**2nd lecture #

# When we finished later from the preparation of the slides ---🡪 Now we put these slides under the microscope to study them ☺ ---🡪 Because that we should study all the types of microscopes to use the appropriate microscope to each slide .

# The types of microscope #

1- Light microscopy ( Optical microscopy) :
 we will talk about Bright field microscopy - Fluorescence microscopy – Phase contrast microscopy – Confocal microscopy – Polarizing microscopy .

a) Bright field microscope ( Students microscope) – The highest magnification of this microscope is X1000 .
# the stained preparations are examined by means of ordinary light that passes through the specimens .
# This microscope includes an optical system and mechanisms to move and focus the specimen ((( The optical components consist of three lenses ) .
- The condenser : collects and focuses a cone of light that illuminates the objects to be observed .
- The objective lenses : enlarge and project the image of the light in the direction of the eyepiece
- The eyepiece ( ocular lens ) : a further magnifies this image and projects it onto the viewers retina or a charge-coupled device (CCD) with monitor and camera**

 **CCD : is highly sensitive picker to the low levels of light ☺

 # The eyepiece magnification = X10 -----🡪 objective lenses = x4 Or x10 Or x40 ☺
# The total magnification is obtained by multiplying the magnification power of objective lens and ocular lens .
 # usually in this microscope when we see in the objective lenses ( in the resolving nosepiece ) a sign : X100 or word : Oil ----🡪 it refers to the Oil immersion lens which has X100 times magnification ( Remember that from the bio lab ☺ ) .
 # The critical factor in obtaining a detailed image in this microscope is" Resolving power" that means the smallest distance between two particles at which they can be seen as separate objects .
# The maximal resolving power of LM is 0.2 micrometer ( A power that permits good images magnified 1000-1500 times ) .
# Objects smaller or thinner than 0.2 Mm such as ( Ribosome – a membrane – filament of actin ) cannot be distinguished with this microscope ☹ .
# The quality of the image depends on the microscope resolving power ---🡪 the magnification is of value only when accompanied by high resolution ----🡪 The resolving power of a microscope depends mainly on the quality of its objective lenses ----🡪 The eyepiece enlarge only the image obtained by the objective lens ( it does not improve the resolution ) ------🡪 For this reason , when objectives of different magnifications are compared , those providing higher magnifications providing higher resolving power .

 # Notice that when the magnification increases means the slides must close to the used lens " bt8rrb mn el 3adasa" ---🡪 means if we move the adjustment of the microscope – in this case – to adjust the distance between the slide and the lens , the lens hit in the slide and break it ☹ .
# The eyepiece magnification = X10 -----🡪 objective lenses = x4 Or x10 Or x40 .

But when we use this microscope for research maybe the eyepiece = x16 or x17 or x20 ----🡪 But because the eyepiece magnification is less than the objective lenses magnification -----🡪 the image – in this case – does not totally appears. ( e3ni msh wa9`7a kteer ☹ ) therefore the last magnifications of the eyepiece = x16 or x17 or x20 are rarely used .

# We use Binocular microscope for researches taught by two researchers at the same time ( means : each one studies from his side ) ----🡪 This microscope contains " laser pointer " to determine the structures on the slide studied ) ----🡪 also it contains " camera lucida : The *camera lucida* performs an**[**optical**](http://en.wikipedia.org/wiki/Optics)**superimposition of the subject being viewed upon the surface upon which the artist is drawing. "
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# The ten-heads microscopy ( used by many medical students at the same time to study same slide )
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# is an**[**optical microscope**](http://en.wikipedia.org/wiki/Optical_microscope)**that uses**[**fluorescence**](http://en.wikipedia.org/wiki/Fluorescence)**and**[**phosphorescence**](http://en.wikipedia.org/wiki/Phosphorescence) **(( instead of / or in addition to))**[**reflection**](http://en.wikipedia.org/wiki/Reflection_%28physics%29)**and**[**absorption**](http://en.wikipedia.org/wiki/Absorption_%28optics%29)**to study properties of organic or**[**inorganic**](http://en.wikipedia.org/wiki/Inorganic)**substances.

 # ( What Doctor said in this microscopy )#

 talking about this point is repeated in the epithelium according to what Dr said ☺

 # certain structures in the body are described as antigen-ices (means---🡪) when they are injected in the body of the human or animal they will form antibodies in response to these antigenic .
# We should Know that these antibodies are specific --🡪 therefore they attack only the antigen-ices formed them
 # In this microscope if we labeled the antibodies (formed firstly in cell ) with fluorescence substances and put these antibodies on another cell to form "antigenic- antibodies reaction" -----🡪 means ☺ ( this cell is similar to the 1st cell ) Because the antibodies are specific .**

**# How do we know that the "antigenic- antibodies reaction" take place ?
when we see the fluorescence ( light ) exit from the slide studied by fluorescence microscopy ☺

# when certain cellular substances are irradiated by light of the proper wavelength , they emit light with longer wavelength -----🡪 This phenomena is called Fluorescence .
# In this microscopy the tissue sections are usually irradiated with Ultra violet (U.V) light and the emission is in visible portion of the spectrum .
# The fluorescent substances appear brilliant on a dark background ( For this method , the microscope has strong U.V light source and special filters that select rays of different wavelengths emitted by the substances ) .
# This microscope commonly is used immunohistochemistry and contributed to the evolution of the histology (Because ---🡪 ) it is accuracy due to the antibodies used are specific in the " antigenic- antibodies reaction" .
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 **# Mainly it is light microscope----🡪 used to study unstained cells and tissue sections which are usually transparent and colorless
# Cellular details are normally difficult to see in unstained tissue because all parts of the specimen have roughly similar optical densities .
# This microscope can be used with living , cultures cells .
# This microscope is based on the principle that " light changes its speed when passing through cellular and extracellular structures with different refractive induces " ----🡪 these changes are used by this microscope to cause the structures to appear lighter and darker in relation to each other .

# In all the info above we should know :
1) ( the light of this microscope if easily penetrate the regions of specimen that means these regions will appear lighter ( No organelles in these region " only normal cytoplasm which is proteins most of the times )
2) ( the light of microscope if difficulty penetrate the regions of the specimen that means these regions will appear darker ( contain organelles such as mitochondria or lysosomes)
 3) By this microscope we can know the organelles ( present or not ) and their sizes , distributions ------🡪 But we cannot know the details such as (If the mitochondrion has membrane or not ) Because this microscope gives general description Not more ☹ .
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# It differs from the Bright –field microscopy in :
 1) the confocal microscopy has computer driven mirror system .
- It has computer driven mirror system (the beam splitter ) to move the point of illumination across the specimen automatically and rapidly
- Digital images are captured at many individual spots in a very thin plane of focus are used to produce an " optical section " of the plane --🡪 Creating such optical sections at a series of focal planes through the specimen allows them to be digitally reconstructed into a 3D image .
2) the source of light is different between two microscopes .

 CONCENTRATE OK ☺

with a Bright-field microscopy , the beam of light is large and fills the specimen --🡪 excess light reduces contrast within the image and the resolving power of the objective lenses ☹ ( Disadvantage point in Bright-field microscopy ) . BUT confocal microscopy avoids these problems and achieves high resolution by :
1) a small point of high-intensity light from a lazar.
2) a plate with a pinhole aperture ( hole ) in front of the image detector .
--🡪 The point light source, the focal point of the lens , and the detectors pinpoint aperture are all optically conjugated to each other in the focal plane (confocal) , and the unfocused light does not pass through the pinhole .

# The details of the specimens do not appear in this microscope But the cytoskeleton of the cell will appear .
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E) Polarizing microscope :
# rarely used in medicine ----🡪 it is used by the workers in the heavy metals, rocks and crystals ( Because they concentrate their studies on the regular arrangements of certain molecules ) .
# it is used to recognize of stained or unstained structures made of highly organized subunits ---🡪 when the light passes polarizing filter , it exit vibrating in only one direction ( IF we put a second filter above the first one , with its main axis perpendicular to the first filter --🡪 No light passes through .
- if tissue structures - that contain oriented macromolecules - are located between two polarizing filters , their repetitive structure rotates the axis of the light emerging from the polarizer and they appear as bright structures against a dark background .
- Birefringence : is the ability to rotate the direction of vibration of polarized light , and is a feature of crystalline substances or (substances that contain highly oriented molecules such as cellulose , collagen or actin filaments) .
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2) Electron microscopy ( We will talk about TEM and SEM )

a) TEM (transmission electron microscope )

# The 1st EM was in 1930 ☺ and was the highest magnification is X1000 ☹ .
# The EM in our faculty magnifies until X500000 ☺ .
# All the steps in the preparation of the slide in LM are same in EM ( The preparation steps are same between LM and EM ) But the difference in embedding step ( we use here Resin instead of paraffin because the Resin is harder than paraffin ) ----🡪 and when start cutting we do not use microtome (with steel knife) ☹ we use Ultra-microtome (glass or diamond knife) ☺ .

# The thickness of specimen in EM ( from 0.1 micron To 1 micron ) ---🡪 this means that the specimens are so small and put them on Cupric cover.

# In the LM we used Light source But in EM we will use electron source (electron beam) that emit from the Tungsten filament .
# In the EM when the Tungsten filament is heated the electrons are emitted scattered in many directions because ( 1. the air currency in the microscope and 2. larger of Tungsten filament ) , therefore we should remove the air currency AND direct the electrons only on the sample ) .

# The (Cylinder of microscope " inside the microscope " ) serves two things :
1) It is isolated from the inside and contains several tubes within them and they contain Electromagnetic tube ( its function " Directing electrons in one direction " )
2) It is connected with Vacuum pump "its function is suction air ---🡪 therefore removing air currency " .
--------🡪 1+2 ---------🡪 The electrons hit the specimen in one direction ☺ ----🡪 when they hit the sample there are two possibilities :-
a) The electrons penetrate the specimen , the areas in the specimen appear whitish color and these areas are called " electron lucent " .
b) The electrons are reflected from the surface of the specimen , the areas in the specimen appear darkish color and these areas are called " electron dense" .
# we should notice that the color ranges from ( dark-- TO---gray--TO---white) in the areas of the specimens ----🡪 more darkish means more electron lucent and less electron dense ----🡪 more whitish means more electron dense and less electron lucent

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transmission electron microscope ) ) The disadvantage of the TEM #
In the biological samples (we do not use the magnification more than X100000 "not accurate but according to what Dr said in the lecture " ) Because when the magnification increases the heating of the filament increases therefore the emission of electrons increases that hit on the sample ----🡪 after few seconds the sample will melt and we cannot see anything under microscope ☹

b) SEM (scanning electron microscope)
# Is a type of** [**electron microscope**](http://en.wikipedia.org/wiki/Electron_microscope) **that produces images of a sample by scanning it with a focused beam of** [**electrons**](http://en.wikipedia.org/wiki/Electron)**. The electrons interact with atoms (heavy metals ) in the sample, producing various signals that can be detected and that contain information about the sample's surface** [**topography**](http://en.wikipedia.org/wiki/Topography) **and composition.
# we use heavy metals ( gold – silver – platinum ) to coat the sample by device called "Coater" .
# This microscope provides a high resolution view of the cells , tissues , and organs .
# SEM is similar TEM in all aspects ( all the info said later) BUT the only difference is (((( the sample does not need cutting ☺ )))) ----🡪 instead of cutting we put the sample on a piece of "Aluminum " and coat with double- face stuck .**

 **# The disadvantage of SEM :
if One drop of water touches the sample 🡪 the sample will desolate ☹ 🡪>>>>>>>>> therefore we put the sample- after all steps of processing - in the device called " Critical point dryer" its function is giving very hard sample.

CONCENTRATE OK !!!
بالمختصر هي القصة كاملة ☺**

**# Like TEM , this microscope produces and focuses a very narrow beam of electrons , but in this microscope the beam does not pass through the specimen , instead , the surface of the specimen is first dried ( by critical point dryer) and coated with a very thin layer of heavy metal such as gold (by Coater) , through which electrons do not pass readily >>>>> the electrons scan on the surface of the specimen and interact with the atoms of the heavy metal >>>>> therefore producing reflected electrons or secondary electrons emitted from the metal ☺
>>>>>> these reflected electrons or secondary electrons are captured by a detector and the resulting signal is processed to produce a black –and-white image on a monitor ☺ .

These are some of the differences between TEM and SEM ### ###
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• SEM is based on scattered electrons while TEM is based on transmitted electrons.
• SEM focuses on the sample’s surface and its composition whereas TEM provides the details about internal composition. Therefore TEM can show many characteristics of the sample, such as morphology, crystallization, stress or even magnetic domains. On the other hand, SEM shows only the morphology of samples.
• The sample in TEM has to be cut thinner whereas there is no such need with SEM sample.
• TEM has much higher resolution than SEM.
• SEM allows for large amount of sample to be analysed at a time whereas with TEM only small amount of sample can be analysed at a time.
• SEM is used for surfaces, powders, polished & etched microstructures, IC chips, chemical segregation whereas TEM is used for imaging of dislocations, tiny precipitates, grain boundaries and other defect structures in solids
• In TEM, pictures are shown on fluorescent screens whereas in SEM, picture is shown on monitor.
• SEM also provides a 3-dimensional image while TEM provides a 2-dimensional picture.
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ملاحظة : يجب النظر إلى جميع الصور الموجودين في الكتاب ضروري جداً لمعرفة كلّ صورة بأي مجهر تمّ دراستها ☺**