Sheet no.

2

Histology

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STAINING AND STAINS

-Most cells and extracellular materials are completely **colorless** (cant be seen under the bright field microscope).

-We have to add a stain (dye) to the cell or tissue to see their image under microscope.

Q / why the dies will stain components differently?

A / Simply, Because of different components might have different charges so they might react with different stain.

For example:

Negative charges found on the nucleic **acid** (DNA and RNA) have higher affinity to **basic** dyes, so acids are basophilic (blue stained).

And bases such as proteins , acidophilic (pink stained).

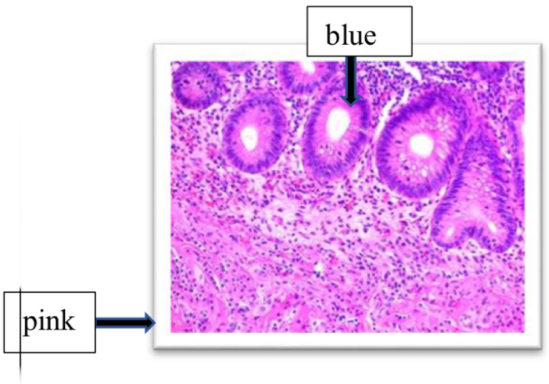
-hematoxylin and eosin (H&E) is the most commonly used stain(is water soluble stain).

- معلومة اضافية

Resin (plastic) is dissolves by propylene oxide.

Hematoxylin	Eosin
Basic dye	Acidic dye
Has positive charge	Has negative charge
Will stain negative (basophilic) structures BLUE	Will stain positive (acidophilic, eosinophilic) structures PINK
Examples: DNA, RNA, ribosomes, rER, GAGs	Examples: proteins, collagen, cytoplasm, mitochondria, secretory granules

-In EM , there is osmium tetroxide , which is used as a stain and a fixative too.

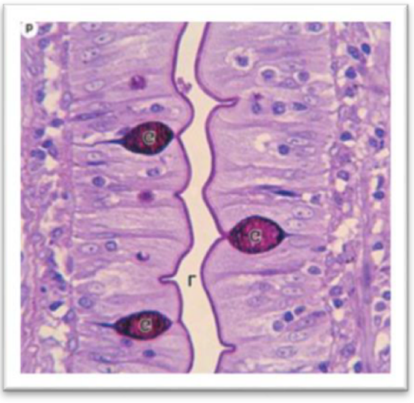


-This is not 100% true with all tissues .
H&E (Hematoxylin and eosin)

these all are cytoplasmic component.
the spindle shape is the nucleus, but why is it blue?
this depend on the dense of the stain when the photo was taken.
these all nuclei are basophilic, on the other hand the cytoplasm stains are acidophilic (have affinity to acidic stains).

-H&E Is a general stain and most widely used .

-If I want to know what structure I am looking at, I use hematoxylin and eosin
-If I want to know more specific structure then I will use ..... but not on the same stain, we don't add it to the same stain (we don't add to the hematoxylin and eosin) we bring a new شريحه(specimen) and stain it due to the investigation we want to do.



*This is example of agony, and this is more specific stain.
As you can see the shades (درجات) they are all nuclei with various sizes and shapes .
The size and shape of nuclei depend on the size and shape of the cell.

-***Periodic acid – Schiff*** (pas) stain is used to look for *carbohydrates*.
If we want to distinguish each carbohydrates that are found in two types of the cell we use (pas) stain. It gives color between pink and purple color this is just an example . After we prepared the specimen we need to look at it under microscope

Microscopes

Before we start with microscopes we need to know some terms:

-Magnification: is the degree to which a lens, mirror, or other device can enlarge an object, or the degree to which the object is enlarged.

-Resolution: is the shortest distance between two points on a specimen that can
still be distinguished by the observer or camera system as separate entities. (dependent on wavelength "λ")

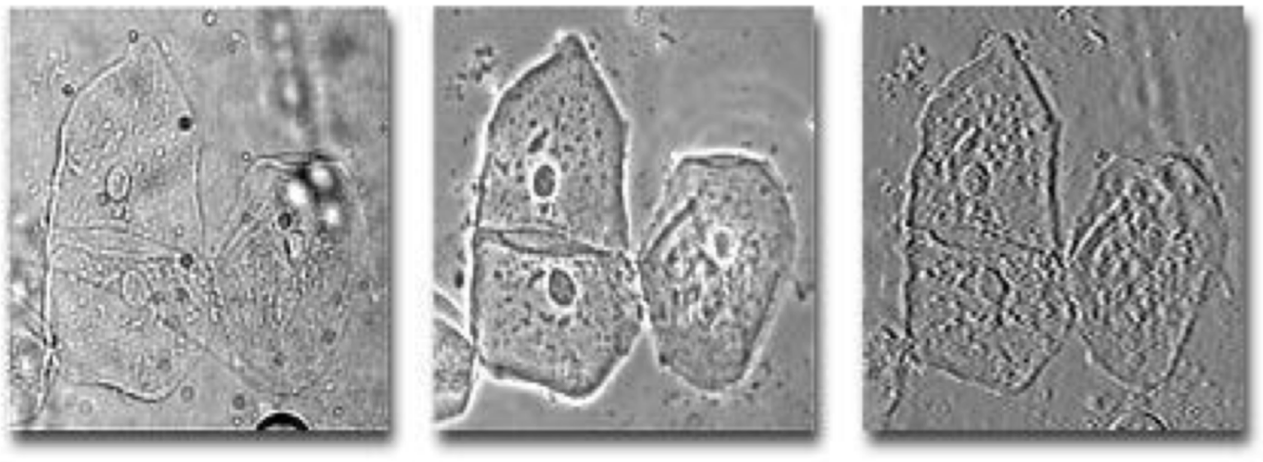
-Contrast: is the difference in light intensity between the image and the adjacent background relative to the overall background intensity.

Resolution

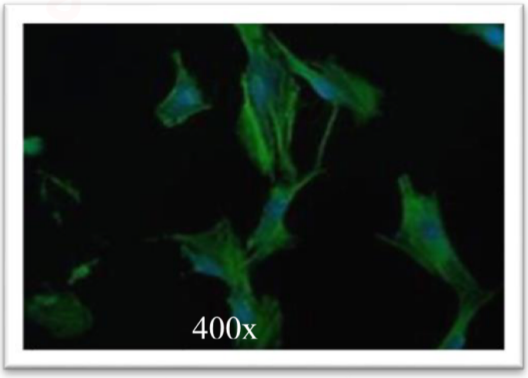
(a) The two dots are resolved--that is, they can clearly be seen as separate structures.

(b) These two dots are not resolved--they appear to be fused.

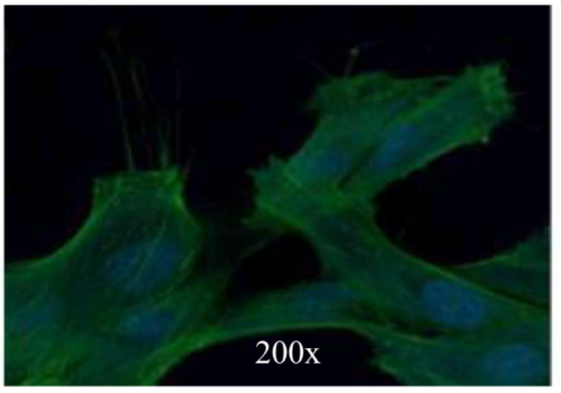
Contrast



(a) (b) (c)



Magnification



-**Techniques used in histology**

1-Light microscope

2-Electron microscope

3-Immunohistochemistry , which depends on antigene-antibody reaction.

If you want to find a specific structure, you add the fluorescent .

-There are two types of microscopes

1- lower resolution power

2-Higher resolution power

Microscopes have very high of precised and resolved images.

You can see and distinguish tiny particles in the cell with microscope.

LIGHT MICROSCOPE

HOW DOES IT WORK?

-The basic functional unit consists of a tube; having an

Objective (موضوعية) lens at one end and an ocular (عينية) lens at the other end.

-The condenser(المكثف) focuses light on the object to be studied.

- The objective lens enlarges the image of the object in the direction of the ocular lens , It can enlarge from 5X to 40X and even 100X but it will need for complicated procedures

- The **ocular** lens further magnifies this image toward the observer's **eye** .

- The total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses

#Light microscope can show tiny particles of the cell but at low resolution.

#Light microscope have other subtypes depending on what investigation we want to do with the microscope .

Light microscopy applications:

-Bright-field microscopy

-Fluorescence microscopy

-Confocal microscopy

-Phase-contrast microscopy

-Polarizing microscopy

They are all based on the interaction of light with tissue components and are used to reveal and study tissue features.

Generally we use the light as a source of energy to illuminate the sample when we look at it.

Light microscope limits:

-What is the critical and main factor in obtaining a crisp (detailed image) with a light microscope is its **resolving power**, defined as 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 um** which can permit clear images magnified 1000-1500 times.

- Objects smaller or thinner than 3um (such as a single ribosome or cytoplasmic microfilament) cannot be distinguished with this instrument.

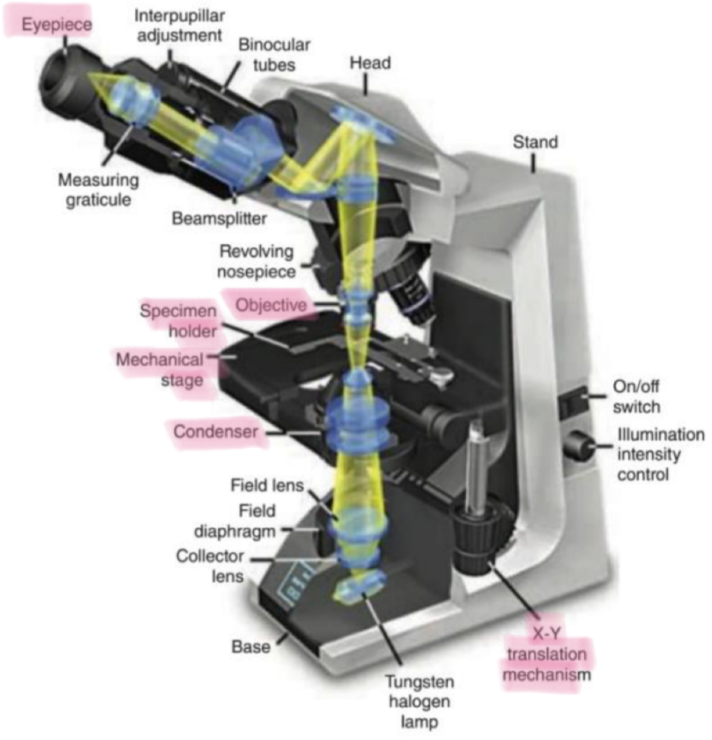
-The low resolution of light microscope come from the wavelength of the light which give disadvantage to light microscope.

However; if we want to see very small objects with higher resolution we should use electron microscope because light microscope won't be enough.

Note : that the resolution is inversely related to the wavelength

You have to know something that Histology is 2D study of 3D structures in histological sections.

"The image of light microscope is very important"



The magnification of objective could be as law as 5X to 40X

Major parts of light microscope :

Tungsten halogen lamp: is source of light (as a dispersed beam) that do illumination of the stained sample. then light passes through

Condensers: focuses the light as a one beam to the sample on the stage.

Mechanical Stage: is place where sample is found. (other name of stage) after light pass the specimen (sample) it goes to

Objective lens: enlarges the image of the object in the direction of the ocular lens. (named objective because it's near to the object sample)

ocular lens : further magnifies this image toward the observer's eye. .
(eyepiece is second name for it because it is near to eyes)

-The total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses.

(the ocular lens is x10 and we choose x4 for objective lense,10*4=x40)

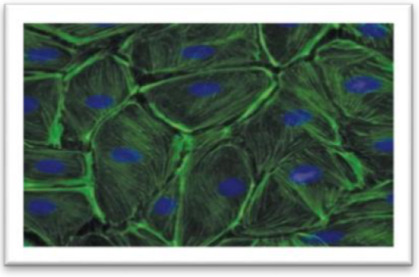
so total magnification is x40, same with other objective lens like x10,x20 or x40

.

the distance between the specimen and objective lens also effects on the resolution of the image .we have **coarse focus** and **fine course** To move it up

and down to resolve the image better .
specimen holder: keep the specimen (sample) in place.
X-Y tr anslation mechanism : are handles used to scan all area of glass light.
(x-y means sample scan to left and right directions).

- Light microscopy has many applications as we said before (page 4)



-this photo was made by immune fluorescence.
-Immuno means antibody (part of immune system).
-Here we have blue and green parts:
BLUE : is the nuclei of the cell
GREEN : let us assume it as "microtubules".

The principle of Fluorescence microscopy

if we want to target a specific protein in the cytoplasm ; how can we target them ?

simply , by antibodies .

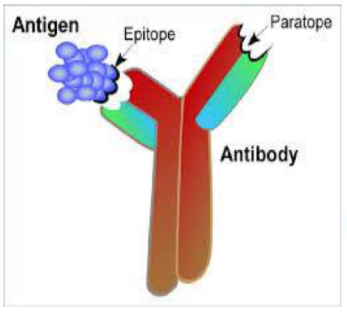
-we have primary antibody that binds only to this microtubules.

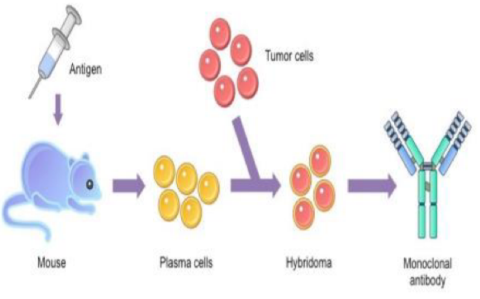
Then how can we distinguish this antibody from other parts?

We cannot project a light to it , so we will put something else called fluorophore

-in the image above we can see the microtubules and epitope (the part of an antigen molecule to which an antibody attaches itself).

And the **primary** antibody. Primary antibody has fluorophore.





monoclonal antibody can help in to attach to different types of cells for identification, and they are also used in certain diagnostic tests for bacteria and viruses.(extra)

an antigen can has one or more epitopes , monoclonal and poly clonal antibodies.

If we infect a mouse for example by human antigens is taken from , the mouse will produce antibodies called mouse anti human antibody. [extra]

-**Fluorophore**: are tiny particles on the antibody that when I do fractionation to the light of any color(wavelength) they will illuminate ;so we will look to the microscope anything negative which didn't bind to primary antibody will appear as a black color , flag that binded to the antibody will show the color that present on this fluorophore; so in the image we had green fluorophore so we had a green color.

-with mitochondria, microfilaments or cytoskeleton present more in cytoplasm primary antibody is enough to get good image.

- sometimes with very tiny and rare protein in the cell; this signal won't be enough to get a good image that's why we need more fluorophore.

-We put primary antibody and design to it a secondary antibody which can binds to more than one primary antibody.

-when we add more fluorophore to the secondary antibody, we will see it much more clear than if I was using only the primary.

(As an idea; a road with 10 limbs of light can be seen better than only 2 limbs
of light, here also same a secondary antibody only make the image butter with the same road).

-more the fluorophore better the image we get

We can see microtubules and microfilaments at the same time but they must have different color ; means fluorophore found on the secondary it's color must be different . each of primary's antibody knows it's epitope and will binds to it and we won't have cross reaction more.(might have rarely).

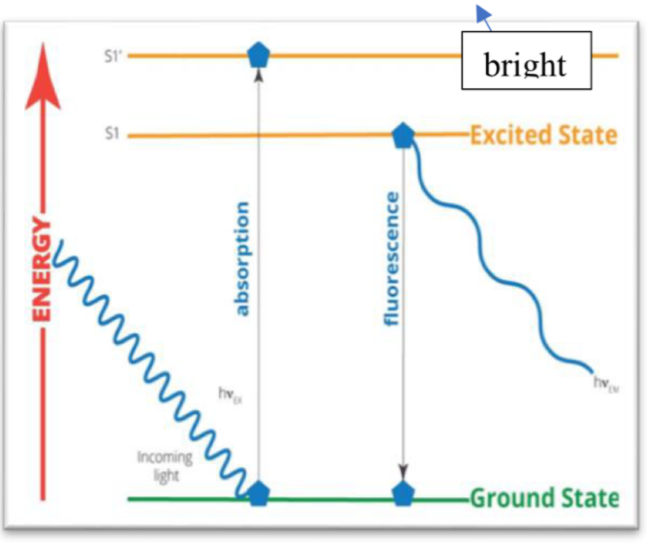
-Another method of immunohistochemistry is by using enzymes as labels, if there is a reaction to convert a substance to product and the product is colorful then whenever there is enzyme the substance will be colorful and whenever there is no enzyme , the substance will be colorless.

Fluorescence microscopy/

When certain cellular substances are irradiated (يتم تعريضها) by light of a proper wavelength, they emit (يصدر) light with a *longer* wavelength— a phenomenon called fluorescence(التألق).

-In fluorescence microscopy, tissue sections are usually irradiated with ultraviolet (UV) light with a long wavelength and the emission is in the visible portion of the spectrum. (يكون مأخوذ من الطيف المرئي)

- The fluorescent substances appear bright on a dark background.

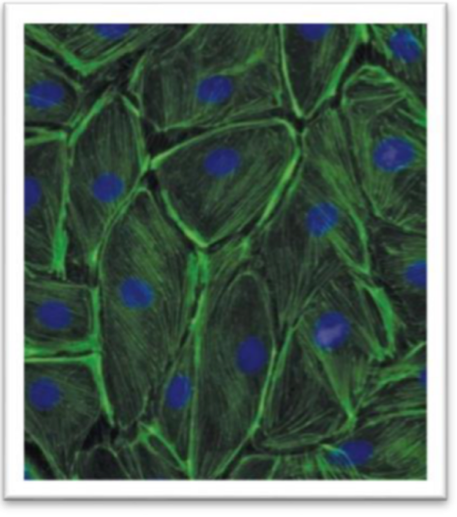


- For fluorescent microscopy the instrument has a source of UV or other light and filters that select rays of different wavelengths emitted by the substances to be visualized.
- Every cell has a flag, a marker on the cell surface, glycoproteins that are antigens it leads to immunological rejection by its epitope against specific marker and attach it, if fluorescence happens then ave the protein.
- S1 has higher wavelength than S1`

- The substance absorbs a proper wavelength and be exited to S1` , then it goes to S1 and emits light with a longer wavelength (to S1) and being bright *fluorescence* .

Blue: DAPI stain (which binds DNA)

Green: fluorescein-phalloidin stain (which binds actin filaments)



Phase contrast microscopy

-Unstained cells and tissue sections, which are usually transparent and colourless, can be studied with these modified light microscopes. (not clear image)

- later; a scientist came with phase contrast microscopy which uses a lens system that produces **visible** images from transparent objects and, importantly, can be used with **living,** cultured cells

Principle of phase contrast microscopy:

-Phase-contrast microscopy is based on the principle that light changes its speed when passing through cellular and extracellular structures with different **refractive indices**.

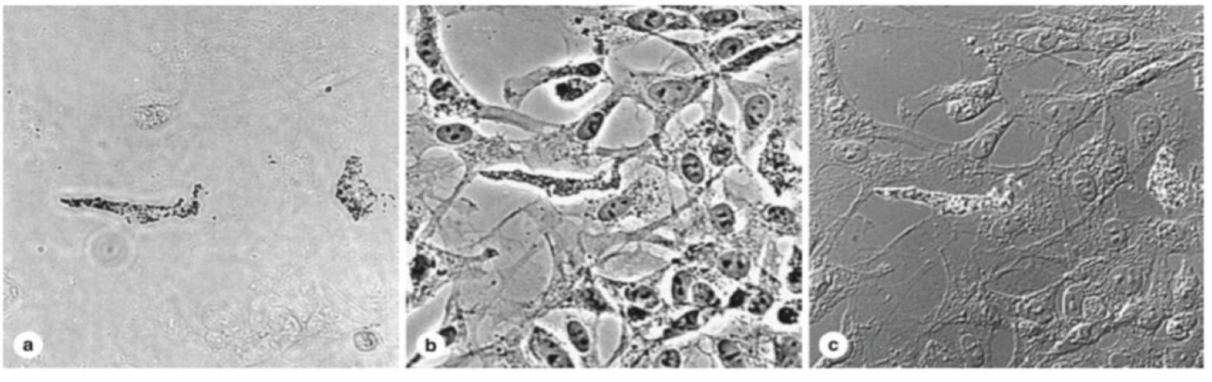
-These changes are used by the phase-contrast system to cause the structures to appear lighter or darker in relation to each other. (show different image) but we need a special lens to have this image.

-Because they allow the examination of cells without fixation or staining, phase-contrast microscopes are prominent tools in all cell culture laboratories .

-Cell culture : means we take a cells and put them in a dish to replicate.

-the cells in the bright field microscope.(a)and phase contrast microscope. are the same cells but details appearance is different. In (b) we can see cells connect to each other, the cytoplasm,……etc.

FIGURE 1–5 Unstained cells' appearance in three types of light microscopy.



Living neural crest cells growing in culture appear differently with various techniques of light microscopy. Here the *same field* of unstained cells, including two differentiating pigment cells, is shown using three different methods (all X200):

(a) Bright-field microscopy: Without fixation and staining, only the two pigment cells can be seen.

(b) Phase-contrast microscopy: Cell boundaries, nuclei, and cytoplasmic structures with different refractive indices affect in-phase light differently and produce an image of these features in *all* the cells.

(c) Differential interference microscopy: Cellular details are highlighted in a different manner using Nomarski optics. Phase-contrast microscopy, with or without differential interference, is widely used to observe live cells grown in tissue culture.

(Used with permission from Dr Sherry Rogers, Department of Cell Biology and Physiology, University of New Mexico, Albuquerque, NM.)

-they did a small modification on phase contrast microscope.

Called **Differential interference microscopy**: same like phase contrast but the lens is more modified to look as more 3-D structure .

Electron Microscopy

In electron microscope, we don`t use glass slice because the whole beam is electrons, so we use a conductive one that is metal (copper is preferable)

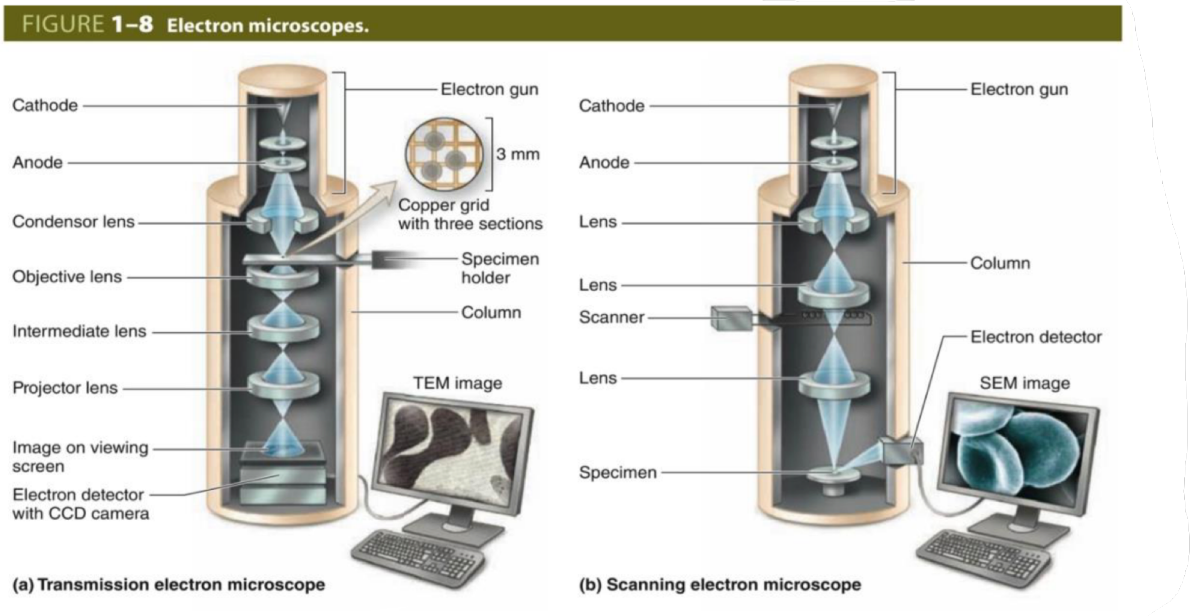
-Transmission and **scanning** electron microscopes are based on the interaction of tissue components with beams of electrons.

-The wavelength in an electron beam is much shorter than that of light, allowing a 1000-fold increase in resolution.

-resolution of electron microscope around 3 nanometer (3 nm).

-Electrons are extremely small, so the resolution of electron microscope is 1000 times more than the light microscope.

-As long as it is 3 nanometer we can distinguish two objects, if smaller they will appear as one structure (unresolved).



Major parts of electron microscope :

Electron gun: is source of electrons. It shoots electron beam. then electron beam hit the anode. here we will have transmission and scanning electron microscope

-both types gives electron beam at the end. After that the electrons passes through.

- **Condensers':** focuses the electrons as a one beam to the specimen.

-fixation, embedding and clearing is same like light microscope except I use different regions with the electron microscope than the light microscope. The material is also different. the process of electron is more complicated but they have same principle.

after the electron beam hit the specimen it has three choices:
Absorption, refraction or penetrate the depending on the density of the structure inside the specimen.
If it is empty it will pass through; If we have dense structure it will absorb it.

- Finally we have electron detector, the beam passed through will appear in the TEM image, other beams will won't appear as TEM image but it will be

recorded because it connected to CCD camera attach that move the image to connected computer next to it.

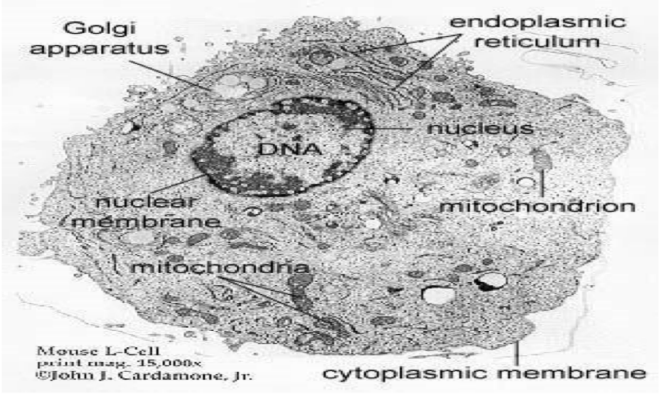
So as a result of interaction between electrons and specimen we capture it in the detector then it will be translated into image.
-from it's name transmission means something will transmit. Opposite of it is scanning

-In scanning, the arrangement of the lens is different from the transmission also the preparation to begin with samples is different.
-scanning means you are doing scan to the topography for structure.
So when I look to topography from outside I will see it's shape from out and it's identification.

The **transmission electron microscope (TEM)** is an imaging system that permits resolution around 3 nm.
This high resolution allows isolated particles magnified as much as 400,000 times to be viewed in detail.
- Very thin (40-90 nm), resin-embedded tissue sections are typically studied by TEM at magnifications up to approximately 120,000 times.

TEM principle

- the components of a TEM and the basic principles of its operation: a beam of electrons focused using electromagnetic "lenses" passes through the tissue section to produce an image with black, white, and intermediate shades of gray regions.
-These regions of an electron micrograph correspond to tissue areas through which electrons passed readily (appearing brighter or electron-lucent) and areas where electrons were absorbed or deflected (appearing darker or more electron-dense).



Scanning Electron Microscopy

Scanning electron microscopy (SEM) provides a high resolution view of the surfaces of cells, tissues, and organs. Like the TEM, this microscope produces and focuses a very narrow beam of electrons, but in this instrument the beam does not pass through the specimen.

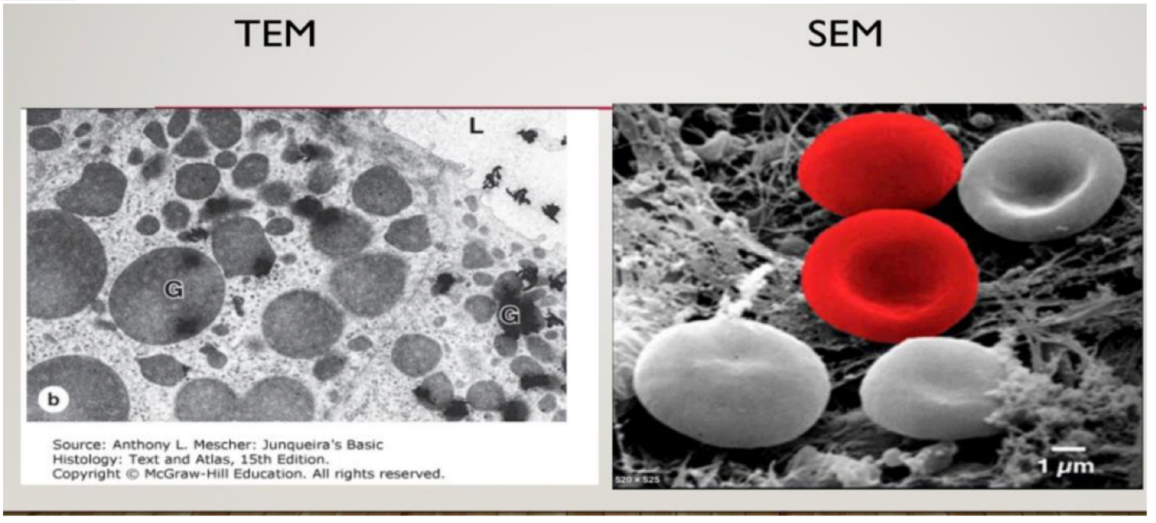
Instead, the surface of the specimen is first dried and spray-coated with a very thin layer of heavy metal (often gold) which reflects electrons in a beam scanning the specimen.

The reflected electrons are c[illegible], producing signals that are processed to produce a black and white

SEM images are usually easy to int[illegible] because they present a three-dimensional view that appears to be illu[illegible]minated in the same way that large objects are seen with highlights and shadows caused by light.

The reflected electrons are captured by a detector, producing signals that are processed to produce a black-and-white image.

SEM images are usually easy to interpret because they present a three-dimensional view that appears to be illuminated in the same way that large objects are seen with highlights and shadows caused by light.



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