For BioResire students

Life sciences Material

Elite Batch

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Techniques

Microscopy: From Magnifying Glasses to Molecular Movies

Basic Principle: At its heart, microscopy is about overcoming the limitations of human sight. The fundamental concepts are magnification (making an object appear larger) and resolution (the ability to distinguish two adjacent points as separate). The theoretical limit of resolution for light microscopy (~200 nm) is defined by the Abbe limit, dictated by the wavelength of visible light and the numerical aperture of the lens.

Engaging the Basics: Light Microscopy

Principle: Uses visible light (400-700 nm wavelength) and a series of glass lenses to magnify images of small samples. Resolution is limited by the wavelength of light.

Maximum Resolution: ~200 nm

Phase Contrast: Imagine trying to see a transparent glass bead in water. It's nearly impossible because it doesn't absorb light. Phase contrast microscopy solves this by converting subtle, invisible shifts in the phase of light waves (caused by the bead's density) into visible contrasts in brightness. This was revolutionary for observing live, unstained cells and watching processes like cell division unfold.

Fluorescence: This technique uses the property of fluorophores—molecules that absorb high-energy light (excitation) and then emit lower-energy light (emission). By tagging specific proteins with fluorescent antibodies or encoding them with fluorescent proteins like GFP (Green Fluorescent Protein, discovered in jellyfish), we can light up specific parts of the cell, like the nucleus or cytoskeleton, against a dark background.

Key Types & Applications:

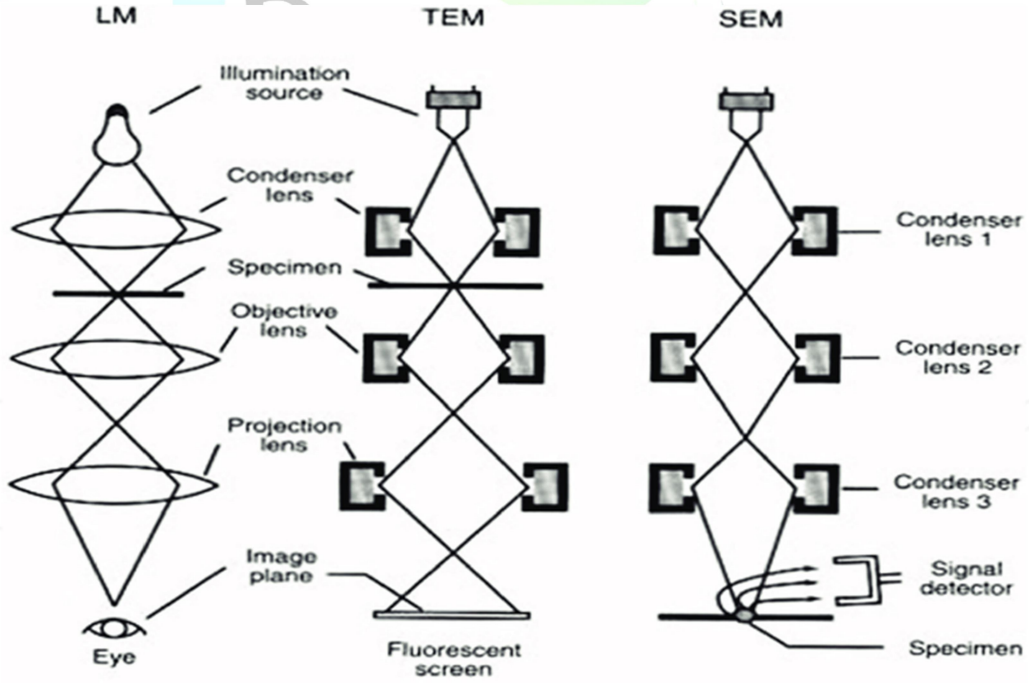
Type	principle	Application
Bright field	Light passes through the sample. Contrast comes from natural pigmentation or staining.	Viewing stained tissue sections, blood smears.

Phase	Converts phase shifts in light waves passing through a transparent specimen into brightness changes.	Observing live, unstained cells (e.g., cell division).
Fluorescence	Uses high-energy light to excite fluorophores in the sample, which then emit lower-energy light.	Detecting specific proteins (immunofluorescence), tracking molecules in live cells.
Confocal	Uses a pinhole to eliminate out-of-focus light, laser for excitation. Creates sharp optical sections.	3D reconstruction of cells and tissues.

. Electron Microscopy (EM)

- Principle: Uses a beam of electrons (wavelength ~0.005 nm) instead of light for illumination. Much higher resolution than light microscopy.

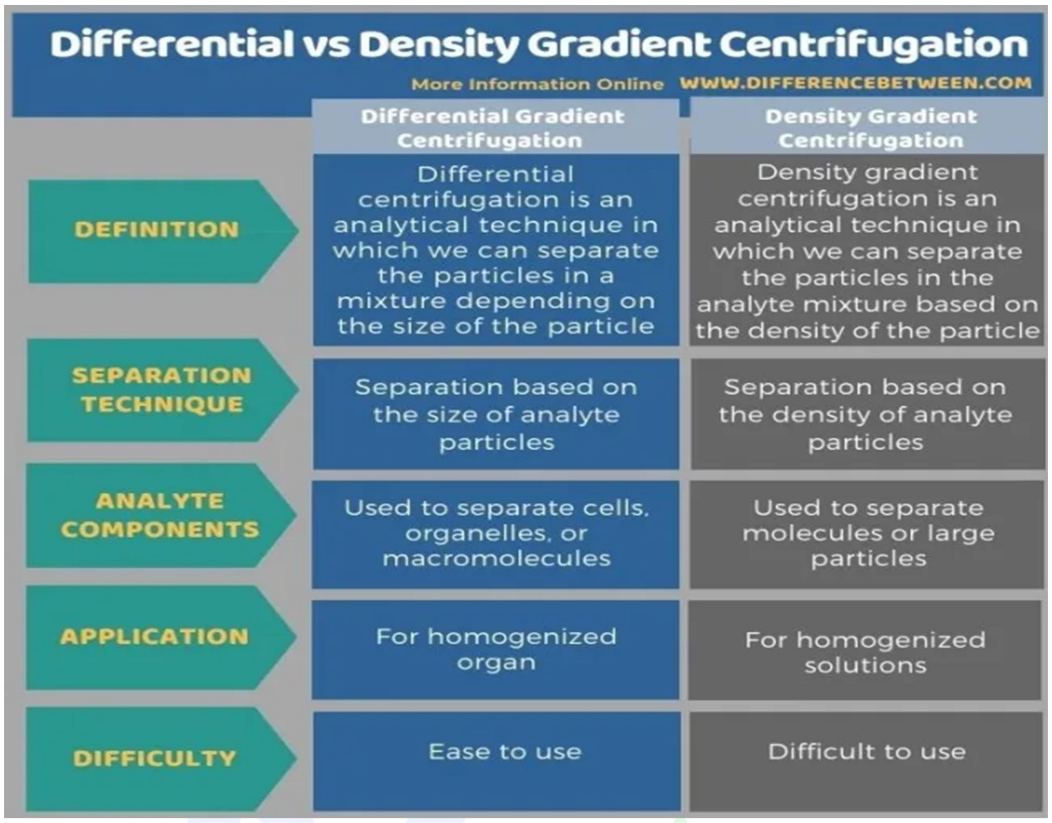
- Maximum Resolution: < 1 nm



Feature	Light microscope	Transmission electron microscope	Scanning electron microscope
Illuminating radiation	Visible light	High speed electrons	High speed electrons
Wavelength of above	400 nm-700 nm	.006 nm (40 kV) – .004 nm (100 kV)	.04 nm (1 kV) – .007 nm (30 kV)
Best resolution	200 nm	.2nm	10 nm
Magnification range	10X - 1000X	500X - 500,000X	20X - 50,000X
Depth of field	.002 – .05 mm	.004 to .006 mm	.003 to 1 mm
High magnification working distance	2 mm	–	12 mm
Lens type	glass	electromagnetic	electromagnetic
Image formation	on eye by lenses	on phosphorescent plate by lenses	Built on cathode ray tube by scanning spot
General use	Surface morphology and sections	Sections (40-150 nm) or small particles on thin membranes	Surface morphology

Centrifugation

- Principle: Separates particles in a suspension based on **size, shape, density, and viscosity** of the mediumby applying a centrifugal force. Denser/heavier particles sediment faster.
- **Centrifugal Force (F_c):** The apparent force that pushes a particle outward in a circular path.
  $F_c = m \omega^2 r$
  - m = mass of the particle
  - ω = angular velocity (in radians/second)
  - r = radial distance from the center of rotation
-
- Key Formula: Relative Centrifugal Force (RCF) is more useful than RPM because it accounts for rotor radius.
  RCF(g)=1.118×r×(RPM/1000)2$RCF(g) = 1.118 \times r \times (RPM/1000)2$
  - r = radial distance in millimeters (from center to tube bottom).



- **The Ultracentrifuge: Analytical Tool**

This is a high-speed centrifuge (up to 150,000 RPM) capable of generating forces over 1,000,000 × *g*. It's used for two main purposes:

1. **Preparative Ultracentrifugation:** The goal is to isolate and purify specific biological particles (e.g., organelles, viruses). This uses the types described above.
2. **Analytical Ultracentrifugation (AUC):** The goal is to **analyze** molecular properties in real-time during the spin. It has an optical system (e.g., UV/Vis, interference) to monitor particle movement.

- **Sedimentation Velocity:** Measures how fast a particle sediments. Provides information on **shape, mass, and size distribution**.
- **Sedimentation Equilibrium:** The centrifugal force is balanced by diffusion. Provides information on **molecular mass, stoichiometry, and binding affinity** of macromolecular complexes.

3. **The Svedberg Equation: Connecting Sedimentation to Mass**

This is a crucial concept for AUC. The **sedimentation coefficient (s)** describes how fast a particle sediments in a centrifugal field.

Formula:

$s = dx/dt \omega^2 x$ $s = \omega^2 x dx/dt$

- dx/dt = sedimentation velocity (dr/dt)
- ω = angular velocity
- x = distance from the center of rotation
- The unit of 's' is the **Svedberg (S)**, where 1 S = 10^{-13} seconds.
- **Why it's important:** The Svedberg value is **not additive**. It depends on both mass and shape. For example:
  - A prokaryotic **70S ribosome** is made of a **50S** large subunit and a **30S** small subunit.
  - This demonstrates that the subunits' shapes contribute to their sedimentation rates; their masses don't simply add up to 80S.

- **Key Applications & Why They're Important**

Application	Centrifugation Type	Purpose & Detail
Cell Fractionation	Differential	Isolate organelles for biochemical study. The order of pelleting: **Nuclei -> Mitochondria/Lysosomes/Peroxisomes -> Microsomes (ER) -> Ribosomes.**
Plasmid DNA Purification	Density Gradient (CsCl-EtBr)	The classic method. Ethidium bromide binds differentially to supercoiled plasmid (less binding) and linear chromosomal DNA (more binding), allowing them to separate based on buoyant density.

Application	Centrifugation Type	Purpose & Detail
Virus Purification	Differential & Density Gradient	Isolate and concentrate viruses from cell culture media for vaccine development or research.
Lipoprotein Separation	Density Gradient (e.g., KBr)	Separate plasma lipoproteins (Chylomicrons, VLDL, LDL, HDL) for clinical research into cardiovascular disease.
Determining Molecular Weight	Analytical Ultracentrifugation	A gold-standard method for determining the native molecular weight and oligomeric state of a protein in solution.

Chromatography

- Principle: Separates components in a mixture based on their differential partitioning between a mobile phase (liquid/gas) and a stationary phase (solid/liquid).
- Key Formula: Retention Factor (Rf) identifies compounds on a chromatogram.
  Rf= Distance travelled by compound/ Distance travelled by solvent front

Fundamental Concepts & Nomenclature

1.1 The Chromatographic Process

Separation occurs because molecules have different **partition coefficients** (K), defined as:

$$K=\frac{C_s}{C_m}$$

where C_s is the concentration of the solute in the stationary phase and C_m is its concentration in the mobile phase. A higher K means stronger interaction with the stationary phase and longer retention.

1.2 Key Performance Parameters (from Skoog et al.)

- **Retention Time (t_R):** The time taken for a solute to elute.
- **Dead Time (t_M):** The time taken for an unretained solute to travel through the column.
- **Adjusted Retention Time (t_R'):** $t_R' = t_R - t_M$
- **Capacity Factor (k):** (Also called retention factor) **Most important parameter** for measuring retention.

  $k = \frac{t_R - t_M}{t_M} = \frac{t_R'}{t_M}$

  A k value between 1 and 10 is generally desirable.
- **Selectivity Factor (α):** Measures the relative separation of two peaks.

  $\alpha = \frac{k_2}{k_1}$ (where $k_2 > k_1$)

  α must be greater than 1.0 for separation to be possible.
- **Resolution (R_s):** The quantitative measure of the separation of two peaks.

  $R_s = \frac{2(t_{R2} - t_{R1})}{w_{b1} + w_{b2}}$

  where w_b is the peak width at base. **$R_s \geq 1.5$** signifies complete baseline resolution.
- **Theoretical Plates (N):** A measure of column efficiency.

  $N = 16\left(\frac{t_R}{w_b}\right)^2$

  Higher N means a more efficient column (sharper peaks).

2. The Van Deemter Equation: Theory of Band Broadening

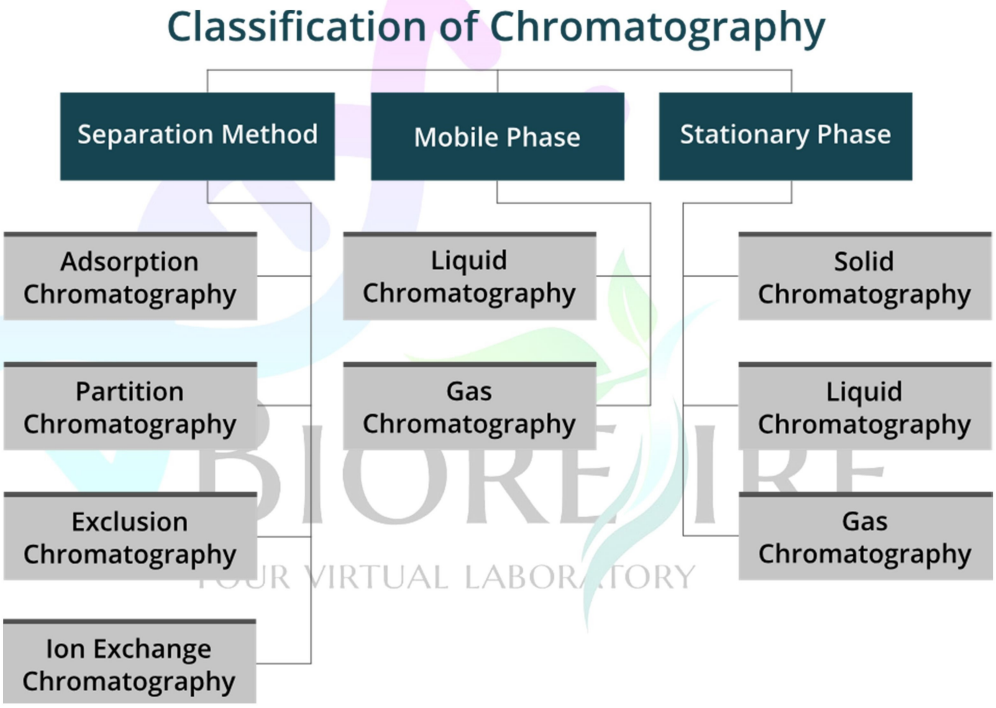
The efficiency of a column is inversely related to the **plate height** ($H = L/N$, where L is column length). The Van Deemter equation describes the factors contributing to band broadening (increased H):

$$H = A + \frac{B}{u} + Cu$$

- **A - Eddy Diffusion (Multi-path effect):** Caused by solute molecules taking different paths of varying lengths through the packed bed. *Independent of mobile phase velocity (u).* Minimized by using uniform, small particles.

- **B - Longitudinal Diffusion:** Caused by the diffusion of solute molecules along the axis of the column from high to low concentration. *Significant at low flow rates.* Minimized by increasing flow rate.
- **C - Mass Transfer Resistance:** Caused by the finite time required for solute molecules to diffuse into and out of the stationary phase. *Significant at high flow rates.* Minimized by using thin stationary phase films and small particles.

This relationship results in a **Van Deemter curve** (plot of H vs. u), which has an optimum flow rate where efficiency is highest (H is minimum).



Major Chromatographic Techniques: A Comparative Table

Technique	Stationary Phase (SP)	Mobile Phase (MP)	Mechanism of Separation	Key Application (from Snyder & Kirkland, Berg et al.)
Adsorption	Solid (Silica, Alumina)	Liquid (Org. Solvents)	Polarity interaction with SP surface	Separation of non-polar/ moderately polar compounds. **Normal-Phase.**
Partition	Liquid coated on inert solid	Liquid	Solubility in SP	**Reversed-Phase HPLC (RP-HPLC):** SP is non-polar (C18, C8); MP is polar. *Most common mode.* Separates peptides, drugs, metabolites.
Ion-Exchange (IEC)	Charged resin (e.g., $-SO_3^-$)	Aqueous Buffer (pH gradient)	Electrostatic attraction to charged SP	Purification of proteins, nucleic acids, antibiotics.
Size-Exclusion (SEC)	Porous beads	Liquid	Size (Hydrodynamic volume)	**Gel Filtration (Aq.) / Gel Permeation (Org.).** Desalting, determining molecular weight, separating complexes. *Large molecules elute first.*
Affinity	Immobilized ligand (Ab, substrate)	Buffer -> Eluent	Specific biological recognition	**Highest purity.** Purification of enzymes (SP: substrate), antibodies (SP: antigen), His-tagged proteins (IMAC: SP:

Technique	Stationary Phase (SP)	Mobile Phase (MP)	Mechanism of Separation	Key Application (from Snyder & Kirkland, Berg et al.)
				Ni^{2+}).
Hydrophobic Interaction (HIC)	Weakly hydrophobic groups	High salt -> Decreasing salt	Hydrophobicity (Salting-out)	Protein separation under mild, non-denaturing conditions.
Gas (GC)	Liquid or solid on column wall	Inert Gas (He, N_2)	Volatility & Polarity	Separation of volatile mixtures (fuels, essential oils, solvents).

Method Development in HPLC (Snyder &Kirkland)

A systematic approach is used to develop an efficient HPLC method:

1. **Select the Mode:** Typically start with **Reversed-Phase (C18 column)**.
2. **Select the Detector: UV-Vis Diode Array Detector (DAD)** is most common.
3. **Choose Initial Conditions:** e.g., 80% Water (with 0.1% Formic Acid) : 20% Acetonitrile.
4. **Adjust Selectivity (α):** This is the most powerful tool.
   - Change the organic modifier (e.g., Acetonitrile vs. Methanol).
   - Adjust pH (for ionizable compounds).
   - Change column chemistry (e.g., C8, Phenyl, Cyano).
5. **Optimize Resolution (R_s):** Use the formula $R_s \propto \sqrt{N} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{k+1}$.
   - To increase N (efficiency): Use a longer column or smaller particles.
   - To increase α (selectivity): Change chemistry (see point 4).
   - To adjust k (retention): Change MP strength (% organic).

5. Advanced Concepts for Competitive Exams

- **Chiral Chromatography:** Uses a chiral stationary phase to separate enantiomers. Critical in pharmacology.
- **Two-Dimensional Chromatography (2D-LC/GC):** Couples two independent separation mechanisms. Dramatically increases peak capacity for complex samples (e.g., proteomics, petroleomics).
- **UPLC/UHPLC:** Ultra-Performance Liquid Chromatography uses smaller particles (<2 µm) and higher pressures to achieve greater speed and resolution.
- **Hyphenated Techniques:**
  - **GC-MS / LC-MS:** Coupling chromatography to mass spectrometry for powerful separation and identification.
  - **LC-NMR:** Coupling liquid chromatography to nuclear magnetic resonance.

Electrophoresis

- Principle: Separates charged molecules (DNA, RNA, proteins) in a gel matrix under an electric field based on their size, charge, and shape.
- Key Formula: The mobility (µ) of a molecule is given by: $\mu = q/6\pi\eta r$
  where q is charge, η is viscosity, and r is the radius of the particle.

Key Factors Affecting Separation

1. **Charge (q):** Higher charge increases mobility. Charge is determined by pH relative to the molecule's pI.
2. **Size (r):** Larger size decreases mobility.
3. **Electric Field Strength (E):** Higher voltage increases speed of separation but can generate excessive heat.
4. **Buffer Properties:**
   - **pH:** Determines the ionization state (charge) of the molecule.

- **Ionic Strength:** Low ionic strength increases mobility but decreases buffer capacity. High ionic strength decreases mobility but can cause overheating (Joule heating).

5. **Support Matrix (Gel):** Acts as a molecular sieve. Pore size retards movement based on size and shape.

Types of Electrophoresis Techniques

	Description	Applications	Advantages	Limitations
Agarose Gel Electrophoresis	Separates DNA by size and charge	Used for sizing DNA fragments	Easy to set up and visualize	Low resolution for small fragments
Polyacrylamide Gel Electrophoresis	Separates proteins and nucleic acids	For protein analysis and purification	High resolution for small molecules	More complex setup and toxic acrylamide
Capillary Electrophoresis	Fast and automated separation method	Used in forensics and genomics	High resolution and sensitivity	Higher cost and needs special equipment
Two-Dimensional Gel Electrophoresis	Combines two different separation techniques	Enhances resolution for complex samples	Very high resolution capabilities	Time-consuming and requires expertise

- **SDS-PAGE: The Workhorse for Protein Molecular Weight**

This is a critical technique. The process involves:

1. **Denaturation:** Proteins are boiled in a sample buffer containing:
   - **SDS:** A detergent that binds to the protein backbone (~1.4 SDS molecules per amino acid), masking the protein's intrinsic charge and imparting a uniform negative charge.
   - **Reducing Agent (β-ME or DTT):** Breaks disulfide bonds to ensure complete unfolding.
2. **Separation:** The SDS-protein complexes are loaded onto a polyacrylamide gel. The gel pore size acts as a molecular sieve. **Smaller proteins migrate faster.**

3. **Molecular Weight Determination:** A standard curve is plotted using known molecular weight markers ($\log(MW)$ vs. migration distance). The unknown protein's MW is extrapolated from this curve.

Visualization and Detection

- **Proteins:**
  - **Coomassie Brilliant Blue:** General stain, detects ~50-100 ng/band.
  - **Silver Staining:** Highly sensitive, detects ~1 ng/band.
  - **Western Blotting:** Transfer to membrane and detect with specific antibodies.
- **Nucleic Acids:**
  - **Ethidium Bromide (EtBr):** Intercalates into DNA/RNA, fluoresces under UV light. (~1 ng sensitivity). **Note: Mutagenic.**
  - **SYBR Safe/Gold:** Safer, non-mutagenic alternatives to EtBr.
  - **GelRed/GelGreen:** Sensitive and safe dyes.

Advanced Concepts for CSIR-NET

- **Pulse-Field Gel Electrophoresis (PFGE):**
  - **Use:** Separates very large DNA fragments (>20 kb up to 10 Mb) like entire chromosomes.
  - **Principle:** The electric field is periodically changed (pulsed) in direction. Larger DNA molecules take longer to reorient and are retarded more than smaller ones.
  - **Application:** "Gold standard" for bacterial strain typing in epidemiology.

- **Capillary Electrophoresis (CE):**
  - **Principle:** Separation occurs inside a thin fused-silica capillary. It has an optical window for on-column detection.
  - **Why it's efficient:** Capillaries dissipate heat very effectively, allowing the use of very high electric fields (~500 V/cm) for fast, high-resolution separations.

- **Modes:** Capillary Zone Electrophoresis (CZE), Capillary Gel Electrophoresis (CGE, for DNA sequencing).
- **Difference from Chromatography:** While both separate mixtures, electrophoresis relies on an **electric field** acting on **charged** analytes. Chromatography relies on **flow** and **partitioning** between phases.
- **Troubleshooting:** Understanding artifacts like **smiling** (caused by overheating in the center of the gel) or **band broadening** (due to too much sample or incorrect buffer ionic strength).

The PCR Revolution and Its Evolution

Basic Principle: The Polymerase Chain Reaction (PCR) is essentially a molecular photocopier. It allows scientists to amplify a specific segment of DNA from a complex mixture, generating millions of copies. The process is cyclical:

Denature: Heat (95°C) separates the double-stranded DNA.

Anneal: Cooler temperature allows short DNA primers to bind to the specific target sequence.

Extend: A heat-stable DNA polymerase (Taq) synthesizes a new DNA strand from the primers.

Repeat 25-40 times for exponential amplification.

Engaging the Basics:

This simple yet powerful technique underpins everything from genetic fingerprinting in forensics to diagnosing genetic diseases and identifying pathogens (like the COVID-19 PCR tests).

Advanced Research: Quantitative and Digital PCR

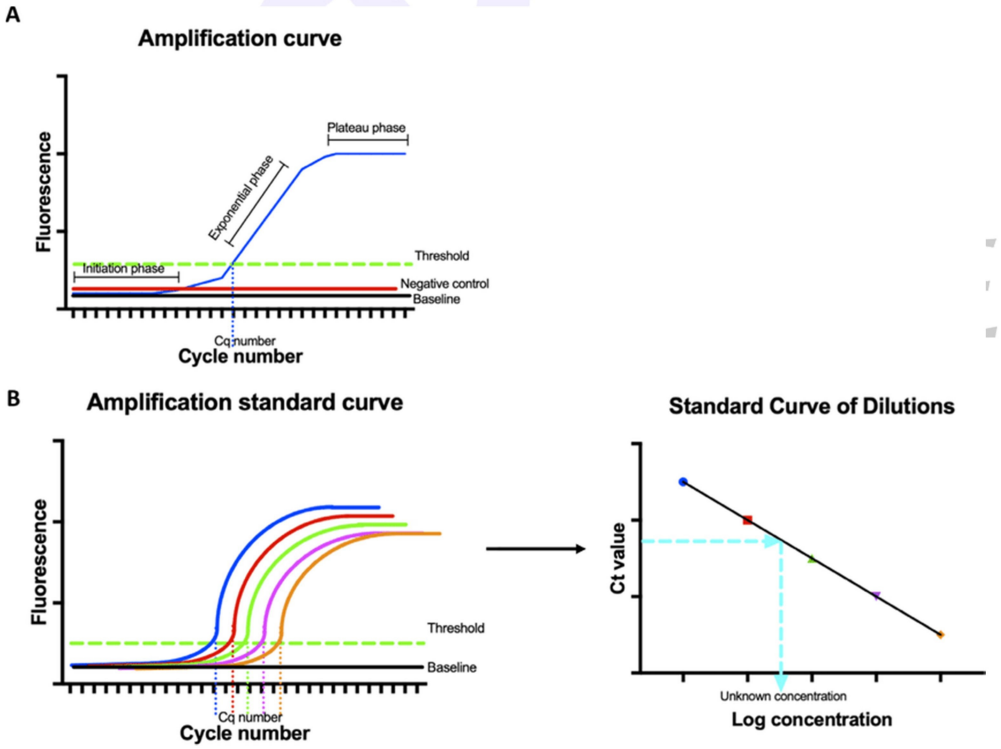
While basic PCR tells you if a sequence is present, advanced variants tell you how much is there with incredible precision.

Quantitative Real-Time PCR (qPCR): This method allows researchers to monitor amplification in "real-time" as it happens. A fluorescent signal increases proportionally to the amount of amplified DNA. The cycle number at which the fluorescence crosses a threshold (Ct value) is used for quantification. Fewer starting copies mean a higher Ct value. This is the gold

standard for measuring gene expression (how much mRNA is present for a specific gene) in response to a drug, disease, or other stimulus.

Digital PCR (dPCR): This is the ultimate quantitation tool. The sample is partitioned into thousands of nanodroplets or microwells, so that each contains either zero, one, or a few molecules. After PCR, each partition is simply scored as "positive" or "negative." By counting the positive partitions, you can achieve absolute quantification without the need for a standard curve. It's exceptionally precise for detecting rare mutations (e.g., in cancer biopsies) or low-level viral loads.

Research Application: qPCR is used in thousands of labs daily to validate results from RNA-Seq experiments. dPCR is used in liquid biopsy research to find one cancer-associated mutant DNA molecule among tens of thousands of healthy ones.



Spectrophotometry

- **Principle:** Measures the amount of light a sample absorbs at a specific wavelength. The amount of absorption is proportional to the concentration of the analyte.

- **Key Formula: Beer-Lambert Law**

  The law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species and the path length of the light through the solution.

  $A=\epsilon.l.c$

  - A = Absorbance (no units)
  - ε = Molar Extinction Coefficient ($M^{-1}cm^{-1}$)
  - l = Pathlength of the cuvette (cm)
  - c = Concentration (M)

- **Transmittance (T):** The fraction of incident light that passes through the sample.

  $T=I/I0$

  - I_0 = Intensity of incident light
  - I = Intensity of transmitted light

- **Relationship between Absorbance and Transmittance:**

  $A=-\log10(T) or T=10-A$

  - *Example:* A = 1.0 means 10% transmittance (T=0.1); A = 2.0 means 1% transmittance (T=0.01).

Key Components of a Spectrophotometer

A spectrophotometer is built to make precise measurements of A or T. Its essential components are:

1. **Light Source:** Provides a broad spectrum of light.
   - **Tungsten Lamp:** For visible region (~400 - 800 nm).
   - **Deuterium Arc Lamp:** For ultraviolet region (~200 - 400 nm).
2. **Monochromator:** Isolates a specific wavelength of light from the source.
   - Consists of an **entrance slit**, a **diffraction grating** (or prism), and an **exit slit**.

- The **bandwidth** is the width of the wavelength range passed by the monochromator. Narrower bandwidth provides better resolution.

3. **Cuvette Holder:** Holds the sample and reference solutions.

- **Cuvette Material:**

- **Glass:** For visible light.
- **Quartz (Fused Silica):** For UV and visible light (transparent down to ~200 nm).
- **Plastic:** Cheap, disposable, for visible light only.

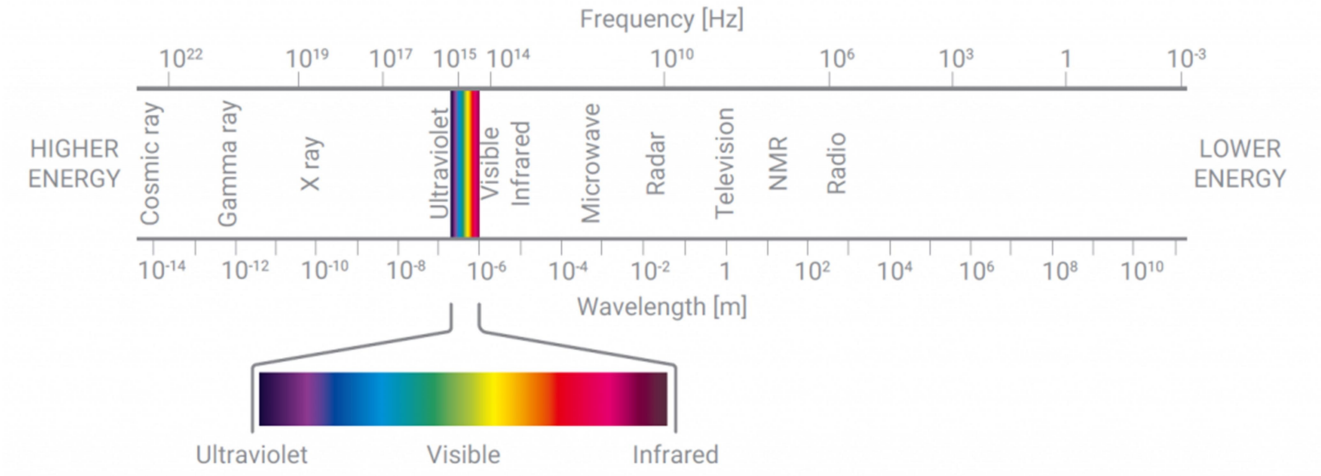
4. **Detector:** Converts light intensity into an electrical signal.

- **Phototube / Photomultiplier Tube (PMT):** Highly sensitive, especially for low-light applications.
- **Photodiode Array (PDA):** Can detect multiple wavelengths simultaneously, enabling very fast scanning.

5. **Readout Device:** A computer or digital display that processes the signal from the detector and outputs the absorbance or transmittance value.

LIST OF VARIOUS SPECTROSCOPIC TECHNIQUES

Types of Energy Transfer	Spectroscopic Technique	Region of Electromagnetic Spectrum	Application
Absorption	UV/Visible spectroscopy	UV/Visible	Detection of functional groups, extent of conjugation, and determines the configurations of geometrical isomers
	Atomic absorption spectroscopy	UV/Visible	Determines the amount of various levels of metals and other electrolytes within samples
	Infrared spectroscopy	Infrared	Determines the functional groups
	Raman spectroscopy	Infrared	Contaminant identification, gemstone and mineral identification
	Nuclear magnetic resonance spectroscopy	Radio wave	Provides information about the structure and chemical environment of atoms
	X-ray absorption spectroscopy	X-ray	Determines the elemental composition and chemical bonding of molecules
Emission	Atomic emission spectroscopy	UV/Visible	Detection of trace metals, minerals, sodium potassium and lithium
	Mass spectrometer		Analysis of proteins, peptides, checking water quality and food contamination
Photolumi nescence	Fluorescence spectroscopy	UV/Visible	Detection of many of organic compounds, numerous aromatic active substances in drug
	Phosphorescence spectroscopy	UV/Visible	used as a tool for analysis and structure determination.



- **Applications:**

Nucleic Acid Quantification and Purity:

- **Concentration:** Pure dsDNA concentration = A_{260} × Dilution Factor × 50 µg/mL.
- **Purity Ratios:**

  A_{260}/A_{280}: ~1.8 for pure DNA; ~2.0 for pure RNA. Lower ratios indicate protein contamination.

  A_{260}/A_{230}: Should be >2.0. Lower values indicate contamination by chaotropic salts or organics.

Protein Quantification:

- **Direct A_{280}:** Uses the intrinsic absorbance of Trp and Tyr. Requires a known extinction coefficient.
- **Colorimetric Assays:** More reliable for complex mixtures.
- **Bradford Assay:** Coomassie dye binds protein, shifts λ_max from 465 to 595 nm.
- **Lowry Assay:** Biuret reaction + Folin-Ciocalteu reagent.
- **BCA Assay:** Similar to Lowry, but more compatible with detergents.

Enzyme Kinetics: Monitor reaction progress by measuring the appearance/disappearance of a light-absorbing product/substrate over time.

Blotting Techniques

- **Principle:** Transferring biomolecules separated by electrophoresis onto a solid membrane for detection with a specific probe.
- **The General Blotting Process**

All blotting techniques follow a similar core workflow:

a) Separation: Molecules are separated by gel electrophoresis (Agarose for nucleic acids, SDS-PAGE for proteins).

b) Transfer: The separated molecules are transferred from the gel onto a thin membrane (usually nitrocellulose or nylon).

c) Blocking: The membrane is treated with a blocking agent (e.g., BSA, non-fat milk) to prevent the probe from binding non-specifically to the membrane.

d) Probing: The membrane is incubated with a labeled probe that binds specifically to the target molecule.

e) Detection: The bound probe is detected, often resulting in a visible or measurable signal (e.g., bands on an X-ray film, chemiluminescent signal).

- **Comparison Table:**

Parameters	Southern Blotting	Western Blotting	Northern Blotting
Target molecule	DNA	Protein	RNA
Membrane used	Nitrocellulose or Nylon membrane	Nitrocellulose or PVDF membrane	diazobenzoxymethyl (DBM) Or Nylon membrane
Separation Technique	Agarose or polyacrylamide gel electrophoresis	PAGE (Polyacrylamide gel electrophoresis)	Agarose gel electrophoresis
Probe used	A nucleic acid probe having a homologous sequence with the target molecules	Primary antibody	DNA, RNA, and oligodeoxynucleotides
Probe label used	Radiolabel enzyme	Enzyme	Radiolabel enzyme
Detection methods	Chemiluminescence, colorimetry, and X-ray film	Infrared imaging system, cooled CCD, or LED imaging system	Chemiluminescence, colorimetry, and X-ray film
Blotting Technique	Capillary transfer	Electroblotting	Capillary transfer
Applications	Used in forensics and study of DNA mutations	Used in identifying specific antigens or proteins from different biological samples	Used in gene expression studies and helps in disease diagnosis

DNA Fingerprinting (DNA Profiling)

- **Principle:** Identifies individuals by analyzing variations in highly variable, non-coding regions of their DNA called **Short Tandem Repeats (STRs)**.
- **Invented by:** Sir Alec Jeffreys in 1984 at the University of Leicester, UK.
- **First Application:** It was first used in 1985 to resolve a UK immigration case and later gained worldwide fame in 1986 for its use in the **Colin Pitchfork case**, the first murder conviction based on DNA evidence.
- **Process:**
    1. **Extract** DNA from a sample (e.g., blood, saliva).
    2. **Amplify** specific STR loci using **Multiplex PCR**.
    3. **Separate** the amplified DNA fragments by **Capillary Electrophoresis** (a high-resolution form).
    4. **Detect** the fragments and generate a unique profile based on the number of repeats at each locus.
- **Application:** Forensics, paternity testing, ancestry tracing.

About us

BioResire (NEET | CSIR NET | Biotech Internships) is a life sciences research and training organization dedicated to bridging the gap between academic learning and industry skills. We provide internships, projects, and programs in Bioinformatics, Biotechnology, Molecular Biology, Cancer Research, Neuroscience, and related fields, helping students build job-oriented scientific careers.

"The future belongs to those who explore the unseen — where biology meets innovation and discovery begins."