Shree H. N. Shukla Institute of science

B. Sc

Semester-6

STUDY MATERIAL

Subject Name: ANALYTICAL TECHNIQUES AND BIOINFORMATICS

Subject Code: 602

UNIT III: MOLECULAR TECHNIQUES AND BIOSENSOR TECHNOLOGY

Contains

Electrophoresis

Electrophoresis is defined as the migration of charged ions in an electric field. In metal conductors, electric current is carried by the movement of electrons, largely along the surface of the metal. In solutions, the electric current flows between electrodes and is carried by ions. The ions that migrate towards the anode, because of their anodic migration, are called “anions”. The ions which will migrate to the cathode are called “cations”.

Principle: When charged molecules are placed in an electric field, they migrate toward either the positive or negative pole according to their charge. In contrast to proteins, which can have either a net positive or net negative charge, nucleic acids have a consistent negative charge imparted by their phosphate backbone, and migrate toward the anode.

An ion placed in such an electric field will experience a force:
Where,
F = electrophoretic force
K = a constant
q = net charge on the protein (atomic charges/protein molecule)

This force will cause the protein to accelerate towards either the cathode or the anode, depending on the sign of its charge. Of course there are other forces such as frictional force when ions move in the electric field. The influence of them cannot be understood easily by a formula, so we omit it.
Electrophoresis exploits the fact that different ions have different mobility in an electric field and so can be separated by this way.

Proteins and nucleic acids are electrophoresed within a matrix or "gel". Most commonly, the gel is cast in the shape of a thin slab, with wells for loading the sample. The gel is immersed within an electrophoresis buffer that provides ions to carry a current and some type of buffer to maintain the pH at a relatively constant value.

The gel itself is composed of either agarose or polyacrylamide, each of which has attributes suitable to particular tasks.

Factors influencing Electrophoresis:
Movement of proteins depends on various aspects. Within the gel the molecules must pass through as they are moving from one pole to another. The smaller molecules can weave in and out of the matrix of the gel with more ease, compared with larger molecules. As a general rule, the molecules move rapid if it has more net charge, has a shape of ball and shorter diameter

1) The buffer pH
It will influence the direction and rapid of the protein migration.
Movement of proteins depends on various aspects; one of them is the charges on the proteins. Proteins are sequence of amino acids that can be ionized depend on their acid or basic character. The protein's net electric charge is the sum of the electric charges found on the surface of the molecule as a function of the environment.

2) The buffer ionic strength
It influences the proportion of the current carried by the proteins
At low ionic strength the proteins will carry a relatively large proportion of the current and so will have a relatively fast migration. At high ionic strength, most of the current will be carried by the buffer ions and so the proteins will migrate relatively slowly.

An analogy might be useful in visualizing this effect of ionic strength. Imagine a bank where there are two counters – one for deposits the anode) and one for withdrawals (= the cathode), with electrons being the money. The ions may be considered as customers waiting to be served at either counter, which one can visualize as being at opposite ends of the banking hall.

In electrophoresis, therefore, a low ionic strength is preferred as it increases the rate of migration of proteins. A low ionic strength is also preferred as it gives a lower heat generation. Assuming a constant voltage, if the ionic strength is increased, the electrical resistance decreases but the

current will increase. A high ionic strength buffer will therefore lead to greater heat generation, and so a low ionic strength is preferred.

3) The voltage gradient

The rate of migration will depend on the voltage gradient: There is more voltage gradient in the electric field, protein will move towards the anode (or the cathode) at a faster rate.

4) Electo-osmosis

Liquid's relative move upon solid medium in an electric field is called electo-osmosis. In applied electric field, electo-osmosis distorts the sample stream and limits the separation. For example, Paper electrophoresis has poor resolution because of electo-osmosis. The surface of paper has -e, so the buffer has +e derived from hydrogen ions because of electrostatic induction.
Then +e drive buffer to cathode in electric field,these flows distort the electrophoretic migration of sample by causing a varying residence time. Thus, sample will move more or less than normal.

- Electrophoresis is basically of two types Free boundary or moving boundary electrophoresis.
- Zone electrophoresis. Moving boundary electrophoresis
- It is a type of electrophoresis without supporting media, in a free solution.
- Tiselius developed this type of electrophoresis in 1937.
- For the separation of different charged molecules in a mixture, sample is placed in glass, which is connected to the electrodes. On applying electric potential across the tube, charged molecule migrates towards one or another electrode.

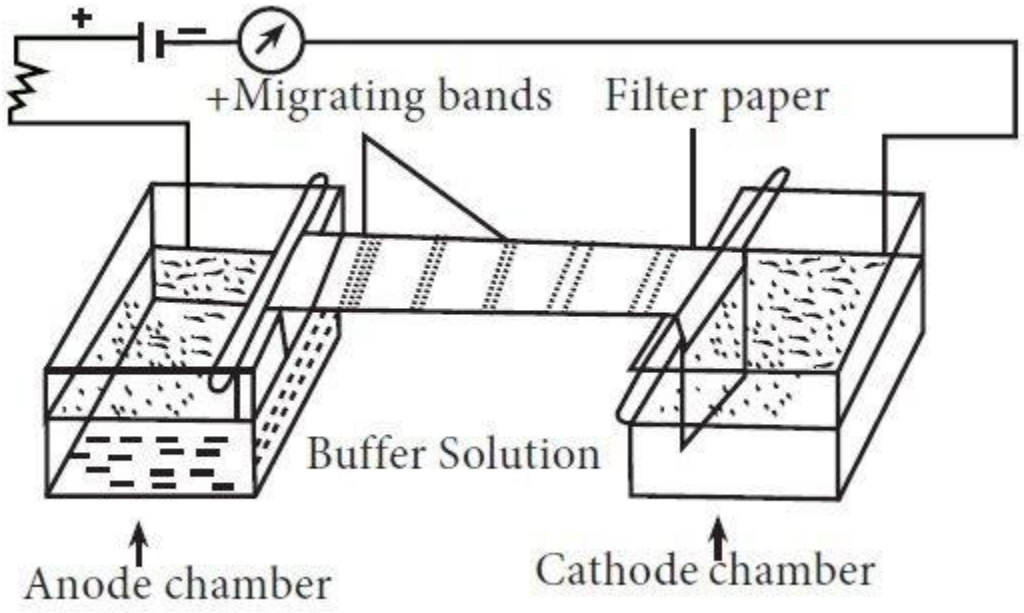
Zone electrophoresis

- It involves the separation of charged particles on inert matrix, or supporting or stabilizing media.
- On the basis of supporting media, it is of following types
- Paper electrophoresis
- Cellulose acetate electrophoresis
- Capillary electrophoresis
- Gel electrophoresis.
- The equipment used for the electrophoresis basically consists of two items:
- An electrophoretic unit and a power pack.

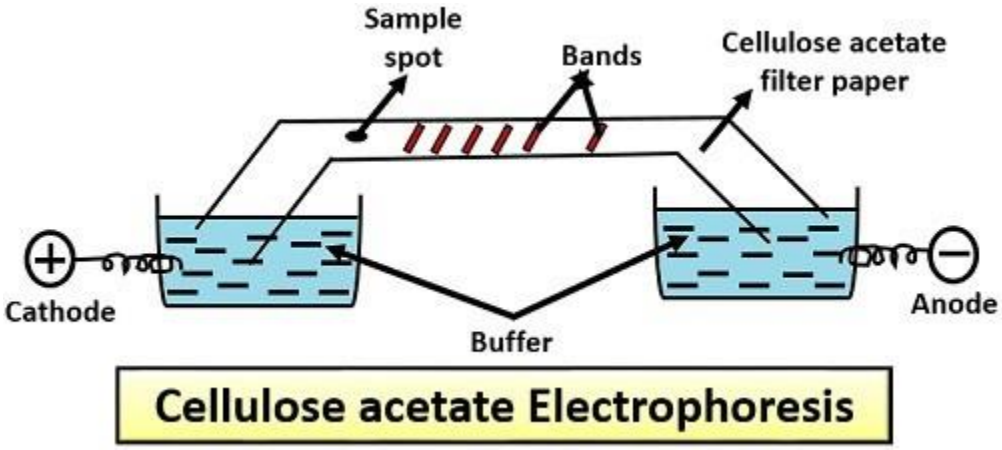
Electrophoretic unit is of two types:

- Vertical electrophoretic unit
- Horizontal electrophoretic unit
- Vertical electrophoretic unit

- It is also known as vertical slab gel units.
- It is available commercially and mostly used to separate proteins in acrylamide gels.
- In this, gel is formed between the two glass plates that are clamped together but uses plastic spacers to hold them apart.
- Dimensions of gel are typically: 8.5 cm wide X 5 cm high, Thickness = 0.51 mm.
- Horizontal electrophoretic unit
- In this, a gel is cast on a plate, horizontally and submerged in a running buffer.
- This apparatus is mostly used to separate nucleic acid or proteins in agarose gel.
- Paper electrophoresis

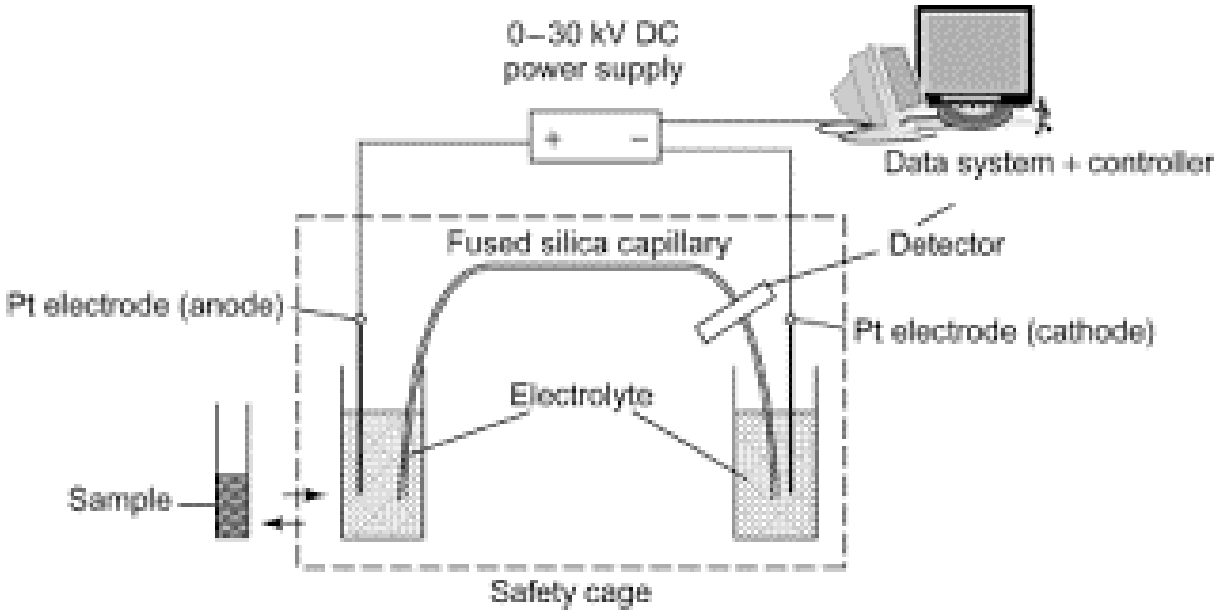


- Filter paper is very popular as a stabilizing media and most commonly used for the study of normal and normal plasma proteins.
- For electrophoresis Chromatography paper is most suitable and it needs no preparation other than to be cut to size.
- Apparatus: It consists basically of two items, a power pack and an electrophoretic cell.
- The power pack provides a stabilized direct current and has controls for both voltage and current output. Power packs, which have an output of 0-500 V and 0-150 rnA are available and can be programmed to give either constant voltage or current.
- Cellulose acetate electrophoresis



Cellulose acetate Electrophoresis

- Kohn in 1958 introduced, Cellulose acetate as a medium for electrophoresis.
- It was developed from bacteriological cellulose acetate membrane filters and is commercially available as high purity cellulose acetate strips, which are thin and have a uniform micropore structure.
- Cellulose acetate is especially used for clinical investigations such as separation of hemoglobin's from blood, lipoproteins and glycoproteins.
- Buffers used in both the electrophoresis i.e., in paper and cellulose acetate electrophoresis are same Capillary electrophoresis

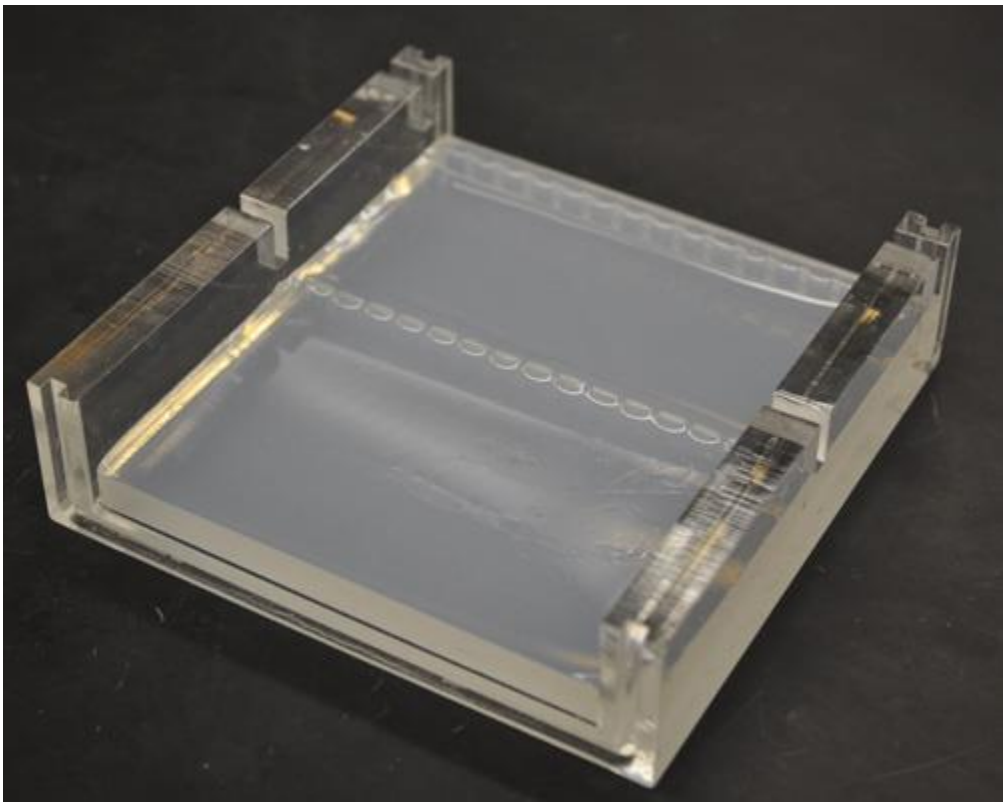


- It can be described as the new generation electrophoretic technique.
- Commercially available CE instruments consist of
- An electrolyte-filled capillary, which passes through the optical center of a detector
- A sample injector

- A high voltage power supply an auto-sampler. The entire instrument is computer controlled.
- This technique of electrophoresis has become very popular over the years because of the many advantages that it has over conventional electrophoresis techniques.
- These advantages are listed below:
- Very high-level automation is possible.
- Fast analysis times.
- Detection of separated peaks is done online; thus, detection is a process that goes hand in hand with separation and not post-separation as is the case with conventional techniques.
- Heat generated inside the capillary is effectively dissipated through the walls of the capillary; therefore, high voltages can be applied.
- High voltages mean a rapid separation.
- This faster separation along with online detection makes this technique considerably faster as compared to the conventional techniques.
- Gel electrophorisis is simple, rapid and sensitive analytical technique for the separation of charged particle.
- The gels, however, are porous and the size of the pores relative to that of the molecule determines whether the molecule will enter the pore and be retarded or will bypass it. The separation thus not only depends on the charge on the molecule but also on its size. Needless to say, that resolution of a sample is sharper and better in a gel than in any other type of medium.
- Agrose gel is used as a supporting media for the separation of DNA, RNA or protein under the influence of electric charge.
- Most of the biomolecules has a net charge at any pH other than at their isoelectric point.
- There is difference in the electrophoretic mobility of these charged molecules due to their difference in size, shape, and charge.
- There are basically two types of materials are used to make gels:
  1) Agarose
  2) Polyacrylamide

Agarose

- Agarose is natural colloid which is isolated from the seaweed.
- It is linear polysaccharide.
- It is made up of repeating units of agarobiose, comprises alternating units of 3,6-anhydrolactose and galactose.
- This gel has generally larger pore size, which makes them suitable to separate larger molecules having molecular mass more than 200 kDa.
- It is most commonly used for the electrophoresis of both protein and nucleic acids.
- Agarose is used in concentration between 1% and 3%.



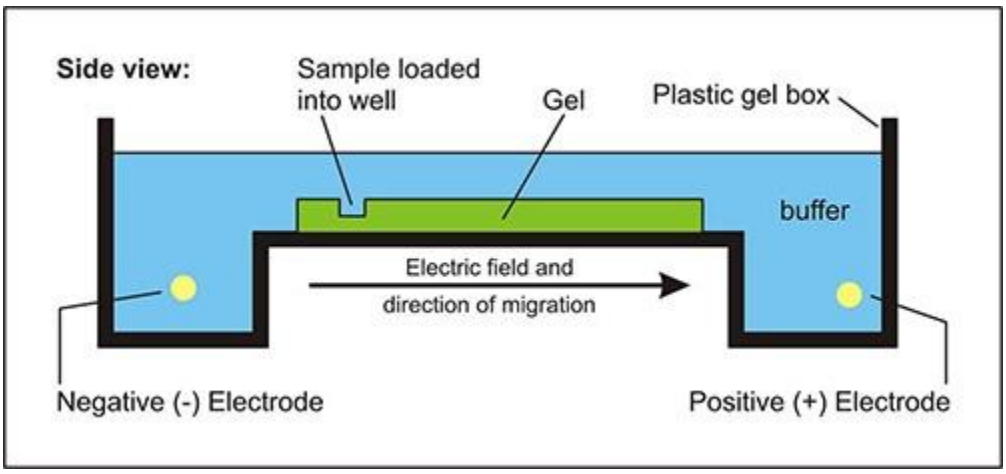
Polyacrylamide gel

- Polyacrylamide gel is consisting of chains of acrylamide monomers crosslinked with N, N'-methylenebisacrylamide units, which is commonly termed as bisacrylamide.
- In this gel, pore size and resolving power is totally depends upon the concentration of acrylamide and bisacrylamide.
- The concentration of the gel normally varies from 5% to 25%.
- This gel is used in electrophoresis for the separation of proteins ranging from molecular weight <5000 to >200,000, and polynucleotides ranges from <5 to ~ 3000 base pairs in size.
- Apparatus of gel electrophoresis
- Vertical gel apparatus: It is commonly used IN sds PAGE for the separation of proteins.
- Horizontal gel apparatus: It is used for immune electrophoresis, iso-electric focusing and electrophoresis of DNA and RNA in the agarose gel.

❖ **Type of gel electrophoresis**

1) Agarose gel electrophoresis
2) SDS-PAGE
3) Pulse field gel electrophoresis (PFGE)
4) 2D gel electrophoresis

- Agarose gel electrophoresis
- Agarose gel the supporting media in the electrophoresis.
- For the electrophoresis of DNA, RNA and Protein agrose gel is used.



Principle

In the agrose gel electrophoresis the potential difference is applied across the electrodes in a horizontal electrophoretic tank containing agarose gel and biomolecules (such as nucleic acid or proteins) is loaded, then molecules migrated to their respective electrodes. The rate of migration of charged particles depends on the size, shape, molecular mass etc.

In this process, larger molecules have difficulty in moving through the pore size of the supporting media, whereas the smaller molecules has more mobility through it. The bands of protein or nucleic acid is visualized by using intercalating dye, i.e., ethidium bromide (Etbr), they are visualized by fluorescence when illuminated with ultraviolet lights.

- Requirement/ instrumentation:
- An electrophoretic unit,
- A power supply,
- Gel casting trays
- Combs
- Agarose gel or media
- Electrophoresis buffer

Composition and ionic strength of electrophoresis buffer is most important factor for the separation of nucleic acids (DNA or RNA).

Most routinely used buffers are:

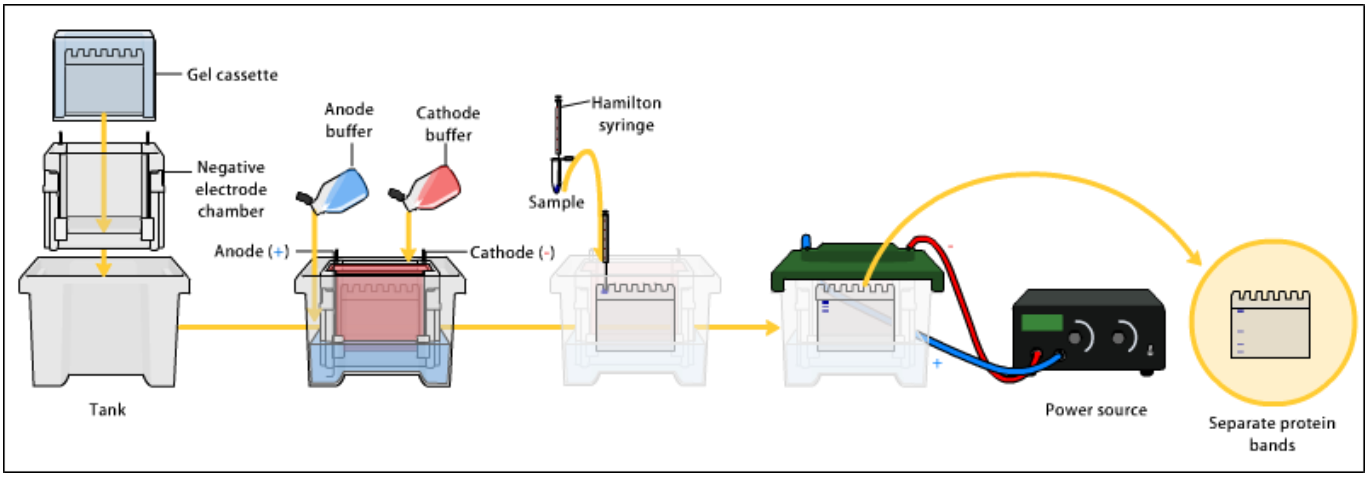
- **TAE- (Tris-acetate-EDTA),** it has lower buffering capacity and generally used to separate larger nucleic acid fragments (>12kb).
- TBE- (Tris-borate-EDTA), it has high buffering capacity and higher ionic strength and generally used for the separation of low molecular weight compound (<1kb).
- **Loading buffer:** Nucleic acid is before loading on to a gel is first mixed with the gel loading buffer, which usually consists of:-
- **Salts:** It creates environment with favorable ionic strength and pH of the sample, e.g., Tris-HCl.
- **Metal chelator**: It prevents nucleases to degrade the nucleic acid such as EDTA.
- **Loading dyes**: It provides color for tracking and easy monitoring of sample. Such as, bromophenol blue, xylene cyanol.
- **Transilluminator**: (An ultraviolet light box), which is used to visualize bands in gels.

Applications

- In molecular genetic diagnosis or genetic fingerprinting for analysis of PCR products.
- For the estimation of size of DNA molecule.
- In the separation of restricted DNA and RNA.
- In addition to providing an excellent medium for fragment size analyses, agarose gels allow purification of DNA fragments.

SDS-PAGE Electrophoresis

- Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis is routinely used for the separation of proteins on the basis of their mass.
- It involves the use of vertical gel apparatus to separate proteins.



Principle

This technique uses anionic detergent sodium dodecyl sulfate (SDS) which disassociates proteins into their individual polypeptide subunits and gives a uniform negative charge along each denatured polypeptide. When these denatured polypeptides are loaded at the cathode end of an electric field, then we get clear bands of proteins arranged in decreasing order of their molecular mass from the cathode to anode.

Requirement/ instrumentation

- Gel or media acrylamide solutions.
- Buffer system the separation and migration patterns of proteins in gel electrophoresis are determined by the chemical composition and pH of the buffer system.

Three basic types of buffers are required:

1) Gel casting buffer to cast gel
2) Sample buffer to prepare the sample
3) Running buffer to fill the electrode reservoirs

Instrumentation of SDS PAGE

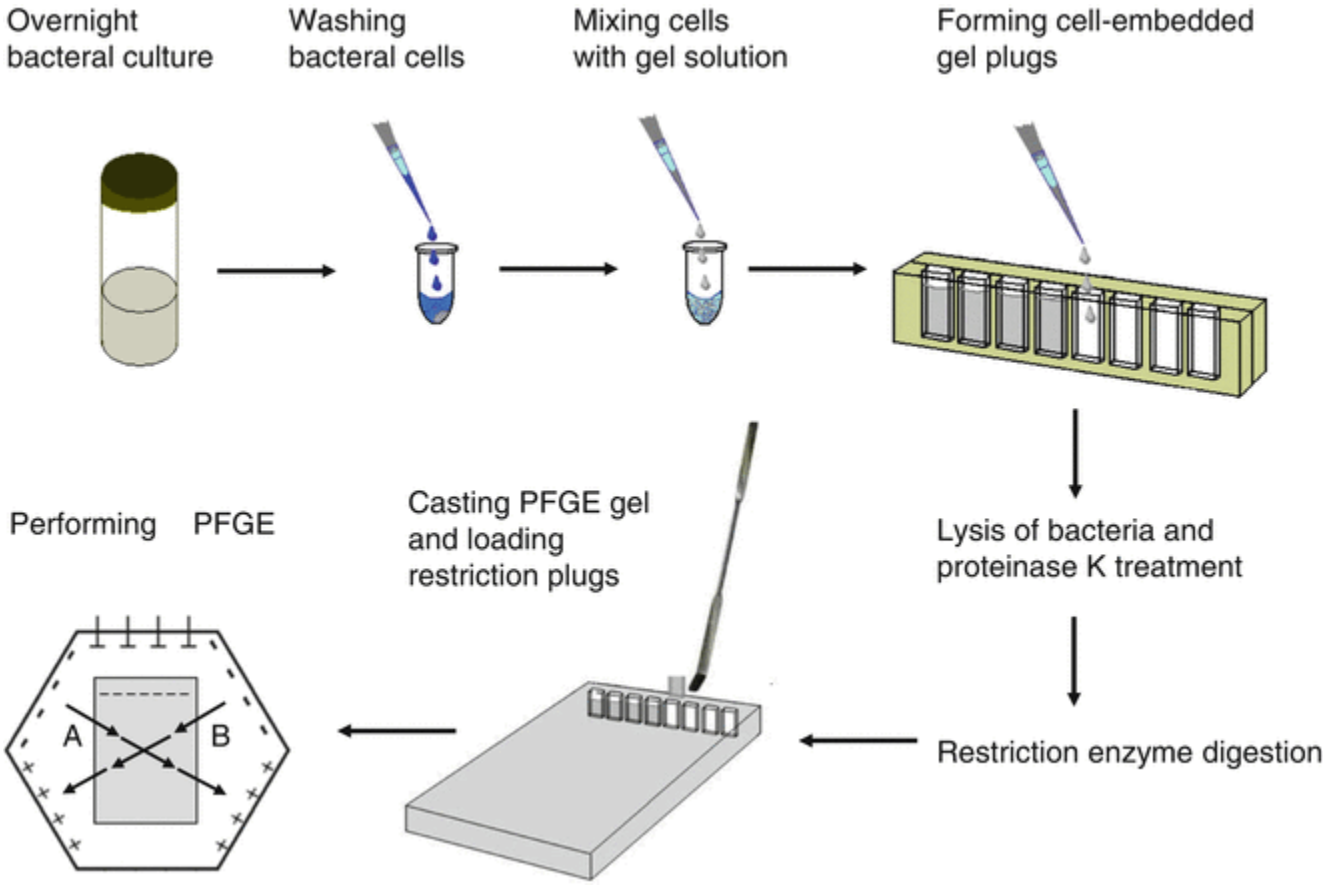
- lower reservoir amine buffer with HCl (running gel)
- Upper reservoir amine buffer with glycine (stack gel)
- Staining or tracking dye
- Protein samples
- Molecular weight markers
- An electrophoresis chamber and power supply
- Glass plates (a short and a top plate)
- Casting frame
- Casting stand
- Combs

Applications

- For measuring molecular weight.
- In Peptide mapping.
- For Estimation of protein size.
- For Determination of protein sub-units or aggregation structures.
- For Estimation of protein purity.
- In Protein quantitation.
- In monitoring protein integrity.

❖ Pulse field gel electrophoresis (PFGE)

- Conventional agarose gel electrophoresis cannot separate linear double stranded DNA molecules that have radius of gyration, which is larger than the pore size of the gel.
- However, with certain changes in the orientation of electric field with respect to the gel, large DNA fragments can be resolved.
- It is used to separate large DNA molecule by applying gel matrix as electric field that periodically changes direction.
- This technique is invented by Schwartz and Cantor in 1984.
- DNA fragments up to 10 mb can be separated by this technique.



Principle

- As opposed to the continuous unidirectional electric fields applied in conventional gel electrophoresis, pulsed-field gel electrophoresis uses pulsed, alternating, orthogonal electric fields. When such a field is applied to a gel, large DNA molecules become trapped into their reptation tubes every time the direction of the electric field is changed.
- These molecules remain immobile till they reorient t themselves along the direction of the new electric field. It is here that different DNA molecules adapt a behavior consonant with their

respective sizes; large DNA molecules take a longer time to reorient themselves and are consequently retarded more in the new electric field as compared to the smaller DNA molecules.

- Thus, all those molecules of DNA whose reorientation times are less than the period of the electric pulse can be fractionated in a size dependent manner. Factors, which are of extreme importance for determining the limit of resolution of pulsed-field gel electrophoresis are given below:
  - → The absolute periods of the electric pulses.
  - → The angles at which the two electric fields are applied to the gel.
  - → The relative field strengths of the two electric fields and the degree of uniformity of the two electric fields.
  - → The ratio of the periods of the electric pulses employed to generate the two electric fields.
  - → Instrumentation
  - → The original apparatus used pulsed electric fields or perpendicular orientations and linear electrodes.

Applications

→ It is used for the separation of DNA more than 10 mb.
→ It generated stable and reproducible DNA restriction patterns.
→ This technique applied to the sub-typing of many pathogenic bacteria and has high concordance with epidemiological relatedness.
→ This technique can be applied as a universal generic method for sub-typing of bacteria.

❖ **2D gel electrophoresis**

- Analysis of sample by one-dimensional electrophoresis is the most common form of protein gel electrophoresis.
- For separation and analysis of hundreds to thousands of proteins in one gel, a powerful electrophoretic method called two-dimensional gel electrophoresis is used.
- 2D gel electrophoresis separates a mixture of proteins according to two properties, one in each dimension.
- The first dimensions involve the separation based on native isoelectric point (pI), using form of electrophoresis called isoelectric focusing (IEF).
- Second dimensions separate mass using SDS-PAGE.
- This technique provides highest resolution for the protein analysis.

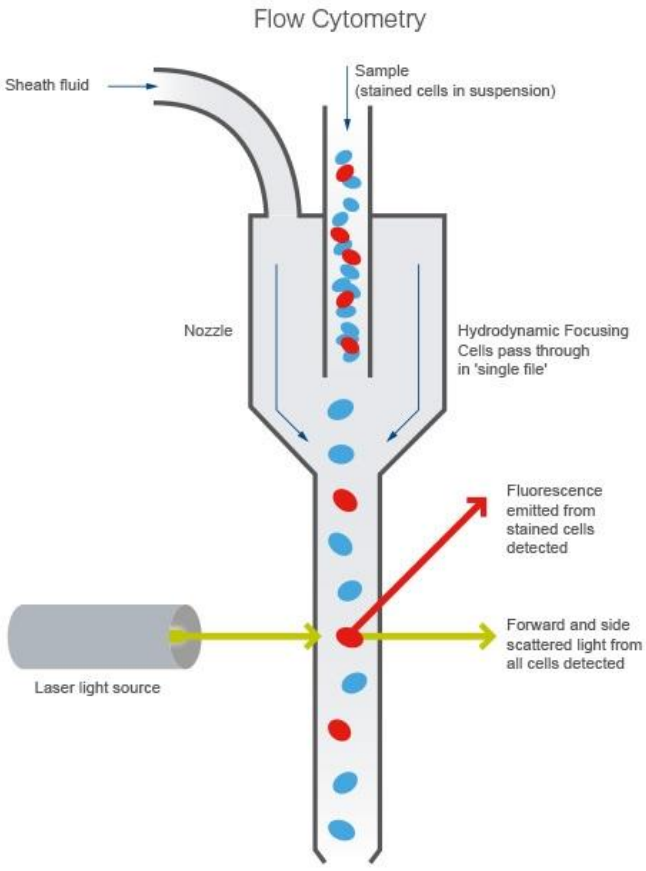
❖ Flow cytometry

Flow cytometry is a widely used method for analyzing the expression of cell surface and intracellular molecules, characterizing and defining different cell types in a heterogeneous cell population, assessing the purity of isolated subpopulations, and analyzing cell size and volume. It allows simultaneous multi-parameter analysis of single cells.

It is predominantly used to measure fluorescence intensity produced by fluorescent-labeled antibodies detecting proteins, or ligands that bind to specific cell-associated molecules such as propidium iodide binding to DNA.

The staining procedure involves making a single-cell suspension from cell culture or tissue samples. The cells are then incubated in tubes or microtiter plates with unlabeled or fluorochrome-labeled antibodies and analyzed on the flow cytometer.

The flow cytometer: fluidics



When a cell suspension is run through the cytometer, sheath fluid is used to hydrodynamically focus the cell suspension through a small nozzle. The tiny stream of fluid takes the cells past the laser light one cell at a time (Figure 1).

Light scattered from the cells or particles is detected as they go through the laser beam. A detector in front of the light beam measures forward scatter (FS) and several detectors to the side measure side scatter (SS). Fluorescence detectors measure the fluorescence emitted from positively stained cells or particles.

The flow cytometer: measurement of forward and side scatter of light Cells or particles passing through the beam scatter light, which is detected as FS and SS. FS correlates with cell size and SS is proportional to the granularity of the cells. In this manner, cell populations can often be distinguished based on differences in their size and granularity alone.

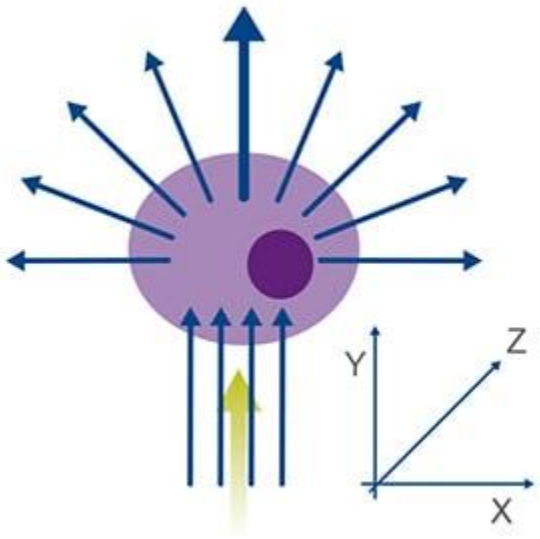


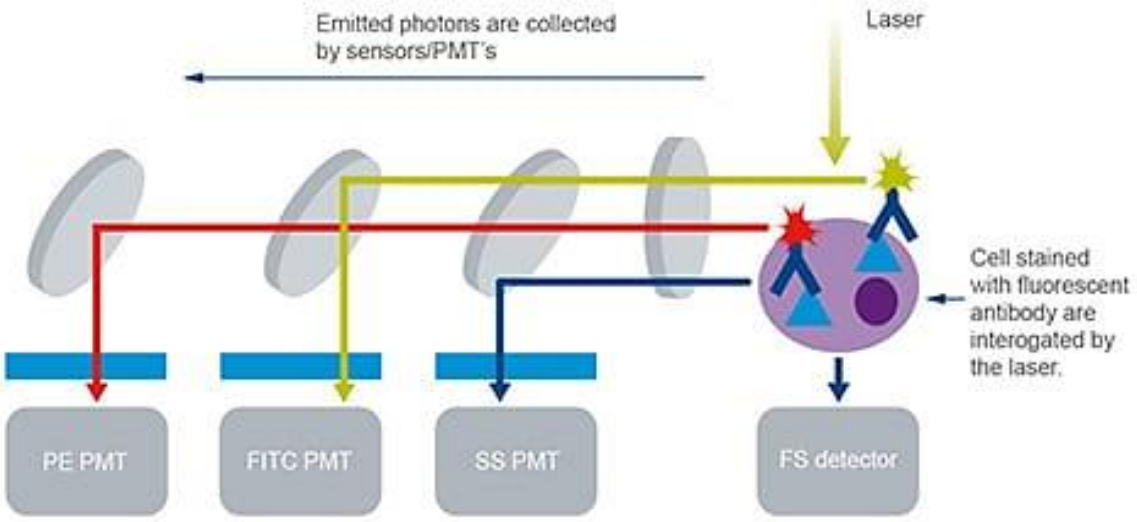
Figure 2. Light scatter as the green laser interrogates the cell. The direction of light scattered by the cell correlates to cell size and granularity.

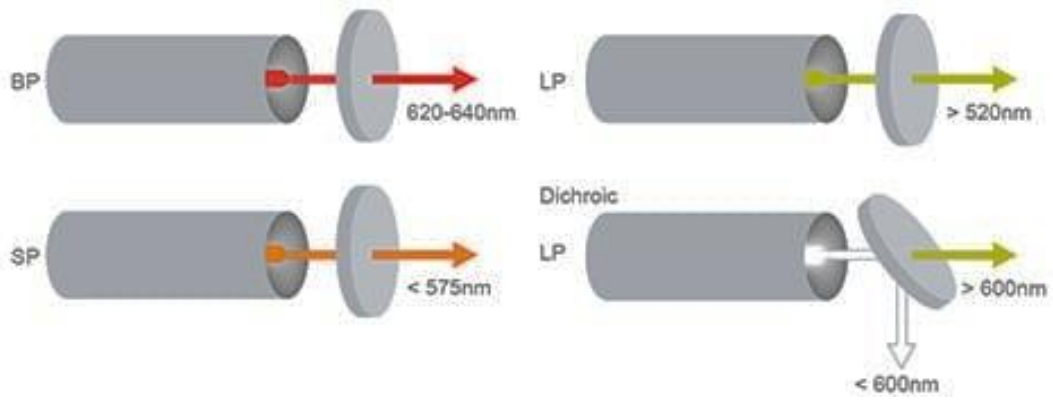
- A useful example of this is when running blood samples on the flow cytometer.
- Larger and more granular granulocyte cells produce a large population with high SS and FS.
- Monocytes are large cells, but not so granular, so these produce a separate population with high FS but lower SS.
- Smaller lymphocytes and lymphoblasts produce a separate population with less FS. They are not granular cells, so also have low SS.

Therefore, these cells can be separated into different populations based on their FS and SS alone.

The flow cytometer: measurement of scattered light and fluorescence

- As well as separating cells based on FS and SS, cells can also be separated by whether they express a particular protein. In this case, a fluorochrome is often used to stain the protein of interest. Fluorochromes used for the detection of target proteins emit light when excited by a laser with the corresponding excitation wavelength. These fluorescent stained cells or particles can be detected individually.
- Forward and side scattered light and fluorescence from stained cells are split into defined wavelengths and channeled by a set of filters and mirrors within the flow cytometer. The fluorescent light is filtered so that each sensor will detect fluorescence only at a specified wavelength. These sensors are called photomultiplying tubes (PMTs).





Long pass (LP) filters allow transmission of photons above a specified wavelength. Dichroic filters/mirrors (such as dichroic LP mirrors) are positioned at a 45° angle to the light beam.Sub heading

In a long pass dichroic filter, photons above a specific wavelength are transmitted straight ahead, whilst photons below the specific wavelength are reflected at a 90° angle.

Measurement of signal

As the fluorescing cell passes through the laser beam, it creates a peak or pulse of photon emission over time. These are detected by the PMT and converted to a voltage pulse, known as an event. The total pulse height and area is measured by the flow cytometer. The measured voltage pulse area will correlate directly to the intensity of fluorescence for that event.

Each event is given a channel number depending on its measured intensity; the more intense the fluorescence, the higher the channel number the event is assigned.

Antibody staining

1) **Directstaining:**

In direct immunofluorescence staining, cells are incubated with an antibody directly conjugated to a fluorochrome (e.g. FITC). This has the advantage of requiring only one antibody incubation step and eliminates the possibility of non-specific binding from a secondary antibody.

This approach is particularly useful for intracellular staining, where large antibody-fluorochrome complexes including secondary antibodies can become trapped causing non-specific binding, or fail to enter the cell preventing primary antibody detection.

2.**Indirectstaining:**

In indirect staining, the primary antibody is not fluorochrome-labeled, but is detected by a fluorochrome-labeled secondary antibody. This second reagent may be an antibody with specificity for the first antibody. Alternatively, the avidin-biotin system can be used, whereby an antibody is conjugated to biotin and detected with fluorochrome-labeled avidin.

With the wide range of conjugated antibodies now available, this method means that unconjugated primary antibodies raised against many different targets can be used in conjunction with a labeled secondary antibody for FACS analysis. This widens the choice of target proteins

Intracellular staining:

Staining of intracellular antigens for flow cytometry protocols depends on various fixation and permeabilization methods to allow access of antibodies to internal cellular proteins. A successful staining procedure in all cases is dependent on optimization of experimental conditions through titering of antibodies, use of appropriate controls to set up the flow cytometer correctly and optimized fixation and permeabilization procedures.

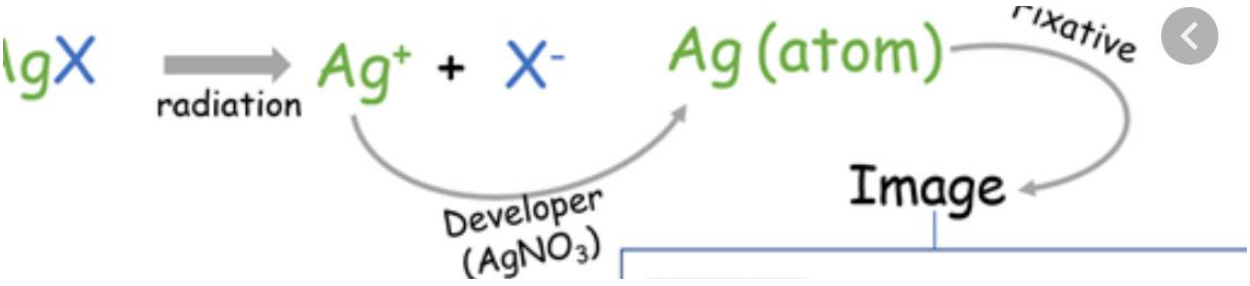
Detection of secreted proteins:

Detection of secreted proteins is difficult as the protein will be released from the cell before detection, or may degrade rapidly. A Golgi block such as Brefeldin A can be used to inhibit secretion of expressed proteins, retaining them in the Golgi apparatus. The intracellular staining method can then be used for detection of the target protein.

❖ **Autoradiography**

Autoradiography is an imaging technique that uses radioactive sources contained within the exposed sample. In vitro autoradiography methods involve the isolation of cellular components such as DNA, RNA, proteins or lipids, followed by labeling with suitable radioisotopes.

In in vivo autoradiography, radioisotopes are coupled with radioactive tracers and administered orally or via injection, and the distribution of radiation is evaluated in thin tissue or whole-body cryosections. In vivo autoradiography using laboratory animals is widely used in metabolic studies, disease monitoring and new drug development experiments.



General Principle Of Autoradiography

- The principle of autoradiographic imaging is the precipitation of silver (Ag) atoms, resulting from the ionization of a silver halide (AgX – silver bromide, chloride, iodide or fluoride – AgBr, AgCl, AgI or AgF, respectively) by radiolabeled samples. AgX are light sensitive compounds commonly used in photography. They are generally suspended in a gelatin photographic emulsion. Each AgX molecule is individually encapsulated in the gelatin, and functions as an independent detector of radioactive decay from the radiolabeled sample. Once radioactive particles hit the gelatin emulsion, AgX is reduced resulting in the production of insoluble silver crystals.
- Gelatin photographic emulsions are used to coat photographic and X-ray films, which are made of a flexible base (usually cellulose acetate). When a radiolabeled sample is in contact with in a coated X-ray film (exposure), it generates a latent (hidden) image corresponding to the radioactivity distribution within the sample.
- To make the image visible, the exposed photographic / X-ray film must be submerged in a developing reagent, a chemical mixture that converts the silver crystals into metallic silver, darkening the gelatin emulsion. Silver nitrate (AgNO3) is highly efficient in the reduction of AgX molecules and is usually a component of developer solutions.
- The reaction is then stopped by a fixative reagent, which removes the excess AgX from the photographic / X-ray film. Highly radioactive areas (e.g. areas with higher concentration of a radiolabeled drug, or with higher metabolic activity) reduce more AgX molecules, resulting in higher optical density in the film (darker areas) Thus, autoradiography must be avoided in samples that are homogeneously labeled. Although it can be quantitative, autoradiography can be a slow process, depending on the half-life of the radioisotopes used.
- In a clinical context, advanced autoradiographic methods like positron-emission tomography (PET) and single-photon emission computerized tomography (SPECT) are used in the diagnosis, staging and monitoring of disease. PET and SPECT rely on the same principle as classic autoradiography but use different radiotracers (suitable for human use) and radioactive decay is detected by gamma cameras (PET / SPECT detectors). Computer algorithms are then used to produce high resolution 3D reconstructions (tomograms) of whole body or parts of an organism.

Autoradiography: Methods

- Classic autoradiography techniques are performed according to the following general sequential steps:
- In vivo autoradiography
- Radioactive labelling of biological sample. Labeling time depends on the type of radioisotope and the radiotracer molecule.
- Injection or oral administration of radioactive tracer in laboratory animals

Sample preparation

- Cryopreservation of euthanized animals and cryosection – whole-body or tissue sections (20-50 µm thick) for microscopy evaluation. Light or electron microscopy can be used, depending on the aim of the study.
- Whole-body or tissue sections are mounted into glass slides and embedded in photographic emulsion to generate a latent image.
- Image development. Here, the incubation time in the developer reagent depends only on the radioisotope used.
- Arrest of image development by exposing the slide to a fixative reagent.
- When analyzing histological sections, a counterstaining step can be added to the protocol, to identify specific tissue structures or cellular components. For example, hematoxylin and eosin staining can be used to define the nucleus and cytoplasm of cells in tissue sections, after autoradiographic detection.

In vitro autoradiography

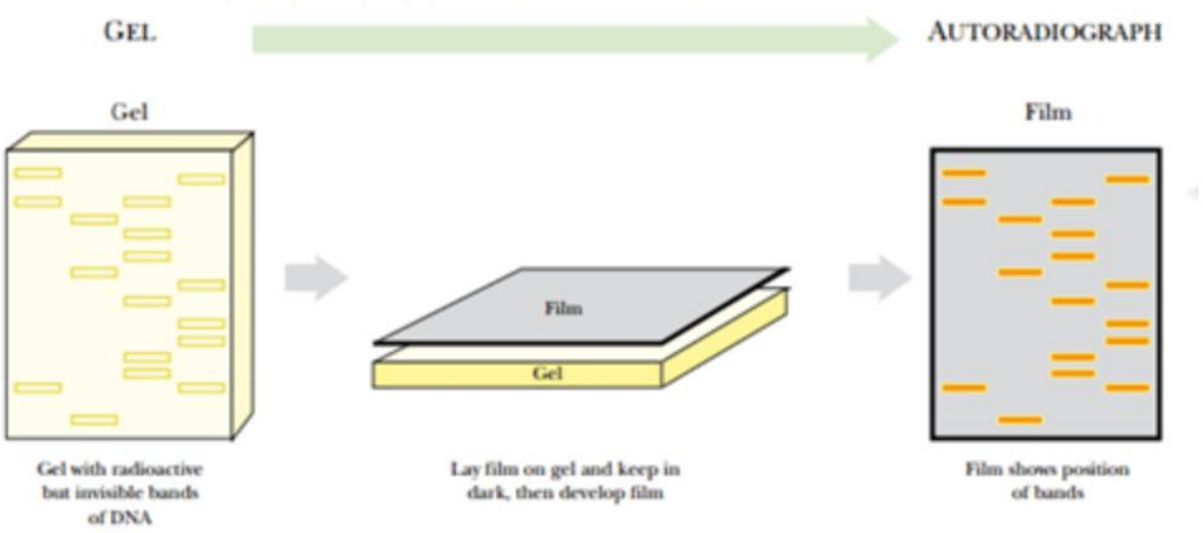
- Sample preparation
- Isolation of cellular components such as proteins, DNA or RNA
- In vitro labeling of isolated cellular components (DNA, RNA, proteins, etc.)
- Loading the sample in a suitable matrix for image development using a photographic / X-ray film. Radiolabeled proteins can be loaded into acrylamide gels, and transferred to a nitrocellulose membrane, which is then put in contact with the film
- Exposure of the photographic / X-ray film to the matrix containing the radiolabeled sample. Exposure time depends of the radioisotope used
- Development of the autoradiographic image by emerging the film in a developer reagent solution.

Arrest of image development by emerging the film in a fixative reagent to remove the excess silver halide in the photographic emulsion

In both in vivo and in vitro autoradiography, the exposure of the radiolabeled sample to AgX-containing photographic emulsion must always be performed in the dark (e.g., in a dark room, or in closed boxes).

- Because the AgX is sensitive to light and radiation, this ensures that the only AgX molecules reduced are the ones where radioactivity is emitted from the sample.
- The resolution of the autoradiographic image is proportional to the ionization capacity of the radioisotopes used.

- Low energy radioisotopes such as 3H reduce only neighboring silver halide molecules, producing a very sharp image, while high energy isotopes such as 125I can reduce a lot more halide molecules, producing darker images with lower specificity.

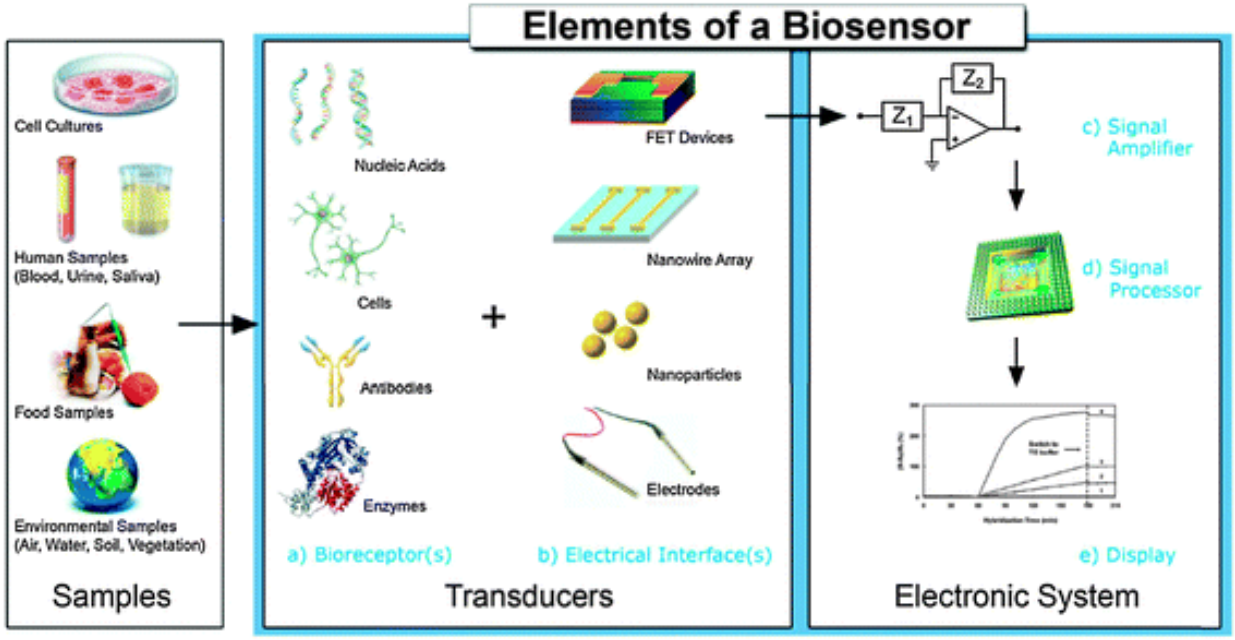


Applications Of Autoradiography

- Whole-body autoradiography (WBA) and Microautoradiography (MARG) – In vivo autoradiography techniques used to determine the tissue distribution of radiolabeled compounds in laboratory animals.
- A radiotracer (usually labeled with 14C and / or 3H) is administered and incubated for specific timepoints. WBA and MARG are especially suitable for the study of receptor biology, as the radioisotopes can be coupled with ligands that are specific to cell membrane receptors.
- Thus, WBA and MARG provide data on the distribution and activity of cellular receptors, making them exceptional techniques in the study of cell biology and cell signaling mechanisms.
- In microbiology, the MARG method has been combined with fluorescence in situ hybridization (FISH), using specific oligonucleotides (DNA probes) to identify organisms.
- WBA can be quantified (quantitative WBA) using dosimetry techniques, i.e., comparing the different radioactivity intensities with radioactive standards of known concentrations. An alternative method to QWBA is autoradioluminography, in which whole-body sections are exposed to a phosphor imaging plate, and scanned by a phosphor image scanner

BIOSENSOR TECHNOLOGY

- A biosensor is a biological detection system consists of a biological component combined with a transducer to perform measurement of a biochemical quantity. The development of biosensors started with the invention of enzyme electrodes by Leland C. Clark in 1962.
- A typical biosensor includes a bioelement such as an enzyme, antibody, or a cell receptor, and a sensing element or a transducer. These two elements are combined together through a number of methods such as covalent bonding, matrix entrapment, physical adsorption and membrane entrapment.
- Working Principle of Biosensors
- Biosensors are operated based on the principle of signal transduction. These components include a bio-recognition element, a biotransducer and an electronic system composed of a display, processor and amplifier.
- The bio-recognition element, essentially a bioreceptor, is allowed to interact with a specific analyte. The transducer measures this interaction and outputs a signal. The intensity of the signal output is proportional to the concentration of the analyte. The signal is then amplified and processed by the electronic system.



Types of Biosensors

Depending on the mechanism of transduction, biosensors are classified as follows:

1) **Resonant Biosensors** – These sensors employ an acoustic wave transducer combined with the bioreceptor. Interaction between the analyte and bioreceptor forces a change in the mass of the

bioreceptor. As a result, the resonant frequency of the acoustic transducer also changes, which is then evaluated and this value can then be interpreted by the end-user.

2) **Optical Detection Biosensors** – These type of sensors include a silicon wafer to which protein molecules are attached through covalent bonds. The wafer is subjected to UV light which makes the antibodies inactive. The wafer is then diced and placed in the analyte, which encourages formation of diffusion grating that generates a signal which can be measured and amplified.
3) **Thermal Detection Biosensors** – In these sensors, the immobilized enzymes are coupled with temperature detectors. A heat reaction is initiated when the analyte is made to interact with the enzyme. The concentration of the analyte can thus be evaluated with respect to the measurement of a heat reaction.
4) **Ion Sensitive Biosensors** – These sensors work on the principle that the interaction of ions with a semiconductor changes the electric potential of the semiconductor surface. The potential changes can then be measured to evaluate the desired parameter.
5) **Electrochemical Biosensors** – The principle behind the operation of these sensors involves the generation of ions by various chemical events that change the electrical properties of the analyte solution. The concentration of the analyte is then measured with respect to this change.

Advantages of Biosensors

- The key benefits of biosensors include the following:
- Rapid and continuous measurement
- High specificity
- Very less usage of reagents required for calibration
- Fast response time
- Ability to measure non-polar molecules that cannot be estimated by other conventional devices.

Applications of Biosensors

- Some of the major applications of biosensors are listed below:
- Monitoring glucose level in diabetes patients
- Food analysis
- Environmental applications
- Protein engineering and drug discovery applications
- Wastewater treatment.