Phase Contrast Microscope

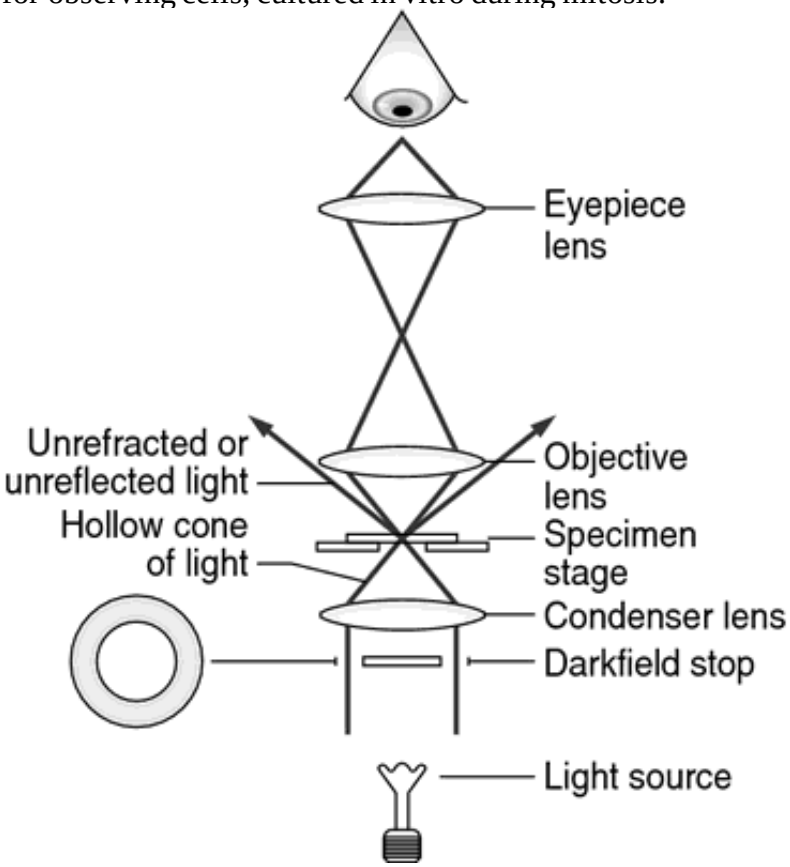
Aim: - To study phase contrast microscope.

Theory: - It was invented by Dutch scientist ―Fredric Zernike‖ in 1935. The source of illumination is ordinary visible light. Thus, its resolving power is same as that of light microscope. It makes highly transfer object much better visible. It is based on the principle that the light transmitted by region of high refractive index in the velocity with respect to light transmitted by region of lower refractive index. This phase contrast is produced deviated and un deviated light rays. So, a transparent object appears in various shape of depending upon the thickness of object and difference between object and medium.

Phase plate: - It is present between objective lens amd eye peice. It has thin and thick region so that ray emerging through the thinner region will be out of phase with rays emerging to the outer thick region of phase plate. This results in grester resolution. Image seen in phase contrast microscope and contrasted between object and background is increased

Structure: - It has an annular diaphragm before condenser and phase plate after the objective lens. The phase contrast microscope has eye piece, objective lens and a condenser comparable to one that is compound microscope. In addition, the lens contains an annular diaphragm. The annular diaphragm permits light to pass through the condenser as a hollow cone and remaining light is focussed on the object to be seen. Phase plate is useful as it gives deviated path of light instead of direct path of light.

Uses: - 1. It is used for the study of organelles in the living cell.
2. To study movement of chromosome during cell division.
3. To study the streaming movement of cytoplasm.
4. It is used to distinguish between normal cell and cell which are fixed and stained.
5. It is valuable for observing cells, cultured in vitro during mitosis.



PCR

PCR or Polymerase Chain Reaction is a technique used in molecular biology to create several copies of a certain DNA segment. This technique was developed in 1983 by Kary Mullis, an American biochemist. PCR has made it possible to generate millions of copies of a small segment of DNA. This tool is commonly used in the molecular biology and biotechnology labs.

Principle of PCR

The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer mediated enzymes. DNA Polymerase synthesises new strands of DNA complementary to the template DNA. The DNA polymerase can add a nucleotide to the pre-existing 3'-OH group only. Therefore, a primer is required. Thus, more nucleotides are added to the 3' prime end of the DNA polymerase.

Components Of PCR

Components Of PCR constitutes the following:

1. **DNA Template**– The DNA of interest from the sample.
2. **DNA Polymerase**– Taq Polymerase is used. It is thermostable and does not denature at very high temperatures.
3. **Oligonucleotide Primer**s- These are the short stretches of single-stranded DNA complementary to the 3' ends of sense and anti-sense strands.
4. **Deoxyribonucleotide triphosphate**– These provide energy for polymerization and are the building blocks for the synthesis of DNA. These are single units of bases.
5. **Buffer System**– Magnesium and Potassium provide optimum conditions for DNA denaturation and renaturation. It is also important for fidelity, polymerase activity, and stability.

Types of PCR

Real-time PCR

In this type, the DNA amplification is detected in real-time with the help of a fluorescent reporter. The signal strength of the fluorescent reporter is directly proportional to the number of amplified DNA molecules.

Nested PCR

This was designed to improve sensitivity and specificity. They reduce the non-specific binding of products due to the amplification of unexpected primer binding sites.

Multiplex PCR

This is used for the amplification of multiple targets in a single PCR experiment. It amplifies many different DNA sequences simultaneously.

Quantitative PCR

It uses the DNA amplification linearity to detect, characterize and quantify a known sequence in a sample.

Arbitrary Primed PCR

It is a DNA fingerprinting technique based on PCR. It uses primers the DNA sequence of which is chosen arbitrarily.

PCR Steps

The PCR involves three major cyclic reactions:

Denaturation

Denaturation occurs when the reaction mixture is heated to 94°C for about 0.5 to 2 minutes. This breaks the hydrogen bonds between the two strands of DNA and converts it into a single-stranded DNA.

The single strands now act as a template for the production of new strands of DNA. The temperature should be provided for a longer time to ensure the separation of the two strands.

Annealing

The reaction temperature is lowered to 54-60°C for around 20-40 seconds. Here, the primers bind to their complementary sequences on the template DNA.

Primers are single-strand sequences of DNA or RNA around 20 to 30 bases in length.

They serve as the starting point for the synthesis of DNA.

The two separated strands run in the opposite direction and consequently there are two primers- a forward primer and a reverse primer.

Elongation

At this step, the temperature is raised to 72-80°C. The bases are added to the 3' end of the primer by the Taq polymerase enzyme.

This elongates the DNA in the 5' to 3' direction. The DNA polymerase adds about 1000bp/minute under optimum conditions.

Taq Polymerase can tolerate very high temperatures. It attaches to the primer and adds DNA bases to the single strand. As a result, a double-stranded DNA molecule is obtained.

These three steps are repeated 20-40 times in order to obtain a number of sequences of DNA of interest in a very short time period.

Applications of PCR
The following are the applications of PCR :

Medicine

- Testing of genetic disease mutations.

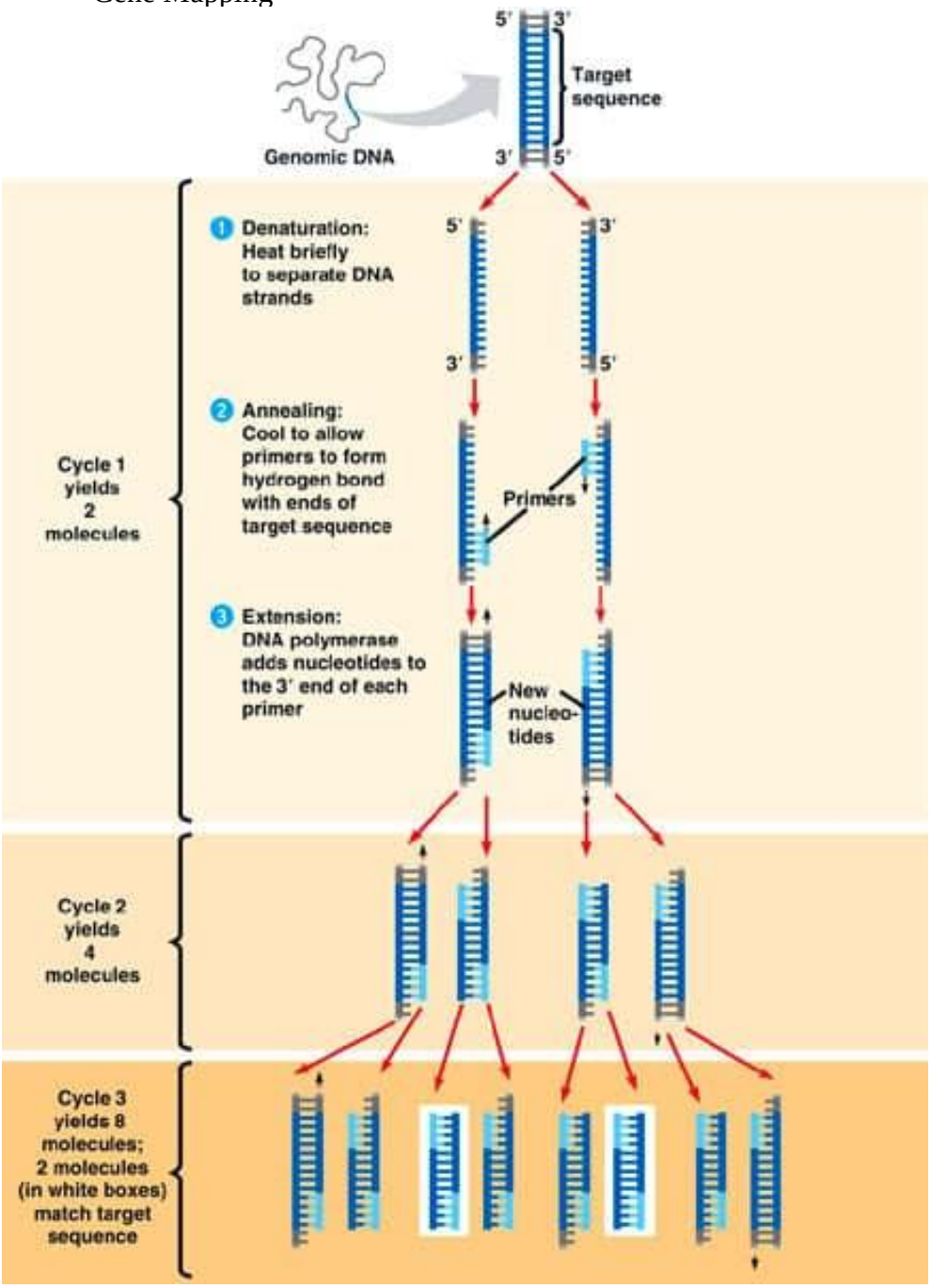
- Monitoring the gene in gene therapy.
- Detecting disease-causing genes in the parents.

Forensic Science

- Used as a tool in genetic fingerprinting.
- Identifying the criminal from millions of people.
- Paternity tests

Research and Genetics

- Compare the genome of two organisms in genomic studies.
- In the phylogenetic analysis of DNA from any source such as fossils.
- Analysis of gene expression.
- Gene Mapping



Centrifuge

A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed. In a centrifuge, the sample is kept in a rotor that is rotated about a fixed point (axis), resulting in strong force perpendicular to the axis. There are different types of centrifuge used for the separation of different molecules, but they all work on the principle of sedimentation.

Principle

Centrifugation is the technique of separating components where the centrifugal force/ acceleration causes the denser molecules to move towards the periphery while the less dense particles move to the center. The process of centrifugation relies on the perpendicular force created when a sample is rotated about a fixed point.

Relative Centrifugal Force (RCF)

- Relative centrifugal force is the measure of the strength of rotors of different types and sizes.
- This is the force exerted on the contents of the rotor as a result of the rotation.
- RCF is the perpendicular force acting on the sample that is always relative to the gravity of the earth.

$$\textbf{RCF (g Force)} = 1.118 \times 10^{-5} \times r \times (RPM)^2$$

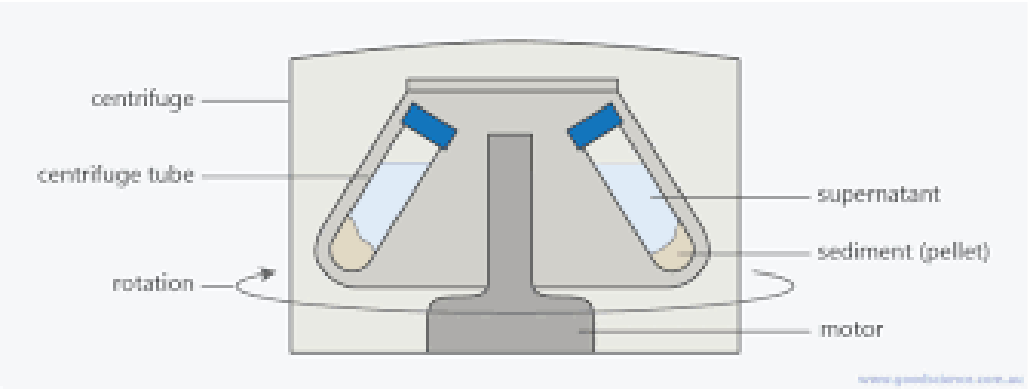
where **r** is the radius of the rotor (in centimeters), and **RPM** is the speed of the rotor in rotation per minute.

Types of Centrifuge:

1. **Low Speed Centrifuge:** The low-speed centrifuge has a maximum speed of 4000-5000rpm
2. **High Speed Centrifuge:** The high-speed centrifuge has a maximum speed of 15,000 – 20,000 RPM
3. **Ultracentrifuge:** Ultracentrifuge has a maximum speed of 65,000 RPM (100,000's x g).

Applications of Centrifugation

1. To separate two miscible substances
2. To analyze the hydrodynamic properties of macromolecules
3. Purification of mammalian cells
4. Fractionation of subcellular organelles (including membranes/membrane fractions) Fractionation of membrane vesicles
5. Separating chalk powder from water
6. Removing fat from milk to produce skimmed milk



Centrifugation

PH meter

- What is pH Measurement?
    - pH Measurement specifies the degree of relative acidity or alkalinity of an aqueous solution at a given temperature.
    - It is generally measured using a pH Meter.
    - All living beings depend on a proper pH level to sustain life and hence pH Measurement becomes an important aspect of our lives.

Theoretically, **pH** is derived from the word "**Pondus Hydrogenii**" which means "**Potential Hydrogen**" or **Power of Hydrogen**

- In an aqueous solution, a pH of 7 describes a neutral solution because the activities of Hydrogen (H+) and Hydroxide ions (OH-) are equal.
- The solution is described as acidic when the pH is below 7,as the activity of Hydrogen ion is dominant when compared to Hydroxide ion.
- Hence a solution is termed more acidic when the Hydrogen ion activity increases and the pH value decreases.
- On the contrary, the solution is described as basic (or alkaline) when the pH is above 7, as the activity of Hydroxide ion is dominant when compared to Hydrogen ion.
- pH is represented in the form of an equation as the negative logarithm of the Hydrogen ion concentration.

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

In pure water or a neutral solution, $[H^+] = 1 \times 10^{-7}M$

$$pH = -\log(1 \times 10^{-7})$$
$$= -(\log 1 \times \log 10^{-7})$$
$$= -(0.0 + (-7.0))$$
$$pH = 7.0$$

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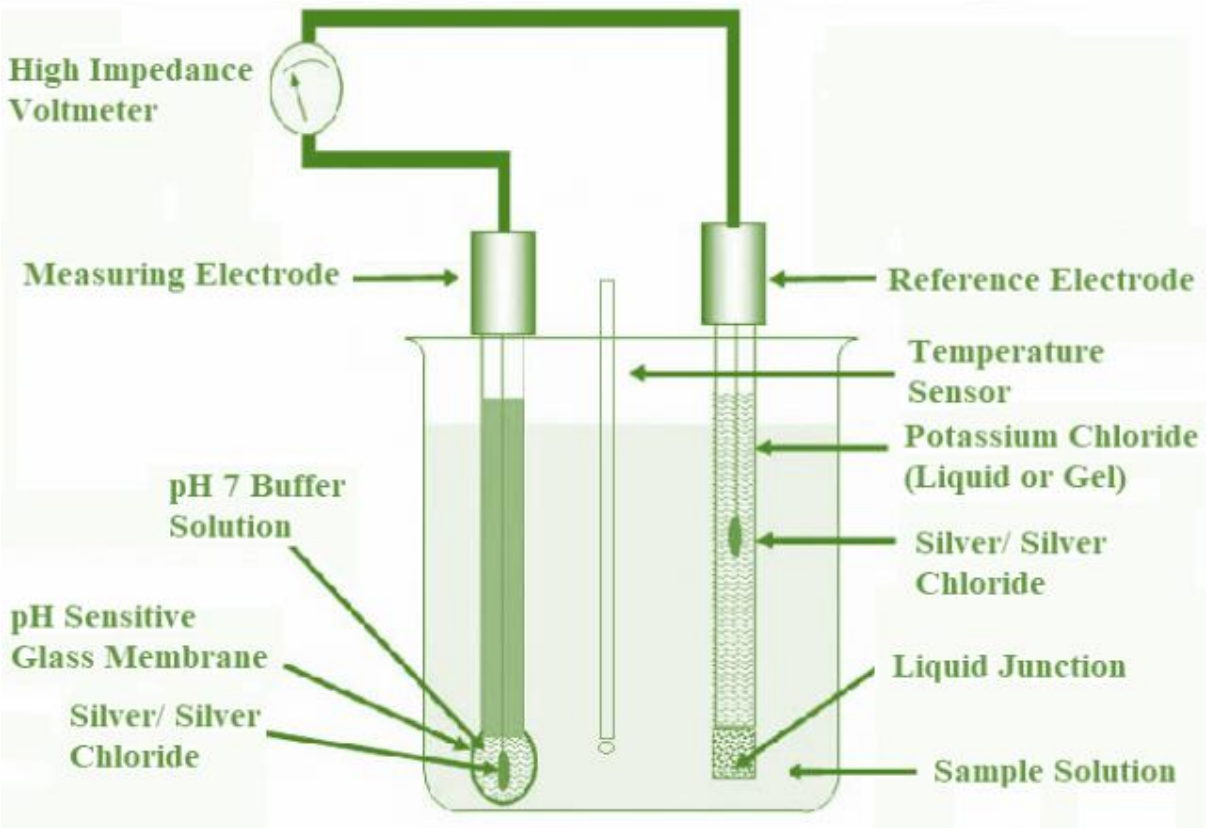
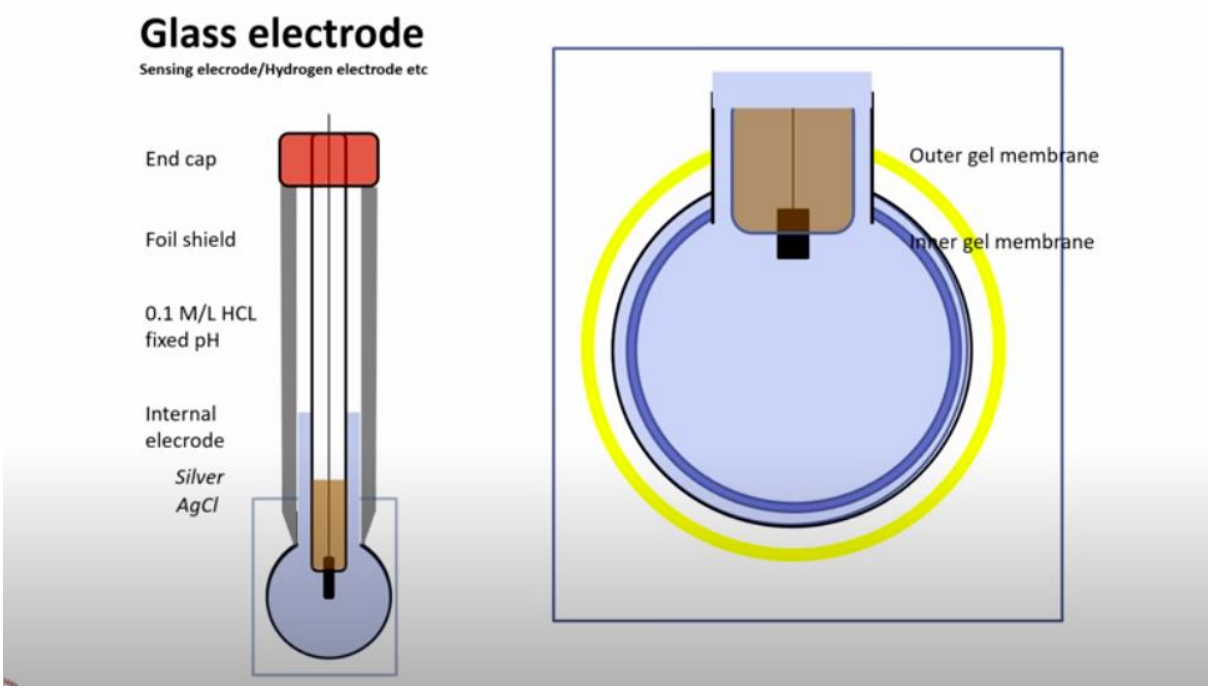
- **Components:**
- Measuring Electrode

- Reference Electrode
- Temperature Sensor
- Sample Solution being measured
- The pH Meter measures the voltage of an electro chemical cell and based on the Temperature Sensor determines the pH of a solution.
- Most of the pH Meters the electrodes and the Temperature Sensor are fabricated into a single body and are called as Combination Electrodes.





Ion-exchange process also takes place on the inner surface of the Glass Electrode from the sample solution.
This creates a potential difference (Hydrogen- ion activity) between them.
The Liquid Junction potential is usually small and relatively constant which mainly depends on the concentration of the ions in the sample solution.
All three potentials are summed up and measured by High Impedance Voltmeter.
The output of the Impedance Voltmeter is Voltage readings and it has to be calibrated to get precise pH Measurement.
Calibration is done by dipping the Measuring Electrode into Buffer Solution of known pH which helps in interpreting millivolt reading as pH measurement of the Sample Solution at the given temperature

The applications include:

- pH Measurement is very crucial in Agriculture industry for soil evaluation. Major crops require alkaline environment and hence pH Measurement becomes necessary.
- It is also used in Food industry especially for dairy products like cheese, curds, yogurts, etc.
- It becomes mandatory for chemical and pharmaceutical industries.
- It becomes a significant factor in the production of detergents.
- pH level monitoring is essential in water treatment plants and RO water purifiers.