بسم الله ارحمن الرحيم

Sheet Information

Subject: Histology 1st And 2nd Lecture Sheet (1st And 2nd Lecture In The 1st Week)

Lecture Date: 1st Lecture: 22/1/2019, Tuesday |||| 2nd Lecture: 23/1/2019, Wednesday

Lecturer, Record And Section: The 1st And 2nd record for Dr. Hanan Jafar in the section number 2

What Lecturer Say 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- We study normal structure of body in order to be able to know the function and to be able to tell the abnormal structure
4- The extra cellular matrix hold cells to together
5- We call (extra cellular matrix + cells) : Tissue
6- Organs made up of tissues that work together to perform a specific activity
7- Multiple organs that work together to perform a specific function is called System
8- We have only four types of tissues in our body
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 is how the image is sharp
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
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)

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
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 then sections there are different methods but the basic principles are similar hardening and sectioning of the tissue (examples paraffin and freezing techniques)
26- We will discuss freezing later on

Micro techniques (steps) for paraffin technique:

27- In order to preserve cellular structure ➔ we use preservative material ➔ the other important thing that we have to do it is fixing our specimen because we need protein to stay and not to denature (degrade)
28- 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) gluteraldehyde (electron microscopy)➔ how paraformaldehyde & gluteraldehyde do fixation ➔ stabilization and cross linking between specimen proteins
29- 2nd step Dehydration: The process to remove the water and replace it with alcohol (using a graded series of alcohol)
30- 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
31- 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 non-polar solvents~
32- 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
33- In order to solidify our sample we use paraffin wax
34- 4th step Infiltration: The process of replacing the clearing agent~(nonpolar solvents (e.g. Xylene))~ (inside the cell) with a material that can harden to support biological tissue (e.g. paraffin wax for LM & resin for EM)
35- 5th step Embedding: Making the mold
36- 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)

37- 7th step Mounting: The process to place (mount) the tissue sections on the adhesive coated glass slides
38- 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)
39- We use freezing technique rather than paraffin technique in urgent cases because paraffin technique may take (5-7 days) but we use 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)
40- We don't use freezing technique in diagnoses but we use it in quick decision
41- Frozen tissues are sectioned by cryostat

Staining techniques:

42- The stain is a chemical substance which reacts with certain tissue components producing a color
43- Types of stains:
    A) Ordinary stains: we use it commonly in histology
    B) Immunohistochemistry and Immunocytochemistry
44- Standard stain (the most commonly used in histology) (dye) : Examples (Hematoxylin & Eosin) ,PAS, Ag, Aldehyde fuchsin, Orcein
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 nucleus 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- But white?? ➔ There was mucus but during preparation it was removed

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~
50- We apply (add) a stained (red) specific anti-body that can't be attracted to anything else the antigen of cancerous cell on the specimen ➔ then we took a look on specimen and found a red color ➔ it means that we have a cancerous cells. ➔ We call this method direct method (primary antibody only), see figure 3
51- If we want to distinguish a type of cells in a specimen (for instance cancerous) and its present is very small ➔ what we can do ➔ We apply (add) specific anti-body (for instance made by goat) that can't be attracted to anything else 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

Types of microscopy

1-Light Microscopy

A- Transmitted light : ~I have already discuss it above but it I will mention what slides tell us~
- The basic functional unit consists of a tube; having an objective lens at one end and an ocular lens at the other
- The objective lens enlarges the image of the object in the direction of the ocular lens
- 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

B- Phase contrast: ~I have already discuss it above but it I will mention what slides tell us~
- It uses a lens system that produces visible images from transparent objects
- The structures appear lighter or darker relative to each other
- The light changes its speed and direction when passing in different media
- Useful in tissue culture

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

D- Confocal ~(lecturer didn't say anything about this microscopy so I will put what slides tell only)
- Uses laser beams
- the laser can be moved (scanned) across the specimen as well as down into the specimen, it can produce 3D images
- Can be used in living and cultured cells and tissue sections
- See figure 7

2- Electron Microscopy features

- Uses electron beams instead of light
- Provides the highest resolution of subcellular structures
- Electromagnets to focus the electrons (versus glass lenses to focus the light)
- Detect by fluorescent screen or photographic emulsion
- Requires ultrathin sections (0.02-0.1 μm)
- Uses hard epoxy resin for embedding instead of paraffin wax

- Ultrathin sections are produced by Ultramicrotome (Diamond or Glass knives)

And its types is

A- Transmission EM

- Views the ultrastructural details in shades of gray
- 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

- Provides information about the surface of a specimen
- 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
- Views only the structure as a 3D image. See figure 8

52- Differences between light and electron microscope In figure 9

Figure 1

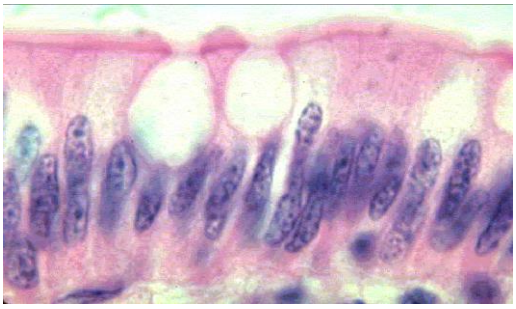

Figure 2

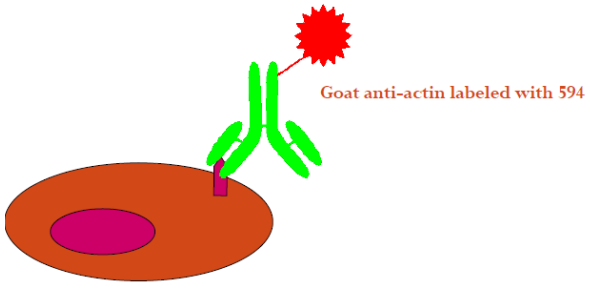

Figure 3

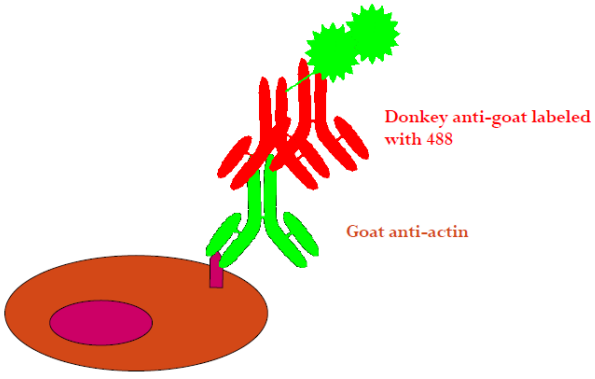

Figure 4

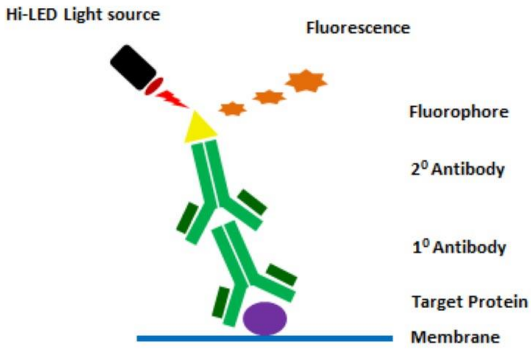

Figure 5

Figure 7

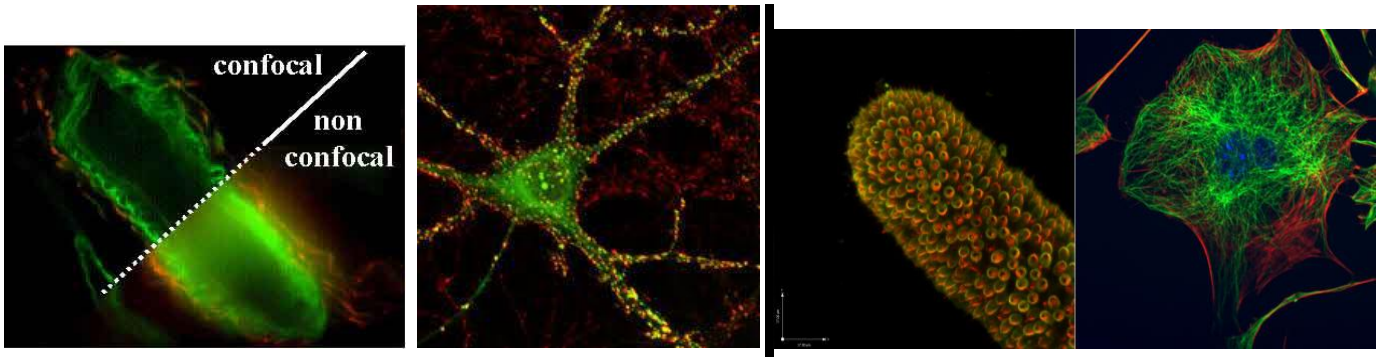

Figure 6

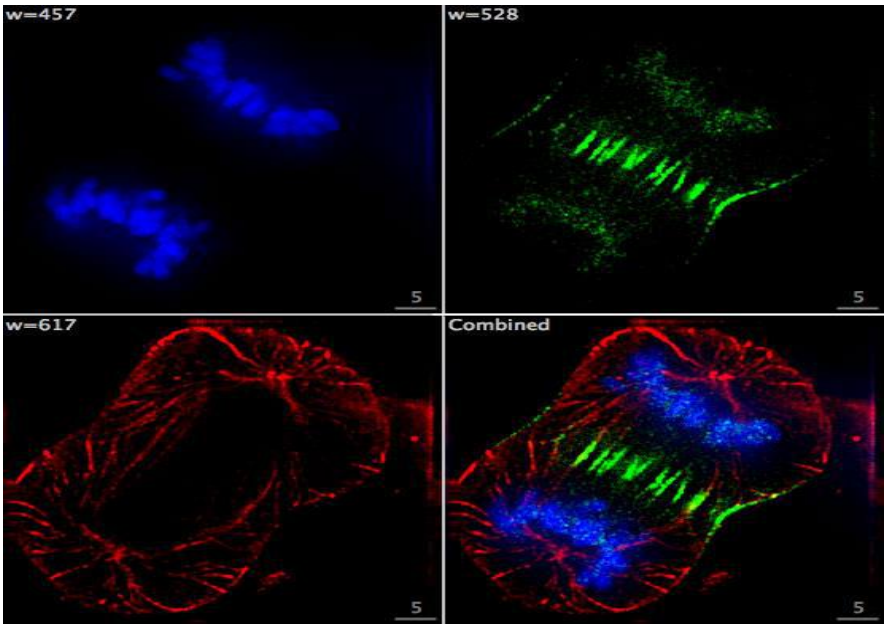

Figure 8

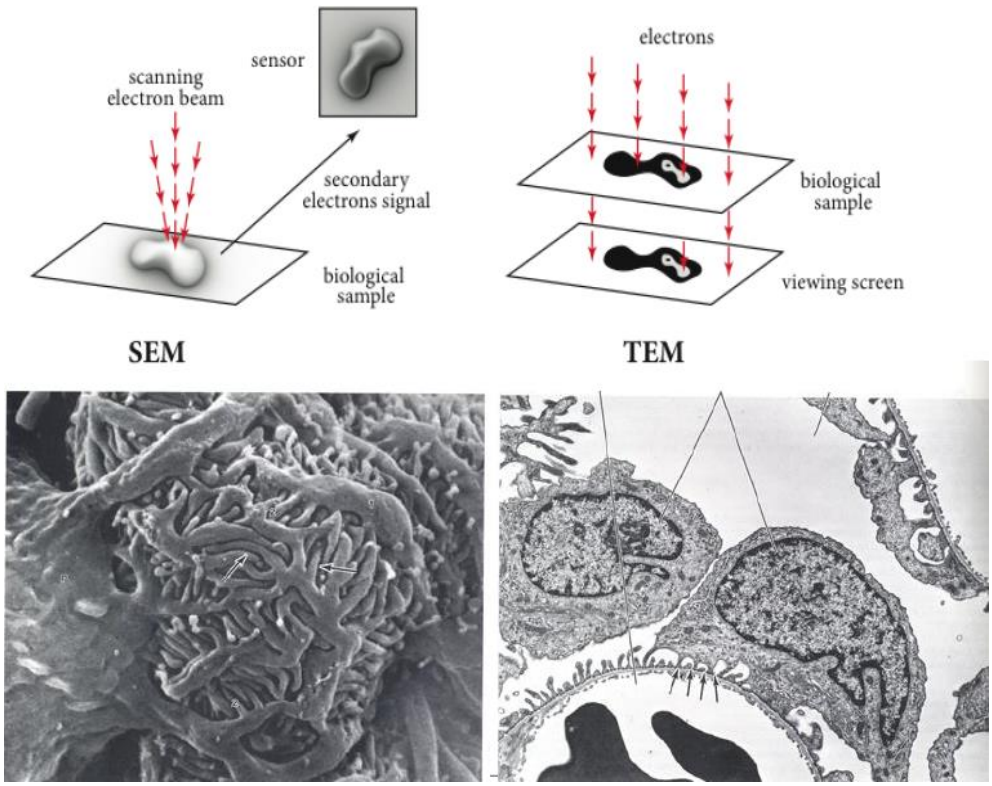

Figure 9

	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 0.25µm	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 µm	Ranges from 0.02-0.1 µm
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 sheet is adequate for understanding & mark purposes.

3rd.This report is corrected by Dr. Hanan Jafar and Jafar Sharabati

Written by: Jafar Sharabati

The End