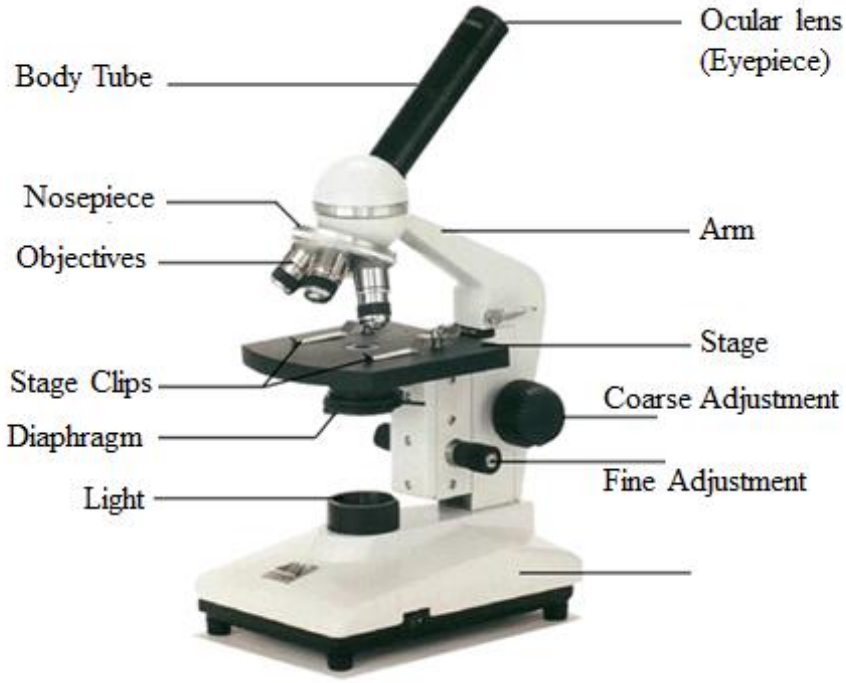
The Microscope



The Microscope is One of the most important tools used to study living things.

"Micro" means very small "Scope" means to look at.

Importance of Microscopes

1. Magnification- the power of the microscope to enlarge the image of an object
2. Resolution- the power of the microscope to show detail clearly

Classification of Microscopes

A. Classification according to number of eye pieces:

1. Monocular (simple microscope) : has just one eyepiece to look through
2. Binocular Microscope (Compound) has two eye pieces.
3. Triocular microscope has three eye pieces

B. Classification according to the source of light

1. Bright field microscope
2. Dark field microscope
3. Polarizing microscope
4. Fluorescence microscope
5. Electrone microscope
4. Phase contrast microscope
5. Stereo or Dissection Microscope

Structure of light microscope

There are three main structure groups:-

1. Mechanical structures
    a. body of microscope (tube ,arm, base, revolving piece)
    b. stage; (Fixed, mechanical stage and stage clips)
    c. 3,focus adjustment knobs: (coarse and fine)
    d. 4.diaphragm
2. **B. Optical structures** (ocular lens :eyepiece, Objective Lenses**,** Condenser lens
3. **Light source**(mirrors) or electrical bulb light

The simple Light Microscope

Microscope parts and their function.

The Microscope has Three main parts:

A. Mechanical parts

1. body of Microscope

The body is the main part of the microscope on which other parts are settled:-

It composed of four parts:-

a) Tube: hollow tube that keeps the ocular lenses and objectives at a set distance. It contain mirrors and prisms that transmit image from the objective lenses to the ocular lenses
b) Arm or neck- supports body tube ,connects it to the base , hold all microscope magnifying lenses and used to safely transport microscope
c) Base or foot Which composed of a heavy metal to give stability to the microscope , hold light source (mirror or electric) , supports entire microscope and used to safely transport microscope
d) Revolving piece (nose piece) :Is a rotating disc fixed at the lower end of the tube . It has four holes in which the objective lenses of different magnifications are fitted and can be turned to quick change of objectives to increase the magnification

2. Stage- It is a platform plate (tray-like structure) that supports slide over stage opening.

- **Mechanical stage,** able to move the slide around. It has two knobs. One moves it left and right, the other moves it up and down.
- **Stage Clips**- two clips that keep specimen/slide tight against stage. Stage has Opening to allow light to pass through and around specimen

3. Focus adjustment knobs:

consist of 2 knobs , fine and coarse adjustment nobs used for changing the distance between the object on the slide and objective lenses.

1. Coarse adjustment- larger knob, that moves the stage up and down quickly for focusing the image.
   The coarse nob must be used with low power objective lenses only.
2. Fine Adjustment- smaller knob, moves the objectives slowly and allows for fine focusing. NOTE: Never use this when viewing on high power

4. Diaphragm-

controls amount of light that passing through the slide. Usually it is better if the amount of light is low.

Many microscopes have a rotating disk under the stage. This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light that is projected upward into the slide.

B. Optical parts

Optical parts are consist of three systems:- ocular lenses, objective lenses , and condenser

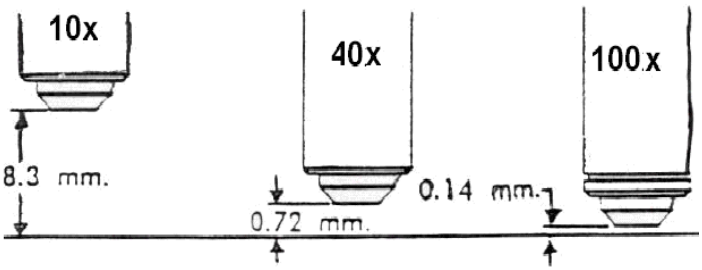
1. Ocular Lens :Eyepiece The part you look at with your eye. Usually 10X magnification.
2. Condenser lens: It is located sub stage contain lenses that focus and condenses the light from light source to tissue section . It can be raised and lowered under the stage , it must be correctly positioned in order to give even illumination.

3. The Objective Lenses These are a set of lenses screwed on the holes in the revolving disc . These lenses enlarge the image of the objet and project it to the ocular lenses increase magnification (usually from 10x to 40x). Three types of objective lenses (low power, high power and oil immersion lenses)
    a. Low Power Objective (L.P) – The first lens you use when doing proper microscope work. Usually red 4x and Medium Power Objective – Usually Blue 10 x
    b. High Power Objective. are retractable. This means that if they hit a slide, the end of the lens will push in (spring loaded) thereby protecting the lens and the slide. (Yellow 40xR)
    c. oil immersion lens It is used with special optical oil. (white lens that magnify 100x.) It makes the image clear at a higher magnification.

NEVER use the course adjustment when using this lens

Use only the fine focus knob with the 40X and 100X lens.

Distance between lens and slide



Total Magnification

- When the image travels through the objective lens and the ocular lens it magnifies the magnification of the objective lens by 10X.

Ocular lens	Objective lens	Total magnification
10X	Red 4X	40X
10X	Blue 10X	100X
10X	Yellow 40X	400X

10X	White 100X	1000X

C. Light source

Light source- usually a sun light (mirrors) or electrical bulb light. In both cases the wave length of the light is limited . Sends light up through the diaphragm and through the slide for viewing

Practical Part

The proper way to carry your microscope

Always carry the microscope with two hands, one on the arm and the other on the base holding it close to your body.

How TO USE THE MICROSCOPE

1. Clean the slide properly & put it on the stage , held in place by the stage clip
2. Switch on the light source
3. Use the smallest objective lens 4x. bring the lens down as close to the specimen as possible using the coarse focus knob without touching it. NEVER use the coarse focus higher than 4X. you'll break the slide!!!
4. Now, look through the eyepiece lens and **focus upward only** until the image is sharp. If you can't get it in focus, repeat the process again.
5. Magnify the image by changing the objective lenses to the high one 10x . Use the fine focus knob to clear the image of objective .
6. Adjust light source (if it's a mirror…don't stand in front of it!)
7. Use the special oil if using oil immersion lens. Place a small drop of oil on the slide in the center of the lighted area. (Take care not to dribble on the stage.). Put the small drop of oil directly over the area of the specimen to be Examined.
8. Always clean this lens by lens paper and xylol
9. Return back the objective lens to the smallest one
10. Take the slide off, clean it & put it in the slide box

Care of microscope

1. Keep the microscope covered when not in use

2. The microscope should always be stored with the 4X (red) lens in place and the stage in its lowest position.
3. The lens should be cleaned by cleaning solution or breathing on the lens and wiping dry with lens paper only.
4. Use lens paper with xylol or alcohol to remove the oil from oil immersion lens
5. Please use only lens paper to clean the lenses, never paper towels ..
6. Make sure it's on a flat table surface at a distance not less than 10 cm from the edge.
7. Always store your microscope with the dust cover in place.
8. Always carry with 2 hands
9. Never touch the lenses with your fingers.
10. When you are finished with your "scope", rotate the nosepiece so that it's on the low power objective, roll the stage down to lowest level, then replace the dust cover.
11. Dry the back of the slide by filter paper to avoid dust formation on the stage of the microscope

Lac:2 The Phase Contrast Microscope

Definition

Phase microscope is a type of light microscopy is an important microscopic technology in biological and medical research that intensifies contrasts of transparent and colorless objects by influencing the optical path of light, and allows one to view internal structures of live organisms cell or bacteria that are difficult to see in a basic light microscope and cell division without staining.

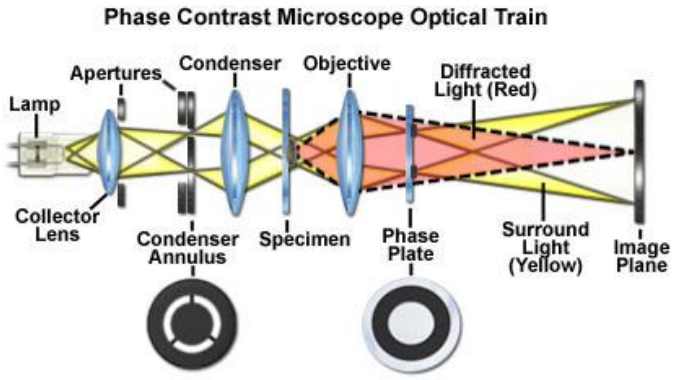
Staining often kills, and can alter specimens. In addition, stained cells are not moving, not feeding, not metabolizing, not dividing, etc.

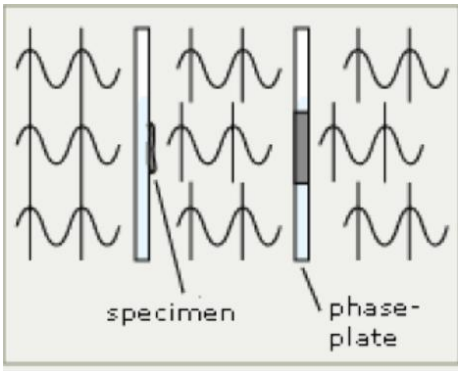
Frederik Zernike invented phase microscopy in the 1930's prior to electron microscopy, so it was an important tool and continues to be. Zernike won the Nobel Prize in Physics in 1953 for his invention.

Importance

using phase microscopy to see bacteria (and may be yeast- (a type of fungi) to observe living cells and their structures.

Principle:





1. The phase contrast microscope uses the fact that the light passing through a transparent part of the specimen travels slower and due to this is shifted compared to the uninfluenced light.
2. This difference in phase is not visible to the human eye. However, the change in phase can be increased to half a wavelength by a transparent phase-plate in

the microscope and thereby causing a difference in brightness. This makes the transparent object shine out in contrast to its surroundings.

3. If you follow the light pathway in a phase scope it first passes through a condenser annulus. Only light in parallel waves passes through the openings in the annulus and illuminates the specimen. Some light passes un-deviated around the specimen. Other light passes through the specimen and is deviated (diffracted) by the specimen.
4. Different parts of the specimen will diffract the light differently because of the relative thickness of different parts of the sample (the diffracted light is also scattered). This causes a "phase shift", because when light is diffracted it slows. The thicker the specimen , the more the light wave is diffracted and thus, slowed.
5. Un-deviated light surrounding the specimen is not slowed because it is not diffracted (no change in the medium the light is passing through), so no bending).
6. The un-deviated light and diffracted light is then collected in the objective and passes through a phase plate in the objective which alters the un-deviated light to further, enhance contrast. Ultimately the, diffracted light and un-deviated light waves, combine and are focused to form the image we see. The image depends on the intensity differences in the un-deviated and diffracted light waves, which causes contrast
7. This technique is strongly dependent on the correct alignment of components in the optical pathway.
8. By using the phase-contrast microscope, transparent and colorless components in a cell and cell division can be clearly studied

Applications:

1. Study live blood cells
2. study cell division and other processes in a living state.
3. Used in microbiological research as virology , bacteriology and parasitology.
4. High-contrast images of transparent specimens, such as micro-organisms, thin tissue slices, living cells in culture, latex and sub-cellular particles, like nuclei and organelles, can be viewed in detail.

Structure

1. **phase plate** increases the phase difference to half a wavelength is located at the focal point of light between the objective lens and the image surface so that only the phase of the direct light changes. This generates contrast on the image surface.
2. **condenser annulus** (Ring aperture on the focal plane of the converging lens). light pathway it first passes through it. Only light in parallel waves passes through the openings in the annulus and illuminates the specimen. Some light passes un-deviated around the specimen. Other light passes through the specimen and is diffracted by the specimen.

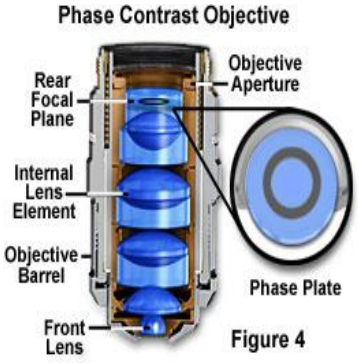
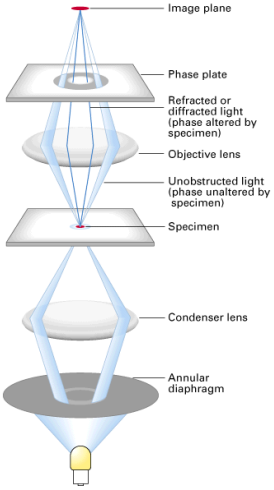
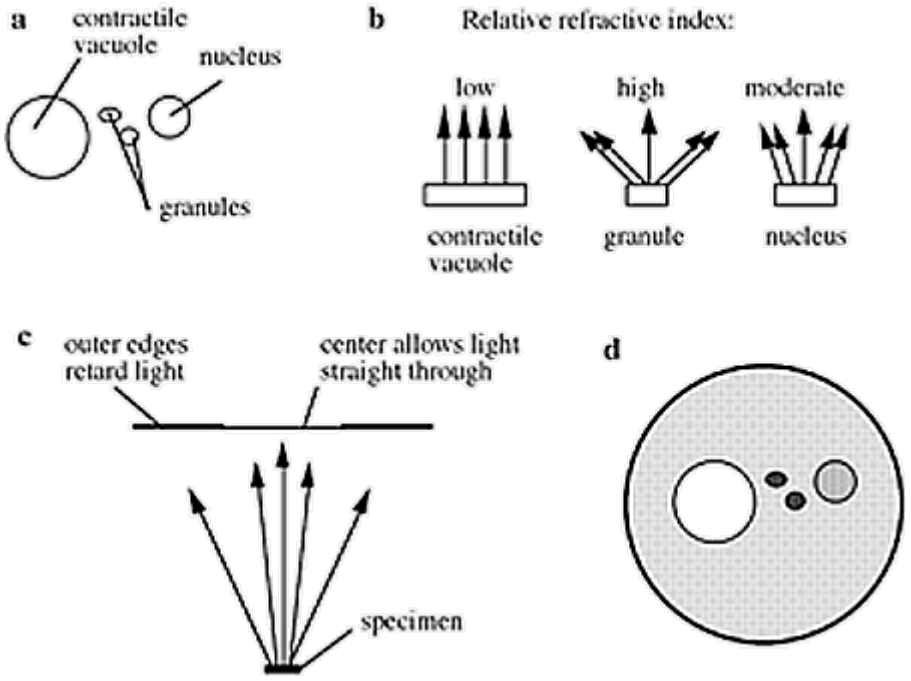


Figure 4





Figures:-

(a) organelles are nearly invisible in bright field although they have different refractive indexes;

(b) light is bent and retarded more by objects with a high refractive index;

(c) in phase contrast a phase plate is placed in the light path. Barely refracted light passes through the center of the plate and is not retarded. Highly refracted light passes through the plate farther from center and is held back another one quarter wavelength.;

(d) The microscope field shows a darker background (in this case the cell cytoplasm has a higher refractive index than the contractile vacuole), with the organelles in sharp contrast.

Lac:3 Dark field microscope

Dark field microscope is type of compound light microscope and one of the best method for viewing transparent specimen without staining .

A dark field mocroscope uses a dark field condenser that contain an opaque disc, which blocks the light that would enter the objective lens directley.

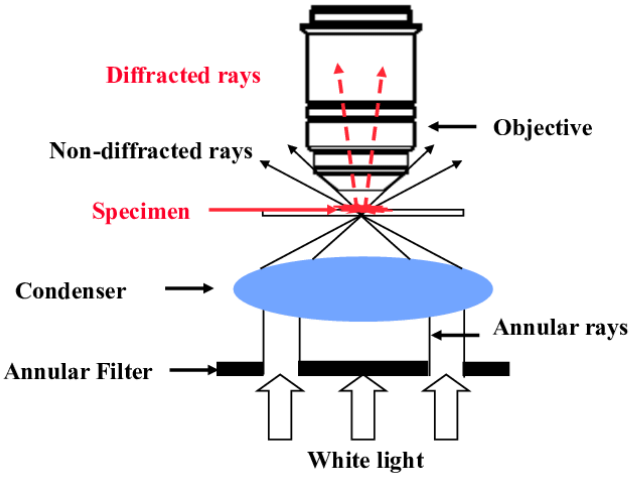
The condenser forms a hollow cone with light travilling around the cone rather than through it . This form of illumination allow only rays of light scattered by the sample and captured in the objective lens. When ther is no sample on the microscope stage , the view is completely dark.

Principle of work

Dark field microscopy work on the idea of oblique illumination . light come to the specimen from the side not directly through the center . The only light that reaches the objective lens is light that scattered by the specimen itself.

The pathway of light

1. The light enter the microsdopefor illumination of the sample
2. A specially sized disc block some light from the light source leaving an outer ring of illumination
3. The condenser lens focuses the light towards the sample.
4. The light enrers the sample . most is directly transmitted and some is scatterd from the sample.
5. The scattered light enter the objective lens . while the directly transmitted light misses the lens and is not collected due to a direct illumination blocked .



Dark field microscope applications

1. Living or lightly stained transparent specimen
2. Single cell organisms
3. Lived blood cells
4. Hay or soil sample
5. Pollen sample
6. Certain molecules such as caffeine crystals

Electron microscope (EM)

Electron microscope (EM) is a type of microscope that uses an electron beam to illuminate يضيء a specimen and produce a magnified image

Characters of EM

1. An EM has greater resolving power than a light microscope and can reveal يكشف the structure of smaller objects because electrons have short wavelengths.
2. They can achieve better resolution and magnifications of up to about 10,000,000X .
3. It is much useful in studying virus and components of cell.

4. The electron microscope uses electrostatic كهربائية and electromagnetic lenses to control the electron beam شعاع and focus it to form an image. These lenses are analogous تناظر to the glass lenses of a light microscope.
5. Modern electron microscope produce electron micrographs, using specialized digital cameras or frame grabbers خطاف to Capture the image

Types electron microscopes:

1. Transmission نافذ electron microscope (TEM)
2. Scanning ماسح electron microscope (SEM)
3. Reflection انعكاسي electron microscope (REM)
4. Scanning transmission electron microscope (STEM)

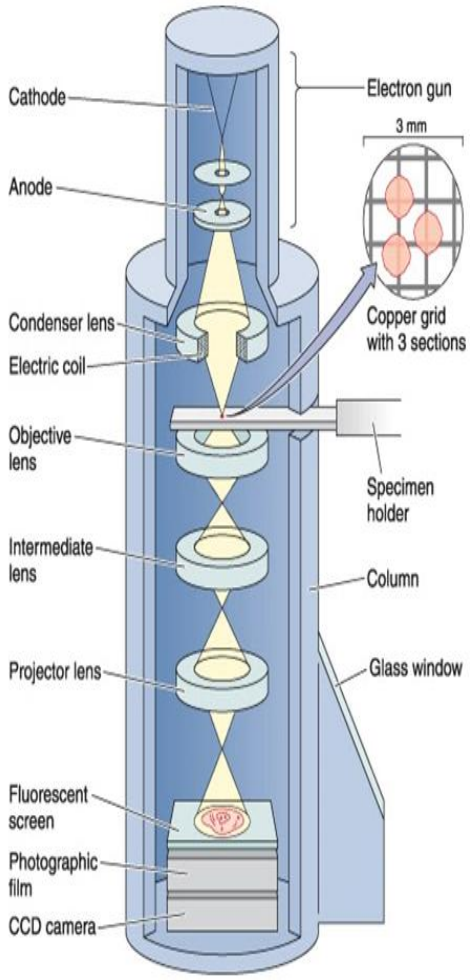
Transmission electron microscope (TEM)

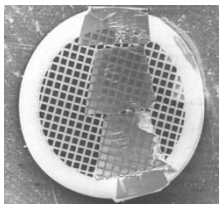
TEM is the original form of electron microscope , uses a high voltage electron beam to create an image. The electron beam is produced by an electron gun

Principles of TEM

1. Illumination - Source is a beam of high velocity electrons accelerated under vacuum, focused by condenser lens (electromagnetic bending of electron beam) onto specimen.
2. Image formation - Emergent electron beam is focused by objective lens. Loss and scattering of electrons by individual parts of the specimen. Final image forms on a fluorescent screen for viewing
3. Image capture – on negative or by digital camera

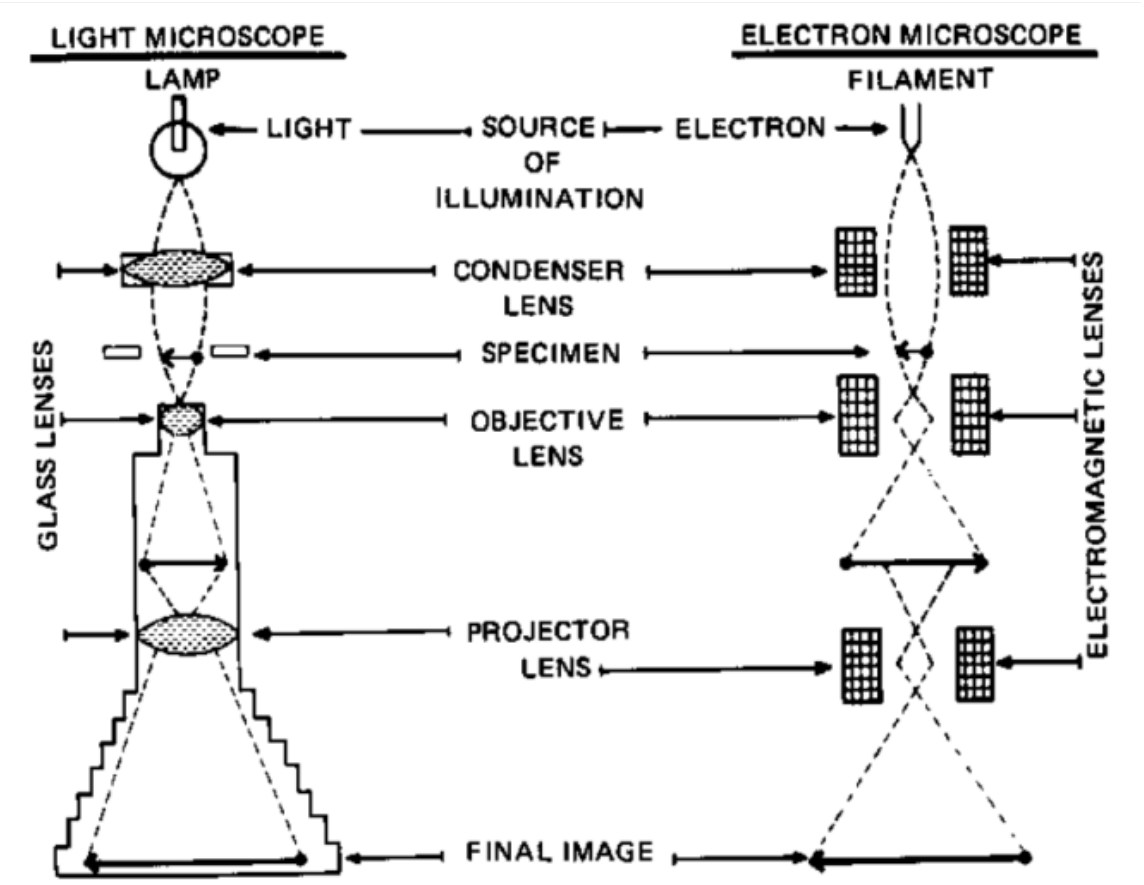
parts and functions of transmission electron microscope (TEM)





Ultra-thin section

Comparison between light and electron microscope



Sample preparation:

Materials to be viewed under an electron microscope may require processing to produce a suitable sample. The technique required varies steps depending on the specimen and the analysis required:

Fixation of tissues for EM

a) Must be prompt[فوري]
b) Cut to 1-2 mm cubes
c) Use sharp razor[حلاقة] blade[شفرة], avoid crushing[سحق]
d) 2.5% glutaraldehyde for 4 to 12 hours
e) Post fixation in 1% osmium tetroxide

Preparation of tissue

1, chemical fixation : for biological specimens aim to stabilize the specimen's mobile macromolecular structure by chemical crosslinking of proteins with aldehydes such as formaldehyde, gluteraldehyde and lipids with osmium tetroxide.

2, Negative stain: suspensions containing nanoparticles or fine biological materials (such as viruses and bacteria) are briefly mixed with a dilute solution of an electron-opaque معتم solution such as ammonium molybdate

3. Cryofixation - freezing a specimen so rapidly, in liquid ethane, and maintained with liquid nitrogen or even liquid helium-temperatures, so that the water forms vitreous (non-crystalline) ice. This preserves the specimen in a snapshot كمية قليلة of solution state.

4.Dehydration نزع الماء freeze drying, or replacement of water with organic solvents such as ethanol or acetone, followed by critical point drying or infiltration with embedding resins.

5.Embedding طمر,(biological specimens - in resin,

6. Sectioning : produces thin slices of specimen, semitransparent These can be cut on an ultramicrotome with a diamond knife to produce ultra-thin slices about 60-90 nm thick.

7 staining -uses heavy metals such as lead, uranium or tungsten to scatter imaging electrons and thus give contrast between different structures

Major Use of TEM in medicine

1. Renal diseases
2. Typing of tumors
3. Muscle diseases
4. Detection of viral particles – in feces, tissue fluids and tissues



The Balance

A laboratory balance is used to measure the mass of reagents or laboratory equipment. Electronic balances have a wide range of accuracy depending on the intended use

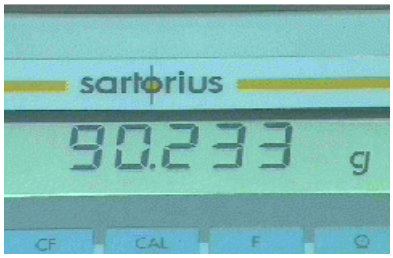
Classification

1. .Analytical balances are used when a high degree of accuracy is needed for the measurement . They may be manual or automatic.
2. Top-loading balances usually provide less sensitive measurement of mass than do analytical balances.

The Draft Shield: A draft shield is required for analytical balances. Common draft shields are either removable glass shells or glass doors. The shield prevents air currents from causing the balance pan to move, which results in fluctuating readings. By protecting the balance pan with a draft shield, a single, constant mass reading can be observed. Some balances have an indicator light to indicate when the balance has stabilized. The balance shown indicates that the mass reading is constant when a "g" lights up to the right of the numerical reading.

The Digital Display

Most electronic balances have a digital display. All of the numbers shown on the display should be recorded in your notebook. You should record 90.233 g in your notebook for the example shown



The Tare Button

Most electronic balances have a tare function. The tare function resets the digital display to zero when pressed

Weighing Hot Objects

Wait for the sample to cool to room temperature before weighing it

Weighing Wet Samples

After Solids collected by suction filtration, to further dry the sample, place it on a large piece of filter paper. Crush the sample with another piece of filter paper using your thumb (be sure to wear gloves if the liquid is hazardous), and allow the sample to dry in the air for several minutes before remeasuring its mass

Weighing Volatile Liquids

If the displayed mass gradually drops while a liquid is being weighed, that liquid is probably volatile. The mass falls because the liquid is evaporating, causing the amount of substance in the weighing vessel to decrease. Take special precautions, such as a placing a rubber stopper in an Erlenmeyer flask, to seal the weighing vessel when weighing a liquid that evaporates quickly. Make sure to account for the mass of whatever you use to seal the weighing vessel

Keeping a Balance Clean (practical part)

Laboratory instrumentation

5

Separation equipment

The centrifuge

Dr.khamael alcheallabi

Overview of separation equipment

Mechanical separations

Mechanical separations can be divided into four groups:-

1. **sedimentation**
   two immiscible liquids, or a liquid and a solid, differing in density, are separated by allowing them to come to equilibrium under the action of gravity, the heavier material falling with respect to the lighter.
2. **centrifugal separation**
   Like sedimentation, but the often speeded up by applying centrifugal forces to increase the rate of sedimentation.
3. **Filtration**
   is the separation of solids from liquids, by causing the mixture to flow through fine pores which are small enough to stop the solid particles but large enough to allow the liquid to pass.
4. **Sieving**
   interposing a barrier through which the larger elements cannot pass, is often used for classification of solid particles.

Centrifuge

It's a simple instrument used in the laboratory separating solid components from liquid suspension by centrifugal action.

Principle:

The centrifuge works using the sedimentation principle, the more dense or solid materials or sediment, separate out and packed at the bottom of the tube sometimes called (precipitate) and the liquid or top portion is called the supernatant. Cells usually collect at the bottom of the centrifuge tube because the particles are heavier than the liquid.

When we uses a centrifuge on blood, the blood cells collect at the bottom while the blood plasma moves to the top.

Centrifugal force depends essentially on three variables:-

1. Mass
2. Speed
3. radius. نصف القطر

Uses

1. It's used for plasma and serum separation from blood samples
2. urine particles sedimentation in microbiological laboratories and other lab.

Types of the centrifuge

1. Ordinary centrifuge.
2. Laboratory centrifuge which has speed 3,000-5,000 RPM (Round per minute)
3. Micro centrifuge (Haematocrit centrifuge) which has speed from (10,000- 12000) RPM
4. Ultra centrifuge (high capacity) which has speed of more than 50,000 RPM

Laboratory instrumentation

Spectrophotometer

Spectrophotometer Is an instrument designed to detect the amount of radiant light energy absorbed by molecules.

Spectrometer: It produces a desired range of wavelength of light. First a collimator (lens) transmits a straight beam of light (photons) that passes through a monochromator (prism) to split it into several component wavelengths (spectrum). Then a wavelength selector (slit) transmits only the desired wavelengths, as shown in Figure 1.

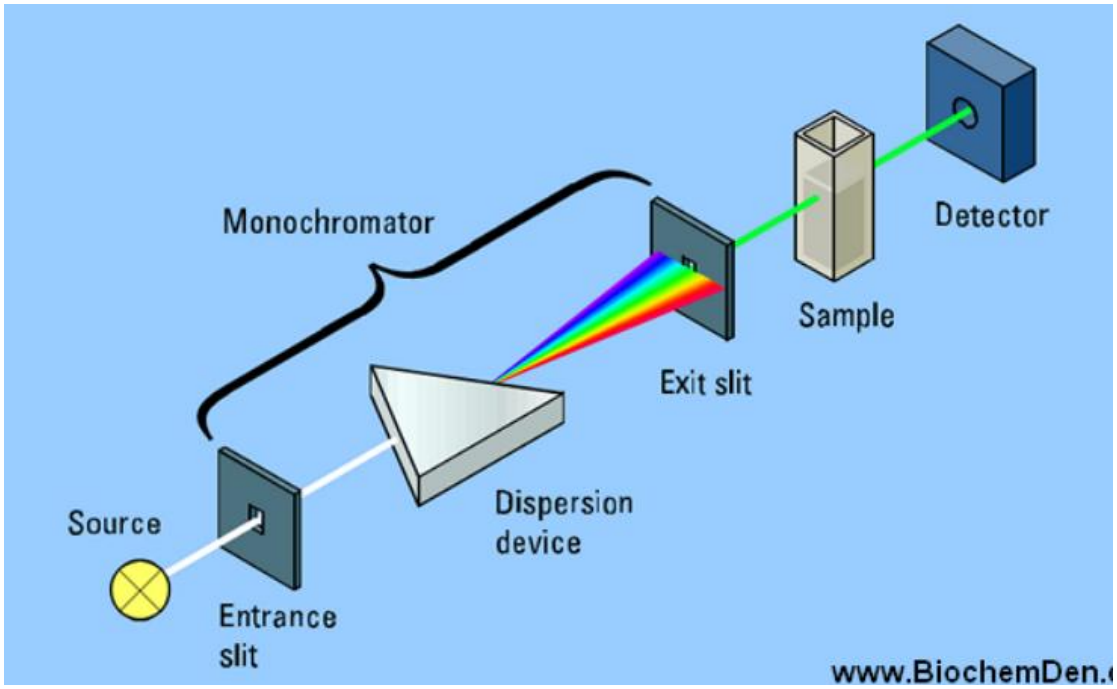


Figure 1: Basic structure of spectrophotometers

Classification of spectrophotometer :

1. Visible region spectrophotometer
2. Ultra violet spectrophotometer.
3. Infrared spectrophotometer.(IR)

1. Visible region spectrophotometer.

Is a tool that analysis quantitatively a molecule depend on how much light is absorbed by colored compounds. It can measure the intensity of any wavelength of visible light. **depending on:-**

a) the range of colors it can transmit through the test sample ,
b) the percentage of sample transmission ,
c) the logarithmic range of sample absorption and
d) percentage of reflectance measurement.

You need spectrophotometer to produce a variety of wave length because different compounds absorbed best at a different wavelengths, e.g. acid forms has a maximum absorbance at 320 nm, and a nitrophenolate (basic form) absorb best at 400 nm .

Uses of spectrophotometer :

uses in clinical tests that comprises the determination of the followings:

1-value of blood sugar.

2-uric acid and urea.

4-hemoglobin of blood (HB).

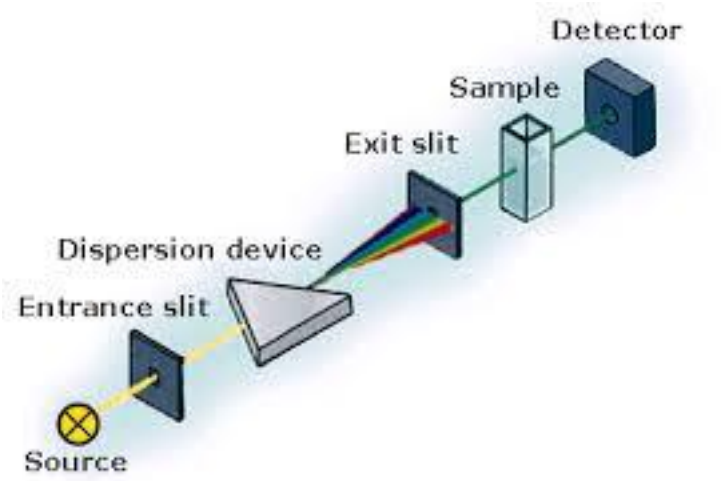
3-creatinine concentration.

5-concentration of some enzyme.

6-bilirubin concentration.

7- concentration of proteins in blood….. And many others.

Parts of spectrophotometer :



a) **Light source:** hydrogen lamp for U.V spectrophotometer and tungsten lamp for visible spectrophotometer.
b) **Lens:** used to focus the beam.

c) **prism:** a straight beam of light that passes through a prism to split it into several component wavelengths (spectrum).
d) **Selector:** Then a wavelength selector (slit) transmits only the desired wavelengths.
e) **Filter:** absorb other undesired wave length.
f) **Cuvatte:** a container to put the sample in. (quartz cuvatte is used In U.V spectrophotometer and glass cuvatte is used in visible region).
g) **Photo cell (detector):** used to convert the light energy transmitted through the filter into electrical energy.
h) **Galvanometer (digital display):** used to measure the amount of electrical energy.

2. Ultra violet spectrophotometer.

Ultraviolet (UV) spectroscopy works on a principle similar to that of colorimetry, except it uses ultraviolet light. It depend on the electrons in the chemical bonds of the sample compound. Researchers use UV spectrometers to:-

a. study chemical bonding
b. to determine the concentrations of substances (nucleic acids for example) that do not interact with visible light.

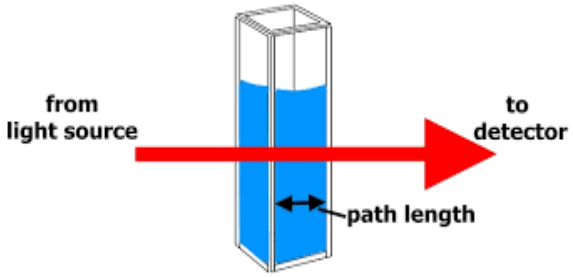
3. Infra red spectrophotometer.(IR)

An infrared spectrophotometer is an analytical instrument used to:-

i) identify materials including organic polymers.

ii) record the relative amount of energy as a function of the wavelength/frequency of the infrared radiation when it passes through a sample

iii) IR spectrometers are used to identify unknown compounds or to confirm their identity since the IR spectrum of a substance serves as a unique "fingerprint."



Beer-Lambert Law

Beer-Lambert Law (also known as Beer's Law) states that there is a linear relationship between the absorbance and the concentration of a sample. For this reason, Beer's Law can *only* be applied when there is a linear relationship. Beer's Law is written as:

$A=\epsilon lcA=\epsilon lc$

where

- AA is the measure of absorbance (no units),
- $\epsilon\epsilon$ is the molar extinction coefficient or molar absorptivity (or absorption coefficient),
- ll is the path length, and
- cc is the concentration.

The molar extinction coefficient is given as a constant and varies for each molecule. Since absorbance does not carry any units, the units for $\epsilon\epsilon$ must cancel out the units of length and concentration. As a result, $\epsilon\epsilon$ has the units: $L \cdot mol^{-1} \cdot cm^{-1}$. The path length is measured in centimeters. Because a standard spectrometer uses a cuvette

that is 1 cm in width, ll is always assumed to equal 1 cm. Since absorption, ϵϵ, and path length are known, we can calculate the concentration cc of the sample.

I) EXAMPLE 1

Guanosine has a maximum absorbance of 275 nm. ϵ275=8400M−1cm−1ϵ275=8400M−1cm−1 and the path length is 1 cm. Using a spectrophotometer, you find the that A275=0.70A275=0.70. What is the concentration of guanosine?

SOLUTION

To solve this problem, you must use Beer's Law.

A=ϵlc(1)(1)A=ϵlc

0.70 = (8400 M^{-1} cm^{-1})(1 cm)(cc)

Next, divide both side by [(8400 M^{-1} cm^{-1})(1 cm)]

cc = 8.33x10^{-5} mol/L

II) EXAMPLE 2

There is a substance in a solution (4 g/liter). The length of cuvette is 2 cm and only 50% of the certain light beam is transmitted. What is the absorption coefficient?

SOLUTION

Using Beer-Lambert Law, we can compute the absorption coefficient. Thus,

−log(ItIo)=−log(0.51.0)=A=8ϵ−log(ItIo)=−log(0.51.0)=A=8ϵ

Then we obtain that

ϵϵ = 0.0376

III) EXAMPLE 3

In example 2 above, how much is the beam of light is transmitted when 8 g/liter ?

SOLUTION

Since we know ϵϵ, we can calculate the transmission using Beer-Lambert Law. Thus,

log(1)−log(It)=0−log(It)log(1)−log(It)=0−log(It) = 0.0376 x 8 x 2 = 0.6016

log(It)log(It) = -0.6016

Therefore, ItIt = 0.2503 = 25%

IV) EXAMPLE 4

In example 2 above, what is the molar absorption coefficient if the molecular weight is 100?

SOLUTION

It can simply obtained by multiplying the absorption coefficient by the molecular weight. Thus,

$\epsilon\epsilon = 0.0376 \times 100 = 3.76\ L \cdot mol^{-1} \cdot cm^{-1}$

V) EXAMPLE 5

The absorption coefficient of a glycogen-iodine complex is 0.20 at light of 450 nm. What is the concentration when the transmission is 40 % in a cuvette of 2 cm?

SOLUTION

It can also be solved using Beer-Lambert Law. Therefore,

−log(It)=−log(0.4)=0.20×c×2(2)(2)−log⁡(It)=−log⁡(0.4)=0.20×c×2

Then cc = 0.9948

7

Sterilization Equipment's

oven and Autoclave

Dr.khamael alchallaebi

Sterilization: is the process of destruction or removal of harmful microorganisms from non living objects by chemical or physical methods.

Equipment of sterilization

1. Hot air oven
2. autoclave
3. Incubator
4. Water bath

Hot Air Oven Or Dry Oven

Hot air oven is an electrical instrument used for sterilization to kill microorganisms and their spors by hot air. Hence it is dry sterilization unit.

Oven structure

1. an insulated cabinet containing heating elements .
2. shelves
3. circulating fan helps to circulate the air
4. Electric heater
5. thermostat
6. thermometer
7. door .

ne

a. at 160 c^o for two hours.
b. at 180 c , for 20 minutes
c. At 120 c . for 8 hours.

Uses of oven

1. Drying glassware (drying temperature is 70 ^oC.
    a. test tube
    b. petri dish
    c. beakers
    d. flasks
2. Dry and sterilize heat resistant materials (metallic tools like forceps, scissor , etc.
3. To dissolve fat waxes

Materials should not be sterilized by oven

1. Labile materials ex.nylon and plastics
2. Inflammable materials ex. Petrolium, paper,

Awareness :تحذير

1. don't open the oven immediately when it very hot because it may break or contaminate the operator.
2. Wait until the temperature reach 25 ^{o}C – 60^{o}C

Hot air ovens – Advantages

a. Moisture is almost negligible
b. the pressure built up inside is much lesser compared to an autoclave.
c. safer than autoclaves,
d. hot air ovens are much smaller in size
e. have equal effectiveness when it comes to sterilization.
f. Temperatures can reach very high levels as in autoclave if it is non-moist;
g. they can sterilize a wider variety of devices and instruments safely.

Hot air ovens Disadvantages

the only disadvantage is that a few tough micro-organisms may not be killed in a .hot air oven all the time

Care and maintenance of drying oven

1. Oven should be frequently clean with light detergent to remove any material precipitate on oven or corrosive materials.
2. If there is any fault in the heater, it should be switched off and replace.

Autoclave

The autoclave is an electrical wet sterilizer apparatus by steam , heat , and high pressure used for sterilization of culture media , glasswares and other tools used in microbiology experiments , at a temperature 121^{o}C for 15 – 20 minutes with 1 bar pressure , this means that all bacteria, viruses , fungi and spores are inactivated.

Structure of Autoclave

1. strong metal jackets (strong enough to withstand high pressure required) double walled cylinder made up of stainless steel or copper , One end is closed and the other end is open. The open end has a lid
2. autoclave door is closed air tight.

3. The door is provided with a pressure gauge for observing the pressure , a steamy valve (exhaust valve) to let out the air and a safety valve
4. timer,
5. water level indicator,
6. Thermometer (give temperature),
7. electrical heater (heat supply) located at the bottom of autoclave which is covered with water .
8. Water is filled below the level of resting plate .
9. The articles to be sterilized are kept in baskets on the resting plate.

Facts about autoclave

1. The temperature inside the autoclave at 15 pounds pressure is about 121 c . This temperature is sufficient to kill all organisms. It is more effective than a hot air oven.
2. Autoclaves are widely used in microbiology, medicine, mycology, dentistry.
3. They vary in size and function.
4. Autoclaves are known to be used to sterilize equepments and other objects that can withstand very high temperatures. include laboratory glassware, surgical instruments and medical waste.

There are two temperature can be used in work :

1. Standard temp. 121 ^{o}C for 15 – 20 minutes for microbes.
2. Allowed temp. 134 ^{o}C foe 3 minutes for stains.

Sterilization in autoclave depend on three factors :

1. Temperiture degree 121 ^{o}C
2. Pressured steam 1 bar
3. Time 15 -20 minutes

Disadvantages of Autoclaves

1. An autoclave also produces a very high pressure due to which materials like glass and powders may break, disintegrate or even get burnt.
2. the consumables and surgical materials like bandages or cotton cannot be sterilized using it.

advantage of autoclave

materials that can take the heat and pressure is that the moist heat produced helps coagulate proteins in micro-organisms and kill them completely.

Method of operation:

1- Fill the bottom of autoclave with distilled water, but below the resting plate .
2- Place the articles within the basket. On the resting plate
3- Close the lid and tighten the screws.
4- Adjust the temperature, pressure and time.
5- Upon completion of the sterilization time discards steam by opening the valve for that then opens the lid.

Laboratory instrumentation

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Incubator

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Incubators

Incubators are insulated boxes with an adjustable heater, typically going up to 60 to 100 °C , used in medical and other laboratories, to grow and maintain bacteria and fungi or cell cultures at temperature $37c^{o}$ for 18-24 hours.

The incubator provides viable growth factors such as :

1. Constant temperature
2. humidity is typically >80% to prevent evaporation
3. carbon dioxide (CO_2) level of 5% to maintaining a slightly acidic pH media.
4. oxygen to maintain life .

Parts of incubator

1. Control Panel.
2. Thermostat.

3. Timer.
4. Thermometer.
5. Electric heater
6. Some units has inner glass doors that permit the contents to be viewed without disrupting the atmosphere of the incubator.

Modern incubator contain additional design improvements that focus on making laboratory incubators easy and more convenient to use.

a. touch-screen displays,
b. programmable alarms,
c. data storage,
d. removable shelves

Uses of Laboratory incubators

A. biology applications, such as

1. cell and tissue cultures, cultures that involves the extraction of fragments of animal or vegetable tissue and to store them in controlled environments to analyze its growth
2. molecular biology .
3. microbiology
4. biochemical and hematological studies,

B. pharmaceutical and hematological studies,

C. food analysis.

General types of incubators

There are many different types of incubators, and the most commonly used are the following:

1. **Standard incubators**. These incubators can be gravity or fan assisted convection and their range of temperature is from ambient to a maximum of 80°C or 100°C
2. **Cooled incubators**: These incubators work at temperatures close to or below ambient temperature
3. **Humidity incubators**: These incubators control both, temperature and humidity, refrigeration system . The humidity is usually obtained from a tray of water which is constantly evaporating.

4. **CO2 (carbon dioxide) incubators**: These incubators are widely used in maintenance and growth of Biological samples when it is necessary to maintain a constant pressure with CO_2 concentration in air ranging from 5 – 20%. The level of CO2 is controlled by a thermal conductivity sensor or infra-red sensor. and digital display of CO_2 pressure monitor.
5. **Shaking incubators**: These incubators shakes in a temperature controlled atmosphere.

Water bath

Water bath is an instrument used for different purposes in the lab, to elevate temp. degree of liquids , . For all water baths, it can be used up to 99.9 °C. When temperature is above 100 °C, alternative methods such as oil bath, silicone bath or sand bath may be used.

Principle of operation

Principle of water bath work depend on an electrical heater which heat water placed in the tank uniformly .

Parts of water bath

1. Control panel
2. Thermostat
3. Electrical heater
4. Easy - to - maintain stainless - steel interior chamber and powder - coated exterior resist most corrosion damages .
5. Circulating pump: direct Water flows around bath perimeter for fast heating and better uniformed temperature control , + 0 . 2°C @ 37°C .
6. Thermometer
7. Low water level alarm .
8. drain valve.

Uses of water bath

1. warming of reagents gently and gradually to fixed temperatures,
2. keep materials warm over a period of time
3. melting of substrates مادة تفاعلية
4. incubation of cell cultures.
5. enable certain chemical reactions to occur at high temperature.
6. heating flammable chemicals instead of an open flame to prevent ignition.

Types of water bath

1. **Circulating Water Baths**

   Circulating the water baths (also called *stirrers)* are ideal for applications when temperature uniformity and consistency are critical, such as enzymatic and serologic experiments. Water is thoroughly circulated throughout the bath resulting in a more uniform temperature.

2. **Non-Circulating Water Baths**

   This type of water bath relies primarily on convection instead of water being uniformly heated. Therefore, it is less accurate in terms of temperature control.

3. **Shaking Water Baths**

   This type of water bath has extra control for shaking, which moves liquids around. This shaking feature can be turned on or off.
   In microbiological practices, constant shaking allows liquid-grown cell cultures grown to constantly mix with the air.

4. Cooled water bath

Laboratory instrumentation

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Biological Safety Cabinets

Dr.khamael alchallabi

Biological safety cabinet

A Biological Safety Cabinet is a ventilated enclosure offering protection to the user, the product and the environment from aerosols arising from the handling of potentially hazardous micro-organisms. The continuous airflow is discharged to the atmosphere via high-efficiency particulate absorber filter (HEPA) and high-energy ultra-violet light units to kill off the live bacteria and viruses trapped by the filter media

Uses of Protection cabinet

1. Personal Protection from harmful agents within the cabinet
2. Product Protection to avoid contamination of the samples.
3. Environmental Protection from contaminants contained within the cabinet.

Classification of biological cabinet

Biological Safety Cabinets are classified into three classes based upon their containment capabilities when working with biological agents.

1. **Class 1 Cabinets**
    a. Provides personal and environment protection.
    b. Used when working with low to moderate risk biological agents.
    c. Biosafety levels: 1, 2 and 3
2. **Class 2 Cabinets**
    a. Provides personnel, environment and product protection.
    b. Used when working with low to moderate risk biological agents.
    c. Biosafety Levels: 1, 2 and 3
3. **Class 3 Cabinets**
    a. A highly specialized laboratory “glovebox”. A Class 3 cabinet provides the same protection as a Class 2 but is designed for working with Biosafety Level 4 highly infectious agents and provides the highest level of protection for the environment, product and user.
    b. Used when you are working with very high risk biological agents.
    c. Biosafety Level: 4

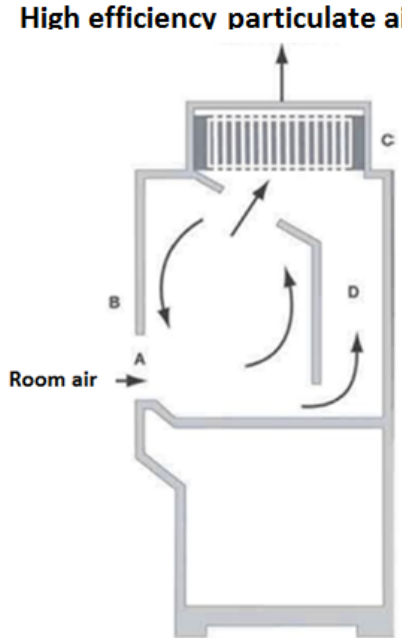
Explanation of the different Biosafety Levels

1. **Biosafety Level 1**
    applies when working with biological agents, which pose a minimal risk/ threat to laboratory personnel and the environment. Work with these types of agents are generally performed in open laboratory cabinets without the use of special equipment.
2. **Biosafety Level 2**
    working with pathogenic or infectious organisms posing a moderate hazard. Examples include Salmonellae, Hepatitis B virus and Measles virus.
3. **Biosafety Level 3**

applies when working with indigenous or exotic agents, which may cause serious or lethal disease via aerosol transmission. Examples include Yellow Fever and Encephalitis.

4. **In Biosafety Level 4**
   applies when working with extremely dangerous, contagious and life-threatening agents. Maximum containment[عزل] and protection is required at all times. Examples include Ebola, the Lassa virus and any sample with unknown risks of pathogenicity and transmission.

Class 1 Biological Safety Cabinets

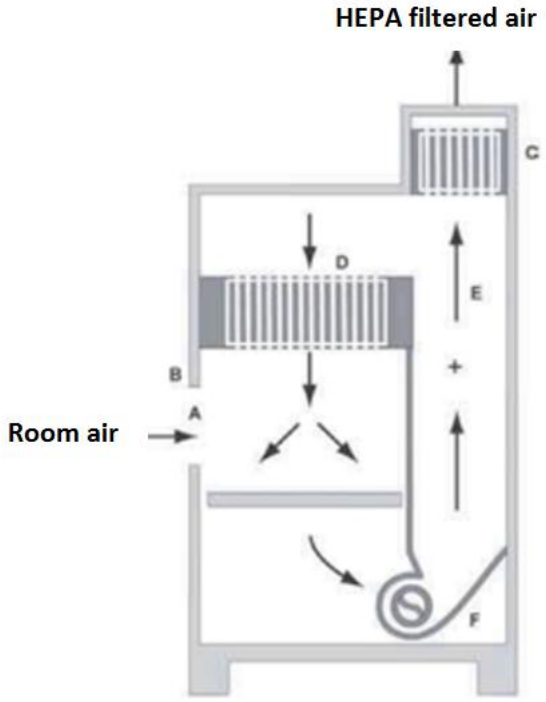


The Class I BSC
(A) front opening;
(B) sash;
(C) exhaust HEPA filter;
(D) exhaust plenum. Note: The cabinet needs to be hard connected to the building exhaust system if toxic vapors are to be used.

The Class II, Type

1. provides personnel and environment protection for the safe handling when working with chemicals and powders.
2. The air enters the cabinet via the front aperture passing through a built-in exhaust fan, HEPA and/or Carbon filter, thus providing operator and environmental protection. The air then exits the cabinet at the rear of the work surface. The escape of any airborne particulates generated within the cabinet are therefore controlled by means of the inward airflow through the front aperture and by filtration/absorption of the exhausted air.
3. HEPA filter in the cabinet protects the environment by filtering the air before it is exhausted.

Class 2 Biological Safety Cabinets



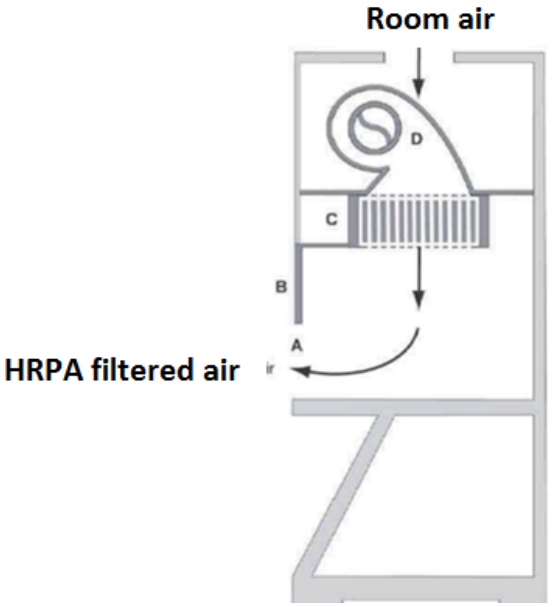
The Class II, Type

(A) front opening;
(B) sash;
(C) exhaust HEPA filter;
(D) supply HEPA filter;
(E) common plenum;
(F) blower.

The Class 2 Biological Safety cabinet must meet the requirements for personnel, environmental and product protection. The air-flow is drawn into the work chamber via the front aperture and continues under the worktop and goes up the back plenum, where 70 % is being recirculated through the main triple HEPA filter to provide down flow and 30 % exits out through the HEPA filter to exhaust.

Class 3 Biological Safety Cabinets

Class 3 Biological Safety Cabinets



The Class II, Type

(A) front opening;
(B) sash;
(C) supply HEPA filter;
(D) blower.

1. is an aerosol-tight enclosure with a non-opening, completely sealed, front window, so the operator is separated from their work by a physical barrier. The cabinet is also referred to as a" Glovebox". Work is conducted via long heavy-duty rubber gloves attached to the ports in the front of the safety cabinet in a gas-tight manner. This allows access and work to be performed on the sample within the cabinet without compromising containment.
2. The cabinet is designed for work with high risk biological agents in Biosafety Level 4 laboratories, where maximum containment and protection are required. Examples include Ebola, the Lassa virus and any agent with unknown risks of pathogenicity and/or transmission.
3. When the Biological Safety Cabinet Class III is installed in a Biosafety Level 4 Laboratory, it offers an additional level of containment and safety for high risk working procedures.

Biological Safety Cabinets (Practical part)

Procedures for the Proper Use of a Class II Biological Safety Cabinet

If users do not properly understand or operate cabinets, they will not maintain an adequate protective barrier between the operator and the experiment. Listed below are procedures for proper use of BSCs.

1. Turn the cabinet fan and the fluorescent light on. Turn off the UV light. Confirm that the drain valve is closed.
2. Wipe the work surface with 70% ethanol or other appropriate disinfectant. Let the unit run for 5 -10 minutes to clean itself before beginning work.
3. Plan the work operation in advance. Place everything needed for the complete procedure in the cabinet before starting. Nothing should pass through the air barrier, either in or out, until the procedure is complete. Arrange materials in a logical manner such that clean and contaminated materials are segregated. Remove from the cabinet all materials or equipment not necessary for the particular procedure.
4. Avoid placing materials on the air intake grille, at the front of cabinet as this disrupts the protective air barrier.
5. Keep equipment at least four inches (10 cm) inside the cabinet work area. Perform manipulations of hazardous materials as far back in the work area as possible.
6. After the procedure is completed, decontaminate all equipment in direct contact with the research agent with an appropriate disinfectant. Run the cabinet at least three minutes with no activity to purge airborne contaminants from the work area.
7. After removal of all materials and equipment, wipe the work surface with 70% ethanol or other appropriate disinfectant. Clean any spilled culture media that may support fungal growth and cause contamination in subsequent experiments.
8. Turn off the cabinet fan. Some researchers prefer to let the cabinet run continuously. You may turn on the UV light if the BSC is so equipped and there are barriers in place to prevent inadvertent UV exposure to other lab personnel.

9. Limit access to BSC to one person at a time. Two people simultaneously moving and working in a BSC can disrupt the protective air curtain at the face of the BSC. This can compromise the safety of the individuals working in the BSC, others in the room as well as product protection, thus negating the engineering control.
10. Do not use an open flame in the cabinet as it disrupts airflow and could damage the HEPA filter.
11. Conduct research in a ‘clean to dirty’ (left to right) work pattern.

Biohazard Spill Control Inside a BSC

1. Keep the BSC on.
2. Put on protective gloves.
3 Spray wipe walls, work surfaces, and equipment with decontamination solution.
4. Flood tray top, drain pans, and catch basins with decontamination solution.
5. Allow to stand for 20 minutes.
6. Drain excess solution into cabinet base.
7. Lift out tray and any removable exhaust grille work.
8. Clean top and bottom surfaces with sponge/cloth soaked in decontamination solution.

9. Replace tray and grille work
10. Place everything that is contaminated into autoclave pan.
11. . Drain decontamination solution from cabinet base into autoclavable containers.
12. Autoclave

Laboratory instrumentation

Lect. 12

pH meter

Dr.khamael alchallaebi

pH

What does the term "ph" mean?

The term pH is derived from “p,” the mathematical symbol رمز رياضي for negative logarithm, and “H,” the chemical symbol for Hydrogen.

pH meter

A pH meter is a scientific instrument that measures the hydrogen-ion activity in water-based solutions, indicating its acidity or alkalinity expressed as pH by special probes

A solution containing more H+ ions remains acidic while the solution containing more OH- ions remains alkaline. pH value of solutions ranges from 1 to 14.

Uses in the clinical laboratory

1. To measure the PH of blood
2. check the PH of certain reagent such as buffer preparation for lab use .
3. to determine the pH of different solutions in pharmaceuticals.

Composition of pH meter

A typical pH meter consists of:-

1. a special measuring probe (a glass electrode)
2. electronic meter that measures and displays the pH reading.

The probe

The pH probe measures pH as the activity of the hydrogen cations surrounding a thin-walled glass bulb at its tip. The probe produces a small voltage (about 6 mV per pH unit) that is measured and displayed as pH units by the meter.

The probe composition

The glass pH probe contains two electrodes, a sensor electrode and a reference electrode القطب المرجع . These electrodes are in the form of glass tubes one contains pH 7 buffer and other contains saturated potassium chloride solution. The sensor electrode bulb is made up of porous glass or permeable glass membrane coated with silica and metal salts. A silver wire coated with silver chloride is immersed in pH 7 buffer in the bulb. Another silver wire coated with silver chloride is immersed in the saturated potassium chloride solution in reference electrode.

Principle of pH meter

Principle of pH sensor and pH meter depends upon the exchange of ions from sample solution to inner solution (pH 7 buffer) of glass electrode through the glass membrane.

When the probe is placed in a solution to measure the pH, hydrogen ions accumulate around the bulb and replace the metal ions from the bulb. This exchange of ions generates some electric flow that is captured by the silver wire. The voltage of this electric flow is measured by the pH meter by converting it into pH value by comparing the generated voltage with the reference electrode.

a. Increase in acidity of solution has greater concentration of hydrogen ions that increases the voltage. This increased voltage decreases the pH reading in pH meter.
b. increase in alkalinity decreases the hydrogen ions or increases in hydroxyl ions concentration also decrease the voltage and increase the pH value in pH meter.

Buffer Solutions

Buffers are solutions that have constant pH values and the ability to resist changes in that pH level. They are used to calibrate the pH measurement system (electrode and meter).

There can be small differences between the output of one electrode and another, over time. Therefore, the system must be periodically calibrated with Buffers.

Buffers are available with a wide range of pH values, and they come in dry powder capsules. Calibration is usually performed near the isopotential point (the signal produced by an electrode at pH 7 is 0 mV at 25°C), and a second is typically performed at either pH 4 or pH 10.

How to calibrate a pH meter?

For very precise work the pH meter should be calibrated before each measurement. For normal use calibration should be performed at the beginning of each day. using at least two standard buffer solutions of known pH values (typically around 4 and 7).

1. The temperature setting on the meter must correspond to the temperature of the buffers used,
2. Turn pH meter to “pH” or “ATC” if automatic temperature compensation is used.
3. Place clean electrode into fresh, room temperature pH 7.00 buffer.
4. Adjust the pH reading to exactly 7.00 using the ZERO OFFSET,STANDARDIZED or SET knob.
5. Rinse the electrode with distilled or deionized water. (This would be the procedure for a one-point calibration. Continue through step 8 for a two-point calibration.)
6. Place electrode into the second buffer, either pH 4.00 or pH 10.00.
7. Adjust the pH reading to display the correct value using the SLOPE, CALIBRATE, or GAIN controls (coarse adjust).
8. Adjust the pH reading to read the correct value using the SLOPE knob (fine adjust).

Why doing calibration?

The reason for this is that the porosity of the glass membrane decreases with the continuous use that decreases the performance of the probe.

Storage conditions of the glass probes

When not in use, the glass probe tip must be kept wet at all times to avoid the pH sensing membrane dehydration.

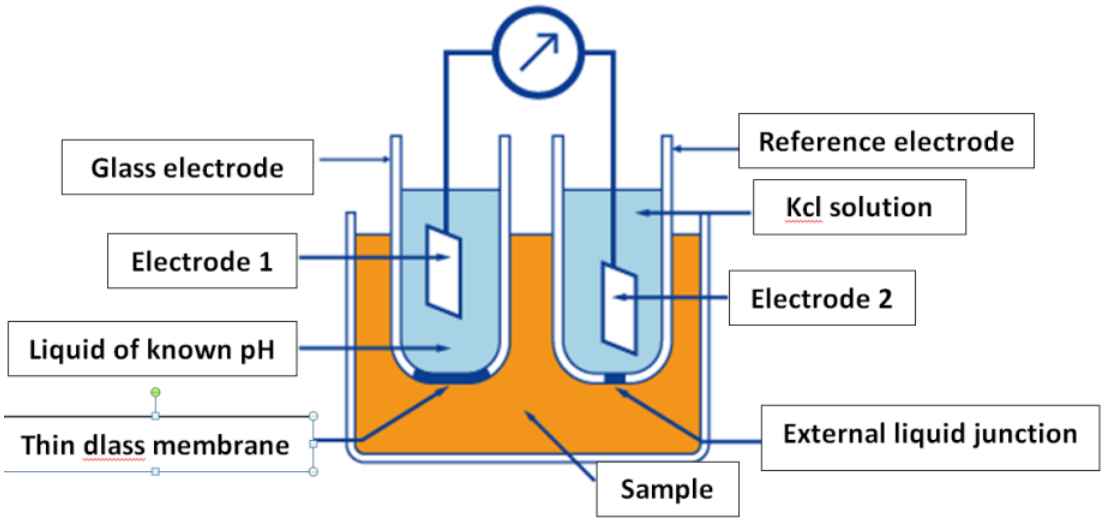
Both (glass electrode + reference electrode) are better stored immersed in the electrolyte solution (often KCl 3 M) .

Cleaning and troubleshooting of the glass probes

Occasionally (about once a month), the probe may be cleaned using pH-electrode cleaning solution; generally a 0.1 M solution of hydrochloric acid (HCl) is used, having a pH of one.

In damaged film of glass. Alternatively a dilute solution of ammonium fluoride (NH4F) can be used. To avoid unexpected problems.

The components of probe



pH meter \ Practical part

PH is defined as the negative log of the hydrogen ion activity

$PH = -\log [H^+]$

The sum of PH and POH = 14

POH=-log[OH^-]

Example: water at 25°C has 0.0000001 mole of hydrogen per liter. This expressed as 10^{-7} , the log of hydrogen ion concentration is -7. The PH of water at 25°C is 7.

* A strong alkaline solution such as NAOH would have a higher concentration of hydroxyl ions and lower concentration of hydrogen ions . An acid have a higher concentration of hydrogen ions .

* **0←← ACID←←← 7 →→→ALKALINE→→14**

Parts of PH meter:

1- Electrode holder.

2- Electrode.

3- Beaker.

4- Switch.

5- Scale runs from 0-14.

6-Temperature knob.

7- Adjustment knob.

Operation of PH meter:

1-Switch on the instrument , allow to warm up for a few minutes

2- Set up the temperature on the actual temperature reading.

3-Rinse the electrode with distilled water and read the reference buffer.

4- Adjust the reading of the reference buffer on the scale according to the actual ph value of the reference.

5- Read your unknown by dipping the electrode into the sample flask.