Chromatography

Chromatography: is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase,* which carries it through a structure holding another material called the *stationary phase.* The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture.

Types :

1. Gel filtration (permeation)chromatography :

are separated protein according their size.

Principle:

Smaller molecules are able to penetrate the pores of gel, therefore, the smaller molecules move through the column more slowly. Whereas larger molecules are unable penetrate the pores of gel, therefore, pass through the column quickly.

2. Ion exchange chromatography

A process that allows the separation of ions and polar molecules based on charged that carry it. By exchanging ions between the article that you want to separated and the surface ions (media) that happens exchange process.

Principle:

It is exchange the sample components that passed in column with ionic components (stationary phase) leading to separated.

Negatively charge exchangers bind with positively charge ions (cations). Similarity positively charged bind with negatively charged ions (anion).

3. Affinity chromatograph:

is a method of separating biochemical mixtures based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand.

Important:

a. Purify and concentrate a substance from a mixture into a buffering solution

b. Reduce the amount of a substance in a mixture

4. HPLC (High liquid chromatography):

Is chromatographic technique is used in biochemistry an analytic chemistry to identify quantify and purify the individual components of the mixture. The stationary phase consist a pump that moves mobile phase and detector that provides a characteristic retention time and also information's about UV data.

Principle:

In HPLC a pump (rather than gravity) provides the higher pressure required to propel the mobile phase and analytic through the density packed column

5. Gas chromatography:

Is a common type of chromatography used in analytic chemistry for separated and analyzing compounds that can be vaporized without composition.

In gas chromatography, the mobile phase is a carrier gas, such as helium or nitrogen. The stationary phase is microscope layer of liquid that coated glass or metal coil tubing called column.

The instrument used to perform gas chromatography is called a gas chromatograph or aerograph, gas separate.

Principle:

The gas compounds being analyzed interact with wall column, which is coated with different stationary phases. This causes each compound to elute at different time, known as the retention time of compound. The comparison of retention times is which gives gas chromatography its analytical usefulness.

Paper chromatography

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of chromatography paper. The paper is placed in a container with a shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture, which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

Incubator

Incubator: is a laboratory apparatus used to grow and maintain mammalian cell cultures, microbes, or plant cells by controlling environmental conditions (temperature, humidity and carbon dioxide (CO2) at optimal levels.

•Changes in the atmospheric CO2can alter the pH of the culture media. A controlled atmosphere is achieved by using a CO2-monitoring device, which draws air from the incubator into a sample chamber, determines the concentration of CO2, and injects pure CO2 into the incubator to make up any deficiency.

• Air is circulated around the incubator by using a fan to keep both the CO2 level and the temperature uniform.

Incubators According To Design:

•Cell incubators usually come in one of three types of designs:

•Air draft incubators: circulate air throughout the interior of the incubator to maintain constant temperatures. This style of incubator is the most commonly used and is used for growing large amounts of cells.

•These incubators are designed to respond quickly to environmental needs of the cells. However, these incubators lose the temperature very quickly and must consistently adjust the air to control the temperature.

•Dry wall: Pass air within the walls of the incubator. These walls then radiate the temperature to the growing chamber. For this reason these incubators are sometimes called radiant incubators. They are better at maintaining a constant temperature because the jacket does not let heat escape through the walls of the incubator.

•These incubators respond slowly to temperature adjustments compared to Air draft incubators.

Water jacket.

•Water-jacket incubators are surrounded by water within the walls of the three sides, the top, and the bottom.

•Advantages include stable temperature and increased security in the event of power failure (due to water's natural insulation abilities).

•These are usually smaller incubators and work by the same principles as dry wall incubators.These incubators also respond more slowly to temperature changes.

•Water bath

• The water bath, like incubator, is an apparatus for controlled temperature incubation of cultures, liquids and many other laboratory tests. The temperature of water bath is adjusted by a thermostat that can be set at any desired temperature ranging from 20-100 c. the water baths are available at various types and capacities. The heating coil may be of immersion type, or enclosed in a case. Some models have propellers that help to circulate the water so that identical temperature is maintained throughout the water bath.

Use and care of water bath

•Maintain the minimum level of water bath with chemically pure water. Avoid using tap water as salts from tap water may get deposited on the coil and affect its function.

•Always use a thermometer to check that temperature is stable at the desired level.

•Be sure that the substance being incubated is below the surface of the water in the bath.

•It is advisable to cover the flasks, plates and tubes during incubation to avoid contamination and dilution as a result of condensing water from the lid of water bath.

•Clean the water bath regularly.

A BIOSAFETY CABINET

A **biosafety cabinet** (**BSC**) — also called a **biological safety cabinet** or **microbiological safety cabinet** — is an enclosed , ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with) pathogensrequiring a defined biosafety level. Several different types of BSC exist, differentiated by the degree of bio containment required. BSCs first became commercially available in 1950.

Purpose:

The primary purpose of a BSC is to serve as a means to protect the laboratory worker and the surrounding environment from pathogens. All exhaust air is HEPA-filtered as it exits the biosafety cabinet, removing harmful bacteria and viruses. This is in contrast to a laminar flow clean bench, which blows unfiltered exhaust air towards the user and is not safe for work with pathogenic agents. Neither are most BSCs safe for use as fume hoods. Likewise, a fume hood fails to provide the environmental protection that HEPA filtration in a BSC would provide. However, most classes of BSCs have a secondary purpose to maintain the sterility of materials inside (the "product").

Classes:

The U.S. Centers for Disease Control and Prevention (CDC) classifies BSCs into three classes. These classes and the types of BSCs within them are distinguished in two ways: **the level of personnel and environmental protection provided** and **the level of product protection provided.**

Maintenance and service

Cabinets need to be maintained on a regular schedule. During this check, the airflow and the filter capacities are controlled. The filters have a limited lifespan. Depending on the laboratory environment and the type of samples used, the filter air flow-through is reduced over time. Newer cabinets measure the air flow-through constantly. If the flow-through is too low, there will be an audial and visual alarm. Changing the filter should be limited to trained persons as the filter is potentially contaminated and a "bag-in/bag-out" procedure needs to be followed. When an UV light is used, this lamp should be checked and changed as well. UV lights decrease in power over time, resulting in suboptimal disinfection of the working area.

is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials. Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect.[1]

Such hoods exist in both horizontal and vertical configurations, and there are many different types of cabinets with a variety of airflow patterns and acceptable uses.

Laminar Flow Cabinets Or Laminar Flow Closet Or Tissue Culture Hood :-

to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials. Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect .

Such hoods exist in both horizontal and vertical configurations, and there are many different types of cabinets with a variety of airflow patterns and acceptable uses.

Laminar flow cabinets may have a UV-C germicidal lamp to sterilize the interior and contents when not in use. (It is important to switch this light off during use, to limit. exposure to skin and eyes as stray ultraviolet light emissions can cause cancer andcataracts.)

A **fume hood** or **fume cupboard** is a type of local ventilation device that is designed to limit exposure to hazardous or toxic fumes,vapors or dusts. A fume hood is typically a large piece of equipment enclosing five sides of a work area, the bottom of which is most commonly located at a standing work height.

Two main types exist, ducted and recirculating (aka ductless). The principle is the same for both types: air is drawn in from the front (open) side of the cabinet, and either expelled outside the building or made safe through filtration and fed back into the room.

Other related types of local ventilation devices include: clean benches, biosafety cabinets, glove boxes and snorkel exhausts. All these devices address the need to control airborne hazards or irritants that are typically generated or released within the local ventilation device. All local ventilation devices are designed to address one or more of three primary goals:

1- to protect the user from inhaling toxic gases (fume hoods, biosafety cabinets, glove boxes);

2- to protect the product or experiment (biosafety cabinets, glove boxes);

3- to protect the environment (recirculating fume hoods, certain biosafety cabinets, and any other type when fitted with appropriate filters in the exhaust airstream).

Secondary functions of these devices may include explosion protection, spill containment, and other functions necessary to the work being done within the device.

Lecture 1-

Introduction to microscopy:

A microscope: is an optical instrument that uses a lens or a combination of lenses to produce highly magnifiedimages of small specimens or objects when they are too small to be seen by the naked (unaided) eye.

• Microscope can be separated into optical microscopes (Light microscope) and electron microscopes.

Historical overview

•There is no one person who invented the microscope as several different inventors experimented with theories and ideasand developed different parts of the concept as they evolved to what is today's microscopes, however, a Dutchman, Anton van Leeuwenhoek, isconsidered the father of microscopes because of the advances he made in microscope design and use.

- In the early 1930's the first electron beam microscopes were developed which were a breakthrough in technology as they increased the magnification from about 1000x or so up to 250,000x or more. These microscopes use electrons rather than light to examine objects.

•Types of microscopes

Various types of microscopes are available for use in the laboratory. The microscopes have varied applications and modifications that contribute to their usefulness.

•Microscopes are classified according to illumination used into:

1. Optical theory (light) microscope: uses light to observe the magnifiedimage of a specimen or object. Optical microscopes function on the basis of optical theory of lenses by which it can magnifies the image obtained by the movement of a light wave through the sample. The waves used in optical microscopes are electromagnetic and that in electron microscopes are electron beams.

Light microscopes can be classified into:

•Bright field microscope.
•Phase contrast microscope.
• Dark field microscope.
• Fluorescence microscope.
•Polarizing microscope.

2. Electron microscope: uses an electron beam to illuminate the specimen to produce a highly magnified image. There is two major types of electron microscope:

•Scanning Electron Microscope(SEM),
•Transmission electron microscope (TEM).

Parts of a Microscope

1.Eyepiece (Ocular) : The eyepiece consists of a series of lenses mounted in a tube at the upper end of the microscope. Its basic function is to look atthe focused, magnified image projected by the objective lens and magnify that image a second time before the eye looks at the image ofthe specimen.The eyepieces are usually 10x but also come in 5x, 12.5x, 15x, and 20x. The "x" refers to the amount of magnification (power) that thislens adds as a multiplier to the magnification of the objective.

2.Head (Body)

•The head is the upper part of the microscope that connects the eyepiece to the nosepiece or turret.

3.Nosepiece (Turret or Revolving Nosepiece)

•The nosepiece is a rotating turret located above the stage on compound microscopes that can hold multiple objective lenses of various magnifications.

4. Objective Lenses

• The objectives are the lens system closest to the specimen Most compound microscopes have three or four (occasionally five) objectives usually of 4x, 10x, 40x, and 100x (oil immersion) which revolveon a nosepiece (turret) to give different magnifying powers. The 4x, 10x, and 40x are called "dry" objectives which means they operate with air between the objective and the specimen. The 100x is called a "wet"

- objective which means it operates with immersion oil between the lens and the specimen.

• The objective lenses are the most important components of microscopes. Their basic function is to gather the light passing through the specimen and then to present the image up into the body of the microscope.

5. Stage:

•The platform beneath the objectives on which the slide or object to be observed is placed is called a stage. It has a smooth, flat surface . On most compound microscopes, the stage moves up and down and thenosepiece is stationary but on some microscopes just the reverse takes place. The stage has an opening for passing ligh.

6. Condenser Lens (Sub-stage Condenser):

•A glass lens or lens system located within or below the stage on compound microscopes. Its basic function is to gather the light coming from the light source and to concentrate that light into a light cone onto the specimen.

7. Diaphragm

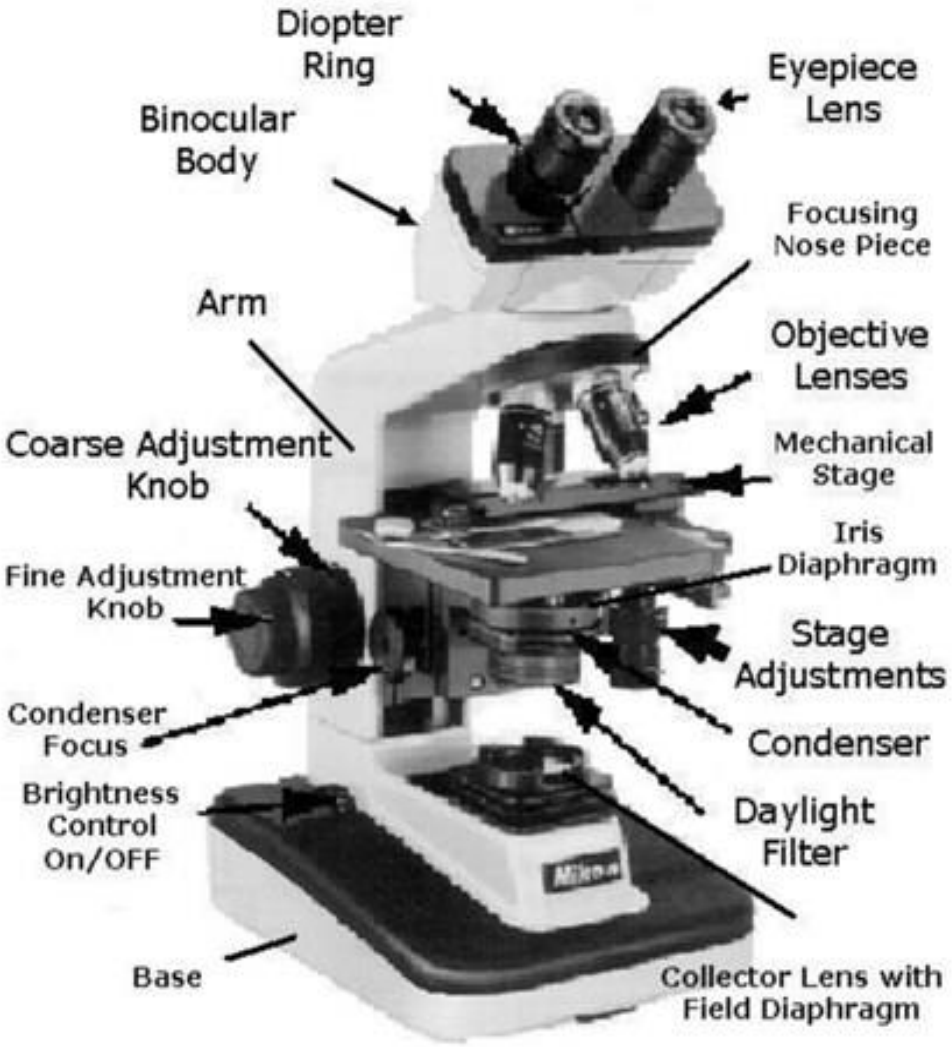
•The diaphragm is also called the sub-stage diaphragm or aperture diaphragm. The diaphragm is normally located under the stage of a microscope and it adjusts the amount of light passing into the slide or specimen.

8. Illumination Systems (Light Source):

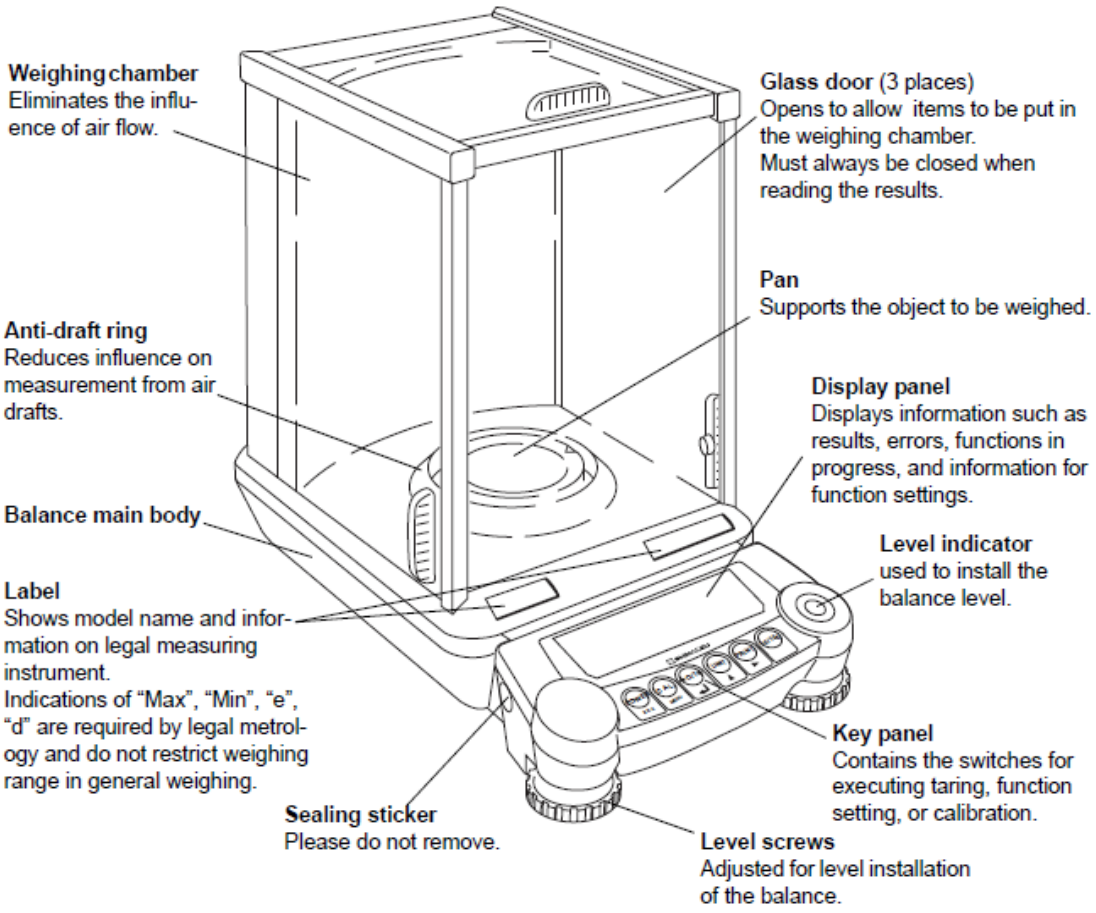
Illumination is the application of light onto an object or specimen in a microscope. The illuminator is the source of light which illuminates the object or specimen to be observed. Illumination of the object or specimen should be bright, free of glare, and evenly dispersed in the field of view.

9.Focus Systems :

•A focus control allows you to adjust the focus of the microscope. Every microscope includes a focusing control (knob) for quick (coarse) focusing of the image.


Diopter Ring
Eyepiece Lens
Binocular Body
Focusing Nose Piece
Arm
Objective Lenses
Coarse Adjustment Knob
Mechanical Stage
Iris Diaphragm
Fine Adjustment Knob
Stage Adjustments
Condenser Focus
Condenser
Brightness Control On/OFF
Daylight Filter
Base
Collector Lens with Field Diaphragm

Analytical balance



An **analytical balance** (often called a "lab balance") is a class of balance designed to measure small mass in the sub-milligram range. The measuring pan of an analytical balance (0.1 mg or better) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation.

Steps of weighting samples

1. Open one of the glass doors of the weighting chamber. Place the weighing container on the pan, and close the glass door again.
2. Wait for the display to stabilize and press tare key. The appearance of the stability mark (➡) indicates a stable state. The display will read Zero.

3. Open the glass door. Place the item to be weighed in the weighing vessel and close the glass door.
4. After the display stabilizes, read the display.

Types of orders

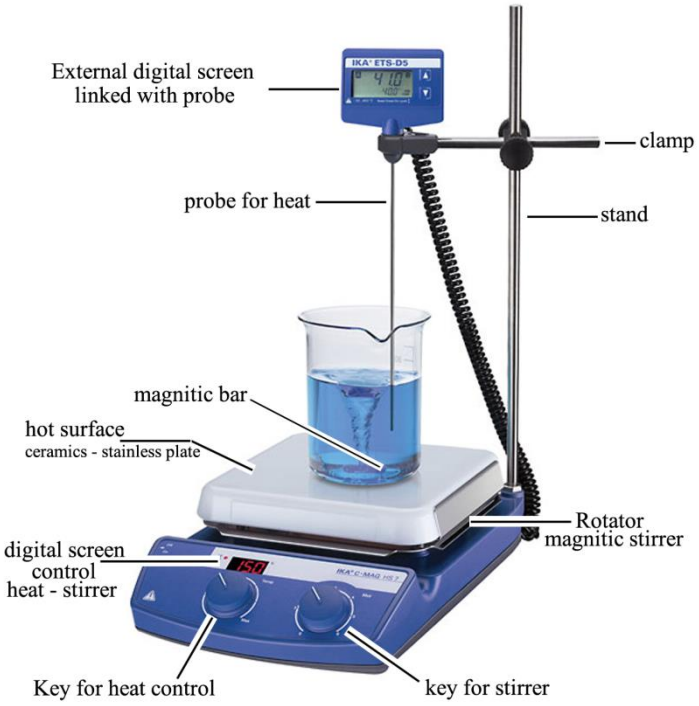
Numeric Decimal	Fractions
0.1 g	**1/10 g**
0.01 g	**1/100 g**
0.001 g	**1/1000 g**
0.0001 g	**1/10000 g (1/10 mg)**
0.00001 g	**1/100000 g (1/100 mg)**

Hotplate device

A hotplate: The variable-temperature hot plate is a very versatile heating unit. The main advantage of using a hot plate is that it provides a flameless variable-temperature heat from a flat surface, so no additional support is normally required when using a flat-bottomed flask.

Hotplate stirrer device

Magnetic stirring is the method of choice if an extended period of continuous agitation is required, since it is easy to set up the apparatus; particularly for small scale set-ups or closed systems.



Heating mantle device

A heating mantle: A heating mantle is a specialized kind of heating device designed to be used only with round-bottomed flasks when liquids are being heated under reflux or are being distilled. There are different heating mantles for each size of flask.

• Dark field and phase contrast microscopes:

Live cells in generally lack sufficient contrast to be studied, since the internal structures of the cell are colorless and transparent.

•The most common way to increase contrast is to stain the different structures with selective dyes, but this often involves killing and fixing the sample. Staining may also introduce **artifacts**(apparent structural details that are caused by the processing of the specimen and are thus not a feature of the specimen).

•The human eye is not sensitive to the difference in light phase when passing into two different media with different refractive indices, but clever optical solutions have been thought out to change this difference in phase into a difference in light intensity.

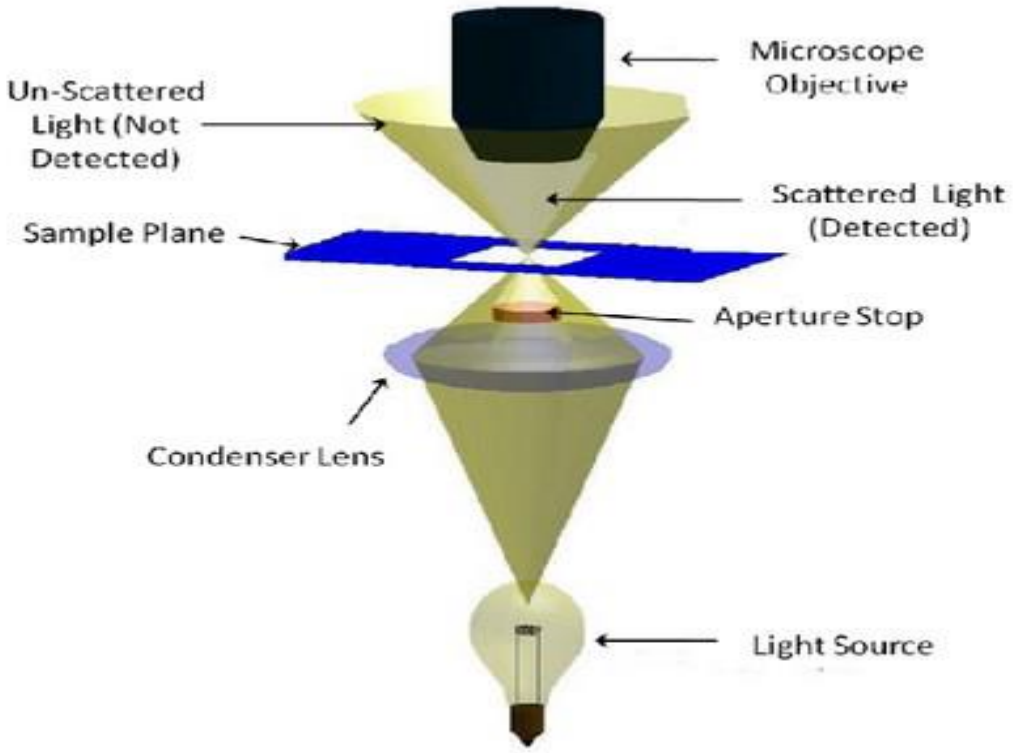
•Illumination techniques

•Many techniques are available which modify the light path to generate an improved contrast image from a sample.

Dark field microscopy: is a technique for improving the contrast of unstained, transparent specimens by using a carefully aligned light source to minimize the quantity of directly transmitted (unscattered) light passing the sample surroundings and collecting only the light scattered by the sample.

•Principal:

•The steps are illustrated in the figure where an upright microscope is used:



(Diagram illustrating the light path through a dark field microscope.)

• Light enters the microscope for illumination of the sample.
• A specially sized disc, the patch stop (see figure) blocks some light from the light source, leaving an outer ring of illumination.
• The condenser lens focuses the light towards the sample.
• The light enters the sample. Most is directly transmitted, while some is scattered from the sample.
• The scattered light (diffracted light) enters the objective lens, while the directly transmitted light simply misses the lens and is not collected.
• Only the scattered light goes on to produce the image, while the directly transmitted light is omitted.

Advantage : Dark field can dramatically improve image contrast – especially of transparent objects – while requiring little equipment setup or sample preparation.

•Disadvantage:

1. The technique suffers from low light intensity in final image of many biological samples.
2. Low resolution.

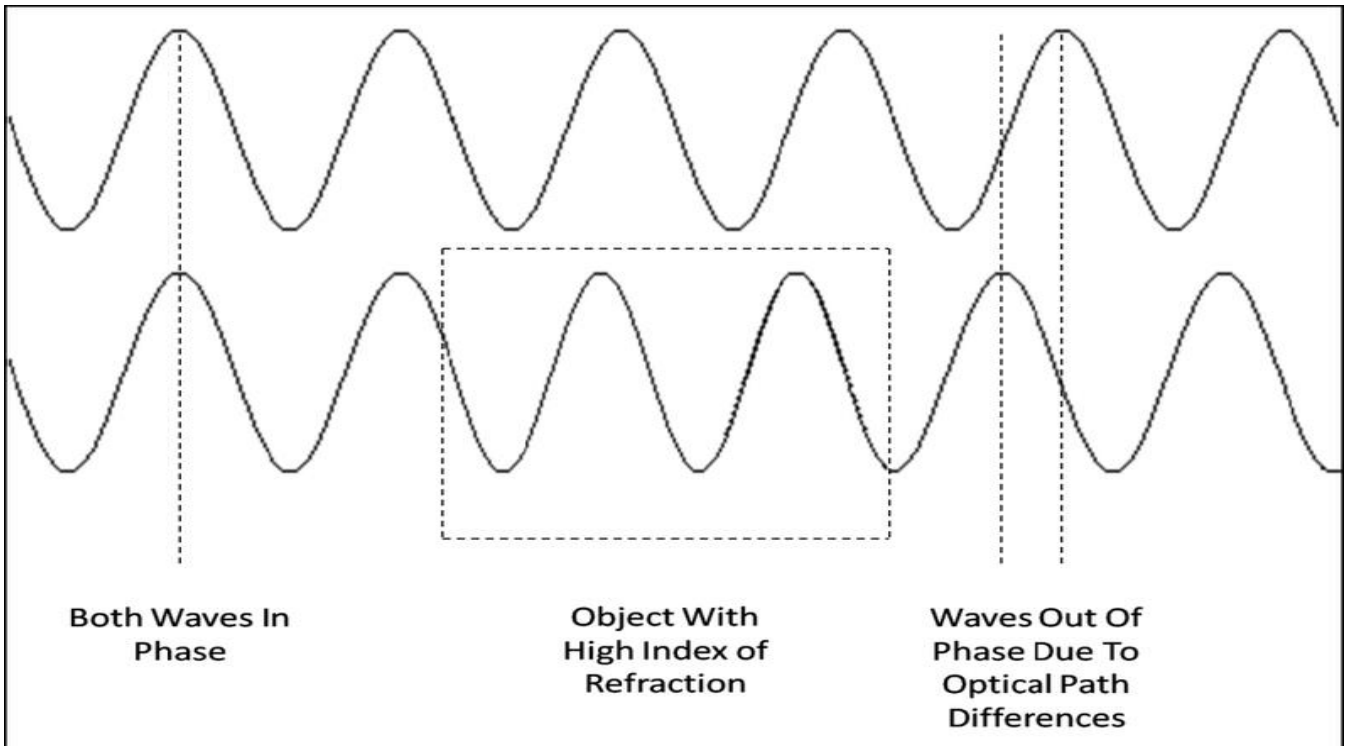
•Phase contrast microscope

•Phase-contrast microscopy: is a method to manipulate light paths through the use of properly placed rings in order to illuminate transparent objects.

•Dutch physicist Fritz Zernike developed the technique in the 1930s, for which he was awarded the Nobel Prize in 1953.

•In this technique, Phase contrast microscopes transforms differences in the phase of light waves diffracted by an

object to differences in the image, making objects appear as if they had been optically stained.



•Internal details and organelles of live unstained organisms can be seen clearly with this microscope (e.g. mitochondria, lysosomes, and the Golgi body).Light passing through cellular structures, such as chromosomes or mitochondria is retarded because they have a higher refractive index than the surrounding medium. Elements of lower refractive index advance the wave.

•**Advantage:**

•Phase-contrast microscopy allows the visualization of living cells in their natural state with high contrast and high resolution.

•**Disadvantage:**

•halo formation called halo-light ring.

Fluorescent Microscopy

•A fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances.

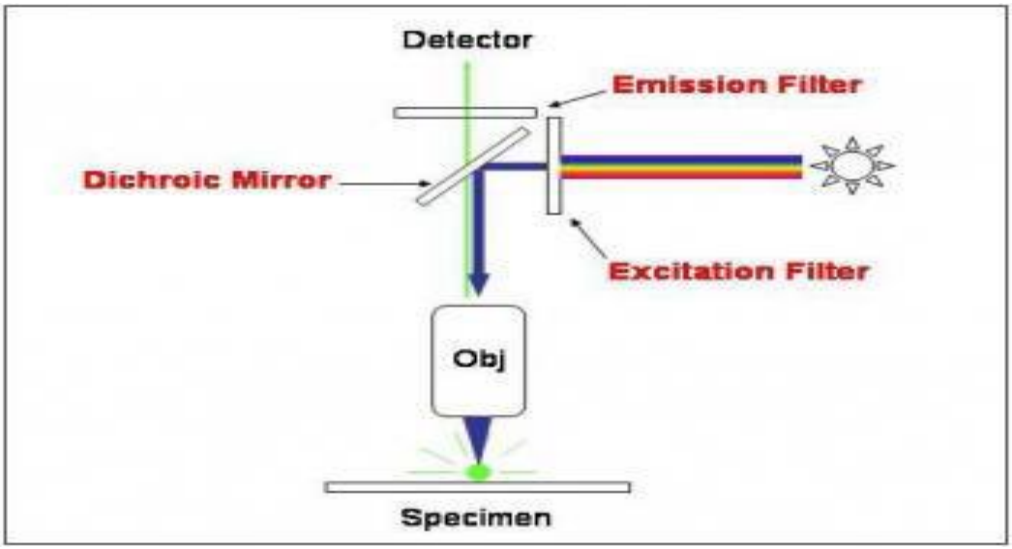
•In most cases the sample of interest is labeled with a fluorescent substance known as a fluorophore and then illuminated through the lens with the higher energy source. The illumination light is absorbed by the fluorophores (now attached to the sample) and causes them to emit a longer lower energy wavelength light. This fluorescent light can be separated from the surrounding radiation with filters designed for that specific wavelength allowing the viewer to see only that which is fluorescing.

Principle:

•The basic task of the fluorescence microscope is to let excitation light radiate the specimen and then sort out the much weaker emitted light from the image. First, the microscope has a filter that only lets through radiation with the specific wavelength that matches your fluorescing material. The radiation interferes with the atoms in your specimen and electrons are excited to a higher energy level. When they relax to a lower level, they emit light. To become detectable (visible to the human eye) the fluorescence emitted from the sample is separated from the much brighter excitation light in a second filter. This works

because the emitted light is of lower energy and has a longer wavelength than the light that is used for illumination.

The majority of fluorescence microscopes, especially those used in the life sciences are of the epifluorescence design .Light of the excitation wavelength is focused on the specimen through the objective lens. The fluorescence emitted by the specimen is focused to the detector by the same objective that is used for the excitation which for greatest sensitivity will have a very high numerical aperture. Since most of the excitation light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light. An additional barrier filter between the objective and the detector can filter out the remaining excitation light from fluorescent light.



Applications:

•The refinement of epi-fluorescent microscopes and advent of more powerful focused light sources, such as lasers, has led to more technically advanced scopes such as the confocal laser scanning microscopes (CLSM) and total internal reflection fluorescence microscopes (TIRF).

•CLSM's are invaluable tools for producing high resolution 3-D images of subsurfaces in specimens such as microbes. Their advantage is that they are able to produce sharp images of thick samples at various depths by taking images point by point and reconstructing them with a computer

•These microscopes are often used for -

•Imaging structural components of small specimens, such as cells

•Conducting viability studies on cell populations (are they alive or dead?)

•Imaging the genetic material within a cell (DNA and RNA)

•Viewing specific cells within a larger population with techniques such as FISH

Electron microscope:

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

•It has a greater resolving power than a light microscope and can reveal the structure of smaller objects. They can achieve better than 50 picometer resolution and magnifications of up to about 10,000,000x whereas ordinary light microscopes are limited to about 200 nm resolution and magnifications below 2000x.

•The electron microscope uses electron optical lenses to control the electron beam and focuses it. The role of electron optical lenses is similar to the glass lenses of a light optical microscope.

•Electron microscopes are used to investigate the structure of a wide range of biological and inorganic specimens including microorganisms, large molecules, biopsy samples, metals, and crystals.

The main component of electron microscope

•**1. Electron gun (cathode):** a heated filament or crystal, made from the metal tungsten that releases electrons when a high voltage is pass through.

•**2. Electromagnetic lens**: is a coil of wire through which current flows, creating magnetic fields that manipulate the electron beam, much the same way that optical lenses focus and direct light.

3. **Vacuum system** to ensure that the microscope is operated under a high vacuum to maintain the integrity of the electron beam.

4. Camera or detector

5. Computer

*** Principle**

•1. The beam is produced by the electron gun at the top of the instrument.

•2. The electron beam then pass down through the vacuum system.

•3. Because electrons change their path when pass in electromagnetic field (electromagnetic lens) the beam can be focused.

•4. First lens is a condenser which focuses the beam of electron on the specimen.

5. Some electrons interact with the specimen and are modified while other crosses the specimen without interacting.

•6. Electron passing through the specimen reach the objective lens, which form a focused magnified images that is then magnified further through other lenses and captured on screen.

•Types

• **1.Transmission electron microscope** is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen that permits resolution around 3 nm, this high resolution allow magnification of up to 400,000 times with isolated molecules or particles

very thin tissue can be observed with detail at magnification of up to about 120 000 times.

•**Scanning electron microscope** permits a three dimensional image of the surfaces of cell, tissue, and organs. Like the transmission electron microscope this microscope produces and focuses a very narrow beam of electrons, but in this type the beam does not pass the specimen. Instead the surface of the specimen is first dried and coated with a very thin layer of metal which electron interact with and produces reflected electrons these are captured by the detector and results a black and white image on monitor.

Centrifuge: A centrifuge is a device for separating particles from a solution according to their size, shape, density by subjecting high gravitational force.

•In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it float to the top. If there is no difference in density the particles stay steady.

• To take advantage of tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful "centrifugal force" provided by a centrifuge.

• A centrifuge is used to separate particles or macromolecules such as :
• -Cells
• -Sub-cellular components (mitochondria, ribosome, membranes)
•-Proteins
• -Nucleic acids (DNA, RNA).
•- salts

Basis of separation:
• -Size
• -Shape
•-Density

Types of Centrifugal Separations ☺

1. Differential centrifugation:

Separation is based on the size of the particles. This type of separation is commonly used in obtaining partially-pure preparation of subcellular organelles and macromolecules. During centrifugation of a cell homogenate, larger particles sediment faster

than smaller ones. A cell homogenate can be centrifuged at a series of higher gravitational-forces and times to generate pellets of partially-purified organelles.

2. Density gradient centrifugation:

Density gradient centrifugation is the preferred method to purify subcellular organelles and macromolecules. Density gradients can be generated by placing layer after layer of gradient media such as sucrose in a tube with the heaviest layer at the bottom and the lightest at the top. The cell fraction to be separated is placed on top of the layer and centrifuged. Density gradient separation can be classified into two categories.

•A. Rate zonal (size) separation

Rate-zonal separation takes advantage of particle size and mass instead of particle density for sedimentation. Examples of common applications include separation of cellular organelles such as separation of proteins, such as antibodies. For example, Antibody classes all have very similar densities, but different masses. Thus, separation based on mass will separate the different classes, whereas separation based on density will not be able to resolve these antibody classes.

B. Isopycnic (density) separation

In this type of separation, a particle of a particular density will sink during centrifugation until a position is reached where the density of the surrounding solution is exactly the same as the density of the particle. Once this is reached, the time length of centrifugation does not have any influence on the movement of the particle. A common example for this method is separation of nucleic acids in a CsCl gradient.

Centrifuge Models

1. swinging-bucket In swinging bucket rotors:

The sample tubes are loaded into individual buckets that hang vertically while the rotor is at rest. When the rotor begins to rotate the buckets swing out to a horizontal position . This rotor is particularly useful when samples are to be isolated in density gradients but relatively inefficient for pelleting.

2.fixed-angle:

The sample tubes are held fixed at the angle of the rotor cavity. This rotor type is most commonly used for pelleting. Examples include pelleting bacteria, yeast, and other mammalian cells.

3- Vertical rotor:

sample tubes are held in vertical position during rotation. This type of rotor is not suitable for pelleting plasmid DNA, RNA, and lipoprotein isolations.

•Principles of Centrifugation

•When a suspension is rotated at a certain speed or revolutions per minute (RPM), centrifugal force causes the particles to move away from the axis of rotation. The force on the particles compared to gravity is called Relative Centrifugal Force (RCF). For example, an RCF of 500 x g indicates that the centrifugal force applied is 500 times greater than Earth gravitational force.

Lectuer 5

Spectrophotometer

spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear range of absorption or reflectance measurement.

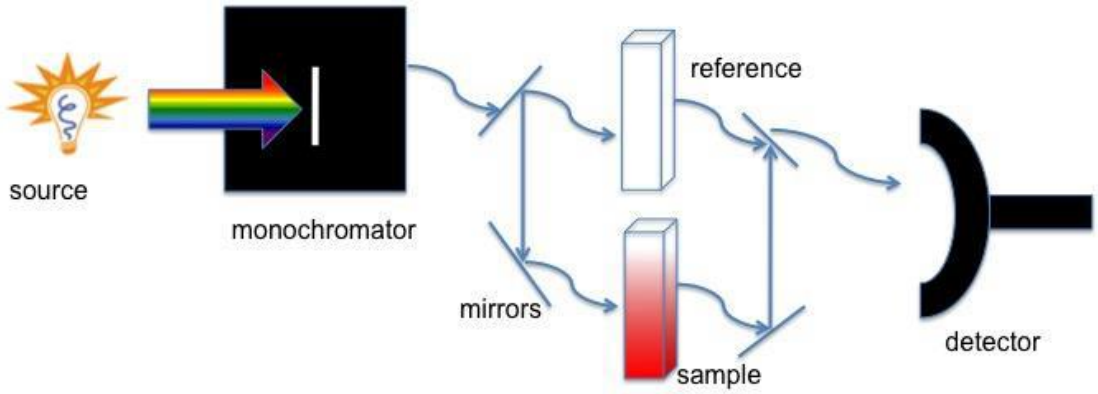
A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases. However they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200 nm – 2500 nm using different controls and calibrations. Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the *photometric determination*.

The use of spectrophotometers spans various scientific fields, such as physics, materials science, chemistry, biochemistry, and molecular biology .

Type of spectrophotometer

There are two major classes of devices: single beam and double beam.

1- double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample.



2- single-beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted.

Although comparison measurements from double-beam instruments are easier and more stable, single-beam instruments can have a larger dynamic range and are optically simpler and more compact. Additionally, some specialized instruments, such as spectrophotometers built onto microscopes or telescopes, are single-beam instruments due to practicality.

Historically, spectrophotometers use a monochromator containing a diffraction grating to produce the analytical spectrum. The grating can either be movable or fixed.

In short, the sequence of events in a modern spectrophotometer is as follows:

1. The light source is imaged upon the sample.

2. A fraction of the light is transmitted or reflected from the sample.

3. The light from the sample is imaged upon the entrance slit of the monochromator

4. The monochromator separates the wavelengths of light and focuses each of them onto the photodetector sequentially.

UV-visible spectrophotometry :

The most common spectrophotometers are used in the UV and visible regions of the spectrum, and some of these instruments also operate into the near-infrared region as well.
Visible region 400–700 nm spectrophotometry is used extensively in colorimetry science.