

Lecturer Talks During Lecture + Slides

- 1- The main difference between histology and anatomy is: Histology: it describes our body at micro level so when we want to study histology we need microscopes, Anatomy: is the study of the structure of the (human) body of what can be seen with the naked eye
- 2- The **cell is smallest structural and functional unit in our body**
- 3- The extra cellular matrix hold cells to together
- 5- **Tissue is made of extra cellular matrix + cells** that are similar in function and structure that work together to perform specific activity
- 6- **Organs made up of tissues** that work together to perform a specific activity
- 7- **System is made up of two or more organs** that work together to perform specific function for the organism
- 8- **Basic tissues in our body: 1- epithelial tissue 2-muscular tissue 3-nervous tissue 4-connective tissue**
- 9- Any organ is covered by epithelium and any hollow is lined with epithelium
- 10- The main function on epithelium is protection
- 11- Epithelium hasn't any space between it cells → no space means → no blood vessels → no food → how epithelium get food and expel it wastes → it needs another tissue to support it → Connective Tissue supply epithelium with food and get it's wastes out
- 12- Also connective tissue support epithelium tissue
- 13- **Magnification is enlargement of specimen** using series of lenses and we express magnification by how many times I enlarge the specimen (for instance 100x means that this lens will enlarge our specimen into 100 times)
- 14- Resolution indicates how sharp the image is.
- 15- When we say that this microscope has resolution limit of 200 micrometer it means that the minimal distance between two separate points you still can resolve them as two separate points is 200 micrometer
- 16- **Resolution is the minimum distance between two separate points you still can resolve them as two separated points**
- 17- Resolution depends on several properties such as (glass you are using, lens, magnification power of lens, microscope, and source of light you are using (wavelength))
- 18- The major factor that resolution depends on is wavelength of light source you using (for instance if you using light source of 200nm wave length then the best resolution of this light you can get is 200nm) ****q****
- 19- If you want to see object that it size is less than 200nm OR it's 200nm away from you then you have to use source that has a higher energy and lower wave length than 200nm
- 20- Contrast is the ability to differentiate light intensity between the image and the adjacent background
- 21- Another definition for **contrast is the difference in light intensity between the image and the adjacent background**
- 22- In microscopes there are two types of light phases one is from a background and the another from the specimen → if we have a lenses that can differentiate between these two phases of light then we will have a clearer image → this method is lenses method → Figure 1
- 23- Another way to get clearer image of specimen is to dye it with pigments see Figure 1
- 24- If we want to see tongue tissues → firstly we will cut it → it is very thick → we have to section it (slice) because we want a very very thin section so the light can transmit it so we can see it clearly

25- In order to get a very thin sections there are different methods but the basic principles are similar hardening and sectioning of the tissue (examples paraffin and freezing techniques)

Micro techniques (steps) for paraffin technique:

26- 1st step **Fixation: Exposing the tissue to chemical agents (fixatives) to preserve biological material as close to its natural state as possible** → **Common fixatives: paraformaldehyde (light microscopy) glutaraldehyde (electron microscopy)** → how paraformaldehyde & glutaraldehyde do fixation → stabilization and cross linking between specimen proteins

27- 2nd step **Dehydration: The process to remove the water and replace it with alcohol (using a graded series of alcohol)**

28- The main reason behind using alcohol in 2nd step (Dehydration), ~as I will discuss in 3rd step~ is using non polar solvents (Xylene for LM & propylene oxide for EM) which is hydrophobic. But at the same time we have a lot of water inside our cells so, we dehydrate cell and replace water with alcohol because alcohol can mix with water and mix with nonpolar solvents

29- 3rd step **Clearing: Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium (e.g. Xylene for LM & propylene oxide for EM)** ~Xylene is a type of wax~

30- ~As I have discussed in point #22~ we want a very thin sections so we have to solidify the specimen to make it possible and easier to slice it into a very thin sections

31- In order to solidify our sample we use wax

32- 4th step **Infiltration: The process of replacing the clearing agent with a material that can harden to support biological tissue (e.g. paraffin wax for LM & resin for EM)**

33- 5th step **Embedding: Making the mold (external)**

34- 6th step **Sectioning: To cut the mold into a very thin sections using Ultramicrotome for Electron microscopy and Microtome for Light microscopy (Ultramicrotome give us thinner sections than Microtome)**

35- 7th step **Mounting: The process to place (mount) the tissue sections on the adhesive coated glass slides**

36- We use heated water (water bath) at 50 Celsius degree (Wax melting point 60 Celsius degree), the idea behind this is to remove air bubbles (iron section)

37- **We use freezing technique rather than paraffin technique in urgent cases** because paraffin technique may take (16 hours) but freezing technique (e.g. When the doctor removes the cancerous tumor and he wants to make sure that no cancerous cells remain, he takes a biopsy → quick technique required to give him an idea about case → freezing technique is used)

38-tissues are frozen using liquid nitrogen and then they are sectioned by cryostat 39-**freezing technique is faster than paraffin technique but it may lead to a false positive judgement**

Staining techniques:

42- The stain is a chemical substance which reacts with certain tissue components producing a color

A) **Ordinary stains:** we use it commonly in histology

B) **Immunohistochemistry and Immunocytochemistry**

43-**Examles of stains:**

44- **A-Standard stain** (the most commonly used in histology) (dye) : Examples (Hematoxylin & Eosin) ,PAS, Ag, Aldehyde fuchsin,

Orcein B-Periodic Acid Schiff C-Masson's Trichrome D-Orcein E-Silver Stain

45- Till now we will focus on **Hematoxylin & Eosin**

A) **Hematoxylin: It is Blue, positively charged and basic stain. It attracts Basophilic negatively charged objects like DNA\RNA\Ribosomes\RER in nucleus so it called nuclear stain.**

B) **Eosin: It is a pink, negatively charged and acidic stain. It attracts positively acidophilic charge objects like proteins\ collagen\cytoplasm. See figure 2**

46- Epithelium cells are illustrated in figure 2 (pink represents cytoplasm and dark blue (violet) represents nucleus.

47-**Immunohistochemistry:**If you want to distinguish the presence of type of cells in a specimen (let's say cancerous) → we know that each type of cell have a marks or tags on its surface (proteins) (antigen) and these antigens is unique → if I find a way to tell the presence of proteins (antigens) then we will be able to tell that the cell is present → this method is called Immunohistochemistry

48- What we do in Immunohistochemistry is finding an anti-body that is able to attach to the antigen 49-

~Note antigen is a part of protein~

DIRECT METHOD:(primary anti-body)

50- We apply (add) a stained anti-body that is specific to the antigen of cancerous cell on the specimen, **see figure 3**

51- **INDIRECT METHOD:** If you want to distinguish a type of cells in a specimen (for instance cancerous) and its present is rare → We apply (add) specific anti-body (for instance made by goat) that is specific to the antigen of cancerous cell → then we add another type of stained anti-body (for instance made by donkey) that is specific for goat antigen (can't be attracted to anything else the antigen of goat anti-body) → more than one of stained anti-body made by donkey attach to one antigen of the anti-body made by goat → so we get **higher light intensity** → this method is called Indirect method (primary and secondary antibodies) **see figure 4**

Note: you don't have to memorize the examples in the immunohistochemistry ; just look at the figures and understand them.

TYPES PF MICROSCOPIES

I-Light Microscopy

a- **Transmitted light** : ~I have already discuss it above but it I will mention what slides tell us~

- 1- **The basic functional unit consists of a tube; having an objective lens at one end and an ocular lens at the other**
- 2- **The objective lens enlarges the image of the object in the direction of the ocular lens**
- 3- **The ocular lens further magnifies this image toward the observer's eye**
- 4- **The total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses**

b- **Phase contrast:** ~I have already discuss it above but it I will mention what slides tell us~

- 1- **It uses a lens system that produces visible images from transparent objects**
- 2- **The structures appear lighter or darker relative to each other**
- 3- **The light changes its speed and direction when passing in different media**
- 4- **Useful in tissue culture**

c- Wide-Field Fluorescence

- 1- Uses ultraviolet light
- 2- When certain fluorescent substances are irradiated with ultra violet light, it emits light
- 3- They appear as shiny particles on a dark background
- 4- Placed in dark room
- 5- ~see Figure 5 (just for understanding) (not included)(I put it from Google)~
- 6- We use it when we want to see a specific object which have a specific wavelength see figure 6

d- Confocal

- 1- Uses laser beams
- 2- the laser can be moved (scanned) across the specimen as well as down into the specimen, it can produce 3D images
- 3- Can be used in living and cultured cells and tissue sections
- 4- See figure 7

2- Electron Microscopy features

- i. Uses electron beams instead of light
- ii. Provides the highest resolution of subcellular structures
- iii. Electromagnets to focus the electrons (versus glass lenses to focus the light)
- iv. Detect by fluorescent screen or photographic emulsion
- v. Requires ultrathin sections (0.02-0.1 μm)
- vi. Uses hard epoxy resin for embedding instead of paraffin wax
- vii. Ultrathin sections are produced by Ultramicrotome (Diamond or Glass knives)

And its types are :

A- Transmission EM

- 1- Views the ultrastructural details in shades of gray
- 2- The bright areas of the images are unstained (the electrons passed through the sample) and the darker regions are areas which have taken up stain and either absorbed or scattered the electrons. See figure 8

B- Scanning EM

- 1- Provides information about the surface of a specimen
- 2- Samples are coated with a gold-carbon film. The electron beam is then scanned across the specimen surface and the electrons that are reflected off of the surface are captured by the detector
- 3- Views only the structure as a 3D image. See figure 8



Figure 1

(a)

(b)

(c)

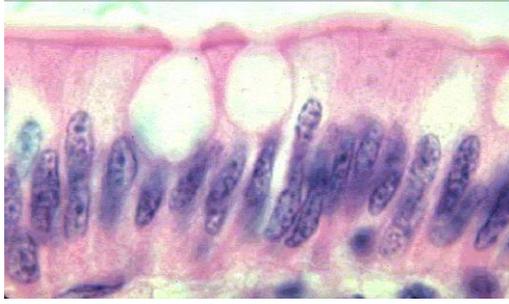


Figure 2

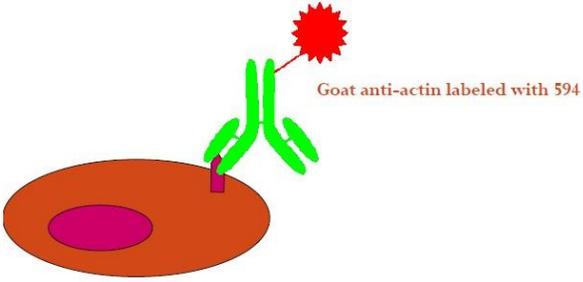


Figure 3

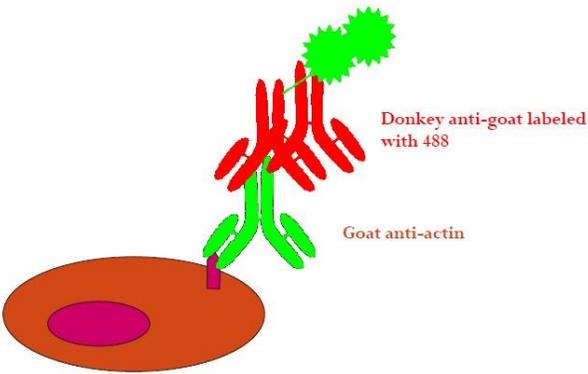


Figure 4

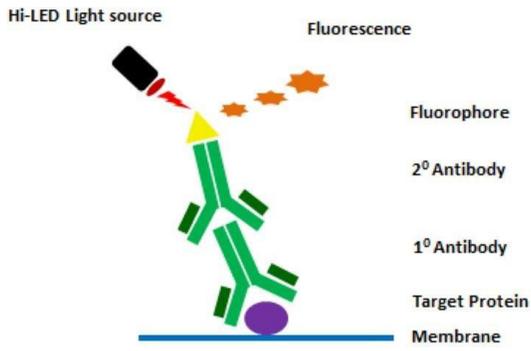


Figure 5

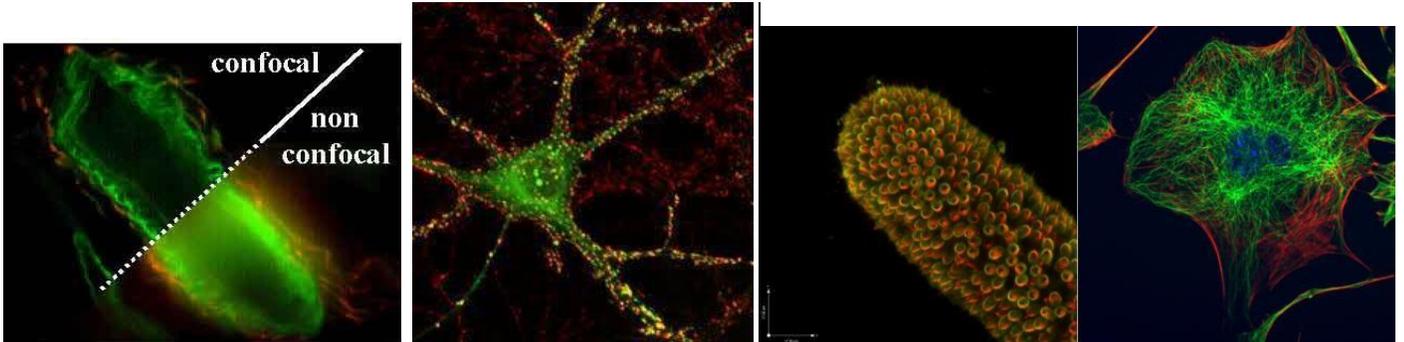


Figure 7

Figure 6

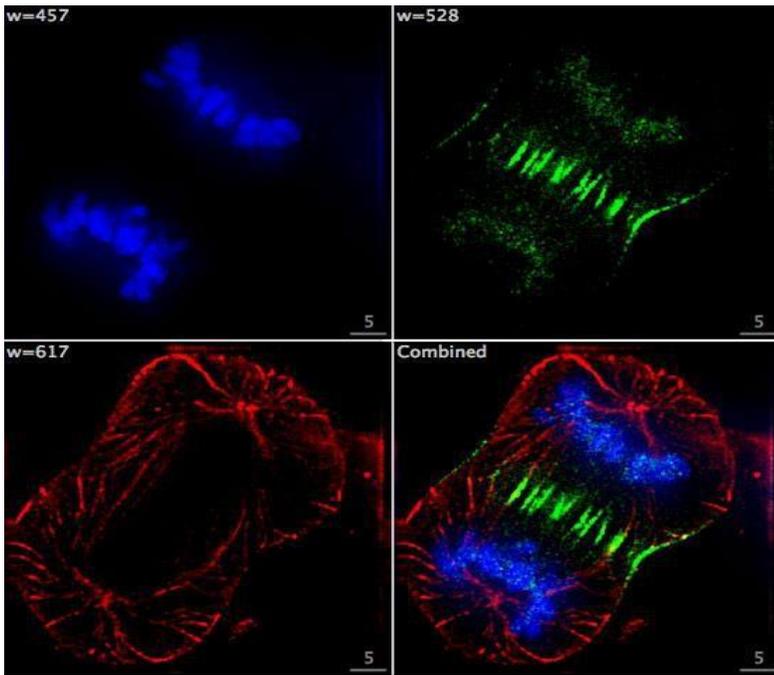
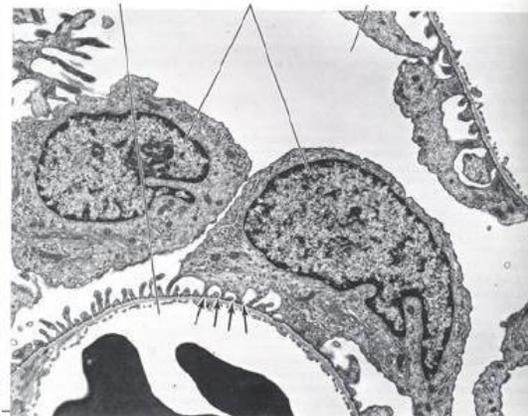
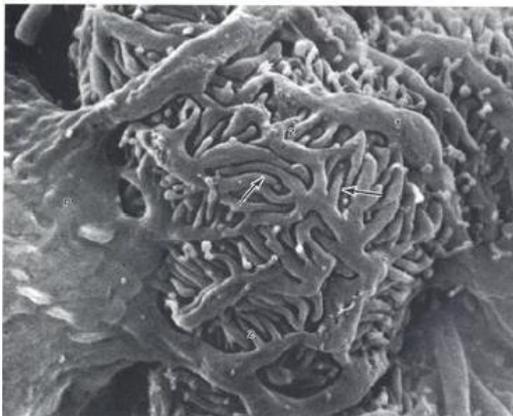
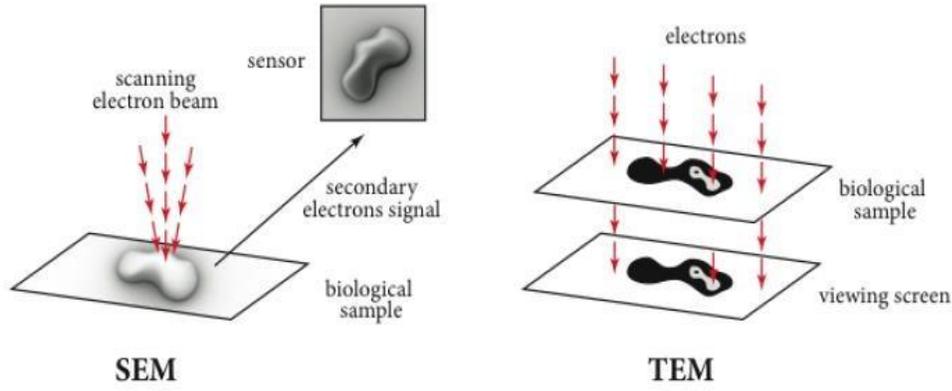


Figure 8



Comparison between LM and EM (from slides):

	Light microscope	Electron microscope
Image	Color images	black and white images
Images produced by	Visible light rays	Electron beam
Magnification	up to 1500x but a wider field of view and easier orientation	Up to 2,000 000x
Resolution	Resolving power to 0.25 μ m	Resolving power to 0.2nm
Time	Frozen sections can yield an image within 20 minutes	One day at least
Section thickness	Ranges from 1-30 μ m	Ranges from 0.02-0.1 μ m
Specimen placed on	Glass slide	Copper mesh

IMPORTANT NOTES:

1st any things between ~ is my talk and lecturer didn't say it, I have just put it for understanding

2nd In my opinion this report is adequate for understanding & mark purposes but I recommend you to take a look at some photos in slide

3rd When I was writing this report I have seeked to absolute accuracy and no mistakes but it may contain mistakes. So if you want to study it study it at your own risk, and if you find any mistake please tell me about it

ملاحظات مهمة

اولا اي كلام موجود بين علامتي ~ فهو كلام لم يقله المحاضر وانما وضعته لغاية الفهم ثانيا في رأيي فان هذا التقرير كاف

للفهم ولغايات الامتحان ولكن انصح بالاطلاع على بعض الصور في السلايدات ثالثا عندما كتبت هذا التقرير تحريت الدقة

المطلقة واللا خطأ لكن قد يحتوي على الاخطاء. لذلك اذا كنت تهتم بدراسته فانك تتحمل كامل المسؤولية. وان وجت اي خطأ

ارجو اخباري بذلك. (جعفر الشرباتي).

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