elecetron Microscope

- Interaction of tissue with a beam of electrons. Reflected or absorbed or pass

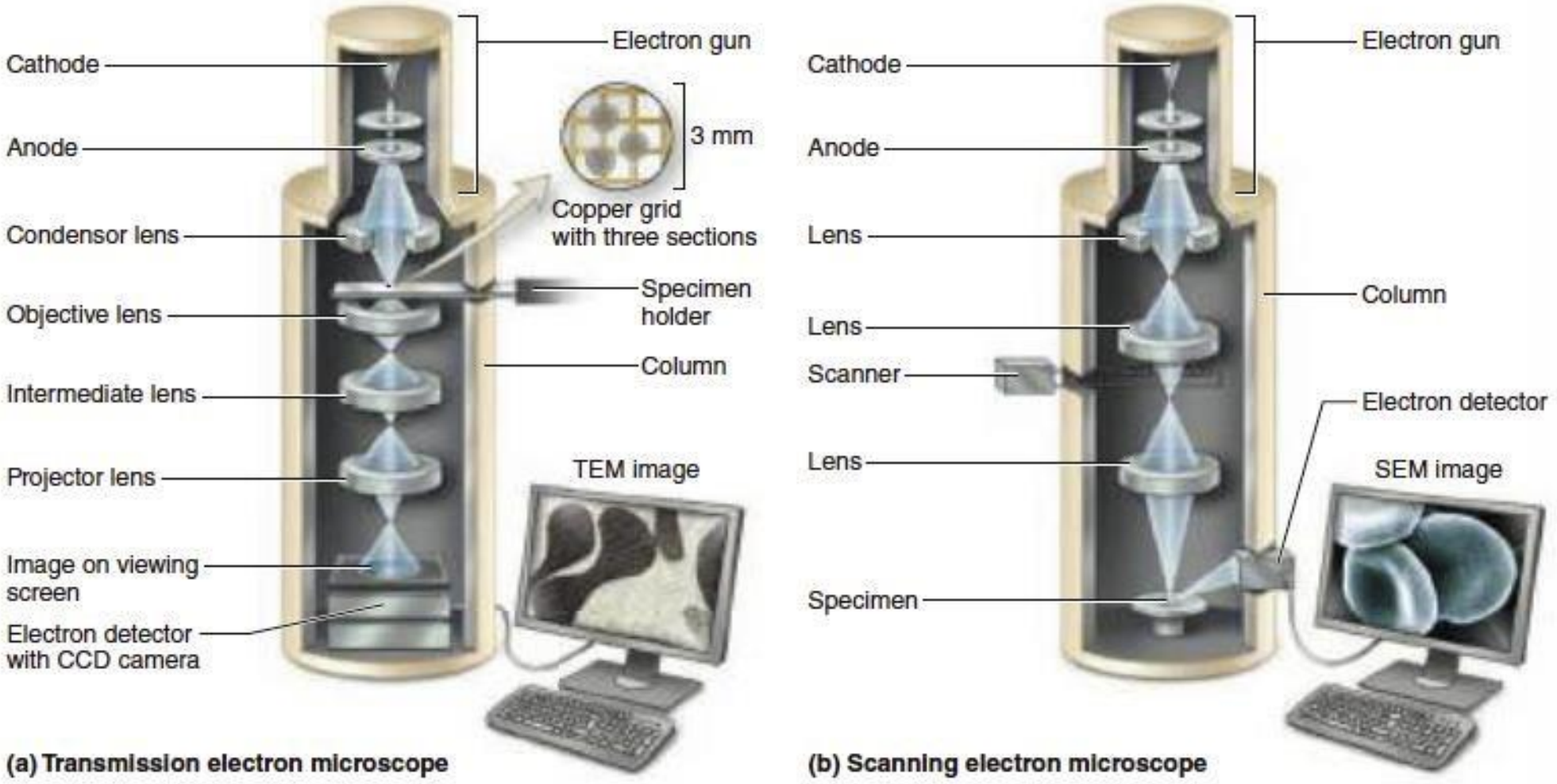
TEM

- The electron beam passes the tissue.
- Very high magnification
- Very thin sections, 40-90 nm.
- Electron beam interact with tissue producing black, white and shades of gray images.

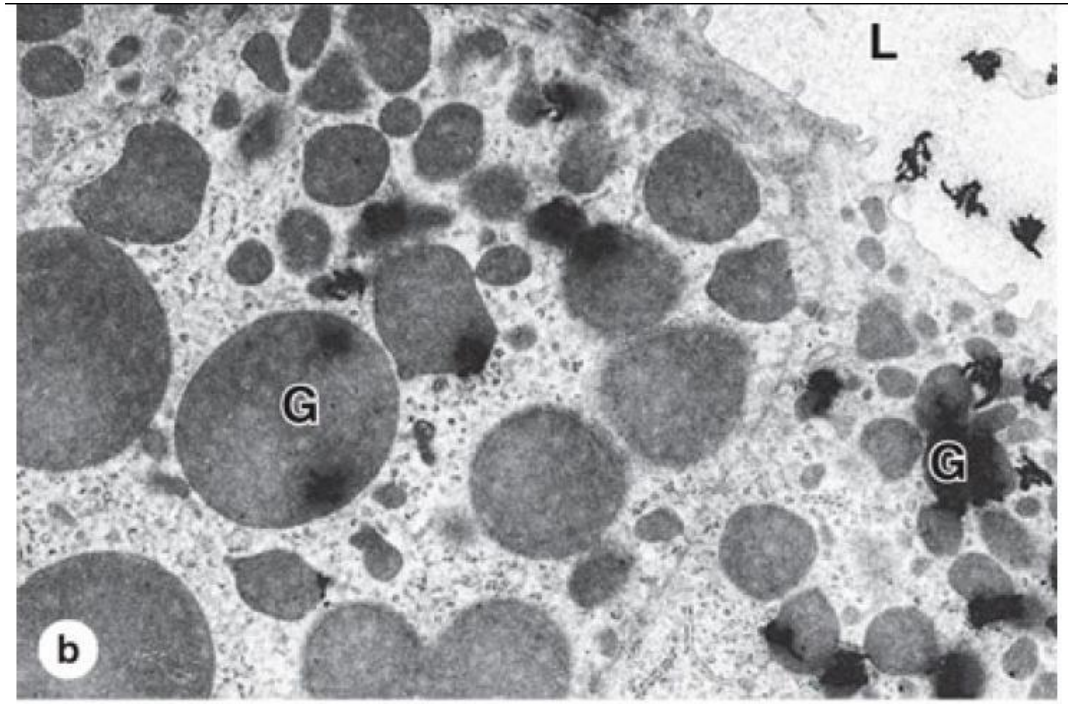
SEM.

- 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.

Electron Microscope

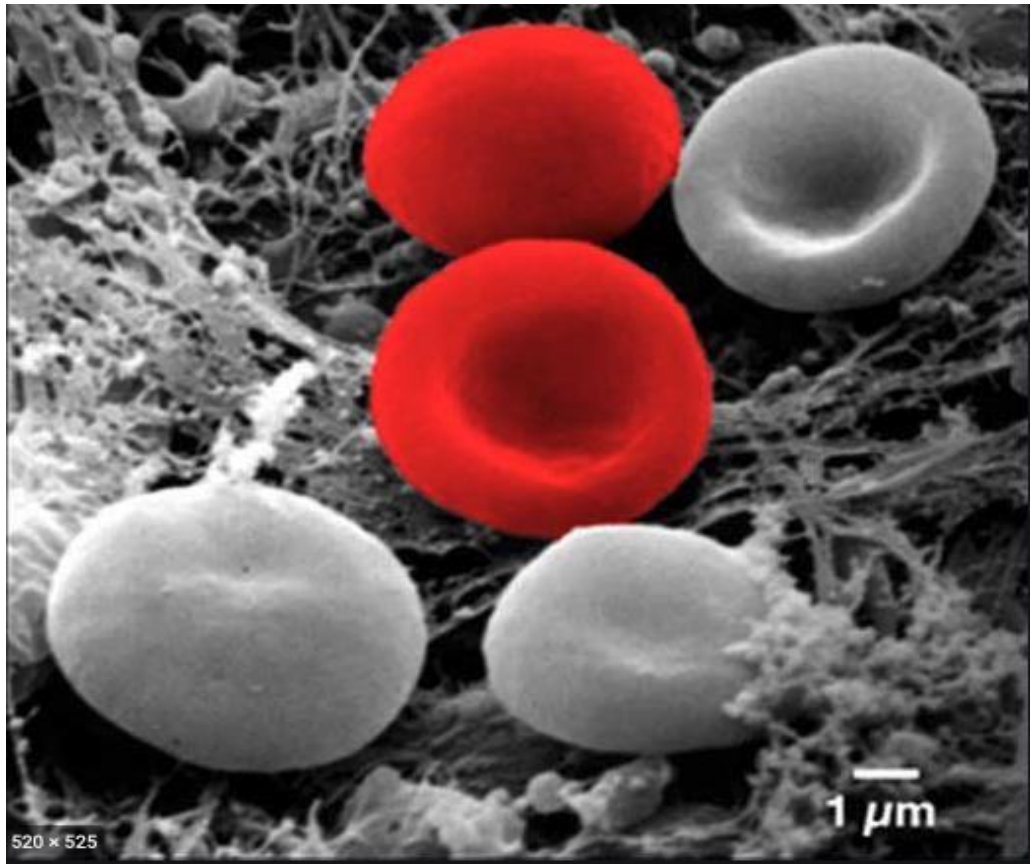


TEM



2D

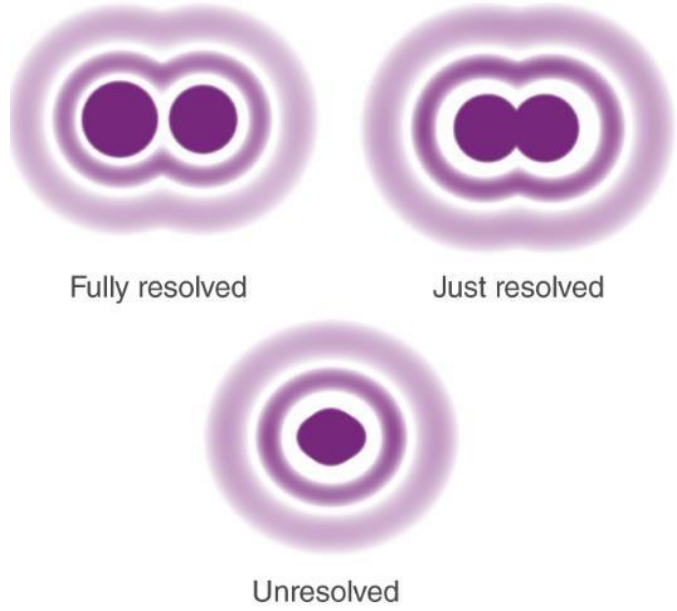
SEM



3D

Resolution

- **Resolution:** how finely detailed the images that we're looking at.
- **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.
- 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		Fluoroforce	Fluorophore
V1 → V2			

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