Histology

Modified n. 2

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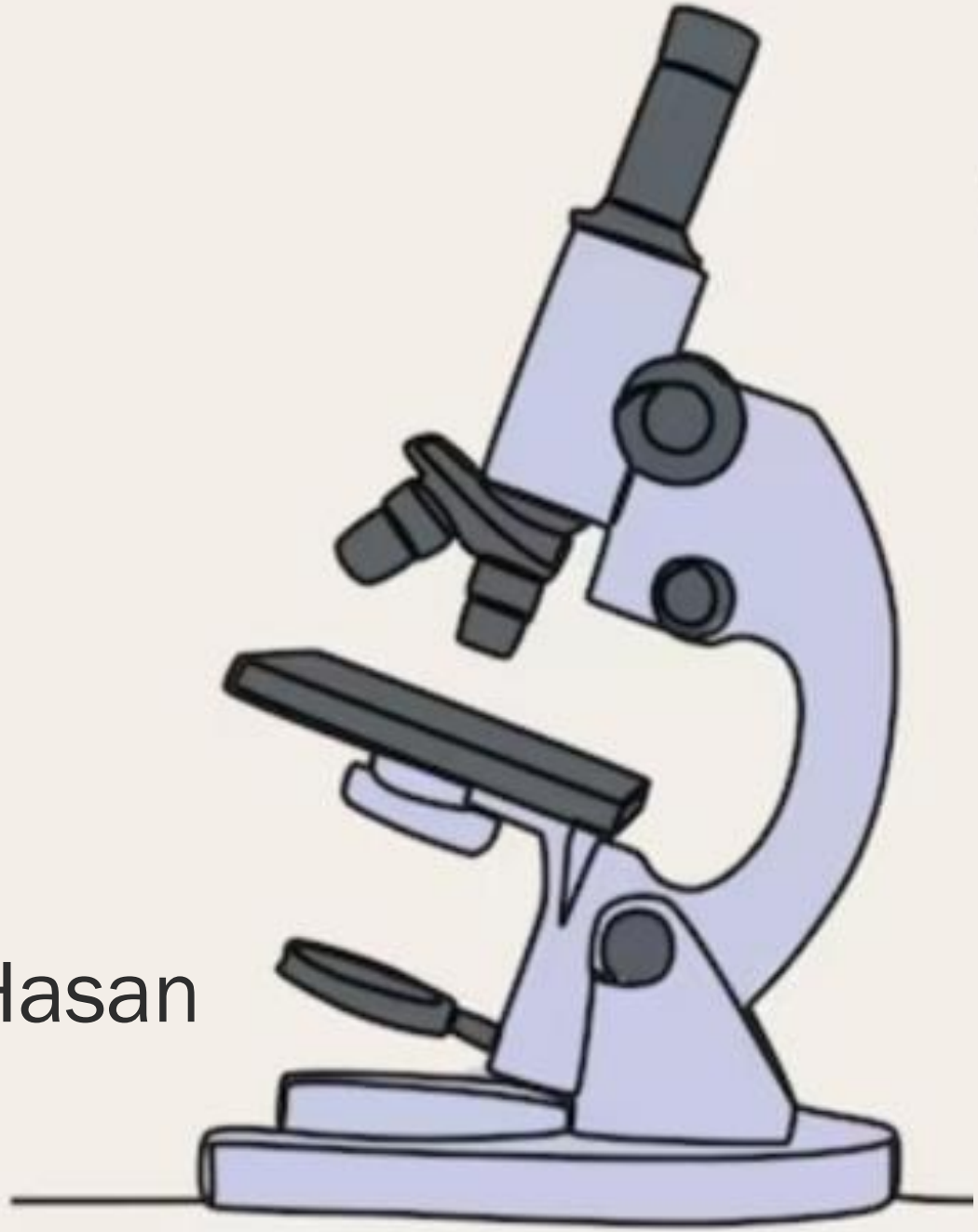


Staining And Stains

We use staining to identify tissues & their structure or molecules.

- Most cells and extracellular material are completely colorless!
- Dyes forming electrostatic (salt) linkages with ionizable radicals of macromolecules in tissues.
- Cell components such as nucleic acids with a net negative charge (anionic) have an affinity for basic dyes and are termed **basophilic.**

Cytoplasm is mainly acidofilic because of the high amount of proteins in it ,it appears pinkish.

- Cationic components, such as proteins with many ionized amino groups, stain more readily with acidic dyes and are termed **acidophilic**.
- Basic dyes include toluidine blue, alcian blue, and methylene blue.
- **Hematoxylin** behaves like a basic dye, staining basophilic tissue components.
- DNA, RNA, and glycosaminoglycans: ionize and react with basic dyes do so because of acids in their composition
- Acid dyes: **eosin**, orange g, and acid fuchsin stains mitochondria, secretory granules, and collagen are acidic.

DNA,RNA,&Glycosaminoglycans are basofilic that's why they appear Reddish or darker.

Staining And Stains-special stains

- **Trichrome** stains allow greater distinctions among various extracellular tissue components, e.g., Masson trichrome.
- **The periodic acid-Schiff (PAS)** reaction utilizes the hexose rings of polysaccharides and other carbohydrate-rich tissue structures and stains such macromolecules distinctly purple or magenta.
- **Sudan black**: lipid-soluble dyes --satins lipids; avoiding the processing steps that remove lipids, such as treatment with heat and organic solvents which can be useful in diagnosis
- **Metal impregnation:** less common methods. Using solutions of silver salts to visual certain ECM fibers and specific cellular elements in nervous tissue.
- Immunostaining: immunofluorescence and immunohistochemistry.

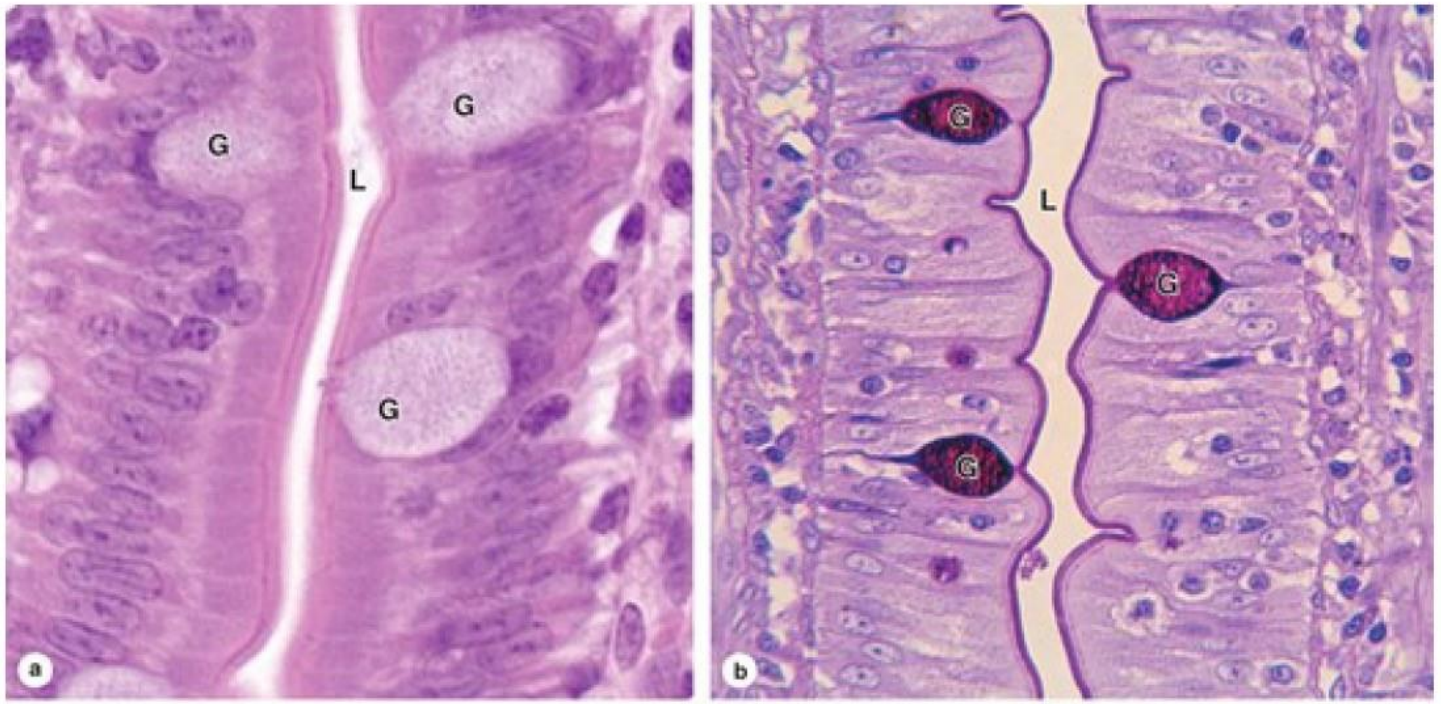
PAS : usually used in distinguishing polysaccharides & they appear purple or magenta.

Immunostaining: we use the concept of antigens & antibodies binding together to observe samples.

Sudan black: usually to stain fats,as we saw in previous lecture (processing),here we won't use the solvent that dissolves lipids so they can be observed.

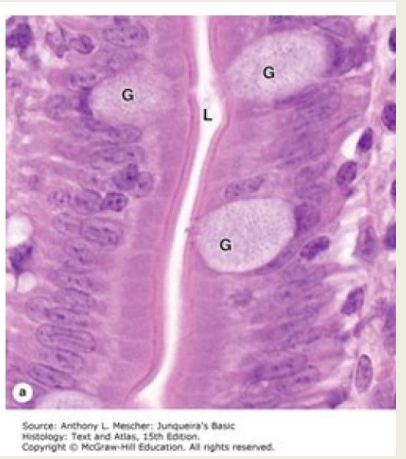
H&E

PAS Staining

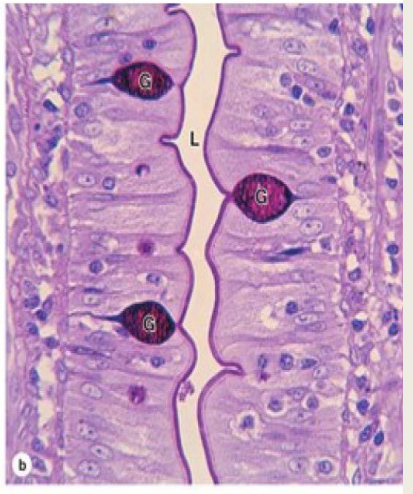


Now let's look together at this slide the image in the left that's a typical hematoxylin and eosin stain (H&E)
What am I looking at?
at this stage we're not going to talk too much about it it's just we need to know where the cells are, (this is an epithelial tissue by the way) I need to localize the nuclei, those are the nuclei which usually appear bluish in color, so all these round the structures are the nuclei and that's the rest of the cytoplasm more pinkish so this will react more readily with the eosin and the nuclei will react more readily with the hematoxylin.
Now, on the epithelial tissue there is a special cell here that we call it goblet cells goblet cells usually have a good amount of polysaccharides inside there cytoplasm and then if we are targeting this we have to use what it's called the PAS stain (periodic acid schiff stain) as you can see the goblet cells inside they are rich with polysaccharides and it shows as a different darker image from the surrounding of the epithelial cells.

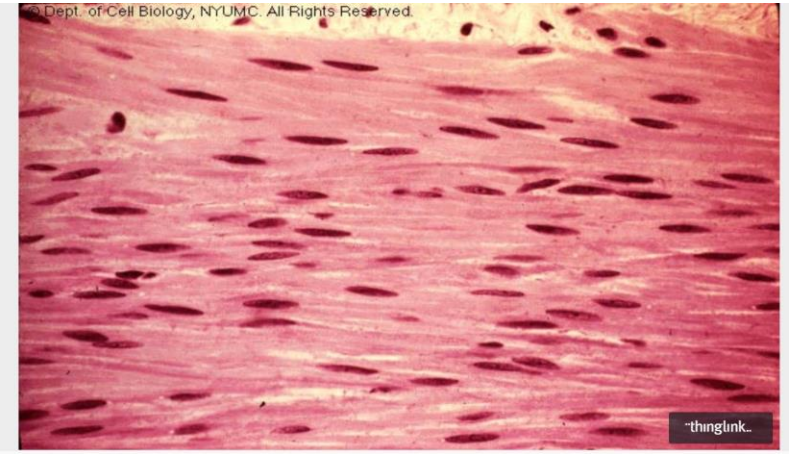
H&E



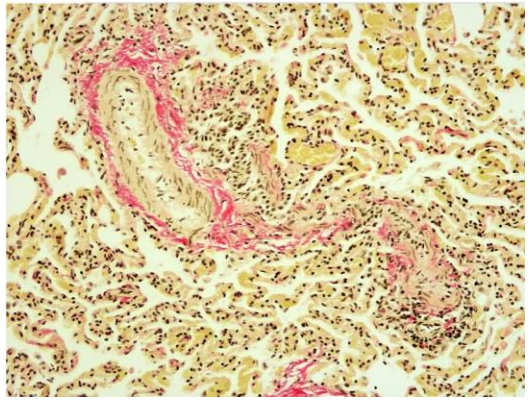
PAS Staining



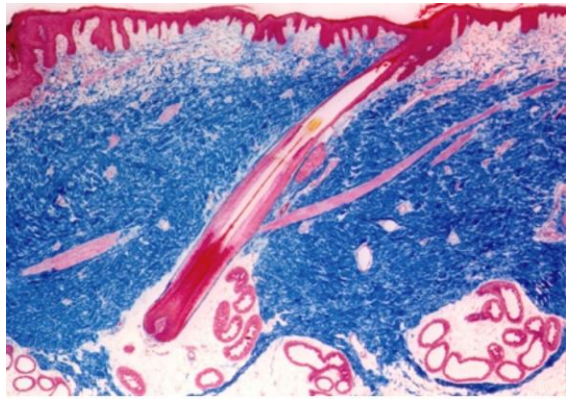
Examples Of Commonly Used Histological Stains



- Hematoxylin and eosin (H&E): nucleus/blue, cytoplasm/pink



- Van Gieson method: collagen/pink, muscle/yellow.



- Trichrome method: three color system to emphasize support fibers: connective tissue/blue, cytoplasm/pink, nuclei/dark brown.

MICROSCOPES

Types of microscope

The use of each microscope depends on the aim of the study.

Light microscope: cheapest and most commonly used.

- **Light microscope.**

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

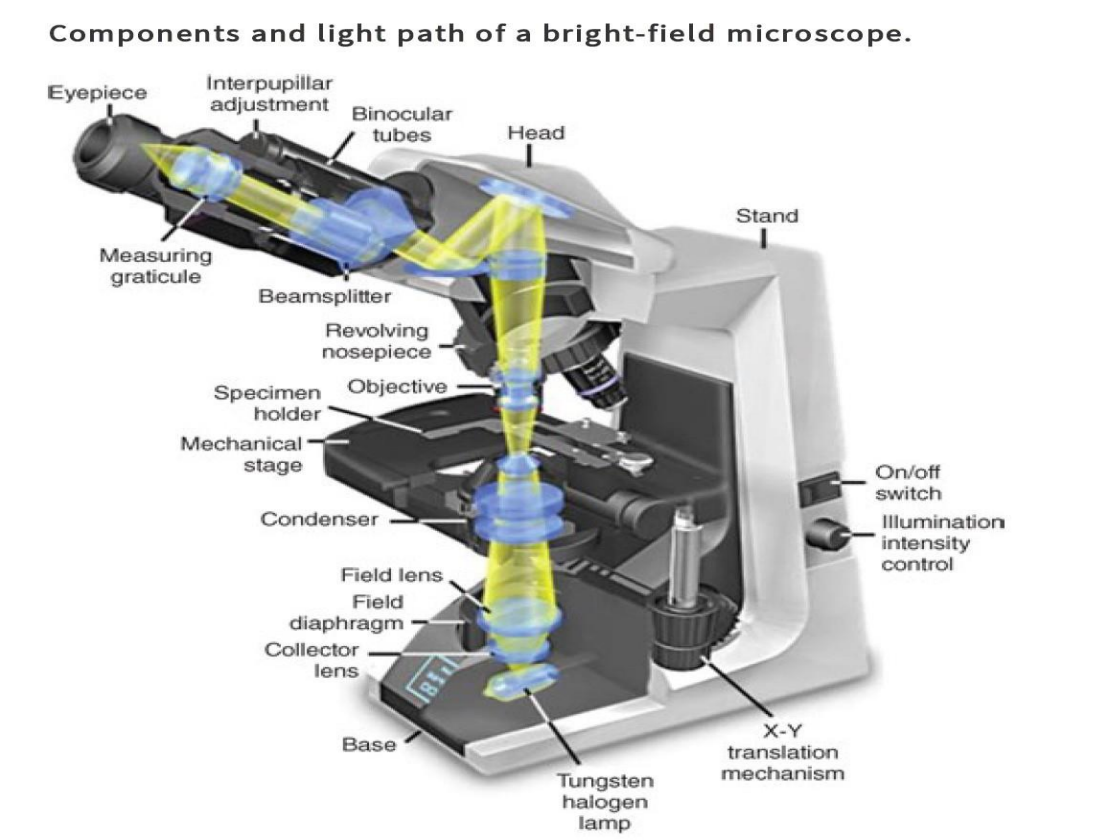
- **Electron microscope**

1. Transmission electron microscopy
2. Scanning electron microscopy

Light microscopes utilize visual light in order to illuminate the tissue which is prepared previously to distinguish its kind and structure.

If we're looking for a specific information of the tissue we use the proper staining method.

Light Microscope (Bright-field)



On the previous slide :

Light microscopes:

- Source of power: electricity.
- Source of light: Tungsten halogen lamp.
- Diaphragm: light is emitted through this opening.
- Mechanical stage: light will ascend upwards till it reaches the glass light on this stage.
- Before the light gets to the tissue we have to narrow down the beam of light in the condenser.
- Through the opening of mechanical stage light hits the tissue on the glass light then it will ascend upwards & enter another station: the objective lens(different objective lenses mean different magnifying intensity.
- Light will travel through the objective lens & reach the Eyepiece where we have

a lens which we put our eyes against so we could see what is on the glass light.

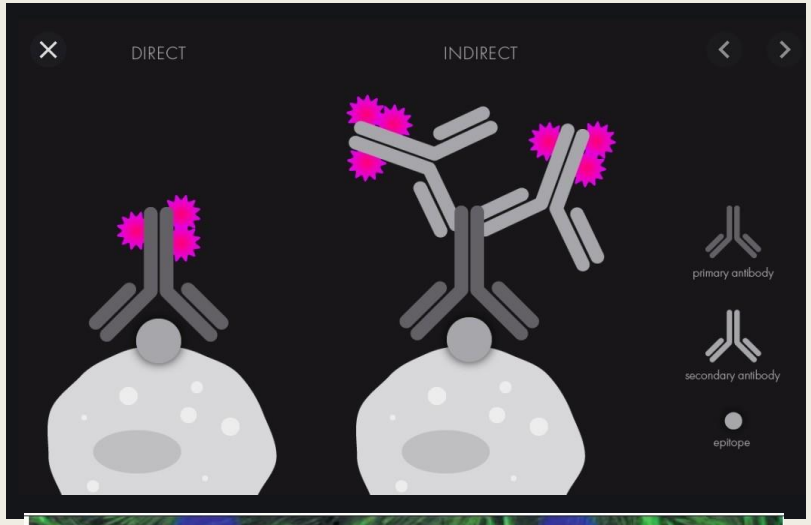
- Eyepiece lens : magnifies by default x 10
- The objective lens: magnifies by different intensity (5,10,20,40,100) depending on viewer.
- Final magnification= (the objective magnifying x eyepiece magnifying).
- Eg. What is the total magnifying when you use a 20x objective lens?
- Ans. 20(obj.) x 10(eyepiece) = 200

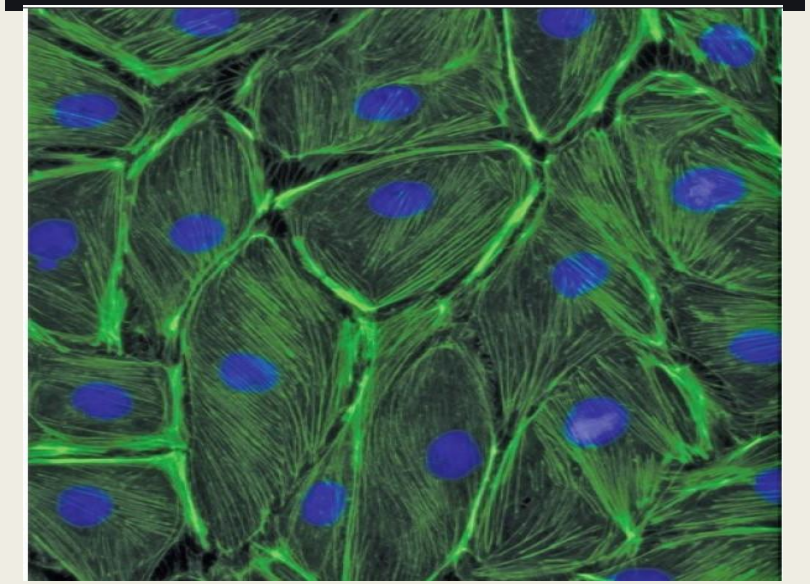
Bright-field Light Microscope

- 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

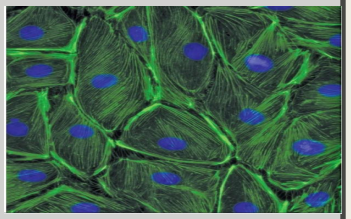
- **Fluorescence**: when certain cellular substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength.
- In fluorescence microscopy, tissue sections are irradiated with
- Ultraviolet (UV) light and the emission is in the visible portion of the spectrum.
- The fluorescent 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.





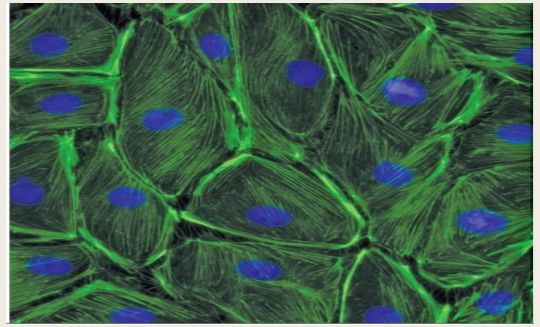
Fluorescence Microscopy

Now the second type that is fluorescence microscopy
This is a different type of light microscope, this type of microscope is actually utilizes the principle of fluorescence It is simply that we have substances when we radiate them with a particular wavelength that's compatible with them Those substances will emit(تبعث)a longer wavelength and that's the part of the wavelength that we see with our eyes when we look through the fluorescence microscope Those microscopes they have a UV lamp unlike the halogen that we saw in the bright field so how does a fluorescence microscope images look like they look like this image on the bottom right



So what am I looking at?
You are simply looking at cells next to each other (those are cultured cells by the way)
What do I mean by cultured cells ?
Those are cells grown in the dishes that we put cells in and we put them in the incubator if we are studying a particular type of cells .

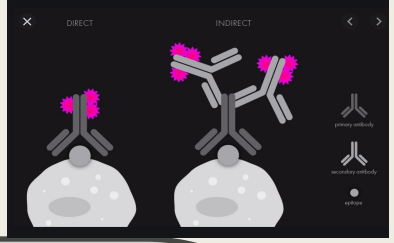
Fluorescence Microscopy



- Now what am I looking at? I'm actually seeing three colors I am seeing a blue and then actually the stained for the nucleus and the background is black and apparently in this image i have targeted something specific so what is this ?
- how is the fluorescence microscope ? is targeting something specific because we use the fluorescence microscope to visualize immune stained section as simple as that immune staining that's one the special stains that we have discussed briefly in the staining section

So in this image you can see they have targeted filamentous structure (it's a protein by the way) And this protein seems to be more concentrated under the plasma regardless of what we are trying to do we just need to discuss the concept of the immune staining here

Fluorescence Microscopy

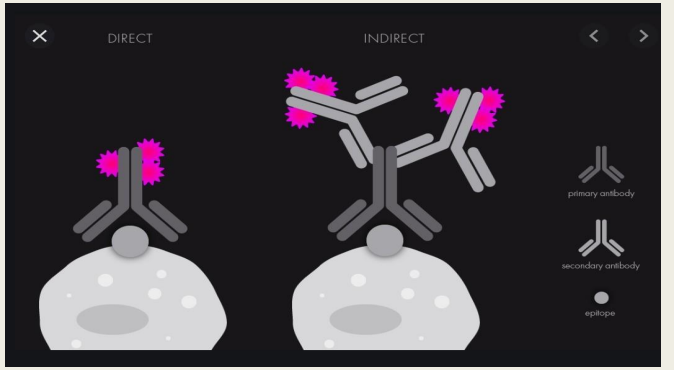


so let move up to the upper image here what am I looking at? This is a cell and this is a nucleus inside on the plasma membrane I have an antigen and when we say antigen usually what jumps into our mind is that antigens usually are bound not usually it's like when we say immune you know antigen means the antibody for it

but it seems that the antibody has something has a fluorescent material attached to it and that's true those are fluorescence those are the elements of the antibody that we are going to irradiate with a specific wavelength and they will emit another wavelength that's longer and that which we see

so how can we combine microscopes with staining or immune staining? simply I'm targeting a specific protein so I purchase the antibody that will bind to this particular protein when this antibody is applied to the tissue it will bind to that particular antigen when it bind to it we already have those fluorescence on their surfaces so I process my sections and i go to the fluorescence microscope and then I choose the wavelength I radiate my prepare samples and then those flouroscense they will emit another wavelength and then I can see .

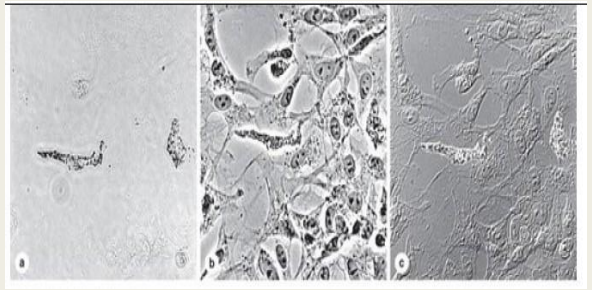
Fluorescence Microscopy



if the protein is there that means the antibody is already attached to the antigen if the protein is not there simply this antibody will not be attached to it and will not see because simply there is no protein

Now I have two structures next to each other what is the difference?
simply both are the same cells same antigen same primary antibody but apparently there is another antibody so the antibody attaches to the antigen is called the primary antibody and the one that attaches to the primary antibody is called the secondary antibody why doing have a secondary antibody? is simply because if you look at using the only one antibody there is a limited space for fluorescence so I have less fluorescence whereas when I use the secondary antibody I can load it with more fluorescence so what will that take us it will take us to that I am having a stronger signal coming from the protein I'm amplifying the signal and therefore and I can visualize the protein easier because the signal is stronger than when I only use the primary antibody.

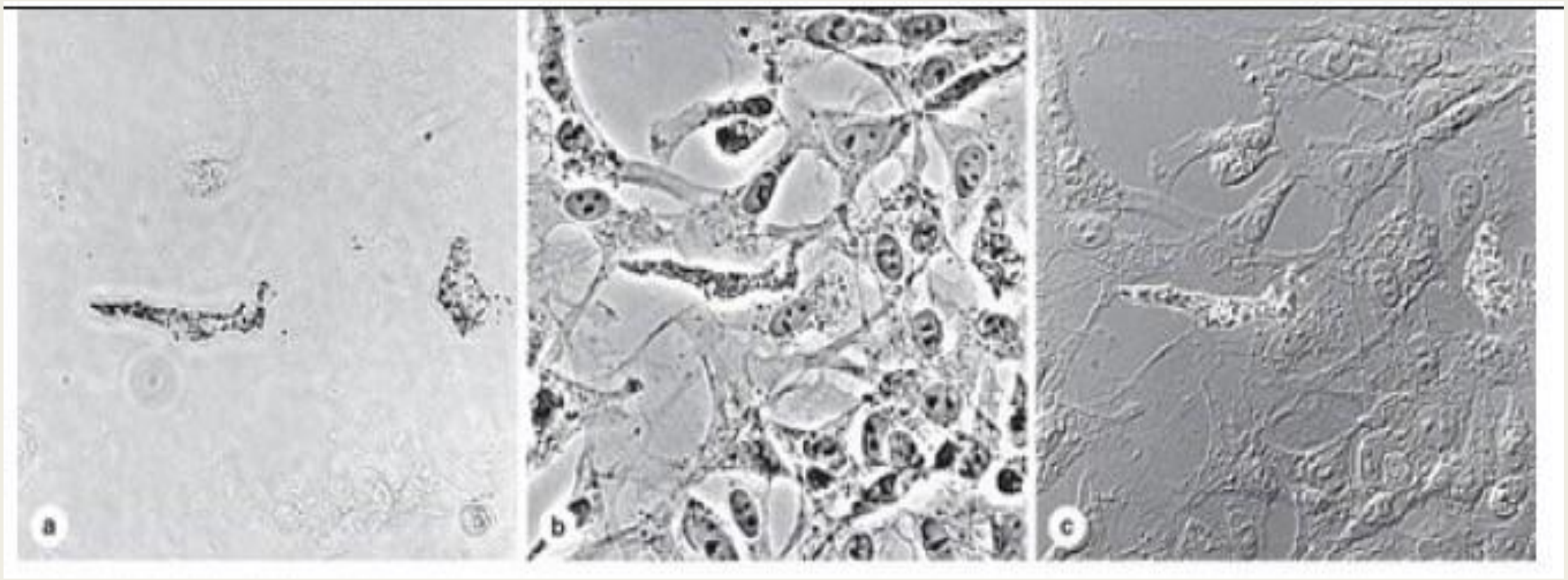
Phase-contrast Microscopy



- 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.

Phase-contrast Microscopy

- Now however invented this microscope is actually a genius, why is that? now I have just mentioned that unstained section simply cannot be visualized using the bright field microscope because simply the image will appear, such there isn't much information that's a colorless with depress here and there that gives us no information, so we usually stain our tissues, but is there a condition where we cannot stain our cells or our tissues? Yes, when does that happen? look at the image on top what is this is called a culture plates we usually use these to grow cells inside and when we say grow cells that means I need them to grow I need them to keep them alive but at the same time I need to be able to check on myself from one time to another so which means I need microscope, and I just said microscopes we cannot use them to visualize and stained cells or tissues and in the same time if i stain these cells i will have to kill them because I need to fix them I need to add a number of chemicals to them and eventually I would just lose the cells so, can I visualize the cells? the answer is Yes, and the phase contrast microscopy solved it through the use of a special lenses and those special lenses they were designed to detect different refractive indices and then an image that I could see before with a face contrast I was able to see and obtain this image where is this image.



- what am I looking at? Simply those are all the culture cells in the culture plat and the round structure here are the nuclei for these cells and those processes are simply the process that these cells they send to the neighboring ones because cells in culture usually it depends on the type they tend to be social so they send these processes and they communicate with each others and that is one method to keep the cells alive actually.

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 producing black, white and shades of gray images.

SEM.

- The electron beam does not passe 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

We have two types of (EM)
1- The transmission electron microscope (TEM)
2- scanning electron microscope (SEM)
They both rely on the concepts of a beam of electrons that we directed toward the tissue that's in our specimen and this beam of electron will react with the tissue accordingly, now why are they different because that principally relies on how the beam of electrons react with the tissues for example the TEM actually the electrons they do pass through the tissue, when it passes those electrons -> might be reflected, might be absorbed or it might pass actually through the tissue, and then the detector in the TEM will record the activity of the electrons and then we get a final image that would usually appear as black white and in between various shades of gray, both microscopes they will give us a very high magnified images and we use usually an extremely thin sections and when we say extremely thin we talk about nanometers (40-90nm)

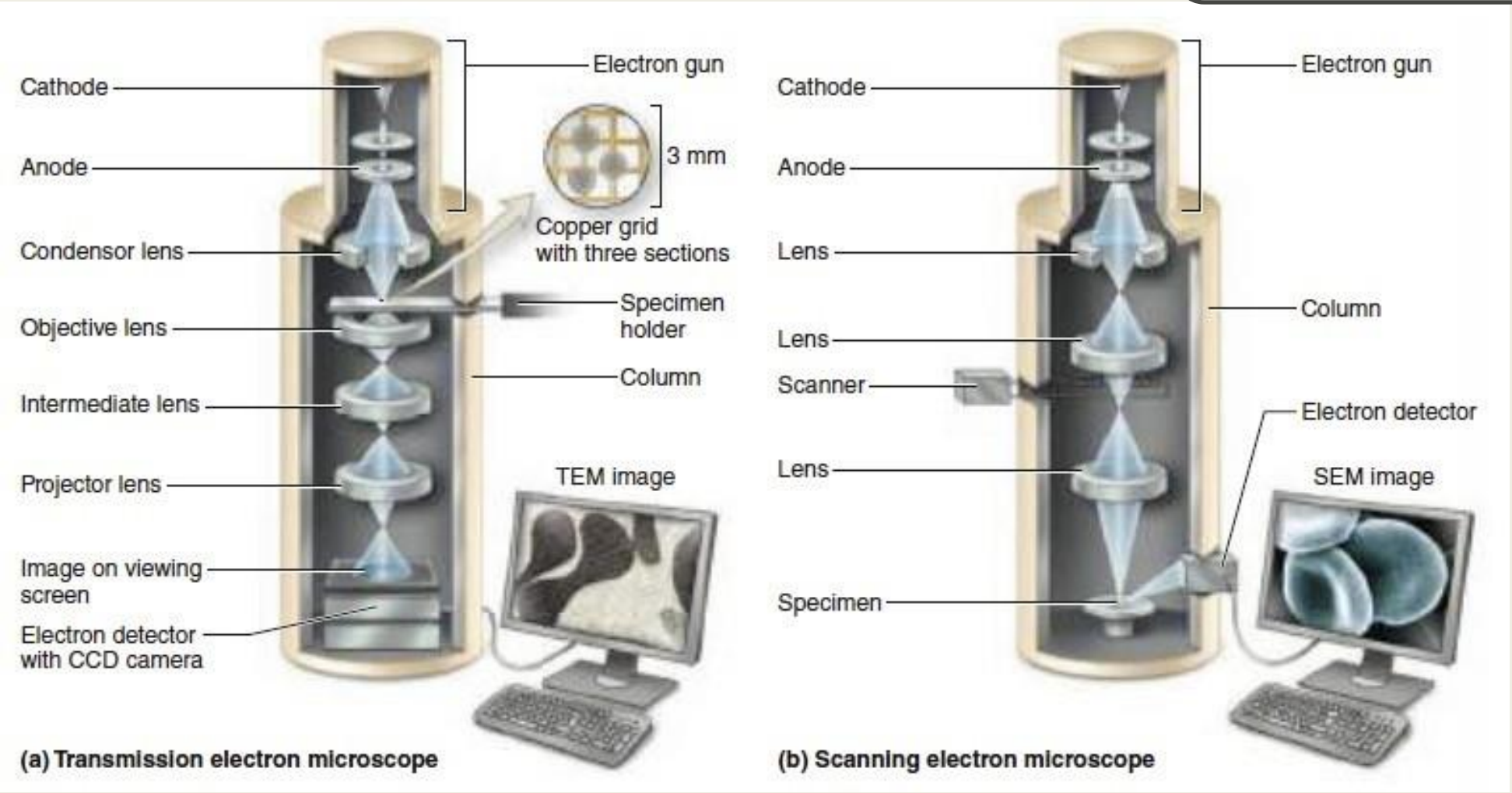
Electron microscope

Now how does the SEM allow the reaction between the beam of electrons with the tissues? now the preparation for the tissue samples differs in the TEM from the SEM and from the electron microscopes from the light microscopes
they're re totally different processes

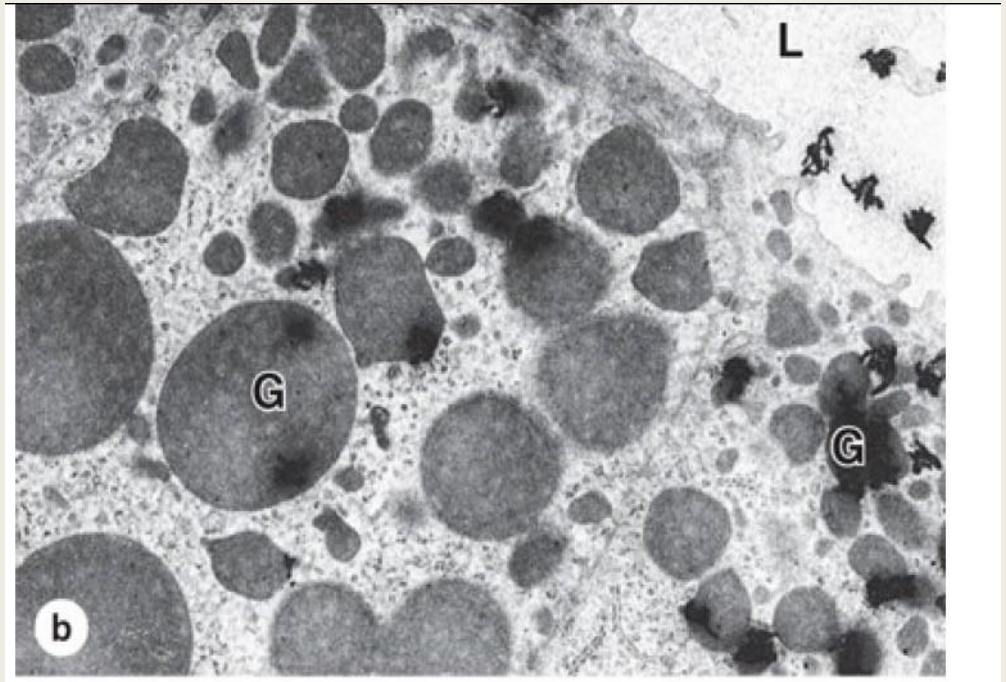
so in the SEM usually they use heavy metals which will cover the outer surface of the cells or the tissues and the most commonly used one is actually gold,
so when we direct the beam of electron toward those specimens that are covered with gold the electrons will be reflected now their reflection will vary because there is a specific topography for different cells different tissues and then the detector in the scanning electron microscope will record that as a 3D image and we usually see the images in the SEM as3D images and that's actually the way how we can tell this is a scanning electron microscope image this is a transmission electron microscope image.

Electron Microscope

- الدكتورة ما حكت شي عن هذا السلايد
- **The doctor didn't say anything about this slide**

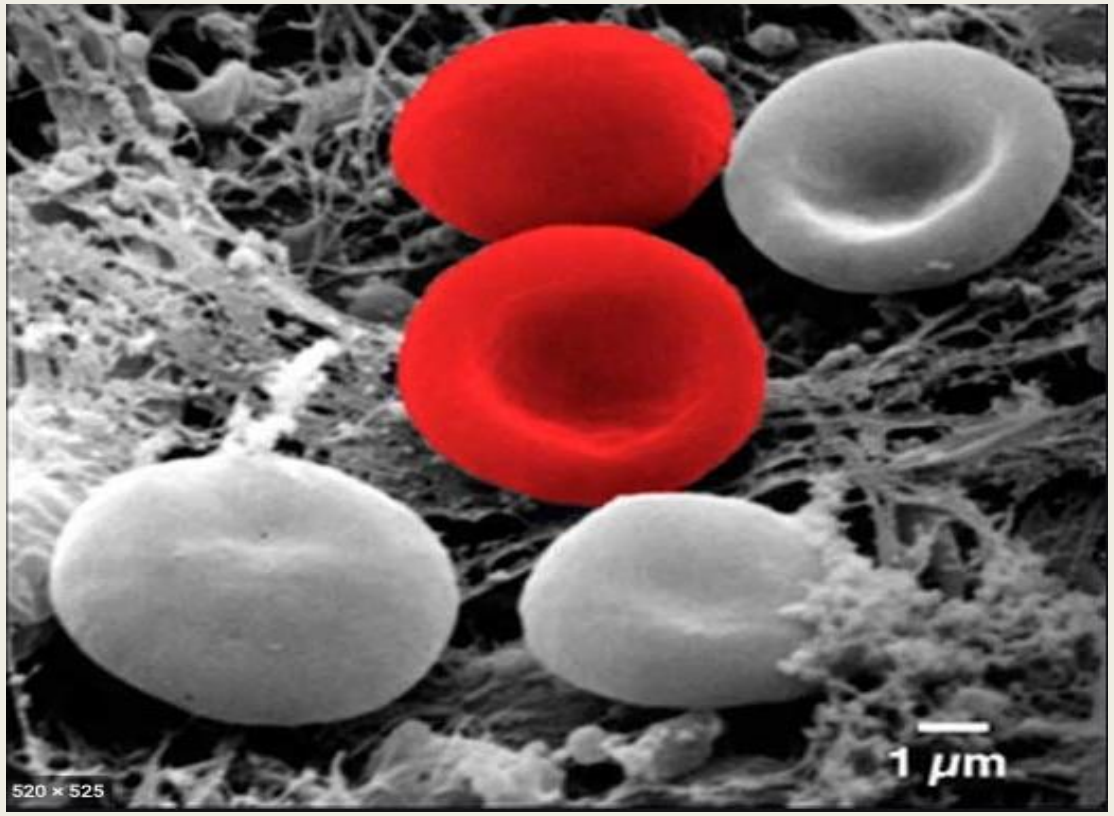


TEM

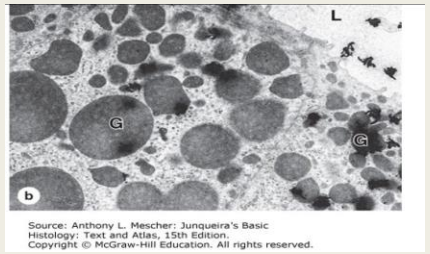


Source: Anthony L. Mescher: Junqueira's Basic Histology: Text and Atlas, 15th Edition.

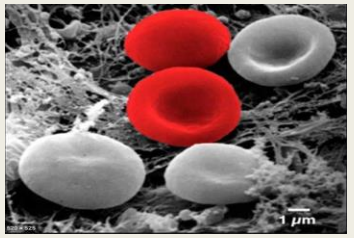
SEM



TEM



SEM

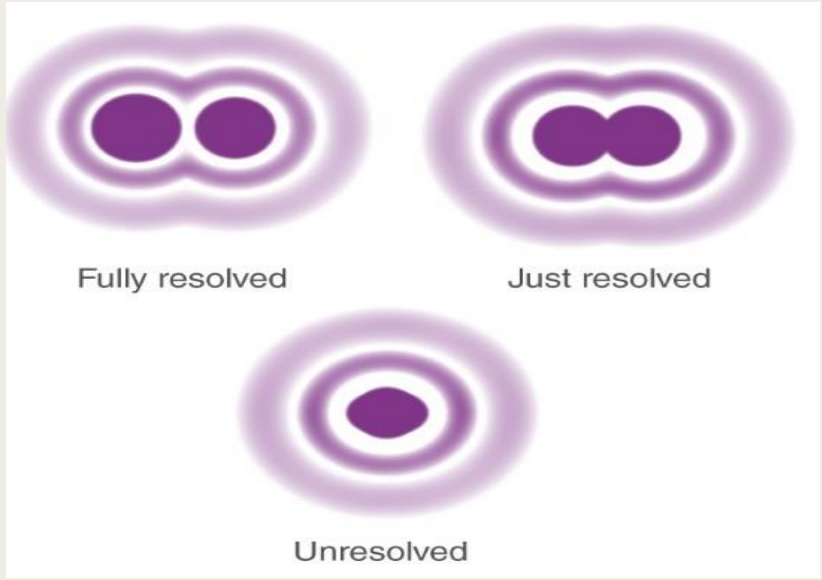


to the one on the left that's a 2d as you can see (TEM) black white and shaded gray, but I see red here (in SEM image) is this accurate? Yes it is accurate, but this red color actually came from photoshop so you can photoshop it and color it (and this is RBC by the way a red blood cell) so look at the image that we obtained from the transmission electron microscope (TEM) you can see easily how finely detailed this images this is simply part of a cell imagine the whole thing it's from a fraction of the cell it shows G stand for granules various sizes and all these fine elements or molecules that they are within the cytoplasm of the cells, so just by looking at this image you can appreciate how finely detailed this image is,

Now there's something that we need to discuss and that's actually one of the reasons that we are not satisfied with the light microscope and we needed a more sophisticated microscope a microscope that gives you more detailed images and this is called resolution.

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.
- 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).

Resolution

What is resolution?
it's how finely detailed the images that we're looking at, if we are zooming inside the image we still get good images because we're getting more and more information, (it is simply the difference between using an older smartphone and the newer generations, the older phones when you use it to take an image if we zoom in we lose the clarity of the image it becomes more hazy whereas the newer phones because they are loaded with bare lenses when we zoom in we still get good images a clear images and that actually stems from resolution, so resolving power
what is the resolving power?
it is simply the smallest distance between two structures these two structures and that is the smallest distance that we can see these two as two
that probably doesn't answer your questions so what does that mean ?

Resolution

What this means Is that each microscope has a certain resolving power which is 0.2 micrometer for the light microscope for the best microscope for the best light microscope we have
so if the distance between these two points is 0.2 micrometers or higher you will be able to see them as two objects if the distance is less than 0.2 micrometer these images will appear touching and that's called just result and if it is way smaller than 0.2 micrometer they will appear as one object or one structure,
so it will appear as unresolved so when scientists realize that the light microscopes are way less in terms of the resolving power they came up with the idea that we cant utilize electrons to visualize tissues and cells because the wavelength of the electrons it's way smaller so it will enable us to see a finer structures within the cells or the tissues as two separate objects because we can have or because we have a higher resulting power, how high? It is simply 3nm.
So you can imagine if these two objects have 3nm or higher you can see and you know the relation between the micrometers and nanometers there is a 10^6 between these two so that means we will get a much better resolved images.

V2 : SLIDE 3 (PAS) instead of trichrome